Synthesis and Protein Kinase C Inhibitory Activities of Balanol Analogs with Replacement of the Perhydroazepine Moiety[1]

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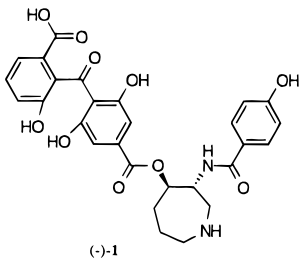
Balanol is a potent protein kinase C (PKC) inhibitor that is structurally composed of a benzophenone diacid, a 4-hydroxybenzamide, and a perhydroazepine ring. A number of balanol analogs in which the perhydroazepine moiety is replaced have been synthesized and their biological activities evaluated against both PKC and cAMP-dependent kinase (PKA). The results suggested that the activity and the isozyme/kinase selectivity of these compounds are largely related to the conformation about this nonaromatic structural element of the molecules.

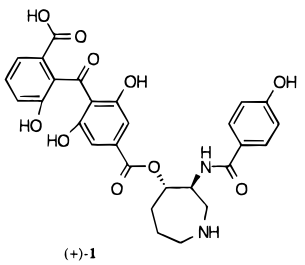
Introduction

Protein kinase C (PKC) is a family of structurally related serine/threonine specific kinases widely distributed in tissues and cells. It is activated by diacylglycerol (DAG) originating from receptor-mediated hydrolysis of membrane inositol phospholipids, a process that is responsible for initiating a cascade of cellular responses to extracellular stimuli such as hormones, neurotransmitters, and growth factors. Activated PKC catalyzes transfer of the γ-phosphate of ATP to substrate proteins and, by doing so, relays transmembrane signals to biological systems involved in a number of cellular processes including gene expression, cell proliferation, and differentiation.[2] PKC is a major receptor for tumor-promoting phorbol ester which activates PKC in a way similar to DAG,[3] and unregulated activation of PKC has been related to a range of disease states including central nervous system (CNS) diseases, cardiovascular disorders, diabetes, asthma, and HIV infections.[4] Given the importance of PKC in cellular biochemistry, inhibitors which are not only specific for this family of enzymes but also selective for only one of the isozymes within this family are desirable as tools for probing pertinent biological systems and, moreover, may allow development of specific therapeutic agents for treatment of human diseases.[5]

Balanol, (−)-**1**, recently isolated in our laboratories from the fungus *Verticillium balanoides*,[6] is a new class of PKC inhibitor that inhibits PKC at low-nanomolar concentrations.[7] Its unique structure was elucidated based on spectroscopic data and confirmed by an asymmetric total synthesis.[8] Balanol may be disassembled



(-)-**1**



(+)-**1**

into three distinctive elements on a retrosynthetic basis, namely, a tetrasubstituted benzophenone diacid, a *trans*-3,4-aminohydroxyperhydroazepine, and a 4-hydroxybenzoic acid. This convenient analysis served well as a guideline in planning the total synthesis of balanol and its analogs and also identified the three major subjects of our investigations regarding the structure−activity relationship (SAR) of these interesting molecules. This manuscipt describes the SAR of a series of azepine replacements[9] in which the other two domains are those found in balanol itself. Modifications include replacement or functionalization of the azepine nitrogen or its equivalent, variation of ring size, and introduction of conformational constraints.

Chemistry

Synthesis of each balanol analog followed a uniform theme in which the azepine replacement was condensed with the 4-hydroxybenzoyl residue followed by coupling of the protected benzophenone (Scheme 1). 4-(Benzyloxy)benzoic acid (**3**) is readily available from 4-hydroxybenzoic acid, and the benzophenone unit is available as **5** by a multigram-scale synthesis developed in our laboratories.[10] All the hydroxyl groups in these two fragments were strategically protected with a benzyl group so the final deprotection step was simplified to a single catalytic hydrogenolysis operation. This tactic was also applied to heteroatoms in the azepine replacement when applicable. Benzoic acid **3** was converted to the corresponding acid chloride or acylimidazole and coupled with an azepine replacement at the C.3 amino site. Occasionally this resulted in concomitant acylation of the vicinal hydroxyl group, and the crude products were treated with NaOH to provide the desired alcohols. Benzophenone acid **5** was usually converted to the corresponding acid chloride immediately before use and was coupled to amido alcohol **4**. With these common synthetic steps to complete the syntheses, the major task was reduced to construction of the desired azepine replacements **2** featuring a *trans*-vicinal amino alcohol substructure. The syntheses of these required elements are shown in Schemes 2−7.

For the most part, the vicinal amino alcohol was secured by stereospecific epoxide opening with an amine or the equivalent. The epoxides were in turn obtained by mCPBA epoxidation of an appropriate olefin. The exceptions are syntheses of analogs **37**−**41** (Scheme 6),

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Scheme 1

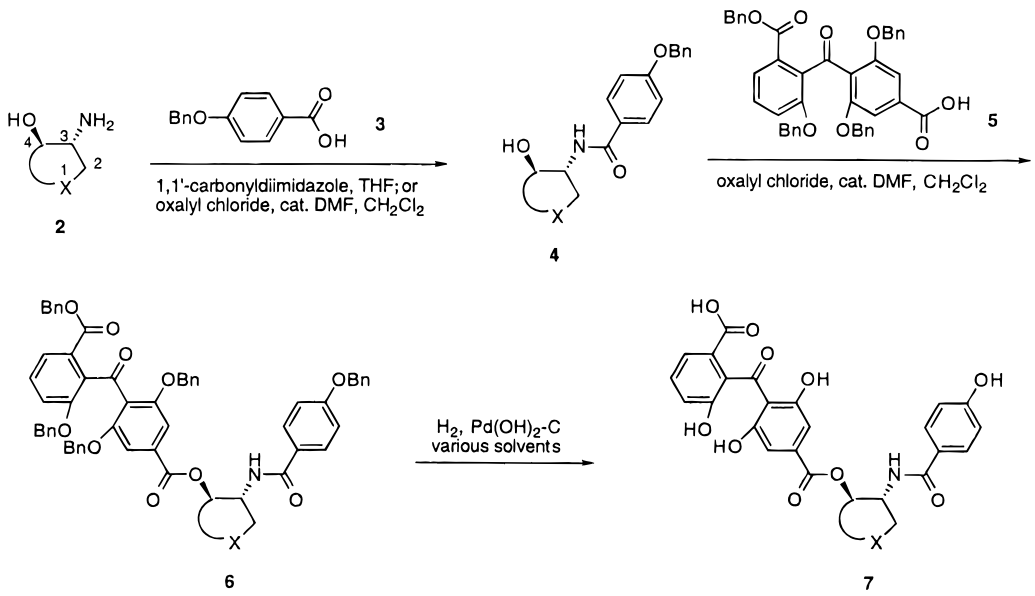


Table 1. Kinase Inhibition by Balanol (IC_{50} values, μM)[a]

compd	α	β1	β2	γ	δ	ϵ	η	ζ	PKA
(±)-**1**	0.07	0.03	0.03	0.03	0.02	0.04	0.02	3.5	
(−)-**1**	0.03	0.01	0.01	0.02	0.004	0.01	0.003	7.4	0.05
(+)-**1**	3.0	0.5	0.88	0.4	0.42	1.0	0.26	>150	5.2

[a] All compounds tested are synthetic.

in which a monooxime of a 1,2-diketone, obtained either from commercial sources or by α-nitrosation of commercial ketones, was reduced with sodium to provide the required substructure. These reduction reactions were usually nonstereospecific, and the *trans* stereoisomers were separated from the *cis* isomers by chromatography after N-acylation with benzoic acid **3**.

Clean separation of **30**α and **30**β proved to be difficult so that only one of the two epimers could be obtained in pure form. Compound **31**β was obtained as a single diastereomer due to the stereospecificity of the chemistry used for its synthesis (step o, Scheme 4), and its epimer **31**α was not prepared. The stereochemical assignments for analogs **30**−**33** and **37**−**41** were based on ^{1}H NMR or established information in the literature.[11] However, due to a lack of unequivocal ^{1}H NMR information and available literature precedent, the relative stereochemistry of **30**α and **30**β and the *exo*/*endo* stereochemistry of **41** were assigned on an arbitrary basis. It should also be noted that analog **39** was derived from optically pure starting material, and as far as the C.3−C.4 region (structure **2**) of the molecule is concerned, the absolute configuration and thus the biological activity of this analog are appropriate for comparison with (−)-balanol.

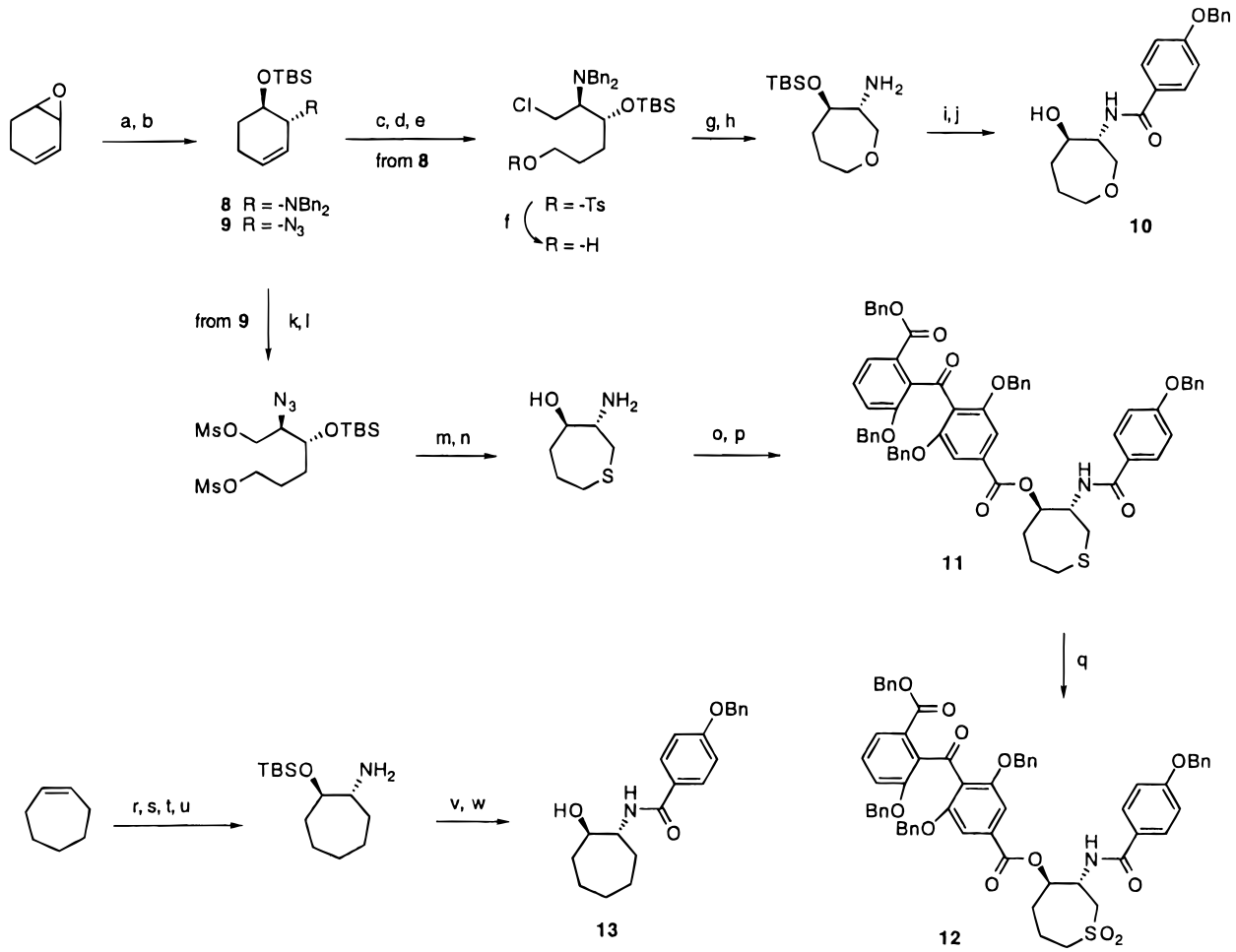
Results and Discussion

Kinase inhibitory activities of balanol and various sets of analogs against PKC isozymes α, β1, β2, γ, δ, ϵ, η, and ζ, as well as cAMP-dependent kinase (PKA), are shown in Tables 1−6. Balanol itself is one of the most potent PKC inhibitors known to date.[12] However, except for its relative inactivity against the ζ isozyme, a property shared by all balanol analogs, balanol falls short of isozyme selectivity as well as kinase selectivity between PKC and PKA. Our SAR studies also showed that the natural, (−)-enantiomer of balanol is at least 20−100-fold more potent than the (+)-enantiomer[8b] and that the two aromatic side chains of the molecule as well as their *trans* relative stereochemistry are indispensable for optimal potency.[13]

The azepine nitrogen atom appeared to be significant for the activity of balanol, as can be seen from Table 2 in which all four analogs with this atom replaced showed loss of activity. The changes in activity typically fell in the range of 50−100-fold and were isozyme-dependent in some cases. Thus, unlike the sulfone analog **16**, which was less active than racemic balanol against all eight isozymes, the sulfide analog **15** and, in particular, the ether analog **14** remained relatively active against the δ and η isozymes. Interestingly the carbocyclic analog **17** was only 2−10-fold reduced in potency compared to racemic balanol, and the IC_{50} values were maintained at a submicromolar level. This strongly suggests that there is an offset of any significant change in electronic properties associated with the −NH− to −CH_2− substitution. The seven-membered ring is generally recognized as a conformationally dynamic ring system. This would allow more conformational variations among these balanol analogs and can lead to major changes in their bioactivities *via* differences in spatial projection of the aromatic side chains. Thus the sulfur and oxygen substituents in analogs **14**−**16** may not otherwise confer poor activities to these analogs. Compounds **14**−**17**, like balanol itself, showed no significant selectivity between PKC and PKA. However, unlike balanol and most of its analogs which inhibit PKC better than PKA, the ether analog **14** appeared to be a better PKA inhibitor than PKC inhibitor, with exceptions in the PKC-δ and -η cases.

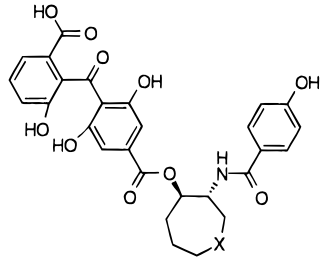
A dramatic fluctuation in potency was observed with changes in ring size (Table 3). Reduction of ring size by one methylene unit to analog **23** was accompanied by a 70−100-fold reduction in activity against six of the eight PKC isozymes. This compound, like analog **14**, is a selective inhibitor for the δ and η isozymes, by a factor of 20−100, and a better inhibitor for PKA over PKC for all isozymes except δ and η. Further shrinkage in ring size produced an opposite effect, rendering the

Scheme 2. Synthesis of Precursors to Analogs **14**−**17**[a]



[a] Conditions: (a) Bn_2NAlEt_2, CH_2Cl_2, rt; or NaN_3, NH_4Cl, MeOH−H_2O, 65 °C; (b) TBDMS-Cl, imidazole, DMF, rt; (c) OsO_4, NMO, acetone−H_2O, rt; (d) $NaIO_4$, THF−H_2O, rt; then $NaBH_4$, Et_2O−MeOH, 5 °C; (e) MeLi, THF; then TsCl, Et_3N, rt; (f) KO_2, 18-crown-6, DMSO, rt; (g) BuLi, $PhCH_3$, reflux; (h) H_2, $Pd(OH)_2$-c, MeOH, rt; (i) 4-(benzyloxy)benzoic acid, 1,1′-carbonyldiimidazole, THF, rt; (j) Bu_4NF, THF, rt; (k) O_3, CH_2Cl_2−MeOH, −78 °C; then $NaBH_4$; (l) $MeSO_2Cl$, Et_3N, CH_2Cl_2, rt; (m) Li_2S, Et_3N, MeOH, reflux; (n) $LiAlH_4$, THF, rt; (o) 4-(benzyloxy)benzoic acid, 1,1′-carbonyldiimidazole, THF; then 1 N aq NaOH, MeOH−THF, rt; (p) **6**, Et_3N, DMAP, CH_2Cl_2, rt; (q) CH_3CO_3H, CH_3CO_2H−CH_2Cl_2, rt; (r) CH_3CO_3H, NaOAc, Na_2CO_3, CH_2Cl_2, 5 °C−rt; (s) NaN_3, NH_4Cl, MeOH−H_2O, reflux; (t) TBDMS-Cl, imidazole, DMF, rt; (u) H_2, 5% Pd−C, MeOH, rt; (v) 4-(benzyloxy)benzoic acid, 1,1′-carbonyldiimidazole, THF, rt; (w) Bu_4NF, THF, rt.

Table 2. Kinase Inhibition by Heteroatom Analogs (IC_{50} values, μM)



14 X = -O-
15 X = -S-
16 X = -SO_2-
17 X = -CH_2-

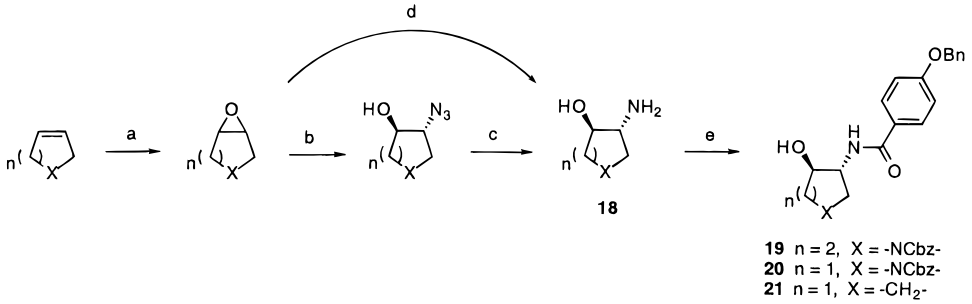
compd	α	β1	β2	γ	δ	ε	η	ζ	PKA
(±)-**1**	0.07	0.03	0.03	0.03	0.02	0.04	0.02	3.5	
14	6.7	2.5	3.3	1.0	0.09	16	0.01	>150	0.45
15	3.3	3.8	2.4	1.0	0.09	5.9	0.1	121	7.5
16	9.6	5.2	3.6	4.3	4.0	45	0.8	>150	38
17	0.27	0.22	0.43	0.06	0.09	0.28	0.06	>150	1.4

pyrrolidine analog **22** a marginally more potent inhibitor than balanol. Consistent with the observation that the azepine nitrogen could be replaced with carbon, analog **24** was found to be as potent as **22** in most cases and more potent than **22** against the δ and η isozymes. In addition, **24** is 5−8 times better in potency than its seven-membered counterpart, analog **17**, further substantiating a ring size preference of 5 ≥ 7 > 6. Both **22** and **24** were more active against the δ and η isozymes than the other isozymes, but neither was as selective as piperidine **23**.

The two five-membered ring analogs **22** and **24** are attractive not only for their impressive potency but also for their ease of preparation. By using aqueous ammonia in the epoxide-opening reactions (step d, Scheme 3), it took only one and three steps, respectively, to reach the required intermediates (**18**, $n = 1$, X = $-CH_2-$ and $-NCbz-$) from commercially available materials. This compared very favorably to a seven-step synthesis of the corresponding azepine amino alcohol.[14] Since large amounts of these intermediates became readily available, we elected to use the five-membered ring system as an advanced lead structure on which much of our later SAR studies were based. Related to this are compounds **30**−**33** which represent analogs of **24** with additional substituents carrying an amino or a hydroxyl group. The amine analogs **31** and **33** can also be treated as extensions of pyrrolidine **22** in which the endocyclic nitrogen atom is repositioned.

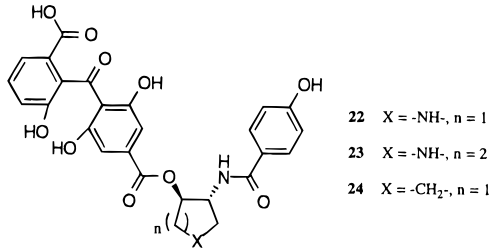
As shown in Table 4, compound **24** remained the most potent PKC-δ and -η inhibitor among all cyclopentane-

Scheme 3. Synthesis of Precursors to Analogs **22**−**24**[a]



[a] Conditions: (a) mCPBA, CH_2Cl_2, rt; (b) NaN_3, NH_4Cl, MeOH−H_2O, reflux; (c) PPh_3, THF, rt; (d) aq NH_3, 65 °C; (e) 4-(benzyloxy)benzoic acid, oxalyl chloride, aq NaOH, CH_2Cl_2, rt.

Table 3. Kinase Inhibition by Ring Size Analogs (IC_{50} values, μM)



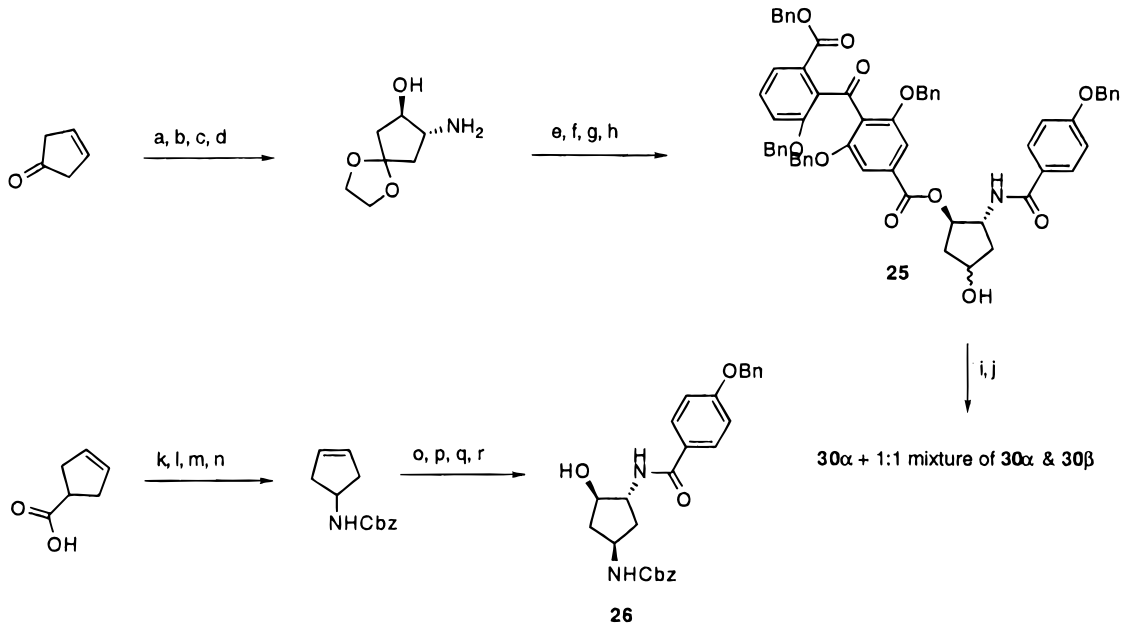
compd	α	β1	β2	γ	δ	ϵ	η	ζ	PKA
(±)-**1**	0.07	0.03	0.03	0.03	0.02	0.04	0.02	3.5	
22	0.022	0.017	0.033	0.013	0.005	0.01	0.004	>0.15	0.07
23	5.1	2.2	4.7	2.2	0.05	1.8	0.09	>150	0.35
24	0.04	0.04	0.05	0.01	0.001	0.05	0.001	22	0.03

based analogs. However, analogs **31**β, **32**α, **33**α, and **33**β were clearly more active than **24** in the β1 and β2 cases and marginally so in some other instances. Analogs **30**−**33** differ from one another in relative stereochemistry as well as nature of the additional fuctional groups, and this seemed to be reflected in their biological activities, albeit in a complex way. The difference caused by stereochemistry is not evident with **31** and the unresolved pair **30**α/**30**β but is manifested in the decreasing potency of **32**α > **24** > **32**β, where the gaps ranged from 5- to 15-fold in magnitude (not including PKC-ϵ). The pair **33**α/**33**β behaved similarly with PKC-γ and -δ but was quite comparable to each other in the other cases. Comparisons of analogs **30** and **31**β, as well as **32**β and **33**β, suggested a preference for an amino group over a hydroxyl group. However there is the exceptional pair **32**α and **33**α. It is likely that the functional group preference is under regulation of the relative stereochemistry of the functionalized substituents. The four amine analogs **22**, **31**β, **33**α, and **33**β inhibited PKC with small and isozyme-dependent differences. In essence the exocyclic amines tend to be better PKC-β1, -β2, and -γ inhibitors and were otherwise comparable to the endocyclic **22**. Chain length may have an influence on activity, as shown by analogs **32**α and **30**, but it is quite obscured whether there is a trend or not. In summary, these additional substituents were effective in fine tuning the biological activity. Stereochemistry around the cyclopentane rings appeared to be important for bioactivity, but it is not as clear whether other factors such as hydrogen bonding are operational in these cases, a similar situation to what was found among balanol and **14**−**17** and between **22** and **24**. These cyclopentane analogs had no significant isozyme selectivity and were found to be as active PKA inhibitors as PKC inhibitors.

A number of conformationally constrained analogs, **37**−**41**, were also examined. Bicyclic analog **37** was, for the most part, 3−7-fold less potent than racemic balanol, and analog **38** was essentially as potent as **24**, except with PKC-δ and -η. These results may not be a fair measure of the steric/lipophilic effect associated with the additional methylene groups in analogs **37** and **38**, since conformational changes from balanol and **24** are expected with these bicyclic analogs. On the other hand, a comparison between **38** and its bulkier congeners **39** and **40** does reveal an activity-reducing steric/lipophilic effect against certain PKC isozymes. Interestingly, the reduction in activity was 2−8 times greater with **39** than **40**; the difference may actually be even greater considering the fact that **39** is a single enantiomer with the correct absolute configuration, while **40** is racemic. Except in the case of the ϵ isozyme, **40** is very close to **38** in activity, suggesting the added volume/hydrophobicity associated with the three extra methylene units in **40** and, perhaps, the two additional methylene units in **38** was not much a significant negative factor for activity. The three methyl groups in **39** are positioned in different regions of the molecule from the three methylene groups of **40**. The poorer activity of **39** seemed indicative of a low tolerance for steric bulk in these regions of the molecules. Also, due to the conformational rigidity and the particular shape of **39**, some of these methyl groups may be sufficiently close in space to the benzophenone to interfere with the local conformation of the aromatic side chains. This is likely to contribute to lowering the activity of **39** relative to **38** and **40** and apparently is not as likely to occur in compounds such as **32** and **33** because of less structural restraint in these molecules. The ϵ isozyme seemed to be especially sensitive to these steric effects in that an exceptional 120- and 30-fold drop in potency relative to **38** was observed with **39** and **40**, respectively. Compound **41** is inactive and may well be another example of the ring size effect which predicts a six-membered ring to be inactive. None of these analogs showed isozyme selectivity, but there is an overall better selectivity for PKC over PKA with these compounds, in particular **39** and **40**.

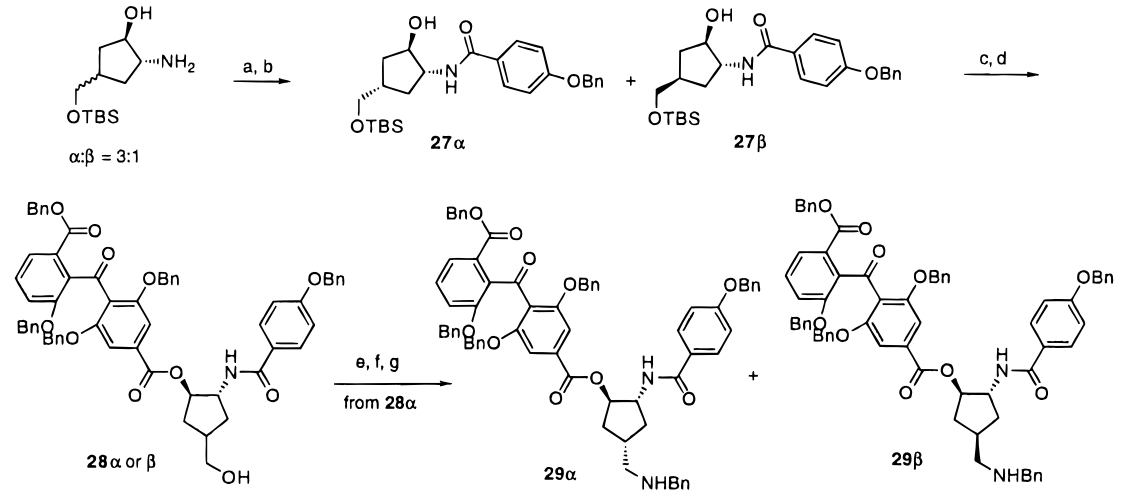
The pyrrolidine nitrogen of analog **22**, though replaceable with a methylene group, is valuable in providing a site of modification to more elaborated and potentially better analogs. Compounds **47**−**52** are examples in which selected functional groups were attached to this nitrogen atom. The *N*-isopropyl analog **47** was 2−10-

Scheme 4. Synthesis of Precursors to Analogs **30** and **31**[a]



[a] Conditions: (a) $HOCH_2CH_2OH$, PPTS, PhH, reflux; (b) mCPBA, CH_2Cl_2, rt; (c) $BnNH_2$, $LiClO_4$, CH_3CN, 60 °C; (d) H_2, 5% Pd−C, EtOAc, rt; (e) **3**, CDI, THF, rt, aq NaOH workup; (f) **5**, Et_3N, DMAP, CH_2Cl_2, rt; (g) $Pd(CH_3CN)_2Cl_2$, $CHCl_3$, rt; (h) $NaBH_4$, MeOH, 0 °C, gave 2:1 mixture; (i) H_2, $Pd(OH)_2$−C, MeOH−THF, rt; (j) HPLC; (k) $(COCl)_2$, DMF, CH_2Cl_2, rt; (l) NH_4OH, rt; (m) $(CF_2CO_2)_2PhI$, CH_3CN, H_2O, rt; (n) BnOCOCl, aq NaOH, CH_2Cl_2, rt; (o) mCPBA, CH_2Cl_2, 0 °C−rt; (p) NaN_3, NH_4Cl, H_2O, MeOH, 50 °C; (q) Zn, AcOH, H_2O, EtOH, rt; (r) **3**, CDI, THF, rt; aq NaOH workup.

Scheme 5. Synthesis of Precursors to Analogs **32** and **33**[a]



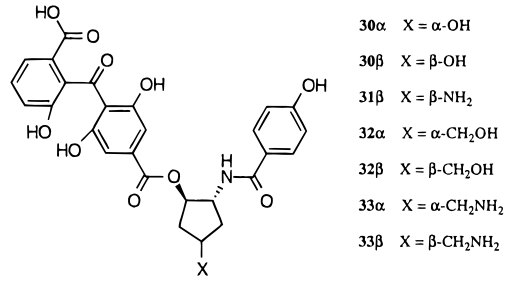
[a] Conditions: (a) 4-(benzyloxy)benzoyl chloride, aq KOH, THF, rt; (b) SiO_2 chromatography; (c) **5**, Et_3N, DMAP, CH_2Cl_2, rt; (d) Bu_4NF, THF, rt; (e) $(COCl)_2$, Me_2SO, Et_3N, 0 °C−rt, SiO_2 chromatography; (f) $BnNH_2$, $NaB(OAc)_3H$, $ClCH_2CH_2Cl$, rt; (g) chromatography on SiO_2.

fold less potent than the parent compound **22**, and analogs **48**−**52** were also found to show loss of activity compared to **22**, typically by a factor of 20−50. The N-substituents in these analogs are comparable in size, so steric bulk is not likely responsible for the reduced activity of **48**−**52** relative to **47**. Rather, the way the pyrrolidine nitrogen is blocked may have more bearing on the reduction in activity. The partial π character of the pyrrolidinium amido N−C bonds of **48**−**52** may influence conformation about the pyrrolidine rings differently from the isopropyl group of **47** and contribute to their lower activity relative to **47**. In addition **47** differs from **48**−**52** in that it has a basic nitrogen and the isopropyl group is relatively nonpolar. The polarity of the N-substituents in **48**−**52** is such that analogs with an exposed partial negative charge and/or hydrogen bond acceptor in that area of the molecule may not be particularly preferred for binding. This also seemed to be consistent with the better activities of analogs **30**−**33**. Finally, none of these N-substituted compounds is significantly isozyme selective, and none of them, but **50**, showed significant kinase selectivity.

Conclusion

The perhydroazepine moiety of balanol possesses special properties that are important to its potency against PKC and PKA. We have carried out SAR studies around this structural element in an attempt to define these properties. It was pointed out that the azepine nitrogen is replaceable as long as the replacement is able to raise the two aromatic side chains in a stereochemically correct manner. A deviated conforma-

Table 4. Kinase Inhibition by Cyclopentane Analogs (IC_{50} values, μM)



compd	α	β1	β2	γ	δ	ϵ	η	ζ	PKA
30α+β	0.04	0.02	0.01	0.02	0.005	0.1	0.003	16	0.13
30β	0.05	0.03	0.02	0.03	0.006	0.15	0.004	13	0.25
31β	0.02	0.004	0.002	0.005	0.005	0.01	0.003	1.2	0.05
32α	0.01	0.004	0.003	0.005	0.004	0.16	0.002	39	0.23
32β	0.08	0.06	0.02	0.05	0.02	0.27	0.02	34	0.22
33α	0.02	0.004	0.004	0.005	0.006	0.03	0.003	2.8	0.17
33β	0.02	0.005	0.005	0.01	0.02	0.03	0.003	3.1	0.09

tion about the azepine replacement may result in decrease in activity but may also be a source of isozyme selectivity for PKC-δ and -η, as shown by analogs **14** and **23**. In terms of ring size, five is optimal for an azepine replacement, seven-membered rings are equally good but are less predictable due to their conformational flexibility, and six-membered rings are inactive against all but the δ and η isozymes. Rational design combining the above replaceability rule and ring size effect may generate simple yet potent inhibitors such as **24**, which is also the most potent inhibitor against the δ and η isozymes among all balanol analogs. Derivatization of a readily available azepine replacement such as pyrrolidine and cyclopentane has been a many faceted issue but has proven to be an effective means of bringing about enhanced activity, such as that of **31**β, **32**α, **33**α, and **33**β, and kinase selectivity, of **39** and **40**, for example. Ultimately these azepine-replaced analogs of balanol provide a model *via* which the nature of the interactions between balanol and PKC, as well as structural requirements for better binding, can be explored. A molecular modeling approach toward the issue of conformation is currently in progress in our laboratories.

Experimental Section

General. Melting points were determined with either a Mel-Temp II or an Electrothermal melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini 300 instrument at 300 MHz. Chemical shifts are reported in part per million (ppm) downfield relative to tetramethylsilane. FTIR spectra were measured with a Mattson Galaxy 5000 spectrometer calibrated against polystyrene standard. Elemental analysis was performed in house with a Carlo Erba EA1108 analyzer. Mass spectra were obtained from Analytical Instrument Group, Raleigh, NC. Flash chromatography was performed on silica gel 60 purchased from EM Science, and preparative HPLC was carried out with a Rainin HPLC system on a C-18 reverse phase column. All anhydrous reactions were run under an atmosphere of N_2 in oven-dried glassware.

(±)-1,1-Dioxo-*trans*-3-[4-(benzyloxy)benzamido]-4-[[4-[2-(benzyloxy)-6-[(benzyloxy)carbonyl]benzoyl]-3,5-bis-(benzyloxy)benzoyl]oxy]perhydrothiepine (12). Peroxyacetic acid (32 wt % in acetic acid, 37 mg, 0.155 mmol) was added to a solution of **11** (75 mg, 0.074 mmol) in CH_2Cl_2 (0.7 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with CH_2Cl_2 (10 mL), and washed with saturated aqueous K_2CO_3 (3 × 5 mL). The organic layer was dried ($MgSO_4$) and evaporated to give a white solid which was recrystallized from a hot mixture of EtOAc−THF−hexanes (10:2:3) to give a white powder (61 mg, 79%): mp 189−193 °C; ^{1}H NMR ($CDCl_3$) δ 7.74 (d, J = 8.8 Hz, 2H), 7.01−7.48 (m, 28H), 6.94 (t, J = 7.9 Hz, 2H), 6.82 (d, J = 8.8 Hz, 2H), 5.47 (t, J = 8.0 Hz, 1H), 5.13 (s, 2H), 5.07 (s, 2H), 4.98 (m, 1H), 4.80 (s, 4H), 4.70 (s, 2H), 3.62 (dd, J = 16.0, 4.5 Hz, 1H), 3.51 (dm, J = 15.7 Hz, 1H), 3.09−3.28 (m, 2H), 2.05−2.29 (m, 4H). Anal. ($C_{63}H_{55}NO_{12}S$) C, H, N.

General Procedure for Hydrogenolysis of 6. 6 (0.1 M, 1 equiv) was dissolved in ethyl acetate−EtOH (1:1 to 1:6) and placed in a round-bottom flask or Parr bottle. The vessel was purged with N_2, and Pearlmann's catalyst (0.01−0.1 equiv) was added. The mixture was either stirred under 1 atm of H_2 (balloon) or charged with H_2 (40−50 psi) and shaken on a Parr apparatus for 16−24 h. The reaction mixture was diluted with ethanol and filtered through Celite, and the filter cake was washed and kept moist with more ethanol. The filtrate and washes were combined and concentrated under reduced pressure. The residue was purified by HPLC using a linear gradient of increasing CH_3CN in H_2O containing 0.1% trifluoroacetic acid as the eluent, and the collected fractions were combined, concentrated under reduced pressure to remove most of the solvents, and lyophilized to give the final product.

(±)-*trans*-3-(4-Hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]-perhydrooxepine (14): yellow solid (61 mg, 94%); mp 204 °C dec; ^{1}H NMR (CD_3OD) δ 7.58 (d, J = 8.6 Hz, 2H), 7.31 (d, J = 7.4 Hz, 1H), 7.15 (t, J = 7.8 Hz, 1H), 6.87 (s, 2H), 6.81 (d, J = 8.1 Hz, 1H), 6.74 (d, J = 8.7 Hz, 2H), 5.20 (m, 1H), 4.34 (m, 1H), 3.75−3.84 (m, 2H), 3.64−3.73 (m, 2H), 1.89−2.02 (m, 2H), 1.79−1.88 (m, 2H); IR (KBr, cm^{-1}) 1702, 1679, 1649, 1641; FABMS m/z = 552 (M + 1). Anal. ($C_{28}H_{25}NO_{11}\cdot 1.4H_2O\cdot 0.4CF_3CO_2H$) C, H, N.

(±)-*trans*-3-(4-Hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]-perhydrothiepine (15): yellow solid (25 mg, 35%); mp 196 °C dec; ^{1}H NMR (CD_3OD) δ 7.62 (d, J = 8.6 Hz, 2H), 7.32 (d, J = 7.1 Hz, 1H), 7.18 (t, J = 7.9 Hz, 1H), 6.88 (s, 2H), 6.84 (d, J = 8.1 Hz, 1H), 6.77 (d, J = 8.6 Hz, 2H), 5.36 (tm, J = 8.6 Hz, 1H), 4.60 (m, 1H), 2.89−2.96 (m, 2H), 2.69−2.79 (m, 2H), 2.11−2.22 (m, 2H), 1.93−2.08 (m, 2H); IR (KBr, cm^{-1}) 1706, 1689, 1633; FABMS m/z = 568 (M + 1). Anal. ($C_{28}H_{25}NO_{10}S\cdot 2H_2O\cdot 0.25CF_3CO_2H$) C, H, N.

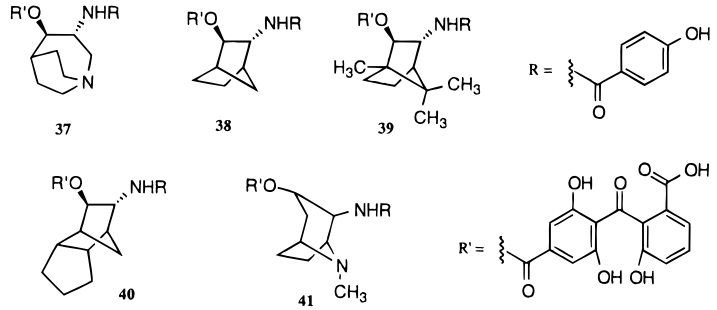
(±)-1,1-Dioxo-*trans*-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]perhydrothiepine (16): yellow solid (33 mg, 95%); mp 198 °C dec; ^{1}H NMR (CD_3OD) δ 7.61 (d, J = 8.7 Hz, 2H), 7.30 (d, J = 7.7 Hz, 1H), 7.19 (t, J = 7.8 Hz, 1H), 6.91 (s, 2H), 6.83 (d, J = 7.7 Hz, 1H), 6.77 (d, J = 8.7 Hz, 2H), 5.50 (tm, J = 8.6 Hz, 1H), 4.61 (m, 1H), 3.77 (dd, J = 15.8, 7.4 Hz, 1H), 3.49 (dm, J = 15.3 Hz, 1H), 3.28−3.46 (m, 2H), 2.06−2.22 (m, 4H); IR (KBr, cm^{-1}) 1718, 1686, 1635; FABMS m/z = 599 (M). Anal. ($C_{28}H_{25}NO_{12}S\cdot 2.4H_2O$) C, H, N.

(±)-*trans*-1-(4-Hydroxybenzamido)-2-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]-cycloheptane (17): yellow solid (83 mg, 95%); mp 186 °C dec; ^{1}H NMR (CD_3OD) δ 7.55 (d, J = 8.7 Hz, 2H), 7.38 (d, J = 7.4 Hz, 1H), 7.19 (t, J = 7.9 Hz, 1H), 6.90 (d, J = 7.8 Hz, 1H), 6.84 (s, 2H), 6.72 (d, J = 8.7 Hz, 2H), 5.13 (m, 1H), 4.29 (m, 2H), 1.53−1.90 (m, 10H). Anal. ($C_{29}H_{27}NO_{10}\cdot H_2O$) C, H, N.

(±)-*trans*-3-(4-Hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]-pyrrolidine (22): yellow solid (88 mg, 72%); mp 197−199 °C; ^{1}H NMR (CD_3OD) δ 7.73 (d, J = 8.7 Hz, 2H), 7.48 (d, J = 7.7 Hz, 1H), 7.24 (t, J = 8.0 Hz, 1H), 7.02 (d, J = 8.2 Hz, 1H), 6.94 (s, 2H), 6.79 (d, J = 8.7 Hz, 2H), 5.58 (m, 1H), 4.66 (m, 1H), 4.93 (dd, J = 13.4, 5.3 Hz, 1H), 3.82 (dd, J = 13.0, 7.0 Hz, 1H), 3.60 (apparent dd, J = 12.6, 4.4 Hz, 2H); IR (KBr, cm^{-1}) 1722, 1665, 1633. Anal. ($C_{26}H_{22}N_2O_{10}\cdot CF_3CO_2H$) C, H, N.

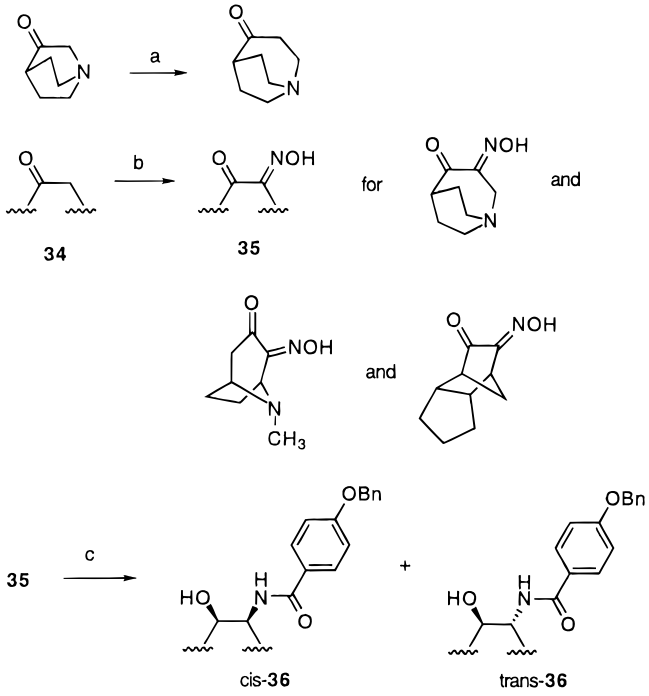
(±)-*trans*-3-(4-Hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]-piperidine (23): yellow solid (60 mg, 50%); mp 216−220 °C dec; ^{1}H NMR (CD_3OD) δ 7.43 (d, J = 8.7 Hz, 2H), 7.29 (d, J =

Table 5. Kinase Inhibition by Conformationally Constrained Analogs (IC_{50} values, μM)



compd	α	β1	β2	γ	δ	ϵ	η	ζ	PKA
37	0.41	0.026	0.069	0.14	0.11	0.17	0.026	22.5	1.7
38	0.06	0.05	0.03	0.04	0.02	0.03	0.02	37	1.9
39	0.41	0.31	0.08	0.21	0.34	3.6	0.31	>50	26
40	0.1	0.15	0.03	0.05	0.05	0.97	0.05	37	32
41	34	4.4	14	4.6	5.0	27	2.8	>50	>50

Scheme 6. Synthesis of Precursors to Analogs **37**−**41**[a]



[a] Conditions: (a) $TMSCHN_2$, THF−MeOH, 5 °C−rt; (b) LiN(TMS)$_2$, *n*-butylnitrile, various solvents, 60 °C−rt; (c) Na, EtOH, heat; 4-(benzyloxy)benzoyl chloride, 1 N NaOH, toluene, rt.

7.6 Hz, 1H), 7.06 (t, J = 8.0 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.70 (s, 2H), 6.58 (d, J = 8.6 Hz, 2H), 5.11 (m, 1H), 4.28 (m, 1H), 3.54 (ddm, J = 12.2, 4.1 Hz, 1H), 3.30 (m, 1H), 2.99−3.11 (m, 2H), 2.08 (ddm, J = 13.7, 3.7 Hz, 1H), 1.87 (m, 1H); IR (KBr, cm^{-1}) 1720, 1677, 1636, 1607, 1510, 1428, 1376, 1234; FABMS *m*/*z* 537 (M + 1). Anal. ($C_{27}H_{24}N_2O_{10}$·2.9 H_2O·0.9 CF_3CO_2H) C, H, N.

(±)-*trans*-1-(4-Hydroxybenzamido)-2-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoy]loxy]-cyclopentane (24): yellow solid (168 mg, 85%); mp 198 °C dec; ^{1}H NMR (CD_3OD) δ 7.48 (d, J = 8.7 Hz, 2H), 7.28 (d, J = 7.7 Hz, 1H), 7.06 (t, J = 7.9 Hz, 1H), 6.81 (d, J = 7.1 Hz, 1H), 6.69 (s, 2H), 6.60 (d, J = 8.8 Hz, 2H), 5.07 (m, 1H), 4.29 (m, 1H), 1.99−2.01 (m, 2H), 1.48−1.70 (m, 4H); IR (KBr, cm^{-1}) 1703, 1633, 1606, 1507, 1425, 1373, 1245, 1200; FABMS *m*/*z* 522 (M + 1). Anal. ($C_{27}H_{23}NO_{10}$·2.0H_2O·0.5C_2H_6O) C, H, N.

(±)-(1*β*,3α,4*β*)-1-Hydroxy-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]cyclopentane (30*β* and 30α + 30*β*). 30*β*: yellow solid; mp 189−192 °C; ^{1}H NMR (CD_3OD) δ 7.70 (d, J = 8.7 Hz, 2H), 7.51 (d, J = 7.7 Hz, 1H), 7.29 (t, J = 7.5 Hz, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.94 (s, 2H), 6.82 (d, J = 8.7 Hz, 2H), 5.32 (dt, J = 12.1, 6.0 Hz, 1H), 4.87 (m, 1H), 4.41 (m, 1H), 2.66 (td, J = 14.5, 6.3 Hz, 1H), 2.20 (tm, J = 8.0 Hz, 1H), 1.97 (ddd, J = 14.5, 8.9, 4.8 Hz, 1H), 1.79 (dm, J = 14.6 Hz, 1H); IR (KBr, cm^{-1}) 1703, 1634, 1606; FABMS *m*/*z* = 538 (M + 1). Anal. ($C_{27}H_{23}NO_{11}$·0.8H_2O·0.1CF_3CO_2H) C, H, N.

30α + 30*β*: yellow solid; mp 184−189 °C; FABMS *m*/*z* = 538 (M + 1). Anal. ($C_{27}H_{23}NO_{11}$·1.0H_2O·0.2CF_3CO_2H) C, H, N. ^{1}H NMR (CD_3OD) for **30**α from spectrum of the mixture: δ 5.45 (m, 1H), 4.53 (m, 1H), 2.55 (m, 1H), 2.26 (m, 1H), 2.08 (m, 1H), 1.75 (m, 1H); the aromatic region was indistinguishable from that of **30***β*.

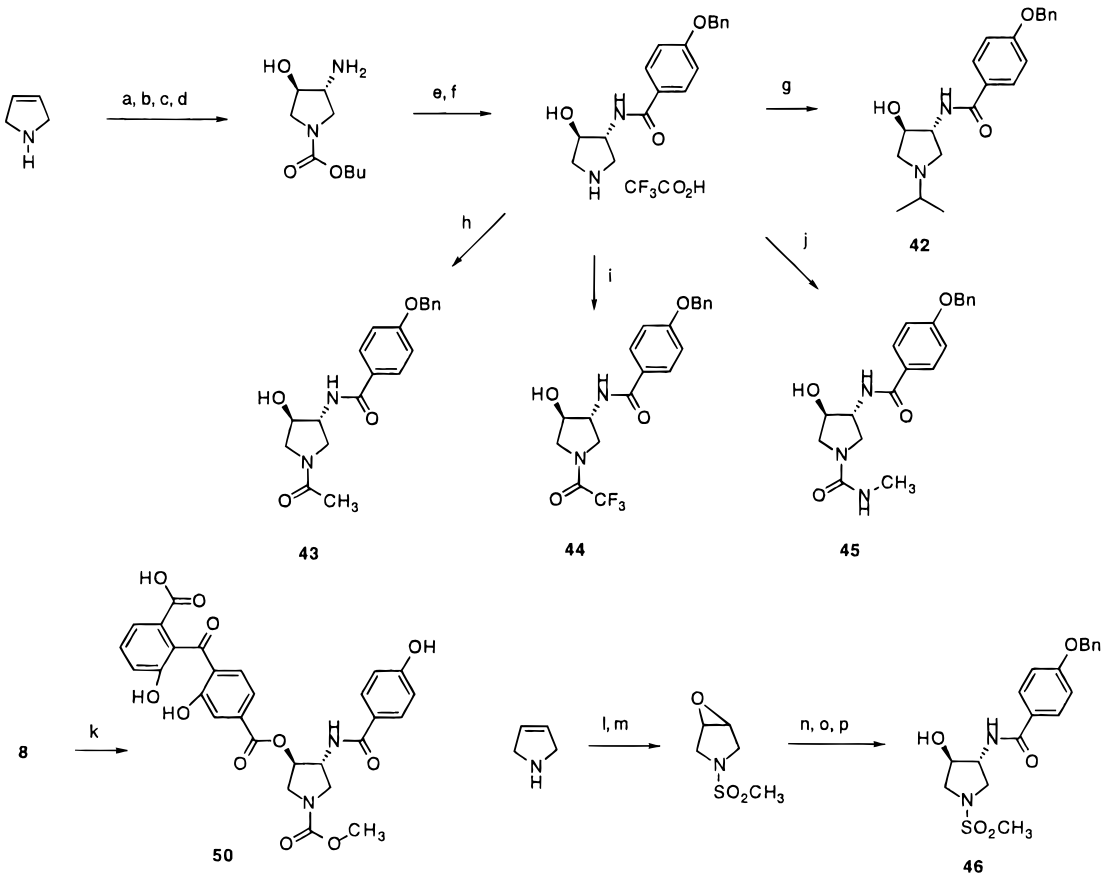
(±)-(1*β*,3α,4*β*)-1-Amino-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]cyclopentane (31*β*): yellow solid (151 mg, 72%); mp 197 °C dec; ^{1}H NMR (CD_3OD) δ 7.90 (d, J = 8.9 Hz, 2H), 7.52 (d, J = 7.6 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.05 (d, J = 6.3 Hz, 1H), 6.99 (d, J = 8.7 Hz, 2H), 6.94 (s, 2H), 5.67 (m, 1H), 5.59 (d, J = 6.5 Hz, 1H), 4.84 (m, 1H), 4.21 (m, 1H), 3.07 (dm, J = 16.8 Hz, 1H), 2.56 (tm, J = 13.3 Hz, 1H), 2.46 (tm, J = 13.4 Hz, 1H); IR (KBr, cm^{-1}) 1776, 1680, 1606; FABMS *m*/*z* = 537 (M + 1). Anal. ($C_{27}H_{24}N_2O_{10}$·1.2H_2O·1.5CF_3CO_2H) C, H, N.

(±)-(1α,3α,4*β*)-1-(Hydroxymethyl)-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]cyclopentane (32α): yellow solid (160 mg, 72%); mp 180−184 °C dec; ^{1}H NMR (CD_3OD) δ 7.47 (d, J = 8.8 Hz, 2H), 7.28 (d, J = 6.6 Hz, 1H), 7.06 (t, J = 7.9 Hz, 1H), 6.80 (d, J = 8.7 Hz, 1H), 6.69 (s, 2H), 6.59 (d, J = 8.8 Hz, 2H), 5.06 (m, 1H), 4.32 (m, 1H), 3.37 (d, J = 5.1 Hz, 2H), 2.30−2.11 (m, 2H), 1.91−1.67 (m, 2H), 1.36−1.25 (m, 1H); IR (KBr, cm^{-1}) 3373, 1704, 1633, 1605, 1507, 1425, 1369, 1244, 1199, 762; FABMS *m*/*z* = 552 (M + 1). Anal. ($C_{28}H_{25}NO_{11}$·1.0H_2O·0.3CF_3CO_2H) C, H, N.

(±)-(1*β*,3α,4*β*)-1-(Hydroxymethyl)-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]cyclopentane (32*β*): yellow solid (85 mg, 79%); mp 159−168 °C dec; ^{1}H NMR (CD_3OD) δ 7.47 (d, J = 8.7 Hz, 2H), 7.28 (d, J = 7.7 Hz, 1H), 7.05 (t, J = 8.0 Hz, 1H), 6.80 (d, J = 7.4 Hz, 1H), 6.68 (s, 2H), 6.58 (d, J = 8.7 Hz, 2H), 5.11 (m, 1H), 4.34 (m, 1H), 3.33 (d, J = 6.0 Hz, 2H), 2.11 (m, 2H), 1.80−1.59 (m, 2H), 1.38 (m, 1H); IR (KBr, cm^{-1}) 3398, 1704, 1632, 1606, 1507, 1426, 1369, 1244, 1200, 763; FABMS *m*/*z* = 552 (M + 1). Anal. ($C_{28}H_{25}NO_{11}$·1.0H_2O·0.3CF_3CO_2H) C, H, N.

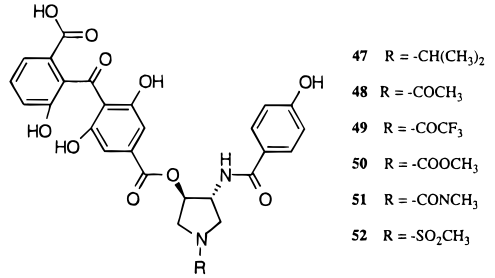
(±)-(1α,3α,4*β*)-1-(Aminomethyl)-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]cyclopentane (33α): yellow solid (60 mg, 50%); mp 164−170 °C dec; ^{1}H NMR (CD_3OD) δ 7.48 (d, J = 8.8 Hz, 2H), 7.28 (d, J = 7.7 Hz, 1H), 7.06 (t, J = 8.0 Hz, 1H), 6.81 (d, J = 8.2 Hz, 1H), 6.70 (s, 2H), 6.60 (d, J = 8.7 Hz, 2H), 5.16 (m, 1H), 4.34 (m, 1H), 2.82 (d, J = 6.6 Hz, 2H), 2.28 (m, 2H), 1.87 (m, 2H), 1.29 (m, 1H); IR (KBr, cm^{-1}) 3391, 3304, 3187, 2978, 1682, 1633, 1606, 1507, 1427, 1371, 1244, 1197,

Scheme 7. Synthesis of Precursors to Analogs **47**−**52**[a]



[a] Conditions: (a) $(^tBoc)_2O$, DMAP, CH_2Cl_2; (b) mCPBA, CH_2Cl_2; (c) NaN_3, NH_4Cl, MeOH−H_2O; (d) H_2, Pd−C, EtOAc; (e) **3**, CDI, THF; then NaOH, H_2O−MeOH; (f) CF_3CO_2H, CH_2Cl_2; (g) acetone, $NaBH_4$, AcOH; (h) Ac_2O; (i) $(CF_3CO)_2O$; (j) CH_3NCO, NEt_3, MeOH; (k) CH_3OCOCl, pyridine; (l) CH_3SO_2Cl, pyridine, CH_2Cl_2; (m) mCPBA, CH_2Cl_2; (n) $TMSN_3$, $Ti(O^iPr)_4$, CH_2Cl_2; 1 N HCl; (o) H_2, 10% Pd−C, MeOH; (p) **3**, $(COCl)_2$, aq NaOH, CH_2Cl_2.

Table 6. Kinase Inhibition by N-Substituted Pyrrolidine Analogs (IC_{50} values, μM)



compd	α	β1	β2	γ	δ	ϵ	η	ζ	PKA
47	0.24	0.06	0.07	0.09	0.04	0.05	0.004	37	<0.33
48	1.2	0.71	0.82	0.39	0.06	3.7	0.03	>150	3.6
49	0.43	0.36	0.26	0.26	0.1	0.25	<0.01	56	2.6
50	0.54	0.81	2.7	0.54	0.1	4.8	0.09	>150	13
51	2.2	0.68	1.8	0.41	0.04	3.6	0.13	>50	3.0
52	0.91	0.33	0.35	0.4	0.035	1.3	0.062	>150	4.5

1141, 764; FABMS *m/z* = 551 (M + 1). Anal. ($C_{28}H_{26}N_2O_{10}$·1.8CF_3CO_2H) C, H, N.

(±)-(1*β*,3α,4*β*)-1-(Aminomethyl)-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]cyclopentane (33*β*): yellow solid (17 mg, 24%); mp 165−169 °C dec; ^{1}H NMR (CD_3OD) δ 7.48 (d, *J* = 8.7 Hz, 2H), 7.28 (d, *J* = 7.8 Hz, 1H), 7.06 (t, *J* = 7.9 Hz, 1H), 6.81 (d, *J* = 8.2 Hz, 1H), 6.69 (s, 2H), 6.60 (d, *J* = 8.7 Hz, 2H), 5.17 (m, 1H), 4.37 (m, 1H), 2.82 (d, *J* = 7.0 Hz, 2H), 2.37 (m, 2H), 1.83 (m, 2H), 1.41 (m, 1H); IR (KBr, cm^{-1}) 3443, 3243, 1680, 1633, 1607, 1508, 1427, 1243, 1199, 1141, 919, 764; FABMS *m/z* = 551 (M + 1). Anal. ($C_{28}H_{26}N_2O_{10}$·2.0H_2O·1.5CF_3CO_2H) C, H, N.

(±)-*trans*-1-Aza-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]bicyclo[3.2.2]nonane (37): yellow solid (7.6 mg, 22%); mp 260−265 °C dec; ^{1}H NMR (DMSO-d_6) δ 11.66 (s, 2H), 10.06 (s, 1H), 9.85 (s, 1H), 8.33 (d, *J* = 8 Hz, 1H), 7.62 (d, *J* = 9 Hz, 2H), 7.36 (d, *J* = 8 Hz, 1H), 7.28 (t, *J* = 8 Hz, 1H), 7.05 (d, *J* = 8 Hz, 1H), 6.80 (m, 4H), 5.28 (d, *J* = 8 Hz, 1H), 4.70 (m, 1H), 3.15−3.70 (m, 6H), 2.70 (m, 1H), 1.80−2.40 (m, 4H); FABMS *m/z* = 577 (M + 1).

(±)-*trans*-2-[[4-[2-Hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]-3-(4-hydroxybenzamido)-bicyclo[2.2.1]heptane (38): yellow solid (110 mg, 68%); mp 185−193 °C dec; ^{1}H NMR (CD_3OD) δ 7.54 (d, *J* = 8.7 Hz, 2H), 7.29 (d, *J* = 7.6 Hz, 1H), 7.06 (t, *J* = 8.1 Hz, 1H), 6.82 (d, *J* = 8.2 Hz, 1H), 6.70 (s, 2H), 6.61 (d, *J* = 8.7 Hz, 2H), 4.59 (s, 1H), 3.98 (br s, 1H), 2.42 (br s, 1H), 2.19 (d, *J* = 4.4 Hz, 1H), 1.65 (d, *J* = 9.9 Hz, 1H), 1.51−1.22 (m, 5H); IR (KBr, cm^{-1}) 3369, 2963, 1703, 1635, 1607, 1505, 1425, 1365, 1238, 1176, 762; FABMS *m/z* = 548 (M + 1). Anal. ($C_{29}H_{25}NO_{10}$) C, H, N.

(+)-*trans*-2-[[4-[2-Hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]-3-(4-hydroxybenzamido)-1,7,7-trimethylbicyclo[2.2.1]heptane (39): yellow solid (26 mg, 46%); $[\alpha]_D$ +101.84° (*c* 0.38, MeOH, room temperature); mp 192−196 °C dec; ^{1}H NMR (CD_3OD) δ 7.53 (d, *J* = 8.7 Hz, 2H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.06 (t, *J* = 8.1 Hz, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 6.70 (s, 2H), 6.61 (d, *J* = 8.7 Hz, 2H), 4.79 (d, *J* = 3.5 Hz, 1H), 4.41 (m, 1H), 1.89 (br s, 1H), 1.56−1.21 (m, 4H), 1.07 (s, 3H), 0.76 (s, 3H), 0.71 (s, 3H); IR (KBr, cm^{-1}) 3387, 3308, 2961, 1703, 1634, 1607, 1505, 1425, 1368, 1238, 1176, 764; FABMS *m/z* = 590 (M + 1). Anal. ($C_{32}H_{31}NO_{10}$·0.5CF_3CO_2H) C, H, N.

(±)-8-*exo*-[[4-[2-Hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]-9-*endo*-(4-hydroxybenzamido)-tricyclo[5.2.1.0^{2,6}]decane (40): yellow solid (166 mg, 82%);

mp 195−200 °C dec; ^{1}H NMR (DMSO-d_6) δ 11.66 (s, 2H), 9.97 (br s, 1H), 9.88 (s, 1H), 8.35 (d, J = 7 Hz, 1H), 7.75 (d, J = 9 Hz, 2H), 7.38 (d, J = 8 Hz, 1H), 7.28 (t, J = 8 Hz, 1H), 7.06 (d, J = 8 Hz, 1H), 6.83 (s, 2H), 6.79 (d, J = 9 Hz, 2H), 4.83 (d, J = 2.5 Hz, 1H), 4.11 (m, 1H), 2.25 (d, J = 4 Hz, 1H), 2.10−2.20 (m, 1H), 2.07 (br s, 2H), 1.60−2.00 (m, 3H), 1.48 (br s, 2H), 1.10−1.30 (m, 1H), 0.90−1.10 (m, 2H); IR (KBr, cm^{-1}) 1703, 1635, 1608; FABMS m/z = 588 (M + 1). Anal. ($C_{32}H_{29}NO_{10}\cdot 1.0H_2O\cdot 0.25CF_3CO_2H$) C, H, N.

(±)-*trans*-3-[[4-[2-Hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]-2-(4-hydroxybenzamido)-8-methyl-8-azabicyclo[3.2.1]octane (41): yellow solid (120 mg, 82%); mp 250 °C dec; ^{1}H NMR (DMSO-d_6) δ 11.50 −12.20 (br s, 2H), 9.50−10.20 (br s, 2H), 8.11 (d, J = 8 Hz, 1H), 7.58 (d, J = 9 Hz, 2H), 7.20−7.35 (m, 2H), 6.98 (d, J = 8 Hz, 1H), 6.86 (s, 2H), 6.75 (d, J = 9 Hz, 2H), 5.34 (m, 1H), 4.67 (m, 1H), 3.60 (m, 2H), 2.59 (s, 3H), 1.90−2.80 (m, 6H); IR (KBr, cm^{-1}) 1712, 1637, 1608; FABMS m/z = 577 (M + 1). Anal. ($C_{30}H_{28}N_2O_{10}\cdot 1.3H_2O\cdot 0.5CF_3CO_2H$) C, H, N.

(±)-1-Isopropyl-*trans*-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]pyrrolidine (47): yellow solid (32 mg, 59%); mp 194−198 °C dec; ^{1}H NMR (CD_3OD) δ 7.78 (d, J = 8.7 Hz, 2H), 7.52 (d, J = 7.7 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.05 (d, J = 8.2 Hz, 1H), 7.00 (s, 2H), 6.86 (d, J = 8.7 Hz, 2H), 5.69 (m, 1H), 4.68 (m, 1H), 3.93−4.04 (m, 2H), 3.55−3.92 (m, 2H), 3.60 (quint, J = 6.5 Hz, 1H), 1.45 (d, J = 6.5 Hz, 3H), 1.44 (d, J = 6.4 Hz, 3H); IR (KBr, cm^{-1}) 1705, 1676, 1636, 1607; FABMS m/z = 565 (M + 1). Anal. ($C_{29}H_{28}N_2O_{10}\cdot 1.0H_2O\cdot 1.0CF_3CO_2H$) C, H, N.

(±)-1-Acetyl-*trans*-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]pyrrolidine (48): yellow solid (43 mg, 59%); mp 196−200 °C dec; ^{1}H NMR (CD_3OD) δ 7.71 (d, J = 8.6 Hz, 2H), 7.48 (d, J = 7.7 Hz, 1H), 7.26 (t, J = 7.9 Hz, 1H), 7.02 (d, J = 7.3 Hz, 1H), 6.91 (s, 2H), 6.80 (d, J = 8.6 Hz, 2H), 5.46 (dm, J = 10.4 Hz, 1H), 4.70 (tm, J = 10.4 Hz, 1H), 4.07 (dt, J = 11.1, 5.3 Hz, 1H), 3.93 (dt, J = 12.4, 5.5 Hz, 1H), 3.60−3.72 (m, 2H), 2.05 and 2.08 (both s, 3H, rotamers); IR (KBr, cm^{-1}) 1716, 1633, 1606; FABMS m/z = 565 (M + 1). Anal. ($C_{28}H_{24}N_2O_{11}\cdot 1.0H_2O$) C, H, N.

(±)-1-(Trifluoroacetyl)-*trans*-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]pyrrolidine (49): yellow solid (51 mg, 59%); mp 167−172 °C dec; ^{1}H NMR (CD_3OD) δ 7.74 (d, J = 8.1 Hz, 2H), 7.49 (d, J = 7.7 Hz, 1H), 7.27 (t, J = 7.9 Hz, 1H), 7.02 (d, J = 8.2 Hz, 1H), 6.93 (s, 2H), 6.83 (d, J = 8.4 Hz, 2H), 5.52 and 5.55 (both m, 1H, rotamers), 4.74 and 4.80 (both m, 1H, rotamers), 4.21 (m, 1H), 4.11 (m, 1H), 3.90 and 3.94 (both br s, 1H, rotamers), 3.77 and 3.82 (both br s, 1H, rotamers); IR (KBr, cm^{-1}) 1688, 1635, 1607; FABMS m/z = 619 (M + 1). Anal. ($C_{28}H_{21}N_2O_{11}F_3\cdot 1.25H_2O$) C, H, N.

(±)-1-(Methoxycarbonyl)-*trans*-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]pyrrolidine (50). Anhydrous pyridine (0.25 mL) was added to a stirred mixture of **22** (0.051 g, 0.080 mmol) and methyl chloroformate (12 μL, 0.160 mmoL) at 0 °C under N_2. The resulting mixture was stirred at 0 °C for 2 h, allowed to warm to room temperature, and stirred for 16 h. The solution was then concentrated *in vacuo*. The residue was chromatographed on a 41 × 300 mm C-18 column (solvent A, 95:5 water/acetonitrile + 0.1% TFA; solvent B, 100% acetonitrile; gradient, 0−100% B over 60 min; flow, 25 mL/min) affording the title compound (4.2 mg, 9%) as a yellow gum; ^{1}H NMR (CD_3OD) δ 7.52 (dd, J = 7.7, 2.1 Hz, 2H), 7.29 (d, J = 7.7 Hz, 1H), 7.07 (t, J = 7.8 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 6.70 (s, 2H), 6.61 (dd, J = 6.9, 2.0 Hz, 2H), 5.21−5.23 (m, 1H), 4.45−4.47 (m, 1H), 3.67−3.75 (m, 2H), 3.51 (s, 3H), 3.36 (dd, J = 12.4, 2.4 Hz, 2H); FABMS m/z = 581 (M + 1).

(±)-1-[(Methylamino)carbonyl]-*trans*-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]pyrrolidine (51): yellow powder (68.7 mg, 77%); mp 178−198 °C dec; ^{1}H NMR (CD_3OD) δ 7.53 (d, J = 8.7 Hz, 2H), 7.30 (d, J = 7.7 Hz, 1H), 7.06 (dd, J = 8.1, 7.9 Hz, 1H), 6.80 (d, J = 8.3 Hz, 1H), 6.70 (s, 2H), 6.62 (d, J = 8.7 Hz, 2H), 5.2−5.3 (m, 1H), 4.4−4.5 (m, 1H), 3.6−3.8 (6-line mult, 2H), 3.2−3.4 (m, 2H), 2.53 (s, 3H); IR (KBr, cm^{-1}) 3385, 1714, 1605, 1236, 763 cm^{-1}; HRMS m/z calcd for $C_{28}H_{25}N_3O_{11}$ 580.1567, found 580.1481. Anal. ($C_{28}H_{25}N_3O_{11}\cdot 0.8H_2O\cdot 0.8CF_3CO_2H$) C, H, N.

(±)-1-(Methylsulfonyl)-*trans*-3-(4-hydroxybenzamido)-4-[[4-[2-hydroxy-6-(hydroxycarbonyl)benzoyl]-3,5-dihydroxybenzoyl]oxy]pyrrolidine (52): yellow solid (137 mg, 74%); mp 179−187 °C dec; ^{1}H NMR (CD_3OD) δ 7.53 (d, J = 8.7 Hz, 2H), 7.29 (d, J = 7.6 Hz, 1H), 7.07 (dd, J = 7.9, 8.1 Hz, 1H), 6.82 (d, J = 8.2 Hz, 1H), 6.73 (s, 2H), 6.62 (d, J = 8.7 Hz, 2H), 5.29 (dt, J = 5.5, 3.1 Hz, 1H), 4.48 (dt, J = 6.2, 4.2 Hz, 1H), 3.73 (dd, J = 12.0, 5.5 Hz, 1H), 3.67 (dd, J = 10.7, 6.9 Hz, 1H), 3.35 (dd, J = 12.0, 2.8 Hz, 1H), 3.28 (dd, J = 10.6, 4.4 Hz, 1H), 2.75 (s, 3H); FABMS m/z = 601 (M + 1); IR (KBr, cm^{-1}) 3394, 1708, 1607, 1235, 762. Anal. ($C_{27}H_{24}N_2O_{12}S\cdot 2.5H_2O$) C, H, N.

Protein Kinase C Expression and Purification. The α, β1, β2, γ, δ, ϵ, η, and ζ recombinant human PKC enzymes were produced using a baculovirus expression system in SF9 cells.[15] The Ca^{2+}-independent isozymes (δ, ϵ, η, and ζ) were purified as described in the literature by Bronson *et al*.[16] The Ca^{2+}-dependent isozymes (α, β1, β2, and γ) were purified using a modification of a method described by Kochs *et al*.[17] 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% to 90% depending on isozyme subtype.

Protein Kinase C and cAMP-Dependent Kinase Assays. 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 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. 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 reaction mixtures 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 0.1 nM to 150 μM and depended on the enzyme employed in the assay and the compound. The assays always started at the highest concentrations, and compounds were retested at lower concentrations until IC_{50} values could be determined. Most of the IC_{50} values were results of single-point determinations at four concentrations. Assay controls included a maximal lipid-activated PKC assay and a no-lipid PKC assay. The no-lipid activity was subtracted 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.[18] The cAMP-dependent protein kinase assay was performed as previously described.[19]

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Supporting Information Available: Listing of the experimental details for the preparation of all intermediates (19 pages). Ordering information is given on any current masthead page.

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(7) One of the reviewers of this manuscript had expressed concerns regarding whether these compounds are irreversible inhibitors, whether they are inhibitors of ATP binding, and the possibility of the inhibition of PKC by these compounds being light-sensitive, as was the case for calphostin, due to the benzophenone moiety. Reversibility experiments were performed on the parent balanol compound. The results suggested that balanol bound very tight in the ATP binding site and the dissociation was very slow. Conclusive experiments could not be performed due to the instability of the enzyme under these assay conditions. The light sensitivity of these compounds was not tested. However, we have no evidence that these compounds are acting like calphostin. Although the assays were not performed in the dark, light was minimal. Calphostin tested under these conditions resulted in IC_{50} values 10−20-fold less than what was reported in the literature. Kinetics were performed on the parent balanol compound, and this compound appeared to be competitive with ATP. Also, for a virtual kinetic study resulting in a similar conclusion, see ref 16.

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