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SYNTHESIS OF A NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR IN THE ALKENYLDIARYLMETHANE (ADAM) SERIES WITH OPTIMIZED POTENCY AND THERAPEUTIC INDEX

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Abstract: A novel alkenyldiarylmethane (ADAM) analog has been synthesized with enhanced potency as an anti-HIV agent. The new compound (ADAM II) inhibits the cytopathic effect of HIV-1_{RF} in CEM-SS cells with an EC_{50} of 13 nM, while it shows cytotoxicity with a CC_{50} of 31.6 μM, providing a therapeutic index of 2430. ADAM II is a non-nucleoside reverse transcriptase inhibitor, displaying an IC_{50} of 0.3 μM with poly(rC)·oligo(dG) as the template/primer.

The non-nucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs) constitute a diverse array compounds that act by an allosteric mechanism involving binding to a pocket adjacent to the deoxyribonucleoside triphosphate binding site.[1,2] Familiar examples of NNRTIs include hydroxyethoxymethylphenylthiothymine (HEPT), tetrahydroimidazobenzodiazepinone (TIBO), dipyridodiazepinone (nevirapine), pyridinone, bis(heteroaryl)piperazine (BHAP), *tert*-butyldimethylsilylspiroaminooxathiole dioxide (TSAO), and α-anilinophenylacetamide (α-APA) derivatives. Nevirapine has recently been approved for clinical use as an anti-AIDS agent.[3] The problem posed by the emergence of resistant viral strains that always result from NNRTI administration is being addressed by various strategies, including switching to another NNRTI, using NNRTI combinations that elicit mutations that counteract one another, and combining NNRTIs with nucleoside RTIs.[4,5] New NNRTIs that cause unique patterns of resistance mutations will be useful in defining the potential clinical utility of these new strategies.

We recently reported the synthesis and biological evaluation of NNRTIs in the alkenyldiarylmethane (ADAM) series that inhibit the cytopathic effect of a wide variety of HIV-1 strains in CEM, MT-4, and monocyte-macrophage cultures.[6,7] The most potent of these compounds proved to be ADAM **1**, which displayed anti-HIV activity vs. a wide range of HIV-1 isolates and was synergistic with AZT. However, the potency of ADAM **1** was lower than generally expected with most NNRTIs, ranging from 0.56 μM vs. HIV-1_{6S} in MT-4 cells to 151 μM vs. HIV-1_{N119} in MT-4 cells. Additional research was therefore undertaken in order to determine whether or not the anti-HIV activity could be optimized.

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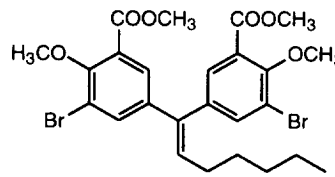

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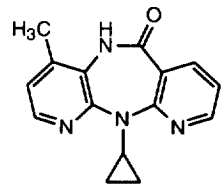

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In order to facilitate the design of additional ADAM analogs, a hypothetical model was constructed of the binding of ADAM **1** to HIV-1 reverse transcriptase. This hypothetical model was derived by overlaying the structure of ADAM **1** "on top" of the ligand in the crystal structure of nevirapine (**2**) bound to RT, so that the initial conformation of the ligand **1** would resemble the structure of bound nevirapine.[8] During this process, the two aromatic rings of the ligand **1** were overlayed on the two pyridine rings of nevirapine, with the hexenyl side chain of **1** pointing in the same direction as the cyclopropyl group of **2**. The structure of nevirapine was then removed, the protein was "frozen", and the energy of the resulting ADAM-RT complex was minimized while the ligand was allowed to move. The validity of this procedure is supported by the fact that nevirapine, α-APA, and TIBO all bind to HIV-1 in a nearly identical mode which conforms to a general "butterfly" structural model for NNRTIs.[9,10] The model is also supported by our prior mutagenesis studies of the ADAM binding site on HIV-1 reverse transcriptase, in which it was determined that the resistance mutations to ADAM **1** circumscribe a well-defined binding pocket.[6] This procedure resulted in the stereomodel displayed in Figure 1.

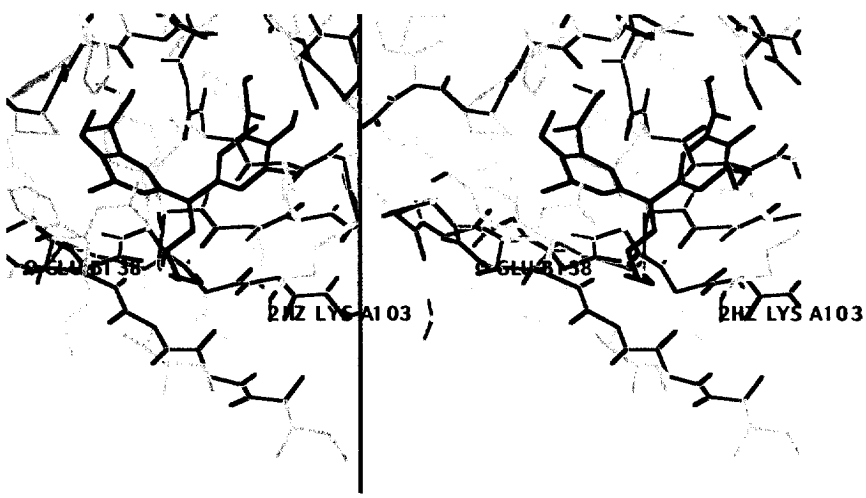
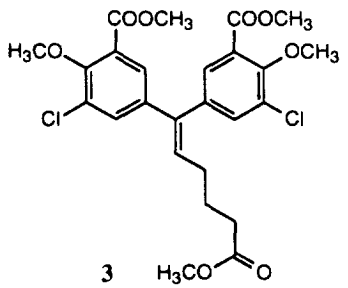


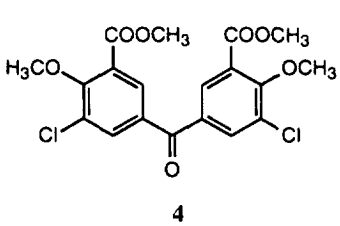
Figure 1. Hypothetical model of ADAM **1** docked in the NNRTI binding site of HIV-1 reverse transcriptase (programmed for walleyed viewing).

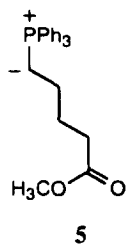
The ligand **1**, Figure 1, is viewed looking down the alkenyl side chain with the end of the chain in front. According to this model, the end of the side chain is located near Glu 138 and Lys 103. Both of these residues contain side chains with functional groups that are capable of acting as hydrogen bond donors. It might be

expected that the incorporation of functional groups at the end of the alkenyl chain of the ligand which are capable of acting as hydrogen bond acceptors might interact favorably with the terminal amino group of Lys 103 or the terminal carboxylic acid group of Glu 138. Based on these findings, it was decided to synthesize an ADAM analog incorporating a methyl ester at the end of the chain.

The desired analog ADAM II (**3**) was prepared in one step from known starting materials. Reaction of the substituted benzophenone **4**[7] with the Wittig reagent **5**[11] in THF at -78 °C for 12 h followed by room temperature for 12 h afforded the desired compound **3** in 45% isolated yield as a pale yellow oil after silica gel chromatography, eluting with hexane:ethyl acetate 3:1.







ADAM II (**3**) was tested for prevention of the cytopathic effect of HIV-1_{RF} in CEM-SS cells. The EC_{50} for prevention of the cytopathic effect of the virus in cell culture was 13 nM, while the CC_{50} for cytotoxicity in uninfected cells was 31.6 μM. This corresponds to a therapeutic index (CC_{50}/EC_{50}) of 2430. ADAM II was also tested as an inhibitor of HIV-1 reverse transcriptase. With poly(rC)·oligo(dG) as the template/primer, ADAM II inhibited the enzyme with an IC_{50} of 0.3 μM, while with poly(rA)·oligo(dT) as the template/primer, the compound exerted an IC_{50} of 1.9 μM. The greater sensitivity to inhibition with poly(rC)·oligo(dG) as the template primer is characteristic of the non-nucleoside HIV-1 reverse transcriptase inhibitors.[12-17] In order to confirm that ADAM II (**3**) was indeed acting as an NNRTI, it was tested in a number of assays representative of important events in the replication of HIV-1. The results indicated that ADAM II (**3**) does not have any significant effect on virus attachment or fusion, integrase, protease, or nucleocapsid protein zinc fingers.

There is a significant difference in potency between **1** and **3** for prevention of the cytopathic effect of HIV-1 in cell culture. For ADAM **1**, the EC_{50} for the prevention of the cytopathic effect of HIV-1_{RF} in CEM-SS cells proved to be 9.2 μM, while the CC_{50} for cytotoxicity in uninfected CEM-SS cells was 138 μM. This corresponds to a therapeutic index (CC_{50}/EC_{50}) of only 15 for **1** vs. 2430 for **3**. The anti-HIV potency of ADAM II (**3**) is greater than that of **1** by a factor of 708. The terminal methoxycarbonyl group present on the side chain of ADAM II (**3**) may in fact be acting as a hydrogen bond acceptor from Glu 138 or Lys 103. However, a definitive answer to this question would have to await the crystal structure of the enzyme complexed with the bound ligand. The difference in activity is most likely not due to the dichloro substitution in **3** vs. the dibromo substitution in **1**, since the analog of ADAM in which the two bromines are replaced by chlorines is less active than **1** vs. HIV-1_{RF} in CEM cells.

The in vitro IC_{50} for compound **3** vs. HIV-1 reverse transcriptase with poly(rC)·oligo(dG) as the template/primer (0.3 μM) is significantly higher than its EC_{50} for inhibition of the cytopathic effect of HIV-1_{RF} in

CEM-SS cell culture (13 nM). This difference is not unusual for the NNRTIs.[12,14-16] As discussed elsewhere, this discrepancy may simply reflect the differences between the in vitro assay, in which synthetic template/primer has been added, and the cellular system.[15]

Future work will concentrate on defining the resistance mutations to ADAM II and also investigating the anti-HIV activity of ADAM II in animal models.

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