Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 833–836

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS OF A COSALANE ANALOG WITH AN EXTENDED POLYANIONIC PHARMACOPHORE CONFERRING ENHANCED POTENCY AS AN ANTI-HIV AGENT

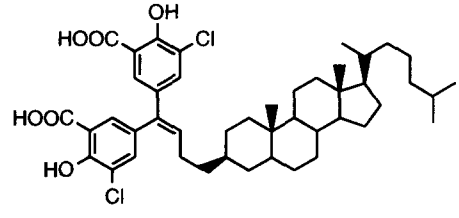
Mark Cushman,*[a] Shabana Insaf,[a] Jeffrey A. Ruell,[a] Catherine A. Schaeffer,[b] and William G. Rice[b]

[a]Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, U.S.A.; [b]Laboratory of Antiviral Drug Mechanisms, SAIC Frederick, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD 21702-1201, U.S.A.

Received 9 January 1998; accepted 23 February 1998

Abstract: A novel cosalane analog having an extended polyanionic pharmacophore was synthesized in order to target specific cationic residues on the surface of CD4. The design rationale is based on a hypothetical binding model of cosalane to the surface of the protein. The new analog displayed an EC_{50} of 0.55 μM as an inhibitor of the cytopathic effect of HIV-1_{RF} in CEM-SS cells, which represents a significant increase in potency over cosalane itself (EC_{50} 5.1 μM). Both cosalane and the new analog are inhibitors of viral entry into target cells.

Cosalane (**1**) is a novel anti-HIV agent with an unusual mechanism of action.[1,2] Although cosalane (**1**) has proven to bind to or inhibit a variety of HIV-1 targets including reverse transcriptase, protease, integrase, gp120, and CD4, the available evidence indicates that its ability to inhibit the cytopathic effect of HIV-1 is primarily due to its capacity to inhibit viral attachment and fusion.[2] Cosalane offers the advantage of being effective against a wide variety of HIV laboratory strains and clinical isolates, but its potency is moderate, ranging from an EC_{50} of 3.4 μM vs. the III_B strain in CEM-SS cells to 80.2 μM in the VIHU (NSI) clinical isolate in CEM-SS cells.[2] Several cosalane analogs have been synthesized with enhanced in vitro potencies as inhibitors of HIV-1 protease and integrase,[3] but in spite of considerable effort in our group and by others, no cosalane analogs have been reported previously which are more potent than cosalane itself as inhibitors of HIV-1 cytopathicity.[3-7]



1

As a possible strategy for obtaining cosalane analogs with enhanced potencies, a hypothetical model was constructed for the binding of the disalicylmethane cosalane "pharmacophore" to CD4 (Figure 1). This model was constructed using Sculpt® 2.5 software by "freezing" the protein and then minimizing the energy of the complex

0960-894X/98/$19.00
PII: S0960-894X(98)00121-8

while allowing the ligand to move. Prior studies have demonstrated that cosalane binds to both gp120 and CD4, resulting in inhibition of gp120 binding to CD4.[8] Since the crystal structure of CD4 has been determined, it is possible to search for likely binding sites for cosalane on the surface of the protein.[9] What appears to be the most likely binding mode features two electrostatic interactions between the positively charged arginine 58 and arginine 59 residues of CD4 with the two negatively charged carboxylate residues of cosalane. In Figure 1, this electrostatic attraction is indicated by the "blue dots" in the two regions of space between the negatively charged carboxylates of the ligand and the two positively charged arginine residues of the protein. The binding site proposed in Figure 1 is located within the D1D2 fragment of CD4, which contains residues that have been implicated in the interaction of CD4 with gp120.[9-12] Residues Lys29, Lys35, Phe43, Leu44, Lys46, Gly47, and Arg59 of CD4 have been implicated as being directly involved in binding to gp120. In one study, it was reported that the alteration of Arg59 "dramatically disrupted the ability of CD4 to bind to gp120".[11] It is apparent from examination of the model portrayed in Figure 1 that there is an additional basic residue in close proximity to the proposed binding site. This is the lysine 72 residue, which could be targeted by additional carboxylate residues that could possibly be added to the cosalane "pharmacophore".

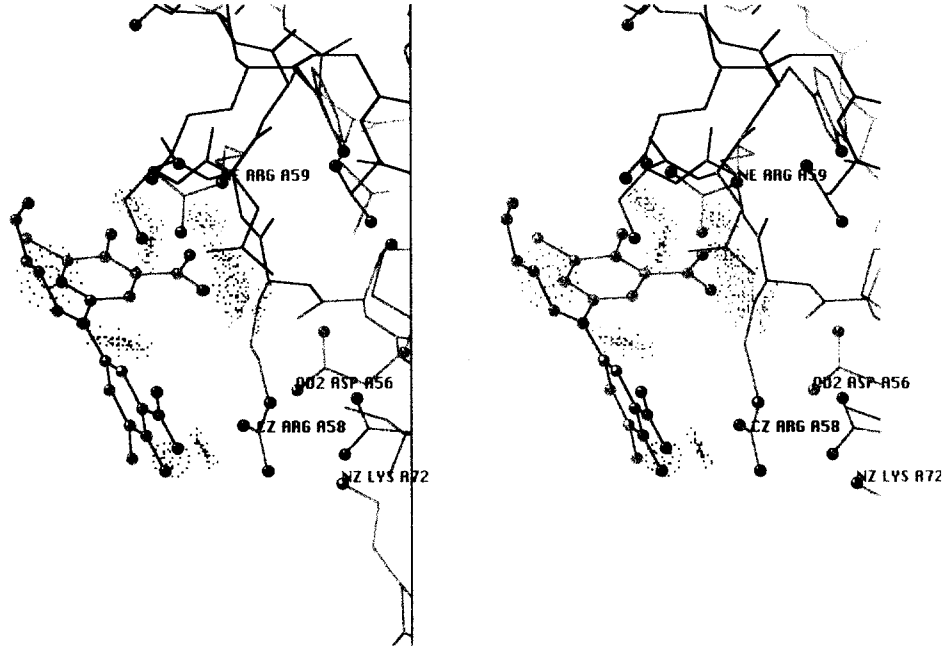
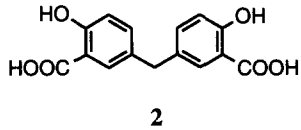


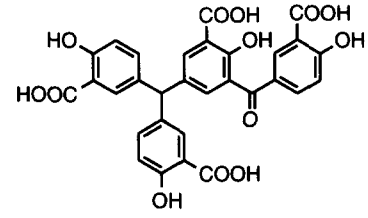
Figure 1. Hypothetical model of the binding of the cosalane "pharmacophore" to CD4 (programmed for walleyed viewing). Blue dots: attraction, red dots: repulsion.

The idea to extend the cosalane "pharmacophore" to higher oligomers containing additional salicylic acid rings is also supported by a second line of reasoning. In our prior work on the fractionation and structure elucidation of low molecular weight aurintricarboxylic acid oligomers, there was an observable increase in potency

in going from the disalicylmethane system **2** to higher oligomers (e.g., **3**).[13] This observation suggests that extension of the disalicylmethane "pharmacophore" of cosalane to contain additional salicylic acid rings, or rings bearing related functionality, would increase potency.



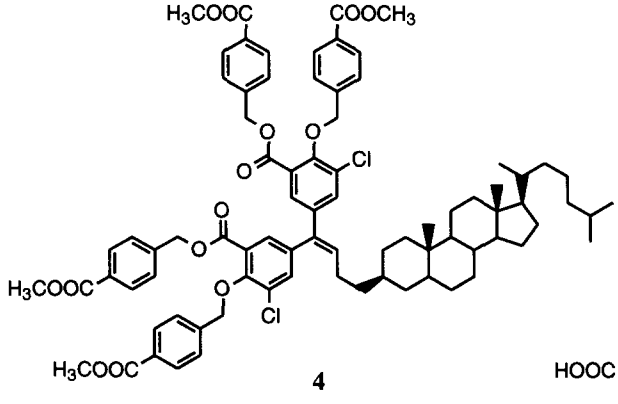
2



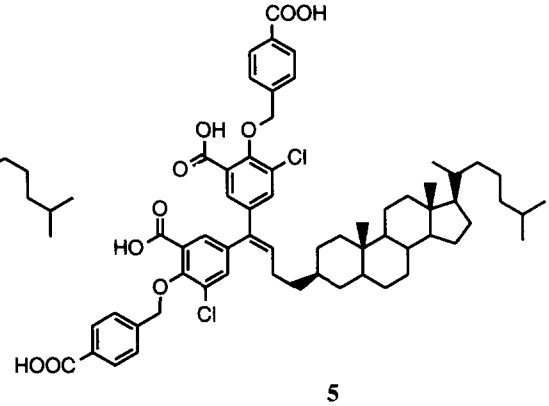
3

The desired analog **5** was synthesized from intermediate **4**, which was obtained in 86% yield by treatment of cosalane (**1**) with *p*-(methoxycarbonyl)benzyl bromide in the presence of K_2CO_3 in DMF at room temperature for 16 h. Hydrolysis of the ester groups of **4** was performed in 81% yield using K_2CO_3 in aqueous ethanol at 105–110 °C for 8 h.

The new analog **5** displayed an EC_{50} of 0.55 μM as an inhibitor of the cytopathic effect of HIV-1_{RF} in CEM-SS cells, which represents a tenfold improvement over cosalane itself when tested in the identical system (EC_{50} 5.1 μM).[2,14] The CC_{50} for cytotoxicity in CEM-SS cells was 72 μM, affording a selectivity index of 131. In contrast to cosalane (**1**), evaluation of compound **5** at a high test of 100 μM in a variety of previously described mechanistic assays revealed no in vitro inhibition of HIV-1 reverse transcriptase, protease or integrase.[15] Compound **5** did inhibit HIV-1 attachment to CEM-SS cells with an IC_{50} of 72 μM, but this did not correlate with the antiviral concentration (EC_{50}) of 0.55 μM. However, the cell-to-cell fusion of gp120 bearing, Tat containing HL-2/3 cells with HeLa-CD4-LTR-β-gal cells was inhibited by compound **5** with an IC_{50} of 2.7 μM. All of the available evidence indicates that **5**, like cosalane, inhibits HIV-1-induced cytopathicity because of its ability to inhibit viral entry into target cells.



4



5

The hypothetical model proposed in Figure 1 for the binding of cosalane (**1**) to CD4 is in accord with a mechanism of action involving the inhibition of gp120 binding to CD4 since the IC_{50} for the inhibition of $HIV\text{-}1_{RF}$ binding to freshly isolated PBLs (5.3 μM) and the IC_{50} for inhibition of gp120 binding to CD4 (7.8 μM) both correlate closely with the EC_{50} values for inhibition of the cytopathic effect of several different strains of HIV-1 in CEM-SS and MT4 cells.[2] However, the observed IC_{50} for compound **5** of 72 μM for inhibition of HIV-1 attachment to CEM-SS cells is not consistent with inhibition of attachment (gp120-CD4 binding) as a mechanism of action for **5**, since the observed EC_{50} for inhibition of viral cytopathicity (0.55 μM) is much lower. In fact, the IC_{50} of 2.7 μM for **5** for inhibition of fusion of HL-2/3 cells with MAGI cells indicates that inhibition of postbinding fusion is a more likely mechanism for this compound. Ongoing studies are investigating if compound **5** selectively affects the interaction of HIV-1 with the cell surface chemokine co-receptors.

Acknowledgment. This research was made possible by NIH Grant NO1-AI-36624.

References

1. Golebiewski, W. M.; Bader, J. P.; Cushman, M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1739.
2. Cushman, M.; Golebiewski, W. M.; McMahon, J. B.; Buckheit, R. W. J.; Clanton, D. J.; Weislow, O.; Haugwitz, R. D.; Bader, J.; Graham, L.; Rice, W. G. *J. Med. Chem.* **1994**, *37*, 3040.
3. Cushman, M.; Golebiewski, W. M.; Pommier, Y.; Mazumder, A.; Reymen, D.; De Clercq, E.; Graham, L.; Rice, W. G. *J. Med. Chem.* **1995**, *38*, 443.
4. Keyes, R. F.; Golebiewski, W. M.; Cushman, M. *J. Med. Chem.* **1996**, *39*, 508.
5. Keyes, R. F.; Cushman, M. *Med. Chem. Res.* **1996**, 372.
6. Golebiewski, W. M.; Keyes, R. F.; Cushman, M. *Bioorg. Med. Chem. Lett.* **1996**, *4*, 1637.
7. Patch, R. J.; Roberts, J. C.; Gao, H.; Shi, Z.; Gopalsamy, A.; Kongsjahju, A.; Daniels, K.; Kowalczyk, P. J.; van Schravendijk, M.-R.; Gordon, K. A.; Pallai, P. V. *Bioorg. Med. Chem.* **1996**, *6*, 2983.
8. Reymen, D.; Witvrouw, M.; Esté, J. A.; Neyts, J.; Schols, D.; Andrei, G.; Snoeck, R.; Cushman, M.; Hejchman, E.; De Clercq, E. *Antiviral Chem. Chemother.* **1996**, *7*, 142.
9. Ryu, S.-E.; Truneh, A.; Sweet, R. W.; Hendrickson, W. A. *Structure* **1994**, *2*, 59.
10. Moebius, U.; Clayton, L. K.; Abraham, S.; Harrison, S.; Reinhertz, E. L. *J. Exp. Med.* **1992**, *176*, 507.
11. Choe, H.; Sodrosky, J. *J. Acquired Immune Defic. Syndr.* **1992**, *5*, 204.
12. Tsui, P.; Sweet, R. W.; Sathe, G.; Rosenberg, M. *J. Biol. Chem.* **1992**, *267*, 9361-9367.
13. Wang, P.; Kozlowski, J.; Cushman, M. *J. Org. Chem.* **1992**, *57*, 3861.
14. Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. *J. Natl. Cancer. Inst.* **1989**, *81*, 577.
15. Rice, W. G.; Baker, D. C.; Schaeffer, C. A.; Graham, L.; Bu, M.; Terpening, S.; Clanton, D.; Schultz, R.; Bader, J. P.; Buckheit, R. W.; Field, L.; Singh, P. K.; Turpin, J. A. *Antimicrob. Agents Chemother.* **1997**, *41*, 419.