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9-[(Hydroxymethyl)phenyl]adenines: New Aryladenine Substrates of Adenosine Deaminase[1]

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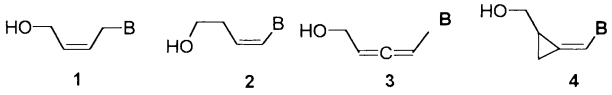
Abstract—New phenyl adenine compounds **5**–**7** were synthesized as analogues of adenosine and studied for their adenosine deaminase (ADA) substrate activity. The 9-[(*o*-hydroxymethyl)phenyl]methyl]adenine **5** and 9-[(*m*-hydroxymethyl)phenyl]adenine **7** were deaminated by ADA, and 9-[(*o*-hydroxyethyl)phenyl]adenine **6** was not deaminated up to 7 days. The ADA substrates **5** and **7** were deaminated quantitatively to their inosine analogues in 10 and 6 h, respectively.

Adenosine deaminase (ADA), is one of the most important enzymes in purine metabolism. It catalyses the hydrolysis of adenosine to inosine and ammonia.[2] It has been well established that several adenosine analogues of chemotherapeutic interest are rapidly deaminated by ADA to their inosine derivatives.[3] Therefore, the structural characteristics governing the ADA substrate specificity of adenosine analogues are of considerable importance in the design of new nucleoside molecules as potential chemotherapeutic agents.

Unsaturated nucleoside analogues such as **1**–**4**, containing one or more double bonds between the nucleic acid base and hydroxymethyl group, have been the focus of several recent studies as potent antiviral agents (Fig. 1). The most important compounds of this series are *Z*-butenols (**1**): the guanine analogue (**1b**) is an antiviral agent,[4] allenols (**3**): adenine analogue is a strong inhibitor of HIV-1,[5] and cyclopropylidenols (**4**): (*Z*)-synadenol (**4a**) and (*Z*)-synguanol (**4b**) both are effective antiviral agents.[6] The adenine analogues of **1**–**4** are also substrates of adenosine deaminase (ADA) with varying degree of efficacy.[7–10]

The benzene ring is a planar and an *ortho*- or *meta*-disubstituted benzene ring can mimic the compounds



1 2 3 4

B = purine base, a = Ade (adenin-9-yl); b = Gua (guanin-9-yl)

Figure 1.

1–**4** with varying degree of π electron cloud (double bond) distribution between the nucleic acid base and hydroxymethyl group. Therefore, we hypothesized that the use of benzene ring as a spacer between the nucleic acid base and the hydroxymethyl group might provide phenyl analogues of **1a**–**4a** as potential ADA substrates and as potential chemotherapeutic agents. For these reasons, we became interested in the design and synthesis of aromatic nucleoside analogues **5**–**7**, wherein the top part of phenyl spacer (bold lines) mimics the unsaturated acyclic chains of *Z*- and *E*-isomers of **1a**–**2a**, as potential substrates of adenosine deaminase (Fig. 2).

In unsaturated analogues **1a**–**4a**, which were designed as acyclic analogues of 2′-3′-dideoxyadenosine, the N9–C5′ distance is also considered to be an important factor for the ADA substrate activity. Therefore, we measured the N9–C5′ distances of designs **5**–**7** and compared with the 2′,3′-dideoxyadenosine (4.61 Å) and *Z*- and *E*-isomers of **1a**–**2a**.[11a,b] Thus, the N9–C5′ distance of phenyladenine **5** (4.10 Å) and **6** (4.41 Å) is comparable to *Z*-isomers **1a**–**2a** (∼4.10 Å), and compound **7** (4.79 Å) is comparable to the *E*-isomers **1a**–**2a** (∼4.70 Å).[5,6]

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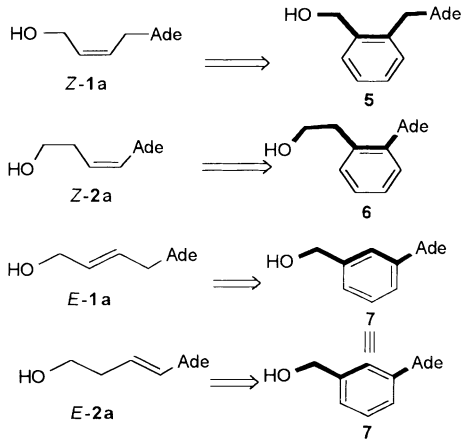
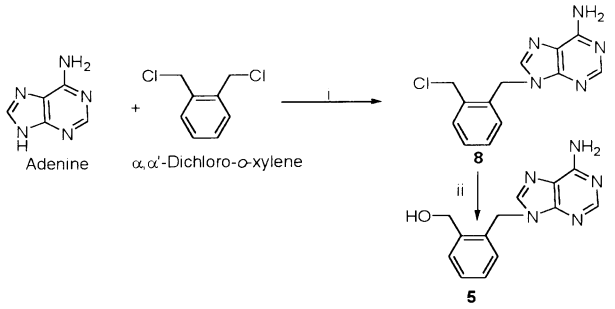


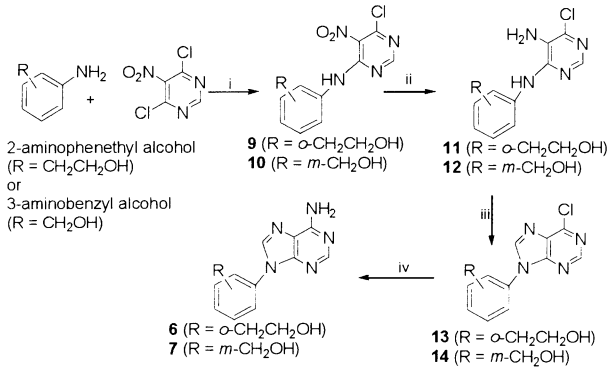
Figure 2.

Synthesis of compound **5** was achieved by the alkylation of adenine with commercially available α,α′-dichloro-*o*-xylene using a previously described procedure.[12,13] The 9-chloromethylphenyl intermediate **8** was hydrolyzed to give the desired product **5** as shown in Scheme 1. The structures of intermediate and final compounds were confirmed by UV, ^{1}H NMR and satisfactory elemental (C, H, N) analysis.[14,15]



Scheme 1. (i) 0.1 M N $(Bu)_4^+ F^-$/THF; (ii) 0.1 N HCl, reflux 2 h.

For the synthesis of **6** and **7**, we had to construct the adenine base starting with an appropriate amino-Ar-alcohol with some modifications in the reported procedures.[16,17] A more reactive 4,6-dichloro-5-nitro pyrimidine was used to react with the 2-aminophenethyl alcohol or 3-aminobenzyl alcohol to get the nitro intermediates **9** and **10**, which were directly used to prepare the final compounds **6** and **7** as described in Scheme 2.



Scheme 2. (i) *N*,*N*-Diisopropylethylamine, DMF, 24 h; (ii) Sodium hyposulfite; (iii) $CH(OEt)_3$, reflux; (iv) $MeOH/NH_3$, pressure.

The structures of the intermediates and the final hydroxypurines **6**–**7** were confirmed with ^{1}H NMR and satisfactory elemental (C, H, and N) analysis.[18–23]

The adenosine deaminase (ADA) experiments of phenyladenines **5**, **6**, and **7** (2 μmol each) and adenosine deaminase from calf intestine (type II, Sigma Chemical Co., St. Louis, MO, 2 units) were conducted in 0.05 M Na_2HPO_4 (pH 7.4, 0.5 mL) under similar conditions used for compounds **1**–**4**.[6–10] The progress of the reaction was monitored by TLC, and UV spectrophotometry at 265 nm (max for adenosine analogue—starting material) and 251 nm (max for inosine analogue—deaminated product) using the reported procedure.[10] The deamination times and % deamination results for **5**, **6**, and **7** are presented in Table 1. Under similar conditions adenosine was completely deaminated to inosine in less than 30 min.[24]

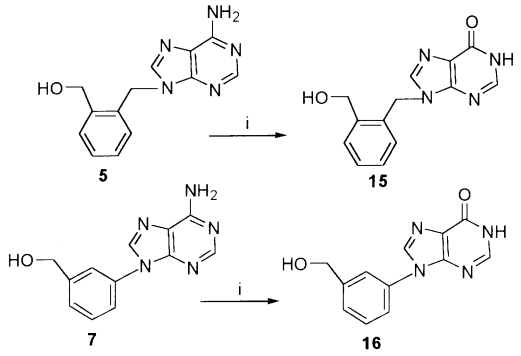
It has been established that the *E*-isomers of **1a** and **2a** are better ADA substrates (deamination time 16–19 h) than the *Z*-isomers of **1a** and **2a** (4–7 days).[7–10] Based on the ADA deamination studies of our phenylpurines **5**–**7**, it is clear that the best ADA substrate compound **7** (deamination time 6 h) which resembles to *E*-**1a** or *E*-**2a** is a better ADA substrate than the *E*-**1a** and *E*-**2a**. This indicates that a 'phenyl' spacer, as compared to an unsaturated acyclic spacer, clearly improves the ADA recognition for the substrate activity. On the other hand, since **6** mimics *Z*-**2a**, it follows that *E*-isomer analogue **7** (6 h) has to be a better substrate than the *Z*-isomer analogue **6** (7 days). Finally the phenylpurine **5**, which mimics *Z*-**1a** is a better ADA substrate (10 h) than both *Z*-**1a** (10 days) and *E*-**1a** (19 h), indicating the 'phenyl' spacer actually improved the ADA substrate activity of **5** as compared to *Z*-**1a** and *E*-**1a**. As expected, the *E*-**1a** or *E*-**2a** analogue phenylpurine **7** is a better substrate than *Z*-**1a** analogue **5** and *Z*-**2a** analogue **6**.

A large scale (0.1 mmol) deamination of phenyladenines **5** and **7** (UV max 261 nm) by ADA was carried out to isolate and confirm the structures of inosine analogues

Table 1. The N9–C5′ distances[a] and ADA deamination results of arylpurines **5**–**7**

Compd	N9–C5′ Distance (Å)	ADA Deamination time	Product
Adenosine	—	<30 min	Inosine (100%)
Z-**1a**[7,10]	—	10 days	100%
E-**1a**	—	19 h	100%
Z-**2a**[9]	—	4 days	Partial
E-**2a**	—	16 h	100%
5	4.10	10 h	**15** (100%)
6	4.41	7 days	0%
7	4.79	6 h	**16** (100%)

[a]Measured by Alchemy 2000 (Tripos Inc., St Louis, MO).



Scheme 3. (i) ADA, 0.05 M Na_2HPO_4 pH 7.4.

15 and **16** (UV max 251 nm) (Scheme 3). The compounds were characterized by the UV, ^{1}H NMR, and satisfactory elemental analysis.[25,26]

We also prepared additional analogues of **5** with larger N9–C5′ distance than 2′,3′-dideoxyadenosine such as *meta*-CH_2OH (N9–C5′ 5.75 Å) and *para*-CH_2OH (N9–C5′ 6.52 Å) and conducted ADA experiments under similar conditions. These *m*- and *p*-CH_2OH analogues of **5** (not shown here) were not deaminated up to 7 days.[27] Similarly an analogue of **7** with smaller N9–C5′ distance than adenosine such as *ortho*-CH_2OH (N9–C5′ 2.98 Å) was also not deaminated by ADA up to 7 days.[27]

In summary, the appropriate substitution of a hydroxymethyl group and adenine base on a benzene ring (*o*- or *m*-) with N9–C5′ distance similar to an adenosine seems to be the most important factor for a substrate binding to adenosine deaminase. Comparative studies with the reported unsaturated compounds **1**–**2**, clearly support the findings that a more stable, π electron rich 'phenyl' spacer improved the ADA recognition as compared to an unsaturated acyclic spacer for the substrate activity. According to our studies, the substitution of a hydroxymethyl function at the appropriate position on the benzene ring allows compounds **5** and **7** to bind to ADA, and in that respect these phenylpurines do resemble to the nucleoside analogues of adenosine. The in vitro antiviral studies of these compounds are under progress. We are currently synthesizing other purine and pyrimidine analogues of **5**–**7** as potential chemotherapeutic agents.

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11. (a) Alchemy 2000 3-D-molecular modeling program was used to measure N9–C5′ distances of **5**–**7**. For each structure, we constructed the 3-D-structure of the molecule, completed molecular mechanics and PM3 single point (stable energy) and geometry optimization energy minimizations, and measured distances between N9–C5′ (Å) of the lowest energy conformation. (b) Lesyng, B.; McCammon, J. *Pharmac. Ther.* **1995**, *60*, 149.
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14. 9-[[*o*-(Chloromethyl)phenyl]methyl]adenine (**8**). Mp 210 °C; ^{1}H NMR (DMSO-d_6, freshly dissolved) δ 8.22 (1H, s, H_8), 8.15 (1H, s, H_2), 7.49 (1H, m, Ar), 7.32 (2H, m, Ar,) 7.27 (2H, br s, NH_2), 7.02 (1H, m, Ar), 5.53 (2H, s, CH_2), 5.07 (2H, s, CH_2). Anal. calcd for $C_{13}H_{12}ClN_5$: C 57.04, H 4.41, N 25.58. Found: C 57.35, H 4.44, N 25.73.
15. 9-[[*o*-(Hydroxymethyl)phenyl]methyl]adenine (**5**). Mp 216 °C; UV (0.01 M Na_2HPO_4, pH 7.4) max 261 nm (ε 13,900); ^{1}H NMR (DMSO-d_6) δ 8.16 (1H, s, H_8), 8.14 (1H, s, H_2), 7.43 (1H, d, Ar), 7.26 (3H, m, NH_2 + Ar), 7.19 (1H, t, Ar), 6.91 (1H, d, Ar), 5.43 (2H, s, CH_2), 5.33 (1H, t, OH), 4.69 (2H, d, CH_2). Anal. calcd for $C_{13}H_{13}N_5O$: C 61.16, H 5.13, N 27.43. Found: C 61.35, H 5.14, N 27.17.
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18. N^6-[*o*-(Hydroxyethyl)phenyl]-4-chloro-5-amino pyrimidine (**11**). Mp 116 °C; ^{1}H NMR (DMSO-d_6) δ 9.28 (1H, s, NH), 8.17 (1H, s, H_2), 7.61 (1H, t, Ar), 7.26 (1H, d, Ar), 7.20 (1H, t, Ar), 7.09 (1H, t, Ar), 6.88 (2H, s, NH_2), 4.53 (1H, t, OH), 3.57 (2H, q, CH_2), 2.78 (2H, t, CH_2). Anal. calcd for $C_{12}H_{13}ClN_4O$: C 54.44, H 4.94, N 21.16. Found: C 54.40, H 5.10, N 21.22.
19. N^6-[*m*-(Hydroxymethyl)phenyl]-4-chloro-5-amino pyrimidine (**12**). Mp 190 °C; ^{1}H NMR (DMSO-d_6) δ 8.58 (1H, s, H_2), 7.85 (1H, d, Ar), 7.63 (2H, m, NH + Ar), 7.24 (1H, m, Ar), 6.96 (1H, t, Ar), 5.44 (2H, br s, NH_2), 5.40 (1H, t, OH), 4.48 (2H, d, CH_2). Anal. calcd for $C_{11}H_{11}ClN_4O$: C 52.70, H 4.42, N 22.35. Found: C 52.69, H 4.55, N 22.53.
20. 9-[*o*-(Hydroxyethyl)phenyl]-6-chloropurine (**13**). Mp 157 °C; ^{1}H NMR (DMSO-d_6) δ 8.86 (1H, s, H_8), 8.77 (1H, s, H_2), 7.58 (2H, m, Ar), 7.48 (2H, m, Ar), 4.55 (1H, t, OH), 3.43 (2H, m, CH_2), 2.48 (part of CH_2 multiplet is overlapped with DMSO). Anal. calcd for $C_{13}H_{11}ClN_4O$: C 56.83, H 4.03, N 20.39. Found: C 57.01, H 4.50, N 20.44.
21. 9-[*m*-(Hydroxymethyl)phenyl]-6-chloropurine (**14**). Mp 138 °C; ^{1}H NMR (DMSO-d_6) δ 9.08 (1H, s, H_8), 8.84 (1H, s, H_2), 7.83 (1H, s, Ar), 7.74 (1H, d, Ar), 7.58 (1H, t, Ar), 7.47 (1H, d, Ar), 5.40 (1H, t, OH), 4.61 (2H, s, CH_2). Anal. calcd

for $C_{12}H_9ClN_4O$: C 55.29, H 3.47, N 21.49. Found: C 55.43, H 3.58, N 21.35.
22. 9-[*o*-(Hydroxyethyl)phenyl]adenine (**6**). Mp 176 °C; UV (0.01 M Na_2HPO_4, pH 7.4) max 261 nm (ε 15,200); ^{1}H NMR (DMSO-d_6) δ 8.25 (1H, s, H_8), 8.10 (1H, s, H_2), 7.51 (2H, m, Ar), 7.41 (1H, m, Ar), 7.36 (3H, m, NH_2 + Ar,), 4.60 (1H, t, OH), 3.40 (2H, q, CH_2), 2.53 (part of CH_2 multiplet is overlapped with DMSO). Anal. calcd for $C_{13}H_{13}N_5O$: C 61.16, H 5.13, N 27.43. Found: C 61.35, H 5.14, N 27.17.
23. 9-[*m*-(Hydroxymethyl)phenyl]adenine (**7**). Mp 180 °C; UV (0.01 M Na_2HPO_4, pH 7.4) max 261 nm (ε 14,000); ^{1}H NMR (DMSO-d_6) δ 8.56 (1H, s, H_8), 8.21 (1H, s, H_2), 7.84 (1H, s, Ar), 7.75 (1H, d, Ar), 7.54 (1H, t, Ar), 7.39 (3H, m, NH_2 + Ar), 5.36 (1H, t, OH), 4.61 (2H, d, CH_2). Anal. calcd. for $C_{12}H_{11}N_5O$: C 59.74, H 4.59, N 29.02. Found: C 59.70, H 4.75, N 29.24.
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25. 9-[[*o*-(Hydroxymethyl)phenyl]methyl]hypoxanthine (**15**). Mp 230 °C; UV (0.01 M Na_2HPO_4, pH 7.4) max 251 nm (ε 17,900); ^{1}H NMR (DMSO-d_6) δ 12.32 (1H, s, NH), 8.11 (1H, s, H_8), 8.04 (1H, s, H_2), 7.42 (1H, d, Ar), 7.27 (1H, t, Ar), 7.21 (1H, t, Ar), 6.88 (1H, d, Ar), 5.45 (2H, s, CH_2), 5.30 (1H, t, OH), 4.68 (2H, d, CH_2). Anal. calcd for $C_{12}H_{12}N_4O_2$: C 59.00, H 4.94, N 22.93. Found: C 59.13, H 5.10, N 23.15.
26. N9-[*m*-(Hydroxymethyl)phenyl]hypoxanthine (**16**). Mp > 200 °C; UV (0.01 M Na_2HPO_4, pH 7.4) max 251 nm (ε 18,500); ^{1}H NMR (DMSO-d_6) δ 12.47 (1H, s, NH), 8.46 (1H, s, H_8), 8.11 (1H, s, H_2), 7.72 (1H, s, Ar), 7.63 (1H, d, Ar), 7.55 (1H, t, Ar,) 7.43 (1H, d, Ar), 5.37 (1H, t, OH), 4.60 (2H, d, CH_2). Anal. calcd for $C_{12}H_{10}N_4O_2$: C 59.49, H 4.16, N 23.12. Found: C 59.35, H 4.05, N 22.69.
27. Unpublished results.