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RESEARCH ARTICLE

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Optimization of norbornyl-based carbocyclic nucleoside analogs as cyclin-dependent kinase 2 inhibitors

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Abstract

We report on the discovery of norbornyl moiety as a novel structural motif for cyclin-dependent kinase 2 (CDK2) inhibitors which was identified by screening a carbocyclic nucleoside analogue library. Three micromolar hits were expanded by the use of medicinal chemistry methods into a series of 16 novel compounds. They had prevailingly micromolar activities against CDK2 and the best compound of the series attained IC_{50} of 190 nM. The binding modes were explored in molecular details by modeling and docking. Quantum mechanics-based scoring was used to rationalize the affinities. In conclusion, the discovered 9-hydroxymethylnorbornyl moiety was shown by joint experimental-theoretical efforts to be able to serve as a novel substituent for CDK2 inhibitors. This finding opens door to the exploration of chemical space towards more effective derivatives targeting this important class of protein kinases.

KEYWORDS

ATP-competitive type I inhibitors, cyclin-dependent kinase 2, protein-ligand binding, quantum mechanical scoring

1 | INTRODUCTION

Cyclin-dependent kinases (CDKs) are ubiquitous enzymes in animals with several isoforms which are essential for numerous cell functions, including cell cycle regulation. Thus, their malfunctioning may lead to various types of cancer. Compounds targeting CDKs can thus be human anticancer therapeutics, as exemplified by three CDK4/CDK6 inhibitors palbociclib, ribociclib, and abemaciclib approved by FDA for the treatment of ER-positive and HER2-negative breast cancer.[1]

Like all other kinases, CDKs phosphorylate their substrates using ATP as a phosphate donor. The ATP molecule binds to the CDK active site located in a cleft ("hinge region"), which is located between the N-terminal β-sheet and C-terminal α-helical domains. The vast majority of known CDK inhibitors are of type I, that is, binding to active conformation of the kinase and directly competing with ATP for the binding site. Cyclin-dependent kinase 2 (CDK2) is the best known and studied member of CDK family.[2] Detailed understanding of the binding of small-molecule inhibitors to CDK2 comes from X-ray structures of co-crystal complexes.[3]

Cemal Köprülüoğlu and Milan Dejmek contributed equally.

Reliable prediction of protein-ligand binding affinity (scoring) is a major but still unsolved task of structure-based drug design. In our laboratory, we have been developing semiempirical quantum mechanics (SQM)-based scoring functions for a general and reliable description of diverse protein–ligand complexes.[4,5] These have successfully been applied to dozens of protein targets, including kinases,[6,7] binding up to thousands of ligands.[8–12]

Small molecule ATP-competitive kinase inhibitors must be adequately sized and shaped in order to fit into the active site. Screening nucleoside libraries might thus prove to be a rich source of new types of scaffolds for design of kinase inhibitors. During our past projects, we have been working on synthesis of numerous new compounds derived mostly from nucleosides. Namely, we have been preparing new carbocyclic and locked nucleoside analogues.[13–18]

These types of compounds have biological effects, most importantly antiviral and cytostatic activities. In addition, various nucleosides, including carboycylic and locked analogues, exert inhibitory potency against diverse medicinally relevant enzymes,[19] such as protein[20] and lipid kinases.[21,22]

Here, we report on a novel structural pattern for CDK2 inhibitors identified and optimized from the three screening hits discovered from our proprietary compound library of mostly nucleoside derivatives. The chemical space around the hits was expanded using our experience in kinase inhibitors design and 16 new compounds were synthesized. The molecular details of their binding were studied by modeling, docking, and quantum mechanics-based scoring.

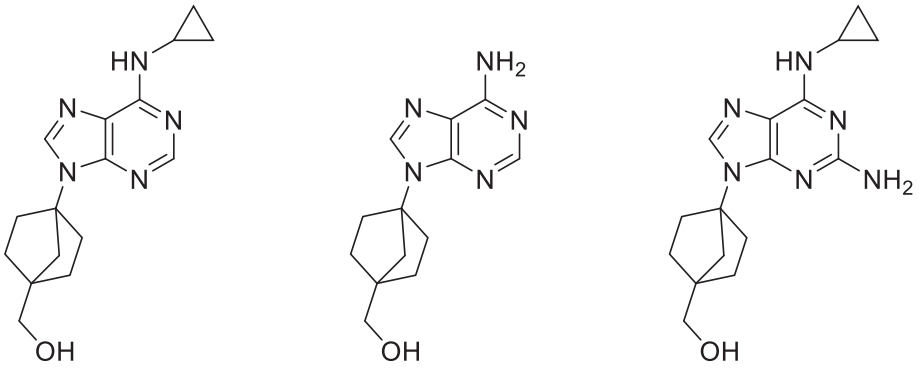
2 | RESULTS AND DISCUSSION

2.1 | Hit discovery and expansion

Previous campaigns launched at the Institute of Organic Chemistry and Biochemistry (IOCB) in Prague focused mostly on diverse nucleoside and nucleotide analogues and resulted in numerous clinically successful antiviral compounds.[23,24] These compounds, after the respective project ends, were collected in the IOCB proprietary library, ready for further exploration. This unique collection of compounds allowed us to perform several screening campaigns, one of which focused on identification of novel protein kinase inhibitors. Over 1000 compounds from this database were taken forward for virtual screening against CDK2 kinase using quantum mechanics-based SQM/COSMO methodology.[5] In total, 200 best-scored structures were selected for activity testing against CDK2. We identified three norbornane-based compounds, **1**, **2**, and **3**, prepared in our lab, as hits for CDK2 inhibition (Figure 1). These compounds are nucleoside analogues and thus can occupy the ATP-binding site of kinases and potentially serve as type I inhibitors.

Molecular docking of these compounds in the CDK2 active site suggested two binding modes. The standard one, very similar to that of the purine-based inhibitor roscovitine,[25–28] featured two hinge-region hydrogen bonds with Leu83 and placed the 2,6,9-substituents into their respective canonical pockets (Figure S1-left). For **1**, we found in addition a reverse binding mode with the two hinge-region hydrogen bonds present but provided by different inhibitor atoms (Figure S1-right). The 2,9-substituents pointed to their respective pockets, yet under different angle. However, the potential 6-substituent would be swapped with the 9-substituent and would thus point toward the gatekeeper, which would hinder a productive hit optimization. Thus, only the canonical-binding mode was used for hit expansion.

Following the known structure-activity relationships (SARs) in purine-isostere-based kinase inhibitors,[29] we have selected 36 modifications of position 2 and 37 in position 6 and combined them with the hydroxynorbornyl moiety in position 9 on a purine scaffold. To prioritize among these 73 compounds for synthesis, we built the modifications on the purine core in the CDK2 active site and scored using SQM/COSMO approach.[5] Eight compounds with high scores and synthetic feasibility were selected for synthesis. Additionally, to explore the importance of the hydroxynorbornyl moiety in position 9, another eight compounds with the cyclohexyl substituent in position 9 were suggested for synthesis.



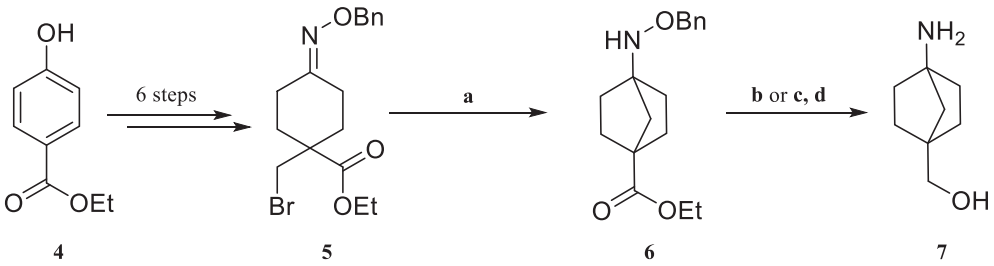
1
IC_{50} = 5.7 μM

2
IC_{50} = 4.7 μM

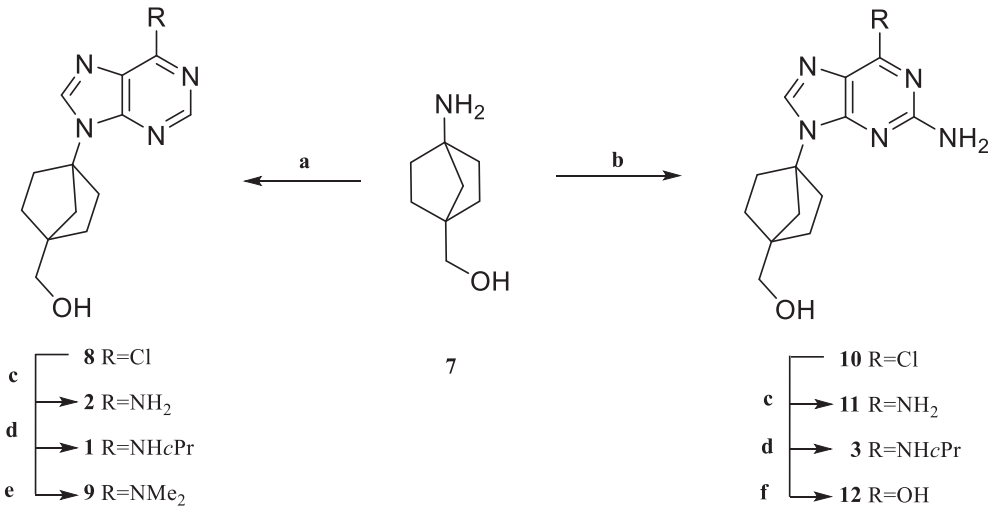
3
IC_{50} = 12.6 μM

FIGURE 1 Three hits for CDK2 inhibition from the IOCB proprietary database

SCHEME 1 (a) Bu_3SnH, AIBN, toluene, reflux, 5 hours, 49%; (b) BH_3-THF, diglyme, 110°C, 24 hours, 95%, (c) $Pd(OH)_2/C$, H_2, MeOH, 24 hours, 85%, (d) $LiAlH_4$, THF, reflux, 5 hours, 67%



SCHEME 2 (a) 4,6-Dichloro-5-formamidopyrimidine, DIPEA, *n*-BuOH, MW, 160°C, 2 hours, 60%; (b) 2-amino-4,6-dichloro-5-formamidopyrimidine, DIPEA, n-BuOH, MW, 160°C, 2 hours, 80%; (c) NH_3-EtOH, MW. 120°C, 30 minutes, 91% for **2** or 65% for **11**; (d) *c*-$PrNH_2$, EtOH, MW 140°C, 30 minutes, 91% for **1** or 80% for **3**; (e) DMF, MW, 200°C, 2 minutes, 87%; (f) TFA, H_2O, 12 hours, 69%



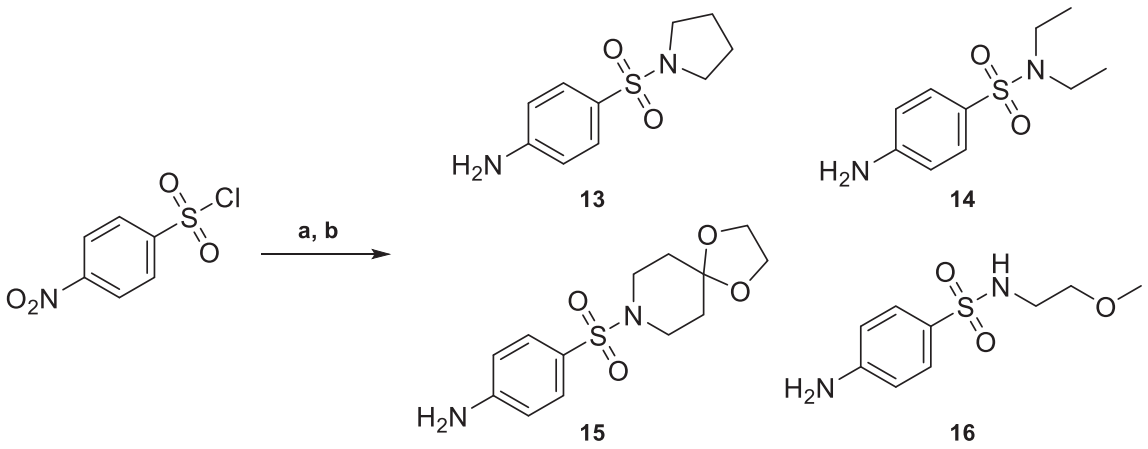
2.2 | Synthesis of nucleoside derivatives as novel CDK2 inhibitors

Synthesis of the original nucleoside analogues started with the preparation of double bridgehead substituted norbornane bicycle (**7**). First, oxime **5** was synthesized in a straightforward manner in 6 steps from ethylparaben **4**. Using Bu_3SnH-mediated radical cyclization of this intermediate, hydroxylamine **6** was obtained (Scheme 1). It is noteworthy that success of this reaction was strongly dependent on the thoroughness of degassing the reaction medium. In standard, undegassed solvent no cyclization occurred, when using simpler degassing procedures such as bubbling inert gas (N_2 or Ar) through the reaction medium yields rose to mediocre 20-50% and after thorough freeze-pump-thaw procedure acceptable 65% yield was achieved. Debrominated **5** was always present in the reaction mixture as a major impurity. The benzyloxy group, together with the ester function, was reduced to obtain key amine **7**, which was used in the following nucleobase construction according to our one-step protocol [30] to prepare the 6-chloropurine derivative **8** and 2-amino-6-chloropurine derivative **10**, respectively (Scheme 2). Simple modifications in the purine 6-position of **8** and **10** lead to a series of simple nucleoside derivatives, around which the chemical space was explored with the aim of establishing structure–activity relationships (SAR) for CDK2 inhibition.

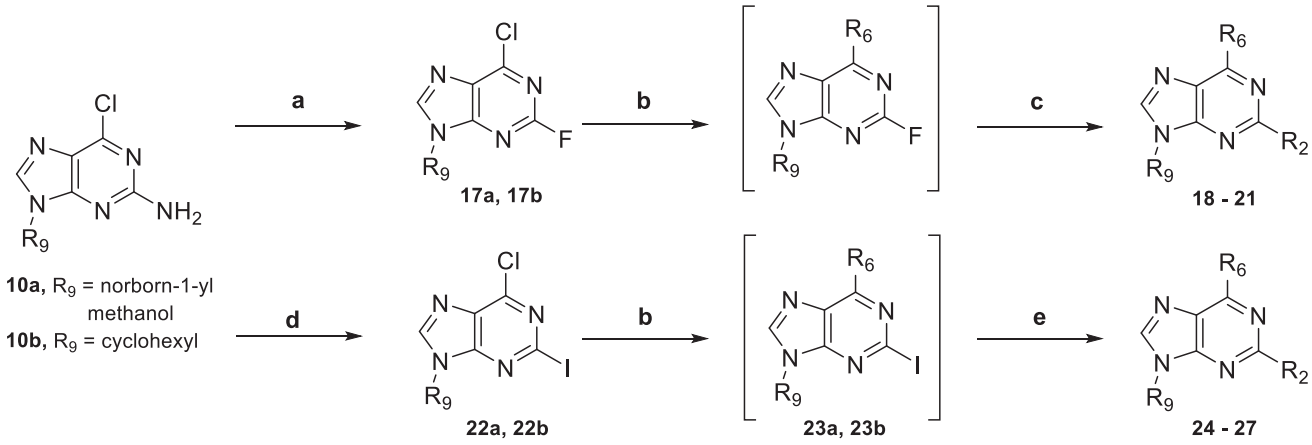
The substituents in positions 2 and 6 suggested by computations (see above) were synthesized as two subseries of compounds. In the first subseries (**18a-21b**), we employed the known trans-cyclohexyldiamine as a substituent in position 2 [29] with a variety of different *p*-benzenesulfonamides in position 6. The sulfonamide-containing sidechains **13-16** were prepared in two simple steps from *p*-nitrobenzenesulfonyl chloride and corresponding amines (Scheme 3). In the second subseries (**24a-27b**), we used the phenylsulfonamidepyrrolidin sidechain in position 6, a moiety that already proved its applicability in other kinase inhibitors of heterocyclic origin,[31] and a variety of aromatic substituents in position 2.

Two general routes were utilized for the synthesis of target compounds (Scheme 4). In the case of compounds bearing an aliphatic substituent in position 2, we exploited different reactivity of halogens in positions 2 and 6 as well as different nucleophilicity of anilines and aliphatic amines in S_NAr reactions. Using the Schiemann reaction, we prepared 2-fluoro-6-chloropurine derivatives bearing in position 9 cyclohexyl and 1-hydroxymethyl-norborn-4-yl, respectively. Both halogens were consecutively exchanged in one pot by sequential addition of appropriate nucleophiles - first the less reactive aniline reacted in position 6 of the purine and then the more reactive aliphatic amine, trans-1,4-cyclohexanediamine, was added to exchange the fluorine in position 2. In the case of sulfonamide **18** final deprotection of a ketal group was necessary.

In the case of aromatic substituents in position 2, we used a similar approach, where the Sandmayer reaction was used to synthesize the corresponding 2-iodo-6-chloropurine intermediates. These were first subjected to S_NAr reaction exchanging the chlorine atom in



SCHEME 3 (a) Amine, TEA, DCM, 0°C-rt, 12 hours; (b) $Pd(OH)_2$, H2, MeOH—AcOEt, rt, 12 hours



SCHEME 4 (a) *i*-pentONO, HF-py, −30°C, 5 minutes 81% for **17a**, 85% for **17b;** (b) Aniline **13-16**, DIPEA, n-BuOH, 150°C, 12 hours; (c) *trans*-1,4,-diaminocyclohexane, n-BuOH, 175°C, 12 hours, 33-49% over two steps; (d) *i*-pentONO, CuI, CH_2I_2, THF, reflux, 4 hours, 74% for **22a**, 81% for **22b**; (e) aromatic amine, Cs_2CO_3, $Pd_2(dba)_3$, XantPhos, toluene-dioxane 1:1, 12 hours, 25–40% over two steps

position 6, followed by the Buchwald reaction coupling aromatic amine to position 2.

For all the 8 synthesized compounds, a series of 9-cyclohexyl analogues was synthesized in the same manner to verify the utility of the norbornane bicycle. All the synthesized compounds were subjected to IC_{50} measurements against CDK2/cyclin E complex (Table 1).

We also performed cytotoxicity assays of four different cell lines (HepG2, HL60, HeLa S3, and CCRF-CEM; Table 2).

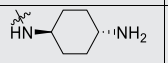
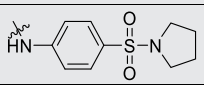
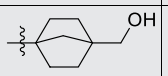
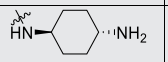
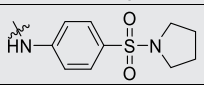
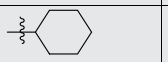
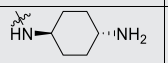
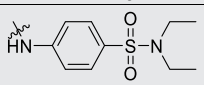
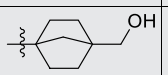
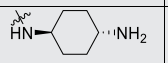
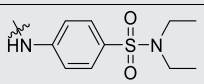
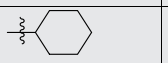
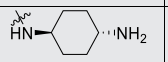
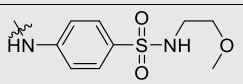
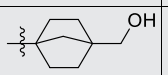
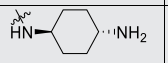
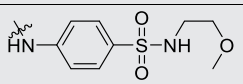
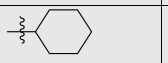
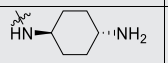
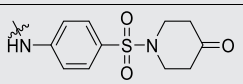
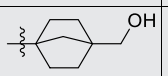
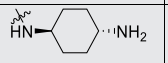
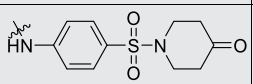
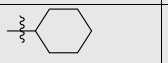
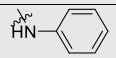
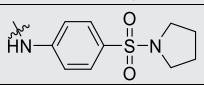
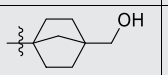
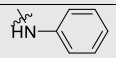
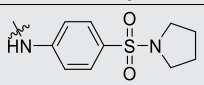
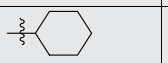
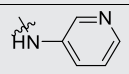
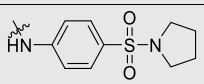
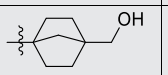
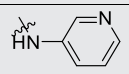
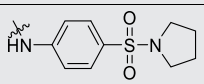
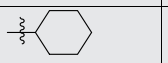
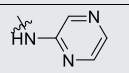
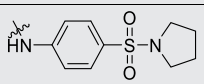
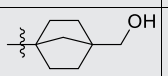
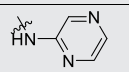
2.3 | The structure–activity relationship

All the compounds with the 9-norbornyl substituent had values in the micro-submicromolar range, while the compounds with the 9-cyclohexyl substituent fell into two classes: (a) in case of trans-1,4-cyclohexanediamine in position 2, the compounds were submicromolar, sometimes even slightly more potent than their 9-norbornyl-substituted counterparts and (b) in case of phenylsulfonamidepyrrolidin in position 6 and various other substituents in position 2, the compounds were inactive (IC_{50} > 15 μM) (Table 1). The strongest-affinity compound **18a** (0.19 μM) combines trans-1,4-cyclohexanediamine at position 2, phenylsulfonamidepyrrolidin at position 6, and norbornyl at position 9. To obtain insight into the binding modes and affinities, we performed docking and scoring.

2.4 | Inhibitor binding modes

The standard binding mode (Figure S1-left) was observed for all the compounds. For **26a**, the reverse binding mode was also found but discarded because it cannot be used further for hit optimization. In the standard binding mode, the purine core featured two hinge-region hydrogen bonds (L83: NH…N(7) and L83:O…N(6)H) similar to roscovitine (Figure 2A) for all the compounds. Docking suggested four types of orientations of the phenylsulfonamidepyrrolidine substituent in position 6 across the compound series. We built all of

TABLE 1 2D structures of the 16 new compounds synthesized in this work and their measured activities against CDK2/cyclin E

Compound	R2	R6	R9	IC50 (μM)	Standard Deviation
18a				0.19	0.04
18b				0.43	0.12
19a				0.39	0.06
19b				0.68	0.14
20a				0.76	0.19
20b				0.58	0.04
21a				1.02	0.14
21b				0.58	0.14
24a				1.35	0.54
24b				>20	n.d.
25a				1.04	0.56
25b				>20	n.d.
26a				0.79	0.13
26b				>20	n.d.
27a				2.79	0.78
27b				14.84	3.40
Roscovitine *				0.17	0.06

*Reference compound.

them into the strongest-affinity compound **18a** (Figure 2A-E). They differed in interactions with Lys89: (a) via sulfonamide oxygen (Figure 2A,B), (b) via pyrrolidine nitrogen and Lys89 (Figure 2C), (c) shifted without any interaction (Figure 2D) and (d) shifted without any interaction, with 5-member ring slightly rotated (Figure 2E).

The first type of interaction was identified as the most favorable based on the SQM/COSMO score values for compound **18a** (a) −63 kcal/mol, (b) −59 kcal/mol, (c) −52 kcal/mol, and (d) −54 kcal/mol score values were obtained. The most favorable orientation was (a) via sulfonamide oxygen and was thus modeled for all the compounds.

TABLE 2 Cytotoxicity of final compounds on HepG2, HL60, HeLa S3, and CCRF-CEM cells

Compound	IC50,μM			
	HepG2	HL60	HeLa S3	CCRF-CEM
18a	>10	1754 ± 0.228	>10	1572 ± 0.103
18b	>10	4866 ± 0.273	4275 ± 0.172	3752 ± 0.218
19a	>10	4347 ± 0.282	6757 ± 0.042	3131 ± 0.02
19b	6289 ± 0.226	3.53 ± 0.251	3646 ± 0.046	1653 ± 0.177
20a	>10	>10	>10	>10
20b	>10	>10	>10	>10
21a	>10	>10	>10	>10
21b	>10	4044 ± 0.321	>10	4299 ± 0.324
24a	2918 ± 0.217	2847 ± 0.194	0.983 ± 0.012	0.823 ± 0.073
24b	>10	>10	>10	>10
25a	1598 ± 0.087	1395 ± 0.045	0.839 ± 0.07	1039 ± 0.086
25b	>10	>10	>10	5265 ± 0.626
26b	>10	>10	2868 ± 0.084	4785 ± 0.089
27a	>10	>10	2442 ± 0.323	4846 ± 0.457
27b	>10	5012 ± 0.545	2.64 ± 0.279	1576 ± 0.107

In position 2, the phenyl, 3-pyridyl, 2,5-pyrimidyl and cyclohexyl substituents fitted into hydrophobic cavity on one side and open to the solvent on the other side. The distal amino group (neutral for the aromatic substituents and charged for the cyclic aliphatic ones) always formed a hydrogen bond with Asp145 (Figure 2A and zoomed in Figure 2F,G). The experimental (Table 1) as well as computational (Figure 3) data show that the charged salt bridge ($-NH_3^+ \ldots {}^-OOC-$) resulted in a stronger affinity than a charge-assisted hydrogen bond ($-NH_2 \ldots {}^-OOC-$). The 9-norbornyl part featured a nonpolar interaction with Phe80 gatekeeper and its terminal hydroxyl made two hydrogen bonds to the Lys33…Glu51 salt bridge (Figure 2A and zoomed in 2H). The 9-cyclohexyl substituent only featured the nonpolar interaction with Phe80 gatekeeper 2.

2.5 | QM-based binding affinities

As an estimate of binding free energy, we calculated the PM6-D3H4X/COSMO score (see Methods) of all the designed compounds (Table 1) complexed with CDK2. It must be stressed that binding entropies are not assessed fully (only solvation entropy is included via the implicit model) and thus the scores are offset by tens of kilocalories per mol to more negative values but should correlate with the experimental binding free energies under the assumption of fortuitous cancellation of errors for similar compounds.[5]

The experimental binding free energies are estimated from the measured IC_{50} values (Table 1) using the Cheng-Prusoff equation for competitive not tight-binding inhibitors.[5] Generally, IC_{50} values are measured in conditions where substrate concentration equals to the Michaelis constant of the enzyme, which transforms the equation into $\Delta G' = RT^*\ln(IC_{50}/2)$.

Figure 3 shows the plot of the PM6-D3H4X/COSMO scores vs experimental binding free energies. Two categories can be distinguished. The active compounds (IC_{50} from 0.19 to 2.79 μM) showed high scores ranging from −64 to 49 kcal/mol (Figure 3, triangles). The inactive compounds (Figure 3, filled circles with arrows representing experimental "worse than") had the scores ranging from −44 to −37 kcal/mol. We can thus see that our score was able to separate the actives from the inactives.

The scoring results also corresponded to the SAR observed above for the 9-norbornyl vs 9-cyclohexyl group. In the first subseries with a potent trans-cyclohexyldiamine as a substituent in position 2 and a variety of different *p*-benzenesulfonamides in position 6, the IC_{50} showed a relative insensitivity to the identity of substituent 9 (2-fold change). The scores for these compounds (**18a-21b**) had high scores ranging from −64 to −50 kcal/mol. The probable structural reason is that in this subseries, the compounds are already bound to CDK2 via two strong interaction motifs: first, in position 2 where trans-cyclohexyldiamine part has electrostatic interaction with Glu145 (Figure 2G) and second, in position 6 with an electrostatic interaction between phenylsulfonpyrrolidin side chain and Lys89, which override the effect of position 9.

In the second subseries with phenylsulfonamidepyrrolidine in position 6 (compounds **24a**, **25a**, **26a**, **27a**), the 9-hydroxymethylnorbornyl group was crucial for activity. The score values ranged from −55 to −49 kcal/mol. On the contrary, the compounds with 9-cyclohexyl in this subseries (**24b**, **25b**, **26b**, **27b**) were inactive (IC_{50} > 14 μM) and their score values ranged from −44 to −37 kcal/mol (Figure 3).

3 | CONCLUSIONS

Herein, we report the hit discovery and expansion *via* medicinal chemistry synthesis, activity measurements, and computational analyses of a series of carbocyclic nucleoside compounds featuring 9-hydroxymethyl norbornyl substituent as CDK2 inhibitors. The

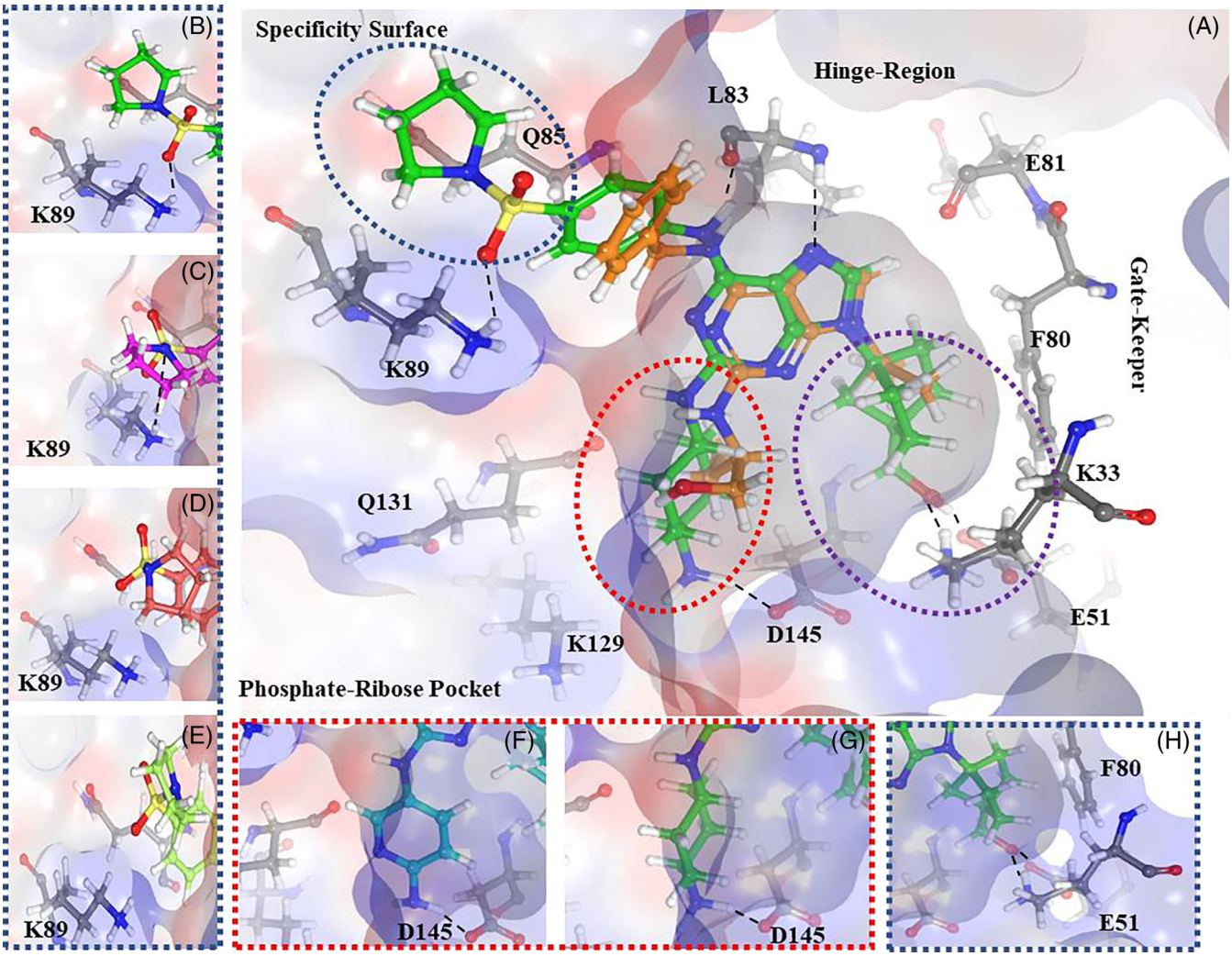


FIGURE 2 (a) The binding mode of **18a** (green sticks for carbon atoms) in CDK2 compared to the crystal structure of roscovitine with hydrogens added (orange sticks for carbon atoms; PDB code: 3DDQ). The ligand is shown in sticks and important CDK2 residues are shown as ball and sticks. Colors of atoms (C: green/orange—ligand/gray—CDK2, N:blue, O:red, S:yellow and H:white). Zoom into position 6 with different orientations distinguished by different carbon colors for the ligands: (b) the interaction between Lys 89 and sulfonamide oxygen (C: green), (c) the interaction between Lys 89 and pyrrolidine nitrogen (C: magenta) (d) shifted without any interaction (C: salmon) and (e) 5-member ring slightly rotated without any interaction (C: light green, f) Zoom into position 2 for compound **27a**, (g) Zoom into position 2 for **18a** (h) Zoom into position 9 of **18a**. The figure was prepared with Maestro (Schrodinger)

measured activities of the 16 newly synthesized compounds against CDK2/cyclin E were in the micromolar/submicromolar range and included as controls inactive compounds. The structure-activity relationships showed that structures with 9-hydroxymethylnorbornyl substituent had all submicromolar potencies.

The compounds with 9-cyclohexyl substituent fell into two classes (active/inactive) depending on the substituent in position 2. For a molecular understanding of the potency determinants, we have carried out computational modeling. Using docking and quantum-based SQM/COSMO scoring, we could separate the compounds into the actives and inactives and link the binding to individual interactions present in the binding cavity. Overall, the discovered 9-hydroxymethyl norbornyl substituent opens way to further explore it in the area of structure-based kinase design.

4 | METHODS

4.1 | Organic synthesis

NMR spectra were recorded on Bruker Avance II 500 (^{1}H at 500 MHz) or Bruker Avance III HD (^{1}H at 400 MHz) spectrometer using DMSO-d_6 or $CDCl_3$ as a solvent and the solvent signal as a reference. Chemical shifts (δ) and coupling constants (J) were expressed in ppm and Hz, respectively. All structures were confirmed and ^{1}H and ^{13}C signals were assigned by a combination of 1D and 2D NMR (H,H-COSY, H,C-HSQC, H,C-HMBC, ROESY) techniques. Standard pulse programs from the library of the spectrometer were used; gradient selection was used in the 2D experiments. Mass spectra were measured on an LTQ Orbitrap XL using electrospray ionization (ESI).

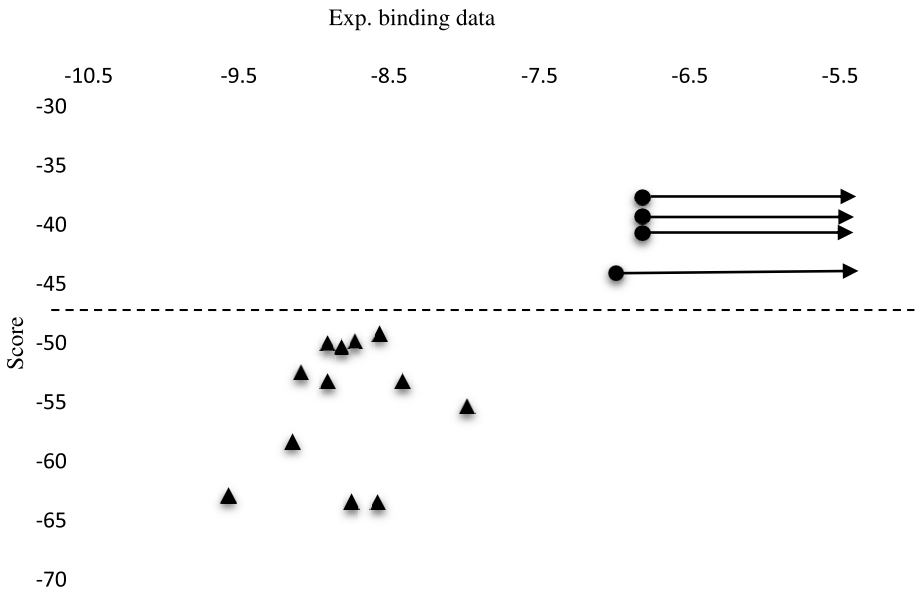


FIGURE 3 PM6-D3H4X/COSMO scores plotted against experimental binding free energies expressed as RT*ln (IC_{50}/2), all in kcal/mol. Triangles are active compounds, filled circles with arrows representing the measured "worse than" for inactives

Elemental analyses were measured on Perkin Elmer CHN Analyzer 2400, Series II Sys or on SPECTRO iQ II. Microwave syntheses were carried out in a CEM Discover instrument with a single-mode cavity and focused microwave heating (microwave power supply 0-300 W, IR temperature sensor, sealed vessel mode). Column chromatography (both normal and reverse phase) was performed on a 40-60 µm silica gel using ISCO flash chromatography system or standard glass columns. Purity of all prepared compounds was higher than 98% unless stated otherwise.

Ethyl 4-[(benzyloxy)amino]bicyclo[2.2.1]heptane-1-carboxylate (**6**): A solution of **5** [15] (17 g, 46.2 mmol) in dry toluene (500 mL, distilled from sodium) was deoxygenated three times using the freeze-pump-thaw degassing method, and then heated to reflux. A solution of Bu_3SnH (37.3 mL, 138.5 mmol) and AIBN (1 g) in dry and deoxygenated toluene (150 mL) was added dropwise to this solution (3 hours), and the resulting reaction mixture was refluxed for further 1 hour. After cooling down, the reaction was quenched with careful addition of methyliodide (30 mL), and volatiles were evaporated and adsorbed on silica. Flash column chromatography (5-30% ethyl acetate in petroleum ether) afforded **3** (8.0 g, 60%) as a clear oil. Spectral characteristics match those described in literature [15].

(4-Aminobicyclo[2.2.1]hept-1-yl)methanol (**7**): To a solution of **6** (7.4 g, 25.6 mmol) in dry diglyme (200 mL) was added BH_3-THF complex (1 M solution in THF, 121 mL) and the mixture was heated in a sealed pressure vessel on 110°C for 48 hours. After cooling to RT, reaction was quenched by careful addition of water (10 mL) and volatiles were evaporated. The residue was adsorbed on silica from ethanol (50 mL) and purified by column chromatography (DCM to DCM: EtOH:(3 M NH_3-EtOH) = 7:2:1 to afford **7** (3.43 g, 95%) as clear oil which solidifies on standing. ^{1}H NMR: 1.12 (s, 2H, H-7), 1.16-1.26 (m, 2H, H-2endo, H-6endo), 1.34-1.50 (m, 4H, H-3, H-5), 1.54-1.59 (m, 2H, H-2exo, H-6exo), 3.34 (s, 2H, CH_2O). ^{13}C NMR: 32.28 (C-2, C-6), 37.40 (C-3, C-5), 48.20 (C-7), 48.98 (C-1), 62.40 (C-4), 65.17 (CH_2O). ESI MS *m/z* (%): 142.1 (100) [M + H]; HRMS ESI ($C_8H_{16}ON$) calculated: 142.12264; found: 142.12256.

[4-(6-Chloro-9H-purin-9-yl)bicyclo[2.2.1]hept-1-yl]methanol (**8**): To a solution of amine **7** (282 mg, 2 mmol) in *n*-BuOH (10 mL), was added 4,6-dichloro-5-formamidopyrimidine (460 mg, 2.4 mmol) and DIPEA (1.05 mL, 6 mmol) and the reaction mixture was microwave irradiated in a sealed vessel on 160°C for 2 hours. Flash chromatography (1-2% methanol in ethyl acetate) followed by crystallization from toluene −cyclohexane mixture afforded **8** (335 mg, 60%) as white crystals (m.p. = 176.5-178°C). ^{1}H NMR: 1.41-1.49 (m, 2H, H-2endo, H-6endo), 1.77-1.85 (m, 2H, H-2exo, H-6exo), 2.05-2.14 (m, 2H, H-3exo, H-5exo), 2.13 (bs, 2H, H-7), 2.24-2.32 (m, 2H, H-3endo, H-5endo), 3.51 (d, 2H, $J_{CH2\text{-}OH}$ = 5.3, CH_2O), 4.68 (t, 1H, $J_{OH\text{-}CH2}$ = 5.3, OH), 8.68 (s, 1H, H-8′), 8.77 (s, 1H, H-2′). ^{13}C NMR: 31.29 (C-2, C-6), 34.55 (C-3, C-5), 43.57 (C-7), 48.51 (C-1), 64.20 (CH_2O), 66.22 (C-4), 131.72 (C-5′), 146.54 (C-8′), 149.37 (C-6′), 151.24 (C-2′), 152.35 (C-4′). ESI MS *m/z* (%): 279.1 (100) [M + H], 301.1 (86) [M + Na]. Anal. calc. For $C_{13}H_{15}N_4OCl$ x 1/5 C_7H_8 (297.17): C 58.20, H 5.63, N 18.85, Cl 11.93; found: C 57.96, H 5.63, N 18.73, Cl 11.73.

[4-(6-Amino-9H-purin-9-yl)bicyclo[2.2.1]hept-1-yl]methanol (**2**): A solution of **8** (150 mg, 0.53 mmol) in ethanolic ammonia (3.5 M, 3 mL) was heated in a microwave reactor at 120°C for 30 minutes. Product was isolated by flash chromatography (5-20% methanol in ethyl acetate) and subsequent crystallization from aqueous methanol. Yield 128 mg, 91%, colorless needles (m.p. = 225-226°C). ^{1}H NMR: 1.38-1.46 (m, 2H, H-2endo, H-6endo), 1.74-1.82 (m, 2H, H-2exo, H-6exo), 2.06 (bs, 2H, H-7), 2.03-2.11 (m, 2H, H-3exo, H-5exo), 2.16-2.24 (m, 2H, H-3endo, H-5endo), 3.50 (d, 2H, $J_{CH2\text{-}OH}$ = 5.3, CH_2O), 4.62 (t, 1H, $J_{OH\text{-}CH2}$ = 5.3, OH), 7.15 (bs, 2H, NH_2), 8.07 (s, 1H, H-8′), 8.11 (s, 1H, H-2′). ^{13}C NMR: 31.38 (C-2, C-6), 34.60 (C-3, C-5), 43.61 (C-7), 48.48 (C-1), 64.39 (CH_2O), 65.37 (C-4), 119.81 (C-5′), 139.59 (C-8′), 150.11 (C-4′), 152.17 (C-2′), 156.29 (C-6′). ESI MS *m/z* (%): 260.2 (100) [M + H], 282.2 (32) [M + Na]. Anal. calc. For

$C_{13}H_{17}N_5O$ (259.31): C 60.21, H 6.61, N 27.01; found: C 59.92, H 6.60, N 26.80.

{4-[6-(Cyclopropylamino)-9H-purin-9-yl]bicyclo[2.2.1]hept-1-yl}methanol (**1**): A solution of **8** (150 mg, 0.53 mmol) and cyclopropylamine (367 μL, 5.3 mmol) in ethanol (5 mL) was heated in a microwave reactor at 140°C for 20 minutes. Volatiles were evaporated, crude product was adsorbed on silica and purified by flash chromatography (1-5% methanol in ethyl acetate), and subsequent crystallization from toluene-cyclohexane mixture to afford **1** (146 mg, 91%) as white crystals (m.p. = 157-158°C). ^{1}H NMR: 0.58-0.62 and 0.68-0.73 (m, 2H, CH_2-cyclop), 1.40-1.46 (m, 2H, H-2endo, H-6endo), 1.73-1.82 (m, 2H, H-2exo, H-6exo), 2.06 (bs, 2H, H-7), 2.03-2.12 (m, 2H, H-3exo, H-5exo), 2.15-2.23 (m, 2H, H-3endo, H-5endo), 3.02 (bs, 1H, CH-cyclop), 3.50 (d, 2H, $J_{CH2\text{-}OH}$ = 5.3, CH_2O), 4.62 (t, 1H, $J_{OH\text{-}CH2}$ = 5.3, OH), 7.81 (bs, 1H, NH), 8.07 (s, 1H, H-8′), 8.22 (bs, 1H, H-2′). ^{13}C NMR: 6.54 (CH_2-cyclop), 31.38 (C-2, C-6), 34.61 (C-3, C-5), 43.63 (C-7), 48.47 (C-1), 64.38 (CH_2O), 65.39 (C-4), 120.18 (C-5′), 139.42 (C-8′), 149.7 (C-4′), 152.07 (C-2′), 155.83 (C-6′). ESI MS *m/z* (%): 300.2 (100) [M + H], 322.2 (3) [M + Na]. Anal. calc. For $C_{16}H_{21}N_5O$ (299.37): C 64.19, H 7.07, N 23.39; found: C 64.11, H 7.02, N 23.40.

{4-[6-(Dimethylamino)-9H-purin-9-yl]bicyclo[2.2.1]hept-1-yl}methanol (**9**): A solution of **8** (150 mg, 0.53 mmol) in DMF (3 mL) was subjected to microwave irradiation (sealed vessel, 200°C, 2 minutes). Volatiles were evaporated, crude product was adsorbed on silica and purified by flash chromatography (1-5% methanol in ethyl acetate), and subsequent crystallization from toluene - ethyl acetate mixture. Yield 135 mg, 87%, colorless crystals (m.p. = 155-156°C). ^{1}H NMR: 1.39-1.46 (m, 2H, H-2endo, H-6endo), 1.73-1.82 (m, 2H, H-2exo, H-6exo), 2.00-2.09 (m, 2H, H-3exo, H-5exo), 2.06 (bs, 2H, H-7), 2.19-2.26 (m, 2H, H-3endo, H-5endo), 3.44 (bs, 6H, CH_3), 3.50 (d, 2H, $'J_{CH2\text{-}OH}$ = 5.3, CH_2O), 4.63 (t, 1H, $J_{OH\text{-}CH2}$ = 5.3, OH), 8.08 (s, 1H, H-8′), 8.19 (s, 1H, H-2′). ^{13}C NMR: 31.37 (C-2, C-6), 34.50 (C-3, C-5), 37.97 (CH_3), 43.60 (C-7), 48.42 (C-1), 64.38 (CH_2O), 65.42 (C-4), 120.36 (C-5′), 138.47 (C-8′), 150.95 (C-4′), 151.48 (C-2′), 154.54 (C-6′). ESI MS *m/z* (%): 288.2 (100) [M + H]. Anal. calc. For $C_{15}H_{21}N_5O$ (287.36): C 62.70, H 7.37, N 24.37; found: C 62.43, H 7.44, N 24.00.

[4-(2-Amino-6-chloro-9H-purin-9-yl)bicyclo[2.2.1]hept-1-yl]methanol (**10**): To a solution of amine **7** (500 mg, 3.5 mmol) in *n*-BuOH (20 mL), was added 2-amino-4,6-dichloro-5-formamidopyrimidine (870 mg, 4.2 mmol) and DIPEA (1.83 mL, 10.5 mmol) and the reaction mixture was microwave irradiated in a sealed vessel on 160°C for 2 hours. Flash chromatography (1%-5% methanol in ethyl acetate) followed by crystallization from ethyl acetate–acetone mixture afforded **10** (846 mg, 81%) as pink crystals (m.p. = 150°C-151°C). ^{1}H NMR: 1.38-1.45 (m, 2H, H-2, H-6endo), 1.72-1.79 (m, 2H, H-2, H-6exo), 2.00-2.06 (m, 2H, H-3exo, H-5exo), 2.04 (bs, 2H, H-7), 2.14-2.23 (m, 2H, H-3endo, H-5endo), 3.46-3.51 (m, 2H, CH_2O), 4.63-4.68 (m, 1H, OH), 6.82 (bs, 2H, NH_2), 8.08 (s, 1H, H-8′). ^{13}C NMR: 31.30 (C-2, C-6), 34.28 (C-3, C-5), 43.34 (C-7), 48.44 (C-1), 64.31 (CH_2O), 65.45 (C-4), 124.27 (C-5′), 142.15 (C-8′), 149.64 (C-6′), 154.55 (C-4′), 159.49 (C-2′). ESI MS *m/z* (%): 294.2 (37) [M + H], 316.2 (19) [M + Na], 608.8 (100) [2 M + Na]; HRMS ESI ($C_{13}H_{17}ON_5Cl$) calculated: 294.11161; found: 294.11167. Anal. calc. For $C_{13}H_{16}ClN_5O$ (293.75): C 53.15, H 5.49, N 23.84, Cl 12.07; found: C 53.33, H 5.56, N 23.61, Cl 12.30.

(4-(2-amino-6-chloro-9H-purin-9-yl)bicyclo[2.2.1]heptan-1-yl)methanol (**10b**): Compound was prepared according to a published procedure reported by author [31].

[4-(2,6-Diamino-9H-purin-9-yl)bicyclo[2.2.1]hept-1-yl]methanol (**11**): A solution of **10** (120 mg, 0.41 mmol) in ethanolic ammonia (3.5 M, 3 mL) was heated in a microwave reactor at 120°C for 30 minutes. Product was isolated by flash chromatography (15%-30% methanol in ethyl acetate) and subsequent crystallization from aqueous methanol. Yield 73 mg, 65%, pale orange crystals (m.p. = 268°C-269°C). ^{1}H NMR: 1.33-1.44 (m, 2H, H-2, H-6endo), 1.69-1.79 (m, 2H, H-2, H-6exo), 2.00 (bs, 2H, H-7), 1.98-2.07 (m, 2H, H-3exo, H-5exo), 2.09-2.17 (m, 2H, H-3endo, H-5endo), 3.48 (d, 2H, $J_{CH2,OH}$ = 5.3, CH_2O), 4.61 (t, 1H, $J_{OH,CH2}$ = 5.3, OH), 5.64 (bs, 2H, 2′-NH_2), 6.58 (s, 2H, 6′-NH_2), 7.64 (s, 1H, H-8′). ^{13}C NMR: 31.39 (C-2, C-6), 34.40 (C-3, C-5), 43.44 (C-7), 48.39 (C-1), 64.47 (CH_2O), 64.83 (C-4), 114.32 (C-5′), 136.17 (C-8′), 152.41 (C-4′), 156.32 (C-6′), 159.90 (C-2′). ESI MS *m/z* (%): 275.3 (100) [M + H]; HRMS ESI ($C_{13}H_{19}ON_6$) calculated: 275.16149; found: 275.16145. Anal. calc. For $C_{13}H_{18}N_6O$ (274.32): C 56.92, H 6.61, N 30.64; found: C 57.00, H 6.49, N 30.64.

{4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]bicyclo[2.2.1]hept-1-yl}methanol (**3**): A solution of **10** (588 mg, 2 mmol) and cyclopropylamine (1.39 mL, 20 mmol) in ethanol (20 mL) was heated in a microwave reactor at 140°C for 30 min. Volatiles were evaporated, crude product was adsorbed on silica and purified by flash chromatography (5%-10% methanol in ethyl acetate), and subsequent crystallization from acetone to afford **3** (502 mg, 80%) as off-white crystals (m.p. = 247-248°C). ^{1}H NMR: 0.53 to 0.61 and 0.61 to 0.68 (m, 2H, CH_2-cyclop), 1.34-43 (m, 2H, H-2, H-6endo), 1.68-1.79 (m, 2H, H-2, H-6exo), 2.00 (bs, 2H, H-7), 1.96-2.07 (m, 2H, H-3exo, H-5exo), 2.09-2.19 (m, 2H, H-3endo, H-5endo), 3.01 (bs, 1H, CH-cyclop), 3.48 (d, 2H, $J_{CH2,OH}$ = 5.0, CH_2O), 4.61 (t, 1H, $J_{OH,CH2}$ = 5.2, OH), 5.70 (bs, 2H, NH_2), 7.18 (bs, 1H, NH), 7.63 (s, 1H, H-8′). ^{13}C NMR: 6.61 (CH_2-cyclop), 31.40 (C-2, C-6), 34.41 (C-3, C-5), 43.46 (C-7), 48.39 (C-1), 64.48 (CH_2O), 64.84 (C-4), 114.58 (C-5′), 135.91 (C-8′), 151.9 (C-4′), 156.10 (C-6′), 159.82 (C-2′).ESI MS *m/z* (%): 315.3 (100) [M + H]; HRMS ESI ($C_{16}H_{23}ON_6$) calculated: 315.19279; found: 315.19268. Anal. calc. For $C_{16}H_{22}N_6O$ (314.39): C 61.13, H 7.05, N 26.73; found: C 60.95, H 7.02, N 26.81.

2-Amino-9-[4-(hydroxymethyl)bicyclo[2.2.1]hept-1-yl]-1,9-dihydro-6H-purin-6-one (**12**): A solution of **10** (120 mg, 0.41 mmol) in TFA–water mixture (2:1, 6 mL) was stirred at RT overnight. Volatiles were evaporated and crude product was codistilled with ethanol (3 × 10 mL), NH_4OH (10 mL), and ethanol (2 × 10 mL), adsorbed on silica gel and purified by flash chromatography (mobile phase: 15-30% methanol in ethyl acetate). Subsequent crystallization from water-methanol mixture afforded **12** (78 mg, 69%) as light brown powder (m.p. > 360°C [decomp.]). ^{1}H NMR: 1.33-1.43 (m, 2H, H-2, H-6endo), 1.67-1.79 (m, 2H, H-2, H-6exo), 1.98 (bs, 2H, H-7), 1.93-2.03 (m, 2H, H-3exo, H-5exo), 2.10-2.19 (m, 2H, H-3endo, H-5endo), 3.47 (d, 2H,

$J_{CH2,OH}$ = 5.3, CH_2O), 4.62 (t, 1H, $J_{OH,CH2}$ = 5.3, OH), 6.36 (bs, 2H, NH_2), 7.62 (s, 1H, H-8′), 10.57 (bs, 1H, H-1′). ^{13}C NMR: 31.38 (C-2, C-6), 34.53 (C-3, C-5), 43.50 (C-7), 48.37 (C-4), 64.40 (CH_2O), 65.13 (C-1), 117.86 (C-5′), 136.17 (C-8′), 151.75 (C-4′), 152.89 (C-2′), 157.04 (C-6′). ESI MS *m/z* (%): 276.2 (14) [M + H], 298.2 (100) [M + Na]; HRMS ESI ($C_{13}H_{18}O_2N_5$) calculated: 276.14550; found: 276.14549. Anal. calc. For $C_{13}H_{17}N_5O_2$ (275.31): C 56.31, H 6.22, N 25.44; found: C 56.24, H 6.30, N 25.31.

General procedure for the preparation of sulfonamides 13-16

A solution of 4-nitrobenzenesulfonyl chloride (665 mg, 3 mmol) in DCM (30 mL) was added dropwise to a stirred solution of amine (3.05 mmol) and triethylamine (418 µL, 3 mmol) in DCM (30 mL) at 0°C and stirred at room temperature for 12 hours. Reaction mixture was diluted with DCM (50 mL) and washed with water, organic phase was dried with sodium sulfate and exaporated in vacuo. This intermediate was, without further purification, dissolved in a MeOH-AcOEt mixture (1:1, 30 mL), Pd/C (10%, 100 mg) was added, and reaction mixture was hydrogenated under balloon for 24 hours. Catalyst was filtered off on a celite pad, and product was purified by flash column chromatography.

4-(Pyrrolidin-1-ylsulfonyl)aniline (**13**): Chromatography: ethyl acetate in petrol ether (40-80%), yield 648 mg (95% over 2 steps). Analytical data are consistent with literature.[32]

4-Amino-N,N-diethylbenzenesulfonamide (**14**): Chromatography: ethyl acetate in petrol ether (30-100%), yield 560 mg (82% over 2 steps). Analytical data are consistent with literature.[33]

4-((1,4-Dioxa-8-azaspiro[4.5]decan-8-yl)sulfonyl)aniline (**15**): Chromatography: ethyl acetate in petrol ether (40-100%), yield 720 mg (80% over 2 steps). ^{1}H NMR: δ 1.61-1.67 (m, 4H, H-6′, H-10′), 2.86-2.92 (m, 4H, H-7′, H-9′), 3.81 (s, 4H, H-2′, H-3′), 6.08 (s, 2H, NH_2), 6.61-6.61 (m, 2H, H-2), 7.32-7.38 (m, 2H, H-3). ^{13}C NMR: δ 33.68 (C-6′, C-10′), 44.39 (C-7′, C-9′), 63.75 (C-2′, C-3′), 105.33 (C-5′), 112.69 (C-2), 119.71 (C-4), 129.48 (C-3), 153.19 (C-1). ESI MS *m/z* (%): 299.1 (4) [M + H], 321.1 (51) [M + Na], 619.2 (100) [2 M + Na]; HRMS ESI ($C_{13}H_{18}O_4N_2NaS$) calculated: 321.08795; found: 321.08806.

4-Amino-N-(2-methoxyethyl)benzenesulfonamide (**16**): Chromatography: ethyl acetate in petrol ether (40-100%), yield 570 mg (83% over 2 steps). Analytical data are consistent with literature.[34]

(4-(6-Chloro-2-fluoro-9H-purin-9-yl)bicyclo[2.2.1]heptan-1-yl)methanol (**17a**): **10a** (100 mg, 0.34 mmol), placed in a plastic falcon tube, was dissolved in 60% HF-pyridine (2 mL) at −50°C. Isoamylnitrite (104 µL, 0.51 mmol) was added, and reaction mixture was stirred at −30°C for 5 minutes, after which it was poured on ice and product was extracted with chloroform. Organic phase was washed with sat. $NaHCO_3$ and water and flash chromatography (5-10% methanol in DCM) afforded **17a** (82 mg, 81%) as a white amorphous solid. ^{1}H NMR: δ 1.41-1.49 (m, 2H, H-2b, H-6endo), 1.75-1.84 (m, 2H, H-2a, H-6exo), 2.00-2.12 (m, 4H, H-3exo, H-5exo, H-7), 2.20-2.29 (m, 2H, H-3endo, H-5endo), 3.50 (d, 2H, $J_{CH2,OH}$ = 5.3, CH_2O), 4.68 (t, 1H, $J_{OH,CH2}$ = 5.3, OH), 8.69 (s, 1H, H-8′). ^{13}C NMR: δ 31.00 (C-2, C-6), 34.27 (C-3, C-5), 43.23 (C-7), 48.29 (C-1), 63.92 (CH_2O), 66.07 (C-4), 130.61 (d, $J_{5',F}$ = 4.8, C-5′), 147.31 (d, $J_{8',F}$ = 3.0, C-8′), 150.37 (d, $J_{6',F}$ = 18.3, C-6′), 153.82 (d, $J_{4',F}$ = 17.6, C-4′), 155.59 (d, $J_{2',F}$ = 212.5, C-2′). ESI MS *m/z* (%): 297.3 (100) [M + H]; HRMS ESI ($C_{13}H_{15}ON_4ClF$) calculated: 297.09129; found: 297.09136.

6-Chloro-9-cyclohexyl-2-fluoro-9H-purine (**17b**): **10b** (100 mg, 0.4 mmol), placed in a plastic falcon tube, was dissolved in 60% HF-pyridine (2 mL) at −50°C. Isoamylnitrite (121 µL, 0.6 mmol) was added, and reaction mixture was stirred at −30°C for 5 minutes, after which it was poured on ice and product was extracted with chloroform. Organic phase was washed with sat. $NaHCO_3$ and water and flash chromatography (30-100% ethyl acetate in petrol ether) afforded **17b** (86 mg, 85%) as a white amorphous solid. ^{1}H NMR: δ 1.27 (tt, 1H, J_{GEM} = 13.0, $J_{4'ax,3'eq}$ = 3.6, H-4′ax), 1.52-1.38 (m, 2H, H-3′ax), 1.64-1.76 (m, 1H, H-4′eq), 1.98-1.80 (m, 4H, H-2′eq, H-3′eq), 1.99-2.07 (m, 2H, H-2′ax), 4.41 (tt, 1H, $J_{1',2'a}$ = 11.9, $J_{1',2'b}$ = 3.8, H-1′), 8.81 (s, 1H, H-8). ^{13}C NMR: δ 24.82 (C-4′), 25.07 (C-3′), 31.97 (C-2′), 55.07 (C-1′), 130.31 (d, $J_{5,F}$ = 4.8, C-5), 146.97 (d, $J_{8,F}$ = 3.1, C-8), 150.39 (d, $J_{6,F}$ = 18.3, C-6), 153.47 (d, $J_{4,F}$ = 17.5, C-4), 156.07 (d, $J_{2,F}$ = 213.5, C-2). ESI MS *m/z* (%): 255.2 (100) [M + H]; HRMS ESI ($C_{11}H_{13}ON_4ClF$) calculated: 255.08073; found: 255.08080.

General procedure for the preparation of compounds 18-21

A mixture of **17a** or **17b**, corresponding aniline (**13-16**, 1.2 eq), DIPEA (2 eq), and *n*-BuOH (2-5 mL) was heated in a sealed pressure vessel to 150°C for 12 hours. When TLC showed complete disappearance of the starting material, *trans*-1,4-cyclohexandiamine (3 eq) was added and the reaction mixture was heated to 175°C for 12 hours. Product was purified by reverse phase column chromatography in H_2O-MeCN gradient (10-100% MeCN, 0,1% TFA) and freeze-dried.

(4-(2-(([1r,4r]-4-Aminocyclohexyl)amino)-6-((4-[pyrrolidin-1-ylsulfonyl]phenyl)amino)-9H-purin-9-yl)bicyclo[2.2.1]heptan-1-yl)methanol (**18a**): Starting from **17a** (80 mg, 0.31 mmol), **13** (85 mg, 0.38 mmol), DIPEA (109 mL, 0.63 mmol), *n*-BuOH (2 mL) in stage 1 and *trans*-1,4-cyclohexandiamine (108 mg, 0.94 mmol) in stage 2, yield 69 mg (32%) as a TFA salt. ^{1}H NMR: δ 1.31-1.51 (m, 6H, H-3″´b, H-3b, H-2b), 1.65 (m, 4H, $NCH_2\mathbf{CH_2}$), 1.77 (m, 2H, H-3a), 2.05 (s, H-5), 2.07-2.22 (m, 6H, H-2a, H-2b, H-2″´a), 3.03 (bm, H-4″´), 3.13 (m, 4H, $N\mathbf{CH_2}CH_2$), 3.51 (s, 2H, $\mathbf{CH_2}OH$), 3.62 (bm, 1H, H-1″´), 6.74 (bs, 2´-NH), 7.69 (m, 2H, H-2″ or H-3″), 7.69 (bd, 3H, 4″´-NH_3^+), 7.98 (s, 1H, H-8′), 8.30 (m, 2H, H-2″ or H-3″), 9.98 (s, 1H, 6´-NH). ^{13}C NMR: δ 24.86 ($NCH_2\mathbf{CH_2}$), 29.53 (C-3″´), 30.09 (C-2″´), 31.44 (C-3), 34.31 (C-2), 43.47 (C-5), 47.98 ($N\mathbf{CH_2}CH_2$), 48.37 (C-4), 49.12 (C-4″´), 49.82 (C-1″´), 64.49 ($\mathbf{CH_2}OH$), 65.28 (C-1), 114.19 (C-5′), 119.39 (C-2″ or C-3″), 128.20 (C-2″ or C-3″), 127.8* (C-1″ or C-4″), 137.6* (C-8′), 144.83 (C-1″ or C-4″), 152.7 (C-4′), 151.56 (C-6′), 157.80 (C-2′). ESI MS *m/z* (%): 581.3 (100) [M + H], 603.3 (15) [M + Na]; HRMS ESI ($C_{29}H_{41}O_3N_8S$) calculated: 581.30168; found: 581.30174.

N^2-([1r,4r]-4-Aminocyclohexyl)-9-cyclohexyl-N^6-(4-[pyrrolidin-1-ylsulfonyl]phenyl)-9H-purine-2,6-diamine (**18b**): Starting from **17b** (80 mg, 0.31 mmol), **13** (85 mg, 0.38 mmol), DIPEA (109 mL, 0.63 mmol), *n*-BuOH (2 mL) in stage 1 and *trans*-1,4-cyclohexandiamine (108 mg, 0.94 mmol) in stage 2, yield 85 mg (42%) as a TFA salt. ^{1}H NMR: δ 1.16-1.56 (m, 7H, H-3"b, H-4"b, H-2‴b, H-3‴b), 1.61-1.67 (m, 4H, $NCH_2\mathbf{CH_2}$), 1.67-1.74 (m, 1H, H-4"a), 1.81-1.93 (m, 4H, H-2"b, H-3"a), 1.94-2.05 (m, 4H, H-2"a, H-3‴a), 2.05-2.17 (m, 2H, H-2‴a), 2.97-3.10 (m, 1H, H-4‴), 3.08-3.19 (m, 4H, $N\mathbf{CH_2}CH_2$), 3.60-3.73 (m, 1H, H-1‴), 4.15-4.27 (m, 1H, H-1"), 6.89 (bs, 1H, 2-NH), 7.67-7.73 (m, 2H, H-3′), 7.83 (bs, 3H, NH_2), 8.10 (s, 1H, H-8), 8.24-8.34 (m, 2H, H-2′), 10.05 (s, 1H, 6-NH). ^{13}C NMR: δ 24.68 ($NCH_2\mathbf{CH_2}$), 24.84 (C-4"), 25.23 (C-3"), 29.32 (C-3‴), 30.01 (C-2‴), 32.06 (C-2"), 47.81 ($N\mathbf{CH_2}CH_2$), 48.86 (C-4‴), 49.6 (C-1‴), 53.2 (C-1"), 113.7 (C-5), 119.15 (C-2′), 127.9 (C-3′), 128.01 (C-4′), 137.00 (C-8), 144.63 (C-1′), 151.32 (C-6), 151.60 (C-4), 157.8 (C-2). ESI MS *m/z* (%): 539.5 (100) [M + H], 561.3 (12) [M + Na]; HRMS ESI ($C_{27}H_{39}O_2N_8S$) calculated: 539.29112; found: 539.29121.

4-((2-(([1r,4r]-4-Aminocyclohexyl)amino)-9-(4-(hydroxymethyl)bicyclo[2.2.1]heptan-1-yl)-9H-purin-6-yl)amino)-N,N-diethylbenzenesulfonamide (**19a**): Starting from **17a** (95 mg, 0.32 mmol), **14** (108 mg, 0.47 mmol), DIPEA (137 mL, 0.79 mmol), *n*-BuOH (4 mL) in stage 1 and *trans*-1,-4-cyclohexandiamine (109 mg, 0.96 mmol) in stage 2. Yield 96 mg (43%) as a TFA salt. ^{1}H NMR: δ 1.04 (t, 6H, $J_{CH3,CH2}$ = 7.1, $NCH_2\mathbf{CH_3}$), 1.28-1.53 (m, 6H, H-3"b, H-5"endo, H-2‴b, H-3‴b), 1.71-1.84 (m, 2H, H-3"a, H-5"exo), 1.97-2.25 (m, 10H, H-2", H-6", H-7", H-2‴a, H-3‴a), 2.98-3.10 (m, 1H, H-4‴), 3.15 (q, 4H, $J_{CH2,CH3}$ = 5.8, $N\mathbf{CH_2}CH_3$), 3.51 (s, 2H, CH_2O), 3.56-3.67 (m, 1H, H-1‴), 6.92 (bs, 1H, 2′-NH), 7.65-7.72 (m, 2H, H-3), 7.92 (bs, 3H, NH_2), 8.19 (s, 1H, H-8′), 8.22-8.27 (m, 2H, H-2), 10.05 (s, 1H, 6′-NH). ^{13}C NMR: δ 14.14 ($NCH_2\mathbf{CH_3}$), 29.34 and 29.88 (C-2‴, C-3‴), 31.24 (C-3", C-5"), 34.05 (C-2", C-6"), 41.81 ($N\mathbf{CH_2}CH_3$), 43.25 (C-7"), 48.21 (C-4"), 48.90 (C-1‴), 49.71 (C-4‴), 64.26 (CH_2O), 65.44 (C-1"), 112.45 (C-5′), 119.46 (C-2), 127.44 (C-3), 131.97 (C-4), 137.33 (C-8′), 144.02 (C-1), 150.95 (C-6′), 152.10 (C-4′), 157.73 (C-2′). ESI MS *m/z* (%): 583.4 (100) [M + H]; HRMS ESI ($C_{29}H_{43}O_3N_8S$) calculated: 583.31733; found: 583.31732.

4-((2-(([1r,4r]-4-Aminocyclohexyl)amino)-9-cyclohexyl-9H-purin-6-yl)amino)-N,N-diethylbenzenesulfonamide (**19b**): Starting from **17b** (100 mg, 0.39 mmol), **14** (108 mg, 0.47 mmol), DIPEA (137 mL, 0.79 mmol), *n*-BuOH (mL) in stage 1 and *trans*-1,4-cyclohexandiamine (135 mg, 1.18 mmol) in stage 2. Yield 123 mg (49%) as a TFA salt. ^{1}H NMR: δ 1.03 (t, 6H, $J_{CH3,CH2}$ = 7.1, $NCH_2\mathbf{CH_3}$), 1.16-1.25 (m, 1H, H-4"b), 1.27-1.44 (m, 4H, H-3"b, H-2‴b), 1.45-1.58 (m, 2H, H-3‴b), 1.61-1.70 (m, 1H, H-4"a), 1.75-2.20 (m, 10H, H-2", H-3"a, H-2‴a, H-3‴a), 2.95-3.06 (m, 1H, H-4‴), 3.13 (q, 4H, $J_{CH2,CH3}$ = 7.0, $N\mathbf{CH_2}CH_3$), 3.60-3.75 (m, 1H, H-1‴), 4.14-4.28 (m, 1H, H-1"), 6.93 (bs, 1H, 2′-NH), 7.59-7.81 (m, 2H, H-3), 8.13 (s, 1H, H-8), 8.18 (bs, 3H, NH_2), 8.24-8.34 (m, 2H, H-2), 10.05 (bs, 1H, 6′-NH). ^{13}C NMR: δ 14.19 ($NCH_2\mathbf{CH_3}$), 24.99 (C-4"), 25.35 (C-3"), 29.44 (C-3‴), 30.23 (C-2‴), 32.18 (C-2"), 41.95 ($N\mathbf{CH_2}CH_3$), 49.08 (C-4‴), 49.83 (C-1‴), 53.5 (C-1"), 113.29 (C-5′), 119.52 (C-2), 127.53 (C-3), 132.01 (C-4), 137.06 (C-8′), 144.36 (C-1), 151.45 (C-4′), 157.96 (C-2′). ESI MS *m/z* (%): 541.4 (100) [M + H], 563.4 (21) [M + Na]; HRMS ESI ($C_{27}H_{41}O_2N_8S$) calculated: 541.30677; found: 541.30679.

4-((2-(([1r,4r]-4-aminocyclohexyl)amino)-9-(4-(hydroxymethyl)bicyclo[2.2.1]heptan-1-yl)-9H-purin-6-yl)amino)-N-(2-methoxyethyl)benzenesulfonamide (**20a**): Starting from **17a** (100 mg, 0.34 mmol), **16** (93 mg, 0.4 mmol), DIPEA (117 mL, 0.67 mmol), *n*-BuOH (3 mL) in stage 1 and *trans*-1,4-cyclohexandiamine (115 mg, 1 mmol) in stage 2. Yield 77 mg (33%) as a TFA salt. ^{1}H NMR: δ 1.28-1.53 (m, 6H, H-3"b, H-5"endo, H-2‴b, H-3‴b), 1.72-1.84 (m, 2H, H-3"a, H-5"exo), 1.94-2.26 (m, 10H, H-2", H-6", H-7", H-2‴a, H-3‴a), 2.89 (q, 2H, $J_{CH2,CH2}$ = $J_{CH2,NH}$ = 5.8, $N\mathbf{CH_2}CH_2$), 2.96-3.11 (m, 1H, H-4‴), 3.17 (s, 3H, OCH_3), 3.31 (t, 2H, $J_{CH2,CH2}$ = 5.8, $NCH_2\mathbf{CH_2}$), 3.51 (s, 2H, CH_2O), 3.56-3.71 (m, 1H, H-1‴), 7.56 (t, 1H, $J_{NH,CH2}$ = 6.0, SNH), 7.63-7.77 (m, 2H, H-2), 7.90 (bs, 3H, NH_2, 2′-NH), 8.14 (s, 1H, H-8′), 8.16-8.28 (m, 2H, H-3), 9.98 (s, 1H, 6′-NH). ^{13}C NMR: δ 29.36 and 29.90 (C-2‴, C-3‴), 31.25 (C-3", C-5"), 34.06 (C-2", C-6"), 42.15 ($N\mathbf{CH_2}CH_2$), 43.26 (C-7"), 48.20 (C-4"), 48.92 (C-4‴), 49.64 (C-1‴), 57.89 (OCH_3), 64.28 (CH_2O), 65.37 (C-1"), 70.57 ($NCH_2\mathbf{CH_2}$), 112.8 (C-5′), 119.29 (C-3), 127.21 (C-2), 132.85 (C-1), 137.2 (C-8′), 143.79 (C-4), 151.09 (C-4′), 157.72 (C-2′). ESI MS *m/z* (%): 585.3 (100) [M + H], 607.3 (43) [M + H]; HRMS ESI ($C_{28}H_{41}O_4N_8S$) calculated: 585.29660; found: 585.29656.

4-((2-(([1r,4r]-4-Aminocyclohexyl)amino)-9-cyclohexyl-9H-purin-6-yl)amino)-N-(2-methoxyethyl)benzenesulfonamide (**20b**): Starting from **17b** (100 mg, 0.39 mmol), 16 (108 mg, 0.47 mmol), DIPEA (137 mL, 0.79 mmol), *n*-BuOH (mL) in stage 1 and *trans*-1,4-cyclohexandiamine (135 mg, 1.18 mmol) in stage 2. Yield 119 mg (46%) as a TFA salt. ^{1}H NMR: δ 1.51-1.20 (m, 7H, H-3"b, H-4"b, H-2‴b, H-3‴b), 1.67-1.74 (m, 1H, H-4"a), 2.14-1.80 (m, 10H, H-2", H-3"a, H-4"a, H-2‴a, H-3‴a), 2.89 (q, 2H, $J_{CH2,CH2}$ = $J_{CH2,NH}$ = 5.8, $N\mathbf{CH_2}CH_2$), 2.99-3.04 (m, 1H, H-4‴), 3.17 (s, 3H, OCH_3), 3.31 (t, 2H, $J_{CH2,CH2}$ = 5.8, $NCH_2\mathbf{CH_2}$), 3.58-3.72 (m, 1H, H-1‴), 4.16-4.31 (m, 1H, H-1"), 7.56 (t, 1H, $J_{NH,CH2}$ = 6.0, SNH), 7.68-7.75 (m, 2H, H-2), 7.90 (bs, 4H, NH_2, 2′-NH), 8.18-8.24 (m, 2H, H-3), 8.29 (s, 1H, H-8′), 10.11 (s, 1H, 6′-NH). ^{13}C NMR: δ 24.83 (C-4"), 25.18 (C-3"), 29.33 and 30.00 (C-2‴, C-3‴), 31.95 (C-2"), 42.16 ($N\mathbf{CH_2}CH_2$), 48.86 (C-4‴), 49.57 (C-1‴), 53.5 (C-1"), 57.89 (OCH_3), 70.57 ($NCH_2\mathbf{CH_2}$), 112.17 (C-5′), 119.41 (C-3), 127.22 (C-2), 133.12 (C-1), 136.95 (C-8′), 143.64 (C-4), 151.13 (C-4′), 157.60 (C-2′). ESI MS *m/z* (%): 543.3 (100) [M + H], 565.2 (36) [M + H]; HRMS ESI ($C_{28}H_{34}O_3N_9S$) calculated: 543.28603; found: 543.28607.

1-((4-((2-(([1r,4r]-4-Aminocyclohexyl)amino)-9-(4-(hydroxymethyl)bicyclo[2.2.1]heptan-1-yl)-9H-purin-6-yl)amino)phenyl)sulfonyl)piperidin-4-one (**21a**): Starting from **17a** (75 mg, 0.25 mmol), **15** (90 mg, 0.30 mmol), DIPEA (88 mL, 0.5 mmol), *n*-BuOH (3 mL) in stage 1 and *trans*-1,4-cyclohexandiamine (87 mg, 0.75 mmol) in stage 2. Final deprotection of the ketal was accomplished by diluting the crude reaction mixture with 5% TFA in H_2O-MeCN (1:1) and stirring this mixture at room temperature for 12 hours. Yield 57 mg (35%) as a TFA salt. ^{1}H NMR: δ 1.27-1.52 (m, 6H, H-2‴b, H-3‴b, H-3"a, H-5"endo), 1.70-1.82 (m, 2H, H-3"b, H-5"exo), 1.95-2.23 (m, 10H, H-2", H-6", H-7", H-3‴a, H-2‴a), 2.38-2.46 (m, 4H, $OC\mathbf{CH_2}$), 3.00-3.10 (m, 1H, H-4‴), 1.23-3.35 (m, 4H, $N\mathbf{CH_2}CH_2$), 3.51 (s, 2H, CH_2O), 3.58-3.68 (m, 1H, H-1‴), 6.78 (bs, 1H, 2′-NH), 7.64-7.74 (m, 2H, H-3), 7.86 (bs, 3H, NH_2), 8.00 (s, 1H, H-8′), 8.27-8.36 (m, 2H, H-2), 10.05 (s, 1H, 6′-NH). ^{13}C NMR: δ 29.35 and 29.88 (C-2‴, C-3‴), 31.25 (C-3",

C-5″), 34.09 (C-2″, C-6″), 39.5 ($NCH_2\mathbf{CH_2}$), 43.26 (C-7″), 45.16 ($N\mathbf{CH_2}CH_2$), 48.20 (C-4″), 48.90 (C-4‴), 49.70 (C-1‴), 64.28 (CH_2O), 65.30 (C-1″), 113.15 (C-5′), 119.44 (C-2), 127.40 (C-4), 128.13 (C-3), 137.4 (C-8′), 144.83 (C-1), 151.09 (C-4′), 152.36 (C-6′), 157.67 (C-2′), 205.58 (CO). ESI MS *m/z* (%): 609.4 (100) [M + H]; HRMS ESI ($C_{30}H_{41}O_4N_8S$) calculated: 609.29660; found: 609.29667.

1-((4-((2-(([1r,4r]-4-Aminocyclohexyl)amino)-9-cyclohexyl-9H-purin-6-yl)amino)phenyl)sulfonyl)piperidin-4-one (**21b**): Starting from **17b** (80 mg, 0.31 mmol), **15** (112 mg, 0.38 mmol), DIPEA (109 mL, 0.63 mmol), *n*-BuOH (2 mL) in stage 1 and *trans*-1,4-cyclohexandiamine (108 mg, 0.94 mmol) in stage 2. Final deprotection of the ketal was accomplished by diluting the crude reaction mixture with 5% TFA in H_2O-MeCN (1:1) and stirring this mixture at room temperature for 12 hours. Yield 81 mg (38%) as a TFA salt. ^{1}H NMR: δ 1.15-1.55 (m, 7H, H-3"b, H-4"b, H-2‴b, H-3‴b), 1.67-1.74 (m, 1H, H-4"a), 1.80-1.95 (m, 4H, H-3"a, H-2"b), 1.95-2.02 (m, 4H, H-3‴a, H-2"a), 2.04-2.15 (m, 2H, H-2‴a), 2.36-2.48 (m, 4H, $OCCH_2$), 2.99-3.09 (m, 1H, H-4‴), 3.21-3.39 (m, 4H, NCH_2), 3.60-3.69 (m, 1H, H-1‴), 4.16-4.28 (m, 1H, H-1″), 6.95 (bs, 1H, 2′-NH), 7.64-7.77 (m, 2H, H-2), 7.84 (bs, 3H, NH_2), 8.12 (s, 1H, H-8′), 8.28-8.37 (m, 2H, H-3), 10.11 (bs, 1H, 6′-NH). ^{13}C NMR: δ 24.83 (C-4″), 25.23 (C-3″), 29.32 (C-3‴), 30.00 (C-2‴), 31.95 (C-2″), 40.3 ($OC\mathbf{CH_2}$), 45.12 (NCH_2), 48.86 (C-4‴), 49.75 (C-1‴), 53.88 (C-1″), 112.1 (C-5′), 119.65 (C-2), 128.15 (C-3), 137.07 (C-8′), 144.90 (C-1), 151.00 (C-4′), 150.74 (C-4′), 157.61 (C-2′), 205.57 (CO). NegESI MS *m/z* (%): 565.3 (100) [M-H]; HRMS negESI ($C_{28}H_{37}O_3N_8S$) calculated: 565.27148; found: 556.27057.

(4-(6-chloro-2-iodo-9H-purin-9-yl)bicyclo[2.2.1]heptan-1-yl)methanol (**22a**): To a mixture of **9** (881 mg, 3 mmol), CuI (571 mg, 3 mmol) and CH_2I_2 (967 µL, 12 mmol) in THF (20 mL) was added isoamyl nitrite (1.22 mL, 6 mmol) dropwise and the reaction mixture was heated to reflux for 4 hours. Even though the TLC retention was almost identical to the starting material (ethyl acetate - methanol 9:1), reaction on *p*-nitrobenzyl pyridine stain was different. Volatiles were evaporated, crude mixture was adsorbed on silica and flash chromatography (0-30%methanol in ethyl acetate) afforded **19** (900 mg, 74%) as pale brown foam. ^{1}H NMR: δ 1.40-1.50 (m, 2H, H-2b, H-6endo), 1.74-1.84 (m, 2H, H-2a, H-6exo), 1.99-2.07 (m, 2H, H-3exo, H-5 endo), 2.08 (s, 2H, H-7), 2.21-2.31 (m, 2H, H-3endo, H-5endo), 3.51 (d, 2H, $J_{CH2,OH}$ = 5.3, CH_2O), 4.69 (t, 1H, $J_{OH,CH2}$ = 5.3, OH), 8.62 (s, 1H, H-8′). ^{13}C NMR: δ 31.36 (C-2, C-6), 34.73 (C-3, C-5), 43.62 (C-7), 48.62 (C-1), 64.30 (CH_2O), 66.49 (C-4), 117.42 (C-2′), 131.89 (C-5′), 146.84 (C-8′), 148.87 (C-6′), 153.43 (C-4′). ESI MS *m/z* (%): 405.1 (100) [M + H]; HRMS ESI ($C_{13}H_{15}N_4ClI$) calculated: 404.99736; found: 404.99743.

6-Chloro-9-cyclohexyl-2-iodo-9H-purine (**22b**): Compound was prepared according to a published procedure.[35]

General procedure for the preparation of compound 22

A solution of **22a** or **22b** (100 mg, 0.25 or 0.28 mmol), DIPEA (2 equiv, 86 µL, 0.5 mmol or 96 µL, 0.55 mmol), and **13** (1.2 equiv, 67 mg, 0.3 mmol or 75 mg, 0.33 mmol) in *n*-BuOH (3 mL) was heated in a pressure vessel to 150°C overnight. Volatiles were thoroughly removed in vacuo, residue was suspended in ethyl acetate, filtered through a plug of celite and evaporated to afford crude intermediate **23a** or **23b**. A sample of the intermediate was isolated and subjected to NMR and HRMS analysis.

In a separate flask, $Pd_2(dba)_3$ (0.05 equiv, 11 mg, 0.012 mmol or 13 mg, 0.014 mmol) and XantPhos (0.1 equiv, 14 mg, 0.025 mmol or 16 mg, 0.028 mmol) were mixed in dry toluene (2 mL) under argon atmosphere and heated to 50°C for 10 minutes (color turns from dark-purple to yellow). To this formed Pd-complex was added Cs_2CO_3 (1.1 equiv, 89 mg, 0.27 mmol or 99 mg, 0.30 mmol) followed by a slow addition of a solution of aniline "(0.32 mmol, 1.3 equiv) and the crude intermediate in dry dioxane (2 mL). Reaction mixture was stirred at room temperature overnight, all volatiles were evaporated, and the product was isolated by a combination of normal phase (MeOH in $CHCl_3$) and reverse phase (10-100% MeCN in H_2O, 0.2% TFA) flash chromatography.

(4-(2-iodo-6-((4-[pyrrolidin-1-ylsulfonyl]phenyl)amino)-9H-purin-9-yl) bicyclo[2.2.1]heptan-1-yl)methanol (**23a**): ^{1}H NMR: δ 1.40-1.50 (m, 2H, H-2b, H-6endo), 1.62-1.69 (m, 4H, $NCH_2\mathbf{CH_2}$), 1.73-1.86 (m, 2H, H-2a, H-6exo), 2.12-1.97 (m, 4H, H-3exo, H-5exo, H-7), 2.19-2.29 (m, 2H, H-3endo, H-5endo), 3.10-3.18 (m, 4H, $N\mathbf{CH_2}CH_2$), 3.51 (d, 2H, $J_{CH2,OH}$ = 5.4, CH_2O), 4.68 (d, 1H, $J_{OH,CH2}$ = 5.5, OH), 7.75-7.82 (m, 2H, H-3″), 8.11-8.19 (m, 2H, H-2″), 8.30 (s, 1H, H-8′), 10.57 (s, 1H, 6′-NH). ^{13}C NMR: δ 24.88 ($NCH_2\mathbf{CH_2}$), 31.30 (C-2, C-6), 34.74 (C-3, C-5), 43.59 (C-7), 47.97 ($N\mathbf{CH_2}CH_2$), 48.50 (C-1), 64.23 (CH_2O), 65.82 (C-4), 118.62 (C-2′), 119.94 (C-2″), 120.01 (C-5′), 128.40 (C-3″), 129.65 (C-4″), 141.45 (C-8′), 143.52 (C-1″), 150.94 (C-4′), 151.26 (C-6′).ESI MS *m/z* (%): 594.2 (100) [M + H]; HRMS ESI ($C_{23}H_{27}IN_6O_3S$) calculated: 594.09100; found: 594.09109.

9-Cyclohexyl-2-iodo-N-(4-[pyrrolidin-1-ylsulfonyl]phenyl)-9H-purin-6-amine (**23b**): ^{1}H NMR: δ 1.23-1.31 (m, 1H, H-4"b), 1.41-1.53 (m, 2H, H-3"a), 1.62-1.68 (m, 4H, H-4"a), 1.69-1.76 (m, 1H, H-4"a), 1.90-1.80 (m, 4H, H-2"a, H-3"a), 1.99-2.07 (m, 2H, H-2"a), 3.11-3.18 (m, 4H, $N\mathbf{CH_2}CH_2$), 4.32-4.42 (m, 1H, H-1″), 7.73-7.81 (m, 2H, H-3′), 8.12-8.18 (m, 2H, H-2′), 8.44 (s, 1H, H-8), 10.60 (bs, 1H, 6-NH). ^{13}C NMR: δ 24.87 ($NCH_2\mathbf{CH_2}$), 24.92 (C-4″), 25.19 (C-3″), 32.52 (C-2″), 47.97 ($N\mathbf{CH_2}CH_2$), 53.95 (C-1″), 118.84 (C-2), 119.95 (C-2′), 120.33 (C-5), 128.40 (C-3′), 129.68 (C-4′), 140.78 (C-8), 143.51 (C-1′), 150.46 (C-4), 150.85 (C-6). ESI MS *m/z* (%): 552.2 (100) [M + H]; HRMS ESI ($C_{21}H_{25}IN_6O_2S$) calculated: 552.08044; found: 552.08031.

(4-(2-(phenylamino)-6-((4-[pyrrolidin-1-ylsulfonyl]phenyl)amino)-9H-purin-9-yl)bicyclo[2.2.1]heptan-1-yl)methanol (**24a**): Aniline (29 µL) was used in the second stage pf reaction, FCC in 1-10% MeOH in $CHCl_3$, yield 54 mg (39%) ^{1}H NMR (401 MHz, DMSO-d_6) δ 1.55-1.41 (m, 2H, H-2a, H-6endo), 1.70-1.61 (m, 4H, $NCH_2\mathbf{CH_2}$), 1.90-1.76 (m, 2H, H-2b, H-6exo), 2.13 (s, 2H, H-7), 2.29-2.16 (m, 4H, H-3, H-5), 3.12-3.17 (m, 4H, NCH_2), 3.53 (s, 2H, CH_2O), 6.94 (t, 1H, *J* = 7.3 Hz,, H-4‴), 7.24-7.32 (m, 2H, H-3‴), 7.66-7.73 (m, 2H, H-3″), 7.80-7.86 (m, 2H, H-2‴), 8.10 (s, 1H, H-8′), 8.32-8.38 (m, 2H, H-2″), 9.25 (s, 1H, 2′-NH), 10.15 (s, 1H, 6′-NH). ^{13}C NMR (101 MHz, DMSO) δ 24.88 ($NCH_2\mathbf{CH_2}$), 31.45 (C-2, C-6), 34.39 (C-3, C-5), 43.52 (C-7), 48.00 (NCH_2), 48.46 (C-1), 64.44 (CH_2O), 65.35 (C-4), 115.76 (C-5″), 118.81

(C-2‴), 119.92 (C-2″), 121.03 (C-4‴), 128.25 (C-3″), 128.49 (C-4″), 128.57 (C-3‴), 138.63 (C-8′), 141.30 (C-1‴), 144.53 (C-1″), 151.60 (C-2′, C-4′), 155.16 (C-6′). NegESI MS *m/z* (%): 558.2 (100) [M-H]; HRMS negESI ($C_{28}H_{37}O_3N_8S$) calculated: 565.27148; found: 565.27112.

9-cyclohexyl-N²-phenyl-N⁶-(4-[pyrrolidin-1-ylsulfonyl]phenyl)-9H-purine-2,6-diamine ((**24b**): Aniline (33 μL) was used in the second stage pf reaction, FCC in 1-3% MeOH in $CHCl_3$, yield 48 mg (34%). ^{1}H NMR: δ 1.26-1.34 (m, 1H, H-4‴a), 1.38-1.50 (m, 2H, H-3‴a), 1.62-1.68 (m, 4H, $NCH_2\mathbf{CH_2}$), 1.71-1.79 (m, 1H, H-4‴b), 1.86-1.93 (m, 2H, H-2‴a), 1.97-2.08 (m, 4H, H-2‴b, H-3‴b), 3.10-3.17 (m, 4H, $N\mathbf{CH_2}CH_2$), 4.27-4.37 (m, 1H, H-1‴), 6.91-6.97 (m, 1H, H-4″), 7.24-7.31 (m, 2H, H-3″), 7.66-7.72 (m, 2H, H-2′), 7.77-7.84 (m, 2H, H-2″), 8.15 (s, 1H, H-8), 8.27-8.34 (m, 2H, H-3′), 9.24 (s, 1H, 2-NH). 10.16 (s, 1H, 6-NH). ^{13}C NMR: δ 24.67 ($NCH_2\mathbf{CH_2}$), 24.93 (C-4‴), 25.24 (C-3‴), 31.99 (C-2‴), 47.79 ($N\mathbf{CH_2}CH_2$), 53.85 (C-1‴), 115.52 (C-5), 118.83 (C-2″), 119.87 (C-2′), 120.85 (C-4″), 127.99 (C-3′), 128.29 (C-4′), 128.32 (C-3″), 138.21 (C-8), 141.14 (C-1″), 144.32 (C-1′), 150.99 (C-4), 151.41 (C-6), 155.21 (C-2).ESI MS *m/z* (%): 518.2 (100) [M + H], 540.2 (12) [M + Na]; HRMS ESI ($C_{27}H_{32}O_2N_7S$) calculated: 518.23327; found: 518.23326.

(4-(2-(Pyridin-3-ylamino)-6-((4-[pyrrolidin-1-ylsulfonyl]phenyl)amino)-9H-purin-9-yl)bicyclo[2.2.1]heptan-1-yl)methanol (**25a**): 3-aminopyridine (30 mg) was used in the second stage pf reaction, FCC in 5-20% MeOH in $CHCl_3$, yield 35 mg (25%). ^{1}H NMR: δ 1.52 (m, 2H, H-2b, H-6endo), 1.66 (m, 4H, $NCH_2\mathbf{CH_2}$), 1.85 (m, 2H, H-2a, H-6exo), 2.20-2.05 (m, 4H, H-3exo, H-5exo, H-7), 2.31 (m, 2H, H-3endo, H-5endo), 3.15 (m, 4H, $N\mathbf{CH_2}CH_2$), 3.55 (s, 2H, CH_2O), 7.74 (m, 2H, H-3″), 7.91 (dd, 1H, $J_{5‴,4‴}$= 8.8, $J_{5‴,6‴}$= 5.3, H-5‴), 8.18 (s, 1H, H-8′), 8.29 (m, 2H, H-2″), 8.47 (dd, 1H, $J_{6‴,5‴}$= 5.4, $J_{6‴,4‴}$= 1.1, H-6‴), 8.65 (ddd, 1H, $J_{4‴,5‴}$= 8.8, $J_{4‴,2‴}$= 2.5, $J_{4‴,6‴}$= 1.2, H-4‴), 9.34 (d, 1H, $J_{2‴,4‴}$= 2.4, H-2‴), 10.16 (s, 1H, 2′-NH), 10.38 (s, 1H, 6′-NH). ^{13}C NMR: δ 24.70 ($NCH_2\mathbf{CH_2}$), 31.31 (C-2, C-6), 34.45 (C-3, C-5), 43.60 (C-7), 47.81 ($N\mathbf{CH_2}CH_2$), 48.21 (C-1), 64.15 (CH_2O), 65.27 (C-4), 117.03 (C-5′), 120.02 (C-2″), 126.49 (C-5‴), 128.14 (C-3″), 128.90 (C-4″), 131.61 (C-4‴), 131.86 (C-2‴), 134.43 (C-6‴), 139.59 (C-8′), 140.32 (C-3‴), 143.99 (C-1″), 150.87 (C-4′), 151.76 (C-2′), 153.48 (C-6′).ESI MS *m/z* (%): 561.2 (100) [M + H]; HRMS ESI ($C_{28}H_{39}O_3N_8S$) calculated: 561.23908; found: 561.23901.

9-Cyclohexyl-N²-(pyridin-3-yl)-N⁶-(4-[pyrrolidin-1-ylsulfonyl]phenyl)-9H-purine-2,6-diamine (**24b**): 3-aminopyridine (34 mg) was used in the second stage pf reaction, FCC in 1-10% MeOH in $CHCl_3$, yield 55 mg (38%). ^{1}H NMR: δ 1.28-1.37 (m, 1H, H-4‴b), 1.44-1.57 (m, 2H, H-3‴b), 1.61-1.69 (m, 4H, $NCH_2\mathbf{CH_2}$), 1.70-1.77 (m, 1H, H-4‴a), 1.85-2.01 (m, 4H, H-2‴b, H-3‴a), 2.02-2.11 (m, 2H, H-2‴a), 3.18-3.11 (m, 4H, $N\mathbf{CH_2}CH_2$), 4.37-4.46 (m, 1H, H-1‴), 7.72-7.77 (m, 2H, H-3′), 7.91 (dd, 1H, $J_{5″,4″}$ = 8.7, $J_{5″,6″}$ = 5.3, H-5″), 8.24-8.30 (m, 2H, H-2′), 8.31 (s, 1H, H-8), 8.47 (dd, 1H, $J_{6″,5″}$ = 5.4, $J_{6″,4″}$ = 1.1, H-6″), 8.62 (ddd, 1H, $J_{4″,5″}$ = 8.7, $J_{4″,2″}$ = 2.6, $J_{4″,6″}$ = 1.2, H-4″), 9.49 (d, 1H, $J_{2″,4″}$ = 2.5, H-2″), 10.22 (bs, 1H, 2-NH), 10.40 (bs, 1H, 6-NH). ^{13}C NMR: δ 24.70 ($NCH_2\mathbf{CH_2}$), 24.88 (C-4‴), 25.10 (C-3‴), 32.52 (C-2‴), 47.81 ($N\mathbf{CH_2}CH_2$), 53.90 (C-1‴), 116.39 (C-5), 120.09 (C-2′), 126.59 (C-5″), 128.15 (C-3′), 128.96 (C-4′), 131.37 (C-2″), 132.11 (C-4″), 134.22 (C-6″), 139.13 (C-8), 140.42 (C-3″), 143.95 (C-1′), 150.29 (C-4), 151.64 (C-2), 153.76 (C-6). ESI MS *m/z* (%): 519.3 (100) [M + H], 563.4 (21) [M + H]; HRMS ESI ($C_{26}H_{31}O_2N_8S$) calculated: 519.22852; found: 519.22853.

(4-(2-(pyrazin-2-ylamino)-6-((4-[pyrrolidin-1-ylsulfonyl]phenyl)amino)-9H-purin-9-yl)bicyclo[2.2.1]heptan-1-yl)methanol (**26a**): Aminopyrazine (31 mg) was used in the second stage pf reaction, FCC in 2-15% MeOH in $CHCl_3$, yield 48 mg (44%) ^{1}H NMR: δ 1.48-1.53 (m, 2H, H-2a, H-6endo), 1.64-1.67 (m, 4H, $NCH_2\mathbf{CH_2}$), 1.81-1.86 (m, 2H, H-2b, H-6exo), 2.11 (bs, 2H, H-7), 2.13-2.19 (m, 2H, H-3a, H-5a), 2.26-2.31 (m, 2H, H-3b, H-5b), 3.12-3.15 (m, 4H, $N\mathbf{CH_2}CH_2$), 3.53 (s, 2H, CH_2O), 7.67-7.70 (m, 2H, H-3″), 8.15 (s, 1H, H-8′), 8.20 (d, 1H, $J_{5‴,6‴}$= 2.5, H-5‴), 8.35 (dd, 1H, $J_{6‴,5‴}$= 2.5, $J_{6‴,3‴}$= 1.5, H-6‴), 8.44-8.47 (m, 2H, H-2″), 9.58 (d, 1H, $J_{3‴,6‴}$= 1.5, H-3‴), 10.09 (s, 1H, 2′-NH), 10.30 (s, 1H, 6′-NH). ^{13}C NMR: δ 24.86 ($NCH_2\mathbf{CH_2}$), 31.51 (C-2, C-6), 34.56 (C-3, C-5), 43.73 (C-7), 47.97 ($N\mathbf{CH_2}CH_2$), 48.37 (C-1), 64.43 (CH_2O), 65.42 (C-4), 117.02 (C-5′), 120.22 (C-2″), 128.21 (C-3″), 128.64 (C-4″), 135.66 (C-3‴), 137.02 (C-5‴), 139.50 (C-8′), 142.46 (C-6‴), 144.35 (C-1″), 150.49 and 151.25 (C-2‴, C-4′), 151.87 and 153.38 (C-2′, C-6′). ESI MS *m/z* (%): 562.2 (13) [M + H], 584.2 (100) [M + H]; HRMS ESI ($C_{27}H_{31}O_3N_9S$) calculated: 584.21628; found: 584.21624.

9-Cyclohexyl-N²-(pyrazin-2-yl)-N⁶-(4-[pyrrolidin-1-ylsulfonyl]phenyl)-9H-purine-2,6-diamine (**26b**): Aminopyrazine (34 mg) was used in the second stage pf reaction, FCC in 1-5% MeOH in $CHCl_3$, yield 41 mg (29%), ^{1}H NMR: δ 1.23-1.36 (m, 1H, H-4‴a), 1.39-1.52 (m, 2H, H-3‴a), 1.63-1.68 (m, 4H, $NCH_2\mathbf{CH_2}$), 1.71-1.78 (m, 1H, H-4‴b), 1.84-1.93 (m, 2H, H-3‴b), 1.95-2.12 (m, 4H, H-2‴), 3.10-3.17 (m, 4H, $N\mathbf{CH_2}CH_2$), 4.33-4.43 (m, 1H, H-1‴), 7.66-7.72 (m, 2H, H-3′), 8.20 (d, 1H, $J_{5″,6″}$ = 2.6, H-5″), 8.27 (s, 1H, H-8), 8.35 (dd, 1H, $J_{6″,5″}$ = 2.6, $J_{6″,3″}$ = 1.5, H-6″), 8.40-8.45 (m, 2H, H-2′), 9.59 (d, 1H, $J_{3″,6″}$ = 1.5, H-3″), 10.13 (s, 1H, 2-NH), 10.32 (s, 1H, 6-NH). ^{13}C NMR: δ 24.67 ($NCH_2\mathbf{CH_2}$), 24.90 (C-4‴), 25.20 (C-3‴), 31.99 (C-2‴), 47.79 ($N\mathbf{CH_2}CH_2$), 54.12 (C-1‴), 116.16 (C-5), 120.14 (C-2′), 128.00 (C-3′), 128.48 (C-4′), 135.61 (C-3″), 136.81 (C-5″), 139.00 (C-8), 142.19 (C-6″), 144.08 (C-1′), 150.32 and 150.43 (C-2″, C-4), 151.49 and 153.42 (C-2, C-6). ESI MS *m/z* (%): 520.5 (100) [M + H], 542.5 (27) [M + Na]; HRMS ESI ($C_{25}H_{30}O_2N_9S$) calculated: 520.22377; found: 520.22387.

(4-(2-([6-aminopyridin-3-yl]amino)-6-((4-[pyrrolidin-1-ylsulfonyl]phenyl)amino)-9H-purin-9-yl)bicyclo[2.2.1]heptan-1-yl)methanol (**27a**): 2,5-diaminopyridine dihydrochloride (59 mg) was used in the second stage pf reaction, ratio of regioisomers 8:1 (HPLC), FCC in 5-20% MeOH in $CHCl_3$, only one isomer isolated with yield 40 mg (23%). ^{1}H NMR: δ 1.44-1.54 (m, 2H, H-2b, H-6endo), 1.61-1.70 (m, 4H, $NCH_2\mathbf{CH_2}$), 1.80-1.90 (m, 2H, H-2a, H-6exo), 2.04-2.13 (m, 4H, H-3exo, H-5exo, H-7), 2.23-2.34 (m, 2H, H-3endo, H-5endo), 3.10-3.16 (m, 4H, $N\mathbf{CH_2}CH_2$), 3.54 (s, 2H, CH_2O), 7.04 (d, 1H, $J_{5‴,4‴}$= 9.4, H-5‴), 7.67-7.75 (m, 2H, H-3″), 7.89 (bs, 2H, NH_2), 8.07 (s, 1H, H-8′), 8.13 (dd, 1H, $J_{4‴,5‴}$= 9.5, $J_{4‴,2‴}$= 2.5, H-2‴), 8.25-8.35 (m, 2H, H-2″), 8.57 (s, 1H, H-2‴), 9.47 (s, 1H, 2′-NH), 10.57 (s, 1H, 6′-NH). ^{13}C NMR: δ 24.70 ($NCH_2\mathbf{CH_2}$), 31.22 (C-2, C-6), 34.40 (C-3, C-5), 43.58 (C-7), 47.82 ($N\mathbf{CH_2}CH_2$), 48.22 (C-1), 64.10 (CH_2O), 65.16 (C-4), 113.53 (C-5‴), 116.36 (C-5′), 119.77 (C-2″), 128.09 (C-3″), 128.38 (C-4″), 128.51 (C-3‴), 138.01 (C-4‴), 138.81 (C-8′), 144.24 (C-1″),

150.28 (C-6‴), 151.21 (C-4′), 151.70 and 154.44 (C-2′, C-6′).ESI MS *m/z* (%): 576.2 (100) [M + H], 598.2 (62) [M + Na]; HRMS ESI ($C_{28}H_{34}O_3N_9S$) calculated: 576.24998; found: 576.24991.

N^2-(6-aminopyridin-3-yl)-9-cyclohexyl-N^6-(4-[pyrrolidin-1-ylsulfonyl] phenyl)-9H-purine-2,6-diamine (**27b**)): 2,5-diaminopyridine dihydrochloride (65 mg) was used in the second stage pf reaction, ratio 3:1 (HPLC), FCC in 2-10% MeOH in $CHCl_3$, separation of isomers on RP FCC, yield 57 mg (32%) of **27b** and 14 mg (8%) of the opposite regioisomer. ^{1}H NMR: δ 1.26-1.37 (m, 1H, H-4‴a), 1.41-1.55 (m, 2H, H-3‴a), 1.61-1.68 (m, 4H, $NCH_2\mathbf{CH_2}$), 1.68-1.75 (m, 1H, H-4‴b), 1.84-1.97 (m, 4H, H-3‴b, H-2‴a), 2.01-2.09 (m, 2H, H-2‴b), 3.09-3.18 (m, 4H, $N\mathbf{CH_2}CH_2$), 4.30-4.39 (m, 1H, H-1‴), 7.04 (d, 1H, $J_{3',4'}$ = 9.5, H-3′), 7.67-7.75 (m, 2H, H-3″), 7.88 (bs, 2H, NH_2), 8.12 (dd, 1H, $J_{4',3'}$ = 9.5, $J_{4',6'}$ = 2.5, H-4′), 8.21-8.29 (m, 2H, H-2″), 8.33 (s, 1H, H-8), 8.63 (bs, 1H, H-6′), 9.56 (bs, 1H, 2-NH), 10.33 (bs, 1H, 6-NH). ^{13}C NMR: δ 24.95 ($NCH_2\mathbf{CH_2}$), 25.10 (C-4‴), 25.38 (C-3‴), 32.38 (C-2‴), 48.09 ($N\mathbf{CH_2}CH_2$), 54.35 (C-1‴), 113.96 (C-3′), 115.10 (C-5), 120.12 (C-2″), 123.20 (C-6′), 128.41 (C-3″), 128.50 (C-5′), 128.91 (C-4″), 138.64 (C-8, C-4′), 144.33 (C-1″), 150.39 (C-2′), 150.74 (C-4), 151.58 and 155.16 (C-2, C-6). ESI MS *m/z* (%): 534.2 (100) [M + H], 556.2 (47) [M + Na]; HRMS ESI ($C_{26}H_{32}O_2N_9S$) calculated: 534.23942; found: 534.23938.

4.2 | Biochemical measurements

The tested compounds were dissolved in DMSO and diluted with water (the concentration of DMSO in the reaction never exceeded 0.2%). The CDK2/Cyclin E complex was produced in Sf9 insect cells via baculoviral infection and purified on a Ni^{2+}NTA column (Qiagen). Kinase (approx. 10 ng) was assayed using a mixture of the following: 1 mg/mL of histone H1, 15 μM of ATP, 0.05 of μCi [γ-^{33}P]ATP, the tested compound, and reaction buffer, in a final volume of 10 μL. The reaction buffer consisted of: 60 mM of HEPES-NaOH, pH 7.5, 3 mM of $MgCl_2$, 3 mM of $MnCl_2$, 3 μM of Na-orthovanadate, 1.2 mM of DTT, and 2.5 μg/50 μL of $PEG_{20.000}$. The reactions were stopped by adding 5 μL of 3% aqueous H_3PO_4. Aliquots were spotted onto P-81 phosphocellulose (Whatman), washed three times with 0.5% aqueous H_3PO_4, and finally air-dried. Kinase inhibition was quantified using a FLA-7000 digital image analyzer (Fujifilm). The concentration of each tested compound required the decrease of the CDK activity by 50%. The IC_{50} values were determined from the dose-response curve.

4.3 | Computational methodology

4.3.1 | Virtual screening and compound design

The final compounds presented in this work issued from computer-aided iterative design. First, conformers of over 1000 compounds from the IOCB proprietary database had been docked into CDK2 structure and scored using SQM/COSMO methodology.[5] We used the CDK2 structure from complex with a large inhibitor staurosporine (PDB: 1AQ1)[36] to allow larger compounds to bind. For docking, the Glide programme of Schrodinger[37] in the standard precision (SP) mode was used with the default settings. Waters beyond 5 Å from the ligand were removed and the bond orders assigned. The receptor grid was created using the default settings. A grid box of $20 \times 20 \times 20$ Å^3 was generated, $10 \times 10 \times 10$ Å^3 inner box was centered on the corresponding ligand. Hydrogen atoms were added to the protein by means of the Protein Preparation Wizard using the default settings. To determine preferable protonation of protein titratable residues, pH was set to 7.0. OPLS3[44] force field was used for hydrogen optimization. Ligand structures were converted from 2D to 3D using the LigPrep module (Schrodinger Suite) with default settings. Glide was set to yield 10 best-scored poses per ligand.

For scoring, we employed a slightly modified setup[39] of the SQM/COSMO method at the PM6-D3H4X level[5] using the linear-scaling MOZYME algorithm[41] in MOPAC 2016 [42] via the Cuby3 interface.[43] To speed up the calculations, only residues within 10 Å of the inhibitor (the union across the whole series, that is, the same for all the complexes) were taken into account. During geometry optimizations, only residues within 8 Å from the inhibitors were allowed to move. The interaction energies were obtained by subtracting the SQM/COSMO energies of the protein and the ligand from those of the complex. Adding the interaction solvation free energies and ligand deformation free energies gave SQM/COSMO scores. Based on the SQM/COSMO scores, selected compounds were put forward for activity testing against CDK2/Cyclin E.

The three hits discovered (compounds **1**, **2**, **3**) defined the 2,6-diamino purine core and 9-norbornyl substituent as a base for further exploration of chemical space to define the structure-activity relationships. We combined this core with selected 36 modifications of position 2 and 37 modifications in position 6 according to literature.[29] For these 73 compounds, we first placed the purine core in the active site to maintain the main hinge region H-bonds using docking with settings as above. The modifications were built manually using PyMol, ver. 0.99[45] so that no steric clashes with the protein resulted. This approach is based on our extensive experience with SQM/COSMO scoring,[8,39,40] which states that docking is useful for exploring various binding modes, while building diminishes the risk of energy variations due to small changes in the structures.[40] SQM/COSMO scoring[5] with settings described above was used to prioritize compounds for synthesis.

Eight compounds with high scores and synthetic feasibility (**18a**, **19a**, **20a**, **21a**, **24a**, **25a**, **26a**, **27a**; Table 1) were suggested for synthesis. To explore the importance of the hydroxynorbornyl moiety in position 9, another eight compounds with the cyclohexyl substituent in position 9 (**18b**, **19b**, **20b**, **21b**, **24b**, **25b**, **26b**, **27b**; Table 1) were additionally suggested for synthesis. Thus, a total of 16 compounds were synthesized based on computational design.

4.3.2 | Refined scoring of CDK2/ligand series

In order to shed light on the molecular reasons of the 16 new compounds' activities or the lack thereof, we carried out a refined scoring.

Most importantly, the protein conformation now was that of CDK2/roscovitin complex (PDB: 3DDQ)[25] because we had learnt that the three hits had purine core. Docking followed the previously published protocol using Glide (see also above).[5,39] Only protein chain A of the CDK2 structure from its complex with roscovitine was used after the ligands and solvent molecules had been discarded. A careful analysis of all the obtained binding modes and their SQM/COSMO scores was performed. The identified best-scoring orientations of modifications in position 6 were built for all the compounds with the modification in question of the series followed by a short molecular dynamics-based quenching for small structural rearrangements. Atomic velocities were assigned following Maxwell–Boltzmann distribution at temperature of 1000 K. The temperature profile was: 1500 K for 1 ps and then cooling down to 0 K over 2 ps.[6,40] The resulting structures were used for SQM/COSMO scoring (see above). The best-scoring orientation of position 6 in compound **18a** was then built into other compounds with the same modification.

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CONFLICT OF INTEREST

The study was partially supported by Gilead Sciences, Inc.

AUTHOR CONTRIBUTIONS

C.K. and M.D. contributed equally to the study. C.K., H.A., J.F., and M.L. performed docking and molecular modeling. M.D., M.Š., and H.H. performed synthesis of compounds. R.J. and V.K. were responsible for biological testing of compounds. M.D and E.P. measured and interpreted analytical data of the prepared compounds. P.H., M.L., and R.N. designed the study, wrote the manuscript, and led the team.

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REFERENCES

1. Klein ME, Kovatcheva M, Davis LE, Tap WD, Koff A. *CDK4/6 inhibitors: the mechanism of action may not be as simple as once thought*. Cancer Cell. 2018;34:9-20.
2. Malumbres M. *Cyclin-dependent kinases*. Genome Biol. 2014;15:122.
3. Huwe A, Mazitschek R, Giannis A. *Cyclin-dependent kinases small molecules as inhibitors of cyclin-dependent kinases*. Angew Chem Int Ed. 2003;42:2122-2138.
4. Fanfrlík J, Bronowska AK, Řezáč J, Přenosil O, Konvalinka J, Hobza P. *A reliable docking/scoring scheme based on the semiempirical quantum mechanical PM6-DH2 method accurately covering dispersion and H-bonding: HIV-1 protease with 22 ligands*. J Phys Chem B. 2010;114:12666-12678.
5. Lepšík M, Řezáč J, Kolář M, Pecina A, Hobza P, Fanfrlík J. *The semiempirical quantum mechanical scoring function for in silico drug design*. ChemPlusChem. 2013;78:921-931.
6. Hylsová M, Carbain B, Fanfrlík J, et al. *Explicit treatment of active-site waters enhances quantum mechanical/implicit solvent scoring: inhibition of CDK2 by new pyrazolo[1,5-a]pyrimidines*. Eur J Med Chem. 2017;126:1118-1128.
7. Nekardová M, Vymětalová L, Khirsariya P, et al. *Structural basis of the interaction of cyclin-dependent kinase 2 with roscovitine and its analogues having bioisosteric central heterocycles*. ChemPhysChem. 2017;18:785-795.
8. Pecina A, Meier R, Fanfrlík J, et al. *The SQM/COSMO filter: reliable native pose identification based on the quantum-mechanical description of protein-ligand interactions and implicit COSMO solvation*. Chem Commun (Camb). 2016;52:3312-3315.
9. Pecina A, Brynda J, Vrzal L, et al. *Ranking power of the SQM/COSMO scoring function on carbonic anhydrase II–inhibitor complexes*. ChemPhysChem. 2018;19:873-879.
10. Pecina A, Haldar S, Fanfrlík J, et al. *SQM/COSMO scoring function at the DFTB3-D3H4 level: unique identification of native protein–ligand poses*. J Chem Inf Model. 2017;57:127-132.
11. Ajani, H., Pecina, A., Eyrilmez, S. M. *et al*. Superior performance of the SQM/COSMO scoring functions in native pose recognition of diverse protein−ligand complexes in cognate docking. 2017
12. Eyrilmez SM, Köprülüoğlu C, Řezáč J, Hobza P. *Impressive enrichment of semiempirical quantum mechanics-based scoring function: HSP90 protein with 4541 inhibitors and decoys*. ChemPhysChem. 2019;20:2759-2766.
13. Nencka R, Sala M, Dejmek M, Dracinsky M, Holy A, Hrebabecky H. *Two convergent approaches toward novel carbocyclic C-nucleosides*. Synthesis. 2010;23:4119-4130.
14. Šála M, De Palma AM, Hřebabecký H, et al. *Design, synthesis, and biological evaluation of novel coxsackievirus B3 inhibitors*. Bioorg Med Chem. 2010;18:4374-4384.
15. Hřebabecký H, Dejmek M, Dračínský M, et al. *Synthesis of novel azanorbornylpurine derivatives*. Tetrahedron. 2012;68:1286-1298.
16. Dejmek M, Hrebabecky H, Sala M, et al. *From norbornane-based nucleotide analogs locked in south conformation to novel inhibitors of feline herpes virus*. Bioorg Med Chem. 2014;22:2974-2983.
17. Šála M, Dejmek M, Procházková E, et al. *Synthesis of locked cyclohexene and cyclohexane nucleic acids (LCeNA and LCNA) with modified adenosine units*. Org Biomol Chem. 2015;13:2703-2715.
18. Hrebabecky H, Dracinsky M, Prochazkova E, Sala M, Mackman R, Nencka R. *Control of alpha/beta Anomer formation by a 2′,5′ bridge: toward nucleoside derivatives locked in the south conformation*. J Org Chem. 2017;82:11337-11347.
19. Hercik K, Kozak J, Sala M, et al. *Adenosine triphosphate analogs can efficiently inhibit the Zika virus RNA-dependent RNA polymerase*. Antiviral Res. 2017;137:131-133.
20. Enkvist E, Raidaru G, Vaasa A, Pehk T, Lavogina D, Uri A. *Carbocyclic 3′-deoxyadenosine-based highly potent bisubstrate-analog inhibitor of basophilic protein kinases*. Bioorg Med Chem Lett. 2007;17:5336-5339.
21. Dejmek M, Sala M, Hrebabecky H, et al. *Norbornane-based nucleoside and nucleotide analogues locked in north conformation*. Bioorg Med Chem. 2015;23:184-191.
22. Klima, M.; Baumlova, A.; Chalupska, D.; Hrebabecky, H.; Dejmek, M.; Nencka, R.; Boura, E. Crystal Structure of Phosphatidyl Inositol 4-Kinase II Alpha in Complex with Nucleotide Analog. *Worldwide Protein Data Bank*, 2015.
23. Holý A. *Antiviral acyclic nucleoside phosphonates structure activity studies*. Antiviral Res. 2006;71:248-253.

24. Eyer L, Nencka R, de Clercq E, Seley-Radtke K, Růžek D. *Nucleoside analogs as a rich source of antiviral agents active against arthropod-borne flaviviruses*. Antiviral Chem Chemother. 2018;26:2040206618761299.
25. Bettayeb K, Oumata N, Echalier A, et al. *CR8, a potent and selective, roscovitine-derived inhibitor of cyclin-dependent kinases*. Oncogene. 2008;27:5797-5807.
26. Gucký T, Jorda R, Zatloukal M, *et al. A novel series of highly potent2,6,9-trisubstituted purine cyclin-dependent kinase inhibitors*. J MedChem. 2013;56:6234-6247.
27. Martin MP, Olesen SH, Georg GI, Schönbrunn E. *Cyclin-dependent kinase inhibitor dinaciclib interacts with the acetyl-lysine recognition site of bromodomains*. ACS Chem Biol. 2013;8:2360-2365.
28. Jorda R, Havlíček L, Šturc A, et al. *3,5,7-substituted pyrazolo[4,3-d] pyrimidine inhibitors of cyclin-dependent kinases and their evaluation in lymphoma models*. J Med Chem. 2019;62:4606-4623.
29. Jorda R, Paruch K, Krystof V. *Cyclin-dependent kinase inhibitors inspired by roscovitine: purine bioisosteres*. Curr Pharm des. 2012;18: 2974-2980.
30. Dejmek M, Kovackova S, Zbornikova E, et al. *One-pot build-up procedure for the synthesis of variously substituted purine derivatives*. RSC Adv. 2012;2:6970-6980.
31. Mojzych M, Šubertová V, Bielawska A, et al. *Synthesis and kinase inhibitory activity of new sulfonamide derivatives of pyrazolo[4,3-e] [1,2,4]triazines*. Eur J Med Chem. 2014;78:217-224.
32. Myers SM, Bawn RH, Bisset LC, et al. *High-throughput screening and hit validation of extracellular-related kinase 5 (ERK5) inhibitors*. ACS Comb Sci. 2016;18:444-455.
33. Di Gioia ML, Leggio A, Guarino IF, Leotta V, Romio E, Liguori A. *A simple synthesis of anilines by LiAlH4/TiCl4 reduction of aromatic nitro compounds*. Tetrahedron Lett. 2015;56:5341-5344.
34. Thomas AP, Breault GA, Beattie JF, Jewsbury PJ. Imidazo[1,2-a]pyridine and Pyrazolo[2,3-a]pyridine Derivatives, WO2001014375A1. 2001.
35. Legraverend M, Ludwig O, Bisagni E, et al. *Synthesis and in vitro evaluation of novel 2,6,9-trisubstituted purines acting as cyclin-dependent kinase inhibitors*. Bioorg Med Chem. 1999;7:1281-1293.
36. Lawrie AM, Noble MEM, Tunnah P, Brown NR, Johnson LN, Endicott JA. *Protein kinase inhibition by staurosporine revealed in details of the molecular interaction with CDK2*. Nat Struct Biol. 1997;4:796-801.
37. Friesner RA, Banks JL, Murphy RB, et al. *Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy*. J Med Chem. 2004;47:1739-1749.
38. Fanfrlík J, Ruiz FX, Kadlčíková A, et al. *The effect of halogen-to-hydrogen bond substitution on human aldose reductase inhibition*. ACS Chem Biol. 2015;10:1637-1642.
39. Ajani H, Jansa J, Köprülüoğlu C, et al. *Imidazo[1,2-c]pyrimidin-5(6H)-one as a novel core of cyclin-dependent kinase 2 inhibitors: synthesis, activity measurement, docking, and quantum mechanical scoring*. J Mol Recognit. 2018;31:e2720.
40. Brahmkshatriya S, Dobes P, Fanfrlik J, et al. *Quantum mechanical scoring: structural and energetic insights into cyclin-dependent kinase 2 inhibition by Pyrazolo[1,5-a]pyrimidines*. Curr Comp Aided-Drug Design. 2013;9:118-129.
41. Stewart JJP. *Application of the PM6 method to modeling proteins*. J Mol Model. 2009;15:765-805.
42. Stewart JJP. MOPAC2012. Stewart Computational Chemistry, Version 7.263W web: HTTP://OpenMOPAC.net
43. Řezáč J. *Cuby: an integrative framework for computational chemistry*. J Comput Chem. 2016;37:1230-1237.
44. Harder E, Damm W, Maple J, et al. *OPLS3: a force field providing broad coverage of drug-like small molecules and proteins*. J Chem Theory Comput. 2016;12:281-296.
45. De Lano WL. *The PyMOL Molecular Graphics System*. San Carlos, CA: DeLano Scientific LLC; 2002.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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