Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 173–178

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis, Computer Modeling and Biological Evaluation of Novel Protein Kinase C Agonists Based on a 7-Membered Lactam Moiety

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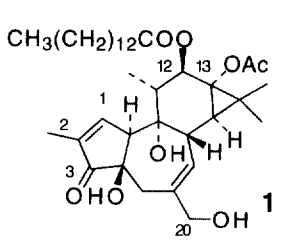
Received 17 October 1998; accepted 26 November 1998

Abstract

4-Hydroxymethyl-5a-methyl-1,3,4,5,5aβ,6,7,8,9,9aα-decahydro-2H-benz[*d*]azepin-2-ones (**4-12**), which were designed to mimic the biologically active conformation of teleocidins and benzolactams, were synthesized and evaluated for the ability to compete with [^{3}H]phorbol 12,13-dibutyrate in a PKCδ binding assay. Among the compounds, **10-12** showed potent binding affinity, with inhibition constants (K_i) of low nanomolar order. Computational docking simulation also indicates that the relative positions of the hydrogen-bonding sites and hydrophobic regions of the compounds are well matched to the PKCδ binding site.

Keywords: Receptors; Agonist; Molecular Modeling

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, diacylglycerol (DAG). [3] Phorbol esters [*e.g.*, 12-*O*-tetradecanoylphorbol-13-acetate, (TPA, **1**)] and teleocidins (*e.g.*, teleocidin B-4, **2**), which have different skeletal structures from phorbol esters, were shown to bind to PKC with extremely high affinities at a site bound by DAG. [4,5,6]



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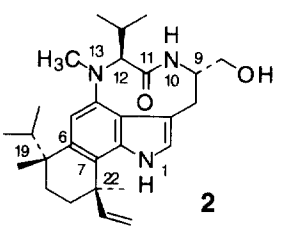

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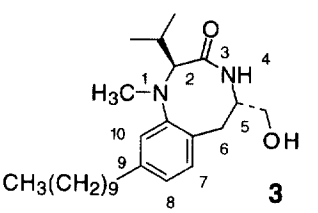

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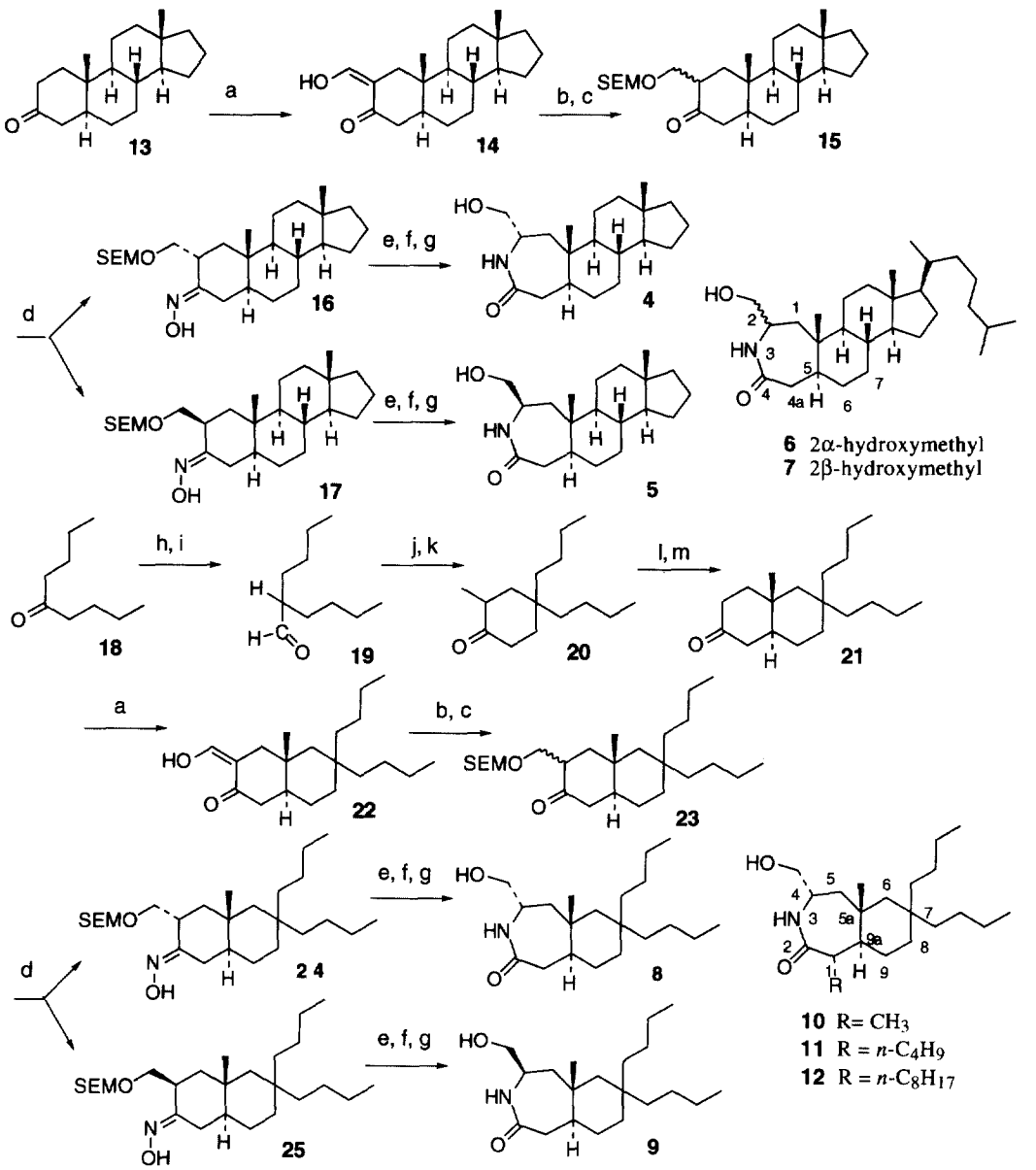
PII: S0960-894X(98)00724-0

Thus, these compounds have been valuable tools for analyzing the role of PKC in biological functions. Definition of common structural requirements for PKC binding, however, has been hindered by the conformational flexibility of teleocidins. [7,8]

We have designed and synthesized conformationally restricted (-)-benzolactam-V8-310 [(-)-BL-V8-310, **3**] [9], which exhibits strong biological activity. [10] We have reported computational docking simulation of teleocidins and benzolactams to the CRD2 domain structure [11,12] observed in the crystalline complex of PKCδ with phorbol-13-acetate. [13] Teleocidin and benzolactams fitted well into the same cavity as phorbol-13-acetate. The hydrogen-bonding pattern and hydrophobic interaction of teleocidin and benzolactams to PKCδ were clarified in the study. The docking model was supported experimentally by the large decrease in the activity of hydrophobically modified benzolactams having a bulky substituent. [12,14] On the other hand, we have reported the synthesis and PKC-binding affinity of simplified molecules with a 6-membered lactam moiety as conformationally constrained analogs of diacylglycerol. [15] These results make it possible to design superior PKC agonists. The relative positions of the *cis*-amide structure and hydroxymethyl group are important for hydrogen bonding to PKC. To achieve appropriate positioning, we selected a 7-membered lactam with a hydroxymethyl group as a hydrogen-bonding site in which the ring conformation is restricted by a 5a-methylperhydrobenz[*d*]- azepin-2-one skeleton. This skeleton may be obtained by transformetion of *trans*-decalin-2-one, which is related to the A, B ring structure of the steroidal skeleton. The hydrophobic moieties of teleocidins and BLs play a critical role in increasing the biological potency. [16,17] The C, D ring structure and C-17 side chain of steroids, with substitution of appropriate flexible alkyl groups on the skeleton, might provide a similar hydrophobic moiety. Thus, we designed 4-hydroxymethyl-5a-methyl-1,3,4,5,5aβ,6,7,8,9,9aα-decahydro-2H-benz[*d*]azepin-2-ones (**4-12**). We report herein the synthesis and activity of these simplified cyclic molecules (**4-12**) with the essential pharmacophore of DAG-site ligands.

Synthesis of the lactams having steroidal skeletons **4-7** was started from steroid-3-one. The scheme shows the preparation of **4** and **5** from androstan-3-one (**13**) as an example. Androstan-3-one (**13**) was condensed with ethyl formate to give 2-hydroxymethylene-5α-androstan-3-one (**14**) (82%). [18] Partial reduction of **14** with $NaBH_4$ followed by protection of the hydroxyl group with trimethylsilylethoxymethyl (SEM) afforded a 6:1 mixture of 2α- and 2β-SEMOCH$_2$-5α-androstan-3-one (**15**) (47%). The 2β-hydroxymethyl isomer was the major product detected on TLC immediately after hydride reduction. However, the isomer ratio of 2α- and 2β-hydroxymethyl isomers was 2:1 after the isolation procedure. The rate of isomerization of the free hydroxyl isomers was too rapid to allow detection of only the 2α-isomer by ^{1}H-NMR in $CDCl_3$ (containing a trace amount of H^+) as a solvent. The isomerization of the SEM ethers was slower than that of the free hydroxyl isomers. Oximation of **15** gave a mixture of two *E*-oxime isomers, which was separated to afford the 2α- (**16**) (70%) and 2β-SEMOCH$_2$-*E*-oxime (**17**) (10%). Beckmann rearrangement of **16** and **17** under mild conditions [19] followed by removal of the SEM group afforded 2α-hydroxymethyl- (**4**) (63%) and 2β-hydroxymethyl-3-aza-*A*-homo-5α-androstan-4-one (**5**) (63%), respectively. 2α-Hydroxymethyl- (**6**) and 2β-hydroxymethyl-3-aza-*A*-homo-5α-cholestan-4-one (**7**) were prepared from

cholestan-3-one in a similar manner. Synthesis of the lactams **9-12** as racemic compounds was started from 5-nonanone (**18**), which is the hydrophobic group of the target molecules. Reaction of **18** with ethoxymethyl Grignard reagent followed by acid hydrolysis gave 2-*n*-butyl-1-hexanal (**19**) (63%). Condensation of **19** with ethyl vinyl ketone followed by catalytic hydrogenation afforded 4,4-di-*n*-butyl-2-methylcyclohexanone (**20**) (53%).



Scheme. Synthesis of **4-12**. Key: a) (CH_3ONa, $HCOOC_2H_5$,benzene; b) $NaBH_4$/ MeOH; c) SEMCl, ($(CH_3)_2CH)_2NC_2H_5$/ CH_2Cl_2 d) NH_2OH, CH_3COONa/ EtOH; e) *p*-TsCl/ pyridine f) HCl aq, heat; g) HF-pyridine/ THF; h) Mg, $ClCH_2OC_2H_5$ / THF; i) HCOOH, heat; j) ethyl vinyl ketone, H_2SO_4/ benzene; k) H_2, Pd-C/ *n*-pentane; l) methyl vinyl ketone, H_2SO_4/ benzene; m) Li/NH_3;

Condensation of **20** with methyl vinyl ketone followed by hydrogenation using lithium afforded the *trans*-decalinone derivative **21** (21%). The *trans*-decalinone **21** was converted into a mixture of 3α- and 3β-SEMOCH$_2$-6,6-di-*n*-butyl-4a-methyl- 1,2,3,4aβ,5,6,7,8,8aα-decahydronaphthalen-2-ones (**23**) (23%). After oximation and separation of the decalinone **23**, ring enlargement rearrangement followed by removal of the SEM group afforded 4α-hydroxymethyl- (**8**) (28%) and 4β-hydroxymethyl-7,7-di-n-butyl-5a-methyl-1,3,4,5,5aβ,6,7,8,9,9aα-decahydro-2H- benz[*d*]azepin-2-ones (**9**) (15%), respectively. The compounds **10-12** with an alkyl group at the 1α-position of the molecule **8** were prepared from **13** in a manner similar to that described for **8** using an appropriate alkyl vinyl ketone instead of methyl vinyl ketone at the step of ring formation (from **20** to **21**). The configurations and the conformations of **4-12** were determined by 1D- and 2D-NMR, including decoupling and NOE experiments.

The biological activities of the lactams (**4-12**) were examined by means of 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. [20] Among the lactams, the compounds with steroidal skeletons (**4-7**) showed growth-inhibitory activity with ED_{50} values of 1 x 10^{-6} to 8 x 10^{-6} M. The compounds with flexible hydrophobic chains (**8** and **9**, racemic) showed ED_{50} values of 3 x 10^{-7} and 7 x 10^{-7} M, respectively, comparable to that of indolactam-V (IL-V). IL-V is the core structure of teleocidins and has moderate tumor-promoting activity. The activity was increased by the introduction of an alkyl group at the 1α-position, and the ED_{50} values for **10-12** were about 1.5 x 10^{-7} M. Assays of inhibition of [^{3}H]PDBu binding (K_d = 0.76 nM) to human recombinant PKCδ (purchased from PanVera Co. Ltd.) were done as previously described. [21] The steroidal lactams **4-7** were weakly active, with K_i's of above 0.5 μM for PKCδ, while the compounds with flexible hydrophobic chains (**8** and **9**) showed strong activity with K_i's of 60 nM and 190 nM, respectively; the potency of **8** is almost the same as that of IL-V itself (80 nM for rat recombinant PKCδ). The activity increased by the introduction of an alkyl group at the 1α-position. The K_i's of **8**, **10**, **11** and **12** for PKCδ were 60 nM, 21 nM, 17 nM and 11 nM, respectively.

The activities of the lactams **4-12** indicate that these compounds provide the requisite structures for hydrogen-bonding to PKC. Conformational analysis suggests that the relative positions of the amide plane and the hydroxymethyl group of these compounds are similar in spite of the differences of configuration of the hydroxymethyl group, such as in **8** and **9**. This is because the angular methyl group of the *trans*-decalin system restricts the ring conformation. The difference of the activities can be attributed to the difference in the direction of the hydrophobic group, including a part of the ring structure. The figure (left) shows the most stable docking model of **10** to the crystal structure of PKCδ CRD2 domain-phorbol-13-acetate obtained by using advanced computational docking. [12] The molecule of **10** forms a stable complex with the same hydrogen-bonding pattern as in teleocidin B-4 (**2**) and BL-V8-310 (**3**). [12] The figure (right) shows superposition of the

most stable docking model of **10** and **3** with the protein surface. The hydrophilic functional groups of **10** are rearranged simlar to those of **3**, and the butyl group of 7-position of **8** corresponds to alkyl substituent of **3**.

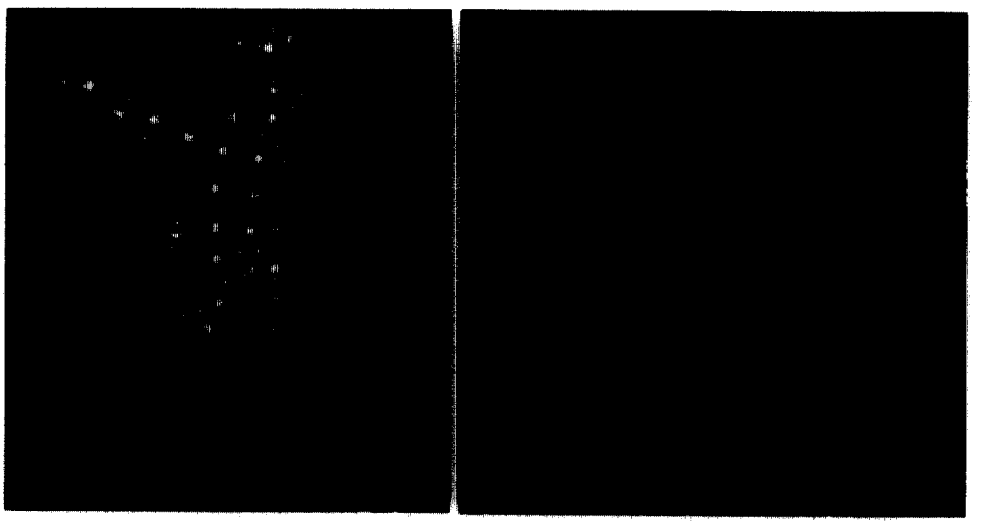


Figure. Drawings of the most stable docking model of **10** (left), and superposition of **10** (pink lines) and **3** (blue lines) in the cavity (right). The decyl group of **3** was shortened for the sake of clarity. Intermolecular hydrogen bonds (distances less than 3.2 Å) are shown with yellow lines.

Although the activity cannot be explained only in terms of the hydrophobic interaction of the hydrophobic moieties on teleocidin-benzolactams with the receptor cavity (the role of external phospholipids must also be considered), the increase in the binding activity of **10-12** can be well explained in terms of the increase of hydrophobic interaction of the additional alkyl group at the 1α-position with the hydrophobic residues of PKC, which seems to be correspond to isopropyl group of **3** as shown in the Figure (right). Thus, the lactams **8**, **10-12** are PKC agonists having a new skeletal structure and should be helpful in the design of further compounds as biological tools for analyzing the mechanisms of signal transduction through PKC and tumor promotion.

Acknowledgment: This work was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the Organization for Drug ADR Relief, R & D Promotion and Product Review of Japan.

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