Carbazate as a Glycine Bioisostere in Restricticin

Sir:

The novel antifungal agent **1** was recently isolated simultaneously in three different laboratories[1~6] and named respectively restricticin, Ro 09-1470, and lanomycin. This structurally unique compound has been shown to inhibit lanosterol 14-α-demethylase in the steroid biosynthetic pathway[4]. As such **1** is the first known natural product to share this mode of action with the commercially successful azoles ketoconazole, itraconazole and fluconazole. In preliminary pharmacokinetic studies in these labs, it was observed that the glycine ester of restricticin **1** is quite labile toward plasma hydrolysis, and the resultant alcohol **2** is biologically inactive.

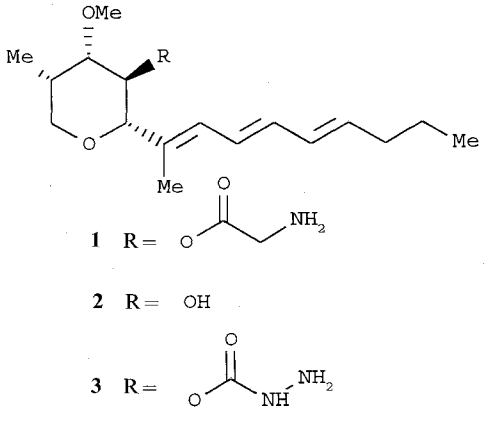
The limited SAR based on congeners implied that the amino moiety was critical to activity and presumably occupies the sixth coordination site of the P_{450} heme[4]. In order to increase the hydrolytic stability of the ester bond while minimizing the change in spatial orientation of the amino functionality, a series of sterically hindered glycine ester analogues was proposed. Initial attempts to esterify **2** with bulky electrophiles were unsuccessful, indicating that the hydroxyl environment is fairly hindered. Successful acylation was accomplished by *in situ* activation of the protected amino acid as the mixed anhydride using isopropenyl chloroformate[7]. After removal of the FMOC group this route resulted in preparation of the more hindered esters of *l*-alanine, **4**, *d*-alanine, **5**, and dimethyl glycine, **6**.

To measure the hydrolytic stability of restricticin and the hindered analogues, these compounds were treated *in vitro* with mouse plasma and the time course of hydrolysis was measured by HPLC. The early observation was confirmed that the glycine ester undergoes rapid hydrolysis. It was found that the *in vitro* plasma half-life of restricticin was 7 to 15 minutes varying with the batch of mouse blood received. The enzymatic nature of the hydrolysis was implied by the observation that the serine protease inhibitor phenyl methyl sulfonyl flouride[8] completely inhibits the observed plasma hydrolysis (data not shown). The dimethyl glycine derivative **6** and the *d*-alanine derivative **5** were considerably more resistant to hydrolysis than restricticin, exhibiting *in vitro* plasma half life values of 80 minutes and 40 minutes respectively.

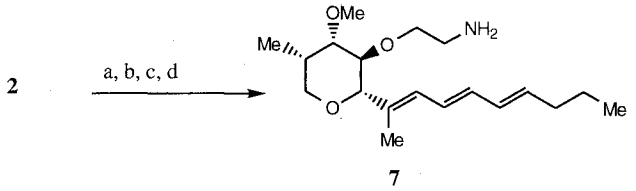
The simplest glycine analogue which would avoid hydrolysis problems was recognized as the amino ether **7**, but its preparation was quite troublesome. Problems associated with the triene instability which caused rearrangements, oxidations, and polymerizations were overcome ultimately in the route outlined below. O-alkylation with more obvious masked amino functions than the OTHP were attempted, but either O-alkylation was not accomplished, or unmasking was unsuccessful. The THP was readily removed under mild conditions, and a Mitsunobu reaction cleanly displaced the alcohol with an azide. The azide was resistant to reduction by triphenyl phosphine and also to reduction by gaseous hydrogen sulfide. However, hydride reduction gave rise to the desired primary amine **7**.

These stabilized derivatives **4**, **5**, **6**, and **7** were tested in several assays for their effect on steroid biosynthesis but none exhibited >1% of the activity of the parent restricticin. At this point the unique activity of the glycine ester was hypothesized to be due to a two point binding involving the carbonyl oxygen and the amine lone pair as well as some sensitive function of the amine pKa.

Scheme 1.



Scheme 2.



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Reagents:

a) i) NaH, 80°C ii) Br⌒⌒OTHP ; b)PPTs; c) $(PhO)_2P(O)N_3$, DIAD, Ph_3P; d)LAH

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Table 1. Minimum fungicidal concentrations (mcg/ml) *vs* selected fungi.

Organism	1	3	AMB
Cryptococcus neoformans MY1051	4	4	4
Cryptococcus neoformans MY1146	2	8	4
Cryptococcus neoformans MY2061	2	8	2
Cryptococcus neoformans MY602	1	2	2
Candida albicans MY1055	2	2	2
Candida pseudotropicalis MY1100	2	8	4
Saccharomyces cerevisiae	4	8	4

* Amphotericin B.

Since only more basic derivatives had been synthesized†, the carbazate derivative was next suggested.

The carbazate was synthesized in good yield by a 2 step procedure[10]. Acylation of **2** with p-nitrophenyl-chloroformate in pyridine proceeded to form the activated carbonate which upon treatment with hydrazine displaced the p-nitro-phenoxide to form the desired **3** in 80% yield. The carbazate **3** showed steroid inhibition activity which was comparable to that of the parent restricticin. Upon *in vitro* incubation with mouse serum, **3** exhibited complete stability toward hydrolysis over a 1 hour period. The azole antifungal agents, and presumably other lanosterol-14-α-demethylase inhibitors, are notorious for exhibiting unimpressive *in vitro* potency and this is the case for **1** and **3**. Some of the organisms which are sensitive to this class of inhibitor are shown in Table 1.

Most promisingly, excellent *in vivo* curative activity was observed in the mouse total organ kidney assay (TOKA)[11]. Carbazate **3** at 100 mg/kg effectively reduced *C. albicans* infection *ca.* 98% from $10^{6.5}$ colony forming units (CFU) to $10^{4.7}$ CFU, whereas the parent restricticin **1** showed no statistically significant antifungal activity in this *in vivo* assay. This experiment was also compromised by aqueous insolubility of the compound, and greater potency could be expected from a more solubilized formulation.

Now that a metabolically stabilized bioactive derivative of the natural product restricticin **1** has been obtained, pertinent biochemical studies can be conducted to compare **3** with the antifungal agents currently being marketed. In addition, the novel structure of restricticin and its derivatives could potentially provide alternative starting points for inhibition of related cytochrome P_{450} targets in other therapeutic areas such as aromatase for breast cancer[12].

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(Received October 16, 1995)

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† The analogues with calculated pKa's in parentheses were: **7** (9.2), **6** (8.2), **4** and **5** (7.9), **1** (7.6) and **3** (1.5). The pKa values for the conjugate acids were calculated using equations in ref 9.

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