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SYNTHESIS AND BIOLOGICAL EVALUATION OF CONFORMATIONALLY CONSTRAINED BICYCLIC AND TRICYCLIC BALANOL ANALOGUES AS INHIBITORS OF PROTEIN KINASE C

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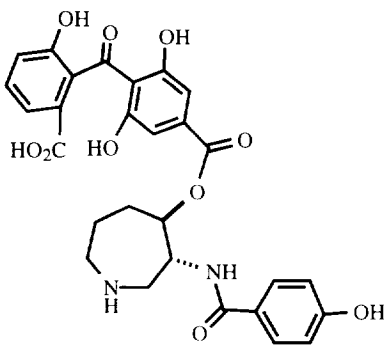
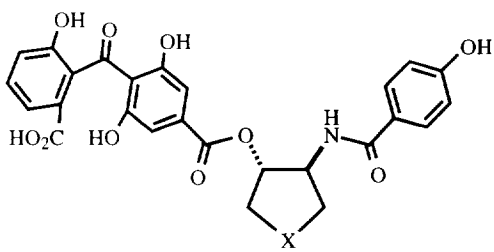
Abstract: A series of conformationally constrained bicyclic (**20**-**25** and **28**) and tricyclic (**26** and **27**) balanol analogues was prepared and evaluated as inhibitors of protein kinase C (PKC). Of special interest are bicyclic balanol analogues **20** and **24** and the tricyclic analogue **26**, which not only retain the nanomolar activity for most PKC isozymes, but also display good selectivity over c-AMP dependent protein kinase (PKA).

Introduction

Protein kinase C (PKC), a family of phospholipid dependent protein kinases, is involved in signal transduction, cellular proliferation, and differentiation.[1] Since the activated enzyme has been implicated in several disease processes, the discovery of specific inhibitors of PKC would be of great value not only to elucidate the physiological role of PKC, but also as potential chemotherapeutic agents in the treatment of human cancer, disorders of the central nervous system, cardiovascular system, diabetes, asthma, and HIV infections.[2] In 1993, our laboratories reported the isolation and structural elucidation of (-)-balanol **1**, a potent PKC inhibitor produced by the fungus *Verticillium balanoides*.[3a] More recently, the total synthesis of (-)-balanol was achieved.[4a-b]



1, (-)-**Balanol**



(±)-**2**, X = NH
(±)-**3**, X = CH_2

In the course of our studies searching for new PKC inhibitors,[5] it was found that the (±)-pyrrolidine and the (±)-cyclopentane containing balanol analogues (compounds **2** and **3**, respectively) were very effective inhibitors of PKC; however, they did not show good selectivity over c-AMP dependent protein kinase (PKA) (Table I). Herein, we report the preparation of a series of conformationally constrained bicyclic and tricyclic

analogues of balanol in order to continue the investigation of the structure-activity relationship as a means of improving the inhibitory activity and enzyme selectivity.

Table I

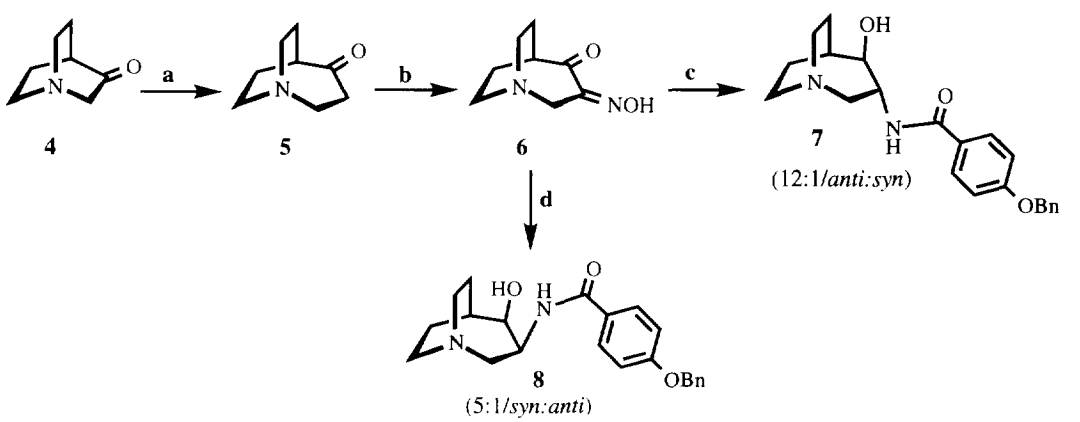
*PKC Isozyme Inhibition by (±)-Balanol and Balanol Analogues:									*PKA Inhibition:
Compound	α	β1	β2	γ	δ	ε	η	ζ	
(±)-**1**[a]	0.074	0.032	0.044	0.034	0.032	0.044	0.022	3.5	0.06
(±)-**2**	0.022	0.017	0.033	0.014	0.005	0.015	0.004	>0.15	0.07
(±)-**3**	0.04	0.04	0.05	0.01	0.001	0.05	0.001	22	0.03

*PKC and PKA inhibition (IC_{50}'s in μM). [a]Synthetic material, see ref 4a and 4c.

Synthesis

The synthesis of the required hydroxy-amido bicyclic intermediates **7** and **8** is outlined in Scheme I. Treatment of quinuclidin-3-one **4** with $TMSCHN_2$ in THF/MeOH gave 1-azabicyclo[3.2.2]nonan-4-one **5**, which was then allowed to react with $LiN(TMS)_2$ and n-butyl nitrite to give the oxime derivative **6** in 41% (two steps) after silica gel chromatography. Reduction of **6** with Na°/EtOH and treatment of the corresponding mixture of amino-alcohols with 4-benzyloxybenzoyl chloride, aqueous 1.0 N NaOH in toluene provided the hydroxy-amido compounds **7** and **8** as a 12:1 mixture of *anti:syn* isomers, respectively. The *syn* isomer **8** was best prepared by treatment of oxime **6** with $NaBH_4$/MeOH followed by hydrogenation (Ra/Ni at 45-50 psi) to give the mixture of amino-alcohols, which after N-acylation afforded compounds **7** and **8** in 52% yield as a 1:5 mixture of *anti:syn* isomers, respectively.[6]

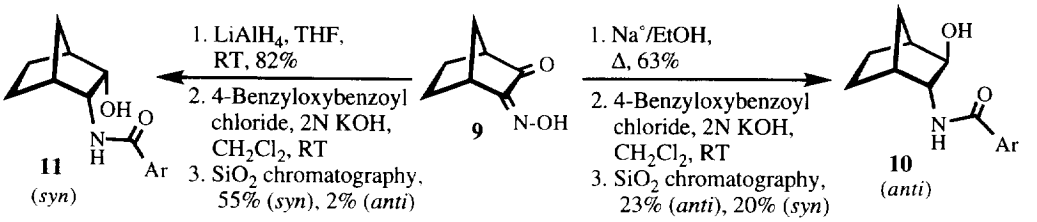
Scheme I



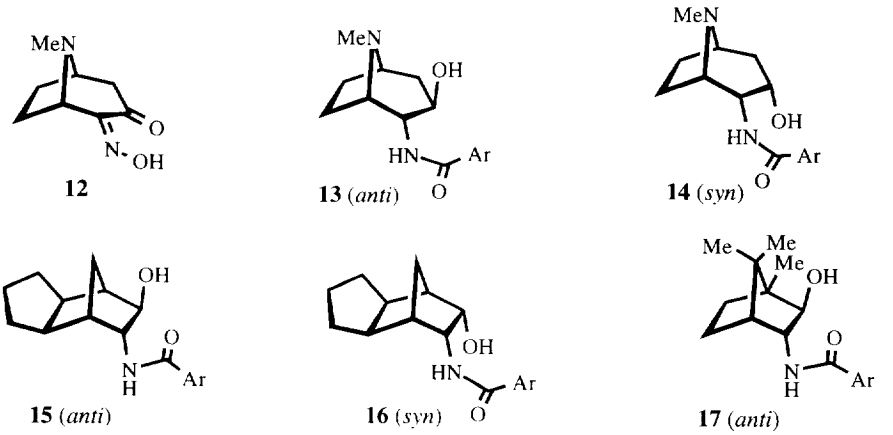
Conditions: a) $TMSCHN_2$, THF/MeOH, 5 °C → RT, 74%. b) $LiN(TMS)_2$, THF, n-butyl nitrite, -60 °C → RT, 55%. c) 1. Na°/EtOH, Δ; 2. 4-benzyloxybenzoyl chloride, 1.0 N NaOH, toluene, RT, 25% from oxime **6** (12:1/*anti:syn*). d) 1. $NaBH_4$, MeOH, RT; 2. H_2, Ra/Ni, MeOH, 45-50 psi, RT; 3. 4-benzyloxybenzoyl chloride, 1.0 N NaOH, toluene, RT, 52% from oxime **6** (5:1/*syn:anti*).

The *anti* and *syn* hydroxy-amido bicyclic intermediates **10** and **11** were prepared using the readily available oxime **9**[7a] (Scheme II). Reduction of **9** with $Na^{\circ}/EtOH$[7b] and treatment of the resulting crude mixture of amino-alcohols with 4-benzyloxybenzoyl chloride in aqueous KOH and CH_2Cl_2 gave stereoisomers **10** (23%) and **11** (20%) after separation by silica gel chromatography. The required *syn* amino-alcohol was best prepared following a literature procedure.[7a] Thus treatment of **9** with $LiAlH_4$ in THF gave predominantly the *syn* amino-alcohol, which without any purification was N-acylated under standard reaction conditions to afford, after silica gel chromatography, the *syn* isomer **11** (48%, two steps).

Scheme II



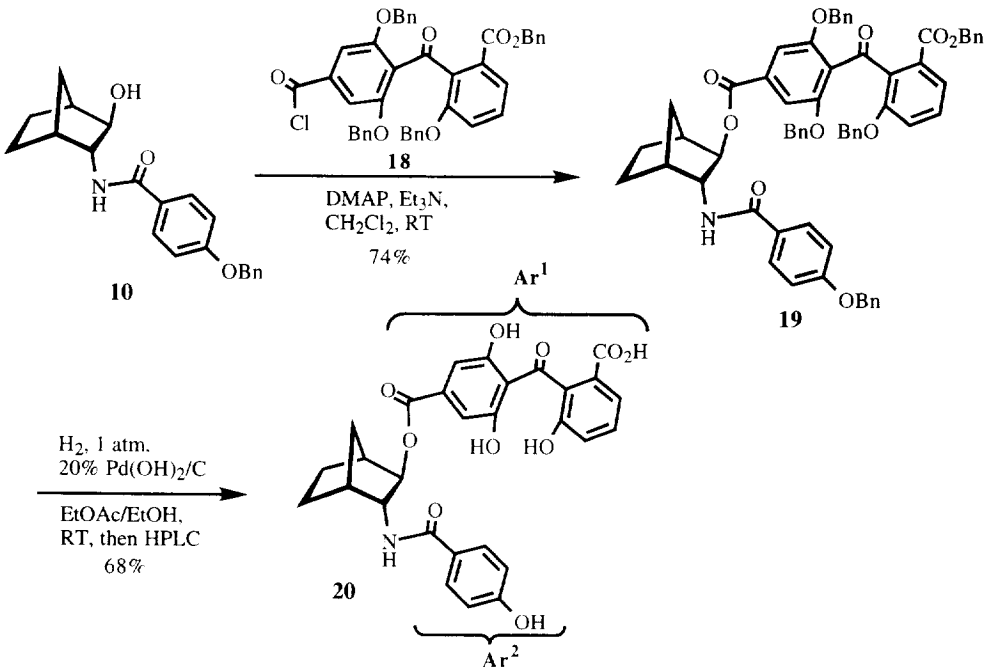
Similarly, the required intermediates **13** and **14** were prepared by hydrogenation (Ra/Ni) of compound **12**, derived from commercially available 8-methyl-8-azabicyclo[3.2.1]octan-3-one[8], followed by LiAl(*tert*-BuO)3H treatment, and N-acylation.[9] The tricyclic hydroxy-amido compounds **15** and**16** were synthesized from 8-ketotricyclo[5.2.1.$0^{2,6}$]decane[8] and the *anti* isomer **17**[7b] was prepared starting from (1R)-(+)-camphorquinone-3-oxime.[8]



Synthesis of Balanol Analogues: The synthesis of bicyclic balanol analogue **20** is illustrated in Scheme III, all the other analogues were prepared similarly. Treatment of the hydroxy-amido intermediate **10** with the benzophenone acid chloride **18**[10] in the presence of DMAP and Et3N in CH_2Cl_2 at room temperature afforded

the corresponding coupled material **19**, which was then submitted to hydrogenolysis to give balanol analogue **20** in 50% overall yield.[11] This synthetic sequence has proven readily amenable to the preparation of a diverse array of balanol analogues.

Scheme III



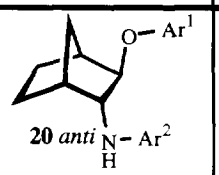
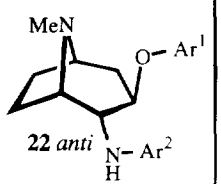
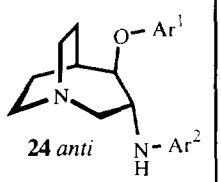
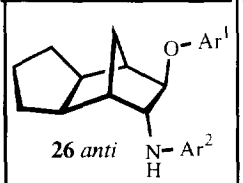
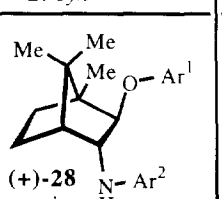
Biological Results and Discussion

Purification of protein kinase C and the enzyme assay were performed as described in the literature.[12]

The results of our SAR studies of bicyclic and tricyclic balanol analogues are illustrated in Table II. The norbornane derivative **20**, in addition to having potency against PKC in the nanomolar range for most enzymes (except ζ), also displayed good selectivity over c-AMP dependent protein kinase (PKA). The 1-azabicyclic[3.2.2]nonane compound **24** showed PKC and PKA inhibition profiles similar to **20**. Interestingly, a decrease in activity against PKC in the 8-methyl-8-azabicyclo[3.2.1]octane analogue **22** was observed. This compound was found to be less active than the other *anti*-derivatives prepared in this study. Biological evaluation of the *anti*-tricyclic analogue **26** revealed a binding affinity for PKC which was similar to that observed for **20** and **24**. Compound **26** not only retained the nanomolar activity for most enzymes, but also showed excellent selectivity. A slight decrease in activity was observed in the bicyclic chiral derivative (+)-**28**. The reduced biological potency of (+)-**28** could be explained in terms of its steric bulk, or perhaps because of a different stereospecificity in binding. Nevertheless, compound **28** showed good selectivity for PKC over PKA.

Similar to previous observations from our laboratories, the *syn*-balanol analogues were found to be less active than the corresponding *anti* compounds. However, it is interesting to note that the *syn*-bicyclic compound **23** displayed similar activity for PKC as its *anti*-analogue **22**.

Table II

Compound	*PKC Isozyme Inhibition by Balanol Analogues: α	β1	β2	γ	δ	ε	η	ζ	*PKA Inhibition:
 **20** *anti*	0.055	0.045	0.03	0.035	0.02	0.03	0.02	37	1.9
21 *syn*	24	17	6.9	10	3.5	1.5	4.5	> 50	> 50
 **22** *anti*	34	4.4	14	4.6	5	27	2.8	> 50	> 50
23 *syn*	35	22	22	16	3.8	49	2.5	> 50	> 50
 **24** *anti*	0.415	0.026	0.069	0.17	0.112	0.17	0.026	22	2.0
25 *syn*	12	6.3	34	4.6	1	14	0.49	>50	> 50
 **26** *anti*	0.10	0.15	0.03	0.05	0.05	0.97	0.05	37	32
27 *syn*	2.7	3.3	2.1	1.6	2.0	40	1.9	> 50	> 50
 **(+)-28** *anti*	0.41	0.31	0.08	0.21	0.34	3.6	0.31	> 50	26

*PKC and PKA inhibition (IC_{50}'s in μM).[13] All balanol analogues are racemic, except for **28** which is a chiral compound.

Ar^1 = Benzophenone subunit of balanol, see Scheme III.

Ar^2 = 4-Hydroxybenzoyl-

In summary, substitution of the perhydroazepine ring of balanol **1** with a bicyclic or a tricyclic ring yields compounds which have been shown to be selective and very effective inhibitors of PKC. Of particular note are bicyclic balanol analogues **20** and **24** and the tricyclic analogue **26**, which not only retain the nanomolar activity for most PKC isozymes, but also display good selectivity over PKA.

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8. Purchased from Aldrich Chemical Co.
9. The *anti/syn* geometry of compounds **13** and **14** was assigned based on ^{1}H NMR chemical shifts upon treatment with trichloroacetyl isocyanate, see: a) Goodlet, V. W. *Anal. Chem.* **1965**, *37*, 431. b) Trehan, I. R.; Monder, C.; Bose, A. K. *Tetrahedron lett.* **1968**, 67. The *exo/endo* relative configuration of **13** and **14** has been assigned tentatively.
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