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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF A NOVEL CLASS OF AZOLE-CONTAINING ANTIFUNGAL AGENTS

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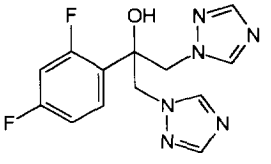
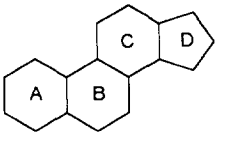
Abstract: A series of novel 1,2,3,4-tetrahydroisoquinoline derived azoles has been designed and synthesized as antifungal agents which might function as inhibitors of cytochrome P-450 dependent lanosterol 14α-demethylase. *In vitro* tests showed that some of these compounds, especially **5b** and **6b**, effectively inhibit the growth of several strains of yeasts as well as molds.

It has been observed that the primary and opportunistic fungal infections have increased.[2] There is an evident need for accelerated development of new and more effective as well as less toxic antifungal agents, especially for treating systemic infections.[2] Although amphotericin B, a polyene macrolide, remains the most useful of the systemic antifungal drugs despite its toxicity, recent works concentrate on the development of triazoles as antifungals.[2] The triazoles affect fungal membrane functions by inhibiting the action of lanosterol 14α-demethylase, a cytochrome P-450 enzyme. Fluconazole is the currently leading triazole drug and has a high degree of specificity for the fungal enzyme. However, the increasing cases of resistance to fluconazole made it necessary to develop new generation of antifungal agents.[2,3]

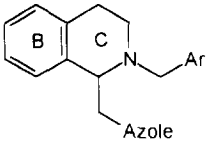
In an effort to find new antifungals, we have designed and synthesized a novel class of azoles such as compound **I** as potential antifungal agents. These compounds are substituted 1,2,3,4-tetrahydroisoquinolines with various N-benzyl groups and azoles, and have a more constrained conformation than that of fluconazole.[3] This approach is based on a hypothesis that the 1,2,3,4-tetrahydroisoquinoline moiety in compound **I** might be a mimetic of the B/C rings of the sterol skeleton. In addition, the two nitrogen atoms, one in the tetrahydroisoquinoline and the other in the azole ring, could complex to the heme iron of fungal cytochrome P-450, and therefore, might inactivate fungal lanosterol 14α-demethylase.



Fluconazole



sterol skelton



I

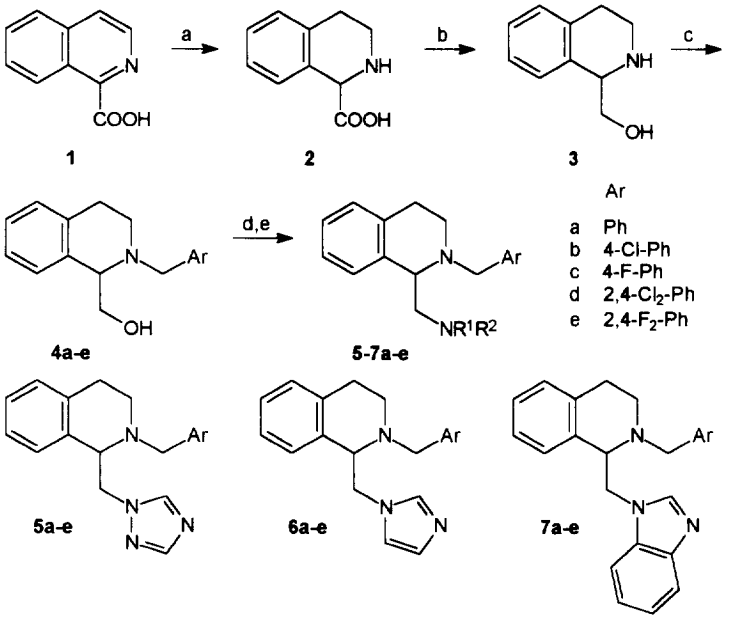
Two series of azole-containing 1,2,3,4-tetrahydroisoquinoline isomers **5** and **13** were synthesized in order to evaluate the structure-activity relationships. These compounds were prepared from α-amino acids **2** and **8** independently via several steps.[4,5] The α-amino acid **2** or **9** was first reduced with lithium aluminum hydride to the corresponding β-amino alcohol **3** or **11**. After N-alkylation with various benzyl bromide or chloride in DMF, the hydroxy group in compound **4** or **12** was then converted to a triazole group in DMF (Schemes 1 and 2).[6] In the later studies, the hydroxy group of compound **4** (the more promising precursor, see below) was also transformed to an imidazole group (compounds **6a-e**) or a benzimidazole group (compounds **7a-e**) as shown in the Scheme 1.

Compounds **5-7** and **13** were evaluated for *in vitro* antifungal activities. The preliminary screening was conducted at a drug concentration of 50 μg/ml.[7] If promising, we then determined its MIC values. The results showed that triazoles **5a-e** and imidazoles **6a-e** effectively inhibited growth of yeasts and molds. Triazoles **13a-e** were less effective than their regioisomers **5a-e**. The benzimidazoles **7a-e** were also less potent than the imidazoles **6a-e**. The *in vitro* values of minimum inhibitory concentration (MIC) for compounds **5** and **6** against selected fungi were shown in the table.[7-9] These results demonstrated that the position and the type of the azole group is crucial for antifungal activity. In contrast to compound **13**, it is evident the triazole group of the compound **5** is in a better arrangement and might effectively inhibit the active site of the fungal enzyme lanosterol 14α-demethylase. On the other hand, imidazoles **6a-e** are more potent than the triazole analogues **5a-e**. In general, the fluoro or chloro substituted N-benzyl groups in compounds **5** and **6** evidently enhance their potency against fungi (e.g., see **5a** and **5b**). It is noteworthy that the chloro-substituted triazole **5b** and imidazoles **6b** as well as **6d** showed strong inhibition comparable to that of fluconzole for yeasts *Cryptococcus neoformans, Candida kefyr*, and molds *Trichophyton mentagrophytes, Aspergillus flavus, Aspergillus fumigatus*. However, these agents are not able to inhibit the growth of *Candida albicans* at a concentration lower than 50 μg/ml.

Table The *in vitro* Susceptibility of Compounds **5** and **6** against Six Selected Fungal Strains[7-9]

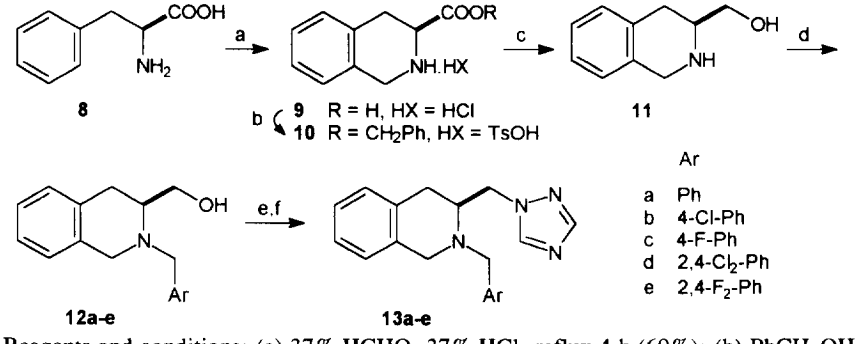
	Minimum Inhibitory Concentration (MIC), μg/ml					
	yeasts			molds		
	Candida albicans	*Candida kefyr*	*Cryptococcus neoformans*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Trichophyton mentagrophytes*
Fluconazole	>50	0.39	3.12	>50	>50	>50
Miconazole	12.50	0.09	0.19	0.39	0.39	0.39
Amp B	0.19	0.19	0.19	12.50	25.00	1.56
5a	>50	>50	>50	>50	>50	4.00
5b	>50	0.39	6.25	25.00	4.00	0.25
5c	>50	>50	>50	>50	>50	1.00
5d	>50	>50	12.50	>50	>50	4.00
5e	>50	0.39	>50	>50	>50	2.00
6a	>50	>50	>50	25.00	25.00	0.19
6b	>50	0.10	0.39	0.19	3.12	0.19
6c	>50	>50	>50	6.25	12.50	0.10
6d	>50	0.19	1.56	0.78	0.39	6.25
6e	>50	>50	>50	0.78	6.25	0.39

Scheme 1



Reagents and conditions: (a) PtO_2, AcOH, H_2, 60 psi, rt, 24 h (80%); (b) $LiAlH_4$, THF, 0 °C, 1h, then 60 °C 16 h (50%); (c) $ArCH_2Br$ or $ArCH_2Cl$, Na_2CO_3, DMF, rt, 2-4 h (55-75%); (d) CH_3SO_2Cl, $N(C_2H_5)_3$, CH_2Cl_2, 0 °C, 2 h (100%); (e) 1,2,4-triazole, (or imidazole, benzimidazole), Na_2CO_3, DMF, 50 °C, 16 h (45-55%).

Scheme 2



Reagents and conditions: (a) 37% HCHO, 37% HCl, reflux 4 h (60%); (b) $PhCH_2OH$, *p*-TsOH, C_6H_6, 80 °C, 16 h (90%); (c) $LiAlH_4$, THF, 0 °C, 1h, then 60 °C 16 h (60%); (d) $ArCH_2Br$ or $ArCH_2Cl$, Na_2CO_3, DMF, rt, 2-4 h (50-75%); (e) CH_3SO_2Cl, $N(C_2H_5)_3$, CH_2Cl_2, 0 °C, 2 h (100%); (f) 1,2,4-triazole, Na_2CO_3, DMF, 50 °C, 16 h (35-55%).

In summary, we have designed and synthesized a series of novel 1,2,3,4-tetrahydro-isoquinoline derived azoles **5** and **6** as antifungal agents which might function as inhibitors of cytochrome P-450 dependent lanosterol 14α-demethylase. *In vitro* tests demonstrated that these

conformationally constrained compounds, especially **5b** and **6b**, effectively inhibit the growth of several yeasts as well as molds.

References and Notes

(1) (a) Division of Natural Product and Medicinal Chemistry (b) Division of Drug Development

(2) (a) Richardson, K.; Marriott, M. In *Annual Reports in Medicinal Chemistry*; Bailey, D. M., Ed.; Academic Press: N.Y., 1987; Vol. 22, Chapter 16, pp 159-167. (b) Koltin, Y. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: N.Y., 1989; Vol. 25, Chapter 15, pp 141-148. (c) Barrett, J. F.; Klaubert, D. H. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: N.Y., 1992; Vol. 27, Chapter 15, pp 149-158. (d) Scrip's Antifungal Report: A Review of Progress in the Development of New Antifungals, PJB Publications: Surrey, UK, 1992.

(3) Some of the conformationally constrained antifungal agents besides itraconazole and ketoconazole have been recently reported: (a) Konosu, T.; Tajima, Y.; Takeda, N.; Miyaoka, T.; Kasahara, M.; Yasuda, H.; Oida, S. *Chem. Pharm. Bull.* **1990**, *38*, 2476. (b) Tsukuda, T.; Watanabe, M.; Ontsuka, H.; Fujimoto, Y.; Shimma, N. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 733. (c) Bartroli, J.; Turmo, E.; Alguero, M.; Boncompte, E.; Vericat, M.; Garcia-Rafanell, J.; Forn, J. *J. Med. Chem.* **1995**, *38*, 3918 and references cited therein. (d) Fromtling, R. A.; Castaner, J. *Drugs Future* **1995**, *20*, 241.

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(6) A typical procedure for the preparation of 2-(4-Chlorophenyl)methyl-1-(1*H*-1,2,4-triazole-1-yl)methyl-1,2,3,4-tetrahydroisoquinoline (**5b**) is described herein. To a CH_2Cl_2 solution of compound **4** and triehylamine (2 equiv) in an ice bath was added methanesulfonyl chloride (1.2 equiv). After stirring at 0 °C for 30 min and rt for 1 h, the mixture was concentrated, filtered through a short pad of silica gel, and eluted with EtOAc. The organic solution was concentrated to dryness. To this pale yellow mass was added 2 equiv of Na_2CO_3 and 1.5 equiv of the triazole and aliquot amount of DMF. The suspension was heated at 50-60 °C overnight. After the reaction was done, the DMF suspension poured into water and extracted with EtOAc. The organic layer was then dried over $MgSO_4$ and the solvent was removed. The crude product was purified by flash chromatography to provide the title compound (45-55% yield) as a viscous liquid, very slowly solidified on standing, mp 97-98 °C: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.98 (s, 1 H), 7.89 (s, 1 H), 7.37-6.90 (m, 8 H), 4.37-4.32 (m, 2 H), 4.03 (dd, J = 8.2, 5.4, Hz, 1 H), 3.71 (d, J = 13.3 Hz, 1 H), 3.58 (d, J = 13.3 Hz, 1 H), 3.40-2.40 (m, 4 H); MS(CI, CH_4) 339/341 (MH^+). Anal. Calcd for $C_{19}H_{19}N_4Cl$: C 67.35; H 5.65; N 16.53. Found: C 67.73; H 5.75; N 16.42.

(7) Preliminary antifungal screenings were performed against six strains of yeast species and four strains of filamentous fungi by using agar dilution method.[9] Susceptibility testings were conducted with standard microdilution and macrodilution methods.[9] The medium used was RPMI 1640 (pH 7.0). The incubation time and temperature were 48 hr and 37 °C separately. The MIC was defined as the lowest concentration of the drug that inhibited multiplication of the yeast and mycelial growth of the mold.

(8) The MIC tests of compounds **7** and **13** were not detected because of their weak activity at a concentration of 50 μg/ml.

(9) Shadomy, S.; Pfaller, M. In *Manual of Clinical Microbiology*, 5th ed.; Balows, A.; Hausler, W.; Herrmann, K.; Isenberg, H. D.; Shadomy, H. J., Ed.; American Society for Microbiology: Washington, D. C., 1991; Chapter 117, pp 1173-1181.

(10) We thank Ms. Ying Chen for operating NMR spectrometers.

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