Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1825–1828

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS OF NOVEL ANTIFUNGAL AGENTS (2)

Takuo Tsukuda, Masami Watanabe, Hitomi Ontsuka, Kazuo Hattori, Michio Shirai, and Nobuo Shimma*

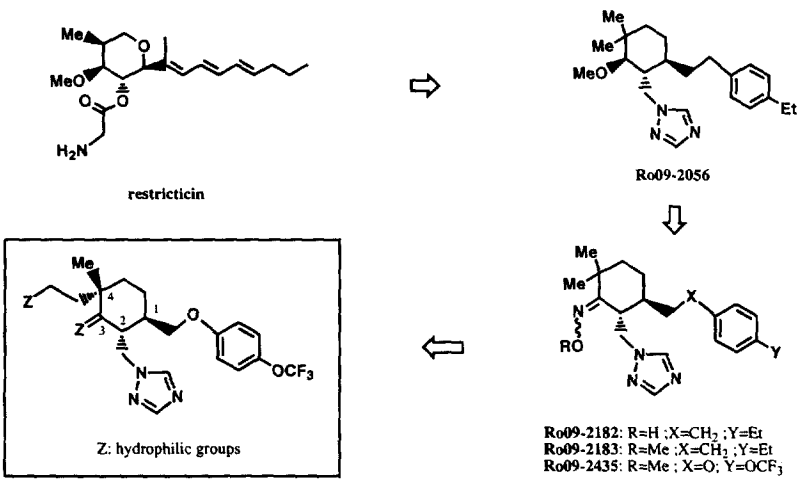
Department of Medicinal Chemistry, Nippon Roche Research Center 200 Kajiwara, Kamakura 247, Japan

Received 15 April 1998; accepted 10 June 1998

Abstract: Synthesis of novel cyclohexyl analogs of restricticin using intramolecular radical cyclization and their *in vitro* and *in vivo* antifungal activities are described.

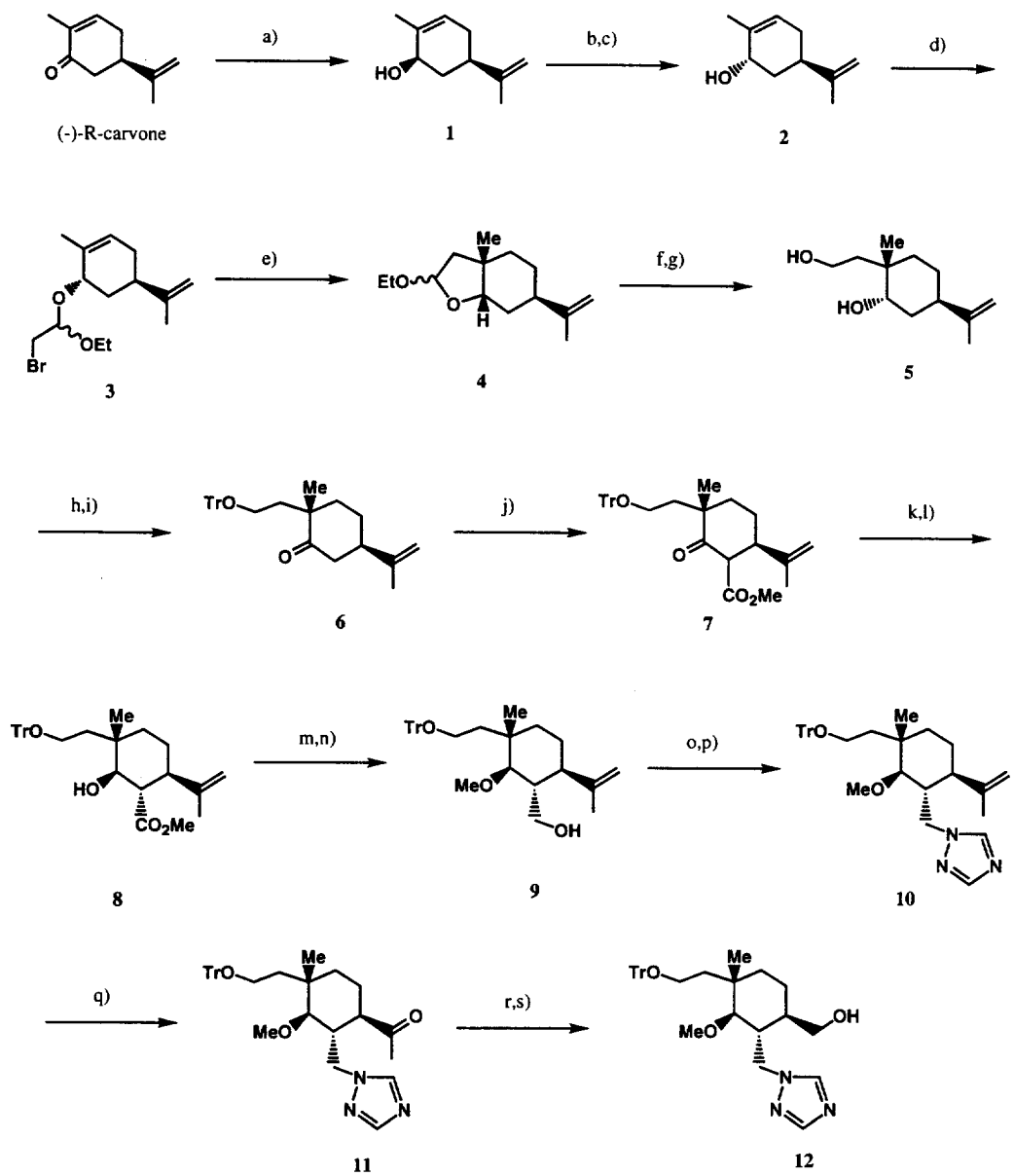
Keyword: Antimicrobial; Chemotherapy; Fungi

The previous paper of this issue deals with the homology modeling of candida lanosterol C-14 demethylase (P450DM), synthesis and the evaluation of the antifungal activity of novel antifungal agents derived from restricticin.[1] In this communication we wish to report the synthesis of the new derivatives at C-4 position which showed suppperior *in vivo* antifungal activity as compared with Ro09-2182, 2183 and 2435.

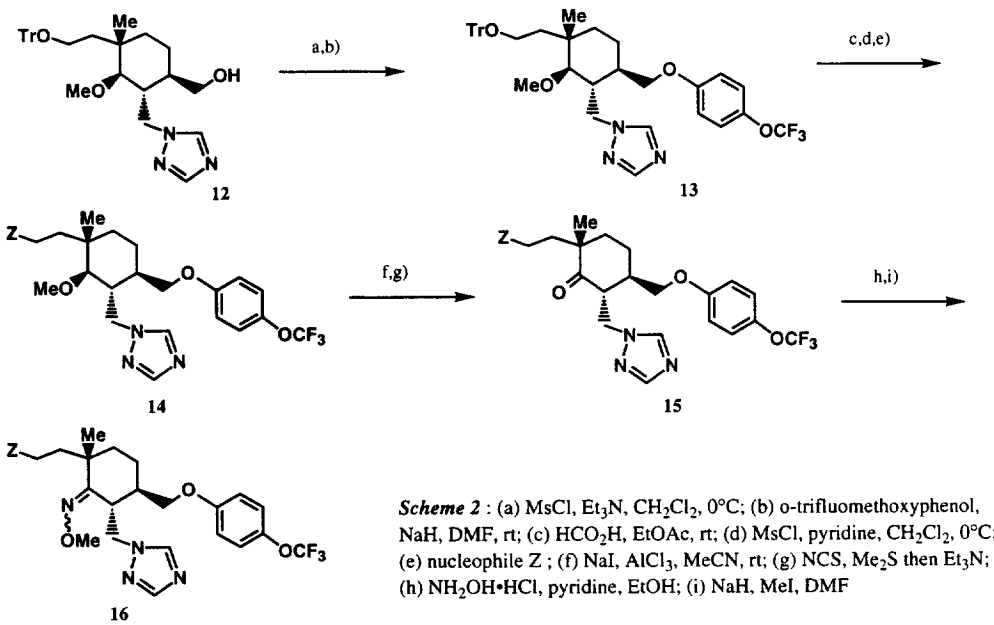


As reported in the previous paper, one of the possible reason for inactivity of Ro09-2182, 2183 and 2435 was their strong serum protein binding property. Thus, we decided to introduce an additional hydrophilic group at the C-4 position to imporove the pharmacokinetic parameters.[2] The homology modeling of P450DM shows that there are several hydrophilic amino acid residues around the C-4 position of Ro09-2056 and that the C-4 substituent is located just below the entrance of the active site pocket of the enzyme.[3] This allows us to introduce hydrophilic functional groups without interfering the hydrophilic interaction between the enzyme and an inhibitor, because they could extend toward the outside of the pocket of the active site.

0960-894X/98/$19.00
PII: S0960-894X(98)00315-1

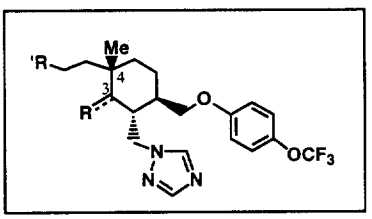


Scheme 1: (a) $LiAlH_4$, ether, -78°C (92%) ; (b) p-nitrobenzoic acid, DMAP, PPh_3, rt ; (c) NaOMe, MeOH, rt (60%, 2 steps) ; (d) ethylvinylether, NBS, THF (83%) ; (e) Bu_3SnCl (cat.), $NaBH_4$, AIBN, t-BuOH, 80°C (79%); (f) THF, H_2O, 1N HCl, 0°C ; (g) $LiAlH_4$, ether, 0°C (81%, 2 steps); (h) tritylchloride, DMAP, DMF, rt (i) PCC, ether, rt (81%, 2 steps); (j) LDA, THF then NCC(O)OMe (71%); (k) $NaBH_4$, MeOH, 0°C; (l) NaOMe, MeOH, reflux (61%, 2 steps); (m) NaH, MeI, DMF, rt; (n) $LiAlH_4$, ether, 0°C (73%, 2 steps); (o) MsCl, Et_3N, CH_2Cl_2, 0°C; (p) 1,2,4-triazole sodium salt, DMF, rt (72%, 2 steps); (q) O_3, MeOH, $CHCl_3$, -78°C then Me_2S, K_2CO_3 (85%); (r) NaOBr, NaOH, dioxane, H_2O; (s) $LiAlH_4$, ether, 0°C (85%, 2 steps).



Scheme 2 : (a) MsCl, Et_3N, CH_2Cl_2, 0°C; (b) o-trifluomethoxyphenol, NaH, DMF, rt; (c) HCO_2H, EtOAc, rt; (d) MsCl, pyridine, CH_2Cl_2, 0°C; (e) nucleophile Z ; (f) NaI, $AlCl_3$, MeCN, rt; (g) NCS, Me_2S then Et_3N; (h) NH_2OH•HCl, pyridine, EtOH; (i) NaH, MeI, DMF

Table 1: Antifungal activity of C-3 and 4 derivatives



Compound			In vitro antifungal activity :IC_{80}(μg/ml)[a]			ED_{50}(mg/kg)[b]
Ro No.	R'	R	*C. albicans* CY 3003	*C. neoformans* CY 1057	*A. fumigatus* CF 1003	
Ro09-2427	-SO_2Me	β-OMe	0.7	0.38	>200	50
Ro09-2473		β-OMe	0.08	0.24	84	50
Ro09-2474		β-OMe	0.5	0.9	36	50
Ro09-2550		β-OMe	0.03	0.02	158	50
Ro09-2711	-OMe	=N-OMe	0.05	1.0	0.6	>100
Ro09-2593		=N-OMe	0.22	13	4.6	>100
Ro09-2687		=N-OMe	0.85	1.1	2.6	>100
Ro09-2684	-SMe	=N-OH	0.18	21	0.45	>100
FCZ	—	—	1.4	3.6	>200	5.4 ± 2.6

a) Broth dilution method; medium:YNBPB(=+1%glucose+0.25%K_2HPO_4), pH 7.0, inoculum size; $1x10^4$ cfu/ml, incubation: 1~2 days at 27°C. b) Systemic Candidosis/mice, ED_{50}(mg/kg) on day 7, p.o. single.

Synthesis of the optically active C-4 derivatives starting from (-)-R-carvone is outlined in ***Scheme 1*** and ***2***. Reduction of (-)-R-carvone with $LiAlH_4$ followed by inversion of the sec. hydroxyl group by Mitsunobu reaction and subsequent hydrolysis gave alcohol **2**. Treatment of the alcohol **2** with ethyl vinyl ether in the presence of NBS produced **3**. Intermolecular radical cyclization of **3** with $NaBH_4$ / catalytic amount of Bu_3SnCl gave desired C-4 derivative **4** in 79% yield.[4] Acid hydrolysis of the acetal group of **4** and subsequent reduction of the resulting aldehyde gave the desired diol **5**[5] in good yield. According to the similar procedure we reproted previously,[6] the diol **5** was then converted to the common intermediate **12** having functional groups at C-3 and C-4 position suitable for introducing hydrophilic functional groups.[6] The *in vitro* and *in vivo* antifungal activity of the C-4 derivatives are summarized in ***Table 1***. Among a number of C-4 derivatives having methoxy grouop at C-3 position, we found that **Ro09-2427**, **2473**, **2474** and **2550** showed oral efficacy in the murine systemic candidosis model, although their *in vivo* efficacy was still one tenth that of Fluconazole. These derivatives were, however, inactive against *Aspergillus fumigatus* . With the expectation of improving the anti-Aspergillus activity as well as *in vivo* efficacy, we attempted to synthesize the C-4 derivatives possessing an oxime group at the C-3 position.[7] The antifungal activities of the C-4 derivatives possessing a methoxyimino group at C-3 position are shown in ***Table 1***. As we expected, they showed anti-Aspergillus activity *in vitro*. In addition, these compounds had a well-balanced antifungal spectrum. However, they were inactive in the murine systemic candidosis models by either the p.o. or i.v. administration. The pharmacokinetic study [8] of **Ro09-2593** suggested that the metabolism and serum protein binding were not major reason for its inactivity *in vivo* but most probably the insufficient drug delivery to the target organ, in this case, the kidney. Further modification studies are required to overcome this problem.

In summary, we demonstrated the design and synthesis of orally active novel antifungal agents (eg.**Ro09-2474** and **2550**) based on the structure of restricticin and the result of homology modeling of the target enzyme, *Candida* P450DM.

References and Notes:

1) a) Matsukuma, S.; Ohtsuka, T.; Kotaki, H.; Shirai, H.; Sano, T.; Watanabe, K.; Nakayama, N.; Itezono, Y.; Fujiu, M.; Shimma, N.; Yokose, K.; Okuda, T. *J. Antibiot.* **1992**, *45*, 151.
b) Aoki, Y.; Yamazaki, T.; Kondo, M.; Sudoh, Y; Nakayama, N.; Sekine, Y.; Shimada, H.; Arisawa, M. *J. Antibiot.* **1992**,*45*,160.
2) Testa, B. In *Burger's Medicinal Chemistry and Drug Discovery* Volume 1; Wolff M.E., Ed.; A Wiely-interscience publication: New York **1995** 129.
3) The previous papaer of this issue
4) Strikrishna, A. Synthetic Commun. **1992**, *22*, 1221.
5) All new compounds gave spectroscopic data in agreement with the assigned structures.
6) Tsukuda, T.; Watanabe, M.; Ontsuka, H.; Fujimoto, Y.; Shimma, N. *Bioorg. Med. Chem. Lett.* , **1994**, *4*, 733.
7) In the previous paper of this issue we found C-3 derivatives possessing oxime group (e.g. Ro09-2183 and 2435) showed broad *in vitro* antifungal spectrum including *Asp. fumigatus.*
8) Pharmacokinetic data of Ro09-2593; i) $T_{1/2}$=0.89hr (mice, 30mg/kg i.v.), ii) ca. 80% of intact Ro09-2593 was recovered after incubation with rat liver microsomes for 24hr. iii) antifungal activity (*C.albicans* CY3003) with 10% mouse serum; IC_{80}=0.24 μg/ml.