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Synthesis and Protein Kinase C Inhibitory Activities of Balanol Analogues with Modification of 4-Hydroxybenzamido Moiety

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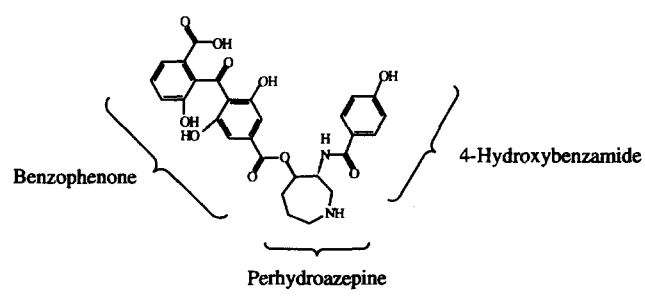
Abstract—A series of racemic balanol analogues with modification of the benzamido moiety of balanol have been synthesized and evaluated for their inhibitory activities against human protein kinase C isozymes (PKC-alpha, -beta I, -beta II, -gamma, -delta, -epsilon, and -eta). The structural modification includes replacement of the 4-hydroxyphenyl group with variously substituted phenyl rings, substitution of the amide linkage with a sulfonamide or an ester, and replacement of the 4-hydroxyphenyl substructure with a hydroxyl substituted indole or a hydroxybenzyl group. In general, these analogues were found to be less potent than balanol, but a number of analogues were identified with improved isozyme selectivity. The structure–activity relationship studies of these analogues also indicated that (1) the optimal general PKC inhibition requires a free 4-hydroxyl group in the benzamido portion of the molecule, (2) the amide linkage of the benzamido moiety is important for PKC inhibition, and (3) the conformation associated with the benzamido moiety seems to have a profound effect on PKC inhibition. The requirement of a free 4-hydroxyl group in conjunction with an appropriate conformation of the benzamido moiety for optimal PKC inhibition suggests that the 4-hydroxyphenyl group may be involved in a specific inhibitor–enzyme interaction important for PKC inhibition. © 1997 Elsevier Science Ltd.

Introduction

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.[1] Inappropriate activation of PKC has been implicated in a variety of human disease processes.[2] Development of PKC inhibitors as novel therapeutics, therefore, has significant value not only for the control of PKC-mediated disorders but also for further elucidation of the biology of PKC. Over the past decade, efforts in developing potent and selective PKC inhibitors have been fruitful. A number of classes of PKC inhibitors have been identified, some of which have demonstrated potential therapeutic applications in both in vitro and in vivo studies.[3]

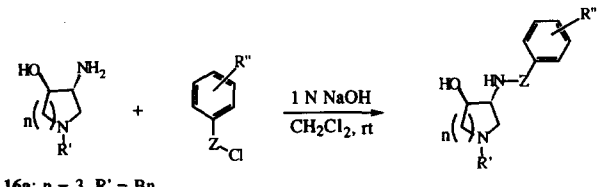
Balanol ((−)-**1**, Fig. 1), a fungal metabolite of *Verticillium balanoides*, was first isolated and identified in 1993 in our laboratories as one of the most potent naturally occurring PKC inhibitors.[4] It inhibited most PKC isozymes at low nanomolar concentrations (Table 1). In addition, balanol is distinct in structure among the known PKC inhibitors. Because of its high potency against PKC, its structural novelty and uniqueness, and its low availability from natural sources, balanol has received considerable attention as a synthetic target[5–9] as well as a new lead compound[10–13] for development of more potent and selective PKC inhibitors.

Balanol (Fig. 1) consists of perhydroazepine core and two side chains, namely highly substituted benzophenone moiety and 4-hydroxybenzamide which are attached to the perhydroazepine ring in a *trans* relationship. Earlier structure–activity relationship studies in our laboratories have established that an intact benzophenone moiety of balanol is essential for maintaining optimum PKC inhibition,[14] and that the balanol perhydroazepine ring could be replaced by a pyrrolidine or cyclopentane ring to provide equally or more potent PKC inhibitors (compounds **2** and **3**,[10] Tables 2 and 3), or by a bicyclic structure to provide more selective inhibitors for PKC in relation to other kinases.[11,12] In order to understand the effect on PKC inhibitory activity of modification of the 4-hydroxybenzamido moiety of balanol, we synthesized and evaluated a series of balanol analogues **4–15**, where the substitution pattern on the phenyl ring, the amide linkage, or the 4-hydroxylphenyl group on the benzamido portion of the molecule was altered. The syntheses of these analogues are illustrated in Schemes 1–4, and their PKC inhibitory activities are presented in Tables 1–4.



Balanol, (−)-1

Figure 1. Structure of (−)-balanol.



16a: n = 3, R' = Bn
16b: n = 1, R' = CBZ

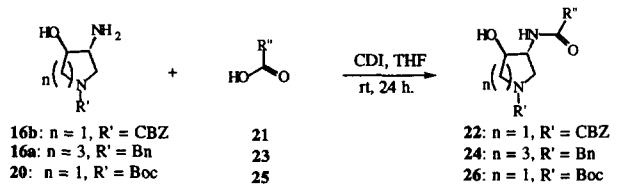
17a–h

18a–f: n = 3, R' = Bn
18g–h: n = 1, R' = CBZ

Compounds	Z	R"
17a** or 18a	-CO-	3-OBn
17b* or 18b	-CO-	4-F
17c** or 18c	-CO-	4-COOBn
17d* or 18d	-CO-	4-OCH_3
17e* or 18e	-CO-	4-CH_3
17f* or 18f	-SO_2-	4-CH_3
17g** or 18g	-CO-	3,4-diOBn
17h* or 18h	-CO-	4-NO_2

*commercially available
**synthesized from their aldehyde precursors

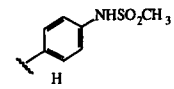
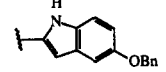
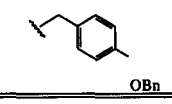
Scheme 1. Synthesis of amidoalcohol precursors to analogues **4–11**.



16b: n = 1, R' = CBZ
16a: n = 3, R' = Bn
20: n = 1, R' = Boc

21
23
25

22: n = 1, R' = CBZ
24: n = 3, R' = Bn
26: n = 1, R' = Boc

Compounds	R"
21* or 22	
23 or 24	
25** or 26	

*synthesized from its anilino precursor
**synthesized from its phenol precursor

Scheme 2. Synthesis of amidoalcohol precursors to analogues **13**, **14**, and **15**.

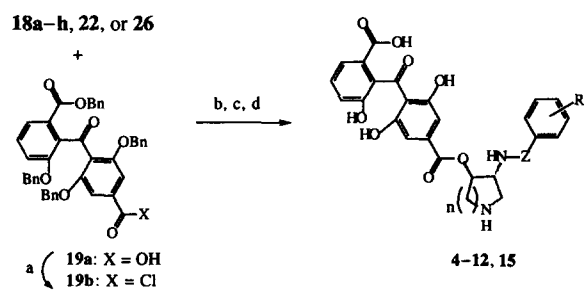
Results and Discussion

Chemistry

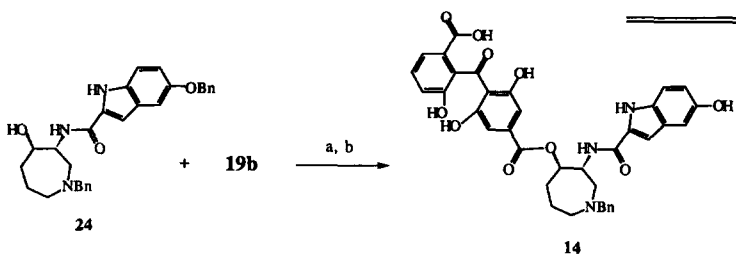
Two general synthetic approaches, as outlined in Schemes 1 and 2, were used for the synthesis of amidoalcohol precusors to balanol analogues. The syntheses of the starting *N*-substituted aminoalcohols of perhydroazepine **16a** and of pyrrolidines **16b** and **20** are described elsewhere.[15]

The first approach (Scheme 1) involved acylation of aminoalcohols **16a** and **16b** with a substituted benzoyl or sulfonyl chloride **17a–h** under Schotten–Baumann conditions to give the corresponding amidoalcohols **18a–h**. Acid chlorides that were not commercially available, namely **17a**, **17c**, and **17g**, were prepared from their corresponding aldehydes. Oxidation of an aldehyde with pyridinium dichromate or Jones reagent and conversion of the resultant acids to the acid chlorides with oxalyl chloride provided the required substituted benzoyl chlorides. The second approach (Scheme 2) employed coupling of aminoalcohols **16b**, **16a**, and **20** with carboxylic acids **21**, **23**, and **25** in the presence of *N,N'*-carbonyldiimidazole to form amidoalcohols **22**, **24**, and **26**, respectively.

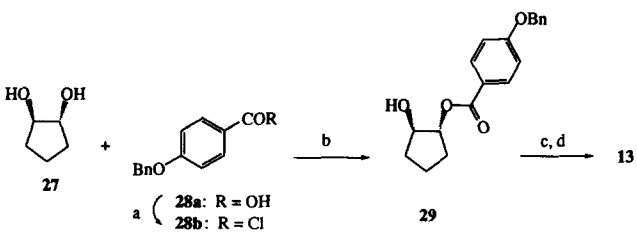
The synthesis of balanol analogues **4–12**, **14**, and **15** (Scheme 3) was completed by subsequent *O*-acylation of amidoalcohols **18a–h**, **22**, **24**, and **26** with benzophenone acid chloride **19b**,[5,16] followed by removal of



Compd	n	Z	R
4	3	-CO-	3-OH
5	3	-CO-	4-F
6	3	-CO-	4-COOH
7	3	-CO-	4-OCH_3
8	3	-CO-	4-CH_3
9	3	-SO_2-	4-CH_3
10	1	-CO-	3,4-diOH
11	1	-CO-	4-NH_2
12	1	-CO-	4-$NHSO_2CH_3$
15	1	-$COCH_2$-	4-OH



Scheme 3. Synthesis of balanol analogues **4–12**, **14**, and **15**. (a) Oxalyl chloride, cat. DMF, CH_2Cl_2, rt; (b) **19b**, Et_3N, DMAP, CH_2Cl_2, rt, 24 h; (c) H_2 (50 psi), $Pd(OH)_2$-C, EtOAc:MeOH (4:1), rt, 15 h; (d) CF_3COOH, rt, applied only to the precursor of the analogue **15**.



Scheme 4. Synthesis of balanol analogue **13**. (a) Oxalyl chloride, cat. DMF, CH_2Cl_2; (b) Et_3N, DMAP, CH_2Cl_2; (c) **19b**, Et_3N, DMAP, CH_2Cl_2; (d) H_2 (50 psi), $Pd(OH)_2$-C, EtOAc:MeOH (4:1), rt.

the benzyl protecting groups with catalytic hydrogenolysis. The synthesis of ester analogue **13** (Scheme 4) was realized from commercially available racemic *trans*-1,2-cyclopentandiol **27** by an analogous method described in Scheme 1.

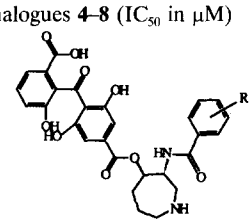
Protein kinase C inhibition

Analogues **4–15** were evaluated against human protein kinase C isozymes (alpha, beta I, beta II, gamma, delta, epsilon, and eta), and their structures and associated IC_{50} values are presented in Tables 1–4.

Improved isozyme selectivity was achieved by altering the substitution pattern on the phenyl ring of the benzamido moiety. As displayed in Tables 1 and 2, compounds **4** and **10** which have 3-hydroxyphenyl and 3,4-dihydroxyphenyl in place of the 4-hydroxyphenyl ring of balanol and analogue **2**, respectively, showed good selectivity for PKC-gamma, -delta, and -eta. Inhibition of these PKC isozymes by **4** and **10** were comparable to balanol. The 4-fluoro substituted analogue **5** demonstrated high potency and good selectivity for PKC-delta and -eta over other isozymes. 4-Methoxy and 4-methyl substituted compounds **7** and **8** inhibited PKC-eta selectively at concentration comparable to balanol.

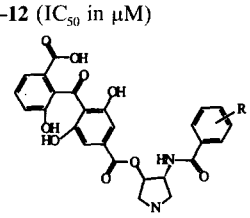
Optimal general PKC inhibition requires a free 4-hydroxyl group in the benzamido portion of the molecule. Compounds **7** and **8** (Table 1), where the 4-hydroxy group was masked or replaced by a methyl, showed a significant decrease in inhibition of most protein kinase C isozymes. Replacement of the phenolic

Table 1. PKC Isozyme inhibition by balanol (±)-**1** and analogues **4–8** (IC_{50} in μM)



		PKC						
Compound	**R**	**Alpha**	**Beta I**	**Beta II**	**Gamma**	**Delta**	**Epsilon**	**Eta**
(±)-**1**	4-OH	0.07	0.03	0.03	0.03	0.023	0.038	0.02
4	3-OH	0.30	0.30	0.40	0.03	0.05	0.28	0.02
5	4-F	3.8	0.32	0.50	0.54	0.06	0.25	0.02
6	4-COOH	>50.0	47.0	>50.0	>50.0	3.00	>50.0	0.49
7	4- OCH_3	7.30	8.80	8.30	3.40	0.23	4.90	0.08
8	4-CH_3	3.00	5.10	6.40	5.70	0.45	7.10	0.06

Table 2. PKC Isozyme inhibition by balanol analogues **10–12** (IC_{50} in μM)



		PKC						
Compound	**R**	**Alpha**	**Beta I**	**Beta II**	**Gamma**	**Delta**	**Epsilon**	**Eta**
2	4-OH	0.015	0.01	0.033	0.12	0.005	0.01	0.004
10	3,4-diOH	0.21	0.23	0.25	<0.05	0.03	0.12	0.04
11	4-NH_2	2.90	4.60	3.00	NA[a]	0.26	24.0	NA[a]
12	4-$NHSO_2CH_3$	3.20	3.60	3.00	1.90	0.50	11.0	0.36

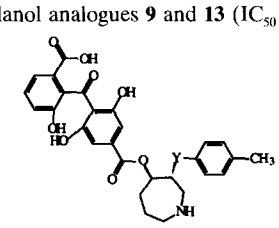
[a]Data not available.

hydroxyl group with hydrogen, as reported by Nicolaou and coworkers,[17] was associated with a 13-fold decrease of PKC inhibition in a purified rat brain PKC assay. Changing 4-hydroxyl group to a carboxyl group on the benzamido portion of the molecule such as compound **6** resulted in virtually no activity. Introduction of an amino or a methanesulfonamido group, compound **11** and **12** (Table 2), led to a remarkable decrease in PKC inhibition in comparison with pyrrolidine analogue **2**. These results show a specific preference for a phenol group at 4-position, not just general polar, acidic, or H-binding substituents. The requirement of a free 4-hydroxyl group for optimal PKC inhibition suggests that the 4-hydroxyl group may be involved in a specific interaction between the inhibitor and the PKC enzyme, presumably serving as a hydrogen bond donor.

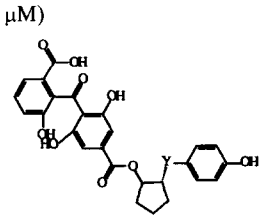
The amide linkage of the benzamido moiety is important for PKC inhibition, but it is not essential for optimal activity. Compared to the 4-methyl substituted compound **8** (Table 1) which showed potent inhibition of PKC-eta and weak inhibition of other isozymes, the corresponding sulfonamide analogue **9** (Table 3) was found to be completely inactive. The compound with isosteric replacement of the amide with an ester functionality, analogue **13**, showed an inhibition profile similar to cyclopentane analogue **3** for the PKC isozymes tested.

The conformation of the amide portion of the molecule seems to have a profound effect on PKC inhibition and isozyme selectivity. Compound **14**, which contains a 5-hydroxyindole as a 4-hydroxyphenyl mimic, showed potent inhibition and improved selectivity for PKC-beta I and -delta. However, compound **15**, which has an additional rotatable bond resulting from insertion of a methylene group between the amide functional group and the 4-hydroxyphenyl ring, showed a significant reduction in inhibition of all PKC enzymes. It may be speculated that the diminished activity displayed by **15** is due partly to a less favorable conformation posed by the 4-hydroxybenzyl group in **15** compared to the 4-hydroxyphenyl group in analogue **2**. It is also possible that the insertion of the methylene group could make

Table 3. PKC Isozyme inhibition by balanol analogues **9** and **13** (IC_{50} in μM)



9: Y = $NHSO_2$

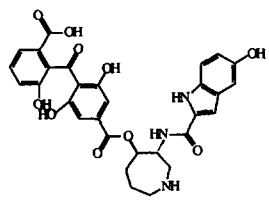


3: Y = NHCO
13: Y = OCO

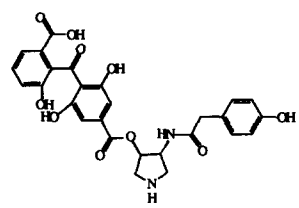
	PKC						
Compound	**Alpha**	**Beta I**	**Beta II**	**Gamma**	**Delta**	**Epsilon**	**Eta**
3	0.04	0.04	0.05	0.01	0.0009	0.05	0.0006
9	>150	>150	>150	7.30	46.0	>150	34.0
13	0.18	0.06	0.032	NA[a]	<0.025	NA[a]	NA[a]

[a]Data not available.

Table 4. PKC Isozyme inhibition by balanol analogues **14** and **15** (IC_{50} in μM)



14



15

	PKC						
Compound	**Alpha**	**Beta I**	**Beta II**	**Gamma**	**Delta**	**Epsilon**	**Eta**
(±)-1	0.07	0.03	0.03	0.03	0.023	0.038	0.02
2	0.015	0.01	0.033	0.12	0.005	0.01	0.004
14	1.30	0.03	0.20	0.32	0.01	0.25	<0.10
15	21.0	2.90	3.60	2.30	0.18	4.20	0.24

the 4-hydroxyl group in **15** less favorable to serve as a hydrogen bond donor in the inhibitor–enzyme interaction.

Conclusion

We have synthesized a series of balanol analogues where the 4-hydroxyl group, the amide functionality, or the 4-hydroxyphenyl subunit in the benzamido portion of the molecule was modified. By evaluation of these analogues against protein kinase C isozymes (PKC-alpha, -beta I, -beta II, -gamma, -delta, -epsilon, and -eta), we have identified a number of analogues with improved PKC isozyme selectivity. For example, 3-hydroxyphenyl or 3,4-dihydroxyphenyl substituted compounds **4** and **10** showed good selectivity for PKC-gamma, -delta, and -eta with IC_{50} values of 20–50 nM. Similarly, the 4-fluoro substituted analogue **5** demonstrated good selectivity for PKC-delta and -eta. 4-Methoxy and 4-methyl substituted compounds **7** and **8** inhibited PKC-eta selectively. The SAR studies of these analogues also indicated that optimal general PKC inhibition requires a free 4-hydroxyl group in the benzamido portion of the molecule. Specifically, replacing the 4-hydroxyl group with 4-fluoro, 4-methoxy, 4-methyl, 4-carboxyl, 4-amino or 4-methanesulfonamido, repositioning the hydroxyl group from 4- to 3-position of the benzamido ring or removal of 4-hydroxyl group led to partial or complete loss of the PKC activity. These studies further showed that the amide linkage of the benzamido moiety is important for PKC inhibition, which is supported by studies on the sulfonamide and ester analogues. Finally, the conformation associated with the amido moiety seems to have a profound effect on PKC inhibition. The less rigid analogue **15**, which has a methylene group inserted between the amide bond and the 4-hydroxyphenyl ring, showed poor PKC inhibition. The requirement of a free 4-hydroxyl group in conjunction with an appropriate conformation of the benzamido moiety for an optimal PKC inhibition suggests that the 4-hydroxyphenyl group may be involved in a specific inhibitor-enzyme interaction important for PKC inhibition.

Experimental

General

Melting points were measured with a MEL-TEMP II capillary melting point apparatus, and are uncorrected. IR spectra were taken on a Mattson Galaxy 2020 FT-IR spectrophotometer. ^1H spectra were recorded on a Varian Gemini-300 instrument (300 MHz) and calibrated with tetramethylsilane as an internal reference. Mass spectra were recorded by Analytical Instrument Group (Raleigh, NC). Flash chromatography was performed with Baker silica-gel (40 μM). The 'standard aqueous workup' involves washing of an organic phase with H_2O and brine, drying over anhydrous Na_2SO_4, filtration and concentration in vacuo on a rotary evaporator. All analogues were purified by high performance liquid chromatography (HPLC) using a Rainin gradient HPLC system equipped with a Rainin UV-1 variable wavelength detector with monitoring at 254 nm. Elemental analyses were determined with a EA 1108 Elemental Analyzer or were performed by Atlantic Microlab Inc. (Norcross, GA). All analogues were characterized by ^1H NMR, FTIR, mass spectroscopic and elemental analyses. Elemental analysis of intermediates or analogues was within 0.4% of theoretical values unless otherwise noted. All analogues were racemic and yields were not optimized.

3-Benzyloxybenzoyl chloride (17a). A mixture of 3-benzyloxybenzaldehyde (Aldrich, 1.0 g, 4.70 mmol) and pyridinium dichromate (7.06 g, 18.4 mmol) in anhydrous DMF (7 mL) was stirred at rt for 48 h. The reaction mixture was then poured into water (50 mL) and extracted with CH_2Cl_2 (100 mL). The CH_2Cl_2 layer was separated, washed with 1 N HCl (2 × 80 mL) and extracted with 1 N NaOH (2 × 100 mL). The basic aqueous solution was acidified with 4 N HCl to pH 1–2 to precipitate the crude product as a brown solid. Upon extractive workup with EtOAc (3 × 100 mL), the crude product was purified by a flash column chromatography (silica-gel, 0.15% HOAc in EtOAc) to afford 3-benzyloxybenzoic acid as a white solid (0.85g, 79%). ^1H NMR (DMSO-d_6) δ 7.52–7.40 (m, 9H), 5.15 (s, 2H). To a solution of 3-benzyloxybenzoic acid (271.97 mg, 1.19 mmol) in anhydrous CH_2Cl_2 (3mL) was added cat. DMF and oxalyl chloride (2.0 M solution in CH_2Cl_2, 1.56 mL, 3.12 mmol) at rt. The mixture was allowed to stir at rt for 2 h. Volatiles were removed, and the 3-benzyloxybenzoyl chloride was dried under vacuum for 1 h and used in the next step without purification.

4-Benzyloxycarbonylbenzoyl chloride (17c). A solution of 4-carboxybenzaldehyde (2.5 g, 16.65 mmol) in DMF (10 mL) was treated with potassium carbonate (2.53 g, 18.32 mmol) and benzyl bromide (3.05 g, 1.94 mL, 17.48 mmol) at rt overnight. To the reaction mixture was added another 0.5 mL of benzyl bromide and the mixture was heated at 50–60 °C for 8 h. The reaction mixture was poured into water (100 mL), extracted with EtOAc (2 × 100 mL), washed with 1 N K_2CO_3 (2 × 50 mL), brine (2 × 50 mL), dried (Na_2SO_4). The crude product after partial evaporation was chromatographed (silica gel, 15% EtOAc in hexane) to afford 4-benzyloxycarbonylbenzaldehyde as white needle-like crystals (3.75 g, 94 %); mp 142–144 °C. ^1H NMR ($CDCl_3$) δ 10.11 (s, 1H), 8.23 (d, 2H, J = 8.3 Hz), 7.95 (d, 2H, J = 8.1 Hz), 7.45–7.41 (m, 5H), 5.40 (s, 2H); IR (KBr) ν cm^{-1} 1715, 1705. Anal. ($C_{15}H_{12}O_3$) C, H. MS (EI) m/z 240 (M). A mixture of 4-benzyloxycarbonylbenzaldehyde (2.5 g, 10.41 mmol) and PDC (10.4 g, 27.71 mmol) in DMF (20 mL) was stirred at rt overnight, poured into water (120 mL), and extracted with EtOAc. The EtOAc layer was washed with 1 N HCl (2 × 50 mL), brine, dried (Na_2SO_4) and concentrated. The crude material was flash chromatographed (silica gel, 1%

AcOH in EtOAc) to give 4-benzyloxycarbonylbenzoic acid as white crytals (2.45 g, 92%); mp 178–179 °C; ^{1}H NMR (DMSO-d_6) δ 8.08 (s, 4H), 7.49–7.37 (m, 5H), 5.47 (s, 2H); IR (KBr) ν cm^{-1} 3410, 1709, 1681. Anal. ($C_{15}H_{12}O_4$) C, H. MS (EI) *m/z* 256 (M). The 4-benzyloxycarbonylbenzaldehyde was converted to the corresponding acid chloride (**17c**) by an analogous method described for the synthesis of **17a**, and the crude **17c** was carried through to acylation without further purification.

3,4-Dibenzyloxybenzoyl chloride (17g). To a solution of 3,4-dibenzyloxybenzaldehyde (Aldrich, 1.0 g, 3.14 mmol) in acetone (20 mL) was added Jones reagent (6 mL, 3.2 mmol in CrVI). After 2 h reaction at rt, the reaction mixture was quenched with *i*-PrOH to consume excess Jones reagent, and volatiles were removed in vacuo. The residue was taken up in EtOAc, washed with brine, dried (Na_2SO_4), and concentrated to afford 3,4-dibenzyloxybenzoic acid as an off-white solid (0.98g, 93%). ^{1}H NMR (DMSO-d_6) δ 12.7 (br s, 1H), 7.62–7.18 (m, 13H), 5.23 and 5.18 (s and s, 2H and 2H). The 3,4-dibenzyloxybenzoic acid was converted to the corresponding acid chloride (**17g**) by an analogous method described for the synthesis of **17a**, and the crude **17g** was carried through to acylation without further purification.

General procedure for the preparation of the amidoalcohols (18a–h) (Scheme 1). To a biphasic mixture of *N*-benzyl-*trans*-3-amino-4-hydroxyperhydroazepine (**16a**) or *N*-benzyloxycarbonyl-*trans*-3-amino-4-hydroxypyrrolidine (**16b**) in CH_2Cl_2: 1.0 N K_2CO_3 (1:1, 10 mL/mmol of **16a** or **16b**) was added a solution of the specified benzoyl or sulfonyl chloride (**17a–h**) (1.1 equiv) in CH_2Cl_2 (5 mL/mol of **16a** or **16b**) at 0 °C. The resulting mixture was vigorously stirred at room temperature for 15 h, followed by standard aqueous workup and purification of the crude product by flash column chromatography (silica gel, 40% hexane in EtOAc).

***N*-Benzyl-*trans*-3-(3-benzyloxybenzamido)-4-hydroxyperhydroazepine (18a)**. This was prepared from *N*-benzyl-*trans*-3-amino-4-hydroxyperhydroazepine (**16a**) and 3-benzyloxybenzoyl chloride (**17a**) as a white solid (43%). ^{1}H NMR ($CDCl_3$) δ 7.52–6.85 (m, 14H), 6.75 (br d, 1H), 5.10 (s, 2H), 3.90–3.82 (m, 3H), 3.73 (d, 1H, *J* = 12 Hz), 3.43 (d, 1H, *J* = 12Hz), 3.08–2.90 (m, 2H), 2.80–2.69 (m, 1H), 2.54–2.45 (m, 1H), 2.09–1.62 (m, 4H).

***N*-Benzyl-*trans*-3-(4-fluorobenzamido)-4-hydroxyperhydroazepine (18b)**. This was prepared from *N*-benzyl-*trans*-3-amino-4-hydroxyperhydroazepine (**16a**) and 4-fluorobenzoyl chloride (**17b**) as a white powder (49%); mp 95 °C. ^{1}H NMR ($CDCl_3$) δ 7.50–7.25 (m, 7H), 7.13 (t, 2H), 6.62 (br d, 1H), 3.90 (d, 1H, *J* = 2.4 Hz, exchangeable), 3.90–3.78 (m, 2H), 3.75 (d, 1H, *J* = 12.8 Hz), 3.42 (d, 1H, *J* = 12.8 Hz), 3.12–2.93 (m, 2H), 2.75 (m, 1H), 2.55 (m, 1H), 2.10–1.68 (m, 4H). Anal. calcd for $C_{20}H_{23}N_2O_2F$: C, 70.16; H, 6.77; N, 8.18; found: C, 70.16; H, 6.78; N, 8.13%.

***N*-Benzyl-*trans*-3-(4-benzyloxycarbonylbenzamido)-4-hydroxyperhydroazepine (18c)**. This was prepared from *N*-benzyl-*trans*-3-amino-4-hydroxyperhydroazepine (**16a**) and 4-benzyloxybenzoyl chloride (**17c**) as a pale-yellow oil (43%). ^{1}H NMR ($CDCl_3$) δ 8.07 (d, 2H, *J* = 8.5 Hz) 7.45–7.25 (m, 12H), 6.77 (br d, 1H, *J* = 5.2 Hz), 5.50 (s, 2H), 3.95–3.82 (m, 2H), 3.74 (d, 1H, *J* = 12.7 Hz), 3.42 (d, 1H, *J* = 12.7 Hz), 3.03 (m, 1H), 2.96 (dd, 1H, *J* = 14.5, 2.3 Hz), 2.72 (dd, 1H, *J* = 14.5, 3.2 Hz), 2.53 (m, 1H), 1.95–1.67 (m, 4H); IR (KBr) ν cm^{-1} 3383, 1716, 1650, 1519; Anal. calcd for C28H30N2O4·0.25H_2O: C, 72.63; H, 6.64; N, 6.05; found: C, 72.82; H, 6.64; N, 5.94%.

***N*-Benzyl-*trans*-3-(4-methoxybenzamido)-4-hydroxyperhydroazepine (18d)**. This was prepared from *N*-benzyl-*trans*-3-amino-4-hydroxyperhydroazepine (**16a**) and 4-methoxybenzoyl chloride (**17d**) as a white solid (48%). ^{1}H NMR ($CDCl_3$) δ 7.45–7.30 (m, 7H), 6.87 (d, 2H, *J* = 8.7 Hz), 6.59 (br s, 1H), 4.20 (br s, 1H), 3.88 (s, 3H), 3.85–3.81 (m, 2H), 3.75 (d, 1H, *J* = 12.7 Hz), 3.47 (d, 1H, *J* = 12.7 Hz), 3.10–2.90 (m, 2H), 2.75 (d, br, 1H), 2.50 (m, 1H), 1.98–1.60 (m, 4H).

***N*-Benzyl-*trans*-3-(4-methylbenzamido)-4-hydroxyperhydroazepine (18e)**. This was prepared from *N*-benzyl-*trans*-3-amino-4-hydroxyperhydroazepine (**16a**) and 4-methylbenzoyl chloride (**17e**) as a white solid (45%). ^{1}H NMR ($CDCl_3$) δ 7.48–7.12 (m, 9H), 6.62 (br s, 1H), 4.03 (br s, 1H), 3.91–3.79 (m, 2H), 3.75 (d, 1H, *J* = 12.9 Hz), 3.40 (d, 1H, *J* = 12.9 Hz), 3.03–2.89 (m, 2H), 2.76 (m, 1H), 2.51–2.44 (m, 2H), 2.41 (s, 1H), 1.94–1.66 (m, 4H).

***N*-Benzyl-*trans*-3-(4-methylbenzenesulfonamido)-4-hydroxyperhydroazepine (18f)**. This was prepared from *N*-benzyl-*trans*-3-amino-4-hydroxyperhydroazepine (**16a**) and 4-methylbenzenesulfonyl chloride (**17f**) as a white solid (58%); mp 69–71 °C. ^{1}H NMR ($CDCl_3$) δ 7.55–7.40 (m, 3H), 7.38–7.30 (m, 4H), 7.14 (d, 2H, *J* = 8.5 Hz), 5.77 (br s, 1H, exchangeable), 3.95 (br d, 1H), 3.61 (d, 1H, *J* = 12.9 Hz), 2.96 (br s, 1H), 3.31 (d, 1H, *J* = 12.9 Hz), 2.85 (m, 1H), 2.70 (d, 1H, *J* = 13.5 Hz), 2.45 (m, 2H), 2.20 (s, 3H), 2.0–1.5 (m, 4H). Anal. calcd for $C_{20}H_{26}N_2O_3S$: C, 64.14; H, 7.00; N, 7.48; S, 8.56; found: C, 63.89; H, 7.12; N, 7.19; S, 8.20%.

***N*-Benzyloxycarbonyl-*trans*-3-(3,4-bisbenzyloxybenzamido)-4-hydroxypyrrolidine (18g)**. This was prepared from *N*-benzyloxycarbonyl-*trans*-3-amino-4-hydroxypyrrolidine (**16b**) and 3,4-dibenzyloxybenzoyl chloride (**17g**) as a white solid (95%). ^{1}H NMR (DMSO-d_6) δ 8.41 (d, 1H, *J* = 8.7 Hz), 7.67 (s, 1H), 7.51–7.28 (m, 15H), 7.12 (d, 1H, *J* = 8.7 Hz), 5.42 (br s, 1H), 5.20 (s, 2H), 5.16 (s, 2H), 5.09 (s, 2H), 4.23 (br s, 1H), 4.18 (br s, 1H), 3.74–3.50 (m, 2H), 3.28 (m, 2H).

***N*-Benzyloxycarbonyl-*trans*-3-(4-nitrobenzamido)-4-hydroxypyrrolidine (18h)**. This was prepared from *N*-benzyloxycarbonyl-*trans*-3-amino-4-hydroxypyrrolidine (**16b**) and 4-nitrobenzoyl chloride (**17h**) as a white solid (93%). ^{1}H NMR (DMSO-d_6) δ 8.67 (d, 1H, J = 8.7 Hz), 8.31 (d, 2H, J = 8.7 Hz), 8.07 (d, 2H, J = 8.0 Hz) 7.40–7.30 (m, 5H), 5.49 (d, 1H, J = 3.0 Hz), 5.01 (s, 2H), 4.28 (br s, 1H), 4.16 (br s, 1H), 3.75–3.50 (m, 2H), 3.30 (m, 2H).

4-Methanesulfonamidobenzoic acid (21). To a cold solution (0 °C) of methyl 4-aminobenzoate (2.0 g, 13.2 mmol) in pyridine (40 mL) was added methanesulfonyl chloride (1.81 g, 1.22 mL, 15.8 mmol). The mixture was allowed to stir at rt overnight. Pyridine was removed in vacuo, and the residue was taken up in EtOAc (100 mL) and washed with brine containing 0.1 N HCl (3 × 60 mL). Crude methyl 4-methanesulfonamidobenzoate was obtained as a light purple solid (2.8 g, 98%) upon drying (Na_2SO_4) and evaporation. A mixture of this material (2.8 g, 12.2 mmol) and KOH (10.2 g, 183.3 mmol) in MeOH:H_2O (4:1, 100 mL) was stirred at rt for 24 h. After removal of methanol in vacuo, the aqueous phase was acidified with 4 N HCl, extracted with EtOAc, and subject to the standard aqueous workup to provide the desired acid as a beige solid (2.34 g, 89%). ^{1}H NMR (DMSO-d_6) δ 7.89 (d, 2H, J = 8.6 Hz), 7.27 (d, 2H, J = 8.7 Hz), 3.09 (s, 3H); IR (KBr) ν cm^{-1} 3276, 1681, 1607. Anal. calcd for $C_8H_9NO_4S$: C, 44.64; H, 4.21; N, 6.51; S, 14.90; found: C, 44.40; H, 4.16; N, 6.34; S, 15.20%.

General procedure for the preparation of the amidoalcohols (22, 24, and 26). Preparation of *N*-benzyloxycarbonyl-*trans*-3-(4-methanesulfonamidobenzamido)-4-hydroxypyrrolidine (22) (Scheme 2). To a solution of 4-methanesulfonamidobenzoic acid (**21**, 219 mg, 1.02 mmol) in anhydrous THF (5 mL) was added 1,1′-carbonyldiimidazole (206 mg, 1.27 mmol). The resulting mixture was stirred at rt for 2 h under nitrogen prior to the addition of a solution of *N*-benzyloxycarbonyl-*trans*-3-amino-4-hydroxypyrrolidine (**16b**, 222 mg, 0.85 mmol) in THF (3 mL). After stirred at rt for overnight, the reaction mixture was diluted with EtOAc, washed with brine, dried (Na_2SO_4), and chromatographed (silica-gel, 80% EtOAc in hexane containing 2% MeOH) to afford the desired acylated product **22** (139 mg) and the corresponding bisacylated product (134 mg). Saponification of the bisacylated product with K_2CO_3 (393 mg, 2.85 mmol) in MeOH:H_2O (1:1, 4 mL) afforded additional 71 mg of **22** (total 210 mg, 57%). ^{1}H NMR (DMSO-d_6) δ 8.40 (d, 1H, J = 6.0 Hz), 7.80 (d, 2H, J = 8.2 Hz), 7.20 (d, 2H, J = 8.2 Hz), 5.4 (br s, 1H), 5.08 (s, 2H), 4.22 (br s, 1H), 4.09 (br s, 1H), 3.71–3.45 (m, 2H), 3.30–3.20 (m, 2H), 3.05 (s, 3H).

***N*-Benzyl-*trans*-3-(5-benzyloxyindol-2-yl)carboxamido-4-hydroxyperhydroazepine (24)**. This was prepared from *N*-benzyl-*trans*-3-amino-4-hydroxyperhydroazepine (**16a**) and 5-benzyloxy-2-carboxyindole (**23**) as a white solid (50%). ^{1}H NMR (DMSO-d_6) δ 8.70 (d, 1H, J = 8.6 Hz), 8.40–7.70 (m, 15H), 5.91 (s, 2H), 5.58 (d, 1H, J = 4.5 Hz), 4.71 (m, 1H), 4.53–4.47 (m, 2H), 4.20 (m, 2H), 3.55–3.29 (m, 3H), 2.78-2.30 (m, 4H).

4-Benzyloxyphenylacetic acid (25). To a mixture of 4-hydroxyphenylacetic acid (3.04 g, 0.02 mol) and anhydrous K_2CO_3 (6.08 g, 0.022 mol) in DMF (60 mL) was added benzyl bromide (5.24 mL, 0.022 mol) at rt. The reaction mixture was stirred for three days at rt and the solid was removed by filtration. The filtrate was diluted with EtOAc and washed with H_2O. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The resulting benzyl-4-benzyloxyphenylacetate (**25**, 5.8 g, 87%) was sufficiently pure and was used directly in the next step without purification. To a solution of benzyl 4-benzyloxyphenylacetate (5.8 g, 17.46 mmol) in THF:MeOH:H_2O (100 mL, 3:1:1) was added LiOH·H_2O (1.76 g). The reaction mixture was stirred for 5 h at rt, then acidified with 50% aq. HCl (8 mL). The solvent was removed in vacuo to approx 20 mL, the solid was filtered and washed with H_2O several times, then dried under vacuum at 60 °C overnight to give 4-benzyloxyphenylacetic acid (4 g, 95%); mp 119–120 °C. ^{1}H NMR ($CDCl_3$) δ 7.45–7.32 (m, 5H), 7.21 (d, 2H, J = 8.6 Hz), 6.96 (d, 2H, J = 8.6 Hz), 5.06 (s, 2H), 3.60 (s, 2H); IR (KBr) ν cm^{-1} 1688, 1512.

***N*-(*tert*-Butyloxycarbonyl)-*trans*-3-(4-benzyloxybenzylcarboxamido)-4-hydroxypyrrolidine (26)**. This was prepared from *N*-(*tert*-butyloxycarbonyl)-*trans*-3-amino-4-hydroxypyrrolidine (**20**) and 4-benzyloxyacetic acid (**25**) as a white solid (220 mg, 52%); mp 157–159 °C; ^{1}H NMR (CD_3OD) δ 7.22–7.08 (m, 5H), 6.97 (d, 2H, J = 8.6 Hz), 6.71 (d, 2H, J = 8.7 Hz), 4.84 (s, 2H), 3.88 (br s, 2H), 3.48–3.27 (m, 2H), 3.21 (s, 2H), 3.08–3.00 (m, 2H), 1.26 (s, 9H). IR (KBr) ν cm^{-1} 3429, 3260, 1677, 1647, 1552. Anal. calcd for $C_{24}H_{30}N_2O_5$: C, 67.59; H, 7.09; N, 6.57; found: C, 67.93; H, 7.23; N, 6.48%.

***trans*-2-(4-Benzyloxybenzoyloxy)cyclopentanol (29) (Scheme 4)**. To a solution of *trans*-1,2-cyclopentandiol (**27**, 613 mg, 6.0 mmol) in CH_2Cl_2 (24 mL) was added DMAP (40 mg), Et_3N (0.6 mL), followed by a slow addition of 4-benzyloxybenzoyl chloride (**28b**, 739.5 mg, 3.0 mmol) at rt under nitrogen atmosphere. The reaction mixture was stirred at rt for 45 min, then the solvent was partially evaporated in vacuo and the crude mixture purified by flash chromatography (silica-gel, 30% hexane in EtOAc) to afford *trans*-2-(4-benzyloxybenzoyloxy)-cyclopentanol (**29**) as a white solid (500 mg, 53%); mp 86–88 °C; ^{1}H NMR ($CDCl_3$) δ 7.99 (d, 2H, J = 8.6 Hz), 7.45–7.35 (m, 5H), 6.99 (d, 2H, J = 8.5 Hz), 5.13 (s, 2H), 5.00 (m, 1H), 4.20 (m, 1H), 3.08 (s, 1H), 2.25–1.67 (m, 6H); IR (KBr) ν cm^{-1} 1680, 1603, 1508. Anal. calcd for $C_{19}H_{20}O_4 \cdot 0.1\ H_2O$: C, 72.64; H, 6.48; found: C, 72.62; H, 6.58%.

General procedure of the preparation of balanol analogues (4–15). Preparation of *trans*-3-(3-hydroxybenzamido)-4-[4-(2-carboxy-6-hydroxybenzoyl)-3,5-dihydroxybenzoyloxy]perhydroazepine (4) (Scheme 3). To a solution of the perbenzyl benzophenone acid (**19a**, 300 mg, 0.442 mmol) in CH_2Cl_2 (3 mL) was added cat. DMF and oxalyl chloride (2.0 M solution in CH_2Cl_2, 0.55 mL, 1.11 mmol) at rt under nitrogen atmosphere. The mixture was allowed to stir at rt for 2 h prior to removal of volatiles in vacuo. The resulting perbenzyl benzophenone acid chloride **19b** was dried under vacuum for 1 h, reconstituted in CH_2Cl_2 (10 mL), and added to a solution of the amidoalcohol (**18a**, 190.3 mg, 0.442 mmol), Et_3N (223.6 mg, 308 mL, 2.21 mmol) and DMAP (54 mg, 0.442 mmol) in CH_2Cl_2 (10 mL) at 5 °C. After reaction at rt overnight, the reaction mixture was partially evaporated and chromatographed (silica-gel, 40% EtOAc in hexane) to afford the coupling product as a white fluffy solid (285 mg, 59%). The coupling product (275 mg, 0.252 mmol) was dissolved in EtOAc:EtOH (3:2, 25 mL) and treated with catalytic CFCOOH followed by 10% $Pd(OH)_2$ on carbon (160 mg, 60 mol %). The mixture was subject to hydrogenolysis on a Parr shaker at 50 psi for 24 h. The reaction mixture was carefully filtered through a pad of Celite® and the Celite cake washed with EtOH (2 × 15 mL). The combined mixture was concentrated in vacuo. The crude product was redissolved in MeOH (1.75 mL) and loaded onto HPLC for purification; conditions: (A) 5% CH_3CN in H_2O + 0.1% trifluoroacetic acid, (B) 100% CH_3CN; 0–50% B over 60 min, 25 mL/min, 41 × 300 mm C18 column. Pure fractions were combined, partially concentrated and lyophilized to afford a pale-yellow fluffy solid (125 mg, 75%); mp 184–186 °C dec; ^{1}H NMR (CD_3OD) δ 7.49 (d, 1H, J = 8.0 Hz), 7.30–7.17 (m, 4H), 7.02 (d, 1H, J = 8.3 Hz), 6.94 (d, 1H, J = 7.87 Hz), 6.90 (s, 2H), 5.45 (m, 1H), 4.49 (m, 1H), 3.50 (d, 2H), 2.30–2.0 (m , 4H); IR (KBr) ν cm^{-1} 1700, 1680, 1650, 1630. Anal. calcd for $C_{28}H_{26}N_2O_{10}\cdot 2.5H_2O\cdot 1.0C_2HF_3O_2$: C, 50.78; H, 4.54; N, 3.95; found: C, 50.45; H, 4.36; N, 3.79%. MS (LRFAB) m/z 551 (M + 1).

***trans*-3-(4-Fluorobenzamido)-4-[4-(2-carboxy-6-hydroxybenzoyl)-3,5-dihydroxybenzoyloxy]perhydroazepine (5).** This was prepared as a yellow solid from *O*-acylation of amidoalcohol **18b** with perbenzyl benzophenone acid chloride **19b** (88%), followed by catalytic debenzylation (62%); mp 175–178 °C dec; ^{1}H NMR (CD_3OD) δ 7.58 (t, 2H, J = 5.5 Hz), 7.28 (d, 1H, J = 7.8 Hz), 7.06 (t, 1H, J = 7.8 Hz), 6.94 (t, 1H, J = 8.7 Hz), 6.80 (d, 1H, J = 7.8 Hz), 6.67 (s, 2H), 5.21 (m, 1H), 4.28 (m, 1H,), 3.29 (d, 2H, J = 5.0 Hz), 2.20–1.70 (m , 4H); IR (KBr) ν cm^{-1} 1704, 1634, 1605. Anal. calcd for $C_{28}H_{25}FN_2O_9\cdot 1.75H_2O\cdot 1.0C_2HF_3O_2$: C, 51.62; H, 4.26; N, 4.01; found: C, 51.44; H, 3.90; N, 4.07%. MS (LRFAB) m/z 553 (M + 1).

***trans*-3-(4-Carboxybenzamido)-4-[4-(2-carboxy-6-hydroxybenzoyl)-3,5-dihydroxybenzoyloxy]perhydroazepine (6).** This was prepared as a yellow solid from *O*-acylation of amidoalcohol **18c** with perbenzyl benzophenone acid chloride **19b** (73%), followed by catalytic debenzylation (74%); mp 204–206 °C dec; ^{1}H NMR (CD_3OD) δ 8.06 (d, 2H, J = 8.4 Hz), 7.80 (d, 2H, J = 8.4 Hz), 7.48 (d, 1H, J = 7.3 Hz), 7.26 (t, 1H, J = 7.3 Hz), 7.01 (d, 1H, J = 7.3 Hz), 6.89 (s, 2H), 5.45 (m, 1H), 4.49 (m, 1H), 3.51 (d, 2H, J = 5.8 Hz), 2.31–2.00 (m , 4H); IR (KBr) ν cm^{-1} 1704, 1676, 1636, 1606. Anal. calcd for $C_{29}H_{26}N_2O_{11}\cdot 1.25H_2O\cdot 1.5C_2HF_3O_2$: C, 49.78; H, 3.92; N, 3.63; found: C, 49.88; H, 3.70; N, 3.68%. MS (LRFAB) m/z 579 (M + 1).

***trans*-3-(4-Methoxybenzamido)-4-[4-(2-carboxy-6-hydroxybenzoyl)-3,5-dihydroxybenzoyloxy]perhydroazepine (7).** This was prepared as a yellow solid from *O*-acylation of amidoalcohol **18d** with perbenzyl benzophenone acid chloride **19b** (58%), followed by catalytic debenzylation (90%); mp 178–180 °C dec; ^{1}H NMR (CD_3OD) δ 7.74 (d, 2H, J = 8.7 Hz), 7.51 (d, 1H, J = 7.2 Hz), 7.29 (t, 1H), 7.04 (d, 1H, J = 8.3 Hz), 6.98 (d, 2H, J = 9.0 Hz), 6.90 (s, 2H), 5.45 (m, 1H), 4.49 (m, 1H), 3.84 (s, 3H), 3.50 (d, 2H, J = 5.8 Hz), 2.38–2.00 (m , 4H); IR (KBr) ν cm^{-1} 1699, 1681, 1650, 1634, 1610. Anal. calcd for $C_{29}H_{28}N_2O_{10}\cdot 2.5H_2O\cdot 1.0C_2HF_3O_2$: C, 51.46; H, 4.74; N, 3.87; found: C, 51.13; H, 4.37; N, 3.82%. MS (LRFAB) m/z 565 (M + 1).

***trans*-3-(4-Methylbenzamido)-4-[4-(2-carboxy-6-hydroxybenzoyl)-3,5-dihydroxybenzoyloxy]perhydroazepine (8).** This as prepared as a yellow solid from *O*-acylation of amidoalcohol **18e** with perbenzyl benzophenone acid chloride **19b** (70%), followed by catalytic debenzylation (62%); mp 172–175 °C dec; ^{1}H NMR (CD_3OD) δ 7.64 (d, 2H, J = 8.3 Hz), 7.50 (d, 1H, J = 7.7 Hz), 7.29–7.23 (m, 4H), 7.03 (d, 1H, J = 8.2 Hz), 6.89 (s, 2H), 5.45 (m, 1H), 4.50 (m, 1H), 3.50 (d, 2H, J = 5.6 Hz), 2.30–2.00 (m , 4H); IR (KBr) ν cm^{-1} 1698, 1680, 1637, 1558. Anal. calcd for $C_{29}H_{28}N_2O_9\cdot 1.5H_2O\cdot 1.25C_2HF_3O_2$: C, 52.69; H, 4.53; N, 3.90; found: C, 52.68; H, 4.28; N, 3.90%. MS (LRFAB) m/z 549 (M + 1).

***trans*-3-(4-Methylbenzenesulfonamido)-4-[4-(2-hydroxy-6-carboxybenzoyl)-3,5-dihydroxy-benzoyloxy]perhydroazepine (9).** Prepared as a yellow solid from *O*-acylation of amidoalcohol **18f** with perbenzyl benzophenone acid chloride **19b** (57%), followed by catalytic debenzylation (30%); mp 162–165 °C dec. ^{1}H NMR (CD_3OD) δ 7.62 (d, 2H, J = 8.3Hz), 7.52 (d, 1H, J = 7.7Hz), 7.28 (t, 1H, J = 7.7Hz), 7.13 (d, 2H, J = 8.1Hz), 7.04 (d, 1H, J = 8.2Hz), 6.58 (s, 2H), 5.05 (m, 1H), 3.72 (m, 1H), 3.51 (m, 2H), 3.25 (m, 2H), 2.10–1.90 (m, 4H); IR (KBr) ν cm^{-1} 1677, 1635, 1602. Anal. calcd for $C_{28}H_{28}N_2O_{10}S\cdot 2.5H_2O\cdot 1.0C_2HF_3O_2$: C, 48.45; H, 4.61; N, 3.77; S, 4.31; found: C, 48.66; H, 4.27; N, 3.49; S, 4.30%. MS (LRFAB) m/z 585 (M + 1).

***trans*-3-(3,4-Dihydroxybenzamido)-4-[4-(2-carboxy-6-hydroxybenzoyl)-3,5-dihydroxybenzoyloxy]pyrrolidine (10).** This was prepared as a yellow fluffy solid from *O*-acylation of amidoalcohol **18g** with perbenzyl benzophenone acid chloride **19b** (70%), followed by

catalytic debenzylation (65%); mp 210–213 °C dec. ^{1}H NMR (CD_3OD) δ 7.50 (d, 2H, J = 8.7 Hz), 7.33–7.25 (m, 3H), 7.03 (d, 1H, J = 7.1 Hz), 6.97 (s, 2H), 6.81 (d, 1H, J = 8.3 Hz), 5.63 (m, 1H), 4.63 (m, 1H), 3.95 (dd, 1H, J = 5.7, 13.5 Hz), 3.85 (dd, 1H, J = 7.2, 12.6 Hz), 3.62 (m, 2H); IR (KBr) ν cm^{-1} 1717, 1676, 1636. Anal. calcd for $C_{26}H_{22}N_2O_{11}\cdot 2.0H_2O\cdot 1.0C_2HF_3O_2$: C, 48.84; H, 3.95; N, 4.07; found: C, 48.75; H, 3.63; N, 4.07%. MS (LRFAB) m/z 579 (M + 1).

***trans*-3-(4-Aminobenzamido)-4-[4-(2-carboxy-6-hydroxybenzoyl)-3,5-dihydroxybenzoyloxy]pyrrolidine (11).** This was prepared as a yellow fluffy solid from *O*-acylation of amidoalcohol **18h** with perbenzyl benzophenone acid chloride **19b** (90%), followed by catalytic debenzylation (6%). The low yield of the debenzylation was due to the incomplete reduction of nitro group under the hydrogenolysis condition with Pearlman's catalyst; mp >184 °C dec. ^{1}H NMR (CD_3OD) δ 7.42 (d, 2H, J = 8.6 Hz), 7.20 (d, 1H, J = 8.4 Hz), 7.03 (t, 1H, J = 8.3 Hz), 6.75 (s, 2H), 6.74 (d, 1H, J = 8.2 Hz), 6.45 (d, 1H, J = 8.7 Hz), 5.35 (m, 1H), 4.40 (m, 1H), 3.72–3.51 (m, 2H), 3.42 (m, 2H); IR (KBr) ν cm^{-1} 1725, 1677, 1631, 1605. Anal. calcd for $C_{26}H_{23}N_3O_9\cdot 1.0C_2HF_3O_2$: C, 52.92; H, 3.81; N, 6.61; found: C, 52.81; H, 4.19; N, 6.48%. MS (LRFAB) m/z 522 (M + 1).

***trans*-3-(4-Methanesulfonamidobenzamido)-4-[4-(2-carboxy-6-hydroxybenzoyl)-3,5-dihydroxybenzoyloxy]-pyrrolidine (12).** This was prepared as a yellow fluffy solid from *O*-acylation of amidoalcohol **22** with perbenzyl benzophenone acid chloride **19b** (32%), followed by catalytic debenzylation (89%); mp 182–184 °C dec; ^{1}H NMR (CD_3OD) δ 7.87 (d, 2H, J = 8.7 Hz), 7.50 (d, 1H, J = 8.3 Hz), 7.31 (d, 2H, J = 8.7 Hz), 7.29 (t, 1H, J = 8.3 Hz), 7.03 (d, 1H, J = 8.3 Hz), 6.98 (s, 2H), 5.64 (m, 1H), 4.67 (m, 1H), 4.00 (dd, 1H), and 3.87 (dd, 1H), 3.64 (m , 2H), 3.04 (s, 3H); IR (KBr) ν cm^{-1} 1721, 1673, 1609. Anal. calcd for $C_{27}H_{25}N_3O_{11}S\cdot 1.7C_2HF_3O_2$: C, 46.02; H, 3.39; N, 5.30; S, 4.04; found: C, 45.85; H, 3.48; N, 5.65; S, 4.03%. MS (LRFAB) m/z 600 (M + 1).

***trans*-1-(4-Hydroxybenzoyloxy)-2-[4-(2-carboxy-6-hydroxybenzoyl)-3,5-dihydroxybenzoyloxy]cyclopentane (13).** This was prepared as a light-yellow powder from further *O*-acylation of monoacylated cyclopentandiol **29** with perbenzyl benzophenone acid chloride **19b** (76%), followed by catalytic debenzylation (89%); mp 160–166 °C. ^{1}H NMR (CD_3OD) δ 7.67 (d, 2H, J = 8.8 Hz), 7.29 (d, 1H, J = 7.7 Hz), 7.06 (t, 1H, J = 8.0 Hz), 6.81 (d, 1H, J = 8.2 Hz), 6.69 (s, 2H), 6.62 (d, 2H, J = 8.8 Hz), 5.15 (m, 2H), 2.06 (m, 2H), 1.70–1.66 (m, 4H). IR (KBr) ν cm^{-1} 1683, 1605. Anal. calcd for $C_{27}H_{22}O_{11}\cdot 1.6H_2O\cdot 0.15\ C_2HF_3O_2$: C, 57.69; H, 4.50; found: C, 57.66; H, 4.31%. MS (LRFAB) m/z 523 (M + 1).

***trans*-3-[(5-Hydroxyindol-2-yl)carboxamido]-4-[4-(2-carboxy-6-hydroxybenzoyl)-3,5-dihydroxybenzoyloxy]-perhydroazepine (14).** This was prepared as a yellow fluffy solid from *O*-acylation of amidoalcohol **24** with perbenzyl benzophenone acid chloride **19b** (64%), followed by catalytic debenzylation (74%); mp 210–213 °C dec. ^{1}H NMR (CD_3OD) δ 7.27 (d, 2H, J = 7.5 Hz), 7.07–7.00 (m, 2H), 6.79 (d, 1H, J = 8.3 Hz), 6.70 (d, 1H, J = 2.3 Hz), 6.68 (s, 2H), 6.66 (s, 1H), 6.59 (dd, 1H, J = 2.5, 8.8 Hz), 5.25 (m, 1H), 4.25 (m, 1H), 3.30 (d, 2H, J = 5.4 Hz), 2.10–1.78 (m, 4H); IR (KBr) ν cm^{-1} 1699, 1682, 1676, 1635. Anal. calcd for $C_{30}H_{27}N_3O_{10}\cdot 0.75H_2O\cdot 1.5C_2HF_3O_2$: C, 51.20; H, 3.91; N, 5.43; found: C, 51.20; H, 4.05; N, 5.52%. MS (LRFAB) m/z 590 (M + 1).

***trans*-3-(4-Hydroxybenzylcarboxamido)-4-[4-(2-carboxy-6-hydroxybenzoyl)-3,5-dihydroxybenzoyloxy]-pyrrolidine (15).** The title compound in a *N*-Boc protected form was prepared as a yellow powder from *O*-acylation of amidoalcohol **26** with perbenzyl benzophenone acid chloride **19b** (53%), followed by catalytic debenzylation (57%); mp 192–204 °C dec. ^{1}H NMR (CD_3OD) δ 7.29 (d, 1H, J = 7.7 Hz), 7.06 (t, 1H, J = 7.8 Hz), 6.87 (d, 2H, J = 8.5 Hz), 6.81 (d, 1H, J = 8.7 Hz), 6.67 (s, 2H), 6.49 (d, 2H, J = 8.4 Hz), 5.03 (m, 1H), 4.22 (m, 1H), 3.55 (m, 2H), 3.26–3.15 (m, 2H), 3.21 (s, 2H), 1.26 (s, 9H). MS (LRFAB) m/z 637 (M + 1). The Boc group was removed with 20% CF_3COOH in CH_2Cl_2 (5 mL/mmoL, 1.5 h) and the desired compound was obtained as a pale-yellow powder; mp 168–176 °C dec. ^{1}H NMR (CD_3OD) δ 7.29 (d, 1H, J = 7.5 Hz), 7.07 (t, 1H, J = 7.9 Hz), 6.89 (d, 2H, J = 8.6 Hz), 6.81 (d, 1H, J = 8.1 Hz), 6.73 (s, 2H), 6.51 (d, 2H, J = 8.6 Hz), 5.25 (m, 1H), 4.25 (m, 1H), 3.66–3.51 (m, 2H), 3.34 (dd, 1H, J = 13.3, 2.3 Hz), 3.25 (s, 2H), 3.21 (m, 1H); IR (KBr) ν cm^{-1} 1673, 1606, 1515. Anal. calcd for $C_{27}H_{24}N_2O_{10}\cdot 1.75\ H_2O\cdot 1.0C_2HF_3O_2$: C, 51.07; H, 4.21; N, 4.11; found: C, 51.18; H, 4.25; N, 4.15%. MS (LRFAB) m/z 536 (M).

Protein kinase C expression and purification

The alpha, beta-I, beta-II, gamma, delta, and epsilon recombinant human PKC enzymes were produced using a baculovirus expression system in SF9 cells.[18] The Ca^{2+}-independent isozymes (delta, epsilon, and eta) were purified as described in the literature by Bronson et al.[19] The Ca^{2+}-dependent isozymes (alpha, beta-I, beta-II, and gamma) were purified using a modification of method described by Kochs et al.[20] After the Ca^{2+}-dependent isozyme was released by EGTA treatment, it was purified on a Poros Q (Perspective Biosystems) anion exchange column using 0–500 mM NaCl. Each fraction was assayed for PKC activity, and the peak activity for each recombinant PKC was pooled and used in these studies. Purities range from 50–90% depending on isozyme subtype.

Protein kinase C assay

PKC was assayed by quantitating the incorporation of ^{32}P from [γ-^{32}P] ATP into histone type IIIS. The reaction mixture (250 μL) contained 30 μg of phosphatidylserine

(Avanti), 20 mM Hepes buffer (pH 7.5, Sigma), 10 mM $MgCl_2$, 47.5 μM EGTA, 100 μM $CaCl_2$, 200 μg/mL histone (Sigma), 10 μL of DMSO or 10 μL of a solution of tested compound in DMSO, 30 μM [^{32}P]-ATP (Dupont), the enzyme, and diacylglycerol. The amount of diacylglycerol necessary for 50% maximal activation of the enzyme was used. This amount was isozyme dependent. The assay was performed for 10 min at 30 °C and terminated with 500 μL of 25% trichloroacetic acid and 100 μL of bovine serum albumin (1 mg/mL; Sigma). The reactions were filtered onto glass fiber filters and quantified by counting in a β-scintillation counter. The concentration of compounds tested to estimate IC_{50} values ranged from 10 nM to 150 μM. Most of the IC_{50} values were results of single-point determinations at four concentrations (1, 10, 50, and 150 μM then 10, 100, 500, 1500 nM, respectively). Assay controls included a maximal lipid-activated PKC assay and a no-lipid PKC assay. The no-lipid activity was substracted from the maximal lipid-dependent activity to account for background nonspecific kinase activities. The PKC inhibitor sphingosine, which inhibits all the PKC isozymes, was included as a control inhibitor for all the PKC assays.[21]

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