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The Synthesis and Antifungal Activity of the Enantiomers of Butoconazole Nitrate[1]

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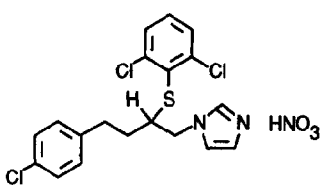
Abstract: The *S* and *R* enantiomers **2** and **3** of the antifungal agent butoconazole nitrate have been prepared in optically pure form, in three steps, from *R*- and *S*-glycidyl tosylates **4** and **5** respectively. No significant difference was found in the *in vitro* activity of butoconazole and its enantiomers *versus Candida albicans.*

Butoconazole nitrate (**1**, Femstat®) is a newer antifungal agent of the imidazole class,[2,3] developed for the treatment of vaginal candidiasis. The mechanism of action is attributed to inhibition of the cytochrome P-450 lanosterol 14α-demethylase,[4] resulting in interruption of cellular ergosterol synthesis and disruption of the cell membrane. Although most azole antifungal agents, including butoconazole, are tested or marketed as racemates,[5] recent work has shown that the stereoisomers of certain antifungal agents exhibit different antifungal activities.[6,7]

For example, although triadimefon shows no significant difference in activity between the *R* and *S* enantiomers,[8] reduction to triadimenol gives four stereoisomers of differing antifungal activity, paralleling the effect on purified cytochrome $P\text{-}450_{14DM}$ from yeast.[9] Similar separation of activity is observed for the stereoisomers of the related diclobutrazole[10] and paclobutrazole,[11] and for the diastereomeric pairs of racemates of the fungicides propiconazole and etaconazole.[12] The superiority of the *R*- or (-)-isomer of several hydroxyphenethyl azole antifungal agents has been demonstrated.[13,14] This same separation of activity is noted in a related acylamino hydroxyphenethyl triazole[15] and the derived oxazolidine,[16] where the active *RR*-isomer is that which most closely mimics the natural stereochemistry of the lanosterol ring system. A different type



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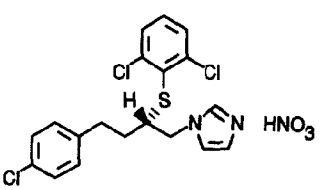

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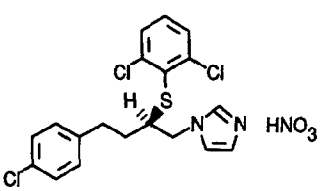

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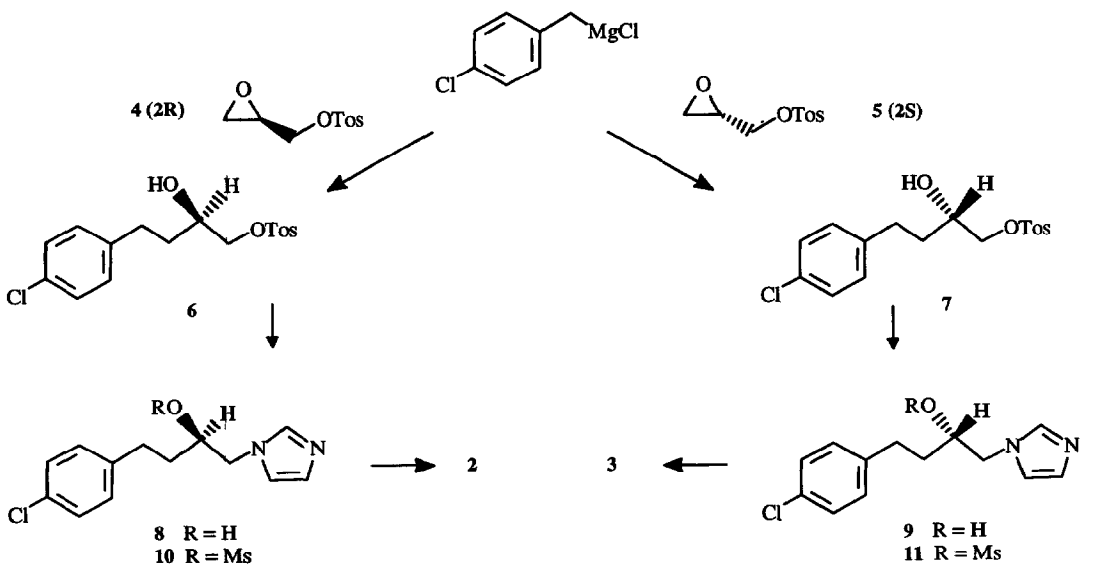
of structure is represented by vibunazole and HWG-1608 which have a four atom bridge between the aromatic ring and the azole, as is found in butoconazole **1**. In the former cases, the *S*-(-) isomer is more active.[17,18] In view of the structural similarity to vibunazole, and the findings reported for other antifungals above, we became interested in determining the relative antifungal activity of the enantiomers of butoconazole.

A method has been developed for preparation of the optically pure enantiomers **2** and **3** of butoconazole from the commercially available *R*- and *S*-glycidyl tosylates **4** and **5**.

The reaction of nonracemic glycidyl tosylates with nucleophiles can lead to loss of optical purity, since products resulting from direct nucleophilic displacement of tosylate have the opposite stereochemistry to those produced by initial epoxide opening followed by internal tosylate displacement. This problem is circumvented if the product of epoxide opening is an alcohol, since direct displacement of tosylate gives an epoxide, which can be separated. Sharpless[19] has used the copper-catalyzed reaction of non-racemic glycidol tosylates with Grignard reagents to give diol monotosylates, thus ensuring that optical purity is maintained. We have utilized the Sharpless procedure to access suitable enantiopure intermediates for preparation of the enantiomers of butoconazole (Scheme).

Separate treatment of (*R*)-(-)- and (*S*)-(+)-glycidyl tosylates with chlorobenzylmagnesium chloride containing a catalytic amount of dilithium tetrachlorocuprate in THF at -35°C for 2.5h afforded the respective 2*R*- and 2*S*- hydroxytosylates **6** and **7** in 68% and 77% yields respectively.



Scheme

Addition of the sodium salt of imidazole to tosylates **6** and **7** in DMF at 75°C for 16h then gave 2*R*- and 2*S*- imidazole alcohols **8** and **9** in 65% and 67% recrystallized yields respectively, in ≥99.5% optical purity.[20] Except for their optical properties, imidazole alcohols **8** and **9** proved identical to authentic racemic compound prepared by a different route.[2] Alcohols **8** and **9** were converted to the corresponding mesylates **10** and **11** by treatment with methanesulfonyl chloride and TEA in THF at 0°C for 1h. Reaction with 2,6-dichlorobenzenethiol and K_2CO_3 in acetone at reflux for 16h then gave the desired butoconazole enantiomers **2** and **3** in 55% and 68% yields respectively (from alcohols **8** and **9**, and in 24% and 35% overall yields from tosylates **4** and **5**). After a single recrystallization, isomers **2** and **3** were analyzed by chiral HPLC[21] and found to be greater than 99.7% optically pure. Thus formation and displacement of the mesylates occurred with full retention of optical purity.

The antifungal activity of the two enantiomers of butoconazole **2** and **3** was determined *in vitro* against *Candida albicans* relative to racemic butoconazole **1** itself, using a broth microdilution assay. No significant differences were found (MIC's: **2**, 6 μg/mL; **3**, 5 μg/mL; **1**, 6 μg/mL).

In conclusion, an efficient straightforward synthesis has been developed providing both enantiomers of butoconazole in good chemical yield and with high optical purity. Butoconazole and its enantiomers had similar inhibitory activities against *Candida albicans* 523 *in vitro*.

Experimental Section

Biology. Test compounds were dissolved in 100% DMSO and diluted in Emmons broth (Difco®) just prior to use. Each compound was tested using 2-fold dilutions ranging from 0.2-200 μg/mL. The final DMSO concentrations ranged from 0.005-5%. A clinical isolate of *Candida albicans* (*Ca* 523) was maintained at -70° until use. The organism was grown overnight on Emmons agar slants, washed off and diluted in Emmons broth, and added to each drug mixture at a final concentration of 7 x 10^4 cells/mL. Two-fold serial dilutions of racemic butoconazole and its enantiomers in Emmons broth were made in 96-well, U-bottom microtiter plates. *C. albicans* was added to one set of these dilutions. A second set was not inoculated so that any drug precipitate could be distinguished from the *C. albicans* pellet. Other controls included a DMSO solvent control which consisted of 2-fold serial dilutions of the DMSO solvent minus test compound in Emmons broth as well as a *C. albicans* growth control consisting of Emmons broth minus both test compound and DMSO.

After inoculation of the appropriate wells, all plates were incubated for 24 h at 35°C. The minimum inhibitory concentration (MIC) was then recorded by visual inspection for each drug alone and in combination. The MIC was defined as the lowest drug concentration without evidence of *C. albicans* growth. The assay was run in triplicate.

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

relative to Me_4Si. Optical rotations were run on a Perkin-Elmer 141 polarimeter. Flash chromatography was performed using 60 Å, 230-400 mesh silica gel from E. Merck. *R*-(-)-glycidyl tosylate, $[\alpha]_D^{19}$ -17 (c 2.7, $CHCl_3$), corresponding to 91% ee, and *S*-(+)-glycidyl tosylate, $[\alpha]_D^{19}$ +17 (c 2.7, $CHCl_3$), corresponding to 91% ee, were obtained from Aldrich.

(2*R*)-1-(*p*-Toluenesulphonyloxy-4-(4-chlorophenyl)butan-2-ol (6). To a solution of Li_2CuCl_4 (0.10 M, 8.8 mL, 0.88 mmol) in dry THF (75 mL) was added dropwise a solution of 4-chlorobenzylmagnesium chloride (17.5 mmol) in Et_2O (15mL) at -35°C. After the reaction mixture was stirred for 45 min, a pre-cooled (-35°C) solution of (*R*)-(-)-glycidyl tosylate **4** (2.0 g, 8.8 mmol) in THF (5 mL) was added via syringe. After 2 h at -35°C, the mixture was quenched with saturated NH_4Cl and extracted with Et_2O. The organic layer was dried (Na_2SO_4), evaporated to dryness and purified by flash chromatography (5% Et_2O in 1:1 CH_2Cl_2/hexane) to afford **6** as a white solid (2.1 g, 68%), m.p. 73.6-75.7°C; $[\alpha]_D^{25}$+0.78 (c 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.71 (m, 2H, CH_2CHOH), 2.45 (s, 3H, CH_3), 2.69 (m, 2H, CH_2Ar), 3.81 (m, 1H, C*H*OH), 3.96 (ddd, 2H, *J* = 10.0, 6.8, 3.0 Hz, CH_2OSO_2), 7.08 (d, 2H, *J* = 8.4 Hz, 2-H,6-H of C_6H_4Cl), 7.23 (d, 2H, *J* = 8.4 Hz, 3-H,5-H of C_6H_4Cl), 7.35 (d, 2H, *J* = 8.3 Hz, 3-H,5-H of C_6H_4Me), 7.78 (d, 2H, *J* = 8.3 Hz, 2-H,6-H of C_6H_4Me). Calcd for $C_{17}H_{19}ClO_4S$: C, 57.54; H, 5.39. Found: C, 57.46; H, 5.42.

(2*S*)-1-(*p*-Toluenesulphonyloxy-4-(4-chlorophenyl)butan-2-ol (7). Prepared as above except for the use of (*S*)-(+)-glycidyl tosylate **5**. After workup and purification by flash chromatography, tosylate **7** was isolated as a white solid (1.9 g, 77%), m.p. 72.7-74.2°C; $[\alpha]_D^{25}$ 0 (c 0.4, $CHCl_3$); ^1H NMR: see spectral data for **6**. Calcd for $C_{17}H_{19}ClO_4S$: C, 57.54; H, 5.39. Found: C, 57.32; H, 5.42.

(2*R*)-1-[2-hydroxy-4-(4-chlorophenyl)butyl]-1*H*-imidazole (8). To a solution of imidazole (0.52 g, 7.61 mmol) in dry DMF (5 mL) at 0°C under N_2 was added NaH (60% dispersion in oil , 0.3 g, 7.61 mmol). The reaction mixture was warmed to room temperature and stirred for 30 min. A solution of **6** in DMF (15 mL) was then added dropwise over 15 min. The reaction mixture was heated at 75°C for 24 h, cooled, poured into water and extracted with EtOAc. The organic phase was dried and evaporated, and the residue purified by flash chromatography. Gradient elution (1-5% MeOH/CH_2Cl_2) afforded **8** as a solid which was recrystallized from EtOH/Et_2O (830 mg, 65%), m.p. 131-133°C; $[\alpha]_D^{25}$+23.63 (c 0.4, $CHCl_3$); ^1H NMR (d_6DMSO) δ 1.53 (m, 2H, CH_2CHOH), 2.66 (m, 2H, CH_2Ar), 3.65 (m, 1H, C*H*OH), 3.93 (ddd, 2H, *J* = 13.9, 6.9, 4.0 Hz, CH_2N), 5.11 (d, 1H, *J* = 5.6 Hz, OH), 6.85 (s, 1H, 4-H of imidazole), 7.11 (s, 1H, 5-H of imidazole), 7.20 (d, 2H, *J* = 8.4 Hz, 2-H,6-H of C_6H_4Cl), 7.31 (d, 2H, *J* = 8.4 Hz, 3-H,5-H of C_6H_4Cl), 7.56 (s, 1H, 2-H of imidazole). Calcd for $C_{13}H_{15}N_2O$: C, 62.27; H, 6.03; N, 11.18. Found: C, 62.60; H, 6.08; N, 11.44.

(2*S*)-1-[2-Hydroxy-4-(4-chlorophenyl)butyl]-1*H*-imidazole (9) was prepared from tosylate **7** as above. After

workup and purification by flash chromatography, imidazole **9** was isolated as a white solid which was recrystallized from EtOAc/Et_2O (236 mg, 67%), m.p. 128-131°C; $[\alpha]_D^{25}$-23.23 (c 0.4, $CHCl_3$); ^1H NMR: see data for **8**. Calcd for $C_{13}H_{15}N_2O{\cdot}1/8H_2O$: C, 61.72; H, 6.08; N, 11.07. Found: C, 61.94; H, 6.08; N, 10.67.

(2*S*)-1-[2-(2,6-Dichlorophenylthio)-4-(4-chlorophenyl)butyl]-1*H*-imidazole nitrate (2). To a solution of alcohol **8** (250 mg, 1 mmol) in THF (5 mL) at 0°C was added triethylamine (0.28 mL, 2.0 mmol), followed by methanesulfonyl chloride (0.15 mL, 2.0 mmol). The reaction mixture was warmed to room temperature and stirred for 1h. The mixture was poured into aq. $NaHCO_3$, extracted with EtOAc, and the organic phase dried and evaporated to dryness. The resulting crude mesylate was dissolved in acetone (50 mL), then 2,6-dichlorobenzenethiol (464 mg, 2.6 mmol) and K_2CO_3 (568 mg, 4.1 mmol) were added. The mixture was heated at reflux under N_2 for 16h, cooled to room temperature, evaporated to dryness and partitioned between water and EtOAc. The organic phase was dried (Na_2SO_4), evaporated, and the residue purified by flash chromatography (1-2% MeOH/CH_2Cl_2 gradient elution) to give an oil which was converted to the nitrate salt. Recrystallization from EtOAc/Et_2O gave **2** (260 mg, 55%), m.p. 120-124°C; $[\alpha]_D^{25}$+22.68 (c 0.4, EtOH); ^1H NMR (d_6DMSO) δ 1.87 (m, 2H, CH_2CHOH), 2.81 (t, 2H, J = 8.0 Hz, CH_2Ar), 3.73 (m, 1H, CHS), 4.52 (ddd, 2H, J = 14.2, 8.7, 5.4 Hz, CH_2N), 7.17 (d, 2H, J = 8.4 Hz, 2-H,6-H of C_6H_4Cl), 7.31 (d, 2H, J = 8.4 Hz, 3-H,5-H of C_6H_4Cl), 7.41 (AB_2, 1H, J = 8.1 Hz, 4-H of ArS), 7.55 (d, 2H, J = 8.1 Hz, 3H,5-H of ArS), 7.61 (s, 1H, 4-H of imidazole), 7.77 (s, 1H, 5-H of imidazole), 9.18 (s, 1H, 2-H of imidazole). Calcd for $C_{19}H_{18}Cl_3N_3O_3S$: C, 48.07; H, 3.82; N, 8.85. Found: C, 48.32; H, 3.91; N, 8.91.

(2*R*)-1-[2-(2,6-Dichlorophenylthio)-4-(4-chlorophenyl)butyl]-1*H*-imidazole nitrate (3) was prepared from alcohol **9** as above. The nitrate salt was recrystallized from MeOH/EtOAc to give **3** (220 mg, 68%), m.p. 123-125°C; $[\alpha]_D^{25}$-23.26 (c 0.4, EtOH); ^1H NMR: see data for **2**. Calcd for $C_{19}H_{18}Cl_3N_3O_3S$: C, 48.07; H, 3.82; N, 8.85. Found: C, 48.29; H, 3.86; N, 8.82.

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20. The optical purity of **8** and **9** was determined by HPLC on a Chiracel OD column (250 x 4.6 mm i.d.) eluting with 5:1 hexane/ethanol. We were unable to obtain an adequate separation of isomers **6** and **7** under a variety of conditions.
21. The optical purity was determined by HPLC on a Chiracel OD column (250 x 4.6 mm i.d.) eluting with 70:30 hexane/ethanol.