On the Protein Kinase C Pharmacophore: Synthesis and Biological Activity of 4-Hydroxylated Analogs of Ingenol

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Dedicated to Professor Gilbert Stork, an inspiring teacher and friend to whom we are very grateful.

Abstract: The synthesis and preliminary biological evaluation of the first C-4 hydroxylated analog of ingenol are described. The activity of 4 demonstrates that the C-4 hydroxyl group is not a critical component of the pharmacophore for protein kinase C binding and activation.

Protein kinase C is the phosphorylating enzyme mediating cellular signal transduction for a large class of hormones and cellular effectors that activate phosphatidylinositol 4,5-bis(phosphate) turnover.3 Several structurally diverse naturally occurring compounds, including the bryostatins, telocidin, aplysia toxin, and esters of phorbol, 1, and ingenol, 2 (Scheme I), mimic the function of diacetyl glycerol, the endogenous activator of protein kinase C (PKC), but possess much greater potency.4

Scheme I

Several models have recently been developed in an effort to identify a pharmacophore, i.e., a similar three-dimensional array of homologous functional groups, in these seemingly unrelated structures, all of which interact at the same regulatory site on protein kinase C.5 The elegant and pioneering studies of Wender5a,b and Jeffrey5c have independently proposed that the C-9, C-20, and either C-3 or C-4 oxygen functionalities of ingenol represent critical elements of the ingenol ester pharmacophore. Kishi and Rando subsequently argued that these models may be flawed, since they propose a key role for the C-4 hydroxyl group in the phorbol esters, while it is known that the C-4 deoxyphorbol esters are still active tumor promoters.6 The study of the structural requirements for the activation of PKC has therefore focused on the synthesis and study of specifically modified derivatives of these natural product leads. We have previously reported the application of the intramolecular dioxonene photocycloaddition7 to the first synthesis of a potent synthetic analog, 3, of ingenol, which contains only the C-3, C-9 and C-20 oxygen functionalities present in the natural product 2.8 We describe herein the synthesis and preliminary biological study of a C-4 hydroxylated analog, 4, the activity of which is ca. 1/3 that of the C-4 deoxy ingenol analog, 3.

The synthesis of the C-4 hydroxylated analog, 4, which is outlined in Schemes II and III, is noteworthy for the highly selective reactions observed in the course of the introduction of the C-4 hydroxyl.9 Reasoning that the dihydroxylation of the Δ3,4 alkene would provide a straightforward route for the stereoselective introduction of the C-4 oxygen functionality, the elimination of the C-3 hydroxyl group in the C-6 epimeric intramolecular dioxonene photoaddition-fragmentation products 58b and 88b was examined (Scheme II). Mitsunobu bromination of 5 (C-β ester) proceeded uneventfully to give the inverted C-3α bromide, 6, which on to standard elimination conditions (LiCl, DMF, reflux) led to the exclusive formation of the Δ3,4 alkene product, 7. In contrast, we found that substitution of the C-6α ester epimer 8 to the same reaction conditions led to the exclusive formation of the Δ2,3 alkene, 10.

Scheme II

In an effort to understand this striking difference in regiochemistry as a function of the C-6 ester stereochirality, the elimination reaction of 11,10 which lacks the ester substituent, was examined. Under reaction conditions identical to those described above, the only product formed was the Δ3,4 alkene product 13. To probe the role of the C-6α ester in the regioselective formation of the Δ2,3 alkene product 10, the elimination reaction of the C-6α tert-butyl diphenylsilyl ether 15 was examined. The Δ2,3 product 16 was the only alkene product observed, albeit in poor yield, once again underscoring the importance of the C-6α substituent in the regioselective formation of the less substituted alkene products.

Dihydroxylation of 7 proceeded exclusively from the sterically less hindered β-face to give the C-3β, C-4β dihydroxy compound 17 (Scheme III), which on ketalization gave 18. Oxidation of 18 to the unsaturated ester, followed by regioselective reduction of the unsaturated ester led to formation of allylic alcohol 20.
Deketalization of 20, followed by selective protection of the primary C-20 hydroxyl of triol 21 gave 22, which on exposure to benzoyl chloride gave C-3 monobenzoate 23. Desilylation of 23 then gave the C-3 monobenzoate product, 4.

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\begin{align*}
\text{17} & \quad \text{a} & \quad \text{18} \\
\text{19} & \quad \text{b} & \quad \text{20} & \quad \text{R=CH}_2\text{OH} \\
\text{21} & \quad \text{R=H} & \quad \text{R=H} & \quad \text{22} & \quad \text{R=TBMS} \\
\text{23} & \quad \text{R=PhCO} & \quad \text{R=TBMS} & \quad \text{R=PhCO} & \quad \text{R=H} \\
\text{24} & \quad \text{R=H} & \quad \text{R=H} & \quad \text{R=H} & \quad \text{R=H} \\
\end{align*}
\]

Scheme III

The C-4 hydroxy monobenzoate, 4, was evaluated for its ability to interact with the regulatory site on protein kinase C, as quantitated by inhibition of [\(^3\)H]PDBU binding to protein kinase C-\(\alpha\) reconstituted in the presence of 100 \(\mu\)g/ml phosphatidylycerine, 0.1 mM Ca\(^{2+}\), and incubated for 5 min at 37°C. These conditions are the same as we have previously described for the analysis of the C-4 deoxyxigenane analog 3,8,11. The curves for inhibition of [\(^3\)H]PDBU binding were consistent with a competitive mechanism. Under these conditions, ingenol 3-monobenzoate, 2 (R=PhCO; R'=H), yielded an apparent \(K_I\) of 0.15 ± 0.03 nM (mean ± SEM, n=4) for protein kinase C-\(\alpha\). The C-4 deoxy analog had a \(K_I\) of 165 ± 21 nM (mean ± SEM, n=3) and the C-4 hydroxylated analog, 4, assayed in parallel, had a \(K_I\) of 561 ± 94 nM (mean ± SEM, n=3).

These data have two important implications for the understanding of structure-activity relations for the ligand binding site on protein kinase C. First, they should permit the role of the 4β-hydroxyl group to finally receive extensive experimental evaluation. For diterpenes of the phorbol series, 4-deoxy derivatives have been obtained by natural product isolation but have not been generally available. In the limited studies conducted, these derivatives were approximately 3-fold weaker in inflammatory potency on the mouse ear than the corresponding 4β-hydroxyl derivatives. 12 Our compounds now provide the first opportunity for the direct, quantitative evaluation of the role of the 4 hydroxy group in binding to protein kinase C. Our present findings emphasize that the loss of the 4-OH causes no diminution of binding affinity, at least in the presence of the 3-ester. A second important implication is that the marked difference in the binding affinities of compounds 2 and 4 suggests that other elements of the structure than those highlighted in the postulated pharmacophore models 5,6 play an important role in the receptor-ligand interactions. Of particular interest is the 1,2,3-double bond of the A ring. In support of a role for this group, Heck and co-workers reported that tetrahydrophorbol 12,13-didecanoate showed a 100-fold decrease in irritant potency 13 and 1,2-dihydroporphobol 12-myristate 13-acetate [β-epoxide showed complete loss of activity. 14 Finally, a number of the unusual features of the biological activity of saptoxina A, 12-O-[2-methylamino-benzoate]-4-deoxyphorbol 13-acetate, have been described, such as lack of activation of protein kinase C-δ and lack of tumor promoting activity despite potent irritancy, that may be related to the absence of the 4-hydroxyl group. 15 Current efforts in synthesis and biological evaluation are underway to pursue these issues.

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References and Notes
1Recipient of the American Cancer Society Young Faculty Award (1989-1992) and a National Institutes of Health Research Career Development Award (1988-1993).
2Address correspondence to this author regarding the biological testing of 2-4.
3For recent reviews, see Blackshear, P. J., J. Biol. Chem. 1993, 268, 1501; b) Dekker, L. V.; Parker, P. J. Trends in Biochem. Sci. 1994, 19, 73.
9All new compounds were characterized by full spectroscopic (NMR, IR, high resolution MS) data. Yields refer to spectroscopically and chromatographically homogeneous (>95%) materials. Selected spectral data-δ 3.4 C6β ester 7: IR (neat): 2950, 2880, 1735, 1450, 1165 cm\(^{-1}\); 1\(^{1}\)H NMR (CDCl\(_3\)): 8 0.98-1.08 (m, 2 H), 1.40-1.65 (m, 5 H), 1.70-2.02 (m, 5 H), 2.14-2.22 (m, 2 H), 2.30-2.40 (m, 1 H), 2.48-2.56 (m, 2 H), 2.71 (d, J = 14 Hz, 1 H), 3.38-3.45 (m, 1 H), 3.63 (s, 3 H), 5.55 (br.s, 1 H); 1\(^{3}\)C NMR (CDCl\(_3\)): δ 25.51 (CH\(_2\)), 28.91 (CH\(_2\)),
29.07 (CH₂), 29.10 (CH₂), 30.28 (CH₂), 30.44 (CH₂), 30.99 (CH₂), 34.11 (CH₂), 43.04 (CH), 46.45 (CH), 51.85 (CH₃), 68.52 (C), 130.31 (CH), 144.08 (C), 175.90 (C), 214.55 (C); exact mass calculated for C₁₆H₂₂O₃: 262.1569, found 262.1553; C-3β monobenzoate 4: IR (neat): 3100-3600, 2960, 2880, 1730, 1700, 1460, 1285, 1130, 720 cm⁻¹; ¹H NMR (CDCl₃): δ 1.00-1.15 (m, 1 H), 1.40-1.50 (m, 1 H), 1.55-1.95 (m, 4 H), 2.05-2.15 (m, 1 H), 2.29 (d, J = 16.6 Hz, 1 H), 2.40-2.50 (m, 1 H), 2.83 (d, J = 16.6 Hz, 1 H), 3.86 (s, 3 H), 4.14-4.20 (m, 2 H), 5.07 (dd, J = 6.6, 5.0 Hz, 1 H), 6.91 (d, J = 4.8 Hz, 1 H), 7.38-7.48 (m, 2 H), 7.52-7.60 (m, 1 H), 7.95-8.05 (m, 2 H); ¹³C NMR (CDCl₃): δ 25.49, 26.93, 27.01, 28.06, 30.53, 34.07, 39.66, 49.71, 55.53, 67.10, 81.01, 81.97, 128.30, 128.56 (two C), 129.61 (two C), 129.80, 133.36, 137.74, 165.93, 210.08; exact mass calculated for C₂₂H₂₆O₅: 370.1780, found 370.1756.
