Pure samples of **17A** and **17B** were prepared by a procedure analogous to that described for **16A** and **16B**. Thus, **17B** di-*p*-toluoyl-D-tartrate and **17A** di-*p*-toluoyl-L-tartrate were prepared and recrystallized to purity. Anal. ($C_{38}H_{43}NO_9$) C, H, N for both samples.

The salts were converted to their free bases **17B** and **17A** as described for **16B** and **16A**. The ^{1}H NMR spectra of **17B** and **17A** were identical to the spectra of **16B** and **16A**, respectively.

Biochemical. σ Receptor Assay. Crude P_2 membrane fractions were prepared from frozen (-80 °C) guinea pig brains (Pel-Freeze, Rogers, AK), minus cerebellum. After removal of cerebella, the brains were allowed to thaw slowly on ice and placed in ice-cold 10 mM Tris-HCl, pH 7.4, containing 320 mM sucrose (Tris-sucrose buffer). The brains were then homogenized in a Potter-Elvehjem homogenizer by 10 strokes of a motor driven Teflon pestle in a volume of 10 mL/g tissue weight. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C, and the supernatants were saved. The pellets were resuspended by vortexing in 2 mL/g of ice-cold Tris-sucrose and centrifuged again at 1000*g* for 10 min. The combined 1000*g* supernatants were centrifuged at 31000*g* for 15 min at 4 °C. The pellets were resuspended by vortexing in 3 mL/g of 10 mM Tris-HCl, pH 7.4, and the suspension allowed to incubate at 25 °C for 15 min. Following centrifugation at 31000*g* for 15 min, the pellets were resuspended by gentle Potter-Elvehjem homogenization to a final volume of 1.53 mL/g in 10 mM Tris-HCl, pH 7.4. Aliquots were stored at -80 °C until use. Protein concentration was determined by the method of Lowry et al.[33] using bovine serum albumin as standard.

σ sites were labeled with [^{3}H]-(+)-pnetazocine (52 Ci/mmol) or [^{3}H]-(+)-3-PPP (98.9 Ci/mmol). Incubations were carried out in 50 mM Tris-HCl, pH 8.0, for 120 min at 25 °C in a volume of 0.5 mL with 500 μg of membrane protein and 3 nM radioligand. Nonspecific binding was determined in the presence of 10 μM (+)-pentazocine ([^{3}H]-(+)-pentazocine) or 1 μM haloperidol ([^{3}H]-(+)-3-PPP). Assays were terminated by the addition of 5 mL of ice-cold 10 mM Tris-HCl, pH 8.0, and filtration through glass fiber filters (Schleicher and Schuell), which were soaked in 0.5% polyethylenimine for at least 30 min at 25 °C prior to use. Filters were then washed twice with 5 mL of ice-cold Tris-HCl buffer. All filtration was carried out using a Brandel cell harvester (Gaithersburg, MD).

PCP Receptor Assay. Adult male Sprague-Dawley rats (Dominion Laboratories, Dublin, VA) were decapitated, and the whole brain minus cerebellum was homogenized in 10 volumes of 5 mM Tris-HCl buffer (pH 7.7) and centrifuged at 45000*g* for 15 min at 4 °C. The pellet was resuspended and recentrifuged as before. The final pellet was resuspended in 10 volumes of buffer. Aliquots (200 μL) of freshly prepared homogenate were incubated in triplicate (25 °C, 20 min) with the appropriate concentration of [^{3}H]TCP. Nonspecific binding was determined in the presence of 1 μM unlabeled TCP. The reaction was terminated by rapid filtration on a Brandel cell harvester (Gaithersburg, MD). Schleicher and Schuell (Keene, NH) #32 glass filters were soaked for 5 min in a 0.05% polyethylenimine solution prior to filtration. Filters were washed two times with 5 mL of cold buffer, suspended in 10 mL of Budgetsolve (Research Products International, Mount Prospect, IL) and shaken for 1 h. Radioactivity was determined by liquid scintillation spectrometry at a counting efficiency of approximately 50% corrected by external standardization. Specific [^{3}H]TCP binding was defined as total binding minus nonspecific binding. Protein concentrations were determined by the method of Bradford.[35] Displacement studies were conducted by incubating the drugs with 1 nM [^{3}H]TCP as described above. K_i values were calculated using the Cheng-Prusoff equation.[34]

μ Opiate Receptor Assay. In the μ-opiate assay, the homogenate was prepared as described above for the PCP receptor assay in a 50 mM Tris-HCl buffer (pH 7.4) and incubated (30 °C, 2.5 h) with 1 nM [^{3}H]DAMGO for displacement studies. Nonspecific binding was determined in the presence of 1 μM levorphanol. The reactions were filtered and counted for radioactivity as described for the PCP assay. K_i values were calculated using the Cheng-Prusoff equation.[34] A K_d of 1.9 ± 0.36 nM for [^{3}H]DAMGO was used.

Molecular Modeling. Molecular modeling studies were performed with the Tripos Associates SYBYL software package (version 5.41) installed on a Silicon Graphics 4D/310VGX graphics workstation. An Apple Macintosh IIcx running the Tripos Associates NITRO terminal emulator was used as the graphics terminal.

Acknowledgment. This work was supported by the National Institute on Drug Abuse under Grant No. DA05721, DA02396, and DA00490.

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Stereoisomers of Ketoconazole: Preparation and Biological Activity[1]

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The four stereoisomers of the antifungal agent ketoconazole (1) were prepared and evaluated for their selectivity in inhibiting a number of cytochrome P-450 enzymes. Large differences in selectivity among the isomers were observed for inhibition of the cytochromes P-450 involved in steroid biosynthesis, whereas little difference was observed for inhibition of those associated with hepatic drug metabolism. The cis-(2*S*,4*R*) isomer **2** was the most effective against rat lanosterol 14α-demethylase, (2*S*,4*R*)-**2** > (2*R*,4*S*)-**4** ≫ (2*R*,4*R*)-**3** = (2*S*,4*S*)-**5**, and progesterone 17α,20-lyase, (2*S*,4*R*)-**2** ≫ (2*S*,4*S*)-**5** > (2*R*,4*R*)-**3** = (2*R*,4*S*)-**4**, whereas the cis-(2*R*,4*S*) isomer **4** was more effective against cholesterol 7α-hydroxylase, (2*R*,4*S*)-**4** > (2*S*,4*S*)-**5** > (2*R*,4*R*)-**3** > (2*S*,4*R*)-**2**, and the trans-(2*S*,4*S*) isomer **5** was the most effective against aromatase, (2*S*,4*R*)-**5** ≫ (2*R*,4*R*)-**3** = (2*R*,4*S*)-**4** > (2*S*,4*R*)-**2**. The cis-(2*S*,4*R*) and trans-(2*R*,4*R*) isomers **2** and **3** are equipotent in inhibiting corticoid 11β-hydroxylase and much more effective than their antipodes. Little selectivity was observed for inhibition of cholesterol side chain cleavage or xenobiotic hydroxylases. These data indicate that the affinity of azoles for cytochrome P-450 enzymes involved in steroid synthesis is highly dependent on the stereochemistry of the entire molecule, whereas binding to drug metabolizing enzymes is a less selective process.

Introduction

Ketoconazole (1) is a potent, orally active, broad-spectrum antifungal agent.[2,3] The basis of the antifungal activity of ketoconazole and related azoles is blockade of the conversion of lanosterol to ergosterol, which is necessary for maintaining the integrity of the organism's cell

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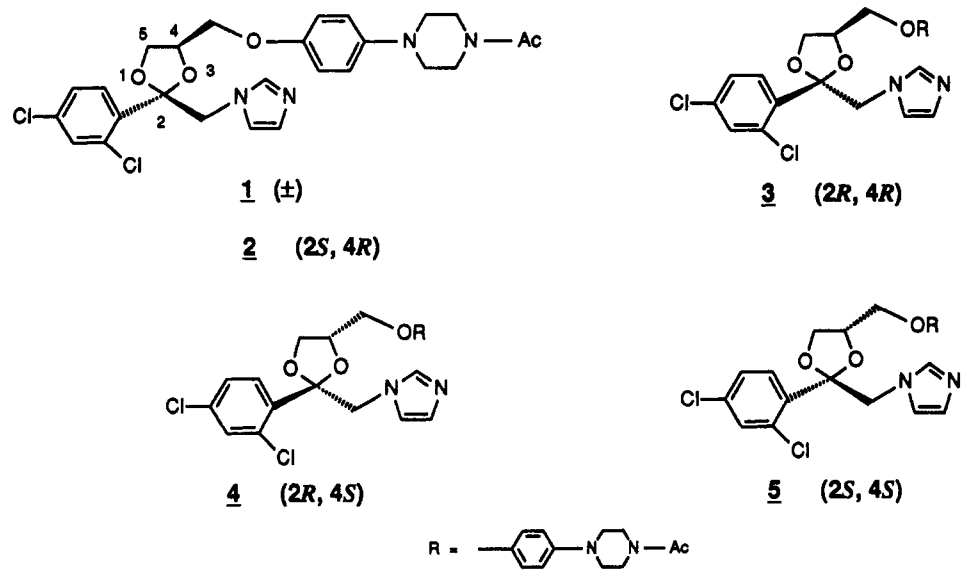
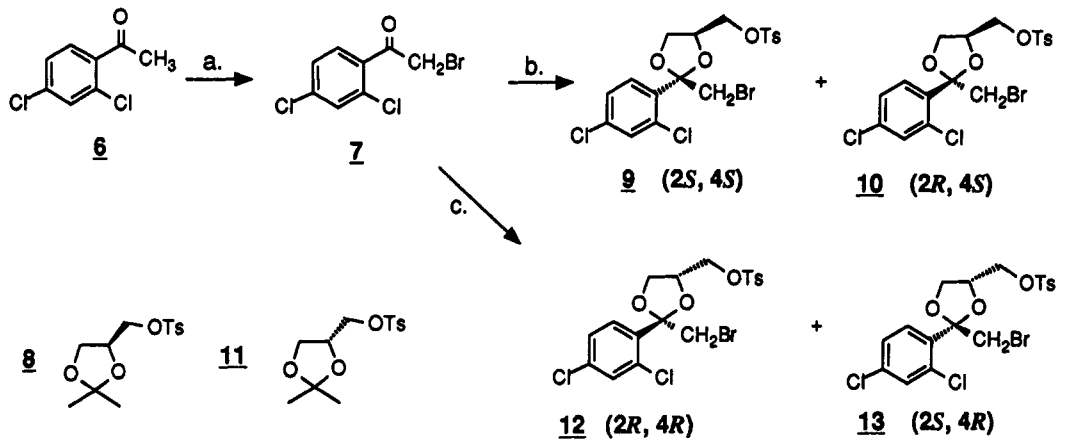
Scheme I



Scheme II



a. $CuBr_2$, EtOAc:CH_2Cl_2, reflux b. 8, p-TsOH, nBuOH, toluene, reflux c. 11, as b.

membrane. The specific point of chemical intervention appears to involve inhibition of the cytochrome P-450 enzyme responsible for the oxidative removal of the C-14 methyl group of lanosterol (lanosterol 14α-demethylase).[4-6] Ketoconazole has shown a similar inhibitory effect on the corresponding enzyme responsible for conversion of lanosterol to cholesterol in mammals[7,8] and has been demonstrated to lower cholesterol in humans.[9-11] In addition, 1 has been shown to inhibit a number of other cytochrome P-450 enzymes involved in steroidogenesis and drug metabolism.[12-17] An example is the well-known clinical

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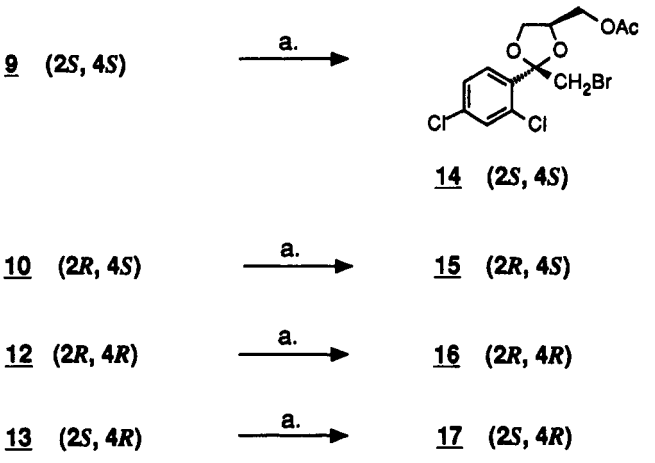
manifestation of decreased androgen levels on long-term use of ketoconazole in man, which is traced to inhibition of progesterone 17α,20-lyase.[18-21] Ketoconazole has also been shown to block adrenal steroidogenesis by inhibition of the corticoid 11β-hydroxylase.[22-24] These properties of ketoconazole have been utilized to treat prostate cancer and Cushing's syndrome, respectively.

Ketoconazole (1) is a racemic mixture of the cis-(2S,4R) and -(2R,4S) enantiomers 2 and 4 (Scheme I). There are numerous known examples of different pharmacological properties between stereoisomers.[25] However, there is only limited information in the literature on the preparation of enantiomers of azole cytochrome P-450 inhibitors and their behavior.[26-30] In particular, the preparation and relative activities of the diastereomeric pairs of enantiomers of ketal-containing antifungal agents such as 1 have not been described except for the simple examples of etaconazole and propiconazole, where antifungal and

Scheme III



a. CsOAc, DMF, reflux

phytotoxic activities were compared.[31-33] We became interested in comparing the activities of the pure enantiomers of ketoconazole and its trans isomer against a series of cytochrome P-450 enzymes. Knowledge of the relationship between biological activity and absolute stereochemistry for these relatively rigid isomers could prove useful in understanding their mode of interaction with the different P-450 enzymes. Therefore, we undertook the synthesis of the optically pure cis enantiomers 2 and 4 and the corresponding trans[34] diastereomeric isomers 3 and 5 and evaluated their effectiveness as P-450 inhibitors.

Chemistry

The reported synthesis of racemic ketoconazole (1) proceeds via the ketalization of 2',4'-dichloroacetophenone with glycerol.[2] Our nonracemic synthesis differed by utilizing transketalization reactions between 2-bromo-2',4'-dichloroacetophenone (7) and the optically pure solketal tosylates 8 and 11 (Scheme II). These reactions required the inclusion of 1-butanol as a catalyst to proceed at a practical rate.

Bromination of 2',4'-dichloroacetophenone (6) with copper(II) bromide in 1:1 CH_2Cl_2–EtOAc gave 2-bromo-2',4'-dichloroacetophenone (7). Transketalization of ketone 7 using (S)-solketal tosylate (8) in the presence of p-TsOH and n-BuOH in refluxing toluene, accompanied by azeotropic removal of water, afforded a 1.2:1 mixture of the cis and trans bromotosylates 9 and 10, which were separable by chromatography. The analogous reaction utilizing (R)-solketal tosylate (11) gave diastereomers 12 and 13.

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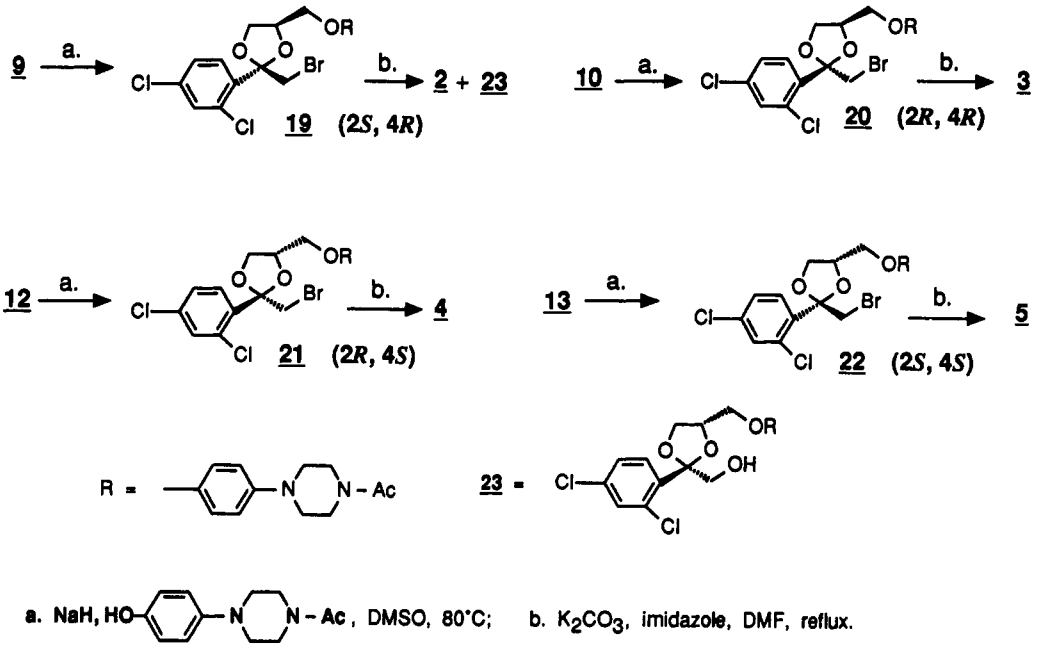
Scheme IV



The cis and trans nature of the isomers was deduced by examination of their ^{1}H NMR spectra. The cis isomers show a characteristic well-separated pair of doublets (separated by ca. 25 Hz at 300 MHz) for the bromomethyl group, caused by restricted rotation due to the proximity of the tosyloxymethylene group. In the trans isomers, the AB system is barely resolved at 300 MHz (separation 0.9 Hz), consistent with much freer rotation of the bromomethyl group. Eventual conversion of the bromotosylates **9**, **10**, **12**, and **13** to the enantiomeric forms of the known racemic compounds also served to confirm the cis/trans assignments. Attempts to determine optical purities of the bromotosylate stereoisomers **9**, **10**, **12**, and **13**, by ^{1}H NMR analysis using chiral shift reagents were unsuccessful. However, it was found that shift studies of the corresponding acetates worked well. Thus, portions of the four tosylates were converted to the corresponding acetates **14**–**17** by treatment with CsOAc/DMF at 100 °C for 1 h (Scheme III). In this case, use of the chiral shift reagent Eu(Hfc)$_3$ caused a significant downfield shift of the C-6 aromatic protons of the *S,S* isomer relative to the *R,R* isomer and the *S,R* isomer relative to the *R,S* isomer and showed the acetates to be optically pure within the limits of the method.[35] By extension, this proved the optical purities of the tosylates themselves and implied that of the final products. Conversion of tosylates **9**, **10**, **12**, and **13** into final products **2**–**5** does not involve any reaction conditions or synthetic manipulations which should adversely effect optical purity.

The synthesis now required differentiation of the primary tosylate from the primary bromide. (In the published synthesis of ketoconazole, imidazole is introduced *prior* to activation of the ketal alcohol as the mesylate.[2]) Treatment of the individual tosylate isomers **9**, **10**, **12**, and **13** with the anion prepared from 4-(*N*-acetylpiperazino)-phenol **18** and NaH in DMSO at 80 °C afforded the anticipated phenyl ethers **19**, **20**, **21**, and **22** respectively (Scheme IV).[36] Literature precedent for displacement of bromine from similar α-bromo ketals with imidazole involves treatment with excess imidazole in refluxing DMA for 4 days.[2] Only a trace of reaction product was observed on applying these conditions to bromides **19**–**22**. However, the addition of 5 equiv of K_2CO_3 to the reaction mixtures in refluxing DMA or DMF led to transformation of **19**–**22** into the desired imidazole products **2**–**5** after 4–16 h in 40–50% yields. A minor side product was isolated from the preparation of **4** (in ~10% yield) and was identified as the hydroxymethyl compound **23**, which probably arose from hydrolysis under the reaction conditions. Similar hydrolysis products were noted in the preparation of **2**, **3**, and **5**, but were not isolated.

Biology

The four stereoisomers of ketoconazole were evaluated for their effectiveness as inhibitors of the cytochromes P-450 involved in cholesterol biosynthesis and degradation (lanosterol 14α-demethylase and cholesterol 7α-hydroxylase), steroid hormone biosynthesis (cholesterol side chain cleavage, progesterone 17α,20-lyase, deoxycorticosterone 11β-hydroxylase, and aromatase) and xenobiotic transformation (lauric acid hydroxylation and progesterone 2α-, 6β-, 15α-, 16α-, and 21-hydroxylation) (see Experimental Section). The activities for the enzymes responsible for cholesterol biosynthesis and degradation and steroid biosynthesis were evaluated with the endogenous substrates, whereas model substrates were used to evaluate the xenobiotic transforming enzymes. Lauric acid hydroxylation has been shown to be characteristic of the CYP4A family,[37] progesterone 2α-hydroxylation for CYP2C11, 6β- for CYP3A, 16α- for a number of enzymes including CYP2B1, CYP2B2, CYP1A1, CYP2C11, and

(35) The addition of 20 mg of shift reagent to 2 mg of a 1:1 mixture of **14** and **16** or **15** and **17** induces separation of the aromatic signals (H_3, H_5, and H_6), with the H_6 being most distinct. Under identical conditions no such effects were observed with any of the separate acetate isomers **14**–**17**. We estimate the limits for the resolution of an enantiomeric impurity in our experiments to be approximately 2%.

(36) The change in stereochemical notation in the formation of **19**–**22** is a consequence of the sequence rule rather than a change in stereochemistry.

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Table I. Inhibition of Cytochromes P450 Activity by the Stereoisomers of Ketoconazole[a]

cytochrome P-450	stereoisomers			
	5 2S,4S	3 2R,4R	2 2S,4R	4 2R,4S
lanosterol 14α-demethylase	1.60 (0.08)[a]	1.37 (0.19)[b]	0.047 (0.003)[a]	0.119 (0.007)[a]
cholesterol 7α-hydroxylase	0.546 (0.161)[c]	1.25 (0.15)[a]	2.40 (0.32)[a]	0.195 (0.037)[a]
aromatase	3.98 (0.24)[a]	38.3 (2.9)[a]	110.4 (24.1)[b]	39.6 (2.5)[a]
progesterone 17α,20 lyase	0.589 (0.127)[a]	2.04 (0.21)[a]	0.050 (0.002)[a]	2.38 (0.28)[a]
corticoid 11β-hydroxylase	0.247 (0.093)[b]	0.135 (0.051)[b]	0.152 (0.040)[b]	0.608 (0.196)[b]
cholesterol side chain cleavage	2.95 (0.27)[a]	3.92 (0.36)[a]	1.24 (0.04)[a]	5.40 (0.41)[a]
hepatic progesterone				
2α-hydroxylase	34.3 (5.5)[b]	20.4 (2.9)[b]	104.3 (5.0)	84.1 (12.4)[b]
6β-hydroxylase	0.570 (0.16)[c]	0.897 (0.343)[b]	1.26 (0.27)[b]	0.786 (0.240)[a]
15α-hydroxylase	1.08 (0.16)[b]	0.728 (0.175)[a]	0.543 (0.149)[b]	0.369 (0.024)[a]
16α-hydroxylase	37.9 (6.7)[a]	32.3 (2.8)[a]	83.9 (9.0)[a]	69.3 (7.1)[b]
21-hydroxylase	4.46 (0.21)[a]	6.57 (0.48)[a]	9.01 (0.37)[a]	11.2 (0.4)[a]
lauric acid hydroxylase	>100	>100	>100	>100

[a] Data are expressed as IC_{50} values in μM ± standard error. IC_{50} were determined by best fit to the following model: percent control activity = $100(1 - (x/(x + IC_{50})))$ where x = concentration; IC_{50} = concentration that corresponds to 50% inhibition of the range 100% to 0%. a indicates $R > 0.99$. b indicates $R > 0.95$. c indicates $R > 0.93$. Statistical analysis was performed using the PCNONLIN software package.

CYP3A, and 21- for CYP2C6.[38] IC_{50} values are presented in Table I.

Results and Discussion

The cis isomers 2 and 4 are more potent inhibitors of mammalian lanosterol 14α-demethylase than the diastereomeric trans isomers 3 and 5, as might be anticipated from their superior antifungal activity (as the racemic mixture). The cis-(2S,4R) isomer 2 is 3 times more active than its antipode 4. This is in contrast to the activity of the stereoisomers of etaconazole and propiconazole on ergosterol synthesis in the fungus *Ustilago maydis*, where 14α-demethylation is inhibited approximately to the same degree by the 2S,4R and 2S,4S isomers (2S,4R ≅ 2S,4S > 2R,4S ≅ 2R,4R).[31]

The largest difference in activity between the stereoisomers of 1 is found in their effects on progesterone 17α,20-lyase. The cis-(2S,4R) isomer 2 is 40 times more potent in inhibition of this enzyme than its (2R,4S) enantiomer 4. In the corresponding pair of trans isomers, the trans-(2S,4S) isomer 5 is 4 times more potent than its (2R,4R) enantiomer 3.

In contrast, most of the 7α-hydroxylase inhibitory activity observed for ketoconazole resides in the cis-(2R,4S) isomer 4, which is 12 times more active than its antipode. Little selectivity was found between the cis and trans stereoisomers.

All four isomers of ketoconazole are relatively poor inhibitors of human placental aromatase, with the greatest activity residing in the trans-(2S,4S) enantiomer 5.

For adrenal 11β-hydroxylase the cis-(2S,4R) enantiomer 2 was observed to be 7.7 times more effective than the cis-(2R,4S) enantiomer 4, while the trans-(2S,4S) enantiomer 3 was ~2 times as effective as the trans-(2R,4R) enantiomer 5.

The four stereoisomers were relatively unselective for inhibition of the side-chain cleavage of cholesterol. Compounds 2 and 5 were 3 and 2 times, respectively, more active than their enantiomers 4 and 3 in causing 50% inhibition of the enzyme.

The stereoisomers differentially inhibited the rat hepatic P-450s involved in drug metabolism. However, almost no selectivity was observed for the ketoconazole isomers. The IC_{50} values for the cis enantiomers were similar to those previously reported for racemic ketoconazole.[39]

Thus the enzymes with strict substrate requirements (those involved in cholesterol biotransformation and steroid biosynthesis) interacted differentially with the stereoisomers of ketoconazole while the drug metabolizing enzymes, which are known to have broad substrate requirements, did not distinguish between the different stereoisomers. These data suggest that binding to the enzyme apoprotein contributes significantly to the binding energy for the cytochromes P-450 involved in steroid biosynthesis, whereas coordination of the azole to the heme of the drug metabolizing enzymes is the overriding influence on enzyme inhibition.

Conclusion

An efficient synthesis for the preparation of all four stereoisomers of ketoconazole has been developed. Studies with a number of important cytochrome P-450 enzymes have revealed significant selectivity between these isomers. While selectivity in vitro may not necessarily parallel in vivo activity, these results suggest that agents with greater potency and selectivity might become available by resolution of racemic azole compounds which act by inhibition of cytochrome P-450 enzymes.

Ketoconazole has been used for treatment of hormone dependent prostate cancer.[17] Unfortunately, it appears that use of the enantiomerically pure cis-(2S,4R) isomer 2, with its enhanced selectivity for the 17α,20-lyase enzyme and decreased affinity for 7α-hydroxylase would offer no advantage over the racemate due to the undesirable inhibition of corticosteroid synthesis found in the same stereoisomer. Neither would it appear that effects on drug metabolizing enzymes would be attenuated by use of a single isomer.

Experimental Section

Biology. Enzyme Source. Microsomes were prepared by established procedures.[40,41] Microsomes for the lanosterol 14α-

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demethylase and cholesterol 7α-hydroxylase assays were obtained from livers of male rats treated with cholestyramine.[42] Microsomes for the lyase assay were obtained from the testes of perinatal pigs (<2 weeks old), mitochondria for the 11β-hydroxylase assay were obtained from bovine adrenals, hepatic microsomes for the drug metabolizing assay were from the male rat, and microsomes for the aromatase assay were from human placenta. Protein concentration was determined by the method of Lowry.[43]

17α,20-Lyase. Incubations with testes microsomes contained protein (0.025–1 mg), NADPH (1 μmol), $MgCl_2$ (3 μmol), potassium phosphate buffer, pH 7.25 (100 μmol), 17α-hydroxyprogesterone (25 nmol in 20 μL of MeOH), and inhibitors (in 20 μL of MeOH) in a total volume of 1 mL and were agitated at 37 °C for 10 min. All reactions were terminated by addition of CH_2Cl_2 (6 mL) followed immediately by 1 nmol of internal standard (11β-hydroxytestosterone in 50 μL of MeOH). After mixing and centrifugation of the samples, the organic phases were evaporated under a stream of N_2, and the residues were dissolved in 200 μL of MeOH and analyzed by HPLC. Separation of substrate (17α-hydroxyprogesterone), product (androstenedione), and internal standard was achieved with a Jones Chromatography 5-μm, 25-cm, ODS column. The column was eluted with MeOH/MeCN/H_2O under the following conditions: 3 min isocratic at 2/13/85, 7 min linear gradient to 20/30/50, 12 min isocratic at those conditions, 3 min linear gradient to 20/75/5, followed by 5 min isocratic at those conditions. Turnover numbers were calculated by comparing peak height of internal standard to peak height of androstenedione.

Cholesterol 7α-Hydroxylase. Incubations contained hepatic microsomes (2 mg of protein; 0.14 μmol of endogenous cholesterol substrate), NADPH (1 μmol), $MgCl_2$ (3 μmol), EDTA (0.1 μmol) cysteamine hydrochloride (20 μmol), and potassium phosphate buffer, pH 7.4 (100 μmol) in a total volume of 1 mL and were agitated for 10 min at 37 °C following addition of NADPH. Inhibitors (in 20 μL of methanol) were added to the incubation tubes, and the solvent was evaporated prior to addition of the other components. After the 10-min incubation period the NADPH-dependent reactions were stopped by the addition of sodium cholate (5 mg) to solubilize the membranes and products were converted to their respective 4-cholesten-3-ones by the addition of cholesterol oxidase (0.23 unit dissolved in 100 μL of 10 mM potassium phosphate buffer containing 20% glycerol, and 1 mM dithiothreitol) and agitation for 20 min at 37 °C. All reactions were terminated by addition of methanol (1 mL) followed by petroleum ether (5 mL). After mixing and centrifugation of the samples, the organic phases were evaporated under a stream of N_2 and the residues were dissolved in 2-propanol (100 μL) and analyzed by HPLC. HPLC analysis of metabolic reactions was performed on a Varian 5000 liquid chromatograph, equipped with a Wisp 710B autosampler and a Kratos SF 769 UV detector (240 nm). Separation of products was achieved with two consecutive 5-μm, 25-cm silica columns (Dupont Zorbax Sil or Beckman Ultrasphere Sil) preceded by a silica packed 3-cm guard column. The columns were eluted with hexane/2-propanol under the following conditions: 12 min isocratic at 95/5, 5 min with a linear gradient to 70/30, and 23 min isocratic at 70/30. All chromatographic separations were performed at room temperature.

Lanosterol Demethylase. The microsomal incubations contained potassium phosphate buffer (20 μmol, pH 7.0), dithiothreitol (0.3 μmol), EDTA (0.01 μmol), $MgCl_2$ (3 μmol), Tween-20 (0.005%), glycerol (20%), glucose 6-phosphate (5 mM), glucose-6-phosphate dehydrogenase (1 unit), microsomal protein (247 μg), inhibitor (in 20 μL of methanol), and [32,32,32-$^3H_3$]-24,25-dihydrolanosterol (40.8 nmol in 20 μL of ethanol) in a total volume of 1 mL. The mixture was vortexed and maintained at 4 °C until the reaction was initiated with NDAPH (1 μmol). Tubes were incubated for 30 min at 37 °C. The incubations were terminated using 250 μL of a 40% TCA solution.

Bond-Elut extraction columns were activated with MeOH (1 × 1 mL) followed by deionized water (2 × 1 mL). The tritiated water-soluble reaction products were separated from the unreacted [32,32,32-$^3H_3$]-24,25-dihydrolanosterol, on the activated Bond-Elut columns. Duplicate 400-μL aliquots of the eluate were mixed with 10 mL of Beta-Blend liquid scintillation fluid and were analyzed for radioactive content.

Progesterone. The progesterone assay for hepatic drug metabolizing enzymes were conducted as previously reported.[38]

Adrenal 11β-Hydroxylase. Incubations with bovine adrenal mitochondria contained protein (0.05 mg), NADPH (1 μmol), $MgCl_2$ (3 μmol), potassium phosphate buffer, pH 7.4 (100 μmol), deoxycorticosterone (5 nmol in 20 μL of MeOH), and inhibitors (in 20 μL of MeOH) in a total volume of 1 mL and were agitated at 37 °C for 10 min. Mitochrondria were sonicated 5 min on ice before addition to the incubation mixture. All reactions were terminated by addition of CH_2Cl_2 (6 mL) followed immediately by 1 nmol of internal standard (11β-hydroxytestosterone in 50 μL of MeOH). After mixing and centrifugation of the samples, the organic phases were evaporated under a stream of N_2, and the residues were dissolved in 200 μL of MeOH and analyzed by HPLC using the same conditions as for the progesterone assay. Turnover numbers were calculated by comparing peak height of internal standard to peak height of the product, corticosterone.

Aromatase. The microsomal incubations contained potassium phosphate buffer (pH 7.4, 5 μmol), dithiothreitol (0.3 μmol), microsomal protein (0.1 mg), inhibitor (in 20 μL of methanol), [1β,2β-$^3H_2$]androst-4-ene-3,17-dione (androstenedione) (1 nmol in 20 μL of methanol), and NADPH (1 μmol) in a total volume of 1 mL. The incubation mixture was maintained at 4 °C on ice until the reaction was initiated. The tubes were incubated for 10 min in a water bath at 37 °C. The reactions were terminated using 250 μL of a 40% TCA solution.

Bond-Elut extraction columns were activated with MeOH (1 × 1 mL) followed by deionized water (2 × 1 mL). The reaction products, tritiated water and tritiated formate, were separated from the unreacted androstenedione by the activated Bond-Elut columns. Duplicate 400-μL aliquots of the eluate were mixed with 10 mL of Beta-Blend liquid scintillation fluid and were analyzed for radioactive content.

Lauric Acid Hydroxylase. Incubations contained microsomal protein (0.05 mg/mL), potassium phosphate buffer (50 μmol, pH 7.4), $MgCl_2$ (3 μmol), EDTA (0.5 μmol), [^{14}C]lauric acid (10 nmol, 6 mCi/mmol), glucose 6-phosphate (5 μmol), glucose-6-phosphate dehydrogenase (2 units), and NADPH (1 μmol) in a total volume of 1 mL. Reactions were agitated for 10 min at 37 °C and then terminated with CH_2Cl_2 (6 mL). The samples were vortexed and centrifuged, the aqueous layer was discarded, and the organic layer (5 mL) was evaporated to dryness under N_2 at 30 °C. The residue was reconstituted in MeOH (200 μL). The rate of lauric acid hydroxylation was determined by a modification of the method of Romano et al.[44] Separation of lauric acid and its metabolites was accomplished with a Rainin 5-μm, 25-cm Microsorb C_{18} column by gradient elution of 1 mL/min with AcOH (1%) and MeCN. Following the sample injection (50 μL), MeCN was held at 38% for 14 min, then increased linearly to 90% over the next 4 min, and held at 90% for 22 min prior to re-equilibration. Radioactivity was monitored with a Radiomutie A-200 radioflow detector. The recovery of radioactivity from the column was >98%.

Cholesterol Side Chain Cleavage. 26-[^{14}C]Cholesterol (10 μmol) in WR-1339 (1:75 w/w ratio) was incubated with bovine adrenal cortex mitochondria (1 mg), bovine adrenal cytosol (1 mg), bovine serum albumen (0.25 mg), potassium phosphate, pH 7.4, (20 μmol), $CaCl_2$ (0.5 μmol), $MgCl_2$ (4 μmol), EDTA (0.5 μmol), glucose-6-phosphate dehydrogenase (2 units), glucose 6-phosphate (5 μmol), and NADPH (1.0 μmol) in a total volume of 1 mL for 1 h. The reactions are stopped with 2 mL of methanol and applied to 3 mL of C_{18} Bond Elut extraction columns previously activated with MeOH (1 × 3 mL) and water (2 × 3 mL). The unreacted

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26-[^{14}C]cholesterol completely binds to the column at pH 7.4, while the [^{14}C]isocaproic acid formed by side-chain cleavage elutes with the 67% methanolic incubation mixture. One milliliter of the eluent is mixed with 10 mL of scintillation cocktail and the total radioactivity determined. Since side-chain cleavage of cholesterol produces equal amounts of progenolone and isocaproic acid, the rate of formation of [^{14}C]isocaproic acid equals the rate of formation of pregnenolone.

Chemistry. All melting points were measured with a Thomas-Hoover capillary melting point apparatus and are uncorrected. The NMR spectra were recorded in ppm on a Brucker WM300 or AM500 spectrometer in $CDCl_3$ relative to Me_4Si. Optical rotations were run on a Perkin-Elmer 141 polarimeter in $CHCl_3$. Flash chromatography was performed using 60 Å, 230–400-mesh silica gel from E. Merck. All reactions were run under a N_2 atmosphere. Organic solutions described as dried during workup utilized sodium sulfate. The reagents (*R*)- and (*S*)-solketal tosylate and 4-(4-acetylpiperazin-1-yl)phenol are commercially available.

2-Bromo-2′,4′-dichloroacetophenone (7). To a stirred suspension of copper(II) bromide (70.9 g, 317 mmol) in EtOAc (250 mL) at reflux under N_2 was added a solution of 2′,4′-dichloroacetophenone (30 g, 159 mmol) in CH_2Cl_2 (250 mL) dropwise over 1 h. After 16 h the mixture was cooled to room temperature, filtered through a pad of Celite, and evaporated to dryness. The residue was dissolved in EtOAc, washed with saturated $NaHCO_3$ and brine, and then dried (Na_2SO_4). The solution was evaporated to dryness and the product isolated as an oil 7 (86 g, 85% yield). An analytical sample was prepared by flash chromatography on silica gel (10% CH_2Cl_2/hexane): ^{1}H NMR δ 4.50 (s 2 H, CH_2Br), 7.36 (dd, 1 H, J = 8.4, 2.1 Hz, H_5 of Ar), 7.47 (d, 1 H, J = 2.1 Hz, H_3 of Ar), 7.56 (d, 1 H, J = 8.4 Hz, H_6 of Ar). Anal. ($C_8H_5BrCl_2O$) C, H.

(2*S*,4*S*)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-[[[(4-methylphenyl)sulfonyl]oxy]methyl]-1,3-dioxolane (9) and (2*R*,4*S*)-*trans*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-[[[(4-methylphenyl)sulfonyl]oxy]methyl]-1,3-dioxolane (10). A mixture of 2-bromo-2′,4′-dichloroacetophenone (7) (6.0 g, 22.4 mmol) and *p*-TsOH·H_2O (7.6 g, 40 mmol) in toluene (75 mL) was dried by reflux through a Dean-Stark trap for 1.5 h. A solution of (*S*)-solketal tosylate (8) (8.0 g, 28 mmol) in toluene (30 mL) was similarly dried. Both reaction solutions were cooled to room temperature, and *n*-BuOH (10 mL) was added to the (*S*)-solketal tosylate solution. The resulting solutions were combined and heated at reflux through a fresh Dean-Stark trap containing 4-Å molecular sieves. After 5 h, the mixture was cooled to room temperature and Et_3N (11.2 mL, 80.6 mmol) added. The mixture was stirred for 15 min and then evaporated to dryness. The residue was partitioned between saturated aqueous $NaHCO_3$ and EtOAc. The organic layer was dried and evaporated and the residue purified by flash chromatography (0.25% Et_2O in 1:1 CH_2Cl_2/hexane) to give the cis (less polar) diasteromer 9 (4.0 g, 41%) and the trans (more polar) diastereomer 10 (3.8 g, 34%) as oils. 9: ^{1}H NMR δ 2.47 (s, 3 H, $ArCH_3$) 3.78 (dd, 2 H, J = 11.4 Hz, CH_2Br), 3.88 (dd, 1 H, J = 6.7, 8.7 Hz) and 3.98 (dd, 1 H, J = 4.7, 8.7 Hz) (OCH_2), 4.19 (m, 2 H, CH_2OS), 4.32 (m, 1 H, CHO), 7.24 (dd, 1 H, J = 8.4, 2.1 Hz, H_5 of $C_6H_3Cl_2$), 7.37 (d, 2 H, J = 8.3 Hz, H_3, H_5 of C_6H_4), 7.40 (d, 1 H, J = 2.1 Hz, H_3 of $C_6H_3Cl_2$), 7.54 (d, 1 H, J = 8.4 Hz, H_6 of $C_6H_3Cl_2$), 7.83 (d, 2 H, J = 8.4 Hz, H_2, H_6 of C_6H_4); $[\alpha]^{25}_D$ = −20.15° (c = 0.4, $CHCl_3$). Anal. ($C_{18}H_{17}BrCl_2O_5S$) C, H. 10: ^{1}H NMR δ 2.46 (s, 3 H, $ArCH_3$), 3.72 (dd, 1 H, J = 7.0, 8.5 Hz) and 4.36 (dd, 1 H, J = 6.4, 8.5 Hz) (OCH_2), 3.81 (dd, 2 H, J = 11.4 Hz, CH_2Br), 3.97 (m, 3 H, CH_2OS), 4.63 (m, 1 H, CH-O), 7.15 (dd, 1 H, J = 8.5, 2.1 Hz, H_5 of $C_6H_3Cl_2$), 7.33 (d, 2 H, J = 8.4 Hz, H_3, H_5 of C_6H_4), 7.34 (d, 1 H, J = 2.1 Hz, H_3 of $C_6H_3Cl_2$), 7.53 (d, 1 H, J = 8.4 Hz, H_6 of $C_6H_3Cl_2$), 7.68 (d, 2 H, J = 8.4 Hz, H_2, H_6 of C_6H_4); $[\alpha]^{25}_D$ = −2.74° (c = 0.4, $CHCl_3$). Anal. ($C_{18}H_{17}BrCl_2O_5S$) C, H.

(2*R*,4*R*)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-[[[(4-methylphenyl)sulfonyl]oxy]methyl]-1,3-dioxolane (12) and (2*S*,4*R*)-*trans*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-[[[(4-methylphenyl)sulfonyl]oxy]methyl]-1,3-dioxolane (13). The transketalization reaction between ketone 7 and (*R*)-solketal tosylate (11) was run as above. After workup and purification by flash chromatography the cis (less polar) diastereomer 12 (4.8 g, 43%) and the trans (more polar) diastereomer 13 (3.87 g, 38%) were isolated as oils. 12: ^{1}H NMR: see spectral data for 9; $[\alpha]^{25}_D$ = +18.98° (c = 0.4, $CHCl_3$). Anal. ($C_{18}H_{17}BrCl_2O_5S$) C, H. 13: ^{1}H NMR: see spectral data for 10; $[\alpha]^{25}_D$ = +5.0° (c = 0.4, $CHCl_3$). Anal. ($C_{18}H_{17}BrCl_2O_5S$) C, H.

(2*S*,4*S*)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-(acetoxymethyl)-1,3-dioxolane (14). A mixture of ketal tosylate 9 (0.40 g, 0.81 mmol) and CsOAc (0.53 g, 2.74 mmol) in DMF (5 mL) was stirred and heated at 100 °C under N_2. After 1 h the mixture was cooled to room temperature, diluted with water, and extracted with EtOAc. The organic layer was dried, evaporated to dryness, and chromatographed. Gradient elution (with 5–15% EtOAc/hexane) afforded 14 as an oil (0.28 g, 90%): ^{1}H NMR δ 2.10 (s, 3 H, $COCH_3$), 3.87 (dd, 2 H, J = 11.4 Hz, CH_2Br), 3.97 (m, 2 H, OCH_2), 4.28 (m, 3 H, CHO, CH_2OAc), 7.24 (dd, 1 H, J = 8.4, 2.1 Hz, H_5 of $C_6H_3Cl_2$), 7.40 (d, 1 H, J = 2.1 Hz, H_3 of $C_6H_3Cl_2$), 7.59 (d, 1 H, J = 8.4 Hz, H_6 of $C_6H_3Cl_2$). Anal. ($C_{13}H_{13}BrCl_2O_4$) C, H.

Bromoacetates 15–17 were prepared by the same procedure.

(2*R*,4*S*)-*trans*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-(acetoxymethyl)-1,3-dioxolane (15) was prepared from 10 in 80% yield as an oil: ^{1}H NMR δ 1.90 (s, 3 H, $COCH_3$), 3.67 (dd, 1 H, J = 7.8, 8.2 Hz) and 4.35 (dd, 1 H, J = 6.4, 8.2 Hz) (OCH_2), 3.83 (dd, 2 *H*, J = 11.2 Hz, CH_2Br), 4.06 (m, 2 H, CH_2OAc), 4.63 (m, 1 H, CHO), 7.23 (dd, 1 H, J = 8.3, 2.0 Hz, H_5 of $C_6H_3Cl_2$), 7.39 (d, 1 H, J = 2.0 Hz, H_3 of $C_6H_3Cl_2$), 7.63 (d, 1 H, J = 8.3 Hz, H_6 of $C_6H_3Cl_2$). Anal. ($C_{13}H_{13}BrCl_2O_4$) C, H.

(2*R*,4*R*)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-(acetoxymethyl)-1,3-dioxolane (16) was prepared from 12 in 90% yield as an oil: ^{1}H NMR, see spectral data for 14. Anal. ($C_{13}H_{13}BrCl_2O_4$) C, H.

(2*S*,4*R*)-*trans*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-(acetoxymethyl)-1,3-dioxolane (17) was prepared from 13 in 90% yield as an oil: ^{1}H NMR, see spectral data for 15. Anal. ($C_{13}H_{13}BrCl_2O_4$) C, H.

(2*S*,4*R*)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-[[4-(4-acetylpiperazin-1-yl)phenoxy]methyl]-1,3-dioxolane (19). To a suspension of NaH (0.33 g, 60% dispersion in oil, 8.22 mmol) in dry DMSO (15 mL) was added phenol 18 (1.66 g, 7.53 mmol). The mixture was stirred at room temperature for 1 h after which tosylate 14 was added. The reaction mixture was heated at 80 °C for 5 h, cooled to room temperature, and poured into water. The mixture was extracted with EtOAc, and the organic phase was dried and evaporated to dryness. Purification by flash chromatography (gradient elution 20–45% acetone/hexane) afforded 19 (2.9 g, 78%) as an oil: ^{1}H NMR δ 2.14 (s, 3 H, $COCH_3$), 3.05, 3.61, and 3.77 (3 m, 8 H, $C_4H_8N_2$), 3.90 (dd, 2 H, J = 11.3 Hz, CH_2Br), 4.06, 4.17 (2 m, 4 H, OCH_2, $ArOCH_2$), 4.43 (m, 1 H, CHO), 6.89 (s, 4 H, C_6H_4), 7.27 (dd, 1 H, J = 8.3, 2.1 Hz, H_5 of $C_6H_3Cl_2$), 7.42 (d, 1 H, J = 2.1 Hz, H_3 of $C_6H_3Cl_2$), 7.65 (d, 1 H, J = 8.3 Hz, H_6 of $C_6H_3Cl_2$); $[\alpha]^{25}_D$ = −30.17° (c = 0.4, $CHCl_3$). Anal. ($C_{23}H_{25}BrCl_2N_2O_4$) C, H, N. Ethers 20–22 were prepared by the same procedure.

(2*R*,4*R*)-*trans*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-[[4-(4-acetylpiperazin-1-yl)phenoxy]methyl]-1,3-dioxolane (20) was prepared from 15 in 70% yield as an oil: ^{1}H NMR δ 2.13 (s, 3 H, $COCH_3$), 3.03, 3.60, and 3.75 (m, 8 H, $C_4H_8N_2$), 3.84 and 4.43 (2 dd, 2 H, OCH_2), 3.89 (dd, 2 H, J = 11.2 Hz, CH_2Br), 3.92 (m, 2 H, CH_2OAr), 4.76 (m, 1 H, CHO), 6.68 (d, 2 H, J = 9.0 Hz, H_3, H_5 of C_6H_4), 6.84 (d, 2 H, J = 9.0 Hz, H_2, H_6 of C_6H_4), 7.21 (dd, 1 H, J = 8.3, 2.1 Hz, H_5 of $C_6H_3Cl_2$), 7.38 (d, 1 H, J = 2.1 Hz, H_3 of $C_6H_3Cl_2$), 7.67 (d, 1 H, J = 8.3 Hz, H_6 of $C_6H_3Cl_2$); $[\alpha]^{25}_D$ = +2.98° (c = 0.4, $CHCl_3$). Anal. ($C_{23}H_{25}BrCl_2N_2O_4$) C, H, N.

(2*R*,4*S*)-*cis*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-[[4-(4-acetylpiperazin-1-yl)phenoxy]methyl]-1,3-dioxolane (21) was prepared from 16 in 70% yield as an oil: ^{1}H NMR, see spectral data for 19; $[\alpha]^{25}_D$ +25.87° (c = 0.4, $CHCl_3$). Anal. ($C_{23}H_{25}BrCl_2N_2O_4$) C, H, N.

(2*S*,4*S*)-*trans*-2-(Bromomethyl)-2-(2,4-dichlorophenyl)-4-[[4-(4-acetylpiperazin-1-yl)phenoxy]methyl]-1,3-dioxolane (22) was prepared from 17 in 67% yield as an oil: ^{1}H NMR, see spectral data for 20; $[\alpha]^{25}_D$ = −9.40° (c = 0.4, $CHCl_3$). Anal. ($C_{23}H_{25}BrCl_2N_2O_4$) C, H, N.

(2*S*,4*R*)-*cis*-2-(1*H*-Imidazol-1-ylmethyl)-2-(2,4-dichlorophenyl)-4-[[4-(4-acetylpiperazin-1-yl)phenoxy]methyl]-1,3-dioxolane (2). A mixture of bromide 19 (1.0 g, 1.84 mmol), imidazole (0.63 g, 9.19 mmol), and anhydrous K_2CO_3 (1.27 g, 9.19

mmol) in DMA (10 mL) was heated under reflux. After 4 h, the mixture was cooled to room temperature, poured into water, and extracted with CH_2Cl_2 (3 × 50 mL). The organic phase was dried and evaporated and the residue purified by flash chromatography (1–4% MeOH/CH_2Cl, gradient elution) to give a solid which crystallized from acetone–EtOAc: mp 155–157 °C; ^1H NMR δ 2.12 (s, 3 H, $COCH_3$), 3.02, 3.61, and 3.72 (3 m, 8 H, $C_4H_8N_2$), 3.26 (dd, 1 H, J = 6.8, 9.6 Hz) and 3.85 (dd, 1 H, J = 6.5, 8.4 Hz) (OCH_2), 3.72 (m, 2 H, CH_2O Ar), 4.32 (m, 1 H, CHO), 4.43 (dd, 2 H, J = 14.7, CH_2N), 6.74 (d, 2 H, J = 9.1 Hz, H_3, H_5 of C_6H_4), 6.86 (d, 2 H, J = 9.1 Hz, H_2, H_6 of C_6H_4), 6.95, 6.98 (2 s, 2 H, H_4, H_5 of imidazole), 7.24 (dd, 1 H, J = 8.4, 2.0 Hz, H_5 of $C_6H_3Cl_2$), 7.44 (d, 1 H, J = 2.0; H_3 of $C_6H_3Cl_2$), 7.51 (s, 1 H, H_2 of imidazole), 7.56 (d, 1 H, J = 8.4 Hz, H_6 of $C_6H_3Cl_2$); $[\alpha]^{25}_D$ = −10.58° (c = 0.4, $CHCl_3$). Anal. ($C_{26}H_{28}Cl_2N_4O_4$) C, H, N.

Imidazoles **3–5** were prepared by the same procedure.

(2*R*,4*R*)-*trans*-2-(1*H*-Imidazol-1-ylmethyl)-2-(2,4-dichlorophenyl)-4-[[4-(4-acetylpiperazin-1-yl)phenoxy]methyl]-1,3-dioxolane (3) was prepared from **20** in 54% yield as an oil: ^1H NMR δ 2.13 (s, 3 H, $COCH_3$), 3.02, 3.60, and 3.75 (3 m, 8 H, $C_4H_8N_2$), 3.80 (m, 4 H, CH_2O, CH_2OAr), 4.15 (m, 1 H, CHO), 4.42 (dd, 2 H, J = 14.6 Hz, CH_2N), 6.64 (d, 2 H, J = 9.1 Hz, H_3, H_5 of C_6H_4), 6.82 (d, 2 H, J = 9.1 Hz, H_2, H_6 of C_6H_4), 6.99, 7.01 (2 s, 2 H, H_4, H_5 of imidazole), 7.18 (dd, 1 H, J = 8.4, 2.1 Hz, H_5 of $C_6H_3Cl_2$), 7.42 (d, 1 H, J = 2.1 Hz, H_3 of $C_6H_3Cl_2$), 7.52 (s, 1 H, H_2 of imidazole), 7.59 (d, 1 H, J = 8.4 Hz, H_6 of $C_6H_3Cl_2$); $[\alpha]^{25}_D$ = +17.66° (c = 0.4, $CHCl_3$). Anal. ($C_{26}H_{28}Cl_2N_4O_4$) C, H, N.

(2*R*,4*S*)-*cis*-2-(1*H*-Imidazol-1-ylmethyl)-2-(2,4-dichlorophenyl)-4-[[4-(4-acetylpiperazin-1-yl)phenoxy]methyl]-1,3-dioxolane (4) was prepared from **21** in 53% yield: mp 154–156 °C (acetone/EtOAc); ^1H NMR, see spectral data for **2**; $[\alpha]^{25}_D$ = +8.22° (c = 0.4, $CHCl_3$). Anal. ($C_{26}H_{28}Cl_2N_4O_4$) C, H, N.

(2*S*,4*S*)-*trans*-2-(1*H*-Imidazol-1-ylmethyl)-2-(2,4-dichlorophenyl)-4-[[4-(4-acetylpiperazin-1-yl)phenoxy]methyl]-1,3-dioxolane (5) was prepared from **22** in 38% yield as an oil: ^1H NMR, see spectral data for **3**; $[\alpha]^{25}_D$ = −19.97° (c = 0.4, $CHCl_3$). Anal. ($C_{26}H_{28}Cl_2N_4O_4$) C, H, N.

(2*R*,4*S*)-*cis*-2-(Hydroxymethyl)-2-(2,4-dichlorophenyl)-4-[[4-(4-acetylpiperazin-1-yl)phenoxy]methyl]-1,3-dioxolane (23): mp 145–151 °C; ^1H NMR δ 2.13 (s, 3 H, $COCH_3$), 3.04, 3.60, and 3.76 (3 m, 8 H, $C_4H_8N_2$), 3.96 (m, 2 H, CH_2OH), 3.98 (dd, 1 H, J = 7.2, 8.3 Hz) and 4.18 (dd, 1 H, J = 4.8, 8.3 Hz) (OCH_2), 4.11 (m, 2 H, $ArOCH_2$), 4.43 (m, 1 H, CHO), 6.88 (s, 4 H, C_6H_4), 7.25 (dd, 1 H, J = 2.1, 8.6 Hz, H_5 of $C_6H_3Cl_2$), 7.41 (d, 1 H, J = 2.1 Hz, H_3 of $C_6H_3Cl_2$), 7.63 (d, 1 H, J = 8.6 Hz, H_6 of $C_6H_3Cl_2$). Anal. ($C_{23}H_{26}Cl_2N_2O_5 \cdot 0.25\ H_2O$) C, H, N.

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