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PROTEIN KINASE C MODULATORS BEARING DICARBA-*CLOSO*-DODECABORANE AS A HYDROPHOBIC PHARMACOPHORE

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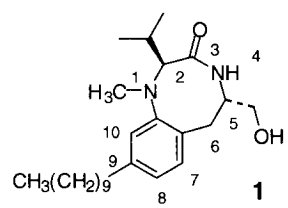
Abstract: The size and position of a hydrophobic moiety on a benzolactam skeleton, which reproduces the active conformation and biological activity of teleocidins, play an important role in the appearance of the activity. We have designed and synthesized benzolactams bearing dicarba-*closo*-dodecaborane. These compounds showed potent binding affinity to protein kinase C, providing a further example of the application of carborane as the hydrophobic pharmacophore of biologically active molecules.

Phorbol esters (*e.g.* 12-*O*-tetradecanoylphorbol-13-acetate: TPA) and teleocidins (*e.g.* teleocidin B-4) are potent tumor promoters and activate protein kinase C (PKC) by binding competitively to the enzyme.[1] The relationship between the chemical structures and the activities of these compounds has attracted much attention because of the marked structural dissimilarities. (-)-Benzolactam-V8-310 ((-)-BL-V8-310, **1**) with an 8-membered lactam ring and benzene ring instead of the 9-membered lactam and the indole ring of teleocidins, reproduces well the active ring conformation and biological activities of teleocidins.[2] Recently, we have reported the synthesis of benzolactams with hydrophobic substituents at various positions.[3] The structure-activity data of benzolactams indicate that the existence of a hydrophobic region between C-2, C-1 and C-9, and the steric factor at C-8 play critical roles in the appearance of biological activity.[4]



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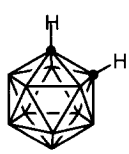

1,2-Dicarba-*closo*-dodecaborane
Closed circles represent carbon atoms and other vertexes represent BH units.

In recent years, the use of carboranes in boron neutron capture therapy (BNCT) has attached much interest, on the basis of their high boron contents.[5] We, on the other hand, have focused on the possibility of using dicarba-*closo*-dodecaboranes (carboranes) as a hydrophobic component in biologically active molecules which interact hydrophobically with receptors. We reasoned that the remarkable thermal and chemical stability, the exceptionally hydrophobic character and spherical geometry of carboranes made them interesting candidates for use as a hydrophobic pharmacophore. Recently, we have reported examples of the design, synthesis and biological evaluation of nuclear receptor ligand, retinoids[6] and estrogens,[7] containing a carborane cage as a hydrophobic pharmacophore.

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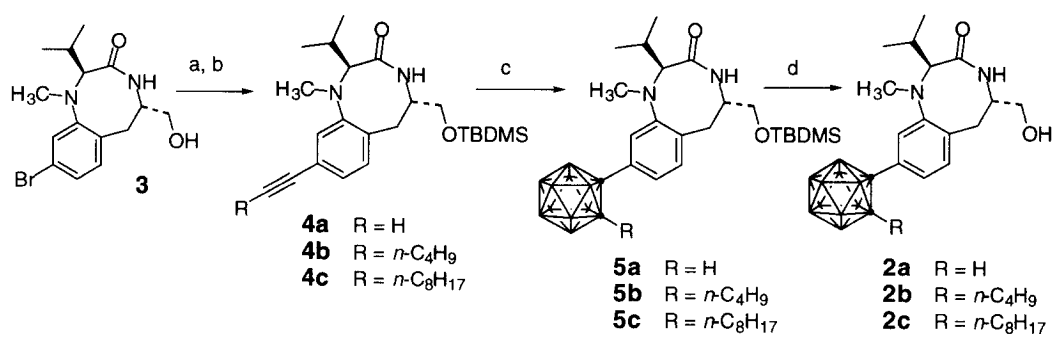
In this article, we describe the synthesis and biological evaluation of PKC modulators having a carborane cage as a hydrophobic pharmacophore.

In connection with the structure-activity studies of BL-V8s, we have reported that substitution of a C10-C14 linear alkyl chain at the 9-position of the aromatic nucleus is optimum for the appearance of biological activity, though substitution of a C8-C16 cyclic alkyl group is also effective.[3] This suggests that the hydrophobic alkyl group on BL-V8s is folded when the molecule binds to a receptor. On the other hand, a bulky substituent at the 8-position remarkably diminished the activities.[4] We have also reported computational docking simulation of teleocidins and benzolactams to the CRD2 domain structure[4,8] observed in the crystalline complex[9] of PKCδ with phorbol-13-aceatate. The docking model explained well the effect on the activity of a bulky substituent at the 8-position. Such a bulky substituent would hinder the fitting of the molecule into the cavity through van der Waals contacts with Ser240 and Met239 of the protein.[4] To investigate the great difference of substituent effects and to examine the usefulness of carborane as a hydrophobic pharmacophore, we designed BL-V8s bearing a carborane cage at the 9-position (**2a**, **2b**, **2c**). Among the three isomers of carboranes (1,2-, 1,7- and 1,12- isomers), we used 1,2-carborane as the hydrophobic pharmacophore. Because, the 2-substituted alkyl chain expect to extend along the protein surface, and 1,2-carborane skeleton is readily constructed from alkyne. As shown in the Scheme, synthesis of the BL-V8s was performed from 9-bromo-BL-V8 (**3**), which was prepared by the method previously described.[3] Protection of the hydroxyl group of **3** with TBDMS, followed by coupling with the terminal alkyne catalyzed by bis(triphenylphosphino)palladium(II) dichloride and copper(I) iodide in diethylamine gave **4b** and **4c** in yields of 70-90 %. *O*-TBDMS-9-Ethynyl-BL-V8 (**4a**) was prepared by the coupling of **3** with ethynyltrimethylsilane, followed by alkaline hydrolysis in a yield of 73%. Construction of the 1,2-carborane cage was performed by reaction of alkynyl-BL-V8s (**4a-4c**) with *nido*-decaborane(14) at 80°C in toluene in the presence of acetonitrile as a Lewis base to give **5a-5c** (55-65%). Deprotection of the TBDMS groups[10] of **5a-5c** with trifluoroacetic acid gave 9-(1,2-carboranyl)-BL-V8s (**2a-2c**)[11] in yields of 75-85%. The benzolactams appear to take similar conformational form to that of **1**, on the basis of the similarity of the ^{1}H NMR spectral data and NOEs.



Scheme. Key: (a) TBDMSCl, imidazole, CH_2Cl_2 (b) terminal alkyne, $(PPh_3)_2PdCl_2$, CuI, Et_2NH or ethynyltrimethylsilane, $(PPh_3)_2PdCl_2$, CuI, Et_2NH then KOH, MeOH (c) *nido*-decaborane(14), CH_3CN, toluene (d) CF_3COOH, H_2O, CH_2Cl_2

The biological activities of the carborane-containing BL-V8s (**2a-2c**) 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 the induction of growth inhibition, cell adhesion, and differentiation to monocytes of HL-60 cells.[12] Among the BL-V8s, the compound without an alkyl substituent on the cage carbon (**2a**) showed growth-inhibitory activity with an ED_{50} value of 3 x 10^{-8} M. The compounds with an alkyl group on the cage carbon (**2b** and **2c**) both showed an ED_{50} value of 7 x 10^{-9} M, comparable to that of BL-V8-310 (**1**, an ED_{50} value of 5 x 10^{-9} M), which is one of the most potent benzolactams known. 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.[13] The steroidal carborane-containing BL-V8s (**2a-2c**) showed strong activity with *Ki*'s of 2.0 nM, 1.4 nM and 1.8 nM, respectively; the potencies of these compounds are almost the same as that of BL-V8-310 itself (K_i =1.8 nM for hPKCδ).

The activities of **2a-2c** indicate that these compounds provide the requisite structures for hydrogen-bonding to and hydrophobic interaction with PKC. The activity cannot be explained only in terms of hydrophobic interaction of the hydrophobic moiety on the teleocidin-benzolactams with the receptor cavity (the role of external phospholipids must also be considered). However, we have demonstrated that the docking simulation of ligands to PKCδ CRD2 explains well the biological activity. The figure shows the most stable docking models of **1** (left) and **2b** (right) to the crystal structure as seen in the PKCδ CRD2 domain-phorbol-13-acetate complex, obtained by using advanced computational docking.[14] The molecule of **2b** forms a stable complex with the same hydrogen-bonding pattern as in the case of **1**, and the bulky carborane cage at the 9-position of **2b** satisfactorily evades contact with the protein surface. In summary, we have developed novel carborane-containing molecules with potent PKC-modulating activity. The results demonstrate that carboranes are applicable as a hydrophobic pharmacophore for biologically active molecules other than nuclear receptor ligands.

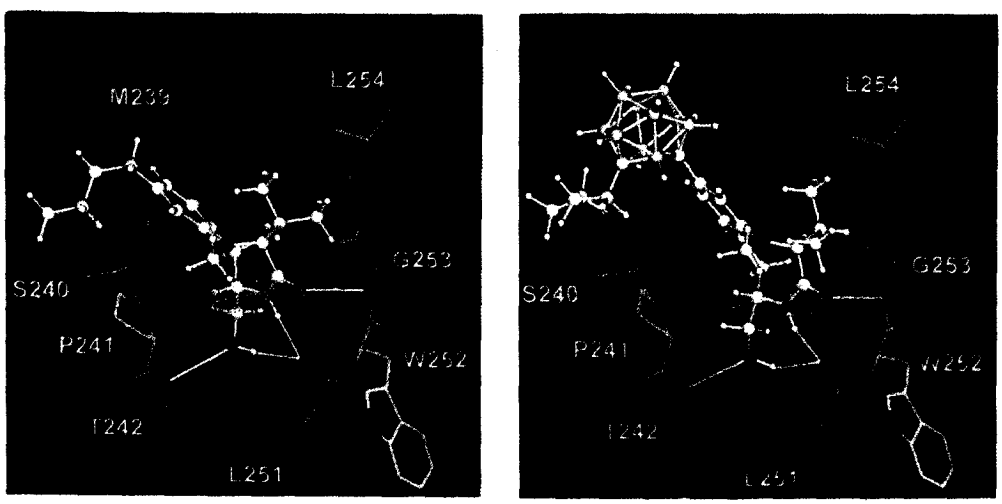


Figure. Drawings of the stable docking models of **1** (left), and **2b** (right) in the PKC cavity. The decyl group of **1** has been shortened for the sake of clarity. Intermolecular hydrogen bonds (distances less than 3.2 Å) are shown with yellow lines.

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References and Notes

1. Ashendel, C. L.; Staller, J. M.; Boutwell, R. K. *Cancer Res.* **1983**, *43*, 4333-4337: Fujiki, H.; Mori, M.; Nakayasu, M.; Terada, M.; Sugimura, T.; Moore, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3872-3876.
2. Endo, Y.; Ohno, M.; Hirano, M.; Itai, A.; Shudo, K. *J. Am. Chem. Soc.* **1996**, *118*, 1841-1855: Endo, Y.; Ohno, M.; Hirano, M.; Takeda, M.; Itai, A.; Shudo, K. *BioMed. Chem. Lett.* **1994**, *4*, 491-494: Ohno, M.; Endo, Y.; Hirano, M.; Itai, A.; Shudo, K. *Tetrahedron Lett.* **1993**, *34*, 8119-8122.
3. Endo Y.; Yamaguchi M.; Hirano M.; Shudo K. *Chem. Pharm. Bull.* **1996**, *44*, 1138-1140: Endo Y.; Ohno, M.; Takehana, S.; Driedger, P. E.; Stabel, S.; Shudo K. *Chem. Pharm. Bull.* **1997**, *45*, 424-426.
4. Endo, Y.; Takehana, S.; Ohno, M.; Driedger, P. E.; Stabel, S.; Mizutani, M. Y.; Tomioka, N.; Itai, A.; Shudo, K. *J. Med. Chem.* **1998**, *41*, 1476-1496.
5. For recent reviews see: Hawthorne, M. F. *Angew. Chem. Int. Ed. Engl.*, **1993**, *32*, 950-984: Soloway A. H.; Tjarks W.; Barnum B. A.; Rong F-G.; Barth R. F.; Codogni I. M.; Wilson J. G. *Chem. Rev.*, **1998**, *98*, 1515-1562.
6. Endo, Y.; Iijima, T.; Ohta, K.; Kagechika, H.; Kawachi, E.; Shudo, K. *Chem. Pharm. Bull.* **1999**, *47*, 585-587: Iijima, T.; Endo, Y.; Tsuji, M.; Kawachi, E.; Kagechika, H.; Shudo, K. *Chem. Pharm. Bull.* **1999**, *47*, 398-404.
7. Endo, Y.; Iijima, T.; Yamakoshi, Y.; Yamaguchi, M.; Fukasawa, H.; Shudo, K. *J. Med. Chem.* **1999**, *42*, 1501-1504.
8. Itai, A.; Matsuo, A.; Mizutani, M.Y.; Tomioka, N.; Endo, Y.; Shudo, K. *Chem. Pharm. Bull.* **1997**, *45*, 573-575.
9. Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. *Cell* **1995**, *81*, 917-924.
10. An attempt of deprotection of TBDMS group with fluoride anion was unsuccessful, affording possibly the *nido*-anion by attack of fluoride anion on the cage boron atoms. Fox, M. A.; MacBride, J. A. H.; Wade, K. *Polyhedron*, **1997**, *16*, 2499-2507.
11. Compound **2a**: colorless viscous liquid, ^{1}H-NMR ($CDCl_3$) 0.84 (d, 3H, J = 7 Hz, -CH_2CH_2 CH_2CH_3), 1.05 (d, 3H, J = 7 Hz, (CH_3)CH-), 1.5-3.3 (br, 10H, B-*H*), 2.47 (m, 1H, C*H*(CH_3)$_2$), 2.78 (d, 1H, J =17 Hz, Ar-CH_2CH-), 2.83 (s, 3H, N-CH_3), 3.13 (dd, 1H, J = 8, 17 Hz, Ar-CH_2CH-), 3.42 (d, 1H, J = 9Hz, CH_3-N-C*H*-CO), 3.55 (dd, 1H, J = 9, 10 Hz, CH_2OH), 3.72 (dd, 1H, J = 4, 10 Hz, CH_2OH), 3.87 (m, 1H, -C*H*CH_2OH), 3.91 (brs, 1H, cage C*H*), 6.28 (bs, 1H, -NHCO-), 6.91 (dd, 1H, J = 2, 8 Hz, Ar*H*), 6.98 (d, 1H, J = 8 Hz, Ar*H*), 7.07(d, 1H, J = 2 Hz, Ar*H*). HRMS: calcd for $C_{17}H_{32}{}^{10}B_2{}^{11}B_8N_2O_2$, 404.3467; found, 404.3461: Compound **2b**: colorless viscous liquid, ^{1}H-NMR ($CDCl_3$) 0.76 (d, 3H, J = 7 Hz, (CH_3)CH-), 0.80 (d, 3H, J = 6 Hz, -CH_2CH_2 CH_2CH_3), 1.05 (d, 3H, J = 6 Hz, (CH_3)CH-), 1.09 (sext, 2H, J = 7 Hz, -CH_2CH_2 CH_2CH_3), 1.36 (m, 2H, -CH_2CH_2 CH_2CH_3), 1.76 (m, 2H, -CH_2CH_2 CH_2CH_3), 1.4-3.1 (br, 10H, B-*H*), 2.45 (m, 1H, C*H*(CH_3)$_2$), 2.81 (d, 1H, J =17 Hz, Ar-CH_2CH-), 2.84 (s, 3H, N-CH_3), 3.16 (dd, 1H, J = 8, 17 Hz, Ar-CH_2CH-), 3.42 (d, 1H, J = 9Hz, CH_3-N-C*H*-CO), 3.56 (dd, 1H, J = 9, 11 Hz, CH_2OH), 3.72 (dd, 1H, J = 4, 11 Hz, CH_2OH), 3.82 (m, 1H, -C*H*CH_2OH), 6.60 (bs, 1H, -NHCO-), 7.04 (dd, 1H, J = 1, 8 Hz, Ar*H*), 7.08 (dd, 1H, J = 1, 8 Hz, Ar*H*), 7.17(d, 1H, J = 1 Hz, Ar*H*). HRMS: calcd for $C_{21}H_{40}{}^{10}B_2{}^{11}B_8N_2O_2$, 460.4093; found, 460.4104: Compound **2c**: colorless viscous liquid, ^{1}H-NMR ($CDCl_3$) 0.80 (d, 3H, J = 6 Hz, -CH_2CH_2 CH_2CH_3), 0.85 (d, 3H, J = 7 Hz, (CH_3)CH-), 1.05 (d, 3H, J = 6 Hz, (CH_3)CH-), 1.1-1.3 (m, 10H, -$CH_2CH_2(CH_2)_5H_3$), 1.36 (m, 2H, -$CH_2CH_2(CH_2)_5CH_3$), 1.75 (m, 2H, -$CH_2CH_2(CH_2)_5CH_3$), 1.4-3.1 (br, 10H, B-*H*), 2.45 (m, 1H, C*H*(CH_3)$_2$), 2.81 (d, 1H, J =17 Hz, Ar-CH_2CH-), 2.84 (s, 3H, N-CH_3), 3.15 (dd, 1H, J = 8, 17 Hz, Ar-CH_2CH-), 3.42 (d, 1H, J = 9Hz, CH_3-N-C*H*-CO), 3.56 (dd, 1H, J = 9, 11 Hz, CH_2OH), 3.71 (dd, 1H, J = 4, 11 Hz, CH_2OH), 3.81 (m, 1H, -C*H*CH_2OH), 6.84 (bs, 1H, -NHCO-), 7.03 (dd, 1H, J = 1, 8 Hz, Ar*H*), 7.08 (dd, 1H, J = 1, 8 Hz, Ar*H*), 7.17(d, 1H, J = 1 Hz, Ar*H*).HRMS: calcd for $C_{25}H_{48}{}^{10}B_2{}^{11}B_8N_2O_2$, 516.4719; found, 516.4712.
12. Fujiki, H.; Mori, M.; Nakayasu, M.; Terada, M.; Sugimura, T. *Biochem. Biophys. Res. Commun.* **1979**, *90*: 976-983; Huberman, E.; Callaham, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 1293-1297: Fujiki, H.; Suganuma, M.; Hakii, H.; Nakayasu, M.; Endo, Y.; Shudo, K.; Irie, K., Koshimizu, K.; Sugimura, T. *Proc. Japan. Acad. Ser. B.* **1985**, *61*, 45-47.
13. Driedger, P. E.; Stabel, S.; Frith, D.; Horgan, T. J. *Receptors and Channels* **1994**, *2*, 99-106.
14. Mizutani, M. Y.; Tomioka, N.; Itai, A. *J. Mol. Biol.* **1994**, *243*, 310-326.