Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 793–798

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS OF C2 ALKYNYLATED PURINES, A NEW FAMILY OF POTENT INHIBITORS OF CYCLIN-DEPENDENT KINASES

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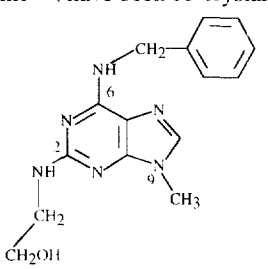
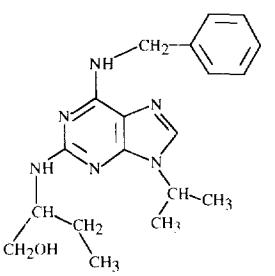
Received 16 December 1997; accepted 12 February 1998

Abstract: The synthesis of a new family of inhibitors of the cell cycle regulating cyclin-dependent kinases (CDK's) is reported. These compounds, related to the purines olomoucine and roscovitine, are characterised by the presence of alkynylated side chains at C2. They inhibit CDK's with IC_{50}'s in the 200 nM range.

Cyclin-dependent kinases (CDK's) constitute a family of highly conserved protein kinases playing key functions in cell cycle regulation[1,2]. Chemical inhibitors of these enzymes have potential applications in a large variety of pathologies, such as cancers, glomerulonephritis, restenosis, proliferation of parasites, and neurodegenerative disorders[3]. The search for inhibitors of CDK's has led to the discovery of a family of C2,N^6,N9-substituted purines with high CDK selectivity. The most studied members of this family, olomoucine[4] and roscovitine[5,6], have been co-crystallised with CDK2[5,7].



Olomoucine



Roscovitine

Both molecules interact with CDK2 at the ATP binding site. Interestingly, the purine ring of these inhibitors lies in the pocket in a totally different orientation than the purine ring of ATP. Furthermore the benzyl ring of these molecules interact with amino acids which are not involved in ATP binding and which are highly conserved in the CDK family. These facts might explain, at least in part, the specificity of this family of purines towards CDK's, as they are largely inactive against a large variety of kinases, including protein kinases C, tyrosine kinases, cyclic nucleotide-dependent kinases,...etc.[4,6]. Analysis of the CDK2/olomoucine and CDK2/roscovitine crystal structures reveals that the C2 substituents bind to an area of the ATP-binding pocket occupied by the ribose in the CDK2/ATP complex. In addition, no interaction is found between the NH at the C2 position and the kinase. This observation led us to investigate the effects of a replacement of this secondary

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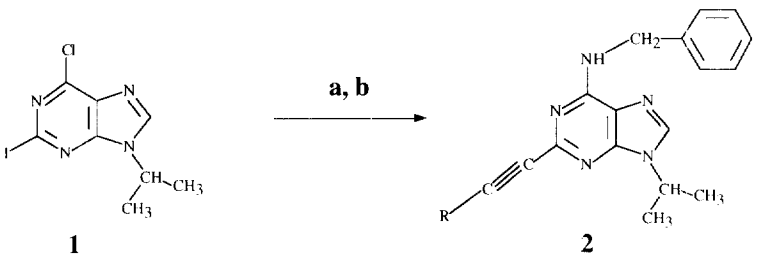
0960-894X/98/$19.00
PII: S0960-894X(98)00097-3

amine by a carbon atom on CDK inhibition. We report herein the synthesis and inhibitory activities of a new purine family of CDK inhibitors, characterised by the presence of an alkynylated side chain at C2. These potent inhibitors may constitute a new avenue for the design of powerful cell cycle inhibitors of potential medicinal interest.

The synthesis of 2-alkynylated purines **2**[9b,9d] was performed from 2,6-dihalogenated purine **1** (Scheme 1), the synthesis of which can be obtained by diazotation-substitution of 9-alkyl-2-amino-6-chloropurine precursor[8] with n-pentyl nitrite in the presence of diiodomethane[9a-c].

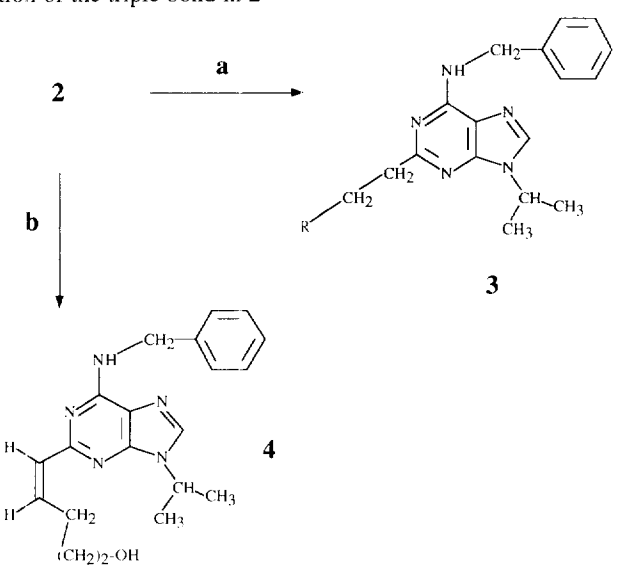
Scheme 1: Synthesis of 2-alkynylated purines **2**.



(a) NH_2-CH_2-Ph, EtOH, 45°C; (b) HC≡C-R, $(PPh_3)_2PdCl_2$, $nBuNH_2$, CuI, DMF, 80°C

Nucleophilic substitution of 6-chloropurine by benzylamine proceeded smoothly near room temperature (40-45°C), while introduction of a hydrocarbon chain at C2 was obtained under the palladium-catalysed cross-coupling reaction with terminal alkynes, a method originally developed by Heck[10] (Scheme 1).

Scheme 2: Hydrogenation of the triple bond in **2**

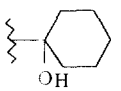
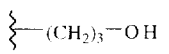
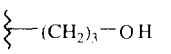
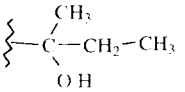
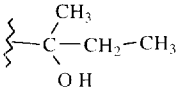
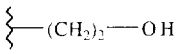
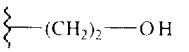
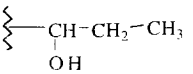
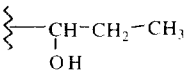
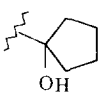
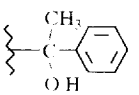
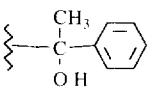
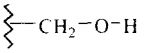
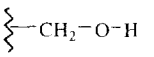


(a) 10% Pd on charcoal, EtOH, H_2, 2-4 h; (b) 10% Pd on charcoal, EtOH, H_2, 1h.

Partial or complete palladium-catalysed reduction under hydrogen atmosphere of the triple bond at C2 was also carried out and led to compounds belonging to the general formulae **3** and **4** (Scheme 2).

The different structures[12,13] which were synthesised are presented in Table 1 along with their IC_{50} on purified CDK1/cyclin B[11].

Table 1. Inhibition of CDK1/cyclin B activity by compounds **2a-h, 3a-f** and **4.**

Compound No	R	IC_{50} (μM)	Compound No	R	IC_{50} (μM)
2a		0.18	**Olomoucine**	-	7
			Roscovitine (R)	-	0.45
2b		0.2	**3b**		2.8
2c(R,S)		0.2	**3c(R,S)**		1.3
2d		0.35	**3d**		3
2e(R,S)		0.5	**3e(R,S)**		3
2f		0.5	**4(Z)**	-	1
2g(R,S)		0.6	**3f(R,S)**		4.5
2h		1.2	**3a**		2.5

Replacement of the C2 amino alcohol substituent in olomoucine and roscovitine by different alkynylated chains led to rather potent CDK1/cyclin B inhibitors. These compounds were equally active on the nervous tissue-specific CDK5/p35 kinase (data not shown). The most active compounds share an unsaturated hydrocarbon chain at the C2 position. Partial or complete hydrogenation of the triple bond decreased about ten times the inhibitory effect of the corresponding purines (compare on Table 1: **2b** and **3b / 4**; **2c** and **3c**; **2d** and **3d**; **2e** and **3e**; **2g** and **3f**; **2h** and **3a**).

In conclusion we feel that we have discovered a new family of potent CDK inhibitors which provides the basis for novel structural developments. An alkynylated side chain at C2 indeed opens the way to a large variety of possible modifications of the parent purine. Combined with modifications at the N^6 position[14], these new molecules should lead to highly potent CDK inhibitors, the enzyme specificity and the pharmacological properties of which should be evaluated for potential applications in various pathological situations.

Acknowledgements. We thankfully acknowledge the financial support of the "Ministère de l'Enseignement Supérieur et de la Recherche (ACC-SV8) (to M.L., E.B. and L.M.), the "Association pour la Recherche sur le Cancer" (ARC9157)(to L.M.) and the "Conseil Régional de Bretagne" (to L.M. and S.L.)

Experimental.

Synthesis of **2a-h**. General Procedure: A solution of 6-benzyl-2-iodo-9*H*-9-isopropyl purine[8,9] (1 mM), CuI (0.05 mM), bis-(triphenylphosphine)palladium dichloride (0.01 mM), n-Butylamine (1.5 mM), and a terminal alkyne (1.5 equiv.), in degassed DMF (25 mL) was stirred under argon atmosphere at 80°C until disappearance of the iodopurine, as judged by TLC in CH_2Cl_2-EtOH: 95-5. After evaporation of the solvant the residue was dissolved in CH_2Cl_2 and H_2S gas was bubbled into the solution for 2 min.The suspension was evaporated to dryness and subjected to silica gel column chromatography.

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11. Starfish CDK1/cyclin B was purified from M oocytes by affinity chromatography on $p9^{CKShs1}$-Sepharose beads, from which it was eluted by free $p9^{CKShs1}$. It was assayed with 1 mg histone H1 (Sigma type III-S)/ml, in the presence of 15 μM [g-^{32}P] ATP (3,000 Ci/mmol; 1 mCi/ml) in a final volume of 30 μl. After 10 min. incubation at 30°C, 25 μl aliquots of supernatant were spotted onto 2.5 x 3 cm pieces of Whatman P81 phosphocellulose paper, and, after 20 sec., the filters were washed five times (for at least 5 min. each time) in a solution of 10 ml phosphoric acid/liter of water. The wet filters were transferred into 6 ml plastic scintillation vials, and counted in the presence of 2 ml ACS (Amersham) scintillation fluid. The kinase activity was expressed in pmoles phosphate incorporated in histone H1/10 min incubation or in % of maximal activity. IC_{50}'s were calculated from inhibitor dose-response curves.

12. Physical data for compounds **2a-h**, **3a-f** and **4** are as shown below:

Compound No	mp (°C)	% yield	formula	analysis	recrystallisation solvent
2a	150-153	75	$C_{23}H_{27}N_5O_1$, 3/4 H_2O	C,H,N	cyclohexane-AcOEt
2b	97	62	$C_{20}H_{23}N_5O_1$, 1/2 H_2O	C,H,N	cyclohexane-AcOEt
2c	141-142	82	$C_{21}H_{25}N_5O_1$	C,H,N	cyclohexane
2d	196	68	$C_{19}H_{21}N_5O_1$	C,H,N	EtOH-water
2e	140-141	60	$C_{20}H_{23}N_5O_1$	C,H,N	EtOH-water
2f	180-182	90	$C_{22}H_{25}N_5O_1$, 1/3 H_2O	C,H,N	cyclohexane-AcOEt
2g	125-126	65	$C_{25}H_{25}N_5O_1$, 1/2 H_2O	C,H,N	cyclohexane-AcOEt
2h	210-211	74	$C_{18}H_{19}N_5O_1$,	C,H,N	Water-EtOH
3c	oily	85	$C_{21}H_{29}N_5O_1$, 1/3 H_2O	C,H,N	-
3a	124-128	67	$C_{18}H_{23}N_5O_1$	C,H,N	Water
3b	108-111	61	$C_{20}H_{27}N_5O_1$, 1/8 H_2O	C,H,N	Water-EtOH
3d	102-105	62	$C_{19}H_{25}N_5O_1$	C,H,N	Water
3e	oil	78	$C_{20}H_{27}N_5O_1$, 1/4 H_2O	C,H,N	-
3f	oil	92	$C_{25}H_{29}N_5O_1$, 1/2 H_2O	C,H,N	-
4	oil	42	$C_{20}H_{25}N_5O_1$, 1/3 EtOH	C,H,N	-

13. Selected ^{1}H NMR data in $CDCl_3$ at 200 MHz are shown below:

2a: 7.85 (s, 1H, H8); 7.38-7.26 (m, 5H,Ph); 4.95-4.85 (m, 3H, **CH2**Ph, **CH**(CH_3)2); 2.00-2.12 (m, 3H, CH_2, CH); 2.82-2.65 (m, 7H,3xCH_2, CH); 1.57 (d, 6H, 2xCH_3, J=6.8 Hz). **2b**: 7.75 (s, 1H, H8); 7.37-7.24 (m, 5H,Ph); 6.32 (s, 1H, NH); 4.93-4.78 (m, 3H,**CH2**Ph, **CH**(CH_3)2); 3.78 (t, 2H, **CH2**OH, J=6.0 Hz); 2.54 (t, 2H, CH_2C≡C, J=7.0 Hz); 2.01 (br s, 1H, OH); 1.90 (quint, CH_2, J=6.5 Hz); 1.50 (d, 6H, (**CH3**)2CH, J=6.7 Hz). **2c**: 7.42-7.25 (m, 6H, H8, Ph); 4.97 (br m, 3H, **CH2**Ph, **CH**(CH_3)2); 2.47 (br m, 2H, OH, NH); 1.85 (q, 2H, **CH2**CH_3, J=7.4 Hz); 1.61 (s, 1H, CH_3); 1.56 (d, 6H, (**CH3**)2CH, J=5.8 Hz); 1.14 (t, 3H, CH_2**CH3**, J=7.3 Hz). **2d**: 7.94 (br s, 1H, H8); 7.38-7.23 (m, 5H,Ph); 5.2 (br s, 1H, NH); 4.89 (quint, **CH**(CH_3)2);, J=6.7 Hz); 3.89 (t, 2H, **CH2**OH,

J=5.9 Hz); 2.72 (t, 2H, $CH_2C{\equiv}C$, J=5.8 Hz); 1.90 (br s, 1H, OH); 1.57 (d, 6H. **(CH3)2**CH, J=6.8); **2e**: 8.0 (br s, 1H, H8); 7.42-7.25 (m, 5H,Ph); 6.35 (br s, 1H, NH); 4.94 (br m, 3H, **CH2**Ph, **CH**$(CH_3)_2$); 4.59 (t, 1H, $CHC{\equiv}C$, J=6.5 Hz); 2.26 (br s, 1H, OH); 1.89 (quint, 2H, **CH2**CH_3, J=7.1 Hz); 1.58 (d, 6H, **(CH3)2**CH, J=6.5 Hz); 1.11 (t, 3H, CH_2**CH3**, J=7.3 Hz). **2f**: 7.95 (br s, 1H, H8); 7.38-7.25 (m, 5H,Ph); 6.1 (br s, 1H, NH); 4.92 (br m, 3H, **CH2**Ph, **CH**$(CH_3)_2$); 2.3 (br s, 1H, OH); 2.18-1.98 (m, 4H, 2xCH_2); 1.98-1.68 (m, 4H, 2xCH_2); 1.57 (d, 6H, **(CH3)2**CH, J=6.7 Hz). **2g**: 7.78 (s, 1H, H8). 7.42-7.25 (m, 10H,2xPh); 4.95 (br m, 3H, **CH2**Ph, **CH**$(CH_3)_2$); 3.24 (br s, 1H, NH); 2.21 (br s, 1H, OH); 1.90 (s, 3H, CH_3); 1.56 (d, 6H, **(CH3)2**CH, J=6.7 Hz). **2h**: 7.79 (s, 1H, H8); 7.37-7.26 (m, 5H, Ph); 6.25 (br s, 1H, NH); 4.89 (m, 3H, **CH2**Ph, **CH**$(CH_3)_2$); 4.52 (d, 2H, $CH_2C{\equiv}C$, J=6.2 Hz); 2.53 (t, 1H, OH); 1.56 (d, 6H, **(CH3)2**CH, J=6.8 Hz). **3c**: 7.74 (s, 1H, H8); 7.42-7.26 (m, 5H, Ph); 6.15 (br s, 1H, NH); 4.88-4.76 (m, 3H, **CH2**Ph, **CH**$(CH_3)_2$); 3.05 (t, 2H, CH_2, J=6.9 Hz); 2.15-1.85 (m, 2H, **CH2**CH_3); 1.57 (d, 6H, **(CH3)2**CH, J=6.8 Hz); 1.21 (s, 3H, **CH3**COH); 0.94 (t, 3H, CH_2**CH3**, J=7.4 Hz); **3a**: 7.71 (s, 1H, H8); 7.40-7.26 (m, 5H, Ph); 6.09 (br s, 1H, NH); 4.86-4.71 (m, 3H, **CH2**Ph, **CH**$(CH_3)_2$); 3.75 (t, 2H, **CH2**OH, J=5.3 Hz); 3.05 (t, 2H, **CH2**-base, J=5.2 Hz); 2.05 (quint, 2H, CH_2, J=6.2 Hz); 1.79 (br s, 1H, OH); 1.57 (d, 6H, **(CH3)2**CH, J=6.0 Hz). **3b**: 7.73 (s, 1H, H8); 7.39-7.20 (m, 5H, Ph); 6.2 (br s, 1H, NH); 4.92-4.80 (m, 3H, **CH2**Ph, **CH**$(CH_3)_2$); 3.64 (t, 2H, **CH2**OH, J=5.3 Hz); 2.85 (t, 2H, **CH2**-base, J=7.3 Hz); 1.91-1.44 (m, 6H, 3xCH_2); 1.57 (d, 6H, **(CH3)2**CH, J=6.8 Hz). **3d**: 7.73 (s, 1H, H8); 7.43-7.26 (m, 5H, Ph); 6.03 (br s, 1H, NH); 4.90-4.79 (m, 3H, **CH2**Ph, **CH**$(CH_3)_2$); 3.67 (t, 2H, **CH2**OH, J=6.1 Hz); 2.90 (t, 2H, **CH2**-base, J=7.0 Hz); 2.64 (br s, 1H, OH); 2.01-1.57 (m, 4H, 2xCH_2); 1.57 (d, 6H, **(CH3)2**CH, J=6.8 Hz). **3e**: 7.72 (s, 1H, H8); 7.41-7.25 (m, 5H, Ph); 6.23 (br s, 1H, NH); 5.00-4.75 (m, 3H, **CH2**Ph, **CH**$(CH_3)_2$); 3.75-3.55 (m, 1H, **CH**OH); 3.2-2.9 (m, 2H, **CH2**-base); 2.12-1.75 (m, 2H, CH_2); 1.70-1.45 (m, 2H, CH_2); 1.57 (d, 6H, **(CH3)2**CH, J=6.8 Hz); 0.94 (t, 3H, CH_3, J=7.4Hz). **3f**: 7.75 (s, 1H, H8); 7.53-7.18 (m, 10H, 2xPh); 5.00-4.70 (m, 3H, **CH2**Ph, **CH**$(CH_3)_2$); 3.05-2.7 (m, 2H, **CH2**-base); 2.45-2.30 (m, 2H, CH_2); 1.60 (s, 3H, CH_3); 1.59 (d, 6H, **(CH3)2**CH, J=6.8 Hz). **4**: 7.69 (s, 1H, H8); 7.40-7.24 (m, 5H, Ph); 6.54 (d, 1H, base-**CH**=CH, J=11.9 Hz); 6.64 (br s, 1H, NH); 5.98-5.85 (dt, 1H, base-CH=**CH**); 5.05-4.75 (br s & quint, 3H, **CH2**Ph, **CH**$(CH_3)_2$, J=6.8 Hz); 3.67 (t, 2H, **CH2**OH, J=5.6 Hz); 2.88 (m, 2H, **CH2**CH=CH); 1.78 (quint, 2H, CH_2); 1.55 (d, 6H, **(CH3)2**CH, J=6.7). A strong nuclear Overhauser effect (NOE) was observed between the olefinic protons. In addition, evidence in favor of the the cis configuration is the observed NOE between the α-CH_2 of the olefinic chain and one methyl of the N-9-isopropyl substituent. Only the cis olefin can adopt a side chain conformation wherein these two substituents are in proximity. This possibility has been substantiated by modelisation using MOPAC.

14. Modifications at the N9 position have been explored in our laboratory. So far an isopropyl residue provides the most active derivatives (unpublished results). We have not yet explored the effects of modifications of the N6 substituent which have been claimed recently to enhance the inhibitory activity of Olomoucine. (Norman, T. C.; Gray, N. S.; Koh, J. T.; Schultz, P. G. *J. Am. Chem. Soc.* **1996**, *118*, 7430-7431.