2,4-Diamino-5-benzylpyrimidines as Antibacterial Agents. 14. 2,3-Dihydro-1-(2,4-diamino-5-pyrimidyl)-1*H*-indenes as Conformationally Restricted Analogues of Trimethoprim[1]

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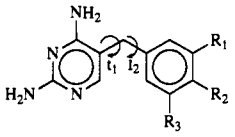
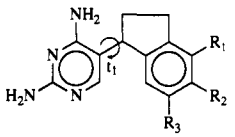
A conformationally restricted analogue of trimethoprim (**1a**) has been prepared by connecting the ortho position of the benzene ring to the methylene linkage with two methylene groups, thus forming a dihydroindene derivative (**2b**). The chemistry involved the condensation of barbituric acid with an indanone derivative, followed by a three-step conversion to a 2,4-diaminopyrimidine. The *S* isomer of **2b** was found to have a minimum-energy conformation very similar to that of **1a** when bound to *Escherichia coli* dihydrofolate reductase, in contrast to that of **1a** in vertebrate DHFR. Theoretically such a derivative might have increased specificity and activity against the bacterial enzyme. Molecular modeling experiments suggested that the actual decreased activity was due to crowding in the enzyme, caused by the extra atoms needed to restrict the conformation.

The importance of trimethoprim (TMP, **1a**)[2] as a species-selective antibacterial agent[3,4] coupled with knowledge of the three-dimensional structure of its target enzyme, dihydrofolate reductase (DHFR, EC 1.5.1.3),[5-9] has presented an unusual opportunity for increasing our basic knowledge about protein–ligand interactions through the design of new inhibitors. This paper describes the synthesis of a conformationally restricted trimethoprim analogue, its biological activity, and modeling of its interactions with *E. coli* DHFR.



1a (R_1 - R_3 = OCH_3) = TMP
1b R_1, R_2 = OCH_3, R_3 = H



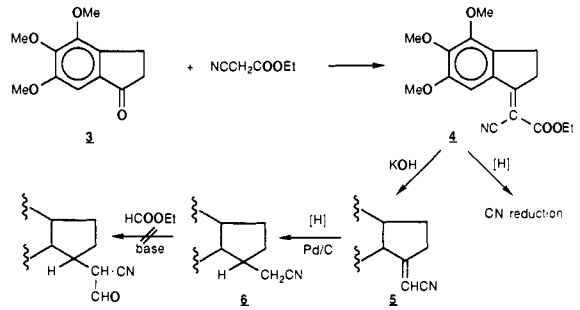
2a R_1-R_3 = OCH_3
2b R_2,R_3 = OCH_3, R_1 = H

This research was initiated before the three-dimensional structure of DHFR was known and was based on knowledge of the X-ray structure of **1a** hydrobromide and acetate salts, published in 1969,[10] as well as that of the free base, which was elucidated shortly thereafter.[11] In the case of the hydrobromide salt the two rings were found to be very nearly at right angles to each other (t_1 and $t_2 \cong$ 155° and 90°, respectively), as illustrated in Figure 1a. The acetate and free base, however, were found to have quite a different conformation in the crystal state, more like that of an open book, with t_1 and t_2 for the free base equal to −89° and 153°, respectively, as shown in Figure 1b.[11] We considered that the former structure might be more likely in combination with *E. coli* DHFR, on the basis of analogue studies. 6-Alkyl derivatives of **1a**, for example, are increasingly less inhibitory to this enzyme as the substituent bulk increases, and steric hindrance would prevent such a conformation.[12]

We reasoned that if we could hold the two rings in the Figure 1a conformation by forming an additional ring, it might not only provide evidence for the shape of **1a** as it interacted with the bacterial enzyme but also the derivative might bind to the enzyme with a lower K_i value than its parent if the conformation were correct, since conformational energy would not be expended in the binding process. Many conformationally restricted analogues of biologically active compounds have been designed on this premise.

Elucidation of the three-dimensional structure of *E. coli* DHFR complexed with **1a** showed that our initial postulate

Scheme I



was essentially correct, in that the two torsional angles for the ligand were found to be 177° and 76°, respectively, upon refinement.[7,9] What was considerably more intriguing, however, was the X-ray crystallography of **1a** with chicken liver DHFR in ternary complex with the coenzyme NADPH.[9] Here the two torsional angles were reported to be −85° and 102°, respectively,[9] which resembled those of the free base of trimethoprim.[11] This created the challenge

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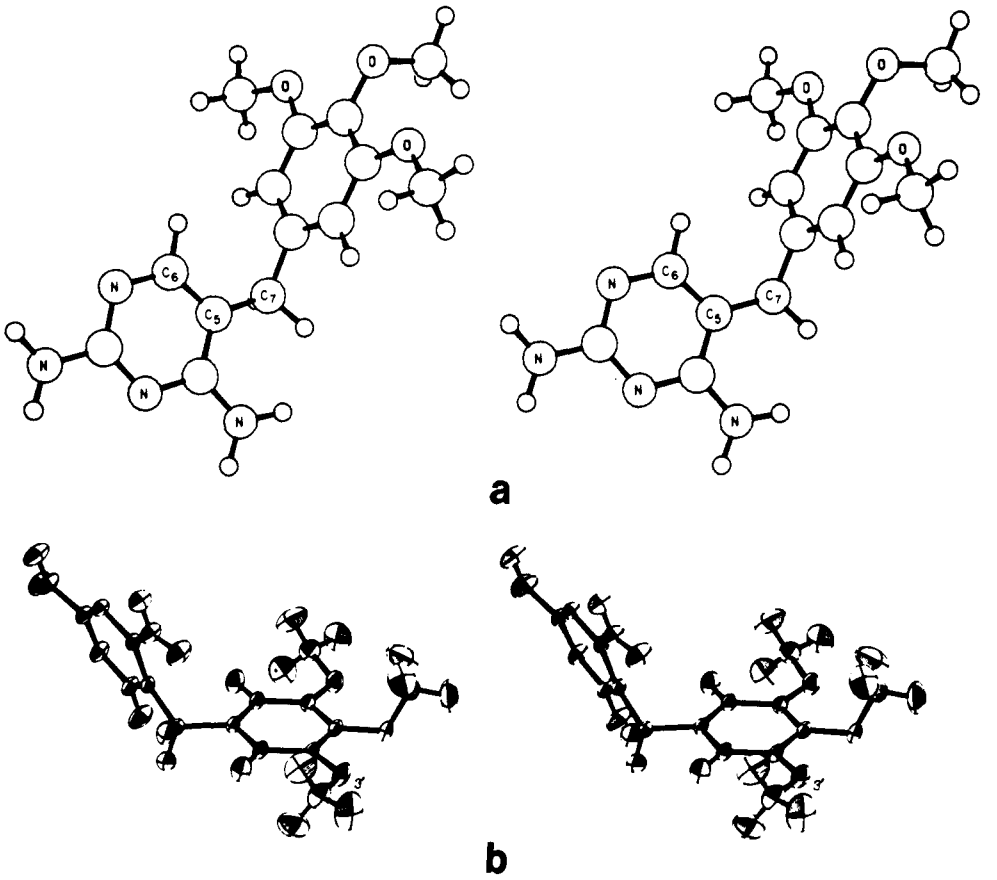


Figure 1. X-ray conformation of trimethoprim (**1a**) shown in stereo views: a, as the hydrobromide salt (see ref 10) (reproduced with permission from Roth, B.; Aig, E.; Lane, K.; Rauckman, B. S. *J. Med. Chem.* **1980**, *23*, 535; copyright 1980 American Chemical Society); b, as the free base (reproduced with permission from Koetzle, T. F.; Williams, G. T. B. *J. Am. Chem. Soc.* **1976**, *98*, 2074; copyright 1976 American Chemical Society).

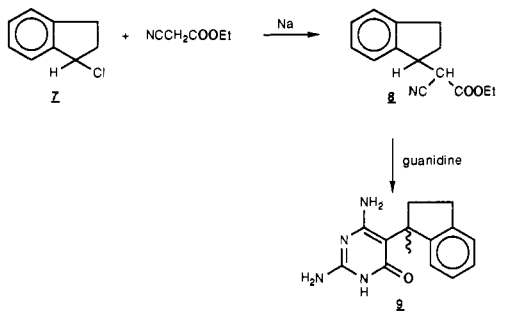
of designing semirigid analogues of **1a** which could not assume the latter conformation. A substantial increase in specificity for the bacterial enzyme might result if one could mimic the one conformation and prevent the other. However, such an experiment might not be definitive if the extra atoms required for conformational restriction were to interfere with the receptor in some way.

We chose structure **2** as an initial target for this type of study, with the intent to resolve the product into its optically active forms if it should prove sufficiently interesting.

Chemistry

Development of a viable route to compounds of type **2** proved to be fraught with problems. The first route to **2a** tried (Scheme I) involved the reaction of 4,5,6-trimethoxyindanone (**3**) with cyanoacetic ester to produce **4**.[13] Various methods were tried for the reduction of the double bond of **4**, including catalytic hydrogenation (Pd/C) at various temperatures, use of Raney nickel, sodium cyanoborohydride, and vitride. In all cases either no reaction occurred, or mixtures of products were obtained, all of which showed reduction of the nitrile prior to the double bond. Compound **4** was then treated with alkali to remove the carbethoxy group, yielding **5**, followed by catalytic reduction to **6**, identified by NMR and IR spectroscopy. Formylation attempts were unsuccessful, possibly due to the low reactivity of **6**. A related approach using malonic ester might have been successful, but in a model reaction it led to the loss of a carbethoxy group, so alternative methods were investigated.

Scheme II



1-Chloro-2,3-dihydro-1*H*-indene (**7**)[14] was treated with ethyl sodium cyanoacetate (Scheme II) to produce **8** as a mixture of isomers; upon treatment with guanidine the pyrimidine **9** was obtained. Since this model reaction was successful, attempts were then made to prepare the 5,6-dimethoxy analogue of **7**. The intermediate 5,6-dimethoxyindene produced a dimer, rather than a chloroindan upon addition of HCl, however. 1-(Tosyloxy)- and 1-(mesyloxy)-5,6-dimethoxy-2,3-dihydro-1*H*-indene were then treated with ethyl sodium cyanoacetate, but elimination rather than displacement occurred.

A successful route to the (dimethoxyindanyl)pyrimidine **2b** involved the use of 5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one (**10**) and barbituric acid (**11**) as starting ma-

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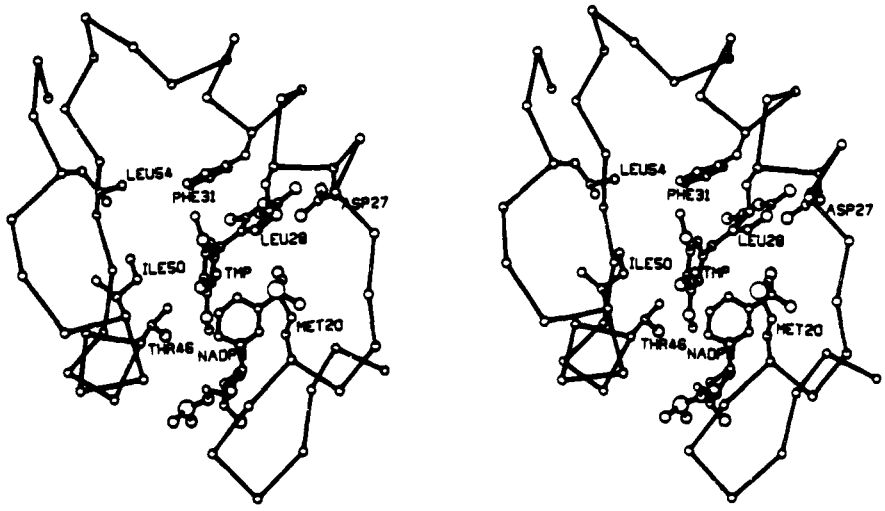
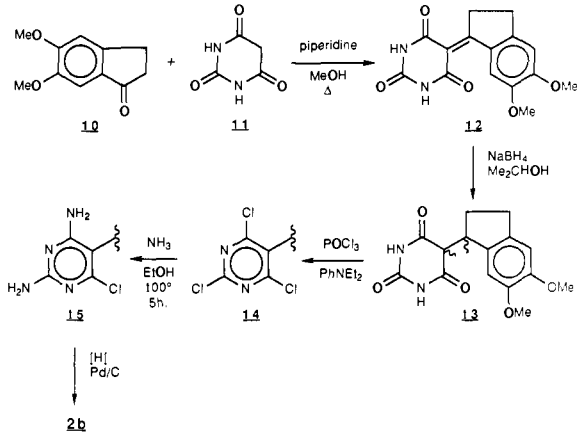


Figure 2. Stereo view of **1a** in ternary complex with *E. coli* DHFR and NADPH. The active site of the enzyme is shown with the α-carbons as small circles and the connections as double lines. **1a** and the coenzyme (shown in part) are drawn with solid lines. Carbon atoms are small circles; nitrogen and oxygen atoms are larger circles; the sulfur of Met-20 is a very large circle. Coordinates were kindly provided by Dr. John Champness (ref 17).

Scheme III



terials, as shown in Scheme III. The condensation product **12** was successfully reduced with sodium borohydride[15] to give **13**, which upon treatment with $POCl_3$ produced trichloropyrimidine **14**. Treatment with ammonia in ethanol at 100 °C yielded 2,4-diamino-6-chloropyrimidine **15**, which was successfully reduced to the target compound **2b**. This was considered a satisfactory test compound for answering our conformational query, to be compared with **1b** and **1a**. Attempts to make a trimethoxy analogue (where R_1–R_3 = OCH_3) were abandoned when it was realized that R_1 would necessarily lie out-of-plane, unlike the corresponding function in **1a**, and that such a shape was probably deleterious to binding.[16]

Biological Data, Molecular Mechanics Calculations, and Discussion

Dihydrofolate reductase inhibitory activities of **2b** compared with those of **1a** and **1b** are shown in Table I. Compound **2b** is more than 1 order of magnitude less active than **1b** and more than 100 times less active than **1a** against *E. coli* DHFR; it is about 20-fold less active against *Neisseria gonorrhoeae* DHFR, about 6 times less active than **1b** against the vertebrate enzyme from rat liver, and slightly less active than **1a** on this enzyme.

Table I. Inhibitory Activities of **1a**, **1b**, and **2b** against Dihydrofolate Reductases[a]

	$I_{50} \times 10^8$ M vs DHFR		
no.	*E. coli*[b]	rat liver[b]	*N. gonorrhoeae*[c]
2b	140	41 000	970
1a	0.5–0.7[d]	26 000–37 000[d]	45
1b	5–10[d]	7 000	53

[a] Although we have not determined whether **2b** is a competitive inhibitor, Lineweaver–Burk plots showed that **1a** and the corresponding unsubstituted benzylpyrimidine both bound competitively with dihydrofolate (ref 22). Furthermore, crystallography of more than 40 2,4-diaminopyrimidines with DHFR has shown that they all bind similarly at the active site of the enzyme (refs 4–9, 20, 21, and unpublished results from the Wellcome Research Laboratories, Beckenham, U.K.). Therefore we assume that **2b** is a competitive inhibitor. [b] These assays were performed as described by Kuyper, L. F.; Roth, B.; Baccanari, D. P.; Ferone, R.; Beddell, C. R.; Champness, J. N.; Stammers, D. K.; Dann, J. G.; Norrington, F. E.; Baker, D. J.; Goodford, P. J. *J. Med. Chem.* **1985**, *28*, 303. [c] The assay method is described by Roth, B.; Baccanari, D. P.; Sigel, C. W.; Hubbell, J. P.; Eaddy, J.; Kao, J. C.; Grace, M. E.; Rauckman, B. S. *J. Med. Chem.* **1988**, *31*, 122. [d] Range of values in many repeat assays over a period of time; the accuracy of this assay has been found to be approximately ±10%.

The conformation of **1a** in ternary complex with *E. coli* DHFR and NADPH is now known[17] and has been found to be very similar to that in binary complex; t_1 and t_2 are approximately 192° and 56°, respectively, as determined from a 3-Å map, yet to be refined. Figure 2 shows a stereo view of this ligand in the active site of the enzyme. Upon forming the ternary complex the "teen" loop moved up about 3 Å to provide a close fit of the ligand in the cleft without materially altering the inhibitor conformation.

If we can assume that the minimum-energy conformation of one of the optical isomers of **2** is similar to that of **1a**, then the poor inhibitory activity of **2b** relative to that of **1** suggests interference with the enzyme. Using MM2 in Macromodel,[18] a conformational search of *S* and *R* optical isomers of **2a** was performed by rotating dihedral angle t_1 from 0° to 360° in increments of ca. 5° to 10°. The

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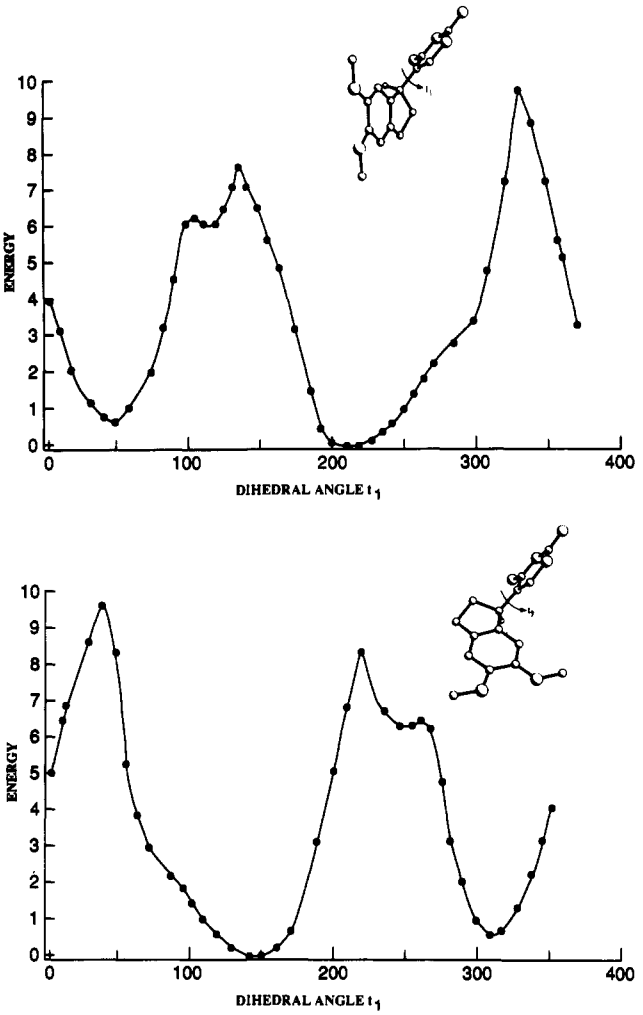

Figure 3. Energy profiles calculated for **2b**, as a function of the dihedral angle, t_1: a, *S*-**2b**; b, *R*-**2b**.

resultant energy profiles are shown in Figure 3, parts a and b, respectively. The *S* isomer, which had been predicted to bind in a fashion similar to that of TMP in its ternary complex with *E. coli* DHFR–NADPH, was found to have a minimum-energy conformation at t_1 = 210°, which is close to the observed t_1 of TMP found in the X-ray ternary complex in *E. coli* DHFR–NADPH (see Figure 2). This low-energy conformation was assumed to be the preferred conformation of *S*-**2b** in *E. coli* DHFR–NADPH. We then replaced TMP by this conformer in the *E. coli* DHFR–NADPH–TMP structure, using coordinates provided by Champness et al.[17] The stereo view of the resultant complex is shown in Figure 4. Inspection of this hypothetical complex showed that crowding occurred between protein and ligand in the conformation shown. The methylene groups of the five-membered ring of *S*-**2a** are ca. 2 Å from parts of the nicotinamide moiety of NADPH.[19] Thus the extra atoms in this semirigid analogue of **1b** may interfere with binding.

A conformational search about dihedral angle t_1 of *R*-**2b** indicated that minimum-energy conformers occur at about 150° and 305°, far from the optimum for **1a**, as might be expected (see Figure 3b). A stereo view of the conformer with t_1 equal to 150° in *E. coli* DHFR–NADPH is shown in Figure 5. This conformation is very different from that of TMP, and crowding is observed to occur between the ligand and the nicotinamide ring as well as Met-20,[19] with some atoms being only about 2 Å apart.

The conformer of TMP in chicken liver DHFR–NADPH corresponds to a high-energy conformer for both the *S* and *R* isomers of **2b**. This conformer of **2b** is therefore probably not the preferred one for interaction with vertebrate DHFR. The low inhibitory activity of **2b** toward rat liver DHFR attests to this. Although the sequence and three-dimensional structure of rat DHFR has not been determined, mouse L1210 lymphoma DHFR and human DHFR structures have been solved recently and have been found to be closely analogous to that found for chicken DHFR.[20,21] Matthews has modeled **1a** in chicken DHFR using the *E. coli* conformation. Although no interference with the enzyme occurred, the different cleft shape suggested poorer van der Waals contacts with the enzyme.[9]

The interactions of the methoxy groups of **1a** with *E. coli* DHFR represent an important part of the binding energy. The methyl moieties all lie in positions for favorable van der Waals interaction with hydrophobic residues in the *E. coli* protein. Each methoxy group adds about 1.4 kcal/mol to the binding energy.[22] The oxygen atoms lie largely in solvent, where they do not disturb the hydrophobic binding, in contrast to their positions in chicken liver DHFR. There one of the methoxy groups is completely buried and the other two oxygens are largely so; furthermore, the methyl functions are in part exposed to solvent. The net result is that the methoxy groups contribute little or nothing to binding in this protein.[9,17,22,23] We do not know the orientation of the methoxy groups of **2b** in the protein. If van der Waals interactions are not optimum, because of interference by the extra ring, this could be a factor in lowering the inhibitory activity. However, the overall effect may be attributed to steric hindrance caused by the additional methylene groups. This could result in higher energy conformations quite different from that of TMP or in adjustments of the enzyme conformation. In our modeling studies, we utilized a static enzyme, realizing of course that side chains can move. However, the interference we observed was in the heart of the active site, where the nicotinamide of the cofactor is quite securely anchored to the protein and where Met-20 and Phe-31 form important hydrophobic contributions to the active site. Any adjustments of these residues would be expected to cost energy.

Experimental Section

Melting points were determined with a Thomas-Hoover or a Mel-Temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Varian FT80A or Varian XL200 spectrometers with tetramethylsilane as the internal standard. Chemical ionization (CI) mass spectra were obtained with Finnigan MAT TSQ and electron impact (EI) with Varian MAT CH5-DF mass spectrometers. Elemental analyses were carried out by Atlantic Microlabs, Inc., Atlanta, GA. These are accurate to within 0.4% of the calculated values.

Ethyl 2-Cyano-2-(4,5,6-trimethoxy-2,3-dihydro-1*H*-inden-1-ylidene)acetate (4). A mixture of 5.15 g (0.023 mol) of 4,5,6-trimethoxy-2,3-dihydro-1*H*-inden-1-one (**3**),[13] 5.64 g (0.054 mol) of ethyl cyanoacetate, 26 mL of benzene, 2.6 g of glacial HOAc, and 2.06 g of NH_4OAc was heated to reflux for 16 h in

(19) For ideal atomic radii, see: (a) Allinger, N. L.; Szkrybalo, W. *J. Org. Chem.* **1962**, *27*, 4601. (b) Hill, T. L. *J. Chem. Phys.* **1948**, *16*, 399.

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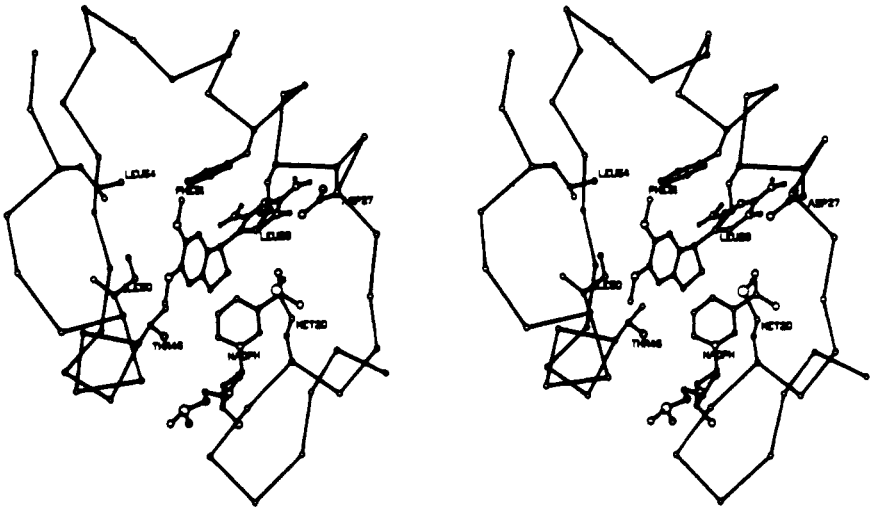

Figure 4. Stereo view of a model of *S*-**2b** in ternary complex with *E. coli* DHFR and NADPH, with the pyrimidine ring placed as for **1a** (see Figure 2) and with the torsional angle t_1 = 210°. The conformations of the methoxy groups are arbitrary.

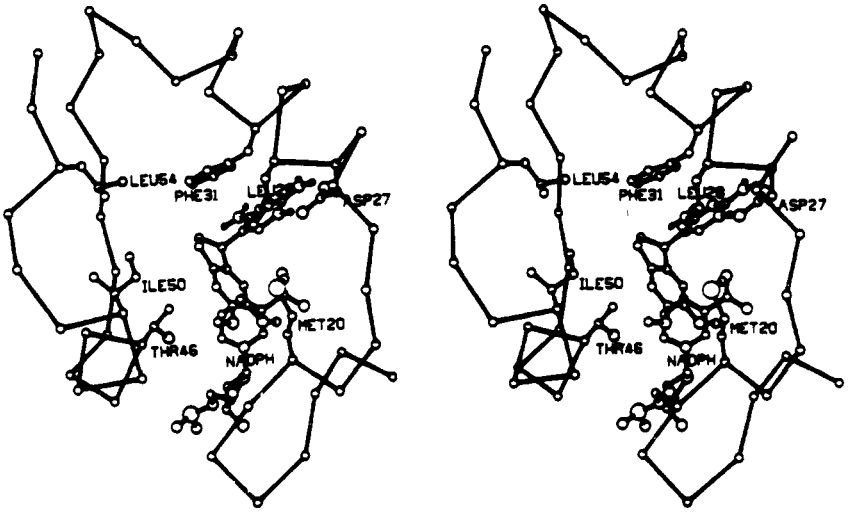

Figure 5. Stereo view of model *R*-**2b** in *E. coli* DHFR, as described for *S*-**2b**; t_1 = 150°.

an apparatus with a Dean–Stark trap. After cooling, the solution was extracted with water, and the benzene layer dried with $MgSO_4$. The solvent was removed under vacuum, and the residual yellow solid recrystallized from absolute EtOH: yield, 5.48 g (75%); mp 112–113 °C. Anal. ($C_{17}H_{19}NO_5$) C, H, N.

Ethyl 2-Cyano-2-(2,3-dihydro-1*H*-inden-1-yl)acetate (8). The sodium salt of ethyl cyanoacetate was prepared from 0.18 g-atom (7.70 mmol) of sodium in 10 mL of dry benzene plus an equivalent of ethyl cyanoacetate (0.87 g). 1-Chloro-2,3-dihydro-1*H*-indene (7,[14] 1.18 g, 7.70 mmol) was added dropwise over 0.5 h, followed by refluxing for 48 h. The mixture was then diluted with water and extracted with CH_2Cl_2. After drying ($MgSO_4$) and solvent removal, the crude product was purified by flash column chromatography on silica gel with CH_2Cl_2 as the eluent. This yielded 0.6 g (34%) of **8** as an oil: NMR (Me_2SO-d_6) δ 1.25 (2 t, 3 H, CH_2CH_3), 1.50–2.50 (m, 2 H, indanyl 2-H), 2.75–3.15 (m, 2 H, indanyl 3-H), 3.65–4.05 (m, 1 H, indanyl 1-H), 4.30 (2 sets of poorly resolved q, 2 H, CH_2CH_3), 4.90* and 4.61† (d, 1 H, J = 4.5 Hz, $NCCHCO_2Et$), 7.0–7.5 (m, 4 H, aromatic) (*major isomer, †minor isomer); MS (EI) m/e 229 (M^+, 9), 117 (indanyl$^+$, 100). Anal. ($C_{14}H_{15}NO_2$) C, H, N.

2,6-Diamino-5-(2,3-dihydro-1*H*-inden-1-yl)-4(3*H*)-pyrimidinone (9). To 0.25 g (2.6 mmol) of guanidine hydrochloride in 20 mL of methoxyethanol was added 0.15 g (2.90 mmol) of NaOMe. This was stirred and filtered into a round-bottomed flask. A 0.2-g portion (0.87 mmol) of **8** was added and the reaction mixture was refluxed overnight. The solvent was then removed in vacuo, and 1 N HCl added until neutral. This was partitioned with EtOAc. The EtOAc layer was separated and dried over $MgSO_4$. After solvent removal, the crude product was purified by flash chromatography on silica gel with 20% MeOH in EtOAc as the eluent; 0.018 g (0.07 mmol, 9%) of **9** was obtained as an off-white solid: mp >200 °C dec; NMR (Me_2SO-d_6) δ 1.85–2.40 (m, 2 H, indanyl 2-H), 2.60–3.00 (m, 2 H, indanyl 3-H), 4.37 (t, 1 H, indanyl 1-H, J = 8 Hz), 5.10 (br s, 2 H, NH_2), 5.97 (br s, 2 H, NH_2), 6.70–7.30 (m, 4 H, aromatic), 9.85 (br s, 1 H, HNCO); MS (EI) m/e 242 (M^+, 100). Anal. ($C_{13}H_{14}N_4O \cdot 1.5H_2O$) C, H, N.

5-(5,6-Dimethoxy-2,3-dihydro-1*H*-inden-1-ylidene)-barbituric Acid (12). In a 1-L round-bottomed flask was refluxed a mixture of 10.00 g (52.0 mmol) of **10** and 6.66 g (52.0 mmol) of **11** in 500 mL of MeOH, using 0.4 mL of piperidine as the catalyst. The reflux was continued for 72 h with addition of 0.4 mL of piperidine every 24 h. Then 150 mL of MeOH was distilled off. The yellow precipitate was collected by filtration, yielding 5.35 g (34%) of **12** as a yellow solid: mp 275–280 °C dec; NMR (Me_2SO-d_6) δ 2.85–3.15 (m, 2 H, indanyl 2-H), 3.40–3.60 (m, 2 H, indanyl 3-H, partially obscured by the H_2O peak), 3.75 (s, 3 H, OMe), 3.90 (s, 3 H, OMe), 7.12 (s, 1 H, aromatic H), 8.42 (s, 1 H, aromatic H), 10.92 (br s, 2 H, HNCO), MS (CI) m/e 303 (M^+ + 1, 100). Anal. ($C_{15}H_{14}N_2O_5$) C, H, N.

5-(5,6-Dimethoxy-2,3-dihydro-1*H*-inden-1-yl)barbituric Acid (13). To a water-cooled suspension of 5.00 g (17 mmol) of **12** stirred in 150 mL of *i*-PrOH was added portionwise 3.13 g (83 mmol) of $NaBH_4$. The mixture was stirred for 18 h and then chilled. Excess $NaBH_4$ was destroyed by the gradual addition of 1 N HCl. The precipitate was collected by filtration and then washed repeatedly with water. After drying, 4.90 g (95%) of **13** was obtained as an off-white solid: mp 241–243 °C; NMR (Me_2SO-d_6) δ 2.00–2.35 (m, 2 H, indanyl 2-H), 2.50–2.80 (m, 2 H,

indanyl 3-H), 3.60–3.71 (m, 1 H, indanyl 1-H, partially obscured by the 2 OMe peaks), 3.70–3.83 (m, 1 H, barbiturate methine H), 3.65 (s, 3 H, OMe), 3.69 (s, 3 H, OMe), 6.66 (s, 1 H, aromatic H), 6.76 (s, 1 H, aromatic H), 11.02 (br s, 1 H, NHCO), 11.30 (br s, 2 H, NHCO); MS (CI) *m/e* 177 (100). Anal. ($C_{15}H_{16}N_2O_5$) C, H, N.

2,4,6-Trichloro-5-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)pyrimidine (14). A 4.50-g portion (14.8 mmol) of **13** was dissolved in 40 mL of *N,N*-diethylaniline and heated to 120 °C. To this was added dropwise 20 mL of $POCl_3$. The resultant mixture was stirred at 120 °C for 15 h. This was then mixed with ice water and neutralized with cold, concentrated NH_4OH, followed by extraction with EtOAc. After drying ($MgSO_4$) and solvent removal, the crude product was purified by flash chromatography on silica gel with 20% EtOAc in hexane as the eluent. A 2.5-g yield (47%) of **14** was obtained as white crystals: mp 125–127 °C; NMR (Me_2SO-d_6) δ 2.10–2.30 (m, 1 H, indanyl 2-H), 2.40–2.60 (m, 1 H, indanyl 2-H, partially obscured in the Me_2SO peak), 2.90–3.10 (m, 2 H, indanyl 3-H), 3.60 (s, 3 H, OMe), 3.73 (s, 3 H, OMe), 5.00 (t, 1 H, indanyl 1-H), 6.69 (s, 1 H, aromatic H), 6.88 (s, 1 H, aromatic H); MS (CI) *m/e* 359 (M^+ + 1, 100), 362 (M^+ + 2, 99), 363 (M^+ + 3, 33). Anal. ($C_{15}H_{13}Cl_3N_2O_2$) C, H, N, Cl.

6-Chloro-2,4-diamino-5-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)pyrimidine (15). A 1.00-g portion (2.78 mmol) of **15** was dissolved in 15 mL of absolute EtOH in a glass-lined bomb. This was chilled in ice/water bath. Ammonia gas was then introduced into the ethanolic solution until saturation, and the resultant mixture was heated in the oven for 5 h at 100 °C. The solvent was removed, and the crude product was purified by flash column chromatography on silica gel with 10% MeOH in CH_2Cl_2 as the eluent, yielding 0.28 g (31%) of **15**; MS (CI) *m/e* 321 (M^+ + 1, 100), 323 (M^+ + 3, 33). This was used directly in the next reaction without analysis.

2,4-Diamino-5-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)pyrimidine (2b). A 0.2-g portion (0.62 mmol) of **15** was dissolved in a mixture of 60 mL of absolute EtOH and 5 mL of DMF in a Parr shaker. 5% Pd/C (0.05 g) was added, and H_2 was introduced. The substance was then dehalogenated overnight. The catalyst was removed and the crude product purified by flash column chromatography on silica gel with 10% MeOH in CH_2Cl_2 as the eluent. A 0.06 g (34%) yield of **2b** was obtained as a white solid: mp 206–208 °C; NMR (Me_2SO-d_6) δ 1.60–1.90 (m, 1 H, indanyl 2-H), 2.30–2.50 (m, 1 H, indanyl 2-H), 2.60–2.95 (m, 2 H, indanyl 3-H), 3.65 (s, 3 H, OMe), 3.73 (s, 3 H, OMe), 4.15 (t, 1 H, indanyl 1-H), 5.68 (br s, 2 H, NH_2), 6.10 (br s, 2 H, NH_2), 6.59 (s, 1 H, aromatic H), 6.88 (s, 1 H, aromatic H), 7.10 (s, 1 H, pyrimidyl 6-H); MS (CI) *m/e* 287 (M^+ + 1, 100). Anal. ($C_{15}H_{18}N_4O_2 \cdot 0.3H_2O$) C, H, N.

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Novel Pyrrolo[2,3-*d*]pyrimidine Antifolates: Synthesis and Antitumor Activities

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New antifolates, characterized by a 6–5 fused ring system, a pyrrolo[2,3-*d*]pyrimidine ring, and a trimethylene bridge at position 5 (**12a,b** and **13a,b**) were designed and efficiently synthesized. The synthetic method included (1) construction of the key intermediary acyclic skeleton, 5-[4-(*tert*-butoxycarbonyl)phenyl]-2-(dicyanomethyl)pentanoates (**6a,b**), (2) cyclization with guanidine, followed by reduction to the pyrrolo[2,3-*d*]pyrimidine derivatives (**8a,b** and **9a,b**), and (3) subsequent glutamate coupling and saponification. These antifolates were more growth-inhibitory by about 1 order of magnitude than methotrexate (MTX) against KB human epidermoid carcinoma cells and A549 human nonsmall cell lung carcinoma cells in in vitro culture. Growth inhibitory IC_{50} values for *N*-[4-[3-(2,4-diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamic acid (**12a**) against KB and A549 were 0.27 and 4.5 ng/mL, while those for MTX were 5.0 and 35 ng/mL, respectively. Other members of this class of antifolates, **12b** and **13a,b**, showed good activities nearly equal to that of **12a**.

Methotrexate (MTX) has been an important drug in cancer chemotherapy, mainly in the treatment of acute lymphocytic leukemia, since its debut in 1953. However, it has limitations in clinical use because of toxicity to patients and lack of efficacy against most human solid tumors.[1] Although many analogues of MTX have been synthesized and tested,[2] none with better therapeutic properties has found its way into clinical practice.

With the specific aim of synthesizing a new anticancer agent with an improved therapeutic index, we concentrated our research on the design and synthesis of new antifolates having new structural features in the heteroaromatic ring and in the bridge region linking the ring to the benzoyl moiety. Because structurally close antifolates inhibit various folate cofactor-requiring enzymes,[3] an antifolate

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