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Article

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Discovery and characterization of 2-nitro-5-(4-(phenylsulfonyl)piperazin-1-yl)-*N*-(pyridin-4-ylmethyl)anilines as novel inhibitors of the *Aedes aegypti* Kir1 (*Ae*Kir1) channel

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Mosquito-borne arboviral diseases such as Zika, dengue fever and chikungunya are transmitted to humans by infected adult female *Aedes aegypti* mosquitoes and affect a large portion of the world's population. The Kir1 channel in *Ae. aegypti* (*Ae*Kir1) is an important ion channel in the functioning of mosquito Malpighian (renal) tubules and one that can be manipulated in order to disrupt excretory functions in mosquitoes. We have previously reported the discovery of various scaffolds that are active against the *Ae*Kir1 channel. Herein we report the synthesis and biological characterization of a new 2-nitro-5-(4-(phenylsulfonyl) piperazin-1-yl)-*N*-(pyridin-4-ylmethyl)anilines scaffold as inhibitors of *Ae*Kir1. This new scaffold is more potent *in vitro* compared to the previously reported scaffolds, and the molecules kill mosquito larvae.

Keywords: Kir channels, *Aedes aegypti*, Zika, vector-borne diseases, larvae

Zika virus (ZIKV) is an arbovirus originating in Africa and Asia that was introduced to South America in 2015 and has since spread throughout Latin America and the Caribbean. This has coincided with the increasingly high number of reported cases of microcephaly and Guillain-Barré syndrome and led the World Health Organization (WHO) to declare the emerging Zika situation a "public health emergency of international concern". The virus is spread primarily through mosquitoes of the genus *Aedes*, with the urban-dwelling, anthropophilic *Aedes aegypti* being suspected as the most important species contributing to the spread of ZIKV throughout Latin America and the Caribbean. In addition to ZIKV, *Ae. aegypti* is the primary vector of the arboviruses that cause chikungunya (CHIKV) and dengue (DENV) fevers, which are diseases that are emerging and reemerging worldwide. Annually, DENV infects hundreds of millions of people, causes hundreds of thousands of hospitalizations, and kills tens of thousands of people.[1] The economic burden is also substantial with estimates over $2 billion in the Americas and over $1 billion in southeast Asia.[2,3] CHIKV had been contained to Africa and Southeast Asia; however,

over the past decade it has spread to Europe and then to the Americas where over 2 million suspected cases and autochthonous transmission have been reported.[4-6]

Unfortunately, there are no effective treatments for ZIKV, CHIKV, or DENV, either as vaccines or therapeutics; thus, the principal strategy of controlling these diseases is to block the vector from biting humans. This is generally accomplished using insecticides or insecticide-treated materials (clothing, nets, etc.). Unfortunately, mosquitoes have evolved resistance to the commonly used classes of insecticides (e.g., pyrethroids)[7,8], and, the use of similar insecticides has also been implicated in the decline of beneficial insects, e.g., the honey bee (*Apis mellifera*).[9] Thus, the confluence of these two issues (resistance and decline of pollinators) has made the identification of novel insecticides with novel mechanisms of action that are selective for mosquitoes an urgent need.

We have previously identified an inwardly-rectifying potassium (Kir1) channel as a novel target for the development of new mosquitocides[10,11]; Kir1 is abundantly expressed in mosquito Malpighian (renal) tubules and ovaries and plays a crucial role in mosquito renal excretory capacity, hemolymph potassium homeostasis, and fecundicity.[11-15] In other insects, Kir1 channels play important roles in renal excretions, salivary gland function, and nervous system function.[16,17] We have published regarding molecules (**1-3**) that inhibit the Kir1 channel of *Ae. aegypti* (*Ae*Kir1) *in vitro*, and after hemolymph injection are toxic to adult female mosquitoes (Figure 1).[11-13] More recently, we have identified a new compound, **4**, that was toxic to mosquitoes *in vivo* after topical application to adult females or addition to the rearing water of larvae.[18,19] Excitingly, **4** was similarly toxic to pyrethroid-susceptible and pyrethroid-resistant lab strains of adult female mosquitoes, showed no apparent toxicity to adult honey bees, *Apis mellifera*, and exhibited toxic efficacy against mosquitoes in semi-field conditions.[18] Although **4**, and the other compounds **1-3**, are important analog milestones in the development of *Ae*Kir1 inhibitors as novel mosquitocides,

these compounds show only micromolar activity against the *Ae*Kir1 channel *in vitro*, and low potency *in vivo* compared to conventional insecticides (e.g., pyrethroids). Thus, the need to develop more potent compounds remains a goal of our laboratories. Herein, we report the discovery and characterization of a new scaffold of *Ae*Kir inhibitors and their activity in a larval toxicity assay.

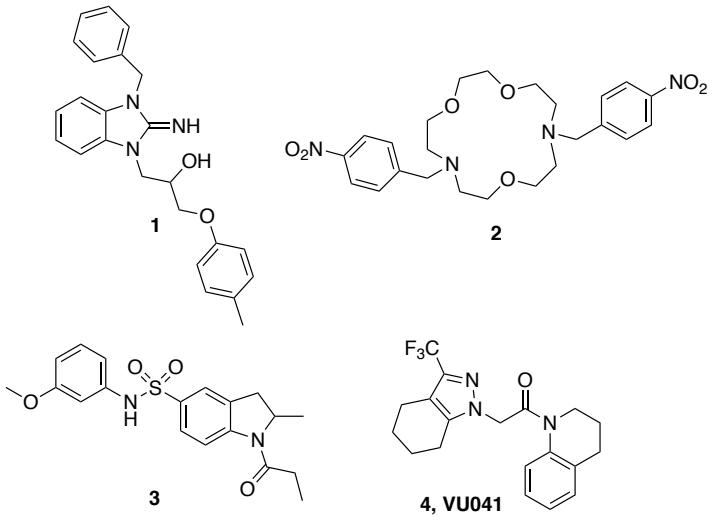
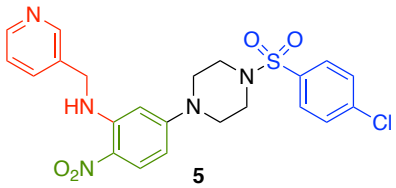


Figure 1. Previously identified *Ae*Kir1 inhibitors.

In order to further develop potent *Ae*Kir1 inhibitors, we performed a high-throughput screening campaign against the Vanderbilt Institute of Chemical Biology compound library and identified a compound, **5**, with a unique and interesting molecular scaffold (Figure 2). This compound, **5**, is characterized by four distinct regions of the molecule that we will exploit in our structure-activity relationship (SAR) campaign. First, the left-hand aminomethyl pyridine region (red) will be investigated to determine the optimal moiety, second, the aryl nitro (green) will be eliminated and replaced, third, the piperazine (black) ring system will be expanded and replaced with spriocycles, and finally, the arylsulfonamide (blue) will be evaluated. Detailed below is the work from our laboratory on the medicinal chemistry campaign, the *in vitro* pharmacology and

lastly, the *in vivo* toxicology which has led to the identification of a new and more efficacious mosquitocide for further evaluation and development.

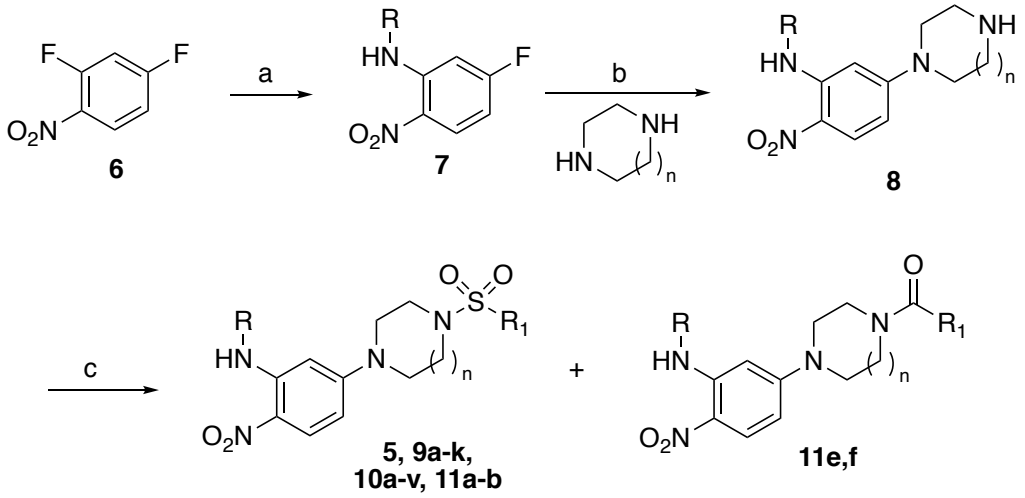


5

Figure 2. Newly identified scaffold from a high-throughput screen and highlighted areas for SAR diversification.

The synthesis of the first analogs to be evaluated is shown in Scheme 1. The 2,4-difluoronitrobenzene, **6**, was reacted with the appropriate amine under basic conditions (Et_3N, DMSO) to give the *ortho*-substituted product exclusively.[20] Next, either piperazine or homopiperazine were reacted via S_NAr with the 4-fluoronitrobenzene analog, **7**, to yield the diamino substituted **8**. The final targets were realized after reaction with an appropriately chosen sulfonyl chloride or acid chloride to yield the desired compounds (**5**, **9a-k**, **10a-v**, **11a-b**, **e-f**). This reaction sequence is robust and allows for scale-up of the final targets (>1 g).

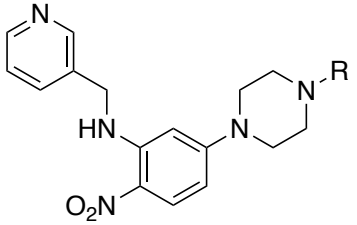
Scheme 1. Synthesis of initial sulfonamide analogs.[a]

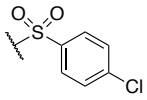
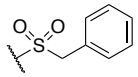
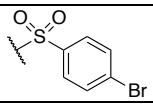
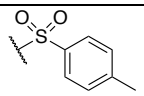
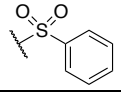
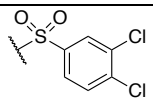
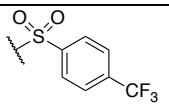
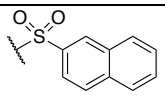
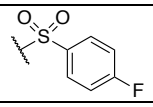
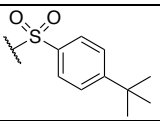


[a]Reagents and conditions. (a) R-NH_2, Et_3N, DMSO; (b) Et_3N, DMSO; (c) R_1SO_2Cl or R_1COCl, Et_3N, CH_2Cl_2

The initial SAR centered around the sulfonamide (right-hand) portion of the molecule (R, Table 1). The original hit molecule, **5**, was resynthesized and tested. This compound was active as an *Ae*Kir1 channel inhibitor (IC_{50} = 1.47 μM) utilizing a thallium flux assay. Addition of a methylene linker between the sulfonyl group and the phenyl resulted in a less active compound, **9a**. Addition of a bromine, **9b**, brought the activity back to the same as **5**; but removing the phenyl group led to a decrease in activity (**9c**, IC_{50} = 21 μM and **9d**, IC_{50} = 44 μM). Further substitution around the phenyl (or no substitution) led to the identification of more potent compounds, highlighted by the phenyl, **9f** (IC_{50} = 0.55 μM), and the 4-fluorophenyl, **9j** (IC_{50} = 0.47 μM) as the most potent compounds. Addition of a large 4-*tert*-butyl group led to a loss of activity (**9k**).

Table 1. Evaluation of the sulfonamide analogs.



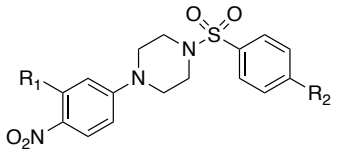
Cmpd	R	cLogP[a]	Thallium Flux IC_{50} ± SEM (μM)[b]	% Inhibition[b]
5		3.37	1.47 ± 0.06	100
9a		3.55	5.66 ± 0.86	67
9b		3.45	1.56 ± 0.20	86
9c		2.29	20.8 ± 1.3	35
9d		1.65	43.7 ± 19	10.8
9e		3.24	1.11 ± 0.016	100
9f		2.88	0.55 ± 0.04	95
9g		4.03	0.98 ± 0.046	97
9h		3.88	1.24 ± 0.01	99
9i		4.05	6.57 ± 0.35	95.3
9j		3.12	0.47 ± 0.018	100
9k		4.29	22.6 ± 6.6	44.8

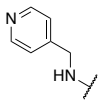
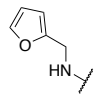
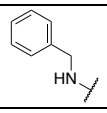
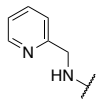
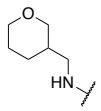
[a]Small-Molecule Drug Discovery Suite 2018-2, Schrödinger, LLC, New York, NY, 2018. [b]Thallium flux *Ae*Kir1 assay; IC_{50} values represent the average (mean ± SEM) of values obtained from at least three individual experiments.

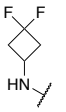
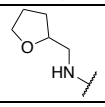
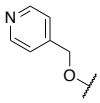
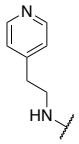
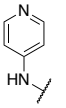
The next round of analog evaluation moved from the sulfonamide portion to the 3-amino group (R_1, Table 2) while maintaining the sulfonamide as either the 4-fluorophenyl or 4-chlorophenyl. The 4-pyridyl group was well tolerated or even produced a slightly more potent analog (**10a**, IC_{50} = 0.39 μM; **10b**, IC_{50} = 0.75 μM), when compared to the 3-pyridyl counterparts.

Substituting the 2-furyl group was also tolerated; however, there was an interesting change of potency where the 4-Cl-phenyl derivative was more potent, although the potencies are in a similar range (4-Cl-phenyl, **10d**, IC_{50} = 0.84 μM; 4-F-phenyl, **10c**, IC_{50} = 1.29 μM). Other replacements led to compounds that were less active than the best compound, **9j**; however, all of the compounds were within 2-fold of each other (**10e-o**). Replacing the methylamino linker with a methylether was not a productive change, but again, there was a divergence between the 4-Cl-phenyl and 4-F-phenyl moieties (4-F-phenyl, **10p**, IC_{50} = 63 μM; 4-Cl-phenyl, **10q**, IC_{50} = 4.3 μM). Finally, extending the linker by a methylene group (**10r,s**) produced equipotent compounds; whereas, reducing the linker (i.e., direct NH linked) was not productive (**10t,u**).

Table 2. Evaluation of the left-hand amine moiety.



Cmpd	R_1	R_2	cLogP[a]	Thallium Flux IC_{50} ± SD (μM)[b]	% Inhibition[b]
10a		F	3.39	0.39 ± 0.24	103
10b		Cl	3.64	0.75 ± 0.23	99
10c		F	3.57	1.29 ± 0.10	96
10d		Cl	3.82	0.84 ± 0.07	93
10e		Cl	4.60	1.23 ± 0.26	19
10f		F	3.72	0.80 ± 0.25	101
10g		Cl	3.98	1.1 ± 0.004	101
10h		F	3.38	1.82 ± 0.40	95
10i		Cl	3.63	1.92 ± 0.03	96

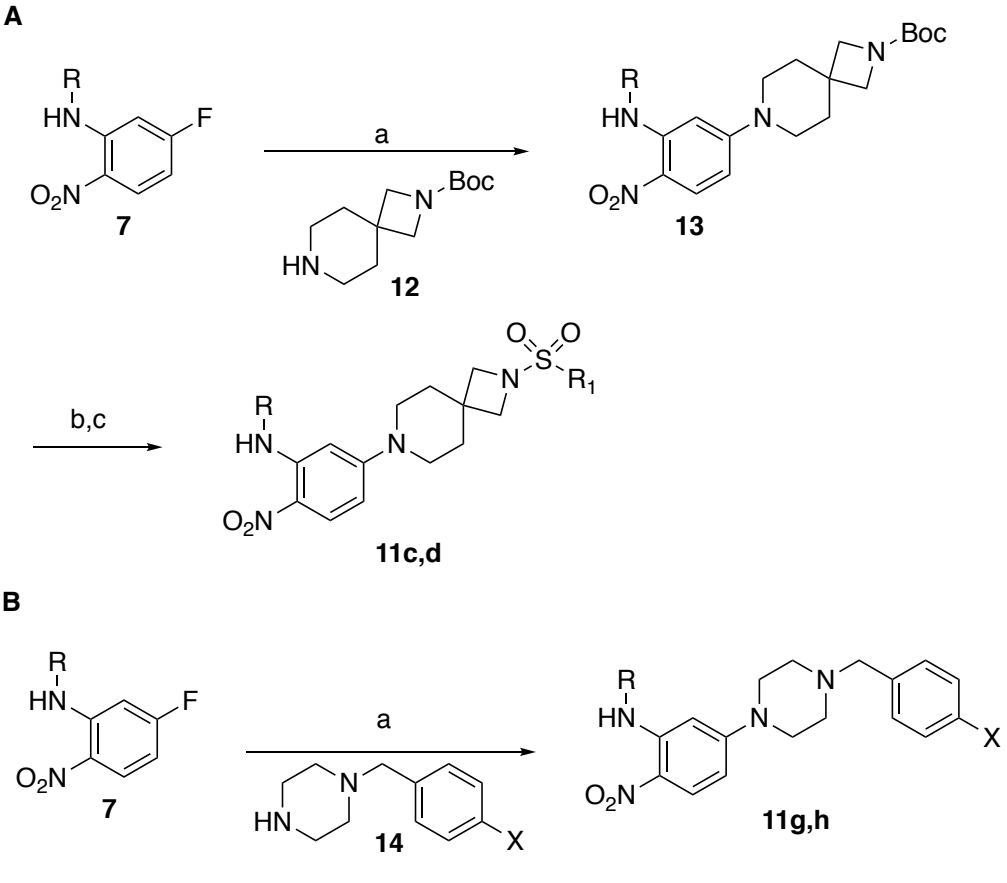
10j		F	3.89	1.77 ± 0.60	31
10k		Cl	4.14	2.17 ± 0.90	14
10l		Cl	3.42	3.94 ± 0.06	76.13
10m		Cl	3.42	4.49 ± 0.31	94.4
10n		Cl	3.32	3.63 ± 0.11	50.58
10o		Cl	3.94	2.61 ± 0.66	13.12
10p		F	3.19	63 ± 13	77
101		Cl	3.46	4.33 ± 0.14	96
10r		F	3.54	0.84 ± 0.07	103
10s		Cl	3.80	1.27 ± 0.05	101
10t		F	3.21	42.6 ± 15.0	69
10u		Cl	3.46	N/A	5

[a]Small-Molecule Drug Discovery Suite 2018-2, Schrödinger, LLC, New York, NY, 2018. [b]Thallium flux *Ae*Kir1 assay; IC_{50} values represent the average (mean ± SEM) of values obtained from at least three individual experiments.

After evaluating the left- and right-side of the molecule, we investigated chemical substitutions at the piperazine moiety. In order to achieve this, we varied the ring size (6- to 7-membered), introduced spirocyclic analogs, and evaluated both amide and methylene linked compounds. The seven-membered ring systems were synthesized as previously shown; however, the spirocyclic analogs and the methylene linked compounds' synthesis is shown in Scheme 2. The spirocyclic analogs, **11c,d**, were made by reacting **7** with the *tert*-butyl 2,7-diazaspiro[3.5]nonane-2-carboxylate, **12** under basic conditions (Et_3N, DMSO). The resulting Boc-protected amine, **13**, was treated with acid in order to remove the protecting group (4 M HCl, dioxane), followed by sulfonamide formation ($ClSO_2R_1$, DMAP, pyridine) to yield the desired

compounds, **11c,d**. The methylene linked compounds were made by S_NAr reaction of the commercially available reagents, **14**, and **7** as described above.

Scheme 2. Synthesis of spirocyclic and methylene linked analogs.[a]

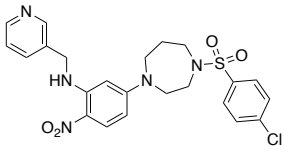
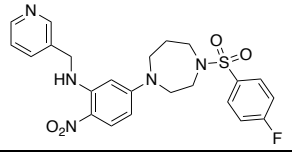
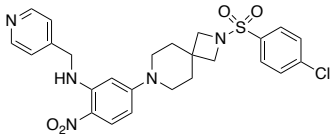
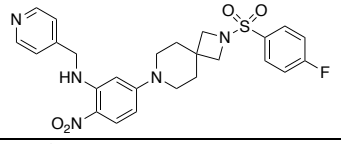
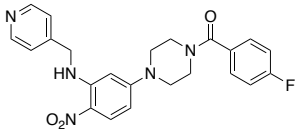
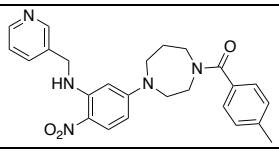
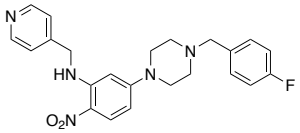
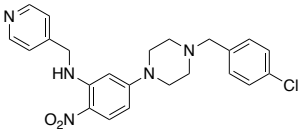


[a]Reagents and conditions. (a) Et_3N, DMSO; (b) 4 M HCl, dioxane; (c) R_1SO_2Cl, DMAP, pyridine.

The piperazine moiety was replaced with a seven-membered ring system (homopiperazine) and the spriocyclic [5.3.0] systems in order to assess the importance of the spatial orientation of the ring system. Either the 3-pyridyl or 4-pyridyl left-hand substituent were used in this study as both were potent. Unfortunately, both the homopiperazine (**11a,b**) and the spirocyclic (**11c,d**) systems were much less potent than the corresponding piperazine. To further evaluate the spatial orientation of the terminal phenyl group (right-hand side) amide bonds were synthesized replacing the sulfonamide systems. The amide bonds were suspected to be in a planar orientation whereas

the sulfonamide group imparts a distinct "kink" in the system. The amide derivatives were also less active (or inactive) (**11e,f**). Lastly, in order to introduce less planarity into the system, the sulfonamide/amide portion was replaced with a simple methylene linker (**11g,h**). Both of these analogs were more active than the amide compounds, and were similar in potency to the sulfonamide analogs, with a slight loss in potency for the 4-fluorophenyl compound (**11g**).

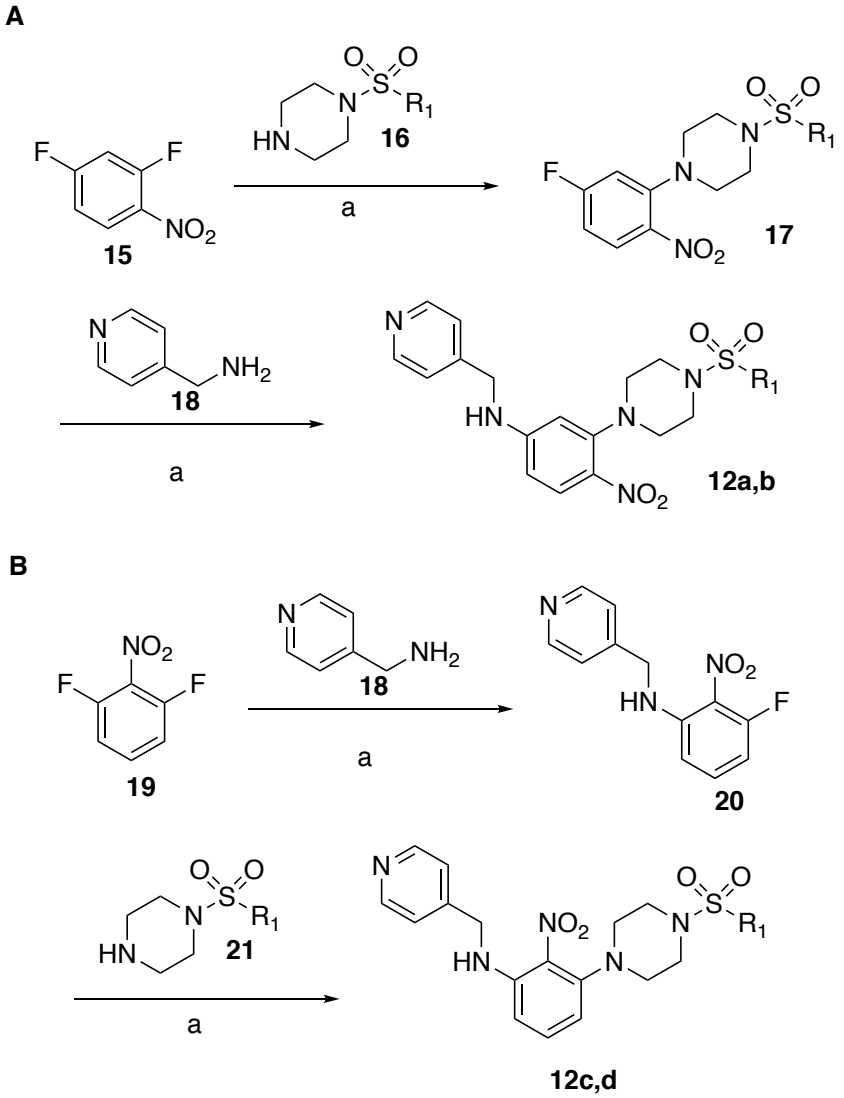
Table 3. SAR of the cyclic sulfonamide derivatives.

Cmpd	Structure	cLogP[a]	Th Flux IC_{50} ± SEM (μM)[b]	% Inhibition[b]
11a		3.76	3.48 ± 0.20	91
11b		3.52	2.74 ± 0.17	98
11c		4.65	6.18 ± 0.08	56
11d		4.16	8.66 ± 3.50	67
11e		3.90	9.33 ± 1.89	23
11f		4.29	>100	27
11g		4.15	1.92 ± 0.019	101
11h		4.41	1.95 ± 0.011	101

[a]Small-Molecule Drug Discovery Suite 2018-2, Schrödinger, LLC, New York, NY, 2018. [b]Thallium flux *Ae*Kir1 assay; IC_{50} values represent the average (mean ± SEM) of values obtained from at least three individual experiments.

Having evaluated the methylpyridine, piperazine and sulfonamide portions of the molecule, we next turned our attention to the nitrophenyl core of the molecule. First, the position of the nitro group was studied. To this end, the compounds **12a-d** were synthesized as outlined in Scheme 3A-B. The 3-(4-(sulfonyl)piperazin-1-yl)-4-nitro-*N*-(pyridin-4-ylmethyl)aniline, **12a,b**, were synthesized starting from the 2,4-difluoro-1-nitrobenzene, **15**, which was reacted with the appropriately substituted piperazinesulfonamide, **16**, to yield **17**. The final targets were realized by reaction of pyridine-4-ylmethanamine, **18**, with **17** under basic conditions (Et_3N, DMSO). In a similar fashion, 3-(4-(sulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-4-ylmethyl)aniline, **12c,d**, were synthesized by a slight change in the ordering of the reactions. Thus, 2,6-difluoro-1-nitrobenzene, **19**, was reacted with pyridine-4-ylmethanamine, **18**, to yield **20** which was then subjected to the piperazinesulfonamide, **21**, yielding the desired compounds, **12c,d**.

Scheme 3. Synthesis of nitro regioisomers, 12a-d.[a]

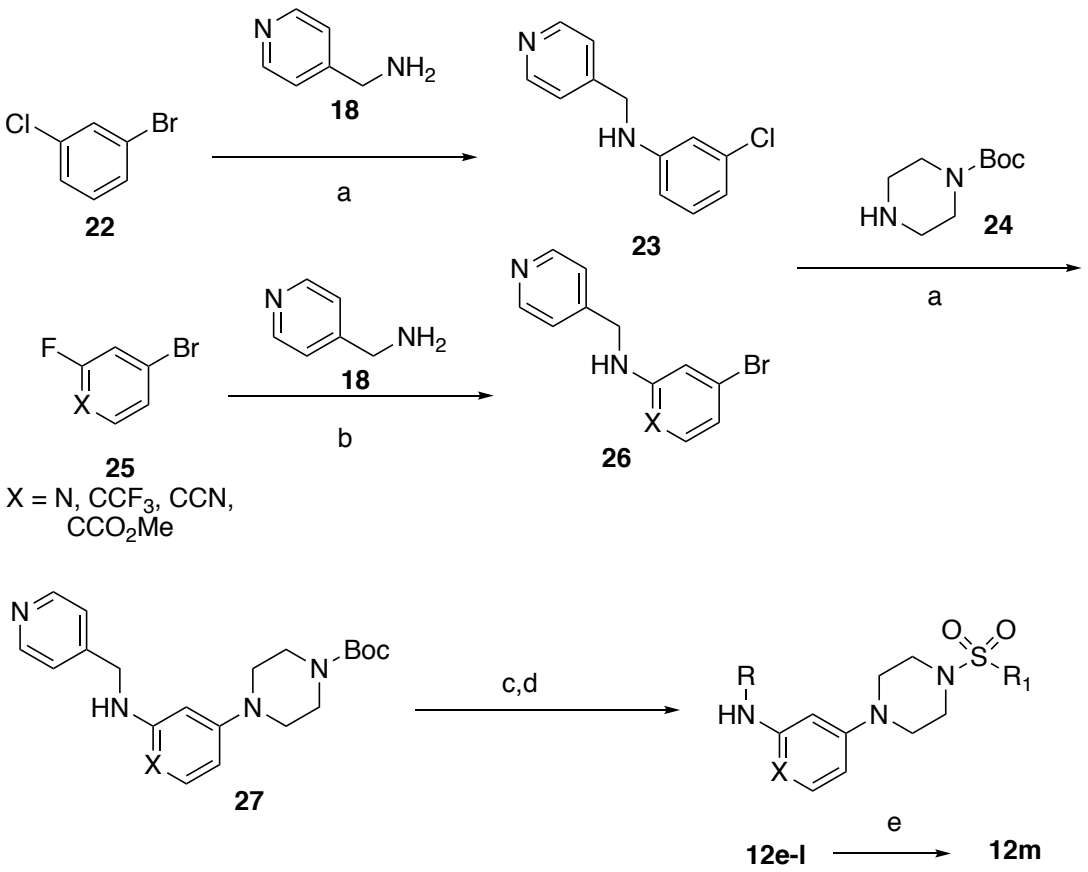


[a]Reagents and conditions. (a) Et_3N, DMSO

Replacement of the nitro group with other electron withdrawing groups, or hydrogen, were also evaluated and the synthesis of these analogs is shown in Scheme 4. The hydrogen analog was synthesized by starting with 3-chloro-bromobenzene, **22**, and reaction with the amine, **18**, under cross-coupling conditions ($Pd_2(dba)_3$, CyJohnPhos, Cs_2CO_3, 120 °C, μW) to yield **23** (selective bromo reaction). The electron withdrawing groups could be synthesized by reaction of **25** with **18** under the normal S_NAr reaction to yield **26**. The next reactions merged the two scaffolds (**23** and **26**) and these could be carried forward under similar conditions. Namely, **23** or **26** was reacted

with N-Bocpiperazine under cross-coupling conditions ($Pd_2(dba)_3$, CyJohnPhos, Cs_2CO_3, 120 °C, μW) to yield **27**. Next, the Boc protecting group was removed (4 M HCl, dioxane) and then the appropriate sulfonamide was formed ($ClSO_2R_1$, DMAP, pyridine) yielding the desired compounds, **12e-l**. Final compound **12m**, was completed by saponification of the ester moiety.

Scheme 4. Synthesis of non-nitro containing analogs, 12e-m.[a]

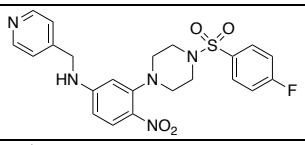
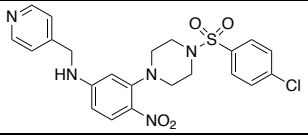
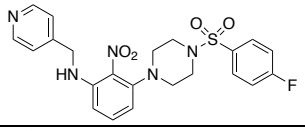
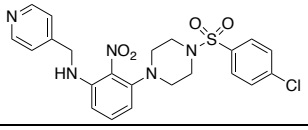
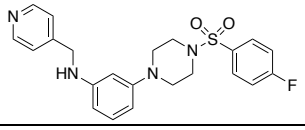
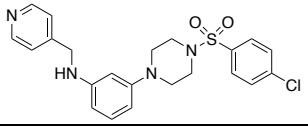
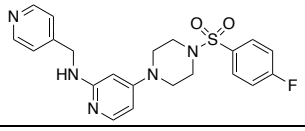
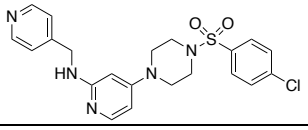
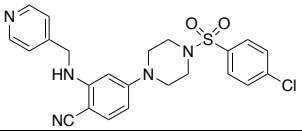


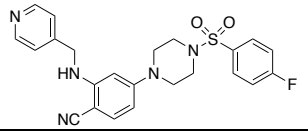
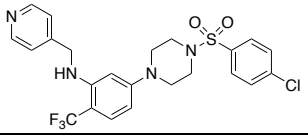
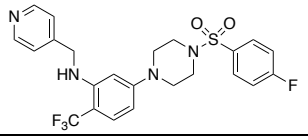
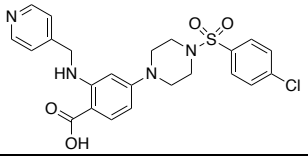
[a]Reagents and conditions. (a) $Pd_2(dba)_3$, CyJohnPhos, Cs_2CO_3, dioxane, 120 °C, μW; (b) Et_3N, DMSO; (c) 4 M HCl, dioxane; (d) R_1SO_2Cl, DMAP, pyridine; (e) LiOH, dioxane

Moving the nitro group ortho to the piperazine group was detrimental to the activity on the 4-fluorophenylsulfonamide, **12a**, but was equipotent with the 4-chlorophenylsulfonamide, **12b**. Placing the nitro group ortho to both amino groups was beneficial. Both **12c** and **12d** were submicromolar in activity. Next, removing the nitro group (**12e,f**) or replacing the nitrophenyl

with a pyridine (**12g,h**) or CF_3 (**12k,l**) were not productive changes as each of these compounds were micromolar (IC_{50}'s = 4 – 9 μM). However, replacing the nitro group with a cyano did produce potent compounds (**12i**, IC_{50} = 0.738 μM and **12j**, IC_{50} = 0.361 μM). Lastly, introduction of the carboxylic acid (**12m**) moiety had no effect on the potency compared to the nitro group.

Table 4. Evaluation of nitro replacements and regioisomers.

Cmpd	Structure	cLogP[a]	Thallium Flux IC_{50} ± SEM (μM)[b]	% Inhibition[b]
12a		3.41	ND	8
12b		3.66	1.20 ± 0.09	101
12c		3.51	0.833 ± 0.06	105
12d		3.76	0.246 ± 0.01	107
12e		3.95	9.63 ± 0.66	81
12f		4.21	4.01 ± 0.19	62
12g		3.59	4.96 ± 0.22	93
12h		3.85	3.69 ± 0.08	96
12i		3.56	0.738 ± 0.045	99

12j		3.31	0.361 ± 0.016	101
12k		5.13	2.70 ± 0.028	95
12l		4.87	3.24 ± 0.13	97
12m		3.87	2.02 ± 0.11	101

[a]Small-Molecule Drug Discovery Suite 2018-2, Schrödinger, LLC, New York, NY, 2018. [b]Thallium flux *Ae*Kir1 assay; IC_{50} values represent the average (mean ± SEM) of values obtained from at least three individual experiments.

Having identified a novel scaffold as *Ae*Kir1 inhibitors, we next evaluated these compounds in a manual patch clamp assay and compared them to our previously disclosed compound, **4**.[18] **4** was the first *Ae*Kir inhibitor that was shown to be active after topical administration to the adult female mosquito or addition to the larval rearing water; however, the *in vitro* potency for this compound was moderate (Thallium Flux = 1.7 μM; Patch clamp, IC_{50} = 238 nM). We have discovered a number of compounds that have significantly improved potency versus **4**. The 3- to 4-fold increase in potency seen in the thallium flux assay translated well to the manual patch clamp assay (Table 5). As we have seen in the past, the compounds are more potent in the patch clamp assay (left-shifted potency) and our best compound, **12j**, is ~9-fold more potent than **4**. In addition, we performed selectivity screening against hKir1.1 and hKir2.1 in thallium flux assays and found that these compounds were inactive, or weakly active (Table 5).

Table 5. Patch clamp and selectivity data for select compounds.

Cmpd	Thallium flux IC_{50} (μM)	Patch Clamp IC_{50} (nM)[a]	95% CI (nM)	hKir1.1, μM (% inhibition)[b]	hKir2.1, μM[b]
9j	0.47 ± 0.018	57.0	49.9-64.8	1.90 (27)	NA
10a	0.39 ± 0.24	49.7	36.2-67.6	NA	NA
10d	0.84 ± 0.07	34.2	23.6-44	NA	NA
10f	0.80 ± 0.25	35.5	29.2-42.7	NA	NA
12i	0.738 ± 0.045	39.0	22.2-64.3	NA	NA
12j	0.361 ± 0.016	27.6	18.3-39.5	7.0 (22)	NA
4	1.7 (0.6 – 2.8)	238	192-294		

[a]Manual patch clamp IC_{50} values represent the average (mean ± SEM) of values obtained from at least three individual experiments. [b]NA = not active

We next screened a selection of the newly developed compounds for larval toxicity at 100 μM and compared their efficacies to **4** and a small molecule insecticide that has been shown to inhibit insect Kir1 channels (flonicamid).[16] As shown in Figure 3A, all of the compounds had limited toxicity (<50% mortality) within 24 h, but **12j** was the most effective in inducing ~42% mortality. **4**, flonicamid, and the parent compound **5** only produced ~6%, 13%, and ~2% mortality, respectively, within 24 h. As we have seen previously with **4**[19], the toxicity of nearly all the compounds increased dramatically between 24 and 48 h, with the exceptions of flonicamid and **5**. Notably, **9j**, **12i**, **12j**, and **12k** all elicited >80% mortality by 48 h, which was significantly greater than flonicamid (12% mortality) and **5** (22% mortality) and statistically similar to **4** (67.5% mortality). In addition to larval toxicity, we compared the 24 h topical toxicity of **9j**, flonicamid, and **4** against adult female mosquitoes using a dose of 12.5 nmol/mosquito. As shown in Figure 3C, the efficacy of **9j** (49%) was statistically similar to that of flonicamid (58.7%) and significantly greater than that of **4** (17.5%). Thus, this new scaffold appears to not only improve the *in vitro* potency but also the *in vivo* efficacy against both larval and adult female mosquitoes.

A **B**

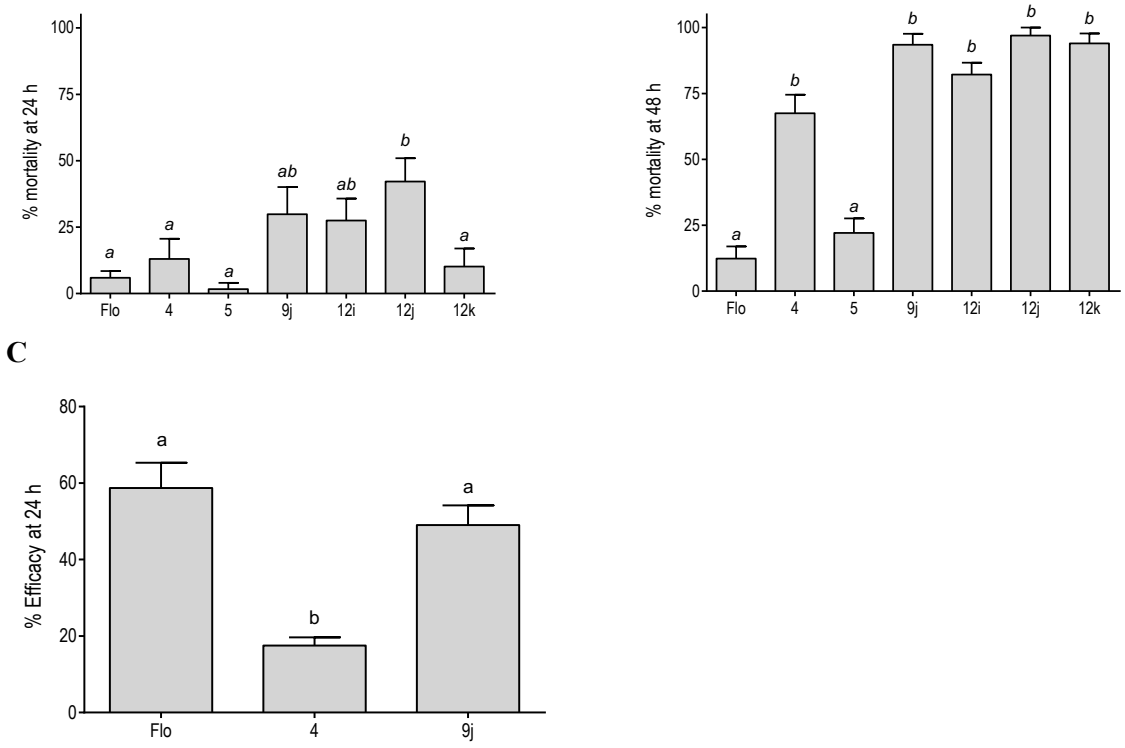


Figure 3. The 24 h (A) and 48 h (B) mortality of 1st instar *Ae. aegypti* after addition of small molecules (100 μM) to the rearing water. Values are means ± SEM based on 6-18 replicates of 6 larvae each. C) 24 h topical efficacy of small molecules (12.5 nmol/mosquito) against adult female *Ae. aegypti*. Values are means ± SEM based on 5-24 replicates of 10 adult females each. In all panels, Abbott's correction[21] was applied to the percent mortalities to account for the negative control mortality, and the lower-case letters indicate statistical categorization of the means as determined by a one-way ANOVA with a Tukey's multiple comparisons test ($P < 0.05$).

In conclusion, we report a novel scaffold as potent and efficacious *Ae*Kir1 channel inhibitors. The new scaffold is superior to the previously reported **4** in both *in vitro* potency and *in vivo* efficacy. Structure-activity relationship studies confirmed that the sulfonamide moiety was critical for activity. In addition, the nitro group was not required and the pyridylmethyl amine could be exchanged for other heterocyclic moieties. Further evaluation in patch clamp assay identified compounds that were ~10-fold more potent than our previously reported inhibitor and

with no activity against the closely related human Kir channels. Lastly, we have shown these compounds to be active against both mosquito larval and adult female mosquitoes, which expands the potential application of these molecules as novel insecticides. However, future studies will be needed to evaluate other chemical and toxicological properties of the molecules to determine their potential suitably as insecticides for field use, such as stability, biodegradability, cuticular penetration, and safety to non-target organisms (e.g,. mammals, beneficial insects, aquatic organisms).

EXPERIMENTAL SECTION

All ^{1}H & ^{13}C NMR spectra were recorded on Bruker AV-400 (500 MHz) instrument. Chemical shifts are reported in ppm relative to residual solvent peaks as an internal standard set to δH 7.26 or δC 77.0 ($CDCl_3$). Data are reported as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (Hz), and integration. Low resolution mass spectra were obtained on an Agilent 1260 LCMS with electrospray ionization, with a gradient of 5-95% MeCN in 0.1% formic acid water over 4 min. Analytical thin layer chromatography was performed on LuxPlate silica gel 60 F254 plates. Visualization was accomplished with UV light, and/or the use of ninhydrin, anisaldehyde and ceric ammonium molybdate solutions followed by charring on a hot-plate. Chromatography on silica gel was performed using Silica Gel 60 Å (230-400 mesh) from Sorbent Technologies. Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased from Aldrich Chemical Co. (or similar) and were used without purification. All reagents and solvents were commercial grade and purified prior to use when necessary. Microwave synthesis was performed on an Anton Paar Monowave 400 equipped with an autosampler.

Final compounds were purified on a Gilson preparative reversed-phase HPLC system comprised of a 322 aqueous pump with solvent-selection valve, 334 organic pump, GX-271 liquid hander, two column switching valves, and a 159 UV detector. UV wavelength for fraction collection was user-defined, with absorbance at 254 nm always monitored. Column: Phenomenex Axia-packed Luna C18, 50 x 21.2 mm, 5 μm. For Acidic Method: Mobile phase: CH_3CN in H_2O (0.1% formic acid). Gradient conditions: 2.0 min equilibration, followed by user-defined gradient (starting organic percentage, ending organic percentage, duration, typically 15 mins), hold at 95% CH_3CN in H_2O (0.1% TFA) for 2 min, 20 mL/min, 23 °C. For Basic Method: Mobile phase: CH_3CN in H_2O (0.05% v/v NH_4OH). Gradient conditions: 0.75 min equilibration, followed by user-defined gradient (starting organic percentage, ending organic percentage, duration), hold at 95% CH_3CN in H_2O (0.05% v/v NH_4OH) for 1 min, 50 mL/min, 23 °C. The purity of all compounds was determined by LCMS to be >95% (or as stated).

General Procedure for the Synthesis of 5, 9a-k, 10a-v, 11a,b,e,f, 12a-d. To a two-dram vial was added 2,4-difluoronitrobenzene, **6**, (10.8 μL, 0.100 mmol), dimethyl sulfoxide (1.00 mL), triethylamine (20.8 μL, 0.150 mmol), followed by the amine (0.100 mmol) of interest. After stirring at room temperature for 16 h, triethylamine (20.8 μL, 0.150 mmol) and the cyclic diamine (0.150 mmol) of interest were added. The reaction was stirred for an additional 16 h at room temperature. The reaction was diluted with ethyl acetate (15 mL) and washed with 1 M HCl (15 mL). The aqueous layer was basified with 1 M NaOH to pH 9 and extracted with ethyl acetate (15 mL) twice. Organic layers were combined and washed with saturated brine (15 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product, **8**, was used without further purification.

To a two-dram vial was added the starting compound, **8**, (0.100 mmol), dichloromethane (3.00 mL), triethylamine (20.8 µL, 0.150 mmol), and the electrophile (R_1SO_2Cl or R_1COCl) (0.150 mmol) of interest. After stirring at room temperature for 16 h, the reaction was diluted with dichloromethane (15 mL) and washed with water (15 mL). The aqueous layer was extracted with dichloromethane (15 mL) twice. Organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude products were purified by flash column chromatography on silica gel (dry loaded using silica/DCM) with a gradient of 0-20% methanol in dichloromethane to yield the final compounds (**5**, **9a-k**, **10a-v**, **11a-b**).

5-(4-((4-chlorophenyl)sulfonyl)piperazine-1-yl)-2-nitro-*N*-(pyridine-3-ylmethyl)aniline (5). Yield = 12 mg; 23%. LCMS: R_T = 2.474 min., >98% @ 215 and 254 nm, *m/z* = 488.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.78 (d, *J* = 5.9 Hz, 1H), 8.56 (s, 1H), 8.43 (d, *J* = 4.0 Hz, 1H), 7.83 (d, *J* = 9.7 Hz, 1H), 7.78 – 7.60 (m, 5H), 7.30 (dd, *J* = 7.6, 4.9 Hz, 1H), 6.28 (dd, *J* = 9.7, 1.9 Hz, 1H), 5.88 (s, 1H), 4.56 (d, *J* = 5.9 Hz, 2H), 3.42 (s, 4H), 2.87 (s, 4H).^{13}C NMR (126 MHz, DMSO-d_6) δ 155.37, 149.83, 149.34, 147.53, 139.36, 136.05, 135.14, 134.57, 130.61, 130.33, 129.26, 124.55, 124.38, 105.60, 95.30, 46.63, 46.01, 44.01.

5-(4-(benzylsulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-3-ylmethyl)aniline (9a). Compound was purchased from ChemDiv. LCMS: R_T = 2.297 min., >98% @ 215 and 254 nm, *m/z* = 468.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.75 (t, *J* = 5.0 Hz, 1H), 8.63 (s, 1H), 8.57 (d, *J* = 3.7 Hz, 1H), 8.09 (d, *J* = 9.6 Hz, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.38 (s, 5H), 7.31 (dd, *J* = 7.7, 4.9 Hz, 1H), 6.18 (dd, *J* = 9.7, 2.3 Hz, 1H), 5.76 (d, *J* = 2.1 Hz, 1H), 4.53 (d, *J* = 5.4 Hz, 2H), 4.25 (s, 2H), 3.18 (dd, *J* = 22.3, 5.3 Hz, 8H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.47, 149.31, 148.80, 146.85, 134.72, 133.03, 130.67, 129.18, 129.02, 128.94, 128.46, 125.26, 123.86, 104.90, 95.18, 57.49, 47.36, 45.36, 44.63.

5-(4-((4-bromophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-3-ylmethyl)aniline (9b). Compound was purchased from ChemDiv. LCMS: R_T = 2.521 min., >98% @ 215 and 254 nm, *m/z* = 532.0 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.74 (t, *J* = 5.1 Hz, 1H), 8.62 (s, 1H), 8.57 (d, *J* = 3.8 Hz, 1H), 8.08 (d, *J* = 9.6 Hz, 1H), 7.68 (dd, *J* = 15.0, 8.2 Hz, 3H), 7.61 (d, *J* = 8.5 Hz, 2H), 7.30 (dd, *J* = 7.7, 4.9 Hz, 1H), 6.17 (dd, *J* = 9.7, 2.2 Hz, 1H), 5.77 (d, *J* = 1.9 Hz, 1H), 4.52 (d, *J* = 5.4 Hz, 2H), 3.40 – 3.32 (m, 4H), 3.14 – 3.04 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.18, 149.32, 148.79, 146.80, 134.69, 134.40, 133.80, 133.02, 132.62, 129.25, 129.21, 128.46, 123.87, 104.75, 95.28, 46.67, 45.38, 44.63.

5-(4-(ethylsulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-3-ylmethyl)aniline (9c). Compound was purchased from ChemDiv. LCMS: R_T = 1.990 min., >98% @ 215 and 254 nm, *m/z* = 406.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.68 (s, 1H), 8.59 (d, *J* = 3.8 Hz, 1H), 8.13 (d, *J* = 9.6 Hz, 1H), 7.76 (d, *J* = 7.7 Hz, 1H), 7.38 (dd, *J* = 7.4, 5.0 Hz, 1H), 6.25 (dd, *J* = 9.6, 2.0 Hz, 1H), 5.82 (d, *J* = 1.7 Hz, 1H), 4.58 (d, *J* = 5.3 Hz, 2H), 3.37 (s, 8H), 2.98 (q, *J* = 7.4 Hz, 2H), 1.38 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.47, 148.40, 147.97, 146.78, 135.58, 133.66, 129.30, 125.35, 124.19, 104.98, 95.23, 47.36, 45.15, 44.55, 44.42, 7.82.

5-(4-(methylsulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-3-ylmethyl)aniline (9d). Yield = 15 mg; 36%. LCMS: R_T = 1.899 min., >98% @ 215 and 254 nm, *m/z* = 392.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.80 (s, 1H), 8.60 (s, 1H), 8.43 (d, *J* = 3.8 Hz, 1H), 7.89 (d, *J* = 9.7 Hz, 1H), 7.76 (d, *J* = 7.7 Hz, 1H), 7.33 (dd, *J* = 7.5, 4.9 Hz, 1H), 6.38 (dd, *J* = 9.7, 1.8 Hz, 1H), 5.98 (s, 1H), 4.61 (d, *J* = 5.9 Hz, 2H), 3.44 (s, 4H), 3.09 (d, *J* = 4.3 Hz, 4H), 2.83 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.75, 149.86, 149.36, 147.67, 136.08, 135.17, 129.28, 124.62, 124.39, 105.78, 95.31, 46.90, 45.64, 44.08, 34.98.

2-nitro-N-(pyridin-3-ylmethyl)-5-(4-tosylpiperazin-1-yl)aniline (9e). Yield = 34 mg; 70%. LCMS: R_T = 2.386 min., >98% @ 215 and 254 nm, *m/z* = 468.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.66 (s, 1H), 8.53 (d, *J* = 3.7 Hz, 1H), 7.91 (d, *J* = 9.7 Hz, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 8.1 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.39 (dd, *J* = 7.5, 4.9 Hz, 1H), 6.35 (dd, *J* = 9.7, 1.8 Hz, 1H), 5.96 (d, *J* = 1.6 Hz, 1H), 4.64 (d, *J* = 5.9 Hz, 2H), 3.51 (s, 4H), 2.89 (s, 4H), 2.43 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.30, 149.84, 149.35, 147.52, 144.84, 136.04, 135.13, 132.55, 130.87, 129.27, 128.48, 124.55, 124.33, 105.55, 95.27, 46.65, 46.06, 44.00, 21.93.

2-nitro-5-(4-(phenylsulfonyl)piperazin-1-yl)-*N*-(pyridin-3-ylmethyl)aniline (9f). Compound was purchased from ChemDiv. LCMS: R_T = 2.289 min., >98% @ 215 and 254 nm, *m/z* = 454.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.76 – 8.69 (m, 1H), 8.62 (s, 1H), 8.57 (d, *J* = 3.8 Hz, 1H), 8.07 (d, *J* = 9.6 Hz, 1H), 7.76 (d, *J* = 7.4 Hz, 2H), 7.64 (dd, *J* = 19.8, 7.6 Hz, 2H), 7.56 (t, *J* = 7.6 Hz, 2H), 7.29 (dd, *J* = 7.7, 4.9 Hz, 1H), 6.16 (dd, *J* = 9.7, 2.3 Hz, 1H), 5.77 (d, *J* = 1.9 Hz, 1H), 4.51 (d, *J* = 5.3 Hz, 2H), 3.45 – 3.30 (m, 4H), 3.12 – 3.02 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.25, 149.31, 148.80, 146.82, 135.32, 134.70, 133.25, 133.02, 129.27, 129.21, 127.76, 125.28, 123.85, 104.73, 95.15, 46.66, 45.44, 44.63.

5-(4-((3,4-dichlorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-3-ylmethyl)aniline (9g). Yield = 34.1 mg; 8%. LCMS: R_T = 2.637 min., >98% @ 215 and 254 nm, *m/z* = 522.0 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.74 (s, 1H), 8.62 (s, 1H), 8.57 (s, 1H), 8.09 (d, *J* = 9.6 Hz, 1H), 7.84 (s, 1H), 7.65 (dd, *J* = 16.8, 8.0 Hz, 2H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.35 – 7.27 (m, 1H), 6.17 (d, *J* = 9.5 Hz, 1H), 5.78 (s, 1H), 4.52 (d, *J* = 4.4 Hz, 2H), 3.37 (s, 4H), 3.11 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.11, 149.32, 148.78, 146.79, 138.33, 135.31, 134.71, 134.17, 133.02, 131.36, 129.52, 129.27, 126.68, 125.43, 123.88, 104.74, 95.36, 46.69, 45.38, 44.63.

2-nitro-N-(pyridin-3-ylmethyl)-5-(4-((4-(trifluoromethyl)phenyl)sulfonyl)piperazin-1-yl)aniline (9h). Yield = 89.1 mg; 21%. LCMS: R_T = 2.551 min., >98% @ 215 and 254 nm, *m/z* = 522.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.73 (s, 1H), 8.62 (s, 1H), 8.57 (s, 1H), 8.08 (d, *J* = 9.6 Hz, 2H), 7.89 (d, *J* = 8.0 Hz, 2H), 7.83 (d, *J* = 8.0 Hz, 2H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.33 – 7.26 (m, 1H), 6.16 (d, *J* = 9.5 Hz, 1H), 5.77 (s, 2H), 4.52 (d, *J* = 4.6 Hz, 2H), 3.36 (s, 4H), 3.12 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.17, 149.33, 148.78, 146.78, 139.18, 134.84, 134.69, 133.02, 129.24, 128.24, 126.47, 125.44, 123.88, 121.98, 104.76, 95.38, 46.73, 45.39, 44.62.

5-(4-(naphthalen-2-ylsulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-3-ylmethyl)aniline (9i). Compound was purchased from ChemDiv. LCMS: R_T = 2.559 min., >98% @ 215 and 254 nm, *m/z* = 504.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.71 (t, *J* = 4.8 Hz, 1H), 8.60 (s, 1H), 8.54 (d, *J* = 3.9 Hz, 1H), 8.34 (s, 1H), 8.04 (d, *J* = 9.7 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 2H), 7.93 (d, *J* = 7.9 Hz, 1H), 7.73 (d, *J* = 8.6 Hz, 1H), 7.67 (dd, *J* = 17.9, 7.7 Hz, 3H), 7.29 – 7.24 (m, 1H), 6.14 (dd, *J* = 9.7, 2.1 Hz, 1H), 5.74 (d, *J* = 1.8 Hz, 1H), 4.49 (d, *J* = 5.3 Hz, 2H), 3.41 – 3.32 (m, 4H), 3.21 – 3.09 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.18, 149.28, 148.79, 146.81, 135.04, 134.68, 133.01, 132.39, 132.20, 129.50, 129.25, 129.20, 129.15, 127.99, 127.82, 125.23, 123.82, 122.76, 104.69, 95.09, 46.68, 45.50, 44.61.

5-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-3-ylmethyl)aniline (9j). Yield = 102.8 mg; 31%. LCMS: R_T = 2.326 min., >98% @ 215 and 254 nm, *m/z* = 472.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.73 (s, 1H), 8.62 (s, 1H), 8.57 (d, *J* = 3.9 Hz, 1H), 8.08 (d, *J* = 9.6 Hz, 1H), 7.82 – 7.73 (m, 2H), 7.67 (d, *J* = 7.3 Hz, 1H), 7.35 – 7.17 (m, 3H), 6.17 (d, *J* = 9.7 Hz, 1H), 5.77 (s, 1H), 4.52 (d, *J* = 5.0 Hz, 2H), 3.36 (s, 4H), 3.08 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.22, 149.30, 148.79, 146.81, 134.70, 133.03, 130.51, 130.43, 129.22, 125.35, 123.87, 116.70, 116.52, 104.75, 95.25, 46.66, 45.40, 44.62.

5-(4-((4-(tert-butyl)phenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-3-ylmethyl)aniline (9k). Compound was purchased from ChemDiv. LCMS: R_T = 2.725 min., >98% @ 215 and 254 nm, *m/z* = 510.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.86 (t, *J* = 5.8 Hz, 1H), 8.66 (s, 1H), 8.52 (d, *J* = 3.5 Hz, 1H), 7.91 (d, *J* = 9.7 Hz, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.74 – 7.61 (m, 4H), 7.38 (dd, *J* = 7.5, 5.0 Hz, 1H), 6.36 (dd, *J* = 9.8, 1.7 Hz, 1H), 5.98 (s, 1H), 4.64 (d, *J* = 5.8 Hz, 2H), 3.51 (s, 4H), 2.91 (s, 4H), 1.30 (d, *J* = 34.1 Hz, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.38, 155.38, 149.84, 149.34, 147.53, 136.04, 135.14, 132.63, 129.26, 128.43, 127.24, 124.53, 124.34, 105.56, 95.26, 46.62, 46.15, 44.01, 35.85, 31.64.

5-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-4-ylmethyl)aniline (10a). Yield = 90.4 mg; 27%. LCMS: R_T = 2.192 min., >98% @ 215 and 254 nm, *m/z* = 472.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.87 (t, *J* = 6.0 Hz, 1H), 8.52 (d, *J* = 5.6 Hz, 2H), 7.90 (d, *J* = 9.7 Hz, 1H), 7.80 (dd, *J* = 8.6, 5.0 Hz, 2H), 7.49 (t, *J* = 8.7 Hz, 2H), 7.35 (d, *J* = 5.3 Hz, 2H), 6.34 (d, *J* = 9.8 Hz, 1H), 5.83 (s, 1H), 4.63 (d, *J* = 6.1 Hz, 2H), 3.43 (s, 4H), 2.88 (s, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 150.24, 150.21, 146.63, 146.21, 131.12, 130.93, 122.75, 117.34, 117.16, 105.20, 95.47, 95.17, 95.01, 46.15, 45.55, 44.99.

5-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-4-ylmethyl)aniline (10b). Yield = 208.7 mg; 60%. LCMS: R_T = 2.286 min., >98% @ 215 and 254 nm, *m/z* = 488.1 $[M + H]^+$. NMR (499 MHz, $CDCl_3$) δ 8.81 (s, 1H), 8.59 (d, *J* = 4.3 Hz, 2H), 8.09 (d, *J* = 9.6 Hz, 1H), 7.72 (s, 1H), 7.67 (d, *J* = 7.9 Hz, 2H), 7.53 (d, *J* = 8.0 Hz, 2H), 6.17 (d, *J* = 9.6 Hz, 1H), 5.62 (s, 1H), 4.53 (d, *J* = 5.1 Hz, 2H), 3.32 (s, 4H), 3.03 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.06, 151.30, 150.39, 146.85, 139.98, 133.77, 129.65, 129.26, 129.12, 125.37, 121.65, 104.85, 95.34, 46.62, 45.98, 45.30.

5-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-*N*-(furan-2-ylmethyl)-2-nitroaniline (10c). Yield = 60 mg; 39%. LCMS: R_t = 2.836 min, >98% @ 215 and 254 nm, *m/z* = 461.1 [M

+ H]$^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.08 (d, *J* = 9.6 Hz, 1H), 7.87 – 7.76 (m, 2H), 7.41 (d, *J* = 11.2 Hz, 2H), 7.26 (d, *J* = 7.7 Hz, 1H), 6.37 (s, 1H), 6.29 (s, 1H), 6.19 (d, *J* = 9.6 Hz, 1H), 6.01 (s, 1H), 4.48 (d, *J* = 4.1 Hz, 2H), 3.48 (s, 4H), 3.16 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.16, 150.91, 146.91, 142.29, 130.50, 130.42, 129.08, 125.30, 125.02, 116.69, 116.51, 110.60, 107.60, 104.66, 95.14, 46.77, 45.46, 40.28, 29.71.

5-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-*N*-(furan-2-ylmethyl)-2-nitroaniline (10d). Yield = 22.2 mg; 29%. LCMS: R_t = 2.942 min, >98% @ 215 and 254 nm, *m/z* = 477.0 [M + H]$^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.06 (d, *J* = 9.6 Hz, 1H), 7.71 (d, *J* = 7.8 Hz, 2H), 7.53 (d, *J* = 7.9 Hz, 2H), 7.37 (s, 1H), 6.35 (s, 1H), 6.26 (s, 1H), 6.16 (d, *J* = 9.6 Hz, 1H), 5.98 (s, 1H), 4.46 (d, *J* = 4.5 Hz, 2H), 3.45 (s, 4H), 3.13 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.14, 150.91, 146.91, 142.30, 139.96, 133.91, 129.63, 129.14, 129.09, 125.32, 110.61, 107.61, 104.67, 95.16, 46.78, 45.45, 40.28.

***N*-benzyl-5-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-2-nitroaniline (10e).** Compound was purchased from ChemDiv. LCMS: R_t = 3.051 min, >98% @ 215 and 254 nm, *m/z* = 487.0 [M + H]$^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.07 (d, *J* = 9.6 Hz, 1H), 7.68 (d, *J* = 8.5 Hz, 2H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.40 – 7.26 (m, 5H), 6.13 (dd, *J* = 9.6, 2.1 Hz, 1H), 5.79 (d, *J* = 1.7 Hz, 1H), 4.49 (d, *J* = 5.2 Hz, 2H), 3.40 – 3.28 (m, 4H), 3.09 – 3.01 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.97, 147.19, 139.94, 137.46, 133.90, 129.61, 129.11, 129.07, 128.96, 127.68, 126.98, 125.21, 104.53, 95.72, 47.22, 46.72, 45.32.

5-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-2-ylmethyl)aniline (10f). Yield = 7.1 mg; 4.6%. LCMS: R_t = 2.615 min, >98% @ 215 and 254 nm, *m/z* = 472.1 [M + H]$^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 9.17 (s, 1H), 8.59 (s, 1H), 7.91 (d, *J* = 9.6 Hz, 1H), 7.83 (dd, *J* = 15.8, 7.2 Hz, 2H), 7.51 (t, *J* = 8.7 Hz, 2H), 7.42 (d, *J* = 7.4 Hz, 1H), 7.35 (d, *J* = 4.8 Hz,

1H), 6.36 (d, *J* = 10.0 Hz, 1H), 6.07 (s, 1H), 4.65 (d, *J* = 4.7 Hz, 2H), 3.52 (s, 4H), 2.96 (s, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.81, 155.52, 149.83, 147.65, 137.95, 131.63, 131.55, 129.18, 124.31, 123.45, 122.89, 117.79, 117.61, 105.50, 95.61, 48.40, 46.63, 46.16.

5-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-2-ylmethyl)aniline (10g). Yield = 6.0 mg; 3.6%. LCMS: R_T = 2.728 min., >98% @ 215 and 254 nm, *m/z* = 488.0 [M + H]$^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 9.20 (t, *J* = 5.2 Hz, 1H), 8.61 (d, *J* = 4.7 Hz, 1H), 7.94 (d, *J* = 9.7 Hz, 1H), 7.89 – 7.72 (m, 5H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.37 (dd, *J* = 7.0, 5.3 Hz, 1H), 6.39 (dd, *J* = 9.8, 2.5 Hz, 1H), 6.09 (d, *J* = 2.4 Hz, 1H), 4.68 (d, *J* = 5.2 Hz, 2H), 3.59 – 3.49 (m, 4H), 3.11 – 2.96 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.82, 155.51, 149.82, 147.65, 139.37, 137.93, 134.61, 130.62, 130.38, 129.17, 124.31, 123.45, 122.89, 105.50, 95.60, 48.40, 46.63, 46.12.

5-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-((tetrahydro-2H-pyran-3-yl)methyl)aniline (10h). Yield = 118.5 mg; 74%. LCMS: R_T = 2.812 min, >98% @ 215 and 254 nm, *m/z* = 479.1 [M + H]$^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.04 (d, *J* = 9.6 Hz, 1H), 7.84 – 7.76 (m, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 6.14 (d, *J* = 9.7 Hz, 1H), 5.87 (s, 1H), 3.91 (d, *J* = 11.4 Hz, 1H), 3.86 – 3.77 (m, 1H), 3.57 – 3.43 (m, 5H), 3.40 – 3.29 (m, 1H), 3.28 – 3.05 (m, 6H), 2.10 – 1.90 (m, 2H), 1.65 (d, *J* = 38.1 Hz, 2H), 1.44 (d, *J* = 9.5 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.49, 164.45, 155.32, 147.64, 131.47, 130.52, 130.45, 129.12, 124.97, 116.70, 116.52, 104.39, 94.57, 70.96, 68.58, 46.78, 45.56, 44.89, 35.53, 27.46, 24.57.

5-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-((tetrahydro-2H-pyran-3-yl) methyl)aniline (10i). Yield = 48.8 mg; 30%. LCMS: R_T = 2.931 min, >98% @ 215 and 254 nm, *m/z* = 495.1 [M + H]$^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.05 (d, *J* = 9.6 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 2H), 7.54 (d, *J* = 8.2 Hz, 2H), 6.14 (d, *J* = 9.6 Hz, 1H), 5.87 (s, 1H), 3.91 (d,

J = 11.4 Hz, 1H), 3.86 – 3.78 (m, 1H), 3.50 (d, *J* = 24.2 Hz, 5H), 3.35 (s, 1H), 3.21 (dd, *J* = 12.6, 6.5 Hz, 2H), 3.16 (s, 4H), 2.06 – 1.89 (m, 2H), 1.66 (d, *J* = 32.7 Hz, 2H), 1.44 (d, *J* = 9.1 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.29, 147.63, 139.96, 133.88, 129.62, 129.15, 129.13, 124.99, 104.38, 94.59, 70.96, 68.58, 46.79, 45.53, 44.89, 35.52, 27.45, 24.55.

***N*-(3,3-difluorocyclobutyl)-5-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-2-nitroaniline (10j).** Yield = 29 mg; 20%. LCMS: R_T = 2.894 min, >98% @ 215 and 254 nm, *m/z* = 471.0 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.33 (d, *J* = 5.2 Hz, 1H), 7.95 (d, *J* = 9.7 Hz, 1H), 7.92 – 7.85 (m, 2H), 7.54 (t, *J* = 8.7 Hz, 2H), 6.44 (d, *J* = 9.8 Hz, 1H), 5.89 (s, 1H), 4.11 (s, 2H), 3.59 (s, 4H), 3.24 – 3.11 (m, 2H), 3.04 (s, 4H), 2.69 (d, *J* = 15.6 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.40, 146.32, 131.61, 131.59, 131.20, 131.12, 128.81, 124.03, 117.33, 117.15, 105.45, 94.79, 46.17, 45.89, 42.70, 36.95.

5-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-N-(3,3-difluorocyclobutyl)-2-nitroaniline (10k). Yield = 79 mg; 51%. LCMS: R_T = 2.983 min, >98% @ 215 and 254 nm, *m/z* = 487.0 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.07 (d, *J* = 9.5 Hz, 1H), 7.72 (d, *J* = 7.6 Hz, 2H), 7.54 (d, *J* = 7.7 Hz, 2H), 6.20 (d, *J* = 9.6 Hz, 1H), 5.66 (s, 1H), 3.91 (s, 1H), 3.47 (s, 4H), 3.24 – 3.04 (m, 6H), 2.67 – 2.50 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.29, 145.87, 140.02, 133.86, 129.65, 129.28, 129.15, 125.37, 104.88, 94.89, 46.73, 45.48, 43.57, 43.39, 43.20.

5-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-*N*-cyclopropyl-2-nitroaniline (10l). Yield = 4 mg; 7%. LCMS: R_T = 3.035 min, >98% @ 215 and 254 nm, *m/z* = 437.0 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.25 (d, *J* = 1.9 Hz, 1H), 7.91 (d, *J* = 9.8 Hz, 1H), 7.84 – 7.78 (m, 3H), 7.80 – 7.74 (m, 2H), 6.47 – 6.40 (m, 2H), 3.65 – 3.53 (m, 4H), 3.13 – 3.03 (m, 4H), 2.62 (td, *J* = 6.6, 2.9 Hz, 1H), 0.89 (dd, *J* = 6.7, 1.9 Hz, 2H), 0.60 (dd, *J* = 3.6, 2.3 Hz, 2H). ^{13}C NMR (126

MHz, DMSO-d_6) δ 155.65, 148.93, 139.37, 134.61, 130.61, 130.40, 128.93, 124.25, 105.99, 95.94, 46.61, 46.28, 25.15, 8.24.

5-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-((tetrahydrofuran-2-yl) methyl)aniline (10m). Yield = 17 mg; 35%. LCMS: R_T = 2.966 min, >98% @ 215 and 254 nm, *m/z* = 481.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.45 (t, *J* = 4.8 Hz, 1H), 7.91 (d, *J* = 9.7 Hz, 1H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.77 (d, *J* = 8.6 Hz, 2H), 6.38 (dd, *J* = 9.8, 1.9 Hz, 1H), 6.09 (d, *J* = 1.6 Hz, 1H), 4.11 (dd, *J* = 6.8, 3.8 Hz, 1H), 3.83 (dd, *J* = 14.5, 7.0 Hz, 1H), 3.71 (dd, *J* = 14.5, 7.3 Hz, 1H), 3.58 (s, 4H), 3.52 – 3.44 (m, 1H), 3.32 – 3.24 (m, 1H), 3.05 (s, 4H), 2.07 – 1.97 (m, 1H), 1.95 – 1.83 (m, 2H), 1.71 – 1.58 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.78, 148.38, 139.37, 134.61, 130.61, 130.39, 129.08, 124.08, 105.62, 94.85, 77.45, 68.34, 47.14, 46.63, 46.29, 29.53, 26.25.

1-((4-chlorophenyl)sulfonyl)-4-(4-nitro-3-(pyrrolidin-1-yl)phenyl)piperazine (10n). Compound was purchased from ChemDiv. LCMS: R_T = 3.000 min, >98% @ 215 and 254 nm, *m/z* = 451.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 7.78 (d, *J* = 9.2 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 2H), 7.54 (d, *J* = 8.5 Hz, 2H), 6.20 (dd, *J* = 9.3, 2.0 Hz, 1H), 6.08 (d, *J* = 1.9 Hz, 1H), 3.45 – 3.35 (m, 4H), 3.27 – 3.11 (m, 8H), 2.05 – 1.92 (m, 4H), 1.56 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.79, 145.12, 139.88, 133.93, 130.18, 129.59, 129.24, 129.17, 104.41, 99.65, 50.78, 47.60, 45.65, 25.73.

5-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-N-isobutyl-2-nitroaniline (10o). Yield = 3 mg; 7%. LCMS: R_T = 3.189 min, >98% @ 215 and 254 nm, *m/z* = 453.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.42 (t, *J* = 5.3 Hz, 1H), 7.91 (d, *J* = 9.8 Hz, 1H), 7.84 – 7.78 (m, 2H), 7.78 – 7.75 (m, 2H), 6.38 (dd, *J* = 9.8, 2.5 Hz, 1H), 6.01 (d, *J* = 2.4 Hz, 1H), 3.62 – 3.53 (m, 4H), 3.20 – 3.12 (m, 2H), 3.08 – 3.02 (m, 4H), 1.96 (dp, *J* = 13.4, 6.7 Hz, 1H), 0.99 (d, *J* = 6.7 Hz, 6H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 155.83, 148.47, 139.37, 134.59, 130.61, 130.39, 129.11, 123.95, 105.61, 94.54, 50.38, 46.62, 46.30, 28.20, 21.09.

1-((4-fluorophenyl)sulfonyl)-4-(4-nitro-3-(pyridin-2-ylmethoxy)phenyl)piperazine (10p). Yield = 8 mg; 14%. LCMS: R_T = 2.209 min, >98% @ 215 and 254 nm, *m/z* = 473.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.65 (d, *J* = 5.9 Hz, 2H), 8.01 (d, *J* = 9.3 Hz, 1H), 7.80 (dd, *J* = 8.8, 5.0 Hz, 2H), 7.44 (d, *J* = 5.6 Hz, 2H), 6.44 (dd, *J* = 9.4, 2.4 Hz, 1H), 6.29 (d, *J* = 2.3 Hz, 1H), 5.18 (s, 2H), 3.48 – 3.41 (m, 4H), 3.20 – 3.12 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.39, 164.35, 154.13, 150.31, 131.72, 131.70, 130.47, 130.39, 129.23, 121.37, 116.58, 116.40, 108.07, 107.53, 45.86, 28.30.

1-((4-chlorophenyl)sulfonyl)-4-(4-nitro-3-(pyridin-2-ylmethoxy)phenyl)piperazine (10q). Yield = 6 mg; 10%. LCMS: R_T = 2.304 min, >98% @ 215 and 254 nm, *m/z* = 489.0 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.66 (d, *J* = 5.8 Hz, 2H), 7.97 (d, *J* = 9.8 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 2H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 5.6 Hz, 2H), 6.62 (dd, *J* = 4.7, 2.3 Hz, 2H), 5.14 (s, 2H), 3.21 (s, 4H), 3.17 – 3.09 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.52, 150.33, 148.40, 144.53, 139.85, 134.08, 129.63, 129.25, 129.15, 121.37, 108.09, 107.57, 68.68, 51.49, 46.07, 30.93.

5-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-(2-(pyridin-4-yl)ethyl)aniline (10r). Yield = 24 mg; 25%. LCMS: R_T = 2.215 min, >98% @ 215 and 254 nm, *m/z* = 486.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.54 (d, *J* = 4.2 Hz, 2H), 8.37 (s, 1H), 8.01 (d, *J* = 17.7 Hz, 1H), 7.79 (dd, *J* = 8.3, 5.1 Hz, 2H), 7.36 – 7.10 (m, 4H), 6.14 (d, *J* = 10.9 Hz, 1H), 5.81 (s, 1H), 3.51 (dd, *J* = 12.2, 6.6 Hz, 2H), 3.45 (d, *J* = 4.8 Hz, 4H), 3.21 – 3.08 (m, 4H), 3.01 (t, *J* = 6.9 Hz, 2H). ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.55, 8.54, 8.37, 8.03, 8.01, 7.81, 7.80, 7.79,

7.78, 7.27, 7.25, 7.24, 7.22, 7.19, 7.18, 6.15, 6.14, 6.13, 5.81, 5.30, 3.53, 3.51, 3.50, 3.49, 3.46, 3.45, 3.44, 3.15, 3.14, 3.13, 3.02, 3.01, 3.00.

5-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-(2-(pyridin-4-yl)ethyl)aniline (10s). Yield = 36 mg; 35%. LCMS: R_T = 2.298 min, >98% @ 215 and 254 nm, *m/z* = 502.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.54 (d, *J* = 3.7 Hz, 2H), 8.36 (s, 1H), 8.01 (d, *J* = 9.6 Hz, 1H), 7.71 (d, *J* = 8.3 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.19 (d, *J* = 4.8 Hz, 2H), 6.14 (d, *J* = 9.6 Hz, 1H), 5.81 (s, 1H), 3.55 – 3.47 (m, 2H), 3.47 – 3.38 (m, 4H), 3.19 – 3.08 (m, 4H), 3.01 (t, *J* = 6.9 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.30, 150.12, 147.36, 147.07, 139.95, 133.82, 129.62, 129.14, 129.11, 124.94, 124.06, 104.57, 94.42, 46.71, 45.51, 43.36, 34.62.

***N*-(5-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-2-nitrophenyl)pyridin-4-amine (10t).** Yield = 11 mg; 12%. LCMS: R_T = 2.109 min, >98% @ 215 and 254 nm, *m/z* = 458.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.56 (s, 1H), 8.26 – 8.20 (m, 2H), 7.86 (dd, *J* = 8.5, 5.2 Hz, 2H), 7.51 (t, *J* = 8.7 Hz, 2H), 7.25 (d, *J* = 1.9 Hz, 1H), 7.16 (d, *J* = 9.7 Hz, 1H), 6.95 (d, *J* = 7.0 Hz, 2H), 3.67 (s, 4H), 3.04 (s, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.61, 160.02, 153.97, 143.74, 138.41, 131.89, 131.21, 131.14, 129.20, 117.36, 117.18, 113.93, 113.72, 109.35, 46.16, 45.76.

***N*-(5-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-2-nitrophenyl)pyridin-4-amine (10u).** Yield = 12 mg; 13%. LCMS: R_T = 2.195 min, >98% @ 215 and 254 nm, *m/z* = 474.0 $[M+H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.87 (s, 1H), 8.56 (s, 1H), 8.21 (dd, *J* = 11.2, 8.5 Hz, 2H), 7.77 (dd, *J* = 26.9, 8.6 Hz, 4H), 7.25 (d, *J* = 2.2 Hz, 1H), 7.18 – 7.12 (m, 1H), 6.95 (d, *J* = 7.1 Hz, 2H), 3.67 (s, 4H), 3.05 (s, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.64, 153.94, 143.71, 138.42, 137.83, 134.20, 131.87, 130.20, 129.96, 129.20, 113.91, 113.70, 109.35, 46.17, 45.71.

5-(4-((4-chlorophenyl)sulfonyl)-1,4-diazepan-1-yl)-2-nitro-*N*-(pyridin-3-ylmethyl)aniline (11a). Yield = 67 mg; 40%. LCMS: R_T = 2.343 min, >98% @ 215 and 254 nm, *m/z* = 502.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.62 (s, 1H), 8.53 (s, 1H), 8.06 (d, *J* = 9.7 Hz, 1H), 7.66 (dd, *J* = 16.5, 8.0 Hz, 3H), 7.43 (d, *J* = 8.1 Hz, 2H), 7.29 (d, *J* = 6.5 Hz, 2H), 6.03 (d, *J* = 9.6 Hz, 1H), 5.50 (s, 1H), 4.52 (d, *J* = 4.8 Hz, 2H), 3.67 – 3.53 (m, 4H), 3.27 (d, *J* = 4.1 Hz, 2H), 3.08 (t, *J* = 5.6 Hz, 2H), 1.81 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.92, 149.16, 148.64, 147.10, 139.29, 137.77, 134.58, 133.37, 129.77, 129.51, 128.17, 124.22, 123.85, 102.48, 92.21, 51.68, 48.27, 48.21, 47.42, 44.67, 26.78.

5-(4-((4-fluorophenyl)sulfonyl)-1,4-diazepan-1-yl)-2-nitro-*N*-(pyridin-3-ylmethyl)aniline (11b). Yield = 28.6 mg; 18%. LCMS: R_T = 2.247 min, >98% @ 215 and 254 nm, *m/z* = 486.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.62 (s, 1H), 8.53 (s, 1H), 8.07 (d, *J* = 9.7 Hz, 1H), 7.72 (dd, *J* = 18.2, 12.9 Hz, 1H), 7.68 (d, *J* = 7.3 Hz, 1H), 7.29 (d, *J* = 5.9 Hz, 1H), 7.16 (t, *J* = 8.0 Hz, 2H), 6.04 (d, *J* = 9.7 Hz, 1H), 5.52 (s, 1H), 4.53 (d, *J* = 4.5 Hz, 2H), 3.65 – 3.53 (m, 4H), 3.26 (s, 2H), 3.06 (t, *J* = 5.4 Hz, 2H), 1.87 – 1.77 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.95, 149.13, 148.61, 147.11, 135.34, 134.60, 133.38, 129.79, 129.48, 129.41, 124.20, 123.86, 116.56, 116.38, 102.49, 92.23, 51.84, 48.27, 47.40, 44.66, 26.77.

(4-fluorophenyl)(4-(4-nitro-3-((pyridin-4-ylmethyl)amino)phenyl)piperazin-1-yl)methanone (11e). Yield = 27 mg; 19%. LCMS: R_T = 2.058 min, >98% @ 215 and 254 nm, *m/z* = 436.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.62 (d, *J* = 4.3 Hz, 2H), 8.16 (d, *J* = 9.5 Hz, 1H), 7.50 – 7.42 (m, 2H), 7.31 (d, *J* = 4.3 Hz, 2H), 7.14 (t, *J* = 8.0 Hz, 2H), 6.26 (d, *J* = 9.6 Hz, 1H), 5.69 (s, 1H), 4.58 (d, *J* = 5.4 Hz, 2H), 3.70 (s, 4H), 3.31 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.14, 150.91, 146.91, 142.30, 139.96, 133.91, 129.63, 129.14, 129.09, 125.32, 110.61, 107.61, 104.67, 95.16, 46.78, 45.45, 40.28.

(4-(4-nitro-3-((pyridin-3-ylmethyl)amino)phenyl)-1,4-diazepan-1-yl)(p-tolyl)methanone (11f). Yield = 28 mg; 28%. LCMS: R_T = 2.287 min, >98% @ 215 and 254 nm, *m/z* = 482.0 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.68 (d, *J* = 1.7 Hz, 1H), 8.50 (dd, *J* = 4.7, 1.5 Hz, 1H), 7.87 (d, *J* = 9.7 Hz, 1H), 7.82 (d, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.39 (dd, *J* = 7.5, 4.7 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 2H), 6.19 (dd, *J* = 9.8, 2.5 Hz, 1H), 5.64 (d, *J* = 2.4 Hz, 1H), 4.64 (d, *J* = 6.0 Hz, 2H), 3.65 (t, *J* = 5.4 Hz, 2H), 3.53 (t, *J* = 5.9 Hz, 2H), 3.22 (t, *J* = 5.3 Hz, 2H), 3.18 – 3.10 (m, 2H), 2.34 (s, 3H), 1.74 – 1.61 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.97, 149.71, 149.31, 147.70, 143.92, 136.80, 135.84, 135.36, 130.57, 129.49, 127.44, 124.58, 123.60, 104.24, 92.67, 50.45, 48.83, 47.56, 47.41, 44.16, 27.73, 21.75.

3-(4-((4-fluorophenyl)sulfonyl)piperazine-1-yl)-4-nitro-*N*-(pyridine-4-ylmethyl)aniline (12a). Yield = 32 mg; 68%. LCMS: R_T = 2.197 min, >98% @ 215 and 254 nm, *m/z* = 472.1 $[M + H]^+$ ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.88 (t, *J* = 5.9 Hz, 1H), 8.52 (d, *J* = 5.0 Hz, 2H), 7.90 (d, *J* = 9.7 Hz, 1H), 7.79 (q, *J* = 8.7 Hz,1H), 7.73 (s, *J* = 10.0 Hz, 4H), 7.35 (d, *J* = 5.0 Hz, 2H), 6.34 (d, *J* = 9.8 Hz, 1H), 5.82 (s, 1H), 4.63 (d, *J* = 5.9 Hz, 2H), 3.43 (s, 4H), 2.90 (s, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.90, 150.22, 148.42, 147.11, 138.91, 134.07, 130.18, 129.86, 128.81, 123.98, 122.75, 105.19, 94.98, 46.15, 45.52, 45.00.

3-(4-((4-chlorophenyl)sulfonyl)piperazine-1-yl)-4-nitro-*N*-(pyridine-4-ylmethyl)aniline (12b). Yield = 22 mg; 45%. LCMS: R_T = 2.299 min, >98% @ 215 and 254 nm, *m/z* = 488.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.55 (d, *J* = 5.5 Hz, 2H), 8.18 (s, 1H), 7.90 (d, *J* = 9.2 Hz, 1H), 7.82 (q, *J* = 8.7 Hz, 3H), 7.74 (s, 1H), 7.36 (d, *J* = 5.2 Hz, 2H), 6.32 (d, *J* = 8.3 Hz, 1H), 6.23 (s, 1H), 4.48 (d, *J* = 5.9 Hz, 2H), 3.05 (d, *J* = 12.5 Hz, 8H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.02, 154.96, 150.70, 150.64, 150.27, 148.91, 139.37, 134.59, 131.12, 130.71, 130.64, 130.44, 123.19, 51.65, 46.89, 45.64.

3-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-4-ylmethyl)aniline (12c). Yield = 26 mg; 28%. LCMS: R_T = 2.233 min, >98% @ 215 and 254 nm, *m/z* = 472.1 [M + H]$^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.80 (t, *J* = 5.3 Hz, 1H), 8.58 (d, *J* = 4.9 Hz, 2H), 8.07 (d, *J* = 9.7 Hz, 1H), 7.76 (dd, *J* = 8.5, 5.0 Hz, 2H), 7.31 – 7.19 (m, 5H), 6.17 (dd, *J* = 9.6, 1.9 Hz, 1H), 5.63 (s, 1H), 4.53 (d, *J* = 5.7 Hz, 2H), 3.36 – 3.27 (m, 4H), 3.09 – 2.99 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.08, 150.34, 146.89, 146.79, 130.48, 130.41, 129.20, 125.27, 121.68, 116.71, 116.53, 104.86, 95.26, 46.57, 45.94, 45.32.

3-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-2-nitro-*N*-(pyridin-4-ylmethyl)aniline (12d). Yield = 17 mg; 17%. LCMS: R_T = 2.364 min, >98% @ 215 and 254 nm, *m/z* = 488.1 [M + H]$^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.80 (t, *J* = 5.4 Hz, 1H), 8.58 (d, *J* = 4.8 Hz, 2H), 8.08 (d, *J* = 9.6 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.34 – 7.21 (m, 2H), 6.17 (dd, *J* = 9.6, 2.1 Hz, 1H), 5.62 (s, 1H), 4.53 (d, *J* = 5.7 Hz, 2H), 3.37 – 3.26 (m, 4H), 3.09 – 2.95 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.05, 150.37, 146.86, 146.79, 139.97, 133.75, 129.65, 129.24, 129.12, 125.32, 121.66, 104.86, 95.31, 46.60, 45.96, 45.30.

General Procedure for the Synthesis of 11c,d,g,h. To a 25 mL round bottom flask was added the starting compound, **13**, (45.3 mg, 0.100 mmol) and 4 M HCl in dioxane (0.500 mL). After stirring for 16 h, the reaction was concentrated under reduced pressure. Pyridine (3.00 mL) was slowly added to the flask followed by DMAP (2.4 mg, 0.020 mmol), and the sulfonyl chloride (0.110 mmol) of interest. After 16 h, the reaction was concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (dry loaded using silica/DCM) with a gradient of 0-20% methanol in dichloromethane to yield the desired compounds, **11c-d**.

The final compounds, **11g,h**, were synthesized as outlined above for compound, **8**.

5-(2-((4-fluorophenyl)sulfonyl)-2,7-diazaspiro[3.5]nonan-7-yl)-2-nitro-*N*-(pyridin-4-yl methyl)aniline (11c). Yield = 27 mg; 5%. LCMS: R_T = 2.311 min, >98% @ 215 and 254 nm, *m/z* = 528.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.81 (s, 1H), 8.44 (d, *J* = 5.6 Hz, 2H), 7.78 (dt, *J* = 25.2, 9.2 Hz, 5H), 7.28 (d, *J* = 5.3 Hz, 2H), 6.29 (dd, *J* = 9.8, 1.9 Hz, 1H), 5.70 (s, 1H), 4.58 (d, *J* = 6.0 Hz, 2H), 3.41 (s, 4H), 3.14 (s, 4H), 1.24 (s, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.76, 164.75, 155.47, 150.63, 148.96, 147.76, 132.16, 132.08, 131.13, 129.30, 123.75, 123.07, 117.71, 117.53, 105.68, 94.66, 61.12, 45.45, 44.46, 34.59, 33.78.

5-(2-((4-fluorophenyl)sulfonyl)-2,7-diazaspiro[3.5]nonan-7-yl)-2-nitro-*N*-(pyridin-4-yl methyl)aniline (11d). Yield = 25 mg; 5%. LCMS: R_T = 2.581 min, >98% @ 215 and 254 nm, *m/z* = 512.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.81 (s, 1H), 8.44 (d, *J* = 5.7 Hz, 2H), 7.93 – 7.76 (m, 3H), 7.50 (t, *J* = 8.7 Hz, 2H), 7.28 (d, *J* = 5.3 Hz, 2H), 6.29 (d, *J* = 9.7 Hz, 1H), 5.69 (s, 1H), 4.58 (d, *J* = 5.9 Hz, 2H), 3.40 (s, 4H), 3.13 (s, 4H), 1.23 (s, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.76, 164.75, 155.47, 150.63, 148.96, 147.76, 132.16, 132.08, 131.13, 129.30, 123.75, 123.07, 117.71, 117.53, 105.68, 94.66, 61.12, 45.45, 44.46, 34.59, 33.78.

5-(4-(4-fluorobenzyl)piperazin-1-yl)-2-nitro-N-(pyridin-4-ylmethyl)aniline (11g). Yield = 244 mg; 72%. LCMS: R_T = min, >98% @ 215 and 254 nm, *m/z* = $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.51 (d, *J* = 5.3 Hz, 2H), 7.92 (d, *J* = 9.8 Hz, 1H), 7.35 (dd, *J* = 16.7, 5.6 Hz, 3H), 7.16 (t, *J* = 8.7 Hz, 2H), 6.40 (d, *J* = 10.1 Hz, 1H), 5.82 (s, 1H), 4.65 (d, *J* = 5.9 Hz, 2H), 3.46 (s, 2H), 3.29 (s, 4H), 2.36 (d, *J* = 4.0 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.09, 150.66, 148.93, 147.69, 131.69, 131.63, 129.18, 124.06, 123.15, 115.92, 115.75, 105.66, 94.76, 61.71, 52.75, 47.12, 45.51.

5-(4-(4-chlorobenzyl)piperazin-1-yl)-2-nitro-N-(pyridin-4-ylmethyl)aniline (11h). Yield = 106.5 mg; 30%. LCMS: R_T = 1.825 min, >98% @ 215 and 254 nm, *m/z* = 422.1 [M +

H]$^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.89 (s, 1H), 8.60 (d, J = 4.4 Hz, 2H), 8.12 (d, J = 9.6 Hz, 1H), 7.29 (m, 4H), 7.03 (t, J = 8.3 Hz, 2H), 6.27 (d, J = 9.7 Hz, 1H), 5.66 (s, 1H), 4.56 (d, J = 5.2 Hz, 2H), 3.49 (s, 2H), 3.26 (s, 4H), 2.47 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.95, 149.13, 148.61, 147.11, 135.37, 134.60, 133.38, 129.79, 129.48, 129.41, 124.20, 123.86, 116.56, 116.38, 102.49, 92.23, 51.84, 48.27, 47.40, 44.66, 26.77.

General Procedure for the Synthesis of 12e-f. To a 25 mL round bottom flask was added 3-chloro-bromobenzene, **22**, (353 μL, 3.00 mmol), $Pd_2(dba)_3$ (82 mg, 0.090 mmol), CyJohnPhos (94.6 mg, 0.270 mmol), Cs_2CO_3 (2.5 g, 7.5 mmol), 4-methylaminopyridine, **18**, (364 μL, 3.60 mmol) and dioxane (15 mL). The reaction was refluxed at 110 °C for 16 h under argon atmosphere. The reaction was cooled to room temperature, Boc-piperazine, **24**, (1.12 g, 6.00 mmol) and Cs_2CO_3 (977 mg, 3.00 mmol) were added to the reaction flask. The reaction was refluxed at 110 °C for an additional 16 h under argon atmosphere. The reaction was cooled to room temperature, filtered through celite with the aid of ethyl acetate, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (dry loaded using silica/DCM) with a gradient of 0-100% ethyl acetate in hexanes yielding **25**.

To a 25 mL round bottom flask was added the starting compound, **25**, (82.9 mg, 0.225 mmol) and 4 M HCl in dioxane (1.12 mL). After stirring for 16 h, the flask was concentrated under reduced pressure. Pyridine (3.00 mL) was slowly added to the flask followed by DMAP (5.5 mg, 0.45 mmol), and the sulfonyl chloride (0.338 mmol) of interest. After 16 h, the reaction was concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (dry loaded using silica/DCM) with a gradient of 0-20% methanol in dichloromethane yielding **12e-f**.

3-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-*N*-(pyridin-4-ylmethyl)aniline (12e). Yield = 36 mg; 28%. LCMS: R_T = 2.115 min, >98% @ 215 and 254 nm, *m/z* = 427.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.50 (d, *J* = 5.7 Hz, 2H), 7.92 – 7.83 (m, 2H), 7.59 – 7.49 (m, 2H), 7.35 (d, *J* = 5.9 Hz, 2H), 6.90 (t, *J* = 8.0 Hz, 1H), 6.25 (t, *J* = 6.3 Hz, 1H), 6.15 (dd, *J* = 8.7, 1.4 Hz, 2H), 6.09 – 6.00 (m, 1H), 4.30 (d, *J* = 6.2 Hz, 2H), 3.19 – 3.08 (m, 4H), 3.05 – 2.95 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.65, 164.64, 152.22, 150.75, 150.36, 149.97, 132.05, 132.02, 131.66, 131.58, 130.28, 123.19, 117.70, 117.52, 105.93, 105.52, 101.55, 49.02, 46.71, 46.31.

3-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-*N*-(pyridin-4-ylmethyl)aniline (12f). Yield = 40 mg; 30%. LCMS: R_T = 2.209 min, >98% @ 215 and 254 nm, *m/z* = 443.1 $[M + H]^+$. ^{1}H NMR (499 MHz, DMSO-d_6) δ 8.50 (dd, *J* = 4.4, 1.6 Hz, 2H), 7.91 – 7.70 (m, 4H), 7.43 – 7.28 (m, 2H), 6.90 (t, *J* = 8.0 Hz, 1H), 6.15 (dd, *J* = 8.8, 1.4 Hz, 2H), 6.08 – 6.01 (m, 1H), 4.30 (s, 2H), 3.16 – 3.05 (m, 4H), 3.05 – 2.95 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.21, 150.90, 150.24, 149.95, 139.27, 134.56, 130.54, 130.41, 130.28, 123.21, 105.94, 105.53, 101.56, 49.04, 46.69, 46.31.

General Procedure for the Synthesis of 12g-l. To a two-dram vial was added the 1-substituted 4-bromo-2-fluorobenzene, **26**, (0.100 mmol), dimethyl sulfoxide (1.00 mL), triethylamine (20.8 μL, 0.150 mmol), and the amine (0.100 mmol). After stirring at room temperature for 16 h, the reaction was diluted with ethyl acetate (15 mL) and washed with water (15 mL) and saturated brine (15 mL). The organic layer dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product, **27**, was taken forward without further purification. To a microwave reaction vial was added the crude product, **27**, (0.100 mmol), $Pd_2(dba)_3$ (0.9 mg, 0.001 mmol), CyJohnPhos (0.4 mg, 0.001 mmol), Cs_2CO_3 (81.5 mg, 0.250 mmol), Boc-piperazine, **24**, (37.3 mg, 0.200 mmol) and dioxane (3.00 mL). The reaction was heated by microwave at 120 °C

for 1 h. The reaction was cooled to room temperature, filtered through celite with aid of ethyl acetate, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (dry loaded using silica/DCM) with a gradient of 0-100% ethyl acetate in hexanes yielding **28**.

To a 25 mL round bottom flask was added the starting compound, **28**, (0.100 mmol) and 4 M HCl in dioxane (0.50 mL). After stirring for 16 h, the flask was concentrated under reduced pressure. Pyridine (3.00 mL) was slowly added to the flask followed by DMAP (2.4 mg, 0.020 mmol), and the sulfonyl chloride (0.110 mmol) of interest. After 16 h, the reaction was concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (dry loaded using silica/DCM) with a gradient of 0-20% methanol in dichloromethane yielding **12g-l**.

4-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-N-(pyridin-4-ylmethyl)pyridin-2-amine (12g). Yield = 5 mg; 5%. LCMS: R_T = 1.706 min, >98% @ 215 and 254 nm, *m/z* = 428.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.52 (d, *J* = 5.7 Hz, 2H), 7.83 (d, *J* = 6.1 Hz, 1H), 7.78 (dd, *J* = 8.6, 5.0 Hz, 2H), 7.23 (dd, *J* = 10.4, 6.6 Hz, 3H), 6.10 (d, *J* = 6.1 Hz, 1H), 5.59 (s, 1H), 4.92 (s, 1H), 4.51 (d, *J* = 5.9 Hz, 2H), 3.35 – 3.26 (m, 4H), 3.13 – 3.03 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.49, 156.31, 149.98, 148.84, 148.76, 130.51, 130.44, 122.00, 116.64, 116.46, 101.62, 90.53, 46.14, 45.47, 45.11.

4-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-N-(pyridin-4-ylmethyl)pyridin-2-amine (12h). Yield = 11 mg; 10%. LCMS: R_T = 1.797 min, >98% @ 215 and 254 nm, *m/z* = 444.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.54 (d, *J* = 4.7 Hz, 2H), 7.84 (d, *J* = 6.1 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.30 – 7.23 (m, 2H), 6.12 (d, *J* = 6.1 Hz, 1H), 5.61 (s, 1H), 4.97 (s, 1H), 4.53 (d, *J* = 5.9 Hz, 2H), 3.36 – 3.26 (m, 4H), 3.17 – 3.03 (m, 4H). ^{13}C

NMR (126 MHz, $CDCl_3$) δ 159.49, 156.28, 149.97, 148.85, 148.74, 139.87, 133.87, 129.58, 129.15, 122.00, 101.61, 90.54, 46.14, 45.44, 45.09.

4-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-2-((pyridin-4-ylmethyl)amino)benzonitrile (12i). Yield = 6 mg; 13%. LCMS: R_T = 2.222 min, >98% @ 215 and 254 nm, *m/z* = 468.1 $[M + H]^+$. ^1H NMR (499 MHz, $CDCl_3$) δ 8.60 (d, *J* = 5.7 Hz, 2H), 7.70 (d, *J* = 8.5 Hz, 2H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.28 (dd, *J* = 10.0, 7.5 Hz, 4H), 6.20 (dd, *J* = 8.7, 2.0 Hz, 1H), 5.73 (d, *J* = 1.9 Hz, 1H), 5.11 (t, *J* = 5.7 Hz, 1H), 4.46 (d, *J* = 5.7 Hz, 2H), 3.26 – 3.18 (m, 4H), 3.11 – 3.02 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.37, 150.75, 150.26, 147.37, 139.88, 134.00, 133.86, 129.59, 129.16, 121.70, 105.47, 96.62, 87.28, 47.21, 46.38, 45.47, 27.52.

4-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-2-((pyridin-4-ylmethyl)amino)benzonitrile (12j). Yield = 10 mg; 17%. LCMS: R_T = 2.126 min, >98% @ 215 and 254 nm, *m/z* = 452.1 $[M + H]^+$. ^1H NMR (499 MHz, $CDCl_3$) δ 8.57 (d, *J* = 4.9 Hz, 2H), 7.81 – 7.72 (m, 2H), 7.35 – 7.17 (m, 4H), 6.18 (dd, *J* = 8.8, 2.0 Hz, 1H), 5.71 (d, *J* = 1.8 Hz, 1H), 5.11 (t, *J* = 5.5 Hz, 1H), 4.44 (d, *J* = 5.7 Hz, 2H), 3.27 – 3.16 (m, 4H), 3.10 – 2.99 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.45, 164.41, 154.40, 150.76, 150.21, 147.45, 133.99, 131.44, 130.51, 130.44, 121.72, 118.39, 116.65, 116.47, 105.46, 96.59, 87.25, 47.19, 46.36, 45.49.

5-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-*N*-(pyridin-4-ylmethyl)-2-(trifluoromethyl) aniline (12k). Yield = 22 mg; 43%. LCMS: R_T = 2.419 min, >98% @ 215 and 254 nm, *m/z* = 511.1 $[M + H]^+$. ^1H NMR (499 MHz, $CDCl_3$) δ 8.56 (d, *J* = 5.8 Hz, 2H), 7.68 (d, *J* = 8.6 Hz, 2H), 7.52 (d, *J* = 8.6 Hz, 2H), 7.31 (d, *J* = 8.8 Hz, 1H), 7.28 – 7.22 (m, 2H), 6.21 – 6.16 (m, 1H), 5.83 (s, 1H), 4.91 (s, 1H), 4.43 (d, *J* = 5.5 Hz, 2H), 3.19 – 3.08 (m, 4H), 3.08 – 2.98 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.95, 150.23, 147.91, 145.80, 139.77, 133.92, 129.54,

129.17, 128.12, 128.08, 121.70, 106.38, 106.14, 105.91, 105.67, 104.47, 99.14, 47.88, 46.48, 45.61.

5-(4-((4-fluorophenyl)sulfonyl)piperazin-1-yl)-*N*-(pyridin-4-ylmethyl)-2-(trifluoromethyl) aniline (12l). Yield = 23 mg; 46%. LCMS: R_T = 2.324 min, >98% @ 215 and 254 nm, *m/z* = 495.1 $[M + H]^+$. ^{1}H NMR (499 MHz, $CDCl_3$) δ 8.56 (d, *J* = 5.8 Hz, 2H), 7.82 – 7.72 (m, 2H), 7.31 (d, *J* = 8.8 Hz, 1H), 7.28 – 7.16 (m, 4H), 6.20 (dd, *J* = 8.7, 1.6 Hz, 1H), 5.83 (d, *J* = 1.2 Hz, 1H), 4.91 (s, 1H), 4.43 (d, *J* = 5.5 Hz, 2H), 3.21 – 3.09 (m, 4H), 3.09 – 2.99 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.42, 164.38, 153.98, 150.21, 147.97, 145.80, 131.48, 130.52, 130.45, 121.72, 116.61, 116.43, 106.38, 106.14, 105.90, 105.66, 104.47, 99.12, 47.87, 46.49, 45.64.

Synthesis of 12m. The synthesis of compound **32** was completed as described above. To a 25 mL round bottom flask was added the methyl ester, **31**, (45 mg, 0.093 mmol), dioxane (5.00 mL), and 10% wt/v aq. LiOH (5.00 mL). The reaction was stirred at room temperature for 24 h. The reaction was acidified to pH 2 using 2 M HCl and extracted with ethyl acetate (15 mL) three times. The combined organic layers were washed with saturated brine solution (15 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (dry loaded using silica/DCM) with a gradient of 0-20% methanol in dichloromethane to yield **12m**.

4-(4-((4-chlorophenyl)sulfonyl)piperazin-1-yl)-2-((pyridin-4-ylmethyl)amino)benzoic acid (12m). Yield = 12.5 mg; 6%. LCMS: R_T = 2.208 min, >98% @ 215 and 254 nm, *m/z* = 487.0 $[M + H]^+$.^{1}H NMR (499 MHz, $CDCl_3$) δ 8.52 (d, *J* = 4.7 Hz, 2H), 7.82 (d, *J* = 6.1 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.24 (d, *J* = 5.1 Hz, 2H), 6.10 (d, *J* = 6.1 Hz, 1H), 5.59 (s, 1H), 4.95 (s, 1H), 4.51 (d, *J* = 5.9 Hz, 2H), 3.34 – 3.25 (m, 4H), 3.13 – 3.02 (m, 4H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 159.49, 156.28, 149.97, 148.85, 148.74, 139.87, 133.87, 129.58, 129.15, 122.00, 101.61, 90.54, 46.14, 45.44, 45.09.

Thallium flux assay development

Thallium flux assays were performed essentially as described previously.[18] Briefly, stably transfected T-Rex-HEK-293-*Ae*Kir1 cells were cultured overnight in 384-well plates in media containing DMEM, 10% dialyzed FBS and 1μg/mL tetracycline to induce channel expression (Fig. S1A). The next day, the cell culture medium was replaced with a dye-loading solution containing assay buffer (Hanks Balanced Salt Solution with 20 mM HEPES, pH 7.3), 0.01% (*w/v*) Pluronic F-127 (Life Technologies, Carlsbad, CA), and 1.2 μM of the thallium-sensitive dye Thallos-AM (TEFlabs, Austin, TX). Following 1 hr incubation at room temperature, the dye- loading solution was washed from the plates and replaced with 20 μL/well of assay buffer. The plates were transferred to a Hamamatsu Functional Drug Screening System 6000 (FDSS6000; Hamamatsu, Tokyo, Japan) or Panoptic kinetic imaging plate reader (Wavefront Bioscience, Franklin, TN, USA) where 20 μL/well of test compounds in assay buffer (9-point concentration response curve starting at 30 μM final concentration) were added and allowed to incubate with the cells for 20 min. After the incubation period, a baseline recording was collected at 1 Hz for 10 s (excitation 470 ± 20 nm, emission 540 ± 30 nm) followed by a thallium stimulus buffer addition (10 μL/well) and data collection for an additional 4 min. The thallium stimulus buffer contains in (mM) 125 $NaHCO_3$, 1.8 $CaSO_4$, 1 $MgSO_4$, 5 glucose, 12 Tl_2SO_4, 10 HEPES, pH 7.4. Fluorescence values for individual wells were normalized by dividing each data point for that well by the initial data point (F/F_0). After normalization of all wells, the fluorescence data (i.e. fluorescence vs. time) from each of the designated vehicle-control wells was averaged and this averaged control wave was subtracted from all wells on the plate to yield normalized, vehicle control-subtracted data. Fluorescence amplitudes (max-min) were extracted from the control-subtracted wells over a range

from 0-200 seconds. To ensure the small-molecule vehicle DMSO had no direct effect on *Ae*Kir1-dependent thallium flux, the assay's tolerance to different doses of DMSO was evaluated. The robustness and reproducibility of the assay was determined by comparing thallium flux through tetracycline-induced and tetracycline- uninduced cells for every plate. The Z' value was calculated as described previously[18], using the following formula:

$$Z' = 1- (3SDp + 3SDn)/|meanp + meann|$$

where SD is standard deviation, p and n are control and uninduced flux values respectively.

To compare the effect of DMSO on *Ae*Kir1-mediated thallium flux, a one-way ANOVA was performed with a Tukey's multiple comparison test. Prism software version 5.01 (GraphPad Software, San Diego, CA) was used to generate CRC from Tl^+ flux. Half-inhibition concentration (IC_{50}) values were calculated from fits using a four-parameter logistic equation.

Patch Clamp Electrophysiology

The stably transfected T-Rex-HEK-293-*Ae*Kir1 cells were grown overnight in presence of 1μg/mL tetracycline to induce channel expression. The cells were dissociated the following day and plated on poly-L-lysine-coated coverslips and allowed to recover for at least 1h in a 37 °C 5% CO2/95% air incubator before starting experiments. Patch clamp experiments were performed essentially as described previously.[22] Briefly, patch electrodes (2-3MΩ) were filled with an intracellular solution containing 135 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, 10 mM HEPES-free acid, and 2 mM Na_2ATP (Roche Diagnostics), pH 7.3, 275 mOsmol/kg water. The standard bath solution contained 135 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, and 10 mM HEPES free acid, pH 7.4. Macroscopic currents were recorded under whole-cell voltage-clamp conditions using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). Cells were voltage clamped at a holding potential of −75 mV and stepped every 5 s to −120 mV for 200

msec before ramping to 120 mV at a rate of 1.2 mV/msec. Data were collected at 5 kHz and filtered at 1 kHz. Data acquisition and analysis were performed using the pClamp 9.2 software suite (Molecular Devices, Sunnyvale, CA). Pharmacology experiments were terminated by applying 2 mM barium (Ba^{2+}) chloride to measure leak current. Cells exhibiting <90% block by Ba^{2+} were excluded from analysis. The mean current amplitude recorded over five successive steps to −120 mV in WT control or mutants at single concentration were expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA with Bonferroni's multiple comparisons test with statistical significance defined at $P < 0.05$. IC_{50} values were determined by fitting the Hill equation to concentration-response curves (CRCs) using variable-slope nonlinear regression analyses. All the analysis was performed with GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA).

LARVAL ASSAY

Mosquito colony

The *Ae. aegypti* colony (Liverpool strain) and rearing of mosquitoes was the same described in our previous studies.[18] In brief, eggs were hatched in distilled H_2O (dH_2O), larvae were fed pulverized fish food flakes (Tetramin, Blacksburg, VA), and adults were maintained on 10% sucrose. Adult females were fed defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA) via a membrane feeder (Hemotek, Blackburn, UK) periodically to produce more eggs. All mosquito rearing and experiments occurred in environmentally-controlled growth chambers held at 28 °C, 80% relative humidity, and 12 h:12 h light:dark.

Larval mosquito toxicity bioassays

The larval toxicity assays were performed exactly as described previously.[19] In brief, six larvae (24 h after hatching) were transferred to wells of a 24-well plate (Falcon Multiwell plate, Becton Dickinson Labware, Franklin Lakes, NJ) containing 985 µl of dH_2O. To each well, 5 µl of a food solution (13 mg/ml of Tetramin flakes in dH_2O) and 10 µl of a small molecule (dissolved in 100% DMSO) were added. Control wells received 10 µl of 100% DMSO to account for solvent effects. The plates were held in a rearing chamber and survival was assessed at 24 h and 48 h. If a larva did not move after being gently prodded with a fine insect pin or pipette tip then it was considered dead.

Adult female mosquito topical toxicity bioassays

The topical toxicity assays were performed exactly as described previously.[18] In brief, 10 adult females were immobilized on ice and 500 nL of a small molecule (dissolved in 100% acetone) was applied to the thorax of each mosquito using a repeating dispenser (PB600-1, Hamilton Company, Reno, NV). A control group of mosquitoes was treated with 100% acetone to account for solvent effects. The treated mosquitoes were transferred to small cages (32 oz. Tupperware containers) in a rearing chamber and provided with 10% sucrose for 24h. The efficacy of a treatment was considered the percentage of mosquitoes in a cage that were flightless or dead at 24 h.[11,18]

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Author Contributions

C.R.H. oversaw and designed the chemistry. C.D.A., M.J.W., A.G.S., A.M.H. and M.V.P. performed the synthetic chemistry work. J.S.D. designed the *in vitro* pharmacology experiments and S.V.K., M.K. and J.B.M. performed the pharmacology experiments. P.M.P. designed the larval lethality experiments and R.R.T. performed the experiments. C.R.H. wrote the manuscript with input from all authors.

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Notes

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Abbreviations

Ae, *Aedes aegypti*; Kir, inwardly rectifying potassium channel; Flo, flonicamide; CHIKV, chikungunya virus; DENV, dengue virus; ZIKV, zika virus; SAR, structure-activity relationship; TEA, triethyl amine; DMSO, dimethyl sulfoxide; DMAP, dimethylamino pyridine; dH_2O, distilled water.

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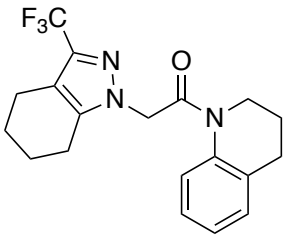
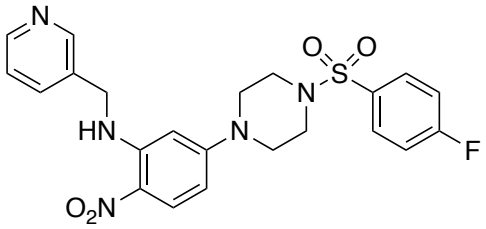


VU041
AeKir inhibitor
Th Flux IC_{50} = 1.7 μM
Patch Clamp IC_{50} = 0.238 μM



9j
AeKir inhibitor
Th Flux IC_{50} = 0.47 μM
Patch Clamp IC_{50} = 0.057 μM