# Structure–Activity Relationships of Dihydrofolate Reductase Inhibitors

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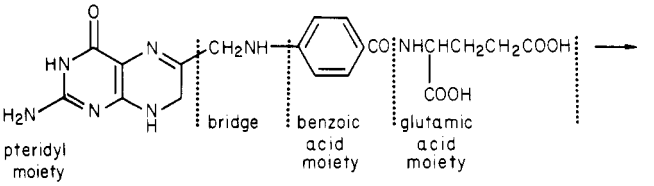
## Contents



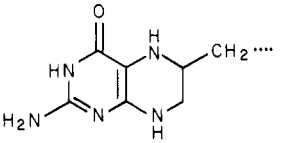
## I. Introduction

Dihydrofolate reductase [5,6,7,8-tetrahydrofolate: $NADP^+$ oxidoreductase (EC 1.5.1.3)] is an enzyme of central importance in biochemistry and medicinal chemistry. It is with the latter area that this review is principally concerned. The function of this enzyme is to reduce dihydrofolic acid (I) to tetrahydrofolic acid (II).

In the view of I the molecule has been divided into the four regions which have been varied in structure in many attempts to develop better inhibitors of dihydrofolate reductase (DHFR) for chemotherapeutic purposes. As of the present over 1700 inhibitors of this reaction have been studied on DHFR from many sources (see Appendix) and many others have been



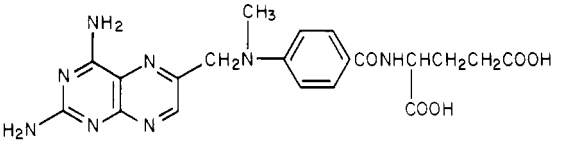

I



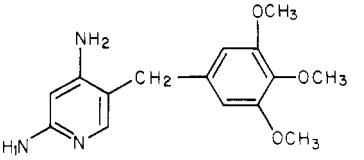
II

tested as antitumor agents or on cell cultures of microorganisms.

Two compounds in particular have provided much of the stimulation for this research. Methotrexate (III), synthesized in 1949 by Seegar et al.,[1] is still one of the most widely used antitumor drugs[2] and is still the most effective antifolate antitumor drug after more than 30 years of clinical use.[3] Trimethoprim (IV), which developed out of the work of Hitchings and Roth[4] and their colleagues, created a storm of publications on the search for better antimicrobial agents. Roth has re-



III (MTX)



IV (TMP)

cently carefully reviewed the history of the development of trimethoprim as well as current ideas on the struc-

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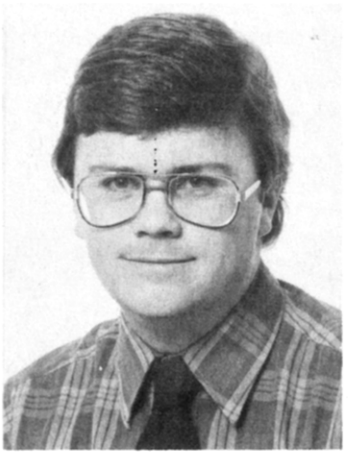
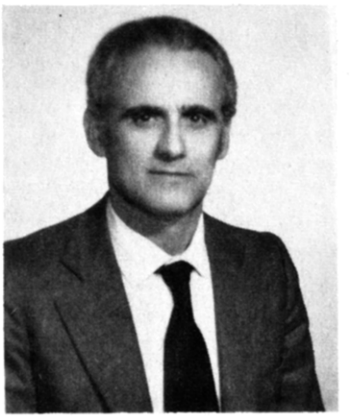
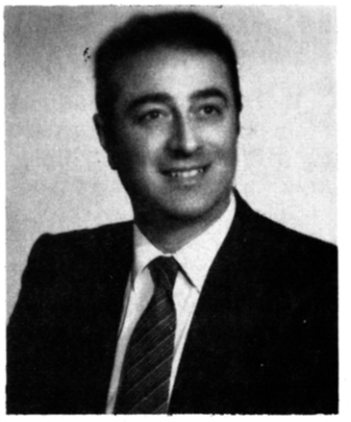


Jeffrey Blaney was born in 1956 in Los Angeles, CA. He received his B.A. in Chemistry/Zoology in 1978 from Pomona College, Claremont, CA, and his Ph.D. in Pharmaceutical Chemistry in 1982 from the University of California at San Francisco. He is currently a research chemist in the computer-assisted drug design group in the Biomedical Products Department at E. I. du Pont de Nemours & Co., Inc. His research interests include structure–activity relationships, drug design, ligand–macromolecule interactions, and the development and application of molecular modeling methods using high-performance computer graphics systems.

Corwin Hansch in 1944 received his Ph.D. from New York University in the field of synthetic organic chemistry, studying under Professor H. G. Lindwall. After a brief postdoctoral period with Professor H. R. Snyder at the University of Illinois, he joined the du Pont Co. and worked first on the Manhattan project at the University of Chicago and Richland, WA, and then at the experimental station in Wilmington, DE. In 1946 he joined the Chemistry Department at Pomona College, where he has remained except for two sabbatical leaves, one in Professor Prelog's laboratory in Zurich and the other in Professor Huisgen's laboratory in Munich. His main interests in research have been the high-temperature dehydrocyclization reaction and the correlation of chemical structure with biological activity.



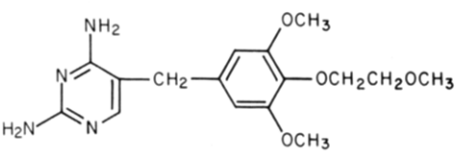
Carlo Silipo received his doctoral degree in Chemistry from the University of Naples. After a postdoctoral study at the Institute of Pharmaceutical and Toxicological Chemistry of Naples, in 1969, he joined the faculty of Pharmacy in Naples as assistant professor in Analytical and Pharmaceutical Chemistry. During 1973–1974 he was a NATO fellow at the Chemistry Department of Pomona College, where he was awarded the title of Visiting Research Professor in Chemistry. Since then he spent as Visiting Scientist several summers at Pomona College working with Corwin Hansch on joint research programs. In 1980 he obtained the full professorship in Pharmaceutical and Toxicological Chemistry at the University of Naples.



Antonio Vittoria received his doctoral degree in Chemistry from the University of Naples. After a postdoctoral study at the Institute of Pharmaceutical and Toxicological Chemistry of Naples, he worked with Mobil Chimica Italiana as an analytical chemist. He was a NATO fellow at the Chemistry Department of Iowa State University, and in 1969 he joined the faculty of Pharmacy in Naples as assistant professor. As a Visiting Scientist at the Chemistry Department of Pomona College, he worked with Corwin Hansch. In 1980 he obtained the full professorship in Pharmaceutical and Toxicological Chemistry at the University of Naples.

ture–activity relationship of antifolates vs. microorganisms.[5]

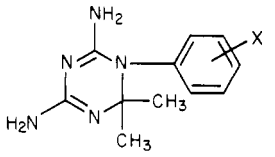
Trimethoprim is used in combination with the sulfa drug sulfamethoxazole.[6] The discovery of the synergistic effect of the sulfas was made by Daniel and Norris in 1947,[7] who recognized that the effect was due to the pteridyl moiety of I in inhibitors competing at one step in the biosynthesis of tetrahydrofolic acid and that the sulfa drugs were competing against *p*-aminobenzoic acid moiety of I at another step. However, the driving force which has created so much excitement about the folates in the antibacterial field was the discovery by Burchall and Hitchings[8] of the great specificity of TMP for bacterial DHFR compared to mammalian DHFR. Trimethoprim in combination with a sulfa drug remains, since its introduction in 1968, by far the most important antibacterial antifolate, although recently tetroxoprim (V) has become a competitor.



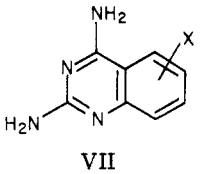

V

The antifolates are also important in the treatment of malaria, although little work has yet been published on DHFR isolated from parasites. Roth and Cheng recently reviewed the antimalarial work as well as the anticancer studies.[9]

Although methotrexate and trimethoprim remain supreme in their respective areas of chemotherapy, there is still an enormous effort to find better antifolates. Many variations of III, IV, VI, and VII have been tested as inhibitors of DHFR in cells and animals.



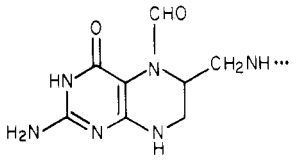
VI



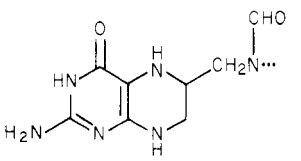
VII

Even in a review primarily directed toward the inhibitors of DHFR it is impossible to cover all that has been written. We have attempted to list all examples of inhibitors and the type of DHFR they were tested on in the Appendix. Blakley[11] wrote an excellent review of the early work on all aspects of DHFR, and since then a variety of short reviews emphasizing certain aspects of the enzyme were published.[3,5,10-20] Several collections of symposia papers also contain much material of interest on DHFR.[6]

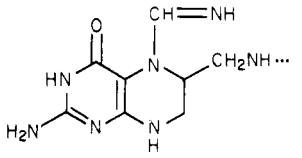
Dihydrofolate reductase's potential for medicine or in the control of plants and insects lies in the great variability of the enzyme from different sources. For example, TMP is 100 000 times more inhibitory of DHFR from *Escherichia coli* than it is of human DHFR. Hitchings[22,23] was one of the first to clearly grasp the potential of selective inhibition of DHFR for medicinal chemistry. The unusual variability in DHFR from various sources became more evident as many studies of small molecule inhibitors showed large variation in potency with reductase from different sources.[8,10,11,21,24-35]



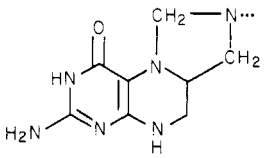
VIII, folinic acid, citrovorum factor, $N^5$-formyl-$FH_4$



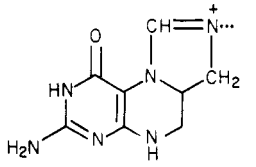
IX, 10-formyltetrahydrofolate $N^{10}$-formyl-$FH_4$



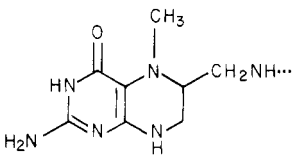
X, $N^5$-formimino-$FH_4$



XI, $N^5$,$N^{10}$-methylene-$FH_4$

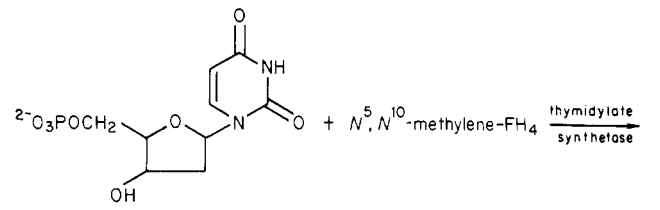


XII, $N^5$,$N^{10}$-methenyl-$FH_4$

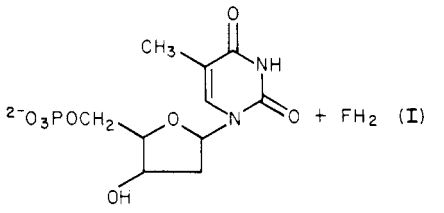


XIII, $N^5$-methyl-$FH_4$

thymidylate synthetase,[39] and this may be an important point for cancer chemotherapy.




dUMP



dTMP

## II. Biochemistry

Dihydrofolate reductase catalyzes the reduction of dihydrofolate ($FH_2$) to tetrahydrofolate ($FH_4$), using NADPH as a cofactor. The tetrahydrofolate is then converted to one-carbon adducts VIII–XIII.

These adducts are utilized in a number of one-carbon transfers in the biosynthesis of thymidylate, purines, serine, and methionine.[11,36] Some aspects of the mechanism of these processes have been discussed by Benkovics.[37] The reaction of most interest in chemotherapy is the synthesis of thymidylate from deoxyuridine monophosphate (dUMP), and this process is vitally dependent on a supply of 5,10-methylene-$FH_4$ (X). Blocking the formation of tetrahydrofolate ($FH_4$) by the inhibition of DHFR or inhibition of thymidylate synthetase can shut off the supply of dTMP necessary for DNA synthesis. In the cell this leads to what is called thymineless death. The inhibition of dTMP synthesis by blocking both DHFR and thymidylate synthetase has attracted the attention of many medicinal chemists.[38] An interesting aspect of this problem is that some inhibitors which block DHFR also inhibit

Figure 1 from Roth et al.[3] summarizes the biochemical pathways involving DHFR. Crucial enzymes are numbered as follows: (1) dihydrofolate reductase (EC 1.5.1.3), (2) thymidylate synthetase (EC 2.1.1.4.5), (3) methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5), (4) phosphoribosyl glucinamide formyltransferase (EC 2.1.2.2), (5) phosphoglutamate methyltransferase (EC 2.1.2.3), (6) tetrahydropteroylglutamate methyltransferase (EC 2.1.1.13). The compounds represented in the boxes are as follows: 5>$NCH_2NH$10 = $N^5$,$N^{10}$-methylene-$FH_4$ (XI), 5>N-$CH_3$ = $N^5$-methyl-$FH_4$ (XIII), 5>$N^+$=CH—N = $N^5$,$N^{10}$-methylenyl-$FH_4$ (XII), 5>N—CHO = $N^5$-formyl-$FH_4$ (VIII), 5>N—CH=NH = $N^5$-formimino-$FH_4$ (X), hCys = homocysteine, FGAR = formylglycinamide ribonucleotide, GAR = glycinamide ribonucleotide, AICAR = aminoimidazolecarboxamide ribonucleotide, FAICAR = 5-formamidoimidazole-4-carboxamide ribonucleotide, dUMP = deoxyuridylate, AMP = adenosine monophosphate (adenylic acid), GMP = guaninemonophosphate (guanylic acid), dTMP = thymidylate, FU

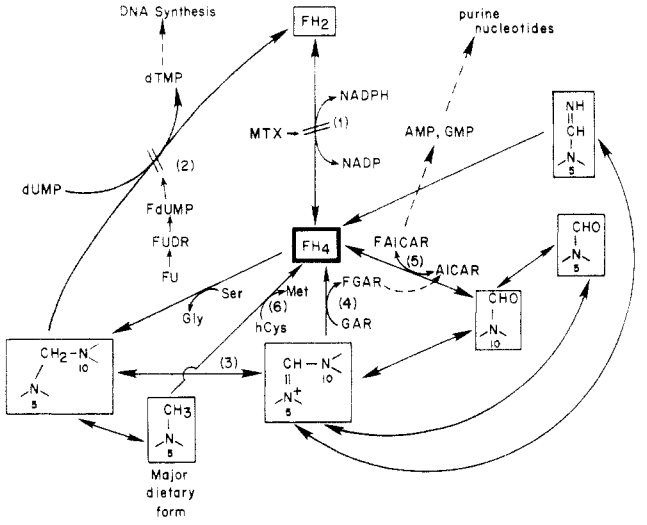



**Figure 1.**

= 5-fluorouracil, FUDR = 5-fluorodeoxyuridine, FdUMP = 5-fluorodeoxyuridylate.

Tetrahydrofolate reaches the cell by active transport[40-44] of especially its 5-methyl and 5-formyl derivatives in mammalian cells. Many bacterial cells synthesize their own $FH_4$. In Figure 1 it can be seen that a major role of DHFR is to reduce $FH_2$ which is produced in the thymidylate conversion of dUMP to dTMP. This is a consequence of the fact that dTMP synthesis (Figure 1, path 2) depletes the $FH_4$ pool more rapidly than path 6 can resupply it.

Since the production of dTMP from dUMP is the only known source of thymidylate for DNA synthesis blocking, this source stops DNA replication in all forms of life. Hence, DHFR inhibitors offer the potential for control of any undesirable form of life.

The exact mechanism of reduction of dihydrofolic acid by DHFR is still not completely understood despite a large effort by many investigators over many years. It is complicated by the fact that both substrates (folic acid or dihydrofolic acid) and cofactor NADPH must be bound to the enzyme for the transfer of hydride from NADPH to the 5,6-double bond of the folate. It now appears that random binding is involved. That is, either the NADPH or the folate may bind first. The same mechanism probably occurs with inhibitors although there is evidence that the binding of NADPH enhances binding of other ligands. Recently Stone and Morrison[45] have examined nine possible mechanisms for the reduction process and concluded that it is a rapid equilibrium random mechanism with a single dead-end enzyme–THF–DHF complex or possibly an additional enzyme–NADPH–THF complex. The reduction product $FH_4$ is so strongly bound that it can act as an inhibitor and when present in significant amounts regulate the reduction rate. Another complicating factor is that $FH_4$ appears to bind at the NADPH binding site. High concentrations of NADPH prevent this complex from forming. Dihydrofolate also appears to be capable of binding to the site of the pyridine nucleotides.

The rapid advances in the study of DHFR in recent years have been made possible by the isolation of highly purified material via affinity chromatography. The purification of DHFR from chicken liver by Kaufman and Pierce[46] and of the $T_4$ phage specified enzyme in

**TABLE I. Sequence Alignment of Seven Dihydrofolate Reductases according to Structural Equivalence**

```
Ec numbering    1       10        20        30              40
                :       :         : I       :          II   :

S. faecium II   MFISMWAQDKNGLIGKDGLLPW-RLPNDMRFFREHTM-------DKIL
L. casei         TAFLWAQNRDGLIGKDGHLPW-HLPDDLHYFRAQTV-------GKIM
E. coli MB1428  MTSLIAALAVDRVIGMENAMPW-NLPADLAWFKRNTL-------NKPV
                  |-βA--|                 |----αB-----|       |--
Chicken liver VRSLNSIVAVCQNMGIGKDGNLPWPPLRNEYKYFQRMTSTSHVEGKQNAV
Bovine liver  VRPLNCIVAVSQNMGIGKNGDLPWPPLRNEFQYFQRMTTVSSVEGKQNLV
Mouse L-1210  VRPLNCIVAVSQNMGIGKNGDLPWPPLRNEFKYFQRMTTTSSVEGKQNLV
Porcine liver VRPLNCIVAVSQNMGIGKNGDLPWPPLRNEYKYFQRMTTTSSVEGKQNLV
                :       :         :         :         :         :
Cl numbering    1       10        20        30        40        50

           41       50        60        70        80
            :       : III     :         : IV      :
          VMGRKTYEGMG--KLSLPYRHIIVLTTQKDFKVEKNAEVLHSIDELLAYA
          VVGRRTYESFP--KRPLPERTNVVLTHQEDYQAQG-AVVVHDVAAVFAYA
          IMGRHTWESIG---RPLPGRKNIILSSQP-GTDDR-VTWVKSVDEAIAAC
          βB-||-αC-|          |-βC-|        |βD| |---αE---
          IMGKKTWFSIPEKNRPLKDRINIVLSRELKEAPKGAHYLSKSLDDALALL
          IMGRKTWFSIPEKNRPLKDRINIVLSRELKEPPKGAHFLAKSLDDALELI
          IMGRKTWFSIPEKNRPLKDRINIVLSRELKEPPRGAHFLAKSLDDALRLI
          IMGRKTWFSIPEKNRPLKDRINIVLSRELKEPPQGAHFLAKSLDDALKLI
          :       :         :         :         :         :
         51       60        70        80        90        100

                   90        100       110       120
                V  :         :    VI   :         :
          KDIPE-----DIYVSGGSRIFQALL--PETKIIWRTLIDAEFEGDTFIGE
          KQHLDQ----ELVIAGGAQIFTAFK--DDVDTLLVTRLAGSFEGDTKMIP
          GNVP------EIMVIGGGRVYEQFL--PKAQKLYLTHIDAEVEGDTHFPD
          |    |αE'| |-βE-| |---αF---|    |---βF--|
          DSPELKSKVDMVWIVGGTAVYKAAMEKPINRRLFVTRILHEFESDTFFPE
          QDPELTNKVDVVWIVGGSSVYKEAMNKPGHVRLFVTRIMQEFEADSFFPE
          EQPELASKVDMVWIVGGSSVYEQAMNEPGHLRLFVTRIMQEFESDTFFPE
          EQPELKDKVDMVWIVGGSSVYKEAMNKPGHIRLFVTRIMKEFESDTFFPE
          :       :         :         :         :         :
        101       110       120       130       140       150

          130             140       150       160
            :           VII :    VIII :         :
          IDFTSFELVEEHEGI--VNQENQY-PHRFQKWQKMSKVV
          LNWDDFTKVSS------RTVEDTN-PALTHTYEVWQKKA
          YEPDDWESVFS------EFHNADAQNSHSYCFKILERR
                |———βC———|       |—βH—|
          IDYDKFKLLTEYPGVPADIQEEDG---IQYKFEVYQKSVLAQ
          IDFEKYKLLPEYPGVPLDVQEEKG---IKYKFEVYEKNN
          IDLEKYKLLPEYPGVLSEVQEEDG---IKYKFEVYEKKD
          IDLEKYKLLSECSGVPSDVQEEKG---IKYKFEVYEKNN
          :       :         :         :
        151       160       170       180
```

*E. coli* by Erickson and Mathews[47] marked the beginning of a new sophistication in DHFR research. The problems involved in obtaining a pure substrate-free enzyme have been discussed by Kaufman and Kemerer.[48]

The properties of the various DHFR which have been purified have been reviewed and compared by Freisheim and Matthews.[16] Table I contains the sequences for those forms of DHFR which have been completed.[16,49]

Data for *Streptococcus faecium* come from Gleisner et al.,[50] *Lactobacillus casei* results are from Bitar et al.,[51] *E. coli* from Bennett et al.,[52] chicken liver from Kumar et al.,[53] bovine liver from Lai et al.,[54] L1210 leukemia from Stone et al.,[55] and porcine liver from Smith et al.[56]

Since the different forms of DHFR contain different numbers of amino acid residues, it is not a straightforward task to line them up according to corresponding residues. Matthew's group employed computer graphics for ease of comparison. Models for DHFR from *E. coli*, *L. casei*, and chicken liver DHFR were constructed from X-ray crystallographic coordinates. The three models were displayed simultaneously and corresponding portions of $\alpha$ helices and $\beta$ sheets were superimposed so as to minimize differences in $\alpha$-carbon positions.[49] Hence, Table I aligns residues according to their known locations in the three reference structures. Of course for structures as different as bacterial and vertebrate DHFR a completely satisfactory correspondence of residues cannot be achieved. The normal bacterial and vertebrate DHFR contain 159–189 amino acid residues with molecular weights in the range of 18 000–22 000.

Protozoal DHFR are distinctly different and have high molecular weights (>100 000).[57-59] Ferone and Roland[60] have made the fascinating discovery that DHFR from the protozoal flagellate *Crithidia faciculata* occurs in a bifunctional form which contains thymidylate synthetase activity! They suggest that association of DHFR and thymidylate synthetase may be a general attribute of Protozoa.

Although some attempts have been made to study insect[61-63] and plant[64-66] DHFR, the amino acid sequences for plant, insect, and protozoal DHFR have not yet been established.

All of the DHFR in Table I as well as other vertebrate and microbial forms are strongly inhibited by methotrexate. However, a completely different bacterial plasmid form of DHFR has been isolated from TMP-resistant bacteria. Two types have been discovered. Type I reductase is inhibited by concentrations of TMP that are several thousand times greater than those required to inhibit the chromosomal enzyme.[67] This form appears to be a dimer with a subunit molecular weight of about 18 000.[68] Surprisingly, both types of plasmid enzyme bind $FH_2$ and NADPH about as tightly as the chromosomal enzyme.[69] The type II plasmid reductase is quite different, with a molecular weight of around 34 000 and a subunit size of about 8500.[68,70,71]

The structural properties of bacterial and vertebrate reductase in particular differ greatly, which provides a firm basis for antibacterial chemotherapy. Bacterial enzyme does not reduce folic acid to tetrahydrofolic acid while animal DHFR does. Dihydrofolate reductases from animals can be activated by mercury salts, have two activity maxima in their pH profile, and exhibit higher activity under acid conditions. Animal DHFR is relatively homologous (>70%), while identities between animal and bacterial sequences are on the order of 25%.

## III. Determination of Inhibition Constants

In attempting to simplify the presentation of test results in the appendix, we have listed a single figure which is either log $1/C$ or log $1/K_{i\,app}$. Of course these parameters are only very roughly comparable and, in fact, even the log $1/K_{i\,app}$ values are not easily compared since the quality of the DHFR used varies so much from laboratory to laboratory. It is only in recent years with the use of affinity chromatography that high-quality enzyme is more generally being studied. Many of the pitfalls involved in getting meaningful $K_i$ values have been discussed over the years.[72-76] The problems with stoichiometric inhibitors have been recognized since the classic studies of Werkheiser et al.[77,78] More recently, Morrison and his colleagues[76,79] and Cha[75] have pointed out the problems of the slow-binding inhibitors. Morrison makes the following classification of types of *reversible* inhibitors. These classifications are based on the equilibrium of the reaction:

$$E \underset{k_2}{\overset{k_1, I_t}{\rightleftharpoons}} EI$$

where $E_t$ is the total enzyme concentration and $I_t$ is the reduction in the inhibitor concentration that occurs on the formation of EI, the enzyme–inhibitor complex. In the classical case (1) studies are made under steady-state conditions where the concentration of enzyme is very much less than that of the inhibitor. It is generally assumed that this is the case with the weak to moderately active reversible DHFR inhibitors. Werkheiser showed that methotrexate inhibits DHFR at concentrations comparable to that of the enzyme. concentrations comparable to that of the enzyme. For such tight-binding inhibitors allowance must be made for the reduction in the inhibitor concentration that occurs on formation of the complex. For many $K_i$ values of highly active compounds in the Appendix, this has not been considered in estimating the $K_i$. Morrison points out that as the strength of interaction between an enzyme and a tight-binding inhibitor increases a point is reached when equilibrium cannot be rapidly established. This slow establishment of full inhibition has long been known; however, it is now clear[76] that slow establishment of equilibrium can occur either with or without tight binding. This appears to be a general problem occurring with many enzymes in addition to DHFR.[79] In studying the slow-binding inhibitors, continuous monitoring of the reaction is necessary if coupling enzymes are used and it is important to use a range of enzyme concentrations to follow the reaction over extended time periods.

For careful SAR work it is recommended that one establish 95% confidence limits on $K_{i\,app}$.[80]

More recently, a number of investigators have turned to radiochemical methods of assay.[81-87] Of course, these methods yield binding constants which may or may not be similar to $K_i$ values.

| class of inhibitor | relationship between $E_t$ and $I_t$ | rate of equilibrium establishment between E, I, and EI |
|---|---|---|
| 1. classical | $I_t >> E_t$ | fast |
| 2. tight-binding | $I_t \simeq E_t$ | fast |
| 3. slow-binding | $I_t >> E_t$ | slow |
| 4. slow, tight-binding | $I_t \simeq E_t$ | slow |

## IV. Qualitative Structure–Activity Relationships of DHFR Inhibitors

### a. General Observations

The discovery of the bacterial inhibitory properties of certain pteridines was made in the 1940s by Daniel et al.[7,88] Since this inhibition could be reversed by folates, the inhibitors were presumed to be antimetabolites of folic acid. The tremendous amount of work since then has shown that all parts of the folic acid molecule can be utilized as models for the design of DHFR inhibitors. The greatest effort has been made studying variations of 2,4-diaminodiazines, hundreds of examples of which are given in the Appendix. Alterations have been made in the four segments of folic acid as shown in I.

The only change needed to convert folic acid to a strong inhibitor is the replacement of the 4-keto group by 4-$NH_2$. The role of the 4-$NH_2$ group has been much discussed. B. R. Baker[10,89] was the first to present evidence for the increased basicity of N1 being crucial. This thinking was supported by the quantum chemical calculations of Perault and Pullman[90] and Neely.[91] Zakrzewski has argued for the importance of hydrogen bonding.[10,92] There is now evidence from X-ray crys-

tallography that the 4-$NH_2$ in *L. casei* DHFR is hydrogen bonded to the carbonyl oxygens of Leu-4 and Ala-97.[93] There is also good NMR evidence that methotrexate is protonated in the bound state and that this probably accounts for the difference in binding between folic acid and MTX.[94] Matthews and his associates have concluded from their crystallography work that the $COO^-$ group of Asp-29 forms a salt bridge to N1 and the 2-$NH_2$ group of MTX. Thus, it appears that both Baker and Zakrzewski were correct. Gready[12] has reviewed many of the details of the various arguments for the importance of protonation at N1 in the binding of 2,4-diaminopyrimidines to DHFR.

Knowing the great importance of protonation for binding of 2,4-diaminopyrimidines, it is easily understood that replacement of either amino group by the less electron releasing groups –SH, –OH, or H results in much weaker inhibitors.

In an early study of a portion of the folic acid molecule Baker found that one could drop the pteridyl ring and still produce compounds with weak inhibitory activity. For example benzoyl glutamate has a $pI_{50}$ of 1.92 with pigeon liver DHFR.[10,95] For example benzoyl glutamate has a $pI_{50}$ of 1.92 with pigeon liver DHFR.[10,95] Adding the hydrophobic fragment $n$-$C_8H_{17}$ to the 4-position of the benzene ring increases $pI_{50}$ to 3.77. Even the simple benzoic acid $C_8H_{17}C_6H_4COOH$ had $pI_{50}$ of 2.25.

Poe[96] has found that simple sulfa drugs act as weak inhibitors (log $1/C$ = 3–4), probably acting at the same site as the benzoic acids. He also noted that pyrimethamine and 4,4′-diaminophenyl sulfone bind simultaneously to DHFR. More recently, Birdsall et al.[97] have shown that 2,4-diaminopyrimidine and analogues of *p*-aminobenzoyl L-glutamate bind in a cooperative fashion to *L. casei* DHFR. It is especially interesting that NADPH increases the affinity of both molecules since NADPH is known to increase the binding of inhibitors to DHFR.[98,99]

Much attention has been devoted to studies of the modification of the bridge (C9–N10) structure between the pteridyl and phenyl moieties. These two atoms have been replaced by (see Appendix Table II for details) S, $CH_2$, $CH_2CH_2$, $CH_2S$, $CH_2O$, CH=CH, $NHCH_2$, $CH_2N(CH_3)$, $CH_2N(C_2H_5)$, $CH_2N(CHO)$, $CH_2N(C_4H_9)$, $CH_2N(C_6H_5)$, $CH_2N(SO_2C_6H_4\text{-}4'\text{-}CH_3)$, $CH_2N(C_3H_7)$, $CH_2N(CH_2C_6H_5)$, $CH_2CH_2NH$, $CH_2CH_2O$, $CH_2CH_2S$, $CH_2NHCH_2$, $CH_2NHNH$, and CH=NNH. While these compounds retain various degrees of inhibitory activity, little can be said with confidence in a general way since in some instances changes have been made simultaneously on the pteridyl ring and the bridge moiety. Also, the various derivatives have been tested under different conditions with use of DHFR from different sources. Nair et al.[100] conclude that the structural changes in the bridge region of analogues of aminopterin (120, Appendix Table II), in which the bridge length is not altered, do not significantly affect DHFR inhibition but do cause changes in transport (i.e., 10-deaza, 10-oxa, 10-thio). The elongation of the bridge region of these analogues results in decreased enzyme inhibition and transport and hence lower antitumor activity.[100] The most studied bridge is that of methotrexate [$CH_2N(CH_3)$].

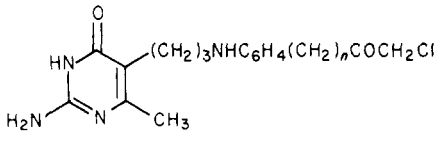
Replacing N by C in the 3-deazo compound does not greatly lower activity (compare 12 and 15, Appendix Table II). Even replacing N1 by carbon produces a compound which is still a strong inhibitor (87, 163, Appendix Table II). See also Elliot et al. for discussion.[101] The N5 and N8 nitrogen atoms are not at all essential for inhibitory activity since the quinazolines of Table III (Appendix), in which both nitrogens are replaced by carbon, are often extremely potent inhibitors.

It is of interest that complete hydrogenation of the one pteridine ring (5,6,7,8-tetrahydro) lowers activity significantly compared to the dihydro or completely unsaturated compounds (compare 152 with 161, 153 with 162, 178 with 179, Appendix Table II). The lower activity of the saturated compound is most probably due to the greatly increased hydrophilicity of the saturated amine (which would be protonated in the usual buffer) compared to that of the unsaturated compound.

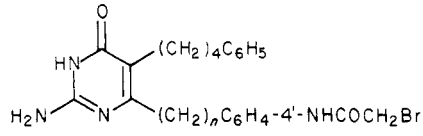
Another such case may be compounds 231 and 232 where the N8–O bond makes for much greater hydrophilic character.

Ever since Baker's intense effort to find what he termed active-site-directed irreversible inhibitors there have been attempts to find such inhibitors of DHFR.[10,102,103] In a recent discussion of the problem, Rosowsky et al.[104] cite extensive evidence that very small amounts (<5%) of intracellular DHFR not blocked by inhibitors can generate enough thymidylate for adequate cell growth. Also, they cite evidence for the presence of small amounts of DHFR with low MTX affinity present in cells. These two factors are serious problems for those concerned with cancer chemotherapy. Therefore, it would be highly desirable to find antifolates which bind to DHFR more strongly than does methotrexate.

Baker's attack on this problem is instructive to those interested in enzyme inhibition. His general approach was to first modify substrates to obtain potent reversible inhibitors. The next step was to attach very large substituents to the positions available on the parent inhibitor molecule (e.g., $(CH_2)_nOC_6H_4X$). From the $I_{50}$ values of such derivatives one could draw qualitative conclusions about the bulk tolerance of the region around the active site. By comparing $\pi$ constants of the substituents with $I_{50}$ values Baker could also assess the surrounding areas for hydrophobic space. In the final phase of the work Baker attached functions ($SO_2F$, $COCH_2Br$, etc.) capable of reacting with nucleophilic groups of the enzyme in such a way that they would not fall into hydrophobic space. He reasoned that there would be few reactive groups in hydrophobic space for covalent binding by nucleophilic substitution. Baker reasoned that if one were to search for differences between enzyme from host and enzyme from pathogenic cell, there would be a higher probability of finding such differences outside of the crucial amino acid residues of the active site. Compounds XIV and XV illustrate how binding in different regions of the enzyme can lead to either reversible or nonreversible inhibitors.[10] Compounds of type XIV proved to be reversible inhibitors while those of type XIII were in some instances irreversible. Baker's modus operandi was to introduce a large hydrophobic group to fit into known hydrophobic space ($(CH_2)_4C_6H_5$ in XV) and then attach a long "arm" carrying a group which could react with



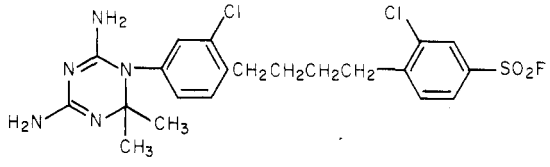
XIV



XV

nucleophiles. He visualized such groups projecting beyond the active site and turning back to react with surface polar groups.

Baker studied the $SO_2F$ substituent in scores of inhibitors.[10] Among these sulfonyl fluorides some turned out to be very effective irreversible agents, while others reacted reversibly with DHFR. One of the difficulties with such an approach to drug design is that the drug must not interact significantly with nucleophilic groups in any of the other macromolecules (or small molecules) it encounters during the random walk in the animal to the enzyme. An attractive feature of the $SO_2F$ function in this respect is its relative inertness. For example, it does not react with so basic a molecule as pyridine or hot ethanol or hot acid solution. It does, however, react with OH groups in cellulose when bound via attachment to an absorbable dye molecule. Fölsch and Bertino[105] discovered that the $SO_2F$ group is rapidly hydrolyzed by mouse serum in vitro but not by several other sera including rat and human. Baker[106] then showed that the hydrolase chymotrypsin would hydrolyze the sulfonyl fluoride function and, moreover, that when triazine VI with a 4-$CH_2CH_2CONHC_6H_3$-3′-Me-4′-$SO_2F$ group labeled with $C_{14}$ was fed to rats, only one product, the sulfonic acid, could be isolated in the urine or feces. Since 80% of the fed material could be accounted for in this form, degradation was rather complete.[107] It was also shown that enzymes in rat liver rapidly hydrolyzed the $SO_2F$ function.[107] Nevertheless, the sulfonyl fluoride XVI designed by Baker has shown promise as



XVI

an antitumor agent in clinical trials and is particularly interesting because of its activity against solid tumors. While experience with fluorosulfonyl compounds suggests that this substance must be rapidly hydrolyzed in vivo, the resulting sulfonic acid, which has a calculated octanol/water log $P$ value of −1.6, would still be 10 times more lipophilic than methotrexate, whose log $P$ is −2.6. Hence, it might enter cells well enough by passive diffusion to be effective.

Recently it has been established that XVI does react irreversibly with the OH of a tyrosine residue in chicken liver DHFR.[103]

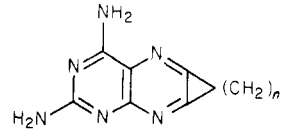
There has been a large amount of experimentation with alteration of the glutamate side chain, especially with MTX. Substitution of the glutamyl group by amino acids,[108-111] peptides,[112] esters,[113,114] or amides[115] did not improve in vivo activity. Examples listed in the Appendix (Table II) which were tested on isolated enzyme have not yielded conclusive results except that transport into cells may be affected.[111,112,115] Polyglutamate derivatives of MTX have been found in various MTX-treated tissues and have been shown to be inhibitors of DHFR[116,117] after being first isolated by Baugh et al.[118]

Even more gross modifications of the glutamate portion of the MTX side chain have been accomplished by the attachment of MTX to polypeptides and proteins[119-121] such as serum albumin,[122-124] immunoglobulins,[114] polylysine,[125] and starch.[120,126] The peptides and protein derivatives appear to be taken up by resistant cells and then hydrolyzed to release MTX inside the cell.[120,121]

The most interesting result of the modification of the benzene ring of the aminobenzoic acid moiety from the viewpoint of chemotherapy is the 3,5-dichloro analogue which has received considerable in vivo and clinical study. It appears to be less toxic to humans than MTX but is metabolized more rapidly. Roth and Cheng[9] review studies of this more lipophilic MTX analogue in the context of other lipophilic antifols which have become more interesting because of their ability to penetrate lipophilic sanctuaries, such as the CNS, in cancer thermotherapy. Greco and Hakala[127] have shown for several antifols of varying degrees of hydrophobicity that the extent of their uptake and growth inhibitory potency of tumor cells, as well as their affinity to DHFR, are correlated with the octanol/water partition coefficients; the more lipophilic, the more potent.[127]

In the important 2,4-diaminopyrimidine, substitution of the amino groups with methyl results in great decrease in inhibitor power.[128]

An interesting set of cycloalka[$g$]pteridines (XVII) has been prepared and tested against DHFR from four sources: rat liver, L1210, *L. casei*, and *T. cruzi*.[34] Two

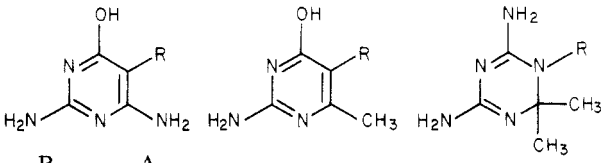


XVII

objectives were involved in this approach; an increase in hydrophobicity while at the same time blocking the 7-position which metabolizes[129-131] to 7-OH in MTX. The 7-OH MTX is much less potent as a DHFR inhibitor than methotrexate.[132] Other analogues of MTX have also been shown to undergo oxidation by liver aldehyde oxidase.[132] As one might expect from their greater lipophilic character, the halogenated derivatives of MTX are more susceptible to oxidation than MTX.[133-135]

By studying different types of pyrimidine rings it became clear to Baker that there must be more than one mode for the binding of the heterocyclic moiety. For example, a comparison of the $\mu MI_{50}$ values obtained from pigeon liver DHFR for the following three series is instructive.[10]

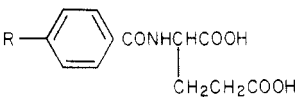
The ethyl group in the A series actually makes for a slightly poorer inhibitor compared to the parent com-



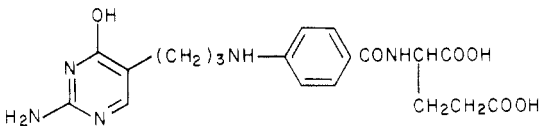
| R | A | B | C |
|---|---|---|---|
| H | 13000 | 19000 | |
| $C_2H_5$ | 17000 | 2200 | 220 |
| $n$-$C_3H_7$ | 450 | 1100 | 11 |
| $n$-$C_4H_9$ | 40 | 260 | 0.36 |
| $n$-$C_6H_{13}$ | 30 | 35 | 0.32 |
| $n$-$C_8H_{17}$ | 48 | 230 | 0.14 |

pound, while in the B series activity is increased about 9-fold. Activity in the two series seems to converge with the hexyl derivatives but changes significantly with the octyl derivatives. For series A the octyl derivative has essentially the same activity as the hexyl, but for series B octyl is considerably less active. In series C the octyl analogue is more active. It is difficult to imagine that in each case R is binding in the same position in enzymic space.[10]

In exploring the nature of hydrophobic space Baker made an interesting comparison between a series of benzoyl glutamates XVIII and the pyrimidine XIX. There is little difference in activity when R of XVIII is $NH_2$ or H; however, when R is increased to octyl, $pI_{50}$ = 3.77, almost the same as XIX ($pI_{50}$ = 4.00).



XVIII



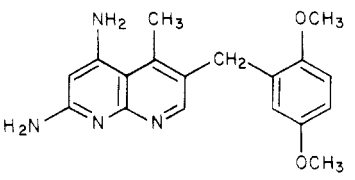
XIX

From these and other examples Baker concluded that the hydrophobic region in DHFR commenced just after the N10 atom in folic acid or MTX.

An approach to the inhibition of DHFR which has been neglected is the design of compounds to compete with NADPH. It has been found by Bertino et al.[136] that NADP is a competitive inhibitor of ascites DHFR with a log $1/K_i$ of 5.31.

## b. Quinazolines

The discovery[137,138] of significant antileukemia activity in the 2,4-diaminoquinazolines prompted a study of their inhibitory action on DHFR. Out of this work has evolved a drug which is halfway between a quinazoline and a pteridine. This compound BW 301U (XX) shows considerable promise as an antitumor agent.[139] One



XX

of the fascinating aspects of XX is that compared to other potent antifolates it is a weak inhibitor of histamine $N$-methyltransferase. Duch et al.[139] concluded from the pattern of clinical side effects of antifolates used in cancer chemotherapy and Cohn's discovery that a number of antimalarials including antifolates were effective inhibitors of the enzyme that it might be possible to make less toxic antifols by studying their activity against histamine $N$-methyltransferase.[140] Besides having low affinity for the methyltransferase, BW 301U is as potent an inhibitor of DHFR from leukemia cells as MTX.

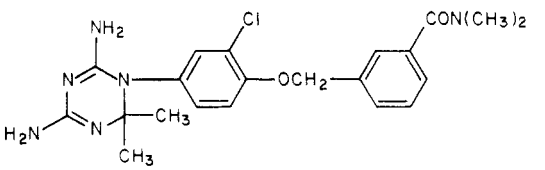
The quinazolines as a class are much more lipophilic than MTX and its ionizable congeners, and BW 301U is another example of the recent interest in lipophilic compounds for cancer chemotherapy, especially of solid tumors and brain tumors. It has been noted that the early standard of using L1210 leukemia as a test system for new drugs tended to select a preponderance of unusually hydrophilic compounds as judged by their octanol/water log $P$ values.[141] Now that there is less preoccupation with leukemia and more interest in using solid tumors for screening, lipophilic agents are attracting more attention.[142]

The quinazolines which have been assayed against DHFR from various sources are listed in the Appendix (Table III). As with the pteridines, it is readily apparent that removal or replacement of the 2-$NH_2$ by OH or SH greatly lowers activity. Replacement of the 4-$NH_2$ by OH results in only a 3–10-fold decrease in activity against rat liver DHFR, while in the case of the pteridines or pyrimidines such a change reduces activity by 2 or 3 orders of magnitude. It is also clear that while small hydrophobic substituents like halogen or methyl increase activity when placed in the 5-position, large groups in this position reduce activity. Contrary to the pteridine system, the 5,6,7,8-tetrahydroquinazolines do not show reduced activity when compared to their unsaturated analogues. This tends to support our above observation that reduction of the pteridine ring lowers activity because of the great concomitant increase in hydrophilicity.

The placement of small substituents in the 7-position in general does not enhance activity but seems to have a slightly deleterious effect.[143,144] The 8-position remains to be explored.

## c. Triazines

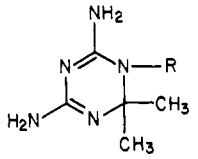
The discovery by Modest and colleagues[145] in 1952 that the 4,6-diamino-2,2-dialkyl-1,2-dihydro-$s$-triazines interfere with folic acid metabolism triggered an enormous amount of research on the antifolate activity of this class of compounds, in particular by B. R. Baker and his students.[10] Although these substances are not very active against bacteria or bacterial DHFR, they have shown definite promise in cancer chemotherapy. Two of the most promising antitumor agents to come out of Baker's extensive studies are XVI and XXI. The



XXI

activity of the triazines against various DHFR are summarized in Tables IV and V of the Appendix.

Baker used qualitative thinking to reach the conclusion that the active site and part of the region nearby is clearly hydrophobic. For example, he compared $I_{50}$ values for variations of XXII. Both the *n*-butyl and



XXII

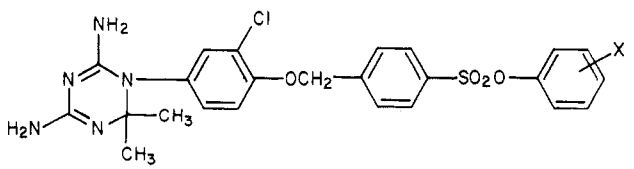
phenyl groups as R were found to make much better inhibitors than methyl or ethyl. The presence of the hydrophobic region was confirmed by showing that when polar groups were placed so as to fall in hydrophobic space, the affinity of the inhibitor was greatly reduced.

Baker's group also reached the conclusion that with phenyltriazines VI there is steric hinderance of rigid or bulky groups attached to the 3- or 4-position of the *N*-phenyl ring.

For example, the 4-$C_6H_5$-VI is 1500 times less active against pigeon liver DHFR than 4-H-VI, but the 4-$CH_2C_6H_5$-VI is much more active than the parent compound. It was also clear to them that 4-CN-VI and 4-$COOC_2H_5$-VI had unusually low activity, but it was not entirely clear whether this was to be attributed to steric or electronic properties of substituents or a combination of both. The steric effect seemed to be more important because of the low activity of 4-$N(CH_3)_2$-VI despite that $+\pi$ and $-\sigma$ values of the $N(CH_3)_2$ group. Unfortunately, Baker did not attempt to use regression analysis to sort out these problems, but later work using this technique showed Baker to be, in general, correct.[146]

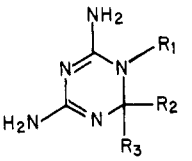
Another important structural feature of VI which was explored by Baker was substitution in the 2-position of the *N*-phenyl group. All of the substituents studied in this position (F, Cl, Br, I, $OCH_3$) yielded analogues much less active than the parent compound. Even the 2-F analogue is about 100 times less active than the parent compound with L-1210 DHFR.[146] Only recently has it been found that the 2-SH analogue is 10 times more active than the parent molecule with both bovine liver and murine L5178Y DHFR.[147]

The most potent type of triazine analogue prepared by Baker's group was XXIII.[146] These substances did not turn out to be useful in chemotherapy possibly because of the easy nucleophilic displacement of the sulfonate groups.



XXIII

Another class of triazines which has received considerable study is XXIV. The results with these antifolates are summarized in Appendix Table V. The most suitable variation of XXIV appears to be derivatives where $R_1 = R_2 = CH_3$. The analogues where $R_1$

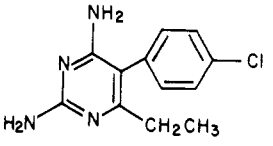


XXIV

$= R_2 = H$ have not been reported. A particularly interesting compound is 22 in Table V, where $R_2 = H$, $R_3 = CH_2C_6H_5$, and $R_1 = C_6H_4$-3′-Cl, which is about equal in activity against bacterial and vertebrate DHFR.

### d. Pyrimidines

Table VI of the Appendix contains pyrimidines, mostly 2,4-diamino, which have been tested against various forms of dihydrofolate reductase. This is the largest class of antifolates studied, probably because the 5-benzyl-2,4-diaminopyrimidines are the most potent inhibitors of bacterial DHFR. The historical aspects as well as current work on this class of compounds has been very well reviewed by Barbara Roth and her associates.[3,5,9] Among this class of compounds trimethoprim is still, although it has been in the public domain since 1959,[148] by far the most widely used antibacterial antifolate.[5] One must remember that although Table VI contains 665 pyrimidines tested on DHFR this is a fraction of the number which have been synthesized and tested against bacteria. The only direct commercial competitor of TMP is tetroxoprim (V).[149,150] Hitchings'[151] discovery of the important antifolate antimalarial pyrimethamine XXV provided strong impetus to the search for antifolate drugs. Elslager and others have extended the search for antimalarials by studying antifolates.[152]



XXV

Roth notes[5] that although there is great difficulty making comparisons among the various antifolates, the 3′,4′,5′-trisubstituted benzylpyrimidines appear to be the most potent antibacterial agents. Moreover, as with other antibacterials[153] the more lipophilic congeners are more active against Gram-positive cells.

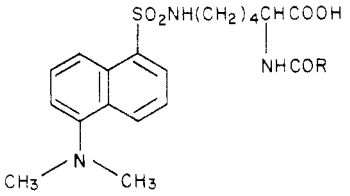
Roth points out that[154,155] among the 3′,4′,5′-trisubstituted benzylpyrimidines, those derivatives which contain a 4′-substituent forced out of plane by adjacent 3,5-substituents are the most selective antibacterial agents. According to Roth the most selective antibacterial antifolate is the 3′,5′-$(OCH_3)_2$-4′-$C(CH_3)=CH_2$ benzylpyrimidine developed by Kompis et al.[156] This substance contains two out of plane groups in the 4′-position. Roth has also suggested that one function of the 4′-substituent may be to force the two *m*-methoxy groups to be oriented away from it and hence favor interactions of the meta substituents with the enzyme. The 3′,5′-dialkyl derivatives have less selectivity for bacterial DHFR than does TMP. Roth notes[5] that this implies that the ether oxygens play a special role in binding the drugs to DHFR. A molecular graphics analysis of this problem offers a suggestion for the

greater activity of the ethers against *E. coli* DHFR compared to be *L. casei* enzyme.[31] Placing substituents in the 2′-position of the benzylpyrimidines also decreases inhibitor potency.[5] An attempt[157] to replace the benzene ring in TMP analogues with pyridine was not successful, as one might have expected from Baker's extensive studies establishing the large hydrophobic binding area in DHFR. Log *P* for pyridine = 0.65, while for benzene log *P* = 2.13.

The many examples of bridges between the diaminopyrimidine ring and the phenyl group in Table VI indicate that the $CH_2$ group is best. Stogryn[158] found that a variety of hydrophilic bridges yielded TMP analogues of low activity against bacteria. Replacing the $CH_2$ with S also gave analogues with low antibacterial activity, which is somewhat surprising since sulfur is not hydrophilic, although oxidation could make it so.

### e. Fluorescent Folate Analogues

A number of DHFR inhibitors carrying fluorescent labels have been synthesized.[159,160] In one instance fluoresceine was attached to MTX to obtain very tight-binding DHFR inhibitors which could be used to label DHFR. Variations of XXVI which are strongly fluorescent are also potent inhibitors. These com-




XXVI

pounds are almost as potent inhibitors of *L. casei*, chicken liver, and human DHFR as MTX, which makes them excellent means for labeling and identifying DHFR.[160] This is rather surprising since XXVI does not contain the usual 2,4-diaminopyrimidine constellation which seems so crucial for DHFR inhibitors. The basic dimethylamino group may react with Asp-26 carboxylate, which seems to be essential for binding. The authors note that the free "alpha" carboxyl of XXVI is vital to activity and no doubt the large hydrophobic naphthalene plays a very important binding role.

Very recently Rosowsky et al. have prepared a very effective complex derivative of methotrexate for fluorescent studies with DHFR.[160a]

## V. Quantitative Structure–Activity Relationships (QSAR)

### a. Introduction

While a great effort has been put into the study of the relationship of the chemical structure of inhibitors to their potency toward various DHFR, we are a long way from a clear understanding of the complex set of forces which promote or hinder the binding of ligands by DHFR. Much of the early work was done with very crude enzyme preparations, small numbers of inhibitors, and without knowledge of the structure of the enzyme. Now that the X-ray crystallographic structures of DHFR from three sources have been determined, and with enzymes from other sources under study, we shall soon have opportunities to see how the various forms of DHFR and inhibitors unite. Of course, even this detailed kind of information is still insufficient to explain the complete mechanism of the enzyme in its reaction with substrates and inhibitors. Matthews and his colleagues in La Jolla have reached the stage where they can determine the difference map between enzyme–cofactor and enzyme–cofactor–inhibitor in a few days in the case of chicken DHFR. Success in this endeavor depends on getting good crystals of DHFR into which inhibitors can be diffused. We believe that the combination of X-ray crystallography, QSAR, and computerized graphics will eventually have a large impact on drug development, starting with dihydrofolate reductase.

Before we discuss the details of QSAR for the various types of antifolates, some general comments about the limitations of this approach should be considered. For a clear general discussion of its use in drug design, the reader is referred to Martin's book,[161] and for a discussion of the basics of computerized regression analysis, Daniel and Wood's book is excellent.[162] Most of the work in QSAR has been done by using regression analysis to separate and delineate the electronic, steric hydrophobic, etc. effects of substituents on inhibitor potency. The basis for the study of SAR of electronic effects of substituents was conceived by Hammett about 1935; hence, a large amount of experience has accumulated about the use of Hammett–Taft $\sigma$ constants. Much less experience has been obtained with the more complex hydrophobic and steric effects, and the latter are particularly difficult to parameterize since in the case of enzymes we have little notion of how rigid and unbending a particular portion of the enzyme with which the inhibitor comes into contact may be. As we shall see below, we are beginning to discover that there seems to be considerable "give" on the part of the enzyme, at least in certain situations. The basic difficulty is that, except in the case of *L. casei* and *E. coli* DHFR where high resolution X-ray crystallography studies have been made, we do not know the exact dimensions of the active site where the inhibitors are binding.

There are three types of steric parameters which have found general use in QSAR. The pioneering work of Taft resulted in the formulation of $E_s$ which was derived from the acid hydrolysis of esters of substituted acetic acids[163,164] ($XCH_2COOR$). The log of the relative rates of hydrolysis of the X derivative compared to that of the parent H compound (assuming the electronic effect of X to be insignificant) is a measure of the steric effect of X on the hydrolysis. Charton has developed a modified form of $E_s$ ($\nu$) which is based on van der Waals radii of substituents.[165,166] The molar refractivity (MR) has been used as a rough measure of the bulk of substituents,[167] and Verloop has devised a set of steric constants based on effective calculated volumes.[168,169]

The hydrophobic parameter $\pi$ is an operationally defined measure of the hydrophobic effect of a substituents: $\pi_X = \log P_X - \log P_H$, where $P_X$ is the octanol/water partition coefficient of a derivative and $P_H$ that of a parent compound (i.e., $\pi_{CH_3} = \log P_{C_6H_5CH_3} - \log P_{C_6H_6}$). Substituents with positive $\pi$ values are hydrophobic, and those with negative values are hydro-

philic. The assumption is made that when binding is positively correlated with $\pi$, the substituent is interacting with hydrophobic space on or within the enzyme. This is a reasonable assumption when the traditional small substituents of physical organic chemistry such as halogen, small alkyl groups, $NH_2$, CN, and the like are under consideration. It is easy to imagine a small region of a macromolecule as being essentially homogeneous. Baker, however, started a trend by making what he called nonclassical antimetabolites. The distinction was that in classical antimetabolites a small structural change was involved, whereas in the nonclassical inhibitors huge changes were made so that much of the substituent would fall outside of the active site. For example, a substituent such as $OCH_2C_6H_4$-4′-$SO_3C_6H_4$-2′-Cl, which is, in fact, larger than the parent triazine VI to which Baker attached it and many others as large or larger, contains large hydrophobic and hydrophilic subsections within itself. Moreover, it seems unlikely that such a large substituent could bind in a homogeneous portion of the enzyme. One must be cautious in interpreting the results of correlation equations containing such mammoth groups. There is virtually no experience in pure physical organic chemistry with such substituents in homogeneous solution to base behavioral expectations on.

The most serious problem in using regression analysis is the collinearity problem.[167] Unless considerable care is taken and some discipline maintained during an analogue synthesis program, vectors such as $\pi$ and MR may turn out to be so collinear that it is impossible to tell whether one or both are involved in the SAR.

Another formidable problem when considered in a general fashion is that of establishing via regression techniques what part of a large substituent fails to contact the enzyme. For example, if one is testing the size of a hydrophobic pocket by increasing the size of substituents binding in this region, the best approach for dealing with the problem is Kubinyi's bilinear model:[170] $\log 1/K_i = a\pi - b \log (\beta \cdot 10^{\pi} + 1) + c$. The disposable parameters $a$, $b$, $c$, and $\beta$ are evaluated by an iterative procedure, since for this nonlinear equation one cannot employ the usual least-squares method. When hydrophobic binding is occurring, one finds a linear increase ($a\pi$) in potency until a breaking point ($\pi_0$) is reached where the second term in the above expression takes over and one finds a new linear relationship with slope of $a - b$. If $a - b$ is essentially zero, the assumption is that at point $\pi_0$ one has reached the edge of the hydrophobic pocket and larger hydrophobic groups must at least in part extend beyond the enzyme into aqueous space. This idea is not hard to apply to a set of homologues of the type $(CH_2)_nCH_3$ or O-$(CH_2)_nCH_3$. However, when one mixes in with such a series compact hydrophobic groups such as *tert*-butyl or adamantyl where the geometry is quite different, the above treatment is too simple. Since the hydrophobicity of an apolar group is proportional to its surface area or volume,[171] the adamantyl group could make much more effective use of a hydrophobic cavity than, say, an extended linear hexyl group. Hence, building the mathematical models of QSAR is not a kind of plug-in problem where the numbers are fed into the computer and one accepts the answer without much thought. The model must be built systematically out of more and more complex changes in the parent compound.

In constructing a model with more complex derivatives, such as Baker's nonclassical inhibitors, structural features may be introduced which cannot yet be parameterized with generalized constants such as $\sigma$, $\pi$, etc. Indicator variables can play an important role in the construction of models of such substances.[162,172,173] These variables take the value of 0 or 1 for some specific structural feature which does not fit the correlation equation. For example, if a data set contained variations of VI in which there was a bridge of two atoms from *N*-phenyl to a second phenyl group which conferred extra activity to such inhibitors beyond that accounted for by the known continuous variables ($\pi$, $\sigma$, etc.), a 1 could be assigned to all congeners containing this feature and 0 to those which do not have it. The above model would be modified to the form:

$$\log 1/K_i = a'\pi - b' \log (\beta \cdot 10^{\pi} + 1) + c'I + d$$

where $I$ is the indicator variable. This implies that we have two sets of congeners, one with and one without the bridge, both behaving in the same way hydrophobically. The question arises why not simply use two correlation equations. To a certain extent it is a matter of bookkeeping. A correlation equation is an extremely compact summary of structure–activity and it is simpler to have one equation rather than several. This becomes more important when dealing with very large data sets based on several hundred congeners where 5–10 indicator variables may be involved.[172,174,175] Before, in effect, merging two data sets via an indicator variable, one should derive the two equations and show that they are parallel except for the intercepts. Sometimes, however, one has only a few examples in one series—not enough to derive a valid correlation equation. If these points are included in the single equation, one can keep track of them and their effect on the structure–activity relationship. One must be cautious in interpreting the quality of the correlation obtained when two or more such sets are merged. If the two sets are rather far apart in data space described by $\pi$, $\sigma$, etc., then one "builds in" a large amount of variance which may yield a much higher correlation coefficient ($r$).[176] The new equation could be a poorer correlation despite the better $r$. The best check is to compare the standard deviations from the two equations.

### b. Triazine Inhibitors of DHFR

The first preliminary DHFR QSAR were formulated with use of results from Baker's group on the inhibition of pigeon liver and *E. coli* reductase by pyrimidines and triazines.[177–179] The first serious QSAR for antifolates was made with a set of about 250 triazines of type VI tested against DHFR from L1210 leukemia cells and Walker 256 rat tumor by Baker's group. From this data the model of eq 1 was derived.[146] In this expression

triazine VI inhibitors of DHFR

$$\log 1/C = 0.68\pi_3 - 0.12(\pi_3)^2 + 0.23MR_4 - 0.024(MR_4)^2 + 0.24I_1 - 2.53I_2 - 1.99I_3 + 0.88I_4 + 0.69I_5 + 0.70I_6 + 6.49 \quad (1)$$

$n = 244$, $r = 0.923$, $s = 0.377$, ideal $\pi_3 =$ 2.9 (2.6–3.3), ideal $MR_4 = 4.7$ (4.2–5.6)

$C$ is the molar concentration of triazine producing 50% inhibition, $n$ represents the number of data points used to derive eq 1, $r$ is the correlation coefficient, $s$ is the standard deviation from the regression, and ideal $\pi_3$ and $MR_4$ are the optimum physical chemical characteristics for substituents in the 3- and 4-positions of VI. The MR values used in deriving eq 1 have been scaled by 0.1 to make them more nearly equiscalar with $\pi$. The $\pi_3$ term represents the hydrophobic effect of 3-substituted VI and the $MR_4$ term represents the role of molar refractivity of 4-substituents on inhibitor potency. Both of these terms have exponential counterparts which show that beyond the ideal values, activity falls off with increasing value of $\pi_3$ or $MR_4$. The indicator variable $I_1$ assumes the value of 1 for log $1/C$ values obtained with use of enzyme from Walker tumor and the value of 0 for L-1210 enzyme. The positive coefficient with this term, although small, brings out the fact that L-1210 DHFR was more difficult for the triazines to inhibit. These results do suggest that there is a small structural difference in the DHFR from the two different types of tumor cells. It is of interest in this context that Walker tumor responds more readily to chemotherapy than L-1210 leukemia does.

Indicator variable $I_2$ takes the value of 1 for all congeners having a substituent in the 2-position of VI. Such derivatives are much less active (about 300 times) than expected. Attempts to correlate the effects of 2-substituents with constants such as $E_s$, $\sigma$, $\pi$, etc. were unsuccessful.[146] All 2-substituents, including the small F group, greatly depressed potency. A further investigation of 2-X-VI also found that all substituents, with one important exception, greatly decrease inhibitory power against DHFR from bovine liver and murine L5178Y tumor cells.[147] The one exception was 2-SH, which was found to be 10 times more potent than the parent structure against both types of DHFR. The fact that the large 2-I group is about 1000 times less active than the parent compound and 2-Cl is about 30 times less suggests that a steric effect is involved. The fact that the small F is more than 10 times less active than the parent molecule would indicate that an electronic effect is also probably involved. Why the large SH group is more active is not clear.

The indicator variable $I_3$ which is given the value of 1 for rigid branched groups ($C_6H_5$, CONH, CH=CHCONH, $>CHC_6H_5$) in either position 3 or 4 brings out detrimental effects of such structures. The coefficient of −1.99 shows that on the *average* these structures are 100 times less active than expected. More recently, it has been found that COOR, $CHOHC_6H_5$, and $C(CH_3)_3$ are usually less active than expected.[27,180-185]

Variable $I_4$ takes the value 1 for those congeners having substituents: 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4X$. Its value of 0.88 shows the unusual contribution to activity made by the $SO_2O$ moiety. It is possible that alkylation of the enzyme could occur via this function. On the other hand, there may be a specific noncovalent reaction of the $SO_3$ group with the enzyme.

I-5 is given the value of 1 for flexible bridges of the type $CH_2$, $CH_2CH_2$, $(CH_2)_4$, $(CH_2)_6$, and $(CH_2)_4O$ between the *N*-phenyl moiety and a second phenyl ring. This flexibility considerably improves activity as shown by the positive coefficient of 0.69.

Amide bridges of the type $CH_2NHCONHC_6H_4X$, $CH_2CH_2CON(R)C_6H_4X$, and $CH_2CH_2CH_2CON(R)C_6H_4X$ (R = H or Me) are correlated by $I_6$. This is a rather arbitrary parameterization since amides of the type $O(CH_2)_nCONHC_6H_4X$, $O(CH_2)_nNHCONHC_6H_4X$, $CH_2CONHC_6H_4X$, and $CH_2CH(R)CONHC_6H_4X$ are excluded from this class. These bridges increase activity on the average by a factor of 5.

It was recognized at the time of the derivation of eq 1 that high collinearity between the $\pi_3$ and $MR_3$ and the $\pi_4$ and $MR_4$ vectors precluded firm statements that enzymatic space binding 3-substituents is hydrophobic and that 4-space is not hydrophobic. Subsequent work has established that there is a rather large hydrophobic region into which both 3- and 4-substituents of limited size can bind and that $\pi$ correlates the effects of substituents on inhibitor potency.[180-185]

Equation 1 has a number of shortcomings. Most serious is the fact that the DHFR employed by Baker was extremely crude, although Baker looked on this as an advantage in drug design. His reasoning was that in vivo DHFR would be surrounded by many other types of macromolecules and hence crude enzymes simulated this condition.

Equation 1 has predictive value showing, for example, what optimum $\pi$ is and which structural features are in general helpful or bad. Terms which do not appear in the equation are of interest. For example, all attempts to find a role for $\sigma$ were unsuccessful, so that we know electronic effects of substituents are not of prime importance. Without regression analysis Baker was never sure of this point.

In addition to predictive values, equations such as eq 1 can be used to characterize enzymic space provided that they are constructed from data obtained from a well-selected set of substituents. Equation 1 structures a huge amount of information so that one can see at a glance the probable activity of a potential new derivative. This bookkeeping function of correlation equations for large data sets of hundreds of congeners is extremely important. Compounds that do not fit the relationship well immediately become the focus of interest and the study of these leads to deeper understanding. Equation 1 can be compared with eq 2 and 3 for the inhibition of highly purified human DHFR by triazines VI.[185] The simpler nature of eq 2 and 3

4-X-VI QSAR from human DHFR

$$\log 1/K_{i\,app} = 0.78\pi'_4 - 0.78 \log (\beta \cdot 10^{\pi'_4} + 1) + 1.26I - 0.88\nu + 5.83 \quad (2)$$

$$n = 35,\ r = 0.953,\ s = 0.361,\ \pi_0 = 3.43$$

3-X-VI QSAR from human DHFR

$$\log 1/K_{i\,app} = 1.07\pi'_3 - 1.10 \log (\beta \cdot 10^{\pi'_3} + 1) + 0.50I + 0.82\sigma + 6.07 \quad (3)$$

$$n = 60,\ r = 0.890,\ s = 0.308,\ \pi_0 = 1.84$$

compared to eq 1 is due to the simpler type of substituent employed in developing the human QSAR. The goodness of fit with the human data is better than that for the tumor DHFR (compare values of $s$). Apparent $K_i$ values were used instead of $I_{50}$ values and

highly purified (affinity chromatography) enzyme [human lymphoblastoid (WIL2)] was used instead of crude material. Considerable effort was made to get reliable $K_i$ values.[80] In eq 1 it seemed acceptable to include all data points in a single QSAR including 3,4-di-X-VI. In the case of human DHFR it was quickly apparent that QSAR for 3- and 4-substituents differed greatly. Equation 2 contains a term in $\nu$ which is Charton's[166] steric parameter. It has been shown to be rather closely related to Taft's $E_s$; however, there are more values available of the $\nu$ constants than of $E_s$. Surprisingly, the QSAR for 3-X-VI contains a term in $\sigma$ and no counterpart could be found for eq 2. It is likely that the higher quality of the data used to derive eq 2 and 3 allows for higher resolution and hence the discovery of more subtle structure–activity features.

Hydrophobic space for 4-X is larger than for 3-X (compare values of $\pi_0$) and the indicator variable $I$ in eq 2 is larger than in eq 3. This variable takes the value of 1 for substituents of the type $CH_2SC_6H_4Y$ or $ZCH_2C_6H_4Y$ (where Z = O or S). The value of $\pi_Y$ for these congeners is set to zero. That is, $\pi_{ZCH_2C_6H_4Y} = \pi_{ZCH_2C_6H_5}$, since Y, regardless of its character, has a negligible effect on log $1/K_i$. Equations 2 and 3 are based on the bilinear model for hydrophobic effects.[170] For eq 2 the right-hand side of the bilinear curve has a slope of zero (0.78 − 0.78 = 0); the same is essentially true for eq 3 (1.07 − 1.10 = −0.03). Initially, inhibitor potency increases with increasing values of $\pi$ until the point of $\pi_0$, where substituents having larger $\pi$ values show a constant effect. It is assumed that part of these larger hydrophobic groups simply project beyond the enzyme into aqueous space and hence have no effect on potency of the inhibitor. The $\pi_0$ for the two equations are different, showing that larger 4-substituents are able to make better hydrophobic contact than 3-substituents.

The negative term in $\nu$ of eq 2 indicates a bad contact between 4-substituents and a portion of the enzyme and the positive term in $I$ brings out the fact that groups of the type $CH_2ZC_6H_4Y$ are on the average more than 10 times as potent as their $\pi$ values alone would lead one to expect. This moiety corresponds to the $CH_2NHC_6H_4CO$ unit in folic acid and the similar group in MTX. The geometry of 4-$CH_2ZC_6H_4Y$ substituents is apparently not so favorable for interaction so that the coefficient with the I term in eq 3 is considerably smaller than in eq 2.

The presence of the $\sigma$ term in eq 3 but not in eq 2 is puzzling, and in fact one might be tempted to ignore it since it is not highly important, except for the fact that it is also found with DHFR from other sources (see below). To our knowledge, the finding of an electronic substituent effect from a meta position but not from the para position is unprecedented in the studies of physical organic chemistry. In some way it must be a consequence of the inhomogeneity of the macromolecular system. If because of the steric effect of 4-X-VI brought out by $-0.88\nu$ in eq 2 by 4-substituents the $XC_6H_4$ moiety is moved slightly, it is conceivable that a dipolar interaction between ligand and enzyme would not play as important a role with 4-X-VI as with 3-X-VI. The $\sigma$ term could also be the result of a high electron density on the substituent, which in the case of 3-substituents promotes a reaction of 3-substituents with an enzymic feature not available to 4-substituents. In any event, the effect seems real and must eventually be accounted for.

In the case of the human DHFR the bilinear model gives a better correlation than the parabolic model ($\pi + \pi^2$) used in eq 1. The complexity of the substituents upon which eq 1 is based is so great that the reasons for the advantage of the parabolic model compared to the bilinear model are obscured. Actually, using the bilinear terms in eq 1 gives almost as good a correlation. However, the $\pi_0$ for 3-substituents is considerably larger for eq 1 than for eq 3. Even if relatively simple 3-X-VI are extracted from the set of 244 congeners used to derive eq 1 and these are treated separately, one finds about the same $\pi_0$ value. Thus, it would seem that the test system used by Baker is the only way to account for the larger $\pi_0$. Enough data has now collected (see below) to establish that 4-substituents also bind in hydrophobic space and that the $MR_4$ terms of eq 1 should be replaced with $\pi_4$ terms.

The QSAR for triazines acting on human DHFR can be compared with a rather extensive study made using highly purified chicken liver DHFR.[184] Equations 4 and 5 are comparable to eq 2 and 3, but the behavior of the 3- and 4-substituted congeners is so similar for the chicken enzyme that the two equations can be merged to yield eq 6, which is almost as good a correlation (compare values of $s$) as the two separate equations. The steric effect brought out by $-0.29\nu$ in eq 4 is so weak that it is completely obscured in eq 6. Equation 6 also correlates ten 3,4-disubstituted analogues including Baker's antifols XIV and XIX, which are all reasonably well fit.

4-X-VI QSAR from chicken DHFR

$$\log 1/K_{i\,app} = 0.73\pi'_4 - 1.40 \log (\beta \cdot 10^{\pi'_4} + 1) - 0.29\nu + 6.49 \quad (4)$$

$n = 32$, $r = 0.949$, $s = 0.280$, $\log \beta = -2.40$, $\pi_0 = 2.44$

3-X-VI QSAR from chicken DHFR

$$\log 1/K_{i\,app} = 1.01\pi'_3 - 1.16 \log (\beta \cdot 10^{\pi'_3} + 1) + 0.86\sigma + 0.633 \quad (5)$$

$n = 59$, $r = 0.906$, $s = 0.286$, $\log \beta = -1.12$, $\pi_0 = 1.80$

3- and 4-X-VI QSAR from chicken liver DHFR

$$\log 1/K_{i\,app} = 0.85\textstyle\sum\pi' - 1.04 \log (\beta \cdot 10^{\sum\pi'} + 1) + 0.57\sigma + 6.36 \quad (6)$$

$n = 101$, $r = 0.910$, $s = 0.294$, $\pi_0 = 2.03$, $\log \beta = -1.38$

The presence of the $\sigma$ term and the less important term in $\nu$ is comparable to eq 2 and 3. The lack of significance of the indicator variable I in eq 4–6 indicates a major structural difference in the two enzymes. The I term is even more important in the QSAR for bacterial DHFR and it is of some importance in the murine QSAR. It will be most interesting to see just which structural feature chicken DHFR has which differentiates it from all other DHFR in this manner. As in eq 4 and 5, $\pi'$ means that $\pi_Y$ for $ZCH_2C_6H_4Y$ or

$CH_2ZC_6H_4Y$ is set to zero. However, in addition $\pi'$ for OR groups was also set to zero. Variation in log $1/K_i$ for both 3-OR and 4-OR, where R is limited to simple alkyl groups, is so small that these groups do not appear to contact the enzyme, or if they do, some sort of steric inhibition must compensate for the hydrophobic interaction. Actually, the alkoxy groups are not well accommodated by eq 4 and 5.

The hydrophobic space available for substituents with the chicken DHFR resembles that for 3-X-VI analogues in the human DHFR (compare values of $\pi_0$).

Equations 1–6 can also be compared with the less extensive QSAR obtained with DHFR from two other vertebrate sources: bovine liver and murine leukemia L5178YR-$C_3$:[180]

3-X-VI QSAR from bovine DHFR[180]

$$\log 1/K_{i\,app} = 1.10\pi'_3 - 1.23 \log (\beta \cdot 10^{\pi'_3} + 1) + 0.61\sigma + 7.08 \quad (7)$$

$$n = 38,\ r = 0.914,\ s = 0.277,\ \pi'_0 = 1.72$$

3-X-VI QSAR from murine tumor DHFR[180]

$$\log 1/K_{i\,app} = 1.19\pi'_3 - 1.38 \log (\beta \cdot 10^{\pi'_3} + 1) + 0.50I + 0.90\sigma + 6.20 \quad (8)$$

$$n = 38,\ r = 0.935,\ s = 0.289,\ \pi'_0 = 1.56$$

The intercepts of eq 2 and 3 are rather close to eq 8 but are considerably different from eq 7 for the bovine reductase. The bovine reductase is about 10 times more sensitive on the average than the human or mouse enzyme.

Human, chicken, bovine, and murine QSAR all contain a term in $\sigma$ for 3-X-VI congeners, and although this is not a variable of major importance, it is clearly significant in each case.

The $\pi_0$ values for human, chicken, bovine, and mouse DHFR QSAR are in rather close agreement for 3-X-VI. The 4-X-VI congeners have not yet been tested with the bovine and murine enzymes. The initial dependence of log $1/K_i$ on $\pi$ is also similar; consequently, the hydrophobic regions of the active sites must be roughly the same.

Like the human equation, a term in $I$ is also found for the murine equation, and in fact eq 8 is remarkably similar to eq 3.

A QSAR for a small set of 3-X-VI has been derived for rat liver DHFR, but the data are not extensive enough to allow for comparisons with the other vertebrate QSAR.[180] In equation 9 only two groups of the

3-X-VI QSAR from rat liver DHFR

$$\log 1/K_{i\,app} = 1.12\pi_3 - 1.34 \log (\beta \cdot 10^{\pi_3} + 1) + 6.80 \quad (9)$$

$$n = 18,\ r = 0.977,\ s = 0.171,\ \pi_0 = 1.68$$

type $OCH_2C_6H_4Y$ were evaluated so that normal $\pi$ values were used for these compounds. Equation 9 is quite similar, as far as it goes, to eq 8.

The results with the vertebrate DHFR can be compared with QSAR from bacterial enzyme.[183]

3-X-VI QSAR from *L. casei* DHFR

$$\log 1/K_{i\,app} = 0.83\pi'_3 - 0.91 \log (\beta \cdot 10^{\pi'_3} + 1) + 0.71I + 4.60 \quad (10)$$

$$n = 38,\ r = 0.961,\ s = 0.244,\ \log \beta = -1.68,\ \pi = 2.69$$

4-X-VI QSAR from *L. casei* DHFR

$$\log 1/K_{i\,app} = 0.44\pi'_4 - 0.65 \log (\beta \cdot 10^{\pi'_4} + 1) - 0.90\nu + 0.69I + 4.67 \quad (11)$$

$$n = 32,\ r = 0.941,\ s = 0.348,\ \log \beta = -4.22,\ \pi_0 = 4.53$$

The QSAR for 3-X-triazines acting on *L. casei* DHFR differs from the corresponding equations with vertebrate DHFR in two ways. The much smaller intercept with eq 10 shows the triazines to be, in general, much less active against the bacterial reductase. Equation 10 lacks a term in $\sigma$ which most of the vertebrate equations contain. The equation for 4-X-triazines has two important differences from that for 3-X-triazines. The slope with $\pi'_4$ is only about half that of eq 10 and $\pi_0$ is much greater, bringing out the point that a larger hydrophobic surface is available with *L. casei* enzyme for four substituents. Equation 11 does contain the term in $\nu$ as do most of the vertebrate equations for 4-X-VI congeners.

Both equations contain the indicator variable $I$ for the special effect of the $ZCH_2C_6H_4Y$ moieties as do the vertebrate equations, except for chicken and bovine DHFR.

Since dihydrofolate reductase like most other enzymes is sensitive to changes in pH, it is of interest to note that in the case of DHFR from bovine liver the same structure–activity relationship is found at pH 6.25 and 7.20 in reacting with 2-X-VI. This suggests that major changes in the structure of the DHFR with pH do not occur.[147]

Examples of results from correlation equations are shown in Table II, where observed and calculated values for log $1/K_{i\,app}$ are present for the inhibition of human and *L. casei* DHFR by triazines. One of the more interesting aspects of $\pi$ constants is that they provide a reasonable model for evaluating the binding of a variety of quite different substituents with the hydrophobic regions of macromolecules. The model in effect says that partitioning of the substituent from the aqueous phase onto the enzyme will parallel the way it partitions from water into octanol. Although macromolecules do not have the same homogeneity that octanol does, the model still works rather well. If one compares observed and calculated values for the first 16 examples in Table II, which contain relatively small substituents and which have a very wide variety of polar bonds, one finds reasonable agreement with a few notable exceptions. Particularly poor correlations with the human enzyme are 6 (4-$COOCH_3$), 7 (4-$COOC_2H_5$), and 16 (4-C≡N). The less-than-expected activity of the two esters is probably due to steric effects (see section on graphics). The 4-CN is much less active than expected in both the human and bacterial examples. The correlation equation alerts us to some kind of specific interaction of the CN group with the enzyme not shown by the other small polar groups, although the basis for this interaction is not yet clear. Follow up on this lead by other means is called for. What is surprising is that sub-

stituents such as 2 and 39 ($SO_2NH_2$), 4 and 40 ($CONH_2$), 5 and 41 ($COCH_3$), 9 (4-$NH_2$), 10 (4-$NHCOCH_3$), and 43 (3-OH), which all have unusual possibilities for hydrogen bonding with the protein, are about as well fit as the apolar groups such as $CH_3$, halogens, and $CF_3$. This suggests that the polar interactions of these substituents with the enzyme are similar to those in octanol.

The large, complex group 17 is poorly correlated and so is the rigid 23 (4-C≡$CC_6H_5$). Also, very poorly correlated is the charged substituent 51. All in all, however, the results are better than one might expect for such a simple model and the correlation equations are of great help in organizing a very complex data set. Inhibition studies with polar and nonpolar substituents established the hydrophobic character of the binding site long before it was confirmed by X-ray crystallographic studies to indeed be hydrophobic.

The quantity log $1/(K_i$ human DHFR) − log $1/(K_i$ *L. casei* DHFR) enables one to see the selectivity of the inhibitors for the two enzymes. There is only one example, 8 4-OH-VI, which is more potent against the bacterial enzyme than the mammalian enzyme. All other inhibitors are more potent against the eucaryotic DHFR than the bacterial DHFR. The most selective substituents are 11 (4-$CF_3$), 22 (4-$C(CH_3)_3$), and 48 (3-I), which are about 100 times more active against the human DHFR.

### c. Quinazoline Inhibitors of DHFR

A class of potent inhibitors which resembles folic acid is the 2,4-diaminoquinazolines (VII) for which QSAR have been derived.

QSAR of VII from rat liver DHFR[186]

$$\log 1/C = 0.81MR_6 - 0.064(MR_6)^2 + 0.77\pi_6 - 0.73I_1 - 2.15I_2 - 0.54I_3 - 1.40I_4 + 0.78I_6 - 0.20MR_6 \cdot I_1 + 4.92 \quad (12)$$

$$n = 101,\ r = 0.961,\ s = 0.441,\ \text{ideal } MR_6 = 6.4$$

The $\pi_5$ and $MR_5$ terms refer to substituents in positions 5 and 6 of VII. The indicator variable $I_1$ is assigned the value of 1 when the 2-$NH_2$ group of VII is replaced by SH or OH. These congeners are on the average 65 times less active than those containing a 2-$NH_2$, other factors being equal. $I_2$ is given the value of 1 when 2-$NH_2$ is replaced by H. These congeners are about 100 times less active than the corresponding 2-$NH_2$ analogues. The variable $I_3$ assumes the value of 1 for congeners having an OH or SH group in place of 4-$NH_2$, which lowers activity by about 30-fold. Thus, the 2-$NH_2$ is seen to be a little more important than the 4-$NH_2$. This is to be expected since there is considerable evidence that the carboxyl of a Glu or Asp residue interacts strongly with the N1 and 2-$NH_2$ groups of inhibitors of this type. In fact, one might expect an even greater effect on removal of the 2-$NH_2$ group. $I_4$ is given the value for 1 of the following bridges from the 5-position to any aryl group: S, SO, $SO_2$, $CH_2S$, CH=CH. Its negative value shows that such an arrangement of atoms greatly depresses activity (by an average factor of 250). $I_6$ is assigned a value of 1 for 6-$SO_2Ar$. This is the only indicator variable which makes a positive contribution to inhibitory potency.

The cross product term of $MR_6 \cdot I$ with its negative coefficient shows that the combination of 2-SH or 2-OH with a large 6-substituent produces less effective inhibitors. Since 2-SH and 2-OH are poor substituents compared to 2-$NH_2$, one assumes that 2-OH holds inhibitors to the enzyme less firmly. This appears to prevent 6-substituents from making their maximum contribution.

The quinazolines have also been studied by Hynes and Freisheim using DHFR from *S. faecium*. This work is summarized in the QSAR of eq 13. Equation

QSAR of quinazolines VII from *S. faecium* DHFR[187]

$$\log 1/C = 1.13\pi_5 - 1.10MR_5 - 2.39I_1 - 4.09I_2 - 2.37I_3 + 8.26 \quad (13)$$

$$n = 67,\ r = 0.926,\ s = 0.672$$

13 is quite different from eq 12 and a much less satisfactory QSAR. The standard deviation of eq 13 is much higher than that of eq 12 and the meaning of the $\pi_5$ and $MR_5$ terms is ambiguous. Since there is high collinearity ($r^2 = 0.86$) between them and their coefficients are almost identical, they tend to cancel each other. The result can be interpreted by postulating a region for hydrophobic binding which is small or sterically hindered. Thus, while large groups give better hydrophobic interaction, this is offset by their bulk.

In the QSAR of eq 12, the apparently limited space in the region occupied by 5-substituents is taken care of by the negative coefficient with $I_4$.

The indicator variables which account for most of the reduction in variance in log $1/C$ have the same meaning as in eq 12; however, they bring out the fact that replacement of $NH_2$ on the pyrimidine ring by H, OH, or SH depresses inhibitor potency much more with the bacterial reductase. The intercept in eq 13 is the estimate for the activity of the parent congener, which actually was not tested. The value of 8.26 is not much different from the value of log $1/C$ for the most active congener (9.15); hence, little has been gained in the extensive modification studies.

An important aspect of eq 13 is that it does not contain any term for 6-substituents despite the fact that many of the derivatives contain them. Structural variation in these substituents is large, but few small, easily parameterizable substituents were studied. The most active congeners are those containing a single large group in position 6; however, the nature of these groups is so complex that no reasonable parameterization could be devised.

From molecular graphics studies (see section X) it is clear that the region into which 6-substituents on quinazolines contact the enzyme is rather open and the 5-position is rather limited. A better selection of small 6-substituents in the data used to formulate eq 13 would have given a better based QSAR. Although data from a few such substituents were used to derive eq 12, the collinearity between $\pi_5$ and $MR_5$ was high ($r^2 = 0.86$). While eq 12 and 13 suggest $MR_6$ is a better parameter than $\pi_6$, we now know from both QSAR and X-ray crystallography studies that this region is largely hydrophobic. This illustrates the great importance of careful substituent selection in SAR studies.

Chen et al.[188] have formulated a QSAR for quinazolines XXVII inhibiting DHFR from human acute lym-

**TABLE II. Comparison of the Inhibition by Triazines VI of Human DHFR with *L. casei* DHFR**

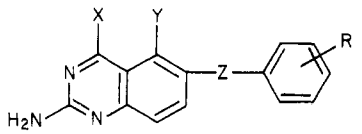
| | | log $1/K_{i\,app}$ | | | | |
|---|---|---|---|---|---|---|
| | | human DHFR[a] | | *L. casei* DHFR[b] | | human – |
| no. | X | obsd | calcd | obsd | calcd | *L. casei*, obsd |
| 1. | H | 5.78 | 5.79 | 4.70 | 4.59 | 1.08 |
| 2. | 4-$SO_2NH_2$ | 3.81 | 3.54 | 2.97 | 2.99 | 0.84 |
| 3. | 4-$SO_2CH_3$ | 4.08 | 3.69 | 2.71 | 3.07 | 1.37 |
| 4. | 4-$CONH_2$ | 3.64 | 4.03 | 3.18 | 3.37 | 0.46 |
| 5. | 4-$COCH_3$ | 4.50 | 4.76 | 3.52 | 3.79 | 0.98 |
| 6. | 4-$COOCH_3$ | 3.85 | 4.46 | 3.39 | 3.31 | 0.46 |
| 7. | 4-$COOC_2H_5$ | 3.46 | 4.79 | 3.41 | 3.54 | 0.05 |
| 8. | 4-OH | 4.57 | 5.02 | 4.91 | 4.09 | −0.34 |
| 9. | 4-$NH_2$ | 4.65 | 4.56 | 3.94 | 3.82 | 0.71 |
| 10. | 4-$NHCOCH_3$ | 4.23 | 4.27 | 3.90 | 3.43 | 0.33 |
| 11. | 4-$CF_3$ | 5.58 | 5.50 | 3.68 | 4.24 | 1.90 |
| 12. | 4-F | 6.15 | 5.65 | 4.65 | 4.49 | 1.50 |
| 13. | 4-Cl | 6.20 | 5.74 | 4.76 | 4.49 | 1.44 |
| 14. | 4-Br | 5.76 | 5.72 | 4.57 | 4.46 | 1.19 |
| 15. | 4-I | 5.51 | 5.70 | 4.43 | 4.46 | 1.08 |
| 16. | 4-CN | 3.69 | 5.02 | 3.30 | 4.06 | 0.39 |
| 17. | 4-$OCH_2CON(CH_2CH_2)_2O$ | 5.66 | 4.20 | 4.27 | 3.50 | 1.39 |
| 18. | 4-$O(CH_2)_2OC_6H_4$-4′-$NH_2$ | 6.00 | 5.54 | 5.12 | 4.32 | 0.88 |
| 19. | 4-$CH_3$ | 5.97 | 5.69 | 4.17 | 4.45 | 1.80 |
| 20. | 4-$(CH_2)_3CH_3$ | 6.27 | 5.93 | 5.05 | 4.99 | 1.22 |
| 21. | 4-$(CH_2)_8CH_3$ | 6.52 | 5.94 | 5.79 | 5.71 | 0.73 |
| 22. | 4-$C(CH_3)_3$ | 5.66 | 5.43 | 3.56 | 4.42 | 2.10 |
| 23. | 4-$C{\equiv}CC_6H_5$ | 5.42 | 5.92 | 3.86 | 5.30 | 1.56 |
| 24. | 4-$OCH_3$ | 5.31 | 5.46 | 4.10 | 4.34 | 1.21 |
| 25. | 4-$O(CH_2)_2CH_3$ | 5.57 | 5.85 | | | |
| 26. | 4-$O(CH_2)_5CH_3$ | 5.53 | 6.00 | 5.25 | 5.26 | 0.28 |
| 27. | 4-$O(CH_2)_{10}CH_3$ | 5.68 | 5.97 | 5.57 | 5.66 | 0.11 |
| 28. | 4-$O(CH_2)_{11}CH_3$ | 5.85 | 5.97 | 5.58 | 5.57 | 0.27 |
| 29. | 4-$OCH_2C_6H_5$ | 6.93 | 7.18 | 5.19 | 5.51 | 1.74 |
| 30. | 4-$OCH_2C_6H_3$-3′,4′-Cl | 6.46 | 7.18 | 5.66 | 5.51 | 0.80 |
| 31. | 4-$OCH_2C_6H_4$-4′-$SO_2NH_2$ | 7.21 | 7.18 | 5.35 | 5.51 | 1.86 |
| 32. | 4-$OCH_2C_6H_4$-4′-$CONH_2$ | 7.23 | 7.18 | 5.63 | 5.51 | 1.60 |
| 33. | 4-$OCH_2C_6H_4$-4′-$CH_2OH$ | 7.12 | 7.18 | | | |
| 34. | 4-$CH_2SC_6H_5$ | 7.33 | 7.07 | 5.61 | 5.63 | 1.72 |
| 35. | 4-$CH_2SC_6H_4$-2′-$CH_3$ | 7.18 | 7.07 | | | |
| 36. | 4-$CH_2SC_6H_4$-3′-$CH_3$ | 7.22 | 7.07 | 5.55 | 5.63 | 1.67 |
| 37. | 4-$SCH_2C_6H_5$ | 7.01 | 6.78 | 5.64 | 5.34 | 1.37 |
| 38. | 4-$SCH_2C_6H_4$-4′-Cl | 6.97 | 6.78 | | | |
| 39. | 3-$SO_2NH_2$ | 4.55 | 4.49 | 2.93 | 3.09 | 1.62 |
| 40. | 3-$CONH_2$ | 4.64 | 4.70 | | | |
| 41. | 3-$COCH_3$ | 5.46 | 5.76 | 4.24 | 4.14 | 1.22 |
| 42. | 3-$COOC_2H_5$ | 4.95 | 6.62 | | | |
| 43. | 3-OH | 5.53 | 5.42 | 3.85 | 4.04 | 1.68 |
| 44. | 3-$CF_3$ | 6.67 | 6.84 | 4.77 | 5.27 | |
| 45. | 3-F | 6.61 | 6.35 | 4.88 | 4.70 | 1.73 |
| 46. | 3-Cl | 7.03 | 6.72 | | | |
| 47. | 3-Br | 7.21 | 6.80 | | | |
| 48. | 3-I | 7.17 | 6.84 | 5.18 | 5.43 | 1.99 |
| 49. | 3-$NO_2$ | 6.09 | 6.29 | 4.74 | 4.36 | 1.35 |
| 50. | 3-CN | 6.30 | 5.88 | 5.31 | 4.12 | 0.99 |
| 51. | 3-$CH_2N^+(CH_3)_3$ | 3.55 | 1.79 | | | |
| 52. | 3-$CH_3$ | 6.74 | 6.29 | 4.96 | 5.03 | 1.78 |
| 53. | 3-$CH_2CH_3$ | 6.93 | 6.47 | 5.40 | 5.36 | 1.53 |
| 54. | 3-$(CH_2)_5CH_3$ | 7.02 | 6.54 | 5.99 | 5.85 | 1.03 |
| 55. | 3-$(CH_2)_8CH_3$ | 6.66 | 6.48 | 6.27 | 5.73 | 0.39 |
| 56. | 3-$(CH_2)_{11}CH_3$ | 6.52 | 6.43 | 5.67 | 5.60 | 0.85 |
| 57. | 3-$C(CH_3)_3$ | 6.39 | 6.54 | | | |
| 58. | 3-$CH(OH)C_6H_5$ | 5.56 | 6.30 | | | |
| 59. | 3-$OCH_3$ | 5.78 | 6.04 | 4.52 | 4.57 | 1.26 |
| 60. | 3-$OCH_2CH_3$ | 5.66 | 6.32 | 5.19 | 4.89 | 0.47 |
| 61. | 3-$O(CH_2)_2CH_3$ | 5.68 | 6.61 | 5.58 | 5.38 | 0.10 |
| 62. | 3-$O(CH_2)_3CH_3$ | 6.08 | 6.69 | | | |
| 63. | 3-$O(CH_2)_4CH_3$ | 6.09 | 6.71 | | | |
| 64. | 3-$O(CH_2)_5CH_3$ | 6.12 | 6.70 | 5.69 | 5.86 | 0.43 |
| 65. | 3-$O(CH_2)_8CH_3$ | 6.78 | 6.65 | 5.64 | 5.77 | 1.14 |
| 66. | 3-$O(CH_2)_{10}CH_3$ | 6.61 | 6.61 | | | |
| 67. | 3-$O(CH_2)_{11}CH_3$ | 6.69 | 6.60 | 5.39 | 5.64 | 1.30 |
| 68. | 3-$O(CH_2)_{12}CH_3$ | 6.54 | 6.58 | | | |
| 69. | 3-$O(CH_2)_{13}CH_3$ | 6.34 | 6.56 | | | |
| 70. | 3-$O(CH_2)_2OC_6H_5$ | 6.82 | 6.70 | | | |
| 71. | 3-$O(CH_2)_2OC_6H_4$-3′-$CF_3$ | 6.92 | 6.70 | 5.87 | 5.82 | 1.05 |
| 72. | 3-$O(CH_2)_4OC_6H_5$ | 6.94 | 6.70 | | | |
| 73. | 3-$O(CH_2)_4OC_6H_4$-3′-$CF_3$ | 6.90 | 6.67 | | | |
| 74 | 3-$OCH_2C_6H_5$ | 6.72 | 7.19 | 5.68 | 5.70 | 1.04 |

TABLE II (Continued)

| no. | X | log $1/K_{i\,app}$ human DHFR[a] obsd | human DHFR[a] calcd | *L. casei* DHFR[b] obsd | *L. casei* DHFR[b] calcd | human – *L. casei*, obsd |
|---|---|---|---|---|---|---|
| 75. | 3-$OCH_2C_6H_3$-3′,4′-$Cl_2$ | 6.83 | 7.19 | 5.57 | 5.70 | 1.26 |
| 76. | 3-$OCH_2C_6H_4$-4′-$CONH_2$ | 6.95 | 7.19 | 5.90 | 5.70 | 1.05 |
| 77. | 3-$OCH_2$-1-admantyl | 6.11 | 6.67 | 5.29 | 5.82 | 0.82 |
| 78. | 3-$CH_2O$-c-$C_6H_{11}$ | 6.64 | 6.65 | 5.69 | 5.60 | 0.95 |
| 79. | 3-$CH_2NHC_6H_3$-3′,5′-$(CONH_2)_2$ | 6.78 | 7.07 | 5.68 | 6.06 | 1.10 |
| 80. | 3-$CH_2NHC_6H_4$-4′-$SO_2NH_2$ | 7.20 | 7.07 | 5.95 | 6.06 | 1.25 |
| 81. | 3-$CH_2OC_6H_5$ | 7.23 | 7.16 | 6.57 | 6.41 | 0.66 |
| 82. | 3-$CH_2OC_6H_4$-3′-Cl | 7.44 | 7.16 | | | |
| 83. | 3-$CH_2OC_6H_4$-3′-CN | 7.44 | 7.16 | 6.44 | 6.41 | 1.00 |
| 84. | 3-$CH_2OC_6H_4$-3′-$OCH_3$ | 7.33 | 7.16 | | | |
| 85. | 3-$CH_2OC_6H_4$-3′-$CH_2OH$ | 7.04 | 7.16 | | | |
| 86. | 3-$CH_2OC_6H_4$-3′-$CH_3$ | 7.22 | 7.16 | | | |
| 87. | 3-$CH_2OC_6H_4$-3′-$C_2H_5$ | 7.37 | 7.16 | 6.33 | 6.41 | 1.04 |
| 88. | 3-$CH_2OC_6H_4$-3′-$CH(CH_3)_2$ | 7.15 | 7.16 | | | |
| 89. | 3-$CH_2OC_6H_4$-3′-$C(CH_3)_3$ | 7.47 | 7.16 | 6.45 | 6.41 | 1.02 |
| 90. | 3-$CH_2OC_6H_4$-3′-$C_6H_5$ | 7.14 | 7.16 | 6.69 | 6.41 | 0.45 |
| 91. | 3-$CH_2OC_6H_4$-3′-$NHCOCH_3$ | 7.30 | 7.16 | 6.61 | 6.41 | 0.69 |
| 92. | 3-$CH_2OC_6H_4$-3′-$NHCSNH_2$ | 7.16 | 7.16 | | | |
| 93. | 3-$CH_2OC_6H_4$-3′-$NHCONH_2$ | 7.39 | 7.16 | | | |
| 94. | 3-$CH_2OC_6H_4$-4′-$(CH_2)_4CH_3$ | 6.73 | 7.16 | 6.09 | 6.41 | 0.64 |
| 95. | 3-$CH_2O$-2-naphthyl | 7.12 | 7.16 | | | |
| 96. | 3-$CH_2O$-1-naphthyl | 6.89 | 7.16 | | | |
| 97. | 3-$CH_2SC_6H_5$ | 6.93 | 7.17 | 6.55 | 6.56 | 0.38 |
| 98. | 3-$CH_2SC_6H_5$-3′-$CH_3$ | 7.12 | 7.17 | | | |
| 99. | 3-$CH_2SeC_6H_5$ | 7.52 | 7.17 | 6.76 | 6.56 | 0.76 |
| 100. | 3-$SCH_2C_6H_5$ | 7.37 | 7.14 | 6.00 | 5.85 | 1.37 |
| 101. | 3-$SCH_2C_6H_4$-4′-Cl | 7.20 | 7.14 | | | |

[a] From ref 185. [b] From ref 184.

phocytic leukemia and mouse L-1210R leukemia. $I_1$



XXVII

QSAR for XXVII inhibition of human leukemia DHFR

$$\log 1/I_{50} = -2.87I_1 + 0.29I_2 - 0.38MR_6 - 0.29\pi_R - 0.19MR_R + 10.12 \quad (14)$$

$$n = 47,\ r = 0.956,\ s = 0.42$$

QSAR for XXVII inhibition of mouse L-1210R DHFR

$$\log 1/I_{50} = 0.49I - 1.23I_3 - 0.30MR_6 - 0.11\pi_R + 9.36 \quad (15)$$

$$n = 24,\ r = 0.904,\ s = 0.235$$

in eq 14 takes the value of 1 for X = OH (no such data points were available for eq 15). As usual, its large negative value brings out the deleterious effect of replacing $NH_2$ with OH. This is the only variable of much significance in eq 14. The variable $I_2$ is assigned the value of 1 for Y = $CH_3$ or Cl, the only substituents studied in this position. It seems likely that this models a moderate hydrophobic effect of small substituents in position 5 as uncovered by eq 12 and 13. However, we know from these equations that this position is sterically hindered and that greater activity cannot be attained by the use of large hydrophobic groups.

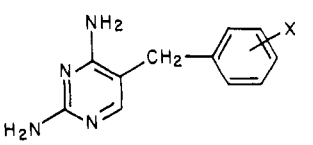
Several bridges Z were studied [$CH_2NH$, $NHCH_2$, $CH_2N(CH_3)$, $CH_2N(CHO)$, $CH_2N(NO)$, $OC_6H_4CH_2NH$, $CH_2N(COCH_3)$] which are parameterized by $MR_6$. The authors interpret the negative coefficient of $MR_6$ to mean that bulky groups on the bridge cause a steric effect at the important 10-position of the folic acid binding site.

Equation 15 is similar to eq 14 but does contain one new variable $I_3$, which is set equal to 1 for Z = $-NHCH_2-$. This structural feature seems to play a different role with murine enzyme not seen in the case of the human enzyme.

Chen et al.[188] also formulated QSAR for the inhibition of thymidylate synthetase from murine L-1210S cells and *L. casei* cells since the quinazolines inhibit both DHFR and thymidylate synthetase. As one would expect, the QSAR for thymidylate synthetase differs from that for DHFR. Drugs which inhibit both are especially interesting for medicinal chemistry.[38,189]

### d. Pyrimidine Inhibitors of DHFR

The class of inhibitors which has received the most extensive study as DHFR inhibitors is that of the pyrimidines. Much of this work has been stimulated by the great success of the clinically important antimicrobial agent trimethoprim (IV). For this reason the benzylpyrimidines (XXVIII) have been intensively studied.



XXVIII

QSAR for XXVIII from bovine DHFR[30]

$$\log 1/K_{i\,app} = 0.48\pi_{3,5} - 1.25 \log (\beta \cdot 10^{\pi_{3,5}} + 1) + 0.13MR_4 + 0.24\sigma + 5.43 \quad (16)$$

$$n = 42,\ r = 0.875,\ s = 0.227\pi_0 = 1.52,\ \log \beta = -1.98$$

QSAR for XXVIII from chicken DHFR[30]

$$\log 1/K_{i\,app} = 0.55\pi_{3,4,5} - 0.42 \log (\beta \cdot 10^{\pi_{3,4,5}} + 1) + 0.20MR_4 + 0.32\sigma + 4.46 \quad (17)$$

$$n = 39,\ r = 0.900,\ s = 0.241,\ \log \beta = -0.22$$

QSAR for XXVIII from human DHFR[21]

$$\log 1/K_{i\,app} = 0.59\pi_{3,5} - 0.63 \log (\beta \cdot 10^{\pi_{3,5}} + 1) + 0.19\pi_4 + 0.19MR_3 + 0.30\sigma + 4.03 \quad (18)$$

$$n = 38,\ r = 0.879,\ s = 0.266,\ \pi_0 = 1.94,\ \log \beta = -0.82$$

Inhibition of the three types of vertebrate DHFR yield QSAR which have similarities as well as differences. In XXVIII X represents mono-, di-, and trisubstitution on the phenyl ring. As noted above with the triazine inhibitors, bovine enzyme is about 10 times more easily inhibited than the chicken or human reductase. In all three equations, there is a rather small but significant electronic effect brought out by the $\sigma$ term. Electron-attracting substituents increase activity which is reminiscent of the triazine equations 3, 5, 7, and 8.

4-Substituents behave in a distinctly different way from 3- or 5-substituted analogues and require individual parameterization. Not much can be concluded about the 5-position since there are only four examples of 3,5-disubstitution and all of these are symmetrical (i.e., 3-X = 5-X).

The initial dependence of log $1/K_i$ on $\pi$ is essentially the same in all three examples. The coefficient with $\pi$ of about 0.5 is distinctly lower than for the triazine equations, suggesting different binding conditions. As with the triazines the chicken DHFR seems to differ from bovine and human. For the avian reductase $\pi_0$ cannot be established with the range of $\pi$ constants possessed by the present set of inhibitors.

For eq 16, ideal $\pi_{3,5}$ is defined as 1.5, but for eq 17 $\pi_0$ could not be determined due to the lack of sufficiently lipophilic congeners. The rather large coefficient of 1.25 in eq 16 for the negative bilinear term seems high and it may be that a steric effect is also involved in determining the rather steep (−0.77) slope of the negative portion of the curve. The three equations are based on relatively few data points so that they serve more to make suggestions for future research than as definitive statements about the SAR.

The benzylpyrimidines (XXVIII) have also been tested on bacterial DHFR with results which are quite different from the vertebrate enzyme. In these equations the most significant variable is MR, while in eq 16–18 $\pi$ is the most important variable. The prime symbol with MR indicates an arbitrary use of this parameter. After considerable analysis it was concluded that only a fraction of a substituent in the 3-, 4-, or 5-position was capable of producing an MR-related effect. The figure of 0.79 (the value of MR for $OCH_3$) appeared to be the breaking point. Thus, the maximum MR effect for all three positions should be 3 × 0.79, regardless of how large the substituents in these positions are. The normal values for MR were used for substituents having values less than 0.79. MR has been scaled by 0.1 to make it more nearly equiscalar with $\pi$.

QSAR for XXVIII from *L. casei* DHFR[31]

$$\log 1/K_i = 0.31\pi_{3,4} - 0.91 \log (\beta \cdot 10^{\pi_{3,4}} + 1) + 0.89MR'_{3,4} - 0.22\sigma_R^- + 5.31 \quad (19)$$

$$n = 42,\ r = 0.889,\ s = 0.214,\ \pi_0 = 1.05$$

QSAR for XXVIII from *E. coli* DHFR[31]

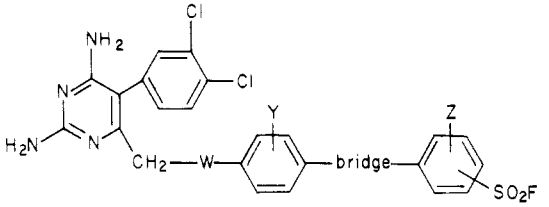
$$\log 1/K_i = 0.43\pi_{3,4,5} - 0.88 \log (\beta \cdot 10^{\pi_{3,4,5}} + 1) + 1.23MR'_{3,5} + 0.80MR'_4 - 0.45\sigma_R^- + 5.81 \quad (20)$$

$$n = 43,\ r = 0.923,\ s = 0.263,\ \pi_0 = 0.64$$

The intercepts of eq 16–20 can be used as a rough estimate of the intrinsic activity of the benzylpyrimidines, yielding the following order: *E. coli* > *L. casei* ≃ bovine > chicken > human. *E coli* is most easily inhibited and human DHFR is most resistant to the benzylpyrimidines. The pattern of individual inhibitors may be very different. Trimethoprim (IV) has the following log $1/K_{i\,app}$: human, 3.77; chicken, 3.98; bovine, 5.51; *L. casei*, 6.88; *E. coli*, 8.87. The selectivity of TMP is outstanding, being 100 000 times more active against *E. coli* than against human DHFR.

The reason for the selectivity of the benzylpyrimidines, as Roth has emphasized, must be steric. The MR term is a measure of bulk and this is the most important term in the bacterial equations. The low $\pi_0$ of 0.25 for the *E. coli* equation denotes no increase in activity of the parent compound can be obtained by the use of hydrophobic substituents. In the eucaryotic QSAR, the most effective compounds are those with hydrophobic substituents (e.g., 3′-$OCH_2C_6H_5$). This lipophilic monosubstituted analogue is only 100 times more active against *E. coli* than against human enzyme. Roth[5,9] has discussed the relationship between 3′,4′,5′-substitution and selectivity for the benzylpyrimidines.

Another class of pyrimidines for which a QSAR has been developed is that of XXIX. In eq 21 *C* is the




XXIX

QSAR for XX IX from four types of mammalian DHFR[190]

$$\log 1/C = 0.37I_1 - 1.01I_8 - 0.78I_9 + 0.42I_{13} - 0.22I_{15} + 0.51I_{20} + 0.67I_4 \cdot I_8 + 7.17 \quad (21)$$

$$n = 105,\ r = 0.903,\ s = 0.229$$

molar concentration of inhibitor causing 50% inhibition. This is a Free–Wilson type analysis[191,192] in which 28 different indicator variables were studied, seven of which turned out to be significant. Three of the 28 parameters were used to explore possible differences in enzyme from four different sources: L-1210/0, L-1210/FR8, L-1210/DF8, liver.

TABLE III. Comparison of Benzylpyrimidines XXVIII Inhibition of Human and *E. coli* DHFR[21,31]

| | | log $1/K_{i\,app}$ | | | | |
|---|---|---|---|---|---|---|
| | | human DHFR | | *E. coli* DHFR | | *L. casei* – |
| no. | X | obsd | calcd | obsd | calcd | human DHFR, obsd |
| 1. | 3,5-$(CH_2OH)_2$ | 2.69 | 2.95 | 6.31 | 6.71 | 3.62 |
| 2. | 3,5-$(OCH_3)_2$, 4-$O(CH_2)_2OCH_3$ | 3.25 | 3.98 | 8.35 | 8.38 | 5.10 |
| 3. | 4-$OCH_2CH_2OCH_3$ | 3.82 | 3.86 | 6.40 | 6.69 | 3.12 |
| 4. | 3,4-$(OH)_2$ | 3.48 | 3.47 | 6.46 | 5.95 | 2.98 |
| 5. | 3-OH | 3.57 | 3.72 | 6.47 | 6.21 | 2.90 |
| 6. | 4-$N(CH_3)_2$ | 3.65 | 3.79 | 6.78 | 6.81 | 3.13 |
| 7. | 4-$OCH_3$ | 3.70 | 3.93 | 6.82 | 6.79 | 3.12 |
| 8. | 4-$NH_2$ | 3.71 | 3.58 | 6.30 | 6.01 | 2.59 |
| 9. | 3-$OCH_2CONH_2$ | 3.75 | 3.56 | 6.57 | 6.46 | 2.82 |
| 10. | 3,4,5-$(OCH_3)_3$ | 3.77 | 4.04 | 8.87 | 8.47 | 5.10 |
| 11. | 4-$NO_2$ | 3.79 | 4.20 | 6.20 | 6.69 | 2.41 |
| 12. | 3-$CH_2OH$ | 3.82 | 3.56 | 6.28 | 6.59 | 2.46 |
| 13. | 3-$OCH_2CH_2OCH_3$ | 3.93 | 4.18 | 6.53 | 7.01 | 2.60 |
| 14. | H | 3.94 | 4.01 | 6.18 | 6.19 | 2.24 |
| 15. | 3,4-$(OCH_2CH_2OCH_3)_2$ | 4.03 | 4.03 | 7.22 | 7.43 | 3.19 |
| 16. | 4-$NHCOCH_3$ | 4.10 | 3.83 | 6.89 | 6.40 | 2.79 |
| 17. | 3-$CH_3$ | 4.13 | 4.32 | 6.70 | 6.81 | 2.57 |
| 18. | 3,5-$(OCH_3)_2$ | 4.15 | 4.26 | 8.38 | 8.07 | 4.23 |
| 19. | 4-$OCF_3$ | 4.17 | 4.32 | 6.57 | 6.70 | 2.40 |
| 20. | 3-$OSO_2CH_3$ | 4.17 | 3.95 | 6.92 | 6.77 | 2.75 |
| 21. | 4-$CH_3$ | 4.18 | 4.07 | 6.48 | 6.60 | 2.30 |
| 22. | 3-$NO_2$, 4-$NHCOCH_3$ | 4.24 | 4.02 | 6.97 | 7.08 | 2.73 |
| 23. | 4-Br | 4.25 | 4.25 | 6.82 | 6.74 | 2.57 |
| 24. | 3-$OCH_3$ | 4.26 | 4.17 | 6.93 | 7.11 | 2.67 |
| 25. | 4-F | 4.29 | 4.06 | 6.35 | 6.19 | 2.06 |
| 26. | 4-$O(CH_2)_3CH_3$ | 4.30 | 4.21 | 6.89 | 6.55 | 2.59 |
| 27. | 3-$CF_3$ | 4.31 | 4.57 | 7.02 | 6.67 | 2.71 |
| 28. | 4-Cl | 4.34 | 4.22 | 6.45 | 6.60 | 2.11 |
| 29. | 3-F | 4.35 | 4.18 | 6.23 | 6.19 | 1.88 |
| 30. | 3-Cl | 4.41 | 4.52 | 6.65 | 6.83 | 2.24 |
| 31. | 4-$O(CH_2)_5CH_3$ | 4.41 | 4.42 | | | |
| 32. | 3-$CH_2OCH_3$ | 4.52 | 3.80 | 6.59 | 6.83 | 2.07 |
| 33. | 3-$CH_2O(CH_2)_3CH_3$ | 4.63 | 4.83 | 6.55 | 7.06 | 1.92 |
| 34. | 3-I | 4.65 | 4.76 | 7.23 | 6.99 | 2.58 |
| 35. | 3,4-$(OCH_3)_2$ | 4.73 | 4.14 | 7.72 | 7.73 | 2.99 |
| 36. | 3-Br | 4.73 | 4.62 | 6.96 | 7.06 | 2.23 |
| 37. | 3-$O(CH_2)_3CH_3$ | 4.99 | 4.88 | 6.82 | 6.87 | 1.83 |
| 38. | 3-$O(CH_2)_5CH_3$ | 5.03 | 5.05 | 6.86 | 6.54 | 1.83 |
| 39. | 3-$OCH_2C_6H_5$ | 5.05 | 5.08 | 6.99 | 6.82 | 1.94 |
| 40. | 3-$CF_3$, 4-$OCH_3$ | 5.10 | 4.48 | 7.69 | 7.28 | 2.59 |

Only one indicator variable proved to be useful in differentiating the enzymes. $I_{20}$ in eq 21 is assigned the value of 1 for log $1/C$ obtained with L-1210/0. All other enzymes were assigned the value of zero since they did not behave in a statistically different way. Although seven different bridges between the two phenyl rings were tested, only three indicator variables were necessary. $I_8 = 1$ for 4-NHCONH, $I_9 = 1$ for 4-NHCO, and $I_{13} = 1$ for 4-$NHSO_2$. The other bridges, 4-$CH_2NHCONH$, 4-$CH_2CH_2NHCONH$, 3-$CH_2NHCONH$, 4-$CH_2NHCO$, 4-$CH_2CH_2NHCO$, 3-$CH_2NHCO$, 4-$CH_2NHSO_2$, 4-$CH_2CH_2NHSO$, 3-$CH_2NHSO_2$, 4-$CH_2CH_2$, 3-$CH_2CH_2$, received no parameterization and hence within the precision of the analysis behave in the same way. Note that the three parameterized bridges all contain heteroatoms which interact strongly electronically with the $\pi$ electrons of the phenyl rings. This interaction stiffens the bridges. Two of the parameterized bridges have negative coefficients with $I$, indicating their depressing effect on log $1/C$. Only one bridge, 4-$NHSO_2$, has a small activity-enhancing effect. The feature $\omega$ has only two forms: $CH_2$ or O. $I_1$ is given the value of 1 for $\omega = CH_2$. The positive coefficient with $I_1$ probably represents a hydrophobic interaction of the $CH_2$. $I_4$ receives the value of 1 for Y = 3-$CH_3$. While there were seven different examples of Y, only one (3-$CH_3$) appeared to differ significantly in its effect on log $1/C$ and this occurred only when the bridge in the congener was 4-NHCONH. The positive coefficient with the cross product term $I_4 \cdot I_8$ shows the activity elevating effect of this combination.

The task of seeking out cooperative effects using cross product terms such as $I_4 \cdot I_8$ is almost endless since in a large data set the possibilities are enormous; nevertheless, this is an area which deserves more serious study in structure–activity analysis.

A comparison of benzylpyrimidines inhibiting DHFR from five sources (chicken, human, bovine, *E. coli*, and *L. casei*) as well as two types of cell cultures (L5178Y and L5178YR tumor cells) was made.[21] Table III compares the inhibitory potency of benzylpyrimidines on human and *E. coli* DHFR. This class of inhibitors is much more selective against bacterial DHFR. As Roth has noted, it is the 3,4,5-trisubstituted benzylpyrimidines which are most selective. Compounds 2 and 10 are over 100 000 times less inhibitory of human DHFR. The least selective are those with lipophilic substituents in the 3-position only. Even these are about 100 times more active against the *E. coli* DHFR.

## VI. Minimal Topological Difference (MTD) Analysis

The most difficult problem in SAR is that of defining

the geometry of the active site. Unfavorable steric interactions between ligand and receptor can have a huge effect on ligand affinity. Naturally such an important as well as difficult problem has elicited a variety of possible solutions. The classic approach to steric problems is that of Taft, which uses the substituent constant $E_s$. Even this elementary method has been of help in the study of macromolecular interactions with small ligands.[164]

A more sophisticated approach is that of Verloop, Hoogenstraaten, and Tipker, who have defined five parameters to describe the size of an organic substituent.[168,169] These parameters were shown, at times, to be better models of steric effects than Taft's $E_s$, which was designed for small organic molecules reacting with each other in homogeneous solution.

A more general attack on the problem of ligand fit to active site has been undertaken by Simon and his colleagues. They have applied their technique to the DHFR problem.[193,194] In this approach the minimal topological difference (MTD) for a molecule M is defined with respect to a standard (S) which is presumably close to an ideal fit for the receptor. There is no completely satisfactory way of deciding which is the best standard. One could choose the most potent inhibitor or one might start with the natural substrate when it is known. In an ongoing investigation it will often be necessary to redefine the standard reference compound as the investigation proceeds. The MTD is defined as the number of unsuperimposable atoms when M is superimposed atom for atom on S, hydrogen atoms being neglected. One first superimposes all $NM_i$ molecules on S to obtain a "hypermolecule" H with an atomic network with M vertices $j = 2, 3, ..., M$. Each $M_i$ molecule is thereby characterized by a vector with $M$ components, $X_{i_1}, X_{i_2}, ..., X_{im}$, and the whole set of $N$ molecules by an $N \times M$ matrix $|X_{i,j}|$ with $X_{ij} = 1$ if vertex $j$ is occupied in $M_i$ and $X_{ij} = 0$ if it is not. The minimal topological difference is given by the following equation:

$$\mathrm{MTD}_i = s + \sum_{j=1}^{M} \epsilon_j X_{ij} \qquad (22)$$

where $s$ = the number of vertices occupied by the standard S when it is superimposed upon H. In this expression $\epsilon_j = -1$ for vertices occupied in the standard S and $\epsilon_j = +1$ for those unoccupied. Of course, not all vertices of H are necessarily relevant for steric fit. Those which project beyond the receptor site into the surrounding aqueous phase have $\epsilon_j$ set equal to zero. While the above definition is straightforward and succinct, in practice it is not always easy to carry out. The $\epsilon_j$ have to be adjusted as the analysis uncovers new features of the SAR.

The $\mathrm{MTD}_i$ are then employed in a regression equation with other suitable parameters

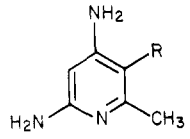
$$A_i^{\mathrm{calcd}} = \alpha_0 + \alpha_i \sigma_{1i} + ... \beta \mathrm{MTD}_i \qquad (23)$$

to correlate activity ($A_i$) with structural features by minimization of

$$Y = \sum_{i=1}^{N} (A_i^{\mathrm{exp}} - A_i^{\mathrm{calcd}})^2 \qquad (24)$$

To find $S_{\mathrm{opt}}$ one must also minimize $Y$ with respect to $\epsilon_j$. Simon et al. have devised algorithms for doing so.[193]

Using $K_i$ values for the inhibition of sarcoma AT/300 DHFR obtained from the work of Zakrzewski et al., they derived the QSAR of eq 25 for pyrimidines XXX.




XXX

$$\log 1/K_i = 0.36\pi - 0.65\mathrm{MTD} + 9.22 \qquad (25)$$

$$n = 15,\ r = 0.981,\ s = 0.287$$

In this study R was limited to hydrocarbon groups in which there was considerable structural variation. Unfortunately for the data available there is rather high collinearity between the $\pi$ and MTD vectors. This may explain the small coefficient with $\pi$ which is out of line with what one would expect for pyrimidines reacting with *mammalian* DHFR. In a second study[194] of 136 triazines VI (tested on DHFR from Walker tumor by Baker) used to derive eq 1, MTD offers some explanation for the variance in log $1/C$ accounted for by indicator variables and $MR_4$ in eq 1.

$$A = 0.89\pi_3 - 0.12(\pi_3)^2 - 0.32\mathrm{MTD} + 14.98 \qquad (26)$$

$$n = 136,\ r = 0.860,\ s = 0.612$$

Although eq 26 has many less terms than eq 1, it has a much higher standard deviation.

## VII. Distance Geometry in QSAR

Recently, Crippen has been applying the method known as distance geometry to the problem of assessing the fit of ligands to DHFR.[195-201] This technique has also been valuable in working out the three-dimensional structure of macromolecules via high-resolution NMR.[202,203] Crippen's approach is to find the "best" partitioning of the total free energy of binding (estimated from $K_i$) of critical points on the ligand interacting with critical points on the enzyme. First the number of such points must be deduced and then a kind of least-squares optimization yields a set of free energies of interaction between the corresponding sets of points. Crippen has outlined the following 10-step approach.[197] (1) The molecules (in the present case quinazolines used to derive eq 13) are constructed from crystallographic data. (2) For each molecule the matrix of upper and lower bounds on the distances between its atoms over all sterically allowed conformations is calculated. To include only acceptable conformations of low energy, no penetration beyond the van der Waals radii or radii of closest approach may be permitted. (3) To reduce use of computer time, some atoms of each molecule must be deleted. (4) A plausible binding mode for each molecule is chosen specifying the binding of atoms to each site point (see Table III). (5) From this the site point distance bounds are evaluated on the assumption that each intersite point distance should be in the range common to the corresponding interatomic distances in all molecules. In some cases no overlapping exists, for example, where the highest of the lower bounds ($l$) is slightly higher than the least of the upper

bounds distance ($u$). Under this condition, two site points can still bind the respective molecular points if one assumes that the site point distance is intermediate between the two limits and the site flexibility, $\delta \geq (l - u)/2$. Binding points should be chosen so that the value of $\delta$ is minimal. (6) The intersite point distances are used to calculate the $3n$ coordinates, where $n$ is the number of site points and there are $n(n-1)/2$ intersite distances. One of the possible sets of coordinates is selected. (7) Since we have no assurance that the site point distances in these derived coordinates will be at the mean of the two respective limits, a reevaluation of $\delta$ is necessary. (8) For molecules with ambiguities about which molecular point goes with which site point, the site point coordinates, $\delta$, and the intramolecular distance bounds may be used to select which molecular points will yield the best fit to these site points. (9) After selecting the site point coordinates, it is necessary to check whether or not these are all geometrically acceptable with no forced contacts. (10) The interaction parameters are evaluated via quadratic programming to minimize the differences between calculated and observed binding energies under the constraint that the fixed modes are energetically more favorable than any other geometrically allowed binding mode.

In treating the quinazolines of Table IV by the above method, they were divided into three classes on the basis of qualitative considerations: "ordinary" quinazolines are loosely bound compounds not containing the 2,4-diamino groups, strongly bound quinazolines containing the 2,4-diamino grouping, and "odd" 2,4-diaminoquinazolines which are loosely bound.

In the preliminary model 11 site points on the enzyme were considered essential, but in the final model just as good results were obtained using only nine points (Table VI). Ghose and Crippen concluded that the quinazolines bind in two different modes, which is not unreasonable in light of the present view of different modes of binding for folic acid and MTX (see section on graphics). The different modes result from the rotation of the quinazoline 180° along the axis connecting points 3 and 4 (Table V). This rotation places substituents at positions 4 and 5 in different space as well as moving $N_1$. In their modeling it seemed to make no difference whether N1 or N3 was protonated; however, it was assumed that the protonated N cannot be attractive to $S_1$, which is for binding of nonprotonated basic N. It was also assumed that N3 could not bind with site point 2 and that the "odd" quinazolines could achieve a reasonably good fit by having C7 at site point 3, N3 at site point 1, and 2-$NH_2$ at site point 4. This mode of binding avoids contact of the SO and $SO_2$ moieties which are present in the "odd" inhibitors with site point 8 or the contact of N1 with site point 1 but maintains the contact of site points 9 and 10 with the phenyl or naphthyl rings. This dual mode of binding requires only four different points for ring nitrogens.

The present distance geometry model was developed from a study of 13 of the 67 quinazolines. The inference which Crippen and Ghose draw from Table VI is that the strong binding of the quinazolines is the result of 4-$NH_2$ binding at site 6 as well as the attraction of the two ring nitrogens with sites 1 and 2 and not just by attraction of a protonated ring nitrogen with site point 2. Site point 10 is concluded to be a strong binding point but not site 9. They suggest that this calls for substitution of electronegative atoms at the 4-position of phenyl rings attached to the 6-position of the quinazolines. Since point 9 seems so unimportant, they point out that aromatic substituents at positions 5 and 6 are probably not worthwhile. This does not seem to be a good generalization since the QSAR of eq 12 and 13 indicate that small 5-substituents make an important binding contribution and one of the most promising antitumor drugs, XVIII, has a 5-substituent. Ghose and Crippen also recommend the study of substituents in position 7 for which as yet no data are available. Position 8, which has also not been explored, seems unattractive in the light of their analysis.

In the end, Ghose and Crippen obtain a model having correlation coefficient of 0.955 and standard deviation of 0.69, which is about the same as eq 13 although they have included one more data point (25, Table IV) not included in eq 13. Their results are in agreement with the conclusion from eq 13 that a hydrophobic region is near position 5 of the quinazoline ring. As no parameter occurs in eq 13 for 6-substituents, it was concluded that this region is open to solvent. In the Ghose–Crippen model the region is postulated to contain a site point which interacts strongly with several groups. That this is a reasonable conclusion is evident from the fact that the most active congeners contain large groups in position 6. Both large polar and nonpolar groups yield highly active compounds, and for this reason attempts to parameterize the varied substituents with $\pi$ or MR do not lead to a simple one-variable explanation. The net result was that no parameter was found for eq 13 for 6-substituents.

More recently[198] Ghose and Crippen have expanded the model to include both quinazolines and triazines (VI). They come to the interesting conclusion that the substituent in 3-X-triazines is involved in a dipolar interaction. The QSAR results of eq 3 and 5–8 all contain terms in $\sigma$, suggesting the importance of a polar reaction. The 3-CN group, which may be the best signal for this effect, is generally more active than expected in eq 3–8; however, it is well predicted in the distance geometry model.

Distance geometry provides a most interesting means for a spatial description of the active site in isolated enzymes and very likely will find an important place in the QSAR paradigm. It is not clear yet how it can be employed with in vivo studies where hydrophobic effects in the random walk process of drugs are so important.

As Crippen has noted, distance geometry is somewhat like the Free–Wilson approach[191,192] to QSAR in that a rather large number of variables compared to data points may be required to define the structurally important features. One has to accept this fact about the complexity of life and judge the value of the distance geometry models on their ultimate value to understanding the SAR. A difficulty with both the Free–Wilson parameters and those from distance geometry is that each number contains the hydrophobic, electronic, steric, and dipolar factors all lumped together. This makes it difficult for one to predict just what kind of substituent should be placed at a given site in order to obtain maximum binding.

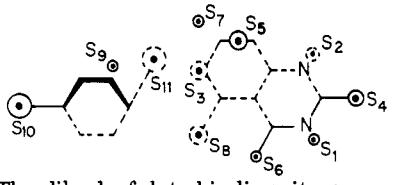
One must bear in mind that all QSAR models, in-

**TABLE IV. Observed and Calculated Free Energies of Binding of Quinazolines to *S. faecium* DHFR[197]**

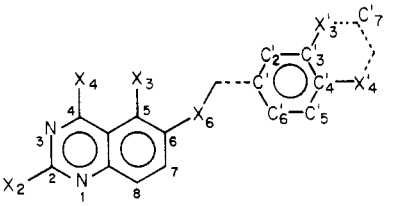
| no. | group | $-\Delta G_{obsd}$,[a] kcal/mol | $-\Delta G_{calcd}$,[b] kcal/mol | | | |
|---|---|---|---|---|---|---|
| | | | I | II | III | IV |
| 1. | 2-H, 4-$NH_2$, 6-$SO_2$(2-$C_{10}H_7$) | 5.8 | 6.5 | 6.7 | 6.7 | 6.9 |
| 2. | 2,4-$(SH)_2$, 6-S(2-$C_{10}H_7$) | 6.0 | 6.3 | 7.0 | 7.0 | 6.9 |
| 3. | 2-SH, 4-OH, 6-S(2-$C_{10}H_7$) | 6.2 | 6.3 | 7.2 | 7.1 | 6.9 |
| 4. | 2,4-$(NH_2)_2$, 5-$SO_2$(2-$C_{10}H_7$) | 6.5 | 8.0 | 7.7 | 10.9 | 7.4 |
| 5. | 2-H, 4-$NH_2$, 6-S(2-$C_{10}H_7$) | 6.5 | 6.8 | 6.7 | 6.3 | 6.8 |
| 6. | 2-$NH_2$, 4-OH, 5-$CH_3$, 6-$NHCH_2$($C_6H_4$-4-$CO_2H$) | 6.5 | 7.9 | 7.4 | 7.1 | 7.4 |
| 7. | 2-OH, 4-SH, 6-S(2-$C_{10}H_7$) | 6.8 | 6.6 | 6.7 | 7.0 | 6.9 |
| 8. | 2,4-$(OH)_2$, 6-S(2-$C_{10}H_7$) | 6.9 | 6.6 | 6.9 | 7.0 | 6.9 |
| 9. | 2-OH, 4-$NH_2$, 6-S(2-$C_{10}H_7$) | 6.9 | 7.3 | 7.1 | 6.6 | 6.9 |
| 10. | 2,4-$(NH_2)_2$, 5-SO(2-$C_{10}H_7$) | 6.9 | 8.0 | 7.7 | 10.9 | 7.4 |
| 11. | 2-$NH_2$, 4-OH, 5-$CH_3$, 6-$NHCH_2$($C_6H_4$-4-$CO_2Et$) | 7.1 | 7.9 | 7.4 | 7.1 | 7.4 |
| 12. | 2-$NH_2$, 4-OH, 6-$NHCH_2$($C_6H_4$-4-$CO_2H$) | 7.2 | 7.9 | 7.4 | 7.1 | 7.4 |
| 13. | 2-H, 4-$NH_2$, 6-SO(2-$C_{10}H_7$) | 7.2 | 6.8 | 7.0 | 6.8 | 6.9 |
| 14. | 2,4-$(NH_2)_2$, 5-SO($C_6H_3$-3,4-$Cl_2$) | 7.3 | 8.2 | 8.2 | 11.2 | 7.6 |
| 15. | 2-$NH_2$, 4-OH, 5-S(2-$C_{10}H_7$) | 7.4 | 8.0 | 7.9 | 7.9 | 7.4 |
| 16. | 2-SH, 4-$NH_2$, 6-S(2-$C_{10}H_7$) | 7.4 | 7.0 | 7.4 | 6.6 | 6.9 |
| 17. | 2-$NH_2$, 4-OH, 6-$CH_2NH$[$C_6H_4$-4-CONHCH($CO_2Et$)$CH_2CH_2CO_2Et$] | 7.9 | 8.9 | 9.2 | 9.3 | 9.0 |
| 18. | 2-$NH_2$, 4-SH, 6-$SO_2$(2-$C_{10}H_7$) | 8.0 | 8.9 | 8.6 | 9.1 | 9.2 |
| 19. | 2-$NH_2$, 4-OH, 6-SO(2-$C_{10}H_7$) | 8.2 | 9.1 | 9.1 | 9.2 | 9.2 |
| 20. | 2-$NH_2$, 4-OH, 6-$SO_2$($C_6H_3$-3,4-$Cl_2$) | 8.2 | 9.1 | 9.3 | 9.4 | 9.4 |
| 21. | 2-$NH_2$, 4-OH, 6-$CH_2N(CH_3)$[$C_6H_4$-4-CONHCH($CO_2Et$)$CH_2CH_2CO_2Et$] | 8.3 | 8.9 | 9.2 | 9.3 | 9.0 |
| 22. | 2-$NH_2$, 4-OH, 6-$NHCH_2$($C_6H_4$-4-$CO_2Et$) | 8.6 | 7.9 | 7.4 | 7.1 | 7.4 |
| 23. | 2-$NH_2$, 4-OH, 6-$CH_2NH$($C_6H_4$-4-$CO_2H$) | 8.8 | 8.9 | 9.2 | 9.3 | 9.0 |
| 24. | 2-$NH_2$, 4-OH, 6-$CH_2NH$[$C_6H_4$-4-CONHCH($CO_2H$)$CH_2CH_2CO_2H$] | 8.9 | 8.9 | 9.2 | 9.3 | 9.0 |
| 25. | 2-$NH_2$, 4-OH, 5-$SO_2$(2-$C_{10}H_7$) | 9.0 | 9.0 | 9.0 | 9.6 | 8.5 |
| 26. | 2,4-$(NH_2)_2$, 5-$SO_2$($C_6H_3$-3,4-$Cl_2$) | 9.0 | 8.1 | 8.2 | 11.2 | 7.6 |
| 27. | 2-$NH_2$, 4-OH, 6-S($C_6H_3$-3,4-$Cl_2$) | 9.1 | 9.4 | 9.3 | 9.0 | 9.3 |
| 28. | 2-$NH_2$, 4-OH, 5-Cl, 6-$NHCH_2$($C_6H_4$-4-$CO_2Et$) | 9.3 | 9.3 | 7.4 | 9.4 | 8.8 |
| 29. | 2-$NH_2$, 4-SH, 6-S(2-$C_{10}H_7$) | 9.3 | 9.2 | 8.6 | 8.7 | 9.0 |
| 30. | 2-$NH_2$, 4-OH, 6-$SO_2$(2-$C_{10}H_7$) | 9.6 | 8.9 | 8.8 | 9.1 | 9.2 |
| 31. | 2-$NH_2$, 4-OH, 6-$CH_2N(CH_3)$[$C_6H_4$-4-CONHCH($CO_2H$)$CH_2CH_2CO_2H$] | 9.7 | 8.9 | 9.2 | 9.3 | 9.0 |
| 32. | 2-$NH_2$, 4-OH, 6-$CH_2N$(CHO)[$C_6H_4$-4-CONHCH($CO_2H$)$CH_2CH_2CO_2H$] | 9.9 | 8.9 | 9.2 | 9.3 | 9.0 |
| 33. | 2,4-$(NH_2)_2$, 5-S($C_6H_3$-3,4-$Cl_2$) | 9.9 | 11.4 | 11.3 | 11.2 | 11.0 |
| 34. | 2-$NH_2$, 4-OH, 6-S(2-$C_{10}H_7$) | 10.2 | 9.2 | 8.8 | 8.8 | 9.0 |
| 35. | 2-$NH_2$, 4-OH, 6-$CH_2NH$($C_6H_4$-4-$CO_2Et$) | 10.6 | 8.9 | 9.2 | 9.3 | 9.0 |
| 36. | 2,4-$(NH_2)_2$, 5-*trans*-CH=CH(2-$C_{10}H_7$) | 10.7 | 10.1 | 10.1 | 10.1 | 10.3 |
| 37. | 2,4-$(NH_2)_2$, 5-$CH_2S$($C_6H_4$-4-Cl) | 10.9 | 11.7 | 11.6 | 11.2 | 11.1 |
| 38. | 2,4-$(NH_2)_2$, 5-S(2-$C_{10}H_7$) | 10.9 | 11.2 | 10.8 | 10.9 | 10.8 |
| 39. | 2,4-$(NH_2)_2$, 6-$NHCH_2$($C_6H_4$-4-$CO_2Et$) | 11.0 | 11.1 | 12.5 | 12.7 | 11.9 |
| 40. | 2,4-$(NH_2)_2$, 5-*cis*-CH=CH(2-$C_{10}H_7$) | 11.1 | 11.2 | 10.9 | 10.9 | 11.5 |
| 41. | 2,4-$(NH_2)_2$, 6-$CH_2NH$($C_6H_4$-4-$CO_2$-*n*-Bu) | 11.2 | 12.1 | 12.1 | 12.4 | 12.0 |
| 42. | 2,4-$(NH_2)_2$, 5-$CH_2S$(2-$C_{10}H_7$) | 11.3 | 11.5 | 11.1 | 10.9 | 10.9 |
| 43. | 2,4-$(NH_2)_2$, 6-$CH_2NH$($C_6H_4$-4-$CO_2Et$) | 11.4 | 12.1 | 12.1 | 12.4 | 12.0 |
| 44. | 2,4-$(NH_2)_2$, 5-Cl, 6-$CH_2NH$($C_6H_4$-4-$CO_2$-*n*-Bu) | 11.4 | 12.5 | 12.2 | 12.4 | 12.3 |
| 45. | 2,4-$(NH_2)_2$, 6-$CH_2NH$[$C_6H_4$-4-CONHCH($CO_2Et$)$CH_2CH_2CO_2Et$] | 11.5 | 12.1 | 12.1 | 12.4 | 12.0 |
| 46. | 2,4-$(NH_2)_2$, 6-$CH_2NH$[$C_6H_4$-4-CONHCH($CO_2Et$)$CH_2CO_2Et$] | 11.6 | 12.1 | 12.1 | 12.4 | 12.0 |
| 47. | 2,4-$(NH_2)_2$, 5-$CH_3$, 6-$CH_2NH$($C_6H_4$-4-$CO_2H$) | 11.8 | 12.4 | 12.4 | 12.4 | 12.6 |
| 48. | 2,4-$(NH_2)_2$, 5-$CH_2CH_2$(2-$C_{10}H_7$) | 11.9 | 11.5 | 11.1 | 10.9 | 10.9 |
| 49. | 2,4-$(NH_2)_2$, 6-S(2-$C_{10}H_7$) | 12.1 | 12.4 | 11.8 | 11.8 | 12.0 |
| 50. | 2,4-$(NH_2)_2$, 5-$CH_3$, 6-$CH_2NH$($C_6H_4$-4-$CO_2$-*n*-Bu) | 12.1 | 12.4 | 12.4 | 12.4 | 12.6 |
| 51. | 2,4-$(NH_2)_2$, 5-Cl, 6-$NHCH_2$($C_6H_4$-4-$CO_2Et$) | 12.2 | 11.5 | 12.6 | 12.7 | 12.2 |
| 52. | 2,4-$(NH_2)_2$, 6-$CH_2NH$[$C_6H_4$-4-CONHCH($CO_2H$)$CH_2CO_2H$] | 12.2 | 12.1 | 12.1 | 12.4 | 12.0 |
| 53. | 2,4-$(NH_2)_2$, 6-S($C_6H_3$-3,4-$Cl_2$) | 12.2 | 12.6 | 12.2 | 12.1 | 12.3 |
| 54. | 2,4-$(NH_2)_2$, 5-$CH_3$, 6-$CH_2NH$[$C_6H_4$-4-CONHCH($CO_2Et$)$CH_2CO_2Et$] | 12.2 | 12.4 | 12.4 | 12.4 | 12.6 |
| 55. | 2,4-$(NH_2)_2$, 5-Cl, 6-$CH_2NH$($C_6H_4$-4-$CO_2H$) | 12.3 | 12.5 | 12.2 | 12.4 | 12.3 |
| 56. | 2,4-$(NH_2)_2$, 5-$CH_3$, 6-$CH_2NH$($C_6H_4$-4-$CO_2Et$) | 12.3 | 12.4 | 12.4 | 12.4 | 12.6 |
| 57. | 2,4-$(NH_2)_2$, 6-$SO_2$(2-$C_{10}H_7$) | 12.4 | 12.1 | 11.7 | 12.2 | 12.2 |
| 58. | 2,4-$(NH_2)_2$, 5-Cl, 6-$CH_2NH$[$C_6H_4$-4-CONHCH($CO_2Et$)$CH_2CO_2Et$] | 12.5 | 12.5 | 12.2 | 12.4 | 12.3 |
| 59. | 2,4-$(NH_2)_2$, 5-Cl, 6-$CH_2NH$[$C_6H_4$-4-CONHCH($CO_2H$)$CH_2CO_2H$] | 12.6 | 12.5 | 12.2 | 12.4 | 12.3 |
| 60. | 2,4-$(NH_2)_2$, 5-$CH_3$, 6-$CH_2NH$[$C_6H_4$-4-CONHCH($CO_2H$)$CH_2CO_2H$] | 12.7 | 12.4 | 12.4 | 12.4 | 12.6 |
| 61. | 2,4-$(NH_2)_2$, 5-Cl, 6-$CH_2NH$($C_6H_4$-4-$CO_2Et$) | 12.8 | 12.5 | 12.2 | 12.4 | 12.3 |
| 62. | 2,4-$(NH_2)_2$, 6-SO(2-$C_{10}H_7$) | 12.8 | 12.3 | 12.1 | 12.2 | 12.2 |
| 63. | 2,4-$(NH_2)_2$, 6-$CH_2NH$($C_6H_4$-4-$CO_2H$) | 13.0 | 12.1 | 12.1 | 12.4 | 12.0 |
| 64. | 2,4-$(NH_2)_2$, 5-$CH_3$, 6-$NHCH_2$($C_6H_4$-4-$CO_2Et$) | 13.1 | 11.4 | 12.8 | 12.7 | 12.5 |
| 65. | 2,4-$(NH_2)_2$, 6-$CH_2NH$[$C_6H_4$-4-CONHCH($CO_2H$)$CH_2CH_2CO_2H$] | 13.1 | 12.1 | 12.1 | 12.4 | 12.0 |
| 66. | 2,4-$(NH_2)_2$, 6-$CH_2N$(CHO)[$C_6H_4$-4-CONHCH($CO_2H$)$CH_2CH_2CO_2H$] | 13.3 | 12.1 | 12.1 | 12.4 | 12.0 |
| 67. | 2,4-$(NH_2)_2$, 6-S($C_6H_4$-3-$CF_3$) | 13.4 | 13.4 | 13.4 | 15.2 | 13.4 |
| 68. | 2,4-$(NH_2)_2$, 6-$SO_2$($C_6H_3$-3,4-$Cl_2$) | 13.4 | 12.3 | 12.2 | 12.5 | 12.4 |

cluding distance geometry, will never reach perfection. Sooner or later large enough changes can be made in the structure of a parent compound so that the derivatives will not fit the best current QSAR. This does not mean the QSAR is an unimportant academic game. These objectively constructed mathematical models are our best means of developing a description of an incredibly complex active site in a macromolecule.

**TABLE V. Correlation of Molecular Site Point Binding with DHFR Site Points[a,b,d]**




The dihydrofolate binding site geometry.[d]




| no. | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $S_6$ | $S_7$ | $S_8$ | $S_9$ | $S_{10}$ | $S_{11}$ | no. | $S_1$ | $S_2$ | $S_3$ | $S_4$ | $S_6$ | $S_8$ | $S_9$ | $S_{10}$ | $S_{11}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $N_1$ | | $C_6$ | | $X_4$ | | | | $C'_2$ | $X'_4$ | $X_6$ | 36 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | | | |
| 2 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | | 37 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_2$ | $X'_4$ | |
| 3 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | | 38 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_2$ | $C'_7$ | |
| 4 | $N_3$ | | $C_7$ | $X_2$ | | | | | $C'_6$ | $X'_4$ | | 39 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_6$ | $X'_4$ | $X_6$ |
| 5 | $N_1$ | | $C_6$ | | $X_4$ | | | | $C'_2$ | $X'_4$ | $X_6$ | 40 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_2$ | $X'_4$ | |
| 6 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | $X_5$ | | $C'_2$ | $X'_4$ | | 41 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_2$ | $X'_4$ | $X_6$ |
| 7 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | $X_6$ | 42 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_1$ | $X'_4$ | |
| 8 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | $X_6$ | 43 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_6$ | $X'_4$ | $X_6$ |
| 9 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | $X_6$ | 44 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_2$ | $X'_4$ | $X_6$ |
| 10 | $N_3$ | | $C_7$ | $X_2$ | | | | | $C'_6$ | $X'_4$ | | 45 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_6$ | $X'_4$ | $X_6$ |
| 11 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | $X_5$ | | $C'_2$ | $X'_4$ | | 46 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_6$ | $X'_4$ | $X_6$ |
| 12 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | | 47 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_6$ | $X'_4$ | $X_6$ |
| 13 | $N_1$ | | $C_6$ | | $X_4$ | | | | $C'_2$ | $X'_4$ | $X_6$ | 48 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_2$ | $X'_4$ | |
| 14 | $N_3$ | | $C_7$ | $X_2$ | | | | | $C'_2$ | $X'_4$ | | 49 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_2$ | $X'_4$ | $X_6$ |
| 15 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | $X_5$ | | $C'_2$ | $X'_4$ | | 50 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_2$ | $X'_4$ | $X_6$ |
| 16 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | | 51 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_6$ | $X'_4$ | $X_6$ |
| 17 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_6$ | $X'_4$ | $X_6$ | 52 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_6$ | $X'_4$ | $X_6$ |
| 18 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | $X_6$ | 53 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_2$ | $X'_4$ | $X_6$ |
| 19 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | $X_6$ | 54 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_6$ | $X'_4$ | $X_6$ |
| 20 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | $X_6$ | 55 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_6$ | $X'_4$ | $X_6$ |
| 21 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_6$ | $X'_4$ | $X_6$ | 56 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_6$ | $X'_4$ | $X_6$ |
| 22 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | | 57 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_2$ | $X'_4$ | $X_6$ |
| 23 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_6$ | $X'_4$ | $X_6$ | 58 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_6$ | $X'_4$ | $X_6$ |
| 24 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_6$ | $X'_4$ | $X_6$ | 59 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_6$ | $X'_4$ | $X_6$ |
| 25 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | $X_5$ | | $C'_2$ | $X'_4$ | | 60 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_6$ | $X'_4$ | $X_6$ |
| 26 | $N_3$ | | $C_7$ | $X_2$ | | | | | $C'_2$ | $X'_4$ | | 61 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_6$ | $X'_4$ | $X_6$ |
| 27 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_6$ | $X'_4$ | $X_6$ | 62 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_2$ | $X'_4$ | $X_6$ |
| 28 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | $X_5$ | | $C'_2$ | $X'_4$ | | 63 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_6$ | $X'_4$ | $X_6$ |
| 29 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | $X_6$ | 64 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | $X_5$ | $C'_6$ | $X'_4$ | $X_6$ |
| 30 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | $X_6$ | 65 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_6$ | $X'_4$ | $X_6$ |
| 31 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_6$ | $X'_4$ | $X_6$ | 66 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_6$ | $X'_4$ | $X_6$ |
| 32 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_6$ | $X'_4$ | $X_6$ | 67[c] | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_2$ | $X'_3$ | $X_6$ |
| 33 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | | $X_4$ | | $X_5$ | $C'_6$ | $X'_4$ | | 68 | $N_3$ | $N_1$ | $C_6$ | $X_2$ | $X_4$ | | $C'_2$ | $X'_4$ | $X_6$ |
| 34 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_2$ | $X'_4$ | $X_6$ | | | | | | | | | | |
| 35 | $N_1$ | | $C_6$ | $X_2$ | $X_4$ | | | | $C'_6$ | $X'_4$ | $X_6$ | | | | | | | | | | |

[a] Table from ref 197. [b] $X_i$ and $X'_i$ are the atoms directly attached to the respective carbon atoms. [c] $X'_3$ for this compound is one fluorine atom. [d] The site points are shown with a strongly bound 2,4-diaminoquinazoline attached. The solid and dotted circles represent above and below the plane, respectively, and their size represents the relative distance from the plane. An "ordinary" quinazoline would bind flipped 180° about the horizontal axis running from $S_3$ to $S_4$.

**TABLE VI. Interaction Energy (kcal/mol) of *S. faecium* DHFR with Quinazoline Site Points**

| ligand | | site points | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| no. | type | 1 | 2 | 3 | 4 | 6 | 7 | 8 | 10 | 11 |
| 1. | C ($sp^3$) | | | | | | −0.001 | −0.550 | | −1.598 |
| 2. | O | | | | −0.112 | | | | | |
| 3. | N | | | | −2.285 | −2.208 | | | | −1.487 |
| 4. | S or SH | | | | −1.790 | | −0.042 | −0.430 | | −1.685 |
| 5. | Cl | | | | | | 1.366 | −0.292 | −1.454 | |
| 6. | F | | | | | | | | −2.585 | |
| 7. | C ($sp^2$) | | | −1.689 | | | | −1.182 | −1.240 | |
| 8. | N (double bond) | −2.138 | | | | | | | | |
| 9. | N (protonated) | | −0.806 | | | | | | | |
| 10. | S (in SO) | | | | | | | | | −1.802 |
| 11. | S (in $SO_2$) | | | | | | −1.134 | | | −1.803 |
| 12. | C (in COOH) | | | | | | | | −1.311 | |

## *VIII. Molecular Shape Analysis*

Another line of attack on the QSAR of DHFR has been initiated by Hopfinger and his colleagues[204-208] using molecular shape analysis (MSA).[206] In this approach the congeners in a data set are first examined by using molecular mechanics to determine the most stable conformers. After this point the approach is somewhat similar to that of Simon et al. in that a reference compound must be selected against which the shape of all other congeners can be compared. The total common overlap volume of the reference molecule and each of the congeners in the data set is defined as $V_0$, which is calculated by a rather complex process.[204] The

parameter $S_0$ ($S_0 = V_0^{2/3}$) is employed in the correlation analysis. The meaning of $S_0$ in QSAR is rather obscure. In attacking the SAR of triazines VI of eq 1, Hopfinger states, "The optimum values of $S_0$ and, correspondingly, $V_0$ have no physical meaning. The $S_0$ represent the relative numerical scales which reflect the need to have an analogue adopt the $\theta = 310°$ conformer state such that the 3 and/or 4 substituents possess size and/or conformational freedom so that specific spaces are occupied." From the molecular mechanics calculations Hopfinger surmised that the most favorable angle $\theta$ between the phenyl ring of VI and the triazine is 310°. In a study of 256 congeners VI upon which eq 1 is based he selected 27 as representatives of 7 distinct classes. Correlation analysis yielded eq 27. In this expression

Inhibition of murine tumor DHFR by VI[206]

$$\Delta \log 1/C = -0.996[S_0] + 0.0145[S_0]^2 + 0.481\Delta\sum\pi + 0.296[\Delta D_4] + 0.528[\Delta D_4]^2 + 16.66 \quad (27)$$

$$n = 27,\ r = 0.940,\ s = 0.49$$

the parameter $D$ was designed to take care of two points which were so badly fit by eq 1 that they were not used in its formulation. The two points, 4-CN and 4-$C_6H_5$, are used in deriving eq 27. $D_4$ is a measure of linear extension of the 4-substituent beyond that of 4-Cl. The steric effect of 4-substituents has also been parameterized via Charton's $\nu$ constants (eq 2, 4). Although eq 27 is an interesting start, it does contain a rather large number of variables for 27 data points and it seems unlikely that eq 27 will correlate all the other 229 molecules in the set. Note that while the correlation coefficient of eq 27 is high, its standard deviation is considerably higher than that of eq 1. Equation 1 uses 10 variables to correlate 244 data points (24/variable) while eq 27 uses 5 terms for 27 points (5/variable). Most of the congeners containing large substituents (a large percentage of the data) were not included in the development of eq 27.

The standard reference molecule for the calculation of $V_0$ was 3,4-$Cl_2$-VI.

Hopfinger has applied the MSA method to other smaller sets of triazine VI data using results from bovine and rat liver DHFR.[207]

3-X-VI inhibition of bovine DHFR

$$\log 1/I_{50} = 0.610\sum\pi - 0.108\sum\pi^2 - 0.246D_3 + 6.38 \quad (28)$$

$$n = 31,\ r = 0.926,\ s = 0.25$$

3-X-VI inhibition of rat DHFR

$$\log 1/I_{50} = 0.707\sum\pi - 0.133\sum\pi^2 - 0.336D_3 + 6.06 \quad (29)$$

$$n = 20,\ r = 0.927,\ s = 0.27$$

In these data sets only 3-substituted VI were tested. The reference molecule for the calculation of $S_0$ was 3-$C(CH_3)_3$; however, $S_0$ did not turn out to be of consequence in the correlation equations. This may be due to high collinearity between $S_0$ and $\pi$. The variable $D_3$ is used to account for a steric effect of 3-substituents which is especially pronounced for branches on the carbon atom attached to the phenyl moiety.[146] Hopfinger's equations can be compared with eq 7 and 9 formulated by the Pomona group, although the use of $\pi$ in eq 7 and 28 is different. In eq 28 $\pi$ for the total substituent has been employed. Also, subsequent to the development of eq 28, 10 more triazine data points were available for deriving eq 7. Three of these were omitted in the derivation of eq 7: 3-$COOC_2H_5$, 3-$CH(OH)C_6H_5$, 3-CN. Equation 28 is a slightly better correlation than eq 7 and in addition includes the three congeners badly fit by eq 7. The 3-CN congener is not well predicted by the Hopfinger equation, being more active than expected, but both of the other two bad fits are well correlated as is 3-$C(CH_3)_3$, which is also not well predicted by eq 7. Thus, Hopfinger's $D_3$ steric parameter seems to be a useful contribution to understanding the QSAR of DHFR. Equation 7 does not contain a term in $\sigma$, which has been found to be important in other QSAR for 3-X-VI. If a term in $\sigma$ is added to eq 28, a slight although significant improvement ($F_{1,26} = 4.12$) in correlation is obtained.

In a more recent application of MSA, Hopfinger and his colleagues have treated quinazoline data collected by Hynes and his group on the inhibition or rat liver DHFR.[204] Their QSAR of eq 30 can be compared with eq 12. In calculating $S_0$, new refinements were employed. For this data set the reference compound used

3-X-VII inhibition of rat liver DHFR

$$\log 1/I_{50} = 0.349S_0 - 0.0021[S_0]^2 + 0.487\sum\pi_{5,6} - 0.0897\Delta\theta - 6.950 \quad (30)$$

$$n = 35,\ r = 0.965,\ s = 0.360$$

to calculate $S_0$ was the most active congener of the set (6-$SO_2$-2′-$C_{10}H_7$). The parameter $\pi_{5,6}$ is the sum of $\pi$ for substituents in position 5 and 6 and $\Delta\theta$ accounts for the intramolecular steric effect of 5-substituents on the 4-$NH_2$ group. It was concluded that the most active congeners result when this group is either parallel or perpendicular to the aromatic ring system. From the calculated normalized optimum $S_0$, it was estimated that the active site requires the ideal inhibitor to occupy 710 Å$^3$.

The complete set of quinazolines comprises 104 congeners, three of which were dropped in the derivation of eq 12. Hopfinger et al. limited their study to 35, all of which contain the 2,4-diamino substituents. Unfortunately, eq 30 cannot be compared to eq 12 since the two are based on such different sets of congeners.

One of the difficulties with MSA analysis which has handicapped Hopfinger's analyses is that of cost in time and money of making the calculations. This has prevented him from studying all of the quinazolines used to derive eq 12.

Hopfinger has used 23 of the 42 compounds on which eq 16 is based to derive the QSAR of eq 31 for the inhibition of bovine DHFR by benzylpyrimidines XXVIII.[205] Although eq 31 is a much sharper correlation than eq 16, it is based on a considerably smaller

$$\log 1/C = -21.31V_0 + 2.39V_0^2 + 0.44\pi_{3,4} + 52.23 \quad (31)$$

$$n = 23,\ r = 0.931,\ s = 0.137$$

set so that the two equations cannot be meaningfully compared. The *cis*-4-$NHCOCH_3$ congener was used as the standard in calculating $V_0$, and Hopfinger concludes that "the less a compound looks like the X = *cis*-4-$NHCOCH_3$ structure, with regard to shape, the more active it will be as a DHFR inhibitor." Obviously this definition cannot be carried very far.

In a reevaluation of eq 31 Hopfinger[208] has combined molecular shape analysis with molecular potential energy fields to formulate eq 32 for the benzylpyrimidines inhibiting bovine DHFR. The common overlap volume

$$\log 1/C = -2.34[F] + 0.29[F]^2 + 0.37\Sigma\pi_{3,4} + 9.39 \quad (32)$$

$$n = 22,\ r = 0.961,\ s = 0.105$$

$V_0$ of eq 31 has been replaced by $F$ in eq 32 to yield a sharper correlation. The parameter $F$ is a simplified potential energy descriptor calculated via molecular mechanics.

## IX. Structure of Dihydrofolate Reductase

The ultimate means for defining the structure of an enzyme is X-ray crystallography. Only recently have structures been elucidated by such means; however, much information about DHFR has been obtained by the sophisticated use of NMR.[209-216] NMR does offer certain advantages not provided by X-ray crystallography. NMR methods recently showed that *L. casei* DHFR exists in three interconverting conformational states which are pH dependent.[216] We shall need all techniques and a great deal of hard thinking to finally understand how DHFR functions in vivo. NMR and X-ray crystallography are of less help for in vivo work than QSAR.[183]

The use of circular dichroism[217-222] proved to be particularly useful in bringing out the difference in DHFR from different sources,[12] although it is difficult to interpret the differences in CD in structural terms. Saperstein et al. studied *E. coli* DHFR using visible and Raman spectroscopy.[223]

UV spectra were used to determine the ionization state of various parts of inhibitors which were UV active.[12] The chemical modification of certain amino acid residues has also afforded interesting clues.[12,16]

X-ray crystallographic studies were carried out on DHFR from three sources: *E. coli*,[224,227-229] *L. casei*,[225,226] and chicken liver.[49] Recently, refined structures (1.7 Å) have been reported for the two bacterial enzymes.[93,230] Our discussion of the structure of DHFR will be largely based on the work of Matthews, Kraut, and their co-workers.[16,93,230]

The backbone of the *E. coli* binary complex shown in Figure 2 contains as the central element an eight-stranded $\beta$ sheet (comprising 35% of the enzyme) starting at the amino end (N) and finishing with an antiparallel section at the carboxy terminus (C). The strands are labeled from left to right as $\beta$G, $\beta$H, $\beta$F, $\beta$A, $\beta$E, $\beta$B, $\beta$C, and $\beta$D with an $\alpha$-helical section labeled $\alpha$F, $\alpha$E, $\alpha$B, and $\alpha$C. The structures of DHFR from *L. casei* and *E. coli* cells appear quite similar despite the fact that the X-ray crystallographic structure for *E. coli* was obtained by using the binary complex with MTX while the *L. casei* structure is based on X-ray diffraction

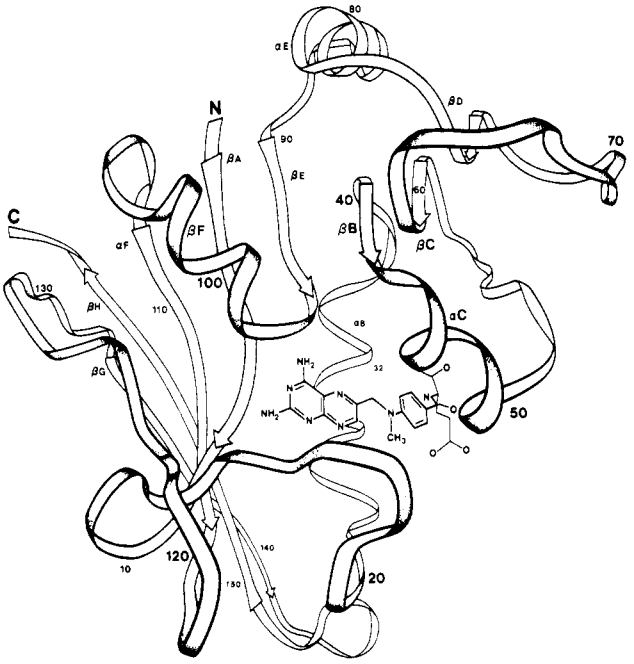



**Figure 2.**

studies of the ternary complex. These bacterial enzymes differ by only three in the number of amino acid residues, but there is less than 30% homology between the two sequences.

Figure 3 compares ternary complexes in stereo, composed from the coordinates of *L. casei* DHFR-NADPH-MTX and chicken DHFR-NADPH-4-$OCH_3$-VI. In this composite structure the light backbone represents the bacterial enzyme and the dark portions the additional residues present in the avian enzyme. The domains labeled by Roman numerals in Table I are also coded in Figure 2 and correspond to the chicken DHFR.

Bolin et al.[93] calculated that, when 142 out of the 159 $\alpha$-carbon coordinates in *E. coli* DHFR are fit via least squares to the corresponding $\alpha$-carbon coordinates for the *L. casei* enzyme, the root mean square deviation is only 1.1 Å. The major difference in the active sites is the replacement of Leu-19 and Phe-49 in *L. casei* DHFR by the corresponding Met-20 and Ile-50 in *E. coli*, which results in a smaller and more constrained active site in *L. casei*. The side chain of Met-20 in the *E. coli* DHFR-MTX binary complex points away from the active site but in the ternary *L. casei* DHFR-NADPH-MTX complex the corresponding Leu-19 side chain projects into the active site. Although it is not strictly proper to compare these two complexes (a binary vs. a ternary), specific differences appear to hold in solution when one compares $K_i$ values for the benzylpyrimidines acting on the two DHFR.[31] A comparative model of the two superimposed active sites (section X) helps to visualize these differences.[31] The greatest differences between the two bacterial DHFR reside in the loops connecting the $\alpha$ and $\beta$ secondary structures. Some of the hairpin turns present in *L. casei* have different geometry or are missing in *E. coli*.[93] Since six of the nine insertions and deletions accounting for differences between bacterial and chicken DHFR coincide with reverse turns in the *L. casei* structure, Bolin et al. conclude that formation of hairpin turns may provide a means for single residue insertions and

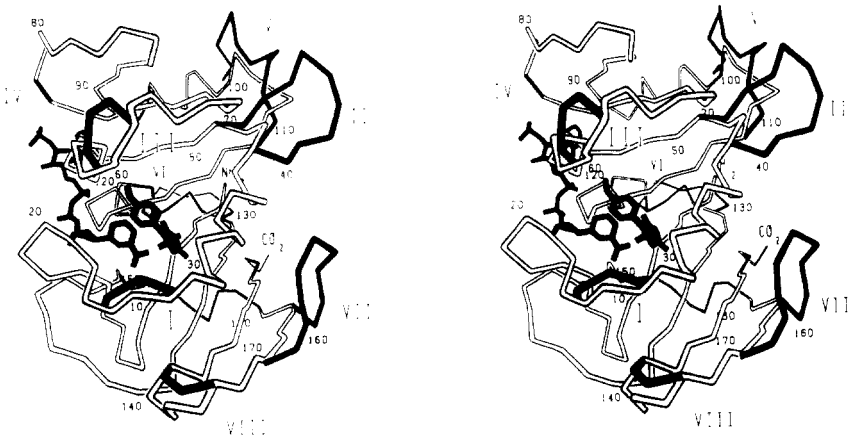


Figure 3.

deletions as suggested by Richardson et al.[231] for $\beta$ bulges and turns are points where further insertions can be accommodated.

The chicken DHFR is readily obtained in highly purified form as the apoenzyme or the holoenzyme so that X-ray structures have been determined for both forms. In fact, X-ray crystallography has been done on a variety of inhibitors bound to the DHFR (triazines, pyrimidines, quinazolines, and pterins).[16]

The four $\alpha$ helices and seven of the eight parallel $\beta$ strands (the eighth is an antiparallel strand) which occur in the bacterial DHFR are present in the chicken liver enzyme, but corresponding elements in the secondary structure may differ by as much as 1–3 Å in the region of the active site.[16] It is these differences which must be primarily responsible for the differences in the QSAR of eq 4 and 6 compared to eq 10 and 11. Over 70% of the extra residues which occur in the chicken DHFR, not counting six residues which only lengthen the chain at the amino and carboxyl ends, occur in three loops rather far from the active site (Figure 3). The avian DHFR contains 189 residues compared to 161 and 159 for *L. casei* and *E. coli*, respectively.

The great similarity of the avian active site and the bacterial active site can be better appreciated from the superpositioning of the two active sites, each holding a TMP molecule (see graphics section). Despite the similarity in the two active sites, the benzyl moiety of TMP in one active site is 90° out of plane with that in the other active site.[21]

One of the most significant differences between chicken and bacterial DHFR is the presence of an extra proline residue in the vertebrate enzyme which is found[49] in a loop (I in Figure 3) connecting $\beta$A to $\alpha$B (Table I) at a point immediately following Trp-24. (Note in this figure the upper set of sequence numbers is associated with the bacteria residues while the lower set codes the vertebrate enzymes.) This segment (Pro-23-Trp-24-Pro-25-Pro-26) forms an almost ideal polyproline 3-fold helix,[49] and this feature is present in all known structures for vertebrate DHFR (Table I) but is not present in the bacterial reductases.

A set of seven amino acids (Thr-40-Ser-41-Ser-42-Val-43-Glu-44-Gly-45-Lys-46) II in Figure 3 occurs in chicken DHFR at the end of $\alpha$B. Most of this segment makes nonbonded contact with segment V which connects $\alpha$E to $\beta$E.

Section III contains two extra residues compared to *L. casei* DHFR and three relative to *E. coli* in the loop connecting $\alpha$C to $\beta$C. Since the loop containing the extra residues in the chicken DHFR (Glu-62-Lys-63-Asn-64) interacts with the NADPH or the inhibitor or both, this could directly affect inhibitor selectivity for the two types of enzymes.

There are small differences in segment IV, but in segment V a large set of residues (101–106) in the avian enzyme expand the loop connecting $\alpha$E to $\beta$E and permit it to fold onto the loop connecting $\alpha$B to $\beta$B.[49] The strand of $\beta$E commences two residues earlier in the chicken DHFR with Met-111-Val-112.

Segment VI constitutes a minor difference of two residues (Glu-126 and Lys-127).

The signal difference between chicken and bacterial enzymes occurs in segment VII. Here the bacterial enzymes have a long strand $\beta$G which makes antiparallel interchain hydrogen bonds with strand $\beta$H. Volz et al.[49] have discovered that the third residue from the lower end of $\beta$G (Val-139 in *L. casei* and Val-136 in *E. coli* DHFR) does not hydrogen bond to $\beta$H and they classify this a $\beta$ bulge according to Richardson et al.[231] The chicken DHFR contains a segment of six residues (III) at the analogous position in the middle of $\beta$G where such a bulge might be expected. Only six of the 11 residues are involved in the classical antiparallel $\beta$ interchain hydrogen bonding. Volz et al.[49] point out that this appears to be the first example of a "$\beta$ blow out" among proteins whose structures are known.

Near the middle of the loop in segment VII a reverse turn (stabilized by a hydrogen bond between the carbonyl of Tyr-162 and the amido nitrogen of Val-165) directs the loop back down toward the $\beta$ sheet plane. Surprisingly, the virtual elimination of one of the three largest $\beta$ strands in the avian enzyme does not greatly change other features of the reductase. Volz et al. postulate that because of the high degree of homology it is likely that all vertebrate DHFR contain such a $\beta$ blowout.

Finally, it is seen from Table I that segment VIII of the chicken enzyme is missing three residues present in *E. coli* and two present in *L. casei* DHFR.

It must be remembered when considering the above discussion that because loop regions vary in conformation from one type of DHFR to another there are serious problems in making comparisons. Just how residues in these loops should be aligned for comparison is not entirely clear.

## X. Molecular Graphics

The recent developments in three-dimensional color computer graphics make the interpretation of the results from X-ray crystallography of macromolecules much easier, especially for those who have not had extensive crystallographic experience with the molecules under consideration. It seems only a matter of time before most laboratories will in one way or another have access to such systems and that the time consuming construction of complex wire models will be a thing of the past. So much can be done with the computer models that is impossible with wires—for example, the excellent visual comparison one can make by superimposing the frames of two proteins or parts thereof. A most important breakthrough in computer models is that of placing a van der Waals surface over the active site or other regions of interest.[232]

The molecular models in this article were constructed and displayed at the Computer Graphics Laboratory of Professor Robert Langridge at the University of California, San Francisco on an Evans and Sutherland color picture system 2 run by a VAX 11/750 computer using the programs CHEM[233] and MIDAS.[234] A few reports have now been published illustrating the value of color stereographics in enlarging our understanding of how ligands interact with macromolecules.[21,31,232,235-241] These molecular models are not intended to provide the definitive structure for the enzyme–ligand complex; rather, the goal is to develop reasonable structural models consistent with experimental results which can be studied to develop new experiments.

The graphics section in this review is intended to give the reader some useful stereoviews in which color coding of the surfaces helps one to understand the type of surface interaction between ligand and macromolecule. Unfortunately, they cannot convey the enormous power of computer graphics seen on the picture tube.

Our models have been constructed from the X-ray crystallographic coordinates of DHFR from three sources. The coordinates were kindly supplied to us by D. A. Matthews. For the construction of the chicken liver DHFR active site the following residues were used: Ser-6 to Val-10, Ile-16 to Gly-17, Lys-18 to Ser-39, Asn-48 to Trp-57, Ser-59 to Pro-66, Leu-57 to Asn-72, Trp-113 to Tyr-121, Thr-136, Ile-138, Asp-145, Thr-146.

For the *L. casei* DHFR active site model we used the following: Leu-4 to Ala-6, Gly-17 to Leu-19, Trp-21 to Arg-31, Val-41 to Ala-57, Ala-97, HOH-201 and for *E. coli* the following were used: Ile-5 to Ala-7, Ile-14 to Gly-15, Met-16 to Met-20, Trp-22 to Pro-25, Asp-27 to Ala-29, Trp-30 to Lys-32, His-45 to Thr-46, Ser-49 to Ile-50, Arg-52, Leu-54, Pro-55, Arg-57, Ile-94 to Gly-97, Tyr-100, Thr-113.

View I (Figure 4) shows the ternary complex of *L. casei* DHFR–NADPH–MTX with water molecules (represented by red O) whose positions have been established by X-ray diffraction studies. (Many of the external waters have high-temperature factors, indicating high mobility or a similar uncertainty in position.[93]) The backbone of the enzyme is colored blue except for the amino acid residues around the active site, which are yellow. Near the center on the left in green is the structure of MTX and just to the left of the green spike representing the 10-$CH_3$ of MTX is the nicotinamide ring which is involved in the transfer of hydride to dihydrofolic acid in its reduction by DHFR. The unit cell of *L. casei* DHFR contains about 1200 water molecules; positions of 264 have been established and are shown in view I.

Out of 178 water molecules bound directly (not through a second water) to *L. casei*, 65 are hydrogen bonded simultaneously to two or more functional groups in the protein and hence may play some role in the overall protein structure. The *E. coli* crystal contains about 800 of which 428 have specified positions.[93] The enzyme is in effect in aqueous solution in the crystal. Bolin et al.[93] note that every fixed water molecule is hydrogen bonded to the protein or to a water molecule which is protein bound. There appear to be no visible clathrate-like water cages around nonpolar groups. Apparently these so-called "flickering clusters" of hydrophobically held water are too unstable to be "seen" by X-ray diffraction studies. The structure of the bound water molecules is quite different in the two similar bacterial enzymes. This feature may be of significance in the binding of inhibitors to DHFR from different sources.

View II is a close-up of view I in which a blue surface has been placed over the active site of the enzyme. This idea for defining the molecular surface of a protein comes from Richards[242a] and was incorporated into a computer program by Connolly.[242b] Instead of calculating van der Waals spheres and solving the hidden surface problem, the program calculates the surface corresponding to the solvent accessible surface. This is done by a probe sphere (representing an idealized water molecule) of 1.4-Å radius traversing the surface of the molecule; a dot is placed at each point of contact of the sphere with the molecular surface or at the inward-facing surface of the sphere when it is simultaneously in contact with more than one atom (reentrant surface). In the resulting model the interstices too small to accommodate the probe are eliminated and the clefts between the atoms are smoothed over.

In view II, red van der Waals surfaces have also been placed on the idealized water molecules, which gives one a better appreciation of their effective size. The "wire" model of MTX is shown in yellow and in the background can be seen in faint yellow the nicotinyl moiety of the NADPH. Water molecules are associated with the two $COO^-$, the carbonyl group of the aminobenzoyl moiety, and the N9 region. Water molecules are also on the surface of the cavity.

The complete backbone of *L. casei* DHFR in blue is displayed in view III, MTX is green, and NADPH is yellow. From the red surface one can see that a relatively large portion of the enzyme is devoted to the active site. This would be even larger if the surface occupied by the NADPH were also included. DHFR is an efficiently constructed molecule with relatively few amino acid residues which are not directly involved in the active site for one or the other of the two ligands.

View IV shows the details of MTX (green) binding to *L. casei* DHFR according to Bolin et al.[93] The nicotinyl portion of the NADPH (red) is hydrogen bonded to the carbonyl and amino group of Ala-6. The carboxylate oxygens of Asp-26 are shown hydrogen bonding to the N1 and 2-$NH_2$ moieties of MTX. The 4-$NH_2$ is hydrogen bonded to the carbonyl oxygen of Leu-4. It also makes a second hydrogen bond to Ala-97 (not shown in view IV). The second H of 2-$NH_2$ is hydrogen

bonded to Thr-116. Since this threonine is conserved in all DHFR, it seems likely that this is an invariant binding feature of the 2,4-diaminopyrimidine type structure.

Vertebrate DHFR contains Glu-30 in a position equivalent to Asp-26 in *L. casei*.

In the *L. casei* DHFR–MTX complex, N5 of MTX makes no hydrogen bonds while N8 is bonded to fixed water molecule (Wat-253), which in turn is hydrogen bonded to oxygen of Asp-26, to Wat-217, and to Nε1 of Trp-21. The nonpolar interactions of the pteridine ring in the ternary complex with *L. casei* DHFR occur mainly through the side chains of Leu-4, Ala-6, Leu-19, Leu-27, Phe-30, and Ala-97. The backbone of Trp-5 and Ala-6 are also in contact with the pteridine ring.

The *p*-aminobenzoyl portion of MTX is hydrophobically bound by the helix αB on one side and by the loop connecting αC to βC on the other side. The side chains of Leu-27 and Phe-30 are important features of this hydrophobic pocket.

The glutamate portion of MTX makes a strong contact between its α-carboxylate and guanidinium group of Arg-57 in both *L. casei* and *E. coli* DHFR. Bolin et al.[93] note that the guanidinium group is located in a narrow hydrophobic pocket formed by side chains of nearby residues and is rather rigidly held by hydrogen bonding to HO of Thr-34 and the carbonyl oxygen of Pro-55. The γ-carboxylate does not appear to interact strongly with the bacterial DHFR.

Another view of MTX (red) and folic acid (yellow) binding to *L. casei* DHFR is presented in view V. This model was constructed from the refined coordinates of the ternary complex. In constructing the model the pteridine ring of the folic acid was superimposed on the coordinates of the pteridine of MTX and then rotated 180° about the $C_6$–$C_9$ bond. So doing brings the two amino benzoylglutamyl units into similar positions of enzymic space. As mentioned above, there is considerable indirect evidence that folic acid does not bind like MTX. Bolin et al.[93] postulate that the pteridine ring can rotate so that N3 is placed in the position of N1 in view IV, which is within hydrogen-bonding distance of an Asp-26 oxygen. This would allow the 4-oxo group of folic acid to hydrogen bond with a nearby water molecule (wat-253), which is in turn hydrogen bonded to Trp-21. This arrangement places N8 of the substrate so that its H can bond to Leu-4 or Ala-97. They point out that in this binding model there is a net loss of one protein–ligand hydrogen bond in the substrate complex compared to the MTX complex. (Because of the replacement of the doubly hydrogen bonded 4-$NH_2$ by 4-oxo, there is still some doubt as to whether or not the Asp-26 carboxylate is protonated or not.)

The association constant between *L. casei* DHFR and dihydrofolate is $2 \times 10^{-6}$ $M^{-1}$ and that for MTX is $2 \times 10^{-10}$ $M^{-1}$. Bolin et al. concluded that this difference is accounted for by the additional hydrogen bond to 4-$NH_2$ and the protonated N1 of MTX reacting with Asp-26. It is clear from the computer graphics analysis that there is room enough in the active site for these two types of binding.

Filman et al.[230] have considered the implications of their above-discussed model of substrate binding for the catalytic step with DHFR. They assume that the geometry of the enzyme is essentially the same when folates bind as when MTX binds. Although it has generally been assumed that a prelude to hydride transfer from the NADPH would be protonation at N5 or N8, they observe that their results show that N5 is not close to any hydrogen-bonding group. However, N8 (view V) is in roughly the same site as 4-$NH_2$ of MTX and hence might interact with carbonyl oxygens of Leu-4 or Ala-97. This model would help to explain folate reduction but not dihydrofolate reduction. Filman et al.[230] are unable at present to offer a mechanism for carbonium ion development at C6 and point out that, unlike MTX, current evidence[243,244] indicates that bound folates are not protonated. Of course, since current thinking is that protonation occurs during the transition state, one would not necessarily expect to see the makings for it in the crystallography of inhibitors bound in the crystal.

Filman et al. visualize that protonation in the transition state might occur by relay of a proton from the aqueous phase along a conserved hydrogen-bonded chain of two water molecules. Wat-253 hydrogen bonds simultaneously to N8 of MTX to Oδ2 of Asp-26 and to Nε1 of Trp-21. With the folates 4-oxo takes the place of N8 and Wat-253 hydrogen bonds to a second water molecule (Wat-217), which also contacts the surrounding solvent.

The binding of the NADPH cofactor occurs in extended form as shown in view VI (Figure 5). The cofactor is red, the *L. casei* backbone is yellow with a blue surface, and in the lower right-hand corner MTX in faint red is adjacent to the nicotinyl moiety. An amino acid side chain with its attendant surface crosses about the middle of the NADPH and appears to help hold it in place.

The nicotinamide ring is fixed between the carboxyl ends of strands βA and βE, being essentially coplanar with the strands, which causes them to separate to accommodate the nucleotide base.[16] A cluster of hydrophobic side chains excludes solvent from the B side of the nicotinamide ring and the A side of the ring is in contact with the folate binding cavity. Filman et al. point out that this geometry is consistent with the finding of other studies[219,243-248] that hydride transfer is A side specific. The A side of the pyridine ring is in contact with the side chains of Leu-19, Trp-21, and Ser-48 and the pyrazine portion of the pteridine ring of the bound folate. The B side of the nicotinyl ring is in hydrophobic contact with Ile-13, Phe-103, and the cis peptide connecting Gly-98 and Gly-99.

The adenine binds in a hydrophobic pocket in contact with five amino acid residues (Leu-62, His-64, Thr-63, Gln-101, Ile-102) of which only one (Leu-62) is conserved in DHFRs of known structure. No direct hydrogen bonding appears to occur between protein and the nitrogen atoms of adenine; however, in two instances (N7 and N1) water molecules bridge from adenine to the reductase.[16]

One of the most interesting discoveries of the La Jolla group[230] is that the nicotinamide interacts via ring hydrogens 2, 4, and 6 with three roughly coplanar oxygen atoms (O:13, O:97, Oγ:145), which places the functional part of the NADPH in a very polar environment. They postulate that these oxygens may play an important role in the delocalization of a positive charge in the cofactor transition-state XXXII.

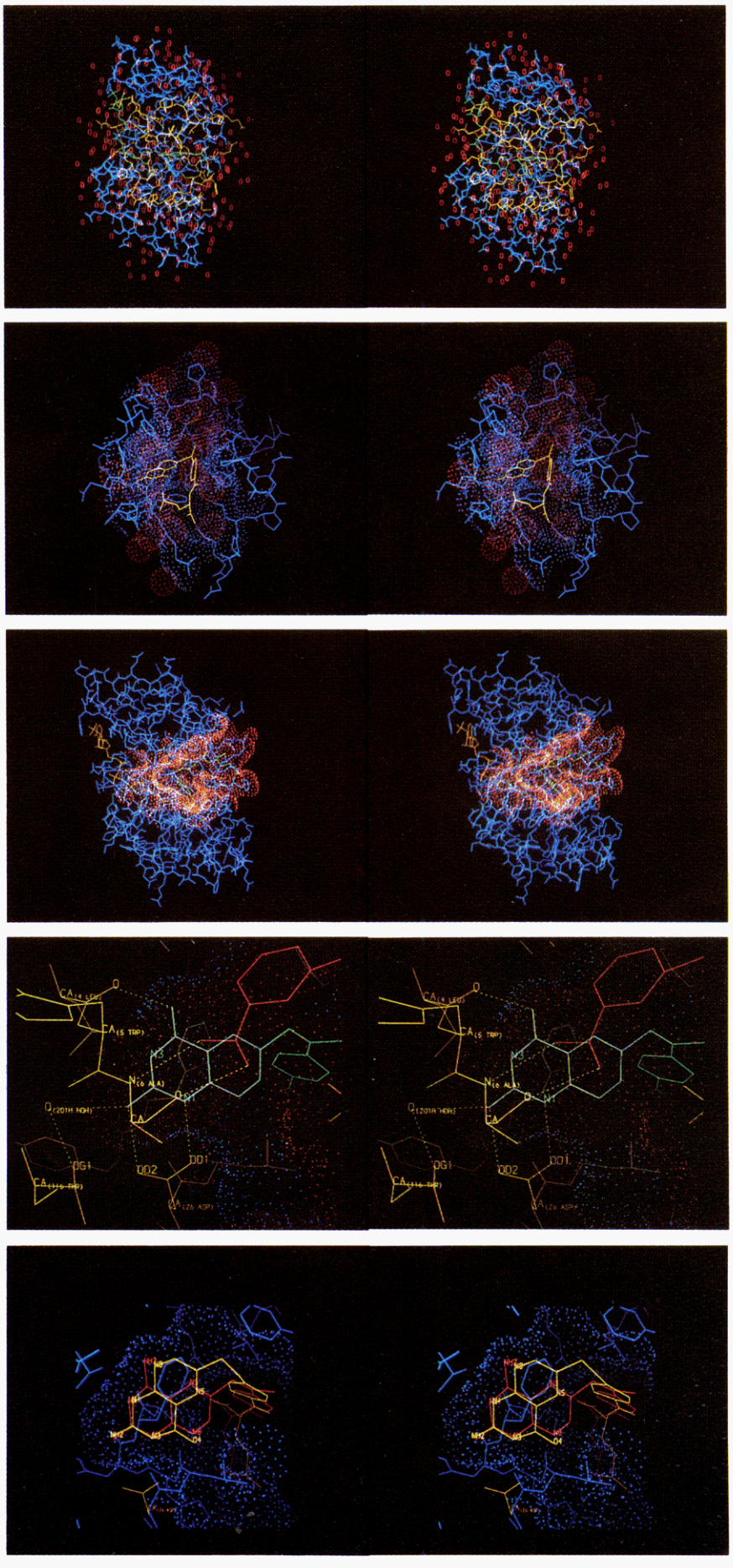


**Figure 4.** Stereoviews I–V.

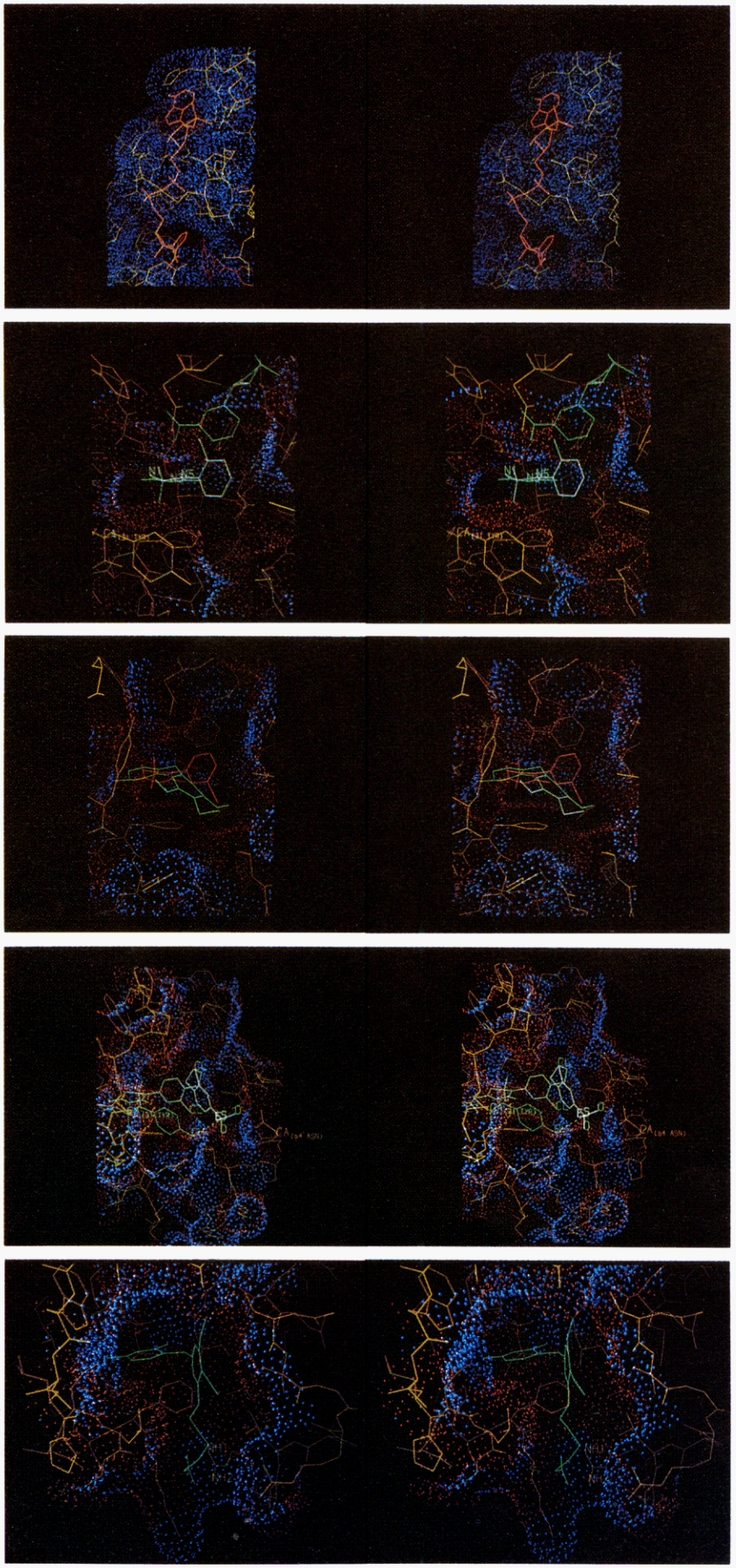
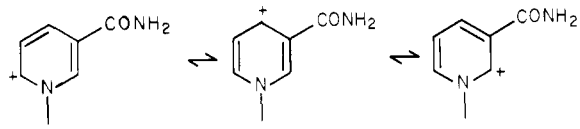


**Figure 5.** Stereoviews VI–X.

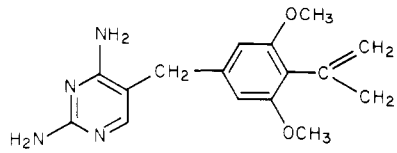


XXXII

A problem of interest is that with the exception of 2-SH all other 2-X-triazines are much less effective inhibitors of vertebrate DHFR than the parent compound (X = H).[146,147] The 2-SH, however, is 10 times more active than the parent. In the model of view VII the 2-I-triazine is shown in green as is the nicotinyl moiety of the NADPH. Coordinates for the 2-I-triazine were derived from crystallographic coordinates of the 4-$OCH_3$ analogue. The enzyme active site is that of chicken DHFR. In this model the 2-I collides with the amide group of the nicotinamide and thus it seems that in the binding competition 2-X-triazines meet with considerable resistance from the NADPH. The 2-I analogue is about 1000 times less active against bovine and murine DHFR, while the SH group of similar size is 10 times more active than the parent. It is hard to find a radically different way for the 2-SH-triazine to bind, so, in fact, it may be able to prevent the NADPH from binding and in this way be both a competitor of NADPH as well as folate. Actually, it might be possible by placing lipophilic substituents in other positions on the phenyl ring of the triazine to increase the holding power of 2-X-triazines so that they are not displaced by the binding of NADPH. This type of NADPH inhibition would be unlikely to interfere with other enzymes employing this cofactor.

View VII shows rather well the large amount of hydrophobic surface (red) in the active site as well as its complex geometry.

The surprising movement of Tyr-31 in chicken DHFR which occurs on binding of trimethoprim (green) is illustrated in view VIII. Hydrophobic surfaces are color coded red and polar surfaces are blue. This model also contains for comparison the 3-I-triazine. The position of both ligands has been established by X-ray diffraction studies (Matthews and Volz, unpublished results) of the ternary complexes. Just behind the red phenyl ring of the triazine the nicotinamide moiety of NADPH is displayed in faint yellow. To appreciate how Tyr-31 has moved upon interaction with TMP, compare view VIII with view VII. In view VII the large yellow Tyr-31 is in the foreground on the lower left while in view VIII it has been raised by almost 180° and the phenyl twisted from its normal position (as found in the native enzyme and with bovine triazine inhibitors). This movement removes one hydrophobic wall of the active site in the chicken DHFR. Exactly what causes this movement is not known, but it would seem to require little energy. Matthews et al.[16] have discovered through their X-ray diffraction studies that while TMP

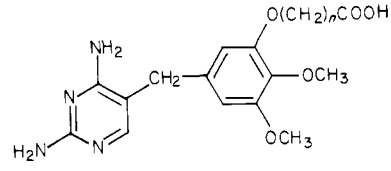


XXXI

causes great change in Tyr-31, a close analogue (XXXI) with greater potential for steric effects binds without causing the large movement of Tyr-31. TMP and XXX are two of the strongest known inhibitors of bacterial DHFR, yet their inhibition constants (log $1/K_i$) of chicken DHFR are weak, being 3.98 and 4.05, respectively (Selassie and Hansch, unpublished results). There is no significant difference in the inhibition of the chicken DHFR by these two substances.

View IX illustrates how Baker's antifol (XVI) can undergo irreversible binding via nucleophilic reaction with the OH of Tyr-31 in chicken DHFR as proposed by Kumar et al.[103] Freisheim and his colleagues have shown that such a reaction does occur and they have proposed that the antifol first binds reversibly and a covalent bond is formed by OH displacement of F from $SO_2F$. In view IX the dotted yellow line shows the postulated path of attack of the OH on the back side of S holding the F leaving group. In this model the oxygens of the sulfonyl group are placed near the polar (blue) surface of Asn-64. This contact may assist in positioning the antifol for the nucleophilic substitution reaction. The X-ray crystallographic study of XVI bound to DHFR has not yet been carried out so that view IX has been constructed on the basis of the crystallographic coordinates for 4-$OCH_3$-VI, deleting the 4-$OCH_3$ group and adding the $-(CH_2)_4-$ bridge so as to position the $SO_2F$ in the proper position for OH attack.

View X is a model representing the binding of inhibitor XXXIII (green) ($n$ = 4) binding to *E. coli* DHFR as pictured by the groups from the Wellcome Laboratories.[229] In a beautiful example of drug design,



XXXIII

the Wellcome group modified trimethoprim by making a series of side chains in the meta position which was varied by using different values of $n$. Derivatives in which $n$ = 3, 4, 5, and 6 were extremely potent inhibitors of *E. coli* DHFR, in fact, more than 30 times as potent as TMP. Such an increase in potency was expected by the Wellcome group since from a study of the crystallographic analyses they assumed that the $COO^-$ of the side chain would be able to interact with Arg-57 as it is shown in view X for the case where $n$ = 4.

The results of Freisheim's group showing the specific reaction of Baker's antifol with Tyr-31 and those of the Wellcome group are very exciting from the point of view of drug design. We have indeed reached the point where highly potent enzyme inhibitors can be designed by using molecular graphics based on coordinates obtained from X-ray crystallographic studies.

While the Wellcome compounds are probably the most potent inhibitors of bacterial DHFR yet made, they are not surprisingly, effective in vivo. No doubt the highly polar carboxylate anion makes these substances too hydrophilic for proper distribution in animals. This should be correctable by converting XXXIII to more lipophilic compounds by the introduction of more lipophilic groups in positions 4 and 5. QSAR results show these positions to be rather free of steric effects.

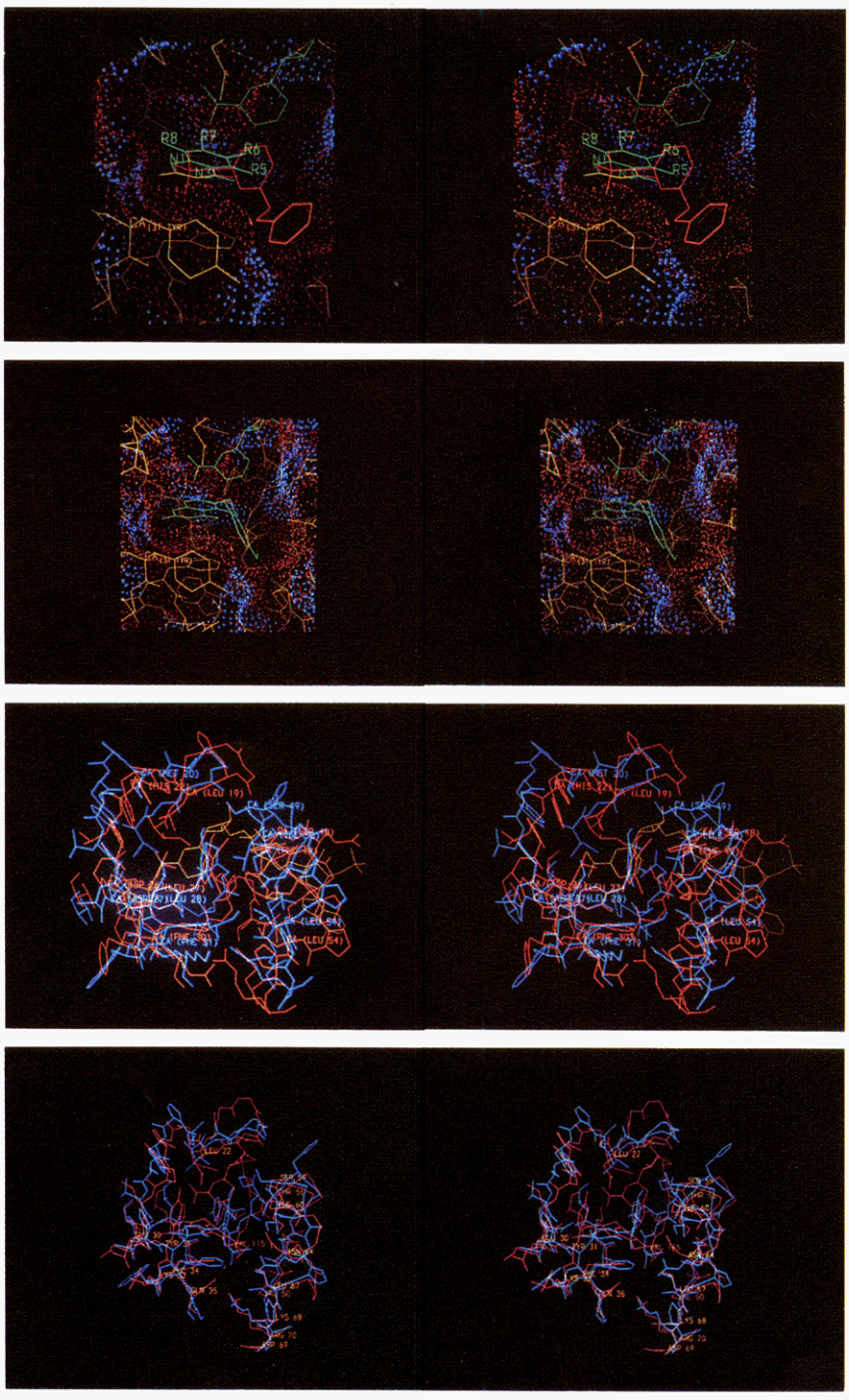


**Figure 6.** Stereoviews XI–XIV.

A similar achievement has been accomplished by Poe et al., who designed 5-(4-$OCH_3$-3-$OCH_2CH_2NHSO_2C_6H_4$-4′-$NH_2$-benzyl)-2,4-diaminopyrimidine to inhibit *E. coli* DHFR.[229] Details have not yet been published.

The quinazolines as well as the triazines are two important classes of inhibitors for cancer chemotherapy. Their comparative binding modes are modeled in view XI (Figure 6). The triazines (red) contains a 3-$CH_2OC_6H_5$ group attached to the 5-phenyl ring and the quinazoline (green) has four substituents attached for reference. The model has been constructed from the X-ray crystallographic coordinates obtained from the ternary complex of chicken DHFR–NADPH–3-$CH_2OC_6H_4$-3′-$NHCOCH_3$-triazine (unpublished results of Volz and Matthews). The 3′-$NHCOCH_3$ group is not included in view XI; however, it is apparent that groups in the 3- and 4-positions of the outer phenyl ring would not contact the enzymes effectively. It is for this reason that in the formulation of the QSAR of eq 2–8, 10, and 11, Y of $CH_2ZC_6H_4$–Y turns out to be best parameterized by 0.

Since the coordinates for the binding of the quinazolines are not available, the model has been constructed by placing the 2,4-diaminopyrimidine moiety of the quinazoline on the binding points occupied by the corresponding nitrogen atoms of the triazine.

The binding geometry of the quinazolines and tri-

azines is quite different. It is clear that there is a large cavity in which 6-substituents on quinazoline can project. For rigid 5-substituents space is limited because of the nearby hydrophobic wall (red). The QSAR of eq 12 and 13 are in accord with these facts. For small 5-substituents, $\pi$ is the parameter of importance and large 6-substituents yield the most active compounds with vertebrate DHFR. So far 7-X-quinazolines have not been studied, but space around the 7-position is limited so that this does not appear to be a promising site for substitution. On the other hand, there is a large hydrophobic cavity just off position 8 which appears opportune for substituent interaction.

A quite promising antitumor drug is BW-301U (XX), developed at the Wellcome laboratories.[139,140] Besides being a potent DHFR inhibitor, it has the interesting characteristic of being a poor inhibitor of histamine *N*-methyltransferase. A number of potent antifolates are strong inhibitors of this enzyme[139] and it would appear that some of the toxic side reactions of the antifolates are due to the inhibition of the histamine *N*-methyltransferase.

In the model of view XII, BW-301U (green) is shown binding to the avian DHFR. It is interesting to see that the 5-$OCH_3$ group can be placed near to a hydrophobic wall, hence it seems that if this somewhat polar group could be replaced with a more lipophilic group such as 5-$CH_2CH_3$, a tighter binding drug might ensue. Of course, making this change would produce an overall more lipophilic drug and how overall lipophilicity would affect antitumor activity is an unknown quantity.

The 2-$OCH_3$ group is also near to a hydrophobic surface so that replacing both methoxy groups with ethyl would also be interesting; however, this would make a large change in log *P*, which for in vivo activity would have to be offset by an additional group(s) with $\pi$ near −2.

In view XIII the active site of the binary *E. coli* DHFR–TMP complex (red) is superimposed on the ternary *L. casei* DHFR–TMP–NADPH complex (blue). The NADPH is color coded yellow. In the center of the picture one can recognize the two color coded trimethoxybenzyl moieties of the TMP by their three $OCH_3$ groups projecting toward the viewer. Phe-30 and -31 provide a hydrophobic "floor" and Leu-27 and -28 a hydrophobic "wall" for the active site. The major difference in the two active sites is that of Leu-19 in *L. casei*, which corresponds to Met-20 in *L. casei* and Phe-49 in *L. casei*, which corresponds to Ile-50 in *E. coli*. The Leu-19 projects into the active site in the *L. casei* enzyme, producing a smaller cavity than that of *E. coli*. The side chain of Met-20 in the *E. coli* complex points away from the active site. Whether this is a consequence of the lack of NADPH in the binary *E. coli* complex or whether it is an intrinsic feature of the structure is not known; however, model building suggests that such a conformational change is not necessary for *E. coli* DHFR to accommodate NADPH. Despite the projection of Leu-19 into the *L. casei* active site, TMP appears to fit very much the same way in each active site.

The presence of Leu-19 does, however, account for difference in the QSAR of eq 19 and 20.[31]

Such a superpositioning of enzymic structures represents an enormous advantage of model building with the computer which is difficult to achieve with models built of wire or plastic.

A similar superimposition of the active sites of the ternary chicken liver DHFR–TMP–NADPH complex (red) and the binary *E. coli* DHFR–TMP complex (blue) is displayed in view XIV. Selected residues are labeled for the avian enzyme only. The strikingly different binding modes are apparent from the two trimethoxybenzyl moieties near the center of the picture. Directly behind Leu-22 one can see in red the nicotinamidoribose of NADPH bound to the chicken enzyme. Matthews et al.[49,225] have suggested that a conformational change in this region of the enzyme induced by NADPH binding may be responsible for enhanced inhibitor binding in the presence of bound NADPH. A direct comparison of the ternary complexes of both enzymes is not possible at present.

The significant differences between *E. coli* and the avian DHFR active sites which may account for the inhibitor selectivity are the substitutions of Leu-28, Lys-32, and Gly-51 in *E. coli* enzyme by Tyr-31, Gln-35, and Pro-61, respectively, in the chicken enzyme and the insertion between Gly-51 and Arg-52 in the *E. coli* DHFR of Glu-62, Lys-63, and Asn-64 in the chicken DHFR. The side chains of Glu-62 and Lys-63 in chicken DHFR (not shown in view XIV) are oriented away from the active site, while the side chain of Asn-64 is in the active site near the position occupied by the side chain of Arg-52 in *E. coli* DHFR. The side chain of the corresponding Arg-65 in chicken DHFR points away from the active site and is not apparent in view XIV. The overall effect of these differences is that a more nonpolar environment is present around the benzyl side chain of XXVIII in chicken DHFR (and presumably in other vertebrate DHFR) than in *E. coli* DHFR. In particular, the replacement of the bacterial Leu-28 by the much larger Tyr-31 in the chicken enzyme results in a narrower, more restricted active site for the avian enzyme.[249] This difference in the more hydrophobic character of the vertebrate DHFR compared to bacterial DHFR, which in part accounts for the selective reaction of the benzylpyrimidines with these enzymes, was first apparent from QSAR studies.[28] For example, trimethoprim is 2300 times less inhibitory of bovine DHFR,[28] 77 000 times less active against chicken DHFR,[30] and 126 000 times (Table III) less potent an inhibitor of human DHFR than of the *E. coli* reductase. The parent benzylpyrimidine (XXVIII, X = H) shows the same trend: 3.2 times less potent with bovine, 30 times less potent against chicken, and 160 times less effective against human than against *E. coli*. Hence, there is an intrinsically different mode of interaction of benzylpyrimidines with vertebrate DHFR compared to *E. coli* DHFR.

The hydrophobic terms in the bacterial DHFR QSAR (eq 19 and 20) are not very significant, and most of the variance is accounted for by MR. The reverse holds for vertebrate QSAR (eq 16–18), where $\pi$ is the significant parameter. The 4-position of the benzyl moiety of the pyrimidines points away from the active site toward solution so that there is little opportunity for contact of 4-X with the enzyme beyond that of 4-$OCH_3$. Both the QSAR and the graphics are in accord on this point. The bacterial QSAR contain the modified MR′ term which is truncated at 0.79, indicating that that part of

the 3- and 5-substituent beyond the MR of 0.79 does not contact the enzyme. For the 3-position (upper substituent on the blue trimethoprim in view XIV) modeling of groups longer than $OCH_3$ (MR = 0.79) shows that these groups will extend beyond the surface of the enzyme into the solvent. Modeling substituents in the 5-position (lowest substituent) indicates that there is only a limited amount of *hydrophobic* active site surface available for contact.[249] Beyond the first two atoms of the 5-substituent there is only very hydrophilic enzyme surface available for contact (Lys-32, Arg-52, Arg-57) so that larger, more hydrophobic 5-substituents have only a negligible effect on activity. This appears to be the case for substituents such as 3-$OSO_2CH_3$ and 3-$CH_2OCH_3$ as well as larger substituents which are well fit by QSAR on the basis of MR′ = 0.79 with $\pi_0 = 0.25$.

Unlike the bacterial DHFR where large hydrophobic substituents on the benzyl moiety of XXVIII promote little additional activity and do not appear to contact the enzyme effectively, similar substituents do provide additional activity with vertebrate enzymes (eq 16–18). Models of the molecular surfaces of DHFR active sites with bound trimethoprim show that binding by chicken DHFR results in more complete desolvation of the benzyl substituents X by the hydrophobic enzyme surface.[249] Because of the different binding mode of the benzyl group (see view XIV), the 3- and 5-substituents are much less exposed to solvent. Tyr-31 in particular constitutes an excellent hydrophobic wall.

The conclusion from the above analysis is that steric effects (MR related) of substituents on trimethoprim must account for a rather large part of the more than 100 000-fold difference in inhibitory power of TMP against *E. coli* compared to human DHFR. It is hard to see how such steric effects could operate other than by producing a conformation change in the bacterial reductase.

Matthews et al. have recently addressed this problem.[250] They point out that TMP binds about 1 Å deeper into the active site of chicken DHFR compared to the *E. coli* enzyme. This results in the avian complex having one less hydrogen bond at the 4-$NH_2$ group of the TMP and decreases solvent access to the salt bridge between the carboxylate of Glu-30 (Asp-27 in *E. coli*) and N1 and 2-$NH_2$ of the TMP. They also point out that there is a major conformational change in TMP binding to avian enzyme in that Tyr-31 makes an average change of position of 5.4 Å. They also note that Baccannari et al.[251] have shown that the inhibitor dissociation constants for ternary complexes of *E. coli* and mouse lymphoma DHFR with TMP and NADPH differ by 4 orders of magnitude and that the affinity of TMP for *E. coli* reductase is strongly dependent on the presence of bound cofactor. This is not true of the mouse DHFR. Hence, cooperativity also appears to play a role in selectivity.

In light of the above thinking about conformational effects brought about by steric effects of substituents being important in the selectivity of TMP analogues for bacterial enzyme, a recent study by Hyde and Roth is most interesting.[252] In comparing the action of benzylpyrimidines on rat liver DHFR with that on *E. coli* enzyme, they confirm that hydrophobic effects are important for the rat enzyme but not for the bacterial enzyme for which MR related effects are more important. They then formulate eq 33. This equation is

$$\log 1/C = 0.83\lambda + 1.15I - 3.10 \qquad (33)$$

$$n = 22,\ r = 0.966$$

based on 10 3-X-XXVIII and 12 3,4-$(OCH_3)_2$-5-X-XXVIII. The indicator variable $I$ takes the value of 1 for the trisubstituted set and the value of 0 for the simple 3-substituted analogues. The introduction of the 3,4-$(OCH_3)_2$ units increases activity, on the average, by a factor of 10. The parameter $\lambda$ is based on the sterimol constants of Verloop et al.[168,169] For simple 3-substitution $\lambda$ corresponds to Verloop's $B_1$ parameter, which would model the minimum in plane steric effect of 3-X. This is a more directionally oriented parameter and for the data of Hyde and Roth yields better results than MR. Hyde and Roth truncate $\lambda$ for alkoxy substituents larger than OH similar to MR′ and correct $B_1$ when substituents ortho to it are present.

Beddell has discussed the results of X-ray crystallography in his laboratory as well as that of Matthews and Kraut's group[253] and reached conclusions similar to those discussed above.

In conclusion, evidence from a variety of sources makes it appear that effects of substituents probably promote a conformational change which accounts for most of the selectivity of TMP for bacterial DHFR.

## XI. From Enzyme Inhibition Studies to Drugs

While it has been abundantly demonstrated that in a short time extremely potent inhibitors of almost any enzyme can be developed, there have been few examples showing that this experience can be used in a straightforward manner to design effective drugs. There are two major problems to consider in this approach to drug discovery. (1) Is there the possibility of finding a significant difference between the enzyme of the host and the enzyme of the parasitic cells? (2) Will a structure–activity relationship developed on purified enzyme bear any relationship to the SAR in the living animal?

The facts of the great success of both methotrexate and trimethoprim are more than enough evidence to supply the courage for the present attempts to design drugs starting with studies on the isolated enzyme; however, what concrete guidance can we get from medicinal chemistry about systematic means for bridging the gap between the biochemistry laboratory and the clinic? The answer is not much, because medicinal chemists have not studied the problem in enough depth in a systematic fashion. It would seem that dihydrofolate reductase will be the first thoroughly worked out system. One reason for this is that the enzyme is not unusually difficult to work with and X-ray crystallographic structures are now in hand with more soon to come.

Two attempts have been made to compare the QSAR of DHFR inhibitors obtained from work with isolated enzyme with the effect of the same inhibitors on cell cultures. In making such comparisons one must assume that inhibition of cell growth is the result of the inhibition of the enzyme in situ. If the inhibitors are active at very low concentrations, $<10^{-5}$ M, in the cell

culture and the QSAR shows definite resemblance to that of the isolated enzyme, then one can have some confidence in comparing the two cases. Equations 34 and 35 correlate the inhibition of murine leukemia (L5178YS) cells sensitive to and resistant to (L5178YR) to methotrexate by triazines VI. In these equations

QSAR of L5178YS cells with triazines 3-X-VI[254]

$$\log 1/C = 1.32\pi - 1.70 \log (\beta \cdot 10^{\pi} + 1) + 0.44I + 8.10 \quad (34)$$

$$n = 37,\ r = 0.929,\ s = 0.274,\ \pi_0 = 0.76$$

QSAR of L5178YR cells with triazines 3-X-VI[255]

$$\log 1/C = 0.57\pi - 0.15\mathrm{MR} - 0.35 \log (\beta \cdot 10^{\pi} + 1) + 5.12 \quad (35)$$

$$n = 42,\ r = 0.932,\ s = 0.288,\ \pi_0 \cong 6$$

$C$ is the molar concentration of triazine required to inhibit the growth of the tumor cells by 50%. Equation 34 can be compared with eq 8. Note first that the hydrophobic effect as parameterized by $\pi$ differs in that for eq 34 $\pi$ for all of X has been used. So doing gives a better correlation and it is assumed that movement of the triazines through lipophilic membranes requires this change. Equation 34 has essentially the same term in $I$ as eq 8, but the addition of a $\sigma$ term to eq 34 did not improve the correlation significantly. The role of $\sigma$ is quite small in eq 8 so that this probably is not of great importance. The $\pi_0$ of eq 34 is definitely lower than for eq 8, but the reasons for this are not clear. There is a large difference in the intercepts, indicating that it is much easier (about a factor of 100) to inhibit cell growth by 50% than to inhibit isolated DHFR by 50%. This finding was surprising since one would assume that in cell culture a certain amount of inhibitor would be lost through binding to macromolecules other than DHFR. The DHFR used to develop eq 8 was obtained from the resistant cells used to obtain eq 35. In this respect comparison of the two sets of results is not strictly proper. However, in the main eq 8 and 34 are not greatly different. Comparison of the outliers gives us some specific insights. The 3-$CH(OH)C_6H_5$ congener is invariably badly fit by QSAR from all forms of DHFR so far studied and it is badly fit by eq 34. The 3-CN congener on the other hand is often much more active on isolated DHFR than the QSAR predict. This congener is well fit by eq 34. The lack of importance of $\sigma$ and the different behavior of the 3-CN analogues suggest that there are small conformational changes in the DHFR in situ. Further work with a larger set of probes is necessary to develop these leads.

Equation 35 for resistant tumor cells is radically different from both eq 8 and 34. The coefficient with $\pi$ is about half that of eq 8 and 34 and the coefficient with the bilinear term is so small that the slope of the right-hand side of the bilinear curve (0.57 − 0.35 = 0.22) is positive. Even more lipophilic substituents would have to be tested in order to set a firm value for $\pi_0$. From an inspection of the data $\pi_0$ would appear to be around 6. This represents a 100 000-fold change in the limiting effect of $\pi$. There is no term in $I$ or $\sigma$ in eq 35, but there is a small negative term in MR not present in eq 8 or 34. The MR term indicates a slight resistance to bulk tolerance by the enzyme of large X groups.

The effect of marker substituents in resistant cell culture is similar to that in the sensitive cell culture. The 3-$CH(OH)C_6H_5$ is badly fit as with isolated DHFR; however, 3-CN normally poorly fit is well fit by both eq 34 and 35.

Again, the lack of a $\sigma$ term and the "normal" activity of the CN group give signals that the DHFR in vivo is not exactly the same DHFR "purified" and in buffer solution. It is not unexpected that the $\sigma$ effect and the behavior of the 3-CN analogue go together. If, as postulated in section V, the $\sigma$ term accounts for a special dipolar interaction of substituent with enzyme, then the 3-CN might well be the epitome type of substituent capable of acting in such a process.

Log $1/C$ for methotrexate for 50% inhibition of sensitive L5178Y cells is 8.89, but for resistant cells it is only 2.90. Thus, it takes 1 000 000 times the concentration of MTX to inhibit the resistant cells compared to the sensitive cells.

A similar effect to that of the triazines has been found for the inhibition of the two types of cells by benzylpyrimidines. The major difference between eq 36 and

QSAR for inhibition of sensitive L5178YS cells by benzylpyrimidines[256] XXVIII

$$\log 1/C = 0.38\pi - 0.79 \log (\beta \cdot 10^{\pi} + 1) + 0.08\mathrm{MR}_3 + 0.18\sigma + 5.23 \quad (36)$$

$$n = 42,\ r = 0.837,\ s = 0.215,\ \pi_0 = 1.38$$

QSAR for inhibition of resistant L5178YR cells by benzylpyrimidines[256] XXVIII

$$\log 1/C = 0.49\pi + 3.76 \quad (37)$$

$$n = 42,\ r = 0.916,\ s = 0.288$$

37 is in the role of hydrophobicity. Equation 36 has a low $\pi_0$ of 1.38 while $\pi_0$ cannot be cleanly established for eq 37, but it would appear to be about 6. The difference in the role of hydrophobicity for eq 36 and 37 is very similar to that of eq 34 and 35. The $\pi_0$ for the sensitive cells is about the same as for the isolated DHFR while there is no clear upper limit to the increase in activity with increase in $\pi$ for the resistant cells.

Clearly there is an important message here for those engaged in cancer chemotherapy using antifolates. Combination chemotherapy using methotrexate and a hydrophobic triazine should prevent the rise in tumor cells resistant to methotrexate and should thus improve the clinical effectiveness of MTX.

In selecting a suitable lipophilic triazine, one must be guided by our knowledge of the passive movement of organic compounds in whole animals. The ideal log $P_0$ for the passive movement of neutral organic compounds in whole animals insofar as we understand it is about 2. The parent structure VI has a log $P$ at pH 7 of −3. Therefore, the $\sum\pi$ to be added to VI needs to be about 5. Baker's antifol II (XVI) has a $\pi$ of 5.13, which would seem to make it an excellent candidate for combination chemotherapy except for the fact that hydrolysis of the $SO_2F$ moiety would lower log $P$ by about 4. There is evidence that the $SO_2F$ moiety un-

dergoes rapid hydrolysis in animals.[105-107]

Browman and Lazarus[257] in fact carried out a combination test using methotrexate and 2,4-diamino-5-(3,4-dichlorophenyl)-6-methylpyrimidine (DDMP), which has a log $P$ of 2.56 at pH 7.2.[258] Greco and Hapala[258] showed that lipophilic antifols, including DDMP, were taken up by tumors thousands of times faster than MTX. However, Browman and Lazarus did not find the combination of MTX and DDMP to be better than MTX. In fact, the DDMP treatment alone gave mice a longer survival time than MTX + DDMP. Resistance developed quicker for DDMP than for MTX. Surprisingly, MTX and DDMP did not show direct cross resistance.

In fact, the choice of DDMP to use in combination with MTX may not have been the best since the data in section V clearly show that triazines are as a class much more potent than pyrimidines against vertebrate DHFR. This is also apparent from a comparison of eq 34 and 35 with 36 and 37. The intercepts of these equations bring out the much greater potency of the triazines for both resistant and sensitive tumor cells. Other evidence is accumulating which shows that the lipophilic character of antifolates is important in their selective uptake by tumor[259] cells of various kinds.[25,127,142,259] Sirotnak et al. have found that murine tumors sensitive to MTX are relatively insensitive to lipophilic agents.[259a]

The triazines when tested against *L. casei* cell cultures sensitive and resistant to MTX show behavior similar to tumor cells. In the case of eq 38, $\pi'$ gives

QSAR for inhibition of sensitive *L. casei* cells by 3-X-VI[183]

$$\log 1/C = 0.80\pi' - 1.06 \log (\beta \cdot 10^{\pi'} + 1) - 0.94MR_Y + 0.80I + 4.37 \quad (38)$$

$$n = 34,\ r = 0.929,\ s = 0.371,\ \pi_0 = 2.94$$

QSAR for inhibition of MTX-resistant *L. casei* cells by 3-X-VI[183]

$$\log 1/C = 0.45\pi + 1.05I - 0.48MR_Y + 3.37 \quad (39)$$

$$n = 38,\ r = 0.964,\ s = 0.264$$

slightly better results even though whole cells are involved. This $\pi'$ term signifies that $\pi_Y$ of $ZCH_2C_6H_4Y$ is set equal to zero. As for the QSAR with purified DHFR and VI, one assumes that Y does not make hydrophobic contact with the DHFR. Equation 38 is rather similar in most respects to eq 10 for isolated *L. casei* DHFR. Even the intercepts of the two equations are close in value. There is one salient difference and that is the term in $MR_Y$ with its negative coefficient in eq 38. This term applies only to Y groups on $ZCH_2C_6H_4Y$, and since MR is a rough measure of bulk, the negative coefficient implies that a steric effect is present in the DHFR in situ, which is missing in DHFR in buffer. This term is also present in eq 39 for resistant bacterial cells. The enzyme in the living cell definitely has a different behavior from the enzyme in buffer. This might be the result of two different conformations of the enzyme or it may be that a nearby macromolecule to which the DHFR might be in contact causes the steric effect by weakly blocking the open region into which Y normally projects. To our knowledge this is the first instance in which specific difference between isolated enzyme and enzyme in situ has been characterized.

The fact that $\pi'$ holds in eq 38 for action taking place in cells reveals that the lipophilic compartments and membranes of the bacterial cells have little influence on the penetration of this set of triazines in *L. casei* cells.

Equation 39 is similar to eq 37 and eq 35. Although eq 35 does contain a bilinear term, this term is not of much significance since it is so small that the right-hand portion of the bilinear model is positive. For all three equations with resistant cells, $\pi_0$ is very high, probably near 6, and all three equations have essentially the same coefficient with the $\pi$ term. Thus, both tumor cells and *L. casei* cells appear to use the same mechanism for protecting themselves from MTX.

Studies of 3-X-triazine inhibiting *E. coli* cells in culture (sensitive and resistant to MTX) do not yield results comparable to those obtained with *L. casei*. Preliminary QSAR are almost identical except for modest differences in the intercepts.[260] Since it is known that *L. casei* cells have an active transport system for folates and *E. coli* do not, this suggests that the major factor responsible for the difference in response of resistant and sensitive tumor cells and *L. casei* cells to hydrophilic triazines and pyrimidines may be the transport system. If this system were inactive in the resistant cells, then very hydrophilic compounds such as MTX would have difficulty penetrating the cells while the hydrophobic triazines and benzylpyrimidines would not.

Reasoning further along this line, if the transport system employs a macromolecular receptor to capture a folate analogue and then transport it across the lipophilic membrane, this receptor must look like dihydrofolate reductase or else one would not see such close similarity between equations 8 and 34 and 10 and 38.

The several possible mechanisms for resistance to MTX have been reviewed by Harper and Kellems.[261] Methotrexate is normally actively transported into mammalian cells, but it is not usually transported actively by bacterial cells (*L. casei* is an exception). The transport problem for normal and resistant cells for folates and methotrexate has been extensively studied and reviewed by Huennekens,[262] Sirotnak,[263] and their colleagues. Sirotnak et al.[264] analyzed the potential of the transport system for the design of antitumor drugs. They point out[264] that structural variation in the transport systems of different cells may be greater than structural differences in the DHFR so that focusing on variations in the transport system may offer a better route for more selective drugs. For example, modifications at the N10 and *p*-carboxyl group of methotrexate have little effect on binding to DHFR but affect transport in L1210 leukemia cells. The presence of methyl or ethyl group at N10 increases $K_m$ for influx by 3- to 4-fold with no effect on eflux.[265] The difference between $K_m$ for influx between tumor cells and intestinal epithelial cells for influx of aminopterin is 6-fold, for methotrexate it is 25-fold, for $N^{10}$-ethylaminopterin it is 40-fold. Sirotnak and Donsbach found[266] that the $K_m$ for MTX influx differed by as much as 4-fold among

a variety of murine tumors and that this was correlated with the responsiveness of these tumors during therapy.

Kamen et al. pointed out that commercial radiolabeled MTX often contains impurities which have compromised some transport studies.[266a]

A second kind of resistance mechanism, which has been known for some time, is that of increased production of dihydrofolate reductase which occurs through mutation of some cells by antifolates.[267,268] Comparison of DHFR from normal and resistant cells reveals no detectable differences, so that it is assumed the DHFR produced by gene amplication in resistant cells is very similar if not identical with normal cells.[269,270] It has been shown that in murine tumor cells (L5178YR) resistant to MTX that a 300-fold increase in DHFR production occurs. This amounts to DHFR constituting 10% of the total soluble cell protein.[271] It was observed that the transport of MTX was unchanged in the resistant cells when compared to the parent line. This observation is at odds with the above discussion on the difference in response between *L. casei* and *E. coli* cells sensitive and resistant to MTX.

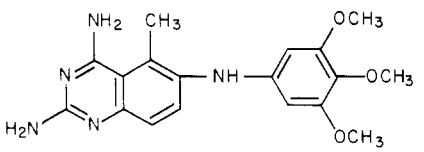
The high rate of production of DHFR in MTX resistant cells is the result of increased levels of DHFR messenger RNA;[272] in fact, seven such RNA have been identified.[273] The increase in RNA is brought about by gene amplification.[274] Recently, Schimke's laboratory has determined that the 558 bases of the dhfr gene are spread over almost 30 kb of DNA.[275]

A surprising discovery of MTX-resistant cells is that the amplified genes may be in an unstable as well as stable state. When MTX is removed from the culture, MTX resistance may be slowly lost.[266,267,276] Both the unstable and the stable resistant cell lines can be derived from the same MTX-sensitive parental cells.[277]

Although the overproduction of DHFR by resistant cells no doubt plays an important role in the resistance of these cells to antifolates, it certainly cannot be the most important factor. L5178Y tumor cells resistant to MTX require 1 000 000 times the concentration of MTX for 50% inhibition. The corresponding figure for *L. casei* cells is $10^{8}$![183]

A third mechanism for cell resistance to MTX is that of altered DHFR with lower affinity for the inhibitor.[250,278-283] Baccanari et al.[251] have shown that two DHFR isozymes from a resistant strain of *E. coli* (RT 5000) differ by only one change in residue 28 from arginine to leucine. However, this small change produces isozymes which differ greatly in their binding and kinetic properties. The TMP dissociation constant is 50-fold greater for one form.

Haber et al.[282] in a study of seven different inhibitors to two forms of DHFR from 3T6 mouse embryo fiberblasts found that there was a large difference in sensitivity of the two enzymes. The most interesting inhibitor JB-11 was 3000 times more active on one form




JB-11

compared to the other. Baker's antifol was 1000 times more effective against one form of the DHFR. Haber et al. concluded that the dramatic change in $ID_{50}$ for the altered enzyme between two folate analogues which bind tightly to normal dihydrofolate reductase and which differ only in the portions equivalent to the *p*-aminobenzoyl moiety of methotrexate suggests that this part of the molecule may be in close contact with the altered enzyme.

It has long been thought that cells could become drug resistant through changes in their membranes. Recently, Burns et al.[284] showed that the lipid composition of L1210 murine leukemia cells was dependent upon the fat fed to the host animal. They also showed that MTX penetrated cells with different lipid composition at different rates.

The results of eq 34 and 39 can be interpreted to suggest that a lipophilic barrier has been erected by the resistant cells to prevent the entry of hydrophilic drugs such as MTX or hydrophilic triazines. This barrier could be nothing other than the normal lipid membrane of the cell if an active transport system for MTX, triazines and other such antifolates were turned off in resistant cells. The definitive work on the possible active transport of these compounds needs to be done.

Other than the above equations comparing resistant and sensitive cells, little QSAR work has been done with living systems. A notable exception is the studies of Wooldridge and his colleagues.[285,286]

Equations 40 and 41 correlate minimum inhibitory concentrations ($C$) for triazines acting on bacterial cells.

3- and 4-X-VI QSAR from *S. aureus* cell culture[286]

$$\log 1/C = 0.60\pi - 1.89 \log (\beta \cdot 10^{\pi} + 1) + 2.84 \quad (40)$$

$$n = 66,\ r = 0.963,\ s = 0.344,\ \pi_0 = 5.86$$

3-X-VI QSAR from *E. coli* cell culture[27]

$$\log 1/C = 0.51\pi - 1.09 \log (\beta \cdot 10^{\pi} + 1) + 2.57 \quad (41)$$

$$n = 22,\ r = 0.960,\ s = 0.307,\ \pi_0 = 5.07$$

While these QSAR are similar to eq 38 in the coefficients with the $\pi$ terms, the additional terms in $MR_Y$ and $I$ of eq 38 are not necessary in eq 40 and 41 because substituents of the type $CH_2ZC_6H_4Y$ were not included in the congener set used to develop those QSAR.

An especially interesting difference between eq 38 and 40 and 41 is the much higher $\pi_0$ one obtains for the latter two organisms. This difference could be associated with the fact that of the three bacteria only *L. casei* has an active transport system. The lower value of $\pi_0$ for the *L. casei* QSAR could be set by the size of the binding site in the transport macromolecule.

Smith et al.[287] have provided some insight in the mechanism of pyrimidine antifolate inhibition of microorganisms. They have used discriminant analysis to classify the ability of folic or folinic acid to reverse the inhibitory effect of 175 pyrimidines acting on *S. faecium*, *L. casei*, and *P. cerevisiae* cells.

Another example of a QSAR from a living system in which DHFR inhibitors have been studied is that of quinazolines in inhibiting *P. berghi* in mice formulated from the many studies by Elslager and his colleagues.[288]

Inhibition of *P. berghei* in mice by VII

$$\log 1/C = 0.88\sum\pi - 0.16(\sum\pi)^2 - 0.68I_6 + 0.37I_8 + 1.53I_9 + 1.18I_{10} + 3.27 \quad (42)$$

$$n = 60,\ r = 0.906,\ s = 0.427,\ \pi_0 = 2.82$$

The two most important indicator variables are $I_9$ and $I_{10}$, which take the value of 1 for substituents of the type 6-$XNCH_2Ar$ ($I_9$) and 6-$CH_2NHAr$ ($I_{10}$). Although there was large variation in X, this did not affect activity greatly. These indicator variables are, of course, reminiscent of the $I$ terms in eq 2, 3, 8, 10, and 11. Thus, it seems that special potency is conferred by structures of the type $CH_2ZC_6H_4$ or $ZCH_2C_6H_4$. $I_8$ assumes the value of 1 for 5-$CH_3$ and 5-Cl. Its positive coefficient, along with our knowledge of the active site of DHFR, suggests an especially effective hydrophobic interaction by these groups which have very similar $\pi$ values (0.56 and 0.71).

There are four examples where alkyl groups have been placed on the two 2,4-diamino groups for which I-6 is given the value of 1. The negative coefficient with this term brings out the deleterious effect of such substitution.

Using the value of log $P$ = 1.00 for the parent 2,4-diaminoquinazoline, we calculate a log $P_0$ of 3.83 for the antimalarial antifolates acting in mice.

The above studies of enzyme inhibitors acting on purified enzymes and on whole cells clearly show that there is a good deal to be learned about the design of drugs from basic research. Needless to say, this type of study is the prelude to studying the same inhibitors in whole animals. Once we understand the SAR at the level of the receptor, in the cell and in the whole animal, we should be able to go about the fine tuning of antifolates to increase their specificity and potency.

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## XII. Glossary

| | |
|---|---|
| CHEM | Computer program for molecular modeling |
| DHFR | Dihydrofolate reductase |
| DDMP | 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine |
| dTMP | Deoxythymidine monophosphate |
| dUMP | Deoxyuridine monophosphate |
| $E_s$ | Taft steric parameter |
| $E_t$ | Total enzyme concentration |
| $FH_2$ | Dihydrofolic acid |
| $FH_4$ | Tetrahydrofolic acid |
| $I_i$ | Indicator variables |
| $I_t$ | Amount of E consumed in formation of EI complex |
| $K_{i\,app}$ | Apparent inhibition constant |
| L-1210 | Mouse leukemia |
| L5178Y | Mouse leukemia |
| MIDAS | Computer program for molecular modeling |
| MR | Molar refractivity |
| MSA | Molecular shape analysis |
| MTD | Minimal topological difference |
| MTX | Methotrexate |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| $P$ | Octanol/water partition coefficient |
| QSAR | Quantitative structure–activity relationship |
| $\pi$ | Hydrophobic substituent constant |
| $\pi_0$ | Optimum hydrophobic substituent constant |
| SAR | Structure–activity relationship |
| $\sigma$ | Hammett electronic substituent constant |
| TMP | Trimethoprim |
| $\nu$ | Charton steric parameter |

## XIII. Appendix

A large number of variations of the 2,4-diaminopyrimidine theme has been synthesized and tested on dihydrofolate reductase from a variety of sources. Table VII lists 81 different sources of DHFR. Tables VIII–XII contain 1709 inhibitors which have been tested against DHFR from the various sources. The inhibitors have been arranged in the tables more or less according to their relationship to folic acid, that is, Table VIII contains the most closely related derivatives containing the pteridine ring with modifications on it as well as on the C9–N10 bridge and the glutamate portion of folic acid. Table IX contains derivatives of quinazoline, while Table X and XI refer to triazines. The largest group of inhibitors is the derivatives of pyrimidine contained in Table XII.

In each table the compounds have been arranged according to increasing substituent complexity. In the case of multiple substitution, arrangement has been in order of increasing substituent complexity in one position, keeping the other positions constant where possible.

Following the formula, data are given in the following sequence, "activity (DHFR source) reference". Activity has been given only when it was reported as $I_{50}$ or $K_i$ and is expressed as log $1/C$ or log $1/K_i$ ($M^{-1}$). No distinction has been made in the tables between the two parameters. In instances where activity was not reported so that it could be expressed in these terms, only source and reference are listed.

The reader must bear in mind that the $1/C$ and $1/K_i$ values have been determined with different degrees of care on enzymes of varying quality under different experimental conditions. In general, the figures are suitable for semiquantitative comparisons only. One can obtain a rough idea of the variation of potency with structure or source.

When a structure contains an amino acid residue, it has usually been abbreviated as follows: Glu = glutamic acid, Glu($R_2$) = $NHCH(COOR)CH_2CH_2COOR$, Asp = aspartic acid, Gly = glycine, Ala = alanine, $\beta$-Ala = $\beta$-alanine, Phe = phenylalanine, Leu = leucine, Abu = aminobutyric acid, Val = valine, Sarc = sarcosine.

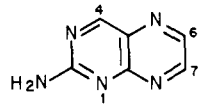
Although we have attempted to include references to all inhibitors which have been tested against the enzyme

**TABLE VII. List of DHFR Sources[a] and Corresponding Symbols Used in Tables VIII-XII**

| | |
|---|---|
| A | human liver |
| B | human erythrocytes |
| C | human placental |
| D | human leukemia cells (AML, ALL, etc.) |
| E | human carcinoma KB/MTX cells |
| F | WI-L2 human lymphoplast |
| G | *Aotus trivirgatus* (owl monkey) red cells |
| H | bovine liver |
| I | porcine liver |
| J | rabbit liver |
| K | guinea pig (liver, small intestine, etc.) |
| L | Chinese hamster ovary cells |
| M | rat liver |
| N | Walker 256 |
| O | sarcoma (Yoshida rat, S-180, etc.) |
| P | Erlich ascites carcinoma cells |
| Q | mouse liver |
| R | mouse intestine |
| S | mouse spleen |
| T | mouse ascites |
| U | L 1210 (murine lymphoma, mouse leukemia, etc.) |
| V | L 5178 Y mouse lymphoma |
| X | murine tumor |
| W | mouse embryo fibroblasts (cells resistant to different MTX concentrations) |
| Y | mouse neuroblastoma (sensitive and resitant cells) |
| Z | mouse erythrocytes |
| AA | chicken liver |
| AB | pigeon liver |
| AC | turtle liver |
| AD | frog liver |
| AE | carp liver |
| AF | tapeworm |
| AG | wheat grain |
| AH | soybean seedlings |
| AI | *Trypanosoma brucei* (culture and rat bloodstream forms) |
| AJ | *Trypanosoma congolense* (culture and rate bloodstream forms) |
| AK | *Trypanosoma cruzi* (culture and rat bloodstream forms) |
| AL | *Trypanosoma equiperdum* (culture and rat bloodstream forms) |
| AM | *Trypanosoma lewisi* (culture and rat bloodstream forms) |
| AN | *Trypanosoma rhodesiense* (culture and rat bloodstream forms) |
| AO | *Trypanosoma vivax* (culture and rat bloodstream forms) |
| AP | *Plasmodium berghei* |
| AQ | *Plasmodium falciparum* (Uganda, Palo Alto, and Vietnam–Oak knoll strains) |
| AR | *Nippostrongylus brasiliensis* |
| AS | *eimeria tenella* |
| AT | *Crithidia fasciculata* |
| AU | *S. mansoni* |
| AV | *Saccharomyces cerevisiae* |
| AX | *Clostridium perfrigens* |
| AW | *Clostridium septicum* |
| AY | *C. tetanomorphum* |
| AZ | *Staphylococcus aureus* |
| BB | *Staphylococcus pyogenes* |
| BC | *Streptococcus faecium* |
| BD | *Streptococcus faecalis* |
| BE | *Diplococcus pneumoniae* (wild type, A and B mutants, recombinant, etc.) |
| BF | *Neisseria sicca* |
| BG | *Proteus vulgaris* |
| BH | *Proteus mirabilis* |
| BI | *Citrobacter* sp. R plasmids |
| BJ | *Escherichia coli* |
| BK | *E. coli* R plasmids |
| BL | *Klebsiella pneumoniae* |
| BM | *Pseudomonas aeruginosa* |
| BN | *Lactobacillus casei* |
| BO | *Plasmodium acnes* |
| BP | *M.* species 607 (drug-response profile similar to that of *M. leprae*) |
| BQ | *Bacteriodes* sp. |
| BR | *Bacillus fragilis* |
| BS | *Caulobacter* sp. |
| BT | *Nocardia restricta* |
| BU | *Nocardia brasiliensis* |
| BV | *D. immitis* |
| BX | *L. carinii* |
| BW | *D. witei* |
| BY | *O. vulvulus* |
| BZ | *C. oncopelti* |
| CC | *Galleria mellonella* |
| CD | $T_2$ phage |
| CE | $T_4$ phage |

[a] Only the main source is generally reported without specification of bacterial strains, cells resistant to different MTX concentrations, etc. Details can be easily obtained from ref in Tables VII–XII.

**TABLE VIII. Inhibition by 2-Aminopteridines**




| no. | $R_4$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|
| 1 | H | $NH_2$ | 5.8 (AA) 4 |
| 2 | H | $C_6H_5$; (8-oxadihydroptd) | (AB) 224 |
| 3 | $NH_2$ | H | 6.3 (AA) 4; 5.2 (BC) 264; 3.6 (M) 104 3.9 (Q) 89 |
| 4 | $NH_2$ | 6-H; 7-$CH_3$ | 5.9 (BC) 231 |
| 5 | $NH_2$ | 6-H, 7-$CH_2OH$ | 5.0 (BC) 34 |
| 6 | $NH_2$ | 6-H; 7-$NH_2$ | 7.0 (M) 68 |
| 7 | $NH_2$ | 6-H; (5-deaza) | 3.7 (M) 82; 3.8 (BJ) 82 |
| 8 | $NH_2$ | 6-H; (5-deaza); 5-$CH_3$ | 4.7 (M) 82; 5.6 (BJ) 82; 5.5 (BM) 82 |
| 9 | $NH_2$ | 6-H; (5-deaza); 5-O-$C_6H_2$-2′-$CH(CH_3)_2$-4′-Cl-5′-$CH_3$ | 6.3 (BC) 128 |
| 10 | $NH_2$ | OH | 4.0 (AA) 66 |
| 11 | $NH_2$ | 6-$NH_2$; 7-$C_6H_5$ | 4.5 (K) 45 |
| 12 | $NH_2$ | $CH_3$ | 3.7 (AB) 20; 5.7 (AA) 4; 5.3 (BP) 165 6.4 (BC) 231; 4.4 (AL) 104; 3.6 (AA) 104 4.1 (M) 104 |
| 13 | $NH_2$ | 6-$CH_3$; 7-$CH_3$ | 6.2 (BC) 231; 5.1 (B) 104; 4.1 (AA) 104 5.7 (M) 68; 3.8 (M) 104; 3.9 (BR) 220 6.8 (BQ) 220; 4.5 (AX) 220 |
| 14 | $NH_2$ | 6-$CH_3$; 7-$NH_2$ | 5.8 (M) 68 |
| 15 | $NH_2$ | $CH_3$; (3-deaza) | 4.0 (AB) 129; 6.2 (BC) 231 |
| 16 | $NH_2$ | $CH_3$; (1-deaza) | 2.0 (AB) 129; (BC) 231 |
| 17 | $NH_2$ | $CH_3$; (8-deaza) | 5.8 (BP) 216 |
| 18 | $NH_2$ | 6-$CH_3$; 7-$CH_3$ (8-oxadihydroptd) | (AB) 224 |

TABLE VIII (Continued)

| no. | $R_4$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|
| 19 | $NH_2$ | CHO | 5.1 (AA) 4 |
| 20 | $NH_2$ | 6-$C_2H_5$; 7-$C_2H_5$ | 8.3 (BC) 231 |
| 21 | $NH_2$ | 6-$C_2H_5$; (5-deaza); 5-*n*-$C_3H_7$ | 6.6 (M) 82; 8.3 (BP) 216 |
| 22 | $NH_2$ | 6-$C_2H_5$; (5-deaza); 7-$C_6H_5$ | 5.6 (M) 82; 6.9 (BJ) 82 |
| 23 | $NH_2$ | 6-$C_3H_7$-*i*; 7-$C_3H_7$-*i* | 6.6 (BQ) 220; 7.8 (BQ) 220 |
| 24 | $NH_2$ | $C_3H_7$; (5-deaza) | 6.0 (BM) 82 |
| 25 | $NH_2$ | 6-$C_3H_7$; (5-deaza); 5-$CH_3$ | 7.1 (M) 82; 7.8 (BJ) 82; 7.5 (BM) 82 |
| 26 | $NH_2$ | 6-$C_3H_7$; (5-deaza); 7-$C_4H_9$ | 6.4 (M) 82; 7.7 (BJ) 82; 6.8 (BM) 82 |
| 27 | $NH_2$ | *n*-$C_4H_9$ | 9.0 (BP) 165 |
| 28 | $NH_2$ | $CH_2CH(CH_3)_2$ | 9.0 (BP) 215; 6.5 (BM) 82 |
| 29 | $NH_2$ | *n*-$C_4H_9$; (5-deaza) | 6.3 (M) 144; 6.3 (M) 82; 7.5 (BJ) 63<br>7.3 (BJ) 34; 6.3 (BJ) 32; 6.3 (BJ) 88<br>6.3 (BJ) 115; 6.3 (BJ) 89; 7.7 (BC) 128<br>7.8 (AP) 144; 7.8 (AP) 115; 7.8 (AP) 88<br>6.2 (AN) 144; 6.2 (AN) 115; 6.2 (AN) 90<br>4.0 (AU) 144; 6.9 (CD) 63; 6.8 (AB) 63<br>6.3 (M) 32; 6.3 (BU) 144; 4.0 (BV) 144<br>5.5 (BX) 144; 5.1 (BW) 144; 5.7 (BY) 144<br>6.1 (BM) 82; 7.1 (BG) 34; 6.3 (BG) 32<br>7.7 (AZ) 34; 7.4 (AZ) 32; 6.0 (J) 32<br>6.3 (AI) 90; 6.3 (AR) 115; 6.4 (AL) 90<br>6.4 (AN) 67; 6.4 (AO) 90; 6.0 (AT) 115<br>6.2 (AJ) 90; 6.1 (AM) 90; 6.5 (AK) 90<br>6.0 (A) 32; 7.4 (K) 32; 6.3 (M) 88 |
| 30 | $NH_2$ | *n*-$C_4H_9$; (5-deaza); 5-$CH_3$ | 7.7 (BJ) 32; 7.7 (BJ) 82; 7.5 (BJ) 63<br>6.6 (A) 32; 8.4 (BJ) 34; 6.6 (M) 82<br>8.9 (BC) 128; 7.1 (AZ) 32; 7.5 (AZ) 34<br>6.6 (M) 32; 8.1 (K) 32; 8.0 (BG) 32<br>7.0 (J) 32; 9.0 (BG) 34; 7.1 (AB) 63<br>7.7 (BM) 82; 7.3 (GD) 63 |
| 31 | $NH_2$ | *n*-$C_4H_9$; (8-deaza) | 7.1 (BP) 216 |
| 32 | $NH_2$ | 6-*i*-$C_6H_9$; (5-deaza); 5-$CH_3$ | 9.8 (BP) 216 |
| 33 | $NH_2$ | *sec*-$C_4H_9$; (5-deaza); 5-$CH_3$ | 9.0 (BJ) 34; 9.0 (BJ) 63; 8.7 (BJ) 82<br>8.0 (CD) 63; 7.6 (AB) 63; 8.1 (D) 266<br>7.5 (AZ) 34; 9.0 (BG) 34; 10.5 (BC) 128<br>8.1 (BM) 82 |
| 34 | $NH_2$ | 6-(2′-furyl); 7-$NH_2$ | 6.6 (K) 45 |
| 35 | $NH_2$ | 6-(3′-thienyl); 7-$NH_2$ | 7.1 (K) 45 |
| 36 | $NH_2$ | *n*-$C_5H_{11}$ | 8.9 (BP) 165 |
| 37 | $NH_2$ | *n*-$C_5H_{11}$; (5-deaza) | 6.4 (BM) 82 |
| 38 | $NH_2$ | *n*-$C_5H_{11}$; (5-deaza); 5-$CH_3$ | 9.7 (BP) 216; 7.2 (M) 82; 8.4 (BJ) 82<br>7.9 (BM) 82 |
| 39 | $NH_2$ | $CH_2CH_2CH(CH_3)_2$ | 9.0 (BP) 165 |
| 40 | $NH_2$ | $CH_2CH_2CH(CH_3)_2$; (5-deaza); 5-$CH_3$ | 8.1 (BM) 82 |
| 41 | $NH_2$ | $CH(CH_3)C_3H_7$; (5-deaza); 5-$CH_3$ | 8.4 (BM) 82 |
| 42 | $NH_2$ | *n*-$C_6H_{13}$; (5-deaza) | 8.1 (BC) 128; 6.4 (BM) 82 |
| 43 | $NH_2$ | 6-*n*-$C_6H_{13}$; (5-deaza); 5-$CH_3$ | 9.5 (BP) 216 |
| 44 | $NH_2$ | *n*-$C_6H_{13}$; (5-deaza); 5-$CH_3$ | 7.5 (M) 82; 8.0 (BJ) 82; 7.8 (BM) 82 |
| 45 | $NH_2$ | 6-(3′-$C_6H_4N$); 7-$NH_2$ | 5.4 (K) 45 |
| 46 | $NH_2$ | S-$C_6H_5$ | (M, BN, U) 183 |
| 47 | $NH_2$ | 6-$C_6H_5$; 7-$NH_2$ | 8.0 (M) 136; 8.0 (Q) 136; 7.7 (C) 136<br>6.6 (K) 45; 8.0 (U) 44; 4.6 (BR) 220<br>7.9 (P) 29; 5.6 (AL) 104; 5.3 (BQ) 220<br>4.5 (AX) 220; 4.7 (AW) 220; 5.8 (AA) 104<br>5.8 (M) 104; 6.2 (BO) 220; 5.4 (BY) 220<br>7.6 (U) 86; 7.1 (BC) 231; 8.0 (M) 68 |
| 48 | $NH_2$ | $C_6H_5$; (5-deaza) | 5.1 (BM) 82 |
| 49 | $NH_2$ | 6-(1′,2′,5′,6′-tetrahydrophenyl); 7-$NH_2$ | 6.0 (M) 68 |
| 50 | $NH_2$ | 6-$C_6H_4$-2′-Br; 7-$NH_2$ | 6.0 (AL) 104; 5.6 (AA) 104; 6.0 (M) 104 |
| 51 | $NH_2$ | 6-$C_6H_4$-4′-Br; 7-$NH_2$ | 4.1 (AL) 104; 3.5 (AA) 104; 4.1 (M) 104 |
| 52 | $NH_2$ | 6-$C_6H_4$-2′-Cl; 7-$NH_2$ | 6.2 (K) 45; 5.4 (AL) 104; 5.6 (AA) 104<br>5.8 (M) 104 |
| 53 | $NH_2$ | 6-$C_6H_4$-3′-Cl; 7-$NH_2$ | 5.9 (AL) 104; 6.0 (AA) 104; 6.6 (M) 104 |
| 54 | $NH_2$ | 6-$C_6H_4$-4′-Cl; 7-$NH_2$ | 3.8 (AL) 104; 4.0 (AA) 104; 4.7 (M) 104 |
| 55 | $NH_2$ | 6-$C_6H_4$-2′-F; 7-$NH_2$ | 5.3 (AL) 104; 5.4 (AA) 104; 5.7 (M) 104 |
| 56 | $NH_2$ | 6-$C_6H_4$-4′-F; 7-$NH_2$ | 4.5 (AL) 104; 4.3 (AA) 104; 4.8 (M) 104 |
| 57 | $NH_2$ | 6-$C_6H_4$-2′-I; 7-$NH_2$ | 5.7 (AL) 104; 6.5 (AA) 104; 5.9 (M) 104 |
| 58 | $NH_2$ | 6-$C_6H_4$-4′-I; 7-$NH_2$ | 3.9 (AL) 104; 4.0 (AA) 104; 4.3 (M) 104 |
| 59 | $NH_2$ | 6-$C_6H_3$-2′,6′-$Cl_2$; 7-$NH_2$ | 5.7 (AL) 104; 5.5 (AA) 104; 6.2 (M) 104 |
| 60 | $NH_2$ | 6-$C_6H_3$-2′,4′-$Cl_2$; 7-$NH_2$ | 4.3 (AL) 104; 3.2 (AA) 104; 5.1 (M) 104 |
| 61 | $NH_2$ | 6-$C_6H_3$-3′,4′-$Cl_2$; 7-$NH_2$ | 3.3 (AL) 104; 4.7 (AA) 104; 5.7 (M) 104 |
| 62 | $NH_2$ | 6-$C_6H_4$-4′-$NH_2$; 7-$NH_2$ | 4.9 (K) 45 |
| 63 | $NH_2$ | 6-$C_6H_5$; 7-$C_6H_5$ | 6.4 (AL) 104; 5.0 (AA) 104; 5.4 (M) 104 |
| 64 | $NH_2$ | 6-$C_6H_5$; 7-$C_6H_5$; (8-oxadihydroptd) | (AB) 224 |
| 65 | $NH_2$ | 6-*n*-$C_7H_{15}$; (5-deaza); 5-$CH_3$ | 7.5 (M) 82; 8.1 (BJ) 82; 7.7 (BM) 82 |
| 66 | $NH_2$ | $CH_2C_6H_5$ | 9.0 (BP) 165 |
| 67 | $NH_2$ | 6-$CH_2C_6H_5$; 7-$NH_2$ | 7.7 (M) 68 |

**TABLE VIII** (Continued)

| no. | $R_4$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|
| 68 | $NH_2$ | $SCH_2C_6H_5$ | (M, BN, U) 183 |
| 69 | $NH_2$ | $SC_6H_4$-4′-$CH_3$ | (M, BN, U) 183 |
| 70 | $NH_2$ | $CH_2C_6H_3$-3′,4′-$Cl_2$ | 8.7 (BP) 165 |
| 71 | $NH_2$ | 6-$CH_2C_6H_5$; (5-deaza) | 6.5 (CD) 63; 8.0 (BJ) 63; 6.3 (AB) 63 6.6 (M) 82; 6.4 (BJ) 82; 6.3 (BM) 82 7.7 (BC) 128; (BN) 232 |
| 72 | $NH_2$ | 6-$CH_2C_6H_5$; (5-deaza); 5-$CH_3$ | 8.0 (BJ) 103; 7.4 (M) 103; 8.5 (BC) 128 8.6 (BM) 82 |
| 73 | $NH_2$ | 6-$CH_2C_6H_5$; (5-deaza); 5-$n$-$C_3H_7$ | 7.2 (M) 82; 5.9 (BJ) 82 |
| 74 | $NH_2$ | 6-$CH_2C_6H_4$-2′-Cl; (5-deaza); 5-$CH_3$ | 7.8 (D) 266 |
| 75 | $NH_2$ | 6-$CH_2C_6H_4$-3′-Cl; (5-deaza); 5-$CH_3$ | 9.6 (BP) 216 |
| 76 | $NH_2$ | 6-$CH_2C_6H_4$-4′-Cl; (5-deaza); 5-$CH_3$ | 9.7 (BP) 216 |
| 77 | $NH_2$ | 6-$C_6H_4$-2′-$CH_3$; 7-$NH_2$ | 6.7 (K) 45; 5.4 (AL) 104; 5.5 (AA) 104 5.9 (M) 104 |
| 78 | $NH_2$ | 6-$C_6H_4$-3′-$CH_3$; 7-$NH_2$ | 5.1 (AL) 104; 5.5 (AA) 104; 5.7 (M) 104 |
| 79 | $NH_2$ | 6-$C_6H_4$-4′-$CH_3$; 7-$NH_2$ | 4.8 (M) 104; 4.0 (AL) 104; 3.9 (AA) 104 |
| 80 | $NH_2$ | 6-$CH_2C_6H_4$-4′-$OCH_3$; (5-deaza); 5-$CH_3$ | 7.3 (D) 266 |
| 81 | $NH_2$ | $CH_2NHNHC_6H_4$-4′-$CO_2H$ | 3.9 (Q) 89 |
| 82 | $NH_2$ | $CH{=}NNHC_6H_4$-4′-$CO_2H$ | 3.9 (Q) 89 |
| 83 | $NH_2$ | $CH_2CH_2C_6H_5$ | 5.4 (AB) 20 |
| 84 | $NH_2$ | 6-$CH_2CH_2C_6H_5$; 7-$NH_2$ | 7.0 (M) 68 |
| 85 | $NH_2$ | $CH_2CH_2C_6H_5$; (5-deaza) | 6.4 (BJ) 82; 5.8 (BM) 82 |
| 86 | $NH_2$ | 6-$C_6H_3$-4′,5′-$(CH_3)_2$; 7-$NH_2$ | 4.0 (AL) 104; 4.5 (AA) 104; 4.1 (M) 104 |
| 87 | $NH_2$ | $CH_2NHC_6H_4$-4′-$CO_2H$; (1-deaza) | 6.2 (BC) 231; 4.9 (AB) 129 |
| 88 | $NH_2$ | $CH_2NHC_6H_4$-4′-$CO_2H$; (3-deaza) | 5.4 (AB) 129 |
| 89 | $NH_2$ | $CH_2N(CH_3)C_6H_5$ | 5.4 (AB) 129 |
| 90 | $NH_2$ | $CH_2N(CH_3)C_6H_5$; (1-deaza) | 4.2 (AB) 129 |
| 91 | $NH_2$ | $CH_2N(CH_3)C_6H_5$; (3-deaza) | 4.2 (AB) 129 |
| 92 | $NH_2$ | $C_9H_{19}$; (5-deaza) | 5.2 (BM) 82 |
| 93 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-$CO_2H$ | 6.5 (BN) 172; 5.5 (AA) 172; 6.1 (AB) 129 |
| 94 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-$CO_2H$; 8-N→O | (BN, AA) 172 |
| 95 | $NH_2$ | $CH_2SC_6H_4$-4′-$CO_2CH_3$ | 5.5 (BN) 190 |
| 96 | $NH_2$ | $SC_6H_4$-2′-$CH(CH_3)_2$ | (M, BN, U) 183 |
| 97 | $NH_2$ | $SC_6H_4$-4′-$C_3H_7$ | (M, BN, U) 183 |
| 98 | $NH_2$ | 6-$CH_2C_6H_3$-2′,5′-$(OCH_3)_2$; (5-deaza); 5-$CH_3$ | 8.3 (D) 241 |
| 99 | $NH_2$ | $CH_2C_6H_2$-3′,4′,5′-$(OCH_3)_3$ | 8.3 (BP) 165 |
| 100 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-$CO_2CH_3$ | 7.5 (BC) 231 |
| 101 | $NH_2$ | $CH_2SC_6H_4$-4′-$CO_2C_2H_5$ | 5.5 (BN) 190 |
| 102 | $NH_2$ | $CH_2NHC_6H_4$-4′-$CO_2C_2H_5$; (8-deaza) | 9.1 (BP) 216 |
| 103 | $NH_2$ | $CH_2NHC_6H_4$-4′-CO-Gly | 6.6 (M) 262 |
| 104 | $NH_2$ | 6-$C_6H_3$-2′,3′-(CH=CHCH=CH); 7-$NH_2$ | 5.9 (AL) 104; 5.1 (AA) 104; 6.0 (M) 104 |
| 105 | $NH_2$ | $CH_2CH_2C_6H_2$-3′,4′,5′-$(OCH_3)_3$ | 7.7 (BP) 165 |
| 106 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Gly | 7.0 (M) 261 |
| 107 | $NH_2$ | 6-H; 7-$CH_2N(CH_3)C_6H_4$-4′-CO-Gly | 5.8 (M) 261 |
| 108 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-$CO_2C_2H_5$ | 6.2 (BN) 172; 5.2 (AA) 172 |
| 109 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-$CO_2C_2H_5$; (8-deaza); 8-thio | (M) 261 |
| 110 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-$CO_2C_2H_5$; 8-N→O | (BN, AA) 172 |
| 111 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-(D,L)-Ala | 7.9 (M) 261 |
| 112 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-$\beta$-Ala | 7.5 (M) 261 |
| 113 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Sarc | 6.3 (M) 261 |
| 114 | $NH_2$ | $CH_2NHC_6H_4$-4′-$CONH(CH_2)_3CO_2H$ | 6.4 (AB) 226 |
| 115 | $NH_2$ | $CH_2N(CH_3)(CH_2)_4$-CO-Glu | 4.7 (AB) 226 |
| 116 | $NH_2$ | $CH_2NH$-5′-$C_5H_3N$-2′-CO-Glu | 7.5 (AB) 226 |
| 117 | $NH_2$ | $CH_2NHC_6H_4$-4′-$SO_2$-Glu | 6.0 (AB) 226 |
| 118 | $NH_2$ | 6-$C_6H_4$-2′-$C_6H_5$; 7-$NH_2$ | 6.0 (AL) 104; 4.0 (AA) 104; 4.8 (M) 104 |
| 119 | $NH_2$ | 6-$C_6H_4$-4′-$C_6H_5$; 7-$NH_2$ | 3.7 (AL) 104; 4.1 (AA) 104; 3.6 (M) 104 |
| 120 | $NH_2$ | $CH_2NHC_6H_4$-4′-CO-Glu | 9.0 (BR) 220; 8.5 (BQ) 220; 8.3 (AX) 220 9.0 (AW) 220; 8.3 (AY) 220; 8.0 (BO) 220 8.3 (M) 262; 7.4 (BC) 231; 7.6 (AB) 226 9.7 (BP) 165; 11.5 (U) 233; 11.3 (M) 136 11.2 (Q) 136; 11.1 (C) 136; 10.0 (BE) 147 9.0 (BJ) 212; 9.1 (AB) 20; 9.0 (AB) 22 6.2 (AH) 195; 7.6 (BN) 265; (CC) 117 (BK, BI) 198; (M) 1; (AG) 85; (BE) 177 |
| 121 | $NH_2$ | $CH_2NHC_6H_4$-4′-CO-Glu; (7-aza) | 4.6 (AB) 179 |
| 122 | $NH_2$ | $CH_2NHC_6H_4$-4′-CO-Glu; 2-$N(CH_3)_2$ | 5.6 (P) 16 |
| 123 | $NH_2$ | $CH_2NHC_6H_4$-4′-$CH_2$-Glu | 6.3 (BN) 265; 7.6 (U) 265 |
| 124 | $NH_2$ | $CH_2NHC_6H_4$-4′-$CH_2$-CO-Asp | 6.7 (M) 262; 6.0 (AB) 226 |
| 125 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Asp | (Q, U) 33 |
| 126 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-NHCO-Asp | 7.3 (BN) 227 |
| 127 | $NH_2$ | 6-H; 7-$CH_2N(CH_3)C_6H_4$-4′-CO-Asp | (M) 261 |
| 128 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-(D,L)-Abu | 8.1 (M) 261 |
| 129 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-$\gamma$-Abu | 6.8 (M) 261 |
| 130 | $NH_2$ | $CH_2NHC_6H_4$-4′-CO-GLU; 7-$CH_3$ | 7.4 (M) 140 |
| 131 | $NH_2$ | $CH_2NHNHC_6H_4$-4′-CO-Glu | 6.1 (Q) 89 |
| 132 | $NH_2$ | $CH{=}NNHC_6H_4$-4′-CO-Glu | 4.3 (Q) 89 |
| 133 | $NH_2$ | $CH_2O$-$C_6H_4$-4′-CO-Glu | 8.3 (BN) 192; 6.3 (AB) 226; 6.6 (U) 233 |

**TABLE VIII** (Continued)

| no. | $R_4$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|
| 134 | $NH_2$ | $CH_2S$-$C_6H_4$-4′-CO-Glu | 7.8 (BN) 190; 8.3 (BN) 180 |
| 135 | $NH_2$ | $NHCH_2C_6H_4$-4′-CO-Glu | 7.8 (BN) 173 |
| 136 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; (MTX) | 9.2 (AU) 144; 8.8 (BU); 9.2 (AP) 144<br>9.0 (AN) 144; 8.9 (BV) 144; 9.0 (BX) 144<br>7.5 (BW) 144; 7.5 (BY) 144; 9.1 (D) 144<br>8.8 (AR) 115; 9.0 (BJ) 115; 9.0 (AN) 115<br>8.1 (AT) 115; 10.1 (AP) 115; 8.6 (AA) 213<br>9.5 (V) 213; 9.2 (O) 213; 10.2 (U) 213<br>11.1 (F) 213; 8.6 (BE) 213; 9.4 (BJ) 213<br>(M) 213; 9.0 (AZ) 32; 8.3 (BG) 32<br>7.0 (A) 32; 8.0 (K) 32; 7.0 (M) 32<br>7.2 (J) 32; 8.2 (BJ) 32; 9.1 (AI) 90<br>9.0 (AN) 90; 9.7 (AL) 90; 9.1 (AO) 90<br>9.1 (AJ) 90; 8.7 (AM) 90; 8.8 (AK) 90<br>9.1 (AP) 88; 8.7 (M) 88; 9.7 (D) 88<br>9.0 (BJ) 88; 7.7 (AB) 112; 9.5 (BD) 112<br>9.0 (U) 163; 9.3 (AK) 163; 8.8 (AS) 181<br>9.6 (AA) 181; 11.0 (M) 38; 9.6 (I) 182<br>8.1 (D) 266; 9.2 (P) 14; 11.2 (C) 136<br>9.6 (BJ) 212; 8.5 (BN) 227; 10.0 (BE) 147<br>7.9 (AB) 129; 11.4 (U) 233; 6.6 (AH) 195<br>7.7 (U) 83; 7.6 (M) 140; 9.0 (U) 153<br>8.5 (U) 167; 7.5 (AA) 172; 9.5 (BP) 165<br>7.5 (Q) 89; 8.8 (U) 175; 8.5 (BN) 168<br>7.6 (BC) 231; 9.0 (D) 214; 8.2 (BZ) 215<br>8.2 (H) 126; 8.3 (M) 261; 7.6 (AB) 226<br>8.2 (U) 252; 7.7 (BN) 252; 7.5 (BN) 222<br>10.0 (O) 143; 8.0 (BJ) 218; 8.5 (BN) 205<br>8.7 (AL) 67; (Y, BN) 164; (O, F) 196<br>(BC) 111; (BN) 238; (M) 1<br>(BE) 177; (BC) 250; (L) 239<br>(BJ) 142; (BJ) 151; (CE) 137<br>(W) 249; (B) 176; (BK, BI) 198 |
| 137 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-(D)-Glu | 7.7 (M) 264; 8.5 (U) 167; 8.5 (D) 167 |
| 138 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-NH-CO-Glu | 7.1 (BN) 227 |
| 139 | $NH_2$ | 6-H; 7-$CH_2N(CH_3)C_6H_4$-4′-CO-Glu | 5.2 (M) 261 |
| 140 | $NH_2$ | 6-H; 7-$CH_2N(CH_3)C_6H_4$-4′-CO-(D)-Glu | 6.4 (M) 261 |
| 141 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH($CONH_2$)$(CH_2)_2CO_2H$ | 5.8 (AB) 217 |
| 142 | $NH_2$ | $CH_2N(CH_3)C_6H_5$-4′-CONHCH($CO_2H$)$(CH_2)_2CONH_2$ | 7.5 (AB) 217; 11.6 (U) 219 |
| 143 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH($CO_2H$)$(CH_2)_2CONHOH$ | 8.3 (U) 252; 7.5 (BN) 252 |
| 144 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH($CO_2H$)$(CH_2)_2CONHNH_2$ | (U, BN) 256 |
| 145 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu$(NHNH_2)_2$ | 7.7 (U) 252; 6.4 (BN) 252 |
| 146 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; 7-OH | 5.4 (M) 140; 8.2 (U) 131 |
| 147 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; 7-$CH_3$ | 7.6 (M) 140; 5.2 (U) 175; 5.0 (BN) 175 |
| 148 | $NH_2$ | $CH_2N(CH_3)C_6H_2$-2′-Br-5′-Cl-4′-CO-Glu | 10.0 (U) 15 |
| 149 | $NH_2$ | $CH_2N(CH_3)C_6H_2$-3′,5′-$Cl_2$-4′-CO-Glu | 11.5 (M) 38; (U) 2 |
| 150 | $NH_2$ | $CH_2N(CH_3)C_6H_2$-2′,6′-$Cl_2$-4′-CO-Glu | 11.6 (C) 136; 11.7 (Q) 136; 11.8 (M) 136<br>9.9 (U) 175; 8.5 (BN) 175 |
| 151 | $NH_2$ | $CH_2N(CH_3)C_6H_2$-2′,6′-$Cl_2$-4′-CO-Glu; 7-$CH_3$ | 5.4 (U) 175; 5.0 (BN) 175 |
| 152 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; (7,8-dihydro) | 8.0 (BC) 231; 7.7 (BN) 205; 7.5 (U) 83 |
| 153 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; 8-$CH_3$; (7,8-dihydro) | 6.8 (BN) 205 |
| 154 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; 8-$C_2H_5$; (7,8-dihydro) | 6.0 (BN) 205 |
| 155 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; 8-*n*-$C_4H_9$; (7,8-dihydro) | 6.4 (BN) 205 |
| 156 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; 8-*n*-$C_6H_{13}$; (7,8-dihydro) | 5.7 (BN) 205 |
| 157 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; 8-$CH_2CH(CH_2CH_2)_2CH_2$; (7,8-dihydro) | 6.1 (BN) 205 |
| 158 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; 8-$CH_2C_6H_5$; (7,8-dihydro) | 6.1 (BN) 205 |
| 159 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; 8-$CH_2C_6H_3$-3′,4′-$Cl_2$; (7,8-dihydro) | 5.4 (BN) 205 |
| 160 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; 8-$CH_2$-1′-$C_{10}H_7$; (7,8-dihydro) | 5.3 (BN) 205 |
| 161 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; (5,6,7,8-tetrahydro) | 6.7 (BN) 205; 7.0 (U) 83 |
| 162 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; 8-$CH_3$; (5,6,7,8-tetrahydro) | 5.6 (BN) 205 |
| 163 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; (1-deaza) | 5.9 (BC) 231; 6.5 (U) 219; 5.3 (AB) 129 |
| 164 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; (3-deaza) | 7.2 (AB) 226; 7.5 (U) 233; 7.2 (AB) 129 |
| 165 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; (3-deaza; 3-Br) | 5.6 (AB) 226 |
| 166 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu; (3-deaza; 3-$NO_2$) | 4.4 (AB) 226 |
| 167 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-glu; (8-deaza); 8-thio | (M) 261 |
| 168 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-(D,L)-Val | 7.9 (M) 261 |
| 169 | $NH_2$ | $CH_2N(CH_3)C_6H_3$-2′-$OCH_3$-4′-CO-Asp | 6.2 (M) 261 |
| 170 | $NH_2$ | $CH_2N(CH_3)C_6H_3$-3′-$OCH_3$-4′-CO-Asp | 6.3 (M) 261 |
| 171 | $NH_2$ | $CH_2NHC_6H_4$-4′-CO-Gly-Asp | 6.0 (M) 261 |
| 172 | $NH_2$ | $CH_2NHC_6H_3$-2′-$OCH_3$-4′-CO-Glu | 6.9 (M) 261 |
| 173 | $NH_2$ | $CH_2NHC_6H_3$-3′-$OCH_3$-4′-CO-Glu | 8.7 (M) 261 |
| 174 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH($CH_2CO_2H$)$_2$ | 8.0 (U) 167; 8.1 (D) 167 |
| 175 | $NH_2$ | $CH_2N(CHO)C_6H_4$-4′-CO-Glu | 5.7 (AH) 195; 7.7 (CE) 148; (BN) 206 |
| 176 | $NH_2$ | $CH_2NHC_6H_4$-4′-$CH_2$-CO-Glu | 7.1 (M) 262; 6.4 (AB) 226 |
| 177 | $NH_2$ | $CH_2CH_2C_6H_4$-4′-CO-Glu | 8.3 (BN) 168; 11.5 (U) 233 |
| 178 | $NH_2$ | $CH_2CH_2C_6H_4$-4′-CO-Glu; (7,8-dihydro) | 8.3 (BN) 168 |
| 179 | $NH_2$ | $CH_2CH_2C_6H_4$-4′-CO-Glu; (5,6,7,8-tetrahydro) | 7.0 (BN) 168 |

**TABLE VIII** (Continued)

| no. | $R_4$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|
| 180 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CH_3)(CH_2)_2CH_3$ | 6.5 (BN) 172; 5.0 (AA) 172 |
| 181 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CH_2OH)(CH_2)_2CH_2OH$ | 6.0 (BN) 172; 4.7 (AA) 172 |
| 182 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CH_3)(CH_2)_2CH_3$; 8-N→O | (BN, AA) 172 |
| 183 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CH_2OH)(CH_2)_2CH_2OH$; 8-N→O | (BN, AA) 172 |
| 184 | $NH_2$ | $CH_2NHCH_2C_6H_4$-4′-CO-Glu | 7.0 (Q) 141 |
| 185 | $NH_2$ | $CH_2CH_2OC_6H_4$-4′-CO-Glu | 6.7 (BN) 254 |
| 186 | $NH_2$ | $CH_2CH_2SC_6H_4$-4′-CO-Glu | 6.4 (BN) 245 |
| 187 | $NH_2$ | $CH_2N(CH_3)C_6H_3$-2′-$OCH_3$-4′-CO-Glu | 7.8 (M) 219 |
| 188 | $NH_2$ | $CH_2N(CH_3)C_6H_3$-3′-$OCH_3$-4′-CO-Glu | 6.2 (M) 219 |
| 189 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Leu | 8.6 (M) 261 |
| 190 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Lys | 8.3 (U) 167; 8.5 (D) 167 |
| 191 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Gly-Asp | 7.3 (M) 261 |
| 192 | $NH_2$ | $CH_2N(C_2H_5)C_6H_4$-4′-CO-Glu | 8.3 (M) 267; 7.3 (AB) 226; 11.4 (U) 233 |
| 193 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-NCOCH$((CH_2)_2CO_2Me)$NHCO | 5.4 (BN) 227 |
| 194 | $NH_2$ | $CH_2N(C_2H_5)C_6H_2$-2′,6′-$Cl_2$-4′-CO-Glu | 7.3 (AB) 226 |
| 195 | $NH_2$ | $CH_2N(CH_3)CH_2C_6H_4$-4′-CO-Glu | 6.1 (AB) 226 |
| 196 | $NH_2$ | $CH_2NHC_6H_4$-4′-$(CH_2)_2$CO-Glu | 6.3 (AB) 226; 6.7 (M) 262 |
| 197 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_2CONHCH_3$ | 7.5 (AB) 217; 11.6 (U) 219 |
| 198 | $NH_2$ | $CH_2NHC_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_4CO_2H$ | 7.9 (AB) 226; 8.6 (M) 262 |
| 199 | $NH_2$ | $CH_2N(C_3H_7)C_6H_4$-4′-CO-Glu | 11.5 (U) 233 |
| 200 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_4NH_2$ | 6.9 (BN) 264; 8.0 (U) 264; (F, AA) 268 |
| 201 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Gly-Glu | 6.9 (M) 261 |
| 202 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH(CO-Gly)$(CH_2)_2CO_2H$ | 6.7 (U) 219 |
| 203 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_2$-CO-Gly | 11.5 (U) 219 |
| 204 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_2CON(CH_3)_2$ | 7.4 (AB) 217; 11.4 (U) 219 |
| 205 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu $(CH_3)_2$ | 8.4 (U) 153; 6.4 (U) 163; 7.0 (AK) 163 |
| 206 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-NHCO-Glu $(CH_3)_2$ | 6.5 (BN) 227 |
| 207 | $NH_2$ | $CH_2N(SO_2C_6H_4$-4′-$CH_3)CH_2C_6H_4$-4″-$CO_2H$ | (BN) 208 |
| 208 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-(D,L)-Ala-Glu | 7.6 (M) 261 |
| 209 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Sarc-Glu | 6.6 (M) 261 |
| 210 | $NH_2$ | $CH_2N(C_4H_9)C_6H_4$-4′-CO-Glu | 4.8 (AB) 226 |
| 211 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_4NHCOCH_2I$ | 7.5 (BN) 264; 8.0 (U) 264 |
| 212 | $NH_2$ | $CH_2S$-$C_6H_4$-4′-CO-Glu-$(C_2H_5)_2$ | 7.8 (BN) 190 |
| 213 | $NH_2$ | $CH_2NHC_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_2$-CO-Glu | 11.4 (U) 219 |
| 214 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Phe | 8.3 (M) 261 |
| 215 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-$(C_2H_5)_2$ | 6.2 (BN) 172; 5.3 (AA) 172; 6.9 (U) 163<br>6.9 (U) 153 |
| 216 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCOCH$(CO_2C_2H_5)(CH_2)_2CO_2H$ | 7.8 (J) 207 |
| 217 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCOCH$(CO_2H)(CH_2)_2CO_2C_2H_5$ | 7.8 (J) 207 |
| 218 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_2CO_2$-$t$-$C_4H_9$ | (U, BN) 256 |
| 219 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_2CONH$-$n$-$C_4H_9$ | (U, BN) 256 |
| 220 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2$-$t$-$C_4H_9)(CH_2)_2CONHNH_2$ | (U, BN) 256 |
| 221 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2$-$t$-$C_4H_9)(CH_2)_2CO_2H$ | (U, BN) 256 |
| 222 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-$(C_2H_5)_2$; 8-N→O | 4.7 (BN) 172; 4.1 (AA) 172 |
| 223 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH(CO-Asp)$(CH_2)_2CO_2H$ | 7.2 (AB) 217; 6.7 (U) 219 |
| 224 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_2$CO-Asp | 7.9 (AB) 217; 11.5 (U) 219 |
| 225 | $NH_2$ | $CH_2N(C_6H_5)C_6H_4$-4′-CO-Glu | 7.2 (M) 262; 6.6 (AB) 226 |
| 226 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_2CONH(CH_2)_4CH_3$ | 7.5 (AB) 217; 11.4 (U) 219 |
| 227 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2Me)(CH_2)_2CO_2$-$t$-$C_4H_9$ | (U, BN) 256 |
| 228 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2$-$t$-$C_4H_9)(CH_2)_2CO_2Me$ | (U, BN) 256 |
| 229 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH(CO-Glu)$(CH_2)_2CO_2H$ | 7.2 (AB) 217; 6.8 (U) 219 |
| 230 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_2$-CO-Glu | (U) 176; 7.9 (AB) 217; 11.4 (U) 219 |
| 231 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONH-adamantyl | 6.7 (BN) 172; 6.7 (AA) 172 |
| 232 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONH-adamantyl; 8-N→O | 4.5 (BN) 172; (AA) 172 |
| 233 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Leu-Glu | 7.4 (M) 261 |
| 234 | $NH_2$ | $CH_2N(CH_2C_6H_5)C_6H_4$-4′-CO-Glu | 5.8 (M) 262; 5.5 (U) 233 |
| 235 | $NH_2$ | $CH_2N(CH_2CH_2C_6H_5)C_6H_4$-4′-CO-Glu | 5.7 (U) 233 |
| 236 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_2CONHCH_2C_6H_5$ | 11.4 (U) 219; (U, BN) 256 |
| 237 | $NH_2$ | $CH_2N(SO_2C_6H_4$-4′-$CH_3)CH_2C_6H_4$-4″-CO-Glu | 5.2 (BN) 208 |
| 238 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Phe-Asp | 6.7 (M) 261 |
| 239 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-$(n$-$C_4H_9)_2$ | 6.9 (J) 207; 8.3 (U) 163 |
| 240 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Phe-Glu | 7.2 (M) 261 |
| 241 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_4$NH-Cbz | 7.3 (BN) 264; 8.0 (U) 264 |
| 242 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-$(n$-$C_5H_{11})_2$ | 8.2 (U) 153; 8.0 (AK) 163 |
| 243 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2CH_2C_6H_5)(CH_2)_2CONH$-$n$-$C_4H_9$ | (U, BN) 256 |
| 244 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu$[N(CH_2CH_2)_2CH_2]_2$ | 7.9 (U) 252; 6.6 (BN) 252 |
| 245 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-$(n$-$C_6H_{13})_2$ | 8.0 (U) 153 |
| 246 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-$(NHC_6H_5)_2$ | 6.0 (BN) 252; 7.1 (U) 252 |
| 247 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CONHCH$(CO_2H)(CH_2)_4NHSO_2$-naphthyl-5″-$N(Me)_2$ | (F, AA, BN) 268 |
| 248 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-$(NHCH_2C_6H_4$-4″-$Cl)_2$ | 6.7 (U) 252; 5.6 (BN) 252 |
| 249 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-$(n$-$C_8H_{17})_2$ | 7.9 (U) 153; 7.7 (AK) 163 |
| 250 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-$[N(CH_3)CH_2C_6H_5]_2$ | 7.6 (U) 252; 6.4 (BN) 252 |
| 251 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-$[NHCH(CH_3)C_6H_5]_2$ | 8.0 (U) 252; 6.5 (BN) 252 |
| 252 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-$(NHCH_2C_6H_4$-4″-$OCH_3)_2$ | 6.9 (U) 252; 6.4 (BN) 252 |
| 253 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-(NH-adamantyl)$_2$ | 5.5 (U) 252; 5.2 (BN) 252 |

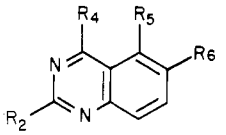
**TABLE VIII** (Continued)

| no. | $R_4$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|
| 254 | $NH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu-$[N(CH_2C_6H_5)_2]_2$ | 6.3 (U) 252; 6.1 (BN) 252 |
| 255 | $NH_2$ | 6,7-$(CH_2)_4$ | 3.7 (M) 204; 3.7 (U) 204; 4.2 (BN) 204 4.6 (AK) 204 |
| 256 | $NH_2$ | 6,7-$(CH_2)_5$ | 4.5 (M) 204; 5.0 (U) 204; 4.7 (AK) 204 (BN) 204 |
| 257 | $NH_2$ | 6,7-$(CH_2)_6$ | 5.0 (M) 204; 5.2 (U) 204; 4.7 (AK) 204 (BN) 204 |
| 258 | $NH_2$ | 6,7-$(CH_2)_8$ | 6.3 (M) 204; 6.5 (U) 204; 4.4 (BN) 204 6.0 (AK) 204 |
| 259 | $NH_2$ | 6,7-$(CH_2)_9$ | 6.6 (M) 204; 6.4 (U) 204; 4.8 (BN) 204 7.0 (AK) 204 |
| 260 | $NH_2$ | 6,7-$(CH_2)_{10}$ | 6.7 (M) 204; 6.7 (U) 204 4.6 (BN) 204 6.8 (AK) 204 |
| 261 | $NH_2$ | 6.7-$(CH_2)_{11}$ | 6.4 (M) 204; 6.5 (U) 204; 4.5 (BN) 204 6.8 (AK) 204 |
| 262 | $NH_2$ | 6,7-$(CH_2)_5$; 1′,2′-benzo | (M, BN, AK, U) 183 |
| 263 | $NH_2$ | 6,7-$(CH_2)_{13}$ | 5.5 (M) 204; 4.8 (U) 204; 4.5 (BN) 204 4.5 (AK) 204 |
| 264 | $NH_2$ | 5,6-$CH_2CH_2CH_2CH_2$; (5-deaza) | 7.9 (BC) 128; 7.8 (M) 82; (BN) 174 |
| 265 | $NH_2$ | 5,6-$CH_2CH_2CH_2CH_2$; (5-deaza); 7-$CH_3$ | (BN) 174 |
| 266 | $NH_2$ | 5,6-$CH_2CH_2CH_2CH_2$; (5-deaza); 7-$(C_2H_5)$ | (BN) 174 |
| 267 | $NH_2$ | 5,6-$CH_2CH_2CH_2CH_2$; (5-deaza); 7-$C_6H_5$ | 5.0 (BN) 174 |
| 268 | $NH_2$ | 5,6-$CH_2CH_2CH_2CH_2$; (5-deaza); 7-$C_6H_4$-4′-Cl | 6.0 (BN) 174 |
| 269 | $NH_2$ | 5,6-$CH_2CH_2CH_2CH_2$; (5-deaza); 7-$CH_2C_6H_5$ | 7.0 (BN) 274 |
| 270 | $N(CH_3)_2$ | $CH_2NHC_6H_4$-4′-CO-Glu | 5.2 (P) 16 |
| 271 | $NHNH_2$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu | (AB) 188 |
| 272 | OH | OH | −1.5 (AA) 4 |
| 273 | OH | $CH_3$ | 0.8 (AA) 4; (P) 29 |
| 274 | OH | $CH_3$; (8-oxadihydroptd) | (AB) 224 |
| 275 | OH | 6-$CH_3$; 7-$CH_3$ (8-oxadihydroptd) | (AB) 224 |
| 276 | OH | 6-$CH_3$; 7-$C_6H_5$ (8-oxadihydroptd) | (AB) 224 |
| 277 | OH | CHO | 3.5 (AA) 5 |
| 278 | OH | $(CH_2)_4CH_3$; (8-oxadihydroptd) | (AB) 224 |
| 279 | OH | $SC_6H_5$ | (M, BN, U) 183 |
| 280 | OH | $C_6H_5$; (8-oxadihydroptd) | (AB) 224 |
| 281 | OH | 6-$C_6H_5$; 7-$CH_3$ (8-oxadihydroptd) | (AB) 224 |
| 282 | OH | 6-$C_6H_5$; 7-$C_6H_5$ (8-oxadihydroptd) | (AB) 224 |
| 283 | OH | $SC_6H_4$-4′-$CH_3$ | (M, BN, U) 183 |
| 284 | OH | $SCH_2C_6H_5$ | (M, BN, U) 183 |
| 285 | OH | $CH_2C_6H_5$; (8-oxadihydroptd) | (AB) 224 |
| 286 | OH | $(CH_2)_2C_6H_5$ | (AB) 20 |
| 287 | OH | $CH_2NHC_6H_4$-4′-$CO_2H$ | 5.1 (AB) 36; 5.7 (C) 136; 5.1 (P) 29 39 (AB) 134; 6.4 (M) 136; 6.4 (Q) 136 |
| 288 | OH | $CH_2SC_6H_4$-4′-$CO_2H$ | (BN) 184 |
| 289 | OH | $(CH_2)_2C_6H_5$; (8-oxadihydroptd) | (AB) 224 |
| 290 | OH | $(CH_2)_3C_6H_5$; (8-oxadihydroptd) | (AB) 224 |
| 291 | OH | $CH_2CH_2C_6H_4$-4′-$CO_2H$ | 4.0 (AB) 134 |
| 292 | OH | CH=$CHC_6H_4$-4′-$CO_2H$ | 4.0 (AB) 134 |
| 293 | OH | $(CH_2)_4C_6H_5$; (8-oxadihydroptd) | (AB) 224 |
| 294 | OH | $CH_2NHC_6H_4$-4′-CO-Glu | 7.6 (C) 136; 7.7 (Q) 136; 7.6 (M) 136 7.6 (D) 136; 7.0 (P) 29; 4.7 (AB) 134 5.7 (U) 219; 6.9 (BJ) 212; 4.4 (BE) 147 5.5 (AB) 22; (BI, BK) 198; (BN) 161 |
| 295 | OH | $CH_2NHC_6H_4$-4′-CO-D-Glu; 7,8-dihydro | (BN) 206 |
| 296 | OH | $CH_2NHC_6H_4$-4′-CO-Glu; (8-diaza) | 5.9 (U) 219 |
| 297 | OH | $CH_2NHC_6H_4$-4′-CO-Glu; 5-$CH_3$ (5,6,7,8-tetrahydro) | 5.6 (U) 219; 5.0 (D) 267 |
| 298 | OH | $CH_2NHC_6H_4$-4′-Glu; 5-CHO (5,6,7,8-tetrahydro) | 5.7 (U) 219; 3.9 (H) 150 |
| 299 | OH | $CH_2NHC_6H_4$-4′-CONHCH($CO_2H$)$CH_2$CH(OH)$CO_2H$ (threo) | (AG) 152; (M) 152 |
| 300 | OH | $CH_2NHC_6H_4$-4′-CONHCH($CO_2H$)$CH_2$CH(OH)$CO_2H$ (erythro) | (AG) 152; (M) 152 |
| 301 | OH | $CH_2NHC_6H_4$-4′-CONHCH($CO_2H$)$CH_2CHFCO_2H$ | (AG) 152; (M) 152 |
| 302 | OH | $CH_2NHC_6H_2$-3′,5′-$Cl_2$-4′-CO-Glu | 7.3 (P) 29 |
| 303 | OH | $CH_2SC_6H_4$-4′-CO-Glu | 4.3 (BN) 190 |
| 304 | OH | 5,10-methynyltetrahydropteroyl glutamate | 4.6 (H) 150 |
| 305 | OH | 5,10-methylenetetrahydropteroyl glutamate | 4.9 (H) 150 |
| 306 | OH | *l*-5,10-methylenetetrahydropteroyl D-glutamate | (BN) 206 |
| 307 | OH | *d*-5,10-methylenetetrahydropteroyl D-glutamate | (BN) 206 |
| 308 | OH | $CH_2CH_2C_6H_4$-4′-CO-Glu | 5.2 (AB) 134 |
| 309 | OH | CH=$CHC_6H_4$-4′-CO-Glu | 4.1 (AB) 134 |
| 310 | OH | $CH_2CH_2SC_6H_4$-4′-CO-Glu | (BN) 225 |
| 311 | OH | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu | 7.9 (D) 267; (AB) 168 |
| 312 | OH | $CH_2N(CH_3)C_6H_2$-3′,5′-$Cl_2$-4′-CO-Glu; 7-OH | (U) 2 |
| 313 | OH | $CH_2N(CHO)C_6H_4$-4′-CO-Glu | 8.2 (AB) 36; 8.2 (P) 29; 7.1 (M) 162 9.0 (D) 267 |
| 314 | OH | $CH_2N(CHO)C_6H_4$-4′-CO-Glu; (5,6,7,8-tetrahydro) | 4.6 (H) 150 |
| 315 | OH | $CH_2NHCH_2C_6H_4$-4′-CO-Glu | 3.2 (Q) 141 |
| 316 | OH | $CH_2NHC_6H_4$-4′-$CH_2$-CO-Glu | (AB) 157 |
| 317 | OH | $CH_2CH_2NHC_6H_2$-2′,6′-$Cl_2$-4′-CO-Glu | 4.1 (U) 167 |

**TABLE VIII** (Continued)

| no. | $R_4$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|
| 318 | OH | $CH_2NHC_6H_4$-4′-$(CH_2)_2$-CO-Glu | (AB) 157 |
| 319 | OH | $CH_2N(SO_2C_6H_4$-4′-$CH_3)CH_2C_6H_4$-4″-$CO_2H$ | (BN) 208 |
| 320 | OH | $CH_2N(SO_2C_6H_4$-4′-$CH_3)CH_2C_6H_4$-4″-$CO_2CH_3$ | (BN) 208 |
| 321 | OH | $CH_2SC_6H_4$-4′-CO-Glu-$(C_2H_5)_2$ | (BN) 184 |
| 322 | OH | $CH_2N(SO_2C_6H_4$-4′-$CH_3)CH_2C_6H_4$-4″-CO-Glu | (BN) 208 |
| 323 | OH | $CH_2NHC_6H_4$-4′-CO-$(Glu)_5$ | 7.3 (D) 267 |
| 324 | OH | $CH_2N(CHO)C_6H_4$-4′-CO-$(Glu)_5$ | (U, BJ) 178 |
| 325 | $CH_3$ | $NH_2$ | 3.7 (AA) 4 |
| 326 | $CH_3$ | 6-$C_6H_5$; 7-$NH_2$ | 4.4 (K) 45 |
| 327 | SH | $CH_2N(CH_3)C_6H_4$-4′-$CO_2H$ | (AB) 188 |
| 328 | SH | $CH_2N(CH_3)C_6H_4$-4′-$CO_2CH_3$ | (AB) 188 |
| 329 | SH | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu | (AB) 188 |
| 330 | $SCH_3$ | $CH_2N(CH_3)C_6H_4$-4′-$CO_2CH_3$ | (AB) 188 |
| 331 | $SCH_3$ | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu | (AB) 188 |
| 332 | $SCH_2C_6H_5$ | $CH_2N(CH_3)C_6H_4$-4′-$CO_2CH_3$ | (AB) 188 |
| 333 | $SCH_2C_6H_5$ | $CH_2N(CH_3)C_6H_4$-4′-$CO_2CH_3$; (7,8-dihydro) | (AB) 188 |

**TABLE IX. Inhibition by Substituted Quinazolines**




| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| 1 | H | H | H | H | 2.2 (M) 156 |
| 2 | H | $NH_2$ | H | H | 3.1 (M) 156 |
| 3 | H | $NH_2$ | H | S-2′-$C_{10}H_7$ | 4.7 (M) 171; 4.1 (BC) 171 |
| 4 | H | $NH_2$ | H | SO-2′-$C_{10}H_7$ | 4.8 (M) 171; 5.6 (BC) 171 |
| 5 | H | $NH_2$ | H | $SO_2$-2′-$C_{10}H_7$ | 5.5 (M) 171; 3.5 (BC) 171 |
| 6 | H | $NH_2$ | $SC_6H_3$-3′,4′-$Cl_2$ | H | (M) 160 |
| 7 | H | OH | H | H | 3.6 (M) 156 |
| 8 | H | SH | H | H | (M) 156 |
| 9 | $NH_2$ | $NH_2$ | H | H | 4.7 (M) 156 |
| 10 | $NH_2$ | $NH_2$ | H | H (5,6,7,8-tetrahydro) | 5.1 (M) 156 |
| 11 | $NH_2$ | $NH_2$ | H | Br | 5.6 (M) 156 |
| 12 | $NH_2$ | $NH_2$ | H | Cl | 5.4 (M) 156 |
| 13 | $NH_2$ | $NH_2$ | H | $NH_2$ | 4.6 (M) 156 |
| 14 | $NH_2$ | $NH_2$ | H | $CH_3$ | 5.7 (M) 156 |
| 15 | $NH_2$ | $NH_2$ | H | $CH_3$ (5,6,7,8-tetrahydro) | 5.7 (M) 156 |
| 16 | $NH_2$ | $NH_2$ | H | CN | 4.7 (M) 156 |
| 17 | $NH_2$ | $NH_2$ | H | $CH_2NH_2$ | 5.0 (M) 156 |
| 18 | $NH_2$ | $NH_2$ | H | CHO | 5.0 (M) 156 |
| 19 | $NH_2$ | $NH_2$ | H | $C_2H_5$ | 7.2 (BJ) 34; 7.8 (AZ) 34<br>7.6 (BG) 34 |
| 20 | $NH_2$ | $NH_2$ | H | pyrrol-1′-yl | 5.4 (M) 170; 5.5 (U) 170 |
| 21 | $NH_2$ | $NH_2$ | H | N-$(CH_2CH_2)_2CH_2$ | 5.8 (M) 170; 5.6 (U) 170 |
| 22 | $NH_2$ | $NH_2$ | H | 3′,5′-dimethylpyrazol-1′-yl | 5.2 (M) 170; 5.2 (U) 170 |
| 23 | $NH_2$ | $NH_2$ | H | furfurylamino | 5.5 (M) 170; 5.9 (U) 170 |
| 24 | $NH_2$ | $NH_2$ | H | 2′-thienylamino | 6.2 (M) 170; 5.3 (U) 170 |
| 25 | $NH_2$ | $NH_2$ | H | (5′-chloro-2′-thienyl)amino | 6.3 (M) 170; 6.0 (U) 170 |
| 26 | $NH_2$ | $NH_2$ | H | 6-$NHC_6H_5$; 7-$CH_3$ | 6.4 (M) 170; 6.0 (U) 170 |
| 27 | $NH_2$ | $NH_2$ | H | 2′,5′-dimethylpyrrol-1′-yl | 5.5 (M) 170; 5.7 (U) 170 |
| 28 | $NH_2$ | $NH_2$ | H | 6,8-bis(2′,5′-dimethylpyrrol-1′-yl) | 5.0 (M) 170<br>6.0 (U) 170 |
| 29 | $NH_2$ | $NH_2$ | H | 2′-pyridylmethylamino | 5.6 (M) 170; 5.3 (U) 170 |
| 30 | $NH_2$ | $NH_2$ | H | 4′-pyridylmethylamino | 5.7 (M) 170; 6.2 (U) 170 |
| 31 | $NH_2$ | $NH_2$ | H | $SC_6H_3$-3′,4′-$Cl_2$ | 8.0 (M) 171; 8.2 (BC) 171 |
| 32 | $NH_2$ | $NH_2$ | H | $SO_2C_6H_3$-3′,4′-$Cl_2$ | 8.0 (M) 171; 9.2 (BC) 171 |
| 33 | $NH_2$ | $NH_2$ | H | $SC_6H_4$-3′-$CF_3$ | 8.4 (AP) 258; 6.6 (M) 258 |
| 34 | $NH_2$ | $NH_2$ | H | (2′,5′-dimethylpyrrol-1′-yl)methyl | 6.6 (M) 170<br>6.9 (U) 170 |
| 35 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-3′-Br | (M, BN, AK) 183; 8.7 (D) 223<br>8.6 (U) 223 |
| 36 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-2′-Cl | (M, BN, AK, U) 183 |
| 37 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-3′-Cl | (M, BN, AK) 183; 8.0 (D) 223<br>8.0 (U) 223 |
| 38 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-4′-Cl | 8.3 (U) 223; 8.2 (D) 223 |
| 39 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_3$-3′,4′-$Cl_2$ | 8.0 (U) 223; 8.4 (D) 223 |
| 40 | $NH_2$ | $NH_2$ | H | $CH_2N(NO)C_6H_3$-3′,4′-$Cl_2$ | 8.0 (U) 223; 8.0 (D) 223 |
| 41 | $NH_2$ | $NH_2$ | H | $N(NO)CH_2C_6H_3$-3′,4′-$Cl_2$ | 6.7 (AN) 144; 6.4 (AU) 144<br>6.7 (BV) 144; 7.0 (BX) 144<br>6.0 (BW) 144; 6.0 (BY) 144<br>6.2 (M) 144 |
| 42 | $NH_2$ | $NH_2$ | H | 2-ethylpiperidino | 7.4 (M) 170; 7.4 (U) 170 |

**TABLE IX** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| 43 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_5$ | 5.8 (M) 170; 6.0 (U) 170 (BN, AK) 183 |
| 44 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-3′-Br | 6.2 (M) 170; 6.2 (U) 170 |
| 45 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-4′-Br | 5.7 (M) 170; 5.8 (U) 170 |
| 46 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-4′-Cl | 5.7 (M) 170; 5.9 (U) 170 |
| 47 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_3$-3′,4′-$Cl_2$ | 6.1 (M) 170; 6.3 (U) 170 5.7 (AN) 144; 5.3 (AU) 144 5.7 (BV) 144; 5.7 (BX) 144 5.2 (BW) 144; 6.0 (BY) 144; (BN, AK) 183 |
| 48 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-4′-F | 6.0 (M) 170; 5.8 (U) 170 |
| 49 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-2′-$NH_2$ | 5.6 (M) 170; 6.0 (U) 170 |
| 50 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-2′-$NO_2$ | 6.5 (M) 170; 6.5 (U) 170 |
| 51 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-2′-OH | 5.7 (M) 170; 5.8 (U) 170 |
| 52 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-3′-OH | 5.5 (M) 170; 5.5 (U) 170 |
| 53 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-4′-OH | 5.0 (M) 170; 5.0 (U) 170 |
| 54 | $NH_2$ | $NH_2$ | H | $NHCOC_6H_4$-4′-Cl | (M) 186 |
| 55 | $NH_2$ | $NH_2$ | H | $NHCOC_6H_3$-3′,4′-$Cl_2$ | (M) 186 |
| 56 | $NH_2$ | $NH_2$ | H | S-$C_6H_4$-3′-$CF_3$ | 7.4 (M) 171; 9.1 (BC) 171 |
| 57 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_3$-3′-$CF_3$-4′-Cl | 8.4 (D) 214 |
| 58 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-4′-COOH | 7.7 (M) 191; 8.8 (BC) 191 |
| 59 | $NH_2$ | $NH_2$ | H | $CH_2N(CH_3)C_6H_3$-3′,4′-$Cl_2$ | 8.3 (D) 214 |
| 60 | $NH_2$ | $NH_2$ | H | $CH_2NHCOC_6H_3$-3′,4′-$Cl_2$ | 7.0 (M) 186 |
| 61 | $NH_2$ | $NH_2$ | H | $CH_2N(CH_3)C_6H_4$-4′-F | 8.5 (D) 214 |
| 62 | $NH_2$ | $NH_2$ | H | 2′-isoindolinyl | (M) 170; 4.6 (U) 170 |
| 63 | $NH_2$ | $NH_2$ | H | $NHCH_2CH_2C_6H_4$-4′-Cl | 6.7 (M) 170; 6.0 (U) 170 |
| 64 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-2′-$CH_3$ | 5.9 (M) 170; 5.8 (U) 170 |
| 65 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-3′-$CH_3$ | 5.6 (M) 170; 5.3 (U) 170 |
| 66 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-4′-$CH_3$ | 5.3 (M) 170; 5.5 (U) 170 |
| 67 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-4′-$CH_2OH$ | 5.0 (M) 170; 5.0 (U) 170 |
| 68 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-4′-COOH | 4.9 (M) 170; 5.3 (U) 170 |
| 69 | $NH_2$ | $NH_2$ | H | $NHCH(CH_3)C_6H_5$ | 6.2 (M) 170; 6.2 (U) 170 |
| 70 | $NH_2$ | $NH_2$ | H | $NHCH(CH_3)C_6H_4$-4′-Cl | 6.2 (M) 170; 6.0 (U) 170 |
| 71 | $NH_2$ | $NH_2$ | H | $N(CH_3)CH_2C_6H_3$-3′,4′-$Cl_2$ | 8.0 (M) 170; 7.8 (U) 170 |
| 72 | $NH_2$ | $NH_2$ | H | $NHCOCH_2C_6H_4$-4′-Cl | 7.6 (M) 186 |
| 73 | $NH_2$ | $NH_2$ | H | $NHCOCH_2C_6H_3$-3′,4′-$Cl_2$ | 7.5 (M) 186 |
| 74 | $NH_2$ | $NH_2$ | H | *N*-phthalimide | (M, U) 170 |
| 75 | $NH_2$ | $NH_2$ | H | 1′,2′,3′,4′-tetrahydro-2′-isoquinolyl | 6.5 (M) 170; 6.5 (U) 170 |
| 76 | $NH_2$ | $NH_2$ | H | $CH_2N(CH_3)C_6H_3$-2′-Cl,4′-$CH_3$ | 7.2 (D) 214 |
| 77 | $NH_2$ | $NH_2$ | H | $CH_2NHCOCH_2C_6H_3$-3′,4′-$Cl_2$ | 5.8 (M) 186 |
| 78 | $NH_2$ | $NH_2$ | H | $CH_2NHCOC_6H_4$-3′-$CF_3$ | 6.6 (M) 186 |
| 79 | $NH_2$ | $NH_2$ | H | $CH_2N(COCH_3)C_6H_3$-3′,4′-$Cl_2$ | 7.3 (D) 223; 8.0 (BN) 214 |
| 80 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-4′-$N(CH_3)_2$ | (M, U) 170 |
| 81 | $NH_2$ | $NH_2$ | H | $NHCO(CH_2)_2C_6H_4$-4′-Cl | 7.3 (M) 186 |
| 82 | $NH_2$ | $NH_2$ | H | $NHCOCH_2C_6H_4$-3′-$CF_3$ | 7.0 (M) 186 |
| 83 | $NH_2$ | $NH_2$ | H | 2′-phenyl-1′-pyrrolidinyl | 8.0 (M) 170; 7.8 (U) 170 |
| 84 | $NH_2$ | $NH_2$ | H | 2′-(*p*-chlorophenyl)-1′-pyrrolidinyl | 7.8 (M) 170 7.9 (U) 170 |
| 85 | $NH_2$ | $NH_2$ | H | 2′-(3″,4″-dichlorophenyl)-1′-pyrrolidinyl | 7.7 (M) 170 8.1 (U) 170 |
| 86 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-4′-$COOC_2H_5$ | 8.2 (M) 191; 7.7 (BC) 191 |
| 87 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_2$-3′,4′,5′-$(OCH_3)_3$ | (M, BN, AK, U) 183; 8.2 (D) 214 |
| 88 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-4′-$COOC_2H_5$ | 5.6 (M) 170; 5.3 (U) 170 7.3 (BC) 191 |
| 89 | $NH_2$ | $NH_2$ | H | S-2′-$C_{10}H_7$ | 8.1 (M) 171; 8.1 (BC) 171 |
| 90 | $NH_2$ | $NH_2$ | H | SO-2′-$C_{10}H_7$ | 8.1 (M) 171; 8.7 (BC) 171 |
| 91 | $NH_2$ | $NH_2$ | H | $SO_2$-2′-$C_{10}H_7$ | 7.3 (M) 258; 8.4 (M) 171 8.4 (B) 171; 9.0 (AP) 258 |
| 92 | $NH_2$ | $NH_2$ | H | $N(CH_2CH_2)_2CH$-$C_6H_5$ | 5.5 (M) 170; 5.5 (U) 170 |
| 93 | $NH_2$ | $NH_2$ | H | 2-methyl-3-phenyl-1-pyrrolidinyl | 5.7 (M) 170 6.0 (U) 170 |
| 94 | $NH_2$ | $NH_2$ | H | $NHCH_2$-2′-$C_{10}H_7$ | 6.1 (M) 170; 6.1 (U) 170 |
| 95 | $NH_2$ | $NH_2$ | H | $NHCH_2$-1′-$C_{10}H_6$-2′-Cl | 7.0 (M) 170; 7.0 (U) 170 |
| 96 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-4′-$CONHCH(CO_2H)_2$ | 8.8 (U) 223; 9.1 (D) 223 |
| 97 | $NH_2$ | $NH_2$ | H | $CH_2S$-2′-$C_{10}H_7$ | 8.0 (M) 160; 7.6 (BC) 171 |
| 98 | $NH_2$ | $NH_2$ | H | $CH_2NHCO$-2′-$C_{10}H_7$ | 7.0 (M) 186 |
| 99 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-4′-$COOC_4H_9$ | 7.6 (M) 191; 7.5 (BC) 191 |
| 100 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-4′-CO-Asp | 8.8 (M) 203; 8.2 (BC) 203 10.9 (U) 219; 8.8 (U) 223 8.9 (D) 223 |
| 101 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_3$-2′-Cl-4′-CO-Asp | 9.1 (U) 223; 8.8 (D) 223 |
| 102 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_3$-2′-Cl-5′-CO-Asp | 8.5 (U) 223; 8.5 (D) 223 |
| 103 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_3$-4′-Cl-3′-CO-Asp | 7.9 (U) 223; 8.4 (D) 223 |
| 104 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-4′-CO-Asp | 7.4 (U) 223 |
| 105 | $NH_2$ | $NH_2$ | H | $NHCOCH_2$-2′-$C_{10}H_7$ | 8.3 (M) 186 |
| 106 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-4′-$CONHCH(CH_2CO_2H)_2$ | 8.1 (U) 223 8.2 (D) 223 |

**TABLE IX** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| 107 | $NH_2$ | $NH_2$ | H | $CH_2N(CH_3)C_6H_4$-4′-CO-Asp | 8.7 (U) 223; 8.7 (D) 223 (BE) 177 |
| 108 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H$-4′-CO-Glu | 8.5 (M) 203; 8.8 (BC) 203 9.2 (D) 223; 8.2 (M) 230 12.2 (U) 219; (BE) 177 |
| 109 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-3′-CO-Glu | 7.4 (D) 114 |
| 110 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-4′-CO-(D)-Glu | 10.5 (U) 219; 8.7 (D) 114 |
| 111 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-4′-CO-(D)-Asp | 10.4 (U) 219 |
| 112 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-4′-$CONHCH(CO_2H)(CH_2)_2CONH_2$ | 10.0 (D) 223 9.6 (U) 223 |
| 113 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_3$-4′-Cl-3′-CO-Glu | 8.0 (U) 223; 6.7 (D) 223 |
| 114 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-4′-CO-Glu | 7.5 (BC) 203; 7.6 (M) 189 6.8 (D) 223 |
| 115 | $NH_2$ | $NH_2$ | H | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu | 8.8 (U) 223; 8.4 (D) 223 7.8 (AB) 112; 9.5 (BD) 112 (Y, BN) 164 |
| 116 | $NH_2$ | $NH_2$ | H | $CH_2N(CHO)C_6H_4$-4′-CO-Glu | 9.0 (BC) 203; 8.8 (M) 203 8.2 (D) 223 |
| 117 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-4′-CO-$Asp(C_2H_5)_2$ | 7.8 (BC) 203; 7.5 (M) 203 |
| 118 | $NH_2$ | $NH_2$ | H | $N{=}CHC_6H_4$-4′-CH=N-6″-quinazoline-2″,4″-$(NH_2)_2$ | 4.3 (M) 170; 4.8 (U) 170 |
| 119 | $NH_2$ | $NH_2$ | H | $CH_2NHC_6H_4$-4′-CO-$Glu(C_2H_5)_2$ | 7.7 (BC) 203; 7.3 (M) 203 |
| 120 | $NH_2$ | $NH_2$ | H | $NHCH_2C_6H_4$-4′-CO-$Glu(C_2H_5)_2$ | 7.5 (BC) 189; 7.5 (M) 189 7.2 (D) 223 |
| 121 | $NH_2$ | $NH_2$ | H | $OC_6H_4CH_2NHC_6H_4$-4′-CO-Asp | 7.7 (U) 223; 7.8 (D) 223 |
| 122 | $NH_2$ | $NH_2$ | H | $OC_6H_4CH_2NHC_6H_4$-4′-CO-Glu | 7.7 (U) 223; 7.8 (D) 223 |
| 123 | $NH_2$ | $NH_2$ | H | $N{=}CH{-}C_6H_4$-4′-O-$(CH_2)_2OC_6H_4$-CH=N-6‴-quinazoline-2‴,4‴-$(NH_2)_2$ | 5.0 (M) 170; 4.9 (U) 170 |
| 124 | $NH_2$ | $NH_2$ | Cl | H | 6.2 (M) 156 |
| 125 | $NH_2$ | $NH_2$ | Cl | Br | 7.0 (M) 156 |
| 126 | $NH_2$ | $NH_2$ | Cl | Cl | 6.7 (M) 156 |
| 127 | $NH_2$ | $NH_2$ | Cl | $NH_2$ | 6.2 (M) 156 |
| 128 | $NH_2$ | $NH_i$ | Cl | $NHCH_2C_6H_5$ | 7.4 (M) 170; 7.4 (U) 170 (BN, AK) 183 |
| 129 | $NH_2$ | $NH_2$ | Cl | $NHCH_2C_6H_3$-3′,4′-$Cl_2$ | 7.9 (M) 170; 8.2 (U) 170 |
| 130 | $NH_2$ | $NH_2$ | Cl | $CH_2NHC_6H_4$-3′-Br | 8.4 (D) 214 |
| 131 | $NH_2$ | $NH_2$ | Cl | $CH_2NHC_6H_3$-3′,4′-$Cl_2$ | 8.7 (U) 183; 9.0 (D) 183 (M, AK, BN) 214 |
| 132 | $NH_2$ | $NH_2$ | Cl | $NHCOCH_2C_6H_4$-3′-Br | 8.1 (M) 186 |
| 133 | $NH_2$ | $NH_2$ | Cl | $NHCOCH_2C_6H_4$-4′-Br | 7.9 (M) 186 |
| 134 | $NH_2$ | $NH_2$ | Cl | $NHCOCH_2C_6H_4$-4′-Cl | 7.9 (M) 186 |
| 135 | $NH_2$ | $NH_2$ | Cl | $NHCOCH_2C_6H_3$-3′,4′-$Cl_2$ | 7.6 (M) 186 |
| 136 | $NH_2$ | $NH_2$ | Cl | $NHCOCH_2OC_6H_3$-3′,4′-$Cl_2$ | 7.6 (M) 186 |
| 137 | $NH_2$ | $NH_2$ | Cl | $CH_2NHC_6H_4$-4′-COOH | 8.2 (M) 191; 8.3 (BC) 191 |
| 138 | $NH_2$ | $NH_2$ | Cl | $NHCOCH(CH_3)C_6H_5$ | 7.8 (M) 186 |
| 139 | $NH_2$ | $NH_2$ | Cl | $NHCOCH_2C_6H_4$-3′-$CF_3$ | 7.6 (M) 186 |
| 140 | $NH_2$ | $NH_2$ | Cl | $NHCO(CH_2)_2C_6H_4$-4′-Cl | 7.4 (M) 186 |
| 141 | $NH_2$ | $NH_2$ | Cl | $NHCH_2C_6H_4$-4′-$COOC_2H_5$ | 7.9 (M) 191; 8.2 (BC) 191 |
| 142 | $NH_2$ | $NH_2$ | Cl | $CH_2NHC_6H_4$-4′-$COOC_2H_5$ | 7.9 (M) 191; 8.6 (BC) 191 |
| 143 | $NH_2$ | $NH_2$ | Cl | $CH_2NHC_6H_4$-4′-$COOC_4H_9$ | 7.9 (M) 191; 7.7 (BC) 191 |
| 144 | $NH_2$ | $NH_2$ | Cl | $CH_2NHC_6H_4$-4′-CO-Asp | 8.6 (BC) 203; 8.2(M) 203 9.4 (D) 223; 9.2 (D) 114 9.2(D) 144; 9.4 (AN) 144 10.0 (AU) 144; 9.5 (BV) 144 9.0 (BX) 144; 8.4 (BW) 144 9.4 (BY) 144; 9.2 (U) 223 |
| 145 | $NH_2$ | $NH_2$ | Cl | $NHCOCH_2$-2′-$C_{10}H_7$ | 8.3 (M) 186 |
| 146 | $NH_2$ | $NH_2$ | Cl | $NHCOCH_2O$-2′-$C_{10}H_7$ | 7.9 (M) 186 |
| 147 | $NH_2$ | $NH_2$ | Cl | $CH_2NHC_6H_4$-4′-CO-Glu | 8.2 (M) 230; 8.1 (M) 203 9.2 (BC) 223; 9.0 (U) 223 9.1 (D) 223; 13.0 (U) 219 |
| 148 | $NH_2$ | $NH_2$ | Cl | $CH_2NHC_6H_4$-4′-CO-$Asp(C_2H_5)_2$ | 7.9 (BC) 203; 8.4 (M) 203 |
| 149 | $NH_2$ | $NH_2$ | Cl | $CH_2NHC_6H_4$-4′-CO-$Glu(C_2H_5)_2$ | 8.8 (BC) 203; 7.9 (M) 203 |
| 150 | $NH_2$ | $NH_2$ | $CH_3$ | H | 6.1 (M) 156; 5.8 (BP) 216 |
| 151 | $NH_2$ | $NH_2$ | $CH_3$ | 6-H; 8-$NHCH_2C_6H_5$ | (M, U) 170 |
| 152 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_3$ | 8.4 (BJ) 34; 6.9 (AZ) 34 8.7 (BG) 34; 6.3 (BP) 216 |
| 153 | $NH_2$ | $NH_2$ | $CH_3$ | $n$-$C_3H_7$ | 8.7 (BP) 216 |
| 154 | $NH_2$ | $NH_2$ | $CH_3$ | $n$-$C_4H_9$ | 9.5 (BP) 216 |
| 155 | $NH_2$ | $NH_2$ | $CH_3$ | $n$-$C_5H_{11}$ | 13.7 (BP) 216 |
| 156 | $NH_2$ | $NH_2$ | $CH_3$ | $i$-$C_5H_{11}$ | (BP) 216 |
| 157 | $NH_2$ | $NH_2$ | $CH_3$ | $n$-$C_6H_{13}$ | 12.7 (BP) 216 |
| 158 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2NHC_6H_4$-3′-Br | 8.8 (U) 223; 8.8 (D) 223 (M, N, AK) 183 |
| 159 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2NHC_6H_3$-3′,4′-$Cl_2$ | 8.6 (D) 223; 8.5 (U) 214 |
| 160 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2N(NO)C_6H_3$-3′,4′-$Cl_2$ | 8.0 (D) 223 |
| 161 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCH_2C_6H_3$-3′,4′-$Cl_2$ | 7.0 (M) 170; 7.2 (U) 170 |

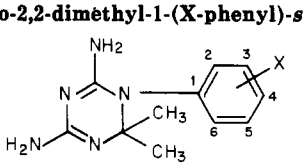
**TABLE IX** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| | | | | | (AK, BN) 183 |
| 162 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCOCH_2C_6H_4$-3′-Br | 7.5 (M) 186 |
| 163 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCOCH_2C_6H_4$-4′-Br | 7.5 (M) 186 |
| 164 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCOCH_2C_6H_4$-4′-Cl | 7.5 (M) 186 |
| 165 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCOCH_2C_6H_3$-3′,4′-$Cl_2$ | 7.8 (M) 186 |
| 166 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2NHC_6H_4$-4′-COOH | 8.1 (M) 191; 8.0 (BC) 191 |
| 167 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCOCH_2C_6H_4$-3′-$CF_3$ | 7.2 (M) 186 |
| 168 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCO(CH_2)_2C_6H_4$-4′-Cl | 7.5 (M) 186 |
| 169 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCH_2C_6H_4$-4′-$COOC_2H_5$ | 8.3 (M) 191; 8.8 (BC) 191 |
| 170 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2NHC_6H_4$-4′-$COOC_2H_5$ | 8.2 (M) 191; 8.3 (BC) 191 |
| 171 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2NHC_6H_2$-3′,4′,5′-$(OCH_3)_3$ | 8.9 (D) 214; 8.9 (U) 214 |
| | | | | | 8.1 (U) 199; (W) 249 |
| | | | | | (M, AK, BN) 183 |
| 172 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2NHC_6H_4$-4′-$COOC_4H_9$ | 7.8 (M) 191; 8.1 (BC) 191 |
| 173 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2NHC_6H_4$-4′-CO-Asp | 9.4 (M) 203; 8.6 (BC) 203 |
| | | | | | 8.9 (U) 223; 8.9 (D) 223 |
| | | | | | (BE) 177; (Y, BN) 164; (BC) 111 |
| 174 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCOCH_2$-2′-$C_{10}H_7$ | 8.4 (M) 186 |
| 175 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCOCH_2O$-2′-$C_{10}H_7$ | 7.8 (M) 186 |
| 176 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCOCH_2S$-2′-$C_{10}H_7$ | 8.3 (M) 186 |
| 177 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCH_2C_6H_4$-4′-CO-Glu | 8.2 (M) 230; 8.1 (BC) 203 |
| | | | | | 9.1 (D) 223 |
| 178 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2NHC_6H_4$-4′-CO-Glu | 8.2 (M) 230; 8.2 (M) 203 |
| | | | | | 12.4 (U) 219; 8.7 (BC) 203 |
| | | | | | 8.8 (D) 223 |
| 179 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2NHC_6H_3$-4′-Cl-3′-CO-Glu | 8.7 (U) 223; 8.5 (D) 223 |
| 180 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2NHC_6H_4$-4′-$CONHCH(CH_2CO_2H)_2$ | 9.2 (U) 223; 8.2 (D) 223 |
| 181 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCOCH[CH(CH_3)_2]S$-2′-$C_{10}H_7$ | 7.7 (M) 186 |
| 182 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2NHC_6H_4$-4′-CO-Asp$(C_2H_5)_2$ | 7.8 (BC) 202 |
| 183 | $NH_2$ | $NH_2$ | $CH_3$ | $CH_2NHC_6H_4$-4′-CO-Glu$(C_2H_5)_2$ | 7.7 (M) 189; 8.6 (BC) 203 |
| | | | | | 8.5 (D) 223 |
| 184 | $NH_2$ | $NH_2$ | $CH_3$ | $NHCH_2C_6H_4$-4′-CO-Glu$(C_2H_5)_2$ | 7.7 (M) 203; 8.4 (BC) 203 |
| 185 | $NH_2$ | $NH_2$ | S-$C_6H_3$-3′,4′-$Cl_2$ | H | 6.8 (M) 160; 6.5 (BC) 171 |
| 186 | $NH_2$ | $NH_2$ | $SOC_6H_3$-3′,4′-$Cl_2$ | H | 4.3 (M) 160; 4.6 (BC) 171 |
| 187 | $NH_2$ | $NH_2$ | $SO_2C_6H_3$-3′,4′-$Cl_2$ | H | 4.2 (M) 160; 5.8 (BC) 171 |
| 188 | $NH_2$ | $NH_2$ | $CH_2SC_6H_4$-4′-Cl | H | 7.7 (M) 160; 7.2 (BC) 171 |
| 189 | $NH_2$ | $NH_2$ | S-2′-$C_{10}H_7$ | H | 6.9 (M) 160; 7.2 (BC) 171 |
| 190 | $NH_2$ | $NH_2$ | SO-2′-$C_{10}H_7$ | H | 4.3 (M) 160; 4.4 (BC) 171 |
| 191 | $NH_2$ | $NH_2$ | $SO_2$-2′-$C_{10}H_7$ | H | 4.8 (M) 160; 3.0 (BC) 171 |
| 192 | $NH_2$ | $NH_2$ | *trans*-CH=CH-2′-$C_{10}H_7$ | H | 5.3 (M) 160; 7.1 (BC) 171 |
| 193 | $NH_2$ | $NH_2$ | *cis*-CH=CH-2′-$C_{10}H_7$ | H | 6.5 (M) 160; 7.4 (BC) 171 |
| 194 | $NH_2$ | $NH_2$ | $CH_2CH_2$-2′-$C_{10}H_7$ | H | 7.7 (M) 160; 8.0 (BC) 171 |
| 195 | $NH_2$ | $NH_2$ | 5,6-CH=CH—NH | | 6.0 (M) 215; 6.4 (BN) 215 |
| | | | | | 5.4 (U) 215; 5.8 (BZ) 215 |
| 196 | $NH_2$ | $NH_2$ | 5,6-CH=CH—$N(CH_3)$ | | 5.8 (M) 215; 5.7 (BN) 215 |
| | | | | | 5.5 (U) 215; 5.6 (BZ) 215 |
| 197 | $NH_2$ | $NH_2$ | 5,6-CH=CH—CH=CH | | 7.0 (M) 130 |
| 198 | $NH_2$ | $NH_2$ | 5,6-CH=CCl—CH=$CH_2$ | | 7.0 (M) 130 |
| 199 | $NH_2$ | $NH_2$ | 5,6-CH=CClCH=CH (7,8-dihydro) | | 7.4 (M) 130 |
| 200 | $NH_2$ | $NH_2$ | 5,6-CH=CHN[$CH_2$-c-$CH(CH_2)_4$] | | 7.2 (M) 215; 7.0 (BN) 215 |
| | | | | | 8.0 (U) 215; 7.7 (BZ) 215 |
| 201 | $NH_2$ | $NH_2$ | 5,6-CH=CHN($CH_2$-2′-$C_5H_4N$) | | 8.1 (M) 215; 7.8 (BN) 215 |
| | | | | | 8.5 (U) 215; 8.0 (BZ) 215 |
| 202 | $NH_2$ | $NH_2$ | 5,6-CH=CHN($CH_2$-4′-$C_5H_4N$) | | 8.0 (M) 215; 8.0 (BN) 215 |
| | | | | | 7.9 (U) 215; 8.6 (BZ) 215 |
| 203 | $NH_2$ | $NH_2$ | 5,6-CH=CHN($CH_2C_6H_5$) | | 8.3 (M) 215; 8.5 (BN) 215 |
| | | | | | 8.6 (U) 215; 8.5 (BZ) 215 |
| 204 | $NH_2$ | $NH_2$ | 5,6-CH=CHN($CH_2C_6H_3$-3′,4′-$Cl_2$) | | 8.5 (M) 215; 7.8 (BN) 215 |
| | | | | | 8.4 (U) 215; 8.7 (BZ) 215 |
| 205 | $NH_2$ | $NH_2$ | 5,6-CH=CHN($CH_2C_6H_4$-4′-$CH_3$) | | 9.0 (M) 215; 8.8 (BN) 215 |
| | | | | | 8.6 (U) 215; 9.1 (BZ) 215 |
| 206 | $NH_2$ | $NH_2$ | 5,6-CH=CHN($CH_2C_6H_4$-4′-CN) | | 8.7 (M) 215; 8.2 (BN) 215 |
| | | | | | 8.4 (U) 215; 9.1 (BZ) 215 |
| 207 | $NH_2$ | $NH_2$ | 5,6-CH=CHN[$CH_2C_6H_2$-3′,4′,5′-$(OCH_3)_3$] | | 8.3 (M) 215; 8.3 (BN) 215 |
| | | | | | 8.5 (U) 215; 8.4 (BZ) 215 |
| 208 | $NH_2$ | OH | H | H | 4.3 (M) 156 |
| 209 | $NH_2$ | OH | H | H (5,6,7,8-tetrahydro) | 4.0 (M) 156 |
| 210 | $NH_2$ | OH | H | 6-H; 7$CF_3$ | 4.3 (M) 156 |
| 211 | $NH_2$ | OH | H | $CH_3$ | 4.4 (M) 156 |
| 212 | $NH_2$ | OH | H | $CH_3$ (5,6,7,8-tetrahydro) | 4.3 (M) 156 |
| 213 | $NH_2$ | OH | H | $SC_6H_3$-3′,4′-$Cl_2$ | 6.9 (M) 171; 5.9 (BC) 171 |
| 214 | $NH_2$ | OH | H | $SO_2C_6H_3$-3′,4′-$Cl_2$ | 7.1 (M) 171; 5.3 (BC) 171 |
| 215 | $NH_2$ | OH | H | $NHCH_2C_6H_5$ | (M, U) 170; (BN, AK) 183 |
| 216 | $NH_2$ | OH | H | $NHCH_2C_6H_4$-4′-COOH | 6.0 (M) 191; 4.6 (BC) 191 |
| 217 | $NH_2$ | OH | H | $CH_2NHC_6H_4$-4′-COOH | 6.8 (M) 191; 5.7 (BC) 191 |
| 218 | $NH_2$ | OH | H | $CH_2NHC_6H_4$-4′-$COOC_2H_5$ | 4.7 (D) 223 |
| 219 | $NH_2$ | OH | H | $NHCH_2C_6H_4$-4′-$COOC_2H_5$ | 5.5 (D) 223 |

**TABLE IX** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| 220 | $NH_2$ | OH | H | S-2′-$C_{10}H_7$ | 7.3 (M) 171; 6.7 (BC) 171 |
| 221 | $NH_2$ | OH | H | SO-2′-$C_{10}H_7$ | 7.4 (M) 171; 5.3 (BC) 171 |
| 222 | $NH_2$ | OH | H | $SO_2$-2′-$C_{10}H_7$ | 8.0 (M) 171; 6.3 (BC) 171 |
| 223 | $NH_2$ | OH | H | $CH_2NHC_6H_4$-4′-CO-Asp | 4.0 (BE) 177; 6.6 (AB) 112<br>6.2 (BD) 112 |
| 224 | $NH_2$ | OH | H | $NHCH_2C_6H_4$-4′-CO-Glu | 6.7 (M) 189; 4.7 (BC) 203<br>5.8 (D) 223 |
| 225 | $NH_2$ | OH | H | $CH_2N(CH_3)C_6H_4$-4′-CO-Asp | 6.1 (AB) 112; 8.8 (BD) 112<br>(BE) 177 |
| 226 | $NH_2$ | OH | H | $CH_2NHC_6H_4$-4′-CO-Glu | 6.6 (M) 230; 7.6 (M) 203<br>6.6 (AB) 112; 6.6 (BD) 112<br>5.8 (BC) 203; (U) 219<br>(BE) 177 |
| 227 | $NH_2$ | OH | H | $CH_2SC_6H_4$-4′-CO-Glu | 4.9 (D) 223 |
| 228 | $NH_2$ | OH | H | $CH_2CH_2C_6H_4$-4′-CO-Glu | 6.3 (D) 223 |
| 229 | $NH_2$ | OH | H | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu | 7.4 (M) 203; 6.4 (BC) 203<br>6.8 (BD) 112; 6.7 (AB) 112<br>6.2 (D) 223; (Y, BN) 164 |
| 230 | $NH_2$ | OH | H | $CH_2N(CHO)C_6H_4$-4′-CO-Glu | 8.8 (M) 203; 9.0 (BC) 203 |
| 231 | $NH_2$ | OH | H | $CH_2N(CH_2C{\equiv}CH)C_6H_4$-4′-CO-Glu | 7.9 (U) 257 |
| 232 | $NH_2$ | OH | H | $CH_2N(CH_2CH{=}CH_2)C_6H_4$-4′-CO-Glu | 7.7 (U) 257 |
| 233 | $NH_2$ | OH | H | $CH_2N(CH_2CH_2CH_3)C_6H_4$-4′-CO-Glu | 7.7 (U) 257 |
| 234 | $NH_2$ | OH | H | $NHCH_2C_6H_4$-4′-CO-Glu$(C_2H_5)_2$ | 6.2 (M) 66; 4.6 (BC) 203<br>5.7 (D) 223 |
| 235 | $NH_2$ | OH | H | $CH_2NHC_6H_4$-4′-CO-Glu$(C_2H_5)_2$ | 6.3 (M) 203; 5.1 (BC) 203 |
| 236 | $NH_2$ | OH | H | $CH_2SC_6H_4$-4′-CO-Glu$(C_2H_5)_2$ | 5.1 (D) 223 |
| 237 | $NH_2$ | OH | H | $CH_2N(CH_3)C_6H_4$-4′-CO-Glu$(C_2H_5)_2$ | 6.0 (M) 203; 5.3 (BC) 203 |
| 238 | $NH_2$ | OH | Cl | H | 4.6 (M) 156 |
| 239 | $NH_2$ | OH | Cl | $NHCH_2C_6H_4$-4′-$COOC_2H_5$ | 6.1 (M) 191; 6.1 (BC) 191 |
| 240 | $NH_2$ | OH | Cl | $CH_2NHC_6H_4$-4′-CO-Glu | 6.2 (M) 229 |
| 241 | $NH_2$ | OH | $CH_3$ | H | 4.8 (M) 156 |
| 242 | $NH_2$ | OH | $CH_3$ | $NHCH_2C_6H_4$-4′-COOH | 5.6 (M) 191; 4.1 (BC) 191 |
| 243 | $NH_2$ | OH | $CH_3$ | $NHCH_2C_6H_4$-4′-$COOC_2H_5$ | 5.5 (M) 191; 5.5 (BC) 191 |
| 244 | $NH_2$ | OH | $CH_3$ | $NHCH_2C_6H_4$-4′-CO-Glu | 6.4 (M) 189; 5.2 (BC) 203<br>6.1 (D) 223 |
| 245 | $NH_2$ | OH | $CH_3$ | $CH_2NHC_6H_4$-4′-CO-Glu | 6.0 (M) 229 |
| 246 | $NH_2$ | OH | $CH_3$ | $NHCH_2C_6H_4$-4′-CO-Glu$(C_2H_5)_2$ | 5.6 (M) 189; 3.9 (BC) 203<br>5.1 (D) 223 |
| 247 | $NH_2$ | OH | S-2′-$C_{10}H_7$ | H | 6.2 (M) 160; 4.7 (BC) 171 |
| 248 | $NH_2$ | OH | $SO_2$-2′-$C_{10}H_7$ | H | 4.9 (M) 160; 5.9 (BC) 171 |
| 249 | $NH_2$ | SH | H | H | 4.4 (M) 156 |
| 250 | $NH_2$ | SH | H | S-2′-$C_{10}H_7$ | 6.6 (M) 171; 6.1 (BC) 171 |
| 251 | $NH_2$ | SH | H | $SO_2$-2′-$C_{10}H_7$ | 7.6 (M) 171; 5.2 (BC) 171 |
| 252 | $N(CH_3)_2$ | $N(CH_3)_2$ | H | $NHCH_2C_6H_3$-3′,4′-$Cl_2$ | (M, U) 170 |
| 253 | $NHC_4H_9$ | $NHC_4H_9$ | H | $NHCH_2C_6H_3$-3′,4′-$Cl_2$ | (M, U) 170 |
| 254 | $N(C_2H_5)_2$ | $N(C_2H_5)_2$ | H | $NHCH_2C_6H_3$-3′,4′-$Cl_2$ | (M, U) 170; (BN, AK) 183 |
| 255 | $NHC_6H_5$ | $NHC_6H_5$ | H | $NHCH_2C_6H_3$-3′,4′-$Cl_2$ | (M, U) 170 |
| 256 | $NHCOCH_3$ | OH | H | H | 4.3 (M) 156 |
| 257 | $NHCOCH_3$ | OH | H | $SO_2$-2′-$C_{10}H_7$ | 6.1 (M) 171; 3.3 (BC) 171 |
| 258 | OH | $NH_2$ | H | H | 4.0 (M) 156 |
| 259 | OH | $NH_2$ | H | S-2′-$C_{10}H_7$ | 5.8 (M) 171; 4.3 (BC) 171 |
| 260 | OH | OH | H | H | 3.9 (M) 156 |
| 261 | OH | OH | H | S-2′-$C_{10}H_7$ | 5.1 (M) 171; 4.3 (BC) 171 |
| 262 | OH | SH | H | S-2′-$C_{10}H_7$ | 5.2 (M) 171; 4.2 (BC) 171 |
| 263 | SH | $NH_2$ | H | H | 3.7 (M) 156 |
| 264 | SH | $NH_2$ | H | S-2′-$C_{10}H_7$ | 5.5 (M) 171; 4.7 (BC) 171 |
| 265 | SH | OH | H | S-2′-$C_{10}H_7$ | 5.0 (M) 171; 3.9 (BC) 171 |
| 266 | SH | SH | H | S-2′-$C_{10}H_7$ | 5.1 (M) 171; 3.7 (BC) 171 |
| 267 | $CH_3$ | $NH_2$ | H | $NHCH_2C_6H_5$ | (M, U) 170 |

**TABLE X. Inhibition by 4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-(X-phenyl)-*s*-triazines**




| no. | X | activity (enzyme) ref |
|---|---|---|
| 1 | H | 7.0 (AB) 9; 5.5 (BJ) 39; 6.9 (N) 61<br>6.7 (U) 61; 6.9 (CD) 63; (AB) 84<br>6.8 (M) 74; 6.3 (H) 247; 5.8 (X) 247<br>6.0 (M) 251; 2.6 (BN) 222 |
| 2 | 2-Br | 4.2 (N) 75; 4.1 (M) 53 |
| 3 | 3-Br | 8.1 (AB) 9; 6.4 (BJ) 9; 5.5 (AL) 104<br>7.4 (AA) 104; 7.5 (M) 104; 7.8 (H) 235 |

**TABLE X** (Continued)

| no. | X | activity (enzyme) ref |
|---|---|---|
| | | 7.3 (X) 235; 7.1 (M) 221; 3.7 (BN) 222 |
| 4 | 4-Br | 7.0 (H) 251; 6.2 (M) 251 |
| 5 | 2-Cl | 3.8 (AB) 9; 3.7 (BJ) 9; 4.6 (CD) 63<br>4.1 (N) 74; 4.1 (M) 74; 4.9 (H) 247<br>4.7 (X) 247 |
| 6 | 3-Cl | 8.1 (Q) 9; 6.2 (BJ) 9; 7.9 (AB) 24<br>7.7 (CD) 63; 7.8 (N) 74; 7.5 (M) 74<br>8.0 (U) 74; 5.7 (AL) 104; 7.3 (AA) 104<br>7.0 (M) 104; 7.9 (H) 235; 7.3 (X) 235<br>6.8 (M) 221; 3.5 (BN) 222 |
| 7 | 4-Cl | 6.2 (Q) 9; 6.4 (AB) 9; 6.4 (BJ) 9<br>7.1 (CD) 63; 6.0 (AL) 104; 6.3 (AA) 104<br>5.9 (M) 104; 9.1 (AP) 116 |
| 8 | 2-F | 4.7 (N) 75; 4.8 (M) 75; 4.9 (H) 247<br>4.4 (X) 247 |
| 9 | 3-F | 7.6 (H) 235; 6.7 (X) 235; 6.4 (M) 221<br>3.3 (BN) 222 |
| 10 | 4-F | 7.2 (H) 251; 6.7 (M) 251 |
| 11 | 2-I | 4.6 (N) 75; 4.6 (M) 75; 3.4 (H) 247<br>2.5 (X) 247 |
| 12 | 3-I | 7.7 (H) 235; 7.2 (X) 235; 7.1 (M) 221<br>3.7 (BN) 222 |
| 13 | 4-I | 7.0 (H) 251; 6.5 (M) 252 |
| 14 | 2-$NH_2$ | 4.1 (H) 247; 3.4 (X) 247 |
| 15 | 4-$NH_2$ | 5.7 (H) 251 |
| 16 | 3-$NO_2$ | 7.1 (CD) 63; 5.5 (BJ) 63; 7.1 (AB) 26<br>7.1 (N) 74; 6.7 (M) 74; 6.7 (U) 74<br>7.3 (H) 235; 6.4 (X) 235; 3.6 (BN) 222 |
| 17 | 2-OH | 3.9 (H) 247; 2.9 (X) 247 |
| 18 | 3-OH | 6.4 (H) 235; 5.4 (X) 235; 3.2 (BN) 222 |
| 19 | 4-OH | 5.8 (H) 251 |
| 20 | 2-SH | 7.2 (H) 247; 6.9 (X) 247 |
| 21 | 3-$SO_2F$ | 7.3 (N) 72; 6.8 (U) 72; 6.4 (H) 221<br>3.2 (BN) 221 |
| 22 | 3-$SO_2NH_2$ | 5.4 (H) 235; 4.7 (X) 235; 4.4 (M) 221<br>1.8 (BN) 222 |
| 23 | 4-$SO_2NH_2$ | 5.0 (H) 251; 4.5 (M) 251 |
| 24 | 2,3-$Cl_2$ | 6.5 (N) 75; 6.4 (M) 75 |
| 25 | 2,5-$Cl_2$ | 3.4 (N) 75; 3.5 (M) 75 |
| 26 | 2,4-$Cl_2$ | 3.8 (N) 75; 3.8 (M) 75 |
| 27 | 3,4-$Cl_2$ | 7.3 (Q) 9; 7.8 (AB) 9; 6.8 (BJ) 9<br>7.7 (CD) 63; 6.8 (BJ) 63; 8.5 (N) 74<br>7.9 (U) 74; 5.5 (AL) 104; 7.6 (AA) 104<br>8.4 (M) 104 |
| 28 | 2,4,5-$Cl_3$ | 4.4 (N) 75; 4.0 (M) 75 |
| 29 | 2-$CH_3$ | 4.4 (N) 75; 4.0 (M) 75; 4.6 (H) 247<br>4.4 (X) 247 |
| 30 | 3-$CH_3$ | 7.1 (AB) 53; 7.7 (H) 235; 6.8 (M) 221<br>6.8 (X) 235; 3.1 (BN) 222 |
| 31 | 4-$CH_3$ | 7.1 (H) 251; 6.4 (M) 251 |
| 32 | 3-$CH_2OH$ | 6.1 (CD) 63; 5.4 (BJ) 63; 6.5 (AB) 63 |
| 33 | 2-$CF_3$ | 3.1 (H) 247; 3.0 (X) 247 |
| 34 | 3-$CF_3$ | 7.1 (AB) 26; 7.5 (CD) 63; 5.6 (BJ) 63<br>7.8 (N) 74; 7.6 (M) 74; 7.4 (U) 74<br>7.6 (H) 235; 7.1 (X) 235; 7.1 (M) 221<br>3.3 (BN) 222 |
| 35 | 4-$CF_3$ | 7.1 (H) 251; 6.1 (M) 251 |
| 36 | 3-CN | 7.5 (H) 235; 7.0 (X) 235; 6.7 (M) 221<br>3.7 (BN) 222 |
| 37 | 4-CN | 5.1 (AB) 26; 5.0 (CD) 63; 4.0 (BJ) 63<br>5.1 (N) 74; 4.8 (M) 74; 5.0 (U) 74 |
| 38 | 4-$CH_2NH_3^+$ | 5.0 (AB) 26; 5.1 (CD) 63; 3.9 (BJ) 63 |
| 39 | 2-$OCH_3$ | 3.7 (N) 75; 3.7 (M) 75; 3.6 (H) 247<br>3.1 (X) 247 |
| 40 | 3-$OCH_3$ | 6.3 (AB) 54; 6.6 (CD) 63; 5.4 (BJ) 63<br>6.2 (N) 74; 6.1 (M) 74; 6.1 (U) 74<br>6.9 (H) 235; 6.0 (X) 235; 6.3 (M) 221<br>3.1 (BN) 222 |
| 41 | 4-$OCH_3$ | 6.2 (U) 79; 6.9 (H) 251; 6.3 (M) 251 |
| 42 | 2-$SCH_3$ | 3.5 (H) 247; 2.5 (X) 247 |
| 43 | 3-COOH | 4.0 (AB) 26; 4.1 (CD) 63; 3.9 (BJ) 63<br>4.7 (N) 74; 4.7 (M) 74; 4.2 (U) 74 |
| 44 | 4-COOH | 3.0 (AB) 26; 3.1 (CD) 63; 2.7 (BJ) 63<br>2.7 (U) 74 |
| 45 | 3-$CONH_2$ | 5.7 (H) 235; 4.7 (X) 235; 2.5 (BN) 222 |
| 46 | 4-$CONH_2$ | 4.8 (H) 251 |
| 47 | 4-$SO_2CH_3$ | 5.7 (H) 251 |

**TABLE X** (Continued)

| no. | X | activity (enzyme) ref |
|---|---|---|
| 48 | 2-$C_2H_5$ | 4.0 (H) 247; 3.4 (X) 247 |
| 49 | 4-$C_2H_5$ | 6.3 (U) 79 |
| 50 | 2-$CH_2CN$ | 4.0 (H) 247; 2.7 (X) 247 |
| 51 | 4-$CH_2CN$ | 6.9 (U) 154 |
| 52 | 3-$CH_2OCH_3$ | 6.7 (AB) 54 |
| 53 | 3-$COCH_3$ | 6.2 (H) 235; 5.4 (X) 235; 5.4 (M) 221<br>2.9 (BN) 222 |
| 54 | 4-$COCH_3$ | 5.9 (H) 251 |
| 55 | 4-$COOCH_3$ | 5.1 (H) 251; 4.3 (M) 251 |
| 56 | 4-$N(CH_3)_2$ | 6.0 (AB) 26 |
| 57 | 3-$COCH_2Cl$ | 5.8 (AB) 24; 5.9 (CD) 63; 5.4 (BJ) 63<br>6.2 (N) 70; 5.9 (U) 70 |
| 58 | 4-$COCH_2Cl$ | 5.9 (AB) 63; 5.8 (CD) 63; 4.6 (BJ) 63<br>6.4 (N) 70; 6.0 (U) 70 |
| 59 | 2,6-$(CH_3)_2$ | 4.5 (CD) 63; 4.0 (BJ) 63; 3.8 (AB) 63<br>4.0 (N) 74; 3.8 (M) 74; 4.1 (U) 74 |
| 60 | 3-$OC_3H_7$-*n* | 5.8 (AB) 53 |
| 61 | 2-$OCH(CH_3)_2$ | 3.2 (AB) 84 |
| 62 | 3-$COOC_2H_5$ | 5.7 (H) 235; 5.1 (X) 235; 5.7 (M) 221<br>3.2 (BN) 221 |
| 63 | 4-$COOC_2H_5$ | 4.4 (AB) 26; 4.5 (CD) 63; 4.4 (BJ) 63<br>4.6 (U) 74; 4.8 (H) 251; 4.4 (M) 251 |
| 64 | 4-$(CH_2)_2CONH_2$ | 6.5 (U) 79 |
| 65 | 4-$CH_2NHCOCH_3$ | 7.0 (AB) 26; 5.9 (CD) 63; 4.5 (BJ) 63 |
| 66 | 3-$CH_2NHCOCH_2Br$ | 6.4 (AB) 70; 6.7 (M) 70; 6.6 (U) 70 |
| 67 | 3-$C_4H_9$-*n* | 7.5 (AB) 53 |
| 68 | 4-$C_4H_9$-*n* | 6.3 (A) 32; 7.4 (K) 32; 6.8 (M) 32<br>6.8 (J) 32; 4.0 (BJ) 32; 3.5 (AZ) 32<br>4.5 (BG) 32; 5.1 (AI) 90; 5.4 (AN) 90<br>4.7 (AL) 90; 5.5 (AO) 90; 5.5 (AK) 90<br>5.4 (AJ) 90; 4.2 (AM) 90; 5.1 (AN) 115<br>4.3 (AT) 115; 8.1 (AP) 115; 4.8 (AR) 115<br>3.2 (BJ) 115; 4.7 (AL) 67; 8.1 (AP) 88<br>6.2 (Z) 88; 6.8 (M) 88; 4.7 (D) 88<br>3.2 (BJ) 88; 7.2 (H) 26; 5.1 (BJ) 39<br>6.9 (I) 63; 7.3 (U) 74; 4.7 (AL) 104<br>6.5 (AA) 104; 7.1 (M) 104; 7.4 (H) 251<br>7.1 (M) 251; 6.2 (A) 146; 3.3 (AZ) 146<br>4.0 (BG) 146; (BK) 198; (BI) 198 |
| 69 | 2-$C(CH_3)_3$ | 6.2 (AB) 84 |
| 70 | 3-$C(CH_3)_3$ | 7.1 (H) 235; 6.4 (X) 235; 6.9 (M) 221<br>3.2 (BN) 222 |
| 71 | 4-$C(CH_3)_3$ | 6.9 (H) 251; 6.4 (M) 251 |
| 72 | 3-$O(CH_2)_3CH_3$ | 7.0 (H) 235; 6.3 (X) 235; 7.0 (M) 221<br>4.2 (BN) 222 |
| 73 | 4-$OCH_2CH(CH_3)_2$ | 5.4 (AB) 84 |
| 74 | 2,3-$CH_2CH_2CH_2CH_2$ | 3.8 (AB) 84 |
| 75 | 2,3-CH=CHCH=CH | 3.9 (AB) 84 |
| 76 | 3,4-CH=CHCH=CH | 5.4 (AL) 104; 6.0 (AA) 104; 6.2 (M) 104 |
| 77 | 4-Cl; 2,3-CH=CHCH=CH | 4.2 (AB) 84 |
| 78 | 3-$(CH_2)_2COCH_2Cl$ | 7.1 (AB) 79; 7.1 (N) 70; 7.5 (U) 70 |
| 79 | 4-$(CH_2)_2COCH_2Cl$ | 7.6 (AB) 70; 7.9 (N) 70; 7.4 (U) 70 |
| 80 | 4-$CH_2CON(CH_3)_2$ | 6.6 (U) 154 |
| 81 | 3-$OCH_2CON(CH_3)_2$ | 5.4 (U) 154 |
| 82 | 4-$OCH_2CON(CH_3)_2$ | 6.3 (U) 154 |
| 83 | 3-Cl; 4-$OCH_2CON(CH_3)_2$ | 7.2 (U) 154 |
| 84 | 4-$(CH_2)_2CON(CH_3)_2$ | 7.0 (U) 154 |
| 85 | 6-$CH_3$; 2,3-CH=CHCH=CH | 2.9 (AB) 84 |
| 86 | 6-$CH_3$; 2,3-$CH_2CH_2CH_2CH_2$ | 3.3 (AB) 84 |
| 87 | 3-$(CH_2)_5CH_3$ | 7.4 (H) 235; 7.1 (X) 235; 5.0 (BN) 222 |
| 88 | 3-$C_6H_5$ | 5.9 (AB) 26; 6.0 (BJ) 39; 8.0 (CD) 63<br>6.8 (N) 74; 6.7 (M) 74; 6.3 (U) 74 |
| 89 | 4-$C_6H_5$ | 3.8 (AB) 26; 5.5 (BC) 128; 5.2 (BJ) 39<br>5.0 (CD) 63; 4.7 (N) 74; 4.7 (M) 74<br>4.3 (U) 74 |
| 90 | 4-$OC_6H_5$ | 5.3 (AB) 84 |
| 91 | 4-$OC_6H_4$-4′-$SO_2F$ | 5.8 (U) 98 |
| 92 | 4-$OCH_2CH_2N(C_2H_5)_2$ | 3.4 (AB) 84 |
| 93 | 4-$OCH_2CO$-c-$N(CH_2CH_2)_2O$ | 6.8 (H) 251 |
| 94 | 3-$(CH_2)_4COCH_2Cl$ | 7.6 (AB) 70; 7.1 (N) 70; 7.6 (U) 70 |
| 95 | 4-$CH_2CON(C_2H_5)_2$ | 6.8 (U) 154 |
| 96 | 4-$CH_2CO$-c-$N(CH_2CH_2)_2O$ | 7.1 (U) 154 |
| 97 | 4-$OCH_2CON(C_2H_5)_2$ | 6.7 (U) 154 |
| 98 | 3-CONH-3′-$C_5H_4N$ | 6.7 (U) 121 |
| 99 | 4-$OCH_2CO$-c-$N(CH_2)_4$ | 6.7 (U) 154 |
| 100 | 3-$OCH_2CO$-c-$N(CH_2CH_2)_2O$ | 4.8 (U) 154 |
| 101 | 4-$OCH_2CO$-c-$N(CH_2CH_2)_2O$ | 7.3 (U) 154 |

**TABLE X** (Continued)

| no. | X | activity (enzyme) ref |
|---|---|---|
| 102 | 3-Cl; 4-$OCH_2CON(C_2H_5)_2$ | 7.6 (U) 154 |
| 103 | 3-Cl; 4-$OCH_2CO$-c-$N(CH_2)_4$ | 7.3 (U) 154 |
| 104 | 3-Cl; 4-$OCH_2CO$-c-$N(CH_2CH_2)_2O$ | 7.8 (U) 154 |
| 105 | 3-$CH_2NHCON(C_2H_5)_2$ | 6.1 (U) 155 |
| 106 | 3-$CH_2NHCO$-c-$N(CH_2CH_2)_2O$ | 6.4 (U) 155 |
| 107 | 3-$CH_2C_6H_5$ | 7.7 (AB) 24; 6.6 (BJ) 39; 8.0 (CD) 63<br>6.8 (N) 74; 6.7 (M) 74; 6.3 (U) 74 |
| 108 | 4-$CH_2C_6H_5$ | 6.7 (BC) 128; 7.2 (AB) 39; 5.3 (BJ) 39<br>5.0 (CD) 63; 4.7 (N) 74; 4.7 (M) 74<br>4.3 (U) 74 |
| 109 | 3,4-($CH_2$-2′-$C_6H_4$)(2-fluorenoyl-1-triazine) | 4.1 (AB) 24; 5.1 (CD) 63; 5.8 (BJ) 63<br>4.8 (N) 74; 4.7 (M) 74; 5.0 (U) 74 |
| 110 | 3-(D,L)-$CH(OH)C_6H_5$ | 6.5 (H) 235 |
| 111 | 3-$CH_2OC_6H_5$ | 7.8 (H) 235; 7.7 (X) 235; 5.4 (BN) 255<br>7.3 (AB) 54 |
| 112 | 3-$CH_2OC_6H_4$-3′-Cl | 7.7 (H) 235; 7.5 (X) 235; 5.0 (BN) 255 |
| 113 | 3-$OCH_2C_6H_5$ | 7.7 (H) 235; 6.8 (X) 235; 6.9 (M) 221<br>4.2 (BN) 222 |
| 114 | 4-$OCH_2C_6H_5$ | 7.0 (AB) 84; 7.3 (H) 251; 7.3 (M) 251 |
| 115 | 3-$OCH_2C_6H_4$-3′,4′-$Cl_2$ | 7.9 (H) 235; 6.4 (X) 235; 6.8 (M) 221<br>4.4 (BN) 222 |
| 116 | 4-$OCH_2C_6H_4$-3′,4′-$Cl_2$ | 7.4 (H) 251; 7.2 (M) 251 |
| 117 | 3-Cl; 4-$OCH_2C_6H_5$ | 7.5 (U) 154 |
| 118 | 3-Cl; 4-$OCH_2C_6H_4$-2′-$SO_2F$ | 7.4 (U) 126 |
| 119 | 3-Cl; 4-$OCH_2C_6H_4$-3′-$SO_2F$ | 7.7 (U) 126 |
| 120 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2F$ | 7.6 (U) 126 |
| 121 | 3-Cl; 4-$OCH_2C_6H_3$-3′-Cl-2′-$SO_2F$ | 7.4 (U) 126 |
| 122 | 3-Cl; 4-$OCH_2C_6H_3$-4′-Cl-2′-$SO_2F$ | 7.5 (U) 126 |
| 123 | 3-Cl; 4-$OCH_2C_6H_3$-5′-Cl-2′-$SO_2F$ | 7.3 (U) 126 |
| 124 | 3-Cl; 4-$OCH_2C_6H_3$-6′-Cl-2′-$SO_2F$ | 7.7 (U) 126 |
| 125 | 3-Cl; 4-$OCH_2C_6H_3$-2′-Cl-3′-$SO_2F$ | 7.5 (U) 126 |
| 126 | 3-Cl; 4-$OCH_2C_6H_3$-4′-Cl-3′-$SO_2F$ | 8.0 (U) 126 |
| 127 | 3-Cl; 4-$OCH_2C_6H_3$-6′-Cl-3′-$SO_2F$ | 7.4 (U) 126 |
| 128 | 3-Cl; 4-$OCH_2C_6H_3$-2′-Cl-4′-$SO_2F$ | 7.4 (U) 126 |
| 129 | 3-Cl; 4-$OCH_2C_6H_3$-3′-Cl-4′-$SO_2F$ | 7.4 (U) 126 |
| 130 | 4-$OCH_2CO$-c-$N(CH_2)_5$ | 7.1 (U) 154 |
| 131 | 3-Cl; 4-$OCH_2CO$-c-$N(CH_2)_5$ | 7.5 (U) 154 |
| 132 | 4-$CH_2CH_2CON(C_2H_5)_2$ | 7.3 (U) 154 |
| 133 | 4-$CH_2CH_2CO$-c-$N(CH_2CH_2)_2O$ | 7.3 (U) 154 |
| 134 | 3-$CH_2NHC_6H_5$ | 7.2 (AB) 53 |
| 135 | 3-$CH_2NHC_6H_4$-4′-$SO_2NH_2$ | 7.7 (H) 235; 6.8 (X) 235; 5.0 (BN) 222 |
| 136 | 3-$COC_6H_5$ | 6.0 (AB) 24; 6.2 (BJ) 39; 7.3 (CD) 63 |
| 137 | 3-$CONHC_6H_4$-4′-$SO_2F$ | 6.0 (N) 64; 5.4 (U) 64; 5.9 (AB) 64 |
| 138 | 4-$CONHC_6H_4$-4′-$SO_2F$ | 4.7 (N) 64; 3.2 (U) 64; 3.8 (AB) 64<br>4.5 (Q) 135; 4.4 (S) 135; 4.0 (U) 135 |
| 139 | 3-$CONHC_6H_4$-3′-$SO_2F$ | 6.6 (N) 72; 6.2 (U) 72 |
| 140 | 3-$CONHCH_2$-3′-$C_5H_4N$ | 7.1 (U) 121 |
| 141 | 4-$CONHC_6H_4$-3′-$SO_2F$ | 4.7 (N) 72; 4.0 (U) 72 |
| 142 | 3-$CH_2CH_2C_6H_5$ | 7.6 (AB) 24; 7.0 (BJ) 39; 7.8 (CD) 63<br>8.2 (U) 110 |
| 143 | 4-$CH_2CH_2C_6H_5$ | 7.9 (U) 110; 7.8 (AB) 53 |
| 144 | 3-$CH_2CH_2C_6H_4$-4′-$SO_2F$ | 8.1 (U) 110 |
| 145 | 4-$CH_2CH_2C_6H_4$-4′-$SO_2F$ | 7.8 (N) 79; 7.4 (U) 79 |
| 146 | 4-Cl; 3-$(CH_2)_2C_6H_4$-4′-$SO_2F$ | 5.8 (U) 125 |
| 147 | 3-Cl; 4-$(CH_2)_2C_6H_4$-4′-$SO_2F$ | 7.8 (U) 98; 8.1 (N) 122; 7.7 (M) 122 |
| 148 | 3-Cl; 4-$(CH_2)_2C_6H_3$-2′-Cl-4′-$SO_2F$ | 8.3 (U) 125 |
| 149 | 3-Cl; 4-$(CH_2)_2C_6H_3$-3′-Cl-4′-$SO_2F$ | 7.8 (U) 125 |
| 150 | 3-Cl; 4-$(CH_2)_2C_6H_3$-4′-Cl-3′-$SO_2F$ | 8.3 (U) 125 |
| 151 | 3-Cl; 4-$(CH_2)_2C_6H_3$-3′-Cl-2′-$SO_2F$ | 8.3 (U) 125 |
| 152 | 3-Cl; 4-$(CH_2)_2C_6H_3$-4′-Cl-2′-$SO_2F$ | 8.0 (U) 125 |
| 153 | 3-Cl; 4-$(CH_2)_2C_6H_3$-5′-Cl-2′-$SO_2F$ | 7.8 (U) 125 |
| 154 | 3-$CH_2OC_6H_4$-3′-$CH_3$ | 8.0 (H) 235; 7.2 (X) 235; 5.3 (BN) 255 |
| 155 | 3-$CH_2OC_6H_4$-3′-$CH_2OH$ | 7.9 (H) 235; 7.4 (X) 235; 5.4 (BN) 255 |
| 156 | 3-$CH_2OC_6H_4$-3′-$OCH_3$ | 8.0 (H) 235; 7.6 (X) 235; 5.6 (BN) 255 |
| 157 | 3-$CH_2OC_6H_4$-3′-CN | 7.8 (H) 235; 7.6 (X) 235; 5.3 (BN) 255 |
| 158 | 3-$CH_2OC_6H_4$-3′-$NHCONH_2$ | 7.8 (H) 235; 7.5 (X) 235; 5.4 (BN) 255 |
| 159 | 3-$CH_2OC_6H_4$-3′-$NHCSNH_2$ | 7.8 (H) 235; 7.3 (X) 235; 4.4 (BN) 255 |
| 160 | 3-$OC_6H_4$-4′-$NHCOCH_2Br$ | 7.1 (AB) 70; 7.9 (N) 70; 7.5 (U) 70 |
| 161 | 3-Cl; 4-$OCH_2C_6H_4$-3′-CN | 7.5 (U) 154 |
| 162 | 3-Cl; 4-$OCH_2C_6H_3$-2′-$CH_3$-4′-$SO_2F$ | 7.4 (U) 126 |
| 163 | 3-$OCH_2CH_2C_6H_5$ | 6.6 (AB) 54 |
| 164 | 3-$O(CH_2)_2OC_6H_5$ | 7.1 (AB) 54; 8.2 (H) 235; 6.8 (X) 140<br>7.2 (M) 221; 3.7 (BN) 222 |
| 165 | 3-$O(CH_2)_2OC_6H_4$-4′-$SO_2F$ | 7.8 (U) 124 |
| 166 | 4-$O(CH_2)_2OC_6H_4$-4′-$SO_2F$ | 7.8 (N) 79; 7.3 (U) 79 |
| 167 | 4-$O(CH_2)_2OC_6H_4$-4′-$NH_2$ | 7.2 (H) 251 |
| 168 | 4-Cl; 3-$O(CH_2)_2OC_6H_4$-4′-$SO_2F$ | 7.3 (U) 124 |

**TABLE X** (Continued)

| no. | X | activity (enzyme) ref |
|---|---|---|
| 169 | 3-Cl; 4-$O(CH_2)_2OC_6H_4$-4′-$SO_2F$ | 7.4 (U) 98; 7.9 (Q) 98 |
| 170 | 3-$CH_2CONHC_6H_5$ | 6.5 (AB) 64 |
| 171 | 3-$CH_2CONHC_6H_4$-4′-$SO_2F$ | 7.3 (N) 64; 7.2 (M) 64; 6.5 (AB) 64<br>6.5 (U) 64 |
| 172 | 4-$CH_2CONHC_6H_4$-4′-$SO_2F$ | 7.7 (N) 64; 7.0 (U) 64; 7.3 (AB) 64<br>7.3 (Q) 135; 7.3 (D) 135; 7.7 (U) 135 |
| 173 | 4-$CH_2CONHC_6H_4$-3′-$SO_2F$ | 8.1 (N) 72; 7.5 (U) 72 |
| 174 | 3-$CH_2NHCONHC_6H_5$ | 7.5 (U) 155 |
| 175 | 3-$CH_2NHCONHC_6H_4$-3′-Cl | 8.0 (U) 155 |
| 176 | 3-$CH_2NHCONHC_6H_4$-3′-$NO_2$ | 8.1 (U) 155 |
| 177 | 3-$CH_2NHCONHC_6H_4$-3′-$SO_2F$ | 7.2 (AB) 72; 7.6 (N) 72; 7.2 (U) 72 |
| 178 | 4-$CH_2NHCONHC_6H_4$-3′-$SO_2F$ | 8.0 (N) 79; 7.5 (U) 79 |
| 179 | 4-$CH_2NHCONHC_6H_4$-4′-$SO_2F$ | 8.0 (N) 79; 7.1 (U) 79; 7.3 (U) 99<br>7.6 (Q) 91 |
| 180 | 3-Cl; 4-$CH_2NHCONHC_6H_4$-4′-$SO_2F$ | 7.9 (U) 91; 8.8 (Q) 135; 8.6 (S) 135<br>8.7 (U) 135 |
| 181 | 3-Cl; 4-$CH_2NHCONHC_6H_3$-3′-Cl-4′-$SO_2F$ | 7.7 (U) 99 |
| 182 | 4-$CH_2CH_2NHSO_2C_6H_4$-3′-$SO_2F$ | 7.6 (N) 79; 6.8 (U) 79 |
| 183 | 4-$CH_2CH_2NHSO_2C_6H_4$-4′-$SO_2F$ | 7.4 (N) 79; 7.0 (U) 79 |
| 184 | 3-$OCH_2CONHC_6H_5$ | 6.8 (U) 154 |
| 185 | 4-$OCH_2CONHC_6H_5$ | 7.9 (U) 154 |
| 186 | 3-$OCH_2CONHC_6H_4$-4′-$SO_2F$ | 6.9 (U) 127 |
| 187 | 4-$OCH_2CONHC_6H_4$-4′-$SO_2F$ | 7.8 (N) 79; 7.3 (U) 79 |
| 188 | 4-$OCH_2CONHC_6H_4$-3′-$SO_2F$ | 8.0 (N) 79; 7.2 (U) 79 |
| 189 | 3-Cl; 4-$OCH_2CONHC_6H_4$-3′-$SO_2F$ | 7.7 (U) 98 |
| 190 | 3-Cl; 4-$OCH_2CONHC_6H_4$-4′-$SO_2F$ | 7.4 (U) 98; 7.6 (N) 122; 7.6 (M) 122 |
| 191 | 3-Br; 4-$OCH_2CONHC_6H_4$-4′-$SO_2F$ | 8.1 (U) 109 |
| 192 | 3-Cl; 4-$OCH_2CONHC_6H_3$-2′-Cl-4′-$SO_2F$ | 7.7 (Q) 109 |
| 193 | 3-Cl; 4-$OCH_2CONHC_6H_3$-3′-Cl-4′-$SO_2F$ | 7.7 (Q) 109 |
| 194 | 3-Cl; 4-$OCH_2CONHC_6H_3$-4′-Cl-3′-$SO_2F$ | 7.8 (Q) 109 |
| 195 | 3-Cl; 4-$OCH_2CONHC_6H_3$-2′-Cl-5′-$SO_2F$ | 7.7 (Q) 109 |
| 196 | 3,5-$Cl_2$; 4-$OCH_2CONHC_6H_4$-4′-$SO_2F$ | 7.7 (U) 109 |
| 197 | 3-Cl; 4-$OCH_2CH_2NHSO_2C_6H_4$-3′-$SO_2F$ | 7.4 (Q) 106 |
| 198 | 3-Cl; 4-$OCH_2CH_2NHSO_2C_6H_4$-4′-$SO_2F$ | 7.4 (Q) 106 |
| 199 | 4-$SCH_2CONHC_6H_4$-4′-$SO_2F$ | 7.5 (U) 127 |
| 200 | 3-Cl; 4-$SCH_2CONHC_6H_4$-3′-$SO_2F$ | 7.7 (U) 127 |
| 201 | 3-Cl; 4-$SCH_2CONHC_6H_4$-4′-$SO_2F$ | 7.4 (U) 127 |
| 202 | 3-$(CH_2)_3C_6H_5$ | 8.2 (AB) 53 |
| 203 | 3-$CH_2OC_6H_4$-3′-$C_2H_5$ | 7.8 (H) 235; 7.1 (X) 235; 5.3 (BN) 255 |
| 204 | 3-$CH_2OC_6H_4$-3′-$NHCOCH_3$ | 8.0 (H) 235; 7.5 (X) 235; 5.5 (BN) 255 |
| 205 | 3-$CH_2NHC_6H_3$-3′,5′-$(CONH_2)_2$ | 7.7 (H) 235; 6.8 (X) 235; 4.7 (BN) 222 |
| 206 | 4-$CH_2CH_2CON(CH_2CH_2CH_3)_2$ | 7.3 (U) 154 |
| 207 | 4-$CH_2CON(CH_3)C_6H_5$ | 7.0 (U) 154 |
| 208 | 3-$(CH_2)_2CONHC_6H_4$-4′-$SO_2F$ | 7.2 (N) 64; 7.6 (M) 64; 7.1 (U) 64<br>7.0 (AB) 64 |
| 209 | 4-$(CH_2)_2CONHC_6H_4$-4′-$SO_2F$ | 7.7 (N) 64; 8.2 (M) 64; 7.1 (U) 64<br>7.9 (U) 91; 7.6 (U) 91; 8.1 (U) 135<br>7.1 (AB) 64; 7.8 (Q) 98; 8.6 (Q) 135<br>8.5 (S) 135 |
| 210 | 4-$(CH_2)_2CONHC_6H_4$-3′-$SO_2F$ | 8.1 (N) 72; 7.4 (U) 72; 7.9 (M) 79 |
| 211 | 4-CH=$CHCONHC_6H_4$-3′-$SO_2F$ | 5.9 (N) 79 |
| 212 | 4-CH=$CHCONHC_6H_4$-4′-$SO_2F$ | 5.2 (N) 79; 5.6 (U) 79 |
| 213 | 3-CH=$CHCONHCH_2$-3′-$C_5H_4N$ | 6.8 (U) 121 |
| 214 | 3-Cl; 4-$(CH_2)_2CONHC_6H_4$-4′-$SO_2F$ | 7.6 (U) 98; 7.8 (Q) 98; 7.8 (M) 122<br>7.8 (N) 122 |
| 215 | 4-$CH_2CH_2NHCOC_6H_4$-4′-$SO_2F$ | 8.1 (N) 79 |
| 216 | 4-$CH_2NHCONHC_6H_4$-3′-$OCH_3$ | 8.0 (U) 155 |
| 217 | 4-$CH_2NHCONHC_6H_4$-3′-CN | 8.2 (U) 155 |
| 218 | 4-$CH_2NHCONHC_6H_3$-2′-$CH_3$-4′-$SO_2F$ | 7.9 (U) 99 |
| 219 | 4-$CH_2NHCONH$-$C_6H_3$-3′-$CH_3$-4′-$SO_2F$ | 7.7 (U) 99 |
| 220 | 3-Cl; 4-$CH_2NHCONHC_6H_3$-3′-$CH_3$-4′-$SO_2F$ | 8.0 (U) 99 |
| 221 | 3-$O(CH_2)_8CH_3$ | 7.4 (H) 235; 6.7 (X) 235; 4.7 (BN) 222 |
| 222 | 3-$O(CH_2)_3C_6H_5$ | 7.1 (AB) 54 |
| 223 | 3-$O(CH_2)_3OC_6H_5$ | 7.0 (AB) 54 |
| 224 | 3-$O(CH_2)_2OC_6H_4$-3′-$CF_3$ | 8.2 (H) 235; 7.0 (X) 235; 7.1 (M) 221<br>4.1 (BN) 222 |
| 225 | 3-$OCH_2CON(CH_3)C_6H_5$ | 6.7 (U) 154 |
| 226 | 4-$OCH_2CON(CH_3)C_6H_5$ | 6.2 (U) 154 |
| 227 | 3-Cl; 4-$OCH_2CON(CH_3)C_6H_5$ | 7.9 (U) 154 |
| 228 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2N(CH_3)_2$ | 7.5 (U) 154 |
| 229 | 3-$OCH_2C_6H_4$-3′-$NHCOCH_2Br$ | 6.7 (AB) 70; 6.9 (N) 70; 7.0 (U) 70 |
| 230 | 3-$O(CH_2)_3OC_6H_4$-4′-$SO_2F$ | 8.0 (U) 124 |
| 231 | 4-$O(CH_2)_3OC_6H_4$-4′-$SO_2F$ | 6.8 (U) 98 |
| 232 | 3-Cl; 4-$O(CH_2)_3OC_6H_4$-4′-$SO_2F$ | 7.1 (U) 98; 7.2 (N) 122; 7.3 (M) 122 |
| 233 | 4-Cl; 3-$O(CH_2)_3OC_6H_4$-4′-$SO_2F$ | 7.5 (U) 124 |
| 234 | 3-Cl; 4-$OCH_2CON(CH_3)C_6H_4$-4′-$SO_2F$ | 7.5 (U) 98 |
| 235 | 3-Cl; 4-$OCH_2CONHC_6H_3$-2′-$CH_3$-4′-$SO_2F$ | 7.5 (Q) 109 |

**TABLE X** (Continued)

| no. | X | activity (enzyme) ref |
|---|---|---|
| 236 | 3-Cl; 4-$OCH_2CONHC_6H_3$-3′-$CH_3$-4′-$SO_2F$ | 7.7 (Q) 109 |
| 237 | 3-Cl; 4-$OCH_2CONH$-$C_6H_3$-4′-$CH_3$-3′-$SO_2F$ | 7.6 (Q) 109 |
| 238 | 3-Cl; 4-$OCH_2CONHC_6H_3$-2′-$CH_3$-5′-$SO_2F$ | 7.6 (Q) 109 |
| 239 | 3-Cl; 4-$OCH_2CONHC_6H_3$-2′-$OCH_3$-5′-$SO_2F$ | 7.8 (Q) 109 |
| 240 | 3-Cl; 4-$OCH(CH_3)CONHC_6H_4$-4′-$SO_2F$ | 7.1 (U) 127 |
| 241 | 3-Cl; 4-$O(CH_2)_2NHCOC_6H_4$-3′-$SO_2F$ | 7.1 (Q) 106 |
| 242 | 3-Cl; 4-$O(CH_2)_2NHCOC_6H_4$-4′-$SO_2F$ | 7.4 (Q) 106 |
| 243 | 3-Cl; 4-$O(CH_2)_2NHCONHC_6H_4$-3′-$SO_2F$ | 7.4 (Q) 106 |
| 244 | 3-Cl; 4-$O(CH_2)_2NHCONHC_6H_4$-4′-$SO_2F$ | 8.0 (U) 106; 7.9 (N) 122; 7.9 (M) 122 |
| 245 | 3-Cl; 4-$O(CH_2)_3NHSO_2C_6H_4$-3′-$SO_2F$ | 7.6 (Q) 106 |
| 246 | 3-Cl; 4-$O(CH_2)_3NHSO_2C_6H_4$-4′-$SO_2F$ | 7.3 (Q) 106 |
| 247 | 3-Cl; 4-$S(CH_2)_2CONHC_6H_4$-4′-$SO_2F$ | 7.4 (U) 127 |
| 248 | 3-$(CH_2)_4C_6H_5$ | 8.6 (AB) 39; 8.3 (U) 110 |
| 249 | 4-$(CH_2)_4C_6H_5$ | 8.0 (AB) 39; 8.0 (U) 110 |
| 250 | 3-$(CH_2)_4OC_6H_5$ | 8.3 (U) 110 |
| 251 | 4-$(CH_2)_4OC_6H_5$ | 8.2 (U) 110 |
| 252 | 3-$CH_2OC_6H_4$-3′-$CH(CH_3)_2$ | 7.8 (H) 235; 7.1 (X) 235; 5.4 (BN) 255 |
| 253 | 3-$(CH_2)_4C_6H_4$-2′-Cl | 8.2 (AB) 53 |
| 254 | 3-$(CH_2)_4C_6H_3$-2′,4′-$Cl_2$ | 8.2 (BC) 128; 8.3 (AB) 53; 8.1 (CD) 63<br>8.0 (U) 110; 6.7 (BJ) 63 |
| 255 | 4-$(CH_2)_4C_6H_3$-2′,4′-$Cl_2$ | 7.0 (BC) 128; 8.3 (AB) 53; 7.3 (CD) 63<br>5.9 (BJ) 63; 7.9 (U) 110 |
| 256 | 3-$(CH_2)_4C_6H_3$-2′,6′-$Cl_2$ | 8.4 (AB) 53 |
| 257 | 3-$(CH_2)_4C_6H_3$-3′,4′-$Cl_2$ | 8.4 (AB) 53 |
| 258 | 3-Cl; 4$(CH_2)_4C_6H_5$ | 8.0 (AB) 53; 7.9 (U) 110 |
| 259 | 3-$(CH_2)_4C_6H_4$-4′-$SO_2F$ | 8.1 (U) 110; 7.7 (M) 122; 7.9 (N) 122 |
| 260 | 4-$(CH_2)_4C_6H_4$-4′-$SO_2F$ | 7.7 (U) 98; 7.7 (M) 122; 7.9 (N) 122 |
| 261 | 4-$(CH_2)_4OC_6H_4$-4′-$SO_2F$ | 8.1 (N) 125 |
| 262 | 3-$(CH_2)_4C_6H_4$-3′-$SO_2F$ | 8.1 (U) 110 |
| 263 | 3-$(CH_2)_4C_6H_3$-4′-Cl-3′-$SO_2F$ | 8.4 (U) 125 |
| 264 | 3-$(CH_2)_4C_6H_3$-5′-Cl-2′-$SO_2F$ | 8.0 (U) 125 |
| 265 | 3-$(CH_2)_4C_6H_3$-3′-Cl-4′-$SO_2F$ | 7.7 (U) 125 |
| 266 | 3-$(CH_2)_4C_6H_3$2′-Cl-4′-$SO_2F$ | 8.0 (U) 125 |
| 267 | 4-$(CH_2)_4C_6H_3$-2′-Cl-4′-$SO_2F$ | 8.0 (U) 125 |
| 268 | 4-Cl; 3-$(CH_2)_4C_6H_4$-4′-$SO_2F$ | 6.7 (U) 125 |
| 269 | 3-Cl; 4-$(CH_2)_4C_6H_4$-4′-$SO_2F$ | 8.1 (U) 110 |
| 270 | 3-Cl; 4-$(CH_2)_4C_6H_4$-3′-$SO_2F$ | 8.0 (U) 125 |
| 271 | 3-Cl; 4-$(CH_2)_4C_6H_4$-2′-$SO_2F$ | 7.7 (U) 125 |
| 272 | 3-Cl; 4-$(CH_2)_4C_6H_3$-2′-Cl-4′-$SO_2F$ | 8.0 (U) 125; (AA) 248 |
| 273 | 3-Cl; 4-$(CH_2)_4C_6H_3$-3′-Cl,4′-$SO_2F$ | 7.7 (U) 125 |
| 274 | 3-Cl; 4-$(CH_2)_4C_6H_3$-4′-Cl-3′-$SO_2F$ | 8.1 (U) 125 |
| 275 | 3-Cl; 4-$(CH_2)_4C_6H_3$-5′-Cl-2′-$SO_2F$ | 7.1 (U) 125 |
| 276 | 3-Cl; 4-$(CH_2)_4C_6H_3$-3′-Cl-2′-$SO_2F$ | 8.2 (U) 125 |
| 277 | 3-Cl; 4-$(CH_2)_4C_6H_3$-4′-Cl-2′-$SO_2F$ | 7.8 (U) 125 |
| 278 | 3-$(CH_2)_2C_6H_4$-4′-$NHCOCH_2Br$ | 8.0 (AB) 70; 8.3 (N) 70; 8.4 (U) 70 |
| 279 | 4-$(CH_2)_3CONHC_6H_4$-2′-$SO_2F$ | 7.8 (N) 72; 7.2 (U) 72 |
| 280 | 4-$(CH_2)_3CONHC_6H_4$-3′-$SO_2F$ | 7.9 (N) 72; 7.2 (U) 72 |
| 281 | 4-$(CH_2)_3CONHC_6H_4$-4′-$SO_2F$ | 7.7 (N) 72; 7.4 (U) 72 |
| 282 | 4-$(CH_2)_2CONHC_6H_3$-2′-$CH_3$-4′-$SO_2F$ | 8.2 (N) 81; 7.7 (U) 81 |
| 283 | 4-$(CH_2)_2CONHC_6H_3$-3′-$CH_3$-4′-$SO_2F$ | 7.9 (N) 81; 7.3 (U) 81; 7.9 (M) 122<br>8.7 (Q) 135; 8.5 (S) 135; 8.6 (U) 135 |
| 284 | 4-$(CH_2)_2CONHC_6H_3$-4′-$CH_3$-3′-$SO_2F$ | 8.0 (N) 81; 7.4 (U) 81 |
| 285 | 4-$(CH_2)_2CONHC_6H_3$-3′-$OCH_3$-4′-$SO_2F$ | 7.5 (N) 81; 7.6 (U) 81 |
| 286 | 4-$(CH_2)_2CONHC_6H_3$-4′-$OCH_3$-3′-$SO_2F$ | 8.2 (N) 81; 7.4 (U) 81 |
| 287 | 4-$(CH_2)_2CONHC_6H_3$-6′-$OCH_3$-3′-$SO_2F$ | 8.1 (N) 81; 7.4 (U) 81 |
| 288 | 4-$CH_2CON(CH_3)CH_2C_6H_5$ | 7.3 (U) 154 |
| 289 | 4-$(CH_2)_2CON(CH_3)C_6H_5$ | 7.6 (U) 154 |
| 290 | 4-$CH(CH_3)CH_2CONHC_6H_4$-4′-$SO_2F$ | 7.3 (N) 80; 7.1 (U) 80; 7.1 (Q) 81<br>8.6 (Q) 135; 8.3 (S) 135; 8.6 (U) 135 |
| 291 | 4-$CH_2CH(CH_3)CONHC_6H_4$-4′-$SO_2F$ | 7.6 (N) 80; 7.3 (U) 80 |
| 292 | 4-$(CH_2)_2CON(CH_3)C_6H_4$-4′-$SO_2F$ | 8.0 (N) 80; 7.4 (U) 80 |
| 293 | 3-$O(CH_2)_4C_6H_5$ | 7.1 (AB) 54 |
| 294 | 3-$O(CH_2)_4OC_6H_5$ | 7.5 (U) 124; 8.0 (H) 235; 6.8 (X) 235<br>7.2 (M) 221; 4.8 (BN) 222 |
| 295 | 3-$O(CH_2)_2OC_6H_4$-2′-$NHCOCH_2Br$ | 7.7 (N) 70; 6.8 (U) 70 |
| 296 | 3-$O(CH_2)_2OC_6H_4$-3′-$NHCOCH_2Br$ | 7.3 (AB) 70; 7.7 (N) 70; 7.1 (U) 70 |
| 297 | 3-$O(CH_2)_2OC_6H_4$-4′-$NHCOCH_2Br$ | 7.3 (AB) 70; 8.1 (N) 70; 7.4 (U) 70 |
| 298 | 4-$O(CH_2)_2OC_6H_4$-4′-$NHCOCH_2Br$ | 7.3 (AB) 70; 7.7 (N) 70; 7.2 (U) 70 |
| 299 | 3-Cl; 4-$OCH_2C_6H_4$-3′-$CON(CH_3)_2$ | 7.7 (U) 154; 4.0 (BN) 222 |
| 300 | 4-$O(CH_2)_4OC_6H_4$-4′-$SO_2F$ | 7.2 (U) 98 |
| 301 | 3-Cl; 4-$O(CH_2)_4OC_6H_4$-4′-$SO_2F$ | 7.6 (U) 124 |
| 302 | 4-Cl; 3-$O(CH_2)_4OC_6H_4$-4′-$SO_2F$ | 7.2 (U) 124 |
| 303 | 3-Cl; 4-$O(CH_2)_2O(CH_2)_2OC_6H_4$-4′-$SO_2F$ | 7.1 (U) 124 |
| 304 | 3-Cl; 4-$O(CH_2)_3CONHC_6H_4$-3′-$SO_2F$ | 7.2 (U) 127 |
| 305 | 3-Cl; 4-$O(CH_2)_3CONHC_6H_4$-4′-$SO_2F$ | 7.2 (U) 127 |
| 306 | 3-Cl; 4-$O(CH_2)_3NHCOC_6H_4$-3′-$SO_2F$ | 7.9 (U) 106; 7.6 (Q) 106 |
| 307 | 3-Cl; 4-$O(CH_2)_3NHCOC_6H_4$-4′-$SO_2F$ | 7.3 (U) 106 |

**TABLE X** (Continued)

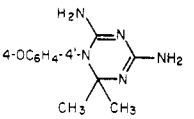
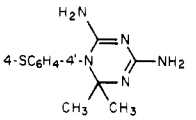
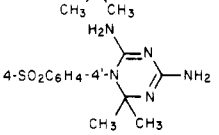
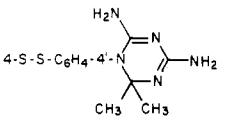
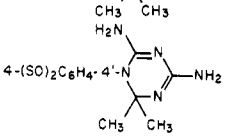
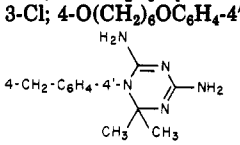
| no. | X | activity (enzyme) ref |
|---|---|---|
| 308 | 4-O$(CH_2)_3NHCONHC_6H_4$-3′-$SO_2F$ | 7.3 (U) 106; 7.5 (Q) 106 |
| 309 | 3-Cl; 4-O$(CH_2)_3NHCONHC_6H_4$-3′-$SO_2F$ | 7.3 (U) 106 |
| 310 | 3-Cl; 4-O$(CH_2)_3NHCONHC_6H_4$-4′-$SO_2F$ | 7.7 (U) 106 |
| 311 | 3-Cl; 4-O$(CH_2)_2NHCONHC_6H_3$-3′-$CH_3$-4′-$SO_2F$ | 7.8 (U) 106; 7.4 (Q) 106 |
| 312 | 3-$(CH_2)_5C_6H_5$ | 8.1 (AB) 54 |
| 313 | 3-$CH_2OC_6H_4$-3′-$C(CH_3)_3$ | 8.0 (H) 235; 7.2 (X) 235; 5.4 (BN) 255 |
| 314 | 4-$(CH_2)_2CON(CH_3)CH_2C_6H_5$ | 7.3 (U) 154 |
| 315 | 3-$CH_2CH(C_6H_5)CH_2NHCOCH_2Br$ | 6.5 (AB) 71; 6.7 (N) 71; 6.7 (U) 71 |
| 316 | 3-$CH_2NHCONHC_6H_4$-3′-$CON(CH_3)_2$ | 7.8 (U) 155 |
| 317 | 3-O$(CH_2)_{10}CH_3$ | 4.5 (BN) 255 |
| 318 | 3-O$(CH_2)_4OC_6H_4$-3′-$CF_3$ | 7.3 (H) 235; 6.8 (X) 235; 6.8 (M) 221<br>4.8 (BN) 255 |
| 319 | 3-O$(CH_2)_3OC_6H_4$-3′-$NHCOCH_2Br$ | 6.9 (AB) 70; 7.6 (N) 70; 7.0 (U) 70 |
| 320 | 3-O$(CH_2)_3OC_6H_4$-4′-$NHCOCH_2Br$ | 6.7 (AB) 70; 7.5 (N) 70; 7.0 (U) 70 |
| 321 | 3-Cl; 4-O$(CH_2)_5OC_6H_4$-4′-$SO_2F$ | 7.6 (U) 124 |
| 322 | 4-Cl; 3-O$(CH_2)_5OC_6H_4$-4′-$SO_2F$ | 6.7 (U) 124 |
| 323 | 3-Cl; 4-O$(CH_2)_4CONHC_6H_4$-4′-$SO_2F$ | 7.2 (U) 127 |
| 324 | 3-Cl; 4-O$(CH_2)_4NHCOC_6H_4$-4′-$SO_2F$ | 7.7 (U) 106 |
| 325 | 3-Cl; 4-O$(CH_2)_4NHCOC_6H_4$-3′-$SO_2F$ | 7.4 (Q) 106 |
| 326 | 3-Cl; 4-O$(CH_2)_4NHCONHC_6H_4$-4′-$SO_2F$ | 7.5 (Q) 106 |
| 327 | 3-Cl; 4-O$(CH_2)_4NHCONHC_6H_4$-3′-$SO_2F$ | 7.7 (U) 106 |
| 328 | 3-Cl; 4-O$(CH_2)_3NHCOC_6H_3$-4′-$CH_3$-3′-$SO_2F$ | 7.3 (Q) 106 |
| 329 | 3-Cl; 4-O$(CH_2)_3NHCONHC_6H_3$-4′-$CH_3$-3′-$SO_2F$ | 8.1 (U) 172; 7.7 (Q) 106 |
| 330 | 3-Cl; 4-O$(CH_2)_3NHCONHC_6H_3$-3′-$CH_3$-4′-$SO_2F$ | 7.2 (Q) 106 |
| 331 | 3-Cl; 4-O$(CH_2)_3NHCONHC_6H_3$-2′-$OCH_3$-5′-$SO_2F$ | 7.3 (Q) 106 |
| 332 | 3-$NHCOCH_2Br$; 4-O$(CH_2)_3C_6H_5$ | 5.2 (AB) 71; 6.1 (N) 71; 6.3 (U) 71 |
| 333 |  | 5.5 (AB) 84 |
| 334 |  | 6.9 (AB) 84 |
| 335 |  | 5.4 (AB) 84 |
| 336 |  | 4.7 (AB) 84 |
| 337 |  | 5.4 (AB) 84 |
| 338 | 3-$(CH_2)_4C_6H_4$-3′-$NHCOCH_2Br$ | 7.9 (AB) 70; 8.4 (N) 70; 7.4 (U) 70 |
| 339 | 3-$(CH_2)_4C_6H_4$-4′-$NHCOCH_2Br$ | 8.0 (AB) 70; 8.4 (N) 70; 8.0 (U) 70 |
| 340 | 4-$(CH_2)_6C_6H_4$-4′-$SO_2F$ | 7.1 (U) 125 |
| 341 | 3-O$(CH_2)_{11}CH_3$ | 7.6 (H) 235; 6.1 (X) 235; 4.6 (BN) 255 |
| 342 | 3-O$(CH_2)_2OC_6H_4$-4′-NHCOCH=CHCOOH | 7.1 (AB) 70; 8.1 (N) 70; 7.7 (U) 70 |
| 343 | 3-Cl; 4-O$CH_2C_6H_4$-3′-$CON(C_2H_5)_2$ | 8.1 (U) 154 |
| 344 | 3-Cl; 4-O$CH_2C_6H_4$-3′-CO-c-$(CH_2)_4$ | 7.8 (U) 154 |
| 345 | 3-Cl; 4-O$CH_2C_6H_4$-3′-CO-c-$N(CH_2CH_2)_2O$ | 7.8 (U) 154 |
| 346 | 3-Cl; 4-O$(CH_2)_6OC_6H_4$-4′-$SO_2F$ | 7.0 (Q) 124; 7.5 (U) 124 |
| 347 |  | 7.2 (AB) 84 |
| 348 | 3-$CH(CH_2CH_2CH_2C_6H_5)CH_2NHCOCH_2Br$ | 6.0 (AB) 71; 6.4 (N) 71; 5.5 (U) 71 |
| 349 | 4-$CH(CH_2CH_2CH_2C_6H_5)CH_2NHCOCH_2Br$ | 5.8 (AB) 71; 6.5 (N) 71; 6.5 (U) 71 |
| 350 | 3-O$(CH_2)_{12}CH_3$ | 4.8 (BN) 255 |
| 351 | 3-O$(CH_2)_3OC_6H_4NHCOCH$=CHCOOH | 7.1 (AB) 70; 8.1 (N) 70; 7.6 (U) 70 |
| 352 | 3-CONH-3′-$C_5H_4N^+CH_2C_6H_4$-3″-$SO_2F$ | 6.1 (U) 121 |
| 353 | 3-Cl; 4-O$CH_2C_6H_4$-3-CO-c-$N(CH_2)_5$ | 8.0 (U) 154 |
| 354 | 3-Cl; 4-O$CH_2C_6H_4$-4′-$SO_2C_6H_5$ | 8.2 (N) 139 |
| 355 | 3-Cl; 4-O$CH_2C_6H_4$-4′-$SO_2OC_6H_4$-2″-Cl | 8.6 (N) 139 |
| 356 | 3-Cl; 4-O$CH_2C_6H_4$-4′-$SO_2OC_6H_4$-3″-Cl | 7.7 (N) 139 |
| 357 | 3-Cl; 4-O$CH_2C_6H_4$-4′-$SO_2OC_6H_4$-4″-Cl | 7.8 (N) 139 |
| 358 | 3-Cl; 4-O$CH_2C_6H_4$-4′-$SO_2OC_6H_4$-2″-F | 8.7 (N) 139 |
| 359 | 3-Cl; 4-O$CH_2C_6H_4$-4′-$SO_2OC_6H_4$-3″-F | 8.5 (N) 139 |
| 360 | 3-Cl; 4-O$CH_2C_6H_4$-4′-$SO_2OC_6H_4$-4″-F | 8.4 (N) 139 |

**TABLE X** (Continued)

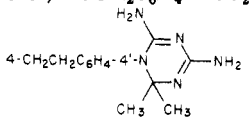
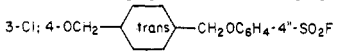
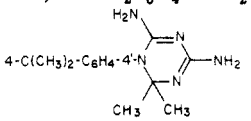
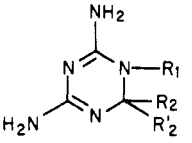
| no. | X | activity (enzyme) ref |
|---|---|---|
| 361 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_3$-3″,4″-$Cl_2$ | 8.2 (N) 139 |
| 362 |  | 7.9 (AB) 84 |
| 363 | 4-$C_6H_3$-2′-$NH_2$-4′-$CONHC_6H_4$-4′-$SO_2F$ | (AB, N, U) 64 |
| 364 | 3-$O(CH_2)_{13}CH_3$ | 4.5 (BN) 255 |
| 365 | 3-CONH-3′-$CH_2C_6H_4N^+CH_2C_6H_4$-4″-$SO_2F$ | 6.3 (U) 121 |
| 366 | 3-Cl; 4-$OCH_2C_6H_4$-3′-$CONHC_6H_5$ | 8.0 (U) 154 |
| 367 |  | 6.4 (U) 124 |
| 368 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$CH_2OC_6H_4$-4″-$SO_2F$ | 6.8 (U) 124 |
| 369 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$CONHC_6H_4$-4″-$SO_2F$ | 6.9 (U) 127 |
| 370 | 3-Cl; 4-$OCH_2C_6H_4$-3′-$CONHC_6H_4$-4″-$SO_2F$ | 6.9 (U) 127 |
| 371 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$CONHC_6H_4$-3″-$SO_2F$ | 7.4 (U) 127 |
| 372 | 3-Cl; 4-$OCH_2C_6H_4$-3′-$CONHC_6H_4$-3″-$SO_2F$ | 7.2 (U) 127 |
| 373 | 3-Cl; 4-$OCH_2C_6H_4$-2′-$CONHC_6H_4$-4″-$SO_2F$ | 7.2 (U) 127 |
| 374 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4$-3″-$CH_3$ | 8.4 (N) 139 |
| 375 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4$-2″-$CF_3$ | 8.3 (N) 139 |
| 376 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4$-3″-$CF_3$ | 8.1 (N) 139 |
| 377 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4$-2″-CN | 8.7 (N) 139 |
| 378 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4$-3″-CN | 8.2 (N) 139 |
| 379 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4$-4″-CN | 8.4 (N) 139 |
| 380 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4$-2″-$OCH_3$ | 8.4 (N) 139 |
| 381 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4$-3″-$OCH_3$ | 8.5 (N) 139 |
| 382 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4$-4″-$OCH_3$ | 8.4 (N) 139 |
| 383 |  | 7.1 (AB) 84 |
| 384 | 3-$(CH_2)_2CONH$-3′-$C_5H_4N^+CH_2C_6H_4$-4″-$SO_2F$ | 6.8 (U) 121 |
| 385 | 4-$CH(C_6H_5)CH_2CONHC_6H_4$-4′-$SO_2F$ | 5.7 (N) 80; 4.9 (U) 80 |
| 386 | 4-$CH_2CH(C_6H_5)CONHC_6H_4$-4′-$SO_2F$ | 6.7 (M) 80; 7.1 (N) 80; 6.7 (U) 80 |
| 387 | 4-$CH_2CH(C_6H_5)CONHC_6H_4$-3′-$SO_2F$ | 6.7 (M) 97; 7.1 (N) 97; 6.7 (U) 97 |
| 388 | 3-$CH_2CH(C_6H_5)CONHC_6H_4$-4′-$SO_2F$ | 6.3 (N) 97; 6.3 (U) 97 |
| 389 | 3-Cl; 4-$OCH_2C_6H_4$-3′-$CON(CH_3)C_6H_5$ | 8.1 (U) 154 |
| 390 | 3-CH=$CHCONHCH_2$-3′-$C_6H_4N^+CH_2C_6H_4$-4″-$SO_2F$ | 6.8 (U) 121 |
| 391 | 4-$CH_2CH(C_6H_4$-2″-$CH_3)CONHC_6H_4$-4′-$SO_2F$ | 7.2 (N) 80; 6.4 (U) 80 |
| 392 | 4-$CH_2CH(C_6H_4$-3″-$CH_3)CONHC_6H_4$-4′-$SO_2F$ | 7.2 (N) 80; 6.7 (U) 80; 6.7 (Q) 97 |
| 393 | 4-$CH_2CH(C_6H_4$-4″-$CH_3)CONHC_6H_4$-4′-$SO_2F$ | 7.2 (N) 80; 6.2 (U) 80 |
| 394 | 4-$CH_2CH(C_6H_4$-2″-$OCH_3)CONHC_6H_4$-4′-$SO_2F$ | 6.3 (U) 97 |
| 395 | 4-$CH_2CH(C_6H_4$-3″-$OCH_3)CONHC_6H_4$-4′-$SO_2F$ | 6.5 (U) 97 |
| 396 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4$-2″-$CON(CH_3)_2$ | 8.6 (N) 139 |
| 397 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4$-3″-$CON(CH_3)_2$ | 8.8 (N) 139 |
| 398 | 3-Cl; 4-$OCH_2C_6H_4$-4′-$SO_2OC_6H_4$-4″-$CON(CH_3)_2$ | 8.6 (N) 139 |
| 399 | 3-Cl; 4-$O(CH_2)_4OC_6H_4$-4′-$SO_2OC_6H_4$-4″-Cl | 6.6 (U) 124 |
| 400 | 4-$CH_2CH(CH_2CH_2C_6H_5)CONHC_6H_4$-4′-$SO_2F$ | 6.2 (N) 80; 5.7 (U) 80 |
| 401 | 4-$CH_2CH(\alpha$-$C_{10}H_7)CONHC_6H_4$-4′-$SO_2F$ | 6.2 (U) 104 |

**TABLE XI. Inhibition by 1-$R_1$-2,2-$R_2$,$R_2$′-4,6-Diamino-1,2-dihydro-*s*-triazines**



| no. | $R_1$ | $R_2$ | $R_2'$ | activity (enzyme) ref |
|---|---|---|---|---|
| 1 | $CH_3$ | $CH_3$ | $CH_3$ | 4.1 (AB) 21; 4.3 (BJ) 39<br>4.2 (CD) 63; 4.5 (N) 74<br>4.4 (M) 74; 4.1 (U) 74 |
| 2 | $C_2H_5$ | $CH_3$ | $CH_3$ | 3.7 (AB) 24; 4.2 (CD) 63<br>4.2 (BJ) 63 |
| 3 | $n$-$C_3H_7$ | $CH_3$ | $CH_3$ | 5.0 (AB) 21; 5.3 (BJ) 39<br>5.5 (CD) 63 |
| 4 | $n$-$C_4H_9$ | $CH_3$ | $CH_3$ | 6.4 (AB) 21; 5.7 (BJ) 39<br>6.0 (CD) 63; 6.9 (N) 74<br>6.4 (M) 74; 6.4 (U) 74 |

**TABLE XI** (Continued)

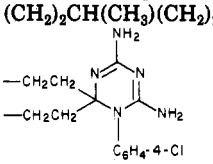
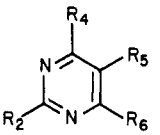
| no. | $R_1$ | $R_2$ | $R_2'$ | activity (enzyme) ref |
|---|---|---|---|---|
| 5 | $i$-$C_5H_{11}$ | $CH_3$ | $CH_3$ | 7.2 (AB) 21; 6.2 (BJ) 39<br>6.5 (CD) 63; 8.2 (N) 74<br>7.1 (BC) 128; 7.1 (M) 74<br>7.1 (U) 74 |
| 6 | $n$-$C_6H_{13}$ | $CH_3$ | $CH_3$ | 6.5 (AB) 21; 6.0 (BJ) 39<br>6.4 (CD) 63 |
| 7 | $CH_2C_6H_5$ | $CH_3$ | $CH_3$ | 5.5 (AB) 24; 6.0 (CD) 63<br>4.5 (BJ) 63 |
| 8 | $n$-$C_8H_{17}$ | $CH_3$ | $CH_3$ | 6.8 (AB) 21; 6.6 (BJ) 39<br>7.1 (CD) 63; 7.9 (Q) 74<br>7.3 (M) 74; 7.2 (U) 74 |
| 9 | $(CH_2)_2C_6H_5$ | $CH_3$ | $CH_3$ | 6.1 (AB) 24; 5.9 (CD) 63<br>5.4 (BJ) 63 |
| 10 | $(CH_2)_3C_6H_5$ | $CH_3$ | $CH_3$ | 7.6 (AB) 24; 7.0 (CD) 63<br>7.6 (BC) 128; 7.2 (BJ) 63<br>7.8 (N) 74; 7.7 (M) 74<br>7.3 (U) 74 |
| 11 | $(CH_2)_4C_6H_5$ | $CH_3$ | $CH_3$ | 7.4 (AB) 24; 7.2 (CD) 63<br>7.7 (BC) 128; 7.7 (BJ) 63<br>8.5 (N) 74; 7.5 (M) 74<br>7.4 (U) 74 |
| 12 | $C_2H_5$ | H | $C_6H_5$ | 1.8 (AB) 24; 3.5 (BJ) 39 |
| 13 | $C_6H_5$ | H | $C_6H_5$ | 4.6 (AB) 84; 5.3 (N) 75<br>5.0 (M) 75 |
| 14 | $C_6H_4$-2′-Br | H | $C_6H_5$ | 2.6 (N) 75; 2.6 (M) 75 |
| 15 | $C_6H_4$-2′-F | H | $C_6H_5$ | 3.8 (N) 75; 4.1 (M) 75 |
| 16 | $C_6H_4$-2′-Cl | H | $C_6H_5$ | 3.8 (N) 75; 3.9 (M) 75 |
| 17 | $C_6H_4$-3′-Cl | H | $C_6H_5$ | 6.7 (CD) 63; 6.0 (BJ) 9<br>5.3 (AB) 9 |
| 18 | $C_6H_4$-4′-Cl | H | $C_6H_5$ | 5.0 (AB) 9; 5.0 (AB) 84<br>5.4 (BJ) 9 |
| 19 | $(CH_2)_4C_6H_5$ | H | $C_6H_5$ | 5.9 (AB) 24 |
| 20 | $C_6H_4$-4′-$OCH_2C_6H_5$ | H | $C_6H_5$ | 4.9 (AB) 84 |
| 21 | $C_6H_4$-4′-Cl | H |  | 3.5 (AB) 84 |
| 22 | $C_6H_4$-3′-Cl | H | $CH_2C_6H_5$ | 6.0 (AB) 24; 6.9 (CD) 63<br>6.0 (BJ) 63; 6.6 (N) 74<br>6.5 (M) 74; 6.8 (U) 74 |
| 23 | $C_6H_4$-3′-Cl | H | $(CH_2)_2C_6H_5$ | 7.1 (AB) 24 |
| 24 | $C_6H_4$-3′-Cl | H | $C_6H_4$-4′-$NHCOCH_3$ | 3.7 (AB) 24; 3.7 (BJ) 39<br>3.9 (N) 74; 4.0 (M) 74<br>3.6 (U) 74 |
| 25 | $(CH_2)_4C_6H_5$ | H | $C_6H_4$-4′-$NHCOCH_3$ | 6.2 (AB) 24; 6.4 (BJ) 39<br>6.3 (N) 74; 6.3 (M) 74<br>6.9 (U) 74 |
| 26 | $C_6H_5$ | $CH_3$ | $CH_2OC_6H_5$ | 5.6 (AB) 60 |
| 27 | $C_6H_5$ | $CH_3$ | $(CH_2)_2C_6H_5$ | 5.6 (AB) 60 |
| 28 | $C_6H_5$ | $CH_3$ | $(CH_2)_3C_6H_4$-4′-$NHCOCH_2Br$ | 5.8 (AB) 61; 6.6 (N) 61<br>5.9 (U) 61 |
| 29 | $C_6H_5$ | $CH_3$ | $(CH_2)_3OC_6H_4$-2′-$NHCOCH_2Br$ | 5.6 (AB) 61; 6.0 (N) 61<br>5.8 (U) 61 |
| 30 | $C_6H_5$ | $CH_3$ | $(CH_2)_3OC_6H_4$-3′-$NHCOCH_2Br$ | 6.4 (AB) 61; 6.7 (N) 61<br>6.5 (U) 61 |
| 31 | $C_6H_5$ | $CH_3$ | $(CH_2)_3OC_6H_4$-4′-$NHCOCH_2Br$ | 6.6 (AB) 61; 6.2 (N) 61<br>6.2 (U) 61 |
| 32 | $C_6H_4$-4′-Cl | | $(CH_2)_2CH(CH_3)(CH_2)_2$ | 5.0 (AB) 84 |
| 33 | $OCH_2C_6H_5$ | | $(CH_2)_2CH(CH_3)(CH_2)_2$ | 5.1 (AB) 84 |
| 34 | $C_6H_3$-2′,3′-$(CH_2CH_2CH_2CH_2)$ | | $(CH_2)_2CH(CH_3)(CH_2)_2$ | 3.3 (AB) 84 |
| 35 | $C_6H_2$-2′,3′-(CH=CHCH=CH)-4′-Cl | | $(CH_2)_2CH(CH_3)(CH_2)_2$ | 3.4 (AB) 84 |
| 36 | $C_6H_4$-4′-Cl | |  | 3.9 (AB) 84 |
| 37 | $C_6H_4$-4′-Cl | | $CH_3$ (1,2-dehydro) | (AB) 27 |
| 38 | $C_6H_4$-4′-Cl | | $n$-$C_5H_{11}$ (1,2-dehydro) | 5.5 (AB) 27 |
| 39 | $C_6H_4$-4′-Cl | | $C_6H_5$ (1,2-dehydro) | 5.6 (AB) 27 |
| 40 | $C_6H_4$-4′-Cl | | $C_6H_4$-3′-Cl (1,2-dehydro) | (AB) 27 |
| 41 | $C_6H_4$-4′-Cl | | $C_6H_4$-4′-Cl (1,2-dehydro) | 5.7 (AB) 27 |
| 42 | $C_6H_4$-4′-Cl | | $CH_2C_6H_5$ (1,2-dehydro) | (AB) 27 |
| 43 | $C_6H_4$-4′-Cl | | $(CH_2)_5C_6H_5$ (1,2-dehydro) | 6.8 (AB) 27 |

**TABLE XII. Inhibition by Substituted Pyrimidines**



| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| 1 | H | $NH_2$ | $i$-$C_5H_{11}$ | $CH_3$ | 3.9 (AB) 57 |
| 2 | H | $NH_2$ | $C_6H_4$-4′-Cl | $CH_3$ | 3.4 (AB) 57 |
| 3 | H | $NH_2$ | $(CH_2)_3NHC_6H_5$ | $CH_3$ | 3.9 (AB) 57 |
| 4 | H | $NH_2$ | $(CH_2)_3N(C_6H_5)SO_2C_6H_4$-4′-$CH_3$ | $CH_3$ | 3.9 (AB) 11 |
| 5 | H | OH | $(CH_2)_3NHC_6H_5$ | $CH_3$ | 2.8 (AB) 6 |
| 6 | H | SH | $(CH_2)_3NHC_6H_5$ | $CH_3$ | 4.1 (AB) 6 |
| 7 | $NH_2$ | H | $CH{=}CH_2$ | $C_6H_5$ | 4.4 (BJ) 218 |
| 8 | $NH_2$ | H | $CH{=}CH_2$ | $C_6H_4$-4′-$OCH_3$ | 4.4 (BJ) 218 |
| 9 | $NH_2$ | H | $C_2H_5$ | $C_6H_4$-4′-$OCH_3$ | 3.9 (BJ) 218 |
| 10 | $NH_2$ | H | $CH{=}CHCH_3$ | $C_6H_4$-4′-$OCH_3$ | 4.2 (BJ) 218 |
| 11 | $NH_2$ | H | $C_3H_7$ | $C_6H_5$ | 4.1 (BJ) 218 |
| 12 | $NH_2$ | H | $CH(CH_3)SCH_3$ | $C_6H_4$-4′-$OCH_3$ | 4.4 (BJ) 218 |
| 13 | $NH_2$ | H | $i$-$C_5H_{11}$ | $CH_3$ | 3.1 (AB) 57 |
| 14 | $NH_2$ | H | $C_6H_4$-4′-Cl | $CH_3$ | 2.8 (AB) 57 |
| 15 | $NH_2$ | H | $(CH_2)_3NHC_6H_5$ | $CH_3$ | 3.3 (AB) 57 |
| 16 | $NH_2$ | H | $(CH_2)_3NHC_6H_5$ | $C_6H_5$ | (AB) 37 |
| 17 | $NH_2$ | $NH_2$ | H | H | 7.0 (BJ) 212; (BI, BK) 198 |
| 18 | $NH_2$ | $NH_2$ | H | $CH_3$ | 3.0 (AB) 21 |
| 19 | $NH_2$ | $NH_2$ | H | $C_6H_5$ | 3.8 (AB) 50 |
| 20 | $NH_2$ | $NH_2$ | H | $C_6H_4$-4′-$C_6H_5$ | 4.5 (AB) 37 |
| 21 | $NH_2$ | $NH_2$ | H | $NH_2$ | 2.9 (AB) 19 |
| 22 | $NH_2$ | $NH_2$ | H | OH | 1.9 (AB) 19 |
| 23 | $NH_2$ | $NH_2$ | $C_2H_5$ | OH | 1.8 (AB) 21 |
| 24 | $NH_2$ | $NH_2$ | $n$-$C_3H_7$ | $CH_3$ | 5.1 (O) 149 |
| 25 | $NH_2$ | $NH_2$ | $n$-$C_3H_7$ | $NH_2$ | 3.5 (AB) 21 |
| 26 | $NH_2$ | $NH_2$ | $n$-$C_3H_7$ | OH | 3.3 (AB) 28 |
| 27 | $NH_2$ | $NH_2$ | $CH_2CH{=}CHCH_3$ | OH | 4.4 (AB) 28 |
| 28 | $NH_2$ | $NH_2$ | $CH_2CH_2CH{=}CH_2$ | OH | 4.0 (AB) 28 |
| 29 | $NH_2$ | $NH_2$ | $n$-$C_4H_9$ | $CH_3$ | 5.7 (AB) 21 |
| 30 | $NH_2$ | $NH_2$ | $n$-$C_4H_9$ | $NH_2$ | 4.6 (AB) 21 |
| 31 | $NH_2$ | $NH_2$ | $n$-$C_4H_9$ | $C_6H_5$ | 4.5 (AB) 37 |
| 32 | $NH_2$ | $NH_2$ | $n$-$C_4H_9$ | OH | 4.4 (AB) 28 |
| 33 | $NH_2$ | $NH_2$ | $t$-$C_4H_9$ | $CH_3$ | 5.5 (O) 149 |
| 34 | $NH_2$ | $NH_2$ | $CH(CH_3)CH_2CH_3$ | OH | 3.2 (AB) 28 |
| 35 | $NH_2$ | $NH_2$ | $n$-$C_5H_{11}$ | $CH_3$ | 6.0 (AB) 27 |
| 36 | $NH_2$ | $NH_2$ | $i$-$C_5H_{11}$ | $CH_3$ | 6.6 (AB) 21 |
| 37 | $NH_2$ | $NH_2$ | $i$-$C_5H_{11}$ | Cl | 4.1 (AB) 57 |
| 38 | $NH_2$ | $NH_2$ | $i$-$C_5H_{11}$ | $NH_2$ | 5.7 (AB) 21 |
| 39 | $NH_2$ | $NH_2$ | $i$-$C_5H_{11}$ | OH | 5.4 (AB) 28 |
| 40 | $NH_2$ | $NH_2$ | $CH_2CH{=}C(CH_3)_2$ | OH | 4.8 (AB) 28 |
| 41 | $NH_2$ | $NH_2$ | $CH(CH_3)CH_2CH_2CH_3$ | OH | 4.1 (AB) 28 |
| 42 | $NH_2$ | $NH_2$ | $CH_2CH(CH_3)CH_2CH_3$ | OH | 4.8 (AB) 28 |
| 43 | $NH_2$ | $NH_2$ | c-$C_5H_9$ | OH | 3.3 (AB) 28 |
| 44 | $NH_2$ | $NH_2$ | $n$-$C_6H_{13}$ | $CH_3$ | 6.9 (O) 149 |
| 45 | $NH_2$ | $NH_2$ | $n$-$C_6H_{13}$ | OH | 4.5 (AB) 21 |
| 46 | $NH_2$ | $NH_2$ | $CH(CH_3)CH_2CH(CH_3)_2$ | OH | 5.1 (AB) 28 |
| 47 | $NH_2$ | $NH_2$ | c-$C_6H_{11}$ | $CH_3$ | 6.9 (O) 149 |
| 48 | $NH_2$ | $NH_2$ | c-$C_6H_{11}$ | OH | 4.4 (AB) 28 |
| 49 | $NH_2$ | $NH_2$ | $CH_2$-c-$C_6H_{11}$ | $CH_3$ | 6.4 (O) 187 |
| 50 | $NH_2$ | $NH_2$ | $CH_2CH_2$-c-$C_6H_{11}$ | $CH_3$ | 6.6 (O) 187 |
| 51 | $NH_2$ | $NH_2$ | $n$-$C_7H_{15}$ | $CH_3$ | 6.7 (O) 149 |
| 52 | $NH_2$ | $NH_2$ | $n$-$C_8H_{17}$ | $CH_3$ | 6.7 (O) 149 |
| 53 | $NH_2$ | $NH_2$ | $n$-$C_8H_{17}$ | OH | 4.3 (AB) 21 |
| 54 | $NH_2$ | $NH_2$ | adamantyl | H | 8.2 (O) 143; 6.7 (O) 138<br>5.7 (BJ) 143 |
| 55 | $NH_2$ | $NH_2$ | adamantyl | $CH_3$ | 6.5 (O) 143; 8.2 (O) 138<br>9.2 (O) 234; 7.1 (BJ) 143<br>9.4 (E) 234 |
| 56 | $NH_2$ | $NH_2$ | adamantyl | $C_2H_5$ | 9.9 (E) 234; 8.7 (O) 158<br>9.9 (O) 234 |
| 57 | $NH_2$ | $NH_2$ | adamantyl | $C_4H_9$ | 6.4 (O) 158 |
| 58 | $NH_2$ | $NH_2$ | NHCO-adamantyl | $CH_3$ | 4.9 (O) 143; 4.9 (O) 138<br>3.6 (BJ) 143 |
| 59 | $NH_2$ | $NH_2$ | $CH_2NHCOCH_2$-adamantyl | H | (O, BJ) 143; (O) 138 |
| 60 | $NH_2$ | $NH_2$ | 1′-$C_{10}H_7$ | $CH_3$ | 4.2 (O) 187 |
| 61 | $NH_2$ | $NH_2$ | 2′-$C_{10}H_7$ | $CH_3$ | 7.1 (O) 187 |
| 62 | $NH_2$ | $NH_2$ | $n$-$C_{10}H_{21}$ | $CH_3$ | 7.6 (O) 149 |
| 63 | $NH_2$ | $NH_2$ | $CH_2NHCO(CH_2)_8CH_3$ | H | 5.0 (O) 143 |
| 64 | $NH_2$ | $NH_2$ | $C_6H_5$ | OH | 5.7 (AB) 28 |
| 65 | $NH_2$ | $NH_2$ | $C_6H_5$ | $CH_2C_6H_5$ | (BN) 238 |
| 66 | $NH_2$ | $NH_2$ | $C_6H_4$-2′-Br | H | 3.9 (M) 104; 3.7 (AA) 104 |

**TABLE XII** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| | | | | | 3.8 (AL) 104 |
| 67 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-$CH_3$ | $CH_3$ | 5.0 (M) 104; 6.8 (AA) 104 |
| | | | | | 6.0 (AL) 104 |
| 68 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | H | 5.8 (M) 104; 5.0 (AA) 104 |
| | | | | | 5.0 (AL) 104; 6.1 (AB) 9 |
| 69 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $CH_3$ | 5.9 (M) 104; 5.6 (AA) 104 |
| | | | | | 6.1 (AB) 104; 5.7 (AL) 104 |
| 70 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $CF_3$ | 4.3 (AB) 27 |
| 71 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $CH_2Br$ | 6.1 (AB) 215 |
| 72 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $COO^-$ | 3.0 (AB) 25 |
| 73 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $C_2H_5$ | 8.4 (M) 69; 6.1 (M) 32 |
| | | | | | 8.9 (M) 136; 6.2 (M) 104 |
| | | | | | 6.1 (M) 88; 6.7 (D) 88 |
| | | | | | 5.7 (A) 32; 7.5 (K) 32 |
| | | | | | 7.3 (K) 45; 6.3 (J) 32 |
| | | | | | 5.6 (BJ) 88; 4.6 (BJ) 32 |
| | | | | | 5.6 (BJ) 115; 9.4 (AP) 116 |
| | | | | | 10.3 (AP) 144; (AP) 132 |
| | | | | | 9.3 (AP) 115; 9.3 (AP) 88 |
| | | | | | 7.5 (BJ) 212; 6.2 (AA) 104 |
| | | | | | 8.4 (AA) 181; 6.7 (AN) 144 |
| | | | | | 6.7 (AN) 115; 6.7 (AN) 90 |
| | | | | | 6.0 (BR) 220; 8.9 (Q) 136 |
| | | | | | 6.1 (BQ) 220; 8.7 (C) 136 |
| | | | | | 8.8 (AQ) 211; 5.1 (AM) 90 |
| | | | | | 6.7 (AI) 90; 6.0 (AK) 90 |
| | | | | | 6.7 (AL) 90; 6.1 (AL) 104 |
| | | | | | 6.7 (AL) 67; 7.6 (I) 182 |
| | | | | | 6.0 (Z) 88; 5.6 (G) 211 |
| | | | | | 5.5 (AT) 115; 8.5 (AS) 181 |
| | | | | | 6.6 (AO) 90; 6.6 (AJ) 90 |
| | | | | | 6.1 (AR) 115; 6.3 (AW) 220 |
| | | | | | 6.6 (BO) 220; 7.6 (BE) 147 |
| | | | | | 4.8 (BG) 32; 5.5 (AZ) 32 |
| | | | | | 5.2 (AU) 144; 4.0 (BX) 144 |
| | | | | | 4.4 (BW) 144; 6.2 (BU) 144 |
| | | | | | 4.8 (BV) 144; 4.4 (BY) 144 |
| | | | | | 5.1 (BC) 128; (BI) 198 |
| | | | | | (BK) 198; (BN) 238 |
| | | | | | (AY) 220; (W) 249 |
| | | | | | (E) 240 |
| 74 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $COOC_2H_5$ | 3.8 (AB) 25 |
| 75 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $OC_2H_5$ | 4.4 (BJ) 218 |
| 76 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $OC_3H_7$ | 5.0 (BJ) 218 |
| 77 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $CH_2C_6H_5$ | (BN) 238 |
| 78 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $(CH_2)_2C_6H_4$-4′-$NHCOCH_2Br$ | 7.0 (BJ) 52; 6.3 (AB) 56 |
| | | | | | 6.6 (N) 73; 6.6 (U) 73 |
| 79 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $(CH_2)_2C_6H_4$-3′-$NHCOC_6H_4$-4″-$SO_2F$ | 5.0 (N) 77; 5.3 (U) 77 |
| 80 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $(CH_2)_2C_6H_4$-3′-$NHCONHC_6H_4$-3″-$SO_2F$ | 5.8 (M) 77; 6.1 (N) 77 |
| | | | | | 5.8 (U) 77 |
| 81 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $(CH_2)_2C_6H_4$-4′-$NHCONHC_6H_4$-3″-$SO_2F$ | 6.4 (M) 77; 6.1 (N) 77 |
| | | | | | 5.8 (U) 77 |
| 82 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $(CH_2)_4NHCOCH_2Br$ | 5.1 (N) 73; 6.2 (U) 73 |
| 83 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $CH_2NHC_6H_4$-4′-$COCH_2Cl$ | 5.2 (N) 73 |
| | | | | | 5.3 (U) 73 |
| 84 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $(CH_2)_2NHCONHC_6H_4$-3″-$SO_2F$ | 5.4 (N) 73 |
| | | | | | 5.1 (U) 73 |
| 85 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $CH_2OC_6H_4$-4′-$NHCOCH_2Br$ | 4.9 (N) 73 |
| | | | | | 5.8 (U) 73 |
| 86 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $CH_2OC_6H_4$-4′-$COCH_2Cl$ | 4.9 (N) 73 |
| | | | | | 6.0 (U) 73 |
| 87 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $CH_2OC_6H_4$-3′-$NHCOC_6H_4$-3″-$SO_2F$ | 4.5 (U) 77 |
| | | | | | (N) 77 |
| 88 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $CH_2OC_6H_4$-4′-$NHCOC_6H_4$-3″-$SO_2F$ | 5.3 (M) 77 |
| | | | | | 5.3 (N) 73 |
| | | | | | 4.3 (U) 77 |
| 89 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $CH_2OC_6H_4$-3′-$NHCONHC_6H_4$-3″-$SO_2F$ | 5.9 (N) 73 |
| | | | | | 6.3 (U) 73 |
| 90 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $CH_2OC_6H_4$-4′-$NHCONHC_6H_4$-3″-$SO_2F$ | 5.8 (M) 77 |
| | | | | | 6.8 (N) 77 |
| | | | | | 5.2 (U) 77 |
| 91 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-Cl | $CH_2OC_6H_4$-4′-$CH_2NHCOC_6H_4$-3″-$SO_2F$ | 6.5 (M) 77; 6.4 (N) 77 |
| | | | | | 6.2 (U) 77 |
| 92 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | Me | 8.1 (K) 45; 8.7 (O) 234 |
| | | | | | 9.0 (E) 234; 7.0 (D) 266 |
| | | | | | 5.0 (M) 104; 5.0 (AA) 104 |
| | | | | | 5.7 (AL) 104; (M) 200 |

**TABLE XII** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| | | | | | 8.0 (Q) 9; 7.5 (AB) 9 |
| | | | | | 6.3 (AA) 9 |
| 93 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $C_2H_5$ | 7.7 (AB) 9; 7.7 (BJ) 9 |
| 94 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH{=}CHC_6H_5$ | 7.6 (Q) 119 |
| 95 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2$-$CH_2C_6H_5$ | 7.5 (Q) 119 |
| 96 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH{=}CHCH_2C_6H_5$ | 7.7 (Q) 119 |
| 97 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2CH_2CH_2C_6H_5$ | 7.5 (Q) 119 |
| 98 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH{=}CH)_2C_6H_5$ | 7.0 (Q) 119 |
| 99 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_4C_6H_5$ | 7.7 (Q) 119 |
| 100 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH{=}CHC_6H_4$-4′-$SO_2F$ | 7.4 (Q) 119 |
| 101 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$SO_2F$ | 7.4 (Q) 119 |
| 102 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$NHCOCH_2Br$ | 6.2 (N) 73; 6.2 (U) 73 |
| 103 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH{=}CH$-1′-$C_{10}H_7$ | 7.4 (Q) 119 |
| 104 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH{=}CH$-2′-$C_{10}H_7$ | 6.9 (Q) 119 |
| 105 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2$-1′-$C_{10}H_7$ | 7.1 (Q) 119 |
| 106 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2$-2′-$C_{10}H_7$ | 6.6 (Q) 119 |
| 107 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_5$ | 7.1 (Q) 119 |
| 108 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-$SO_2F$ | 7.7 (Q) 119 |
| 109 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2O$-1′-$C_{10}H_7$ | 7.5 (Q) 119 |
| 110 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2O$-2′-$C_{10}H_7$ | 6.6 (Q) 119 |
| 111 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$(CH_2)_2C_6H_5$ | 6.8 (Q) 119 |
| 112 | $NH_2$ | $NH_2$ | $C_6H_3$-3′4′-$Cl_2$ | $CH{=}CHC_6H_4$-4′-$CH{=}CH$-$C_6H_4$-4″-$SO_2F$ | 6.5 (Q) 119 |
| 113 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH{=}CHC_6H_4$-3′-$CH{=}CHC_6H_4$-4″-$SO_2F$ | 6.5 (Q) 119 |
| 114 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$(CH_2)_2C_6H_4$-4″-$SO_2F$ | 7.3 (U) 119 |
| 115 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$(CH_2)_2$-$C_6H_4$-3″-$SO_2F$ | 7.4 (U) 119 |
| 116 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-3′-$(CH_2)_2C_6H_4$-4″-$SO_2F$ | 6.5 (U) 119 |
| 117 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2$-4′-$C_5H_4N^+CH_2C_6H_4$-3″-$SO_2F$ | 6.8 (U) 121 |
| 118 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2$-4′-$C_5H_4N^+CH_2C_6H_4$-4″-$SO_2F$ | 6.4 (U) 121 |
| 119 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2$-3′-$C_5H_4N^+CH_2C_6H_4$-3″-$SO_2F$ | 6.3 (U) 121 |
| 120 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2$-3′-$C_5H_4N^+CH_2C_6H_4$-4″-$SO_2F$ | 6.7 (U) 121 |
| 121 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$NHCOC_6H_4$-3″-$SO_2F$ | 6.6 (N) 77 |
| | | | | | 6.1 (U) 77 |
| | | | | | 7.5 (Q) 94 |
| 122 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$NHCOC_6H_4$-4″-$SO_2F$ | 7.2 (U) 94 |
| 123 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$CH_2NHCOC_6H_4$-3″-$SO_2F$ | 7.6 (U) 96 |
| 124 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$CH_2NHCOC_6H_4$-4″-$SO_2F$ | 7.6 (Q) 96 |
| 125 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-3′-$CH_2NHCOC_6H_4$-4″-$SO_2F$ | 7.2 (Q) 108 |
| 126 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$NHCONHC_6H_4$-4″-$SO_2F$ | 6.1 (U) 94 |
| 127 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-3′-$CH_2NHCONHC_6H_4$-4″-$SO_2F$ | 7.1 (Q) 108 |
| 128 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-3′-$CH_2NHCONHC_6H_4$-3″-$SO_2F$ | 7.1 (Q) 108 |
| 129 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$CH_2NHCONHC_6H_4$-4″-$SO_2F$ | 7.5 (U) 96 |
| 130 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$CH_2NHCONHC_6H_4$-3″-$SO_2F$ | 7.5 (U) 96 |
| 131 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-3′-$CH_2NHSO_2C_6H_4$-4″-$SO_2F$ | 7.3 (Q) 108 |
| 132 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-3′-$CH_2NHSO_2C_6H_4$-3″-$SO_2F$ | 7.0 (Q) 108 |
| 133 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_4C_6H_4$-4′-$NHCOC_6H_4$-3″-$SO_2F$ | 6.8 (Q) 108 |
| 134 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_4$-3′-$C_5H_4N^+CH_2C_6H_4$-4″-$SO_2F$ | 7.3 (U) 121 |
| 135 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$NHCOC_6H_4$-4″-$SO_2F$ | 6.7 (U) 94 |
| 136 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$NHSO_2C_6H_4$-3″-$SO_2F$ | 7.7 (U) 94 |
| 137 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$NHSO_2C_6H_4$-4″-$SO_2F$ | 7.3 (U) 94 |
| 138 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2NHC_6H_4$-4′-$COCH_2Cl$ | 6.6 (N) 73; 6.0 (U) 73 |
| 139 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$NHCOC_6H_4$-3″-$SO_2F$ | 6.7 (N) 77; 6.1 (U) 77 |
| 140 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$CH_2NHCOC_6H_4$-3″-$SO_2F$ | 6.1 (U) 95; 6.5 (Q) 91 |
| 141 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-3′-$CH_2NHCONHC_6H_4$-3″-$SO_2F$ | 7.2 (Q) 108 |
| 142 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$NHCONHC_6H_4$-3″-$SO_2F$ | 6.8 (M) 77; 6.8 (N) 77 |
| | | | | | 6.0 (U) 77 |
| 143 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-3′-$NHCONHC_6H_4$-3″-$SO_2F$ | 6.1 (M) 77; 6.2 (N) 77 |
| | | | | | 5.8 (U) 77 |
| 144 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2C_6H_4$-4′-$NHCOC_6H_4$-4″-$SO_2F$ | 5.7 (U) 93 |
| 145 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-3′-$NHCONHC_6H_4$-3″-$SO_2F$ | 6.2 (N) 73; 5.8 (U) 73 |
| 146 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-3′-$CH_2NHCOC_6H_4$-3″-$SO_2F$ | 7.2 (U) 108 |
| 147 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-3′-$CH_2NHCOC_6H_4$-4″-$SO_2F$ | 7.2 (U) 108 |
| 148 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-3′-$CH_2NHSO_2C_6H_4$-3″-$SO_2F$ | 7.0 (U) 108 |
| 149 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-3′-$CH_2NHSO_2C_6H_4$-4″-$SO_2F$ | 7.2 (U) 108 |
| 150 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$CH_2NHSO_2C_6H_4$-3″-$SO_2F$ | 7.2 (U) 95 |
| 151 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$CH_2NHSO_2C_6H_4$-4″-$SO_2F$ | 6.8 (U) 95 |
| 152 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-3′-$CH_2NHCONHC_6H_4$-4″-$SO_2F$ | 7.1 (U) 108 |
| 153 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-3′-$CH_2NHCONHC_6H_4$-3″-$SO_2F$ | 6.8 (U) 95 |
| 154 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$CH_2NHCONHC_6H_4$-4″-$SO_2F$ | 6.8 (U) 95 |
| 155 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$NHCOC_6H_4$-3″-$SO_2F$ | 6.3 (U) 94 |
| 156 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$NHCOC_6H_4$-4″-$SO_2F$ | 6.5 (U) 94 |
| 157 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$NHCOC_6H_4$-3″-$SO_2F$ | 6.5 (U) 94 |
| 158 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$NHCOC_6H_4$-4″-$SO_2F$ | 6.9 (U) 94 |
| 159 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$NHCONHC_6H_4$-4″-$SO_2F$ | 5.1 (U) 93 |
| 160 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$NHCONHC_6H_4$-4″-$SO_2F$ | 6.5 (U) 93 |

**TABLE XII** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| 161 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Cl-4′-$NHCONHC_6H_4$-4″-$SO_2F$ | 6.0 (U) 93 |
| 162 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$NHCONHC_6H_4$-3″-$SO_2F$ | 6.5 (U) 92; 7.2 (Q) 92 |
| 163 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Cl-4′-$NHCONHC_6H_4$-3″-$SO_2F$ | 6.5 (U) 92 |
| 164 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$NHCONHC_6H_4$-3″-$SO_2F$ | 5.7 (U) 92 |
| 165 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′,6′-$(Me)_2$-4′-$NHCONHC_6H_4$-3″-$SO_2F$ | 5.5 (U) 92 |
| 166 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$NHSO_2C_6H_4$-3″-$SO_2F$ | 7.5 (Q) 108 |
| 167 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$NHSO_2C_6H_4$-3″-$SO_2F$ | 7.7 (Q) 108 |
| 168 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$NHSO_2C_6H_4$-4″-$SO_2F$ | 7.3 (Q) 108 |
| 169 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$NHSO_2C_6H_4$-4″-$SO_2F$ | 7.3 (Q) 108 |
| 170 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-OMe-4′-$CH_2NHCOC_6H_4$-3″-$SO_2F$ | 7.3 (Q) 107 |
| 171 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$CH_2NHCOC_6H_4$-3″-$SO_2F$ | 7.3 (Q) 107 |
| 172 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$CH_2NHCOC_6H_4$-3″-$SO_2F$ | 7.0 (Q) 107 |
| 173 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-OMe-4′-$CH_2NHCOC_6H_4$-4″-$SO_2F$ | 7.0 (Q) 107 |
| 174 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$CH_2NHCOC_6H_4$-4″-$SO_2F$ | 7.3 (Q) 107 |
| 175 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$CH_2NHCOC_6H_4$-4″-$SO_2F$ | 7.0 (Q) 107 |
| 176 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-OMe-4′-$CH_2NHCOC_6H_4$-3″-$SO_2F$ | 7.3 (Q) 107 |
| 177 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$CH_2NHCOC_6H_4$-3″-$SO_2F$ | 7.2 (Q) 107 |
| 178 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$CH_2NHCOC_6H_4$-3″-$SO_2F$ | 6.8 (U) 107 |
| 179 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-OMe-4′-$CH_2NHCONHC_6H_4$-4″-SO2F | 7.0 (Q) 107 |
| 180 | $NH_2$ | $NH_2$ | $C_6$H3-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$CH_2NHCONHC_6H_4$-4″-$SO_2F$ | 7.2 (Q) 107 |
| 181 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$CH_2NHCONHC_6H_4$-4″-$SO_2F$ | 7.1 (Q) 107 |
| 182 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$CH_2NHCONHC_6H_3$-3″-Me-4″-$SO_2F$ | 6.9 (Q) 107 |
| 183 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-OMe-4′-$CH_2NHSO_2C_6H_4$-3″-$SO_2F$ | 7.4 (Q) 107 |
| 184 | $NH_2$ | $NH_2$ | $C_6H_3$-3′-,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$CH_2NHSO_2C_6H_4$-3″-$SO_2F$ | 7.4 (Q) 107 |
| 185 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$CH_2NHSO_2C_6H_4$-3″-$SO_2F$ | 7.2 (Q) 107 |
| 186 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-OMe-4′-$CH_2NHSO_2C_6H_4$-4″-$SO_2F$ | 7.1 (Q) 107 |
| 187 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$CH_2NHSO_2C_6H_4$-4″-$SO_2F$ | 6.9 (Q) 107 |
| 188 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$CH_2NHSO_2C_6H_4$-4″-$SO_2F$ | 7.0 (Q) 107 |
| 189 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$NHCOC_6H_3$-4″-Me-3″-$SO_2F$ | 6.8 (U) 94 |
| 190 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$NHCOC_6H_3$-2″-Cl-5″-$SO_2F$ | 7.1 (U) 94 |
| 191 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$NHCONHC_6H_3$-3″-$SO_2F$-4″-OMe | 6.8 (U) 92 |
| 192 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$NHCONHC_6H_3$-3″-$SO_2F$-4″-OEt | 6.3 (U) 92 |
| 193 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$NHCONHC_6H_3$-3″-$SO_2F$-6″-OMe | 6.3 (U) 92 |
| 194 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$NHCONHC_6H_3$-3″-$SO_2F$-6″-Cl | 6.3 (U) 92 |
| 195 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$NHCONHC_6H_3$-2″-Cl-5″-$SO_2F$ | 7.1 (U) 94 |
| 196 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$NHCONHC_6H_3$-3″-Cl-4″-$SO_2F$ | 6.0 (U) 94 |
| 197 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$CH_2NHCOC_6H_3$-2″-Cl-5″-$SO_2F$ | 7.7 (U) 96 |
| 198 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $(CH_2)_2C_6H_4$-4′-$CH_2NHCOC_6H_3$-3″-$SO_2F$-4″-Me | 7.6 (U) 96 |
| 199 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$CH_2NHCOC_6H_3$-3″-$SO_2F$-4″-Cl | 7.5 (Q) 95<br>7.0 (U) 95 |
| 200 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$CH_2NHCOC_6H_3$-3″-$SO_2F$-4″-Me | 7.7 (U) 95<br>(Q) 95 |
| 201 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$CH_2NHCOC_6H_3$-3″-$SO_2F$-4″-*i*-$C_3H_7$ | 6.9 (U) 95<br>(Q) 95 |
| 202 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$CH_2NHCOC_6H_3$-2″-Cl-5″-$SO_2F$ | 7.0 (U) 95 |
| 203 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2SC_6H_4$-4′-$NHCONHC_6H_4$-3″-$SO_2F$ | (Q) 108 |
| 204 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$NHCONHC_6H_3$-3″-Me-4″-$SO_2F$ | 5.7 (U) 93 |
| 205 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$CH_2NHCONHC_6H_3$-3″-$SO_2F$-4″-Me | 7.0 (U) 95 |
| 206 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$CH_2NHCONHC_6H_3$-2″-Cl-5″-$SO_2F$ | 6.4 (U) 95 |
| 207 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$CH_2NHCONHC_6H_3$-3″-Me-4″-$SO_2F$ | 6.4 (U) 95 |
| 208 | $NH_2$ | $NH_2$ | $C_6H_3$-3′-,4′-$Cl_2$ | $CH_2OC_6H_4$-3′-$CH_2NHCONHC_6H_3$-3″-$SO_2F$-4″-Me | 7.3 (U) 108 |
| 209 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-3′-$CH_2NHCONHC_6H_3$-2″-Cl-5″-$SO_2F$ | 6.5 (Q) 108 |
| 210 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$NHCOC_6H_3$-2″-Cl-5″-$SO_2F$ | 6.8 (U) 94 |
| 211 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$NHCOC_6H_3$-2′-Cl-5″-$SO_2F$ | 7.0 (U) 94 |
| 212 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$CH_2NHCONHC_6H_3$-2′-Cl-5″-$SO_2F$ | 7.4 (Q) 107 |
| 213 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$CH_2NHCONHC_6H_3$-3″-$SO_2F$-4″-Me | 6.8 (Q) 107 |
| 214 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$NHCOC_6H_3$-4″-Me-3″-$SO_2F$ | 6.3 (U) 94 |
| 215 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$NHCONHC_6H_3$-3″-Me-4″-$SO_2F$ | 6.8 (U) 93 |
| 216 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Cl-4′-$NHCONHC_6H_3$-3″-Me-4″-$SO_2F$ | 6.0 (U) 93 |
| 217 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Cl-4′-$NHCONHC_6H_3$-3″-Cl-4″-$SO_2F$ | 5.7 (U) 93 |
| 218 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Cl-4′-$NHCONHC_6H_3$-3″-OMe-4″-$SO_2F$ | 6.3 (U) 93 |
| 219 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Cl-4′-$NHCONHC_6H_3$-3″-$SO_2F$-4″-OMe | 6.7 (U) 92 |
| 220 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Cl-4′-$NHCONHC_6H_3$-3″-$SO_2F$-6″-OMe | 6.6 (U) 92 |
| 221 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$NHCONHC_6H_3$-3″-$SO_2F$-6″-OMe | 6.9 (U) 92 |
| 222 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Cl-4′-$NHCONHC_6H_3$-3″-$SO_2F$-6″-Cl | 6.2 (U) 92 |
| 223 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-3′-Me-4′-$NHCONHC_6H_3$-3″-$SO_2F$-6″-Cl | 6.9 (U) 92 |
| 224 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$CH_2NHCOC_6H_3$-2″-Cl-5″-$SO_2F$ | 7.0 (Q) 107 |
| 225 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_3$-2′-Cl-4′-$CH_2NHCOC_6H_3$-4″-Me-3″-$SO_2F$ | 6.9 (Q) 107 |
| 226 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$(CH_2)_2NHCOC_6H_4$-3″-$SO_2F$ | 7.3 (Q) 108 |
| 227 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$(CH_2)_2NHCOC_6H_4$-4″-$SO_2F$ | 6.8 (Q) 108 |
| 228 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$(CH_2)_2NHCONHC_6H_4$-3″-$SO_2F$ | 7.0 (Q) 108 |
| 229 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$(CH_2)_2NHCONHC_6H_4$-4″-$SO_2F$ | 6.8 (Q) 108 |
| 230 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$(CH_2)_2NHSO_2C_6H_4$-3″-$SO_2F$ | 7.3 (Q) 108 |
| 231 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$Cl_2$ | $CH_2OC_6H_4$-4′-$(CH_2)_2NHSO_2C_6H_4$-4″-$SO_2F$ | 7.0 (Q) 108 |
| 232 | $NH_2$ | $NH_2$ | $C_6H_3$-3′-$NH_2$-4′-Cl | $C_2H_5$ | 5.8 (M) 104 |

**TABLE XII** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| | | | | | 5.8 (AA) 104 |
| | | | | | 5.6 (AL) 104 |
| 233 | $NH_2$ | $NH_2$ | $C_6H_3$-3′-$NO_2$-4′-Cl | $C_2H_5$ | 7.0 (M) 104 |
| | | | | | 8.2 (AA) 104 |
| | | | | | 7.8 (AL) 104 |
| 234 | $NH_2$ | $NH_2$ | $C_6H_3$-3′,4′-$(OMe)_2$ | $CH_3$ | 3.5 (M) 104 |
| | | | | | 5.5 (AA) 104 |
| | | | | | 5.1 (AL) 104 |
| 235 | $NH_2$ | $NH_2$ | $C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | 3.6 (BJ) 258 |
| 236 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-$CH_2NHCONHC_6H_5$ | $CH_3$ | 7.5 (U) 155 |
| 237 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-$CH_2NHCONHC_6H_4$-3″-Cl | $CH_3$ | 7.7 (U) 155 |
| 238 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-$CH_2NHCONHC_6H_4$-3″-OMe | $CH_3$ | 7.8 (U) 155 |
| 239 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-$CH_2NHCONHC_6H_4$-3″-$NO_2$ | $CH_3$ | 8.0 (U) 155 |
| 240 | $NH_2$ | $NH_2$ | $C_6H_4$′-$CH_2NHCONHC_6H_4$-3″-CN | $CH_3$ | 7.7 (U) 155 |
| 241 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-$CH_2NHCONHCH_6H_4$-3″-$CONMe_2$ | $CH_3$ | 7.9 (U) 155 |
| 242 | $NH_2$ | $NH_2$ | $C_6H_4$-4′-$CH_2NHCONHC_6H_3$-3″-Me-4″-$SO_2F$ | $CH_3$ | 8.0 (U) 155 |
| 243 | $NH_2$ | $NH_2$ | 2′-$CH_2$-pyridyl | H | 3.5 (BJ) 201 |
| 244 | $NH_2$ | $NH_2$ | 3′-$CH_2$-pyridyl | H | 5.3 (BJ) 201 |
| 245 | $NH_2$ | $NH_2$ | 4′-$CH_2$-pyridyl | H | 4.6 (BJ) 201 |
| 246 | $NH_2$ | $NH_2$ | 3′-$CH_2$-pyridyl-(N→O) | H | 4.3 (BJ) 201 |
| 247 | $NH_2$ | $NH_2$ | 4′-$CH_2$-pyridyl-(N→O) | H | 4.2 (BJ) 201 |
| 248 | $NH_2$ | $NH_2$ | 3′-$CH_2$-pyridyl-6′-$OCH_3$ | H | 5.7 (BJ) 201 |
| 249 | $NH_2$ | $NH_2$ | 3′-$CH_2$-pyridyl-5′-Br,6′-$OCH_3$ | H | 6.2 (BJ) 201 |
| 250 | $NH_2$ | $NH_2$ | 3′-$CH_2$-pyridyl-5′-$OCH_3$-6′-OH | H | 6.0 (BJ) 201 |
| 251 | $NH_2$ | $NH_2$ | 2′-$CH_2$-pyridyl-4′,6′-$(OCH_3)_2$ | H | 5.4 (BJ) 201 |
| 252 | $NH_2$ | $NH_2$ | 3′-$CH_2$-pyridyl-5′,6′-$(OCH_3)_2$ | H | 6.8 (BJ) 201 |
| 253 | $NH_2$ | $NH_2$ | 3′-$CH_2$-pyridyl-2′,6′-$(OCH_3)_2$ | H | 6.2 (BJ) 201 |
| 254 | $NH_2$ | $NH_2$ | 4′-$CH_2$-pyridyl-2′,6′-$(OCH_3)_2$ | H | 6.7 (BJ) 201 |
| 255 | $NH_2$ | $NH_2$ | $CH_2C_6H_5$ | H | 5.2 (BN) 263; 6.2 (BJ) 259 |
| | | | | | 5.4 (BJ) 34; 5.6 (AZ) 34 |
| | | | | | 6.0 (BG) 34; 5.7 (H) 246 |
| | | | | | 3.9 (F) 260 |
| 256 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-Br | H | 7.0 (BJ) 263; 6.2 (BN) 263 |
| | | | | | 4.7 (F) 260; 6.1 (H) 246 |
| 257 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-Br | H | 6.8 (BJ) 263; 6.2 (BN) 263 |
| | | | | | 4.2 (F) 260; 5.7 (H) 246 |
| 258 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-Cl | H | 6.6 (BJ) 263; 5.9 (BN) 263 |
| | | | | | 4.4 (F) 260; 6.0 (H) 246 |
| 259 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-Cl | H | 6.4 (BJ) 263; 6.2 (BN) 263 |
| | | | | | 4.3 (F) 260; 5.9 (M) 104 |
| | | | | | 4.1 (AA) 104; 5.6 (H) 246 |
| | | | | | 3.7 (AL) 104 |
| 260 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-Cl | $CH_3$ | 5.6 (M) 104; 4.6 (AA) 104 |
| | | | | | 4.3 (AL) 104 |
| 261 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-F | H | 5.4 (BN) 263; 4.3 (F) 260 |
| | | | | | 5.8 (H) 246; 6.2 (BJ) 263 |
| 262 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-F | H | 6.3 (BJ) 263; 5.7 (BN) 263 |
| | | | | | 4.3 (F) 260; 5.7 (H) 246 |
| 263 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-I | H | 7.2 (BJ) 263; 6.7 (BN) 263 |
| | | | | | 4.6 (F) 260 |
| 264 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$NH_2$ | H | 6.3 (BJ) 263; 5.5 (BN) 263 |
| | | | | | 6.3 (BJ) 259; 5.1 (H) 246 |
| | | | | | 3.7 (F) 260 |
| 265 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$NO_2$ | H | 6.0 (BN) 263; 6.2 (BJ) 259 |
| | | | | | 6.2 (BJ) 263; 5.5 (H) 246 |
| | | | | | 3.8 (F) 260 |
| 266 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$NO_2$ | $CH_3$ | 6.0 (M) 104; 4.3 (AA) 104 |
| | | | | | 4.9 (AL) 104 |
| 267 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-OH | H | 6.5 (BJ) 263; 5.8 (BN) 263 |
| | | | | | 3.6 (F) 260 |
| 268 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$CH_3$ | H | 6.7 (BJ) 263; 5.8 (BN) 263 |
| | | | | | 4.1 (F) 260; 5.7 (H) 246 |
| 269 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$CH_3$ | H | 6.5 (BJ) 263; 5.8 (BN) 263 |
| | | | | | 4.2 (F) 260; 5.2 (H) 246 |
| 270 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$CH_2OH$ | H | 6.3 (BJ) 263; 5.7 (BN) 263 |
| | | | | | 3.8 (F) 260 |
| 271 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$CF_3$ | H | 7.0 (BJ) 263; 6.2 (BN) 263 |
| | | | | | 4.3 (F) 260; 6.0 (H) 246 |
| 272 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$OCH_3$ | H | 6.6 (BJ) 263; 6.3 (BN) 263 |
| | | | | | 4.2 (F) 260; 5.5 (H) 246 |
| 273 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$OCH_3$ | H | 3.9 (BJ) 263; 5.9 (BN) 263 |
| | | | | | 4.3 (F) 260; 5.5 (H) 246 |
| 274 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$OCH_3$ | H | 6.8 (BJ) 263; 6.2 (BN) 263 |
| | | | | | 3.7 (F) 260; 5.4 (H) 246 |
| 275 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$OSO_2CH_3$ | H | 6.9 (BJ) 263; 5.9 (BN) 263 |
| | | | | | 4.2 (F) 260 |
| 276 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$N(CH_3)_2$ | H | 6.8 (BJ) 263; 6.2 (BN) 263 |

**TABLE XII** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| | | | | | 3.6 (F) 260; 5.5 (H) 246 |
| 277 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$NHCOCH_3$ | H | 6.9 (BJ) 263; 6.0 (BN) 263<br>4.1 (F) 260; 5.6 (H) 246 |
| 278 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$CH_2OCH_3$ | H | 6.6 (BJ) 263; 5.6 (BN) 263<br>4.5 (F) 260 |
| 279 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$OCH_2CONH_2$ | H | 5.6 (BJ) 263; 6.0 (BN) 263<br>3.7 (F) 260 |
| 280 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$O(CH_2)_2OCH_3$ | H | 6.5 (BJ) 263; 6.1 (BN) 263<br>3.9 (F) 260 |
| 281 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$O(CH_2)_2OCH_3$ | H | 6.4 (BJ) 263; 6.0 (BN) 263<br>3.3 (F) 260 |
| 282 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$O(CH_2)_3CH_3$ | H | 6.8 (BJ) 263; 6.1 (BN) 263<br>5.0 (F) 260 |
| 283 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$O(CH_2)_3CH_3$ | H | 6.9 (BJ) 263; 6.4 (BN) 263<br>4.3 (F) 260 |
| 284 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$CH_2O(CH_2)_3CH_3$ | H | 6.5 (BJ) 263; 5.5 (BN) 263<br>4.6 (F) 260 |
| 285 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$O(CH_2)_5CH_3$ | H | 6.9 (BJ) 263; 5.8 (BN) 263 |
| 286 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$O(CH_2)_5CH_3$ | H | 6.1 (BJ) 263; 5.7 (BN) 263<br>5.0 (F) 260 |
| 287 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$O(CH_2)_6CH_3$ | H | 6.4 (BJ) 263; 5.6 (BN) 263 |
| 288 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$O(CH_2)_6CH_3$ | H | 5.6 (BJ) 263; 5.4 (BN) 263 |
| 289 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$O(CH_2)_7CH_3$ | H | 6.2 (BJ) 263; 5.3 (BN) 263 |
| 290 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-3′-$OCH_2C_6H_5$ | H | 7.0 (BJ) 263; 6.1 (BN) 263<br>5.0 (F) 260; 6.6 (H) 246 |
| 291 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$NHC_6H_4$-4″-CO-Asp | H | 10.0 (BE) 147 |
| 292 | $NH_2$ | $NH_2$ | $CH_2C_6H_4$-4′-$NHC_6H_4$-4″-CO-Asp | $CH_3$ | 10.0 (BE) 147 |
| 293 | $NH_2$ | $NH_2$ | $CH_2C_6H_3$-3′,4′-$(OH)_2$ | H | 4.8 (H) 246; 6.5 (BJ) 259<br>5.5 (BJ) 263; 5.8 (BN) 263<br>3.5 (F) 260 |
| 294 | $NH_2$ | $NH_2$ | $CH_2C_6H_3$-3′,5′-$(OH)_2$ | H | 3.0 (BJ) 263; 3.4 (BN) 263 |
| 295 | $NH_2$ | $NH_2$ | $CH_2C_6H_3$-3′-$NO_2$-4′-$NHCOCH_3$ | H | 7.0 (BJ) 263; 6.0 (BN) 263<br>4.2 (F) 260; 5.7 (H) 246 |
| 296 | $NH_2$ | $NH_2$ | $CH_2C_6H_3$-3′-$CH_3$-4′-$OCH_3$ | H | 6.3 (H) 246; 7.7 (BJ) 259 |
| 297 | $NH_2$ | $NH_2$ | $CH_2C_6H_3$-3′,5′-$(CH_2OH)_2$ | H | 6.3 (BJ) 263; 5.7 (BN) 263<br>2.7 (F) 260 |
| 298 | $NH_2$ | $NH_2$ | $CH_2C_6H_3$-3′-$CF_3$-4′-$OCH_3$ | H | 7.7 (BJ) 263; 7.3 (BN) 263<br>5.1 (F) 260 |
| 299 | $NH_2$ | $NH_2$ | $CH_2C_6H_3$-3′,4′-$(OMe)_2$ | H | 6.1 (M) 104; 3.8 (AA) 104<br>7.7 (BJ) 263; 6.9 (BN) 263<br>4.7 (F) 260; 5.7 (H) 246<br>4.7 (AL) 104; (BN) 238 |
| 300 | $NH_2$ | $NH_2$ | $CH_2C_6H_3$-3′,4′-$(OMe)_2$ | $CH_3$ | 5.6 (M) 104; 3.6 (AA) 104<br>3.6 (AL) 104; (BN) 238 |
| 301 | $NH_2$ | $NH_2$ | $CH_2C_6H_3$-3′,5′-$(OMe)_2$ | H | 8.4 (BJ) 263; 6.4 (BN) 263<br>7.2 (BJ) 253; 7.2 (BJ) 197<br>4.0 (M) 253; 4.1 (F) 260 |
| 302 | $NH_2$ | $NH_2$ | $CH_2C_6H_3$-3′,4′-$[O(CH_2)_2OCH_3]_2$ | H | 7.2 (BJ) 263; 6.5 (BN) 263<br>4.0 (F) 260 |
| 303 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-Cl-4′-OH; 5-OMe | H | 4.1 (H) 237; 8.3 (BJ) 237 |
| 304 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(CH_3)_2$-4′-OH | H | 7.1 (BJ) 259; 4.1 (M) 259 |
| 305 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-2′-Br-4′,5′-$(OMe)_2$ | H | 6.1 (M) 104; 3.9 (AA) 104<br>4.1 (AL) 104 |
| 306 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-Br-4′,5′-$(OMe)_2$ | H | 6.7 (M) 104; 3.6 (AA) 104<br>7.3 (BJ) 236 |
| 307 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-Cl-4′,5′-$(OMe)_2$ | H | 4.0 (M) 236; 7.4 (BJ) 236 |
| 308 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-$NH_2$-4′,5′-$(OMe)_2$ | H | 3.1 (M) 236; 6.0 (BJ) 236 |
| 309 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-I | H | 3.9 (M) 236; 7.6 (BJ) 236<br>7.6 (BJ) 197 |
| 310 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-OH | H | 8.0 (BJ) 230; 4.0 (M) 230<br>4.3 (H) 237; 3.6 (M) 244<br>8.3 (BJ) 244; 6.9 (AP) 244 |
| 311 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-OH | H | (BJ) 242 |
| 312 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-OH | $CH_3$ | 6.5 (BJ) 244; 6.3 (AP) 244 |
| 313 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-OH | $n$-$C_3H_7$ | 6.1 (BJ) 244; 5.8 (AP) 244 |
| 314 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-OH | $C_6H_5$ | 5.7 (BJ) 244; (M) 244 |
| 315 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$NH_2$ | H | 7.8 (BJ) 236; (M) 236 |
| 316 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-Br | H | 5.7 (BQ) 236; 8.9 (AZ) 236<br>5.3 (AK) 236; 8.0 (BJ) 197<br>8.5 (BG) 236; 6.4 (BF) 236<br>5.5 (BT) 236; (M) 236 |
| 317 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-Cl | H | 8.5 (BJ) 197; (M) 236 |
| 318 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-F | H | 7.7 (BJ) 197; 4.1 (M) 236 |
| 319 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-SMe-4′-OMe-5′-Br | H | 8.0 (BJ) 259 |
| 320 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(CH_3)_2$-4′-OMe | H | 6.8 (BJ) 259; 3.6 ( M) 259 |
| 321 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-2′-$CH_3$-4′,5′-$(OMe)_2$ | H | 7.3 (BJ) 258 |
| 322 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-$CH_3$-4′,5′-$(OMe)_2$ | H | 4.0 (M) 236; 7.4 (BJ) 236 |

**TABLE XII** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| 323 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$CH_3$ | H | 3.9 (M) 236; 8.1 (BJ) 236 |
| 324 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-2′,3′,4′-$(OMe)_3$ | H | 6.0 (BJ) 258 |
| 325 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-2′,4′,5′-$(OMe)_3$ | H | 5.7 (M) 104; 3.4 (AA) 104<br>4.2 (AL) 104 |
| 326 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | 6.0 (M) 104; 3.6 (M) 32<br>8.0 (BJ) 201; 9.3 (BJ) 115<br>8.3 (BJ) 185; 8.4 (BJ) 210<br>8.3 (BJ) 88; 8.9 (BJ) 263<br>8.3 (BJ) 32; 8.3 (BJ) 253<br>8.0 (BJ) 197; 8.6 (BJ) 34<br>8.1 (AP) 116; 7.1 (AP) 115<br>7.1 (AP) 88; 3.6 (M) 88<br>5.6 (H) 246; 5.0 (AK) 90<br>7.2 (BQ) 220; (AP) 132<br>8.9 (AP) 244; 4.8 (AK) 236<br>3.5 (A) 32; 5.0 (AR) 115<br>6.1 (AX) 220; 8.1 (AZ) 236<br>8.3 (AZ) 32; 8.4 (AZ) 51<br>6.0 (AW) 220; 4.8 (BT) 236<br>5.6 (BR) 236; 6.9 (BN) 263<br>6.6 (AN) 115; 6.6 (AN) 90<br>3.4 (J) 32; (BN) 238<br>6.6 (AN) 144; 4.7 (K) 67<br>4.7 (K) 146; 4.5 (K) 45<br>8.1 (BG) 236; 4.7 (AY) 220<br>7.7 (BC) 171; 6.2 (CD) 63<br>3.8 (F) 260; (BK) 198<br>4.7 (AT) 115; 7.5 (BO) 220<br>4.8 (AB) 63; 5.1 (H) 221<br>7.2 (BP) 165; 8.8 (BG) 34<br>8.4 (BG) 32; 4.0 (AU) 144<br>5.6 (BN) 222; 7.5 (BM) 146<br>6.0 (AL) 67; 6.0 (AL) 90<br>4.0 (BU) 144; 4.2 (AV) 144<br>6.3 (AI) 90; (Z) 88<br>4.7 (BV) 144; 3.4 (J) 146<br>3.5 (A) 146; 8.4 (BE) 147<br>4.0 (BX) 144; 6.5 (BS) 146<br>3.7 (AD) 146; (W) 249<br>6.1 (AO) 90; 4.2 (BW) 144<br>4.2 (AE) 146; 5.3 (AF) 146<br>6.0 (I) 182; (BI) 198<br>6.1 (AJ) 90; 4.7 (AM) 90<br>4.4 (BY) 144; 5.0 (AC) 146<br>3.4 (AA) 104; 3.9 (AL) 104<br>6.0 (D) 88 |
| 327 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,4′,5′-$(OMe)_3$ | Cl | (BJ) 244 |
| 328 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,4′,5′-$(OMe)_3$ | $NH_2$ | 5.0 (BJ) 244 |
| 329 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,4′,5′-$(OMe)_3$ | $CH_3$ | 7.0 (BJ) 244; 6.5 (AP) 244 |
| 330 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,4′,5′-$(OMe)_3$ | $SCH_3$ | (BJ) 244 |
| 331 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,4′,5′-$(OMe)_3$ | $N(CH_3)_2$ | 2.3 (BJ) 244; (BK) 198<br>(BI) 198 |
| 332 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,4′,5′-$(OMe)_3$ | $n$-$C_3H_7$ | 2.8 (M) 244; 5.8 (BJ) 244 |
| 333 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,4′,5′-$(OMe)_3$ | $i$-$C_3H_7$ | 5.7 (BJ) 244; (M) 244 |
| 334 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,4′,5′-$(OMe)_3$ | $C_6H_5$ | 5.5 (BJ) 244; (M) 244 |
| 335 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,4′,5′-$(OMe)_3$ | $OC_6H_5$ | (M) 244; (BJ) 244 |
| 336 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$SCH_3$ | H | 3.7 (H) 237; 3.6 (M) 236<br>3.4 (M) 259; 8.4 (BJ) 236<br>8.5 (BJ) 259 |
| 337 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-$SCH_3$-4′,5′-$(OMe)_2$ | H | 8.3 (BJ) 259; 3.6 (M) 236 |
| 338 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(SMe)_2$-4′-OMe | H | 8.1 (BJ) 259 |
| 339 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,4′,5′-$(SMe)_3$ | H | 8.3 (BJ) 259 |
| 340 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-OMe-4′-Br-5′-OEt | H | 4.3 (M) 236; 8.3 (BJ) 236 |
| 341 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-4′,5′-$(OMe)_2$-3′-$CH_2OH$ | H | 6.9 (BJ) 258 |
| 342 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$CH_2OH$ | H | 3.2 (M) 228; 7.7 (BJ) 228 |
| 343 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-CN | H | 3.7 (M) 228; 7.3 (BJ) 228 |
| 344 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$CH_2NH_2$ | H | 6.2 (BJ) 228; (M) 228 |
| 345 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-COOH | H | 5.8 (BJ) 228; (M) 228 |
| 346 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$CONH_2$ | H | 7.6 (BJ) 228; (M) 228 |
| 347 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$NHCH_3$ | H | 7.9 (BJ) 236; (M) 236 |
| 348 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-$CH_3$-4′-OMe-5′-$CH_2CH_3$ | H | 7.3 (BJ) 259; 4.1 (M) 259 |
| 349 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OEt)_2$-4′-Br | H | 4.7 (M) 236; 8.4 (BJ) 236 |
| 350 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$C_2H_5$ | H | 8.0 (BJ) 259; 8.0 (BJ) 253<br>3.7 (M) 259; 3.7 (M) 253 |
| 351 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-SEt | H | 8.7 (BJ) 259 |
| 352 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$N(CH_3)_2$ | H | 7.5 (BJ) 236; (M) 236 |
| 353 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$COOCH_3$ | H | 3.1 (M) 228; 8.0 (BJ) 228 |

**TABLE XII** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| 354 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$CH_2OCH_3$ | H | 7.7 (BJ) 228; (M) 228 |
| 355 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$COCH_3$ | H | 3.1 (M) 228; 7.7 (BJ) 228 |
| 356 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$CH(OH)CH_3$ | H | 3.0 (M) 228; 7.3 (BJ) 228 |
| 357 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$C(=NOH)CH_3$ | H | 7.0 (BJ) 228; (M) 228 |
| 358 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2CH_2Cl$ | H | 8.3 (BJ) 259; 7.8 (BJ) 253<br>3.6 (M) 259; 3.2 (M) 253 |
| 359 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OC_2H_5$ | H | 8.1 (BJ) 253; 3.2 (M) 253 |
| 360 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2CH_2OH$ | H | 7.7 (BJ) 253; (M) 253 |
| 361 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2CH_2NH_2$ | H | 7.7 (BJ) 253; (M) 253 |
| 362 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2CO_2H$ | H | 6.5 (BJ) 253; (M) 253 |
| 363 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$SCH_2CH_2OMe$ | H | 7.0 (BJ) 259 |
| 364 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2CH_2OMe$ | H | 7.7 (BJ) 259; 7.7 (BJ) 253<br>8.4 (BJ) 237; 8.5 (BJ) 210<br>8.4 (BJ) 263; (M) 259<br>(M) 253; 3.6 (H) 237<br>6.9 (BN) 263; 3.3 (F) 260 |
| 365 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OC_3H_7$ | H | 7.9 (BJ) 253; 3.6 (M) 253 |
| 366 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH(CH_3)_2$ | H | 7.6 (BJ) 253; (M) 253 |
| 367 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2\overline{CHCH_2O}$ | H | 7.8 (BJ) 253; (M) 253 |
| 368 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2CH{=}CH_2$ | H | 8.3 (BJ) 253; 3.3 (M) 253 |
| 369 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2CH_2CH_2OH$ | H | 8.4 (BJ) 253; (M) 253 |
| 370 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2CH(OH)CH_2OH$ | H | 7.8 (BJ) 253 |
| 371 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2CH_2CH_2Cl$ | H | 8.3 (BJ) 253; 3.6 (M) 253 |
| 372 | $NH_2$ | NH2 | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2CH_2CH_2NH_2$ | H | 7.3 (BJ) 253; (M) 253 |
| 373 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$CON(Me)_2$ | H | 4.6 (BJ) 228; (M) 228 |
| 374 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′5′-$(OMe)_2$-4′-$C(OH)(Me)_2$ | H | 7.8 (BJ) 236; 3.1 (M) 236<br>8.1 (BG) 236; 7.1 (AZ) 236<br>4.6 (BF) 236; 5.1 (BT) 236<br>5.8 (BR) 236; 4.6 (AK) 236 |
| 375 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-COOEt | H | 7.9 (BJ) 228; 3.1 (M) 228 |
| 376 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$C(=CH_2)Me$ | H | 8.5 (BJ) 236; 8.6 (BJ) 259<br>2.8 (M) 236; 2.8 (M) 259<br>9.1 (BG) 236; 8.8 (AZ) 236<br>5.6 (BF) 236; 4.8 (BT) 236<br>5.4 (BR) 236; 4.6 (AK) 236 |
| 377 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-CH(OH)Et | H | 7.9 (BJ) 228; 3.2 (M) 228 |
| 378 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$COCH_2SO_2Me$ | H | 7.1 (BJ) 228; (M) 228 |
| 379 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$CH(Me)_2$ | H | 7.7 (BJ) 228; (M) 228 |
| 380 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(Et)_2$-4′-OMe | H | 7.9 (BJ) 259; 4.2 (M) 259 |
| 381 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(Et)_2$-4′-OMe | $SCH_3$ | (BJ) 244 |
| 382 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-Et-4′-OH-5′-$C_3H_7$ | H | 8.2 (BJ) 259; 5.4 (M) 259 |
| 383 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OC_4H_9$-*n* | H | 8.0 (BJ) 253; 3.2 (M) 253 |
| 384 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OC_4H_9$-*i* | H | 7.7 (BJ) 253 |
| 385 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OC_4H_9$-*sec* | H | 7.8 (BJ) 253 |
| 386 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-O-$CH_2CO_2C_2H_5$ | H | 7.8 (BJ) 253; (M) 253 |
| 387 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$CO_2$-$CH(Me)_2$ | H | 6.7 (BJ) 228; 3.2 (M) 228 |
| 388 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-Et-4′-OMe-5′-$C_3H_7$ | H | 7.9 (BJ) 259; 4.5 (M) 259 |
| 389 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-OMe-4′-$C(OH)(Me)_2$-5′-OEt | H | 8.0 (BJ) 236; 4.1 (M) 236 |
| 390 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-OMe-4′-$C(=CH_2)CH_3$-5′-OEt | H | 8.5 (BJ) 236; (M) 236 |
| 391 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-OMe-4′-$CO_2$Et-5′-OEt | H | (BJ) 236; (M) 236 |
| 392 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-pyrrol-1″-yl | H | 8.3 (BJ) 236; 4.3 (M) 236 |
| 393 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(n\text{-}C_3H_7)_2$-4′-OH | H | 8.0 (BJ) 259; 5.7 (M) 259 |
| 394 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OEt)_2$-4′-$C(CH_3)_2$-OH | H | 8.4 (BJ) 236; 3.4 (M) 236 |
| 395 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OEt)_2$-4′-$C(=CH_2)CH_3$ | H | 8.7 (BJ) 236; 3.4 (M) 236 |
| 396 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OEt)_2$-4′-$CO_2$Et | H | 8.3 (BJ) 236; 3.7 (M) 236 |
| 397 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$CO_2C_4H_9$ | H | 7.8 (BJ) 228; 3.4 (M) 228 |
| 398 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OC_5H_{11}$ | H | 7.8 (BJ) 253; 3.6 (M) 253 |
| 399 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$C(OH)(Et)_2$ | H | 8.0 (BJ) 228; (M) 228<br>7.1 (AZ) 236 |
| 400 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(n\text{-}C_3H_7)_2$-4′-OMe | H | 7.4 (BJ) 259; 4.7 (M) 259 |
| 401 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(i\text{-}C_3H_7)_2$-4′-OMe | H | 6.8 (BJ) 259; 4.9 (M) 259 |
| 402 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′-OMe-4′-pyrrol-1″-yl-5′-OEt | H | 8.5 (BJ) 236; (M) 236 |
| 403 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(t\text{-}C_4H_9)_2$-4′-OH | H | 4.5 (BJ) 259; 5.7 (M) 259 |
| 404 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-$(OEt)_2$-4′-pyrrol-1″-yl | H | 8.7 (BJ) 236; 3.9 (M) 236<br>5.3 (AK) 236; 8.6 (AZ) 236<br>8.7 (BG) 236; 6.3 (BF) 236<br>4.7 (BT) 236; 5.9 (BR) 236 |
| 405 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-O-*n*-$C_6H_{13}$ | H | 8.4 (BJ) 253; 3.7 (M) 253 |
| 406 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$O(CH_2)_5CO_2H$ | H | 7.7 (BJ) 253; 3.5 (M) 253 |
| 407 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$O(CH_2)_6$-$NH_2$ | H | 7.8 (BJ) 253; 3.2 (M) 253 |
| 408 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2CH_2N(CH_2CH_2)_2O$ | H | 7.8 (BJ) 253; (M) 253 |
| 409 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OC_6H_4$-4″-$NH_2$ | H | 8.2 (BJ) 253 |
| 410 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OC_6H_4$-4″-$NO_2$ | H | 8.1 (BJ) 253; 4.3 (M) 253 |
| 411 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(t\text{-}C_4H_9)_2$-4′-OMe | H | 6.4 (BJ) 259; 4.5 (M) 259 |
| 412 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(t\text{-}C_4H_9)_2$-4′-OMe | $NH_2$ | 4.2 (BJ) 244 |
| 413 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$O(CH_2)_5CO_2Me$ | H | 7.2 (BJ) 253; 3.2 (M) 253 |

**TABLE XII** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| 414 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$O(CH_2)_3$N-c-$(CH_2)_4$ | H | 7.3 (BJ) 253; (M) 253 |
| 415 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2C_6H_5$ | H | 7.8 (BJ) 253; 4.3 (M) 253 |
| 416 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2C_6H_4$-4″-$NO_2$ | H | 8.1 (BJ) 253; (M) 253 |
| 417 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-O-*n*-$C_8H_{17}$ | H | 8.4 (BJ) 253 |
| 418 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$O(CH_2)_3$N-$(CH_2CH_2)_2NCH_3$ | H | 7.7 (BJ) 253; (M) 253 |
| 419 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OC_6H_4$-4″-$NHCOCH_2Br$ | H | 8.4 (BJ) 253; (M) 253 |
| 420 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$O(CH_2)_2$N-1″,2″-$(CO)_2C_6H_4$ | H | 7.7 (BJ) 253 |
| 421 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$OCH_2C_6H_2$-3″,4″,5″-$(OMe)_3$ | H | 7.7 (BJ) 253; 4.8 (M) 253 |
| 422 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$O(CH_2)_4$-N-1″,2″-$(CO)_2C_6H_4$ | H | 7.6 (BJ) 253; 4.3 (M) 253 |
| 423 | $NH_2$ | $NH_2$ | $CH_2C_6H_2$-3′,5′-$(OMe)_2$-4′-$O(CH_2)_6$-N-1″,2″-$(CO)_2C_6H_4$ | H | 7.4 (BJ) 253 |
| 424 | $NH_2$ | $NH_2$ | $C(=CH_2)C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | 5.6 (BJ) 185 |
| 425 | $NH_2$ | $NH_2$ | $CH(CH_3)C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | 5.3 (BJ) 185 |
| 426 | $NH_2$ | $NH_2$ | $COH(CH_3)C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | 4.0 (BJ) 185 |
| 427 | $NH_2$ | $NH_2$ | $CH(C_2H_5)C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | (BJ) 185 |
| 428 | $NH_2$ | $NH_2$ | $C(=CHCH_3)C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | 6.3 (BJ) 185 |
| 429 | $NH_2$ | $NH_2$ | $COH(C_2H_5)C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | 5.4 (BJ) 185 |
| 430 | $NH_2$ | $NH_2$ | $COH(CH{=}CH_2)C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | 4.1 (BJ) 185 |
| 431 | $NH_2$ | $NH_2$ | $C(=CHCH_2CH_3)C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | (BJ) 185 |
| 432 | $NH_2$ | $NH_2$ | $CH(C_3H_7)C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | 5.7 (BJ) 185 |
| 433 | $NH_2$ | $NH_2$ | $COH(C_3H_7)C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | 4.0 (BJ) 185 |
| 434 | $NH_2$ | $NH_2$ | $COH(CH_2CH{=}CH_2)C_6H_2$-3′,4′,5′-$(OMe)_3$ | H | 5.7 (BJ) 185 |
| 435 | $NH_2$ | $NH_2$ | $CH_2C_6H$-2′-Br-3′,4′,5′-$(OMe)_3$ | H | 6.0 (M) 104; 3.4 (AA) 104 3.6 (AL) 104 |
| 436 | $NH_2$ | $NH_2$ | $(CH_2)_3C_6H_5$ | $CH_3$ | 6.7 (AB) 21 |
| 437 | $NH_2$ | $NH_2$ | $(CH_2)_3C_6H_5$ | $NH_2$ | 5.1 (AB) 19 |
| 438 | $NH_2$ | $NH_2$ | $(CH_2)_3C_6H_5$ | OH | 4.2 (AB) 19 |
| 439 | $NH_2$ | $NH_2$ | $(CH_2)_4C_6H_5$ | $CH_3$ | 7.6 (AB) 19; 7.6(AB) 21 |
| 440 | $NH_2$ | $NH_2$ | $(CH_2)_4C_6H_5$ | *n*-$C_3H_7$ | 7.7 (AB) 37 |
| 441 | $NH_2$ | $NH_2$ | $(CH_2)_4C_6H_5$ | $C_6H_5$ | 6.0 (AB) 37 |
| 442 | $NH_2$ | $NH_2$ | $(CH_2)_4C_6H_5$ | $CH_2C_6H_5$ | 6.5 (AB) 37 |
| 443 | $NH_2$ | $NH_2$ | $(CH_2)_4C_6H_5$ | $NH_2$ | 5.5 (AB) 19 |
| 444 | $NH_2$ | $NH_2$ | $(CH_2)_4C_6H_5$ | OH | 5.1 (AB) 19 |
| 445 | $NH_2$ | $NH_2$ | $(CH_2)_4C_6H_5$ | $(CH_2)_2C_6H_4$-4′-$NHCOCH_2Br$ | 7.4 (AB) 56; 7.0 (BJ) 52 |
| 446 | $NH_2$ | $NH_2$ | $(CH_2)_4C_6H_4$-4′-$NHCOC_6H_4$-3″-$SO_2F$ | H | 8.2 (U) 102 |
| 447 | $NH_2$ | $NH_2$ | $(CH_2)_4C_6H_4$-4′-$NHCOC_6H_4$-4″-$SO_2F$ | H | 7.3 (U) 102 |
| 448 | $NH_2$ | $NH_2$ | $(CH_2)_4C_6H_4$-4′-$NHCOC_6H_4$-3″-$SO_2F$ | $CH_3$ | 8.0 (U) 102 |
| 449 | $NH_2$ | $NH_2$ | $(CH_2)_4C_6H_4$-4′-$NHCONHC_6H_4$-4″-$SO_2F$ | H | 7.3 (U) 102 |
| 450 | $NH_2$ | $NH_2$ | $(CH_2)_4C_6H_4$-4′-$NHCOC_6H_4$-3″-$SO_2F$ | $NH_2$ | 7.2 (Q) 105; 8.0 (U) 105 |
| 451 | $NH_2$ | $NH_2$ | $NHCH_2C_6H_4$-3′-$NHCOCH_2Br$ | $C_6H_5$ | 4.6 (N) 76; 4.3(U) 76 |
| 452 | $NH_2$ | $NH_2$ | $NHCH_2C_6H_4$-4′-$NHCOCH_2Br$ | $C_6H_5$ | 4.7 (N) 76; 3.8 (U) 76 |
| 453 | $NH_2$ | $NH_2$ | $NH(CH_2)_3C_6H_4$-4′-$NH_2$ | $C_6H_5$ | 6.4 (AB) 50 |
| 454 | $NH_2$ | $NH_2$ | $NH(CH_2)_3C_6H_4$-4′-$NHCOCH_2Br$ | $C_6H_5$ | 7.3 (N) 76; 6.7 (U) 76 6.1 (AB) 50 |
| 455 | $NH_2$ | $NH_2$ | $NHCO(CH_2)_2C_6H_5$ | $C_6H_5$ | 3.6 (AB) 50 |
| 456 | $NH_2$ | $NH_2$ | $CH_2NHC_6H_4$-4′-$COCH_2Cl$ | $C_6H_4$-4′-Cl | 5.6 (M) 65; 4.3 (AB) 65 5.3 (N) 65; 5.2 (U) 65 |
| 457 | $NH_2$ | $NH_2$ | $CH_2NHC_6H_4$-4′-$CH{=}CHCOCH_2Cl$ | $C_6H_4$-4′-Cl | 5.2 (N) 65; 5.1 (U) 65 |
| 458 | $NH_2$ | $NH_2$ | $CH_2NHC_6H_4$-4′-$CH_2CH_2COCH_2Cl$ | $C_6H_4$′-4′-Cl | 6.0 (U) 65; 5.1 (U) 65 |
| 459 | $NH_2$ | $NH_2$ | $CH_2NHC_6H_4$-4′-$(CH_2)_4COCH_2Cl$ | $C_6H_4$-4′-Cl | 6.5 (N) 65; 5.4 (U) 65 |
| 460 | $NH_2$ | $NH_2$ | $(CH_2)_3NHC_6H_5$ | $CH_3$ | 5.7 (AB) 37 |
| 461 | $NH_2$ | $NH_2$ | $(CH_2)_3NHC_6H_5$ | $C_6H_5$ | 6.1 (AB) 37 |
| 462 | $NH_2$ | $NH_2$ | $(CH_2)_3NHC_6H_5$ | $CH_2C_6H_5$ | 5.4 (AB) 37 |
| 463 | $NH_2$ | $NH_2$ | $(CH_2)_3NHCOC_6H_4$-4′-$NH_2$ | $CH_3$ | 3.1 (AB) 17 |
| 464 | $NH_2$ | $NH_2$ | $(CH_2)_3NHCOC_6H_4$-4′-$NO_2$ | $CH_3$ | 3.5 (AB) 17 |
| 465 | $NH_2$ | $NH_2$ | $(CH_2)_3NHSO_2C_4H_9$-n | $CH_3$ | 2.2 (AB) 17 |
| 466 | $NH_2$ | $NH_2$ | $(CH_2)_3NHSO_2C_6H_4$-4′-$CH_3$ | $CH_3$ | 3.2 (AB) 17 |
| 467 | $NH_2$ | $NH_2$ | $(CH_2)_3N(C_4H_9)SO_2C_6H_4$-4′-$CH_3$ | $CH_3$ | 3.7 (AB) 17 |
| 468 | $NH_2$ | $NH_2$ | $(CH_2)_3N(C_6H_5)SO_2C_6H_4$-4′-$CH_3$ | $CH_3$ | 5.7 (AB) 18 |
| 469 | $NH_2$ | $NH_2$ | $OC_6H_4$-4′-Cl | H | 5.6 (M) 104; 3.8 (AA) 104 4.1 (AL) 104 |
| 470 | $NH_2$ | $NH_2$ | $OC_6H_4$-4′-Cl | $CH_3$ | 5.7 (M) 104; 4.3 (AA) 104 4.7 (AL) 104; 4.6 (AB) 9 6.0 (BJ) 9 |
| 471 | $NH_2$ | $NH_2$ | $OC_6H_4$-4′-OMe | H | 5.8 (M) 104; 4.6 (AA) 104 4.2 (AL) 104 |
| 472 | $NH_2$ | $NH_2$ | $OC_6H_4$-4′-$NO_2$ | $CH_3$ | 4.8 (M) 104; 4.1 (AA) 104 5.2 (AL) 104; (BN) 238 |
| 473 | $NH_2$ | $NH_2$ | $OC_6H_2$-2′-$CH(CH_3)_2$-4′-Cl-5′-$CH_3$ | H | (BN) 238 |
| 474 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_5$ | $C_6H_5$ | 5.5 (AB) 51 |
| 475 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NO_2$ | $C_6H_5$ | 5.5 (AB) 51 |
| 476 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NH_2$ | $NH_2$ | 4.2 (AB) 49 |
| 477 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NH_2$ | $C_6H_5$ | 5.1 (AB) 51 |
| 478 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCOCH_2Br$ | $C_6H_5$ | 6.8 (N) 76; 6.5 (U) 76 5.1 (AB) 51 |
| 479 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-$NHCOC_6H_4$-3″-$SO_2F$ | $CH_3$ | 7.7 (Q) 101; 7.8 (U) 101 |

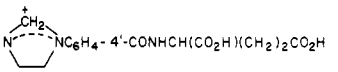
**TABLE XII** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| 480 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCOC_6H_4$-4″-$SO_2F$ | $CH_3$ | 7.6 (U) 101 |
| 481 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCOC_6H_3$-3″-$SO_2F$-4″-$CH_3$ | $CH_3$ | 7.7 (U) 101 |
| 482 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCONHC_6H_3$-3″-$SO_2F$-4″-$CH_3$ | $CH_3$ | 7.8 (U) 101 |
| 483 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCOCH_2Br$ | $NH_2$ | 4.6 (AB) 49; 5.5 (N) 76<br>5.1 (U) 76 |
| 484 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCO(CH_2)_2NHCOCH_2Br$ | $NH_2$ | 4.0 (AB) 49 |
| 485 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCH_2C_6H_4$-3″-$SO_2F$ | $NH_2$ | 5.6 (M) 76; 5.8 (U) 76 |
| 486 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCOC_6H_4$-3″-$SO_2F$ | $NH_2$ | 5.4 (Q) 105; 6.2 (M) 76<br>6.0 (AA) 76; 5.2 (U) 76 |
| 487 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCOC_6H_4$-4″-$SO_2F$ | $NH_2$ | 6.3 (N) 76; 5.4 (U) 76 |
| 488 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCONHC_6H_4$-3″-$SO_2F$ | $NH_2$ | 6.2 (M) 76; 6.2 (N) 76<br>5.4 (U) 76 |
| 489 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCONHC_6H_4$-4″-$SO_2F$ | $NH_2$ | 5.8 (N) 76; 5.4 (U) 76 |
| 490 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCONHC_6H_4$-3″-$SO_3H$ | $NH_2$ | 6.2 (N) 76; 6.1 (U) 76 |
| 491 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_3$-2′-Cl-4′-$NHCOC_6H_4$-3″-$SO_2F$ | $NH_2$ | 5.8 (Q) 100; 5.9 (U) 100 |
| 492 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCOC_6H_3$-3″-$SO_2F$-4″-Me | $NH_2$ | 5.3 (Q) 100; 5.9 (U) 100 |
| 493 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCOC_6H_3$-2″-Me-5″-$SO_2F$ | $NH_2$ | 5.5 (U) 100 |
| 494 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCOC_6H_3$-3″-$SO_2F$-4″-*i*-$C_3H_7$ | $NH_2$ | 6.1 (U) 100 |
| 495 | $NH_2$ | $NH_2$ | $(CH_2)_3OC_6H_4$-4′-$NHCO(CH_2)_3C_6H_5$-4″-$NHCOCH_2Br$ | $NH_2$ | 5.9 (N) 76; 4.8 (U) 76<br>4.9 (AB) 49 |
| 496 | $NH_2$ | $NH_2$ | $(CH_2)_3C_6H_4$-4′-$NHCOC_6H_4$-3″-$SO_2F$ | $NH_2$ | 7.2 (Q) 105; 7.9 (U) 105 |
| 497 | $NH_2$ | $NH_2$ | $NH_2$ | $C_6H_5$ | 3.0 (AB) 50 |
| 498 | $NH_2$ | $NH_2$ | $N{=}NC_6H_5$ | $NH_2$ | 5.0 (M) 104; 4.8 (AA) 104<br>5.1 (AL) 104 |
| 499 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-3′-F | $NH_2$ | (AA) 133 |
| 500 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-2′-Cl | $NH_2$ | (AA) 133 |
| 501 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-3′-Cl | $NH_2$ | (AA) 133 |
| 502 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-Cl | $NH_2$ | (AA) 133 |
| 503 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-3′-Br | $NH_2$ | (AA) 133 |
| 504 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-Br | $NH_2$ | (AA) 133 |
| 505 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-2′-I | $NH_2$ | (AA) 133 |
| 506 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-3′-I | $NH_2$ | (AA) 133 |
| 507 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-I | $NH_2$ | (AA) 133 |
| 508 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-3′-$NO_2$ | $NH_2$ | (AA) 133 |
| 509 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$SO_2NH_2$ | $NH_2$ | (M) 31 |
| 510 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-2′,5′-$Cl_2$ | $NH_2$ | (AA) 133 |
| 511 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-2′,5′-$Cl_2$ | $NH(CH_2)_3N(Et)(CH_2)_2Cl$ | 5.6 (Q) 113; 5.7 (R) 113<br>5.5 (T) 113 |
| 512 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-2′,4′-$Cl_2$ | $NH(CH_2)_3N(Et)(CH_2)_2Cl$ | 6.0 (Q) 113; 5.9 (R) 113<br>5.7 (T) 113 |
| 513 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-2′,6′-$Cl_2$ | $NH_2$ | (AA) 133 |
| 514 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-2′,6′-$Cl_2$ | $NH(CH_2)_3N(Et)(CH_2)_2Cl$ | 6.2 (Q) 113; 6.7 (R) 113<br>6.5 (T) 113 |
| 515 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-2′,3′-$Cl_2$ | $NH_2$ | (AA) 133 |
| 516 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-2′,3′-$Cl_2$ | $NH(CH_2)_3N(Et)(CH_2)_2Cl$ | 7.0 (Q) 113; 7.0 (R) 113<br>6.7 (T) 113 |
| 517 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-3′,4′-$Cl_2$ | $NH_2$ | (AA) 133 |
| 518 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-3′,4′-$Cl_2$ | $NH(CH_2)_3N(Et)(CH_2)_2Cl$ | 6.1 (Q) 113; 5.7 (R) 113<br>5.5 (T) 113 |
| 519 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-3′,5′-$Cl_2$ | $NH_2$ | 5.1 (AL) 104; (AA) 133 |
| 520 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-3′,5′-$Cl_2$ | $NH(CH_2)_3N(Et)(CH_2)_2Cl$ | 6.5 (Q) 113; 6.5 (R) 113<br>6.3 (T) 113 |
| 521 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-2′-$CH_3$ | $NH_2$ | (AA) 133 |
| 522 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-3′-$CH_3$ | $NH_2$ | (AA) 133 |
| 523 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$CH_3$ | $NH_2$ | (AA) 133 |
| 524 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-3′-$OCH_3$ | $NH_2$ | (AA) 133 |
| 525 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$OCH_3$ | $NH_2$ | (AA) 133 |
| 526 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-2′-$CF_3$ | $NH_2$ | (AA) 133 |
| 527 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-3′-$CF_3$ | $NH_2$ | (AA) 133 |
| 528 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$CF_3$ | $NH_2$ | (AA) 133 |
| 529 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-2′-$CH_3$-3′-Cl | $NH_2$ | (AA) 133 |
| 530 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-COONa | $NH_2$ | (M) 31 |
| 531 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-2′-$C_2H_5$ | $NH_2$ | (AA) 133 |
| 532 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-3′-$C_2H_5$ | $NH_2$ | (AA) 133 |
| 533 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$C_2H_5$ | $NH_2$ | (AA) 133 |
| 534 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-2′,6′-$(CH_3)_2$ | $NH_2$ | (AA) 133 |
| 535 | $NH_2$ | $NH_2$ | $N{=}NC_6H_3$-3′,5′-$(CH_3)_2$ | $NH_2$ | (AA) 133 |
| 536 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-2′-COOMe | $NH_2$ | (AA) 133 |
| 537 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-3′-COOEt | $NH_2$ | (AA) 133 |
| 538 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-COOEt | Cl | (M) 31 |
| 539 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-COOEt | $NH_2$ | (AA) 133; (M) 31 |
| 540 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$CO_2Et$ | $N(Et)_2$ | (M) 31 |
| 541 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$CO_2Et$ | $N(CH_2CH_2)_2N(CH_2)_2OH$ | (M) 31 |
| 542 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$CO_2Et$ | $N(CH_2CH_2)_2N(CH_2)_2Cl$ | (M) 31 |

**TABLE XII** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| 543 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$CO_2Et$ | $NH(CH_2)_2N(Et)CH_2CH_2Cl$ | 4.6 (Q) 78; 4.4 (R) 78 4.7 (T) 78; (M) 38 |
| 544 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-COOEt | $NH(CH_2)_3N(Et)CH_2CH_2Cl$ | (M) 43 |
| 545 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-COOEt | $NH(CH_2)_4N(Et)CH_2CH_2Cl$ | (M) 43 |
| 546 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-COOEt | $NH(CH_2)_5N(Et)CH_2CH_2Cl$ | (M) 43 |
| 547 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-COOEt | $NH(CH_2)_3NHC_6H_5$ | (M) 43 |
| 548 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-COOEt | $NHCH_2CHClCH_2NHC_6H_5$ | (M) 43 |
| 549 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-COOEt | $NHCH_2CH(OH)CH_2NHC_6H_5$ | (M) 43 |
| 550 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-COOEt | $NH(CH_2)_6N(Et)CH_2CH_2Cl$ | (M) 43 |
| 551 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-2′-$CH(CH_3)_2$ | $NH_2$ | (AA) 133 |
| 552 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$N(Et)_2$ | $NH_2$ | (M) 31 |
| 553 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$N(CH_2CH_2Cl)_2$ | $NH_2$ | (M) 31 |
| 554 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$N(CH_2CH_2Br)_2$ | $NH_2$ | (M) 31 |
| 555 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$N(Et)CH_2CH_2Cl$ | $NH_2$ | (M) 31 |
| 556 | $NH_2$ | $NH_2$ | $N{=}N(CH_2)_2C_6H_4$-2′-$CH_3$ | $NH_2$ | (M) 31 |
| 557 | $NH_2$ | $NH_2$ | $N{=}N(CH_2)_2C_6H_4$-4′-$CH_3$ | $NH_2$ | (M) 31 |
| 558 | $NH_2$ | $NH_2$ | $N{=}N(CH_2)_3C_6H_4$-4′-$CH_3$ | $NH_2$ | (AA) 133 |
| 559 | $NH_2$ | $NH_2$ | $N{=}N$-1′-$C_{10}H_7$ | $NH_2$ | (AA) 133 |
| 560 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-CO-Glu | Cl | (M) 31 |
| 561 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-CO-Glu | $NH_2$ | (M) 31 |
| 562 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-CO-Glu | $N(Et)_2$ | (M) 31 |
| 563 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-2′-$C_6H_5$ | $NH_2$ | (AA) 133 |
| 564 | $NH_2$ | $NH_2$ | $N{=}NC_6H_4$-4′-$C_6H_5$ | $NH_2$ | (AA) 133 |
| 565 | $NH_2$ | $NH_2$ | $N{=}N(CH_2)_6C_6H_4$-2′-$CH_3$ | $NH_2$ | (AA) 133 |
| 566 | $NH_2$ | $NH_2$ | $N{=}N(CH_2)_8C_6H_4$-4′-$CH_3$ | $NH_2$ | (AA) 133 |
| 567 | $NH_2$ | $NH(CH_2)_2C_6H_5$ | *i*-$C_5H_{11}$ | $CH_3$ | 3.2 (AB) 57 |
| 568 | $NH_2$ | $NH(CH_2)_3C_6H_5$ | *i*-$C_5H_{11}$ | $CH_3$ | 3.8 (AB) 57 |
| 569 | $NH_2$ | $NH(CH_2)_2C_6H_5$ | $C_6H_4$-4′-Cl | $CH_3$ | 3.1 (AB) 57 |
| 570 | $NH_2$ | $NH(CH_2)_3C_6H_5$ | $C_6H_4$-4′-Cl | $CH_3$ | 3.7 (AB) 57 |
| 571 | $NH_2$ | $NH(CH_2)_3C_6H_5$ | *i*-$C_6H_{13}$ | $NH_2$ | 4.5 (AB) 57 |
| 572 | $NH_2$ | OH | H | $CH_3$ | 1.7 (AB) 19 |
| 573 | $NH_2$ | OH | $CH_3$ | $CH_3$ | 2.1 (AB) 21 |
| 574 | $NH_2$ | OH | $C_2H_5$ | $CH_3$ | 2.7 (AB) 21 |
| 575 | $NH_2$ | OH | $C_3H_7$ | $CH_3$ | 3.0 (AB) 21 |
| 576 | $NH_2$ | OH | $CH_2CH{=}CH_2$ | $CH_3$ | 2.7 (AB) 21 |
| 577 | $NH_2$ | OH | $CH_2CH_2CO_2H$ | $CH_3$ | (M) 12 |
| 578 | $NH_2$ | OH | *n*-$C_4H_9$ | $CH_3$ | 3.6 (AB) 21 |
| 579 | $NH_2$ | OH | *n*-$C_5H_{11}$ | $CH_3$ | 4.5 (AB) 21 |
| 580 | $NH_2$ | OH | *i*-$C_5H_{11}$ | $CH_3$ | 5.1 (AB) 21 |
| 581 | $NH_2$ | OH | *n*-$C_6H_{13}$ | $CH_3$ | 4.5 (AB) 21 |
| 582 | $NH_2$ | OH | *n*-$C_8H_{17}$ | $CH_3$ | 3.6 (AB) 21 |
| 583 | $NH_2$ | OH | adamantyl | H | 4.5 (O) 143 |
| 584 | $NH_2$ | OH | adamantyl | $CH_3$ | 5.8 (L) 138 |
| 585 | $NH_2$ | OH | NHCOCH=N-adamantyl | $CH_3$ | 3.1 (O) 143 |
| 586 | $NH_2$ | OH | $(CH_2)_3{}^+NH_2$-*n*-$C_4H_9$ | $CH_3$ | (AB) 48 |
| 587 | $NH_2$ | OH | $(CH_2)_3NHCOCH_3$ | $CH_3$ | 2.9 (AB) 17 |
| 588 | $NH_2$ | OH | $(CH_2)_3NHCOCH_2Br$ | $CH_3$ | 2.5 (AB) 58 |
| 589 | $NH_2$ | OH | $(CH_2)_3NHCOCH_2Br$ | $C_6H_5$ | 2.9 (AB) 58 |
| 590 | $NH_2$ | OH | $C_6H_4$-4′-Cl | $CH_3$ | 4.5 (AB) 57 |
| 591 | $NH_2$ | OH | $(CH_2)_3C_6H_5$ | $CH_3$ | 3.8 (AB) 21 |
| 592 | $NH_2$ | OH | $(CH_2)_4C_6H_5$ | $CH_3$ | 4.5 (AB) 19 |
| 593 | $NH_2$ | OH | $(CH_2)_4C_6H_5$ | $COO^-$ | 2.4 (AB) 25 |
| 594 | $NH_2$ | OH | $(CH_2)_4C_6H_5$ | $COOC_2H_5$ | (AB) 25 |
| 595 | $NH_2$ | OH | $(CH_2)_4C_6H_5$ | $COOCH_2CN$ | (AB) 25 |
| 596 | $NH_2$ | OH | $(CH_2)_4C_6H_5$ | CHO | (AB) 25 |
| 597 | $NH_2$ | OH | $(CH_2)_4C_6H_5$ | $C_6H_5$ | 6.3 (AB) 51 |
| 598 | $NH_2$ | OH | $(CH_2)_4C_6H_5$ | $(CH_2)_2C_6H_4$-4′-$NHCOCH_2Br$ | 3.7 (AB) 56; 3.0 (BJ) 52 |
| 599 | $NH_2$ | OH | $(CH_2)_4C_6H_5$ | $(CH_2)_4C_6H_4$-4′-$NHCOCH_2Br$ | 4.4 (M) 40; 3.3 (BJ) 52 |
| 600 | $NH_2$ | OH | $(CH_2)_4C_6H_4$-4′-COOH | $CH_3$ | 5.6 (AB) 36 |
| 601 | $NH_2$ | OH | $(CH_2)_4C_6H_4$-4′-$COOC_2H_5$ | $CH_3$ | 4.9 (AB) 36 |
| 602 | $NH_2$ | OH | $(CH_2)_3NH$-3′-pyridine | $CH_3$ | 3.0 (AB) 55 |
| 603 | $NH_2$ | OH | $(CH_2)_3NHC_6H_5$ | $CH_3$ | 3.1 (AB) 46; (AB) 11 (M) 12 |
| 604 | $NH_2$ | OH | $(CH_2)_3NHC_6H_5$ | $C_6H_5$ | 5.7 (M) 10 |
| 605 | $NH_2$ | OH | $(CH_2)_3NHC_6H_5$ | $CH_2C_6H_5$ | 5.4 (M) 10 |
| 606 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-F | $CH_3$ | 3.2 (AB) 55 |
| 607 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-3′-$CF_3$ | $CH_3$ | 3.8 (AB) 55 |
| 608 | $NH_2$ | OH | $(CH_2)_3NHCH_2C_6H_5$ | $CH_3$ | 4.5 (AB) 7 |
| 609 | $NH_2$ | OH | $(CH_2)_2CONHC_6H_4$-4′-$CONHCH(CO_2H)(CH_2)_2CO_2H$ | $CH_3$ | (M) 12 |
| 610 | $NH_2$ | OH | $(CH_2)_3NH$-2′-naphthyl | $CH_3$ | 3.6 (AB) 55 |
| 611 | $NH_2$ | OH | $NH(CH_2)_3C_6H_5$ | $CH_3$ | 3.2 (AB) 19 |
| 612 | $NH_2$ | OH | $NH(CH_2)_2NHC_6H_4$-4′-CO-Glu | H | 4.3 (BN) 13 |
| 613 | $NH_2$ | OH | $NHCOCH_2NHC_6H_5$ | $CH_3$ | (AB) 8 |
| 614 | $NH_2$ | OH | $NHCOCH_2NHC_6H_4$-4′-CO-Glu | $CH_3$ | (AB) 8 |
| 615 | $NH_2$ | OH | $(CH_2)_3N(COCH_3)CH_2C_6H_5$ | $CH_3$ | 3.2 (AB) 55 |
| 616 | $NH_2$ | OH | $(CH_2)_3N(COCH_3)CH_2$-2′-pyridine | $CH_3$ | 2.7 (AB) 55 |
| 617 | $NH_2$ | OH | $(CH_2)_3N(COCH_3)CH_2$-3′-pyridine | $CH_3$ | 2.4 (AB) 55 |

**TABLE XII** (Continued)

| no. | $R_2$ | $R_4$ | $R_5$ | $R_6$ | activity (enzyme) ref |
|---|---|---|---|---|---|
| 618 | $NH_2$ | OH | $(CH_2)_3N(COCH_3)CH_2$-4′-pyridine | $CH_3$ | 2.4 (AB) 55 |
| 619 | $NH_2$ | OH | $(CH_2)_3N(COCH_3)CH_2$-2′-furyl | $CH_3$ | 3.7 (AB) 55 |
| 620 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-COOH | $CH_3$ | 4.1 (AB) 30; (M) 12 |
| 621 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-$COCH_2Br$ | $CH_3$ | (AB) 58 |
| 622 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-$CONHCH_2COOH$ | $CH_3$ | 3.8 (AB) 30; (M) 12 |
| 623 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-$CH{=}CHCOCH_2Cl$ | $CH_3$ | 4.7 (AB) 30 |
| 624 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-$(CH_2)_2COCH_2Cl$ | $CH_3$ | 4.0 (AB) 30 |
| 625 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-$(CH_2)_2NHCOCH_2Br$ | $C_6H_5$ | 3.9 (AB) 47 |
| 626 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-$CH{=}CHCOCH_2Cl$ | $C_6H_5$ | 3.7 (AB) 47 |
| 627 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-$(CH_2)_2COCH_2Cl$ | $C_6H_5$ | 4.3 (AB) 47 |
| 628 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-$(CH_2)_2NHCOCH_2Br$ | $C_6H_5$ | 3.7 (AB) 47 |
| 629 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-$(CH_2)_4COCH_2Cl$ | $CH_3$ | 4.6 (AB) 30 |
| 630 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-$CONH(CH_2)_3COOH$ | $CH_3$ | (AB) 30; (M) 12 |
| 631 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-CO-Glu | $CH_3$ | 4.0 (AB) 30; 5.7 (M) 10 (P) 3 |
| 632 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-$CONHCH(CO_2H)(CH_2)_2CONH_2$ | $CH_3$ | (M) 12 |
| 633 | $NH_2$ | OH | $(CH_2)_3NHC_6H_4$-4′-$(CH_2)_6COCH_2Cl$ | $CH_3$ | (AB) 235 |
| 634 | $NH_2$ | OH | $(CH_2)_3NHCOC_6H_5$ | $CH_3$ | 3.3 (AB) 58 |
| 635 | $NH_2$ | OH | $(CH_2)_3NHCOC_6H_4$-3′-$CH_2Br$ | $CH_3$ | 3.4 (AB) 58 |
| 636 | $NH_2$ | OH | $(CH_2)_3NHCOC_6H_4$-4′-$CH_2Br$ | $CH_3$ | 3.4 (AB) 58 |
| 637 | $NH_2$ | OH | $(CH_2)_3NHCOC_6H_4$-4′-$CH_2Br$ | $C_6H_5$ | 3.4 (AB) 58 |
| 638 | $NH_2$ | OH | $(CH_2)_3NHCOC_6H_4$-4′-$COCH_2Br$ | $CH_3$ | 4.1 (AB) 58 |
| 639 | $NH_2$ | OH | $(CH_2)_3NHCOC_6H_4$-4′-$COCH_2Br$ | $C_6H_5$ | (AB) 58 |
| 640 | $NH_2$ | OH | $(CH_2)_3NHCOC_6H_4$-3′-$CH_2OEt$ | $CH_3$ | 3.0 (AB) 58 |
| 641 | $NH_2$ | OH | $(CH_2)_3NHCOC_6H_4$-4′-COOH | $CH_3$ | 3.3 (AB) 36 |
| 642 | $NH_2$ | OH | $(CH_2)_3NHCOCH_2C_6H_5$ | $CH_3$ | 3.0 (AB) 17 |
| 643 | $NH_2$ | OH | $(CH_2)_3NHSO_2C_6H_4$-4′-$CH_3$ | $CH_3$ | 2.6 (AB) 17 |
| 644 | $NH_2$ | OH | $(CH_2)_3NHSO_2C_6H_4$-4′-COOH | $CH_3$ | 2.5 (AB) 36 |
| 645 | $NH_2$ | OH | $(CH_2)_3N(n\text{-}C_4H_9)COC_6H_5$ | $CH_3$ | 3.7 (AB) 17 |
| 646 | $NH_2$ | OH | $(CH_2)_3N(COCH_3)$-$C_6H_4$-4′-COOH | $CH_3$ | 4.3 (AB) 36 |
| 647 | $NH_2$ | OH | $(CH_2)_3N(n\text{-}C_4H_9)SO_2C_6H_4$-4′-$CH_3$ | $CH_3$ | 3.7 (AB) 17 |
| 648 | $NH_2$ | OH | $(CH_2)_3OC_6H_5$ | $CH_3$ | (AB) 7 |
| 649 | $NH_2$ | OH | $(CH_2)_3OC_6H_4$-4′-$NHCOCH_2Br$ | $CH_3$ | 4.1 (AB) 41 |
| 650 | $NH_2$ | OH | $(CH_2)_3OC_6H_4$-4′-$NHCOCH_2Br$ | $C_6H_5$ | 4.0 (AB) 41 |
| 651 | $NH_2$ | OH |  | H | 4.3 (BN) 13 |
| 652 | $NH_2$ | $OCH_2C_6H_5$ | $i\text{-}C_5H_{11}$ | $CH_3$ | 3.5 (AB) 57 |
| 653 | $NH_2$ | SH | $i\text{-}C_5H_{11}$ | $CH_3$ | 5.6 (AB) 57 |
| 654 | $NH_2$ | SH | $C_6H_4$-4′-Cl | $CH_3$ | 5.8 (AB) 57 |
| 655 | $NH_2$ | SH | $(CH_2)_3NHC_6H_5$ | $CH_3$ | 4.4 (AB) 55 |
| 656 | $NH_2$ | SH | $(CH_2)_3NHC_6H_4$-4′-Cl | $CH_3$ | 4.9 (AB) 55 |
| 657 | $NH_2$ | SH | $(CH_2)_3NHC_6H_4$-4′-$N(CH_3)_2$ | $CH_3$ | 4.5 (AB) 55 |
| 658 | $NH_2$ | SH | $(CH_2)_3N(COCH_3)C_6H_5$ | $CH_3$ | 4.7 (AB) 18 |
| 659 | $NH_2$ | SH | $(CH_2)_3N(COC_6H_5)C_6H_5$ | $CH_3$ | 4.3 (AB) 18 |
| 660 | $NH_2$ | SH | $(CH_2)_3N(COOC_6H_5)C_6H_5$ | $CH_3$ | (AB) 18 |
| 661 | $NH_2$ | SH | $(CH_2)_4C_6H_5$ | $C_6H_5$ | 3.5 (AB) 37 |
| 662 | $NH(CH_2)_3C_6H_5$ | $NH_2$ | $i\text{-}C_5H_{11}$ | $NH_2$ | 2.8 (AB) 57 |
| 663 | $N(CH_3)_2$ | OH | $(CH_2)_3NHC_6H_5CH_3$ | $CH_3$ | (AB) 11 |
| 664 | OH | $NH_2$ | $C_6H_4$-4′-Cl | $CH_3$ | 2.5 (AB) 57 |
| 665 | SH | $NH_2$ | $C_6H_4$-4′-Cl | $CH_3$ | 2.4 (AB) 57 |

dihydrofolate reductase, we have no doubt missed some examples. The literature is so extensive and publication has occurred in such a wide variety of places that the probability for missing reports is high.

**Registry No.** Dihydrofolate reductase, 9002-03-3.

## XIV. References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