CONCISE ARTICLE

6-Alkyl-, 6-aryl- or 6-hetaryl-7-deazapurine ribonucleosides as inhibitors of human or MTB adenosine kinase and potential antimycobacterial agents†

Pavla Perlíková,‡[a] Petr Konečný,‡[b] Petr Nauš,[a] Jan Snášel,[a] Ivan Votruba,[a] Petr Džubák,[b] Iva Pichová,[a] Marián Hajdúch[b] and Michal Hocek*[ac]

Title 6-alkyl-, 6-aryl- and 6-hetaryl-7-deazapurine ribonucleosides previously known as nanomolar cytostatics were found to be potent inhibitors of either human or mycobacterial (MTB) adenosine kinase (ADK). Several new derivatives bearing bulky substituents at position 6 were non-cytotoxic but selectively inhibited MTB ADK. However, most of the nucleosides (ADK inhibitors) as well as their octadecylphosphate prodrugs were inactive in the whole cell assay of inhibition of *Mycobacterium bovis* growth. 6-Methyl-7-deazapurine ribonucleoside was found to be a potent antimycobacterial agent.

Introduction

Modified purine nucleoside derivatives and analogs display a wide range of biological activities. Their antiviral[1] and antitumor[2] properties are particularly important and used in clinical therapeutics. However, purine derivatives[3] and nucleosides[4] also are potent antimycobacterial agents, *i.e.* for treatment of *Mycobacterium tuberculosis* (MTB). Out of the variety of possible target enzymes from the purine salvage pathway in MTB,[5] adenosine kinase (ADK)[6] is considered as a promising target for drug development since it is structurally very different from the human ADK.[7]

Recently, we have discovered 6-hetaryl-7-deazapurine ribonucleosides 1–3 with nanomolar cytostatic activities towards a wide panel of leukemia and cancer cell-lines.[8] Their *cyclo*Sal-phosphate[9] and phosphoramidate prodrugs[10] were less active due to increased efflux from the cells. However, *cyclo*Sal-phosphates were also found[9] to be potent inhibitors of human and moderate inhibitors of MTB ADKs. Since also other 7-deazapurine nucleosides are known as inhibitors of ADKs,[11] we have revisited the whole class of 7-deazapurine nucleosides with diverse aryl and hetaryl groups at position 6 and systematically studied their activity toward human and MTB ADKs.

Results and discussion

The synthesis of a series of nineteen 6-alkyl, 6-aryl- and 6-hetaryl-7-deazapurine ribonucleosides **1b–t**, nine 7-fluoro derivatives **2e, j–m, o–r** and six 7-chloro derivatives **3e, g, j–m** has been reported earlier,[8] as well as the 6-methyl derivative **1a** (ref. 12) (Chart 1). Most of these derivatives exhibited strong cytostatic or cytotoxic activities.[8,12]

In order to get less cytotoxic derivatives, we have extended the series by synthesis of other six derivatives bearing bulky (het)aryl groups at position 6 (Scheme 1). The dibenzofuryl and benzofuryl derivatives **1u** and **v** were prepared by the Suzuki coupling of isopropylidene-protected nucleoside **4** followed by deprotection. The other derivatives **1w–z** were synthesized by direct aqueous Suzuki coupling of 6-chloro-7-deazapurine ribonucleoside with the corresponding hetarylboronic acid in the presence of Pd(OAc)$_2$, triphenylphosphine-3,3′,3″-trisulfonate (TPPTS) and Na$_2$CO$_3$. In the case of indole derivative **1x**, the coupling was performed with the Boc-protected indole-2-boronic acid and the TFA treatment was used for deprotection. In all cases, the products were obtained in good 71–85% yields.

A synthetic path to 6-(het)aryl-7-deazapurine ribonucleoside-5′-octadecylphosphates, as potential lipophilic phosphate prodrugs, was developed starting from isopropylidene-protected ribonucleoside **4**. An octadecylphosphate group was attached by reaction with octadecylphosphate in the presence of 2,4,6-trimethylbenzene-1-sulphonyl chloride (MtsCl) in pyridine. Nucleoside-5′-octadecylphosphate **7** was obtained in 58% yield. Deprotection by treatment with 90% aqueous TFA provided free
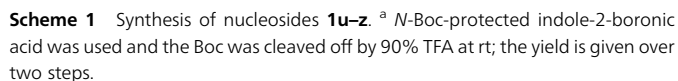
[a]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Gilead Sciences & IOCB Research Center, Flemingovo nam. 2, CZ-16610 Prague 6, Czech Republic. E-mail: hocek@uochb.cas.cz; Tel: +420 220183324*

[b]*Institute of Molecular and Translational Medicine, Laboratory of Experimental Medicine, Palacky University and University Hospital in Olomouc, Faculty of Medicine and Dentistry, Hněvotínská 5, CZ-775 15 Olomouc, Czech Republic*

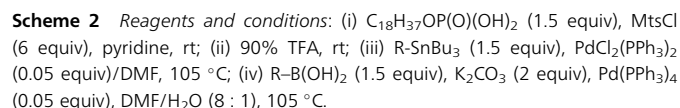[c]*Department of Organic Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, CZ-12843 Prague 2, Czech Republic*

† Electronic supplementary information (ESI) available: Experimental part and characterization data for all new compounds. See DOI: 10.1039/c3md00232b

‡ These authors contributed equally.

This journal is © The Royal Society of Chemistry 2013

Med. Chem. Commun., 2013, **4**, 1497–1500 | **1497**

Chart 1 Structures of previously known nucleosides **1a–t**.

Scheme 1 Synthesis of nucleosides **1u–z**. [a] N-Boc-protected indole-2-boronic acid was used and the Boc was cleaved off by 90% TFA at rt; the yield is given over two steps.

nucleoside-5'-octadecylphosphate **8** (94%), which was used as a starting material for a series of Stille and Suzuki cross-coupling reactions. Stille cross-coupling reactions with hetaryltributyl-stannanes were performed in the presence of $PdCl_2(PPh_3)_2$ in DMF at 105 °C. As 5'-octadecylphosphate **8** is insoluble in toluene, standard conditions for the Suzuki cross-coupling reaction had to be slightly modified. The reactions with (het) arylboronic acids were performed in the presence of potassium carbonate and $Pd(PPh_3)_4$ in DMF/water (8 : 1) at 105 °C. 6-(Het) aryl-7-deazapurine ribonucleoside-5'-octadecylphosphates **9e, j–m** and **v** were obtained in 62–85% yields (Scheme 2).

All the title nucleosides **1a–z**, **2e, j–m, o–r, 3e, g, j–m** and nucleotides **9e, j–m, v** were tested for the inhibition of human and MTB ADKs (for cloning expression and purification of these enzymes, see ref. 9) and most of them also for substrate activity to the kinases, i.e. for phosphorylation. The results were correlated with their in vitro cytotoxicity (MTT) against non-malignant BJ and MRC-5 human fibroblast cell lines. The cytotoxicity strongly depended on the bulkiness of the substituent at position 6. Most active were derivatives bearing five-membered heterocycles, whereas derivatives bearing bulky aryl groups were generally not cytotoxic, which was consistent with previously published[8] cytostatic and cytotoxic activities on leukemia and solid tumor cell lines. Most of the nucleosides did not show significant inhibition to human ADK with the exception of bulky derivatives **1u–z**, which showed inhibition with micromolar IC_{50} values. On the other hand, most of the nucleosides were moderate to good substrates for the human ADK and were readily phosphorylated to nucleoside 5'-O-monophosphates, which is a necessary step in their activation for eventual inhibition of RNA synthesis in their cytostatic or cytotoxic effect.[8] On the other hand, none of the compounds was found to be a substrate for MTB ADK and most compounds efficiently inhibited this enzyme. While the alkyl-substituted derivatives **1a–d** were weak inhibitors of MTB ADK, all the aryl-

Scheme 2 Reagents and conditions: (i) $C_{18}H_{37}OP(O)(OH)_2$ (1.5 equiv), MtsCl (6 equiv), pyridine, rt; (ii) 90% TFA, rt; (iii) R-SnBu$_3$ (1.5 equiv), PdCl$_2$(PPh$_3$)$_2$ (0.05 equiv)/DMF, 105 °C; (iv) R–B(OH)$_2$ (1.5 equiv), K$_2$CO$_3$ (2 equiv), Pd(PPh$_3$)$_4$ (0.05 equiv), DMF/H$_2$O (8 : 1), 105 °C.

1498 | Med. Chem. Commun., 2013, **4**, 1497–1500

This journal is © The Royal Society of Chemistry 2013

Concise Article

Table 1 Cytotoxicity, human and MTB ADK inhibition and antimycobacterial activity

Compd	BJ[a] CC$_{50}$ (μM)	MRC-5 CC$_{50}$ (μM)	ADK substrate[b] (%) Human	ADK inhibition IC$_{50}$ (μM) Human	MTB	*Mycobacterium bovis*[c] IC$_{50}$ (μM)
1a	0.098	0.081	54	>10	8.8 ± 0.03	0.30
1b	2.70	50	n.d.	>20	8.1 ± 0.9	88.84
1c	0.34	0.28	26	>10	>5.00	32.61
1d	36.92	>50		>20	>20	>100
1e	44.15	>50	n.d.	>10	0.12 ± 0.03	75.16
1f	>50	>50	n.d.	>10	0.058 ± 0.003	56.88
1g	>50	>50	17	>10	0.08 ± 0.008	93.82
1h	1.74	>50	39	>10	0.12 ± 0.02	38.22
1i	50	>50	n.d.	>10	1.00 ± 0.11	>100
1j	0.23	11.83	56	1.30 ± 0.25	0.32 ± 0.05	>100
1k	1.20	45.38	32	>10	0.32 ± 0.04	83.67
1l	0.31	>50	n.d.	>5	0.046 ± 0.003	>100
1m	21.33	>50	56	>10	0.195 ± 0.05	81.00
1n	1.52	>50	43	>10	0.030 ± 0.005	>100
1o	>50	>50	40	>10	0.073 ± 0.007	56.44
1p	1.87	40.15	70	>10	0.30 ± 0.04	98.23
1q	0.26	37.00	41	>20	0.023 ± 0.003	80.53
1r	0.21	>50	28	>10	0.78 ± 0.10	15.55
1s	43.93	1.16	31	>20	0.059 ± 0.005	91.10
1t	42.13	>50	7	>20	0.67 ± 0.10	>100
1u	>50	>50	n.d.	5.26 ± 0.66	2.35 ± 0.35	>100
1v	>50	>50	n.d.	2.10 ± 0.03	0.0145 ± 0.001	>100
1w	>50	>50	n.d.	0.30 ± 0.02	0.0075 ± 0.0007	>100
1x	>50	>50	n.d.	8.35 ± 0.75	0.04 ± 0.005	>100
1y	>50	43.76	n.d.	15.4 ± 1.2	0.15 ± 0.007	>100
1z	>50	>50	n.d.	3.07 ± 0.40	1.46 ± 0.07	>100
2e	>50	>50	17	>10	0.20 ± 0.05	74.81
2j	0.31	29.04	50	1.30 ± 0.25	0.19 ± 0.10	73.20
2k	0.35	15.38	n.d.	>10	0.15 ± 0.05	91.19
2l	0.17	47.21	52	1.10 ± 0.20	0.035 ± 0.005	>100
2m	0.62	50	51	>10	0.063 ± 0.003	>100
2o	2.99	44.45	76	>10	0.070 ± 0.010	45.78
2p	2.02	48.94	97	>10	0.40 ± 0.06	>100
2q	0.63	>50	75	>20	0.028 ± 0.007	68.99
2r	>50	>50	11	>20	1.00 ± 0.12	11.73
3e	44.49	>50	29	>10	0.22 ± 0.05	>100
3g	49.52	>50	n.d	>20	0.40 ± 0.02	89.63
3j	37.25	>50	77	0.29 ± 0.03	0.11 ± 0.01	89.23
3k	21.62	>50	100	3.4	0.30 ± 0.03	97.10
3l	4.97	>50	100	1.20 ± 0.20	0.07 ± 0.007	96.38
3m	1.72	>50	97	>10	0.25 ± 0.03	99.57
9e	>50	>50	—	4.40 ± 0.10	>20	>100
9j	>50	45.26	—	2.4 ± 0.11	>20	>100
9k	44.20	>50	—	5.15 ± 0.35	>20	56.56
9l	n.d.	n.d.	—	1.97 ± 0.16	>20	n.d.
9m	>50	>50	—	6.35 ± 0.34	>20	82.78
9v	>50	>50	—	2.9 ± 0.30	>20	93.39

[a] Cytotoxicity (MTT test) in BJ and MRC-5 fibroblasts. [b] ADK substrate activity, conversion to 5′-phosphate (%). [c] 50% growth inhibitory concentration of *in vitro* cultivated *Mycobacterium bovis* BCG.

and hetaryl-substituted 7-deazapurine ribonucleosides (1e–z), including 7-fluoro- (2) and 7-chloro-derivatives (3) were strong inhibitors of this enzyme with submicromolar to low nanomolar IC$_{50}$ values. Most of the derivatives bearing phenyl and five-membered hetaryl groups at position 6 (1e–i, k–t, 2e, k, m–r and 3e, g, m) were selective inhibitors of the MTB ADK and did not significantly inhibit the human enzyme (but were strongly cytotoxic). 6-Furyl derivatives 1j and 2j and the thienyl derivative

2l were less selective, inhibited both enzymes and were cytotoxic. The derivatives bearing bulky aryl groups 1u–z inhibited the MTB ADK in low nanomolar concentrations while the inhibition of human enzyme was observed at micromolar concentrations, and so the selectivity index was 2–3 orders of magnitude. These bulky derivatives were not cytotoxic. The octadecylphosphate prodrugs 9 were moderate inhibitors of the human ADK and inactive against MTB enzyme.

This journal is © The Royal Society of Chemistry 2013

Med. Chem. Commun., 2013, **4**, 1497–1500 | **1499**

All the derivatives were also tested for *in vivo* inhibition of *Mycobacterium bovis* growth (Table 1). From all tested nucleosides only compound **1a** displayed very significant antimycobacterial activity ($IC_{50} = 0.3$ μM) but showed the highest cytotoxicity. This derivative, however, only weakly inhibited *in vitro* MTB ADK ($IC_{50} = 8.8$ μM) which may indicate that the mode of antimycobacterial activity of this compound is independent of MTB ADK and rather suggests a more general cytotoxic mechanism. Two 6-(imidazolyl)deazapurine nucleosides **1r** and **2r** exerted moderate antimycobacterial activity ($IC_{50} = 15.6$ and 11.7 μM, respectively) accompanied by preferential inhibition of MTB ADK and low cytotoxicity, whereas all other nucleosides were virtually inactive. The octadecylphosphate prodrugs **9e**, **j–m** and **v**, which were designed as lipophilic derivatives with increased penetration through the mycobacterial cell wall, did not show any antimycobacterial activity either.

Conclusions

It can be concluded that the 6-(het)aryl-7-deazapurine ribonucleosides are strong and mostly selective inhibitors of MTB ADK but not the human ADK. Nonetheless, they showed only limited potential to inhibit growth of mycobacteria. One reason could be their poor penetration through the mycobacterial cell wall. Alternatively, the mycobacterial ADK may not be a suitable target for therapy since AMP also can be biosynthesized by the salvage pathway from adenine utilizing adenine phosphoribosyl transferase or by a reaction sequence from IMP.[5]

Acknowledgements

Notes and references

1 Reviews: (*a*) E. De Clercq, *J. Med. Chem.*, 2010, **53**, 1438–1450; (*b*) E. De Clercq, *Nucleosides, Nucleotides Nucleic Acids*, 2012, **31**, 339–352.

2 Reviews: (*a*) W. B. Parker, J. A. Secrist, III and W. R. Waud, *Curr. Opin. Invest. Drugs*, 2004, **5**, 592–596; (*b*) C. M. Galmarini, F. Popowycz and B. Joseph, *Curr. Med. Chem.*, 2008, **15**, 1072–1082; (*c*) W. B. Parker, *Chem. Rev.*, 2009, **109**, 2880–2893.

3 Examples: (*a*) A. Scozzafava, A. Mastrolorenzo and C. T. Supuran, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 1675–

1678; (*b*) A. K. Pathak, V. Pathak, L. E. Seitz, W. J. Suling and R. C. Reynolds, *J. Med. Chem.*, 2004, **47**, 273–276; (*c*) L.-L. Gundersen, J. Nissen-Meyer and B. Spilsberg, *J. Med. Chem.*, 2002, **45**, 1383–1386; (*d*) A. K. Bakkestuen, L.-L. Gundersen and B. T. Utenova, *J. Med. Chem.*, 2005, **48**, 2710–2723; (*e*) M. Braendvang and L.-L. Gundersen, *Bioorg. Med. Chem.*, 2005, **13**, 6360–6373.

4 Reviews: (*a*) M. C. Long and W. B. Parker, *Biochem. Pharmacol.*, 2006, **71**, 1671–1682; (*b*) M. C. Long, S. C. Shaddix, O. Moukha-Chafiq, J. A. Maddry, L. Nagy and W. B. Parker, *Biochem. Pharmacol.*, 2008, **75**, 1588–1600.

5 Reviews: (*a*) W. B. Parker and M. C. Long, *Curr. Pharm. Des.*, 2007, **13**, 599–608; (*b*) R. G. Ducati, A. Breda, A. Basso, L. A. Basso and D. S. Santos, *Curr. Med. Chem.*, 2011, **18**, 1258–1275.

6 M. C. M. Reddy, S. K. Palaninathan, N. D. Shetty, J. L. Owen, M. D. Watson and J. C. Sacchettini, *J. Biol. Chem.*, 2007, **282**, 27334–27342.

7 S. W. Muchmore, R. A. Smith, A. O. Stewart, M. D. Cowart, A. Gomtsyan, M. A. Matulenko, H. Yu, J. M. Severin, S. S. Bhagwat, C.-H. Lee, E. A. Kowaluk, M. F. Jarvis and C. L. Jakob, *J. Med. Chem.*, 2006, **49**, 6726–6731.

8 P. Nauš, R. Pohl, I. Votruba, P. Džubák, M. Hajdúch, R. Ameral, G. Birkus, T. Wang, A. S. Ray, R. Mackman, T. Cihlar and M. Hocek, *J. Med. Chem.*, 2010, **53**, 460–470.

9 P. Spáčilová, P. Nauš, R. Pohl, I. Votruba, J. Snášel, H. Zábranská, I. Pichová, R. Ameral, G. Birkuš, T. Cihlář and M. Hocek, *ChemMedChem*, 2010, **5**, 1386–1396.

10 P. Perlíková, R. Pohl, I. Votruba, R. Shih, G. Birkuš, T. Cihlář and M. Hocek, *Bioorg. Med. Chem.*, 2011, **19**, 229–242.

11 Examples: (*a*) B. G. Ugarkar, J. M. DaRe, J. J. Kopcho, C. E. Browne, III, J. M. Schanzer, J. B. Wiesner and M. D. Erion, *J. Med. Chem.*, 2000, **43**, 2883–2893; (*b*) B. G. Ugarkar, A. Castellino, J. M. DaRe, J. J. Kopcho, J. B. Wiesner, J. M. Schanzer and M. D. Erion, *J. Med. Chem.*, 2000, **43**, 2894–2905; (*c*) B. G. Ugarkar, A. J. Castellino, J. S. DaRe, M. Ramirez-Weinhouse, J. J. Kopcho, S. Rosengren and M. D. Erion, *J. Med. Chem.*, 2003, **46**, 4750–4760; (*d*) B. C. Bookser, M. C. Matelich, K. Ollis and B. G. Ugarkar, *J. Med. Chem.*, 2005, **48**, 3389–3399; (*e*) S. H. Boyer, B. G. Ugarkar, J. Solbach, J. J. Kopcho, M. C. Matelich, K. Ollis, J. E. Gomez-Galeno, R. Mendonca, M. Tsuchiya, A. Nagahisa, M. Nakane, J. B. Wiesner and M. D. Erion, *J. Med. Chem.*, 2005, **48**, 6430–6441; (*f*) Y. A. Kim, A. Sharon, C. K. Chu, R. H. Rais, O. N. Al Safarjalani, F. N. Naguib and M. H. el Kouni, *J. Med. Chem.*, 2008, **51**, 3934–3945.

12 R. Wu, E. D. Smidansky, H. S. Oh, R. Takhampunya, R. Padmanabhan, C. E. Cameron and B. R. Peterson, *J. Med. Chem.*, 2010, **53**, 7958–7966.

1500 | *Med. Chem. Commun.*, 2013, **4**, 1497–1500

This journal is © The Royal Society of Chemistry 2013