Tele-Substitution Reactions in the Synthesis of a Promising Class of Antimalarials

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Tele-substitution Reactions in the Synthesis of a Promising Class of Antimalarials

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Abstract

We have discovered and studied a *tele*-substitution reaction in a biologically important heterocyclic ring system. Conditions that favour the *tele*-substitution pathway were identified: the use of increased equivalents of the nucleophile or decreased equivalents of base, or the use of softer nucleophiles, less polar solvents and larger halogens on the electrophile. Using results from X-ray crystallography and isotope labelling experiments a mechanism for this unusual transformation is proposed. We focused on this triazolopyrazine as it is the core structure of the *in vivo* active anti-plasmodium compounds of Series 4 of the Open Source Malaria consortium.

1 Introduction

Nucleophilic substitution is a widely employed method for functionalising electron deficient aromatic systems. Most commonly, a halide or other leaving group is simply displaced by an incoming nucleophile, known as direct or *ipso*-substitution.¹ Under some circumstances however, a leaving group may be displaced from an aromatic system by a nucleophile entering

at a different position on the ring, for example at the carbon adjacent to the leaving group (*cine*-substitution²) or even further away (*tele*-substitution,³ Figure 1A). We report here our discovery, and mechanistic studies, of a *tele*-substitution reaction in a [1,2,4]triazolo[4,3-*a*]pyrazine system, which is at the core of a series of molecules with significant potential for the future treatment of malaria.⁴

The first example of a *tele*-substitution reaction was reported in 1930 (Figure 1B).⁵ In this case, the reaction of 2-(chloromethyl)furan (**1**) with NaCN resulted in the attachment of the nitrile group not in place of the chlorine atom, but instead distant from the expected electrophilic site on the opposite side of the furan ring (**2**). Other examples of *tele*-substitution reactions have since been reported for a variety of aromatic systems ranging from simple pyrazine rings⁶ (Figure 1C) to more complex triazolopyrazine ring systems^{7,8} (Figures 1D and 1E), the latter of which are of particular relevance to the present work. Despite these and other reports,⁹⁻¹² *tele*-substitution reactions are not well understood; they remain hard to predict and appear to be strongly substrate dependent. Interestingly, many of the known examples of *tele*-substitution involve aza-aromatic ring systems which are common in medicinal chemistry

Figure 1: A) Possible positions for nucleophilic aromatic substitution of X. B) First reported case of a *tele*-substitution reaction in 1930. Further reports of *tele*-substitution in C) pyrazine, D) [1,2,4]triazolo[1,5-a]pyrazine and E) [1,2,4]triazolo[4,3-a]pyrazine ring systems.

and drug discovery campaigns. Given the isomeric nature of the *ipso*- and *tele*-substituted products, and the sometimes cursory level of characterisation in medicinal chemistry articles (where compound identity may be demonstrated using only a ^1H NMR spectrum and an LCMS trace) it is important, as we have discovered, to be aware of the possibility of this underappreciated reaction in order to avoid drawing conclusions from erroneous SAR data.

Here, we illustrate this with our studies on the *tele*-substitution reactions of the [1,2,4]triazolo[4,3-a]pyrazine (hereafter referred to as ‘triazolopyrazine’) heterocyclic system. These nitrogen-rich, electron-deficient heterocycles are important building blocks for the development of new medicines and have shown a wide variety of biological activities (Figure 2). We have an interest in this motif because it forms the core of Series 4 of the Open Source Malaria (OSM) consortium,¹³ represented here by compound **10** which possesses *in vitro*¹⁴ ($\text{IC}_{50} = 38 \text{ nM}$) and *in vivo*¹⁵ antimalarial activity. Compound **11** has been reported to have nanomolar potency as an inhibitor of the kidney urea transporter UT-A1.¹⁶ Compound

12 was recently patented in 2016 as a renal outer medullary potassium channel (ROMK) inhibitor.¹⁷ Sitagliptin (**13**) was approved by the FDA in 2006 as an antidiabetic drug (dipeptidyl peptidase (DPP)-IV inhibitor).¹⁸ Compound **14** is a lead molecule ($\text{IC}_{50} < 100 \text{ nM}$), that acts as an inhibitor of bromodomain and extra-terminal motif (BET) proteins for cancer treatment.¹⁹ Compound **15** is patented as an *N*-methyl-D-aspartate subtype 2B (NMDAR2B) receptor antagonist.²⁰

Figure 2: Examples of bioactive molecules that include a triazolopyrazine motif or close derivative. **10** is an example compound from OSM Series 4; **11** is an inhibitor of the UT-A1 transporter; **12** is a ROMK inhibitor; Sitagliptin (**13**) is an FDA approved antidiabetic drug; **14** is a BET inhibitor with potential in cancer treatment; **15** is an NMDAR2B receptor antagonist.

2 Results and discussion

The synthesis of members of OSM Series 4 relies on a routine $\text{S}_{\text{N}}\text{Ar}$ reaction involving the nucleophilic displacement of a chlorine atom from a triazolopyrazine core (e.g. **16**). When the synthesis of thioether analogue **17** was attempted using the standard conditions for this reaction (Figure 3A), in addition to this expected product, a compound with a significantly lower TLC retention factor was observed and isolated. This was later identified to be

Figure 3: A) Reaction used to make an OSM Series 4 compound **17**, and its *tele*-substituted isomer **18**. B) Proposed mechanisms for *ipso*- and *tele*-substitution.²¹ R = CH₂CH₂Ph.

the *tele*-substituted isomer **18**. Since the 8-isomer **18** is a main product that was formed in 83% yield and due to the similarity of the ¹H NMR spectra of these two isomers (Figure 4), the *tele*-substituted isomer **18** was initially mis-assigned as the desired product **17**. After the reaction had been repeated and examined more thoroughly compound **17** was successfully isolated as a minor product with 8% yield. The diagnostic spectroscopic difference between these isomers lies in the peaks arising from the hydrogen atoms at positions 5 and 8 on the triazolopyrazine ring; the correspondence between the NMR spectra and the structures was confirmed using X-ray crystallography (*vide infra*) and deuteration experiments. In a medicinal chemistry context, this spectroscopic similarity is a hazard for the understanding of structure activity relationships: the original evaluation of this synthetic product had concluded that **17** was inactive (IC₅₀ > 10 μM) in a malaria parasite killing assay (*in vitro* against *P. falciparum* 3D7 strain), when in fact it was **18** that had been evaluated in its place. Compound **17** was later tested and found to have reasonable potency (IC₅₀ = 1.04 μM).

According to the generally accepted *ipso*-substitution reaction mechanism, the first step is nucleophile attack on the carbon atom to

which halogen is attached (**19**, Figure 3B). The resulting intermediate (**20**) expels chloride, leading to the *ipso*-substituted product (**21**). On the other hand, a plausible mechanism for the *tele*-substitution reaction could involve the initial attack of the nucleophile at the 8-position (**22**, Figure 3B), followed by loss of the 8-position proton as part of the elimination of the chloride (**23**). Since mechanistic studies on *tele*-substitution reactions are scarce in the literature, we sought better understanding of the process operating in this case.

To better define the scope of *tele*-substitution in this triazolopyrazine system, 8- and 6-halogenated variants of the triazolopyrazine core were synthesised from the corresponding dihalopyrazines following literature procedures²² and subjected to the same reaction conditions as the original 5-chloro triazolopyrazine. The 8-halogenated cores (**25-27**, Figure 5A) reacted to give the expected *ipso*-substituted products only (**28-36**), while the 6-halogenated analogues (**37** and **38**, Figure 5B) resulted only in degradation of starting material without formation of any substituted product. While there is limited literature precedence, dihalopyrazines (e.g. **39-41**, Figure 5C) have been shown to give exclusively *ipso*-substituted products (**42-44** respectively). With these experiments showing that the *tele*-substitution reaction is observed only with the 5-halogenated cores (Figure 3A), the following mechanistic discussion will focus on that system.

Factors influencing *ipso*- vs. *tele*-substitution.

A) *Influence of triazolopyrazine structure and nucleophile.*^a The nature of the nucleophile plays a crucial role in the outcome of the reaction (Table 1). When compared to reactions with alcohols, the use of more nucleophilic amines and thiols led to significantly more *tele*-substituted products (Entries 1-6, 12-17 and 21-26). This trend may explain why *tele*-substituted isomers were apparently not seen in the literature synthesis of related structures²³ in which the incoming nucleophile was restricted to alcohols.

^aWhen the conditions employed with alcohols and thiols (KOH, 18-crown-6) were used with amine nucle-

Figure 4: ^1H NMR spectra of **17** and **18** in CDCl_3 . The hydrogen atoms on the pyrazine ring for the 5-substituted isomers (highlighted in green and orange in **17**) give rise to sharp singlets ($\delta \sim 7.5$ and ~ 9.0 ppm), while those in the 8-substituted isomers (highlighted in pink and blue in **18**) give well-defined doublets ($\delta \sim 7.2$ – 7.7 ppm, $J = 4.6$ Hz).

The nature of the leaving halogen also influences the outcome, with *tele*-substitution favoured in the order $\text{I} > \text{Br} > \text{Cl}$ (compare ratio in Entries 4, 15 and 24).

In cases where a larger substituent is in position 3 of the triazolopyrazine core (e.g. a (4-OMe)Ph group compared to a hydrogen atom), and the leaving halogen is either a Br or I atom, the distribution of *ipso*- to *tele*-substituted products is favoured towards the latter (compare Entries 12 and 15 or 21 and 24). Similar experiments in which the leaving halogen is a Cl atom show little to no change in distribution of products (compare Entries 1 and 4). Further investigation of the substituent at the

3-position led to the conclusion that bulkiness does not affect the reaction (i.e. substitution with (4-OMe)Ph is comparable to that of the larger (3,5-*t*Bu)Ph or 9-anthracene; Entries 4, 10 and 11 respectively).

Substrates with electron donating (EDG) and electron withdrawing (EWG) groups on the phenyl ring at the 3-position of the core were studied in order to evaluate the influence of electronic effects on the distribution of products. Experiments on bromotriazolopyrazines showed that EDGs tend to promote the *tele*-substitution pathway of the reaction, while EWGs lead to *ipso*-products only (Entries 15 and 18–20). Interestingly, chloro-triazolopyrazines do not follow this pattern and show no dependence on the electronic effects from the substituent in the 3-position (Entries 4, 7, 8 and 9).

From the experiments summarised in Table 1, two gave surprising results. The reaction be-

ophiles, the reaction progress was comparatively slow so the base was replaced with silica, which gave better conversion; for convenience the rate was made comparable to those seen with the other nucleophiles by raising the reaction temperature, as the reaction at room temperature was not complete after 2 weeks.

Table 1: Influence of triazolopyrazine structure, leaving halogen X and nucleophile on the reaction outcome. Conditions: ^aKOH, 18-crown-6, toluene, room temperature; ^bsilica, toluene, reflux. ^cDehalogenation by-product **49** was isolated as well in 74% yield. ^dDehalogenation by-product **49** was isolated in 11% yield along with ring opening product **50** in 17% yield (refer to Figure 6 for details). R₁ = CH₂CH₂Ph. ND: not determined.

Entry	X	R	45	Nucleophile	46 yield [%]	47 yield [%]	48 yield [%]
1	Cl	H	45a	R ₁ OH	46a , 75	ND	-
2	Cl	H	45a	R ₁ SH	46b , 51	ND	-
3	Cl	H	45a	R ₁ NH ₂ ^b	ND	30 , 65	-
4	Cl	(4-OMe)Ph	45b	R ₁ OH	46c , 70	47a , 3	-
5	Cl	(4-OMe)Ph	45b	R ₁ SH	46d , 8	47b , 83	-
6	Cl	(4-OMe)Ph	45b	R ₁ NH ₂ ^b	ND	47c , 89	-
7	Cl	(4-NO ₂)Ph	45c	R ₁ OH	46e , 77	34 , 2	-
8	Cl	(2-OMe)Ph	45d	R ₁ OH	46f , 58	ND	-
9	Cl	(2-NO ₂)Ph	45e	R ₁ OH	46g , 65	ND	-
10	Cl	(3,5- <i>t</i> Bu)Ph	45f	R ₁ OH	46h , 82	47d , 3	-
11	Cl	9-anthracenyl	45g	R ₁ OH	46i , 66	47e , 2	-
12	Br	H	45h	R ₁ OH	46a , 66	ND	-
13	Br	H	45h	R ₁ SH	46b , 34	29 , 17	-
14	Br	H	45h	R ₁ NH ₂ ^b	ND	30 , 72	-
15	Br	(4-OMe)Ph	45i	R ₁ OH	46c , 32	47a , 10	48a , 20
16	Br	(4-OMe)Ph	45i	R ₁ SH	ND	47b , 93	-
17	Br	(4-OMe)Ph	45i	R ₁ NH ₂ ^b	ND	47c , 66	-
18	Br	(4-NO ₂)Ph	45j	R ₁ OH	46e , 60	ND	48b , 30
19	Br	(2-OMe)Ph	45k	R ₁ OH	46f , 26	47f , 9	48c , 49
20	Br	(2-NO ₂)Ph	45l	R ₁ OH	46g , 76	ND	48d , 12
21	I	H	45m	R ₁ OH	46a , 34	ND	-
22	I	H	45m	R ₁ SH	46b , 7	29 , 7	-
23	I	H	45m	R ₁ NH ₂ ^b	ND	30 , 40	-
24	I	(4-OMe)Ph	45n	R ₁ OH	46c , 23	47a , 53	-
25 ^c	I	(4-OMe)Ph	45n	R ₁ SH	ND	47b , 13	-
26 ^d	I	(4-OMe)Ph	45n	R ₁ NH ₂ ^b	ND	47c , 58	-

tween the iodo-triazolopyrazine core **45n** and the thiol nucleophile (Entry 25) in addition to the 8-substituted compound **47b**, isolated in 13% yield, gave dehalogenated product **49** in 74% yield. This product was not observed for any other reaction substrates bearing a chlorine or bromine atom. This type of the dehalogenation reaction has not previously been reported

in the literature. The other unexpected product was isolated from the reaction between the iodo-triazolopyrazine core **45n** and the amine nucleophile (Entry 26). In addition to the isolation of the major *tele*-substituted isomer **47c** and dehalogenation product **49**, a minor by-product was obtained in 17% yield, the structure was determined by single crystal X-ray

Figure 5: Reactions of halogenated triazolopyrazine isomers and pyrazines. A) 8-Isomer; B) 6-Isomer; C) Pyrazine; Conditions: ^aKOH, 18-crown-6, toluene, room temperature (reactions involve measuring small amounts of hygroscopic KOH, which can contribute to reproducibility challenges, thus experiments were performed in duplicate and are reported as average values); ^bsilica, toluene, reflux (more details in Table 1).

diffraction to be based on a 5-(1H-imidazol-2-yl)-1H-1,2,4-triazole core instead of triazolopyrazine (**50**, Figure 6). It is possible that compound **50** could be formed *via* initial nucleophile attack at the 8-position of the pyrazine ring (**51**), followed by the pyrazine ring opening (**52**) and rearrangement (**53**) leading to **50**. While the analogous reaction utilising the chlorine-substituted triazolopyrazine (Entry 6) did not lead to this rearranged product, it was formed in trace amounts when the bromo-substituted triazolopyrazine was employed (Entry 17). This trend may either be due to a sub-optimal bond geometry (i.e. pseudo-equatorial

I atom) arising from the larger halogen atom or from a better match of orbital energies for elimination (in the case of the chlorine leaving group).

B) Influence of solvent. With the reaction between **45i** and the alcohol nucleophile (Table 1, Entry 1) giving significant quantities of both isomers, this was used as the model reaction to investigate further the influence of solvent on the reaction outcome (Table 2). A screen of aprotic solvents clearly showed that solvents with higher dielectric constants lead to less *tele*-substitution and also lowers the overall yield of the reaction. Protic solvents are inherently unsuitable for this reaction as they can easily themselves react with the halogenated triazolopyrazine. This was demonstrated when water was used as the solvent, giving the product **48a** in 94% yield, by result of *tele*-substitution with H_2O .

C) Influence of excess alcohol and base. By using the same model reaction above, the effect of alcohol and base equivalents was investigated. It was found that the use of an excess of nucleophile resulted in a shift of the reaction outcome drastically towards the formation of the 8-isomer (**47a**, Figure 7A). These observations suggest that the use of a softer nucleophile (here one in which the anion is surrounded by a "solvent shell" of OH bonds arising from excess nucleophile) leads to greater formation of the 8-isomer. Similarly, when fewer equivalents of base were used, a higher proportion of *tele*-substitution was again observed (Figure 7B).

D) Influence of water and temperature. In order to evaluate the impact of the level of water present on *tele*-substitution, the reaction between **45a** (unsubstituted on the triazole ring) and piperidine was conducted in toluene with various levels of water, as well as in water itself (H_2O and D_2O). The isolated yields of the 5- (**55**) and 8-isomer (**56**) were identical for experiments in both wet and dry toluene (Table 3, Entries 1 and 3). At room temperature the reaction took 14 days to complete (Entry 2), but the outcome was comparable to that when heating under reflux conditions. When molecular sieves were included in the reaction mixture (using dried toluene) the ratio of products

Figure 6: Unexpected product **50** of ring opening and rearrangement from the reaction of iodotriazolopyrazine and an amine nucleophile, with an X-ray structure and proposed mechanism for this product (see SI for more details, Figure S2).

Figure 7: Crude ^1H NMR comparison of the reaction outcome depending on A) amount of alcohol; B) amount of base. Structures of isomers placed next to corresponding signals from CH_2 groups which are indicated by arrow.

changed, though it is possible that this could arise from catalytic activity at the zeolite surface itself (Entry 4).^{24,25} Performing the reaction in H_2O (Entry 5) gave a comparable result to that in wet toluene. This is counter to the example where the alcohol nucleophile was out-competed by the solvent water to give the *tele*-substitution product (*vide supra*). It could be concluded that the presence of water in the solvent and the reaction temperature do not al-

ter the distribution of products in the studied reaction.

E) Isotope labeling experiments. Following the observation that no hydroxy-substituted product was identified in the reaction between the halogenated triazolopyrazine core **45a** and an amine nucleophile in the presence of water, deuteration experiments were performed to gain insight into the reaction mechanism. This reaction was carried out in D_2O giving two compounds, **57** and **58** (Figure 8A). The examination of products with ^1H NMR and ^2H NMR spectroscopy showed incorporation of one D atom in **57** and two in **58**. Both molecules underwent deuterium exchange of the triazole H atom. The deuteration of triazole rings has been reported in a handful of cases,^{26,27} but not for the triazolopyrazine system investigated here. In order to prove that deuteration at the 3-position occurs as a parallel reaction to the main substitution, compounds **45a**, **55** and **56** were heated under reflux in D_2O without piperidine to give corresponding mono-deuterated products **59**, **57** and **60** respectively (Figure 8B). The second D atom in **58** was at the 5-position, thus confirming that the proton which takes the place of the leaving group in the *tele*-substitution reaction comes from the

Table 2: A) Reaction used to study the influence of solvent; B) Product isolated when H₂O was employed as a solvent. Results of the reaction in different solvents (reactions performed in duplicate). All solvents were dried over molecular sieves (3 Å) for 48 h before application. All reactions proceed to complete consumption of bromo-triazolopyrazine as indicated by TLC. Total yield reported is the sum of both isomers. Product **48a** typically observed to form in ~15% yield but was not isolated in these reactions. R = CH₂CH₂Ph.

A

45m **46c** **47a**

B

48a

Entry	Solvent	46c (5-isomer) yield [%]	47a (8-isomer) yield [%]	Total yield [%]	Dielectric constant
1	Cyclohexane	35	24	58	2.02
2	Toluene	31	8	40	2.38
3	Dioxane	20	9	29	2.25
4	THF	19	3	22	7.58
5	Acetonitrile	43	3	45	37.5
6	DMF	25	2	27	36.7

solvent and not from the substrate (see the proposed mechanism for **19** in Figure 3B).

Importantly, the amine products **55** and **56** were found to be not interconvertible when each product separately was subjected to the reaction conditions for 3 days, as no conversion of one isomer into another could be detected by TLC. Thus the ratios of products observed in these telesubstitution reactions arise from a kinetic difference rather than one that has a thermodynamic origin.

3 Biological activity

As mentioned above, 5-substituted triazolopyrazines (e.g. **17**) showed antiparasmodium activity, while an 8-substituted isomer (**18**) proved to be inactive. Based on the structural similarity of these triazolopyrazines to kinase inhibitors,²⁸ we evaluated several compounds in the preliminary KINOMEScan[®] assay (at 1 µM concentration). The results revealed complementary activity of *ipso*- and *tele*-isomers, for example **47b** has higher potency against serine/threonine-protein kinase 3 (STK3) com-

pared to **46d** (Figure 9, see SI for full screening results). Thus the occurrence of this *tele*-substitution reaction allows the generation of two biologically active compounds with complementary activities from a single reaction.

Figure 9: Compounds evaluated in KINOMEScan[®] assay.

4 Conclusion

Tele-substitution reactions are simple to achieve in the triazolopyrazine ring system, and it is important to be aware of the possi-

Table 3: Results of the reaction with wet and dry solvent. 3Å molecular sieves were used to dry the toluene. Water levels were measured with a Karl-Fischer titration apparatus immediately before the experiment. ^aReaction time 14 days. ^bProducts were partially deuterated (Figure 8).

X-ray structure of 45a 45a solvent, reflux 55 56 X-ray structure of 56

Entry	Solvent	Water level (ppm)	55 (5-isomer) yield [%]	56 (8-isomer) yield [%]	Total yield [%]
1	Toluene commercial	136	16	71	87
2 ^a	Toluene commercial at rt	136	7	86	93
3	Toluene dry	6	16	71	87
4	Toluene dry with sieves in rxn	6	36	40	76
5	H ₂ O	-	21	57	78
6 ^b	D ₂ O	-	24	59	83

Figure 8: A) Reaction between simplified chloro-substituted core **45a** and piperidine, performed in D₂O as the solvent; B) Verification that H/D exchange on the triazole, but not the pyrazine, is a parallel reaction to the main substitution reaction. ^aD₂O, heating at reflux.

bility of such isomers forming, given the wide biological relevance of many of these structures. The *tele*-substitution reaction occurs only in 5-halogenated triazolo-pyrazine cores, while 8- or 6-halogenated cores tend to give *ipso*-substitution or degradation respectively. The *tele*-substitution pathway of the reaction is also made more likely by the use of stronger nu-

cleophiles, triazolo-pyrazines with bulkier halogens and the use of less polar solvents. As concluded from the isotope labeling experiments, the hydrogen atom that takes the place of the halogen derives from solvent and not from substrate. The product ratios arise from a kinetic difference in the reactions rather than a thermodynamic difference in product energies, where, broadly, a combination of hard nucleophile and hard electrophile promotes *ipso*-substitution while a softer combination promotes *tele*-substitution.

5 Experimental

5.1 General Procedures

General Procedure A. Preparation of halogen-hydrazinylpyrazines

Mono or dihalogenopyrazine (70 mmol, 1 equiv.) was dissolved in ethanol (100 mL), then hydrazine monohydrate was added (140 mmol, 2 equiv.) and the mixture was heated at reflux overnight. The solvent was removed under reduced pressure. Equal amounts of EtOAc (100 mL) and H₂O (100 mL) were added, the EtOAc

layer was separated and the aqueous layer was washed with EtOAc (30 mL $\times$ 3). The combined organic phases were washed with brine (30 mL), dried over Na₂SO₄ and evaporated under reduced pressure to give the desired compound, which was used in the subsequent reaction without further purification.

General Procedure B. Preparation of halogeno-[1,2,4]triazolo[4,3-a]pyrazine

To a suspension of halogen-hydrazinylpyrazine (70.0 mmol, 1.0 equiv.) in toluene (200 mL) triethyl orthoformate or trimethyl orthoformate (140 mmol, 2.0 equiv.) was added followed by *p*-toluenesulfonic acid monohydrate (14.0 mmol, 0.2 equiv.). The mixture was heated at reflux for 5 h. The solvent was removed under reduced pressure and the residue purified by flash column chromatography (FCC) on silica using a gradient of EtOAc (20% to 100%) in hexanes to give the desired product.

General Procedure C. Preparation of halogeno-3-aryl-[1,2,4]triazolo[4,3-a]pyrazine

Adopted from the literature procedures.²² To a stirred suspension of halogeno-hydrazinylpyrazine (7.0 mmol, 1.0 equiv.) in ethanol (100 mL) was added aldehyde (7.7 mmol, 1.1 equiv.) and the mixture heated at reflux overnight. After the full consumption of starting material as indicated by TLC, the reaction was cooled in an ice bath and chloramine T trihydrate (9.1 mmol, 1.3 equiv.) was added portionwise while stirring over 1 h. After consumption of the intermediate was confirmed by TLC, cold H₂O (100 mL) was added to the reaction mixture. The solution was stirred for 10 min, then filtered through a sintered glass filter (P3 porosity) and washed with H₂O (30 mL $\times$ 3) followed by Et₂O (30 mL). The solid was dried *in vacuo* to give desired product that was used without further purification.

General Procedure D. Coupling of alcohol or thiol with halogen-heterocycle

To a suspension of halogen-heterocycle (0.40 mmol, 1 equiv.) in toluene (10 mL) was added 18-crown-6 (0.032 mmol, 0.08 equiv.) and alcohol or thiol (0.40 mmol, 1 equiv.) followed by KOH (1.20 mmol, 3.0 equiv.). The reaction mixture stirred for 2-24 h at room temperature. Upon completion as indicated by TLC, the reaction mixture was directly subjected to the purification by FCC on silica and flushed at the beginning with hexanes (in order to wash out toluene from the column) followed by a gradient of EtOAc (30% to 100%) in hexanes (unless specified in the compound preparation) to give the desired product.

General Procedure E. Coupling of amine with halogen-heterocycle

To a suspension of halogen-heterocycle (0.40 mmol, 1.0 equiv.) in toluene (10 mL) was added amine (1.20 mmol, 3.0 equiv.) followed by silica (0.5 g). The reaction was heated at 80 °C overnight. Upon completion of the reaction as indicated by TLC, the solvent was evaporated *in vacuo* and the mixture purified by FCC on silica using a gradient of EtOAc EtOAc (30% to 100%) in hexanes (unless specified in the compound preparation) to give the desired product.

5.2 Synthesis

2-Chloro-6-hydrazinylpyrazine (S1). General Procedure A was applied using 2,6-dichloropyrazine (35.0 g, 235 mmol) to give **S1** as a yellow solid (29.2 g, 202 mmol, 86%). mp 137–139 °C (lit.⁸ 136–139 °C). ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.42 (s, 1H), 8.05 (s, 1H), 7.70 (s, 1H), 4.37 (s, 2H). ¹³C{¹H} NMR (50 MHz, DMSO-*d*₆): δ 157.1, 145.7, 129.0, 128.6.

The spectroscopic data and melting point were in agreement with those in the literature.^{8,29}

2-Bromo-6-hydrazinylpyrazine (S2). General Procedure A was applied using 2,6-dibromopyrazine (8.09 g, 34.0 mmol) to give **S2** as an orange solid (5.45 g, 28.9 mmol, 85%). mp 142–144 °C. ¹H NMR (500 MHz, CDCl₃): δ 8.16 (s, 1H), 7.98 (s, 1H), 6.28 (s, 1H), 3.72 (s, 2H). ¹³C{¹H} NMR (126 MHz, CDCl₃): δ 156.8, 138.1, 135.4, 129.2. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₄H₆⁷⁹BrN₄ 188.97704; found 188.97728.

2-Iodo-6-hydrazinylpyrazine (S3). General Procedure A was applied using **41** (8.37 g, 25.2 mmol) to give **S3** as a yellow solid (4.87 g, 20.7 mmol, 82%). mp 154–156 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.31 (s, 1H), 8.05 (s, 1H), 7.91 (s, 1H), 4.33 (s, 2H). ¹³C{¹H} NMR (75 MHz, DMSO-*d*₆): δ 157.8, 137.8, 128.9, 115.9. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₄H₆IN₄ 236.96317; found 236.96297.

2-Chloro-3-hydrazinylpyrazine (S4). General Procedure A was applied using 2,3-dichloropyrazine (10.2 g, 68.3 mmol) to give **S4** as a yellow solid (6.61 g, 45.7 mmol, 67%). mp 156–158 °C (lit.³⁰ mp 154 °C). ¹H NMR (200 MHz, DMSO-*d*₆): δ 8.23 (s, 1H), 8.04 (d, *J* = 2.7 Hz, 1H), 7.55 (d, *J* = 2.8 Hz, 1H), 4.34 (s, 2H). ¹³C{¹H} NMR (50 MHz, DMSO-*d*₆): δ 152.6, 140.6, 132.6, 130.0. The spectroscopic data and melting point were in agreement with those in the literature.^{22,30}

2-Chloro-5-hydrazinylpyrazine (S5). Compound was prepared following literature procedures.³¹ 2,5-Dichloropyrazine (2.00 g, 13.4 mmol, 1.0 equiv.) was added to H₂O (12.5 mL) followed by 28% aq. ammonia solution (2.63 mL, 38.9 mmol, 2.9 equiv.) and hydrazine monohydrate (1.57 mL, 1.61 g, 32.2 mmol, 2.4 equiv.). The mixture was heated at reflux overnight, then cooled in an ice bath for 15 min, filtered through a sintered funnel and washed with cold H₂O (25 mL × 3), then dried *in vacuo* to give **S5** as a pale yellow solid (1.62 g, 11.2 mmol, 83%). mp 168–170 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.14 (s, 1H), 8.02 (d, *J* = 1.4 Hz, 1H), 7.93 (d, *J* = 1.4 Hz, 1H), 4.32 (s, 2H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 156.6, 140.3, 133.9,

129.5. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₄H₆ClN₄ 145.02755; found 145.02749. The spectroscopic data were in agreement with those in the literature.³²

5-Chloro-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (16). General Procedure C was applied using **S1** (1.51 g, 10.4 mmol, 1.0 equiv.) and 4-(difluoromethoxy)benzaldehyde (1.98 g, 11.5 mmol, 1.1 equiv.) to give **16** as a brown solid (2.26 g, 7.62 mmol, 73%). mp 124–126 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 9.47 (s, 1H), 8.08 (s, 1H), 7.79 (d, *J* = 8.2 Hz, 2H), 7.41 (t, *J* = 73.6 Hz, 1H), 7.36 (d, *J* = 8.2 Hz, 2H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 153.3 – 152.1 (m), 147.0, 146.7, 142.7, 133.3, 129.2, 124.0, 121.8, 117.4, 116.2 (t, *J* = 258.0 Hz) (OCHF₂). HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₁₂H₈ClF₂N₄O 297.03492; found 297.03460.

3-(4-(Difluoromethoxy)phenyl)-5-(phenethylthio)-[1,2,4]triazolo[4,3-*a*]pyrazine (17). General Procedure D was applied using **16** (101 mg, 0.341 mmol, 1.0 equiv.) and 2-phenylethane-1-thiol (47.1 mg, 0.341 mmol, 1.0 equiv.). Fractions corresponding to the second peak were evaporated to give **17** as a yellow solid (11.0 mg, 0.0276 mmol, 8%). mp 78–83 °C. ¹H NMR (400 MHz, CDCl₃): δ 9.21 (s, 1H), 7.76 (s, 1H), 7.68 – 7.60 (m, 2H), 7.28 – 7.15 (m, 5H), 7.02 – 6.94 (m, 2H), 6.64 (t, *J* = 73.1 Hz, 1H), 2.92 (t, *J* = 7.5 Hz, 2H), 2.76 (t, *J* = 7.4 Hz, 2H). ¹³C{¹H} NMR (101 MHz, CDCl₃): δ 153.10 (t, *J* = 2.8 Hz), 147.6, 146.4, 142.3, 138.3, 133.5, 131.3, 128.8, 128.6, 128.4, 127.1, 124.1, 118.3, 115.65 (t, *J* = 261.3 Hz), 35.8, 34.6. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₂₀H₁₇F₂N₄OS 399.10856; found 399.10801.

3-(4-(Difluoromethoxy)phenyl)-8-(phenethylthio)-[1,2,4]triazolo[4,3-*a*]pyrazine (18). Isolated from the same reaction as for **17**. Fractions corresponding to the first peak were evaporated to give **18** as an off-white solid (113 mg, 0.284 mmol, 83%). mp 156–158 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.32 (d, *J* = 4.8 Hz, 1H), 8.04 – 7.96 (m, 2H), 7.83 (d, *J* = 4.8 Hz, 1H), 7.58 – 7.39 (m, 5H), 7.37 – 7.29 (m, 4H), 7.30 – 7.20 (m, 1H), 3.59 (dd, *J* = 8.4, 6.7 Hz, 2H), 3.05 (dd, *J* = 8.4, 6.7

Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 153.0, 152.4 (t, $J = 3.3$ Hz), 146.9, 143.8, 139.9, 130.2, 129.5, 128.6, 128.4, 126.4, 122.5, 119.2, 116.1 (t, $J = 258.5$ Hz), 113.2, 34.4, 29.4. ^{19}F NMR (471 MHz, DMSO- d_6): δ -82.8. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{17}\text{F}_2\text{N}_4\text{OS}$ 399.10856; found 399.10827.

8-Chloro-[1,2,4]triazolo[4,3-*a*]pyrazine (25). General Procedure B was applied using **S4** (2.71 g, 18.8 mmol) to give **25** as a yellow solid (0.870 g, 5.63 mmol, 30%). mp 203–206 °C (lit.³⁰ mp 205 °C). ^1H NMR (200 MHz, CDCl_3): δ 9.00 (s, 1H), 8.05 (d, $J = 4.7$ Hz, 1H), 7.74 (d, $J = 4.7$ Hz, 1H). HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_5\text{H}_3\text{ClN}_4\text{Na}$ 176.99384; found 176.99367. The spectroscopic data and melting point were in agreement with those in the literature.^{30,33}

8-Chloro-3-phenyl-[1,2,4]triazolo[4,3-*a*]pyrazine (26). General Procedure C was applied using **S4** (0.768 g, 5.31 mmol, 1.0 equiv.) and benzaldehyde (0.620 g, 5.84 mmol, 1.1 equiv.) to give **26** as a white solid (0.976 g, 4.23 mmol, 80%). mp 192–195 °C (lit.³⁴ mp 193–195 °C). ^1H NMR (200 MHz, CDCl_3): δ 8.16 (d, $J = 4.8$ Hz, 1H), 7.92 – 7.78 (m, 2H), 7.72 (d, $J = 4.8$ Hz, 1H), 7.64 (q, $J = 3.1$ Hz, 3H). HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{11}\text{H}_7\text{ClN}_4\text{Na}$ 253.02514; found 253.02522. The spectroscopic data and melting point were in agreement with those in the literature.³⁴

8-Chloro-3-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (27). General Procedure C was applied using **S4** (0.655 g, 4.53 mmol, 1.0 equiv.) and 4-nitrobenzaldehyde (0.754 g, 4.99 mmol, 1.1 equiv.) to give **27** as a yellow solid (1.15 g, 4.16 mmol, 92%). m.p. 231–234 °C (decomp.) (lit.²² mp 201–204 °C). ^1H NMR (500 MHz, DMSO- d_6): δ 8.77 (d, $J = 4.8$ Hz, 1H), 8.47 (d, $J = 8.6$ Hz, 2H), 8.26 (d, $J = 8.6$ Hz, 2H), 7.89 (d, $J = 4.8$ Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 148.9, 147.6, 144.6, 142.5, 132.0, 130.1, 129.6, 124.8, 118.4. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{11}\text{H}_7\text{ClN}_5\text{O}_2$ 298.01022; found 298.01025. The spectroscopic data were in agreement with the literature, but the melting point was significantly higher.²²

8-Phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine

(28). General Procedure D was applied using **25** (104 mg, 0.673 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (82.2 mg, 0.673 mmol, 1.0 equiv.) to give **28** as an off-white solid (83.0 mg, 0.345 mmol, 51%). mp 161–162 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 9.36 (s, 1H), 8.19 (d, $J = 4.7$ Hz, 1H), 7.42 (d, $J = 4.7$ Hz, 1H), 7.38 – 7.27 (m, 4H), 7.26 – 7.19 (m, 1H), 4.72 (t, $J = 6.9$ Hz, 2H), 3.16 (t, $J = 6.9$ Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 152.6, 138.7, 138.4, 137.9, 128.9, 128.4, 126.6, 126.4, 113.2, 113.2, 67.2, 34.2. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{13}\text{H}_{12}\text{N}_4\text{ONa}$ 263.09033; found 263.08997.

8-(Phenethylthio)-[1,2,4]triazolo[4,3-*a*]pyrazine (29). General Procedure D was applied using **25** (104 mg, 0.673 mmol, 1.0 equiv.) and 2-phenylethane-1-thiol (93.0 mg, 0.673 mmol, 1.0 equiv.) to give **29** as an off-white solid (154 mg, 0.602 mmol, 90%). mp 148–150 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 9.38 (d, $J = 0.8$ Hz, 1H), 8.33 (dd, $J = 4.6, 0.8$ Hz, 1H), 7.79 (dd, $J = 4.7, 0.8$ Hz, 1H), 7.31 (d, $J = 4.4$ Hz, 4H), 7.23 (h, $J = 4.0$ Hz, 1H), 3.59 – 3.53 (m, 2H), 3.06 – 3.00 (m, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 152.2, 142.7, 139.9, 138.1, 128.7, 128.6, 128.4, 126.4, 114.3, 34.4, 29.4. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{13}\text{H}_{12}\text{N}_4\text{SNa}$ 279.06749; found 279.06714.

***N*-Phenethyl-[1,2,4]triazolo[4,3-*a*]pyrazin-8-amine (30).** Preparation 1: General Procedure E was applied using **25** (104 mg, 0.654 mmol) and 2-phenylethan-1-amine (244 mg, 2.01 mmol, 3.0 equiv.) to give **30** as an off-white solid (135 mg, 0.564 mmol, 84%). Preparation 2: General Procedure E was applied using **45a** (100 mg, 0.649 mmol, 1.0 equiv.) and 2-phenylethan-1-amine (235 mg, 1.95 mmol, 3.0 equiv.) to give **30** as an off-white solid (102 mg, 0.424 mmol, 65%). mp 191–193 °C (decomp.). ^1H NMR (500 MHz, DMSO- d_6): δ 9.19 (s, 1H), 8.16 (t, $J = 5.8$ Hz, 1H), 7.74 (d, $J = 4.7$ Hz, 1H), 7.32 – 7.23 (m, 5H), 7.23 – 7.15 (m, 1H), 3.71 (q, $J = 6.9$ Hz, 2H), 2.99 – 2.92 (m, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 147.4, 139.5, 138.6, 138.1, 129.1, 128.6, 128.3, 126.0, 107.2, 41.6, 34.5. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{14}\text{N}_5$ 240.12437; found 240.12406.

*8-Phenethoxy-3-phenyl-[1,2,4]triazolo[4,3-*a*]pyrazine (31)*. General Procedure D was applied using **26** (115 mg, 0.499 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (60.9 mg, 0.499 mmol, 1.0 equiv.) to give **31** as a white solid (91.0 mg, 0.288 mmol, 58%). mp 145–147 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.19 (d, *J* = 4.9 Hz, 1H), 7.94 – 7.88 (m, 2H), 7.68 – 7.59 (m, 3H), 7.47 (d, *J* = 4.9 Hz, 1H), 7.41 – 7.29 (m, 4H), 7.24 (t, *J* = 7.3 Hz, 1H), 4.76 (t, *J* = 6.8 Hz, 2H), 3.19 (t, *J* = 6.9 Hz, 2H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 153.2, 148.0, 139.7, 138.0, 130.5, 129.3, 129.0, 128.4, 128.1, 127.4, 126.4, 125.9, 112.1, 67.4, 34.2. HRMS (ESI/FTICR) *m/z*: [M + Na]⁺ calcd for C₁₉H₁₆N₄ONa 339.12163; found 339.12165.

*8-(Phenethylthio)-3-phenyl-[1,2,4]triazolo[4,3-*a*]pyrazine (32)*. General Procedure D was applied using **26** (107 mg, 0.464 mmol) and 2-phenylethane-1-thiol (65.1 mg, 0.464 mmol, 1.0 equiv.) to give **32** as an off-white solid (145 mg, 0.440 mmol, 94%). mp 154–156 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.33 (d, *J* = 4.8 Hz, 1H), 7.97 – 7.88 (m, 2H), 7.83 (d, *J* = 4.8 Hz, 1H), 7.69 – 7.59 (m, 3H), 7.33 (d, *J* = 5.0 Hz, 4H), 7.24 (ddd, *J* = 8.8, 5.3, 3.5 Hz, 1H), 3.63 – 3.56 (m, 2H), 3.09 – 3.02 (m, 2H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 153.0, 147.6, 143.8, 140.0, 130.5, 129.5, 129.3, 128.6, 128.4, 128.2, 126.4, 125.7, 113.2, 34.4, 29.4. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₁₉H₁₇N₄S 333.11684; found 333.11641.

*N-Phenethyl-3-phenyl-[1,2,4]triazolo[4,3-*a*]pyrazin-8-amine (33)*. General Procedure E was applied using **26** (102 mg, 0.442 mmol) and 2-phenylethan-1-amine (161 mg, 1.33 mmol, 3.0 equiv.) to give **33** (120 mg, 0.381 mmol, 86%). mp 206–209 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.27 (t, *J* = 5.7 Hz, 1H), 7.92 – 7.86 (m, 2H), 7.75 (d, *J* = 4.8 Hz, 1H), 7.67 – 7.57 (m, 3H), 7.36 (d, *J* = 4.8 Hz, 1H), 7.34 – 7.25 (m, 4H), 7.25 – 7.17 (m, 1H), 3.79 – 3.71 (m, 2H), 3.02 – 2.95 (m, 2H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 147.9, 147.7, 139.6, 139.5, 130.2, 130.2, 129.3, 128.7, 128.3, 128.0, 126.3, 126.1, 106.0, 41.6, 34.5. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₁₉H₁₈N₅ 316.15567; found 316.15526.

3-(4-Nitrophenyl)-8-phenethoxy-[1,2,4]tria-

*zolo[4,3-*a*]pyrazine (34)*. Preparation 1: General Procedure D was applied using **27** (113 mg, 0.410 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (50.1 mg, 0.410 mmol, 1.0 equiv.) to give **34** as a yellow solid (125 mg, 0.346 mmol, 84%). Preparation 2: isolated from the same reaction as for **46e** preparation 1: fractions correspond to the first peak were evaporated to give **34** as a yellow solid (2.05 mg, 5.51 μmol, 2%). mp 238–240 °C (decomp.). ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.45 (d, *J* = 8.3 Hz, 2H), 8.32 (d, *J* = 4.9 Hz, 1H), 8.24 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 4.9 Hz, 1H), 7.38 (d, *J* = 7.6 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.24 (t, *J* = 7.2 Hz, 1H), 4.79 (t, *J* = 6.8 Hz, 2H), 3.20 (t, *J* = 6.8 Hz, 2H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 153.2, 148.2, 146.5, 140.1, 137.9, 132.0, 129.4, 128.9, 128.4, 127.9, 126.4, 124.3, 112.4, 67.5, 34.2. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₁₉H₁₆N₅O₃ 362.12477; found 362.12462.

*3-(4-Nitrophenyl)-8-(phenethylthio)-[1,2,4]triazolo[4,3-*a*]pyrazine (35)*. Preparation 1: General Procedure D was applied using **27** (107 mg, 0.390 mmol) and 2-phenylethane-1-thiol (65.7 mg, 0.390 mmol, 1.0 equiv.) to give **35** as a yellow solid (103 mg, 0.273 mmol, 70%). Preparation 2: Isolated from the same reaction as for **46j**. Fractions corresponding to the first peak were evaporated to give **35** as a yellow solid (66.2 mg, 0.175 mmol, 44%). mp 236–238 °C (decomp.). ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.46 (dd, *J* = 6.9, 2.0 Hz, 3H), 8.28 – 8.22 (m, 2H), 7.92 (d, *J* = 4.8 Hz, 1H), 7.37 – 7.30 (m, 4H), 7.29 – 7.21 (m, 1H), 3.61 (dd, *J* = 8.4, 6.7 Hz, 2H), 3.07 (dd, *J* = 8.4, 6.7 Hz, 2H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 153.1, 148.3, 146.2, 144.2, 139.9, 131.8, 130.0, 129.5, 128.6, 128.4, 126.4, 124.3, 113.5, 34.4, 29.5. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₁₉H₁₆N₅O₂S 378.10192; found 378.10180.

*3-(4-Nitrophenyl)-N-phenethyl-[1,2,4]triazolo[4,3-*a*]pyrazin-8-amine (36)*. Preparation 1: General Procedure E was applied using **27** (112 mg, 0.406 mmol, 1.0 equiv.) and 2-phenylethan-1-amine (148 mg, 1.22 mmol, 3.0 equiv.) to give **36** (127 mg, 0.352 mmol, 87%). Preparation 2: General Procedure E was applied using **45c** (103 mg, 0.374 mmol,

1.0 equiv.) and 2-phenylethan-1-amine (136 mg, 1.12 mmol, 3.0 equiv.) to give **36** as a yellow solid (133 mg, 0.369 mmol, 99%). mp 236–238 °C (decomp.). ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.47 – 8.41 (m, 2H), 8.38 (t, *J* = 5.8 Hz, 1H), 8.29 – 8.20 (m, 2H), 7.88 (d, *J* = 4.8 Hz, 1H), 7.45 (d, *J* = 4.8 Hz, 1H), 7.30 (h, *J* = 5.9 Hz, 4H), 7.21 (tt, *J* = 5.9, 2.1 Hz, 1H), 3.76 (q, *J* = 6.8 Hz, 2H), 3.02 – 2.96 (m, 2H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 148.0, 147.9, 146.1, 139.9, 139.5, 132.4, 130.8, 129.1, 128.7, 128.3, 126.1, 124.3, 106.2, 41.6, 34.4. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₁₉H₁₇N₆O₂ 361.14075; found 361.14041.

*6-Chloro-[1,2,4]triazolo[4,3-*a*]pyrazine (37)*. General Procedure B was applied using **S5** (1.53 g, 10.6 mmol) to give **37** as an orange solid (0.800 g, 5.18 mmol, 49%). mp 215–217 °C (decomp.). ¹H NMR (500 MHz, DMSO-*d*₆): δ 9.41 (d, *J* = 0.7 Hz, 1H), 9.36 (dd, *J* = 1.5, 0.7 Hz, 1H), 8.90 (d, *J* = 1.5 Hz, 1H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 143.9, 143.0, 137.3, 133.4, 116.3. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₅H₄ClN₄ 155.01190; found 155.01178.

*6-Chloro-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (38)*. General Procedure C was applied using **S5** (1.33 g, 9.23 mmol, 1.0 equiv.) and 4-(difluoromethoxy)benzaldehyde (1.22 mL, 1.59 g, 9.23 mmol, 1.1 equiv.) to give **27** as a pale brown solid (1.75 g, 5.89 mmol, 64%). mp 159–161 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 9.41 (s, 1H), 8.85 (s, 1H), 8.05 (d, *J* = 8.6 Hz, 2H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.41 (t, *J* = 73.5 Hz, 1H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 152.5 (t, *J* = 3.3 Hz), 146.2, 145.2, 143.4, 134.6, 130.3, 122.1, 119.2, 116.1 (t, *J* = 258.6 Hz), 115.2. ¹⁹F NMR (471 MHz, DMSO-*d*₆): δ -82.8. HRMS (ESI/FTICR) *m/z*: [M + Na]⁺ calcd for C₁₂H₇ClF₂N₄ONa 319.01687; found 319.01690.

2,6-Diiodopyrazine (41). Compounds was prepared following literature procedures.³⁵ Hydroiodic acid (50% solution, 25 mL, 5.0 equiv.) was added to 2,6-dichloropyrazine (5.07 g, 34.0 mmol, 1.0 equiv.) and NaI (6.63 g, 44.2 mmol, 1.3 equiv.) in a sealed tube and heated at 100 °C for 3 h. The reaction was cooled to room

temperature and diluted with Et₂O (200 mL). The solution was washed with H₂O (100 mL × 2), sat. aq. NaHCO₃ (50 mL), sat. aq. Na₂S₂O₃ (50 mL), brine (30 mL), dried (Na₂SO₄), filtered and concentrated under reduced pressure to give **41** as a white solid (9.91 g, 29.9 mmol, 88%). mp 90–92 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.74 (s, 2H). ¹³C{¹H} NMR (75 MHz, CDCl₃): δ 151.2, 116.8. The spectroscopic data were in agreement with those in the literature.³⁵

2-Chloro-6-phenethoxypyrazine (42). General Procedure D was applied using 2,6-dichloropyrazine (107 mg, 0.718 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (87.8 mg, 0.718 mmol, 1.0 equiv.). The reaction mixture was purified by FCC on silica using a gradient of EtOAc (0% to 6%) in hexanes to give **42** as a colourless oil (137 mg, 0.582 mmol, 81%). ¹H NMR (500 MHz, CDCl₃): δ 8.13 (s, 1H), 8.11 (s, 1H), 7.36 – 7.20 (m, 5H), 4.56 (t, *J* = 7.0 Hz, 2H), 3.11 (t, *J* = 7.0 Hz, 2H). ¹³C{¹H} NMR (126 MHz, CDCl₃): δ 159.3, 145.5, 137.8, 135.3, 133.3, 129.1, 128.7, 126.8, 67.8, 35.2. HRMS (ESI/FTICR) *m/z*: [M + Na]⁺ calcd for C₁₂H₁₁ClN₂ONa 257.04521; found 257.04508.

2-Bromo-6-phenethoxypyrazine (43). General Procedure D was applied using 2,6-dibromopyrazine (127 mg, 0.534 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (65.2 mg, 0.534 mmol, 1.0 equiv.). The reaction mixture was purified by FCC on silica using a gradient of EtOAc (0% to 6%) in hexanes to give **43** as a colourless oil (122 mg, 0.436 mmol, 82%). ¹H NMR (500 MHz, CDCl₃): δ 8.21 (s, 1H), 8.12 (s, 1H), 7.35 – 7.20 (m, 5H), 4.55 (t, *J* = 7.0 Hz, 2H), 3.09 (t, *J* = 7.0 Hz, 2H). ¹³C{¹H} NMR (126 MHz, CDCl₃): δ 159.4, 138.3, 137.8, 136.5, 133.5, 129.1, 128.7, 126.8, 68.0, 35.2. HRMS (ESI/FTICR) *m/z*: [M + Na]⁺ calcd for C₁₂H₁₁⁷⁹BrN₂ONa 300.99470; found 300.99465.

2-Iodo-6-phenethoxypyrazine (44). General Procedure D was applied using **41** (108 mg, 0.325 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (39.8 mg, 0.325 mmol, 1.0 equiv.). The reaction mixture was purified by FCC on silica using a gradient of EtOAc (0% to 6%) in hexanes

to give **44** as a colourless oil (83.0 mg, 0.254 mmol, 78%). ^1H NMR (500 MHz, CDCl_3): δ 8.38 (s, 1H), 8.11 (s, 1H), 7.37 – 7.21 (m, 5H), 4.54 (t, J = 7.0 Hz, 2H), 3.09 (t, J = 7.0 Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, CDCl_3): δ 159.5, 144.2, 137.8, 133.7, 129.1, 128.7, 126.8, 112.7, 68.0, 35.2. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{12}\text{H}_{11}\text{IN}_2\text{ONa}$ 348.98083; found 348.98065.

5-Chloro-3-(2-methoxyphenyl)-[1,2,4]triazolo[4,3-a]pyrazine (45a). General Procedure B was applied using **S1** (25.4 g, 176 mmol) to give **45a** as a yellow solid (12.3 g, 79.8 mmol, 45%). mp 169–171 °C (lit.⁸ 167–172 °C). ^1H NMR (500 MHz, CDCl_3): δ 9.27 (s, 1H), 9.04 (s, 1H), 7.93 (s, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, CDCl_3): δ 145.8, 141.9, 134.7, 128.3, 121.3. The spectroscopic data and melting point were in agreement with those in the literature.⁸ X-ray single crystal data can be found in the supporting information.

5-Chloro-3-(4-methoxyphenyl)-[1,2,4]triazolo[4,3-a]pyrazine (45b). General Procedure C was applied using **S1** (1.01 g, 6.97 mmol, 1.0 equiv.) and 4-methoxybenzaldehyde (1.04 g, 7.66 mmol, 1.1 equiv.) to give **45b** as an off-white solid (1.34 g, 5.16 mmol, 74%). mp 145–147 °C (decomp.). ^1H NMR (200 MHz, CDCl_3): δ 9.31 (s, 1H), 7.84 (s, 1H), 7.63 – 7.47 (m, 2H), 7.11 – 6.95 (m, 2H), 3.91 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, $\text{DMSO}-d_6$): δ 160.8, 147.4, 146.9, 142.7, 132.8, 129.1, 121.8, 119.1, 113.1, 55.3. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{12}\text{H}_{10}\text{ClN}_4\text{O}$ 261.05377; found 261.05348.

5-Chloro-3-(4-nitrophenyl)-[1,2,4]triazolo[4,3-a]pyrazine (45c). General Procedure C was applied using **S1** (1.06 g, 7.33 mmol, 1.0 equiv.) and 4-nitrobenzaldehyde (1.21 g, 8.07 mmol, 1.1 equiv.) to give **45c** as an off-white solid (1.91 g, 6.93 mmol, 95%). mp 238–240 °C (decomp.). ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ 9.53 (s, 1H), 8.41 (d, J = 8.8 Hz, 2H), 8.15 (s, 1H), 8.05 (d, J = 8.7 Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, $\text{DMSO}-d_6$): δ 148.6, 147.2, 145.8, 142.7, 133.7, 132.9, 129.4, 122.7, 121.9. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{11}\text{H}_7\text{ClN}_5\text{O}_2$ 276.02828; found 276.02784.

5-Chloro-3-(2-methoxyphenyl)-[1,2,4]triazolo[4,3-a]pyrazine (45d). General Procedure C was applied using **S1** (400 mg, 2.77 mmol, 1.0 equiv.) and 2-methoxybenzaldehyde (414 mg, 3.04 mmol, 1.1 equiv.) to give **45d** as an off-white solid (430 mg, 1.65 mmol, 60%); **m.p.** 142–145 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ 9.47 (s, 1H), 8.08 (s, 1H), 7.63 (ddd, J = 8.3, 7.5, 1.7 Hz, 1H), 7.54 (dd, J = 7.5, 1.7 Hz, 1H), 7.20 (dd, J = 8.5, 1.0 Hz, 1H), 7.13 (td, J = 7.5, 1.0 Hz, 1H), 3.73 (s, 3H); $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, $\text{DMSO}-d_6$): δ 158.4, 146.9, 144.7, 142.8, 132.7, 132.0, 129.0, 121.8, 120.1, 116.3, 111.0, 55.4; HRMS (ESI/FTICR+) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{12}\text{H}_{10}\text{ClN}_4\text{O}$ 261.05377; found 261.05389.

5-Chloro-3-(2-nitrophenyl)-[1,2,4]triazolo[4,3-a]pyrazine (45e). General Procedure C was applied using **S1** (1.04 g, 7.20 mmol, 1.0 equiv.) and 2-nitrobenzaldehyde (1.20 g, 7.92 mmol, 1.1 equiv.) to give **45e** as a grey solid (1.74 g, 6.29 mmol, 87%). mp 224–228 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ 9.57 (s, 1H), 8.44 (dd, J = 7.9, 1.6 Hz, 1H), 8.17 (s, 1H), 8.06 – 7.95 (m, 2H), 7.93 (dd, J = 7.1, 2.0 Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, $\text{DMSO}-d_6$): δ 148.0, 146.8, 143.8, 143.0, 134.5, 134.3, 132.9, 129.2, 125.0, 122.4, 121.4. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{11}\text{H}_6\text{ClN}_5\text{O}_2\text{Na}$ 298.01022; found 298.01092.

5-Chloro-3-(3,5-di-tert-butylphenyl)-[1,2,4]triazolo[4,3-a]pyrazine (45f). General Procedure C was applied using **S1** (1.05 g, 7.26 mmol, 1.0 equiv.) and 3,5-di-tert-butylbenzaldehyde (1.74 g, 7.99 mmol, 1.1 equiv.) to give **45f** as a grey solid (1.68 g, 4.90 mmol, 67%). mp 133–135 °C. ^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ 9.46 (s, 1H), 8.06 (s, 1H), 7.61 (t, J = 1.8 Hz, 1H), 7.56 (d, J = 1.8 Hz, 2H), 1.34 (s, 18H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, $\text{DMSO}-d_6$): δ 149.6, 148.1, 147.0, 142.7, 129.2, 126.3, 125.8, 123.6, 121.8, 34.6, 31.1. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{24}\text{ClN}_4$ 343.16840; found 343.16865.

3-(Anthracen-9-yl)-5-chloro-[1,2,4]triazolo[4,3-a]pyrazine (45g). General Procedure C was applied using **S1** (1.08 g, 7.47 mmol, 1.0 equiv.) and anthracene-9-carbaldehyde (1.69 g, 8.22 mmol, 1.1 equiv.) to give **45g** as a bright

yellow solid (1.62 g, 4.90 mmol, 66%). mp 218–221 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 9.64 (s, 1H), 8.96 (s, 1H), 8.24 (d, J = 8.4 Hz, 2H), 8.02 (s, 1H), 7.58 (ddd, J = 8.2, 6.6, 1.1 Hz, 2H), 7.49 (ddd, J = 8.8, 6.5, 1.2 Hz, 2H), 7.34 (dd, J = 8.7, 1.1 Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 147.8, 144.0, 143.4, 132.4, 130.6, 130.3, 129.2, 128.6, 127.5, 125.8, 125.5, 121.1, 120.5. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{12}\text{ClN}_4$ 331.07450; found 331.07449.

*5-Bromo-[1,2,4]triazolo[4,3-*a*]pyrazine (45h)*. General Procedure B was applied using **S2** (2.35 g, 12.4 mmol) to give **45h** as an orange solid (1.75 g, 8.81 mmol, 71%). mp 167–170 °C (decomp.) (lit.⁸ 214–217 °C). ^1H NMR (300 MHz, DMSO- d_6): δ 9.62 (s, 1H), 9.43 (s, 1H), 8.20 (s, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (75 MHz, DMSO- d_6): δ 145.3, 142.0, 137.5, 131.0, 109.9. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_5\text{H}_3^79\text{BrN}_4\text{Na}$ 220.94333; found 220.94309. The spectroscopic data were in agreement with the literature, but the melting point was significantly different.⁸

*5-Bromo-3-(4-methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (45i)*. General Procedure C was applied using **S2** (1.03 g, 5.46 mmol, 1.0 equiv.) and 4-methoxybenzaldehyde (0.818 g, 6.01 mmol, 1.1 equiv.) to give **45i** as a pale brown solid (1.00 g, 3.27 mmol, 60%). mp 156–157 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 9.44 (s, 1H), 8.10 (s, 1H), 7.66 – 7.57 (m, 2H), 7.13 – 7.06 (m, 2H), 3.86 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 160.8, 148.1, 146.6, 143.0, 133.1, 132.7, 119.1, 113.1, 110.2, 55.3. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{12}\text{H}_{10}^{79}\text{BrN}_4\text{O}$ 305.00325; found 305.00303.

*5-Bromo-3-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (45j)*. General Procedure C was applied using **S2** (0.65 g, 3.4 mmol, 1.0 equiv.) and 4-nitrobenzaldehyde (0.57 g, 3.8 mmol, 1.1 equiv.) to give **45j** as a yellow solid (0.93 g, 2.9 mmol, 85%). mp 200–205 °C (decomp.). ^1H NMR (500 MHz, DMSO- d_6): δ 9.54 (s, 1H), 8.41 (d, J = 8.0 Hz, 2H), 8.20 (s, 1H), 8.04 (d, J = 8.2 Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 148.7, 146.9, 146.4, 143.0, 133.9, 133.2, 133.0, 122.6, 110.4. HRMS (ESI/FTICR) m/z :

$[\text{M} + \text{H}]^+$ calcd for $\text{C}_{11}\text{H}_7^{79}\text{BrN}_5\text{O}_2$ 319.97776; found 319.97811.

*5-Bromo-3-(2-methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (45k)*. General Procedure C was applied using **S2** (0.66 g, 3.5 mmol, 1.0 equiv.) and 2-methoxybenzaldehyde (0.52 g, 3.8 mmol, 1.1 equiv.) to give **45k** as a white solid (0.75 g, 2.5 mmol, 71%). mp 137–139 °C. ^1H NMR (500 MHz, CDCl_3): δ 9.34 (s, 1H), 7.95 (s, 1H), 7.58 (ddd, J = 8.4, 7.5, 1.7 Hz, 1H), 7.54 (dd, J = 7.5, 1.7 Hz, 1H), 7.11 (td, J = 7.5, 1.0 Hz, 1H), 6.97 (dd, J = 8.4, 1.0 Hz, 1H), 3.73 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, CDCl_3): δ 159.2, 147.1, 146.6, 143.4, 133.1, 133.0, 132.5, 120.5, 116.3, 110.5, 110.1, 55.4. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{12}\text{H}_{10}^{79}\text{BrN}_4\text{O}$ 305.00325; found 305.00359.

*5-Bromo-3-(2-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (45l)*. General Procedure C was applied using **S2** (0.62 g, 3.3 mmol, 1.0 equiv.) and 2-nitrobenzaldehyde (0.54 g, 3.6 mmol, 1.1 equiv.) to give **45l** as a yellow solid (0.84 g, 2.6 mmol, 81%). mp 210–213 °C. ^1H NMR (200 MHz, CDCl_3): δ 9.41 (s, 1H), 8.50 – 8.36 (m, 1H), 8.00 (s, 1H), 7.94 – 7.80 (m, 2H), 7.72 (d, J = 6.6 Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 148.1, 146.4, 144.6, 143.2, 134.6, 134.2, 132.9, 132.7, 124.9, 122.6, 109.9. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{11}\text{H}_7^{79}\text{BrN}_5\text{O}_2$ 319.97776; found 319.97803.

*5-Iodo-[1,2,4]triazolo[4,3-*a*]pyrazine (45m)*. General Procedure B was applied using **S3** (1.54 g, 6.52 mmol, 1.0 equiv.) to give **45m** as a brown solid (1.08 g, 4.39 mmol, 67%, contains 0.5% DCM). mp 180–185 °C (decomp.). ^1H NMR (500 MHz, DMSO- d_6): δ 9.54 (s, 1H), 9.36 (s, 1H), 8.24 (s, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 144.4, 142.2, 140.2, 137.7, 83.9. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_5\text{H}_4\text{IN}_4$ 246.94752; found 246.94745.

*5-Iodo-3-(4-methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine (45n)*. General Procedure C was applied using **S3** (1.47 g, 6.21 mmol) and 4-methoxybenzaldehyde (0.930 g, 6.83 mmol, 1.1 equiv.) to give **45n** as an off-white solid (1.55 g, 4.39 mmol, 71%). mp 229–230 °C (decomp.). ^1H NMR (500 MHz, DMSO- d_6): δ 9.40 (s, 1H), 8.22 (s, 1H), 7.60 – 7.54 (m, 2H), 7.15 – 7.09 (m, 2H), 3.86 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR

(126 MHz, DMSO- d_6): δ 161.6, 149.5, 146.2, 143.9, 140.6, 134.4, 119.4, 113.6, 84.1, 55.8. HRMS (ESI/FTICR) m/z : $[M + H]^+$ calcd for $C_{12}H_{10}IN_4O$ 352.98938; found 352.98910.

*5-Phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine (46a)*. General Procedure D was applied using **45a** (107 mg, 0.692 mmol) and 2-phenylethanol (84.5 mg, 0.692 mmol, 1.0 equiv.). The reaction mixture was purified by FCC on silica using a gradient of EtOAc (20 to 100%) in hexanes to give **46a** as an off-white solid (125 mg, 0.520 mmol, 75%). mp 143–146 °C (decomp.). 1H NMR (500 MHz, DMSO- d_6): δ 9.38 (d, J = 0.7 Hz, 1H), 9.02 (t, J = 0.7 Hz, 1H), 7.63 (s, 1H), 7.43 – 7.37 (m, 2H), 7.35 – 7.28 (m, 2H), 7.26 – 7.19 (m, 1H), 4.63 (t, J = 6.7 Hz, 2H), 3.19 (t, J = 6.7 Hz, 2H). $^{13}C\{^1H\}$ NMR (126 MHz, DMSO- d_6): δ 145.8, 142.4, 137.3, 134.4, 133.0, 129.2, 128.4, 126.5, 108.3, 71.3, 34.4. HRMS (ESI/FTICR) m/z : $[M + H]^+$ calcd for $C_{13}H_{13}N_4O$ 241.10839; found 241.10813.

*5-(Phenethylthio)-[1,2,4]triazolo[4,3-*a*]pyrazine (46b)*. General Procedure D was applied using **45a** (105 mg, 0.681 mmol, 1.0 equiv.) and 2-phenylethane-1-thiol (94.2 mg, 0.681 mmol, 1.0 equiv.) to give **46b** as an off-white solid (88.6 mg, 0.346 mmol, 51%). mp 108–110 °C. 1H NMR (500 MHz, DMSO- d_6): δ 9.55 (d, J = 0.8 Hz, 1H), 9.33 (s, 1H), 8.03 (s, 1H), 7.27 – 7.20 (m, 4H), 7.20 – 7.12 (m, 1H), 3.48 (dd, J = 7.9, 7.0 Hz, 2H), 2.95 (t, J = 7.4 Hz, 2H). $^{13}C\{^1H\}$ NMR (126 MHz, DMSO- d_6): δ 144.6, 141.5, 139.0, 136.1, 131.5, 128.6, 128.2, 126.4, 126.1, 34.8, 33.5. HRMS (ESI/FTICR) m/z : $[M + H]^+$ calcd for $C_{13}H_{13}N_4S$ 257.08554; found 257.08534.

*3-(4-Methoxyphenyl)-5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine (46c)*. Preparation 1: General Procedure D was applied using **45b** (103 mg, 0.395 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (48.3 mg, 0.395 mmol, 1.0 equiv.) to give **46c** as a yellow solid (95.1 mg, 0.275 mmol, 69%). Preparation 2: General Procedure D was applied using **45i** (122 mg, 0.400 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (48.9 mg, 0.400 mmol, 1.0 equiv.). Fractions corresponding to the second peak were evaporated to give **46c** (first run: 45.4 mg, 0.127 mmol, 33%, second run: 43.0 mg, 0.124 mmol, 31%,

average yield is 32%). mp 162–163 °C. 1H NMR (500 MHz, DMSO- d_6): δ 9.00 (s, 1H), 7.67 – 7.60 (m, 2H), 7.55 (s, 1H), 7.22 – 7.12 (m, 3H), 7.07 – 7.01 (m, 2H), 6.96 – 6.88 (m, 2H), 4.48 (t, J = 6.4 Hz, 2H), 3.83 (s, 3H), 2.89 (t, J = 6.4 Hz, 2H). $^{13}C\{^1H\}$ NMR (126 MHz, DMSO- d_6): δ 160.4, 147.3, 146.3, 143.9, 137.4, 135.0, 132.2, 128.7, 128.2, 126.3, 120.0, 113.0, 108.6, 71.2, 55.3, 33.9. HRMS (ESI/FTICR) m/z : $[M + H]^+$ calcd for $C_{20}H_{19}N_4O_2$ 347.15025; found 347.14983.

*3-(4-Methoxyphenyl)-5-(phenethylthio)-[1,2,4]triazolo[4,3-*a*]pyrazine (46d)*. General Procedure D was applied using **45b** (100 mg, 0.384 mmol, 1.0 equiv.) and 2-phenylethane-1-thiol (53.0 mg, 0.384 mmol, 1.0 equiv.). The reaction mixture was purified by FCC on silica using a gradient of MeOH (0% to 10%) in DCM, fractions corresponding to the second peak were evaporated to give **46d** as a yellow solid (11.3 mg, 0.0312 mmol, 8%). mp 202–205 °C. 1H NMR (500 MHz, CD_3CN): δ 9.12 (s, 1H), 7.78 (s, 1H), 7.59 – 7.51 (m, 2H), 7.27 – 7.19 (m, 2H), 7.21 – 7.14 (m, 1H), 7.09 – 6.99 (m, 4H), 3.89 (s, 3H), 2.94 (t, J = 7.3 Hz, 2H), 2.72 (t, J = 7.3 Hz, 2H). $^{13}C\{^1H\}$ NMR (126 MHz, CD_3CN): δ 162.5, 149.2, 147.5, 142.8, 140.2, 134.1, 132.3, 129.59, 129.56, 129.4, 127.5, 120.7, 113.9, 56.2, 36.3, 35.1. HRMS (ESI/FTICR) m/z : $[M + H]^+$ calcd for $C_{20}H_{19}N_4OS$ 363.12741; found 363.12700.

*3-(4-Nitrophenyl)-5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine (46e)*. Preparation 1: General Procedure D was applied using **45c** (104 mg, 0.377 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (46.1 mg, 0.377 mmol, 1.0 equiv.). Fraction corresponding to the second peak were evaporated to give **46e** as a yellow solid (105 mg, 0.290 mmol, 77%). Preparation 2: General Procedure D was applied using **45j** (128 mg, 0.400 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (48.8 mg, 0.400 mmol, 1.0 equiv.) to give **46e** (first run: 85.6 mg, 0.237 mmol, 59%, second run: 87.8 mg, 0.243 mmol, 61%, average yield is 60%). m.p. 168–170 °C. 1H NMR (500 MHz, DMSO- d_6): δ 9.11 (s, 1H), 8.29 – 8.22 (m, 2H), 7.99 – 7.93 (m, 2H), 7.70 (s, 1H), 7.15 (dd, J = 5.0, 1.9 Hz, 3H), 6.95 (dd, J = 6.6, 2.9 Hz, 2H), 4.58 (t,

$J = 6.4$ Hz, 2H), 2.93 (t, $J = 6.4$ Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 147.9, 147.6, 144.6, 143.8, 137.1, 134.9, 134.1, 131.9, 128.4, 128.1, 126.3, 122.6, 109.3, 70.9, 33.5. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{16}\text{N}_5\text{O}_3$ 362.12477; found 362.12448.

3-(2-Methoxyphenyl)-5-phenethoxy-[1,2,4]-triazolo[4,3-*a*]pyrazine (**46f**). General Procedure D was applied using **45k** (122 mg, 0.400 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (48.8 mg, 0.400 mmol, 1.0 equiv.). Fractions corresponding to the second peak were combined and evaporated to give **46f** as a yellow solid (first run: 36.1 mg, 0.104 mmol, 26%, second run: 34.2 mg, 0.100 mmol, 25%, average yield is 26%). mp 147–150 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 9.02 (s, 1H), 7.62 (ddd, $J = 8.3, 7.5, 1.8$ Hz, 1H), 7.53 (s, 1H), 7.50 (dd, $J = 7.5, 1.8$ Hz, 1H), 7.22 (dd, $J = 8.4, 1.0$ Hz, 1H), 7.20 – 7.10 (m, 4H), 6.79 – 6.73 (m, 2H), 4.37 (s, 2H), 3.72 (s, 3H), 2.65 (s, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 158.2, 147.1, 143.9, 143.2, 137.2, 135.1, 132.0, 131.4, 128.8, 128.2, 126.3, 119.9, 117.4, 110.9, 108.8, 71.4, 55.4, 34.1. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{19}\text{N}_4\text{O}_2$ 347.15025; found 347.15041.

3-(2-Nitrophenyl)-5-phenethoxy-[1,2,4]-triazolo[4,3-*a*]pyrazine (**46g**). Preparation 1: General Procedure D was applied using **45e** (110 mg, 0.399 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (48.8 mg, 0.399 mmol, 1.0 equiv.). The reaction mixture was purified by FCC on silica using a gradient of EtOAc (30% to 100%) in hexanes, then MeOH (0% to 5%) in EtOAc to give **46g** as a yellow solid (first run: 123 mg, 0.341 mmol, 86%, second run: 113 mg, 0.313 mmol, 79%, average yield is 83%). Preparation 2: General Procedure D was applied using **45l** (128 mg, 0.400 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (48.8 mg, 0.400 mmol, 1.0 equiv.) to give **46g** (first run: 108 mg, 0.299 mmol, 75%, second run: 111 mg, 0.307 mmol, 77%, average yield is 76%). mp 178–181 °C (decomp.). ^1H NMR (500 MHz, DMSO- d_6): δ 9.13 (s, 1H), 8.37 (dd, $J = 8.1, 1.4$ Hz, 1H), 7.96 (td, $J = 7.5, 1.4$ Hz, 1H), 7.90 (td, $J = 7.8, 1.6$ Hz, 1H), 7.85 (dd, $J = 7.5, 1.6$ Hz, 1H), 7.63 (s, 1H), 7.18 – 7.11 (m,

3H), 6.83 – 6.76 (m, 2H), 4.38 (t, $J = 6.5$ Hz, 2H), 2.68 (t, $J = 6.4$ Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 147.7, 147.0, 143.1, 142.5, 136.7, 135.3, 134.0, 133.8, 131.9, 128.4, 128.2, 126.4, 124.7, 123.1, 109.1, 71.2, 33.6. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{16}\text{N}_5\text{O}_3$ 362.12477; found 362.12469.

3-(3,5-Di-*tert*-butylphenyl)-5-phenethoxy-[1,2,4]-triazolo[4,3-*a*]pyrazine (**46h**). General Procedure D was applied using **45f** (137 mg, 0.400 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (48.8 mg, 0.400 mmol, 1.0 equiv.). Fractions corresponding to the second peak were combined and evaporated to give **46h** as a yellow solid (first run: 139 mg, 0.325 mmol, 81%, second run: 140 mg, 0.326 mmol, 82%, average yield is 82%). mp 175–177 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 9.01 (s, 1H), 7.65 (t, $J = 1.9$ Hz, 1H), 7.57 (d, $J = 1.8$ Hz, 2H), 7.54 (s, 1H), 7.16 – 7.01 (m, 3H), 6.75 – 6.68 (m, 2H), 4.43 (t, $J = 6.3$ Hz, 2H), 2.76 (t, $J = 6.2$ Hz, 2H), 1.37 (s, 18H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 149.6, 147.2, 147.0, 143.9, 137.4, 135.0, 128.6, 128.1, 127.4, 126.3, 124.7, 123.7, 108.8, 71.4, 34.7, 34.1, 31.3. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{27}\text{H}_{32}\text{N}_4\text{ONa}$ 451.24683; found 451.24708.

3-(Anthracen-9-yl)-5-phenethoxy-[1,2,4]-triazolo[4,3-*a*]pyrazine (**46i**). General Procedure D was applied using **45g** (132 mg, 0.399 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (48.8 mg, 0.399 mmol, 1.0 equiv.). Fractions corresponding to the second peak were combined and evaporated to give **46i** as a yellow solid (first run: 110 mg, 0.264 mmol, 66%, second run: 107 mg, 0.258 mmol, 65%, average yield is 66%). mp 207–211 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 9.22 (s, 1H), 8.95 (s, 1H), 8.27 (d, $J = 8.5$ Hz, 2H), 7.62 (ddd, $J = 8.3, 6.6, 1.1$ Hz, 2H), 7.56 – 7.47 (m, 3H), 7.39 (dd, $J = 8.7, 1.1$ Hz, 2H), 6.96 – 6.89 (m, 1H), 6.78 – 6.70 (m, 2H), 6.08 – 6.03 (m, 2H), 3.94 (t, $J = 6.2$ Hz, 2H), 1.57 (t, $J = 6.2$ Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 147.7, 143.6, 142.4, 136.7, 135.7, 131.8, 130.5, 129.8, 128.5, 128.2, 127.7, 127.1, 126.0, 125.6, 125.5, 121.8, 109.2, 71.4, 33.3. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{21}\text{N}_4\text{O}$ 417.17099; found 417.17134.

*3-(4-Methoxyphenyl)-8-phenethoxy-[1,2,4]-triazolo[4,3-*a*]pyrazine (47a)*. Preparation 1: General Procedure D was applied using **45n** (132 mg, 0.375 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (45.8 mg, 0.375 mmol, 1.0 equiv.). Fraction corresponding to the first peak were evaporated to give **47a** as an off-white solid (70.0 mg, 0.202 mmol, 54%). Preparation 2: isolated from the same reaction as for **46c** preparation 2. Fractions corresponding to the first peak were evaporated to give **47a** (first run: 13.0 mg, 0.0375 mmol, 9%, second run: 15.5 mg, 0.0447 mmol, 11%, average yield is 10%). mp 208–211 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.17 – 8.12 (m, 1H), 7.88 – 7.81 (m, 2H), 7.47 – 7.42 (m, 1H), 7.40 – 7.29 (m, 4H), 7.27 – 7.23 (m, 1H), 7.22 – 7.15 (m, 2H), 4.76 (t, *J* = 6.8 Hz, 2H), 3.87 (s, 2H), 3.18 (t, *J* = 6.9 Hz, 2H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 160.8, 153.2, 147.9, 139.5, 138.0, 129.7, 128.9, 128.4, 127.2, 126.4, 118.1, 114.8, 112.1, 67.3, 55.4, 34.2. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₂₀H₁₉N₄O₂ 347.15025; found 347.14994.

*3-(4-Methoxyphenyl)-8-(phenethylthio)[1,2,4]-triazolo[4,3-*a*]pyrazine (47b)*. Preparation 1: General Procedure D was applied using **45i** (110 mg, 0.360 mmol) and 2-phenylethane-1-thiol (50.0 mg, 0.360 mmol, 1.0 equiv.) to give **47b** as a yellow solid (122 mg, 0.337 mmol, 93%). Preparation 2: General Procedure D was applied using **45n** (108 mg, 0.307 mmol, 1.0 equiv.) and 2-phenylethane-1-thiol (43.0 mg, 0.307 mmol, 1.0 equiv.) to give **47b** (14.0 mg, 0.0390 mmol, 13%). Preparation 3: isolated from the same reaction as for **46d**. Fractions corresponding to the first peak were evaporated to give **47b** (116 mg, 0.319 mmol, 83%). mp 192–194 °C. ¹H NMR (200 MHz, CDCl₃): δ 7.85 (d, *J* = 4.8 Hz, 1H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.69 (d, *J* = 4.8 Hz, 1H), 7.32 (d, *J* = 4.3 Hz, 4H), 7.11 (d, *J* = 8.8 Hz, 2H), 3.91 (s, 3H), 3.69 – 3.52 (m, 2H), 3.20 – 3.02 (m, 2H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 160.9, 152.9, 147.6, 143.7, 140.0, 129.8, 129.3, 128.6, 128.4, 126.4, 117.9, 114.8, 113.2, 55.5, 34.5, 29.4. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₂₀H₁₉N₄OS 363.12741;

found 363.12676.

*3-(4-Methoxyphenyl)-N-phenethyl-[1,2,4]-triazolo[4,3-*a*]pyrazin-8-amine (47c)*. Preparation 1: General Procedure E was applied using **45b** (106 mg, 0.407 mmol, 1.0 equiv.) and 2-phenylethan-1-amine (148 mg, 1.22 mmol, 3.0 equiv.) to give **47c** as a yellow solid (126 mg, 0.365 mmol, 90%). Preparation 2: General Procedure E was applied using **45i** (101 mg, 0.331 mmol, 1.0 equiv.) and 2-phenylethan-1-amine (120 mg, 0.993 mmol, 3.0 equiv.) to give **47c** (75.0 mg, 0.217 mmol, 66%). Preparation 3: General Procedure E was applied using **45n** (341 mg, 0.968 mmol) and 2-phenylethan-1-amine (350 mg, 2.91 mmol, 3.0 equiv.) to give **47c** (195 mg, 0.564 mmol, 58%). mp 193–196 °C. ¹H NMR (500 MHz, DMSO-*d*₆): δ 8.23 (t, *J* = 5.8 Hz, 1H), 7.86 – 7.80 (m, 2H), 7.69 (d, *J* = 4.8 Hz, 1H), 7.34 (d, *J* = 4.9 Hz, 1H), 7.32 – 7.25 (m, 4H), 7.21 (dd, *J* = 6.8, 2.1 Hz, 1H), 7.19 – 7.15 (m, 2H), 3.86 (s, 3H), 3.74 (q, *J* = 6.9 Hz, 2H), 2.98 (t, *J* = 7.5 Hz, 2H). ¹³C{¹H} NMR (126 MHz, DMSO-*d*₆): δ 160.6, 147.9, 147.6, 139.5, 139.4, 129.9, 129.5, 128.7, 128.3, 126.0, 118.5, 114.7, 105.9, 55.4, 41.6, 34.5. HRMS (ESI/FTICR) *m/z*: [M + H]⁺ calcd for C₂₀H₂₀N₅O 346.16624; found 346.16572.

*3-(3,5-Di-*tert*-butylphenyl)-8-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine (47d)*. Isolated from the same reaction as for **46h**. Fractions corresponding to the first peak were combined and evaporated to give **47d** as a yellow sticky solid (first run: 5.0 mg, 11.6 μmol, 3%, second run: 5.00 mg, 11.6 μmol, 3%, average yield is 3%). ¹H NMR (500 MHz, CDCl₃): δ 7.76 (d, *J* = 4.8 Hz, 1H), 7.65 – 7.60 (m, 3H), 7.39 – 7.27 (m, 5H), 7.24 (t, *J* = 7.2 Hz, 1H), 4.81 (t, *J* = 7.5 Hz, 2H), 3.28 (t, *J* = 7.5 Hz, 2H), 1.39 (s, 18H). ¹³C{¹H} NMR (126 MHz, CDCl₃): δ 154.4, 152.3, 149.8, 140.5, 137.7, 129.3, 128.7, 128.0, 126.8, 125.4, 125.1, 122.8, 110.8, 68.3, 35.3, 35.2, 31.5. HRMS (ESI/FTICR) *m/z*: [M + Na]⁺ calcd for C₂₇H₃₂N₄ONa 451.24683; found 451.24714.

*3-(Anthracen-9-yl)-8-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine (47e)*. Isolated from the same reaction as for **46i**. Fractions corresponding to the first peak were combined and

evaporated to give **47e** as a yellow solid (first run: 2.50 mg, 6.00 μ mol, 2%, second run: 3.00 mg, 7.20 μ mol, 2%, average yield is 2%). mp 175–180 °C (decomp.). ^1H NMR (500 MHz, DMSO- d_6): δ 9.00 (s, 1H), 8.28 (d, J = 8.5 Hz, 2H), 7.74 – 7.68 (m, 1H), 7.61 (ddd, J = 8.3, 6.6, 1.1 Hz, 2H), 7.51 (ddd, J = 8.9, 6.6, 1.3 Hz, 2H), 7.47 – 7.42 (m, 2H), 7.41 – 7.34 (m, 4H), 7.32 (d, J = 4.8 Hz, 1H), 7.30 – 7.23 (m, 2H), 4.83 (t, J = 6.8 Hz, 2H), 3.25 (t, J = 6.8 Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 153.4, 145.4, 141.8, 141.4, 140.0, 138.1, 131.0, 130.83, 130.79, 129.3, 129.0, 128.9, 128.4, 127.8, 127.5, 126.5, 125.9, 125.6, 124.6, 118.1, 111.8, 67.5, 34.4. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{27}\text{H}_{21}\text{N}_4\text{O}$ 417.17099; found 417.17089.

*3-(2-Methoxyphenyl)-8-phenethoxy-[1,2,4]-triazolo[4,3-*a*]pyrazine (47f)*. Isolated from the same reaction as for **46f**. Fractions corresponding to the first peak were combined and evaporated to give **47f** as a white solid (first run: 13.1 mg, 37.8 μ mol, 9%, second run: 12.2 mg, 35.2 μ mol, 9%, average yield is 9%). mp 124–128 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 7.71 (d, J = 4.8 Hz, 1H), 7.65 (ddd, J = 8.5, 7.4, 1.8 Hz, 1H), 7.59 (dd, J = 7.5, 1.8 Hz, 1H), 7.42 (d, J = 4.9 Hz, 1H), 7.40 – 7.37 (m, 2H), 7.36 – 7.28 (m, 3H), 7.27 – 7.20 (m, 1H), 7.18 (td, J = 7.4, 0.9 Hz, 1H), 4.76 (t, J = 6.9 Hz, 2H), 3.82 (s, 3H), 3.19 (t, J = 6.9 Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 156.9, 152.9, 146.7, 139.4, 138.0, 132.7, 131.9, 128.9, 128.4, 126.5, 126.4, 120.9, 114.2, 113.3, 112.1, 67.3, 55.6, 34.2. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{20}\text{H}_{18}\text{N}_4\text{O}_2\text{Na}$ 369.13220; found 369.13255.

*3-(4-Methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-8-ol (48a)*. General Procedure D was applied (with following modification: H_2O was used as a solvent) using **45i** (107 mg, 0.341 mmol) and 2-phenylethan-1-ol (41.7 mg, 0.341 mmol, 1.0 equiv.). The reaction mixture was purified by FCC on silica using a gradient of MeOH (0% to 20%) in EtOAc to give **48a** as a pale brown solid (80.0 mg, 0.330 mmol, 94%). mp 312–316 °C (decomp.). ^1H NMR (500 MHz, DMSO- d_6): δ 11.42 (s, 1H), 7.81 – 7.74 (m, 2H), 7.39 (d, J = 5.7 Hz, 1H), 7.21 –

7.14 (m, 2H), 6.89 (d, J = 5.8 Hz, 1H), 3.86 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 160.9, 153.0, 149.2, 145.0, 129.9, 118.4, 117.9, 114.8, 103.8, 55.4. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{12}\text{H}_{10}\text{N}_4\text{O}_2\text{Na}$ 265.06960; found 265.06955.

*3-(4-Nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-8-ol (48b)*. General Procedure D was applied using **45j** (128 mg, 0.400 mmol, 1.0 equiv.) and 2-phenylethan-1-ol (48.8 mg, 0.400 mmol, 1.0 equiv.). The reaction mixture was purified by FCC on silica using a gradient of EtOAc (30% to 100%) in hexanes, then MeOH (0% to 20%) in EtOAc, fractions corresponding to the third peak were combined and evaporated to give **48b** as a yellow solid (first run: 28.8 mg, 0.112 mmol, 28%, second run: 31.9 mg, 0.124 mmol, 31%, average yield is 30%). mp 207–210 °C (decomp.). ^1H NMR (500 MHz, DMSO- d_6): δ 8.48 – 8.36 (m, 2H), 8.20 – 8.14 (m, 2H), 7.41 (d, J = 4.7 Hz, 1H), 7.10 (d, J = 4.7 Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 159.9, 147.4, 146.3, 144.6, 133.7, 132.7, 128.3, 124.3, 99.8. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{11}\text{H}_7\text{N}_5\text{O}_3\text{Na}$ 280.04411; found 280.04439.

*3-(2-Methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-8-ol (48c)*. Isolated from the same reaction as for **46f**. Fractions corresponding to the third peak were combined and evaporated to give **48c** as a yellow solid (first run: 47.6 mg, 0.197 mmol, 49%, second run: 46.3 mg, 0.191 mmol, 48%, average yield is 49%). mp 116–119 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 11.45 (s, 1H), 7.65 (ddd, J = 8.9, 7.4, 1.8 Hz, 1H), 7.56 (dd, J = 7.5, 1.7 Hz, 1H), 7.29 (dd, J = 8.5, 0.9 Hz, 1H), 7.17 (td, J = 7.5, 1.0 Hz, 1H), 7.00 (d, J = 5.7 Hz, 1H), 6.86 (d, J = 5.8 Hz, 1H), 3.83 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 156.9, 152.9, 147.9, 144.9, 132.8, 131.9, 120.9, 117.7, 114.2, 112.1, 104.8, 55.7. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{12}\text{H}_{10}\text{N}_4\text{O}_2\text{Na}$ 265.06960; found 265.06995.

*3-(2-Nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-8-ol (48d)*. Isolated from the same reaction as for **46g** preparation 2. Fractions corresponding to the third peak were combined and evaporated to give **48d** as an orange solid (first run: 11.4 mg, 44.3 μ mol, 11%, second run: 13.8

mg, 53.7 μ mol, 13%, average yield is 12%). mp 124–127 °C (decomp.). ^1H NMR (500 MHz, DMSO- d_6): δ 11.65 (s, 1H), 8.37 (dd, J = 8.1, 1.3 Hz, 1H), 8.02 (td, J = 7.5, 1.4 Hz, 1H), 7.97 (td, J = 7.8, 1.6 Hz, 1H), 7.87 (dd, J = 7.5, 1.6 Hz, 1H), 7.20 (d, J = 5.6 Hz, 1H), 6.93 (d, J = 5.7 Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 152.7, 148.1, 146.2, 144.9, 134.6, 133.1, 132.7, 125.4, 119.9, 118.9, 103.7. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{11}\text{H}_7\text{N}_5\text{O}_3\text{Na}$ 280.04411; found 280.04426.

*3-(4-Methoxyphenyl)-[1,2,4]-triazolo[4,3-*a*]pyrazine (49)*. Preparation 1: General Procedure E was applied using **45n** (341 mg, 0.968 mmol, 1.0 equiv.) and 2-phenylethan-1-amine (352 mg, 2.91 mmol, 3.0 equiv.). Fractions corresponding to the second peak were re-purified by RP-FCC on C18 using a gradient of MeOH (5% to 80%) in H_2O . Fractions corresponding to the first peak were combined and evaporated to give **49** as a white solid (24.0 mg, 0.106 mmol, 11%). Preparation 2: General Procedure D was applied using **45n** (108 mg, 0.307 mmol, 1.0 equiv.) and 2-phenylethane-1-thiol (43.0 mg, 0.307 mmol, 1.0 equiv.). The reaction mixture was purified by FCC on silica using a gradient of MeOH (0% to 10%) in DCM, fractions corresponding to the second peak were evaporated to give **49** as a yellow solid (51.0 mg, 0.225 mmol, 74%). mp 202–205 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 9.45 (d, J = 1.6 Hz, 1H), 8.57 (dd, J = 4.9, 1.6 Hz, 1H), 7.94 (d, J = 4.9 Hz, 1H), 7.93 – 7.87 (m, 2H), 7.23 – 7.16 (m, 2H), 3.87 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 160.9, 146.5, 145.5, 144.1, 129.8, 129.7, 117.8, 116.9, 114.8, 55.4. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{12}\text{H}_{10}\text{N}_4\text{ONa}$ 249.07468; found 249.07465.

3-(4-Methoxyphenyl)-5-(1-phenethyl-1H-imidazol-2-yl)-4H-1,2,4-triazole (50). Isolated from the same reaction as for **49** preparation 1. Fractions corresponding to the second peak, after RP-FCC were combined and evaporated to give **50** as a white solid (57.0 mg, 0.165 mmol, 17%). mp 143–146 °C. ^1H NMR (500 MHz, CD_3OD): δ 8.00 (d, J = 8.8 Hz, 2H), 7.29 – 7.17 (m, 2H), 7.19 – 7.12 (m, 4H), 7.07 (d, J = 8.8 Hz, 2H), 7.05 (s, 1H), 4.78 (t, J = 7.4 Hz, 2H), 3.87 (s, 3H), 3.12 (t, J = 7.3

Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, CD_3OD): δ 162.9, 139.3, 129.9, 129.5, 129.1, 127.7, 124.0, 115.4, 55.9, 50.0, 38.6. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{20}\text{N}_5\text{O}$ 346.16624; found 346.16560. X-ray single crystal data can be found in the supporting information.

*5-(Piperidin-1-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (55)*. General Procedure E was applied using **45a** (101 mg, 0.652 mmol, 1.0 equiv.) in toluene (10 mL) and piperidine (167 mg, 1.96 mmol, 3.0 equiv.) and heated at reflux for 72 h. The reaction mixture was purified by FCC on silica using a gradient of EtOAc (50% to 100%) in hexanes, then MeOH (0% to 5%) in EtOAc. Fractions corresponding to the second peak were evaporated to give **55** as an orange crystalline solid (20.7 mg, 0.102 mmol, 16%). mp 158–161 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 9.39 (d, J = 0.8 Hz, 1H), 9.05 (d, J = 0.7 Hz, 1H), 7.48 (s, 1H), 3.23 – 3.05 (m, 4H), 1.76 (p, J = 5.8 Hz, 4H), 1.67 – 1.58 (m, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 145.6, 138.4, 135.7, 134.5, 116.4, 50.2, 25.0, 23.6. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{10}\text{H}_{14}\text{N}_5$ 204.12437; found 204.12426.

*8-(Piperidin-1-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine (56)*. Isolated from the same reaction as for **55**. Fractions corresponding to the first peak were evaporated to give **56** as an orange crystalline solid (93.4 mg, 0.460 mmol, 71%). mp 181–183 °C. ^1H NMR (500 MHz, CD_3CN): δ 8.82 (s, 1H), 7.56 (d, J = 4.5 Hz, 1H), 7.26 (d, J = 4.6 Hz, 1H), 4.25 (t, J = 5.4 Hz, 4H), 1.78 – 1.70 (m, 2H), 1.66 (dd, J = 7.6, 3.9 Hz, 4H). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, CD_3CN): δ 148.8, 141.5, 138.3, 129.9, 108.3, 48.1, 26.9, 25.5. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{10}\text{H}_{14}\text{N}_5$ 204.12437; found 204.12412. X-ray single crystal data can be found in the supporting information.

*5-(Piperidin-1-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine-3-*d* (57)*. General Procedure E was applied using **45a** (101 mg, 0.652 mmol, 1.0 equiv.) and piperidine (167 mg, 1.96 mmol, 3.0 equiv.) in D_2O (5 mL). The reaction mixture was heated at reflux for 72 h and purified by FCC on silica using a gradient of EtOAc (50% to 100%) in hexanes then MeOH (0% to 5%) in EtOAc. Fractions corresponding to the

second peak were evaporated to give **57** as an orange crystalline solid (31.1 mg, 0.153 mmol, 23%). mp 158–161 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 9.05 (s, 1H), 7.48 (s, 1H), 3.19 – 3.13 (m, 4H), 1.75 (p, J = 5.7 Hz, 4H), 1.66 – 1.58 (m, 2H). ^2H NMR (77 MHz, DMSO- d_6): δ 9.44 (s, 1D). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 145.6, 138.3, 135.7, 134.7 – 133.9 (m), 116.3, 50.2, 25.0, 23.6. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{10}\text{H}_{13}\text{DN}_5$ 205.13065; found 205.13038.

8-(Piperidin-1-yl)-[1,2,4]triazolo[4,3-a]pyrazine-3,5-d₂ (**58**). Isolated from the same reaction as for **57**. Fractions corresponding to the first peak were evaporated to give **58** as an orange crystalline solid (78.7 mg, 0.387 mmol, 59%). mp 181–183 °C. ^1H NMR (500 MHz, DMSO- d_6): δ 7.31 (s, 1H), 4.21 (t, J = 5.5 Hz, 4H), 1.68 (tt, J = 6.4, 2.4 Hz, 2H), 1.61 (tq, J = 8.4, 5.3, 4.2 Hz, 4H). ^2H NMR (77 MHz, DMSO- d_6): δ 9.28 (s, 1D), 7.88 (s, 1D). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, DMSO- d_6): δ 147.1, 139.6, 137.86 – 137.14 (m), 128.6, 107.41 (t, J = 29.3 Hz), 46.6, 25.7, 24.2. HRMS (ESI/FTICR) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{10}\text{H}_{12}\text{D}_2\text{N}_5$ 206.13693; found 206.13662.

5-Chloro-[1,2,4]triazolo[4,3-a]pyrazine-3-d (**59**). Compound **45a** (227 mg, 1.47 mmol) was stirred in D_2O (5 mL) at 80 °C for 2 days. The solvent was evaporated and the reaction mixture was purified by FCC on silica using a gradient of EtOAc (20 to 100%) in hexanes to give **59** as a white solid (197 mg, 1.27 mmol, 86%). mp 170–173 °C. ^1H NMR (500 MHz, CDCl_3): δ 9.30 (s, 1H), 7.95 (s, 1H). ^2H NMR (77 MHz, CDCl_3): δ 9.10 (s, 1D). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, CDCl_3): δ 145.9, 142.0, 134.96 – 134.04 (m), 128.4, 121.3. LRMS (ESI/IT) m/z : $[\text{M} + \text{H}]^+$ 156.0.

8-(Piperidin-1-yl)-[1,2,4]triazolo[4,3-a]pyrazine-3-d (**60**). **56** (10 mg, 49 μmol) was dissolved in D_2O (5 mL) and heated at reflux for 72 h. Solvent was evaporated to give **60** as an orange solid (10 mg, 49 μmol , 100%). mp 181–183 °C. ^1H NMR (500 MHz, CDCl_3): δ 7.37 (d, J = 4.5 Hz, 1H), 7.31 (d, J = 4.5 Hz, 1H), 4.30 (s, 4H), 1.72 (d, J = 7.5 Hz, 6H). ^2H NMR (77 MHz, CDCl_3): δ 8.75 (s, 1D). $^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, CDCl_3): δ 148.1, 140.7, 137.1 –

136.1 (m), 130.0, 106.0, 47.6, 26.4, 24.9. LRMS (ESI/IT) m/z : $[\text{M} + \text{Na}]^+$ 227.1.

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Supporting Information Available

The Supporting Information is available free of charge on the ACS Publications website at DOI:

The following files are available free of charge.

- ORTEP diagrams for the X-ray structures and crystal data; experimental details for biological activity evaluations and copies of ^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of novel compounds. (PDF)
- The archive of laboratory notebook with all experiments described in the article and raw NMR data for all novel compounds. (ZIP)
- The KINOMEScan[®] assay report on the biological activity of compounds **46d** and **47b**. (XLS)
- X-ray crystal data of **45a**, **50**, **56**. (CIF)
- The structural information in strings format for all compounds described in the article with reference codes to the laboratory notebook. (XLS)

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Supporting information

Tele-substitution Reactions in the Synthesis of a Promising Class of Antimalarials

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Contents

S1 Biological activity experimental details	S3
S1.1 <i>In Vitro</i> Antiplasmodial Activity	S3
S1.2 KINOMEScan [®] Assay	S3
S2 General synthetic route to make studied triazolopyrazines	S4
S3 Mechanism of transformation that leads to the ring opening product 50	S4
S4 NMR spectra of compounds	S4
2-Bromo-6-hydrazinylpyrazine S2	S5
2-Iodo-6-hydrazinylpyrazine S3	S6
2-Chloro-3-hydrazinylpyrazine S4	S7
2-Chloro-5-hydrazinylpyrazine S5	S7
5-Chloro-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 16	S9
3-(4-(Difluoromethoxy)phenyl)-5-(phenethylthio)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 17	S10
3-(4-(Difluoromethoxy)phenyl)-8-(phenethylthio)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 18	S11
8-Chloro-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 25	S12
8-Chloro-3-phenyl-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 26	S13
8-Chloro-3-(4-nitrophenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 27	S14
8-Phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 28	S15
8-(Phenethylthio)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 29	S16
<i>N</i> -Phenethyl-[1,2,4]triazolo[4,3- <i>a</i>]pyrazin-8-amine 30	S17
8-Phenethoxy-3-phenyl-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 31	S18
8-(Phenethylthio)-3-phenyl-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 32	S19
<i>N</i> -Phenethyl-3-phenyl-[1,2,4]triazolo[4,3- <i>a</i>]pyrazin-8-amine 33	S20
3-(4-Nitrophenyl)-8-phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 34	S21
3-(4-Nitrophenyl)-8-(phenethylthio)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 35	S22
3-(4-Nitrophenyl)- <i>N</i> -phenethyl-[1,2,4]triazolo[4,3- <i>a</i>]pyrazin-8-amine 36	S23
6-Chloro-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 37	S24
6-Chloro-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 38	S25
2-Chloro-6-phenethoxypyrazine 42	S26
2-Bromo-6-phenethoxypyrazine 43	S27
2-Iodo-6-phenethoxypyrazine 44	S28
5-Chloro-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45a	S29
5-Chloro-3-(4-methoxyphenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45b	S31
5-Chloro-3-(4-nitrophenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45c	S32
5-Chloro-3-(2-methoxyphenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45d	S33
5-Chloro-3-(2-nitrophenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45e	S34
5-Chloro-3-(3,5-di- <i>tert</i> -butylphenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45f	S35
3-(Anthracen-9-yl)-5-chloro-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45g	S36
5-Bromo-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45h	S37
5-Bromo-3-(4-methoxyphenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45i	S38
5-Bromo-3-(4-nitrophenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45j	S39
5-Bromo-3-(2-methoxyphenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45k	S40
5-Bromo-3-(2-nitrophenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45l	S41
5-Iodo-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45m	S42
5-Iodo-3-(4-methoxyphenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 45n	S43

5-Phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 46a	S44
5-(Phenethylthio)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 46b	S45
3-(4-Methoxyphenyl)-5-phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 46c	S46
3-(4-Methoxyphenyl)-5-(phenethylthio)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 46d	S47
3-(4-Nitrophenyl)-5-phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 46e	S48
3-(2-Methoxyphenyl)-5-phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 46f	S49
3-(2-Nitrophenyl)-5-phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 46g	S50
3-(3,5-Di- <i>tert</i> -butylphenyl)-5-phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 46h	S51
3-(Anthracen-9-yl)-5-phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 46i	S52
3-(4-Methoxyphenyl)-8-phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 47a	S53
3-(4-Methoxyphenyl)-8-(phenethylthio)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 47b	S54
3-(4-Methoxyphenyl)- <i>N</i> -phenethyl-1,2,4]triazolo[4,3- <i>a</i>]pyrazin-8-amine 47c	S55
3-(3,5-Di- <i>tert</i> -butylphenyl)-8-phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 47d	S56
3-(Anthracen-9-yl)-8-phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 47e	S57
3-(2-Methoxyphenyl)-8-phenethoxy-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 47f	S58
3-(4-Methoxyphenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazin-8-ol 48a	S59
3-(4-Nitrophenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazin-8-ol 48b	S60
3-(2-Methoxyphenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazin-8-ol 48c	S61
3-(2-Nitrophenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazin-8-ol 48d	S62
3-(4-Methoxyphenyl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 49	S63
3-(4-Methoxyphenyl)-5-(1-phenethyl-1H-imidazol-2-yl)-4H-1,2,4-triazole 50	S64
5-(Piperidin-1-yl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 55	S65
8-(Piperidin-1-yl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine 56	S66
5-(Piperidin-1-yl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine-3- <i>d</i> 57	S67
8-(Piperidin-1-yl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine-3,5- <i>d</i> ₂ 58	S69
5-Chloro-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine-3- <i>d</i> 59	S71
8-(Piperidin-1-yl)-[1,2,4]triazolo[4,3- <i>a</i>]pyrazine-3- <i>d</i> 60	S73
S5 X-ray structures	S75
S5.1 Crystal structure determination experimental parameters	S75
S5.2 X-ray data and structure for 45a	S76
S5.3 X-ray data and structure for 50	S78
S5.4 X-ray data and structure for 56	S80
References	S82

S1 Biological activity experimental details

S1.1 *In Vitro* Antiplasmodial Activity

Evaluation of in vitro Antiplasmodial Activity was conducted at Drug Discovery Unit, University of Dundee following literature procedure.¹ Cultures of the widely-used malaria reference strain of chloroquine-sensitive *P. falciparum* strain 3D7 were maintained in a 5% suspension of human red blood cells cultured in RPMI 1640 medium supplemented with 0.5% Albumax II (available from Gibco Life Technologies, San Diego, CA, cat. no. 11021-037), 12 mM sodium bicarbonate, 0.2 mM hypoxanthine, (pH 7.3), and 20 mg/L gentamicin at 37 °C, in a humidified atmosphere of 1% O₂, 3% CO₂ with a gas balance of nitrogen. Growth inhibition of the *P. falciparum* cultures was quantified in a 10-point dose response curve with a 1 in 3 dilution series from a top assay concentration of 50 µM. This 384 well plate based fluorescence assay utilises the binding of SYBRgreen I (Thermo Fisher Scientific/Invitrogen cat. no. S7585) to double stranded DNA, which greatly increases the fluorescent signal at 528 nm after excitation at 485 nm. Mefloquine was used as a drug control to monitor the quality of the assay ($Z' = 0.6$ to 0.8 , where Z' is a measure of the discrimination between the positive and negative controls on a screen plate). Dose-response curves were determined from a minimum of 3 independent experiments. Compound bioactivity was expressed as IC₅₀, the concentration of compound causing 50% inhibition. IC₅₀ values were determined from a minimum of 3 independent experiments. All data was processed using IDBS ActivityBase[®] raw data was converted into per cent inhibition through linear regression by setting the high inhibition control as 100% and the no inhibition control as 0%. Quality control criteria for passing plates were as follows: $Z' > 0.5$, S:B > 3, % CV (no inhibition control) < 15. The formula used to calculate $Z' = 1 - (3(StDev_{high} + StDev_{low})) / (ABS(Mean_{high} - Mean_{low}))$. All IC₅₀ curve fitting was undertaken using XLFit version 4.2 using Model 205 with the following 4 parametric equation: $y = A + (B - A) / (1 + (C/x)^D)$, where A = % inhibition at bottom, B = % inhibition at top, C = IC₅₀, D = slope, x = inhibitor concentration and y = % inhibition. If curve did not reach 100% of inhibition, B was fixed to 100 only when at least 50% of inhibition was reached.

S1.2 KINOMEscan[®] Assay

Evaluation was performed following literature procedure.² All compounds were dissolved and stored at -20 °C as 10 mM DMSO stocks. Compounds were screened at a single concentration of 1 µM using binding assays as described previously.^{3,4} Briefly, extracts containing kinases (expressed either as T7 phage fusion proteins or DNA-tagged) are incubated with an immobilized bait compound (known inhibitor like staurosporine) and the test compound (at 1 µM). Test compounds that displace the immobilized bait compound are detected by quantitative PCR. The results are expressed as the percentage of kinase bound to the bait compound bead relative to a DMSO control (% control). Compounds with high affinity have % control of 0–1 whereas compounds with lower affinity have higher scores.

S2 General synthetic route to make studied triazolopyrazines

Figure S1: Synthetic pathway for studied [1,2,4]triazolo[4,3-a]pyrazines.

S3 Mechanism of transformation that leads to the ring opening product 50

Figure S2: Detailed mechanism of transformation that leads to the ring opening product **50**. This mechanism involves a 5-endo-trig cyclisation though this type of cyclisation is disfavoured according to Baldwin's rules.⁵ Examples of disfavoured reactions are known and were reported by Baldwin.⁶ Such cyclisations can be made more feasible if they involve stabilisation of an intermediate anion (on one of the carbons of the ring double bond after the first step) by adjacent groups, such as the C-I sigma star ("exocyclic hyperconjugation") or by virtue of the charge being part of an extended aromatic system involving the adjacent ring.⁷ As for other apparent violations of Baldwin's rules, there is no competing ring closure pathway available.

S4 NMR spectra of compounds

2-Bromo-6-hydrazinylpyrazine S2

¹H NMR (CDCl₃, 500.13 MHz)
1D

¹³C NMR (CDCl₃, 125.77 MHz)
1D

2-Iodo-6-hydrazinylpyrazine S3

¹H NMR (DMSO, 300.13 MHz)
1D

¹³C NMR (DMSO, 75.48 MHz)
1D

2-Chloro-3-hydrazinylpyrazine S4

2-Chloro-5-hydrazinylpyrazine S5

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

5-Chloro-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine 16

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

3-(4-(Difluoromethoxy)phenyl)-5-(phenethylthio)-[1,2,4]triazolo[4,3-*a*]-pyrazine 17

3-(4-(Difluoromethoxy)phenyl)-8-(phenethylthio)-[1,2,4]triazolo[4,3-*a*]-pyrazine 18

8-Chloro-[1,2,4]triazolo[4,3-*a*]pyrazine 25

8-Chloro-3-phenyl-[1,2,4]triazolo[4,3-a]pyrazine 26

8-Chloro-3-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine 27

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

8-Phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 28

8-(Phenethylthio)-[1,2,4]triazolo[4,3-*a*]pyrazine 29

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

N-Phenethyl-[1,2,4]triazolo[4,3-*a*]pyrazin-8-amine 30

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

8-Phenethoxy-3-phenyl-[1,2,4]triazolo[4,3-*a*]pyrazine 31

8-(Phenethylthio)-3-phenyl-[1,2,4]triazolo[4,3-*a*]pyrazine 32

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

N-Phenethyl-3-phenyl-[1,2,4]triazolo[4,3-*a*]pyrazin-8-amine 33

3-(4-Nitrophenyl)-8-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 34

3-(4-Nitrophenyl)-8-(phenethylthio)-[1,2,4]triazolo[4,3-*a*]pyrazine 35

3-(4-Nitrophenyl)-*N*-phenethyl-[1,2,4]triazolo[4,3-*a*]pyrazin-8-amine 36

6-Chloro-[1,2,4]triazolo[4,3-*a*]pyrazine 37

6-Chloro-3-(4-(difluoromethoxy)phenyl)-[1,2,4]triazolo[4,3-a]pyrazine 38

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

2-Chloro-6-phenethoxypyrazine 42

2-Bromo-6-phenethoxypyrazine 43

2-Iodo-6-phenethoxypyrazine 44

5-Chloro-[1,2,4]triazolo[4,3-*a*]pyrazine 45a

190403_mkor7275_50041251615.1.fid
1H NMR (CDCl₃, 500.13 MHz)
1D

190403_mkor7275_50041251615.4.fid
13C NMR (CDCl₃, 125.77 MHz)
1D

5-Chloro-3-(4-methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine 45b

¹H NMR (CDCl₃, 200.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

5-Chloro-3-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine 45c

5-Chloro-3-(2-methoxyphenyl)-[1,2,4]triazolo[4,3-a]pyrazine 45d

5-Chloro-3-(2-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine 45e

5-Chloro-3-(3,5-di-*tert*-butylphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine 45f

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

3-(Anthracen-9-yl)-5-chloro-[1,2,4]triazolo[4,3-*a*]pyrazine 45g

5-Bromo-[1,2,4]triazolo[4,3-*a*]pyrazine 45h

5-Bromo-3-(4-methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine 45i

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

5-Bromo-3-(4-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine 45j

5-Bromo-3-(2-methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine 45k

5-Bromo-3-(2-nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine 45l

5-Iodo-[1,2,4]triazolo[4,3-*a*]pyrazine 45m

5-Iodo-3-(4-methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine 45n

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

5-Phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 46a

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

5-(Phenethylthio)-[1,2,4]triazolo[4,3-*a*]pyrazine 46b

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

3-(4-Methoxyphenyl)-5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 46c

3-(4-Methoxyphenyl)-5-(phenethylthio)-[1,2,4]triazolo[4,3-*a*]pyrazine 46d

3-(4-Nitrophenyl)-5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 46e

3-(2-Methoxyphenyl)-5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 46f

3-(2-Nitrophenyl)-5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 46g

3-(3,5-Di-*tert*-butylphenyl)-5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 46h

3-(Anthracen-9-yl)-5-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 46i

3-(4-Methoxyphenyl)-8-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 47a

3-(4-Methoxyphenyl)-8-(phenethylthio)-[1,2,4]triazolo[4,3-*a*]pyrazine 47b

¹H NMR (CDCl₃, 200.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

3-(4-Methoxyphenyl)-*N*-phenethyl-[1,2,4]triazolo[4,3-*a*]pyrazin-8-amine 47c

3-(3,5-Di-*tert*-butylphenyl)-8-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 47d

3-(Anthracen-9-yl)-8-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 47e

3-(2-Methoxyphenyl)-8-phenethoxy-[1,2,4]triazolo[4,3-*a*]pyrazine 47f

3-(4-Methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-8-ol 48a

3-(4-Nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-8-ol 48b

3-(2-Methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-8-ol 48c

3-(2-Nitrophenyl)-[1,2,4]triazolo[4,3-*a*]pyrazin-8-ol 48d

3-(4-Methoxyphenyl)-[1,2,4]triazolo[4,3-*a*]pyrazine 49

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

50

5-(Piperidin-1-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine 55

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

8-(Piperidin-1-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine 56

5-(Piperidin-1-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine-3-*d* 57

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

2H NMR (DMSO, 76.77 MHz)
1D

8-(Piperidin-1-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine-3,5-*d*₂ 58

¹H NMR (DMSO, 500.13 MHz)
1D

¹³C NMR (DMSO, 125.77 MHz)
1D

²H NMR (DMSO, 76.77 MHz)
1D

5-Chloro-[1,2,4]triazolo[4,3-*a*]pyrazine-3-*d* 59

8-(Piperidin-1-yl)-[1,2,4]triazolo[4,3-*a*]pyrazine-3-*d* 60

2H NMR (CDCl₃, 76.77 MHz)
1D

S5 X-ray structures

S5.1 Crystal structure determination experimental parameters

Single crystals of samples were obtained by slow evaporation from the corresponding solvent, which is specified for each sample below. A suitable crystal was selected and attached with Paratone to a nylon loop. A SuperNova Dual equipped with an Atlas detector and employing mirror monochromated Cu ($K\alpha$) radiation from a micro-source was used for the X-ray data collections. The crystal was kept at 100(2) K during data collection. Using Olex2,⁸ the structure was solved with the Olex2.solve⁹ structure solution program using Charge Flipping and refined with ShelXL¹⁰ and ShelXT¹¹ refinement packages using Least Squares minimisation. Full information on crystal structure available free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif using corresponding CCDC numbers provided for each compound.

S5.2 X-ray data and structure for 45a

Table S1: Crystal data and structure refinement for **45a**

Crystallisation method	Slow evaporation from EtOAc solution
Empirical formula	C ₅ H ₃ N ₄ Cl
Formula weight	154.56
Temperature/K	100(2)
Crystal system	monoclinic
Space group	P2 ₁ /m
a/Å	6.9527(2)
b/Å	6.30259(16)
c/Å	7.5416(2)
$\alpha/^\circ$	90
$\beta/^\circ$	114.963(4)
$\gamma/^\circ$	90
Volume/Å ³	299.600(17)
Z	2
$\rho_{\text{calc}}/\text{g}/\text{cm}^3$	1.713
μ/mm^{-1}	4.929
F(000)	156.0
Crystal size/mm ³	0.249 × 0.072 × 0.065
Radiation	CuK α (λ = 1.54184)
2 θ range for data collection/ $^\circ$	12.948 to 151.992
Index ranges	-8 ≤ h ≤ 8, -7 ≤ k ≤ 7, -8 ≤ l ≤ 9
Reflections collected	5100
Independent reflections	686 [R _{int} = 0.0178, R _{sigma} = 0.0086]
Data/restraints/parameters	686/0/61
Goodness-of-fit on F ²	1.123
Final R indexes [I ≥ 2 σ (I)]	R ₁ = 0.0319, wR ₂ = 0.0832
Final R indexes [all data]	R ₁ = 0.0319, wR ₂ = 0.0832
Largest diff. peak/hole/ e Å ⁻³	0.95/-0.25
CCDC Number	1917945

Figure S3: ORTEP depiction of 45a

S5.3 X-ray data and structure for 50

Table S2: Crystal data and structure refinement for **50**

Crystallisation method	Slow evaporation from EtOAc solution
Empirical formula	C ₂₀ H ₁₉ N ₅ O
Formula weight	345.40
Temperature/K	100(2)
Crystal system	triclinic
Space group	P-1
a/Å	7.6546(6)
b/Å	10.4869(7)
c/Å	11.4484(8)
$\alpha/^\circ$	71.511(6)
$\beta/^\circ$	82.118(6)
$\gamma/^\circ$	82.695(6)
Volume/Å ³	859.90(11)
Z	2
$\rho_{\text{calc}}/\text{cm}^3$	1.334
μ/mm^{-1}	0.693
F(000)	364.0
Crystal size/mm ³	0.247 × 0.061 × 0.037
Radiation	CuK α (λ = 1.54184)
2 Θ range for data collection/ $^\circ$	8.186 to 145.928
Index ranges	-9 ≤ h ≤ 9, -12 ≤ k ≤ 12, -13 ≤ l ≤ 14
Reflections collected	12353
Independent reflections	3334 [R _{int} = 0.0299, R _{sigma} = 0.0241]
Data/restraints/parameters	3334/0/239
Goodness-of-fit on F ²	1.031
Final R indexes [I ≥ 2 σ (I)]	R ₁ = 0.0358, wR ₂ = 0.0938
Final R indexes [all data]	R ₁ = 0.0417, wR ₂ = 0.0987
Largest diff. peak/hole/ e Å ⁻³	0.32/-0.25
CCDC Number	1917944

Figure S4: ORTEP depiction of **50**

S5.4 X-ray data and structure for **56**

Table S3: Crystal data and structure refinement for **56**

Crystallisation method	Slow evaporation from EtOAc solution
Empirical formula	C ₁₀ H ₁₃ N ₅
Formula weight	203.25
Temperature/K	100(2)
Crystal system	monoclinic
Space group	P2 ₁ /c
a/Å	9.27494(16)
b/Å	8.81723(13)
c/Å	12.0155(2)
$\alpha/^\circ$	90
$\beta/^\circ$	92.8415(17)
$\gamma/^\circ$	90
Volume/Å ³	981.41(3)
Z	4
$\rho_{\text{calc}}/\text{g}/\text{cm}^3$	1.376
μ/mm^{-1}	0.722
F(000)	432.0
Crystal size/mm ³	0.246 × 0.166 × 0.1
Radiation	CuK α (λ = 1.54184)
2 Θ range for data collection/ $^\circ$	9.548 to 152.624
Index ranges	-11 ≤ h ≤ 11, -11 ≤ k ≤ 11, -15 ≤ l ≤ 14
Reflections collected	19400
Independent reflections	2040 [R _{int} = 0.0264, R _{sigma} = 0.0118]
Data/restraints/parameters	2040/0/136
Goodness-of-fit on F ²	1.068
Final R indexes [I ≥ 2 σ (I)]	R ₁ = 0.0323, wR ₂ = 0.0863
Final R indexes [all data]	R ₁ = 0.0347, wR ₂ = 0.0889
Largest diff. peak/hole/ e Å ⁻³	0.30/-0.24
CCDC Number	1917948

Figure S5: ORTEP depiction of **56**

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Paper code	ELN code	SMILES
S1	MK001	<chem>C1C=CN=CC(NN)=N1</chem>
S2	MK102	<chem>NNC1=NC(Br)=CN=C1</chem>
S3	MK124	<chem>IC1=CN=CC(NN)=N1</chem>
S4	MK092	<chem>NNC1=NC=CN=C1Cl</chem>
S5	MK097	<chem>NNC1=NC=C(Cl)N=C1</chem>
16	MK004	<chem>C1C=CN=CC2=NN=C(C3=CC=C(OC(F)F)C=C3)N21</chem>
17	MK087a	<chem>FC(F)OC(C=C1)=CC=C1C2=NN=C3C=NC=C(SCCC4=CC=CC=C4)N32</chem>
18	MK087b	<chem>FC(F)OC(C=C1)=CC=C1C2=NN=C3C(SCCC4=CC=CC=C4)=NC=CN32</chem>
25	MK093	<chem>C1C=NC=CN2C1=NN=C2</chem>
26	MK134	<chem>C1C=NC=CN2C1=NN=C2C3=CC=CC=C3</chem>
27	MK133	<chem>[O-][N+](C1=CC=C(C2=NN=C3C(Cl)=NC=CN32)C=C1)=O</chem>
28	MK096	<chem>C1(OCCC2=CC=CC=C2)=NC=CN3C1=NN=C3</chem>
29	MK094	<chem>C1(SCCC2=CC=CC=C2)=NC=CN3C1=NN=C3</chem>
30	MK085	<chem>C1(NCCC2=CC=CC=C2)=NC=CN3C1=NN=C3</chem>
31	MK113b	<chem>C1(OCCC2=CC=CC=C2)=NC=CN3C1=NN=C3C4=CC=CC=C4</chem>
32	MK110b	<chem>C1(SCCC2=CC=CC=C2)=NC=CN3C1=NN=C3C4=CC=CC=C4</chem>
33	MK116	<chem>C1(NCCC2=CC=CC=C2)=NC=CN3C1=NN=C3C4=CC=CC=C4</chem>
34	MK115b	<chem>O=[N+](C(C=C1)=CC=C1C2=NN=C3C(OCCC4=CC=C(C=C4)=NC=CN32)[O-]</chem>
35	MK112b	<chem>O=[N+](C(C=C1)=CC=C1C2=NN=C3C(SCCC4=CC=C(C=C4)=NC=CN32)[O-]</chem>
36	MK118	<chem>O=[N+](C(C=C1)=CC=C1C2=NN=C3C(NCCC4=CC=C(C=C4)=NC=CN32)[O-]</chem>
37	MK098	<chem>C1C(N=C1)=CN2C1=NN=C2</chem>
38	MK145	<chem>C1C(N=C1)=CN2C1=NN=C2C3=CC=C(OC(F)F)C=C3</chem>
41	MK123	<chem>IC1=NC(I)=CN=C1</chem>
42	MK141a	<chem>C1C=NC(OCCC2=CC=CC=C2)=CN=C1</chem>
43	MK143	<chem>BrC1=NC(OCCC2=CC=CC=C2)=CN=C1</chem>
44	MK144	<chem>IC1=NC(OCCC2=CC=CC=C2)=CN=C1</chem>

45a	MK002	C1C1=CN=CC2=NN=CN21
45b	MK108	C1C1=CN=CC(N12)=NN=C2C3=CC=C(OC)C=C3
45c	MK109	C1C1=CN=CC(N12)=NN=C2C3=CC=C([N+])([O-])=O)C=C3
45d	SSP 2019D1	C1C1=CN=CC(N12)=NN=C2C3=C(OC)C=CC=C3
45e	MK168	C1C1=CN=CC(N12)=NN=C2C3=C([N+])([O-])=O)C=CC=C3
45f	MK175	C1C1=CN=CC2=NN=C(C3=CC(C(C)(C)C)=CC(C(C)(C)C)=C3)N21
45g	MK170	C1C1=CN=CC(N12)=NN=C2C3=C(C=CC=C4)C4=CC5=C3C=CC=C5
45h	MK103	BrC1=CN=CC2=NN=CN21
45i	MK119	BrC1=CN=CC(N12)=NN=C2C3=CC=C(OC)C=C3
45j	MK183	BrC1=CN=CC(N12)=NN=C2C3=CC=C([N+])([O-])=O)C=C3
45k	MK181	BrC1=CN=CC(N12)=NN=C2C3=C(OC)C=CC=C3
45l	MK185	BrC1=CN=CC(N12)=NN=C2C3=C([N+])([O-])=O)C=CC=C3
45m	MK129	IC1=CN=CC2=NN=CN21
45n	MK125	IC1=CN=CC(N12)=NN=C2C3=CC=C(OC)C=C3
46a	MK006	C12=NN=CN1C(OCCC3=CC=CC=C3)=CN=C2
46b	MK086	C12=NN=CN1C(SCCC3=CC=CC=C3)=CN=C2
46c	MK114a	COC(C=C1)=CC=C1C2=NN=C3C=NC=C(OCCC4=CC=CC=C4)N32
46d	MK111a	COC(C=C1)=CC=C1C2=NN=C3C=NC=C(SCCC4=CC=CC=C4)N32
46e	MK115a	O=[N+](C(C=C1)=CC=C1C2=NN=C3C=NC=C(OCCC4=CC=CC=C4)N32)[O-]
46f	MK182a	COC1=C(C2=NN=C3C=NC=C(OCCC4=CC=CC=C4)N32)C=CC=C1
46g	MK169	O=[N+](C(C=CC=C1)=C1C2=NN=C3C=NC=C(OCCC4=CC=CC=C4)N32)[O-]
46h	MK176a	CC(C)(C)C1=CC(C2=NN=C3C=NC=C(OCCC4=CC=C4)N32)=CC(C(C)(C)C)=C1
46i	MK171a	C12=NN=C(C3=C4C=CC=CC4=CC5=C3C=CC=C5)N1C(OCCC6=CC=CC=C6)=CN=C2
47a	MK114b	COC(C=C1)=CC=C1C2=NN=C3C(OCCC4=CC=CC=C4)=NC=CN32

47b	MK111b	<chem>COC(C=C1)=CC=C1C2=NN=C3C(SCCC4=CC=CC=C4)=NC=CN32</chem>
47c	MK117	<chem>COC(C=C1)=CC=C1C2=NN=C3C(NCCC4=CC=CC=C4)=NC=CN32</chem>
47d	MK176b	<chem>CC(C)(C)C1=CC(C2=NN=C3C(OCCC4=CC=CC=C4)=NC=CN32)=CC(C(C)(C)C)=C1</chem>
47e	MK171b	<chem>C1(OCCC2=CC=CC=C2)=NC=CN3C1=NN=C3C4=C(C=CC=C5)C5=CC6=C4C=CC=C6</chem>
47f	MK182b	<chem>COC(C=CC=C1)=C1C2=NN=C3C(OCCC4=CC=CC=C4)=NC=CN32</chem>
48a	MK120-5	<chem>COC(C=C1)=CC=C1C2=NN=C3C(O)=NC=CN32</chem>
48b	MK184c	<chem>OC1=NC=CN2C1=NN=C2C3=CC=C([N+])([O-])=O)C=C3</chem>
48c	MK182c	<chem>OC1=NC=CN2C1=NN=C2C3=C(OC)C=CC=C3</chem>
48d	MK186c	<chem>OC1=NC=CN2C1=NN=C2C3=C([N+])([O-])=O)C=CC=C3</chem>
49	MK128c	<chem>COC(C=C1)=CC=C1C2=NN=C3C=NC=CN32</chem>
50	MK128d	<chem>COC(C=C1)=CC=C1C2=NN=C(N2)C3=NC=CN3CCC4=CC=CC=C4</chem>
55	MK026a	<chem>C12=NN=CN1C(N3CCCCC3)=CN=C2</chem>
56	MK026b	<chem>C1(N2CCCCC2)=NC=CN3C1=NN=C3</chem>
57	MK026-7a	<chem>[2H]C1=NN=C2C=NC=C(N3CCCCC3)N21</chem>
58	MK026-7b	<chem>[2H]C1=NN=C2C(N3CCCCC3)=NC=C([2H])N21</chem>
59	MK002-10	<chem>C1C1=CN=CC2=NN=C([2H])N21</chem>
60	MK026-9	<chem>[2H]C1=NN=C2C(N3CCCCC3)=NC=CN21</chem>

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Compound
structure and
number

46d

DiscoverX Gene Symbol	Entrez Gene Symbol	Percent Control	C (nM)
AURKC	AURKC	0	1000
BIKE	BMP2K	0	1000
BMX	BMX	0	1000
FLT1	FLT1	0	1000
FLT3(ITD)	FLT3	0	1000
PIK3CD	PIK3CD	0	1000
RIOK3	RIOK3	0	1000
RIOK1	RIOK1	11	1000
FLT3(ITD,F691L)	FLT3	39	1000
TSSK1B	TSSK1B	46	1000
TNK1	TNK1	47	1000
EGFR(L861Q)	EGFR	50	1000
IKK-alpha	CHUK	53	1000
BRAF	BRAF	54	1000
EGFR(L747-T751del,Sins)	EGFR	56	1000
EPHA2	EPHA2	61	1000
MKNK1	MKNK1	64	1000
ERBB2	ERBB2	65	1000
ULK3	ULK3	65	1000
PRKD3	PRKD3	67	1000
EGFR(L858R,T790M)	EGFR	70	1000
KIT(D816H)	KIT	70	1000
DCAMKL1	DCLK1	71	1000

47b

DiscoverX Gene Symbol	Entrez Gene Symbol	Percent Control	C (nM)
RIOK1	RIOK1	16	1000
MST2	STK3	42	1000
TSSK1B	TSSK1B	42	1000
EGFR(L861Q)	EGFR	48	1000
BRAF	BRAF	51	1000
FLT3(ITD,F691L)	FLT3	54	1000
IKK-alpha	CHUK	57	1000
ERBB2	ERBB2	60	1000
RAF1	RAF1	61	1000
SBK1	SBK1	65	1000
KIT(D816H)	KIT	67	1000
PAK2	PAK2	67	1000
JAK2(JH1domain-catalytic)	JAK2	68	1000
EGFR(L747-T751del,Sins)	EGFR	69	1000
PIK3CA(H1047Y)	PIK3CA	69	1000
PIP5K2C	PIP4K2C	69	1000
PRKCE	PRKCE	69	1000
TYRO3	TYRO3	69	1000
MYLK4	MYLK4	71	1000
PKMYT1	PKMYT1	71	1000
TYK2(JH2domain-pseudokinas	TYK2	71	1000
MEK5	MAP2K5	72	1000
TNK1	TNK1	72	1000

DLK	MAP3K12	71	1000
EGFR(S752-I759del)	EGFR	72	1000
AURKB	AURKB	73	1000
DYRK1B	DYRK1B	73	1000
FLT3(ITD,D835V)	FLT3	74	1000
SRMS	SRMS	74	1000
GCN2(Kin.Dom.2,S808G)	EIF2AK4	76	1000
HPK1	MAP4K1	76	1000
JNK1	MAPK8	76	1000
MINK	MINK1	76	1000
NDR1	STK38	76	1000
PIK3CA(E545K)	PIK3CA	76	1000
PIK4CB	PI4KB	76	1000
CDK8	CDK8	77	1000
DDR2	DDR2	77	1000
MAP3K4	MAP3K4	77	1000
PLK2	PLK2	77	1000
CDK4-cyclinD1	CDK4	78	1000
CDKL3	CDKL3	78	1000
SRPK2	SRPK2	78	1000
BRSK1	BRSK1	79	1000
ERN1	ERN1	79	1000
FYN	FYN	79	1000
BMPR2	BMPR2	80	1000
FGFR2	FGFR2	80	1000
FLT3(R834Q)	FLT3	80	1000
ICK	ICK	80	1000
PIK3CA(H1047Y)	PIK3CA	80	1000
ROCK2	ROCK2	80	1000
RPS6KA4(Kin.Dom.2-C-term	RPS6KA4	80	1000
VPS34	PIK3C3	80	1000
ABL1-nonphosphorylated	ABL1	81	1000
ALK	ALK	81	1000
JNK2	MAPK9	81	1000
JNK3	MAPK10	81	1000
MAP3K15	MAP3K15	81	1000
MEK5	MAP2K5	81	1000
MST2	STK3	81	1000
NIK	MAP3K14	81	1000
RIPK5	DSTYK	81	1000
SYK	SYK	81	1000
TAOK2	TAOK2	81	1000
TRPM6	TRPM6	81	1000

ULK3	ULK3	72	1000
ERBB3	ERBB3	73	1000
PIK3CA(E545K)	PIK3CA	73	1000
STK33	STK33	73	1000
TGFBR1	TGFBR1	73	1000
AURKB	AURKB	75	1000
BRSK1	BRSK1	75	1000
TNNI3K	TNNI3K	75	1000
YSK4	MAP3K19	76	1000
CAMK2D	CAMK2D	77	1000
PIP5K1C	PIP5K1C	77	1000
SRMS	SRMS	77	1000
ABL1-nonphosphorylated	ABL1	78	1000
PIKFYVE	PIKFYVE	79	1000
ASK2	MAP3K6	80	1000
MAK	MAK	80	1000
MKNK1	MKNK1	80	1000
DCAMKL1	DCLK1	81	1000
MARK1	MARK1	81	1000
NDR1	STK38	81	1000
KIT(L576P)	KIT	82	1000
MAP3K2	MAP3K2	82	1000
RSK2(Kin.Dom.2-C-terminal)	RPS6KA3	82	1000
ANKK1	ANKK1	83	1000
DDR2	DDR2	83	1000
PRKD3	PRKD3	83	1000
RIPK4	RIPK4	83	1000
CASK	CASK	84	1000
FLT3(ITD,D835V)	FLT3	84	1000
MINK	MINK1	84	1000
PIK3CA(E542K)	PIK3CA	84	1000
PIK4CB	PI4KB	84	1000
PLK3	PLK3	84	1000
RSK1(Kin.Dom.1-N-terminal)	RPS6KA1	84	1000
TAOK2	TAOK2	84	1000
WNK2	WNK2	84	1000
ABL1(M351T)-phosphorylated	ABL1	85	1000
BMX	BMX	85	1000
CSF1R	CSF1R	85	1000
PIK3CA(I800L)	PIK3CA	85	1000
SNRK	SNRK	85	1000
ABL1(T315I)-phosphorylated	ABL1	86	1000
FLT3(R834Q)	FLT3	86	1000

CDC2L5	CDK13	82	1000
HIPK4	HIPK4	82	1000
MAP3K1	MAP3K1	82	1000
MEK1	MAP2K1	82	1000
OSR1	OXSRI	82	1000
PLK3	PLK3	82	1000
FLT3(K663Q)	FLT3	83	1000
MAP4K2	MAP4K2	83	1000
PFCDPK1(P.falciparum)	CDPK1	83	1000
ULK1	ULK1	83	1000
ANKK1	ANKK1	84	1000
MAP3K2	MAP3K2	84	1000
MEK3	MAP2K3	84	1000
MEK4	MAP2K4	84	1000
PIK3CA(E542K)	PIK3CA	84	1000
PIK3CA(I800L)	PIK3CA	84	1000
ROCK1	ROCK1	84	1000
TYRO3	TYRO3	84	1000
WNK2	WNK2	84	1000
YSK4	MAP3K19	84	1000
ZAP70	ZAP70	84	1000
BLK	BLK	85	1000
FAK	PTK2	85	1000
FLT3(D835V)	FLT3	85	1000
MAK	MAK	85	1000
MAP4K3	MAP4K3	85	1000
PIM2	PIM2	85	1000
SGK3	SGK3	85	1000
EGFR(T790M)	EGFR	86	1000
MEK2	MAP2K2	86	1000
PIK3CA(H1047L)	PIK3CA	86	1000
PRKG2	PRKG2	86	1000
RSK2(Kin.Dom.2-C-terminal)	RPS6KA3	86	1000
ADCK3	CABC1	87	1000
BUB1	BUB1	87	1000
CDK4	CDK4	87	1000
CDKL1	CDKL1	87	1000
CDKL5	CDKL5	87	1000
JAK3(JH1domain-catalytic)	JAK3	87	1000
MARK2	MARK2	87	1000
TAOK1	TAOK1	87	1000
TAOK3	TAOK3	87	1000
ULK2	ULK2	87	1000

PIP5K1A	PIP5K1A	86	1000
QSK	KIAA0999	86	1000
RIPK5	DSTYK	86	1000
ZAP70	ZAP70	86	1000
FLT3(D835V)	FLT3	87	1000
HASPIN	GSG2	87	1000
IKK-beta	IKBKB	87	1000
JNK2	MAPK9	87	1000
PHKG2	PHKG2	87	1000
PLK2	PLK2	87	1000
RSK4(Kin.Dom.2-C-terminal)	RPS6KA6	87	1000
BRSK2	BRSK2	88	1000
GRK1	GRK1	88	1000
JNK3	MAPK10	88	1000
MAP3K15	MAP3K15	88	1000
MEK3	MAP2K3	88	1000
SGK3	SGK3	88	1000
SYK	SYK	88	1000
ULK1	ULK1	88	1000
MARK2	MARK2	89	1000
NIM1	MGC42105	89	1000
PAK7	PAK7	89	1000
DLK	MAP3K12	90	1000
IRAK4	IRAK4	90	1000
PIM2	PIM2	90	1000
SGK	SGK1	90	1000
TAOK1	TAOK1	90	1000
CDK8	CDK8	91	1000
EGFR(L858R,T790M)	EGFR	91	1000
FYN	FYN	91	1000
MAP3K4	MAP3K4	91	1000
MEK1	MAP2K1	91	1000
NEK10	NEK10	91	1000
PIK3CD	PIK3CD	91	1000
PRKD2	PRKD2	91	1000
CAMK1G	CAMK1G	92	1000
DYRK2	DYRK2	92	1000
ICK	ICK	92	1000
KIT(V559D,T670I)	KIT	92	1000
MAPKAPK5	MAPKAPK5	92	1000
RPS6KA4(Kin.Dom.2-C-terminal)	RPS6KA4	92	1000
TLK1	TLK1	92	1000
KIT(V559D)	KIT	93	1000

DMPK	DMPK	88	1000
DYRK1A	DYRK1A	88	1000
EGFR(G719C)	EGFR	88	1000
ERBB3	ERBB3	88	1000
IRAK4	IRAK4	88	1000
PIK3C2B	PIK3C2B	88	1000
PKN1	PKN1	88	1000
RPS6KA5(Kin.Dom.2-C-term	RPS6KA5	88	1000
SBK1	SBK1	88	1000
SIK2	SIK2	88	1000
TYK2(JH2domain-pseudokin	TYK2	88	1000
ABL1(H396P)-nonphosphor	ABL1	89	1000
ABL1(M351T)-phosphorylat	ABL1	89	1000
CASK	CASK	89	1000
FLT3-autoinhibited	FLT3	89	1000
KIT(V559D)	KIT	89	1000
NEK11	NEK11	89	1000
S6K1	RPS6KB1	89	1000
SRPK1	SRPK1	89	1000
TBK1	TBK1	89	1000
TNNI3K	TNNI3K	89	1000
BTk	BTk	90	1000
EGFR(L747-E749del, A750P	EGFR	90	1000
FGFR3	FGFR3	90	1000
MAPKAPK5	MAPKAPK5	90	1000
MKK7	MAP2K7	90	1000
SRC	SRC	90	1000
AKT1	AKT1	91	1000
CHEK2	CHEK2	91	1000
CSF1R-autoinhibited	CSF1R	91	1000
NIM1	MGC42105	91	1000
PIP5K1C	PIP5K1C	91	1000
SNARK	NUAK2	91	1000
TRKC	NTRK3	91	1000
ABL1-phosphorylated	ABL1	92	1000
ASK1	MAP3K5	92	1000
BRSK2	BRSK2	92	1000
GRK1	GRK1	92	1000
HIPK3	HIPK3	92	1000
IRAK1	IRAK1	92	1000
MST4	MST4	92	1000
NDR2	STK38L	92	1000
PAK2	PAK2	92	1000

MKK7	MAP2K7	93	1000
NDR2	STK38L	93	1000
PIK3CA(E545A)	PIK3CA	93	1000
RIPK2	RIPK2	93	1000
TBK1	TBK1	93	1000
ABL1(Q252H)-nonphosphoryla	ABL1	94	1000
AKT1	AKT1	94	1000
BMPR2	BMPR2	94	1000
BRAF(V600E)	BRAF	94	1000
CDK4-cyclinD1	CDK4	94	1000
DMPK	DMPK	94	1000
DYRK1A	DYRK1A	94	1000
MST3	STK24	94	1000
MST4	MST4	94	1000
SNARK	NUAK2	94	1000
ABL1-phosphorylated	ABL1	95	1000
AKT2	AKT2	95	1000
CLK1	CLK1	95	1000
CLK2	CLK2	95	1000
DAPK3	DAPK3	95	1000
LRRK2	LRRK2	95	1000
PFTAIRE2	CDK15	95	1000
TAOK3	TAOK3	95	1000
TRKB	NTRK2	95	1000
VRK2	VRK2	95	1000
BTk	BTk	96	1000
CAMKK2	CAMKK2	96	1000
EGFR(E746-A750del)	EGFR	96	1000
EGFR(G719C)	EGFR	96	1000
PRKR	EIF2AK2	96	1000
ROCK1	ROCK1	96	1000
TRKA	NTRK1	96	1000
WEE1	WEE1	96	1000
WEE2	WEE2	96	1000
CDKL1	CDKL1	97	1000
CLK4	CLK4	97	1000
EGFR(S752-I759del)	EGFR	97	1000
EPHA4	EPHA4	97	1000
FLT3-autoinhibited	FLT3	97	1000
JNK1	MAPK8	97	1000
MAP4K3	MAP4K3	97	1000
MARK4	MARK4	97	1000
MEK2	MAP2K2	97	1000

PRKCE	PRKCE	92	1000
TNIK	TNIK	92	1000
TYK2(JH1domain-catalytic)	TYK2	92	1000
IKK-beta	IKKBK	93	1000
MYLK	MYLK	93	1000
PCTK1	CDK16	93	1000
VRK2	VRK2	93	1000
YANK1	STK32A	93	1000
FLT3(N841I)	FLT3	94	1000
KIT(L576P)	KIT	94	1000
LCK	LCK	94	1000
PDGFRB	PDGFRB	94	1000
PIK3CA(E545A)	PIK3CA	94	1000
PIM1	PIM1	94	1000
PKMYT1	PKMYT1	94	1000
PRKCI	PRKCI	94	1000
STK33	STK33	94	1000
TXK	TXK	94	1000
EGFR(G719S)	EGFR	95	1000
EIF2AK1	EIF2AK1	95	1000
FLT3(D835Y)	FLT3	95	1000
HASPIN	GSG2	95	1000
LIMK1	LIMK1	95	1000
MLK2	MAP3K10	95	1000
RIPK4	RIPK4	95	1000
SLK	SLK	95	1000
SNRK	SNRK	95	1000
ABL1(F317I)-phosphorylate	ABL1	96	1000
ABL1(T315I)-phosphorylate	ABL1	96	1000
BMPR1B	BMPR1B	96	1000
BRAF(V600E)	BRAF	96	1000
CSNK1A1	CSNK1A1	96	1000
KIT(A829P)	KIT	96	1000
LRRK2	LRRK2	96	1000
PIK3CA(C420R)	PIK3CA	96	1000
PIM3	PIM3	96	1000
PRKD2	PRKD2	96	1000
EPHB2	EPHB2	97	1000
GRK3	ADRBK2	97	1000
GSK3B	GSK3B	97	1000
HIPK1	HIPK1	97	1000
LYN	LYN	97	1000
MEK6	MAP2K6	97	1000

MEK6	MAP2K6	97	1000
PIK3CA(Q546K)	PIK3CA	97	1000
RSK4(Kin.Dom.1-N-terminal)	RPS6KA6	97	1000
TNIK	TNIK	97	1000
YANK2	STK32B	97	1000
YANK3	STK32C	97	1000
AMPK-alpha2	PRKAA2	98	1000
GCN2(Kin.Dom.2,S808G)	EIF2AK4	98	1000
GSK3B	GSK3B	98	1000
MAP3K1	MAP3K1	98	1000
MAP4K2	MAP4K2	98	1000
MTOR	MTOR	98	1000
OSR1	OXS1	98	1000
PAK1	PAK1	98	1000
PFCDPK1(P.falciparum)	CDPK1	98	1000
RIPK1	RIPK1	98	1000
RSK2(Kin.Dom.1-N-terminal)	RPS6KA3	98	1000
RSK3(Kin.Dom.2-C-terminal)	RPS6KA2	98	1000
TRPM6	TRPM6	98	1000
CDKL2	CDKL2	99	1000
DAPK2	DAPK2	99	1000
PDGFRB	PDGFRB	99	1000
RPS6KA5(Kin.Dom.2-C-terminal)	RPS6KA5	99	1000
RSK1(Kin.Dom.2-C-terminal)	RPS6KA1	99	1000
S6K1	RPS6KB1	99	1000
TAK1	MAP3K7	99	1000
AAK1	AAK1	100	1000
ABL1(E255K)-phosphorylated	ABL1	100	1000
ABL1(F317I)-nonphosphorylated	ABL1	100	1000
ABL1(F317I)-phosphorylated	ABL1	100	1000
ABL1(F317L)-nonphosphorylated	ABL1	100	1000
ABL1(F317L)-phosphorylated	ABL1	100	1000
ABL1(H396P)-nonphosphorylated	ABL1	100	1000
ABL1(H396P)-phosphorylated	ABL1	100	1000
ABL1(Q252H)-phosphorylated	ABL1	100	1000
ABL1(T315I)-nonphosphorylated	ABL1	100	1000
ABL1(Y253F)-phosphorylated	ABL1	100	1000
ABL2	ABL2	100	1000
ACVR1	ACVR1	100	1000
ACVR1B	ACVR1B	100	1000
ACVR2A	ACVR2A	100	1000
ACVR2B	ACVR2B	100	1000
ACVRL1	ACVRL1	100	1000

MYLK2	MYLK2	97	1000
NEK5	NEK5	97	1000
TTK	TTK	97	1000
VEGFR2	KDR	97	1000
CAMKK2	CAMKK2	98	1000
CDK7	CDK7	98	1000
GRK7	GRK7	98	1000
KIT	KIT	98	1000
KIT(D816V)	KIT	98	1000
RSK1(Kin.Dom.1-N-termina	RPS6KA1	98	1000
SGK	SGK1	98	1000
SIK	SIK1	98	1000
WNK4	WNK4	98	1000
CAMK2D	CAMK2D	99	1000
DMPK2	CDC42BPG	99	1000
EGFR(E746-A750del)	EGFR	99	1000
HIPK2	HIPK2	99	1000
KIT(V559D,V654A)	KIT	99	1000
LZK	MAP3K13	99	1000
PKAC-alpha	PRKACA	99	1000
QSK	KIAA0999	99	1000
RIOK2	RIOK2	99	1000
ROS1	ROS1	99	1000
SGK2	SGK2	99	1000
TAK1	MAP3K7	99	1000
WNK1	WNK1	99	1000
AAK1	AAK1	100	1000
ABL1(E255K)-phosphorylate	ABL1	100	1000
ABL1(F317I)-nonphosphory	ABL1	100	1000
ABL1(F317L)-nonphosphory	ABL1	100	1000
ABL1(F317L)-phosphorylate	ABL1	100	1000
ABL1(H396P)-phosphorylate	ABL1	100	1000
ABL1(Q252H)-nonphosphor	ABL1	100	1000
ABL1(Q252H)-phosphorylat	ABL1	100	1000
ABL1(T315I)-nonphosphory	ABL1	100	1000
ABL1(Y253F)-phosphorylate	ABL1	100	1000
ABL2	ABL2	100	1000
ACVR1	ACVR1	100	1000
ACVR1B	ACVR1B	100	1000
ACVR2A	ACVR2A	100	1000
ACVR2B	ACVR2B	100	1000
ACVRL1	ACVRL1	100	1000
ADCK4	ADCK4	100	1000

ADCK3	CABC1	100	1000
ADCK4	ADCK4	100	1000
AKT3	AKT3	100	1000
ALK	ALK	100	1000
ALK(C1156Y)	ALK	100	1000
ALK(L1196M)	ALK	100	1000
AMPK-alpha1	PRKAA1	100	1000
ARK5	NUAK1	100	1000
ASK1	MAP3K5	100	1000
AURKA	AURKA	100	1000
AURKC	AURKC	100	1000
AXL	AXL	100	1000
BIKE	BMP2K	100	1000
BLK	BLK	100	1000
BMPR1A	BMPR1A	100	1000
BMPR1B	BMPR1B	100	1000
BRK	PTK6	100	1000
BUB1	BUB1	100	1000
CAMK1	CAMK1	100	1000
CAMK1B	PNCK	100	1000
CAMK1D	CAMK1D	100	1000
CAMK2A	CAMK2A	100	1000
CAMK2B	CAMK2B	100	1000
CAMK2G	CAMK2G	100	1000
CAMK4	CAMK4	100	1000
CAMKK1	CAMKK1	100	1000
CDC2L1	CDK11B	100	1000
CDC2L2	CDC2L2	100	1000
CDC2L5	CDK13	100	1000
CDK11	CDK19	100	1000
CDK2	CDK2	100	1000
CDK3	CDK3	100	1000
CDK4	CDK4	100	1000
CDK4-cyclinD3	CDK4	100	1000
CDK5	CDK5	100	1000
CDK7	CDK7	100	1000
CDK9	CDK9	100	1000
CDKL3	CDKL3	100	1000
CDKL5	CDKL5	100	1000
CHEK1	CHEK1	100	1000
CHEK2	CHEK2	100	1000
CIT	CIT	100	1000
CLK3	CLK3	100	1000

AKT2	AKT2	100	1000
AKT3	AKT3	100	1000
ALK(C1156Y)	ALK	100	1000
ALK(L1196M)	ALK	100	1000
AMPK-alpha1	PRKAA1	100	1000
AMPK-alpha2	PRKAA2	100	1000
ARK5	NUAK1	100	1000
ASK2	MAP3K6	100	1000
AURKA	AURKA	100	1000
AXL	AXL	100	1000
BMPR1A	BMPR1A	100	1000
BRK	PTK6	100	1000
CAMK1	CAMK1	100	1000
CAMK1B	PNCK	100	1000
CAMK1D	CAMK1D	100	1000
CAMK1G	CAMK1G	100	1000
CAMK2A	CAMK2A	100	1000
CAMK2B	CAMK2B	100	1000
CAMK2G	CAMK2G	100	1000
CAMK4	CAMK4	100	1000
CAMKK1	CAMKK1	100	1000
CDC2L1	CDK11B	100	1000
CDC2L2	CDC2L2	100	1000
CDK11	CDK19	100	1000
CDK2	CDK2	100	1000
CDK3	CDK3	100	1000
CDK4-cyclinD3	CDK4	100	1000
CDK5	CDK5	100	1000
CDK9	CDK9	100	1000
CDKL2	CDKL2	100	1000
CHEK1	CHEK1	100	1000
CIT	CIT	100	1000
CLK1	CLK1	100	1000
CLK2	CLK2	100	1000
CLK3	CLK3	100	1000
CLK4	CLK4	100	1000
CSF1R	CSF1R	100	1000
CSK	CSK	100	1000
CSNK1A1L	CSNK1A1L	100	1000
CSNK1D	CSNK1D	100	1000
CSNK1E	CSNK1E	100	1000
CSNK1G1	CSNK1G1	100	1000
CSNK1G2	CSNK1G2	100	1000

CSF1R-autoinhibited	CSF1R	100	1000
CSK	CSK	100	1000
CSNK1A1	CSNK1A1	100	1000
CSNK1A1L	CSNK1A1L	100	1000
CSNK1D	CSNK1D	100	1000
CSNK1E	CSNK1E	100	1000
CSNK1G1	CSNK1G1	100	1000
CSNK1G2	CSNK1G2	100	1000
CSNK1G3	CSNK1G3	100	1000
CSNK2A1	CSNK2A1	100	1000
CSNK2A2	CSNK2A2	100	1000
CTK	MATK	100	1000
DAPK1	DAPK1	100	1000
DCAMKL2	DCLK2	100	1000
DCAMKL3	DCLK3	100	1000
DDR1	DDR1	100	1000
DMPK2	CDC42BPG	100	1000
DRAK1	STK17A	100	1000
DRAK2	STK17B	100	1000
DYRK1B	DYRK1B	100	1000
EGFR	EGFR	100	1000
EGFR(G719S)	EGFR	100	1000
EGFR(L747-E749del, A750P)	EGFR	100	1000
EGFR(L747-S752del, P753S)	EGFR	100	1000
EGFR(L858R)	EGFR	100	1000
EGFR(T790M)	EGFR	100	1000
EIF2AK1	EIF2AK1	100	1000
EPHA1	EPHA1	100	1000
EPHA2	EPHA2	100	1000
EPHA3	EPHA3	100	1000
EPHA5	EPHA5	100	1000
EPHA6	EPHA6	100	1000
EPHA7	EPHA7	100	1000
EPHA8	EPHA8	100	1000
EPHB1	EPHB1	100	1000
EPHB2	EPHB2	100	1000
EPHB3	EPHB3	100	1000
EPHB4	EPHB4	100	1000
EPHB6	EPHB6	100	1000
ERBB4	ERBB4	100	1000
ERK1	MAPK3	100	1000
ERK2	MAPK1	100	1000
ERK3	MAPK6	100	1000

CSNK1G3	CSNK1G3	100	1000
CSNK2A1	CSNK2A1	100	1000
CSNK2A2	CSNK2A2	100	1000
CTK	MATK	100	1000
DAPK1	DAPK1	100	1000
DAPK2	DAPK2	100	1000
DAPK3	DAPK3	100	1000
DCAMKL2	DCLK2	100	1000
DCAMKL3	DCLK3	100	1000
DDR1	DDR1	100	1000
DRAK1	STK17A	100	1000
DRAK2	STK17B	100	1000
DYRK2	DYRK2	100	1000
EGFR	EGFR	100	1000
EGFR(L747-S752del, P753S)	EGFR	100	1000
EGFR(L858R)	EGFR	100	1000
EPHA1	EPHA1	100	1000
EPHA3	EPHA3	100	1000
EPHA4	EPHA4	100	1000
EPHA5	EPHA5	100	1000
EPHA6	EPHA6	100	1000
EPHA7	EPHA7	100	1000
EPHA8	EPHA8	100	1000
EPHB1	EPHB1	100	1000
EPHB3	EPHB3	100	1000
EPHB4	EPHB4	100	1000
EPHB6	EPHB6	100	1000
ERBB4	ERBB4	100	1000
ERK1	MAPK3	100	1000
ERK2	MAPK1	100	1000
ERK3	MAPK6	100	1000
ERK4	MAPK4	100	1000
ERK5	MAPK7	100	1000
ERK8	MAPK15	100	1000
FER	FER	100	1000
FES	FES	100	1000
FGFR1	FGFR1	100	1000
FGFR3(G697C)	FGFR3	100	1000
FGFR4	FGFR4	100	1000
FGR	FGR	100	1000
FLT3	FLT3	100	1000
FLT3(D835H)	FLT3	100	1000
FLT4	FLT4	100	1000

ERK4	MAPK4	100	1000
ERK5	MAPK7	100	1000
ERK8	MAPK15	100	1000
ERN1	ERN1	100	1000
FAK	PTK2	100	1000
FER	FER	100	1000
FES	FES	100	1000
FGFR1	FGFR1	100	1000
FGFR2	FGFR2	100	1000
FGFR3	FGFR3	100	1000
FGFR3(G697C)	FGFR3	100	1000
FGFR4	FGFR4	100	1000
FGR	FGR	100	1000
FLT1	FLT1	100	1000
FLT3	FLT3	100	1000
FLT3(D835H)	FLT3	100	1000
FLT3(D835Y)	FLT3	100	1000
FLT3(ITD)	FLT3	100	1000
FLT3(K663Q)	FLT3	100	1000
FLT3(N841I)	FLT3	100	1000
FLT4	FLT4	100	1000
FRK	FRK	100	1000
GAK	GAK	100	1000
GRK2	ADRBK1	100	1000
GRK3	ADRBK2	100	1000
GRK4	GRK4	100	1000
GRK7	GRK7	100	1000
GSK3A	GSK3A	100	1000
HCK	HCK	100	1000
HIPK1	HIPK1	100	1000
HIPK2	HIPK2	100	1000
HIPK3	HIPK3	100	1000
HIPK4	HIPK4	100	1000
HPK1	MAP4K1	100	1000
HUNK	HUNK	100	1000
IGF1R	IGF1R	100	1000
IKK-epsilon	IKBKE	100	1000
INSR	INSR	100	1000
INSRR	INSRR	100	1000
IRAK1	IRAK1	100	1000
IRAK3	IRAK3	100	1000
ITK	ITK	100	1000
JAK1(JH1domain-catalytic)	JAK1	100	1000

FRK	FRK	100	1000
GAK	GAK	100	1000
GRK2	ADRBK1	100	1000
GRK4	GRK4	100	1000
GSK3A	GSK3A	100	1000
HCK	HCK	100	1000
HUNK	HUNK	100	1000
IGF1R	IGF1R	100	1000
IKK-epsilon	IKBKE	100	1000
INSR	INSR	100	1000
INSRR	INSRR	100	1000
IRAK3	IRAK3	100	1000
ITK	ITK	100	1000
JAK1(JH1domain-catalytic)	JAK1	100	1000
JAK1(JH2domain-pseudokinase)	JAK1	100	1000
JAK2(JH1domain-catalytic)	JAK2	100	1000
KIT(V559D,T670I)	KIT	100	1000
KIT-autoinhibited	KIT	100	1000
LATS1	LATS1	100	1000
LATS2	LATS2	100	1000
LIMK2	LIMK2	100	1000
LKB1	STK11	100	1000
LOK	STK10	100	1000
LRRK2(G2019S)	LRRK2	100	1000
LTK	LTK	100	1000
MAP3K3	MAP3K3	100	1000
MAP4K4	MAP4K4	100	1000
MAP4K5	MAP4K5	100	1000
MAPKAPK2	MAPKAPK2	100	1000
MARK1	MARK1	100	1000
MARK3	MARK3	100	1000
MARK4	MARK4	100	1000
MAST1	MAST1	100	1000
MELK	MELK	100	1000
MERTK	MERTK	100	1000
MET	MET	100	1000
MET(M1250T)	MET	100	1000
MET(Y1235D)	MET	100	1000
MKNK2	MKNK2	100	1000
MLCK	MYLK3	100	1000
MLK1	MAP3K9	100	1000
MLK3	MAP3K11	100	1000
MRCKA	CDC42BPA	100	1000

JAK1(JH2domain-pseudokinase)	JAK1	100	1000
JAK3(JH1domain-catalytic)	JAK3	100	1000
KIT	KIT	100	1000
KIT(A829P)	KIT	100	1000
KIT(D816V)	KIT	100	1000
KIT(V559D,V654A)	KIT	100	1000
KIT-autoinhibited	KIT	100	1000
LATS1	LATS1	100	1000
LATS2	LATS2	100	1000
LCK	LCK	100	1000
LIMK1	LIMK1	100	1000
LIMK2	LIMK2	100	1000
LKB1	STK11	100	1000
LOK	STK10	100	1000
LRRK2(G2019S)	LRRK2	100	1000
LTK	LTK	100	1000
LYN	LYN	100	1000
LZK	MAP3K13	100	1000
MAP3K3	MAP3K3	100	1000
MAP4K4	MAP4K4	100	1000
MAP4K5	MAP4K5	100	1000
MAPKAPK2	MAPKAPK2	100	1000
MARK3	MARK3	100	1000
MAST1	MAST1	100	1000
MEK4	MAP2K4	100	1000
MELK	MELK	100	1000
MERTK	MERTK	100	1000
MET	MET	100	1000
MET(M1250T)	MET	100	1000
MET(Y1235D)	MET	100	1000
MKNK2	MKNK2	100	1000
MLCK	MYLK3	100	1000
MLK1	MAP3K9	100	1000
MLK2	MAP3K10	100	1000
MLK3	MAP3K11	100	1000
MRCKA	CDC42BPA	100	1000
MRCKB	CDC42BPB	100	1000
MST1	STK4	100	1000
MST1R	MST1R	100	1000
MUSK	MUSK	100	1000
MYLK	MYLK	100	1000
MYLK2	MYLK2	100	1000
MYO3A	MYO3A	100	1000

MRCKB	CDC42BPB	100	1000
MST1	STK4	100	1000
MST1R	MST1R	100	1000
MST3	STK24	100	1000
MTOR	MTOR	100	1000
MUSK	MUSK	100	1000
MYLK4	MYLK4	100	1000
MYO3A	MYO3A	100	1000
MYO3B	MYO3B	100	1000
NEK1	NEK1	100	1000
NEK10	NEK10	100	1000
NEK2	NEK2	100	1000
NEK3	NEK3	100	1000
NEK4	NEK4	100	1000
NEK6	NEK6	100	1000
NEK7	NEK7	100	1000
NEK9	NEK9	100	1000
NLK	NLK	100	1000
p38-alpha	MAPK14	100	1000
p38-beta	MAPK11	100	1000
p38-delta	MAPK13	100	1000
p38-gamma	MAPK12	100	1000
PAK1	PAK1	100	1000
PAK3	PAK3	100	1000
PAK4	PAK4	100	1000
PAK6	PAK6	100	1000
PAK7	PAK7	100	1000
PCTK2	CDK17	100	1000
PCTK3	CDK18	100	1000
PDGFRA	PDGFRA	100	1000
PDPK1	PDPK1	100	1000
PFPK5(P.falciparum)	MAL13P1.279	100	1000
PFTAIRE2	CDK15	100	1000
PFTK1	CDK14	100	1000
PHKG1	PHKG1	100	1000
PHKG2	PHKG2	100	1000
PIK3C2G	PIK3C2G	100	1000
PIK3CA	PIK3CA	100	1000
PIK3CA(M1043I)	PIK3CA	100	1000
PIK3CA(Q546K)	PIK3CA	100	1000
PIK3CB	PIK3CB	100	1000
PIK3CG	PIK3CG	100	1000
PIKFYVE	PIKFYVE	100	1000

MYO3B	MYO3B	100	1000
NEK1	NEK1	100	1000
NEK11	NEK11	100	1000
NEK2	NEK2	100	1000
NEK3	NEK3	100	1000
NEK4	NEK4	100	1000
NEK5	NEK5	100	1000
NEK6	NEK6	100	1000
NEK7	NEK7	100	1000
NEK9	NEK9	100	1000
NIK	MAP3K14	100	1000
NLK	NLK	100	1000
p38-alpha	MAPK14	100	1000
p38-beta	MAPK11	100	1000
p38-delta	MAPK13	100	1000
p38-gamma	MAPK12	100	1000
PAK3	PAK3	100	1000
PAK4	PAK4	100	1000
PAK6	PAK6	100	1000
PCTK1	CDK16	100	1000
PCTK2	CDK17	100	1000
PCTK3	CDK18	100	1000
PDGFRA	PDGFRA	100	1000
PDPK1	PDPK1	100	1000
PFPK5(P.falciparum)	MAL13P1.	100	1000
PFTK1	CDK14	100	1000
PHKG1	PHKG1	100	1000
PIK3C2B	PIK3C2B	100	1000
PIK3C2G	PIK3C2G	100	1000
PIK3CA	PIK3CA	100	1000
PIK3CA(C420R)	PIK3CA	100	1000
PIK3CA(H1047L)	PIK3CA	100	1000
PIK3CA(M1043I)	PIK3CA	100	1000
PIK3CB	PIK3CB	100	1000
PIK3CG	PIK3CG	100	1000
PIM1	PIM1	100	1000
PIM3	PIM3	100	1000
PIP5K2B	PIP4K2B	100	1000
PKAC-alpha	PRKACA	100	1000
PKAC-beta	PRKACB	100	1000
PKN1	PKN1	100	1000
PKN2	PKN2	100	1000
PKNB(M.tuberculosis)	pknB	100	1000

PIP5K1A	PIP5K1A	100	1000
PIP5K2B	PIP4K2B	100	1000
PIP5K2C	PIP4K2C	100	1000
PKAC-beta	PRKACB	100	1000
PKN2	PKN2	100	1000
PKNB(M.tuberculosis)	pknB	100	1000
PLK1	PLK1	100	1000
PLK4	PLK4	100	1000
PRKCD	PRKCD	100	1000
PRKCH	PRKCH	100	1000
PRKCQ	PRKCQ	100	1000
PRKD1	PRKD1	100	1000
PRKG1	PRKG1	100	1000
PRKR	EIF2AK2	100	1000
PRKX	PRKX	100	1000
PRP4	PRPF4B	100	1000
PYK2	PTK2B	100	1000
RAF1	RAF1	100	1000
RET	RET	100	1000
RET(M918T)	RET	100	1000
RET(V804L)	RET	100	1000
RET(V804M)	RET	100	1000
RIPK1	RIPK1	100	1000
RIPK2	RIPK2	100	1000
RPS6KA4(Kin.Dom.1-N-term	RPS6KA4	100	1000
RPS6KA5(Kin.Dom.1-N-term	RPS6KA5	100	1000
RSK1(Kin.Dom.2-C-terminal	RPS6KA1	100	1000
RSK2(Kin.Dom.1-N-terminal	RPS6KA3	100	1000
RSK3(Kin.Dom.1-N-terminal	RPS6KA2	100	1000
RSK3(Kin.Dom.2-C-terminal	RPS6KA2	100	1000
RSK4(Kin.Dom.1-N-terminal	RPS6KA6	100	1000
RSK4(Kin.Dom.2-C-terminal	RPS6KA6	100	1000
SgK110	SgK110	100	1000
SRPK3	SRPK3	100	1000
STK16	STK16	100	1000
STK35	STK35	100	1000
STK36	STK36	100	1000
STK39	STK39	100	1000
TEC	TEC	100	1000
TESK1	TESK1	100	1000
TGFBR1	TGFBR1	100	1000
TGFBR2	TGFBR2	100	1000
TIE1	TIE1	100	1000

PLK1	PLK1	100	1000
PLK4	PLK4	100	1000
PRKCD	PRKCD	100	1000
PRKCH	PRKCH	100	1000
PRKCI	PRKCI	100	1000
PRKCQ	PRKCQ	100	1000
PRKD1	PRKD1	100	1000
PRKG1	PRKG1	100	1000
PRKG2	PRKG2	100	1000
PRKX	PRKX	100	1000
PRP4	PRPF4B	100	1000
PYK2	PTK2B	100	1000
RET	RET	100	1000
RET(M918T)	RET	100	1000
RET(V804L)	RET	100	1000
RET(V804M)	RET	100	1000
RIOK2	RIOK2	100	1000
RIOK3	RIOK3	100	1000
ROCK2	ROCK2	100	1000
ROS1	ROS1	100	1000
RPS6KA4(Kin.Dom.1-N-termin	RPS6KA4	100	1000
RPS6KA5(Kin.Dom.1-N-termin	RPS6KA5	100	1000
RSK3(Kin.Dom.1-N-terminal)	RPS6KA2	100	1000
SgK110	SgK110	100	1000
SGK2	SGK2	100	1000
SIK	SIK1	100	1000
SIK2	SIK2	100	1000
SLK	SLK	100	1000
SRC	SRC	100	1000
SRPK1	SRPK1	100	1000
SRPK2	SRPK2	100	1000
SRPK3	SRPK3	100	1000
STK16	STK16	100	1000
STK35	STK35	100	1000
STK36	STK36	100	1000
STK39	STK39	100	1000
TEC	TEC	100	1000
TESK1	TESK1	100	1000
TGFBR2	TGFBR2	100	1000
TIE1	TIE1	100	1000
TIE2	TEK	100	1000
TLK2	TLK2	100	1000
TNK2	TNK2	100	1000

TIE2	TEK	100	1000
TLK1	TLK1	100	1000
TLK2	TLK2	100	1000
TNK2	TNK2	100	1000
TRKA	NTRK1	100	1000
TRKB	NTRK2	100	1000
TSSK3	TSSK3	100	1000
WEE1	WEE1	100	1000
WEE2	WEE2	100	1000
WNK3	WNK3	100	1000
YANK2	STK32B	100	1000
YANK3	STK32C	100	1000
YES	YES1	100	1000
YSK1	STK25	100	1000
ZAK	ZAK	100	1000

TRKC	NTRK3	100	1000
TSSK3	TSSK3	100	1000
TTK	TTK	100	1000
TXK	TXK	100	1000
TYK2(JH1domain-catalytic)	TYK2	100	1000
ULK2	ULK2	100	1000
VEGFR2	KDR	100	1000
VPS34	PIK3C3	100	1000
WNK1	WNK1	100	1000
WNK3	WNK3	100	1000
WNK4	WNK4	100	1000
YANK1	STK32A	100	1000
YES	YES1	100	1000
YSK1	STK25	100	1000
ZAK	ZAK	100	1000

Kinases inhibited
<50% annotated
with **46d** shown
in black circles
and **47b** shown in
blue triangles

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46d

OSM-S-626

468 Assays Tested
8 Interactions Mapped
S-Score(35) = 0.01

Legend

Percent Control

ATYPICAL

MUTANT

LIPID

PATHOGEN

47b

OSM-S-627
 468 Assays Tested
 1 Interactions Mapped
 S-Score(35) = 0

ATYPICAL

MUTANT

LIPID

PATHOGEN

KinomeScan_data_SI.pdf (482.21 KiB)

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Other files

X-ray data.zip (2.53 MiB)

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