J. Med. Chem. **1991,** *34,* 1383–1394

(s, 26 H, $(CH_2)_{13}$), 1.57 (m, 2 H, CH_2CH_2O), 2.8 (m, 2 H, CH_2P), 3.38–3.78 (m, 8 H, $CH_3OCHCH_2OCH_2$), 4.38 (bs, 2 H, $POCH_2$), 5.09 (m, 2 H, CH_2N), 8.03 (m, 2 H, pyridine), 8.40 (m, 1 H, pyridine), 9.49 (d, 2 H, pyridine). Anal. ($C_{27}H_{50}NO_5P\cdot 1H_2O$) C, H, N.

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Registry No. **2**, 131973-30-3; **3**, 131933-48-7; 4, 127642-24-4; **5**, 112989-00-1; **6**, 112989-01-2; **7**, 88876-07-7; **8**, 112989-02-3; **9**, 103304-64-9; **10**, 103304-65-0; 11, 112989-09-0; **12**, 131933-49-8; **13**, 22598-16-9; 14, 131973-31-4; **15**, 131933-50-1; **16**, 131933-51-2; **16** trityl derivative, 131933-60-3; **16** 2-bromoethyl phosphate derivative, 131933-61-4; **17**, 131933-52-3; **18**, 124581-78-8; **19**, 124581-94-8; **20**, 124581-81-3; **21**, 124581-79-9; **22**, 131933-53-4; **23**, 23248-47-7; **24**, 131933-54-5; **25**, 111-57-9; **26**, 82755-92-8; **27**, 131933-56-7; **28**, 119980-18-6; **29**, 119980-19-7; **30**, 92758-87-7; **31**, 131933-57-8; **32**, 126614-08-2; **33**, 126614-06-0; **34**, 131933-58-9; **35**, 126614-21-9; **36**, 131933-59-0; **36** dimethyl ester, 131933-63-6; **37**, 131933-64-7; Et-18-OMe, 70641-51-9; Et-18-OEt, 78858-43-2; AZT, 30516-87-1; 1-*O*-hexadecyl-2-*O*-ethylglycerol, 92758-87-7; *rac*-1-*O*-tosyl-2-*O*-ethylglycerol, 131973-32-5; *rac*-3-(hexadecylthio)-2-ethoxy-1-bromopropane, 124581-76-6; *N*,*N*-dimethyl-*N*-(2,3-dihydroxypropyl)amine, 98923-15-0; 2-(octadecanamido)ethyl 2′-bromoethyl phosphate, 131933-62-5; 1-(octadecyloxy)-2-iodoethane, 90339-56-3; *N*-(*β*-hydroxyethyl)pyridinium bromide, 31678-16-7; reverse transcriptase, 9068-38-6.

Receptor-Based Design of Novel Dihydrofolate Reductase Inhibitors: Benzimidazole and Indole Derivatives

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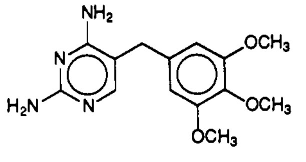
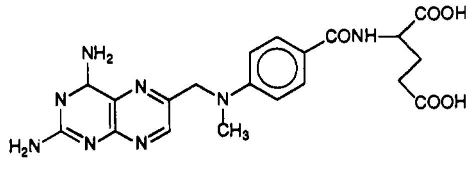
Although many thousands of inhibitors of the enzyme dihydrofolate reductase (DHFR) have been synthesized, all of the very active compounds have been 2,4-diaminopyrimidines or very close analogues. This paper describes 2,4-diamino-6-benzylbenzimidazole (**3b**) and the corresponding indole (**4**), as well as more complex tri- and tetracyclic derivatives (**5** and **6**). These were designed on the basis of molecular modeling to the known X-ray structure of *Escherichia coli* DHFR, in an effort to determine whether one could drastically alter the diamino configuration by placing one amino substituent in a 5-membered nitrogen-containing ring and the second in the ortho position of a fused ring and still inhibit DHFR significantly. Although the electronics and bond angles are quite different from that of a 2,4-diaminopyrimidine, the pK_a values are in an appropriate range, and hydrogen-bond distances appear to be quite reasonable. The most active compound, **4**, was very unstable and active only in the 10^{-4} M range. Dihydroindenoimidazole derivatives such as **6** showed quite a good fit to the enzyme by modeling studies, but had low activity. Since the most active compound made was 2 orders of magnitude weaker as an inhibitor of bacterial DHFR than the unsubstituted 5-benzyl-2,4-diaminopyrimidine, we concluded that such a ring system was unlikely to produce the high inhibitory potency of trimethoprim (**1**), even with greatly improved hydrophobic contacts. Thus the 2,4-diaminopyrimidine system remains unparalleled to date for the competitive inhibition of this enzyme.

Successful inhibitors of dihydrofolate reductase (DHFR, EC 1.5.1.3), such as trimethoprim (**1**) and methotrexate (**2**), have in almost every case been based on the 2,4-diaminopyrimidine skeleton or on closely allied 1,2,4-triazine or 1,3,5-dihydrotriazine analogues.[1] Prior to elucidation of the 3-dimensional structure of this enzyme many other substituent patterns, as well as other ring systems, were examined for their inhibitory properties, but none possessed the apparent unique properties of this original prototype.



1 (Trimethoprim, TMP)



2 (MTX)

The 3-dimensional structures of DHFR from *Escherichia coli*, *Lactobacillus casei*, chicken liver, mouse liver lymphoma, and human DHFR have been solved and refined in the presence of several ligands,[2-7] and it is now known that a very complex hydrogen-bonding pattern exists between a protonated diaminopyrimidine and the protein, involving all of the available hydrogen atoms.[5] In

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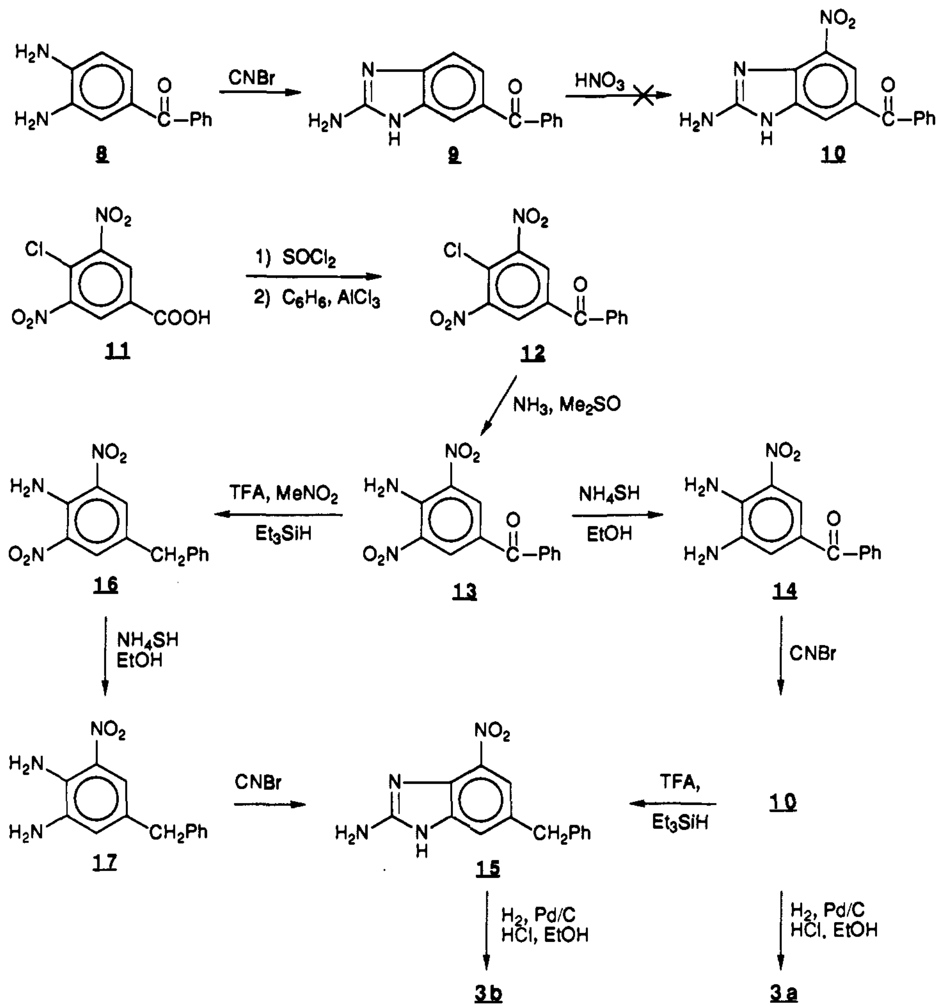
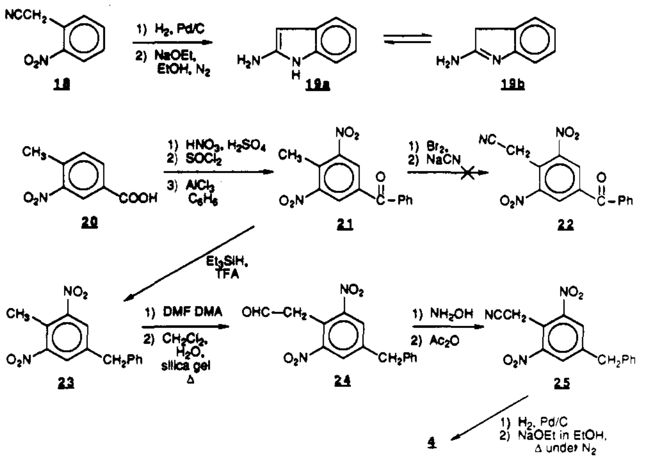
Scheme I



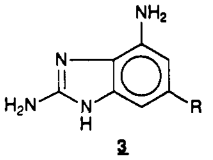
Scheme II



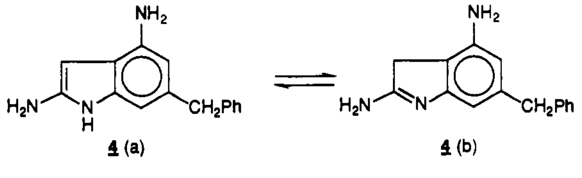
addition, tight binding requires a hydrophobic moiety which fits into an adjacent hydrophobic pocket.

The object of the research reported here was to test other types of ring systems which might conceivably fit into the active site of the enzyme by using the same hydrogen-bonding atoms, but in a system which would of necessity involve a different geometry and electron density pattern, and which would also have a hydrophobic moiety.

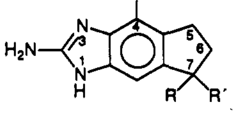
The compounds chosen for initial study were the benzimidazoles **3a** and **3b**, and the related indole **4**. Molecular modeling, chemical synthesis, and enzyme inhibitory data are described below. The results led to the synthesis (or attempted synthesis) of more complex semirigid derivatives, which are also discussed.



3
a R = C(=O)Ph
b R = CH2Ph



4 (a) 4 (b)



5 R, R' = H2
6 R, R' = -S(CH2)2S
7 R, R' = -O(CH2)2O

Chemistry

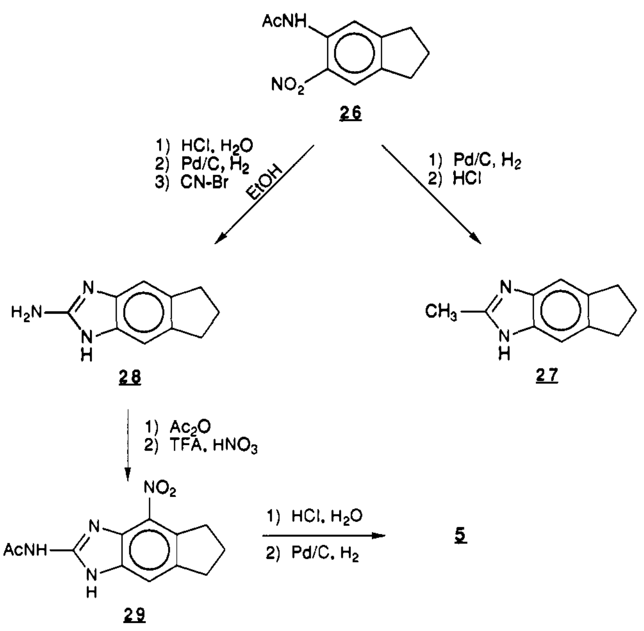
The syntheses of compounds **3a**,**b**, and **4** are summarized in Schemes I and II. It was initially anticipated that the synthesis of compounds **3a** and **3b** could be accomplished from 2-amino-5-benzoylbenzimidazole (**9**).[8] We prepared compound **9** from the commercially available 3,4-diaminobenzophenone (**8**) and cyanogen bromide. However, several attempts to nitrate **9** were unsuccessful, due to insolubility of the acetylated derivative in appropriate solvents.

An alternate route for synthesis of **3a** and **3b** involved the Friedel–Crafts reaction of 4-chloro-3,5-dinitrobenzoyl chloride (from **11**) and benzene to give **12**. The highly activated chloro group of **12** was then displaced with ammonia in dimethyl sulfoxide to give **13**. Selective reduction of one of the nitro groups was accomplished by the Zinin procedure,[9] to give the diamino derivative **14**. This was then converted to the benzimidazole **10**, followed by reduction of the ketone with trifluoroacetic acid and triethylsilane to give **15**. Alternatively the ketone group of **13** could be reduced first, followed by the Zinin reduction of the nitro group. The resultant diamino derivative **17** was then treated with cyanogen bromide to give **15**. This procedure gave slightly better yields in the final step. Catalytic reduction of the nitro groups of **10** and **15** gave the products **3a** and **3b**, respectively.

In the case of the aminoindoles, it was considered desirable to establish which tautomer (**19a** or **19b**, Scheme II) was the predominant structure in solution, due to past controversy on this point.[10,11] Following the procedure of Pschorr and Hope,[11] *o*-nitrophenylacetonitrile (**18**) was reduced with Pd/C and hydrogen to the amino derivative, which was then cyclized in deoxygenated sodium ethoxide solution to give a highly unstable compound. This product was isolated under nitrogen and immediately dissolved in deoxygenated deuterochloroform for ^{1}H NMR studies. The 3-H of 2-methylindole has been reported to occur at 6.05 ppm (δ) in $CDCl_3$,[12] and thus we expected a signal in this region if **19a** was present in solution. However, in $CDCl_3$ a strong signal appeared at 3.60 ppm which integrated for approximately 2 H, suggesting structure **19b**, which has two nonaromatic protons at position 3. A small sharp peak was also present at 5.60 ppm (about 6% of that at 3.60) which suggested that a small amount of tautomer **19a** was present in solution.

Compound **21** was synthesized from 4-methyl-3-nitrobenzoic acid (**20**) by nitration followed by Friedel–Crafts acylation.[13] Several attempts to brominate the methyl group with the intent to convert the brominated derivative to a nitrile (**22**) were unsuccessful, probably due to inactivation of the side chain by the neighboring electron-withdrawing groups. The problem was circumvented by first reducing the ketone function of **21** with TFA and triethylsilane to give **23**, followed by reaction with dimethylformamide dimethyl acetal to give the enamine, which was hydrolyzed with silica gel and water to give the aldehyde **24**. This product was then converted to the oxime, followed by dehydration with acetic anhydride to produce the nitrile **25**. The dinitro groups of **25** were reduced catalytically with Pd/C and hydrogen, followed by cyclization with sodium ethoxide to give a highly unstable product **4**. Attempts to purify the product either by recrystallization or by column chromatography led to polymeric products. The substance was finally isolated as the hydrochloride salt by precipitation with HCl gas from an ethanol solution and characterized without further purification. Its NMR spectrum showed the structure to be that of a 3*H*-indol-2-amine (**4b**).

Scheme III



Synthetic routes to compounds **5** and **6** are found in Schemes III–V. Compound **5** (Scheme III) was synthesized from 5-nitro-6-acetamido-2,3-dihydroindene (**26**),[14] which was hydrolyzed with HCl in dilute ethanol, followed by catalytic hydrogenation with Pd/C, and finally condensation with CNBr to give **28**. Initially hydrolysis of **26** failed because of its poor solubility in water. Attempts to reduce the nitro group, followed by hydrolysis, led to the formation of the 2-methyldihydroindenoimidazole **27**. Compound **28** was nitrated in trifluoroacetic acid with 70% nitric acid, after protection of the 2-amino group with acetic anhydride, thus producing **29**. Hydrolysis of the amide, followed by reduction of the nitro group with hydrogen and palladium on charcoal gave **5**.

Two routes (Schemes IV and V) were designed for the synthesis of compounds **6** and **7**. In the first route, 4-chloro-3-nitrophenylcinnamic acid (**30**)[15] was reduced with diimide[16-18] to give **31**. Attempted nucleophilic displacement of the 4-chloro group of **31** or its esterified derivative with ammonia in dimethyl sulfoxide under several conditions did not produce **32**. However, the chloro group of the esterified derivative of **30** was easily displaced to give **33**, which was then converted to the benzimidazole **34**. Unexpectedly, the double bond of **33** survived catalytic hydrogenation with 10% palladium on charcoal at 50 psi for 48 h. The double bond of **34** was reduced with diimide[17,18] after hydrolysis of the ester and protection of the 2-amino group with acetic anhydride, to give **35**.

Cyclization of **35** in polyphosphoric acid afforded a very unstable compound which could not be purified by either chromatography or recrystallization. Mass spectral analysis of the crude derivative suggested the formation

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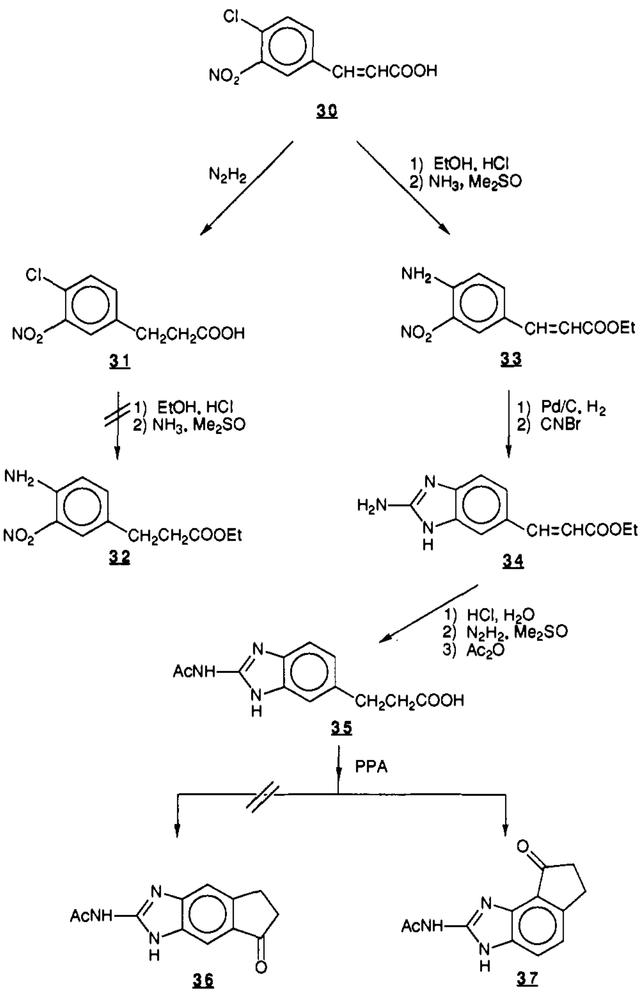
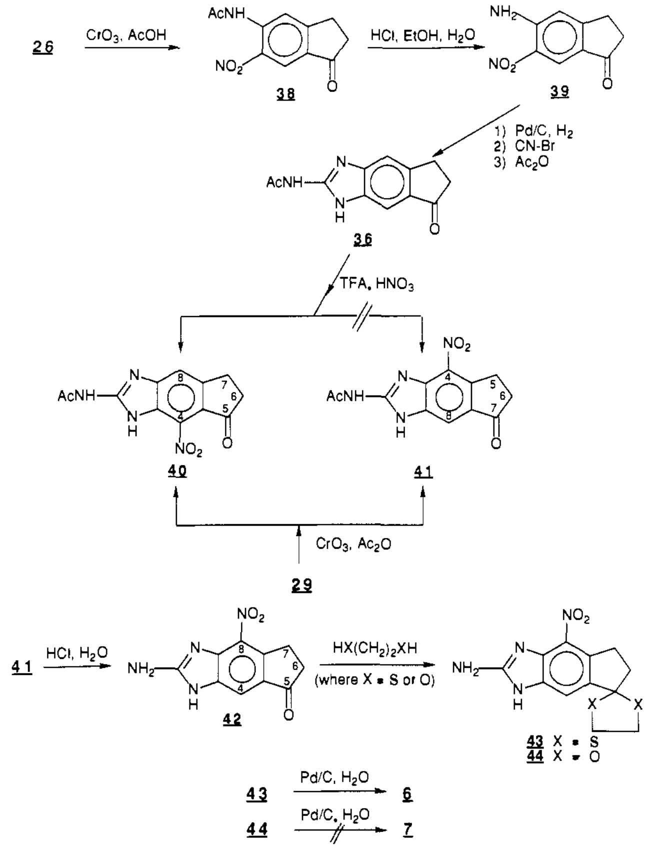
Scheme IV



Scheme V



of the expected product **36**. However, NMR spectroscopy of the crude substance showed a splitting in the aromatic region which was consistent with **37**, the unexpected isomer. This route was therefore abandoned.

An alternate route (Scheme V) involved oxidation of **26** with chromic oxide in acetic acid to give **38**. The position of the resultant keto function was not proven unequivocally, but was probably the expected isomer based on the meta-directing effect of the nitro group; the aromatic NMR signals were consistent with this interpretation. This was followed by hydrolysis to **39**. Catalytic hydrogenation followed by ring closure with CNBr and acetylation gave **36**. It was anticipated that the ketone function in **36** would direct electrophilic aromatic substitution to give the nitro derivative **41**. However, nitration of **36** with nitric acid in trifluoroacetic acid gave only the unexpected isomer **40**. This structure was proven by NMR spectroscopy by investigating the NOE effect on the 7-methylene protons and the aromatic proton. The distribution of the electrons on the aromatic ring could not easily explain this result. However, it is possible that a complex might form between the nitrating agent with the 1-NH or the ketone, which would favor the nitration in the position adjacent to the ketone function on the benzene ring.

This problem was circumvented by oxidizing compound **29** with chromic oxide in acetic anhydride, which gave a mixture of **41** and **40** in a ratio of 2:1. Separation, followed by hydrolysis of **41** with dilute acid, gave **42**. This was converted to **43** and **44** with ethanethiol and ethylene glycol, respectively. All attempts to reduce **44** to give **7** led to polymeric materials. However, **43** was successfully reduced to **6** with palladium/charcoal and hydrogen.

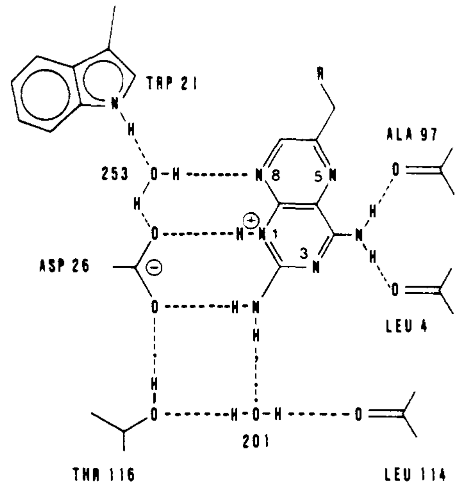


Figure 1. Hydrogen bonding pattern observed for the pteridine moiety of methotrexate (2) in *L. casei* DHFR. This is reproduced from Bolin et al. (ref 5) with permission (Journal of Biological Chemistry).

Molecular Modeling, Enzyme Binding, and Discussion

The hydrogen-bonding pattern for the pteridine moiety of methotrexate (**2**) in ternary complex with *Lactobacillus casei* DHFR and NADPH has been elucidated by Bolin et al.,[5] as shown in Figure 1. The pyrimidine ring of trimethoprim (**1**) binds in an identical manner to *Escherichia coli* DHFR in binary complex,[3] so we felt confident of using this structure for modeling. The structure of the ternary complex, with coenzyme, has not been refined.[2] In designing non-pyrimidine inhibitors to fit into the active site of the *E. coli* enzyme, we started from the premise that we should attempt to retain the five direct

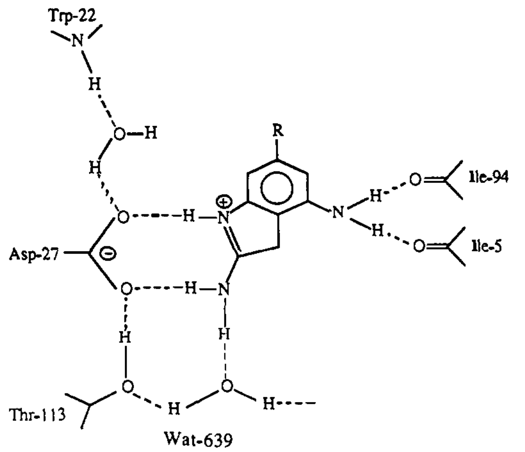


Figure 2. Postulated hydrogen bonding pattern for compound 4b in *E. coli* DHFR. Residues which are equivalent in *E. coli*/*L. casei* DHFR are as follows: Trp-22/Trp-21; Asp-27/Asp-26; Thr-113/Thr-116; Ile-5/Leu-4; Ile-94/Ala-97; Wat-639/Wat-201.

hydrogen bonds from the amino groups and the protonated nitrogen to the enzyme side chains or backbone, and furthermore that we should maintain an electrostatic interaction with Asp-27. Methotrexate, TMP, and pyrimethamine are protonated in their complexes with DHFR, as has been well documented by NMR and UV studies.[19-24] Furthermore, analogous pyrimidine derivatives with lower pK_a values have been found to bind poorly to the enzyme when tested near physiological pH.[25] With certain isosteres of low pK_a, that activity is restored by testing in media of low pH.[26,27]

We were further constrained by the knowledge that thousands of heterocyclic compounds, as well as guanidines, biguanides, and related nonheterocyclic bases, have been tested as DHFR inhibitors during the past 40 years and were found to have little or no activity if they lacked the diaminopyrimidine skeleton or that of certain close isosteres. Even 3-deaza-TMP, which retains high basicity and loses no potential hydrogen bond on the exchange of a 3-N= by —CH= is 300-fold less active than its parent,[28] possibly due to steric interference by the added hydrogen atom.[29]

A few unrelated compounds have been claimed to inhibit DHFR at concentrations of 10^{-3} to 10^{-5} M, including various tricyclic antidepressants,[30] CoA,[31] certain pyrrolopyrimidones,[32] sulfonamides,[33] and others. The CoA binding was competitive with the cofactor NADPH, rather than the substrate. In the other cases no attempt to define a mechanism was described. The experiments in most cases were carried out in an effort to explain weak anticancer activity of certain drugs used for entirely different purposes. None of these compounds have properties which should lead to strong binding in the substrate cavity.

Table I. Distances between Hydrogen-Bonding Groups on 1 or 4 and Those in the Active Site of *E. coli* DHFR.

E. coli DHFR residue	H-bonding substituent on 1 or 4	distance, Å	
		1[a]	4
Asp 27	1	1.5665	1.5192
Asp 27	2	1.6630	1.2710
Water-639	2	2.6567	2.4639
Ile-94	4	2.1501	1.5732
Ile-5	4	2.2330	2.6070

[a] Data from ref 3.

Table II. Inhibitory Activities (I_{50}) of Benzimidazole and Indole Derivatives against Dihydrofolate Reductase Enzymes Compared to Trimethoprim (1)

compd no	I_{50} vs dihydrofolate reductase, μM	
	E. coli	rat liver
1	0.005–0.007[a]	260–370[a]
3a	100 (I_{14})	100 (I_{16})
3b	300 (I_{42})	300 (I_5)
4	100	500 (I_{28})[b]
5	360 (I_{13})	360 (I_7)
6	140 (I_{31})	14 (I_{14})

[a] Range of many tests over a long period of time. [b] Very steep slope.

The target compounds **3b** and **4** which we chose for initial study seemed to fulfill the requirements for sufficient basicity. 2-Aminobenzimidazole is reported to have a pK_a value of 7.51,[34] and 2-aminoindole a pK_a of 8.15, although the structure is mainly 3*H*-indol-2-amine.[10] The actual pK_a values which we found for **3b** and **4** were 7.21 and 8.25, respectively. The compounds also possessed sufficient hydrogen-bonding atoms, but the question remained as to whether these would fit properly into the active site. The different bond angles, and the entirely different electronic properties of the electron-rich 5-membered rings compared to the diaminopyrimidines, was a major cause for concern.

Figure 2 depicts a possible fitting of compound **4** into the DHFR active site cavity according to the hydrogen-bonding pattern of Figure 1. We decided to carry out our initial computerized graphics fitting by simply superimposing the five-membered ring of **3** or **4** over the pyrimidine ring of **1** in its X-ray conformation in *E. coli* DHFR binary complex.[3] The protonated small molecules were built by using PROPHET[35] and optimization of the hydrogen-bonding fit was then carried out by using the program MATCHMOL.[36] The resultant hydrogen-bond distances to the protein atoms for **4** compared to **1** are listed in Table I. The results were found to compare very favorably with one exception. The 1.27 Å distance from Asp-27 to the

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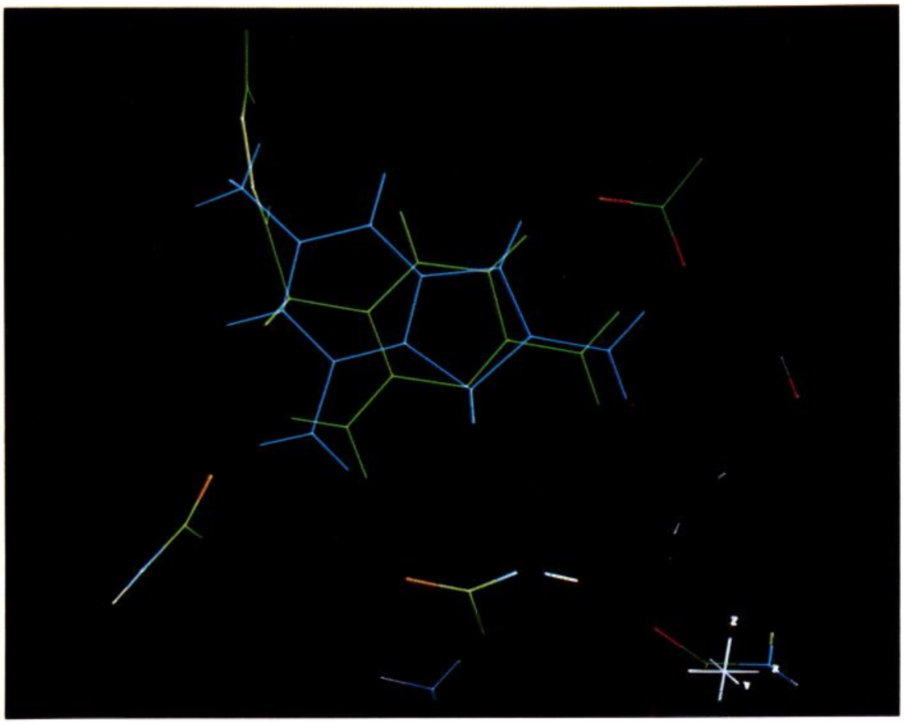

Figure 3. Superposition of the heterocyclic rings of 1 and 4 in *E. coli* DHFR. Compound 1 is in green, and 4 in blue. Asp-27 is in the upper right, with the carboxylate oxygen atoms shown in red; the backbone carbonyl atoms of Ile-5 and Ile-94 are at the bottom, left and middle. Representation is on Evans and Sutherland picture screen. Coordinates for the complex of *E. coli* DHFR with 1 (ref 3) are available from the Brookhaven protein data bank.

2-amino group of **4** is too short by about 0.2 Å, which would require some adjustments of the ligand. Figure 3 shows the actual superposition by using the Evans and Sutherland PS 300 graphics systems.

To compare the location of the aromatic moieties of **3a,b**, **4**, and **1** in the hydrophobic pocket, we fitted the compounds by constraining the benzimidazole moiety into the position described above, followed by energy minimization with AMBER.[37] The results are shown for **3b** in Figure 4, in which a surface representation (with double van der Waals radius) for the protein atoms of the hydrophobic pocket is depicted as dots, with the benzyl group shown as a stick figure which clearly fits into the pocket. Compound **3a**, not shown, cannot achieve the appropriate torsional angles with its sp^2 bridge carbon and appears to bump into the protein backbone unless the whole molecule is shifted into an unfavorable location. It then was not expected to bind appreciably, but was available for comparison, since it was an intermediate to **3b**.

Table II shows the inhibitory activities of **3a,b** and **4** against *E. coli* and rat liver DHFR compared to **1**. These are expressed as I_{50} values where possible. Low activity, coupled with low solubilities, precluded obtaining accurate I_{50} values in some instances. Compound **3a** had negligible activity against the two enzymes. Compound **3b**, on the other hand, nearly reached an I_{50} at 3×10^{-4} M against the bacterial enzyme, but was very inactive against mammalian DHFR. Compound **4**, despite its instability, was the most active of the three compounds. The very steep slope observed when reacting with rat liver DHFR suggested that an irreversible reaction might be taking place with the enzyme. We have no further information on this point. The rat liver DHFR has not been sequenced, so one can only speculate as to a conceivable reaction site.

These activities, while low, were nonetheless encouraging. The relative data suggested that the increased binding of **4** compared to **3b** might result from its greater charge localization at N1, coupled with the fact that the 3-carbon may lie close to 5-Ile side chain, which could provide a hydrophobic contact.

In using an unsubstituted benzyl moiety with **3b** and **4** in these initial studies we were of course mindful of the fact that **1** owes its very high inhibitory activity to its three methoxy functions, which form vital contacts with *E. coli* DHFR and its coenzyme NADPH.[2,3,38] Comparative values of K_i (nM) for **1** and its unsubstituted benzyl derivative are 1.3 and 671, respectively.[39] The importance of a hydrophobic moiety is illustrated by the fact that 2,4-diaminopyrimidine itself has a K_i value of only 760 000 nM.[40]

We compared the positions of the aromatic ring of **1** and **3b** superimposed in the enzyme pocket, as illustrated in Figure 5, and noted that the benzene ring of **3b** extended farther out toward the edge of the cleft than was the case with **1**. It appears to be nearly superimposed on the methoxy groups of the latter, and the oxygen atoms of these are in partial contact with solvent.[2,38] However, we also compared the refined X-ray structures (2.3 vs 1.7 Å, respectively) of the binary complexes of **1** and methotrexate (**2**) in *E. coli* DHFR by superposition and found that the benzene moiety of **2** also extended farther out than that of **1**, although there appears to be a partial overlap.[3,5] It makes contacts with Leu-28 and Ile-50, as well as Leu-54 and Ile-94; **3b** and **4** as modeled appear to do so as well. We cannot then ascribe the lower activity of **3b** and **4**,

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(37) Weiner, P. K.; Kollman, P. A. *J. Comp. Chem.* **1981**, *2*, 287.

(38) Roth, B.; Rauckman, B. S.; Ferone, R.; Baccanari, D. P.; Champness, J. N.; Hyde, R. M. *J. Med. Chem.* **1987**, *30*, 348.

(39) Baccanari, D. P.; Daluge, S.; King, R. *Biochemistry* **1982**, *21*, 5068.

(40) Ferone, R. Unpublished data.

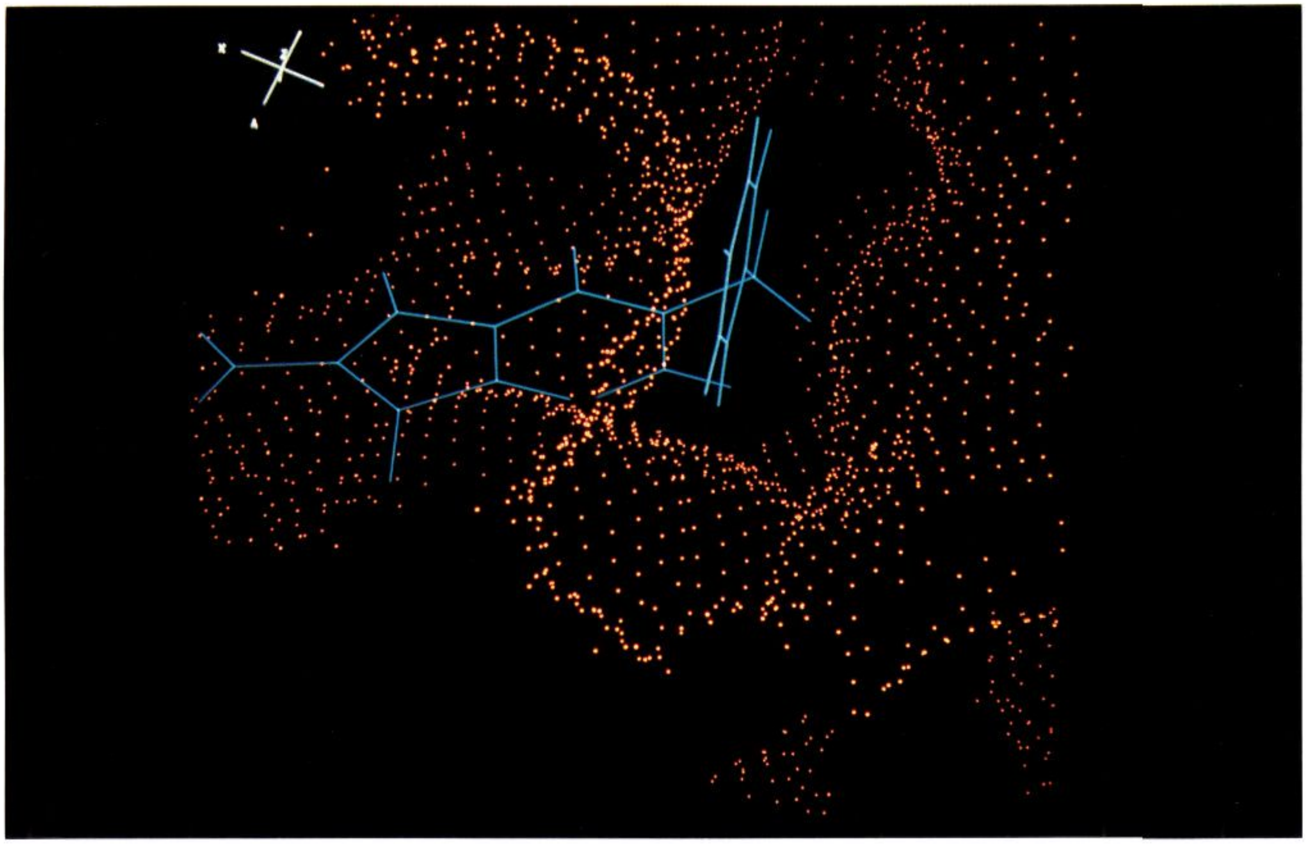


Figure 4. Compound 4 fitted into hydrophobic activity of *E. coli* DHFR, shown as a black hole, upper center, surrounded by protein surface, shown with orange dots. The benzyl moiety, shown in blue, fits in this pocket, with the benzene ring facing the viewer edge on.

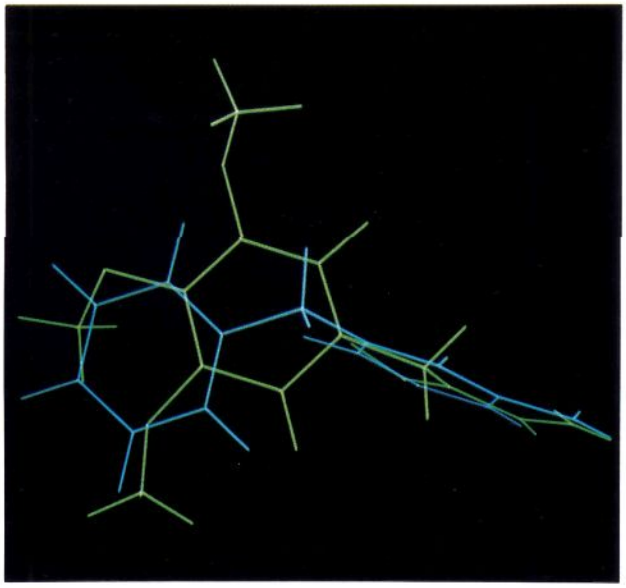
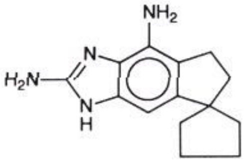


Figure 5. Superposition of the hetero rings of 1 and 4 shown edge on, on the right, and resultant positioning of the benzene ring of 4 (in blue) on the trimethoxybenzyl moiety of 1 (in green). Note that the benzene ring of 4 is nearly superimposed on two methoxy groups of 1.

relative to the unsubstituted benzyl derivative of 1, to the different hydrophobic milieu. However, additional hydrophobic contacts certainly appeared warranted, which necessitated a revision in design.

A study of the hydrophobic pocket of the enzyme in the presence of **3b** suggested that a better fit to the enzyme would be obtained by fusing a 5-membered ring to the benzimidazole to form a linear 5,6-dihydroindenoimidazole such as **5**. This per se did not fill the pocket sufficiently, but the addition of a tetramethylene function to form a spiro ring, as in **45**, appeared to fit the pocket very well.



45

This semirigid structure, energy minimized in the active site of the protein (held rigid) using the program MACROMODEL[41] is shown in Figure 6, and in Figure 7 compound 1 is superimposed on **45** in the enzyme. The spiro ring appears to be nearly superimposed on the benzene ring of 1. Figure 8 depicts this superposition looking down on the aromatic moiety of 1. This basic structure seemed a good starting point for devising new syntheses, with embellishments to be provided later. As an initial target we rationalized that a thioketal, or possibly a ketal, derivative such as **6** or **7** might serve as a guide to structure–activity, since the ketone intermediate **42** might provide an appropriate entry. As described above, we did obtain targets **5** and **6**. Inhibitory activities against DHFR are shown in Table II, and no improvement over the initial compounds

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(41) Still, W. C.; Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liscamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrikson, T. Macromodel V2.5, Dept. of Chemistry, Columbia University.

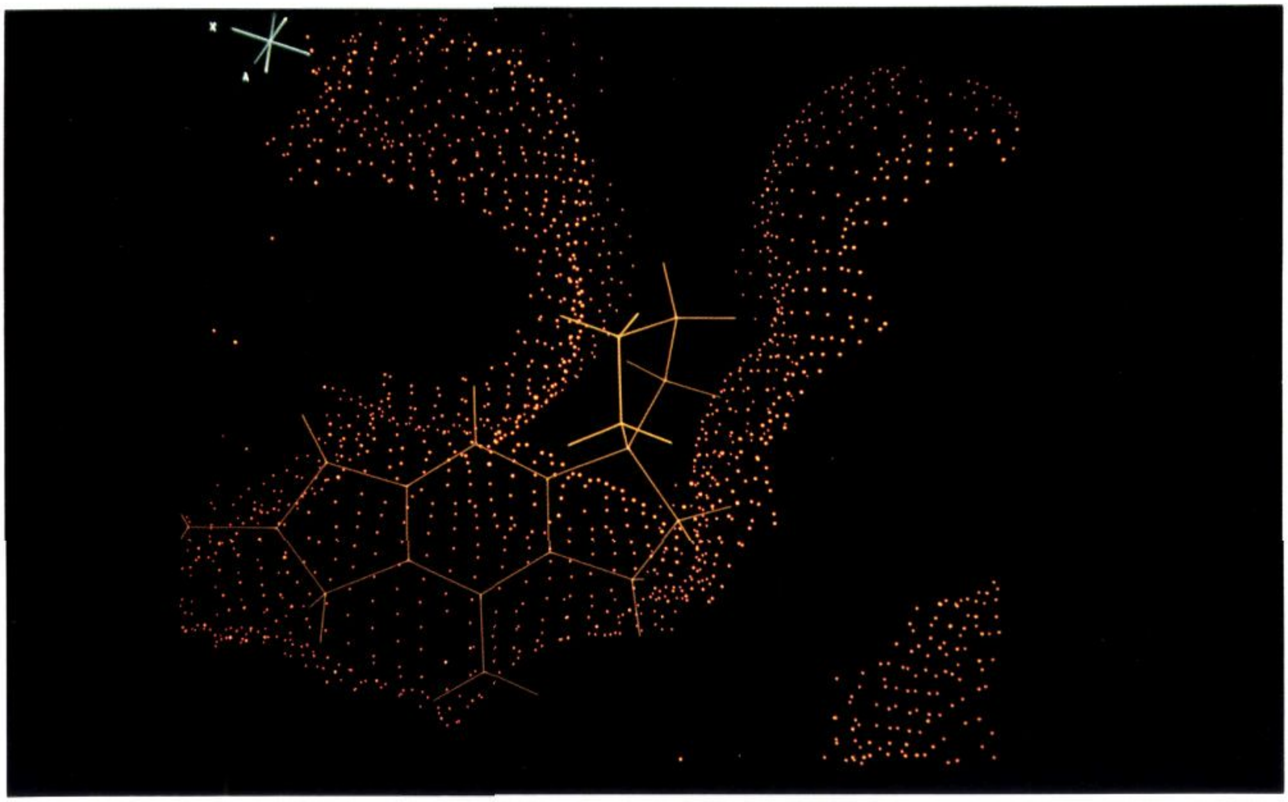

Figure 6. Hypothetical compound **45** fitted into the hydrophobic cavity of *E. coli* DHFR, as in Figure 4.

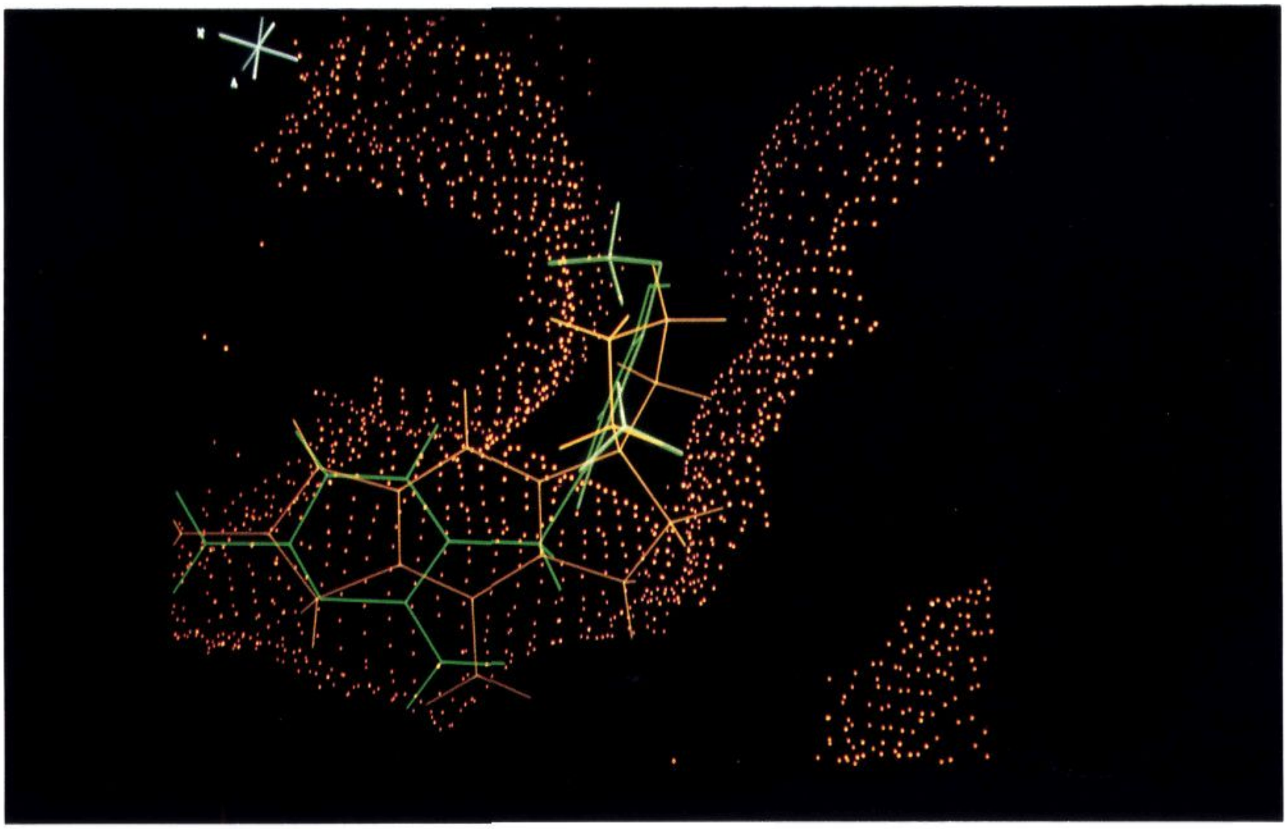

Figure 7. Superposition of **45** (yellow) and **1** (blue) in the hydrophobic cavity of *E. coli* DHFR.

is seen. Compound **6**, with an additional ring, is more active than **5**, but low solubility precludes accurate comparison. None of the compounds showed any antibacterial activity at 100 μg/mL in vitro against a spectrum of 24 organisms, and toxicity was noted in other tests.

We have insufficient information to explain these low activities unequivocally. Initial results with **3b** and **4** suggested that the indoles would be superior candidates

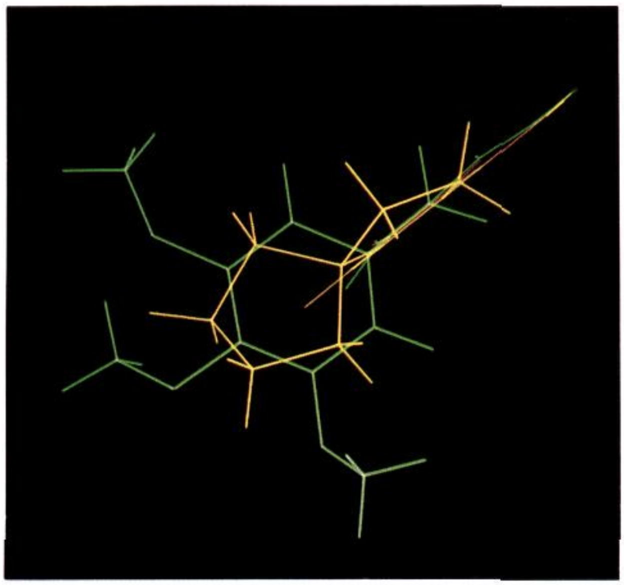

Figure 8. Superposition of **45** (yellow) and **1** (blue) in the manner of Figure 5, showing the locus of the spiro ring relative to the aromatic moiety of **1**.

for further study, but synthetic pitfalls precluded this as an initial choice. The fact that the charge on the protonated 2,4-diaminobenzimidazoles may be distributed between the 1- and 3-nitrogen atoms may create a hydration sphere which hinders a fit in the pocket around the Ile-5 side chain.

Another cause for concern was the close contact calculated for the one hydrogen bond listed in Table I between Asp-27 and the 2-amino group. A referee has pointed out that this might force the ligand back several tenths of an Ångstrom, so that it might lose a hydrogen bond with the backbone carbonyl atom of Ile-5, as well as creating bad geometry for a hydrogen bond from Asp-27 to protonated N1.

We carried out energy calculations with various modifications of **45**, with additional hydrophobic atoms, which suggested that it might be possible to increase DHFR inhibition significantly—particularly with indoles, but the previously mentioned concerns rendered the project impractical for us to pursue.

The design of non-diaminopyrimidine inhibitors of DHFR remains a very challenging problem, which may possibly be solved with the newer molecular mechanics and dynamics approaches and with supercomputers. It is not often that we have the X-ray structure of our target receptor and a vast multitude of ligands that have been studied.

Experimental Section

Melting points were determined on a Meltemp apparatus and are uncorrected. All ^1H NMR spectra were determined on Varian 90, XL-100, and 300-MHz spectrometers, and values are reported in ppm (δ) from Me_4Si. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA and are within 0.4% of the theoretical values unless otherwise indicated. Thin-layer chromatography (TLC) was performed on silica gel plates with a fluorescent indicator, and visualized with light at 254 nm. All final products showed a single spot on TLC. Mass spectral data were carried out under the supervision of Dr. David Brent, using electron impact, and NMR spectra by Dr. Stuart Hurlbert and his staff. Biological assays were carried out under the supervision of Robert Ferone using methods previously described.[42] The dissociation constants of compounds **3b** and **4** were measured as described in ref 43.

2-Amino-5-benzoylbenzimidazole (9).[8] To a stirred suspension of 3,4-diaminobenzophenone (**8**) (21.23 g, 0.10 mol) in H_2O (200 mL) was added CNBr (10.6 g, 0.1 mol). Most of the starting material remained undissolved initially, but as the reaction proceeded a solution was obtained. The stirring was continued for 24 h, after which the mixture was filtered. The filtrate was made strongly basic with concentrated NH_4OH, and the resultant syrup was left to stand without stirring for 1 h, during which time it crystallized. The solid material was washed with water until neutral and dried over P_2O_5, giving 20 g (83%) of **9** as a yellow product: mp 170–171 °C (EtOAc/hexane, 1:1); TLC (silica gel, EtOAc/hexane, 1:1) R_f 0.13; MS (M^+) 237. Anal. ($C_{14}H_{11}N_3O\cdot0.25H_2O$) C, H, N.

4-Chloro-3,5-dinitrobenzophenone (12). A suspension of 4-chloro-3,5-dinitrobenzoic acid (**11**) (50 g, 0.2 mol) in $SOCl_2$ (100 mL) was refluxed for 15 h. The $SOCl_2$ was then distilled off. Dried thiophene-free benzene (150 mL) was added and 30 mL was distilled off. The clear solution was added dropwise over 30 min to an ice-cooled suspension of $AlCl_3$ (40.6 g) in benzene (100 mL), with stirring. A yellowish precipitate formed immediately. The mixture was stirred for an additional 3 h and poured onto a mixture of ice (150 g) and concentrated HCl (90 mL), along with 45 mL of additional benzene used to rinse the flask. The mixture was stirred for 30 min, followed by separation of the organic phase. The aqueous layer was extracted twice with CH_2Cl_2 (50 mL each). The combined organic extracts were washed with saturated $NaHCO_3$ (200 mL), followed by H_2O, and dried over $MgSO_4$. The solvent was removed in vacuo, and the residue was crystallized from *i*-PrOH, giving 40 g (64%) of **12** as a yellowish crystalline solid: mp 98–100 °C; MS (M^+) 306. Anal. ($C_{13}H_7ClN_2O_5$) H, N; C: calcd, 50.92; found, 51.50; Cl: calcd, 11.56; found, 12.08.

4-Amino-3,5-dinitrobenzophenone (13). Anhydrous ammonia was bubbled through Me_2SO (80 mL) for 15 min, followed by the addition of **12** (13.1 g, 43 mmol). The resultant solution immediately turned red. The temperature was gradually raised to 100 °C, and ammonia was then bubbled through the mixture continuously for 12 h. The mixture was poured into ice–water (200 mL) and the resultant yellow precipitate filtered, air dried, and crystallized from *i*-PrOH to give 10.2 g (82.7%) of **13** as a yellow powder; mp 115–116 °C; MS (M^+) 287; ^1H NMR (80 MHz, Me_2SO-d_6) δ 7.70 (m, 5, C_6H_5), 8.72 (s, 2, 2- and 6-H), 8.80 (br s, 2 NH_2). Anal. ($C_{13}H_9N_3O_5$) C, H, N.

3,4-Diamino-5-nitrobenzophenone (14). Hydrogen sulfide was bubbled through a mixture of 2 N NH_4OH (80 mL) and absolute EtOH (80 mL) until a constant weight was obtained. Then **13** (7 g, 2.4 mmol) was added with stirring. Most of the material remained undissolved initially, but after about 10 min a clear solution was formed, followed by immediate precipitation of a brown solid. The mixture was stirred for an additional 30 min, and the precipitate was filtered. The filtrate was chilled to 10 °C, which resulted in the precipitation of additional product. The combined precipitates were air dried and crystallized from PrOH, yielding 5.14 g (79%) of **14** as reddish brown needle-like crystals: mp 128–130 °C; MS (M^+) 257. Anal. ($C_{13}H_{11}N_3O_3\cdot0.8H_2O$) C, H, N.

4-Benzyl-2,6-dinitroaniline (16). To a solution of **13** (2 g, 6.97 mmol) in CF_3COOH (7 mL) and $MeNO_2$ (5 mL) (to aid solution) was added Et_3SiH (2 g) over 5 min. The mixture was stirred at room temperature for 96 h, after which time a yellowish precipitate had formed on the sides of the flask. Acetone (20 mL) was added to dissolve the precipitate, followed by 30 mL of H_2O, and the solution clarified. The filtrate was basified at 15 °C to pH 8 with concentrated NH_4OH, and the resultant precipitate filtered, washed with water, and dried, followed by recrystallization from anhydrous Et_2O. This produced 1.86 g (98%) of **16** as a yellow powder: mp 82–84 °C; MS (M^+) 273; ^1H NMR (80 MHz, $CDCl_3$) δ 3.96 (s, 2, CH_2), 7.24 (m, 5, C_6H_5), 8.37 (br s, 4, NH_2 and 2,6-H_2). Anal. ($C_{13}H_{11}N_3O_4$) C, H, N.

2-Amino-6-benzoyl-4-nitrobenzimidazole Hydrochloride (10). To a suspension of **14** (3.78 g, 14.0 mmol) in 95% EtOH (150 mL) plus H_2O (50 mL) was added a 5 M solution of CNBr

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[42] Roth, B.; Strelitz, J. Z.; Rauckman, B. S. *J. Med. Chem.* **1980**, *23*, 379.

[43] Roth, B.; Strelitz, J. Z. *J. Org. Chem.* **1969**, *34*, 821.

in MeCN (10 mL) over a 15-min period with stirring. The mixture was stirred at room temperature for 48 h, and then filtered. A TLC analysis of the filtered material indicated that it was identical with the starting material. The filtrate was basified to pH 8 with NH_4OH and cooled to 15 °C. The resultant precipitate was filtered and dissolved in EtOH plus a few drops of concentrated HCl. The solution was evaporated to dryness under reduced pressure and the residue crystallized from absolute EtOH, resulting in 0.92 g (20%) of **10**: mp 251–252 °C; TLC (silica gel; EtOAc/EtOH, 5:1) $R_f = 0.45$; MS (M^+) 282; ^1H NMR (80 MHz, Me_2SO-d_6) δ 7.7 (m, 5, C_6H_5), 8.09 (d, 1, 7-H), 8.26 (d, 1, 5-H), 8.79 (br s, 2, 2-NH_2). Anal. ($C_{14}H_{10}N_4O_3$·HCl), C, H, N, Cl.

2-Amino-6-benzyl-4-nitrobenzimidazole (15). Method A. Compound **15** was synthesized from **10** (0.9 g, 2.8 mmol), TFA (5 mL), and Et_3SiH (1 g) by using the method described for **16**: yield 0.76 g (97%); mp 186–188 °C; TLC (silica gel, EtOH/EtOAc, 1:5) R_f 0.47; MS (M^+) 268; ^1H NMR (80 MHz, Me_2SO-d_6) δ 6.76 (br s, 2 NH2), 7.28 (s, 5, C_6H_5), 7.37 (d, 1, 7-H), 7.61 (d, 1, 5-H). Anal. ($C_{14}H_{12}N_4O_2$·0.6H_2O) C, H, N.

Method B. Compound **15** was prepared from **17** (1.5 g) and BrCN (0.93 g, 8.76 mmol) and 50% EtOH (60 mL) in 75% yield by using the procedure for **10**.

2-Amino-4-benzyl-6-nitroaniline (17). This compound was prepared by a procedure similar to that used for **14**, from **16** (4 g, 0.015 mol) in a mixture of 2 N NH_4OH and absolute EtOH (50 mL), by bubbling H_2S into the solution for 1 h. After workup, it was found that the compound was unstable on storage, so it was used directly without purification. The crude yield was 3.2 g; MS (M^+) 243.

2,4-Diamino-6-benzylbenzimidazole Dihydrochloride (3b). A mixture of **15** (0.5 g, 1.80 mmol), 5% Pd/C (0.4 g), 12 N HCl (1 mL), and EtOH (41 mL) was hydrogenated at 30 psi for 15 min, giving a total pressure drop of 5 psi. The catalyst was removed and the solvent evaporated under reduced pressure. The residue was dissolved in hot BuOH, a few drops of hexane was added, and the mixture was chilled, which deposited 0.3 g of **3b** (51%): mp 190–195 °C dec; TLC (silica gel, EtOH/EtOAc/1 N NH_4OH, 20:9:1) $R_f = 0.55$; MS (M^+) 268; ^1H NMR (80 MHz, Me_2SO-d_6) δ 3.90 (s, 2, CH_2), 6.57 (d, 2, 5- and 7-H), 7.24 (s, 5, C_6H_5), 8.40 (br s, 2, NH_2); $pK_{a1} = 2.09 \pm 0.10$; $pK_{a2} = 7.21 \pm 0.06$ (20 °C).[43] Anal. ($C_{14}H_{14}N_4$·2HCl·0.7H_2O) C, H, N, Cl.

2,4-Diamino-6-benzoylbenzimidazole Hydrochloride (3a) (Incomplete dihydrochloride). A mixture of **10** (0.2 g, 0.63 mmol), EtOH (5 mL), 12 N HCl (2 mL), and Pd/C (5%), 0.1 g, was hydrogenated at 30 psi until 3 equiv of hydrogen were consumed. The catalyst was removed and the solvent evaporated. The resultant syrup was suspended in 50 mL of EtOAc, heated to the boil, and filtered, followed by chilling, which resulted in the crystallization of 0.12 g (57%) of **3a** as a yellow powder: mp 260–262 °C dec; MS (M^+) 252; TLC (silica gel, EtOAc/EtOH/NH_4OH, 9:20:1) $R_f = 0.64$; ^1H NMR (80 MHz, Me_2SO-d_6) δ 7.11 (s, 2, 5- and 7-H), 7.63 (m, 5, C_6H_5), 8.60 (br s, 5, $(NH_2)_2$ and 1-H). Anal. ($C_{14}H_{12}N_4O$·1.68HCl·0.9H_2O) C, H, N, Cl.

4-Benzyl-2,6-dinitrotoluene (23). A mixture of compound **21**[13] (24 g, 0.084 mol), Et_3SiH (24 g), and TFA (84 mL) was treated as for compound **15** to produce **23**. The crude product was separated on a silica gel column, eluting with CH_2Cl_2/hexane (1:3), which gave 12.5 g (54.6%) of **23** as a transparent syrup which solidified on standing to a white product: mp 34–35 °C; MS (M^+) 272; TLC (silica gel, hexane/CH_2Cl_2, 3:1) $R_f = 0.13$. Anal. ($C_{14}H_{12}N_2O_4$) C, H, N.

2-(4-Benzyl-2,6-dinitrophenyl)acetaldehyde (24). A solution of **23** (4.75 g, 0.017 mol) and DMFDMA (6.43 g) in DMF (20 mL) was heated at 120–130 °C for 12 h under N_2 atmosphere. The DMF was removed under reduced pressure, and the residue was dissolved in a mixture of CH_2Cl_2 (100 mL) and H_2O (50 mL). Silica gel (50 g) was then added, and the mixture was refluxed for 4 h, followed by removal of the silica gel, which was washed several times with CH_2Cl_2. The aqueous layer was separated and discarded. The combined CH_2Cl_2 fractions were washed with H_2O and dried with $MgSO_4$, followed by removal of the solvent. The residue was purified by column chromatography with use of a silica gel column which was eluted with CH_2Cl_2/EtOAc (1:3) to give 2.12 g (42%) of **24**: mp 134–135 °C; MS (M^+) 300; TLC (silica gel, hexane/CH_2Cl_2, 3:1) $R_f = 0.13$; ^1H NMR (80 MHz, $CDCl_3$) δ 4.13 (s, 2, $ArCH_2Ar$), 4.23 (s, 2, CH_2CO), (m, 5, C_6H_5), 7.98 (s, 2, 3- and 5-ArH), 9.77 (s, 1, CHO). Anal. ($C_{15}H_{12}N_2O_5$) C, H, N.

2-(4-Benzyl-2,6-dinitrophenyl)acetonitrile (25). A warm solution of **24** (1.86 g, 6.2 mmol) in 95% EtOH (15 mL) was mixed with a solution of NH_2OH·HCl (0.52 g) in 1 mL of H_2O. To this was added a solution of NaOH (3.72 mg) in 0.2 mL of H_2O with stirring. The mixture was stirred continuously for 2.5 h, during which time a white precipitate formed. It was then poured on ice (50 g) and stirred until the ice melted. The precipitated oxime was filtered and dried (2.3 g). This was mixed with acetic anhydride (5 mL) and gently warmed to initiate dehydration. The mixture was then refluxed for 1.5 h, quenched in 50 g of ice, extracted twice with CH_2Cl_2 (25 mL), and washed with 50 mL of water. The organic phase was concentrated to dryness and purified by column chromatography with use of a silica gel column with CH_2Cl_2 as eluent: yield 0.6 g (32.6%) of **25**; mp 110–111 °C; MS (M^+) 297; ^1H NMR (80 MHz, $CDCl_3$) δ 4.14 (d, 4, CH_2), 7.29 (m, 5, C_6H_5), 8.06 (s, 2, 3- and 5-ArH). Anal. ($C_{15}H_{11}N_3O_4$) C, H, N.

4-Amino-6-benzyl-3*H*-indol-2-amine Dihydrochloride (4b). A suspension of **25** (0.76 g (2.50 mmol) and 10% Pd/C (0.12 g) in absolute EtOH (40 mL) was hydrogenated at 49 psi until a pressure drop of 15.5 psi occurred. The catalyst was removed and the filtrate evaporated to dryness. A TLC analysis indicated that the residue was homogenous. The subsequent steps of the procedure were carried out in a dry box in an oxygen-free atmosphere with use of deoxygenated solvents. A solution of NaOEt was prepared from deoxygenated EtOH (15 mL) and Na metal (0.28 g) under nitrogen. The above product was added, which caused the mixture to turn brown immediately. This was then refluxed for 2 h under nitrogen and the excess EtOH distilled off. Deoxygenated water (50 mL) was then added and the mixture stirred to a homogeneous suspension, followed by filtration under nitrogen. The precipitate was dissolved in deoxygenated EtOH (10 mL) and filtered, followed by bubbling HCl gas through the filtrate. This resulted in the precipitation of a brown product. Deoxygenated ether (300 mL) was then added, followed by chilling for 15 min and filtration. The precipitate was washed with deoxygenated ether (10 mL), followed by drying in vacuo over P_2O_5 which gave 410 mg (52%) of **4b**: 235–240 °C dec; TLC (silica gel, EtOH/EtOAc/NH_4OH, 20:9:1) $R_f = 0.84$; MS (M^+) 237; ^1H NMR (300 MHz, Me_2SO-d_6) 3.93 (s, 2, $ArCH_2Ar$), 4.16 (s, 2, 3-CH_2), 6.75 (d, 2, 5- and 7-H), 7.25 (m, 5, C_6H_5), 9.90 (s, 1, 2-NH), 10.22 (s, 1, 2-NH), 12.3 (br s, 1, 1-H); $pK_{a1} = 2.35 \pm 0.07$; $pK_{a2} = 8.25 \pm 0.04$ (20 °C).[43] Anal. ($C_{15}H_{15}N_3$·2HCl·0.2H_2O) C, H, N, Cl.

2-Methyl-1,5,6,7-tetrahydroindeno[5,6-*d*]imidazole (27). A suspension of **26**[14] (6.3 g, 28 mmol) in 95% EtOH (100 mL) was hydrogenated until 3 equiv of H_2 was consumed. The catalyst was removed and the filtrate concentrated to dryness. The residue was suspended in 100 mL of 2 N HCl and refluxed until no more starting material remained, as shown by TLC. The solution was cooled and the resultant precipitate isolated and recrystallized from EtOAc/EtOH plus a few drops of NH_4OH to neutrality: wt 2.83 g (58%) of **27**; mp, 250 °C; MS ($M^+ + 1$) 173; NMR (Me_2SO-d_6) δ 2.00 (m, 2, 6-CH_2), 2.40 (s, 3, CH_3), 2.85 (t, 4, 5- and 7-CH_2), 7.22 (s, 2, 4- and 8-H). Anal. ($C_{11}H_{12}N_2$·0.2H_2O) C, H, N.

2-Amino-1,5,6,7-tetrahydroindeno[5,6-*d*]imidazole (28). Compound **26** (5 g, 23 mmol) was hydrolyzed with 2 N HCl (60 mL) and EtOH (20 mL) by refluxing for 1.5 h and chilling. The precipitate was collected and dissolved in 50 mL of Me_2CO. The solution was neutralized with NH_4OH and taken to dryness under reduced pressure. The residue was crystallized from dilute EtOH, giving 3.5 g of 5-amino-6-nitroindan, mp 129–130 °C.[14] This substance (2.5 g, 14 mmol) and 500 mg of 10% Pd/C in 95% EtOH was hydrogenated at 40 psi until 3 equiv of H_2 was consumed. The catalyst was removed and the solution taken to dryness. The residue was suspended in 20 mL of H_2O and solid CNBr (2.0 g) added, followed by stirring for 30 min, filtering, and neutralizing the filtrate to pH 8 with ammonia. The resultant precipitate was isolated, washed well with water, dried, and dissolved in EtOAc. The solution was dried over $MgSO_4$ and then partially concentrated, followed by chilling. A crystalline product separated: 1.1 g (44%) of **28**; mp 200–201 °C; MS ($M^+ + 1$) 174; NMR (Me_2SO-d_6) δ 2.0 (m, 2, 6-CH_2), 2.70 (t, 4, 5- and 7-CH_2), 5.93 (s, 2, 2-NH_2), 6.92 (s, 2, 4- and 8-H), 10.5 (br s, 1, 1-NH). Anal. ($C_{10}H_{11}N_3$·0.2H_2O) C, H, N.

***N*-(1,5,6,7-Tetrahydro-4-nitroindeno[5,6-*d*]imidazol-2-yl)acetamide (29).** Compound 28 (5.2 g, 0.029 mol) was acetylated by stirring in Ac_2O (150 mL) at 25 °C for 18 h, followed by refluxing for 30 min. The mixture was then cooled to 15 °C with an icewater mixture. The resultant precipitate was filtered and suspended in 100 mL of water at 5 °C and the pH adjusted to 8 with concentrated NH_4OH. The mixture was extracted three times with 50 mL portions of EtOAc. The combined organic extracts were washed with water (100 mL) and dried ($MgSO_4$), and the solvent was removed, wt residue, 3.9 g. To a solution of the crude acetylated derivative (2.1 g, 0.01 mol) in CF_3COOH (30 mL) cooled to −4 °C was added dropwise 1.5 mL of 70% HNO_3 at a rate that kept the temperature below 0 °C. The total addition time was 2 h. The CF_3COOH was evaporated off and 5 g of ice was added, followed by stirring until the ice melted. The solution was made alkaline with NH_4OH and stirred for 10 min. The resultant precipitate was isolated, washed with H_2O, and recrystallized from EtOH and CH_2Cl_2: wt, 1.8 g (71%) of **29**; mp >300 °C; MS ($M^+ + 1$) 261; NMR (Me_2SO-d_6) δ 1.9 (m, 2, 6-CH_2), 2.1 (s, 3, CH_3CO), 3.0 (m, 4-H, 5- and 7-CH_2), 7.5 (s, 1, 8-H). Anal. ($C_{12}H_{12}N_4O_3$) C, H, N.

2,4-Diamino-1,5,6,7-tetrahydroindeno[5,6-*d*]imidazole Dihydrochloride (5). A suspension of **29** (1.5 g, 5.7 mmol) was hydrolyzed with 2 N HCl (20 mL) by refluxing for 1.5 h. The solution was chilled and the precipitate isolated and hydrogenated over Pd/C (200 mg) in 50 mL of 95% EtOH until 3 equiv of H_2 was consumed. The catalyst was removed and the EtOH evaporated. The residue was crystallized from 50% EtOH plus a few drops of 10 N HCl: wt 0.85 g (57%) of **5**; mp 300 °C dec; MS (M^+) 188; NMR (Me_2SO-d_6) δ 2.49 (m, 2, 6-CH_2), 2.75 (m, 4, 5- and 7-CH_2), 4.88 (br s, 4, NH_2, $(NH)_2$), 6.63 (s, 1, 8-H), 8.33 (s, 2, NH_2). Anal. ($C_{10}H_{12}N_4 \cdot 2HCl$) C, H, N, Cl.

3-(4-Chloro-3-nitrophenyl)propionic Acid (31). A solution of **30**[15] (10 g, 0.044 mol) in deoxygenated MeOH (150 mL) was added to a well-stirred suspension of potassium azodicarboxylate[16-18] (17.1 g, 61.6 mmol) in deoxygenated MeOH (150 mL) under N_2. A mixture of 8 g of AcOH and 20 mL of MeOH was then added dropwise to the mixture over 30 min, followed by stirring for 6 h. A second mixture of 8 g AcOH and 20 mL of MeOH was added over 20 min and stirring continued for another 18 h. The solution was clarified and the solvents removed, giving a yellow solid. This was dissolved in 50 mL of water and filtered and the pH of the filtrate adjusted to 6 with 2 N H_2SO_4, which resulted in the precipitation of a pink solid: 8.05 g (80%) of **31**; mp 65–67 °C (50% EtOH); MS (M^+) 229; NMR ($CDCl_3$) δ 2.72 (t, 2, 2′-CH_2), 3.00 (t, 2, 3′-CH_2), 7.38 (dd, 1, 6-ArH), 7.47 (d, 1, 5-ArH), 7.74 (d, 1, 2-ArH). Anal. ($C_9H_8ClNO_4$) C, H, N, Cl.

Ethyl 4-Amino-3-nitrocinnamate (33). Compound **30**[15] was esterified with anhydrous EtOH and HCl, which was slowly bubbled through for 48 h. The solvent was removed, and the residue (5.0 g, 0.02 mol) was then added to a solution of anhydrous NH_3 in 50 mL of Me_2SO, prepared by saturating the solvent at 25 °C by bubbling NH_3 through for 15 min. The mixture was then heated at 100 °C while continuously adding NH_3 for a 48-h period. The solution was then poured into 50 mL of ice/water and the yellow precipitate isolated and recrystallized from *i*-PrOH: 4.65 g (98.5%) of **33** was obtained; mp 135–137 °C; MS (M^+) 236; NMR ($CDCl_3$) δ 1.33 (t, 3, CH_3), 4.26 (q, 2, CH_2) 6.30 (d, 1, C=CH), 6.40 (br s, 2, NH_2), 6.84 (d, 1, 5-ArH), 7.55 (m, 2, 6-ArH and Ar*CH*=), 8.27 (d, 1, 2-ArH). Anal. ($C_{11}H_{12}N_2O_4$) C, H, N.

Ethyl 3-(2-Aminobenzimidazol-5-yl)acrylate (34). A suspension of **33** (10 g, 0.042 mol) in a mixture of 150 mL of 95% EtOH, 5 mL of 12 N HCl, and 50 mL of H_2O was hydrogenated at 40 psi over 10% Pd/C (3 g) until 3 equiv of H_2 was consumed. The product was in solution at the end of this time. The catalyst was removed and the filtrate made basic with NH_4OH, followed by evaporation under reduced pressure, which produced a syrupy residue. This was added to 100 mL of water, and solid CNBr (6 g) was added in 2-g portions at 30-min intervals while stirring at 25 °C. The mixture was then stirred at 25 °C for 1 h, followed by filtration. The filtrate was made basic with NH_4OH and the waxy precipitate isolated, dissolved in EtOH, and clarified. Removal of the EtOH and purification by chromatography on silica gel, eluting with EtOAc/EtOH (3:1), gave 7 g (72%) of **34**: NMR (Me_2SO-d_6) δ 1.25 (t, 3, CH_3), 4.15 (q, 2, CH_2), 6.3 (d, 1, 2′-CH), 6.87 (d, 1, 3′-CH), 7.6 (m, 3, ArH), 8.30 (br s, 3, 2-NH_2 and 1-NH). Anal. ($C_{12}H_{13}N_3O_2$) C, H, N.

3-(2-Acetamidobenzimidazol-5-yl)propionic Acid (35). A suspension of 7.5 g (0.032 mol) of **34** in 250 mL of 2 N HCL was refluxed for 2 h. Analysis by TLC indicated the complete disappearance of the starting material. The mixture was cooled in ice and the solid (6.4 g) separated. This was reduced in Me_2SO with 18 g of potassium azodicarboxylate as described for **31**.[16-18] The product was then refluxed in Ac_2O (100 mL) for 3 h and poured into 900 mL of ice water, and the precipitate was isolated. This was dissolved in 100 mL of EtOAc, washed twice with water, and dried over $MgSO_4$, followed by removal of the drying agent. Eventually crystals formed on chilling: 2.4 g (28%) of **35**; MS ($M^+ + 1$) 248; NMR (Me_2SO-d_6) δ 2.6 (s, 3, CH_3), 3.0 (t, 2, 3-CH_2), 3.2 (t, 2, 2-CH_2), 7.62 (m, 3, ArH), 8.2 (br s, 2, $(NH)_2$). Anal. ($C_{12}H_{13}N_3O_3 \cdot 1.2H_2O$) C, H, N.

5-(or 6-)Acetamido-6-(or 5-)nitro-1-indanone (38). A solution of CrO_3 (26.5 g) in a mixture of 15 mL of H_2O and 235 mL of AcOH was prepared by sonicating the suspension for 45 min. This was added dropwise to a cooled solution of **26** (22 g, 0.1 mol) in Ac_2O (2.5 L) at such a rate that the temperature remained between 15–20 °C, while stirring mechanically. After the addition was completed, the mixture was stirred at 25 °C for 4 h, poured into 10 L of water, and stirred for 1 h. The solution was then extracted with two 2-L portions of CH_2Cl_2, and the combined CH_2Cl_2 fractions were then concentrated to 500 mL, washed with two 50-mL portions of 10% NaOH followed by water, and then dried ($MgSO_4$). The solvent was removed, leaving a yellow powder, which was purified on a silica gel column, eluted with CH_2Cl_2. Unreacted starting material (2.5 g) was recovered first, followed by 12 g (50%) of **38**: mp 98–100 °C; MS (M^+) 235; NMR (Me_2SO-d_6) δ 2.30 (s, 3, CH_3), 2.77 (m, 2, 2-CH_2) 3.25 (m, 2, 3-CH_2), 8.60 (s, 1, 4-H), 8.94 (s, 1, 7-H), 10.62 (br s, 1, NH). Anal. ($C_{11}H_{10}N_2O_4 \cdot 0.2H_2O$) C, H, N.

5-Amino-6-nitro-1-indanone (39). A suspension of **38** (10 g, 0.042 mol) in 2 N HCl (200 mL) and EtOH (100 mL) was refluxed for 30 min with stirring. The reaction was cooled to 15 °C and the resultant precipitate isolated and recrystallized from dilute EtOH: 7.9 g (97.5%) of **39**; mp 248 °C; MS ($M^+ + 1$) 193; NMR (Me_2SO-d_6) δ 2.52 (m, 2, 2-CH_2), 3.00 (m, 2, 3-CH_2), 7.00 (s, 1, 4-H), 7.93 (s, 2, NH_2), 8.20 (s, 1, 7-H). Anal. ($C_9H_8N_2O_3$) C, H, N.

***N*-[1,5,6,7-Tetrahydro-7-oxoindeno[5,6-*d*]imidazol-2-yl]acetamide (36).** A suspension of **39** (13.25 g, 0.069 mol) and 10% Pd/C (3.3 g) in 95% EtOH (100 mL) was hydrogenated at 30 psi until 3 equiv of H_2 was consumed. The catalyst was removed and NH_4OH (5 mL) added to the filtrate, which was then evaporated to dryness; a yellow solid remained. This was suspended in 150 mL of H_2O, and solid CNBr (8 g, 0.075 mol) was added. The mixture was stirred for 30 min, and a second quantity of CNBr (1 g) was then added. Stirring was continued for another 20 min, followed by filtration. The filtrate was made alkaline to pH 8 with NH_4OH, and the resultant precipitate filtered and dried. This was suspended in 200 mL of Ac_2O and stirred at 25 °C for 18 h. The insoluble material was collected, washed well with ether, and dried: 7.3 g (AcOH) of **36** was obtained; mp >300 °C; MS ($M^+ + 1$) 230; NMR (Me_2SO-d_6) δ 2.17 (s, 3, CH_3), 2.61 (t, 2, 6-CH_2), 3.12 (t, 2, 5-CH_2), 7.49 (s, 1, 4-H), 7.65 (s, 1, 8-H), 11.85 (s, 1, CONH), 12.20 (s, 1, NH). Anal. ($C_{12}H_{11}N_3O_2 \cdot 1.0AcOH$) C, H, N.

***N*-(1,5,6,7-Tetrahydro-4-nitro-5-oxoindeno[5,6-*d*]imidazol-2-yl)acetamide (40).** A solution of **36** (0.9 g, 3.1 mmol) in CF_3COOH (30 mL) was cooled to −7 °C. To this was added dropwise a mixture of 0.5 mL of 70% HNO_3 and 2 mL of Ac_2O at such a rate that the temperature did not exceed 0 °C. The mixture was then stirred for about 1 h, until the temperature rose to room temperature. The solvent was removed, and the residue was washed with water and dried: yield 0.53 g (58.9%) of **40**, a yellow powder which melted at 180 °C dec; MS (M^+) 274; NMR (Me_2SO-d_6) δ 2.07 (s, 3, CH_3), 2.70 (m, 2, 6-CH_2), 3.18 (m, 2, 7-CH_2), 7.7 (s, 1, 8-H). Anal. ($C_{12}H_{10}N_4O_4 \cdot 0.2HNO_3 \cdot 0.2H_2O$) C, H, N.

***N*-(1,5,6,7-Tetrahydro-4-nitro-7-oxoindeno[5,6-*d*]imidazol-2-yl)acetamide (41).** A solution of CrO_3 (2.6 g) in 500 mL of Ac_2O was prepared by sonicating the suspension for 1.5 h. A solution of **29** (2.61 g, 0.01 mol) in Ac_2O (500 mL) was prepared by heating the suspension to boiling and filtering. The filtrate was then cooled to 10 °C. The CrO_3 solution was then added to a cooled solution of **29** at such a rate that the temperature did not rise above 15 °C. The mixture was then stirred at room temperature for 4 h, diluted to 3 times its volume with icewater, and then cooled to 25 °C. This was followed by extraction three times with EtOAc. The combined fractions were concentrated to 150 mL and washed twice with 10% NaOH and twice with water, followed by drying over $MgSO_4$ and concentration to dryness. Analysis by TLC (silica gel, EtOAc) showed the presence of three compounds, with R_f values of 0.88, 0.43, and 0.35. The mixture was then fractionated on a silica gel column, which was eluted with EtOAc. The first compound eluted was the starting material. This was followed by 0.6 g (22%) of **40** and then 1.2 g (44%) of **41**. The latter melted at 249–250 °C: MS (M^+ + 1) 275; NMR (Me_2SO-d_6) δ 2.21 (s, 3, CH_3), 2.68 (m, 2, 6-CH_2), 3.41 (m, 2, 5-CH_2), 7.91 (s, 1, 8-H). Anal. ($C_{12}H_{10}N_4O_4$), C, H, N.

2-Amino-6,7-dihydro-8-nitroindeno[5,6-*d*]imidazol-5-(1*H*)-one Hydrochloride (42). A suspension of **41** (0.5 g, 1.8 mmol) in 2 N HCl (15 mL) was heated to boiling. The material went into solution after 20 min. After another 15 min the mixture was chilled and the precipitate was isolated and recrystallized from dilute EtOH plus a few drops of HCl: wt 460 mg (97%) of **42**; mp 250 °C dec; MS (M^+ + 1) 233; NMR (Me_2SO-d_6) δ 3.52 (m, 2, 6-CH_2), 3.76 (m, 2, 7-CH_2), 7.80 (s, 1, 4-H), 8.82 (br s, 2, NH_2). Anal. ($C_{10}H_8N_4O_3$·HCl) C, H, N, Cl.

2′-Amino-1′,5′,6′,7′-tetrahydro-8′-nitrospiro[1,3-dithiolane-2,5′-indeno[5,6-*d*]imidazole] (43). To a solution of **42** (0.4 g, 1.5 mmol) in CF_3COOH was added 6 mL of ethanedithiol. The mixture was stirred at room temperature for 2 h, and the solvents were evaporated under vacuum. The resultant syrup was added to 50 mL of EtOAc and a few drops of concentrated NH_4OH was added to adjust to pH to 8. The solution was shaken three times with 50-mL portions of water and dried over $MgSO_4$, and the solvent was removed. The yellowish solid was recrystallized from EtOAc: wt 0.35 g (73%) of **43**; mp 240 °C; MS (M^+ + 1) 309; NMR (Me_2SO-d_6) δ 2.45 (t, 2, 6′-CH_2), 2.60 (t, 2, 7′-CH_2), 3.42 (m, 4, $S(CH_2)_2S$), 6.70 (s, 2, NH_2) 7.32 (s, 1, 4′-H), 11.50 (br s, 1, NH). Anal. ($C_{12}H_{12}N_4O_2S_2$) C, H, N, S.

2′-Amino-1′,5′,6′,7′-tetrahydro-8′-nitrospiro[1,3-dioxolane-2,5′-indeno[5,6-*d*]imidazole] (44). A solution of **42** (1.0 g, 3.7 mmol) in 10 mL of ethylene glycol was prepared by heating the mixture, and 30 mL of benzene was then added. The biphasic mixture was refluxed for 20 h, with continuous removal of water, with use of a Dean Stark trap. The mixture was then poured into 10 mL of icewater and extracted three times with 50-mL portions of EtOAc. The combined extracts were washed with water and evaporated to dryness under reduced pressure. The residue was extracted with EtOAc: wt residue 1.0 g (95%) of **44**; mp 300 °C dec; MS (M^+ + 1) 277; NMR (Me_2SO-d_6) δ 2.25 (t, 2, 6-CH_2), 3.1 (t, 2, 7-CH_2), 4.05 (m, 4, $O(CH_2)_2O$), 6.47 (br s, 2, NH_2), 7.35 (s, 1, 4′-H), 11.5 (br s, 1, 1′-NH). Anal. ($C_{12}H_{12}N_4O_4$·$0.4H_2O$) C, H, N.

2′,4′-Diamino-1′,5′,6′,7′-tetrahydrospiro[1,3-dithiolane-2,7′-indeno[5,6-*d*]imidazole] (6). A solution of **43** (0.1 g, 0.3 mmol) in EtOH (20 mL) was hydrogenated over 10% Pd/C (60 mg) at 30 psi until 3 equiv of H_2 was consumed. The catalyst was removed and the filtrate evaporated to dryness. The resulting solid was recrystallized from 50% aqueous EtOH to give 45 mg (53%) of **6**: mp 210–212 °C dec; MS (FAB) (M^+ + 1) 279; NMR (Me_2SO-d_6) δ 2.58 (m, 2, 5′, and 6′-CH_2), 3.40 (m, 4, $S(CH_2)_2S$), 4.50 (br s, 2, NH_2), 7.9 (s, 2, NH_2), 8.60 (s, 1, 8′-H), 11.30 (br s, 1, NH). Anal. ($C_{12}H_{14}N_4S_2$·0.2 H_2O) C, H, N, S.

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Synthesis and Anti-HIV Activity of 2-, 3-, and 4-Substituted Analogues of 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT)

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Several analogues of a new lead for anti-HIV-1 agents, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT), in which the C-2, N-3, or C-4 position was modified were synthesized. These involve 2-thiothymine (11), 2-thiouracil (12), 4-thiothymine (17), 4-thiouracil (18), 5-methylcytosine (27), and cytosine (28) derivatives. Preparation of N-3-substituted derivatives (29 and 30) of HEPT was also carried out. Among these analogues, compound 11 exhibited excellent activity against HIV-1 HTLV-III_B strain with an EC_{50} value of 0.98 μM, which is 7-fold more potent than that of HEPT. Removal of the 5-methyl group in compound 11 results in total loss of activity. Other compounds did not show any anti-HIV-1 activity. The 4-thio derivatives 17 and 18 were found to be rather cytotoxic. When compound 11 was evaluated for its inhibitory effects on another HIV-1 strain, HTLV-III_{RF}, and two HIV-2 strains, LAV-2_{ROD} and LAV-2_{EHO}, it proved equally inhibitory to HTLV-III_{RF}, whereas both HIV-2 strains were insensitive to the compound.

In the search for more selective and effective agents against human immunodeficiency virus (HIV),[1,2] which is the causative agent of the acquired immunodeficiency syndrome (AIDS), a large number of nucleoside analogues have been synthesized and investigated for their antiviral activities.[3,4] Among these, 3′-azido-3′-deoxythymidine[5] (AZT) has already been approved for use for patients with AIDS. 2′,3′-Dideoxyinosine (DDI), which is less toxic than

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