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NOVEL PKC INHIBITORY ANALOGS OF BALANOL WITH REPLACEMENT OF THE ESTER FUNCTIONALITY[1]

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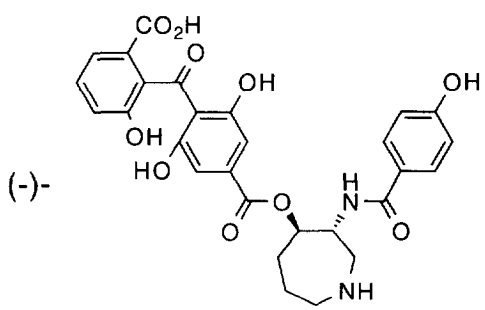
ABSTRACT: Balanol ((-)-**1**) is a potent protein kinase inhibitory natural product isolated from the fungus *Verticillium balanoides.* The presence of an ester functionality in this molecule has prompted concern over the metabolic stability of balanol, and has led to a search for active analogs lacking this moiety. The synthesis and serum stability for some of these is presented herein.

Balanol ((-)-**1**) is a potent protein kinase C (PKC) inhibitory natural product isolated from the fungus *Verticillium balanoides.*[2] PKC is a family of phospholipid-dependent serine/threonine-specific protein kinases involved in cellular growth control, regulation, and differentiation.[3] Activation of PKC is a key step in processes such as cellular proliferation and gene expression,[4] and the enzyme has been implicated in the progress of a number of diseases.[5] The presence of an ester functionality has prompted concern over the metabolic stability of the compound,[6] and has led to a search for active analogs with linking groups other than esters.



(-)-**1**

Completion of the total synthesis of balanol[7] has facilitated the preparation of analogs in which the ester group has been replaced with functionality less susceptable to metabolic degradation. These have included the corresponding amide and ether derivatives. An increase in the steric demands of the ester would be expected to increase resistance to esterases; thus, the tertiary alcohol ester **6** was also prepared. PKC isozyme inhibition data are summarized in Table 1.

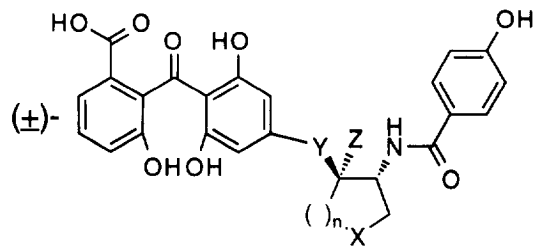


Table 1. PKC Isozyme Inhibition[8] by Racemic Balanol Analogs (IC-50's in μM).

Compound	X	Y	Z	n	α	βI	βII	γ	δ	ε	η	ζ
1(-balanol)	NH	C(O)O	H	3	0.030	0.010	0.010	0.010	0.016	0.020	0.003	5.9
1(±balanol)	NH	C(O)O	H	3	0.067	0.030	0.030	0.030	0.023	0.038	0.020	3.5
2	NH	C(O)O	H	1	0.022	0.010	0.033	0.012	0.005	0.010	0.004	6.8
3	NH	C(O)NH	H	3	3.2	0.630	1.2	0.420	0.220	0.670	0.050	>50
4	NH	C(O)N(CH_3)	H	3	>50	>50	>50	>50	42	>50	31	>50
5	NH	C(O)NH	H	1	0.920	0.220	0.330	0.330	0.012	0.080	0.003	9.6
6	NH	C(O)O	CH_3	3	0.220	0.120	0.070	0.040	0.030	0.050	0.020	42
7	CH_2	C(O)O	H	1	0.040	0.040	0.050	0.010	0.001	0.050	0.001	22
8	CH_2	CH_2O	H	1	0.460	0.430	0.290	1.1	0.020	0.370	0.030	>50
9	CH_2	C(O)OCH_2	H	1	0.040	0.040	0.030	0.300	0.020	0.340	0.020	>50
10	CH_2	C(O)$NHCH_2$	H	1	35	17	4.9	46	0.330	>50	2.3	>50

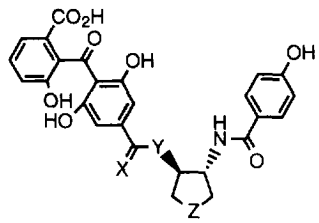
Results and Discussion

The azepine portion of balanol **1** can be replaced by certain other cyclic arrays such as a pyrrolidine (**2**) or cyclopentane (**7**) without significant loss of PKC activity.[9] Bisamides **3** and **5** represented the initial attempts to prepare non-ester analogs of balanol. Unfortunately, PKC activity dropped off by one order of magnitude or more, probably due to structural rigidification about the carbonyl-nitrogen bond.[10] Similar loss in potency was observed for the less rigid ether analog **8**.[10] N-methylation of the amide (see **4**) further diminished activity, while tertiary ester **6** maintained a high level of PKC inhibition. Diamide **10**, with increased conformational flexibility over the parent diamide **5**, was nevertheless 10-100 fold less active than **5**. Ester **9** maintained good activity, thus the loss in activity with **10** was not caused solely by the homologation.

In order to investigate the issue of plasma stability, compounds **2**, **5**, and **8** were added to human plasma at a concentration of 150 nM/mL and incubated at 37°C for 0h, 4h, and 24h. Samples were treated with methanol, and an aliquot of the plasma supernatant was injected onto HPLC for quantification of parent compound and detection of any breakdown peaks. Under the HPLC conditions used, **2**, **5**, and **8** eluted at 11.5, 9.4, and 13.6 min, respectively.

Although all three compounds were found to be stable in human plasma when incubated for 4h, the concentration of **2** decreased by an average of 12% over 24 h (Table 2). No significant changes were found when **5** and **8** were incubated in plasma for 24 h.

Table 2. Summary of Plasma Stability Results (% of reference, average of two replicates, <3% deviation).

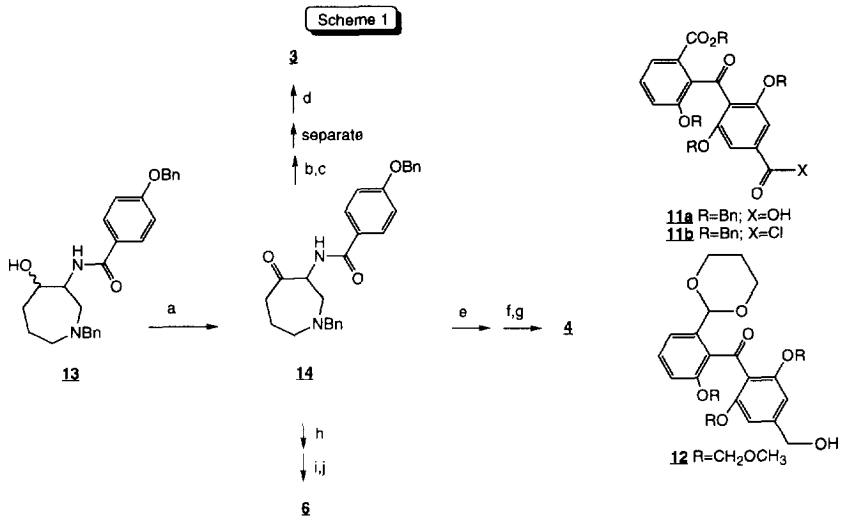


Compound	Incubation 4h	24h
2 (X=Y=O; Z=NH)	103%	88%
5 (X=O; Y=NH; Z=NH)	104%	95%
8 (X=H_2; Y=O; Z=CH_2)	100%	100%

The compounds were prepared as outlined in Schemes 1-4. Intermediates **11a** and **12** have been previously described,[11] and acid chloride **11b** was obtained in the standard fashion (oxalyl chloride/CH_2Cl_2/DMF). Several methods of preparation for amide/alcohol **13** have been detailed;[12] oxidation product **14** proved to be useful in synthesizing bisamides **3** and **4** *via* reductive aminations, and **6** after treatment with trimethylaluminum/methyl magnesium chloride. Carbocyclic analogues **7** and **8** were similarly prepared by elaboration of the appropriate hydroxyamide **16**, although **8** was somewhat problematic due to the need for an alternative protection strategy in order to avoid cleaving the benzophenone from the cyclopentane ring upon deprotection (Scheme 2). As outlined in Scheme 3, pyrrolidine analogs **2** and **5** could be obtained from a common starting material, 3-pyrroline, which was conveniently prepared in high purity by a recently reported method.[13] Finally, homologues **9** and **10** were prepared from intermediates **25** and **26**, respectively, which could be obtained by reductive amination of commercially available **24** (Scheme 4).

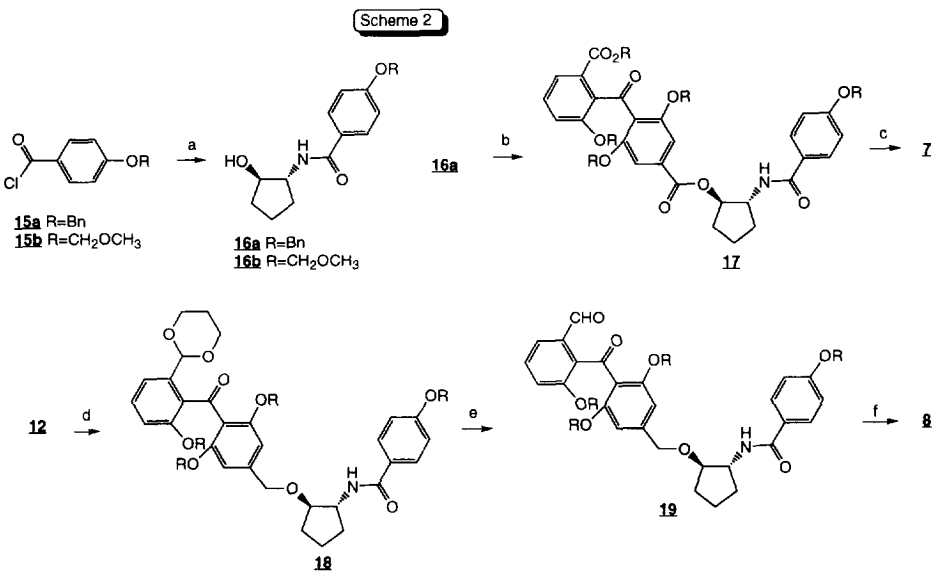
In conclusion, two analogs disclosed in this paper (compounds **5** and **8**) have been demonstrated to have slightly greater stability to serum esterases than the amido-ester with which they were compared (**2**). However, the magnitude of this enhancement, the trade-off in activity, and the reasonable and somewhat surprising serum stability of balanoid esters make it unlikely that any of the subject analogs are preferable compounds for development based solely on this information.

Scheme 1

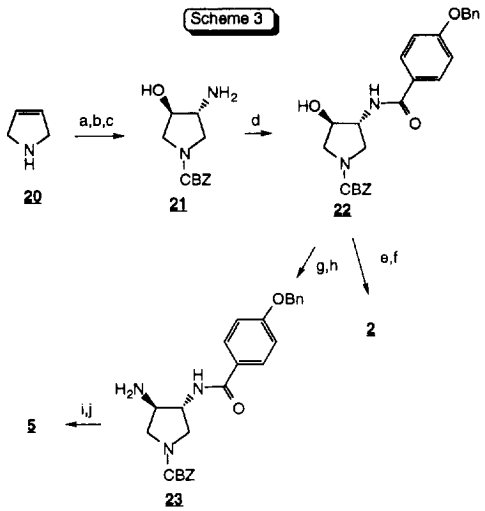


Reagents and conditions: a) $(COCl)_2$, DMSO, CH_2Cl_2, -60 °C, 80%; b) NH_2OH, EtOH, 50 °C, 99%; c) i. H_2, RaNi, MeOH; ii. **11b**, 1N NaOH, CH_2Cl_2, 35%; d) H_2, 20% $Pd(OH)_2/C$, EtOH, EtOAc, 60%; e) i. MeNHOH, EtOH; ii. H_2, RaNi, EtOH, 32%; f) **11b**, 1N NaOH, CH_2Cl_2, 88%; g) H_2, 20% $Pd(OH)_2/C$, EtOH, EtOAc, 68%; h) Me_3Al/MeMgCl, CH_2Cl_2, 40%; i) **11b**, Et_3N, DMAP, CH_2Cl_2, 85%; j) H_2, 20% $Pd(OH)_2/C$, EtOH, EtOAc, 47%.

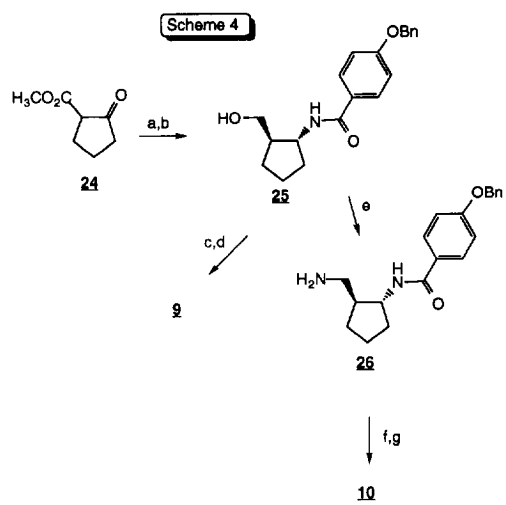
Scheme 2



Reagents and conditions: a) trans-2-aminocyclopentanol, 1N NaOH, CH_2Cl_2, 51% for **16a**, 31% for **16b**; b) **11b**, Et_3N, DMAP, CH_2Cl_2, 78%; c) H_2, 20% $Pd(OH)_2/C$, EtOH, EtOAc, 85%; d) i. MesCl; ii. NaI; iii. Na salt of **16b**, 71%; e) i. 2.5% H_2SO_4, SiO_2, CH_2Cl_2; ii. CH_3OCH_2Cl, EtN(i-Pr)$_2$, 65%; f) i. $NaClO_2$, H_2NSO_3H, CH_3CN; ii. conc. HCl/MeOH, 90%.



Reagents and conditions: a) BnOCOCl, pyridine, CH_2Cl_2, 90%; b) MCPBA, CH_2Cl_2, 86%; c) NH_4OH, 90%; d) **15a**, 1N NaOH, CH_2Cl_2, 85%; e) **11b**, Et_3N, DMAP, CH_2Cl_2, 40%; f) H_2, 20% $Pd(OH)_2/C$, MeOH, THF, 72%; g) i. PPh_3, DEAD, THF, 83%; ii. NaN_3, DMF, 95%; h) Zn, AcOH, H_2O, EtOH, 57%; i) **11b**, Et_3N, DMAP, CH_2Cl_2, 51%; j) H_2, 20% $Pd(OH)_2/C$, MeOH, THF, 96%.



Reagents and conditions: a) NH_2OH•HCl, NaOAc, MeOH, 100%; b) i. LAH, THF; ii. **15a**, 1N NaOH, CH_2Cl_2; iii. KOH, MeOH, H_2O, 37% (20% syn also obtained); c) **11b**, Et_3N, DMAP, THF, DMF, 94%; d) H_2, 20% $Pd(OH)_2/C$, EtOH, EtOAc, 90%; e) i. MesCl, pyridine; ii. NaN_3, DMSO; iii. Zn, EtOH, AcOH, H_2O, 92%; f) **11b**, 1N NaOH, CH_2Cl_2, 78%; g) H_2, 20% $Pd(OH)_2/C$, EtOH, EtOAc, 67%.

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