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Cytotoxic and Antibacterial Activity of 2-Oxopurine Derivatives

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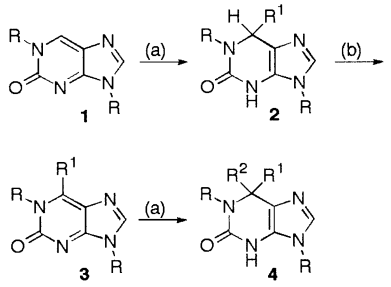
Abstract—Initial screening of the cytotoxic and antibacterial properties of 6-substituted 2-oxopurines and dihydro-2-oxopurines revealed that several compounds exhibited cytotoxicity against K-562 cells in the same range as the well known antileukemic drug 6-mercaptopurine. Most compounds were also tested for inhibitory effect on a Gram-positive bacterium, *Lactobacillus casei*, as well as the mycobacterium *Mycobacterium tuberculosis*. Generally the 2-oxopurines exhibited low antibacterial effect. © 2002 Published by Elsevier Science Ltd.

We have previously reported that Grignard reagents add to the 6-position of 2-oxopurines with complete regioselectivity and that the adducts are readily oxidized to 6-substituted 2-oxopurine derivatives.[1] Employing this synthetic strategy we have prepared plant growth stimulators,[2] antioxidants[3] and potential anti-HIV compounds.[4] Purines carrying a carbon substituent at C-6 have recently been reported to exhibit profound cytotoxic[5] and antimycobacterial activity.[6] As a continuation of our studies on 2-oxopurines, we have now performed an initial screening of the cytotoxic and antibacterial properties of these compounds.

The purines examined were prepared following the general strategy outlined in Scheme 1 for the synthesis of 1,9-dibenzyl-2-oxopurines **2**, **3** and **4**, and the actual structures are shown in Figure 1.[7,8]

The compounds **1**–**6** were screened for cytotoxic activity against K-562 cells[9] (a human chronic myelogenous leukemia cell line)[10] using a [^{3}H]-thymidine incorporation assay.[11]

In the initial screening, 10 μg/mL purine concentration was used and inhibition of [^{3}H]-thymidine incorporation was determined after 5 and 48 h exposure to the purine. For the most active purines IC_{50} was also determined (Table 1). 1,9-Dibenzylpurine **1** was only weakly active, but when a substituent was introduced in the purine 6-position, several derivatives exhibit cytotoxicity against K-562 cells after 48 h in the same range as the well known antileukemic drug 6-mercaptopurine. Dihydro adducts **2c**, **2e** and **2f**, compounds with a phenyl or alkyl substituent in the 6-position, were substantially more active than the corresponding aromatic structures **3c**, **3f**, and **3g**. Comparison of the isomeric phenyl adducts **2c**, **5** and **6** shows that the 1,9-dialkylation pattern is preferred and the data for the adducts **4b** and **4c** show that the nitrogens should definitely be alkylated. Only when the purine 6-position carries an alkynyl group was comparable cytotoxicity observed for aromatic compounds (**3a** and **3b**) compared to the related adducts **2a**, **2b** and **4b**. The IC_{50} value found for 6-mercaptopurine after 48 h was substantially lower than the IC_{50} found after only 5 h. However, for the 2-oxopurine derivatives **2a**, **2b**, **2d**, **3a**, **3b** and **4b** there was



Scheme 1. (a) Grignard reagent, −78 °C, THF, aqueous work-up; (b) DDQ or MnO_2.

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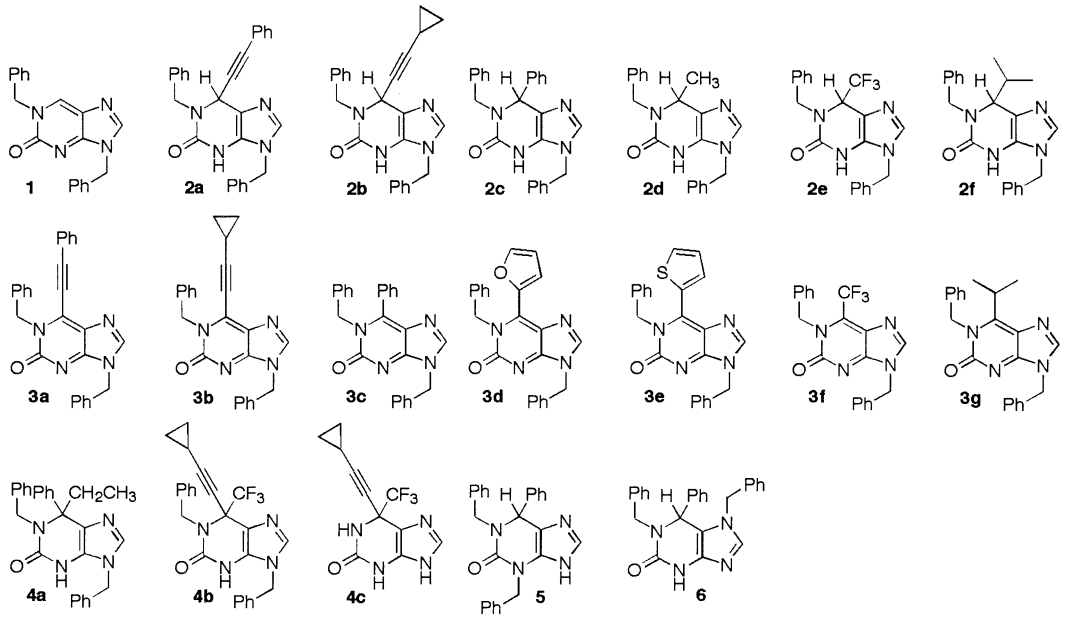


Figure 1. Structures of the 2-oxopurine derivatives studied.

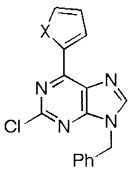
Table 1. Cytotoxicity against chronic myelogenous leukemia cells, cell line K-562, for compounds **1**–**6**

Compd	Inhibition of [^{3}H]-thymidine incorporation after 48 h			Inhibition of [^{3}H]-thymidine incorporation after 5 h		
	% Inhibition at 10.0 μg/mL concn. (±SD)[a]	% Inhibition at 1.25 μg/mL concn. (±SD)[a]	IC_{50} (μg/mL)[b] (±SD)[a]	% Inhibition at 10.0 μg/mL concn. (±SD)[a]	% Inhibition at 1.25 μg/mL concn. (±SD)[a]	IC_{50} (μg/mL)[b] (±SD)[a]
1	37(±21)	5.7(±1)	>10	16(±9)	4.4(±0.2)	>10
2a	100(±0)	59(±0)[c]	0.8(±0.1)	n.d.	20(±5.4)[c]	1.4(±0.1)
3a	100(±0)	90(±0)[c]	0.7(±0.05)	n.d.	48(±3.3)[c]	1.0(±0.05)
2b	98(±1)	42(±6)	1.3(±0.05)	90(±11)	35(±0.4)	1.6(±0.2)
3b	97(±2)	100(±0)	0.2(±0.02)	98(±0)	98(±0.5)	0.4(±0.05)
2c	90(±3)	85(±1)	0.2(±0.01)	69(±4)	9.1(±3)	5.1(±0.1)
3c	70(±6)	n.d.	n.d.	n.d.	n.d.	n.d.
4a	80(±3)	n.d.	n.d.	n.d.	n.d.	n.d.
5	32(±7)	n.d.	>10	n.d.	n.d.	n.d.
6	48(±1)	n.d.	>10	n.d.	n.d.	n.d.
2d	98(±2)	33(±1)	1.4(±0.1)	76.4(±1.2)	n.d.	2.5(±0.1)
2e	97(±0.3)	26(±0.9)	2.0(±0.1)	51(±27)	15.4(±17)	10(±3)
3f	11(±6)	n.d.	>10	n.d.	n.d.	n.d..
4b	100(±0)	99(±0.5)	0.2(±0.01)	94(±1)	66(±3.7)	0.4(±0.05)
4c	n.d.[d]	0.8(±4)[c]	>1	n.d.	0.3(±3)[c]	>1
2f	51(±6)	n.d.	ca. 10	n.d.	n.d.	n.d.
3g	18(±5)	n.d.	>10	n.d.	n.d.	n.d.

[a]Standard deviation is given in parentheses.
[b]The concentration that reduces [^{3}H]-thymidine incorporation by 50%, IC_{50}, for 6-mercaptopurine was 0.4 μg/mL (48 h assay) and 8.5 μg/mL (5 h assay).
[c]1.00 μg/mL concn.
[d]Not determined.

only a small difference in the level of thymidine incorporation after 5 and 48 h, and these compounds were significantly more active than 6-mercaptopurine in the 5 h assay.

We have recently reported high activity against *Mycobacterium tuberculosis* for 6-substituted 9-benzylpurines.[6] The structures of two of the most active compounds are shown in Figure 2. Most compounds described herein were tested as antimycobacterials against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA).[12] All adducts **2** and **4** examined were completely inactive and the same was true for most aromatic compounds **3**. However the phenylethynylpurine **3a** exhibited a minimum inhibitory concn. (MIC) against the tuberculosis bacteria at 12.5 μg/mL (Table 2) and the alkyne was also active against *Mycobacterium avium*, an organism causing opportunistic infections in AIDS patients, but toxicity (IC_{50} against VERO cells <0.2 μg/mL) makes this 2-oxopurine an unsuitable candidate for an antimycobacterial drug. The 6-aryl-2-oxopurines **3c**–**3e**, which bear structural resemblance to the active compounds shown in Figure 2, had



X = O; MIC against *M. tuberculosis* 0.78 μg/mL
X = S; MIC against *M. tuberculosis* 1.56 μg/mL

Figure 2. Structures of other antimycobacterial purines.

Table 2. Antibacterial activity for selected compounds **3**

Compd	% Inhibition of *M. tuberculosis* at 12.5 μg/mL	MIC *M. tuberculosis* (μg/mL)	% Inhibition of *M. avium* at 12.5 μg/mL	MIC *L. casei* (μg/mL)
3a	95	12.5	60	>10
3c	0	n.d.	n.d.	>10
3d	0	n.d.	n.d.	>10
3e	0	n.d.	n.d.	>10

no inhibitory effect on *M. tuberculosis*. Most compounds described herein were also tested for inhibitory effect on a Gram-positive bacterium; *Lactobacillus casei*.[13] The MICs were found to be higher than 10 μg/mL except for the 1,3-dibenzylpurine **5**, where MIC was found to be 10 μg/mL.

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References and Notes

1. Andresen, G.; Gundersen, L.-L.; Rise, F. *Tetrahedron* **1996**, *52*, 12979.
2. Andresen, G.; Dalhus, B.; Eriksen, A. B.; Gundersen, L.-L.; Rise, F. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1662.
3. Bråthe, A.; Andresen, G.; Gundersen, L.-L.; Malterud, K. E.; Rise, F. *Bioorg. Med. Chem.* (in press).
4. Andresen, G.; Gundersen, L.-L.; Rise, F. *ARKIVOC* **2001**, *2* (http://www.arkat-usa.org) (in press).
5. (a) Hocek, M.; Holy, A.; Votruba, I.; Dvorakova, H. *J. Med. Chem.* **2000**, *43*, 1817. (b) Hocek, M.; Holy, A.; Votruba, I.; Dvorakova, H. *Collect. Czech. Chem. Commun.* **2001**, *66*, 483.
6. Bakkestuen, A. K.; Gundersen, L.-L.; Langli, G.; Liu, F.; Nolsøe, J. M. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1207.
7. Compounds **1**, **2a**, **2c**, **2d**, **2f**, **3a**, **3c**, **3g, 4a, 5** and **6** were prepared according to ref 1 and the syntheses of compounds **2b**, **2e**, **3b**, **3f**, **4b** and **4c** are reported in ref 4.
8. **1,9-Dibenzyl-1,9-dihydro-6-(2-furanyl)-2H-purin-2-one 3d**. To a solution of 1,9-dibenzyl-1,9-dihydro-2*H*-purin-2-one (100 mg, 0.32 mmol) in dry Et_2O (8 mL) under N_2 at −78 °C was added dropwise a solution of Grignard reagent [5.0 mL, 5.0 mmol, generated from 2-iodofuran (4 g, 20 mmol) and magnesium (1 g, 41 mmol) in dry Et_2O (to a total volume of 20 mL) under N_2]. The mixture was stirred for 1 h at −78 °C and 1 h at room temperature and diluted with dichloromethane (10 mL). The solution was washed with saturated aqueous ammonium chloride (10 mL) and saturated aqueous sodium hydrogen carbonate (10 mL), dried ($MgSO_4$) and evaporated in vacuo. The crude product was dissolved in dry benzene (30 mL), DDQ (72 mg, 0.32 mmol) was added and the resulting mixture was stirred at room temperature for 3 h under N_2 before the mixture was filtered and the solvent evaporated in vacuo. The product was purified by flash chromatography on silica gel eluting with $CHCl_3/CH_3CN$ (7:1) to give the *furanylpurine* (80 mg, 66%), yellow crystals; mp 178–181 °C; (found: C, 72.33; H, 4.76. $C_{23}H_{18}N_4O_2$ requires: C, 72.24; H, 4.74%); δ_H (300 MHz; $CDCl_3$) 5.21 [2H, s, N(9)CH_2], 5.76 [2H, s, N(1)CH_2], 6.60 (1H, dd, J = 3.6, 1.8 Hz, CH in furanyl), 7.1–7.4 (10H, m, CH in Ph), 7.45 (1H, dd, J = 3.6, 0.7 Hz, CH in furanyl), 7.67 (2H, m, H-8 and CH in furanyl); δ_C (75 MHz; $CDCl_3$) 46.4 [N(9)CH_2], 50.9 [N(1)CH_2], 112.7 (CH in furanyl), 121.4 (C-5 and CH in furanyl), 126.5 (CH in Ph), 127.2 (CH in Ph), 128.1 (CH in Ph), 128.4 (CH in Ph), 128.4 (CH in Ph), 129.0 (CH in Ph), 134.9 (C in Ph), 136.7 (C in Ph), 140.1 (C in furanyl), 141.9 (C-6), 145.9 (C-8), 146.0 (CH in furanyl), 156.4 (C-2) and 157.9 (C-4); m/z (EI) 382 (M^+, 28%), 353 (7), 292 (11), 291 (52), 282 (9), 280 (10), 198 (7), 92 (8), 91 (100) and 65 (26). **1,9-Dibenzyl-1,9-dihydro-6-(2-thienyl)-2H-purin-2-one 3e** was prepared from 2-thienylmagnesium bromide using the same procedure as described above; yield 66%, yellow oil; (found: C, 69.49; H, 4.56. $C_{23}H_{18}N_4OS$ requires: C, 69.32; H, 4.55%); δ_H (300 MHz; $CDCl_3$) 5.11 [2H, s, N(9)CH_2], 5.39 [2H, s, N(1)CH_2], 6.9–7.0 (3H, m), 7.1–7.3 (9H, m), 7.52 (1H, dd, J = 4.9, 1.0 Hz, CH in thienyl), 7.65 (1H, s, H-8); δ_C (75 MHz; $CDCl_3$) 46.4 [N(9)CH_2], 51.1 [N(1)CH_2], 123.4 (C-5), 126.1 (CH in Ph), 127.2 (CH in Ph), 127.5 (CH in Ph), 127.9 (C in thienyl), 128.0 (CH in thienyl), 128.3 (CH in Ph), 128.5 (CH in Ph), 128.9 (CH in Ph), 131.1 (CH in thienyl), 132.3 (CH in thienyl), 134.8 (C in Ph), 136.7 (C in Ph), 146.0 (C-6), 146.4 (C-8), 156.4 (C-2) and 157.7 (C-4); m/z (EI) 398 (M^+, 39%), 308 (14), 307 (45), 198 (14), 183 (16), 141 (25), 91 (38), 32 (26) and 28 (100).
9. K-562 cells were maintained in RPMI 1640 medium with 10% (vol/vol) fetal calf serum, 2 mM L-glutamine and 0.1 mg/mL kanamycin in a humidified atmosphere containing 5% CO_2.
10. Lozzio, C. B.; Lozzio, B. B. *Blood* **1975**, *45*, 321.
11. 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-well 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).
12. Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.
13. Activity against *L. casei* (NCDO 2713) was determined as reported before: Fimland, G.; Blingsmo, O. R.; Sletten, K.; Jung, G.; Nes, I. F.; Nissen-Meyer, J. *Appl. Environ. Microbiol.* **1996**, *62*, 3313.