Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 3015–3019

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Binding Selectivity of 7- and 15-Decylbenzolactone-V8 for the C1 Domains of Protein Kinase C Isozymes

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Received 12 March 2002; accepted 10 May 2003

Abstract—Benzolactone-V8 (**4**) is a lactone analogue of the artificial tumor promoter benzolactam-V8 (**1**). To investigate the effect of hydrophobic substituents at positions 7 and 15 of **4** on binding selectivity for protein kinase C (PKC) isozymes, 7- and 15-decylbenzolactone-V8 (**7**, **8**) were synthesized and their binding affinities for synthetic PKC isozyme C1 peptides were examined. Compound **8** showed moderate selectivity for novel PKC isozymes similar to 9-decylbenzolactone-V8 (**5**), while **7** was less selective. Compounds **7** and **8** showed no significant selectivity among novel PKC isozymes unlike 8-decylbenzolactone-V8 (**6**). These results indicate that the introduction of a hydrophobic substituent at position 8 of **4** is most effective in the development of PKCε- and PKCη-selective binders.

Protein kinase C (PKC) isozymes are serine/threonine-specific protein kinases involved in cellular signal transduction.[1] PKC isozymes are subdivided into three groups; conventional PKCs (α, βI, βII, γ), novel PKCs (δ, ε, η, θ), and atypical PKCs (ζ, ι/λ) (Fig. 1).[2,3] Conventional and novel PKCs are also major receptors of tumor promoters and thus serve as therapeutic targets in cancer. Tumor promoters such as 12-*O*-tetradecanoylphorbol 13-acetate (TPA),[4] teleocidin B-4,[5] and aplysiatoxin[5] activate these PKC isozymes by binding to two C1 domains (C1A, C1B) in the regulatory region, which are also the binding sites of the endogenous second messenger 1,2-*sn*-diacylglycerol and antineoplastic bryostatin 1.[6] Recent studies revealed that novel PKC isozymes (δ, ε, η) are involved in tumor promotion,[7–10] and that the role of each C1 domain is different among PKC isozymes in response to tumor promoters.[11–14] The design of agents with not only PKC isozyme-selectivity but also C1 domain-selectivity is thus indispensable to understand the precise mechanism of tumor promotion and to develop anticancer drugs.

The artificial tumor promoter benzolactam-V8 (**1**)[15] is a promising lead compound for PKC isozyme- and C1 domain-selective agents since it has a simple structure, and since its binding affinity for PKC isozymes can be easily enhanced by the introduction of hydrophobic side chains (Fig. 2). It is reported that the position of the hydrophobic chain of benzolactam-V8s might affect the selectivity of binding to PKC isozymes.[16,17] The binding affinities of 8-decylbenzolactam-V8 (**2**) were slightly higher for conventional PKCs than novel PKCs, while 7-decylbenzolactam-V8 (**3**) showed moderate selectivity for novel PKCs rather than conventional PKCs. Such substituent effect was more significant in benzolactone-V8 (**4**), the lactone analogue of **1**.[18] We have recently synthesized 8- and 9-decylbenzolactone-V8s (**5**, **6**) and evaluated the selectivity of their binding to C1 domains of PKC isozymes using synthetic PKC C1 peptides.[19] The binding affinities of 9-decylbenzolactone-V8 (**5**) were considerably higher for the C1B peptides of novel PKCs than the other PKC C1 peptides. The selectivity was especially improved in **6**, which showed significant selective binding of PKCε and η. These results suggest that benzolactone-V8s with hydrophobic substituents at positions other than 8 and 9 might be candidates for new agents with PKC isozyme- and C1 domain-selectivity.

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doi:10.1016/S0960-894X(03)00637-1

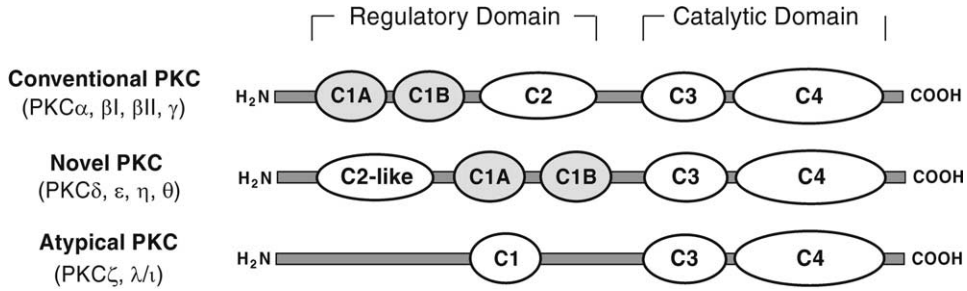


Figure 1. Structures of PKC isozymes.

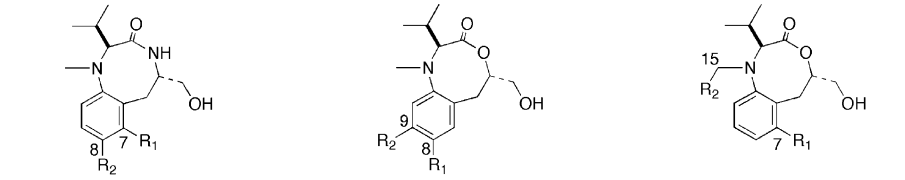
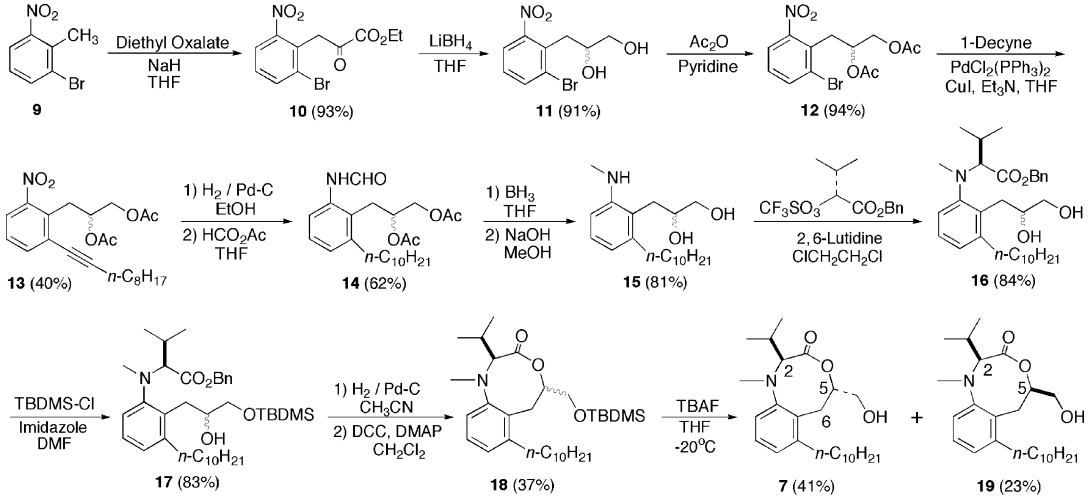


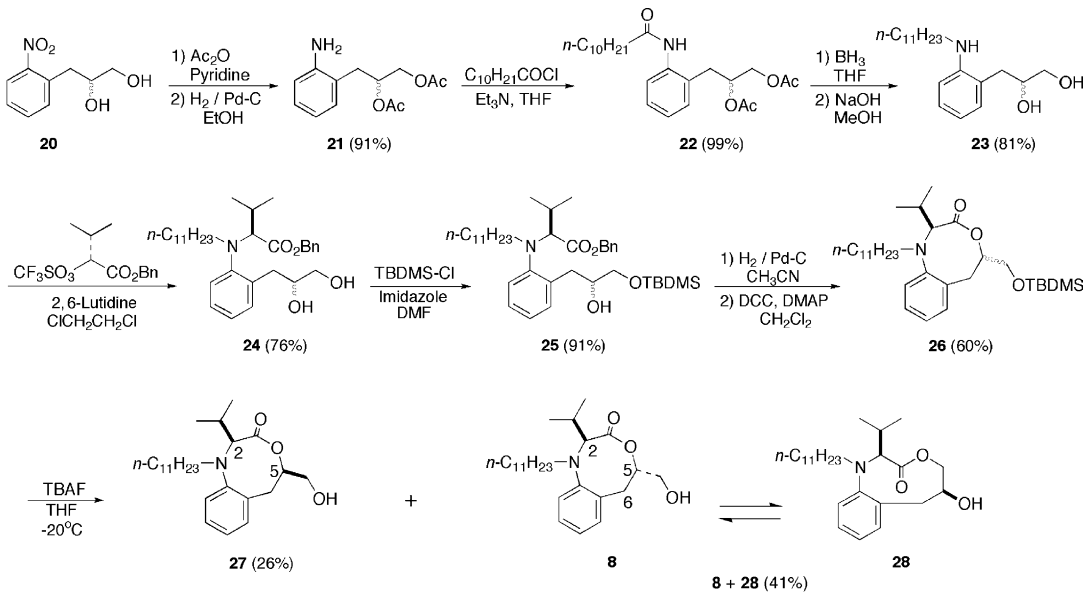
Figure 2. Structures of benzolactam-V8s and benzolactone-V8s.

We report here the synthesis of 7- and 15-decylbenzolactone-V8 (**7** and **8**) and their binding affinities for the C1 peptides of all PKC isozymes.

7-Decylbenzolactone-V8 (**7**) was synthesized from 2-bromo-6-nitrotoluene (**9**) as shown in Scheme 1. S_N2 substitution of **9** with diethyl oxalate gave the ethyl pyruvate derivative **10** (93%). After reduction of both the ketone and the ester groups of **10** (91%), the resulting two hydroxyl groups were protected with acetyl groups (94%). Modified Sonogashira coupling[20] of **12** with 1-decyne gave **13** in a slightly lower yield (40%) because of steric hindrance with the *ortho* substituent. Hydrogenation of **13** followed by *N*-formylation gave **14** (62%), the formyl group of which was reduced with borane, followed by alkaline hydrolysis to yield **15** (81%). The valine subunit was introduced by substitution of **15** with D-valine-derived triflate[21] to give two diastereomeric esters **16** (84%). The primary hydroxyl group of **16** was selectively protected with a TBDMS group at 0 °C (83%). Hydrogenolysis of the benzyl group of **17**, followed by intramolecular esterification with 1,3-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP), gave diastereomeric lactones **18** (37%). To avoid intramolecular transesterification observed in the synthesis of benzolactone-V8 (**4**),[18] deprotection of the TBDMS group of **18** was carried out with tetrabutylammonium fluoride (TBAF) at −20 °C. The two diastereomers were easily separated by column chromatography to give **7** (41%)[22] and its C-5 epimer **19** (23%),[23] which were identified according to the NOESY spectra; significant NOE enhancement between the H-2 (δ 3.22) and H-5 (δ 4.51) protons was observed in **19** but not in **7**.



Scheme 1. Synthesis of 7-decylbenzolactone-B8 (**7**).



Scheme 2. Synthesis of 15-decylbenzolactone-V8 (**8**).

Synthesis of **8** is shown in Scheme 2. The starting material **20** was prepared as reported previously.[18] After the diacetylation of **20**, the nitro group was reduced by hydrogenation to give **21** (91%). Undecanoylation of **21** (99%) and reduction of the amide group with borane gave the undecyl aniline derivative, two acetyl groups of which were deprotected by alkaline hydrolysis (81%). S_N2 substitution of **23** with D-valine-derived triflate (76%), followed by selective TBDMS protection of the primary hydroxyl group, gave **25** (91%). After removal of the benzyl group of **25**, intramolecular esterification easily proceeded (60%) since the carboxyl group could access the diol moiety due to steric repulsion between the undecyl group and the TBDMS group. Deprotection of the TBDMS group of **26** with TBAF at −20 °C gave three products **27**,[24] **8**,[25] and **28**[26] (26% for **27**, 41% for **8** and **28**). ^{1}H NMR and NOESY spectra showed that **27** was *epi*-15-decylbenzolactone-V8 with the *R* configuration at position 5 because significant NOE enhancement between the H-2 (δ 3.26) and H-5 (δ 4.72) protons was observed. Unexpectedly, **8** and **28** interconverted easily at room temperature. The ratio of **8** to **28** was 1:3 in deuterioacetonitrile. The free hydroxylmethylene signal (δ 3.59) was observed in **8**, indicating that **8** was 15-decylbenzolactone-V8. On the other hand, the hydroxymethylene signals of **28** were shifted downfield (δ 3.85, 4.40). These results indicated that **28** was a nine-membered lactone deduced to be formed by intramolecular transesterification. Since the nine-membered lactone adopted a quite different conformation from

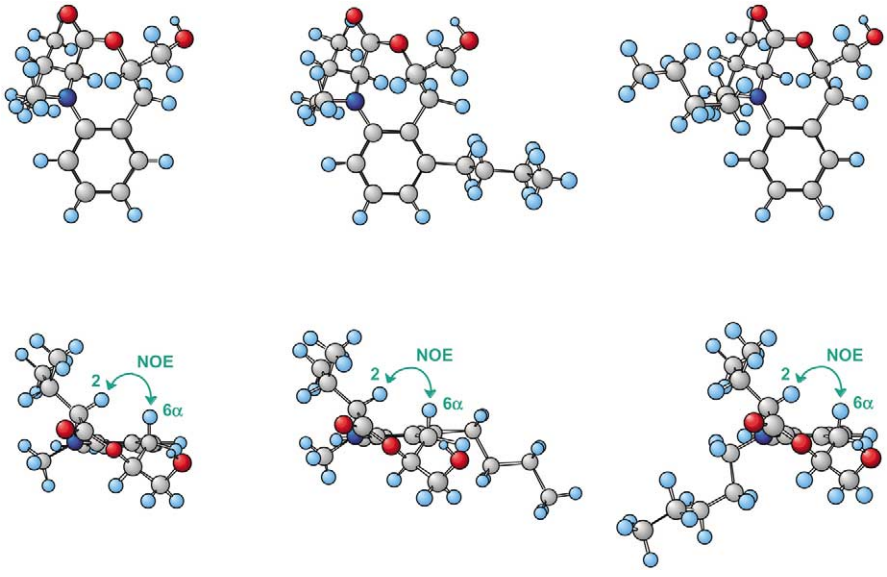


Figure 3. Stable conformations of benzolactone-V8 (**4**, left), 7-decylbenzolactone-V8 (**7**, center) and 15-decylbenzolactone-V8 (**8**, right). The decyl groups of **7** and **8** were displayed as butyl groups for convenience. The initial structures were determined by MM2 calculation on the condition that the distance between H-2 and H6α was fixed to 2 Å followed by PM3 optimization. Further optimization was carried out by Hartree-Fock calculation with 6-31G* basis set.

Table 1. K_i values for inhibition of the specific binding of [^{3}H]PDBu by 7-decylbenzolactone-V8 (**7**), 15-decylbenzolactam-V8 (**8**), 9-decylbenzolactone-V8 (**5**) and 8-decylbenzolactone-V8 (**6**)

PKC C1 peptide	K_i (nM)				K_d (nM)
	7-Decylbenzolactone-V8 (**7**)	15-Decylbenzolactone-V8 (**8**)	9-Decylbenzolactone-V8 (**5**)[a]	8-Decylbenzolactone-V8 (**6**)[a]	PDBu
Conventional PKC					
α-C1A (72-mer)[b]	870 (340)[c]	4580 (982)	412 (6)	>10,000	1.12 (0.04)
α-C1B	>10,000	>10,000	4050 (153)	>10,000	7.44 (0.27)
β-C1A (72-mer)	539 (57)	4690 (390)	1140 (216)	>10,000	1.31 (0.26)
β-C1B	2690 (330)	2070 (157)	610 (24)	22,000 (1900)	1.34 (0.41)
γ-C1A	970 (45)	5750 (198)	2360 (187)	6320 (536)	1.50 (0.57)
γ-C1B	1720 (320)	2820 (146)	675 (23)	17,600 (1650)	1.19 (0.29)
Novel PKC					
δ-C1A	>10,000	30,300 (1480)	6150 (304)	70,600 (3320)	51.9 (15.6)
δ-C1B	92 (8)	415 (49)	149 (1)	1160 (46)	0.53 (0.14)
ε-C1A	7880 (1330)	5360 (626)	4830 (112)	>10,000	5.60 (0.62)
ε-C1B	540 (70)	411 (31)	83 (4)	262 (4)	0.81 (0.03)
η-C1A	4250 (780)	6330 (2580)	1590 (95)	>10,000	4.30 (0.18)
η-C1B	111 (5)	189 (5)	37 (1)	120 (7)	0.45 (0.12)
θ-C1A	NT[d]	NT	NT	NT	>200
θ-C1B	167 (14)	358 (33)	62 (5)	1100 (53)	0.72 (0.14)

[a]These data are cited from ref 19.
[b]Ten residues from both N and C-termini of the previous α-C1A and β-C1A were elongated as the solubility of the original 52-mer peptides was extremely low.
[c]Standard deviation of at least two separate experiments.
[d]Not tested. The K_d value of [^{3}H]PDBu for θ-C1A could not be measured because of a very weak binding affinity.

those of eight-membered lactones, and since the 10-decyl derivative of the nine-membered lactone did not bind to C1 peptides of novel PKC isozymes at all (unpublished data), **28** would lack the ability to bind to PKC isozymes. Therefore, the binding selectivity of **8** for the C1 peptides of PKC isozymes would be estimated without separation of **8** from **28** though the precise binding constants of **8** for each PKC C1 peptides cannot be determined.

Conformational analyses of **7** and **8** were performed using molecular mechanics and quantum mechanics calculations. Since significant NOE enhancement between the H-2 (δ 3.40 for **7**, δ 3.32 for **8**) and H-6α (δ 2.97 for **7**, δ 2.96 for **8**) protons was observed in both compounds, the initial structures were determined by MM2 calculation on the condition that the distance between H-2 and H-6α was fixed to 2 Å followed by PM3 optimization. Further optimization was carried out by Hartree–Fock calculation with 6-31G* basis set. The calculated structures of **7** and **8** along with **4**[18] are shown in Figure 3. The ring conformation of both compounds was quite similar to that of **4**. These results suggest that introduction of the decyl group at position 7 or 15 does not influence the lactone ring conformation though the decyl group at position 15 of **8** lowered the relative thermochemical stability of the eight-membered lactone ring.

Binding affinities of **7** and **8** for PKC isozymes were evaluated by inhibition of the specific binding of [^{3}H]phorbol 12,13-dibutyrate (PDBu) to the synthetic C1 peptides of all PKC isozymes as reported previously.[27–29] PKC C1 peptides consisting of about 50 amino acids are PKC C1 domain surrogates, which exhibit PDBu binding affinities comparable to the whole PKC isozymes.[28] Using the PKC C1 peptides, the concentration required to cause 50% inhibition of the [^{3}H]PDBu binding (IC_{50}) was measured. The binding affinities of **7** and **8** for each PKC C1 peptide were expressed as K_i values calculated from the IC_{50} and the K_d value of [^{3}H]PDBu as reported by Sharkey and Blumberg.[29] Table 1 summarizes the K_i values of **7** and **8** along with those of 9- and 8-decylbenzolactone-V8 (**5**, **6**).[19]

The binding affinities of 15-decylbenzolactone-V8 (**8**) were more than 10-fold higher for the C1B peptides of novel PKCs (δ, ε, η, θ) than for the other PKC C1 peptides. This selectivity of **8** was similar to that of 9-decylbenzolactone-V8 (**5**) though the binding affinities of **8** were 10-fold weaker than those of **5**. In contrast, the selectivity of 7-decylbenzolactone-V8 (**7**) for the C1B peptides of novel PKCs was relatively low because of strong binding to the C1A peptides of conventional PKCs. This result indicates that the decyl group at position 7 could effectively interact with the C1A peptides of conventional PKCs. On the other hand, the decyl group at position 8 might evoke steric hindrance in binding with the C1 peptides of conventional PKCs since 8-decylbenzolactone-V8 (**6**) exhibited poor binding affinity for these C1 peptides. Moreover, significant binding selectivity for the C1B peptides of PKCε and η was also observed in **6** although **7** and **8** along with **5** showed little selectivity among novel PKCs. Since the ring conformation of four decylbenzolactone-V8s (**5**, **6**, **7**, **8**) was quite similar to each other, these results indicate that the position of the hydrophobic substituent in the benzolactone-V8 skeleton plays a critical role in the binding selectivity for PKC isozymes.

In summary, we have synthesized 7- and 15-decylbenzolactone-V8 (**7**, **8**) to investigate the effect of the hydro-

phobic substituent at positions 7 and 15 of benzolactone-V8 (**4**) on the binding selectivity for PKC isozymes. Although **7** and **8** along with 9-decylbezolactone-V8 (**5**)[16] bound more strongly to the C1B domains of novel PKCs (δ, ε, η, θ) than the other PKC C1 domains, significant selectivity among novel PKCs was not observed unlike 8-decylbenzolactone-V8 (**6**),[18,19] which shows high selectivity for PKCε and PKCη. The present results indicate that the introduction of a hydrophobic substituent at position 8 of **4** is most effective in increasing the PKC isozyme- and C1 domain-selectivity and to develop PKCε- and PKCη-selective binders, which might be useful for analyzing the mechanism of tumor promotion.

Acknowledgements

This work was partly supported by a Grant-in-Aid for Scientific Research on Priority Areas (2) (No. 13024245) (for K.I.) and Scientific Research (A) (2) (No. 15208012) (for H.O.) from the Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government, and from the Japan Society for the Promotion of Science for Young Scientists (Y.N.).

References and Notes

1. Nishizuka, Y. *FASEB. J.* **1995**, *9*, 484.
2. Ron, D.; Kazanietz, M. G. *FASEB. J.* **1999**, *13*, 1658.
3. Newton, A. C. *Chem. Rev.* **2001**, *101*, 2353.
4. Hoppe, W.; Brandel, F.; Strell, I.; Röhrl, M.; Grassmann, J.; Hecker, E.; Bartsch, H.; Kreibich, G.; Szczepanski, C. *Angew. Chem.* **1967**, *79*, 824.
5. Fujiki, H.; Sugimura, T. *Adv. Cancer Res.* **1987**, *49*, 223.
6. Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L. *J. Am. Chem. Soc.* **1982**, *104*, 6846.
7. Lu, Z.; Hornia, A.; Jiang, Y.-W.; Zang, Q.; Ohno, S.; Foster, D. A. *Mol. Cell. Biol.* **1997**, *17*, 3418.
8. Reddig, P. J.; Dreckschimdt, N. E.; Ahrens, H.; Simsiman, R.; Tseng, C.-P.; Zou, J.; Oberley, T. D.; Verma, A. K. *Cancer Res.* **1999**, *59*, 5710.
9. Chida, K.; Hara, T.; Hirai, T.; Konishi, C.; Nakamura, K.; Nakao, K.; Katsumi, M.; Kuroki, T. *SEIKAGAKU* **2000**, *72*, 674.
10. Reddig, P. J.; Dreckschmidt, N. E.; Bourguignon, S. E.; Oberley, T. D.; Verma, A. K. *Cancer Res.* **2000**, *60*, 595.
11. Bögi, K.; Lorenzo, P. S.; Acs, P.; Szallasi, Z.; Wagner, G. S.; Blumberg, P. M. *FEBS Lett.* **1999**, *456*, 27.
12. Hunn, M.; Quest, A. F. G. *FEBS Lett.* **1997**, *400*, 226.
13. Szallasi, Z.; Bögi, K.; Gohari, S.; Biro, T.; Acs, P.; Blumberg, P. M. *J. Biol. Chem.* **1996**, *271*, 18299.
14. Irie, K.; Nakahara, A.; Nakagawa, Y.; Ohigashi, H.; Shindo, M.; Fukuda, H.; Konishi, H.; Kikkawa, U.; Kashiwagi, K.; Saito, N. *Pharmacol. Ther.* **2002**, *93*, 271.
15. Endo, Y.; Ohno, M.; Hirano, M.; Itai, A.; Shudo, K. *J. Am. Chem. Soc.* **1996**, *118*, 1841.
16. Kozikowski, A. P.; Wang, S.; Ma, D.; Yao, J.; Ahmad, S.; Glazer, R. I.; Bögi, K.; Acs, P.; Modarres, S.; Lewin, N. E.; Blumberg, P. M. *J. Med. Chem.* **1997**, *40*, 1316.
17. Ma, D.; Tang, G.; Kozikowski, A. P. *Org. Lett.* **2002**, *4*, 2377.
18. Nakagawa, Y.; Irie, K.; Nakamura, Y.; Ohigashi, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 723.
19. Nakagawa, Y.; Irie, K.; Masuda, A.; Ohigashi, H. *Tetrahedron* **2002**, *58*, 2101.
20. Thorand, S.; Krause, N. *J. Org. Chem.* **1998**, *63*, 8551.
21. Kogan, T. P.; Somers, T. C.; Venuti, M. C. *Tetrahedron* **1990**, *46*, 6623.
22. 7-Decylbenzolactone-V8 (**7**): $[\alpha]_D$ −88.0° (*c* 0.45, MeOH, 29.7 °C); UV λ_{max} (MeOH) nm (ε) 255 (5100), 212 (15,600); ^{1}H NMR (500 MHz, $CDCl_3$, 0.079 M, 27 °C) δ 0.88 (3H, t, *J* = 7.0 Hz), 0.96 (3H, d, *J* = 6.6 Hz), 1.02 (3H, d, *J* = 6.5 Hz), 1.28–1.36 (14H, m), 1.53 (2H, m), 2.27 (1H, m), 2.34 (1H, br.s), 2.57 (2H, m), 2.83 (3H, s), 2.97 (1H, dd, *J* = 17.2, 6.5 Hz), 3.12 (1H, dd, *J* = 17.1, 2.7 Hz), 3.40 (1H, d, *J* = 9.6 Hz), 3.72 (2H, m), 4.80 (1H, m), 6.90 (1H, d, *J* = 7.5 Hz), 6.95 (1H, d, *J* = 7.7 Hz), 7.12 (1H, t, *J* = 7.7 Hz); HR-FABMS *m*/*z* 404.3159 (MH^+ calcd for $C_{25}H_{42}NO_3$, 404.3165).
23. *epi*-7-Decylbenzolactone-V8 (**19**): $[\alpha]_D$ −121.0° (*c* 0.79, MeOH, 29.7 °C); UV λ_{max} (MeOH) nm (ε) 256 (4900), 213 (14,800); ^{1}H NMR (500 MHz, $CDCl_3$, 0.079 M, 27 °C) δ 0.87 (3H, t, *J* = 6.5 Hz), 0.89 (3H, d, *J* = 6.9 Hz), 1.03 (3H, d, *J* = 6.6 Hz), 1.27–1.39 (14H, m), 1.50 (2H, m), 2.43 (2H, m), 2.57 (2H, m), 2.77 (1H, dd, *J* = 15.9, 5.5 Hz), 2.89 (3H, s), 3.00 (1H, dd, *J* = 15.9, 1.8 Hz), 3.22 (1H, d, *J* = 10.5 Hz), 3.83 (1H, dd, *J* = 11.7, 9.0 Hz), 3.89 (1H, dd, *J* = 11.7, 7.3 Hz), 4.51 (1H, m), 6.94 (1H, d, *J* = 7.3 Hz), 7.05 (1H, d, *J* = 7.7 Hz), 7.12 (1H, t, *J* = 7.7 Hz); HR-FABMS *m*/*z* 404.3161 (MH^+ calcd for $C_{25}H_{42}NO_3$, 404.3165).
24. *epi*-15-Decylbenzolactone-V8 (**27**): $[\alpha]_D$ −59.0° (*c* 0.51, EtOH, 22.1 °C); UV λ_{max} (EtOH) nm (ε) 260 (2700), 206 (11,700); ^{1}H NMR (500 MHz, $CDCl_3$, 0.049 M, 27 °C) δ 0.86 (3H, d, *J* = 6.5 Hz), 0.87 (3H, t, *J* = 7.1 Hz), 1.10 (3H, d, *J* = 6.5 Hz), 1.19–1.32 (18H, m), 2.07 (1H, br.s), 2.21 (1H, m), 2.87 (2H, m), 3.22 (2H, m), 3.26 (1H, d, *J* = 10.6 Hz), 3.73 (2H, m), 4.72 (1H, m), 7.10 (2H, m), 7.22 (2H, m); HR-FABMS *m*/*z* 404.3164 (MH^+ calcd for $C_{25}H_{42}NO_3$, 404.3165).
25. 15-Decylbenzolactone-V8 (**8**): $[\alpha]_D$ −58.0° (*c* 0.79, EtOH, 22.1 °C); UV λ_{max} (EtOH) nm (ε) 260 (2500), 205 (11,100); ^{1}H NMR (500 MHz, CD_3CN, 0.071 M, 27 °C) δ 0.87 (3H, t, *J* = 6.9 Hz), 0.92 (3H, d, *J* = 6.5 Hz), 1.08 (3H, d, *J* = 6.7 Hz), 1.15–1.29 (18H, m), 2.07 (1H, m), 2.96 (4H, m), 3.04 (1H, br.t, *J* = 6.2 Hz), 3.32 (1H, d, *J* = 10.0 Hz), 3.59 (2H, m), 4.85 (1H, m), 7.09–7.25 (4H, m); HR-FABMS *m*/*z* 404.3161 (MH^+ calcd for $C_{25}H_{42}NO_3$, 404.3165).
26. Nine-membered benzolactone (**28**): ^{1}H NMR (500 MHz, CD_3CN, 0.071 M, 27 °C) δ 0.87 (3H, t, *J* = 7.0 Hz), 0.98 (3H, d, *J* = 6.6 Hz), 1.08 (3H, d, *J* = 6.7 Hz), 1.15–1.30 (18H, m), 2.28 (1H, m), 2.65 (1H, dd, *J* = 13.1, 2.7 Hz), 2.88 (1H, m), 2.96 (2H, m), 3.12 (1H, d, *J* = 9.3 Hz), 3.40 (1H, br.d, *J* = 5.2 Hz), 3.85 (1H, dd, *J* = 11.1, 5.4 Hz), 4.06 (1H, m), 4.40 (1H, br.s), 7.12–7.24 (4H, m).
27. Irie, K.; Oie, K.; Nakahara, A.; Yanai, Y.; Ohigashi, H.; Wender, P. A.; Fukuda, H.; Konishi, H.; Kikkawa, U. *J. Am. Chem. Soc.* **1998**, *120*, 9159.
28. Shindo, M.; Irie, K.; Nakahara, A.; Ohigashi, H.; Konishi, H.; Kikkawa, U.; Fukuda, H.; Fukuda, H.; Wender, P. A. *Bioorg. Med. Chem.* **2001**, *9*, 2073.
29. Sharkey, N. A.; Blumberg, P. M. *Cancer Res.* **1985**, *45*, 19.