Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 5, pp. 497-500, 1995

0960-894X/95 $9.50+0.00

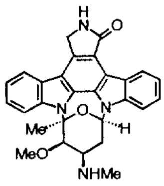
0960-894X(95)00060-7

PROTEIN KINASE C-α INHIBITORS; STRUCTURE-ACTIVITY RELATIONSHIPS IN BIS-INDOLE SERIES

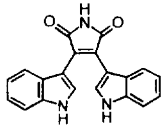
Guojian Xie,[1] Jürg Zimmermann,[2] Thomas Meyer[2] and J. William Lown*[1]
[1]*Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2*
[2]*Pharmaceuticals Division, Ciba-Geigy Ltd., CH-4002 Basel, Switzerland*

Abstract: To assess the role of the amine and ester containing oligopeptide side-chains in bis-indole protein kinase C inhibitors, six novel hybrids in this series were synthesized. These new compounds displayed effective inhibitory activity against protein kinase C-α.

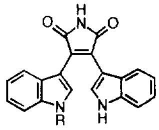
Protein kinase C (PKC) is a member of the serine/threonine specific protein kinase family[1] which is implicated in the pathogenesis of a variety of diseases.[2] Over the past decade, regulation of this family of enzymes has generated considerable interest. One of the most informative approaches to study the role played by PKC in cellular processes is to inhibit the enzymatic activity of the kinase in intact cells. For this purpose, cell-permeable, potent and selective compounds are required. Among a variety of agents[3,13] which inhibit PKC and other protein kinases, the naturally occuring glycosylated indolocarbazole staurosporine (**1**)[4] is the most potent, and has been modified[5] in attempts to control kinase selectivity and toxicity. The structurally simpler Arcyriarubins[6] (**2**: Arcyriarubin A) also inhibit PKC.[7] Recent investigations by Toullec,[8a] Davis[8b] and co-workers have revealed that derivatives of this class, such as compound **3** in which a dimethylamine containing side chain is attached to the indole nitrogen, are not only highly potent but also quite selective inhibitors of PKC.



1

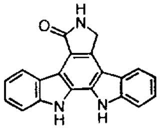


2

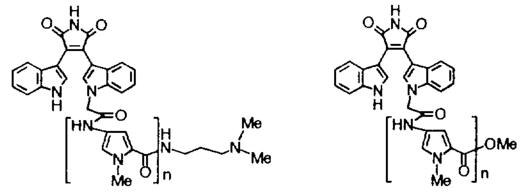


R = $(CH_2)_3NMe_2$

3

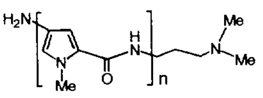


4

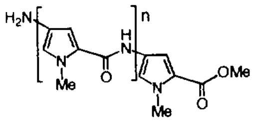


5 n = 1
6 n = 2
7 n = 3

8 n = 1
9 n = 2
10 n = 3



11 n = 1
12 n = 2
13 n = 3



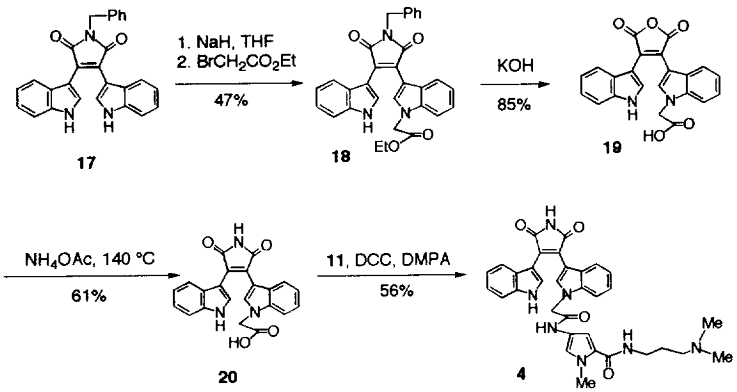
14 n = 0
15 n = 1
16 n = 2

In the course of structure-activity studies on PKC inhibitors in this series, we wished to determine if amine or ester containing polypyrrolamide side-chains contribute to biological activity. We report here the synthesis and activity of the compounds **5-10**, in which one of the hydrogen atoms attached to the nitrogen atom of the indole ring is substituted by such oligopeptide chains. Within this group of target molecules, compounds **5-7** contain a dimethylamine as a peptide terminal, while, for comparison, compounds **8-10** contain a methyl ester as a terminal group.

Chemistry: We recently established a very efficient procedure to the staurosporine aglycone **4**,[9] in which compound **17** was conveniently prepared in two steps from the easily available dibromomaleic acid. Here, we took **17** as the starting material and treated it with sodium hydride, followed by addition of ethyl bromoacetate, to obtain **18** in a moderate yield. Since the deprotection of the N-benzyl group has not proved to be possible,[9,10] we utilized an indirect method.[9] Thus, treatment of **18** with strong base, 5N potassium hydroxide, readily afforded the anhydride **19** in high yield, and meanwhile, the ester group of **18** was also converted to the desired carboxylic acid function. Subsequently, conversion of the anhydride to the imide was achieved by heating with ammonium acetate overnight, .yielding the key intermediate **20** in good yield. Finally, **20** was coupled with the amine containing oligopeptide **11**, prepared from N-methyl pyrrole according to the method previously developed in this laboratory,[11] in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 4-

dimethylaminopyridine (DMAP) to give the target compound **5**[12] in a reasonable yield. Similarly, coupling of **20** with other oligopeptides **12-16**, afforded the products **6-10** in moderate to good yields.

Scheme I



In Vitro Biological Evaluation: These compounds were evaluated for their inhibition of PKC-α.[14] The results are summarized in **Table 1**. All of the compounds proved to be effective inhibitors of PKC, although none of them was as active as staurosporine 1. In addition, the methyl ester containing compounds **8-10** proved to be more potent than the dimethylamine containing compounds **5-7**. In comparison with the structural simpler derivative **3** (IC_{50} = 0.01 μM), our compounds showed less PKC

Table 1. Inhibition of PKC-α[a]

Compounds	IC_{50} (μM)
Staurosporine 1	0.004
5	1.00
6	0.63
7	0.46
8	0.50
9	0.28
	0.17

[a] The PKC-α was purified and assayed as described in the literature.[15]

inhibitory effects, which suggests that the structurally more complex oligopeptide substituents do not enhance PKC inhibitory activity in this series of compounds.

Further studies on the effects of these compounds on cellular topoisomerases and development of even more potent inhibitors are ongoing.

Acknowledgement: The authors would like to thank the National Cancer Institute of Canada and Department of Chemistry, University of Alberta for support of this research.

References and notes:

1. Nishizuka, Y. *Nature* **1984**, 308, 693.
2. Bradshaw, D.; Hill, C. H.; Nixon, J.. S.; Wilkinson, S. E. *Agents and Actions* **1993**, 38, 135.
3. Hidaka, H.; Hagiwara, M. *Trends Pharmacol. Sci.* **1987**, 8, 162.
4. Furusaki, A.; Hashiba, N.; Matsumoto, T.; Hirano, A.; Iwai, Y.; Omura, S. *Bull. Chem. Soc. Jpn.* **1982**, 55, 3681.
5. Nakanishi, S.; Yamada, K.; Iwahashi, K.; Kuroda, K.; Kase, H. *Molecular Pharmacology.* **1990**, 37, 482.
6. Steglich, W.; Steffan, B.; Kopanski, L.; Eckhardt, G. *Angew. Chem. Int. Ed. Eng.* **1980**, 19, 459.
7. Gribble, G. W.; Berthel, S. J. *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: New York, **1993**; Vol. 12, pp. 365.
8. (a) Toullec, D.; Pianetti, P.; Coste, H.; Bellevergue, P.; Grand-Perret, T.; Ajakne, M.; Baudet, V.; Boissin, P.; Boursier, E.; Loriolle, F.; Duhamel, L.; Charon, D.; Kirilovsky, J. *J. Biol. Chem.* **1991**, 266, 15771. (b) Davis, P. D.; Hallam, T. J.; Harris, W.; Hill, C. H.; Lawton, G.; Nixon, J, S.; Smith, J. S.; Vesey, D. R.; Wilkinson S. E. *Bioorg. Med. Chem Lett.* **1994**, 4, 1303.
9. Xie, G.; Lown, J. W. *Tetrahedron Lett.* **1994**, 35, 5555.
10. Hughes, I.; Nolan, W. P.; Raphael, R. A. *J. Chem. Soc. Perkin Trans I* **1990,** 2475.
11. (a) Xie, G.; Morgan, A. R.; Lown, J. W. *Bioorg. Med.Chem Lett.* **1993**, 13, 1565. (b) Lown, J. W.; Krowicki, K. *J. Org. Chem.* **1985**, 50, 3774.
12. Spectroscopic data for **5**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.5 (s, 1H),10.90 (s, 1H), 10.30 (s, 1H), 8.08 (t, J = 6.0 Hz, 1H), 7.90 (s, 1H), 7.68 (d, J = 2.0 Hz, 1H), 7.37 (s, 1H), 7.35 (s, 1H), 7.10 (d, J = 2.0 Hz, 1H), 6.89-7.04 (m, 3H), 6.55-6.70 (m, 4H), 5.08 (s, 2H), 3.75 (s, 3H), 3.15 (q, J = 7.5 Hz, 2H), 2.23 (t, J = 7.5 Hz, 2H), 2.18 (s, 6H), 1.60 (m, 2H); HRMS (FAB): Calcd. for $C_{33}H_{34}N_7O_4$ 592.2672. Found 592.2652 (M+H).
13. Bishop, W. R.; Petrin, J.; Wang, L.; Ramesh, U.; Doll, R. J. *Biochem. Pharmacol.* **1990**, 40, 2129.
14. Diwu, Z.; Zimmermann, J.; Meyer, T.; Lown, J. W. *Biochem. Pharmacol.* **1994**, 47, 373.
15. Marte, B. M; Meyer, T.; Stabel, S.; Standke, G. J. R.; Jacken, S.; Fabbro, D.; Hynes, N. E. *Cell Growth and Differentiation* **1994**, 5, 239.

(*Received in USA* 12 *December* 1994; *accepted* 23 *January* 1995)