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SYNTHESIS AND PKC INHIBITORY ACTIVITIES OF BALANOL ANALOGS WITH A CYCLOPENTANE SUBSTRUCTURE

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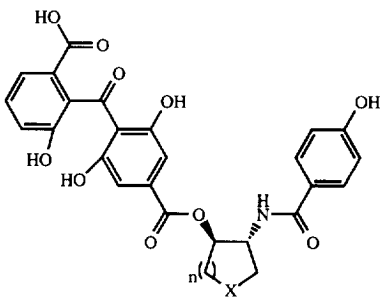
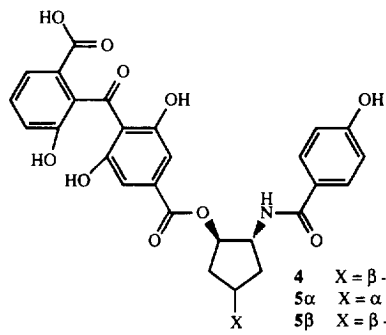
Abstract: Compounds **4-7**, analogs of the potent protein kinase C (PKC) inhibitor balanol (-)-**1**, were synthesized and their potency against PKC was compared with racemic balanol and other related analogs. These cyclopentane-based analogs were found to be, in general, more potent PKC inhibitors than balanol.

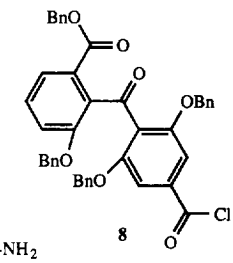
Activation of the protein kinase C (PKC) family of enzymes leads to modulation of a number of cellular processes[1] and is thought to underlie several disease states.[2] Thus, compounds that inhibit PKC may prove useful as chemotherapeutic agents for human diseases. Balanol (-)-**1** is a potent PKC inhibitor recently isolated in our laboratories from the fungus *Verticillium balanoides*.[3] As a result of further studies intended to determine the structure-activity relationships of balanol analogs we identified compounds **2** and **3**, two equally potent balanol analogs in which the balanol perhydroazepine is replaced with a pyrrolidine and cyclopentane ring, respectively.[4] This aroused an interest in evaluating more analogs having a similar five-membered ring substructure in which the pyrrolidine heteroatom is moved to an exocyclic position. Such compounds can be viewed as analogs of **3** and were expected to reveal the influence, if any, of the substitution pattern around the five-membered ring on the potency of these molecules against PKC. We describe herein the syntheses, shown in **Scheme 1-4**, and the biological activities of seven such analogs, namely **4-7**.[5]



1 n = 3 X = -NH-
2 n = 1 X = -NH-
3 n = 1 X = $-CH_2-$



4 X = β -NH_2
5α X = α -OH
5β X = β -OH
6α X = α -CH_2OH
6β X = β -CH_2OH
7α X = α -CH_2NH_2
7β X = β -CH_2NH_2



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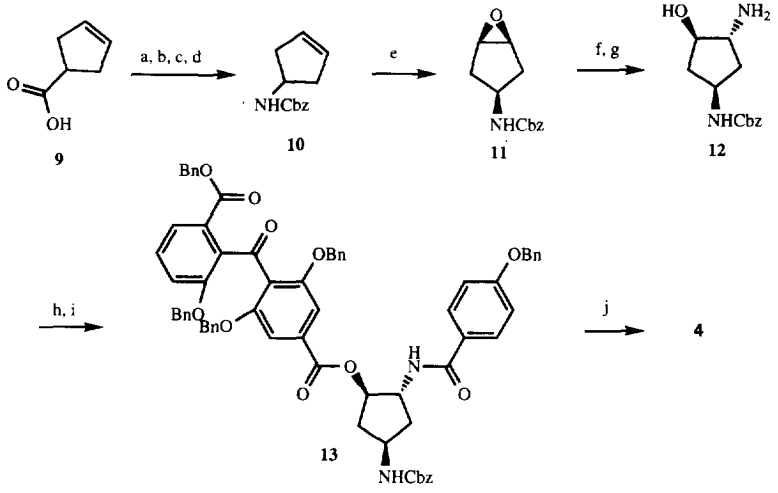
Cyclopentene-4-carboxamide, derived from **9**[6] by acid chloride formation and condensation with ammonia, was allowed to undergo a modified Hoffman degradation[7] with [bis(trifluoroacetoxy)iodo]benzene, and the resultant amine was protected with a Cbz group to give **10**. Epoxidation of **10** using mCPBA was highly stereoselective giving only one stereoisomer, **11**.[8] Treatment of **11** with NaN_3[9] gave the desired trans azidoalcohol which was reduced to the aminoalcohol **12**. Compound **12** was coupled with 4-benzyloxybenzoyl chloride, derived from the corresponding acid by treatment with oxalyl chloride, and with **8**[10] to give **13**, which was subsequently debenzylated to give analog **4**.

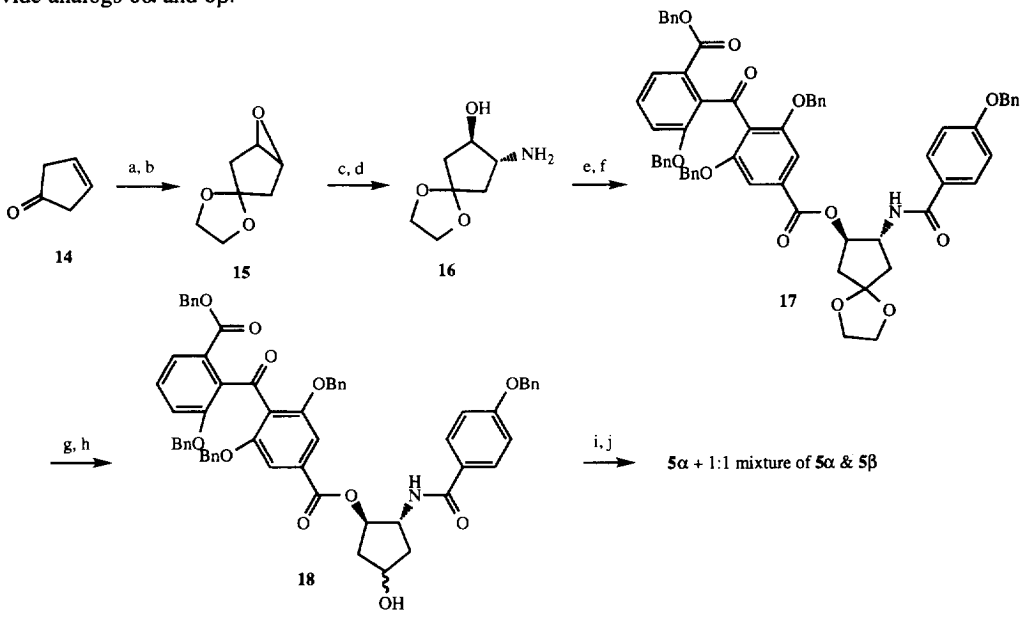


Scheme 1: (a) $(COCl)_2$, DMF, CH_2Cl_2, rt; (b) NH_4OH, rt, 72% 2 steps; (c) $(CF_2CO_2)_2PhI$, CH_3CN, H_2O, rt, 91%; (d) BnOCOCl, aq. NaOH, CH_2Cl_2, rt, 67%; (e) mCPBA, CH_2Cl_2, 0 °C-rt, 87%; (f) NaN_3, NH_4Cl, H_2O, MeOH, 50 °C, 94%; (g) Zn, AcOH, H_2O, EtOH, rt, quant.; (h) 4-benzyloxybenzoic acid, CDI, THF, rt; aq. NaOH workup, 77%; (i) **8**, Et_3N, DMAP, CH_2Cl_2, rt, 76%; (j) H_2, $Pd(OH)_2$-C, MeOH, THF, rt, 72%.

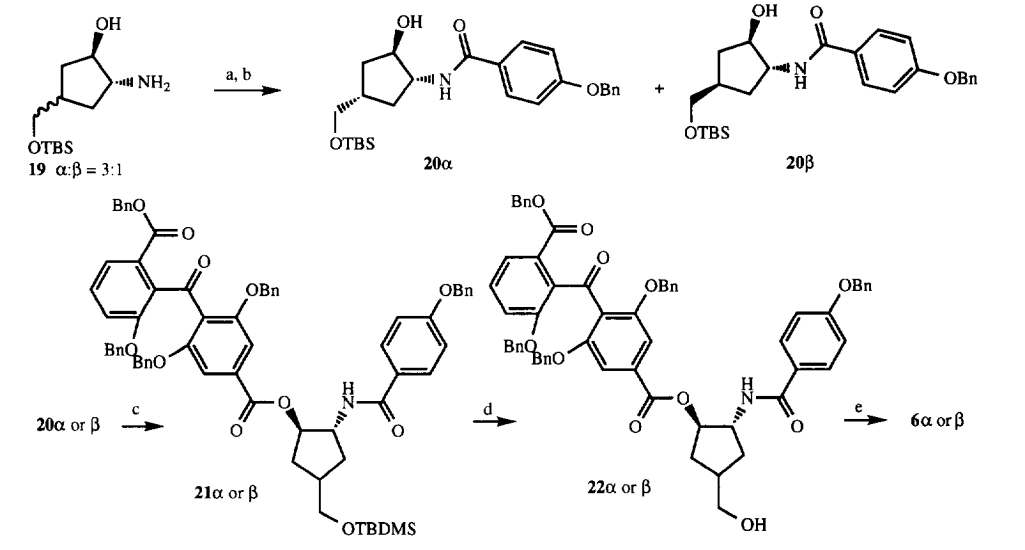
3-Cyclopentenone **14**[11] was protected as its dioxalane derivative under standard conditions and epoxidized to **15**. Epoxide opening of **15** with benzylamine in the presence of $LiClO_4$[12] followed by N-debenzylation gave **16**, which was coupled similarly with the two aryl side chains to give **17**. Treatment of **17** with a catalytic amount of $Pd(CH_3CN)_2Cl_2$[13] followed by reductive work up using methanolic $NaBH_4$ gave a mixture of two epimeric alcohols **18**. This mixture was debenzylated by hydrogenolysis and the products were separated by HPLC to give one of the two epimers in pure form, which was arbitrarily assigned the structure **5α** since an unambiguous structural assignment using available ^1H NMR data could not be reached. The other epimer, **5β**, was obtained as a 1:1 mixture with **5α**.

Compounds **6** and **7** were all derived from the readily available **19**,[14] which was obtained as a 3:1 mixture of two stereoisomers. N-acylation of this mixture with 4-benzyloxybenzoyl chloride enabled us to cleanly separate the two stereoisomers **20α** and **20β** by silica gel chromatography. These two compounds were

then individually carried through a sequence of (i) coupling with **8**, (ii) desilylation, and (iii) debenzylation to provide analogs **6α** and **6β**.

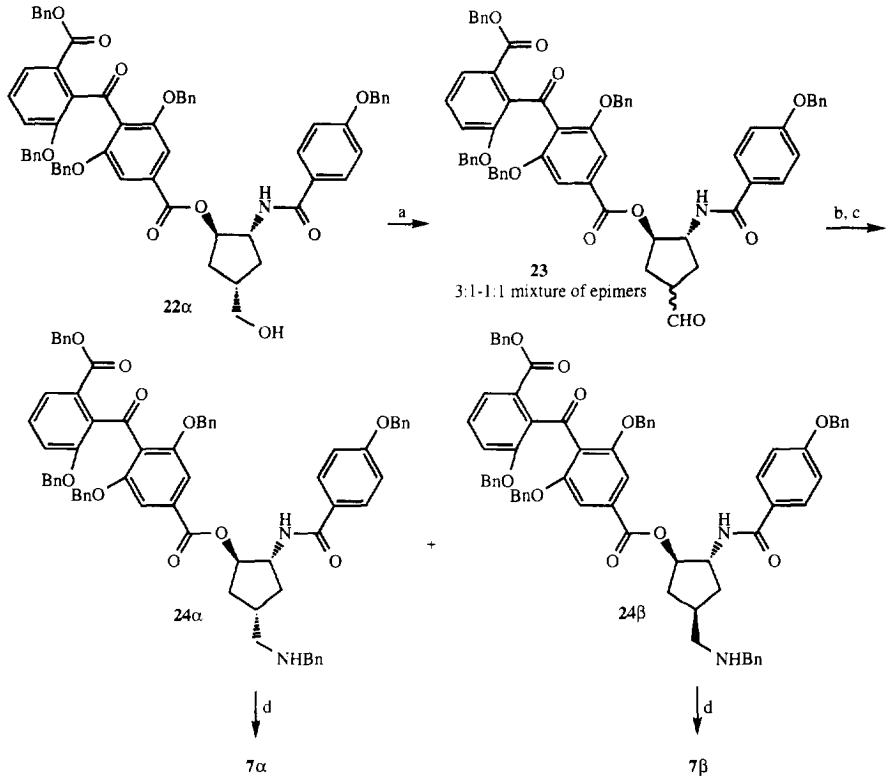


Scheme 2: (a) $HOCH_2CH_2OH$, PPTS, PhH, reflux, 67%; (b) mCPBA, CH_2Cl_2, rt, 89%; (c) $BnNH_2$, $LiClO_4$, CH_3CN, 60 °C, quant.; (d) H_2, 5% Pd-C, EtOAc, rt, 93%; (e) 4-benzyloxybenzoic acid, CDI, THF, rt; aq. NaOH workup, 71%; (f) **8**, Et_3N, DMAP, CH_2Cl_2, rt, 95%; (g) $Pd(CH_3CN)_2Cl_2$, $CHCl_3$, rt; (h) $NaBH_4$, MeOH, 0 °C, gave 2:1 mixture, 50% two steps; (i) H_2, $Pd(OH)_2$-C, MeOH-THF, rt, 82%; (j) HPLC



Scheme 3: (a) 4-benzyloxybenzoyl chloride, aq. KOH, THF, rt, 80%; (b) SiO_2 chromatography; (c) **8**, Et_3N, DMAP, CH_2Cl_2, rt, **20α**, 81%, **20β**, 91%; (d) Bu_4NF, THF, rt, **21α**, 91%, **21β**, 89%; (e) H_2, $Pd(OH)_2$-C, EtOAc, EtOH, rt, **6α**, 80%, **6β**, 79%.

Swern oxidation[15] of **22α** gave a mixture of isomeric aldehydes **23** after silica gel chromatography. Separation of the two isomers was not achieved until the aldehydes were reductively aminated with benzylamine and sodium triacetoxyborohydride. The resultant **24α** and **24β** were then hydrogenated to give **7α** and **7β** respectively.[16]



Scheme 4: (a) $(COCl)_2$, Me_2SO, Et_3N, 0 °C-rt, SiO_2 chromatography, 75%; (b) $BnNH_2$, $NaB(OAc)_3H$, $ClCH_2CH_2Cl$, rt; (c) chromatography on SiO_2, **23α**, 58%, **23β**, 36%; (d) H_2, $Pd(OH)_2$-C, TFA, EtOAc, EtOH, rt, **7α**, 50%, **7β**, 24%.

Compounds **4-7** were screened against human PKC isozymes α, β-1, β-2, γ, δ, ε, η, and ζ using standard protocols[17] and the results are shown in **Table 1** together with those of compounds **1-3**.

Except **6β**, compounds **4-7** are all more potent than racemic balanol in essentially every assay. A comparison of the biological activity of the parent cyclopentane analog **3** with that of analogs **4**, **7α**, and **7β** suggests an enhancement in potency by the exocyclic amino groups, regardless of chain length. A similar enhancement in potency by extra substitution on the cyclopentane ring, however, was not shown unambiguously by either the hydroxyl analogs **5α**, **5β**, **6α**, and **6β** as a group or the endocyclic amine **2**. The amino group also appears to be favored over the hydroxyl group in terms of potency by a comparison of amine **4** with alcohol **5α**,

and of amine **7β** with alcohol **6β**. The amine/alcohol pair **7α** and **6α** is an obvious exception to this trend. However, since both **6α** and, to a lesser extent, **7α** are more potent than their β stereoisomers, this discrepancy may simply be a reflection on the difference in degree of this stereochemical effect. Unfortunately this stereochemical effect can not be clearly assessed on compounds **5** due to the uncertainty in structural assignment and the fact that **5β** was not assayed in its pure form.

Table 1: PKC Isozyme Inhibition by Balanol Analogs **1-7** (IC_{50} values in μM)

entry	compd	α	β-1	β-2	γ	δ	ε	η	ζ
1	**(±)-1***	0.07	0.03	0.04	0.03	0.03	0.05	0.02	3.5
2	**2**	0.022	0.017	0.033	0.013	0.005	0.01	0.004	>0.15
3	**3**	0.04	0.04	0.05	0.01	0.0009	0.05	0.0006	22
4	**4**	0.02	0.004	0.002	0.005	0.005	0.01	0.002	1.2
5	**5α**	0.05	0.02	0.02	0.03	0.006	0.15	0.004	12.5
6	**5α+5β**	0.04	0.02	0.01	0.02	0.005	0.10	0.003	16
7	**6α**	0.01	0.004	0.003	0.005	0.004	0.16	0.002	39
8	**6β**	0.08	0.06	0.02	0.045	0.02	0.26	0.02	34
9	**7α**	0.02	0.004	0.004	0.005	0.006	0.03	0.003	2.8
10	**7β**	0.02	0.005	0.005	0.01	0.02	0.03	0.003	3.1

* synthetic material, see ref. 10a and 18.

In summary, we have successfully synthesized seven balanol analogs in which the perhydroazepine ring is replaced with a substituted cyclopentane ring. In general, these analogs are found to be more potent PKC inhibitors relative to racemic balanol. The biological results also indicate that attachment of an exocyclic amino or aminomethyl group to the core cyclopentane ring increases the potency against PKC, but the same can not be said definitively for their hydroxyl counterparts. The relative stereo-orientation of the substituents on the cyclopentane ring appears to have an influence on the PKC inhibitory activity of these analogs, and this may complicate the total effect of substitution pattern around the cyclopentane ring. We are currently using these compounds to evaluate the dependency of their potency against PKC on some of the conformational indices via a computational approach.

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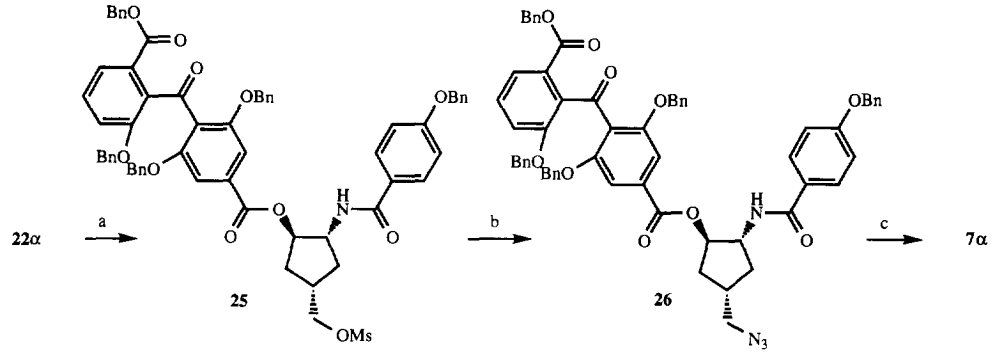
(16) The relative stereochemistry of **7α** was established by chemical correlation with **6α** as shown below.



Conditions: (a) $MeSO_2Cl$, Et_3N, CH_2Cl_2, rt, 91%; (b) NaN_3, DMF, rt-65 °C, 84%; (c) H_2, $Pd(OH)_2$-C, EtOAc, EtOH, rt, 63%.

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