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Rapamycin analogs with reduced systemic exposure

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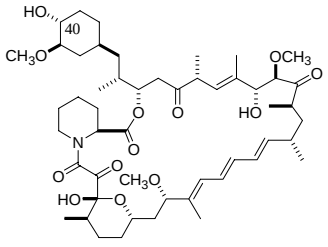
Abstract—The synthesis and biological activities of rapamycin (**I**) analogs modified at the C-40 position are reported. Emphasis placed on compounds that potentially have an improved safety profile on account of their shorter in vivo half-life when compared with rapamycin.

Rheumatoid arthritis (RA) is an autoimmune disease afflicting over 2 million individuals in the United States and 5.7 million worldwide. RA is a painful disease that results in progressive joint destruction, deformity, and immobility, and can eventually lead to death. Pain relief using non-steroidal anti-inflammatory drugs (NSAIDS) is the most popular treatment for RA. Unfortunately, this treatment cannot prevent disease progression. Other disease modifying antirheumatic drugs (DMARDS) are also currently available, but their use as first-line therapy is normally avoided due to slow onset of action, modest efficacy, and frequent toxic side effects.[1] The potent immunosuppressant, cyclosporine, has seen increased use in the treatment of severe RA, despite its publicized liabilities, demonstrating the marketability of more efficacious agents.[1] Indeed, new classes of macromolecules, targeting TNF-α and IL-1, have been introduced to the market with astonishing success. While these compounds demonstrate an improved benefit/risk profile when compared to DMARDS, they do present patients with a unique side effect profile, and therefore provide additional opportunity for the development of alternative treatments.[2] The criteria guiding our search for a potent immunosuppressant for the treatment of RA require demonstration of efficacy and an improved safety profile with respect to currently available drugs. Furthermore, this agent should act immediately and produce complete relief from the disease by arresting the disease process itself.



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Rapamycin (**1**), a secondary fungal metabolite, has shown comparable efficacy when substituted for cyclosporine in clinical trials for renal transplantation.[3] Moreover, the side-effect profile is reported to be relatively benign compared to that of cyclosporine, with elevated triglyceride and cholesterol levels as the principal dose limiting side-effects seen in some patients. However, rapamycin is also beset with an overly long half-life of 63 h.[4] Prolonged exposure of patients to drug levels becomes problematic once toxicity is encountered. Patients suffering from autoimmune diseases, such as RA, historically have not tolerated as much risk as the

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transplant community has been willing to accept. We therefore viewed rapamycin as an excellent starting point for synthesizing an improved agent, emphasizing similar in vivo efficacy, prompt measurable effect, and a shorter half-life. This communication briefly describes the synthesis and biological characterization of our most promising compounds.

Treatment of rapamycin (**1**) with trifluoromethanesulfonic anhydride and 2,6-lutidine in CH_2Cl_2, followed by filtration through a pad of silica gel (Et_2O), provided **2** in 95% yield (Scheme 1). The triflate **2** is used immediately in the subsequent reaction. 40-Epi-tetrazolyl-rapamycins **3** (ABT-578) and **4** were obtained in a 2:1 ratio in 34% yield after the reaction of **2** with tetrazole in isopropyl acetate.

Another promising analog, carbamate **6**, was synthesized, according to Scheme 2. Rapamycin was treated with finely ground *p*-nitrophenylcarbonate and pyridine at 0 °C, and then warmed to room temperature. After purification by flash chromatography, pure carbonate **5** was isolated in 73% yield. This key intermediate was treated with *N*,*O*-dimethylhydroxylamine hydrochloride in pyridine at room temperature, followed by heating to 50 °C. Chromatographic purification provided pure **6** in 90% yield.

Rapamycin (**1**) exerts its immunosuppressive activity by the formation of a trimolecular complex, imitating the first step in the biochemical mechanism of action of FK506.[5] The drug is required to bind to FKBP12, a peptidyl-prolyl-isomerase, before this molecular partnership inhibits the serine–threonine kinase TOR (or RAFT1).[6] The binding assay and MLR (mixed lymphocyte reaction) assays used below have been described previously.[7] Rapamycin (**1**) inhibits FKBP12 with an IC_{50} of 1.6 nM (Table 1).

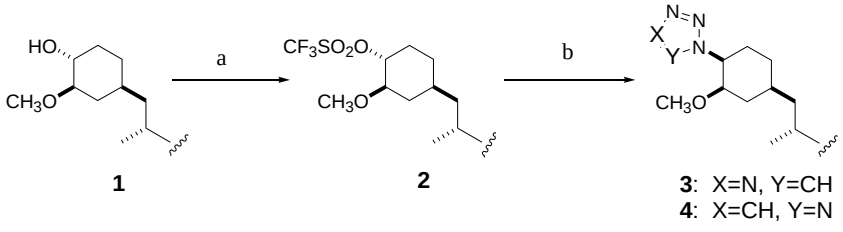
Table 1. IC_{50}, nM (*n*)[a]

Compound	FKBP12 binding	MLR Hu inhibition[b]	MLR Rt inhibition[c]
1	1.6 ± 0.9 (14)	1.6 ± 2.2 (72)	2600 ± 1900 (14)
3	3.3 ± 2.2 (3)	2.0 ± 4.4 (17)	1400 ± 600 (9)
4	4.8 ± 1.0 (3)	3.3 ± 2.2 (3)	1500 ± 600 (6)
6	5.8 ± 4.4 (8)	5.9 ± 6.1 (36)	2500 ± 1800 (11)

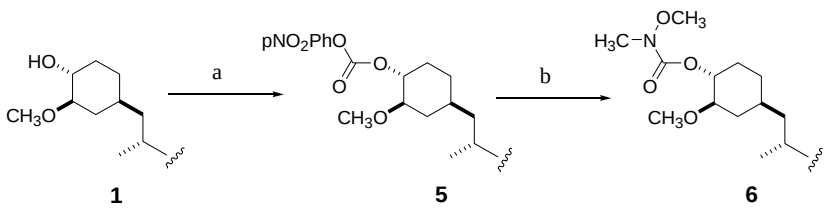
[a] Data reported as the mean ± SEM for (*n*) determinations as noted.
[b] Human mixed lymphocyte reaction, serum containing.
[c] Rat mixed lymphocyte reaction, serum free, Lewis rat lymph node cells stimulated by Brown–Norway splenocytes.

Likewise, analogs **3**, **4**, and **6**, exhibit IC_{50}s in the range from 3.3 to 5.8 nM. In vitro T-cell antiproliferative ability was assessed by comparison of the analogs to **1**, in the human (MLR Hu) and rat (MLR Rt) mixed lymphocyte reaction. The ability of all semi-synthetic rapamycin analogs to inhibit alloantigen-induced T-cell proliferation in the MLR Hu was similar to that of rapamycin (**1**), which measured 1.6 nM. The weakest inhibitor was carbamate **6**, with a potency of 5.9 nM. All compounds were much weaker inhibitors in the rat, with no differentiation shown between them. The low apparent potency with rat cells in vitro has been observed by others, but is little understood.[8]

Having established reasonable efficacy in vitro, the next stage required the evaluation of the compounds in autoimmune disease rat models. The delayed-type hypersensitivity (DTH Rt) response to antigen challenge is a T-cell-mediated inflammatory response that may mimic the pathologic response to altered self-proteins or microbial antigens involved in RA, and may thus serve as a reasonable RA model (Table 2).[9] Tetrazoles **3** and **4**, as well as carbamate **6**, demonstrated nearly equal efficacy with rapamycin **1**, with the weakest compound, **4**, only being fourfold less potent than **1**.



Scheme 1. Reagents and conditions: (a) i. 2,6-Lutidine (4.2 equiv), CH_2Cl_2 −78 °C, ii. $(CF_3SO_2)_2O$ added dropwise (2 equiv) −78 °C 15 min RT 20 min, 95%; (b) isopropyl acetate, tetrazole (2 equiv), diisopropylethylamine (2 equiv), RT 18 h, 34%, isomers **3** and **4** separated by silica gel chromatography eluting sequentially with hexane, hexane/ether (4:1, 3:1, 2:1, 1:1), ether, hexane/acetone (1:1).



Scheme 2. Reagents and conditions: (a) Bis(*p*-nitrophenyl)carbonate (2 equiv) 0 °C, pyridine addition, warmed to RT (1 h), 73%; (b) *N*,*O*-dimethylhydroxylamine hydrochloride (4.4 equiv), pyridine, RT (18 h), 50 °C (1 h), 90%.

Table 2. ED_{50}, mpk/day PO (*n*) (95% range)[a]

Compound	DTH Rt[b]	EAE Rt[c]	AA Rt[d]
1	0.5 (0.4–0.7) (35)	0.3 (0.2–0.4) (10)	0.2 (0.2–0.3) (20)
3	1.7 (0.9–7.7) (21)	1.2 (0.3–1.7) (10)	0.7 (0.5–0.9) (10)
4	2.0 (1.3–4.5) (7)	NA	NA
6	0.4 (0.1–1.0) (21)	0.5 (0.3–0.7) (10)	0.3 (0.2–0.5) (10)

[a] 95% confidence limits for *n* determinations as noted.
[b] Sprague–Dawley rat delayed-type hypersensitivity.
[c] Lewis rat experimental autoimmune encephalomyelitis.
[d] Lewis rat developing adjuvant-induced arthritis.

The rat experimental autoimmune encephalomyelitis (EAE Rt) model is used as a model of multiple sclerosis. EAE is an acute experimental neurological disease mediated by $CD4^+$ T cells, whose target is the central nervous system.[10] The disease is artificially induced by subcutaneously injecting the rat with homogenized isologous spinal cord in modified complete Freund's adjuvant. This disease is effectively abrogated by the three agents tested, with rapamycin (**1**) being most potent with an ED_{50} of 0.3 mpk/day and **3** being the least potent at 1.2 mpk/d.

The pathogenesis of human rheumatoid arthritis and adjuvant arthritis in the rat (AA Rt) shares several important features.[11] Among these are included T-cell mediation of the disease, deposition of fibrin in the joints, leukocyte influx into the synovium, and destruction of cartilage and bone. While this pathogenic process requires as long as 30–50 years in human RA, the AA Rt model accelerates the disease progression to end-stage disease after a mere 30–40 days post-antigen administration. Using a prophylactic approach, rapamycin (**1**) effectively blocks the development of AA with an ED_{50} of 0.2 mpk/day, whereas **3** and **6** show ED_{50}'s of 0.7 and 0.3 mpk/day, respectively. Abrogation of the soft tissue destruction in AA Rt is shown in Figure 1.[12] All three models, DTH Rt, EAE Rt, and AA Rt, therefore establish the efficacy of rapamycin (**1**), as well as **3** and **6**, in autoimmune disease models, despite the apparent weak rat whole-cell activity shown in Table 1. Since we have demonstrated efficacy in a species with weak whole cell activity, we are optimistic that efficacy can be demonstrated in human subjects since the whole cell humam MLR response is more sensitive to all three compounds.

The most favorable profiles for **3** and **6** would also include decreased risk of toxicity for the patient population. One aspect of this is a more controllable exposure of the drug toward the patient, which could be influenced by an attenuation of some key pharmacokinetic parameter. One can observe that in rats (Table 3), compounds **3** and **6** both have dramatically shorter half-lives than rapamycin (**1**), as well as lower C_{max} and AUC values upon a single oral drug dosing of 2.5 mpk. The oral bioavailability (*F*) of both new compounds is significantly lower than **1**, 12.1% (**3**) and 6.2% (**6**) versus 43.2% for **1**. Nevertheless, sufficient levels of each drug are achievable in the DTH, EAE, and AA rat models to demonstrate a robust biological effect, and it should be noted that the oral bioavailability of an oral solution of **1** is advertised to be 14% in humans.[13] It is gratifying that the rat PK model has been a reliable comparative predictor of the PK behavior of **1** versus **3** in human subjects. Indeed, in a multiple dose escalation study, the half-life of **3** in humans was determined to be 25–31 h, significantly shorter than the 63 h reported for **1**.[14]

Unsurprisingly, it was observed that rapamycin (**1**) was a metabolite of **6**. In a separate experiment, oral dosing

Table 3. Male Sprague–Dawley rat pharmacokinetic parameters (2.5 mpk PO, solution)

Compound	*n*	$t_{1/2}$ (h),	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0-\infty}$ (ng h/mL)	*F* (%)
1	7	33.4	50.8	0.5	559	43.2
3	3	7.9	46.7	0.4	163	12.1
6	3	8.7	29.7	0.4	132	6.2

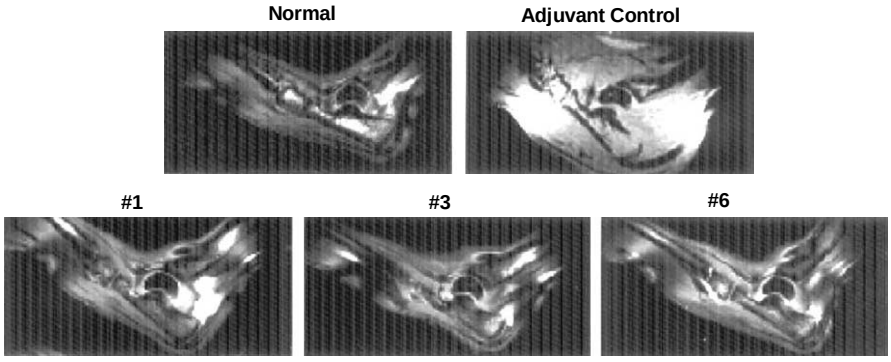


Figure 1. Magnetic resonance images showing soft tissue changes in sagittal sections from left hindpaws from normal, vehicle dosed 15 day adjuvant controls, and rats dosed from day 0 to 15 with compounds **1** (1 mpk), **3** (3 mpk) and **6** (1 mpk).

Table 4. Male Sprague–Dawley rat pharmacokinetic parameters (2.5 mpk PO, solution)

Compound	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0-\infty}$ (ng h/mL)
6	23.2	0.3	127
1	9.9	1.0	83

of rats with 2.5 mpk of **6** revealed that the maximal concentration of **1** was significantly lower than the C_{max} for **6** (Table 4). Yet since the oral half-life of **1** is significantly longer than **6**, the AUCs are very similar, yet the sum of both is still much lower than the AUC generated by an oral administration of 2.5 mpk of **1** alone. Whether or not this attenuated exposure of the animal to **1** via the active 'pro-drug' **6** translates to a less insulting side effect profile would need to be established by clinical trials in human patients.

In summary, we have identified two promising analogs of rapamycin that show impressive efficacy in several models of autoimmune disease, comparing favorably in efficacy to rapamycin. Furthermore, it is possible that these agents may offer a safety advantage with respect to exposure toward parent drug. Indeed, it has been found that significant circulating levels of rapamycin were found in rats that were dosed with **6**. While **6** is several fold less potent than **1**, it has yet to be shown that suppression of the maximal concentrations of either compound (**1** or **6**) via this strategy can result in the reduction of adverse side effects. Further characterization of drug candidate **3** has already occurred in human subjects and will be the subject of future reports, especially in regard to its use on drug-coated stents. The use of **3** in autoimmune diseases, however, and continuing toxicological studies, is clearly a tantalizing prospect. Establishment of efficacy in rat autoimmune disease models has provided a cause for optimism toward its eventual use in the treatment of diseases of the human immune system.

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