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Synthesis of 6-(het) ary Xylocydine analogues and evaluating their inhibitory activities of CDK1 and CDK2 in vitro

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ABSTRACT

A series of purine nucleoside analogues bearing an aryl and hetaryl group in position 6 were prepared and their biological activities were assessed by in vitro CDK1/Cyclin B1 and CDK2/Cyclin A2 kinase assay. From the synthesized chemicals, three Xylocydine derivatives **3h**, **3i**, and **3j** exhibited specific inhibitory activities on CDK2/Cyclin A2 with IC_{50} values of 4.6, 4.8, and 55 μM, respectively. Those three compounds all induced G1/S phase arrest in Human epithelial carcinoma cell line (HeLa), and the results suggested they may inhibit CDK2 activity in vitro. Furthermore, molecular modeling study, their docking into Cyclin Dependant Kinase 2 (CDK2) active site showed high docking scores. Taken together, these data suggest that, those three compounds are good inhibitors of CDK2 for studying this kinase signal transduction pathway in cell system.

1. Introduction

Cyclin-dependent kinases (CDKs), which belong to this Ser/Thr kinase family,[1] play an important role in cell-cycle regulation.[2,3] Specific CDKs are sequentially activated during different phases of the cell cycle by their association with Cyclins and the activity of several CDK/Cyclins has been identified.[4,5] The mammalian cell cycle is governed by the sequential activation of CDKs.[6] Progression through G1 and entry into S phase are regulated by Cdk2 complexed with Cyclin A and Cyclin E, and the G2/M transition is driven by Cdc2 complexed with Cyclin B.[7] CDK activity is frequently upregulated in proliferative disease,[8] and CDKs have been to be proved important targets for treating hyperproliferating diseases such as cancer.[9,10] Currently, much effort has focused on the discovery of new chemical inhibitors of CDKs that could act as anticancer agents. Purines, pyrimidines, flavonoids, indolocarbazole analogues, and other heterocyclic compounds have been reported as potent CDK inhibitors.[11,12] Particularly, most of the already known CDK inhibitors (natural products or/and synthetic molecules) are flat, small heterocycles that act by competing with ATP for the kinase ATP-binding site. However, inhibitor specificity for CDKs over other kinases and selectivity for a specific target CDK within the CDK family of enzymes remain major challenges.[13]

As CDK2 is an important target in cancer therapy,[13–16] much effort has been devoted to find more specific and potent CDK2 inhibitors.[17–20] Purine nucleoside analogues Xylocydine (**2**, Fig. 1C), as a novel inhibitor of CDKs, can selectively inhibit CDK1 and CDK2,[21] and has been implicated to be useful for treatment of various CDK activity-related diseases.[22] Purines ring system was used in a number of CDK inhibitors, such as Olomoucine (Fig. 1A) which was identified as an active lead compound through the screening of substituted purines with modest potency.[23–25] Purine nucleosides and their analogues (Fig. 1B) represent an important class of biologically active compounds that have been extensively explored in the last five decades for their antitumor properties.[26–30] On this basis, our work was to explore new purine nucleosides to achieve better specificity inhibition to CDK2 kinase activity. We were interested whether the replacement of the Br by aryl or hetaryl substituent in position 6 would result in a specific inhibition to CDK2/CyclinA2 kianses. Here we report on the synthesis and evaluation of inhibitory activities of these 6-substituted Xylocydine analogues **3, 4** (Fig. 1C).

2. Result and discussion

2.1. Chemistry

Our synthesis of 6-substituted Xylocydine analogues **3a**–**j**, **4a**–**j** relied on palladium catalyzed Suzuki reactions of protected 4-amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-L-xylofuranosyl-) pyrrolo[2,3-*d*]pyrimidine **11**.

As shown in Scheme 1, the first half of the convergent synthesis required preparation of the 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine **7**. Initially, tetracyanoethylene **5** was charged with

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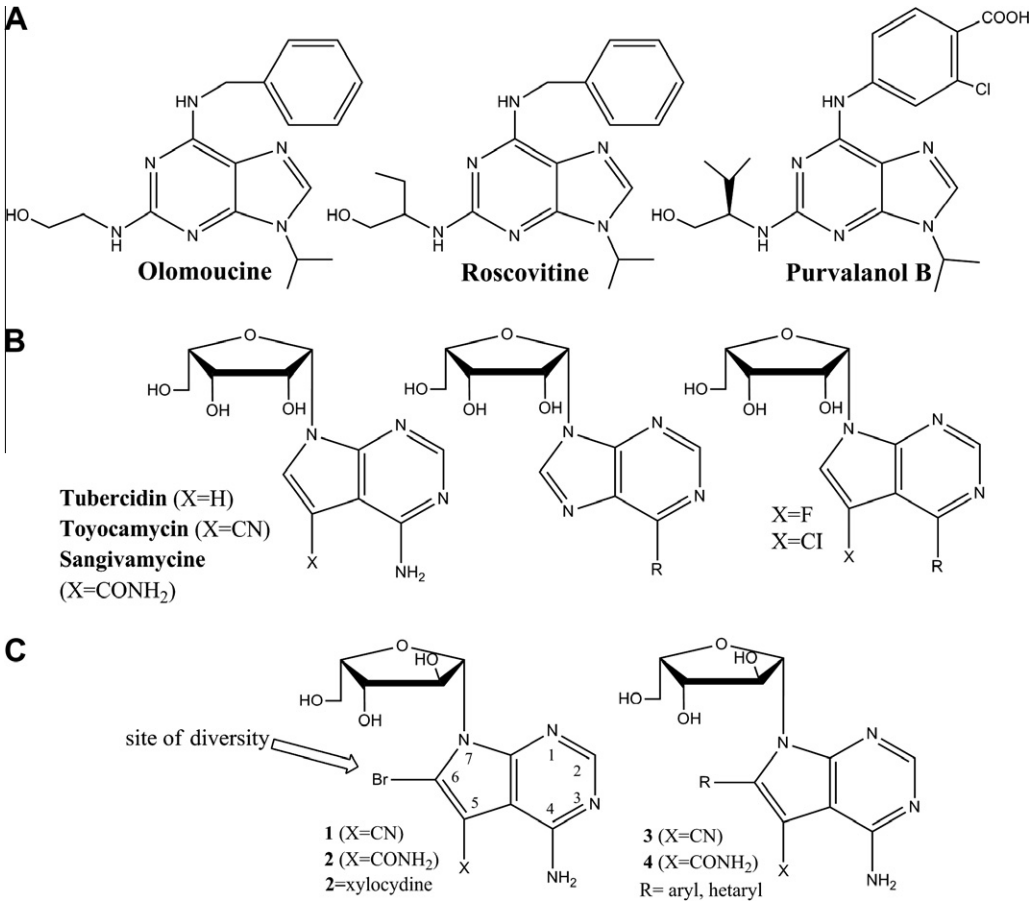
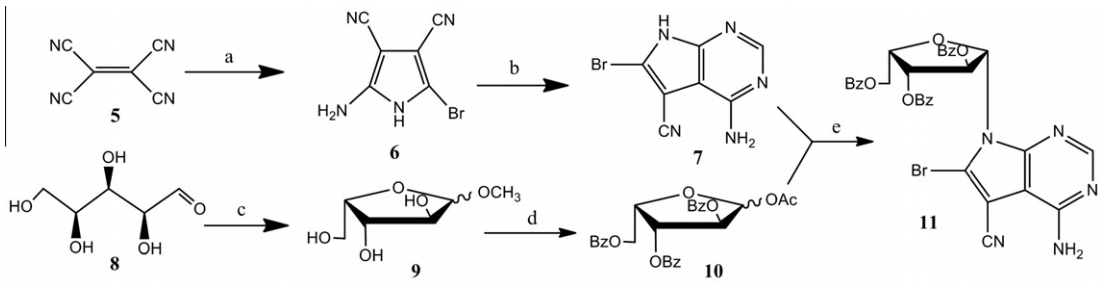


Figure 1. (A) Chemical structures of some potent purine CDK2 inhibitors. (B) Chemical structures of some potent purine nucleosides. (C) Modification position and structure of target product.

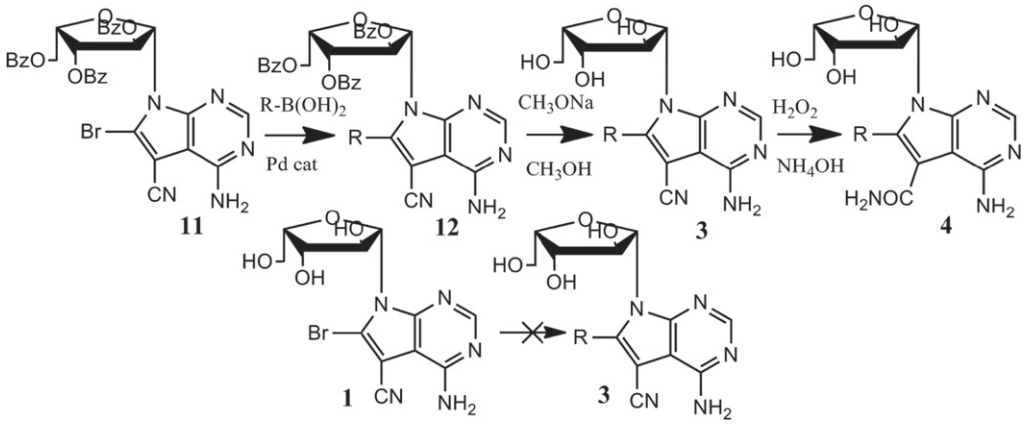


Scheme 1. Reagents and conditions: (a) HBr/HOAc, 0 °C, 2 h; (b) HN=CHNH$_2$·CH$_3$COOH, CH$_3$CH$_2$OCH$_2$CH$_2$OH; (c) CH$_3$OH/H$_2$SO$_4$; (d) BzCl, pyridine; AcOH, Ac$_2$O; (e) BSA, CH$_3$CN, then 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-L-ribofuranose, TMSOTf, 80 °C.

33% HBr in acetic acid at 0 °C, that gave 2-amino-5-bromo-3,4-dicyanopyrrole **6**.[31] Treatment of **6** with fomamidine acetate in 2-ethoxyethanol at reflux temperature gave 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine **7**. Synthesis of the compound **10** began from L (−)-xylose **8**. Compound **8** was easily converted into **9** by methylation. It should be noted that the operation of this method is simple than reference reported.[32] Then compound **10** was prepared from **9** by benzoylation and acetylation. Finally, glycosylation of compound **7** and **10** gave compound **11**, which was accomplished in a similar procedure as previously described for the synthesis of toyocamycin.[33,34]

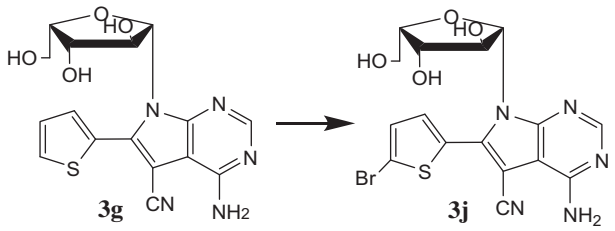
Cross-coupling reactions conditions: (A) Pd(OAc)$_2$, K$_2$CO$_3$, dioxane, 100 °C; (B) Pd(PPh$_3$)$_4$, K$_2$CO$_3$, toluene, 105 °C; (C) PdCl$_2$(PPh$_3$)$_2$, K$_2$CO$_3$, THF, reflux; (D) Pd(PPh$_3$)$_4$, K$_2$CO$_3$, DMF, 110 °C; (E) Pd(PPh $_3$)$_4$, Na$_2$CO$_3$, DME/H$_2$O, 80 °C.

Palladium catalyzed cross-coupling reactions of compound **11** (Scheme 2) with the corresponding arylboronic acids were performed to provide protected 6-substituted Xylocydine analogues **12a–i** in good yield.[35–38] According to the stability and activity of these arylboronic acids, we had tried several palladium catalyst systems and determined their own optimal reaction conditions. For arylboronic acid with an electrondonating group (entries **b** and **c**) and electronically neutral arylboronic acid (entries **a** and **d**), we applied Pd(OAc)$_2$/PPh$_3$-dioxane system as an efficient catalytic medium for the Suzuki coupling reaction. As to 4-bromophenyl boronic acid (entry **e**) and 3-bromophenyl boronic acid (entry **f**), it was very possible to generate multi-coupling products. Therefore, we applied Pd(PPh$_3$)$_4$-toluene system with the bromophenyl boronic acid being 1.5 equiv of material **11**. Compound **12g** was obtained from 1-*N*-(Boc)-pyrrole-2-boronic



12, 3, 4	R	Product (yiled)		
a	*p*-tolyl	**12a** (82%)	**3a** (85%)	**4a** (89%)
b	4-methoxyphenyl	**12b** (86%)	**3b** (80%)	**4b** (90%)
c	3-methoxyphenyl	**12c** (83%)	**3c** (80%)	**4c** (84%)
d	4-(tert-butyl)phenyl	**12d** (81%)	**3d** (86%)	**4d** (88%)
e	4-bromophenyl	**12e** (78%)	**3e** (82%)	**4e** (85%)
f	3-bromophenyl	**12f** (76%)	**3f** (83%)	**4f** (84%)
g	1*H*-pyrrol-2-yl	**12g** (75%)	**3g** (76%)	**4g** (70%)
h	thiophen-2-yl	**12h** (70%)	**3h** (75%)	**4h** (68%)
i	furan-2-yl	**12i** (72%)	**3i** (73%)	**4i** (79%)
j	5-bromothiophen-2-yl	—	**3j** (81%)	**4j** (89%)

Scheme 2. Synthesis of compounds **12**, **3**, and **4**.



Scheme 3. Reagents and conditions: NBS, CH_3CN, 0 °C to rt, 16 h.

acid in $Pd(PPh_3)_4$-DMF-Na_2CO_3 system.[39] As to instability of the active hetaryl boronic acid (entries **h** and **i**), the reaction was accomplished in $PdCl_2(PPh_3)_2$–THF system.

The deprotection step was performed by treatment with methanolic sodium methoxide to afford the desired 6-substituted Xylocydine analogues **3a–3i**. Alternative **3j** was prepared from **3g**, using NBS bromination (Scheme 3). Compound **3a–i** also had been experimented to prepared directly from unprotected 4-amino-6-bromo-5-cyano-7-(β-L-xylofuranosyl) pyrrolo[2,3-*d*] pyrimidine **13** (Scheme 2). Unfortunately, the reaction was multiproduct and difficult to purify, therefore this route is unreasonable. In accordance with literature reports, conversion of the cyano group of compounds **3a–j** to an carboxamide group afforded Xylocydine analogues 4-amino-6-aryl-7-(β-L-xylofuranosyl)pyrrolo[2,3-*d*] pyrimidine-5-carboxamide **4a–j**, using 30% H_2O_2 in concentrated ammonia.[40] But the reaction carried uneasily, because of poor solubility of reactants and products in 30% H_2O_2-concentrated ammonia system. After adding a certain amount of methanol to improve solubility of reactants and products and a certain amount of aqueous sodium hydroxide as a catalyst, the reaction time decreased from an average of more than three days to a few hours.

2.2. Biological profiling

The inhibitory actions of all prepared compounds were evaluated by kinase assays in vitro with CDK1/Cyclin B1 and CDK2/Cyclin A2 kianses originating in HeLa cells as well as the cell cycle study against the HeLa cell line.

Table 1
CDK2 inhibition and docking result of newly synthesized compounds

Compound	CDK inhibition in vitro, IC_{50} (μM)		Docking result	
	CDK2/Cyclin A2	CDK1/Cyclin B	Docking value (kcal/mol)	H-bonds
3h	4.6	>100	−8.8	4
3i	4.8	>100	−8.9	4
3j	55	>100	−8.2	2
2	0.061[a]	0.0014[a]	−8.6	5
1, **3a–g**, **4a–j**	—[b]	—[b]	—	—

[a] The biological value of Xylocydine **2** as reported in lit.[21]
[b] No significant activity.

2.2.1. In vitro CDK1/Cyclin B1 and CDK2/Cyclin A2 inhibition activity

In general, 5-carboxamide substituted purine nucleosides modified with a (het) aryl ring either with or without substitution (compounds **4a–j**) showed no significant activity against the tested kianses at the micro molar level (Table 1). In contrast, three 5-cyano substituted purine nucleosides containing 5-member heterocyclic aryl in 6-position (compounds **3h**, **3i**, and **3j**) exhibited inhibitory activity. We found that the three modified compounds inhibit CDK2 kinase activity to a higher degree than CDK1 kinase activity, though the IC_{50} values are at the micro molar level (from 4.6 μM to 55 μM). Comparing to Xylocydine **2**,[21] we have synthesized three new inhibitors (**3h–j**) specificity for CDK2/Cyclin A2.

In order to get CDK1/Cyclin B1 and CDK2/Cyclin A2 kianses, HeLa cells were treated by Paclitaxel with 80nM and Etoptoside with 50 μg/ml in DMEM with 5% newborn calf serum afer cell seeding for 24 h, 200 μg of protein extract of Hela cells were immunoprecipitated for 6 h ant 4 °C with an antibody specific for CDK1 and CDK2 respectly. The kinase was treated with 0–200 μM inhibitor in a final volume of 25 μl for 30 min. Then kinase assay were performed at 30 °C for 15 min in reaction buffer that contains 5 μg Histone H1 as a specific substrate for each kinase together with 10 μCi of [γ-^{32}P] ATP and 15 μM ATP in a final volume of 50 μl. The reaction was stopped by adding 5× SDS–PAGE Sample Loading Buffer. The kinase activity was detected by autoradiograph.

2.3. Measuring cell analysis by flow cytometry

Progression through G1 and entry into S phase are regulated by Cdk2 complexed with Cyclin A and Cyclin E, and the G2/M transition is driven by Cdc2 complexed with Cyclin B. To evaluate the inhibitory activity of CDK2 of compound **3h**, 3i, and **3j** in vivo, HeLa cell were treated with indicated concentration of compound **3h**, 3i, and **3j** for 72 h. The cell number of different phase was determined by flow cytometry. As shown in Figure 2, at the concentration of 6.25 μM, compound **3h** shows obvious G1/S phase arrest not including **3i** and **3j**. But after increasing the compound concentration to 12.5 μM, the amount of G1/S phase fraction were increased 13.6% (**3h**), 6.47% (**3i**) and 13.52% (**3j**) respectively contrast to untreated cell, while G2 phase cells were only 0.13%, 7.26% and 0.22% of total cells number in the same time. These results indicated that compound **3h**, **3i**, and **3j** may inhibit CDK2 activity and thus arrest cell cycle in G1/S phase in HeLa cells.

2.4. Molecular docking studies of CDK2 inhibitors and binding conformation

All dock runs were conducted using Autodock Vina software. An automated docking study was carried out using the crystal structure of Olomoucine/CDK2 complex (PDB code: 1W0X, available from the Protein Data Bank) which was successfully obtained with good resolution 2.2 Å and this co-crystallized structure allowed a good understanding of the necessary selectivity requirements. The prepared protein was used in the determination of the important amino in the predicted binding pocket. The docking process was carried out for compounds (Xylocydine **2**, **3h**, **3i**, **3j**) using the affinity energy as a scoring function. The docking results are recorded in the Table 1. The docking of Xylocydine **2** and active molecule **3h–j** into the active site of CDK2 was performed (Fig. 3).

The docking results revealed that: Compound **3h** and **3i** formed four hydrogen bonds, three with Gln131, Asn132 and Glu12 through theirs –OH and the other hydrogen bond with Leu83 through theirs $-NH_2$ moiety and the docking energy of them were −8.8 kcal/mol and −8.9 kcal/mol respectively. While compound **3j** formed only two hydrogen bonds with two Glu12 through its –OH and had a higher docking energy (−8.2 kcal/mol). In addition, **3h** and **3i** have the same spatial orientation while **3j** taken place a great change to lead to the hydrogen bonding between $-NH_2$ with Leu83 was absolutely destroyed. The in vitro CDK2 inhibitory activity of the designed three compounds was found to approximately match with the docking values, as **3h** and **3i** had close inhibitory effect, IC_{50} 4.6 μM and 4.8 μM respectively, with higher docking score meanwhile **3j** had lower docking score with IC_{50} 55 μM. Therefore, this result suggested that hydrogen bond between $-NH_2$ and Leu83 may play a very important role in the regulation of inhibitory activity for CDK2 inhibitors. Alignment study of docked compound **3h**, **3i**, and Xylocydine **2** with the binding pocket of CDK2 protein (Fig. 3E) suggested that newly synthesized compound **3h** and **3i** was perfectly aligned with Xylocydine **2**. Both the Xylocydine **2** and compound **3h** and **3i** make the same hydrogen bonding interaction with Leu83, Gln131 and Glu12. All of the three compounds (Xylocydine **2**, **3h**, **3i**) make the same hydrogen bonding interaction with Leu83, Gln131, and Glu12. This result indicated that spatial orientation of purine ring should be fully considered in optimizing the lead compound.

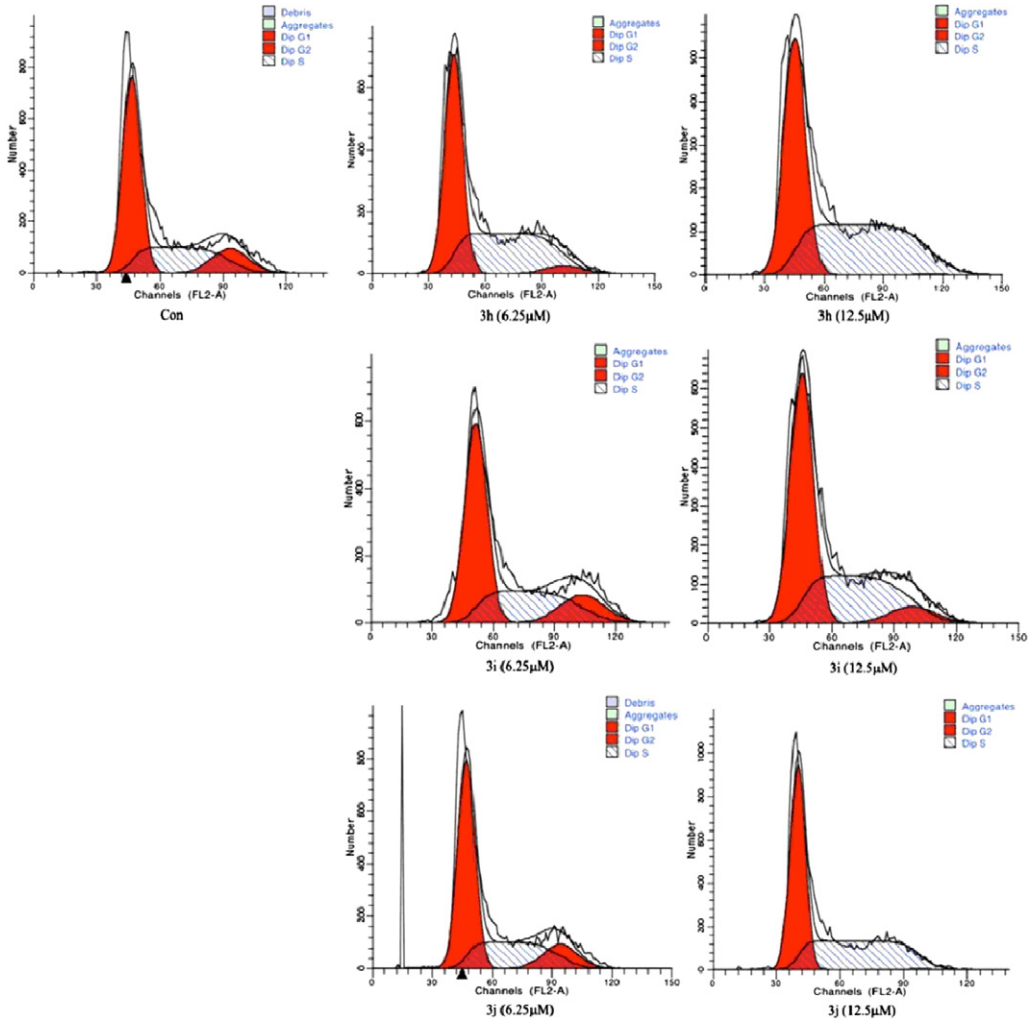
Analyzing the docking data, Xylocydine **2** forms five hydrogen bonds in the CDK2 binding pocket: two with two Leu83 through its $-NH_2$ and $-CONH_2$ moiety, three with Gln131, Asp145, and Glu12 through its –OH (Fig. 3A). However, we found that 6-Br is in front of the triad (Lys33, Glu51, and Asp145) of CDK2 which is involved in ATP phosphate orientation and magnesium coordination, and is thought to be critical for catalysis.[41] It was noteworthy that the 6-Br occupies a pocket composed by Lys33, Glu51, Asp145, Phe80, and Leu55. This area is thought to confer a very important opportunity for selectivity.[42] Subsequent in vitro kinase assay showed excellent selectivity to CDK2/Cyclin A2 (Table 1), that appeared coherent with the theories above.

The molecular docking study provides useful information for understanding the structural features of CDK2 inhibitors. Three new purine nucleoside analogues **3h–j** showed good docking scores values, and this was worth expanding to design potent activity and selectivity purine analogues.

3. Experimental section

3.1. Chemistry: General

The ^{1}H and ^{13}C NMR data were recorded with a Varian Mercury 300 NMR spectrometer, using TMS as an internal standard. Chemical shifts (δ) are given in parts per million and coupling constants are given as absolute values expressed in Hertz. Mass spectra were obtained using LC/MS 1100 of Agilent Technology Corporation and Alltech ELSD 2000 instrument. Melting points were determined by a X-4 digital microscope. Column chromatography was generally performed on silica gel (300–400 mesh) and TLC inspections on silica gel GF254 plates.



Phase	Control	3h		3i		3j	
		6.25μM	12.5μM	6.25μM	12.5μM	6.25μM	12.5μM
G1/S	86.27	96.3	99.87	85.13	92.74	86.83	99.79
G2	13.73	3.7	0.13	14.87	7.26	13.17	0.22

Figure 2. The cell cycle was blocked at G1/S phase by compound **3h**, **3i**, and **3j**. HeLa cells were treated with indicated concentrations of compound **3h**, **3i**, and **3j** for 72 h. Cells were collected, stained with DAPI, analyzed by flow cytometry and calculated by measuring the area of G1, S and G2 peak.

3.1.1. 2-Amino-5-bromo-3,4-dicyanopyrrole (6)

A solution of tetracyanoethylene (29.0 g, 226.0 mmol) in acetone (160 mL) and ethyl acetate (340 mL) was treated with a solution of 33% HBr in acetic acid (162 mL) while maintaining the internal temperature at 0 °C. The reaction mixture was stirred for an additional 1 h and the resulting yellow solid was filtered. The solid was then suspended in water and the pH of the suspension was adjusted to 11 with 50% NaOH to achieve solution, stirred for 15 min and then treated with glacial acetic acid to pH 5 to yield a precipitate, which was filtered and dried in vacuo over KOH to yield 37.2 g (78%) of **6**. Analytical data of compound **6** is in agreement with published data.[31]

3.1.2. 4-Amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (7)

2-Amino-5-bromo-3,4-dicyanopyrrole (21.0 g, 0.20 mol) and formamidine acetate (21.0 g, 0.20 mol) were dissolved in ethyleneglycolmonoethyl ether (380 mL). The mixture was heated at reflux temperature for 24 h, the dark solution treated with charcoal, and the charcoal removed from the hot solution by filtration. After washing the filter cake several times with 30 mL hot ethanol, the filtrate was cooled to room temperature to yield a brown precipitate, which was washed with water and collected by filtration. Recrystallization of the crude product afforded 12.14 g (50%) of pale yellow product **7**. mp >300 °C. MS (ESI): *m/z* 240.0 [M+H$^+$]. ^{1}H NMR (300 MHz, DMSO-d_6): 13.84(s, 1H), 8.21(s, 1H), 7.20(s, 2H, NH_2).

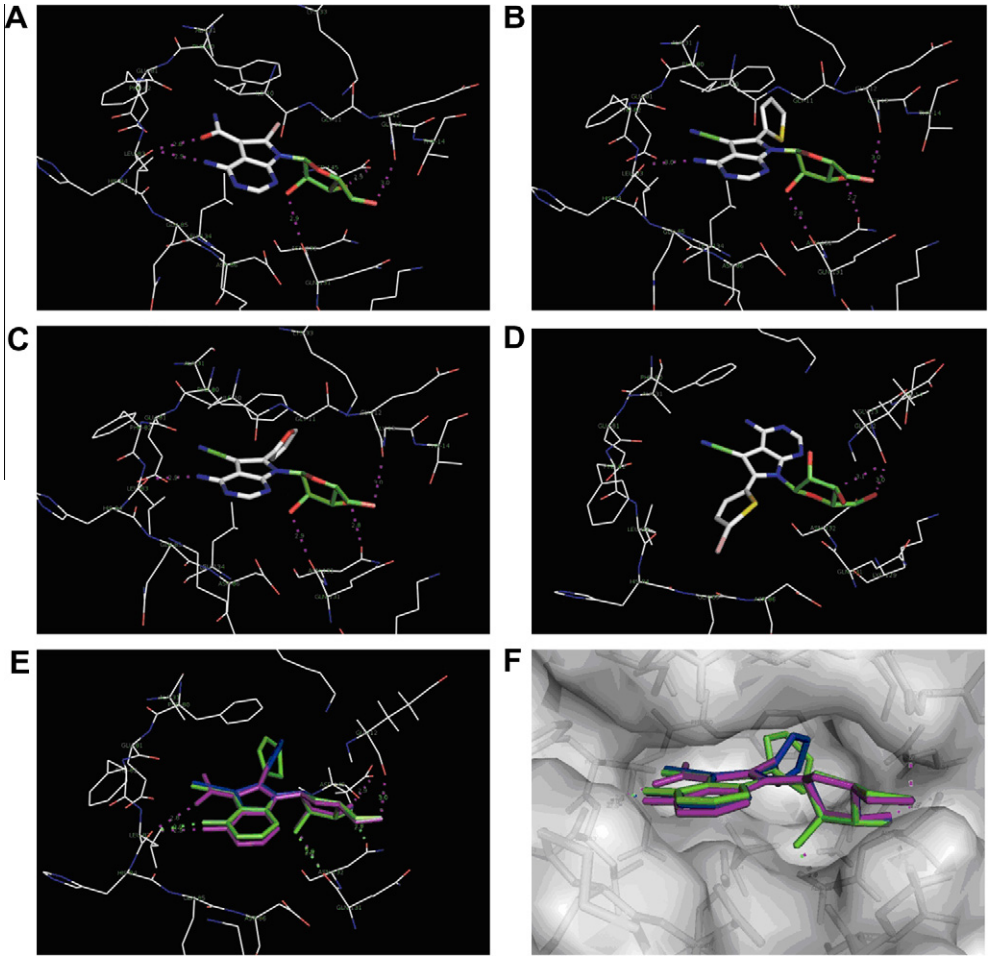


Figure 3. (A–D) The proposed binding mode of compound Xylocydine, **3h**, **3i**, and **3j** inside the active site of CDK2 kinase resulting from docking respectively. The most important amino acids are shown together with their respective numbers. Xylocydine (A) forms five hydrogen bonds, two with two Leu83, three with Gln131, Asp145 and Glu12. Both **3h** (B) and **3i** (C) form four hydrogen bonds, three with Gln131, Asn132 and Glu12 and the other with Leu83. Compound **3j** (D) forms two hydrogen bonds with two Glu12. (E) Alignment of docked compound **3h** (green color), **3i** (blue color) and Xylocydine (magenta color) with the binding pocket of CDK2. (F) The alignment was shown as surface colored gray.

3.1.3. 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-L-ribofuranose (10)

A suspension of the L(−)-xylose **8** (30.0 g, 0.20 mol) in methanol (500 mL) was slowly added concentrated sulfuric acid (2 mL). The mixture was stirred for 5 h at room temperature and then anhydrous potassium carbonate (5.2 g, 0.037 mol) was added to adjust the pH to 7. The solid was filtered and washed well with methanol. The filtrate was evaporated to dryness, witch was removed water by azeotropic distillation with toluene to afford crud compound **9**.

To a solution of above-mentioned crud compound **9** in pyridine (200 mL) at ice bath was added dropwise Benzoyl chloride (90 mL, 0.773 mol). The reaction was warmed to ambient temperature, and stirred overnight. The reaction mixture was poured into water and extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to give a fulvous oil.

This fulvous oil compound was dissolved in acetic acid (160 mL) and acetic anhydride (40.0 mL, 0.411 mol). The mixture was cooled to 0 °C before slowly adding concentrated sulfuric acid (25.0 mL, 0.469 mol). After dropping, the reaction was stirred at ambient temperature overnight. The reaction mixture was poured into water (200 mL) and neutralized with sodium acetate (40 g) and then extracted with chloroform (4 × 50 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed using petroleum ether and ethyl acetate (5:1, v/v) as an eluent. The product **10** was obtained as a white solid (91.0 g, yield 71%). mp 128–130 °C. Analytical data are identical to those reported earlier.[43]

3.1.4. 4-Amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-L-xylofuranosyl-)pyrrolo[2,3-*d*]pyrimidine(11)

A stirred suspension of 4-amino-6-bromo-5-cyanopyrrolo* [2,3-*d*] pyrimidine **7** (4.8 g, 20.0 mmol) in 200 mL dry acetonitrile was added *N*,*O*-Bis(trimethylsily1)acetamide (BSA, 8.2 g, 40.0 mmol) at room temperature under N_2. After 30 min, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-L-ribofuranose **10** (15.0 g, 30.0 mmol) was added along with trimethylsilyl trifluoromethanesulfonate (TMSOTf, 6.66 g, 30.0 mmol). The suspension was stirred at room temperature for 10 min, heated at 80 °C for 3 h and then cooled to room temperature. The solution was diluted with ethyl acetate (200 mL) and water (100 ml). The aqueous layer was separated and discarded and the organic layer was washed with aqueous sodium bicarbonate, brine, and dried over magnesium sulfate. The solvent was removed and the residue was chromatographed using a solvent system of ethyl acetate and chloroform (1:9, v/v). The product **11** was obtained as a white solid (10.2 g, 73% yield). mp 150–152 °C. MS (ESI): *m/z* 683.1 [M+H$^+$]. ^{1}H NMR (300 MHz, $CDCl_3$): 8.11–8.14 (m, 2H), 7.92–7.03 (m, 5H), 7.40–7.62 (m, 9H), 6.82–6.84 (m, 1H), 6.23 (d, 1H), 5.98–6.00 (m, 1H), 5.60 (s, 2H, NH_2), 4.83–4.90 (m, 3H). Analytical data are identical to those reported earlier.[33]

3.1.5. 4-Amino-6-(*p*-tolyl)-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-L-xylofuranosyl-)pyrrolo[2,3-*d*]pyrimidine (12a)

4-Amino-6-bromo-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-L-xylofura nosyl-)pyrrolo[2,3-*d*]pyrimidine **11** (0.136 g, 0.20 mmol) was suspended in dioxane (2.0 mL). The corresponding 4-methylphenylboronic acid (0.054 mg, 0.40 mmol), K_2CO_3 (0.082 g, 0.60 mmol) was added and the solution was degassed with N_2 for 5 min. Then, $Pd(OAc)_2$ (5 mol %, 2.5 mg,0.01 mmol) was added along with triphenylphosphine (15.5 mg, 0.06 mmol) and the mixture was further degassed. The reaction was heated at 100 °C for 12 h or until completion of the reaction as shown by TLC. The reaction mixture was then cooled to room temperature and extracted with ethyl acetate (3 × 10 ml). The organic layer was washed with brine (30 mL) and dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was chromatographed using a solvent system of ethyl acetate and chloroform (1:9, v/v), and the product **12a** was obtained as a white solid (0.133 g, 82% yield). mp 196–198 °C. IR (KBr): 3363 (NH_2), 2220 (CN), 1720, 1632. ^{1}H NMR (300 MHz, $CDCl_3$): 812–8.15 (m, 2H), 7.92–8.01 (m, 5H), 7.38–7.62 (m, 13H), 6.95–6.97 (m, 1H), 5.86–5.88 (m, 2H), 5.68 (s, 2H, NH_2), 4.96–4.90 (m, 3H), 2.38 (s, 3H). Anal. Calcd for $C_{40}H_{31}N_5O_7$: C, 69.26; H, 4.50; N, 10.10. Found: C, 69.29; H, 4.46; N, 10.07.

3.1.6. 4-Amino-6-(4-methoxyphenyl)-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-L-xylofuranosyl-)pyrrolo[2,3-*d*]pyrimidine (12b)

The reaction was conducted according to the procedure described for **12a** to afford **12b** as a white solid (0.139 g, 86% yield). Mp 226–228 °C. IR (KBr): 3354 (NH_2), 2218 (CN), 1726, 1629. ^{1}H NMR (300 MHz, $CDCl_3$): 8.11–8.14 (m, 2H), 7.92–8.01 (m, 5H), 7.40–7.61 (m, 11H), 6.94–6.99 (m, 3H), 5.87–5.90 (m, 2H), 5.58 (s, 1H, NH_2), 4.76–4.86 (m, 3H), 3.81 (s, 3H). Anal. Calcd for $C_{40}H_{31}N_5O_8$: C, 67.69; H, 4.40; N, 9.87. Found: C, 67.72; H, 4.46; N, 9.80.

3.1.7. 4-Amino-6-(3-methoxyphenyl)-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-L-xylofuranosyl-)pyrrolo[2,3-*d*]pyrimidine (12c)

The reaction was conducted according to the procedure described for **12a** to afford **12c** as a white solid (0.134 g, 83% yield). Mp 218–220 °C. IR (KBr): 3336 (NH_2), 2221 (CN), 1723, 1628. ^{1}H NMR (300 MHz, $CDCl_3$): 8.15 (d, 2H), 7.93–8.00 (m, 5H), 7.38–7.62 (m, 10H), 7.12–7.19 (m, 2H), 7.01–7.03 (m, 2H), 5.84–5.89 (m, 2H), 5.56 (s, 2H, NH_2), 4.76–7.87 (m, 3H), 3.71 (s, 3H). Anal. Calcd for $C_{40}H_{31}N_5O_8$: C, 67.69; H, 4.40; N, 9.87. Found: C, 67.74; H, 4.44; N, 9.91.

3.1.8. 4-Amino-6-(4-(tert-butyl)phenyl)-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-L-xylofuranosyl-)pyrrolo[2,3-*d*]pyrimidine (12d)

The reaction was conducted according to the procedure described for **12a** to afford **12c** as a white solid (0.131 g, 81% yield). Mp 201–202 °C. IR (KBr): 3348 (NH_2), 2220 (CN), 1724, 1626.^{1}H NMR (300 MHz, $CDCl_3$): 8.15–8.18 (m, 2H), 7.92–8.00 (m, 5H), 7.40–7.61 (m, 13H), 7.09–7.11 (m, 1H), 5.90–5.93 (m, 1H), 5.81(d, 1H), 5.62 (s, 2H, NH_2), 4.78–4.88 (m, 3H), 1.33(s, 9H). Anal. Calcd for $C_{43}H_{37}N_5O_7$: C, 70.19; H, 5.07; N, 9.52. Found: C, 70.12; H, 5.06; N, 9.60.

3.1.9. 4-Amino-6-(4-bromophenyl)-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-L-xylofuranosyl-)pyrrolo[2,3-*d*]pyrimidine (12e)

To a 25 ml round-bottom flask were charged **11** (0.136 g, 0.20 mmol) and 4-bromophenyl boronic acid (0.083 g, 0.4 mmol) together with palladium tetrakis-triphenylphosphine (11.6 mg, 0.01 mmol) and K_2CO_3 (0.082 g, 0.6 mmol) in toluene (5.0 mL) under an atmosphere of N_2. The reaction was heated at reflux for 24 h and cooled to room temperature. The mixture was diluted with water (10 mL) and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by flash chromatography, using a solvent system of ethylacetate and chloroform (1:9, v/v). The product **12e** was obtained as a white solid (0.118 g, 78% yield). Mp 182–184 °C. IR (KBr): 3358(NH_2), 2924, 2219(CN), 1722, 1628. MS (ESI): *m/z* 758.0 [M+H$^+$]. ^{1}H NMR (300 MHz, $CDCl_3$): 8.11–8.13 (d, 2H), 7.92–8.00 (m, 5H), 7.39–7.64 (m, 13H), 6.89–6.90 (m, 1H), 5.73–5.90 (m, 4H), 4.77–4.85 (m, 3H). Anal. Calcd for $C_{39}H_{28}BrN_5O_7$: C, 61.75; H, 3.72; N, 9.23. Found: C, 61.71; H, 3.66; N, 9.28.

3.1.10. 4-Amino-6-(3-bromophenyl)-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-L-xylofuranosyl-)pyrrolo[2,3-*d*]pyrimidine (12f)

The reaction was conducted according to the procedure described for **12e** to afford **12f** as a white solid (0.117 g, 76% yield). Mp 180–183 °C. IR (KBr): 3361(NH_2), 2924, 2221(CN), 1723, 1628. MS (ESI): *m/z* 758.0 [M+H$^+$]. ^{1}H NMR (300 MHz, $CDCl_3$): 8.11–8.13 (m, 2H), 7.95–8.02 (m, 5H), 7.78–7.79 (m, 1H), 7.41–7.61 (m, 12H), 6.84–6.87 (m, 1H), 5.85–5.88 (m, 1H), 5.83 (d, 1H), 5.69 (s, 2H, NH_2), 4.79–4.89 (m, 3H). Anal. Calcd for $C_{39}H_{28}BrN_5O_7$: C, 61.75; H, 3.72; N, 9.23. Found: C, 61.72; H, 3.68; N, 9.26.

3.1.11. 4-Amino-6-(1*H*-pyrrol-2-yl)-5-cyano-7-(2,3,5-tri-*O*-benzoyl-β-L-xylofuranosyl-)pyrrolo[2,3-*d*]pyrimidine (12g)

A solution of **11** (0.272 g, 0.40 mmol) in DMF (2.5 ml) was added sequentially 1-*N*-(Boc)-pyrrole-2-boronic acid (0.126 g, 0.60 mmol) and $PdCl_2(PPh_3)_2$ (5 mol %, 0.02 mmol, 24.0 mg). The mixture was heated to 70 °C and 0.675 g (3.60 mmol) of sodium carbonate dissolved in minimal water was added. The reaction was then heated to 110 °C for 14 h. After cooling to room temperature, the reaction was diluted with water (15 mL) and extracted with ethyl acetate (3 × 15 mL). The organic layers were dried with magnesium sulfate, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography, using a solvent system of ethylacetate and chloroform (1:6, v/v). The product **12g** was obtained as a white solid (0.123 g, 75% yield). Mp 176–178 °C. IR (KBr): 3425 (NH_2), 2923, 2218 (CN), 1726, 1635. ^{1}H NMR(300 MHz, $CDCl_3$): 9.76 (s, 1H), 7.91–8.07 (m, 16 H), 7.41–7.63 (m, 9H), 7.07 (s, 1H), 6.94 (s, 1H), 6.71 (d, 1H), 6.41 (d, 2H), 6.33 (d, 1H), 5.90 (d, 1H), 5.54 (s, 2H, NH_2), 5.04–5.11 (m, 1H), 4.87–4.92 (m, 1H), 4.65–4.70 (m, 1H). Anal. Calcd for $C_{37}H_{28}N_6O_7$: C, 66.46; H, 4.22; N, 12.57. Found: C, 66.49; H, 4.27; N, 12.52.

3.1.12. 4-Amino-6-(thiophen-2-yl)-5-cyano-7-(2,3,5-tri-*O*benzoyl-β-L-xylofuranosyl-)pyrrolo[2,3-*d*]pyrimidine (12h).

To a 25 mL round-bottom flask were charged **11** (0.272 g, 0.40 mmol) and thiophen-2-ylboronic acid (0.115 g, 0.90 mmol) together with $PdCl_2$ $(PPh_3)_2$ (13.8 mg, 0.02 mmol) and K_2CO_3 (0.082 g, 0.6 mmol) in 5 mL of tetrahydrofuran under an atmosphere of N_2. The reaction was heated at reflux for 24 h and cooled to room temperature. The mixture was diluted with water and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by flash chromatography using a solvent system of ethylacetate and chloroform (1:9, v/v). The product **12h** was obtained as a white solid (0.205 g, 75% yield). Mp 165–167 °C. IR (KBr): 3345 (NH_2), 2926, 2219 (CN), 1720, 1634. MS (ESI): *m/z* 685.9 [M+H$^+$]. ^{1}H NMR (300 MHz, $CDCl_3$): 8.13–8.16 (d, 2H), 7.96–8.13 (m, 5H), 7.38–7.62 (m, 11), 7.15–7.18 (m, 1H), 7.03–7.06 (m, 1H), 6.03 (d, 1H), 5.90–5.93 (m, 1H), 5.58 (s, 2H), 4.81–4.88 (m, 3H). Anal. Calcd for $C_{37}H_{27}N_5O_7S$: C, 64.81; H, 3.97; N, 10.21; S, 4.68. Found: C, 64.84; H, 3.96; N, 10.23; S, 4.72.

3.1.13. 4-Amino-6-(furan-2-yl)-5-cyano-7-(2,3,5-tri-*O*-benz-oyl-β-L-xylofuranosyl-)pyrrolo[2,3-*d*]pyrimidine (12i)

The reaction was conducted according to the procedure described for **12g** to afford **12i** as a white solid (0.123 g, 76% yield).

Mp 162–164 °C. IR (KBr): 3358 (NH_2), 2220 (CN), 1720, 1632. ^1H NMR (300 MHz, $CDCl_3$): 8.13–8.16 (m, 2H), 7.96–8.02 (m, 5H), 7.38–7.61 (m, 10H), 7.11–7.13 (m, 2H), 6.56–6.57 (m, 1H), 6.32 (d, 1H), 5.99–6.00 (d, 1H), 5.59 (s, 2H, NH_2), 4.81–4.90 (m, 3H). Anal. Calcd for $C_{37}H_{27}N_5O_8$: C, 66.36; H, 4.06; N, 10.46. Found: C, 66.31; H, 4.11; N, 10.43.

3.2. General procedure A for the deprotection of compounds 11, 12

Compound **11**, **12** (0.20 mmol) was treated with NaOMe (32.00 mg, 0.60 mmol) in MeOH (10 mL) for 12 h at room temperature or until completion of the reaction as shown by TLC. The mixture was co-evaporated with silica and chromatographed using a solvent system of methanol and chloroform (1:9, v/v) on a column of silica to afford **1**, **3**.

3.2.1. 4-Amino-6-bromo-5-cyano-7-(β-L-xylofuranosyl)pyrrolo [2,3-*d*]pyrimidine (1)

The reaction was conducted according to the general procedure A and the compound **1** was obtained as a white solid. IR (KBr): 3460 (OH), 3332 (NH_2), 3162, 2921, 2217 (CN), 16437, 1596. ^1H NMR (300 MHz, DMSO-d_6): 8.24 (s, 1H), 7.25 (s, 2H, NH_2), 6.47 (d, 1H, J = 9.0 Hz), 5.91 (d, 1H, J = 4.8 Hz), 5.74 (d, 1H, J = 3.6 Hz), 4.74–4.82 (m, 2H), 4.02–4.06 (m, 2H), 3.59–3.74 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 156.22, 153.00, 148.81, 142.73, 121.70, 114.09, 102.11, 92.31, 82.49, 79.27, 76.31, 59.64. Anal. Calcd for $C_{12}H_{12}BrN_5O_4$: C, 38.94; H, 3.27; N, 18.92. Found: C, 39.00; H, 3.28; N, 18.88.

3.2.2. 4-Amino-6-(*p*-tolyl)-5-cyano-7-(β-L-xylofuranosyl)pyrrolo [2,3-*d*]pyrimidine (3a)

The reaction was conducted according to the general procedure A and the compound 3a was obtained as a white solid (85% yield). Mp 142–144 °C. IR (KBr): 3416 (OH), 3311 (NH_2), 2918, 2221 (CN), 1649, 1595. ^1H NMR (300 MHz, DMSO-d_6): 8.28 (s, 1H), 7.45–7.53 (m, 4H), 7.15 (s, 2H, NH_2), 6.72 (d, 1H, J = 9.6 Hz), 5.83 (d, 1H, J = 4.8 Hz), 5.39 (d, 1H, J = 3.3 Hz), 4.76–4.81 (m, 2H), 3.88–3.97 (m, 2H), 3.57–3.73 (m, 2H), 2.43 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): 157.12, 152.77, 148.02, 146.39, 140.72, 130.18, 129.99, 120.82, 124.18, 115.37, 101.71, 91.24, 82.31, 79.55, 76.61, 55.56, 21.02. Anal. Calcd for $C_{19}H_{19}N_5O_4$: C, 59.84; H, 5.02; N, 18.36. Found: C, 59.96; H, 5.08; N, 18.28.

3.2.3. 4-Amino-6-(4-methoxyphenyl)-5-cyano-7-(β-L-xylofuranosyl)pyrrolo[2,3-*d*]pyrimidine (3b)

The reaction was conducted according to the general procedure A and the compound **3b** was obtained as a white solid. Mp 170–172 °C. IR (KBr): 3426 (OH), 3337 (NH_2), 2895, 2221(CN), 1638, 1606. MS (ESI): m/z 398.0 [M+H$^+$]. ^1H NMR (300 MHz, DMSO-d_6): 8.28 (s, 1H), 7.57 (d, 2H), 7.22 (d, 2H), 7.12 (s, 2H, NH_2), 6.71 (d, 1H, J = 9.6 Hz), 5.83 (d, 1H, J = 4.5 Hz), 5.41 (d, 1H, J = 3.3 Hz), 4.76–4.80 (m, 2H), 3.90–3.95 (m, 2H), 3.88 (s, 3H), 3.58–3.71 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 161.00, 157.03, 152.67, 148.56, 146.32, 131.64, 119.02, 115.49, 114.76, 101.66, 91.18, 82.35, 79.52, 76.60, 59.54, 55.51. Anal. Calcd for $C_{19}H_{19}N_5O_5$: C, 57.43; H, 4.82; N, 17.62. Found: C, 57.50; H, 4.87; N, 17.54.

3.2.4. 4-Amino-6-(3-methoxyphenyl)-5-cyano-7-(β-L-xylofuranosyl)pyrrolo[2,3-*d*]pyrimidine (3c)

The reaction was conducted according to the general procedure A and the compound **3c** was obtained as a white solid. Mp 167–168 °C. IR (KBr): 3434 (OH), 3329 (NH_2), 2898, 2219(CN), 1642, 1613. MS (ESI): m/z 398.0 [M+H$^+$]. ^1H NMR (300 MHz, DMSO-d_6): 8.29 (s, 1H), 7.55–7.60 (m, 1H), 7.19–7.22 (m, 5H), 6.68 (d, 1H, J = 9.3 Hz), 5.85 (d, 1H, J = 4.8 Hz), 5.44 (d, 1H, J = 3.6 Hz), 4.79 (d, 2H, J = 3.0 Hz), 3.91–3.93 (m, 2H), 3.84 (s, 3H), 3.32–3.73 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 159.49, 157.27, 152.98, 148.75, 146.04, 130.66, 128.32, 122.30, 116.52, 115.67, 115.38, 101.82, 91.31, 82.46, 79.71, 76.70, 59.66, 55.51. Anal. Calcd for $C_{19}H_{19}N_5O_5$: C, 57.43; H, 4.82; N, 17.62. Found: C, 57.54; H, 4.86; N, 17.53.

3.2.5. 4-Amino-6-(4-(tert-butyl)phenyl)-5-cyano-7-(β-L-xylofuranosyl)pyrrolo[2,3-*d*]pyrimidine (3d)

The reaction was conducted according to the general procedure A and the compound **3d** was obtained as a white solid. Mp 144–146 °C. IR (KBr): 3427 (OH), 3335 (NH_2), 2902, 2220(CN), 1648, 1611. MS (ESI): m/z 424.1 [M+H$^+$]. ^1H NMR (300 MHz, DMSO-d_6): 8.29 (s, 1H), 7.57–7.71 (m, 4H), 7.16 (s, 2H, NH_2), 6.72 (d, 1H, J = 9.3 Hz), 5.86 (d, 1H, J = 4.5 Hz), 5.43 (d, 1H, J = 3.3 Hz), 4.80 (s, 2H), 3.94–4.10 (m, 2H), 3.61–3.75 (m, 2H), 1.37(s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6): 157.11, 152.77, 148.60, 146.18, 129.80, 126.14, 115.45, 101.72, 91.21, 82.41, 79.56, 76.63, 59.54, 34.79, 30.93. Anal. Calcd for $C_{22}H_{22}N_5O_4$: C, 62.40; H, 5.95; N, 16.54. Found: C, 62.34; H, 5.86; N, 16.61.

3.2.6. 4-Amino-6-(4-bromophenyl)-5-cyano-7-(β-L-xylofuranosyl) pyrrolo[2,3-*d*]pyrimidine (3e)

The reaction was conducted according to the general procedure A and the compound **3e** was obtained as a white solid. Mp 160 °C. IR (KBr): 3439 (OH), 3320 (NH_2), 2923, 2221 (CN), 1637, 1597. MS (ESI): m/z 445.9 [M+H$^+$]. ^1H NMR (300 MHz, DMSO-d_6): 8.30 (s, 1H), 7.90 (d, 2H), 7.58 (d, 2H), 7.19 (s, 2H, NH_2), 6.61 (d, 1H, J = 9.3 Hz), 5.82 (d, 1H, J = 4.8 Hz), 5.36 (d, 1H, J = 3.6 Hz), 4.75–4.82 (m, 2H), 3.91–3.99 (m, 2H), 3.58–3.74 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 157.15, 152.96, 148.81, 144.84, 132.25, 132.07, 126.34, 124.49, 115.00, 101.68, 91.11, 82.27, 79.37, 76.39, 59.53. Anal. Calcd for $C_{18}H_{16}BrN_5O_4$: C, 48.45; H, 3.61; N, 15.69. Found: C, 48.51; H, 3.66; N, 17.63.

3.2.7. 4-Amino-6-(3-bromophenyl)-5-cyano-7-(β-L-xylofuranosyl)pyrrolo[2,3-*d*]pyrimidine (3f)

The reaction was conducted according to the general procedure A and the compound **3f** was obtained as a white solid. Mp 156–157 °C. IR (KBr): 3435(OH), 3331 (NH_2) 2913, 2218 (CN), 1633, 1599. MS (ESI): m/z 445.9 [M+H$^+$]. ^1H NMR (300 MHz, DMSO-d_6): 8.30 (s, 1H), 7.85–7.88 (m, 2H), 7.62–7.66 (m, 2H), 7.02 (s, 2H, NH_2), 6.55 (d, 1H, J = 1.8 Hz), 5.84 (d, 1H, J = 4.5 Hz), 5.37 (d, 1H, J = 3.6 Hz), 4.74 (s, 2H), 3.94–3.96 (m, 2H), 3.58–3.73 (m, 2H). Anal. Calcd for $C_{18}H_{16}BrN_5O_4$: C, 48.45; H, 3.61; N, 15.69. Found: C, 48.49; H, 3.66; N, 17.59.

3.2.8. 4-Amino-6-(1*H*-pyrrol-2-yl)-5-cyano-7-(β-L-xylofuranosyl)pyrrolo[2,3-*d*]pyrimidine (3g)

The reaction was conducted according to the general procedure A and the compound **3g** was obtained as a white solid. Mp 146–147 °C. IR (KBr): 3425 (OH), 3324 (NH_2), 2923, 2216 (CN), 1635, 1599. MS (ESI): m/z 356.8 [M+H$^+$]. ^1H NMR (300 MHz, DMSO-d_6): 11.71 (s, 1H), 8.25 (s, 1H), 7.18 (d, 1H), 7.08 (s, 2H, NH_2), 6.71–6.73 (m, 1H), 6.55 (s, 1H), 6.35 (d, 1H, J = 3.0 Hz), 5.89 (d, 1H, J = 4.5 Hz), 5.73 (d, 1H, J = 3.3 Hz), 4.73 (s, 2H), 3.96 (d, 2H, J = 3.6 Hz), 3.60–3.70 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 156.80, 152.53, 148.52, 139.33, 122.77, 116.91, 115.56, 113.16, 109.81, 101.75, 90.93, 82.25, 79.67, 76.65, 59.49. Anal. Calcd for $C_{16}H_{16}N_6O_4$: C, 53.95; H, 4.53; N, 23.85. Found: C, 53.87; H, 4.50; N, 23.92.

3.2.9. 4-Amino-6-(thiophen-2-yl)-5-cyano-7-(β-L-xylofuranosyl)pyrrolo[2,3-*d*]pyrimidine (3h)

The reaction was conducted according to the general procedure A and the compound **3h** was obtained as a white solid. Mp 180 °C.

IR (KBr): 3434 (OH), 3332 (NH_2), 2926, 2855, 2219 (CN), 1646, 1600. MS (ESI): *m/z* 373.9 [M+H$^+$]. ^{1}H NMR (300 MHz, DMSO-d_6): 8.29 (s, 1H), 8.02–8.04 (m, 1H), 7.52–7.53 (m, 1H), 7.37–7.40 (m, 1H), 7.21 (s, 2H, NH_2), 6.63 (d, 1H, *J* = 9.3 Hz), 5.86 (d, 1H, *J* = 4.5 Hz), 5.61 (d, 1H, *J* = 3.3 Hz), 4.78–4.82 (m, 2H), 3.94–3.99 (m, 2H), 3.59–3.72 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 157.08, 153.12, 148.85, 138.79, 132.07, 131.34, 128.48, 126.38, 114.99, 101.75, 90.98, 82.37, 79.34, 76.49, 59.53. Anal. Calcd for $C_{16}H_{15}N_5O_4S$: C, 51.47; H, 4.05; N, 18.76; S, 8.59. Found: C, 51.41; H, 4.06; N, 18.63; S, 8.65.

3.2.10. 4-Amino-6-(furan-2-yl)-5-cyano-7-(β-L-xylofuranosy-l) pyrrolo[2,3-*d*]pyrimidine (3i)

The reaction was conducted according to the general procedure A and the compound **3i** was obtained as a white solid. Mp 165–167 °C. IR (KBr): 3445 (OH), 3339 (NH_2), 2937, 2872, 2223 (CN), 1640, 1612. MS (ESI): *m/z* 357.9 [M+H$^+$]. ^{1}H NMR (300 MHz, DMSO-d_6): 8.28 (s, 1H), 8.12 (d, 1H), 7.22 (s, 1H, NH_2), 7.14 (d, 1H), 6.85–6.86 (m, 1H), 6.64 (d, 1H, *J* = 9.3 Hz), 5.87 (d, 1H, *J* = 4.5 Hz), 5.77 (d, 1H, *J* = 3.3 Hz), 4.75–4.81 (m, 2H), 3.99–4.02 (m, 2H), 3.62–3.75 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 157.27, 153.24, 148.97, 146.42, 140.90, 134.84, 115.06, 112.57, 102.04, 91.70, 82.48, 79.69, 76.56, 59.55. Anal. Calcd for $C_{16}H_{15}N_5O_5$: C, 53.78; H, 4.23; N, 19.60. Found: C, 53.85; H, 4.26; N, 19.53.

3.2.11. 4-Amino-6-(5-bromothiophen-2-yl)-5-cyano-7-(β-L-xylofuranosyl)pyrrolo[2,3-*d*]py rimidine (3j)

A solution of **3g** (45.1 mg, 0.10 mmol) in acetonitrile (20 mL) was added NBS (42.8 mg, 0.20 mmol) in small portions under N_2 at 0 °C. The resulting mixture was then warmed to room temperature and stirred for 16 h. The solvent was removed under reduced pressure. The residue was chromatographed using a solvent system of methanol and chloroform (1:9, v/v) and the product **3i** was obtained as a white solid. Mp 185–186 °C. IR (KBr): 3435 (OH), 3331 (NH_2), 2960, 2221 (CN), 1638, 1595. MS (ESI): *m/z* 453.7 [M+H$^+$]. ^{1}H NMR (300 MHz, DMSO-d_6): 8.29 (s, 1H), 7.53 (d, 1H, *J* = 3.9 Hz), 7.37 (d, 1H, *J* = 3.6 Hz), 7.24 (s, 1H, NH_2), 6.51 (d, 1H, *J* = 9.9 Hz), 5.83 (d, 1H, *J* = 4.8 Hz), 5.58 (d, 1H, *J* = 3.9 Hz), 4.77–4.81 (m, 2H), 3.99 (d, 2H, *J* = 6.3 Hz), 3.61–3.71 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6):157.16, 153.38, 149.02, 137.27, 133.14, 131.80, 128.11, 116.45, 114.70, 101.74, 90.87, 82.34, 79.21, 76.36, 59.58. Anal. Calcd for $C_{16}H_{14}BrN_5O_4S$: C, 42.49; H, 3.12; N, 15.48; S, 7.09. Found: C, 42.45; H, 3.16; N, 15.53; S, 7.12.

3.3. General procedure B for the synthesis of compound 4

A solution of **3** (0.2 mmol) in concentrated ammonium hydroxide (5 ml) was added 30% H_2O_2 (0.5 mL) and methanol (0.3 mL), the mixture was stirred at room temperature until completion of the reaction as shown by TLC. The solvent was removed under reduced pressure. The residue was chromatographed using a solvent system of methanol and chloroform (1:8, v/v) and the product **4** were obtained as white solid.

3.3.1. 4-Amino-6-(*p-tolyl)-7-(β-L-xylofuranosyl)pyrrolo[2,3-d*] pyrimidine-5-carboxamide (4a)

The reaction was conducted according to the general procedure B and the compound **4a** was obtained as a white solid. Mp 238–240 °C. IR (KBr): 3463(OH), 3325(NH_2), 3168, 2919, 2846, 1634, 1598. MS (ESI): *m/z* 399.9 [M+H$^+$]. ^{1}H NMR (300 MHz, DMSO-d_6): 8.17 (s, 1H), 7.53 (s, 2H, NH_2),7.37–7.44 (m, 4H), 7.08 (d, 1H, *J* = 10.2 Hz), 5.80(s,1H, NH_2), 5.71 (d, 1H, *J* = 3.8 Hz), 5.06 (d, 1H, *J* = 3.0 Hz), 4.77–4.81 (m, 1H), 4.63–465 (m, 1H), 3.76–3.89 (m, 2H), 3.52–3.66 (m, 2H), 2.42(s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): 166.73, 158.29, 151.88, 147.97, 140.07, 138.51, 131.22, 131.17, 131.05, 129.81, 125.83, 105.18, 101.59, 91.02, 82.17, 80.04, 76.86, 59.48, 21.02. Anal. Calcd for $C_{19}H_{21}N_5O_5$: C, 57.14; H, 5.30; N, 17.53. Found: C, 57.21; H, 5.28; N, 17.48.

3.3.2. 4-Amino-6-(4-methoxy)-7-(β-L-xylofuranosyl) pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (4b)

The reaction was conducted according to the general procedure B and the compound **4b** was obtained as a white solid. Mp 234–235 °C. IR (KBr): 3473 (OH), 3327 (NH_2), 3169, 2892, 2839, 1626, 1596. MS (ESI): *m/z* 415.9 [M+H$^+$]. ^{1}H NMR (300 MHz, DMSO-d_6): 8.14 (s, 1H), 7.52 (s, 2H, NH_2), 7.41–7.52 (m, 2H), 7.15–7.19 (m, 2H), 7.07 (d, 1H, *J* = 10.8 Hz), 5.80 (s, 1H, NH_2), 5.70 (d, 1H, *J* = 4.5 Hz), 5.08 (d, 1H, *J* = 3.0), 4.76–4.82 (m, 1H), 4.62–4.65 (m, 1H), 3.86 (s, 3H), 3.70–3.82 (m, 2H), 3.50–3.67 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 166.76, 160.55, 158.24, 151.81, 147.89, 138.42, 132.60, 120.46, 114.70, 109.15, 90.94, 82.13, 80.00, 76.85, 59.47, 55.39. Anal. Calcd for $C_{19}H_{21}N_5O_6$: C, 54.94; H, 5.10; N, 16.86. Found: C, 54.91; H, 5.03; N, 16.95.

3.3.3. 4-Amino-6-(3-methoxy)-7-(β-L-xylofuranosyl) pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (4c)

The reaction was conducted according to the general procedure B and the compound **4c** was obtained as a white solid. Mp 230–232 °C. IR (KBr): 3468 (OH), 3326 (NH_2), 3168, 2902, 2845, 1628, 1594. MS (ESI): *m/z* 415.9 [M+H$^+$]. ^{1}H NMR (300 MHz, DMSO-d_6): 8.15 (s, 1H), 7.50–7.55 (m, 3H), 7.16–7.20 (m, 1H), 7.02–7.08 (m, 3H), 5.92 (s, 1H, NH_2), 5.73 (d, 1H, *J* = 4.5 Hz), 5.10 (d, 1H, *J* = 2.7 Hz), 4.79–4.82 (m, 1H), 4.65(s, 1H), 3.8–3.89 (m, 5H), 3.53–3.70 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 166.68, 158.32, 151.96, 147.95, 137.98, 130.40, 123.24, 116.75, 115.84, 109.25, 101.53, 91.01, 82.17, 80.08, 76.82, 59.48, 55.32. Anal. Calcd for $C_{19}H_{21}N_5O_6$: C, 54.94; H, 5.10; N, 16.86. Found: C, 54.89; H, 5.04; N, 16.93.

3.3.4. 4-Amino-6-(4-(tert-butyl)phenyl)-7-(β-L-xylofuranos-yl) pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (4d)

The reaction was conducted according to the general procedure B and the compound **4d** was obtained as a white solid. Mp 254–256 °C. IR (KBr): 3456 (OH), 3332 (NH_2), 3171, 2894, 1629, 1600. MS (ESI): *m/z* 441.9 [M+H$^+$]. ^{1}H NMR (300 MHz, DMSO-d_6): 8.15 (s, 1H), 7.64 (d, 2H, *J* = 8.1 Hz), 7.51 (s, 1H, NH_2), 7.44 (d, 2H, *J* = 8.1 Hz), 7.11 (d, 1H, *J* = 10.2 Hz), 5.87 (s, 1H, NH_2), 5.76 (d, 1H, *J* = 4.5 Hz), 5.10 (d, 1H, 2.4 Hz), 4.77–4.80 (m, 1H), 4.66 (s, 1H), 3.79–3.88 (m, 2H), 3.52–3.70 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 166.77, 158.24, 152.76, 151.83, 147.91, 138.30, 130.91, 125.94, 125.82, 109.36, 101.59, 90.99, 82.21, 80.11, 76.93, 59.51, 34.71, 31.02. Anal. Calcd for $C_{22}H_{27}N_5O_5$: C, 59.85; H, 6.16; N, 15.86. Found: C, 59.90; H, 6.13; N, 15.74.

3.3.5. 4-Amino-6-(4-bromophenyl)-7-(β-L-xylofuranosyl) pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (4e)

The reaction was conducted according to the general procedure B and the compound **4e** was obtained as a white solid. Mp 241–243 °C. IR (KBr): 3461 (OH), 3327 (NH_2), 3171, 2925, 2851, 1629, 1602. ^{1}H NMR (300 MHz, DMSO-d_6): 8.16 (s, 1H), 7.81 (d, 2H), 7.52 (s, 1H, NH_2), 7.44 (d, 2H), 6.99 (d, 1H, *J* = 9.9 Hz), 6.22 (s, 1H, NH_2), 5.73 (d, 1H, *J* = 4.8 Hz), 5.08 (d, 1H, *J* = 3.0 Hz), 4.78–4.82 (m, 1H), 4.65 (s, 1H), 3.80–3.90 (m, 2H), 3.51–3.72 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 166.71, 158.18, 151.95, 148.03, 136.66, 133.29, 131.88, 127.91, 123.77, 109.90, 101.52, 90.91, 82.08, 79.86, 76.68, 59.44. Anal. Calcd for $C_{18}H_{18}BrN_5O_5$: C, 46.57; H, 3.91; N, 15.08. Found: C, 46.51; H, 3.89; N, 15.13.

3.3.6. 4-Amino-6-(3-bromophenyl)-7-(β-L-xylofuranosyl) pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (4f)

The reaction was conducted according to the general procedure B and the compound **4f** was obtained as a white solid. Mp 220 °C. IR

(KBr): 3458 (OH), 3328 (NH_2), 2942, 2864, 1630, 1612. ^{1}H NMR (300 MHz, DMSO-d_6): 8.17 (s, 1H), 7.78–7.81 (m, 1H), 7.71 (s, 2H, NH_2), 7.51–7.58 (m, 3H), 6.98 (d, 1H, J = 9.3 Hz), 6.32 (s, 1H, NH_2), 5.76 (d, 1H, J = 4.8 Hz), 5.09 (d, 1H, J = 3.0 Hz), 4.80–4.81 (m, 1H), 4.64–4.66 (m, 1H), 3.82–3.89 (m, 2H), 3.56–3.71 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 166.79, 158.28, 152.10, 148.06, 136.12, 132.90, 131.02, 130.89, 130.49, 121.88, 110.27, 101.53, 91.01, 82.21, 80.01, 76.75, 59.48. Anal. Calcd for $C_{18}H_{18}BrN_5O_5$: C, 46.57; H, 3.91; N, 15.08. Found: C, 46.54; H, 3.90; N, 15.15.

3.3.7. 4-Amino-6-(1*H*-pyrrol-2-yl)-7-(β-L-xylofuranosyl)pyrrolo [2,3-*d*]pyrimidine-5-carboxamide (4g)

The reaction was conducted according to the general procedure B and the compound **4g** was obtained as a white solid. Mp 225–226 °C. IR (KBr): 3439 (OH), 3345(NH_2), 2922, 1636, 1445. ^{1}H NMR (300 MHz, DMSO-d_6): 11.56 (s, 1H), 8.13 (s, 1H), 7.59 (s, 2H, NH_2), 7.10–7.14 (m, 2H), 6.43–6.45 (m, 1H), 6.31–6.35 (m, 1H), 5.68 (d, 1H, J = 4.5 Hz), 5.57 (s, 1H), 5.23 (d, 1H, J = 2.7 Hz), 4.72–4.75 (m, 1H), 4.58–4.60 (m, 1H), 3.80–3.90 (m, 2H), 3.66–3.71 (m, 1H), 3.51–3.57 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): 166.41, 158.32, 152.44, 148.30, 133.09, 131.51, 130.08, 128.29, 127.89, 111.99, 101.65, 90.99, 82.28, 80.06, 76.90, 59.59. Anal. Calcd for $C_{16}H_{18}N_6O_5$: C, 51.33; H, 4.85; N, 22.45. Found: C, 51.37; H, 4.87; N, 22.32.

3.3.8. 4-Amino-6-(thiophen-2-yl)-7-(β-L-xylofuranosyl)pyrrolo [2,3-*d*] pyrimidine-5-carboxamide (4h)

The reaction was conducted according to the general procedure B and the compound **4h** was obtained as a white solid. Mp 228–230 °C. IR (KBr): 3455 (OH), 3324(NH_2), 2879, 1638, 1600. ^{1}H NMR (300 MHz, DMSO-d_6): 8.16 (s, 1H), 7.98 (d, 1H), 7.69 (s, 1H, NH_2), 7.42–7.44 (m, 1H), 7.31–7.34 (m, 1H), 6.98 (d, 1H, J = 9.9 Hz), 6.15 (s, 1H, NH_2), 5.72 (d, 1H, J = 4.8 Hz), 5.18(d, 1H, J = 3.0 Hz), 4.75–4.79 (m, 1H), 4.63–4.66 (m, 1H), 3.82–3.89 (m, 2H), 3.55–3.72 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 166.27, 158.37, 152.13, 147.97, 131.68, 121.89, 116.48, 113.11, 110.52, 109.35, 101.63, 91.00, 82.16, 80.29, 76.91, 59.46. Anal. Calcd for $C_{16}H_{17}N_5O_5S$: C, 49.10; H, 4.38; N, 17.89; S, 8.19. Found: C, 49.11; H, 4.36; N, 17.83; S, 8.25.

3.3.9. 4-Amino-6-(furan-2-yl)-7-(β-L-xylofuranosyl)pyrrolo-[2,3-*d*]pyrimidine-5-carboxamide (4i)

The reaction was conducted according to the general procedure B and the compound **4i** was obtained as a white solid. Mp 208–209 °C. IR (KBr): 3461 (OH), 3332(NH_2), 2929, 2832, 1634, 1596. ^{1}H NMR (300 MHz, DMSO-d_6): 8.18 (s, 1H), 8.02–8.03 (m, 1H), 7.71 (s, 1H, NH_2), 6.99–7.02 (m, 1H), 6.95 (d, 1H, J = 3.6 Hz), 6.76–6.78 (m, 1H), 6.56 (s, 1H, NH_2), 5.78 (d, 1H, J = 4.5 Hz), 5.25 (d, 1H, J = 3.0 Hz), 4.74–4.77 (m, 1H), 4.61–4.64 (m, 1H), 3.88 (s, 2H), 3.53–3.72 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6):166.32, 158.40, 152.54, 148.15, 145.85, 140.62, 126.77, 115.83, 112.29, 111.78, 101.58, 91.36, 82.26, 80.16, 76.81, 59.46. Anal. Calcd for $C_{16}H_{17}N_5O_6$: C, 51.20; H, 4.57; N, 18.66. Found: C, 51.25; H, 4.53; N, 18.63.

3.3.10. 4-Amino-6-(5-bromothiophen-2-yl)-7-(β-L-xylofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (4j)

The reaction was conducted according to the general procedure B and the compound **4j** was obtained as a white solid. Mp 232–234 °C. IR (KBr): 3455 (OH), 3338 (NH_2), 2925, 1633, 1598. ^{1}H NMR (300 MHz, DMSO-d_6): 8.18 (s, 1H), 7.73 (s, 2H, NH_2), 7.46 (d, 1H, J = 3.9 Hz), 7.26 (d, 1H, J = 3.9 Hz), 6.91 (d, 1H, J = 9.6 Hz), 6.58 (s, 1H, NH_2), 5.76 (d, 1H, J = 4.5 Hz), 5.24 (d, 1H, J = 3.0 Hz), 4.80–4.83 (m, 1H), 4.64–4.66 (m, 1H), 3.85–3.93 (m, 2H), 3.31–3.71 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): 166.30, 158.24, 152.61, 148.24, 133.99, 131.42, 129.73, 128.23, 115.78, 112.70, 101.47, 90.81, 82.23, 79.89, 76.71, 59.51. Anal. Calcd for $C_{16}H_{16}BrN_5O_5S$: C, 40.86; H, 3.43; N, 14.89; S, 6.82. Found: C, 40.89; H, 3.41; N, 14.83; S, 6.92.

3.4. Biology

3.4.1. Materials and reagents

Histone H1 were purchased from Calbiochem, dissolved in water, and stored at −80 °C as 1 μg/μl stock. Hela cells were maintained at 37 °C in 5% CO_2 in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% newborn calf serum (Inbitrogen) and aitibiotics/antimycotics (Invitrogen).

3.4.2. In vitro kinase assay

The kinase assays were performed at 30 °C for 15 min in reaction buffer with a final volume of 50 μl that contains Histone H1 as a specific substrate for each kinase. In order to get CDK1/Cyclin B1 and CDK2/Cyclin A2 kianses, after cell seeding for 24 h, HeLa cells were treated by Paclitaxel with 80 nM and Etoptoside with 50 μg/ml in DMEM with 5% newborn calf serum for 12 h, 200 μg of protein extract of HeLa cells were immunoprecipitated for 6 h at 4 °C with an antibody specific for CDK1 and CDK2 respectively. Then the protein was washed for several times as kinase for experiment.

3.4.3. Cell cycle assay

In brief, after treatment with indicated concentrations of compound **3h**, 3i, and **3j (6.25 μM, 12.5 μM)** for 72 h, HeLa cells were pelleted and washed with PBS. Then cells were fixed in 75% ethanol at 4 °C overnight. After being washed twice with PBS, the cells were stained with 1.0 mL DAPI, 1 mg/mL RNase A (Sigma), and Triton X-100 in 3.8 mmol/L sodium citrate, and incubated on ice for 30 min in the dark. Analysis was carried out using a Becton Dickinson FACSCalibur cytometer, and cells were quantitated by measurement the area of G1, S and G2 peak.

3.4.4. Molecular docking studies of CDK2 inhibitors

Autodock vina was chosen as docking program.[44] The 3D structure of the test compound was constructed by InsightII/Builder and the geometry of the inhibitor was further optimized using AM1 method. After determining the binding site based on the crystal structure of CDK2/Cyclin A (PDB code: 1W0X), the inhibitor (Xylocydine, **3h**, 3i, and **3j**) was docked into the protein CDK2/Cyclin A. Finally, the docked complex was selected by the criterion of interacting energy combined with the geometrical matching.

4. Conclusion

A novel and simple route to syntheses 6-substituted purine nucleoside analogues with tetracyanoethylene and L(−)-xylose as starting molecules has been developed. Three synthesized compounds (**3h–j**), which have specific CDK2 inhibitory activity, were identified by in vitro CDK1/Cyclin B1 and CDK2/Cyclin A2 kinase assay. Those three new compounds induce cell cycle arrest obviously at G1/S phase in HeLa cells. Molecular docking study provides useful information for understanding the structural features of CDK2 inhibitors and appears coherent with the obtained biological data.

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Supplementary data

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