Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 5, pp. 733-736, 1994

0960-894X/94 $6.00+0.00

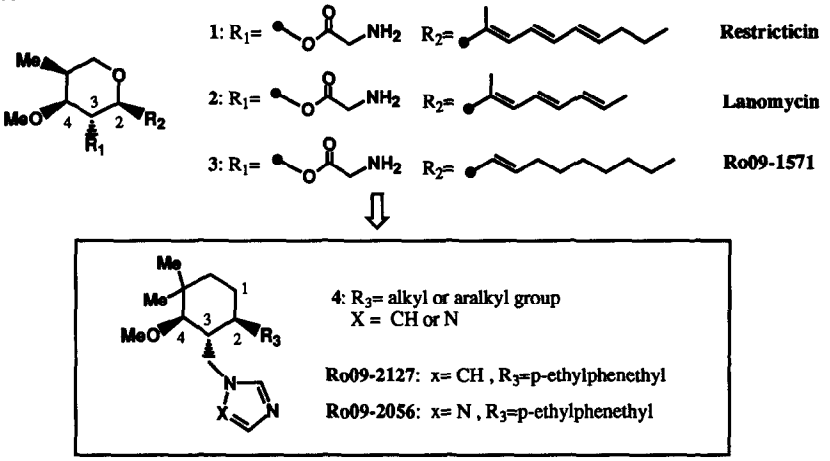
0960-894X(94)E0039-H

SYNTHESIS OF CYCLOHEXYL ANALOGS OF RESTRICTICIN

Takuo Tsukuda, Masami Watanabe, Hitomi Ontsuka, Yasuko Fujimoto, and Nobuo Shimma*
Department of Medicinal Chemistry, Nippon Roche Research Center
200 Kajiwara, Kamakura 247, Japan

Abstruct: Synthesis of cyclohexyl analogs of restricticin, a novel type of antifungal agent from (-) R-carvone and their *in vitro* antifungal activity are described.

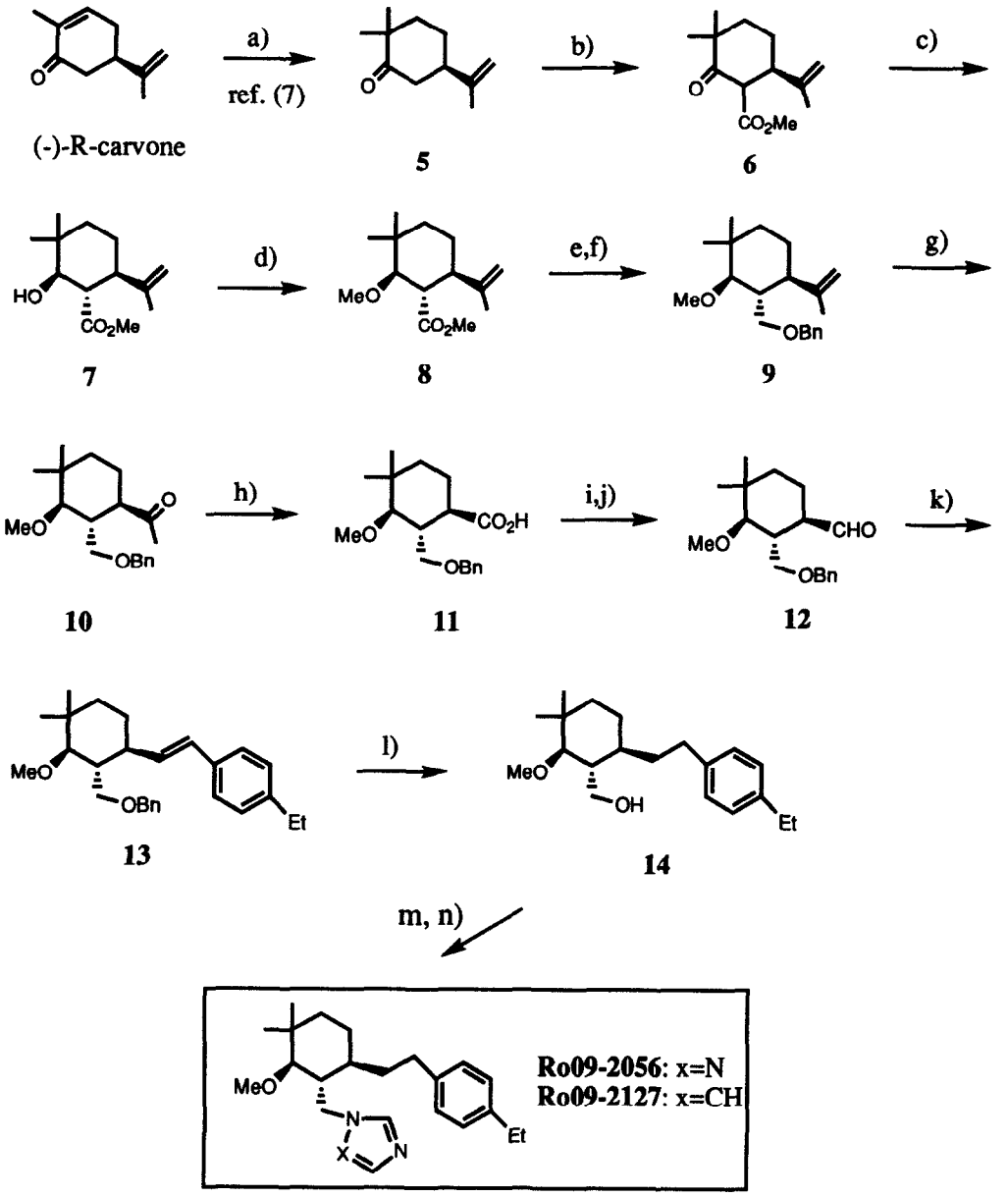
Restricticin (**1**), and lanomycin (**2**) were isolated from cultured broth of *Penicillium* sp. and *Pycnidiophara* sp. by Merck,[1] Bristol-Myers Squibb,[2] and Roche [3] as novel antifungal substances which inhibit lanosterol C_{14} demethylase.[2,4]



In a previous paper,[5] we reported that the synthesis of restricticin derivatives led to the identification of Ro09-1571 (**3**) which showed improved chemical stability and *in vitro* antifungal activity relative to restricticin (**1**).

In this communication, we wish to report the design and synthesis of cyclohexyl analogs (**4**) of (**1**) with superior antifungal properties relative to (**1**) and (**3**).

The mechanism by which (**1**) and its analogs inhibit P_{450} lanosterol C_{14} demethylase involves binding of their primary amino group to the P_{450} heme iron atom.[4] However, the glycine ester of (**1**) and its analogs were easily hydrolyzed both chemically and enzymatically. Thus, the chemical modification of this group was needed to develop a clinically useful antifungal agent. We introduced an azolylmethyl moiety at the C-3[6] position as a conformationally restricted and stable bioisostere of the glycine ester moiety where amino nitrogen and carbonyl oxygen atoms in the glycine moiety are fixed with one additional carbon atom forming a five membered ring. Actually, we synthesized cyclohexyl analogs (**4**) of (**1**) having an azolylmethyl group at the C-3 position. Many azole derivatives are known to inhibit lanosterol C_{14} demethylase and some of these are clinically useful antifungal agents.



Scheme1: (a) K-selectride, THF, -78°C then MeI (78%) ; (b) dimethyl carbonate, NaH, pyridine, 80°C (89%) ; (c) $NaBH_4$, $CeCl_3.7H_2O$, MeOH, 0°C ; (d) MeI, NaH, DMF, rt (74%, 2 steps) ; (e) $LiAlH_4$, ether , 0°C ; (f) BnBr, NaH, DMF, rt (82%, 2 steps) ; (g) O_3, MeOH, -78°C then Me_2S (78%) ; (h) NaOBr, dioxane, H_2O, 0°C (92%) ; (i) $LiAlH_4$, ether, 0°C (89%) ; (j) PCC, 4AMS, CH_2Cl_2, rt (79%); (k)*p*-ethylbenzyltriphenylphosphonium bromide, n-BuLi, THF, 0°C (71%) ; (l) H_2, Pd-C, MeOH, rt (85%) ; (m) MsCl, TEA, CH_2Cl_2, 0°C ; (n) 1,2,4-triazole sodium salt or imidazole sodium salt, DMF, rt (73%, 2 steps).

We chose (-)-R-carvone as a starting material, which has a cyclohexane ring with absolute configuration corresponding to that of C-2[6] of target compound (**4**). Treatment of (-)-R-carvone with K-selectride followed by methylation of the resulting enolate with methyl iodide afforded (**5**).[7] Introduction of a carbomethoxy group at the C-3[6] position was carried out by the use of dimethyl carbonate and sodium hydride in pyridine at 80°C to give (**6**) in 89% yield. Reduction of ketone (**6**) with sodium borohydride in the presence of cerium (III) chloride followed by O-methylation gave the desired equatorial methyl ether (**8**) in 74% yield. After the reduction of the carbomethoxy group of compound (**8**) by lithium aluminum hydride, the resulting primary alcohol was protected with a benzyl group to give a benzyl ether (**9**) in 82% yield. For the introduction of various aralkyl groups at the C-2[6] position, (**9**) was converted into aldehyde (**12**) by following four-step procedure. Thus, the ozonolysis of compound (**9**) followed by the haloform reaction of the resulting methyl ketone (**10**) with sodium hypobromite gave carboxylic acid (**11**) in a good yield. The reduction of carboxylic acid (**11**) with lithium aluminum hydride followed by oxidation of the resulting primary alcohol with PCC gave aldehyde (**12**). Wittig olefination of aldehyde (**12**) gave an olefin (**13**) in 71% yield. After removal of the benzyl group of (**13**) by catalytic hydrogenolysis, the resulting alcohol (**14**) was converted to the mesylate . Finally, the mesylate group was substituted with the 1,2,4-triazole sodium salt or the imidazole sodium salt in DMF to give the desired derivative Ro 09-2056 and 2127 respectively.

The *in vitro* antifungal activity of Ro 09-2056 and 2127 is summarized in Table 1 in comparison with those of itraconazole, fluconazole and Ro 09-1571. Ro 09-2056 and 2127 were found to have much more potent *in vitro* antifungal activity against *Candida albicans* and *Cryptococcus neoformans* when compared with fluconazole and Ro 09-1571, but were somewhat inferior to those of itraconazole. Detailed structure-activity relationships of a series of derivative (**4**) will be reported elsewhere.

In summary, we have accomplished the synthesis of cyclohexyl analogs of restricticin (**1**), Ro 09-2056 and 2127 with potent antifungal activity starting from (-)-R-carvone.

Table 1. *In vitro* antifungal activity (IC_{80}:μg/ml) and enzyme inhibitory activity (IC_{50}:μg/ml) of Ro 09-2056 and 2127

antifungal activity[a)]	Itraconazole	Fluconazole	Ro09-1571	Ro09-2056	Ro09-2127
C. albicans CY1005	0.0056	2.8	1.58	0.042	0.012
C. albicans CY3003	0.0012	1.1	0.97	0.012	0.0025
C. albicans CY1002	0.03	2.6	1.80	0.098	0.059
C. neoformans CY1057	0.022	3.8	2.19	0.82	0.12
C. neoformans CY1059	0.046	11	3.20	0.37	0.14
A. fumigatus CF1003	0.02	200	0.73	>200	0.37
A. fumigatus CF1004	<0.0004	180	0.52	>200	0.19
Enzyme inhibitory activity [b)]	0.060	0.042	0.080	0.010	0.032

a)Broth dilution method; medium: YNBPB (=YNB+1% glucose+ 0.25% K_2HPO_4), pH 7.0, inoculun size; $1x10^4$ cfu/ml, incubation: 1~2 days at 27°C. b)P_{450} lanosterol C_{14} demethylase (*C.albicans* CY1005).

REFERENCE AND NOTE

1) a) Schwartz, R.E.; Dufresne, C.; Flor, J.E.; Kempf, A.J.; Wilson, K.E.; Lam,T.; Onishi, J.; Milligan, J.; Fromtling, R.A.; Abruzzo, G.K.; Jenkis, R.; Glazomitsky, K.; Bills, G.; Zitano, L.; Del Val, S.M.;Omstead, M.N. *J. Antibiot.***1991**, *44*, 463. b) Hensens, O.D.; Wichmann, C.S.; Liesch, J.M.; Van Middlesworth,F.L. Wilson, K.E.; Schwartz, R.E.*Tetrahedron*, **1991**,*47*, 3915.

2) a) O'Sullivan, J.; Phillipson, D.W.; Kirsch, D.R.; Fisher, S.M.; Lai, M.H.; Trejo,W.H.*J.Antibiot.***1992**,*45*, 306. b) Phillipson, D.W.; O'Sullivan, J.; Johnson, J.H.;Bolgar, M.S.; Kahle, A.D. *J.Antibiot.***1992**,*45*, 313.

3) 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.

4) Aoki, Y.; Yamazaki, T.; Kondoh, M.; Sudoh, Y.; Nakayama, N.; Sekine, Y.; Shimada, H.; Arisawa, M. *J.Antibiot.***1992**, *45*, 160.

5) Tsukuda, T.; Umeda, I.; Masubuchi, K.; Shirai, M.; Shimma, N. *Chem.Pharm. Bull.* **1993**,*41*,1191.

6) This numbering corresponds to that of Restricticin **1**.

7) Ganem, B. *J.Org.Chem.* **1975**, *40*, 147.

8) All the intermediates and final products were characterized by stereoscopic methods. Representative physical data is shown bellow.

7: ^{1}H-NMR($CDCl_3$) δ:0.91(s, 3H), 1.02(s, 3H), 1.3~1.5(m, 4H), 1.71(s, 3H), 2.31(m, 1H), 2.58(t, J= 11Hz, 1H), 3.56(d, J=11Hz, 1H), 3.65(s, 3H), 4.69(br.s, 1H), 4.71(br.s,1H); MS(EI) m/z 224(M^+); *Anal.* Calcd for $C_{13}H_{22}O_3$: C, 68.98; H, 9.80. Found: C, 69.00; H, 9.88.

10: ^{1}H-NMR($CDCl_3$) δ:0.89(s, 3H), 1.01(s, 3H), 1.2~1.6(m, 5H), 1.97(m, 1H), 2.07(s, 3H), 2.67 (dt, J=4 and 12Hz, 1H), 2.85(d, J=11Hz, 1H), 3.39(dd, J=3 and 10Hz, 1H), 3.42(s, 3H), 3.59(dd, J=4 and 10Hz, 1H), 4.45(d, J=12Hz, 1H), 4.85(d, J=12Hz, 1H), 7.2~7.4(m, 5H); MS(EI) m/z 304(M^+).

11: ^{1}H-NMR($CDCl_3$) δ: 0.90(s, 3H), 1.01(s, 3H), 1.2~ 2.0(m, 5H), 2.61(dt, J=4 and 12Hz, 1H), 2.91 (d, J=11Hz, 1H), 3.44(s, 3H), 3.5(m, 1H), 3.69(dd, J=3 and 10Hz, 1H), 4.45(d, J=12Hz, 1H), 4.49(d, J=12Hz, 1H), 7.3(br.s, 5H); MS(EI) m/z 306(M^+).

13: ^{1}H-NMR($CDCl_3$) δ: 0.90(s, 3H), 1.03(s, 3H), 1.23(t, J=7Hz, 3H), 1.2~1.5(m, 5H), 2.34(m, 1H), 2.62(q, J=7Hz, 2H), 3.02(d, J=11Hz, 1H), 3.47(s, 3H), 3.50(dd, J=2 and 9Hz, 1H), 3.65(dd, J=2 and 9Hz, 1H), 4.39(d, J=12Hz, 1H), 4.48(d, J=12Hz, 1H), 5.96(dd, J=9 and 16Hz, 1H), 6.36(d, J=16Hz, 1H), 7.05~7.35(m, 9H); MS(FAB) m/z 393(M^++1).

Ro09-2127: ^{1}H-NMR($CDCl_3$) δ: 0.90(s, 3H), 1.04(s, 3H), 1.16(m, 2H), 1.24(t, J=7Hz, 3H), 1.36(m, 1H), 1.40(m, 1H), 1.58(m, 1H), 1.72(m, 1H), 1.94(m, 2H), 2.46(m, 1H), 2.56(d, J=10Hz, 1H), 2.63 q, J=7Hz, 2H), 2.67(m, 1H), 3.58(s, 3H), 4.04(dd, J=3 and 14Hz, 1H), 4.16(dd, J=3 and 14 Hz ,1H), 6.78(s, 1H), 7.01(s, 1H), 7.10(d, J=8Hz, 2H), 7.11(d, J=8Hz, 2H), 7.46(s, 1H); MS(EI) m/z 354(M^+); $[\alpha]_D^{25}$ -53.8 (c=2.2, EtOH).

Ro09-2056: ^{1}H-NMR($CDCl_3$) δ:0.89(s, 3H), 1.05(s, 3H), 1.14(m, 1H), 1.23(t, J=7Hz, 3H),1.37(dd, J=3 and 13Hz, 1H), 1.53(m, 1H), 1.67(m, 4H), 1.98(m, 1H), 2.42(m, 1H), 2.63(q, J=8Hz, 2H), 2.69 (m, 1H), 2.88(d, J=10Hz, 1H), 3.63(s, 3H), 4.27(dd, J=4 and 14Hz, 1H), 4.40(dd, J=2 and 14Hz), 7.08(d, J=8Hz, 2H), 7.13(d, J=8Hz, 2H), 8.00(s,1H), 8.14(s, 1H); MS(EI) m/z 354(M^+); $[\alpha]_D^{25}$-49.3 (c=1.4,EtOH).

(*Received in USA* 24 *November* 1993; *accepted* 19 *January* 1994)