Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 2989–2992

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Pyrazolo[4,3-*d*]pyrimidines as New Generation of Cyclin-Dependent Kinase Inhibitors

Daniela Moravcová,[a,†] Vladimír Kryštof,[b,†] Libor Havlíček,[a] Jiří Moravec,[a] René Lenobel[b] and Miroslav Strnad[b,*]

[a]*Isotope Laboratory, Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Vídeňská 1083, 14220 Prague 4, Czech Republic*

[b]*Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany, Šlechtitelů 11, 783 71 Olomouc, Czech Republic*

Received 25 February 2003; accepted 11 March 2003

Abstract—A search among analogues of anti-CDK purines led to the identification of substituted pyrazolo[4,3-*d*]pyrimidines as novel inhibitors of CDK1/cyclin B. Some of these derivatives also show antiproliferative activity on cancer cell line K-562, thus may find an application as anticancer agents.

While searching for new groups of CDK inhibitors we found that substituted pyrazolo[4,3-*d*]pyrimidines, studied in early 1970s by plant physiologists as cytokinin antagonists with antiproliferative effects on plant cells,[1] also potently inhibit human CDK1. In contrast to C2, C6, N9-trisubstituted purine inhibitors,[2] the pyrazolo[4,3-*d*]pyrimidines demonstrate the comparable inhibitory effect with only two side chains at positions 3 and 7, respectively. Hence, we prepared several 3,7-disubstituted pyrazolo[4,3-*d*]pyrimidines and compared their CDK1 inhibitory properties and antiproliferative activity against cancer cell lines with corresponding 6,9-disubstituted purines. Novel compounds showed significantly higher biological activities.

Chemistry of Pyrazolo[4,3-*d*]pyrimidines

A series of new 3,7-disubstituted pyrazolo[4,3-*d*]pyrimidines was synthesised using a general synthetic route outlined in Figure 1. The starting material,[3] 3-isopropyl-4-nitropyrazolecarboxylic acid (**I**), was esterified in methanol saturated with HCl. The resulting nitroester **II** was reduced with hydrogen on RaNi under atmospheric pressure to the corresponding aminoester **III**. Substituted pyrazole **III** was then cyclised with formamidin acetate in the presence of Et_3N to 7-hydroxy-3-isopropylpyrazolo[4,3-*d*]pyrimidine (**IV**). The 7-hydroxy derivative **IV** was chlorinated with a mixture of $SOCl_2$-DMF to 7-chloro derivative **V**, which did not have to be isolated as a pure compound and thus can be immediately used for nucleophilic substitution with different amines to obtain selected 7-substituted-3-isopropylpyrazolo[4,3-*d*]pyrimidines (**VI**). In total, 11 new pyrazolo[4,3-*d*]pyrimidine derivatives have been prepared by two different methods.[4] **Method A:** The substitution of Cl–C[7] was achieved in $CHCl_3$ by reaction of **V** with 5 equivalents of the appropriate amine at 60 °C for 1 h. **Method B:** Five equivalents of the selected amines in 3 mL of ethanol and 3 equiv of *N*-ethyldiisopropylamine were added to the $CHCl_3$ solution of **V**. The mixture was stirred at 60 °C for 1 h. The crude products were purified by column chromatography in both cases.

Synthesis of 6,9-Disubstituted Purines

The straightforward synthesis of 9-isopropyl-6-substituted purines started from 6-chloro-9-isopropylpurine.[5] The 6-chloro atom was displaced by refluxing with a primary amine in butan-1-ol in the presence of *N*-ethyldiisopropylamine at 112 °C for 1 h. Compounds obtained by this method were also purified by column chromatography and data characterising the prepared purines are

*Corresponding author. Tel. +420-58-563-4850; fax: +420-58-563-4870; e-mail: strnad@aix.upol.cz
†Both authors contributed equally to this work.

0960-894X/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00631-0

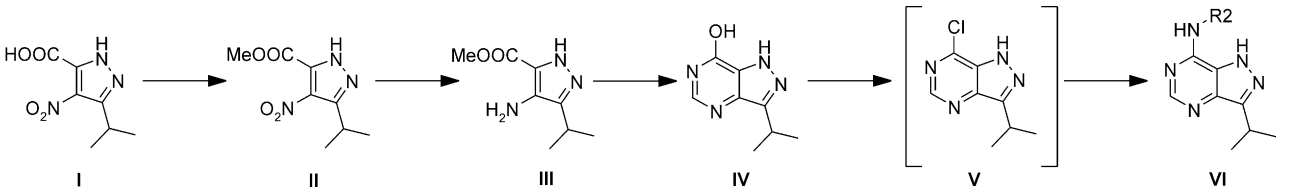

Figure 1. Synthesis of 3,7-disubstituted pyrazolo[4,3-*d*]pyrimidines.

given in ref 4 Compounds **VIIa** and **VIIc** were prepared as described previously.[6,7]

Results and Discussion

As a part of our ongoing study on structure–activity relationships of purine CDK inhibitors, we prepared and tested disubstituted pyrazolo[4,3-*d*]pyrimidines for their ability to block the activity of CDK1/cyclin B and proliferation of myeloid leukemia cell line K-562 according to a published method.[8] The results are summarised in Table 1.

All synthesised pyrazolo[4,3-*d*]pyrimidine derivatives strongly inhibited CDK1 kinase at concentrations approximately 2–10 times lower than the respective 6,9-disubstituted purines. In the present study, the first modification of **VIa** to be examined was introduction of *o*-Br (**VIb**), which however led to a big loss of activity. Introduction of an *o*-MeO group to give the analogue **VIc** resulted in a slight loss in activity, whereas the hydroxybenzyl derivatives **VId–f** were usually more potent. The most active substance **VId**, bearing a 2-hydroxybenzylamino moiety at C7 like the trisubstituted purine olomoucine II,[8] demonstrated the impact and the power of hydroxylated aromatic substituents. Introduction of a second MeO group to 3-hydroxy group on the aromatic ring gave 3-hydroxy-4-methoxybenzyl analogue **VIg** with a minimal gain in activity. Interestingly, certain losses of activity were observed after substitution of the C7 position with effective cytokinin or anticytokinin side chains like furfuryl (**VIh**), pentyl (**VIi**) and isopent-2-en-(1-yl) (**VIj**). The preparation of compound **VIk** was undertaken on the basis of independent reports by Gray et al.[2c] which showed that trisubstituted purines possessing a substituted arylamine at C6 were strongly reactive. Relative to the parent compound purvalanol A, good levels of activity were also observed for the equivalent pyrazolo[4,3-*d*]pyrimidine analogue **VIk**. Cytotoxicity of new derivatives usually increased in relation to the improved CDK inhibitory activity. The pyrazolo[4,3-*d*]pyrimidine derivatives were again much more potent than appropriate 6,9-disubstituted purine analogues **VIIa–k** (Table 1).

Table 1. CDK1/cyclin B inhibitory and in vitro antiproliferative activities of 6,9-disubstituted purines and corresponding 3,7-disubstituted pyrazolo[4,3-*d*]pyrimidines. IC_{50} values (given in μmol/dm^3) are means of three experiments (olomoucine added for comparison)

Pyrazolo[4,3-*d*] pyrimidine	IC_{50}		Purine	IC_{50}	
	CDK1	K562		CDK1	K562
VIa	1.2	66	**VIIa**	3.6	113
VIb	11	29	**VIIb**	23	49
VIc	2.3	46	**VIIc**	3.8	>167
VId	0.44	54	**VIId**	4.4	125
VIe	1.7	88	**VIIe**	3.1	>167
VIf	1.8	112	**VIIf**	4.0	>167
VIg	1.1	26	**VIIg**	4.5	104
VIh	2.5	>167	**VIIh**	9.1	>167
VIi	1.2	61	**VIIi**	6.9	144
VIj	4.5	152	**VIIj**	17	>167
VIk	0.9	18.4	**VIIk**	5.9	54
			Olomoucine	7	163

To summarise the data, the best results were obtained with *o*-hydroxy or *m*-chloro groups on the benzylamino and aminophenyl substituents, respectively. These results confirm that the capacity of new pyrazolo[4,3-*d*]pyrimidines can be modulated through variation of the C7 position aromatic ring substituents. Furthermore, their increased CDK inhibitory activity and antiproliferative capacity brings further potential to the area of development of novel CDK inhibitors.

Acknowledgements

We thank D. P. Lane for baculoviruses, K. Nováková, and E. Friesnerová for technical assistance, and L. H. Jones for English corrections. The work was supported by Czech Grant Agency (303/02/0875) and Grant Agency of the ASCR (KJB4038301).

References and Notes

1. Hecht, S. M.; Bock, R. M.; Schmitz, R. Y.; Skoog, F.; Leonard, N. J. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *68*, 2608.
2. (a) Veselý, J.; Havlíček, L.; Strnad, M.; Blow, J. J.; Donella-Deana, A.; Pinna, L.; Letham, D. S.; Kato, J.; Detivaud, L.; LeClerc, S.; Meijer, L. *Eur. J. Biochem.* **1994**, *224*, 771. (b) Havlíček, L.; Hanuš, J.; Veselý, J.; LeClerc, S.; Meijer, L.; Shaw, G.; Strnad, M. *J. Med. Chem.* **1997**, *40*, 408. (c) Gray, N. S.; Wodicka, L.; Thunnissen, A. M.; Norman, T. C.; Kwon, S.; Espinoza, F. H.; Morgan, D. O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S. H.; Lockhart, D. J.; Schultz, P. G. *Science* **1998**, *281*, 533.
3. Baraldi, P. G.; Cacciari, B.; Leoni, A.; Recanatini, M.; Roberti, M.; Rossi, M. *Farmaco* **1991**, *46*, 1337.
4. Data for prepared compounds: (**II**): 5-Isopropyl-4-nitropyra-zole-3-carboxylic acid was added to a 4.5 M solution of HCl in absolute methanol. The reaction mixture was heated at 60 °C for 7 h and evaporated to dryness. The title compound was crystallised from ethyl acetate; yield 91%; mp = 78–80 °C. MS ESI+: 214.1 (100, $M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$): 1.39 d (6H, J = 7.1 Hz); 3.64 sept (1H, J = 7.1 Hz), 3.98 s (3H). Anal. ($C_8H_{11}N_3O_4$) C, H, N. **Methyl 4-amino-5-isopropylpyrazol-3-carboxylate (III)**: To a solution of methyl 5-isopropyl-4-nitropyrazol-3-carboxylate (4.34 g, 24 mmol) in

20 mL ethanol and 5 mL water was added 1 g RaNi (an activity W5). The mixture was stirred under hydrogen atmosphere (760 torr) for 12 h. The RaNi was filtered off and the filtrate was evaporated in vacuo. The residue crystals were washed with cold ethyl acetate; yield 95%; mp = 122–123 °C. MS ESI+: 184.1 (100, $M+H^+$). ^1H NMR (400 MHz, $CDCl_3$): 1.31 d (6H; $J=6.9$ Hz), 2.93 sept (1H; $J=6.9$ Hz), 3.9 s (3H). Anal. ($C_8H_{13}N_3O_2$) C, H, N. **7-Hydroxy-3-isopropylpyrazolo[4,3-*d*]pyrimidine (IV)**: A mixture of amino ester III (1.5 g, 8.42 mmol), formamidine acetate (2.47 g, 24 mmol) and triethylamine (5.25 mL) in 32 mL of 2-ethoxyethanol was heated for 2 h at 90 °C under argon atmosphere. The excess of triethylamine was evaporated from 2-ethoxyethanol (Cellosolve) solution in vacuo, crystallised product was filtered off and washed with $CHCl_3$. An analytical sample was obtained by recrystallisation from ethanol. Yield 96%; mp = 302–304 °C. MS ESI+: 178.3 (100, $M+H^+$). ^1H NMR (300 MHz, CD_3OD): 1.41 d (6H, $J=7.15$ Hz), 3.40 sept (1H, $J=7.15$ Hz), 7.82 s (1H). ^{13}C NMR (400 MHz, DMSO-d_6 + AcOD): 21.912, 25.985, 141.85, 172.17. Anal. ($C_8H_{10}N_4O$) C, H, N. **7-Chloro-3-isopropylpyrazolo[4,3-*d*]pyrimidine (V)**: 7-hydroxy-3-isopropylpyrazolo[[4,3-*d*]]pyrimidine IV (200 mg, 1.122 mmol) was dissolved in the mixture of 0.81 mL (11 mmol) $SOCl_2$, 0.12 mL (1.56 mmol) of dimethylformamide and 5 mL $CHCl_3$. This mixture was heated under reflux for 3 h. The solution was evaporated to dryness in vacuo and the residue was dissolved in $CHCl_3$. This solution was extracted twice with a small portions of water and combined chloroform extract was dried by Na_2SO_4. Column chromat. 1.5% MeOH in $CHCl_3$; mp 84–86 °C; yield 62%. MS ESI+: 197.2 (100, $M+H^+$). ^1H NMR (300 MHz, $CDCl_3$): 1.513 d (6H, $J=7.2$ Hz, $(C\underline{H}_3)_2$–CH); 3.591 sept (1H, $J=7.2$ Hz, $C\underline{H}(CH_3)_2$); 7.925 bs (1H, =N$\underline{H}$); 8.878 s (1H, H–C^5). Anal. ($C_8H_9N_4Cl$) C, H, N, Cl. **7-Benzylamino-3-isopropylpyrazolo[4,3-*d*]pyrimidine (VIa)**: Method A. Column chromat.: 1.5% MeOH in $CHCl_3$; mp 153–154 °C; yield 82%. MS ESI+: 268.3 (100, $M+H^+$). ^1H NMR (400 MHz, $CDCl_3$): 1.403 d (6H, $J=7.0$ Hz, $(C\underline{H}_3)_2CH$); 3.407 sept (1H, $J=7.0$ Hz, $C\underline{H}(CH_3)_2$); 4.791 s (2H, $C\underline{H}_2NH$), 6.530 bs (1H, C^7–N$\underline{H}$–), 7.214–7.284 m (5H, H–Ar), 8.405 s (1H, H–C^5). Anal. ($C_{15}H_{17}N_5$) C, H, N. **7-(2-Brombenzyl)amino-3-isopropylpyrazolo[4,3-*d*]py-rimidine (VIb)**: Method B. Column chromatography 1.5% MeOH in $CHCl_3$; crystallised from Et_2O; mp 194–196 °C; yield 42%. MS ESI+: 246.2 (100, $M+H^+$). ^1H NMR (300 MHz, CH_3OD): 1.435 d (6H, $J=6.9$ Hz, $(C\underline{H}_3)_2CH$); 3.468 hept (1H, $J=6.9$ Hz, $C\underline{H}(CH_3)_2$); 4.893 s (2H, $C\underline{H}_2NH$); 7.203 t (1H, $J=7.2$ Hz, Ar–$\underline{H}^{4'}$); 7.322 t (1H, $J=7.2$ Hz, Ar–$\underline{H}^{5'}$); 7.451 m (1H, Ar–$\underline{H}^{6'}$); 7.618 d (1H, $J=7.2$ Hz, Ar–$\underline{H}^{3'}$); 8.238 bs (1H, H–C^5). Anal. ($C_{15}H_{16}BrN_5$) C, H, N, Br. **7-(4-Methoxybenzyl)-amino-3-isopropylpyrazolo[4,3-*d*]pyrimidine (VIc)**: Method A. Column chromat.: 2% MeOH in $CHCl_3$; crystallised from Et_2O; mp 143–144 °C; yield 42%. MS ESI+: 298.3 (100, $M+H^+$). ^1H NMR (300 MHz, $CDCl_3$): 1.358 d (6H, $J=6.9$ Hz, $(C\underline{H}_3)_2CH$); 3.457 sept (1H, $J=6.9$ Hz, $C\underline{H}(CH_3)_2$); 3.693 s (3H, $OC\underline{H}_3$), 4.862 d (2H, $J=3.6$ Hz, $C\underline{H}_2NH$); 6.721 d (2H, $J=8.8$ Hz, Ar–H); 7.295 d (2H, $J=8.8$ Hz, Ar–H); 8.340 s (1H, H–C^5). Anal. ($C_{16}H_{19}N_5O$) C, H, N. **7-(2-Hydroxybenzyl)amino-3-isopropylpyrazolo[4,3-*d*]pyrimidine (VId)**: Method B. Column chromat.: $CHCl_3$/methanol/AcOH (20:0.4:0.1); mp 214–217 °C; yield 40%. MS ESI+: 274.3(100, $M+H^+$). ^1H NMR (400 MHz, DMSO-d_6): 1.358 d (6H, $J=7.0$ Hz, $(C\underline{H}_3)_2CH$); 3.297 sept (1H, $J=7.0$ Hz, $C\underline{H}(CH_3)_2$); 4.644 s (2H, $C\underline{H}_2NH$), 6.775 ddd (1H, $J=8.1$ Hz, $J=7.2$ Hz, $J=1.3$ Hz, Ar–$\underline{H}^{5'}$), 6.861 d (1H, $J=8.0$ Hz, Ar–$\underline{H}^{3'}$), 7.119 ddd (1H, $J=7.7$ Hz, $J=1.7$ Hz, $J=1.4$ Hz, Ar–$\underline{H}^{4'}$), 7.259 dd (1H, $J=7.5$ Hz, $J=1.3$ Hz, Ar–$\underline{H}^{6'}$), 8.232 s (1H, H–C^5). Anal. ($C_{15}H_{17}N_5O$) C, H, N. **7-(3-Hydroxybenzyl)amino - 3 - isopropylpyrazolo[4,3 - *d*]pyrimidine (VIe)**: Method B. Column chromat.: $CHCl_3$/methanol/AcOH (20:0.6:0.1); mp 220–221 °C; yield 48%. MS ESI+: 274.3 (100, $M+H^+$). ^1H NMR (300 MHz, DMSO-d_6): 1.380 d (6H, $J=7.14$ Hz, $(C\underline{H}_3)_2CH$); 3.340 sept (1H, $J=7.14$ Hz, $C\underline{H}(CH_3)_2$); 4.678 s (2H, $C\underline{H}_2NH$), 6.631–7.173 m (4H, Ar–H), 8.221 s (1H, H–C^5). Anal. ($C_{15}H_{17}N_5O$) C, H, N. **7-(4-Hydroxybenzyl)amino-3-isopropylpyrazolo[4,3-*d*]pyrimidine (VIf)**: Method B. Column chromat.: $CHCl_3$/methanol/AcOH (20:1:0.1); mp 234–236 °C; yield 49%. MS ESI+: 274.3 (100, $M+H^+$). ^1H NMR (300 MHz, MeOD): 1.420 d (6H, $J=6.9$ Hz, $(C\underline{H}_3)_2CH$); 3.449 sept (1H, $J=6.9$ Hz, $C\underline{H}(CH_3)_2$); 4.683 s (2H, $C\underline{H}_2NH$); 6.780 d (2H; $J=8.3$ Hz, Ar–H), 7.247 d (2H; $J=8.3$ Hz, Ar–H); 8.261 bs (1H, H–C^5). Anal. ($C_{15}H_{17}N_5O$) C, H, N. **7-(3-Hydroxy-4-methoxybenzyl)-amino-3-isopropylpyrazolo[4,3-*d*]pyrimidine (VIg)**: Method B. Column chromat.: $CHCl_3$/methanol/aq NH_4OH (94:6:0.2); crystallised from a mixture $CHCl_3/Et_2O$; mp 197–199 °C; yield 62%. MS ESI+: 314.3 (100, $M+H^+$). ^1H NMR (300 MHz, CH_3OD): 1.427 d (6H, $J=7.2$ Hz, $(C\underline{H}_3)_2CH$); 3.446 hept (1H, $J=7.2$ Hz, $C\underline{H}(CH_3)_2$); 3.833 s (3H, –O–$C\underline{H}_3$); 4.670 bs (2H, $C\underline{H}_2NH$), 6.820–6.904 m (3H, Ar–H), 8.244 s (1H, H–C^5). Anal. ($C_{16}H_{19}N_5O_2$) C, H, N. **7-Furfurylamino-3-isopropylpyrazolo[4,3-*d*]pyrimidine (VIh)**: Method A. Column chromat.: $CHCl_3$/MeOH/aq NH_4OH (98:2:0.2); mp 179–182 °C; yield 43%. MS ESI+: 258.3 (100, $M+H^+$). ^1H NMR (500 MHz, MeOD): 1.422 d (6H, $J=7.0$ Hz, $(C\underline{H}_3)_2CH$); 3.455 sept (1H, $J=7.0$ Hz, $C\underline{H}(CH_3)_2$); 4.802 s (2H, $C\underline{H}_2NH$); 6.373 s (2H, furfuryl-(3′ + 4′)); 7.468 s (1H, furfuryl-(5′)); 8.273 s (1H, H–C^5). Anal. ($C_{13}H_{15}N_5O$) C, H, N. **7-Pentylamino-3-isopropylpyrazolo[4,3-*d*]pyrimidine (VIi)**: Method A. Column chromat.: 1% MeOH in $CHCl_3$; mp 73–75 °C; yield 52%. MS ESI+: 248.2 (100, $M+H^+$). ^1H NMR (500 MHz, MeOD): 0.933 t (3H, $J=7.0$ Hz, –$(CH_2)_4$–$C\underline{H}_3$); 1.374–1.388 m (4H, –$(C\underline{H}_2)_2$–CH_3); 1.418 d (6H, $J=6.9$ Hz, $(C\underline{H}_3)_2CH$); 1.715 pent (2H, $J=7.0$ Hz, –$C\underline{H}_2$–$(CH_2)_2$–CH_3), 3.447 hept (1H, $J=6.9$ Hz, $C\underline{H}(CH_3)_2$); 3.583t (2H, $J=7.0$ Hz, –NH–$C\underline{H}_2$–); 8.207 s (1H, H–C^5). Anal. ($C_{13}H_{21}N_5$) C, H, N. **7-(Isopent-2-en-1-ylamino)-3-isopropylpyrazolo[4,3-*d*] pyrimidine (VIj)**: Method B. Column chromat.: $CHCl_3$/methanol/aq NH_4OH (98:2:1); syrup; yield 48%. MS ESI+: 246.5 (100, $M+H^+$). ^1H NMR (400 MHz, $CDCl_3$): 1.449 d (6H, $J=7.0$ Hz, $(C\underline{H}_3)_2CH$); 1.647 d (6H, $J=1.3$ Hz, =C–$(C\underline{H}_3)_2$), 3.467 sept (1H, $J=7.0$ Hz, $C\underline{H}(CH_3)_2$); 4.178 d (2H, $J=6.8$ Hz, –$C\underline{H}_2$–CH=), 5.250 m (1H, –CH_2–C$\underline{H}$=), 6.252 s (1H, –N$\underline{H}$–C^7), 8.430 s (1H, $\underline{H}$–C^5). COSY [1.45 d (6H, $J=6.96$ Hz); 3.47 sept (1H, $J=6.96$ Hz)], COSY [1.65 d (6H, $J=1.28$ Hz); 4.18 d (2H, $J=1.28$ Hz); 5.25 m (1H, $J=1.28$ Hz)], COSY [4.18 d (2H); 6.25 s (1H)]. Anal. ($C_{13}H_{19}N_5$) C, H, N. **7-(3-Chloroanilino)-3-isopropylpyrazolo[4,3-*d*]pyrimi-dine (VIk)**: Method A. The product was precipitated when the reaction mixture was cooled at room temperature. The colourless crystals were washed with Et_2O; the analytical sample was obtained by re-crystallisation from mixture EtOH/Et_2O. Yield 58%; mp = 213–216 °C. MS ESI+: 288.5 (100, $M+H^+$). ^1H NMR (400 MHz, DMSO-d_6): 1.400 d (6H, $J=6.9$ Hz, $(C\underline{H}_3)_2CH$); 3.482 sept (1H, $J=6.9$ Hz, $C\underline{H}(CH_3)_2$); 7.299 dd (1H, $J=7.8$ Hz, $J=1.7$ Hz, Ar–$\underline{H}^{6'}$), 7.488 dd (1H, $J=8.0$ Hz, $J=8.0$ Hz, Ar–$\underline{H}^{5'}$), 7.862 dd (1H, $J=7.8$ Hz, $J=1.7$ Hz, Ar–$\underline{H}^{3'}$), 8.202 bs (1H, H–C^5); 8.750 s (1H, Ar–$\underline{H}^{2'}$). Anal. ($C_{14}H_{14}N_5Cl{\cdot}HCl{\cdot}H_2O$) C, H, N, Cl. **6-(2-Bromobenzyl)amino-9-isopropylpurine (VIIb)**: Column chromat. in $CHCl_3$; crystallised from Et_2O; mp 112–114 °C; yield 79%. MS ESI+: 346.2 (100, $M+H^+$). ^1H NMR (300 MHz, $CDCl_3$): 1.629 d (6H, $J=6.9$ Hz, $(C\underline{H}_3)_2CH$); 3.870 hept (1H, $J=6.6$ Hz, $C\underline{H}(CH_3)_2$); 5.46 bs (2H, $C\underline{H}_2NH$); 7.124–7.580 m (4H, Ar–H); 7.938 bs (1H, H–C^8); 8.320 bs (1H, H–C^2). Anal. ($C_{15}H_{16}N_5Br$) C, H, N, Br. **6-(2-Hydroxybenzyl)amino-9-isopropylpurine (VIId)**: Column chromat. 2% MeOH in $CHCl_3$; crystallised from a mixture EtOH/Et_2O; mp 162–163 °C; yield 85%. MS ESI+: 284.2 (100, $M+H^+$). ^1H NMR (300 MHz, MeOD): 1.603 d (6H, $J=6.7$ Hz,

($C\underline{H}_3)_2CH$); 4.715 bs (2H, $C\underline{H}_2NH$); 4.814 sept (1H, $J=6.7$ Hz, $C\underline{H}(CH_3)_2$); 6.758–6.828 m (2H, Ar–H); 7.109 dd (1H, $J=7.6$ Hz, $J=7.6$ Hz, Ar–$\underline{H}^{4'}$); 7.256 (1H, $J=7.4$ Hz, Ar–$\underline{H}^{6'}$), 8.159 bs (1H, H–C^8), 8.270 s (1H, H–C^2). Anal. ($C_{15}H_{17}N_5O$) C, H, N. **6-(3-Hydroxybenzyl)amino-9-isopropylpurine** (**VIIe**): Column chromat. 3% MeOH in $CHCl_3$; crystallised from a mixture EtOH/Et_2O; mp 170–171 °C; yield 61%. MS ESI+: 284.2 (100, $M+H^+$). ^{1}H NMR (300 MHz, MeOD): 1.613 d (6H, $J=6.6$ Hz, ($C\underline{H}_3)_2CH$), 4.756 bs (2H, $C\underline{H}_2NH$), 4.824 sept (1H, $J=6.6$ Hz, $C\underline{H}(CH_3)_2$), 6.737 dd (1H, $J=8.0$ Hz, $J=2.2$ Hz, Ar–$\underline{H}^{3'}$), 6.804–6.843 m (2H, Ar–H), 7.122 dd (1H, $J=8.0$ Hz, $J=8.0$ Hz, Ar–$\underline{H}^{5'}$), 8.164 s (1H, H–C^8), 8.248s (1H, H–C^2). Anal. ($C_{15}H_{17}N_5O$) C, H, N. **6-(4-Hydroxybenzyl)amino-9-isopropylpurine** (**VIIf**): Column chromat. 3.5% MeOH in $CHCl_3$; crystallised from a mixture EtOH/Et_2O; mp 162–163 °C; yield 85%. MS ESI+: 284.2 (100, $M+H^+$). ^{1}H NMR (300 MHz, MeOD): 1.610 d (6H, $J=6.9$ Hz, ($C\underline{H}_3)_2CH$); 4.70 bs (2H, $C\underline{H}_2NH$); 4.819 sept (1H, $J=6.9$ Hz, $C\underline{H}(CH_3)_2$); 6.737 d (2H, d, $J=8.4$ Hz, Ar–H); 7.208 d (2H, $J=8.4$ Hz, Ar–H); 8.154 s (1H, H–C^8), 8.252 s (1H, H–C^2). Anal. ($C_{15}H_{17}N_5O$) C, H, N. **6-(3-Hydroxy-4-methoxybenzyl)amino-9-isopropylpu-rine** (**VIIg**): Column chromat.: 3.5% MeOH in $CHCl_3$; crystallised from a mixture $CHCl_3$/Et_2O; mp 163–164 °C; yield 61%. MS ESI+: 314.3 (100, $M+H^+$). ^{1}H NMR (300 MHz, CH_3OD): 1.601 d (6H, $J=6.9$ Hz, ($C\underline{H}_3)_2$–CH); 3.821 s (3H, –O–$C\underline{H}_3$); 4.69 bs (2H, $C\underline{H}_2NH$), 4.821 hept (1H, $J=6.9$ Hz, $C\underline{H}(CH_3)_2$); 6.800–6.882 m (3H, Ar–H), 8.166 s (1H, H–C^8); 8.261 s (1H, H–C^2). Anal. ($C_{16}H_{19}N_5O_2$) C, H, N. **6-Furfurylamino-9-isopropylpurine** (**VIIh**): Column chromat. 2.5% MeOH in $CHCl_3$; crystallised from Et_2O; mp 128–130 °C; yield 68%. MS ESI+: 258.2 (100, $M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$): 1.631 d (6H, $J=6.9$ Hz, ($C\underline{H}_3)_2CH$); 4.866 sept (1H, $J=6.9$ Hz, $C\underline{H}(CH_3)_2$); 4.934 bs (2H, $C\underline{H}_2NH$); 6.308–6.339 m (2H, furfuryl-(3′+4′)); 7.362 s (1H, furfuryl-(5′)); 7.882 bs (1H, H–C^8); 8.192 bs (1H, H–C^2). Anal. ($C_{13}H_{15}N_5O$) C, H, N. **6-Pentylamino-9-isopropylpurine** (**VIIi**): Column chromat. 1% MeOH in $CHCl_3$; crystallized from $CHCl_3$; mp 48–50 °C; yield 78%. MS ESI+: 248.2 (100, $M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$): 0.926 (3H, t, $J=6.9$ Hz), 1.408 (4H, m, $J=6.9$ Hz) 1.635 (6H, d, $J=6.9$ Hz), 1.709 (2H, m, $J=6.9$ Hz), 4.609 (2H, t, $J=7.1$ Hz) 4.863 (1H, sept, $J=6.9$ Hz), 7.885 (1H, s), 8.192 (1H, s). Anal. ($C_{13}H_{21}N_5$) C, H, N. **6-(Isopent-2-en-1-ylamino)-9-isopropylpurine** (**VIIj**): Column chromat. 0.6% MeOH in $CHCl_3$; crystallised from Et_2O; mp 99–100 °C; yield 89%. MS ESI+: 246.2 (100, $M+H^+$). ^{1}H NMR (300 MHz, $CDCl_3$): 1.631 d (6H, $J=6.9$ Hz, ($C\underline{H}_3)_2CH$); 1.764 s (6H, CH=C–($C\underline{H}_3)_2$); 4.245 bs (1H, –C$\underline{H}$=); 4.860 sept (1H, $J=6.9$ Hz, $C\underline{H}(CH_3)_2$), 5.385 t (2H, $J=7.0$ Hz, $C\underline{H}_2CH$=); 7.84 bs (1H, H–C^8), 8.36 bs (1H, H–C^2). Anal. ($C_{13}H_{19}N_5$) C, H, N. **6-(3-Chloroanilino)-9-isopropylpurine** (**VIIk**): Column chromat. $CHCl_3$; crystallised from Et_2O; mp 72–74 °C; yield 33%. MS ESI+: 288.1 (100, $M+H^+$). ^{1}H NMR (300 MHz, MeOD): 1.638 d (6H, $J=6.6$ Hz, ($C\underline{H}_3)_2CH$); 4.921 sept (1H, $J=6.6$ Hz, $C\underline{H}(CH_3)_2$); 7.111 dd (1H, $J=8.0$ Hz, $J=1.9$ Hz, Ar–$\underline{H}^{6'}$); 7.300 dd (1H, $J=8.0$ Hz, $J=8.0$ Hz, Ar–$\underline{H}^{5'}$); 7.597 dd (1H, $J=8.1$ Hz, $J=2.0$ Hz, Ar–$\underline{H}^{4'}$); 7.975 bs (1H, Ar–$\underline{H}^{2'}$); 8.425 bs (1H, H–C^8), 8.514 s (1H, H–C^2). Anal. ($C_{14}H_{14}N_5Cl$) C, H, N,Cl.

5. Kelley, J. L.; Bullock, R. M.; Krochmal, M. P.; McLean, E. W.; Linn, J. A.; Durcan, M. J.; Cooper, B. R. *J. Med. Chem.* **1997**, *40*, 3207.
6. Otyepka, M.; Kryštof, V.; Havlíček, L.; Siglerová, V.; Strnad, M.; Koča, J. *J. Med. Chem.* **2000**, *43*, 2506.
7. Franěk, F.; Havlíček, L.; Siglerová, V.; Strnad, M.; Eckschlager, T.; Weigl, E. *Collect. Czech. Chem. Commun.* **2002**, *67*, 257.
8. Kryštof, V.; Lenobel, R.; Havlíček, L.; Kuzma, M.; Strnad, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3283.