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SYNTHESIS AND PHOSPHATASE INHIBITORY ACTIVITY OF ANALOGS OF SULFIRCIN

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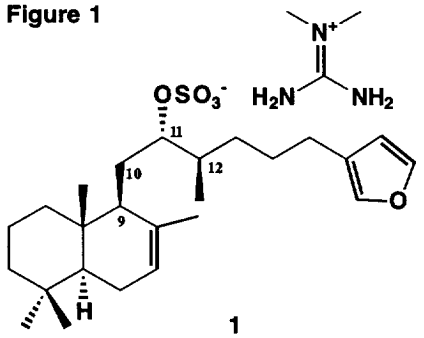
Abstract: Analogs of sulfircin (1) were synthesized and tested for inhibitory activity against a panel of phosphatases. We attempted to optimize the potency and selectivity of sulfircin for Cdc25A by modifying three structural areas of the molecule. An anionic group is required for potency and malonate was found to be an effective substitute for sulfate. © 1997 Elsevier Science Ltd.

Sulfircin (1) is a marine natural product isolated from a deep-water sponge of the genus *Ircinia*. Its isolation and structure elucidation were reported in 1989 by Wright and coworkers.[1,2] Key features of this molecule (Figure 1) include a bicyclic sesquiterpene moiety and an aliphatic sidechain terminating in a furyl residue flanking a sulfate group. While sulfircin was initially discovered in an antifungal assay to have a minimum inhibitory concentration against *Candida albicans* of 25 μg/mL,[2] subsequent testing[3] (*vide infra*) against a panel of phosphatases including PTP1B,[4] VHR,[5] and Cdc25A[6] indicated that sulfircin was a nonspecific protein phosphatase inhibitor. Cdc25A is a member of the dual-specificity family of protein phosphatases and is responsible for the activation of the important cell cycle regulatory complex cyclin A/cdk2 by dephosphorylation of pThr14 and pTyr15 of the cdk2 subunit. In an effort to discover more potent and selective inhibitors for Cdc25A, we prepared several analogs of sulfircin. The synthesis and biological activity of these analogs is described herein.

Figure 1



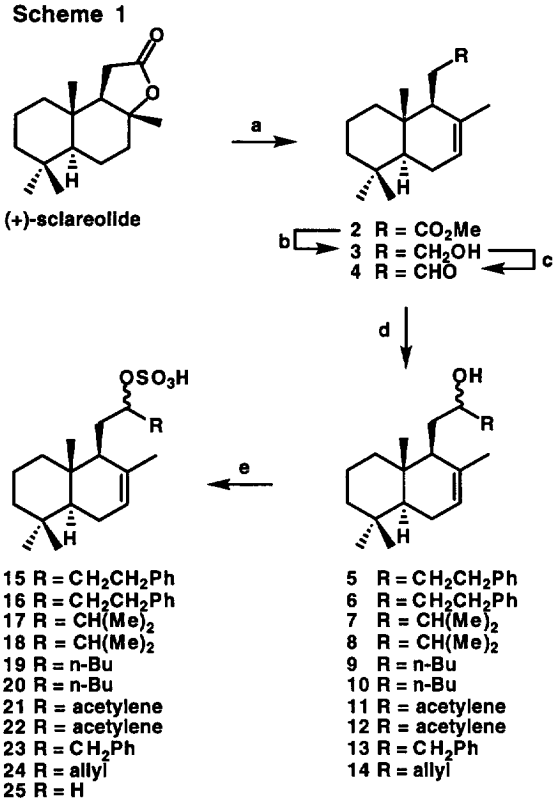
1

enzyme	IC_{50} (μM)
Cdc25A	**7.8**
***Candida* Cdc25**	**12.7**
PTP1B	**29.8**
VHR	**4.7**

Our synthetic goal was to reduce the structural and stereochemical complexity of this molecule. Sulfircin can be divided into three structural regions: the bicyclic sesquiterpene core, the alkylfuryl sidechain, and the sulfate group. We first turned our attention to the sidechain. The synthesis of sidechain analogs that vary in both the length and steric bulk (Scheme 1) began with commercially available (+)-sclareolide. The lactone was opened and concomitantly dehydrated to yield the unsaturated methyl ester **2**.[7] The ester was then reduced to the alcohol

3[8] and subsequently oxidized to the aldehyde **4**.[9] The aldehyde was treated with a variety of Grignard reagents to give the resulting diastereomeric alcohols **5** - **14**. In some cases these diastereomeric alcohols were chromatographically separable and were functionalized as single isomers; however, compounds that were not separable were carried on as the diastereomeric mixture (isomeric ratio was ~1:1). The alcohols, including compound **3**, were then treated with SO_3•pyridine to yield the desired sulfated analogs **15**-**25**.

Scheme 1

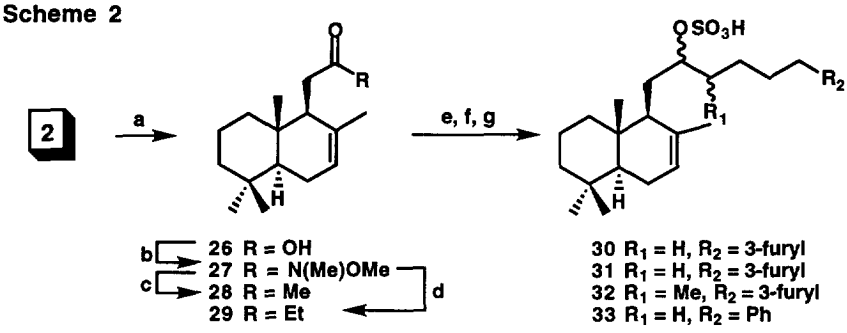


Reagents and conditions: a. H_2SO_4, MeOH, reflux, 20 h, 59 %; b. $LiAlH_4$, Et_2O, 0 °C, 15 min, 100 %; c. $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C to rt, 1 h, 91 %; d. RMgX, THF, rt, 1 h, 48 - 72 %; e. SO_3•pyridine, pyridine, rt, 24 h, 40 - 87 %.

To investigate the importance of the C-12 methyl group, methyl ester **2** was converted (Scheme 2) to the methyl **28**[10] and ethyl **29** ketones via the Weinreb amide **27**. Regiospecific lithiation at the less hindered position followed by alkylation with the appropriate alkyl iodides afforded the extended chain compounds. The ketones were then reduced and the resulting alcohols were sulfated as previously described to obtain analogs **30** - **33**. The alcohols corresponding to sulfates **30** and **31** were chromatographically separable, whereas those corresponding to **32** and **33** (diastereomeric ratio of **33** was ~1:1) were not.

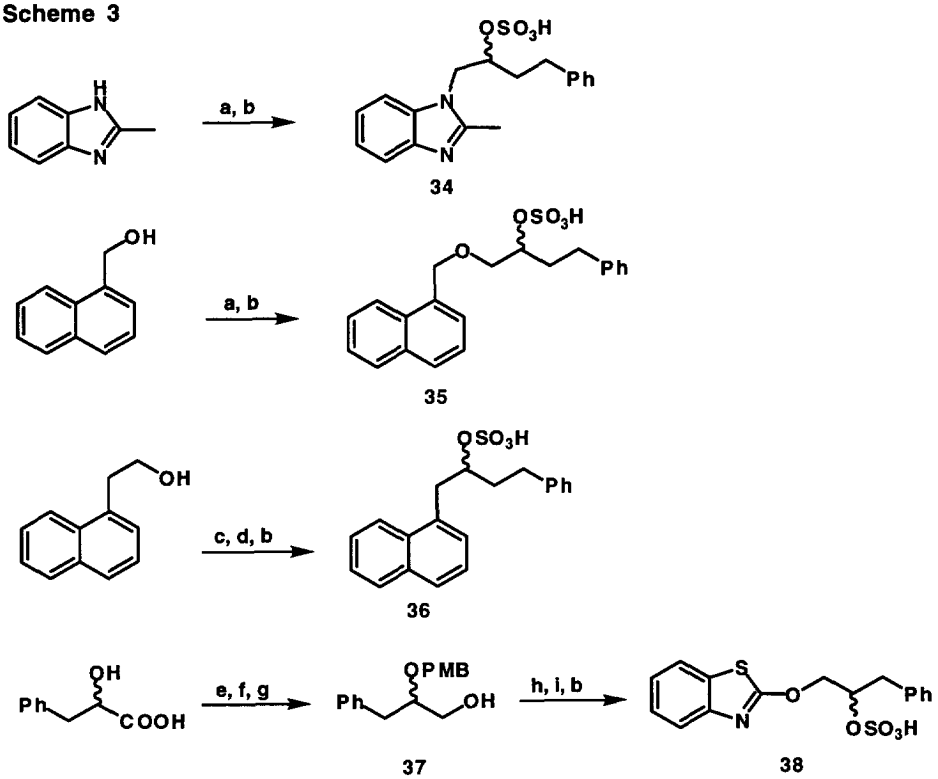
Replacement of the bicyclic sesquiterpene system with aromatic cores allowed us to explore whether the sesquiterpene nucleus was involved in specific interactions with the enzyme. The synthesis of these analogs is described in Scheme 3.

Scheme 2



Reagents and conditions: a. LiOH, MeOH, H_2O, reflux 48 h, 100 %; b. HN(Me)OMe•HCl, HOBT, HBTU, DIEA, DMF, rt, 1 h, 82 %; c. MeMgBr, THF, rt, 3 h, 89 %; d. EtMgBr, THF, rt, 1 h, 75 %; e. LDA, 3-(3-iodopropyl)furan or (3-iodopropyl)benzene, THF:DMPU (9:1), 0 °C to rt, 16 h, 64 %; f. $NaBH_4$, MeOH, rt, 48 h, 66 %; g. SO_3•pyridine, pyridine, rt, 24 h, 95 %.

Scheme 3

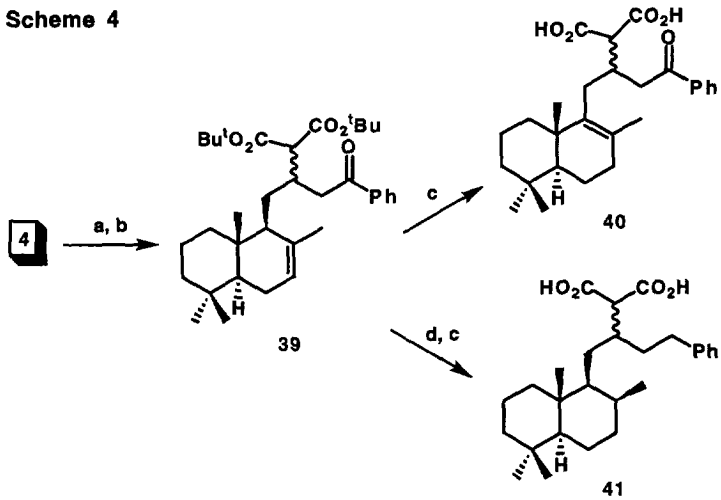


Reagents and conditions: a. NaH, (3,4-epoxybutyl)benzene, $Ti(OiPr)_4$, THF, rt, 20 h, 35 %; b. SO_3•pyr, pyridine, rt, 24 h, 67 %; c. $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C to rt, 4 h, 33 %; d. $PhCH_2CH_2MgCl$, THF, rt, 4 h, 70 %; e. MeOH, H_2SO_4, reflux, 16 h; f. NaH, p-methoxybenzyl chloride, Bu_4NI, DMF, rt, 16 h, 74 % (2 steps); g. $LiBEt_3H$, THF, 0 °C, 1 h, 89 %; h. NaH, 2-chlorobenzothiazole, DMF, rt, 16 h, 59 %; i. CAN, CH_3CN, H_2O, rt, 15 min.

Regiospecific alkylation of (3,4-epoxybutyl)benzene with either 2-methylbenzimidazole or 1-naphthalenemethanol followed by sulfation of the intermediate alcohol provided analogs **34** and **35**. Another analog was made by first oxidizing 1-(2-hydroxyethyl)naphthalene to the aldehyde followed by reaction with phenethylmagnesium chloride and sulfation of the resulting alcohol to give compound **36**. The synthesis of the benzothiazole derivative began with (+/-)-phenyllactic acid. The acid was converted to the methyl ester, the remaining alcohol was alkylated with p-methoxybenzyl chloride, and the ester was reduced to give the alcohol **37**. The alcohol was then alkylated with 2-chlorobenzothiazole, the *p*-methoxybenzyl ether was then oxidatively removed, and the resulting alcohol was sulfated to yield compound **38**.

Because the anionic nature of the sulfate group may hinder the ability of sulfircin to penetrate the cell membrane, as well as the susceptibility of sulfate to hydrolysis, we were interested in finding stable replacements for this group. The carboxyl group has been reported to be isosteric with the sulfate group.[11] We expected that the malonyl group would also serve as a surrogate for the sulfate moiety. As shown in Scheme 4, aldehyde **4** was treated with the required ylide to give an α,β-unsaturated ketone, which was then treated with the anion of di-*tert*-butyl malonate to give the Michael product **39** as a mixture of isomers (diastereomeric ratio of ~3:1). Attempted deprotection with TFA resulted in concomitant isomerization of the olefin to produce the tetrasubstituted olefin analog **40**. Another analog was prepared by reduction of compound **39**, followed by TFA treatment to yield compound **41**. Hydrogenation not only reduces the ketone, but also saturates the bicyclic double bond. Hydrogenation from the less hindered α face was confirmed by ^{1}H NMR.

Scheme 4



Reagents and conditions: a. benzoylmethylenetriphenylphosphorane, toluene, rt, 24 h, 88 %; b. di-*t*-butyl malonate, NaH, THF, 0 °C to rt, 5 h, 74 %; c. TFA, CH_2Cl_2, rt, 4 h, 95 %; d. H_2, Pd on C (10%), EtOAc, 16 h, 95 %.

The synthetic compounds[12] were then tested for inhibitory activity against Cdc25A, PTP1B, and VHR.[13] The activities for the sidechain analogs are shown in Table 1. The overall length of the aliphatic sidechain was important to the inhibitory activity of these analogs. The analogs that had the smallest groups **21**, **22** (both acetylene), and **25** (H) were the least potent. The compounds with slightly larger groups **17** - **20**, **23**, and **24** were somewhat more potent with the most potent compounds being those with the longest chains **30** - **33**. The

sulfate moiety was critical for inhibitory activity, possibly by binding in either the pThr or pTyr binding pocket of Cdc25. However, the stereochemistry of this group seemed to have no effect on IC_{50} since none of the single diastereomer sulfates had significantly greater potency than the corresponding epimer (**15** vs. **16**; **17** vs. **18**; **19** vs. **20**; **21** vs. **22**; **30** vs. **31**). Removal of the C-12 methyl group had no effect on potency as compounds **30** and **31** were equipotent to sulfircin. Replacement of the furyl substituent with phenyl **33** had little effect, although shortening the alkyl chain (**15**, **16**, and **23**) again reduced the potency.

Table 1. IC_{50}s (μM) for sulfircin and sidechain analogs.

compound	Cdc25A	PTP1B	VHR
sulfircin (1)	7.8	29.8	4.7
15	16.5	40.5	25.5
16	10.6	31	10
17	46.3	110	52
18	98	170	73
19	41	96.5	37.5
20	32	50	21
21	115	215	96
22	74	160	39
23	29	87	28
24	60	185	47
25	170	>320	275
30	2.8	4.4	4.6
31	4.2	11	10.8
32	8.4	17	7.9
33	3.8	5.4	4.6

The results of the analogs with variations in the sulfate region (Table 2) were of interest. Analogs with a free hydroxyl group (**5**, **13**, and **14**) were all inactive which led us to believe that a negatively charged group was necessary for potency. We, therefore, attempted to find a stable replacement for the sulfate moiety. The malonate compounds (**40** and **41**) were then synthesized and found to be equipotent with sulfircin itself.

Table 2. IC_{50}s (μM) for sulfate analogs.

compound	Cdc25A	PTP1B	VHR
5	>290	>290	45
13	>310	>310	66
14	>360	>360	120
40	31	68	8.6
41	6.3	21	5.6

In the hope that a less lipophilic bicyclic nucleus would simplify the synthesis of analogs, we prepared the benzimidazole, benzothiazole, and naphthyl analogs. These compounds are considerably more planar than the bicyclic sesquiterpene nucleus. Unfortunately, all of these analogs resulted in a loss of potency. The IC_{50}s for **34**, **35**, **36**, and **38** against each of the enzymes (Cdc25A, PTP1B, and VHR) were >100 μM.

In summary, we have described efficient synthetic routes to analogs of the natural product sulfircin. Analogs of each of the three important regions of the molecule have been synthesized and assayed. The results indicate that we have found replacements for the furyl sidechain and the sulfate moiety. The replacement of the sulfate moiety with a malonate group appears to be advantageous since the latter is synthetically accessible, stable to hydrolysis, and amenable to the synthesis of prodrugs.

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