Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 14, pp. 1479-1482, 1995

0960-894X/95 $9.50+0.00

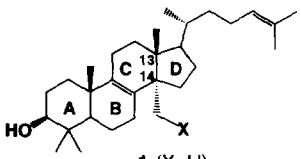
0960-894X(95)00241-3

STRUCTURE-ACTIVITY RELATIONSHIPS OF SULFUR-CONTAINING TRIAZOLE ANTIFUNGALS

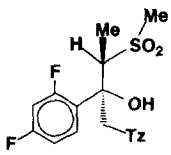
Hiroshi MIYAUCHI*, Koichi KOZUKI, Tomoharu TANIO, and Naohito OHASHI
Research Center, Sumitomo Pharmaceuticals Co., Ltd.,
1-98, Kasugadenaka 3-chome, Konohana-ku, Osaka 554, Japan

Abstract: Alkylthio and alkylsulfonyl derivatives of antifungal SM-8668 (**3**) were synthesized and estimated for their activities *in vitro* and *in vivo*. Derivatives having pentylthio, heptylthio or nonylthio group showed potent activities against both candidiasis and aspergillosis. The introduction of hydroxyl group at the end of their alkyl chain made their activities stronger.

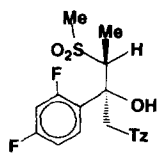
Imidazole and triazole antifungals are known as potent inhibitors of the cytochrome P_{450} monooxygenase in the process of fungal biosynthesis of ergosterol,[1,2] which is an important constituent of fungal cell membrane. This enzyme oxidatively removes 14-α-methyl group of lanosterol (**1**) by using O_2 and NADPH.[3] Inhibition of this enzyme causes the accumulation of precursor 14-α-methyl sterols (lanosterol and its biosynthetic derivatives), which leads to the destruction of cell membrane integrity.[4] Imidazole and triazole antifungals are believed to inhibit this enzyme by binding of heterocyclic nitrogen atom (at 3-position of imidazole or at 4-position of triazole) to the protoheme iron atom and exclude oxygen atom which would normally take part in the reaction.[2,5] Since the target of the enzyme is 14-α-methyl group of lanosterol, a logical inhibitor could be a lanosterol derivative with a heme binding component at the 14-α-methyl position. Indeed, such a derivative **2** was reported to be an inhibitor of fungal ergosterol biosynthesis and was active *in vitro* against *Candida* and dermatophyte strains.[6]



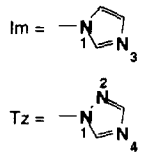
1 (X=H)
2 (X=Im)



3



4



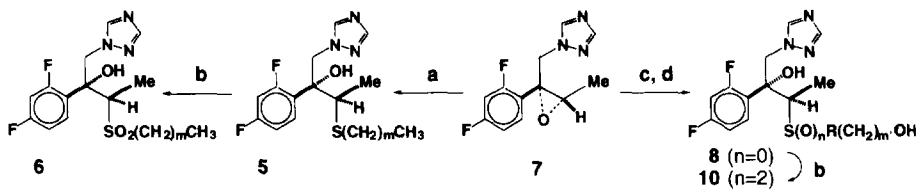
On the other hand, our research group previously reported that SM-8668 (**3**)[7,8] was demonstrated to have higher potency against a wide range of mycoses in animal experiments than fluconazole.[9] Interestingly, its *erythro*-isomer **4** showed much lower activity than **3** (*dl-threo*-isomer) both *in vitro* and *in vivo*.[7] Moreover, the potent antifungal activity of **3** was only depended on (2*R*,3*R*)-isomer and was scarcely depended on (2*S*,3*S*)-isomer, as we recently reported.[10] It seems that (2*R*,3*R*)-**3** has a similar structure to

lanosterol as well as **2**, since the aromatic ring of **3** can be regarded as the B ring of **1**, and methyl group at 4-position and methylene group at 1-position of **3** can also be regarded as 13-β-methyl group and 14-α-methyl group of **1**, respectively. On the basis of these results, we considered (2*R*,3*R*)-**3** as a logical inhibitor of the cytochrome P_{450} monooxygenase. As part of our search for active antifungal agents, we synthesized a new series of analogs of **3** having a long alkyl group on sulfur atom. Such a kind of substituents should be reasonable because they are regarded as the side chain of **1**.

Synthesis

Corresponding long alkyl derivatives **5a-j** and **6b-j** were synthesized as shown below, in order to investigate the structure-activity relationships on the length of R. Sulfides **5a-j** were obtained by reaction of epoxide **7**[8] with sodium alkylthiolate in dimethylformamide. Sulfides **5a-j** were respectively oxidized under acidic conditions with hydrogen peroxide in presence of catalytic amount of sodium tungstate to give sulfones **6b-j** in high yields.[8]

In order to increase the hydrophilicity of **5d-h**, we also synthesized derivatives **8d-h** which had hydroxyl group at the end of R group. It is expected that log P values of them become 1.5-1.7 point lower by the introduction of hydroxyl group.[11,12] Such derivatives **8d-h** were respectively obtained by reaction of epoxide **7** with protected (ω-hydroxyalkyl)thioacetates **9d-h** in methanol in presence of pottasium carbonate followed by treatment with tetrabutylammonium fluoride in tetrahydrofuran. Sulfones **10f-h** were prepared by the same method for **6**.



a) NaH, $CH_3(CH_2)_mSH$, DMF b) H_2O_2, conc HCl, Na_2WO_4, MeOH
c) $AcS(CH_2)_mOSiMe_2{}^tBu$ (**9**), K_2CO_3, MeOH d) Bu_4NF, THF

Antifungal activities

The minimum inhibitory concentration values (MIC, μg/ml) against *Candida albicans* KB-8 and *Aspergillus fumigatus* MTU6001 are presented in Table.[13] All the derivatives showed higher activity against *C. albicans* than **3**. In contrast, the highest activity against *A. fumigatus* was observed when the number of carbon atoms on R was six (**5f**, in case of sulfide series) or eight (**6h**, in case of sulfone series). However, these *in vitro* activity couldn't be directly reflected in their *in vivo* activities.

The results of the prophylactic efficiencies against murine systemic candidiasis and aspergillosis are also summarized in Table.[14] Almost all control mice died within 3 days after infection, whereas a considerable number of mice treated by oral administration of the triazole derivatives (10 mg/kg/dose for candidiasis or 50 mg/kg/dose for aspergillosis) survived appreciably longer. Sulfides having odd carbon atoms on R (**5a,e,g,i**) and sulfones having less than two carbon atoms on R (**3** and **6b**) were shown to have good efficiencies against candidiasis and aspergillosis. Especially, **3**, **5a** and **6b** were shown to have potent activity, even though their *in vitro* activity was lower than those of longer side-chain derivatives. Perhaps because of their excellent

pharmacokinetics, they could show such a strong activity in spite of their relatively weak *in vitro* activity.[15] They should show higher serum concentration in protein-free form, owing to their low affinities for serum proteins based on their hydrophilic properties.[16] On the other hand, it is surprising that sulfides **5e,g,i** showed remarkable activity against both candidiasis and aspergillosis with reflecting their *in vitro* activity though they were much more hydrophobic than **3**, **5a** or **6b**.

On the basis of these results, we prepared and tested the activities of their hydrophilic analogs **8d-h** having hydroxyl group at the end of R group. As expected, hydroxyl analogs **8e** and **8g** showed better efficiencies against both candidiasis and aspergillosis than their alkyl analogs **5e** and **5g**. The synthesis and evaluation of their further derivatives, which have other hydrophilic substituents such as amino, substituted amino or carboxyl group, are now on investigating.

[Table] Antifungal activities of sulfur-containing triazole derivatives **5**, **6** and **8**

Compound	R or R'	MIC (μg/ml)		Mean survival days (d.) [a]	
		C. albicans	*A. fumigatus*	*C. albicans* [b]	*A. fumigatus* [c]
fluconazole [d]		0.78	400	10 (0.7)	2.8 (2.0) [e]
SM-8668 (**3**)	methyl	0.20	12.5	10 (1.6)	10 (2.1)
Sulfides **5**					
5a	methyl	0.025	1.56	10 (0.8)	9.8 (1.3)
5b	ethyl	0.10	12.5	4.4 (0.6)	1.0 (1.0)
5c	n-propyl	≤ 0.013	0.78	9.9 (0.6)	2.5 (1.0)
5d	n-butyl	≤ 0.013	0.78	9.9 (0.6)	2.5 (1.0)
5e	n-pentyl	≤ 0.013	0.39	9.2 (2.4)	6.8 (1.8)
5f	n-hexyl	≤ 0.013	≤ 0.20	10 (0.7)	2.2 (2.5)
5g	n-heptyl	≤ 0.013	0.39	8.6 (0.0)	7.2 (1.5)
5h	n-octyl	≤ 0.013	3.13	5.0 (0.8)	1.8 (1.0)
5i	n-nonyl	≤ 0.013	0.78	9.1 (0.6)	8.0 (2.1)
5j	n-decyl	≤ 0.013	25	5.8 (0.8)	1.6 (1.3)
Sulfones **6**					
6b	ethyl	0.20	6.25	8.3 (2.0)	10 (2.1)
6c	n-propyl	0.20	12.5	7.6 (0.6)	4.1 (3.7)
6d	n-butyl	0.20	25	0.4 (0.8)	3.6 (3.7)
6e	n-pentyl	0.10	12.5	2.6 (0.8)	1.6 (2.6)
6f	n-hexyl	≤ 0.013	6.25	0.9 (0.4)	1.1 (2.5)
6g	n-heptyl	≤ 0.013	3.13	1.3 (0.8)	1.6 (1.5)
6h	n-octyl	≤ 0.013	0.78	0.6 (0.8)	1.0 (1.0)
6i	n-nonyl	≤ 0.013	≤ 0.20	3.9 (0.6)	1.5 (2.1)
6j	n-decyl	≤ 0.013	3.13	1.1 (0.8)	1.6 (1.3)
Sulfides **8**					
8d	$(CH_2)_4OH$	0.20	6.25	2.5 (0.1)	3.2 (2.6)
8e	$(CH_2)_5OH$	0.025	0.78	9.8 (1.6)	10 (3.5)
8f	$(CH_2)_6OH$	≤ 0.013	0.78	2.8 (1.1)	2.8 (2.3)
8g	$(CH_2)_7OH$	≤ 0.013	0.78	10 (0.1)	9.7 (1.5)
8h	$(CH_2)_8OH$	≤ 0.013	≤ 0.20	4.3 (2.0)	3.5 (5.3)
Sulfones **10**					
10f	$(CH_2)_6OH$	0.39	100	1.4 (0.9)	3.1 (1.8)
10g	$(CH_2)_7OH$	0.39	25	1.0 (2.0)	1.2 (1.5)
10h	$(CH_2)_8OH$	0.10	6.25	0.5 (0.4)	3.0 (2.9)

[a] In vivo activity was determined in mice. The triazole derivative was administrated orally. Mean survival days of control mice on the same conditions are given in parentheses. [b] 10 mg/kg/dose of the triazole derivative was used. [c] 50 mg/kg/dose of the triazole derivative was used. [d] See ref. 17. [e] Only in this case, 100 mg/kg/dose of fluconazole was used.

References and notes

1) Aoyama, Y.; Yoshida, Y.; Sato, R., *J. Biol. Chem.*, **1984**, *259*, 1661.

2) Aoyama, Y.; Yoshida, Y., *Biochem Pharmacol.*, **1987**, *36*, 229.

3) a) Trzaskos, J. M.; Bowen, W. D.; Shafiee, A.; Fisher, R. T.; Gaylor, J. T., *J. Biol. Chem.*, **1984**, *259*, 13402. b) Schroeper, G. T., *Ann. Rev. Biochem.*, **1982**, *51*, 603.

4) a) Baldwin, B. C., *Biochem. Soc. Trans.*, **1983**, *11*, 659. b) Mercer, E. I., *ibid.*, **1983**, *11*, 663. c) Vanden Bossche, H.; Williamsens, G.; Cools, W.; Marichal, P.; Lauwers, W., *ibid.*, **1983**, *11*, 665.

5) Gadher, P.; Mercer, E. I.; Baldwin, B. C.; Wiggens, T. E., *Pest. Biochem. Physiol.*, **1983**, *19*, 1.

6) Cooper, A. B.; Wright, J. J.; Ganguly, A. K.; Desai, J.; Loebenberg, D.; Parmegiani, R.; Feingold, D. S.; Sud, I. J., *J. Chem. Soc., Chem. Commun.*, **1989**, 898.

7) a) Saji, I.; Tamoto, K.; Tanio, T.; Okuda, T.; Atsumi, T., *Abstracts of 8th Medicinal Chemistry Symposium*, Osaka, Nov. 1986, pp.9-12. b) Saji, I.; Tanaka, Y.; Ichise, K.; Tanio, T.; Okuda, T.; Atsumi, T., *Europian Patent*, **1986**, 178533; *Chem. Abstr.*, **1986**, *105*, 35613.

8) Saji, I.; Tamoto, K.; Tanaka, Y.; Miyauchi, H.; Fujimoto, K.; Ohashi, N., *Bull. Chem. Soc. Jpn.*, **1994**, *67*, 1427.

9) Tanio, T.; Ichise, K.; Nakajima, T.; Okuda, T., *Antimicrob. Agents Chemother.*, **1990**, *34*, 980.

10) Miyauchi, H.; Tanio, T.; Ohashi, N., *Biomed. Chem. Lett.*, in press.

11) Rekker, R. F.; de Kort, H. M., *Eur. J. Med. Chem.*, **1979**, *14*, 479.

12) Log P values of **5g** and **8g** are expected to be about 5.3 and 3.7, respectively. These log P values were calculated by the reported method (see ref. 11) and corrected by the observed values for **5a** (log P*=2.16).

13) The *in vitro* activity was tested as follows: *C. albicans* KB-8 was grown at 37 °C on Sabouraud dextrose agar (SDA) for 24 h and transferred in glucose polypeptone yeast-extractbroth for 24 h. *A. fumigatus* MTU6001 was grown at 30 °C on potatodextrose agar for 5 days. Approximately 10^3 saline-washed cells of *C. albicans* or conidia of *A. fumigatus* were inoculated into 1 ml of synthetic amino acid medium fungal (GIBCO) contained serially diluted triazole compound, and those were incubated at 37 °C for 24 h for *C. albicans* or 30 °C for 2 days for *A. fumigatus*. The MIC was determined as the lowest concentration of compound preventing visible fungal growth.

14) The prophylactic efficiencies against murine systemic candidiasis and aspergillosis were tested as follows: Male albino ddY mice, five-week old, were inoculated via tail vein with 2.0×10^6 cells of *C. albicans* KB-8 or 2×10^7 conidia of *A. fumigatus* MTU6001. Appropriate dose of each compounds in 0.5 % methylcellulose or saline were orally administered to groups of 10 mice at 0, 24 and 48 h after infection. The survival rates were recorded for a period of 10 days.

15) Pharmacokinetic parameters of **3** were reported in ref. 9.

16) Affinities of compounds **3** and **5h** for human serum albumin (HSA) were measured to be 10% and 95%, respectively.

17) a) Richardson, K.; Brammer, K. W.; Marriott, M. S.; Troke, P. F., *Antimicrob. Agents Chemother.*, **1985**, *27*, 832. b) Richardson, K.; Cooper, K.; Marriott, M. S.; Tarbit, M. H.; Troke, P. F.; Whittle, P. J., *N. Y. Acad. Sci.*, **1988**, *544*, 12.

(*Received in Japan* 24 *April* 1995; *accepted* 29 *May* 1995)