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POTENT AND SELECTIVE PKC INHIBITORY 5-MEMBERED RING ANALOGS OF BALANOL WITH REPLACEMENT OF THE CARBOXAMIDE MOIETY

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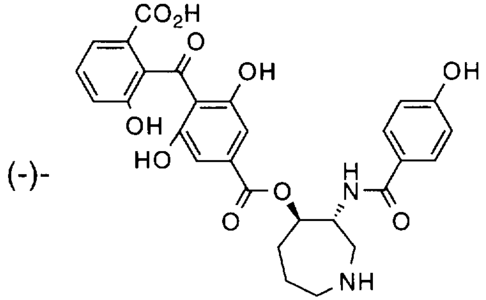
Abstract: Balanol ((-)-**1**) is a potent protein kinase inhibitory natural product isolated from the fungus *Verticillium balanoides*. The lack of cellular activity and protein kinase C selectivity in balanol has prompted a search for analogs that incorporate these properties. This paper describes the synthesis and biological activity of such compounds with substitution similar to balanol, but with a single atom bridge in place of the carboxamide moiety. Copyright © 1996 Elsevier Science Ltd

Balanol ((-)-**1**) is a potent protein kinase C (PKC) inhibitory natural product isolated from the fungus *Verticillium balanoides.*[1] PKC is a family of phospholipid-dependent serine/threonine-specific protein kinases involved in cellular growth control, regulation, and differentiation.[2] The role of PKC in a variety of biological processes is well documented,[3] and we recently reported our attempts to probe the SAR,[4] metabolic stability,[5] and cellular PKC inhibitory activity[6] of balanol and many analogs. We found that the ester functionality in balanol was highly resistant to degradation by esterases,[5] and that the benzophenone acid unit coupled through this ester was vital,[6] but that the carboxylate group could be substituted by isosteres[6a] (in some cases) or esterified[6b] with nominal loss of activity. The seven membered azepine ring could be replaced by a five membered ring (but not six),[4b,4c] or with a configurationally constrained equivalent,[4d] and the presence of additional heteroatoms within or external to the skeletal ring had no major effect on PKC inhibitory activity.[4a,4c]



(-)-**1**

Several total syntheses of balanol have been achieved,[7] and this, together with efficient methodology for preparing the benzophenone acid unit on a multigram scale,[8] has facilitated our search for stable PKC selective cellularly active analogs of this compound. A prodrug approach has led to some success in this regard,[6] but we also hoped to obtain a stable selective methyl or ethyl ester with intrinsic PKC inhibitory activity which might be expected to hydrolyze after cell penetration. We wish to report herein the synthesis and biological activity of a family of analogs including one of this description. Biological data for these are summarized in Table 1.

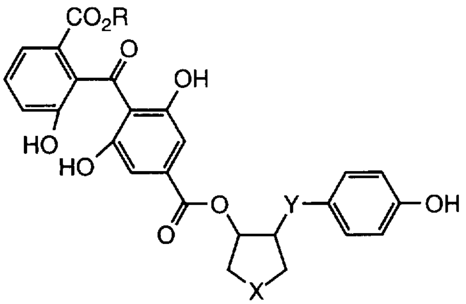


Table 1. Kinase Inhibition[9] by Balanol and Racemic Analogs (IC50's in μM).

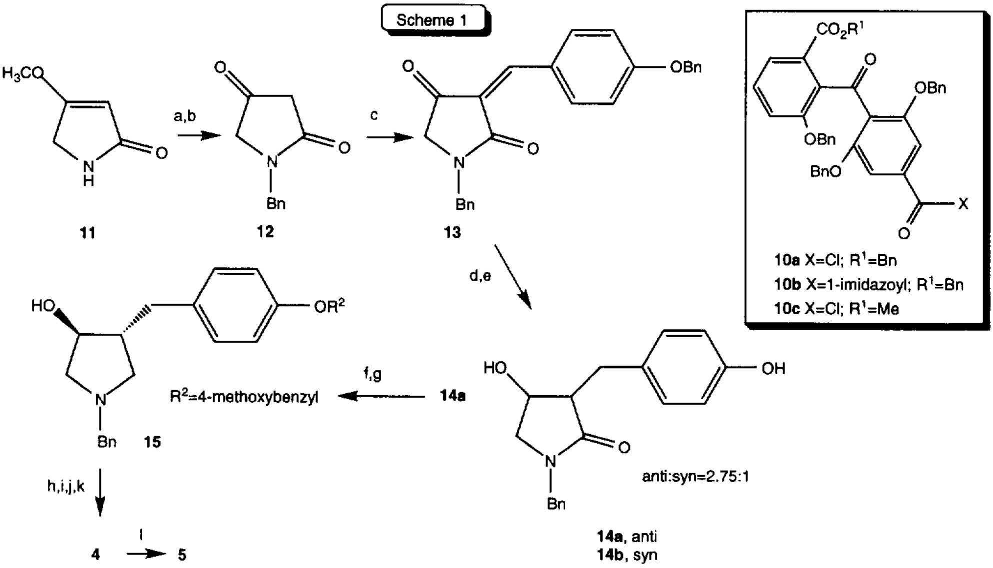
Cmpd	X	Y	geom	R	PKC enzyme assays							PKA	Neutrophil
					α	βI	βII	γ	δ	ε	η	assay	assay
(-)-1	CH_2CH_2NH	NHCO	*anti*	H	0.030	0.010	0.010	0.010	0.016	0.020	0.003	0.040	>10
(±)-1	CH_2CH_2NH	NHCO	*anti*	H	0.067	0.030	0.030	0.030	0.023	0.038	0.020	0.060	>10
2	NH	NHCO	*anti*	H	0.022	0.010	0.033	0.012	0.005	0.010	0.004	0.070	>10
3	CH_2	NHCO	*anti*	H	0.040	0.040	0.050	0.010	0.001	0.050	0.001	0.030	>10
4	NH	CH_2	*anti*	H	0.260	0.010	0.030	0.040	0.005	1.8	0.020	0.320	>10
5	NH	CH_2	*anti*	CH_3	2.2	0.110	0.250	0.380	0.100	4.0	0.050	4.1	4.3
6	CH_2	O	*anti*	H	0.220	0.020	0.020	0.250	0.020	0.240	0.010	50	>10
7	CH_2	CH_2	*anti*	H	0.030	0.004	0.003	0.040	0.005	0.120	0.003	>50	3.1
8	CH_2	CH_2	*syn*	H	0.160	0.020	0.020	0.130	0.030	0.500	0.010	>50	10.0
9	CH_2	CH_2	*anti*	CH_3	1.4	0.100	0.090	1.7	0.050	4.5	0.040	>50	0.26[a]

[a] One hour preincubation at 4 °C.[9]

Results and Discussion

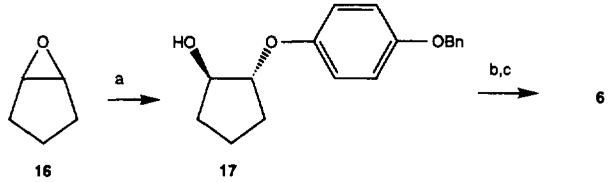
We chose to examine replacement of the carboxamide unit of balanol (**1**) by a methylene bridge and by an oxygen bridge on pyrrolidine and cyclopentane skeletons, since the substitution of a 5- for a 7-membered ring backbone has not significantly diminished PKC isozyme inhibition to date.[4b] Additionally, the 5-membered ring intermediates are more synthetically accessible. Previously reported[4b] carboxamide analogs **2** and **3** are included in Table 1 for comparison. Pyrrolidine **4** has nearly the same PKC isozyme inhibitory activity as pyrrolidine **2**, but has not gained any selectivity for PKC over cAMP dependant protein kinase A (PKA). Cyclopentane ether **6** is less potent than counterpart **3** against five of seven isozymes, but is considerable more selective for PKC's. Methylene bridged cyclopentane **7**, however, has a comparable PKC isozyme inhibitory profile to **3**, as well as excellent selectivity. This PKC/PKA potency ratio exceeds that previously reported for some conformationally constrained bicyclic and tricyclic balanol analogs.[4d] It is also noteworthy that the syn[10] isomer of **7** (compound **8**) has unusually high activity relative to other syn isomers we have obtained.[4d]

Cellular activity, measured by the neutrophil assay (PMA induced superoxide release),[9] was observed for all methylene bridged analogs, but was best when the side chain carboxylic acid (in **7**) was converted to a methyl ester (compound **9**). Such cellular activity is comparable with the best obtained for prodrug forms of **3**.[6] A prodrug form of **7** has not been prepared in our laboratories, but such a compound could be expected to display excellent PKC inhibitory activity in cells.


Scheme 1

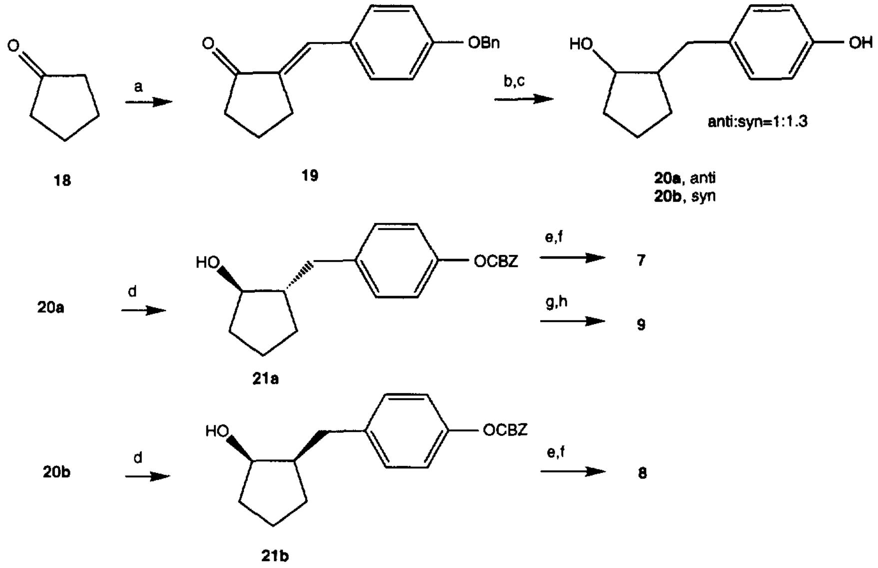
Reagents and Conditions:(a) KOtBu, BnBr, THF, 50 °C, 68%; (b) 50% H_2SO_4/H_2O, CH_2Cl_2, 97%; (c) 4-benzyloxybenzaldehyde, $LiN(SiMe_3)_2$, THF, -50 °C to 35 °C, 46%; (d) $NaBH_4$, CH_2Cl_2, MeOH; (e) H_2, Raney Ni, MeOH, EtOH, 94% (syn+anti) from **13**; (f) 4-methoxybenzyl chloride, K_2CO_3, DMF, 65 °C, 81%; (g) LAH, THF, reflux, 81%; (h) BuLi, THF, -20 °C; (i) **10b**; (j) H_2, 20% $Pd(OH)_2/C$, EtOH, EtOAc; (k) TFA, 55 °C, 40% from **15**; l) $SOCl_2$, MeOH, -20 °C to 50 °C, 52%.

Scheme 2



Reagents and Conditions: (a) 4-benzyloxyphenol, DBU, 100 °C, 42%; (b) **10a**, $(iPr)_2NEt$, DMAP, CH_2Cl_2, 65%; (c) H_2, 20% $Pd(OH)_2/C$, EtOH EtOAc, 64%.

Scheme 3



Reagents and Conditions: (a) 4-benzyloxybenzaldehyde, KOH, H_2O, reflux, 61%; (b) $NaBH_4$, THF, MeOH; (c) H_2, Raney Ni, EtOH, 81% (syn+anti) from **19**; (d) $PhCH_2OCOCl$, Et_3N, CH_2Cl_2, 99%; (e) **10a**, Et_3N, DMAP, CH_2Cl_2; (f) H_2, 20% $Pd(OH)_2/C$, EtOH, EtOAc, 30% from **21a**, 37% from **21b**; (g) **10c**, Et_3N, DMAP, CH_2Cl_2; (h) H_2, 20% $Pd(OH)_2/C$,TFA, EtOAc, 37% from **21a**.

The subject compounds were prepared as outlined in Schemes 1-3. The carboxylic acid precursors to compounds **10** have been previously reported.[8,6b] The pyrrolidine analogs (Scheme 1) were prepared by condensation of dicarbonyl **12** with 4-benzyloxybenzaldehyde, followed by sequential reduction of the keto group and the exocyclic double bond. This resulted in the unexpected loss of one benzyl protecting group, which was replaced by an acid labile 4-methoxybenzyl substituent before lithium aluminum hydride reduction of the lactam to pyrrolidine **15**. Acylation and deprotection of **15** afforded target **4**; ester **5** was obtained upon treatment of **4** with thionyl chloride in methanol.

The cyclopentane ether analog **6** was prepared by opening cyclopentene oxide (**16**) with 4-benzyloxyphenol (**17**), followed by standard acylation and reductive deprotection. Methylene bridged cyclopentanes **7-9** were prepared by condensation of cyclopentanone (**18**) with 4-benzyloxybenzophenone, followed by sequential reduction of the keto group and the exocyclic double bond of compound **19**, with loss of a benzyl unit to give **20**. In this case, reprotection with a carbobenzyloxy group was followed by appropriate acylation/deprotection of compounds **21** to yield the desired targets.

In conclusion, balanol analogs containing a cyclopentane skeleton and a single atom bridging unit in place of the carboxamide moiety are potent inhibitors of PKC isozymes and display excellent selectivity for PKC over PKA. Analogs wherein the bridging unit is a methylene group display good cellular activity; this is further enhanced when the carboxylate moiety is presented as a methyl ester.

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9. The PKC, PKA, and Neutrophil (PMA/lucigenin + human neutrophil cells) assays have been previously described; see (a) Osita, K.; Ono, Y.; Kikkawa, U.; Nishizuka, Y. *Methods in Enzymology* **1991**, *200*, 228. (b) Kashiwada, Y.; Huang, L.; Ballas, L. M.; Jiang, J. B.; Janzen, W. P.; Lee, K.-H. *J. Med. Chem.* **1994**, *37*, 195. (c) Kulanthaivel, P.; Janzen, W. P.; Ballas, L. M.; Jiang, J. B.; Hu, C.; Darges, J. W.; Seldin, J.; Cofield, D.; Adams, L. *Planta Medica* **1995**, *61*, 41, and references 6a and 6b.

10. The ring geometries of the balanol analogs reported herein were determined by examination of the NMR coupling constants of the ring-bound methine protons of precursor alcohols (Fig. 1) in $CDCl_3$ or d_6-DMSO. Coupling constants for *syn* protons were 4-5 Hz, and coupling constants for *anti* protons were 0.5-1.5 Hz.

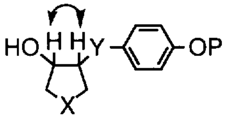

Fig. 1

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