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NOVEL SUBSTITUTED INDOLOCARBAZOLES AS POTENT AND SELECTIVE INHIBITORS OF PROTEIN KINASE C

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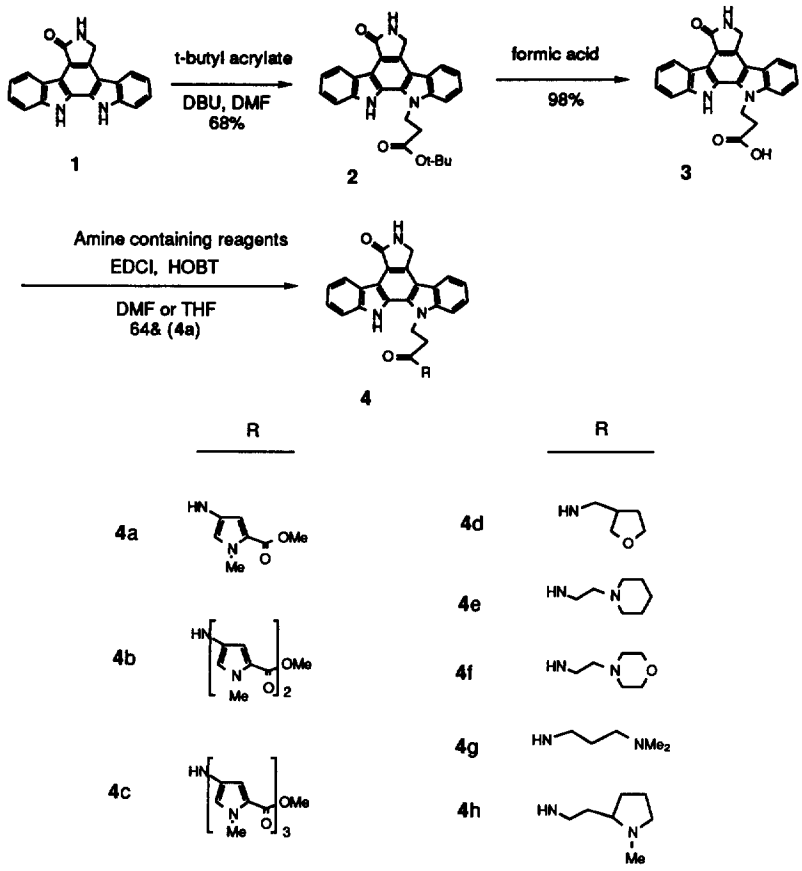
Abstract: Novel oligopeptide and amino alkyl substitued indolocarbazoles were synthesized and characterized with respect to inhibition of protein kinases C and A. In both series potent and selective PKC inhibitors could be identified. Structure activity relationships are discussed.

Protein kinase C (PKC),[1] a key enzyme in transmembrane signal transduction, has been implicated in a variety of diseases such as cancer or psoriasis,[2] as well as an array of cellular functions and inflammation. Thus, inhibitors of this enzyme might be useful for therapeutic application.[3] In the past decade, a large number of widely diverse compounds have been found to inhibit PKC activity. Among these agents, staurosporine, the microbial alkaloid, has been identified to be the most potent inhibitor.[4] Nevertheless, this natural product is poorly selective against other protein kinases. As a consequence, the therapeutic use of this compound is limited. In order to obtain specific PKC inhibitors, compounds structurally related to staurosporine were isolated from microbial origin, e.g. K-252a[5] and UCN-01,[6] or were synthesized by modification of the natural product, e.g. Go-6976[7] and Ro-32-0432.[8] This approach resulted in the discovery of PKC inhibitors with improved selectivity as compared to staurosporine.

The approach towards the development of more selective inhibitors has been aimed at the identification of substructures of staurosporine, and substitution at one of the indole nitrogen atoms by an amino alkyl side chain appears to mimic the amino alkyl function present in the sugar moiety of staurosporine. Therefore, it has been postulated that this side chain by providing a cationic binding site, is essential for inhibitory activity.[9] In our structure-activity studies on PKC inhibitors in this series, we have found that the indolocarbazoles containing oligopeptides or amino alkyl as side chains, with an amide function as a linker, can also be potent and selective PKC inhibitors.

Chemistry: Staurosporine aglycone **1** was synthesized by a very efficient procedure as described in our recent work.[10] To conjugate the aglycone with the amine containing side chains,

we first required introduction of a carboxylic function onto one indole nitrogen of the aglycone. Thus , by a Michael type reaction,[7] the adduct **2** was obtained regioselectively by reacting aglycone **1** with an excess of t-butyl acrylate and a catalytic amount of DBU in DMF at room temperature. A selectivity of 92:8 was observed in this case. Acidic cleavage of the t-butyl ester group with formic acid smoothly afforded the acid **3** which was isolated in pure form by crystallization.



With the acid **3** in hand, we started to assemble the target molecules. Accordingly, by using a typical coupling procedure, compounds **4a-4c**[11] were obtained in moderate yields by reacting **3** with the corresponding oligopeptides[12] in the presence of 1.2 equivalent of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole

hydrate (HOBT). Compounds 4d-4h were synthesized using this procedure in moderate to good yields by coupling of 3 with the appropriate amino alkyl agents.

In Vitro Biological Evaluation: These compounds were evaluated for their inhibition of PKC and PKA. The results are summarized in Table 1 and staurosporine was tested as reference. All of the compounds proved to be effective inhibitors of PKC, although none of them was as active as staurosporine. Significantly, all these compounds proved to be inactive against PKA. Of the oligopeptide substituted indolocarbazoles 4a-4c, the longer side chain is accompanied with an increase of inhibitory activity towards PKC. This trend is consistent with the finding that the longer chain compounds appear to fit the binding site better.[9] In comparison of 4e with 4f, an oxa-function in the piperidine ring of the side chain decreases the inhibitory potency by 50%. Compound 4g containing a dimethylamine group in the side chain proved to be the best representative with an IC_{50} value at 0.35 μM.

Table I. Inhibitory potencies for the compounds 4a–4h.

Compound	PKC IC_{50} (μM)[a]	PKA % Inhibition at 10 μM
4a	3.0	40
4b	0.5	43
4c	0.36	40
4d	0.55	41
4e	0.45	42
4f	0.9	49
4g	0.35	41
4h	0.8	25
Staurosporine	0.003	0.0065[b]

[a]The PKC and PKA were purified and assayed as described in the literature.[13]
[b]IC_{50} (μM).

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

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11. Spectroscopic and analytical data for compound **3**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.84 (2H, t, J = 8.0 Hz), 4.95 (2H, s), 5.15 (2H, t, J = 8.0 Hz), 7.24 (1H, t, J = 7.5 Hz), 7.35 (1H, t, J = 7.5 Hz), 7.42 (1H, t, J = 7.5 Hz), 7.53 (1H, t, J = 7.5 Hz), 7.72 (1H, d, J = 8.0 Hz), 7.81 (1H, d, J = 8.0 Hz), 8.05 (1H, d, J = 8.0 Hz), 8.50 (1H,s), 9.35(1H, d, J = 8.0 Hz), 11.92 (1H, s), 12.40 (1H, br.); HRMS (EI): Calcd. for $C_{23}H_{17}N_3O_3$ 383.12698, Found 383.12658. Compound **4a**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.95 (2H, t, J = 7.5 Hz), 3.70 (3H, s), 3.78 (3H, s), 4.95 (2H, s), 5.23 (2H, t, J = 7.5 Hz), 6.62 (1H, d, J = 2.0 Hz), 7.20-7.60 (5H, m), 7.72 (1H, d, J = 7.5 Hz), 7.80 (1H, d, J = 7.5 Hz), 8.52 (1H, s), 9.45(1H, d, J = 7.5 Hz), 9.95 (1H, s), 11.72 (1H, s); HRMS (FAB): Calcd. for $C_{30}H_{25}N_5O_4$ (M^+) 520.1985, Found 520.1968.
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