Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 955–959

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Rational Design, Synthesis, and Biological Evaluation of Rigid Pyrrolidone Analogues as Potential Inhibitors of Prostate Cancer Cell Growth

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Received 20 October 2000; accepted 23 January 2001

Abstract—In view of its role in tumor promotion and signal transduction, protein kinase C (PKC) has proven to be an exciting target for cancer therapy. With the aid of molecular modeling, we rationally designed and stereoselectively synthesized a new class of rigidified pyrrolidone-based PKC activators. Pyrrolidone **15** was found to exhibit reasonable affinity for PKCδ, with lower affinity for the other isozymes tested. Pyrrolidone **2** causes the dose-dependent induction of apoptosis in LNCaP prostate cancer cells. This apoptotic effect could be markedly potentiated by the use of LNCaP cells overexpressing the PKCα or δ isozymes.

Cancer is the second leading cause of death in the United States after cardiovascular disease, and it is projected that cancer will become the leading cause of death within the coming years.[1] After lung cancer, prostate cancer is the second most fatal cancer for American men. As an important signal transducing enzyme that is crucial for all aspects of cellular development, differentiation, and transformation,[2] PKC has been proposed to play an important role in carcinogenesis, metastasis,[3] and chemotherapy-associated multidrug resistance.[4] The PKC activator 12-*O*-tetradecanoylphorbol-13-acetate (TPA) functions differently in androgen-sensitive and androgen-independent human prostate cancer cells.[5] TPA activates cell death in androgen-sensitive LNCaP cells, in contrast to androgen-independent DU-145 or PC-3 cells. This inhibitory effect in androgen-sensitive cells was significantly decreased by the competitive PKC inhibitor, staurosporine. A recent review[6] summarizes other lines of evidence that the expression of constitutive PKCα activity is required for the survival and growth of androgen-independent human prostate cancer cells. Moreover, systemic delivery of PKCα antisense oligonucleotides (ISIS 3521) has been shown to result in the marked growth inhibition of U-87 xenografts without affecting the levels of other PKC isoforms.[7] Taken together, these studies imply that PKCα may serve as a novel target in the treatment of prostate cancer. In this report, we describe our results with a rigidified pyrrolidone-based PKC activator **2**, which is shown to cause the dose-dependent induction of apoptosis in LNCaP prostate cancer cells. This apoptotic effect is shown to be potentiated in LNCaP cells overexpressing the PKCα or δ isozymes.

The PKC gene family consists presently of 11 genes, which are divided into four subgroups. Each PKC isozyme contains a regulatory domain and a catalytic domain. Within the regulatory domain, both classical (α, β, and γ) and novel (δ, ε, η, and θ) PKC isozymes

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0960-894X/01/$ - see front matter
PII: S0960-894X(01)00097-X

contain two cysteine-rich zinc finger domains (C1A and C1B), which constitute the diacylglycerol/phorbol ester binding site. In 1995, the X-ray crystal structures of PKCδ C1B in complex with phorbol 13-acetate revealed precisely how phorbol ester binds to PKC[8] and provided a solid structural basis for the rational design of new PKC modulators.[9,10] Based upon the structure of the potent PKC activator 8-decynylbenzolactam (**1**), and with the aid of molecular modeling, we designed a series of pyrrolidone analogues as a new class of PKC modulators.[11] These pyrrolidone analogues maintain the hydrogen bonding network and hydrophobic interactions considered crucial for interaction with PKC, and were found to possess reasonably good affinity for PKC and, in a few cases, some isozyme selectivity. Upon taking into consideration both the rotational flexibility of the aromatic ring in these pyrrolidone analogues together with the structure of the more potent benzolactams, we designed the rigidified pyrrolidone structures with the aim to possibly reach comparable potency by reducing conformational mobility. Thus, the benzolactam N-1 atom was replaced by a carbon atom, followed by shifting the isopropyl group from the previous C-2 position to the new carbon. C-2 and C-6 were subsequently joined to give the pyrrolidone system. Finally, the stereochemistry of all chiral centers was adjusted based upon results derived from molecular modeling to arrive at the structure of pyrrolidone **2** shown in Figure 1. After energy minimization, the resulting conformation of compound **2** was superimposed upon the benzolactam **1**. As depicted in Figure 2, the crucial hydroxyl group and amide residue are preserved in compound **2**, and these groups engage in a strong hydrogen bonding network with PKCδ C1B. The front methyl group on the marked chiral center mimics the N–Me in benzolactam **1**, interacting with Leu250 of PKC (Fig. 3). Similarly, the rear isopropyl group of pyrrolidone **2** interacts with the side chain of Leu254, thereby mimicking the isopropyl group of benzolactam **1**. The same orientation of the phenyl group in compound **2** allows for strong hydrophobic interactions with Pro241 of PKC.

Retrosynthetic analysis revealed that pyrrolidone **2** could be easily assembled via conjugate addition and radical cyclization as the key steps. The bromide **6** required for preparation of the corresponding Grignard reagent is readily available from 3-hydroxybenzaldehyde. Bromination of 3-hydroxybenzaldehyde (**3**) with bromine in dichloromethane gave rise to 2-bromo-5-hydroxybenzaldehyde (**4**) in quantitative yield. Subsequent nucleophilic addition of 2 equiv of *t*-butylmagnesium chloride provided the crystalline benzyl alcohol **5** in good yield. Next, a cationic rearrangement induced by

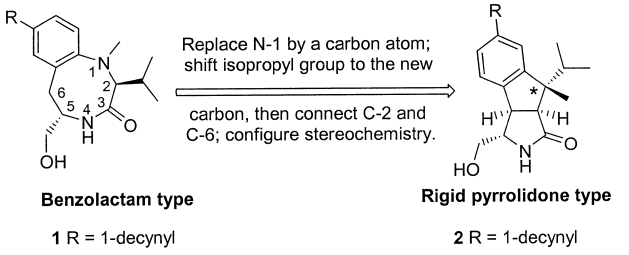


Figure 1. Design concept of pyrrolidone **2**.

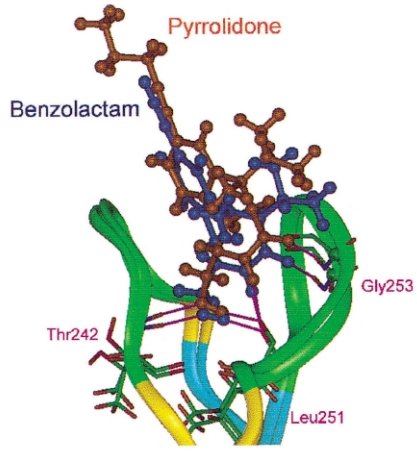


Figure 3. Superimposition of the parent structure of pyrrolidone **2** upon the benzolactam **1** in PKCδ C1B.

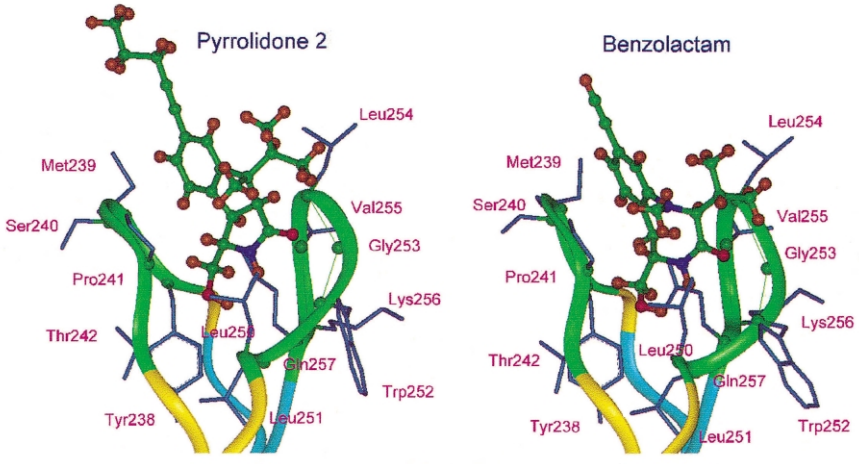
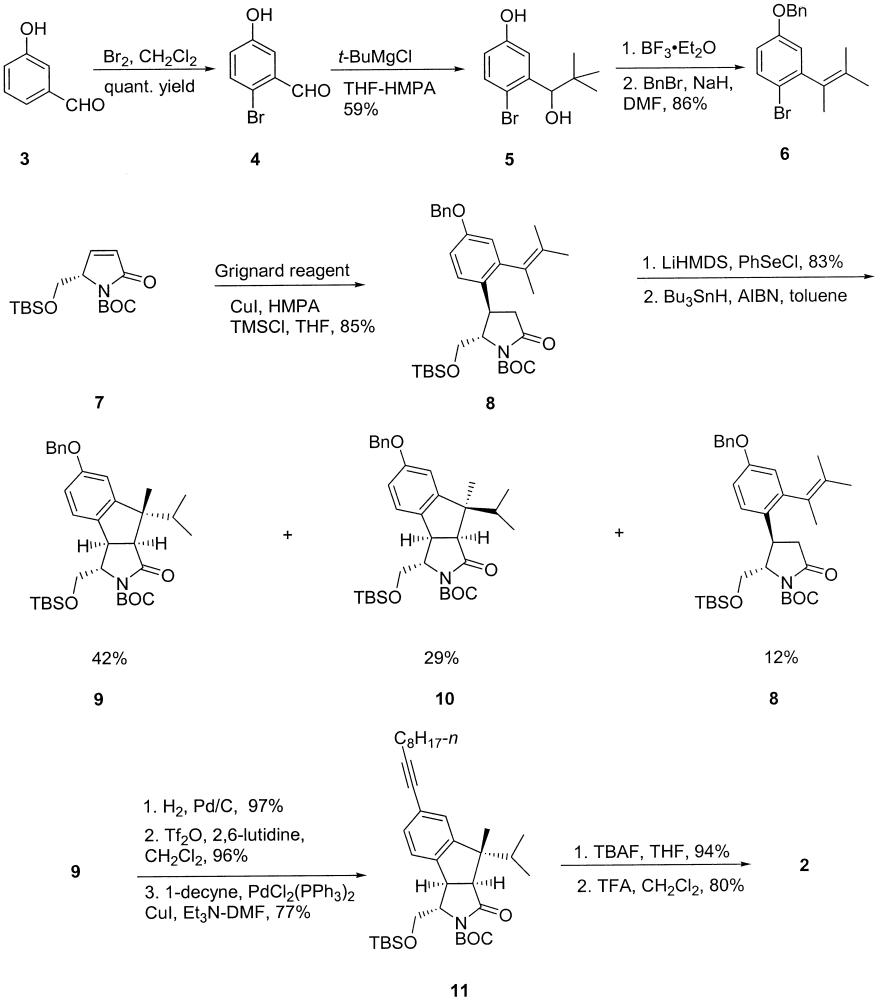


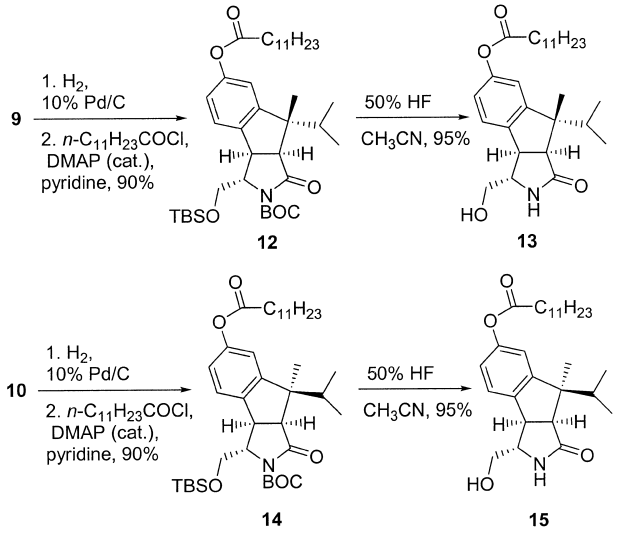
Figure 2. Overall features of the binding model for pyrrolidone **2** and benzolactam **1** in complex with PKCδ C1B.

boron trifluoride etherate followed by *O*-benzylation furnished the desired bromide **6** bearing a tetrasubstituted olefin. Copper(I)-catalyzed conjugate addition of Grignard reagent derived from bromide **6** to lactam **7** furnished compound **8** stereoselectively. The steric hindrance provided by the tetrasubstituted olefin prevents free rotation of the phenyl ring. Hence, lactam **8** was observed to exist as a pair of conformers by NMR, presumably with the olefin located on one side or the other of the pyrrolidone ring. Introduction of the ring constraint, which involves creation of a new five-membered ring, was then accomplished through radical cyclization initiated by tin hydride reduction of a phenylselenyl group introduced α to the carbonyl group. The cyclized products **9** and **10**, and the recovered product **8** were separated by careful column chromatography on silica gel. The stereochemistry of lactams **9** and **10** were assigned by ^1H–^1H COSY and ^1H–^1H NOESY as well as by X-ray diffraction of lactam **10**. Following the general procedures reported previously,[11] intermediate **9** was then readily transformed into the target molecule, pyrrolidone **2** (Scheme 1). Compound **13**, which contains an ester function in place of the decynyl appendage of **2**, was also prepared from intermediate **9**. For purposes of comparison, compound **15**, the diastereoisomer of compound **13**, was synthesized in a similar manner from intermediate **10** (Scheme 2).

Pyrrolidones **2**, **13**, and **15**, as well as their progenitor, benzolactam **1**, were evaluated for their ability to displace phorbol 12,13-dibutyrate (PDBu) binding from five recombinant PKC isozymes, namely, PKCα, PKCβ, PKCγ, PKCδ, and PKCε (Table 1).[12] Compared to the benzolactam **1**, these three new rigid pyrrolidones exhibited 5- to 30-fold lower potency in binding to the classical and novel PKC isozymes. These results suggest that some important hydrophobic interactions that exist between the benzolactam **1** and PKC are likely to have been lost upon conversion to the rigid pyrrolidone structures. The methyl group and the isopropyl group at the starred chiral center of pyrrolidone **2** (Fig. 1) may thus only partially mimic the *N*-methyl and isopropyl groups in benzolactam **1**. Interestingly, the PKC δ binding affinity of pyrrolidone **15** is almost identical to



Scheme 1. Synthetic route to pyrrolidone **2**.



Scheme 2. Preparation of pyrrolidones **13** and **15** from intermediates **9** and **10**, respectively.

that of the benzolactam **1**, and it is greater than that found for compounds **2** and **13**. This result indicates that the gain in binding affinity stemming from the hydrophobic interaction of the isopropyl group in compound **15** with Pro241 of PKCδ may override the loss in the interaction with Leu250 and Leu254 of PKCδ (Fig. 3). Accordingly, it may be possible to further modify this particular chiral center in pyrrolidone **2** to enhance such hydrophobic interactions with PKC.

Since the chemical yield of intermediate **9** is better than that of its diastereoisomer **10**, we scaled up the synthesis of pyrrolidone **2** to allow for further biological studies. Pyrrolidone **2** is also preferable over analogues **13** and **15**, as the latter may be subject to the action of esterases, resulting in cleavage of the hydrophobic side-chain residue. The capability of inducing apoptosis by pyrrolidone **2** was evaluated in a model of LNCaP prostate cancer cells. It is well established that in this model phorbol esters (e.g., PMA) activate PKC to induce apoptosis.[13] LNCaP cells were treated with pyrrolidone **2** for 1 h, and apoptosis was assessed 24 h later. As depicted in Figure 4A, pyrrolidone **2** (1–30 μM) induces a dose-dependent apoptotic effect in LNCaP cells. For comparison, a representative experiment using the phorbol ester PMA is shown in Figure 4B.

Control cells or cells infected for 14 h with adenoviruses for LacZ, PKCα, or PKCδ (multiplicity of infection = 10 pfu/cell) were treated with different concentrations of pyrrolidone **2** or PMA for 1 h. Cells were collected 24 h later and stained with DAPI. The incidence of apoptosis in each preparation was analyzed by counting 500 cells and determining the percentage of apoptotic cells. Results are the mean ± standard error of three independent experiments.

Table 1. K_i values (nM) for the inhibition of [^{3}H]PDBu binding to human recombinant PKC isozymes by the compounds tested

Compound	PKCα	PKCβ	PKCγ	PKC δ	PKCε
1	23±4	70±18	43±7	114±37	40±14
2	580±214	701±217	279±50	1630±391	469±140
13	395±71	593±178	209±38	1370±329	515±155
15	674±249	1052±284	327±65	150±64	228±43
PMA	2.5±0.5	1.8±0.6	4.9±0.9	0.8±0.2	0.45±0.15

It is well established that PKCα and PKCδ are the isozymes that mediate phorbol ester-induced apoptosis in LNCaP cells.[13c–e] A model that had been used to assess isozyme-specificity is the overexpression of specific PKCs by an adenoviral system. Infection of LNCaP cells with adenoviruses for PKCs leads to a high expression of the corresponding PKC isozyme, as determined by Western-blot analysis, kinase activity, or [^{3}H]PDBu binding.[13e] Using this system, we found a marked potentiation of PMA-induced apoptosis in LNCaP cells by overexpression of PKC isozymes,[13e] as also shown in Figure 4B. Remarkably, a similar effect was observed with pyrrolidone **2**, where overexpression of either PKCα or PKCδ markedly potentiates the apoptotic response in LNCaP prostate cancer cells (Fig. 4A). As expected, a control adenovirus (LacZ adenovirus) did not affect the apoptotic effect of either pyrrolidone **2** or PMA.

The present efforts reveal that modification of the parent benzolactam **1** to produce the rigid pyrrolidone analogues leads to pyrrolidone **15** that shows comparable binding affinity at PKCδ, while at the same time exhibiting reduced affinity for the other isozymes tested. This result provides structural information for improving PKCδ isozyme selectivity. Moreover, while the pyrrolidone **2** was found to be less potent than PMA at binding to PKC, it still induces levels of apoptosis in LNCaP cells overexpressing either the PKCα or δ isozymes. As other evidence indicates that lower affinity ligands may prove to be safer when administered systemically, the present results may be relevant to anticancer drug development.

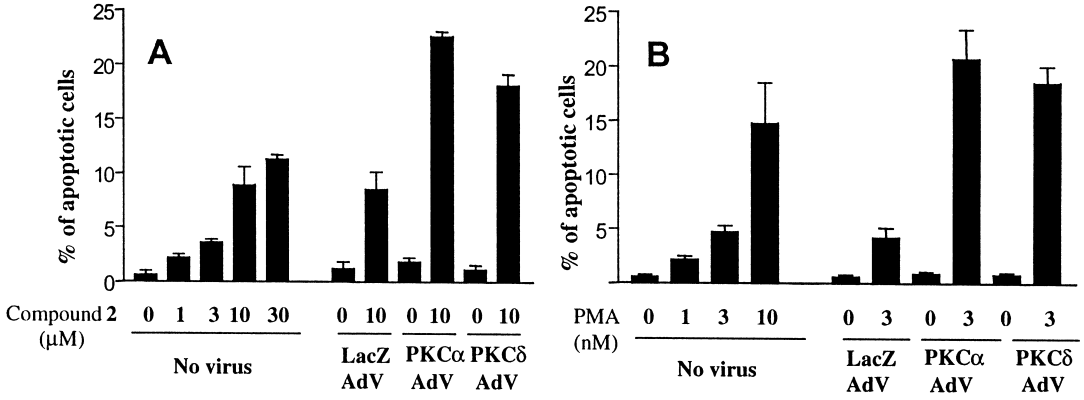


Figure 4. Induction of apoptosis in LNCaP prostate cancer cells by pyrrolidone **2** (Panel A) or PMA (Panel B).

Acknowledgements

This work was supported by NIH grant CA79601 (to A. P.K.), the Department of Defense DMAD 17-93-V-3018 (to A.P.K.), the NIMH Psychoactive Drug Screening Program (MH 01366 to B.L.R.), the Department of Defense Prostate Cancer Research Program (to M.G.K.), and in part by NIDA and the Office of Naval Research (to J.F.-A.).

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