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Inhibition of tubulin polymerization by select alkenyldiarylmethanes

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Abstract—During studies on the alkenyldiarylmethane (ADAM) class of non-nucleoside reverse transcriptase inhibitors (NNRTIs), analogues were discovered that exhibit low micromolar and submicromolar cytotoxicities. Since the ADAMs are structurally related to the tubulin polymerization inhibitor CC-5079, a set of 14 ADAMs were tested for inhibition of tubulin polymerization in an attempt to identify the biological target responsible for their cytotoxicity. The results indicate that, overall, the ADAMs are poor inhibitors of tubulin polymerization. However, the two most cytotoxic compounds, **15** and **16**, are in fact active as inhibitors of tubulin assembly with IC_{50} values of 3.7 ± 0.3 and 2.8 ± 0.2 μM, respectively, and they both inhibit the binding of colchicine to tubulin. Both compounds were investigated for anticancer activity in the National Cancer Institute's panel of 60 human cancer cell lines, and both compounds consistently displayed submicromolar cytotoxicities with mean-graph midpoint (MGM) values of 0.31 ± 0.08 and 0.47 ± 0.09 μM, respectively.

Over the past 25 years, infection by the human immunodeficiency virus (HIV) has reached pandemic proportions, and an estimated 41 million people are believed to be carriers of the acquired immune deficiency syndrome's (AIDS) etiological agent.[1] HIV has caused the deaths of more than 25 million people since its first major appearance in 1981,[1] and developing a cure for HIV-infection is one of the major challenges currently facing medical science. Several FDA-approved drugs are available to combat HIV infections and AIDS progression. Unfortunately, the rapid mutation rate of HIV allows the virus to develop resistance to many antiviral agents as early as 2 months after initial anti-HIV treatment. Thus, until a cure is discovered, development of antiviral therapeutics that are active against both the wild-type and drug-resistant forms of HIV is a primary goal for AIDS researchers.[2–4]

The alkenyldiarylmethane (ADAM) class of non-nucleoside reverse transcriptase inhibitors (NNRTIs) was discovered over 10 years ago. The lead compounds **1** and **2** retain antiviral activity against the common HIV-1 reverse transcriptase (RT) drug-resistance mutations K103N and Y188C.[5–8] For this reason, the development of the ADAMs as potential antiviral therapeutics has been pursued. It has been established that the ADAMs exert their antiviral properties through the allosteric inhibition of HIV-1 RT. However, the observation that some ADAM analogues do not inhibit HIV-1 RT and yet still exhibit anti-HIV activity indicates that, at least, certain ADAMs interact with another viral or cellular entity, and this has led us to investigate other molecular targets.[9]

Another series of ADAMs has recently been developed by scientists at Celgene Corp. as potent inhibitors of inflammation, phosphodiesterase type 4 activity, and

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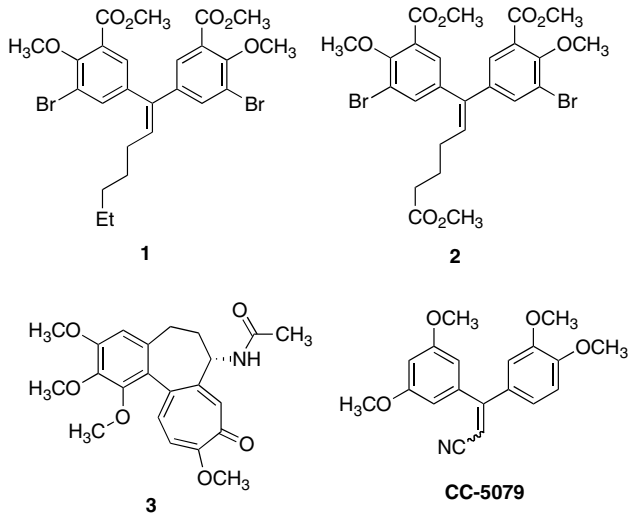


Chart 1.

tubulin polymerization, where tubulin inhibition involves binding of the inhibitor to the same site as the natural antimitotic agent, colchicine (**3**, Chart 1).[10–13]

The structural similarity between Celgene's inhibitor CC-5079 (Chart 1) and our own antiviral agents led us to consider that the ADAMs may also exhibit one or more of the properties displayed by the Celgene inhibitors. It is well known that disrupting microtubule homeostasis causes cells to undergo apoptosis,[11] and the low micromolar toxicity generally displayed by the ADAM class of NNRTIs led to the hypothesis that the ADAMs' cytotoxic properties may originate from the inhibition of tubulin polymerization by binding to the colchicine binding site. Additionally, inhibition of tubulin polymerization may also account for the RT-independent antiviral activity displayed by many ADAM analogues. The viral HIV protein Tat is known to, among other things, stabilize the microtubule framework of HIV-infected cells by binding to microtubule-associated protein (MAP) binding sites.[14] It has been proposed that the interactions between Tat and microtubules help facilitate the replication of HIV and may also contribute to the mechanism of HIV-related cell death.[14] In light of this information, the inhibition of tubulin polymerization by a select group of ADAMs was investigated. Herein we report the syntheses, antiviral activity, and tubulin inhibitory effects of ADAMs **4–17** (Chart 2).

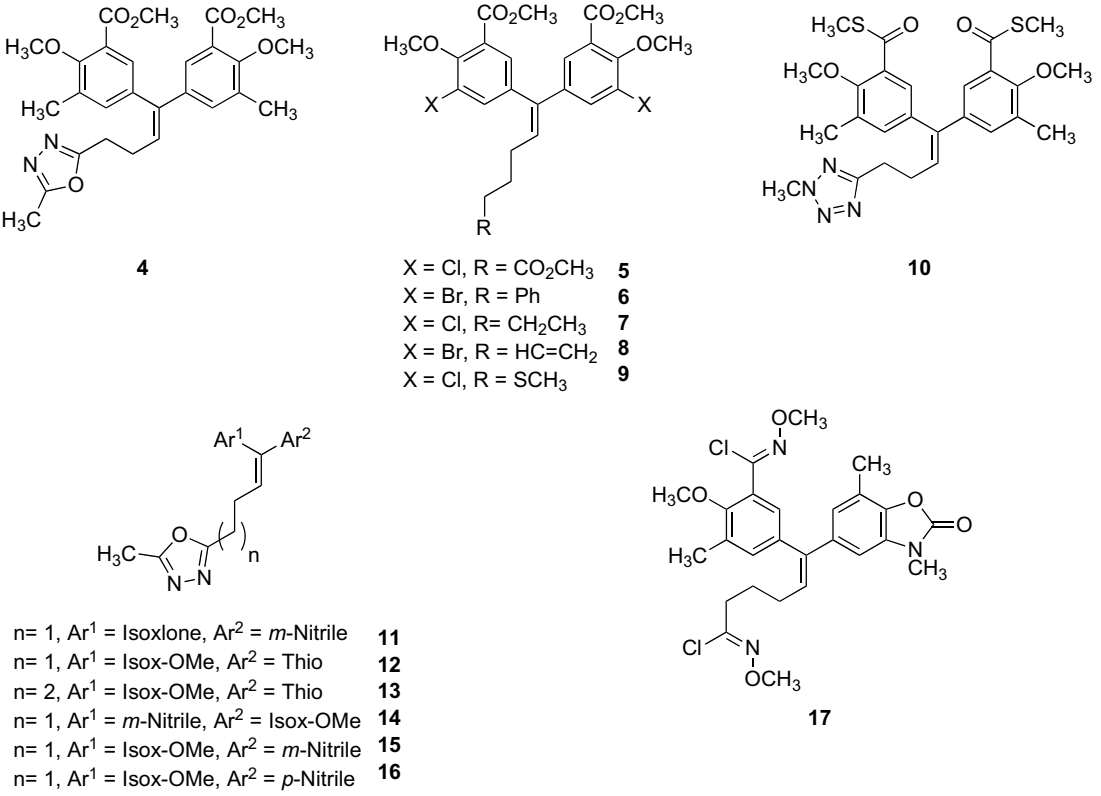
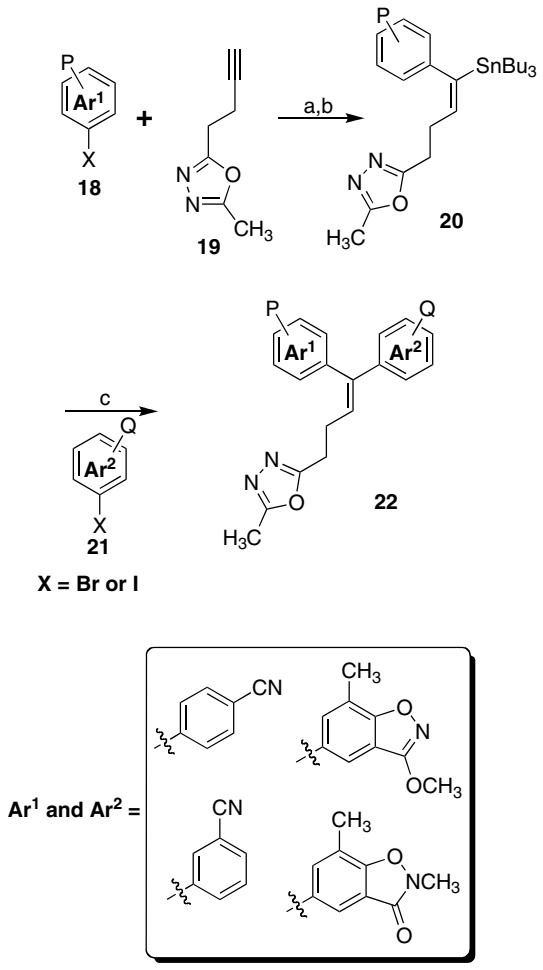


Chart 2.



Scheme 1. Reagents and conditions: (a) 5 mol% $PdCl_2(PPh_3)_2$, 10 mol% CuI, Et_3N, THF; (b) 2 mol% $Pd(PPh_3)_4$, Bu_3SnH, THF, 0 °C; (c) 10 mol% $Pd(PPh_3)_4$, 20–100 mol% CuI, CsF, DMF, 60 °C.

A number of methods have been developed for the synthesis of the ADAM scaffold, and the syntheses of ADAMs **4–10**,[†,15] **12**,[15] **13**,[15] and **17** [16] have been published. ADAMs **11**, **14**, **15**, and **16** were constructed via the general cross-coupling route depicted in Scheme 1. Sonogashira coupling of aryl halide **18**[†,17] and terminal alkyne **19**,[15] followed by hydrostannation, affords stannane intermediate **20**. The stannane and aryl halide **21** are coupled via the Stille reaction to obtain the desired analogue.

The tubulin polymerization inhibitory data[18–20] for ADAMs **4–17** are presented in Table 1, together with the antiviral data[*,6,21–23] associated with the compounds. Nevirapine is included for antiviral activity comparisons, while colchicine and combretastatin A-4 are well-known inhibitors of tubulin polymerization, with the latter compound being an exceptionally potent inhibitor of the binding of radiolabeled colchicine to tubulin. The majority of the compounds studied for inhibition of tubulin polymerization were chosen on the basis of their acute cytotoxicity, which one would expect to correlate with tubulin destabilizing activity. To our surprise, only 2 of the 14 analogues investigated were capable of inhibiting tubulin polymerization at concentrations lower than 40 μM, despite the structural similarities observed between the compounds and Celgene's inhibitors of tubulin polymerization. These data refute the hypothesis that inhibition of tubulin polymerization is the general source of ADAMs' cytotoxicity. However, the tubulin IC_{50}s observed for ADAMs **15** and **16** are not substantially greater than that of the potent antimitotic agent combretastatin A-4, indicating the extreme cytotoxicity observed with these two analogues most likely results from disruption of the microtubule network. Inhibitors of tubulin polymerization often induce cell death at concentrations much lower than their in vitro tubulin IC_{50} values in cell-free systems, which accounts for the 1000-fold difference in tubulin inhibitory activity and cytotoxicity observed for ADAMs **15** and **16**. The ADAMs **12** and **13**, which are structurally related to **15** and **16**, were completely inactive as inhibitors of tubulin polymerization. Evidently, the relatively small structural change involving the movement of the nitrile from the para position in **16** to the meta position in **15** only causes a small drop in activity, but the more drastic alterations embodied in **12** and **13** are not tolerated.

In order to confirm that ADAMs **15** and **16** are in fact binding to the colchicine site of tubulin, the inhibition of colchicine binding to tubulin was determined at 37 °C in reaction mixtures incubated for 10 min and containing tubulin (1 μM), **15** or **16** (5 μM), and [^{3}H]colchicine (5 μM). ADAMs **15** and **16** inhibited [^{3}H]colchicine binding by 24 ± 1% and 42 ± 3%, respectively. Combretastatin A-4, which was used as a positive control, inhibited [^{3}H]colchicine binding by 98 ± 0.3%.

The ADAMs **15** and **16** were examined for antiproliferative activity against the human cancer cell lines in the National Cancer Institute screen, in which the activity of each compound was evaluated with approximately 55 different cancer cell lines of diverse tumor origins. The GI_{50} values obtained with selected cell lines, along with the mean-graph midpoint (MGM) values, are summarized in Table 2. The MGM is based on a calculation of the average GI_{50} for all of the cell lines tested (approximately 55) in which GI_{50} values below and above the test range (10^{-8} to 10^{-4} molar) are taken as the minimum (10^{-8} molar) and maximum (10^{-4} molar) drug concentrations used in the screening test. Both ADAMs **15** and **16** consistently produced submicromolar GI_{50} values in these human cancer cell lines, resulting in MGM values of 0.31 ± 0.08 and 0.47 ± 0.09 μM, respectively.

[†] Syntheses for aryl iodides corresponding to the isoxazole and isoxazolone were reported in Ref. 17. The aryl bromides corresponding to the benzonitriles are commercially available.

[*] The ability of target compounds to inhibit the enzymatic activity of recombinant HIV-1 RT (p66/51 dimer) was evaluated as previously described.[6] Evaluation of antiviral activity against HIV-1_{RF} was performed in infected CEM-SS cells while using the XTT cytoprotection assay, as previously described.[6,22] 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.[20,23]

Table 1. Antiviral and tubulin polymerization inhibitory activities of ADAMs **4–17**

Compound	HIV-1 RT IC_{50}[a] (μM)	EC_{50}[b] (μM)			CC_{50}[c] (μM)		Tubulin IC_{50}[d] (μM)
		1_{RF}	1_{IIIB}	2_{ROD}	CEM-SS	MT-4	
4	71	2.3	3.2	N.A.[e]	40	36	>40
5	0.3	0.013	0.60	2.5	32	160	>40
6	>100	13	2.6	29	>200	>198	>40
7	N.T.[f]	N.A.[e]	N.T.[f]	N.T.[f]	29	N.T.[f]	>40
8	99	8.2	3.0	N.A.[e]	>100	62	>40
9	100	53	N.T.[f]	N.T.[f]	20	N.T.[f]	>40
10	67	2.9	N.A.[e]	N.A.[e]	33	7.0	>40
11	40	N.A.[e]	N.A.[e]	N.A.[e]	1.1	0.97	>40
12	33	N.A.[e]	N.A.[e]	N.A.[e]	1.2	1.6	>40
13	5.2	N.A.[e]	N.A.[e]	N.A.[e]	0.78	1.1	>40
14	73	N.A.[e]	N.A.[e]	N.A.[e]	2.1	4.2	>40
15	93	N.A.[e]	N.A.[e]	N.A.[e]	0.004	0.20	3.7 ± 0.3
16	>100	N.A.[e]	N.A.[e]	N.A.[e]	0.004	0.34	2.8 ± 0.2
17	0.60	N.A.[e]	1.2	N.A.[e]	2.9	12	>40
Nevirapine	0.084[24]	0.0015	0.053	N.A.[e]	N.T.[f]	15	N.T.[f]
Combretastatin A-4	N.T.[f]	N.T.[f]	N.T.[f]	N.T.[f]	N.T.[f]	N.T.[f]	1.2 ± 0.1
Colchicine	N.T.[f]	N.T.[f]	N.T.[f]	N.T.[f]	N.T.[f]	N.T.[f]	3.8 ± 0.1

[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 cause cell death for 50% of the mock-infected CEM-SS or MT-4 cells.
[d] The concentration required to inhibit 50% of tubulin polymerization in vitro. The reaction mixtures contained 10 μM tubulin, and the extent of assembly after 20 min at 30 °C in 0.8 M monosodium glutamate + 0.4 mM GTP was measured.
[e] Not active.
[f] Not tested.

Table 2. Cytotoxicities of Alkenyldiarylmethanes **15** and **16**

Cell line or MGM[b]	Compound GI_{50}[a] (μM)	
	15	**16**
Lung (HOP-62)	0.76	0.55 ± 0.08
Colon (HCT-116)	0.49	0.46 ± 0.08
CNS (SF-539)	0.24 ± 0.06	0.30 ± 0.04
Melanoma (LOX IMVI)	0.59 ± 0.17	0.72 ± 0.19
Ovarian (OVCAR-3)	0.10 ± 0.06	0.20 ± 0.03
Renal (SN12C)	0.50 ± 0.07	0.78 ± 0.32
Prostate (DU-145)	0.32 ± 0.08	0.57 ± 0.14
Breast (MDA-MB-435)	0.089 ± 0.051	0.19 ± 0.02
MGM	0.31 ± 0.08	0.47 ± 0.09

[a] Concentration for 50% growth inhibition.
[b] Mean-graph midpoint for growth inhibition of all human cancer cell lines found to be sensitive.

The results with analogues **4**, **6**, **8**, and **10** are consistent with the conclusion that inhibition of tubulin polymerization is not required for the ADAMs' RT-independent antiviral mechanism. These compounds each exhibited RT-independent antiviral activity, as can be seen by comparing their in vitro RT inhibitory activities with their respective cytoprotective activities; yet, none of these four analogues had a significant effect on tubulin polymerization at 40 μM.

In summary, select ADAMs were evaluated for inhibition of tubulin polymerization, and the results indicate that most members of this structural class of compounds are poor inhibitors. These results do not support the hypothesis that disruption of microtubule stability is the origin of the ADAMs' cytotoxicity, nor do the results support an important role for tubulin in the ADAMs' RT-independent antiviral mechanism. Additional studies are required to elucidate the so far unknown antiviral mechanism. Despite the poor tubulin inhibition generally exhibited by most members of the ADAM class described here, the highly cytotoxic analogues **15** and **16** were identified as potent tubulin destabilizing agents with activities not substantially greater than the activity of the natural antimitotic agent combretastatin A-4. In light of this information, **15** and **16** could potentially be used as leads in the development of ADAM-based inhibitors of tubulin polymerization for the treatment of cancer.

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Supplementary data

Physical constants, elemental analysis data, and experimental procedures for the synthesis of compounds **11**, **14**, **15**, and **16** are included in the Supporting Information section of this report. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.114.

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