Monatshefte für Chemie - Chemical Monthly (2021) 152:109–120
https://doi.org/10.1007/s00706-020-02727-x

ORIGINAL PAPER

Synthesis and biological evaluation of seliciclib derivatives as potent and selective CDK9 inhibitors for prostate cancer therapy

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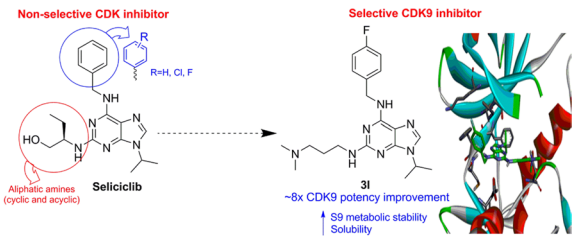
Received: 7 May 2020 / Accepted: 30 December 2020 / Published online: 13 January 2021

Abstract

Seliciclib is a cyclin-dependent kinase (CDK) inhibitor that has been assayed in phase II clinical trials as an anticancer agent. This paper describes the synthesis of novel derivatives of seliciclib with improved potency, metabolic stability, aqueous solubility, and anti-proliferative activity. The new derivatives showed a novel CDKs selectivity profile. Replacement of ethyl alcohol at position 2 of purine with dimethylaminopropyl and fluorination of benzyl at position 6 of purine of seliciclib resulted in the formation of a derivative that potently and selectively inhibited CDK9 (26 nM vs. CDK9 and > 60-fold selectivity vs. CDK2/5/7). In comparison to seliciclib, this derivative shows lower metabolic clearance (25% lower in Cl_{int}), higher aqueous solubility and is more cytotoxic in androgen-independent prostate cancer cells.

Graphic abstract



Keywords Cyclin-dependent kinase · Purine · Metabolic stability · Roscovitine · CYC202

Introduction

Cyclin-dependent kinase (CDK) enzymes form heterodimers with a specific family of proteins called cyclins. These CDK–cyclin complexes regulate the cell division cycle and modulate gene transcription [1]. In cancer cells, the activity of CDK–cyclins is deregulated by various mechanisms including hyperactivity of CDKs, overexpression of cyclins and loss or inactivation of inhibitory proteins, e.g. p16 [2, 3]. CDK9 is a member of the CDK family which dimerises with cyclin T to form positive transcription elongation factor b (p-TEFb), an elongation factor during RNA transcription [4, 5]. CDK9 conducts its function by phosphorylating the C-terminal domain (CTD) subunit of RNA polymerase II [6].

There is trong evidence that indicates that CDK9 is a promising therapeutic opportunity in cancer through the regulation of anti-apoptotic proteins that initiate cancer cell immortality. Its pathogenic role has been established in a number of malignancies including prostate cancer [6–14]. Prostate cancer is the most common cancer and the second cause of death among men in the world [15, 16]. The current treatment options are surgery, chemotherapy, radiotherapy, and hormone deprivation with highly successful results. However, 20% of prostate cancer patients develop castration-resistance prostate cancer (CRPC), which is unresponsive to

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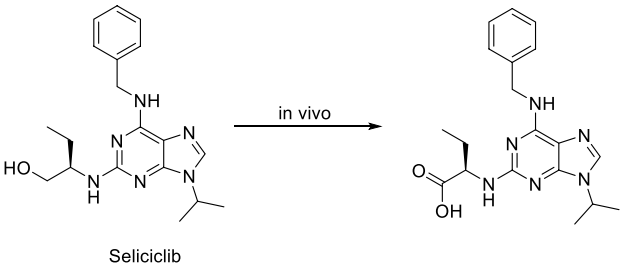
conventional therapy and associated with poor prognosis. Recently, CDK9 has been identified as a key component in CRPC through modulating the activity of androgen receptor [10, 17–19]. This highlights the role of CDK9 in CRPC.

Since the identification of CDK9 as a valid therapeutic target in cancer, a large number of small molecules have been developed as CDK9 inhibitors. These inhibitors show good anti-tumour activity and drug-likeness properties, several of which have progressed into clinical trials as anticancer agents in various types of cancer [12, 14, 20–27]. The first generation evaluated in clinical trials is pan-CDK inhibitors including flavopiridol, seliciclib, dinaciclib, SNS-032, and RGB-286638, which can inhibit CDK9 as well as other CDK isoforms and other kinases, as shown in Table 1. Flavopiridol is the most studied CDK9 inhibitor and although it is not a selective CDK9 inhibitor, its main mechanism of action is believed to be through inhibition of CDK9 [28, 29]. Their clinical results show many adverse effects and events [6, 23, 24, 30], which could be due to the lack of selectivity. In 2017, researchers at Bayer identified the first highly selective CDK9 inhibitor, atuveciclib, which is currently under evaluation in phase I clinical trials for the treatment of acute leukemia [31].

Seliciclib (also known as CYC202 or roscovitine) is a CDK inhibitor, mainly CDK2/E inhibitor with lower potency versus CDK9/T, CDK7/H, CDK5/p53, and CDK4/D [32, 35]. It demonstrates a good selectivity profile against a panel of other kinases [39]. Its evaluation in phase II clinical trials did not show any adverse effects associated with its use, but also did not show complete response to the patient's treatment [22, 40, 41]. Its anti-tumour effect is associated with the inhibition of RNA polymerase II and Rb protein phosphorylation, and reduction of the expression of cyclin D1 protein [22, 30, 42]. Seliciclib has sub-optimum water solubility but well-absorbed post-oral administration. It shows high metabolic clearance, which was reported as the major issue associated with its use in clinical studies [22, 34, 43, 44].

Metabolic identification studies of seliciclib have shown that 86% of the parent drug is readily metabolised by oxidation and 60% of this metabolism is contributed to the formation of the carboxylate metabolite (Scheme 1) [45, 46]. The carboxylate metabolite is seven times less active than the parent drug as a CDK2 inhibitor and, therefore, it is unlikely to contribute to the observed anti-proliferative efficacy of seliciclib [47].

Scheme 1



Seliciclib

The objective of the present study is to develop derivatives of seliciclib with improved potency and selectivity toward CDK9, as well as enhanced metabolic stability and aqueous solubility. To achieve this, we designated and synthesized derivatives with terminal amines to replace the metabolically labile 2-hydroxyl of seliciclib. The majority of the synthesized compounds contain ionisable amines at physiological pHs and, therefore, can be formulated as salts.

Results and discussion

Chemistry

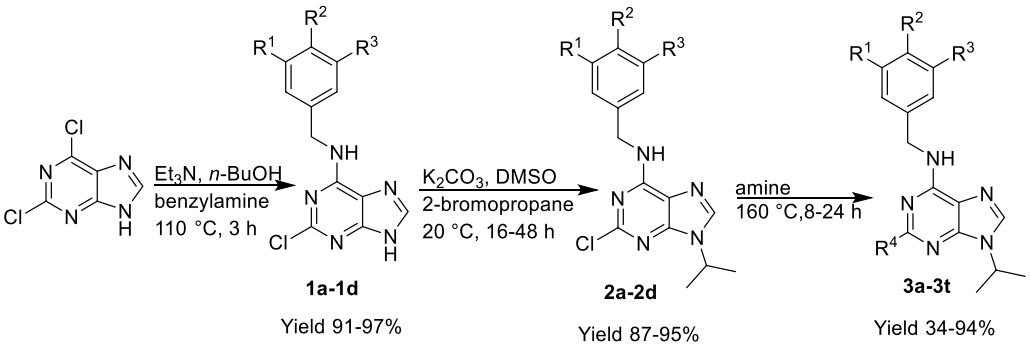
In this work, seliciclib and its derivatives were synthesized using the straightforward three-step procedure starting from commercially available 2,6-dichloropurine. First, replacement of the reactive chlorine at C6 of purine with benzylamines. Second, alkylation of N9 of purine using 2-bromopropane. Third, substitution of the less reactive 2-chloropurine with various amines (Scheme 2).

In the first step, 2,6-dichloropurine was treated with the desired substituted benzylamines to replace the chlorine at position C6 of purine. This is achieved by heating the reaction mixture to 110 °C for 3 h using Et_3N and *n*-butanol as a solvent. The crude intermediates were recrystallized with butanol and hexane to obtain 2-chloro-6-(substituted benzylamino)purines **1a**–**1d** in excellent yield (91–97%).

Table 1 Selectivity profile of CDKs inhibitors in clinical trials (IC_{50}/nM)

Inhibitor	CDK9/T	CDK2/E	CDK5/p53	CDK7/H	CDK4/D	References
Flavopiridol	11	282	–	514	132	[32–34]
Seliciclib	950	100	160	540	13,500	[32, 35]
Dinaciclib	4	1	1	–	–	[32, 36]
SNS-032	4	48	340	62	925	[32, 37]
RGB-286638	1	3	5	44	4	[34, 38]
Atuveciclib	6	1000	1600	> 10,000	–	[31]

Scheme 2



In the second step, the 2-chloro-6-(substituted benzylamino)purines **1a**–**1d** were alkylated at position N9 of purine using 2-bromopropane and K_2CO_3 as a base in DMSO at 20 °C for 16–48 h. The crude products were purified by recrystallization with isopropanol to yield 2-chloro-6-(substituted benzylamino)-9-isopropylpurines **2a**–**2d** in good-to-excellent yield (87–95%).

The first two steps of the synthesis were switched (i.e. alkylation of the 2,6-dicholorpurine) in an attempt to keep the derivatization steps as the last two steps and minimize the number of reactions required. This resulted in the formation of undesired N7 regioisomer in considerable amounts (~ 40%), which requires column chromatography to separate it from the desired N9 isomer. Therefore, it was decided to replace the 6-chloro with benzylamine first, then alkylate the N9.

In the final step, the 2-chloro-6-(substituted benzylamino)-9-isopropylpurines **2a**–**2d** were treated with appropriate amines. The reaction temperature was 160 °C for 8–24 h depending on the reactivity of the amines. The final products were purified by recrystallization using EtOAc:hexane (1:1). A further purification by column chromatography was required in some cases to obtain 2-amino-6-(substituted benzylamino)-9-isopropylpurines **3a**–**3t**. The yield was varied from 34 to 94% depending on nucleophilicity and size of the amine used.

Structure–activity relationship in biological assays

The main objective of this work was to develop derivatives of seliciclib with improved potency and selectivity toward CDK9 isoform with potential utility in prostate cancer therapy. In terms of chemical structure, the newly synthesized compounds have two different substituents in comparison to the standard compound, seliciclib: ionisable amino group which replaces the 2-hydroxyl of seliciclib and the benzylamines at position 6 substituted with halogens in various positions. Previous studies of the purine scaffold showed that halogen substituents on the benzyl ring are associated with high cytotoxic activity [48, 49]. Isopropyl has not been changed in the new compounds as it showed the maximum CDK activity in previous studies. Havlicek et al. showed that N9-substitution by a hydrophobic residue is probably important for a positive binding, and isopropyl appears to be the most active group at position 9. Removal or change of this side chain dramatically decreased the inhibitory activity of the seliciclib and its derivatives [46, 48–53].

All new compounds were tested against CDK9/2. In addition, six of these compounds were selected to examine their activity against CDK5/7. Also, the cytotoxic effect of all compounds was assessed in PC3 cell line. The biological results are illustrated in Tables 2 and 3.

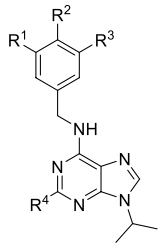
The biological data indicate that the majority of the new compounds showed better separation between CDK9 and CDK2 activity in comparison to seliciclib, which is essentially equipotent against the two isoforms. Several examples show great selectivity (10–70-fold) for CDK9 over CDK2, e.g. **3f**, **3 g**, **3j**, **3l**, and **3o**, whereas other compounds exhibited improved CDK2 selectivity over CDK9 but not exceeding tenfold, e.g. **3d**, **3h,** and **3k**.

Some of these compounds were assessed for their CDK5/7 inhibitory activities as shown in Table 3. With the exception of **3f** and **3i** activity against CDK7, all tested compounds exhibit better selectivity for CDK5/7 than seliciclib. In the PC3 cell line, most of the newly prepared compounds show enhanced anti-proliferative activity in comparison to seliciclib (Table 4).

The impact of insertion of halogens to benzylamines was examined. The results showed that 3,5-difluorobenzylamine has unfavourable CDK9 activity and PC3 cytotoxicity in comparison to 4-fluorobenzylamine, e.g. **3l** vs. **3q**, **3m** vs. **3r**, **3h** vs. **3s**, and **3o** vs. **3t**. The molecular docking study revealed that the binding mode of the new compounds with CDK9 shows a lysine residue (Lys35) close to *m*-fluorobenzylamine at position 6 of purine, which could account for the lower activity of *meta*-fluorinated compounds (Fig. 1).

The impact of the replacement of a neutral hydroxyl at position 2 of purine ring of seliciclib with positively charged amines was investigated. Various amines with different steric hindrances and nucleophilicity were examined

Table 2 CDK9/2 biochemical assay results of seliciclib and its derivatives (data are means (± SD) of three individual experiments)



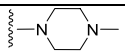
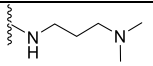
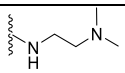
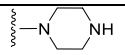
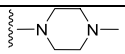
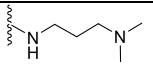
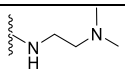
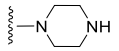
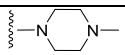
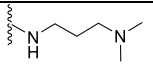
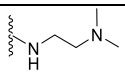
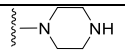
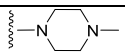
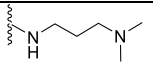
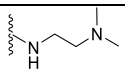
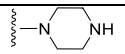
Comp.	Structure				IC_{50} /nM		
	R^1	R^2	R^3	R^4	CDK9	CDK2	CDK9 :2 ratio
Seliciclib	H	H	H		199.7(±12.9)	200.8(±7.9)	1:1
3a	H	H	H		239.1(±14.0)	120(±6.9)	2:1
3b	H	H	H		288.9(±12.8)	874.8(32.3)	1:3
3c	H	H	H		2785.7(±96.9)	1066.8(±47.5)	2.6:1
3d	H	H	H		608.38(±27.8)	93.5(±5)	6.4:1
3e	H	H	H		498.2(±20.6)	2370.8 (±84.5)	1:4.7
3f	H	Cl	H		150.7(±7.8)	3673.7(±80.2)	1:24
3g	H	Cl	H		388.5(±15.5)	6174(±180.1)	1:16
3h	H	Cl	H		508.8(±19.5)	330.6(±13.1)	1.5:1
3i	H	Cl	H		304.7(±11.2)	238.5(±8.8)	1.3:1
3j	H	Cl	H		112.4(±6.1)	1961.6(±67.9)	1:17
3k	H	F	H		2288(±81.8)	267.5(±16)	8.4:1
3l	H	F	H		26(±1.7)	1852.6(±56.3)	1:71
3m	H	F	H		835(±34)	2119.2(±64.8)	1:2.5
3n	H	F	H		392.7(±15.8)	70.5(±4.6)	5.5:1
3o	H	F	H		131.1(±5.8)	17774.8(±61)	1:13
3p	F	H	F		288.7(±17.2)	350.7(±12)	1:1.2
3q	F	H	F		444.4(±21.8)	242.6(±8.4)	1.8:1
3r	F	H	F		1863.2(±69.5)	565.7(±34.1)	3.3:1
3s	F	H	F		3578.6(±157.5)	778.8(±24.3)	4.5:1
3t	F	H	F		443.2(±16.5)	1294.1(±44.2)	1:2.9

Table 3 CDK activity of selected compounds expressed as IC_{50}/nM (data are means (± SD) of three individual experiments)

Comp	CDK9	CDK2	CDK5	CDK7
3d	608.38 (±27.8)	93.5 (±5)	7093.4 (±283.7)	5986.8 (±226.9)
3f	150.7 (±7.8)	3673.7 (±80.2)	1725.1 (±50)	300 (±25)
3i	304.7 (±11.2)	238.5 (±8.8)	2843.2 (±103.4)	852.9 (±25.8)
3j	112.4 (±6.1)	1961.6 (±67.9)	932.8 (±51.8)	1813.9 (±77.7)
3l	26 (±1.7)	1852.6 (±56.3)	2723.8 (±103.7)	1452.6 (±51.8)
3n	392.7 (±15.8)	70.5 (±4.6)	2213.6 (±26.9)	7774.7 (±323.9)
Seliciclib	199.7 (±12.9)	200.8 (±7.9)	507.8 (±28.2)	1608 (±56.4)

Table 4 PC3 cytotoxicity results of seliciclib and its derivatives (data are means (± SD) of three individual experiments) and predicted topological polar surface area (tPSA), log *P* (calculated using ChemBioDraw 15.0) and log *D* at pH 7.4 (calculated using ChemAxon)

Compounds	PC3 cytotoxicity IC_{50}/μM	tPSA/Å^2	log *P*	Log *D*@ pH = 7.4
Seliciclib (literature [54])	30	84.6	2.75	2.3
Seliciclib (in house)	39.7 (±1.3)			
3a	22.6 (±0.7)	58.8	3.01	2.6
3b	41.4 (±1.3)	67.6	2.57	0.35
3c	14.1 (±0.4)	67.6	2.46	1.0
3d	20.6 (±0.6)	64.8	2.85	2.8
3e	33.4 (±1)	67.6	2.63	1.1
3f	40.8 (±1.3)	58.8	3.57	3.2
3g	50.2 (±1.6)	67.6	3.13	0.45
3h	67.8 (±2.2)	67.6	3.02	1.6
3i	8 (±0.2)	64.8	3.41	3.5
3j	10.7 (±0.3)	67.6	3.19	1.6
3k	8.4 (±0.2)	58.8	3.17	2.7
3l	32.5 (±1)	67.6	2.73	0.5
3m	58.5 (±1.9)	67.6	2.62	1.1
3n	21.6 (±0.7)	64.8	3.01	3.0
3o	53.3 (±1.7)	67.6	2.79	3.0
3p	18 (±0.5)	58.8	3.33	2.8
3q	10 (±0.3)	67.6	2.89	0.6
3r	249 (±8.1)	67.6	2.78	1.3
3s	149 (±4.9)	64.8	3.17	3.0
3t	64 (±2.1)	67.6	2.95	1.3

including cyclic versus acyclic, high versus low pK_A, secondary vs. tertiary and hydrogen bond acceptor versus hydrogen bond donor amines. The results indicate that both hydroxyl and amine substituents are good for CDKs activity and cytotoxicity, suggesting that both groups are well tolerated and interact with the binding site. However, amines give better separation between CDK2 and CDK9 activity than the hydroxyl group. Compounds with morpholinyl substituents show favourable CDK2 activity and cytotoxicity in comparison to compounds with piperazinyl and methyl piperazinyl substituents, e.g. **3d** vs. **3e** and **3a**, **3i** vs. **3j** and **3f**, **3h** vs. **3o** and **3k**, and **3s** vs. **3t** and **3p**. Figure 1C shows the binding mode of **3l** and seliciclib with CDK9 and CDK2. The terminal amino group of **3l** and hydroxyl of seliciclib interact with Asp167 and Asp145 residues in CDK9 and CDK2, respectively (distances are 2.038 and 2.277 Å, respectively). When the free rotatable amino and hydroxyl groups adopt a flipped pose, they will interact with acidic residue (Asp109) in CDK9, which will bind more tightly with the basic amino group of **3l** than the hydroxyl of seliciclib. This residue is replaced by neutral Gln131 in CDK2, which shows no preference for the amino group of **3l** over hydroxyl group of seliciclib and this could account for the better separation between CDK9 and CDK2 observed with derivatives with a basic amino group (in flip pose, the distances of cationic centre of **3l** and Asp109 of CDK9 is 3.067 Å and between 3 l and Gln131 of CDK2 is 3.935 Å, respectively).

Derivatives with a three-carbon distance of appendant amine from purine core (dimethylpropylamine) showed better activity than derivatives with two-carbon distanced amine (dimethylethylamine) in terms of kinase activities and cytotoxicity in PC3, e.g. **3q** vs. **3r**, **3l** vs. **3m**, **3g** vs. **3h**, and **3b** vs. **3c**. This could be due to the distance between the terminal amines and Asp167 residue, the amine is closer to Asp167 in propyl linker derivatives than in ethyl linker derivatives (Fig. 1).

The cytotoxic effects of seliciclib and the new compounds were determined in androgen-independent prostate cancer cells (PC3 cells) by MTT assay. In this assay, seliciclib had an IC_{50} = 39.7 μM (Table 4), which is comparable with the reported IC_{50} of seliciclib in PC3 cells of 30 μM determined by Arisan et al. [54]. In other cell lines, the average IC_{50} = 16 μM of a 60 cell line panel (range from 8 to 30 μM) [40, 55]. This low cellular activity of seliciclib could be due to fast metabolic clearance and sub-optimum water solubility. Most of the new compounds in the present work show improved cellular potency in comparison to seliciclib, with a few compounds showing weak cellular cytotoxicity, e.g. **3g**, **3o**, and **3r**. However, this weak cellular activity does not correlate with in vitro CDK9 activity, which may be attributed to the physicochemical properties, e.g. lipophilicity and chargeable amines, which could affect the permeability and

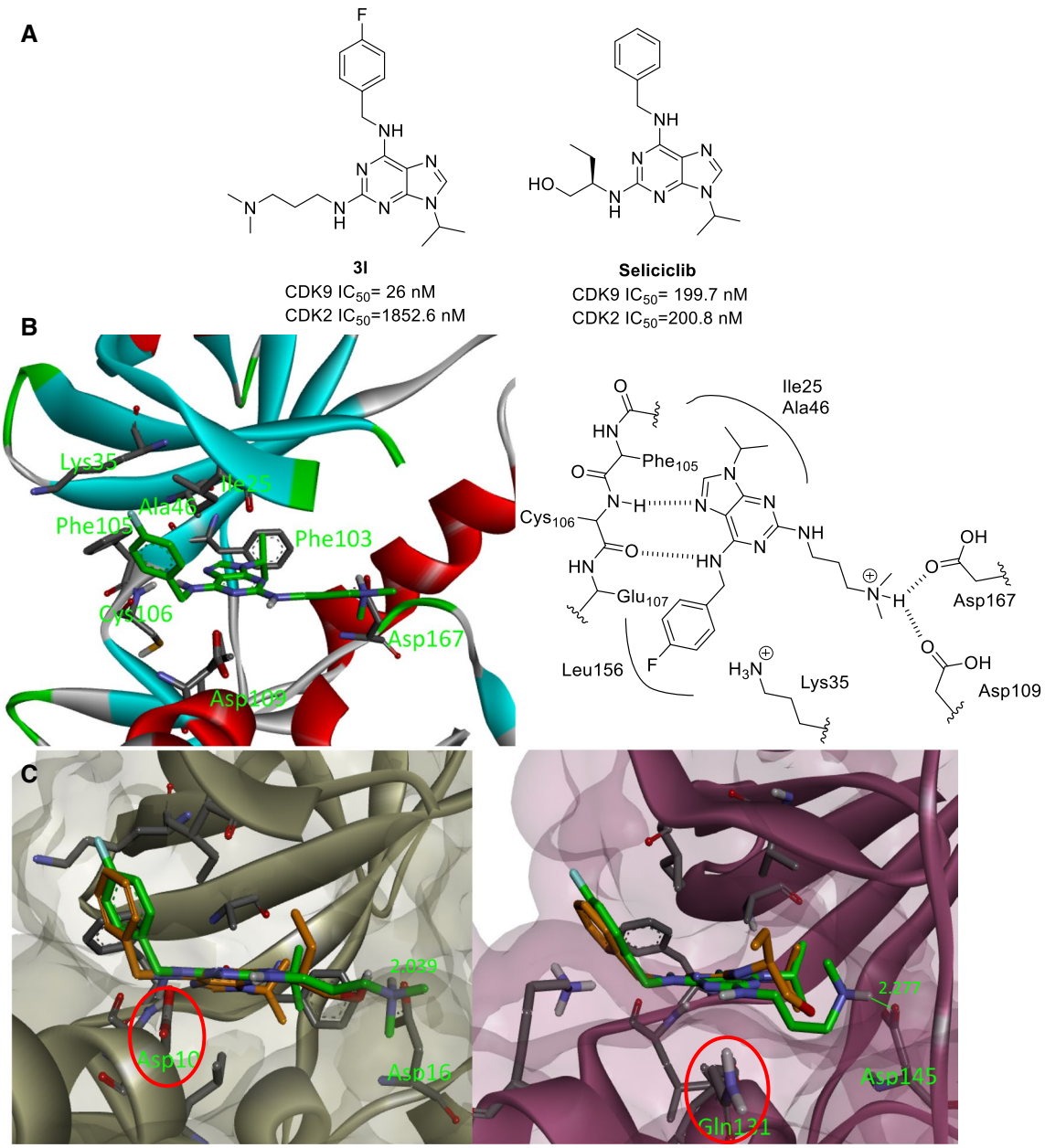


Fig. 1 **a** Structures of **3l** and seliciclib CDK9/2 inhibitory data expressed IC_{50} values. **b** Proposed binding mode of **3l** with CDK9-ATP binding site (left) and schematic representation of **3l** with CDK9 showing the key binding interactions (right). **c** Overlaid **3l** (green) and seliciclib (orange) with CDK9 (right) and CDK2 (left) showing the key difference in amino acid residues between the two isoforms that could account for selectivity (color figure online)

account for the low cellular potency of these compounds (Table 4).

Metabolic stability and water solubility

The major metabolic pathway of seliciclib is oxidation of the terminal hydroxyl group to a carboxylic acid (Scheme 1), which is rapidly formed during first-pass metabolism [45, 46]. This high metabolic clearance has been reported as a major issue associated with the progression of seliciclib in clinical trials. Protecting the terminal hydroxyl group against oxidation can enhance the metabolic stability as reported by Wilson et al. [43]. In this work, we replaced the metabolic labile hydroxyl group on position 2 of purine with cyclic amines, e.g. morpholine, piperazine, and methylpiperazine, which are known to resist oxidative metabolism [56, 57] or sterically hinder appendant tertiary amines, e.g. dimethylpropylamine and dimethylethylamine. Table 5 shows S9

Table 5 S9 metabolic stability and kinetic aqueous solubility for selected compounds

Compounds	$t_{1/2}$/min	Cl_{int} mm^3/min/im cells	Aqueous solubility/ μM
3d	ND	ND	56
3f	64.3	0.010784	50
3j	62.5	0.011091	12
3l	69.3	0.009998	52
3q	ND	ND	50
Seliciclib	56.9	0.01218	12

ND not determined

metabolic stability results of selected examples (S9 assay assesses the metabolic stability against both phase I and phase II enzymes). All tested examples, with different types of terminal amines, show lower metabolic clearance and prolonged half-life than seliciclib.

Almost all tested compounds showed better aqueous solubility than seliciclib. Furthermore, most of the synthesized compounds contain ionisable amines at physiological pHs and, therefore, can be formulated as salts.

Conclusion

This paper describes the synthesis and anti-CDK activity of novel derivatives of seliciclib. Most of the prepared compounds demonstrated better separation between CDK9 and CDK2 inhibitory activity with enhanced metabolic stability. Compound **3l** is a potent and selective CDK9 inhibitor (IC_{50} for CDK9 = 26 nM, CDK2 = 1852.6 nM, CDK5 = 2723.8 nM, and CDK7 = 1452.6 nM), showing enhanced cytotoxicity against prostate cancer cells (IC_{50} = 32.5 μM), improved metabolic stability against phase I and phase II metabolic pathways (S9 $t_{1/2}$ = 60 min, 25% increase in $t_{1/2}$ comparing to seliciclib), and improved water solubility (52 μM). Further mechanistic studies of this compound in prostate cancer cells are currently ongoing.

Experimental

Chemicals such as 2,6-dichloropurine, 2-bromopropane, aliphatic and benzylamines were purchased from Sichuan Benepure and Aldrich Chemical Companies. The melting point measurements were carried out using the electrothermal melting point apparatus (UK). Bruker Ascend 700 NMR spectrometer (Fällanden, Switzerland) was used to obtain the NMR spectra at 700.17 MHz for ^{1}H and 176.08 MHz for ^{13}C and the solvent used was DMSO-d_6 in all samples. TLC was performed for monitoring the reactions using silica gel-precoated aluminium sheets (60 F254, Merck) with UV visualization at 365 and 254 nm. A LC–MS purity check was performed at King Abdullah International Medical Research Center.

2-Chloro-6-(substituted benzylamino) purines 1a–1d Benzylamine (6.35 mmol) and 1.2 cm^3 triethylamine (8.6 mmol) were added to a solution of 1 g 2,6-dichloropurine (5.29 mmol) in 10 cm^3 *n*-butanol. The reaction mixture was kept at 110 °C for 3 h with continuous stirring. After cooling to ambient temperature, the solid was filtered and washed with cold *n*-butanol and hexane then dried.

***N*-Benzyl-2-chloro-9*H*-purin-6-amine (1a)** M.p.: 235–236 °C (Ref. [58] 239–241 °C).

2-Chloro-*N*-(4-chlorobenzyl)-9*H*-purin-6-amine (1b, $C_{12}H_9Cl_2N_5$) Yield 91%; m.p.: 249–250 °C; ^{1}H NMR (700 MHz, DMSO-d_6): δ = 4.63 (br s, 2H), 7.38 (br s, 4H), 8.16 (br s, 1H), 8.75 (br s, 1H), 13.12 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): δ = 43.00, 118.35, 128.73, 129.70, 131.87, 138.85, 140.10, 151.09, 153.32, 155.17 ppm; HRMS (ESI): *m/z* calcd for $C_{12}H_9Cl_2N_5$ 294.03078, found 294.03641.

2-Chloro-*N*-(4-fluorobenzyl)-9*H*-purin-6-amine (1c, $C_{12}H_9ClFN_5$) Yield 91%; m.p.: 239–241 °C; ^{1}H NMR (700 MHz, DMSO-d_6): δ = 4.63 (br s, 2H), 7.15 (br s, 2H), 7.40 (br s, 2H), 8.14 (br s, 1H), 8.74 (br s, 1H), 13.12 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): δ = 42.90, 115.49 (d, *J* = 69.94 Hz), 118.35, 129.84, 136.02, 140.07, 151.05, 153.35, 155.17, 160.98, 162.36 ppm; HRMS (ESI): *m/z* calcd for $C_{12}H_9ClFN_5$ 278.06033, found 278.06359.

2-Chloro-*N*-(3,5-difluorobenzyl)-9*H*-purin-6-amine (1d, $C_{12}H_8ClF_2N_5$) Yield 97%; m.p.: 240–242 °C; ^{1}H NMR (700 MHz, DMSO-d_6): δ = 4.68 (br s, 2H), 7.05–7.20 (m, 2H), 7.24–7.34 (m, 1H), 8.18 (br s, 1H), 8.74 (br s, 1H), 13.15 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): δ = 37.55, 115.57, 116.14, 117.15 (dd, *J* = 33.44, 8.8 Hz), 118.39, 128.60, 140.28, 151.17, 153.19, 155.06, 115.96, 157.32, 157.86, 159.21 ppm; HRMS (ESI): *m/z* calcd for $C_{12}H_8ClF_2N_5$ 296.05091, found 296.05723.

2-Chloro-6-(substituted benzylamino)-9-isopropylpurines 2a–2d Dropwise addition of 0.85 cm^3 2-bromopropane (9 mmol) to a solution of 2-chloro-6-(substituted benzylamino)purine **1** (3.6 mmol) and 1.5 g potassium carbonate (10.8 mmol) in 10 cm^3 DMSO and the temperature did not exceed 19 °C using isopropanol bath for 16–48 h. After the reaction was completed, 25 cm^3 of cold H_2O was added then the mixture was extracted with ethylacetate (3 × 15 cm^3). The organic layers were combined and washed

with brine (3×10 cm^3) then dried over $MgSO_4$ and evaporated to dryness. In some cases, the product crystallized using isopropanol.

***N*-Benzyl-2-chloro-9-isopropyl-9*H*-purin-6-amine (2a, $C_{15}H_{16}ClN_5$)** Yield 87%; m.p.: 177–179 °C; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.49$ (d, $J = 6.67$ Hz, 6H), 4.62–4.71 (m, 3H), 7.20–7.25 (m, 1H), 7.28–7.36 (m, 4H), 8.27 (s, 1H), 8.77 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.54$, 43.55, 47.26, 118.84, 127.31, 127.74, 128.76, 139.73, 139.89, 149.82, 153.21, 155.35 ppm; HRMS (ESI): *m/z* calcd for $C_{15}H_{16}ClN_5$ 302.11670, found 302.12223.

2-Chloro-*N*-(4-chlorobenzyl)-9-isopropyl-9*H*-purin-6-amine (2b, $C_{15}H_{15}Cl_2N_5$) Yield 95%; m.p.: 144–146 °C; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.51$ (d, $J = 6.45$ Hz, 6H), 4.62 (d, $J = 5.81$ Hz, 2H), 4.68 (dt, $J = 13.44$, 6.83 Hz, 1H), 7.37 (q, $J = 8.60$ Hz, 4H), 8.30 (s, 1H) 8.84 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.58$, 42.96, 47.22, 118.92, 128.71, 129.64, 131.83, 138.85, 140.02, 149.90, 153.15, 155,29 ppm; HRMS (ESI): *m/z* calcd for $C_{15}H_{15}Cl_2N_5$ 336.07773, found 336.08606.

2-Chloro-*N*-(4-fluorobenzyl)-9-isopropyl-9*H*-purin-6-amine (2c, $C_{15}H_{15}ClFN_5$) Yield 95%; m.p.: 152–154 °C; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.50$ (d, $J = 7.10$ Hz, 6H), 4.62 (d, $J = 5.81$ Hz, 2H), 4.68 (dt, $J = 13.55$, 6.78 Hz, 1H), 7.15 (t, $J = 8.82$ Hz, 2H), 7.39 (t, $J = 6.56$ Hz, 2H), 8.30 (s, 1H), 8.82 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.58$, 42.90, 47.21, 115.41 (d, $J = 39.42$ Hz), 118.90, 129.81 (d, $J = 8.90$ Hz), 135.99, 139.96, 149.87, 153.16, 155.27, 160.97, 162.35 ppm; HRMS (ESI): *m/z* calcd for $C_{15}H_{15}ClFN_5$ 320.10728, found 320.11303.

2-Chloro-*N*-(3,5-difluorobenzyl)-9-isopropyl-9*H*-purin-6-amine (2d, $C_{15}H_{14}ClF_2N_5$) Yield 92%; m.p.: 182–184 °C; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.51$ (d, $J = 6.45$ Hz, 6H), 4.62–4.75 (m, 3H), 7.11–7.20 (m, 2H), 7.22–7.29 (m, 1H), 8.33 (s, 1H), 8.83 (br s, 1 H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.57$, 37.49, 47.24, 115.61 (dd, $J = 33.44$, 8.8 Hz), 116.18, 117.14 (dd, $J = 33.44$, 7.04 Hz), 119.03, 128.55, 140.19, 150.01, 153.02, 155.26, 155.91, 157.27, 157.86, 159.21 ppm; HRMS (ESI): *m/z* calcd for $C_{15}H_{14}ClF_2N_5$ 338.09786, found 338.10403.

Amination of 2-chloro-6-(substituted benzylamino)-9-isopropylpurines (synthesis of 3a–3t) 2-Chloro-6-(substituted benzylamino)-9-isopropylpurine **2** (3.3 mmol) and appropriate amine (49.5 mmol) were stirred at 160 °C for 8–24 h depending on amine's reactivity. After the reaction was completed, the mixture was allowed to cool and treated with 7 cm^3 cold water for an hour. After that, it was extracted with ethyl acetate (3×5 cm^3). The organic layers were combined and dried using $MgSO_4$ then the organic solvent was evaporated to get the dry product. The sticky residue was triturated with hexane:EtOAc (1:1) to give the solid pure product. In some cases, column chromatography was used to get pure product.

***N*-Benzyl-9-isopropyl-2-(4-methylpiperazin-1-yl)-9*H*-purin-6-amine (3a, $C_{20}H_{27}N_7$)** Yield 83%; m.p.: 138–140 °C; LC–MS purity 99.9%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 2.18 (s, 3H), 2.30 (br s, 4H), 3.65 (br s, 4H), 4.51–4.68 (m, 3H), 7.17–7.23 (m, 1H), 7.29 (t, $J = 7.53$ Hz, 2H), 7.36 (d, $J = 7.53$ Hz, 2H), 7.85 (s, 1H), 7.97 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.55$, 44.46, 46.17, 46.38, 54.97, 66.82, 114.13, 126.94, 127.90, 128.53, 136.27, 141.21, 150.95, 154.47, 158.69 ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{27}N_7$ 366.24007, found 366.2474.

***N*^{6}-Benzyl-*N*^{2}-[3-(dimethylamino)propyl]-9-isopropyl-9*H*-purine-2,6-diamine (3b, $C_{20}H_{29}N_7$)** Yield 44%; LC–MS purity 93.6%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.88$ Hz, 6H), 1.62 (br s, 2H), 2.10 (s, 6H), 2.22 (t, $J = 6.67$ Hz, 2H), 3.23 (q, $J = 6.60$ Hz, 2H), 4.54 (dt, $J = 13.34$, 6.67 Hz, 1H), 4.62 (br s, 2H), 6.30 (br s, 1H), 7.18–7.23 (m, 1H), 7.28 (t, $J = 7.64$ Hz, 2H), 7.35 (d, $J = 7.31$ Hz, 2H), 7.78 (s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.54$, 27.84, 43.19, 45.68, 46.04, 57.69, 79.64, 114.03, 126.89, 127.78, 128.52, 135.39, 141.27, 151.22, 154.89, 159.56 ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{29}N_7$ 368.25572, found 368.26201.

***N*^{6}-Benzyl-*N*^{2}-[2-(dimethylamino)ethyl]-9-isopropyl-9*H*-purine-2,6-diamine (3c, $C_{19}H_{27}N_7$)** Yield 34%; m.p.: 87–89 °C; LC–MS purity 96%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 2.16 (br s, 6H), 2.40 (br s, 2H), 3.30–3.33 (m, 2H), 4.54 (dt, $J = 13.50$, 6.70 Hz, 1H), 4.63 (br s, 2H), 6.10 (br s, 1H), 7.18–7.22 (m, 1H), 7.29 (t, $J = 7.53$ Hz, 2H), 7.34 (d, $J = 7.74$ Hz, 2H), 7.80 (s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.52$, 33.41, 43.11, 45.59, 46.12, 58.81, 114.15, 126.88, 127.69, 128.53, 135.54, 141.22, 151.20, 154.94, 159.38 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{27}N_7$ 354.24007, found 354.24650.

***N*-Benzyl-9-isopropyl-2-morpholino-9*H*-purin-6-amine (3d, $C_{19}H_{24}N_6O$)** Yield 78%; m.p.: 158–159 °C; LC–MS purity 96.7%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = $(d, $J = 6.88$ Hz, 6H), 3.61 (s, 8H), 4.53–4.67 (m, 3H), 7.18–7.22 (m, 1H), 7.29 (t, $J = 7.64$ Hz, 2H), 7.36 (d, $J = 7.53$ Hz, 2H), 7.88 (s, 1H), 8.03 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.54$, 43.50, 45.19, 46.24, 66.52, 114.38, 126.95, 127.89, 128.55, 136.46, 141.17, 150.84, 154.51, 158.74 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{24}N_6O$ 353.20844, found 353.21753.

***N*-Benzyl-9-isopropyl-2-(piperazin-1-yl)-9*H*-purin-6-amine (3e, $C_{19}H_{25}N_7$)** Yield 72%; m.p.: 116–118 °C; LC–MS purity 98.8%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 2.68 (br s, 4H), 3.54–3.62 (m, 4H), 4.51–4.68 (m, 3H), 7.17–7.22 (m, 1H), 7.28 (t, $J = 7.53$ Hz, 2H), 7.36 (d, $J = 7.53$ Hz, 2H), 7.84 (s, 1H), 7.94 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.54$, 43.50, 45.79, 46.00, 46.15, 114.03, 126.92, 127.90, 128.52, 136.17, 141.27, 151.01, 154.45, 158.85 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{25}N_7$ 352.22441, found 352.23135.

***N*-(4-Chlorobenzyl)-9-isopropyl-2-(4-methylpiperazin-1-yl)-9*H*-purin-6-amine (3f, $C_{20}H_{26}ClN_7$)** Yield 56%; m.p.: 97–99 °C; LC–MS purity 95.9%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 2.18 (s, 3H), 2.29 (br s, 4H), 3.63 (br s, 4H), 4.49–4.66 (m, 3H), 7.36 (q, $J = 8.53$ Hz, 4H), 7.86 (s, 1H), 8.02 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.54$, 42.94, 44.46, 46.17, 46.38, 54.96, 114.13, 128.48, 129.69, 131.42, 136.38, 140.30, 150.94, 154.36, 158.66 ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{26}ClN_7$ 400.20116, found 400.20871.

***N*^{6}-(4-Chlorobenzyl)-*N*^{2}-[3-(dimethylamino)propyl]-9-isopropyl-9*H*-purine-2,6-diamine (3g, $C_{20}H_{28}ClN_7$)** Yield 81%; LC–MS purity 96.7%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.46$ (d, $J = 6.88$ Hz, 6H), 1.60 (br s, 2H), 2.09 (s, 6H), 2.20 (br s, 2H), 3.15–3.26 (m, 2H), 4.42–4.57 (m, 2H), 4.59 (br s, 1H), 6.31 (br s, 1H), 7.35 (q, $J = 8.53$ Hz, 4H), 7.79 (s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.53$, 27.84, 42.60, 45.66, 46.05, 57.6, 79.64, 114.01, 128.46, 129.59, 131.38, 135.39, 140.33, 151.25, 154.79, 159.53 ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{28}ClN_7$ 402.21675, found 402.22476.

***N*^{6}-(4-Chlorobenzyl)-*N*^{2}-[2-(dimethylamino)ethyl]-9-isopropyl-9*H*-purine-2,6-diamine (3h, $C_{19}H_{26}ClN_7$)** Yield 80%; m.p.: 99–101 °C; LC–MS purity 98.3%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 2.12 (br s, 6H), 2.34 (br s, 2H), 3.29 (q, $J = 6.02$ Hz, 2H), 4.48–4.57 (m, 1H), 4.59 (br s, 2H) 6.09 (br s, 1H), 7.35 (s, 4H), 7.80 (s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.51$, 37.42, 42.55, 45.71, 46.14, 58.89, 114.14, 128.48, 129.51, 131.38, 135.62, 140.30, 151.21, 154.82, 159.36 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{26}ClN_7$ 388.20110, found 388.20881.

***N*-(4-Chlorobenzyl)-9-isopropyl-2-morpholino-9*H*-purin-6-amine (3i, $C_{19}H_{23}ClN_6O$)** Yield 79%; m.p.: 142–144 °C; LC–MS purity 95.8%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 3.60 (br s, 8H), 4.50–4.66 (m, 3H), 7.36 (q, $J = 8.60$ Hz, 4H), 7.88 (s, 1H), 8.08 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.53$, 42.90, 45.16, 46.26, 86.51, 114.37, 128.50, 129.70, 131.44, 136.56, 140.24, 150.90, 154.41, 158.70 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{23}ClN_6O$ 387.1646, found 387.17714.

***N*-(4-Chlorobenzyl)-9-isopropyl-2-(piperazin-1-yl)-9*H*-purin-6-amine (3j, $C_{19}H_{24}ClN_7$)** Yield 69%; LC–MS purity 94.2%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.46$ (d, $J = 6.67$ Hz, 6H), 2.68 (br s, 4H), 3.57 (br s, 4H), 4.56 (dt, $J = 13.18$, 6.64 Hz, 3H), 7.32–7.39 (m, 4H), 7.85 (s, 1H), 7.99 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.52$, 42.92, 45.67, 45.89, 46.18, 113.99, 128.47, 129.68, 131.41, 136.30, 140.32, 151.04, 154.32, 158.79 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{24}ClN_7$ 386.18545, found 386.19246.

***N*-(4-Fluorobenzyl)-9-isopropyl-2-(4-methylpiperazin-1-yl)-9*H*-purin-6-amine (3k, $C_{20}H_{26}FN_7$)** Yield 88%; m.p.: 107–109 °C; LC–MS purity 99.4%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.88$ Hz, 6H), 2.19 (s, 3H), 2.27–2.36 (m, 4H), 3.65 (br s, 4H), 4.57 (dt, $J = 13.50$, 6.70 Hz, 3H), 7.11 (t, $J = 8.93$ Hz, 2H), 7.39 (dd, $J = 8.39$, 5.81 Hz, 2H), 7.86 (s, 1H), 7.99 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.54$, 42.83, 44.47, 46.17, 46.39, 54.98, 114.13, 115.16 (d, $J = 55.95$ Hz), 129.79 (d, $J = 7.63$ Hz), 136.33, 137.41, 150.96, 154.39, 158.69, 160.79, 162.16 ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{26}FN_7$ 384.23065, found 384.23702.

***N*^{2}-[3-(Dimethylamino)propyl]-*N*^{6}-(4-fluorobenzyl)-9-isopropyl-9*H*-purine-2,6-diamine (3l, $C_{20}H_{28}FN_7$)** Yield 85%; LC–MS purity 98.7%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.46$ (d, $J = 6.67$ Hz, 6H), 1.62 (br s, 2H), 2.10 (s, 6H), 2.21 (br s, 2H), 3.23 (q, $J = 6.53$ Hz, 2H), 4.53 (dt, $J = 13.34$, 6.67 Hz, 2H), 4.59 (br s, 1H), 6.31 (br s, 1H), 7.10 (t, $J = 8.82$ Hz, 2H), 7.39 (dd, $J = 8.28$, 5.92 Hz, 2H), 7.79 (s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.53$, 27.84, 37.44, 42.48, 45.87, 46.04, 57.69, 114.01, 115.20 (d, $J = 29.25$ Hz), 129.69 (d, $J = 7.63$ Hz), 135.44, 137.43, 151.10, 154.80, 159.55, 160.77, 162.14 ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{28}FN_7$ 386.2463, found 386.25355.

***N*^{2}-[2-(Dimethylamino)ethyl]-*N*^{6}-(4-fluorobenzyl)-9-isopropyl-9*H*-purine-2,6-diamine (3m, $C_{19}H_{26}FN_7$)** Yield 42%; m.p.: 95–97 °C; LC–MS purity 97.9%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 2.13 (br s, 6H), 2.35 (br s, 2H), 3.31 (q, $J = 6.45$ Hz, 2H), 4.54 (dt, $J = 13.34$, 6.67 Hz, 1H), 4.59 (br s, 2H), 6.09 (br s, 1H), 7.11 (t, $J = 8.82$ Hz, 2H), 7.35–7.40 (m, 2H), 7.79 (s, 1H), 7.84 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.51$, 40.48., 42.48, 45.72, 46.13, 58.91, 114.14, 115.22 (d, $J = 40.69$ Hz), 129.61 (d, $J = 8.90$ Hz), 135.57, 137.39, 151.27, 154.84, 159.38, 160.77, 162.15 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{26}FN_7$ 372.23065, found 372.23750.

***N*-(4-Fluorobenzyl)-9-isopropyl-2-morpholino-9*H*-purin-6-amine (3n, $C_{19}H_{23}FN_6O$)** Yield 79%; m.p.: 149–151 °C; LC–MS purity 98.5%; ^{1}H NMR (700 MHz, DMSO-d_6):

$\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 3.61 (s, 8H), 4.58 (quin, $J = 6.67$ Hz, 3H), 7.11 (t, $J = 8.82$ Hz, 2H), 7.39 (dd, $J = 8.17$, 5.81 Hz, 2H), 7.88 (s, 1H), 8.05 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.54$, 42.80, 45.18, 46.25, 66.52, 114.37, 115.26 (d, $J = 48.32$ Hz), 129.79 (d, $J = 26.70$ Hz), 136.52, 137.35, 150.87, 154.40, 158.72, 160.79, 162.17 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{23}FN_6O$ 371.19901, found 371.20758.

***N*-(4-Fluorobenzyl)-9-isopropyl-2-(piperazin-1-yl)-9*H*-purin-6-amine (3o, $C_{19}H_{24}FN_7$)** Yield 94%; LC–MS purity 95.3%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 2.64–2.74 (m, 4H), 3.54–3.63 (m, 4H), 4.49–4.63 (m, 3H), 7.11 (t, $J = 8.82$ Hz, 2H), 7.39 (dd, $J = 8.17$, 5.81 Hz, 2H), 7.84 (s, 1H), 7.96 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.54$, 42.79, 45.78, 45.99, 46.15, 114.02, 115.24 (d, $J = 36.88$ Hz), 129.77 (d, $J = 7.63$ Hz),136.23, 137.44, 151.04, 154.34, 158.83, 160.77, 162.15 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{24}FN_7$ 370.2150, found 370.2269.

***N*-(3,5-Difluorobenzyl)-9-isopropyl-2-(4-methylpiperazin-1-yl)-9*H*-purin-6-amine (3p, $C_{20}H_{25}F_2N_7$)** Yield 84%; m.p.: 103–105 °C; LC–MS purity 99.1%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 2.17 (s, 3H), 2.27 (br s, 4H), 3.62 (br s, 4H), 4.54–4.70 (m, 3H), 7.06–7.14 (m, 1H), 7.14–7.19 (m, 1H), 7.22 (td, $J = 9.20$, 4.41 Hz, 1H), 7.89 (s, 1H), 8.03 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.53$, 36.98, 44.38, 46.22, 46.36, 54.91, 114.14, 115.14 (dd, $J = 33.44$, 8.8 Hz), 115.96, 116.91(dd, $J = 35.20$, 8.8 Hz), 130.13, 136.59, 151.09, 154.27, 155.83, 157.20, 157.88, 158.58, 159.23 ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{25}F_2N_7$ 402.22123, found 402.22991.

***N*^{6}-(3,5-Difluorobenzyl)-*N*^{2}-[3-(dimethylamino)propyl]-9-isopropyl-9*H*-purine-2,6-diamine (3q, $C_{20}H_{27}F_2N_7$)** Yield 83%; LC–MS purity 91.3%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 1.58 (br s, 2H), 2.08 (s, 6H), 2.18 (br s, 2H), 3.14–3.25 (m, 2H), 4.54 (dt, $J = 13.28$, 6.59 Hz, 1H), 4.65 (br s, 2H), 6.36 (br s, 1H), 7.06–7.15 (m, 2H), 7.22 (td, $J = 9.14$, 4.52 Hz, 1H), 7.82 (s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.51$, 27.79, 36.89, 40.43, 45.62, 46.10, 57.63, 114.04, 114.55 (dd, $J = 33.44$, 10.56 Hz), 115.80, 116.87 (dd, $J = 33.44$, 8.8 Hz), 130.14, 135.73, 151.38, 154.76, 155.78, 157.14, 157.91, 159.26, 159.48 ppm; HRMS (ESI): *m/z* calcd for $C_{20}H_{27}F_2N_7$ 404.23688, found 404.24336.

***N*^{6}-(3,5-Difluorobenzyl)-*N*^{2}-[2-(dimethylamino)ethyl]-9-isopropyl-9*H*-purine-2,6-diamine (3r, $C_{19}H_{25}F_2N_7$)** Yield 79%; LC–MS purity 97.1%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 2.11 (br s, 6H), 2.32 (br s, 2H), 3.22–3.32 (m, 2H), 4.54 (dt, $J = 13.39$, 6.75 Hz, 1H), 4.65 (br s, 2H), 6.14 (br s, 1H), 7.11 (dd, $J = 7.42$, 3.98 Hz, 2H), 7.22 (td, $J = 9.41$, 3.98 Hz, 1H), 7.83 (s, 1 H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.50$, 36.93, 45.63, 46.18, 58.84, 79.63, 114.13, 115.03 (dd, $J = 31.68$, 7.04 Hz), 115.65, 116.90 (dd, $J = 33.44$, 8.8 Hz), 130.19, 135.67, 151.33, 154.75, 155.75, 157.10, 157.92, 159.27, 159.32 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{25}F_2N_7$ 390.22123, found 290.22781.

***N*-(3,5-Difluorobenzyl)-9-isopropyl-2-morpholino-9*H*-purin-6-amine (3s, $C_{19}H_{22}F_2N_6O$)** Yield 93%; m.p.: 141–143 °C; LC–MS purity 98.8%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.88$ Hz, 6H), 3.59 (s, 8H), 4.55–4.72 (m, 3H), 7.07–7.13 (m, 1H), 7.14–7.19 (m, 1H), 7.22 (td, $J = 9.20$, 4.41 Hz, 1H), 7.91 (s, 1H), 8.08 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.52$, 36.94, 45.08, 46.30, 66.46, 114.38, 115.18 (dd, $J = 33.44$, 8.8 Hz), 116.07, 116.93 (dd, $J = 33.44$, 7.04 Hz), 129.99, 136.79, 151.02, 154.31, 155.31, 155.82, 157.18, 157.88, 159.24 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{22}F_2N_6O$ 389.1859, found 389.19707.

***N*-(3,5-Difluorobenzyl)-9-isopropyl-2-(piperazin-1-yl)-9*H*-purin-6-amine (3t, $C_{19}H_{23}F_2N_7$)** Yield 92%; m.p.: 167–169 °C; LC–MS purity 98.5%; ^{1}H NMR (700 MHz, DMSO-d_6): $\delta = 1.47$ (d, $J = 6.67$ Hz, 6H), 2.66 (br s, 4H), 3.55 (br s, 4H), 4.57–4.61 (m, 3H), 7.07–7.13 (m, 1H), 7.14–7.19 (m, 1H), 7.22 (td, $J = 9.14$, 4.52 Hz, 1H), 7.87 (s, 1H), 8.00 (br s, 1H) ppm; ^{13}C NMR (176 MHz, DMSO-d_6): $\delta = 22.52$, 36.97, 45.71, 45.94, 46.20, 114.03, 115.12 (dd, $J = 33.44$, 8.8 Hz), 116.07, 116.90 (dd, $J = 33.44$, 8.8 Hz), 130.21, 136.49, 151.20, 154.26, 155.83, 157.19, 157.86, 158.74, 159.23 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{23}F_2N_7$ 388.20558, found 388.21513.

Kinase inhibition assays

The CDK2 assay kit was purchased from Promega Corporation (Madison, WI, USA) and the anti-CDK2 effect was measured following the manufacturer's instructions (CDK2/CyclinA2 Kinase Assay Catalogue# V2971). The remaining kinase assay kits (CDK5, 7, and 9) crystallized from BPS Biosciences (San Diego, CA, USA) and the anti-CDK effect was measured following the manufacturer's instructions (CDK5 Kinase Assay Kit Catalogue# 79,600, CDK7 Kinase Assay Kit Catalogue# 79,603, CDK9 Kinase Assay Kit Catalogue# 79,628). The kinase inhibitory activity was expressed as IC_{50} for three individual experiments. In all kinase assays, seliciclib was used as positive control and DMSO was used as negative control.

MTT cytotoxicity assay

PC3 cells were treated with compounds in a concentration range from 0.1 to 10 μM for 48 h, 10% v/v reconstituted MTT was added and incubated for 3 h before reading the plates on Wallac Victor2 1420 multilabel counter in fluorescence mode at a wavelength of 460/590 nm (ex/em). Cytotoxicity was expressed as IC_{50} for three individual experiments. Seliciclib was used as positive control and DMSO was used as negative control in this assay.

S9 metabolic stability assay

The tested inhibitors (5 μM) were incubated with Tris-buffer (0.1 M), mouse liver S9, NADPH, PAPS, and UDPGA at 37 °C for 1 h. Then the protein in the samples was participated, centrifuged, and filtered. The disappearance of the parent compounds was analysed using LC–MS/MS.

Kinetic solubility assay

The tested compounds (30 μM) in DMSO were diluted with aqueous buffer and turbidimetry was measured as end point at 450 nm absorbance.

Molecular docking

The three-dimensional crystal structure of the proteins (PDB: 3LQ5, 2A4L) was obtained from RCSB Protein Data Bank. The programs utilized for performing the molecular docking were Discovery Studio, ChemBio3D Ultra 14.0 (PerkinElmer informatics), and PyRx (the Scripps Research Institute, La Jolla, CA, USA). The preparation of protein was conducted using Discovery Studio, which involved removing water and sulfate molecules as well as the co-crystallized compound, and it was saved as a PDB file. Then, the AutoDock Tools in PyRx were used to introduce the polar hydrogens and it was saved as a PDBQT file. The ligands (compound **3 l** and seliciclib) were prepared using ChemBio3D Ultra 14.0 and saved as PDB files. Lastly, Grid box and AutoDock Vina in PyRx software were used to perform the docking. The minimum energy pose of **3 l** was compared with that of seliciclib.

Acknowledgements This research was funded by the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University, through the Research Funding Program (Grant No#FRP-1440-8).

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