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# Investigation of the alkenyldiarylmethane non-nucleoside reverse transcriptase inhibitors as potential cAMP phosphodiesterase-4B2 inhibitors

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**Abstract**—The alkenyldiarylmethanes (ADAMs) are currently being investigated as non-nucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs) of potential value in the treatment of HIV infection and AIDS. During the course of these studies, a number of ADAM analogues have been identified that protect HIV-infected cells from the cytopathic effects of the virus by an unknown, HIV-1 RT-independent mechanism. Since the phosphodiesterase 4 family is required for HIV infection, the effect of various ADAMs on the activity of PDE4B2 was investigated in an effort to determine if the ADAMs could possibly be targeting phosphodiesterases. Six compounds representative of the ADAM class were tested for inhibition of cAMP hydrolysis by PDE4B2 enzymatic activity. Four ADAMs were found to be weak inhibitors of PDE4B2 and two of them were inactive. The experimental results are consistent with an antiviral mechanism that does not include inhibition of PDE4 isoforms.


Acquired immune deficiency syndrome (AIDS) is estimated to have claimed more than 25 million lives since it was first described in 1981, making it one of the most deadly epidemics in history.[1] Increasing appreciation of the complex biology involved with human immunodeficiency virus (HIV) infection has led to the successful development of antiviral agents that are used clinically to combat the progression of AIDS. However, a cure for AIDS does not appear to be on the horizon, and HIV infection continues to spread on a pandemic scale.[1] It is quite clear that finding a solution to the problem of HIV infection will be one of this century's greatest challenges in medical science.

Until a cure is discovered, clinicians will have to rely on the various therapeutic agents that have been developed to combat HIV infection and replication. Unfortunately, the low polymerase fidelity of HIV reverse transcriptase allows the virus to rapidly mutate and develop resistance to the existing spectrum of anti-HIV agents.[2–4] In fact, it has been reported that when antiviral-naive patients begin highly active antiretroviral therapy (HAART), it is possible to detect drug-resistant strains of HIV in the patients' circulation as early as two months after initial treatment.[5–7] HIV's rapid mutability has recently led to the emergence of multi-drug-resistant viral strains, and thus the latest challenge has been to develop antiviral agents that are active against both the wild type form of the virus as well as the most common drug-resistant strains.

The alkenyldiarylmethane (ADAM) non-nucleoside reverse transcriptase inhibitors (NNRTIs) inhibit HIV-1 reverse transcriptase (RT) by an allosteric mecha-

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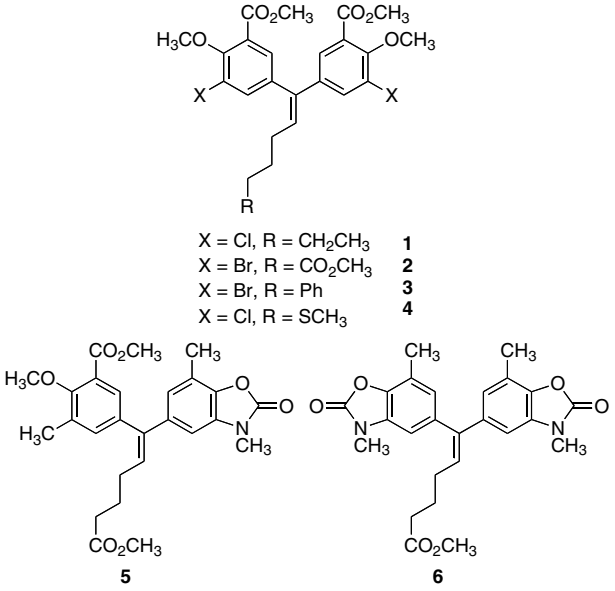
nism.[8–14] Early investigations revealed that a number of the leading compounds, such as ADAM **2**, retained antiviral activity against several common reverse transcriptase mutants (for example Y188C and K103N)[11] and development of the ADAMs as potential AIDS therapeutics has therefore been pursued. During these studies, several ADAM analogues were identified that do not inhibit the enzymatic activity of HIV-1 RT in vitro, but do protect HIV-1-infected cells from the cytopathic effect of the virus at micromolar and submicromolar concentrations. Examples include ADAMs **3** and **4** (Table 1). Inhibition of HIV-1 RT is the ADAMs' usual mechanism of action, and the analogues that exhibit RT-independent antiviral activity must exert their antiviral effects by an alternative mechanism. Efforts have therefore been made to elucidate this unknown mechanism. A variety of alkenyldiarylmethanes that are structurally related to those with anti-HIV activity have been developed at Celgene Corp. as inhibitors of tubulin polymerization, inflammation, and phosphodiesterase 4 enzymatic activity.[15] Structural similarities between the ADAM NNRTIs and Celgene's inhibitors suggested that some of the anti-HIV ADAMs may exhibit additional pharmacological properties besides inhibition of RT. This hypothesis led directly to consideration of inhibition of phosphodiesterase 4 as a potential antiviral mechanism for ADAM analogues that exhibit RT-independent anti-HIV activity.

Studies have shown that infection of a T4 cell by HIV-1 requires the cell to be 'activated', and this immunological response is highly regulated by intracellular levels of cAMP.[16–18] The phosphodiesterase family of hydrolases is one group of enzymes that is responsible for regulating cellular cAMP levels.[19] Expression of the phosphodiesterase 4 (PDE4) family is absolutely required for HIV infection to occur, suggesting that inhibition of PDE4 isoforms by a small molecule is a potential therapeutic strategy for the treatment of AIDS.[16] Indeed, inhibitors of PDE4 isoforms are capable of attenuating the virulence of HIV and it has long been suggested that PDE4 inhibitors be included in the HAART of AIDS.[20] In light of this information, we decided to investigate the inhibitory activities of various ADAMs on PDE4B2, a member of the PDE4 family that has been implicated as a key target for anti-inflammatory agents. The aim of the presently reported investigation was to establish if the ADAMs' RT-independent antiviral mechanism involves the inhibition of the PDE4 family. This communication details the PDE4B2 inhibitory and antiviral activities of ADAMs **1**–**6**.

The syntheses of ADAMs **1**–**6** have been previously published, and thus a brief, general depiction of their construction is outlined in Scheme 1. ADAMs **1**–**4**[8,11,21] were synthesized according to general synthetic Route 1, in which a functionalized salicylic acid **7** was condensed with formaldehyde to afford a diarylmethane intermediate, which was subsequently alkylated and oxidized to obtain benzophenone **8**. Coupling of **8** with a suitably functionalized aldehyde **9** under standard McMurry conditions yielded the desired ADAM analogues **10**. The palladium-catalyzed cross-coupling chemistry depicted in Route 2 was utilized for the syntheses of ADAMs **5**[22] and **6**.[23] Sonogashira coupling of aryl iodide **11** and terminal alkyne **12**, followed by hydrostannation, afforded stannane intermediate **13**. The stannane and aryl iodide **14** were coupled via the Stille reaction to obtain the desired analogues **15**.

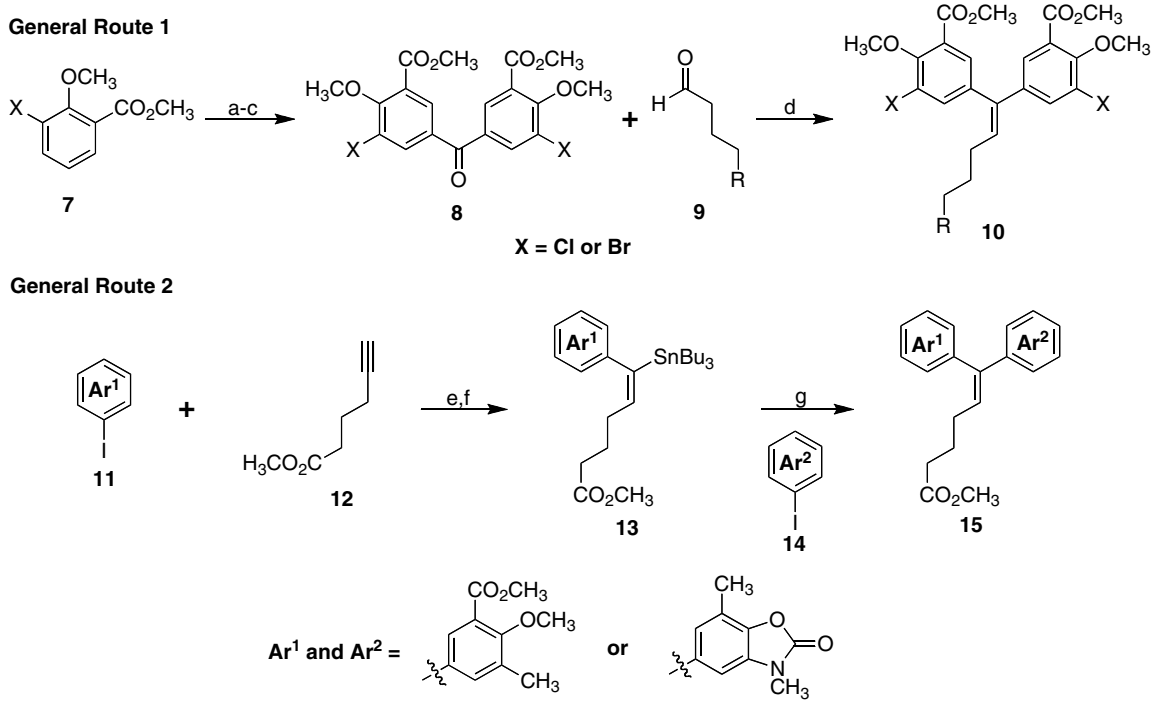



The in vitro PDE4B2 inhibitory data[24] for ADAMs **1**–**6** are presented in Table 1, along with the antiviral data[9,12,25–27] associated with the compounds. Nevirapine is included for antiviral comparisons. Inhibition of PDE4B2 enzymatic activity was observed for four of the six compounds under investigation, suggesting that inhibition of PDE4 isoforms by the ADAMs may be a general phenomenon, given the strong similarities of PDE4 isoforms in sequence homology and structure.[28] However, despite the structural similarities with Celgene's potent PDE4 inhibitors, ADAMs **1**–**6** display either weak PDE4B2 inhibitory activity or no activity, with the most potent PDE4 inhibitor exhibiting 72% inhibition at a concentration of 100 μM.[29–35] One of the main goals of the present study was to determine if inhibition of PDE4 activity was integral to the RT-independent antiviral mechanism of ADAMs such as **3** and **4**. Little to no inhibition of PDE4B2 was observed for compounds **3** and **4** at a concentration 10- to 20-fold higher than their respective cytoprotective efficacies ($EC_{50}$ values). These data are consistent with an RT-independent antiviral mechanism that does not involve the inhibition of PDE4 isoforms. Additionally, although other analogues in this study (**2**, **5**, and **6**) displayed significant inhibition of PDE4B2 at a 100 μM concentration, PDE4 inhibition also does not contribute to their antiviral efficacies, since inhibition of cAMP hydrolysis would be negligible at the minimal concentrations required for ADAMs **2**, **5**, and **6** to protect cells from the cytopathic effect of HIV-1. It is clear that an alternative viral or cellular entity is being targeted when the ADAMs display an RT-independent antiviral mechanism.

**Table 1.** Antiviral and PDE4B2 inhibitory activities of ADAMs **1**–**6**

| Compound | $IC_{50}$[a] (μM) | $EC_{50}$[b] (μM) | | | $CC_{50}$[c] (μM) | | % PDE4 inhibition[d] |
|---|---|---|---|---|---|---|---|
| | | $1_{RF}$ | $1_{IIIB}$ | $2_{ROD}$ | CEM-SS | MT-4 | |
| **1** | NT[e] | 16 | NT[e] | NT[e] | >29 | NT[e] | NI[f] |
| **2** | 0.30 | 0.001 | 0.3 | NA[g] | 13 | 91 | 40 |
| **3** | >100 | 13 | 2.6 | 21 | >200 | >198 | NI[f] |
| **4** | >100 | 5.3 | NT[e] | NT[e] | >20 | NT[e] | 20 |
| **5** | 0.02 | 0.03 | 0.09 | NA[g] | 5.1 | 17 | 72 |
| **6** | 0.5 | 0.62 | 0.22 | NA[g] | 31 | 33 | 71 |
| Nevirapine | 0.084[36] | 0.0015 | 0.053 | NA[g] | NT[e] | 15 | NT[e] |
| Rolipram | NT[e] | NT[e] | NT[e] | NT[e] | NT[e] | NT[e] | 100[g] |

[a] Inhibitory activity versus HIV-1 RT with poly(rC).oligo(dG) as the template primer.
[b] $EC_{50}$ is the concentration required to inhibit 50% of the cytopathic effect of HIV-$1_{RF}$ in CEM-SS cells, HIV-$1_{IIIB}$ in MT-4 cells, or HIV-$2_{ROD}$ in MT-4 cells.
[c] $CC_{50}$ is the cytotoxic concentration required to induce cell death for 50% of the mock-infected CEM-SS or MT-4 cells.
[d] The percent inhibition of PDE4B2 enzymatic activity observed when the compound was tested at a concentration of 100 μM.
[e] Not tested.
[f] No inhibition observed at 100 μM.
[g] The $IC_{50}$ of rolipram is 105 ± 8 nM under the assay conditions used in the present study.




**Scheme 1.** Reagents and conditions: (a) formaldehyde, methanol, 0 °C; (b) $K_2CO_3$, $Me_2SO_4$, acetone, reflux; (c) $CrO_3$, methanol; (d) i—Zn dust, $TiCl_4$·2 THF, THF, reflux; ii—**8**, **9**, THF, reflux; (e) 5 mol% $PdCl_2(PPh_3)_2$, 10 mol% CuI, $Et_3N$, THF; (f) 2 mol% $Pd(PPh_3)_4$, $Bu_3SnH$, THF, 0 °C; (g) 10 mol% $Pd(^tBu_3P)_2$, CsF, toluene, reflux.

In summary, the PDE4B2 inhibitory activities of select ADAMs were determined. The weak to negligible inhibition of PDE4B2 observed for ADAMs **3** and **4** demonstrates that PDE4 isoforms are not integral to these ADAMs' RT-independent antiviral mechanism.

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## References and notes

1. In *AIDS Epidemic Update*; World Health Organization; Joint United Nations Programme on HIV/AIDS (UNAIDS): Geneva, 2006, p 1.

2. Gotte, M.; Kameoka, M.; McLellan, N.; Cellai, L.; Wainberg, M. A. *J. Biol. Chem.* **2001**, *276*, 6711.
3. Kerr, S. G.; Anderson, K. S. *Biochemistry* **1997**, *36*, 14056.
4. Kati, W. M.; Johnson, K. A.; Jerva, L. F.; Anderson, K. S. *J. Biol. Chem.* **1992**, *267*, 25988.
5. De Clercq, E. *Chem. Biodiversity* **2004**, *1*, 44.
6. Tomasselli, A. G.; Heinrikson, R. L. *Biochim. Biophys. Acta Protein Struct. Mol. Enzymol.* **2000**, *1477*, 189.
7. Vivet-Boudou, V.; Didierjean, J.; Isel, C.; Marquet, R. *Cell. Mol. Life Sci.* **2006**, *63*, 163.
8. Cushman, M.; Golebiewski, M.; Buckheit, R. W., Jr.; Graham, L.; Rice, W. G. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2713.
9. Cushman, M.; Golebiewski, W. M.; Graham, L.; Turpin, J. A.; Rice, W. G.; Fliakas-Boltz, V.; Buckheit, R. W., Jr. *J. Med. Chem.* **1996**, *39*, 3217.
10. Casimiro-Garcia, A.; Micklatcher, M.; Turpin, J. A.; Stup, T. L.; Watson, K.; Buckheit, R. W.; Cushman, M. *J. Med. Chem.* **1999**, *42*, 4861.
11. Xu, G.; Micklatcher, M.; Silvestri, M. A.; Hartman, T. L.; Burrier, J.; Osterling, M. C.; Wargo, H.; Turpin, J. A.; Buckheit, R. W., Jr.; Cushman, M. *J. Med. Chem.* **2001**, *44*, 4092.
12. Silvestri, M. A.; Nagarajan, M.; De Clercq, E.; Pannecouque, C.; Cushman, M. *J. Med. Chem.* **2004**, *47*, 3149.
13. Deng, B.-L.; Hartman, T. L.; Buckheit, R. W., Jr.; Pannecouque, C.; De Clercq, E.; Fanwick, P. E.; Cushman, M. *J. Med. Chem.* **2005**, *48*, 6140.
14. Deng, B.-L.; Hartman, T. L.; Buckheit, R. W., Jr.; Pannecouque, C.; De Clercq, E.; Cushman, M. *J. Med. Chem.* **2006**, *49*, 5316.
15. Muller, G. W.; Man, H.-W. United States of America Patent US 2006052596, 20050901, 2006, 1.
16. Sun, Y. L. L.; Lau, F.; Beavo, J. A.; Clark, E. A. *J. Immun.* **2000**, *165*, 1755.
17. Stevenson, M.; Stanwick, T. L.; Dempsey, M. P.; Lamonica, C. A. *EMBO J.* **1990**, *9*, 1551.
18. Zhang, L.-H.; Wu, L.; Raymon, H. K.; Chen, R. S.; Corral, L.; Shirley, M. A.; Krishna Narla, R.; Gamez, J.; Muller, G. W.; Stirling, D. I.; Bartlett, J. B.; Schafer, P. H.; Payvandi, F. *Cancer Res.* **2006**, *66*, 951.
19. Soderling, S. H.; Beavo, J. A. *Curr. Opin. Cell. Biol.* **2000**, *12*, 174.
20. Angel, J. B.; Saget, B. M.; Walsh, S. P.; Greten, T. F.; Dinarello, C. A.; Skolnik, P. R.; Endres, S. *AIDS* **1995**, *9*, 1137.
21. Cushman, M.; Casimiro-Garcia, A.; Hejchman, E.; Ruell, J. A.; Huang, M.; Schaeffer, C. A.; Williamson, K.; Rice, W. G.; Buckheit, R. W., Jr. *J. Med. Chem.* **1998**, *41*, 2076.
22. Deng, B.-L.; Cullen, M. D.; Zhou, Z.; Hartman, T. L.; Buckheit, R. W.; Pannecouque, C.; De Clercq, E.; Fanwick, P. E.; Cushman, M. *Bioorg. Med. Chem.* **2006**, *14*, 2366.
23. Xu, G.; Loftus, T. L.; Wargo, H.; Turpin, J. A.; Buckheit, R. W., Jr.; Cushman, M. *J. Org. Chem.* **2001**, *66*, 5958.
24. Transfection of a plasmid encoding PDE4B[29] was performed using a COS7 SV40-transformed monkey kidney cell line maintained at 37 °C in an atmosphere of 5% $CO_2$/95% air, in complete growth medium containing DMEM supplemented with 0.1% penicillin/streptomycin (10,000 U/mL), glutamine (2 mM), and 10% FCS. As described previously, COS7 cells were transfected using DEAE–dextran. The DNA to be transfected (10 μg) was mixed and incubated for 15 min with 200 μL of 10 mg $mL^{-1}$ DEAE–dextran in PBS to give a 'DNA–dextran' mix.[30–33] When the cells reached 70% confluency, in 100 mm dishes, the medium was removed and the cells were given 10 mL of fresh DMEM containing 0.1 mM chloroquine and the DNA–dextran mix (450 μL). The cells were then incubated for 4 h at 37 °C, after which the medium was removed and the cells were shocked with 10% DMSO in PBS. After PBS washing, the cells were returned to normal growth medium and left for a further two days before use. For determination of PDE activity the cells were homogenized in KHEM buffer (50 mM KCl, 10 mM EGTA, 1.92 mM $MgCl_2$, 1 mM dithiothreitol, 50 mM Hepes, final pH 7.2) containing 'complete' protease inhibitors (Boehringer–Mannheim) of final concentrations 40 μg/mL PMSF, 156 μg/mL benzamine, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin A, and 1 μg/mL antipain. In such transfected cells, >98% of the total PDE activity is due to the recombinant PDE4 isoform.[30–33]
PDE4 inhibitory activity, using 1 μM cAMP as substrate, was assayed as described previously.[32,34,35] All assays were conducted at 30 °C, and, in all experiments, a freshly prepared slurry of Dowex/$H_2O$/ethanol (1:1:1) was used. Initial rates were taken from linear time courses of activity. Dose-dependent inhibition by inhibitor compounds was determined in the presence of 1 μM concentrations of cAMP over a range of inhibitor compound concentrations. Inhibitor compounds were dissolved in 100% dimethylsulfoxide (DMSO) as a 1 mM stock and diluted in 20 mM Tris–Cl, pH 7.4, 10 mM $MgCl_2$ to provide a range of concentrations in the assay. The residual levels of DMSO were shown not to affect PDE activity over the ranges used in this study.
25. Rice, W. G.; Bader, J. P. *Adv. Pharmacol. (San Diego)* **1995**, *33*, 389.
26. Pauwels, R. B. J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309.
27. The ability of target compounds to inhibit the enzymatic activity of recombinant HIV-1 RT (p66/51 dimer) was evaluated as previously described.[9] Evaluation of antiviral activity against HIV-$1_{RF}$ was performed in infected CEM-SS cells while using the XTT cytoprotection assay, as previously described.[9] Evaluation of antiviral activity against the HIV-$1_{IIIB}$ and HIV-$2_{ROD}$ strains was performed in infected MT-4 cells using the previously described MTT assay.[13]
28. Manallack, D. T.; Hughes, R. A.; Thompson, P. E. *J. Med. Chem.* **2005**, *48*, 3449.
29. Cheung, Y.-F.; Kan, Z.; Garrett-Engele, P.; Gall, I.; Murdoch, H.; Baillie, G. S.; Camargo, L. M.; Johnson, J. M.; Houslay, M. D.; Castle, J. C. In *J. Pharmacol. Exp. Ther.*, **2007**, *322*, 600.
30. Huston, E.; Lumb, S.; Russell, A.; Catterall, C.; Ross, A. H.; Steele, M. R.; Bolger, G. B.; Perry, M. J.; Owens, R. J.; Houslay, M. D. *Biochem. J.* **1997**, *328*, 549.
31. Huston, E.; Pooley, L.; Julien, P.; Scotland, G.; McPhee, I.; Sullivan, M.; Bolger, G.; Houslay, M. D. *J. Biol. Chem.* **1996**, *271*, 31334.
32. Rena, G.; Begg, F.; Ross, A.; MacKenzie, C.; McPhee, I.; Campbell, L.; Huston, E.; Sullivan, M.; Houslay, M. D. *Mol. Pharmacol.* **2001**, *59*, 996.
33. Wallace, D. A.; Johnston, L. A.; Huston, E.; MacMaster, D.; Houslay, T. M.; Cheung, Y.-F.; Campbell, L.; Millen, J. E.; Smith, R. A.; Gall, I.; Knowles, R. G.; Sullivan, M.; Houslay, M. D. *Mol. Pharmacol.* **2005**, *67*, 1920.
34. Marchmont, R. J.; Houslay, M. D. *Biochem. J.* **1980**, *187*, 381.
35. Sullivan, M.; Rena, G.; Begg, F.; Gordon, L.; Olsen, A. S.; Houslay, M. D. *Biochem. J.* **1998**, *333*, 693.
36. Hargrave, K. D.; Proudfoot, J. R.; Grozinger, K.; Cullen, E.; Kapadia, S. R.; Patel, U. R.; Fuchs, V. U.; Mauldin, S. C.; Vitous, J.; Behnke, M. L.; Klunder, J. M.; Plal, K.; Skiles, J. W.; McNeil, D. W.; Rose, J. M.; Chow, G. C.; Skoog, M. T.; Wu, J. C.; Schmidt, G.; Engel, W. W.; Eberlein, W. G.; Saboe, T. D.; Campbell, S. J.; Rosenthal, A. S.; Adams, J. *J. Med. Chem.* **1991**, *34*, 2231.