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A 2,6,9-hetero-trisubstituted purine inhibitor exhibits potent biological effects against multiple myeloma cells

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ABSTRACT

A focused library of hetero-trisubstituted purines was developed for improving the cell penetrating and biological efficacy of a series of anti-Stat3 protein inhibitors. From this SAR study, lead agent **22e** was identified as being a promising inhibitor of MM tumour cells (IC_{50}'s <5 μM). Surprisingly, biophysical and biochemical characterization proved that **22e** was not a Stat3 inhibitor. Initial screening against the kinome, prompted by the purine scaffold's history for targeting ATP binding pockets, suggests possible targeting of the JAK family kinases, as well for ABL1 (nonphosphorylated F317L) and AAK1.

1. Introduction

Purines feature in biological molecules that are critically involved in many essential cellular processes. Considered a privileged structure in biological systems, the purine heterocycle represents an intriguing molecular starting point for new pharmaceutical agents.[1] Purines have been incorporated into therapeutic structures for applications in nucleotide anti-metabolites and play a key structural role in inhibitors of cancer-promoting dysregulated proteins in cancer.[2–6] For example, hetero-tri-substituted purines have been used successfully to inhibit cell cycle initiators, cyclin-dependant kinases which have been implicated in cancer.[7] In addition, purine inhibitors have successfully suppressed the activity of Heat Shock Protein 90, a protein critical for tumour survival.[8]

We have demonstrated that purine scaffolds could be functionalized as part of a pharmacophore model to bind and inhibit the Src Homology 2 (SH2) domain of the signal transducer and activator of transcription 3 (Stat3) protein. Stat3 has been shown to play a key role in regulating cancer cell growth and differentiation. Hyperactivation of Stat3 protein levels has been shown to be transformative, leading to uncontrolled cell proliferation and apoptotic resistance in a multitude of human cancer cells.[9,10] Many types of cancer cells exhibit Stat3 'addiction' and are widely acknowledged to be hypersensitive to Stat3 inhibition that leads to programmed cancer cell death. Thus, potent Stat3 inhibitors have tremendous potential as novel therapeutics.[11,12]

In previous work, a quantitative structure activity relationship (QSAR) of a 2,6,9-hetero-trisubstituted purine core was conducted for targeting the Src Homology 2 domain 'hot spot' of Stat3 (Fig. 1).[13] Computational docking studies suggested that trisubstituted purine inhibitors may best access the sub-pockets of Stat3's SH2 domain and thus prevent Stat3 cellular function. Specifically, inhibitors were prepared to access two predominantly hydrophobic pockets (various appendages at R_1 and the cyclohexylbenzyl substituent at the N2 position), as well as the polar phosphotyrosine (pTyr) binding site with an N9-carboxylate substituent. The purines prepared showed potent Stat3 protein binding in vitro, as assessed by surface plasmon resonance (SPR: Biacore 3000), with K_d values ranging from 1 to 10 μM. However, lead Stat3 binders **8a–g** (Fig. 1) displayed only modest anti-proliferative effects in cancer cell lines. To determine the origins of the lower than expected IC_{50} values, experiments were conducted to assess the cell penetrating properties of lead purine inhibitors. In particular, Caco2 cell penetrating studies using a Waters Xevo quadrupole time-of-flight (QTof) mass spectrometer and an ACQUITY UPLC system revealed that inhibitors poorly traversed the cell membrane.

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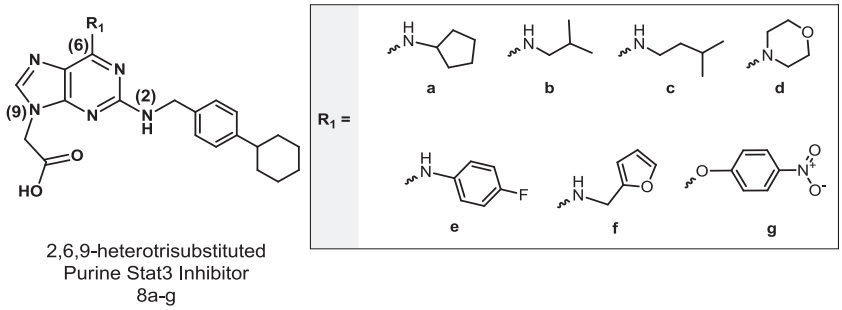


Figure 1. 1st generation 2,6,9-heterotrisubstituted purine inhibitors of Stat3.

Herein, synthetic efforts to prepare second-generation anti-Stat3, purine-based inhibitors, with improved cell permeability and enhanced cytotoxicity toward cancer cells are presented. Specifically, synthetic efforts were undertaken to mask the anionic N9-carboxylic acid substituent, present on lead Stat3 inhibitors prepared in the previous study. Prodrug and bioisostere strategies were employed to furnish a family of purine-based small molecules that retained the top ranked R1 and R2 substituents from the previous studies. However, while non-carboxylate containing analogs did not exhibit Stat3 binding affinity, compound **22e**, substituted with a sulfamate group, exhibited potent cytoxicity in multiple myeloma (MM) whole cell tumour studies. Efforts to delineate the intracellular targets are described.

2. Results and discussion

2.1. Inhibitor design

To overcome the poor cell penetrating properties of first generation purines, two design strategies were adopted. First, the N9 carboxylic acid substituent was masked using prodrug strategies.[14] Since lead purine compounds were considered relatively non-polar ($c\log P = -0.2$–4.1), and that masking the charged appendage might significantly reduce water solubility, we prepared a range of prodrugs with varying polarity to circumvent possible inhibitor aggregation. We reasoned that a successful prodrug approach will facilitate inhibitor cell membrane penetration, improve inhibitor half-life, and increase cellular potency.

Second, bioisosteres of the carboxylic acid were introduced for purposes of increasing inhibitor lipophilicity, reducing anionic character and improving binding potency through potentially more favorable and increased intermolecular interactions with the protein surface. In this study, the N9-carboxylic acid appendage was replaced with either a tetrazole or sulfamate appendage. Tetrazole, while possessing a similarly acidic proton to the carboxylate, possesses significantly greater lipophilicity, and potentially improved cell penetrative properties.[15] In addition, a neutral, hydrogen bonding sulfamate group was selected for making contacts with the pTyr binding pocket of Stat3's SH2 domain.

2.2. Preparation of 2,6,9-tri-heterosubstituted purines

Access to final molecules was achieved through four synthetic routes each starting from 2-amino-6-chloropurine (Fig. 2). Near quantitative BOC protection of the N9 position was achieved using BOC anhydride and catalytic dimethylamino-pyridine to give **2** (Scheme 1).[16] As previously reported by our group,[17] treatment with NaH efficiently mediated BOC group transfer from N9 to N2 (**3**). Mitsunobu reaction conditions using ethyl glycolate afforded the N9 alkylated product **4**, over the less reactive N7 position. Next, **4** was subjected to a successive round of Mitsunobu conditions, this time with cyclohexyl benzyl alcohol, to furnish the N2 alkylated product **5**. Using microwave mediated nucleophilic aromatic substitution, **5** was treated with a select series of previously identified alkylamines,[13] to give **6a**–**g** in good yields. Quantitative BOC deprotection of **6a**–**g** was achieved using standard trifluoroacetic acid/CH_2Cl_2 conditions to give the ethyl ester prodrug final molecules, **7a**–**g**. To access acyloxymethyl ester classes of carboxylic acid prodrug, the ethyl ester of compounds, **7a**–**g** was hydrolysed using LiOH mediated saponification to give carboxylic acids **8a**–**g**, then intermediates subjected to treatment with either acetoxymethyl bromide to yield the acyloxymethyl ester prodrugs (**9a**–**g**), or pivaloyloxymethyl iodide to yield the pivaloyloxymethyl ester prodrugs (**10a**–**g**). Due to stability issues, all prodrug analogs were used immediately after HPLC and lyophilisation. Purity levels were confirmed by analytical HPLC prior to in vitro or biological testing.

Preparation of N9-sulfamate-containing purine inhibitors were prepared by Mitsunobu-mediated installation of the sulfamate substituent (Scheme 2). Briefly, compound **3** was selectively alkylated on N9 with freshly prepared monosilyated ethylene glycol using Mitsunobu conditions as previously reported to acquire **17** in good yields.[18] Next, **17** was subjected to a subsequent Mitsunobu reaction with cyclohexyl benzyl alcohol to yield the N2 alkylated product **18**. Analogous to the prodrug approach, nucleophilic aromatic substitution was employed to incorporate alkylamines at C6 to yield compounds **19a**–**g**. TBDMS deprotection using TBAF in THF afforded the free alcohol **20a**–**g** in excellent yields. Finally, primary alcohols **20a**–**g** were subjected to NaH and treated with sulfonamide chloride (as prepared from chlorosulfonyl isocyante **23**)[19] to yield the sulfamate products **21a**–**g** in good yields (35–66%).[20] Finally, BOC deprotection using trifluoroacetic acid provided final molecules **22a**–**g** in excellent yields (73–89%).

Derivatives featuring the lipophilic tetrazole bioisostere utilized a modified alkylation procedure. Precursor **3**, was alkyated with trityl protected tetrazole **30**, proceeding through an S_N2 reaction after Mitsunobu conditions proved poor yielding (Scheme 3). The resulting mixture of N7 and N9 alkylated products were separated by chromatography, with the N9 product isolated in good yields providing **31**. Formation of **32** follows conventional N2 alkylation using Mitsunobu chemistry, and **33a**–**g** were formed using an aromatic substitution diversification step. Final molecules **34a**–**g** was obtained in good yields following a global de-protection of BOC and trityl groups using trifluoroacetic acid.

Several lead derivatives of the past library possessed a cyclohexyl carbonyl moiety in the N2 position To successfully acylate these molecules, the N2 nitrogen was liberated of protecting groups using trifluoroacetic acid or a Lewis acid ($AlCl_3$). The remaining steps follow logically from the methods just described and are detailed in Supplementary data.

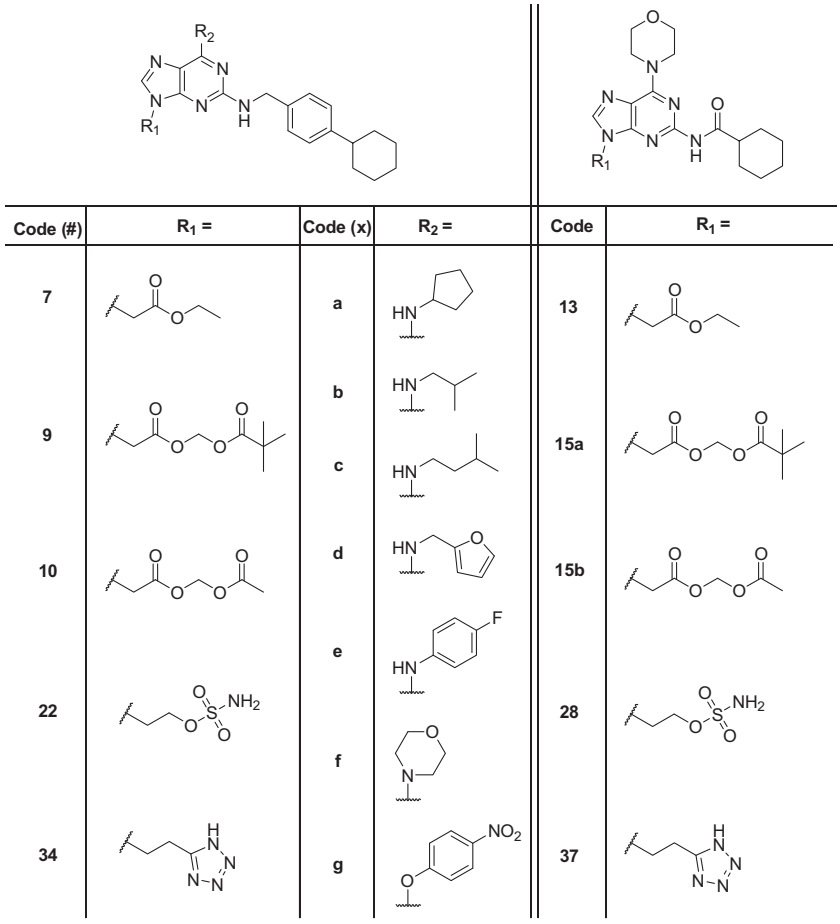
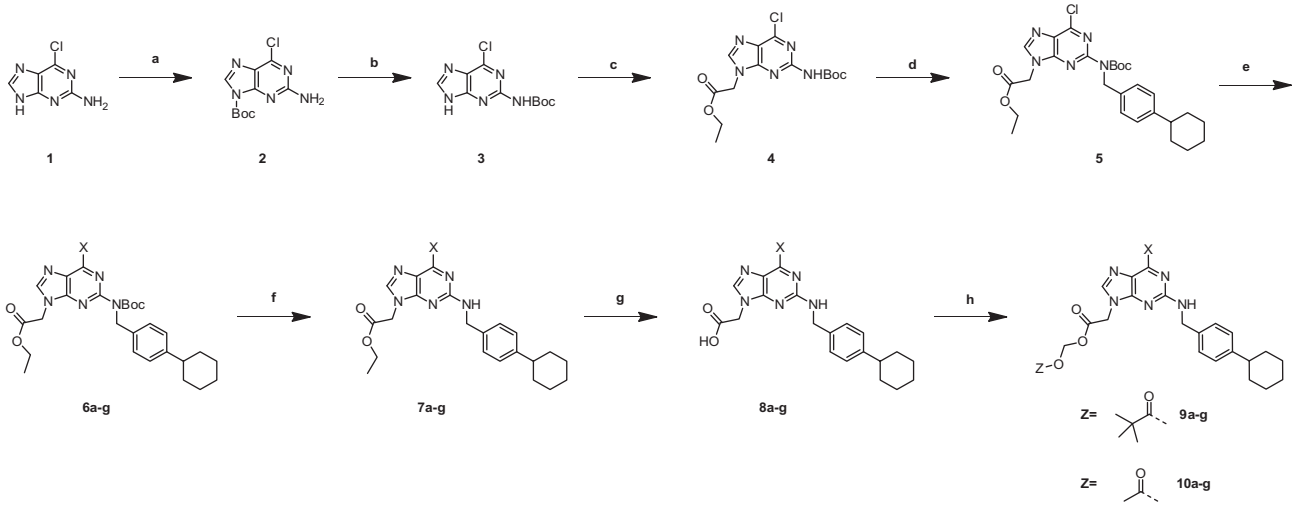


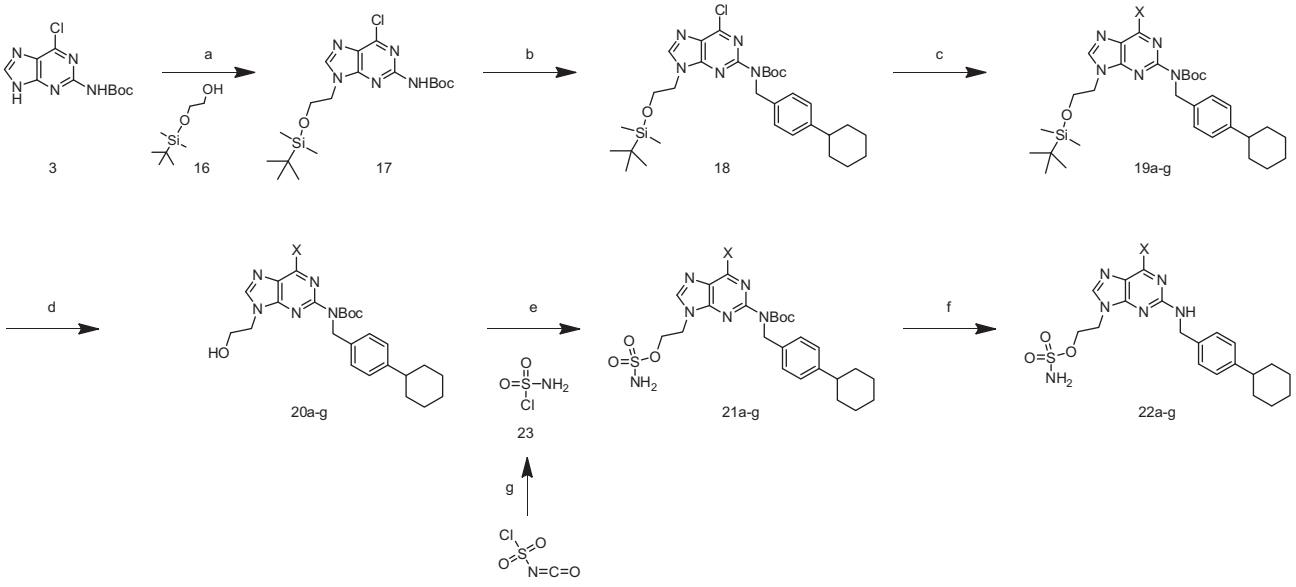
Figure 2. Summary and coding system for the 2nd generation library of purine inhibitors of Stat3.



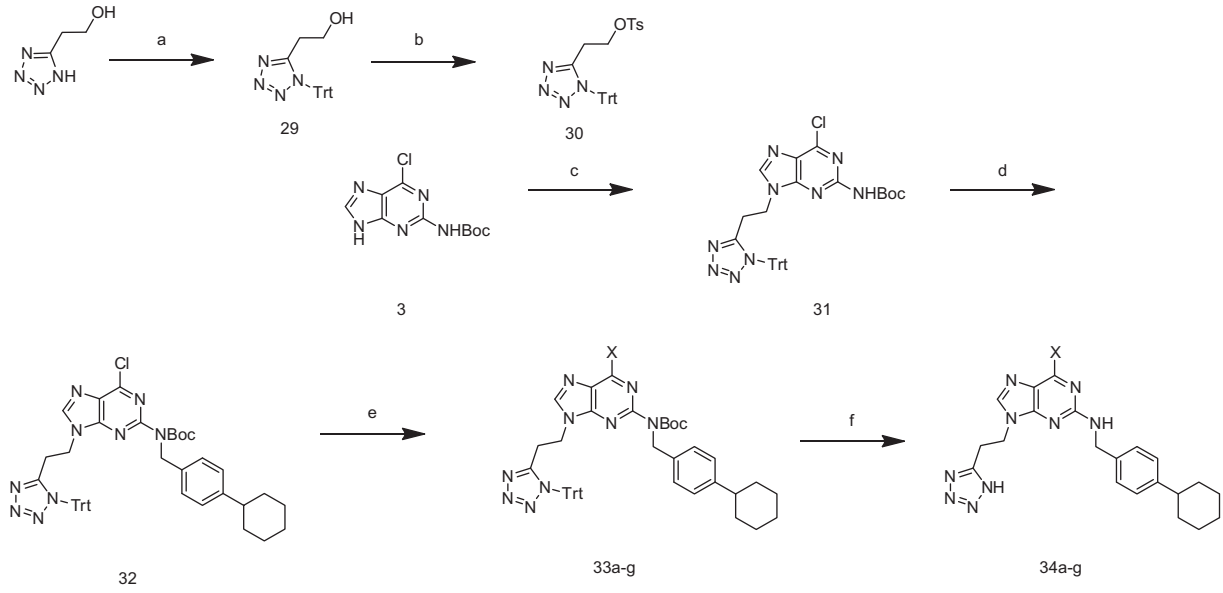
Scheme 1. Reagents and conditions: (a) BOC anhydride, DMSO, DMAP (cat), 0 °C to rt, 30 min, 75%; (b) NaH, THF, rt, 30 min, 95%; (c) (i) ethyl 2-hydroxyacetate, PPh_3, THF, rt, 2 min, (ii) DIAD, rt, 15 min, 83%; (d) (i) 4-cyclohexylbenzyl alcohol, PPh_3, THF, rt, 2 min, (ii) DIAD, rt, 15 min, 82–74%; (e) X (HNR′R″), DIPEA, DMSO, 105 °C, 40 min, microwave assisted, 65–97%; (f) TFA:CH_2Cl_2 (1:1), rt, 1 h, 63–95%; (g) LiOH, THF:H_2O (3:1), rt, 15 min, 75–93%; (h) acetoxymethyl bromide, DIPEA, DMF, rt, 6 h; or for **10a–g**; (h) pivoyloxymethyl iodide, DIPEA, DMF, rt, 6 h.

2.3. IC_{50} determination in tumour cell studies

To quantify improved cellular efficacy, cell lines known to harbor elevated Stat3 levels including, breast cancer (MDA-MB-468),[21] human prostate carcinoma (DU 145),[22] and acute myeloid leukemia 2 (OCI-AML2)[23] were subjected to purine analogs and assessed for inhibitor-induced cytotoxicity using an MTS colorimetric assay.[24] Significantly, no biological activity was observed amongst the library members, with the exception of **22e**, substituted at N9 with a sulphamate group. In MDA-MB-468 cells, **22e** showed



Scheme 2. Reagents and conditions: (a) (i) monosilyated ethylene glycol, PPh_3, THF, rt, 2 min, (ii) DIAD, rt, 15 min, 83%; (b) (i) 4-cyclohexylbenzyl alcohol, PPh_3, THF, rt, 2 min, (ii) DIAD, rt, 15 min, 82–74%; (c) X (HNR′R″), DIPEA, DMSO, 105 °C, 40 min, microwave assisted, 65–97%; (d) TBAF, THF, rt, 20 min, 80–88%; (e) sulfamoyl chloride, NaH, THF, rt, 30 min, 75–89%; (f) TFA:CH_2Cl_2 (1:1), rt, 1 h, 63–95%; (g) formic acid, N_2, 0 °C–rt, 81%.



Scheme 3. Reagents and conditions: (a) trityl chloride, DBU, DCM, rt, 16 h, 96%; (b) tosylchloride, DMAP, DIPEA, CH_2Cl_2, 0 → rt, 16 h, 85%; (c) OTs-trt-tetrazole, DMF, K_2CO_3, 60 °C, 62%; (d) (i) cyclohexylbenzyl alcohol, PPh_3, THF, rt, 2 min, (ii) DIAD, rt, 3 h, 68%; (e) X (HNR′R″), DIPEA, DMSO, 65 °C, 40 min, 32–86 %; (f) TFA:CH_2Cl_2 (1:1), rt, 1 h, 67–79%.

promising cell cytotoxicity (EC_{50} = 19.9 ± 0.9 μM). Lower potency was observed in the other cell lines treated. For further cellular evaluation, **22e** was incubated with a panel of eleven multiple myeloma (MM) primary cancer cell lines for 48 h and assessed for induced cell death using an MTT cytotoxicity assay. Encouragingly, **22e** demonstrated potent, dose-dependant cytotoxic effects in a number of cell lines, whilst being ineffective to others (Fig. 3). The most sensitive cell line to **22e**, XG6, possessing high levels of activated Stat3, was shown to be completely inhibited at 10 μM of inhibitor. A surprising result was the success of **22e** against SKMM2 cells, which do not harbor pStat3. Similarly, JJN3 cells, possessing elevated pStat3 levels were not sensitive to **22e**. Thus, we postulated that **22e**'s biological activity was likely a result of off-target effects and not due to Stat3 inhibition.

MTT assays directly measure levels of cellular metabolism with decreased levels indicating cell death. However, it is possible that compound **22e** functions as a metabolism suppressor. To evaluate for compound induced apoptosis, we next conducted an Annexin V apoptosis assay in the previously indicated cell lines, XG6 and JJN3. Cells were treated with **22e** for 48 h and directly compared to the activity of the anti-tumor mustard drug, mephalan. Results from this assay paralleled the results of the MTT, with XG6 cells experiencing complete late stage apoptosis after 48 h exposure to **22e** at a concentration of 15 μM (Fig. 4). Moreover, **22e** appears to have significantly more potent effects than mephalan. Biological effects in JJN3 cells treated with **22e** were significantly less than in XG6, as was previously observed in the MTT assay. With promising IC_{50} values of sub-10 μM against lethal multiple myeloma cancer cell

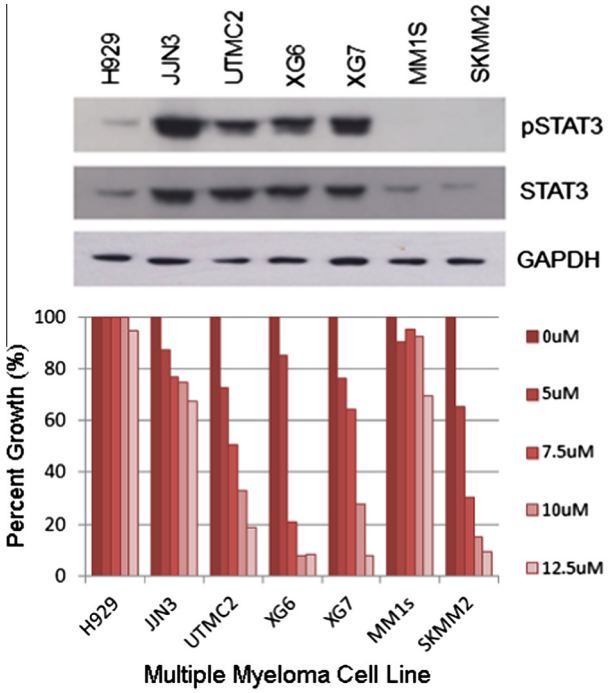


Figure 3. Western blot and the differential cellular response observed in the MTT assay when multiple myeloma cells are treated with **22e**.

lines, we elected to try and identify the intracellular target responsible for **22e**'s cytotoxicity.

2.4. Fluoresence polarization (FP) Stat3 binding assay

To assess for Stat3 SH2 domain binding affinities, purines were subjected to a competitive Stat SH2 domain FP assay.[25] Non-phosphorylated, full-length Stat3 monomers were incubated with fluorescently labeled Stat3 SH2 domain binding peptide, FAM-GpYLPQTV-NH_2, and treated with inhibitors and positive control pYLPQTV. Dose-dependant inhibitor-mediated displacement of the fluorescent peptide and resultant decreased polarization signal, facilitates IC_{50} determination (Fig. 5). Of the 38 inhibitors synthesized, we observed negligible disruption of the Stat3-phosphopeptide complex at inhibitor concentrations of up to 400 μM. Notably, lead anti-cancer compound, **22e** (determined by MTS assay), failed to displace the fluorescent peptide even at 100 μM, suggesting that the Stat3 SH2 domain was not the cellular target in MM cells.

2.5. Stat3 phosphorylation measured through flow cytometry

While **22e** may not inhibit Stat3 SH2 domain complexation events, it feasibly could be inhibiting de novo Stat3 phosphorylation in vitro by binding Stat3's SH2 domain and/or binding elsewhere on Stat3 and prevent recruitment to the target kinase and subsequent phosphorylation. Thus, we conducted phospho-flow experiments with **22e** to rule out a Stat3-related mode of inhibitory action. Thus, MM tumour cells, XG6 and OPM2 were starved overnight and then treated with various concentrations of **22e** (Fig. 6).[26] Starved OPM2 cells were then treated with **22e** and stimulated with human interleukin-6. After 30 min, cells were fixed and stained with mouse anti-pStat3-PE(BD). Samples were run on FACSCalibur(BD) flow cytometer and analyzed using FlowJo software. As expected, and in support of our in vitro FP data, **22e** failed to inhibit Stat3 phosphorylation at concentrations above cytotoxic IC_{50} values.

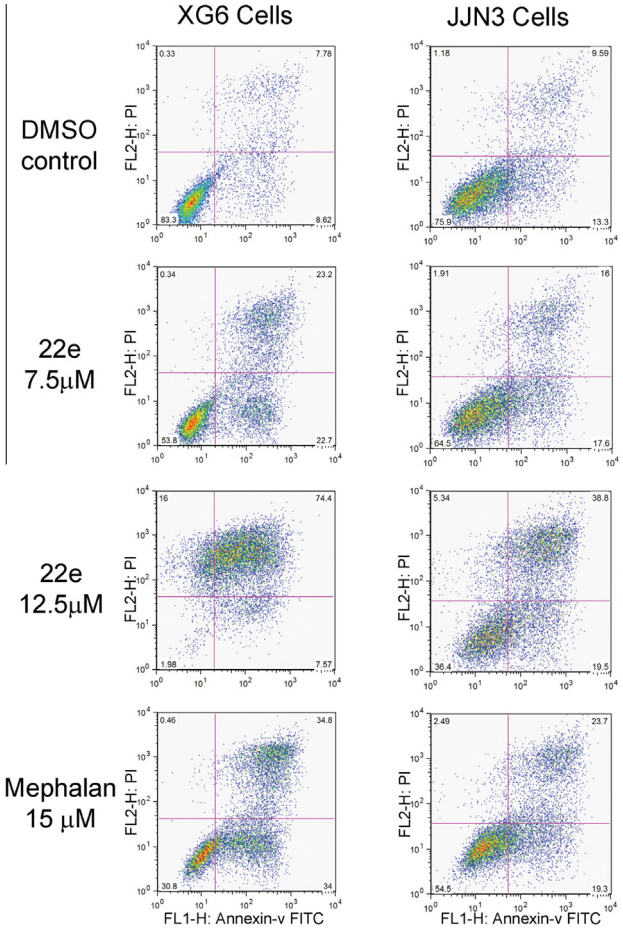


Figure 4. Annexin assay output developed using Flowo software following 48 h treatment with **22e** alongside positive control, mephalan. Bottom left quandrant indicates healthy cells, bottom right quandrant visualizes early stage apoptosis, and top quandrants display late stage apoptosis. Signal strength increases from blue to red.

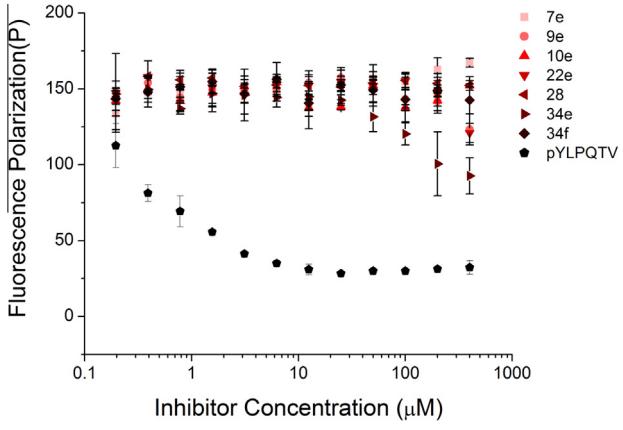


Figure 5. Representative purine agents performed poorly in a competitive fluorescence polarization assay.

2.6. Selected panel kinase screen and complete kinome screen

Given **22e**'s limited in vitro activity against Stat3, we elected to explore other likely cellular targets to help delineate the observed potency in myeloma cells. Traditionally, purine therapeutics have been utilized to great effect for inhibiting protein kinases through ATP binding site occupation. Since select kinases activate many of

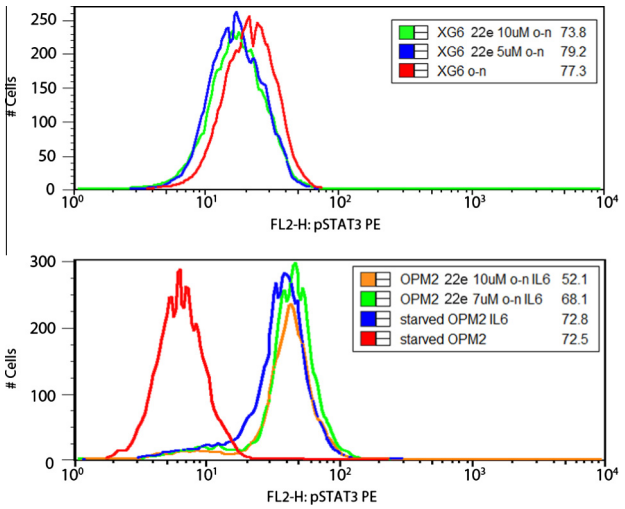


Figure 6. Phosphorylated Stat3 levels measured by phospho-flow cytometry.

the intracellular factors that promote the cancer phenotype, we explored **22e**'s potency toward kinases via a kinase filtration binding assay.[27] Briefly, a panel of ten cancer related kinases, ABL1, AKT1, c-Src, CDK1/cyclin B, CDK2/cyclin A, ERK2/MAPK1, FLT3, JAK2, JAK3, and LCK were tested at a single concentration (50 μM). The experiment measures performance of the target kinase in the presence of ^{32}P-γ-ATP or ^{33}P-γ-ATP. Of the ten kinases screened, JAK2 and JAK3 were 50% and 85% inhibited by **22e**, respectively. The JAK tyrosine kinases are often implicated in the phosphorylation of oncogenic proteins.[28,29] Multi-dose experiments were performed on the four JAK kinases to establish IC_{50} concentrations. As shown in Figure 7, **22e** exhibited modest activity against TYK1, JAK2 and JAK3 kinases with IC_{50}'s of 10.6, 16.6, and 10.5 μM, respectively. JAK1 was negligibly inhibited by **22e** (100 μM). Given the potency in whole cells, the levels of JAK inhibition exhibited may contribute to the observed biological effect but are likely not the only target.

Given the structural homology, and the promising activity against the JAK family of kinases, we reasoned that other kinases in the greater kinome family may be more strongly inhibited by **22e**. Thus, to screen **22e**'s inhibitory activity against other cellular kinases, we employed competitive qPCR screening to identify activity against a comprehensive 'DiscoveRx KINOMEscan' library of 456 human kinases. In this assay, kinases labeled with DNA were treated with **22e** (2.5 μM single concentration) and incubated with an immobilized ligand designed to capture its target kinase. Ultrasensitive quantitative PCR (qPCR) is employed to measure levels of immobilized kinases when treated with **22e** and relative kinase

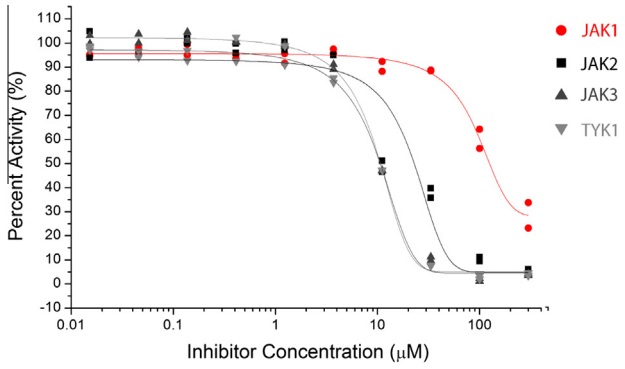


Figure 7. Compound **22e** inhibition of JAK family members kinase activity.

levels compared to control samples. In this screen, hits are classified as compounds where captured kinase levels fall below a 30% threshold. Images were generated using TREEspot software tool and reprinted with permission from KINOMEscan, a division of DiscoveRx Corporation, Discoverx Corporation 2010.

Surprisingly, of the 456 kinases treated with **22e**, only mutant abelson murine leukemia viral oncogene homolog 1 (ABL1 (F317L)) and Adapter-associated protein kinase 1 (AAK1) were substantially inhibited at 50% and 52%, respectively. Inhibitor **22e** did not, with the remaining kinases inhibit the remaining kinases below the critical range required for designation as a hit compound (Fig. 8). JAK families kinases, as expected, were minimally inhibited at the testing concentrations (Supporting Information). ABL1 is one half of the chimeric protein, BCR-ABL, which results from a reciprocal translocation between chromosome 9 and 22.[30] The discovery that fusion oncoprotein BCR-ABL drives the formation and proliferation of chronic myeloid leukemia (CML) produced potent tyrosine kinase inhibitors (TKI), specifically the blockbuster drug imatinib.[31] Unfortunately, 20% of patients treated with imatinib develop

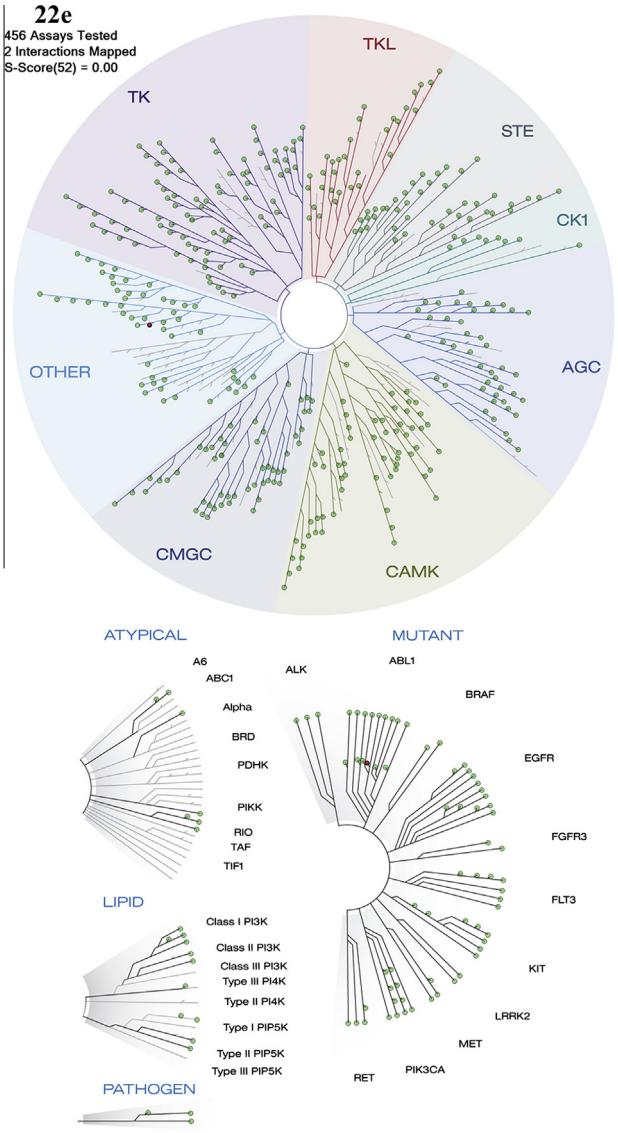


Figure 8. A complete kinome scan highlights insensitivity to treatment with **22e**. Highlighted in red are ABL1and AAK1. Images were generated using TREEspot software tool and reprinted with permission from KINOMEscan, a division of DiscoveRx Corporation, Discoverx Corporation 2010.

protein mutations leading to drug-resistance.[32] One such mutant is ABL1 (F317L), a variant which demonstrated sensitivity to treatment with **22e**. Agents that bind ABL1 mutants and prevent their activity are potential useful for treatment of CML patients.[33] Third generation TKIs currently in development boast IC_{50}'s 100-fold lower than that of **22e**.[34] In addition, aberrant ABL activity is unrelated to the progression of multiple myeloma and thus unlikely to be our biological target in MM cells. Investigation of **22e**'s potency in treating imatinib resistant CML tumour cells may be an interesting line of research.

AAK1 plays an up-regulatory role in the Notch pathway.[35] Studies show that dysregulation of Notch signaling can lead to various human diseases, including cancer.[36] A recent study demonstrated that Dll1/Notch interaction increases MM-cell proliferation. Thus, we postulate that **22e** may be acting as a Notch signaling inhibitor in MM cells investigated with additional off-target effects against JAK kinases.[37] However, the lack of Stat3 knockdown suggests that the inhibition of upstream JAK kinases is minimal, as greater inhibition of Stat3 phosphorylation would have been expected. Further investigation into AAK1's role in multiple myeloma, and **22e**'s inhibitory effect upon this kinase in MM cells is required before we can more accurately assign the origin of **22e**'s biological activity.

3. Conclusions

A focused library of hetero-trisubstituted purines was developed for improving the cell penetrating and biological efficacy of a series of anti-Stat3 protein inhibitors. From this SAR study, lead agent **22e** was identified as being a promising inhibitor of MM tumour cells (IC_{50}'s <5 μM). Surprisingly, biophysical and biochemical characterization proved that **22e** was not a Stat3 inhibitor. Initial screening against the kinome, prompted by the purine scaffold's history for targeting ATP binding pockets, suggests possible targeting of the JAK family kinases, as well for ABL1 (nonphosphorylated F317L) and AAK1. At 2.5 μM the remaining 454 kinases evaluated were insensitive to **22e**. However, the exact molecular target responsible for **22e**'s promising cytotoxicity in MM remains to be defined, and is part of an ongoing investigation within our lab.

4. Experimental section

4.1. Chemistry: final molecule characterization

4.1.1. Ethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-(cyclopentylamino)-9*H*-purin-9-yl)acetate (7a)

IR (KBr, cm^{-1}) 3373, 2924, 2851, 1753, 1607, 1488, 1384 1261, 1201; δ_H (400 MHz, $CDCl_3$) 1.18–1.46 (m, 5H, (cyclohexyl)), 1.28 (t, *J* = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 1.46–1.70 (m, 8H, 5H (cyclohexyl) & 3H (cyclopentyl)), 1.71–1.85 (m, 5H, (cyclopentyl)), 2.03 (br s, 2H (cyclopentyl)), 2.43–2.50 (m, 1H, CH), 4.24 (q, *J* = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 4.58 (d, *J* = 5.8 Hz, 2H, CH_2Ar), 4.78 (s, 2H, CH_2CO_2Et), 5.12 (br s, 1H, NH), 5.47 (br s, 1H, NH), 7.15 (d, *J* = 8.1 Hz, 2H, 2CH (Ar)), 7.28 (d, *J* = 8.1 Hz, 2H, 2CH (Ar)), 7.52 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 14.1, 23.8, 26.2, 26.9, 33.4, 34.5, 43.8, 44.3, 45.7, 62.0, 113.4, 126.7, 127.5, 136.8, 137.4, 146.6, 151.1, 155.0, 159.5, 167.6; HRMS (MS-ES), calcd for $C_{27}H_{37}N_6O_2$ [M+H] *m*/*z* = 477.2978, found: 465.2990; *rp*HPLC t_R: condition (I) 14.784 (II) 37.297 min, purity 97.4% and 91.7%.

4.1.2. Ethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-(isobutylamino)-9*H*-purin-9-yl)acetate (7b)

IR (KBr, cm^{-1}) 3263, 2957, 2923, 2851, 1750, 1627, 1602, 1550, 1384, 1261, 1222, 1126; δ_H (400 MHz, $CDCl_3$) 0.96 (d, *J* = 6.6 Hz, 6H, $CH_2CH(CH_3)_2$), 1.24–1.47 (m, 8H, 5H (cyclohexyl) and $CO_2CH_2CH_3$), 1.53–1.95 (m, 6H, $CH_2CH(CH_3)_2$ and 5H (cyclohexyl)), 2.40–2.47 (m, 1H, $CH(CH_3)_2$), 3.38 (br s, 2H, $CH_2CH(CH_3)_2$), 4.24 (q, *J* = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.58 (d, *J* = 5.8 Hz, 2H, CH_2Ar), 4.78 (s, 2H, CH_2CO_2Et), 5.07 (br s, 1H, NH), 5.56 (br s, 1H, NH), 7.15 (d, *J* = 7.9 Hz, 2H, 2CH (Ar)), 7.29 (d, *J* = 7.8 Hz, 2H, 2CH (Ar)), 7.50 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 14.0, 20.1, 26.0, 26.8, 28.5, 29.6, 34.4, 43.6, 44.1, 45.5, 61.8, 113.4, 126.7, 127.5, 136.8, 137.4, 146.6, 151.1, 155.0, 159.5, 167.6; HRMS (MS-ES), calcd for $C_{26}H_{37}N_6O_2$ [M+H] *m*/*z* = 465.2978, found: 465.2977; *rp*HPLC t_R: condition (I) 18.274 (II) 38.507 min, purity 96.8% and 92.7%.

4.1.3. Ethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-(isopentylamino)-9*H*-purin-9-yl)acetate (7c)

IR (KBr, cm^{-1}) 3265, 2959, 2926, 2852, 1737, 1681, 1650, 1610, 1522, 1429, 1384, 1261, 1231, 1202, 1127, 1098, 1021; δ_H (400 MHz, $CDCl_3$) 0.92 (d, *J* = 6.5 Hz, 6H, $(CH_2)_2CH(CH_3)_2$), 1.25–1.39 (m, 9H, 5H (cyclohexyl), $CO_2CH_2CH_3$, and CH_2CH_2CH), 1.49–1.65 (m, 2H, $CH_2CH_2CH(CH_3)_2$), 1.65–1.89 (m, 7H, $CH_2CH_2CH(CH_3)_2$ and 5H (cyclohexyl)), 2.40–2.47 (m, 1H, CH), 3.58 (br s, 1H, NH), 4.02 (s, 1H, NH), 4.24 (q, *J* = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.58 (s, 2H, CH_2Ar), 4.59 (s, 2H, CH_2CO_2Et), 7.15 (d, *J* = 8.1 Hz, 2H, 2CH (Ar)), 7.26 (d, *J* = 8.1 Hz, 2H, 2CH (Ar)), 7.61 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 14.0, 22.4, 25.6, 26.0, 26.8, 29.6, 34.4, 38.6, 43.6, 44.1, 45.5, 61.8, 113.4, 126.6, 127.5, 136.7, 137.6, 146.6, 151.2, 155.0, 159.8, 167.6; HRMS (MS-ES), calcd for $C_{27}H_{39}N_6O_2$ [M+H] *m*/*z* = 479.3134, found: 479.3140; *rp*HPLC t_R: condition (I) 20.092 (II) 39.919 min, purity 94.4% and 93.1%.

4.1.4. Ethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-morpholino-9*H*-purin-9-yl)acetate (7d)

IR (KBr, cm^{-1}) 3255, 3096, 2925, 2855, 2589, 1787, 1759, 1677, 1608, 1555, 1436, 1215, 1167; δ_H (400 MHz, $CDCl_3$) 1.27–1.40 (m, 8H, 5H (cyclohexyl) and $CO_2CH_2CH_3$), 1.67–1.88 (m, 5H (cyclohexyl)), 2.42–2.48 (m, 1H, CH), 3.73–3.80 (m, 4H, $2CH_2$, (morpholine)), 3.94 (br s, 2H, 2 CH_2, (morpholine)), 4.27 (q, *J* = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 4.56 (s, 2H, CH_2Ar), 4.64 (br s, 2H, $2CH_2$, (morpholine)), 4.97 (s, 2H, CH_2CO_2Et), 7.13 (d, *J* = 8.1 Hz, 2H, 2CH (Ar)), 7.23 (d, *J* = 8.2 Hz, 2H, 2CH (Ar)), 7.40 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 14.1, 26.1, 26.9, 34.5, 43.8, 44.3, 45.7, 62.0, 67.1, 113.4, 126.9, 127.6, 136.1, 137.4, 146.9, 151.3, 155.2, 159.1, 167.7; HRMS (MS-ES), calcd for $C_{26}H_{34}N_6O_3$ [M+H] *m*/*z* = 479.2692, found: 479.2648; *rp*HPLC t_R: condition (I) 15.691 (II) 36.602 min, purity 92.8% and 94.0%.

4.1.5. Ethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-((4-fluoro phenyl)amino)-9*H*-purin-9-yl)acetate (7e)

IR (KBr, cm^{-1}) 3357, 2924, 2851, 1747, 1681, 1629, 1594, 1508, 1384, 1208, 1140; δ_H (400 MHz, $CDCl_3$) 1.26–1.48 (m, 8H, 5H (cyclohexyl) and $CO_2CH_2CH_3$), 1.67–1.91 (m, 5H (cyclohexyl)), 2.43–2.49 (m, 1H, CH), 4.26 (q, *J* = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 4.60 (s, 2H, CH_2Ar), 4.85 (s, 2H, CH_2CO_2Et), 6.97–7.01 (m, 2H, 2CH (Ar)), 7.17 (d, *J* = 7.9 Hz, 2H, 2CH (Ar)), 7.27 (d, *J* = 8.0 Hz, 2H, 2CH (Ar)), 7.45–7.78 (m, 4H, 3CH (Ar) and CH (H-8)); δ_C (100 MHz, $CDCl_3$) 14.1, 26.1, 26.9, 34.5, 43.8, 44.3, 45.8, 62.1, 113.7, 115.2, 115.4, 121.6, 121.7, 126.9, 127.5, 135.1, 137.1, 137.8, 147.0, 151.7, 152.2, 157.4, 159.5, 159.8, 167.5; HRMS (MS-ES), calcd for $C_{28}H_{32}N_6O_2$ [M+H] *m*/*z* = 503.2571, found: 503.2583; *rp*HPLC t_R: condition (I) 20.913 (II) 40.496 min, purity 92.7% and 85.0%.

4.1.6. Ethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-((furan-2-ylmethyl)amino)-9*H*-purin-9-yl)acetate (7f)

IR (KBr, cm^{-1}) 3263, 2923, 2850, 1739, 1678, 1611, 1384, 1200, 1141; δ_H (400 MHz, $CDCl_3$) 1.25–1.39 (m, 5H, cyclohexyl), 1.63–1.82 (m, 5H (cyclohexyl)), 2.41–2.49 (m, 1H, CH), 4.16 (q, *J* = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.53 (d, *J* = 5.7 Hz, 2H, CH_2NH), 4.65–4.75 (m, 4H, CH_2 (furfuryl) and CH_2Ar), 5.19 (br s, 1H, NH), 6.02

(br s, 1H, NH (furfuryl)), 6.14–6.16 (m, 1H, CH (furfuryl)), 6.21–6.22 (m, 1H, CH (furfuryl)), 7.10 (d, J = 8.0 Hz, 2H, 2CH (Ar)), 7.21 (d, J = 8.0 Hz, 2H, 2CH (Ar)), 7.25–7.26 (m, 1H, CH (furfuryl)), 7.43 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 14.1, 26.2, 26.9, 34.5, 43.8, 44.3, 45.7, 62.0, 107.3, 110.4, 126.9, 127.6, 137.3, 137.4, 142.0, 146.9, 152.1, 154.4, 167.6; HRMS (MS-ES), calcd for $C_{27}H_{33}N_6O_3$ [M+H] m/z = 489.2614, found: 489.2623; *rp*HPLC t_R: condition (I) 17.405 (II) 37.814 min, purity 92.8% and 93.24%.

4.1.7. Ethyl2-(2-((4-cyclohexylbenzyl)amino)-6-(4-nitro phenoxy)-9*H*-purin-9-yl)acetate (7g)

IR (KBr, cm^{-1}) 3422, 3231, 3074, 2926, 2853, 1748, 1630, 1581, 1526, 1406, 1384, 1251, 1225; δ_H (400 MHz, $CDCl_3$) 1.29–1.45 (m, 8H, 5H (cyclohexyl) and $CO_2CH_2CH_3$), 1.73–1.85 (m, 5H (cyclohexyl)), 2.43–2.49 (m, 1H, CH), 4.27 (q, J = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.44 (s, 2H, CH_2Ar), 4.86 (s, 2H, CH_2CO_2Et), 5.28 (br s, 1H, NH), 7.14 (s, 4H, 4CH (Ar)), 7.36 (d, J = 9.9 Hz, 2H, 2CH (Ar)), 7.74 (s, 1H, CH (H-8)), 8.23 (d, J = 8.7 Hz, 2H, 2CH (Ar)); HRMS (MS-ES), calcd for $C_{28}H_{31}N_6O_5$ [M+H] m/z = 531.2356, found: 531.2357; *rp*HPLC t_R: condition (I) 25.147 (II) 43.779 min, purity 99.2% and 99.3%.

4.1.8. Ethyl 2-(2-(cyclohexanecarboxamido)-6-morpholino-9*H*-purin-9-yl)acetate (13)

Mp = 142–147 °C; IR (KBr, cm^{-1}) 3551, 3415, 3238, 2928, 2852, 1755, 1669, 1604, 1585, 1514, 1448, 1407, 1327, 1266; δ_H (400 MHz, $CDCl_3$) 1.29 (t, J = 7.2 Hz, 3H, $CO_2CH_2CH_3$), 1.28–1.32 (m, 3H, CH_2 (cyclohexyl)), 1.49–1.58 (m, 2H, (cyclohexyl)), 1.69–1.71 (m, 1H, (cyclohexyl)), 1.82–1.84 (m, 2H, (cyclohexyl)), 1.96–1.99 (m, 2H, (cyclohexyl)), 2.86–2.91 (m, 1H, CH), 3.82 (t, J = 4.9 Hz, 4H, $2CH_2$ (morpholine)), 4.25 (q, J = 7.2 Hz, 2H, $CO_2CH_2CH_3$), 4.27 (br s, 4H, $2CH_2$ (morpholine)), 4.87 (s, 2H, CH_2CO_2Et), 7.69 (br s, 1H, NH), 7.70 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 14.3, 26.0, 29.5, 44.2, 45.3, 45.4, 45.5, 62.4, 67.2, 116.4, 138.5, 152.4, 152.7, 154.1, 167.5, 175.9; HRMS (MS-ES), calcd for $C_{20}H_{29}N_6O_4$ [M+H] m/z = 417.2250, found: 417.2258; *rp*HPLC t_R: condition (I) 4.012 (II) 26.231 min, purity 97.4% and 95.5%.

4.1.9. (2-(2-((4-Cyclohexylbenzyl)amino)-6-(cyclopentyl amino)-9*H*-purin-9-yl)acetoxy)methyl pivalate (9a)

IR (KBr, cm^{-1}) 3404, 2927, 2853, 1762, 1686, 1637, 1437, 1384, 1201.7, 1138, 1110; δ_H (400 MHz, $CDCl_3$) 1.20 (s, 9H, $C(CH_3)_3$), 1.22–1.43 (m, 9H, 5H (cyclohexyl) and 4H (cyclopentyl)), 1.67–1.75 (m, 5H, cyclohexyl), 2.00–2.15 (m, 3H, cyclopentyl), 2.43–2.48 (m, 1H, CH), 4.56 (s, 2H, CH_2Ar), 4.83 (s, 2H, CH_2CO_2H), 5.80 (s, 2H, OCH_2), 6.99 (br s, 1H, NH), 7.15 (d, J = 8.1 Hz, 2H, 2CH (Ar)), 7.22 (d, J = 7.9 Hz, 2H, 2CH (Ar)), 7.65 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 23.9, 26.3, 27.0, 27.1, 33.7, 34.7, 39.0, 44.0, 44.5, 44.8, 56.2, 80.7, 111.2, 127.3, 127.7, 135.2, 139.5, 147.7, 152.5, 153.6, 163.6, 165.9, 177.1; HRMS (MS-ES), calcd for $C_{31}H_{43}N_6O_4$ [M+H] m/z = 563.3346, found: 563.3351; *rp*HPLC t_R: condition (I) 22.558 (II) 41.740 min, purity 88.2% and 85.4%.

4.1.10. (2-(2-((4-Cyclohexylbenzyl)amino)-6-(isobutylamino)-9*H*-purin-9-yl)acetoxy)methyl pivalate (9b)

IR (KBr, cm^{-1}) 3282, 2961, 2927, 2852, 1761, 1686, 1641, 1433, 1384, 1202, 1109; δ_H (400 MHz, $CDCl_3$) 1.01 (d, J = 6.0 Hz, 6H, $CH_2CH(CH_3)_2$), 1.21 (s, 9H, $C(CH_3)_3$), 1.24–1.47 (m, 5H, cyclohexyl), 1.53–1.95 (m, 5H, cyclohexyl), 2.02 (heptet, J = 6.6 Hz, $CH(CH_3)_2$), 2.43–2.50 (m, 1H, $CH(CH_3)_2$), 3.84–3.87 (m, 2H, $CH_2CH(CH_3)_2$), 4.58 (s, 2H, CH_2Ar), 4.82 (s, 2H, CH_2CO_2H), 5.81 (s, 2H, OCH_2), 7.05 (br s, 1H, NH), 7.16 (d, J = 7.9 Hz, 2H, 2CH (Ar)), 7.26 (d, J = 7.8 Hz, 2H, 2CH (Ar)), 7.45 (br s, 1H, NH), 7.61 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 19.6, 26.0, 26.6, 26.7, 28.9, 34.3, 38.6, 43.6, 44.1, 44.5, 51.2, 80.3, 110.9, 126.9, 127.3, 134.8, 138.9, 147.4, 152.1, 165.5, 176.8; HRMS (MS-ES), calcd for $C_{30}H_{43}N_6O_4$ [M+H] m/z = 551.3346, found: 551.3346; *rp*HPLC t_R: condition (I) 22.228 (II) 41.547 min, purity 99.3% and 98.5%.

4.1.11. (2-(2-((4-Cyclohexylbenzyl)amino)-6-(isopentylamino)-9*H*-purin-9-yl)acetoxy)methyl pivalate (9c)

IR (KBr, cm^{-1}) 3417, 2955, 2917, 2849, 1765, 1691, 1430, 1384, 1261, 1205, 1108, 1029; δ_H (400 MHz, $CDCl_3$) 0.95 (d, J = 7.2 Hz, 6H, $(CH_2)_2CH(CH_3)_2$), 1.21 (s, 9H, $C(CH_3)_3$), 1.24–1.44 (m, 5H, cyclohexyl), 1.55–1.85 (m, 8H, $CH_2CH_2CH(CH_3)_2$ and 5H (cyclohexyl)), 2.43–2.50 (m, 1H, CH), 4.01 (br s, 2H, $CH_2CH_2CH(CH_3)_2$), 4.57 (s, 2H, CH_2Ar), 4.82 (s, 2H, CH_2CO_2H), 5.80 (s, 2H, OCH_3), 7.03 (br s, 1H, NH), 7.16 (d, J = 8.0 Hz, 2H, 2CH (Ar)), 7.24 (d, J = 8.0 Hz, 2H, 2CH (Ar)), 7.61 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 19.6, 22.3, 26.0, 26.6, 26.7, 28.9, 34.3, 38.6, 43.6, 44.1, 44.5, 51.2, 80.4, 99.8, 110.4, 126.9, 127.3, 134.8, 139.1, 149.4, 152.1, 169.2, 182.6; HRMS (MS-ES), calcd for $C_{31}H_{45}N_6O_4$ [M+H] m/z = 565.3502, found: 565.3496; *rp*HPLC t_R: condition (I) 24.309 (II) 43.125 min, purity 100% and 99.0%.

4.1.12. (2-(2-((4-Cyclohexylbenzyl)amino)-6-morpholino-9*H*-purin-9-yl)acetoxy)methyl pivalate (9d)

IR (KBr, cm^{-1}) 3421, 2924, 2851, 1636, 1448, 1384, 1200; δ_H (400 MHz, $CDCl_3$) 1.20 (s, 9H, $(CH_3)_3$), 1.32–1.45 (m, 5H, cyclohexane), 1.72–1.83 (m, 5H, cyclohexane), 2.44–2.47 (m, 1H, CH (cyclohexane)), 3.76–3.78 (m, 2H, $O(CH_2)_2$), 4.20 (m, 2H, $N(CH_2)_2$), 4.54 (d, J = 5.0 Hz, 2H, CH_2NH), 4.85 (s, 2H, NCH_2CO), 5.80 (s, 1H, OCH_2O), 7.14 (d, J = 8.0 Hz, 1H, CH (Ar)), 7.26 (d, J = 8.0 Hz 1H, CH (Ar)), 7.48 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 26.0, 26.6, 26.7, 34.3, 38.6, 44.1, 45.1, 66.6, 80.8, 126.7, 127.1, 135.4, 136.0, 147.0, 152.1, 163.0, 163.3, 165.4, 176.8; HRMS (MS-ES), calcd for $C_{30}H_{41}N_6O_4$ [M+H] m/z = 565.3138, found: 565.3124; *rp*HPLC t_R: condition (I) 20.347 (II) 40.128 min, purity 95.8% and 94.1%.

4.1.13. (2-(2-((4-Cyclohexylbenzyl)amino)-6-((4-fluorophenyl) amino)-9*H*-purin-9-yl)acetoxy)methyl pivalate (9e)

IR (KBr, cm^{-1}) 3415, 2924, 2851, 1757, 1681, 1594, 1508, 1384, 1203, 1131; δ_H (400 MHz, $CDCl_3$) 1.22 (s, 9H, $C(CH_3)_3$), 1.31–1.45 (m, 5H, cyclohexyl), 1.72–1.88 (m, 5H, cyclohexyl), 2.44–2.53 (m, 1H, CH), 4.60 (s, 2H, CH_2Ar), 4.95 (br s, 2H, CH_2), 5.83 (s, 2H, CH_2), 7.02–7.08 (m, 2H, C_6H_4F), 7.17 (d, J = 8.0 Hz, 2H, C_6H_4), 7.23 (d, J = 7.8 Hz, 2H, C_6H_4), 7.45–7.51 (m, 2H, C_6H_4F), 7.61 (br s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 26.0, 26.6, 26.7, 34.3, 38.6, 44.1, 115.5, 115.7, 126.9, 165.2; HRMS (MS-ES), calcd for $C_{32}H_{37}FN_6O_4$ [M+H] m/z = 589.2933, found: 589.2960; *rp*HPLC t_R: condition (I) 25.875 (II) 38.964 min, purity 95.2% and 98.6%.

4.1.14. (2-(2-((4-Cyclohexylbenzyl)amino)-6-((furan-2-ylmethyl)amino)-9*H*-purin-9-yl)acetoxy)methyl pivalate (9f)

IR (KBr, cm^{-1}) 3424, 2961, 2924, 1762, 1685, 1648, 1612, 1429, 1384, 1261; δ_H (400 MHz, $CDCl_3$) 1.20 (s, 9H, $(CH_3)_3$), 1.37–1.45 (m, 5H, cyclohexane), 1.72–1.83 (m, 5H, cyclohexane), 2.45–2.48 (m, 1H, CH (cyclohexane)), 4.77 (m, 2H, CH_2 (furan), 4.83 (s, 2H, CH_2CO), 5.80 (s, 2H, OCH_2O),), 6.22 (m, 1H, CH (furan)), 6.28–6.29 (m, 1H, CH (furan)), 7.15 (d, J = 8.0 Hz, 1H, CH (Ar)), 7.26 (d, J = 8.1 Hz 1H, CH (Ar)), 7.33 (m, 1H, CH (furan)), 7.50 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 26.0, 26.6, 26.7, 34.3, 38.6, 40.4, 44.1, 45.0, 80.3, 99.5, 108.4, 110.3, 123.6, 126.9, 127.3, 139.3, 142.7, 159.1, 177.7; HRMS (MS-ES), calcd for $C_{31}H_{39}N_6O_5$ [M+H] m/z = 575.2982, found: 575.2979; *rp*HPLC t_R: condition (I) 21.615 (II) 41.022 min, purity 96.1% and 94.8%.

4.1.15. (2-(2-((4-Cyclohexylbenzyl)amino)-6-(4-nitrophenoxy)-9*H*-purin-9-yl)acetoxy)methyl pivalate (9g)

IR (KBr, cm^{-1}) 3441, 2925, 2851, 1770, 1751, 1627, 1581, 1407, 1384, 1348, 1258, 1235, 1114; δ_H (400 MHz, $CDCl_3$) 1.22 (s, 9H, $C(CH_3)_3$), 1.33–1.45 (m, 5H, cyclohexyl), 1.73–1.85 (m, 5H,

cyclohexyl), 2.46–2.50 (m, 1H, CH), 4.42 (s, 2H, CH_2), 4.94 (s, 2H, CH_2Ar), 5.84 (s, 2H, CH_2), 7.09–7.16 (m, 4H, CH (Ar)), 7.36 (d, J = 8.8 Hz, 2H, CH (Ar)), 7.77 (s, 1H, CH (H-8)), 8.22–8.25 (d, J = 8.8 Hz, 2H, CH_2 (Ar)); δ_C (100 MHz, $CDCl_3$) 26.3, 27.0, 27.1, 34.7, 39.0, 44.2, 44.4, 45.9, 80.6, 114.34, 122.7, 125.3, 127.2, 127.6, 127.6, 136.2, 140.5. 145.1, 147.6, 157.6, 158.8, 159.5, 166.2, 177.2; HRMS (MS-ES), calcd for $C_{32}H_{37}N_6O_7$ [M+H] m/z = 617.2724, found: 617.2726; *rp*HPLC t_R: condition (I) 29.638 (II) 47.065 min, purity 87.4% and 91.0%.

4.1.16. (2-(2-(cyclohexanecarboxamido)-6-morpholino-9H-purin-9-yl)acetoxy)methyl pivalate (15a)

IR (KBr, cm^{-1}) 3441, 3229, 2928, 2851, 1774, 1751, 1665, 1604, 1586, 1515, 1384, 1315, 1262, 1113; δ_H (400 MHz, $CDCl_3$) 1.24 (s, 9H, $C(CH_3)_3$), 1.27–1.33 (m, 3H, CH_2 (cyclohexyl)), 1.48–1.56 (m, 2H, (cyclohexyl)), 1.68–1.70 (m, 1H, (cyclohexyl)), 1.81–1.83 (m, 2H, (cyclohexyl)), 1.97–2.00 (m, 2H, (cyclohexyl)), 2.88–2.93 (m, 1H, CH), 3.83 (t, J = 4.9 Hz, 4H, 2 CH_2 (morpholine)), 4.28 (br s, 4H, 2CH_2 (morpholine)), 4.88 (s, 2H, CH_2), 5.85 (s, 2H, CH_2), 7.68 (br s, 1H, NH), 7.76 (s, 1H, CH (H-8)); HRMS (MS-ES), calcd for $C_{24}H_{35}N_6O_6$ [M+H] m/z = 503.2618, found: 503.2615; *rp*HPLC t_R: condition (I) 3.745 (II) 24.977 min, purity 80.8% and 80.1%.

4.1.17. Acetoxymethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-(cyclopentylamino)-9*H*-purin-9-yl)acetate (10a)

δ_H (400 MHz, $CDCl_3$) 1.22–1.43 (m, 9H, 5H (cyclohexyl) and 4H (cyclopentyl)), 1.55 (s, 3H, $COCH_3$), 1.67–1.75 (m, 5H, cyclohexyl), 2.00–2.15 (m, 3H, cyclopentyl), 2.43–2.48 (m, 1H, CH), 4.56 (s, 2H, CH_2Ar), 4.83 (s, 2H, CH_2CO_2H), 5.80 (s, 2H, OCH_2), 6.99 (br s, 1H, NH), 7.15 (d, J = 8.1 Hz, 2H, 2CH (Ar)), 7.22 (d, J = 7.9 Hz, 2H, 2CH (Ar)), 7.65 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 13.8, 21.7, 27.8, 27.9, 28.3, 43.0, 43.7, 47.3, 79.3, 115.7, 126.5, 127.0, 127.1, 128.0, 140.0, 141.3, 149.8, 153.9, 155.2, 169.2; HRMS (MS-ES), calcd for $C_{27}H_{37}N_6O_2$ [M+H] m/z = 477.2978, found: 477.2991; *rp*HPLC t_R: condition (I) 17.105 (II) 37.622 min, purity 52.54% and 53.85%.

4.1.18. Acetoxymethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-(isobutylamino)-9*H*-purin-9-yl)acetate (10b)

IR (KBr, cm^{-1}) 3386, 2927, 2853, 1774, 1685, 1642, 1515, 1433.53, 1367, 1202, 1139; δ_H (400 MHz, $CDCl_3$) 1.19–1.28 (m, 2H, CH_2CH), 1.29–1.33 (d, J = 6.7 Hz, 6H, CH(CH_3)$_2$), 1.32–1.43 (m, 5H, cyclohexyl), 1.70–1.90 (m, 5H, cyclohexyl), 1.96–2.08 (m, 1H, CH(CH_3)$_2$), 2.11 (s, 3H, $COCH_3$), 2.43–2.49 (m, 1H, CH), 3.84 (t, J = 6.6 Hz, 2H, NHBn), 4.56 (s, 1H, NHCH_2CH), 4.57 (s, 2H, CH_2), 4.83 (s, 2H, CH_2CO_2), 5.80 (s, 2H, OCH_2O), 7.16 (d, J = 7.7 Hz, 2H, C_6H_4), 7.24 (d, J = 7.9 Hz, 2H, C_6H_4), 7.49–7.53 7.63 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 19.7, 20.4, 26.0, 26.7, 28.9, 34.3, 43.5, 44.1, 44.5, 51.2, 79.9, 110.9, 126.9, 127.4, 134.9, 138.9, 147.4, 149.6, 152.1, 165.6, 169.2; HRMS (MS-ES), calcd for $C_{27}H_{37}N_6O_4$ [M+H] m/z = 509.2876, found: 509.2887; *rp*HPLC t_R: condition (I) 15.851 (II) 36.589 min, purity 97.2% and 95.6%.

4.1.19. Acetoxymethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-(isopentylamino)-9*H*-purin-9-yl)acetate (10c)

δ_H (400 MHz, $CDCl_3$) 0.91 (d, J = 7.1 Hz, 6H, $(CH_2)_2CH(CH_3)_2$), 1.23–1.27 (m, 9H, 5H (cyclohexyl), 1.47–1.53 (m, 2H, $CH_2CH_2CH(CH_3)_2$), 1.64–1.83 (m, 7H, $CH_2CH_2CH(CH_3)_2$ and 5H (cyclohexyl)), 2.10 (s, 3H, $COCH_3$), 2.40–2.52 (m, 1H, CH), 3.56 (br s, 1H, NH), 4.02 (s, 1H, NH), 4.58 (s, 2H, CH_2Ar), 4.83 (s, 2H, CH_2CO_2), 5.79 (s, 2H, OCH_2), 7.14 (d, J = 8.2 Hz, 2H, 2CH (Ar)), 7.26 (d, J = 8.1 Hz, 2H, 2CH (Ar)), 7.48 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 14.1, 20.5, 20.9, 22.4, 22.6, 26.0, 26.8, 34.3 38.5, 43.3, 44.1, 45.4, 60.6, 76.6, 76.9, 77.3, 79.7, 126.7, 127.5; HRMS (MS-ES), calcd for $C_{31}H_{45}N_6O_4$ [M+H] m/z = 565.3502, found: 565.3496.

4.1.20. Acetoxymethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-morpholino-9*H*-purin-9-yl)acetate (10d)

IR (KBr, cm^{-1}) 3424, 2924, 2852, 1766, 1606, 1583, 1546, 1450, 1384, 1220, 1164; δ_H (400 MHz, $CDCl_3$) 1.21–1.44 (m, 5H, cyclohexyl), 1.72–1.83 (m, 5H, cyclohexyl), 2.91 (s, 3H, $COCH_3$), 2.45–2.50 (m, 1H, CH), 3.77 (t, J = 4.8 Hz, 4H, 2 CH_2 (morpholine)), 4.19 (br s, 4H, 2 CH_2, (morpholine)), 4.55 (d, J = 5.8 Hz, 2H, CH_2Ar), 4.85 (s, 2H, CH_2CO_2), 5.03 (br s, 1H, NH), 5.79 (s, 2H, OCH_2), 7.15 (d, J = 8.0 Hz, 2H, 2 CH (Ar)), 7.27 (d, J = 8.0 Hz, 2H, 2 CH (Ar)), 7.50 (s, 1H, CH, (H-8)); HRMS (MS-ES), calcd for $C_{27}H_{35}N_6O_3$ [M+H] m/z = 523.2669, found: 523.2681; *rp*HPLC t_R: condition (I) 13.798 (II) 35.975 min, purity 91.4% and 92.1%.

4.1.21. Acetoxymethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-((4-fluorophenyl)amino)-9*H*-purin-9-yl)acetate (10e)

IR (KBr, cm^{-1}) 3416, 2925, 2852, 1770, 1682, 1626, 1508, 1203; δ_H (400 MHz, $CDCl_3$) 1.26–1.45 (m, 5H, cyclohexyl), 1.72–1.88 (m, 5H, cyclohexyl), 2.12 (s, 3H, $COCH_3$), 2.42–2.48 (m, 1H, CH), 3.45–3.50 (m, 2H, cyclohexyl), 4.60 (s, 2H, CH_2Ar), 4.97 (s, 2H, CH_2CO_2), 5.82 (s, 2H, OCH_2O), 7.03 (t, J = 8.3 Hz, 2H, C_6H_4F), 7.17 (d, J = 8.0 Hz, 2H, C_6H_4), 7.25 (d, J = 8.0 Hz, 2H, C_6H_4), 7.49–7.53 (m, 2H, C_6H_4F), 7.58 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 20.8, 26.3, 27.1, 34.7, 44.5, 45.5, 80.35, 115.8, 116.0, 117.9, 124.5, 127.3, 127.6, 135.5, 147.6, 147.7, 163.9, 164.25, 165.8, 169.6; HRMS (MS-ES), calcd for $C_{29}H_{31}FN_6O_4$ [M+H] m/z = 547.2469, found: 547.2478; *rp*HPLC t_R: condition (I) 20.286 (II) 40.006 min, purity 97.4% and 93.8%.

4.1.22. Acetoxymethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-((furan-2-ylmethyl)amino)-9*H*-purin-9-yl)acetate (10f)

IR (KBr, cm^{-1}) 3416, 2924, 2851, 1764, 1685, 1612, 1384, 1261, 1201, 1127; δ_H (400 MHz, $CDCl_3$) 1.27–1.41 (m, 5H, 5H (cyclohexyl)), 1.69–1.89 (m, 5H (cyclohexyl)), 2.12 (s, 3H, CH_3), 2.45–2.50 (m, 1H, CH), 4.60 (br s, 2H, CH_2), 4.76 (s, 1H, NH), 4.88 (br s, 2H, CH_2Ar), 5.24 (br s, 2H, CH_2), 5.80 (s, 2H, OCH_2), 6.29–6.33 (m, 1H, CH (furfuryl)), 6.35 (br s, 1H, CH (furfuryl)), 7.16 (d, J = 8.1 Hz, 2H, C_6H_4), 7.25 (d, J = 7.9 Hz, 2H, C_6H_4), 7.26 (br s, 1H, CH), 7.96 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 13.9, 20.5, 22.5, 26.0, 26.8, 29.6, 34.4, 36.6, 43.4, 44.1, 45.5, 63.5, 79.7. 107.1, 110.2, 126.8, 127.5, 136.7, 141.8, 146.8, 152.0, 166.4, 169.2; HRMS (MS-ES), calcd for $C_{28}H_{32}N_6O_5$ [M+H] m/z = 533.2512, found: 533.2521; *rp*HPLC t_R: condition (I) 15.849 (II) 36.522 min, purity 96.8% and 96.7%.

4.1.23. Acetoxymethyl 2-(2-((4-cyclohexylbenzyl)amino)-6-(4-nitrophenoxy)-9*H*-purin-9-yl)acetate (10g)

IR (KBr, cm^{-1}) 3423, 2926, 2851, 1769, 1627, 1577, 1524, 1384, 1346, 1237; δ_H (400 MHz, $CDCl_3$) 1.33–1.43 (m, 5H, cyclohexyl), 1.73–1.84 (m, 5H, cyclohexyl), 2.13 (s, 3H, CH_3) 2.46–2.50 (m, 2H, CH), 4.42 (s, 2H, CH_2), 4.96 (s, 2H, CH_2) 5.70 (s, 1H, NH), 5.83 (s, 2H, CH_2), 7.02–7.12 (m, 4H, CH (Ar)), 7.35 (d, J = 8.6 Hz, 2H, CH (Ar)), 7.86 (s, 1H, CH (H-8)), 8.23 (d, J = 8.6 Hz, 2H, CH (Ar)); δ_C (100 MHz, $CDCl_3$) 20.8, 26.3, 27.1, 34.7, 44.4, 45.8, 80.2, 122.7, 125.3, 127.0, 127.2, 127.6, 136.0, 140.6, 145.2, 147.6, 157.4, 158.8, 159.5, 166.1, 169.6; HRMS (MS-ES), calcd for $C_{29}H_{31}N_6O_7$ [M+H] m/z = 575.2254, found: 575.2253; *rp*HPLC t_R: condition (I) 23.736 (II) 42.657 min, purity 87.0% and 83.6%.

4.1.24. Acetoxymethyl 2-(2-(cyclohexanecarboxamido)-6-morpholino-9*H*-purin-9-yl)acetate (15b)

δ_H (400 MHz, $CDCl_3$) 1.18–1.38 (m, 3H, cyclohexyl), 1.44–1.55 (m, 2H, cyclohexyl), 1.66–1.72 (m, 1H, cyclohexyl), 1.77–1.84 (m, 2H, cyclohexyl), 1.92–1.99 (m, 2H, cyclohexyl), 2.13 (s, 3H, $COCH_3$), 2.62–2.71 (m, 1H, cyclohexyl), 3.84–3.89 (m, 4H, morpholine), 4.34 (br s, 4H, morpholine), 5.03 (s, 2H, CH_2CO_2), 5.81 (s, 2H, OCH_2), 7.71 (s, 1H, CH (H-8)); δ_C (100 MHz, $CDCl_3$) 20.4, 25.3, 25.5, 29.0, 44.6,

45.4, 66.6, 79.9, 115.7, 138.7, 148.4, 149.6, 152.1, 165.7, 169.3, 176.7; HRMS (MS-ES), calcd for $C_{21}H_{29}N_6O_6$ [M+H] m/z = 461.2143, found: 461.2134; *rp*HPLC t_R: condition (I) 4.491 (II) 27.159 min, purity 94.8% and 88.1%.

4.1.25. 2-(2-((4-Cyclohexylbenzyl)amino)-6-(cyclopentylamino)-9*H*-purin-9-yl)ethyl sulfamate (22a)

IR (KBr, cm^{-1}) 3384, 2924, 2851, 1609, 1541, 1513, 1489, 1351, 1179, 1017, 912, 787, 551; δ_H (400 MHz, DMSO) 1.12–1.25 (m, 2H, cyclohexyl), 1.25–1.41 (m, 4H, cyclohexyl), 1.41–1.57 (m, 4H, cyclopentyl), 1.58–1.68 (m, 9H, cyclopentyl and cyclohexyl), 2.35–2.46 (m, 1H, cyclohexyl), 4.27 (t, J = 4.8 Hz, 2H, $H_2NSO_3CH_2CH_2N$), 4.33 (t, J = 4.9 Hz, $H_2NSO_3CH_2CH_2N$), 4.39 (d, J = 6.1 Hz, Ar-CH_2), 6.85 (br s, 1H, cyclopentyl-NH), 7.05 (br s, 1H, cyclohexylbenzyl-NH), 7.09 (d, J = 7.9 Hz, 2H, ArCH), 7.23 (d, J = 7.9 Hz, 2H, ArCH), 7.55 (s, 2H, H_2NSO_3), 7.64 (s, 1H, ArCH); δ_C (100 MHz, DMSO) 23.4, 25.6, 26.3, 32.1, 34, 41.6, 43.5, 44.3, 66.6, 126.2, 127.4, 137.0, 138.9, 145.5, 159.1; HRMS (MS-ES), calcd for $C_{25}H_{36}N_7O_3S$ [M+H] m/z = 514.2600, found: 514.2588; *rp*HPLC t_R: condition (I) 14.026 (II) 35.274 min, purity 91.7% and 92.2%.

4.1.26. 2-(2-((4-Cyclohexylbenzyl)amino)-6-(isobutylamino)-9*H*-purin-9-yl)ethyl sulfamate (22b)

IR (KBr cm^{-1}) 3463, 3426, 3314, 2923, 2851, 1616, 1522, 1384, 1175, 1020, 919, 786, 550; δ_H (400 MHz, $CDCl_3$) 0.96 (d, J = 6.6 Hz, 6H, $CH(CH_3)_2$), 1.06–1.49 (m, 5H, cyclohexyl), 1.70–2.10 (m, 7H, cyclohexyl and alkyl), 2.48 (s, 1H, cyclohexyl), 3.37 (s, 2H, alkyl), 4.36 (t, J = 4.9 Hz, 2H, $NH_2SO_3CH_2CH_2$)), 4.51 (t, J = 5.0 Hz, 2H, $NH_2SO_3CH_2CH_2$)), 4.57 (d, J = 5.7 Hz, 2H, NH-CH_2), 5.23 (s, 1H, N*H*), 5.78 (s, 1H, NH), 7.17 (d, J = 7.8 Hz, 2H, 2 CH (Ar)), 7.29 (s, 2H, 2 CH (Ar)), 7.45 (d, 1H, CH (H-8)); LRMS (MS-ES) calcd for $C_{24}H_{35}N_7O_3S$ [M+H] m/z = 502.25, found: 502.42.

4.1.27. 2-(2-((*tert*-Butoxycarbonyl)(4-cyclohexylbenzyl)amino)-6-(isopentylamino)-9*H*-purin-9-yl)ethyl sulfamate (22c)

IR (KBr, cm^{-1}) 3425, 3275, 2980, 2940, 2868, 1761, 1705, 1625, 1495, 1390, 1270, 1208; δ_H (400 MHz, MeOD) 0.92 (d, J = 6.6 Hz, 6H, $CH(CH_3)_2$) 1.18–2.00 (m, 15H, alkyl and cyclopentyl), 2.46 (m, 1H, cyclohexyl), 3.44–3.60 (m, 2H, –NH_2), 4.40 (dt, J = 8.3, 4.0 Hz, 4H, $SOCH_2CH_2$), 4.56 (s, 2H, ArNH-CH_2), 7.13 (d, J = 8.1 Hz, 2H, 2Ar (C*H*)), 7.26 (d, J = 7.8 Hz, 2H, 2Ar (C*H*)), 7.69 (s, 1H, Ar (C*H*)); δ_c NMR (100 MHz, MeOD) 20.8, 23.0, 27.0, 27.3, 28.0, 35.8, 39.7, 43.5, 45.7, 46.2, 49.7, 54.8, 68.3, 114.2, 127.7, 128.4, 139.0, 139.38, 147.7, 156.2, 161.2; HRMS (MS-ES), calcd for $C_{25}H_{37}N_7O_3S$ [M+H] m/z = 516.2757, found: 516.2758; *rp*HPLC t_R: condition (I) 15.891 (II) 36.656 min, purity 99.5% and 99.6%.

4.1.28. 2-(2-((4-Cyclohexylbenzyl)amino)-6-morpholino-9*H*-purin-9-yl)ethyl sulfamate (22d)

δ_H (400 MHz, DMSO-d_6) 0.99–1.49 (m, 5H, cyclohexyl), 1.57–1.87 (m, 5H, cyclohexyl), 2.42–2.45 (m, 1H, cyclohexyl), 3.98–4.03 (m, 6H, morpholine and OCH_2CH_2N), 4.24–4.53 (m, 6H, morpholine and OCH_2CH_2N), 7.11 (d, J = 7.8 Hz, 2H, 2CH (Ar)), 7.24 (d, J = 7.8 Hz, 2H, 2CH (Ar)), 7.74 (s, 1H, CH (H-8)); δ_C (100 MHz, DMSO-d_6) 25.5, 26.3, 34.0, 38.6, 38.8, 41.8, 43.5, 44.2, 45.1, 47.0, 47.2, 47.5, 47.7, 47.9, 66.1, 66.4, 126.2, 127.4, 137.11, 138.3,; HRMS (MS-ES), calcd for $C_{24}H_{34}N_7O_4S$ [M+H] m/z = 516.2393, found: 516.2396; *rp*HPLC t_R: condition (I) 10.709 (II) 32.895 min, purity 98.4% and 98.6%.

4.1.29. 2-(2-((4-Cyclohexylbenzyl)amino)-6-((4-fluorophenyl) amino)-9H-purin-9-yl)ethyl sulfamate (22e)

δ_H (400 MHz, MeOD) 1.30 (m, 5H, cyclohexyl), 1.83 (m, 5H, cyclohexyl), 2.49 (s, 1H, cyclohexyl), 4.50 (t, J = 5.0 Hz, 2H, OCH_2CH_2N), 4.59 (d, J = 5.0 Hz, 2H, OCH_2CH_2N), 5.11 (s, 2H, CH_2Ar), 6.97 (t, J = 8.6 Hz, 2H, CH (Ar)), 7.16 (d, J = 8.0 Hz, 2H, 2 CH (Ar)), 7.27 (d, J = 7.7 Hz, 2H (Ar)), 7.68 (dd, J = 8.9 and 4.8 Hz, 2H, 2 CH (Ar))), 8.15 (s, 1H, CH (H-8)); δ_c NMR (100 MHz, MeOD) 27.3, 28.0, 28.4, 35.8, 44.1, 45.7, 48.4, 49.6, 52.3, 68.3, 83.5, 101.4, 116.2, 116.4, 123.9, 127.7, 128.0, 128.2, 137.3, 143.6, 148.1, 156.2; HRMS (MS-ES), calcd for $C_{26}H_{31}FN_7O_3S$ [M+H] m/z = 540.2193, found: 540.2205; *rp*HPLC t_R: condition (I) 15.449 (II) 36.313 min, purity 97.3% and 96.8%.

4.1.30. 2-(2-((4-Cyclohexylbenzyl)amino)-6-((furan-2-yl methyl)amino)-9*H*-purin-9-yl)ethyl sulfamate (22f)

δ_H (400 MHz, DMSO-d_6) 1.03–1.48 (m, 5H, cyclohexyl), 1.59–1.88 (m, 5H, cyclohexyl), 2.43–2.49 (m, 1H, cyclohexyl), 4.29 (t, J = 5.2 Hz, 2H, OCH_2CH_2N), 4.35 (t, J = 5.2 Hz, 2H, OCH_2CH_2N), 4.42 (d, J = 5.4 Hz, 2H, CH_2Ar), 4.58 (s, 1H, ArNH), 6.15 (s, 1H, ArNH), 6.31 (t, J = 2.6 Hz, 1H, furylamine), 7.10 (d, J = 7.8 Hz, 2H, 2CH (Ar)), 7.24 (d, J = 7.7 Hz, 2H, 2CH (Ar)), 7.50 (d, J = 1.8 Hz, 1H, furylamine), 7.56 (s, 1H, furylamine), 7.69 (s, 1H, CH (H-8)); δ_C (100 MHz, DMSO-d_6) 25.6, 26.3, 34.0, 38.8, 39.0, 41.6, 43.4, 44.1, 47.3, 47.5, 47.7, 66.5, 106.5, 110.3, 126.2, 127.5, 137.5, 141.5; HRMS (MS-ES), calcd for $C_{26}H_{38}N_7O_3S$ [M+H] m/z = 526.2237, found: 526.2239; *rp*HPLC t_R: condition (I) 12.579 (II) 34.259 min, purity 99.3% and 98.1%.

4.1.31. 2-(2-(Cyclohexanecarboxamido)-6-morpholino-9*H*-purin-9-yl)ethyl sulfamate (28)

IR (KBr, cm^{-1}) 3299, 2924, 2854, 1667, 1594, 1578, 1467, 1384, 1306, 1263, 1235, 1179; δ_H (400 MHz, DMSO-d_6) 1.08–1.39 (m, 5H, (cyclohexyl)), 1.60–1.79 (m, 5H, (cyclohexyl)), 2.62–2.68 (m, 1H, (cyclohexyl)), 3.69–3.71 (m, 4H, (morpholine)), 4.18 (br s, 2H, CH_2N), 4.38–4.42 (m, 4H, (morpholine)), 6.73 (br s, 2H, NH_2), 7.55 (s, 2H, OCH_2), 8.04 (s, 1H, CH (H-8)), 9.99 (s, 1H, NH); δ_C (100 MHz, DMSO-d_6) 25.6, 25.8, 29.3, 42.5, 44.3, 66.5, 66.8, 116.2, 140.0, 151.9, 152.4, 153.1, 158.4, 174.9, 194.8; HRMS (MS-ES), calcd for $C_{18}H_{27}N_7O_5S$ [M+H] m/z = 454.1873, found: 454.1886; *rp*HPLC t_R: condition (I) 3.209 (II) 24.516 min, purity 92.6% and 92.4%.

4.1.32. 9-(2-(1*H*-Tetrazol-5-yl)ethyl)-N2-(4-cyclohexylbenzyl)-N6-cyclopentyl-9*H*-purine-2,6-diamine (34a)

IR (KBr, cm^{-1}) 3384, 2924, 2851, 1676, 1636, 1448, 1384, 1351, 1203, 1139; δ_H (400 MHz, DMSO-d_6) 1.28–1.53 (m, 9H, 5H (cyclohexyl) and 4H (cyclopentyl)), 1.65–1.91 (m, 10 H, 5H (cyclohexyl) and 5H (cyclopentyl)), 2.41–2.45 (m, 1H, CH (cyclohexyl), 3.41–3.45 (m, 2H, NCH_2CH_2), 4.39–4.43 (m, 2H, NCH_2CH_2), 4.41 (br s, 2H, CH_2 (benzyl)), 7.09 (br s, 1H, NH), 7.11 (d, J = 7.9 Hz, 2H C_6H_4), 7.26 (d, J = 7.9 Hz, 2H, C_6H_4), 7.65 (s, 1H, CH (H-8)); δ_C (100 MHz, DMSO-d_6) 23.4, 23.5, 25.6, 26.4, 32.1, 32.2, 34.1, 40.7, 43.5, 44.4, 126.3, 126.3, 127.7, 127.7, 129.4, 137.4, 138.3, 145.9, 145.9, 153.7; HRMS (MS-ES), calcd for $C_{26}H_{35}N_{10}$ [M+H] m/z = 487.3046, found: 487.3044; *rp*HPLC t_R: condition (I) 12.326 (II) 34.008, purity 96.6% and 94.6%.

4.1.33. 9-(2-(1*H*-Tetrazol-5-yl)ethyl)-N2-(4-cyclohexylbenzyl)-N6-isobutyl-9*H*-purine-2,6-diamine (34b)

IR (KBr, cm^{-1}) 3404, 2924, 2851, 1615, 1514, 1447, 1384, 1351, 1262; δ_H (400 MHz, DMSO-d_6) 0.82 (d, J = 6.3 Hz, 6H, $CH_2CH(CH_3)_2$), 1.23–1.37 (m, 5H, cyclohexyl), 1.66–1.76 (m, 5H, cyclohexyl), 1.83–1.87 (m, 1H, $CH(CH_3)_2$), 2.43–2.50 (m, 1H, $CH(CH_3)_2$), 3.17 (br s, 2H, $CH_2CH(CH_3)_2$), 3.39 (t, J = 6.9 Hz, 2H NCH_2CH_2), 4.37 (t, J = 7.1 Hz, 2H, NCH_2CH_2), 4.37 (br s, 2H, CH_2 (benzyl)), 6.88 (br s, 1H, NH), 7.09 (d, J = 8.1 Hz, 2H, 2CH (Ar)), 7.25 (d, J = 8.0 Hz, 2H, 2CH (Ar)), 7.23 (br s, 1H, NH), 7.61 (s, 1H, CH (H-8)); δ_C (100 MHz, DMSO-d_6) 20.1, 23.68, 25.6, 26.3, 34.0, 43.4, 44.3, 78.6, 78.9, 79.2, 126.1, 127.4, 127.5, 127.5, 136.7, 138.9, 145.5, 154.0, 154.8, 159.1; HRMS (MS-ES), calcd for

$C_{25}H_{35}N_{10}$ [M+H] m/z = 475.3046, found: 475.3037; *rp*HPLC t_R: condition (I) 12.328 (II) 34.028 min, purity 97.0% and 96.8%.

4.1.34. 9-(2-(1*H*-Tetrazol-5-yl)ethyl)-N2-(4-cyclohexylbenzyl)-N6-isopentyl-9*H*-purine-2,6-diamine (34c)

IR (KBr, cm^{-1}) 3417, 2924, 2851, 1643, 1603, 1514, 1384, 1261, 1126; δ_H (400 MHz, DMSO-d_6) 0.95 (d, J = 6.6 Hz, 6H, $CH(CH_3)_2$), 1.31–1.43 (m, 5H, cyclohexyl), 1.55–1.58 (m, 1H, $CH(CH_3)_2$), 1.64–1.78 (m, 7H, 5H (cyclohexyl) and 2H ($CH_2CH(CH_3)_2$)), 2.39–2.44 (m, 1H, cyclohexyl), 3.33 (br s, 2H, CH_2CH_2CH), 3.38–3.42 (m, 2H, NCH_2CH_2), 4.36–4.41 (m, 4H, 2H (NCH_2CH_2) and CH_2 (benzyl)), 6.86 (br s, 1H, NH), 7.09 (d, J = 8.0 Hz, 2H, C_6H_4), 7.18 (s, 1H, NH), 7.25 (d, J = 7.9 Hz, 2H, C_6H_4), 7.52 (s, 1H, CH (H-8)); HRMS (MS-ES), calcd for $C_{26}H_{37}N_{10}$ [M+H] m/z = 489.3203, found: 489.3197; *rp*HPLC t_R: condition (I) 14.342 (II) 35.483 min, purity 98.3% and 98.1%.

4.1.35. 9-(2-(1*H*-Tetrazol-5-yl)ethyl)-*N*-(4-cyclohexylbenzyl)-6-morpholino-9*H*-purin-2-amine (34d)

IR (KBr, cm^{-1}) 3424, 3082, 1924, 2851, 2359, 1682, 1602, 1550, 1439, 1384, 1198, 1184; δ_H (400 MHz, DMSO-d_6) 1.23–1.37 (m, 5H, cyclohexyl), 1.66–1.76 (m, 5H, cyclohexyl), 2.39–2.44 (m, 1H, cyclohexyl), 3.50 (t, J = 6.8 Hz, 2H, NCH_2CH_2), 3.60–3.70 (m, 4H, morpholine), 4.11 (br s, 4H, morpholine), 4.44–4.48 (m, 4H, $NHCH_2CH_2$ and CH_2Ar), 7.12 (d, J = 8.0 Hz, 2H, C_6H_4), 7.26 (d, J = 8.0 Hz, 2H, C_6H_4), 7.75 (s, 1H, CH (H-8)); δ_C (100 MHz, DMSO-d_6) 23.4, 25.6, 26.3, 34.0, 40.8, 43.4, 44.3, 45.3, 113.0, 114.3, 117.2, 126.4, 137.3, 137.8, 145.9, 158.3, 158.7, 159.1; HRMS (MS-ES), calcd for $C_{25}H_{33}N_{10}O$ [M+H] m/z = 489.2839, found: 489.2849; *rp*HPLC t_R: condition (I) 9.251 (II) 31.975 min, purity 98.5% and 98.2%.

4.1.36. 9-(2-(1*H*-tetrazol-5-yl)ethyl)-N2-(4-cyclohexylbenzyl)-N6-(4-fluorophenyl)-9*H*-purine-2,6-diamine (34e)

IR (KBr, cm^{-1}) 3444.9, 2962, 2924, 2851, 1629, 1592, 1507, 1384, 1262, 1223, 1098; δ_H (400 MHz, DMSO-d_6) 1.25–1.37 (m, 5H, (cyclohexyl)), 1.63–1.75 (m, 5H, (cyclohexyl)), 2.36–2.45 (m, 1H, (cyclohexyl)), 4.39 (s, 2H, $ArCH_2$), 4.41 (t, J = 6.3 Hz, 2H, NCH_2CH_2), 6.99 (t, J = 8.9 Hz, 2H, C_6H_4F), 7.11 (d, J = 8.0 Hz, 2H, C_6H_4), 7.25 (d, J = 7.8 Hz, C_6H_4), 7.70 (s, 1H, CH (H-8)), 7.79–7.90 (m, 2H, C_6H_4F); HRMS (MS-ES), calcd for $C_{27}H_{30}F_1N_6$ [M+H] m/z = 513.2639, found: 513.26567; *rp*HPLC t_R: condition (I) 14.524 (II) 35.649 min, purity 93.5% and 92.8%.

4.1.37. 9-(2-(1*H*-Tetrazol-5-yl)ethyl)-*N*-(4-cyclohexylbenzyl)-6-(4-nitrophenoxy)-9*H*-purin-2-amine (34g)

IR (KBr, cm^{-1}) 3415, 2924, 2851, 1631, 1576, 1447, 1384, 1231 1162; δ_H (400 MHz, $CDCl_3$) 1.35–1.40 (m, 5H, cyclohexyl), 1.65–1.83 (m, 2H, $(CH_2)_3CH_2CH_3$), 2.46–2.50 (m, 2H, $CH_2(CH_2)_3CH_3$), 3.59–3.63 (m, 2H, NCH_2CH_2), 4.51 (br s, 2H, CH_2Ar), 4.59–4.63 (m, 2H, NCH_2CH_2) = 7.18 (br s, 1H, NH), 7.25–7.33 (m, 6H, 4CH (benzyl) and 2CH (nitrophenol)), 7.73 (s, 1H, CH (H-8)), 8.24–8.26 (m, 2H, CH (nitrophenol)); δ_C (100 MHz, DMSO-d_6) 23.4, 25.6, 26.37, 34.0, 41.0, 43.5, 44.4, 80.58, 113.6, 122.7, 125.3, 126.3, 126.7, 127.8, 137.6, 141.4, 144.3, 145.8, 147.8, 157.7, 158.4, 158.4; HRMS (MS-ES), calcd for $C_{27}H_{29}N_{10}O_3$ [M+H] m/z = 541.2417, found: 541.2418; *rp*HPLC t_R: condition (I) 17.407 (II) 37.851 min, purity 95.2% and 94.1%.

4.1.38. *N*-(9-(2-(1*H*-Tetrazol-5-yl)ethyl)-6-morpholino-9*H*-purin-2-yl)cyclohexanecarboxamide (37)

IR (KBr, cm^{-1}) 2929, 2853, 1697, 1596, 1450, 1384, 1263, 1195, 1113; δ_H (400 MHz, DMSO-d_6) 1.19–1.26 (m, 5H, cyclohexyl), 1.64–1.81 (m, 5H, cyclohexyl), 2.68–2.72 (m, 1H, cyclohexyl), 3.45 (t, J = 7.0 Hz, 2H, NCH_2CH_2), 3.69 (t, J = 4.6 Hz, 4H, morpholine), 4.18 (br s, 4H, morpholine), 4.50 (t, J = 7.0 Hz, 2H, NCH_2CH_2), 7.92 (s, 1H, CH (H-8)), 9.88 (s, 1H, CONH); HRMS (MS-ES), calcd for $C_{19}H_{26}N_{10}O_2$ [M+H] m/z = 427.2318, found: 427.2318; *rp*HPLC t_R: condition (I) 2.859 (II) 22.922 min, purity 98.2% and 96.1%.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.04.080.

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