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Synthesis of alkenyldiarylmethanes (ADAMs) containing benzo [d]isoxazole and oxazolidin-2-one rings, a new series of potent non-nucleoside HIV-1 reverse transcriptase inhibitors

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Abstract

As a continuation of efforts to replace the metabolically labile methyl esters of the lead alkenyldiarylmethanes (ADAMs) with stable bioisosteres, compounds bearing benzo[d]isoxazole and oxazolidine-2-one rings were designed and evaluated as a new series of potent HIV-1 non-nucleoside reverse transcriptase inhibitors with anti-HIV activity. All of the resulting ADAMs were found to inhibit HIV-1 RT with poly(rC)-oligo(dG) as the template primer. The most promising compound in this series was ADAM **3**, with EC₅₀ values of 40 nM (vs HIV-1_{RF}) and 20 nM (vs HIV-1_{IIIB}). Compound **3** also inhibited HIV-1 reverse transcriptase with an IC₅₀ of 0.91 μM. ADAM **4** has an antiviral EC₅₀ of 0.6 μM in CEM-SS cells and a plasma half-life of 51.4 min.

Keywords

alkenyldiarylmethanes (ADAMs); anti-HIV; non-nucleoside reverse transcriptase inhibitors (NNRTIs)

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) is the etiological agent of acquired immunodeficiency syndrome (AIDS), one of the world's most serious health problems with about 33 million people infected worldwide in 2007. The reverse transcriptase (RT) of HIV-1 is an essential enzyme in HIV replication and has been a key target in anti-AIDS drug discovery. The non-nucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine, delavirdine, and efavirenz have been approved by the Food and Drug Administration (FDA) for the treatment of AIDS [1]. They are very useful drugs in combination therapy with nucleoside analogs (NRTIs) and protease inhibitors (PIs) [2,3] for the treatment of AIDS. However, a number of problems still remain with these agents. In particular, significant resistance has developed against these drugs. Therefore, considerable effort has been expended to develop new NNRTIs

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that would overcome the current drug resistance. More than 30 structurally different classes of molecules have been reported as NNRTIs [1–3]. Recently, the NNRTI etravirine was approved by the FDA for treatment of antiretroviral drug-resistant HIV infections.

The alkenyldiarylmethanes (ADAMs) are a unique class of potent and highly specific HIV NNRTIs [4–15]. Certain ADAMs have been found to inhibit the cytopathic effect of HIV-1 in cell culture at low nanomolar concentrations. In addition, some of the ADAMs have displayed synergistic activity with AZT and have shown enhanced activity when tested against AZT-resistant strains of HIV-1 [5]. Unfortunately, most of the ADAMs are too unstable toward hydrolysis in blood plasma to be interesting as possible therapeutic candidates [12]. Therefore, one of the current research objectives is to optimize the ADAMs by replacing the metabolically unstable methyl ester moieties with stable isosteres while maintaining the antiviral potency.

Recently, we synthesized ADAM **1** bearing a 3-fluoro-5-trifluoromethylphenyl group and a benzo[*d*]isoxazole ring, which was inactive as an inhibitor of the cytopathic effect of HIV-1_{RF} in CEM-SS cells and HIV-1_{IIB} in MT-4 cells [13]. However, replacement of the 3-fluoro-5-trifluoromethylphenyl group in compound **1** with an *N*-methyl benzoxazolone ring resulted in ADAM **2**, which displayed significant antiviral activity but was unstable in rat blood plasma [14]. Compound **2** inhibited the cytopathic effects of two strains of HIV-1 (RF and IIB) in cell cultures with values EC₅₀ of 260 nM (vs HIV-1_{RF} in CEM-SS cells) and 330 nM (vs HIV-1_{IIB} in MT-4 cells), and it also inhibited HIV-1 reverse transcriptase with an IC₅₀ of 250 nM. Encouraged by the strong anti-HIV activity of compound **2**, a new series of ADAMs bearing a benzo[*d*]isoxazole ring were designed and synthesized to investigate the structure-activity relationships of ADAMs. First of all, it was hypothesized that compound **3**, having a methyl ester on the aryl ring, would possess anti-HIV activity. Compound **4**, in which the methyl ester moiety in the side chain of the ADAM **3** is replaced with an oxazolidin-2-one group, might have greater stability to plasma esterases. It is established that the stereochemistry of the alkene in the ADAM system has a strong influence on the anti-HIV activity [15]. Therefore, compound **5**, an isomeric analogue of compound **4**, was also considered. Incorporation of halogens in the inhibitors has been shown in various examples to result in biologically active RT inhibitors [13,15,16]. Accordingly, the replacement of the methyl group in compound **5** with a chlorine atom, as shown in compound **6**, was also planned. It would also be very interesting to synthesize ADAM analogue **7**, having two identical benzo[*d*]isoxazole rings. A number of ADAM analogues incorporating an *N*-methyl benzoxazolone ring inhibit HIV-1 RT with submicromolar IC₅₀ values and exhibit anti-HIV-1 activity in low micromolar to submicromolar concentration ranges in cellular assays [14]. Therefore, replacement of the methyl ester moieties of compound **5** with an *N*-methyl benzoxazolone ring, as shown in compound **8**, was also considered. ADAMs having a cyanophenyl trans to the side chain generally retain or increase the inhibitory activity [13]. ADAM **9**, having a cyanophenyl trans to the side chain, was therefore synthesized.

2. Chemistry

The syntheses of these compounds were accomplished via the Stille cross-coupling of an arylstannane with an aryl iodide or bromide in the presence of Pd(PBu₃)₂ and CsF in toluene at reflux temperature (Scheme 1). This method is a general and practical one to synthesize alkenyldiarylmethanes having non-identical aromatic substituents and has been used to synthesize number of ADAM analogues [13–15]. The key intermediate, 5-iodo-3-methoxy-7-methylbenzo[*d*]isoxazole (**12**), was prepared in a multi-step fashion from 3-methyl salicylic acid (**10**) via methyl 2-hydroxy-5-iodo-3-methylbenzoate (**11**) as described previously [13]. The syntheses of aryl stannanes **13–15** and aryl iodides **16–18** have also been reported previously [13,15]. Compounds **3** and **4** were synthesized from the same iodide **12** with different vinylstannanes **13** and **14** by the Stille cross-coupling reaction in the presence of Pd

(PBU^t₃)₂ and CsF in toluene at reflux temperature. The same reaction conditions were employed to synthesize ADAMs **5–9**.

3. Biological results and discussion

The cytotoxicities of the newly synthesized ADAMs were determined along with their abilities to inhibit the cytopathic effect of HIV-1 in cell culture. The inhibition of HIV-1 RT by the ADAMs, and their metabolic stabilities in rat plasma were also investigated. The data are shown in Table 1. Five of the nine ADAMs (compounds **1–4** and **7**) were found to inhibit HIV-1_{RT} with poly(rC)-oligo(dG) as the template primer with IC₅₀ values ranging from 0.25 to 0.93 μM. The others had intermediate IC₅₀ values from 3.60 to 39.2 μM. Only compounds **2**, **3** and **4** inhibited the cytopathic effect of the virus at concentrations that were not cytotoxic. ADAM **3** was the most promising compound, and exhibited very potent inhibitory activity against the cytopathic effect of HIV-1 with EC₅₀ values of 40 nM (vs HIV-1_{RF} in CEM-SS cells) and 20 nM (vs HIV-1_{IIIB} in MT-4 cells) [17]. The EC₅₀ of compound **3** was lower than the IC₅₀. In this case, the discrepancy may simply reflect the differences between the in vitro enzymatic assay, which uses a synthetic template/primer, and the cellular system, in which natural template/primers are utilized by RT [17]. Although the replacement of the metabolically unstable methyl ester moieties in the ADAM system with metabolically stable bioisosteres led to several very stable compounds, such as ADAMs **7–9**, they turned out to be inactive as antiviral agents. However, active compound **4** (EC₅₀ = 0.6 μM) had improved stability relative to ADAMs **1–3** in rat plasma with a half-life value of 51.4 min. Replacement of the side chain in compound **3** (EC₅₀ = 40 nM) with an oxazolidinonyl group afforded compound **4** (EC₅₀ = 600 nM) with a significant drop in antiviral potency. These results suggest further modification of the side chain of the ADAMs with other methyl esters bioisosteres [18,19,20].

The ADAMs **1–4**, having the alkenyl side chain trans to the benzo[*d*]isoxazole ring, were submicromolar inhibitors of HIV-1 RT, while those with a cis relationship (ADAMs **5**, **6**, **8** and **9**) were much less active. This structure-activity trend carried over to their antiviral activities as well. DAM **7**, having benzy[*d*]isoxazole rings both cis and trans to the alkenyl side chain, was active as an RT inhibitor but was inactive as an antiviral agent.

4. Conclusion

A series of alkenyldiarylmethanes (ADAMs) with a benzo[*d*]isoxazole ring in place of metabolically unstable methyl ester moiety and an adjacent methoxyl group were synthesized using the Stille cross coupling of a vinylstannane with an aryl halide. The anti-HIV activities and metabolic stabilities of these compounds were investigated. All of the resulting ADAMs were found to inhibit HIV-1 RT with poly(rC)-oligo(dG) as the template primer. Among these, ADAM **3** also exhibited anti-HIV-1 activity with EC₅₀ values in the 20–40 nanomolar range. These initial results indicate that the benzo[*d*]isoxazole ring is an effective bioisosteric replacement of the metabolically labile methyl ester moiety in ADAMs. The replacement of methyl esters with fused benzo[*d*]isoxazole and oxazolidine-2-one rings could prove to be generally useful in situations that require alternatives to hydrolytically unstable methyl esters.

5. Experimental

Melting points were obtained in capillary tubes and uncorrected. NMR spectra were obtained at 300 MHz (¹H) and 75 MHz (¹³C) in CDCl₃ using CHCl₃ as the internal standard. IR spectra were recorded using a Perkin-Elmer 1600 series FT-IR. Electrospray mass spectra were obtained using a FinniganMATT LCQ (Thermoquest Corp., San Jose, CA) instrument at the Purdue Campus-Wide Mass Spectrometry Center. Microanalyses were performed at the Purdue University Microanalysis Laboratory. All yields given refer to isolated yields. Flash

chromatography was performed with 230–400 mesh silica gel. TLC was carried out using Baker-flex silica gel IB2-F plates of a 2.5 mm thickness. Unless otherwise stated, chemicals and solvents were of reagent grade and used as obtained from commercial sources without further purification. Lyophilized rat plasma (lot 052K7609) was obtained from Sigma Chemical Co., St. Louis, MO.

5.1. General procedure for the synthesis of alkenyldiarylmethanes by the Stille cross-coupling reaction of vinylstannanes with aromatic iodides or bromides

A mixture of vinylstannane (1 equiv), aryl halide (1.2–1.5 equiv), cesium fluoride (3.0–4.5 equiv), and $\text{Pd}(\text{P}^t\text{Bu}_3)_2$ (~10 mol%) in toluene (1 mL) was stirred under argon at different temperatures for various periods of time. The reaction mixture was cooled to room temperature, filtered through a short column of silica gel (5 g), and the column was washed with ethyl acetate. The organic solution was concentrated.

5.1.1. (Z)-2-Methoxy-5-[5-methoxycarbonyl-1-(3-methoxy-7-methylbenzo[d]isoxazol-5-yl)-pent-1-enyl]-3-methylbenzoic acid methyl ester (**3**)—

The general procedure was followed using methyl 6-(tributylstannyl)-6-[4-methoxy-5-methoxycarbonyl-3-methylphenyl]-hex-5-enoate (**13**) (349 mg, 0.59 mmol), 5-iodo-3-methoxy-7-methylbenzo[d]isoxazole (**12**) (253 mg, 0.88 mmol), cesium fluoride (320 mg, 2.09 mmol), and $\text{Pd}(\text{P}^t\text{Bu}_3)_2$ (39 mg, 0.08 mmol). The mixture was stirred at room temperature for 5 h, at 60 °C for 16 h, at 80 °C for 8 h, and at 110 °C for 16.5 h. The residue was purified by column chromatography on silica gel (15 g), eluting with EtOAc-hexanes (0–10%), to afford the product **3** (91 mg) as an oil in 33% yield. IR (KBr) 2950, 1732, 1548, 1496, 1436, 1395, 1318, 1255, 1203, 1141, 1009, 910, 765 cm^{-1} ; ^1H NMR δ 7.41 (d, $J = 2.1$ Hz, 1 H), 7.18 (broad singlet, 1 H), 7.16 (broad singlet, 1 H), 7.10 (broad singlet, 1 H), 5.97 (t, $J = 7.4$ Hz, 1 H), 4.13 (s, 3 H), 3.89 (s, 3 H), 3.86 (s, 3 H), 3.61 (s, 3 H), 2.45 (s, 3 H), 2.31 (s, 3 H), 2.34–2.29 (m, 2 H), 2.18–2.11 (dt, $J = 7.4$ Hz, 2 H), 1.83–1.73 (pentet, $J = 7.4$ Hz, 2 H); ^{13}C NMR δ 173.83, 167.32, 166.73, 162.64, 157.40, 140.64, 138.41, 136.28, 135.11, 132.76, 130.46, 130.25, 129.54, 124.31, 120.38, 116.56, 113.54, 61.49, 57.26, 52.16, 51.46, 33.43, 29.12, 24.99, 16.13, 14.65; ESIMS m/z (rel intensity) 467.99 (MH^+ , 100). Anal. Calcd for $\text{C}_{26}\text{H}_{29}\text{NO}_7$: C, 66.80; H, 6.25; N, 3.00. Found: C, 66.52; H, 6.32; N, 3.05.

5.1.2. (Z)-2-Methoxy-5-[1-(3-methoxy-7-methylbenzo[d]isoxazol-5-yl)-4-(2-oxo-oxazolidin-3-yl)-but-1-enyl]-3-methylbenzoic acid methyl ester (**4**)—

The general procedure was followed using 1-(tributylstannanyl)-2-methoxy-3-methyl-5-[4-(2-oxo-oxazolidin-3-yl)-but-1-enyl] benzoic acid methyl ester (**14**) (329 mg, 0.54 mmol), 5-iodo-3-methoxy-7-methylbenzo[d]isoxazole (**12**) (232 mg, 0.80 mmol), cesium fluoride (320 mg, 2.09 mmol), and $\text{Pd}(\text{P}^t\text{Bu}_3)_2$ (28 mg, 0.05 mmol). The mixture was stirred at room temperature for 22.5 h, at 60 °C for 29 h, and at 110 °C for 19.5 h. The residue was purified by column chromatography on silica gel (30 g), eluting with EtOAc-hexanes (0–50%) to afford the product **4** (161 mg) as foam in 62% yield: mp 53–54 °C. IR (KBr) 2947, 1755, 1729, 1614, 1548, 1495, 1435, 1396, 1318, 1263, 1229, 1204, 1146, 1121, 1044, 1009, 911, 800, 763, 732, 686 cm^{-1} ; ^1H NMR δ 7.36 (d, $J = 1.2$ Hz, 1 H), 7.15 (broad singlet, 1 H), 7.10 (broad singlet, 1 H), 7.07 (d, $J = 1.2$ Hz, 1 H), 5.92 (t, $J = 7.5$ Hz, 1 H), 4.19 (t, $J = 7.8$ Hz, 2 H), 4.05 (s, 3 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.33 (t, $J = 6.6$ Hz, 2 H), 3.30 (t, $J = 7.8$ Hz, 2 H), 2.38 (s, 3 H), 2.34 (dt, $J = 7.5$ Hz and 6.6 Hz, 2 H), 2.26 (s, 3 H); ^{13}C NMR δ 167.17, 166.50, 162.50, 158.32, 157.40, 142.08, 137.92, 136.03, 134.77, 132.90, 130.45, 129.89, 126.06, 124.34, 120.35, 116.72, 113.41, 61.43, 57.10, 52.09, 44.16, 43.75, 27.79, 16.00, 14.51; ESIMS m/z (rel intensity) 481.01 (MH^+ , 100), 503.12 (MNa^+ , 55). Anal. Calcd for $\text{C}_{26}\text{H}_{28}\text{N}_2\text{O}_7$: C, 64.99; H, 5.87; N, 5.83. Found: C, 64.91; H, 5.97; N, 5.60.

5.1.3. (E)-2-Methoxy-5-[1-(3-methoxy-7-methylbenzo[d]isoxazol-5-yl)-4-(2-oxoxazolidin-3-yl)-but-1-enyl]-3-methylbenzoic acid methyl ester (5)—The general procedure was followed using 3-[4-(tributylstannanyl)-4-(3-methoxy-7-methylbenzo[d]isoxazol-5-yl)-but-3-enyl]-oxazolidin-2-one (**15**) (404 mg, 0.68 mmol), 5-iodo-2-methoxy-3-methylbenzoic acid methyl ester (**16**) (265 mg, 0.87 mmol), cesium fluoride (324 mg, 2.11 mmol), and Pd(PBu^t₃)₂ (42 mg, 0.05 mmol). The mixture was stirred at room temperature for 23 h, at 60 °C for 24 h, and at 110 °C for 23 h. The residue was purified by column chromatography on silica gel (20 g), eluting with EtOAc-hexanes (0–50%), to afford the product **5** (81 mg) as an oil in 25% yield. IR (KBr) 2946, 1752, 1727, 1615, 1548, 1498, 1425, 1317, 1266, 1208, 1142, 1043, 1007, 910, 764 cm⁻¹; ¹H NMR δ 7.37 (d, *J* = 2.4 Hz, 1 H), 7.22 (s, 1 H), 7.13 (d, *J* = 2.4 Hz, 1 H), 7.04 (s, 1 H), 5.99 (t, *J* = 7.5 Hz, 1 H), 4.23 (t, *J* = 7.5 Hz, 2 H), 4.15 (s, 3 H), 3.85 (s, 3 H), 3.78 (s, 3 H), 3.35 (t, *J* = 6.9 Hz, 2 H), 3.31 (t, *J* = 7.5 Hz, 2 H), 2.48 (s, 3 H), 2.34 (dt, *J* = 6.9 Hz and 7.5 Hz, 2 H), 2.24 (s, 3 H); ¹³C NMR δ 167.14, 166.76, 162.47, 158.29, 157.47, 142.07, 137.51, 134.55, 133.69, 132.44, 130.50, 127.62, 125.97, 124.20, 121.03, 118.71, 113.65, 61.47, 61.37, 57.27, 52.09, 44.24, 43.82, 27.81, 16.02, 14.58; ESIMS *m/z* (rel intensity) 480.75 (MH⁺, 100). Anal. Calcd for C₂₆H₂₈N₂O₇: C, 64.99; H, 5.87; N, 25.83. Found: C, 65.24; H, 5.92; N, 5.59.

5.1.4. (E)-3-Chloro-2-methoxy-5-[1-(3-methoxy-7-methyl-benzo[d]isoxazol-5-yl)-4-(2-oxoxazolidin-3-yl)-but-1-enyl]-benzoic acid methyl ester (6)—The general procedure was followed using the vinylstannane **15** (385 mg, 0.65 mmol), 3-chloro-5-iodo-2-methoxybenzoic acid methyl ester (**17**) (266 mg, 0.82 mmol), cesium fluoride (410 mg, 2.67 mmol), and Pd(PBu^t₃)₂ (33 mg, 0.06 mmol). The mixture was stirred under argon at room temperature for 25 h, at 65 °C for 24 h, and at 100 °C for 6 h. The residue was purified by column chromatography on silica gel (20 g), eluting with EtOAc-hexanes (0–50%), to afford the product **6** (225 mg) as white foam in 69% yield: mp 59–60 °C. IR (KBr) 2946, 1749, 1616, 1548, 1498, 1476, 1425, 1316, 1263, 1206, 1096, 1043, 998, 911, 765, 733, 663 cm⁻¹; ¹H NMR δ 7.46 (d, *J* = 2.4 Hz, 1 H), 7.30 (t, *J* = 2.4 Hz, 1 H), 7.21 (s, 1 H), 7.03 (s, 1 H), 6.03 (t, *J* = 7.5 Hz, 1 H), 4.22 (t, *J* = 7.8 Hz, 2 H), 4.13 (s, 3 H), 3.90 (s, 3 H), 3.86 (s, 3 H), 3.35 (t, *J* = 6.9 Hz, 2 H), 3.31 (t, *J* = 7.8 Hz, 2 H), 2.49 (s, 3 H), 2.35 (dt, *J* = 6.9 Hz and 7.5 Hz, 2 H); ESIMS *m/z* (rel intensity) 522.82/524.78 (MNa⁺, 100/32). Anal. Calcd for C₂₅H₂₅ClN₂O₇: C, 59.84; H, 5.03; N, 5.59, Cl, 7.08. Found: C, 59.86; H, 5.23; N, 5.70, Cl, 7.01.

5.1.5. 3-[4,4-Bis-(3-methoxy-7-methyl-benzo[d]isoxazol-5-yl)-but-3-enyl]-oxazolidin-2-one (7)—The general procedure was followed using the vinylstannane **15** (392 mg, 0.66 mmol), 5-iodo-3-methoxy-7-methylbenzo[d]isoxazole (**12**) (243 mg, 0.84 mmol), cesium fluoride (350 mg, 2.28 mmol), and Pd(PBu^t₃)₂ (38 mg, 0.07 mmol) in toluene (1 mL). The mixture was stirred under argon at room temperature for 23 h, at 65 °C for 24 h, and at 110 °C for 23 h. The residue was purified by column chromatography on silica gel (20 g), eluting with EtOAc-hexanes (0–50%), to afford the product **7** (51 mg) as an oil in 17% yield. IR (KBr) 2942, 1750, 1614, 1548, 1497, 1425, 1387, 1354, 1315, 1267, 1225, 1105, 1043, 954, 911, 764, 732, 695, 642 cm⁻¹; ¹H NMR δ 7.24 (s, 1 H), 7.18 (s, 1 H), 7.12 (s, 1 H), 7.05 (s, 1 H), 6.01 (t, *J* = 7.2 Hz, 1 H), 4.22 (t, *J* = 7.8 Hz, 2 H), 4.12 (s, 3 H), 4.07 (s, 3 H), 3.37 (t, *J* = 9 Hz, 2 H), 3.29 (t, *J* = 7.8 Hz, 2 H), 2.47 (s, 3 H), 2.42 (s, 3 H), 2.36 (dt, *J* = 6.9 Hz and 7.2 Hz, 2 H); ¹³C NMR δ 167.27, 167.16, 162.68, 162.50, 158.35, 142.58, 138.20, 135.03, 132.48, 130.45, 126.00, 121.11, 120.48, 118.77, 116.79, 113.70, 113.52, 61.49, 57.32, 57.18, 44.20, 43.84, 27.84, 14.61; ESIMS *m/z* (rel intensity) 486.18 (MNa⁺, 31), 464.16 (MH⁺, 29). Anal. Calcd for C₂₅H₂₅N₃O₆: C, 67.79; H, 5.44; N, 9.07. Found: C, 64.40; H, 5.52; N, 8.80.

5.1.6. (E)-5-[1-(3-Methoxy-7-methylbenzo[d]isoxazol-5-yl)-4-(2-oxoxazolidin-3-yl)-but-1-enyl]-3,7-dimethyl-3H-benzoxazol-2-one (8)—The general procedure was followed using the vinylstannane **15** (390 mg, 0.66 mmol), 5-iodo-3,7-dimethyl-3H-

benzoxazol-2-one (**18**) (229 mg, 0.79 mmol), cesium fluoride (355 mg, 2.31 mmol), and Pd(PBu^t₃)₂ (35 mg, 0.07 mmol) in toluene (1 mL). The mixture was stirred under argon at room temperature for 21 h, at 60 °C for 24 h, and at 110 °C for 25 h. The residue was purified by column chromatography on silica gel (20 g), eluting with EtOAc-hexanes (0–50%), to afford the product **8** (140 mg) as solid in 46% yield: mp 187–188 °C. IR (KBr) 2925, 1775, 1618, 1548, 1496, 1448, 1426, 1359, 1333, 1305, 1267, 1226, 1153, 1104, 1065, 1043, 973, 955, 912, 881 cm⁻¹; ¹H NMR δ 7.23 (s, 1 H), 7.05 (s, 1 H), 6.67 (s, 1 H), 6.64 (s, 1 H), 5.97 (t, *J* = 7.5 Hz, 1 H), 4.21 (t, *J* = 8.1 Hz, 2 H), 4.14 (s, 3 H), 3.37 (t, *J* = 6.6 Hz, 2 H), 3.32 (s, 3 H), 3.25 (t, *J* = 8.1 Hz, 2 H), 2.48 (s, 3 H), 2.39 (dt, *J* = 6.6 Hz and 7.5 Hz, 2 H), 2.28 (s, 3 H); ¹³C NMR δ 167.19, 162.52, 158.49, 154.99, 142.91, 140.63, 138.68, 134.99, 132.50, 131.31, 126.14, 123.80, 121.09, 119.87, 118.79, 113.68, 104.88, 61.52, 57.37, 44.10, 43.79, 28.15, 27.74, 14.64, 14.40; ESIMS *m/z* (rel intensity) 486.00 (MNa⁺, 100). Anal. Calcd for C₂₅H₂₅N₃O₆: C, 64.79; H, 5.44; N, 9.07. Found: C, 65.01; H, 5.41; N, 9.00.

5.1.7. (Z)-3-[1-(3-Methoxy-7-methylbenzo[d]isoxazol-5-yl)-4-(2-oxoxazolidin-3-yl)-but-1-enyl]-benzonitrile (9**)**—The general procedure was followed using the vinylstannane **15** (396 mg, 0.669 mmol), 3-bromobenzonitrile (**19**) (117 mg, 0.91 mmol), cesium fluoride (350 mg, 2.28 mmol), and Pd(PBu^t₃)₂ (37 mg, 0.07 mmol) in toluene (1 mL). The mixture was stirred under argon at room temperature for 4 h, at 55 °C for 41.5 h, and at 95 °C for 5 h. The residue was purified by column chromatography on silica gel (15 g), eluting with EtOAc-hexanes (0–20%), to afford the product **9** (23 mg) as an oil in 9% yield. ¹H NMR δ 7.50 (m, 2 H), 7.40–7.31 (m, 2 H), 7.23 (s, 1 H), 7.03 (s, 1 H), 6.11 (t, *J* = 7.5 Hz, 1 H), 4.25 (t, *J* = 7.8 Hz, 2 H), 4.14 (s, 3 H), 3.38 (t, *J* = 6.6 Hz, 2 H), 3.29 (t, *J* = 7.8 Hz, 2 H), 2.50 (s, 3 H), 2.38 (dt, *J* = 6.6 Hz and 7.5 Hz, 2 H); ¹³C NMR δ 167.18, 162.70, 158.60, 143.26, 141.53, 133.82, 132.32, 131.50, 130.98, 130.19, 129.22, 128.20, 121.61, 118.97, 118.73, 114.00, 112.40, 61.54, 57.46, 44.30, 43.75, 27.97, 14.72; ESIMS *m/z* (rel intensity) 403.70 (MH⁺, 100). Anal. Calcd for C₂₃H₂₁N₃O₄: C, 68.47; H, 5.25; N, 10.42. Found: C, 68.24; H, 5.24; N, 10.31.

5.2. RT inhibition assay

Analysis of the effects of the compounds on recombinant HIV-1 RT enzyme (p66/51 dimer) was performed as previously described [22]. Briefly, inhibition of purified recombinant reverse transcriptase enzyme was measured by the incorporation of [³²P]GTP into poly(rC)-oligo(dG) (rCdG) homopolymer template primers [4].

5.3. In vitro antiviral assays

Evaluation of the antiviral activity of compounds against HIV-1_{RF} infection in CEM-SS cells was performed using the MTS cytoprotection assay as previously described [22]. Evaluation of the antiviral activity of the compounds against HIV-1 strain III_B and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described [11,23].

5.4. In vitro hydrolytic stability study in rat plasma

The alkenyldiarylmethanes **3–9** were tested for their hydrolytic stability, utilizing rat plasma in vitro using methods as previously described [11]. 1,1-Diphenylethylene was used as an internal standard.

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Figure 1.
Structures of ADAMs non-nucleoside reverse transcriptase inhibitors.

Scheme 1.

Reagents and conditions: (a) $\text{Pd}(\text{P}^t\text{Bu}_3)_2$, CsF, toluene, reflux; (b) 3-but-3-ynyl-1,3-oxazolidin-2-one, $\text{PdCl}_2(\text{PPh}_3)_2$, Cu(I)I , Et_3N , THF; (c) Bu_3SnH , $\text{Pd}(\text{PPh}_3)_4$, THF, room temperature.

Table 1
Anti-HIV activities, cytotoxicities, and metabolic stabilities of ADAM analogues

Compound	IC ₅₀ (μM) ^d	EC ₅₀ (μM) ^b			CC ₅₀ (μM) ^c			TT ^d		Rat Plasma t _{1/2} (min ± SD)
		HIV-1 _{RF}	HIV-1 _{IIIB}	HIV-2 _{ROD}	CEM-SS	MT-4	CEM-SS	MT-4	MT-4	
1	0.90	>2.1	>5.76	>5.76	2.1	5.76	<1	<1	<1	4.26 ± 0.13
2	0.25	0.26	0.33	>7.26	2.08	7.26	8	22	22	1.58 ± 0.13
3	0.91	0.04	0.02	>1.09	0.5	1.09	12.5	54.5	54.5	3.46 ± 0.31
4	0.63	0.6	>1.1	>1.1 ^d	1.2	>1.1	2	<1	<1	51.4 ± 1.52
5	18.2	>1.88	>3.79	>3.79	1.88	3.79	<1	<1	<1	2.96 ± 0.01
6	5.88	>8.25	>4.73	>4.73	8.25	4.73	<1	<1	<1	NT ^e
7	0.93	>1.85	>1.86	>1.86	1.85	1.86	<1	<1	<1	>1440
8	3.6	>2.99	>9.54	>9.54	2.99	9.54	<1	<1	<1	>1440
9	39.2	>0.91	NT ^e	NT ^e	0.91	NT ^e	<1	NT ^e	NT ^e	>1440
Nevirapine [21]			0.25	NT ^e		>200		>800		NT ^e

^aInhibitory activity vs HIV-1 reverse transcriptase with poly(rC)-oligo(dG) as the template primer.

^bEC₅₀ is the 50% inhibitory concentration for inhibition of cytopathicity of HIV-1_{RF} in CEM-SS cells, and HIV-1_{IIIB} or HIV-2_{ROD} in MT-4 cells.

^cThe CC₅₀ is the 50% cytotoxic concentration for mock-infected CEM-SS cells or MT-4 cells.

^dTT: Therapeutic Index: ratio CC₅₀/EC₅₀.

^eNot tested. All data represent mean values for at least two separate experiments.