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Original article

Inhibitors of the TAM subfamily of tyrosine kinases: Synthesis and biological evaluation

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ABSTRACT

The TAM subfamily of Receptor Tyrosine Kinases (RTKs) contains three human proteins of therapeutical interest, Axl, Mer, and Tyro3. Our goal was to design a type II inhibitor specific for this family, i.e. able to interact with the allosteric pocket and with the hinge region of the kinase. We report the synthesis of several series of purine analogues of BMS-777607. The structural diversity of the designed inhibitors was expected to modify the interactions formed in the binding site and consequently to modulate their selectivity profiles. The most potent inhibitor **6g** exhibits K_ds of 39, 42, 65 and 200 nM against Axl, Mer, Met and Tyro3 respectively. Analysis of the affinity of **6g** for active and inactive forms of Abl1, an RTK protein that does not belong to the TAM subfamily, together with the binding modes of **6g** predicted by docking studies, indicates that **6g** displays some selectivity for the TAM family and may act as a type II inhibitor.

1. Introduction

The receptor tyrosine kinases (RTKs) are transmembrane proteins which transduce signals from extracellular medium to the cytoplasm. They regulate cellular functions such as growth, differentiation or motility. Abnormal overexpression levels and/or enhanced activities of RTKs have been associated to a variety of human cancers, leading to a strong interest in the development of inhibitors against these human cancers. There are 58 RTKs in the human genome, which have been classified into 20 families based on sequence similarities within the kinase domain and structural similarities within their extracellular regions [1].

Mer, Axl and Tyro3 are the three human RTKs that belong to the TAM subfamily, and that share the vitamine K-dependent ligands Gas6 and Protein S [2]. Among RTKs, the TAM subfamily is defined by the presence of the KWIAIES signature motif. Recently, these three RTKs have emerged as potential oncology targets, due to their over expression and/or involvement in several types of cancers [3]. Mer (UniProt ID: Q12866) is expressed in at least 50% of acute lymphoblastic leukemia (ALL), with a poor prognosis in 10–15% of pediatric ALL cases while it is not expressed in normal human T- and B-lymphocytes at any stage of development. Mer has therefore a tumor specific pattern which makes this tyrosine kinase an interesting therapeutic target. Axl (UniProt ID: P30530) is overexpressed in numerous tumor types such as chronic myelogenous leukemia (CML), breast, brain, lung, pancreatic, prostate, esophageal, and renal tumors and hence is also considered a potential therapeutic target for the treatment of cancer. High levels of Axl and of Gas6, as well as Mer have been found in NSCLC cell lines [4]. Tyro3 (UniProt ID: Q06418) is the least studied of the TAM receptors [2]. Tyro3 is significantly elevated in human primary melanoma tissue samples and melanoma cell lines where it is an upstream regulator of ITF. Tyro3 knockdown represses cellular proliferation, colony formation and tumorigenesis in vivo of melanoma cells indicating [5] that Tyro3 may serve as a therapeutic target for melanoma. Kinases share a similar overall folding topology and enzymatic mechanism. They consist of a mainly β-stranded N-terminal lobe, and a mainly helical C-terminal lobe and the two terminal lobes are connected by a short strand named the hinge

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region. The ATP binding site is sandwiched between these two lobes. In the active kinase, a characteristic DFG motif located immediately before the activation loop, adopts the so-called DFG-in conformation in which the D and F residues are oriented toward the active site. In its active form, ATP binds to the active site and the terminal phosphate group of ATP is transferred to the substrate.

The most common approach to conceive kinase inhibitors is to design ATP competitive compounds, i.e. compounds that bind to the active site when the kinase is in the DFG-in conformation. These inhibitors are called type I inhibitors. Typically, type I inhibitors form between one and three hydrogen bonds with the backbone hinge aminoacid residues, and hydrophobic interactions with a hydrophobic pocket belonging to the active site [6]. Although a few examples of selective type I inhibitors have been reported, the highly conserved nature of the ATP-binding region often results in poor kinase selectivity.

The second broad class of kinase inhibitors, named type II inhibitors, targets an inactive form of the kinase, in which the protein adopts the DFG-out conformation [7,8]. In this conformation, a large conformational change of the conserved DFG motif and of the activation loop creates a large hydrophobic pocket adjacent to the active site, the allosteric site, into which type II inhibitors can bind (Fig. 1). The phenylalanine side chain of the DFG motif blocks the approach of ATP, and the kinase is trapped in an inactive conformation. In addition to hydrophobic interactions within the active site and allosteric pocket and to the stacking interaction with the Phe side chain of the DFG motif, the majority of known type II inhibitors make two pairs of conserved hydrogen bonds to their respective kinase targets: one pair to an aminoacid of the hinge region, and a second pair to the side chain carboxylate of a conserved glutamate in the α-C helix and to the backbone NH of the aspartate of the DFG motif [6].

Several type I inhibitors for proteins of the TAM subfamily have been reported in the literature (Fig. 2), although their selectivity profiles are poor since they also target kinases outside of the TAM subfamily. A complete review of available type I inhibitors is out of the scope of this paper, but we will recall some of these studies. For example, Sunitinib inhibits multiple RTKs such as platelet-derived growth factor receptors (PDGFRs), vascular endothelium growth factor receptors (VEGFRs), and Kit (also called Mast/stem cell growth factor receptor) [11]. It also inhibits proteins from the TAM subfamily such as Axl, Mer and Tyro3 [12]. Despite this lack of specificity, Sunitinib is approved by FDA for renal cell carcinoma as well as gastrointestinal stromal tumor [13]. Similarly, Vandetanib is approved for the treatment of unresectable locally advanced or metastatic medullary thyroid cancer, although this drug displays inhibitory activity on many kinases among the 109 kinases tested, including mutants [14], and is also able to bind Axl, Mer and Tyro3 with moderate affinities [14]. Another example of type I inhibitor for a protein of the TAM subfamily is compound C-52, a weak inhibitor of Mer. It has been co-crystallized with Mer (PDB ID: 3BPR), giving a good starting point for the rational design of more potent type I inhibitors for this protein [15]. However, its selectivity profile within the TAM subfamily is unknown. A type I specific inhibitor of Axl has been reported in the literature, as a diaminopyrimidine derivative (Fig. 2) and has been patented recently [16]. Finally, using *Mus musculus* Sky (UniProt ID: P55144), the murine orthologue of human Tyro3, a novel selective type I inhibitor for Tyro3 (Fig. 2) and with lower affinity for Mer and Axl has been recently reported. The X-ray structure of the kinase domain of *Mm*Sky in complex with this spiroindoline-based molecule is available at the PDB under the 3QUP entry [17].

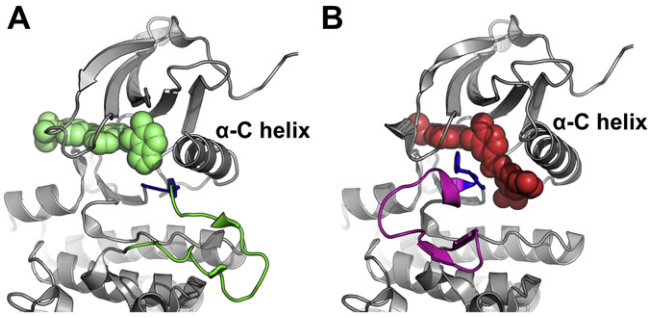


Fig. 1. Ribbon representations of the "active DFG-in form" (Panel A) and "in-active DFG-out form" (Panel B) of a kinase domain. The activation loops are shown in green in DFG-in conformation (A), and pink in DFG-out conformation (B). The phenylalanine side chain of DFG motif is highlighted in blue, underlining its reorientation between the two states. An ATP-competitive Src-Abl type I inhibitor (AP24283) is presented in green spheres (A) while a Src-Abl type II inhibitor (AP24163) overhanging the allosteric binging site is pictured with red spheres (B). (Figures generated using PyMol from X-ray structures of Abl1; PDB ID: 3KF4 and 3KFA) [9,10]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

As opposite to type I inhibitors, to our knowledge, only one type II inhibitor has been reported to be active against Tyro3, Mer and Axl: the BMS-777607 molecule, described by Schroeder [18] (Fig. 2). This molecule also inhibits Met (UniProt ID: P08581), a protein whose sequence does not contain the TAM signature. However, Met presents strong sequence similarity with proteins of the TAM subfamily (in the range of 40% sequence identity), and Ron. This similarity between Met and proteins of the TAM subfamily can be illustrated by the sequence alignment and by the phylogenetic tree respectively shown in Figs. 1 and 2 of the supplementary materials. X-ray crystal structure of the BMS-777607/Met complex (PDB ID: 3F82) demonstrates that this compound is a type II inhibitor. BMS-777607 is presently in Phase I/II of clinical studies [2,18].

At this point, it should be emphasized that given the high sequence identity between the kinase domains of Axl, Mer, Tyro3, the design of a specific inhibitor of either member of the TAM (or even Met) subfamilies remains a challenge, although such inhibitors would be of great interest to better understand the role of these kinases. Optimization of type II inhibitors targeting mainly the TAM family or Met is a strategy that could be adopted to design inhibitors with even higher specificity.

In a context where only a few type II inhibitor are available today for the Met family [19], the purpose of this study was to synthesize and evaluate new type II inhibitors for this family of RTKs. These inhibitors could then be optimized in a later stage of the project.

We will first present the strategy that was used to conceive these inhibitors and detail their chemical synthesis. Then, the results of inhibitory assays and biological tests will be presented. Finally, structural analysis based on available X-Ray structures, homology modelling and docking experiments will be used to discuss the binding modes and selectivity of the tested molecules.

2. Results and discussion

2.1. Chemistry

Our goal was to design a type II inhibitor of the TAM subfamily and therefore to build a structure able to interact both with the allosteric pocket, on one side, and, with the hinge region of the kinase on the other side. As a starting point of our project, we focused on the Met inhibitor BMS-777607 which is also a potent inhibitor of the TAM subfamily but more importantly is a type II

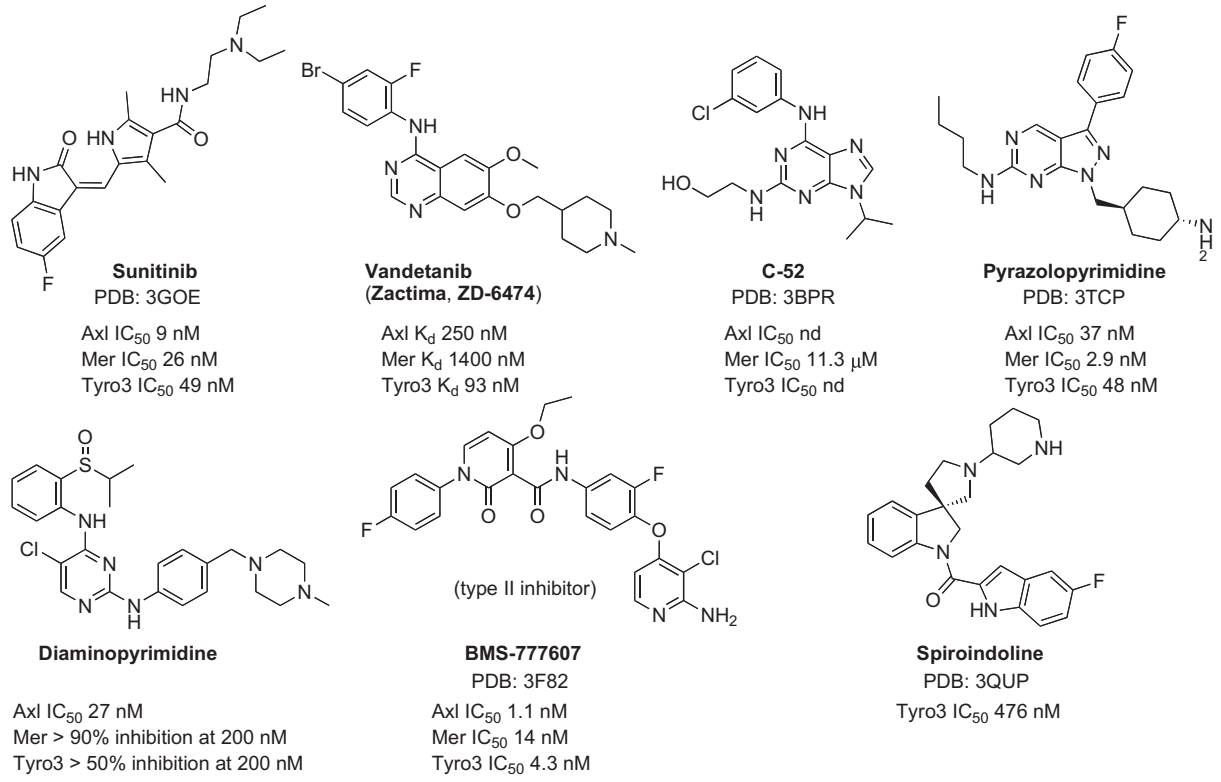


Fig. 2. Reported type I inhibitors of the TAM subfamily, with the exception of BMS-777607 (type II inhibitor).

inhibitor. Indeed, the X-ray crystal structure of the complex between BMS-777607 and Met showed that the 2-aminopyridine core (Fig. 2) displays two key hydrogen bonds with the hinge backbone of the kinase, and the pyridone lateral chain interacts with the allosteric pocket created by the rearrangement of the protein in a DFG-out conformation [18]. Therefore, we synthesized analogues of BMS-777607 by i) replacing the 2-aminopyridine moiety by a purine ring that could also allow hydrogen bonds with the hinge region ii) by keeping the lateral chain of BMS-777607 or replacing it by another chain potentially interacting with the allosteric site. The conservation of this lateral chain is of utmost importance to target the inactive conformation of the TAM subfamily. Introduction of structural diversity using various types of connections to link the side chain to the purine scaffold was expected to slightly modify the interactions formed between the proteins and the inhibitors, and therefore to change their selectivity profiles.

Thus, three series of inhibitors have been synthesized, which differ by the connection at the position 6, bearing an ether linkage (**6**), an amino bonding (**13**/**14**) or a direct carbon–carbon bridge (**18**), linked to a chain that mimics the chain found in BMS-777607 (Fig. 3). A fluorine atom was maintained in the *ortho* position of the first phenyl group, both in the 6-oxo series **6** and in the 6-amino series **14** since it was found important in several close tyrosine kinase inhibitors [18,20]. A fourth series (**26**–**29**) is characterized by an aniline at position 6 and an additional phenylbenzamide at position 8. The synthesis of the targeted molecules (series **6**, **13**/**14**, **18**, **26-29**) was achieved by coupling various aryl carboxylic acids (molecules **4a-i**-CO_2H) with aminophenylpurine intermediates **3**, **9**, **10**, **16** and **21** which were all synthesized from precursor **1** (6-chloro-9-THP-purine), as described in Scheme 2 for intermediates **3**, **9**, **10**, in Scheme 3 for intermediate **16**, and in Scheme 4 for intermediates **21a**–**d**.

The aryl carboxylic acid derivatives **4a**–**e** were commercially available, while the **4f**–**i** derivatives were synthesized separately following the experimental procedure described in the literature [21] (Scheme 1). The carboxylic acids derivatives of **4f**–**i** were synthesized from 2-hydroxynicotinic acid methyl ester, in the

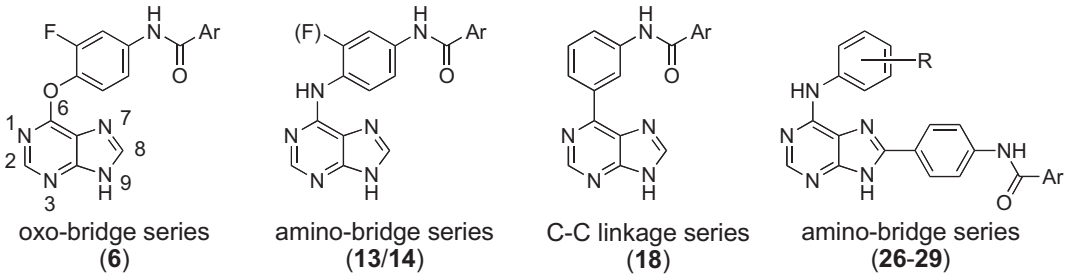
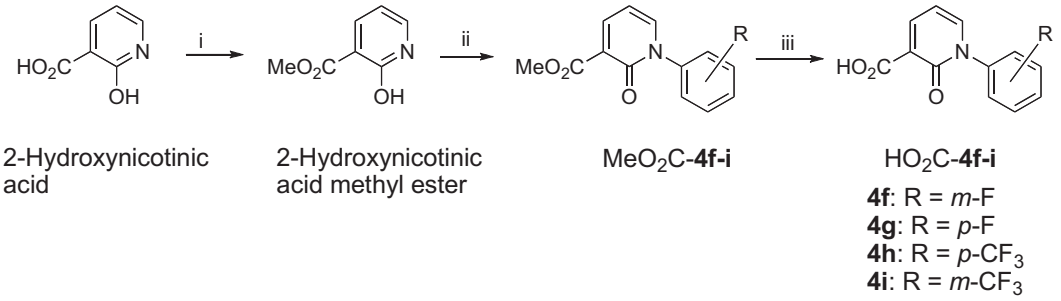


Fig. 3. Proposed library for novel inhibitors of the TAM subfamily.



Scheme 1. Synthesis of **4f**–**i** carboxylic acid derivatives. (i) 1) $SOCl_2$, CH_2Cl_2, 75 °C, 4 h; 2) MeOH, r.t., 12 h (92%); (ii) ArI, CuBr, 2-ethylester cyclohexanone, Cs_2CO_3, DMSO, 60 °C, 18 h (51–57%); (iii) LiOH (1N aq sol), THF, r.t., 3 h (58–74%).

presence of the corresponding aryl iodide, CuBr, 2-ethylester-cyclohexanone and Cs_2CO_3 in DMSO, according to the literature [21]. Saponification of the resulting methylesters was achieved in THF and an aqueous 1N solution of LiOH, leading to **4f**–**i**-carboxylic acids in short reaction time and good yields (58–74%).

This strategy was adopted because the method used by Schroeder for the synthesis of BMS-777607 (Fig. 2) [18] for the Ullmann-type arylation of the 2-pyridone with the carboxylic acid in the 3 position (2-hydroxy-nicotinic acid) was assayed, but in our hands, poor yields were obtained for the desired product **4f**–**i**-CO_2H (for these free carboxylic acids and the corresponding methyl esters).

In parallel, starting from the known precursor 6-chloro-9-THP-purine **1** [22,23], the first step differed in the four series (**6**, **13/14**, **18** or **26-29**), depending on the kind of connection to be reached. The tetrahydropyrane group was chosen as protecting group for the purine N9 (**1**) due to its liability, envisaging the use of mild conditions for the deprotection of the final products. Then, for series **6**, nucleophilic substitution of the 6 chlorine atom of **1** with 2-fluoro-4-nitrophenol was followed by nitro reduction by iron in aqueous ethanol (Scheme 2), leading to the purin-6-yloxyaniline derivative **3**. For series **13/14**, Buchwald–Hartwig coupling [24] of the 6-chloro-9-THP-purine **1** with (2-fluoro)-4-nitroaniline was followed by nitro reduction leading to the 6-aminophenylaminopurine derivatives **9** and **10** (Scheme 2). For series **18**, the 6-nitrophenylpurine derivative **15** was obtained by a Suzuki coupling [25], between **1** and 3-nitrophenyl boronic acid with $Pd(PPh_3)_4$, and K_2CO_3 at 100 °C (Scheme 3). It was then reduced to **16** in the usual way.

Series **26**–**29** was obtained by aromatic nucleophilic substitution of the 6 chlorine atom of **1** with various aniline derivatives and followed by iodination in position 8 of the purine moiety, leading to intermediates **20a**–**d** which gave compounds **21a**–**d** after a Suzuki coupling with 3-aminophenylboronic acid (Scheme 4).

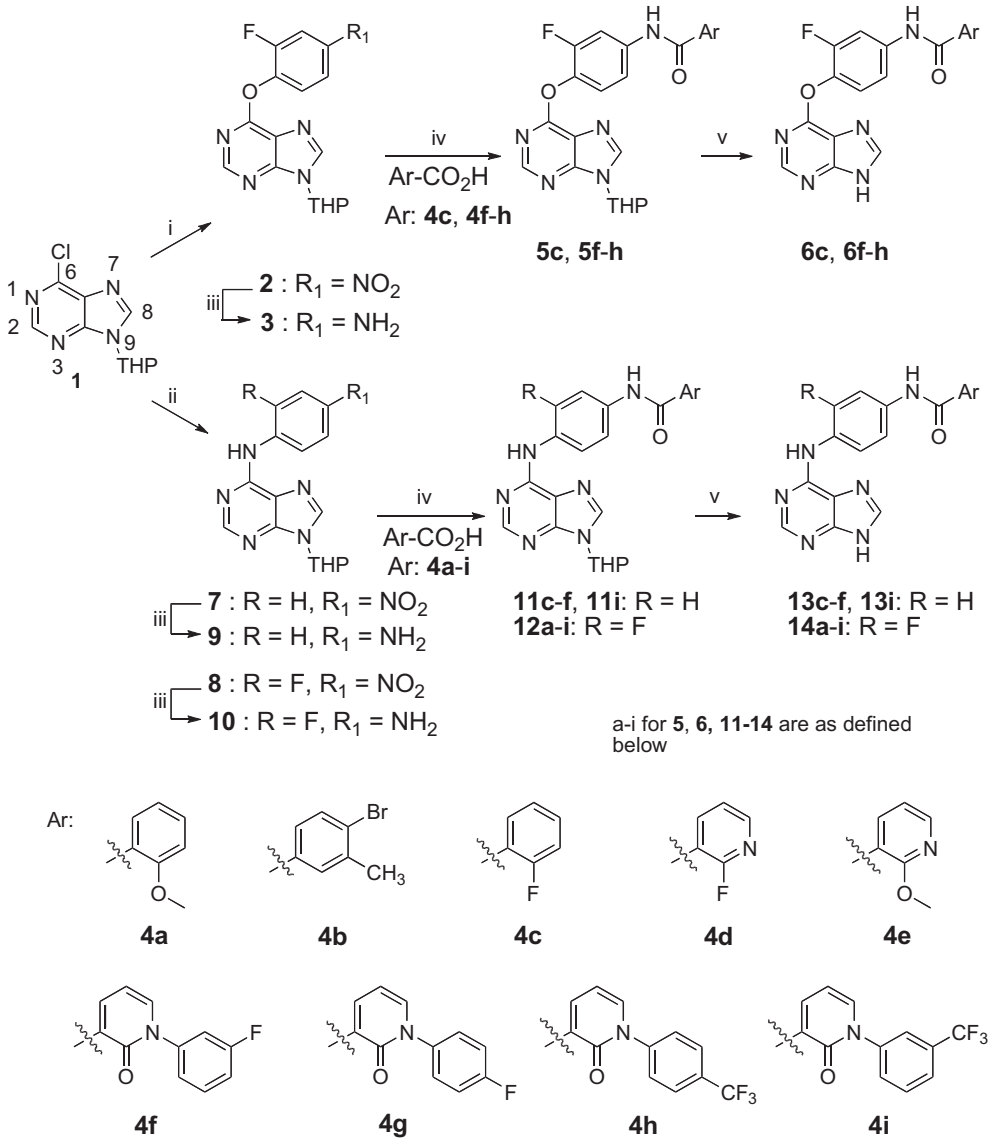
The final two steps of these convergent syntheses were the connection (amide formation) between the various carbocyclic acids **4a**–**i**-CO_2H and the different aminophenyl purine counterparts **3**, **9**, **10**, **16** and **21** followed by the purine deprotection. For the coupling between the different amino purine derivatives (**3**, **9**, **10**, **16** and **21**) with aryl carboxylic acids (both commercially available **4a**–**e**-COOH or compounds **4f**–**i**-COOH), the procedure of Okram was used, employing HATU as the coupling reagent in the presence of DIPEA as base, affording the corresponding products **5**, **11**, **12**, **17** and **22**–**25** in high to excellent yield (71–99%) [8]. Finally these intermediates were efficiently deprotected by treatment with trifluoroacetic acid at room temperature, leading to the desired pure final products (**6**, **13**, **14**, **18** and **26**–**29**) in good yields after chromatography (65–98% yields; >95% purity by HPLC) [26].

2.2. Tyrosine kinase inhibitory assays

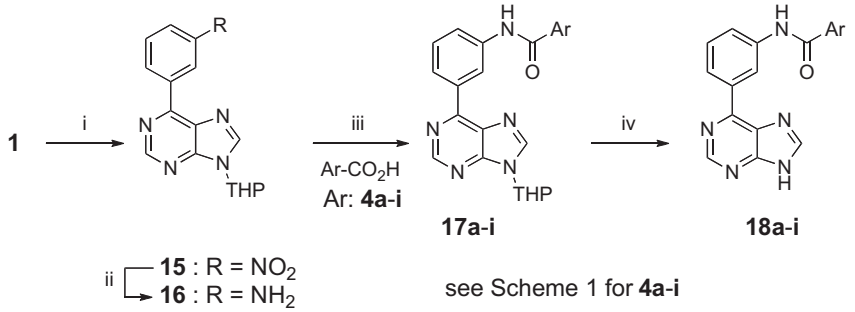
The entire library of the synthesized molecules was evaluated against five tyrosine kinases by the Kinomescan company (see Experimental section) [27], namely Tyro3, Axl, Mer, Met, and Abl1. Abl1 was included in these tests because it belongs to the RTK family, but is not a member of the TAM subfamily. Moreover, type II inhibitors have been well characterized, including Gleevec®. Thus, analysis of the inhibition profiles of the tested molecules against these five kinases should allow to discuss their selectivities against the TAM subfamily. The phosphorylated and the unphosphorylated forms of Abl1 were tested. Indeed, phosphorylated-Abl1 is characterized by a DFG-in conformation, while unphosphorylated-Abl1 being in a DFG-out conformation. In other words, inhibitor affinity for unphosphorylated-Abl1 and not for phosphorylated-Abl1 is an indirect indication that studied inhibitors might be type II inhibitors. The most active compounds at a concentration of 1 and 75 μM are presented on Table 1.

The complete results of all tested molecules are given in Table 1 of the supplementary material. Some potent inhibitors of Axl, Mer, Met and Tyro3 were identified (Tables 1 and 2). Furthermore, as shown in Table 1, molecules **6g** and **6f** that present some inhibition for the TAM subfamily also inhibit the activity of unphosphorylated-Abl1. This indicates that we might indeed be in the presence of type II inhibitors. In addition, some of the compounds (**6h**, and **14h** for example) are able to strongly inhibit proteins from the TAM subfamily, their inhibition activity being much lower against Abl1. This shows that the designed compounds using our strategy may achieve specificity for the TAM subfamily. It is interesting to notice that the presence of a trifluoromethyl group at the *para* position of the terminal phenyl in **6h** and **14h**, strongly decreased the inhibition of unphosphorylated Abl1 while the same group in *meta* position (**14i**) gave a good inhibition of unphosphorylated Abl1, as in **6g**, **6f**, **14f**, **14g** or **13f** where a small fluorine atom is present in the *meta* or *para* positions.

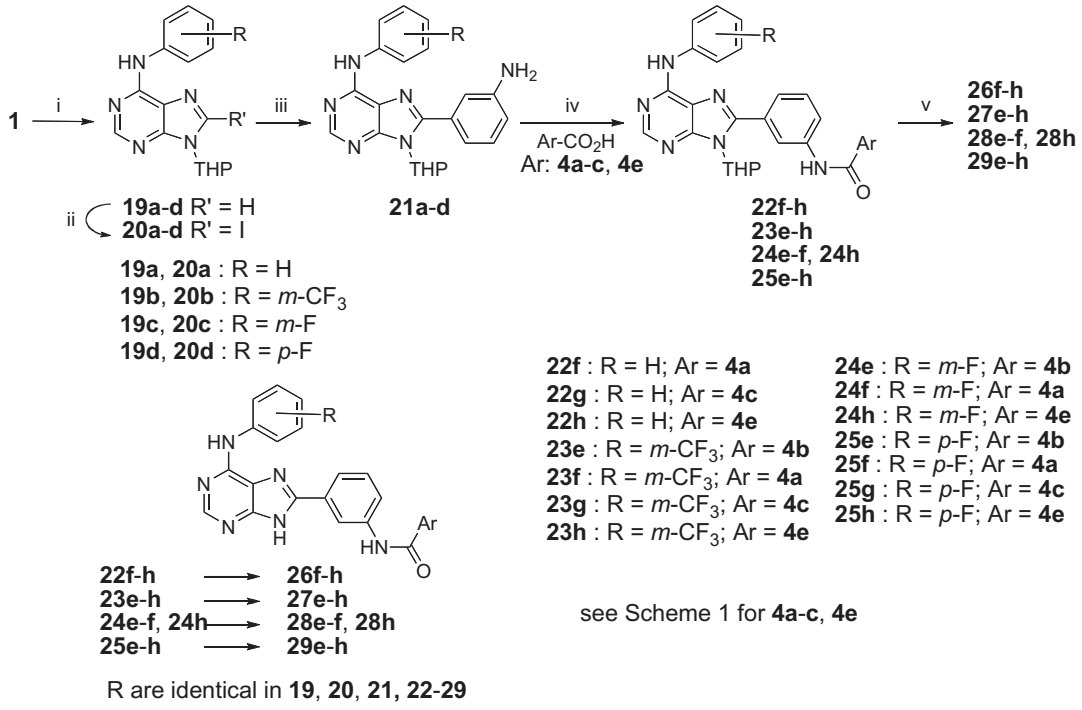
Interestingly, analysis of the inhibition activities within proteins of the TAM subfamily shows that the inhibitors do not have the same inhibition profiles, which illustrates that slight variations on the molecular scaffold could achieve further selectivity within the TAM subfamily. For example, only compounds carrying an oxygenated linkage at position 6 of the purine showed Tyro3 inhibition (**6g**, **6f**, **6h**). Compounds with no bridge at position 6 (C–C bond, in **18a**–**i**) were inactive on Tyro3, Axl and Mer at 1 μM, and marginally active at 75 μM (see supplementary material, Table 1). Replacement of the *para* fluoro atom on the terminal phenyl (**6g**) group by a *meta* fluorine (**6f**) or a *para*-trifluoromethyl group (**6h**) decreased the Tyro3 inhibition, but had almost no effect on Axl, Mer and Met inhibition.



Scheme 2. Synthesis of 6-phenoxy **6** and 6 aminophenyl **13**, **14** derivatives. Reaction conditions : i) 2-fluoro-4-nitrophenol, K_2CO_3, DMF, 100 °C, 12 h (58%); ii) $Pd(OAc)_2$, *X-phos*, 4-nitroaniline or 2-fluoro-4-nitroaniline, *t*-BuOH/H_2O, 110 °C, 15 h (91 or 94%); iii) iron powder, NH_4Cl, EtOH/H_2O, 90 °C, (65–89%) 2 h; iv) carboxylic acid, HATU, CH_2Cl_2, DIPEA, r.t., 3 h (71–99%); v) TFA, CH_3CN/H_2O, rt, 4 h (65–98%).



Scheme 3. Synthesis of the 6-phenylpurine derivatives **18**. Reaction conditions : i) $Pd(PPh_3)_4$, K_2CO_3, 3-nitrophenyl boronic acid, dioxane/H_2O, 100 °C, 15 h (73%); ii) iron powder, NH_4Cl, EtOH/H_2O, 90 °C, 2 h, 65–89%); iii) carboxylic acid, HATU, CH_2Cl_2, DIPEA, 0 °C to r.t., 3 h (71–99%); iv) TFA, CH_3CN/H_2O, rt, 4h (65–98%).



Scheme 4. Synthesis of derivatives **26**–**29**. i) $Pd(OAc)_2$, *X-phos*, *t*-BuOH/H_2O are introduced into a preheated bath at 110 °C for 1 min. Then, **1**, aniline compound, K_2CO_3; 110 °C for 15 h 84–97%); ii) LDA, THF, −78 °C, 1 h, then I_2, −78 °C, 1 h (48–84%); iii) $Pd(PPh_3)_4$, K_2CO_3, dioxane/water 4/1, 85 °C, 5 min, then 3-aminophenylboronic acid, 100 °C, 3 h (71–84%). iv) HATU, DIPEA, CH_2Cl_2, 0 °C to r.t., 12 h (29–92%); v) TFA, CH_3CN/H_2O, r.t., 3 h (39–99%).

Another example of selectivity modulation is provided by comparing **6c**, where the pyridone motif is lacking, and **6g** (or **6f**). The former looses activity against Tyro3, Axl or Mer, whereas inhibition of Met is preserved. The same observation can be made for compounds **14a**–**e** (see supplementary material).

K_ds were determined for the most active inhibitors against proteins of the TAM family, namely **6g**, **6f** and **6h** (Table 2). It is interesting to notice the good K_d of **6g** on Axl (39 nM), Mer (42 nM) and Met (65 nM) and the somewhat increased K_d on Tyro3 (200 nM).

Furthermore, **6g** and **6f** bind preferentially the inactive non-phosphorylated form of Abl1, as compared to the phosphorylated active enzyme (K_d of 6600 and 320 vs 40,000 and 3300, respectively on the phosphorylated enzyme). Again, this suggests that these inhibitors recognize preferentially the inactive form of the enzyme, and therefore could be type II inhibitors, although this could only be demonstrated by solving the X-ray structure of the complex. However, analysis of the known DFG-out structure of Met, together with modelling studies presented below seem to confirm that we indeed designed type II inhibitors.

A this point, one should note that K_ds shown in Table 2 were determined on the phosphorylated forms of Axl, Mer and Met, because the inactive (unphosphorylated) forms of these enzymes were not available. As shown in Table 2 in the case of Abl1, a type II inhibitor does present some affinity for the active form of the protein, although this affinity is several orders of magnitude weaker than for the inactive form. Therefore, the affinities of the **6g**, **6f** and **6h** molecules for proteins of the TAM subfamily in the inactive form (unphosphorylated DFG-out conformation) are expected to be much stronger (i.e. smaller K_ds) than those reported in Table 2.

2.3. In vitro cytotoxicity assays

The antiproliferative activities of the compounds were evaluated at 10^{-5} M against four human tumor cell lines, including KB (oral carcinoma), HT29 (colorectal carcinoma), MCF7 (breast carcinoma) and PC3 (prostate adenocarcinoma) and one non tumoral cell line, Vero cells (Kidney Vero cells). No cytotoxicity could be detected at 10^{-5} M for all compounds listed on these five cell lines. The growth inhibition of the cell lines was smaller than 10% at the concentration tested.

2.4. Molecular modelling studies

A deeper structural interpretation of the above biological results is required for future optimization of the designed inhibitors. Therefore, we undertook a series of molecular modelling studies. Since no DFG-out structure is available in the TAM subfamily, we first built a model of structure for one of the three human members of the TAM subfamily, namely Tyro3. We modelled a DFG-out structure for Tyro3 because the context of this study is to conceive type II inhibitors (see Experimental section for a description of the modelling protocol and Fig. 1 in supplementary materials for sequence alignment). The quality of the topology of this model, including bond lengths and bond angles, was carefully checked, as detailed in the Experimental section.

Among all the proteins for which a DFG-out structure in complex with a type II inhibitor is available, Met is the closest one to proteins of the TAM subfamily. This structure (PDB ID: 3F82) corresponds to a Met DFG-out structure in complex with the BMS-777607 inhibitor [18]. In addition, the inhibition tests presented in the previous section included the Met protein. Therefore, we first compared the structure of the 3F82 complex to that of **6g** docked in

Table 1
Active inhibitors of the TAM subfamily. Percent of the enzyme captured from the solid support in the presence of 1 μM or 75 μM inhibitor.

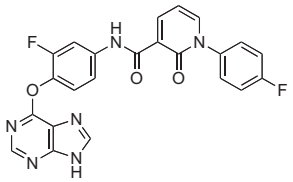
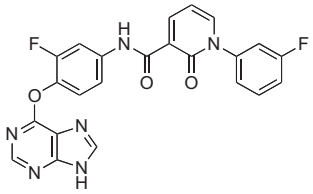
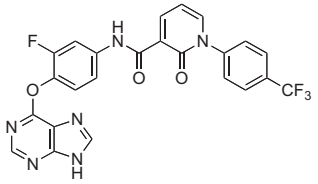
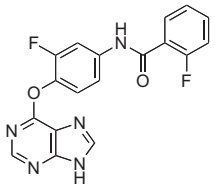
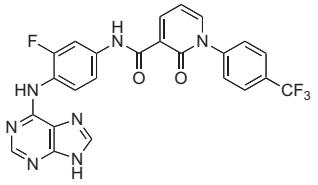
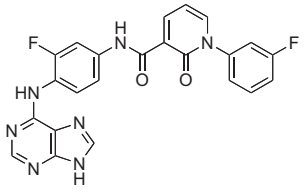
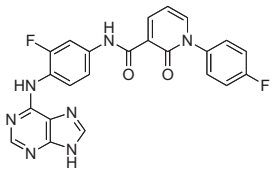
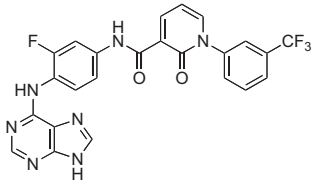
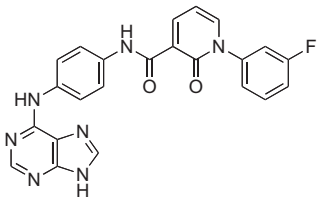
Cmpd	Structure	% Binding affinity at 1 or 75 μM											
		Abl1 unphos.		Abl1 phos.		Axl		Mer		Met		Tyro3	
		1	75	1	75	1	75	1	75	1	75	1	75
6g		40	80	0	35	99	99	99	99	96	99	83	100
6f		75	73	4	18	93	99	89	97	51	98	20	94
6h		0	35	0	0	88	99	84	99	92	99	0	92
6c		30	89	10	37	0	4	0	40	34	98	0	18
14h		18	22	0	0	45	88	33	66	53	90	0	10
14f		38	41	4	0	81	90	75	81	76	86	0	4
14g		84	78	18	8	45	57	39	55	6	22	0	0
14i		84	92	12	24	34	61	36	59	22	33	0	0

Table 1 (*continued*)

Cmpd	Structure	% Binding affinity at 1 or 75 μM											
		Abl1 unphos.		Abl1 phos.		Axl		Mer		Met		Tyro3	
		1	75	1	75	1	75	1	75	1	75	1	75
13f		88	86	26	22	4	32	28	24	0	6	0	0
	Sunitinib	100	100	73	99	100	100	99	100	5	96	9	99
	Vandetanib	98	100	99	100	82	99	59	99	0	99	90	100

an empty DFG-out structure of Met. The **6g** molecule was chosen because, according to the biological results presented in the previous section, it is one of the most active tested compounds against the Met subfamily.

In the 3F82 structure, within the active site of Met, BMS-777607 forms a double hydrogen bond with the backbone of M1160 from the hinge region, and hydrophobic interactions with M1211, both interactions being also found in type I inhibitors. In addition, it also binds to Met via several expected interactions for type II inhibitors: a hydrogen bond with D1222 (of the DFG motif), hydrophobic stacking with F1223 (of the DFG motif), and hydrophobic interactions in the allosteric site with residues M1131, A1221, and F1134. A hydrogen bond is also observed between the inhibitor and the side chain of K1110 (Fig. 4A–C).

After removal of the BMS-777607 inhibitor from 3F82, we docked the **6g** molecule in the resulting empty DFG-out structure of Met (see the Experimental section for details about the docking protocol). Overall, the docked inhibitor occupies the same position in the pocket than BMS-777607 does in 3F82. In addition, most of the interactions observed between BMS-777607 and Met in 3F82 are conserved in the complex formed by **6g** docked in Met. The **6g** molecule binds to active site residues via the double hydrogen bond with the M1160 backbone, and hydrophobic interactions with M1211. It also binds via typical type II interactions: the hydrogen bond with D1222, the hydrophobic stacking with F1223, the hydrophobic interplay with the allosteric pocket via M1131, A1221 and F1134 (see Fig. 4D–F). Therefore, the docked complex is compatible with a type II binding mode for the **6g** molecule.

Using the same docking protocol, we docked BMS-777607 (which inhibits Tyro3) in the DFG-out model of Tyro3. We observed that the binding mode of BMS-777607 docked in Tyro3 is similar to its binding mode in the 3F82 structure of Met (see Fig. 5A–C). In particular, the main interactions observed between the docked BMS-777607 and Tyro3 are similar to the key interactions in 3F82. In the active site, the inhibitor forms a double hydrogen bond with M606 (of the hinge region, equivalent to M1160 in Met), and a hydrophobic interaction with L524 and L603 (comparable to the interaction with M1211 in Met). In addition, the interactions typical of type II inhibitors are also conserved: a double hydrogen bond with D673 (of the DFG motif, equivalent to D1222 in Met), hydophobic stacking with F674 (of the DFG motif, equivalent to F1223 in Met), and hydrophobic interactions in the allosteric pocket with F575 and A672 (respectively equivalent to F1134, A1221 in Met). These interactions are in agreement with the strong inhibitory activity of BMS-777607 against Tyro3 (IC_{50} = 4.3 nM) [18], and they indicate that Tyro3 could be inhibited by a type II inhibitor. At this point, it should be recalled that there is a direct relation between the K_d and the IC_{50} (see experimental section, kinase assays).

We docked all the synthesized and tested molecules (see previous sections) in the DFG-out model of Tyro3, and analysed the docking scores. We observed that all molecules active against Tyro3 obtained best docking scores than any of the inactive molecules. In other words, the top ranked molecules were active against Tyro3 (Table 3). The complete docking scores for all the tested molecules are given in Table 1 of the supplementary materials.

These results validate the docking protocol used in this study and allow further analysis of the docked complexes.

In particular, the complex formed by Tyro3 and the (docked) **6g** molecule provides an example of how the active molecules can bind to their target (Fig. 5D–F). In the active site, the purine ring of **6g** binds to M606 via a double hydrogen bond, and a hydrophobic interaction is also formed with L524. In addition, typical interactions for type II inhibitors are also observed in this docked complex: hydrophobic stacking with F674, a double hydrogen bond with D672, and a hydrophobic interaction in the allosteric pocket with F575 and M571. Overall, the interactions observed between **6g** and Tyro3 are very similar to those observed between BMS-777607 and Tyro3, and between BMS-777607 or **6g** and Met (Fig. 4). Therefore, the active molecules synthesized in this study are indeed expected to be type II inhibitors.

Table 2
K_d values of the most potent inhibitors, **6g**, **6f** and **6h**. nd, not determined.

Kinase target/inhibitor	**6g**	**6f**	**6h**
	K_d (nM)	K_d (nM)	K_d (nM)
Abl1-nonphosphorylated	6600	320	nd
Abl1-phosphorylated	40,000	3300	nd
Axl	39	210	170
Mer	42	190	260
Met	65	440	150
Tyro3	200	900	2900

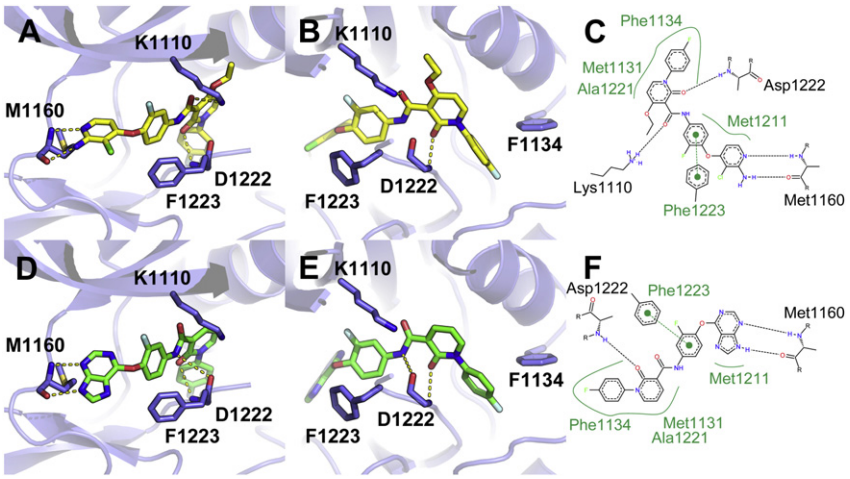


Fig. 4. Panels A and B: binding site of Met (in blue ribbon and in sticks for the side chains) with BMS-777607 (yellow sticks) in the 3F82 crystal structure, viewed from the active site (Panel A) and from the allosteric site (Panel B). Panel C: 2D representation showing polar (black labelled residues) and hydrophobic (green labelled residues) contacts in 3F82. Panels D and E: binding site of Met (in blue ribbon and in sticks for side chains) with the docked compound **6g** (green sticks) viewed from the active site (Panel D) and from the allosteric site (Panel E). Panel F: 2D representation of the interactions in the Met-**6g** docked complex. Figures generated using PyMol, 2D schemes generated and modified from PoseView analysis) [28]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

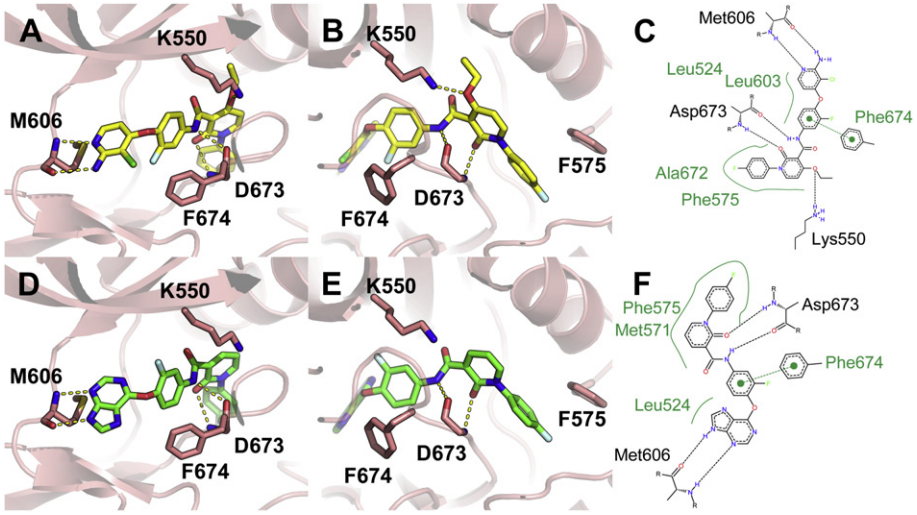


Fig. 5. Panels A and B: schematic view of BMS-777607 (yellow sticks) docked in the Tyro3 model (in pink ribbon and in sticks for the side chains), viewed from the active site (Panel A) and from the allosteric site (Panel B). Panel C: 2D representation of the polar (black labels) and hydrophobic (green labels) contacts in the docked Tyro3- BMS-777607 complex. Panels D and E: compound **6g** (green sticks) docked in Tyro3 viewed from the active site (panel C) and from the allosteric site (panel D). Panel F: 2D representation of polar (black labels) and hydrophobic (green labels) contacts in the docked Tyro3-**6g** complex. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Top scored molecules according to the ChemGauss score used in this study [29]. The best ranked molecules correspond to the most active compounds according to the experimental tests shown in Table 1.

Name	Docking score
6g	−133.6
BMS-777607	−130.1
6f	−129.4
6h	−121.0
14h	−115.7
14g	−114.5
14f	−111.4
14i	−108.3
13i	−106.6
14a	−104.5

It is also interesting to analyse the docking results of inactive molecules, since this could also guide the optimization of the most active molecules (namely, **6g**, **6f** and **6h**). The biological tests showed that **6g** strongly inhibits Tyro3, while **14g** does not, although these two molecules present only a single and subtle structural difference: in **14g**, an amino group is bound to position 6 of the purine, while it is replaced by an oxygen in **6g**. In the docked complex of **14g**, the conserved hydrogen bond with M606 in the hinge region is lost because of a rotation of the purine moiety (Fig. 6, Panels A, B and C). This might explain why compound **14g** is inactive against Tyro3. It also suggests that docking could help to guide the design of type II inhibitors able to present some selectivity within the TAM subfamily.

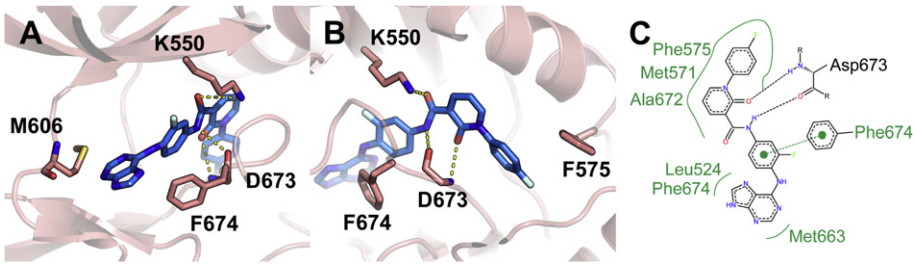


Fig. 6. Panels A and B: schematic view of the **14g** compound (blue sticks) docked in the Tyro3 model (in pink ribbon and in sticks for the side chains), viewed from the active site (Panel A) and from the allosteric site (Panel B). Panel C: 2D representation of polar (in black) and hydrophobic (in green) contacts in the docked Tyro3-**14g** complex. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Conclusions

We have synthesized a new series of purine derivatives, using a convergent synthetic route, which allowed us to introduce efficiently structural diversity at several different points of the molecule, and to access rapidly to a library of potential TAM inhibitors. The best inhibitor **6g**, exhibits K_ds of 39, 42, 65 and 200 nM against Axl, Mer, Met and Tyro3 respectively. Much lower activity was observed against unphosphorylated Abl1 (K_d = 6600 nM) and phosphorylated Abl1 (K_d = 40,000 nM).

However, as mentioned in the result section, due to the activated form of Axl, Mer, Tyro3 and Met used in the kinase assays, the activity of inhibitors like **6f**, **6g** and **6h** may be underestimated, since it is now well established that type II inhibitors bind preferentially the DFG-out conformation of the enzyme [6,30] as Imatinib (Gleevec) which exhibits a clear preference (>30 fold) for the non-phosphorylated form of Abl1 [30]. The lower K_d of **6g** and **6f** for the inactive unphosphorylated form (DFG-out) of Abl1 than for its active phosphorylated form (DFG-in), suggests that **6g** and **6f** act as type II inhibitors of Abl1. Furthermore, compound **6g** is also active against Met and Tyro3, and the docking studies into the known DFG-out structure of Met, or the modelled DFG-out structure of Tyro3 are also in agreement with a type II binding mode for this molecule. This inhibitor is most active on Axl, Mer and Met, and slightly less potent on Tyro3. In addition, the absence of cytotoxicity against several tumor cell lines in culture makes this inhibitor a good candidate for the growth inhibition of tumor cells that would overexpress a gene belonging to the TAM subfamily.

Numerous selective protein kinase inhibitors are known to date, but the design of specific inhibitors of each kinase still remains a real challenge. In fact receptor tyrosine kinase inhibitors are often less specific for the intended target, which may be of benefit if the additional targets contribute to oncogenesis and metastasis or detrimental if additional adverse effects are elicited. In this context, specific inhibitors remain highly desirable and useful, at least in pre-clinical studies, in order to help delineate the individual contributions of the different kinases to tumorigenesis and metastasis [4]. A way to improve the selectivity of kinase inhibitors, during the last few years, has been to design type II inhibitors, which target an inactive form of the kinase and are therefore less ATP competitive [31]. These inhibitors specifically exploit the DFG-out conformation of the kinase, giving access to a deep hydrophobic pocket which is less conserved than the ATP binding site and thus open the possibility of creating more specific interactions with each kinase [32,33].

In this context, we are presently developing new structural modifications, necessary to improve the selectivity profile of the most promising molecules reported in this study. They will be based on the first structure–actvity relationships as well as molecular modelling studies reported therein.

4. Experimental section

4.1. Chemistry

NMR spectra were recorded at 300 MHz (^{1}H NMR) or 75.3 MHz (^{13}C NMR) with $CDCl_3$ or DMSO-d_6 as solvents and tetramethylsilane as internal standard on a Bruker AC300 spectrometer. Chemical shifts are expressed in ppm (δ) downfield from TMS. J values are expressed in Hertz (Hz). The following abbreviations are used for the multiplicities: s, singlet; d, doublet; t, triplet; m, multiplet; b, broad. MS spectra were recorded on a Waters ZQ2000 mass spectrometer with direct injection. HRMS spectra were performed by the mass spectrometry service Imagif at the ICSN (Gif-sur-Yvette). Melting points were recorded into a Stuart SMP30 melting point apparatus unless otherwise stated and are uncorrected. A Kofler Heizbank system was used in specified cases. Carboxylic acids **4a** (Janssen), **4b** (Aldrich), **4c**, **4e** (Acros) and **4d** (Alfa Aesar) are commercially available. Carboxylic acids **4f**–**i** were synthesized according to reported procedures [21]. Numbering of the purine heterocycle is indicated on Scheme 2.

4.2. HPLC chromatographies were performed on a Waters Alliance apparatus equipped with a diode array and a XterraMS column

4.2.1. HPLC method I

A linear gradient of acetonitrile versus water was used ranging from 98% of acetonitrile to 55% over 5 min then to 100% over 5 min at a flow rate of 1 mL/min.

4.2.2. HPLC method II

A linear gradient of acetonitrile versus water containing 0.1% of TFA was used ranging from 98% of acetonitrile to 55% over 2 min then to 5% over 4 min and finally, to 100% over 4 min at the flow 1.4 mL/min.

4.3. 6-(2-Fluoro-4-nitrophenoxy)-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (**2**)

In a sealed tube purged with argon, 6-chloro-9-THP-purine **1** (100 mg, 0.42 mmol), 2-fluoro-4-nitrophenol (79 mg, 0.50 mmol) and K_2CO_3 (174 mg, 1.26 mmol) were suspended into DMF (5 mL). The reaction mixture was heated at 100 °C during 12 h. The reaction mixture was then allowed to reach room temperature, diluted in EtOAc and washed with a small fraction of brine. The organic phase was separated and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo*, and the oily residue purified by column chromatography over silica gel, using EtOH/CH_2Cl_2 mixtures (from 1 to 5%) as eluent. The product was obtained in 58% yield. M.p. 142–144 °C. R_f (2% EtOH/CH_2Cl_2): 0.46. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.67–1.85 (m, 3H), 2.05–2.21 (m, 3H), 3.81 (dt, J = 12.3 Hz,

1H), 4.21 (dd, J = 12.3 Hz, 1H), 5.82 (dd, J = 9.3 Hz, 1H), 7.54 (t, J = 9 Hz, 1H), 8.12–8.19 (m, 2H), 8.31 (s, 1H), 8.49 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.7, 24.8, 31.8, 68.9, 82.5, 113.2, 120.4, 121.5, 124.5, 141.9, 145.2, 151.7, 152.3, 152.9, 155.7, 158.2. MS (EI) ES$^+$: 360.2 ([M$^+$+H], 20).

4.4. General method A for the alkylation of 2-hydroxynicotinic acid methyl ester (MeO_2C-**4f**-**i**) [21]

2-Hydroxynicotinic acid methyl ester (CAS number 10128-91-3) (100–200 mg scale), the corresponding aryl iodide (0.95 equiv), CuBr (10 mol%), 2-ethylester cyclohexanone (20 mol%) and Cs_2CO_3 (2.2 equiv) were suspended in DMSO (anhydrous, 2–3 mL) in a sealed tube. The reaction was complete after about 18 h heating at 60 °C. The mixture was diluted in EtOAc and washed with brine. The aqueous phase was re-extracted once with EtOAc. The combined organic phases were dried over anhydrous $MgSO_4$, and the solids filtered off. The solvent was removed *in vacuo* and the resulting crude material purified by column chromatography over silica gel, EtOH/CH_2Cl_2. The products were isolated in good yields (53–58%).

4.4.1. Methyl 1-(3-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylate (MeO_2C-**4f**)

Following the general method A, the desired product was isolated in 55% yield. R_f (7% EtOH/CH_2Cl_2): 0.57. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.91 (s, 3H), 6.35 (dd, J = 9.6 Hz, 1H), 7.17 (dd, J = 9.3 Hz, 3H), 7.43–7.48 (m, 1H), 7.57 (dd, J = 6.3 Hz, 1H), 8.25 (dd, J = 6.3 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 52.4, 105.1, 114.6, 116.1, 122.4, 130.6, 141.4, 142.1, 145.4, 159.0, 160.9, 164.2, 165.5.

4.4.2. Methyl 1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylate (MeO_2C-**4g**)

Following the general method A, the desired product was isolated in 58% yield. R_f (7% EtOH/CH_2Cl_2): 0.58. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.90 (s, 3H), 6.34 (t, J = 6 Hz, 1H), 7.17 (t, J = 9 Hz, 2H), 7.33–7.38 (m, 2H), 7.57 (dd, J = 6.3 Hz, 1H), 8.25 (dd, J = 9.3 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 52.6, 105.5, 116.2, 116.5, 122.0, 128.5, 128.6, 136.1, 143.3, 145.0, 160.8, 164.1.

4.4.3. Methyl 2-oxo-1-(4-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxylate (MeO_2C-**4h**)

Following the general method A, the desired product was isolated in 54% yield. R_f (7% EtOH/CH_2Cl_2): 0.62 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.91 (s, 3H), 6.35 (dd, J = 9.6 Hz, 1H), 7.15–7.19 (m, 3H), 7.43–7.51 (m, 1H), 7.57 (dd, J = 6.3 Hz, 1H), 8.25 (dd, J = 6.3 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 52.5, 105.3, 122.2, 125.4, 126.6, 127.3, 131.3, 141.9, 143.3, 145.7, 158.9, 165.5.

4.4.4. Methyl 2-oxo-1-(3-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxylate (MeO_2C-**4i**)

Following the general method A, the desired product was isolated in 53% yield. R_f (7% EtOH/CH_2Cl_2): 0.60 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.91 (s, 3H), 6.35 (dd, J = 9.6 Hz, 1H), 7.15–7.19 (m, 3H), 7.43–7.51 (m, 1H), 7.57 (dd, J = 6.3 Hz, 1H), 8.25 (dd, J = 6.3 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 52.5, 105.3, 122.0, 123.9, 125.8, 130.0, 130.2, 131.9, 140.7, 142.2, 145.8, 165.5.

4.5. General method B for the saponification of methyl esters MeO_2C-**4f**–**i**

The methyl 2-oxo-1-aryl-1,2-dihydropyridine-3-carboxylates (MeO_2C-**4f**–**i**) were deprotected to the corresponding carboxylic acids. The methyl ester compounds (500 mg scale) were suspended in THF (15 mL) and an aqueous 1N solution of LiOH (1.5 equiv) was added slowly. The reaction mixture was becoming rapidly darker; it was stirred at room temperature for 3 h. After that time, the conversion showed to be complete by TLC. The reaction mixture was acidified by addition of 1N HCl, till pH 2–3. The crude product was diluted in EtOAc and washed with brine. The aqueous phases were re-extracted with EtOAc (2 × 50 mL), and the combined organic phases were dried over anhydrous $MgSO_4$. The crude products were purified by column chromatography over silica gel, eluting with EtOH/CH_2Cl_2 mixtures (2%–10%), to afford the pure corresponding carboxylic acids of the *N*-arylated-2-pyridones in high yields (55–74%).

4.5.1. 1-(3-Fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid (HO_2C-**4f**)

Following the general method B described previously, the desired product was isolated in 60% yield. R_f (7% EtOH/CH_2Cl_2): 0.6 ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.82 (bs, 1H), 7.83–7.92 (m, 3H), 8.06 (s, 1H), 8.28 (d, J = 3 Hz, 1H), 8.50 (d, J = 6 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 108.3, 114.7, 116.2, 117.5, 123.2, 131.0, 140.4, 144.9, 146.4, 163.4, 164.5. MS (EI) ES$^+$: 256 ([M$^+$ + Na], 100); MS (EI) ES$^-$: 232 ([M$^+$-H], 100).

4.5.2. 1-(4-Fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid (HO_2C-**4g**)

Following the general method B described previously, the desired product was isolated in 74% yield. R_f (7% EtOH/CH_2Cl_2): 0.59 ^{1}H NMR (DMSO-d_6, 300 MHz) d (ppm): 6.79 (dd, J = 9.6 Hz, 1H), 7.43 (t, J = 9 Hz, 2H), 7.63 (dd, J = 9.6 Hz, 2H), 8.22 (dd, J = 6.3 Hz, 1H), 8.49 (dd, J = 9.3 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 108.3, 116.0, 116.3, 117.4, 129.0, 129.2, 135.4, 145.2, 146.3, 160.4, 163.7, 164.6. MS (EI) ES$^+$: 256 ([M$^+$ + Na], 100); MS (EI) ES$^-$: 232 ([M$^+$-H], 100).

4.5.3. 2-Oxo-1-(4-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxylic acid (HO_2C-**4h**)

Following the general method B described previously, the desired product was isolated in 55% yield. R_f (7% EtOH/CH_2Cl_2): 0.45 ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.82 (t, J = 6 Hz, 1H), 7.82 (d, J = 6 Hz, 2H), 7.99 (d, J = 9 Hz, 2H), 8.26 (d, J = 6 Hz, 1H), 8.50 (d, J = 6 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 108.4, 117.7, 121.8, 126.4, 128.0, 129.6, 142.5, 144.6, 146.5, 163.3, 164.5. MS (EI) ES$^+$: 306 ([M$^+$ + Na], 100); MS (EI) ES$^-$: 282 ([M$^+$-H], 95), 238 ([M$^+$-CO_2H], 100).

4.5.4. 2-Oxo-1-(3-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxylic acid (HO_2C-**4i**)

Following the general method B described previously, the desired product was isolated in 71% yield. R_f (7% EtOH/CH_2Cl_2): 0.57 ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.81 (t, J = 6 Hz, 1H), 7.80–7.95 (m, 3H), 8.06 (s, 1H), 8.28 (dd, J = 6.3 Hz, 1H), 8.50 (dd, J = 9.3 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 108.3, 117.6, 124.2, 126.2, 129.9, 130.6, 131.2, 139.7, 144.9, 146.4, 163.4, 164.6. MS (EI) ES$^+$: 306 ([M$^+$ + Na], 100); MS (EI) ES$^-$: 282 ([M$^+$-H], 95), 238 ([M$^+$-CO_2H], 100).

4.5.5. N-(4-nitrophenyl)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (**7**)

The source of palladium, $Pd(OAc)_2$ (19 mg, 0.08 mmol), and the ligand, *X-phos* [2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl] (80 mg, 0.17 mmol), were mixed into a sealed tube with a deoxygenated mixture of *t*-BuOH/H_2O (3 + 1 mL), and introduced into a preheated bath at 110 °C for 1 min. Afterwards, the 6-Cl-9-THP-purine **1** (200 mg, 0.84 mmol), 4-nitroaniline (174 mg, 1.26 mmol) and K_2CO_3 (290 mg, 2.09 mmol) were added to the reaction mixture and it was additionally heated at 110 °C for 15 h.

The reaction mixture was allowed to reach room temperature and diluted into EtOAc. It was filtered through a short pad of celite, which was washed with EtOAc. The filtrate was extracted with brine and the organic phase dried over anhydrous $MgSO_4$. The solids were filtered off and the solvent removed *in vacuo*. The residue was purified by column chromatography over silica gel, using as eluent EtOH/CH_2Cl_2 mixtures (from 2% to 8%), obtaining the product as a yellow powder (261 mg, 91% isolated yield). M.p. >300 °C. R_f (2% EtOH/CH_2Cl_2): 0.19. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.69–1.80 (m, 3H), 2.05–2.17 (m, 3H), 3.80 (dt, J = 12. 3 Hz, 1H), 4.20 (dd, J = 12.3 Hz, 1H), 5.78 (dd, J = 12.3 Hz, 1H), 8.06 (d, J = 9 Hz, 2H), 8.13 (s, 1H), 8.19 (bs, 1H), 8.26 (d, J = 9 Hz, 2H), 8.64 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 68.9, 82.1, 118.8, 120.8, 125.2, 139.5, 142.5, 144.9, 149.6, 151.2, 152.6. MS (EI) ES$^+$: 341 ([M+H], 30).

4.5.6. N-(2-fluoro-4-nitrophenyl)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (**8**)

Following the same experimental procedure than for the synthesis of the compound **7**, the Buchwald–Hartwig coupling of the 6-chloro-THP-purine **1** (400 mg, 1.68 mmol) with 2-fluoro-4-nitroaniline (393 mg, 2.52 mmol) led to the desired product **4** in 94% isolated yield (567 mg), after purification of the crude material by column chromatography over silica gel using EtOH/CH_2Cl_2 mixtures as eluent. M.p. 176 °C. R_f (4% EtOH/CH_2Cl_2): 0.47. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.70–1.89 (m, 3H), 2.06–2.15 (m, 3H), 3.81 (dt, J = 12.3 Hz, 1H), 4.21 (d, J = 12 Hz, 1H), 5.78 (dd, J = 9.3 Hz, 1H), 8.06 (dd, J = 12.3 Hz, 1H), 8.13 (bs, 1H), 8.16 (s, 1H), 8.66 (s, 1H), 9.17 (t, J = 9 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 68.9, 82.2, 111.0, 119.8, 120.9, 121.3, 134.0, 139.9, 141.5, 149.7, 149.7, 150.7, 152.3, 152.5. MS (EI) ES$^-$: 357 ([M$^+$-H], 100).

4.6. General procedure C for the preparation of **3**, **9**, **10** and **16** (NO_2 reduction)

To a suspension of the corresponding nitro compound (**2**, **7**, or **8**, 100 mg scale) in a mixture of EtOH/H_2O (3 + 1 mL), iron (325 mesh powder, 2.5 equiv) and NH_4Cl (6 equiv) were added. The reaction mixture was heated at 90 °C in a round bottom flask equipped with a reflux system. After 2 h, the reaction mixture had become black and the conversion showed to be complete by TLC, giving the desired compound as the major product. The resulting mixture was filtered through a short pad of celite to remove the metal residues, and the solids were carefully washed several times with EtOAc and with a final fraction of EtOH. The filtrates were concentrated *in vacuo* and the residue was directly purified by column chromatography, using EtOH/CH_2Cl_2 mixtures (from 2% to 10%) as eluent. This purification led to the pure desired products in good to high yields (65–89% yields).

4.6.1. 3-Fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yloxy)aniline (**3**)

According to procedure C in 69% yield. Slightly coloured white solid. M.p. (decomposition) 120 °C. R_f (6% EtOH/CH_2Cl_2): 0.40. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.68–1.75 (m, 3H), 2.08–2.14 (m, 3H), 3.71–3.79 (m, 1H), 4.17–4.22 (m, 1H), 5.79 (dd, J = 9.3 Hz, 1H), 6.47–6.56 (m, 2H), 7.08 (t, J = 9 Hz, 1H), 8.24 (s, 1H), 8.52 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.8, 68.8, 82.2, 103.5, 110.7, 121.3, 124.2, 131.1, 140.9, 145.7, 152.2, 153.3, 156.5, 160.0. MS (EI) ES$^+$: 352 ([M$^+$ + Na], 65), 330 ([M$^+$ + H], 100).

4.6.2. N1-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)benzene-1,4-diamine (**9**)

According to procedure C in 82% yield. Brownish solid. M.p. 210–212 °C. R_f (5% EtOH/CH_2Cl_2): 0.18. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.67–1.78 (m, 3H), 2.04–2.17 (m, 3H), 3.79 (bt, J = 9 Hz, 1H), 4.18 (bd, J = 12 Hz, 1H), 5.73 (dd, J = 9.3 Hz, 1H), 6.73 (d, J = 9 Hz, 2H), 7.48 (d, J = 9 Hz, 1H), 7.53 (bs, 1H), 8.03 (s, 1H), 8.48 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.9, 24.9, 31.9, 68.8, 81.9, 115.6, 119.9, 123.1, 129.6, 138.0, 143.2, 148.7, 152.8, 153.2. MS (EI) ES$^+$: 311 ([M$^+$ + H], 100), 333 ([M$^+$ + Na], 42).

4.6.3. 2-Fluoro-N1-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl) benzene-1,4-diamine (**10**)

According to procedure C in 89% yield. Brownish solid. M.p. 130–132 °C. R_f (5% EtOH/CH_2Cl_2): 0.22; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.66–1.77 (m, 3H), 2.07–2.11 (m, 3H), 3.78 (dd, 1H), 4.19 (bd, J = 12 Hz, 1H), 5.74 (d, J = 15 Hz, 1H), 6.49 (bs, 1H), 6.52 (s, 1H), 7.49 (bs, 1H), 7.98 (t, J = 9 Hz, 1H), 8.04 (s, 1H), 8.49 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.9, 24.9, 31.9, 68.8, 81.9, 102.5, 110.7, 117.0, 120.3, 125.4, 138.3, 144.4, 148.9, 152.0, 152.7, 157.1. MS (EI) ES$^+$: 329 ([M$^+$ + H], 55), 351 ([M$^+$ + Na], 100).

4.6.4. 3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)aniline (**16**)

According to procedure C in 81% yield. Light yellow solid. M.p. 180–182 °C. R_f (5% EtOH/CH_2Cl_2): 0.37 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.69–1.75 (m, 3H), 2.08–2.14 (m, 3H), 3.78–3.82 (m, 1H), 4.18–4.22 (bd, J = 15 Hz, 1H), 5.85 (bd, J = 9 Hz, 1H), 6.86 (d, J = 9 Hz, 1H), 7.35 (t, J = 6 Hz, 1H), 8.09 (bs, 1H), 8.20 (d, J = 6 Hz, 1H), 8.33 (s, 1H), 9.00 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.8, 31.8, 68.9, 81.9, 115.8, 117.8, 120.4, 129.6, 131.1, 136.5, 142.0, 146.8, 151.6, 152.3, 155.13. MS (EI) ES$^+$: 296 ([M$^+$ + H], 95), 318 ([M$^+$ + Na], 65).

4.7. 6-(3-nitrophenyl)-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (**15**) [25]

A sealed tube containing a suspension of the 6-chloro-9-THP purine **1** (300 mg, 1.26 mmol) and $Pd(PPh_3)_4$ (145 mg, 0.13 mmol) in a degassed mixture of dioxane/H_2O (4 + 1 mL), was preheated at 85 °C for 5 min. Next, K_2CO_3 (520 mg, 3.77 mmol) and 3-nitrophenyl boronic acid (315 mg, 1.88 mmol), were added to the mixture and the reaction was additionally heated at 100 °C in the sealed tube for 15 h. Afterwards, the crude material was allowed to reach room temperature. It was diluted in EtOAc and filtered through a short pad of celite, washing the solids with EtOAc. The filtrate was washed with brine and the organic phase separated and dried over anhydrous $MgSO_4$. After filtration of the solids and evaporation *in vacuo*, the resulting oily residue was purified by column chromatography over silica gel, using EtOH/CH_2Cl_2 mixtures (from 0% to 3%) as eluent. The purification gave the desired pure product in high yield (299 mg, 73% yield). Light yellow solid. M.p. 190–192 °C. R_f (2% EtOH/CH_2Cl_2): 0.41; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.64–1.87 (m, 3H), 2.05–2.23 (m, 3H), 3.84 (dd, J = 12.3 Hz, 1H), 4.22 (dd, J = 12.3 Hz, 1H), 5.88 (dd, J = 9.3 Hz, 1H), 7.74 (dd, J = 9.6 Hz, 1H), 8.37 (dd, J = 9.3 Hz, 1H), 8.41 (s, 1H), 9.07 (s, 1H), 9.22 (d, J = 9 Hz, 1H), 9.72 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 69.0, 82.2, 124.7, 125.3, 129.6, 131.3, 135.5, 137.4, 142.9, 148.7, 151.9, 152.1, 152.4. MS (EI) ES$^+$: 326 ([M$^+$ + H], 78), 242 ([M$^+$+H-THP], 100).

4.8. General procedure D for the reaction of phenylaminopurines with phenyl carboxylic acids

An experimental procedure previously described, that employs HATU as the coupling reagent [8] was used for the reaction between the four different amino purine derivatives (**3**, **9**, **10** and **16**) with several aryl carboxylic acids (both commercially available **4a**–**e** or compounds **4f**–**i**). The corresponding amino purine derivative (50 mg scale) was dissolved in 3 mL of anhydrous DMF or CH_2Cl_2

under argon, and the corresponding commercial or previously synthesized arylcarboxilic acid (1.15 equiv) and HATU (1.2 equiv) were added. The mixture was cooled at 0 °C (ice/water bath) and DIPEA (1.2 equiv) was added dropwise via a syringe. The reaction mixture was allowed to reach room temperature, and the reaction was already complete after 3 h. The crude product was concentrated *in vacuo* and redissolved in EtOAc. It was washed twice with saturated aqueous NH_4Cl and once with brine. The organic phase was dried over anhydrous $MgSO_4$. The solids were filtered off and the solvent was removed *in vacuo*. This crude material was purified by column chromatography over silica gel, using EtOH/CH_2Cl_2 mixtures (0%–3% ethanol) as eluent, giving the desired products in high yields (71–99%).

4.8.1. 2-Fluoro-N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yloxy)phenyl)benzamide (**5c**)

According to procedure D. Yield 82%. Brownish solid. M.p. 232–234 °C. R_f (5% EtOH/CH_2Cl_2): 0.48. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.69–1.80 (m, 3H), 2.09–2.15 (m, 3H), 3.81 (dd, J = 12.9 Hz, 1H), 4.20 (d, J = 12 Hz, 1H), 5.81 (dd, J = 9.3 Hz, 1H), 7.17–7.24 (m, 2H), 7.32–7.36 (m, 2H), 7.52–7.59 (m, 1H), 7.89 (dd, J = 12.3 Hz, 1H), 8.19 (dt, J = 6.3 Hz, 1H), 8.29 (s, 1H), 8.52 (s, 1H), 8.58 (bs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 68.9, 82.3, 109.6, 116.1, 116.4, 121.0, 124.0, 125.3, 132.3, 134.2, 135.9, 136.5, 141.2, 152.1, 152.6, 156.0, 158.8, 159.4, 161.3, 162.0. MS (EI) ES$^+$: 474 ([M$^+$ + Na], 100), 452 ([M$^+$ + H], 2), 390 ([M$^+$ + Na-THP + H), 45).

4.8.2. N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yloxy)phenyl)-1-(3-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (**5f**)

According to procedure D. Yellowish solid. Yield 92%. M.p. >250 °C. R_f (7% EtOH/CH_2Cl_2): 0.39 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.69–1.79 (m, 3H), 2.08–2.17 (m, 3H), 3.80 (dt, J = 12.3 Hz, 1H), 4.19 (bd, J = 12 Hz, 1H), 5.80 (dd, J = 9.3 Hz, 1H), 6.62 (t, J = 6 Hz, 1H), 7.19–7.28 (m, 4H), 7.38 (dd, J = 9.3 Hz, 1H), 7.53–7.59 (m, 1H), 7.63 (dd, J = 6.3 Hz, 1H), 7.96 (dd, J = 12.3 Hz, 1H), 8.25 (s, 1H), 8.51 (s, 1H), 8.76 (dd, J = 6.3 Hz, 1H), 11.93 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 68.9, 82.3, 107.4, 109.2, 109.5, 114.4, 114.7, 116.1, 116.7, 121.2, 122.3, 122.4, 123.8, 131.2, 135.4, 135.6, 137.1, 141.1, 141.3, 145.3, 152.1, 152.4, 159.6, 161.3, 162.2. MS (EI) ES$^+$: 567 ([M$^+$ + Na], 100), 545 ([M$^+$ + H], 2), 483 ([M$^+$ + Na-THP + H), 35).

4.8.3. N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yloxy)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (**5g**)

According to procedure D. White solid. Yield 99%. M.p. (decomposition) > 220 °C. R_f (5% EtOH/CH_2Cl_2): 0.41 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.69–1.79 (m, 3H), 2.08–2.14 (m, 3H), 3.80 (t, J = 9 Hz, 1H), 4.20 (bd, J = 12 Hz, 1H), 5.80 (dd, J = 12.3 Hz, 1H), 6.61 (dd, J = 9.6 Hz, 1H), 7.24–7.30 (m, 3H), 7.36–7.42 (m, 3H), 7.62 (dd, J = 6.3 Hz, 1H), 7.95 (dd, J = 12.3 Hz, 1H), 8.25 (s, 1H), 8.51 (s, 1H), 8.75 (dd, J = 6.3 Hz, 1H), 11.96 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 68.9, 82.3, 107.3, 109.5, 116.1, 116.7, 117.0, 121.2, 122.1, 123.8, 128.4, 128.5, 135.9, 137.3, 141.1, 141.8, 145.2, 152.1, 152.4, 159.6, 161.4, 162.5, 174.9. MS (EI) ES$^+$: 567 ([M$^+$ + Na], 80), 483 ([M$^+$ + Na-THP + H), 45).

4.8.4. N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yloxy)phenyl)-2-oxo-1-(4-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxamide (**5h**)

According to procedure D. Yield 71%. Slightly coloured solid (beige) M.p. > 260 °C. R_f (5% EtOH/CH_2Cl_2): 0.40; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.69–1.80 (m, 3H), 2.11–2.15 (m, 3H), 3.80 (t, J = 9 Hz, 1H), 4.20 (bd, J = 12 Hz, 1H), 5.80 (bd, J = 12 Hz, 1H), 6.66 (dd, J = 9.6 Hz, 1H), 7.23-7.29 (m, 1H), 7.38 (bd, J = 9Hz, 1H), 7.60 (d, J = 9 Hz, 2H), 7.60-7.64 (m, 1H), 7.87 (d, J = 9 Hz, 2H), 7.96 (dd, J = 12.3 Hz, 1H), 8.25 (s, 1H), 8.51 (s, 1H), 8.77 (dd, J = 9.3 Hz, 1H), 11.87 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 68.9, 82.3, 107.6, 109.2, 116.1, 121.2, 122.4, 123.8, 127.1, 127.2, 135.7, 137.1, 141.0, 141.1, 145.5, 152.1, 152.4, 155.9, 159.5, 161.1, 162.2. MS (EI) ES$^+$: 617 ([M$^+$ + Na], 100), 595 ([M$^+$ + H], 2), 533 ([M$^+$+Na-THP+H), 20).

4.8.5. 2-Fluoro-N-(4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)benzamide (**11c**)

According to procedure D. Yield 82%. Oil. R_f (5% EtOH/CH_2Cl_2): 0.4. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.68–1.79 (m, 3H), 2.04–2.13 (m, 3H), 3.80 (t, J = 9 Hz, 1H), 4.19 (d, J = 12 Hz, 1H), 5.76 (d, J = 9 Hz, 1H), 7.19 (dd, J = 12.9 Hz, 1H), 7.32 (t, J = 6 Hz, 1H), 7.51 (m, 1H), 7.69 (d, J = 9 Hz, 1H), 7.85 (d, J = 9 Hz, 1H), 8.00 (s, 1H), 8.09 (s, 1H), 8.18 (dd, J = 9.6 Hz, 1H), 8.48 (d, J = 15 Hz, 1H), 8.55 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 68.9, 81.9, 116.2, 120.0, 121.0, 121.3, 121.4, 125.1, 132.2, 133.4, 133.7, 135.5, 138.4, 148.8, 152.1, 153.0, 158.7, 161.2, 162.0. MS (EI) ES$^+$: 455 ([M$^+$ + Na], 100), 371 ([M$^+$ + Na-THP + H], 65).

4.8.6. 2-Fluoro-N-(4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)pyridine-3-carboxamide (**11d**)

According to procedure D. Yield 88%. Brownish solid. M.p. 156–158 °C. R_f (5% EtOH/CH_2Cl_2): 0.67. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.68–1.78 (m, 3H), 2.05–2.13 (m, 3H), 3.81 (dd, J = 12.9 Hz, 1H), 4.19 (d, J = 12 Hz, 1H), 5.76 (d, J = 9 Hz, 1H), 7.41–7.45 (m, 1H), 7.70 (d, J = 9 Hz, 2H), 7.85 (d, J = 9 Hz, 1H), 8.08 (s, 1H), 8.39 (d, J = 3 Hz, 1H), 8.56 (dd, J = 9.6 Hz, 1H), 8.68 (dt, J = 9.3 Hz, 1H). MS (EI) ES$^+$: 456 ([M$^+$ + Na], 100), 434 ([M$^+$ + H], 2), 372 ([M$^+$ + H + Na-THP], 50).

4.8.7. 2-Methoxy-N-(4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)pyridine-3-carboxamide (**11e**)

According to procedure D. Yield 89%. White solid. M.p. (decomposition) 170 °C. R_f (5% EtOH/CH_2Cl_2): 0.33 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.68–1.84 (m, 3H), 2.09–2.13 (m, 3H), 3.81–3.96 (m, 1H), 4.18–4.21 (m, 1H), 4.21 (s, 1H), 5.76 (d, J = 9 Hz, 1H), 7.13 (m, 1H), 7.71 (d, J = 6 Hz, 2H), 7.80 (t, J = 9 Hz, 3H), 8.07 (s, 1H), 8.33 (bs, 1H), 8.55 (s, 1H), 8.61 (d, J = 6 Hz, 1H), 9.84 (s, 1H). MS (EI) ES$^+$: 468 ([M$^+$ + Na], 100), 384 ([M$^+$ + Na-THP + H], 45); ES$^-$: 444 ([M$^+$-H], 100).

4.8.8. 1-(3-fluorophenyl)-2-oxo-N-(4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)-1,2-dihydropyridine-3-carboxamide (**11f**)

According to procedure D. Yield 91%. R_f (5% EtOH/CH_2Cl_2): 0.35. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.67–1.78 (m, 3H), 1.96–2.17 (m, 3H), 3.76–3.80 (m, 1H), 4.19 (d, J = 9 Hz, 1H), 5.74 (dd, J = 9.3 Hz, 1H), 6.61 (dd, J = 9.6 Hz, 1H), 7.21–7.24 (m, 3H), 7.55–7.61 (m, 2H), 7.76 (bs, 5H), 8.06 (s, 1H), 8.54 (s, 1H), 8.75 (dd, J = 9.3 Hz, 1H), 11.76 (s, 1H). MS (EI) ES$^+$: 548 ([M$^+$ + Na], 100), 526 ([M$^+$ + H], 10), 464 ([M$^+$ + Na-THP + H], 48).

4.8.9. 2-Oxo-N-(4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)-1-(3-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxamide (**11i**)

According to procedure D. Yield 84%. Yellow solid. M.p. 210–230 °C. R_f (5% EtOH/CH_2Cl_2): 0.38 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.64–1.78 (m, 3H), 2.08–2.12 (m, 3H), 3.76–3.83 (m, 1H), 4.19 (d, J = 12 Hz, 1H), 5.75 (d, J = 9 Hz, 1H), 6.64 (dd, J = 9.6 Hz, 1H), 7.58–7.64 (m, 4H), 7.76 (bs, 4H), 7.87 (d, J = 9 Hz, 2H), 8.06 (s, 1H), 8.54 (s, 1H), 8.77 (dd, J = 6.3 Hz, 1H), 11.70 (s, 1H). MS (EI) ES$^+$: 598 ([M$^+$ + Na], 100), 576 ([M$^+$ + H], 30), 514 ([M$^+$ + Na-THP+H], 45).

*4.8.10. N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)-2-methoxybenzamide (**12a**)*

According to procedure D. Yield 82%. White solid. M.p. 166–168 °C. R_f (5% EtOH/CH_2Cl_2): 0.36 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.67–1.78 (m, 3H), 2.09–2.17 (m, 3H), 3.71–3.83 (m, 1H), 4.07 (s, 3H Me), 4.17–4.21 (m, 1H), 5.75 (d, *J* = 9 Hz, 1H), 7.02–7.05 (d, *J* = 9 Hz, 1H), 7.11–7.16 (m, 1H), 7.20–7.28 (m, 1H), 7.50 (m, 1H), 7.81 (bs, 1H), 7.93 (d, *J* = 12 Hz, 1H), 8.08 (s, 1H), 8.27 (d, *J* = 6 Hz, 1H), 8.52 (m, 1H), 8.55 (s, 1H), 9.87 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 56.3, 68.8, 82.0, 108.2, 111.5, 115.7, 120.5, 121.4, 121.7, 122.6, 122.8, 122.9, 132.4, 133.4, 134.5, 138.7, 149.1, 152.0, 152.8, 157.2, 163.2. MS (EI) ES$^+$: 463 ([M$^+$ + H], 8), 485 ([M$^+$ + Na], 100); ES$^-$: 461 ([M$^+$-H], 100).

*4.8.11. 4-Bromo-N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)-3-methylbenzamide (**12b**)*

According to procedure D. Slightly brownish solid. Yield 80%. M.p. >250 °C (Kofler). R_f (5% EtOH/CH_2Cl_2): 0.33. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.65–1.78 (m, 3H), 2.09–2.17 (m, 3H), 2.46 (s, 3H), 3.80 (dd, *J* = 12.9 Hz, 1H), 4.19 (bd, *J* = 12 Hz, 1H), 5.75 (dd, *J* = 9.3 Hz, 1H THP), 7.24 d, *J* = 9 Hz, 1H), 7.52 (bd, *J* = 9 Hz, 1H), 7.62 (d, *J* = 6 Hz, 1H), 7.75 (d, *J* = 12 Hz, 2H), 7.85 (dd, *J* = 12.3 Hz, 1H), 8.01 (bs, 1H), 8.09 (s, 1H), 8.55 (s, 1H), 8.60 (d, *J* = 9 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 23.0, 24.9, 31.9, 68.9, 82.0, 107.8, 108.2, 115.6, 120.6, 122.4, 123.6, 125.6, 129.1, 129.4, 132.8, 133.7, 138.8, 149.1, 151.9, 152.7, 164.9. MS (EI) ES$^+$: 547 ([M$^+$ + Na], 90), 549 (100); ES$^-$: 523 ([M$^+$-H], 100).

*4.8.12. 2-Fluoro-N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)benzamide (**12c**)*

According to procedure D. Yield 86%. White solid. M.p. (decomposition) 170 °C. R_f (5% EtOH/CH_2Cl_2): 0.32. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.68–1.79 (m, 3H), 2.09–2.17 (m, 3H), 3.77–3.84 (m, 1H), 4.18–4.21 (m, 1H), 5.76 (d, *J* = 12 Hz, 1H), 7.16–7.35 (m, 3H), 7.52 (bs, 1H), 7.89 (bt, *J* = 12 Hz, 2H), 8.02 (s, 1H), 8.15 (m, 1H), 8.56 (s, 1H), 8.49–8.62 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 68.9, 82.0, 108.2, 115.8, 116.2, 120.6, 121.0, 122.5, 123.6, 125.2, 132.3, 133.6, 134.0, 138.8, 149.1, 151.5, 152.3, 154.7, 158.7, 161.2.

*4.8.13. 2-Fluoro-N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6 ylamino)phenyl)pyridine-3-carboxamide (**12d**)*

According to procedure D. Yield 89%. Slightly coloured solid (beige). M.p. (decomposition) 110 °C. R_f (5% EtOH/CH_2Cl_2): 0.24; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.72–1.79 (m, 3H), 2.10–2.17 (m, 3H), 3.77–3.84 (m, 1H), 4.18–4.22 (m, 1H), 5.76 (d, *J* = 9 Hz, 1H), 7.25 (m, 1H), 7.44 (bs, 1H), 7.79 (s, 1H), 7.90 (d, *J* = 12 Hz, 1H), 8.10 (s, 1H), 8.40 (bs, 1H), 8.57 (s, 1H), 8.61–8.70 (m, 3H). MS (EI) ES$^+$: 452 ([M$^+$ + H], 10), 474 ([M$^+$ + Na], 100); ES$^-$: 450 ([M$^+$-H], 100).

*4.8.14. N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)-2-methoxypyridine-3-carboxamide (**12e**)*

According to procedure D. Yield 83%. White solid. M.p. 166–168 °C. R_f (5% EtOH/CH_2Cl_2): 0.34. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.68–1.79 (m, 3H), 2.09–2.17 (m, 3H), 3.69–3.84 (m, 1H), 4.18–4.20 (m, 1H), 4.21 (s, 3H Me), 5.77 (d, *J* = 9 Hz, 1H), 7.13 (dd, *J* = 6.3 Hz, 1H), 7.26 (m, 1H), 7.79 (bs, 1H), 7.92 (bd, *J* = 12 Hz, 1H), 8.09 (s, 1H), 8.33 (d, *J* = 3 Hz, 1H), 8.56-8.61 (m, 1H), 8.61 (s, 1H), 9.89 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 54.5, 68.9, 82.0, 108.1, 115.8, 116.0, 118.2, 120.6, 122.5, 123.2, 134.0, 138.7, 141.8, 149.1, 150.1, 151.9, 152.7, 154.8, 160.2, 161.7. MS (EI) ES$^+$: 464 ([M$^+$ + H], 18), 486 ([M$^+$ + Na], 100); ES$^-$: 462 ([M$^+$-H], 85).

*4.8.15. 4-Fluoro-N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)benzamide (**12f**)*

According to procedure D. Yield 93%. Yellow solid. M.p. (decomposition) > 250 °C (Kofler). R_f (5% EtOH/CH_2Cl_2): 0.34. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.68–1.78 (m, 3H), 2.09–2.12 (m, 3H), 3.76–3.83 (m, 1H), 4.17–4.21 (m, 1H), 5.76 (d, *J* = 9 Hz, 1H), 6.60 (dd, *J* = 9.6 Hz, 1H), 7.25–7.30 (m, 2H), 7.39–7.43 (m, 2H), 7.61 (d, *J* = 6 Hz, 1H), 7.71 (bs, 1H), 7.99 (d, *J* = 12 Hz), 8.07 (s, 1H), 8.51–8.55 (m, 1H), 8.55 (bs, 1H), 8.74 (d, *J* = 6 Hz, 1H), 11.89 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 68.8, 81.9, 107.3, 108.1, 115.9, 116.7, 117.0, 120.6, 122.3, 123.1, 128.4, 128.5, 134.3, 135.9, 138.6, 141.5, 145.0, 149.1, 151.9, 152.8, 161.2, 162.5. MS (EI) ES$^+$: 544 ([M$^+$ + H], 100).

*4.8.16. N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (**12g**)*

According to procedure D. Yield 87%. Yellow solid. M.p. (decomposition) 166 °C. R_f (5% EtOH/CH_2Cl_2): 0.32. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.68–1.78 (m, 3H), 2.09–2.17 (m, 3H), 3.76–3.84 (m, 1H), 4.17–4.21 (m, 1H), 5.76 (d, *J* = 9 Hz, 1H), 6.62 (dd, *J* = 9.6 Hz, 1H), 7.21–7.26 (m, 2H), 7.53–7.61 (m, 2H), 7.72 (bs, 1H), 7.99 (d, *J* = 12 Hz, 1H), 8.07 (s, 1H), 8.52–8.55 (m, 2H), 8.55 (bs, 1H), 8.75 (bd, *J* = 6 Hz, 1H), 11.85 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 68.9, 82.0, 100.0, 107.4, 107.8, 108.1, 114.8, 115.9, 120.6, 122.2, 122.2, 122.4, 123.0, 131.1, 131.2, 134.3, 138.7, 141.2, 145.1, 149.1, 151.9, 152.8, 154.7, 161.1, 162.2. MS (EI) ES$^+$: 566 ([M$^+$ + Na], 100), 544 ([M$^+$ + H], 20); ES$^-$: 542 ([M$^+$-H], 65).

*4.8.17. N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)-2-oxo-1-(4-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxamide (**12h**)*

According to procedure D. Yield 82%. White solid. M.p. (decomposition) > 250 °C (Kofler). R_f (5% EtOH/CH_2Cl_2): 0.35. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.68–1.78 (m, 3H), 2.09–2.12 (m, 3H), 3.76–3.83 (m, 1H), 4.17–4.21 (m, 1H), 5.76 (d, *J* = 9 Hz, 1H), 6.65 (dd, *J* = 9.6 Hz, 1H), 7.24–7.27 (m, 1H), 7.58–7.60 (m, 3H), 7.72 (bs, 1H), 7.86 (d, *J* = 9 Hz, 2H), 8.07 (d, *J* = 15 Hz, 1H), 8.08 (s, 1H), 8.52–8.55 (m, 1H), 8.55 (bs, 1H), 8.76 (d, *J* = 6 Hz, 1H), 11.80 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.9, 68.9, 82.0, 107.6, 108.1, 115.8, 120.6, 122.3, 122.5, 123.1, 127.1, 127.2, 134.3, 138.7, 140.8, 142.8, 145.2, 149.1, 151.9, 152.8, 161.0, 162.2. MS (EI) ES$^+$: 616 ([M$^+$ + Na], 100), 594 ([M$^+$ + H], 20); ES$^-$: 592 ([M$^+$-H], 100).

*4.8.18. N-(3-fluoro-4-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-ylamino)phenyl)-2-oxo-1-(3-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxamide (**12i**)*

According to procedure D. Yellow solid. Yield 98%. M.p. (decomposition) > 250 °C (Kofler). R_f (5% EtOH/CH_2Cl_2): 0.35 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.67–1.85 (m, 3H), 2.09–2.17 (m, 3H), 3.76–3.83 (m, 1H), 4.17–4.21 (m, 1H), 5.75 (d, *J* = 12 Hz, 1H), 6.64 (t, *J* = 6 Hz, 1H), 7.27 (m, 1H), 7.61–7.63 (m, 1H), 7.68 (bs, 1H), 7.72–7.76 (m, 2H), 7.82 (m, 1H), 7.98 (d, *J* = 12 Hz, 1H), 8.08 (s, 1H), 8.52–8.55 (m, 1H), 8.55 (bs, 1H), 8.76 (d, *J* = 6 Hz, 1H), 11.79 (s, 1H). MS (EI) ES$^+$: 616 ([M$^+$ + Na], 100), 594 ([M$^+$ + H], 15); ES$^-$: 592 ([M$^+$-H], 100).

4.9. General method E for the THP deprotection of 9-N-THP-purines

The precursors (50 mg scale) were suspended into a mixture of CH_3CN/H_2O (2 + 2 mL) and trifluoroacetic acid (1 mL/mmol of s.m.) was added slowly at 0 °C via a syringe. The reaction mixture was allowed to reach room temperature. After 4 h [23], the crude was concentrated till dryness and directly purified through a short pad

of silica gel, using mixtures EtOH/H_2O (from 3% to 10%) as eluent. The desired final products were isolated as pure compounds with high recovery after chromatography.

*4.9.1. N-(4-(9H-purin-6-yloxy)-3-fluorophenyl)-2-fluorobenzamide (**6c**)*

According to method E, **6c** was obtained as a slightly coloured solid (beige). Yield 65%. R_f (7% EtOH/CH_2Cl_2): 0.18. M.p. 159.8–162.3 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.58 (dd, J = 9.6 Hz, 1H), 7.77 (bs, 2H), 8.02 (bs, 2H), 8.67 (bs, 1H), 8.98 (s, 1H), 9.12 (bs, 1H), 10.54 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 108.5, 108.6, 116.4, 116.7, 116.8, 118.5, 124.8, 125.0, 125.1, 130.3, 133.2, 135.5, 144.5, 151.7, 155.4, 156.3, 158.0. MS (EI) EI$^+$: 390 ([M$^+$ + Na], 100), 368 ([M$^+$ + H], 30). HRMS-ESI (m/z) calcd for $C_{18}H_{12}F_2N_5O_2$ (M + 1) 368.0959, found: 368.0958. HPLC purity (method I) 100%.

*4.9.2. N-(4-(9H-purin-6-yloxy)-3-fluorophenyl)-1-(3-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (**6f**)*

6f was obtained according to method E. Yield 82%. M.p. > 250 °C (Kofler). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.75 (dd, J = 9.6 Hz, 1H), 7.41–7.47 (m, 4H), 7.55–7.65 (m, 2H), 7.99 (dd, J = 12.3 Hz, 1H), 8.16 (dd, J = 6.3 Hz, 1H), 8.44 (s, 1H), 8.55 (bs, 1H), 8.61 (dd, J = 6.3 Hz, 1H), 12.06 (s, 1H), 13.69 (bs, 1H NH$_9$ purine). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.7, 108.6, 108.8, 115.7, 116.7, 117.1, 120.6, 123.5, 125.1, 131.6, 135.5, 137.4, 138.2, 141.7, 143.8, 144.5, 145.5, 145.6, 151.7, 162.0, 162.8. MS (EI) EI$^+$: 483 ([M$^+$ + Na], 100), 461 ([M$^+$ + H], 2); EI$^-$: 459 ([M$^+$-H], 100). HRMS-ESI (m/z) calcd for $C_{23}H_{15}F_2N_6O_3$ (M + 1) 461.1174, found: 461.1157. HPLC purity (method I) 99%.

*4.9.3. N-(4-(9H-purin-6-yloxy)-3-fluorophenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (**6g**)*

According to method E, **6g** was obtained as white solid. Yield 92%. R_f (6% EtOH/CH_2Cl_2): 0.17. M.p. 232.2–234.8 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.74 (dd, J = 9.6 Hz, 1H), 7.40–7.46 (m, 4H), 7.60–7.63 (m, 2H), 7.98 (d, J = 15 Hz, 1H), 8.14 (d, J = 6 Hz, 1H), 8.44 (s, 1H), 8.55 (s, 1H), 8.60 (d, J = 6 Hz, 1H), 12.10 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 54.1, 107.5, 108.7, 116.3, 116.6, 116.7, 129.8, 144.8, 145.6, 151.6. MS (EI) EI$^+$: HRMS-ESI (m/z) calcd for $C_{23}H_{15}F_2N_6O_3$ (M + 1) 461.1174; found: 461.1168. HPLC purity (method I) 99%.

*4.9.4. N-(4-(9H-purin-6-yloxy)-3-fluorophenyl)-2-oxo-1-(4-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxamide (**6h**)*

According to method E, **6h** was obtained as a slightly coloured solid (beige). Yield 98%. R_f (7% EtOH/CH_2Cl_2): 0.22. M.p. 276.5–279.9 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.78 (dd, J = 9.6 Hz, 1H), 7.46 (bs, 2H), 7.83 (d, J = 9 Hz, 2H), 7.99 (d, J = 9 Hz, 3H), 8.20 (dd, J = 9.3 Hz, 1H), 8.43 (s, 1H), 8.52 (bs, 1H), 8.62 (dd, J = 6.3 Hz, 1H), 12.02 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.8, 108.6, 108.7, 116.7, 117.4, 121.2, 125.0, 127.1, 127.1, 128.7, 130.0, 143.7, 143.9, 144.1, 143.9, 145.6, 145.8, 151.7, 161.9, 162.4. MS (EI) EI$^+$: 533 ([M$^+$ + Na], 50), 511 ([M$^+$ + H], 2); EI$^-$: 509 ([M$^+$-H], 100). HRMS-ESI (m/z) calcd for $C_{24}H_{15}F_4N_6O_3$ (M + 1) 511.1142, found: 511.1151. HPLC purity (method I) 100%.

*4.9.5. N-(4-(9H-purin-6-ylamino)phenyl)-2-fluorobenzamide (**13c**)*

According to method E. Slightly coloured white solid. Yield 94%. R_f (8% EtOH/CH_2Cl_2): 0.18 m.p.: 219.7–223.5 °C 1H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.12 (s, 1H), 7.29–7.38 (m, 2H), 7.57–7.59 (m, 1H), 7.68 (d, J = 6 Hz, 3H), 7.91 (d, J = 9 Hz, 2H), 8.30 (s, 1H), 8.38 (s, 1H), 9.81 (s, 1H), 10.37 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 116.4, 116.7, 120.2, 120.2, 121.2, 121.5, 125.1, 125.4, 130.6, 132.9, 134.1, 136.3, 140.7, 151.5, 152.3, 162.3. MS (EI) EI$^+$: 371 ([M$^+$ + Na], 100), 349 ([M$^+$ + H], 85); EI$^-$: 347 ([M$^+$-H], 100). HRMS-ESI (m/z) calcd for $C_{18}H_{14}FN_6O$ (M + 1) 349.1213, found: 349.1213. HPLC purity (method I) 100%.

*4.9.6. N-(4-(9H-purin-6-ylamino)phenyl)-2-fluoropyridine-3-carboxamide (**13d**)*

According to method E. Light yellow solid. Yield 96%. R_f (7% EtOH/CH_2Cl_2): 0.11. M.p. 228.2–230.5 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.52 (dt, J = 6.3 Hz, 1H), 7.70 (d, J = 9 Hz, 2H), 7.89 (d, J = 9 Hz, 2H), 8.26 (dt, J = 9.3 Hz, 1H), 8.40 (s, 1H), 8.41 (bs, 1H), 8.46 (s, 1H), 10.13 (bs, 1H), 10.58 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 117.5, 120.0, 120.5, 120.6, 121.9, 122.6, 122.9, 134.9, 136.0, 141.6, 142.0, 149.7, 151.0, 151.0, 151.2, 163.8. MS (EI) EI$^+$: 372 ([M$^+$ + Na], 5), 350 ([M$^+$ + H], 100). HPLC purity (method I) 98%.

*4.9.7. N-(4-(9H-purin-6-ylamino)phenyl)-2-methoxypyridine-3-carboxamide (**13e**)*

According to method E. Yield 89%. Yellow solid. R_f (8% EtOH/CH_2Cl_2): 0.16. M.p. 325.2–335.5 °C (decomposition). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 4.00 (s, 3H), 7.15 (t, J = 6 Hz, 2H), 7.68 (d, J = 9 Hz, 2H), 7.93 (bd, J = 9 Hz, 2H), 8.07 (dd, J = 6.3 Hz, 1H), 8.27 (s, 1H), 8.32–8.36 (m, 2H), 9.78 (s, 1H), 10.15 (s, 1H), 13.16 (bs, 1H NH9-purine). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 54.3, 117.6, 117.7, 119.8, 120.6, 120.7, 121.2, 136.3, 139.7, 139.8, 140.4, 149.3, 149.4, 152.3, 160.2, 160.3. MS (EI) EI$^+$: 384 ([M$^+$ + Na], 85), 362 ([M$^+$ + H], 100). HPLC purity (method I) 99%.

*4.9.8. N-(4-(9H-purin-6-ylamino)phenyl)-1-(3-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (**13f**)*

According to method E. Yield 68%. Yellow solid. R_f (7% EtOH/CH_2Cl_2): 0.14. M.p.: 323.5–328.1 °C (decomposition). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.73 (dd, J = 9.6 Hz, 1H), 7.39–7.44 (m, 2H), 7.54–7.62 (m, 2H), 7.67 (bd, J = 9 Hz, 2H), 7.91 (bd, J = 9 Hz, 2H), 8.12 (dd, J = 9.3 Hz, 1H), 8.30 (s, 1H), 8.38 (s, 1H), 8.59 (dd, J = 9.3 Hz, 1H), 9.86 (s, 1H), 11.84 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.3, 115.4, 116.7, 118.8, 120.3, 120.4, 121.0, 121.6, 123.8, 131.5, 133.7, 137.1, 141.0, 143.4, 143.9, 145.2, 151.4, 151.8, 152.0, 161.8, 162.1. MS (EI) EI$^+$: 464 ([M$^+$ + Na], 80), 442 ([M$^+$ + H], 100); EI$^-$: 440 ([M$^+$-H], 100). HPLC purity (method I) 97.5%.

*4.9.9. N-(4-(9H-purin-6-ylamino)phenyl)-2-oxo-1-(3-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxamide (**13i**)*

According to method E. Yield 98%. Yellow solid. R_f (7% EtOH/CH_2Cl_2): 0.18. M.p. 288.5–292.5 °C (decomposition). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.74 (t, J = 6 Hz, 1H), 6.98 (s, 1H), 7.15 (s, 1H), 7.32 (s, 1H), 7.67 (d, J = 9 Hz, 1H), 7.83–7.92 (m, 4H), 8.04 (bs, 1H), 8.16 (dd, J = 6.3 Hz, 1H), 8.29 (s, 1H), 8.37 (s, 1H), 8.60 (dd, J = 9.3 Hz, 1H), 9.82 (s, 1H), 11.79 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.7, 118.9, 120.2, 120.3, 121.1, 121.6, 124.8, 126.1, 126.4, 130.3, 131.0, 132.2, 133.3, 136.3, 140.7, 141.1, 143.9, 144.1, 145.2, 151.5, 152.3, 162.4. MS (EI) EI$^+$: 514 ([M$^+$ + Na], 55), 492 ([M$^+$ + H], 100); EI$^-$: 490 ([M$^+$-H], 100). HPLC purity (method I) 100%.

*4.9.10. N-(4-(9H-purin-6-ylamino)-3-fluorophenyl)-2-methoxybenzamide (**14a**)*

According to method E. Yield 94%. Slightly coloured white solid. R_f (8% EtOH/CH_2Cl_2): 0.19. M.p. 275.6–278.9 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.90 (s, 3H), 7.08 (dd, J = 9.6 Hz, 1H), 7.19 (d, J = 6Hz, 1H), 7.49 (t, J = 9 Hz, 2H), 7.63 (d, J = 9 Hz, 2H), 7.81 (d, J = 15 Hz, 1H), 8.26 (d, J = 3 Hz, 2H), 9.39 (bs, 1H), 10.30 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 56.2, 107.7, 112.5, 112.6, 115.7, 118.6, 120.9, 120.9, 125.0, 125.1, 127.8, 130.0, 132.9, 141.0, 152.0, 152.3, 157.0, 165.4. MS (EI) ES$^+$: 379 ([M$^+$ + H], 12), 401 ([M$^+$ + Na], 100). HPLC purity (method I) 100%.

*4.9.11. N-(4-(9H-purin-6-ylamino)-3-fluorophenyl)-4-bromo-3-methylbenzamide (**14b**)*

According to method E. Yield 85%. Light yellow solid. R_f (8% EtOH/CH_2Cl_2): 0.19. M.p. 323.2–325.4 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.45 (s, 3H), 7.56 (d, J = 9 Hz, 1H), 7.66–7.86 (m, 4H), 7.95 (s, 1H), 8.36 (d, J = 3Hz, 2H), 9.68 (bs, 1H), 10.50 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 22.9, 107.7, 108.3, 116.3, 116.4, 117.6, 121.8, 127.4 (CH), 127.5, 130.6, 132.6, 134.2, 138.0, 141.6, 151.5, 151.3, 155.1, 165.2, 165.3. MS (EI) ES$^+$: 441 ([M$^+$ + H], 100). HPLC purity (method A) 97.5%. HPLC purity (method I) 97.5%.

*4.9.12. N-(4-(9H-purin-6-ylamino)-3-fluorophenyl)-2-fluorobenzamide (**14c**)*

According to method E. Yield 88%. White solid. R_f (8% EtOH/CH_2Cl_2): 0.19. M.p.: 288.6–289.4 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.33–7.41 (m, 2H), 7.46 (d, J = 6 Hz, 1H), 7.57–7.78 (m, 4H), 8.23 (bs, 2H), 9.36 (s, 1H), 10.62 (s, 1H), 13.13 (bs, 1H NH9-purine). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.6, 107.7, 107.8, 115.4, 115.6, 116.7, 117.0, 122.6, 125.1, 127.8, 130.0, 130.3, 133.2, 140.3, 151.6, 152.6, 163.4. MS (EI) ES$^+$: 367 ([M$^+$ + H], 35), 389 ([M$^+$ + Na], 100). HPLC purity (method I) 100%.

*4.9.13. N-(4-(9H-purin-6-ylamino)-3-fluorophenyl)-2-fluoropyridine-3-carboxamide (**14d**)*

According to method E. Yield 97%. White solid. R_f (8% EtOH/CH_2Cl_2): 0.17. M.p. 298.5–299.6 °C (decomposition). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.45 (d, J = 9 Hz, 1H), 7.54 (dd, J = 6.3 Hz, 1H), 7.68–7.80 (m, 2H), 8.29 (m, 2H), 8.31 (s, 1H), 8.42 (d, J = 3 Hz, 1H), 9.52 (bs, 1H), 10.79 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.6, 107.7, 115.8, 115.9, 118.1, 119.4, 122.7, 122.8, 122.9, 127.5, 127.7, 141.6, 150.0, 151.9, 152.0, 152.0, 161.9. MS (EI) ES$^+$: 368 ([M$^+$ + H], 25), 390 ([M$^+$ + Na], 100). HPLC purity (method I) 100%.

*4.9.14. N-(4-(9H-purin-6-ylamino)-3-fluorophenyl)-2-methoxypyridine-3-carboxamide (**14e**)*

According to method E. Yield 95%. White solid. R_f (8% EtOH/CH_2Cl_2): 0.19. M.p. 207.3–210.5 °C (decomposition). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 4.00 (s, 3H), 7.17 (t, J = 6 Hz, 1H), 7.50 (t, J = 9 Hz, 1H), 7.68 (dd, J = 9.6 Hz, 1H), 7.83 (d, J = 15 Hz, 1H), 8.07 (d, J = 6 Hz, 1H), 8.36 (bs, 3H), 9.73 (bs, 1H), 10.42 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 54.3, 55.3, 107.6, 107.7, 115.8, 115.9, 117.6, 117.7, 119.4, 127.7, 139.7, 141.9, 149.6, 151.5, 151.9, 160.2, 160.3, 163.9. MS (EI) EI$^+$: 380 ([M$^+$ + H], 100). HPLC purity (method I) 100%.

*4.9.15. N-(4-(9H-purin-6-ylamino)-3-fluorophenyl)-1-(3-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (**14f**)*

According to method E. Yield 84%. Yellow solid. R_f (8% EtOH/CH_2Cl_2): 0.21. M.p. 308.8–309.8 °C (decomposition). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.73 (t, J = 6 Hz, 1H), 7.35 (d, J = 6 Hz, 2H), 7.43 (dd, J = 9.6 Hz, 2H), 7.61 (m, 1H), 7.68 (m, 1H), 7.91 (d, J = 12 Hz, 1H), 8.12 (d, J = 6 Hz, 1H), 8.30 (s, 2H), 8.59 (d, J = 6 Hz, 1H), 9.48 (bs, 1H), 12.06 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.3, 107.7, 107.8, 108.0, 115.8, 116.2, 116.4, 118.0, 118.2, 120.7, 128.2, 129.7, 136.5, 136.7, 141.3, 144.5, 144.7, 145.3, 145.5, 151.9, 152.0, 162.1, 162.3. MS (EI) EI$^+$: 460 ([M$^+$ + H], 85), 482 ([M$^+$ + Na], 100). HPLC purity (method I) 97.9%.

*4.9.16. N-(4-(9H-purin-6-ylamino)-3-fluorophenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (**14g**)*

According to method E. Yield 70%. Yellow solid. R_f (8% EtOH/CH_2Cl_2): 0.18. M.p. 314.0–320.9 °C (decomposition). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.74 (s, 1H), 7.33-7.42 (m, 4H), 7.58 (bdd, J = 12.9 Hz, 3H), 7.89 (bd, J = 12 Hz, 1H), 8.13 (bs, 1H), 8.25 (s, 1H), 8.58 (bs, 1H), 9.33 (s, 1H), 12.00 (s, 1H), 13.14 (bs, 1H NH9-pur). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.5, 107.7, 107.7, 114.4, 115.5, 115.6, 115.9, 116.5, 120.8, 122.5, 123.6, 128.4, 131.4, 140.5, 141.8, 144.1, 144.2, 144.3, 145.4, 145.4, 152.4, 161.9, 162.2. MS (EI) ES$^+$: 460 ([M$^+$ + H], 12), 482 ([M$^+$ + Na], 100). HPLC purity (method I) 91.2%.

*4.9.17. N-(4-(9H-purin-6-ylamino)-3-fluorophenyl)-2-oxo-1-(4-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxamide (**14h**)*

According to method E. Yellow solid. Yield 72%. R_f (8% EtOH/CH_2Cl_2): 0.19. M.p. 316.8–318.9 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.77 (t, J = 6 Hz, 1H), 7.32 (d, J = 9 Hz, 1H), 7.68 (dd, J = 9.6 Hz, 1H), 7.81 (d, J = 9 Hz, 2H), 7.89 (d, J = 12 Hz, 1H), 7.98 (d, J = 9 Hz, 2H), 8.16 (bd, J = 3 Hz, 1H), 8.26 (s, 2H), 8.61 (bd, J = 6 Hz, 1H), 9.37 (bs, 1H), 11.96 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.8, 107.9, 108.0, 115.6, 115.8, 120.6, 120.9, 127.5, 127.1, 128.6, 128.7, 129.6, 141.0, 143.8, 143.9, 144.0, 145.5, 152.1, 152.3, 161.8, 162.3. MS (EI) EI$^+$: 510 ([M$^+$ + H], 55), 532 ([M$^+$ + Na], 100). HPLC purity (method I) 92.1%.

*4.9.18. N-(4-(9H-purin-6-ylamino)-3-fluorophenyl)-2-oxo-1-(3-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxamide (**14i**)*

According to method E. Yield 81%. Yellow solid. R_f (8% EtOH/CH_2Cl_2): 0.23. M.p. 307.4–309.2 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.75 (dd, J = 9.6 Hz, 1H), 7.36 (d, J = 6 Hz, 1H), 7.69 (t, J = 9 Hz, 1H), 7.83–7.93 (m, 4H), 8.04 (s, 1H), 8.16 (dd, J = 6.3 Hz, 1H), 8.34 (s, 2H), 8.60 (dd, J = 6.3 Hz, 1H), 11.97 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.7, 108.0, 108.1, 115.9, 116.1, 117.6, 120.7, 124.8, 125.8, 127.7, 130.7, 131.9, 136.7, 141.6, 144.1, 144.2, 145.4, 145.5, 151.3, 151.9, 161.9, 162.4. MS (EI) EI$^+$: 509.9 ([M$^+$ + H], 35), 532 ([M$^+$ + Na], 100). HPLC purity (method I) 97.2%.

*4.9.19. 2-Methoxy-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)phenyl)benzamide (**17a**)*

According to procedure D. Yield 85%. R_f (5% EtOH/CH_2Cl_2): 0.54 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.70–1.80 (m, 3H), 2.10–2.16 (m, 3H), 3.68–3.71 (m, 1H), 3.83 (dd, J = 12.9 Hz, 1H), 4.10 (s, 3H), 5.86 (d, J = 9 Hz, 1H), 7.04–7.16 (m, 3H), 7.46–7.60 (m, 3H), 8.10 (d, J = 9 Hz, 1H), 8.28 (dd, J = 9 Hz, 2H), 8.38 (s, 1H), 8.60 (d, J = 6 Hz, 1H), 8.83 (s, 1H), 9.04 (s, 1H), 10.00 (s, 1H). MS (EI) ES$^+$: 452 ([M$^+$ + Na], 95), 430 ([M$^+$ + H], 2); ES$^-$: 344 ([M$^+$-THP], 100).

*4.9.20. 4-Bromo-3-methyl-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)phenyl)benzamide (**17b**)*

According to procedure D. Yield 99%. White solid. M.p. 214–216 °C. R_f (5% EtOH/CH_2Cl_2): 0.54 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.70–1.82 (m, 3H), 2.10–2.17 (m, 3H), 2.46 (s, 3H), 3.82 (dd, J = 12.9 Hz, 1H), 4.21 (dd, J = 12.3 Hz, 1H), 5.86 (dd, J = 9.3 Hz, 1H), 7.55–7.63 (m, 3H), 7.76 (bs, 1H), 8.14 (s, 1H), 8.17 (bs, 1H), 8.34 (s, 1H), 8.60 (d, J = 9 Hz, 1H), 8.80 (t, J = 3 Hz, 1H), 9.02 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 23.0, 24.9, 31.9, 68.9, 82.0, 120.9, 122.9, 125.7, 126.0, 129.0, 129.5, 129.7, 131.1, 132.7, 133.9, 136.4, 138.3, 138.7, 142.3, 151.8, 152.4, 154.1, 165.1. MS (EI) ES$^+$: 492 ([M$^+$ + H], 100); ES$^-$: 490 ([M$^+$-H], 100).

*4.9.21. 2-Fluoro-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)phenyl)benzamide (**17c**)*

According to procedure D. Yield 94%. R_f (5% EtOH/CH_2Cl_2): 0.70 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.70–1.86 (m, 3H), 2.09–2.21 (m, 3H), 3.82 (dd, J = 12.9 Hz, 1H), 4.22 (bd, J = 9 Hz, 1H), 5.87 (dd, J = 9.3 Hz, 1H), 7.15–7.21 (m, 2H), 7.29–7.34 (m, 1H), 7.52–7.62 (m, 2H), 7.98–8.04 (m, 1H), 8.11–8.21 (m, 2H), 8.41 (s, 1H), 8.60 (d, J = 9 Hz, 1H), 8.70 (d, J = 15 Hz, 1H), 8.83 (s, 1H), 9.05 (s, 1H). MS (EI) ES$^+$: 440 ([M$^+$ + Na], 25), 356 (100); ES$^-$: 332 ([M$^+$-THP], 100).

*4.9.22. 2-Fluoro-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)phenyl)pyridine-3-carboxamide (**17d**)*

According to procedure D. Yield 96%. R_f (5% EtOH/CH_2Cl_2): 0.60 ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.70–1.80 (m, 3H), 2.10–2.17 (m, 3H), 3.82 (dd, J = 12.9 Hz, 1H), 4.22 (d, J = 9 Hz, 1H), 5.87 (d, J = 12 Hz, 1H), 7.43 (m, 1H), 7.60 (dd, J = 9.6 Hz, 1H), 8.06 (bd, J = 9 Hz, 1H), 8.37 (bs, 2H), 8.67 (bd, J = 9 Hz, 2H), 8.71–8.79 (m, 1H), 8.92 (s, 1H), 9.04 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): MS (EI) ES$^+$: 441 ([M$^+$ + Na], 100), 419 ([M$^+$ + H], 2); ES$^-$: 333 ([M$^+$-THP], 100).

*4.9.23. 2-Methoxy-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)phenyl)pyridine-3-carboxamide (**17e**)*

According to procedure D. Yield 86%. White solid. M.p. 150–152 °C. R_f (5% EtOH/CH_2Cl_2): 0.50; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.71–1.80 (m, 3H), 2.10–2.17 (m, 3H), 3.15–3.19 (m, 1H), 3.84 (dd, J = 12.9 Hz, 1H), 4.24 (s, 3H), 5.88 (d, J = 9 Hz, 1H), 7.13 (dd, J = 9.6 Hz, 1H), 7.59 (t, J = 9 Hz, 1H), 8.08 (d, J = 9 Hz, 1H), 8.34 (bd, J = 3 Hz, 1H), 8.38 (s, 1H), 8.63 (dd, J = 9.3 Hz, 2H), 8.88 (s, 1H), 9.04 (s, 1H), 10.01 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.8, 55.8, 68.9, 82.0, 116.2, 118.2, 121.3, 123.3, 126.2, 129.5, 131.2, 136.4, 138.4, 141.9, 142.4, 150.0, 152.4, 154.3, 160.3, 162.0. MS (EI) ES$^+$: 453 ([M$^+$ + Na], 100), 431 ([M$^+$ + H], 3); ES$^-$: 429 ([M$^+$-H], 25), 313 ([M$^+$-H-THP-OMe], 100).

*4.9.24. 1-(3-Fluorophenyl)-2-oxo-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)phenyl)-1,2-dihydropyridine-3-carboxamide (**17f**)*

According to procedure D. Yield 91%. White solid. M.p. 196–198 °C. R_f (5% EtOH/CH_2Cl_2): 0.68; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.69–1.81 (m, 3H), 2.09–2.15 (m, 3H), 3.78–3.86 (m, 1H), 4.19–4.22 (m, 1H), 5.85 (d, J = 9 Hz, 1H), 6.59 (t, J = 6 Hz, 1H), 7.24–7.29 (m, 2H), 7.40–7.45 (m, 2H), 7.50–7.61 (m, 1H), 8.01 (m, 1H), 8.34 (s, 1H), 8.57 (d, J = 6 Hz, 1H), 8.77 (d, J = 6 Hz, 1H), 8.99 (bs, 1H), 9.03 (s, 1H), 11.96 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.8, 68.9, 81.9, 107.2, 116.7, 117.0, 121.4, 122.5, 123.0, 125.9, 128.4, 128.6, 129.3, 131.2, 136.3, 138.7, 141.4, 142.2, 145.1, 151.7, 152.3, 154.5, 161.5, 162.5, 164.4, 208.4. MS (EI) ES$^+$: 533 ([M$^+$ + Na], 85), 511 ([M$^+$ + H], 5).

*4.9.25. 1-(4-Fluorophenyl)-2-oxo-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)phenyl)-1,2-dihydropyridine-3-carboxamide (**17g**)*

According to procedure D. Yield 94%. White solid. M.p. (decomposition) > 200 °C. R_f (5% EtOH/CH_2Cl_2): 0.68; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.69–1.81 (m, 3H), 2.09–2.15 (m, 3H), 3.78–3.86 (m, 1H), 4.19–4.22 (m, 1H), 5.85 (d, J = 9 Hz, 1H), 6.59 (t, J = 6 Hz, 1H), 7.24–7.29 (m, 2H), 7.40–7.45 (m, 2H), 7.50–7.61 (m, 1H), 8.01 (m, 1H), 8.34 (s, 1H), 8.57 (d, J = 6 Hz, 1H), 8.77 (d, J = 6 Hz, 1H), 8.99 (bs, 1H), 9.03 (s, 1H), 11.96 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.8, 68.9, 81.9, 107.2, 116.7, 117.0, 121.4, 122.5, 123.0, 125.9, 128.4, 128.6, 129.3, 131.2, 136.3, 138.7, 141.4, 142.2, 145.1, 151.7, 152.3, 154.5, 161.5, 162.5, 164.4, 208.4. MS (EI) ES$^+$: 533 ([M$^+$ + Na], 85), 511 ([M$^+$ + H], 5).

*4.9.26. 2-Oxo-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)phenyl)-1-(4-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxamide (**17h**)*

According to procedure D. Yield 87%. White solid. M.p. 190–192 °C. R_f (5% EtOH/CH_2Cl_2): 0.46; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.71–1.81 (m, 3H), 2.11–2.15 (m, 3H), 3.82 (dd, J = 12.9 Hz, 1H), 4.20 (bd, J = 9 Hz, 1H), 5.86 (dd, J = 6.3 Hz, 1H), 6.62 (t, J = 6 Hz, 1H), 7.54 (dd, J = 9.6 Hz, 2H), 7.60 (d, J = 6 Hz, 2H), 7.87 (d, J = 9 Hz, 2H), 8.03 (d, J = 6 Hz, 1H), 8.33 (s, 1H), 8.58 (d, J = 9 Hz, 1H), 8.79 (dd, J = 9.3 Hz, 1H), 8.99 (s, 1H), 9.03 (s, 1H), 11.87 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 22.8, 24.9, 31.8, 68.9, 81.9, 107.5, 121.4, 122.8, 122.9, 125.9, 127.0, 127.3, 129.3, 131.2, 136.4, 138.7, 140.7, 142.2, 142.9, 145.3, 151.7, 152.3, 154.5, 161.2, 162.2. MS (EI) ES$^+$: 583 ([M$^+$ + Na], 100), 561 ([M$^+$ + H], 2), 499 ([M$^+$ + Na-THP+H), 45).

*4.9.27. 2-Oxo-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl)phenyl)-1-(3-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxamide (**17i**)*

According to procedure D. Yield 89%. Slightly coloured solid (beige). M.p. 200–202 °C. R_f (5% EtOH/CH_2Cl_2): 0.47; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.69–1.91 (m, 3H), 2.08–2.14 (m, 3H), 3.82 (dd, J = 12.9 Hz, 1H), 4.20 (bd, J = 12 Hz, 1H), 5.85 (dd, J = 12.3 Hz, 1H), 6.63 (dd, J = 9.6 Hz, 1H), 7.54–7.79 (m, 6H), 8.02 (d, J = 9 Hz, 1H), 8.33 (s, 1H), 8.58 (d, J = 9 Hz, 1H), 8.79 (dd, J = 6.3 Hz, 1H), 8.99 (s, 1H), 9.03 (s, 1H), 11.87 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): MS (EI) ES$^+$: 583 ([M$^+$ + Na], 100), 561 ([M$^+$ + H], 5), 499 ([M$^+$ + Na-THP + H), 15).

*4.9.28. N-(3-(9H-purin-6-yl)phenyl)-2-methoxybenzamide (**18a**)*

According to method E. Yield 65%. White solid. R_f (8% EtOH/CH_2Cl_2): 0.34. M.p. 189.5–192.1 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.92 (s, 3H), 7.08 (t, J = 6 Hz, ^{1}H), 7.20 (d, J = 9 Hz, 1H), 7.49–7.58 (m, 2H), 7.66 (d, J = 6 Hz, 1H), 7.90 (d, J = 9 Hz, 1H), 8.66 (bs, 2H), 8.97 (s, 1H), 9.10 (bs, 1H), 10.34 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 56.2, 112.4, 112.5, 120.6, 120.7, 120.9, 121.7, 125.3, 125.8, 129.6, 130.0, 132.2, 136.5, 139.8, 145.5, 152.3, 157.0, 153.7. MS (EI) EI$^+$: 346 ([M$^+$ + H], 15), 368 ([M$^+$ + Na], 100); MS (EI) EI$^-$: 344 ([M$^+$-H], 100). HRMS-ESI (m/z) calcd for $C_{19}H_{16}N_5O_2$ (M + 1) 346.1304, found: 346.1295. HPLC purity (method I) 97.8%.

*4.9.29. N-(3-(9H-purin-6-yl)phenyl)-4-bromo-3-methylbenzamide (**18b**)*

According to method E. White solid. Yield 94%. R_f (8% EtOH/CH_2Cl_2): 0.23. M.p. 323.1–324.7 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.45 (s, 3H), 7.58 (dd, J = 9.6 Hz, 1H), 7.77 (s, 2H), 8.01 (s, 2H), 8.66 (s, 2H), 8.97 (s, 1H), 9.10 (s, 1H), 10.53 (s, 1H), 13.70 (bs, 1H NH9-purine). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 22.9, 121.6, 122.4, 123.5, 126.1, 127.4, 128.1, 129.3, 129.8, 130.6, 132.6, 134.6, 136.7, 139.8, 145.5, 152.8, 152.3, 154.5, 165.4. MS (EI) EI$^+$: 408 ([M$^+$ + H], 25), 430 ([M$^+$ + Na], 100); MS (EI) EI$^-$: 406 ([M$^+$-H], 85). H], 85). HRMS-ESI (m/z) calcd for $C_{19}H_{15}BrN_5O$ (M + 1) 408.0460, found: 408.0451. HPLC purity (method II) 100%.

*4.9.30. N-(3-(9H-purin-6-yl)phenyl)-2-fluorobenzamide (**18c**)*

According to method E. White solid. Yield 98%. R_f (8% EtOH/CH_2Cl_2): 0.23. M.p. 275.0–282.5 °C (decomposition) ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.32–7.39 (m, 2H), 7.58 (dd, J = 9.6 Hz, 2H), 7.71 (dt, J = 6.3 Hz, 1H), 7.93 (d, J = 6 Hz, 1H), 8.65 (s, 2H), 8.97 (s, 1H), 9.09 (bs, 1H), 10.68 (s, 1H), 13.64 (bs, 1H NH9-purine). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 116.6, 116.7, 120.9, 121.9, 122.9, 125.1, 125.8, 129.7, 130.3, 130.4, 132.9, 136.5, 139.5, 145.5, 152.3, 153.0, 154.1, 163.6. MS (EI) EI$^+$: 334 ([M$^+$ + H], 15), 356 ([M$^+$ + Na], 100); MS (EI) EI$^-$: 332 ([M$^+$-H], 100). HRMS-ESI (m/z) calcd for $C_{19}H_{13}FN_5O$ (M + 1) 334.1104, found: 334.1104. HPLC purity (method I) 100%.

*4.9.31. N-(3-(9H-purin-6-yl)phenyl)-2-fluoropyridine-3-carboxamide (**18d**)*

According to method E. Yield 98%. Yellow solid. R_f (8% EtOH/CH_2Cl_2): 0.13. M.p. 268.5–272.3 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.52 (dd, J = 6.3 Hz, 1H), 7.60 (t, J = 9 Hz, 1H), 7.92 (d, J = 9 Hz, 1H), 8.28 (t, J = 9 Hz, 1H), 8.40 (d, J = 3 Hz, 1H), 8.63 (s, 1H), 8.63–8.67 (m, 1H), 8.96 (s, 1H), 9.09 (s, 1H), 10.85 (s, 1H), 13.69 (bs, 1H NH9-purine). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 119.6, 120.9, 121.8, 122.5, 122.9, 126.0, 129.7, 137.0, 139.2, 141.9, 145.5,

149.8, 150.0, 150.1, 152.2, 152.3, 152.8. MS (EI) EI$^+$: 335 ([M$^+$ + H], 35), 357 ([M$^+$ + Na], 100); MS (EI) EI$^-$: 333 ([M$^+$-H], 100). HRMS-ESI (m/z) calcd for $C_{17}H_{12}FN_6O$ (M + 1) 335.1057, found: 335.1048. HPLC purity (method I) 100%.

4.9.32. N-(3-(9H-purin-6-yl)phenyl)-2-methoxypyridine-3-carboxamide (**18e**)

According to method E. Yield 94%. Slightly coloured beige solid. R_f (8% EtOH/CH_2Cl_2): 0.24. M.p. 233.5–241.3 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 4.00 (s, 3H), 7.17 (dd, J = 9.3 Hz, 1H), 7.58 (t, J = 9 Hz, 1H), 7.92 (d, J = 6 Hz, 1H), 8.08 (d, J = 6 Hz, 1H), 8.35 (dd, J = 6.3 Hz, 1H), 8.66 (bs, 2H), 8.97 (s, 1H), 9.12 (bs, 1H), 10.45 (s, 1H), 13.67 (bs, 1H NH9-purine). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 54.2, 117.7, 119.6, 120.8, 121.6, 122.8, 125.9, 129.5, 138.2, 139.5, 139.6, 145.3, 149.4, 152.2, 152.3, 160.3, 160.5, 163.9. MS (EI) EI$^+$: 347 ([M$^+$ + H], 55), 369 ([M$^+$ + Na], 100). HRMS-ESI (m/z) calcd for $C_{18}H_{15}N_6O_2$ (M + 1) 347.1256, found: 347.1250. HPLC purity (method I) 95.8%.

4.9.33. N-(3-(9H-purin-6-yl)phenyl)-1-(3-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (**18f**)

According to method E, **18f** was obtained in 85% yield. R_f (8% EtOH/CH_2Cl_2): 0.28. M.p. 315.5–316.7 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.74 (dd, J = 9.6 Hz, 1H), 7.43 (t, J = 9 Hz, 2H), 7.55–7.65 (m, 3H), 8.03 (d, J = 6 Hz, 1H), 8.13 (dd, J = 6.3 Hz, 1H), 8.61 (dt, J = 6.3 Hz 2H), 8.67 (s, 1H), 8.96 (s, 1H), 9.02 (s, 1H), 12.15 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.7, 116.4, 120.9, 120.9, 121.5, 121.6, 122.5, 125.5, 129.7, 129.8, 129.9, 136.7, 137.7, 144.1, 144.5, 145.3, 152.3, 154.4, 154.5, 162.0. MS (EI) EI$^+$: 427 ([M$^+$ + H], 25), 449 ([M$^+$ + Na], 100); MS (EI) EI$^-$: 425 ([M$^+$-H], 100). HRMS-ESI (m/z) calcd for $C_{23}H_{16}FN_6O_2$ (M + 1) 427.1319, found: 427.1316. HPLC purity (method I) 96.9%.

4.9.34. N-(3-(9H-purin-6-yl)phenyl)-1-(4-fluorophenyl)-2-oxo-1,2-dihydropyridine-3-carboxamide (**18g**)

According to method E, **18g** was obtained in 61% yield. R_f (8% EtOH/CH_2Cl_2): 0.23. M.p. 314.7–319.2 °C (decomposition) ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.74 (dd, J = 9.6 Hz, 1H), 7.42 (t, J = 9 Hz, 2H), 7.56–7.62 (m, 3H), 7.98 (d, J = 9 Hz, 1H), 8.08 (d, J = 6 Hz, 1H), 8.61 (m, 3H), 8.94 (s, 1H), 9.07 (s, 1H NH), 12.16 (s, 1H CH), 13.66 (bs, 1H NH9-purine). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.0, 115.9, 116.2, 120.4, 121.9, 124.9, 129.2, 129.4, 130.0, 136.3, 136.4, 138.5, 143.9, 144.8, 151.6, 151.7, 153.5, 160.2, 161.3, 161.9, 163.5. MS (EI) EI$^+$: 427 ([M$^+$ + H], 2), 449 ([M$^+$ + Na], 100). HRMS-ESI (m/z) calcd for $C_{23}H_{16}FN_6O_2$ (M + 1) 427.1319, found: 427.1318. HPLC purity (method I) 100%.

4.9.35. N-(3-(9H-purin-6-yl)phenyl)-2-oxo-1-(4-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxamide (**18h**)

According to method E, **18h** was obtained in 81% yield. R_f (5% EtOH/CH_2Cl_2): 0.13. M.p.: 315.5–317.4 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.77 (t, J = 6 Hz, 1H), 7.58 (dd, J = 9.6 Hz, 1H), 7.81 (d, J = 6 Hz, 2H), 7.96–8.01 (m, 3H), 8.14 (dd, J = 6.3 Hz, 1H), 8.57 (d, J = 9 Hz, 1H), 8.61 (d, J = 3 Hz, 1H), 8.63 (s, 1H), 8.94 (s, 1H), 8.98 (bs, 1H), 12.05 (s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 108.0, 120.9, 121.1, 121.4, 122.5, 125.5, 127.1, 127.1, 128.7, 130.0, 130.2, 137.6, 143.7, 143.9, 145.2, 145.5, 152.3, 161.9, 162.4. MS (EI) EI$^+$: 499 ([M$^+$ + Na], 100), 477 ([M$^+$ + H], 5). HRMS-ESI (m/z) calcd for $C_{24}H_{16}F_3N_6O_2$ (M + 1) 477.1297, found: 477.1274. HPLC purity (method I) 100%.

4.9.36. N-(3-(9H-purin-6-yl)phenyl)-2-oxo-1-(3-(trifluoromethyl)phenyl)-1,2-dihydropyridine-3-carboxamide (**18i**)

According to method E, **18i** was obtained in 78% yield. R_f (5% EtOH/CH_2Cl_2): 0.14. M.p.: 301.5–306.6 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.78 (dd, J = 9.6 Hz, 1H), 7.59 (dd, J = 9.6 Hz, 1H), 7.85 (dd, J = 9.6 Hz, 1H), 7.94 (dd, J = 9.6 Hz, 2H), 8.03 (bd, J = 9 Hz, 1H), 8.08 (bs, 1H), 8.21 (dd, J = 6.3 Hz, 1H), 8.64 (dd, J = 9.3 Hz, 2H), 8.67 (s, 1H), 8.96 (s, 1H), 9.06 (bs, 1H), 12.07 (s, 1H), 13.64 (bs, 1H NH9-purine). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 107.7, 120.6, 121.6, 121.9, 122.4, 124.1, 122.7, 126.2, 129.7, 130.2, 131.0, 132.2, 133.3, 138.0, 141.8, 144.0, 144.2, 145.6, 152.2, 154.4, 154.7, 163.8. MS (EI) EI$^+$: 499 ([M$^+$ + Na], 100). HRMS-ESI (m/z) calcd for $C_{24}H_{16}F_3N_6O_2$ (M + 1) 477.1287, found: 477.1289. HPLC purity (method I) 98%.

4.10. General procedure F of palladium-anilination of 6-chloro-9-THP-purine [24]

In a flamed sealed tube, $Pd(OAc)_2$ (19 mg, 0.08 mmol), and *X-phos* (80 mg, 0.17 mmol) were introduced in a deoxygenated mixture of *t*-BuOH/H_2O (3/1 mL). The tube was heated at 110 °C for 1 min. Afterwards, **1** (200 mg, 0.84 mmol), the aniline compound (1.26 mmol) and K_2CO_3 (290 mg, 2.09 mmol) were added to the reaction mixture and heated at 110 °C for 15 h. At room temperature, the reaction mixture was diluted into EtOAc and filtered through a short pad of celite. The filtrate was extracted with brine and the organic phase dried over anhydrous $MgSO_4$ and filtered off. After concentration *in vacuo*, the residue was purified by column chromatography over silica gel, using EtOH: CH_2Cl_2 mixtures as eluent.

4.10.1. N-Phenyl-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (**19a**)

Following the general procedure F, **19a** was obtained in 100% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (s, 1H), 8.07 (s, 1H), 7.79 (t, J = 7.7 Hz, 3H), 7.39 (t, J = 7.9 Hz, 1H), 7.12 (t, J = 7.4 Hz, 1H), 5.75 (dd, J = 10.0, 2.7 Hz, 1H), 4.15 (dd, J = 19.1, 10.4 Hz, 1H), 3.93–3.66 (m, 1H), 1.99 (m 3H), 1.68 (m 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.0, 152.3, 148.9, 138.6, 138.4, 129.1, 123.6, 120.3, 81.9, 68.9, 31.9, 24.9, 22.8. MS (ES$^+$) m/z (%): 296(100) [M + H]$^+$, 297(20) [M-H]$^+$, 318(20) [M + Na]$^+$, 319(5) [M + Na]$^+$.

4.10.2. 9-(Tetrahydro-2H-pyran-2-yl)-N-(3-(trifluoromethyl)phenyl)-9H-purin-6-amine (**19b**)

Following the general procedure F, **19b** was obtained in 97% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.26 (d, J = 18.2 Hz, 2H), 8.08 (s, 1H), 7.98 (d, J = 8.2 Hz, 1H), 7.47 (dd, J = 10.2, 5.6 Hz, 1H), 7.34 (d, J = 7.7 Hz, 1H), 7.00 (s, OH), 5.77 (dd, J = 10.2, 2.5 Hz, 1H), 4.27–4.15 (m, 1H), 3.80 (td, J = 11.6, 2.9 Hz, 1H), 2.25–1.95 (m, 3H), 1.90–1.56 (m, 6H), 1.33–1.22 (m, 3H), 0.96 (d, J = 6.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.8, 151.9, 149.1, 145.9, 139.5, 138.8, 129.4, 123.1, 120.3, 119.7, 116.7, 82.0, 68.9, 31.9, 24.8, 22.8. MS (ES$^+$) m/z (%): 364(100) [M + H]$^+$, 365(15) [M + H]$^+$.

4.10.3. N-(3-fluorophenyl)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (**19c**)

Following the general procedure F **19c** was obtained in 87% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (s, 1H), 8.25 (d, J = 14.7 Hz, 1H), 8.06 (s, 1H), 7.90 (dt, J = 11.4, 2.1 Hz, 1H), 7.40–7.33 (m, 1H), 7.31–7.22 (m, 1H), 6.77 (td, J = 8.2, 1.6 Hz, 1H), 5.74 (dd, J = 10.0, 2.5 Hz, 1H), 4.21–4.07 (m, 1H), 3.77 (td, J = 11.4, 2.7 Hz, 1H), 2.22–1.93 (m, 3H), 1.84–1.55 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.6, 161.4, 152.8, 151.9, 149.0, 140.5, 140.4, 138.7, 130.0, 129.9, 120.3, 115.3, 110.0, 109.7, 107.6, 107.3, 82.0, 68.8, 31.8, 24.9, 22.8. MS (ES$^+$) m/z (%): 314 (100) [M + H]$^+$.

4.10.4. N-(4-fluorophenyl)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (**19d**)

Following the general procedure F **19d** was obtained in 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (s, 1H), 8.38 (s, 1H), 8.01 (s,

1H), 7.76–7.63 (m, 2H), 7.03 (t, $J = 8.7$, 2H), 5.72 (dd, $J = 10.2$, 2.4 Hz, 1H), 4.13 (dd, $J = 13.3$, 9.7 Hz, 1H), 3.76 (td, $J = 11.5$, 2.7 Hz, 1H), 2.15–1.94 (m, 3H), 1.71 (ddd, $J = 26.3$, 11.7, 8.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.7, 157.6, 153.3–152.3 (m), 148.9, 138.6–138.2 (d), 134.6, 122.6–122.14 (m), 120.0, 116.1–115.1 (m), 81.9, 68.8, 31.9, 24.9, 22.8. MS (ES^+) m/z (%): 314(100) $[M + H]^+$.

4.11. General procedure G for the 8-iodination of 6-chloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine **1** [22,23]

In a dry flask, a solution of lithium amide (LDA) was prepared *in situ* from *n*-butyllithium (*n*-BuLi, 1.6 M in hexane) and diisopropylamine in freshly distilled THF (Na/benzophenone). The mixture was stirred for 30 min at −78 °C under positive pressure of argon. A solution of the appropriate purine in freshly distilled THF was then added dropwise and stirred over 1 h with LDA at −78 °C. A solution of the electrophile (I_2) in THF was then added dropwise (unless mentioned otherwise) and the resulting mixture was stirred over 1 h while maintaining the temperature at −78 °C, before it was warmed to room temperature. Water was added and the mixture extracted twice with AcOEt. The organic layers were combined, dried over $MgSO_4$ and evaporated under *vacuum*. The crude material was purified by flash chromatography on silica gel eluting with a gradient of DCM/EtOH (0–1 %).

4.11.1. 8-Iodo-N-phenyl-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (**20a**)

Following the general procedure G from **19a**, **20a** was obtained in 70% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.79 (d, $J = 7.6$ Hz, 2H), 7.64 (s, 1H), 7.40 (t, $J = 8.0$ Hz, 2H), 7.13 (t, $J = 7.4$ Hz, 1H), 5.65 (dd, $J = 11.2$, 2.4 Hz, 1H), 4.25 (d, $J = 11.9$ Hz, 1H), 3.76 (dd, $J = 12.8$, 10.8 Hz, 1H), 3.15 (ddd, $J = 24.3$, 12.7, 4.0 Hz, 1H), 2.14 (s, 1H), 1.90 (d, $J = 12.2$ Hz, 2H), 1.83–1.71 (m, 3H), 1.65 (d, $J = 14.4$ Hz, 1H). MS (ES^+) m/z (%): 422 (100)$[M + H]^+$, 338 (25))$[M\text{-}THP]^+$.

4.11.2. 8-Iodo-9-(tetrahydro-2H-pyran-2-yl)-N-(3-(trifluoromethyl)phenyl)-9H-purin-6-amine (**20b**)

Following the general procedure G from **19b**, **20b** was obtained in 48% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.22 (s, 1H), 7.95 (d, $J = 8.2$ Hz, 1H), 7.71 (s, 1H), 7.50 (t, $J = 7.9$ Hz, 1H), 7.37 (d, $J = 7.7$ Hz, 1H), 5.67 (dd, $J = 11.2$, 2.4 Hz, 1H), 4.25 (d, $J = 11.7$ Hz, 1H), 3.78 (td, $J = 11.8$, 2.7 Hz, 1H), 3.15 (ddd, $J = 24.2$, 12.8, 4.2 Hz, 1H), 2.17 (d, $J = 10.8$ Hz, 1H), 1.97–1.73 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.4, 151.0, 150.4, 139.1, 131.3, 129.5, 123.6, 122.9, 120.0, 116.7, 116.6, 99.0, 86.8, 69.3, 29.1, 24.7, 23.4.

4.11.3. N-(3-fluorophenyl)-8-iodo-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (**20c**)

Following the general procedure G from **19c**, **20c** was obtained in 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.89 (d, $J = 11.1$ Hz, 1H), 7.66 (s, 1H), 7.41–7.20 (m, 3H), 6.79 (d, $J = 7.7$ Hz, 1H), 5.64 (d, $J = 11.1$ Hz, 1H), 4.24 (d, $J = 10.8$ Hz, 1H), 3.75 (t, $J = 11.5$ Hz, 1H), 3.26–3.03 (m, 1H), 1.99–1.59 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.7, 161.5, 152.4, 150.9, 150.4, 140.1, 140.0, 130.2, 130.0, 123.6, 115.2, 110.3, 110.0, 107.6, 107.2, 98.9, 86.8, 77.5, 77.0, 76.6, 69.3, 29.0, 24.7, 23.4. MS (ES^+) m/z (%): 440(100) $[M + H]^+$.

4.11.4. N-(4-fluorophenyl)-8-iodo-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (**20d**)

Following the general procedure G from **19d**, **20d** was obtained in 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (s, 1H), 7.71 (d, $J = 4.5$ Hz, 2H), 7.54 (s, 1H), 7.26 (s, 1H), 7.07 (t, $J = 7.4$ Hz, 2H), 5.63 (d, $J = 11.0$ Hz, 1H), 4.23 (d, $J = 10.2$ Hz, 1H), 3.75 (t, $J = 11.4$ Hz, 1H), 3.25–3.04 (m, 1H), 2.24–2.09 (m, 1H), 2.02–1.59 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.8, 157.6, 152.6, 150.8, 150.7, 134.3, 123.4, 122.2, 122.1, 115.9, 115.6, 98.4, 86.8, 69.3, 29.1, 24.7, 23.4. MS (ES^+) m/z (%): 440(100) $[M + H]^+$, 441(20) $[M + H]^+$, 356(100) $[M\text{-}THP]^+$, 357(20) $[M\text{-}THP]^+$.

4.12. General procedure H for the Suzuki reaction with 8-iodo-9-(tetrahydro-2H-pyran-2-yl)-9H-purin compounds [34]

In a flamed sealed tube under argon, the appropriate compound (**20a**–**d**) (1eq), tetrakis(triphenylphosphine)palladium (10 mol%) and potassium carbonate (3eq) in a mixture of dioxane/water 4/1 were warmed at 85 °C for 5 min. Then 3-aminophenylboronic acid (1.5equiv) was added and the reaction mixture stirred at 100 °C till complete disappearance of the starting material on TLC plate. The crude material was purified by column chromatography over silica gel, using cyclohexane/ethyl acetate as eluent to afford the desired products **21a**–**d**.

4.12.1. 8-(3-Aminophenyl)-N-phenyl-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (**21a**)

Following the general procedure H from **20a**, **21a** was obtained in 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (s, 1H), 7.95 (s, 1H), 7.80 (d, $J = 7.8$ Hz, 2H), 7.33 (dt, $J = 23.7$, 6.7 Hz, 4H), 7.20–7.01 (m, 3H), 6.85 (d, $J = 7.4$ Hz, 1H), 5.54 (d, $J = 10.0$ Hz, 1H), 4.20 (t, $J = 17.8$ Hz, 1H), 4.01 (s, 2H), 3.66 (t, $J = 11.4$ Hz, 1H), 3.16 (dd, $J = 22.5$, 10.4 Hz, 1H), 2.06–1.49 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.4, 152.1, 152.0, 151.0, 147.0, 138.9, 130.9, 129.8, 129.0, 123.3, 120.2, 120.1, 119.5, 117.0, 115.9, 84.3, 68.9, 28.6, 24.7, 23.5. MS (ES^+) m/z (%): 409(30) $[M + Na]^+$, 387(100) $[M + H]^+$, 353(100) $[M\text{-}THP]^+$.

4.12.2. 8-(3-Aminophenyl)-9-(tetrahydro-2H-pyran-2-yl)-N-(3-(trifluoromethyl)phenyl)-9H-purin-6-amine (**21b**)

Following the general procedure H from **20b**, **21b** was obtained in 75% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.20 (s, 1H), 7.98 (s, 2H), 7.47 (t, $J = 7.3$ Hz, 1H), 7.39–7.24 (m, 3H), 6.88 (d, $J = 7.3$ Hz, 1H), 5.56 (d, $J = 10.7$ Hz, 1H), 4.25 (d, $J = 9.8$ Hz, 1H), 3.98 (s, 2H), 3.68 (t, $J = 11.6$ Hz, 1H), 3.17 (d, $J = 11.6$ Hz, 1H), 1.97–1.52 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.6, 152.3, 151.6, 151.2, 147.0, 139.6, 130.8, 129.8, 129.5, 122.7, 120.4, 119.5, 117.1, 116.4, 115.9, 84.3, 68.9, 28.6, 24.7, 23.4. MS (ES^+) m/z (%): 455 (100)$[M + H]^+$.

4.12.3. 8-(3-Aminophenyl)-N-(3-fluorophenyl)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (**21c**)

Following the general procedure H from **20c**, **21c** was obtained in 71% yield. Mp 208 °C (Kofler). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (s, 1H), 7.98–7.82 (m, 2H), 7.43–7.22 (m, 4H), 6.82 (dd, $J = 32.5$, 7.0 Hz, 2H), 5.55 (d, $J = 10.9$ Hz, 1H), 4.25 (d, $J = 10.8$ Hz, 1H), 3.96 (s, 2H), 3.67 (t, $J = 11.7$ Hz, 1H), 3.17 (dd, $J = 24.5$, 12.0 Hz, 1H), 2.06–1.67 (m, 4H), 1.60 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.8, 161.5, 152.5, 152.3, 151.6, 151.1, 147.0, 140.6, 130.8, 130.1, 129.8, 120.4, 119.5, 117.0, 115.8, 115.0, 109.9, 109.6, 107.4, 107.0, 84.3, 68.9, 28.6, 24.7, 23.4. MS (ES^-) m/z (%): 403(80) $[M - H]^-$, 404(30) $[M\text{-}H]^-$.

4.12.4. 8-(3-Aminophenyl)-N-(4-fluorophenyl)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine (**21d**)

Following the general procedure H from **20d**, **21d** was obtained in 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, $J = 7.7$ Hz, 1H), 7.61–7.53 (m, 2H), 7.49 (dd, $J = 7.6$, 2.8 Hz, 3H), 7.34 (t, $J = 7.8$ Hz, 1H), 7.21–7.03 (m, 4H), 6.91–6.85 (m, 1H), 5.56 (t, $J = 11.2$, 2.1 Hz, 1H), 4.26 (d, $J = 8.6$ Hz, 1H), 3.75–3.62 (m, 1H), 3.31–3.04 (m, 1H), 2.04–1.62 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.0, 161.6, 158.8, 152.4, 151.7, 151.2, 139.5, 138.2, 134.1, 134.0, 132.3, 131.5, 131.1, 130.6, 129.6, 129.4, 129.3, 125.9, 125.8, 125.2, 122.8, 122.5, 122.3, 121.8, 121.1, 121.0, 120.6, 120.4, 119.5, 116.5, 116.4, 116.1, 84.3, 68.9, 28.7, 24.7, 23.4. MS (ES^-) m/z (%): 403 (100) $[M\text{-}H]^-$.

*4.12.5. 2-Methoxy-N-(3-(6-(phenylamino)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-8-yl)phenyl)benzamide (**22f**)*

Following the general procedure D from **21a**, **22f** was obtained in 74% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.00 (s, 1H), 8.60 (s, 1H), 8.32 (d, J = 7.5 Hz, 1H), 8.10–7.96 (m, 2H), 7.88–7.74 (m, 3H), 7.55 (dd, J = 15.5, 8.3 Hz, 3H), 7.39 (t, J = 7.2 Hz, 2H), 7.12 (dt, J = 17.0, 8.0 Hz, 3H), 5.63 (d, J = 11.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.48, 157.3, 152.6, 152.0, 151.5, 151.0, 139.0, 138.8, 133.6, 132.7, 130.7, 129.6, 129.1, 125.1, 123.4, 122.5, 121.8, 121.6, 121.4, 120.3, 120.1, 111.6, 100.0, 84.3, 77.5, 77.0, 76.6, 68.9, 56.3, 30.2, 28.8, 26.9, 24.7, 23.4. MS (ES^+) m/z (%): 543(45) $[M + Na]^+$, 521(50) $[M + H]^+$, 437(100) $[M\text{-}THP]^+$. HPLC purity (method I): 100%.

*4.12.6. 2-Fluoro-N-(3-(6-(phenylamino)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-8-yl)phenyl)benzamide (**22g**)*

Following the general procedure D from **21a**, **22g** was obtained in 68% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.59 (s, 1H), 8.20 (t, J = 7.5 Hz, 1H), 8.14 (s, 1H), 7.93 (d, J = 7.3 Hz, 1H), 7.82 (d, J = 7.5 Hz, 2H), 7.74 (s, 1H), 7.66 (d, J = 6.8 Hz, 1H), 7.57 (t, J = 6.8 Hz, 2H), 7.44–7.31 (m, 3H), 7.20 (d, J = 10.9 Hz, 1H), 7.10 (t, J = 6.8 Hz, 1H), 5.64 (d, J = 10.9 Hz, 1H), 4.29 (d, J = 10.6 Hz, 1H), 3.75 (t, J = 11.9 Hz, 1H), 3.20–3.02 (m, 1H), 1.90–1.72 (m, 3H), 1.66–1.50 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.5, 158.8, 152.6, 152.1, 151.3, 151.0, 138.8, 138.3, 134.2, 134.1, 132.4, 131.0, 129.7, 129.1, 125.8, 125.3, 123.4, 122.4, 121.8, 121.1, 120.9, 120.3, 120.2, 116.4, 116.1, 84.2, 68.9, 28.8, 24.7, 23.4. MS (ES^-) m/z (%): 507(100) $[M\text{-}H]^-$. HPLC purity (method I) 100%.

*4.12.7. 2-Methoxy-N-(3-(6-(phenylamino)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-8-yl)phenyl)nicotinamide (**22h**)*

Following the general procedure D from **21a**, **22h** was obtained in 39% yield. Mp 245 °C (Kofler). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.01 (s, 1H), 8.62 (d, J = 12.3 Hz, 2H), 8.35 (s, 1H), 8.09 (s, 1H), 8.00 (d, J = 7.5 Hz, 1H), 7.83 (d, J = 7.4 Hz, 2H), 7.76 (s, 1H), 7.59 (dd, J = 14.9, 7.2 Hz, 2H), 7.39 (t, J = 7.2 Hz, 2H), 7.19–7.05 (m, 2H), 5.63 (d, J = 11.0 Hz, 1H), 4.34–4.14 (m, 4H), 3.75 (t, J = 11.5 Hz, 1H), 3.12 (q, J = 11.3 Hz, 1H), 2.02 (s, 1H), 1.73 (dd, J = 52.2, 28.3 Hz, 7H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 162.1, 160.3, 152.6, 152.1, 151.4, 151.0, 150.3, 142.0, 138.8, 138.6, 130.9, 129.7, 129.1, 125.5, 123.4, 122.5, 121.7, 120.3, 120.1, 118.3, 116.0, 84.2, 68.9, 54.5, 28.8, 24.7, 23.4. MS (ES^+) m/z (%): 544(90) $[M + Na]^+$, 522(40) $[M + H]^+$, 438(100) $[M\text{-}THP]^+$. HPLC purity (method I): 94%.

*4.12.8. 4-Bromo-3-methyl-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-6-(3-(trifluoromethyl)phenylamino)-9H-purin-8-yl)phenyl) benzamide (**23e**)*

Following the general procedure D from **21b**, **23e** was obtained in 77% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.18 (s, 1H), 8.15–8.03 (m, 3H), 7.95 (d, J = 8.6 Hz, 1H), 7.80–7.76 (m, 1H), 7.69–7.40 (m, 6H), 7.31 (d, J = 7.6 Hz, 1H), 5.62 (d, J = 11.2 Hz, 1H), 4.25 (d, J = 9.6 Hz, 1H), 3.73 (t, J = 11.4 Hz, 1H), 3.22–3.00 (m, 1H), 2.49 (s, 3H), 2.09–1.94 (m, 1H), 1.71–1.49 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.2, 152.4, 151.7, 151.3, 139.5, 138.9, 138.3, 133.6, 132.9, 130.6, 129.5, 129.4, 125.7, 122.7, 122.5, 121.6, 120.5, 119.6, 116.5, 84.3, 69.0, 28.7, 24.7, 23.4, 23.1. HPLC purity (method I) 100%.

*4.12.9. 2-Methoxy-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-6-(3-(trifluoromethyl)phenylamino)-9H-purin-8-yl)phenyl)benzamide (**23f**)*

Following the general procedure D from **21b**, **23f** was obtained in 92% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.99 (s, 1H), 8.63 (s, 1H), 8.32 (d, J = 7.1 Hz, 1H), 8.24 (s, 1H), 8.11 (s, 1H), 8.03–7.87 (m, 3H), 7.65–7.40 (m, 4H), 7.33 (d, J = 6.3 Hz, 1H), 7.18 (d, J = 7.1 Hz, 1H), 7.07 (d, J = 7.7 Hz, 1H), 5.64 (d, J = 11.0 Hz, 1H), 4.28 (d, J = 10.6 Hz, 1H), 4.09 (s, 4H), 3.76 (t, J = 12.2 Hz, 1H), 3.15 (d, J = 11.7 Hz, 1H), 1.94 (s, 1H), 1.82 (t, J = 18.0 Hz, 2H), 1.65 (d, J = 22.3 Hz, 2H), 1.43 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5, 157.3, 152.4, 152.0, 151.6, 151.2, 139.5, 139.0, 133.6, 132.7, 130.6, 129.6, 129.5, 125.1, 122.7, 122.6, 121.8, 121.7, 121.4, 120.4, 116.5, 111.6, 84.3, 77.2, 69.0, 56.3, 28.7, 24.7, 23.4. MS (ES^-) m/z (%): 587 (100) $[M\text{-}H]^-$, 588 (30) $[M\text{-}H]^-$. HPLC purity (method I) 98%.

*4.12.10. 2-Fluoro-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-6-(3-(trifluoromethyl)phenylamino)-9H-purin-8-yl)phenyl)benzamide (**23g**)*

Following the general procedure D from **21b**, **23g** was obtained in 73% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68–8.56 (m, 2H), 8.26–8.15 (m, 3H), 8.00 (d, J = 7.9 Hz, 1H), 7.96–7.86 (m, 2H), 7.66 (d, J = 7.7 Hz, 1H), 7.56 (dd, J = 10.5, 5.0 Hz, 2H), 7.48 (t, J = 7.9 Hz, 1H), 7.35 (t, J = 7.6 Hz, 2H), 7.24–7.16 (m, 1H), 5.65 (d, J = 9.5 Hz, 1H), 4.29 (d, J = 9.3 Hz, 1H), 3.76 (t, J = 11.8 Hz, 1H), 3.11 (dd, J = 16.1, 7.5 Hz, 1H), 2.05 (d, J = 16.4 Hz, 1H), 1.78 (d, J = 12.6 Hz, 4H), 1.56 (dd, J = 17.1, 10.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.5, 152.4, 151.7, 151.3, 139.5, 138.3, 134.2, 132.4, 131.6, 130.8, 129.7, 129.5, 125.8, 125.3, 122.8, 122.6, 121.8, 120.4, 116.6, 116.1, 84.3, 77.2, 69.0, 28.7, 24.7, 23.4. MS (ES^-) m/z (%): 575 (100) $[M - H]^-$, 491 (40) $[M\text{-}THP]^-$. HPLC purity (method I): 99%.

*4.12.11. 2-Methoxy-N-(3-(9-(tetrahydro-2H-pyran-2-yl)-6-(3-(trifluoromethyl)phenylamino)-9H-purin-8-yl)phenyl)nicotinamide (**23h**)*

Following the general procedure D from **21b**, **23h** was obtained in 62% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.03 (s, 1H), 8.64 (s, 2H), 8.37 (s, 1H), 8.26 (s, 1H), 8.15 (s, 1H), 7.98 (dd, J = 17.7, 8.6 Hz, 3H), 7.61 (d, J = 14.9 Hz, 2H), 7.49 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 7.2 Hz, 1H), 7.16 (s, 1H), 5.66 (d, J = 11.2 Hz, 1H), 4.29 (s, 1H), 4.24 (s, 3H), 3.78 (t, J = 11.7 Hz, 1H), 3.15 (d, J = 11.4 Hz, 1H), 2.05 (s, 1H), 1.85 (s, 4H), 1.63 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.1, 160.3, 152.4, 151.9, 151.7, 151.3, 150.3, 142.0, 139.5, 138.6, 130.7, 129.6, 129.5, 125.5, 122.7, 122.6, 121.7, 120.4, 118.3, 116.5, 116.0, 84.3, 77.2, 69.0, 54.5, 28.7, 24.7, 23.4. MS (ES^-) m/z (%): 588 (100) $[M\text{-}H]^-$. HPLC purity (method I): 98%.

*4.12.12. 4-Bromo-N-(3-(6-(3-fluorophenylamino)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-8-yl)phenyl)-3-methylbenzamide (**24e**)*

Following the general procedure D from **21c**, **24e** was obtained in 39% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.25 (d, J = 19.6 Hz, 2H), 8.08 (s, 1H), 7.92 (d, J = 11.1 Hz, 1H), 7.87–7.77 (m, 2H), 7.67–7.51 (m, 4H), 7.43 (d, J = 8.1 Hz, 1H), 6.78 (t, J = 8.2 Hz, 1H), 5.63 (d, J = 10.5 Hz, 1H), 4.26 (d, J = 8.4 Hz, 1H), 3.74 (t, J = 11.8 Hz, 1H), 3.12 (q, J = 23.7, 12.0 Hz, 1H), 2.47 (s, 3H), 1.88–1.73 (m, 2H), 1.71–1.54 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 168.9, 165.7, 161.8, 157.5, 156.3, 155.8, 146.6, 146.5, 144.7, 137.5, 135.0, 134.9, 134.6, 129.7, 127.3, 125.8, 123.4, 121.6, 117.3, 114.3, 112.7, 112.3, 61.1, 33.3, 23.4, 19.4, 18.1. MS (ES^+) m/z (%) 601 (80) $[M + H]^+$. HPLC purity (method I): 90%.

*4.12.13. N-(3-(6-(3-fluorophenylamino)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-8-yl)phenyl)-2-methoxybenzamide (**24f**)*

Following the general procedure D from **21c**, **24f** was obtained in 52% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.00 (s, 1H), 8.63 (s, 1H), 8.32 (dd, J = 7.8, 1.4 Hz, 1H), 8.10 (s, 1H), 8.03–7.89 (m, 3H), 7.65–7.51 (m, 3H), 7.42 (d, J = 8.1 Hz, 1H), 7.37–7.26 (m, 1H), 7.17 (t, J = 7.6 Hz, 1H), 7.07 (d, J = 8.3 Hz, 1H), 6.80 (t, J = 8.4 Hz, 1H), 5.65 (d, J = 11.1 Hz, 1H), 4.35–4.24 (m, 1H), 4.09 (s, 3H), 3.77 (t, J = 11.8 Hz, 1H), 3.15 (qd, J = 12.6, 3.6 Hz, 1H), 2.12–2.01 (m, 1H), 1.92–1.75 (m, 2H), 1.71–1.55 (m, 2H). MS (ES^+) m/z (%): 561 (100) $[M + Na]^+$, 539 (40) $[M + H]^+$. HPLC purity (method I): 96%.

*4.12.14. N-(3-(6-(3-fluorophenylamino)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-8-yl)phenyl)-2- methoxynicotinamide (**24h**)*

Following the general procedure D from **21c**, **24h** was obtained in 52% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.03 (s, 1H), 8.64 (t, J = 3.8 Hz, 2H), 8.37 (dd, J = 4.8, 2.0 Hz, 1H), 8.13 (s, 1H), 8.06–7.92 (m, 2H), 7.81 (s, 1H), 7.67–7.55 (m, 2H), 7.17 (dd, J = 7.6, 4.8 Hz, 2H), 6.82 (t, J = 8.3 Hz, 1H), 5.65 (dd, J = 11.5, 2.4 Hz, 1H), 4.24 (s, 3H), 3.77 (t, J = 11.1 Hz, 1H), 3.16 (q, J = 24.6, 12.8 Hz, 1H), 2.07 (d, J = 11.6 Hz, 2H), 1.94–1.72 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 161.8, 157.3, 155.1, 144.7, 140.9, 137.5, 134.9, 129.7, 128.1, 127.2, 125.8, 123.4, 120.4, 120.1, 117.3, 108.2, 99.9, 61.18, 33.3, 23.4, 19.4, 18.1. MS (ES^-) m/z (%): 538 (30) $[M - H]^-$, 454 (100) $[M\text{-}THP]^-$. HPLC purity (method I): 94%.

*4.12.15. 4-Bromo-N-(3-(6-(4-fluorophenylamino)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-8-yl)phenyl)-3-methylbenzamide (**25e**)*

Following the general procedure D from **21d**, **25e** was obtained in 29% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, J = 6.2 Hz, 1H), 8.19 (s, 1H), 8.08 (s, 1H), 7.89 (s, 1H), 7.80–7.69 (m, 4H), 7.66–7.50 (m, 5H), 7.05 (t, J = 8.7 Hz, 2H), 5.61 (dd, J = 11.2, 2.0 Hz, 1H), 4.24 (d, J = 7.6 Hz, 1H), 3.73 (t, J = 11.2 Hz, 1H), 3.10 (qd, J = 12.7, 4.0 Hz, 1H), 2.56–2.32 (m, 3H), 2.01 (s, 3H), 1.21–0.82 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 165.4, 160.6, 157.3, 152.5, 152.0, 151.3, 151.0, 138.7, 138.5, 134.7, 133.7, 132.7, 132.1, 132.0, 132.0, 131.6, 130.6, 129.6, 129.4, 129.2, 128.6, 128.4, 125.8, 125.5, 122.3, 122.0, 121.9, 121.7, 120.1, 115.8, 115.5, 84.2, 68.9, 38.6, 30.2, 26.9, 23.0. MS (ES^-) m/z (%): 599 (100) $[M\text{-}H]^-$, 601 (100) $[M\text{-}H]^-$, 514 (30) $[M\text{-}THP]^-$, 516 (30) $[M\text{-}THP]^-$. HPLC purity (method I) : 70%.

*4.12.16. N-(3-(6-(4-fluorophenylamino)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-8-yl)phenyl)-2-methoxybenzamide (**25f**)*

Following the general procedure D from **21d**, **25f** was obtained in 29% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.99 (s, 1H), 8.57 (s, 1H), 8.32 (d, J = 7.8 Hz, 1H), 8.10 (s, 1H), 7.97 (d, J = 7.5 Hz, 1H), 7.90 (s, 1H), 7.77 (dd, J = 8.9, 4.7 Hz, 2H), 7.63–7.47 (m, 3H), 7.16 (t, J = 7.5 Hz, 1H), 7.07 (t, J = 8.6 Hz, 3H), 5.64 (d, J = 11.1 Hz, 1H), 4.28 (d, J = 9.7 Hz, 1H), 4.08 (s, 2H), 3.76 (t, J = 11.7 Hz, 1H), 3.24–3.06 (m, 1H), 1.94–1.76 (m, 2H), 1.62 (dd, J = 23.0, 12.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.6, 160.7, 157.5, 157.4, 152.7, 152.1, 151.7, 151.1, 139.1, 134.9, 134.9, 133.7, 132.8, 130.8, 129.7, 125.2, 122.6, 122.2, 122.1, 121.9, 121.7, 121.5, 120.2, 115.9, 115.6, 111.7, 84.3, 69.1, 56.4, 31.1, 28.9, 24.8, 23.5. MS (ES^-) m/z (%): 537 (100) $[M\text{-}H]^-$, 453 (30) $[M\text{-}THP]^-$. HPLC purity (method I): 100%.

*4.12.17. 2-Fluoro-N-(3-(6-(4-fluorophenylamino)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-8- yl)phenyl)benzamide (**25g**)*

Following the general procedure D from **21d**, **25g** was obtained in 57% yield.^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (d, J = 15.8 Hz, 1H), 8.58 (s, 1H), 8.27–8.15 (m, 2H), 8.00 (s, 1H), 7.94 (d, J = 7.6 Hz, 1H), 7.80 (dd, J = 8.9, 4.8 Hz, 2H), 7.67 (d, J = 7.3 Hz, 1H), 7.59 (t, J = 7.4 Hz, 2H), 7.36 (t, J = 7.6 Hz, 1H), 7.23 (dd, J = 12.6, 8.5 Hz, 1H), 7.10 (t, J = 8.6 Hz, 2H), 5.66 (d, J = 11.3 Hz, 1H), 4.30 (d, J = 8.7 Hz, 1H), 3.77 (t, J = 12.1 Hz, 1H), 3.13 (d, J = 11.9 Hz, 1H), 2.04 (s, 1H), 1.94–1.61 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.5, 160.6, 157.4, 152.6, 152.0, 151.3, 151.0, 138.2, 134.8, 134.2, 134.1, 132.4, 130.8, 129.7, 125.8, 125.3, 122.5, 122.1, 122.0, 121.8, 121.1, 120.9, 120.0, 116.4, 116.1, 115.8, 115.5, 84.2, 68.9, 28.7, 24.7, 23.4. MS (ES^+) m/z (%): 525 (50) $[M + H]^+$, 441 (30) $[M\text{-}THP]^+$. HPLC purity (method I): 90%.

*4.12.18. N-(3-(6-(4-fluorophenylamino)-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-8-yl)phenyl)-2-methoxynicotinamide (**25h**)*

Following the general procedure D from **21d**, **25h** was obtained in 60% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.00 (s, 1H), 8.62 (dd, J = 7.6, 1.9 Hz, 1H), 8.56 (s, 1H), 8.35 (dd, J = 4.8, 1.9 Hz, 1H), 8.11 (s, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.80–7.71 (m, 3H), 7.68–7.52 (m, 2H), 7.18–7.02 (m, 3H), 5.62 (dd, J = 11.2, 2.0 Hz, 1H), 4.27 (d, J = 11.4 Hz, 1H), 4.21 (s, 3H), 3.74 (t, J = 11.1 Hz, 1H), 3.11 (qd, J = 12.8, 3.9 Hz, 1H), 2.04 (d, J = 14.2 Hz, 1H), 1.81–1.53 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.2, 160.7, 160.4, 157.5, 152.7, 152.1, 151.6, 151.1, 150.4, 142.1, 138.7, 134.9, 130.9, 129.8, 125.6, 122.6, 122.2, 122.1, 121.8, 120.3, 118.4, 116.1, 116.0, 115.7, 84.4, 69.1, 54.6, 30.3, 28.9, 24.8, 23.6. MS (ES^+) m/z (%): 562 (100) $[M + H]^+$, 563 (40) $[M + H]^+$, 456 (60) $[M\text{-}THP]^+$, 457 (15) $[M + THP]^+$. HPLC purity (method I): 96%.

*4.12.19. 2-Methoxy-N-(3-(6-(phenylamino)-9H-purin-8-yl)phenyl) benzamide (**26f**)*

Following the general method E from **22f**, **26f** was obtained as a solid in 88% yield. M.p. > 250 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.35 (s, 1H), 10.05 (s, 1H), 8.75 (s, 1H), 8.45 (s, 1H), 8.03–7.83 (m, 3H), 7.81–7.64 (m, 2H), 7.62–7.46 (m, 2H), 7.37 (t, J = 7.8 Hz, 2H), 7.22 (d, J = 8.3 Hz, 1H), 7.14–6.83 (m, 2H), 3.94 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.6, 158.4, 157.9, 156.5, 152.1, 150.9, 150.6, 150.1, 139.5, 139.2, 132.2, 129.8, 129.6, 129.4, 128.5, 124.5, 122.9, 122.0, 121.0, 120.5, 118.1, 112.0, 55.9. MS (ES^+) m/z (%): 437 (100) $[M + H]^+$. HRMS (EI) calcd. for $C_{25}H_{20}N_6O_2$: 437.1726, found: 437.1717 (M^+) HPLC purity (method II): 100%.

*4.12.20. 2-Fluoro-N-(3-(6-(phenylamino)-9H-purin-8-yl)phenyl) benzamide (**26g**)*

Following the general method E from **22g**, **26g** was obtained as a yellow solid in 84% yield. M.p. > 250 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.70 (s, 1H), 10.65 (s, 1H), 9.89 (s, 1H), 8.75 (s, 1H), 8.42 (s, 1H), 7.98 (d, J = 7.9 Hz, 2H), 7.92 (d, J = 7.9 Hz, 1H), 7.72 (t, J = 7.7 Hz, 2H), 7.58 (dd, J = 16.8, 8.8 Hz, 2H), 7.44–7.29 (m, 4H), 7.05 (t, J = 7.2 Hz, 1H), 3.85 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.9, 160.6, 157.3, 151.6, 139.6, 139.2, 132.7, 132.6, 130.1, 129.9, 129.4, 128.4, 124.8, 124.6, 122.5, 122.2, 121.9, 120.7, 118.3, 116.3, 116.0. MS (ES^+) m/z (%): 447 (30) $[M + Na]^+$, 425 (100) $[M + H]^+$. HPLC purity (method II): 98%.

*4.12.21. 2-Methoxy-N-(3-(6-(phenylamino)-9H-purin-8-yl)phenyl) nicotinamide (**26h**)*

Following the general method E from **22h**, **26h** was obtained in 66% yield. M.p. > 250 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.69 (s, 1H), 10.42 (s, 1H), 9.88 (s, 1H), 8.71 (s, 1H), 8.48–8.32 (m, 2H), 8.17–8.06 (m, 1H), 7.95 (dd, J = 17.3, 7.8 Hz, 4H), 7.74 (d, J = 7.9 Hz, 1H), 7.56 (t, J = 7.9 Hz, 1H), 7.35 (t, J = 7.8 Hz, 2H), 7.19 (dd, J = 7.2, 5.1 Hz, 1H), 7.05 (t, J = 7.3 Hz, 2H), 3.97 (d, J = 3.53 Hz, 4H), 3.42 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 200.4, 178.7, 163.3, 159.9, 151.7, 149.1, 139.7, 139.3, 139.2, 130.1, 129.5, 128.4, 122.5, 122.2, 121.9, 120.7, 119.0, 118.3, 117.3, 53.8. MS (ES^+) m/z (%): 460 (100) $[M + Na]^+$, 438 (90) $[M + H]^+$. HRMS (EI) calcd. for $C_{24}H_{19}N_7O_2$ (M^+): 438.1660, found: 438.1678. HPLC purity (method II): 100%.

*4.12.22. 4-Bromo-3-methyl-N-(3-(6-(3-(trifluoromethyl) phenylamino)-9H-purin-8-yl)phenyl)benzamide (**27e**)*

Following the general method E from **23e**, **27e** was obtained in 94% yield. M.p. > 250 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.53 (s, 1H), 10.30 (s, 1H), 8.79 (s, 1H), 8.52 (d, J = 8.3 Hz, 2H), 8.26 (d, J = 8.1 Hz, 1H), 8.07–7.88 (m, 2H), 7.80 (d, J = 8.3 Hz, 3H), 7.58 (dd, J = 7.8, 4.3 Hz, 2H), 7.38 (d, J = 7.6 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.8, 152.5, 151.4, 150.7, 150.1, 140.6, 139.5, 137.6, 133.9, 132.3, 130.2, 129.8, 129.6, 129.5, 129.4, 129.0, 127.9, 127.0, 126.1, 124.0, 122.7, 122.5, 122.3, 119.2, 118.6, 116.5, 22.5. MS (ES^+) m/z (%): 567 (100) $[M + H]^+$, 569 (100) $[M + H]^+$. HRMS (EI) calcd. for $C_{26}H_{18}BrF_3N_6O$ (M^+) : 567.0756, found: 567.0737. HPLC purity (method II): 100%.

*4.12.23. 2-Methoxy-N-(3-(6-(3-(trifluoromethyl)phenylamino)-9H-purin-8-yl)phenyl)benzamide (**27f**)*

Following the general method E from **23f**, **27f** was obtained as a solid in 81% yield. M.p. 242 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.34 (s, 2H), 8.76 (s, 1H), 8.52 (d, J = 7.8 Hz, 2H), 8.27 (d, J = 8.2 Hz, 1H), 7.92 (d, J = 7.5 Hz, 1H), 7.71 (t, J = 8.3 Hz, 2H), 7.63–7.49 (m, 3H), 7.38 (d, J = 7.5 Hz, 1H), 7.22 (d, J = 8.2 Hz, 1H), 7.10 (t, J = 7.2 Hz, 1H), 3.95 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.6, 156.6, 152.5, 151.4, 150.7, 150.2, 140.6, 139.5, 132.3, 129.9, 129.7, 129.6, 129.4, 129.4, 129.0, 126.1, 124.5, 124.0, 122.5, 122.1, 120.6, 118.6, 118.6, 118.3, 116.6, 116.6, 116.5, 112.1, 55.9. MS (ES$^+$) m/z (%): 527 (15) [M + Na]$^+$, 505 (100) [M + H]$^+$. HPLC purity (method II): 100%.

*4.12.24. 2-Fluoro-N-(3-(6-(3-(trifluoromethyl)phenylamino)-9H-purin-8-yl)phenyl)benzamide (**27g**)*

Following the general method E from **23g**, **27g** was obtained as a solid in 90% yield. M.p. > 250 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.65 (s, 1H), 10.34 (s, 1H), 8.78 (s, 1H), 8.51 (d, J = 5.4 Hz, 2H), 8.25 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.80–7.51 (m, 6H), 7.38 (q, J = 7.9 Hz, 4H), 4.60 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 203.4, 164.8, 143.8, 140.6, 139.5, 139.4, 137.6, 133.9, 132.3, 132.0, 130.1, 129.82, 129.6, 129.4, 127.9, 127.0, 124.0, 122.7, 122.3, 119.2, 118.6, 116.5, 22.5. MS (ES$^+$) m/z (%): 493 (100) [M + H]$^+$. HRMS (EI) calcd. for $C_{25}H_{18}F_3N_7O_2$ (M$^+$): 493.1400, found: 493.1382. HPLC purity (method II): 100%.

*4.12.25. 2-Methoxy-N-(3-(6-(3-(trifluoromethyl)phenylamino)-9H-purin-8-yl)phenyl) nicotinamide (**27h**)*

Following the general method E from **23h**, **27h** was obtained as a solid in 94% yield. M.p. 240–242 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.42 (s, 1H), 10.33 (s, 1H), 8.74 (s, 1H), 8.51 (d, J = 7.4 Hz, 2H), 8.37 (dd, J = 4.9, 1.8 Hz, 1H), 8.27 (d, J = 8.5 Hz, 1H), 8.16–8.07 (m, 1H), 7.94 (d, J = 7.5 Hz, 1H), 7.74 (d, J = 8.1 Hz, 1H), 7.58 (dd, J = 13.3, 7.8 Hz, 2H), 7.38 (d, J = 7.3 Hz, 1H), 7.19 (dd, J = 7.3, 5.0 Hz, 1H), 4.03 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.3, 159.9, 158.5, 158.0, 152.5, 151.4, 150.7, 150.0, 149.1, 140.6, 139.3, 139.2, 130.0, 129.6, 129.5, 129.0, 126.1, 124.0, 122.5, 122.3, 122.1, 118.9, 118.6, 118.3, 117.3, 116.6, 116.5, 54.9, 53.8. MS (ES$^-$) m/z (%): 504 (100) [M-H]$^-$. HRMS (EI) calcd. for $C_{25}H_{18}F_3N_7O_2$ (M$^-$): 504.1396, found: 504.1394. HPLC purity (method II): 100%.

*4.12.26. 4-Bromo-N-(3-(6-(3-fluorophenylamino)-9H-purin-8-yl) phenyl)-3-methylbenzamide (**28e**)*

Following the general method E from **24e**, **28e** was obtained as a solid in 99% yield. M.p. > 250 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.76 (s, 1H), 10.52 (s, 1H), 10.12 (s, 1H), 8.77 (s, 1H), 8.46 (s, 1H), 8.07 (d, J = 12.3 Hz, 1H), 7.99 (s, 1H), 7.92 (d, J = 7.7 Hz, 1H), 7.78 (dd, J = 12.3, 8.5 Hz, 4H), 7.56 (t, J = 7.8 Hz, 1H), 7.36 (d, J = 7.8 Hz, 1H), 6.86 (d, J = 7.6 Hz, 1H), 2.46 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.8, 163.7, 160.5, 152.0, 150.5, 139.7, 137.7, 134.5, 132.3, 130.1, 129.5, 128.3, 128.1, 127.0, 124.9, 122.9, 122.3, 119.1, 116.5, 20.9. MS (ES$^-$) m/z (%): 515 (100) [M-H]$^-$, 517 (100) [M-H]$^-$. HPLC purity (method II): 94%.

*4.12.27. N-(3-(6-(3-fluorophenylamino)-9H-purin-8-yl)phenyl)-2-methoxybenzamide (**28f**)*

Following the general method E from **24f**, **28f** was obtained as a solid in 77% yield. M.p. 250 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.34 (s, 1H), 10.21 (s, 1H), 8.75 (s, 1H), 8.49 (s, 1H), 8.05 (d, J = 12.2 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 8.6 Hz, 1H), 7.70 (dd, J = 13.1, 5.5 Hz, 2H), 7.61–7.50 (m, 2H), 7.38 (dd, J = 15.4, 8.2 Hz, 1H), 7.22 (d, J = 8.3 Hz, 1H), 7.10 (t, J = 7.5 Hz, 1H), 6.87 (dd, J = 9.6, 7.3 Hz, 1H), 3.94 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.7, 163.7, 160.5, 158.0, 156.6, 152.3, 151.1, 150.6, 150.2, 141.4, 141.3, 139.5, 132.3, 130.1, 129.9, 129.8, 129.7, 129.4, 124.5, 122.1, 120.5, 118.2, 116.4, 112.1, 109.1, 108.8, 107.4, 107.1, 55.9. MS (ES$^+$) m/z (%): 477 (20) [M + H]$^+$, 455 (100) [M + H]$^+$. HRMS (EI) calcd. for $C_{25}H_{19}FN_6O_2$ (M$^+$) : 455.1632, found: 455.1623. HPLC purity (method II): 100%.

*4.12.28. N-(3-(6-(3-fluorophenylamino)-9H-purin-8-yl)phenyl)-2-methoxynicotinamide (**28h**)*

Following the general method E from **24h**, **28h** was obtained as a solid in 40% yield. M.p. > 250 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.42 (s, 1H), 10.17 (s, 1H), 8.72 (s, 1H), 8.48 (s, 1H), 8.37 (dd, J = 4.9, 1.9 Hz, 1H), 8.15–8.02 (m, 2H), 7.92 (d, J = 7.8 Hz, 1H), 7.76 (dd, J = 21.3, 8.6 Hz, 2H), 7.56 (t, J = 7.9 Hz, 1H), 7.37 (dd, J = 15.4, 8.1 Hz, 1H), 7.19 (dd, J = 7.4, 5.0 Hz, 1H), 6.85 (dd, J = 8.5, 6.2 Hz, 1H), 4.03 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.7, 163.4, 160.5, 159.9, 152.5, 151.5, 150.8, 149.9, 149.1, 141.7, 141.5, 139.3, 139.2, 130.0, 129.9, 129.5, 122.3, 122.1, 118.9, 118.3, 117.3, 116.2, 108.8, 108.6, 107.3, 106.9, 53.8. MS (ES$^+$) m/z (%): 456 (100) [M + H]$^+$. HRMS (EI) calcd. for $C_{24}H_{18}FN_7O_2$ (M+): 456.1577, found: 456.1584. HPLC purity (method II): 94%.

*4.12.29. 4-Bromo-N-(3-(6-(4-fluorophenylamino)-9H-purin-8-yl) phenyl)-3-methylbenzamide (**29e**)*

Following the general method E from **25e**, **29e** was obtained in 39% yield. M.p. > 250 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.51 (s, 1H), 9.93 (s, 1H), 8.76 (s, 1H), 8.39 (s, 1H), 8.04–7.90 (m, 4H), 7.77 (q, J = 8.3 Hz, 3H), 7.56 (t, J = 7.9 Hz, 1H), 7.19 (t, J = 8.9 Hz, 2H), 2.46 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.8, 159.4, 156.3, 151.5, 139.5, 137.6, 135.9, 133.9, 132.3, 130.2, 129.9, 129.4, 127.9, 127.0, 122.6, 122.6, 122.5, 122.2, 119.1, 115.1, 114.8, 100.2, 22.5. MS (ES$^+$) m/z (%): 517 (70) [M + H]$^+$, 519 (70) [M + H]$^+$. HRMS (EI) calcd. for $C_{25}H_{18}F_3N_7O_2$ (M$^-$): 515.0631, found: 515.0615. HPLC purity (method II): 81%.

*4.12.30. N-(3-(6-(4-fluorophenylamino)-9H-purin-8-yl)phenyl)-2-methoxybenzamide (**29f**)*

Following the general method E from **25f**, **29f** was obtained in 92% yield. M.p. 250 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.34 (s, 1H), 10.10 (s, 1H), 8.74 (s, 1H), 8.42 (s, 1H), 8.06–7.84 (m, 3H), 7.70 (t, J = 8.1 Hz, 2H), 7.54 (t, J = 8.1 Hz, 2H), 7.21 (t, J = 7.7 Hz, 3H), 7.10 (t, J = 7.4 Hz, 1H), 3.94 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.6, 156.6, 156.4, 152.1, 151.1, 150.8, 149.9, 139.5, 135.7, 132.3, 129.9, 129.7, 129.4, 124.5, 122.9, 122.8, 122.0, 120.6, 118.2, 115.2, 114.9, 112.1, 55.9. MS (ES$^+$) m/z (%): 455 (100) [M + H]$^+$. HRMS (EI) calcd. for $C_{25}H_{19}FN_6O_2$ (M$^+$): 455.1632, found: 455.1619. HPLC purity (method II): 92%.

*4.12.31. 2-Fluoro-N-(3-(6-(4-fluorophenylamino)-9H-purin-8-yl) phenyl)benzamide (**29g**)*

Following the general method E from **25g**, **29g** was obtained in 73% yield. M.p. > 250 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.64 (s, 1H), 10.05 (s, 1H), 8.75 (s, 1H), 8.40 (s, 1H), 8.02–7.89 (m, 3H), 7.79–7.52 (m, 4H), 7.37 (dd, J = 15.1, 7.7 Hz, 2H), 7.19 (t, J = 8.9 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.04, 160.67, 159.65, 157.38, 152.20, 151.30, 150.94, 149.86, 139.39, 135.82, 132.86, 132.77, 130.00, 129.59, 124.89, 124.74, 124.69, 122.93, 122.83, 122.37, 122.09, 118.42, 116.45, 116.16, 115.28, 114.98. MS (ES$^+$) m/z (%): 443 (100) [M + H]$^+$. HRMS (EI) calcd. for $C_{24}H_{16}F_2N_6O_2$ (M$^+$) : 443.1432, found: 443.1427. HPLC purity (method II): 100%.

*4.12.32. N-(3-(6-(4-fluorophenylamino)-9H-purin-8-yl)phenyl)-2-methoxynicotinamide (**29h**)*

Following the general method E from **25h**, **29h** was obtained as a solid in 82% yield. M.p. 250 °C (Kofler). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.43 (s, 1H), 10.19 (s, 1H), 8.73 (s, 1H), 8.45 (s, 1H), 8.36 (dd, J = 4.8, 1.5 Hz, 1H), 8.10 (dd, J = 7.3, 1.5 Hz, 1H), 7.98–7.87 (m, 3H),

7.73 (d, $J = 8.1$ Hz, 1H), 7.57 (t, $J = 7.9$ Hz, 1H), 7.20 (dt, $J = 9.0$, 6.9 Hz, 3H), 4.02 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.4, 159.9, 151.8, 150.7, 150.2, 149.1, 139.3, 135.4, 129.8, 129.5, 123.1, 123.0, 122.2, 122.1, 118.9, 118.2, 117.3, 115.3, 115.0, 53.8. MS (ES^+) m/z (%): 456 (100) $[M + H]^+$. HRMS (EI) calcd. for $C_{24}H_{18}FN_7O_2$ (M^+): 456.1584, found: 456.1578. HPLC purity (method II): 91%.

4.13. Molecular modelling and docking

Experimental crystallographic coordinates of human Met (PDB ID: 3F82, resolution 2.50 Å) and Kit (PDB ID: 1T46, resolution 1.60 Å) without water molecules were used as templates to obtain the Tyro3 modelled structure. The homology modelling of human Tyro3 was performed using the software MODELLER [35] with an automated protocol for optimization and minimization of the models.

The sequence alignement of Met, Kit and Tyro 3 was accomplished with BlastP [36] utilizing BLOSUM62 matrix algorithm [37], T-Coffee and Expresso [38,39] and resulting in Tyro3-Met identity of 44% and similary of 60%, and identity of 31% and similarity of 43% for Tyro3-Kit comparison. Although Met presents a higher sequence similarity with Tyro3, three solvent exposed loops involving respectively 17, 5 and 4 residues, are absent in the 3F82 structure, whereas they are present in the 1T46 structure of Kit. Therefore, we used both proteins in order to increase the number of residues in the Tyro3 sequence for which a template structure was available. The resulting sequence alignment presented only a small gap corresponding to a 5 residue loop in insertion in the Tyro3 sequence (Fig. 2 in supplementary materials).

The model with the best MODELLER target function was further analysed according to its Ramachandran plot, QMEAN server analysis [40], MolProbity [41] in order to check its overall topological quality. This model was retained to perform docking experiments.

Docking experiments were performed with FRED [42] using Chemgauss3 scoring function [29]. A conformational database of listed compounds was generated with OMEGA [43] and subsequently used as input to FRED. OMEGA generates high quality 3D conformers with low energies. Bond lengths and angles remain fixed, but rotation around rotatable torsion angles is allowed. The required receptor file was built with FRED_Receptor using multiple molecular probes detection. No constraint was used in order not to restrict the pose selection. The best ranked pose for each compound was retained and analysed in order to clarify the interactions involved in the ligand binding.

4.14. Kinase assays

Kinase assays were carried out at Kinomescan, a division of DiscoveRex, 11180 Roselle St. Suite D, San Diego, CA 92121, as follows. For most assays, kinase-tagged T7 phage strains were grown in parallel in 24-well blocks in an *Escherichia coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32 °C until lysis (90–150 min). The lysates were centrifuged (6000 × g) and filtered (0.2 μm) to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 min at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1% BSA, 0.05% Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific phage binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1× binding buffer (20% SeaBlock, 0.17× PBS, 0.05% Tween 20, 6 mM DTT). Test compounds were prepared as 40× stocks in 100% DMSO and directly diluted into the assay. All reactions were performed in polypropylene 384-well plates in a final volume of 0.04 mL. The assay plates were incubated at room temperature with shaking for 1 h and the affinity beads were washed with wash buffer (1× PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1× PBS, 0.05% Tween 20, 0.5 μM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 min. The kinase concentration in the eluates was measured by qPCR.

Compounds that bind the kinase active site and directly (sterically) or indirectly (allosterically) prevent kinase binding to the immobilized ligand, will reduce the amount of kinase captured on the solid support. Conversely, test molecules that do not bind the kinase have no effect on the amount of kinase captured on the solid support. Screening "hits" are identified by measuring the amount of kinase captured in test versus control samples by using a quantitative, precise and ultra-sensitive qPCR method that detects the associated DNA label. In a similar manner, dissociation constants (K_ds) for test compound–kinase interactions are calculated by measuring the amount of kinase captured on the solid support as a function of the test compound concentration. The values of K_d and IC_{50} are related by the Cheng–Prusoff equation: $K_d = (IC_{50})/(1 + ([S]/[K_m]))$, where K_m is the Michaelis constant of the enzyme, and $[S]$ is the substrate's concentration [44].

4.15. Cell culture and cell proliferation assays

Cytotoxicity assays were carried out in Gif sur Yvette, France at the Imagif/CNRS/ICSN platform.

The human cell lines were originated from ATCC, except when otherwise stated. The human cell line KB (mouth epidermoid carcinoma) and Vero (epithelial monkey kidney) were grown in D-MEM medium supplemented with 10% fetal calf serum (InVitrogen), in the presence of 100 UI/mL penicilline, 100 μg/mL streptomycine and 1.5 μg/mL fungizone in 75 cm^2 flask under 5% CO_2, whereas HT29 (colon adenocarcinoma), MCF7 (breast adenocarcinoma) from Matthias Kassac (Bonn) and PC-3 (prostate adenocarcinoma) were grown in RPMI medium.

Cells were plated in 96-well tissue culture plates in 200 μl medium and treated 24 h later with compounds dissolved in DMSO using a Biomek 3000 (Beckman). Controls received the same volume of DMSO (1% final volume). After 72 h exposure MTS reagent (Celltiter 96Aqueous One solution, Promega) was added and incubated for 3 h at 37 °C: the absorbance was monitored at 490 nm and results expressed as the inhibition of cell proliferation calculated as the ratio [(1 − (OD490 treated/OD490 control)) × 100] in triplicate experiments.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.06.005.

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