Synthesis and Biological Evaluation of Novel Cyclosporin A Analogues: Potential Soft Drugs for the Treatment of Autoimmune Diseases

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Abstract: Cyclosporin A is effective in the treatment of asthma patients, but its chronic use is limited by toxicity. Novel cyclosporin A analogues were synthesized utilizing the olefin metathesis reaction and evaluated in a calcineurin A inhibition assay. The novel analogues demonstrated activity comparable to activity of the parent molecule and are potential soft drugs.

Introduction. Cyclosporin A[1] (**1**, Chart 1) is a neutral cyclic undecapeptide that belongs to the class of calcineurin[2] inhibitors (CNIs). Cyclosporin A (Sandimmune, Neoral) is widely used to prevent and treat organ transplant rejection and has potential therapeutic use in the treatment of autoimmune diseases such as asthma, psoriasis, atopic dermatitis, and rheumatoid arthritis.[3] When given orally, cyclosporin A improves lung function and lessens symptom exacerbations in corticosteroid-dependent asthma patients.[4] Short-term inhaled cyclosporin A is well tolerated by humans, but its chronic use is limited by toxicity.[5] The most serious side effect, nephrotoxicity, is believed to be mechanism-based, i.e., caused by the inhibition of calcineurin.[6] A potential approach to circumvent the mechanism-based toxicity of cyclosporin A is to develop "soft drug" analogues that can be administered locally and are subsequently biotransformed to an inactive (and thus nontoxic) metabolite.

Since the soft drug approach has been used extensively with steroid drugs,[7] we decided to apply the same principle to the design of a novel series of cyclosporin A analogues. We aimed to design analogues that retain the biological activity of the parent compound but are rapidly inactivated under physiological conditions, thus minimizing systemic exposure and thereby minimizing toxicity. One common soft drug strategy is the incorporation of an ester functionality into the drug. The ester can then be hydrolyzed (and thus inactivated) by ubiquitous esterases[8] (Scheme 1).

Extensive structure−activity relationship studies on cyclosporin A have shown that the side chain of the unusual amino acid *N*,4-dimethyl-4(*R*)-[2(*E*)-butenyl]-L-threonine (MeBmt, **2**, Chart 2) is essential (but not sufficient) for biological activity.[9] Since this side chain plays an essential role and is the only side chain with readily modifiable functional groups, it became the focus of our semisynthetic efforts.

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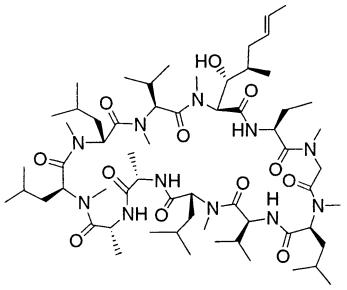
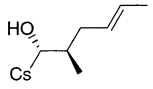
Chart 1



1, Cyclosporin A, abbreviated as



Scheme 1. Ester Soft Drug Model

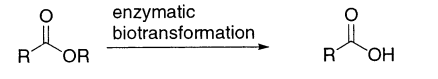
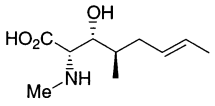
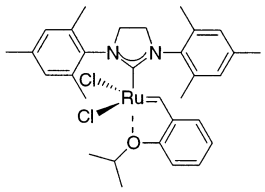


Chart 2



2, MeBmt

Chart 3. Hoveyda's Second-Generation Metathesis Catalyst



3

Chemistry. We decided to explore the olefin cross metathesis reaction[10] as an efficient one-step transformation of cyclosporin A into suitable soft drug analogues. We screened several ruthenium catalysts, using the cross metathesis of cyclosporin A and dimethyl maleate as a test reaction. Hoveyda's second-generation metathesis catalyst[11] (**3**, Chart 3) proved to be the best catalyst for this cross metathesis. Upon further optimization, we developed a highly scalable process that routinely affords 85−90% yield of >95% pure compound. NMR studies showed that the metathesis reaction proceeds regioselectively, affording the trans isomer exclusively.

We sought to synthesize esters of varying steric bulk and electronics, reasoning that modification of the ester moiety would provide a simple means of "titrating" the hydrolytic half-life of the soft drug. We also sought to synthesize the corresponding acid to demonstrate that the product of ester hydrolysis would be inactive. The cross metathesis reaction of cyclosporin A with 15 equiv of the appropriate maleate diester in the presence of

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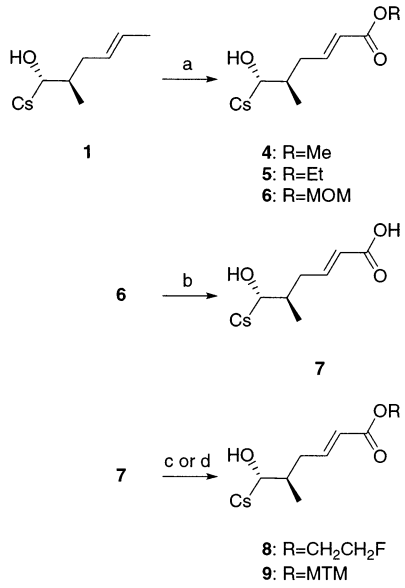
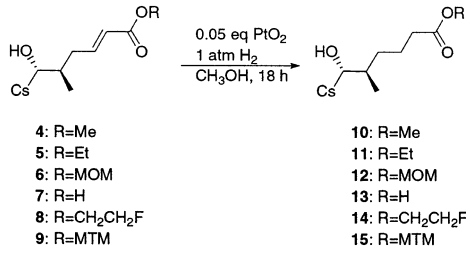
Scheme 2. Synthesis of Unsaturated Esters and Acid[a]



[a] Reagents: (a) 15 equiv of maleate diester, 0.08 equiv of **3**, CH_2Cl_2, 40 °C, 18 h, 85−90%; (b) 2% concentrated aqueous HBr, 28% H_2O, 70% ^iPrOH, gradually heated until disappearance of **6**, 80%; (c) 10 equiv of $BrCH_2CH_2F$, 20 equiv of $NaHCO_3$, DMF, 24 h, 100%; (d) 5 equiv of MTMCl, 10 equiv of $^iPr_2NEt$, CH_2Cl_2, 12 h, 100%.

Scheme 3. Synthesis of Saturated Analogues



0.08 equiv of catalyst **3** in refluxing dichloromethane for 18 h afforded methyl ester **4**, ethyl ester **5**, and methoxymethyl (MOM) ester **6**. Standard MOM deprotection conditions afforded acid **7**. Acid **7** was esterified under standard alkylation conditions to afford 2-fluoroethyl ester **8** and under standard methylthiomethyl (MTM) protection conditions to afford MTM ester **9** (Scheme 2).

We also prepared the corresponding saturated analogues to determine whether these compounds retain biological activity (Scheme 3). Hydrogenation of the unsaturated analogues under standard conditions with catalytic platinum(IV) oxide readily afforded saturated methyl ester **10**, ethyl ester **11**, MOM ester **12**, acid **13**, 2-fluoroethyl ester **14**, and MTM **15** in quantitative yield.

Results and Discussion. The novel analogues were evaluated for biological activity in a calcineurin inhibition assay. Calcineurin inhibition was monitored by measuring the catalytic release of phosphate from a phosphopeptide substrate using the BioMOL Green detection reagent.[12] The measured IC_{50} values are shown in Table 1.

Acids **7** and **13** show no calcineurin inhibition activity. If the toxicity of cyclosporin A and its analogues truly is mechanism-based, then with no calcineurin inhibition activity, acids **7** and **13** should not exhibit toxicity characteristic of the parent molecule. A preliminary toxicology study comparing acid **7** with cyclosporin A supported the nontoxic nature of acid **7**.

Table 1. Calcineurin Inhibition Data for Cyclosporin A Analogues

cyclosporin A analogues	IC_{50} (nM)
cyclosporin A (**1**)	183
unsaturated esters	
methyl (**4**)	581
ethyl (**5**)	363
2-fluoroethyl (**8**)	636
mom (**6**)	742
mtm (**9**)	348
unsaturated acid (**7**)	>5000
saturated esters	
methyl (**10**)	890
ethyl (**11**)	840
2-fluoroethyl (**14**)	1539
MOM (**6**)	1916
MTM (**9**)	2147
saturated acid (**13**)	>5000

Unsaturated esters **4**−**6**, **8**, and **9** show calcineurin inhibition activity comparable to the activity of cyclosporin A. Cleavage of any of these esters by an esterase would yield acid **7** as a metabolite.

Saturated esters **10**−**12**, **14**, and **15** show significantly reduced calcineurin inhibition activity relative to their unsaturated analogues. Nonetheless, methyl ester **10** and ethyl ester **11** show significant calcineurin inhibition activity. Cleavage of any of these esters would yield acid **13** as a metabolite.

In conclusion, we have developed a rapid and efficient semisynthetic route to a family of cyclosporin A analogues that may yield useful soft drugs. In our biochemical screen, analogues containing the ester functionality show comparable activity compared to cyclosporin A. The corresponding acids (the presumed metabolites) are inactive and appear to demonstrate significantly less toxicity than cyclosporin A. More members of this family of analogues are being synthesized and evaluated, some of which show improved calcineurin inhibition. Studies to establish the efficacy and pharmacokinetics of our analogues are in progress.

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Supporting Information Available: Detailed experimental procedures for syntheses and biological assay conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Absorbance at 620 nm is proportional to free phosphate concentration.

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