monitored continuously by a Berthold LB506C radiochemical detector fitted with a 1-mL flow cell. Hydroxylase activity was measured as the production of 17α-hydroxyprogesterone, androstenedione, and testosterone while lyase activity was measured as the production of androstenedione and testosterone alone.

All the compounds were tested at least four concentrations and the enzyme activities measured were fitted by linear regression to the Dixon equation.[22] The results are given as IC_{50} (the concentration of the compound required to inhibit the reaction by 50%) with 95% confidence limits.

The IC_{50} for **4** was estimated at several concentrations of progesterone in order to ascertain the nature of the enzyme inhibition.[22]

Comparative Hydrolysis Rates of 4, 25, and 26 by Hog Liver Esterase. Samples of hog liver esterase (2 μg, Boehringer) were preincubated for 2 min in 10 mM sodium borate buffer, pH 8.0 (0.9 mL) at 30 °C. Reactions were started in parallel by the addition of solutions of the esters (1 μmol) in the above buffer (0.1 mL). Aliquots (0.1 mL) were taken at intervals of 0, 5, 10, and 15 min and added to ice-cold CH_3CN (0.1 mL). Samples (0.05 mL) were analyzed by HPLC by using a 15-cm Apex octadecyl (5-μm) stationary phase and 10 mM sodium phosphate buffer (pH 6.8)/CH_3CN 1:1 as mobile phase at a flow rate of 1.5 mL min^{-1}. The pyridylacetic acids formed were detected by their UV absorption at 254 and 229 nm. No hydrolysis took place when the esters were incubated in the absence of enzyme. In the 15-min period, the ester **4** was hydrolyzed to an extent of 12% and graphs of amounts of the 4-pyridylacetic acid formed against time were essentially linear. Rates of hydrolysis of the esters expressed in nmol/min ± SEM (determined from regression analysis) were for **4**, 7.3 ± 0.2; **25**, 8.2 ± 1.0; **26**, 1.3 ± 0.3.

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Inhibitors of the P450 Enzymes Aromatase and Lyase. Crystallographic and Molecular Modeling Studies Suggest Structural Features of Pyridylacetic Acid Derivatives Responsible for Differences in Enzyme Inhibitory Activity

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Derivatives of 4-pyridylacetic acid are known to be inhibitors of the cytochrome P450 enzymes aromatase and lyase (17α-hydroxylase/C_{17-20}lyase), and are therefore of interest in the treatment of hormone dependent breast and prostate cancers. We report the determination of the crystal structure of one such derivative, the 4-*tert*-butyl cyclohexyl ester, and molecular modeling studies on two related inhibitors, the cyclohexyl ester and its α-methyl derivative. These latter two compounds show a marked difference in their relative activities against aromatase and lyase. Two models are proposed for the interaction of these molecules with the target enzymes on the basis of their ability to adopt conformations that partially mimic steroid substrates. From these models an explanation can be advanced for the fact that, compared with the unmethylated analogue, the (racemic) α-methylated compound is seven times poorer as an inhibitor of aromatase but seven times better as an inhibitor of lyase. The model proposed for binding to aromatase places the α-carbon of the ester group in the position occupied by C(2) of steroid substrates. In contrast, that proposed for binding to lyase places this atom in the position occupied by C(17) of steroid substrates. The introduction of steric bulk at C(2) is known to be unfavorable for aromatase inhibition, while its introduction at C(17) may lead to a better mimicry of the steroid D-ring and so improve lyase inhibition.

Introduction

A proportion of breast tumors require the presence of estradiol for their growth. The reduction of estrogen levels therefore represents a desirable goal in the treatment of such cancers. One approach to this has been the use of inhibitors of the enzyme aromatase, which is responsible for the last step in the biosynthesis of the estrogens from the androgens.[1,2] Aromatase is a cytochrome P-450 enzyme, as are several other of the steroid-processing enzymes; one of the major problems that has been encountered in the design and use of aromatase inhibitors has been a lack of selectivity in their inhibitory action. For instance the aromatase inhibitor aminoglutethimide also inhibits the P-450 enzyme desmolase (cholesterol side chain cleavage enzyme); this enzyme is necessary for the synthesis of all steroid hormones and therefore corticosteroid replacement therapy must accompany its use.[3] However, inhibitors of other P-450 enzymes, if selective, are also of interest. For example inhibitors of the enzyme 17α-hydroxylase/C_{17-20}lyase, which is involved in the biosynthesis of the androgens from pregnanes, may be of use in the treatment of androgen-dependent prostate cancer.[1,4]

In the absence of an X-ray crystal structure for aromatase, studies of the natural substrates of the enzyme and of its steroidal and non-steroidal inhibitors have lead to the development of a model for the active site.[5]

Two features of this model, those utilized by the natural substrates, are a hydrophobic region that interacts with the steroid skeleton and a hydrogen-bond donor that interacts with an acceptor grouping in the C(3) position of the steroid. Two further features of the model are the heme and an additional hydrophobic pocket extending from the region occupied by C(4) of steroid substrates.

There is no direct interaction between the heme and natural substrates, as this site is required to bind the di-

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oxygen for the redox reaction. This does not appear to be the case for certain inhibitors bearing basic substituents where a binding interaction between the substituent and the heme iron has been inferred from the observation of "Type II" difference binding spectra, characterized by a peak at about 425 nm and a trough at about 390 nm. In contrast, inhibitors or substrates unable to provide a strong sixth ligand to the iron give rise to "Type I" difference binding spectra with a peak at about 390 nm and a trough near 420 nm.[6] On the basis of the sites of oxidation, modeling studies, and the inhibitory activity of C(19) substituted steroids, the heme is believe to occupy a position in the region of the C(1), C(2), and C(19) positions.[7,8] The existence of the additional hydrophobic pocket has been inferred from modeling studies on the inhibitory activity of steroids bearing substituents in the 4-position[9] where considerable bulk (e.g. phenyl) appears to be tolerated.

On the basis that androstenedione is a somewhat barrier substrate for aromatase than is testosterone, a further hydrogen-bonding group in the active site has been proposed capable of interacting with C(17) substituents. However, its importance appears to be secondary, on the basis of an investigation of D-ring modified steroids.[10] In addition to the binding interactions in the model, one negative feature is a lack of tolerance of steric bulk in the region occupied by C(1) and C(2) of steroid substrates.[11] The importance of this feature, combined with the existence of the hydrophobic pocket at C(4), has recently been illustrated in the activity of N(1)-substituted derivatives of 3-ethyl-3-(4-pyridyl)piperidine-2,6-dione, where alkylation causes the transfer of inhibitory activity from the *R* to the *S* enantiomer.[12]

In contrast to the wealth of information that has accumulated regarding the active site of aromatase, lyase has received little attention. Again, no crystal structure has been determined. By analogy with aromatase we may predict a hydrophobic cleft to accommodate the steroid skeleton with a hydrogen-bond donor and the heme to one end. The hydrogen-bond donor may be positioned to interact with the C(20) carbonyl group of the substrate (pregnenolone) and the heme should be in the vicinity of the sites of oxidation, C(17) and C(20).

Recently, the synthesis and biological testing of a new class of inhibitors of aromatase and lyase, derivatives of 4-pyridylacetic acid,[13,14] have been reported. These are of interest in that there appears to be scope for considerable control of both the absolute and relative activities of these compounds against the two enzymes, opening up the possibility of new, potent and selective inhibitors of both.

We are interested in attempting to rationalize the available structure-activity information on these compounds to aid the search for superior inhibitors and to provide further insight into important features of the active sites of both aromatase and lyase.

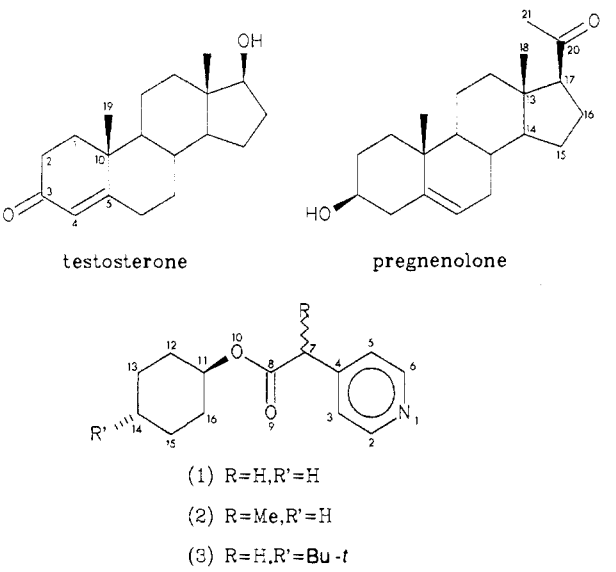

Figure 1. Structures of testosterone, pregnenolone, and the 4-pyridylacetates investigated in this study. The definitions of the torsion angles are α = 3-4-7-8; β = 4-7-8-10; γ = 7-8-10-11; and δ = 8-10-11-H11.

We report here a molecular modeling study of the aromatase and lyase inhibitor cyclohexyl 4-pyridylacetate **1** and its α-methyl derivative **2** (Figure 1). The choice of these two analogues for study was promoted by the observation that, relative to analogue **1**, the introduction of the α-methyl group into **2** results in an 7-fold reduction in its aromatase inhibitory activity, but an 8-fold increase in its lyase inhibitory activity.[14] Thus the concentration of **1** producing a 50% decrease in the activity of the aromatase enzyme (IC_{50}) was found to be 0.30 μM, while for racemic **2** against the same enzyme the IC_{50} was 2.1 μM. For comparison, the IC_{50} of aminoglutethimide, an aromatase inhibitor in clinical use, is 8 μM.[15] Against lyase on the other hand, **1** has an IC_{50} of 20 μM and **2**, 2.2 μM. The IC_{50} of ketoconazole, a lyase inhibitor that has been employed in the treatment of prostate cancer, is 10 μM.[16] The aim of the present study is to identify low-energy regions of conformational space for each analogue and to compare molecular shape/volume features with those of the natural substrates for aromatase and lyase, testosterone and pregnenolone, respectively.

In association with this study we report on a crystal structure analysis of the related aromatase and lyase inhibitor 4-*tert*-butylcyclohexyl 4-pyridylacetate (**3**). The crystal structure analysis of **3** was performed alongside the molecular mechanics calculations so that the results of the former could be used as a test of the quality of the conformational search procedures involved in the latter.

Experimental Section

Crystallography. Compound **3** crystallized as irregular thin plates. Unit cell dimensions were obtained by least-squares refinement of 25 θ values measured on an Enraf-Nonius CAD4 diffractometer. They are a = 12.347 (4), b = 6.125 (1), c = 22.058 (7) Å, β = 100.88 (3)°. The space group was unambiguously assigned as $P2_1/c$, with four molecules of $C_{17}H_{25}NO_2$ in the cell. Intensity data were collected in the range $1.5 < \theta < 60°$ with graphite-monochromated Cu K_α radiation by using the diffrac-

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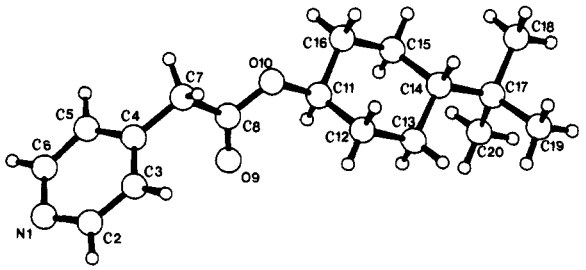

Figure 2. Crystal structure conformation of compound **3**.

Table I. Positional and Averaged Thermal Parameters for the Non-H Atoms of **3**[a]

atom	x	y	z	B (Å)^2
O9	0.2016 (3)	−0.135 (1)	0.3533 (5)	14.7 (2)
O10	0.1910 (2)	0.1189 (8)	0.4739 (3)	5.9 (1)
N1	0.0589 (3)	−0.266 (1)	0.0154 (4)	6.0 (2)
C2	0.0877 (4)	−0.070 (1)	0.0204 (5)	6.6 (2)
C3	0.1044 (3)	0.052 (1)	0.1137 (5)	5.7 (2)
C4	0.0923 (3)	−0.025 (1)	0.2126 (5)	4.4 (1)
C5	0.0620 (3)	−0.224 (1)	0.2089 (5)	5.8 (2)
C6	0.0468 (3)	−0.334 (1)	0.1123 (6)	6.0 (2)
C7	0.1130 (3)	0.097 (1)	0.3179 (5)	5.8 (2)
C8	0.1741 (3)	0.018 (1)	0.3795 (5)	7.2 (2)
C11	0.2499 (3)	0.058 (1)	0.5423 (5)	5.2 (2)
C12	0.2786 (4)	0.263 (1)	0.5961 (6)	6.1 (2)
C13	0.3401 (3)	0.210 (1)	0.6700 (6)	6.1 (2)
C14	0.3338 (3)	0.039 (1)	0.7581 (5)	4.6 (1)
C15	0.3015 (3)	−0.161 (1)	0.7010 (6)	5.8 (2)
C16	0.2403 (3)	−0.109 (1)	0.6248 (6)	6.5 (2)
C17	0.3948 (3)	−0.011 (1)	0.8387 (5)	4.7 (2)
C18	0.4222 (4)	0.196 (1)	0.8952 (6)	7.2 (2)
C19	0.4430 (3)	−0.115 (1)	0.7798 (6)	7.0 (2)
C20	0.3829 (4)	−0.170 (1)	0.9296 (6)	7.1 (2)

[a] Anisotropically refined atoms are given in the form of the isotropic equivalent thermal parameter defined as $^4/_3[a^2B(1,1) + b^2B(2,2) + c^2B(3,3) + ab(\cos\gamma)B(1,2) + ac(\cos\beta)B(1,3) + bc(\cos\alpha)B(2,3)]$.

tometer in an $\omega - 2\theta$ scan mode for a crystal of dimensions 0.3 × 0.5 × 0.03 mm. A total of 2419 unique reflections were obtained, of which 1259 were considered significant, with $I > 2\sigma(I)$. There was a 24% crystal decay during the data collection; an anisotropic correction was applied to take account of this by using intensities from three standard reflections. The correction factor was obtained by interpolating between successive standard reflections, weighted to allow for the relative distance between reciprocal lattice points. An empirical absorption correction was also applied. The structure was solved by direct methods and refined by full-matrix least squares. The positions of the hydrogen atoms were calculated geometrically and were kept fixed during the refinement. The final R factor is 0.0993, with a weighting scheme of the form $1/[\sigma^2(F) + 0.04(F)^2]$. This relatively high R factor is most likely due to the poor quality of the crystal and its decay during data collection. Final atomic coordinates are given in Table I and the structure shown in Figure 2.

Molecular Modeling. All molecular mechanics calculations were performed with use of the program MMP2(85)[17] running on a VAX 11/751. Graphical display and manipulation were performed with use of the program GEMINI[18] running on an IRIS 3130 workstation. The molecular structure of cyclohexyl 4-pyridylacetate (**1**) and the α-methyl derivative **2** were constructed from fragments in the GEMINI database. The coordinates of the *tert*-butylcyclohexyl derivative **3** were taken from the crystal structure reported here, and the structures of testosterone[19] and pregnenolone[20] were obtained from the Cambridge crystallographic databank.

All molecular structures were subjected to an initial minimization by using MMP2(85) prior to conformational and overlap analysis. For this purpose lone pairs were included on the pyridine nitrogen and the ester oxygen atoms (one and two, respectively) of the 4-pyridylacetates. No extra parameterization was required. The definition of the four torsion angles $\alpha - \delta$ in the chain between the pyridyl and cyclohexyl rings is shown in Figure 1. During the four-dimensional conformational grid-search calculations, minimization of the generated conformations was suppressed.

Results

A. Analysis of the Crystal-Structure Conformation of 4-*tert*-Butylcyclohexyl 4-Pyridylacetate (3). The crystal structure conformation of the molecule (Figure 2) is close to the fully extended one (α, 86°; β, −178°, γ, 180°; δ, 22°). The crystal-structure analysis is of relatively low accuracy, due to problems with crystal decay/size, and hence the temperature factors in particular are subject to some uncertainty. The oxygen atom O(9) has apparent high anisotropic motion resulting in a large averaged B factor (Table I). Nonetheless, the essential conformational features of the structure are unequivocal. The molecular mechanics energy of this conformation is 272.3 Kcal/mol, but most of this is concentrated in the C–H bond lengths. Minimization reduced the energy to 21.6 Kcal/mol with very little change in the torsion angles other than δ (α, 86°; β, 168°; γ, −179°; δ, 41°). RMS shifts were 0.175 Å for all atoms, 0.119 Å for non-H atoms. Restoring the torsion angles to their crystal-structure values raised the energy by just 0.51 Kcal/mol.

B. Conformational Analysis of Cyclohexyl 4-Pyridylacetate (1). The molecular structure of cyclohexyl 4-pyridylacetate (**1**) was generated with use of GEMINI and then subjected to an initial MMP2 minimization. The initial values of α and β were −120°, the initial values of γ and δ were 180°. Final values were α, −117°; β, −143°; γ, 176°; and δ, 179°.

Local minima in the conformational space defined by the torsion angles $\alpha - \delta$ were located by using a three-stage procedure. The first step involved a grid search over all values in $\alpha - \delta$ in 60° intervals (30° intervals for α). Local minima in this (four-dimensional) conformational space were taken as conformations, having all eight nearest neighbors of higher energy, a nearest neighbor having just one torsion angle differing by one angle increment. In the second stage each area of conformational space located in stage one was searched again using a finer grid (20° increments apart from 10° increments in α). Approximate minima found were defined as before. In the third stage the minima thus found were subjected to a full molecular mechanics minimization.

Stage one led to the identification of seven conformations. After stage two this had risen to 16. After stage three and the removal of duplicates and mirror-images the list was reduced to six (conformers 1A–1F, Table II part a). Note that minimum 1D corresponds closely to the crystal structure conformation of the *tert*-butyl analogue **3** and that in addition to the local minima listed, there exist their equi-energetic mirror images. In the discussion these are identified by a starred superscript so that, for instance, conformer 1A* is the mirror image of 1A and its torsion angles $\alpha - \delta$ are obtained from those of 1A by reversing their signs. The choice of a two-stage grid-search proce-

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Table II. Energies (in Kcal/mol) and Torsion Angles (in Degrees) of Local Minima (For definitions of the torsion angles, see Figure 1)

conformer	energy	torsion angle α	β	γ	δ
a. For 1 Calculated with Use of MM2(85)[a]					
1A	13.92	-71	-60	175	42
1B	14.11	-83	-64	175	-41
1C	14.15	-89	78	178	43
1D	14.94	-87	-168	179	-41
1E	15.10	-74	-61	174	-178
1F	15.94	-90	161	-179	-180
b. S Enantiomer of 2 Calculated with Use of MM2(85)[b]					
2A	14.90	-47	-61	177	41
2B	15.12	-46	-56	179	-40
2C	16.05	-45	-60	178	180
2D	16.27	-49	103	-176	-43
2E	16.31	-83	67	-178	-42
2F	16.47	-59	95	-175	39
2G	16.50	-85	55	-178	38
2H	17.31	-76	-145	177	-41
2I	17.46	-50	102	-176	179
2J	17.57	-75	59	-179	179
2K	18.15	-67	-167	-179	179

[a] A symmetry-related set of local minima is obtained by changing the signs of all the torsions angles. [b] Changing the signs of all the torsion angles yields the local minima for the (R) enantiomer.

dure was dictated by computer resources, a full search at the resolution of the finer grid would have generated some 46 656 conformers whose energy would need to be calculated. However, on the basis of the results, it was decided that for 2, a single search at intermediate resolution would suffice.

C. Conformational Analysis of the Cyclohexyl Ester of α-Methyl-4-pyridylacetic Acid (2). As before, the structure (as the (S) isomer) was built up from fragments in the GEMINI database and minimized by using MM2P(85). The initial conformation was the same as for 1. Final torsion angles were α, -110°; β, -148°; γ, 174°; and δ, 180°. For the conformational search a two-stage procedure was employed; first a grid search over all values of α - δ (30° increments apart from 20° increments for α) with approximate minima being located as before on the nearest-neighbor principle, followed by full molecular mechanics minimization of each conformation thus identified.

The grid search identified 16 local minima of energy <25 Kcal/mol; after minimization and the removal of duplicates, the list reduced to 11 (local minima 2A–2K, Table II part b). Note that in this case the mirror-image conformers, 2A*–2K*, for which the signs of all the torsion angles are changed, correspond to the local minimum energy conformations of the (R) enantiomer of 2.

D. Fitting of Cyclohexyl 4-Pyridylacetate (1) and the Methyl Analogue 2 to Testosterone. Both 1 and 2 show Type II binding spectra in their interaction with aromatase.[14,21] This is characteristic of inhibitors able to provide a strong sixth ligand to the heme iron.[6] Such ligands are typically nitrogen-containing aliphatic or aromatic compounds. We therefore concluded that the pyridyl nitrogen was involved in direct interaction with the heme in the active site. On the basis of a previous modeling study[5] this was expected to require the positioning of the pyridyl nitrogen atom above the steroid A-ring, in the vicinity of C(19). The cyclohexyl ring is expected to occupy one of the two hydrophobic pockets, either that defined by the steroid backbone or that hypothesized as

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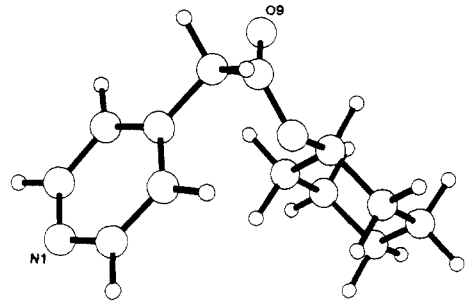

Figure 3. Conformation 1A of 1, predicted as being suitable for binding to aromatase.

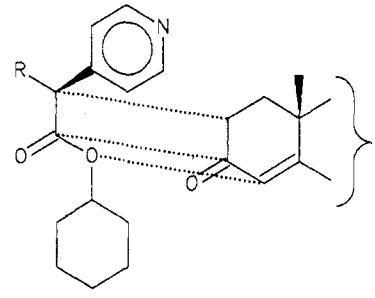

Figure 4. Mode of superposition of testosterone and the pyridylacetates for analysis as aromatase inhibitors.

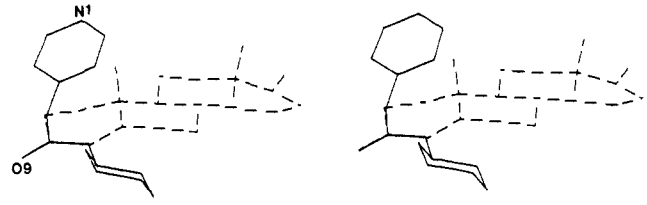

Figure 5. Stereoplot of fitting of 1 in conformation 1A to testosterone (dashed lines).

extending from the C(4) position. Taking the second alternative, we discovered that for the lowest energy conformation of 1, 1A, both requirements could be satisfied in an overlap mode that in addition places the ester carbonyl group over that of the steroid. This mode corresponds approximately to that previously developed for the cyclohexyl amide of (R)-2-(4-aminophenyl)propanoic acid, FCE24328.[5] This conformation of 1 is shown in Figure 3, while the overlap is illustrated diagrammatically in Figure 4 and in the stereoplot Figure 5. The O(9), C(8), O(8), and C(7) atoms of 1 are matched to the C(4), C(3), O(3), and C(2) atoms of testosterone. The goodness of fit depends only on the values of β and γ; it can be seen from Table II part b that the lowest energy conformation of (S)-2 (2A) and the fifth lowest energy conformation of the (R) enantiomer of 2, (2E*), are both suitable. However, it is known that aromatase inhibition is impaired in molecules whose orientation in the binding site places steric bulk in the region occupied by C(2) of a steroid.[11] The overlap model used here places the α-methyl group of 2 in just such a position.

E. Fitting of Cyclohexyl 4-Pyridylacetate (1) and the α-Methyl Analogue 2 to Pregnenolone. In attempting to overlap pregnenolone with the various conformers of 1 and 2 the question arose as to the preferred conformation of the steroid C(17) side chain. The preferred conformation of the pregnane side-chain has been subject to some study. Previous MM2 studies[22] have shown

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us to compare the molecular weight of the labeled protein with those reported by Emerit et al. and Shih et al.

Since the consequences of modifications of the 1,2,3,4-tetrahydronaphthalene nucleus have not been explored extensively, it is of interest to determine the structure–activity relationships in functional receptor preparations and the binding discrimination for these compounds. These experiments are currently underway. Some preliminary conclusions can be drawn, however, regarding both affinities and photochemical behavior of these novel 1,2,3,4-tetrahydronaphthalenes. Experimental and computational lines of evidence support the idea that the volume of the C-8 substituent (with a maximum around 24 Å^3) rather than the electronic properties contributes to 5-HT_{1A} receptor affinity.[18] Functionalization of the 8-phenolic group as either an ether (**2**) or ester (**3**) decreases the affinity of the substituted 1,2,3,4-tetrahydronaphthalene to a similar extent (12–20-fold) at 5-HT_{1A} binding sites. Elimination of the oxygen at C-8 altogether provides a smaller group at this position and restores much of the receptor affinity for the modified ligand. The proposed inverse relationship of size to affinity is bolstered by the observation that the IC_{50} of the ester 8-carbomethoxy-DPAT (**9**) for specific [^{3}H]-8-OH-DPAT sites is 5 nM, which is comparable to the affinity of the receptor for 8-OH-DPAT.

In summary, a probe has been developed that shows good potential as a photoaffinity label for the 5-HT_{1A} receptor. The results of the ^{3}H-labeling and functional receptor studies will be reported elsewhere.

Experimental Section

Chemistry. All reactions were conducted under nitrogen or argon. Tetrahydrofuran (THF) was distilled from benzophenone ketyl immediately prior to use. Other reaction solvents and reagents were purified as described below or were the highest grade commercially available. Thin-layer chromatography (TLC) analyses of reactions were run on Polygram SilG/UV 254 plates and visualized under ultraviolet light, with (2,4-dinitrophenyl)-hydrazine, Ehrlich's reagent, and/or dichlorotolidine. Unless otherwise indicated, preparative chromatography was done on silica gel under flash conditions.[19] Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian FT 80-A instrument, in $CDCl_3$, unless otherwise indicated. Infrared spectra were recorded on a Beckman IR8 instrument. Ultraviolet spectra were recorded on a Beckman Model 34 instrument. Low-resolution mass spectra were obtained on a 5980-A Hewlett Packard mass spectrometer and high-resolution mass spectra on an MS-30 Kratos spectrometer, by Dr. Chalres Iden of the Department of Chemistry at State University of New York at Stony Brook. Elemental analyses were obtained from the Microanalytical Service at the Rockefeller University.

8-[[(Diazomethyl)carbonyl]methoxy]-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (2). A solution of bromodiazoacetone[15] (235.6 mg, 1.58 mmol), K_2CO_3 (515 mg, 3.74 mmol), and **1**[20] (296 mg, 1.2 mmol base) in a 5 mL of 4:1 THF/MeOH was allowed to stir at room temperature for 72 h in the dark. The reaction mixture was poured into water, the pH adjusted to 9, and the basic fraction extracted into ether. The organic phase was dried, and the solvent was removed in vacuo to give a dark oily residue. Chromatography (5:95 MeOH–$CHCl_3$ plus 5 drops of NH_4OH/100 mL) gave 160 mg of **2** as a yellow oil (50% of converted starting material) and 54 mg of recovered 1 that could be recycled. Anal. ($C_{19}H_{27}N_3O_2$) Calcd: C, 69.30; H, 8.21; N, 12.77. Found: C, 69.57; H, 8.45; N, 12.80. NMR: δ 6.49–7.17 (3 H, ArH), 5.78 (s, 1 H, CH=N=N), 4.54 (2 H, s, OCH_2CO), 2.70–3.32 (5 H, m, NCH over NCH_2), 2.40–2.70 (4 H, m, $ArCH_2$), 1.10–2.35 (6 H, m, CH_2CH_2), 0.90 (6 H, t, CH_3). IR (neat oil): 2120, 1670 cm^{-1}. UV (EtOH): λ_{max} 206 nm (ϵ = 16500), 262 (ϵ = 10200). MS: m^+/z 330 (MH^+), 329 (M), 300 (M – C_2H_5). HRMS: 329.2111.

8-(Diazoacetoxy)-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (3). To a 0 °C solution of 1 (241 mg, 0.98 mmol) in 8 mL of THF was added a hexane solution of *n*-butyllithium (1.53 mL of a 1.6 M solution, 2.45 mmol). The anion solution was stirred at 0 °C for 10 min and then at room temperature for 20 min, before being transferred under nitrogen to a 0 °C solution of glyoxal chloride (*p*-tolylsulfonyl)hydrazone (524 mg, 2.8 mmol) in 8 mL of methylene chloride. The reaction mixture was allowed to come to room temperature and stirred for 2.5 h before the first 4 equiv of triethylamine was added. The reaction mixture was then stirred 1 additional h, a second 4 equiv of triethylamine was added, and stirring was continued for 30 min before the reaction was quenched by the addition of excess cold water. The crude reaction mixture was partly concentrated in vacuo and then taken up in ethyl acetate. The crude basic fraction was extracted and purified by chromatography (2:98 MeOH/$CHCl_3$ plus 10 drops of NH_4OH/100 mL) to give 98.7 mg of **3** (57% of converted **1**) and 107.3 mg of **1**. Anal. $C_{18}H_{25}N_3O_2$ (C, H, N). NMR: δ 6.84–7.19 (3 H, ArH), 4.97 (1 H, s, CH=N=N), 2.68–3.12 (5 H, m, NCH over NCH_2), 2.19–2.59 (4 H, m, $ArCH_2$), 1.09–2.16, (6 H, m, CH_2CH_2), 0.88 (6 H, t, J = 7 Hz, CH_3). IR (neat): 2120, 1710 cm^{-1}. UV (EtOH): λ_{max} 249 nm (ϵ = 19600), 210 (ϵ = 16600). MS: m^+/z: 315 (M), 285 (M – C_2H_5). HRMS: 315.1963.

8-Bromo-1,2,3,4-tetrahydronaphthalen-2-one (5). To a 0 °C solution of (2-bromophenyl)acetic acid (3.1 g, 14.4 mmol) in 10 mL of methylene chloride was added, in small portions, solid PCl_5 (3.75 g, 18 mmol), and the reaction mixture was allowed to stir for 15 min at 0 °C and then 30 min at room temperature. The acid chloride was then transferred under nitrogen over the course of 1 h to a −78 °C suspension of $AlCl_3$ (3.83 g, 28.7 mmol) in 20 mL of methylene chloride. The reaction mixture was stirred 1 h at −78 °C and maintained at this temperature while ethylene was bubbled vigorously through the solution for 30 min. The cooling bath was removed, and the solution was stirred overnight before being poured into excess ice water. The methylene chloride phase was washed successively with 2 N HCl, $NaHCO_3$, and NaCl, then dried, and concentrated in vacuo to a residual oil. Chromatography (0.5:99.5 ethyl acetate/hexane followed by 1:99 ethyl acetate/hexane) gave 786 mg (24%) of **5** as a white solid. NMR: δ 7.13–7.62 (3 H, ArH), 3.74 (2 H, s, $ArCH_2CO$), 3.18 (2 H, t, J = 6.2, 7.0 Hz, $ArCH_2$), 2.65 (2 H, t, J = 7.1, 6.4 Hz, $COCH_2CH_2$). IR ($CHCl_3$): 1740 cm^{-1}. This material was used immediately in the subsequent reaction.

8-Bromo-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (6). A solution of **5** (402 mg, 1.79 mmol), *N*,*N*-dipropylamine (1404 mg, 14 mmol), and *p*-toluenesulfonic acid monohydrate (37 mg, 0.2 mmol) in 25 mL of freshly distilled benzene was refluxed with a Dean-Stark water separator for 72 h and then concentrated in vacuo. The residual oil was dissolved in 100 mL of absolute ethanol and shaken over 100 mg of PtO_2 under 55 psi H_2 in a Parr hydrogenation apparatus for 24 h. the catalyst was filtered off, the solvent evaporated in vacuo, and the basic fraction isolated to give crude **6** as a purple oil. Chromatography on neutral alumina (1:99 ethyl acetate/hexane) gave 185 mg (53%) of **6** as a colorless oil. NMR: δ 6.92–7.13 (3 H, ArH), 2.59–2.89 (5 H, m, NCH over NCH_2), 2.25–2.50 (4 H, m, $ArCH_2$), 1.1–2.0 (4 H, q, J = 7.6 Hz, CH_2CH_3), 0.89 (6 H, t, J = 6.9, 7.2 Hz, CH_3). IR (neat): 2980 cm^{-1} (C–H stretch). Anal. $C_{16}H_{24}BrN$ (C, H, N).

8-Carboxy-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (8). To a −78 °C solution of **6** (185 mg, 0.71 mmol) in 20 mL of THF was added, dropwise, a hexane solution of *n*-butyllithium (2.6 mL of a 1.6 M solution, 4.2 mmol). The bright red-orange solution was allowed to stir at −78 °C for 1 h and then warmed to −10 °C. Carbon dioxide gas was bubbled through, immediately decolorizing the solution, and the bubbling was continued for 1 h; the reaction mixture was stirred 1 additional h after the CO_2 inlet was removed and then neutralized with 1 equiv of trifluoroacetic acid (81 mg, 55 μL, 0.71 mmol). The volatiles were removed in vacuo, the residue dissolved in water,

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to produce less severe steric clashes and so to be the more effective inhibitor.

To our knowledge there has so far been no detailed model presented for the binding of reversible lyase inhibitors to the enzyme. The model developed here permits the rationalization of the lyase inhibition data without the postulation of features of the binding site not evident from the substrate structure, i.e. a second hydrophobic pocket. That the binding of the ester moiety does not involve a pocket related to that postulated for aromatase inhibition is supported in part by the observation that, for chiral esters (menthyl, borneyl and isopinocampheyl), the enantiomer that was more inhibitory against aromatase was the less inhibitory against lyase.[14] This suggests that the hydrophobic pockets are quite different in the two enzymes. The positioning of the pyridyl group was based on the relationship between oxidation site, postulated heme position and pyridyl orientation developed for aromatase inhibition. The model requires torsion angle β to take a value of about 60°. Such an angle is readily accessible to **1** and the *R* enantiomer of **2**, and only slightly less so for (*S*)-**2**. The root of the increased lyase inhibitory potential of **2** is therefore hypothesized as stemming largely from the ability of the methyl group to substitute either for C(16) or for C(20) of a steroid substrate. The observation that the dimethylated derivative is a poorer lyase inhibitor than racemic **2** suggests that in fact the mimicry of only one of these two sites is favorable. However, at this stage the model does not provide any predictions as to which of these it will be and so as to which of the two enantiomers of **2** will be the best lyase inhibitor.

The results presented here provide further support for the model of aromatase inhibition that has previously been developed[5] and which has already been used predictively with success.[12] The model presented here for lyase inhibition can only be regarded as preliminary since its validation will require the examination of a broader range of structurally diverse lyase inhibitors. However, it is clear that it provides enough detail to suggest avenues of structural modification which should lead to both more potent and more selective inhibitors of lyase based on the pyridylacetate framework.

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Registry No. 1, 129175-15-1; (*S*)-**2**, 129175-16-2; (*R*)-**2**, 129175-18-4; **3**, 129175-17-3; steroid 17-20-lyase, 9044-50-2; aromatase, 9039-48-9.

Supplementary Material Available: Tables of crystallographic data for compound **3** including temperature factors, H-atom coordinates, and bond lengths and angles (4 pages). Ordering information is given on any current masthead page.

Quinazoline Antifolate Thymidylate Synthase Inhibitors: Alkyl, Substituted Alkyl, and Aryl Substituents in the C2 Position

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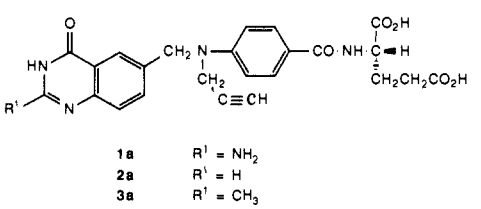
Modification of the potent thymidylate synthase (TS) inhibitor *N*-[4-[*N*-[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid (**1a**) has led to the synthesis of quinazoline antifolates bearing alkyl, substituted alkyl, and aryl substituents at C2. In general the synthetic route involved the coupling of the appropriate diethyl *N*-[4-(alkylamino)benzoyl]-L-glutamate with a C2-substituted 6-(bromomethyl)-3,4-dihydro-4-oxoquinazoline followed by deprotection using mild alkali. Good enzyme inhibition and cytotoxicity were found with compounds containing small nonpolar groups in the C2 position with the 2-desamino-2-methyl analogue **3a** being the most potent. Larger C2 substituents were tolerated by the enzyme, but cytotoxicity was reduced. Highly potent series were followed up by the synthesis of a number of analogues in which the N10 substituent was varied. In this manner a number of interesting TS inhibitors have been prepared. Although none of these was more potent than **1a** against the isolated enzyme, over half of the compounds prepared were more potent as cytotoxic agents against L1210 cells in culture. The potential of such compounds as useful antitumor agents was further enhanced by the finding that the improved aqueous solubilities of compounds such as **3a** over **1a** were reflected in vivo in that **3a** was at least 5 times less toxic to mice than **1a**.

The potent antifolate thymidylate synthase (TS) inhibitor *N*-[4-[*N*-[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid (**1a**)[1,2] has shown encouraging antitumor activity against breast and ovarian cancers[3,4] and hepatomas[5] in recent clinical trials. However unacceptable liver and kidney toxicities have prevented its widespread use.[6] The hypothesis has been that these toxicities are the result of the physicochemical properties of **1a**, in particular its poor water solubility, rather than being intrinsic to this



1a $R^1 = NH_2$
2a $R^1 = H$
3a $R^1 = CH_3$

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