Targeting of fumarate hydratase from *Mycobacterium tuberculosis* using allosteric inhibitors with a dimeric-binding mode

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Abstract

With the growing worldwide prevalence of antibiotic-resistant strains of tuberculosis (TB), new targets are urgently required for the development of treatments with novel modes of action. Fumarate hydratase (fumarase), a vulnerable component of the citric acid cycle in *Mycobacterium tuberculosis* (*Mtb*), is a metabolic target that could satisfy this unmet demand. A key challenge in the targeting of *Mtb* fumarase is its similarity to the human homolog, which shares an identical active site. A potential solution to this selectivity problem was previously found in a high-throughput screening hit that binds in a non-conserved allosteric site. In this work, a structure-activity relationship study was carried out with the determination of further structural biology on the lead series, affording derivatives with sub-micromolar inhibition. Further, the

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screening of this series against *Mtb in vitro* identified compounds with potent minimum inhibitory concentrations (MIC).

Keywords

INTRODUCTION

Mycobacterium tuberculosis (*Mtb*), the causative agent of the disease tuberculosis (TB), infects 10 million people per year and is responsible for the deaths of 1.6 million annually, of which 300,000 are HIV-positive.[1] Further, a quarter of the global population, 1.7 billion individuals, are thought to be infected with asymptomatic latent TB and hence at risk of active infection.[2] Despite the scope of the disease, treatment remains intensive and long-term with multi- and extensively-drug resistant TB strains a growing problem, hence there is a continuing urgent need for novel improved anti-TB therapeutics.[3]

Mtb fumarate hydratase (fumarase) is a component of the citric acid cycle in *Mtb*,[4] where it catalyzes the reversible interconversion of fumarate and L-malate. When normoxic cells are rapidly dividing fumarase operates in the forward direction (fumarate to L-malate) and is involved in energy generation and the synthesis of precursors for biosynthetic pathways. Under conditions of lowered oxygen availability fumarase operates in the reverse direction allowing fermentative production and secretion of succinate.[5,6] During pathogenesis of tuberculosis anoxia develops in the center of caseous lesions prior to the development of cavitary lesions, where sudden reaeration allows rapid bacterial growth to promote bacterial transmission from host-to-host.[7] *Mtb* fumarase is a non-redundant and therefore vulnerable component of the citric acid cycle,[8] a situation that is not guaranteed in bacteria.[9] The regulation of fumarase is therefore central to the realignment of bacterial metabolism that occurs during normal disease progression in *Mtb*, which makes it a very high value target for drugs that might allow faster bacterial clearance. *Mtb* fumarase has been shown to be essential for *Mtb* survival with depletion linked to impaired growth due to accumulation of fumarate, which can react with cysteine thiols.[10] This modification adversely impacts the antioxidants catalase and mycothiol, inducing hypersensitivity to oxidative stress and ultimately *Mtb* death both *in vitro* and *in vivo*.

Mtb fumarase is a 210 kDa class II fumarase,[11] characterized by thermostability, iron-independence and a homotetrameric structure.[9,12] Each subunit is defined by an *N*-terminal domain (residues 1–137), a central domain (residues 138–393) whose α-helices pack with those from other subunits to form a central 20-helix bundle that holds together the quaternary structure, and a conformationally flexible *C*-terminal domain (residues 394–474). The protein possesses four symmetry-related active sites, each located in clefts formed by three subunits and covered by an 'SS' loop (residues 316–325) that can switch between 'open' and 'closed' states.[11] The selective targeting of *Mtb* fumarase is challenging due to its 53% overall sequence identity with the human homolog, which was recently the focus

of a study on cancer cell lines.[13] In our previous work, the conservation of active site residues between *Mtb* and human fumarase was circumvented through the discovery by high-throughput screening (HTS) of an allosteric inhibitor **1** of *Mtb* fumarase (IC$_{50}$ 2.5 ± 1.1 μM) (Figure 1a).[14]

X-ray crystallography showed that inhibitor **1** binds dimerically to *Mtb* fumarase at the interface between two adjacent *C*-terminal domains in an induced pocket (Figure 1b), locking the nearest active site in an 'open' conformation, with the bound molecules interacting with each other through π-stacking of their central phenyl rings (Figures 1c and S8a).[14] A second symmetry-related allosteric site exists in the homotetramer however this is unoccupied in the X-ray crystal structure (Figure 1b), likely due to its participation in a crystal contact. Whilst **1** did not exhibit bactericidal properties it was able to exert inhibition of *Mtb* growth *in vitro* (65% inhibition at 250 μM) that, in combination with the demonstrated essentiality of *Mtb* fumarase,[8,10] warranted additional investigation into inhibitor **1**. Due to the dimeric-binding mode of **1**, a possible strategy would be to link the molecules together into one inhibitor, however the geometry of the π-stacking interaction makes this route challenging. One way of circumventing this problem could be the use of a deconstruction-reconstruction approach,[15] where the HTS hit is defragmented into fragment-like molecules allowing the identification of key binding motifs. This would allow the use of fragment-based methods to more effectively sample the ligand-binding site and develop a sub-micromolar inhibitor with activity against *Mtb in vitro*.[16]

RESULTS AND DISCUSSION

Defragmentation of HTS hit 1.

The defragmentation of HTS hit **1** into 'fragment-like' molecules resulted in the assembly of a focused library (Table 1), with members spanning a range of molecular weights (204 to 326 Da) and incorporating various binding motifs of **1**. The screening of the library with a biochemical assay afforded no measurable inhibition at concentrations of 100 μM or 1 mM (data not shown). Similarly, the use of differential scanning fluorimetry (DSF) showed either negligible change in the melting temperature (ΔT$_m$ +0.1 °C for **10**) or negative thermal shifts (ΔT$_m$ −1.6 °C for **7**) at a ligand concentration of 5 mM (Table 1), in contrast to the thermal shift of +3.9 °C afforded by **1** at 0.625 mM. This includes the higher molecular weight compounds in the library **4** (ΔT$_m$ −0.4 °C) and **6** (ΔT$_m$ −0.6 °C), which retain the substituted phenyl ring of **1** in addition to either of the sulfonamide or phthalazinone motifs. These results suggest that the inhibitory capability of **1** for *Mtb* fumarase is dependent upon all its binding motifs, with high sensitivity to truncation. The negative impact of defragmentation is not surprising due to the large number of interactions that doubly-binding **1** makes in the allosteric site with the two *C*-terminal domains of *Mtb* fumarase (in addition to itself through the π-stacking interaction of its phenyl ring), as well as the induced nature of the site with cryptic pockets not easily targeted by weak fragments.

Structure-activity Relationship Study of HTS hit 1.

Due to lack of success in defragmentation of the HTS hit **1**, attention was shifted to a structure-activity relationship study. Initial modification of the central phenyl ring of

1 through the removal of its methoxy group, which indirectly engages Leu303, Gly305, Leu306 and His397 through hydrogen bonds with two ordered water molecules (Figures 2 and S8b), was not tolerated in **12** (16% inhibition at 50 μM) (Table 2). A similar result was seen upon replacement of the methoxy group with a methyl group in **13** (<10% inhibition at 50 μM), illustrating the sensitivity of the central phenyl ring of **1** to alteration. The replacement of the sulfonamide group with an amide, and the resultant rigidification and rotation of the azepanyl ring was also not tolerated in **14** (14% inhibition at 50 μM).

The replacement of the phthalazinone ring of HTS hit **1** with an indole in **15a** (27% inhibition at 50 μM) resulted in a significant drop in inhibition (Table 3), despite X-ray crystallography showing the maintenance of the cation-π interaction with Arg432 and a hydrogen-bond with the backbone carbonyl of Leu429 (Figure 3a). The substitution of the ring in **15b** (38% inhibition at 50 μM) or extension of the linker in **15c** (12% inhibition at 50 μM) did not result in a significant improvement on **15a**. The use of alternative 5–6 fused rings including benzofuran (**15d**), benzisoxazole (**15e**) and benzimidazole (**15f**) derivatives failed to provide inhibition in the biochemical assay at 50 μM. In contrast, the screening of quinoline derivative **15g** (IC_{50} 4.1 μM) resulted in a comparable IC_{50} value to **1** (IC_{50} 2.0 μM), with the X-ray crystal structure of the *Mtb* fumarase-**15g** complex showing the quinoline ring hydrogen-bonding to a water molecule as opposed to the protein backbone (Figure 3b). Truncation of the quinoline ring of **15g** to a pyridine in **15i** eliminated inhibitory activity at 50 μM, in a similar manner to the defragmentation of **1**.

The truncation of HTS hit **1** to the *N*-methyl sulfonamide **16a** (IC_{50} 57 μM) afforded the lowest molecular weight derivative (402 Da) to give a measurable IC_{50} value (Table 4). An X-ray crystal structure was obtained of the complex of **16a** with *Mtb* fumarase, illustrating a 5 Å movement in the Arg400 side chain (at Cε) relative to the complex with **1** to form a salt-bridge with Glu396, significantly reducing the apparent volume for growth around the sulfonamide (Figures 4a and S9b). Heterocyclic derivatives of **1** were also produced to explore the volume defined by His397, Arg400 and Arg432, beginning with the azocanyl and piperidinyl analogues **16b** (IC_{50} 4.0 μM) and **16c** (IC_{50} 4.4 μM). These 8- and 6-membered derivatives possessed slightly attenuated inhibition in comparison to **1** (IC_{50} 2.0 μM), however were not sufficiently different to preclude testing of alternative heterocycles of similar size. X-ray crystallography showed no significant difference in the binding mode of **16b** in *Mtb* fumarase in comparison to **1** except for Arg400 located above the larger 8-membered ring, which showed evidence of a second alternative conformation (Figures 4b and S10a). The *N*-methyl piperazinyl derivative **16d** (IC_{50} 38 μM), along with morpholino and thiomorpholine dioxide analogues **16e** (IC_{50} 12 μM) and **16g** (IC_{50} 13 μM) afforded IC_{50} values weaker than **1** or the thiomorpholino derivative **16f** (IC_{50} 4.7 μM).

The replacement of the methyl group of **16a** with a phenyl ring in **16h** (IC_{50} 17 μM) (Table 4) partially restored the conformation of the Arg400 side chain, with observation of a cation-π interaction by X-ray crystallography (Figures S9a and S10b). The addition of more flexibility to **16h** in **16i** through insertion of a methylene linker had a detrimental impact on inhibition (53% inhibition at 50 μM). The rigidification of **16i** through incorporation of its benzyl group into a tetrahydroisoquinolyl ring system in **16j** (IC_{50} 2.2 μM) was

more successful, with comparable inhibition to **1** (IC$_{50}$ 2.0 μM). X-ray crystallography showed the tetrahydroisoquinolyl ring of **16j** lying between Arg400 and Arg432, with the side chain of Arg400 maintaining the conformation exhibited in the *Mtb* fumarase-**16h** structure through a cation-π interaction with the phenyl portion of the ring (Figure 5a). Ring expansion of **16j** to improve the interaction with Arg432 in **16l** (IC$_{50}$ 0.67 μM) resulted in a sub-micromolar IC$_{50}$ value, 3-fold lower than that of **1** (IC$_{50}$ 2.0 μM) (Figure 5b). Addition of a methoxy group in **16m** (IC$_{50}$ 0.67 μM) maintained the sub-micromolar inhibitory profile, however further modification of **16l** through addition of a methylene bridge to the tetrahydrobenzoazepanyl ring in **16n** was not tolerated (44% inhibition at 50 μM), revealing the limits of occupancy of this pocket in the allosteric site.

Screening against H37Rv *Mycobacterium tuberculosis*.

As previously reported,[14] no bactericidal activity was observed for HTS hit **1** (Table 5). This was also found with derivatives **15g-i**, **16c**-**h** and **16j**-**m** (data not shown). However, activity was observed with **15a-d** and **16b** (Table 5). The balance of metabolic intermediates in *Mtb* can be programmed by feeding the bacterium various carbon sources. As such, *Mtb* growing under glucose as the sole carbon source relies on glycolysis while bacteria that grow on dipalmitoyl phosphatidylcholine (DPPC) will rely heavily on β-oxidation to supply intermediates for the central carbon metabolism. Both glucose and DPPC are carbon sources predicted to be relevant during the *in vivo* pathogenesis of *Mtb*.[17] Because pyruvate from glycolysis will go on to feed several other anabolic pathways, on balance bacteria get more acetyl-CoA equivalence under growth in DPPC. These acetyl-CoA feed directly into the citric acid cycle (among other pathways) in which fumarase lies. Therefore, the flux of intermediates in the citric acid cycle is greater under growth in DPPC and *Mtb* are more reliant on the activity of enzymes in this pathway for growth. Hence, it is reassuring that compounds **15a-d** and **16b** were highly active in DPPC-containing medium. In particular, the benzofuran analogue **15d** gave the lowest MIC value against *Mtb* at 6.3 μM in DPPC-containing medium. Of these active compounds **16b** (IC$_{50}$ 4.0 μM, MIC 19 μM in 7H9/DPPC) was the most potent against purified *Mtb* fumarase, and all were more lipophilic than **1** (cLogP 1.6) with the majority possessing a cLogP between 3.6 and 4.1. The poor activity in 7H9 medium supplemented with ADC was likely due to high protein binding since this medium contains 0.4% bovine serum albumin. This suggests overcoming protein binding will be an important component of future optimization.

Synthetic Chemistry.

The synthesis of **1** was achieved in a convergent manner with the T3P®-mediated amide coupling of carboxylic acid **3** and aniline **5** (60% yield) (Scheme 1), each produced as previously reported.[14] Synthesis of aniline **5** involved the trifluoroacetyl-protection of the amino group of o-anisidine **19** as **20** (99% yield), followed by S$_E$Ar of a sulfonyl chloride group by treatment with chlorosulfonic acid (77% yield). Reaction of **21** with hexamethyleneimine and sodium hydride converted the sulfonyl chloride to a sulfonamide, with the resultant intermediate heated under reflux in an aqueous mixture of ethanol and HCl to remove the trifluoroacetyl protecting group, affording **5** (83% yield). Carboxylic acid **3** was obtained by a route beginning with the Horner-Wadsworth-Emmons reaction

of phthalic anhydride **17** and (carbethoxymethylene)triphenylphosphorane to give **18** (67% yield), followed by heating under reflux with hydrazine in ethanol to afford the phthalazinone ester **2** (99% yield). Hydrolysis of **2** produced acid **3** (44% yield), which was also used in the synthesis of **4** through T3P®-mediated amide coupling with o-anisidine **19** (82% yield) (Scheme 1). The application of acetic anhydride and pyridine to aniline **5** also allowed the production of **6** (79% yield).

Fragment **7** was synthesized in three steps, beginning with the treatment of phenol **22** with chlorosulfonic acid in a similar manner to **20** (78% yield) (Scheme 2). The resultant sulfonyl chloride **23** was reacted with hexamethyleneimine in the presence of DIPEA to afford sulfonamide **24** (64% yield), whose nitro group was reduced by a mixture of sodium borohydride and nickel(II) chloride (62% yield). The production of fragment **8** and the corresponding methyl analogue **29** involved the functionalization of commercial sulfonyl chlorides **25** and **26** with hexamethyleneimine and sodium hydride, affording sulfonamides **27** and **28** (42–72% yield) (Scheme 2). The nitro groups of **27** and **28** were reduced in the same manner as **24** to the corresponding anilines **8** and **29** (82–88% yield). Aniline **8** was also acetylated to fragment **9** in the same manner as **5** (86% yield). Anisole **30** was used to make fragment **10** through stirring with chlorosulfonic acid, with the resultant sulfonyl chloride taken forwards crude for reaction with hexamethyleneimine and sodium hydride (49% yield overall) (Scheme 2). The sulfonyl chloride **21** was converted to *N*-methyl sulfonamide **31a** by heating under reflux with methylamine in THF (82% yield), with **31a** also acetylated in the same manner as **5** to afford fragment **11** (56% yield).

Compounds **12** and **13** were synthesized using anilines **8** and **29** (Scheme 2), which were coupled with carboxylic acid **3** using T3P® as with the synthesis of **1** (45–50% yield) (Scheme 3). In contrast, the route for **14** required the initial T3P®-mediated amide coupling of carboxylic acid **32** with hexamethyleneimine, affording phenol **33** (24% yield) that was methylated with methyl 21 iodide (77% yield) (Scheme 3). The nitro group of **34** was reduced in the same manner as **27** and **28** (64% yield), with the resultant aniline **35** coupled with acid **3** to afford **14** (45% yield).

Compounds **15a**-**f** and **15h** were synthesized in one step from aniline **5** via T3P®-mediated amide coupling with commercially available carboxylic acids (21–67% yield), whilst **15i** required alternative amide-coupling conditions with the use of EDC (64% yield) (Scheme 4). In the production of **15g**, 4-methylquinoline **36** was converted using LDA and carbon dioxide to 4-quinolylacetic acid, which was taken forwards crude for reaction with aniline **5** (5% yield overall). The synthesis of compounds **16a**-**n** involved the T3P®-mediated amide coupling of anilines **31a**-**n** with carboxylic acid **3** as with the synthesis of **1** (10–43% yield) (Scheme 4). Anilines **31b**-**f** and **31h**-**l** were produced by the reaction of sulfonyl chloride **21** with commercially available amines in the presence of sodium hydride, followed by trifluoroacetyl deprotection via heating under reflux with ethanol and HCl as in **5** (37–81% yield). In comparison, whilst the trifluoroacetyl deprotection step in the synthesis of anilines **31g** and **31m-n** was the same as **31b**-**f** and **31h**-**l**, the prior reaction of sulfonyl chloride **21** with an amine was carried out with triethylamine and DMAP (65–80% yield overall).

CONCLUSIONS

The application of a deconstruction-reconstruction approach with the previously identified HTS hit **1** did not lead to the development of improved inhibitors, with defragmentation failing to afford fragments with either measurable inhibition in the biochemical assay or a positive result by DSF. This points to the essentiality of all components of **1** for its unique dimeric, induced-fit binding mode in the allosteric site of *Mtb* fumarase. A subsequent SAR study of **1** showed that modifications including replacement of both the phthalazinone and azepanyl ring systems were possible, with benzoazepanyl derivatives **16l-m** affording sub-micromolar IC_{50} values (0.67 μM) three-fold stronger than that of **1**. Derivatives of **1** maintained the dimeric-binding mode in the allosteric site of *Mtb* fumarase under X-ray crystallography soaking conditions, including in the case of attenuated inhibition as in **16a** (IC_{50} 57 μM). In contrast to **1**, a subset of the derivatives afforded measurable MIC values against *Mtb in vitro*. These derivatives ranged from conservative expansion of the azepanyl ring of **1** with a subsequent rise in lipophilicity in **16b** (MIC 19 μM in 7H9/DPPC) to complete replacement of the phthalazinone ring with alternative heterocyclic systems in **15a-d** (MIC 6.3–12.5 μM in 7H9/DPPC). These results represent a significant improvement on the original HTS hit **1** and encourage further work on *Mtb* fumarase as a target for the development of compounds with bactericidal activity.

EXPERIMENTAL SECTION

General Chemistry.

All reactions were carried out in oven-dried glassware under a positive pressure of dry nitrogen atmosphere. Temperatures of 0 and −78 °C were obtained by submerging the reaction vessel in a bath containing either ice or a mixture of solid CO_2 pellets and acetone respectively. The solvents DCM, ethyl acetate, acetonitrile, methanol, petroleum ether and toluene were distilled over calcium hydride under a dry nitrogen atmosphere prior to use, with THF distilled over a mixture of lithium aluminium hydride, calcium hydride and triphenylphosphine. DMF was purchased as anhydrous from commercial suppliers, with ethanol obtained in the absolute form. All purchased chemicals were used as received. Solutions of Na_2CO_3 and NaCl (brine) were aqueous and saturated.

Flash column chromatography was performed using automated Biotage® Isolera™ Spektra purification systems with appropriately sized Biotage® SNAP cartridges, containing either KP 50 μm silica in 'normal phase' purification or HP-sphere 25 μm C18 silica in 'reverse phase' purification. Analytical thin layer chromatography (TLC) was performed using Merck glass-backed silica plates, with visualization by 254 or 365 nm ultraviolet light.

Liquid chromatography mass spectrometry (LCMS) was carried out using a Waters® Acquity UPLC® H-Class system, with samples run on a solvent gradient from 0 to 95% acetonitrile in water (+ 0.1% formic acid) over 4 minutes. Peaks corresponding to desired product are described, including the retention time (rt) and % purity by integration. High-resolution mass spectrometry (HRMS) was mainly performed using ThermoFinnigan Orbitrap Classic, Waters® LCT Premier™ or Waters® Vion™ IMS QTof systems. A Perkin-Elmer® Spectrum One FT-IR spectrometer fitted with a universal attenuated total reflectance

accessory was used to record infrared spectra, with wavelengths of maximum absorbance (ν_{max}) quoted in wavenumbers (cm^{-1}) for signals outside of the fingerprint region (br = broad). Only peaks corresponding to key functional groups were characterized. Nuclear magnetic resonance (NMR) spectra were recorded in the indicated deuterated solvents with Avance™ III HD (400 MHz), QNP Cryoprobe (400 MHz) or DCH Cryoprobe (500 MHz) Bruker spectrometers. ^{1}H NMR data are presented in the following order: chemical shift (in ppm on a δ scale relative to the residual solvent resonance peak), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sep = septet, m = multiplet) and coupling constant (J, in Hz). ^{13}C NMR spectra were proton-decoupled, with chemical shifts presented.

A combination of TLC and LCMS analysis was used to monitor reactions. All tested compounds possessed a purity of at least 95% as determined by LCMS analysis.

N-(5-(Azepan-1-ylsulfonyl)-2-methoxyphenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (1).

T3P® (50 wt. % in DMF, 1.3 mL, 2.2 mmol) and *N,N*-diisopropylethylamine (0.64 mL, 3.7 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid 3 (0.150 g, 0.735 mmol) and 5-(azepan-1-ylsulfonyl)-2-methoxyaniline 5 (0.209 g, 0.735 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 1 hour. The reaction mixture was diluted with water (15 mL), adjusted to pH 1 and extracted into ethyl acetate (3 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 20% methanol in DCM) afforded 1 (0.206 g, 60% yield). LCMS (ESI+): m/z 471.3 [M + H]$^+$, (ESI-): m/z 469.1 [M - H]$^-$, rt 1.92 minutes, >99%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 12.62 (1H, s), 9.86 (1H, s), 8.45 (1H, d, J = 2.1 Hz), 8.27 (1H, d, J = 7.9 Hz), 7.99–7.91 (2H, m), 7.90–7.82 (1H, m), 7.49 (1H, dd, J = 8.5, 2.2 Hz), 7.24 (1H, d, J = 8.8 Hz), 4.21 (2H, s), 3.96 (3H, s), 3.12 (4H, t, J = 5.9 Hz), 1.64–1.51 (4H, m), 1.50–1.39 (4H, m); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) 168.4, 159.5, 152.0, 142.1, 133.5, 131.6, 130.4, 129.8, 127.7, 127.6, 125.9, 125.5, 123.4, 119.1, 111.2, 56.3, 47.6, 28.5, 26.3 (1 peak missing); spectroscopic data consistent with literature.[14]

Ethyl 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetate (2).[14,18]

Hydrazine monohydrate (0.47 mL, 9.0 mmol) was added dropwise to a solution of ethyl (E)-2-(3-oxoisobenzofuran-1(3H)-ylidene)acetate **18** (1.97 g, 9.03 mmol) in ethanol (10 mL). The reaction mixture was stirred at 50 °C over 2 hours. The reaction mixture was cooled to room temperature and left to stand for 12 hours. The resulting precipitate was obtained by vacuum filtration and washed with ethanol (10 mL) to afford **2** (2.21 g, 99% yield). LCMS (ESI+): m/z 233.2 [M + H]$^+$, rt 1.53 minutes, >99%; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 12.63 (1H, s), 8.30–8.24 (1H, m), 7.98–7.91 (1H, m), 7.90–7.83 (2H, m), 4.11 (2H, q, J = 7.1 Hz), 4.05 (2H, s), 1.17 (3H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 169.8, 159.4, 140.9, 133.6, 131.7, 129.4, 127.5, 125.9, 125.5, 60.7, 37.9, 14.0; ^{1}H NMR spectroscopic data consistent with literature.[18]

2-(4-Oxo-3,4-dihydrophthalazin-1-yl)acetic acid (3).[14]

Ethyl 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetate **2** (0.260 g, 1.12 mmol) was dissolved in a mixture of aqueous NaOH (10% w/v, 10 mL) and THF (10 mL). The reaction mixture was heated under reflux for 1 hour. The reaction mixture was adjusted to pH 1 by the addition of aqueous HCl (2 M) at 0 °C, then extracted into diethyl ether (3×100 mL). The combined organic extracts were washed (brine), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 10% methanol in DCM) afforded **3** (0.101 g, 44% yield). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) 12.61 (1H, s), 8.26 (1H, d, J = 7.7 Hz), 7.94 (1H, ddd, J = 8.3, 6.9, 1.4 Hz), 7.90–7.82 (2H, m), 3.95 (2H, s); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) 171.4, 159.5, 141.5, 133.5, 131.6, 129.6, 127.5, 125.9, 125.6, 38.2; ^{1}H NMR spectroscopic data consistent with literature.[18]

N-(2-Methoxyphenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (4).

T3P® (50 wt. % in DMF, 0.35 mL, 0.59 mmol) and *N*,*N*-diisopropylethylamine (0.10 mL, 0.59 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (40 mg, 0.20 mmol) and o-anisidine **19** (33 μL, 0.29 mmol) in DMF (1 mL). The reaction mixture was heated to 40 °C over 2 hours. The reaction mixture was diluted with water (15 mL) and extracted into DCM (3×20 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was dissolved in toluene (10 mL) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 20% methanol in DCM) afforded **4** (50 mg, 82% yield). LCMS (ESI+): m/z 332.1 [M + Na]+, rt 1.64 minutes, >99%; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) 12.61 (1H, s), 9.56 (1H, s), 8.27 (1H, d, J = 7.9 Hz), 8.01–7.79 (4H, m), 7.11–7.00 (2H, m), 6.92–6.83 (1H, m), 4.16 (2H, s), 3.86 (3H, s); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) 167.6, 159.5, 149.6, 142.3, 133.5, 131.5, 129.8, 127.6, 127.2, 125.8, 125.6, 124.6, 121.8, 120.2, 111.2, 55.7 (1 peak missing); ν_{max}/cm^{-1} 2905, 1665 (C=O), 1646, 1597, 1539; HRMS (ESI)+: m/z calculated for $[C_{17}H_{15}N_3O_3 + H]^+$ = 310.1186, observed 310.1175.

5-(Azepan-1-ylsulfonyl)-2-methoxyaniline (5).[14]

Hexamethyleneimine (0.212 mL, 1.89 mmol) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.189 g, 4.72 mmol) in DMF (2 mL). The reaction mixture was stirred at 0 °C over 30 minutes. A solution of 4-methoxy-3-(2,2,2-trifluoroacetamido)benzenesulfonyl chloride **21** (0.500 g, 1.57 mmol) in DMF (3 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 3 hours. Ethanol (10 mL) was added dropwise at 0 °C to the reaction mixture, followed by water (10 mL) and aqueous HCl (37.5% w/v, 10 mL). The reaction mixture was heated under reflux for 20 hours. The reaction mixture was concentrated *in vacuo* to remove ethanol, then adjusted to pH 9 by the dropwise addition of aqueous NaOH (10% w/v). The mixture was diluted with ethyl acetate (50 mL), and the resultant aqueous layer discarded. The organic layer was washed with water (3×50 mL) and brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash column chromatography (20 – 50% ethyl acetate in petroleum ether) afforded **5** (0.373 g, 83% yield). LCMS (ESI+): m/z 285.2 [M + H]+, rt 1.85 minutes, >99%; ^{1}H NMR (400 MHz,

CDCl$_3$) 7.17 (1H, dd, J = 8.4, 2.3 Hz), 7.09 (1H, d, J = 2.2 Hz), 6.81 (1H, d, J = 8.4 Hz), 3.98 (2H, br s), 3.90 (3H, s), 3.23 (4H, t, J = 5.9 Hz), 1.75–1.64 (4H, m), 1.62–1.53 (4H, m); ^{13}C NMR (100 MHz, CDCl$_3$) 150.1, 136.7, 131.6, 118.1, 112.7, 109.7, 55.8, 48.3, 29.3, 27.1; ν_{max}/cm^{-1} 3484 (N-H), 3379 (N-H), 2932, 2849, 1610, 1577, 1513; HRMS (ESI)+: m/z calculated for [C$_{13}$H$_{20}$N$_2$O$_3$S + K]$^+$ = 323.0826, observed 323.0835.

N-(5-(Azepan-1-ylsulfonyl)-2-methoxyphenyl)acetamide (6).

Acetic anhydride (10 µL, 0.11 mmol) was added to a solution of 5-(azepan-1-ylsulfonyl)-2-methoxyaniline **5** (15 mg, 0.053 mmol) and pyridine (8.5 µL, 0.11 mmol) in DCM (2 mL). The reaction mixture was stirred over 90 minutes. The reaction mixture was diluted with water (10 mL) and extracted into DCM (3 × 15 mL). The combined organic extracts were dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 80% ethyl acetate in petroleum ether) afforded **6** (14 mg, 79% yield). LCMS (ESI+): m/z 327.3 [M + H]$^+$, (ESI-): m/z 325.2 [M - H]$^-$, rt 1.90 minutes, 96%; ^{1}H NMR (400 MHz, CDCl$_3$) 8.78 (1H, d, J = 1.8 Hz), 7.76 (1H, br s), 7.53 (1H, dd, J = 8.5, 2.3 Hz), 6.91 (1H, d, J = 8.6 Hz), 3.94 (3H, s), 3.30 (4H, t, J = 6.0 Hz), 2.22 (3H, s), 1.80–1.67 (4H, m), 1.65–1.53 (4H, m); ^{13}C NMR (125 MHz, CDCl$_3$) 168.4, 150.4, 132.1, 128.2, 123.5, 118.0, 109.5, 56.2, 48.5, 29.3, 27.1, 25.0; ν_{max}/cm^{-1} 3349, 2930, 2855, 1673 (C=O), 1592, 1518; HRMS (ESI)+: m/z calculated for [C$_{15}$H$_{22}$N$_2$O$_4$S + H]$^+$ = 327.1373, observed 327.1375.

2-Amino-4-(azepan-1-ylsulfonyl)phenol (7).

Sodium borohydride (28 mg, 0.75 mmol) was added portionwise at 0 °C to a suspension of NiCl$_2$·6H$_2$O (59 mg, 0.25 mmol) in methanol (2 mL). The reaction mixture was warmed to room temperature and stirred over 20 minutes. A solution of 4-(azepan-1-ylsulfonyl)-2-nitrophenol **24** (0.170 g, 0.498 mmol) in methanol (2 mL) was added at 0 °C to the reaction mixture, followed by further sodium borohydride (94 mg, 2.5 mmol). The reaction mixture was warmed to room temperature and stirred over 2 hours. Water (15 mL) was added at 0 °C and the reaction mixture filtered through celite. The product was extracted into DCM (3 × 25 mL). The combined organic extracts were washed (brine), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (20 – 50% ethyl acetate in petroleum ether) afforded **7** (83 mg, 62% yield). LCMS (ESI+): m/z 271.2 [M + H]$^+$, (ESI-): m/z 269.2 [M - H]$^-$, rt 1.71 minutes, >99%; ^{1}H NMR (400 MHz, CD$_3$CN) 7.45 (1H, br s), 7.04 (1H, d, J = 2.3 Hz), 6.94 (1H, dd, J = 8.2, 2.2 Hz), 6.80 (1H, d, J = 8.3 Hz), 4.26 (2H, br s), 3.18 (4H, t, J = 5.9 Hz), 1.74–1.60 (4H, m), 1.59–1.50 (4H, m); ^{13}C NMR (100 MHz, CD$_3$CN) 148.0, 137.6, 131.9, 117.8, 114.8, 113.6, 48.9, 29.8, 27.6; ν_{max}/cm^{-1} 3397, 3350, 3320, 3285, 2927, 2855, 1593, 1510; HRMS (ESI)-: m/z calculated for [C$_{12}$H$_{18}$N$_2$O$_3$S - H]$^-$ = 269.0965, observed 269.0965.

3-(Azepan-1-ylsulfonyl)aniline (8).

Sodium borohydride (51 mg, 1.4 mmol) was added portionwise at 0 °C to a suspension of NiCl$_2$ (59 mg, 0.45 mmol) in methanol (2 mL). The reaction mixture was warmed to room temperature and stirred over 30 minutes. 1-((3-Nitrophenyl)sulfonyl)azepane **27** (0.257 g, 0.904 mmol) was added at 0 °C to the reaction mixture, followed by further methanol (4 mL) and sodium borohydride (0.171 g, 4.52 mmol). The reaction mixture was warmed to

room temperature and stirred over 45 minutes. Water (10 mL) was added at 0 °C and the mixture filtered through celite, eluted with methanol (10 mL) and water (15 mL). The filtrate was concentrated *in vacuo* to remove methanol, then extracted into ethyl acetate (3×25 mL). The combined organic extracts were washed (brine), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (20 – 50% ethyl acetate in petroleum ether) afforded **8** (0.188 g, 82% yield). LCMS (ESI+): m/z 255.2 [M + H]$^+$, rt 1.77 minutes, >99%; ^{1}H NMR (400 MHz, CDCl$_3$) 7.24 (1H, t, J = 7.8 Hz), 7.15–7.10 (1H, m), 7.08 (1H, t, J = 2.0 Hz), 6.81 (1H, ddd, J = 7.9, 2.3, 0.7 Hz), 3.89 (2H, br s), 3.26 (4H, t, J = 5.9 Hz), 1.77–1.65 (4H, m), 1.63–1.54 (4H, m); ^{13}C NMR (100 MHz, CDCl$_3$) 147.2, 140.4, 130.0, 118.6, 116.7, 113.0, 48.4, 29.3, 27.0; ν_{max}/cm^{-1} 3391 (N-H), 3326 (N-H), 2928, 2851, 1639, 1596; HRMS (ESI)+: m/z calculated for [C$_{12}$H$_{18}$N$_2$O$_2$S + H]$^+$ = 255.1162, observed 255.1166.

N-(3-(Azepan-1-ylsulfonyl)phenyl)acetamide (9).

Acetic anhydride (11 μL, 0.12 mmol) was added to a solution of 3-(azepan-1-ylsulfonyl)aniline **8** (15 mg, 0.059 mmol) and pyridine (10 μL, 0.12 mmol) in DCM (2 mL). The reaction mixture was stirred over 5 hours. The reaction mixture was diluted with water (10 mL) and extracted into DCM (3×15 mL). The combined organic extracts were dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (35 – 70% ethyl acetate in petroleum ether) afforded **9** (15 mg, 86% yield). LCMS (ESI+): m/z 297.2 [M + H]$^+$, (ESI-): m/z 295.2 [M - H]$^-$, rt 1.87 minutes, >99%; ^{1}H NMR (500 MHz, CDCl$_3$) 7.98 (1H, d, J = 7.9 Hz), 7.91 (1H, br s), 7.83–7.78 (1H, m), 7.52–7.47 (1H, m), 7.45 (1H, t, J = 7.9 Hz), 3.27 (4H, t, J = 6.0 Hz), 2.21 (3H, s), 1.76–1.66 (4H, m), 1.65–1.53 (4H, m); ^{13}C NMR (125 MHz, CDCl$_3$) 169.0, 139.9, 139.1, 130.0, 123.7, 122.2, 117.9, 48.5, 29.3, 27.0, 24.7; ν_{max}/cm^{-1} 3305, 3257, 3192, 3117, 2929, 2851, 1670 (C=O), 1592, 1545; HRMS (ESI)+: m/z calculated for [C$_{14}$H$_{20}$N$_2$O$_3$S + H]$^+$ = 297.1267, observed 297.1268.

1-((4-Methoxyphenyl)sulfonyl)azepane (10).

Chlorosulfonic acid (0.25 mL, 3.7 mmol) was added dropwise at 0 °C to a mixture of anisole **30** (0.201 mL, 1.85 mmol) and chloroform (5 mL). The reaction mixture was warmed to room temperature and stirred over 30 minutes. Water (15 mL) was added dropwise at 0 °C to the reaction mixture. The mixture was extracted into DCM (3×20 mL). The combined organic extracts were washed (brine), dried (MgSO$_4$) and concentrated *in vacuo* to afford a crude residue. Hexamethyleneimine (0.141 mL, 1.26 mmol) was added dropwise at 0 °C to a suspension of sodium hydride (60% in mineral oil, 0.137 g, 3.43 mmol) in DMF (1 mL). The reaction mixture was stirred at 0 °C over 20 minutes. A solution of the crude residue in DMF (2 mL) was added dropwise at 0 °C to the reaction mixture. The reaction mixture was warmed to room temperature and stirred over 1 hour. Water (15 mL) was added dropwise at 0 °C to the reaction mixture. The product was extracted into DCM (3×20 mL). The combined organic extracts were washed (brine), dried (MgSO$_4$) and concentrated *in vacuo*. The residue was dissolved in toluene (5 mL) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 35% ethyl acetate in petroleum ether) afforded **10** (0.243 g, 49% yield). LCMS (ESI+): m/z 270.2 [M + H]$^+$, rt 2.15 minutes, >99%; ^{1}H NMR (400 MHz, CDCl$_3$) 7.75–7.70 (2H, m), 6.99–6.93 (2H, m), 3.86 (3H, s), 3.25 (4H, t, J = 5.9 Hz), 1.75–1.66 (4H, m), 1.62–1.53 (4H, m); ^{13}C NMR (100 MHz, CDCl$_3$) 162.6, 131.5, 129.1,

114.2, 55.7, 48.3, 29.2, 27.1; ν_{max}/cm^{-1} 2929, 2848, 1595, 1579, 1501; HRMS (ESI)+: m/z calculated for $[C_{13}H_{19}NO_3S + H]^+$ = 270.1158, observed 270.1153.

N-(2-Methoxy-5-(N-methylsulfamoyl)phenyl)acetamide (11).

Acetic anhydride (18 μL, 0.19 mmol) was added to a solution of 3-amino-4-methoxy-N-methylbenzenesulfonamide **31a** (42 mg, 0.19 mmol) and pyridine (16 μL, 0.19 mmol) in DCM (2 mL). The reaction mixture was stirred over 2 days. The reaction mixture was diluted with water (15 mL) and extracted into DCM (3 × 20 mL). The combined organic extracts were dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether) afforded **11** (28 mg, 56% yield). LCMS (ESI+): m/z 259.2 [M + H]$^+$, (ESI-): m/z 257.2 [M - H]$^-$, rt 1.31 minutes, >99%; ^{1}H NMR (400 MHz, CD$_3$CN) 8.74 (1H, d, J = 2.0 Hz), 8.30 (1H, br s), 7.51 (1H, dd, J = 8.5, 2.2 Hz), 7.10 (1H, d, J = 8.6 Hz), 5.43–5.27 (1H, m), 3.94 (3H, s), 2.47 (3H, d, J = 5.3 Hz), 2.14 (3H, s); ^{13}C NMR (100 MHz, CD$_3$CN) 170.0, 152.2, 131.6, 129.5, 124.0, 118.7, 111.2, 56.9, 29.5, 24.7; ν_{max}/cm^{-1} 3422 (N-H), 3168, 1672 (C=O), 1594, 1530; HRMS (ESI)+: m/z calculated for $[C_{10}H_{14}N_2O_4S + Na]^+$ = 281.0566, observed 281.0569.

N-(3-(Azepan-1-ylsulfonyl)phenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (12).

T3P$^®$ (50 wt. % in ethyl acetate, 0.35 mL, 0.59 mmol) and *N,N*-diisopropylethylamine (0.17 mL, 0.98 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (47 mg, 0.20 mmol) and 3-(azepan-1-ylsulfonyl)aniline **8** (50 mg, 0.20 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 2 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 90% ethyl acetate in petroleum ether) afforded **12** (40 mg, 45% yield). LCMS (ESI+): m/z 441.3 [M + H]$^+$, (ESI-): m/z 439.2 [M - H]$^-$, rt 1.82 minutes, 98%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.63 (1H, s), 10.67 (1H, s), 8.30–8.25 (1H, m), 8.13 (1H, t, J = 1.8 Hz), 7.98–7.92 (2H, m), 7.86 (1H, ddd, J = 8.1, 5.7, 2.4 Hz), 7.78 (1H, dq, J = 8.2, 1.0 Hz), 7.54 (1H, t, J = 8.0 Hz), 7.44 (1H, dq, J = 7.8, 0.9 Hz), 4.10 (2H, s), 3.18 (4H, t, J = 5.9 Hz), 1.66–1.56 (4H, m), 1.53–1.44 (4H, m); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.1, 159.5, 141.8, 139.7, 139.5, 133.6, 131.6, 130.0, 129.8, 127.6, 125.8, 125.6, 122.6, 121.2, 116.7, 47.7, 28.5, 26.3 (1 peak missing); ν_{max}/cm^{-1} 2929, 2852, 1644 (C=O), 1591, 1541; HRMS (ESI)+: m/z calculated for $[C_{22}H_{24}N_4O_4S + H]^+$ = 441.1591, observed 441.1613.

N-(5-(Azepan-1-ylsulfonyl)-2-methylphenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (13).

T3P$^®$ (50 wt. % in ethyl acetate, 0.33 mL, 0.56 mmol) and *N,N*-diisopropylethylamine (0.16 mL, 0.93 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (44 mg, 0.19 mmol) and 5-(azepan-1-ylsulfonyl)-2-methylaniline **29** (50 mg, 0.19 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 2 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 90% ethyl acetate in petroleum ether, 0 – 5% methanol in DCM) afforded **13** (42 mg, 50% yield). LCMS (ESI+): m/z 455.3 [M + H]$^+$, (ESI-): m/z 453.2

[M - H]$^-$, rt 1.82 minutes, >99%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.64 (1H, s), 9.84 (1H, s), 8.28 (1H, d, J = 7.7 Hz), 8.02–7.92 (3H, m), 7.87 (1H, ddd, J = 8.0, 6.7, 1.5 Hz), 7.49–7.41 (2H, m), 4.15 (2H, s), 3.14 (4H, t, J = 6.0 Hz), 2.33 (3H, s), 1.65–1.53 (4H, m), 1.51–.1.42 (4H, m); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.0, 159.5, 142.0, 136.8, 136.5, 135.8, 133.5, 131.6, 131.3, 129.8, 127.6, 125.9, 125.5, 122.8, 122.1, 47.7, 28.5, 26.3, 18.0 (1 peak missing); ν_{max}/cm^{-1} 3177, 3045, 2915, 2853, 1651 (C=O), 1612, 1600, 1582, 1553; HRMS (ESI)+: m/z calculated for [C$_{23}$H$_{26}$N$_4$O$_4$S + H]$^+$ = 455.1748, observed 455.1769.

N-(5-(Azepane-1-carbonyl)-2-methoxyphenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (14).

T3P$^®$ (50 wt. % in ethyl acetate, 0.12 mL, 0.20 mmol) and *N,N*-diisopropylethylamine (58 µL, 0.33 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (14 mg, 0.067 mmol) and (3-amino-4-methoxyphenyl)(azepan-1-yl)methanone **35** (17 mg, 0.067 mmol) in DMF (1 mL). The reaction mixture was heated to 70 °C over 2 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 10 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 10% methanol in DCM) afforded **14** (13 mg, 45% yield). LCMS (ESI+): m/z 435.3 [M + H]$^+$, (ESI-): m/z 433.3 [M - H]$^-$, rt 1.68 minutes, >99%; ^{1}H NMR (400 MHz, (CD$_3$)$_2$CO) 11.69 (1H, br s), 9.01 (1H, br s), 8.39–8.32 (2H, m), 8.04 (1H, d, J = 8.1 Hz), 7.94 (1H, td, J = 7.7, 1.5 Hz), 7.89–7.82 (1H, m), 7.09 (1H, dd, J = 8.4, 2.0 Hz), 7.04 (1H, d, J = 8.4 Hz), 4.21 (2H, s), 3.91 (3H, s), 3.56 (2H, br s), 3.40 (2H, br s), 1.81–1.47 (8H, m); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 169.9, 167.9, 159.5, 149.7, 142.2, 133.5, 131.5, 129.8, 129.1, 127.6, 126.8, 125.8, 125.5, 122.9, 119.9, 110.8, 55.9, 49.2, 45.5, 28.9, 27.2, 26.8, 25.8 (1 peak missing); ν_{max}/cm^{-1} 3191, 2929, 2856, 1674, 1648, 1618, 1548; HRMS (ESI)+: m/z calculated for [C$_{24}$H$_{26}$N$_4$O$_4$ + H]$^+$ = 435.2027, observed 435.2025.

N-(5-(Azepan-1-ylsulfonyl)-2-methoxyphenyl)-2-(1H-indol-3-yl)acetamide (15a).

T3P$^®$ (50 wt. % in ethyl acetate, 0.15 mL, 0.26 mmol) and *N,N*-diisopropylethylamine (75 µL, 0.43 mmol) were added to a solution of 3-indoleacetic acid (15 mg, 0.086 mmol) and 5-(azepan-1-ylsulfonyl)-2-methoxyaniline **5** (24 mg, 0.086 mmol) in DMF (1 mL). The reaction mixture was heated to 70 °C over 4 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 10 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (40 – 80% ethyl acetate in petroleum ether) afforded **15a** (8 mg, 21% yield). LCMS (ESI+): m/z 442.3 [M + H]$^+$, (ESI-): m/z 440.2 [M - H]$^-$, rt 2.13 minutes, 97%; ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO) 10.98 (1H, s), 9.34 (1H, s), 8.50 (1H, d, J = 2.2 Hz), 7.61 (1H, d, J = 7.9 Hz), 7.45 (1H, dd, J = 8.6, 2.4 Hz), 7.37 (1H, d, J = 8.1 Hz), 7.30 (1H, d, J = 2.3 Hz), 7.18 (1H, d, J = 8.8 Hz), 7.09 (1H, ddd, J = 8.1, 7.0, 1.1 Hz), 6.99 (1H, ddd, J = 7.9, 7.0, 1.0 Hz), 3.87 (2H, s), 3.85 (3H, s), 3.12 (4H, t, J = 5.9 Hz), 1.65–1.52 (4H, m), 1.51–1.38 (4H, m); ^{13}C NMR (100 MHz, (CD$_3$)$_2$SO) 170.4, 151.7, 136.2, 130.4, 127.9, 127.2, 124.3, 123.1, 121.2, 118.7, 118.6, 118.5, 111.5, 111.1, 108.2, 56.3, 47.7, 33.5, 28.5, 26.4; ν_{max}/cm^{-1} 3345 (br, N-H), 2928, 2856, 1673 (C=O), 1594, 1524; HRMS (ESI)+: m/z calculated for [C$_{23}$H$_{27}$N$_3$O$_4$S + Na]$^+$ = 464.1614, observed 464.1615.

N-(5-(Azepan-1-ylsulfonyl)-2-methoxyphenyl)-2-(5-methoxy-1H-indol-3-yl)acetamide (15b).

T3P® (50 wt. % in ethyl acetate, 0.31 mL, 0.53 mmol) and *N,N*-diisopropylethylamine (0.15 mL, 0.88 mmol) were added to a solution of 5-methoxy-3-indoleacetic acid (36 mg, 0.18 mmol) and 5-(azepan-1-ylsulfonyl)-2-methoxyaniline **5** (50 mg, 0.18 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 2 hours. The reaction mixture was diluted with ethyl acetate (100 mL), washed with water (3 × 100 mL) and brine (100 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (30 – 70% ethyl acetate in petroleum ether) afforded **15b** (32 mg, 38% yield). LCMS (ESI+): m/z 472.3 [M + H]$^+$, (ESI-): m/z 470.2 [M - H]$^-$, rt 2.09 minutes, 98%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 10.81 (1H, d, J = 1.6 Hz), 9.29 (1H, s), 8.51 (1H, d, J = 2.1 Hz), 7.45 (1H, dd, J = 8.7, 2.3 Hz), 7.29–7.24 (2H, m), 7.17 (1H, d, J = 8.8 Hz), 7.13 (1H, d, J = 2.4 Hz), 6.74 (1H, dd, J = 8.7, 2.4 Hz), 3.84 (3H, s), 3.83 (2H, s), 3.74 (3H, s), 3.13 (4H, t, J = 6.0 Hz), 1.64–1.54 (4H, m), 1.51–1.42 (4H, m); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 170.4, 153.2, 151.6, 131.3, 130.4, 127.8, 127.4, 124.9, 123.0, 118.5, 112.1, 111.2, 111.0, 107.9, 100.5, 56.2, 55.3, 47.7, 33.6, 28.4, 26.3; ν_{max}/cm^{-1} 3350 (br, N-H), 2933, 1719, 1675 (C=O), 1593, 1522; HRMS (ESI)+: m/z calculated for [C$_{24}$H$_{29}$N$_3$O$_5$S + H]$^+$ = 472.1901, observed 472.1890.

N-(5-(Azepan-1-ylsulfonyl)-2-methoxyphenyl)-3-(1H-indol-3-yl)propanamide (15c).

T3P® (50 wt. % in ethyl acetate, 0.25 mL, 0.42 mmol) and *N,N*-diisopropylethylamine (0.12 mL, 0.70 mmol) were added to a solution of 3-indolepropionic acid (27 mg, 0.14 mmol) and 5-(azepan-1-ylsulfonyl)-2-methoxyaniline **5** (40 mg, 0.14 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 1 hour. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (30 – 70% ethyl acetate in petroleum ether) afforded **15c** (26 mg, 41% yield). LCMS (ESI+): m/z 456.3 [M + H]$^+$, (ESI-): m/z 454.3 [M - H], rt 2.17 minutes, >99%; ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO) 10.78 (1H, s), 9.37 (1H, s), 8.51 (1H, d, J = 1.9 Hz), 7.57 (1H, d, J = 7.8 Hz), 7.47 (1H, dd, J = 8.7, 2.4 Hz), 7.33 (1H, d, J = 8.0 Hz), 7.19 (1H, d, J = 8.7 Hz), 7.14 (1H, d, J = 2.0 Hz), 7.06 (1H, t, J = 7.5 Hz), 6.97 (1H, t, J = 7.4 Hz), 3.89 (3H, s), 3.16 (4H, t, J = 5.9 Hz), 3.01 (2H, t, J = 7.5 Hz), 2.82 (2H, t, J = 7.5 Hz), 1.70–1.56 (4H, m), 1.55–1.41 (4H, m); ^{13}C NMR (100 MHz, (CD$_3$)$_2$SO) 171.8, 152.0, 136.2, 130.3, 127.8, 127.0, 123.1, 122.3, 120.9, 119.4, 118.5, 118.2, 113.6, 111.3, 111.0, 56.2, 47.7, 36.8, 28.5, 26.4, 20.7; ν_{max}/cm^{-1} 3360 (br, N-H), 2927, 2856, 1674 (C=O), 1594, 1524; HRMS (ESI)+: m/z calculated for [C$_{24}$H$_{29}$N$_3$O$_4$S + H]$^+$ = 456.1952, observed 456.1970.

N-(5-(Azepan-1-ylsulfonyl)-2-methoxyphenyl)-2-(benzofuran-3-yl)acetamide (15d).

T3P® (50 wt. % in ethyl acetate, 0.31 mL, 0.53 mmol) and *N,N*-diisopropylethylamine (0.15 mL, 0.88 mmol) were added to a solution of benzo[b]furan-3-ylacetic acid (31 mg, 0.18 mmol) and 5-(azepan-1-ylsulfonyl)-2-methoxyaniline **5** (50 mg, 0.18 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 90 minutes. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (30 – 60% ethyl acetate in petroleum ether) afforded **15d** (50 mg, 64% yield). LCMS (ESI+):

m/z 443.3 [M + H]$^+$, (ESI-): m/z 441.3 [M - H]$^-$, rt 2.24 minutes, >99%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 9.70 (1H, s), 8.46 (1H, d, J = 2.0 Hz), 7.92 (1H, s), 7.69 (1H, d, J = 7.6 Hz), 7.57 (1H, d, J = 8.2 Hz), 7.49 (1H, dd, J = 8.5, 2.3 Hz), 7.32 (1H, td, J = 7.7, 1.1 Hz), 7.27 (1H, t, J = 7.5 Hz), 7.22 (1H, d, J = 8.7 Hz), 3.94 (3H, s), 3.92 (2H, s), 3.13 (4H, t, J = 5.9 Hz), 1.64–1.53 (4H, m), 1.51–1.42 (4H, m); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 169.0, 154.5, 152.1, 143.5, 130.4, 127.8, 127.6, 124.4, 123.4, 122.6, 120.2, 119.3, 114.6, 111.3, 111.2, 56.3, 47.6, 31.2, 28.4, 26.3; ν_{max}/cm^{-1} 3353, 2929, 2855, 1680 (C=O), 1593, 1523; HRMS (ESI)+: m/z calculated for [C$_{23}$H$_{26}$N$_2$O$_5$S + H]$^+$ = 443.1635, observed 443.1652.

N-(5-(Azepan-1-ylsulfonyl)-2-methoxyphenyl)-2-(benzo[d]isoxazol-3-yl)acetamide (15e).

T3P® (50 wt. % in ethyl acetate, 81 μL, 0.14 mmol) and *N,N*-diisopropylethylamine (39 μL, 0.23 mmol) were added to a solution of 2-(1,2-benzisoxazol-3-yl)acetic acid (8 mg, 0.05 mmol) and 5-(azepan-1-ylsulfonyl)-2-methoxyaniline **5** (13 mg, 0.045 mmol) in DMF (1 mL). The reaction mixture was heated to 70 °C over 3 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 15 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (30 – 70% ethyl acetate in petroleum ether) followed by reverse phase column chromatography (0 – 100% acetonitrile in water (+ 0.1% NH$_3$)) afforded **15e** (11 mg, 55% yield). LCMS (ESI+): m/z 444.3 [M + H]$^+$, (ESI-): m/z 442.2 [M - H]$^-$, rt 2.15 minutes, >99%; ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO) 10.03 (1H, s), 8.44 (1H, d, J = 1.9 Hz), 7.91 (1H, d, J = 8.0 Hz), 7.75 (1H, d, J = 8.5 Hz), 7.66 (1H, t, J = 7.7 Hz), 7.52 (1H, dd, J = 8.6, 2.2 Hz), 7.41 (1H, t, J = 7.4 Hz), 7.25 (1H, d, J = 8.7 Hz), 4.34 (2H, s), 3.97 (3H, s), 3.13 (4H, t, J = 5.9 Hz), 1.67–1.52 (4H, m), 1.51–1.39 (4H, m); ^{13}C NMR (100 MHz, (CD$_3$)$_2$SO) 166.8, 162.4, 154.2, 152.2, 130.4, 127.4, 123.7, 122.6, 121.5, 119.5, 111.3, 109.7, 56.3, 47.6, 32.8, 28.5, 26.3 (2 peaks missing); ν_{max}/cm^{-1} 2923, 2856, 1689 (C=O), 1595, 1527; HRMS (ESI)+: m/z calculated for [C$_{22}$H$_{25}$N$_3$O$_5$S + H]$^+$ = 444.1588, observed 444.1592.

N-(5-(Azepan-1-ylsulfonyl)-2-methoxyphenyl)-2-(1H-benzo[d]imidazol-1-yl)acetamide (15f).

T3P® (50 wt. % in ethyl acetate, 0.25 mL, 0.42 mmol) and *N,N*-diisopropylethylamine (0.12 mL, 0.70 mmol) were added to a solution of 2-(1H-benzimidazol-1-yl)acetic acid (25 mg, 0.14 mmol) and 5-(azepan-1-ylsulfonyl)-2-methoxyaniline **5** (40 mg, 0.14 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 1 hour. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 5% methanol in DCM) afforded **15f** (33 mg, 53% yield). LCMS (ESI+): m/z 443.3 [M + H]$^+$, (ESI-): m/z 441.2 [M - H]$^-$, rt 1.62 minutes, >99%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 10.00 (1H, s), 8.45 (1H, d, J = 2.2 Hz), 8.23 (1H, s), 7.70–7.65 (1H, m), 7.54 (1H, d, J = 7.8 Hz), 7.51 (1H, dd, J = 8.7, 2.3 Hz), 7.29–7.19 (3H, m), 5.31 (2H, s), 3.98 (3H, s), 3.11 (4H, t, J = 5.9 Hz), 1.62–1.51 (4H, m), 1.49–1.40 (4H, m); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 166.5, 152.0, 145.0, 143.2, 134.4, 130.4, 127.3, 123.7, 122.4, 121.6, 119.4, 119.1, 111.3, 110.3, 56.4, 47.6, 47.3, 28.4, 26.3; ν_{max}/cm^{-1} 2930, 2853, 1694 (C=O), 1596, 1533; HRMS (ESI)+: m/z calculated for [C$_{22}$H$_{26}$N$_4$O$_4$S + H]$^+$ = 443.1748, observed 443.1769.

N-(5-(Azepan-1-ylsulfonyl)-2-methoxyphenyl)-2-(quinolin-4-yl)acetamide (15g).

Lithium diisopropylamide (2 M in THF/heptane/ethylbenzene, 1.5 mL, 3.0 mmol) was added dropwise over 30 minutes at −78 °C to a mixture of 4-methylquinoline **36** (0.33 mL, 2.5 mmol) and THF (5 mL). The reaction mixture was stirred at −78 °C over 1 hour, then solid CO_2 pellets (1 g) were added. The reaction mixture was stirred at −78 °C over 10 minutes, then warmed to room temperature and stirred over 1 hour. Water (20 mL) was added dropwise at 0 °C, followed by aqueous NaOH (10% w/v, 5 mL). The mixture was washed with DCM (3 × 25 mL), then adjusted to pH 6 and washed with DCM/methanol (9:1, 3 × 50 mL). The aqueous phase was concentrated *in vacuo* to afford a crude residue. 5-(azepan-1-ylsulfonyl)-2-methoxyaniline **5** (0.140 g, 0.492 mmol) and DMF (10 mL) were added to the crude residue, followed by T3P® (50 wt. % in ethyl acetate, 3.5 mL, 5.9 mmol) and *N,N*-diisopropylethylamine (1.7 mL, 9.9 mmol). The reaction mixture was heated to 70 °C over 40 minutes. The reaction mixture was diluted with ethyl acetate (100 mL), washed with water (3 × 100 mL) and brine (100 mL), dried (MgSO₄) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 10% methanol in DCM) followed by reverse phase column chromatography (0 – 50% acetonitrile in water (+ 0.1% NH₃)) afforded **15g** (11 mg, 5% yield). LCMS (ESI+): m/z 454.3 [M + H]⁺, (ESI-): m/z 452.3 [M - H]⁻, rt 1.70 minutes, >99%; ¹H NMR (400 MHz, (CD₃)₂SO) 9.90 (1H, s), 8.85 (1H, d, J = 4.3 Hz), 8.42 (1H, d, J = 2.2 Hz), 8.19 (1H, d, J = 8.4 Hz), 8.05 (1H, dd, J = 8.4, 0.7 Hz), 7.77 (1H, ddd, J = 8.4, 6.9, 1.4 Hz), 7.65 (1H, ddd, J = 8.4, 6.9, 1.3 Hz), 7.53–7.46 (2H, m), 7.24 (1H, d, J = 8.8 Hz), 4.39 (2H, s), 3.97 (3H, s), 3.11 (4H, t, J = 5.9 Hz), 1.63–1.51 (4H, m), 1.50–1.39 (4H, m); ¹³C NMR (125 MHz, CDCl₃) 167.1, 150.6, 150.5, 148.8, 140.2, 132.2, 130.6, 130.0, 127.63, 127.58, 127.4, 124.0, 123.7, 122.8, 118.1, 109.6, 56.2, 48.5, 42.1, 29.3, 27.0; ν$_{max}$/cm⁻¹ 3295, 2923, 1663 (C=O), 1593, 1537; HRMS (ESI)+: m/z calculated for $[C_{24}H_{27}N_3O_4S + H]^+$ = 454.1795, observed 454.1781.

N-(5-(Azepan-1-ylsulfonyl)-2-methoxyphenyl)-2-(naphthalen-1-yl)acetamide (15h).

T3P® (50 wt. % in ethyl acetate, 0.31 mL, 0.53 mmol) and *N,N*-diisopropylethylamine (0.15 mL, 0.88 mmol) were added to a solution of 1-naphthaleneacetic acid (33 mg, 0.18 mmol) and 5-(azepan-1-ylsulfonyl)-2-methoxyaniline **5** (50 mg, 0.18 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 2 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO₄) and concentrated *in vacuo*. Purification by flash column chromatography (0 – 50% ethyl acetate in petroleum ether) afforded **15h** (53 mg, 67% yield). LCMS (ESI+): m/z 453.3 [M + H]⁺, (ESI-): m/z 451.3 [M - H]⁻, rt 2.37 minutes, >99%; ¹H NMR (400 MHz, (CD₃)₂SO) 9.69 (1H, s), 8.44 (1H, d, J = 2.3 Hz), 8.12 (1H, d, J = 7.9 Hz), 7.98–7.91 (1H, m), 7.86 (1H, dd, J = 7.2, 2.2 Hz), 7.59–7.44 (5H, m), 7.22 (1H, d, J = 8.8 Hz), 4.30 (2H, s), 3.94 (3H, s), 3.11 (4H, t, J = 5.9 Hz), 1.62–1.51 (4H, m), 1.49–1.40 (4H, m); ¹³C NMR (125 MHz, CDCl₃) 169.0, 150.6, 134.2, 132.2, 132.1, 130.6, 129.0, 128.9, 128.6, 127.9, 127.1, 126.4, 125.8, 123.9, 123.6, 117.9, 109.4, 56.0, 48.5, 43.1, 29.4, 27.0; ν$_{max}$/cm⁻¹ 3360, 2925, 2862, 1675 (C=O), 1592, 1519; HRMS (ESI)+: m/z calculated for $[C_{25}H_{28}N_2O_4S + Na]^+$ = 475.1662, observed 475.1652.

N-(5-(Azepan-1-ylsulfonyl)-2-methoxyphenyl)-2-(pyridin-4-yl)acetamide (15i).

EDC.HCl (83 mg, 0.43 mmol), *N,N*-diisopropylethylamine (0.15 mL, 0.86 mmol) and DMAP (5 mg, 0.04 mmol) were added to a solution of 4-pyridylacetic acid hydrochloride (50 mg, 0.29 mmol) and 5-(azepan-1-ylsulfonyl)-2-methoxyaniline **5** (98 mg, 0.35 mmol) in DCM (2 mL). The reaction mixture was stirred over 90 minutes, then diluted with water (25 mL) and extracted into DCM (3×25 mL). The combined organic extracts were washed (brine), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 5% methanol in DCM) afforded **15i** (74 mg, 64% yield). LCMS (ESI+): m/z 404.3 [M + H]$^+$, (ESI-): m/z 402.2 [M - H]$^-$, rt 1.54 minutes, >99%; ^{1}H NMR (400 MHz, (CD$_3$)$_2$SO) 9.73 (1H, s), 8.54–8.48 (2H, m), 8.45 (1H, d, J = 2.2 Hz), 7.49 (1H, dd, J = 8.6, 2.3 Hz), 7.38–7.31 (2H, m), 7.23 (1H, d, J = 8.8 Hz), 3.94 (3H, s), 3.86 (2H, s), 3.14 (4H, t, J = 5.9 Hz), 1.66–1.54 (4H, m), 1.52–1.41 (4H, m); ^{13}C NMR (125 MHz, CDCl$_3$) 167.2, 150.6, 150.5, 143.1, 132.3, 127.6, 124.7, 124.0, 118.2, 109.6, 56.3, 48.5, 44.2, 29.3, 27.0; ν_{max}/cm^{-1} 3300, 2931, 2858, 1658 (C=O), 1596, 1536; HRMS (ESI)+: m/z calculated for [C$_{20}$H$_{25}$N$_3$O$_4$S + Na]$^+$ = 426.1458, observed 426.1447.

N-(2-Methoxy-5-(N-methylsulfamoyl)phenyl)-2-(4-oxo-3,4-dihydrophthalazin-1yl)acetamide (16a).

T3P$^®$ (50 wt. % in ethyl acetate, 93 μL, 0.16 mmol) and *N,N*-diisopropylethylamine (46 μL, 0.26 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (11 mg, 0.052 mmol) and 3-amino-4-methoxy-N-methylbenzenesulfonamide **31a** (11 mg, 0.052 mmol) in DMF (1 mL). The reaction mixture was heated to 70 °C over 5 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3×15 mL) and brine (15 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 10% methanol in DCM) afforded **16a** (6 mg, 29% yield). LCMS (ESI+): m/z 403.2 [M + H]$^+$, (ESI-): m/z 401.1 [M - H]$^-$, rt 1.44 minutes, >99%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.61 (1H, s), 9.83 (1H, s), 8.47 (1H, d, J = 2.2 Hz), 8.27 (1H, d, J = 7.5 Hz), 8.00–7.92 (2H, m), 7.86 (1H, ddd, J = 8.0, 6.3, 1.9 Hz), 7.50 (1H, dd, J = 8.7, 2.3 Hz), 7.31–7.22 (2H, m), 4.21 (2H, s), 3.96 (3H, s), 2.35 (3H, d, J = 5.0 Hz); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.2, 159.5, 152.0, 142.2, 133.5, 131.6, 130.7, 129.8, 127.6, 127.5, 125.8, 125.6, 123.5, 119.5, 111.1, 56.3, 28.6 (1 peak missing); ν_{max}/cm^{-1} 3277, 3173, 3016, 2904, 1660 (C=O), 1594, 1537; HRMS (ESI)+: m/z calculated for [C$_{18}$H$_{18}$N$_4$O$_5$S + H]$^+$ = 403.1071, observed 403.1064.

N-(5-(Azocan-1-ylsulfonyl)-2-methoxyphenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16b).

T3P$^®$ (50 wt. % in ethyl acetate, 0.24 mL, 0.40 mmol) and *N,N*-diisopropylethylamine (0.12 mL, 0.67 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (32 mg, 0.13 mmol) and 5-(azocan-1-ylsulfonyl)-2-methoxyaniline **31b** (40 mg, 0.13 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 2 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3×25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 5% methanol in DCM)

afforded **16b** (24 mg, 37% yield). LCMS (ESI+): m/z 485.3 [M + H]$^+$, (ESI-): m/z 483.2 [M - H]$^-$, rt 1.94 minutes, >99%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.62 (1H, s), 9.86 (1H, s), 8.45 (1H, d, J = 2.2 Hz), 8.30–8.25 (1H, m), 7.98–7.92 (2H, m), 7.89–7.83 (1H, m), 7.48 (1H, dd, J = 8.6, 2.3 Hz), 7.24 (1H, d, J = 8.8 Hz), 4.21 (2H, s), 3.97 (3H, s), 3.01 (4H, t, J = 5.8 Hz), 1.67–1.44 (10H, m); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.4, 159.5, 152.1, 142.1, 133.5, 131.6, 129.8, 129.5, 127.7, 127.6, 125.9, 125.5, 123.5, 119.2, 111.2, 56.3, 48.0, 27.2, 26.2, 24.6 (1 peak missing); ν_{max}/cm^{-1} 3307, 2923, 1693, 1644 (C=O), 1596, 1530; HRMS (ESI)+: m/z calculated for [C$_{24}$H$_{28}$N$_4$O$_5$S + H]$^+$ = 485.1853, observed 485.1848.

N-(2-Methoxy-5-(piperidin-1-ylsulfonyl)phenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16c).

T3P$^®$ (50 wt. % in ethyl acetate, 0.14 mL, 0.23 mmol) and *N,N*-diisopropylethylamine (66 μL, 0.38 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (16 mg, 0.076 mmol) and 2-methoxy-5-(piperidin-1-ylsulfonyl)aniline **31c** (21 mg, 0.076 mmol) in DMF (1 mL). The reaction mixture was heated to 70 °C over 2 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 10 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 10% methanol in DCM) afforded **16c** (14 mg, 40% yield). LCMS (ESI+): m/z 457.3 [M + H]$^+$, (ESI-): m/z 455.2 [M - H]$^-$, rt 1.76 minutes, 98%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.62 (1H, s), 9.88 (1H, s), 8.39 (1H, d, J = 2.2 Hz), 8.28 (1H, d, J = 7.9 Hz), 7.99–7.92 (2H, m), 7.86 (1H, ddd, J = 8.0, 5.7, 2.5 Hz), 7.45 (1H, dd, J = 8.6, 2.3 Hz), 7.28 (1H, d, J = 8.7 Hz), 4.22 (2H, s), 3.98 (3H, s), 2.81 (4H, t, J = 5.2 Hz), 1.50 (4H, quin, J = 5.7 Hz), 1.39–1.28 (2H, m); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.4, 159.5, 152.4, 142.1, 133.5, 131.6, 129.8, 127.59, 127.56, 126.7, 125.9, 125.5, 124.3, 119.9, 111.2, 56.4, 46.5, 24.6, 22.8 (1 peak missing); ν_{max}/cm^{-1} 3176, 3057, 2923, 1674, 1646 (C=O), 1594, 1542; HRMS (ESI)+: m/z calculated for [C$_{22}$H$_{24}$N$_4$O$_5$S + H]$^+$ = 457.1540, observed 457.1537.

N-(2-Methoxy-5-((4-methylpiperazin-1-yl)sulfonyl)phenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16d).

T3P$^®$ (50 wt. % in ethyl acetate, 0.24 mL, 0.41 mmol) and *N,N*-diisopropylethylamine (0.12 mL, 0.68 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (32 mg, 0.14 mmol) and 2-methoxy-5-((4-methylpiperazin-1-yl)sulfonyl)aniline **31d** (40 mg, 0.14 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 2 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 10% methanol in DCM) followed by reverse phase column chromatography (0 – 30% acetonitrile in water (+ 0.1% NH$_3$)) afforded **16d** (10 mg, 16% yield). LCMS (ESI+): m/z 472.3 [M + H]$^+$, (ESI-): m/z 470.2 [M - H]$^-$, rt 1.23 minutes, >99%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.62 (1H, s), 9.91 (1H, s), 8.40 (1H, d, J = 2.2 Hz), 8.30–8.25 (1H, m), 7.99–7.92 (2H, m), 7.86 (1H, ddd, J = 8.0, 5.8, 2.4 Hz), 7.46 (1H, dd, J = 8.6, 2.3 Hz), 7.30 (1H, d, J = 8.7 Hz), 4.22 (2H, s), 3.99 (3H, s), 2.81 (4H, br s), 2.38–2.28 (4H, m), 2.10 (3H, s); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.4, 159.4, 152.6, 142.1, 133.5, 131.6, 129.8, 127.62, 127.56, 126.0, 125.8, 125.5, 124.5, 120.0, 111.3, 56.4, 53.5, 45.7, 45.2 (1 peak missing); ν_{max}/cm^{-1} 3376, 2922, 1684, 1655

(C=O), 1594, 1533; HRMS (ESI)+: m/z calculated for $[C_{22}H_{25}N_5O_5S + H]^+$ = 472.1649, observed 472.1662.

N-(2-Methoxy-5-(morpholinosulfonyl)phenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16e).

T3P® (50 wt. % in ethyl acetate, 0.26 mL, 0.44 mmol) and *N,N*-diisopropylethylamine (0.13 mL, 0.73 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (35 mg, 0.15 mmol) and 2-methoxy-5-(morpholinosulfonyl)aniline **31e** (40 mg, 0.15 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 2 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO₄) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 10% methanol in DCM) followed by reverse phase column chromatography (0 – 60% acetonitrile in water (+ 0.1% NH₃)) afforded **16e** (7 mg, 10% yield). LCMS (ESI+): m/z 459.3 $[M + H]^+$, (ESI-): m/z 457.3 $[M - H]^-$, rt 1.55 minutes, >99%; ^{1}H NMR (400 MHz, (CD₃)₂SO) 12.62 (1H, s), 9.93 (1H, s), 8.41 (1H, d, J = 2.0 Hz), 8.27 (1H, d, J = 7.8 Hz), 8.01–7.91 (2H, m), 7.86 (1H, ddd, J = 8.0, 5.7, 2.5 Hz), 7.47 (1H, dd, J = 8.7, 2.2 Hz), 7.31 (1H, d, J = 8.7 Hz), 4.23 (2H, s), 3.99 (3H, s), 3.59 (4H, t, J = 4.5 Hz), 2.79 (4H, t, J = 4.4 Hz); ^{13}C NMR (100 MHz, (CD₃)₂SO) 168.5, 159.5, 152.7, 142.1, 133.6, 131.6, 129.8, 127.7, 127.6, 125.9, 125.6, 125.5, 124.6, 120.1, 111.4, 65.2, 56.4, 45.9 (1 peak missing); ν_{max}/cm^{-1} 3175, 3027, 2924, 2854, 1672, 1646 (C=O), 1593, 1538; HRMS (ESI)+: m/z calculated for $[C_{21}H_{22}N_4O_6S + Na]^+$ = 481.1152, observed 481.1143.

N-(2-Methoxy-5-(thiomorpholinosulfonyl)phenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16f).

T3P® (50 wt. % in ethyl acetate, 0.24 mL, 0.40 mmol) and *N,N*-diisopropylethylamine (0.12 mL, 0.67 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (32 mg, 0.13 mmol) and 2-methoxy-5-(thiomorpholinosulfonyl)aniline **31f** (40 mg, 0.13 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 2 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO₄) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 10% methanol in DCM) afforded **16f** (23 mg, 36% yield). LCMS (ESI+): m/z 475.2 $[M + H]^+$, (ESI-): m/z 473.2 $[M - H]^-$, rt 1.72 minutes, >99%; ^{1}H NMR (500 MHz, (CD₃)₂SO) 12.62 (1H, s), 9.92 (1H, s), 8.41 (1H, d, J = 2.3 Hz), 8.27 (1H, d, J = 7.9 Hz), 8.00–7.92 (2H, m), 7.86 (1H, ddd, J = 8.0, 5.8, 2.3 Hz), 7.47 (1H, dd, J = 8.7, 2.3 Hz), 7.29 (1H, d, J = 8.7 Hz), 4.23 (2H, s), 3.99 (3H, s), 3.13 (4H, t, J = 4.7 Hz), 2.63 (4H, t, J = 5.1 Hz); ^{13}C NMR (125 MHz, (CD₃)₂SO) 168.4, 159.5, 152.6, 142.1, 133.6, 131.6, 129.8, 127.8, 127.6, 127.2, 125.9, 125.6, 124.2, 119.6, 111.4, 56.4, 47.8, 26.3 (1 peak missing); ν_{max}/cm^{-1} 3391, 2929, 2849, 1674, 1645, 1607, 1516; HRMS (ESI)+: m/z calculated for $[C_{21}H_{22}N_4O_5S_2 + H]^+$ = 475.1104, observed 475.1104.

N-(5-((1,1-Dioxidothiomorpholino)sulfonyl)-2-methoxyphenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16g).

T3P® (50 wt. % in ethyl acetate, 0.67 mL, 1.1 mmol) and *N,N*-diisopropylethylamine (0.33 mL, 1.9 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (89 mg, 0.38 mmol) and 4-((3-amino-4-methoxyphenyl)sulfonyl)thiomorpholine 1,1-dioxide **31g** (0.120 g, 0.375 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 1 hour. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 20% methanol in DCM) followed by reverse phase column chromatography (0 – 60% acetonitrile in water (+ 0.1% NH$_3$)) afforded **16g** (55 mg, 28% yield). LCMS (ESI+): m/z 507.2 [M + H]$^+$, (ESI-): m/z 505.2 [M - H]$^-$, rt 1.61 minutes, 97%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.62 (1H, s), 9.96 (1H, s), 8.47 (1H, s), 8.27 (1H, d, J = 7.8 Hz), 8.02–7.82 (3H, m), 7.55 (1H, d, J = 8.8 Hz), 7.32 (1H, d, J = 8.7 Hz), 4.23 (2H, s), 4.00 (3H, s), 3.27–3.19 (4H, m) (1 peak suspected to be obscured by H$_2$O signal); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.6, 159.5, 152.9, 142.1, 133.6, 131.6, 129.8, 128.0, 127.6, 126.8, 125.9, 125.6, 124.2, 119.5, 111.7, 56.5, 49.9, 45.1 (1 peak missing); ν_{max}/cm^{-1} 3403, 3001, 2933, 1663 (C=O), 1597, 1525; HRMS (ESI)-: m/z calculated for [C$_{21}$H$_{22}$N$_4$O$_7$S$_2$ - H]$^-$ = 505.0857, observed 505.0851.

N-(2-Methoxy-5-(N-phenylsulfamoyl)phenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16h).

T3P® (50 wt. % in ethyl acetate, 0.16 mL, 0.27 mmol) and *N,N*-diisopropylethylamine (79 µL, 0.45 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (19 mg, 0.090 mmol) and 3-amino-4-methoxy-N-phenylbenzenesulfonamide **31h** (25 mg, 0.090 mmol) in DMF (1 mL). The reaction mixture was heated to 70 °C over 2 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 10 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 10% methanol in DCM) afforded **16h** (14 mg, 33% yield). LCMS (ESI+): m/z 465.2 [M + H]$^+$, (ESI-): m/z 463.2 [M - H]$^-$, rt 1.71 minutes, 98%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.61 (1H, s), 10.17 (1H, s), 9.78 (1H, s), 8.55 (1H, d, J = 2.2 Hz), 8.31–8.25 (1H, m), 7.98–7.91 (2H, m), 7.90–7.84 (1H, m), 7.47 (1H, dd, J = 8.7, 2.4 Hz), 7.22–7.14 (3H, m), 7.07–7.03 (2H, m), 7.00–6.94 (1H, m), 4.20 (2H, s), 3.91 (3H, s); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.2, 159.4, 152.2, 142.1, 137.9, 133.5, 131.6, 131.0, 129.8, 129.1, 127.6, 127.5, 125.8, 125.6, 123.7, 123.6, 119.6, 119.3, 111.0, 56.3 (1 peak missing); ν_{max}/cm^{-1} 3175, 3016, 1674, 1647 (C=O), 1592, 1538; HRMS (ESI)+: m/z calculated for [C$_{23}$H$_{20}$N$_4$O$_5$S + H]$^+$ = 465.1227, observed 465.1223.

N-(5-(N-Benzylsulfamoyl)-2-methoxyphenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16i).

T3P® (50 wt. % in ethyl acetate, 0.26 mL, 0.43 mmol) and *N,N*-diisopropylethylamine (0.12 mL, 0.71 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (30 mg, 0.14 mmol) and 3-amino-N-benzyl-4-methoxybenzenesulfonamide **31i** (45 mg, 0.14 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 2 hours. The

reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 8% methanol in DCM) afforded **16i** (25 mg, 37% yield). LCMS (ESI+): m/z 479.3 [M + H]$^+$, (ESI-): m/z 477.2 [M - H]$^-$, rt 1.74 minutes, >99%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.62 (1H, s), 9.81 (1H, s), 8.51 (1H, d, J = 2.2 Hz), 8.27 (1H, d, J = 7.8 Hz), 8.03–7.92 (3H, m), 7.86 (1H, ddd, J = 8.0, 6.6, 1.6 Hz), 7.53 (1H, dd, J = 8.6, 2.3 Hz), 7.28–7.17 (6H, m), 4.22 (2H, s), 3.96 (3H, s), 3.89 (2H, d, J = 6.5 Hz); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.1, 159.5, 152.0, 142.2, 137.7, 133.5, 132.1, 131.6, 129.8, 128.2, 127.6, 127.53, 127.49, 127.1, 125.8, 125.6, 123.4, 119.4, 111.1, 56.3, 46.1 (1 peak missing); ν_{max}/cm^{-1} 3287, 3176, 3026, 1672, 1647 (C=O), 1593, 1538; HRMS (ESI)+: m/z calculated for [C$_{24}$H$_{22}$N$_4$O$_5$S + H]$^+$ = 479.1384, observed 479.1381.

N-(5-((3,4-Dihydroisoquinolin-2(1H)-yl)sulfonyl)-2-methoxyphenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16j).

T3P® (50 wt. % in ethyl acetate, 0.28 mL, 0.47 mmol) and *N,N*-diisopropylethylamine (0.14 mL, 0.79 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (37 mg, 0.16 mmol) and 5-((3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-2-methoxyaniline **31j** (50 mg, 0.16 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 1 hour. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 5% methanol in DCM) afforded **16j** (35 mg, 43% yield). LCMS (ESI-): m/z 503.3 [M - H]$^-$, rt 1.90 minutes, 98%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.62 (1H, s), 9.89 (1H, s), 8.50 (1H, d, J = 2.2 Hz), 8.28 (1H, d, J = 7.9 Hz), 7.99–7.92 (2H, m), 7.86 (1H, ddd, J = 8.0, 5.9, 2.3 Hz), 7.56 (1H, dd, J = 8.6, 2.3 Hz), 7.28 (1H, d, J = 8.7 Hz), 7.17–7.05 (4H, m), 4.22 (2H, s), 4.11 (2H, s), 3.97 (3H, s), 3.22 (2H, t, J = 6.0 Hz), 2.83 (2H, t, J = 5.8 Hz); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.4, 159.5, 152.5, 142.1, 133.5, 133.0, 131.6, 131.5, 129.8, 128.6, 127.7, 127.6, 126.9, 126.6, 126.4, 126.1, 125.8, 125.6, 124.4, 119.9, 111.4, 56.4, 47.2, 43.6, 28.0 (1 peak missing); ν_{max}/cm^{-1} 3354, 2929, 2853, 1679 (C=O), 1592, 1523; HRMS (ESI)+: m/z calculated for [C$_{26}$H$_{24}$N$_4$O$_5$S + H]$^+$ = 505.1540, observed 505.1534.

N-(5-((6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-2-methoxyphenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16k).

T3P® (50 wt. % in ethyl acetate, 0.38 mL, 0.63 mmol) and *N,N*-diisopropylethylamine (0.18 mL, 1.1 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (50 mg, 0.21 mmol) and 5-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)sulfonyl)-2-methoxyaniline **31k** (80 mg, 0.21 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 1 hour. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 7% methanol in DCM) followed by reverse phase column chromatography (0 – 50% acetonitrile in water (+ 0.1% NH$_3$)) afforded **16k** (48 mg, 40% yield). LCMS (ESI+): m/z 565.4 [M + H]$^+$, (ESI-): m/z 563.2 [M - H]$^-$, rt 1.78 minutes, >99%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.62 (1H, s), 9.89 (1H, s), 8.50 (1H, d, J = 2.2 Hz), 8.28 (1H, d, J = 7.7 Hz),

7.99–7.92 (2H, m), 7.86 (1H, ddd, J = 8.0, 6.0, 2.1 Hz), 7.54 (1H, dd, J = 8.6, 2.3 Hz), 7.28 (1H, d, J = 8.9 Hz), 6.71 (1H, s), 6.65 (1H, s), 4.22 (2H, s), 4.01 (2H, s), 3.97 (3H, s), 3.67 (3H, s), 3.65 (3H, s), 3.18 (2H, t, J = 5.9 Hz), 2.74 (2H, t, J = 5.8 Hz); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.4, 159.5, 152.5, 147.5, 147.3, 142.1, 133.5, 131.6, 129.8, 127.7, 127.6, 127.0, 125.8, 125.6, 124.7, 124.4, 123.1, 119.8, 111.7, 111.3, 109.8, 56.4, 55.5, 55.4, 46.9, 43.7, 27.7 (1 peak missing); ν_{max}/cm^{-1} 3299, 3010, 2938, 2837, 1695, 1641 (C=O), 1596, 1520; HRMS (ESI)+: m/z calculated for [C$_{28}$H$_{28}$N$_4$O$_7$S + Na]$^+$ = 587.1571, observed 587.1543.

N-(2-Methoxy-5-((1,2,4,5-tetrahydro-3H-benzo[d]azepin-3-yl)sulfonyl)phenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16l).

T3P$^®$ (50 wt. % in ethyl acetate, 0.38 mL, 0.63 mmol) and *N,N*-diisopropylethylamine (0.18 mL, 1.1 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (50 mg, 0.21 mmol) and 2-methoxy-5-((1,2,4,5-tetrahydro-3H-benzo[d]azepin-3-yl)sulfonyl)aniline **31l** (70 mg, 0.21 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 2 hours. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 80% ethyl acetate in petroleum ether, 0 – 5% methanol in DCM) followed by reverse phase column chromatography (0 – 100% acetonitrile in water (+ 0.1% NH$_3$)) afforded **16l** (20 mg, 18% yield). LCMS (ESI+): m/z 519.3 [M + H]$^+$, (ESI-): m/z 517.2 [M - H]$^-$, rt 2.06 minutes, >99%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.61 (1H, s), 9.85 (1H, s), 8.42 (1H, d, J = 2.1 Hz), 8.27 (1H, d, J = 7.8 Hz), 7.97–7.90 (2H, m), 7.89–7.83 (1H, m), 7.48 (1H, dd, J = 8.7, 2.3 Hz), 7.23 (1H, d, J = 8.8 Hz), 7.12–7.04 (4H, m), 4.19 (2H, s), 3.94 (3H, s), 3.18–3.04 (4H, m), 2.96–2.83 (4H, m); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.4, 159.4, 152.3, 142.1, 140.4, 133.5, 131.6, 129.8, 129.1, 128.7, 127.7, 127.6, 126.5, 125.9, 125.5, 123.9, 119.3, 111.3, 56.3, 48.1, 35.5 (1 peak missing); ν_{max}/cm^{-1} 3302, 3173, 3011, 2906, 1687, 1650 (C=O), 1595, 1530; HRMS (ESI)-: m/z calculated for [C$_{27}$H$_{26}$N$_4$O$_5$S - H]$^-$ = 517.1551, observed 517.1549.

N-(2-Methoxy-5-((7-methoxy-1,2,4,5-tetrahydro-3H-benzo[d]azepin-3-yl)sulfonyl)phenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16m).

T3P$^®$ (50 wt. % in ethyl acetate, 0.50 mL, 0.84 mmol) and *N,N*-diisopropylethylamine (0.24 mL, 1.4 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (66 mg, 0.28 mmol) and 2-methoxy-5-((7-methoxy-1,2,4,5-tetrahydro-3H-benzo[d]azepin-3-yl)sulfonyl)aniline **31m** (0.101 g, 0.279 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 1 hour. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO$_4$) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 100% ethyl acetate in petroleum ether, 0 – 7% methanol in DCM) afforded **16m** (48 mg, 30% yield). LCMS (ESI+): m/z 549.4 [M + H]$^+$, (ESI-): m/z 547.3 [M - H]$^-$, rt 1.96 minutes, 96%; ^{1}H NMR (500 MHz, (CD$_3$)$_2$SO) 12.61 (1H, s), 9.85 (1H, s), 8.41 (1H, d, J = 2.1 Hz), 8.27 (1H, d, J = 7.9 Hz), 7.97–7.90 (2H, m), 7.89–7.83 (1H, m), 7.47 (1H, dd, J = 8.7, 2.3 Hz), 7.22 (1H, d, J = 8.8 Hz), 6.98 (1H, d, J = 8.2 Hz), 6.67 (1H, d, J = 2.6 Hz), 6.62 (1H, dd, J = 8.2, 2.8 Hz), 4.20 (2H, s), 3.94 (3H, s), 3.66 (3H, s), 3.15–3.02 (4H, m), 2.91–2.76 (4H, m); ^{13}C NMR (125 MHz, (CD$_3$)$_2$SO) 168.4, 159.5, 157.8, 152.3, 142.1, 141.7, 133.6, 132.4, 131.6,

130.2, 129.8, 128.7, 127.7, 127.6, 125.9, 125.5, 123.9, 119.3, 115.0, 111.3, 111.2, 56.3, 55.0, 48.6, 48.1, 35.7, 34.7 (1 peak missing); ν_{max}/cm^{-1} 3308, 2913, 1691, 1642 (C=O), 1595, 1529; HRMS (ESI)+: m/z calculated for $[C_{28}H_{28}N_4O_6S + Na]^+$ = 571.1622, observed 571.1615.

N-(2-Methoxy-5-((1,2,4,5-tetrahydro-3H-1,5-methanobenzo[d]azepin-3yl)sulfonyl)phenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (16n).

T3P® (50 wt. % in ethyl acetate, 0.26 mL, 0.43 mmol) and *N,N*-diisopropylethylamine (0.13 mL, 0.72 mmol) were added to a solution of 2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetic acid **3** (34 mg, 0.14 mmol) and 2-methoxy-5-((1,2,4,5-tetrahydro-3H-1,5-methanobenzo[d]azepin-3-yl)sulfonyl)aniline **31n** (55 mg, 0.14 mmol) in DMF (2 mL). The reaction mixture was heated to 70 °C over 45 minutes. The reaction mixture was diluted with ethyl acetate (25 mL), washed with water (3 × 25 mL) and brine (25 mL), dried (MgSO₄) and concentrated *in vacuo*. Purification by flash column chromatography (50 – 80% ethyl acetate in petroleum ether, 0 – 5% methanol in DCM) followed by reverse phase column chromatography (0 – 50% acetonitrile in water (+ 0.1% NH₃)) afforded **16n** (26 mg, 33% yield). LCMS (ESI+): m/z 531.3 [M + H]⁺, (ESI-): m/z 529.3 [M - H]⁻, rt 1.98 minutes, 96%; ¹H NMR (500 MHz, (CD₃)₂SO) 12.64 (1H, s), 9.82 (1H, s), 8.28 (1H, d, J = 7.9 Hz), 8.22 (1H, d, J = 1.6 Hz), 8.01–7.92 (2H, m), 7.87 (1H, ddd, J = 8.0, 6.3, 1.9 Hz), 7.22–7.06 (6H, m), 4.23 (2H, s), 3.97 (3H, s), 3.40 (2H, d, J = 10.6 Hz), 3.18–3.12 (2H, m), 2.80 (2H, d, J = 10.8 Hz), 2.11–2.01 (1H, m), 1.51 (1H, d, J = 10.9 Hz); ¹³C NMR (125 MHz, (CD₃)₂SO) 168.2, 159.5, 152.1, 144.3, 142.1, 133.5, 131.6, 129.8, 128.1, 127.6, 127.5, 126.9, 125.9, 125.6, 123.6, 122.4, 119.6, 111.0, 56.3, 49.2, 41.2 (2 peaks missing); ν_{max}/cm^{-1} 3177, 3020, 2937, 2908, 2851, 1673, 1646 (C=O), 1597, 1540; HRMS (ESI)+: m/z calculated for $[C_{28}H_{26}N_4O_5S + Na]^+$ = 553.1516, observed 553.1517.

Expression and Purification of *Mycobacterium tuberculosis* fumarate hydratase.

A single freshly transformed *Escherichia coli* strain BL21 (DE3) colony was transferred to LB media (20 mL) with ampicillin (100 μg mL⁻¹) and incubated overnight (37 °C, 200 rpm). The starter culture was used to inoculate 2 flasks, each containing TB media (1 L) with ampicillin (100 μg mL⁻¹), with incubation (37 °C, 200 rpm) until an optical density (A₆₀₀ₙₘ) of 1.1 was reached. Protein expression was induced by the addition of isopropyl β-D-1-thiogalactopyranoside (0.5 mM), followed by overnight incubation (15 °C, 200 rpm). Cells were harvested by centrifugation (4 °C, 4000 g, 20 minutes), then frozen.

The cells were resuspended in 50 mL lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 20 mM imidazole) with a tablet of cOmplete™ Protease Inhibitor Cocktail (Roche). The suspension was sonicated (15 minutes, 10 seconds on/ 20 seconds off) and centrifuged (4 °C, 30000 g, 20 minutes). The lysate was loaded onto a 7.5 mL nickel Sepharose™ fast flow column (GE Healthcare), pre-equilibrated with lysis buffer. The column was washed with 5 column volumes of lysis buffer and eluted with buffer B (50 mM Tris pH 8.0, 150 mM NaCl, 300 mM imidazole) in 8 × 5 mL aliquots. Protein-containing aliquots, as determined by SDS-PAGE, were combined and concentrated (30 kDa cutoff), then loaded onto a Superdex 200 Hiload™ 26/60 column (GE Healthcare) pre-equilibrated with filtration

buffer (10 mM Tris pH 8.0, 150 mM NaCl, 0.5 mM TCEP). Chromatography was conducted using an ÄKTA™ FPLC system (GE Healthcare), with protein-containing fractions, as determined by SDS-PAGE, combined and concentrated to 27.9 mg mL^{-1} (24.3 mg L^{-1} yield), then flash-frozen in liquid nitrogen and stored at −80 °C. The identity of the protein was confirmed by LCMS analysis.

Differential Scanning Fluorimetry.

DSF was performed using a BioRad CFX Connect™ system, from 25 to 95 °C in 0.5 °C increments of 30 seconds duration. Samples were run in 96-well plates, with each well containing a final volume of 50 μL. Screening was conducted with 100 mM Tris pH 7.5, 50 mM NaCl, 2.5x SyproOrange®, 2.5 μM *Mtb* fumarase and either 5% DMSO (control) or 5% ligand stock solution in DMSO.

Biochemical Assay.

The enzymatic activity of *Mtb* fumarase was followed by a CLARIOstar® microplate spectrometer (BMG Labtech) in a 96-well UV plate (Greiner). 2 μL of either DMSO (control) or ligand stock solution in DMSO was pipetted per well. 150 μL of a solution containing acetyl coenzyme A sodium salt (267 μM), β-nicotinamide adenine dinucleotide hydrate (200 μM), malate dehydrogenase (13.3 units mL^{-1}), citrate synthase (1.33 units mL^{-1}) and *Mtb* fumarase (33.3 nM) in buffer (250 mM Tris pH 8.0, 5 mM MgCl$_2$) was pipetted per well. The plate was left to equilibrate for 5 minutes at 25 °C, then 48 μL of either buffer (negative control) or fumaric acid (1.67 mM) (positive control or ligand) was pipetted per well. After 2 minutes the plate was read at a wavelength of 340 nm at intervals of 24 seconds over 10 minutes.

Inhibition values for each ligand at a particular concentration were calculated from the gradient of the assay read over the 10 minutes, with correction by the negative control and normalization by the positive control. Inhibition experiments at 50 μM ligand concentration were performed at least twice (n   2), with % inhibition and standard error of the mean reported. IC$_{50}$ experiments (n = 6) were performed with 10 ligand concentrations, obtained through serial dilution. Dose-response curves were calculated using Origin software (OriginLab, Northampton, MA, USA), with IC$_{50}$ and standard error reported.

Soaking of *Mycobacterium tuberculosis* fumarate hydratase Crystals.

Crystals were grown in Intelli-Plate® 24–4 well sitting drop plates (Art Robbins Instruments), incubated at 19 °C in a ROCK IMAGER® 1000 system (FORMULATRIX®). A sitting drop was set up with 2 μL protein solution (14 mg mL^{-1} *Mtb* fumarase, 150 mM NaCl, 10 mM Tris pH 8.0 and 0.5 mM TCEP) and 1 μL reservoir solution (10% w/v PEG3350, 5% DMSO and 0.30 M magnesium formate), equilibrated against 400 μL reservoir solution. After 3 weeks the resultant crystals were used to make seed stocks to produce reliable crystals for ligand screening. Sitting drops were set up with 3 μL protein solution (14 mg mL^{-1} *Mtb* fumarase, 150 mM NaCl, 10 mM Tris pH 8.0 and 0.5 mM TCEP), 1 μL reservoir solution (10 – 11.4% w/v PEG3350, 5% DMSO and 0.30 M magnesium formate) and 0.5 μL seed stock, equilibrated against 400 μL reservoir solution. After 1 – 2 weeks drops were treated with 2 μL soaking solution (0.5 – 3 mM ligand, 7.5%

DMSO, 26.25% w/v PEG3350 and 0.20 M magnesium formate) and incubated overnight. Crystals were mounted into loops and flash frozen in liquid nitrogen.

X-ray Data Collection and Processing.

X-ray data were collected on beamlines I03 and I04 at the Diamond Light Source synchrotron. Data was processed using autoPROC.[19] Molecular replacement for the structure of *Mtb* fumarase in complex with **16b** was carried out using PHASER,[20] accessed through the CCP4 software suite,[21] with the previously published **1**-bound *Mtb* fumarase structure (PDB code 5F91) used as a search model.[14] For structures of *Mtb* fumarase in complex with **15a**, **15g**, **16a**, **16h**, **16j** or **16l**, the solved **16b**-bound structure was used as a search model. Models were manually rebuilt using the COOT molecular graphics software package,[22] and refined with REFMAC5,[23] accessed through CCP4.[21] All coordinates have been deposited to the Protein Data Bank and the accession numbers, with data collection statistics compiled using PHENIX,[24] are presented in Table S1.

Screening against H37Rv *Mycobacterium tuberculosis.*

Antimicrobial susceptibility testing against *Mycobacterium tuberculosis* H37Rv was performed in various media including: GAST-Fe, 7H9/ADC, 7H9/DPPC (4.7 g/L 7H9 base, 14 mg/L DPPC, 0.81 g/L NaCl, 0.3 g/L Casitone, and 0.05% Tyloxapol) and 7H9/Glucose (4.7 g/L 7H9 base, 4 g/L glucose, 0.81 g/L NaCl, 0.3 g/L Casitone, and 0.05% Tyloxapol). H37Rv was grown in the corresponding media to OD 0.2–0.4 prior to use. 50 μL of a two-fold serial dilution series was placed in each well of a sterile 96-well round bottom plate, then 50 μL of H37Rv diluted to OD 0.0002 was added. Plates were incubated at 37 °C for two weeks prior to determining MIC. The MIC is defined here as the drug concentration that completely inhibits growth of cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ABBREVIATIONS

T_m	change in melting temperature
cLogP	partition coefficient

DSF	differential scanning fluorimetry
IC$_{50}$	half-maximal inhibitory concentration
MIC	minimum inhibitory concentration
Mtb	Mycobacterium tuberculosis
TB	tuberculosis

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a)

1

IC$_{50}$ 2.5 ± 1.1 μM

b)

c)
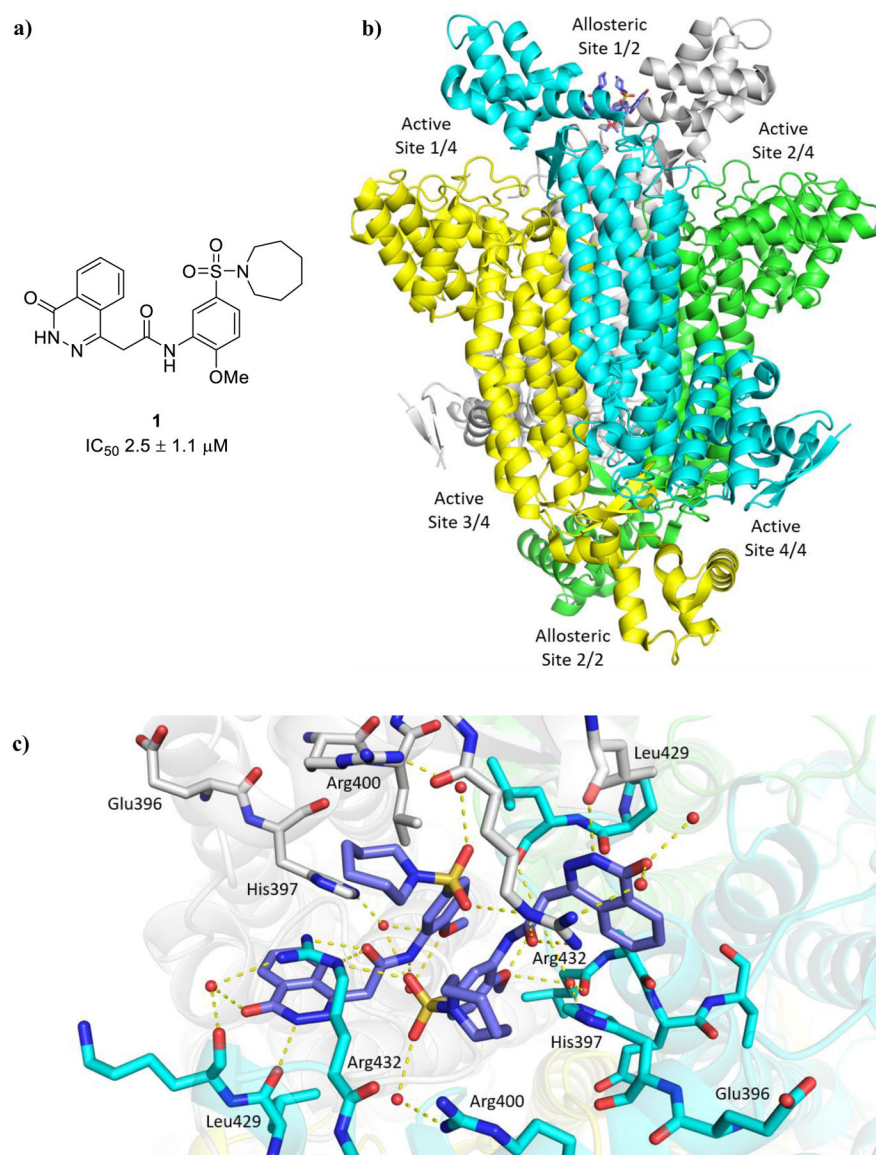
Figure 1.

a) Allosteric inhibitor **1** of *Mtb* fumarase.[14] b) X-ray crystal structure of *Mtb* fumarase in complex with **1** (PDB code 5F91, subunit A = white, subunit B = green, subunit C = cyan, subunit D = yellow),[14] illustrating relative locations of the allosteric and active sites in the homotetramer. c) The interactions (yellow dashed lines) of **1** (lilac) in the allosteric site
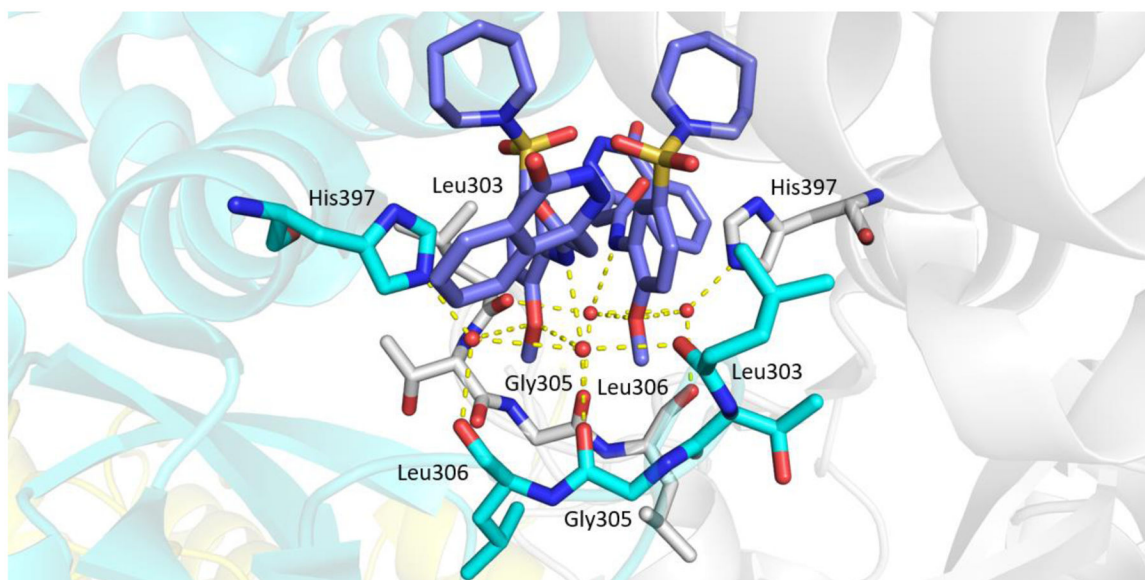
Figure 2.
X-ray crystal structure of *Mtb* fumarase in complex with **1** (lilac) (PDB code 5F91, subunit A = white, subunit B = green, subunit C = cyan, subunit D = yellow),[14] illustrating the interactions (yellow dashed lines) of the methoxy group of **1** in the allosteric site.
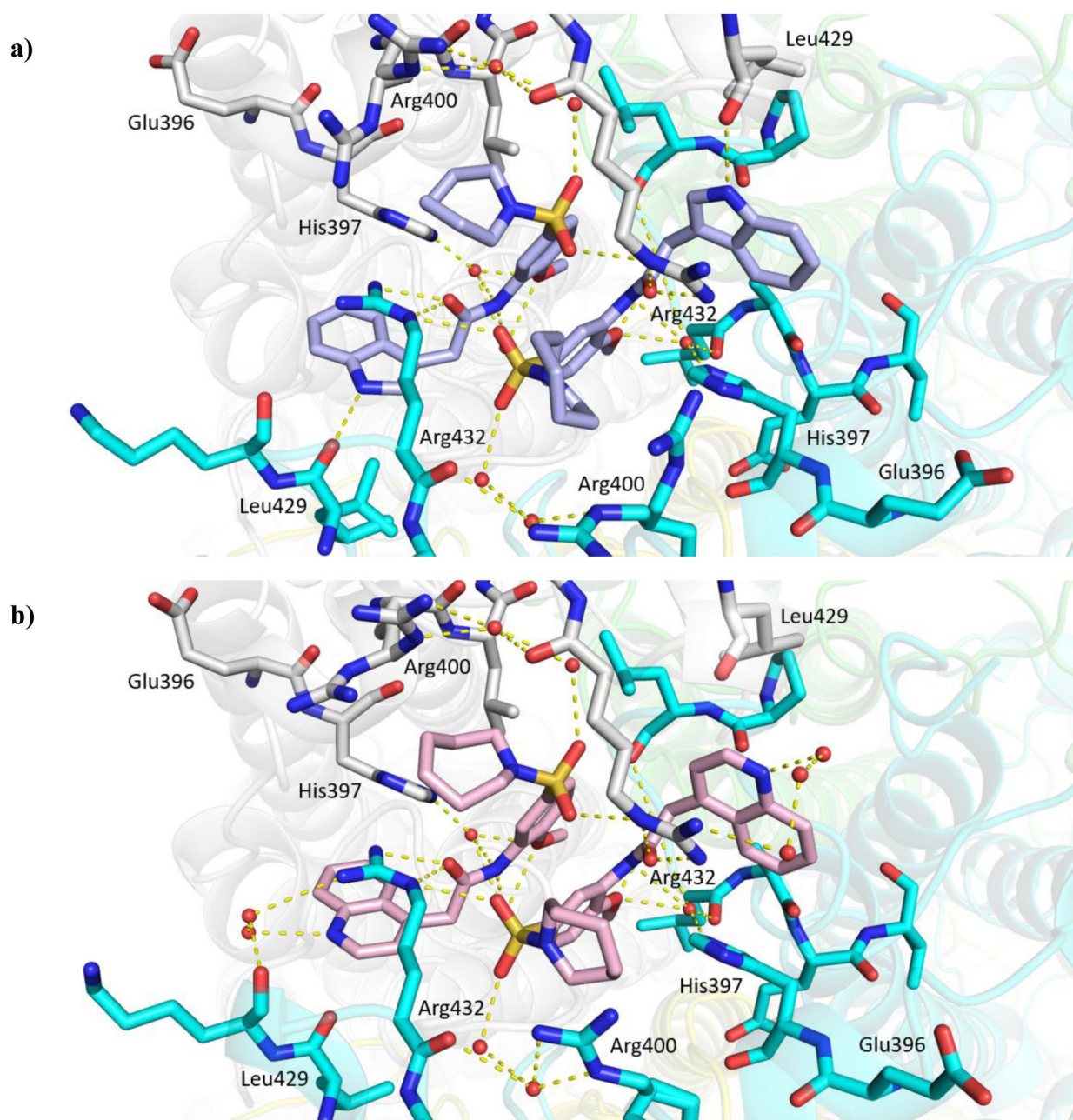
Figure 3.
X-ray crystal structures of *Mtb* fumarase (subunit A = white, subunit B = green, subunit C = cyan, subunit D = yellow) in complex with a) **15a** (blue) (PDB code 6S7U) and b) **15g** (pink) (PDB code 6S7W), illustrating the interactions (yellow dashed lines) of the ligands in the allosteric site.
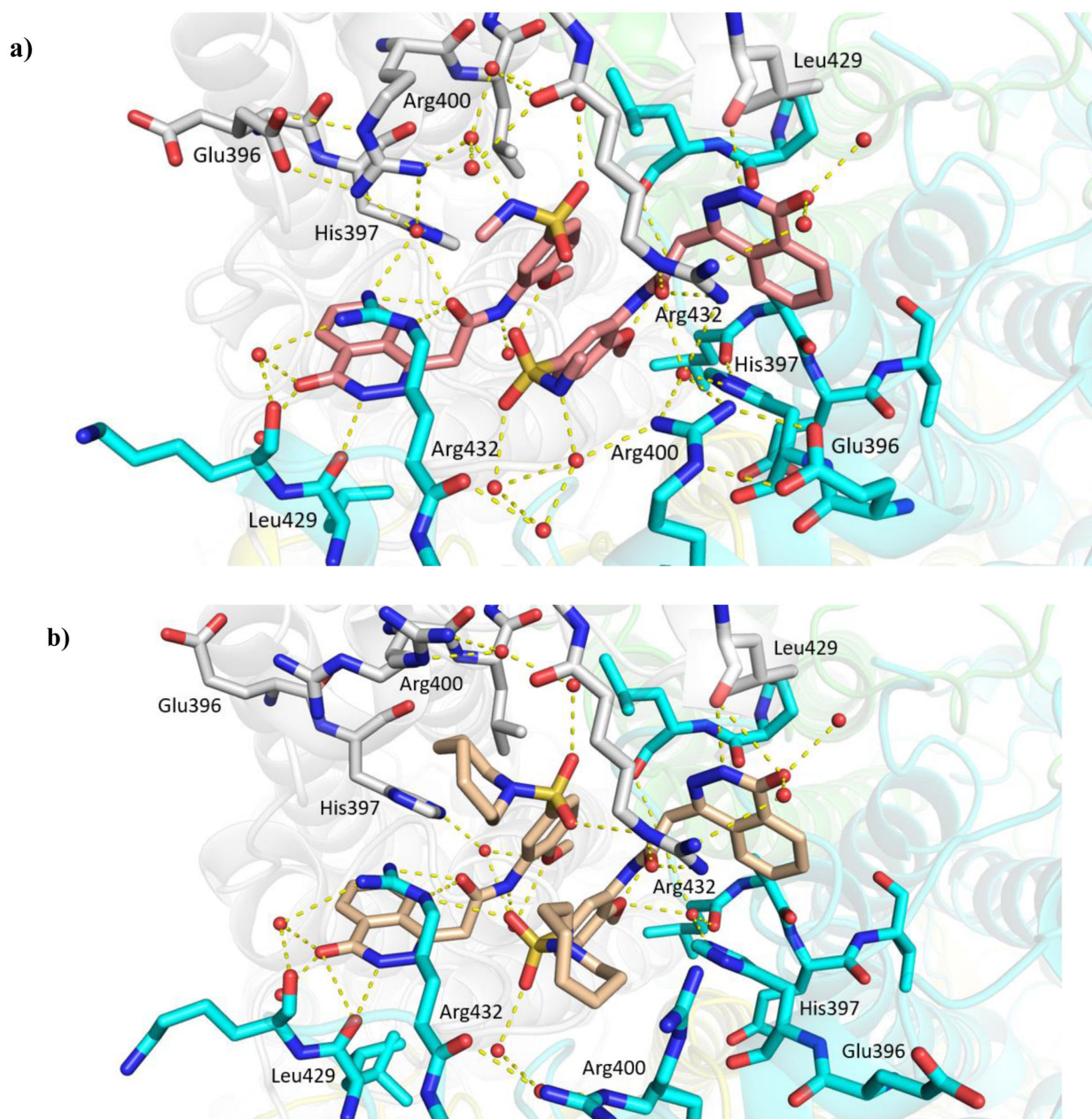
Figure 4.
X-ray crystal structure of *Mtb* fumarase (subunit A = white, subunit B = green, subunit C = cyan, subunit D = yellow) in complex with a) **16a** (light pink) (PDB code 6S7K) and b) **16b** (beige) (PDB code 6S43), illustrating the interactions (yellow dashed lines) of the ligands in the allosteric site.
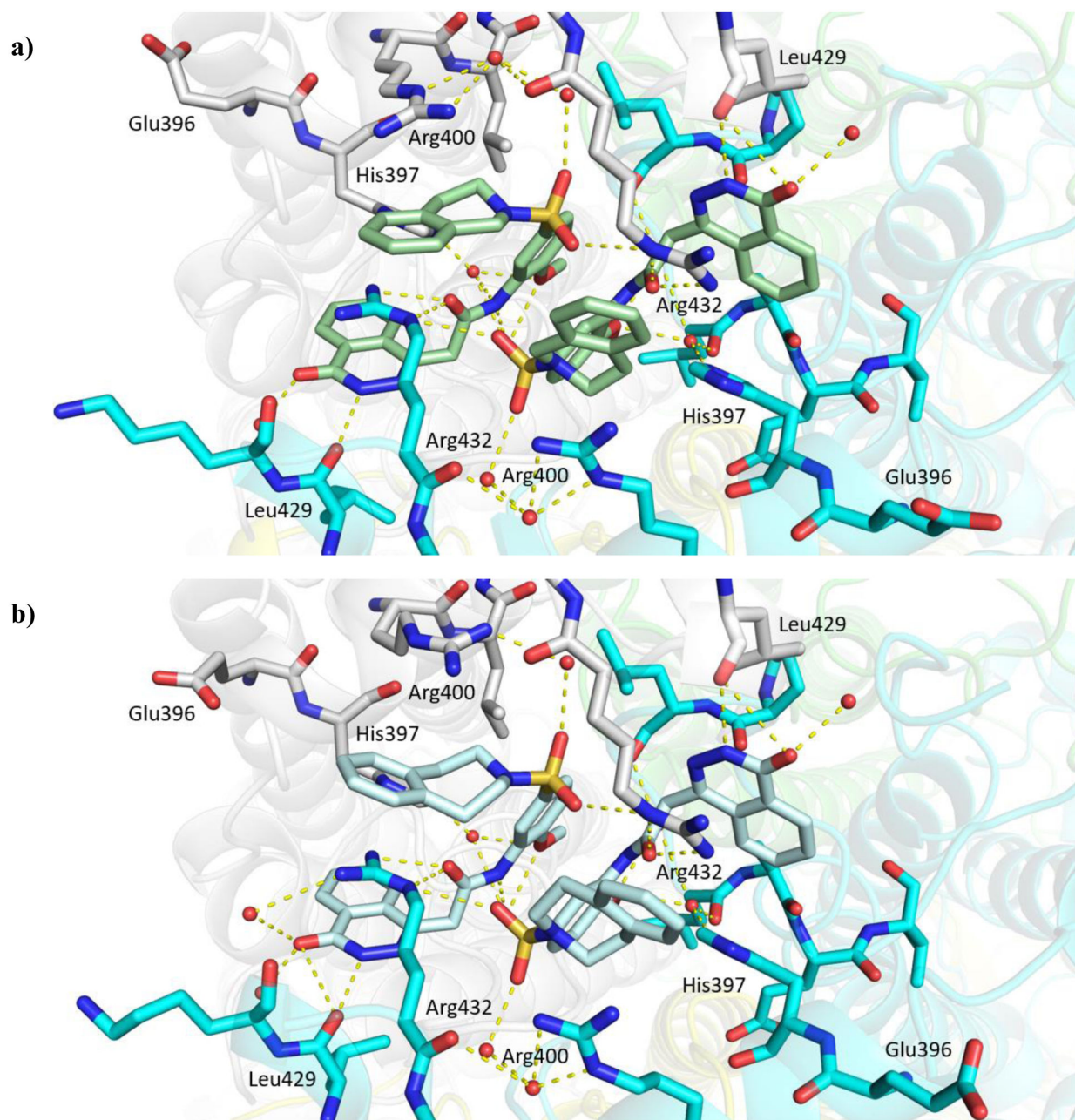
Figure 5.
X-ray crystal structure of *Mtb* fumarase (subunit A = white, subunit B = green, subunit C = cyan, subunit D = yellow) in complex with a) **16j** (light green) (PDB code 6S7Z) and b) **16l** (light blue) (PDB code 6S88), illustrating the interactions (yellow dashed lines) of the ligands in the allosteric site.
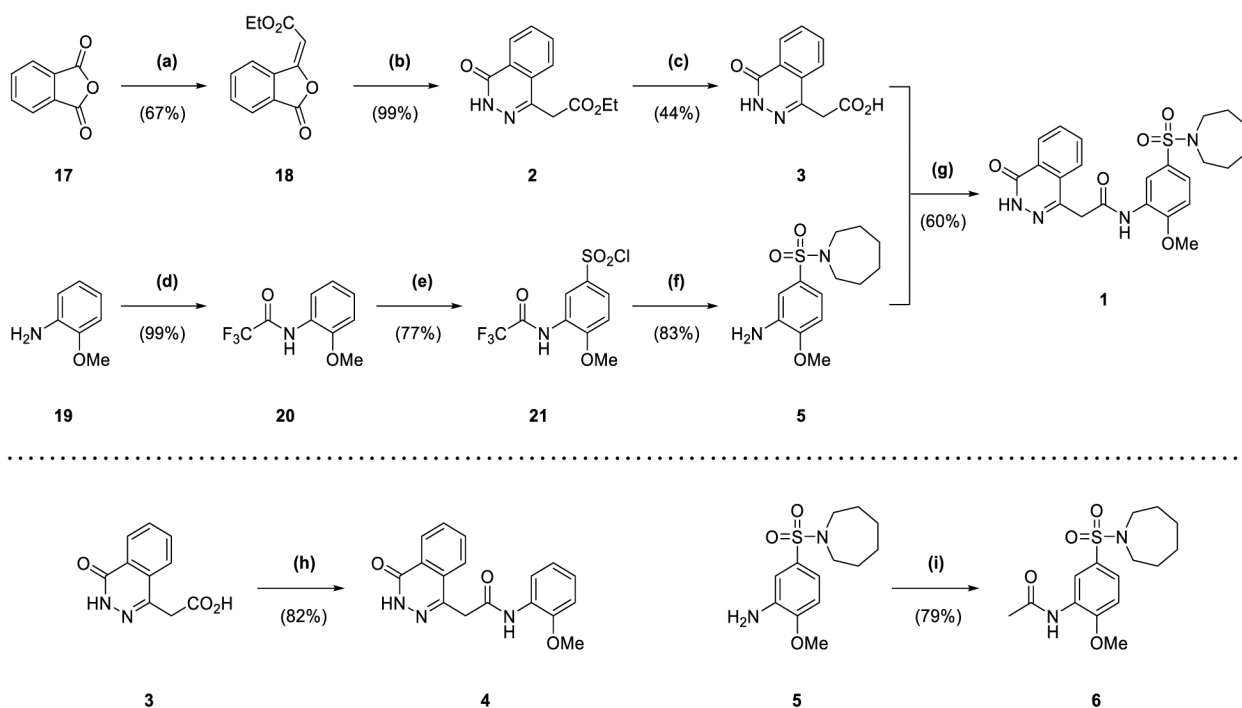
Scheme 1.

Synthesis of Compounds **1**-**6** and **21**.

Reagents and Conditions: (a) (carbethoxymethylene)triphenylphosphorane, CHCl$_3$, reflux, 3 h; (b) N$_2$H$_4$·H$_2$O, EtOH, 50 °C, 2 h; (c) NaOH (10% w/v), THF, reflux, 1 h; (d) TFAA, pyridine, DCM, 0 °C to rt, 3 d; (e) HSO$_3$Cl, DCM, 0 °C to rt, 16 h; (f) (i) hexamethyleneimine, NaH, DMF, 0 °C to rt, 3 h (ii) EtOH, HCl (37.5% w/v), H$_2$O, reflux, 20 h; (g) T3P® (50 wt. % in DMF), DIPEA, DMF, 70 °C, 1 h; (h) **19**, T3P® (50 wt. % in DMF), DIPEA, DMF, 40 °C, 2 h; (i) Ac$_2$O, pyridine, DCM, 90 min.
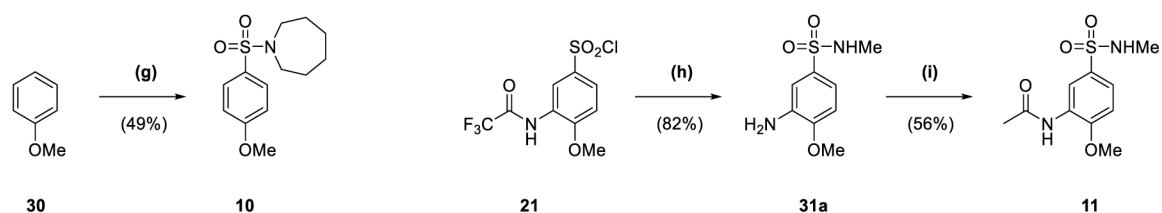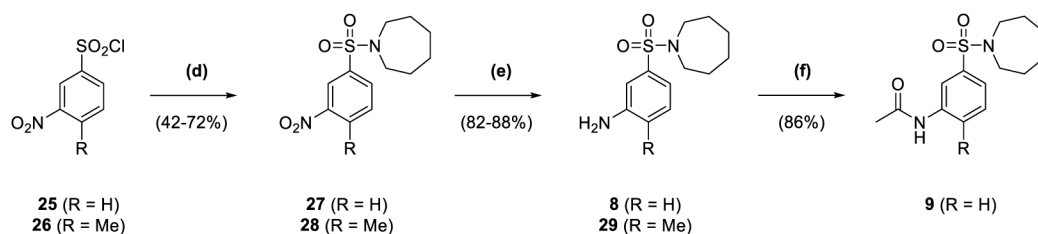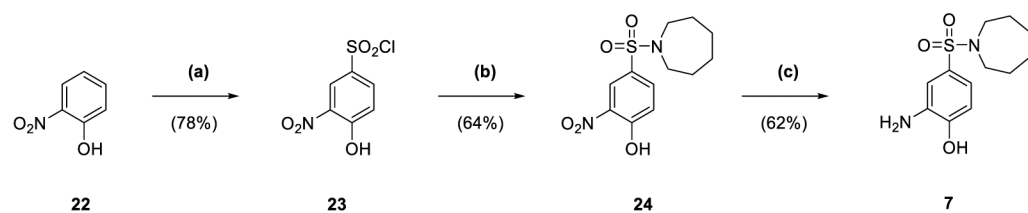
Scheme 2.

Synthesis of Compounds **7-11**, **29** and **31a**.

Reagents and Conditions: (a) HSO_3Cl, $CHCl_3$, 0 °C to reflux, 90 min; (b) hexamethyleneimine, DIPEA, DCM, 15 h; (c) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, MeOH, 0 °C to rt, 2 h; (d) hexamethyleneimine, NaH, DMF, 0 °C to rt, 1 h to 90 min; (e) $NaBH_4$, $NiCl_2$, MeOH, 0 °C to rt, 45 min; (f) Ac_2O, pyridine, DCM, 5 h; (g) (i) HSO_3Cl, $CHCl_3$, 0 °C to rt, 30 min (ii) hexamethyleneimine, NaH, DMF, 0 °C to rt, 1 h; (h) (i) methylamine (2 M in THF), THF, reflux, 90 min (ii) EtOH, HCl (37.5% w/v), H_2O, reflux, 3 h 30 min; (i) Ac_2O, pyridine, DCM, 2 d.
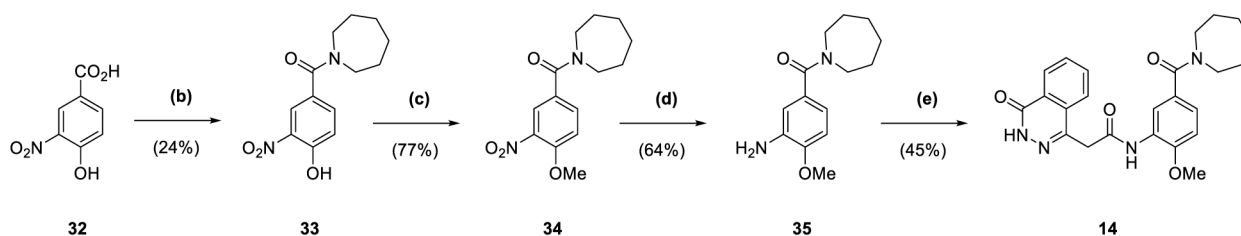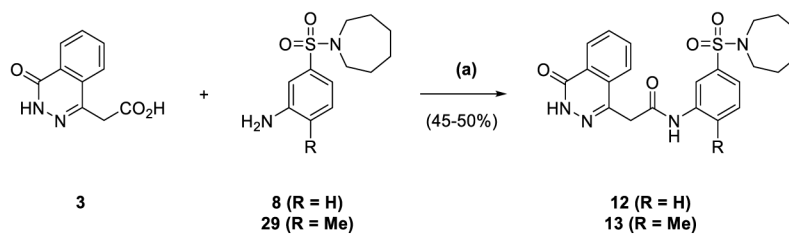
Scheme 3.

Synthesis of Compounds **12**-**14**.

Reagents and Conditions: (a) T3P® (50 wt. % in EtOAc), DIPEA, DMF, 70 °C, 2 h; (b) hexamethyleneimine, T3P® (50% in DMF), DIPEA, DMF, 1 d; (c) Me_2SO_4, K_2CO_3, acetone, reflux, 2 h; (d) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, MeOH, 0 °C to rt, 90 min; (e) **3**, T3P® (50 wt. % in EtOAc), DIPEA, DMF, 70 °C, 2 h.
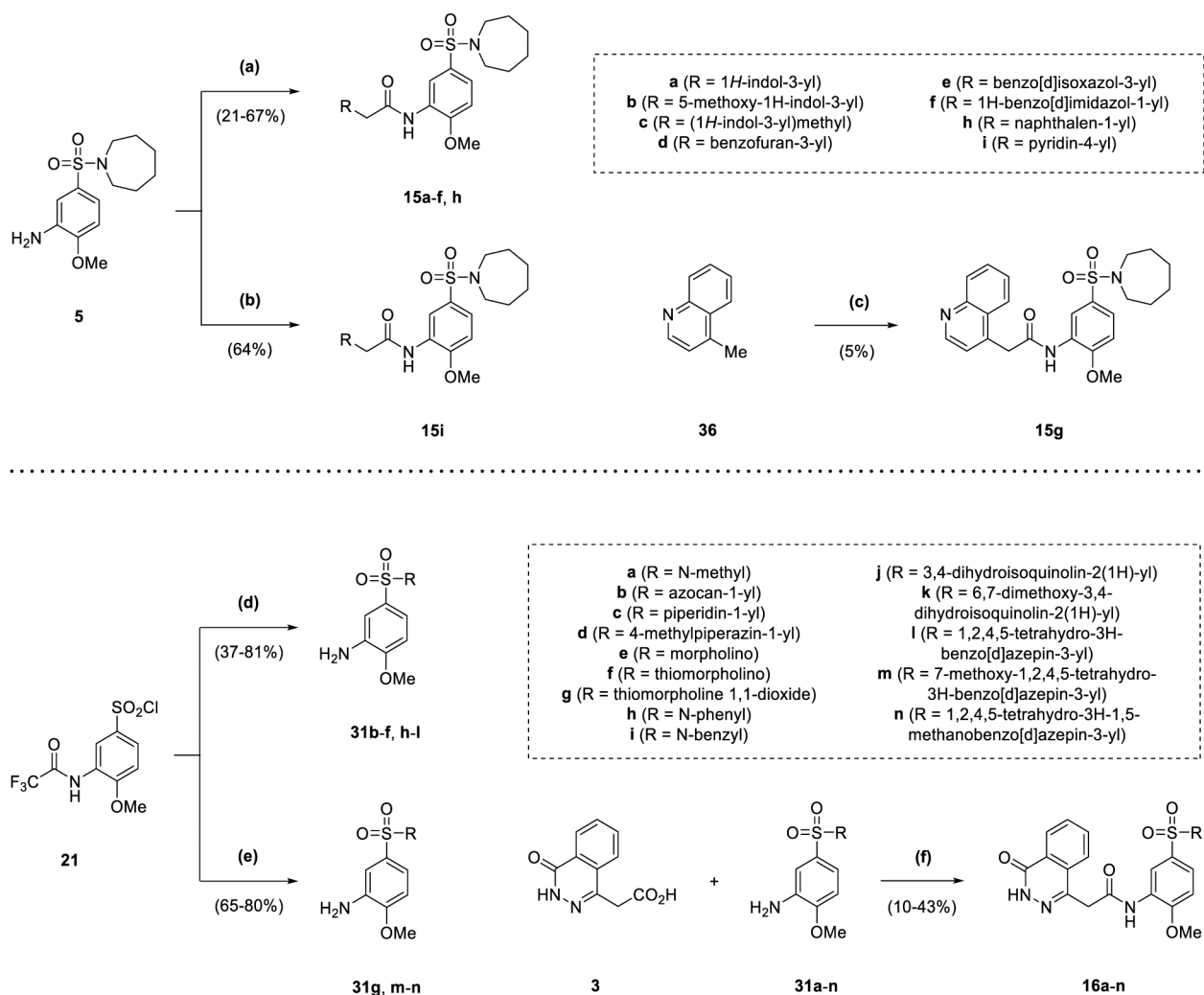
Scheme 4.

Synthesis of Compounds **15-16**.

Reagents and Conditions: (a) RCH$_2$CO$_2$H, T3P® (50 wt. % in EtOAc), DIPEA, DMF, 70 °C, 1 to 4 h; (b) 4-pyridylacetic acid HCl, EDC.HCl, DIPEA, DMAP, DCM, 90 min; (c) (i) CO$_2$ (s), LDA (2 M in THF/heptane/ethylbenzene), THF, −78 °C to rt, 1 h (ii) **5**, T3P® (50 wt. % in EtOAc), DIPEA, DMF, 70 °C, 40 min; (d) (i) RH, NaH, DMF, 0 °C to rt, 30 min to 20 h (ii) EtOH, HCl (37.5% w/v), H$_2$O, reflux, 9 h to 2 d; (e) (i) RH, NEt$_3$, DMAP, DCM, 30 min to 1 h (ii) EtOH, HCl (37.5% w/v), H$_2$O, reflux, 3 to 16 h; (f) T3P® (50 wt. % in EtOAc), DIPEA, DMF, 70 °C, 45 min to 5 h.

Table 1.

The change in the melting temperatures (ΔT_m) of compounds **2-11**.

1
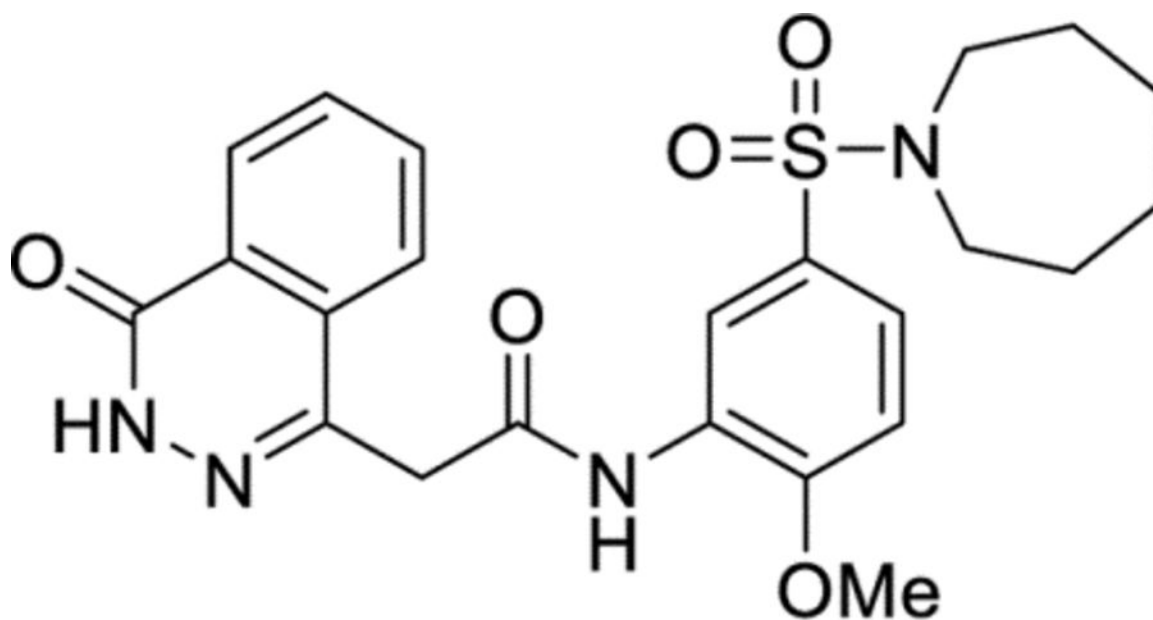
ΔT_m +3.9 °C [a]

Compound	$T_m{}^b$ (°C)	Compound	$T_m{}^b$ (°C)
2	−0.6	7	−1.6
3	−0.4	8	−1.2

1

ΔT_m +3.9 °C [a]

Compound		T_m[b] (°C)	Compound		T_m[b] (°C)
4		−0.4	9		−0.4
5		−0.7	10		+0.1

1
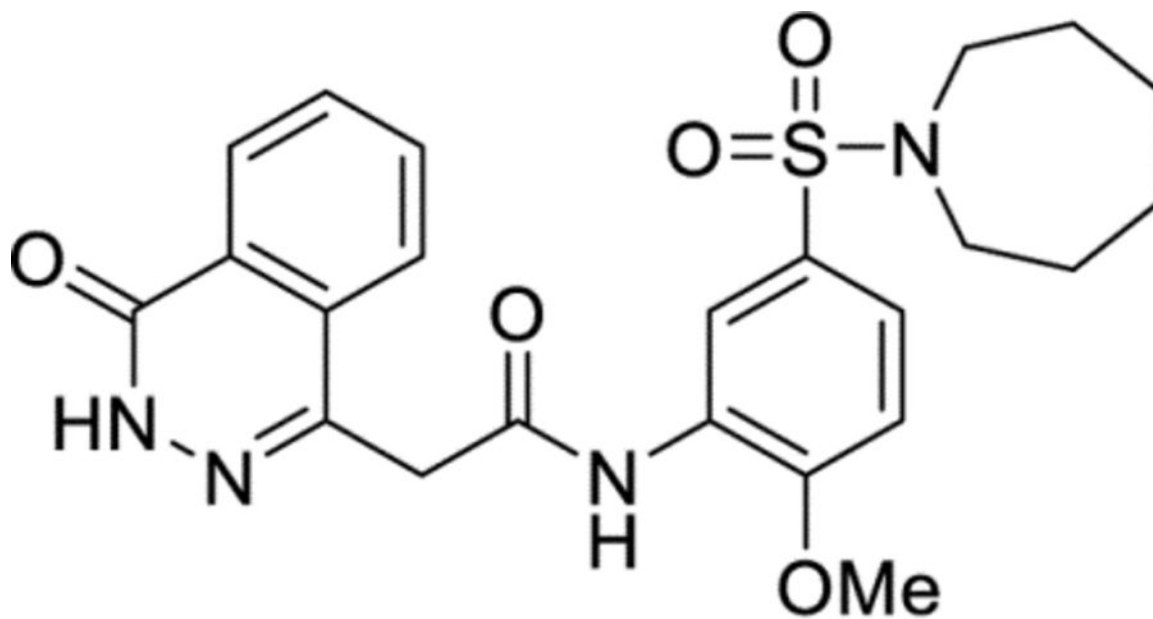
ΔT_m +3.9 °C [a]

Compound	T_m^{b} (°C)	Compound	T_m^{b} (°C)
6	−0.6	11	−0.4

[a]0.625 mM ligand and 2.5 μM *Mtb* fumarase.

[b]5 mM ligand and 2.5 μM *Mtb* fumarase.

Table 2.

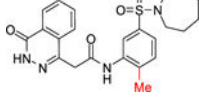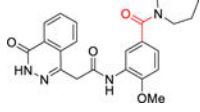The inhibition at 50 μM ligand concentration afforded by compounds **1** and **12**–**14**.
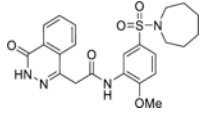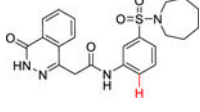
Compound	Structure	Inhibition (%) at 50 μM
1		> 90
12		16 ± 1
13		< 10
14		14 ± 1

Table 3.

The inhibition at 50 μM ligand concentration or half-maximal inhibitory concentrations (IC$_{50}$) afforded by compounds **1** and **15a-i**.
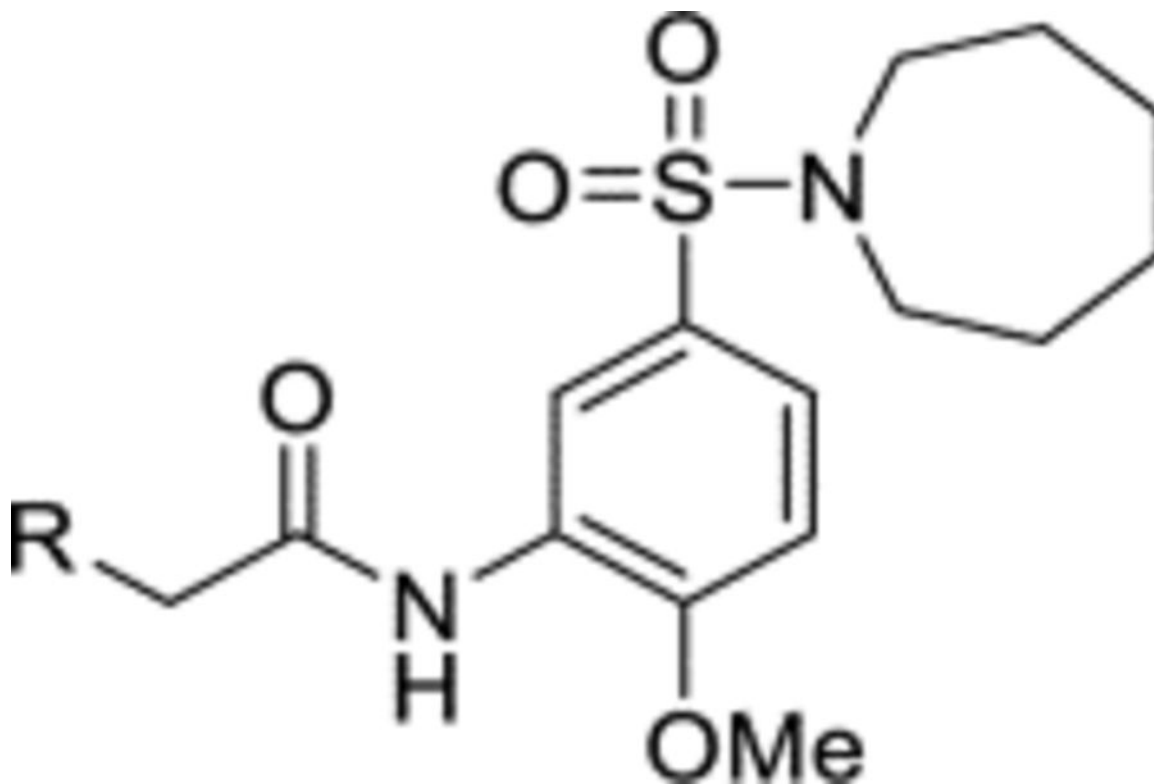
Compound	R	Inhibition (%) at 50 μM	Compound	R	Inhibition (%) at 50 μM
1		> 90^a	15e		< 10
15a		27 ± 6	15f		< 10
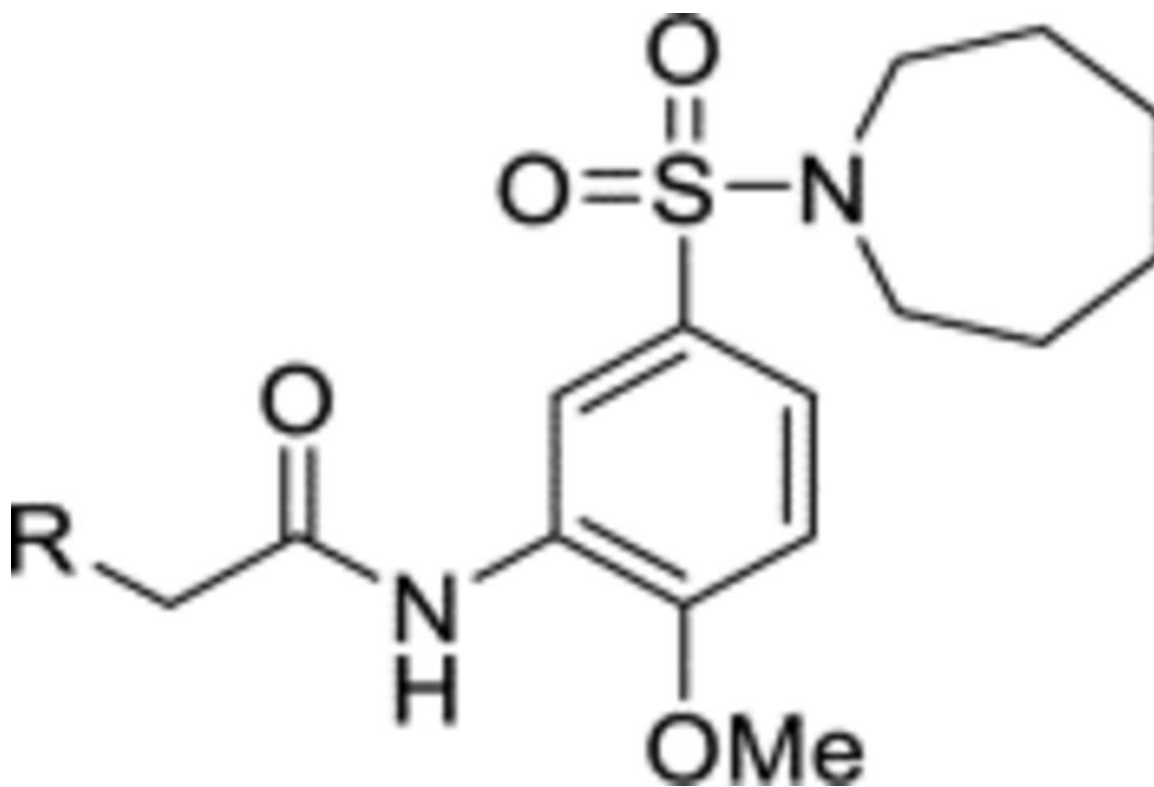
Compound	R	Inhibition (%) at 50 μM	Compound	R	Inhibition (%) at 50 μM
15b		38 ± 5	15g		86 ± 2[b]
15c		12 ± 9	15h		21 ± 17
15d		< 10	15i		< 10

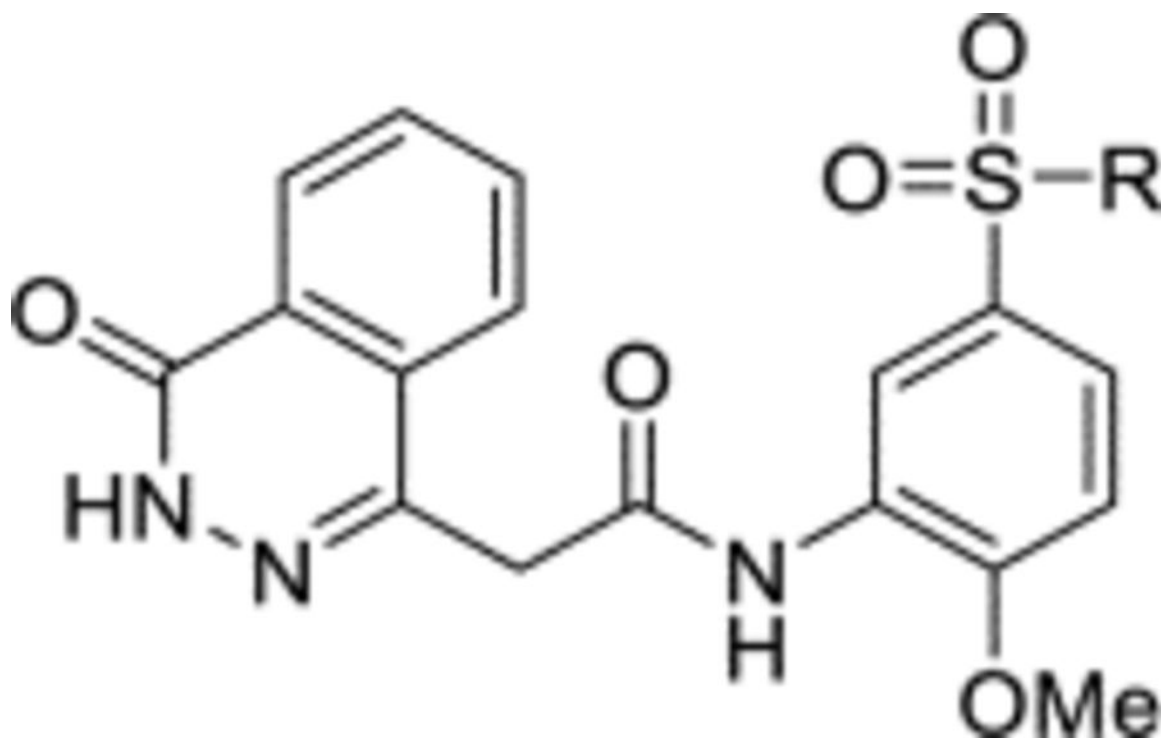[a]IC$_{50}$ 2.0 ± 0.1 μM, consistent with previously reported value.[14]

[b]IC$_{50}$ 4.1 ± 0.3 μM.

Table 4.

The inhibition at 50 μM ligand concentration or half-maximal inhibitory concentrations (IC_{50}) afforded by compounds **16a-n**

Compound	R	IC_{50} (μM)	Compound	R	IC_{50} (μM)
16a	—NHMe	57 ± 3	16h	(NH-phenyl)	17 ± 3
16b	(azepane)	4.0 ± 0.1	16i	(NH-benzyl)	ND[a]
16c	(piperidine)	4.4 ± 0.1	16j	(tetrahydroisoquinoline)	2.2 ± 0.2

Compound	R	IC$_{50}$ (μM)	Compound	R	IC$_{50}$ (μM)
16d		38 ± 2	16k		3.4 ± 0.2
16e		12 ± 1	16l		0.67 ± 0.03
16f		4.7 ± 0.2	16m		0.67 ± 0.01
16g		13 ± 1	16n		ND[b]

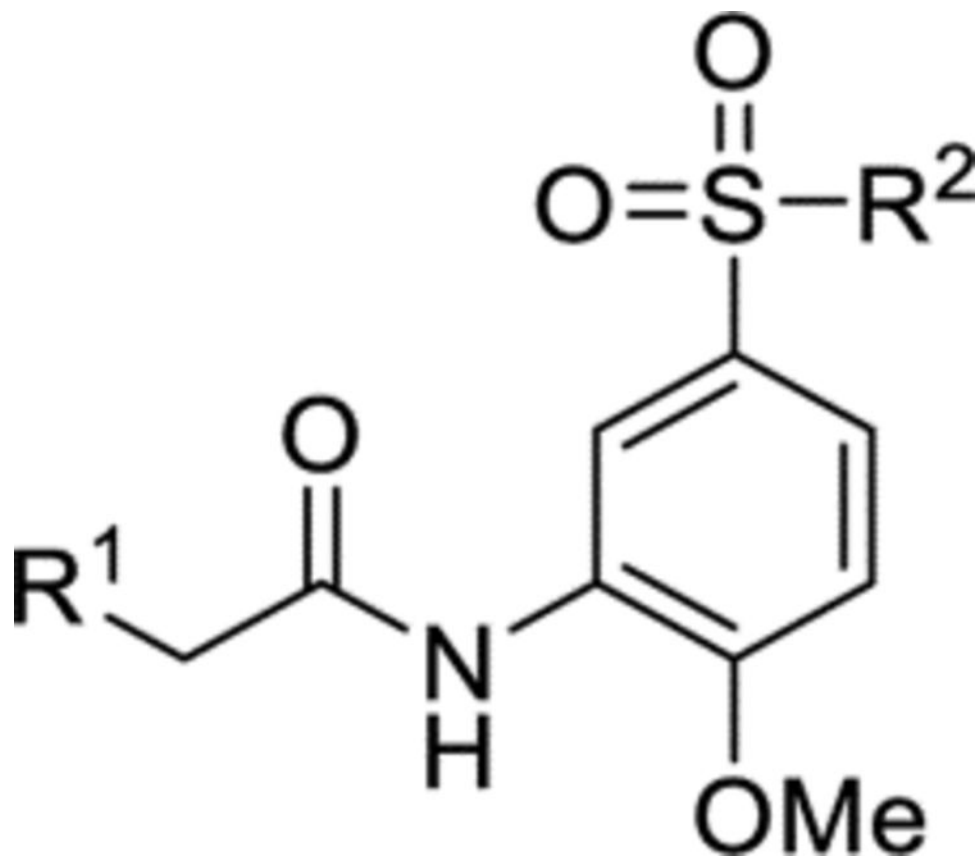[a] 53 ± 3% inhibition at 50 μM concentration.

[b] 44 ± 5% inhibition at 50 μM concentration.

Table 5.

The minimum inhibitory concentrations (MIC) against H37Rv *Mycobacterium tuberculosis* (*Mtb*) afforded by compounds **1**, **15[1]-d** and **16b**.
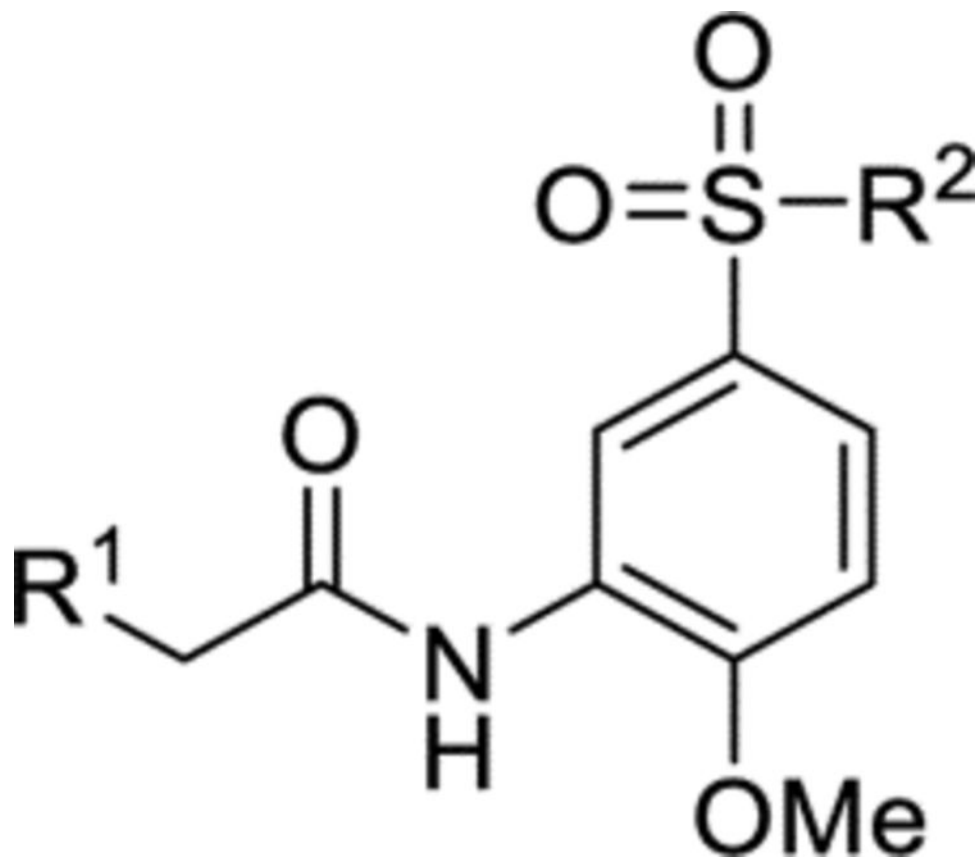
Compound	R[1]	R[2]	cLogP[a]	H37Rv *Mtb* MIC (μM)			
				GAST-Fe	7H9/ADC	7H9/glucose	7H9/DPPC
1			1.6	> 100	> 100	> 100	> 100
15a			3.6	> 100	> 100	100	9.4

Compound	R^1	R^2	cLogP^a	H37Rv *Mtb* MIC (μM)			
				GAST-Fe	7H9/ADC	7H9/glucose	7H9/DPPC
15b			3.6	> 100	> 100	> 100	12.5
15c			4.1	> 100	> 100	25	9.4
15d			4.1	> 100	> 100	9.4	6.3
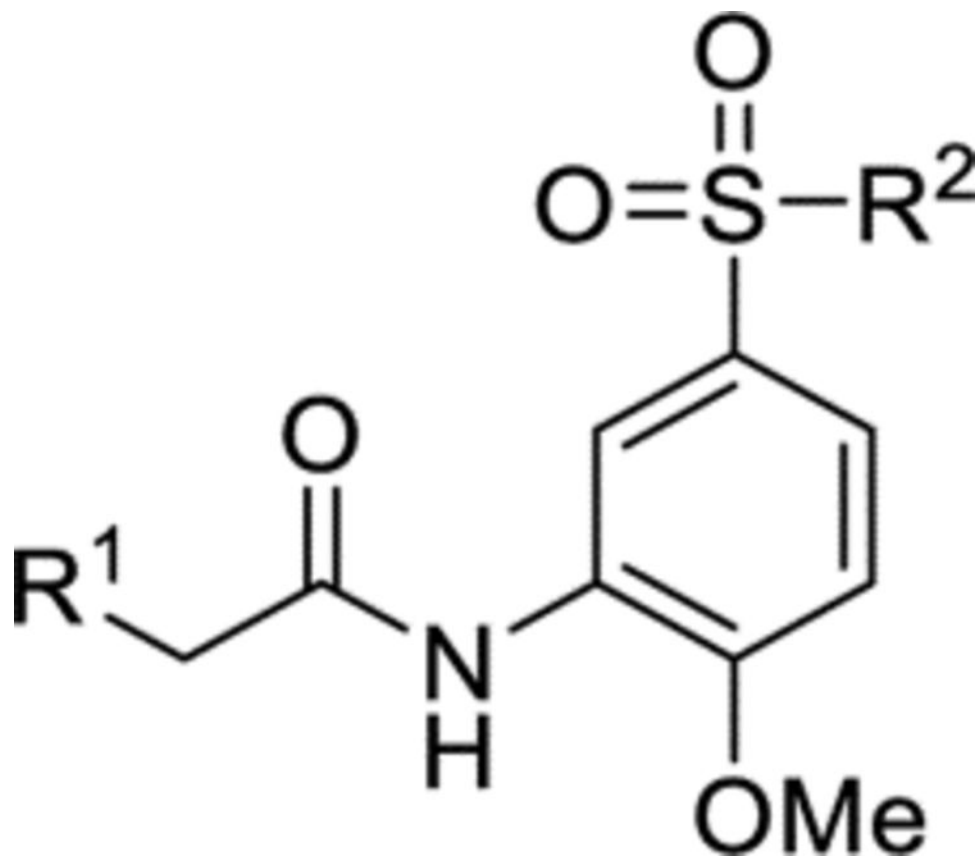
Compound	R¹	R²	cLogP[a]	H37Rv *Mtb* MIC (μM)			
				GAST-Fe	7H9/ADC	7H9/glucose	7H9/DPPC
16b			2.1	> 100	> 100	25	19

[a] *Calculated* using ChemDraw (PerkinElmer, Waltham MA).