Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 8, pp. 973-978, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00151-5

SYNTHESIS AND PROTEIN KINASE C INHIBITORY ACTIVITIES OF INDANE ANALOGS OF BALANOL[1]

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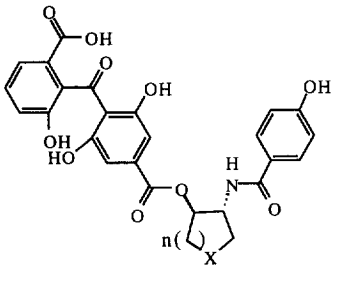
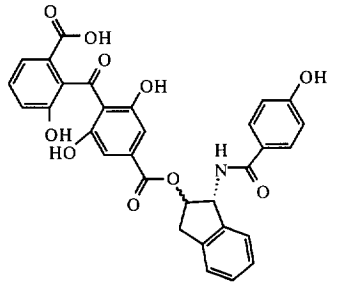
Abstract: Regio- and stereoisomeric indane analogs (**4-6**) of balanol (-)-**1**, a potent protein kinase C (PKC) inhibitor, were synthesized in which the perhydroazepine of balanol was replaced by an indane nucleus. Analog (-)-**4** and its racemic regioisomer **6** were found to have highly potent PKC inhibitory activities. In addition, compound (-)-**4** displayed excellent kinase selectivity for PKC over PKA. Copyright © 1996 Elsevier Science Ltd

Protein kinase C (PKC) is a family of phospholipid-dependent serine/threonine kinases which regulate a variety of cellular responses, including cell proliferation and differentiation. [2] Activated PKC has been implicated in the pathogenesis of diseases such as cancer,[3] asthma,[4] AIDS,[5] hypertension,[6] psoriasis,[7] and rheumatoid arthritis.[8] Development of potent and selective PKC inhibitors as novel therapeutics, therefore, have significant value not only for further revelation of the biology of PKC but also for the control of PKC-mediated disorders.

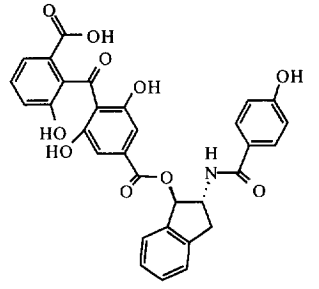
Balanol (-)-**1**, isolated from the fungus *Verticillium Balanoides*,[9] was recently discovered in our laboratories as one of the most potent PKC inhibitors. It has IC_{50}'s in the low nM range against most of the PKC isozymes tested. The discovery of balanol has advanced the development of PKC inhibitors as balanol



1, (-) n = 3, X = NH
2, (±) n = 1, X = NH
3, (±) n = 1, X = CH_2



4, (±) *anti*
4, (-) *anti*
4, (+) *anti*
5, (±) *syn*

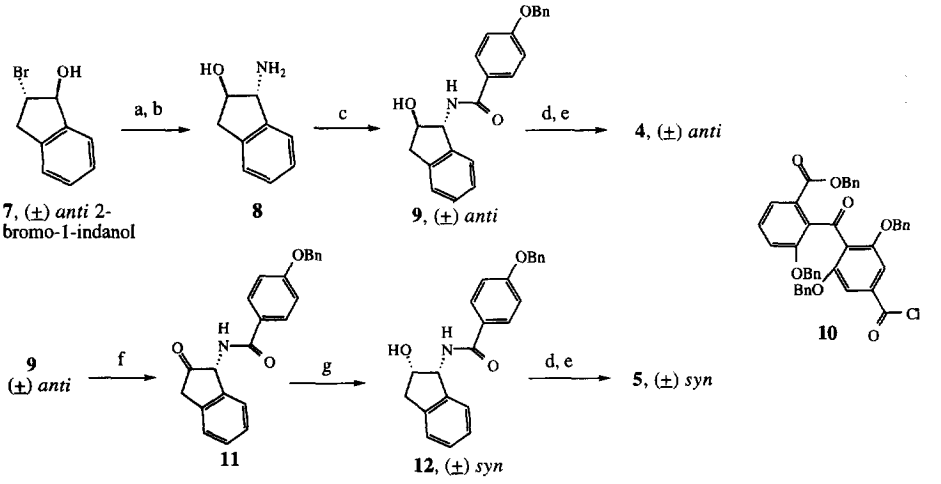


6, (±) *anti*

represents a novel and unique chemical class among known PKC inhibitors. The total synthesis of (-)-**1** was accomplished in our laboratories[10,11] as well as others[12] shortly after its isolation and structure elucidation.

Although balanol possesses significant PKC inhibitory activities, it inhibits cAMP-dependent protein

kinase (PKA) at a similar concentration (Table I). Our initial SAR studies established that the benzophenone ester of balanol was essential for optimum PKC inhibition.[13] As a result, part of our efforts were focused on replacement of the balanol perhydroazepine moiety in order to identify compounds with improved potency, better kinase selectivity, and increased synthetic efficiency. Two racemic five-membered ring analogs **2** and **3**[14] were shown to have highly potent PKC inhibitory activities. However, there was essentially no selectivity of these analogs for PKC over PKA, as was found with balanol (Table I). We report herein the syntheses and kinase inhibition of a family of indane analogs of balanol **4-6**.[15] The syntheses of these analogs are illustrated in Schemes I-IV.

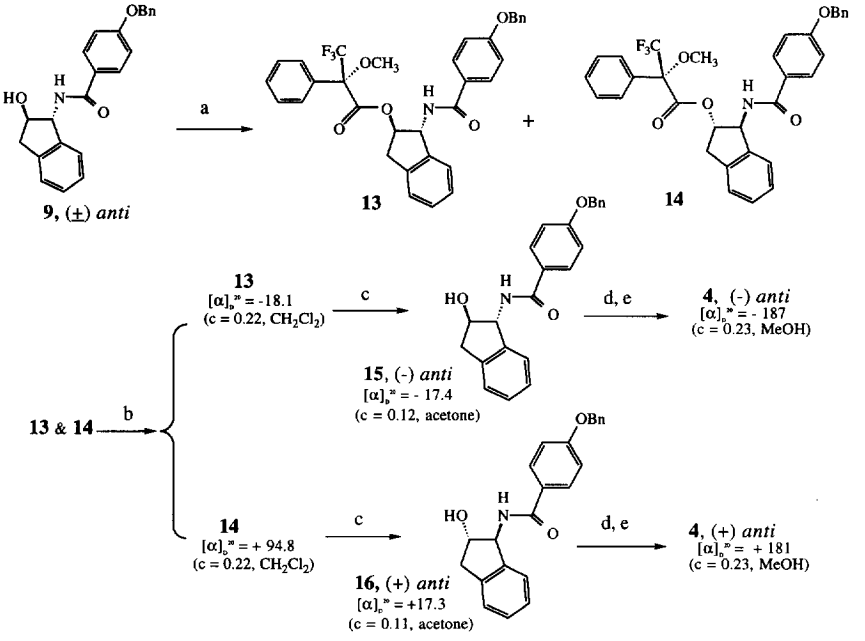


Scheme I: (a) DBU, CH_2Cl_2, rt, 63%. (b) aq. NH_4OH, rt, 82%. (c) (i). 4-Benzyloxybenzoyl chloride, NaOH, CH_2Cl_2, 5°C-rt. (ii). NaOH, MeOH, rt, 85%. (d) **10**, Et_3N, DMAP, CH_2Cl_2, rt. (e) H_2, $Pd(OH)_2$-C, EtOAc-MeOH (1:1), rt, 39% of **4** from **9** and 66% of **5** from **12**. (f) Jones reagent, acetone, rt, 46%. (g) $LiAl(^tBuO)_3H$, THF, 5°C-rt, 62% (*syn*:*anti* = 1.75:1, separated by column chromatography (silica gel, 5% acetone in CH_2Cl_2)).

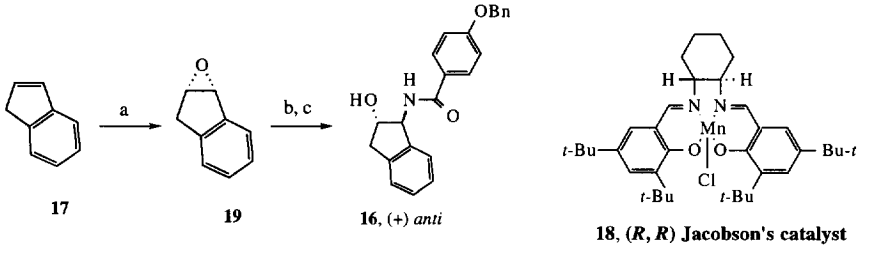
(±)-*anti*-2-Bromo-1-indanol **7** (purchased from Aldrich) was treated with DBU to provide the corresponding indene oxide which was subsequently treated with ammonium hydroxide to generate (±)-*anti*-1-amino-2-indanol **8** (Scheme I). The intermediate, (±)-*anti*-1-(4-benzyloxybenzamido)-2-indanol **9**, was prepared by condensation of **8** with 4-benzyloxybenzoyl chloride. (±)-*syn*-1-(4-Benzyloxybenzamido)-2-indanol **12** was derived from the (±)-*anti* precursor **9** by Jones oxidation followed by reduction with $LiAl(^tBuO)_3H$. Respective O-acylation of **9** and **12** with benzophenone acid chloride **10**[11] followed by hydrogenolysis with Pearlman's catalyst afforded indane analogs **4** and **5**.

Resolution of **9** (Scheme II) via its Mosher's esters **13** and **14** provided enantiomerically pure (-)-*anti*- and (+)-*anti*-1-(4-benzyloxybenzamido)-2-indanols **15** and **16**, which underwent O-acylation and debenzylation to afford (-)-**4** and (+)-**4**, respectively. The asymmetric synthesis of compound **16** also proved

to be feasible using (a) asymmetric epoxidation of indene **17** with (R, R) Jacobson's catalyst **18**,[16] (b) epoxide ring opening with NH_4OH, and (c) N-acylation with 4-benzyloxybenzoyl chloride, as shown in Scheme III.



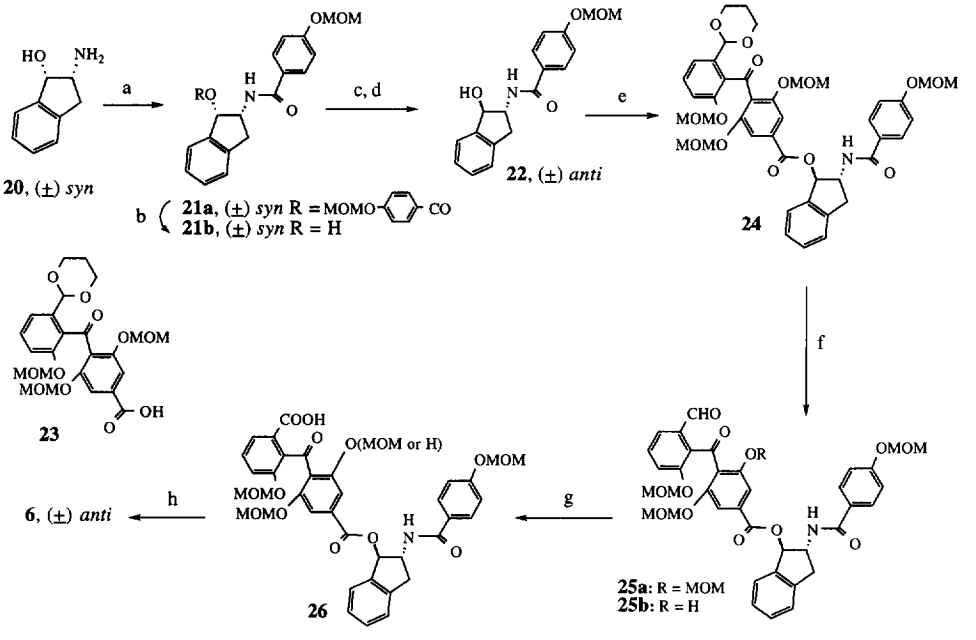
Scheme II: (a) (*S*)-(+)-Mosher's acid chloride, DMAP, Et_3N, CH_2Cl_2, 5°C-rt, 95%. (b) (i). recrystallization from MeOH gave **13**; (ii). chromatography of mother liquor (silica gel, 2% EtOAc in toluene) afforded **14**. Analyses of **13** and **14** by HPLC gave diastereometric purities of >99.9% de.[15] (c) KOH, MeOH-THF-H_2O. (d) **12**, Et_3N, DMAP, CH_2Cl_2, rt. (e) H_2, $Pd(OH)_2$-C, EtOAc-MeOH (1:1), rt, 79% of (-)-**4** from **15** and 73% of (+)-**4** from **16**.



18, (*R, R*) Jacobson's catalyst

Scheme III: (a) **18**, NaOCl, 4-phenylpyridine-N-oxide, 83%. (b) aq. NH_4OH (neat), 81%. (c) p-benzyloxybenzoyl chloride, NaOH, CH_2Cl_2, 62%. The Mosher's ester derived from **16** and (S)-(+)-Mosher's acid chloride was shown to have >98% de by HPLC.[15]

The synthesis of indane analog **6**, in which the positions of the amide and the ester are exchanged relative to **4**, is shown in Scheme IV. Since hydrogenolysis may not be a suitable method for the final deprotection of benzyl groups employed in the synthesis of **4** and **5** due to the presence of a benzylic oxygen at the C.1 position of the indane ring, all phenols on both side chains were protected with a MOM group instead of a benzyl group. Attempts to couple **20**[17] to either 4-methoxymethenyloxybenzoyl chloride (prepared from lithium salt of 4-methoxymethenyloxybenzoic acid with oxalyl chloride) or 4-methoxymethenyloxybenzoic acid in the presence of 1,1'-carbonyldiimidazole failed to give product **21b**. The acylation of **20** with 4-methoxymethenyloxybenzoic acid mediated by $P(O)(EtO)_2CN$ in the presence of Et_3N was successful, giving the bisacylated product **21a**. Interestingly, the bisacylation occurred even with only 0.5 equivalent of 4-methoxymethenyloxybenzoic acid. Compound **21a** was treated with KOH to unmask the indanolic hydroxyl group for oxidation and subsequent reduction to **22.** O-Acylation of **22** with acid **23**[18] in the presence of CDI and DBU provided intermediate **24**. Acidolysis of the dioxane in **24**, followed by oxidation of the resulting aldehyde **25a** to the corresponding acid **26** and removal of the MOM groups in **26** completed the synthesis of analog **6.**



Scheme IV: (a) 4-Methoxymethenyloxybenzoic acid (2 eq), $P(O)(OEt)_2CN$, Et_3N, DMF. (b) KOH, MeOH-H_2O, 50% from **20**. (c) Jones reagent, acetone, rt, quantitative. (d) $NaBH_4$, MeOH, rt, 75% (syn isomer < 2%). (e) **23**, CDI, DBU, DMF, rt, 97%. (f) 15%H_2SO_4 absorbed on SiO_2, CH_2Cl_2, rt, 43% of **25a** and 4.6% of **25b**. (g) H_2NSO_3H, $NaClO_2$, CH_3CN-H_2O, rt. (h) HCl, THF, rt, 63% from **25a**.

Analogs **4-6** were screened against human protein kinase C isozymes (Alpha, Beta I, Beta II, Gamma, Delta, Epsilon, and Eta) and cAMP-dependent protein kinase (PKA).[19] The IC_{50} values are illustrated in

Table I. The *anti* racemic indane analog **4**, was found to have high potency against most of PKC isozymes. The (-)-enantiomer of **4** proved to be two orders of magnitude more potent than the (+)-form. Compound **5**, the *syn* stereoisomer of (±)-**4**, was found to be essentially inactive against all PKC isozymes. These results appears to be consistent with the studies on *syn*- and optically active balanols (Table I). Interestingly, the inhibitory activity against PKC Beta I, Beta II, and Gamma isozymes was enhanced at least 10-fold compared with (±)-**4** by switching the positions of the amide and ester on the indane ring, as shown by compound **6**. In addition, indane analog (-)-**4** not only exhibited potent PKC inhibitory activity, but also displayed significant kinase selectivity for PKC over PKA. Since both balanol and its 5-membered ring analogs (compounds **2** and **3**) lacked kinase selectivity, the studies on indane analogs may provide a novel lead structure to develop therapeutically useful agents by selective inhibition of the protein kinase C.

Table I PKC Isozyme Inhibition by Balanol **1**[a] and Its Analogs **2-6** (IC_{50} in μM[b])

Compound	Alpha	Beta I	Beta II	Gamma	Delta	Epsilon	Eta	PKA
(-)-**1**	0.03	0.01	0.01	0.01	0.004	0.01	0.003	0.08
(+)-**1**	3.00	0.50	0.88	0.40	0.42	<1.00	0.26	5.20
(±)-*syn*-**1**	0.37	0.08	0.26	0.14	0.05	0.34	<0.10	0.79
2	0.022	0.01	0.033	0.012	0.005	0.01	0.004	0.07
3	0.04	0.04	0.05	0.01	0.0009	0.05	0.0006	0.03
(±)-**4**[20]	0.16	0.20	0.17	0.16	0.03	0.31	<0.01	50.0
(-)-**4**	0.29	0.06	0.05	0.05	0.05	1.90	<0.05	50.0
(+)-**4**[c]	>50.0	>50.0	39.0	32.0	32.0	>50.0	35.0	NA[d]
5	>50.0	37.0	37.0	34.0	21.0	NA[d]	25.0	>100
6	0.07	<0.029	<0.025	<0.03	<0.025	0.48	<0.025	NA[d]

(a) synthetic material.[11b] (b) IC_{50} values of **4-6** were calculated from four concentrations at 0.01, 0.1, 0.5 and1.5 μM or 0.05, 0.5, 5 and 50 μM; (c) synthesized by Scheme II; (d) data not available.

In summary, the syntheses of regio- and stereoisomeric indane analogs in which the perhydroazepine ring of balanol was replaced by an indane nucleus provided analogs (-)-**4** and **6** with highly potent PKC inhibitory activity and excellent selectivity for PKC over PKA, as seen in compound (-)-**4**. In addition, the racemic and asymmetric syntheses of functionalized indane moieties can be achieved more efficiently than those of perhydroazepine ring of balanol.[21]

Acknowledgements The authors wish to thank Joseph W. Wilson for providing the corresponding acid of **10**, and Thomas Mitchell for performing HPLC on Mosher's esters, FTIR and elemental analyses on intermediates and analogs described in the study.

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20. The data shown by (±)-**4** and its enantiomers in Table I were generated in two different experiments at different times employing different PKC enzyme preparations. We believed in this specific case that the difference in inhibitory activity on some isozymes (such as PKC-Alpha) between the racemate and the (-) enantiomer may be too slim to outweigh the experimental error. Upon reviewer's comments, the original materials of (±)-**4**, (-)-**4**, and (+)-**4** were retested side by side against the PKC-Alpha and shown to have IC_{50} of 0.46, 0.24, and >>0.50 μM, respectively. Since additional material is not available, we are unable to repeat the experiment with all the PKC isozymes listed in Table I.
21. For asymmetric synthesis of the azepine ring of balanol, see references 11 and 12. For racemic synthesis of the azepine, see Hu, H.; Jagdmann, G. E. Jr.; Hughes, P. F.; Nichols, J. B. *Tetrahedron Lett.* **1995**, *36*, 3659.

(*Received in USA* 17 *January* 1996; *accepted* 18 *March* 1996)