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NOVEL CONFORMATIONALLY CONSTRAINED ANALOGUES OF DIACYLGLYCEROL. PROTEIN KINASE C BINDING AFFINITY OF SIMPLIFIED COMPOUNDS BASED ON A 6-MEMBERED LACTAM MOIETY

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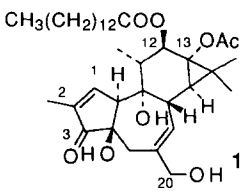
Abstract: Four configurational isomers of 6-hydroxymethyl-3-isopropyl-4-tetradecylpiperazin-2-ones (**4**-**7**), which were designed based on information obtained from the biologically active conformation of teleocidins and benzolactams, were synthesized and evaluated for their ability to compete with [^{3}H]phorbol 12,13-dibutyrate in a PKCδ binding assay. Among the compounds, the 3*S*,6*S*-isomer (**5**) showed moderate binding affinity, 8-30 fold more potent than for the other isomers. This indicates that the relative position of the hydrogen-bonding sites and hydrophobic regions of **5** fits into the cavity of PKCδ binding site. Compound **5** provides a conformationally constrained analogue of diacylglycerol. © 1997 Elsevier Science Ltd.

Protein kinase C (PKC) plays an important role in cellular signal transduction and related regulation of cell growth and differentiation.[1,2] Physiologically, signals are generated by various ligands which produce the lipid second messenger, diacylgycerol (DAG).[3] Phorbol esters [*e.g.*, 12-*O*-tetradecanoylphorbol-13-acetate, (TPA, **1**)] were shown to bind to PKC with extremely high affinities at a site bound by DAG.[4,5] Thus, phorbol esters have been valuable tools for analyzing the role of PKC in biological functions.

Although several molecular-modeling studies have been based on ligand structures of phorbol esters and DAG,[6,7] the flexibility of DAG makes it difficult to assign a preferred conformation for binding to PKC. The design and synthesis of conformationally constrained analogues of DAG is one of the effective method for studying these problems. Marquez *et al.* reported several conformational restricted analogues of DAG which contain various DAG-like features[8] and have obtained the potent PKC agonists, 5-disubstituted tetrahydro-2-furanones.[9]



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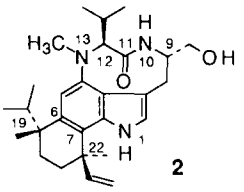

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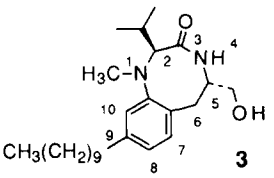

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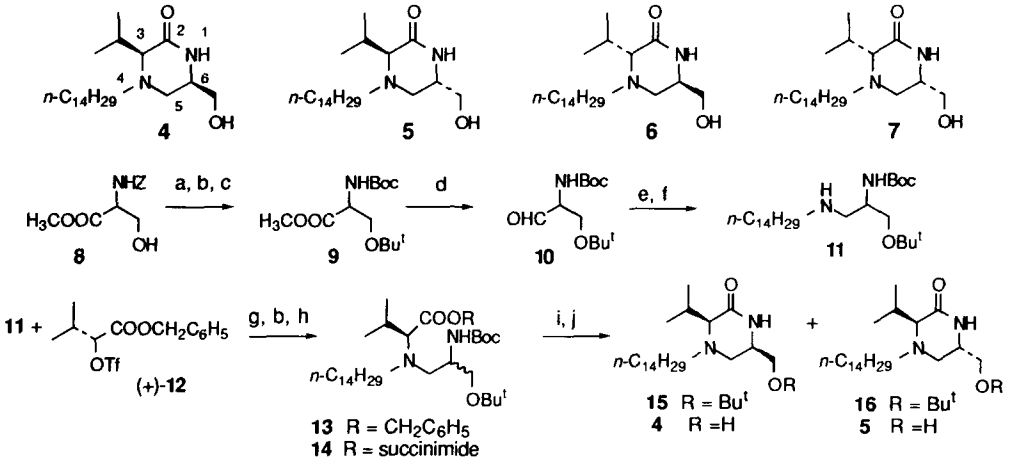
On the other hand, teleocidins (*e.g.*, teleocidin B-4, **2**) which have different skeletal structures from phorbol esters, have exhibited PKC agonist activity as potent as phorbol esters.[10] Definition of common structural requirements for PKC binding, however, has been hindered by the conformational flexibility of the

lactam ring conformations[11] of teleocidins. Teleocidins exist in an equilibrium between at least two conformational states, the twist and sofa forms, in solution.[12] We have designed and synthesized (-)-benzolactam-V8-310 [(-)-BL-V8-310, **3**] which is a conformationally restricted molecule having a benzene ring and a simplified linear alkyl group instead of the indole ring and terpenoid side chain of teleocidins.[13] (-)-BL-V8-310 (**3**), which exists only in the twist form with a *cis*-amide in solution, exhibits strong biological activity.[14] This finding clearly indicated that the twist form of teleocidin is the biologically active form and appears to solve the conformational flexibility problem that has bedeviled structure-activity studies of teleocidins.

The benzolactams (BLs) make it possible to design evolutional PKC agonists. The conformation of the lactam ring is considerably restricted by the planarity of the amide bond as compared to the lactones. Among the various 1,2-diacylglycerol analogues in structure-activity studies of PKC agonists, compounds replaced the 1-ester or 2-ester with an amide linkage have been reported. The 1-amide caused loss of activity and the 2-amide showed weak activity, with a potency at least 10 times less than that of DAG.[15] Design of DAG analogues with cyclic amide has not been reported. The relative positions of the *cis*-amide structure and hydroxymethyl group, which are important for hydrogen bonding to PKC, seem to be favorably arranged on a six-membered lactam. Furthermore, the hydrophobic moieties of teleocidins and BLs play a critical role in increasing their biological potency.[16, 17] Thus, we designed 2-piperazinones with a hydroxymethyl group as a hydrogen-bonding site and iso-propyl and *N*-linear alkyl groups as hydrophobic moieties. We report herein the synthesis and activity of simplified cyclic molecules (**4**, **5**, **6**, **7**) with the essential pharmacophore of DAG-site ligands.



Scheme. Synthesis of **4**-**7**. Key: a) $(CH_3)_2C{=}CH_2$, H_2SO_4/ CH_2Cl_2 b) H_2, Pd-C/ MeOH c) Boc_2O, Et_3N/ CH_2Cl_2 d) DIBAH/ toluene e) n-$C_{14}H_{29}NH_2$/ benzene, heat f) $NaBH_3CN$/ MeOH g) 2,6-lutidine/ CH_2ClCH_2Cl h)N-hydroxysuccinimide, DCC/ CH_3CN i)CF_3COOH/ CH_2Cl_2 then $NaHCO_3$aq/ CH_3COOEt j) CF_3COOH/ CH_2Cl_2, heat

Synthesis of the lactams **4**-**7** started with two amino acid components as shown in the Scheme. *N*-Cbz-D,L-serine methyl ester (**8**) was converted to *N*-Cbz-*O*-*tert*-butyl-D,L-serine methyl ester (**9**) (97 %). Exchange of the *N*-protective group from the carbobenzoxy group of **9** to a Boc group, followed by reduction of the ester group to an aldehyde gave 2-(*N*-Boc-amino)-3-*tert*-butoxypropanal (**10**) (68 %). Condensation of **10** with *n*-tetradecylamine followed by hydride reduction with $NaBH_3CN$ afforded 3-(*N*-*n*-tetradecylamino)-2-(*N*-Boc-amino)propyl *tert*-butyl ether (**11**) (99 %). The other fragments, *R*-(+)-benzyl 2-((trifluoromethylsulfonyl)oxy)isovalerate (*R*-**12**) and *S*-(-)-benzyl 2-[(trifluoromethylsulfonyl)oxy]isovalerate (*S*-**12**), were

prepared from D-valine and L-valine, respectively, by the methods reported by Kogan *et al.*[18] Reaction of the amine **11** with the triflate *R*-**12** gave diastereomeric ester **13** (57%). After hydrogenolysis of the benzyl ester, condensation with N-hydroxysuccinimide using DCC gave the activated esters **14** (84%). After removal of the Boc group using CF_3COOH, cyclization was carried out under dilute conditions to give **15** (35%) and the epimer **16** (35%), which were separated at this stage. Removal of the *tert*-butyl group on the hydroxyl groups of **15** and **16** afforded **4**[19] with *3S,6R* (70%) and **5**[20] with *3S,6S* (56%), respectively. The other two isomers,[21] **6** with *3R,6R* and **7** with *3R,6S* , were prepared from **11** and *S*-**12**. The configurations of **4**-**7** were determined by 1D- and 2D-NMR including decoupling and NOE experiments. The conformations of the compounds were deduced from the NMR experiments as describes in the Figure.

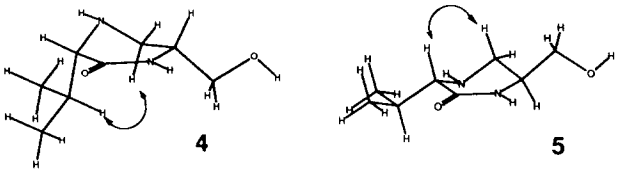


Figure. Possible Conformational structures of **4** and **5**.
The *N*-alkyl side chain is omitted for the sake of clarity. The arrows indicate characteristic NOE enhancements.

The biological activities of the lactams (**4**,**5**,**6**,**7**) were examined by two bioassays related to *in vivo* tumor promotion. One of the most sensitive and specific biological activities of the TPA-type tumor promoters is induction of growth inhibition, cell adhesion, and differentiation to monocytes of HL-60 cells.[22] Among the lactams, the *3S,6S* isomer (**5**) showed growth inhibitory activity with an ED_{50} of 2 x 10^{-7} M, comparable to indolactam-V (IL-V). IL-V is the core structure of teleocidins and has moderate tumor promoting activity.[23] The *3S,6R* isomer (**4**) showed weaker activity, with an ED_{50} of 10^{-6} M, and the *3R,6R* - (**6**) and the *3R,6S* isomers were almost inactive (ED_{50} >5 x 10^{-6}). Assays of inhibition of [^{3}H]PDBu binding were done as previously described.[24] The *3S,6S* isomer was moderately active, with K_i's of 240-1,100 nM for PKCδ and seven other isotypes; this potency range is only about 10-fold weaker than that of IL-V itself on the same isotypes (30-250 nM), and this compound is substantially more potent than typical diacylglycerols (K_i values of 0.5-1.3 μM for glycerol-1-myristate-2-acetate in the binding to PKC mixture has been reported[25]). The other isomers showed weaker binding activity (range: 1.8 μM to 50 μM). K_i's for IL-V, **4**,**5**,**6** and **7** on PKCδ were 80 nM, 2.7 μM, 290 nM, 12 μM and 6.5 μM, respectively.

The activities of the *3S,6S* isomer (**5**) differ significantly from the other three isomers. We have reported advanced computational docking of teleocidin-benzolactam congeners to the cys2 domain of PKCδ[26] based on the crystal structure of PKCδ cys2 domain-phorbol-13-acetate complex.[27] The conformations of **4**-**7** suggest that the relative positions of hydrogen-bonding functional groups of these isomers are arranged well for bonding to PKC. Although the activity can not be explained only by the hydrophobic interaction of the hydrophobic moieties on phorbol esters and teleocidin-benzolactams with the receptor cavity but with external phospholipids, the present relative activities of the simplified lactams **4**-**7** are best explained the differences in the fitting of the molecules into the cavity through van der Waals contacts with PKCδ. The lactam 6*S*-hydroxymethyl-3*S*-isopropyl-4-tetradecylpiperazin-2-one (**5**) is a PKC agonist having a new skeletal structure and should be helpful in the design of further compounds as biological tools for analyzing the mechanism of signal transduction through PKC and tumor promotion.

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19. Compound **4**: colorless oil, $[\alpha]^{22}_{D}$ -16.5 ° (*c* = 1.01, $CHCl_3$) ^{1}H-NMR ($CDCl_3$) 0.88 (t, 3H, *J* = 6.6 Hz, $CH_3(CH_2)_{11}CH_2CH_2$-N), 1.04 (d, 3H, *J* = 6.6 Hz, (CH_3)CH-), 1.05 (d, 3H, *J* = 6.6 Hz, (CH_3)CH-), 1.26 (m, 22H, $CH_3(CH_2)_{11}CH_2CH_2$-N), 1.45 (m, 2H, $CH_3(CH_2)_{11}CH_2CH_2$-N), 2.11 (dsept, 1H, *J* = 6.0, 6.6 Hz, (CH_3)C*H*-), 2.50 (dt, 1H, *J* = 7.0, 12.8 Hz, $CH_3(CH_2)_{11}CH_2CH_2$-N), 2.60 (dt, 1H, *J* = 7.3, 12.8 Hz, $CH_3(CH_2)_{11}CH_2CH_2$-N), 2.71 (dd, *J* = 4.4, 13.6 Hz, -NCH_2CH), 2.76 (d, 1H, *J* = 6.0 Hz, N-*CH*CO), 2.91 (dd, 1H, *J* = 8.4, 13.6 Hz, -NCH_2CH), 3.53 (dd,1H, *J* = 6.4, 10.6 Hz, CH_2OH), 3.65 (dd,1H, *J* = 3.7, 10.6 Hz, CH_2OH), 3.68 (m, 1H, NHC*H*CH_2OH), 6.79 (bs, 1H, CON*H*); HRMS Calcd for $C_{22}H_{44}N_2O_2$ 368.3405, Found 368.3381.
20. Compound **5**: colorless oil, $[\alpha]^{22}_{D}$ +25.2 ° (*c* = 1.01, $CHCl_3$) ^{1}H-NMR ($CDCl_3$) 0.88 (t, 3H, *J* = 6.6 Hz, $CH_3(CH_2)_{11}CH_2CH_2$-N), 0.98 (d, 3H, *J* = 6.6 Hz, (CH_3)CH-), 1.12 (d, 3H, *J* = 6.6 Hz, (CH_3)CH-), 1.26 (m, 22H, $CH_3(CH_2)_{11}CH_2CH_2$-N), 1.44 (m, 2H, $CH_3(CH_2)_{11}CH_2CH_2$-N), 2.02 (dsept, 1H, *J* = 2.9, 6.6 Hz, (CH_3)C*H*-), 2.14 (dd, *J* = 9.5, 11.4 Hz, -NCH_2CH), 2.34 (dt, 1H, *J* = 8.0, 12.8 Hz, $CH_3(CH_2)_{11}CH_2CH_2$-N), 2.58 (dt, 1H, *J* = 7.7, 12.8 Hz, $CH_3(CH_2)_{11}CH_2CH_2$-N), 2.83 (d, 1H, *J* = 2.9 Hz, N-*CH*CO), 2.97 (dd, 1H, *J* = 3.7, 11.4 Hz, -NCH_2CH), 3.50 (dd,1H, *J* = 7.7, 11.0 Hz, CH_2OH), 3.63 (m, 1H, NHC*H*CH_2OH), 3.70 (dd,1H, *J* = 3.3, 11.0 Hz, CH_2OH), 7.46 (bs, 1H, CON*H*); HRMS Calcd for $C_{22}H_{44}N_2O_2$ 368.3405, Found 368.3418.
21. Compound **6**: colorless oil, $[\alpha]^{22}_{D}$ -25.2° (*c* = 1.02, $CHCl_3$) HRMS Calcd for $C_{22}H_{44}N_2O_2$ 368.3405, Found 368.3398. Compound **7**: colorless oil, $[\alpha]^{22}_{D}$ +16.45° (*c* = 1.00, $CHCl_3$) HRMS Calcd for $C_{22}H_{44}N_2O_2$ 368.3405, Found 368.3392.
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