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Synthesis and Biological Activity of Olomoucine II

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Abstract—Based on our previous experiences with synthesis of purines, novel 2,6,9-trisubstituted purine derivatives were prepared and assayed for the ability to inhibit CDK1/cyclin B kinase. One of newly synthesized compounds designated as olomoucine II, 6-[(2-hydroxybenzyl)amino]-2-{[1-(hydroxymethyl)propyl]amino}-9-isopropylpurine, displays 10 times higher inhibitory activity than roscovitine, potent and specific CDK1 inhibitor. Olomoucine II in vitro cytotoxic activity exceeds purvalanol A, the most potent CDK inhibitor, as it kills the CEM cells with IC_{50} value of 3.0 μM.

A number of reports during the past decade witness the crucial role of cyclin dependent kinases (CDK) in cell division and proliferation. Their discovery as enzymes driving progress through cell division was followed closely with revelation of frequent alterations of cell cycle regulatory genes in many primary tumours and cancer cells. Acting in the centre of all particular processes during cell division, CDK's became promising targets for specific inhibitors able to block cell division at defined points.[1] The potential use of these substances in the therapy of cancer and other proliferative diseases initiated an intensive search for chemical CDK inhibitors.

Systematic screening of purine derivatives of cytokinin origin leading to the discovery of 2,6,9-trisubstituted purines revealed a large number of highly active CDK inhibitors.[2] One of the first CDK specific inhibitors olomoucine, 2-(2-hydroxyethylamino)-6-benzylamino-9-methylpurine, was found to block selectively CDK1, CDK2 and CDK5 kinases at micromolar concentrations.[2] Its structure modifications led to the development of roscovitine, 2-{[(1-hydroxymethyl) propyl]amino}-6-benzylamino-9-isopropylpurine,[3] a novel substance with enhanced inhibitory activity towards CDK1, increased selectivity and antimitotic activity.[4]

Crystal structures of CDK2–purine complexes revealed inhibitors fixed in the ATP binding pocket, occupying approximately the same region, but demonstrating different orientation with respect to the protein.[5] Detailed analysis of molecular and cellular effects of purine CDK inhibitors, supported by co-crystal analysis, has motivated several scientific teams to continue with the synthesis and biological testing of 2,6,9-trisubstituted purines.[6] The research contributed to the identification of purvalanol A, the compound with about 100-fold increase of inhibitory potency over roscovitine.[7]

Comparing the previously published purine inhibitors of CDK1, the most potent compound purvalanol B bears a 3-chlorophenylamino substituent at position 6 in contrast to the parental benzylamino moiety of cytokinins, from which purine inhibitors have been originally developed. Our initial experiments suggested that the hydroxy substituent on the benzyl ring enhances slightly the ability of purines to block CDK1 activity.[2] That idea has been recently confirmed by Legraverend and co-workers, who have found different substituents at benzyl ring (Cl, OCH_3) to be favourable.[8] We show here that hydroxy substitutions of the benzyl ring modifies very positively the affinity of purine derivatives to CDK1. Hence prepared derivatives of bohemine and other trisubstituted purines bearing various hydroxylated benzylamino groups at position C-6 are about 10 times more active than unmodified parental

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Table 1. CDK1/cyclin B inhibitory activity of 2,6,9-trisubstituted purines

Compd	Substitution			IC_{50} (μM)[a]
	C-2	C-6	N-9	
Olomoucine	2-Hydroxyethylamino	Benzylamino	Methyl	7[b]
Bohemine	3-Hydroxypropylamino	Benzylamino	Isopropyl	1.1[b]
B2	3-Hydroxypropylamino	2-Hydroxybenzylamino	Isopropyl	0.1
B3	3-Hydroxypropylamino	3-Hydroxybenzylamino	Isopropyl	1.0
B4	3-Hydroxypropylamino	4-Hydroxybenzylamino	Isopropyl	2.6
Roscovitine	[1-(Hydroxymethyl)propyl]amino	Benzylamino	Isopropyl	0.45[b]
Olomoucine II	[1-(Hydroxymethyl)propyl]amino	2-Hydroxybenzylamino	Isopropyl	0.02
R3	[1-(Hydroxymethyl)propyl]amino	3-Hydroxybenzylamino	Isopropyl	0.2
R4	[1-(Hydroxymethyl)propyl]amino	4-Hydroxybenzylamino	Isopropyl	0.3
Purvalanol A	[1-(Hydroxymethyl)-2-methylpropyl]amino	3-Chloroanilino	Isopropyl	0.05[b]
P2	[1-(Hydroxymethyl)-2-methylpropyl]amino	2-Hydroxybenzylamino	Isopropyl	0.08
P3	[1-(Hydroxymethyl)-2-methylpropyl]amino	3-Hydroxybenzylamino	Isopropyl	0.12
P4	[1-(Hydroxymethyl)-2-methylpropyl]amino	4-Hydroxybenzylamino	Isopropyl	0.15

[a]Values are means of three experiments.
[b]Olomoucine, bohemine, roscovitine and purvalanol A IC_{50} values measured in our laboratory.

compounds. Together with synthesis of the described purine derivatives, their ability to block recombinant CDK1/cyclin B kinase activity and their in vitro cytotoxic properties are also presented.

Chemistry

The straightforward two-step synthesis of 2,6,9-trisubstituted purines started from 2,6-dichloro-9-isopropylpurine,[9] which has to be used as a parental compound to avoid alkylation of phenolic OH during introduction of *N*-isopropylgroup via classic alkylation. The starting compounds were reacted with appropriate hydroxybenzylamines in butan-1-ol in the presence of triethylamine (112 °C, 2 h). Resulting 2-chloro-6-(hydroxybenzyl)amino-9-isopropylpurines were further coupled with 3-aminopropan-1-ol (160 °C, 2 h), (*R*/*S*)-2-aminobutan-1-ol (160 °C, 3 h) or (*R*/*S*)-2-amino-3-methylbutan-1-ol (160 °C, 20 h), respectively. The products were purified by column chromatography. Data characterizing prepared purine derivatives are given in ref 10.

Table 2. In vitro cytotoxicity of selected trisubstituted purines

Compd	IC_{50} (μM)[a]				
	MCF7	K562	CEM	HOS	G361
Olomoucine	132	>167	62	149	160
Bohemine	28	113	27	58	45
B2	12.4	24	10.7	17.2	13.3
B3	14.7	56	17.6	26	26
B4	12.7	57	18.2	26	23
Roscovitine	11.1	40	18.2	32	34
Olomoucine II	5.3	11.1	3.0	6.3	6.3
R3	13.7	26	9.9	11.0	19.4
R4	7.8	24	11.0	13.0	11.1
Purvalanol A	10.7	9.0	7.4	22	24
P2	5.7	10.9	4.4	8.3	7.8
P3	14.8	16.1	6.7	12.0	12.0
P4	9.3	14.8	5.3	12.0	11.1

[a]Values are means of three experiments.

Compounds P2 and P3 were prepared as described previously.[9,11]

Results and Discussion

All newly synthesized trisubstituted purines were tested in a CDK1/cyclin B kinase inhibition assay as described previously,[9] with enzyme purified on NiNTA column (according to Qiagen manual). Resulting data presented in Table 1, including IC_{50} values of olomoucine, bohemine, roscovitine and purvalanol A for comparison, demonstrate that hydroxybenzylamino moiety at position C-6 increase the activity of almost all derivatives. The 6-benzylamino group is thought to be responsible for the specificity of purine CDK inhibitors. Addition of a hydroxyl group at the C-6 benzylamino substituent of bohemine and other related analogues improved CDK1 inhibition. Now it becomes clear that the activity grows with the addition of one hydroxyl group. The most significant increase of CDK1 inhibition is obvious when decorating benzyl positions 2 and 3, respectively. In contrast to that, 6-(4-hydroxybenzylamino) derivatives display only slight change in activity.

When the compounds were assayed for in vitro antitumour activity against various cancer cell lines,[12] we obtained data showing that drug activity rises remarkably with enhancement of its CDK1 inhibitory potency (Table 2). These results suggest that the CDK1 inhibition may be at least partly responsible for the antiproliferative activity of the compounds.[13] 2-Hydroxybenzylamino compounds were again the most active from the tested series. Cytotoxicity values exceeded their respective 6-benzylaminopurines more than 2-fold.

Olomoucine, the first cytokinin CDK inhibitor, has only weak cytotoxic activity on cancer cell lines in comparison to olomoucine II, being effective in low micromolar concentrations. On the other hand, olomoucine II is more potent in vitro against tumour cells than purvalanol A, the most active CDK1 inhibitor. The results provide valuable information for the design of CDK1 inhibiting compounds with enhanced cytotoxic effect.

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10. Data for prepared compounds: **2-chloro-6-(2-hydroxybenzylamino)-9-isopropylpurine:** Column chromatography stepwise 1, 2, 3, 4% MeOH in $CHCl_3$; crystallized from Et_2O; mp 170–171 °C; yield 83%. MS ESI+: 318.3 (100, $M+H^+$), 319 (18), 320 (25). ^1H NMR (300 MHz, $CDCl_3$): 1.636 (6H, d, $J=7.0$ Hz, $(CH_3)_2CH$), 4.682 (2H, bd, $J=5.3$ Hz, CH_2NH), 4.916 (1H, sept, $J=6.8$, $CH(CH_3)_2$), 6.866 (1H, ddd, $J=6.7$, $J=6.7$, $J=1.1$ Hz, H-5′), 6.966 (1H, dd, $J=8.2$, $J=1.1$ Hz, H-3′), 7.198 (1H, ddd, $J=8.0$, $J=8.1$, $J=1.6$ Hz, H-4′), 7.280 (1H, dd, $J=7.5$, $J=1.7$ Hz, H-6′) 8.44 (1H, bs, OH), 8.592 (1H, s, HC^8). Anal. ($C_{15}H_{16}ClN_5O$) C, H, N. **2-chloro-6-(3-hydroxybenzylamino)-9-isopropylpurine:** The title compound crystallized from 1-butanol after reaction and cooling; recrystallization from $CHCl_3$ gave mp 217–218 °C; yield 92%. MS ESI+: 318.3 (100, $M+H^+$), 319 (18), 320 (25). ^1H NMR (300 MHz, $CDCl_3$): 1.61 (6H, d, $J=6.7$ Hz, $(CH_3)_2CH$), 4.72 (2H, bs, CH_2NH), 4.83 (1H, sept, $J=6.7$ Hz, $CH(CH_3)_2$), 6.70–7.20 (4H, m, H–Ar), 7.92 (1H, s, HC^8). Anal. ($C_{15}H_{16}ClN_5O$) C, H, N. **2-chloro-6-(4-hydroxybenzylamino)-9-isopropylpurine:** Column chromatography stepwise 1, 2, 3, 4% MeOH in $CHCl_3$; crystallized from benzene; mp (dec) 110–122 °C; yield 80%. MS: 319 (25), 317 (62, $C_{15}H_{16}N_5OCl$, −0.6) 276 (12), 274 (32), 213 (12), 211 (40), 171 (31), 169 (100), 134 (28), 122 (60, C_7H_8NO, −1.8), 107 (60, C_7H_7O, −1.5), 77 (31). ^1H NMR (300 MHz, $CDCl_3$): 1.638 (6H, d, $J=6.6$ Hz, $(CH_3)_2CH$), 4.682 (2H, bd, $J=4.8$ Hz, CH_2NH), 4.913 (1H, sept, $J=6.7$ Hz, $CH(CH_3)_2$), 6.685 (2H, d, $J=8.2$ Hz, H–Ar), 7.129 (2H, d, $J=8.5$ Hz, H–Ar), 8.173 (1H, bs, OH), 8.686 (1H, s, HC^8). Anal. ($C_{15}H_{16}ClN_5O$) C, H, N. **6-(2-hydroxybenzylamino)-2-[(3-hydroxypropyl)amino]-9-isopropylpurine (B2)**: Column chromatography stepwise 0.5, 1, 2% MeOH in $CHCl_3$; amorphous glass-like product; yield 70%. MS: 356 (57, M^+), 325 (6), 312 (8), 298 (7), 250 (69), 219 (65), 206 (71), 205 (100), 177 (37), 163 (72), 150 (37), 134 (50), 108 (38), 106 (28), 78 (53). ^1H NMR (300 MHz, $CDCl_3$): 1.520 (6H, d, $J=6.6$ Hz, $(CH_3)_2CH$), 1.782 (2H, m, $CH_2CH_2CH_2$), 3.660 (4H, m, $CH_2CH_2CH_2$), 4.54–4.60 (2H, bs, CH_2Ph), 4.575 (1H, sept, $J=6.6$, $CH(CH_3)_2$), 5.11 (1H, bt, exch H), 6.53 (1H, exch H), 6.82–6.93 (2H, m, H-Ar), 7.17–7.27 (2H, m, H–Ar), 7.520 (1H, s, HC^8). Anal. ($C_{18}H_{24}N_6O_2$) C, H, N. **6-(2-hydroxybenzylamino)-2-{[1(*R*/*S*)-(hydroxymethyl)propyl]amino}-9-isopropylpurine (R2, olomoucine II)**: Column chromatography stepwise 1, 2, 3, 4% MeOH in $CHCl_3$; amorphous glass-like product; mp 110–114 °C; yield 76%. MS ESI+: 371.3 (100, $M+H^+$), 372 (20). ^1H NMR (300 MHz, $CDCl_3$): 1.055 (3H, t, $J=7.1$, CH_3CH_2), 1.532 and 1.538 (6H, 2 × d, $J=6.6$ Hz, $CH(CH_3)_2$), 1.67 (2H, m, CH_2CH_3), 3.70 (1H, m, CHHOH), 3.84 (1H, dd, $J=2.9$, $J=11$ Hz, CHHOH), 3.985 (1H, m, NHC*H), 4.597 (1H, sept, $J=6.6$ Hz, $(CH_3)_2CH$), 4.60 (2H, bs, CH_2Ph), 5.57 (1H, bs, NH), 6.78–6.94 (2H, m, H-Ar), 7.12–7.20 (2H, m, H-Ar), 7.567 (1H, s, HC^8). Anal. ($C_{19}H_{26}N_6O_2$) C, H, N. **6-(3-hydroxybenzylamino)-2-[(3-hydroxypropyl)amino]-9-isopropylpurine (B3)**: Column chromatography stepwise 1, 2, 3, 4% MeOH in $CHCl_3$; mp 134–136 °C; yield 77%. MS: 356 (100, $C_{18}H_{24}N_6O_2$, −0.1, M^+), 325 (43), 312 (42), 311 (43), 298 (24), 191 (17), 122 (24), 107 (62). ^1H NMR(400 MHz, $CDCl_3$): 1.562 (6H, d, $J=6.8$ Hz, $CH(CH_3)_2$), 1.747 (2H, m, $CH_2CH_2CH_2$), 3.65 (4H, m, $CH_2CH_2CH_2$, 4.59 (2H, bd, CH_2Ph), 4.66 (1H, sept, $J=6.8$ Hz, $(CH_3)_2CH$), 6.4 (1H, bs, exch H), 6.73–6.82 (3H, m, H–Ar), 7.06–7.12 (1H, m, H–Ar), 7.265 (1H, s, HC^8), 7.605 (1H, s, phenolic H). The proton 2D-COSY spectrum was used for the assignment of signals. Anal. ($C_{18}H_{24}N_6O_2$) C, H, N. **6-(3-hydroxybenzylamino)-2-{[1(R/S)-(hydroxymethyl)propyl]amino}-9-isopropylpurine (R3)**: Column chromatography stepwise 1, 2, 3, 4% MeOH in $CHCl_3$; crystallized from EtOAc; mp 150–152 °C; yield 81%. MS: 370 (25, $C_{19}H_{26}N_6O_2$, 0.7, M^+), 352 (13), 339 (100, $C_{18}H_{23}N_6O$, +0.4), 323 (20), 309 (7), 298 (9), 217 (7), 134 (8), 122 (6), 107 (33), 43 (13), 41 (11). ^1H NMR (400 MHz, $CDCl_3$): 1.032 (3H, t, $J=7.5$ Hz, CH_3CH_2), 1.50–1.70 (3H, m, $CH_2OH+CH_2CH_3$), 1.555 (6H, d, $J=6.8$ Hz, $CH(CH_3)_2$), 3.637 (1H, dd, $J=7.8$, $J=10.7$ Hz, CHHOH), 3.830 (1H, dd, $J=2.8$, 10.7 Hz, CHHOH), 3.95 (1H, m, NHC*H), 4.59 (2H, bd, CH_2Ph), 4.64 (1H, sept, $J=6.8$ Hz, $(CH_3)_2CH$), 4.937 (1H, d, $J=6.4$ Hz, exch H), 6.4 (1H, bs, NH), 6.746 (1H, ddd, $J=1.1$, 2.5, 8.1 Hz, H–Ar), 6.848 (1H, ddd, $J=1.0$, 1.7, 7.5 Hz, H–Ar), 6.871 (1H, m, H–Ar), 7.131 (1H, dd, $J=7.5$, 8.1 Hz, H–Ar), 7.579 (1H, s, HC^8). Anal. ($C_{19}H_{26}N_6O_2$) C, H, N. **6-(4-hydroxybenzylamino)-2-[(3-hydroxypropyl)amino]-9-isopropylpurine (B4)**: Column chromatography stepwise 1, 2, 3, 4% MeOH in $CHCl_3$; crystallized from EtOAc–Et_2O; mp 169–170 °C; yield 79%. MS: 356 (43, $C_{18}H_{24}N_6O_2$, +0.3, M^+), 250 (46, $C_{11}H_{18}N_6O$, +1.5), 219 (62, $C_{10}H_{15}N_6O$, +0.4), 164 (45), 163 (100), 150 (55), 134 (73), 122 (91), 108 (48), 107 (65), 78 (48), 77 (47), 51 (44), 43 (72). ^1H NMR (200 MHz, $CDCl_3$): 1.509 (6H, d, $J=6.8$ Hz, $CH(CH_3)_2$), 1.70 (2H, m, $CH_2CH_2CH_2$), 3.6 (4H, m, $CH_2CH_2CH_2$, 4.6 (3H, m, $CH_2Ph+CH(CH_3)_2$), 6.71 (2H, d, $J=7.5$ Hz, H–Ar), 7.10 (2H, d, $J=7.5$ Hz, H–Ar), 7.512 (1H, s, HC^8). Anal. ($C_{18}H_{24}N_6O_2$) C, H, N. **6-(4-hydro-**

xybenzylamino)-2-{[1(*R*/*S*)-(hydroxymethyl)propyl]amino}-9-isopropylpurine (**R4**): Column chromatography stepwise 1, 2, 3% MeOH in $CHCl_3$; crystallized from $CHCl_3$–Et_2O; mp 135–140 °C; yield 70%. MS: 370 (M^+, 11), 352 ($M^+ - H_2O$, 11), 340 (13), 339.1942 ($C_{18}H_{23}N_6O$, −0.9, 40), 246.1605 ($C_{12}H_{18}N_6$, −1.2, 100), 233 (72), 217 (59), 204 (40), 175 (30). ^{1}H NMR (300 MHz, $CDCl_3$): 1.02 (3H, t, J = 7.4 Hz, CH_3CH_2), 1.54 (6H, d, J = 6.8 Hz, $CH(CH_3)_2$), 1.60 (2H, m, CH_2CH_3), 3.66 (1H, dd, J = 10.9, 7.7 Hz, CHHOH), 3.83 (1H, dd, J = 10.9, 2.7 Hz, CHHOH), 3.99 (1H, m, C^8HNH), 4.63 (3H, bs + sept, J = 6.8 Hz, CH_2NH + $CH(CH_3)_2$), 6.63 (2H, d, J = 7.8 Hz, H–Ar), 6.92 (2H, d, J = 7.8 Hz, H–Ar), 7.57 (1H, s, HC^8). Anal. ($C_{19}H_{26}N_6O_2$) C, H, N. **6-(4-hydroxybenzylamino)-2-{[1(*R*/*S*)-(hydroxymethyl)}-2-(methyl)propyl]amino}-9-isopropylpurine** (**P4**): Column chromatography stepwise 0, 0.5, 1.0, 2.0% MeOH in $CHCl_3$; the syrup-like product; crystallized from $CHCl_3$–Et_2O; mp 176–178; yield 40%. MS ESI+: 385 (100, $M + H^+$), 386 (20). ^{1}H NMR (300 MHz, $CDCl_3$): 0.980 (3H, d, J = 6.9 Hz, $(CH_3)_2$CHC*), 1.003 (3H, d, J = 6.9 Hz, $(CH_3)_2$CHC*), 1,547 (3H, d, J = 6.9 Hz, $(CH_3)_2$CHN9), 1.558 (3H, d, J = 6.9 Hz, $(CH_3)_2$CHN9), 2.023 (1H, m, $(CH_3)_2$CHC*), 3.660–3.700 (2H, m, CH_2OH), 3.90–3.98 (1H, m, CH_2C*H), 4.54–4.68 (3H, m, CH_2Ph + N9CH$(CH_3)_2$), 6.728 (2H, d, J = 8.5 Hz, H–Ar), 7.193 (2H, d, J = 8.5 Hz, H–Ar), 7.760 (1H, s, HC^8). The proton 2D-COSY experiments were used for the assignment of signals. Anal. ($C_{20}H_{28}N_6O_2$) C, H, N.

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