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Structure activity refinement of phenylsulfonyl piperazines as antimalarials that block erythrocytic invasion

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ABSTRACT

The emerging resistance to combination therapies comprised of artemisinin derivatives has driven a need to identify new antimalarials with novel mechanisms of action. Central to the survival and proliferation of the malaria parasite is the invasion of red blood cells by *Plasmodium* merozoites, providing an attractive target for novel therapeutics. A screen of the Medicines for Malaria Venture Pathogen Box employing transgenic *P. falciparum* parasites expressing the nanoluciferase bioluminescent reporter identified the phenylsulfonyl piperazine class as a specific inhibitor of erythrocyte invasion. Here, we describe the optimization and further characterization of the phenylsulfonyl piperazine class. During the optimization process we defined the functionality required for *P. falciparum* asexual stage activity and determined the alpha-carbonyl *S*-methyl isomer was important for antimalarial potency. The optimized compounds also possessed comparable activity against multidrug resistant strains of *P. falciparum* and displayed weak activity against sexual stage gametocytes. We determined that the optimized compounds blocked erythrocyte invasion consistent with the asexual activity observed and therefore the phenylsulfonyl piperazine analogues described could serve as useful tools for studying *Plasmodium* erythrocyte invasion.

1. Introduction

Malaria remains a substantial global health burden causing significant morbidity and mortality each year [1]. The World Health Organization (WHO) estimates that in 2018, 228 million cases of malaria occurred causing 405,000 deaths, with sub-Saharan Africa bearing the major proportion of the global burden [2]. Malaria is a disease caused by protozoan parasites belonging to the genus *Plasmodium* that are transmitted by female *Anopheles* mosquitoes [3]. Of the five species of *Plasmodium* that infect humans, *P. falciparum* is the deadliest, accounting for 99.7% of malaria cases in sub-Saharan Africa [2].

Artemisinin-based combination therapies (ACT) have significantly reduced malaria burden and deaths worldwide. However, *P. falciparum* resistance to artemisinins and their partner drugs have emerged in regions of southeastern Asia [4–6] and resistance mutations have recently been observed in parasites in Rwanda [7]. Beginning with the isolation of quinine in the 1820's, an array of antimalarial drugs have been developed, however they target only a small handful of biochemical pathways within the parasite [8]. Gratifyingly, there has been a concerted effort in recent years by many organisations to develop novel scaffolds with unique mechanisms of action [9]. Despite this endeavor and coupled with the spread of resistance to ACT, there is an ongoing need for therapies

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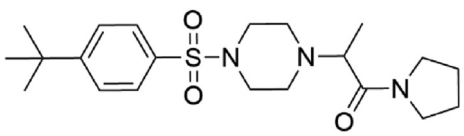
that have novel and complementary mechanisms of action that ideally have efficacy against different stages of the parasite life cycle [8,10].

Malaria in humans begins with the bite of an infected *Anopheles* mosquito that transfers sporozoite forms of *Plasmodium* parasites to the bloodstream. The sporozoites then migrate to the liver and infect hepatocytes. Within hepatocytes, parasites grow and divide over 7–10 days into merozoites which are then released into the bloodstream [11]. These merozoites then immediately invade red blood cells (RBCs) and grow and replicate for 48 h to form a schizont stage parasite containing about 20 merozoites. The merozoites then egress from their schizonts and host blood cells and invade new RBCs in a rapid process that takes just a few minutes [12]. The egress and invasion of merozoites are dependent on a number of cell signaling events, proteolytic cascades, receptor-ligand interactions and an actomyosin invasion motor. As these events occur in the bloodstream, they are optimally exposed to inhibition with small molecule drugs [13,14]. Drugs which could strongly reduce invasion efficiency would result in a dramatic reduction in parasite amplification and disease progression. Furthermore, delaying merozoite egress and RBC invasion would expose the merozoites to enhanced destruction by innate and humoral immune responses [15,16]. Drugs known to target these processes include kinase, subtilisin-like protease 1, plasmepsin 9 and actin inhibitors, and is comprehensively reviewed by Burns et al. [13].

To detect compounds that interfere with either erythrocytic egress or invasion, Dans et al. recently reported the development of an assay employing transgenic *P. falciparum* parasites which express the bioluminescent nanoluciferase (Nluc) reporter [14,17]. This assay platform was utilized to screen the Medicines for Malaria Venture (MMV) Pathogen Box, a collection of 400 compounds with activity against a variety of neglected tropical disease pathogens including 125 compounds with antimalarial activities. The screen of the Pathogen Box identified a small set of compounds that inhibited either erythrocyte invasion or egress and were validated by further synchronized parasite phenotype assays and live cell microscopy. One of the invasion-specific hit compounds discovered was MMV020291 (**1**) (Fig. 1). Microscopy studies demonstrated hit compound **1** blocked merozoite RBC internalization but had minimal effects on other stages of the asexual lifecycle. MMV020291 has a unique phenylsulfonyl piperazine scaffold, that is structurally divergent from other previously described phenylsulfonyl piperazine antimalarial series [18,19], thus providing a promising starting point for the development of an invasion-specific antimalarial.

In this study we define the structure–activity relationship (SAR) and optimize the antimalarial activity of the phenylsulfonyl piperazine class (**1**). During this process the antimalarial activity of analogues was iteratively determined utilizing the previously reported lactate dehydrogenase (LDH) asexual *P. falciparum* 3D7 assay [20]. Human HepG2 cell cytotoxicity and physicochemical



MMV020291 (**1**)
asexual Pf EC_{50} 0.90 μM
human HepG2 >10 μM

Fig. 1. Structure and asexual activity of hit MMV020291 from the screen of the Pathogen Box using the invasion assay described in Dans et al. [14] Asexual blood stage *P. falciparum* EC_{50} taken from MMV Pathogen Box data [24].

parameters were monitored in parallel [21]. Analogues with suitable physicochemical properties and asexual stage activity were further evaluated against *P. falciparum* multi-drug resistant lines, *P. knowlesi* parasites and NF54 gametocytes to ultimately determine their target candidate profile (TCP) suitability [22].

2. Results and discussion

The initial focus for optimizing the hit compound **1** was to establish the structure-activity relationship (SAR) and improve antimalarial activity based upon the LDH viability-based assay. This assay involves culturing asexual *P. falciparum* 3D7 parasites in the presence of compound for 72 h. Therefore, this assay allows the completion of one full 48 h asexual cycle and will reveal compounds that reduce parasite viability by blocking erythrocytic invasion. Subsequently selected compounds were then further evaluated in a previously described synchronized schizont release to RBC transition assay to confirm an invasion blocking phenotype [23]. Human cellular cytotoxicity was monitored for parasite active compounds against human HepG2 cells using a Cell Titre Glo growth inhibition assay [21]. The hit compound **1** exhibited an EC_{50} of 0.69 μM against asexual *P. falciparum* and no human HepG2 cytotoxicity at the highest concentration tested (EC_{50} > 40 μM) (Table 1).

The initial focus of the SAR study was to establish the functionality required for antimalarial activity at each position of the hit scaffold. A particularly important facet of the SAR study was to determine which stereoisomer at the alpha-center adjacent to the carboxamide of **1** was essential for antimalarial activity. The hit compound (**1**) was reported to be rapidly degraded by mouse liver microsomes (CL_{int} > 126 L/h/kg protein) [24] (Table S1) and therefore the physicochemical properties were taken into consideration during the optimization process.

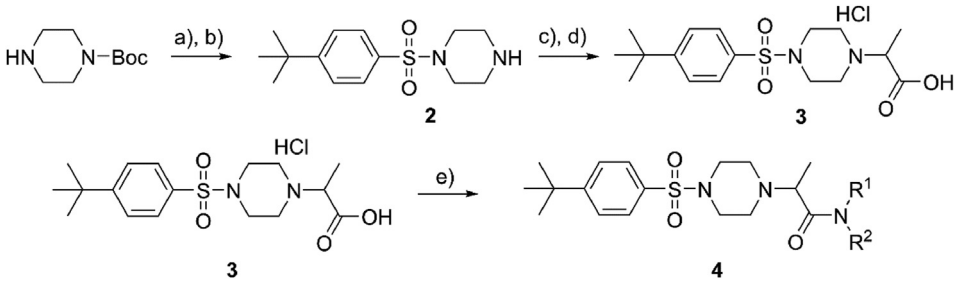
2.1. Chemistry

The synthesis of the phenylsulfonyl piperazine analogues started with *N*-sulfonylation of Boc protected piperazine with 4-*tert*-butylbenzenesulfonyl chloride followed by Boc deprotection to yield the sulfonamide **2**. Intermediate **2** was then alkylated with *tert*-butyl 2-bromopropanoate and subjected to acidic deprotection conditions to afford the carboxylic acid as a hydrochloride salt (**3**). Finally, the carboxylic acid **3** was coupled with the appropriate amine using HBTU to afford the carboxamide analogues **4** (Scheme 1).

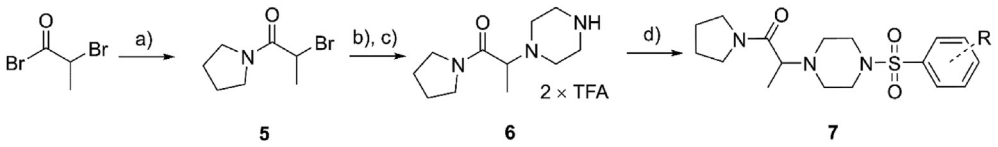
The synthetic route to generate analogues with varying substitutions on the phenyl ring began with acylation of pyrrolidine with 2-bromopropanoyl bromide to form the carboxamide (**5**). Intermediate (**5**) was then *N*-alkylated with Boc protected piperazine under basic conditions followed by Boc deprotection to yield the piperazine intermediate **6**. Finally, **6** was coupled with the appropriate phenylsulfonyl chloride to furnish the phenylsulfonyl analogues **7** (Scheme 2).

The synthesis to generate analogues, whereby the piperazine was replaced with another heterocycle, began with the alkylation of a selected *N*-Boc protected heterocycle with the alkyl halide **5** followed by Boc deprotection to yield intermediate **8**. **8** was then coupled with 4-*tert*-butylbenzenesulfonyl chloride to afford the heterocyclic analogues **9** (Scheme 3).

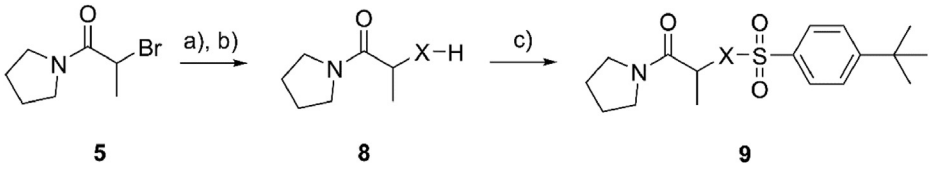
The synthesis to generate analogues with differing carboxamide substitution began with the alkylation of intermediate **2** with *tert*-butyl bromoacetate to yield intermediate **10**. The carboxamide functionality of **10** was then enolized under lithiation conditions and alkylated *in situ* with the appropriate alkyl halide, followed by *tert*-butyl deprotection to produce the alpha-substituted carboxylic



Scheme 1. Universal synthetic route to generate carboxamide derivatives. *Reagents and conditions:* (a) 4-*tert*-butylbenzenesulfonyl chloride, DIPEA, DCM, 40 °C; (b) TFA/DCM, 20 °C; (c) *tert*-butyl 2-bromopropanoate, K_2CO_3, ACN, reflux; (d) 4 M HCl, dioxane, 90 °C; (e) HBTU, NHR^1 R^2, DMF, 20 °C.



Scheme 2. Synthesis to afford substituted aryl compounds. *Reagents and conditions:* (a) pyrrolidine, DCM, 0 °C – 24 °C; (b) Boc-piperazine, DIPEA, EtOH, 70 °C; (c) TFA/DCM, 20 °C; (d) substituted benzenesulfonyl chloride, DIPEA, DCM, reflux.



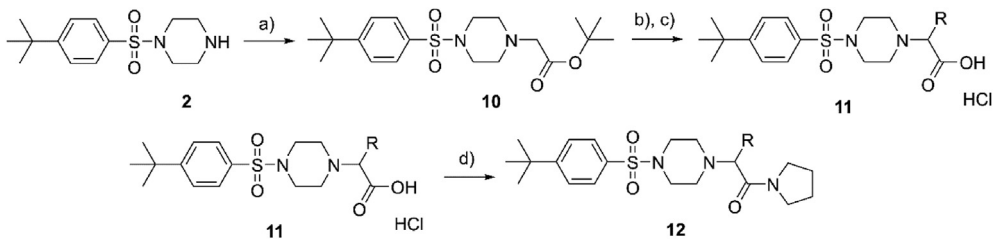
Scheme 3. Synthetic pathway to yield analogues incorporating piperazine variations or isosteres. *Reagents and conditions:* (a) Boc-X-H, DIPEA, EtOH, 70 °C; (b) TFA/DCM, 20 °C; (c) 4-*tert*-butylbenzenesulfonyl chloride, DIPEA, DCM, 40 °C.

acids (**11**). **11** was then coupled with the appropriate amine using HBTU conditions to produce the alpha-substituted analogues **12** (Scheme 4).

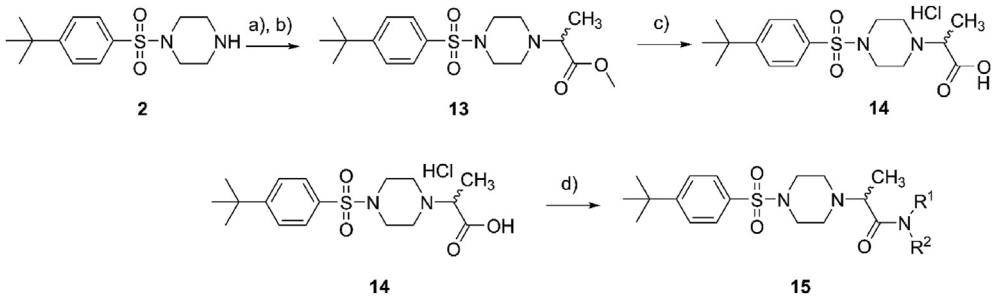
The synthesis of enantiopure alpha-methyl carboxamide analogues was performed following an adaption of the procedure by Aastrand et al. [25] The synthesis was initiated with the nucleophilic substitution of intermediate (**2**) with triflate methyl (*R*)-(+)- or (*S*)-(−)-lactate to afford the alkylated product **13** with inverted stereochemistry. The carboxylic ester **13** was then deprotected to afford the carboxylic acid as a hydrochloride salt (**14**). Finally, the carboxylic acid was coupled to an appropriate amine using HBTU to afford the enantiopure analogues **15** (Scheme 5).

2.2. Structure and activity relationship

To define the SAR, we systematically surveyed each moiety on the hit structure and determined its impact on asexual *P. falciparum* 3D7 parasite viability. We first examined the importance of the *N*-pyrrolidine group on the carboxamide of **1** by replacing it with groups of varying size and polarity (Table 1). The data showed exchanging pyrrolidine with a mono *N*-ethyl substitution (**16**) on the carboxamide was not tolerated (EC_{50} > 10 μM) whereas a *N,N*-diethyl substitution (**17**) was 3-fold less active (EC_{50} 1.8 μM) compared to **1** (EC_{50} 0.69 μM) suggesting that disubstitution of the carboxamide nitrogen is required for activity. Introduction of a bridged carbon between the 3- and 4-position of the pyrrolidine ring (**18**) resulted in a 2-fold improvement in activity (EC_{50} 0.33 μM) as well as a moderate improvement in LipE compared to **1** (LipE 4.2 and 3.7 respectively) however a larger 3-carbon bridge to afford an octahydrocyclopenta[*c*]pyrrole (**19**) had potency (EC_{50} 0.81 μM) comparable to **1**. Replacement of the pyrrolidine with a piperidine (**20**) resulted in a marginal improvement in activity (EC_{50} 0.5 μM), while the homopiperidine (**21**) had similar activity (EC_{50} 0.65 μM) compared to **1**. Analogues **22** and **25** with difluoro and cyclopropyl substitution in the 4-position of the piperidine respectively, have comparable activity (EC_{50} 0.38 and 0.30 μM) to



Scheme 4. Synthesis to yield compounds with mono-substitution alpha to the carboxamide. *Reagents and conditions:* (a) *tert*-butyl bromoacetate; (b) LiHMDS, RI or RBr, THF, −78 °C – 24 °C. K_2CO_3, ACN, reflux; (c) 4 M HCl, dioxane, 90 °C; (d) HBTU, pyrrolidine, DMF, 24 °C.



Scheme 5. Synthetic pathway to afford enantiopure analogues. *Reagents and conditions:* (a) methyl (*R*)-(+)-lactate or methyl (*S*)-(−)-lactate, triflic anhydride, 2,6-lutidine, DCM, −78 °C − 24 °C; (b) 1-((4-(*tert*-butyl)phenyl)sulfonyl) piperazine (**2**), DCM, 0 °C–24 °C; (c) 4 M HCl, dioxane, 90 °C; (d) HBTU, NHR^1R^2, DMF, 24 °C.

20, while a mono and dimethyl substitution at the 4-position in **23** and **24** respectively, resulted in a 2- and 6-fold loss in activity (EC_{50} 1.3 and 4.3 μM). A selection of 6-membered heterocycles were also trialed in place of the piperidine substitution on the carboxamide. This selection included an 8-azabicyclo[3.2.1]octane (**26**) that resulted in a 2-fold loss (EC_{50} 0.92 μM) in activity compared to unsubstituted piperidine (**20**). Introduction of heterocycles with increased polarity such as a morpholine (**27**) and methyl piperazine (**28**) resulted in a >10-fold loss for both compounds (EC_{50} 4.4 and 6.1 μM), while an unprotected piperazine **29** resulted in a significant loss in activity (EC_{50} > 10 μM), suggesting that hydrophobicity at this region of the pharmacophore is important for activity. While a number of the analogues in Table 1 either maintain (**19**–**21**, **24** and **26**) or were 2-fold more active (**18**, **22**, **25**) than the hit compound **1**, the marginal increase in cLogP of these analogues resulted in slightly increased LipE values, with the exception of **18**.

We next examined aryl and heteroaromatic systems in place of pyrrolidine on the carboxamide of **1**. The activity data for these analogues presented in Table 2 show the N-methylaniline substitution (**30**) was detrimental to activity (EC_{50} > 10 μM). This suggested the aryl ring directly linked to the carboxamide was not tolerated, however activity was mostly reinstated when the *N*-alkyl substitution was cyclized to the aryl group in the form of a 1,2,3,4-tetrahydroquinoline (**31**) and indoline (**32**) (EC_{50} 1.9 and 0.93 μM, respectively). Isoindoline (**33**), 2-methylbenzylamine (**34**) and 1,2,3,4-tetrahydroisoquinoline (**36**) systems were trialed and all exhibited activity in the EC_{50} range between 0.99 and 1.2 μM. Extending the distance between the aryl group and the carboxamide by one carbon (**35**) resulted in a marginal loss in activity (EC_{50} 2.1 μM). This trend indicated that *N*-benzylic or 1,2,3,4-tetrahydroisoquinoline substitution on the carboxamide was preferred, however *N*-benzyl groups are notorious metabolic liabilities due to potential hydroxylation of the benzylic carbon atom and formation of a carbinolamine intermediate which can result in further oxidation or dealkylation [26–28]. In order to restrict this potential metabolic liability, we introduced a monomethyl (**37**) and gem-dimethyl (**38**) substitution to the benzylic carbon of the 1,2,3,4-tetrahydroisoquinoline present in **36**. It was found that monomethyl substitution (**37**) was tolerated (EC_{50} 1.2 μM), while the gem-dimethyl substitution (**38**) resulted in a 5-fold improvement in potency (EC_{50} 0.18). Substitution at the benzylic position was further investigated with gem diethyl (**39**), cyclopentyl (**40**) and cyclohexyl (**41**) substitution, however these substitutions resulted in a 5- to 20-fold loss in activity when compared to **38**. Although **38** was 3-fold more potent than **1** (Table 2), **38** also had higher lipophilicity (cLogP) resulting in a decreased LipE (2.2 compared with 3.7 for **1**). To reduce the hydrophobicity and with the additional aim to improve aqueous solubility and reduce metabolic oxidation of the aryl group, an endocyclic nitrogen was introduced into the aryl ring (**42** and **43**). Both endocyclic iterations resulted in activity comparable to **36** but both have improved LipE values (3.0 and 2.9).

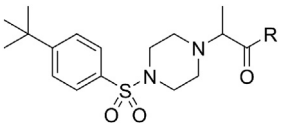
The hit compound **1** contains a *tert*-butyl substitution which is susceptible to metabolic degradation. *In vitro* and *in vivo* studies of *tert*-butylbenzene containing compounds have established that the methyl group is oxidized to form a 2,2-dimethyl-2-phenylethanol metabolite. Further oxidative metabolism of the primary metabolite results in the α,α-dimethyl-phenylacetic acid metabolite [29,30]. To investigate whether we could overcome this potential metabolic liability, we replaced the *tert*-butyl group with different functional groups (Table 3) and bioisosteres (Table 4) to determine their impact on *P. falciparum* parasite viability.

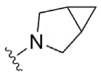
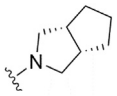
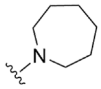
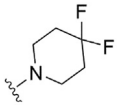
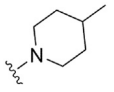
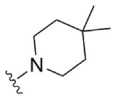
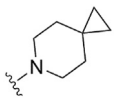
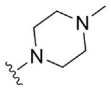
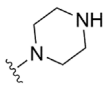
The first set of analogues focused on replacing the 4-*tert*-butyl group with different functional groups (Table 3). The results showed deletion of the *tert*-butyl group (**44**) resulted in a marked decrease in activity (EC_{50} > 10 μM). Decreasing the steric bulk of the 4-aryl substituent from a *tert*-butyl group to an isopropyl (**45**), trifluoromethyl or methyl group (**46**) significantly impacted activity (EC_{50} > 10 μM). Reciprocally increasing the steric bulk 4-aryl substituent from a *tert*-butyl group to *sec*-butyl (**47**), cyclopentyl (**49**) or phenyl (**50**) group also resulted in a loss of activity (EC_{50} > 10 μM). A variety of polar functionality and electron withdrawing substituents were also introduced into the 4-position in place of the *tert*-butyl including trifluoromethyl (**48**), bromo (**50**), methoxy (**51**) and acetyl (**52**) groups, however all examples were inactive at the highest concentration tested (EC_{50} > 10 μM). Moving the 4-*tert*-butyl substitution to the 3-position (**54**) was also detrimental to activity (EC_{50} of 8.4 μM). Taken together, these results suggest that the 4-*tert*-butyl group is encompassed by a hydrophobic pocket of a protein essential for parasite erythrocytic invasion and survival.

The second set of analogues introduced groups considered as *tert*-butyl isosteres [31] in place of the 4-*tert*-butyl substituent (Table 4). The *tert*-butyl isosteres attempted have been successfully employed to mitigate metabolic degradation whilst maintaining biological activity [30,32,33]. The bioisosteres trialed included the pentafluorosulfanyl (**55**), trimethylsilyl (**56**), trifluoromethyl oxetane (**57**), trifluoroethane (**58**), trifluoromethoxy (**59**) and cyclopropyl (**60**). All these bioisoteres resulted in marked decreased activity (EC_{50} > 10 μM) except for the trimethylsilyl group (**56**) which showed a 2-fold loss in activity (EC_{50} 1.3 μM) compared to **1**. The metabolite of *tert*-butyl in the first step of oxidative degradation, 2,2-dimethyl-2-phenylethanol was also incorporated in an analogue (**61**) to determine if the *tert*-butyl group was acting as prodrug in a similar manner to terfenadine [34], however this change resulted in a loss in activity (EC_{50} > 10 μM). This data set further highlights the critical requirement of the *tert*-butyl group at the 4-position on the aryl ring for antimalarial activity.

We next investigated the impact of iterations around the piperazine ring and replacing the piperazine ring with isosteric

Table 1
Activity of analogues with carboxamide Substitutions.



Cmpd	R group	Pf parasite EC_{50} (SD) μM[a]	HepG2 CC_{50} (SD) μM[b]	cLogP[c]	PSA (Å^2)[c]	LipE
1		0.69 (0.06)	>40	2.5	61	3.7
16		>10	–	2.2	70	–
17		1.8 (0.2)	>40	2.8	61	2.9
18		0.33 (0.06)	>40	2.3	61	4.2
19		0.81 (0.1)	>40	3.2	61	2.9
20		0.50 (0.04)	>40	3.0	61	3.3
21		0.65 (0.04)	>40	3.4	61	2.8
22		0.38 (0.13)	>40	3.2	61	3.2
23		1.3 (0.08)	>40	3.2	61	2.7
24		4.3 (2.1)	>40	3.5	61	1.9
25		0.30 (0.05)	>40	3.1	61	3.4
26		0.92 (0.03)	>40	3.5	61	2.5
27		4.4 (1.3)	>40	1.9	70	3.5
28		6.1 (1.2)	>40	2.0	64	3.2
29		>10	–	1.6	73	–

[a] EC_{50} data represents averages and SDs for three or more independent experiments. Artesunate EC_{50} 8 (1) nM; Chloroquine EC_{50} 21 (7) nM.
[b] CC_{50} data represents means and SDs for three or more experiments.
[c] Calculated using ChemAxon software [56].

heterocycles (Table 5). It was shown that *S*-methyl substitution in the 3-position (**62**) was 3-fold less active (EC_{50} 1.9 μM) than **1**, while the *R*-methyl isomer (**63**) was inactive (EC_{50} > 10 μM). Accordingly, dimethyl substitution in the same position possessed similar activity (EC_{50} 1.6 μM) to **62**. The analogue **65** with a methylene bridge linking the 2- and 5-positions of the piperazine was synthetically tractable, but it was not active at the highest concentration tested (EC_{50} > 10 μM), while a two-carbon bridge linker in the 3,8-diazabicyclo[3.2.1]octane core of **66** resulted in comparable activity (EC_{50} 0.92 μM) to **1**. Replacement of the piperazine ring with a homopiperazine (**67**) or octahydropyrrolo[3,4-*c*]pyrrole (**68**) resulted in a significant decrease in activity (EC_{50} 6.3 and 4.3 μM) while replacement with a 2,6-diazaspiro[3.3]heptane system (**69**) resulted in a loss of activity (EC_{50} > 10 μM). Replacement of the *N*1 tertiary nitrogen with a carbon (**70**, an inseparable mixture of diastereomers) and additional replacement of the alpha-carbon with a nitrogen (**71**) both resulted in a loss of activity (EC_{50} > 10 μM). Incorporation of a carbonyl into the 2-position of the piperazine (**72**) was also detrimental to activity (EC_{50} > 10 μM). Overall, these results highlight the importance of maintaining the 6-atom configuration of the piperazine and basicity at the *N*1 position for activity.

We next explored the effect of the substitution at the alpha-carbon of the acyl moiety (Table 6). At this stage of the SAR exploration, the substituents were not installed at the alpha-carbon using a stereoselective synthesis (Scheme 4), therefore analogues were evaluated as a mixture of enantiomers and served to provide an early indication of functional group tolerance at this position. The data shows that removal of the methyl substitution at the alpha-carbon position (**73**) resulted in a loss of activity (EC_{50} > 10 μM). Ethyl substitution at the alpha-carbon position (**74**) resulted in a 3-fold loss in activity (EC_{50} 1.9 μM) compared to **1**, while the larger substitution such as *n*-propyl (**75**), *iso*-propyl (**76**) and benzyl (**78**) groups eliminated activity (EC_{50} > 10 μM), while phenyl substitution (**77**) was 7-fold less active (EC_{50} 5.1 μM) compared to **1**. These results highlight the requirement for a methyl substituent in this position and that space for further derivatization is limited. With this knowledge an analogue with gem-dimethyl substitution at the alpha-carbon of the carbonyl (**79**) was generated and showed a 10-fold loss in activity (EC_{50} 7.5 μM) compared to **1**. This data provided evidence that one enantiomer at this position was likely imparting antimalarial activity.

To determine importance of stereochemistry at alpha-carbon of the carboxamide each enantiomer of **1** was synthesized (Scheme 5) and evaluated in the LDH parasite assay (Table 7). Here, the *S*-isomer (***S*-1**) was found to be twice as active (EC_{50} 0.30 μM) as **1** (EC_{50} 0.69 μM), while the *R*-isomer (***R*-1**) was inactive within the concentration range of the titration (EC_{50} > 10 μM). To reaffirm these findings, the two stereoisomers of **20** were also produced and confirmed the *S*-isomer (***S*-20**) was found to be twice as active (EC_{50} 0.20 μM) as **20** (EC_{50} 0.50 μM), while the *R*-isomer (***R*-20**) was 14-fold less active (EC_{50} 6.9 μM). Consequently, these results clearly define the *S*-isomer as being the active stereoisomer.

We next synthesized the *S*-isomers of **18**, **22** and **38** which were the most potent analogues from the SAR study (EC_{50} 0.33, 0.38 and 0.18 μM, respectively). The *S*-isomers of these compounds (***S*-18**, ***S*-22** and ***S*-38**) showed 1.5 to 2-fold increases in activity (EC_{50} 0.13, 0.20 and 0.11 μM, respectively) when compared to their enantiomeric mixtures. Furthermore, the *S*-isomer (***S*-56**) of the trimethylsilyl bioisostere (**56**) was also 2-fold more active than its enantiomeric mixture (EC_{50} 0.49 versus 1.3 μM, respectively). We reasoned that the *S*-isomer is more active than the racemic mixture because the *S*-isomer binds more favorably to the parasite protein target than the *R*-isomer. It is also possible that the *S*-isomer is mimicking the natural form of alanine found in the substrate of the parasite protein target, providing a possible reason why the *S*-isomer preferred. As a result of defining the active stereoisomer, the LipE values of compounds accordingly improved with the activity, for example the *S*-isomer of **1** (***S*-1**) had a LipE 4.0 compared to a LipE of 3.7 for ***R*/*S*-1**.

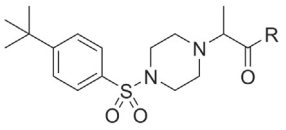
The solubility and *in vitro* metabolism of selected compounds from the phenylsulfonyl piperazine series was assessed to determine if they have suitable properties for dosing *in vivo* models. The *S*-isomer of **1** (***S*-1**) was found to possess moderate aqueous solubility (25−50 μM at pH 6.5) (Table S1) while **38** showed poor solubility (<1.6 at pH 6.5) due to high lipophilicity (Table S2). Both ***S*-1** and **38** were rapidly degraded by mouse liver microsomes (Cl_{int} > 866 μL/min/mg protein) (Tables S1 and S2). We reasoned there were several sites on the scaffold susceptible to metabolic turnover, including 1) oxidation of the *tert*-butyl group, 2) oxidation of the tertiary amine and subsequent dealkylation and 3) amide bond hydrolysis. We hypothesized one or more of these metabolic events likely contributed to the poor stability of this series. Replacement of the *tert*-butyl in ***S*-1** with trimethylsilyl (***S*-56**) did not improve clearance (Cl_{int} > 866 μL/min/mg protein) implying the major metabolic liability lies elsewhere in the scaffold. **70** possessed slightly improved stability in mouse microsomes (Cl_{int} 532 μL/min/mg protein), suggesting the tertiary basic nitrogen was likely one metabolic liability. **66** also possessed improved stability (Cl_{int} 473 μL/min/mg protein) signifying there were avenues to overcome the metabolic turnover at the tertiary amine while maintaining antimalarial activity. It is unlikely the intrinsic metabolic stability of this series can be addressed precluding the evaluation of this compound series from *in vivo* malaria efficacy studies. Nevertheless, the compounds series has suitable aqueous solubility for investigating erythrocyte invasion by parasites *in vitro*.

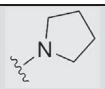
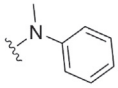
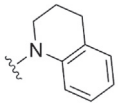
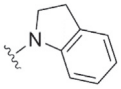
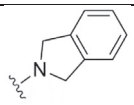
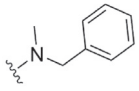
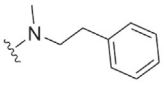
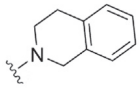
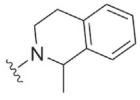
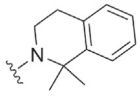
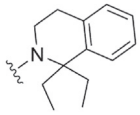
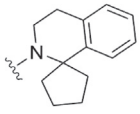
In summary of the SAR (Fig. 2), we determined the critical nature of several moieties on the phenylsulfonyl piperazine scaffold for antimalarial activity. The *tert*-butyl group was essential and could not be replaced with other functionality or bioisostere, except for the trimethylsilyl group (**56**) that had reduced potency (Tables 3 and 4). The piperazine moiety was also sensitive to modification (Table 5), except for a two-carbon bridge piperazine replacement (**66**). A tertiary substituted carboxamide was also required for activity with pyrrolidine and piperidine systems preferred (Table 1), while tetrahydroquinoline at this position was also tolerated but introduced lipophilicity resulting in low LipE (Table 2). The methyl group at the alpha-carbon of the carbonyl was also highly sensitive to alteration and did not tolerate larger substituents than a methyl group (Table 6). Furthermore, the *S*-configuration of the methyl group accounted for the observed antimalarial activity in their enantiomeric mixtures (Table 7). Overall, future optimization of the phenylsulfonyl piperazine class will be largely focused on improving the metabolic stability while maintaining or improving activity and aqueous solubility to develop compounds with suitable attributes for dosing in *in vivo* malaria models.

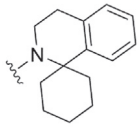
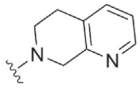
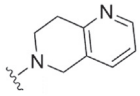
2.3. Evaluation of HepG2 cellular cytotoxicity

To determine whether the activity observed in the LDH viability-based assay was associated with cytotoxic to human cells, we assessed parasite active analogues in a human HepG2 cell assay employing Cell Titer-Glo [21]. Of the compounds evaluated, only five compounds (**35**−**38**, ***S*-38**) exhibited weak HepG2 growth inhibition (EC_{50} range of 17−31 μM). The toxicity of ***S*-38** was overcome by changing the tetrahydroisoquinoline carboxamide substitution to aliphatic heterocycles (***S*-18**, ***S*-20** and ***S*-22**) which have comparable *P. falciparum* parasite activity (EC_{50} 0.15, 0.20, 0.22 μM, respectively). This data shows there is no correlation between the activities of compounds in the parasite assay and the cell

Table 2
Activity of Analogues with Aryl Carboxamide Substitutions.



Cmpd	R group	Pf parasite EC_{50} (SD) μM [a]	HepG2 CC_{50} (SD) μM [b]	cLogP [c]	PSA ($Å^2$) [c]	LipE
1		0.6	>40	2.5	61	3.7
30		>10	-	3.8	61	-
31		1.9 (0.2)	>40	4.2	61	1.5
32		0.93 (0.14)	>40	3.8	61	2.2
33		1.0 (0.2)	>40	3.6	61	2.4
34		1.2 (0.3)	>40	3.8	61	2.1
35		2.1 (0.1)	17 (2.5)	4.1	61	1.6
36		0.99 (0.25)	29 (1.0)	3.9	61	2.1
37		1.2 (0.2)	28 (0.9)	4.3	61	1.6
38		0.18 (0.01)	31 (4.6)	4.6	61	2.2
39		1.2 (0.1)	>40	5.6	61	0.3
40		0.81 (0.21)	>40	5.1	61	1.0

41		2.9 (1.5)	>40	5.6	61	-0.2
42		1.9 (0.2)	>40	2.7	74	3.0
43		2.6 (0.4)	>40	2.7	74	2.9

[a]EC_{50} data represents averages and SDs for three or more independent experiments. Artesunate EC_{50} 8 (1) nM; Chloroquine EC_{50} 21 (7) nM.
[b]CC_{50} data represents means and SDs for three or more experiments.
[c]Calculated using ChemAxon software [56].

growth inhibition assay and therefore the cell growth inhibition exhibited by several compounds is likely promiscuous. Overall, the HepG2 activity observed with several analogues was modest and it was unlikely these concentrations would be relevant physiologically in *in vivo* models.

2.4. Invasion phenotype evaluation

We evaluated a selection of compounds (***S*-18**, ***S*-20**, ***S*-22** and ***S*-38**) with promising asexual stage activity to determine if they were primarily killing the parasite by blocking invasion as was originally observed for the hit compound **1** [14]. Here, we employed two previously described assays. The first is a schizont rupture assay [35,36] and the second uses nanoluciferase (NLuc) expressing parasites [14] to distinguish the invasion (or egress) blocking capacity of compounds. In the schizont rupture assay, late stage schizont *P. falciparum* D10 parasites expressing GFP [37] (44—48 h post erythrocyte invasion) were treated with either vehicle control or compound at four times the asexual EC_{90} to ensure a robust phenotypic response was observed. The subsequent 6 h of parasite stage development, including merozoite exit from the schizont and RBC re-entry, was quantified by flow cytometry relative to the vehicle control. The results showed that the majority of the compound and the cytochalasin D invasion control [12,23] treated schizonts had ruptured and released merozoites (Fig. 3A), but only a low percentage of merozoites were able to invade the RBC and develop into ring stage parasites. This implies the compounds were blocking merozoite entry into the RBC and preventing further parasite development. Notably, this effect by compounds ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38** was phenocopied by cytochalasin D, which is known to bind to actin and inhibit the actomyosin motor from driving the parasite into the RBC.

To confirm the result of the schizont rupture assay, NLuc-expressing merozoites that had been mechanically released from late stage schizonts were incubated with new RBCs in the presence of compounds ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38** or a vehicle control. The compounds were used at five times the EC_{90} of the 72 h asexual assay to provoke a quantifiable phenotypic response, and heparin was employed as a positive control for invasion inhibition. After a 30 min incubation period to allow RBC invasion, free or non-invaded merozoites were removed by washing. The newly invaded parasites were cultured for 24 h, after which their bioluminescence was measured to quantify the capacity of compounds to inhibit invasion versus the vehicle control. The data showed that compounds ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38** phenocopied the heparin positive control, largely preventing parasite invasion (Fig. 3B). The data from both assays confirmed the optimized compounds retained the invasion inhibitory capacity originally detected with compound **1** [14].

2.5. Evaluation against P. knowlesi and multi-drug resistant strains of P. falciparum

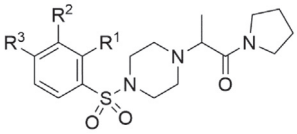
A representative selection of compounds ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38** were profiled against multi-drug resistant strains of *P. falciparum* (W2mef, Dd2, 7G8, Cam1.3) to determine cross resistance to clinically used antimalarials. The W2mef strain of *P. falciparum* expresses a mutant PfCRT resulting in chloroquine (CQ) resistance as well as augmented levels of the *pfmdrl* gene that confers resistance to mefloquine [38,39]. The other *P. falciparum* resistant parasites include the Dd2 and 7G8 strains (CQ, quinine, pyrimethamine and sulfadoxine resistant) and the artemisinin-resistant strain, Cam1.3 [40,41]. The results showed that compounds ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38** exhibited a relatively similar level of activity (within the levels of assay variation) against the multidrug resistance parasites compared to *P. falciparum* 3D7 (Table 8, Fig. S2). The one exception was compound ***S*-20**, which has an 8-fold difference in activity against 7G8 compared to 3D7. Overall, these results suggested that the compounds were not susceptible to the mechanisms of resistance observed with the clinically used antimalarials.

Compounds ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38** were also evaluated against *P. knowlesi* a species endemic to Southeast Asia [42]. In comparison to *P. falciparum* 3D7, the selected compounds were 2- to 5- fold less potent against *P. knowlesi* YH1 (Table 8, Fig. S3). This data suggests there could be a divergence in amino acid composition between the *P. knowlesi* and *P. falciparum* protein target of these compounds responsible for either the invasion phenotype observed or a resistance mechanism. Identification of the mechanism of action of this compound class is required to delineate between the differences in *P. knowlesi* and *P. falciparum* activity observed.

2.6. Evaluation against P. falciparum sexual stage gametocytes and liver stage schizonts

The sexual stage of the parasite lifecycle is where asexual parasites commit to gametocytogenesis. During this morphogenesis event micro-(male) and macro-(female) gametocytes have five morphologically distinct stages (I to V) of development before transmission of mature gametocytes to a mosquito via a blood meal [43,44]. The identification of novel agents which eliminate late stage gametocytes and therefore block transmission of the parasite

Table 3
Activity of Analogues with Aryl Substitutions Replacing the *tert*-Butyl Group.



Cmpd	R^2	R^3	Pf parasite EC_{50} (SD) μM [a]	HepG2 CC_{50} (SD) μM [b]	cLogP [c]	PSA ($Å^2$) [c]	LipE
1	H	*t*-Bu	0.69 (0.06)	>40	2.5	61	3.7
44	H	H	>10	-	1.0	61	-
45	H	*i*-Pr	>10	>40	2.2	61	-
46	H	Me	>10	-	1.6	61	-
47	H	*sec*-Bu	9.1 (0.4)	>40	2.7	61	2.3
48	H	CF_3	>10	-	1.8	61	-
49	H	CyPentyl	>10	-	1.7	61	-
50	H	Br	>10	-	1.7	61	-
51	H	OMe	>10	-	0.8	70	-
52	H	Ac	>10	-	0.5	78	-
53	H	Ph	>10	-	2.6	61	-
54	*t*-Bu	H	8.4 (0.7)	>40	2.5	61	2.6

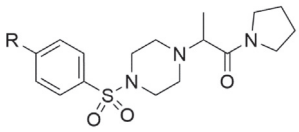
[a]EC_{50} data represents averages and SDs for three or more independent experiments. Artesunate EC_{50} 8 (1) nM; Chloroquine EC_{50} 21 (7) nM.
[b]CC_{50} data represents means and SDs for three or more experiments.
[c]Calculated using ChemAxon software [56].

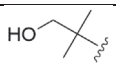
to a mosquito is crucial for long term disease control and eradication of malaria. To determine if the phenylsulfonyl piperazines series were suitable as a transmission blocking agent, representative analogues ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38** were evaluated against a highly synchronous culture of *P. falciparum* $NF54^{pfs16-LUC-GFP}$ [45] parasites at either the early (I-III) or late (IV–V) stages of gametocyte development, using a high content imaging assay [46]. The results indicated that ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38** demonstrate weak activity against both early and late stage gametocytes (approximately 50% at 20 μM) (Table 9, Figs. S4 and S5), which is consistent with the lack of gametocyte activity previously observed with compound **1** (Table S1) [24,47]. The weak gametocidal activity observed with these analogues is likely promiscuous and suggests that the mechanism by which this antimalarial class blocks invasion is not required for gametocyte development. Additionally, Pathogen Box data previously afforded on compound **1** indicates it does not impact liver schizont development (Table S1) [24]. Future studies will investigate whether sporozoite entry into the hepatocyte is impacted by the phenylsulfonyl piperazine class by the same mechanism that erythrocyte invasion is inhibited, and this data will ultimately be required to determine the potential target candidate profile (TCP) of this antimalarial class.

3. Conclusions

Merozoite invasion of the RBC is an essential step in the asexual development of *Plasmodium* parasites. We previously exploited a novel phenotypic assay capable of detecting RBC invasion to screen the MMV Pathogen Box and identified that MMV020291 (compound **1**) blocks invasion [14]. In this study, we explored the structure activity relationship of compound **1** and determined several key structural motifs were necessary for *P. falciparum*

Table 4
Activity of Analogues with *tert*-Butyl Isosteres.



Cmpd	R	Pf parasite EC_{50} (SD) μM [a]	HepG2 CC_{50} (SD) μM [b]	cLogP [c]	PSA ($Å^2$) [c]	LipE
1	*t*-Bu	0.69 (0.06)	>40	2.5	61	3.7
55	SF_5	>10	-	2.9	61	-
56	$(Me)_3Si$	1.3 (0.1)	>40	2.6	61	3.3
57		>10	-	2.6	61	-
58	CF_3CH_2	>10	-	2.1	61	-
59	CF_3O	>10	-	2.4	70	-
60	CyPr	>10	-	1.7	61	-
61		>10	>40	0.9	81	-

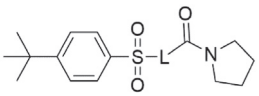
[a]EC_{50} data represents averages and SDs for three or more independent experiments. Artesunate EC_{50} 8 (1) nM; Chloroquine EC_{50} 21 (7) nM
[b]CC_{50} data represents means and SDs for three or more experiments.
[c]Calculated using ChemAxon software [56].

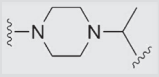
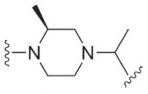
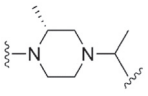
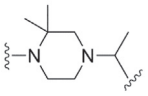
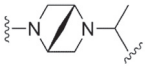
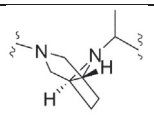
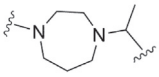
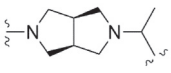
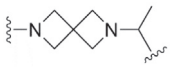
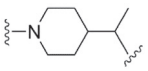
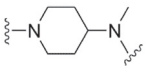
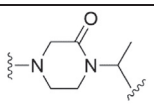
asexual parasite activity. Most notably, the *t*-butyl group, phenyl sulfonyl piperazine and the alpha-methyl group in the *S*-configuration were all essential for asexual activity, while a hydrophobic substituent on the carboxamide was found to be important for modulating asexual activity (Fig. 2). The optimization process generated compounds ***S*-18**, ***S*-22** and ***S*-38** with asexual activity in the 100–200 nM potency range, while maintaining selectivity against the human HepG2 cell line (Table 7). In addition, compounds ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38** also exhibited a similar level of asexual activity against multi-drug resistant lines relative to 3D7 parasites (Table 8) suggesting they were not susceptible to resistance mechanisms similar to those of clinically used antimalarials. Compounds ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38** were found to have weak activity against early and late stage gametocytes (Table 9), which is consistent with the lack of activity previously observed with compound **1** (Table S1) [47], and implies the mechanism of the target related to RBC invasion is not required for gametocytogenesis.

The optimized analogues ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38** were found to block invasion consistent with our previous findings with compound **1** [14]. The schizont rupture assay showed compound treated merozoites were able to engage with the RBC surface, but do not enter the RBC to initiate ring stage development (Fig. 3A). ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38** were shown to phenocopy the invasion inhibitory effect of the actin myosin motor inhibitor, CytoD. However, there are a number of actin independent mechanisms by which this antimalarial class could be preventing RBC invasion, such as proteolytic cleavage of surface ligands, Ca^{2+} release and essential protein-ligand complexes [13]. The mechanism of action of this antimalarial class appears conserved across *P. falciparum and P. knowlesi* against asexual parasites (Table 8), although the activity against *P. knowlesi* was reduced, implying there maybe differences in amino acid composition of the phenylsulfonyl piperazine target binding site between *Plasmodium* species.

Future studies will be directed at identifying the protein target associated with the invasion phenotype of the phenylsulfonyl piperazine series. Identifying the target of this antimalarial class in the future may enable a structure guided approach that would be valuable in further optimization. The antimalarial series described here displays potent *in vitro* activity against *Plasmodium* parasites but its intrinsic issue with metabolic turnover is likely to limit its use in *in vivo* without further development. Nevertheless, the analogues from the phenylsulfonyl piperazine class could serve as useful probes for studying *Plasmodium* erythrocyte invasion.

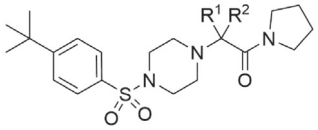
Table 5
Activity of Analogues with Piperazine Replacements.



Cmpd	X	Pf parasite EC_{50} (SD) μM [a]	HepG2 CC_{50} (SD) μM [b]	cLogP [c]	PSA ($Å^2$) [c]	LipE
1		0.69 (0.06)	>40	2.5	61	3.7
62		1.9 (0.2)	>40	2.9	61	2.8
63		>10	-	2.9	61	-
64		1.6 (0.2)	>40	3.2	61	2.6
65		>10	>40	2.5	61	-
66		0.92 (0.08)	>40	3.0	61	3
67		6.3 (1.3)	>40	2.6	61	2.6
68		4.3 (0.2)	>40	2.2	61	3.2
69		>10	-	2.2	61	-
70		>10	>40	3.4	58	1.7
71		>10	-	2.3	61	-
72		>10	-	1.6	78	-

[a] EC_{50} data represents averages and SDs for three or more independent experiments. Artesunate EC_{50} 8 (1) nM; Chloroquine EC_{50} 21 (7) nM.
[b] CC_{50} data represents means and SDs for three or more experiments.
[c] Calculated using ChemAxon software [56].

Table 6
Activity Analogues with Substitutions at the alpha-Carbon of the Carboxamide.



Cmpd	R^1	R^2	Pf parasite EC_{50} (SD) μM [a]	HepG2 CC_{50} (SD) μM [b]	cLogP [c]	PSA ($Å^2$) [c]	LipE
1	Me	H	0.69 (0.06)	>40	2.5	61	3.7
73	H	H	>10	-	1.9	61	-
74	Et	H	1.9 (0.04)	>40	3.0	61	2.7
75	*n*-Pr	H	>10	-	3.5	61	-
76	*i*-Pr	H	>10	-	3.3	61	-
77	Ph	H	5.1 (0.5)	>40	3.9	61	1.4
78	Bzl	H	>10	-	4.2	61	-
79	Me	Me	7.5 (2.1)	>40	2.9	61	2.2

[a] EC_{50} data represents averages and SDs for three or more independent experiments. Artesunate EC_{50} 8 (1) nM; Chloroquine EC_{50} 21 (7) nM.
[b] CC_{50} data represents means and SDs for three or more experiments.
[c] Calculated using ChemAxon software [56].

4. Experimental section

***P. falciparum* Asexual Stage Parasite Assay.** Parasite viability assays were performed as previously described [48,49].

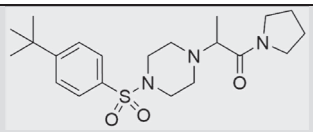
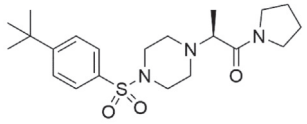
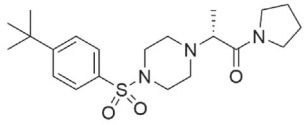
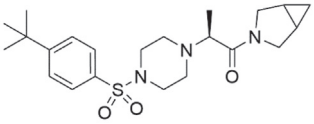
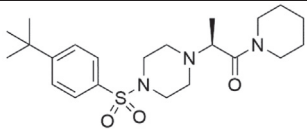
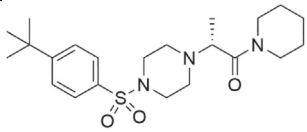
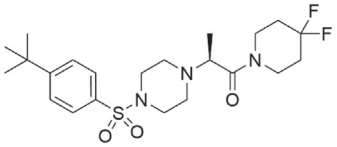
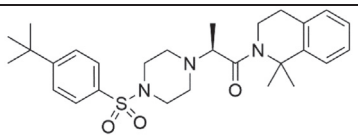
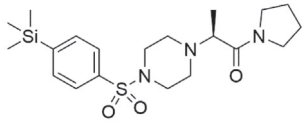
HepG2 Cell Growth Inhibition Assay. The HepG2 cellular assay was undertaken according to previously described protocol [21].

***P. falciparum* Multidrug Resistant and *P. knowlesi* Asexual Stage Parasite Viability Assays**. Mefloquine resistant W2mef, chloroquine resistant DD2 and 7G8, artemisinin resistant Cam3.I^{2539T} Cambodian isolate [50], and *P. knowlesi* YH1, were cultured according to established protocols [51]. *P. falciparum* 3D7 and resistant lines, and *P. knowlesi* (Pk YHI), were synchronized with 5% w/v sorbitol treatment at ring stage. An 8-point titration series of compounds were added at the ring stage in a 96 well plate and parasite growth measured at late trophozoite/schizont stages in the next cycle of growth (72 h post-invasion for *P. falciparum*; 48 h post-invasion for *P. knowlesi*). Parasite growth was quantified using flow cytometry of parasites stained with ethidium bromide (EtBr) (5 μg/mL for 1 h). Parasitaemia was measured on a BD Accuri C6 Plus Flow Cytometer (Becton Dickinson) using a 96-well plate reader. For parasite growth assays, late trophozoite/schizont stage parasites were gated with a forward scatter-high (FSC) and FL-2-high (EtBr) gate. Typically, 40,000 RBCs were counted in each well. All samples were analyzed using FlowJo software and growth of drug treatments were normalized against growth of DMSO treated parasites to calculate the percent survival of drug treated parasites. IC_{50} values were determined for each drug nonlinear regression of a log-(inhibitor)-versus-response curve in GraphPad Prism (version 8.0.1).

Schizont Rupture assay. D10-PfPHG parasites [37] were cultured according to established protocols [51] and maintained in an atmosphere of 1% O_2, 4% CO_2 and 95% N_2. Tight synchronization of GFP-fluorescent D10-PfPHG parasites for use in schizont rupture assays was achieved using sodium heparin [35,52]. Tightly synchronized D10-PfPHG schizont-stage (42–46 h post-invasion) parasites at 2% parasitaemia were incubated with a 4 times EC_{90} of compound until flow cytometry analysis of parasite populations 4 h after the expected completion of schizont rupture and merozoite invasion was completed. The assays were treated with 5 μg/mL ethidium bromide for 5 min prior to flow cytometry assessment of parasitaemia. Gating of newly invaded rings, free merozoites, non-invaded merozoites (those in close proximity to, but have not invaded, a RBC) and unruptured late stages was achieved as per published methods [36]. D10-PfPHG ring stage parasites (<6 h post invasion) were counted using Fl-1-high (GFP; excitation wavelength, 488 nm) and Fl-2-low (EtBr; excitation wavelength, 488 nm) gates. D10-PfPHG non-invaded merozoites were gated using Fl-1-low and Fl-2-high, while free merozoites were gated with a forward scatter-low (FSC) and FL-2-high gate. Typically, 40,000 RBCs were counted in each well. All samples were analyzed using FlowJo software and compound treatments were normalized against growth of DMSO treated parasites to calculate the percent survival of drug treated parasites. IC_{90} values were determined using GraphPad Prism (version 8.0.1) using nonlinear regression of a log-(inhibitor)-versus-response curve.

NLuc Invasion Assay. Merozoite invasion assays were performed as previously described in Ref. [14] whereby PEXEL-Nluc schizonts were isolated by magnet purification and incubated with 10 μM E64 for 4 h to allow the merozoites to mature but not egress. The schizonts were mechanically broken by passing them through a 1.2 μm filter to release the merozoites which were subsequently aliquoted into a 96-well plate into 5 times the EC_{90} of

Table 7
Activity of Analogues Showing the Importance of alpha-Methyl Stereochemistry.

Cmpd	Structure	Pf parasite EC_{50} (SD) μM [a]	HepG2 CC_{50} (SD) μM [b]	cLogP [c]	PSA ($Å^2$) [c]	LipE
1		0.69 (0.06)	>40	2.5	61	**3.7**
***S*-1**		0.30 (0.05)	>40	2.5	61	4.0
***R*-1**		>11	>40	2.5	61	-
***S*-18**		0.13 (0.02)	>40	2.3	61	4.6
***S*-20**		0.20 (0.03)	>40	3.0	61	3.7
***R*-20**		6.9 (2.1)	>40	3.0	61	2.2
***S*-22**		0.20 (0.02)	>40	3.2	61	3.5
***S*-38**		0.11 (0.04)	26 (3.0)	4.6	61	2.4
***S*-56**		0.49 (0.01)	>40	2.6	61	3.7

[a] EC_{50} data represents averages and SDs for three or more independent experiments. Artesunate EC_{50} 8 (1) nM; Chloroquine EC_{50} 21 (7) nM.
[b] CC_{50} data represents means and SDs for three or more experiments.
[c] Calculated using ChemAxon software [56].

growth of each compound (4 μM compound **1**, 62.75 μg/mL heparin, 2.5 μM ***S*-20** and ***S*-38** 0.25 μM, ***S*-18** 0.5 μM and ***S*-22** 0.5 μM) and with a final hematocrit of 1%. After a 30 min incubation at 37 °C to allow invasion, cell pellets were washed 3 times and incubated at 37 °C for a further 24 h. To determine the rate of invasion, Nluc activity was measured by resuspending the parasite infected RBCs in 1 x NanoGlo Lysis Buffer with NanoGlo substrate (Promega, 1:1000). A luminometer (CLARIOstar) measured relative light units

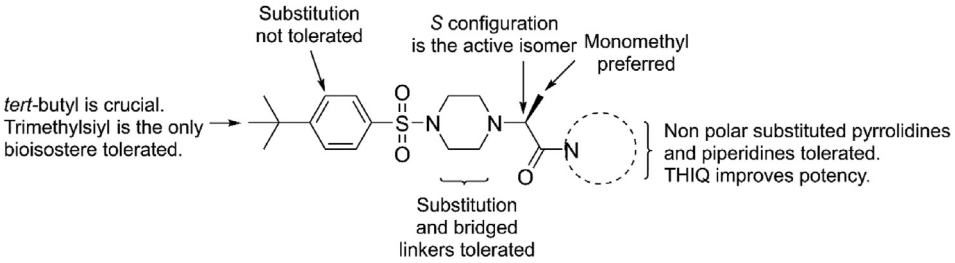


Fig. 2. Summary of the structure activity relationship.

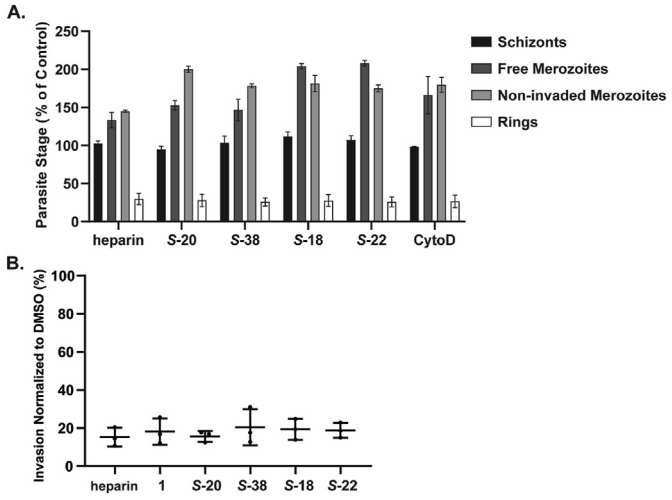


Fig. 3. Invasion phenotyping for representative compounds **1**, ***S*-18**, ***S*-20**, ***S*-22** and ***S*-38**. **A.** Late schizont stage parasites (44–48 h post invasion) were allowed to rupture in the presence of compound or invasion inhibitory controls heparin and cytochalasin D (CytoD) at 4 times the asexual EC_{90} and parasite populations assessed 6 h later by flow cytometry. Parasite populations quantitated were newly invaded ring stages (white bar), free merozoites (dark grey bar), non-invaded merozoites in close proximity to the RBC (light grey bar) and unruptured late stage parasites (black bar). **B.** Compounds demonstrated invasion inhibitory activity against purified merozoites comparable to the control heparin. Data represents the NLuc activity of compounds at 5 times the asexual EC_{90}. Data shown represents means for 3 independent experiments expressed as a percentage of the DMSO control population. Error bars shown are standard deviation.

(RLU). Invasion values were expressed as a percentage of the 0.1% DMSO vehicle control.

Gametocyte Assays. The sexual stage activity of compounds were evaluated using the established high content imaging assay according to the protocol of Duffy et al. [46] using $NF54^{pfs16-LUC-GFP}$ transgenic parasites [45].

***In vitro* metabolic stability using mouse liver microsomes.** Metabolic stability was performed according to a previously described protocol [53,54].

Aqueous Solubility. The aqueous solubility range of compounds were analyzed via nephelometry following a previously escribed method [55].

General Chemistry Procedures. Chemistry methods are analogous to those previously described by Nguyen et al. [49] NMR spectra were recorded on either a Bruker Avance DRX 300 or an Agilent MR400. Chemical shifts are reported in ppm on the δ scale and referenced to the appropriate solvent peak. MeOD, DMSO-d_6, Acetone-d_6 and $CDCl_3$ contain H_2O. LCMS were recorded on an Agilent LCMS system composed of an Agilent G6120B Mass Detector, 1260 Infinity G1312B Binary pump, 1260 Infinity G1367E HiPALS autosampler and 1260 Infinity G4212B Diode Array Detector (Method B). Conditions for LCMS Method A were as follows, column: Kinetex TM XB-C18 5 μm 4.6 × 50 mm, injection volume 10 μL, 5–100% B over 3 min (solvent A: water 0.1% formic acid; solvent B: AcCN 0.1% formic acid), flow rate: 1.5 mL/min, detection: 100–600 nm, acquisition time: 6 min. Conditions for LCMS Method B were as follows, column: Poroshell 120 EC-C18, 2.1 × 50 mm 2.7 μm at 20 °C, injection volume 2 μL, gradient: 5–100% B over 3 min (solvent A: water 0.1% formic acid; solvent B: AcCN 0.1% formic acid), flow rate: 0.8 mL/min, detection: 254 nm, acquisition time: 5 min. LCMS conditions used to assess purity of final compounds were as follows, column: Phenomenex Gemini C18, 2.0 × 50 mm; injection volume 20 μL; gradient: 0–100% Buffer B over 6 min (buffer A: 0.1% formic acid in autoclaved MilliQ water; buffer B: 0.1% formic acid in 100% acetonitrile), flow rate: 1.0 mL/min, detection: 214 or 224 nm. Unless otherwise noted, all compounds were found to be >95% pure by this method. HRMS were acquired through The Bio21 Mass Spectrometry and Proteomics Facility using a Thermo Scientific™ nano-LC Q Exactive™ Plus Mass spectrometer. Compounds **20**, **44**, **45** and **47** were procured from commercial vendors and used without further purification.

4.1. Synthesis methods

2-(4-((4-(tert-Butyl)phenyl)sulfonyl)piperazin-1-yl)*-1-(pyrrolidin*-1-yl)*propan-1-one* (**1**). General Method A. **6** (45 mg, 0.10 mmol), 4-*tert*-butylbenzenesulfonyl chloride (24 mg, 0.10 mmol) and

Table 8
Activity of Selected Compounds Against Asexual Blood Stage (ABS) *P. falciparum* 3D7 and Multi-Drug Resistant, and *P. knowlesi* parasites.[a]

Cmpd	ABS Pk YH1 EC_{50} (SD) μM	ABS Pf 3D7 EC_{50} (SD) μM	ABS Pf resistant lines EC_{50} (SD) μM			
			W2mef	Dd2	7G8	Cam3.1[b]
***S*-18**	0.14 (0.02)	0.03 (<0.01)	0.06 (0.01)	0.07 (<0.01)	0.08 (0.02)	0.06 (<0.01)
***S*-20**	1.3 (0.6)	0.61 (0.13)	0.49 (0.21)	0.86 (0.41)	5.1 (3.3)	1.3 (1.3)
***S*-22**	0.26 (0.02)	0.05 (0.02)	0.09 (0.01)	0.11(<0.01)	0.11 (0.03)	0.08 (0.02)
***S*-38**	0.09 (0.02)	0.01 (<0.01)	0.04 (0.02)	0.06 (0.05)	0.03 (<0.01)	0.02 (0.01)
CQ	0.01 (<0.01)	0.004 (0.002)	0.25 (0.08)	0.58 (0.31)	0.32 (0.12)	0.56 (0.25)

[a] EC_{50}s were determined for *P. falciparum* 3D7 and multidrug resistant strains (72 h) and *P. knowlesi* (Pk YH1) (48 h) asexual stage using flow cytometry. Data represents the means (SDs) of three experiments.
[b] Artemisinin resistant Cam3.I DHA resistant (R539T) parasites.

Table 9
Evaluation of selected analogues against stage I-III and stage VI-V gametocytes.[a]

Cmpd	Early stage I–III		Late stage IV–V	
	EC_{50} (SD) μM	% (SD) inhibition at 20 μM	EC_{50} (SD) μM	% (SD) inhibition at 20 μM
***S*-18**	>10	44 (2)	>10	36 (1)
***S*-20**	>10	49 (3)	>10	41 (4)
***S*-22**	>10	59 (2)	>10	47 (6)
***S*-38**	>10	79 (2)	>10	59 (3)
CQ	0.097 (0.003)	–	>10	99 (1)
ART	0.002 (0.001)	–	0.024 (0.001)	–
Pyr	0.053 (0.008)	–	1.24 (<0.01)	–

[a] EC_{50} data represent the averages and SDs for three or more experiments. CQ = chloroquine; ART = Artesunate; PYR = pyronaridine.

DIPEA (107 μL, 0.62 mmol) were dissolved in DCM (2 mL) and stirred at 40 °C for 16 h. The reaction was then concentrated *in vacuo* and the crude material was then purified by column chromatography eluting with 100% DCM to 5% MeOH/DCM to afford **1** as a solid (16.7 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71–7.59 (m, 2H), 7.57–7.48 (m, 2H), 3.63–3.52 (m, 1H), 3.52–3.29 (m, 4H), 3.19–2.95 (m, 4H), 2.95–2.77 (m, 2H), 2.77–2.59 (m, 2H), 2.00–1.72 (m, 4H), 1.35 (s, 9H), 1.24 (d, *J* 6.7 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.5, 156.6, 132.3, 127.6, 126.3, 125.9, 61.1, 48.7, 46.4, 45.9, 45.8, 35.1, 31.0, 26.1, 24.0, 23.9, 12.3. MS, *m/z* 408 (100) $[M+H]^+$. HRMS acquired: (M + H) 408.2313; $C_{21}H_{33}N_3O_3S$ requires (M + H), 408.2314.

1-((4-(tert-Butyl)phenyl)sulfonyl)piperazine (**2**). 1-Boc-piperazine (2.20 g, 11.8 mmol), 4-*tert*-butylbenzenesulfonyl chloride (1.96 g, 8.42 mmol) and DIPEA (4.40 mL, 25.3 mmol) were dissolved in DCM (10 mL) and stirred for 16 h at 20 °C under N_2. The reaction was then washed with cold 1 M HCl (10 mL), saturated $NaHCO_3$ (10 mL), brine (10 mL), dried with anhydrous Na_2SO_4, filtered and concentrated to afford Boc-protected product. This material was then dissolved in DCM/TFA (3:1, 8 mL), stirred for 1 h and then concentrated *in vacuo*. The residue was the dissolved in EtOAc (30 mL) and washed with saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was then dried with Na_2SO_4 and concentrated to afford **2** as a white solid (3.20 g, 99%). ^{1}H NMR (300 MHz, MeOD): δ 7.73 (q, *J* 8.1 Hz, 4H), 3.29–3.20 (m, 8H), 1.37 (s, 9H). MS, *m/z* 283 (100) $[M+H]^+$.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]propanoic acid hydrochloride* (**3**). *tert*-Butyl 2-bromopropanoate (464 μL, 2.77 mmol), potassium carbonate (0.87 g, 6.31 mmol) and **2** (0.91 g, 2.52 mmol) were dissolved in ACN (2 mL) and stirred at 80 °C for 16 h. The reaction was then concentrated and diluted with EtOAc (30 mL). The organic layer was washed with H_2O (10 mL) and brine (10 mL), and then dried with anhydrous Na_2SO_4, filtered and concentrated. The crude material was then purified by column chromatography eluting with 100% DCM to 5% MeOH/DCM to afford *tert*-butyl 2-(4-((4-(*tert*-butyl)phenyl)sulfonyl)piperazin-1-yl)propanoate as a solid (980 mg, 99%). The protected product was then stirred in 4 M HCl in dioxane (5 mL) and stirred for 48 h. Concentration afforded **3** as a solid (1.03 g, 100%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72–7.69 (m, 4H), 3.37–3.30 (m, 9H), 1.38–1.34 (s, 12H). MS, *m/z* 411 (100) $[M+H]^+$.

2-Bromo-1-pyrrolidin-1-yl-*propan-1-one* (**5**). Pyrrolidine (2.28 mL, 27.8 mmol) was added dropwise to a stirred solution of 2-bromopropanoyl bromide (1.46 mL, 13.9 mmol) in DCM (20 mL) at 0 °C and stirred for 30 min. The reaction was then warmed to 20 °C and stirred for a further 1 h. Saturated ammonium chloride solution (1 mL) was then added, and DCM removed *in vacuo*. The aqueous mixture was then extracted with Et_2O (2 × 30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to afford **5** as an oil (2.35 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.45 (q, *J* 6.6 Hz, 1H), 3.75–3.63 (m, 1H), 3.56–3.47 (m, 2H), 3.41 (td, *J* 7.0, 10.1 Hz, 1H), 2.07–1.95 (m, 2H), 1.95–1.85 (m, 2H), 1.83 (d, *J* 6.6 Hz, 3H). MS, *m/z* 206 (100) $[M+H]^+$.

2-Piperazin-1-yl-1-pyrrolidin-1-yl-*propan-1-one trifluoroacetate* (**6**). **5** (320 mg, 1.55 mmol), 1-Boc-piperazine (347 mg, 1.86 mmol) and DIPEA (482 μL, 3.11 mmol) in EtOH (5 mL) were stirred at 70 °C for 16 h. The reaction was concentrated and the crude material purified by column chromatography eluting with 100% DCM to 5% MeOH/DCM to afford *tert*-butyl 4-(1-methyl-2-oxo-2-pyrrolidin-1-yl-ethyl)piperazine-1-carboxylate as an oil (428 mg, 89%). This material was then dissolved in DCM/TFA (3:1, 8 mL) and stirred for 1 h and then concentrated to afford **6** as a solid (600 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.72 (q, *J* 4.6 Hz, 1H), 3.59–3.30 (m, 8H), 2.73–2.40 (m, 4H), 2.02–1.80 (m, 4H), 1.46 (s, 9H), 1.32–1.15 (m, 3H). MS, *m/z* 212 (100) $[M+H]^+$.

tert-Butyl 2-[4-(4-tert-butylphenyl)sulfonylpiperazin-1-yl*]acetate* (**10**). *tert*-Butyl bromoacetate (0.122 mL, 0.83 mmol), potassium carbonate (209 mg, 1.51 mmol) and **2** (211 mg, 0.76 mmol) were dissolved in ACN (2 mL) and stirred at reflux for 16 h. The mixture was then concentrated, and the residue was dissolved in EtOAc (10 mL). The organic layer was washed with H_2O (10 mL) and brine (10 mL), then dried with anhydrous Na_2SO_4, filtered and concentrated. The crude material was then purified by column chromatography eluting with 100% DCM to 5% MeOH/DCM to afford **10** as a solid (295 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71–7.61 (m, 2H), 7.57–7.48 (m, 2H), 3.12–2.97 (m, 6H), 2.70–2.55 (m, 4H), 1.43 (s, 9H), 1.34 (s, 9H). MS, *m/z* 397 (100) $[M+H]^+$.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-N-ethyl-propanamide* (**16**). General Method B: **3** (22 mg, 0.056 mmol), ethanamine hydrochloride (9 mg, 0.11 mmol), DIPEA (0.05 mL, 0.28 mmol) and HBTU (0.12 mL, 0.080 mmol) were dissolved in DMF (2 mL) and allowed to stir for 3 h at 20 °C. The reaction was concentrated *in vacuo* and dissolved in EtOAc (15 mL) and washed with 10% $NaHCO_3$ (10 mL), dried with anhydrous Na_2SO_4, filtered and concentrated. The crude material was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to afford **16** as a solid (8.3 mg, 39%). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 8.07 (s, 1H), 7.59–7.81 (m, 4H), 3.04–3.27 (m, 7H), 2.70–2.99 (m, 4H), 1.37 (s, 9H), 1.28 (d, *J* 6.9 Hz, 3H), 1.08 (t, *J* 7.3 Hz, 3H). MS, *m/z* 382 (100) $[M+H]^+$. HRMS acquired: (M + H) 382.2165; $C_{19}H_{31}N_3O_3S$ requires (M + H), 382.2159.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-N,N-diethyl-propanamide* (**17**). General Method B was replicated employing **3** (22 mg, 0.056 mmol) and *N,N*-diethylamine (11 μL, 0.12 mmol) to afford **17** as a solid (11 mg, 48%). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 7.85–7.65 (m, 4H), 4.35–4.12 (m, 1H), 3.77–3.59 (m, 6H), 3.30–2.56 (m, 6H), 1.44 (d, *J* 6.7 Hz, 3H), 1.35–1.41 (m, 9H), 1.24 (t, *J* 7.2 Hz, 3H), 1.14 (t, *J* 7.1 Hz, 3H). MS, *m/z* 410 (100) $[M+H]^+$. HRMS acquired: (M + H) 410.2469; $C_{21}H_{35}N_3O_3S$ requires (M + H), 410.2472.

1-(3-Azabicyclo[3.1.0]hexan-3-yl*)-2-[4-(4-tert-butylphenyl)sulfonylpiperazin*-1-yl*]propan-1-one* (**18**). General Method B was

replicated employing **3** (25 mg, 0.064 mmol) and 3-azabicyclo [3.1.0]hexane hydrochloride (11 mg 0.096 mmol) to afford **18** as a solid (24 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.75–7.60 (m, *J* 8.1 Hz, 2H), 7.60–7.49 (m, *J* 8.1 Hz, 2H), 3.90–3.72 (m, 1.5H), 3.67–3.20 (m, 3.5H), 3.06 (app s, 4H), 2.80–2.64 (m, 2H), 2.64–2.48 (m, 2H), 1.61–1.46 (m, 2H), 1.37 (s, 9H), 1.17 (d, *J* 6.6 Hz, 3H), 0.79–0.58 (m, 1H), 0.06 (dd, *J* 4.2, 17.3 Hz, 1H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 171.8, 171.7, 156.6, 132.5, 132.4, 127.7, 126.0, 125.9, 61.6, 61.1, 49.2, 48.7, 48.6, 48.4, 48.1, 46.2, 46.1, 35.1, 31.1, 16.0, 15.9, 14.5, 14.4, 12.0, 11.8, 9.5, 9.2. MS, *m/z* 420 (100) $[M+H]^+$. HRMS acquired: 420.2318 (M + H); $C_{22}H_{33}N_3O_3S$ requires (M + H), 420.2315.

*1-[(3aR,6aS)-3,3a,4,5,6,6a-Hexahydro-1H-cyclopenta[c]pyrrol-2-*yl*]-2-[4-(4-tert-butylphenyl)sulfonylpiperazin-*1-yl*]propan-1-one* (**19**). General Method B was replicated employing **3** (25 mg, 0.064 mmol) and (3a*R*,6a*S*)-octahydrocyclopenta[*c*]pyrrole hydrochloride (14 mg 0.096 mmol) to afford **19** as a solid (27 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.75–7.60 (m, *J* 7.6 Hz, 2H), 7.60–7.45 (m, *J* 8.0 Hz, 2H), 3.80–3.13 (m, 4H), 3.23–2.93 (m, 4H), 2.77 (as, 2H), 2.64 (as, 4H), 1.91–1.51 (m, 5H), 1.51–1.25 (m, 11H), 1.25–1.11 (m, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.6, 170.4, 156.6, 132.3, 132.0, 127.7, 127.7, 126.0, 125.9, 61.2, 61.1, 52.6, 52.3, 51.8, 51.7, 48.9, 48.6, 46.2, 46.0, 43.7, 43.5, 41.4, 41.3, 35.1, 32.3, 32.0, 32.0, 31.9, 31.0, 25.5, 25.4, 12.4, 11.6. MS, *m/z* 448 (100) $[M+H]^+$. HRMS acquired: (M + H) 448.2629; $C_{24}H_{37}N_3O_3S$ requires (M + H), 448.2628.

*1-(Azepan-*1-yl*)-2-[4-(4-tert-butylphenyl)sulfonylpiperazin-*1-yl*]propan-1-one* (**21**). General Method B was replicated employing **3** (10 mg, 0.026 mmol) and azepane (14 μL 0.13 mmol) to afford **21** as a solid (27 mg, 95%). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 7.80–7.63 (m, 4H), 4.08–3.92 (m, 1H), 3.71–3.33 (m, 6H), 3.16–2.85 (m, 6H), 1.63–1.81 (m, 4H), 1.22–1.45 (m, 12H). MS, *m/z* 436 (100) $[M+H]^+$. HRMS acquired: (M + H) 436.2635; $C_{23}H_{37}N_3O_3S$ requires (M + H), 436.2628.

*2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-*1-yl*]-1-(4,4-difluoro-1-piperidyl)propan-1-one* (**22**). General Method B was replicated employing **3** (25 mg, 0.064 mmol) and 4,4-difluoropiperidine hydrochloride (20 mg, 0.19 mmol) to afford **22** as a solid (26 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.71–7.63 (m, *J* 8.0 Hz, 2H), 7.60–7.50 (m, *J* 7.6 Hz, 2H), 4.00–3.82 (m, 1H), 3.75–3.59 (m, 1H), 3.59–3.39 (m, 3H), 3.02 (as, 4H), 2.79–2.62 (m, 2H), 2.62–2.41 (m, 2H), 2.07–1.72 (m, 4H), 1.35 (s, 9H), 1.18 (d, *J* 6.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, rotamers) δ 170.3, 156.8, 132.4, 127.6, 126.0, 121.4, 60.0, 48.1, 46.2, 42.3, 39.0, 35.2, 35.0, 34.7, 34.4, 34.2, 33.9, 33.6, 31.0, 10.3. MS, *m/z* 458 (100) $[M+H]^+$. HRMS acquired: (M + H) 458.2286; $C_{22}H_{33}F_2N_3O_3S$ requires (M + H), 458.2283.

*2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-*1-yl*]-1-(4-methyl-1-piperidyl)propan-1-one* (**23**). General Method B was replicated employing **3** (22 mg, 0.056 mmol) and 4-methylpiperidine hydrochloride (14 mg, 0.11 mmol) to afford **23** as a solid (1.4 mg, 6%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.73–7.62 (m, 2H), 7.61–7.49 (m, 2H), 4.01–3.84 (m, 1H), 3.69–3.35 (m, 6H), 3.30–3.09 (m, 2H), 3.09–2.96 (m, 2H), 2.92–2.52 (m, 2H), 2.09–1.76 (m, 5H), 1.76–1.54 (m, 3H), 1.44–1.27 (m, 9H), 1.02–0.78 (m, 3H). MS, *m/z* 436 (100) $[M+H]^+$. HRMS acquired: (M + H) 436.2627; $C_{23}H_{37}N_3O_3S$ requires (M + H), 436.2628.

*2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-*1-yl*]-1-(4,4-dimethyl-1-piperidyl)propan-1-one* (**24**). General Method B was replicated employing **3** (22 mg, 0.056 mmol) and 4,4-dimethylpiperidine hydrochloride (17 mg, 0.11 mmol) to afford **24** as a solid (1.4 mg, 6%). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 7.78–7.64 (m, 4H), 3.91–3.79 (m, 1H), 3.77–3.68 (m, 1H), 3.61–3.52 (m, 1H), 3.46–3.35 (m, 2H), 3.14–2.96 (m, 4H), 2.90–2.69 (m, 4H), 1.38 (s, 9H), 1.35–1.24 (m, 4H), 1.20 (d, *J* 6.7 Hz, 3H), 0.99 (s, 3H), 0.93 (s, 3H). MS, *m/z* 450 (100) $[M+H]^+$. HRMS acquired: (M + H) 450.2782; $C_{24}H_{39}N_3O_3S$ requires (M + H), 450.2785.

*1-(6-Azaspiro[2.5]octan-*6-yl*)-2-[4-(4-tert-butylphenyl)sulfonylpiperazin-*1-yl*]propan-1-one* (**25**). General Method B was replicated employing **3** (22 mg, 0.056 mmol) and 6-azaspiro[2.5]octane hydrochloride (17 mg, 0.11 mmol) to afford **25** as a solid (1.4 mg, 6%). ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ 7.72–7.57 (m, *J* 8.6 Hz, 2H), 7.56–7.43 (m, *J* 8.2 Hz, 2H), 3.79–3.66 (m, 1H), 3.59–3.34 (m, 4H), 3.03 (app s, 4H), 2.74–2.61 (m, 2H), 2.61–2.48 (m, 2H), 1.41–1.14 (m, 16H), 0.19–0.38 (m, 4H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.3, 156.6, 132.6, 127.7, 125.9, 59.6, 48.4, 46.1, 45.7, 42.2, 35.9, 35.1, 34.9, 31.0, 17.7, 11.8, 11.5, 11.4. MS, *m/z* 448 (100) $[M+H]^+$. HRMS acquired: (M + H) 448.2623; $C_{24}H_{37}N_3O_3S$ requires (M + H), 448.2628.

*1-(8-Azabicyclo[3.2.1]octan-*8-yl*)-2-(4-((4-(tert-butyl)phenyl)sulfonyl)piperazin-*1-yl*)propan-1-one* (**26**). General Method B was replicated employing **3** (25 mg, 0.064 mmol) and 8-azabicyclo [3.2.1]octane hydrochloride (14 mg, 0.096 mmol) to afford **26** as a solid (26 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.72–7.61 (m, *J* 8.4 Hz, 2H), 7.58–7.47 (m, *J* 8.0 Hz, 2H), 4.75–4.56 (m, 1H), 4.39–4.23 (m, 0.5H), 4.23–4.10 (m, 0.5H), 3.48–3.20 (m, 1H), 3.16–2.85 (m, 4H), 2.82–2.48 (m, 4H), 1.88–1.82 (m, 1H), 1.79–1.45 (m, 9H), 1.35 (s, 9H), 1.27–1.10 (m, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 167.7, 167.3, 156.6, 132.3, 132.2, 127.7, 127.7, 125.9, 61.0, 60.2, 54.9, 51.9, 51.8, 48.9, 48.6, 46.3, 46.1, 35.1, 32.8, 32.5, 31.3, 31.0, 30.8, 28.6, 28.5, 26.8, 26.6, 16.7, 16.7, 13.1, 12.6. MS, *m/z* 448 (100) $[M+H]^+$. HRMS acquired: (M + H) 448.2632; $C_{24}H_{37}N_3O_3S$ requires (M + H), 448.2628.

*2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-*1-yl*]-1-morpholino-propan-1-one* (**27**). General Method B was replicated employing **3** (22 mg, 0.056 mmol) and morpholine (0.11 mL, 0. 11 mmol) to afford **27** as a solid (8 mg, 34%). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 7.77–7.61 (m, 4H), 3.74–3.35 (m, 9H), 3.00 (t, *J* 4.8 Hz, 4H), 2.77–2.53 (m, 4H), 1.40–1.33 (m, 9H), 1.15 (d, *J* 6.7 Hz, 3H). MS, *m/z* 424 (100) $[M+H]^+$. HRMS acquired: (M + H) 424.2271; $C_{21}H_{33}N_3O_4S$ requires (M + H), 424.2265.

*2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-*1-yl*]-1-(4-methylpiperazin-*1-yl*)propan-1-one* (**28**). General Method B was replicated employing **3** (10 mg, 0.026 mmol) and 1-methylpiperazine (94 μL, 0. 11 mmol) to afford **28** as a solid (4.1 mg, 33%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.70 (d, *J* 7.9 Hz, 2H), 7.57 (d, *J* 8.1 Hz, 2H), 4.26–3.38 (m, 7H), 3.50–2.21 (m, 13H), 1.37 (s, 9H), 1.20 (d, *J* 5.5 Hz, 3H). MS, *m/z* 437 (100) $[M+H]^+$. HRMS acquired: (M + H) 437.2588; $C_{22}H_{36}N_4O_4S$ requires (M + H), 437.2588.

*2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-*1-yl*]-1-piperazin-*1-yl*-propan-1-one trifluoroacetate* (**29**). **80** (20.5 mg, 0.039 mmol) was stirred in a 1:3 mixture of TFA/DCM (4 mL) for 2 h. The reaction was then concentrated to afford **29** as a solid (21.2 mg, 83%). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 7.84–7.68 (m, 4H), 4.55 (q, *J* 6.7 Hz, 1H), 4.07–3.67 (m, 4H), 3.67–3.35 (m, 8H), 3.31–3.04 (m, 4H), 1.53 (d, *J* 6.9 Hz, 3H), 1.39 (s, 9H). MS, *m/z* 423 (100) $[M+H]^+$. HRMS acquired: (M + H) 423.2428; $C_{21}H_{34}N_4O_3S$ requires (M + H), 423.2424.

*2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-*1-yl*]-N-methyl-N-phenyl-propanamide* (**30**). General Method B was replicated employing **3** (10 mg, 0.026 mmol) and N-methylaniline (13 μL, 0.10 mmol) to afford **30** as a solid (2.3 mg, 20%). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 7.74–7.64 (m, 4H), 7.43–7.32 (m, 3H), 7.29–7.21 (m, 2H), 3.59–3.53 (m, 1H), 3.23 (s, 3H), 2.95–2.81 (m, 4H), 2.63–2.52 (m, 2H), 2.46–2.36 (m, 2H), 1.40 (s, 9H), 1.10 (d, *J* 6.8 Hz, 3H). MS, *m/z* 444 (100) $[M+H]^+$. HRMS acquired: (M + H) 444.2319; $C_{24}H_{33}N_3O_3S$ requires (M + H), 444.2315.

*2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-*1-yl*]-1-(3,4-dihydro-2H-quinolin-*1-yl*)propan-1-one* (**31**). General Method B was replicated employing **3** (30 mg, 0.077 mmol) and 1,2,3,4-

tetrahydroquinoline (20 mg, 0. 15 mmol) to afford **31** as a solid (29.3 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.69–7.58 (m, 2H), 7.57–7.46 (m, 2H), 7.21–6.90 (m, 4H), 4.05–3.75 (m, 2H), 3.68–3.44 (m, 1H), 2.91 (app s, 4H), 2.76–2.38 (m, 6H), 2.09–1.76 (m, 2H), 1.39–1.31 (m, 9H), 1.20 (d, *J* 6.7 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 171.2, 156.1, 138.8, 133.6, 131.8, 127.9, 127.3, 125.7, 125.5, 125.1, 123.6, 58.0, 47.6, 45.8, 42.0, 34.7, 30.6, 26.2, 23.6, 12.3. MS, *m/z* 370 (100) $[M+H]^+$. HRMS acquired: (M + H) 470.2477; $C_{26}H_{35}N_3O_3S$ requires (M + H), 470.2471.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-indolin*-1-yl-*propan-1-one* (**32**). General Method B was replicated employing **3** (30 mg, 0.077 mmol) and indoline (17 μL, 0. 15 mmol) to afford **32** as a solid (35 mg, 100%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 8.24 (d, *J* 7.9 Hz, 1H), 7.73–7.62 (m, *J* 8.4 Hz, 2H), 7.58–7.48 (m, *J* 8.4 Hz, 2H), 7.26–7.11 (m, 2H), 7.09–6.97 (m, 1H), 4.39–4.23 (m, 1H), 4.09–3.92 (m, 1H), 3.59–3.42 (m, 1H), 3.24–2.93 (m, 6H), 2.88–2.55 (m, 4H), 1.36 (s, 9H), 1.28 (d, *J* 6.6 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.1, 156.6, 143.0, 132.4, 131.2, 127.6, 127.4, 126.0, 124.4, 123.9, 117.2, 61.9, 48.3, 47.6, 46.2, 35.1, 31.0, 28.1, 10.5. MS, *m/z* 456 (100) $[M+H]^+$. HRMS acquired: (M + H) 456.2320; $C_{25}H_{33}N_3O_3S$ requires (M + H), 456.2315.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-isoindolin*-2-yl-*propan-1-one* (**33**). General Method B was replicated employing **3** (25 mg, 0.064 mmol) and isoindoline (15 μL, 0. 15 mmol) to afford **33** as a solid (34.9 mg, 100%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.72–7.63 (m, 2H), 7.56–7.47 (m, 2H), 7.33–7.16 (m, 4H), 5.09–4.94 (m, 1H), 4.83–4.70 (m, 3H), 3.55–3.36 (m, 1H), 3.07 (app s, 4H), 2.91–2.51 (m, 4H), 1.39–1.18 (m, 12H). MS, *m/z* 456 (100) $[M+H]^+$. HRMS acquired: (M + H) 456.2320; $C_{25}H_{33}N_3O_3S$ requires (M + H), 456.2315.

N-Benzyl-2-[4-(4-tert-butylphenyl)sulfonylpiperazin-1-yl*]-N-methyl-propanamide* (**34**). General Method B was replicated employing **3** (30 mg, 0.077 mmol) and *N*-methylbenzylamine (20 μL, 0. 15 mmol) to afford **34** as a solid (31 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.68 (t, *J* 8.5 Hz, 2H), 7.55 (d, *J* 8.3 Hz, 2H), 7.34–7.04 (m, 5H), 4.72–4.43 (m, 2H), 3.63–3.48 (m, 1H), 3.15–2.89 (m, 7H), 2.82–2.48 (m, 4H), 1.38 (d, *J* 6.0 Hz, 9H), 1.21 (dd, *J* 6.7, 10.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, rotamers) δ 172.4, 172.1, 156.5, 156.5, 137.3, 136.9, 132.5, 128.6, 128.5, 127.8, 127.7, 127.4, 127.3, 126.2, 125.9, 59.5, 53.0, 51.1, 48.4, 48.2, 46.2, 46.0, 35.1, 34.7, 34.3, 31.1, 31.0, 11.4, 10.9. MS, *m/z* 458 (100) $[M+H]^+$. HRMS acquired: (M + H) 458.2477; $C_{25}H_{35}N_3O_3S$ requires (M + H), 458.2471.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-N-methyl-N-(2-phenylethyl)propenamide* (**35**). General Method B was replicated employing **3** (20 mg, 0.051 mmol) and *N*-methyl-2-phenylethanamine (15 μL, 0. 10 mmol) to afford **35** as a solid (22.7 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.67 (t, *J* 6.9 Hz, 2H), 7.48–7.58 (m, 2H), 7.11–7.32 (m, 4H), 7.07 (d, *J* 6.9 Hz, 1H), 3.88–3.56 (m, 1H), 3.53–3.15 (m, 2H), 3.08–2.91 (m, 5H), 2.90–2.72 (m, 4H), 2.68–2.37 (m, 4H), 1.33 (s, 4.5H), 1.37 (s, 4.5H), 1.12 (d, *J* 6.3 Hz, 1.5H), 1.00 (d, *J* 6.5 Hz, 1.5H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 171.4, 156.6, 138.8, 138.0, 132.5, 132.4, 128.8, 128.8, 128.6, 128.3, 127.6, 126.8, 126.3, 126.0, 59.4, 58.9, 51.2, 49.5, 48.2, 46.0, 35.7, 35.1, 35.1, 34.9, 33.6, 33.5, 31.0, 31.0, 11.6, 11.3. MS, *m/z* 472 (100) $[M+H]^+$. HRMS acquired: (M + H) 472.2632; $C_{26}H_{37}N_3O_3S$ requires (M + H), 472.2628.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-(3,4-dihydro-1H-isoquinolin*-2-yl*)propan-1-one* (**36**). General Method B was replicated employing **3** (30 mg, 0.077 mmol) and 1,2,3,4-tetrahydroisoquinoline (20 μL, 0. 153 mmol) to afford **36** as a solid (24.2 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.72–7.57 (m, 2H), 7.57–7.46 (m, 2H), 7.24–7.02 (m, 3H), 6.97–6.83 (m, 1H), 4.78 (dd, *J* 10.2, 16.5 Hz, 1H), 4.58 (dd, *J* 7.2, 16.6 Hz, 1H), 4.01–3.88 (m, 0.5H), 3.86–3.75 (m, 0.5H), 3.70–3.49 (m, 2H), 3.04 (t, *J* 4.1 Hz, 2H), 2.96–2.87 (m, 1H), 2.86–2.52 (m, 7H), 1.42–1.32 (m, 9H), 1.24–1.14 (m, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.6, 156.6, 156.5, 134.7, 133.8, 133.2, 132.8, 132.5, 128.9, 128.2, 127.7, 127.6, 126.8, 126.7, 126.6, 126.5, 126.0, 125.8, 60.3, 60.0, 48.2, 47.6, 46.1, 45.8, 44.5, 43.2, 40.7, 35.1, 31.1, 29.6, 28.1, 11.1. MS, *m/z* 470 (100) $[M+H]^+$. HRMS acquired: (M + H) 470.2476; $C_{26}H_{35}N_3O_3S$ requires (M + H), 470.2471.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-(1-methyl-3,4-dihydro-1H-isoquinolin*-2-yl*)propan-1-one* (**37**). General Method B was replicated employing **3** (20 mg, 0.051 mmol) and 1-methyl-1,2,3,4-tetrahydroisoquinoline (15 mg, 0. 10 mmol) to afford **37** as a solid mixture of diastereomers (24.2 mg, 98%). ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ 7.73–7.61 (m, 2H), 7.61–7.45 (m, 2H), 7.22–6.94 (m, 3.5H), 6.90–6.81 (m, 0.5H), 5.68–5.53 (m, 1H), 5.38–5.24 (m, 0.5H), 5.14–5.05 (m, 0.5), 4.71–4.55 (m, 1H), 4.04 (d, *J* 14.1 Hz, 0.5H), 3.96 (d, *J* 10.6 Hz, 0.5H), 3.70–3.41 (m, 1H), 3.34 (dt, *J* 3.5, 12.3 Hz, 1H), 3.18–3.05 (m, 2H), 3.05–2.92 (m, 2H), 2.90–2.78 (m, 2H), 2.76–2.50 (m, 5H), 1.43–1.34 (m, 9H), 1.24–1.15 (m, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.0, 156.6, 156.3, 138.5, 138.3, 133.8, 133.1, 132.9, 132.9, 132.7, 132.5, 132.3, 129.3, 129.1, 128.5, 127.7, 127.6, 127.5, 127.2, 127.1, 126.8, 126.5, 126.4, 126.4, 126.3, 126.3, 126.1, 126.0, 125.9, 125.8, 60.3, 59.9, 59.6, 51.8, 48.7, 48.6, 48.5, 48.1, 47.9, 46.1, 45.8, 39.4, 39.1, 35.4, 35.1, 31.0, 29.6, 28.2, 23.3, 23.0, 21.6, 21.3, 11.9, 10.7. MS, *m/z* 484 (100) $[M+H]^+$. HRMS acquired: (M + H) 484.2622; $C_{27}H_{37}N_3O_3S$ requires (M + H), 484.2628.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-(1,1-dimethyl-3,4-dihydroisoquinolin*-2-yl*)propan-1-one* (**38**). General Method B was replicated employing **3** (20 mg, 0.051 mmol) and 1,1-dimethyl-1,2,3,4-tetrahydroisoquinoline (16.5 mg, 0. 10 mmol) to afford **38** as a solid (18.3 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.75–7.63 (m, *J* 8.4 Hz, 2H), 7.61–7.49 (m, *J* 8.3 Hz, 2H), 7.35–7.20 (m, 2H), 7.14 (t, *J* 7.2 Hz, 1H), 7.01 (d, *J* 7.3 Hz, 1H), 3.77–3.55 (m, 2H), 3.51–3.36 (m, 1H), 3.06 (app s, 4H), 2.82–2.67 (m, 4H), 2.67–2.54 (m, 2H), 1.80 (s, 6H), 1.38 (s, 9H), 1.20 (d, *J* 6.4 Hz, 3H). MS, *m/z* 498 (100) $[M+H]^+$. HRMS acquired: (M + H) 498.2790; $C_{28}H_{39}N_3O_3S$ requires (M + H), 498.2785.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-(1,1-diethyl-3,4-dihydroisoquinolin*-2-yl*)propan-1-one* (**39**). General Method B was replicated employing **3** (20 mg, 0.051 mmol) and **82** (19.4 mg, 0. 10 mmol) to afford **39** as a solid (2.5 mg, 9%). ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ 7.72–7.61 (m, *J* 7.4 Hz, 2H), 7.58–7.47 (m, *J* 7.8 Hz, 2H), 7.26–7.19 (m, 2H), 7.16–7.10 (m, 1H), 7.02 (d, *J* 7.4 Hz, 1H), 3.76–3.53 (m, 3H), 3.14–3.02 (m, 4H), 2.79–2.57 (m, 6H), 1.78–1.63 (m, 4H), 1.35 (s, 9H), 1.21 (d, *J* 6.7 Hz, 3H), 0.59–0.41 (m, 6H). MS, *m/z* 526 (100) $[M+H]^+$. HRMS acquired: (M + H) 526.3088; $C_{30}H_{43}N_3O_3S$ requires (M + H), 526.3098.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-spiro[3,4-dihydroisoquinoline-1,1′-cyclopentane]*-2-yl-*propan-1-one* (**40**). General Method B was replicated employing **3** (12 mg, 0.031 mmol) and **84** (6.5 mg, 0. 035 mmol) to afford **40** as a solid (10.6 mg, 66%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.73–7.61 (m, *J* 8.4 Hz, 2H), 7.59–7.49 (m, *J* 8.4 Hz, 2H), 7.24–7.07 (m, 3H), 6.99 (d, *J* 7.2 Hz, 1H), 3.86–3.69 (m, 1H), 3.65–3.46 (m, 2H), 3.03 (app s, 4H), 2.82–2.46 (m, 8H), 2.30–2.03 (m, 2H), 1.96–1.71 (m, 4H), 1.42–1.30 (m, 9H), 1.18 (d, *J* 6.5 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 171.1, 156.7, 145.6, 134.2, 132.7, 127.6, 126.6, 126.1, 125.8, 125.5, 70.5, 61.7, 48.1, 46.1, 44.2, 40.6, 40.4, 35.2, 31.1, 30.9, 26.3, 26.1, 11.1. MS, *m/z* 524 (100) $[M+H]^+$. HRMS acquired: (M + H) 524.2950; $C_{30}H_{41}N_3O_3S$ requires (M + H), 524.2959.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-spiro[3,4-dihydroisoquinoline-1,1′-cyclohexane]*-2-yl-*propan-1-one* (**41**). General Method B was replicated employing **3** (25 mg, 0.064 mmol) and **86** (26 mg, 0.13 mmol) to afford **41** as a solid (1.9 mg, 6%). ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ 7.73–7.63 (m, 2H), 7.60–7.49 (m, 2H), 7.33 (d, *J* 7.8 Hz, 1H), 7.19–7.04 (m, 2H), 6.83 (d, *J* 7.0 Hz, 1H),

3.96–3.72 (m, 2H), 3.12–2.49 (m, 12H), 2.01–1.48 (m, 8H), 1.41–1.33 (m, 9H), 1.23–1.09 (m, 3H). MS, *m/z* 538 (100) $[M+H]^+$. HRMS acquired: (M + H) 538.3100; $C_{31}H_{43}N_3O_3S$ requires (M + H), 538.3098.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-(6,8-dihydro-5H-1,7-naphthyridin*-7-yl*)propan-1-one* (**42**). General Method B was replicated employing **3** (15 mg, 0.038 mmol) and 5,6,7,8-tetrahydro-1,7-naphthyridine dihydrochloride (16 mg, 0.077 mmol) to afford **42** as a solid (17 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 8.45 (d *J* 4.1, 0.3H), 8.06 (d, *J* 4.1 Hz, 0.7H), 7.72–7.50 (m, 4H), 7.47–7.34 (m, 1H), 7.15–7.08 (m, 0.3H), 6.98 (dd, *J* 4.9, 7.6 Hz, 0.7H), 4.95–4.80 (m, 1H), 4.76–4.57 (m, 1H), 4.08–3.92 (m, 0.7H), 3.87–3.79 (0.3H), 3.74–3.59 (m, 2H), 3.16–2.86 (m, 1H), 3.03–2.66 (m, 6H), 2.58 (app s, 3H), 1.43–1.33 (m, 9H), 1.19 (d, *J* 6.6 Hz, 2H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.7, 156.7, 156.4, 153.2, 147.9, 146.9, 137.0, 136.3, 132.8, 132.7, 130.1, 129.0, 127.6, 126.0, 121.9, 121.7, 60.3, 59.8, 50.4, 47.9, 47.1, 45.7, 42.7, 40.1, 38.6, 35.1, 31.1, 29.6, 28.7, 27.1, 10.0. MS, *m/z* 471 (100) $[M+H]^+$. HRMS acquired: (M + H) 471.2425; $C_{25}H_{34}N_4O_3S$ requires (M + H), 471.2424.

2-(4-((4-(tert-Butyl)phenyl)sulfonyl)piperazin-1-yl*)-1-(7,8-dihydro-1,6-naphthyridin-6(5H)-yl)propan-1-one* (**43**). General Method B was replicated employing **3** (20 mg, 0.051 mmol) and 5,6,7,8-tetrahydro-1,6-naphthyridine dihydrochloride (21 mg, 0.10 mmol) to afford **43** as a solid (23.2 mg, 96%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 8.43 (d, *J* 4.4 Hz, 0.7H), 8.32 (d, *J* 4.4 Hz, 0.3H), 7.73–7.59 (m, 2H), 7.50–7.59 (m, 2H), 7.46 (d, *J* 7.6 Hz, 0.7H), 7.25–7.20 (m, 0.3H), 7.17 (dd, *J* 4.8, 7.8 Hz, 0.7H), 6.87–6.80 (m, 0.3H), 4.86–4.74 (m, 1H), 4.74–4.53 (m, 1H), 4.22–4.08 (m, 0.3H), 3.96–3.56 (m, 2.7H), 3.10 (app s, 3H), 3.04–2.78 (m, 3H), 2.73 (br. s., 1H), 2.69–2.39 (m, 3H), 1.43–1.34 (m, 9H), 1.30–1.17 (m, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.8, 170.3, 156.6, 156.5, 154.8, 153.6, 147.8, 147.6, 134.6, 133.6, 132.7, 128.7, 127.6, 127.5, 126.0, 126.0, 121.8, 121.0, 60.3, 60.1, 48.3, 48.0, 46.8, 46.0, 45.7, 43.6, 42.9, 40.4, 35.1, 32.6, 31.3, 31.1, 31.0, 29.6, 11.0, 10.3. MS, *m/z* 471 (100) $[M+H]^+$. HRMS acquired: (M + H) 471.2433; $C_{25}H_{34}N_4O_3S$ requires (M + H), 471.2425.

2-[4-(p-Tolylsulfonyl)piperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**46**). General Method A was replicated employing **6** (18 mg, 0.041 mmol) and 4-methylbenzenesulfonyl chloride (7.8 mg, 0.041 mmol) to afford **46** as a solid (13.4 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.67–7.56 (m, 2H), 7.37–7.29 (m, 2H), 3.64–3.27 (m, 5H), 3.09 (t, *J* 4.8 Hz, 4H), 2.95–2.80 (m, 2H), 2.78–2.64 (m, 2H), 2.43 (s, 3H), 2.01–1.72 (m, 4H), 1.25 (d, *J* 6.8 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.1, 143.7, 132.5, 129.7, 127.8, 61.1, 48.6, 46.5, 45.9, 45.8, 26.1, 24.0, 21.5, 12.1. MS, *m/z* 366 (100) $[M+H]^+$. HRMS acquired: (M + H) 366.1860; $C_{18}H_{27}N_3O_3S$ requires (M + H), 366.1854.

1-Pyrrolidin-1-yl-2-[4-[4-(trifluoromethyl)phenyl]sulfonylpiperazin-1-yl*]propan-1-one* (**48**). General Method A was replicated employing **6** (18 mg, 0.041 mmol) and 4-methylbenzenesulfonyl chloride (7.8 mg, 0.041 mmol) to afford **48** as a solid (13.4 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.92–7.86 (m, *J* 8.3 Hz, 2H), 7.84–7.77 (m, *J* 8.5 Hz, 2H), 3.61–3.50 (m, 1H), 3.50–3.28 (m, 4H), 3.08 (br. s., 4H), 2.88–2.49 (m, 4H), 1.99–1.75 (m, 4H), 1.20 (d, *J* 6.7 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.4, 139.4, 134.7, 134.3, 128.6, 128.2, 126.2, 126.2, 125.0, 121.4, 117.7, 61.1, 48.6, 46.4, 46.1, 45.9, 26.2, 24.0, 11.8. MS, *m/z* 420 (100) $[M+H]^+$. HRMS acquired: (M + H) 420.1564; $C_{18}H_{24}F_3N_3O_3S$ requires (M + H), 420.1563.

2-[4-(4-Cyclopentylphenyl)sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**49**). **50** (11.0 mg, 0.026 mmol), 1-cyclopentenylboronic acid pinacol ester (6.45 mg, 0.033 mmol), sodium carbonate (13.5 mg, 0.13 mmol) and $Pd(PPh_3)_4$ (2.4 mg, 0.002 mmol) were suspended in a mixture of toluene (1 mL) and H_2O (0.25 mL) which had been purged with nitrogen for 15 min. This was then stirred at reflux under N_2 for 4 h. The reaction was then filtered through Celite and concentrated. The crude material was then purified by column chromatography eluting with 100% DCM to 5% MeOH/DCM to afford crude intermediate 2-[4-[4-(cyclopenten-1-yl)phenyl]sulfonylpiperazin-1-yl]-1-pyrrolidin-1-yl-propan-1-one (9.1 mg, 85%). MS, *m/z* 418 (100) $[M+H]^+$. This material was dissolved in MeOH (3 mL) and Pd/C (3 mg) was added, and the mixture stirred under H_2 atmosphere for 14 h. The reaction was then filtered through Celite, washed with MeOH (10 mL) and concentrated. The crude material was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to afford **49** as a solid (4.7 mg, 51%). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 7.72–7.62 (m, 2H), 7.55–7.45 (m, 2H), 3.67–3.56 (m, 1H), 3.52–3.34 (m, 4H), 3.18–3.05 (m, 1H), 2.99 (t, *J* 5.0 Hz, 4H), 2.81–2.55 (m, 4H), 2.19–2.04 (m, 2H), 1.96–1.56 (m, 10H), 1.16 (d, *J* 6.7 Hz, 3H). MS, *m/z* 420 (100) $[M+H]^+$. HRMS acquired: (M + H) 420.2315; $C_{22}H_{33}N_3O_3S$ requires (M + H), 420.2315.

2-[4-(4-Bromophenyl)sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**50**). General Method A was replicated employing **6** (100 mg, 0.23 mmol) and 4-bromobenzenesulfonyl chloride (58 mg, 0.23 mmol) to afford **50** as a solid (27.7 mg, 28%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.72–7.64 (m, 2H), 7.64–7.58 (m, 2H), 3.61–3.53 (m, 1H), 3.50–3.41 (m, 2H), 3.36 (td, *J* 6.8, 10.1 Hz, 2H), 3.05 (app s, 4H), 2.83 (d, *J* 3.6 Hz, 2H), 2.79–2.50 (m, 2H), 1.96–1.80 (m, 4H), 1.21 (d, *J* 6.4 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.6, 134.7, 132.4, 129.3, 129.0, 128.2, 127.9, 125.3, 65.5, 61.2, 48.6, 46.4, 46.2, 45.9, 29.7, 26.2, 26.1, 24.1, 23.9, 21.5, 20.6, 11.7. MS, *m/z* 430 (100) $[M+H]^+$. HRMS acquired: (M + H) 430.0797 and 432.0773; $C_{17}H_{24}BrN_3O_3S$ requires (M + H), 430.0795 and 432.0775.

2-[4-(4-Methoxyphenyl)sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**51**). General Method A was replicated employing **6** (18 mg, 0.041 mmol) and 4-methoxybenzenesulfonyl chloride (8.5 mg, 0.041 mmol) to afford **51** as a solid (13.2 mg, 84%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.72–7.63 (m, 2H), 7.06–6.93 (m, 2H), 3.88 (s, 3H), 3.66–3.28 (m, 5H), 3.11 (t, *J* 4.7 Hz, 4H), 3.03–2.89 (m, 2H), 2.83–2.67 (m, 2H), 2.02–1.74 (m, 4H), 1.27 (d, *J* 6.8 Hz, 3H). MS, *m/z* 382 (100) $[M+H]^+$. HRMS acquired: (M + H) 382.1794; $C_{18}H_{27}N_3O_4S$ requires (M + H), 382.1794.

2-[4-(4-Acetylphenyl)sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**52**). General Method A was replicated employing **6** (18 mg, 0.041 mmol) and 4-acetylbenzenesulfonyl chloride (9.0 mg, 0.041 mmol) to afford **52** as a solid (10.8 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 8.13–8.05 (m, 2H), 7.88–7.79 (m, 2H), 3.61–3.30 (m, 5H), 3.20–3.04 (m, 4H), 2.98–2.81 (m, 2H), 2.78–2.67 (m, 2H), 2.66 (s, 3H), 1.98–1.75 (m, 4H), 1.25 (d, *J* 6.6 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 196.7, 140.3, 139.7, 128.9, 128.0, 60.9, 48.5, 46.6, 46.0, 45.5, 26.9, 26.4, 26.2, 24.0, 12.1 (missing amide carbonyl carbon at ~170 ppm). MS, *m/z* 394 (100) $[M+H]^+$. HRMS acquired: (M + H) 394.1801; $C_{19}H_{27}N_3O_4S$ requires (M + H), 394.1800.

2-[4-(4-Phenylphenyl)sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**53**). General Method A was replicated employing **6** (18 mg, 0.041 mmol) and 4-phenylbenzenesulfonyl chloride (10.4 mg, 0.041 mmol) to afford **53** as a solid (2.0 mg, 11%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.86–7.80 (m, 2H), 7.79–7.73 (m, 2H), 7.66–7.59 (m, 2H), 7.54–7.41 (m, 3H), 4.26–4.09 (m, 2H), 4.05–3.86 (m, 2H), 3.67–3.29 (m, 9H), 2.12–1.90 (m, 4H), 1.68 (d, *J* 7.0 Hz, 3H). MS, *m/z* 428 (100) $[M+H]^+$. HRMS acquired: (M + H) 428.2000; $C_{23}H_{29}N_3O_3S$ requires (M + H), 428.1999.

2-[4-(3-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**54**). General Method A was replicated employing **6** (15 mg, 0.034 mmol) and 3-*tert*-butylbenzenesulfonyl chloride (8.0 mg, 0.034 mmol) to afford **54** as a solid (10.5 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.76–7.71 (m, 1H), 7.65–7.51 (m, 2H),

7.50–7.38 (m, 1H), 3.61–3.50 (m, 1H), 3.45 (t, *J* 6.9 Hz, 2H), 3.42–3.21 (m, 2H), 3.05 (app s, 4H), 2.83–2.47 (m, 4H), 1.95–1.78 (m, 4H), 1.34 (s, 9H), 1.21 (d, *J* 6.7 Hz, 3H). MS, *m/z* 408 (100) $[M+H]^+$. HRMS acquired: (M + H) 408.2323; $C_{21}H_{33}N_3O_3S$ requires (M + H), 408.2315.

2-[4-[4-(Pentafluoro-6-sulfanyl)phenyl]sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**55**). General Method A was replicated employing **6** (15 mg, 0.034 mmol) and 4-(pentafluorosulfur) benzenesulfonyl chloride (8.0 mg, 0.034 mmol) to afford **55** as a solid (13.1 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 8.06–7.78 (m, 4H), 3.63–3.52 (m, 1H), 3.52–3.30 (m, 5H), 3.10 (app s, 4H), 2.91–2.53 (m, 4H), 2.01–1.81 (m, 4H), 1.22 (d, *J* 6.5 Hz, 3H). MS, *m/z* 478 (100) $[M+H]^+$. HRMS acquired: (M + H) 478.1263; $C_{17}H_{24}F_5N_3O_3S_2$ requires (M + H), 478.1252.

1-(Pyrrolidin-1-yl*)-2-(4-((4-(trimethylsilyl)phenyl)sulfonyl)piperazin*-1-yl*)propan-1-one* (**56**). General Method C. **5** (4.2 mg, 0.020 mmol), **88** (7.0 mg, 0.017 mmol) and DIPEA (0.016 mL, 0.10 mmol) were stirred in EtOH (1 mL) and heated at 70 °C for 16 h. The reaction mixture was concentrated *in vacuo* and the crude material purified by column chromatography eluting with 100% DCM to 5% MeOH/DCM to afford the product. The product was then further purified by reverse phase preparatory HPLC using a of 95% water/ACN to 100% ACN to afford **56** as a solid (1.0 mg, 14% yield). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 7.89–7.59 (m, 4H), 3.67–3.56 (m, 1H), 3.48–3.34 (m, 4H), 2.98 (t, *J* 4.7 Hz, 4H), 2.73–2.52 (m, 4H), 1.95–1.78 (m, 4H), 1.14 (d, *J* 6.7 Hz, 3H), 0.31 (s, 9H). MS, *m/z* 424 (100) $[M+H]^+$. HRMS acquired: (M + H) 424.2093; $C_{20}H_{33}N_3O_3SiS$ requires (M + H), 424.2085.

1-Pyrrolidin-1-yl-2-[4-[4-[1-(trifluoromethyl)cyclopropyl]phenyl] sulfonylpiperazin-1-yl*]propan-1-one* (**57**). General Method A was replicated employing **6** (15 mg, 0.034 mmol) and **89** (15 mg, 0.051 mmol) to afford **57** as a solid (14.2 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.77–7.68 (m, 2H), 7.65–7.57 (m, *J* 8.4 Hz, 2H), 3.62–3.50 (m, 1H), 3.50–3.24 (m, 4H), 3.06 (app s, 4H), 2.87–2.68 (m, 2H), 2.68–2.48 (m, 2H), 2.02–1.74 (m, 4H), 1.49–1.39 (m, 2H), 1.20 (d, *J* 6.6 Hz, 3H), 1.12–0.99 (m, 2H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.6, 141.2, 135.6, 131.9, 127.9, 127.7, 124.0, 61.2, 48.7, 46.5, 46.1, 45.9, 28.3, 27.9, 27.4, 26.1, 24.0, 12.2, 10.0, 9.9. MS, *m/z* 460 (100) $[M+H]^+$. HRMS acquired: (M + H) 460.1888; $C_{21}H_{28}F_3N_3O_3S$ requires (M + H), 460.1876.

1-Pyrrolidin-1-yl-2-[4-[4-(2,2,2-trifluoroethyl)phenyl]sulfonylpiperazin-1-yl*]propan-1-one* (**58**). General Method A was replicated employing **6** (15 mg, 0.034 mmol) and **90** (13 mg, 0.051 mmol) to afford **58** as a solid (13.7 mg, 93%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.81–7.72 (m, 2H), 7.49 (d, *J* 8.1 Hz, 2H), 3.62–3.51 (m, 1H), 3.51–3.41 (m, 4H), 3.36 (dd, *J* 6.8, 10.1 Hz, 2H), 3.08 (br. s., 4H), 2.88–2.68 (m, 2H), 2.68–2.50 (m, 2H), 2.02–1.75 (m, 4H), 1.21 (d, *J* 6.4 Hz, 3H). MS, *m/z* 434 (100) $[M+H]^+$. HRMS acquired: (M + H) 434.1730; $C_{19}H_{26}F_3N_3O_3S$ requires (M + H), 434.1720.

1-Pyrrolidin-1-yl-2-[4-[4-(trifluoromethoxy)phenyl]sulfonylpiperazin-1-yl*]propan-1-one* (**59**). General Method A was replicated employing **6** (15 mg, 0.034 mmol) and 4-(trifluoromethoxy)benzenesulfonyl chloride (13 mg, 0.051 mmol) to afford **59** as a solid (34 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.85–7.74 (m, 2H), 7.41–7.34 (m, 2H), 3.63–3.52 (m, 2H), 3.47 (t, *J* 6.8 Hz, 2H), 3.38 (td, *J* 6.7 10.1 Hz, 1H), 3.15 (t, *J* 4.9 Hz, 4H), 2.98 (td, *J* 5.2, 10.8 Hz, 2H), 2.86–2.70 (m, 2H), 2.04–1.78 (m, 4H), 1.28 (d, *J* 6.7 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.4, 152.3, 134.1, 129.9, 125.3, 121.9, 120.9, 118.5, 115.0, 61.1, 48.6, 46.5, 46.0, 45.9, 26.2, 24.0, 12.0. MS, *m/z* 436 (100) $[M+H]^+$. HRMS acquired: (M + H) 426.1524; $C_{18}H_{24}F_3N_3O_4S$ requires (M + H), 436.1512.

2-(4-((4-Cyclopropylphenyl)sulfonyl)piperazin-1-yl*)-1-(pyrrolidin*-1-yl*)propan-1-one* (**60**). The procedure used for **49** was replicated employing **50** (11 mg, 0.026 mmol) and cyclopropylboronic acid (2.9 mg, 0.033 mmol) to afford **60** as a solid (4.4 mg, 44%). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 7.67–7.58 (m, 2H), 7.35–7.24 (m, 2H), 3.68–3.56 (m, 1H), 3.52–3.42 (m, 2H), 3.42–3.34 (m, 2H), 2.97 (t, *J* 4.8 Hz, 4H), 2.76–2.50 (m, 4H), 2.10–1.96 (m, 1H), 1.96–1.77 (m, 4H), 1.21–1.03 (m, 5H), 0.87–0.75 (m, 2H). MS, *m/z* 392 (100) $[M+H]^+$. HRMS acquired: (M + H) 392.2000; $C_{20}H_{29}N_3O_3S$ requires (M + H), 392.2002.

2-[4-[4-(2-Hydroxy-1,1-dimethyl-ethyl)phenyl]sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**61**). General Method C was replicated employing **5** (17.2 mg, 0.084 mmol) and **93** (23 mg, 0.056 mmol) to afford **61** as a solid (15.2 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.76–7.68 (m, 2H), 7.60–7.53 (m, 2H), 3.67 (s, 2H), 3.60–3.50 (m, 1H), 3.49–3.40 (m, 2H), 3.40–3.30 (m, 2H), 3.11 (t, *J* 4.1 Hz, 4H), 2.82–2.50 (m, 4H), 1.96–1.80 (m, 4H), 1.38 (s, 6H), 1.21 (d, *J* 6.7 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.8, 152.5, 133.5, 127.7, 127.0, 72.5, 61.1, 48.5, 46.5, 46.1, 45.9, 40.5, 26.1, 25.2, 24.0, 12.4. MS, *m/z* 424 (100) $[M+H]^+$. HRMS acquired: (M + H) 424.2261; $C_{21}H_{33}N_3O_4S$ requires (M + H), 424.2264.

2-[(3S)-4-(4-tert-Butylphenyl)sulfonyl-3-methyl-piperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**62**). General Method C was replicated employing **5** (19 mg, 0.091 mmol) and **94** (25 mg, 0.061 mmol) to afford **62** as a solid (15.2 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ 7.83–7.62 (m, *J* 8.2 Hz, 2H), 7.59–7.40 (m, *J* 8.2 Hz, 2H), 4.15–4.00 (m, 1H), 3.88–3.60 (m, 2H), 3.59–3.29 (m, 5H), 3.27–3.11 (m, 1H), 2.67–2.51 (m, 2H), 2.50–2.36 (m, 2H), 1.98–1.76 (m, 4H), 1.34 (s, 9H), 1.22–0.95 (m, 6H). MS, *m/z* 422 (100) $[M+H]^+$. HRMS acquired: (M + H) 422.2466; $C_{22}H_{35}N_3O_3S$ requires (M + H), 422.2472.

2-[(3R)-4-(4-tert-Butylphenyl)sulfonyl-3-methyl-piperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**63**). General Method C was replicated employing **5** (19 mg, 0.091 mmol) and **95** (25 mg, 0.061 mmol) to afford **63** as a solid (15.2 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ 7.81–7.60 (m, *J* 8.6 Hz, 2H), 7.58–7.39 (m, *J* 8.2 Hz, 2H), 4.17–3.98 (m, 1H), 3.86–3.54 (m, 2H), 3.50–3.30 (m, 4H), 3.25–3.10 (m, 1H), 2.66–2.36 (m, 4H), 1.95–1.77 (m, 4H), 1.33 (s, 9H), 1.24–1.14 (m, 2H), 1.22–0.95 (m, 6H). ^{13}C NMR (75 MHz, CDCl3, rotamers) 170.2, 156.2, 137.5, 127.0, 126.0, 61.5, 52.0, 50.3, 49.4, 49.2, 47.1, 46.3, 46.1, 45.9, 45.8, 40.8, 35.1, 31.2, 31.1, 26.2, 24.1, 24.0, 23.9, 15.6, 15.3, 9.8, 9.2. MS, *m/z* 422 (100) $[M+H]^+$. HRMS acquired: (M + H) 422.2467; $C_{22}H_{35}N_3O_3S$ requires (M + H), 422.2472.

2-[4-(4-tert-Butylphenyl)sulfonyl-3,3-dimethyl-piperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**64**). General Method C was replicated employing **5** (9.5 mg, 0.046 mmol) and **96** (15 mg, 0.035 mmol) to afford **64** as a solid (3.5 mg, 23%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.78–7.68 (m, *J* 8.5 Hz, 2H), 7.55–7.41 (m, *J* 8.5 Hz, 2H), 3.87–3.72 (m, 1H), 3.63–3.25 (m, 6H), 2.78–2.53 (m, 2H), 2.41–2.21 (m, 2H), 2.01–1.74 (m, 4H), 1.34 (s, 9H), 1.32–1.26 (m, 6H), 1.15 (d, *J* 6.8 Hz, 3H). MS, *m/z* 436 (100) $[M+H]^+$. HRMS acquired: (M + H) 436.2630; $C_{23}H_{37}N_3O_3S$ requires (M + H), 436.2628.

1-Pyrrolidin-1-yl-2-[rac-(1S,4S)-5-(4-tert-butylphenyl)sulfonyl-2,5-diazabicyclo[2.2.1]heptan-2-yl*]propan-1-one* (**65**). General Method C was replicated employing **5** (23 mg, 0.11 mmol) and **97** (30 mg, 0.074 mmol) to afford **65** as a solid (6.7 mg, 22%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.81–7.69 (m, 2H), 7.58–7.46 (m, 2H), 4.34–4.21 (m, 1H), 3.73–3.33 (m, 7H), 3.11 (dd, *J* 2.2, 9.8 Hz, 1H), 2.99 (dd, *J* 2.2, 9.8 Hz, 1H), 2.77 (d, *J* 9.9 Hz, 1H), 2.07–1.71 (m, 5H), 1.35 (s, 9H), 1.28–1.14 (m, 3H), 1.11–1.01 (m, 1H). MS, *m/z* 420 (100) $[M+H]^+$. HRMS acquired: (M + H) 420.2318; $C_{22}H_{33}N_3O_3S$ requires (M + H), 420.2315.

2-[(1S,5R)-3-(4-tert-Butylphenyl)sulfonyl-3,8-diazabicyclo[3.2.1] octan-8-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**66**). General Method C was replicated employing **5** (15 mg, 0.073 mmol) and **99** (15 mg, 0.049 mmol), to afford **66** as a solid (8.6 mg, 41%). ^{1}H NMR

(300 MHz, MeOD, rotamers): δ 7.77–7.63 (m, 4H), 3.78–3.66 (m, 1H), 3.56–3.35 (m, 8H), 2.83–2.69 (m, 2H), 2.14–1.77 (m, 8H), 1.39 (s, 9H), 1.29–1.22 (m, 3H). MS, *m/z* 434 (100) $[M+H]^+$. HRMS acquired: (M + H) 434.2481; $C_{23}H_{35}N_3O_3S$ requires (M + H), 434.2472.

2-[4-(4-tert-Butylphenyl)sulfonyl-1,4-diazepan-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**67**). General Method B was replicated employing **101** (38 mg, 0.094 mmol) and pyrrolidine (31 μL, 0.38 mmol) to afford **67** as a solid (12.9 mg, 33%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.74–7.65 (m, 2H), 7.56–7.45 (m, 2H), 3.81 (dt, *J* 6.6, 10.4 Hz, 1H), 3.60–3.21 (m, 8H), 3.02–2.64 (m, 4H), 1.96–1.77 (m, 6H), 1.35 (s, 9H), 1.20 (d, *J* 6.6 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.3, 155.6, 135.6, 126.4, 125.6, 61.3, 51.5, 50.4, 48.7, 46.5, 45.8, 45.5, 34.6, 30.6, 28.6, 25.8, 23.7, 10.4. MS, *m/z* 422 (100) $[M+H]^+$. HRMS acquired: (M + H) 422.2481; $C_{22}H_{35}N_3O_3S$ requires (M + H), 422.2480.

2-[(3aR,6aS)-5-(4-tert-Butylphenyl)sulfonyl-1,3,3a,4,6,6a-hexahydropyrrolo[3,4-c]pyrrol-2-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**68**). General Method C was replicated employing **5** (11 mg, 0.053 mmol) and **102** (16 mg, 0.035 mmol) to afford **68** as a solid (12.7 mg, 82%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.76–7.68 (m, 2H), 7.59–7.50 (m, 2H), 3.65–3.54 (m, 1H), 3.53–3.34 (m, 4H), 3.30–2.90 (m, 6H), 2.89–2.71 (m, 2H), 2.51–2.29 (m, 2H), 1.98–1.80 (m, 4H), 1.38–1.24 (m, 12H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.4, 156.6, 131.9, 127.9, 125.9, 59.6, 56.3, 56.2, 52.9, 52.9, 46.5, 46.0, 41.1, 41.1, 35.1, 31.0, 26.2, 24.0, 14.4. MS, *m/z* 434 (100) $[M+H]^+$. HRMS acquired: (M + H) 434.2480; $C_{23}H_{35}N_3O_3S$ requires (M + H), 434.2472.

2-[2-(4-tert-Butylphenyl)sulfonyl-2,6-diazaspiro[3.3]heptan-6-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (**69**). General Method C was replicated employing **5** (11 mg, 0.053 mmol) and **103** (15 mg, 0.037 mmol) to afford **69** as a solid (6.6 mg, 43%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.80–7.70 (m, 2H), 7.62–7.53 (m, 2H), 3.89 (s, 4H), 3.56–3.17 (m, 9H), 1.99–1.80 (m, 4H), 1.37 (s, 9H), 1.14 (d, *J* 6.7 Hz, 3H). MS, *m/z* 420 (100) $[M+H]^+$. HRMS acquired: (M + H) 420.2323; $C_{22}H_{33}N_3O_3S$ requires (M + H), 420.2315.

2-[1-(4-tert-Butylphenyl)sulfonyl-4-piperidyl]-1-pyrrolidin-1-yl-*propan-1-one* (**70**). Lithium bis(trimethylsilyl)amide (0.16 mL, 0.16 mmol) was added to a solution of **105** (31 mg, 0.079 mmol) in THF (1 mL) under a N_2 atmosphere at −78 °C and stirred at this temperature for 30 min. Iodomethane (0.015 mL, 0.24 mmol) was then added dropwise and the reaction warmed to 0 °C and stirred at this temperature for 1 h. The reaction was then quenched with saturated solution of NH_4Cl (10 mL). The reaction was then extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine (10 mL), dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude material was then purified by column chromatography eluting with 100% CyHex to 100% EtOAc to afford **70** as a solid mixture of diastereomers (13.8 mg, 43%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.71–7.60 (m, 2H), 7.57–7.47 (m, 2H), 3.89–3.69 (m, 2H), 3.56–3.32 (m, 4H), 2.32–2.13 (m, 3H), 2.01–1.77 (m, 5H), 1.75–1.65 (m, 1H), 1.60–1.44 (m, 1H), 1.35 (s, 9H), 1.33–1.19 (m, 2H), 1.06 (d, *J* 6.8 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 174.3, 156.4, 132.7, 127.6, 125.9, 46.8, 46.5, 46.4, 45.7, 43.1, 38.2, 35.1, 31.1, 30.2, 28.5, 26.0, 24.3, 14.7. MS, *m/z* 407 (100) $[M+H]^+$. HRMS acquired: (M + H) 407.2365; $C_{22}H_{34}N_2O_3S$ requires (M + H), 407.2370.

N-[1-(4-tert-Butylphenyl)sulfonyl-4-piperidyl]-N-methyl-pyrrolidine-1-carboxamide (**71**). Sodium hydride (4.37 mg, 0.11 mmol) was added to a stirred solution of **108** (21.5 mg, 0.055 mmol) in DMF (2 mL) and stirred for a further 20 min. Iodomethane (6.8 μL, 0.11 mmol) was then added and the reaction heated at 80 °C for 2 h. The reaction was then concentrated *in vacuo* and dissolved in EtOAc (20 mL). The organic layer was washed with H_2O (10 mL) and brine (10 mL), then dried with anhydrous Na_2SO_4, filtered and concentrated. The crude material was then purified by column chromatography eluting with 100% DCM to afford **71** as a solid (17.1 mg, 77%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72–7.62 (m, 2H), 7.58–7.48 (m, 2H), 3.88 (td, *J* 2.0, 11.6 Hz, 2H), 3.73–3.60 (m, 1H), 3.35–3.20 (m, 4H), 2.69 (s, 3H), 2.35 (td, *J* 3.1, 11.8 Hz, 2H), 1.90–1.68 (m, 8H), 1.35 (s, 9H). ^{13}C NMR (75 MHz, CDCl3) δ 163.2, 156.5, 133.0, 127.5, 126.0, 53.2, 48.5, 46.1, 35.1, 31.1, 30.8, 28.3, 25.5. MS, *m/z* 408 (100) $[M+H]^+$. HRMS acquired: (M + H) 408.2316; $C_{21}H_{33}N_3O_3S$ requires (M + H), 408.2317.

4-(4-tert-Butylphenyl)sulfonyl-1-(1-methyl-2-oxo-2-pyrrolidin-1-yl-*ethyl)piperazin-2-one* (**72**). General Method A was replicated employing **110** (15 mg, 0.044 mmol) and 4-*tert*-butylbenzenesulfonyl chloride (15.4 mg, 0.066 mmol) to afford **72** as a solid (18.4 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.75–7.66 (m, 2H), 7.60–7.51 (m, 2H), 5.35 (q, *J* 7.0 Hz, 1H), 3.86 (dd, *J* 1.1, 16.6 Hz, 1H), 3.75–3.66 (m, 1H), 3.58–3.38 (m, 6H), 3.23–3.09 (m, 1H), 2.01–1.76 (m, 5H), 1.36 (s, 9H), 1.29 (d, *J* 7.2 Hz, 3H). MS, *m/z* 422 (100) $[M+H]^+$. HRMS acquired: (M + H) 422.2108; $C_{21}H_{31}N_3O_4S$ requires (M + H), 422.2108.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*ethanone* (**73**). General Method B was replicated employing **111** (15.5 mg, 0.041 mmol) and pyrrolidine (13.5 μL, 0.164 mmol) to afford **73** as a solid (6.4 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.74–7.63 (m, 2H), 7.59–7.49 (m, 2H), 3.46 (t, *J* 6.8 Hz, 2H), 3.38 (t, *J* 6.7 Hz, 2H), 3.24–3.00 (m, 6H), 2.84–2.62 (m, 4H), 2.01–1.78 (m, 5H), 1.36 (s, 9H). MS, *m/z* 394 (100) $[M+H]^+$. HRMS acquired: (M + H) 394.2165; $C_{20}H_{31}N_3O_3S$ requires (M + H), 394.2163.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*butan-1-one* (**74**). General Method B was replicated employing **112** (14 mg, 0.035 mmol) and pyrrolidine (14 μL, 0.17 mmol) to afford **74** as a solid (3.1 mg, 21%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.72–7.62 (m, 2H), 7.60–7.48 (m, 2H), 3.72–3.32 (m, 6H), 3.30–2.92 (m, 4H), 2.90–2.52 (m, 3H), 2.07–1.73 (m, 6H), 1.37 (s, 9H), 0.97–0.71 (m, 3H). MS, *m/z* 422 (100) $[M+H]^+$. HRMS acquired: (M + H) 422.2467; $C_{22}H_{35}N_3O_3S$ requires (M + H), 422.2472.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*pentan-1-one* (**75**). General Method B was replicated employing **113** (13 mg, 0.026 mmol) and pyrrolidine (11 μL, 0.13 mmol) to afford **75** as a solid (1.2 mg, 10%). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 7.72–7.63 (m, 4H), 3.62–3.46 (m, 3H), 2.95 (t, *J* 5.0 Hz, 4H), 2.78–2.70 (m, 2H), 2.66–2.58 (m, 2H), 1.94–1.82 (m, 4H), 1.74–1.63 (m, 2H), 1.58–1.47 (m, 2H), 1.36 (s, 9H), 1.25–1.18 (m, 2H), 0.90 (t, *J* 7.4 Hz, 3H). MS, *m/z* 436 (100) $[M+H]^+$. HRMS acquired: (M + H) 436.2627; $C_{23}H_{37}N_3O_3S$ requires (M + H), 436.2628.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-3-methyl-1-pyrrolidin*-1-yl-*butan-1-one* (**76**). General Method B was replicated employing **115** (4.6 mg, 0.012 mmol) and pyrrolidine (7 μL, 0.072 mmol) to afford **76** as a solid (1.8 mg, 34%). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 7.74–7.61 (m, 4H), 3.62–3.38 (m, 4H), 3.16–3.05 (m, 1H), 3.04–2.85 (m, 4H), 2.73–2.67 (m, 4H), 2.11–1.81 (m, 5H), 1.41–1.32 (m, 9H), 0.91 (d, *J* 6.6 Hz, 3H), 0.82 (d, *J* 6.6 Hz, 3H). MS, *m/z* 436 (100) $[M+H]^+$. HRMS acquired: (M + H) 436.2638; $C_{23}H_{37}N_3O_3S$ requires (M + H), 436.2628.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-2-phenyl-1-pyrrolidin*-1-yl-*ethanone* (**77**). General Method B was replicated employing **116** (14 mg, 0.031 mmol) and pyrrolidine (13 μL, 0.16 mmol) to afford **77** as a solid (11.4 mg, 79%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.66–7.60 (m, 2H), 7.54–7.48 (m, 2H), 7.41–7.28 (m, 5H), 4.00 (s, 1H), 3.57–3.43 (m, 2H), 3.43–3.31 (m, 2H), 3.18–2.94 (m, 4H), 2.69–2.43 (m, 4H), 2.01–1.67 (m, 4H), 1.36 (s, 9H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 168.6, 156.6, 134.5, 131.9, 129.4, 128.7, 128.5, 127.7, 126.0, 72.1, 50.4, 47.9, 46.2, 46.2, 46.0, 35.1, 31.1, 29.7, 26.1, 25.5, 23.8. MS, *m/z* 470 (100) $[M+H]^+$. HRMS acquired: (M + H) 470.2484; $C_{26}H_{35}N_3O_3S$ requires (M + H), 470.2472.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-3-phenyl-1-pyrrolidin*-1-yl-*propan-1-one* (**78**). General Method B was replicated employing **117** (8 mg, 0.015 mmol) and pyrrolidine (6 μL, 0.074 mmol) to afford **78** as a solid (3.5 mg, 49%). ^{1}H NMR (300 MHz, MeOD, rotamers): δ 7.76–7.61 (m, 4H), 7.29–7.12 (m, 5H), 3.59 (dd, *J* 5.3, 9.5 Hz, 1H), 3.29–3.12 (m, 2H), 3.02–2.91 (m, 6H), 2.91–2.82 (m, 2H), 2.79–2.59 (m, 4H), 1.78–1.66 (m, 2H), 1.61–1.47 (m, 2H), 1.38 (s, 9H). MS, *m/z* 484 (100) $[M+H]^+$. HRMS acquired: (M + H) 484.2633; $C_{27}H_{37}N_3O_3S$ requires (M + H), 484.2628.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-2-methyl-1-pyrrolidin*-1-yl-*propan-1-one* (**79**). General Method A was replicated employing **119** (20 mg, 0.044 mmol) and 4-*tert*-butylbenzenesulfonyl chloride (12 mg, 0.053 mmol) to afford **79** as a solid (17.5 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74–7.65 (m, 2H), 7.58–7.50 (m, 2H), 3.73 (t, *J* 6.0 Hz, 2H), 3.43 (t, *J* 6.8 Hz, 2H), 3.04 (app s, 4H), 2.54 (t, *J* 4.8 Hz, 4H), 1.81–1.64 (m, 5H), 1.35 (d, *J* 0.9 Hz, 9H), 1.21 (s, 6H). ^{13}C NMR (75 MHz, CDCl3) δ 173.1, 156.6, 133.1, 127.6, 125.9, 64.2, 47.7, 47.4, 46.3, 45.5, 35.1, 31.0, 26.8, 22.9, 19.7. MS, *m/z* 422 (100) $[M+H]^+$. HRMS acquired: (M + H) 422.2478; $C_{22}H_{35}N_3O_3S$ requires (M + H), 422.2477.

(2S)-2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (***S*-1**). General Method B was replicated employing **121** (12 mg, 0.031 mmol) and pyrrolidine (7.6 μL, 0.092 mmol) to afford ***S*-1** as a solid (7.6 mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.72–7.63 (m, 2H), 7.57–7.48 (m, 2H), 3.63–3.50 (m, 1H), 3.50–3.23 (m, 4H), 3.05 (t, *J* 4.4 Hz, 4H), 2.80–2.69 (m, 2H), 2.69–2.48 (m, 2H), 2.00–1.76 (m, 4H), 1.35 (s, 9H), 1.20 (d, *J* 6.7 Hz, 3H). MS, *m/z* 408 (100) $[M+H]^+$. HRMS acquired: (M + H) 408.2321; $C_{21}H_{33}N_3O_3S$ requires (M + H), 408.2315.

(2R)-2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-pyrrolidin*-1-yl-*propan-1-one* (***R*-1**). General Method B was replicated employing **123** (12 mg, 0.031 mmol) and pyrrolidine (7.6 μL, 0.092 mmol) to afford ***R*-1** as a solid (9.0 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.72–7.63 (m, 2H), 7.58–7.49 (m, 2H), 3.56 (td, *J* 6.6, 10.1 Hz, 1H), 3.45 (t, *J* 6.8 Hz, 2H), 3.41–3.26 (m, 2H), 3.05 (t, *J* 4.2 Hz, 4H), 2.85–2.70 (m, 2H), 2.70–2.53 (m, 2H), 2.00–1.73 (m, 4H), 1.35 (s, 9H), 1.21 (d, *J* 6.7 Hz, 3H). MS, *m/z* 408 (100) $[M+H]^+$. HRMS acquired: (M + H) 408.2320; $C_{21}H_{33}N_3O_3S$ requires (M + H), 408.2315.

(2S)-1-(3-Azabicyclo[3.1.0]hexan-3-yl*)-2-[4-(4-tert-butylphenyl) sulfonylpiperazin*-1-yl*]propan-1-one* (***S*-18**). General Method B was replicated employing **121** (20 mg, 0.051 mmol) and 3-azabicyclo [3.1.0]hexane hydrochloride (9.2 mg, 0.077 mmol) to afford ***S*-18** as a solid (13.1 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ 7.73–7.59 (m, *J* 8.6 Hz, 2H), 7.56–7.45 (m, *J* 8.6 Hz, 2H), 3.84–3.77 (m, 1.5H), 3.62 (dd, *J* 3.7, 10.4 Hz, 0.5H), 3.52 (d, *J* 10.6 Hz, 0.5H), 3.44 (dd, *J* 3.9, 9.8 Hz, 0.5H), 3.35 (ddd, *J* 3.9, 8.6, 12.1 Hz, 1H), 3.28–3.17 (m, 1H), 3.10–2.95 (m, 4H), 2.74–2.62 (m, 2H), 2.62–2.48 (m, 2H), 1.51 (tq, *J* 3.7, 7.8 Hz, 2H), 1.35 (s, 9H), 1.15 (dd, *J* 3.5, 6.7 Hz, 3H), 0.75–0.58 (m, 1H), 0.11–0.01 (m, 1H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 171.8, 171.8, 156.6, 132.5, 132.4, 127.7, 126.0, 126.0, 61.6, 61.1, 49.2, 48.7, 48.7, 48.4, 48.1, 46.2, 46.1, 35.1, 31.1, 16.0, 15.9, 14.5, 14.4, 12.0, 11.9, 9.5, 9.2. MS, *m/z* 420 (100) $[M+H]^+$. HRMS acquired: (M + H) 420.2317; $C_{22}H_{33}N_3O_3S$ requires (M + H), 420.2315.

(2S)-2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-(1-piperidyl)propan-1-one* (***S*-20**). General Method B was replicated employing **121** (12 mg, 0.031 mmol) and piperidine (9 μL, 0.092 mmol) to afford ***S*-20** as a solid (7.8 mg, 60%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.72–7.63 (m, 2H), 7.59–7.48 (m, 2H), 3.74–3.61 (m, 1H), 3.57–3.27 (m, 4H), 3.04 (t, *J* 4.2 Hz, 4H), 2.78–2.49 (m, 4H), 1.62–1.43 (m, 6H), 1.35 (s, 9H), 1.17 (d, *J* 6.5 Hz, 3H). MS, *m/z* 422 (100) $[M+H]^+$. HRMS acquired: (M + H) 422.2474; $C_{22}H_{35}N_3O_3S$ requires (M + H), 422.2471.

(2R)-2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-(1-piperidyl)propan-1-one* (***R*-20**). General Method B was replicated employing **123** (12 mg, 0.031 mmol) and piperidine (9 μL, 0.092 mmol) to afford ***R*-20** as a solid (5.6 mg, 43%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.72–7.64 (m, 2H), 7.58–7.46 (m, 2H), 3.72–3.61 (m, 1H), 3.54–3.43 (m, 2H), 3.43–3.27 (m, 2H), 3.04 (t, *J* 4.2 Hz, 4H), 2.75–2.63 (m, 2H), 2.63–2.48 (m, 2H), 1.65–1.43 (m, 6H), 1.35 (s, 9H), 1.17 (d, *J* 6.7 Hz, 3H). MS, *m/z* 422 (100) $[M+H]^+$. HRMS acquired: (M + H) 422.2472; $C_{22}H_{35}N_3O_3S$ requires (M + H), 422.2471.

(2S)-2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-(4,4-difluoro-1-piperidyl)propan-1-one* (***S*-22**). General Method B was replicated employing **121** (20 mg, 0.051 mmol) and 4,4-difluoropiperidine hydrochloride (12.1 mg, 0.077 mmol) to afford ***S*-22** as a solid (14.2 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ 7.76–7.60 (m, *J* 8.6 Hz, 2H), 7.60–7.48 (m, *J* 8.6 Hz, 2H), 3.96–3.84 (m, 1H), 3.71–3.59 (m, 1H), 3.57–3.39 (m, 3H), 3.02 (app s, 4H), 2.73–2.61 (m, 2H), 2.61–2.48 (m, 2H), 2.00–1.77 (m, 4H), 1.35 (s, 9H), 1.17 (d, *J* 6.7 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 170.3, 156.8, 132.4, 127.7, 126.0, 121.4, 60.0, 48.1, 46.3, 42.3, 39.0, 35.2, 35.1, 34.7, 34.3, 34.0, 33.6, 31.1, 10.4. MS, *m/z* 458 (100) $[M+H]^+$. HRMS acquired: (M + H) 458.2284; $C_{22}H_{33}F_2N_3O_3S$ requires (M + H), 458.2283.

(2S)-2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-1-(1,1-dimethyl-3,4-dihydroisoquinolin*-2-yl*)propan-1-one* (***S*-38**). General Method B was replicated employing **121** (12 mg, 0.031 mmol) and 1,1-dimethyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (7.3 mg, 0.037 mmol) to afford ***S*-38** as a solid (6.2 mg, 41%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.72–7.64 (m, 2H), 7.58–7.51 (m, 2H), 7.34–7.28 (m, 1H), 7.27–7.19 (m, 1H), 7.17–7.08 (m, 1H), 7.04–6.96 (m, 1H), 3.76–3.56 (m, 2H), 3.49–3.36 (m, 1H), 3.05 (app s, 4H), 2.86–2.47 (m, 6H), 1.78 (s, 6H), 1.37 (s, 9H), 1.18 (d, *J* 6.5 Hz, 3H).). ^{13}C NMR (75 MHz, CDCl3, rotamers) δ 171.8, 156.5, 144.9, 134.1, 132.6, 127.8, 127.6, 126.7, 126.3, 125.9, 125.6, 61.9, 60.5, 48.0, 46.3, 42.8, 35.1, 31.0, 31.0, 28.6, 27.0, 10.5. MS, *m/z* 498 (100) $[M+H]^+$. HRMS acquired: (M + H) 498.2791; $C_{28}H_{39}N_3O_3S$ requires (M + H), 498.2785.

(2S)-1-Pyrrolidin-1-yl-2-[4-(4-trimethylsilylphenyl)sulfonylpiperazin-1-yl*]propan-1-one* (***S*-56**). General Method B was replicated employing **125** (12 mg, 0.030 mmol) and pyrrolidine (7.3 μL, 0.089 mmol) to afford ***S*-56** as a solid (5.6 mg, 45%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.76–7.60 (m, 4H), 3.63–3.50 (m, 1H), 3.45 (t, *J* 6.7 Hz, 2H), 3.41–3.25 (m, 2H), 3.06 (app s, 4H), 2.88–2.49 (m, 4H), 2.01–1.72 (m, 4H), 1.20 (d, *J* 6.0 Hz, 3H), 0.31 (s, 9H). MS, *m/z* 424 (100) $[M+H]^+$. HRMS acquired: (M + H) 424.2086; $C_{20}H_{33}N_3O_3SSi$ requires (M + H), 424.2085.

tert-Butyl 4-[2-[4-(4-*tert*-butylphenyl)sulfonylpiperazin-1-yl] propanoyl]piperazine-1-carboxylate (**80**). General Method B was replicated employing **3** (30 mg, 0.077 mmol) and 1-Boc-piperazine (29 mg, 0. 15 mmol) to afford **80** as a solid (35.3 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.74–7.62 (m, *J* 8.5 Hz, 2H), 7.59–7.48 (m, *J* 8.5 Hz, 2H), 3.74–3.63 (m, 1H), 3.57–3.33 (m, 6H), 3.33–3.17 (m, 2H), 3.05 (app s, 4H), 2.75–2.63 (m, 2H), 2.63–2.49 (m, 2H), 1.46 (s, 9H), 1.36 (s, 9H), 1.18 (d, *J* 6.6 Hz, 3H). MS, *m/z* 523 (100) $[M+H]^+$.

1,1-Diethyl-2,4-dihydroisoquinolin-3-one (**81**). Polyphosphoric acid (5 g) was heated to 140 °C and 2-phenylacetonitrile (0.99 mL, 8.54 mmol) was added. After stirring for 5 min, pentan-3-one (2.72 mL, 25.6 mmol) was added dropwise, and the mixture was stirred for 1 h at 140 °C. The viscous mixture is poured into cold H_2O (10 mL) and extracted with $CHCl_3$ (3 × 15 mL). The organic layers were combined and washed with H_2O (15 mL), saturated $NaHCO_3$ (15 mL) and brine (15 mL) and the organic layer dried with Na_2SO_4. The crude material was then purified by column chromatography eluting with 100% DCM to 5% MeOH/DCM to **81** as a solid (1.04 g,

60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31–7.18 (m, 2H), 7.18–7.07 (m, 2H), 3.63 (s, 2H), 1.97 (qd, *J* 7.3, 14.3 Hz, 2H), 1.74 (qd, *J* 7.3, 14.3 Hz, 2H), 0.71 (t, *J* 7.2 Hz, 6H). MS, *m/z* 204 (100) $[M+H]^+$.

1,1-Diethyl-3,4-dihydro-2H-isoquinoline (**82**). **81** (769 mg, 3.78 mmol) was dissolved in anhydrous THF (5 mL) and stirred under N_2. To this 2 M borane dimethyl sulfide complex (0.72 mL, 7.57 mmol) was added dropwise. The reaction mixture was then heated to reflux for 3 h. The reaction was then quenched with MeOH (2 mL) and stirred at 40 °C for 30 min. The reaction was then concentrated and 1 M HCl (10 mL) added. This mixture was then washed with EtOAc (10 mL) and then basified with 1 M NaOH. The aqueous layer was then extracted with EtOAc (3 × 15 mL). This organic layer was then washed with brine (20 mL), dried with anhydrous Na_2SO_4, filtered and concentrated. The crude material was then purified by column chromatography eluting with 100% DCM to 12% MeOH/DCM to **82** as am oil (336 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20–7.02 (m, 4H), 3.64 (s, 1H), 3.10 (t, *J* 5.9 Hz, 2H), 2.76 (t, *J* 5.7 Hz, 2H), 1.80–1.54 (m, 4H), 0.79 (t, *J* 7.4 Hz, 6H). MS, *m/z* 190 (100) $[M+H]^+$.

Spiro[2,4-dihydroisoquinoline-1,1′-cyclopentane]-3-one (**83**). The procedure used for **81** was replicated employing cyclopentanone (1.1 mL, 12.8 mmol) and 2-phenylacetonitrile (500 mg, 4.3 mmol) to afford **83** as a solid (362 mg, 42%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34–7.28 (m, 2H), 7.25–7.15 (m, 2H), 3.64 (s, 2H), 2.21–2.07 (m, 2H), 2.02–1.87 (m, 6H). MS, *m/z* 202 (100) $[M+H]^+$.

Spiro[3,4-dihydro-2H-isoquinoline-1,1′-cyclopentane] (**84**). The procedure used for **82** was replicated employing **83** (180 mg, 0.89 mmol) and to afford **84** as a solid (6.5 mg, 4%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25–7.16 (m, 3H), 7.13–7.07 (m, 1H), 3.42 (t, *J* 6.1 Hz, 2H), 3.19 (t, *J* 6.0 Hz, 2H), 2.55–2.37 (m, 2H), 2.30–2.11 (m, 4H), 2.05–1.89 (m, 2H). MS, *m/z* 188 (100) $[M+H]^+$.

Spiro[2,4-dihydroisoquinoline-1,1′-cyclohexane]-3-one (**85**). The procedure used for **81** was replicated employing cyclohexanone (2.7 mL, 25.6 mmol) and 2-phenylacetonitrile (1 g, 8.53 mmol) to afford **85** as a solid (1.05 g, 57%).^{1}H NMR (300 MHz, $CDCl_3$, rotamers): δ 7.38–7.28 (m, 2H), 7.27–7.22 (m, 1H), 7.21–7.10 (m, 1H), 6.50 (app s, 1H), 3.66 (s, 2H), 1.91–1.77 (m, 8H), 1.66–1.49 (m, 2H). MS, *m/z* 216 (100) $[M+H]^+$.

Spiro[3,4-dihydro-2H-isoquinoline-1,1′-cyclohexane] (**86**). The procedure used for **82** was replicated employing **85** (500 mg, 2.3 mmol) and to afford **86** as a solid (302 mg, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37–7.28 (m, 1H), 7.25–7.04 (m, 3H), 3.10 (t, *J* 5.8 Hz, 2H), 2.83 (t, *J* 5.8 Hz, 2H), 2.34 (app s, 1H), 1.91–1.55 (m, 10H). MS, *m/z* 202 (100) $[M+H]^+$.

tert-Butyl 4-(4-bromophenyl)sulfonylpiperazine-1-carboxylate (**87**). General Method A was replicated employing 1-Boc-piperazine (50 mg, 0.27 mmol) and 4-bromobenzenesulfonyl chloride (82.3 mg, 0.32 mmol) to afford **87** (105 mg, 97%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74–7.66 (m, 2H), 7.66–7.55 (m, 2H), 3.60–3.44 (m, 4H), 3.07–2.90 (m, 4H), 1.42 (s, 9H). MS, *m/z* 305 (100) [M-Boc].

Trimethyl-(4-piperazin-1-ylsulfonylphenyl)silane trifluoroacetate (**88**). A solution of *n*-butyllithium in THF (1 M, 0.090 mL, 0.090 mmol) was added to a stirred solution of **87** (33.0 mg, 0.0814 mmol) in THF (1 mL) at −78 °C under nitrogen. The reaction was then stirred for 5 min before the addition of chloro(trimethyl) silane (0.0114 mL, 0.090 mmol). The reaction was then stirred to 20 °C and stirring continued for 12 h. The reaction was then concentrated *in vacuo* and dissolved in EtOAc (5 mL). The organic layer was then washed with H_2O (2 × 5 mL), dried with anhydrous Na_2SO_4, filtered and concentrated. The crude material was then purified by column chromatography eluting with 100% CyHex to 20% EtOAc/CyHex to *tert*-butyl 4-((4-(trimethylsilyl)phenyl)sulfonyl)piperazine-1-carboxylate as a solid (6.80 mg, 21%). The Boc-protected product was then dissolved in DCM/TFA (3:1, 8 mL) and stirred for 1 h. The reaction was then concentrated *in vacuo* to afford **88** (7.0 mg, 21%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75–7.62 (m, 4H), 3.57–3.45 (m, 4H), 3.04–2.91 (m, 4H), 1.41 (s, 9H), 0.31 (s, 9H). MS, *m/z* 299 (100) $[M+H]^+$.

4-[1-(Trifluoromethyl)cyclopropyl]benzenesulfonyl chloride (**89**). To a solution of [1-(trifluoromethyl)cyclopropyl]benzene (0.039 mL, 0.27 mmol) in DCM (2 mL) at 0 °C was added sulfurochloridic acid (0.091 mL, 1.37 mmol) dropwise. The reaction was then stirred at 20 °C for 4 h. The reaction was then concentrated *in vacuo* and suspended between EtOAc (20 mL) and H_2O (20 mL). The organic layer was separated and dried with anhydrous Na_2SO_4, filtered and concentrated. The crude material was then purified by column chromatography eluting with 100% CyHex to 30% EtOAc/CyHex to **89** as a solid (75.0 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10–7.97 (m, 2H), 7.80–7.67 (m, *J* 8.4 Hz, 2H), 1.64–1.39 (m, 2H), 1.17–1.02 (m, 2H). MS, *m/z* 265 (100) [M-19 (acid intermediate generated)]$^-$.

4-(2,2,2-Trifluoroethyl)benzenesulfonyl chloride (**90**). The procedure used for **89** was replicated employing 2,2,2-trifluoroethylbenzene (50 mg, 0.31 mmol) to afford **90** as a solid (43.1 mg, 53%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10–8.03 (m, 2H), 7.59 (dd, *J* 0.6, 8.7 Hz, 2H), 3.53 (q, *J* 10.4 Hz, 2H). MS, *m/z* 239 (100) [M-19 (acid intermediate generated)]$^-$.

Methyl 2-(4-chlorosulfonylphenyl)-2-methyl-propanoate (**91**). The procedure used for **89** was replicated employing methyl 2-methyl-2-phenyl-propanoate (524 mg, 2.49 mmol) to afford **91** as a solid (468 mg, 57%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05–7.95 (m, 2H), 7.64–7.52 (m, 2H), 3.70 (s, 3H), 1.64 (s, 6H). MS, *m/z* 277 (100) $[M+H]^+$.

tert-Butyl 4-[4-(2-methoxy-1,1-dimethyl-2-oxo-ethyl)phenyl]sulfonylpiperazine-1-carboxylate (**92**). The procedure for **2** was replicated employing **92** (130 mg, 0.47 mmol) and 1-Boc-piperazine (131 mg, 0.70 mmol) to afford **92** as a solid (200 mg, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82–7.61 (m, 2H), 7.39–7.61 (m, 2H), 3.69 (s., 3H), 3.61–3.48 (m, 4H), 3.14–2.89 (m, 4H), 1.62 (s, 6H), 1.42 (s, 9H). MS, *m/z* 277 (100) [M-Boc]$^+$.

2-*Methyl-2-(4-piperazin-1-ylsulfonylphenyl)propan-1-ol* (**93**). To a suspension of $LiAlH_4$ (15.6 mg, 0.46 mmol) in anhydrous THF (1 mL) at 0 °C was added **92** (35 mg, 0.082 mmol), and the resulting reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched by a dropwise addition of saturated sodium sulfate until the evolution of hydrogen gas had ceased. The reaction was then diluted with EtOAc (10 mL), dried with anhydrous sodium sulfate and filtered through Celite to afford *tert*-butyl 4-[4-(2-hydroxy-1,1-dimethyl-ethyl)phenyl]sulfonylpiperazine-1-carboxylate as an oil (32 mg, 98%). This material was then dissolved in a 4:1 mixture of DCM/TFA and stirred for 1 h. The reaction was then concentrated and dissolved in EtOAc (10 mL) which was then washed with saturated $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was then dried with anhydrous Na_2SO_4, filtered, and concentrated to afford **93** (17 mg, 35%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80–7.70 (m, 2H), 7.67–7.53 (m, 2H), 3.70 (s, 2H), 3.18–2.91 (m, 8H), 1.39 (s, 6H). MS, *m/z* 299 (100) $[M+H]^+$.

(2S)-1-(4-tert-Butylphenyl)sulfonyl-2-methyl-piperazine trifluoroacetate (**94**). General Method D. *tert*-butyl (3*S*)-3-methylpiperazine-1-carboxylate (50 mg, 0.25 mmol), 4-*tert*-butylbenzenesulfonyl chloride (58.1 mg, 0.25 mmol) and DIPEA (131 μL, 0.75 mmol) were dissolved in DCM (2 mL) and stirred for 16 h at 20 °C under N_2. The reaction mixture was then washed with cold 1 M HCl (10 mL), saturated $NaHCO_3$ (10 mL) and brine (10 mL), then dried with anhydrous Na_2SO_4, filtered and concentrated to afford the Boc-protected product. This material was then dissolved in DCM/TFA (3:1, 8 mL) and stirred for 1 h. The mixture was then concentrated *in vacuo* to afford **94** as a solid (66 mg, 65%).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82–7.63 (m, *J* 8.6 Hz, 2H), 7.62–7.44 (m, *J* 8.6 Hz, 2H), 4.44–4.18 (m, 1H), 3.84 (app d, *J* 14 Hz, 1H), 3.56–3.28

(m, 2H), 3.28–3.10 (m, 2H), 3.10–2.91 (m, 1H), 1.35 (s, 9H), 1.24 (d, *J* 7.0 Hz, 3H). MS, *m/z* 297 (100) $[M+H]^+$.

(2R)-1-(4-tert-Butylphenyl)sulfonyl-2-methyl-piperazine trifluoroacetate (**95**). General Method D was replicated employing *tert*-butyl (3*R*)-3-methylpiperazine-1-carboxylate (50 mg, 0.25 mmol) and 4-*tert*-butylbenzenesulfonyl chloride (58 mg, 0.25 mmol) to afford **95** as a solid (75 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.78–7.61 (m, *J* 8.6 Hz, 2H), 7.61–7.45 (m, *J* 8.2 Hz, 2H), 4.43–4.25 (m, 1H), 3.83 (app d, *J* 14.5 Hz, 1H), 3.54–3.34 (m, 2H), 3.22 (app s, 2H), 3.17–2.96 (m, 1H), 1.34 (s, 9H), 1.22 (d, *J* 7.0 Hz, 3H). MS, *m/z* 297 (100) $[M+H]^+$.

1-(4-tert-Butylphenyl)sulfonyl-2,2-dimethyl-piperazine trifluoroacetate (**96**). General Method D was replicated employing *tert*-butyl 3,3-dimethylpiperazine-1-carboxylate (46 mg, 0.21 mmol) and 4-*tert*-butylbenzenesulfonyl chloride (50 mg, 0.21 mmol) to afford **96** as a solid (32 mg, 35%). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.78–7.65 (m, *J* 8.3 Hz, 2H), 7.58–7.49 (m, *J* 8.2 Hz, 2H), 3.86 (br s, 2H), 3.28 (br s, 2H), 2.96 (br s, 2H), 1.49–1.22 (m, 15H). MS, *m/z* 311 (100) $[M+H]^+$.

(1S,4S)-2-(4-tert-Butylphenyl)sulfonyl-2,5-diazabicyclo[2.2.1]heptane trifluoroacetate (**97**). General Method D was replicated employing *tert*-butyl (1*S*,4*S*)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (50 mg, 0.25 mmol) and 4-*tert*-butylbenzenesulfonyl chloride (58 mg, 0.25 mmol) to afford **97** as a solid (100 mg, 97%). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.81–7.68 (m, *J* 8.3 Hz, 2H), 7.65–7.51 (m, *J* 8.3 Hz, 2H), 4.57 (app s, 1H), 4.44 (app s, 1H), 3.76–3.51 (m, 2H), 3.48–3.34 (m, 2H), 1.95 (d, *J* 11.6 Hz, 1H), 1.61–1.45 (m, 1H), 1.35 (s, 9H). MS, *m/z* 295 (100) $[M+H]^+$.

(1S,5R)-3-(4-tert-Butylphenyl)sulfonyl-8-methyl-3,8-diazabicyclo [3.2.1]octane (**98**). 8-Methyl-3,8-diaza-bicyclo[3.2.1]octane dihydrochloride (50 mg, 0.25 mmol), 4-*tert*-butylbenzenesulfonyl chloride (70 mg, 0.30 mmol) and DIPEA (262 μL, 1.51 mmol) were dissolved in DCM (2 mL) and heated at reflux for 16 h. The crude material was then purified by column chromatography eluting with 100% DCM to 5% MeOH/DCM to afford **98** as a solid (71 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.67–7.59 (m, 2H), 7.54–7.44 (m, 2H), 3.43 (dd, *J* 3.0, 11.0 Hz, 2H), 3.24–3.03 (m, 2H), 2.60 (d, *J* 10.3 Hz, 2H), 2.21 (s, 3H), 2.06–1.80 (m, 4H), 1.32 (s, 9H). MS, *m/z* 323 (100) $[M+H]^+$.

(1S,5R)-3-(4-tert-Butylphenyl)sulfonyl-3,8-diazabicyclo[3.2.1]octane (**99**). 1-Chloroethyl carbonochloridate (1.19 mL, 11.0 mmol) was dissolved in DCE (2 mL) and cooled to 0 °C. **98** (71.0 mg, 0.22 mmol) was then added portion wise under an atmosphere of N_2 and the reaction stirred at reflux for 144 h. The reaction was then cooled to 20 °C and filtered through a short column of silica washing with 50% EtOAc in cyclohexane (10 mL). The mixture was then concentrated *in vacuo* to afford 1-chloroethyl (1*S*,5*R*)-3-(4-*tert*-butylphenyl)sulfonyl-3,8-diazabicyclo[3.2.1]octane-8-carboxylate as a clear oil (91 mg, 100%). MS, *m/z* 415 (100) $[M+H]^+$, 417 (30). This intermediate was then was dissolved in MeOH (4 mL) and stirred at reflux for 2 h. The reaction was then concentrated to afford **99** (67 mg, 99% yield). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.69–7.47 (m, 4H), 4.16–3.91 (m, 2H), 3.82–3.57 (m, 2H), 3.36–3.05 (m, 2H), 2.33–2.03 (m, 4H), 1.35 (s, 9H). MS, *m/z* 311 (100) $[M+H]^+$.

1-(4-tert-Butylphenyl)sulfonyl-1,4-diazepane trifluoroacetate (**100**). General Method D was replicated employing 1-Boc-homopiperazine (50 mg, 0.25 mmol) and 4-*tert*-butylbenzenesulfonyl chloride (64 mg, 0.27 mmol) to afford **100** as a solid (102 mg, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.76–7.65 (m, 2H), 7.60–7.50 (m, 2H), 3.64–3.54 (m, 2H), 3.54–3.30 (m, 6H), 2.30–2.11 (m, 2H), 1.36 (s, 9H). MS, *m/z* 297 (100) $[M+H]^+$.

2-[4-(4-tert-Butylphenyl)sulfonyl-1,4-diazepan-1-yl*]propanoic acid hydrochloride* (**101**). The procedure used for **3** was replicated employing **100** (102 mg, 0.25 mmol) and *tert*-butyl 2-bromopropanoate (41 μL, 0.25 mmol) to afford **101** as a solid (38 mg, 38%). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.75–7.65 (m, 2H), 7.54–7.46 (m, 2H), 3.48–3.21 (m, 5H), 2.99–2.67 (m, 4H), 1.94–1.66 (m, 2H), 1.44 (s, 9H), 1.34 (s, 9H), 1.21 (d, *J* 7.2 Hz, 3H). MS, *m/z* 369 (100) $[M+H]^+$.

(3aR,6aS)-5-(4-tert-Butylphenyl)sulfonyl-2,3,3a,4,6,6a-hexahydro-1H-pyrrolo[3,4-c]pyrrole trifluoroacetate (**102**). General Method D was replicated employing *tert*-butyl (3a*R*,6a*S*)-2,3,3a,4,6,6a-hexahydro-1H-pyrrolo[3,4-*c*]pyrrole-5-carboxylate (20 mg, 0.094 mmol) and 4-*tert*-butylbenzenesulfonyl chloride (24 mg, 0.10 mmol) to afford **102** as a solid (39 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.78–7.71 (m, 2H), 7.58–7.51 (m, 2H), 3.60–3.31 (m, 4H), 3.10 (dd, *J* 3.9, 10.0 Hz, 4H), 2.91–2.68 (m, 2H), 1.43 (s, 9H), 1.36 (s, 9H). MS, *m/z* 309 (100) $[M+H]^+$.

2-(4-tert-Butylphenyl)sulfonyl-2,6-diazaspiro[3.3]heptane trifluoroacetate (**103**). General Method D was replicated employing *tert*-butyl 2,6-diazaspiro[3.3]heptane-2-carboxylate (10 mg, 0.050 mmol) and 4-*tert*-butylbenzenesulfonyl chloride (13 mg, 0.056 mmol) to afford **103** as a solid (15 mg, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.72 (d, *J* 8.4 Hz, 2H), 7.60 (d, *J* 8.4 Hz, 2H), 4.16 (br s, 4H), 3.94 (br s, 4H), 1.36 (s, 9H). MS, *m/z* 295 (100) $[M+H]^+$.

2-[1-(4-tert-Butylphenyl)sulfonyl-4-piperidyl]acetic acid (**104**). Methyl 2-(4-piperidyl)acetate (50 mg, 0.32 mmol), 4-*tert*-butylbenzenesulfonyl chloride (81 mg, 0.35 mmol) and DIPEA (0.11 mL, 0.64 mmol) were dissolved in DCM (5 mL) and stirred for 5 h. The reaction was then diluted with more DCM (10 mL) and washed with saturated $NaHCO_3$ (10 mL), brine (10 mL), dried with Na_2SO_4, filtered and concentrated. 100% CyHex to 20% EtOAc/CyHex to afford methyl 2-[1-(4-*tert*-butylphenyl)sulfonyl-4-piperidyl]acetate as a solid (49 mg, 44%). This product was then dissolved in a stirred mixture of sodium hydroxide (28 mg, 0.70 mmol) were dissolved in EtOH (4 mL) and H_2O (4 mL) and stirred for 16 h at reflux. The reaction was then acidified to pH 2 with 1 M HCl and filtered to afford **104** as a solid (47 mg, 44%). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.71–7.64 (m, 2H), 7.57–7.48 (m, 2H), 3.85–3.69 (m, 2H), 2.37–2.19 (m, 4H), 1.88–1.61 (m, 3H), 1.48–1.29 (m, 11H). MS, *m/z* 340 (100) $[M+H]^+$.

2-[1-(4-tert-Butylphenyl)sulfonyl-4-piperidyl]-1-pyrrolidin-1-yl-*ethanone* (**105**). General Method B was replicated employing **104** (47 mg, 0.14 mmol) and pyrrolidine (46 μL, 0.55 mmol) to afford **105** as a solid (33 mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$, rotamers): *δ* 7.71–7.63 (m, 2H), 7.57–7.48 (m, 2H), 3.83–3.72 (m, 2H), 3.40 (td, *J* 6.9, 16.3 Hz, 4H), 2.28 (dt, *J* 2.3, 12.0 Hz, 2H), 2.16 (d, *J* 6.4 Hz, 2H), 2.02–1.76 (m, 7H), 1.46–1.23 (m, 11H). MS, *m/z* 393 (100) $[M+H]^+$.

1-(4-tert-Butylphenyl)sulfonylpiperidin-4-amine trifluoroacetate (**106**). General Method D was replicated employing 4-Boc-aminopiperidine (516 mg, 2.58 mmol) and 4-*tert*-butylbenzenesulfonyl chloride (500 mg, 2.15 mmol) to afford **106** as a solid (700 mg, 100%). ^{1}H NMR (300 MHz, MeOD): *δ* 7.76–7.65 (m, 4H), 3.90–3.81 (m, 2H), 3.12–3.05 (m, 1H), 2.48–2.37 (m, 2H), 2.10–2.01 (m, 2H), 1.76–1.60 (m, 2H), 1.38 (s, 9H). MS, *m/z* 297 (100) $[M+H]+$.

Phenyl N-[1-(4-tert-butylphenyl)sulfonyl-4-piperidyl]carbamate (**107**). Phenyl chloroformate (544 μL, 1.08 mmol) was added dropwise to a stirred solution of **106** (515 mg, 0.98 mmol) in pyridine (5 mL) at 0 °C under N_2. The reaction was warmed to 20 °C and then stirred for a further 2 h. Additional phenyl chloroformate (408 μL, 3.24 mmol) was then added and the reaction was stirred for a further 2 h. The reaction was then diluted with H_2O (30 mL) which precipitated crude product which was then filtered off and washed with H_2O. The crude solid was then purified by column chromatography eluting with 100% CyHex to 100% EtOAc to afford **106** as a solid (348 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.72–7.65 (m, 2H), 7.58–7.52 (m, 2H), 7.38–7.32 (m, 2H), 7.24–7.16 (m, 1H), 7.13–7.07 (m, 2H), 3.82–3.72 (m, 2H), 3.64–3.43 (m, 1H), 2.53–2.42 (m, 2H), 2.16–2.03 (m, 2H), 1.73–1.57 (m, 2H), 1.36 (s, 9H). MS, *m/z* 417 (100) $[M+H]+$.

N-[1-(4-tert-Butylphenyl)sulfonyl-4-piperidyl]pyrrolidine-1-carboxamide (**108**). Phenyl *N*-[1-(4-*tert*-butylphenyl)sulfonyl-4-piperidyl]carbamate (348 mg, 0.84 mmol) and pyrrolidine (0.686 mL, 8.35 mmol) were dissolved in 1,4-dioxane (10 mL) and stirred at reflux under a N_2 atmosphere for 2 h. The reaction was then concentrated *in vacuo* and dissolved in DCM (30 mL). The organic layer was washed with H_2O (10 mL) and brine (10 mL), then dried with anhydrous Na_2SO_4, filtered and concentrated. The crude material was then purified by column chromatography eluting with 100% DCM to 5% MeOH/DCM to afford **108** as a solid (289 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72–7.63 (m, 2H), 7.59–7.50 (m, 2H), 4.18–3.97 (m, 1H), 3.84–3.73 (m, 2H), 3.69–3.53 (m, 1H), 3.39–3.20 (m, 4H), 2.38 (dt, *J* 2.7, 12.0 Hz, 2H), 2.09–1.96 (m, 2H), 1.92–1.85 (m, 4H), 1.51 (dq, *J* 4.2, 12.14 Hz, 2H), 1.36 (s, 9H). MS, *m/z* 394 (100) $[M+H]^+$.

tert-Butyl 4-(2-ethoxy-1-methyl-2-oxo-ethyl)-3-oxo-piperazine-1-carboxylate (**109**). Sodium hydride (13.2 mg, 0.33 mmol) was added to a stirred solution of *tert*-butyl 3-oxopiperazine-1-carboxylate (33 mg, 0.17 mmol) in DMF (1 mL) and stirred for 10 min. Ethyl 2-bromopropanoate (0.026 mL, 0.20 mmol) was then added and the reaction stirred at 50 °C for 6 h. The reaction was then quenched with saturated NH_4Cl solution (1 mL) and concentrated. The crude material was dissolved in EtOAc (10 mL) and washed with H_2O (10 mL) and brine (10 mL). The organic layer was then dried with anhydrous Na_2SO_4, filtered and concentrated. The crude material was then purified by column chromatography eluting with 100% DCM to 5% MeOH/DCM to afford *tert*-butyl 4-(2-ethoxy-1-methyl-2-oxo-ethyl)-3-oxo-piperazine-1-carboxylate as an oil (28.0 mg, 57%). This Boc-protected product was then dissolved in a solution of LiOH (11.2 mg, 0.466 mmol) in EtOH (2 mL) and stirred at 60 °C for 2 h. The reaction was then concentrated and diluted with cold 0.5 M HCl (10 mL). The product was then extracted with EtOAc (2 × 10 mL) and the combined organic layers were dried with anhydrous Na_2SO_4, filtered and concentrated to afford **109** as a white solid (25 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.24 (q, *J* 7.48 Hz, 1H), 4.24 (app d, *J* 18.5 Hz, 1H), 4.07 (app d, *J* 18.5 Hz, 1H), 3.87–3.75 (m, 1H), 3.62–3.49 (m, 1H), 3.45–3.33 (m, 2H), 1.51–1.44 (m, 12H). MS, *m/z* 271 (100) $[M-H]^-$.

1-(1-Methyl-2-oxo-2-pyrrolidin-1-yl-*ethyl)piperazin-2-one trifluoroacetate* (**110**). General Method B was replicated employing **109** (25 mg, 0.092 mmol) and pyrrolidine (15 μL, 0.18 mmol) to afford 2-(4-*tert*-butoxycarbonyl-2-oxo-piperazin-1-yl)propanoic acid as a solid (25 mg, 0.092 mmol). The protected product was then dissolved in DCM/TFA (3:1, 8 mL) and stirred for 1 h. The reaction was then concentrated to afford **110** (31 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.46–5.27 (m, 1H), 4.00–3.84 (m, 2H), 3.77–3.63 (m, 2H), 3.54–3.39 (m, 6H), 2.08–1.82 (m, 4H), 1.41 (d, *J* 7.2 Hz, 3H). MS, *m/z* 226 (100) $[M+H]^+$.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]acetic acid hydrochloride* (**111**). **10** (21 mg, 0.053 mmol) was stirred in 4 M HCl in dioxane (2 mL) and stirred for 16 h. The mixture was then concentrated *in vacuo* to afford **111** as a solid (19 mg, 95%).^{1}H NMR (300 MHz, MeOD): δ 7.81–7.67 (m, 4H), 4.12 (s, 2H), 3.62–3.33 (m, 7H), 3.28–3.12 (m, 1H), 1.37 (s, 9H). MS, *m/z* 339 (100) $[M-H]^-$.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]butanoic acid hydrochloride* (**112**). Lithium bis(trimethylsilyl)amide (111 μL, 0.11 mmol) was added dropwise to a cooled solution (0 °C) of **10** (40.0 mg, 0.10 mmol) in THF (1 mL) at −78 °C. After stirring for 1 h, iodoethane (9.0 μL, 0.111 mmol) was added and the reaction stirred at 20 °C for 14 h. The reaction was then quenched with a solution of NH_4Cl (2 mL), and the mixture basified with saturated $NaHCO_3$ solution (10 mL). The solution was extracted with EtOAc (3 × 10 mL) and the combined organic layer washed with brine (10 mL), then dried with anhydrous Na_2SO_4 and concentrated. The crude material was then purified by column chromatography eluting with 100% DCM to 5% MeOH/DCM to afford *tert*-butyl 2-[4-(4-*tert*-butylphenyl)sulfonylpiperazin-1-yl]butanoate as a solid (16 mg, 37%). This solid was then dissolved in 3 mL of HCl in dioxane (4 M) and stirred for 3 h. The mixture was then concentrated *in vacuo* to afford **112** as a solid (14 mg, 34%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.69–7.65 (m, 2H), 7.56–7.51 (m, 2H), 3.51–3.44 (m, 1H), 3.11–2.86 (m, 6H), 2.83–2.61 (m, 4H), 1.36–1.34 (m, 8H), 0.97–0.75 (m, 3H). MS, *m/z* 369 (100) $[M+H]^+$.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]pentanoic acid hydrochloride* (**113**). The procedure used for **112** was replicated employing **10** (40 mg, 0.10 mmol) and 1-iodopropane (10 μL, 0.12 mmol) to afford **113** as a solid (13 mg, 27%). This material was taken directly to the next step. MS, *m/z* 383 (100) $[M+H]^+$.

1-(4-tert-Butylphenyl)sulfonylpiperazine trifluoroacetate (**114**). General Method D was replicated employing 1-Boc-piperazine (500 mg, 2.69 mmol) and 4-*tert*-butylbenzenesulfonyl chloride (625 mg, 2.69 mmol) to afford **114** as a solid (1.1 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70–7.62 (m, 2H), 7.62–7.51 (m, 2H), 3.32 (app s, 8H), 1.35 (s, 9H). MS, *m/z* 283 (100) $[M+H]^+$.

2-(4-((4-(tert-Butyl)phenyl)sulfonyl)piperazin-1-yl*)-3-methylbutanoic acid* (**115**). **114** (20 mg, 0.051 mmol), ethyl 2-bromo-3-methyl-butanoate (12 μL, 0.076 mmol) and potassium carbonate (21 mg, 0.15 mmol) and potassium iodide (13 mg, 0.076 mmol) were dissolved in MeCN (1 mL) was then added and the reaction heated at reflux for a further 72 h. The reaction was then filtered and concentrated. The crude material was then purified by column chromatography eluting with 100% CyHex to 20% EtOAc/CyHex to afford ethyl 2-[4-(4-*tert* butylphenyl)sulfonylpiperazin-1-yl]-3-methyl butanoate as a solid (5.0 mg, 24%). This product was then dissolved in a stirred mixture of lithium hydroxide (1.5 mg, 0.061 mmol) were dissolved in EtOH (0.50 mL) and H_2O (0.50 mL) and stirred for 16 h at reflux. The reaction was then neutralized with 1 M HCl and concentrated. The crude was then dissolved in MeOH (2 mL) and filtered and concentrated to afford **115** as a solid (4.6 mg, 24%). ^{1}H NMR (300 MHz, MeOD): δ 7.72–7.65 (m, 4H), 4.00–3.92 (m, 1H), 2.93 (app s, 4H), 2.76–2.45 (m, 5H), 1.37 (s, 9H), 0.90–0.81 (m, 6H). MS, *m/z* 383 (100) $[M+H]^+$.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-2-phenyl-acetic acid hydrochloride* (**116**). The procedure used for **115** was replicated employing **114** (20 mg, 0.051 mmol) and ethyl 2-bromo-2-phenyl-acetate (13.2 μL, 0.078 mmol) to afford **116** as a solid (14 mg, 61%). ^{1}H NMR (300 MHz, MeOD): δ 7.77–7.68 (m, 4H), 7.57–7.49 (m, 5H), 5.17 (s, 1H), 3.65–3.33 (m, 4H), 3.29–3.06 (m, 4H), 1.37 (s, 9H). MS, *m/z* 417 (100) $[M+H]^+$.

2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]-3-phenyl-propanoic acid hydrochloride* (**117**). The procedure used for **112** was replicated employing **10** (40 mg, 0.101 mmol) and bromomethylbenzene (13.2 μL, 0.12 mmol) to afford **117** as a solid (8 mg, 15%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71–7.65 (m, 2H), 7.58–7.52 (m, 2H), 7.42–7.06 (m, 5H), 3.80–3.47 (m, 3H), 3.21–2.97 (m, 4H), 2.86–2.46 (m, 4H), 1.39–1.33 (m, 12H). MS, *m/z* 431 (100) $[M+H]^+$.

tert-Butyl 4-(2-ethoxy-1,1-dimethyl-2-oxo-ethyl)piperazine-1-carboxylate (**118**). Ethyl 2-bromo-2-methyl-propanoate (0.090 mL, 0.62 mmol), potassium carbonate (255 mg, 1.85 mmol) and 1-Boc-piperazine (206 mg, 1.11 mmol) in ACN (2 mL) were stirred at reflux for 48 h. The reaction was then filtered and washed with DCM and concentrated *in vacuo*. The crude material was then purified by column chromatography eluting with 100% CyHex to 45% EtOAc/CyHex to afford **118** as an oil (119 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.18 (q, *J* 7.2 Hz, 2H), 3.44 (app s, 4H), 2.56 (app s, 4H), 1.46 (s, 9H), 1.41–1.23 (m, 9H). MS, *m/z* 301 (100) $[M+H]^+$.

2-Methyl-2-piperazin-1-yl-1-pyrrolidin-1-yl-*propan-1-one trifluoroacetate* (**119**). *n*-Butyllithium in hexanes (1 M, 0.40 mL, 0.40 mmol) was added to a stirred solution of pyrrolidine (0.033 mL, 0.40 mmol) in THF (2 mL) at 0 °C and stirred at this

temperature for 5 min. **118** (60 mg, 0.20 mmol) in THF (2 mL) was then added to this mixture and stirred for 24 h. The mixture was quenched with saturated NH_4Cl solution (10 mL) and extracted with EtOAc (15 mL). The reaction was then dried with anhydrous Na_2SO_4 and concentrated. The crude material was then purified by column chromatography eluting with 100% CyHex to 15% EtOAc/CyHex to afford *tert*-butyl 4-(1,1-dimethyl-2-oxo-2-pyrrolidin-1-yl-ethyl)piperazine-1-carboxylate (29.5 mg, 45%). The residue was then dissolved in 4:1 DCM/TFA (5 mL) and stirred for 1 h. The mixture was concentrated *in vacuo* to afford **119** as a solid (59 mg, 45%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.02–3.65 (m, 3H), 3.63–3.39 (m, 6H), 3.32–3.09 (m, 4H), 2.04–1.77 (m, 4H), 1.49 (s, 6H). MS, *m/z* 226 (100) $[M+H]^+$.

Methyl (2S)-2-[4-(4-tert-butylphenyl)sulfonylpiperazin-1-yl*] propanoate* (**120**). Trifluoromethanesulfonic anhydride (444 μL, 0.264 mmol) was added dropwise to a stirred solution of methyl (2*R*)-2-hydroxypropanoate (23 μL, 0.24 mmol) and 2,6-dimethylpyridine (306 μL, 0.26 mmol) in DCM (1 mL) over 30 min at −78 °C. The reaction was then stirred at this temperature for a further 30 min. The reaction mixture was then warmed to 20 °C and stirred for a further 30 min. The reaction solution was then washed with 1 N HCl (1 mL), dried with anhydrous Na_2SO_4, filtered and concentrated. The residue was then dissolved in DCM (1 mL) and cooled to 0 °C. **2** (136 mg, 0.48 mmol) was then added in portions. H_2O (1 mL) was then added dropwise, and the reaction stirred for 16 h at 20 °C. The reaction was then washed with brine (2 mL), dried with anhydrous Na_2SO_4, filtered and concentrated. The crude material was then purified by column chromatography eluting with 100% CyHex to 50% EtOAc/CyHex to **120** as a solid (52 mg, 59%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70–7.62 (m, *J* 8.4 Hz, 2H), 7.57–7.48 (m, *J* 8.4 Hz, 2H), 3.28 (q, *J* 6.8 Hz, 1H), 3.05 (app s, 4H), 2.84–2.51 (m, 4H), 1.35 (s, 9H), 1.26 (d, *J* 6.9 Hz, 3H). MS, *m/z* 369 (100) $[M+H]^+$.

(2S)-2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]propanoic acid hydrochloride* (**121**). **120** (41 mg, 0.11 mmol) was dissolved in 1 mL of 4 N HCl and stirred at 90 °C in a sealed tube for 7 h. The completed reaction was then concentrated to afford **121** (42.0 mg, 98%). ^{1}H NMR (300 MHz, MeOD): δ 7.75 (q, *J* 8.5 Hz, 5H), 4.21 (q, *J* 7.2 Hz, 1H), 3.64–3.33 (m, 7H), 3.29–3.02 (m, 1H), 1.60 (d, *J* 7.2 Hz, 4H), 1.37 (s, 9H). MS, *m/z* 355 (100) $[M+H]^+$.

Methyl (2R)-2-[4-(4-tert-butylphenyl)sulfonylpiperazin-1-yl*] propanoate* (**122**). The procedure used for **120** was replicated employing methyl (2*S*)-2-hydroxypropanoate (18 μL, 0.19 mmol) and **2** (109 mg, 0.38 mmol) to afford **122** as a solid (55 mg, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71–7.62 (m, *J* 8.4 Hz, 2H), 7.57–7.47 (m, *J* 8.3 Hz, 2H), 3.70 (s, 3H), 3.28 (q, *J* 6.8 Hz, 1H), 3.05 (br. s., 4H), 2.78–2.58 (m, 4H), 1.35 (s, 9H), 1.26 (d, *J* 6.9 Hz, 3H). MS, *m/z* 369 (100) $[M+H]^+$.

(2R)-2-[4-(4-tert-Butylphenyl)sulfonylpiperazin-1-yl*]propanoic acid hydrochloride* (**123**). The procedure used for **121** was replicated employing **122** (55 mg, 0.15 mmol) to afford **123** as a solid (42 mg, 72%). ^{1}H NMR (300 MHz, MeOD): δ 7.75 (q, *J* 8.4 Hz, 4H), 4.29–4.10 (m, 1H), 3.72–3.33 (m, 6H), 3.29–3.09 (m, 2H), 1.69–1.51 (m, 3H), 1.37 (s, 9H). MS, *m/z* 355 (100) $[M+H]^+$.

Methyl (2S)-2-[4-(4-trimethylsilylphenyl)sulfonylpiperazin-1-yl*] propanoate* (**124**). The procedure used for **120** was replicated employing methyl (2*R*)-2-hydroxypropanoate (21 μL, 0.19 mmol) and **88** (66 mg, 0.22 mmol) to afford **124** as a solid (27.1 mg, 32%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89–7.54 (m, 4H), 3.70 (s, 3H), 3.35–3.23 (m, 1H), 3.14–2.99 (m, 4H), 2.69 (app s, 4H), 1.26 (d, *J* 6.6 Hz, 3H), 0.31 (s, 9H). MS, *m/z* 385 (100) $[M+H]^+$. HRMS acquired: (M + H) 424.2090; $C_{20}H_{33}N_3O_3SiS$ requires (M + H), 424.2085.

(2S)-2-[4-(4-Trimethylsilylphenyl)sulfonylpiperazin-1-yl*]propanoic acid hydrochloride* (**125**). The procedure used for **121** was replicated employing **124** (27.1 mg, 0.071 mmol) to afford **125** as a solid (28 mg, 98%). ^{1}H NMR (300 MHz, MeOD): δ 7.89–7.75 (m, 4H), 4.21 (q, *J* 7.2 Hz, 1H), 3.91–3.59 (m, 3H), 3.59–3.33 (m, 5H), 1.60 (d, *J* 7.0 Hz, 3H), 0.32 (s, 9H). MS, *m/z* 371 (100) $[M+H]^+$.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113253.

Abbreviations

ABS	asexual blood stage
ACT	artemisinin combination therapy
ART	artemisinin
Cl_{int}	intrinsic clearance
cLogP	calculated partition coefficient
CQ	chloroquine
DHFR	dihydrofolate reductase
DIPEA	diisopropylethylamine
DMEM	Dulbecco's modified Eagle's medium
EH	hepatic extraction ratio
FCS	fetal calf serum
GFP	green fluorescent protein
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
LDH	lactate dehydrogenase
LipE	lipophilic efficiency
MMV	Medicines for Malaria Venture
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NLuc	nano luciferase
PBS	phosphate buffered saline
Pk	*P. knowlesi*
Pf	*P. falciparum*
PSA	polar surface area
pyr	pyrimethamine
RBC	red blood cell
SAR	structure-activity relationship
TFA	trifluoracetic acid
WHO	World Health Organization

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