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Antifungal Rapamycin Analogues with Reduced Immunosuppressive Activity

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Abstract—Several 1,2,3,4-tetrahydro- and 7-*N*-hydroxycarbamate derivatives of the natural product rapamycin were prepared and assayed for their immunosuppressive and antifungal profiles. Substitutions at the 7-position indicate the possibility of a differentiated immunosuppressive to antifungal profile, whereas 40-position variants of the tetrahydro-analogues did not show similar differentiated activity.

Over the last decade, the incidence and types of life-threatening fungal infections have increased due to greater numbers of immunocompromised patients who are at risk for acquiring fungal infections from HIV infection, chemotherapy-induced neutropenia, organ transplantation, hemodialysis, and from the use of broad-spectrum antibiotics and glucocorticosteroids.[1] As troublesome, is the increasing rate at which drug-resistant *Candida* species are being reported.[2] At present, clinically used systemic antifungal agents are the polyene antibiotic amphothericin B and its liposome variants, azoles, allylamines, thiocarbamates, and fluorocytosine.[3] The need for new fungicidal, broad-spectrum, orally active antifungal agents is driven by several shortcomings that existing drugs suffer from: severe toxicity/side effects, fungistatic mode of action, development of drug resistance, unfavorable routes of administration, and undesirable drug/drug interactions. Two new azoles[4] (Voriconazole:UK-109,496; SCH5692) and two cyclic lipopeptides[5] (MK-0991; LY303366) are being clinically evaluated for the treatment of severe human mycoses.

Rapamycin, **1**, (Fig. 1) a macrolide antibiotic produced by *Streptomyces hygroscopicus*, was initially discovered as an antifungal agent[6] and subsequently shown to exhibit potent antitumor and immunosuppressive activities.[7] Its therapeutic potential in organ transplantation grew as a result of its ability to prolong graft survival in man and has recently been approved as an immunosuppressive agent.[8] Total syntheses of this natural product have been reviewed[9] and its entire biosynthetic gene cluster has been sequenced.[10] Rapamycin is suggested to inhibit T-cell and B-cell proliferation in the middle to late G1 phase of the cell cycle, by forming a complex between FKBP12 (FK-506-binding proteins) and FRAP proteins, which results in downstream phosphorylation of elongation factor 4E-BP1, alteration of protein translation processes, and ultimately shutdown of protein synthesis.[11] Several mechanistic and structural studies continue to delineate the details of this signaling pathway that appears to be conserved, in yeast, plants, flies and mammals.[12]

We present herein data on some novel rapamycin analogues that displayed good in vitro antifungal potency and reduced immunosuppressive activity as compared to the parent natural product. Early reports on 1,2,3,4-tetrahydrorapamycin, **2**, and substituent variations at the 7-position showed analogues with this differentiated profile.[13] This encouraged us to further investigate this possibility with the aim of developing less lipophilic and orally active antifungal agents from the available natural product.

Early SAR work on immunosuppressive analogues of rapamycin focused on the cyclohexanyl moiety with particular interest on the 40-position.[14] By analogy, 1,2,3,4-tetrahydrorapamycin **2** was prepared and modified at the 40-position, giving compounds **3**–**11**. Results displayed in Table 1, show that such changes on **2** generally suppressed both antifungal and immunosuppressive

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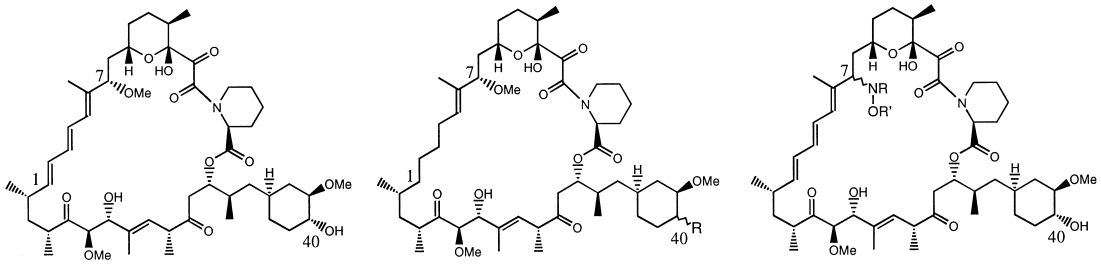

Rapamycin-1 | 40- *Sub.*-1,2,3,4-Tetrahydrorapamycin 2 | 7-*Sub.* rapamycin Analogues

Figure 1.

Table 1. Antifungal and immunosuppressive activities of 1,2,3,4-tetrahydrorapamycin derivatives (**2–11**)

Compound		Minimum inhibitory concentration (μg/mL)[a,b]					*H*-ConA:IC_{50}
	40-Position Sub.	*CA*10231	*CA*579a	*CA*62376	*CT*750	*CK*28838	(nM) (b)
1	Rapamycin	0.2	0.2	0.2	0.2	0.78	1.00
2	(*R*)-OH	1.56	1.56	1.56	1.56	1.56	1592
3	(*S*)-OH	1.56	1.56	0.78	1.56	0.78	3395
4	(*R*)-OCO–*p*NO_2Ph	>100	>100	>100	>100	>100	—
5	(*S*)-Tetrazole	>100	>100	>100	>100	>100	—
6	(*R*)-OCONMeOMe	50	>100	—	>100	>100	2468
7	(*R*)-OCO-Morpholine	>100	>100	—	>100	>100	—
8	(*R*)-OCO-Piperazine	>100	>100	—	>100	>100	—
9	(*R*)-OCO-Piperidine	>100	>100	—	>100	>100	—
10	(*R*)-OCO-NME_2	50	50	—	>100	6.25	485
11	(R)-OCO-N(OH)Me	3.12	3.12	—	3.12	1.56	434

[a]*CA* = *Candida albicans* (*CA*442 and *CA*38247 were also screened and gave similar results); *CT* = *Candida tropicalis*; *CK* = *Candida krusei*; Control: Amp.B MIC = 0.78 mg/mL.
[b]H-Concanavalin-A proliferation assay on rapamycin: IC_{50} values ranged from 0.6 nM to 4.3 nM and were normalized to 1.0 nM.

activities. Compound **3** showed the highest antifungal potency and the greater differential in immunosuppressive profile, whereas analogues **4**–**11** did not show promising results. We were thus prompted to investigate reactions of novel substrates at the 7-position of rapamycin, a site whose reactivity with nucleophiles was studied under Lewis acid catalysis to give previously synthesized compounds **12** and **13**.[13a,b,15]

Thus, reaction of **1** with various *N*-hydroxy-acyl, -carbamoyl, and-urea substrates at the 7-position under Lewis acid catalysis gave epimeric 7-substituted rapamycin derivatives **12** through **36**, in moderate to high yields.[16] These were shown to be *N*-bonded to the rapamycin skeleton by carbon NMR chemical shift data. Absolute configuration (7*R* or 7*S*), was determined by analogy to earlier work performed with proton NMR coupling constant data.[13b,15] Table 2 shows compounds with low immunosuppressive and potent antifungal activities. Analogues with substituents having the natural 7-*S* configuration were the most active antifungal and immunosuppresssive agents. As a class, the 7-(*S*)-*N*-hydroxy-carbamates were the most active antifungal agents with compounds **18**, **22**, **26**, and **32** having MICs consistently lower than 3 μg/mL over several fungal strains. Of this group, derivatives **18** and **32** showed IC_{50}'s in the Human Concanavalin-A proliferation assay that were 288- and 786-fold less active than rapamycin as immunosuppressive agents, respectively. The 7-*N*-hydoxy-derivatives of **1** generally showed a much greater decrease in immunosuppressive activity than they did in their antifungal activity when compared to **1** (Table 2).

In order to explore the mechanism of action of **2** (tetrahyrdo-**1**), both **1** and **2** were tested against *S. cerevisiae* strains that had known mutations in the rapamycin pathway. Strains **329** (a leu2 ura3 rme1 trp1 his4 GAL4 HMLa ade2D fpr1:ADE2-2; JH3-3d) and **496** (a tor2-1 leu2 ura3 rme1 trp1 his4 GAL4 HMLa; JH12-17b) were resistant to both **1** and **2** as determined by a disc diffusion assay.[17] The wild-type strain **281** (a leu2-3 ura3-52 rme1 trp1 his4 GAL+ HMLa; JK9-3da) was susceptible to both **1** and **2**. These results suggest that the mechanism of action of **2** was similar to that of **1** against fungi. Further, compounds **6**, **7**, **22**, and **24** were evaluated for binding to FKBP12 using **1** as a control in a competition binding assay with an ascomycin conjugate of alkaline phosphatase. Data in Table 3 show that the binding of **6** and **7** to FKBP12 are at least 100–1000 less than that of compounds **22** and **24**.

In conclusion, several novel *N*-hydroxy-acyl,-carbamoyl, and-urea rapamycin analogues were synthesized and evaluated for differential immunnosuppressive and antifungal profiles. Compounds **18** and **32** showed potent antifungal activities with reduced immunosuppressive profiles compared to **1**, and compare favorably with published compounds **12** and **13**.[13,18] Our experimental data also suggests that the antifungal activities

Table 2. Antifungal and immunosuppressive activities of 7-substituted rapamycin analogues (**12–36**)[a]

Compound		Minimum inhibitory concentration (μg/mL)[b]					H-ConA:IC_{50} (nM)[b]
	7-Position Substitutions	CA10231	CA579a	CT750	CK28838	TG15545	
1	Rapamycin	0.2	0.2	0.2	0.78	<=0.1	1.00
12	(*S*)-$NHCO_2OMe$	1.56	1.56	1.56	0.78	0.78	1383
13	(*S*)-NOHAc	6.25	6.25	6.25	3.12	1.56	95
14	(*S*)-$NOCONH_2$	12.5	25	3.12	3.12	1.56	379
15	(*R*)-$NOHCONH_2$	100	100	12.5	3.12	1.56	758
16	(*S*)-NOHCOOMe	3.12	6.25	3.12	0.39	0.78	16
17	(*R*)-NOHCOOMe	25	50	25	1.56	1.56	1047
18	(*S*)-2-Me-Thienyl	3.12	12.5	1.56	1.56	0.78	221
19	(*S*)-NOHCOOiBu	1.56	3.12	1.56	0.78	0.78	196
20	(*R*)-NOHCOOiBu	3.12	12.5	3.12	0.78	0.78	760
21	(*S*)-NOHCOOPh	1.56	3.12	0.78	0.78	0.78	170
22	(*R*)-NOHCOOPh	12.5	25	12.5	1.56	1.56	1100
23	(*S*)-NOHCON-Piperidyl	1.56	1.56	6.25	1.56	1.56	786
24	(*S*)-OH	1.56	3.12	1.56	0.78	0.78	6.3
25	(*S*)-NOHCO-Morpholine	25	50	25	6.25	6.25	1740
26	(*R*)-NOHCO-Morpholine	25	50	25	6.25	3.12	1616
27	(S)-NOHCO-NMe_2	100	100	50	3.12	3.12	2028
28	(*R*)-NOHCO-NMe_2	100	100	50	312	3.12	2028
29	(*S*)-$NOMeCONMe_2$	50	50	50	1.56	1.56	6.5
30	(*R*)-$NOMeCONMe_2$	>100	>100	50	3.12	3.12	3611
31	(*S*)-NOHCOO-*n*-Pr	1.56	3.12	1.56	0.78	1.56	288
32	(*R*)-NOHCOO-*n*-Pr	12.5	12.5	6.25	0.78	1.56	900
33	(*S*)-NOHCOO-*i*-Pr	6.25	12.5	25	0.78	0.78	220
34	(*R*)-NOHCOO-*i*-Pr	6.25	12.5	25	0.78	0.78	820
35	(*S*)-NOHCOO-*n*-Bu	1.56	1.56	1.56	0.78	0.78	83
36	(*R*)-NOHCOO-*n*-Bu	12.5	12.5	12.5	1.56	1.56	523

[a]See Table 1 for footnotes; TG = *T. glabrata*.
[b]*CA*442, *CA*38247, *CA*62376 were also screened and gave MICs in similar range to or weaker than *CA*10231. *CrA*34140 and *Aspergillus niger* were not affected by these compounds (MICs ~100 μg/mL).

Table 3. Binding Affinities for FKBP12

Compound	**1**	**6**	**7**	**22**	**24**
IC_{50} (nM)	1.6	>1000	>1000	2.1	14

of our active compounds proceeds along a similar signaling pathway as their more immunosuppressive analogues. Further work is required to provide orally bioavailable and systemically efficacious rapamycin analogues with this preferred profile.

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16. **General experimental**: Tetrahydrorapamycin **2** was prepared from **1** by controlled hydrogenation. Compounds **3**–**11** were prepared by reaction of **2** with the appropriate substrates as described in the literature (see ref 14) for their corresponding unsaturated rapamycin analogues. Compounds **13**–**36**: 7-*N*-hydroxamic acid, -hydoxyurea, and-hydroxycarbamate analogues were prepared according to the reported method for compounds **12** and **18** from **1** in the presence of TFA.[13b] The epimeric products were purfied by preparative HPLC (acetone:hexane gradient from 1:10 to 20:1 ratios). Compounds **13**, **14** and **15** were synthesized from **1** and acetyl hydroxamic acid and *N*-hydroxyurea. Substituted *N*-hydroxycarbamates and *N*-hydroxyureas were prepared by reaction of the corresponding chloroformates in a stirred biphasic water–dichloromethane solution in the presence of 2.0 equiv of hydroxylamine-HCl and 3.0 equiv sodium bicarbonate 12 h; the aqueous layer was acidified with phosphoric acid and the organic layer was concentrated to yield the *N*-hydroxycarbamate. Compounds **23** and **25**, were prepared by reaction of *N*-carbonylphenoxyhydroxylamine with piperidine and morpholine, respectively. Compound **24** was formed as a side product when water was present in the Lewis-acid catalyzed reaction mixture. All compounds were characterized by NMR and HRMS.

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