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Cytotoxic Activity of 6-Alkynyl- and 6-Alkenylpurines

Anders Bråthe,[a] Lise-Lotte Gundersen,[a,*] Jon Nissen-Meyer,[b] Frode Rise[a] and Bjørn Spilsberg[a]

[a]*Department of Chemistry, University of Oslo, PO Box 1033, Blindern, 0315 Oslo, Norway*
[b]*Department of Biochemistry, University of Oslo, PO Box 1041, Blindern, 0316 Oslo, Norway*

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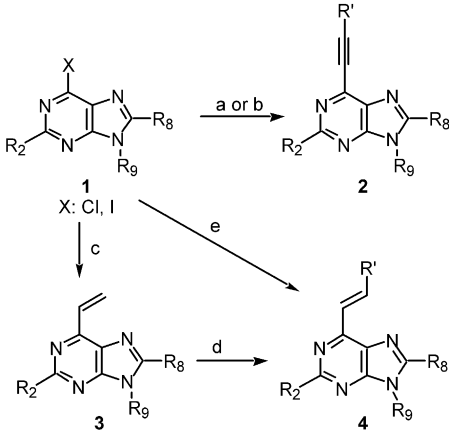
Abstract—6-Alkynyl- and 6-alkenylpurines have been screened for cytotoxic activity against a human chronic myelogenous leukemia cell line; K-562 cells using a [^{3}H]-thymidine incorporation assay. Most alkynes displayed cytotoxicity comparable to, or better than, the known anticancer drugs 6-mercaptopurine and fludarabine. The 6-alkenylpurines, which are promising plant growth stimulators and 15-lipoxygenase inhibitors, exhibited only low toxicity.

We have recently reported that certain 6-alkynyl- and 6-alkenylpurines have profound inhibitory activity against 15-lipoxygenase (15-LO) from soy beans.[1] If these compounds are also active against human lipoxygenase, they may have a therapeutic potential against atherosclerosis[2–4] or other diseases linked to free radicals.[5–7] We have also shown that 6-alkynyl- and 6-alkenylpurines are cytokinin analogues with profound plant growth stimulating effect.[8] However, we have found high cytotoxicity for 6-alkynyl-2-oxopurines,[9] and 6-arylpurinenucleosides[10] and certain bis(purin-6-yl)alkynes[11] are also reported to be cytotoxic. Before initiating structure optimalization on 6-substituted purines as 15-LO inhibitors and plant growth stimulators, we were interested in the potential toxicity of these compounds.

The 6-alkynylpurines **2** were prepared by Sonogashira or Stille coupling, and 6-alkenylpurines **3** and **4**[12–19] were prepared by Stille coupling, followed by Heck coupling as outlined in Scheme 1.

The purines **2–4** (Fig. 1) were screened for cytotoxic activity against a human chronic myelogenous leukemia cell line;[20] K-562 cells[21] using a [^{3}H]-thymidine incorporation assay.[22] In the initial screening 10 μg/mL purine concd was used and inhibition of [^{3}H]-thymidine incorporation was determined after 48 h exposure to the purine. For compounds exhibiting at least ca. 90% inhibition in the initial screening, IC_{50} values after 5 h and after 48 h exposure were determined. The results are presented in Table 1.



Scheme 1. (a) PhC≡CSnBu$_3$, cat. Pd etc. (Stille conditions); (b) R′C≡CH, cat. Pd etc. (Sonogashira conditions); (c) CH$_2$=CHSnBu$_3$, cat. Pd etc. (Stille conditions); (d) R′I, cat. Pd etc. (Heck conditions); (e) PhCH=CHSnBu$_3$, cat. Pd etc. (Stille conditions).

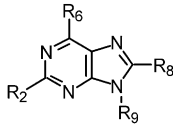


Figure 1. General structure of the purines **2–4**.

*Corresponding author. Tel.: +47-22-85-7019; fax: +47-22-85-55507; e-mail: l.l.gundersen@kjemi.uio.no

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All phenylacetylenes **2a**–**2g** and also the enyne **2h** are highly toxic against K-562 cells. These compounds exhibit IC_{50} comparable to 6-mercaptopurine and fludarabine in the 48 h assay and they are significantly more active than 6-mercaptopurine after 5 h.

The 8-chloro substituent in compound **2c** apparently reduces toxicity somewhat compared to the other phenylacetylenes. The 2-chloro-9-benzylalkynylpurine **2e** and the nucleoside analogue **2g** are even more active than the well known anticancer drugs fludarabine and 6-mercaptopurine. Compounds **2a** and **2b** are profound 15-LO inhibitors, but their cytotoxicity makes these compounds less suitable as antioxidants. The plant growth hormone analogues[8] and 15-LO inhibitors[1] **2i** and **2j** are however, of low toxicity as judged by the results reported herein.

The 6-vinylpurines **3a** and **3b** were highly cytotoxic Attack by nucleophilic species to the alkynyl or alkenyl groups in the purines studied may be involved in the mode of action for these compounds as cytostatica. It is previously reported that 6-vinylpurines have a potential as cross-linking agents. They form adducts with guanosine and cytidine at the *N*-7 and *N*-4 positions, respectively.[23,24] We have also studied nucleophilic attack to vinylpurines, and we have found higher reactivity for the 7-benzylated isomer **3b** than for the 9-benzyl purine **3a**,[25] and we now observe higher toxicity for **3b** than the 9-alkylated isomer **3a** (Fig. 2). The need for an electrophilic double or triple bond in the purine 6-position is also indicated by the fact that at least in the alkyne series, an electron withdrawing chlorine in the purine 2-position enhances cytotoxic activity (compound **4e** vs **4d**; Fig. 2). In general, the 6-alkenylpurines studied were less toxic than their alkyne analogues, and relatively low cytotoxicity were found for the promising lipoxygenase inhibitors and plant growth stimulators **4a**, **4c**, and **4i**–**4s**.

We find it remarkable that while purine nucleoside analogues possessing a carbon substituent in the purine

Table 1. Cytotoxicity against chronic myelogenous leukemia cells, cell line K-562 for compounds **2**–**4**

Compd	-R_2	-R_6[a]	-R_8	-R_9	% Inhibition 10 μg/mL (±SD),[b] 48 h	IC_{50} (μg/mL)[c] (±SD),[b] 48 h	IC_{50} (μg/mL)[c] (±SD),[b] 5 h
2a	–H	–C≡CPh	–H	–H	100 (±1)	0.7 (±0.07)	5.9 (±0.3)
2b	–H	–C≡CPh	–H	–THP[d]	100 (±1)	0.5 (±0.03)	3.2 (±0.01)
2c	–H	–C≡CPh	–Cl	–THP	85 (±8)	6.1 (±0.5)	7.0 (±0.6)
2d	–H	–C≡CPh	–H	–Bn[e]	98 (±2)	1.3 (±0.07)	6.0 (±0.3)
2e	–Cl	–C≡CPh	–H	–Bn	100 (±1)	0.12(±0.005)	0.8 (±0.02)
2f	–Ph	–C≡CPh	–H	–Bn	100 (±1)	1.4 (±0.02)	2.4 (±0.02)
2g	–H	–C≡CPh	–H	–Rib[f]	100 (±1)	0.1 (±0.005)	0.2 (±0.03)
2h	–H	–C≡CCH=C(CH_3)CO_2CH_3	–H	–H	100 (±1)	1.6 (±0.04)	3.2 (±0.2)
2i	–H	–C≡CCH=C(CH_3)CH_2OH	–H	–H	17 (±1)	n.d.	n.d.[g]
2j	–H	–C≡CCH=C(CH_3)$_2$	–H	–H	30 (±2)	n.d.	n.d.
3a	–H	–CH=CH_2	–H	–Bn	100 (±1)	1.1 (±0.03)	2.2 (±0.1)
3b	–H	–CH=CH_2	–H	–Bn[h]	100 (±1)	0.5 (±0.01)	1.4 (±0.1)
4a	–H	–CH=CHPh	–H	–H	28 (±5)	n.d.	n.d.
4b	–NH_2	–CH=CHPh	–H	–H	2 (±1)	n.d.	n.d.
4c	–H	–CH=CHPh	–H	–THP	66 (±4)	n.d.	n.d.
4d	–H	–CH=CHPh	–H	–Bn	93 (±2)	n.d.	n.d.
4e	–Cl	–CH=CHPh	–H	–Bn	99 (±1)	2.0 (±0.1)	6.0 (±0.2)
4f	–NH_2	–CH=CHPh	–H	–Bn	91 (±1)	1.2 (±0.01)	2.0 (±0.04)
4g	–Ph	–CH=CHPh	–H	–Bn	34 (±5)	n.d.	n.d.
4h	–H	–CH=CHPh	–H	–Rib	79 (±1)	2.0 (±0.02)	10 (±4)
4i	–H	–CH=CH–C_6H_4–*p*–OCH_3	–H	–H	54 (±4)	n.d.	n.d.
4j	–H	–CH=CH–C_6H_4–*p*–OCH_3	–H	–THP	58 (±5)	n.d.	n.d.
4k	–H	–CH=CH–(2-furyl)	–H	–H	24 (±3)	n.d.	n.d.
4l	–H	–CH=CH–(2-furyl)	–H	–THP	35 (±5)	n.d.	n.d.
4m	–H	–CH=CH–(2-thienyl)	–H	–H	50 (±2)	n.d.	n.d.
4n	–H	–CH=CH–(2-thienyl)	–H	–THP	65 (±5)	n.d.	n.d.
4o	–H	–CH=CH–(3-thienyl)	–H	–H	32 (±6)	n.d.	n.d.
4p	–H	–CH=CH–(3-thienyl)	–H	–THP	58 (±6)	n.d.	n.d.
4q	–H	–C=CCH=C(Me)CO_2Me	–H	–H	20 (±1)	n.d.	n.d.
4r	–H	–C=CH=C(Me)CH_2OH	–H	–H	6 (±4)	n.d.	n.d.
4s	–H	–C=CH=C(Me)CH_2OH	–H	–H	27 (±10)	n.d.	n.d.
6-MP[i]	–H	–SH	–H	–H	80 (±1)	0.6 (±0.3)	>10
Flud[j]	–F	–NH_2	–H	–Ara[k]	98 (±1)	0.6 (±0.03)	0.6 (±0.06)

[a]All alkenes have *E*-configuration.
[b]Standard deviation is given in parentheses.
[c]The concd that reduces [^{3}H]-thymidine incorporation by 50%.
[d]THP, Tetrahydropyranyl.
[e]Bn, Benzyl.
[f]Rib, β-D-ribofuranosyl.
[g]Not determined.
[h]Bn in the purine 7-position.
[i]6-MP, 6-mercaptopurine.
[j]Flud, fludarabine.
[k]Ara, β-D-arabinofuranosyl.

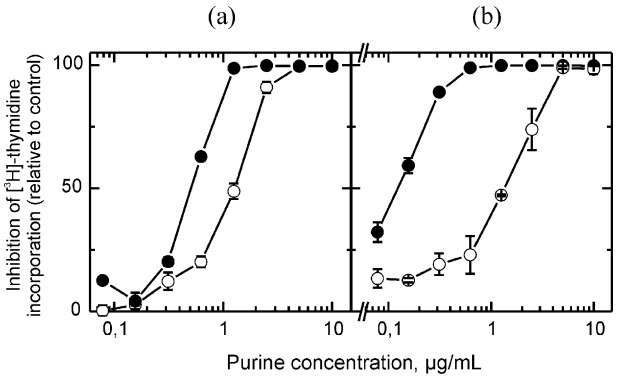

Figure 2. Difference in cytotoxicity (a) for the 6-vinylpurines **3a** (○) and **3b** (●); (b) 6-phenylalkynylpurines **2d** (○) and **2e** (●).

6-position have attracted considerable interest as potential anticancer compounds,[10,11,26] the highly cytotoxic 6-phenylalkynyl- and 6-*trans*-styrylpurinenucleosides, **2g** and **4h** have never before been examined in this context. In the assay employed, these compounds displayed toxicity comparable to well known anticancer drugs.

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12. Compounds **2a**, **2b** and **2j** were prepared according to ref 1, and **2h**, **2i**, **4a**, **4c**, **4i**–**4s** were prepared as described in ref 8.
13. For synthesis of **2d** and **4f** see: Bakkestuen, A. K.; Gundersen, L.-L.; Langli, G.; Liu, F.; Nolsøe, J. M. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1207.
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19. Synthesis of compound **4h**: Vinyltributyltin (175 µL, 0.60 mmol) was added to a solution of 6-chloro-2′,3′,5′-tris-*O*-(*tert*-butyldimethylsilyl)-9-β-D-ribofuranosyl-9*H*-purine[27] (315 mg, 0.50 mmol) and $(Ph_3P)_2PdCl_2$ (17.5 mg, 0.025 mmol) in dry DCE (10 mL) and the resulting mixture was stirred at reflux under Ar for 4.5 h, cooled to ambient temperature and evaporated under reduced pressure. The residue was dissolved in satd. KF in MeOH (20 mL), stirred for 1 h and evaporated. The crude product was purified by flash chromatography on silica gel eluting with 0–6% acetone in hexane to give the 6-vinylpurine, pale yellow oil, which was dissolved in dry DMF (3 mL). Iodobenzene (67 µL, 0.6 mmol), palladium acetate (5.6 mg, 0.025 mg) and ethyldiisopropylamine (260 µL, 1.5 mmol) were added and the resulting mixture was stirred at 60 °C under Ar for 15 h. The reaction mixture was evaporated under reduced pressure, and crude product was purified by flash chromatography on silica gel eluting with 0–6% acetone in hexane. Yield 225 mg (65%) of the *O*-silylated styrylpurine, coloress oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.93 (s, 1H, H-2), 8.46 (s, 1H, H-8), 8.42 (d, 1H, J = 17.0 Hz), 7.77 (m, 3H), 7.33 (m, 3H), 6.17 (d. 1H, J = 5.2 Hz), 4.75 (t, 1H, J = 4.6 Hz), 4.38 (t, 1H, J 4.0 Hz), 4.19 (m, 1H), 4.07 (dd, 1H, J = 11.3 and 4.0 Hz), 3.83 (dd, 1H, J = 11.3 and 2.7 Hz), 0.99 (s, 9H), 0.97 (s, 9H), 0.82 (s, 9H), 0.18 (s, 3H), 0.17 (s, 3H), 0.14 (s, 6H), −0.02 (s, 3H), −0.16 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 153.6, 152.3, 151.6, 143.0, 139.4, 136.0, 131.5, 130.2, 129.3, 128.7, 127.8, 126.0, 122.1, 88.1, 85.5, 71.9, 62.4, 26.0, 25.8, 25.6, 18.4, 18.0, 17.7, −4.5, −4.7, −4.5, −5.2, −5.4; MS (CI): 698 (53, $M^+ + 1$), 697 (100, M^+), 682 (13), 681 (31), 641 (20), 640 (44), 639 (91), 379 (25), 343 (17), 261 (7), 223 (12), 89 (11), 73 (29). A solution of TBAF (621 mg, 1.97 mmol) in dry THF (4 mL) was added to the *O*-silylated styrylpurineriboside (162 mg, 0.395 mmol) and the resulting mixture was stirred at ambient temperature under Ar for 16 h. Saturated NH_4Cl (1 mL) was added, and the mixture was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel eluting with 1:1 acetone/hexane, followed by 5% EtOH in EtOAc and 10% EtOH in EtOAc. Yield 66 mg (47%) of *trans*-6-styryl-9-β-D-ribofuranosyl-9*H*-purine **4h**, colorless powdery crystals, mp 157–160 °C. ^{1}H NMR (200 MHz, CD_3OD) δ 8.74 (s, 1H, H-2), 8.66 (s, 1H, H-8), 8.30 (d, 1H, J 16.1 Hz, CH=), 7.67–7.57 (m, 3H, Ph and CH=), 7.35 (m, 3H, Ph), 6.06 (d, 1H, J 5.8 Hz), 4.70 (t, 1H, J 5.5 Hz), 4.30 (dd, 1H, J 5.0 and 3.5 Hz), 4.11 (q, 1H, J 3.0 Hz), 3.78 (dd, 2H, J 12.5 and 3.0 Hz); ^{13}C NMR (50 MHz, CD_3OD) δ 155.2, 153.1, 152.7, 146.1, 142.0, 137.4, 132.7, 130.8, 130.0, 128.9, 122.7, 90.8, 87.7, 75.7, 72.3, 63.1; MS (ESI): 355 (100, $M^+ + 1$), 339 (5), 283 (5), 225 (5), 224 (30); HRMS found 355.1383, calcd for $C_{18}H_{18}N_4O4$ 355.1400.
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22. K-562 cells in logarithmic growth at 4×10^4 cells/mL and

2×10^4 cells/mL for the 5 and 48 h assay, respectively, were seeded out in 200 μL RPMI 1640 medium with supplements in 96-cell culture plates. The cell cultures were exposed to various purine concentrations ranging from 10 to 0.078 μg/mL for 5 and 48 h, pulsed for 2 h with 1 μCi/well [^{3}H]-thymidine and immobilized on fiberglass filters with a semiautomatic cell harvester (Skatron) and the cell associated radioactivity was counted. IC_{50} (defined as the concentration that inhibits [^{3}H]-thymidine incorporation by 50%) was determined for the most active purines. The purines were initially dissolved in DMSO and the final DMSO concentration in the assay was 0.05% (vol/vol). This DMSO concentration did not inhibit [^{3}H]-thymidine incorporation for the K-562 cells used (results not shown).

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