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2,6,8,9-Tetrasubstituted Purines as New CDK1 Inhibitors

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Abstract—Purine inhibitors of cyclin-dependent kinases attract attention as potential anticancer drugs because their first representative roscovitine recently entered clinical trials. Although well described in terms of structure–activity relationships, we still present here a novel modification of the purine scaffold influencing their inhibitory properties. The introduced C-8 substituents, however, lowered the CDK inhibitory activity of roscovitine, whereas the antiproliferative potential of several derivatives remained high.

2,6,9-Trisubstituted purines belong to the first and the best characterised inhibitors of cyclin-dependent kinases (CDK), the protein kinase family currently being explored as a potential target of novel anticancer drugs based on specific inhibition of their catalytic functions.[1] The first CDK specific purine inhibitor olomoucine,[2] was followed by derivatives with enhanced efficiency, like roscovitine, purvalanol, olomoucine II and others.[1,3] Several of them have already undergone in vivo efficacy studies, preclinical and clinical evaluation, and thus confirmed the selected CDKs as a proper target for development of new anticancer drugs.[4]

Up to now the advancement of olomoucine has been based mainly on modifications of substituents at positions 2, 6 and 9, respectively. A similar strategy was used for optimisation of related O^6-substituted guanines.[5] In the effort to enhance the affinity of purine inhibitors to CDKs (besides rearranging the purine heteroatoms described elsewhere[6]), we had the idea to extend the inhibitor–CDK mutual interactions by introduction of another side chain. Such modification could also lead to improvement of solubility of hydrophobic roscovitine-like substaces. Therefore we prepared several 2,6,8,9-tetrasubstituted purines as analogues of potent CDK inhibitors and analysed in details their structure–activity relationships with respect to C8 substituents. The impact of numerous 2,6,8,9-modifications of the purine skeleton on CDK inhibitory activity was consequently verified by molecular modelling.

Results

The straightforward syntheses of 2,6,8,9-tetrasubstituted purines started from previously synthesised 2,6,9-trisubstituted purine CDK inhibitors. 8-Chloro compounds **1**, **2** and **11** were synthesised by reaction of respective 2,6,9-trisubstituted purines with a small excess of *N*-chlorosuccinimide in dry dimethylformamide at various temperatures.[7] No side reactions on the aromatic ring were observed and all products were obtained in yields over 60%. Compound **3** was prepared

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in a 95% yield by reaction of roscovitine with 1.1 equiv of bromine in chloroform.[8] The resulting 8-bromoroscovitine was subsequently transformed to compounds **4**–**6** and **9**–**10**. 8-Nitroroscovitine (**7**) was prepared in 79% yield by nitration of roscovitine with diluted HNO_3 over 4 days at room temperature.[9] The results of chlorination, bromination and nitration suggest that 2,6,9-trisubstituted purines such as roscovitine and purvalanol readily undergo electrophilic substitution in position 8. 8-Substituted derivatives of roscovitine **4**–**6**, **9** and **10** were synthesised by nucleophilic displacement of 8-bromoroscovitine hydrobromide (**3**).[8] All of these reactions need heating to about 150 °C, except for the reactions with sulphur nucleophiles (NaSH and CH_3SNa), where 90 and 40 °C gave satisfactory results, respectively. 8-Aminoroscovitine (**4**) was surprisingly isolated in a high yield after reaction of **3** with 80% hydrazine hydrate.[10] The same compound (**4**) was obtained by reduction of 8-nitroroscovitine (**7**) with zinc in acetic acid.

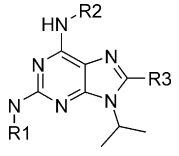
8-Methylpurines **8** and **12** were prepared from 8-methylxanthine which was chlorinated with pyrophosphoryl tetrachloride to give 2,6-dichloro-8-methylpurine. 2,6-Dichloro-8-methylpurine was alkylated with isopropyliodide in DMSO and then subsequently treated with appropriate amines to give target compounds **8** and **12**. Data characterizing the compounds are given in ref 16.

To verify the effect of an additional substituent at C-8 of active purine inhibitors, new derivatives (summarised in Table 1), were screened for the ability to inhibit recombinant CDK1. The results showed that the attachment of the fourth group to the purine ring reduces CDK1 inhibitory activity in all cases. In particular, the larger C-8 side chains of derivatives **9**–**10** decreased the inhibitory activity to below a detectable level. Although smaller groups (Cl, OH, SH, NH_2, NO_2) also decreased the affinity of tetrasubstituted purines to CDK1, some compounds (**2**, **8**) still retained IC_{50} values on olomoucine level (7 μM). On the other hand, approximately 3-fold increase of inhibitory power was observed with derivative **2** over compound **1**, which lacks a phenolic hydroxyl group on N^6-benzyl ring. A similar impact of hydroxy decoration on roscovitine activity has already been described.[3d]

New tetrasubstituted derivatives were also examined for their cytotoxic properties against MCF7 and K562 cancer cell lines. A rapid drop in anti-CDK activity, previously shown to correlate with the antiproliferative effect of 2,6,9-trisubstituted purines,[11] was also obvious from the in vitro anticancer test (Table 1). However, a 200-fold decrease of CDK inhibitory potency of **11** versus parental purvalanol A and even more detrimental effect of C-8 methylation on CDK inhibition by **12**, was unexpectedly not followed by so intensive reduction of cytotoxic properties of the respective tetrasubstituted derivatives, active in a micromolar range. Moreover, the derivatives **9** and **10** with relatively bulky C-8 side chains surprisingly exerted even slightly stronger cytotoxicity on K562 cells than parental roscovitine.

In computer simulation of molecular binding the 2,6,8,9-tetrasubstituted purine derivatives were docked into the active site of CDK2 (for comutational details see ref 12). All of them occupy the roscovitine-like binding mode, but due to a sterical repulsion between the C-8 substituent and the 'back' part of the active site (Fig. 1) their interactions with protein is disfavoured compared to roscovitine (the interaction energy of **5** is less favourable by 2.2 kcal mol^{-1} than roscovitine).

Table 1. Structure–activity relationships of 2,6,8,9-tetrasubstituted purines[a]



Compd	Substitution			IC_{50} (μM)		
	R1	R2	R3	CDK1	MCF7	K562
Roscov.	1-(Hydroxymethyl)propyl	Benzyl	H	0.45	11.1	40
1	1-(Hydroxymethyl)propyl	Benzyl	Chloro	20	54	91
2	1-(Hydroxymethyl)propyl	3-Hydroxybenzyl	Chloro	6	27	27
3	1-(Hydroxymethyl)propyl	Benzyl	Bromo	>100	53	27
4	1-(Hydroxymethyl)propyl	Benzyl	Amino	70	60	80
5	1-(Hydroxymethyl)propyl	Benzyl	Hydroxy	65	65	74
6	1-(Hydroxymethyl)propyl	Benzyl	Mercapto	10	55	154
7	1-(Hydroxymethyl)propyl	Benzyl	Nitro	16	117	117
8	1-(Hydroxymethyl)propyl	Benzyl	Methyl	8	94	58
9	1-(Hydroxymethyl)propyl	Benzyl	Propyloxy	>100	50	20
10	1-(Hydroxymethyl)propyl	Benzyl	(3-Hydroxypropyl)amino	>100	40	30
Purval.	[1-(Hydroxymethyl)-2-methyl]propyl	3-Chlorophenyl	H	0.05	10.7	9.0
11	[1-(Hydroxymethyl)-2-methyl]propyl	3-Chlorophenyl	Chloro	13	45	21
A.2.I.41	4-Aminocyclohexyl	3-Chlorophenyl	H	0.03	1.1	1.4
12	4-Aminocyclohexyl	3-Chlorophenyl	Methyl	14	8.0	3.5

[a]Biological activities were tested according to methods already described.[3d] All the values are means of three experiments, performed in our laboratory. Roscovitine (Roscov.), purvalanol A (Purval.) and A.2.I.41 included for comparison.

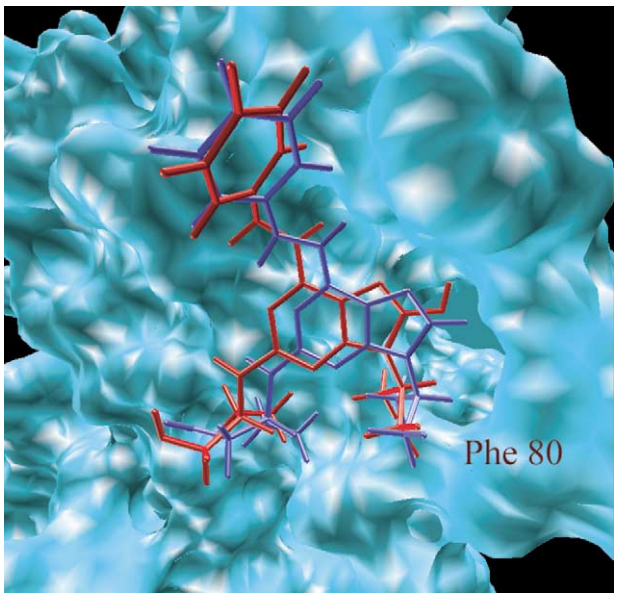


Figure 1. Overlay of roscovitine (blue) and **5** (red) in the active site of CDK2 represented as water accessible surface; **5** occupies the roscovitine-like binding mode but its position in the active site is not optimal compared to roscovitine.

These results confirm the lower affinity of the purine ligands to CDK2 protein and hereby explain our in vitro experiments.

It is well known that the binding mode of purine derivatives is controlled by the number and type of purine substituents and that trisubstituted purines prefer the roscovitine-like binding mode.[12–14] As mentioned above, the CDK active site can accept a wide range of inhibitors and seems to be very flexible. But considering the bilobal structure of CDK2, which has a hinge located in the loop between β5-α2 (residues 80–85), and taking into account the fact that the 'back' part of the active site (residues 80–82) remains fairly rigid,[14] it thus cannot accept an extra C-8 substituent. Therefore, the prepared tetrasubstituted purines proved the negative action of a fourth group on biological activity. Correlation coefficients between CDK inhibition by roscovitine-like inhibitors and their antiproliferative activity demonstrated evident connection.[11] On the other hand, the values were not very high giving us to speculate that CDKs, once believed to be specifically inhibited by 2,6,9-trisubstituted purines, seem not to be the one and only targets responsible for the antiproliferative activity of purine CDK inhibitors. Moreover, several other enzymes were recently identified to interact with purine as well as other types of CDK inhibitors.[15] In the light of the above findings the possibility of targeting other structures besides CDKs by 2,6,8,9-tetrasubstituted purines remains the subject of our further work.

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16. **Data for prepared compounds**: (**1**) Yield 61%. MS-ESI+: 389.2 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$): 1.01 (3H, t, $J=7.7$ Hz, CH_2CH_3), 1.20 (3H, d, $J=7.1$ Hz, $(CH_3)_2CH$), 1.24 (3H, d, $J=7.1$ Hz, $(CH_3)_2$ CH), 1.62 (2H, m, CH_2CH_3), 3.61 (1H, m, NHCH), 3.82 (2H, m CH_2OH), 4.71 (1H, sept, $J=7.1$ Hz, $CH(CH_3)_2$), 4.78 (1H, m, CH_2Ph), 4.87 (2H, d,

$J=6.6$ Hz, C^2NH), 5.81 (1H, br s, C^6NH), 7.31 (5H, m, Ph). (**2**) Yield 69%. MS-ESI+: 403.3 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$): 1.02 (3H, t, $J=7.7$ Hz, CH_2CH_3), 1.56 (2H, m, CH_2CH_3), 1.61 (6H, d, $J=7.1$ Hz, $(CH_3)_2CH$), 3.62 (1H, m, NHCH), 3.83 (2H, m, CH_2OH), 3.99 (1H, br s, CH_2OH), 4.60 (1H, br s, C^2NH), 4.75 (1H, sept, $J=7.1$ Hz, $CH(CH_3)_2$), 4.88 (2H, d, $J=6.60$ Hz, CH_2Ph), 6.10 (1H, br s, C^6NH), 6.75 (1H, m, Ph-H4), 6.78 (1H, m, Ph-H2), 6.85 (1H, m, Ph-H6), 7.15 (1H, t, $J=7.7$ Hz, Ph-H5). (**3**) MS-ESI+: 433.3 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$): 1.00 (3H, t, $J=6.6$ Hz, CH_2CH_3), 1.64 (6H, d, $J=6.9$ Hz, $(CH_3)_2CH$), 1.74 (2H, m, CH_2CH_3), 3.68 (1H, m, NHCH), 3.82 (2H, m, CH_2OH), 4.06 (1H, br s, CH_2OH), 4.74 (1H, sept, $J=6.9$ Hz, $CH(CH_3)_2$), 5.08 (1H, br s, C^2NH), 5.17 (2H, d, $J=6.6$ Hz, CH_2Ph), 6.92 (1H, br s, C^6NH), 7.39 (5H, m, Ph). (**4**) Yield 88%. MS-ESI+: 370.2 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$): 0.97 (3H, t, $J=7.7$ Hz, CH_2CH_3), 1.52 (6H, d, $J=6.9$ Hz, $(CH_3)_2CH$), 1.55 (2H, m, CH_2CH_3), 3.57 (1H, m, NHCH), 3.77 (2H, m, CH_2OH), 3.84 (1H, br s, CH_2OH), 4.55 (1H, sept, $J=6.9$ Hz, $CH(CH_3)_2$), 4.66 (1H, br s, C^2NH), 4.92 (2H, br s, CH_2Ph), 6.62 (1H, br s, C^6NH), 7.02 (2H, br s, C^8NH_2), 7.26 (5H, m, Ph). (**5**) Yield 89%. MS-ESI+: 371.2 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$): 0.96 (3H, t, $J=7.1$ Hz, CH_2CH_3), 1.33 (6H, t, $J=7.1$ Hz, $(CH_3)_2CH$), 1.54 (2H, m, CH_2CH_3), 3.54 (1H, m, NHCH), 3.73 (2H, m, CH_2OH), 4.29 (1H, sept, $J=7.1$ Hz, $CH(CH_3)_2$), 4.68 (2H, br s, CH_2Ph), 4.90 (1H, d, $J=6.0$ Hz, C^2NH), 6.58 (1H, br s, C^6NH), 7.26 (5H, m, Ph), 10.2 (1H, br s, imidazole-NH). (**6**) Yield 95%. MS-ESI+: 387.3 ($M+H^+$). ^1H NMR (400 MHz, $CDCl_3$): 0.99 (3H, t, $J=7.4$ Hz, CH_2CH_3), 1.52 (3H, d, $J=6.9$ Hz, $(CH_3)_2CH$), 1.56 (3H, d, $J=6.9$ Hz, $(CH_3)_2CH$), 1.59 (2H, m, CH_2CH_3), 3.27 (1H, br s, SH), 3.59 (1H, m, CH_AHOH), 3.78 (1H, m, CHH_BOH), 3.87 (1H, m, NHCH), 4.66 (1;H, m, CH_2Ph), 4.80 (2H, d, $J=7.3$ Hz, C^2NH), 5.12 (1H, sept, $J=6.9$ Hz, $CH(CH_3)_2$), 6.02 (1H, t, $J=5.7$ Hz, C^6NH), 7.29 (5H, m, Ph). (**7**) Yield 79%. MS-ESI+: 400.2 ($M+H^+$). ^1H NMR (400 MHz, CD_3OD): 0.980 (3H, t, $J=7.2$ Hz, H-5″), 1.570 (1H, m, H-4″a), 1.716 (1H, m, H-4″b), 1.716 (6H, d, $J=6.9$H, H-2′), 3.642 (2H, m, H-3″), 4.013 (1H, dq, $J=5.3$, 8.1 Hz, H-2″), 4.709 (1H, d, $J=15.4$ Hz, CH_2-a), 4.772 (1H, d, $J=15.4$ Hz, CH_2-b), 5.445 (1H, sept, $J=6.9$ Hz, H-1′), 7.234 (1H, m, H-*para*), 7.308 (2H, m, H-*meta*), 7.373 (2H, m, H-*ortho*). (**8**) Yield 79%. MS-ESI+: 369.2 ($M+H^+$). ^1H NMR (300 MHz, $CDCl_3$): 0.996 (3H, t, $J=7.4$ $C\underline{H}_3CH_2$), 1.582–1.675 (8H, m, $(C\underline{H}_3)_2CH+CH_3C\underline{H}_2$), 2.545 (3H, s, CH_3C8), 3.62 dd (1H, $J=10.9$, $J=7.7$, $C\underline{H}CHOH$), 3.78 dd (1H, $J=10.9$, $J=2.7$, $CH\underline{H}OH$), 3.95 (1H, bs, C*H), 4.54 (1H, sept, $J=6.9$ Hz, $C\underline{H}(CH_3)_2$), 4.94 (2H, bs, $C\underline{H}_2Ph$), 7.21–7.41 (5H, m, H–Ar). (**9**) Yield 88%. MS-ESI+: 413.2 ($M+H^+$). ^1H NMR (400 MHz, $CDCl_3$): 1.008 (3H, t, $J=7.5$ Hz, H-5″), 1.037 (3H, t, $J=7.4$ Hz, H-3‴), 1.502 (3H, d, $J=6.9$H, H-2′a), 1.511 (3H, d, $J=6.9$H, H-2′b), 1.593 (1H, m, H-4″a), 1.681 (1H, m, H-4″b), 1.835 (2H, m, H-2‴), 3.638 (1H, dd, $J=7.0$, 11.2 Hz, H-3″a), 3.788 (1H, dd, $J=3.6$, 11.2 Hz, H-3″b), 3.976 (1H, m, H-2″), 4.366 (2H, t, $J=6.5$ Hz, H-1‴), 4.628 (1H, sept, $J=6.9$ Hz, H-1′), 4.956 (2H, b r s, CH_2), 7.278 (1H, m, H-*para*), 7.337 (2H, m, H-*meta*), 7.406 (2H, m, H-*ortho*). (**10**) Yield 96%. MS-ESI+: 428.3 ($M+H^+$). ^1H NMR (500 MHz, $CDCl_3$): 0.99 (3H, t, $J=7.4$ Hz, CH_3CH_2), 1.51 (3H, d, $J=6.9$ Hz, $(CH_3)_2CH$), 1.53 (3H, d, $J=6.9$ Hz, $(CH_3)_2CH$), 1.59 (2H, m, CH_2CH_3), 1.79 (2H, pent, $J=5.6$ Hz, $CH_2CH_2CH_2$), 3.52 (2H, t, $J=5.9$ Hz, CH_2CH_2OH), 3.57 (2H, m, $CHCH_2OH$), 3.67 (2H, t, $J=5.4$ Hz, $NHCH_2$), 3.77 (1H, m, NHCH), 3.82 (1H, br s, C^8NH), 4.41 (1H, sept, $J=6.9$ Hz, $CH(CH_3)_2$), 4.68 (2H, m, CH_2Ph), 4.75 (1H, d, $J=5.5$ Hz, C^2NH), 5.99 (1H, br s, C^6NH), 7.25 (5H, m, Ph). (**11**) Yield 74%. MS-ESI+: 423.3 ($M+H^+$). ^1H NMR (400 MHz, $CDCl_3$): 1.042 (6H, d, $J=6.8$ Hz, H-5″), 1.638 (3H, d, $J=6.5$ Hz, H-2′a), 1.650 (3H, d, $J=6.9$ Hz, H-2′b), 2.019 (1H, m, H-4″), 3.755 (1H, dd, $J=7.2$, 11.1 Hz, H-3″a), 3.893 (1H, dd, $J=3.5$, 11.1 Hz, H-3″b), 3.976 (1H, m, H-2″), 4.772 (1H, sept, $J=6.9$ Hz, H-1′), 7.044 (1H, ddd, $J=0.9$, 2.0, 8.0 Hz, H-4‴), 7.229 (1H, dd, $J=8.0$, 8.1 Hz, H-5‴), 7.365 (1H, dd, $J=1.4$, 8.1 Hz, H-6‴), 7.928 (1H, br s, H-2‴). (**12**) Yield 75%. MS-ESI+: 400.2 ($M+H^+$). ^1H NMR (400 MHz, $CDCl_3$): 1.291 (2H, m, H-2′a, H-6′a), 1.479 (2H, m, H-3′a, H-5′a), 1.621 (6H, d, $J=7.0$ Hz, C-2″), 2.045 (2H, m, H-3′b, H-5′b), 2.261 (2H, m, H-2′b, H-6′b), 2.512 (3H, s, CH_3-8), 2.868 (1H, m, $\Sigma J=29.9$ Hz, H-4′), 3.824 (1H, m, $\Sigma J=37.9$ Hz, H-1′), 4.576 (1H, septet, $J=7.0$ Hz, H-1″), 4.718 (1H, d, $J=7.7$ Hz, NH-2), 6.973 (1H, ddd, $J=0.9$, 2.0, 8.0 Hz, H-4‴), 7.204 (dd, $J=8.0$, 8.2 Hz, H-5‴), 7.408 (1H, ddd, $J=0.8$, 2.1, 8.2 Hz, H-6‴), 7.654 (1H, br s, NH-6), 8.136 (1H, br s, H-2‴). ^{13}C NMR: 15.1 (q, CH_3-8), 21.2 (q, C-2″), 31.8 (t, C-2′), 34.1 (t, C-3′), 50.1 (d, C-1′), 50.4 (d, C-4′), 113.1 (s, C-5), 117.3 (d, C-6‴), 119.5 (s, C-2‴), 122.2 (d, C-4‴), 129.7 (d, C-5‴), 134.3 (s, C-3‴), 140.7 (s, C-1‴), 145.8 (s, C-8), 150.9 (s, C-2 or C-6), 153.0 (s, C-4), 157.7 (s, C-2 or C-6). (**A.2.I.41**) Yield 18%. MS-ESI+: 400.2 ($M+H^+$). ^1H NMR (400 MHz, $CDCl_3$): 1.316 (1H, m, H-3′a), 1.562 (6H, d, $J=6.8$ Hz, C-2″), 1.645 (1H, m, H-2′a), 2.163 (1H, m, H-3′b), 2.306 (1H, m, H-2′b), 3.021 (1H, m, $\Sigma J=30.6$ Hz, H-4′), 3.905 (1H, m, $\Sigma J=38.2$ Hz, 1H, H-1′), 4.676 (1H, septet, $J=6.8$ Hz, H-1″), 4.786 (1H, d, $J=7.7$ Hz, NH-2), 7.001 (1H, ddd, $J=1.0$, 2.1, 7.9 Hz, H-4‴), 7.231 (dd, $J=7.9$, 8.1 Hz, H-5‴), 7.407 (1H, ddd, $J=0.8$, 2.1, 8.1 Hz, H-6‴), 7.590 (1H, s, H-8), 7.636 (1H, br s, NH-6), 8.161 (1H, br s, H-2‴).