site-specific delivery of intravenously administered radiodiagnostics or highly potent drugs (e.g., anticancer agents) appears promising. Only the results of ongoing studies will determine the practicality of this approach.

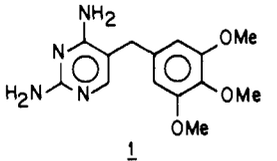
Acknowledgment. This investigation was supported by PHS Grant CA-08349, awarded by the National Cancer Institute, and by Training Grant GM-07767, awarded by the National Institute of General Medical Sciences, DHHS.

Communications to the Editor

Receptor-Based Design of Dihydrofolate Reductase Inhibitors: Comparison of Crystallographically Determined Enzyme Binding with Enzyme Affinity in a Series of Carboxy-Substituted Trimethoprim Analogues

Sir:

The biochemical basis for the chemotherapeutic effect of trimethoprim (TMP, 1), a widely used antibacterial



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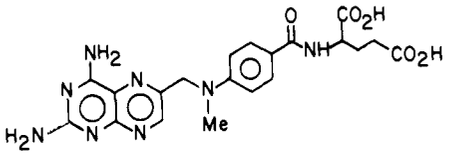

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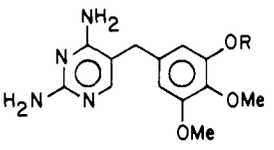
agent,[1] is the specific potent inhibition of dihydrofolate reductase (DHFR) in a broad spectrum of bacteria.[2] Determination of the three-dimensional molecular structure of DHFR, as defined by X-ray crystallography, led to the challenge of using this information to design analogues of TMP. X-ray studies have been reported by Matthews et al. on the binary complex of *Escherichia coli* DHFR and methotrexate (MTX, 2),[3] the ternary complex of *Lactobacillus casei* DHFR, NADPH, and MTX,[4] and chicken liver DHFR in ternary complex with NADPH and a series of inhibitors including TMP.[5] In addition, one of our laboratories recently reported the crystal structures of *E. coli* DHFR in binary complex with TMP[6] and two closely related analogues.[7]

Table I. Affinity Constants from TMP and Compounds 3-13 for *E. coli* DHFR



compd	R	rel binary K_D[a]	$K_I \times 10^9$,[b] M
1 (TMP)	CH_3	1.0	1.3
3	CH_2CO_2H	1.2	2.6
4	$(CH_2)_2CO_2H$	0.29	0.37
5	$(CH_2)_3CO_2H$	0.15	0.035
6	$(CH_2)_4CO_2H$	0.13	0.066
7	$(CH_2)_5CO_2H$	0.063	0.024
8	$(CH_2)_6CO_2H$	0.13	0.050
9	$CH_2CO_2CH_3$		11.0
10	$(CH_2)_3CO_2CH_2CH_3$		0.47
11	$(CH_2)_4CO_2CH_3$		0.76
12	$(CH_2)_5CO_2CH_3$		0.86
13	$(CH_2)_6CO_2CH_3$		1.9

[a] Relative K_D values are derived from competition experiments with MTX by spectrophotometric analysis and are normalized to the value of TMP such that a value less than one indicates higher affinity than that of TMP. Values of multiple determinations agreed within ±20%. This constant measures dissociation of inhibitor from the enzyme-inhibitor binary complex. [b] K_I values for compounds 1 and 3-8 were determined with the Henderson analysis (Henderson, P. J. F. *Biochem. J.* **1973**, *135*, 101). For the weaker binding compounds 9-13, K_I values were calculated with Cha's equation for competitive inhibitors (Cha, S. *Biochem. Pharmacol.* **1975**, *24*, 2177). For each method, values of multiple determinations agreed within ±15%. Equivalent K_I values were obtained from these two methods for TMP and several closely related analogues. This constant effectively measures dissociation of the inhibitor from the enzyme-NADPH-inhibitor ternary complex.

We report here the design, synthesis, DHFR affinity, and X-ray crystallographic binding analysis of a series of 3′-carboxyalkoxy analogues of TMP. At the time this work began only the unrefined structure of *E. coli* DHFR–MTX was available; the design of these compounds was therefore based solely on that structure. The goal of this design effort was not only to provide TMP analogues with higher affinity for *E. coli* DHFR but also to gain information on

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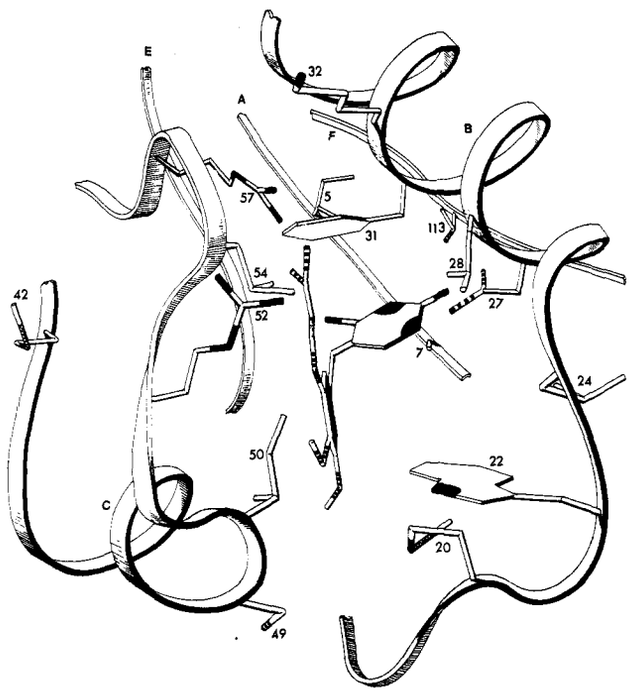

Figure 1. Schematic illustration of the binding site for compound **4** in *E. coli* DHFR. Nitrogen atoms are shown in black, oxygen striped, and sulfur crosshatched. Segments of β-sheet strands A, E, and F (in the nomenclature of ref 3) form the rear of the binding cleft. The left-hand side of the cleft is formed by an irregular peptide chain and the right-hand side contains an irregular region and contiguous helix B. Binding of the TMP portion of compound **4** is essentially identical with that described for TMP itself in ref 6. The pyrimidine ring is bound to Asp-27, and the benzyl moiety is partially enclosed by Phe-31 above, Ile-50 on the left, and Leu-28 on the right. The carboxy group of **4** has been modeled as interacting with the guanidinium group of Arg-57 through one hydrogen bond.

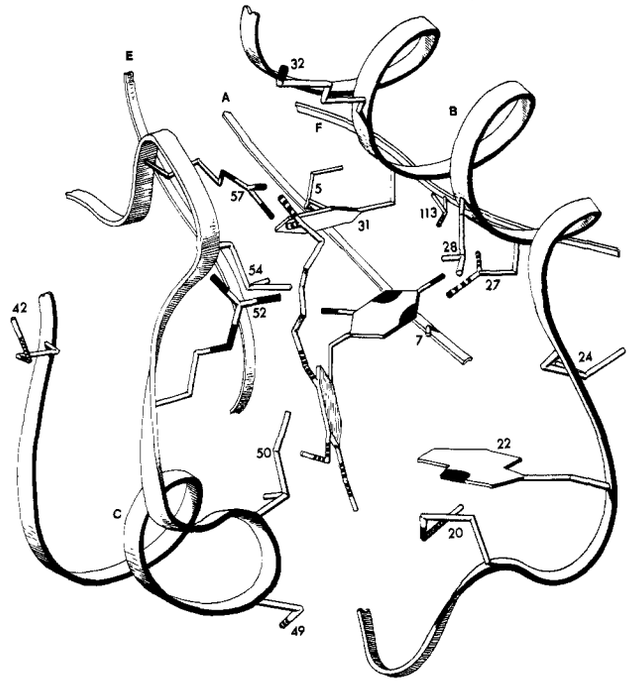

Figure 2. Schematic illustration of the binding site for compound **7** in *E. coli* DHFR. Nitrogen atoms are shown in black, oxygen striped, and sulfur crosshatched. The carboxy group of **7** has been modeled as interacting with the guanidinium moiety of Arg-57 through two approximately parallel hydrogen bonds.

the binding interactions for this class of inhibitors.

In the crystalline complex of *E. coli* DHFR and MTX, the α-carboxy group of MTX forms an ionic linkage to the guanidinium moiety of Arg-57, and the γ-carboxy group is in the vicinity of the aminoalkyl side chain of Lys-32 and the side chain of Arg-52.[8] The presence of these three basic residues in the active site of *E. coli* DHFR suggested to us that analogues of TMP containing appropriately placed carboxylic acid substituents might interact with one or more of these positively charged sites and thereby increase affinity for the enzyme and possibly give useful information on the mode of binding. To test this hypothesis, we prepared compounds **3–13**. Table I shows affinity data from these compounds and *E. coli* DHFR. The significantly higher affinity of acids **5–8**, compared with that of TMP and the corresponding esters **10–13**, implied that the desired ionic interaction did occur for these carboxy-containing compounds.

These data, in conjunction with affinity data from α- and γ-amide analogues of MTX[9] and molecular modeling experiments utilizing conformational implications drawn from a series of 6-substituted analogues of TMP,[10] led us to postulate a binding mode for TMP and compounds **4–8** in which the pyrimidine ring of TMP was bound in a manner identical with that of MTX, and the carboxy groups of **4–8** were ionically bonded to Arg-57. The side chain of Arg-57 is substantially buried in a hydrophobic region of the enzyme and, because of this environment, should form stronger ionic bonds than residues, such as Lys-32 and Arg-52, that are on the enzyme surface with side chains extending into the solvent.[11] This hypothesis was subsequently found to be completely in accord with the crystallographically determined binding of TMP[6] and with one of the two postulated binding modes proposed by Cayley et al. from elegant NMR studies of TMP in solution with this enzyme.[12]

The X-ray structures of compounds **4** and **7** in binary complex with *E. coli* DHFR, as solved by difference Fourier methods, further confirmed the above postulates. These structures, depicted schematically in Figures 1 and 2, showed that the binding interactions of the benzyl and diaminopyrimidine moieties of these two compounds were essentially identical with that of TMP.[6] The carboxylic acid substituents interacted with Arg-57 as predicted. The short chain length of **4** did not allow positioning of the carboxy group close enough to the guanidinium group of Arg-57 for an interaction that is optimal (two approximately parallel hydrogen bonds[13]), whereas the greater chain length of compound **7** allowed optimal juxtaposition of the two ionic groups. These structural observations

(8) In the unrefined structure of *E. coli* DHFR–MTX described in ref 3, the γ-carboxy group of MTX was modeled within hydrogen-bonding distance of the amino group of Lys-32. However, in the refined structure (J. Bolin et al., unpublished results), the γ-carboxy group is modeled near the side chains of Lys-32 and Arg-52, but interactions are mediated through intervening water molecules.

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agreed well with the measured affinity constants. The resolution of the electron density did not clearly define the conformation of the alkoxy linkage of **7**, but an approximately helical folded conformation, as shown in Figure 2, accommodated the electron density.

In summary, we have used three-dimensional molecular models of the *E. coli* DHFR–MTX complex to design analogues of TMP that not only had significantly higher affinity for DHFR than that of TMP but also furnished useful information on the binding mode of this class of inhibitor in solution. The postulated binding mode was then verified by X-ray crystallographic studies of TMP and two of these analogues in complex with *E. coli* DHFR. Although these analogues were not as effective as TMP as broad-spectrum antibacterials, we feel that this study amply demonstrates the considerable potential of this approach to inhibitor design.

Acknowledgment. The authors thank Drs. D. Matthews and J. Kraut for generously providing coordinates of the *E. coli* DHFR–MTX structure. We thank Drs. D. Henry and J. Burchall for their encouragement during this work.

Lee F. Kuyper,* Barbara Roth
David P. Baccanari, Robert Ferone
Wellcome Research Laboratories, Burroughs Wellcome Co.
Research Triangle Park, North Carolina 27709

Christopher R. Beddell, John N. Champness
David K. Stammers, John G. Dann
Frank E. A. Norrington, Dorothea J. Baker
Peter J. Goodford
Wellcome Research Laboratories
Langley Court, Beckenham
Kent BR3 3BS, England

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Two Clonidine-like Compounds with Substituents at the 2-, 3-, and 6-Position of the Phenyl Ring Possessing Pronounced Hypotensive Potencies

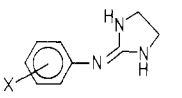
Sir:

The discovery of clonidine (**1**; Chart I) as a potent, centrally acting, hypotensive drug[1-4] has led to detailed studies on the relationship between structure and hypotensive activity in the class of 2-(phenylimino)-imidazolidines of the clonidine type. The majority of these investigations has considered variations of the substitution in the phenyl moiety of the molecules.[5-17] An interesting observation has been the approximately equal hypotensive activities found for 2,3- and 2,6-disubstituted analogues,[7,11-17] of which clonidine itself is one of the most active representatives. This knowledge has prompted us to synthesize 2,3,6-trisubstituted derivatives with the objective of obtaining potent hypotensive drugs in the clonidine series.

The present communication reports on the hypotensive activities of 2-[(2,3,6-trichlorophenyl)imino]imidazolidine (**2**) and 2-[(2,3-dichloro-6-methylphenyl)imino]-imidazolidine (**3**), demonstrating the potential of this newly substituted class of centrally acting, hypotensive imidazolidine compounds.

The trisubstituted 2-(phenylimino)imidazolidines **2**[18] and **3**[18] resulted from a reaction of the corresponding *N*-(di-

Chart I. Structural Formulas of Clonidine and Two of Its 2,3,6-Trisubstituted 2-(Phenylimino)imidazolidines[a]



1 (X = 2,6-Cl_2; Clonidine)
2 (X = 2,3,6-Cl_3)
3 (X = 2,3-Cl_2,6-CH_3)

[a] The numbering refers to Table I.

Table I. Hypotensive Activities, pC_{20}, and Apparent Partition Coefficients, Log P', of Clonidine and Two of Its 2,3,6-Trisubstituted Derivatives

compd	pC_{20}[a] anesthetized normotensive rat[b]	pC_{20}[a] anesthetized cat[c]	log P'[d]
1[e]	7.96	8.72	0.91
2	8.39	9.17	1.71
3	7.79	7.92	1.06

[a] –Log dose (moles/kilogram) required for 20% decrease in mean arterial pressure following systemic administration. The data reported were calculated from log dose-depressor response curves. At least six animals were used for each dose level, and a minimum of five dose levels was employed for determination of a dose-response curve. [b] Artificially ventilated, male Wistar, normotensive rats (200–250 g) anesthetized with pentobarbital (75 mg/kg, intraperitoneally) were used. The compounds dissolved in saline were administered as single bolus injections (0.5 mL/kg) via a cannulated jugular vein (see ref 12 and 16). [c] Artificially ventilated mongrel cats of either sex (2–4.5 kg) anesthetized with α-glucochloralose (60 mg/kg, intraperitoneally) were used. The compounds dissolved in saline were infused via the left vertebral artery in a volume of 140 μL over a period of 1 min (see ref 13). [d] Octanol/buffer (pH 7.4; 37 °C) partition coefficients (see ref 16 and 21). Mean P' values were obtained from six partition experiments (SEM < 5%). [e] Clonidine.

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(18) Compound **2** (base), formula $C_9H_8Cl_3N_3$: mp 153–156 °C (uncorrected); compound **3** (HCl salt), formula $C_{10}H_{12}Cl_3N_3$: mp 241–243 °C (uncorrected); both substances were analyzed for C, H, and N, and the results were within ±0.4% of theory.

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