Irreversible Enzyme Inhibitors. CLXIII.[1,2] Active-Site-Directed Irreversible Inhibitors of Cytosine Nucleoside Deaminase Derived from 1-Phenoxypropyl-5-arylcytosines

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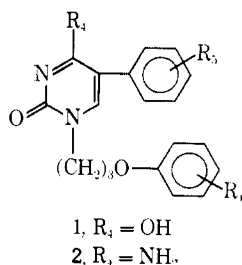
Eighteen candidate irreversible inhibitors of cytosine nucleoside deaminase were synthesized: fifteen (**3–17**) were bridged to a terminal sulfonyl fluoride group from the phenoxy moiety of 5-(3,4-dichlorophenyl)-1-phenoxypropylcytosine and three (**18–20**) from the 5-phenyl moiety of 1-phenoxypropyl-5-phenylcytosine. Of these 18 compounds, 15 showed no irreversible inhibition, one (**9**) showed poor irreversible inhibition, one (**18**) showed good irreversible inhibition, and one (**3**) showed excellent irreversible inhibition. 5-(3,4-Dichlorophenyl)-1-[*p*-(*p*-fluorosulfonylbenzamido)phenoxypropyl]cytosine (**3**) had a $K_i \sim 50\ \mu M$; at 100 μM, **3** gave complete inactivation of the enzyme with a half-life of <2 min; that **3** inactivated the enzyme *via* a reversible enzyme-inhibitor complex, the so-called active-site-directed mechanism, was strongly supported by structure-activity relationships.

The cytosine nucleoside deaminase from *Escherichia coli* B can be inhibited by 1,5-disubstituted uracils (**1**)[3,4] and cytosines (**2**).[2] Inhibition by **2** can be enhanced sevenfold when the 5-aryl group is substituted by R_5 = 3,4-Cl_2; furthermore, the 5-aryl group could be substituted with R_5 = AcNH with some enhancement of inhibition.[2] The binding of the phenoxy moiety of **2** could also be enhanced two- to threefold by substitution with R_1 = halogen or benzamido.[2]



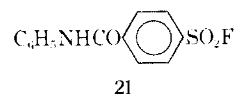
The bulk tolerance for R_5 = acetamido or R_1 = benzamido on **2** suggested that active-site-directed irreversible inhibitors[5] of cytosine nucleoside deaminase could be constructed by having either R_1 or R_5 bear a terminal sulfonyl fluoride;[6] the latter group has been found to be particularly effective for formation of a covalent bond with the enzyme outside the active site, presumably with an appropriately located serine.[6,7] The synthesis and enzymic evaluation of such candidate active-site-directed irreversible inhibitors of cytosine nucleoside deaminase is the subject of this paper.

Enzyme Assays.—Since the position of an amino acid residue capable of forming a covalent bond with an SO_2F moiety on an inhibitor, such as the hydroxyl of a serine, is unknown, a random search must be made until an effective compound is found. When an amino group on an inhibitor is available, we have standardized on six combinations of bridges to benzenesulfonyl fluoride; these bridges are carbamoyl, ureido, and sulfamoyl. The fluorosulfonyl group is then positioned *meta* or *para* to the bridge. The carboxamido and sulfonamido bridges have different bond angles in the ground state, therefore positioning the SO_2F group differently; similarly, the carboxamido and ureido bridges will position the SO_2F moiety differently due to the difference in bridge lengths and relatively fixed ground-state conformations.

Three positions for the amino group (R_1) on the phenoxy moiety of **2** are feasible. Thus the total number of compounds that could be made is 18 for a preliminary search; of these 18, all six *para* compounds were made, but only five of the *meta* and four of the *ortho* compounds; in all cases the R_5 group on **2** was 3,4-Cl_2. The enzyme results with these 15 compounds are listed in Table I. Of these fifteen compounds, only one (**3**) was a good irreversible inhibitor, one (**9**) was a poor irreversible inhibitor, and the remaining 13 showed no irreversible inhibition.

When the *p*-fluorosulfonylbenzamido moiety was attached to the *para* position of the phenoxy group of **2** (R_5 = 3,4-Cl_2), the resultant **3** had I_{50} = 0.1 m*M*; a Dixon plot of $1/V$ *vs.* I at two substrate concentrations gave K_i = 50 ± 15 μM for **3** and the kinetics were "competitive." The enzyme was completely inactivated in 60 min when incubated with 0.1 m*M* of **3**. Although this concentration is twice the solubility of the compound, actually only a slight turbidity was present; since an incubation aliquot is diluted tenfold for assay, no problem was encountered in the assay of the resultant clear solution.

That **3** inactivated the enzyme by the active-site-directed mechanism was clearly shown with **21**; the



latter at 0.1 m*M* showed no inactivation of the enzyme. If **3** had inactivated the enzyme by a simple bimolecular process, then **21**, which is the terminal moiety of **3**, should have inactivated the enzyme even more rapidly than **3**. Thus it is highly probable that the inactiva-

(1) The generous support of this work by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service, is gratefully acknowledged.

(2) For the previous paper in this series see B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **12**, 1039 (1969).

(3) B. R. Baker and J. L. Kelley, *ibid.*, **11**, 682 (1968), paper CXXX of this series.

(4) B. R. Baker and J. L. Kelley, *ibid.*, **11**, 686 (1968), paper CXXXI of this series.

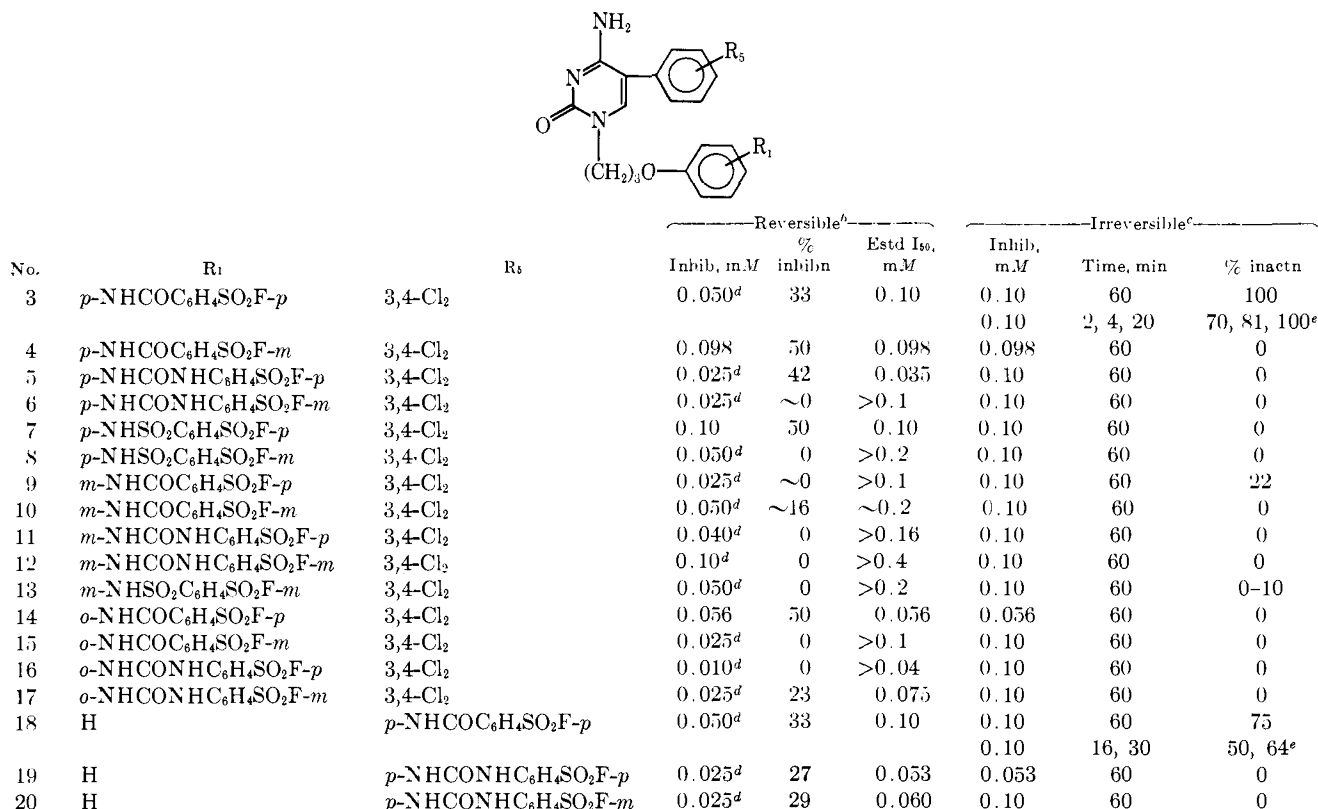
(5) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley and Sons, Inc., New York, N. Y., 1967.

(6) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **10**, 1113 (1967), paper CV of this series.

(7) B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 233 (1968), paper CXIII of this series.

TABLE I

REVERSIBLE AND IRREVERSIBLE INHIBITION[a] OF CYTOSINE NUCLEOSIDE DEAMINASE BY



No.	R_1	R_5	Reversible[b]: Inhib, mM	Reversible[b]: % inhibn	Reversible[b]: Estd I_{50}, mM	Irreversible[c]: Inhib, mM	Irreversible[c]: Time, min	Irreversible[c]: % inactn
3	*p*-$NHCOC_6H_4SO_2F$-*p*	3,4-Cl_2	0.050[d]	33	0.10	0.10	60	100
						0.10	2, 4, 20	70, 81, 100[e]
4	*p*-$NHCOC_6H_4SO_2F$-*m*	3,4-Cl_2	0.098	50	0.098	0.098	60	0
5	*p*-$NHCONHC_6H_4SO_2F$-*p*	3,4-Cl_2	0.025[d]	42	0.035	0.10	60	0
6	*p*-$NHCONHC_6H_4SO_2F$-*m*	3,4-Cl_2	0.025[d]	~0	>0.1	0.10	60	0
7	*p*-$NHSO_2C_6H_4SO_2F$-*p*	3,4-Cl_2	0.10	50	0.10	0.10	60	0
8	*p*-$NHSO_2C_6H_4SO_2F$-*m*	3,4-Cl_2	0.050[d]	0	>0.2	0.10	60	0
9	*m*-$NHCOC_6H_4SO_2F$-*p*	3,4-Cl_2	0.025[d]	~0	>0.1	0.10	60	22
10	*m*-$NHCOC_6H_4SO_2F$-*m*	3,4-Cl_2	0.050[d]	~16	~0.2	0.10	60	0
11	*m*-$NHCONHC_6H_4SO_2F$-*p*	3,4-Cl_2	0.040[d]	0	>0.16	0.10	60	0
12	*m*-$NHCONHC_6H_4SO_2F$-*m*	3,4-Cl_2	0.10[d]	0	>0.4	0.10	60	0
13	*m*-$NHSO_2C_6H_4SO_2F$-*m*	3,4-Cl_2	0.050[d]	0	>0.2	0.10	60	0-10
14	*o*-$NHCOC_6H_4SO_2F$-*p*	3,4-Cl_2	0.056	50	0.056	0.056	60	0
15	*o*-$NHCOC_6H_4SO_2F$-*m*	3,4-Cl_2	0.025[d]	0	>0.1	0.10	60	0
16	*o*-$NHCONHC_6H_4SO_2F$-*p*	3,4-Cl_2	0.010[d]	0	>0.04	0.10	60	0
17	*o*-$NHCONHC_6H_4SO_2F$-*m*	3,4-Cl_2	0.025[d]	23	0.075	0.10	60	0
18	H	*p*-$NHCOC_6H_4SO_2F$-*p*	0.050[d]	33	0.10	0.10	60	75
						0.10	16, 30	50, 64[e]
19	H	*p*-$NHCONHC_6H_4SO_2F$-*p*	0.025[d]	27	0.053	0.053	60	0
20	H	*p*-$NHCONHC_6H_4SO_2F$-*m*	0.025[d]	29	0.060	0.10	60	0

[a] The technical assistance of Julie Leseman and Sharon Lafler is acknowledged. [b] The enzyme from *E. coli* B was assayed with 0.1 mM 2'-deoxycytidine in pH 7.4 Tris buffer containing 10% DMSO as previously described.[3] [c] The enzyme was incubated at 37° with the inhibitor at the concentration and time indicated in pH 7.4 Tris buffer containing 10% DMSO, then the amount of remaining enzyme was assayed as described in the Experimental Section. [d] Maximum solubility. [e] From a six-point time study.

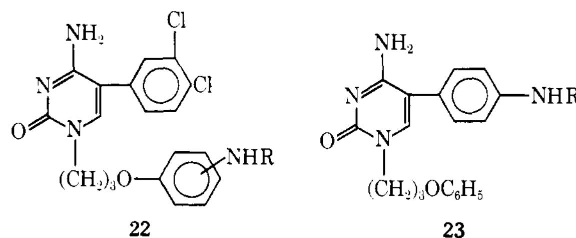
tion of the enzyme by **3** proceeds through a reversible enzyme-inhibitor complex, the concentration of the latter being the rate-determine species;[8] with a K_i = 0.05 mM, 0.1 mM of **3** would reversibly complex about 67% of the enzyme.[8] A more detailed time study showed that 70% of the enzyme was inactivated in 2 min, 84% in 4 min and 100% in 20 min.

The only other sulfonyl fluoride bridged to the phenoxy moiety of **2** (R_5 = 3,4-Cl_2) that showed irreversible inhibition was the *m*-(*p*-fluorosulfonylbenzamido) derivative (**9**); **9** was much less effective than **3**, since **9** gave only 22% inactivation in 60 min.

The most accessible amino derivative on the 5-phenyl group of **2** is the *para* isomer.[2] Three of the six basic bridges to an SO_2F moiety were synthesized from this *p*-NH_2 derivative (Table I). Of these three, only **18** showed irreversible inhibition with a half-life of 16 min.

The discovery that **3** and **18** are active-site-directed irreversible inhibitors of cytosine nucleoside deaminase from *E. coli* B completes this phase in the search for irreversible inhibitors of this enzyme that might be useful adjuncts to cytosine β-arabinoside in cancer chemotherapy.[9] The next phase is the design and synthesis of irreversible inhibitors of cytosine nucleoside deaminase that could inactivate a tumor enzyme, but not the enzyme in normal tissues;[9] such studies have been hampered by the instability of the mammalian enzymes.[10]

Chemistry.—The synthesis of the amine intermediates (**22a**, **23a**) were described in the previous paper.[2] The



a, R = H
b, R = $CONHC_6H_4SO_2F$
c, R = $COC_6H_4SO_2F$
d, R = $SO_2C_6H_4SOF$

urea-bridged sulfonyl fluorides (**22b**, **23b**) were synthesized by condensation with the appropriate O-(*p*-nitrophenyl)urethan derivative[11] of aminobenzenesulfonyl fluoride; these urethans have the advantage that they are insensitive to traces of moisture. However, the synthesis of the carboxamide (**23c**) from the corresponding acid chloride was hampered by traces of

(8) For the kinetics and types of irreversible inhibition see ref 5, pp 122–129.

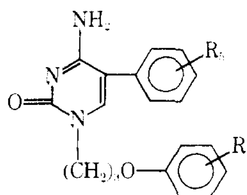
(9) For a detailed discussion of the possible utility of selective irreversible inhibitors of this enzyme see ref 3.

(10) (a) W. A. Creasey, *J. Biol. Chem.*, **238**, 1772 (1963); (b) R. Tomchick, L. D. Saslaw, and V. S. Waravdekar, *ibid.*, **243**, 2534 (1968); (c) G. B. Wisdom and B. A. Orsi, *European J. Biochem.*, **7**, 223 (1969); (d) the human liver enzyme is stable according to G. W. Camiener and C. G. Smith, *Biochem. Pharmacol.*, **14**, 1405 (1965).

(11) B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 79 (1969), paper CXXXV of this series.

TABLE II

PHYSICAL PROPERTIES OF



No.	R_1	R_5	Method	Yield,[a] %	Mp, °C	Formula[b]
3	p-$NHCOC_6H_4SO_2F$-p	3,4-Cl_2	B	57[c]	237–239[d]	$C_{26}H_{21}Cl_2FN_4O_5S$
4	p-$NHCOC_6H_4SO_2F$-m	3,4-Cl_2	A	18[e]	234–238	$C_{26}H_{21}Cl_2FN_4O_5S$
5	p-$NHCONHC_6H_4SO_2F$-p	3,4-Cl_2	B[f]	12[g]	235–238 dec	$C_{26}H_{22}Cl_2FN_5O_5S$
6	p-$NHCONHC_6H_4SO_2F$-m	3,4-Cl_2	A[f]	30[e]	222–223	$C_{26}H_{22}Cl_2FN_5O_5S$
7	p-$NHSO_2C_6H_4SO_2F$-p	3,4-Cl_2	B	8[e]	251–254 dec (245)[i]	$C_{25}H_{21}Cl_2FN_4O_6S_2$
8	p-$NHSO_2C_6H_4SO_2F$-m	3,4-Cl_2	B	11[j]	214–216 dec (207)[i]	$C_{25}H_{21}Cl_2FN_4O_6S_2$
9	m-$NHCOC_6H_4SO_2F$-p	3,4-Cl_2	A	32[g]	249–252	$C_{26}H_{21}Cl_2FN_4O_5S$
10	m-$NHCOC_6H_4SO_2F$-m	3,4-Cl_2	B	36[e]	200–202	$C_{26}H_{21}Cl_2FN_4O_5S$
11	m-$NHCONHC_6H_4SO_2F$-p	3,4-Cl_2	B[f]	10[g]	254–257	$C_{26}H_{22}Cl_2FN_5O_5S$
12	m-$NHCONHC_6H_4SO_2F$-m	3,4-Cl_2	B[f]	73[g]	201–203	$C_{26}H_{22}Cl_2FN_5O_5S$
13	m-$NHSO_2C_6H_4SO_2F$-m	3,4-Cl_2	A	7[h]	177–179	$C_{25}H_{21}Cl_2FN_4O_6S_2$
14	o-$NHCOC_6H_4SO_2F$-p	3,4-Cl_2	A	17[h]	231–234	$C_{26}H_{21}Cl_2FN_4O_5S$
15	o-$NHCOC_6H_4SO_2F$-m	3,4-Cl_2	A[k]	51[e]	225–228[l]	$C_{26}H_{21}Cl_2FN_4O_5S$
16	o-$NHCONHC_6H_4SO_2F$-p	3,4-Cl_2	A[m,f]	58[g]	237–238	$C_{26}H_{22}Cl_2FN_5O_5S$
17	o-$NHCONHC_6H_4SO_2F$-m	3,4-Cl_2	A[m,f]	26[h]	207–208	$C_{26}H_{22}Cl_2FN_5O_5S$
18	H	p-$NHCOC_6H_4SO_2F$-p	C	12[h,j]	232–236	$C_{26}H_{23}FN_4O_5S$
19	H	p-$NHCONHC_6H_4SO_2F$-p	A[f]	22[n]	257–260	$C_{26}H_{24}FN_5O_5S$
20	H	p-$NHCONHC_6H_4SO_2F$-m	A[m,f]	18[o,h]	234–236	$C_{26}H_{24}FN_5O_5S$

[a] Yield of analytically pure material and is minimum. [b] Analyses for C, H, F. [c] Recrystallized from EtOH–MeOEtOH. [d] Initially melts at 170–179°, resolidified ~190°, then remelts. [e] Recrystallized from EtOH–MeOEtOH–H_2O. [f] See ref 11 for intermediate carbamate. [g] Recrystallized from DMF–H_2O. [h] Recrystallized from MeOEtOH–H_2O. [i] Number in parenthesis refers to temperature of preheated block; slow heating from 25° gave gradual decomposition over a wide range. [j] Recrystallized from EtOH–H_2O. [k] 1,5-Diazabicyclo[4.3.0]non-5-ene (DBN) used in place of Et_3N. [l] Higher melting dimorph as white granules; a lower melting dimorph obtained earlier as needles, mp 151–153°. [m] Et_3N omitted in reaction. [n] Recrystallized from MeOEtOH–DMF–H_2O, then DMF–EtOH. [o] Leached with hot EtOAc to remove p-nitrophenol.

water and the poor solubility of the starting amine; these difficulties were overcome by treatment of the reaction mixture with molecular sieves prior to addition of the acid chloride. This procedure effectively removed the last traces of H_2O and afforded easily purified **23c** (*para*). Attempted activation of p-fluorosulfonylbenzoic acid as the water-stable p-nitrophenyl[12] or N-hydroxysuccinimide[13] esters failed due to the lack of reactivity of these esters toward aromatic amines such as aniline, **22a**, or **23a**.

Experimental Section[14]

5-(3,4-Dichlorophenyl)-1-[p-(m-fluorosulfonylbenzamido)phenoxypropyl]cytosine (4) (Method A).—To the cooled solution of 0.202 g (0.50 mmole) of **22a** (*para*) in 4 ml of DMF was added 0.056 g (0.55 mmole) of Et_3N. To the stirred solution, protected from moisture and cooled on an ice bath, was added 0.111 g (0.50 mmole) of m-fluorosulfonylbenzoyl chloride in 1 ml of DMF. After 10 min at 0°, the reaction was left overnight at ambient temperature, then poured over 30 g of crushed ice. The resultant dispersion was acidified to pH 1 with 1 N HCl, stirred for several hours, then filtered, and the precipitate was washed with H_2O. This solid was dissolved in a few milliliters of MeOEtOH or DMF and cooled on an ice bath. The solution was adjusted to pH 7–8 with 5% aqueous $NaHCO_3$, then diluted with 40 ml of iced water.[15] The resultant precipitate was collected, washed with H_2O, and recrystallized from EtOH–MeOEtOH–H_2O; yield, 52 mg (18%) of a light brown powder, mp 234–238°, which gave a negative Bratton–Marshall test.[16] See Table II for additional data and other compounds prepared by this method.

In **method B**, the reaction mixture was added to a stirred mixture of 15 ml of $CHCl_3$ and 30 ml of 3 N H_2SO_4. After a few minutes 50 g of crushed ice was added to effect solidification of the salt. The product was collected, washed extensively with H_2O, then processed as in A.

5-[p-(p-Fluorosulfonylbenzamido)phenyl]-1-phenoxypropylcytosine (18) (Method C).—A mixture of 0.336 g (1.0 mmole) of **23a**, 0.124 g (1.0 mmole) of 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), 150 mg of molecular sieves (Type 3A), and 4 ml of DMF was stirred at ambient temperature for 2 hr. To the stirred mixture was added 0.222 g (1.0 mmole) of p-fluorosulfonylbenzoyl chloride in 1 ml of DMF. After 15 min the resultant solution was decanted from the sieves into a solution of 30 ml of ice water and 5 ml of 1 N HCl, then stirred for 30 min. The product was collected and washed with H_2O. The product was dissolved in about 2 ml of DMF and cooled on an ice bath. The solution was adjusted to pH 8–9 with 5% aqueous $NaHCO_3$, then diluted with 40 ml of ice water. The product was collected, washed with H_2O, and recrystallized; yield, 61 mg (12%) of yellow micro needles, mp 232–236°, which gave a negative Bratton–Marshall test.[16] See Table II for additional data.

***p*-Fluorosulfonylbenzanilide (21).**—To a stirred solution of 0.945 g (10.1 mmoles) of aniline in 10 ml of dioxane was added a solution of 2.23 g (10.0 mmoles) of p-fluorosulfonylbenzoyl chloride in 15 ml of dioxane. The resultant mixture was diluted with 200 ml of water. The product was collected and washed

(12) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).

(13) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *ibid.*, **86**, 1839 (1964).

(14) Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had ir and uv spectra compatible with their assigned structures and moved as a single spot on tlc with C_6H_6–EtOH (3:1). The analytical samples gave combustion values for C, H, and F within 0.4% of theory.

(15) When the original precipitate was purified, it analyzed as the one-half hydrochloride; thus neutralization was an essential step.

(16) (a) B. R. Baker, D. V. Santi, J. K. Coward, H. S. Shapiro, and J. H. Jordaan, *J. Heterocycl. Chem.*, **3**, 425 (1966); (b) A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, **128**, 537 (1939).

with H_2O; yield 2.67 g (96%), mp 206–207.5°. Recrystallization from *i*-PrOH gave 2.27 g (81%) of white spears with unchanged melting point. *Anal.* ($C_{13}H_{10}FNO_3S$) C, H, F.

Irreversible Enzyme Assays.—The velocity of the reaction with 0.1 m*M* 2′-deoxycytidine was found to be proportional to the cytosine nucleoside deaminase concentration. No spontaneous inactivation of the enzyme in 10% DMSO and pH 7.4 Tris buffer occurred after 1 hr at 37°. The incubation method for detection of irreversible inhibition was the same as described for trypsin.[17]

(17) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **11**, 245 (1968); paper CXV of this series.

Irreversible Enzyme Inhibitors. CLXIV.[1,2] Proteolytic Enzymes. XIV.[2] Inhibition of Guinea Pig Complement by *meta*-Substituted Benzamidines

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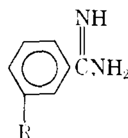
A series of 33 *meta*-substituted benzamidines were evaluated as inhibitors of guinea pig complement–sheep red blood cell–antibody system in order to determine the optimum substituents for inhibition. Since some of the benzamidines with *meta*-hydrocarbon substituents caused severe lysis of the red blood cells in the absence of complement, optimum hydrophobic interaction with minimal lysis was determined. The activity peaked at *n*-amyloxy (**15**) and isoamyloxy (**16**) which showed 50% inhibition at 0.5 m*M*; *n*-butoxy (**14**) showed less inhibition and *n*-hexyloxy (**17**) caused extensive lysis of the red blood cells. *m*-Alkyl substituents were less effective than *m*-alkoxy. Similar results were seen with phenylalkyl and phenylalkoxy substituents; *m*-phenylbutyl (**30**) and *m*-phenylbutyloxy (**20**) showed extensive lysis of the red blood cells, but *m*-phenylpropoxy (**19**) was an effective inhibitor at 0.5 m*M*. The best inhibitor showing no lysis was still the previously described *m*-phenoxypropyloxybenzamidine (**21**)[4] which at 0.5 m*M* showed 50% inhibition of complement.

Complement is a complex mixture of eleven serum proteins with proteolytic activity that has a number of biological actions including rejection of foreign cells.[3] Since complement has both "tryptic" and "chymotryptic" properties,[3] it can be inhibited by some compounds that inhibit trypsin[4] or chymotrypsin[2,5] when measured by the sheep red blood cell–hemolysin–serum method.[4,6] Effective "tryptic" type inhibitors were found in the benzamidine series (**1**), particularly when



R was isoamyloxy or phenoxypropoxy.[4] The hydrocarbon nature of the R group leading to enhanced activity suggested that further studies be performed to determine the nature and dimensions of this hydrophobic interaction, studies that have proved highly successful with other single enzyme systems.[7] Such a study with compounds of structure **1** is the subject of this paper.

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper in this series, see B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **12**, 1046 (1969).

(3) (a) H. J. Müller-Eberhard, *Advan. Immunol.*, **8**, 1 (1968); (b) P. H. Schur and K. R. Austen, *Ann. Rev. Med.*, **19**, 1 (1968).

(4) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **12**, 408 (1969), paper CLII of this series.

(5) (a) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 415 (1969), paper CLIII of this series; (b) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 677 (1969), paper CLVI of this series.

(6) E. A. Kabat and M. M. Mayer, "Experimental Immunochemistry," 2nd ed, Charles C Thomas, Publisher, Springfield, Ill., 1967, pp 149–153.

(7) For a review see B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley and Sons, Inc., New York, N. Y., 1967.

Inhibition Results.—The inhibition of complement by a given concentration of compound is determined by comparison with a control lysis of sheep red blood cells (RBC) with no compound (Table I). In some cases acceleration of lysis occurred which is expressed as a minus amount of inhibition. The increase in rate of lysis can be due to one of two factors; the compound can cause lysis in the absence of complement, which is recorded as the percentage of total lysis possible (0.7 OD) (see **30**), or the compound can directly accelerate the complement system as shown by a negative inhibition or a decrease in inhibition as concentration is increased, but no lysis in the absence of complement[4] (see **19**).

A strict comparison of the effects of substitution on benzamidine for inhibition of complement is complicated by the multitude of enzymes involved in the complement system; with a single enzyme such as trypsin[8] or chymotrypsin,[9] good comparisons of substituent effects can be made. Nevertheless, the substitutions giving the best increments in inhibition of complement can be determined. Little variation in inhibition of complement was seen with single small *meta* substituents (**3–8**), except for NO_2 (**7**) where inhibition was poor; the effect of the NO_2 group does not appear to be electronic since CH_3 (**4**) and CF_3 (**6**) are nearly the same. The best inhibitor of this group was *m*-methoxybenzamidine (**5**); therefore higher alkoxy groups were studied as discussed below.

Five disubstituted benzamidines were investigated. The 3,4-Me_2 derivative (**10**) was slightly more effective than 3-Me (**4**), being equivalent to the parent benzamidine (**2**). The 3,5-Me_2 derivative (**11**) was three to four

(8) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **10**, 1123 (1967), paper CVI of this series.

(9) B. R. Baker and J. A. Hurlbut, *ibid.*, **10**, 1129 (1967), paper CVII of this series.