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HETEROATOM EFFECT IN THE PKC INHIBITORY ACTIVITIES OF PERHYDROAZEPINE ANALOGS OF BALANOL[1]

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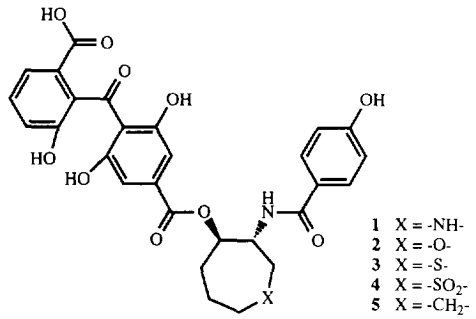
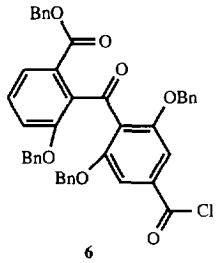
Abstract: Analogs **2-5** of balanol (-)-**1**, a potent protein kinase C (PKC) inhibitor, were prepared in which the perhydroazepine N atom was replaced with O, S, or C. Compounds **2** and **3** are found to show enhanced isozyme selectivity, despite the general trend of these analogs being less potent PKC inhibitors relative to balanol.

Protein kinase C (PKC) is a family of Ca^{++}/phospholipid dependent, serine/threonine specific kinases that plays a key role in signal transduction as well as cellular proliferation, differentiation, and various regulatory events.[2] Specific inhibition of PKC appeals to us as a potential means for treating human diseases because of the body of evidence which implicates the activation of PKC in a range of pathologic states.[3] Balanol, (-)-**1**, is a potent PKC inhibitor recently isolated in our laboratories from the fungus *Verticillium Balanoides*.[4] During our efforts to determining structure-activity relationships in balanol-like compounds, the perhydroazepine moiety of this molecule elicited special interest due to its structural distinction from the other parts of the molecule, namely the two aromatic side chains. We report here the preparation and PKC inhibitory activities of four balanol anologs **2-5** that differ from balanol only in the azepine heteroatom. The syntheses of these analogs are shown in **Scheme 1-3**.[5]



1 X = -NH-
2 X = -O-
3 X = -S-
4 X = $-SO_2-$
5 X = $-CH_2-$



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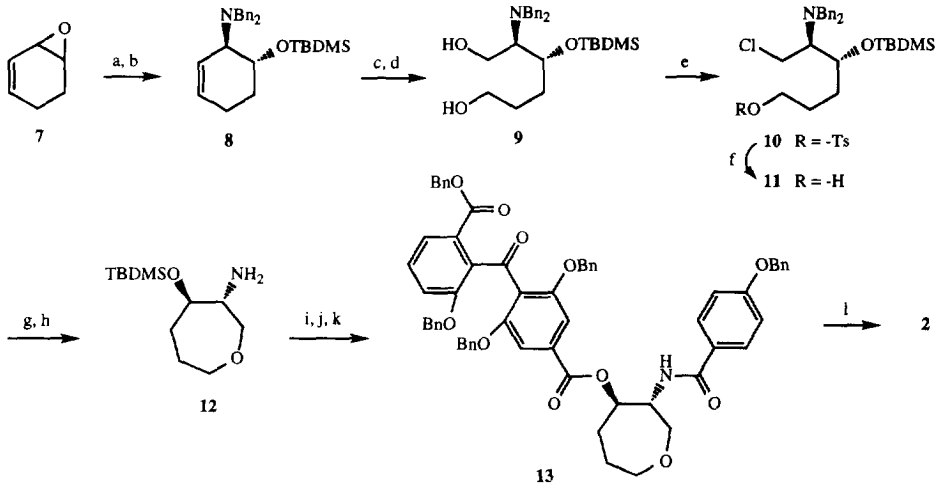
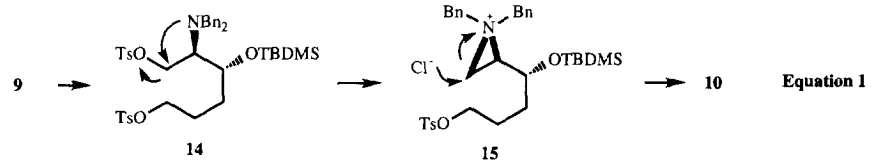
Treatment of epoxide **7**[6] with diethylaluminum-N,N-dibenzylamide[7] followed by O-protection with a tert-butyldimethylsilyl group provided **8**. Oxidative cleavage of alkene **8** with $OsO_4/NaIO_4$ and reduction of the resultant dialdehyde with $NaBH_4$ gave diol **9**. This was deprotonated and treated with 2 eq of p-toluenesulfonylchloride to give **10**, in which the two termini were differentiated, presumably via participation of

the dibenzylamino group as shown in **Equation 1**. Bis-tosylate **14** was also isolated in 17% yield. Reaction of **10** with KO_2[8] produced **11**, which cyclized to the required ether scaffold upon deprotonation and heating. Subsequent debenzylation by catalytic hydrogenolysis gave **12**, which was (i) N-acylated with 4-benzyloxybenzoyl imidazole; (ii) desilylated; and (iii) O-acylated with acid chloride **6**[9] to give **13**. Compound **13** was then debenzylated in one hydrogenation operation to give ether analog **2**.



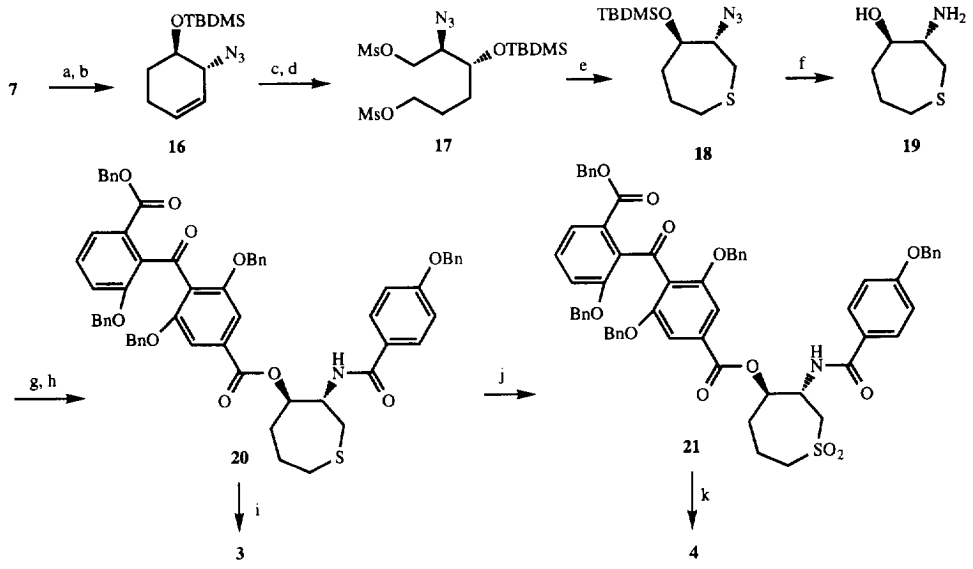
Scheme 1: (a) Bn_2NAlEt_2, CH_2Cl_2, rt, 91%; (b) TBDMS-Cl, imidazole, DMF, rt, 98%; (c) OsO_4, NMO, acetone-H_2O, rt, 80%; (d) $NaIO_4$, THF-H_2O, rt; then $NaBH_4$, Et_2O-MeOH, 5 °C, 70% total; (e) MeLi, THF; then TsCl, Et_3N, rt, 72%; (f) KO_2, 18-crown-6, DMSO, rt, 83%; (g) BuLi, $PhCH_3$, reflux, 57%; (h) H_2, $Pd(OH)_2$-c, MeOH, rt, 83%; (i) 4-benzyloxybenzoic acid, 1,1'-carbonyldiimidazole, THF, rt, 65%; (j) Bu_4NF, THF, rt, 61%; (k) **6**, Et_3N, DMAP, CH_2Cl_2, rt, 80%; (l) H_2, $Pd(OH)_2$-C, THF, MeOH, rt, 94%.



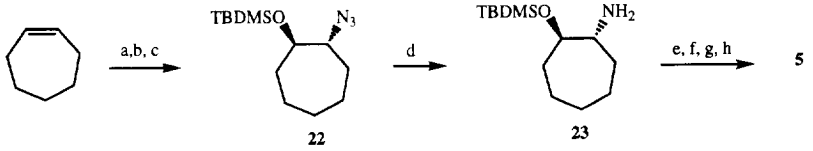
Equation 1

Compound **3** was prepared similarly from **7** with bis-mesylate **17** as a key intermediate. Cyclization of this compound to the desired sulfide **18** was effected with lithium sulfide, though in low yield. Reduction of azide **18** with $LiAlH_4$, followed by basic workup, concomitantly removed the tert-butyldimethylsilyl group; this gave **19**, which was coupled with 4-benzyloxybenzoyl imidazole and acid chloride **6**. The resultant **20** was hydrogenated using 2 eq of Pearlman's catalyst to provide the sulfide analog **3** in low yield. Alternatively, **20** was oxidized to sulfone **21**, which was debenzylated to give analog **4**.

The synthesis of **5**, as shown in **Scheme 3**, was essentially achieved using the chemistry described above. In contrast to the catalytic hydrogenation of azide **18**, which failed presumably due to catalyst poisoning, azide **22** was reduced to **23** under standard catalytic hydrogenation conditions.



Scheme 2: (a) NaN_3, NH_4Cl, MeOH-H_2O, 65 °C, 53%; (b) TBDMS-Cl, imidazole, DMF, rt, 97%; (c) O_3, CH_2Cl_2-MeOH, -78 °C; then $NaBH_4$, 78%; (d) $MeSO_2Cl$, Et_3N, CH_2Cl_2, rt, 96%; (e) Li_2S, Et_3N, MeOH, reflux, 36%; (f) $LiAlH_4$, THF, rt, 95%; (g) 4-benzyloxybenzoic acid, 1,1'-carbonyldiimidazole, THF; then 1N aq. NaOH, MeOH-THF, rt, 67%; (h) **6**, Et_3N, DMAP, CH_2Cl_2, rt, 76%; (i) H_2, $Pd(OH)_2$-C (2 eq), THF, MeOH, rt, 35%; (j) CH_3CO_3H, CH_3CO_2H-CH_2Cl_2, rt, 79%; (k) H_2, $Pd(OH)_2$-C, THF, MeOH, rt, 94%.



Scheme 3: (a) CH_3CO_3H, NaOAc, Na_2CO_3, CH_2Cl_2, 5 °C-rt, quant.; (b) NaN_3, NH_4Cl, MeOH- H_2O, reflux, 83%; (c) TBDMS-Cl, imidazole, DMF, rt, 93%; (d) H_2, 5% Pd-C, MeOH, rt, 72%; (e) 4-benzyloxybenzoic acid, 1,1'-carbonyldiimidazole, THF, rt, 82%; (f) Bu_4NF, THF, rt, 89%; (g) **6**, Et_3N, DMAP, CH_2Cl_2, rt, 32%; (h) H_2, $Pd(OH)_2$-C, MeOH, rt, 95%.

Screening of analogs **2-5** against human PKC isozymes α, β–1, β–2, γ, δ, ε, η, and ζ was carried out with standard protocols[10] and the IC_{50} values are shown in **Table 1**, together with racemic balanol.

Table 1: PKC Isozyme Inhibition by Balanol And Analogs **2-5** (IC_{50} values in μM)

Compd	α	β–1	β–2	γ	δ	ε	η	ζ
(±)-1*	0.074	0.032	0.044	0.034	0.032	0.049	0.022	3.5
2	6.7	2.5	3.3	1.0	0.09	16	0.01	>150
3	3.3	3.8	2.4	1.0	0.09	5.9	0.10	121
4	9.6	5.2	3.6	4.3	4.0	45	0.83	>150
5	0.27	0.22	0.43	0.06	0.09	0.28	0.06	>150

*synthetic material, see ref. 9a and 11.

One feature of balanol, as remarkable as its high potency against PKC, is its lack of isozyme selectivity. Interestingly, our results showed that significant isozyme selectivity can be obtained by single structural modification of this novel molecule. Thus, replacement of the perhydroazepine nitrogen atom with oxygen resulted in a 30 (γ) to 300 (ε) fold drop in potency against, not all, but six of the eight isozymes tested, as demonstrated by the ether analog **2**. In assays against the δ and η isozymes compound **2** appeared to be as potent as racemic balanol itself. Altogether this uneven change in potency among isozymes renders analog **2** a selective inhibitor for the δ and η isozymes. The sulfide analog **3** showed a profile similar to the ether analog in terms of potency, and selectivity for the η isozyme was significantly diminished. The sulfone analog **4** showed further decreased activity against all eight isozymes and, as a result, isozyme selectivity is completely absent. Somewhat unexpectedly the carbocycle analog **5** was found to be only 3-10 fold less potent than balanol and remained a submicromolar inhibitor against all but the ζ isozymes. This suggests that the perhydroazepine nitrogen is not necessarily required for good activity.

In summary, we have successfully synthesized four balanol analogs in which the perhydroazepine nitrogen was replaced with a different atom. In general, these modifications tend to degrade the potency of the resultant compounds against PKC. However, there is an isozyme-dependency in the degree of this negative effect which amounts to significant isozyme selectivety of analogs **2** and **3**. In addition, the reduction in potency was found to be minimal with the carbocycle analog, which demonstrated that the perhydroazepine substructure is not crucial for potency.

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