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Tozasertib analogues as inhibitors of necroptotic cell death

Sam Hofmans [a], Lars Devisscher [a], Sofie Martens [b,c], Dries Van Rompaey [a], Kenneth Goossens [a], Tatyana Divert [b,c], Wim, Nerinckx [d,e,], Nozomi Takahashi [b,c], Hans De Winter [a], Pieter Van Der Veken [a], Vera Goossens [b,c], Peter Vandenabeele [b,c,f], Koen Augustyns [a]

[a] Laboratory of Medicinal Chemistry, University of Antwerp, Universiteitsplein 1, Wilrijk-Antwerp 2610, Belgium

[b] Molecular Signaling and Cell Death unit, VIB Center for Inflammation Research, Technologiepark 927, Zwijnaarde-Ghent 9052, Belgium

[c] Department of Biomedical Molecular Biology (DBMB), Ghent University, Technologiepark 927, Zwijnaarde-Ghent 9052, Belgium

[d] Unit for Medical Biotechnology, Center for Medical Biotechnology, VIB, Technologiepark 927, Zwijnaarde-Ghent 9052, Belgium

[e] Laboratory for Protein Biochemistry and Biomolecular Engineering, Department of Biochemistry and Microbiology, Ghent University, K.L.-Ledeganckstraat 35, Ghent 9000, Belgium

[f] Methusalem Program, Ghent University, Ghent 9000, Belgium

Keywords: necroptosis, regulated necrosis

ABSTRACT

Receptor interacting protein kinase 1 (RIPK1) plays a crucial role in tumor necrosis factor (TNF)-induced necroptosis, suggesting that this pathway might be druggable. Most inhibitors of RIPK1 are classified as either type II or type III kinase inhibitors. This opened up some interesting perspectives for the discovery of novel inhibitors that target the active site of RIPK1. Tozasertib, a type I pan-aurora kinase (AurK) inhibitor, was found to show a very high affinity for RIPK1. Since tozasertib presents the typical structural elements of a type I kinase inhibitor, the development of structural analogues of tozasertib is a good starting point for identifying novel type I RIPK1 inhibitors. In this paper we identified interesting inhibitors of mTNF-induced necroptosis with no significant effect on AurK A and B, resulting in no nuclear abnormalities as is the case for tozasertib. Compouds 71 and 72 outperformed tozasertib in an in vivo TNF-induced systemic inflammatory response syndrome (SIRS) mouse model.

INTRODUCTION

Cell death upon stimulation by tumor necrosis factor (TNF) mainly occurs by two major forms, namely apoptosis and necrosis. These two different forms of cell death are each characterized by their own typical morphological features.[1,2] Apoptosis is the major cell death pathway used to remove unwanted and harmful cells in an immunologically "silent".[3] The main executioners of the apoptotic pathway are the so-called caspases, a class of proteolytic enzymes that can cleave various intracellular proteins.[4] Since the execution of apoptosis is highly dependent on caspase-activity, it can be inhibited by using the pan-caspase inhibitor zVAD.fmk.[5,6] In contrast, necroptosis is characterized by a lack of caspase activation, cytoplasmic and mitochondrial swelling, random DNA degradation and irreversible plasma membrane damage. This in turn results in the leakage of intracellular content and the so-called "damage associated molecular patterns" (DAMPs) in the surrounding tissue which then initiate an inflammatory response.[7,8]

Necrosis has historically been considered to be an uncontrolled form of cell death that is refractory to therapy. However, cell death research was revitalized by the understanding that necrosis can occur in a programmed and tightly regulated manner leading to the introduction of the term necroptosis.[9] Multiple forms of regulated necrosis have been characterized so far, including necroptosis, ferroptosis, oxytosis and pyroptosis. One of the most studied forms of regulated necrosis is tumour necrosis factor receptor-1 (TNFR1) mediated necroptosis which can be induced by TNF.[9] This process of TNF-mediated necroptosis is highly dependent on the function of receptor interacting protein kinase 1 (RIPK1) which was identified as a crucial kinase in the necroptotic core machinery. [10–14] It should be noted that stimulation of TNFR1 by TNF does not irrevocably result in cell death. The binding of TNF to its receptor results in the formation of a receptor proximal complex I in which RIPK1 fulfills an important scaffolding function. Post-translational effects to this complex I can result in the downstream activation of the transcription factor NF-kB. This in turn results in the upregulation of various pro-survival genes. To maintain a pro-survival status of the cell, the kinase activity of RIPK1 is supressed. However, when the kinase activity of RIPK1 is not supressed, multiple cellular outcomes such as apoptosis and necroptosis become possible. This makes RIPK1 an important enzyme that functions at the crossroads between cell death and survival, having a scaffolding function that promotes cell survival while its kinase function is important for cell death signalling (both apoptosis and necroptosis).[7,14–17] Since the discovery that TNF-induced necroptosis is dependent on the kinase activity of RIPK1, it became clear that this pathway might be druggable. The design of chemical inhibitors of RIPK1 could further elucidate the complex signalling cascade that drives this form of regulated necrosis.

The first inhibitors of necroptosis were discovered in 2005 in a phenotypical screening to inhibit necroptotic cell death.[14,18–20] This lead to the identification of both necrostatin-1 (Nec-1, **1**, Figure 1A) and necrostatin-1 stable (Nec-1s, **2**, Figure 1A) which were able to inhibit the kinase activity of RIPK1. No other molecule has been used as extensively as Nec-1s as an important tool compound in cell death research. More importantly, the discovery of Nec-1s demonstrated that the inhibition of RIPK1 could ameliorate certain conditions and diseases.[20–27] Following the success of Nec-1s,

significant follow-up research has been conducted which lead to the identification of multiple other molecules that were subsequently classified as necrostatins.[20,28–30] Despite their excellent kinase selectivity, all of the reported necrostatins struggle with multiple drawbacks such as a narrow SAR profile, moderate potency and non-ideal pharmacokinetic properties. [14,18,28,31–33] These findings underline the need for the discovery of novel inhibitors of necroptosis.

Cocrystallization of Nec-1s with the RIPK1 kinase domain showed that it can be classified as a type III kinase inhibitor by occupying an allosteric hydrophobic pocket created by the DLG-out conformation without interacting with any of the residues in the hinge region of RIPK1.[28,34–37] Other cocrystallization studies that revealed that all necrostatins bind in a similar manner and can therefore all be classified as type III inhibitors.[28]

In addition to the necrostatins, another very promising RIPK1 inhibitor was recently discovered by GlaxoSmithKline (GSK). Screening of RIPK1 against GSK's property collection of DNA-encoded small-molecule libraries identified GSK'481 (**3,** Figure 1A) which was able to selectively inhibit the kinase activity of RIPK1. Upon investigation of the binding mode of GSK'481 (**3**) it was found that the benzylic moiety occupied the same allosteric lipophilic pocket as the necrostatins while the benzoxazepinone moiety was located near the ATP binding site. It is thus difficult to classify this compound as either a pure type II or type III kinase inhibitor.[38] Lead optimization of GSK'481 resulted in the development of GSK2982772 (**4**, Figure 1A) which is currently in phase 2a clinical studies for psoriasis, rheumatoid arthritis and ulcerative colitis.[39]

Less work has been reported considering type II RIPK1 inhibitors, however some compounds that use this binding mode have been reported by various research groups together with some very interesting *in vivo* activity. (**5-7**, Figure 1B)[28,35]

In an attempt to discover novel necroptosis inhibitors, we decided to use a drug repurposing approach. The investigation whether well-characterized drugs can be used in an entirely different context has received increased interest over the last years, mainly due to the rising costs of traditional drug development. Also drugs selected for drug repurposing have often been through several stages of

clinical development and therefore have a well-characterized safety profile and pharmacokinetic profile.[40–42] A paper by Davis *et al*. published a kinase inhibitor selectivity study back in 2011 reporting a screening of 72 preclinical and clinical kinase inhibitors against 442 kinases, covering more than 80% of the human catalytic protein kinome. In this screening tozasertib (also known as VX-680 or MK-0457) had the strongest affinity for RIPK1 of all tested kinase inhibitors (**8**, Figure 1C) with a K_d of 20 nM.[43] In addition to its high affinity for RIPK1 we also considered tozasertib to be opportune due to its scaffold that is divergent from currently used type II and type III RIPK1 inhibitors.[37] The development and screening of structural analogues of tozasertib may thus lead to new insights in the synthesis of novel RIPK1 inhibitors. A first objective in the framework of drug repurposing was thus to evaluate if tozasertib and structural analogues of tozasertib were indeed able to effectively inhibit necroptosis.

Tozasertib was originally identified as a pan aurora-kinase (AurK) inhibitor. Since these kinases play an important role during mitosis and meiosis it is obvious that these enzymes are integral for correct cell proliferation. Inhibition of AurK by tozasertib subsequently leads to cellular abnormalities which can be observed as an inhibition of cellular growth and changes in nuclear morphology and DNA content of the cells (increased nuclear area).[44–46] However in the context of necroptosis inhibition these effects are unfavourable and should thus be preferably removed. The second objective in the framework of drug respurposing was thus to investigate whether it was possible to decrease or even completely remove these cellular and nuclear effects associated with AurK inhibition by introducing structural variations to the tozasertib scaffold. Therefore a library of tozasertib analogues with a 2,4,6-trisubstituted pyrimidine or triazine core was synthesized.

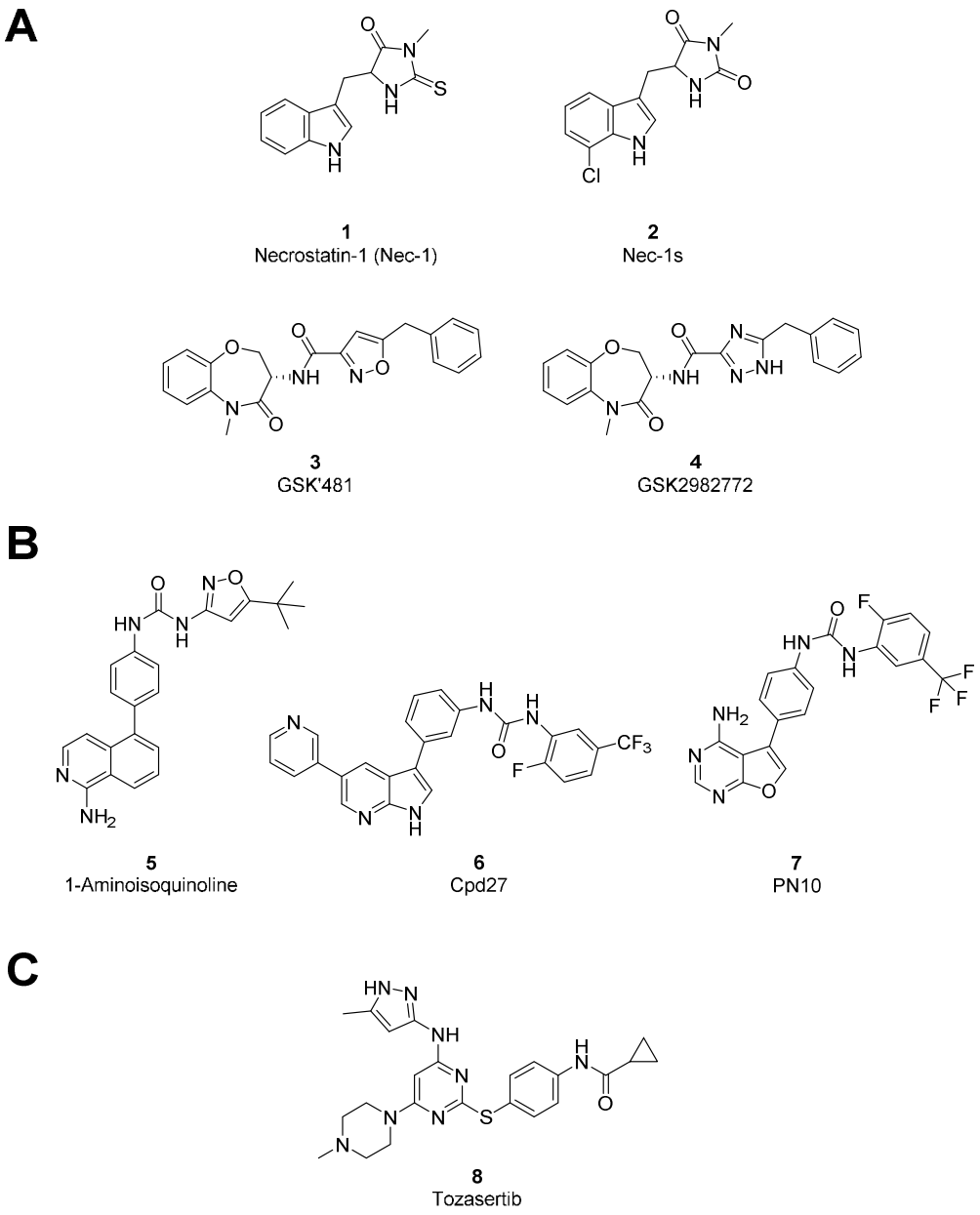


Figure 1. Reported inhibitors of necroptosis: (A) Type III RIPK1 inhibitors; (B) Type II RIPK1 inhibitors; (C) Tozasertib, a type I pan-aurora kinase inhibitor

RESULTS AND DISCUSSION

Compound design

Prior to the synthesis of the first library of inhibitors, the potential of tozasertib to inhibit TNF-induced necroptosis was validated. Next, a library of 30 compounds that are structurally similar to tozasertib was synthesized in order to investigate whether novel analogues of tozasertib were still able to inhibit TNF-induced necroptosis in a dose-dependent manner. On the other hand this also helped to identify which structural modifications were preferred to possibly increase the potency of these analogues. The structural variations were introduced at three different positions. Firstly, the amide-linked aliphatic substituent at R_1 was varied between different cyclic and aliphatic groups with divergent steric properties. Secondly, the pyrazole moiety at the R_2 position was preserved but its substitution pattern was varied. Finally, the solubility-improving moiety at R_3 was varied with different cyclic amines. (Figure 2) These 30 compounds were tested in a phenotypical assay in which their inhibitory potential against TNF-induced necroptosis was studied. (Supporting information S1-S3) The results from this initial series allowed us to formulate a preliminary structure-activity relationship (SAR) to see which substituents in R_1, R_2 and R_3 were beneficial for improving the inhibitory potency of this type of compounds. This newly found SAR was then used as a guideline in the design of a second library of tozasertib analogues. Next to these most preferred functional groups in R_1, R_2 and R_3, additional variations were introduced to the central heterocyclic core and linker atom connecting the two six-membered aromatic systems. (Figure 2, respectively shown as X and Y). The introduction of these modifications provided a set of 46 final compounds **8, 43, 44, 46, 48, 50, 52, 53, 55, 57, 70-81, 89-100, 107-118**. These compounds were evaluated for their potential to inhibit mTNF-induced necroptosis in the presence of zVAD.fmk. Since tozasertib analogues can still potentially interfere with cell proliferation and induce nuclear abnormalities, the effect of these 46 compounds on cellular growth and nuclear area was also investigated.

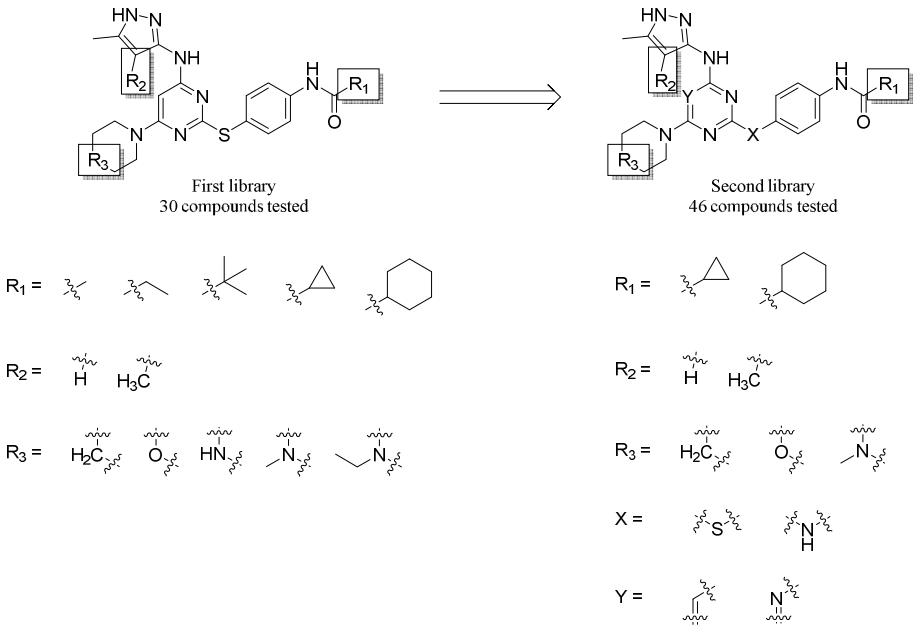
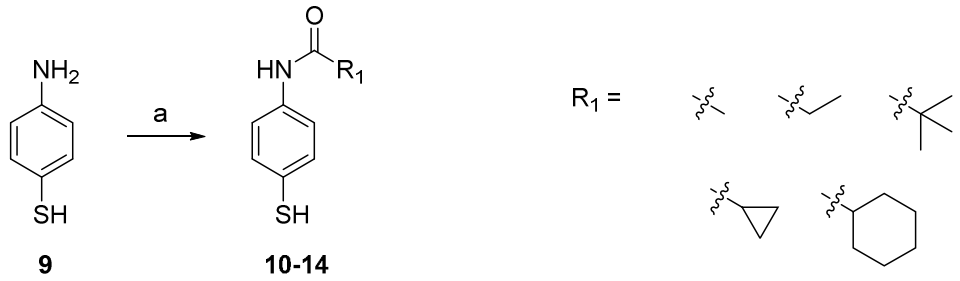


Figure 2. Overview of tozasertib analogues. The results from the phenotypical assay of the first series of inhibitors (Supplementary info S1-S3) were used to narrow down the selection of substituents for the second series of compounds. For this second series the most preferred functional groups were used and extra variations were additionally introduced in X and Y.

Chemistry

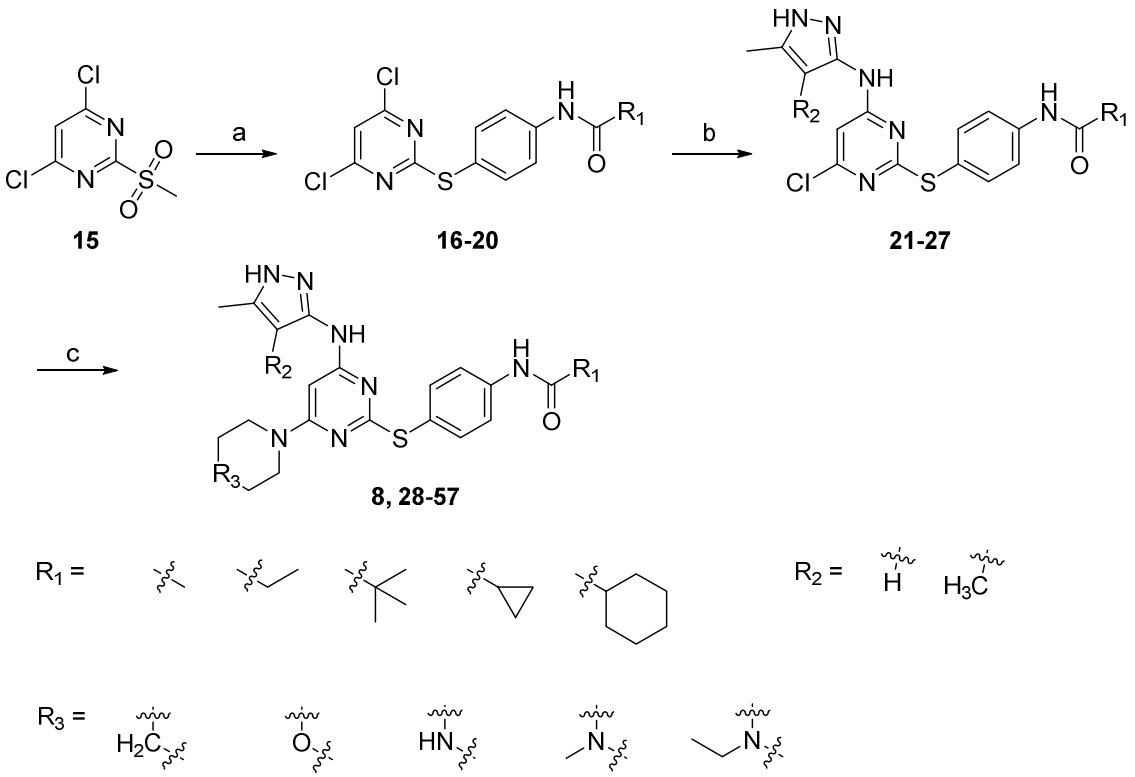
All compounds in this study were prepared following the general strategies in Schemes 1-6. In general, nearly all final compounds were prepared using nucleophilic aromatic substitution reactions.

Prior to the synthesis of the first library of 30 tozasertib analogues, several *N*-(4-mercaptophenyl)amide intermediates **10-14** were synthesized (Scheme 1). These molecules were synthesized from the commercially available 4-aminobenzothiol **9** which was acylated using appropriate acylchlorides to form the corresponding aromatic amides.



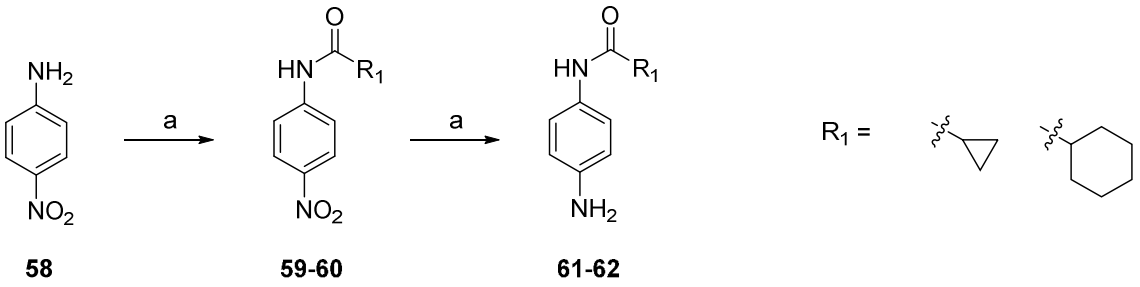
Scheme 1: Reagents and conditions: (a) 4-aminobenzothiol, substituted acylchloride, triethylamine, tetrahydrofuran, 2h, 0 °C[47]

Treatment of 4,6-dichloro-2-(methylsulfonyl)pyrimidine **15** with intermediates **10-14** resulted in compounds **16-20**. These compounds were subsequently treated with 5-methyl-1H-pyrazol-3-amine or 4,5-dimethyl-1H-pyrazol-3-amine, providing compounds **21-27**. Target compounds **8** and **28-57** were obtained by substituting the 6-position of the pyrimidine scaffold with a a piperidine, morpholine, piperazine, N-methyl- or N-ethylpiperazine.



Scheme 2: Reagents and conditions: (a) 4,6-dichloro-2-(methylsulfonyl)pyrimidine, triethylamine, acetonitrile, 2h; -10 °C to rt; (b) 5-methyl-1*H*-pyrazol-3-amine or 4,5-dimethyl-1*H*-pyrazol-3-amine, DIPEA, DMF, 18h; 90 °C; (c) piperidine, morpholine, piperazine, N-methylpiperazine or N-ethylpiperazine, 2h, 110 °C;[47–49]

In order to investigate the effects of the heteroatom that links both aromatic six-membered rings in tozasertib, we prepared a set of various N-(4-aminophenyl)cycloalkyl carboxamide intermediates which are described in Scheme 3. These compounds were prepared by N-acylation of **58** using either cyclopropanecarbonyl chloride or cyclohexanecarbonyl chloride to afford the corresponding amide analogues **59** and **60** respectively. Catalytic hydrogenation of the nitro-functional group resulted in compounds **61-62**.

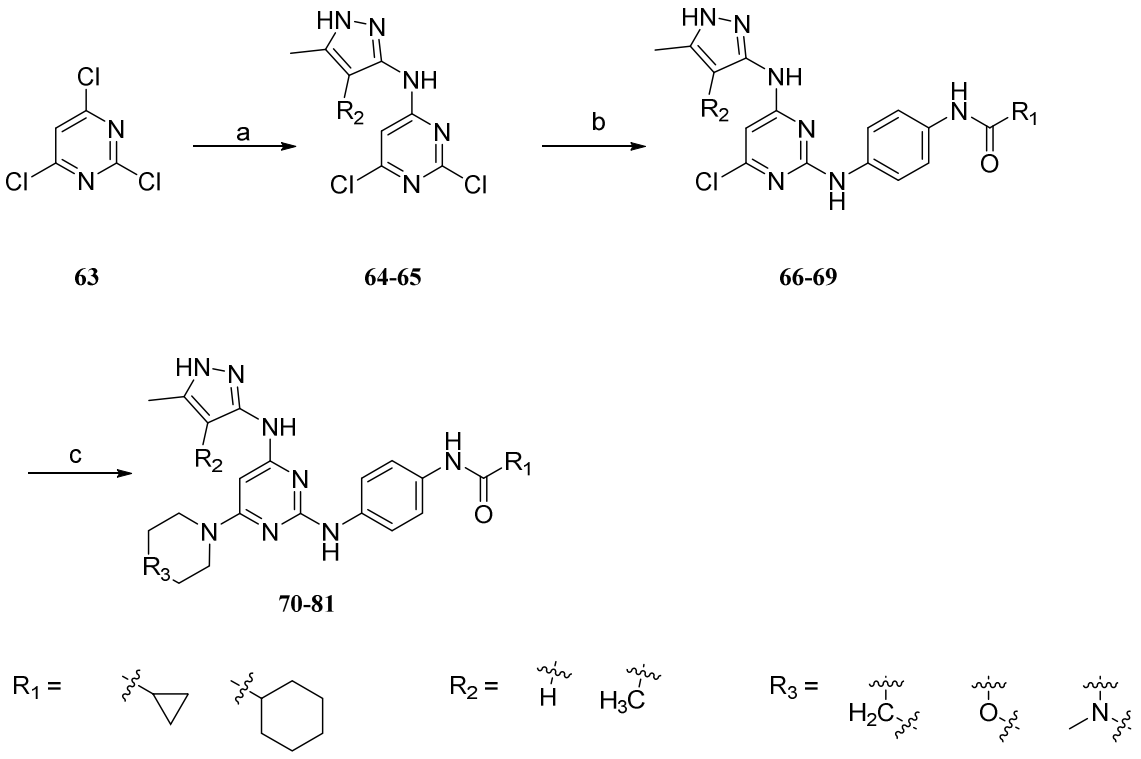


58 **59-60** **61-62**

Scheme 3: Reagents and conditions: (a) substituted acylchloride, DIPEA, DCM, 3h, rt; (b) $Pd(OH)_2$, H_2, methanol, 17h, rt[50]

For the sake of clarity the second series can be divided into 3 smaller groups of 12 compounds, of which the structures can be described as 2,4,6-triaminosubstituted pyrimidines, 2,6-diamino-4-sulfosubstituted triazines and 2,4,6-triaminosubstituted triazines. The synthesis of these compounds is discussed in Schemes 4-6 respectively.

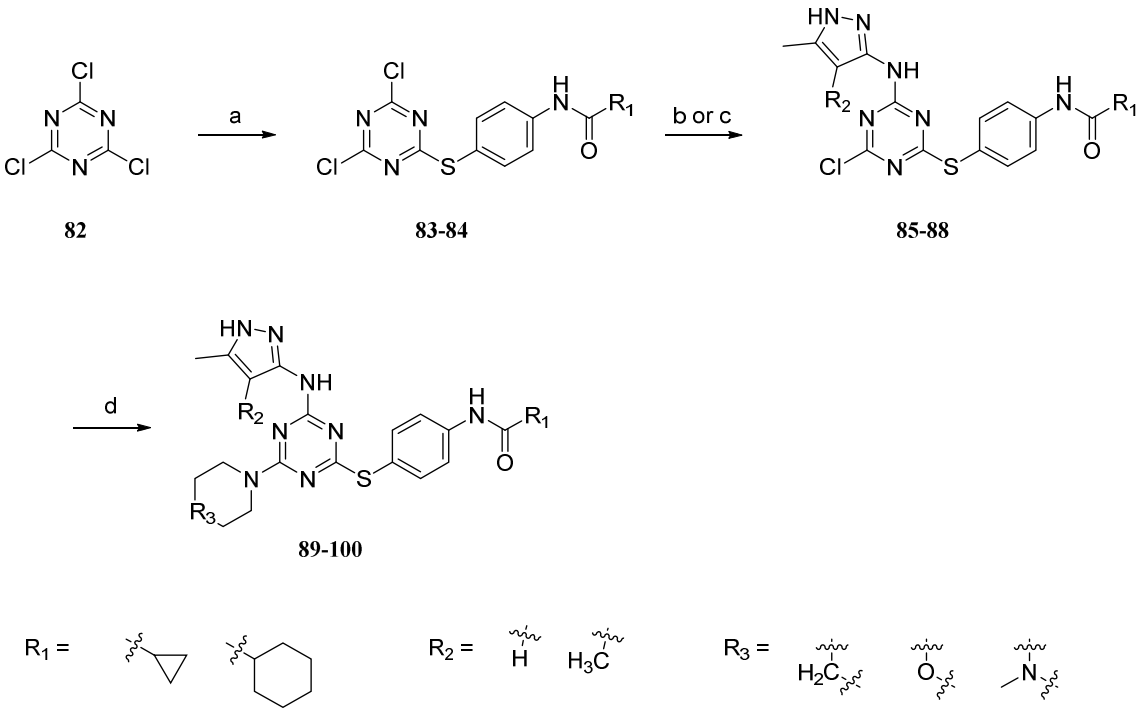
The synthesis of 2,4,6-triaminosubstituted pyrimidines is described in Scheme 4. The pyrimidine derivates were synthesized starting from 2,4,6-trichloropyrimidine **63**. Firstly the 2-position was substituted by treating **63** with the corresponding 5-methyl-1*H*-pyrazol-3-amine or 4,5-dimethyl-1*H*-pyrazol-3-amine to give intermediates **64-65**. These compounds were in turn coupled to the previously prepared substituents **61-62** to give **66-69**. Finally compounds **66-69** were treated with either *N*-methylpiperazine, morpholine or piperidine using the same conditions as described earlier to yield final compounds **70-81**.



Scheme 4: Reagents and conditions: (a) 5-methyl-1*H*-pyrazol-3-amine or 4,5-dimethyl-1*H*-pyrazol-3-amine, dioxane, 12h, rt;[51] (b) **61-62**, *p*-toluene sulfonic acid monohydrate, *n*-butanol, 12h, reflux;[52] (c) piperidine, morpholine, *N*-methylpiperazine, 2h, 110°C[49]

The synthesis of 2,6-diamino-4-sulfosubstituted triazines is described in Scheme 5. All derivatives of this type were synthesized from the 2,4,6-trichlorotriazine **82**. Firstly, intermediates **13** and **14** were

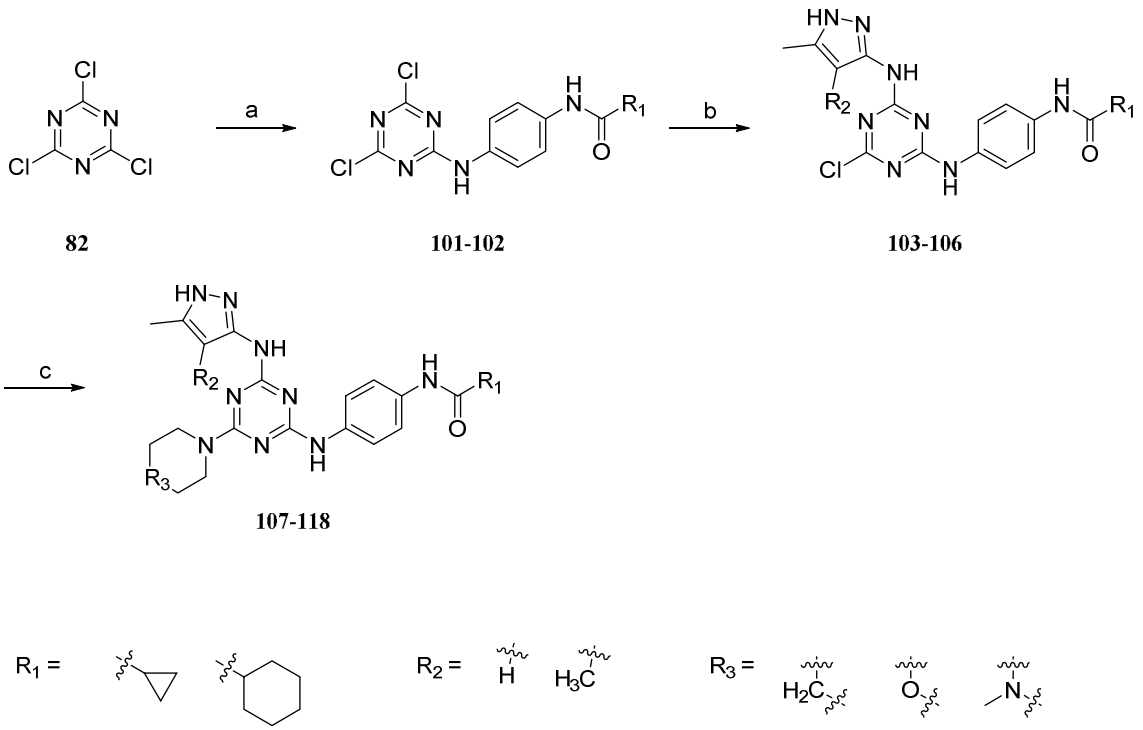
introduced to the triazine core which resulted in the intermediates **83-84**. Subsequently these intermediates were treated with either 5-methyl-1H-pyrazol-3-amine or 4,5-dimethyl-1H-pyrazol-3-amine resulting in intermediates **85-88**. Once again, the N-methylpiperazine, morpholine or piperidine moiety was introduced at the 6-position of the triazine core of compounds **85-88** which resulted in the formation of the final compounds **89-100**.



Scheme 5: Reagents and conditions: (a) Compound **13** or **14**, DIPEA, 0 °C, dropwise addition (b) 5-methyl-1H-pyrazol-3-amine, DIPEA, dioxane, 16 h, 120 °C (c) 4,5-dimethyl-1*H*-pyrazol-3-amine, DIPEA, dioxane, 16 h, rt (d) piperidine, morpholine or N-methylpiperazine, 30 min, rt

The synthesis of 2,4,6-triaminosubstituted triazines is described in Scheme 6. The triazine derivates were synthesized from 2,4,6-trichlorotriazine **82**. In the first step intermediates **61-62** were coupled to **82** to give compounds **101-102.** . Next, overnight treatment of **101-102** with the appropriate 5-methyl-1H-pyrazol-3-amine or 4,5-dimethyl-1H-pyrazol-3-amine yielded compounds **103-106**. Treatment of

103-106 with either *N*-methylpiperazine, morpholine or piperidine using conditions as described in previous schemes afforded target triazines **107-118.**



Scheme 6: Reagents and conditions: (a) **61-62**, K_2CO_3, dioxane, rt; (b) 5-methyl-1*H*-pyrazol-3-amine or 4,5-dimethyl-1*H*-pyrazol-3-amine, DIPEA, dioxane, 16h, 120° C; (c) piperidine, morpholine, N-methylpiperazine, 2h, 110°C[49]

Evaluation and characterization of the synthesized tozasertib analogues

Phenotypical screening of the compounds in L929 cells

The research was initiated by synthesizing a preliminary library of 30 compounds in order to investigate which substituents in R_1, R_2 and R_3 were favourable. Exact IC_{50}-values and assay conditions are reported in table S1 (Supporting information S1-S3). In general, it was observed that compounds containing a cyclopropyl and cyclohexyl moiety in R_1 showed the best potential towards the inhibition of TNF-induced necroptosis. Regarding R_2, both hydrogen and methyl substituents were well tolerated, but generally the compounds containing a hydrogen substituent instead of an extra methyl group showed to be more potent for inhibiting TNF-induced necroptosis. At R_3 it was clear that compounds which contained an N-methylpiperazine, morpholine or piperidine moiety were most potent in inhibiting TNF-induced necroptosis. (Figure 3)

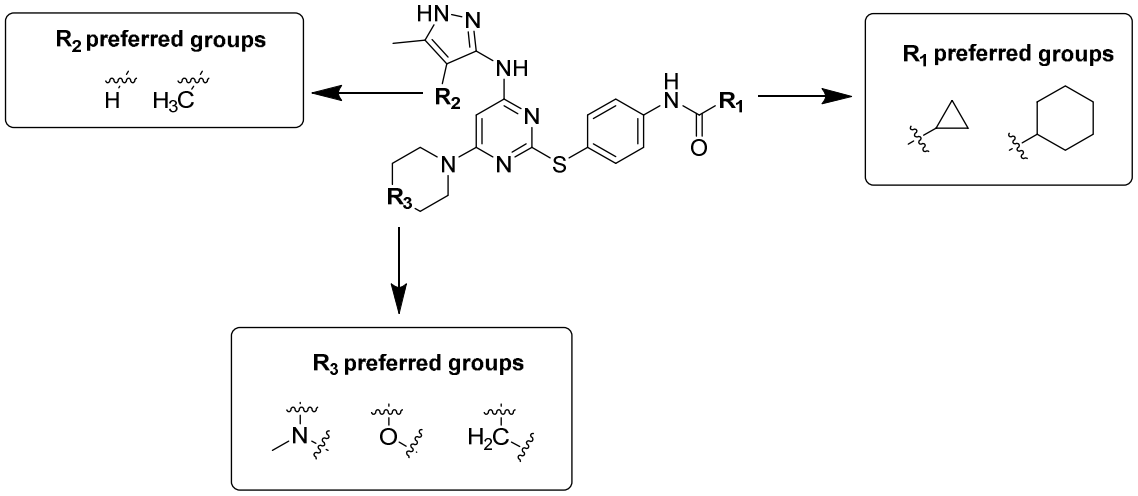
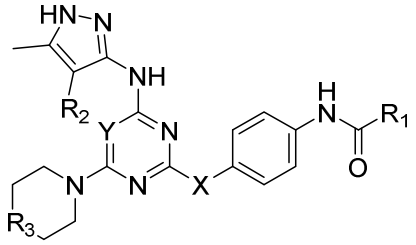


Figure 3. Summary of the results from the first phenotypical assay in determining the preferred substituents in R_1, R_2 and R_3.

With these preferred groups at R_1, R_2 and R_3 in place, additional structural variations were introduced to the central heterocyclic core and the linker connecting the two aromatic six-membered rings. A

second compound library was synthesized in which nearly all possible and preferred aforementioned combinations were introduced. This library consists of 46 compounds of which **8, 43, 44, 46, 48, 50, 52, 53, 55, 57** were re-used from the initial series, and of which **70-81, 89-100, 107-118** were synthesized additionally. These 46 compounds were evaluated in a phenotypical assay for their potential to inhibit necroptosis stimulated by mTNF. Additional data on different cell lines and the mechanism of action of selected compounds will be published elsewhere.[53]

As mentioned earlier, treatment of cells with tozasertib results in an inhibition of TNF-induced necroptosis, but also affects cellular growth and induces nuclear abnormalities with similar IC_{50}-values. With this second library, we investigated whether it was possible for a molecule to be a potent inhibitor of TNF-induced necroptosis without affecting the cellular growth and/or the nuclear area. The results of this assay are reported in table 1. The 95% confidence interval for the active compounds is reported in the supporting information (Table S2, S5-S9).



8, 43, 44, 47, 48, 50, 52, 53, 55, 57, 70-81, 89-100, 107-118

Compound	Core Y	Linker X	R_1	R_2	R_3	IC_{50} -values (μM)			
						-0.5h[a]	-24h[b]	Cell growth[c]	Nuclear area[d]
8, Tozasertib (Reference)	CH	S	Cyclopropyl	H	NMe	0.98	1.02	0.97	1.06
43	CH	S	Cyclopropyl	H	CH_2	0.75	1.74	1.32	1.00
44	CH	S	Cyclopropyl	H	O	0.39	0.81	1.00	1.04

47	CH	S	Cyclohexyl	H	CH_2	1.75	5.04	0.813	>3
48	CH	S	Cyclohexyl	H	O	0.38	0.36	0.214	<0.6
50	CH	S	Cyclohexyl	H	NMe	>3	>3	<0.6	>3
52	CH	S	Cyclohexyl	Me	CH_2	>3	>3	>3	>3
53	CH	S	Cyclohexyl	Me	O	>3	>3	>3	>3
55	CH	S	Cyclohexyl	Me	NMe	>3	>3	>3	>3
57	CH	S	Cyclopropyl	Me	NEt	>3	>3	>3	>3
70	CH	NH	Cyclopropyl	H	CH_2	0.53	0.68	>3	>3
71	CH	NH	Cyclopropyl	H	O	0.62	0.43	>3	>3
72	CH	NH	Cyclopropyl	H	NMe	1.04	0.64	>3	>3
73	CH	NH	Cyclopropyl	Me	CH_2	>3	>3	>3	>3
74	CH	NH	Cyclopropyl	Me	O	>3	>3	>3	>3
75	CH	NH	Cyclopropyl	Me	NMe	>3	>3	>3	>3
76	CH	NH	Cyclohexyl	H	CH_2	>3	1.94	>3	>3
77	CH	NH	Cyclohexyl	H	O	0.64	1.50	2.25	>3
78	CH	NH	Cyclohexyl	H	NMe	0.91	1.75	>3	>3
79	CH	NH	Cyclohexyl	Me	CH_2	>3	>3	>3	>3
80	CH	NH	Cyclohexyl	Me	O	>3	>3	>3	>3
81	CH	NH	Cyclohexyl	Me	NMe	>3	>3	>3	>3
89	N	S	Cyclopropyl	H	CH_2	>3	>3	>3	>3
90	N	S	Cyclopropyl	H	O	0.70	1.45	>3	>3
91	N	S	Cyclopropyl	H	NMe	>3	1.14	0.44	0.80
92	N	S	Cyclopropyl	Me	CH_2	>3	>3	>3	>3
93	N	S	Cyclopropyl	Me	O	>3	>3	>3	>3
94	N	S	Cyclopropyl	Me	NMe	>3	>3	>3	>3
95	N	S	Cyclohexyl	H	CH_2	>3	>3	>3	>3
96	N	S	Cyclohexyl	H	O	>3	1.63	2.17	>3

97	N	S	Cyclohexyl	H	NMe	>3	>3	0.33	0.40
98	N	S	Cyclohexyl	Me	CH_2	>3	>3	>3	>3
99	N	S	Cyclohexyl	Me	O	>3	>3	>3	>3
100	N	S	Cyclohexyl	Me	NMe	>3	>3	>3	>3
107	N	NH	Cyclopropyl	H	CH_2	>3	>3	>3	>3
108	N	NH	Cyclopropyl	H	O	1.06	2.64	>3	>3
109	N	NH	Cyclopropyl	H	NMe	>3	>3	>3	>3
110	N	NH	Cyclopropyl	Me	CH_2	>3	>3	>3	>3
111	N	NH	Cyclopropyl	Me	O	>3	>3	>3	>3
112	N	NH	Cyclopropyl	Me	NMe	>3	>3	>3	>3
113	N	NH	Cyclohexyl	H	CH_2	>3	>3	>3	>3
114	N	NH	Cyclohexyl	H	O	>3	>3	2.30	>3
115	N	NH	Cyclohexyl	H	NMe	>3	>3	>3	>3
116	N	NH	Cyclohexyl	Me	CH_2	>3	>3	>3	>3
117	N	NH	Cyclohexyl	Me	O	>3	>3	>3	>3
118	N	NH	Cyclohexyl	Me	NMe	>3	>3	>3	>3

Table 1. Evaluation of the anti-necroptotic activity and observation of possible effects on cellular growth and nuclear area of the synthesized tozasertib analogues. Compound is classified as inactive when less than 50% inhibition is observed at a concentration of 3 μM of inhibitor. The IC_{50}-value is then shown as >3 μM in table 4.1.

[a] Inhibition of mTNF-induced necroptosis in the presence of pan-caspase inhibitor zVAD.fmk. L929sAhFAS cells have been pre-treated with compound for 0.5h followed by stimulation with mTNF (2500 IU/ml) and zVAD.fmk (1 μM) for 3h in the presence of Hoechst (1 μM) and propidium iodide (PI) (3 μM). IC_{50}-values were calculated through a dose-response curve with the percentage of cell death as quantitative read-out.

[b] Inhibition of mTNF-induced necroptosis in the presence of pan-caspase inhibitor zVAD.fmk. L929sAhFAS cells have been pre-treated with compound for 24h followed by stimulation with mTNF (2500 IU/ml) and zVAD.fmk p(1 μM) for 3h in the presence of Hoechst (1 μM) and propidium iodide (PI) (3 μM). IC_{50}-values were calculated through a dose-response curve with the percentage of cell death as quantitative read-out.

[c] Value is calculated via dose-response curve of the concentration of the individual compound in function of the cellular growth after 24h. For curve fitting and IC_{50} determination, the bottom value equals the total amount of cells in the highest concentration of tozasertib and the top value equals the total amount of cells in untreated conditions.

[d] Value is calculated via dose-response curve of the concentration of the individual compound in function of the mean nuclear area. For curve fitting and IC_{50}-determination, this results in a sigmoidal curve of which the bottom value equals the normal nuclear area and the top value equals the maximal nuclear area at the highest concentration of tozasertib.

Because tozasertib was initially identified as a pan-AurK inhibitor to treat multiple types of leukemia, it is to be expected that novel analogues that are derived from tozasertib can still display similar properties.[48,49] As can be viewed in table 1, tozasertib clearly reduced cellular growth ($IC_{50,\ growth}$ = 0.97 μM) and also interfered with normal cell division which can be verified by morphological analysis of the nuclei of the cells ($IC_{50,\ nuclear\ area}$ = 1.06 μM). Tozasertib is able to inhibit necroptosis in the low micromolar range ($IC_{50,\ -0.5h}$ = 0.98 μM and $IC_{50,\ -24h}$ = 1.02 μM). However due to its effects on cell division, tozasertib can be classified as an aselective compound for the inhibition of necroptosis. In order to meet the aforementioned goals, it was important that tozasertib analogues were free from these undesirable effects on cell growth and nuclear area whilst still maintaining a potent inhibition of TNF-induced necroptosis.

Out of the 46 compounds that were tested under these conditions, seven compounds (**70, 71, 72, 77, 78, 90** and **108**) had the potential to inhibit TNF-induced necroptosis without affecting cell growth and/or nuclear area. Out of these seven compounds **70-72** were able to inhibit necroptosis in a slightly more potent manner than tozasertib after both 0.5h and 24h pre-treatment. Compounds **77**, **78**, **90** and **108** also provided the desired inhibitory profile albeit in a slightly less significant manner than compounds **70-72**. In addition to compounds that favour necroptosis inhibition without affecting cell growth and/or nuclear area, there were also compounds with the opposite effect. These compounds did not inhibit TNF-induced necroptosis, but did inhibit cellular growth and nuclear area in a very similar

manner as tozasertib. This is most clearly exemplified by compound **97** which affects cell growth and nuclear area in a more potent fashion than tozasertib.

The results of this assay led to another interesting remark that can be made towards the nature of the linker atom X between the two aromatic six-membered rings. In compounds **70-72**, which were deemed the most interesting compounds regarding both potency and absence of nuclear abnormalities, the sulfur linker connecting the two six-membered aromatic systems was replaced by a nitrogen linker. In general, most compounds in which the sulfur linker was replaced by a nitrogen no longer affect cellular growth and nuclear area. This strongly suggests that the choice of the linker atom could be important for diminishing the unwanted cellular effects that are classically associated with AurK inhibition by tozasertib. Overall a trend was observed that compounds containing a central triazine core, a 4,5-dimethylated pyrazole moiety at R_2 or the combination of both, no longer affected cell growth and nuclear area. This was clearly exemplified by compounds **73-75, 79-81, 92-94**, and **110-112**. It should be noted however that these compounds do no longer possess any activity in the phenotypical assays.

In order to validate the findings of the phenotypical assay, the most favourable necroptosis inhibitors **70-72** were first evaluated in an enzymatic assay against recombinant hAurK A and B, and hRIPK1. Compound **97** was also included in this assay since it showed a strong potential for induction of nuclear abnormalities without showing any potency to inhibit TNF-induced necroptosis. In this enzymatic assay some interesting observations were seen regarding the nature of the linker atom between the two six-membered aromatic rings. In order to investigate this further, the structural effects of the substitution of a sulfur to a nitrogen linker were studied through a molecular modelling study.

Evaluation of the compounds against the recombinant enzymes

In addition to compounds **70-72** and **97**, Nec-1s (**2**) and tozasertib (**8**) were also included as reference compounds. (Table 2)

In this enzymatic assay Nec-1s (**2**) potently inhibited hRIPK1 but did not affect the hAurK enzymes. Tozasertib (**8**) on the other hand was shown to be a potent inhibitor of all the enzymes included in this assay.

In this experiment compounds **70-72** were able to inhibit hRIPK1 with low micromolar IC_{50}-values. These compounds are even 2- to 4-fold more potent for inhibition of hRIPK1 than Nec-1s (**2**). Tozasertib (**8**) was also shown to be a very potent inhibitor of both hAurK A and hAurK B. It should be noted that the most potent compounds **70-72** still inhibited both hAurK enzymes, nevertheless in a significantly less potent manner than tozasertib. Compound **97**, which did not demonstrate any potency towards the inhibition of TNF-induced necroptosis in the aforementioned phenotypic assay, showed to be a significantly less potent inhibitor for hRIPK1 in the enzymatic assay. Compound **97** still maintained its inhibition of the hAurK enzymes, but in a slightly more potent manner in comparison to compound **70-72**. In general it can be concluded that a correlation is observed between the results of both the phenotypic and the enzymatic assay.

Compound	hAurK A (μM)	hAurK B (μM)	hRIPK1 (μM)
Nec-1s (**2**)	>3	>3	0.754
Tozasertib (**8**)	0.030	0.068	0.208
70	0.310	0.959	0.295
71	0.430	0.853	0.167
72	0.293	1.345	0.178
97	0.210	0.229	2.468

Table 2. IC_{50}-values from the enzymatic assay. Compounds were evaluated for their potency to inhibit recombinant hAurK A, hAurK B and hRIPK1. The results were obtained through a luminescence-based ADP-Glo kinase™ assay. When a compound was inactive the IC_{50}-value is shown as >3 μM. The confidence intervals and statistical validation of the results presented in table 2 are reported in the supplementary information table S3. [53]

Possible effects of the nature of the linking atom X

Inspection of the tozasertib:aurora kinase A:TPX2 cocrystal structure (PDB 3E5A) reveals that the linker atom in tozasertib (**8**) is rather solvent-exposed and does not engage in highly specific interactions with the protein.[54] It thus seems unlikely that contacts with the protein are responsible for the difference in assay outcomes. However, the linker atoms do differ substantially in electronic properties. The nitrogen linker is more strongly conjugated to the aromatic ring systems, which may increase the rigidity of the general structure and influence the conformational preferences of the system. In order to investigate this effect, two model systems were designed: *N*-phenylpyrimidin-2-amine and 2-(phenylthio)pyrimidine. The two torsion angles around the nitrogen or sulfur linker atoms controlling the orientation of the two rings are examined through relaxed dihedral scans at the PW6B95-D3(BJ)[55–57]/def2-TVZP[58,59] level of theory in ORCA.[60] For the first dihedral, depicted in figure 4 (upper image), both profiles are similar in shape. Mimima are located at 0° (or 360°) and 180°. In the tozasertib:aurora kinase A:TPX2 cocrystal structure, this dihedral is approximately 336°. As this is quite close to a minimum, we do not anticipate this dihedral to be responsible for the differences observed in the cellular assay. For the second dihedral, illustrated in figure 4 (lower image), marked differences between the energy profiles are observed. For the sulfur-linked system, mimima are located at 90° and 270°. The angle in the crystal structure is 98°, corresponding to a minimum. In the most stable conformation of the sulfur-linked system, the two aromatic rings are approximately peripendicular to one another. In the nitrogen-linked system, energetic minima are located at 0° (and 360°) and 180° degrees. The 98° torsion angle in the crystal structure of tozasertib (**8**) is highly unfavourable for this system. In contrast to the sulfur-linked system, the most stable conformation of the nitrogen-linked system is a fully planar conformation. We thus propose that the nitrogen-linked compounds are not able to bind in the same conformation as tozasertib (**8**), due to the difference in their respective conformational preferences. This may explain the lower affinity of the nitrogen-linked analogs for AurK A.

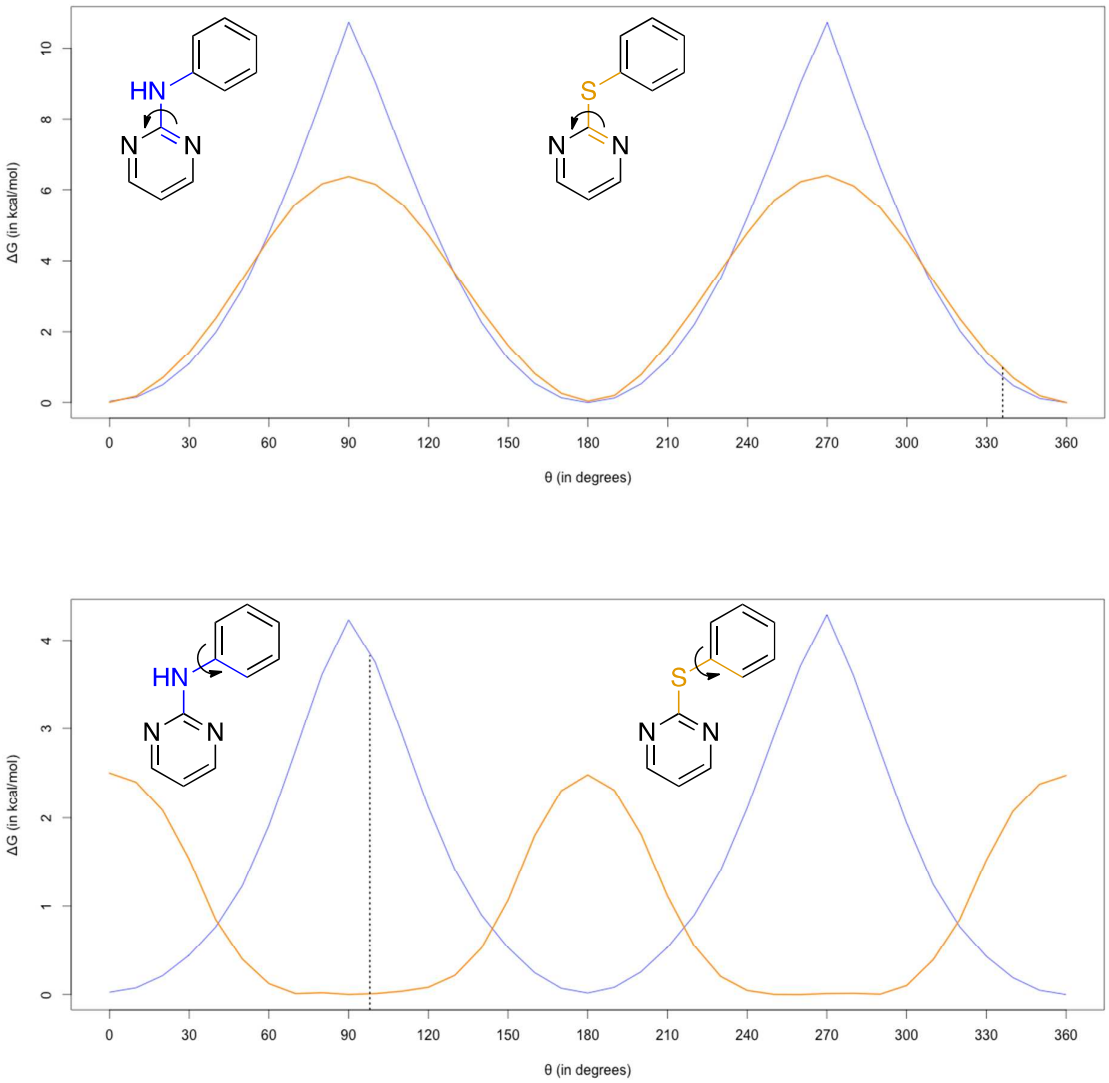


Figure 4: DFT relaxed surface scan of both dihedrals. **(Upper image)** Relaxed surface scan for the first dihedral. **(Lower Image)** Relaxed surface scan for the second dihedral. The energies for the nitrogen-linked model compound are shown in blue, the energies for the sulfur-linked model compound are shown in orange. The arrow denotes the dihedral angle being scanned. Dihedral angle values corresponding to the crystal structure are marked by a dotted line.

Investigation of the binding mode of tozasertib in RIPK1

A docking experiment was performed to propose a possible binding mode for tozasertib in RIPK1 (Figure 5). Tozasertib was docked into a cocrystal structure 4NEU of RIPK1 complexed with a 1-aminoisoquinoline inhibitor 4NEU.[35] The proposed binding mode is very similar to the binding mode observed in the tozasertib:AurA cocrystal structure. The three hydrogen bonds to the hinge motif are conserved and the phenyl ring interacts with the G-loop.

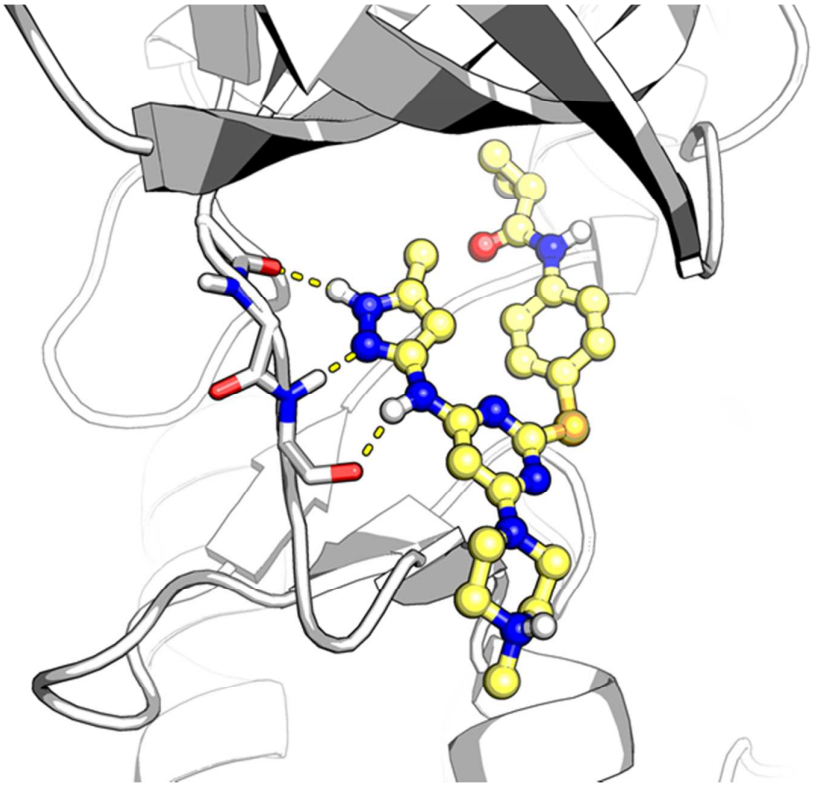


Figure 5. Predicted binding mode of tozasertib on RIPK1. The protein is shown in grey cartoon representation. Key hinge backbone residues are shown in stick representation. The ligand is shown in yellow ball-and-stick representation. Key hydrogen bonds with the hinge are denoted with a yellow dashed line.

In vivo investigation of tozasertib and selected analogues in a TNF-induced SIRS model

Tozasertib **8** and compounds **70-72** and **97** were tested in the TNF-induced systemic inflammatory response syndrome (SIRS) model in order to evaluate their potential to protect against RIPK kinase-driven inflammation *in vivo*, a model that relies on RIPK1 and RIPK3 kinase activity.[27,61–63] Based on the *in vitro* results (both phenotypical and enzymatic assays), compounds **70-72** were selected as more TNF-induced necroptosis specific inhibitors, as well as compound **97** that favours AurK over necroptosis inhibition. This experiment was conducted to investigate whether the most interesting tozasertib analogues **70-72** have an improved profile over tozasertib for inhibition of TNF-induced necroptosis *in vivo*. All compounds were administered by oral gavage (50 mg/kg) 1.5h before mTNF treatment (i.v., 500 µg/kg). Tozasertib protected mice significantly from hypothermia and death caused by TNF ($p=0.0035$ compared to vehicle, $p= 0.0014$ compared to **97**), though in a less potent manner than Nec-1s. (Figure 6) The mice pre-treated with compounds **71** and **72**, survived the TNF-treatment better than those treated with tozasertib ($p_{72}=0.0133$ and $p_{71}=0.0199$ compared to vehicle and $p_{72}=0.0195$ and $p_{71}=0.0174$ compared to **97**). Suprisingly, compound **70** did not exert any protective effect.

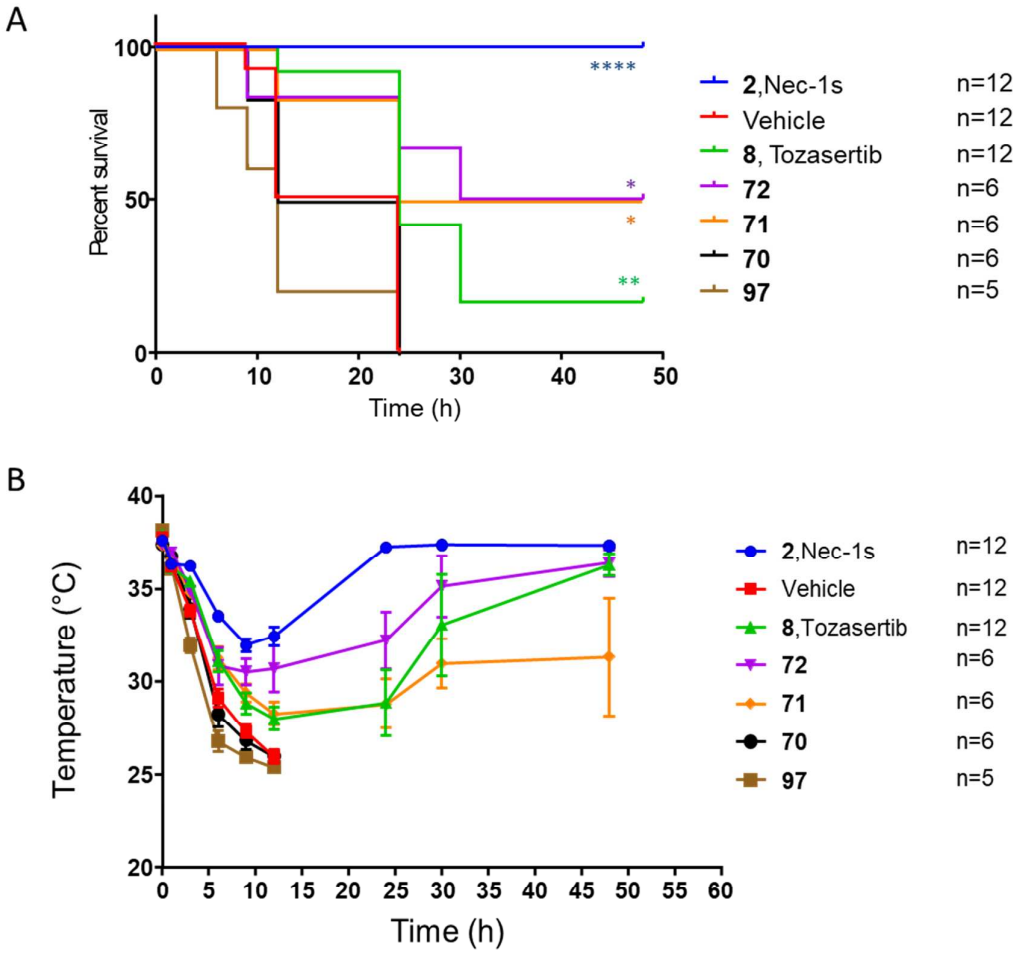


Figure 6. Tozasertib and its analogues **71** and **72** protect against TNF-induced SIRS. Mice were challenged with 10 µg mTNF (500 µg/kg) in the presence or absence of tozasertib/analogue. All compounds were given by oral gavage 1.5h before mTNF challenge at a dose of 50 mg/kg. Mantel-Cox test was performed as statistical analysis on the survival curves. P-values <0.05 = *, P-values <0.005 = **, P-values <0.0005 = **** (compared to vehicle). Temperature curves represent mean and standard error of the mean. Data from two independent experiments are shown.

CONCLUSION

Tozasertib, a pan-aurora kinase inhibitor, was originally developed to treat multiple forms of leukemia.[49] Due to AurK inhibition tozasertib induces nuclear abnormalities like inhibition of cell proliferation and increased nuclear area. In a kinase selectivity screen tozasertib was reported to have a high affinity for RIPK1 (K_d = 20 nM.).[43] In the framework of drug repurposing we started this research with the confirmation that tozasertib indeed potently inhibited TNF-induced necroptosis in a dose-dependent manner using a cellular assay. This prompted the design and synthesis of a set of 30 compounds with very strong structural similarities to tozasertib to investigate the potential of this scaffold for the design of novel inhibitors of TNF-induced necroptosis. Different variations at multiple positions were introduced in order to investigate which structural modifications of the original tozasertib scaffold were well tolerated. Upon analysis of the results of this first set of molecules we were able to formulate a preliminary SAR that was used as a base for a follow-up series. After the most desirable substituents were identified, a second library of 46 tozasertib analogues was synthesized. These 46 novel compounds were once again tested in a phenotypical screen using mTNF as a stimulus of necroptotic cell death whilst preventing the cells from undergoing apoptosis by using the pan-capase inhibitor zVAD.fmk. Whilst compounds **70-72** were considered to be good inhibitors for TNF-induced necroptosis, they did not result in visible malfunctions in normal cell division which is a characteristic for treatment with AurK-inhibitors such as tozasertib. In order to validate these findings, compounds **70-72** were tested in an enzymatic assay against recombinant hAurK A, hAurK B and hRIPK1. Nec-1s (**2**) and tozasertib (**8**) were used as reference molecules. In this assay compounds **70-72** were able to inhibit recombinant hRIPK1 in the low micromolar range. These compounds were even shown to be 2- to 4-fold more potent for inhibition of hRIPK1 than Nec-1s (**2**), a compound extensively used in necrotic cell death research. Another interesting feature of compounds **70-72** was that they inhibited both hAurK in a significantly less potent manner than the reference compound tozasertib (**8**). For compounds **70-72** the linker atom between the two six-membered aromatic rings was modified from a sulfur to a nitrogen. Molecular modelling studies suggested that nitrogen-linked compounds are not able to bind with AurK in the same conformation as tozasertib (**8**)

due to the difference in their respective conformational preferences. In order to get a better insight of the binding mode of tozasertib, it was docked into a cocrsytal structure of RIPK1 complexed with a 1-aminoisoquinoline inhibitor. This showed that three hydrogen bonds to the hinge motif are conserved and the phenyl ring interacts with the G-loop. Finally, the *in vivo* effect of tozasertib and four tozasertib analogues (**70-72** and **97**) was studied in a TNF-induced SIRS model. In this study, compounds **71** and **72** had an increased survival to TNF-treatment compared to tozasertib.

Concludingly, we were able to report for the first time that tozasertib analogues can potently inhibit TNF-induced necroptosis. By applying some structural modifications to the tozasertib scaffold, we were able to fine-tune the inhibitory profile of this type of compound in order to reduce the off-target effects that are commonly associated with AurK inhibition. The more selective compounds **71** and **72** were able to inhibit TNF-induced necroptosis *in vitro* and *in vivo* in a similar or even more potent manner than tozasertib (**8**).

EXPERIMENTAL SECTION

Unless otherwise stated, laboratory reagent grade solvents were used. Reagents were obtained from Sigma-Aldrich, Acros Organics or Fluorochem and were used without further purification. Characterization of all compounds was done with ^{1}H and ^{13}C NMR and mass spectrometry. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker Avance III Nanobay spectrometer with Ultrashield and analysed by use of MestReNova analytical chemistry software. Chemical shifts are in ppm, and coupling constants are in hertz (Hz). ES mass spectra were obtained from an Esquire 3000plus ion trap mass spectrometer from Bruker Daltonics. Purities were determined with two diverse HPLC systems based either on mass determination or on UV detection. A Waters acquity UPLC system coupled to a Waters TQD ESI mass spectrometer (HPLC System A) or a Waters SQD ESI mass spectrometer (HPLC system B) was used both in combination with a Waters TUV detector. The same methods on both HPLC system A and B was used for compound detection and purity determination. Water (A) and CH_3CN (B) were used as eluents. Waters Acquity UPLC BEH C18 1.7 μm, 2.1 mm × 50 mm column was used. Solvent A consisted of water with 0.1% formic acid. Solvent B consisted of acetonitrile with 0.1% formic acid. Method I involved the following: 0.15 min 95% A, 5% B, then in 1.85 min from 95% A, 5% B to 95% B, 5% A, then 0.25 min (0.350 mL/min), 95% B, 5% A. The wavelength for UV detection was 254 nm. Method II involved the following: flow 0.4 mL/min, 0.25 min 95% A, 5% B, then in 4.75 min to 95% B, 5% A, then 0.25 min 95% B, 5% A, followed by 0.75 min 95% A, 5% B. The wavelength for UV detection was 214 nm. Where necessary, flash purification was performed on a Biotage ISOLERA One flash system equipped with an internal variable dualwavelength diode array detector (200−400 nm). For normal phase purifications SNAP cartridges (10−340 g, flow rate of 10−100 mL/min) were used, and reversed phase purifications were done making use of KP-C18 containing cartridges. Dry sample loading was done by self-packing samplet cartridges using silica and Celite 545, respectively, for normal and reversed phase purifications. Gradients used varied for each purification. Mouse and rat plasma came from Innovative Research. The turbidity in the kinetic solubility experiments was measured using the UV/vis spectrophotometer Synergy MX, Biotek with Gen5.

The following section comprises the synthetic procedures and analytical data for all compounds reported in this manuscript. Several synthesis procedures that were used in the preparation of intermediates and final products are summarized here as "General Procedures". The purities of all final products were found to be > 95%.

General procedure A for compounds 10-14

To a solution of 4-aminobenzothiol (1 equiv.) in THF was added triethylamine (2.2 equiv). After cooling down the mixture to 0°C, the appropriate acyl chloride (2.2 equiv.) was added dropwise in order to keep the temperature below 10°C. The reaction mixture was stirred at 0°C for 20 minutes then warmed up to room temperature for 1 hour. The obtained solid was filtered off and the filtrate was concentrated under reduced pressure. The concentrated residue was treated with NaOH (3 equiv.) which was first dissolved in a mixture of 6:1 water:ethanol. The reaction mixture was heated to 100°C for 1 hour and cooled down to room temperature. The crude was filtered and concentrated under reduced pressure. The residue was diluted with water and filtered through a path of celite. The obtained filtrate was acidified with concentrated HCl upon reaching a pH of 3 and a white precipitate was obtained. The resulting precipitate was filtered, dissolved in ethyl acetate and washed with brine. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to the appropriate amidobenzothiol in good yields.

General procedure B for compounds 16-20

A solution of 4,6-dichloro-2-(methylsulfonyl)pyrimidine (1 equiv.) and the appropriate *N*-(4-mercaptophenyl)amide analogue (1.05 equiv.) in ACN was cooled down to -10°C. Next triethylamine (1 equiv.) was added dropwise over 20 minutes while maintaining the temperature at -10°C. Once added, the solution was stirred at that temperature for 20 more minutes. Afterwards the reaction mixture was allowed to warm up to room temperature while stirring and concentrated. Water was added to the crude and a precipitate was formed. This solid was collected by filtration and dried by

suction. After drying, the precipitate was washed with a minimal amount of ethyl acetate. An off white solid was collected by filtration and dried in vacuo. The process was repeated to yield more solid. The batches were combined to afford compounds **16-20**.

General procedure C for compounds 21-27

A solution of compound **16-20** (1 equiv.), the appropriate pyrazole moiety (1.05 equiv.), NaI (1.17 equiv.) and *N,N*-di-iso-propylethylamine (3.14 equiv.) in DMF was heated at 90°C for 18 hours. The reaction mixture was cooled down to room temperature, dissolved in ethyl acetate, washed with a $NaHCO_3$ aqueous solution (3 times) and brine. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. Next the residue was purified by flash-column chromatography on silica gel to provide the desired intermediates **21-27.**

General procedure D for compounds 8, 28-57, 70-81 and 107-118

Intermediates **21-27** , **66-69** and **103-106** were treated with the appropriate cycloaminoderivate (excess) and the mixture was stirred at 110° for 2 hours. The reaction mixture was cooled down, dissolved in ethyl acetate and washed with slightly acidified water (3 times) (pH = 5-6). The organic phases were combined, dried over $MgSO_4$ and concentrated under reduced pressure. The concentrate was purified by flash-column chromatography on silica gel (0-100% EtOAc in heptane) to provide the desired target compounds **8, 28-57, 70-81** and **107-118**.

General procedure E for compounds 59-60

4-nitroaniline (1 equiv.) was dissolved in DCM and to this solution was added dropwise to a mixture of DIPEA (1.2 equiv.) and corresponding acyl chloride (1.2-2.0 equiv.) in DCM. The reaction was stirred for 3h at room temperature. After reaction, the solution was washed with slightly acidified water (3 times) to remove the excess of aniline. The organic phase was dried over $MgSO_4$ and

concentrated under reduced pressure. The concentrate was purified by flash-column chromatography on silica gel (0-10% MeOH in DCM) to provide the desired target compounds **59-60.**

General procedure F for compounds 61-62

Intermediates **59-60** were dissolved in methanol, flushed with argon, and hydrogenated (H_2 gas) over 10% palladium hydroxide (1.5 equiv.) for 17h at room temperature. The solution was filtered through a path of Celite, and the volatiles were removed under reduced pressure. The concentrate was purified by flash-column chromatography on silica gel (10% MeOH in DCM) to provide the desired target compounds **61-62.** [50]

General procedure G for compounds 64-65

To a solution of the appropriate pyrazole moiety (1.04 equiv.) in absolute ethanol were added triethylamine (3.1 equiv.) and 2,4,6-trichloropyrimidine **63** (1 equiv.). The solution was stirred for 12h at room temperature. Next the solution was diluted in EtOAc and extracted with H_2O. The organic phase was washed with brine, dried over over $MgSO_4$ and concentrated under reduced pressure. Further purification was conducted using flash column chromatography on silica gel (0-100% EtOAc in heptane) to provide the desired target compounds **64-65** [51]

General procedure H for compounds 66-69

Intermediates **64-65** (1 equiv.), *N*-(4-aminophenyl)amide analogue intermediates **61-62** and *p*-toluene sulfonic acid monohydrate were dissolved in *n*-butanol and refluxed at 130°C for 12h. The solution was cooled down and neutralized using a saturated $NaHCO_3$ aqueous solution. The crude was extracted with EtOAc (3 times) and the collected organic phases were dried over over $MgSO_4$ and concentrated under reduced pressure. The concentrate was purified by flash-column chromatography, unless stated otherwise, on silica gel (0-100% EtOAc in heptane) to provide the desired target compounds **66-69**.[64]

General procedure I for compounds 83-84

A solution containing **82** (1.1 equiv.) was in THF was cooled down to 0 °C. A separate solution containing **13** or **14** (1 equiv.) and DIPEA (1 equiv.) in THF was added dropwise to the cooled solution containing **77**. After addition was complete, the reaction mixture was diluted with EtOAc and washed with water, acidified water (pH =4-5) and finally brine. The organic phase was dried using anhydrous $NaSO_4$ and then filtered. After concentration of the filtered solution compounds **83-84** were obtained. These intermediates were used without any further purification in the next step of the synthesis.

General procedure J for compounds 85-88

DIPEA (1.5 equiv.) was added to a solution containing **83-84** (1 equiv.) and the appropriate pyrazole moiety (1 equiv.) in dioxane. This solution was then stirred overnight at either room temperature or 120°C. The resulting mixture was then diluted with EtOAc and washed with acidified water (pH = 4-5), saturated $NaHCO_3$ and brine. The organic layer was dried using anhydrous $NaSO_4$ and then filtered. The organic layer was concentrated under reduced pressure to yield compounds **85-88**. These intermediates were used in the following step of the synthesis without any further purification.

General procedure K for compounds 89-100

Intermediates **85-88** were treated with the appropriate cycloaminoderivate (excess). This mixture was allowed to stir at room temperature for 30 minutes before being diluted with EtOAc. The organic layer was washed with slightly acidic water (pH = 4-5) and then it was dried using anhydrous $NaSO_4$. The organic layer was concentrated under reduced pressure. This crude product was then further purified using flash column chromatography on silica gel. (0-100% EtOAc in heptane) to provide the final compounds **89-100.**

General procedure L for compounds 101-102

To a homogenous solution of 2,4,6-trichloropyrimidine **77** (1 equiv.) in dioxane was added K_2CO_3 (1.1 equiv.) and *N*-(4-aminophenyl)amide analogue intermediates **61-62** (1 equiv.)**.** This solution was allowed to stir at room temperature for 36 h. Solvents were evaporated and water was added, extracting the aqueous phase with EtOAc (3 times). The organic layers were washed with $NaHCO_3$ dried over over $MgSO_4$ and concentrated under reduced pressure to provide intermediates **101-102**.

General procedure M for compounds 103-106

To a solution of **101-102** (1 equiv.) in dioxane was added DIPEA (1.5 equiv.) and the appropriate pyrazole moiety (1.5 mmol). The reaction was refluxed overnight. Solvents were evaporated and water was added, extracting the aqueous phase with EtOAc (3 times). The combined organic phases were washed brine, dried over over $MgSO_4$ and concentrated under reduced pressure. Further purification was conducted, unless stated otherwise, using flash column chromatography on silica gel (0-100% EtOAc in heptane) to provide the desired target compounds **103-106.**

***N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (8)**

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide **24** (0.100g, 0.249 mmol) and *N*-methylpiperazine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (0.010g, 0.022 mmol) as an amorphous powder. (Yield : 8.63%)

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 0.78 - 0.85 (m, 4H), 1.82 (p, J = 6.27 Hz, 1H), 2.02 (s, 3H), 2.19 (s, 3H), 2.31 (t, J = 5.11 Hz, 4H), *3.44 (m, 4H)*, 5.44 (s, 1H), 6.04 (s, 1H), 7.46 - 7.49 (m, 2H), 7.67 - 7.72 (m, 2H), 9.22 (s, 1H), 10.39 (s, 1H), 11.71 (s, 1H).

t_R 1.44 min, MS (ESI) m/z 465 [M +H] (100%) (HPLC System A)

N-(4-mercaptophenyl)acetamide (10)

Following **general procedure A**, using acetylchloride (5.02 ml, 70.3 mmol) as the appriopriate acylchloride. *N*-(4-mercaptophenyl)acetamide was obtained as a white solid. (Yield: 56.1%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.03 (s, 3H), 5.21 (s, 1H), 6.99 - 7.36 (m, 2H), 7.37 - 7.56 (m, 2H), 9.92 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 24.49, 120.14, 129.81, 130.62, 139.99, 168.96.

t_R 1.35 min, MS (ESI) m/z 167 [M +H] (95%) (HPLC System B)

***N*-(4-mercaptophenyl)propionamide (11)**

Following **general procedure A**, using propionylchloride (6.14 ml, 70.3 mmol) as the appriopriate acylchloride. *N*-(4-mercaptophenyl)propionamide was obtained as a white solid. (Yield: 51.8%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.07 (t, J = 7.55 Hz, 3H), 2.32 (q, J = 7.56 Hz, 2H), 5.24 (s, 1H), 7.18 - 7.26 (m, 2H), 7.49 - 7.57 (m, 2H), 9.98 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.06, 30.00, 120.17, 129.68, 130.68, 140.14, 172.72.

t_R 1.47 min, MS (ESI) m/z 182 [M +H] (100%) (HPLC System A)

***N*-(4-mercaptophenyl)pivalamide (12)**

Following **general procedure A**, using pivaloyl chloride (8,66 ml, 70,3 mmol) as the appriopriate acylchloride. *N*-(4-mercaptophenyl)pivalamide was obtained as a white solid. (Yield: 61.8%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21 (s, 9H), 5.20 (s, 1H), 7.19 - 7.25 (m, 2H), 7.51 - 7.58 (m, 2H), 9.16 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 27.67, 121.56, 125.61, 129.48, 137.38, 176.79.

t_R 1.76 min, MS (ESI) m/z 210 [M +H] (100%) (HPLC System B)

***N*-(4-mercaptophenyl)cyclopropanecarboxamide (13)**

Following **general procedure A**, using cyclopropanecarbonyl chloride (2.64 ml, 29.0 mmol) as the appriopriate acylchloride. *N*-(4-mercaptophenyl)cyclopropanecarboxamide was obtained as a white solid. (Yield: 82.0%)

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 0.71 - 0.87 (m, 4H), 1.67 - 1.81 (m, 1H), 5.20 (s, 1H), 7.18 - 7.26 (m, 2H), 7.46 - 7.55 (m, 2H), 10.16 (s, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 7.59, 14.99, 120.25, 125.24, 129.85, 137.43, 171.92.

t_R 1.57 min, MS (ESI) m/z 194 [M +H] (100%) (HPLC System A)

_N_-(4-mercaptophenyl)cyclohexanecarboxamide (14)

Following **general procedure A**, using cyclohexanecarbonyl chloride (9.45 ml, 32.0 mmol) as the appriopriate acylchloride. *N*-(4-mercaptophenyl)cyclopropanecarboxamide was obtanied as a white solid. (Yield: 39.7%)

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 1.33 - 1.46 (m, 3H), 1.61 - 1.70 (m, 2H), 1.71 - 1.84 (m, 5H), 2.25 - 2.35 (m, 1H), 5.18 (s, 1H), 7.16 - 7.26 (m, 2H), 7.47 - 7.54 (m, 2H), 9.77 (s, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 25.71, 25.89, 29.59, 42.66, 120.32, 125.15, 129.77, 137.56, 174.62.

t_R 1.90 min, MS (ESI) m/z 236 [M +H] (91%) (HPLC System A)

_N_-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)acetamide (16)

Following **general procedure B**, using *N*-(4-mercaptophenyl)acetamide **10** (1.547g, 9.25 mmol) to afford *N*-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)acetamide (Yield: 52.8%)

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 2.09 (s, 3H), 7.53 (d, J = 8.39 Hz, 2H), 7.62 - 7.81 (m, 3H), 10.20 (s, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 24.59, 117.75, 120.09, 120.48, 136.45, 141.52, 161.63, 169.21, 173.33.

MS (ESI) m/z 314 [M +H] (HPLC System A)

_N_-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)propionamide (17)

Following **general procedure B,** using *N*-(4-mercaptophenyl)propionamide **11** (2.498 g, 13.78 mmol) to afford *N*-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)propionamide. (Yield: 58.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (t, J = 7.55 Hz, 3H), 2.37 (q, J = 7.52 Hz, 2H), 7.53 (d, J = 8.20 Hz, 2H), 7.69 (s, 1H), 7.74 (d, J = 8.25 Hz, 2H), 10.17 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.02, 30.09, 117.73, 120.11, 120.33, 136.45, 141.61, 161.62, 172.90, 173.36.

MS (ESI) m/z 328 [M +H] (HPLC System A)

_N_-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)pivalamide (18)

Following **general procedure B,** using *N*-(4-mercaptophenyl)pivalamide **12** (3.87 g, 18.50 mmol) to afford *N*-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)pivalamide (Yield: 34.4%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25 (s, 9H), 7.51 - 7.55 (m, 2H), 7.70 (s, 1H), 7.78 - 7.83 (m, 2H), 9.43 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 27.58, 117.75, 120.63, 121.24, 136.17, 141.66, 161.63, 173.35, 177.28.

MS (ESI) m/z 356 [M +H] (HPLC System A)

_N_-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (19)

Following **general procedure B,** using *N*-(4-mercaptophenyl)cyclopropanecarboxamide **13** (3.290 g, 17.02 mmol) to afford *N*-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (Yield: 42.7%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (d, J = 6.82 Hz, 5H), 1.81 (dd, J = 6.12, 11.82 Hz, 1H), 2.09 (s, 3H), 7.54 (d, J = 8.27 Hz, 2H), 7.73 (d, J = 9.08 Hz, 3H), 10.46 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.93, 15.16, 117.77, 120.11, 120.40, 136.50, 141.52, 161.63, 172.55, 173.33.

MS (ESI) m/z 341 [M +H] (HPLC System A)

_N_-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (20)

Following **general procedure B,** using *N*-(4-mercaptophenyl)cyclohexanecarboxamide **14** (4.35 g, 18.50 mmol) to afford *N*-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (Yield: 63.8%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.35 (m, 3H), 1.37 - 1.49 (m, 2H), 1.63 - 1.70 (m, 1H), 1.73 - 1.88 (m, 4H), 2.36 (tt, J = 3.59, 11.63 Hz, 1H), 7.48 - 7.55 (m, 2H), 7.71 (s, 1H), 7.72 - 7.77 (m, 2H), 10.07 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 25.68, 25.85, 29.54, 45.41, 117.75, 120.19, 120.32, 136.44, 141.73, 161.63, 173.36, 175.19.

MS (ESI) m/z 382 [M +H] (HPLC System A)

_N_-(4-((4-chloro-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)acetamide (21)

Following **general procedure C,** using *N*-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)acetamide **16** (1 g, 3.18 mmol) and 5-methyl-1*H*-pyrazol-3-amine (0.325 g, 3.34 mmol) to obtain *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)acetamide (Yield: 43.6%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.99 (s, 3H), 2.09 (s, 3H), 5.26 (s, 1H), 6.47 (s, 1H), 7.51 - 7.57 (m, 2H), 7.67 - 7.83 (m, 2H), 10.23 (s, 2H), 11.92 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.65, 24.57, 96.03, 100.84, 119.81, 122.06, 137.45, 141.29, 152.78, 159.91, 169.14, 170.79, 172.71.

MS (ESI) m/z 375 [M +H] (HPLC System A)

_N_-(4-((4-chloro-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)propionamide (22)

Following **general procedure C,** using *N*-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)propionamide **17** (1 g, 3.05 mmol) and 5-methyl-1*H*-pyrazol-3-amine (0.311 g, 3.20 mmol) to obtain *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)propionamide (Yield: 76%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (t, J = 7.55 Hz, 3H), 1.89 - 2.05 (m, 3H), 2.36 (q, J = 7.53 Hz, 2H), 6.47 (s, 1H), 5.25 (s, 1H), 7.50 - 7.58 (m, 2H), 7.71 - 7.82 (m, 2H), 10.16 (s, 1H), 10.22 (s, 1H), 11.91 (s, 1H) **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.18, 10.64, 30.21, 96.03, 100.82, 119.85, 121.94, 137.47, 138.49, 141.37, 147.72, 157.35, 159.91, 162.76, 172.87.

MS (ESI) m/z 388 [M +H] (HPLC System A)

***N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)pivalamide (23)**

Following **general procedure C,** using *N*-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)pivalamide **18** (0.600 g, 1.684 mmol) and 5-methyl-1*H*-pyrazol-3-amine (0.172 g, 1.768 mmol) to obtain *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)pivalamide (Yield: 81.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (s, 3H), 1.25 (s, 9H), 5.24 (s, 1H), 6.47 (s, 1H), 7.47 - 7.60 (m, 2H), 7.79 - 8.01 (m, 2H), 9.46 (s, 1H), 10.23 (s, 1H), 11.89 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.75, 27.56, 31.76, 96.10, 100.79, 120.71, 122.09, 137.22, 138.45, 141.50, 147.70, 157.30, 159.89, 172.81, 177.21.

MS (ESI) m/z 388 [M +H] (HPLC System A)

***N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (24)**

Following **general procedure C,** using *N*-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide **19** (0.180 g, 0.529 mmol) and 5-methyl-1*H*-pyrazol-3-amine (0.054 g, 0.556 mmol) to obtain *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (Yield: 9.43%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (d, J = 6.14 Hz, 4H), 1.78 - 1.87 (m, 1H), 1.98 (s, 3H), 5.12 - 5.33 (m, 1H), 6.47 (s, 1H), 7.50 - 7.60 (m, 2H), 7.69 - 7.84 (m, 2H), 10.22 (s, 1H), 10.49 (d, J = 10.43 Hz, 1H), 11.95 (d, J = 37.80 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.77, 10.58, 15.14, 96.00, 100.82, 119.81, 121.93, 137.49, 141.35, 147.71, 157.29, 159.91, 163.46, 172.50, 173.62.

MS (ESI) m/z 401 [M +H] (HPLC System A)

***N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (25)**

Following **general procedure C,** using *N*-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide **20** (0.500 mg, 1.308 mmol) and 5-methyl-1*H*-pyrazol-3-amine (0.140 mg, 1.439 mmol) to obtain *N*-(4-((4-chloro-6-((5-methyl-1H-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (Yield: 51.8%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21 - 1.36 (m, 3H), 1.37 - 1.51 (m, 2H), 1.62 - 1.69 (m, 1H), 1.73 - 1.85 (m, 5H), 1.89 - 1.98 (m, 2H), 2.32 - 2.42 (m, 1H), 5.25 (s, 1H), 6.47 (s, 1H), 7.48 - 7.56 (m, 2H), 7.73 - 7.85 (m, 2H), 10.10 (s, 1H), 10.23 (s, 1H), 11.89 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.75, 25.68, 25.84, 29.53, 45.46, 96.06, 100.79, 119.92, 121.88, 137.46, 138.46, 141.50, 147.54, 157.31, 159.89, 170.79, 175.12.

MS (ESI) m/z 443 [M +H] (HPLC System A)

_N_-(4-((4-chloro-6-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (26)

Following **general procedure C,** using *N*-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide **19** (1 g, 2.94 mmol) and 5-methyl-1*H*-pyrazol-3-amine (0.343 g, 3.09 mmol) to obtain *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (Yield: 65.6%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.78 - 0.91 (m, 4H), 1.51 - 1.91 (m, 4H), 2.11 (s, 3H), 6.81 (s, 1H), 7.49 (s, 2H), 7.60 - 7.75 (m, 2H), 9.62 (s, 1H), 10.41 (s, 1H), 12.14 (s, 1H).**^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.42, 7.87, 9.77, 15.14, 99.85, 104.43, 119.76, 122.03, 136.39, 137.00, 140.86, 145.36, 159.31, 162.28, 171.40, 172.42.

MS (ESI) m/z 414 [M +H] (HPLC System B)

_N_-(4-((4-chloro-6-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (27)

Following **general procedure C,** using *N*-(4-((4,6-dichloropyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide **20** (1 g, 2.62 mmol) and 4,5-dimethyl-1*H*-pyrazol-3-amine

(0.305 g, 2.75 mmol) to obtain *N*-(4-((4-chloro-6-((4,5-dimethyl-1H-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (Yield: 41.8%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23 - 1.35 (m, 3H), 1.37 - 1.50 (m, 2H), 1.62 - 1.87 (m, 8H), 2.11 (s, 3H), 2.30 - 2.41 (m, 1H), 6.73 (s, 1H), 7.41 - 7.53 (m, 2H), 7.64 - 7.74 (m, 2H), 9.59 (s, 1H), 10.01 (s, 1H), 12.14 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.48, 9.86, 25.69, 25.86, 29.57, 45.41, 99.90, 100.84, 119.85, 121.98, 136.31, 140.09, 141.04, 162.30, 164.61, 170.79, 171.46, 175.06.

MS (ESI) m/z 457 [M +H] (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)thio)phenyl)acetamide (28)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)acetamide **21** (0.100 g, 0,267 mmol) and piperidine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)thio)phenyl)acetamide (0.0811g, 0.191 mmol) as an amorphous powder. (Yield: 71.8%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42 - 1.50 (m, 4H), 1.55 - 1.61 (m, 2H), 2.04 (s, 3H), 2.08 (s, 3H), 3.35 - 3.38 (m, 4H), 5.48 (s, 1H), 6.07 (s, 1H), 7.45 - 7.52 (m, 2H), 7.65 - 7.73 (m, 2H), 9.15 (s, 1H), 10.14 (s, 1H), 11.71 (s, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 10.04, 22.65, 23.70, 25.15, 44.93, 79.54, 94.49, 119.72, 120.23, 125.24, 129.76, 136.32, 139.41, 160.28, 170.32, 170.47, 171.66.

t_R 1.71 min, MS (ESI) m/z 424 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)thio)phenyl)acetamide (29)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)acetamide **21** (0.100 g, 0,267 mmol) and morpholine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)thio)phenyl)acetamide (0.0855g, 0.201mmol) as an amorphous powder. (Yield: 75.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.03 (s, 3H), 2.08 (s, 3H), 3.31 - 3.36 (m, 4H), 3.59 - 3.64 (m, 4H), 5.46 (s, 1H), 6.03 (s, 1H), 7.46 - 7.52 (m, 2H), 7.65 - 7.72 (m, 2H), 9.28 (s, 1H), 10.15 (s, 1H), 11.73 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.84, 24.55, 44.46, 66.17, 80.78, 95.52, 119.49, 123.80, 130.62, 136.84, 140.52, 143.68, 160.34, 162.76, 169.03, 169.94.

t_R 1.48 min, MS (ESI) m/z 426 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperazin-1-yl)pyrimidin-2-yl)thio)phenyl)acetamide (30)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)acetamide **21** (0.100 g, 0,267 mmol) and piperazine (0.023g, 1.335 mmol) in DMF (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(piperazin-1-yl)pyrimidin-2-yl)thio)phenyl)acetamide (0.0047g, 0.011 mmol) as an amorphous powder. (Yield: 4.15%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.03 (s, 3H), 2.07 (s, 3H), 2.47 (s, 1H), 2.62 - 2.74 (m, 4H), 3.29 (d, J = 4.96 Hz, 4H), 5.46 (s, 1H), 6.03 (s, 1H), 7.44 - 7.53 (m, 2H), 7.63 - 7.73 (m, 2H), 9.18 (s, 1H), 10.14 (s, 1H), 11.70 (s, 1H).

t_R 1.23 min, MS (ESI) m/z 425 [M +H] (94%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)acetamide (31)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)acetamide **21** (0.100 g, 0,267 mmol) and *N*-methylpiperazine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)acetamide (0.0323g, 0.074 mmol) as an amorphous powder. (Yield: 27.6%).

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 2.03 (s, 3H), 2.08 (s, 3H), 2.19 (s, 3H), 2.31 (t, J = 5.03 Hz, 4H), 3.35 (s, 4H), 5.45 (s, 1H), 6.04 (s, 1H), 7.43 - 7.50 (m, 2H), 7.64 - 7.72 (m, 2H), 9.22 (s, 1H), 10.16 (s, 1H), 11.71 (s, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 13.13, 22.67, 43.28, 44.75, 54.04, 80.00, 94.94, 119.75, 125.09, 129.74, 136.40, 139.44, 160.35, 162.40, 170.29, 170.63.

t_R 1.25 min, MS (ESI) m/z 439 [M +H] (100%) (HPLC System A)

_N_-(4-((4-(4-ethylpiperazin-1-yl)-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)acetamide (32)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)acetamide **21** (0.100 g, 0,267 mmol) and N-ethylpiperazine (2 ml) to affordthe desired *N*-(4-((4-(4-ethylpiperazin-1-yl)-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)acetamide (0.106g, 0.235 mmol) as an amorphous powder. (Yield : 88.0%).

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 1.01 (t, J = 7.10 Hz, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 2.28 - 2.42 (m, 6H), 3.36 (d, J = 5.62 Hz, 4H), 5.46 (s, 1H), 6.04 (s, 1H), 7.44 - 7.56 (m, 2H), 7.64 - 7.78 (m, 2H), 9.22 (s, 1H), 10.16 (s, 1H), 11.74 (s, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 10.87, 12.38, 24.55, 31.76, 44.06, 52.06, 52.35, 80.69, 95.46, 119.46, 123.90, 136.82, 138.59, 140.48, 160.32, 162.48, 169.02, 169.90.

t_R 1.22 min, MS (ESI) m/z 453 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)thio)phenyl)propionamide (33)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)propionamide **22** (0.100 g, 0.257 mmol) and piperidine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)thio)phenyl)propionamide (0.030g, 0.069 mmol) as an amorphous powder. (Yield : 26.7)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.07 - 1.14 (m, 5H), 1.46 (tt, J = 4.40, 8.43 Hz, 5H), 1.52 - 1.63 (m, 3H), 2.03 (s, 3H), 2.35 (q, J = 7.52 Hz, 2H), 5.46 (s, 1H), 6.06 (s, 1H), 7.44 - 7.52 (m, 2H), 7.65 - 7.74 (m, 2H), 9.15 (s, 1H), 10.06 (s, 1H), 11.69 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.16, 10.89, 24.66, 25.40, 31.77, 45.03, 80.39, 95.40, 119.44, 123.91, 136.82, 140.50, 149.36, 160.34, 162.16, 169.87, 172.70.

t_R 1.80 min, MS (ESI) m/z 483 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)thio)phenyl)propionamide (34)

Following **general procedure D,** using _N_-(4-((4-chloro-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)propionamide **22** (0.100 g, 0.257 mmol) and morpholine (2 ml) to afford the desired _N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)thio)phenyl)propionamide (0.100g, 0.228 mmol) as an amorphous powder. (Yield: 88%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.06 - 1.14 (m, 3H), 2.02 (s, 3H), 2.35 (q, J = 7.55 Hz, 2H), 3.34 (s, 4H), 3.62 (dd, J = 3.88, 5.78 Hz, 4H), 5.44 (s, 1H), 6.00 (s, 1H), 7.46 - 7.51 (m, 2H), 7.67 - 7.73 (m, 2H), 9.27 (s, 1H), 10.07 (s, 1H), 11.69 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.16, 10.84, 30.15, 44.46, 66.18, 80.76, 95.53, 119.52, 123.67, 136.89, 140.59, 149.05, 160.33, 162.76, 170.02, 172.73.

t_R 1.57 min, MS (ESI) m/z 440 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperazin-1-yl)pyrimidin-2-yl)thio)phenyl)propionamide (35)

Following **general procedure D,** using _N_-(4-((4-chloro-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)propionamide **22** (0.100 g, 0.257 mmol) and piperazine (0.066g, 0.771 mmol) in DMF (2 ml) to afford the desired _N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-

(piperazin-1-yl)pyrimidin-2-yl)thio)phenyl)propionamide (0.0367g, 0.084 mmol) as an amorphous powder. (Yield : 32.5%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.07 - 1.14 (m, 3H), 1.92 (s, 1H), 2.02 (s, 3H), 2.35 (q, J = 7.53 Hz, 2H), 2.74 (dd, J = 3.63, 6.25 H028z, 4H), 3.32 (d, J = 10.60 Hz, 4H), 5.46 (s, 1H), 6.02 (s, 1H), 7.45 - 7.51 (m, 2H), 7.67 - 7.74 (m, 2H), 9.17 (s, 1H), 10.04 (s, 1H), 11.69 (s, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 8.84, 9.88, 29.78, 43.51, 44.18, 79.94, 94.70, 119.74, 124.93, 136.44, 139.58, 160.44, 162.45, 170.77, 174.03, 178.48.

t_R 1.30 min, MS (ESI) m/z 439 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)propionamide (36)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)propionamide **22** (0.100 g, 0.257 mmol) and *N*-methylpiperazine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)propionamide (0.089g, 0.197 mmol) as an amorphous powder. (Yield : 76%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.06 - 1.15 (m, 3H), 2.02 (s, 3H), 2.19 (s, 3H), 2.27 - 2.42 (m, 6H), 3.36 (d, J = 8.93 Hz, 4H), 5.44 (s, 1H), 6.02 (s, 1H), 7.45 - 7.52 (m, 2H), 7.67 - 7.75 (m, 2H), 9.22 (s, 1H), 10.07 (s, 1H), 11.70 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.17, 10.86, 30.15, 43.95, 46.19, 54.55, 80.70, 95.49, 119.50, 123.76, 136.88, 140.56, 149.12, 160.33, 162.47, 169.98, 172.73.

t_R 1.27 min, MS (ESI) m/z 453 [M +H] (100%) (HPLC System A)

_N_-(4-((4-(4-ethylpiperazin-1-yl)-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)propionamide (37)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)propionamide **22** (0.100 g, 0.257 mmol) and *N*-ethylpiperazine

(2 ml) to afford the desired *N*-(4-((4-(4-ethylpiperazin-1-yl)-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)propionamide (0.0987g, 0.212 mmol) as an amorphous powder. (Yield : 82%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.01 (t, J = 7.11 Hz, 3H), 1.06 - 1.14 (m, 3H), 2.02 (s, 3H), 2.28 - 2.42 (m, 8H), 3.37 (s, 4H), 5.44 (s, 1H), 6.02 (s, 1H), 7.47 - 7.50 (m, 2H), 7.69 - 7.74 (m, 2H), 9.22 (s, 1H), 10.07 (s, 1H), 11.69 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.17, 10.87, 12.39, 30.15, 44.07, 52.07, 52.36, 80.67, 95.49, 119.50, 123.77, 136.87, 140.56, 149.17, 160.31, 162.48, 169.96, 172.72**.**

t_R 1.30 min, MS (ESI) m/z 467 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)thio)phenyl)pivalamide (38)

Following **general procedure D,** using *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)pivalamide **23** (0.100g, 0.240 mmol) and piperidine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)thio)phenyl)pivalamide (0.0637g, 0.137 mmol) as an amorphous powder. (Yield : 57.0%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25 (s, 9H), 1.43 - 1.53 (m, 4H), 1.54 - 1.65 (m, 2H), 2.00 (s, 3H), 3.40 (t, J = 5.48 Hz, 4H), 5.42 (s, 1H), 6.02 (s, 1H), 7.45 - 7.50 (m, 2H), 7.77 - 7.83 (m, 2H), 9.15 (s, 1H), 9.37 (s, 1H), 11.72 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.94, 24.66, 25.41, 28.88, 31.78, 45.04, 80.37, 95.46, 120.41, 124.05, 136.61, 138.45, 140.63, 160.30, 162.15, 163.49, 169.95, 177.07.

t_R 1.98 min, MS (ESI) m/z 466 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)thio)phenyl)pivalamide (39)

Following **general procedure D,** using *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)pivalamide **23** (0.100g, 0.240 mmol) and morpholine

(2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)thio)phenyl)pivalamide (0.0657g, 0.141 mmol) as an amorphous powder. (Yield : 58.6%)

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 1.25 (s, 9H), 1.99 (s, 3H), 3.34 (m, 4H), 3.60 - 3.69 (m, 4H), 5.40 (s, 1H), 5.97 (s, 1H), 7.45 - 7.51 (m, 2H), 7.78 - 7.83 (m, 2H), 9.28 (s, 1H), 9.38 (s, 1H), 11.69 (s, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 10.91, 27.60, 31.78, 44.48, 66.20, 80.74, 95.60, 120.47, 123.83, 136.69, 138.39, 140.73, 160.32, 162.76, 170.13, 177.09.

t_R 1.77 min, MS (ESI) m/z 468 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperazin-1-yl)pyrimidin-2-yl)thio)phenyl)pivalamide (40)

Following **general procedure D,** using *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)pivalamide **23** (0.100g, 0.240 mmol) and piprazine (0.069g, 0.720 mmol) in DMF (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(piperazin-1-yl)pyrimidin-2-yl)thio)phenyl)pivalamide (0.0132g, 0.028 mmol) as an amorphous powder. (Yield : 11.79%)

^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 1.25 (s, 9H), 1.91 (s, 1H), 1.99 (s, 3H), 2.73 (dt, J = 1.90, 6.76 Hz, 4H), 3.29 - 3.34 (m, 4H), 5.40 (s, 1H), 5.96 (s, 1H), 7.41 - 7.52 (m, 2H), 7.75 - 7.85 (m, 2H), 9.20 (s, 1H), 9.38 (s, 1H), 11.67 (s, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 10.02, 26.39, 39.30, 44.39, 44.69, 79.82, 95.76, 120.99, 125.31, 136.22, 139.48, 160.29, 162.59, 170.69, 171.59, 178.37.

t_R 1.46 min, MS (ESI) m/z 467 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)pivalamide (41)

Following **general procedure D,** using *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)pivalamide **23** (0.150g, 0.360 mmol) and *N*-

methylpiperazine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)pivalamide (0.156g, 0.325 mmol) as an amorphous powder. (Yield : 90%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (s, 2H), 1.25 (s, 9H), 1.99 (s, 2H), 2.20 (s, 3H), 2.29 - 2.35 (m, 3H), 3.35 - 3.40 (m, 4H), 5.40 (s, 1H), 5.99 (s, 1H), 7.44 - 7.54 (m, 2H), 7.76 - 7.87 (m, 2H), 9.22 (s, 1H), 9.38 (s, 1H), 11.68 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.89, 27.61, 31.78, 43.98, 46.22, 54.58, 67.41, 80.68, 95.60, 120.44, 123.92, 136.66, 140.70, 160.31, 162.48, 170.06, 177.08.

t_R 1.41 min, MS (ESI) m/z 481 [M +H] (100%) (HPLC System A)

_N_-(4-((4-(4-ethylpiperazin-1-yl)-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)pivalamide (42)

Following **general procedure D,** using *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)pivalamide **43** (0.100g, 0.240 mmol) and *N*-ethylpiperazine (2 ml) to afford the desired *N*-(4-((4-(4-ethylpiperazin-1-yl)-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)pivalamide (0.085g, 0.172 mmol) as an amorphous powder. (Yield : 71.6%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02 (t, J = 7.11 Hz, 3H), 1.25 (s, 9H), 1.99 (s, 3H), 2.30 - 2.41 (m, 6H), 3.36 - 3.40 (m, 4H), 5.40 (s, 1H), 5.99 (s, 1H), 7.45 - 7.53 (m, 2H), 7.76 - 7.85 (m, 2H), 9.22 (s, 1H), 9.38 (s, 1H), 11.67 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.92, 12.42, 27.60, 28.87, 31.78, 44.10, 52.08, 52.38, 80.65, 95.55, 120.44, 123.93, 136.67, 140.69, 160.30, 162.48, 177.08.

t_R 1.47 min, MS (ESI) m/z 495 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (43)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide **24** (0.100g, 0.249 mmol) and

piperidine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (0.030g, 0.067mmol) as an amorphous powder. (Yield : 26.8%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.79 - 0.84 (m, 4H), 1.46 (m, 4H), 1.54 - 1.63 (m, 2H), 1.78 - 1.85 (m, 1H), 2.03 (s, 3H), 3.36 - 3.40 (m, 4H), 5.46 (s, 1H), 6.07 (s, 1H), 7.45 - 7.51 (m, 2H), 7.66 - 7.73 (m, 2H), 9.14 (s, 1H), 10.38 (s, 1H), 11.69 (s, 1H).

t_R 1.89 min, MS (ESI) m/z 450 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-_1H_-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (44)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide **24** (0.100g, 0.249 mmol) and morpholine (2 ml) to affored the desired *N*-(4-((4-((5-methyl-*1H*-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (0.0932g, 0.306 mmol) as an amorphous powder. (Yield : 83.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.78 - 0.86 (m, 4H), 1.77 - 1.86 (m, 1H), 2.02 (s, 3H), 3.31 - 3.39 (m, 4H), 3.62 (m, 4H), 5.45 (s, 1H), 6.02 (s, 1H), 7.46 - 7.52 (m, 2H), 7.67 - 7.73 (m, 2H), 9.28 (s, 1H), 10.39 (s, 1H), 11.69 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.72, 10.84, 15.11, 28.87, 44.46, 67.41, 80.77, 95.53, 119.49, 123.68, 127.11, 130.65, 136.90, 140.56, 160.34, 162.76, 172.34.

t_R 1.65 min, MS (ESI) m/z 452 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (45)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide **24** (0.050g, 0.125 mmol) and

piperazine (0.021g, 0.249 mmol) in DMF (1.5 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(piperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (0.0117g, 0.026 mmol) as an amorphous powder. (Yield: 20.82%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.76 - 0.85 (m, 4H), 1.76 - 1.86 (m, 1H), 1.91 (s, 1H), 2.02 (s, 3H), 2.70 (q, J = 5.99, 6.89 Hz, 4H), 3.29 (m, 4H), 5.45 (s, 1H), 6.02 (s, 1H), 7.42 - 7.55 (m, 2H), 7.63 - 7.76 (m, 2H), 9.19 (s, 1H), 10.39 (s, 1H), 11.70 (s, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 6.69, 7.95, 14.32, 44.17, 44.57, 79.79, 119.58, 122.35, 124.84, 136.46, 139.69, 160.41, 162.58, 170.72, 171.58, 173.55.

t_R 1.37 min, MS (ESI) m/z 451 [M +H] (100%) (HPLC System A)

_N_-(4-((4-(4-ethylpiperazin-1-yl)-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (46)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide **24** (0.100g, 0.249 mmol) and *N*-ethylpiperazine (2 ml) to affored the desired *N*-(4-((4-(4-ethylpiperazin-1-yl)-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (0.03850g, 0.079 mmol) as an amorphous powder. (Yield : 31.8%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.77 - 0.84 (m, 4H), 1.02 (t, J = 7.14 Hz, 3H), 1.82 (p, J = 6.26 Hz, 1H), 2.02 (s, 3H), 2.29 - 2.45 (m, 6H), 3.35 - 3.43 (m, 4H), 5.44 (s, 1H), 6.04 (s, 1H), 7.44 - 7.52 (m, 2H), 7.67 - 7.73 (m, 2H), 9.21 (s, 1H), 10.40 (s, 1H), 11.70 (s, 1H).

t_R 1.42 min, MS (ESI) m/z 479 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (47)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide **25** (0.100g 0.226 mmol) and piperidine (2 ml) to affored the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.109g, 0.222 mmol) as an amorphous powder. (Yield : 98.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δδ 1.16 - 1.83 (m, 16H), 2.00 (s, 3H), 2.36 (tt, J = 3.48, 11.64 Hz, 1H), 3.39 (t, J = 5.55 Hz, 4H), 5.42 (s, 1H), 6.02 (s, 1H), 7.44 - 7.49 (m, 2H), 7.69 - 7.75 (m, 2H), 9.14 (s, 1H), 10.01 (s, 1H), 11.66 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 24.66, 25.40, 25.69, 25.85, 29.56, 31.78, 45.03, 45.43, 67.41, 80.35, 95.48, 119.52, 123.83, 136.86, 140.66, 160.32, 162.15, 170.00, 174.98.

t_R 2.07 min, MS (ESI) m/z 492 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (48)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide **25** (0.100g 0.226 mmol) and morpholine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.0855g, 0.173 mmol) as an amorphous powder. (Yield : 77.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 - 1.35 (m, 6H), 1.37 - 1.51 (m, 2H), 1.66 (d, J = 11.02 Hz, 1H), 1.73 - 1.87 (m, 5H), 1.99 (s, 3H), 2.36 (tt, J = 3.45, 11.67 Hz, 1H), 3.56 - 3.68 (m, 4H), 5.40 (s, 1H), 5.97 (s, 1H), 7.44 - 7.52 (m, 2H), 7.68 - 7.79 (m, 2H), 9.28 (s, 1H), 10.02 (s, 1H), 11.67 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.89, 25.69, 29.56, 31.77, 44.47, 45.44, 66.20, 67.42, 80.74, 95.62, 119.61, 123.62, 130.69, 136.94, 140.75, 160.32, 162.76, 170.16, 175.01.

t_R 1.84 min, MS (ESI) m/z 494 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (49)

Following **general procedure D,** using _N_-(4-((4-chloro-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide **25** (0.100g 0.226 mmol) and piperazine (0.058g 0.677 mmol) in DMF (2 ml) to afford the desired _N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.0385g, 0.078 mmol) as an amorphous powder. (Yield : 34.6%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.19 - 1.36 (m, 4H), 1.37 - 1.53 (m, 2H), 1.67 (d, J = 11.49 Hz, 1H), 1.71 - 1.84 (m, 4H), 2.00 (s, 3H), 2.36 (tt, J = 3.46, 11.64 Hz, 1H), 2.67 - 2.80 (m, 4H), 3.17 - 3.52 (m, 4H), 5.42 (s, 1H), 5.98 (s, 1H), 7.43 - 7.49 (m, 2H), 7.65 - 7.77 (m, 2H), 9.16 (s, 1H), 9.99 (s, 1H), 11.63 (s, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 10.00, 25.39, 25.49, 29.31, 44.29, 44.63, 45.89, 79.82, 99.99, 119.82, 124.95, 136.45, 139.65, 160.35, 162.59, 170.73, 171.61, 176.32.

t_R 1.59 min, MS (ESI) m/z 493 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (50)

Following **general procedure D,** using _N_-(4-((4-chloro-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide **25** (0.270g 0.610 mmol) and _N_-methylpiperazine (2 ml) to afford the desired _N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.195g, 0.384 mmol) as an amorphous powder. (Yield : 63.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (s, 3H), 1.16 - 1.36 (m, 4H), 1.37 - 1.50 (m, 2H), 1.66 (d, J = 11.07 Hz, 1H), 1.73 - 1.85 (m, 4H), 1.99 (s, 3H), 2.19 (s, 3H), 2.29 - 2.41 (m, 5H), 5.40 (s, 1H), 5.99 (s, 1H), 7.45 - 7.50 (m, 2H), 7.70 - 7.76 (m, 2H), 9.23 (s, 1H), 10.02 (s, 1H), 11.67 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 25.70, 27.59, 29.56, 31.77, 43.97, 45.44, 46.21, 54.57, 67.41, 80.68, 95.63, 119.57, 121.31, 123.70, 136.91, 140.72, 160.31, 162.47, 170.07, 174.99.

t_R 1.47 min, MS (ESI) m/z 507 [M +H] (100%) (HPLC System A)

_N_-(4-((4-(4-ethylpiperazin-1-yl)-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (51)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide **25** (0.100g 0.226 mmol) and *N*-ethylpiperazine to afford the desired *N*-(4-((4-(4-ethylpiperazin-1-yl)-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.0976g, 0.18) as an amorphous powder. (Yield : 84.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.02 (t, J = 7.13 Hz, 3H), 1.13 - 1.52 (m, 7H), 1.66 (d, J = 11.16 Hz, 1H), 1.72 - 1.87 (m, 5H), 1.99 (s, 3H), 2.28 - 2.44 (m, 8H), 5.40 (s, 1H), 5.98 (s, 1H), 7.44 - 7.50 (m, 2H), 7.70 - 7.76 (m, 2H), 9.22 (s, 1H), 10.02 (s, 1H), 11.67 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 10.92, 12.39, 25.69, 29.56, 31.77, 44.07, 45.43, 52.07, 52.36, 80.65, 95.53, 119.58, 123.70, 136.92, 140.72, 145.95, 160.29, 162.47, 170.08, 175.00.

t_R 1.47 min, MS (ESI) m/z 521 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (52)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide **27** (0.100g, 0.219 mmol) and piperidine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.0849g, 0.168 mmol) as an amorphous powder. (Yield : 77.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25 (dqd, J = 9.12, 12.16, 24.53 Hz, 4H), 1.35 - 1.51 (m, 5H), 1.54 (d, J = 5.35 Hz, 2H), 1.66 (d, J = 10.88 Hz, 1H), 1.74 - 1.85 (m, 7H), 2.09 (s, 3H), 2.33 (dt, J = 3.53,

11.75 Hz, 1H), 3.23 - 3.32 (m, 4H), 6.08 (s, 1H), 7.40 - 7.48 (m, 2H), 7.60 - 7.68 (m, 2H), 8.47 (s, 1H), 9.94 (s, 1H), 11.90 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.53, 11.33, 24.63, 25.34, 25.70, 25.87, 29.59, 45.00, 45.38, 67.40, 79.57, 95.28, 119.40, 124.17, 135.90, 140.25, 161.82, 162.32, 174.93.

t_R 2.09 min, MS (ESI) m/z 506 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (53)

Following **general procedure D,** using _N_-(4-((4-chloro-6-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide **27** (0.100g, 0.219 mmol) and morpholine (2 ml) to afford the desired _N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.0940g, 0.185 mmol) as an amorphous powder. (Yield : 85.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 - 1.35 (m, 3H), 1.36 - 1.49 (m, 2H), 1.66 (d, J = 11.02 Hz, 1H), 1.73 - 1.85 (m, 7H), 2.09 (s, 3H), 2.35 (tt, J = 3.51, 11.50 Hz, 1H), 3.20 - 3.29 (m, 4H), 3.51 - 3.58 (m, 4H), 6.04 (s, 1H), 7.42 - 7.49 (m, 2H), 7.61 - 7.68 (m, 2H), 8.58 (s, 1H), 9.94 (s, 1H), 11.92 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.55, 9.86, 25.70, 25.87, 29.59, 44.38, 45.38, 66.10, 79.90, 104.43, 119.50, 123.97, 127.83, 135.90, 140.29, 161.97, 162.98, 169.00, 170.80, 174.95.

t_R 1.94 min, MS (ESI) m/z 508 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(piperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (54)

Following **general procedure D,** using _N_-(4-((4-chloro-6-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide **27** (0.240g, 0.525 mmol) and piperazine (0.090 g, 1.05 mmol) in DMF (4 ml) to afford the desired _N_-(4-((4-((4,5-dimethyl-1_H_-

pyrazol-3-yl)amino)-6-(piperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.095g, 0.188 mmol) as an amorphous powder. (Yield : 35.7%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.14 - 1.34 (m, 4H), 1.36 - 1.51 (m, 2H), 1.60 - 1.68 (m, 1H), 1.70 - 1.87 (m, 7H), 2.09 (s, 3H), 2.35 (tt, J = 3.38, 11.76 Hz, 1H), 2.57 - 2.66 (m, 4H), 3.19 (d, J = 5.20 Hz, 4H), 6.00 (s, 1H), 7.38 - 7.48 (m, 2H), 7.59 - 7.69 (m, 2H), 8.52 (s, 1H), 9.95 (s, 1H), 11.88 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.54, 10.11, 25.70, 25.87, 29.59, 45.26, 45.38, 45.63, 79.69, 104.00, 119.45, 124.09, 135.90, 140.27, 144.80, 161.82, 162.77, 168.93, 174.94.

t_R 1.51 min, MS (ESI) m/z 507 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (55)

Following **general procedure D,** using _N_-(4-((4-chloro-6-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide **27** (0.100g, 0.219 mmol) and _N_-methylpiperazine (2 ml) to afford the desired _N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.0890g, 0.171 mmol) as an amorphous powder. (Yield : 78.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 - 1.35 (m, 3H), 1.36 - 1.49 (m, 2H), 1.61 - 1.86 (m, 9H), 2.05 - 2.13 (m, 3H), 2.13 - 2.19 (m, 3H), 2.19 - 2.29 (m, 4H), 2.35 (ddt, J = 4.07, 8.18, 16.36 Hz, 1H), 3.28 (d, J = 5.64 Hz, 4H), 6.05 (s, 1H), 7.46 (dd, J = 3.76, 9.38 Hz, 2H), 7.64 (dd, J = 3.05, 6.47, 7.64 Hz, 2H), 8.54 (s, 1H), 9.95 (s, 1H), 11.91 (s, 1H). **^{13}C NMR (101 MHz, DMSO)** δ 7.54, 9.82, 25.70, 25.87, 29.59, 40.41, 43.87, 45.39, 46.15, 54.48, 79.85, 104.19, 119.36, 119.45, 124.04, 135.91, 140.28, 161.91, 162.66, 169.00, 174.94.

t_R 1.66 min, MS (ESI) m/z 521 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(4-ethylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (56)

Following **general procedure D,** using _N_-(4-((4-chloro-6-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide **27** (0.100g, 0.219 mmol) and _N_-ethylpiperazine (2 ml) to afford the desired _N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(4-ethylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.0932g, 0.174 mmol) as an amorphous powder. (Yield : 80.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.99 (t, J = 7.14 Hz, 3H), 1.15 - 1.34 (m, 4H), 1.36 - 1.49 (m, 2H), 1.62 - 1.69 (m, 1H), 1.70 - 1.88 (m, 7H), 2.09 (s, 3H), 2.24 - 2.42 (m, 7H), 3.27 (t, J = 4.98 Hz, 4H), 6.05 (s, 1H), 7.41 - 7.49 (m, 2H), 7.60 - 7.70 (m, 2H), 8.54 (s, 1H), 9.94 (s, 1H), 11.91 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.54, 12.36, 25.70, 25.87, 29.60, 43.99, 45.39, 52.03, 52.29, 79.82, 99.99, 119.46, 124.06, 128.67, 135.91, 140.29, 161.88, 162.66, 168.95, 174.94.

t_R 1.49 min, MS (ESI) m/z 535 [M +H] (100%) (HPLC System A)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (57)

Following **general procedure D,** using _N_-(4-((4-chloro-6-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide **26** (0.250g, 0.603 mmol) and _N_-ethylpiperazine (2 ml) to afford the desired _N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)thio)phenyl)cyclopropanecarboxamide (0.150g, 0.313 mmol) as an amorphous powder. (Yield : 52.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.78 - 0.87 (m, 4H), 1.77 (s, 3H), 1.79 - 1.85 (m, 1H), 2.09 (s, 3H), 2.15 (s, 3H), 2.20 - 2.28 (m, 4H), 3.18 - 3.31 (m, 4H), 6.07 (s, 1H), 7.42 - 7.53 (m, 2H), 7.59 - 7.71 (m, 2H), 8.58 (s, 1H), 10.37 (s, 1H), 11.94 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.52, 7.75, 10.00, 15.09, 31.78, 43.89, 46.14, 54.49, 79.87, 104.14, 119.38, 124.13, 135.98, 140.13, 146.82, 161.93, 162.67, 169.00, 172.26.

t_R 1.47 min, MS (ESI) m/z 479 [M +H] (100%)

***N*-(4-nitrophenyl)cyclopropanecarboxamide (59)**

Following **general procedure E,** using 4-nitroaniline **58** (5.61 g, 43.4 mmol), cyclopropanecarbonyl chloride (7.57 g, 72.4 mmol) and DIPEA (7.57 ml, 72.4 mmol) to afford the desired *N*-(4-nitrophenyl)cyclopropanecarboxamide (5.1 g, 24.73 mmol). (Yield : 68.3 %)

^{1}H NMR (400 MHz, Acetone-d_6) δ 0.83 - 0.89 (m, 2H), 0.94 - 0.99 (m, 2H), 1.83 (tt, J = 4.54, 7.80 Hz, 1H), 7.86 - 7.95 (m, 2H), 8.16 - 8.24 (m, 2H), 10.01 (s, 1H). **^{13}C NMR (101 MHz, Acetone-d_6)** δ 7.59, 14.92, 118.58, 124.74, 142.64, 145.47, 172.59.

MS (ESI) m/z 207 [M +H] (HPLC System B)

***N*-(4-nitrophenyl)cyclohexanecarboxamide (60)**

Following **general procedure E,** using 4-nitroaniline **58** (5.61 g, 43.4 mmol), cyclohexanecarbonyl chloride (7.57 g, 72.4 mmol) and DIPEA (7.57 ml, 72.4 mmol) to afford the desired *N*-(4-nitrophenyl)cyclohexanecarboxamide (6.43 g, 25.9 mmol). (Yield : 71.5 %)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 - 1.34 (m, 3H), 1.34 - 1.48 (m, 2H), 1.60 - 1.70 (m, 1H), 1.72 - 1.79 (m, 2H), 1.79 - 1.87 (m, 2H), 2.38 (tt, J = 3.48, 11.58 Hz, 1H), 7.80 - 7.88 (m, 2H), 8.16 - 8.23 (m, 2H), 10.45 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 25.60, 25.79, 29.41, 45.44, 119.08, 125.40, 142.34, 146.16, 175.70.

MS (ESI) m/z 249 [M +H] (HPLC System B)

***N*-(4-aminophenyl)cyclopropanecarboxamide (61)**

Following **general procedure F,** using *N*-(4-nitrophenyl)cyclopropanecarboxamide **59** (5.00 g, 24.3 mmol) and palladium hydroxide (5.11 g, 36.4 mmol). (Yield : 86.0 %)

^{1}H NMR (400 MHz, Acetone-d_6) δ 0.63 - 0.76 (m, 2H), 0.80 - 0.93 (m, 2H), 1.69 (tt, J = 4.57, 7.85 Hz, 1H), 4.44 (s, 2H), 6.53 - 6.66 (m, 2H), 7.27 - 7.40 (m, 2H), 9.09 (s, 1H) **^{13}C NMR (101 MHz, Acetone-d_6)** δ 6.30, 14.42, 114.26, 120.85, 129.82, 144.41, 170.79.

MS (ESI) m/z 177 [M +H] (HPLC System B)

_N_-(4-aminophenyl)cyclohexanecarboxamide (62)

Following **general procedure F,** using *N*-(4-nitrophenyl)cyclohexanecarboxamide **60** (6.43 g, 25.9 mmol) and palladium hydroxide (5.46 g, 38.8 mmol). (Yield : 86.0 %)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.31 (m, 3H), 1.32 - 1.45 (m, 2H), 1.59 - 1.69 (m, 1H), 1.70 - 1.79 (m, 4H), 2.23 (tq, J = 3.29, 10.06 Hz, 1H), 4.82 (s, 2H), 6.41 - 6.53 (m, 2H), 7.16 - 7.28 (m, 2H), 9.36 (s, 1H). **^{13}C NMR (101 MHz, Acetone-d_6)** δ 25.58, 25.72, 29.56, 45.65, 114.19, 120.82, 129.86, 144.33, 173.32.

MS (ESI) m/z 219 [M +H] (HPLC System B)

2,6-dichloro-_N_-(5-methyl-1_H_-pyrazol-3-yl)pyrimidin-4-amine (64)

Following **general procedure G,** using 2,4,6-trichlorotriazine **63** (0.878 ml, 7.63 mmol), 5-methyl-1*H*-pyrazol-3-amine (0.771 g, 7.94 mmol) and triethylamine (3.30 ml, 23.66 mmol) to afford the desired 2,6-dichloro-N-(5-methyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (0.900 g, 3.69 mmol). (Yield : 62.6 %)

^{1}H NMR (400 MHz, Acetone-d_6) δ 2.33 (d, J = 0.76 Hz, 3H), 5.98 (s, 1H), 7.93 (s, 1H), 9.54 (s, 1H), 11.51 (s, 1H). **^{13}C NMR (101 MHz, Acetone-d_6)** δ 10.01, 95.41, 99.99, 103.07, 139.79, 147.70, 159.04, 161.69.

MS (ESI) m/z 244, 248 [M +H] (HPLC System B)

2,6-dichloro-*N*-(4,5-dimethyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine (65)

Following **general procedure G,** using 2,4,6-trichlorotriazine **63** (0.752 ml, 6.54 mmol), 4,5-dimethyl-1*H*-pyrazol-3-amine (0.727 g, 6.54 mmol) and triethylamine (2.83 ml, 20.28 mmol) to afford the desired 2,6-dichloro-*N*-(5-methyl-1H-pyrazol-3-yl)pyrimidin-4-amine (0.883 g, 3.42 mmol). (Yield : 52.3 %)

^{1}H NMR (400 MHz, Acetone-d_6) δ 2.00 (s, 3H), 2.25 (s, 3H), 7.49 (s, 1H), 8.94 (s, 1H), 11.51 (s, 1H). **^{13}C NMR (101 MHz, Acetone-d_6)** δ 6.02, 8.73, 102.19, 102.33, 137.20, 159.03, 160.30, 163.10, 174.92.

MS (ESI) m/z 258, 262 [M +H] (HPLC System B)

***N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (66)**

Following **general procedure H,** using 2,6-dichloro-*N*-(5-methyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine **64** (1g, 4.10 mmol), *N*-(4-aminophenyl)cyclopropanecarboxamide **61** (0.722 g, 4.10 mmol) and *p*-toluene sulfonic acid monohydrate (0.779 g, 4.10 mmol) to afford the desired *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (0,9 g, 2,345 mmol). (Yield : 57,2 %)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.71 - 0.84 (m, 4H), 1.76 (tt, J = 4.75, 7.62 Hz, 1H), 2.22 (s, 3H), 5.58 - 7.23 (m, 2H), 7.32 - 7.86 (m, 4H), 9.44 (s, 1H), 9.80 (s, 1H), 10.09 (s, 1H), 12.07 (d, J = 25.79 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.42, 14.55, 14.93, 95.32, 96.39, 119.69, 120.53, 134.32, 135.75, 148.38, 159.61, 161.22, 170.81, 171.58.

MS (ESI) m/z 384, 386 [M +H] (HPLC System B)

***N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (67)**

Following **general procedure H,** using 2,6-dichloro-*N*-(4,5-dimethyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine **65** (0.850g, 3.90 mmol), *N*-(4-aminophenyl)cyclopropanecarboxamide **61** (0.580 g, 3.29 mmol) and *p*-toluene sulfonic acid monohydrate (0.626 g, 3.29 mmol) to afford the desired *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide. The compound was immediately introduced in the next step without further purification.

MS (ESI) m/z 398, 400 [M +H] (HPLC System B)

_N_-(4-((4-chloro-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (68)

Following **general procedure H,** using 2,6-dichloro-*N*-(5-methyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine **64** (0.240g, 1.1 mmol), *N*-(4-aminophenyl)cyclohexanecarboxamide **62** (0.240 g, 1.1 mmol) and *p*-toluene sulfonic acid monohydrate (0.152 g, 0.8 mmol) to afford the desired *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.174 g, 0.409 mmol). (Yield : 51.1 %)

^{1}H NMR (400 MHz, Acetone-d_6) δ 1.18 - 1.38 (m, 3H), 1.44 - 1.61 (m, 2H), 1.62 - 1.72 (m, 1H), 1.75 - 1.82 (m, 2H), 1.85 - 1.91 (m, 2H), 2.22 - 2.39 (m, 4H), 6.17 (s, 1H), 7.54 - 7.78 (m, 4H), 8.52 (s, 1H), 8.96 (s, 1H), 9.00 (s, 1H), 11.34 (s, 1H).

MS (ESI) m/z 426, 428 [M +H] (HPLC System B)

_N_-(4-((4-chloro-6-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (69)

Following **general procedure H,** using 2,6-dichloro-*N*-(4,5-dimethyl-1*H*-pyrazol-3-yl)pyrimidin-4-amine **65** (0.883 g, 3.42 mmol), *N*-(4-aminophenyl)cyclohexanecarboxamide **63** (0.747 g, 3.42 mmol) and *p*-toluene sulfonic acid monohydrate (0.651 g, 3.42 mmol) to afford the desired *N*-(4-((4-chloro-6-

((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide. (0.627 g, 1,425 mmol). (Yield : 41.7%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.32 (m, 3H), 1.34 - 1.46 (m, 2H), 1.59 - 1.70 (m, 1H), 1.71 - 1.89 (m, 7H), 2.18 (s, 3H), 2.25 - 2.34 (m, 1H), 6.26 (s, 1H), 7.46 (s, 2H), 7.61 (s, 2H), 9.12 (s, 1H), 9.42 (s, 1H), 9.66 (s, 1H), 12.13 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.70, 9.95, 25.77, 25.91, 29.68, 45.30, 94.22, 105.64, 119.47, 119.68, 134.01, 136.05, 136.84, 146.00, 159.48, 163.45, 174.25.

MS (ESI) m/z 440, 442 [M +H] (HPLC System B)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (70)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide **66** (0.200 g, 0.521 mmol) and piperidine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.080 g, 0.185 mmol) as an amorphous powder. (Yield : 33.5%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.68 - 0.88 (m, 4H), 1.54 (q, J = 5.71 Hz, 4H), 1.62 (dt, J = 4.83, 11.50 Hz, 2H), 1.78 (tt, J = 4.81, 7.72 Hz, 1H), 2.18 (s, 3H), 3.50 (t, J = 5.26 Hz, 4H), 5.94 (s, 2H), 7.47 (d, J = 8.83 Hz, 2H), 7.63 (d, J = 8.53 Hz, 2H), 8.77 (s, 1H), 8.97 (d, J = 51.66 Hz, 1H), 10.06 (s, 1H), 11.95 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.34, 11.96, 14.90, 24.80, 25.53, 45.35, 76.84, 94.90, 119.28, 119.81, 133.10, 137.23, 159.09, 160.76, 163.37, 171.44.

t_R 1.47 min, MS (ESI) m/z 433 [M +H] (100%) (HPLC System B)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (71)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide **66** (0.200 g, 0.521 mmol) and morpholine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.108 g, 0.248 mmol) as an amorphous powder. (Yield : 47.6%)

^{1}H NMR (400 MHz, DMSO-d*6*) δ 0.70 - 0.82 (m, 4H), 1.79 (tt, J = 4.82, 7.61 Hz, 1H), 2.18 (s, 3H), 3.38 - 3.50 (m, 4H), 3.59 - 3.75 (m, 4H), 5.34 - 6.23 (m, 2H), 7.43 - 7.57 (m, 2H), 7.57 - 7.73 (m, 2H), 8.75 (s, 1H), 9.12 (s, 1H), 10.11 (s, 1H), 11.95 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d*6*)** δ 7.34, 11.73, 14.88, 44.83, 66.35, 77.19, 119.33, 119.82, 133.16, 137.16, 159.26, 161.04, 162.20, 164.11, 171.46.

t_R 1.34 min, MS (ESI) m/z 435 [M +H] (95%) (HPLC System B)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (72)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide **66** (0.200 g, 0.521 mmol) and *N*-methylpiperazine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.106 g, 0.236 mmol) as an amorphous powder. (Yield : 45.2%)

^{1}H NMR (400 MHz, DMSO-d*6*) δ 0.69 - 0.84 (m, 4H), 1.76 (tt, J = 4.99, 7.81 Hz, 1H), 2.18 (s, 3H), 2.21 (s, 3H), 2.31 - 2.42 (m, 4H), 3.42 - 3.52 (m, 4H), 5.22 - 6.53 (m, 2H), 7.34 - 7.54 (m, 2H), 7.57 - 7.74 (m, 2H), 8.61 (s, 1H), 8.96 (s, 1H), 10.02 (s, 1H), 11.86 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d*6*)** δ 7.34, 11.16, 14.90, 44.28, 46.29, 54.76, 77.21, 95.97, 119.30, 119.83, 133.05, 137.27, 159.30, 161.00, 162.08, 163.82, 164.64, 171.42.

t_R 1.09 min, MS (ESI) m/z 448 [M +H] (100%) (HPLC System B)

***N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (73)**

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide **67** (0.200 g, 0.503 mmol) and piperidine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.122 g, 0.273 mmol) as an amorphous powder. (Yield : 53.4%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.68 - 0.86 (m, 4H), 1.47 - 1.55 (m, 4H), 1.57 - 1.66 (m, 2H), 1.71 - 1.80 (m, 1H), 1.85 (s, 3H), 2.12 (s, 3H), 3.40 - 3.56 (m, 4H), 5.69 (s, 1H), 7.43 (d, J = 8.83 Hz, 2H), 7.62 (d, J = 8.91 Hz, 2H), 8.18 (s, 1H), 8.70 (s, 1H), 9.98 (s, 1H), 11.97 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.32, 7.73, 10.79, 14.90, 24.82, 25.52, 45.35, 76.23, 118.89, 119.82, 132.85, 137.48, 159.32, 162.18, 163.48, 171.38.

t_R 1.68 min, MS (ESI) 447 m/z [M +H] (100%)

***N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (74)**

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide **67** (0.200 g, 0.503 mmol) and morpholine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.130 g, 0.291 mmol) as an amorphous powder. (Yield : 57.8%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.72 - 0.82 (m, 4H), 1.71 - 1.78 (m, 1H), 1.85 (s, 3H), 2.13 (s, 3H), 3.39 - 3.47 (m, 4H), 3.65 - 3.72 (m, 4H), 5.67 (s, 1H), 7.45 (d, J = 7.94 Hz, 2H), 7.60 (d, J = 8.54 Hz, 2H), 8.44 (s, 1H), 8.89 (s, 1H), 10.00 (s, 1H), 12.03 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.35,

7.71, 10.89, 14.90, 44.84, 66.33, 76.42, 119.24, 119.86, 133.16, 137.03, 158.76, 164.03, 164.24, 171.43.

t_R 1.47 min, MS (ESI) 449 m/z [M +H] (96%) (HPLC System B)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (75)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide **67** (0.200 g, 0.503 mmol) and *N*-methylpiperazine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.133 g, 0.289 mmol) as an amorphous powder. (Yield : 57.4%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.68 - 0.83 (m, 4H), 1.72 - 1.81 (m, 1H), 1.85 (s, 3H), 2.12 (s, 3H), 2.28 (s, 3H), 2.45 - 2.50 (m, 4H), 3.47 - 3.52 (m, 4H), 5.83 (d, J = 119.93 Hz, 1H), 7.44 (d, J = 8.93 Hz, 2H), 7.61 (d, J = 8.58 Hz, 2H), 8.40 (s, 1H), 8.80 (s, 1H), 10.03 (s, 1H), 11.98 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.33, 7.57, 7.76, 10.92, 43.94, 45.80, 54.43, 76.55, 104.12, 118.99, 119.84, 132.96, 137.32, 159.26, 162.22, 163.76, 164.65, 171.41.

t_R 1.25 min, MS (ESI) m/z 462 [M +H] (100%) (HPLC System B)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (76)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide **68** (0.100 g, 0.178 mmol) and piperidine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.085 g, 0.178 mmol) as an amorphous powder. (Yield : 76.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.34 (m, 3H), 1.34 - 1.48 (m, 2H), 1.48 - 1.58 (m, 4H), 1.58 - 1.70 (m, 3H), 1.72 - 1.84 (m, 4H), 2.09 - 2.23 (m, 3H), 2.30 (tt, J = 3.44, 11.66 Hz, 1H), 3.44 - 3.55 (m, 4H), 5.43 (d, J = 36.05 Hz, 1H), 6.12 (s, 1H), 7.36 - 7.79 (m, 4H), 8.49 (s, 1H), 8.79 (s, 1H), 9.61 (s, 1H), 11.79 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 11.20, 24.83, 25.53, 25.77, 25.92, 29.70, 31.78, 45.28, 67.41, 95.78, 119.26, 119.80, 132.92, 135.92, 137.47, 138.39, 150.11, 159.49, 161.22, 163.51, 174.17.

t_R 1.89 min, MS (ESI) m/z 475 [M +H] (100%) (HPLC System B)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (77)

Following **general procedure D,** using _N_-(4-((4-chloro-6-((5-methyl-1_H_-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide **68** (0.100 g, 0.253 mmol) and morpholine (2 ml) to afford the desired _N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.084 g, 0.177 mmol) as an amorphous powder. (Yield : 75.0%)

^{1}H NMR (400 MHz, Acetone-d_6) δ 1.23 - 1.37 (m, 3H), 1.45 - 1.60 (m, 2H), 1.63 - 1.70 (m, 1H), 1.73 - 1.82 (m, 2H), 1.83 - 1.91 (m, 2H), 2.25 (s, 3H), 2.32 (tt, J = 3.56, 11.72 Hz, 1H), 3.50 (dd, J = 3.75, 5.71 Hz, 4H), 3.69 (dd, J = 3.58, 6.25 Hz, 4H), 6.00 (s, 2H), 7.53 - 7.62 (m, 2H), 7.62 - 7.70 (m, 2H), 8.58 (s, 1H), 8.94 (s, 1H), 9.08 (s, 1H), 11.55 (s, 1H). **^{13}C NMR (101 MHz, Acetone-d_6)** δ 10.90, 25.55, 25.70, 29.77, 44.66, 45.74, 66.23, 76.62, 94.34, 119.44, 119.65, 133.34, 136.99, 142.74, 149.56, 159.39, 161.49, 164.38, 173.89.

t_R 1.74 min, MS (ESI) m/z 477 [M +H] (100%) (HPLC System B)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (78)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide **68** (0.100 g, 0.235 mmol) and *N*-methylpiperazine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.065 g, 0.132 mmol) as an amorphous powder. (Yield : 56.2%)

^{1}H NMR (400 MHz, Acetone-d_6) δ 1.23 - 1.36 (m, 3H), 1.46 - 1.58 (m, 2H), 1.63 - 1.69 (m, 1H), 1.74 - 1.81 (m, 2H), 1.82 - 1.91 (m, 2H), 2.23 (s, 3H), 2.25 (s, 3H), 2.31 (dt, J = 3.40, 11.54 Hz, 1H), 2.39 (t, J = 5.04 Hz, 4H), 3.34 (s, 1H), 3.55 (t, J = 5.09 Hz, 4H), 5.99 (t, J = 2.25 Hz, 2H), 7.54 - 7.62 (m, 2H), 7.62 - 7.71 (m, 2H), 8.53 (s, 1H), 8.96 (s, 1H), 9.03 (s, 1H). **^{13}C NMR (101 MHz, Acetone-d_6)** δ 10.96, 25.55, 25.71, 29.60, 44.11, 45.53, 45.73, 54.59, 76.67, 94.00, 119.35, 119.67, 133.27, 137.09, 141.08, 148.14, 159.40, 161.41, 164.03, 173.91.

t_R 1.34 min, MS (ESI) m/z 490 [M +H] (100%) (HPLC System B)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (79)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide **69** (0.200 g, 0.455 mmol) and piperidine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.157 g, 0.321 mmol) as an amorphous powder. (Yield : 70.7%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 - 1.33 (m, 3H), 1.35 - 1.48 (m, 2H), 1.53 (d, J = 5.61 Hz, 4H), 1.57 - 1.69 (m, 3H), 1.71 - 1.81 (m, 4H), 1.86 (s, 3H), 2.12 (s, 3H), 2.30 (tt, J = 3.31, 11.52 Hz, 1H), 3.44 - 3.56 (m, 4H), 5.69 (s, 1H), 7.45 (d, J = 8.61 Hz, 2H), 7.59 (d, J = 8.52 Hz, 2H), 8.35 (s, 1H), 8.80 (s, 1H), 9.61 (s, 1H), 12.08 (s, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 5.77, 9.13, 24.51, 25.23, 25.45, 25.54, 29.38, 45.23, 45.72, 75.59, 98.36, 118.94, 120.55, 132.05, 137.49, 159.26, 163.60, 175.95, 176.05.

t_R 1.80 min, MS (ESI) m/z 489 [M +H] (100%) (HPLC System B)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (80)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide **69** (0.200 g, 0.455 mmol) and morpholine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-morpholinopyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.175 g, 0.357 mmol) as an amorphous powder. (Yield : 78.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 - 1.34 (m, 3H), 1.33 - 1.48 (m, 2H), 1.66 (d, J = 10.96 Hz, 1H), 1.77 (td, J = 3.46, 10.31, 11.65 Hz, 4H), 1.85 (s, 3H), 2.12 (s, 3H), 2.29 (tt, J = 3.42, 11.47 Hz, 1H), 3.38 - 3.45 (m, 4H), 3.58 - 3.73 (m, 4H), 5.66 (s, 1H), 7.45 (d, J = 8.54 Hz, 2H), 7.58 (d, J = 8.57 Hz, 2H), 8.39 (s, 1H), 8.77 (s, 1H), 9.61 (s, 1H), 12.07 (s, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 5.79, 9.26, 25.45, 25.54, 29.37, 44.57, 45.72, 66.27, 75.91, 97.29, 119.08, 120.55, 132.18, 137.32, 159.22, 164.27, 175.96, 176.06.

t_R 1.63 min, MS (ESI) m/z 491 [M +H] (100%) (HPLC System B)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (81)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide **69** (0.200 g, 0.455 mmol) and *N*-methylpiperazine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)pyrimidin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.180 g, 0.357 mmol) as an amorphous powder. (Yield : 79.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 - 1.34 (m, 3H), 1.36 - 1.50 (m, 2H), 1.65 (d, J = 11.12 Hz, 1H), 1.70 - 1.82 (m, 4H), 1.85 (s, 3H), 2.12 (s, 3H), 2.21 (s, 3H), 2.28 (dt, J = 3.46, 11.72 Hz, 1H), 2.37 (t, J = 4.83 Hz, 4H), 3.23 - 3.45 (m, 4H), 5.67 (s, 1H), 7.45 (d, J = 8.56 Hz, 2H), 7.60 (d, J = 8.56 Hz, 2H), 8.37 (s, 1H), 8.73 (s, 1H), 9.61 (s, 1H), 11.87 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.75, 10.86, 25.76, 25.92, 29.70, 44.28, 45.27, 46.27, 54.75, 76.49, 101.74, 118.91, 119.91, 132.98, 135.20, 137.31, 159.25, 162.18, 163.83, 174.18.

t_R 1.26 min, MS (ESI) m/z 504 [M +H] (100%) (HPLC System B)

N-(4-((4,6-dichloro-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (83)

Following **general procedure I**, a solution of 2,4,6-trichloro-1,3,5-triazine **82** (2,099 g, 11,38 mmol) in THF (50 mL) was cooled down to 0°C. A second solution of N-(4-mercaptophenyl)cyclopropanecarboxamide **13** (2 g, 10,35 mmol) and N-ethyl-N-isopropylpropan-2-amine (1,710 ml, 10,35 mmol) in THF (80 mL) was added dropwise to the cooled solution. After the addition was complete, the mixture was allowed to heat to room temperature. The reaction mixture was diluted with EtOAc (300 mL) and washed with water (300 mL), slightly acidic water (300 mL) and brine (300 mL). The organic phase was dried with anhydrous sodium sulfate and then filtered. The organic solvent was removed under reduced pressure.

MS (ESI) m/z 340, 344 [M +H] (HPLC System B)

N-(4-((4,6-dichloro-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (84)

Following **general procedure I**, a solution of 2,4,6-trichloro-1,3,5-triazine **82** (1,810 g, 9,82 mmol) in THF (43 mL) was cooled down to 0°C. Another solution of N-(4-mercaptophenyl)cyclohexanecarboxamide **14** (2.1 g, 8,92 mmol) and N-ethyl-N-isopropylpropan-2-amine (1,475 ml, 8,92 mmol) in THF (69 mL) was added dropwise to this cooled solution. After the addition was complete, the mixture was allowed to heat to room temperature. The reaction mixture was diluted with EtOAc (200 mL) and washed with water (pH 4-5, 150 mL), saturated NaHCO3 (150

mL) and brine (150 mL). The organic phase was dried with anhydrous sodium sulfate and then filtered. The organic solvent was removed under reduced pressure.

MS (ESI) m/z 384, 386 [M +H] (HPLC System B)

N-(4-((4-chloro-6-((5-methyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (85)

Following **general procedure J**, N-ethyl-N-isopropylpropan-2-amine (1.453 ml, 8.79 mmol) was added to a solution of N-(4-((4,6-dichloro-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide **83** (2 g, 5.86 mmol) and 5-methyl-1H-pyrazol-3-amine (0.569 g, 5.86 mmol) in Dioxane (Volume: 69.8 ml). This solution was heated to 120 °C overnight. After the mixture was cooled to room temperature, it was diluted with EtOAc (500 mL) and washed with water (pH 4, 200 mL), saturated NaHCO3 (200 mL) and brine (200 mL). The organic phase was dried with anhydrous sodium sulfate and then filtered. The organic solvent was removed under reduced pressure.

MS (ESI) m/z 402 [M +H] (HPLC System B)

N-(4-((4-chloro-6-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (86)

Following **general procedure J**, N-ethyl-N-isopropylpropan-2-amine (0,291 ml, 1,758 mmol) was added to a solution of N-(4-((4,6-dichloro-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide **83** (0,600 g, 1,758 mmol) and 4,5-dimethyl-1H-pyrazol-3-amine (0,195 g, 1,758 mmol) in Dioxane (Volume: 20,93 ml). The solution was left to stir overnight at room temperature. The mixture was diluted with EtOAc (120 mL) and washed with water (pH 4-5, 3 x 100 mL), saturated NaHCO3 (100 mL) and brine (100 mL). The organic phase was dried over anhydrous sodium sulfate and then filtered. The solvent was removed under reduced pressure.

MS (ESI) m/z 416 [M +H] (HPLC System B)

N-(4-((4-chloro-6-((5-methyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (87)

Following **general procedure J**, N-ethyl-N-isopropylpropan-2-amine (1.294 ml, 7.83 mmol) was added to a solution of N-(4-((4,6-dichloro-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide **84** (2 g, 5.22 mmol) and 5-methyl-1H-pyrazol-3-amine (0.507 g, 5.22 mmol) in Dioxane (Volume: 62.1 ml). The solution was refluxed at 120°C overnight. After the mixture was allowed to cool to room temperature, it was diluted with EtOAc (500 mL) and washed with water (pH 4-5, 200 mL), saturated NaHCO3 (200 mL) and brine (200 mL). The organic phase was dried with anhydrous sodium sulfate and then filtered. The organic solvent was removed under reduced pressure.

MS (ESI) m/z 444, 446 [M +H] (HPLC System B)

N-(4-((4-chloro-6-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (88)

Following **general procedure J**, N-ethyl-N-isopropylpropan-2-amine (0,454 ml, 2,61 mmol) was added to a solution N-(4-((4,6-dichloro-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide **83** (1,000 g, 2,61 mmol) and 4,5-dimethyl-1H-pyrazol-3-amine (0,290 g, 2,61 mmol) in Dioxane (Volume: 31,1 ml). The solution was left to stir overnight at room temperature. The crude mixture was diluted with EtOAc (120 mL) and washed with water (pH 4-5, 3 x 100 mL), saturated NaHCO3 (1 x 100 mL) and brine (1 x 100 mL); The organic phase was dried over anhydrous sodium sulfate and then filtered. The organic solvent was removed under reduced pressure.

MS (ESI) m/z 458, 460 [M +H] (HPLC System B)

N-(4-((4-((5-methyl-1H-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (89)

Following **general procedure K**, using N-(4-((4-chloro-6-((5-methyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide **85** (0,3 g, 0,747 mmol) and piperidine (Volume: 2,5 ml) to afford the desired N-(4-((4-((5-methyl-1H-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide. (0.221 g, 0.491 mmol) as an amorphous powder. (Yield = 65.7%)

1H NMR (400 MHz, DMSO-d_6) δ 0.79 - 0.86 (m, 4H), 1.47 (s, 4H), 1.55 - 1.64 (m, 2H), 1.78 - 1.87 (m, 1H), 1.92 - 2.23 (m, 3H), 3.67 (s, 4H), 5.25 - 6.34 (m, 1H), 7.50 (d, 2H), 7.74 (s, 2H), 9.46 (s, 1H), 10.43 (s, 1H), 11.71 (s, 1H). **13C NMR (101 MHz, DMSO-d_6) δ** 7.76, 10.71, 15.13, 24.65, 25.79, 44.04, 96.55, 119.48, 122.03, 137.28, 141.07, 147.95, 162.49, 162.90, 172.40, 180.79.

t_R 1.85 min, MS (ESI) m/z 451 [M +H] (100%) (HPLC System B)

N-(4-((4-((5-methyl-1H-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (90)

Following **general procedure K,** using N-(4-((4-chloro-6-((5-methyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide **85** (0,300 g, 0,747 mmol) and Morpholine (Volume: 2,5 ml) to afford the desired N-(4-((4-((5-methyl-1H-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide. (0.184 g, 0.407 mmol) as an amorphous powder. (Yield = 54.5%)

1H NMR (400 MHz, DMSO-d_6) δ 0.80 - 0.86 (m, 4H), 1.83 (p, J = 5.88, 6.45, 12.70 Hz, 1H), 1.93 - 2.25 (m, 3H), 3.64 (d, J = 22.88 Hz, 8H), 5.24 - 6.34 (m, 1H), 7.50 (d, J = 8.43 Hz, 2H), 7.74 (s, 2H), 9.62 (s, 1H), 10.43 (s, 1H), 11.78 (s, 1H). **13C NMR (101 MHz, DMSO-d_6)** δ 7.77, 10.78, 15.13, 43.69, 66.31, 96.48, 119.54, 121.82, 137.31, 141.13, 147.45, 162.38, 163.31, 172.43, 181.04.

t_R 1.60 min, MS (ESI) m/z 453 [M +H] (100%) (HPLC System B)

N-(4-((4-((5-methyl-1H-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (91)

Following **general procedure K,** using N-(4-((4-chloro-6-((5-methyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide **85** (0.3 g, 0.747 mmol) and 1-methyl-piperazine (Volume : 2.5 mL) to afford the desired N-(4-((4-((5-methyl-1H-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (0.222 g, 0.477 mmol) as an amorphous powder. (Yield = 63.9%)

1H NMR (400 MHz, DMSO-d_6) δ 0.79 - 0.86 (m, 4H), 1.85 (p, J = 5.92, 6.76, 12.76 Hz, 1H), 1.91 - 2.14 (m, 3H), 2.19 (s, 3H), 2.30 (s, 4H), 3.68 (s, 4H), 5.24 - 6.34 (m, 1H), 7.50 (d, J = 8.39 Hz, 2H), 7.76 (d, J = 8.57 Hz, 2H), 9.59 (d, J = 9.75 Hz, 1H), 10.51 (s, 1H), 11.80 (s, 1H). **13C NMR (101 MHz, DMSO-d_6)** δ 7.76, 10.82, 15.12, 43.06, 46.16, 54.69, 96.42, 119.53, 121.83, 137.30, 141.14, 147.47, 162.41, 163.15, 172.45, 180.98.

t_R 1.29 min, MS (ESI) m/z 466 [M +H] (100%) (HPLC System B)

N-(4-((4-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (92)

Following **general procedure K**, using N-(4-((4-chloro-6-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide **86** (0.300 g, 0,721 mmol) and piperidine (Volume: 2,5 ml) to afford the desired N-(4-((4-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (0.122 g, 0.262 mmol) as an amorphous powder. (Yield = 36.3%)

1H NMR (400 MHz, DMSO-d_6) δ 0.79 - 0.85 (m, 4H), 1.40 (s, 4H), 1.55 (d, J = 6.02 Hz, 2H), 1.68 (s, 3H), 1.81 (tt, J = 5.14, 7.29 Hz, 1H), 2.09 (s, 3H), 3.50 (s, 4H), 7.44 (d, J = 8.16 Hz, 2H), 7.63 (d, J = 8.24 Hz, 2H), 9.01 (s, 1H), 10.36 (s, 1H). **13C NMR (101 MHz, DMSO-d_6)** δ 7.81, 8.10, 10.28, 15.11, 24.60, 25.75, 43.89, 106.87, 119.30, 122.25, 136.17, 140.49, 162.94, 164.13, 172.33, 179.48.

t_R 1.89 min, MS (ESI) m/z 465 [M +H] (100%) (HPLC System B)

N-(4-((4-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (93)

Following **general procedure K**, using N-(4-((4-chloro-6-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide **86** (0,234 g, 0,563 mmol) and Morpholine (Volume: 2,5 mL) to afford the desired N-(4-((4-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (0.130 g, 0.279 mmol) as an amorphous powder. (Yield = 49.5%)

1H NMR (400 MHz, DMSO-d_6) δ 0.79 - 0.86 (m, 4H), 1.68 (s, 3H), 1.81 (tt, J = 5.16, 7.21 Hz, 1H), 2.09 (s, 3H), 3.41 - 3.62 (m, 8H), 7.45 (s, 2H), 7.63 (d, J = 8.56 Hz, 2H), 9.05 (s, 1H), 10.36 (s, 1H), 11.93 (s, 1H). **13C NMR (101 MHz, DMSO-d_6)** δ 7.81, 8.16, 10.09, 15.12, 43.52, 66.24, 107.35, 119.36, 122.06, 136.17, 140.53, 163.40, 164.15, 172.33, 179.65.

t_R 1.63 min, MS (ESI) m/z 467 [M +H] (100%) (HPLC System B)

N-(4-((4-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (94)

Following **general procedure K**, using N-(4-((4-chloro-6-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide **86** (0.300 g, 0,721 mmol) and 1-methylpiperazine (Volume: 2,5 ml) to afford the desired N-(4-((4-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclopropanecarboxamide (0.167 g, 0.348 mmol) as an amorphous powder. (Yield = 48.3%)

1H NMR (400 MHz, DMSO-d_6) δ 0.79 - 0.85 (m, 4H), 1.68 (s, 3H), 1.81 (tt, J = 5.10, 7.17 Hz, 1H), 2.09 (s, 3H), 2.19 (s, 3H), 2.28 (s, 4H), 3.50 (s, 4H), 7.44 (s, 2H), 7.57 - 7.69 (m, 2H), 9.03 (s, 1H),

10.36 (s, 1H), 11.91 (s, 1H). **13C NMR (101 MHz, DMSO-d_6)** δ 7.81, 8.15, 10.24, 15.12, 42.72, 45.92, 54.49, 107.00, 119.34, 122.12, 136.18, 140.53, 163.23, 164.18, 172.33, 179.68.

t_R 1.41 min, MS (ESI) m/z 480 [M +H] (100%) (HPLC System B)

N-(4-((4-((5-methyl-1H-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (95)

Following **general procedure K,** using N-(4-((4-chloro-6-((5-methyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide **87** (0,3 g, 0.676 mmol) and Piperidine (Volume: 2,5 ml) to afford the desired N-(4-((4-((5-methyl-1H-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.198 g, 0.402 mmol) as an amorphous powder. (Yield = 59.5%)

1H NMR (400 MHz, DMSO-d_6) δ 1.14 - 1.35 (m, 3H), 1.37 - 1.53 (m, 6H), 1.64 (d, J = 29.37 Hz, 3H), 1.73 - 1.84 (m, 4H), 1.88 - 2.14 (m, 3H), 2.31 - 2.41 (m, 1H), 3.67 (s, 4H), 5.19 - 6.34 (m, 1H), 7.49 (d, J = 8.62 Hz, 2H), 7.75 (d, J = 7.70 Hz, 2H), 9.50 (s, 1H), 10.06 (s, 1H), 11.90 (s, 1H). **13C NMR (101 MHz, DMSO-d_6)** δ 10.97, 24.65, 25.69, 25.80, 25.85, 29.55, 44.03, 45.44, 67.41, 96.41, 119.60, 121.94, 137.24, 141.24, 147.42, 162.45, 162.92, 175.05, 180.87.

t_R 2.07 min, MS (ESI) m/z 493 [M +H] (100%) (HPLC System B)

N-(4-((4-((5-methyl-1H-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (96)

Following **general procedure K**, using N-(4-((4-chloro-6-((5-methyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide **87** (0.300 g, 0.676 mmol) and morpholine (2.5 mL) to afford the desired N-(4-((4-((5-methyl-1H-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.128 g, 0.259 mmol) as an amorphous powder. (Yield = 38.3%)

1H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.36 (m, 3H), 1.36 - 1.50 (m, 2H), 1.66 (d, J = 11.00 Hz, 1H), 1.73 - 1.84 (m, 4H), 1.88 - 2.26 (m, 3H), 2.37 (tt, J = 3.25, 11.81 Hz, 1H), 3.51 - 3.73 (m, 8H), 5.11 - 6.36 (m, 1H), 7.46 - 7.53 (m, 2H), 7.65 - 7.82 (m, 2H), 9.61 (s, 1H), 10.08 (s, 1H), 11.75 (s, 1H). **13C NMR (101 MHz, DMSO-d_6)** δ 10.87, 25.69, 25.85, 29.55, 43.69, 45.45, 66.32, 96.50, 119.65, 121.74, 137.30, 141.30, 147.61, 162.35, 163.30, 175.06, 181.08.

t_R 1.86 min, MS (ESI) m/z 495 [M +H] (100%) (HPLC System B)

N-(4-((4-((5-methyl-1H-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (97)

Following **general procedure K**, using N-(4-((4-chloro-6-((5-methyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide **87** (0,3 g, 0.676 mmol) and 1-methylpiperazine (Volume: 2,5 ml) to afford the desired N-(4-((4-((5-methyl-1H-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.201 g, 0.396 mmol) as an amorphous powder. (Yield = 58.5%)

1H NMR (400 MHz, DMSO-d_6) δ 1.17 - 1.35 (m, 3H), 1.36 - 1.50 (m, 2H), 1.66 (d, J = 11.22 Hz, 1H), 1.72 - 1.86 (m, 4H), 1.87 - 2.03 (m, 3H), 2.21 (s, 3H), 2.27 - 2.43 (m, 5H), 3.69 (s, 4H), 5.15 - 6.32 (m, 1H), 7.37 - 7.60 (m, 2H), 7.62 - 7.97 (m, 2H), 9.56 (s, 1H), 10.10 (d, J = 13.03 Hz, 1H), 11.74 (s, 1H). **13C NMR (101 MHz, DMSO-d_6)** δ 10.89, 25.69, 25.85, 29.55, 42.99, 45.42, 46.09, 54.64, 67.41, 96.47, 119.64, 121.75, 137.30, 141.30, 147.42, 162.38, 163.15, 175.09, 181.03.

t_R 1.50 min, MS (ESI) m/z 508 [M +H] (100%) (HPLC System B)

N-(4-((4-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (98)

Following **general procedure K**, using N-(4-((4-chloro-6-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide **88** (0.300 g, 0,655 mmol) and piperidine

(Volume: 2,5 ml) to afford the desired N-(4-((4-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.180 g, 0.354 mmol) as an amorphous powder. (Yield = 54.1%)

1H NMR (400 MHz, DMSO-d_6) δ 1.19 - 1.34 (m, 3H), 1.35 - 1.49 (m, 6H), 1.56 (d, J = 5.57 Hz, 2H), 1.62 - 1.72 (m, 4H), 1.73 - 1.85 (m, 4H), 2.09 (s, 3H), 2.36 (tt, J = 3.45, 11.66 Hz, 1H), 3.44 - 3.63 (m, 4H), 7.43 (d, J = 7.91 Hz, 2H), 7.65 (d, J = 8.26 Hz, 2H), 9.07 (s, 1H), 10.00 (s, 1H). **13C NMR (101 MHz, DMSO-d_6)** δ 8.03, 10.25, 24.59, 25.69, 25.76, 25.87, 29.58, 43.92, 45.38, 106.87, 119.42, 122.12, 136.08, 140.70, 162.92, 164.04, 175.01, 179.53.

t_R 2.12 min, MS (ESI) m/z 507 [M +H] (100%) (HPLC System B)

N-(4-((4-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (99)

Following **general procedure K**, using N-(4-((4-chloro-6-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide **88** (0.300 g, 0,655 mmol) and morpholine (Volume: 2,5 ml) to afford the desired N-(4-((4-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.131 g, 0.258 mmol) as an amorphous powder. (Yield = 39.4%)

1H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.34 (m, 3H), 1.42 (qd, J = 2.78, 12.28 Hz, 2H), 1.66 (d, J = 10.90 Hz, 4H), 1.79 (ddt, J = 3.12, 12.38, 15.77 Hz, 4H), 2.10 (s, 3H), 2.36 (tt, J = 3.43, 11.68 Hz, 1H), 3.54 (s, 8H), 7.43 (d, J = 8.83 Hz, 2H), 7.66 (d, J = 8.27 Hz, 2H), 9.24 (s, 1H), 10.02 (s, 1H). **13C NMR (101 MHz, DMSO-d_6)** δ 7.98, 10.21, 25.69, 25.86, 29.58, 43.58, 45.37, 66.24, 106.98, 119.50, 121.85, 136.10, 140.78, 163.35, 163.94, 175.04, 179.81.

t_R 1.90 min, MS (ESI) m/z 509 [M +H] (100%) (HPLC System B)

N-(4-((4-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (100)

Following **general procedure K**, using N-(4-((4-chloro-6-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide **88** (0.280 g, 0,611 mmol) and 1-methylpiperazine (Volume: 2,5 ml) to afford the desired N-(4-((4-((4,5-dimethyl-1H-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)thio)phenyl)cyclohexanecarboxamide (0.158 g, 0.303 mmol) as an amorphous powder. (Yield = 49.6%)

1H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.34 (m, 3H), 1.36 - 1.49 (m, 2H), 1.51 - 1.85 (m, 8H), 2.07 (s, 3H), 2.27 - 2.42 (m, 4H), 2.51 (s, 4H), 3.57 (s, 4H), 7.43 (s, 2H), 7.66 (d, J = 8.2 Hz, 2H), 9.06 (s, 1H), 10.04 (s, 1H), 11.92 (s, 1H). **13C NMR (101 MHz, DMSO-d_6)** δ 8.13, 10.22, 25.69, 25.87, 29.58, 42.24, 45.32, 45.36, 54.05, 107.01, 119.24, 121.96, 136.08, 140.76, 163.28, 164.19, 175.04, 179.75.

t_R 1.66 min, MS (ESI) m/z 522 [M +H] (100%) (HPLC System B)

***N*-(4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (101)**

Following **general procedure L,** using 2,4,6-trichloropyrimidine **82** (1.5g, 8.13 mmol), *N*-(4-aminophenyl)cyclopropanecarboxamide **61** (1.433g, 8.13 mmol) and K_2CO_3 (1.237g, 8.95 mmol) to afford the desired *N*-(4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (1.9 g, 5.86 mmol). (Yield: 72.1%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.76 - 0.84 (m, 4H), 1.78 (tt, J = 5.00, 7.48 Hz, 1H), 7.46 - 7.52 (m, 2H), 7.57 - 7.64 (m, 2H), 10.27 (s, 1H), 11.07 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.64, 14.96, 119.72, 122.58, 132.09, 137.00, 164.01, 169.07, 170.08, 171.99.

MS (ESI) m/z 325, 327 [M +H] (HPLC System B)

***N*-(4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (102)**

Following **general procedure L,** using 2,4,6-trichloropyrimidine **82** (1.5g, 8.13 mmol), *N*-(4-aminophenyl)cyclohexanecarboxamide **62** and K_2CO_3 (1.237g, 8.95 mmol) to afford the desired *N*-(4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (1.83 g, 5.00 mmol). (Yield: 61.4 %)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.14 - 1.33 (m, 3H), 1.34 - 1.48 (m, 2H), 1.60 - 1.71 (m, 1H), 1.70 - 1.84 (m, 4H), 2.32 (tt, J = 3.37, 11.63 Hz, 1H), 7.43 - 7.52 (m, 2H), 7.58 - 7.65 (m, 2H), 9.88 (s, 1H), 11.06 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 25.72, 25.88, 29.61, 45.29, 119.78, 122.55, 132.04, 137.15, 164.03, 169.06, 170.08, 174.67.

MS (ESI) m/z 367, 369 [M +H] (HPLC System B)

_N_-(4-((4-chloro-6-((5-methyl-1_H_-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (103)

Following **general procedure M,** using *N*-(4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide **101** (1.0 g, 3.08 mmol), 5-methyl-1*H*-pyrazol-3-amine (0.300 g, 3.08 mmol) and DIPEA (0.806 ml, 4.63 mmol) to afford the desired *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.933 g, 2.424 mmol). (Yield: 79.0 %)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.74 - 0.83 (m, 4H), 1.77 (ddd, J = 5.05, 7.67, 12.59 Hz, 1H), 2.22 (s, 3H), 3.18 (d, J = 3.63 Hz, 1H), 6.26 (d, J = 26.28 Hz, 1H), 7.37 - 7.69 (m, 4H), 10.16 (s, 1H), 10.27 (s, 1H), 12.11 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.53, 14.55, 14.97, 97.81, 119.66, 122.22, 133.76, 135.85, 152.19, 164.09, 164.33, 168.45, 170.81, 171.79.

MS (ESI) m/z 385, 387 [M +H] (HPLC System B)

_N_-(4-((4-chloro-6-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (104)

Following **general procedure M,** using *N*-(4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide **101** (0.750 g, 2.31 mmol), 4,5-dimethyl-1*H*-pyrazol-3-amine (0.257 g, (2.31 mmol) and DIPEA (0.604 ml, 3.47 mmol) to afford the desired *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide. The compound was immediately introduced in the next step without further purification.

MS (ESI) m/z 399, 401 [M +H] (HPLC System B)

_N_-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (105)

Following **general procedure M,** using *N*-(4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide **102** (1.00 g, 2.73 mmol), 5-methyl-1*H*-pyrazol-3-amine (0.265 g, 2.73 mmol) and DIPEA (0.73 ml, 4.10 mmol) to afford the desired *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide. The compound was immediately introduced in the next step without further purification.

MS (ESI) m/z 427, 429 [M +H] (HPLC System B)

_N_-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (106)

Following **general procedure M,** using *N*-(4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide **102** (0.800 g, 2.18 mmol), 4,5-dimethyl-1*H*-pyrazol-3-amine (0.243 g, 2.18 mmol) and DIPEA (0.571 ml, 3.28 mmol) to afford the desired *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide. The compound was immediately introduced in the next step without further purification.

MS (ESI) m/z 441, 443 [M +H] (HPLC System B)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (107)

Following **general procedure D,** using _N_-(4-((4-chloro-6-((5-methyl-1_H_-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide **103** (0.120 g, 0.312 mmol) and piperidine (2 ml) to afford the desired _N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.037g, 0.086 mmol) as an amorphous powder. (Yield : 27.5%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.73 - 0.87 (m, 4H), 1.53 (t, J = 7.05 Hz, 4H), 1.63 (q, J = 6.12 Hz, 2H), 1.75 (td, J = 3.74, 7.39 Hz, 1H), 2.19 (s, 3H), 3.74 (t, J = 5.39 Hz, 4H), 6.32 (s, 1H), 7.34 - 7.86 (m, 5H), 9.04 (s, 1H), 9.86 - 10.22 (m, 1H), 11.87 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.43, 11.19, 14.93, 24.85, 25.88, 44.20, 96.44, 119.68, 120.48, 134.17, 136.02, 138.66, 164.41, 164.82, 168.22, 171.11, 171.56.

t_R 1.69 min, MS (ESI) m/z 434 [M +H] (100%) (HPLC System B)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (108)

Following **general procedure D,** using _N_-(4-((4-chloro-6-((5-methyl-1_H_-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide **103** (0.120 g, 0.312 mmol) and morpholine (2 ml) to afford the desired _N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.052 g, 0.119 mmol) as an amorphous powder. (Yield : 38.1%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.70 - 0.83 (m, 4H), 1.77 (ddd, J = 4.75, 7.65, 9.46 Hz, 1H), 1.97 - 2.23 (m, 3H), 3.58 - 3.70 (m, 4H), 3.69 - 3.85 (m, 4H), 6.27 (s, 1H), 7.36 - 7.88 (m, 5H), 9.14 (s, 1H), 10.15 (d, J = 28.00 Hz, 1H), 11.93 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.43, 11.53, 14.92, 43.88, 66.47, 95.92, 119.71, 120.46, 134.20, 135.80, 151.31, 163.80, 164.31, 165.29, 171.59, 171.73.

t_R 1.50 min, MS (ESI) m/z 436 [M +H] (100%) (HPLC System B)

***N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (109)**

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide **103** (0.120 g, 0.312 mmol) and *N*-methylpiperazine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.061 g, 0.135 mmol) as an amorphous powder. (Yield : 43.3%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.71 - 0.86 (m, 4H), 1.75 (td, J = 3.92, 7.47 Hz, 1H), 1.98 - 2.25 (m, 6H), 2.35 (t, J = 4.95 Hz, 4H), 3.74 (t, J = 5.00 Hz, 4H), 6.28 (s, 1H), 7.36 - 7.83 (m, 5H), 9.07 (s, 1H), 10.13 (d, J = 27.51 Hz, 1H), 11.91 (s, 1H). **^{13}C NMR (101 MHz, Acetone-d_6)** δ 6.63, 13.32, 14.57, 43.30, 45.55, 54.72, 96.95, 119.39, 121.35, 134.49, 135.66, 151.65, 164.15, 164.45, 164.84, 165.03, 171.35.

t_R 1.26 min, MS (ESI) m/z 449 [M +H] (100%) (HPLC System B)

***N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (110)**

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide **103** (0.200 g, 0.501 mmol) and piperidine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.082g, 0.184 mmol) as an amorphous powder. (Yield : 36.6%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.68 - 0.84 (m, 4H), 1.43 - 1.56 (m, 4H), 1.63 (dt, J = 4.93, 11.60 Hz, 2H), 1.71 - 1.78 (m, 1H), 1.81 (s, 3H), 2.13 (s, 3H), 3.70 (s, 4H), 7.50 (d, J = 56.86 Hz, 4H), 8.68

(s, 1H), 9.06 (s, 1H), 10.03 (s, 1H), 12.01 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.39, 8.14, 10.51, 14.91, 24.85, 25.92, 44.04, 119.62, 120.06, 133.86, 136.13, 164.35, 164.78, 165.27, 171.52.

t_R 1.57 min, MS (ESI) m/z 448 [M +H] (95%) (HPLC System B)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (111)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide **104** (0.200 g, 0.501 mmol) and morpholine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.097 g, 0.216 mmol) as an amorphous powder. (Yield : 43.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.71 - 0.83 (m, 4H), 1.70 - 1.80 (m, 1H), 1.81 (s, 3H), 2.13 (s, 3H), 3.54 - 3.66 (m, 4H), 3.67 - 3.77 (m, 4H), 7.51 (d, J = 50.63 Hz, 4H), 8.79 (s, 1H), 9.15 (s, 1H), 10.04 (s, 1H), 12.06 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.41, 8.13, 10.58, 14.92, 43.80, 66.48, 119.65, 120.26, 134.02, 135.91, 164.22, 165.21, 171.56.

t_R 1.39 min, MS (ESI) m/z 450 [M +H] (100%) (HPLC System B)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (112)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide **104** (0.200 g, 0.501 mmol) and *N*-methylpiperazine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclopropanecarboxamide (0.130 g, 0.281 mmol) as an amorphous powder. (Yield : 56.0%)

^{1}H NMR (400 MHz, DMSO-d_6) δ 0.77 (tt, J = 3.10, 7.98 Hz, 4H), 1.72 - 1.78 (m, 1H), 1.81 (s, 3H), 2.13 (s, 3H), 2.23 (s, 3H), 2.36 (s, 4H), 3.72 (s, 4H), 7.17 - 7.91 (m, 4H), 8.70 (s, 1H), 9.08 (s, 1H), 10.05 (s, 1H), 12.00 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 7.39, 8.15, 10.77, 14.91, 42.98, 46.16, 54.82, 119.63, 120.12, 133.93, 136.04, 164.41, 165.09, 171.53.

t_R 1.24 min, MS (ESI) m/z 463 [M +H] (95%) (HPLC System B)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (113)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide **105** (0.100 g, 0.234 mmol) and piperidine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.059g, 0.124 mmol) as an amorphous powder. (Yield : 53.0 %)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.18 - 1.33 (m, 3H), 1.36 - 1.47 (m, 2H), 1.47 - 1.59 (m, 4H), 1.59 - 1.70 (m, 3H), 1.70 - 1.85 (m, 4H), 2.13 (d, J = 46.40 Hz, 3H), 2.26 - 2.40 (m, 1H), 3.74 (q, J = 5.23, 7.46 Hz, 4H), 6.23 (s, 1H), 6.94 (d, J = 23.12 Hz, 1H), 7.24 - 7.89 (m, 4H), 9.18 (s, 1H), 9.82 (d, J = 32.48 Hz, 1H), 11.98 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 14.44, 24.61, 24.83, 25.73, 25.88, 29.67, 44.21, 45.22, 88.44, 119.77, 134.23, 134.88, 135.80, 151.23, 151.78, 164.59, 164.77, 174.37, 174.49.

t_R 1.89 min, MS (ESI) m/z 476 [M +H] (95%) (HPLC System B)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (114)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide **105** (0.100 g, 0.234 mmol) and morpholine (2 ml)

to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.052g, 0.108 mmol) as an amorphous powder. (Yield : 46.1 %)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 - 1.35 (m, 3H), 1.35 - 1.47 (m, 2H), 1.60 - 1.70 (m, 1H), 1.70 - 1.84 (m, 4H), 2.13 (d, J = 46.55 Hz, 3H), 2.31 (tt, J = 3.60, 11.53 Hz, 1H), 3.57 - 3.84 (m, 9H), 6.21 (s, 1H), 7.39 - 7.74 (m, 4H), 9.34 (s, 1H), 9.72 (s, 1H), 12.00 (s, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 14.33, 25.75, 25.90, 31.78, 43.89, 45.26, 66.47, 99.97, 119.78, 119.92, 120.32, 120.68, 134.36, 135.73, 165.23, 174.34, 174.46, 184.72.

t_R 1.72 min, MS (ESI) m/z 478 [M +H] (95%) (HPLC System B)

_N_-(4-((4-((5-methyl-1_H_-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (115)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((5-methyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide **105** (0.100 g, 0.234 mmol) and *N*-methylpiperazine (2 ml) to afford the desired *N*-(4-((4-((5-methyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.051g, 0.103 mmol) as an amorphous powder. (Yield : 43.9 %)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.34 (m, 3H), 1.34 - 1.49 (m, 2H), 1.60 - 1.70 (m, 1H), 1.72 - 1.82 (m, 4H), 2.16 - 2.24 (m, 4H), 2.25 - 2.43 (m, 5H), 3.62 - 3.89 (m, 4H), 6.31 (s, 1H), 7.53 (d, J = 20.18 Hz, 4H), 9.04 (s, 1H), 9.69 (s, 1H), 11.90 (s, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 12.60, 25.44, 25.53, 29.36, 42.46, 44.70, 45.73, 54.22, 88.60, 120.36, 133.36, 135.90, 151.88, 152.61, 163.81, 164.32, 164.90, 165.02, 176.03, 176.07.

t_R 1.69 min, MS (ESI) m/z 491 [M +H] (100%) (HPLC System B)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (116)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide **106** (0.200 g, 0.454 mmol) and piperidine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-(piperidin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.180 g, 0.368 mmol) as an amorphous powder. (Yield : 81.0 %)

1H NMR (400 MHz, DMSO-d_6) δ 1.17 - 1.32 (m, 3H), 1.34 - 1.45 (m, 2H), 1.44 - 1.54 (m, 4H), 1.57 - 1.69 (m, 3H), 1.70 - 1.90 (m, 7H), 2.13 (s, 3H), 2.29 (tt, J = 3.43, 11.68 Hz, 1H), 3.71 (s, 4H), 7.50 (d, J = 50.27 Hz, 4H), 8.47 (s, 1H), 8.92 (s, 1H), 9.63 (s, 1H), 11.93 (s, 1H). **13C NMR (101 MHz, MeOD)** δ 6.10, 8.55, 24.52, 25.45, 25.55, 29.38, 44.11, 45.73, 107.50, 119.74, 120.34, 133.05, 136.10, 137.23, 146.20, 164.17, 164.59, 175.98.

t_R 1.69 min, MS (ESI) m/z 490 [M +H] (100%)(HPLC System B)

_N_-(4-((4-((4,5-dimethyl-1_H_-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (117)

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide **106** (0.200 g, 0.454 mmol) and morpholine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-morpholino-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.180 g, 0.366 mmol) as an amorphous powder. (Yield : 81.0 %)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.13 - 1.33 (m, 3H), 1.34 - 1.49 (m, 2H), 1.60 - 1.70 (m, 1H), 1.71 - 1.86 (m, 7H), 2.13 (s, 3H), 2.29 (tt, J = 3.31, 11.68 Hz, 1H), 3.58 - 3.67 (m, 4H), 3.66 - 3.76 (m, 4H), 7.25 - 7.83 (m, 4H), 8.76 (s, 1H), 9.11 (s, 1H), 9.65 (s, 1H), 12.04 (s, 1H). **^{13}C NMR (101 MHz,**

MeOD) δ 6.06, 8.53, 25.44, 25.52, 29.35, 43.57, 45.73, 66.40, 107.71, 119.90, 120.31, 133.21, 135.95, 137.26, 146.01, 164.30, 165.21, 176.02.

t_R 1.54 min, MS (ESI) m/z 492 [M +H] (100%) (HPLC System B)

***N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (118)**

Following **general procedure D,** using *N*-(4-((4-chloro-6-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide **106** (0.200 g, 0.454 mmol) and *N*-methylpiperazine (2 ml) to afford the desired *N*-(4-((4-((4,5-dimethyl-1*H*-pyrazol-3-yl)amino)-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl)cyclohexanecarboxamide (0.180 g, 0.357 mmol) as an amorphous powder. (Yield : 79.0 %)

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 - 1.34 (m, 3H), 1.34 - 1.49 (m, 2H), 1.61 - 1.69 (m, 1H), 1.71 - 1.87 (m, 7H), 2.13 (s, 3H), 2.23 (s, 3H), 2.28 (dt, J = 3.49, 11.84 Hz, 1H), 2.37 (s, 4H), 3.71 (t, J = 6.37 Hz, 4H), 7.29 - 7.76 (m, 4H), 8.67 (s, 1H), 9.04 (s, 1H), 9.65 (s, 1H), 11.99 (s, 1H). **^{13}C NMR (101 MHz, MeOD)** δ 6.14, 9.32, 25.44, 25.53, 29.36, 42.45, 44.76, 45.73, 54.29, 119.99, 120.34, 133.25, 135.94, 164.31, 165.02, 176.01.

t_R 1.37 min, MS (ESI) m/z 505 [M +H] (100%) (HPLC System B)

Inhibition of mTNF+zVAD.fmk-induced necroptosis in L929sAhFas cells

In this assay a different stimulus was applied to induce cell death. By using the pan-caspase inhibitor zVAD.fmk in conjunction with mTNF the cells are rendered more sensitive to necroptosis. By inhibiting caspases the read-out of this cell death assay is also more specific for RIPK1-mediated necroptosis since it studies necrotic cell death completely independent of any caspase activity, which is essential for apoptosis execution. Compounds **8, 43, 44, 46, 48, 50, 52, 53, 55, 57, 70-81, 89-100** and **107-118** were evaluated *in vitro* for their potential to inhibit mTNF+zVAD.fmk induced

necroptosis in L929sAhFas cells. The L929sAhFas cells were pre-treated with inhibitor for either 0.5h or 24h and subsequently stimulated for 3h with mTNF (2500 IU/ml) and zVAD.fmk (1 μM). The cells were stained with PI (3 μM) and Hoechst 33342 (1 μM) and imaged using a BD Pathway™ 855 automated imaging platform. In order to determine the anti-necroptotic activity of these newly synthesized tozasertib analogues, the percentage of PI positive nuclei (% of the control) was calculated as a measure of cell death. DMSO was used as a control solvent and its outcome was assimilated to 100%. An inhibitor inhibits necroptosis if the percentage PI positive cells is less than 100%. All inhibitors were tested at different concentrations following a 1/2 serial dilution ranging from 3 μM to 0.188 μM. The calculated IC_{50}-values are reported in table 2.

To analyse nuclear abnormalities in the treated cells an automated microscopy platform BD Pathway™ 855 and Columbus™ image analysis software is used. Hoechst 33342 staining enables quantification of the amount of nucleï and nuclear area with Columbus™ software. Inhibition of AurK results in reduced cell growth and increased nuclear area. When treatment of L929sAhFas cells with the tozasertib analogues results in these morphological changes, the analogue was classified as inducer of nuclear abnormalities.

For determination of the different IC_{50} values (cell death, cell growth and nuclear area) a dose-response curve of the compounds (range 3 μM – 0.188 μM) was designed. All IC50 values were calculated using nonlinear curve fitting (4 parameters) with fixed top and bottom in GraphPad Prism 7. Depending on the parameter a different top and bottom was used: percentage of cell death (bottom = 0%, top = 100%), cell growth (bottom = total amount of cells in the highest concentration of tozasertib, top = total amount of cells in untreated condition) and nuclear area (bottom = normal nuclear area, top = maximal nuclear area at the highest concentration of tozasertib). If less than 50% inhibition is observed at a concentration of 3 μM of inhibitor, the IC_{50} was described as >3μM and the compound was deemend inactive.

ADP-Glo kinase™ assay: evaluation of the compounds against the recombinant enzymes

Compounds (concentrations as indicated) were co-incubated with recombinant hRIPK1 (100 nM) (produced in our laboratory), hAurora kinase A (25 nM) or hAurora kinase B (25 nM) (ADP-Glo™ kinase assay + Aurora A/Aurora B kinase enzyme system, Promega, V9081 and V9181). For hRIPK1, the kinase assay buffer (2x) contains 50 mM HEPES pH 7.5, 30 mM MgCl2, 50 mM NaCl, 0.5 mg/mL BSA, 0.02% CHAPS and 1 mM DTT. For the Aurora kinases, the kinase assay buffer was used from the promega assay kit (V9081, V9181). Next, the in vitro ADP-Glo™ kinase assay (promega, V6930) was used to determine kinase activity. The assay was performed according to manufacturer's protocol. The primary kinase reaction was carried out for 4h at room temperature and a 2:2:1 ratio of kinase reaction volume to ADP-Glo reagent volume to kinase detection reagent volume was used. [53] The confidence intervals and statistical validation of the results presented in table 2 are elaborated in the supplementary information table S3.

Computational methods

The coordinates for the RIPK1 crystal structure were obtained from the protein data bank with accession code 4NEU. [35] The MOE 2016.08 program suite was used for all steps unless mentioned otherwise. The structure was processed using the protein preparation wizard and protonation states were assigned corresponding to pH 7.4 with the protonate3d. [65] Docking was then performed using the triangle matcher – London dG placement method and the GBVI/VWSA dG induced fit refinement protocol. The energy of the protein:ligand complex was then minimized with the Amber10:EHT forcefield. Figures were generated using PyMOL 1.7.6.0 Open-Source. [66]

The model systems were built using Avogadro 1.1.1 [67] and starting geometries were optimized on the HF-3c [68] level with Orca. For the sulfur-linked model system, a separate geometry optimization for each dihedral scan was performed whereby the dihedral to be scanned was constrained to 0°. Due to hysteretic behavior of the nitrogen-linked model system during the scanning of the first dihedral, an additional scan in the reverse direction was performed. The minimum value of the two scans was obtained for each value of the dihedral angle. Relaxed dihedral scans were performed from 0° to 360°

in 37 steps in ORCA [60] at the PW6B95-D3(BJ) [55–57]/def2-TVZP [58,59] level of theory. The def2-TVZP/J auxiliary basis set was used in combination with the resulation of identity approximation. Grid5 and FinalGrid6 keywords were used to control numerical precision. Data processing was performed in RStudio [69] using the R programming language. [70]

In vivo experimentation of tozasertib and selected analogues

All *in vivo* experiments were conducted according to institutional, national and European regulations. Animal protocols were approved by the ethics committee of Ghent University (SIRS model). Female C57BL/6J mice (8-9 weeks old) were purchased from Janvier (Le Genest, France) for all SIRS experiments. To study the effect of tozasertib and its analogues on TNF-induced SIRS in mice, mice were challenged with 10 µg mTNF (500 µg/kg) in the presence or absence of tozasertib/analogue. Nec-1s served as positive control, since it was shown to protect against TNF-induced SIRS. All compounds were given by oral gavage 1.5h before mTNF challenge at a concentration of 50 mg/kg. The compounds were dissolved in an aqueous solution containing 8.75% ethanol and 12.5% Chremophor EL and administrated in a total volume of 200 µL. Rectal body temperature was recorded with an electric thermometer. In GraphPad7, the Mantel-Cox test was performed as statistical analysis on the survival curves. . Statistical significance is indicated in the graphs by P-values: P-values <0.05 = *, P-values <0.005 = **, P-values <0.00005 = ****. Temperature curves are illustrated with standard error of the mean.

ABBREVIATIONS USED

AurK, aurora kinase; DAMP, damage-associated molecular pattern; K_d, dissociation constant; MOE, molecular operating environment; Nec-1s, necrostatin-1 stable; Nec, necrostatin; RIPK, receptor-interacting protein kinase; zVAD.fmk, carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone

ANCILLARY INFORMATION

Supporting Information. The phenotypical evaluation and assay conditions of the first series of 30 compounds (**8**, **28-56**) is reported in the supporting information. The 95-percent confidence intervals for the IC_{50}-values from the phenotypical assay (compounds **8, 43, 44, 47, 48, 50, 52, 53, 55, 57, 70-81, 89-100, 107-118**) are reported in table S2. The 95-percent confidence intervals for the IC_{50}-values from the enzymatic screening (compounds **2, 8, 70-72, 97**) and a statistical validation of these values is reported in table S3. The molecular formula strings data file for all chemical structures mentioned in the manuscript is also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

Corresponding Author

*Koen Augustyns, Laboratory of Medicinal Chemistry (UAMC), University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk. E-mail: koen.augustyns@uantwerpen.be.

Author Contributions

The manuscript and synthesis of all reported tozasertib analogues was produced by Sam Hofmans and Lars Devisscher. The biological assays were conducted by Sofie Martens and Vera Goossens. The molecular modelling experiments were done by Dries Van Rompaey and Hans De Winter. All authors have given approval to the final version of the manuscript. The overall work leading to these results was supervised by Peter Vandenabeele, Pieter Van der Veken and Koen Augustyns.

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