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Research paper

Activity of 2,6,9-trisubstituted purines as potent PDGFRα kinase inhibitors with antileukaemic activity

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ABSTRACT

Receptor tyrosine kinase PDGFRα is often constitutively activated in various tumours and is regarded as a drug target. Here, we present a collection of 2,6,9-trisubstituted purines with nanomolar potency against PDGFRα and strong and selective cytotoxicity in the human eosinophilic leukaemia cell line EOL-1 that expresses the *FIP1L1-PDGFRA* oncogene. In treated EOL-1 cells, the example compound **14q** inhibited the autophosphorylation of PDGFRα and the phosphorylation of STAT3 and ERK1/2. Interestingly, we observed pronounced and even increased effects of **14q** on PDGFRα and some of its downstream signalling pathways after drug washout. In accordance with suppressed PDGFRα signalling, treated cells were arrested in the G1 phase of the cell cycle and eventually underwent apoptosis. Our results show that substituted purines can be used as specific modulators of eosinophilic leukaemia.

1. Introduction

The primary role of eosinophils is to defend against parasitic infections as a part of the innate immune system. However, eosinophils also contribute to different disorders when they expand, either due to mutations that directly cause eosinophil lineage expansion or upon cytokine stimulation from other cell types, referred to as primary or secondary hypereosinophilia, respectively. Hypereosinophilia, is a heterogeneous group of rare myeloproliferative disorders characterized by peripheral eosinophilia, which may be associated with tissue and end-organ damage [1,2]. Clonal hypereosinophilia, and chronic eosinophilic leukaemia are subtypes of primary hypereosinophilia, caused by a pre-malignant or malignant clones of eosinophils often driven by mutations or rearrangements of genes encoding protein kinases, including *KIT*, *JAK2*, *ETV6-PDGFRB* or *ETV6-ABL1*; however, the most frequent chromosomal abnormality involves *FIP1L1-PDGFRA* fusion [1].

PDGFRα and PDGFRβ, together with KIT, FLT3 and CSF1R, are members of the family of class III receptor tyrosine kinases that are frequently deregulated in various neoplasms, for which many small molecule inhibitors have been developed as therapeutics (Fig. 1) [3,4]. In addition to other cancers, the application of tyrosine kinase inhibitors dramatically altered the survival of patients with *PDGFRA*- and *PDGFRB*-rearranged neoplasms, including clonal hypereosinophilia and chronic eosinophilic leukaemia [5]. In particular, imatinib has been shown to be more effective and less toxic than conventional therapies, which are usually based on cytotoxic, glucocorticoid or immunomodulatory agents with poorly predictable efficacy [6]. Unfortunately, in some cases of *PDGFRA*-induced eosinophilic leukaemia, imatinib leads to the development of secondary resistance. Moreover, hypereosinophilic patients with *FGFR1*, *JAK2*, *FLT3* or *ABL1* rearrangements display a more aggressive disease with variable sensitivity to kinase inhibitors [2]. These patients therefore still lack an effective therapy, and new PDGFRα inhibitors are still actively sought [7–9].

We previously described a collection of 2,6,9-trisubstituted purines with submicromolar inhibitory activities against PDGFRα and showed that the compounds are selective for the *FIP1L1-*

Abbreviations: ERK, extracellular signal-regulated kinase; FLT3, fms-like tyrosine kinase 3; ITD, internal tandem duplication; MAPK, mitogen-activated protein kinase; PDGFR, platelet derived growth factor receptor; STAT, signal transducer and activator of transcription.

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0223-5234/

PDGFRA-positive leukaemia cell line EOL-1 [7]. In parallel, we also prepared purine derivatives as nanomolar FLT3 inhibitors with high potency and selectivity against the acute myeloid leukaemia cell line MV4-11 [10]. The compounds exhibited substantially reduced cytotoxic activity (at least 2-3 orders of magnitude) in most cancer cell lines, but not in the lines bearing FLT3-ITD mutations. Interestingly, kinase profiling of the lead compound also revealed strong inhibition of the receptor tyrosine kinases PDGFRβ and PDGFRα, which have proven to be viable drug targets for the management of corresponding subtypes of eosinophilic leukaemia. Molecular docking of these compounds to the FLT3 and PDGFRα kinases predicted a type I binding mode [10]. Furthermore, molecular docking revealed that modifications at the 6 and 9 positions of the purine core should be well tolerated and may lead to compounds with higher affinity for PDGFRα. We therefore expanded the library of 2,6,9-trisubstituted purines and studied their inhibitory potency against PDGFRα.

2. Results and discussion

2.1. Chemistry

The multistep synthesis of the target compounds **11a**-**11k**, **12a**-**12g**, **13a**-**13e** and **14a**-**14s** began with 2,6-dichloro-9*H*-purine, which was alkylated in the first step with cyclopentanol, 1,3-dihydroxypropane and 1,3-dihydroxycyclopentane. For the purposes of this study, we did not investigate the influence of the geometric isomery of the 1,3-disubstitution of the cyclopentyl ring at position 9 of the purine moiety. Thus, we used a mixture of *cis* and *trans* 1,3-dihydroxycyclopentane for alkylation. The alkylation was performed under Mitsunobu conditions and proceeded smoothly with high yield and purity in cases with these diols (see Scheme 1). The formation of additional bis derivatives was negligible.

The next step was a substitution at position 6 with various aromatic amines, namely, **3a**-**3b**, **6a-6d** and 4-bromoaniline, to yield the derivatives **9a-9t**. Some of the aromatic amines used were commercially available, and the aromatic amines **3a**-**3b** and **6a-6d** were synthesized. The 2-(morpholinomethyl)aniline **3a** and 3-(morpholinomethyl)aniline **3b** were synthesized by the reaction of 1-(bromomethyl)-2-nitrobenzene **1a** or 1-(bromomethyl)-3-nitrobenzene **1b** with morpholine to obtain nitroderivatives **2a**-**2b,** which were reduced with hydrazine hydrate on Raney nickel to derivatives **3a**-**3b** (see Scheme 2).

Similarly, the aromatic amines **6a**-**6d** were prepared from 2-bromo-5-nitropyridine via Suzuki coupling with corresponding arylboronic acids leading to the nitroderivatives **5a**-**5d**. The derivatives **5a**-**5d** were then reduced with hydrogen on palladium to yield the corresponding aromatic amines **6a-6d** (see Scheme 3).

The substitution at position 6 was accomplished in the presence of *N,N*-diisopropyl-*N*-ethylamine at 120 °C in *n*-propanol. Finally, the substitution at position 2 of the purine ring with selected primary amines or morpholine was performed in a large excess of amine in 1,2-ethanediol at 160 °C (see Scheme 1). Compounds **11a** and **11c** were directly prepared by Suzuki coupling of bromoderivative **10** with corresponding 2-methoxyphenylboronic or 2-aminophenylboronic acids. Finally, the hydroxyderivatives **11b** and **11j** were synthesized from the corresponding methoxyderivatives **11a** and **11i** by the reaction with boron tribromide.

2.2. In vitro structure–activity relationships

All of the prepared compounds, **11a-11k**, **12a-12g**, **13a-13e** and **14a-14s**, were tested in biochemical assays to determine their kinase inhibitory activities against recombinant PDGFRα kinase. The cytotoxic activities were studied in the EOL-1 cell line (chronic eosinophilic leukaemia, *FIP1L1-PDGFRA*-positive) and other cell lines, including CEM (acute lymphoblastic leukaemia), K562 (chronic myeloid leukaemia), HCC-827 (lung adenocarcinoma), and BJ (nontransformed human fibroblast cell line), to determine the potential selectivity. The results of the biological assays testing these compounds are summarized in Table 1-4.

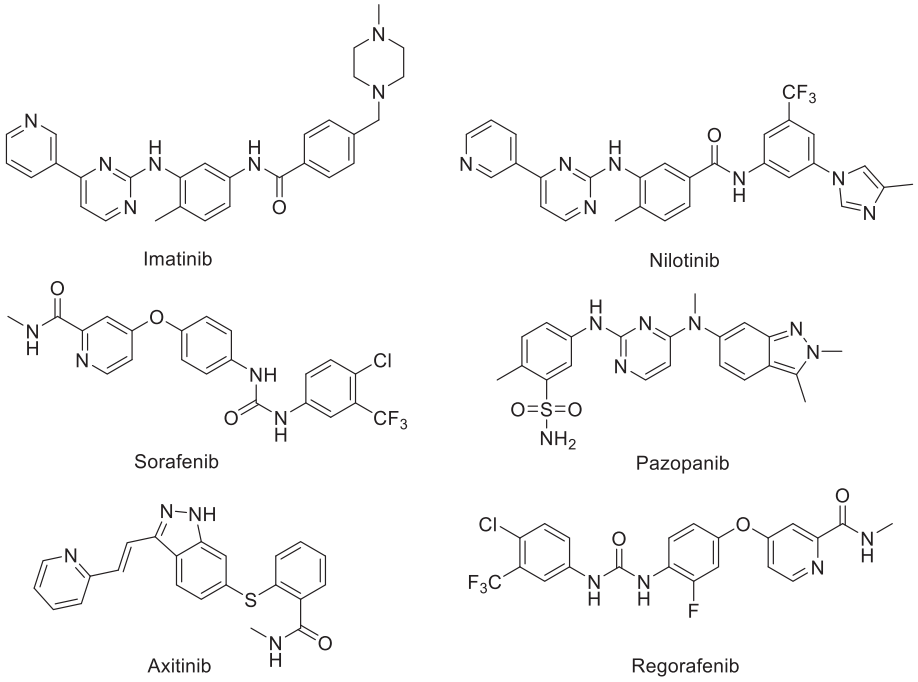
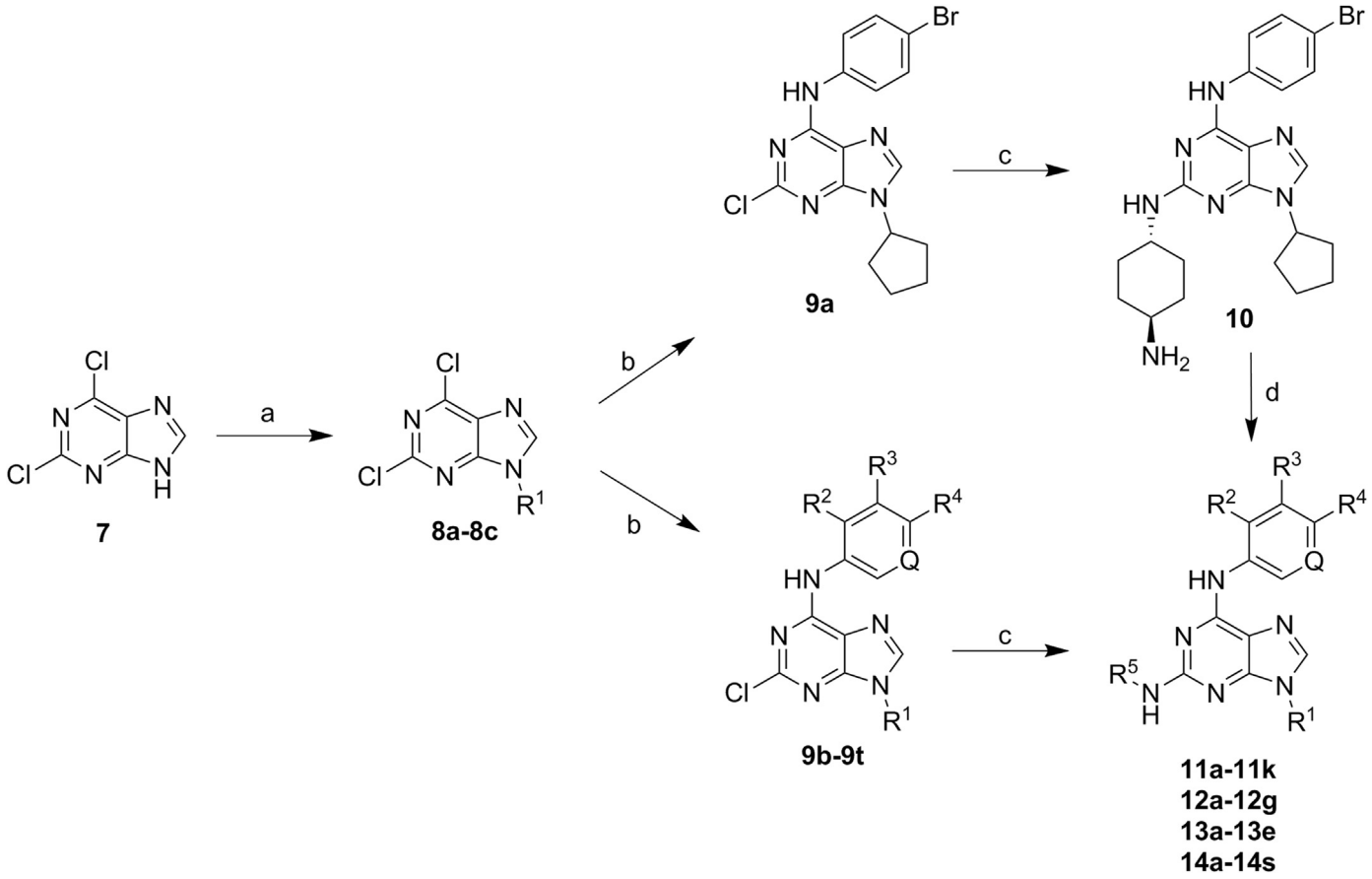
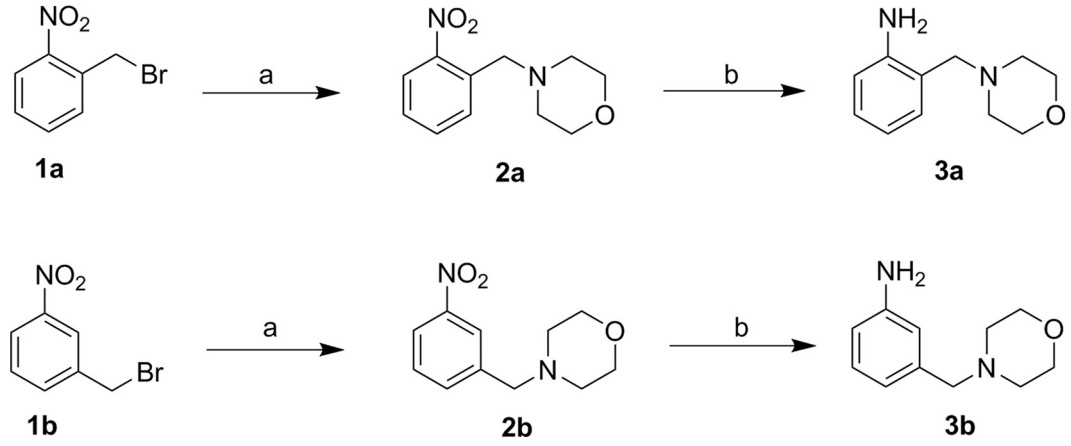


Fig. 1. Structures of selected PDGFR multikinase inhibitors.

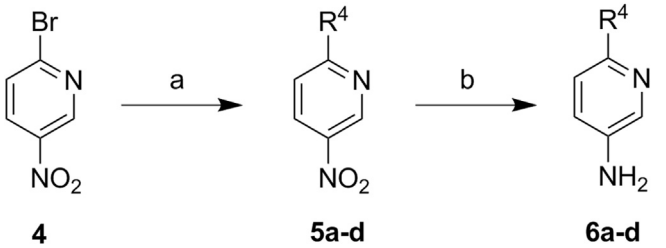


Scheme 1. The reaction conditions were as follows: a) corresponding alcohol, DIAD, PPh_3, rt; b) corresponding aromatic amine, DIPEA, *n*-propanol, 120 °C (sealed tube); c) corresponding primary amine, 1,2-ethanediol, 160 °C (sealed tube); and d) corresponding arylboronic acid, $Pd(PPh_3)_4$, K_3PO_4, THF.

First, we studied the influence of 6-biarylamino and 6-heterobiarylamino substituents on biological activities while we conserved the substituents at positions 2 and 9. Thus, we started our study with a series of 9-cyclopentyl-2-(4-aminocyclo hexylamino)-6-(4-aryl-phenyl)amino-9*H*-purines, **11a**-**11f,** and a series of 9-cyclopentyl-2-(4-aminocyclohexylamino)-6-(6-aryl-pyridin-3-yl)amino-9*H*-purines, **11g**-**11k**. All these compounds exhibited significant inhibitory activities against PDGFRα kinase and antiproliferative activities in EOL-1 cells in a nanomolar range. Other tested cell lines displayed GI_{50} values in the submicromolar or low micromolar range. The differences between the biological activities of phenyl and corresponding pyridine derivatives slightly favoured the latter. The most promising biological activities in this group of compounds were observed in the **11d** and **11e** molecules, which displayed the strongest inhibition of PDGFRα kinase and antiproliferative activities in *FIP1L1-PDGFRA*-positive EOL-1 cells; the other cancer and nontransformed cells were far less sensitive to these compounds. However, compounds **11d** and **11e** contain 1,2,4-triazole and imidazole rings, which could lead to chemical instability, and these molecules are susceptible to oxidative cleavage.



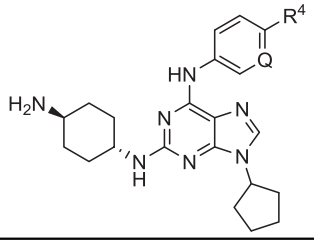
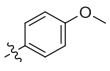
Scheme 2. The reaction conditions were as follows: a) morpholine, K_2CO_3, ethanol, 100 °C (sealed tube); and b) Raney nickel, hydrazine hydrate 80%, 40 °C.



Scheme 3. The reaction conditions were as follows: a) appropriate arylboronic acid, $Pd(OAc)_2$, $K_3PO_4.3H_2O$, tetrabutylammonium bromide, DMF, H_2O; and b) 5% wt, Pd/C.

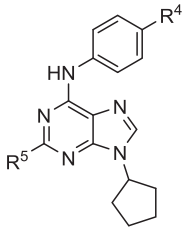
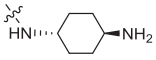
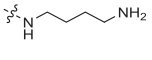
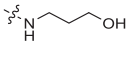
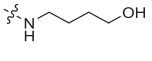
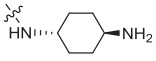
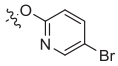
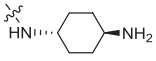
Based on the previous results, we prepared another group of compounds containing 4-(heteroaryloxy)phenylamino substituents at position 6 of the purine moiety. The starting molecule **12a** exhibited good cell growth inhibition in the EOL-1 cell line, while other cancer cell lines were approximately 80 times less sensitive. Therefore, we decided to study the influence of various substituents at position 2 of the purine moiety in a group of compounds with 6-(4-thiazol-2-yl-phenyl)amino substitution (**12a-12e**). These results are consistent with our previously described series of FLT3-ITD kinase inhibitors [10]. The substitution with a linear 4-aminobutylamino chain in **12b** led to an approximately ten-fold decrease in cytotoxicity in all tested cell lines. The PDGFRα kinase inhibitory activities also decreased. The substitution with a 3-hydroxypropylamino chain in **12c**, with a 4-hydroxybutylamino chain in **12d** or a morpholine ring in **12e** caused significant loss of antiproliferative and kinase inhibitory activities. Compound **12a**, the most active compound among **12a-12g,** still demonstrated relatively high cytotoxicity against the tested normal BJ cells. The biological activities of compounds **12a-12g** are summarized in Table 2.

Table 1
Summary of synthesized derivatives **11a-11k** and their kinase inhibitory activities and cytotoxicities.

compound	substituents		IC_{50} (μM)[a]	GI_{50} (μM)[a]				
								
	R^4	Q	PDGFRα	EOL-1	CEM	K562	HCC-827	BJ
11a		CH	0.065	0.117	2.35	2.32	2.30	22.8
11b		CH	0.073	0.053	1.49	2.08	2.53	9.6
11c		CH	0.005	0.018	0.68	1.07	0.81	12.5
11d		CH	0.007	0.008	0.93	0.98	0.85	10.4
11e		CH	0.006	0.005	0.67	1.12	0.87	12.8
11f		CH	0.011	0.008	0.87	1.56	1.42	14.7
11g		N	0.032	0.021	0.48	0.57	0.65	2.94
11h		N	0.025	0.017	0.62	0.88	0.63	13.1
11i		N	0.036	0.014	0.46	0.55	0.64	6.20
11j		N	0.026	0.085	0.62	0.50	0.65	6.35
11k		N	0.039	0.025	0.54	0.70	0.58	7.23

[a] Data for control compounds (imatinib and quizartinib) and all SD values are available from supplementary material.

Table 2
Summary of the synthesized derivatives **12a-12g** and their kinase inhibitory activities and cytotoxicities.

compound	substituents		IC_{50} (μM)[a]			GI_{50} (μM)[a]		
								
	R^4	R^5	PDGFRα	EOL-1	CEM	K562	HCC-827	BJ
12a			0.014	0.008	0.650	0.676	0.692	6.85
12b			0.12	0.12	8.64	5.99	6.31	18.6
12c			1.13	0.44	22.9	11.3	6.49	34.1
12d			0.69	0.49	13.4	6.08	6.05	23.5
12e			1.25	0.35	>40	>40	15.4	>40
12f			0.047	0.020	1.15	2.27	2.72	16.9
12g			0.18	0.12	6.16	6.08	3.73	>40

[a] Data for control compounds (imatinib and quizartinib) and all SD values are available from supplementary material.

Another aim of this study was to discover the impact of hydroxy groups on aliphatic or alicyclic chains at position 9 of the purine moiety. For this purpose, we synthesized compounds **14a-14h** and **14i-14o** bearing 3-hydroxypropyl and 3-hydroxycyclopentyl groups, respectively. The substituents at positions 6 and 2 were chosen based on our previous series of potent FLT3-ITD kinase inhibitors. The 4-(morpholinomethyl-phenyl)-amino and 4-(4-benzylpiperazin-1-yl)phenylamino motifs used for the substitution at position 6 have already been verified as potent pharmacophores for FLT3-ITD kinase inhibition. The comparison of biological activities of the compound triads **14q**/**14c**/**14j** and **14p**/**14f**/**14**m indicates that the replacement of cyclopentyl at position 9 by the 3-hydroxycyclopentyl group decreases the toxicity in non-transformed cells, while the GI_{50} values in cancer cell lines remain unaltered. Interestingly, we did not note a similar trend for the pairs **14s**/**14k** and **14r**/**14l** with 4-aminobutylamino or 4-hydroxybutylamino groups at position 2.

The last part of our study was modifying the substitution position of the 6-aminophenyl ring with a morpholinomethyl group. We synthesized the *ortho*-disubstituted derivatives **13a**, **13c** and **14a** and the *meta*-disubstituted derivatives **13b**, **13d**, **13e**, **14b** and **14i**. In the case of all of the tested *o*-disubstituted derivatives, extreme loss of kinase inhibitory activities and cellular growth inhibition were observed. On the other hand, the derivatives **13b**, **13d**, **13e**, **14b** and **14i** bearing a 3-(morpholinomethyl)phenylamino moiety at position 6 generally retained low nanomolar kinase inhibitory activities comparable to analogous *para*-disubstituted derivatives.

Generally, we found a strong correlation between PDGFRα kinase inhibitory activity and EOL-1 cytotoxicity (see Table S2). On the other hand, we did not observe a significant correlation between inhibition of PDGFRα kinase and cytotoxicity against HCC-827, K562, CEM and BJ, which suggests that the inhibition of PDGFRα may be a predominant mechanism of antiproliferative activities.

In order to rationalize inhibition of PDGFRα by prepared compounds, we generated a homology model based on the c-KIT cocrystal structure (PDB ID: 1PKG) and used it to predict the binding modes of **11e** and **14q** (Fig. 2). Examination of the predicted docking modes suggested that the binding modes are consistent with type I inhibitors. In the hinge region, C677 backbone forms two classical hydrogen bonds with both compounds. The binding pose of compound **11e** indicates that compound makes also a salt bridge between the terminal amine of 1,4-diaminocyclohexyl group and carboxylate of D681. The terminal amine of 1,4-diaminocyclohexyl group forms a salt bridge interaction with the D836. In addition, the secondary amine of diaminocyclohexyl moiety of **14q** interacts with carbonyl of L599 near the top of adenine pocket.

2.3. Activity of *14q* in the EOL-1 cell line

The EOL-1 cell line is characterized by the expression of the *FIP1L1-PDGFRA* fusion gene, which encodes the 110-kDa oncogenic variant of PDGFRα. This genetic background makes this cell line a commonly used in vitro model of *FIP1L1-PDGFRA*-positive chronic eosinophilic leukaemia [11]. The high sensitivity of the EOL-1 cell line to our compounds encouraged us to describe their mechanism of action in this cellular model in more detail. EOL-1 cells were treated with increasing concentrations of the most potent compound, **14q**, for 1 h, lysed and subsequently analyzed by immunoblotting. As shown in Fig. 3, the analysis revealed a dose-dependent decrease in PDGFRα autophosphorylation at three different

Table 3
Summary of synthesized derivatives **13a**-**13e**, **14a**-**14s** and their kinase inhibitory activities and cytotoxicities.

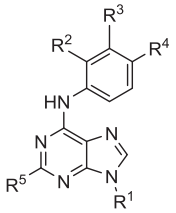
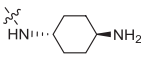
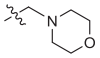
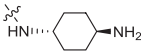
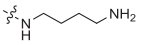
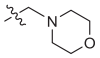
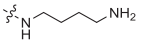
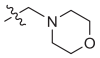
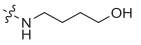
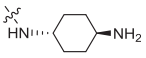
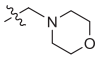
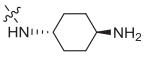
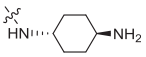
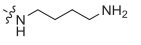
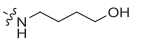
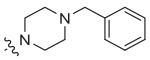
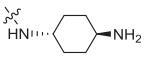
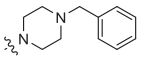
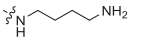
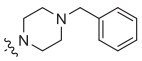
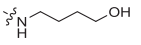
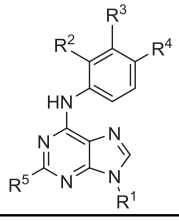
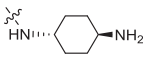
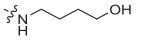
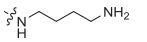
compound	substituents					IC_{50} (μM)[a]	GI_{50} (μM)[a]				
											
	R^1	R^2	R^3	R^4	R^5	PDGFRα	EOL-1	CEM	K562	HCC-827	BJ
13a			H	H		>20	4.52	18.1	4.11	22.2	30.4
13b		H		H		0.035	0.012	1.36	1.28	1.19	14.7
13c			H	H		>20	6.41	25.7	12.2	26.3	37.5
13d		H		H		0.211	0.061	8.19	8.40	5.37	22.0
13e		H		H		1.29	0.282	11.1	30.4	8.13	25.7
14a			H	H		>20	10.6	>40	>40	>40	>40
14b		H		H		0.156	0.075	>40	>40	25.5	>40
14c		H	H			0.094	0.164	34.5	9.93	18.4	>40
14d		H	H			0.167	0.138	8.20	9.37	19.0	35.0
14e		H	H			1.85	0.294	5.17	4.74	10.6	20.8
14f		H	H			0.121	0.058	4.56	2.67	2.38	16.5
14g		H	H			0.236	0.158	7.57	2.57	6.70	28.5
14h		H	H			1.35	1.01	27.9	8.67	9.80	>40
14i		H		H		0.037	0.009	1.65	1.85	1.67	>40
14j		H	H			0.047	0.008	1.42	1.04	0.801	>40
14k		H	H			0.074	0.036	5.51	2.23	3.24	4.90
14l		H	H			1.02	0.162	6.43	3.78	3.26	26.1
14m		H	H			0.033	0.011	0.540	0.652	0.840	16.9
14n		H	H			0.128	0.046	3.67	1.63	2.30	13.2
14o		H	H			1.81	0.223	9.09	3.73	3.96	23.8
14p		H	H			0.035	0.022	0.582	0.970	0.918	10.0

Table 3 (*continued*)

compound	substituents					IC_{50} (μM)[a]	GI_{50} (μM)[a]				
											
	R^1	R^2	R^3	R^4	R^5	PDGFRα	EOL-1	CEM	K562	HCC-827	BJ
14q		H	H			0.027	0.007	0.820	1.12	1.01	12.3
14r		H	H			1.03	0.202	3.48	1.45	2.51	22.8
14s		H	H			0.188	0.049	3.04	2.79	2.04	9.54

[a] Data for control compounds (imatinib and quizartinib) and all SD values are available from supplementary material.

tyrosine residues (Y848 and Y1018) and inhibition of the activity of two PDGFRα downstream signalling pathways, namely, MAPK/ERK and STAT3. At a concentration as low as 10 nM, **14q** reduced the T202/Y204 phosphorylation on ERK1/2 and Y705 phosphorylation on STAT3, while higher doses completely blocked these signalling pathways. As the disruption of activated oncogenic kinase signalling usually results in the inhibition of proliferation and the induction of apoptosis, we treated EOL-1 cells with compound **14q** for 24 h and performed flow cytometry analysis of the cell cycle. The concentration of 10 nM induced marked G1 arrest of the cell cycle, with a slight increase in the subG1 population corresponding to apoptotic cells. Doses higher than 10 nM significantly affected the viability of EOL-1 cells, as evidenced by the predominant subG1 population (Fig. 4).

Next, we performed a drug washout experiment to gain some insight into target vulnerability (Fig. 5). EOL-1 cells were treated with three different doses of compound **14q** for 1 h. After the incubation period, the cells were transferred to drug-free medium and cultivated for an additional 2, 6 or 24 h. Subsequent immunoblotting analysis revealed that 100 nM compound **14q** was sufficient to reduce the autophosphorylation of PDGFRα at Y849 and to decrease the phosphorylation of downstream targets STAT3 and ERK1/2. This effect remained stable even after 2 and 6 h of cultivation in drug-free medium. Interestingly, after the cells were treated with 100 nM compound **14q** for 1 h, the drug was washed out, and the cells were cultivated for an additional 24 h, we observed a further increase in the inhibitory effect of our compound, which completely blocked the phosphorylation of PDGFRα, STAT3 and ERK1/2. In the cells treated with 1000 nM **14q** at the beginning of the experiment, this effect was coupled with the degradation of the proteins PDGFRα and STAT3. This pronounced effect suggests that exposure of EOL-1 cells to the candidate compound **14q** for even a short period of time is sufficient to induce the inhibition of PDGFRα signalling, which persists even in the absence of **14q**.

3. Conclusion

The receptor tyrosine kinase PDGFRα has been validated as a drug target in several myeloproliferative disorders and neoplasms. Our collection of 2,6,9-trisubstituted purines demonstrated nanomolar potency against PDGFRα in biochemical and cellular experiments and showed selective activity in the human eosinophilic leukaemia cell line EOL-1, expressing an oncogenic variant of the mentioned kinase. The cytotoxicity of these compounds against EOL-1 cells was significantly correlated with PDGFRα inhibition, confirming the proposed cellular mechanism of action. The effect of the example compound was time-dependent and even increased after drug removal; prolonged signalling inhibition following washout indicated a high level of PDGFRα vulnerability. The results suggest that substituted purines can be used as specific modulators of eosinophilic leukaemia.

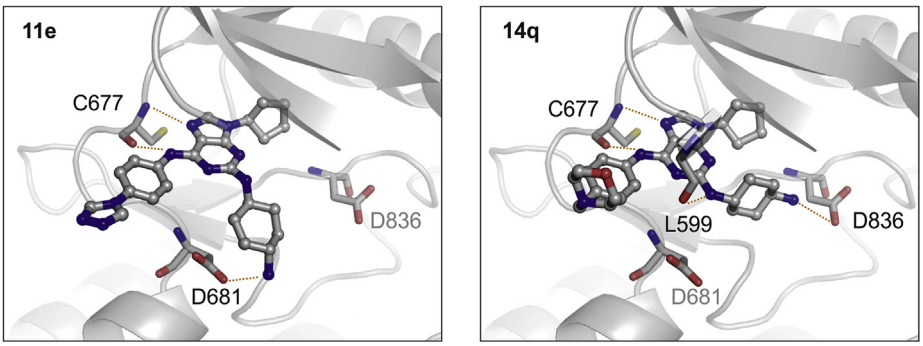


Fig. 2. Docked binding poses of **11e** and **14q** in PDGFRα. The kinase is modeled in active conformation. The orange dashed lines indicate H-bond and salt-bridge interactions. Discussed amino acids are shown as sticks. The atoms are colored gray for carbon, blue for nitrogen, red for oxygen and yellow for sulfur. Figure prepared with Pymol, Delano Scientific. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

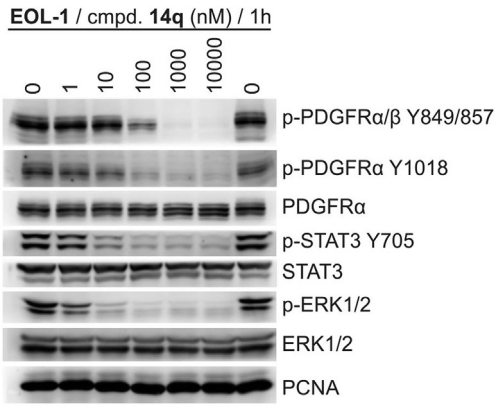


Fig. 3. Effect of **14q** on PDGFRα and some downstream signalling pathways in EOL-1 cells treated for 1 h. PCNA was used as a control for protein loading.

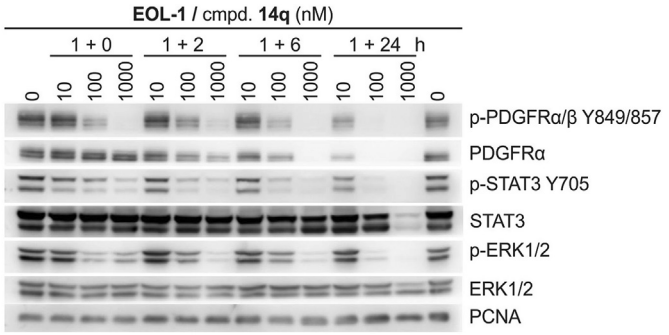


Fig. 5. Pronounced effect of **14q** on PDGFRα and some downstream signalling pathways in EOL-1 cells after drug washout. EOL-1 cells were treated with different doses of compound **14q** for only 1 h (labelled as 1 + 0) or treated for 1 h and subsequently cultivated for an additional 2 h (1 + 2), 6 h (1 + 6) or 24 h (1 + 24) in a drug-free medium.

4. Experimental section

4.1. Reagents and general methods

Melting points were determined on a Büchi Melting Point B-540 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol 500 ECA instrument operating at 500 MHz for ^{1}H and 126 MHz for ^{13}C or on a Bruker Avance 300 spectrometer (300 MHz) operating at 300 MHz for ^{1}H and 76 MHz for ^{13}C at ambient temperature in DMSO-d_6 or $CDCl_3$. Chemical shifts are reported in ppm. Coupling constants (*J*) are reported in Hertz (Hz), and the following abbreviations are used: broad (b), singlet (s), doublet (d), triplet (t), quartet (q), quintet (qui), sextet (sex), septet (sep), and multiplet (m). Mass spectra were recorded using an LCQ ion trap mass spectrometer (Finnigan MAT, San Jose, CA, USA). The chromatographic purity of the compounds was determined using HPLC-DAD-MS. An Alliance 2695 separations module (Waters) linked to both a PDA 996 (Waters) and a Q-Tof micro (Waters) benchtop quadrupole orthogonal acceleration time-of-flight tandem mass spectrometer were used. Samples were dissolved in methanol and diluted to a concentration of 10 $\mu g \cdot mL^{-1}$ in the mobile phase (initial conditions). Then, 10 μL of the solution was injected on an RP column (150 mm × 2.1 mm; 3.5 μm; Symmetry C18, Waters). The column was kept in a thermostat at 25 °C. Solvent (A) consisted of 15 mM formic acid adjusted to a pH of 4.0 with ammonium hydroxide. Methanol was used as the organic modifier (solvent B). At a flow rate of 0.2 $mL \cdot min^{-1}$, the following binary gradient was used: 0 min, 10% B; 0–24 min, a linear gradient to 90% B; and 10 min isocratic elution of 90% B. At the end of the gradient, the column was re-equilibrated to the initial conditions for 10 min. The effluent was introduced into the DAD (scanning range 210–400 nm, with 1.2 nm resolution), and an electrospray source was applied (source temperature 110 °C, capillary voltage +3.0 kV, cone voltage +20 V, and desolvation temperature 250 °C). Nitrogen was used as both the desolvation gas (500 $L \cdot h^{-1}$) and the cone gas (50 $L \cdot h^{-1}$). The mass spectrometer was operated in positive (ESI+) ionization mode, and data were acquired in the 50–1000 m/z range. Elemental analyses were performed using an EA1112 CHN analyser (Thermo Finnigan); the results obtained for C, H, and N were within acceptable limits of the expected values. Merck silica gel Kieselgel 60 (230–400 mesh) was used for column chromatography. The purity of biologically evaluated compounds was >95%, as determined by HPLC-DAD-MS and elemental analysis.

4.2. General procedures for the preparation of the derivatives 2a, 2b, 3a, 3b, 5a-5d, 6a-6d, 8b, 8c, 9a-9t, 11a-11k, 12a-12g, 13a-13e and 14a-14p

4.2.1. General procedure for the preparation of compounds 3a and 3b

The mixture of 2-nitrobenzylbromide (**1a**) or 3-nitrobenzylbromide (**1b**) (1.00 mmol), morpholine (1.05 mmol), and potassium carbonate (2.00 mmol) in ethanol (10 mL) was heated under an argon atmosphere in a sealed tube at 100 °C for 4 h. The completion of the reaction was checked with TLC on silica (chloroform/methanol, 19:1). After cooling to room temperature, the reaction mixture was evaporated under reduced pressure. The residue was partitioned between dichloromethane (25 mL) and water (25 mL). The water phase was extracted twice with dichloromethane (25 mL). The combined organic phases were washed with water and brine, dried over sodium sulphate, and concentrated under reduced pressure. The crude product (**2a** or **2b**) was then directly used without further purification.

The crude product (**2**) from the previous step (0.75 mmol) was reduced on Raney nickel (approx. 50 mg) by the dropwise addition of hydrazine hydrate 80% (7.50 mmol) over a period of 20 min at

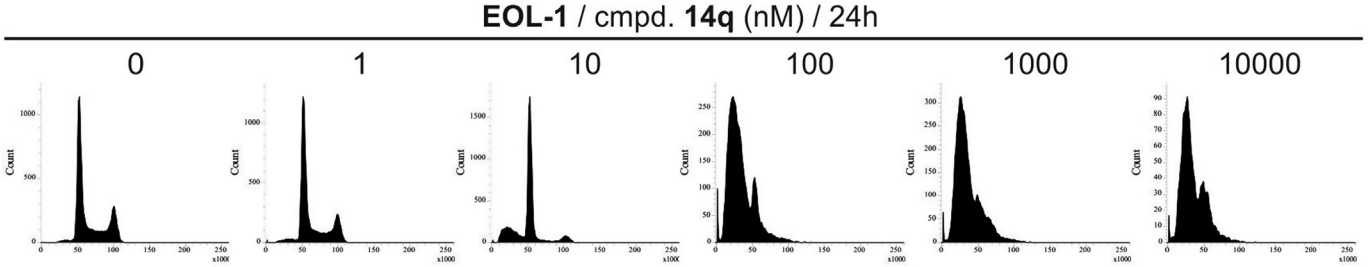


Fig. 4. Effect of **14q** on the cell cycle in EOL-1 cells treated with the indicated doses for 24 h.

40 °C with stirring. The reaction mixture was filtered through Celite, washed with methanol and evaporated under reduced pressure. The crude products **3a**-**3b** were dissolved in 2 M hydrochloric acid (50 mL) and extracted with dichloromethane (25 mL). The water phase was neutralized with 5% sodium hydrogen carbonate, and the precipitate was filtered off and washed with water. The crude product was dried in a vacuum desiccator.

*4.2.1.1. 2-(morpholinomethyl)aniline (**3a**).* Yield: 72%. Elemental analysis: Calcd for $C_{11}H_{16}N_2O$ (192.26): C, 68.72; H, 8.39; N, 14.57. Found: C, 68.54; H, 8.69; N, 14.21. HPLC-MS (ESI+): 193.12 (98.5%). ^{1}H NMR (500 MHz, DMSO-d_6): 2.28−2.32 (m, 4H), 3.41 (s, 2H, CH_2), 3.42−3.46 (m, 4H), 4.55 (bs, 2H, NH_2), 6.93 (t, J = 7.5 Hz, 1H, ArH), 7.12 (d, J = 7.5 Hz, 1H, ArH), 7.23 (t, J = 7.5 Hz, 1H, ArH), 8.62 (d, J = 7.5 Hz, 1H, ArH).

*4.2.1.2. 3-(morpholinomethyl)aniline (**3b**).* Yield: 84%. Elemental analysis: Calcd for $C_{11}H_{16}N_2O$ (192.26): C, 68.72; H, 8.39; N, 14.57. Found: C, 68.66; H, 8.61; N, 14.36. HPLC-MS (ESI+): 193.38 (96%). ^{1}H NMR (500 MHz, DMSO-d_6): 2.28−2.32 (m, 4H), 3.43 (s, 2H, CH_2), 3.52−3.59 (m, 4H), 4.85 (bs, 2H, NH_2), 6.42−6.49 (m, 2H, ArH), 6.53 (s, 1H, ArH), 6.93 (t, J = 7.5 Hz, 1H, ArH).

4.2.2. General procedure for the preparation of compounds 6a-6d

The mixture of 2-bromo-5-nitropyridine (**4**) (1.00 mmol), the appropriate arylboronic acid (1.05 mmol), palladium diacetate (10.0 μmol), potassium phosphate trihydrate (2.00 mmol) and tetrabutylammonium bromide (10.0 μmol) was suspended in a mixture of *N,N*-dimethylformamide (5.0 mL) and water (2.5 mL). The mixture was heated at 80 °C for 4 h under an argon atmosphere with stirring in a sealed tube. The completion of the reaction was checked with TLC on silica (hexane/ethylacetate, 5:1). After cooling to room temperature, the reaction mixture was evaporated under reduced pressure. The residue was partitioned between dichloromethane (25 mL) and water (25 mL). The water phase was extracted twice with dichloromethane (25 mL). The combined organic phases were washed with water and brine, dried over sodium sulphate, and concentrated under reduced pressure. The crude product (**5**) was purified by flash column chromatography on silica using mobile phase hexan/ethylacetate (9:1, v/v).

The crude nitroderivative (**5**) from the previous step (0.75 mmol) was hydrogenated under atmospheric pressure in methanol (50 mL) with 5% wt. palladium on charcoal (50 mg). After the consumption of hydrogen, the reaction mixture was filtered through Celite, washed with methanol and evaporated under reduced pressure. The crude product was dissolved in 2 M hydrochloric acid (50 mL) and extracted with dichloromethane (25 mL). The water phase was neutralized with 5% sodium hydrogen carbonate, and the precipitate was filtered off and washed with water. The crude product was dried in a vacuum desiccator and finally purified by flash column chromatography using mobile phase chloroform/methanol (4:1, v/v).

*4.2.2.1. 6-(thiophen-2-yl)pyridin-3-amine (**6a**).* Yield: 71%. Elemental analysis: Calcd for $C_9H_8N_2S$ (176.24): C, 61.34; H, 4.58; N, 15.89. Found: C, 61.05; H, 4.84; N, 15.63. HPLC-MS (ESI+): 177.25 (97%). ^{1}H NMR (500 MHz, DMSO-d_6): 5.41 (bs, 2H, NH_2), 7.02−7.06 (m, 2H, ArH), 7.25 (t, J = 4.5 Hz, 1H, ArH), 7.40 (d, J = 4.0 Hz, 1H, ArH), 7.47 (d, J = 8.5 Hz, 1H, ArH), 8.05 (d, J = 4.0 Hz, 1H, ArH).

*4.2.2.2. 6-(furan-2-yl-pyridin-3-ylamine (**6b**).* Yield: 52%. Elemental analysis: Calcd for $C_9H_8N_2O$ (160.18): C, 67.49; H, 5.03; N, 17.49. Found: C, 67.58; H, 4.89; N, 17.11. HPLC-MS (ESI+): 161.43 (98.9%). ^{1}H NMR (500 MHz, DMSO-d_6): 5.21 (bs, 2H, NH_2), 6.91 (d, J = 3.2 Hz, 1H, ArH), 7.25 (s, 1H, ArH), 7.42−7.53 (m, 2H, ArH), 7.56 (s, 1H, ArH), 7.73 (d, J = 3.4 Hz, 1H, ArH).

*4.2.2.3. 6-(2-methoxyphenyl)pyridin-3-amine (**6c**).* Yield: 89%. Elemental analysis: Calcd for $C_{12}H_{12}N_2O$ (200.24): C, 71.98; H, 6.04; N, 13.99. Found: C, 72.14; H, 5.79; N, 13.55. HPLC-MS (ESI+): 201.36 (99.3%). ^{1}H NMR (500 MHz, DMSO-d_6): 3.82 (s, 3H, CH_3), 5.19 (bs, 2H, NH_2), 7.06 (d, J = 8.5 Hz, 1H, ArH), 7.14 (t, J = 7.5 Hz, 1H, ArH), 7.43 (t, J = 8.5 Hz, 1H, ArH), 7.60 (s, 1H, ArH), 7.75−7.85 (m, 3H, ArH).

*4.2.2.4. 6-(4-methoxyphenyl)pyridin-3-amine (**6d**).* Yield: 89%. Elemental analysis: Calcd for $C_{12}H_{12}N_2O$ (200.24): C, 71.98; H, 6.04; N, 13.99. Found: C, 71.69; H, 5.84; N, 14.24. HPLC-MS (ESI+): 201.33 (97.2%). ^{1}H NMR (500 MHz, DMSO-d_6): 3.84 (s, 3H, CH_3), 5.25 (bs, 2H, NH_2), 6.92 (d, J = 8.5 Hz, 2H, ArH), 7.05 (dd, J = 8.5 Hz, J = 3.0 Hz, 1H, ArH), 7.49 (dd, J = 8.5 Hz, J = 1.0 Hz, 1H, ArH), 7.85 (d, J = 8.5 Hz, 2H, ArH), 8.15 (s, 1H, ArH).

4.2.3. General procedure for the preparation of compounds 8a-8c

Compounds **8a-8c** were prepared according to a previously described procedure [12] via Mitsunobu alkylation. The crude products **8b** and **8c** were purified by column chromatography on silica using mobile phase hexan/ethylacetate (3:1, v/v).

*4.2.3.1. 9-Cyclopentyl-2,6-dichloro-9H-purin (**8a**).* Yield: 56%, mp: 118−120 °C. Elemental analysis: Calcd. for $C_{10}H_{10}Cl_2N_4$ (257.12): C, 46.71; H, 3.92; N, 21.79. Found: C, 46.95; H, 3.81; N, 21.70. HPLC-MS (ESI+): 288.10 (99.6%). ^{1}H NMR (DMSO-d_6): 1.64−1.69 (m, 2H), 1.81−1.96 (m, 4H), 2.09−2.15 (m, 2H), 4.92 (qui, J = 7.53 Hz, 1H, CH), 8.82 (s, 1H, CH).

*4.2.3.2. 3-(2,6-dichloro-9H-purin-9-yl)propan-1-ol (**8b**).* Yield: 65%, mp: 142−144 °C. Elemental analysis: Calcd for $C_8H_8Cl_2N_4O$ (247.08): C, 38.89; H, 3.26; N, 22.68. Found: C, 38.56; H, 2.98; N, 22.79. HPLC-MS (ESI+): 247,36 (96%). ^{1}H NMR (500 MHz, DMSO-d_6): 1.95 (qui, J = 6.0 Hz, 2H, CH_2), 3.39 (q, J = 6.0 Hz, 2H, CH_2), 4.28 (t, J = 7.0 Hz, 2H, CH_2), 4.61 (t, J = 5.0 Hz, 1H, OH), 8.68 (s, 1H, CH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 32.24, 42.14, 58.19, 131.06, 149.21, 150.0, 151.31, 154.06.

*4.2.3.3. 3-(2,6-dichloro-9H-purin-9-yl)cyclopentan-1-ol (**8c**).* Yield: 58%, mp: 133−135 °C. Elemental analysis: Calcd for $C_{10}H_{10}Cl_2N_4O$ (273.12): C, 43.98; H, 3.69; N, 20.51. Found: C, 43.88; H, 3.84; N, 20.43. HPLC-MS (ESI+): 274.18 (95.2%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.71−1.76 (m, 2H), 1.82−1.86 (m, 1H), 1.96−2.03 (m, 1H), 2.15−2.19 (m, 1H), 2.38−2.43 (m, 1H), 3.47−3.50 (m, 1H, CH), 4.85 (qui, J = 6.5 Hz, 1H, CH), 4.99 (bs, 1H, OH).

4.2.4. General procedure for the preparation of compounds 9a-9t

To a suspension of 9-substituted-2,6-dichloro-9*H*-purine (**8**) (2.00 mmol) in a mixture of *n*-propanol (10 mL) and *N,N*-diisopropyl-*N*-ethylamine (6.00 mmol), appropriate amine (2.05 mmol) was added. The suspension was heated while stirring in a sealed tube under an argon atmosphere at 100 °C for 2−6 h. The reaction was checked by TLC using mobile phase toluene-ethylacetate (1:1, v/v). After the completion of the reaction, the reaction mixture was cooled to room temperature and evaporated under reduced pressure. The residue was partitioned between water (50 mL) and dichloromethane (50 mL), and the water phase was extracted two additional times with the same volume of dichloromethane. The combined organic phases were washed with water and brine and evaporated under reduced pressure. The crude product was crystallized from petroleum ether/ethylacetate (3:1).

*4.2.4.1. 4-(Bromo-phenyl)-(2-chloro-9-cyclopentyl-9H-purin-6-yl)-amine (**9a**).* Yield: 85%, mp: 193−194 °C. Elemental analysis:

Calcd.for $C_{16}H_{15}BrClN_5$ (392.69): C, 48.94; H, 3.85; N, 17.83. Found: C, 49.06; H, 3.94; N, 17.49. HPLC-MS (ESI+): 393.7 (98.4%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.87–1.92 (m, 2H), 1.92–1.99 (m, 4H), 2.01–2.23 (m, 2H), 4.85 (qui, $J = 7.17$ Hz, 1H), 7.54 (d, $J = 8.73$ Hz, 2H), 7.84 (d, $J = 8.73$ Hz, 2H), 8.45 (s, 1H), 10.42 (s, 1H).

*4.2.4.2. (2-Chloro-9-cyclopentyl-9H-purin-6-yl)-(4-pyrazol-1-yl-phenyl)-amine (**9b**).* Yield: 84%, mp: 214–216 °C. Elemental analysis: Calcd for $C_{19}H_{18}ClN_8$ (379.86): C, 60.08; H, 4.78; N, 25.81. Found: C, 60.11; H, 4.51; N, 25.32. HPLC-MS (ESI+): 380.94 (95.8%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.86–1.91 (m, 2H), 1.92–1.99 (m, 4H), 2.00–2.24 (m, 2H), 4.87 (qui, $J = 7.5$ Hz, 1H), 7.95 (d, $J = 7.50$ Hz, 2H, ArH), 7.86 (d, $J = 6.5$ Hz, 1H, ArH), 8.11 (t, $J = 7.0$ Hz, 1H, ArH), 8.20 (s, 1H, CH), 8.50 (d, $J = 7.5$ Hz, 2H, ArH), 9.22 (d, $J = 7.0$ Hz, 1H, ArH), 9.25 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.14, 32.45, 56.27, 119.78, 120.29, 121.56, 122.30, 132.55, 139.02, 141.58, 142.52, 151.34, 152.44, 152.74.

*4.2.4.3. N-(4-(1H-1,2,4-triazol-1-yl)phenyl)-2-chloro-9-cyclopentyl-9H-purin-6-amine (**9c**).* Yield: 89%, mp: 183–185 °C. Elemental analysis: Calcd for $C_{18}H_{17}ClN_8$ (380.84): C, 56.77; H, 4.50; N, 29.42. Found: C, 56.84; H, 4.26; N, 29.04. HPLC-MS (ESI+): 381.96 (98.5%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.61–1.69 (m, 2H), 1.79–1.87 (m, 2H), 1.91–1.98 (m, 2H), 2.11–2.17 (m, 2H), 4.81 (qui, $J = 7.5$ Hz, 1H, CH), 7.79 (d, $J = 9.0$ Hz, 2H, ArH), 8.00 (d, $J = 9.0$ Hz, 2H, ArH), 8.18 (s, 1H, ArH), 8.41 (s, 1H, CH), 9.20 (s, 1H, ArH), 10.48 (s, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.02, 32.54, 56.15, 119.78, 120.30, 122.30, 132.55, 139.02, 141.58, 142.52, 151.34, 152.44, 152.64, 152.74.

*4.2.4.4. (2-Chloro-9-cyclopentyl-9H-purin-6-yl)-(4-thiophen-2-yl-phenyl)-amine (**9d**).* Yield: 73%, mp: 194–196 °C. Elemental analysis: Calcd for $C_{20}H_{18}ClN_5S$ (395.92): C, 60.68; H, 4.58; N, 17.69. Found: C, 60.59; H, 4.64; N, 17.84. HPLC-MS (ESI+): 396.96 (96.3%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.60–1.68 (m, 2H), 1.79–1.87 (m, 2H), 1.91–1.98 (m, 2H), 2.11–2.17 (m, 2H), 4.78 (qui, $J = 7.0$ Hz, 1H, CH), 7.03–7.08 (m, 2H, ArH), 7.32 (t, $J = 5.0$ Hz, 1H, ArH), 7.43 (d, $J = 5.0$ Hz, 1H, ArH), 7.43 (d, $J = 8.0$ Hz, 1H, ArH), 8.03 (d, $J = 4.5$ Hz, 1H, ArH), 8.85 (s, 1H, CH), 9.65 (bs, 1H, NH).

*4.2.4.5. 2-chloro-9-cyclopentyl-N-(6-(furan-3-yl)pyridin-3-yl)-9H-purin-6-amine (**9e**).* Yield: 78%, mp: 201–203 °C. Elemental analysis: Calcd for $C_{19}H_{17}ClN_6O$ (380.12): C, 59.92; H, 4.50; N, 22.07. Found: C, 59.84; H, 4.12; N, 21.84. HPLC-MS (ESI+): 381.22 (%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.85–1.90 (m, 2H), 1.94–1.98 (m, 4H), 2.01–2.23 (m, 2H), 4.83 (qui, $J = 7.0$ Hz, 1H), 6.90 (d, $J = 3.2$ Hz, 1H, ArH), 7.27 (s, 1H, ArH), 7.43–7.51 (m, 2H, ArH), 7.57 (s, 1H, ArH), 7.73 (d, $J = 3.4$ Hz, 1H, ArH), 8.03 (s, 1H, CH), 8.96 (s, 1H, NH).

*4.2.4.6. 2-Chloro-9-cyclopentyl-9H-purin-6-yl)-[6-(2-methoxy-phenyl)-pyridin-3-yl]-amine (**9f**).* Yield: 79%, mp: 172–175 °C. Elemental analysis: Calcd for $C_{22}H_{21}ClN_6O$ (420.91): C, 62.78; H, 5.03; N, 19.97. Found: C, 62.94; H, 5.12; N, 19.64. HPLC-MS (ESI+): 422.05 (97.6%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.85–1.90 (m, 2H), 1.94–1.98 (m, 4H), 2.01–2.23 (m, 2H), 3.43 (s, 3H, CH_3), 4.83 (qui, $J = 6.5$ Hz, 1H, CH), 7.02 (d, $J = 8.2$ Hz, 1H, ArH), 7.10 (t, $J = 7.5$ Hz, 1H, ArH), 7.40 (t, $J = 8.2$ Hz, 1H, ArH), 7.60 (s, 1H, ArH), 7.75–7.85 (m, 2H, ArH), 8.05 (s, 1H, CH), 9.29 (bs, 1H, NH).

*4.2.4.7. (2-Chloro-9-cyclopentyl-9H-purin-6-yl)-[6-(4-methoxy-phenyl)-pyridin-3-yl]-amine (**9g**).* Yield: 84%, mp: 183–185 °C. Elemental analysis: Calcd for $C_{22}H_{21}ClN_6O$ (420.91): C, 62.78; H, 5.03; N, 19.97. Found: C, 62.85; H, 5.22; N, 19.59. HPLC-MS (ESI+): 422.05 (95.1%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.87–1.90 (m, 2H), 1.92–1.96 (m, 4H), 2.01–2.23 (m, 2H), 3.21 (s, 3H, CH_3), 4.78 (qui, $J = 7.0$ Hz, 1H, CH), 6.58 (d, $J = 8.0$ Hz, 1H, ArH), 8.01 (s, 1H, CH), 7.85 (d, $J = 9.0$ Hz, 2H, ArH), 8.42 (d, $J = 8.0$ Hz, 1H, ArH), 9.58 (bs, 1H, NH).

*4.2.4.8. 2-chloro-9-cyclopentyl-N-(4-(thiazol-2-yloxy)phenyl)-9H-purin-6-amine (**9h**).* Yield: 97%, mp: 143–144 °C. Elemental analysis: Calcd for $C_{19}H_{17}ClN_6OS$ (412.90): C, 55.27; H, 4.15; N, 20.35. Found: C, 55.45; H, 3.89; N, 20.01. HPLC-MS (ESI+): 412.88/414.92 (93.45%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.87–1.92 (m, 2H), 1.92–1.99 (m, 4H), 2.01–2.23 (m, 2H), 4.58 (qui, $J = 7.5$ Hz, 1H, CH), 6.85 (d, $J = 9.0$ Hz, 2H, ArH), 7.67 (d, $J = 9.0$ Hz, 2H, ArH), 7.96 (s, 1H, CH), 9.12 (s, 1H, ArH), 9.22 (s, 1H, ArH).

*4.2.4.9. (2-Chloro-9-cyclopentyl-9H-purin-6-yl)-[4-(pyrimidin-2-yloxy)-phenyl]-amine (**9i**).* Yield: 65%, mp: 133–135 °C. Elemental analysis: Calcd for $C_{20}H_{18}ClN_7O$ (407.87): C, 58.90; H, 4.45; N, 24.04. Found: C, 58.61; H, 4.72; N, 24.28. HPLC-MS (ESI+): 408.92 (96.8%).^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.87–1.90 (m, 2H), 1.92–1.96 (m, 4H), 2.01–2.23 (m, 2H), 4.78 (qui, $J = 7.0$ Hz, 1H, CH), 6.45 (t, $J = 5.0$ Hz, 1H, ArH), 6.74 (d, $J = 9.0$ Hz, 2H, ArH), 7.41 (d, $J = 9.0$ Hz, 2H, ArH), 8.02 (s, 1H, CH), 8.25 (d, $J = 5.0$ Hz, 2H, ArH), 9.12 (bs, 1H, NH).

*4.2.4.10. [4-(6-Bromo-pyridin-3-yloxy)-phenyl]-(2-chloro-9-cyclopentyl-9H-purin-6-yl)-amine (**9j**).* Yield: 71%, mp: 108–111 °C. Elemental analysis: Calcd for $C_{21}H_{18}BrClN_6O$ (485.77): C, 51.92; H, 3.73; N, 17.30. Found: C, 51.83; H, 3.99; N, 17.02. HPLC-MS (ESI+): 486.81 (95.1%).^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.87–1.90 (m, 2H), 1.92–1.96 (m, 4H), 2.01–2.23 (m, 2H), 4.78 (qui, $J = 7.0$ Hz, 1H, CH), 6.45 (t, $J = 5.0$ Hz, 1H, ArH), 6.74 (d, $J = 9.0$ Hz, 2H, ArH), 7.41 (d, $J = 9.0$ Hz, 2H, ArH), 8.02 (s, 1H, CH), 8.25 (d, $J = 5.0$ Hz, 2H, ArH), 9.12 (bs, 1H, NH).

*4.2.4.11. 2-chloro-9-cyclopentyl-N-(2-(morpholinomethyl)phenyl)-9H-purin-6-amine (**9k**).* Yield: 64%, m.p.: 113–116 °C. Elemental analysis: Calcd for $C_{21}H_{25}ClN_6O$ (412.92): C, 61.08; H, 6.10; N, 20.35. Found: C, 61.12; H, 6.25; N, 20.09. HPLC-MS (ESI+): 413.80 (95.8%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.62–1.71 (m, 2H), 1.80–1.88 (m, 2H), 1.91–1.98 (m, 2H), 2.10–2.17 (m, 2H), 2.40–2.46 (m, 4H), 3.66 (s, 2H, CH_2), 3.50–3.73 (m, 4H), 4.80 (qui, $J = 7.5$ Hz, 1H, CH), 7.02 (dt, $J = 7.5$ Hz, $J = 1.0$ Hz, 1H, ArH), 7.22 (dd, $J = 7.5$ Hz, $J = 1.5$ Hz, 1H, ArH), 7.33 (dt, $J = 7.5$ Hz, $J = 1.5$ Hz, 1H, ArH), 8.33 (d, $J = 7.5$ Hz, 1H, ArH), 8.39 (s, 1H, CH), 11.59 (s, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.18, 32.59, 53.02, 56.13, 61.51, 66.42, 120.26, 120.81, 123.50, 126.47, 128.52, 130.60, 138.96, 141.85, 150.79, 152.41, 152.63.

*4.2.4.12. 2-chloro-9-cyclopentyl-N-(3-(morpholinomethyl)phenyl)-9H-purin-6-amine (**9l**).* Yield: 79%, mp: 169–171 °C. Elemental analysis: Calcd for $C_{21}H_{25}ClN_6O$ (412.92): C, 61.08; H, 6.10; N, 20.35. Found: C, 61.46; H, 5.87; N, 20.62. HPLC-MS (ESI+): 413.78 (98.43%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm:1.63–1.71 (m, 2H), 1.80–1.88 (m, 2H), 1.91–1.98 (m, 2H), 2.11–2.17 (m, 2H), 2.32–2.40 (m, 4H), 3.43 (s, 2H, CH_2), 3.54–3.58 (m, 4H), 4.80 (qui, $J = 7.5$ Hz, 1H, CH), 6.98 (d, $J = 7.5$ Hz, 1H, ArH), 7.26 (t, $J = 8.0$ Hz, 1H, ArH), 7.65 (dd, $J = 8.0$ Hz, $J = 1.5$ Hz, 1H, ArH), 7.76 (t, $J = 7.5$ Hz, 1H, ArH), 8.37 (s, 1H, CH), 10.22 (s, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.05, 32.56, 53.67, 56.09, 62.92, 66.79, 119.66, 120.50, 122.40, 124.52, 128.81, 138.65, 139.17, 141.30, 151.22, 152.55, 152.95, 162.84.

*4.2.4.13. [4-(4-Benzyl-piperazin-1-yl)-phenyl]-(2-chloro-9-cyclopentyl-9H-purin-6-yl)-amine (**9m**).* Yield: 79%, mp: 181–182 °C. Elemental analysis: Calcd.for $C_{27}H_{30}ClN_7$ (488.03): C, 66.45; H, 6.20; N, 20.09. Found: C, 66.71; H, 6.41; N, 19.84. HPLC-MS (ESI+): 490.6 (99.9%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ ppm

1.62–1.70 (m, 2H), 1.79–1.86 (m, 2H), 1.89–1.97 (m, 2H), 2.10–2.16 (m, 2H), 3.06–3.09 (m, 4H), 3.29–3.31 (m, 4H), 3.49 (s, 2H), 4.78 (qui, $J = 7.5$ Hz, 1H), 6.88 (d, $J = 9.0$ Hz, 2H), 7.21–7.25 (m, 1H), 7.27–7.31 (m, 4H), 7.57 (d, $J = 9.0$ Hz, 2H), 8.32 (s, 1H), 10.03 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 24.03, 32.56, 49.14, 53.09, 56.01, 62.60, 115.99, 119.38, 123.13, 127.51, 128.74, 129.46, 130.91, 138.61, 140.84, 148.10, 150.85, 152.76, 152.99.

*4.2.4.14. 3-(2-chloro-6-((2-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)propan-1-ol (**9n**).* Yield: 89%, mp: 187–189 °C. Elemental analysis: Calcd for $C_{19}H_{23}ClN_6O_2$ (402,88): C, 56.64; H, 5.75; N, 20.86. Found: C, 56.87; H, 5.41; N, 20.84. HPLC-MS (ESI+): 403,68 (95.7%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.93 (qui, $J = 6.0$ Hz, 2H, CH_2), 2.40–2.45 (m, 4H), 3.39 (q, $J = 6.0$ Hz, 2H, CH_2), 3.66 (s, 2H, CH_2), 3.73–3.75 (m, 4H), 4.19 (t, $J = 7.0$ Hz, 2H, CH_2), 4.64 (t, $J = 5.0$ Hz, 1H, OH), 7.02 (td, $J = 7.5$ Hz, $J' = 1.0$ Hz, 1H, ArH), 7.22 (dd, $J = 8.0$ Hz, $J' = 1.5$ Hz, 1H, ArH), 7.33 (td, $J = 7.8$ Hz, 1H, ArH), 8.28 (s, 1H, CH), 8.34 (d, $J = 8.0$ Hz, 1H, ArH), 11.59 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 32.75, 41.30, 53.01, 58.17, 61.68, 66.41, 119.91, 120.78, 123.50, 126.46, 128.52, 130.60, 138.96, 143.52, 150.92, 152.38, 152.79.

*4.2.4.15. 3-(2-chloro-6-((3-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)propan-1-ol (**9o**).* Yield: 81%, mp: 114–116 °C. Elemental analysis: Calcd for $C_{19}H_{23}ClN_6O_2$ (402,88): C, 56.64; H, 5.75; N, 20.86. Found: C, 56.31; H, 5.41; N, 20.64. HPLC-MS (ESI+): 403,65 (98.4%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.93 (qui, $J = 6.0$ Hz, 2H, CH_2), 2.33–2.39 (m, 4H), 3.39 (q, $J = 6.0$ Hz, 2H, CH_2), 3.43 (s, 2H, CH_2), 3.55–3.57 (m, 4H), 4.19 (t, $J = 7.0$ Hz, 2H, CH_2), 4.63 (t, $J = 5.0$ Hz, 1H, OH), 6.98 (d, $J = 7.5$ Hz, 1H, ArH), 7.26 (t, $J = 7.5$ Hz, 1H, ArH), 7.65 (d, $J = 8.5$ Hz, 1H, ArH), 7.76 (s, 1H, ArH), 8.27 (s, 1H, CH), 10.22 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 32.73, 41.26, 53.67, 58.17, 62.92, 66.79, 119.36, 120.51, 122.42, 124.54, 128.81, 138.66, 139.16, 143.01, 151.36, 152.71, 152.93.

*4.2.4.16. 3-(2-chloro-6-((4-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)propan-1-ol (**9p**).* Yield: 86%, mp: 187–189 °C. Elemental analysis: Calcd for $C_{19}H_{23}ClN_6O_2$ (402.88): C, 56.64; H, 5.75; N, 20.86. Found: C, 56.85; H, 6.01; N, 20.69. HPLC-MS (ESI+): 403.69 (98%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.93 (qui, $J = 6.0$ Hz, 2H, CH_2), 2.28–2.33 (m, 4H), 3.34–3.42 (m, 4H), 3.50–3.56 (m, 4H), 4.19 (t, $J = 7.0$ Hz, 2H, CH_2), 4.63 (t, $J = 4.5$ Hz, 1H, OH), 7.23 (d, $J = 8.5$ Hz, 2H, ArH), 7.73 (d, $J = 8.5$ Hz, 2H, ArH), 8.27 (s, 1H, CH), 10.23 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 32.72, 41.26, 53.68, 58.17, 62.57, 66.74, 119.33, 121.60, 129.64, 133.35, 138.16, 142.99, 151.29, 152.74, 152.85.

*4.2.4.17. 3-(6-((4-(4-benzylpiperazin-1-yl)phenyl)amino)-2-chloro-9H-purin-9-yl)propan-1-ol (**9q**).* Yield: 79%, mp: 158–160 °C. Elemental analysis: Calcd for $C_{25}H_{28}ClN_7O$ (478.00): C, 62.82; H, 5.90; N, 20.51. Found: C, 62.93; H, 5.62; N, 20.12. HPLC-MS (ESI+): 478.84 (97%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.92 (qui, $J = 6.0$ Hz, 2H, CH_2), 3.07–3.10 (m, 4H), 3.38 (q, $J = 6.0$ Hz, 2H, CH_2), 3.49 (s, 2H, CH_2), 4.17 (t, $J = 7.0$ Hz, 2H, CH_2), 4.63 (t, $J = 5.0$ Hz, 1H, OH), 6.89 (d, $J = 9.0$ Hz, 2H, ArH), 7.21–7.32 (m, 5H, ArH), 7.57 (d, $J = 9.0$ Hz, 2H, ArH), 8.21 (s,1H, CH), 10.02 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 32.72, 41.26, 53.68, 58.17, 62.57, 66.74, 119.33, 121.60, 129.64, 133.35, 138.16, 142.99, 151.29, 152.74, 152.85.

*4.2.4.18. 3-(2-chloro-6-((3-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)cyclopentan-1-ol (**9r**).* Yield: 92%, mp: 187–189 °C. Elemental analysis: Calcd for $C_{21}H_{25}ClN_6O_2$ (428.92): C, 58.81; H, 5.88; N, 19.59. Found: C, 58.49; H, 5.51; N, 19.42. HPLC-MS (ESI+): 430.00 (97.4%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.72–1.78 (m, 2H), 1.83–1.87 (m, 1H), 1.98–2.05 (m, 1H), 2.14–2.21 (m, 1H), 2.38–2.43 (m, 1H), 3.32–3.36 (m, 4H), 3.41 (s, 2H, CH_2), 3.49 (t, $J = 4.5$ Hz, 1H, CH), 3.50–3.54 (m, 4H), 4.88 (qui, $J = 6.5$ Hz, 1H, CH), 4.97 (bs, 1H, OH), 6.49 (s, 1H, ArH), 6.98 (d, $J = 7.5$ Hz, 1H, ArH), 7.26 (t, $J = 7.5$ Hz, 1H, ArH), 7.65 (d, $J = 7.5$ Hz, 1H, ArH), 8.41 (s, 1H, CH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 31.69, 34.61, 42.20, 53.78, 66.71, 70.82, 113.24, 114.91, 117.04, 119.39, 120.50, 122.40, 123.05, 124.51, 133.66, 139.18, 141.23, 149.08, 151.11.

*4.2.4.19. 3-(2-chloro-6-((4-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)cyclopentan-1-ol (**9s**).* Yield: 84%, mp: 195–196 °C. Elemental analysis: Calcd for $C_{21}H_{25}ClN_6O_2$ (428,92): C, 58.81; H, 5.88; N, 19.59. Found: C, 58.64; H, 5,93; N, 19.57. HPLC-MS (ESI+): 429,84 (96.7%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.74–1.80 (m, 2H), 1.84–1.88 (m, 1H), 1.99–2.07 (m, 1H), 2.16–2.23 (m, 1H), 2.36–2.42 (m, 1H), 3.32–3.36 (m, 4H), 3.38 (s, 2H, CH_2), 3.49 (t, $J = 4.5$ Hz, 1H, CH), 3.52–3.53 (m, 4H), 4.87 (quid, $J = 6.0$ Hz, $J' = 2.0$ Hz, 1H, CH), 4.98 (bs, 1H, OH), 7.23 (d, $J = 8.5$ Hz, 2H, ArH), 7.74 (d, $J = 8.5$ Hz, 2H, ArH), 8.41 (s, 1H, CH), 10.25 (s, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 31.60, 34.62, 42.10, 53.72, 62.56, 66.72, 70.84, 114.11, 119.35, 121.56, 129.63, 130.40, 133.27, 138.19, 141.40, 151.09, 152.64.

*4.2.4.20. 3-(2-((4-aminocyclohexyl)amino)-6-((2-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)propan-1-ol (**9t**).* Yield: 88%, mp: 252–254 °C. Elemental analysis: Calcd for $C_{25}H_{36}N_8O_2$ (480.62): C, 62.48; H, 7.55; N, 23.32. Found: C, 62.11; H, 7.74; N, 23.02. HPLC-MS (ESI+): 481.62 (99.9%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.11–1.28 (m, 4H), 1.74–1.97 (m, 6H), 2.43 (m, 4H), 2.51–2.60 (m, 1H, CH), 3.33–3.37 (m, 4H), 3.57–3.61 (m, 1H, CH), 3.63 (s, 2H, CH_2), 3.73–3.75 (m, 4H), 4.02 (t, $J = 6.5$ Hz, 2H, CH_2), 4.64 (bs, 1H, OH), 6.53 (bs, 1H, NH), 6.91 (td, $J = 7.25$ Hz, $J' = 1.5$ Hz, 1H, ArH), 7.77 (s, 1H, CH), 8.66 (d, $J = 8.0$ Hz, 1H, ArH), 10.92 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 29.55, 31.51, 31.82, 32.85, 33.75, 50.09, 53.76, 58.14, 63.31, 66.76, 114.12, 119.26, 121.00, 122.84, 128.55, 138.40, 138.80, 140.80, 141.01, 152.51, 158.88.

4.2.5. General procedure for the preparation of compounds 10, 11d-11i, 11k, 12a-12g, 13a-13e, 14a-14r

The mixture of 2-chloro-9-substituted-9*H*-purin-6-subst.amino derivative (**9**) (1.00 mmol) and corresponding primary amine or morpholine (10.0 mmol) in 1,2-ethanediol (5.0 mL) was heated with stirring at 160 °C for 4 h under an argon atmosphere. After cooling to room temperature, the mixture was diluted with ethyl acetate (40 mL) and washed with water (40 mL). The organic phase was washed with brine, dried over sodium sulphate and concentrated under reduced pressure. The crude product was purified by column chromatography on silica using mobile phase chloroform/methanol/concentrated ammonium hydroxide (4:1:0.025, v/v) in case of derivatives **10**, **11d-11i**, **11k**, **12a**, **12b**, **12e-12g**, **13a**, **13b**, **13d**, **14a-14d**, **14f**, **14g**, **14i-14k**, **14m**, **14n** and **14p-14t** or chloroform/methanol/concentrated ammonium hydroxide (9:1:0.05, v/v) in case of derivatives **12c-12e**, **13e**, **14e**, **14h**, **14l**, **14o** and **14r**.

*4.2.5.1. N^2-(4-amino-cyclohexyl)-N^6-(4-bromo-phenyl)-9-cyclopentyl-9H-purine-2,6-diamine (**10**).* Yield: 79%, mp: 162–164 °C. Elemental analysis: Calcd. For $C_{22}H_{28}BrN_7$ (470.42): C, 56.17; H, 6.00; N, 20.84. Found: C, 56.39; H, 5.63; N, 20.51. HPLC-MS (ESI+): 473.0 (99.8%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.09–1.32 (m, 4H), 1.64–1.68 (m, 2H), 1.78–2.08 (m, 12H), 2.57 (m, 1H), 3.58–3.61 (m, 1H), 4.69 (qui, $J = 7.11$ Hz, 1H), 6.50 (d, $J = 7.41$ Hz, 1H), 7.42 (d, $J = 8.85$ Hz, 2H), 7.90 (s, 1H), 8.04 (d, $J = 8.85$ Hz, 2H), 9.58 (bs, 1H). ^{13}C NMR (76 MHz, DMSO-d_6) δ ppm 24.18, 24.48, 31.77, 32.28, 35.67, 49.12, 50.63, 133.53, 122.22, 123.80, 131.42, 137.46, 140.55, 150.85, 152.26, 158.52.

*4.2.5.2. N^2-(4-Amino-cyclohexyl)-9-cyclopentyl-N^6-(4-pyrazol-1-yl-phenyl)-9H-purine-2,6-diamine (**11d**).* Yield: 79%, mp: 247–248 °C. Elemental analysis: Calcd for $C_{25}H_{31}N_9$ (457.59): C, 65.62; H, 6.83; N, 27.55. Found: C, 65.41; H, 6.99; N, 27.19. HPLC-MS (ESI+): 458.64 (96.2%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.10–1.18 (m, 2H), 1.27–1.36 (m, 2H), 1.61–1.70 (m, 2H), 1.77–1.89 (m, 6H), 1.93–2.00 (m, 2H), 2.11–2.17 (m, 2H), 2.80–2.90 (m, 1H, CH), 3.27–3.33 (m, 1H, CH), 4.88 (qui, J = 7.5 Hz, 1H, CH), 5.78 (bs, 2H, NH_2), 6.38 (t, J = 2.0 Hz, 1H, ArH), 6.66 (d, J = 9.0 Hz, 2H, ArH), 7.42 (d, J = 9.0 Hz, 2H, ArH), 7.56 (d, J = 1.5 Hz, 1H, ArH), 8.17 (d, J = 1.5 Hz, 1H, ArH), 8.17 (d, J = 2.5 Hz, 1H, ArH), 8.80 (s, 1H, CH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.05, 28.87, 32.35, 33.37, 49.18, 56.94, 68.07, 107.92, 119.12, 119.57, 120.22, 122.35, 127.93, 131.33, 140.89, 147.63, 150.00, 151.16, 152.71, 153.80.

*4.2.5.3. N^2-(4-Amino-cyclohexyl)-9-cyclopentyl-N^6-(4-[1,2,4]triazol-4-yl-phenyl)-9H-purine-2,6-diamine (**11e**).* Yield: 84%, mp: 212–215 °C. Elemental analysis: Calcd for $C_{24}H_{30}N_{10}$ (458.57): C, 62.86; H, 6.59; N, 30.54. Found: C, 62.94; H, 6.71; N, 30.23. HPLC-MS (ESI+): 459.62 (97.4%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.09–1.17 (m, 2H), 1.28–1.36 (m, 2H), 1.61–1.69 (m, 2H), 1.78–1.89 (m, 4H), 1.92–2.00 (m, 2H), 2.11–2.17 (m, 2H), 2.80–2.90 (m, 1H, CH), 3.27–3.33 (m, 1H, CH), 4.88 (qui, J = 7.5 Hz, 1H, CH), 5.54 (bs, 2H, NH_2), 6.64 (d, J = 9.0 Hz, 2H, ArH), 7.39 (d, J = 9.0 Hz, 2H, ArH), 8.05 (s, 1H, CH), 8.11 (s, 2H, ArH), 8.79 (s, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.07, 28.86, 32.34, 33.36, 49.08, 56.94, 68.07, 116.18, 120.35, 121.52, 122.30, 128.29, 131.31, 141.99, 147.61, 150.03, 152.22, 153.78.

*4.2.5.4. N^6-(4-(1H-1,2,4-triazol-1-yl)phenyl)-N^2-(4-aminocyclohexyl)-9-cyclopentyl-9H-purine-2,6-diamine (**11f**).* Yield: 67%, mp: 221–223 °C. Elemental analysis: Calcd for $C_{24}H_{30}N_{10}$ (458.57): C, 62.86; H, 6.59; N, 30.54. Found: C, 62.95; H, 6.41; N, 30.28. HPLC-MS (ESI+): 459.89 (98.76%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.17–1.27 (m, 4H), 1.58–1.68 (m, 4H), 1.76–1.84 (m, 4H), 1.92–1.98 (m, 4H), 2.02–2.12 (m, 2H), 2.59–2.69 (m, 1H), 3.39 (bs, 2H, NH_2), 3.58–3.66 (m, 1H), 4.67 (qui, J = 6.0 Hz, 1H, CH), 6.55 (d, J = 6.5 Hz, 1H, NH), 7.70 (d, J = 8.0 Hz, 2H, ArH), 7.89 (s, 1H, ArH), 8.17 (s, 1H, CH), 8.20 (d, J = 8.0 Hz, 2H, ArH), 9.19 (s, 1H, ArH), 9.64 (bs, 1H, NH).

*4.2.5.5. N^2-(4-Amino-cyclohexyl)-9-cyclopentyl-N^6-(6-thiophen-2-yl-pyridin-3-yl)-9H-purine-2,6-diamine (**11g**).* Yield: 68%, mp: 233–235 °C. Elemental analysis: Calcd for $C_{25}H_{30}N_8S$ (474.64): C, 63.27; H, 6.37; N, 23.61. Found: C, 63.59; H, 6.12; N, 23.14. HPLC-MS (ESI+): 475.62 (97.2%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.64–1.72 (m, 2H), 1.81–1.89 (m, 2H), 1.92–2.00 (m, 2H), 2.12–2.19 (m, 2H), 4.83 (qui, J = 7.0 Hz, 1H, NH), 7.60 (dd, J = 5.0 Hz, J' = 3.0 Hz, 1H, ArH), 7.86 (d, J = 9.0 Hz, 1H, ArH), 8.07 (dd, J = 3.0 Hz, J' = 1.5 Hz, 1H, ArH), 7.86 (d, J = 9.0 Hz, 1H, ArH), 8.07 (dd, J = 3.0 Hz, J' = 1.5 Hz, 1H, ArH), 8.27 (dd, J = 9.0 Hz, J' = 3.0 Hz, 1H, ArH), 8.43 (s, 1H, CH), 9.00 (d, J = 2.5 Hz, 1H, ArH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.06, 32.56, 56.19, 119.83, 120.39, 123.4, 126.71, 127.48, 129.23, 134.77, 141.81, 142.20, 142.72, 148.14, 151.48, 152.43, 152.71.

*4.2.5.6. N^2-(4-aminocyclohexyl)-9-cyclopentyl-N^6-(6-(furan-3-yl) pyridin-3-yl)-9H-purine-2,6-diamine (**11h**).* Yield: 74%, mp: 256–258 °C. Elemental analysis: Calcd for $C_{25}H_{30}N_8O$ (458.57): C, 65.48; H, 6.59; N, 24.44. Found: C, 65.62; H, 6.84; N, 24.11. HPLC-MS (ESI+): 459.62 (98.6%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.10–1.28 (m, 4H), 1.59–1.68 (m, 2H), 1.76–1.89 (m, 4H), 1.92–1.98 (m, 4H), 2.03–2.11 (m, 2H), 2.54–2.59 (m, 1H, CH), 3.55–3.62 (m, 1H, CH), 4.67 (qui, J = 7.0 Hz, 1H, CH), 6.97 (d, J = 1.5 Hz, 1H, ArH), 7.57 (d, J = 8.5 Hz, 1H, ArH), 7.71 (t, J = 1.5 Hz, 1H, ArH), 7.88 (s, 1H, ArH), 8.20 (s, 1H, ArH), 8.42 (d, J = 6.5 Hz, 1H, ArH), 9.13 (bs, 1H, NH), 9.65 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.54, 31.57, 32.25, 35.02, 50.40, 55.66, 109.23, 114.76, 119.97, 127.37, 127.67, 136.21, 137.65, 141.03, 141.84, 144.59, 144.69, 152.80, 158.51, 164.84.

*4.2.5.7. N^2-(4-aminocyclohexyl)-9-cyclopentyl-N^6-(6-(2-methoxyphenyl)pyridin-3-yl)-9H-purine-2,6-diamine (**11i**).* Yield: 84%, mp: 258–259 °C. Elemental analysis: Calcd for $C_{28}H_{34}N_8O$ (498.63): C, 67.45; H, 6.87; N, 22.47. Found: C, 67.09; H, 7.02; N, 22.69. HPLC-MS (ESI+): 499.41 (99.12%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.11–1.28 (m, 4H), 1.60–1.68 (m, 2H), 1.77–1.90 (m, 4H), 1.95–1.98 (m, 4H), 2.04–2.09 (m, 2H), 2.53–2.60 (m, 1H), 3.54–3.64 (m, 1H), 3.81 (s, 3H, CH_3), 4.67 (qui, J = 6.0 Hz, 1H, CH), 6.57 (d, J = 7.5 Hz, 1H, ArH), 7.01 (t, J = 7.0 Hz, 1H, ArH), 7.09 (d, J = 8.0 Hz, 1H, ArH), 7.32 (td, J = 7.75 Hz, J' = 1.5 Hz, 1H, ArH), 7.72 (d, J = 7.5 Hz, 1H, ArH), 7.76 (d, J = 9.0 Hz, 1H, ArH), 7.89 (s, 1H, ArH), 8.50 (s, 1H, CH), 9.16 (bs, 1H, NH), 9.70 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 31.64, 32.30, 35.14, 50.43, 55.68, 56.07, 112.39, 114.66, 117.00, 121.13, 124.48, 126.74, 128.77, 129.88, 130.80, 136.16, 137.68, 141.64, 148.32, 152.35, 157.14, 158.52.

*4.2.5.8. N^2-(4-aminocyclohexyl)-9-cyclopentyl-N^6-(6-(4-methoxyphenyl)pyridin-3-yl)-9H-purine-2,6-diamine (**11k**).* Yield: 70%, mp: 228–230 °C. Elemental analysis: Calcd for $C_{28}H_{34}N_8O$ (498.62): C, 67.45; H, 6.87; N, 22.47. Found: C, 67.14; H, 6.95; N, 22.31. HPLC-MS (ESI+): 498,98 (97,70%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.14–1.68 (m, 4H), 1.59–1.68 (m, 2H), 1.75–2.01 (m, 8H), 2.02–2.11 (m, 2H), 2.52–2.57 (m, 1H, CH), 3.54–3.62 (m, 1H, CH), 3.76 (s, 3H, CH_3), 4.67 (qui, J = 7.0 Hz, 1H, CH), 6.55 (d, J = 7.50 Hz, 1H, ArH), 7.88 (s, 1H, CH), 7.95 (d, J = 9.0 HZ, 2H, ArH), 8.46 (d, J = 8.0 Hz, 1H, ArH), 9.17 (bs, 1H, NH), 9.67 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.29, 24.49, 24.81, 31.69, 32.26, 35.28, 50.47, 55.69, 63.29, 114.58, 119.12, 127.86, 131.84, 136.18, 137.70, 141.72, 149.35, 152.30, 158.54, 159.99.

*4.2.5.9. N^2-(4-aminocyclohexyl)-9-cyclopentyl-N^6-(4-(thiazol-2-yloxy)phenyl)-9H-purine-2,6-diamine (**12a**).* Yield: 85%, mp: 189–191 °C. Elemental analysis: Calcd for $C_{25}H_30N_8OS$ (490.63): C, 61.20; H, 6.16; N, 22.84. Found: C, 61.48; H, 5.93; N, 22.56. HPLC-MS (ESI+): 490.92 (99,47%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.08–1.26 (m, 4H), 1.59–1.67 (m, 2H), 1.73–1.80 (m, 2H), 1.82–2.05 (m, 6H), 2.07–2.14 (m, 2H), 2.48–2.53 (m, 1H, CH), 3.54–3.61 (m, 1H, CH), 4.66 (qui, J = 6.5 Hz, 1H, CH), 6.45 (d, J = 8.0 Hz, 1H, NH), 7.13 (d, J = 3.5 Hz, 1H, ArH), 7.22 (d, J = 9.0 Hz, 2H, ArH), 7.24 (d, J = 3.5 Hz, 1H, ArH), 7.87 (s, 1H, CH), 8.08 (d, J = 9.0 Hz, 2H, ArH), 9.54 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.21, 31.75, 32.28, 32.49, 35.55, 50.50, 55.40, 63.30, 114.64, 120.87, 121.66, 137.33, 138.13, 139.30, 149.94, 152.32, 158.57, 174.79.

*4.2.5.10. N^2-(4-aminobutyl)-9-cyclopentyl-N^6-(4-(thiazol-2-yloxy) phenyl)-9H-purine-2,6-diamine (**12b**).* Yield: 84%, mp: 174–176 °C. Elemental analysis: Calcd for $C_{23}H_{28}N_8OS$ (464.59): C, 59.46; H, 6.08; N, 24.12. Found: C, 59.13; H, 6.23; N, 23.87. HPLC-MS (ESI+): 465.83 (99.6%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.54–1.61 (m, 4H), 1.62–1.69 (m, 2H), 1.81–1.89 (m, 2H), 1.93–1.99 (m, 2H), 2.06–2.12 (m, 2H), 2.48–2.50 (m, 2H), 2.72–2.80 (m, 2H), 4.69 (qui, J = 7.0 Hz, 1H, CH), 6.81 (bs, 1H, NH), 7.15 (d, J = 3.5 Hz, 1H, ArH), 7.24-.726 (m, 3H, ArH), 7.66 (bs, 2H, NH_2), 8.02 (s, 1H, CH), 8.06 (d, J = 9.0 Hz, 2H, ArH), 8.70 (bs, 1H, NH).

*4.2.5.11. 3-((9-cyclopentyl-6-((4-(thiazol-2-yloxy)phenyl)amino)-9H-purin-2-yl)amino)propan-1-ol (**12c**).* Yield: 32%, mp: 169–172 °C. Elemental analysis: Calcd for $C_{22}H_{25}N_7O_2S$ (451.55): C, 58.52; H, 5.58; N, 21.71. Found: C, 58.69; H, 5.54; N, 21.53. HPLC-MS (ESI+): 452.79 (99.09%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.59–1.71 (m, 2H), 1.81–1.89 (m, 2H), 1.92–1.98 (m, 2H), 2.03–2.11

(m, 2H), 3.28−3.35 (m, 2H), 3.45 (q, $J = 6.0$ Hz, 2H, CH_2), 4.44 (t, $J = 5.5$ Hz, 1H, OH), 4.67 (qui, $J = 7.5$ Hz, 1H, CH), 6.62 (t, $J = 5.5$ Hz, 1H, NH), 7.14 (d, $J = 3.5$ Hz, 1H, ArH), 7.21 (d, $J = 9.5$ Hz, 2H, ArH), 7.24 (d, $J = 3.5$ Hz, 1H, ArH), 7.89 (s, 1H, CH), 8.10 (d, $J = 9.5$ Hz, 2H, ArH), 9.54 (bs, 1H, NH).^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.24., 32.34, 33.05, 55.36, 59.40, 65.46, 114.57, 114.65, 120.84, 121.77, 137.16, 138.13, 139.21, 150.00, 152.28, 152.38, 159.35, 174.74.

*4.2.5.12. 4-((9-cyclopentyl-6-((4-(thiazol-2-yloxy)phenyl)amino)-9H-purin-2-yl)amino)butan-1-ol (**12d**).* Yield: 24% mp: 141−142 °C. Elemental analysis: Calcd for $C_{23}H_{27}N_7O_2S$ (465.58): C, 59.34; H, 5.85; N, 21.06. Found: C, 59.55; H, 5.41; N, 21.17. HPLC-MS (ESI+): 466.80 (96.92%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm. 1.44 (qui, $J = 6.5$ Hz, 2H, CH_2), 1.55 (qui, $J = 6.5$ Hz, 2H, CH_2), 1.61−1.68 (m, 2H), 1.81−1.89 (m, 2H), 1.92−1.99 (m, 2H), 2.04−2.11 (m, 2H), 3.24 (q, $J = 6.0$ Hz, 2H, CH_2), 3.37 (q, $J = 5.5$ Hz, 2H, CH_2), 4.35 (t, $J = 5.5$ Hz, 1H, OH), 4.67 (qui, $J = 7.5$ Hz, 1H, CH), 6.66 (t, $J = 5.5$ Hz, 1H, NH), 7.14 (d, $J = 4.0$ Hz, 1H, ArH), 7.22 (dd, $J = 9.0$ Hz, $J = 2.0$ Hz, 2H, ArH), 7.24 (d, $J = 4.0$ Hz, 1H, ArH), 7.88 (s, 1H, CH), 8.08 (d, $J = 9.0$ Hz, 2H, ArH), 9.53 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.28, 26.69, 30.77, 32.31, 41.80, 55.42, 61.38, 114.56, 120.57, 121.73, 137.20, 138.13, 139.25, 149.97, 152.20, 152.37, 159.19, 159.31, 174.76.

*4.2.5.13. 9-cyclopentyl-2-morpholino-N-(4-(thiazol-2-yloxy) phenyl)-9H-purin-6-amine (**12e**).* Yield: 74%, mp: 153−155 °C. Elemental analysis: Calcd for $C_{23}H_{25}N_7O_2S$ (463.56): C, 59.59; H, 5.44; N, 21.15. Found: C, 60.13; H, 5.12; N, 21.07. HPLC-MS (ESI+): (98.10%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.59−1.68 (m, 2H), 1.79−1.87 (m, 2H), 1.90−1.97 (m, 2H), 2.05−2.11 (m, 2H), 3.56−3.65 (m, 8H), 4.69 (qui, $J = 7.5$ Hz, 1H, CH), 6.66 (d, $J = 9.0$ Hz, 2H, ArH), 7.54 (d, $J = 9.0$ Hz, 2H, ArH), 7.91 (s, 1H, CH), 9.07 (s, 1H, ArH), 9.22 (s, 1H, ArH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.30, 32.14, 45.38, 55.39, 66.74, 114.64, 115.27, 122.94, 131.89, 137.67, 151.66, 152.36, 153.23, 158.80.

*4.2.5.14. N^2-(4-aminocyclohexyl)-9-cyclopentyl-N^6-(4-(pyrimidin-2-yloxy)phenyl)-9H-purine-2,6-diamine (**12f**).* Yield: 59%, mp: 171−173 °C. Elemental analysis: Calcd for $C_{26}H_{31}N_9O$ (485.60): C, 64.31; H, 6.43; N, 25.96. Found: C, 64.02; H, 6.75; N, 25.74. HPLC-MS (ESI+): (96.05%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.06−1.25 (m, 4H), 1.58−1.66 (m, 2H), 1.72−1.83 (m, 4H), 1.90−1.97 (m, 4H), 2.01−2.11 (m, 2H), 3.52−3.59 (m, 1H, CH), 2.51−2.60 (m, 1H, CH), 4.63 (qui, $J = 6.5$ Hz, 1H, CH), 6.26 (bs, 1H, NH), 6.46 (t, $J = 5.0$ Hz, 1H, ArH), 6.65 (d, $J = 9.0$ Hz, 2H, ArH), 7.69 (d, $J = 9.0$ Hz, 2H, ArH), 7.80 (s, 1H, CH), 8.19 (d, $J = 5.0$ Hz, 2H, ArH), 9.04 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.19, 31.69, 32.30, 35.11, 49.42, 50.42, 55.30, 110.13, 114.30, 115.21, 122.24, 132.55, 136.68, 151.97, 152.59, 152.91, 158.39, 158.68, 162.23.

*4.2.5.15. N^2-(4-aminocyclohexyl)-N^6-(4-((5-bromopyridin-2-yl)oxy) phenyl)-9-cyclopentyl-9H-purine-2,6-diamine (**12g**).* Yield: 77%, mp: 231−233 °C. Elemental analysis: Calcd for $C_{27}H_{31}BrN_8O$ (563.50): C, 57.55; H, 5.55; N, 19.89. Found: C, 57.36; H, 5.28; N, 20.12. HPLC-MS (ESI+): 564.81 (%).^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.14−1.68 (m, 4H), 1.59−1.68 (m, 2H), 1.75−2.01 (m, 8H), 2.02−2.11 (m, 2H), 2.52−2.57 (m, 1H, CH), 3.54−3.62 (m, 1H, CH), 3.76 (s, 3H, CH_3), 4.67 (qui, $J = 7.0$ Hz, 1H, CH), 6.55 (d, $J = 7.50$ Hz, 1H, ArH), 7.88 (s, 1H, CH), 7.95 (d, $J = 9.0$ HZ, 2H, ArH), 8.46 (d, $J = 8.0$ Hz, 1H, ArH), 9.17 (bs, 1H, NH), 9.67 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.29, 24.49, 24.81, 31.69, 32.26, 35.28, 50.47, 55.69, 63.29, 114.58, 119.12, 127.86, 131.84, 136.18, 137.70, 141.72, 149.35, 152.30, 158.54, 159.99.

*4.2.5.16. N^2-(4-aminocyclohexyl)-9-cyclopentyl-N^6-(2-(morpholinomethyl)phenyl)-9H-purine-2,6-diamine (**13a**).* Yield: 88%, mp: 127−129 °C. Elemental analysis: Calcd for $C_{27}H_{38}N_8O$ (490.66): C, 66.09; H, 7.81; N, 22.84. Found: C, 66.12; H, 7.97; N, 22.56. HPLC-MS (ESI+): 491.68 (98.25%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.22−1.30 (m, 2H), 1.59−1.67 (m, 2H), 1.78−2.10 (m, 8H), 2.02−2.06 (m, 2H), 2.41 (s, 2H, CH2), 2.70−2.78 (m, 1H), 3.58−3.62 (m, 4H), 3.72−3.74 (m, 4H), 4.65 (qui, $J = 7.5$ Hz, 1H, CH), 6.46 (bs, 1H, NH), 6.90 (t, $J = 7.5$ Hz, 1H, ArH), 7.15 (d, $J = 7.0$ Hz, 1H, ArH), 7.25 (t, $J = 7.5$ Hz, 1H, ArH), 7.85 (s, 1H, CH), 8.65 (d, $J = 7.5$ Hz, 1H, ArH), 10.88 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.24, 31.23, 32.34, 32.94, 49.11, 50.02, 53.04, 55.48, 62.07, 66.43, 115.22, 120.23, 121.75, 125.21, 128.21, 130.45, 137.67, 140.45, 152.19, 158.66.

*4.2.5.17. N^2-(4-aminocyclohexyl)-9-cyclopentyl-N^6-(3-(morpholinomethyl)phenyl)-9H-purine-2,6-diamine (**13b**).* Yield: %, mp: 154−155 °C. Elemental analysis: Calcd for $C_{27}H_{38}N_8O$ (490.66): C, 66.09; H, 7.81; N, 22.84. Found: C, 66.36; H, 7.48; N, 22.97. HPLC-MS (ESI+): 492.02 (98.39%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.46−1.52 (m, 2H), 1.56−1.62 (m, 2H), 1.66−1.70 (m, 2H), 1.84−1.91 (m, 2H), 1.94−2.01 (m, 2H), 2.07−2.13 (m, 2H), 2.33−2.39 (m, 4H), 3.31 (q, $J = 6.5$ Hz, 2H, CH_2), 3.41 (q, $J = 6.5$ Hz, 2H, CH2), 3.43 (s, 2H, CH_2), 3.56−3.58 (m, 4H), 4.38 (t, $J = 5.0$ Hz, 1H, CH), 4.69 (qui, $J = 7.0$ Hz, 1H, CH), 6.60 (bs, 1H, NH), 6.91 (d, $J = 7.0$ Hz, 1H, ArH), 7.21 (t, $J = 7.0$ Hz, 1H, ArH), 7.82−7.90 (m, 2H, ArH), 9.31 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.20, 26.56, 30.90, 32.32, 41.94, 53.78, 55.33, 61.26, 63.36, 66.76, 119.17, 121.08, 122.76, 128.55, 136.89, 138.50, 140.88, 152.55, 159.47.

*4.2.5.18. N^2-(4-aminobutyl)-9-cyclopentyl-N^6-(2-(morpholinomethyl)phenyl)-9H-purine-2,6-diamine (**13c**).* Yield: %, mp: 167−169 °C. Elemental analysis: Calcd for $C_{25}H_{36}N_8O$ (464.62): C, 64.63; H, 7.81; N, 24.12. Found: C, 64.95; H, 7.52; N. HPLC-MS (ESI+): 465.74 (98.5%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.39−1.42 (m, 2H), 1.51−1.59 (m, 2H), 1.62−1.66 (m, 2H), 1.80−1.85 (m, 2H), 1.88−1.98 (m, 2H), 2.02−2.08 (m, 2H), 2.30−2.36 (m, 4H), 2.56 (t, $J = 6.5$ Hz, 2H, CH_2), 3.24 (q, $J = 6.5$ Hz, 2H, CH_2), 3.42 (s, 2H, CH_2), 3.51−3.58 (m, 4H), 4.64 (qui, $J = 7.0$ Hz, 1H, CH), 6.42 (bs, 1H, NH), 6.85 (t, $J = 7.5$ Hz, 1H, ArH), 7.12 (d, $J = 7.5$ Hz, 1H, ArH), 7.28 (t, $J = 7.5$ Hz, 1H, ArH), 7.80 (s, 1H, CH), 8.60 (d, $J = 7.5$ Hz, 1H, ArH), 10.64 (bs, 1H, NH).

*4.2.5.19. N^2-(4-aminobutyl)-9-cyclopentyl-N^6-(3-(morpholinomethyl)phenyl)-9H-purine-2,6-diamine (**13d**).* Yield: 40%, mp: 187−189 °C. Elemental analysis: Calcd for $C_{25}H_{36}N_8O$ (464.62): C, 64.63; H, 7.81; N, 24.12. Found: C, 64.32; H, 7.51; N, 24.00. HPLC-MS (ESI+): 465.74 (98.7%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.38−1.44 (m, 2H), 1.52−158 (m, 2H), 1.60−1.68 (m, 2H), 1.80−1.87 (m, 2H), 1.90−1.98 (m, 2H), 2.04−2.10 (m, 2H), 2.30−2.36 (m, 4H), 2.60 (t, $J = 7.0$ Hz, 2H, CH_2), 3.28 (q, $J = 6.5$ Hz, 2H, CH_2), 3.35 (bs, 2H, NH_2), 3.40 (s, 2H, CH_2), 3.53−3.55 (m, 4H), 4.66 (qui, $J = 7.5$ Hz, 1H, CH), 6.58 (t, $J = 6.5$ Hz, 1H, NH), 6.88 (d, $J = 7.5$ Hz, 1H, ArH), 7.18 (t, $J = 7.5$ Hz, 1H, ArH), 7.82−7.88 (m, 2H, ArH), 7.94 (bs, 1H, NH), 9.28 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.19, 27.30, 30.30, 32.32, 41.60, 53.78, 55.33, 63.36, 66.76, 70.31, 114.62, 119.18, 121.09, 122.77, 128.55, 137.01, 138.39, 140.93, 152.55, 159.36.

*4.2.5.20. 4-((9-cyclopentyl-6-((3-(morpholinomethyl)phenyl) amino)-9H-purin-2-yl)amino)butan-1-ol (**13e**).* Yield: 84%, mp: 178−181 °C. Elemental analysis: Calcd for $C_{25}H_{35}N_7O_2$ (465.60): C, 64.49; H, 7.58; N, 21.06. Found: C, 64.87; H, 7.29; N, 20.74. HPLC-MS (ESI+): 466.93 (99.44%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.43−1.48 (m, 2H), 1.52−1.58 (m, 2H), 1.61−1.68 (m, 2H), 1.80−1.88 (m, 2H), 1.91−1.98 (m, 2H), 2.04−2.10 (m, 2H), 2.30−2.36 (m, 4H), 3.28 (q, $J = 6.5$ Hz, 2H, CH_2), 3.37 (q, $J = 6.5$ Hz, 2H), 3.40 (s, 2H, CH_2), 3.53−3.55 (m, 4H), 4.35 (t, $J = 5.0$ Hz, 1H, OH), 4.66 (qui, $J = 7.0$ Hz, 1H, CH), 6.56 (t, $J = 6.5$ Hz, 1H, NH), 6.88 (d, $J = 7.0$ Hz, 1H, ArH), 7.17

(t, $J = 8.0$ Hz, 1H, ArH), 7.82−7.82 (m, 2H, ArH), 7.94 (s, 1H, CH), 9.27 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.20, 26.56, 30.79, 32.32, 41.84, 53.11, 55.33, 61.26, 63.36, 66.76, 114.07, 114.57, 119.17, 121.08, 122.76, 128.55, 137.01, 138.38, 140.88, 152.55, 159.38.

*4.2.5.21. 3-(2-((4-aminocyclohexyl)amino)-6-((2-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)propan-1-ol (**14a**).* Yield: 78%, mp: 252−254 °C. Elemental analysis: Calcd for $C_{25}H_{36}N_8O_2$ (480.62): C, 62.48; H, 7.55; N, 23.32. Found: C, 62.15; H, 7.21; N, 23.54. HPLC-MS (ESI+): 481.62 (99.9%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.11−1.28 (m, 4H), 1.74−1.97 (m, 6H), 2.43 (m, 4H), 2.51−2.60 (m, 1H, CH), 3.33−3.37 (m, 4H), 3.57−3.61 (m, 1H, CH), 3.63 (s, 2H, CH_2), 3.73−3.75 (m, 4H), 4.02 (t, $J = 6.5$ Hz, 2H, CH_2), 4.64 (bs, 1H, OH), 6.53 (bs, 1H, NH), 6.91 (td, $J = 7.25$ Hz, $J = 1.5$ Hz, 1H, ArH), 7.16 (d, $J = 7.5$ Hz, 1H, ArH), 7.23 (td, $J = 7.75$ Hz, $J = 1.5$ Hz, 1H, ArH), 7.77 (s, 1H, CH), 8.66 (d, $J = 8.0$ Hz, 1H, ArH), 10.92 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 29.55, 31.51, 31.82, 32.85, 33.75, 50.09, 53.76, 58.14, 63.31, 66.76, 114.12, 119.26, 121.00, 122.84, 128.55, 138.40, 138.80, 140.80, 141.01, 152.51, 158.88.

*4.2.5.22. : 3-(2-((4-aminocyclohexyl)amino)-6-((3-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)propan-1-ol (**14b**).* Yield: 66%, mp: 231−233 °C. Elemental analysis: Calcd for $C_{25}H_{36}N_8O_2$ (480.62): C, 62.48; H, 7.55; N, 23.32. Found: C, 62.74; H, 7.21; N, 22.94. HPLC-MS (ESI+): 481.99 (96.7%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.19−1.27 (m, 6H), 1.82−1.90 (m, 4H), 1.92−1.96 (m, 2H), 2.28−2.36 (m, 4H), 2.65−2.69 (m, 1H, CH), 5.53−5.55 (m, 4H), 3.60−3.64 (m, 1H, CH), 4.02 (t, $J = 7.0$ Hz, 2H, CH_2), 6.49 (bs, 1H, NH), 6.70 (d, $J = 8.0$ Hz, 1H, ArH), 7.17 (t, $J = 8.0$ Hz, 1H, ArH), 7.77−7.81 (m, 2H, ArH, CH), 7.95 (d, $J = 7.5$ Hz, 1H, ArH), 9.33 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 29.55, 31.51, 31.82, 32.85, 33.75, 50.09, 53.76, 58.14, 63.31, 66.76, 114.12, 119.26, 121.00, 122.84, 128.55, 138.40, 138.80, 140.80, 141.00, 152.51, 158.88.

*4.2.5.23. 3-(2-((4-aminocyclohexyl)amino)-6-((4-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)propan-1-ol (**14c**).* Yield: 85%, mp: 108−109 °C. Elemental analysis: Calcd for $C_{25}H_{36}N_8O_2$ (480.62): C, 62.48; H, 7.55; N, 23.32. Found: C, 62.41; H, 7.16; N, 23.07. HPLC-MS (ESI+): 482.15 (99.6%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.07−1.13 (m, 2H), 1.19−1.30 (m, 4H), 1.72−1.79 (m, 2H), 1.83−1.95 (m, 4H), 2.35−2.49 (m, 4H), 3.37 (s, 2H, CH_2), 3.52−3.54 (m, 4H), 3.56−3.62 (m, 1H, CH), 4.02 (t, $J = 7.0$ Hz, 2H, CH_2), 4.61−4.68 (m, 1H, CH), 6.46 (bs, 1H, NH), 7.15 (d, $J = 8.5$ Hz, 2H, ArH), 7.77 (s, 1H, CH), 7.92 (d, $J = 8.5$ Hz, 2H, ArH), 9.34 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 31.87, 32.85, 35.92, 50.55, 53.74, 58.15, 62.85, 66.87, 113.99, 120.25, 129.43, 131.30, 135.49, 138.68, 139.88, 134.42, 152.44, 158.92, 159.94.

*4.2.5.24. 3-(2-((4-aminobutyl)amino)-6-((4-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)propan-1-ol (**14d**).* Yield: 75%, mp: 197−198 °C. Elemental analysis: Calcd for $C_{23}H_{34}N_8O_2$ (454.58): C, 60.77; H, 7.54; N, 24.65. Found: C, 61.08; H, 7.32; N, 24.49. HPLC-MS (ESI+): 455.99 (98.6%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.38 (qui, $J = 7.0$ Hz, 2H, CH_2), 1.53 (qui, $J = 7.0$ Hz, 2H, CH_2), 1.88 (qui, $J = 6.5$ Hz, 2H, CH_2), 2.28−2.33 (m, 4H), 2.52 (t, $J = 7.0$ Hz, 2H, CH_2), 3.22−3.26 (m, 4H), 3.35−3.37 (m, 4H), 3.52−3.54 (m, 4H), 4.04 (t, $J = 7.0$ Hz, 2H, CH_2), 6.64 (bs, 1H, NH), 7.15 (d, $J = 9.0$ Hz, 2H, ArH), 7.91 (d, $J = 9.0$ Hz, 2H, ArH), 9.31 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 27.34, 28.00, 31.26, 32.86, 42.00, 49.09, 53.64, 58.14, 62.65, 66.75, 114.10, 120.30, 129.45, 131.31, 138.62, 139.84, 152.37, 152.49, 159.60.

*4.2.5.25. 4-((9-(3-hydroxypropyl)-6-((4-(morpholinomethyl)phenyl)amino)-9H-purin-2-yl)amino)butan-1-ol (**14e**).* Yield: 76%, mp: 143−145 °C. Elemental analysis: Calcd for $C_{23}H_{33}N_7O_3$ (445.56): C, 60.64; H, 7.30; N, 21.52. Found: C, 60.96; H, 7.04; N, 21.86. HPLC-MS (ESI+): 456.94 (97%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.45 (qui, $J = 7.5$ Hz, 2H, CH_2), 1.54 (qui, $J = 7.5$ Hz, 2H, CH_2), 1.88 (qui, $J = 6.5$ Hz, 2H, CH_2), 2.28−2.32 (m, 4H), 3.24 (q, $J = 6.0$ Hz, 2H, CH_2), 3.34−3.37 (m, 6H), 3.52−3.54 (m, 4H), 4.03 (t, $J = 7.0$ Hz, 2H, CH_2), 4.45 (t, $J = 4.5$ Hz, 1H, CH), 4.68 (t, $J = 5.0$ Hz, 1H, OH), 6.65 (bs, 1H, NH), 7.15 (d, $J = 8.5$ Hz, 2H, ArH), 7.78 (s, 1H, CH), 7.92 (d, $J = 8.5$ Hz, 2H, ArH), 9.32 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 26.54, 30.77, 32.83, 41.83, 53.65, 58.14, 61.23, 62.66, 63.31, 66.74, 114.10, 120.29, 129.29, 129.45, 131.30, 138.62, 139.84, 152.38, 152.54, 159.61.

*4.2.5.26. 3-(2-((4-aminocyclohexyl)amino)-6-((4-(4-benzylpiperazin-1-yl)phenyl)amino)-9H-purin-9-yl)propan-1-ol (**14f**).* Yield: 68%, mp: 101−103 °C. Elemental analysis: Calcd for $C_{31}H_{41}N_9O$ (555.73): C, 67.00; H, 7.44; N, 22.68. Found: C, 67.09; H, 7.31; N, 22.51. HPLC-MS (ESI+): 557.20 (99.7%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.73−1.75 (m, 2H), 1.83−1.91 (m, 5H), 2.48−2.50 (m, 4H), 3.02−3.04 (m, 4H), 3.34−3.36 (m, 4H), 3.49 (s, 2H, CH_2), 3.55−3.59 (m, 1H, CH), 4.01 (t, $J = 6.5$ Hz, 2H, CH_2), 4.65 (bs, 1H, OH), 6.34 (bs, 1H, NH), 6.81 (d, $J = 9.0$ Hz, 2H, ArH), 7.20−7.33 (m, 5H, ArH), 7.73 (s, 1H, CH), 7.78 (d, $J = 9.0$ Hz, 2H, ArH), 9.12 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 31.92, 32.88, 35.88, 49.63, 50.58, 53.18, 56.55, 58.13, 62.62, 79.71, 113.86, 116.22, 121.54, 127.50, 128.73, 129.45, 133.20, 138.31, 138.64, 146.84, 152.10, 152.49, 158.99.

*4.2.5.27. 3-(2-((4-aminobutyl)amino)-6-((4-(4-benzylpiperazin-1-yl)phenyl)amino)-9H-purin-9-yl)propan-1-ol (**14g**).* Yield: 79%, mp: 106−108 °C. Elemental analysis: Calcd for $C_{29}H_{39}N_9O$ (529.69): C, 65.76; H, 7.42; N, 23.80. Found: C, 65.49; H, 7.30; N, 23.47. HPLC-MS (ESI+): 531.18 (99.9%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.32−1.39 (m, 2H), 1.46−1.54 (m, 2H), 1.87 (qui, $J = 6.5$ Hz, 2H, CH_2), 2.48−2.51 (m, 4H), 3.02−3.04 (m, 4H), 3.21 (q, $J = 7.5$ Hz, 2H, CH_2), 3.34−3.36 (m, 4H), 3.48 (s, 2H, CH_2), 4.02 (t, $J = 6.5$ Hz, 2H, CH_2), 6.54 (bs, 1H, NH), 6.82 (d, $J = 9.0$ Hz, 2H, ArH), 7.21−7.32 (m, 5H, ArH), 7.73 (s, 1H, CH), 7.77 (d, $J = 9.0$ Hz, 2H, ArH), 9.10 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 27.38, 29.56, 31.44, 32.99, 42.16, 49.53, 53.12, 58.12, 62.63, 116.05, 121.71, 123.91, 127.50, 128.73, 129.45, 132.96, 138.25, 138.55, 146.82, 152.14, 152.50, 157.78, 159.67.

*4.2.5.28. 4-((6-((4-(4-benzylpiperazin-1-yl)phenyl)amino)-9-(3-hydroxypropyl)-9H-purin-2-yl)amino)butan-1-ol (**14h**).* Yield: 84%, mp: 146−148 °C. Elemental analysis: Calcd for $C_{29}H_{38}N_8O_2$ (530.68): C, 65.64; H, 7.22; N, 21.12. Found: C, 65.32; H, 7.06; N, 20.87. HPLC-MS (ESI+): 532.17 (99.9%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.41−1.47 (m, 2H), 1.50−1.55 (m, 2H), 1.84−1.90 (m, 2H), 3.02−3.04 (m, 4H), 3.22 (q, $J = 7.0$ Hz, 2H), 3.28−3.30 (m, 4H), 3.35−3.39 (m, 10H), 3.49 (s, 2H, CH_2), 4.02 (t, $J = 7.5$ Hz, 2H, CH_2), 4.34 (t, $J = 5.0$ Hz, 1H, OH), 4.41−4.48 (m, 2H, CH_2), 4.65 (t, $J = 5.5$ Hz, 1H, OH), 6.53 (bs, 1H, NH), 6.82 (d, $J = 9.5$ Hz, 2H, ArH), 7.21−7.25 (m, 1H, ArH), 7.29−7.32 (m, 4H, ArH), 7.73 (s, 1H, CH), 7.77 (d, $J = 9.5$ Hz, 2H, ArH), 9.09 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 30.76, 32.87, 49.53, 53.18, 58.12, 61.25, 62.63, 116.15, 121.68, 127.49, 128.73, 129.45, 133.10, 138.26, 138.65, 146.81, 152.55, 159.68.

*4.2.5.29. 3-(2-((4-aminocyclohexyl)amino)-6-((3-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)cyclopentan-1-ol (**14i**).* Yield: 73%, mp: 228−231 °C. Elemental analysis: Calcd for $C_{27}H_{38}N_8O_2$ (506.65): C, 64.01; H, 7.56; N, 22.12. Found: C, 63.75; H, 7.32; N, 22.46. HPLC-MS (ESI+): 507.72 (98.62%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.15−1.29 (m, 2H), 1.72−1.85 (m, 5H), 1.92−1.94 (m, 2H), 2.10 (sep, $J = 6.0$ Hz, 1H, CH), 2.26−2.37 (m, 5H), 2.57−2.64 (m, 1H, CH), 3.41 (s, 2H, CH_2), 3.53−3.55 (m, 4H), 4.20

(qui, $J = 5.0$ Hz, 1H, CH), 4.71 (qui, $J = 5.0$ Hz, 1H, CH), 6.35 (bs, 1H, NH), 6.89 (d, $J = 7.5$ Hz, 1H, ArH), 7.19 (t, $J = 7.5$ Hz, 1H, ArH), 7.78 (s, 1H, ArH), 7.87–8.01 (m, 2H, ArH, CH), 9.33 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 23.94, 31.60, 34.24, 34.71, 41.91, 50.12, 52.88, 53.76, 63.32, 66.76, 70.92, 114.32, 119.27, 121.01, 122.82, 128.56, 137.33, 138.39, 140.80, 152.57, 158.66.

*4.2.5.30. 3-(2-((4-aminocyclohexyl)amino)-6-((4-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)cyclopentan-1-ol (**14j**).* Yield: 75%, mp: 197–198 °C. Elemental analysis: Calcd for $C_{27}H_{38}N_8O_2$ (506.65): C, 64.01; H, 7.56; N, 22.12. Found: C, 64.28; H, 7.14; N, 22.27. HPLC-MS (ESI+): 508.07 (99.9%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.70–1.83 (m, 5H), 1.90–1.97 (m, 2H), 2.05–2.16 (m, 1H), 2.26–2.39 (m, 5H), 2.52–2.62 (m, 2H), 3.13 (s, 2H, CH_2), 3.52–3.54 (m, 4H), 3.56–3.63 (m, 1H, CH), 4.20 (qui, $J = 5$,0 Hz, 1H, CH), 4.71 (qui, $J = 7.5$ Hz, 1H, CH), 5.08 (bs, 1H, OH), 6.38 (d, $J = 7.5$ Hz, 1H, NH), 7.15 (d, $J = 8.0$ Hz, 2H, ArH), 7.88–7.94 (m, 3H, ArH, CH), 9.36 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 31.70, 34.72, 35.02, 41.90, 49.09, 49.69, 50.41, 53.65, 56.55, 62.68, 66.74, 70.95, 120.25, 129.45, 130.13, 131.40, 137.27, 139.86, 152.54, 152.53, 157.60, 158.66.

*4.2.5.31. 3-(2-((4-aminobutyl)amino)-6-((4-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)cyclopentan-1-ol (**14k**).* Yield: 80%, mp: 142–144 °C. Elemental analysis: Calcd for $C_{25}H_{36}N_8O_2$ (480.62): C, 62.48; H, 7.55; N, 23.32. Found: C, 62.74; H, 7.29; N, 23.01. HPLC-MS (ESI+): 481.9 (98%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.93 (qui, $J = 6.0$ Hz, 2H, CH_2), 2.40–2.45 (m, 4H), 3.39 (q, $J = 6.0$ Hz, 2H, CH_2), 3.66 (s, 2H, CH_2), 3.73–3.75 (m, 4H), 4.19 (t, $J = 7.0$ Hz, 2H, CH_2), 4.64 (t, $J = 5.0$ Hz, 1H, OH), 7.02 (td, $J = 7.5$ Hz, $J' = 1.0$ Hz, 1H, ArH), 7.22 (dd, $J = 8.0$ Hz, $J' = 1.5$ Hz, 1H, ArH), 7.33 (td, $J = 7.8$ Hz, 1H, ArH), 8.28 (s, 1H, CH), 8.34 (d, $J = 8.0$ Hz, 1H, ArH), 11.59 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 32.75, 41.30, 53.01, 58.17, 61.68, 66.41, 119.91, 120.78, 123.50, 126.46, 128.52, 130.60, 138.96, 143.52, 150.92, 152.38, 152.79.

*4.2.5.32. 3-(2-((4-hydroxybutyl)amino)-6-((4-(morpholinomethyl)phenyl)amino)-9H-purin-9-yl)cyclopentan-1-ol (**14l**).* Yield: 78%, mp: 222–224 °C. Elemental analysis: Calcd for $C_{25}H_{35}N_7O_3$ (481.60): C, 62.35; H, 7.33; N, 20.36. Found: C, 62.58; H, 6.94; N, 20.69. HPLC-MS (ESI+): 482.92 (98.4%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.73–1.77 (m, 2H), 1.80–1.84 (m, 1H), 1.99–2.15 (m, 2H), 2.22–2.38 (m, 5H), 3.25 (q, $J = 7.0$ Hz, 2H, CH_2), 3.35–3.40 (m, 4H), 3.50–3.61 (m, 4H), 4.20 (sex, $J = 4.5$ Hz, 1H, CH), 4.36 (t, $J = 5.5$ Hz, 1H, OH), 4.74 (qui, $J = 8.0$ Hz, 1H, CH), 5.10 (d, $J = 4.0$ Hz, 1H, OH), 6.59 (t, $J = 6.0$ Hz, 1H, NH), 7.15 (d, $J = 8.0$ Hz, 2H, ArH), 7.91–7.93 (m, 3H, ArH, CH), 9.34 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 23.05, 26.50, 30.75, 31.16, 34.76, 41.92, 52.90, 53.62, 61.23, 62.60, 66.71, 70.97, 114.37, 120.31, 129.49, 137.04, 139.90, 151.93, 152.52, 159.39, 172.11.

*4.2.5.33. 3-(2-((4-aminocyclohexyl)amino)-6-((4-(4-benzylpiperazin-1-yl)phenyl)amino)-9H-purin-9-yl)cyclopentan-1-ol (**14m**).* Yield: 65%, mp: 176–178 °C. Elemental analysis: Calcd for $C_{33}H_{43}N_9O$ (581.77): C, 68.13; H, 7.45; N, 21.67. Found: C, 68.24; H, 7.49; N, 21.22. HPLC-MS (ESI+): 583.23 (99.9%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.09–1.26 (m, 4H), 1.72–1.80 (m, 4H), 1.90–1.93 (m, 2H), 2.01–2.13 (m, 2H), 2.31–2.37 (m, 1H, CH), 2.51–2.57 (m, 1H, CH), 3.02–3.04 (m, 4H), 3.49 (s, 2H, CH_2), 3.54–3.61 (m, 1H, CH), 4.19 (qui, $J = 4.5$ Hz, 1H, CH), 4.70 (qui, $J = 7.5$ Hz, 1H, CH), 5.08 (bs, 1H, OH), 6.25 (d, $J = 7.5$ Hz, 1H, NH), 6.81 (d, $J = 9.0$ Hz, 2H, ArH), 7.21–7.25 (m, 1H, ArH), 7.27–7.32 (m, 4H, ArH), 7.78 (d, $J = 9.0$ Hz, 2H, ArH), 7.87 (s, 1H, CH), 9.12 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 31.72, 34.81, 35.03, 41.89, 48.31, 49.08, 49.63, 50.43, 53.71, 62.62, 70.96, 116.22, 121.62, 127.49, 128.73, 129.45, 132.57, 133.17, 136.98, 138.63, 146.86, 152.58, 154.81, 158.73.

*4.2.5.34. 3-(2-((4-aminobutyl)amino)-6-((4-(4-benzylpiperazin-1-yl)phenyl)amino)-9H-purin-9-yl)cyclopentan-1-ol (**14n**).* Yield: 81%, mp: 271–273 °C. Elemental analysis: Calcd for $C_{31}H_{41}N_9O$ (555.73): C, 67.00; H, 7.44; N, 22.68. Found: C, 66.95; H, 7.01; N, 22.39. HPLC-MS (ESI+): 557.28 (97.7%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.42 (qui, $J = 6.5$ Hz, 2H, CH_2), 1.53 (qui, $J = 6.5$ Hz, 2H, CH_2), 1.72–1.77 (m, 2H), 1.79–1.84 (m, 1H), 2.01–2.15 (m, 2H), 2.32–2.38 (m, 1H, CH), 2.47–2.49 (m, 2H), 2.58 (t, $J = 7.0$ Hz, 2H, CH_2), 3.02–3.05 (m, 4H), 3.22 (q, $J = 6.5$ Hz, 4H, 2xCH_2), 3.49 (s, 2H, CH_2), 4.19 (qui, $J = 4.5$ Hz, 1H, CH), 4.72 (qui, $J = 7.5$ Hz, 1H, CH), 6.48 (t, $J = 6.0$ Hz, 1H, NH), 6.82 (d, $J = 9.0$ Hz, 2H, ArH), 7.21–7.25 (m, 1H, ArH), 7.29–7.31 (m, 4H, ArH), 7.76 (d, $J = 9.0$ Hz, 2H, ArH), 7.87 (s, 1H, CH), 9.11 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 27.15, 29.34, 31.11, 34.79, 41.15, 41.89, 49.12, 49.53, 52.92, 53.19, 62.64, 70.97, 114.28, 116.16, 121.75, 127.51, 128.74, 129.45, 133.05, 136.05, 138.64, 146.86, 151.63, 152.60, 159.43.

*4.2.5.35. 3-(6-((4-(4-benzylpiperazin-1-yl)phenyl)amino)-2-((4-hydroxybutyl)amino)-9H-purin-9-yl)cyclopentan-1-ol (**14o**).* Yield: 74%, mp: 218–220 °C. Elemental analysis: Calcd for $C_{31}H_{40}N_8O_2$ (556.72): C, 66.88; H, 7.24; N, 20.13. Found: C, 66.62; H, 7.19; N, 20.46. HPLC-MS (ESI+): 558.03 (95.4%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.44 (qui, $J = 7.5$ Hz, 2H, CH_2), 1.53 (qui, $J = 7.5$ Hz, 2H, CH_2), 1.72–1.76 (m, 2H), 1.79–1.84 (m, 1H, CH), 1.99–2.15 (m, 2H), 2.32–2.38 (m, 1H, CH), 2.47–2.49 (m, 2H), 2.98–3.08 (m, 4H), 3.22 (q, $J = 6.5$ Hz, 2H, CH_2), 3.37 (q, $J = 6.5$ Hz, 2H, CH_2), 3.47–3.53 (m, 2H), 4.19 (sex, $J = 4.0$ Hz, 1H, CH), 4.34 (t, $J = 5.0$ Hz, 1H, OH), 4.72 (qui, $J = 7.5$ Hz, 1H, CH), 5.12 (d, $J = 4.0$ Hz, 1H, OH), 6.47 (t, $J = 5.5$ Hz, 1H, NH), 6.82 (d, $J = 9.0$ Hz, 2H, ArH), 7.21–7.26 (m, 1H, ArH), 7.29–7.33 (m, 4H, ArH), 7.77 (d, $J = 9.0$ Hz, 2H, ArH), 7.87 (s, 1H, CH), 9.11 (bs, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 26.51, 30.74, 31.13, 34.78, 41.73, 41.91, 49.49, 52.89, 53.12, 61.25, 62.60, 70.97, 116.20, 121.70, 127.55, 128.76, 129.50, 131.98, 132.60, 133.12, 136.88, 146.80, 151.65, 152.58, 159.45.

*4.2.5.36. N^2-(4-Amino-cyclohexyl)-N^6-[4-(4-benzyl-piperazin-1-yl)-phenyl]-9-cyclopentyl-9H-purine-2,6-diamine (**14p**).* Yield: 92%. m.p.: 149–150 °C. Elemental analysis: Calcd.for $C_{33}H_{43}N_9$ (565.77): C, 70.06; H, 7.66; N, 22.28. Found: C, 69.82; H, 7.48; N, 22.01. HPLC-MS (ESI+): 568.8 (99.9%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.28–1.12 (m, 8H), 1.70–1.58 (m, 4H), 2.11–1.74 (m, 19H), 2.65–2.57 (m, 2H), 3.02 (t, $J = 9.8$ Hz, 7H), 3.49 (d, $J = 3.3$ Hz, 9H), 3.62–3.54 (m, 4H), 4.69–4.59 (m, 2H), 6.29 (bs, 1H), 6.82 (d, $J = 8.8$ Hz, 4H), 7.26–7.19 (m, 2H), 7.32–7.28 (m, 8H), 7.84–7.74 (m, 6H), 9.10 (bs, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 31.49, 32.29, 34.18, 49.62, 50.29, 53.16, 62.61, 116.25, 121.62, 127.51, 128.73, 128.73, 129.47, 133.19, 138.60, 146.85, 158.66.

*4.2.5.37. N^2-(4-Amino-cyclohexyl)-9-cyclopentyl-N^6-(4-morpholin-4-ylmethyl-phenyl)-9H-purine-2,6-diamine (**14q**).* Yield: 94%. m.p.: 133–135 °C. Elemental analysis: Calcd.for $C_{27}H_{38}N_8O$ (490.64): C, 66.09; H, 7.81; N, 22.84. Found: C, 66.04; H, 7.59; N, 22.61. HPLC-MS (ESI+): 491.65 (98.2%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.30–1.05 (m, 4H), 1.69–1.56 (m, 2H), 2.12–1.72 (m, 10H), 2.27–2.32 (m, 4H), 2.53 (t, $J = 10.4$ Hz, 1H), 3.36 (s, 2H), 3.50–3.56 (m, 4H), 3.68–3.56 (m, 1H), 4.72–4.59 (sep, $J = 7.5$ Hz, 1H), 6.39 (bs, 1H), 7.15 (d, $J = 8.0$ Hz, 2H), 7.84 (s, 1H), 7.92 (d, $J = 8.0$ Hz, 2H), 9.32 (bs), 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 24.31, 31.76, 32.29, 35.57, 50.53, 53.65, 55.34, 62.67, 66.74, 114.51, 120.25, 129.42, 131.24, 137.12, 139.90, 152.50, 158.63.

*4.2.5.38. 4-[9-Cyclopentyl-6-(4-morpholin-4-ylmethyl-phenylamino)-9H-purin-2-ylamino]-butan-1-ol (**14r**).* Yield: 88%. m.p.:

119–121 °C. Elemental analysis: Calcd.for $C_{25}H_{35}N_7O_2$ (451.56): C, 64.49; H, 7.58; N, 21.06. Found: C, 64.49; H, 7.62; N, 20.85. HPLC-MS (ESI+): 466.49 (98.4%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.45 (qui, J = 6.5 Hz, 2H), 1.53 (qui, J = 6.5 HZ, 2H), 1.69–1.60 (m, 2H), 1.89–1.77 (m, 2H), 2.00–1.89 (m, 2H), 2.12–2.02 (m, 2H), 2.28–2.32 (m, 4H), 3.24 (q, J = 6.5 HZ, 2H), 3.48 (s, 2H), 3.51–3.57(m, 4H), 4.38 (t, J = 5.0 Hz, 1H), 4.66 (qui, J = 7.0 Hz, 1H), 6.58 (t, J = 5.4 Hz, 1H), 7.15 (d, J = 8.3 Hz, 2H), 7.85 (s, 1H), 7.91 (d, J = 8.3 Hz, 2H), 9.29 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 24.25, 26.52, 30.76, 32.30, 41.77, 53.63, 55.36, 61.25, 62.72, 66.73, 112.78, 114.55, 120.24, 129.49, 131.26, 137.00, 139.92, 152.48, 159.37.

*4.2.5.39. N^2-(4-Amino-butyl)-9-cyclopentyl-N^6-(4-morpholin-4-ylmethyl-phenyl)-9H-purine-2,6-diamine (**14s**).* Yield: 85%. m.p.: 140–141 °C. Elemental analysis: Calcd.for $C_{25}H_{36}N_8O$ (464.61): C, 64.63; H, 7.81; N, 24.12. Found: C, 64.47; H, 7.43; N, 23.88. HPLC-MS (ESI+): 465.8 (96.7%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.38–1.66 (m, 6H), 1.87–1.92 (m, 6H), 2.07–2.09 (m, 4H), 2.53 (t, J = 6.78 Hz, 2H), 3.17 (q, J = 6.39 Hz, 2H), 3.30 (s, 2H), 3.39–3.55 (m, 4H), 4.69 (qui, J = 7.11 Hz, 1H), 6.64 (t, J = 5.34 Hz, 1H), 7.18 (d, J = 8.28 Hz, 2H), 7.88 (s, 1H), 7.95 (d, J = 8.28 Hz, 2H), 9.34 (bs, 1H).

4.2.6. General procedure for the preparation of derivatives 11a and 11c

The mixture of N^2-(4-amino-cyclohexyl)-N^6-(4-bromo-phenyl)-9-cyclopentyl-9*H*-purine-2,6-diamine (1,00 mmol), corresponding arylboronic acid (1.50 mmol), palladium diacetate (0.06 mmol), tetrabutylammonium bromide (0.02 mmol), potassium phosphate trihydrate (3.0 mmol) was heated with stirring in *N*,*N*-dimethylformamide (5.0 mL) at 100 °C for 8 h under an argon atmosphere in a sealed tube. After cooling to room temperature the reaction mixture was diluted with 50 mL of water and the suspension was extracted three times with dichloromethane (25 mL). The combined organic phases were washed with water, brine, dried over anhydrous sodium sulphate and evaporated under reduced pressure. The crude product was purified by column chromatography on silica using mobile phase chloroform/methanol/conc.ammonium hydroxide (4:1:0.025, v/v).

*4.2.6.1. N^2-(4-aminocyclohexyl)-9-cyclopentyl-N^6-(2′-methoxy-[1,1′-biphenyl]-4-yl)-9H-purine-2,6-diamine (**11a**).* Yield: 68%, mp: 239–241 °C. Elemental analysis: Calcd for $C_{29}H_{35}N_7O$ (497.65): C, 69.99; H, 7.09; N, 19.70. Found: C, 70.14; H, 6.85; N, 19.41. HPLC-MS (ESI+): 498.25 (98.6%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm:1.21–1.41 (m, 4H), 1.72–1.80 (m, 2H), 1.84–1.99 (m, 8H), 2.01–2.27 (m, 4H), 2.74–2.81 (m, 1H, CH), 3.84 (s, 3H, CH_3), 4.75 (qui, J = 7.35 Hz, 1H, CH), 4.81 (d, J = 7.5 Hz, 1H, NH), 6.98–7.07 (m, 2H, ArH), 7.29–7.37 (m, 2H, ArH), 7.53 (d, J = 8.55 Hz, 2H, ArH), 7.58 (s, 1H, CH), 7.65 (bs, 1H, NH), 7.85 (d, J = 8.55 Hz, 2H, ArH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.26, 32.00, 32.74, 35.34, 50.39, 55.56, 111.30, 114.99, 119.22, 120.94, 128.36, 130.01, 130.44, 130.80, 132.83, 136.00, 138.42, 151.85, 152.28, 156.60, 158.73.

*4.2.6.2. N^6-(2′-amino-[1,1′-biphenyl]-4-yl)-N^2-(4-aminocyclohexyl)-9-cyclopentyl-9H-purine-2,6-diamine (**11c**).* Yield: 62%, mp: 145–147 °C. Elemental analysis: Calcd for $C_{28}H_{34}N_8$ (482.64): C, 69.68; H, 7.10; N, 23.22. Found: C, 69.83; H, 7.13; N, 22.87. HPLC-MS (ESI+): 483.74 (97.3%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.20–1.39 (m, 4H), 1.75–2.29 (m, 10H), 2.68–2.82 (m, 1H, CH), 3.83 (bs, 2H, NH2), 4.75 (qui, J = 7.32 Hz, 1H, CH), 4.85 (d, J = 7.5 Hz, 1H, NH), 6.77 (dd, J = 8.1 Hz, J' = 0.84 Hz, 1H, ArH), 6.81 (t, J = 7.4 Hz, 1H, ArH), 7.13–7.18 (m, 2H, ArH), 7.42 (d, J = 8.58 Hz, 2H, ArH), 7.58 (s, 1H, NH), 7.78–7.90 (m, 3H, ArH, CH). ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 24.27, 32.00, 32.72, 35.39, 50.36, 53.53, 55.60, 114.96, 115.62, 118.71, 119.97, 127.46, 128.35, 129.55, 130.54, 133.69, 136.12, 138.61, 143.76, 151.94, 152.25, 158.69.

4.2.7. General procedure for the preparation of derivatives 11b and 11j

The methoxyderivative **11a** or **11i** (0.15 mmol) was dissolved in dichloromethane (10 mL) and cooled to 0–5 °C. A boron tribromide solution in dichloromethane (0.47 M, 2.0 mL) was added dropwise so that the temperature was maintained between 0 and 5 °C. The reaction mixture was stirred at room temperature for further 20 h. Methanol (10 mL) was added dropwise to the reaction mixture and the mixture was evaporated under reduced pressure to obtain crude product which was purified by column chromatography on silica using mobile phase chloroform/methanol/conc.ammonium hydroxide (4:1:0.025, v/v).

*4.2.7.1. 4'-((2-((4-aminocyclohexyl)amino)-9-cyclopentyl-9H-purin-6-yl)amino)-[1,1′-biphenyl]-2-ol (**11b**).* Yield: 67%, mp: 258–260 °C. Elemental analysis: Calcd for $C_{28}H_{33}N_7O$ (483.62): C, 69.54; H, 6.88; N, 20.27. Found: C, 69.88; H, 6.49; N, 20.00. HPLC-MS (ESI+): 483.85 (99.2%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.30–1.49 (m, 4H), 1.65–1.72 (m, 2H), 1.78–2.20 (m, 10H), 2.96–3.05 (m, 1H, CH), 3.61–3.72 (m, 1H, CH), 4.70 (qui, J = 7.5 Hz, 1H, CH), 6.60 (bs, 1H, NH), 6.86 (t, J = 7.26 Hz, 1H, ArH), 6.92 (d, J = 7.9 Hz, 1H, ArH), 7.13 (dt, J = 6.6 Hz, J' = 1.41 Hz, 1H, ArH), 7.24 (dd, J = 7.5 Hz, J' = 1.32 Hz, 1H, ArH), 7.45 (d, J = 8.6 Hz, 2H, ArH), 7.93 (s, 1H, CH), 8.04 (d, J = 8.6 Hz, 2H, ArH), 9.45 (bs, 1H, NH).

*4.2.7.2. 2-(5-((2-((4-aminocyclohexyl)amino)-9-cyclopentyl-9H-purin-6-yl)amino)pyridin-2-yl)phenol (**11j**).* Yield: 64%, mp: 283–284 °C. Elemental analysis: Calcd for $C_{27}H_{32}N_8O$ (484.61): C, 66.92; H, 6.66; N, 23.12. Found: C, 66.68; H, 6.32; N, 22.87. HPLC-MS (ESI+): (98.28%). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.12–1.30 (m, 4H), 1.60–1.70 (m, 2H), 1.75–1.88 (m, 4H), 1.94–1.99 (m, 4H), 2.04–2.09 (m, 2H), 2.55–2.58 (m, 1H), 3.62–3.66 (m, 1H), 4.72 (qui, J = 6.5 Hz, 1H, CH), 6.62 (d, J = 7.5 Hz, 1H, ArH), 7.12–7.16 (m, 2H, ArH), 7.34 (t, J = 7.5 Hz, 1H, ArH), 7.78 (d, J = 8.0 Hz, 1H, ArH), 7.80 (d, J = 8.5 Hz, 1H, ArH), 7.85 (s, 1H, ArH), 8.30 (s, 1H, CH), 9.21 (bs, 1H, NH), 9.35 (bs, 1H, NH), 10.02 (s, 1H, OH).

4.3. Kinase assays

PDGFRα was purchased from ProQinase. The kinase reactions were assayed with AGLT peptide (1 mg/mL) in the presence of 1 μM ATP, 0.05 μCi [γ-^{33}P]ATP, and the test compound in a final volume of 10 μL, all in a reaction buffer (60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 μM Na-orthovanadate, 1.2 mM DTT, 2.5 μg/50 μL $PEG_{20.000}$). The reactions were stopped by adding 5 μL of 3% aq. H_3PO_4. Aliquots were spotted onto P-81 phosphocellulose (Whatman), washed 3 × with 0.5% aq. H_3PO_4 and finally air-dried. Kinase inhibition was quantified using a FLA-7000 digital image analyser. The concentration of the test compounds required to reduce the kinase's activity by 50% was determined from dose-response curves and reported as the IC_{50} value.

4.4. Cell Cultures

Human cell lines were obtained from the American Type Culture Collection or the German Collection of Microorganisms and Cell Cultures and were cultivated according to the provider's instructions. Briefly, EOL-1 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum. HCC-827 and CEM were maintained in RPMI-1640 medium supplemented with 20% fetal bovine serum. K562 and BJ cell lines were cultivated in DMEM medium supplemented with 10% fetal bovine serum. All media were supplemented with penicillin (100 U/mL) and

streptomycin (100 μg/mL) and cell lines were cultivated at 37 °C in 5% CO_2.

4.5. Cell viability assays

For the viability assays, cells were seeded into 96-well plates in the appropriate densities and after preincubation period, were treated in triplicates with six different doses of each compound for 72 h. After treatments, resazurin (Sigma Aldrich) solution was added for 4 h, and fluorescence of resorufin corresponding to live cells was measured at 544 nm/590 nm (excitation/emission) using a Fluoroskan Ascent microplate reader (Labsystems). The GI_{50} value, the drug concentration lethal to 50% of the cells, was calculated from the dose response curves that resulted from the assays.

4.6. Flow cytometry

Asynchronous cells were seeded and, after a preincubation period, treated with test compound for 24 h. After the staining with propidium iodide, DNA content was analyzed by flow cytometry using a 488 nm laser (BD FACS Verse with software BD FACSuite™, version 1.0.6.). Cell cycle distribution was analyzed using ModFit LT (Verity Software House).

4.7. Immunoblotting

Cell lysates were separated on SDS-polyacrylamide gels and electroblotted onto nitrocellulose membranes. After blocking, overnight incubation with specific primary antibodies, and incubation with peroxidase-conjugated secondary antibodies, peroxidase activity was detected with SuperSignal West Pico reagents (Thermo Scientific) using a CCD camera LAS-4000 (Fujifilm). The following specific antibodies were purchased from Cell Signaling: anti-PDGFRα (D1E1E), anti-phospho-PDGFRα/β Y849/857 (C43E9), anti-phospho-PDGFRα Y1018, anti-STAT3 (79D7), anti-phospho-STAT3 Y705 (D3A7), anti-ERK1/2, anti-phospho-ERK1/2 T202/Y204. Anti-PCNA (clone PC-10) was generously gifted by Dr. B. Vojtěšek.

4.8. Homology modeling and molecular docking

The active conformation of PDGFRα (DFG-in) was built by homology modeling based on the c-KIT kinase template (PDB ID: 1PKG). The dimer of active c-KIT contains two identical chains; chain A was selected. With default setting of Prime in Schrodinger 2018-4 we built the homology model and subjected it to energy minimization using the OPLS3e all-atom force field [13]. All ligands were converted from 2D to 3D using Ligprep module (Schrodinger Suite). Grids were generated using a receptor site that was defined by the centroid of the cognate ligand and forming a hydrogen bond impose hydrogen bond constrain on backbone of C677 residue (oxygen of carbonyl and hydrogen of amine). Docking using the Glide (version 81012) in standard precision (SP) mode with flexible ligand docking utilizes precomputed grids [14,15]. The docking hierarchy starts with the systematic conformational expansion of the ligand, followed by placement in the receptor site. Minimization of the ligand in the field of the receptor is then carried out using the OPLSe force field with a distance-dependent dielectric (default 2.0). The best pose for a given ligand was determined by the composite Glide G-score. Default van der Waals scaling was used (1.0 for the receptor and 0.8 for the ligand).

Author contributions

E. Ř. and T.G. contributed equally. E.Ř. and R.J. performed biochemical and cellular experiments, T.G. and V.M. prepared and analyzed compounds, H.A. and V.K. analyzed binding modes, E.Ř, T.G. and V.K. designed the study and wrote the manuscript.

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Appendix A. Supplementary data

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