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Potent Kv1.3 inhibitors from correolide—modification of the C18 position

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Abstract—Kv1.3, the voltage-gated potassium channel in human T cells, represents a new target for treating immunosuppression and autoimmune diseases. Correolide (**1**), a pentacyclic natural product, is a potent and selective Kv1.3 channel blocker. Simplification of correolide via removal of its E-ring generates enone **4**, whose modification produced a new series of tetracyclic Kv1.3 blockers. The structure–activity relationship for this class of compounds in two functional assays, Rb_Kv and human T cell proliferation, is presented herein. The most potent analog **43** is 15-fold more potent than correolide as inhibitor of human T cell proliferation.

The voltage-gated potassium channel, Kv1.3, sets the resting membrane potential of human T lymphocytes.[1,2] Blockade of Kv1.3 channels causes membrane depolarization and this leads to attenuation of the influx of extracellular calcium that occurs upon activation of the T cell receptor complex. Since this increase in calcium concentration is necessary for T cell proliferation, a selective Kv1.3 channel blocker may represent a new class of immunosuppressant agent.[3–5] Levels of Kv1.3 channels are significantly and specifically up-regulated in pathogenic myelin-reactive T cells from patients with multiple sclerosis (MS) and Kv1.3 inhibitors prevent and reverse the symptoms of the disease in animal models of MS. These data suggest that Kv1.3 inhibitors will have utility in MS and other autoimmune disorders.[6]

Correolide **1**, a pentacyclic nor-triterpenoid isolated from the plant *Spachea correa*, is a selective, bioavailable inhibitor of the Kv1 family of potassium channels and inhibits human T cell proliferation in a dose-dependent manner.[7–9] Correolide's complexity and limited supply warrant attempts toward simplification of the molecule with the goal of preparing synthetic analogs. The results of SAR studies at C18 of enone **4** in the Kv1.3 functional assay (Rb_Kv) that measures depolarization-induced $^{86}Rb^{+}$ efflux from CHO cells stably transfected with the human Kv1.3 channel,[8] and in human T cell[9] proliferation are presented herein.[10–12]

The conversion of **1** to oxepin **2** and the excision of the E-ring via a double-retroaldol fragmentation reaction of the C20 ketone **3** to tetracyclic enone **4** were described in an earlier publication (Scheme 1).[13]

This process eliminates five stereocenters but the simplification is accompanied by a significant drop in potency. The challenge now is to restore potency.

Keywords: Potassium channel; Kv1.3; Correolide; Ion channel blocker; T cell; Immunosuppressant.
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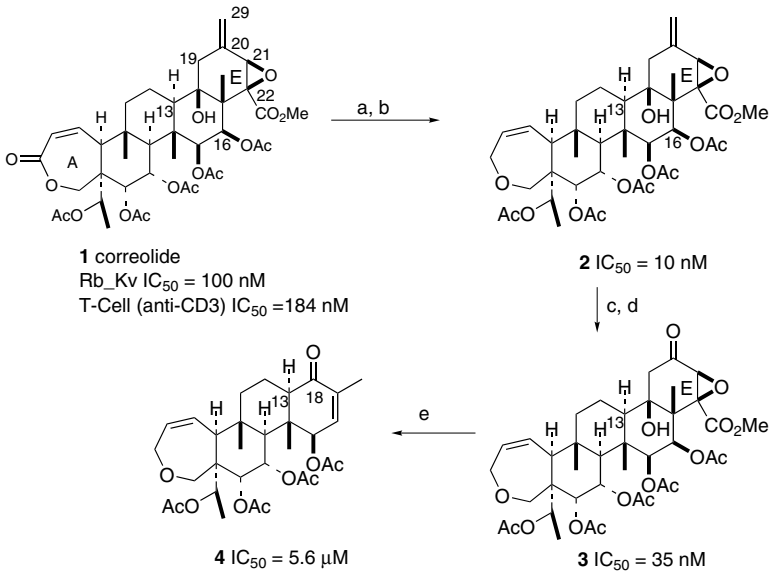


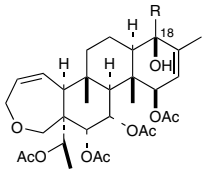
Scheme 1. Reagents and conditions: (a) LiHAl(O*t*Bu)$_3$, CH_2Cl_2, 0 °C–rt, 18 h, 97%; (b) BF_3–Et_2O, Et_3SiH, CH_2Cl_2, rt, 0.5 h, 88%; (c) OsO_4, NMMO; (d) $NaIO_4$, 95%, two steps; (e) LiCl, 125 °C, DMSO, 5 h, 66%.

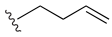
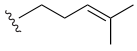
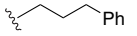
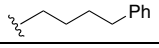
Models of **4** suggest that carbanion addition to the C18 carbonyl would occur from the α-face of the tetracycle since approach from the β-face would be blocked by the C14 methyl group. In the product of this addition, the hydroxyl group (Fig. 1) would have the same orientation as the C18 hydroxy group of correolide and the carbon chain would occupy the same space as the E-ring.

Addition of Grignard reagents to enone **4** at C18 was stereospecific. The stereochemistry of the adducts was confirmed to be as predicted by extensive NMR studies, which showed a strong NOE between the C18 hydroxy proton and the C14 methyl group. It was also determined that there was no epimerization at H13 during the reaction.

Two series of analogs were prepared from Grignard addition reactions and their data are listed in Table 1. In the alkyl series, the 2-methyl-2-pentenyl analog **9** is the most potent. In the second series, aryl groups are attached to C18 via tethers of varying lengths. That compound **12**, in which the aryl group is attached to C18 by a 2-carbon tether, is the most potent is consistent with the SAR of the alkyl series. It is also consistent with computer modeling studies, which show the ethylene group of compound **12** overlapping well with C19 and C20 of correolide (Fig. 2). Compound **12** is 4-fold more potent than correolide in the Rb_Kv assay.

Table 1. C18 SAR in Rb_Kv assay[8]



Compd	R	IC_{50} (nM)
6		1814
7		143
8		664
9		21
10		2000
11		380
12		26
13		150
14		270

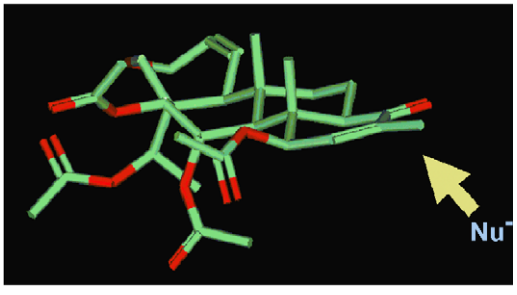


Figure 1. Nucleophilic addition to enone 4.

In the models presented in Figure 2, the phenyl group of **12** occupies the same space as the epoxide and methyl ester groups on C21 and C22 of correolide. Therefore, a series of substituted phenyl analogs were prepared. The activities of these analogs (**12**, **16**–**24**) are listed in Table 2. In general, they are more potent than correolide in the Rb_Kv assay; substituents at the *para*-position of the phenyl ring reduce potency (**24**). In the T cell assay,

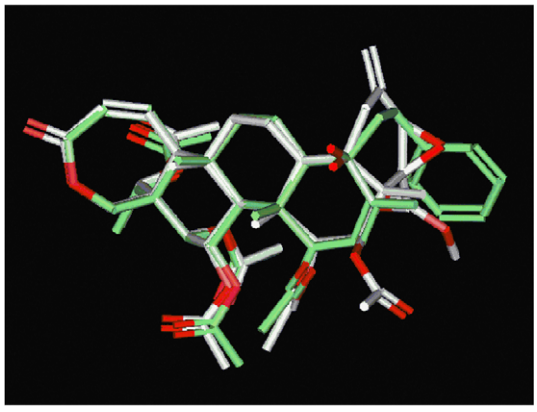
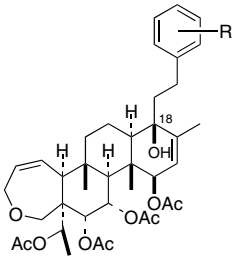


Figure 2. Superposition of correolide and **12**.

Table 2. C18 SAR in Rb_Kv and T cell[9] assays (IC_5, nM)

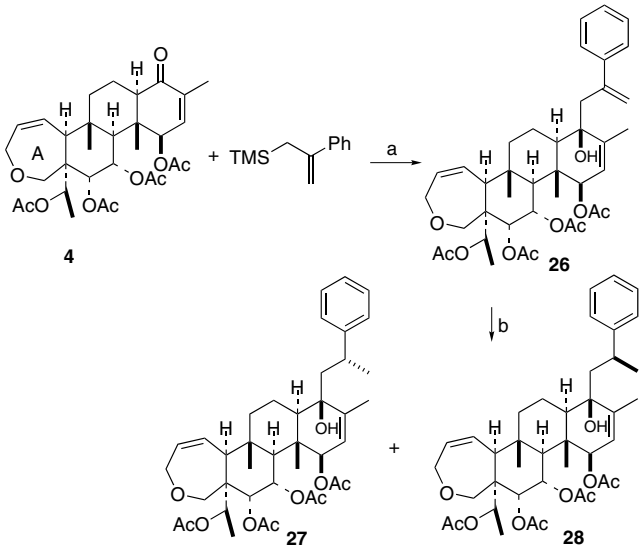


Compd	R	Rb_Kv	T cell	Ratio[a]
12	H	26	460	0.40
16	2-F	74	245	0.75
17	2-CF_3	87	1840	0.10
18	2-Me	41	194	0.95
19	2-OH	26	428	0.43
20	2-OAc	31	—	—
21	2-OMe	14	142	1.30
22	2-OEt	34	80	2.30
23	3-OMe	24	1314	0.14
24	4-OMe	130	—	—

[a] IC_{50} ratio of correolide to compound (bigger is more potent).

2-alkoxy analogs **21** and **22** are the most potent. Although certain compounds are more potent than correolide in the Rb_Kv assay (i.e., **12**, **19** and **23**), this potency does not translate into inhibition of T cell proliferation. The differences may be to due to the differences in incubation times used in the assays. Since compounds access the channel from the inside of the cell, parameters such as cell penetration can contribute to the apparent compound affinity in the short incubation time of the Rb_Kv assay, but they may not be as important in the T cell assay, where compounds are present for longer time.

A double bond was introduced at the carbon next to the phenyl in **12** to mimic the C20–C29 double bond of correolide. Lewis acid catalyzed addition of 2-phenylallylsilane to enone **4** occurs from the less-hindered face to afford **26** (Scheme 2), which has the same C18 configuration as the Grignard addition products and correolide. Compound **26** is 3.5-fold more potent than **12** in the T



Scheme 2. Reagents and conditions: (a) $TiCl_4$, −40 °C, CH_2Cl_2, 12 h, 80%; (b) Wilkinson's catalyst, H_2 (50 psi), THF, 23 h, rt, 90%.

cell assay and equivalent to correolide. This result is consistent with modeling studies indicating that the double bond at **26** overlaps well with the C20–C29 alkene of correolide (Fig. 3).

The double bond of the C18 substituent in **26** was selectively reduced with hydrogen in presence of Wilkinson's catalyst to provide **27** and **28** in a 5:1 ratio. The stereochemistry of the newly generated methyl center in **27** and **28** was assigned based on independent synthesis (Scheme 3).

The SAR data of the compounds with substitutions in the ethylene tether are summarized in Table 3. Although compound **25** is a 1:1 mixture of diastereomers, its activity suggests that any substitution at the carbon next to C18 has a detrimental effect on the Rb_Kv activity (**25**).

Substitution at the carbon next to the phenyl affects the orientation of the C18 substituent. Models show that the phenyl group and olefin of **26** overlap closely with

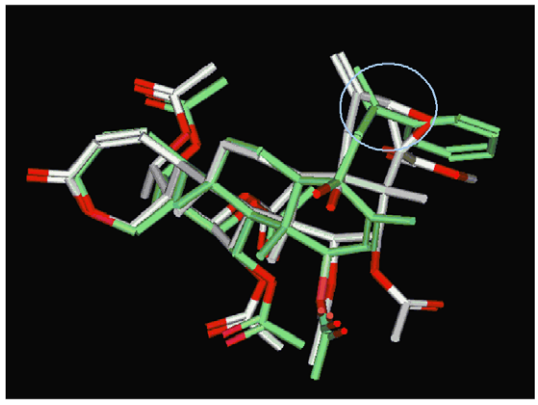
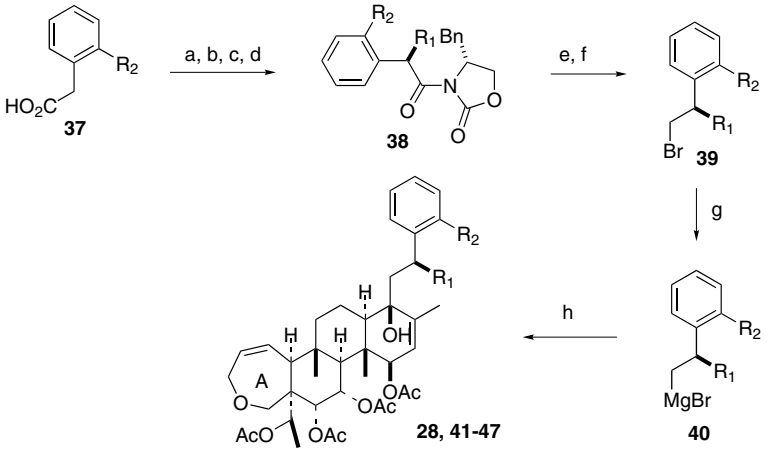
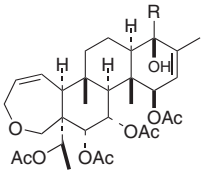


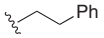
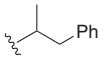
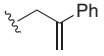
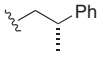
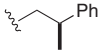
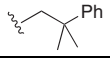
Figure 3. Superposition of correolide and **26**.



Scheme 3. Reagents and conditions: (a) *t*-BuCOCl, Et_3N, −78 °C, THF; (b) (*R*)-4-benzyl-2-oxazolidinone, BuLi, −78 °C, THF; (c) $NaN(TMS)_2$, −78 °C, THF; (d) R_1I; (e) LAH, rt, THF; (f) CBr_4, PPh_3, rt, ether; (g) Mg, ether; (h) **4**, 70 °C, THF.

Table 3. C18 SAR in Rb_Kv and T cell assays (IC_{50}, nM)



Compd	R	Rb_Kv	T cell	T cell ratio[a]
12		26	460	0.4
25		200	—	—
26		19	131	1.4
27		120	613	0.3
28		12	102	1.8
29		19	184	1.0

[a] IC_{50} ratio of correolide to compound.

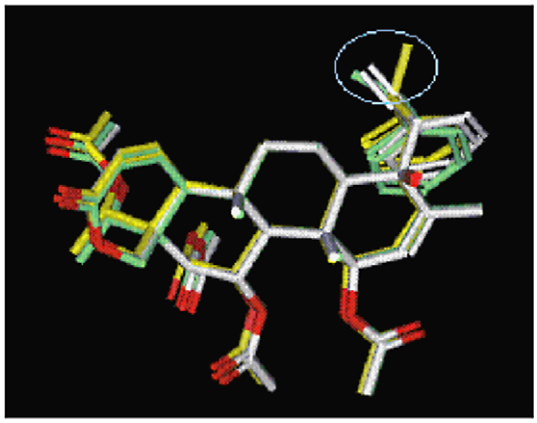


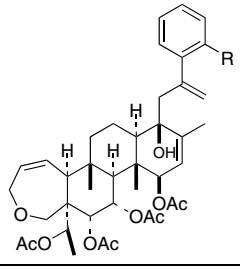
Figure 4. Superposition of **26**, **27** and **28**.

the phenyl group and (*S*)-Me of **28** (Fig. 4).Vinyl analog **26**, (*S*)-Me analog **28** and *gem*-dimethyl analog **29** are potent blockers of the Kv1.3 channel in both the Rb_Kv and the T cell proliferation assays. The (*R*)-Me compound **27** is significantly less potent. These results suggest that (*S*)-Me and vinyl groups at the carbon next to the phenyl enhance the T cell potency. The potency enhancement of the (*S*)-Me group is not diminished by the presence of an (*R*) substituent (**28** vs **29**).

Analogs containing the two potency enhancing substitution patterns (substitution at the *ortho*-position of phenyl and a vinyl group at the carbon next to the phenyl) were then prepared (Table 4). Hybrid analogs with lipophilic phenyl substituents are potent inhibitors of T cell proliferation. The most potent compound in this series, thioanisole **32**, is 12.5-fold more potent than correolide in the T cell proliferation assay. The two potency enhancing substitution patterns have a synergistic effect on inhibition of T cell proliferation.

Phenethyl analogs (**41**–**47**) with (*S*)-substitution on the tether were prepared according to Scheme 3. Substituted

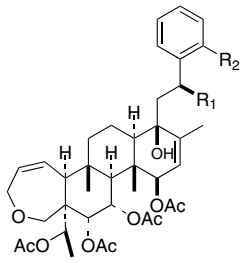
Table 4. C18 SAR in Rb_Kv and T cell assays (IC_{50}, nM)



Compd	R	Rb_Kv	T cell	T cell ratio[a]
26	H	19	131	1.4
30	Et	44	25.5	7.2
31	OMe	11	51.1	3.6
32	SMe	14	14.7	12.5
33	SEt	17	31.7	5.8
34	SOMe	145	—	—
35	SOMe	259	—	—
36	SO_2Me	352	—	—

[a] IC_{50} ratio of correolide to compound.

Table 5. C18 SAR in Rb_Kv and T cell assays (IC_{50}, nM)



Compd	R1	R2	Rb_Kv	T cell	T cell ratio[a]
28	Me	H	12	102	1.8
41	Me	OMe	19	25.6	7.2
42	Me	Et	28	36.8	5.0
43	Me	OEt	37	12.3	15
44	Et	OMe	28	29.2	6.3
45	Et	Et	12	—	—
46	Allyl	OMe	21	32.9	5.6
47	Allyl	Et	48	—	—

[a] IC_{50} ratio of correolide to compound.

phenyl acetic acid **37** was attached to (*R*)-4-benzyl-2-oxazolidinone via a mixed anhydride. Stereoselective alkylation of the resulting oxazolidinone provided **38**. Reduction of **38** with LAH and bromination of the resulting alcohol afforded bromide **39**. Treatment of **39** with Mg generated Grignard reagent **40** and addition of **40** to enone **4** gave compounds **41**–**47**.

Data for these compounds are presented in Table 5. Increasing the size of the (*S*)-substituent had little effect on activity in either assay. Ethoxy analog **43** is the most potent of this series, with activity 15-fold greater than correolide in the T cell proliferation assay. As in the vinyl series, combination of the two potency enhancing substitution patterns (substitution at the *ortho*-position of phenyl and an alkyl group at the carbon next to the phenyl) has a synergistic effect on inhibition of T cell proliferation.

In summary, several series of tetracyclic analogs have been synthesized. These compounds are structurally simpler than correolide. Two substitution patterns that enhance the compounds' inhibition of T cell proliferation were identified. A synergistic effect in potency was found in compounds that incorporate these substitution patterns. The most potent compound **43** is as much as 15-fold more potent than correolide as inhibitor of human T cell proliferation.

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