Synthesis and Evaluation of 1-((Benzo[b]thiophen-2-yl)cyclohexyl)piperidine (BTCP) Analogues as Inhibitors of Trypanothione Reductase

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Thirty two analogues of phencyclidine were synthesised and tested as inhibitors of trypanothione reductase (TryR), a potential drug target in trypanosome and leishmania parasites. The lead compound BTCP (1, 1-(1-benzo[b]thiophen-2-yl-cyclohexyl)-piperidine) was found to be a competitive inhibitor of the enzyme \( (K_i = 1 \mu M) \) and biologically active against bloodstream \( T. brucei \) \( (EC_{50} = 10 \mu M) \), but with poor selectivity against mammalian MRC5 cells \( (EC_{50} = 29 \mu M) \). Analogues with improved enzymatic and biological activity were obtained. The structure–activity relationships of this novel series are discussed.

Introduction

Parasites of the order Kinetoplastida are the causative agents of a number of human and animal diseases including Human African Trypanosomiasis (HAT) (caused by \( Trypanosoma brucei rhodesiense \) and \( T. b. gambiense \)), Chagas’ disease \( (T. cruzi) \) and the leishmaniases \( (Leishmania \) sp.). Collectively these diseases have a large unmet disease burden, with the current therapeutics used to treat them possessing severe limitations. All of these trypanosomatid parasites use a trypanothione-based redox metabolism, which is absent in humans. The enzymes of this redox pathway are therefore considered to be attractive targets for the development of new antitrypanosomatid drugs.

One component of the trypanothione-based redox pathway is trypanothione reductase (TryR), which is responsible for reducing trypanothione disulfide to the diithiol trypanothione and in doing so provides reducing equivalents to protect the parasites from oxidative damage. In \( T. brucei \) it has been demonstrated that TryR activity is required for parasites to grow in culture and to be infective in a mouse disease model. Therefore, TryR is a validated drug target, and there are a number of recent reports outlining the discovery and development of inhibitors of this key enzyme.

A recently reported high-throughput screening (HTS) of known bioactive compounds against \( T. cruzi \) TryR identified a number of novel TryR inhibitors including the arylcyclohexylamine BTCP (1, 1-(1-benzo[b]thiophen-2-yl-cyclohexyl)-piperidine). BTCP (1) is an analogue of the anaesthetic drug PCP (2, 1-(1-phenyl-cyclohexyl)-piperidine, phenylcyclidine). However, despite the structural similarity between compounds 1 and 2, they have been shown to possess a different pharmacological selectivity. BTCP (1) is a more potent dopamine uptake inhibitor and has a much lower affinity for the PCP receptor.

BTCP (1) was considered to be a promising screening hit for further development due to its low molecular weight \( (299) \), low micromolar potency against \( T. cruzi \) TryR \( (IC_{50} = 3.7 \mu M) \), a promising ligand efficiency \( (0.35 \text{ kcal mol}^{-1} \text{L}) \), lack of activity against the human homologue of TryR, glutathione reductase (GR), and the fact that phencyclidines are known to cross the blood–brain barrier, an essential property for the successful treatment of stage 2 HAT. BTCP (1) also has the advantage of being a druglike molecule, in contrast to some of the more potent reported TryR inhibitors, many of which are polyamine analogues designed to mimic the spermidine moiety of the enzyme substrate trypanothione. In addition, there are a number of publications relating to BTCP (1) and other phencyclidines detailing both synthetic strategies for analogue synthesis and their associated pharmacological activities.

Due to the limitations of the current treatments for HAT, there is a need for the identification of new compound classes displaying antitrypanosomal activity. Therefore, a systematic structure–activity relationship (SAR) analysis of BTCP (1) was undertaken to optimise activity against both TryR and the intact parasite \( T. brucei \). The results of these investigations are reported herein.

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Results and Discussion

Biological characterisation of BTCP

In order to determine the validity of BTCP (1) as a starting point for a target-driven approach towards the identification of a lead compound for the treatment of HAT, the inhibitory activity of BTCP against T. brucei TryR had to be determined. BTCP (1) was assayed against T. brucei TryR using a HTS format based on a published nonenzymatically coupled assay\[10] and found to have an EC\textsubscript{50} value of 3.3 \mu M, confirming its suitability for further investigation. There is no significant difference between the IC\textsubscript{50} values for 1 against T. cruzi (IC\textsubscript{50} = 3.7 \mu M) and T. brucei TryR (IC\textsubscript{50} = 3.3 \mu M), which is as expected given the high degree of sequence identity between TryR in the two species (83\% at the amino acid level). A more detailed kinetic analysis established that BTCP is a linear competitive inhibitor of TryR (with respect to trypanothione), with a \( K_i \) value of 1.00 \pm 0.08 \mu M, in good agreement with the IC\textsubscript{50} value determined in the HTS-format TryR assay.

BTCP (1) was assayed against bloodstream form T. brucei brucei cells in a HTS-assay format and found to have an EC\textsubscript{50} value of 10 \mu M, in close agreement with the previously published EC\textsubscript{50} value of 14 \mu M.\[17] BTCP (1) was screened against MRC-5 cells in the same 96-well format as for the trypanosome assay giving an EC\textsubscript{50} value of 29 \mu M. Unfortunately, the threefold selectivity between MRC-5 and T. brucei is suboptimal, but the selectivity is sufficient to warrant further development of the compound series.

Synthesis of BTCP analogues

There are insufficient commercially available analogues of BTCP (1) to establish a comprehensive SAR. Therefore, a chemical synthesis programme was required to support the development of the hit compound. Initial synthetic studies focussed on preparing a diverse collection of BTCP analogues systematically modifying the benzo[b]thiophene group, the piperidine ring and the cyclohexyl ring (Table 1). In particular we were interested in carrying out the following modifications to probe for new interactions with the protein: changing the benzo[b]-thiophene to other aromatic rings, both monocyclic and bicyclic; modifying the size of the piperidine ring and putting heteroatoms into the ring; modifying the size of the cyclohexyl ring and adding substituents to it.

Two different synthetic methodologies were employed to prepare the initial collection: first, addition of aryl lithiis to the benzotriazole addsucts of enamines\[11] (Scheme 1, route A); and second, the reaction of aryl Grignards with \( \alpha \)-amino nitriles (the Bruylants reaction,\[12] Scheme 1, route B). Route A was successfully employed in reactions where the aryl group was an unsubstituted monomeric aromatic (2 & 3), or when the aryl group was a 5/6 fused bicyclic aromatic (e.g. benzo[\( \beta \)]thiophene, compounds 10, 13–15 & 17). The only exception to the latter observation was that when 1-methylindole was employed in the reaction only a trace amount of the target mole-

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### Table 1. Analogues of BTCP (1) and their inhibitory activities against T. brucei TryR and in cell-based assays. See Scheme 1 for the structure of analogues 1–19 and Scheme 2 for 23–25.

| Compd | Ar | X | Y | \( n_1 \) | \( n_2 \) | TryR IC\textsubscript{50} [\mu M] | T. brucei IC\textsubscript{50} [\mu M] |
|-------|----|---|---|---------|---------|----------------|----------------|
| 1 (BTCP) | 2-Benzol[b]thiophene | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | 3.3\textsuperscript{[a]} | 10\textsuperscript{[b]} |
| 2 (PCP) | Benzene | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | 1.1 | ND |
| 3 | 2-Thiophene | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | >100 | ND |
| 4 | 4-Phenyl-benzene | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | >100 | ND |
| 5 | 2-Benzol[b]furan | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | 4.4\textsuperscript{[c]} | 18 |
| 6 | 1-Naphthylene | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | >100 | ND |
| 7 | 2-Naphthylene | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | 28 | ND |
| 8 | (1-Methylindole) | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | 36 | ND |
| 9 | 2-Benzol[b]thiazole | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | >100 | ND |
| 10 | 3-Benzol[b]thiophene | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | 60 | ND |
| 11 | 2-(3-Bromobenzol[b]thiophene) | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | 16 | ND |
| 12 | 2-(5-Bromobenzol[b]thiophene) | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | >100 | ND |
| 13 | 2-Benzol[b]thiophene | CH\textsubscript{3} | CH\textsubscript{3} | 0 | 1 | 0.91\textsuperscript{[d]} | 5.0 |
| 14 | 2-Benzol[b]thiophene – CH\textsubscript{3} | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | 5.0 | 13\textsuperscript{[d]} |
| 15 | 2-Benzol[b]thiophene | O | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | 11 | 37 |
| 16 | 2-Benzol[b]thiophene | NCH\textsubscript{3} | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | 10 | 2.1\textsuperscript{[e]} |
| 17 | 2-Benzol[b]thiophene | CH\textsubscript{2} | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 0 | 11 | 35 |
| 18 | 2-Benzol[b]thiophene | CH\textsubscript{3} | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | 0.93 \textsuperscript{[f]} | 15\textsuperscript{[f]} |
| 19 | 2-Benzol[b]thiophene | CH\textsubscript{3} | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | 15 | 27 |
| 23 | n/a | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | >100 | ND |
| 24 | n/a | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | >100 | ND |
| 25 | n/a | CH\textsubscript{3} | CH\textsubscript{3} | 1 | 1 | >100 | ND |

[a] TryR \( K = 1.00 \mu M \). [b] MRC-5 EC\textsubscript{50} 29 \mu M. [c] TryR \( K = 1.46 \mu M \). [d] TryR \( K = 0.26 \mu M \). [e] MRC-5 EC\textsubscript{50} 22 \mu M. [f] MRC-5 EC\textsubscript{50} 50 \mu M. [g] MRC-5 EC\textsubscript{50} 15 \mu M. ND = not determined. n/a not applicable, structures shown in full.

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Scheme 1. Routes to BTCP analogues 2–19\[11, 14, 15\]. See Table 1 for details of analogue structures. Reagents and conditions: a) 1, H-benzotriazole, Et\textsubscript{2}O, 25 °C, 1 h; b) AcLi, Et\textsubscript{2}O, 0–25 °C, 16 h; c) acetone cyanohydrin, DMF, MgSO\textsubscript{4}, 50 °C, 2–4 d; d) ArMgBr, Et\textsubscript{2}O, 35 °C, 16 h.
Analogues 2–25 were tested for their ability to inhibit *T. brucei* TryR (Table 1) using the HTS assay format previously employed to assay BTCP (1). None of the aryl analogues (compounds 2–12) showed an improvement in potency over the hit compound 1. Analogues where the benzo[b]thiophene was replaced with a monocyclic aromatic (compounds 2–4) showed a dramatic reduction in potency against TryR (IC_{50} values 57 to >100 μM), suggesting a requirement for a fused bicyclic aromatic moiety for optimal inhibitor binding. The inhibition values from analogues containing alternative fused bicyclic systems (compounds 5–10) suggest that there is a very specific requirement for a 2-benzo[b]thiophene substitution, as demonstrated by testing close isosteres such as 2-naphthyl (compound 7, IC_{50} = 28 μM vs 3.3 μM) and analogues containing minor changes in inhibitor structure for example, compound 9 where the benzo[b]thiophene is replaced with a benzo[b]thiazole (IC_{50} > 100 μM). Indeed, with the exception of replacing 2-benzo[b]thiophene with 2-benzo[b]furan (compound 5) all of the aromatic analogues of BTCP (1) were at least one order of magnitude less potent against *T. brucei* TryR (IC_{50} values 28 to >100 μM). The screening results for analogues 11 and 12 demonstrate that it is not possible to substitute 2-benzo[b]thiophene at the 5 position, but that substitution at the 3-position gives analogues that retain some activity, albeit reduced. Given these results no further exploration of the aromatic moiety was conducted and all subsequent analogues would incorporate the 2-benzo[b]thiophene functionality.

Analogues 13–16 were prepared to investigate the effect of changing the piperidine ring of BTCP (1). Exchanging the piperidine for a morpholine or pipеразине ring (compounds 15 & 16) results in a threefold reduction in potency (Table 1), possibly due to the attenuated basicity of the nitrogen atom, or due to the introduction of an additional polar atom (or a combination of both). The acyclic diethylamino analogue (14) is of approximately equal potency to the hit compound 1 (IC_{50} = 5.0 μM vs 3.3 μM). Unfortunately, attempts to prepare more highly substituted acyclic analogues of 1 using route B (Scheme 1) proved unsuccessful. The pyrrolidine-containing analogue 13 was marginally more potent than the hit compound (1) (IC_{50} = 0.91 μM vs 3.3 μM). A full kinetic analysis of analogue 13 showed it to be a linear competitive inhibitor with respect to trypanothione (K_{i} = 0.26±0.01 μM vs 1 μM for BTCP), confirming this mode of inhibition within the BTCP compound series (Figure 1). However, this fourfold increase in potency did not warrant any additional investigation into replacing the piperidine moiety.

The investigation of BTCP cyclohexyl-analogues was limited by synthetic considerations, with just three analogues (17–19) being prepared. Altering the cyclohexyl moiety by either ring contraction to a cyclopentane ring (17), or by replacement with a gem dimethyl substitution (19) gave analogues that were three or fivefold less potent, respectively. This suggests that the cyclohexane ring contributes to inhibitory activity by either hydrophobic interactions, or by controlling the orientation by which the other moieties are presented to the protein. The amine-containing analogue 18 showed a slight improvement in potency (IC_{50} = 0.93 μM vs 3.3 μM) suggesting that it may be possible to introduce a substituted nitrogen at the 4-position of the cyclohexane moiety. Additionally, it may be possible to substitute a carbon atom at the 4 position.

The “spacer”-containing analogues 23–25 were all found to be inactive in the *T. brucei* TryR assay (IC_{50} > 100 μM). Therefore,
direct attachment of the aromatic moiety to the cyclohexyl-piperidine core is probably an absolute requirement for TryR inhibition within this series. The inactivity of these analogues combined with the failure to significantly increase potency by substitution of the aromatic, or piperidine moieties, meant that substitution at the 4-position of the cyclohexyl ring became the only focus of further investigations (see below).

Cell-based assays of BTCP analogues

A subset of the analogues prepared as part of the initial diverse BTCP analogue collection (compounds 1, 5 & 13–19) were assayed for their ability to inhibit the growth of T. brucei in culture (Table 1). With the exception of compound 16, the analogues displayed a decrease in potency between the enzyme and cellular assays of between 2- and 15-fold. Although it is not possible to draw a reliable correlation with this small subset, this level of decrease and its consistency between analogues suggests that inhibition of TryR could be the cause of the inhibition of parasite growth and that it is not the result of an off-target effect.

Additional analogues (14, 16 & 18) were subjected to the MRC-5 counter screen and their selectivity between MRC-5 cells and T. brucei was found to be ~1- to >20-fold. Although this low selectivity is disadvantageous, it may increase in analogues with improved inhibitory activity against TryR.

Synthesis and TryR assay of BTCP analogues substituted at the 4-position of the cyclohexyl ring

Two strategies were employed to functionalise the 4-position of the cyclohexyl moiety; first, preparation of a bipiperidinyl analogue (28), with subsequent derivatisation of the nitrogen atom, allowing the synthesis of a number of analogues with a minimal number of synthetic transformations (Scheme 3); and second, a stepwise preparation of cis and trans 38 containing a tert-butyl substitution at C4 of the cyclohexane ring (Scheme 4).

In order to prepare the bipiperidinyl 28 it was necessary to employ a suitable protecting group for the nitrogen atom. Previously it has been reported that both the benzyl and benzoyl nitrogen protecting groups are unsuitable for the preparation of substituted phencyclidines. Therefore, the Boc protecting group was employed during the Bruylants reaction giving the key protected intermediate 27 (Scheme 3). The Boc group of 27 was deprotected under acidic conditions to yield the secondary amine 28, which subsequently underwent either acylation or alkylation reactions to give the substituted analogues 29–
However, the alkylation reactions proved problematic leading to the formation of significant quantities of quaternary ammonium salts as side products, which proved difficult to separate from the tertiary amines by column chromatography. Therefore, LiAlH₄ reduction of the amide analogues 30 and 32 was used to prepare the tertiary amine analogues 34 and 35, respectively.

Analogues 27–35 were assayed for their ability to inhibit T. brucei TryR as described above and the results are displayed in Table 2. The free amine 28 was approximately equal in activity to BTCP (IC₅₀ = 5.1 μM vs 3.3 μM), suggesting that the increased activity of the N-methyl analogue 18 is derived from the introduction of the methyl group, not through the introduction of a hydrogen bond donor. However, analogues containing larger hydrophobic amide or alkyl substitutions (analogues 29–31 & 34) all possessed reduced inhibitory activity (IC₅₀ = 6.6–19 μM). Similarly the Boc protected precursor 27 proved to be completely inactive in the TryR assay (IC₅₀ > 100 μM). This demonstrates that the 4-position of the cyclohexane ring of BTCP (1) is not fully occluded by TryR upon inhibitor binding, but that the protein region around this position does not form favourable hydrophobic interactions. This conclusion is supported by the fact that analogues 32 and 33 containing polar substitutions were found to be approximately equipotent with BTCP (1) (IC₅₀ = 2.6 μM and 4.4 μM, respectively vs 3.3 μM), and of similar potency to the N-methyl analogue 18. Analogue 35 was found to be inactive in the TryR assay consistent with the results observed for 32 and 33. However, this lack of activity could be due to 35 being the only analogue to contain three highly basic atoms.

Analogues 28 and 30–34 were assayed against T. brucei parasites and MRC-5 cells (Table 2). With the exception of compound 32, all of the analogues showed some degree of selectivity against the parasites (>2-fold). However, as observed with the N-methyl analogue 16, compounds 28, 30, 31 and 34 showed improved potency in the T. brucei assay over the enzyme assay. This is suggestive of either selective uptake, or an off-target effect for these analogues.

Analogues containing alkyl substitutions at C4 of the cyclohexyl ring have been previously prepared by employing either the Bruylants reaction (Scheme 1, route B), or in a stepwise sequence from tertiary benzylic alcohols (e.g. 36) (Scheme 4). It has been demonstrated that the Bruylants reaction gives only a single isomer (cis) when 4-substituted α-aminonitriles are used as the substrates for the reaction. However, there was an interest in assaying both isomers of 38 as they have been shown to possess a different pharmacological selectivity and could offer an insight into the optimal arrangement of the piperidine ring, aromatic group and 4-cyclohexyl substituent relative to each other for the inhibition of TryR. Therefore, in order to access both isomers, a modification of the published synthetic route outlined in Scheme 4 was employed. The two isomers, cis- and trans-38, were separated by column chromatography at the final step. It has been demonstrated that the cis isomer elutes first when the mixture is purified with silica as the stationary phase.

Cis- and trans-38 were assayed for their ability to inhibit TryR under the standard assay conditions and found to have IC₅₀ values of >100 μM and 3.6 μM, respectively. This demonstrates that there is an absolute requirement for the piperidine moiety to be equatorial and conversely for the aromatic moiety to be in an axial conformation in order for BTCP analogues to inhibit TryR. Additionally, these results show that substituting BTCP with a bulky tert-butyl group at the 4-position of the cyclohexane ring leads to no appreciable change in TryR inhibitory activity (3.6 μM vs 3.3 μM for 1), supporting the conclusion that the 4-position is not occluded by the protein structure upon binding of the inhibitor with TryR. Trans-38 was also screened in the cell assay and found to have an EC₅₀ value of 3.2 μM against T. brucei and inactive against the mammalian cell line (EC₅₀ > 15 μM), again comparable to 1.

Conclusion

The investigations reported herein have confirmed that analogues of BTCP (1) represent a new class of TryR inhibitors, which are to our knowledge structurally distinct from inhibitors previously reported in the literature. Enzyme and cellular assays have demonstrated that analogues of this series are competitive inhibitors with respect to the natural TryR substrate, trypanothione, and that the analogues are marginally more potent against trypanosomes than mammalian cells in culture.

Synthesis and screening of a diverse analogue collection has allowed a detailed SAR to be established for all moieties of the arylocylohexylamine pharmacophore (Figure 2). However, although the essential structural features for maintaining the inhibitory activity of BTCP analogues have been determined, no functional group changes that significantly increase the potency against TryR have been identified.

From the rough correlation between T. brucei TryR IC₅₀ and T. brucei EC₅₀ values it is expected that TryR inhibitors in the single nanomolar range will be a requisite for adequate inhibition of parasite growth. However, given the preliminary SAR this goal is unlikely to be realised without the aid of a protein–ligand structure to identify potentially beneficial binding interactions. However, no noncovalent protein–ligand structures...
have been reported for TryR. Although it has been demonstrated that submicromolar inhibitors of TryR can be developed, these inhibitors are not considered druglike (e.g. MW > 500). This requirement for high molecular weight compounds to efficiently inhibit TryR may be a direct consequence of TryR possessing a large, solvent-exposed active site. To date, druglike molecules have only achieved potencies in the low micromolar range, unfortunately this remains true for the BTCP series.

Experimental Section

Biology

TryR enzyme assay

A nonenzymatically coupled assay for detecting TryR activity was used. In this assay, the activity of TryR is coupled to the reduction of DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) to 2TNB by dihydrotrepanothione (TSH\(_2\)). Formation of 2TNB is measured as an increase in absorbance at 412 nm (Figure 3). The TryR screening assay was miniaturised and optimised to a 384-well plate format. Assessment of the assay for robustness in an automated environment yielded the following typical performance statistics: \(Z' = 0.84 \pm 0.001\); %CV (plate) = 3.65 ± 0.4; signal to background = 10 ± 0.25; clomipramine IC\(_{50}\) = 12.4 ± 0.14 \(\mu\)M.

Potency was determined as independent duplicates for all compounds tested. Serial titrations (10 half log increments) of test compounds from 30 \(\mu\)M to 1 \(n\)M were created in DMSO using the Janus automated 8 channel pipettor (Perkin–Elmer). A serial titration of clomipramine was used as a positive control in each assay plate; BTCP was used as an additional control in some screening plates. Using a Platemate Plus (Thermofisher Scientific), 500 nL of each test compound was transferred into assay plates (384 clear polystyrene plates) along with standard inhibitor and DMSO in the appropriate control wells. A TryR/DTNB/TrySH mixture (3.75 \(\mu\)L in buffer containing 40 mm HEPES and 1 mm Na\(_2\)EDTA, pH 7.4) was then added to each well (Platemate Plus, Thermofisher Scientific) such that final assay concentrations were 3 \(n\)M, 50 \(\mu\)M and 6 \(\mu\)M, respectively. The reaction was started by addition of 4 \(\mu\)L NADPH (4 \(\mu\)L buffer for LO controls), to yield a final assay concentration 150 \(\mu\)M. The reaction was incubated for 35 min at room temperature. The absorbance was then measured at 405 ± 8 nm using the Envision plate reader (Perkin–Elmer).

ActivityBase from IDBS was used for all data processing and analysis. Database querying and report creation was undertaken using SARgen version 3.4 and SARPview version 6.1 from IDBS.

Cell-based assays

Trypanosomes (T. b. brucei, BSF 427 vsg221) were seeded in 96-well plates at 2000 cells per well in a volume of 200 \(\mu\)L of HMI-9 containing 10% FCS. MRC-5 cells were seeded at 2000 cells per well in a volume of 200 \(\mu\)L of DMEM containing 10% FCS and allowed to adhere for 24 h prior to use. For compound assessment, compounds were serially diluted in 100% DMSO through a ten-point, one in three dilution curve, in row orientation using a Janus 8 channel Varispan. This produced a working stock of 200 \(\times\) final concentration in the assay. Compound plates contained six test compounds and one standard compound occupying columns 1–10: row A was omitted from screening due to potential edge effect and row H contained the standard compound. Each compound working stock (1 \(\mu\)L) was then stamped into duplicate clear 96-well polystyrene assay plates using a PlateMate 2 (Matrix-Thermofisher) to achieve the final assay concentration of 0.5% DMSO level. Plates were read for fluorescence at an excitation wavelength of 528 nm and an emission wavelength of 590 nm.

Mode of inhibition studies

An assay mixture consisting of TryR, NADPH and DTNB was made up in 40 mm HEPES; 1 mm EDTA (pH 7.4). Aliquots of the assay mixture (180 \(\mu\)L) containing three different concentrations of test compound were added to three rows of a microtitre plate, a fourth row contained only the assay mixture. The test compound concentration ranged from –0.25 to 1 times the IC\(_{50}\) value. Trypanothione disulfide was serially diluted across a fifth row of the plate to produce a 12-point range from 500 \(\mu\)M to 5.8 \(\mu\)M. The assay was initiated by transferring 20 \(\mu\)L of trypanothione disulfide row to each of the assay rows. The final 200 \(\mu\)L assay contained 150 \(\mu\)M NADPH; 50 \(\mu\)M DTNB and 20 \(m\)M TryR. The linear rate of increase in absorbance at 412 nm was determined using a Molecular Devices Thermomax plate reader. Each data set was fitted by nonlinear regression to the Michaelis–Menten equation using GraFit 5.0 (Erthacaus software). The resulting individual fits were examined as Lineweaver–Burke transformations and the graphs inspected for diagnostic inhibition patterns. The entire dataset was then globally fitted to the appropriate equation (competitive, mixed or uncompetitive inhibition).

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**Figure 2.** SAR summary for the inhibition of T. brucei TryR by BTCP (1) analogues.
Chemistry

General: Chemicals and solvents were purchased from the Aldrich Chemical Company, Fluka, ABCR, VWR, Acros, Fisher Chemicals and Alfa Aesar and were used as received unless otherwise stated. Air and moisture sensitive reactions were carried out under an inert atmosphere of Ar in oven-dried glassware. Analytical thin-layer chromatography (TLC) was performed with precoated TLC plates (0.20 mm silica gel60 with fluorescent indicator UV 254) (Merck). Plates were air-dried and visualized under a UV lamp (UV254/365 nm), and where necessary, stained with a solution of ninhydrin or iodine on silica to aid identification. Flash column chromatography was performed using precoated silica gel cartridges (230–400 mesh, 40–63 μm) (Silicycle) using a Teledyne ISCO CombiFlash Companion or CombiFlash Retrieve. 1H NMR, 13C NMR, and 2D-NMR spectra were recorded on a Bruker Avance DPX 500 spectrometer (1H at 500.1 MHz, 13C at 125.8 MHz). Chemical shifts (δ) are expressed in ppm recorded using the residual solvent as the internal reference in all cases. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), or a combination thereof. Coupling constants (J) are quoted to the nearest 0.5 Hz. LC/MS analyses were performed with either an Agilent Technologies 1100 series liquid chromatography system or an Agilent Technologies 1200 series HPLC connected to an Agilent MSD for the mass spectra. Coupling constants (J) are expressed in Hz. LC/MS analyses were performed with either an Agilent Technologies 1100 series HPLC system or a combination thereof. HRMS analyses were performed with either a Bruker Daltonics MicrOTOF mass spectrometer.

Procedures for the synthesis of BTCP analogues

Method A (compounds 3, 5, 9–15, 1714) nBuLi (1.6 M in hexanes, 4 eq) was added to a solution of the corresponding heteroaromatic compound (4 eq) in anhyd THF (10 mL) at –78 °C and stirred for 1 h. The resultant ArLi solution was then added via a cannula to an ice-cooled solution of the relevant benzotriazoyl adduct prepared by stirring the corresponding enamine (1 eq) and benzotriazole in an anhydrous MeCN (5 mL) for 1 h. The reaction mixture was then cooled to 0 °C prior to the addition of phenyllithium (1.8 M in dibutyl ether, 4 eq, 2.2 mL). The reaction mixture was allowed to warm to RT and stirred for 15 h. The reaction was worked up and purified as described for method A above to give a clear gum (36 mg, 15%), which was further purified by titration of the HCl salt from Et2O. The reported analysis is for the HCl salt. 1H NMR (500 MHz, CD3OD): δ = 1.20–1.31 (3H, m, CH2CH2CH2, & 2 × CH2), 1.66–1.68 (1H, m, CH2), 1.78–1.81 (1H, m, CH2), 1.88–2.03 (8H, m, 2 × CH2CH = CH2, 3 × CH2), 2.55–2.59 (2H, m, 2 × NHCCH), 2.67–2.98 (2H, m, 2 × CH2CH), 3.78–3.80 (2H, m, 2 × NHCCH), 7.27 (1H, d, J = 5.0, 4.0 Hz, thiophene H4), 7.44 (1H, d, J = 4.0 Hz, thiophene H3), 7.74 ppm (1H, d, J = 4.0 Hz, thiophene H2). 13C NMR (125 MHz, CD3OD): δ = 23.1 (CH3), 24.3 (CH3), 24.8 (CH3), 25.6 (CH3), 34.7 (CH2Cl), 48.5 (CH2), 88.5 (s, CH2CH, DEPT135 & HSQC), 71.3 (C), 126.2 (thiophene C4), 130.1 (thiophene C5), 132.5 (thiophene C3), 136.8 ppm (thiophene C2). MS (LCMS ES+): m/z (%) 165 (50) [M–Piperidine]+, 244 (100) [M+H]+. HREMS (ES+): calcd for C17H16N2, [M+H]+ 244.2060, found 244.2059 (0.28 ppm).

1-(1-Thiophen-2-yl)cyclohexyl)piperidine 3: Prepared by meth- od A from thioephene (4 mmol, 337 mg) and 1-(1-piperidino)cyclohexene (1 mmol, 165 mg). The product was obtained as an brown oil (158 mg, 63%). The reported analysis is for the HCl salt. 1H NMR (500 MHz, CD3OD): δ = 1.30–1.45 (4H, m, CH2CH2CH2, & 2 × CH2), 1.66–1.68 (1H, m, CH2), 1.78–1.81 (1H, m, CH2), 1.88–2.03 (8H, m, 2 × CH2CH = CH2, 3 × CH2), 2.55–2.59 (2H, m, 2 × NHCCH), 2.67–2.98 (2H, m, 2 × CH2CH), 3.78–3.80 (2H, m, 2 × NHCCH), 7.27 (1H, d, J = 5.0, 4.0 Hz, thiophene H4), 7.44 (1H, d, J = 4.0 Hz, thiophene H3), 7.74 ppm (1H, d, J = 4.0 Hz, thiophene H2). 13C NMR (125 MHz, CD3OD): δ = 23.1 (CH3), 24.3 (CH3), 24.8 (CH3), 25.6 (CH3), 34.7 (CH2Cl), 48.5 (CH2), 88.5 (s, CH2CH, DEPT135 & HSQC), 71.3 (C), 126.2 (thiophene C4), 130.1 (thiophene C5), 132.5 (thiophene C3), 136.8 ppm (thiophene C2). MS (LCMS ES+): m/z (%) 165 (50) [M–Piperidine]+, 250 (100) [M+H]+. HREMS (ES+): calcd for C17H16N2, [M+H]+ 250.1624, found 250.1622 (0.89 ppm).

1-(1-Pheophen-2-yl)cyclohexyl)piperidine 4: Prepared by meth- od B1 from 4-bromobiphenyl (15.5 mmol, 365 mg). The product was obtained as a colourless crystalline solid (1.04 g, 21%). The reported analysis is for the free base. 1H NMR (500 MHz, CDCl3): δ = 1.28–1.40 (4H, m, 2 × CH2CH=CH2, 1.45–1.57 (6H, m, 2 × CH2, & 2 × CHH), 1.73–1.80 (2H, m, 2 × CHH), 2.00–2.07 (2H, m, 2 × CH2CH), 2.16–2.22 (2H, m, 2 × CH2CH), 2.27–2.39 (2H, m, 2 × NHCCH), 3.75–3.79 (3H, m, AA′BB′-p-PPh3), 7.47 (2H, t, J = 8.0 Hz, 2 × Ph2), 7.60–7.61 (2H, m, AA′BB′), 7.66 ppm (2H, dd, J = 8.0, 1.0 Hz, 2 × O2Ph2). 13C NMR (125 MHz, CDCl3): δ = 22.5 (CH2), 25.0 (CH2), 26.5 (CH3), 27.2 (CH3), 33.7 (CH2Cl), 46.5 (CH2), 60.9 (C), 126.0 (biphenyl CH), 127.0 (biphenyl CH), 127.1 (biphenyl CH), 127.8 (biphenyl CH), 128.7 (biphenyl CH), 138.6 (biphenyl C), 139.1 (biphenyl C), 140.9 ppm (biphenyl C). MS (LCMS ES+): m/z (%) 320 (100) [M+H]+. HREMS (ES+): calcd for C23H26N2, [M+H]+ 320.2373, found 320.2375 (−0.86 ppm).

Trypanothione Reductase Inhibitors

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1-(1-Benzoyl)[furan-2-yl]cyclohexyl)piperidine 5: Prepared by method A from benzo[b]furan (4 mmol, 473 mg) and 1-(1-piperidinomethyl)cyclohexene (10 mmol, 165 mg). The product was obtained as a yellow oil (239 mg, 84%). The reported analysis is for the HCl salt.

1H NMR (500 MHz, CD3OD): 6 = 1.21–1.40 (4 H, m, CH2CH2CH2), 1.48–1.57 (8 H, m, CH2CH2CH2, 2 × CH3, 2 × CH2, 1.48–1.57 (8 H, m, CH2CH2CH2, 2 × CH3, 1.48–1.57 (8 H, m, CH2CH2CH2, 2 × CH3, 1.63–1.71 (3 H, m, 3 × CH2), 1.83–1.90 (2 H, m, 2 × CH2CH), 2.22–2.27 (2 H, m, 2 × CH2CH), 2.55–2.57 (4 H, m, 2 × NCH2), 4.09 (1H, s, CH2), 6.52 (1H, s, indole H3), 7.10 (1H, ddd, J = 7.5, 1.0 Hz, indole H5), 7.20 (1H, ddd, J = 8.0, 7.5, 1.0 Hz, indole H6), 7.32 (1H, d, J = 8.0 Hz, indole H7), 7.57 (1H, d, J = 7.5 Hz, indole H4). 13C NMR (125 MHz, CDCl3): 6 = 2.00 (CH3), 29.8 (CH2), 31.5 (CH2), 42.8 (NCH2), 72.5 (CH2), 106.6 (benzo[b]furan CH), 122.0 (CH2), 122.6 (CH2), 123.0 (CH2), 136.0 (CH2), 148.6 (CH), 149.7 (CH), 150.8 (CH), 165.3 (CO). MS (LCMS ES+): m/z (%) 309 (100) [M+H]+. HRMS (ES+): calcd for C19H17N5S [M+H]+ 309.2028, found 309.2029 (1.0 ppm).

1-(1-Benzoyl)[thiazoyl-2-yl]cyclohexyl)piperidine 9: Prepared by method A from benzo[b]thiazole (4 mmol, 541 mg) and 1-(1-piperidinocyclohexene (1 mmol, 165 mg). The product was obtained as a yellow semisolid (164 mg, 55%). The reported analysis is for the free base.

1H NMR (500 MHz, CDCl3): 6 = 1.38–1.48 (2 H, m, 2 × CH2), 2.01–2.08 (2 H, m, 2 × CH2), 2.68–2.73 (2 H, m, 2 × CH2), 3.84–3.87 (2 H, m, 2 × NCH2), 7.35–7.37 (1 H, s, indole H3), 7.57 (1H, d, J = 7.5 Hz, indole H4). 13C NMR (125 MHz, CDCl3): 6 = 26.7 (CH2), 32.4 (CH2), 46.8 (NCH2), 62.3 (CH), 104.8 (indole CH), 108.9 (benzo[b]furan CH), 119.0 (indole CH), 119.8 (benzo[b]furan CH), 120.8 (indole CH), 127.0 (indole C), 138.7 (indole C), 142.5 ppm (indole C) [Note, two of the CH3 carbons have an identical chemical shift]. MS (LCMS ES+): m/z (%) 322 (100) [M−piperidine]+. HRMS (ES+): calcd for C18H16N3 [M+H]+ 297.2325, found 297.2313 (4.16 ppm).

1-(1-Benzoyl)[thiazoyl-2-yl]cyclohexyl)piperidine 10 & 1-(1-[3-Bromo-benzo[b]thiazoyl-2-yl]cyclohexyl)piperidine 11: Prepared by method A from 3-bromobenzo[b]thiazole (4 mmol, 852 mg) and 1-(1-piperidinocyclohexene (1 mmol, 165 mg). The reaction gave two products that could be separated by column chromatography. The reported analysis is for the free bases.

For 10: Rf = 0.20 (EtOAc/hexanes, 1:1), clear oil (143 mg, 48%).

1H NMR (500 MHz, CDCl3): 6 = 1.38–1.40 (2 H, m, 2 × CH2), 1.50–1.80 (4 H, m, 2 × CH2), 2.00–2.05 (2 H, m, 2 × CH2), 2.50–2.55 (4 H, m, 2 × NCH2), 3.00–3.05 (2 H, m, 2 × NCH2), 7.28–7.33 (2 H, m, 2 × CH2), 7.38–7.43 (2 H, m, 2 × CH2), 7.53–7.58 (2 H, m, 2 × CH2), 7.60–7.65 (2 H, m, 2 × CH2), 7.69–7.74 (2 H, m, 2 × CH2), 7.79–7.84 (1 H, d, J = 8.0 Hz, benzo[b]furan H7), 8.08 (1H, d, J = 7.5 Hz, benzo[b]furan H8). 13C NMR (125 MHz, CDCl3): 6 = 26.7 (CH2), 32.5 (CH2), 46.8 (NCH2), 62.3 (CH), 104.8 (indole CH), 108.9 (benzo[b]furan CH), 119.0 (indole CH), 119.8 (benzo[b]furan CH), 120.8 (indole CH), 127.0 (indole C), 138.7 (indole C), 142.5 ppm (indole C) [Note, two of the CH3 carbons have an identical chemical shift]. MS (LCMS ES+): m/z (%) 322 (100) [M−piperidine]+. HRMS (ES+): calcd for C18H16N3S [M+H]+ 297.2325, found 297.2313 (4.16 ppm).

1-(1-Benzoyl)[thiazoyl-2-yl]cyclohexyl)piperidine 10 & 1-(1-[3-Bromo-benzo[b]thiazoyl-2-yl]cyclohexyl)piperidine 11: Prepared by method A from 3-bromobenzo[b]thiophene (4 mmol, 852 mg) and 1-(1-piperidinocyclohexene (1 mmol, 165 mg). The reaction gave two products that could be separated by column chromatography. The reported analysis is for the free bases.

For 11: Rf = 0.49 (EtOAc/hexanes, 1:1), clear oil (58 mg, 15%).

1H NMR (500 MHz, CDCl3): 6 = 1.21–1.26 (2 H, m, CH2), 1.35–1.49 (8 H, m, 2 × CH2), 1.64–1.73 (2 H, m, 2 × CH2), 1.93–2.00 (2 H, m, 2 × CH2), 2.49–2.62 (6 H, m, 2 × CH2CH2 & 2 × NCH2), 7.27–7.36 (2 H, m, benzo[b]thiophene H5 & H6), 7.67 (1H, d, J = 8.0 Hz, benzo[b]thiophene H7), 7.80 ppm (1H, d, J = 8.0 Hz, benzo[b]thiophene H8). MS (LCMS ES+): m/z (%) 237 (100) [M−piperidine]+, 255 (100) [M+H]+. HRMS (ES+): calcd for C18H16N3S [M+H]+ 309.2028, found 309.2029 (1.0 ppm).
1-(5-Bromo-benzo[thieno-2-yl])cyclohexyl)piperidine 12: Pre pared by method A from 5-bromobenzo[thieno (4 mmol, 852 mg, 3.7 molar equiv) and 2-cyclohexene (1 mmol, 165 mg). The product was obtained as a white solid (43 mg, 11%). The reported analysis is for the HCl salt. 1H NMR (500 MHz, CD3OD): δ = 1.37–1.43 (1H, m, CH(C=CH2)), 1.45–1.55 (2H, m, CH(C=CH2)), 1.69–1.74 (1H, m, CH(C=CH2)), 1.96–2.01 (2H, m, 2 × CH2CH2), 2.07–2.13 (2H, m, 2 × CH2CH2), 2.88–2.93 (2H, m, 2 × CH2CH2), 2.99–3.05 (2H, m, 2 × CH2CH2), 3.66–3.71 (2H, m, 2 × CH2CH2), 3.88–3.94 (2H, m, 2 × NCCH2), 7.49–7.53 (2H, m, 2 × benzothiophene H), 7.81 (1H, s, benzo[b] thiophene H3), 7.97–8.01 ppm (2H, m, 2 × benzothiophene H). 13C NMR (125 MHz, CD3OD): δ = 24.3 (CH2), 25.5 (CH3), 34.1 (CH2), 48.1 (OCH3), 65.2 (NCH3), 72.1 (C), 124.3 (benzo[b]thiophene CH), 125.9 (benzo[b]thiophene CH), 126.3 (benzo[b]thiophene CH), 127.3 (benzo[b]thiophene CH), 130.5 (benzo[b]thiophene CH3), 140.7 (benzo[b]thiophene CH), 141.7 ppm (benzo[b]thiophene CH). MS (LCMS ES+): m/z (%) 215 (100) [M–morpholine]+, 302 (12) [M+H]+. HRMS (ES+): calc. for C19H27N2S1 [M+H]+ 302.1573, found 302.1562 (3.71 ppm).

1-(Benzo[b]thieno-2-yl)cyclohexyl)-4-methylpiperazine 16: Prepared by method B2 from benzothiophene (27.5 mmol, 3.69 g) and nitrile 21 (10 mmol, 2.07 g). The product was obtained as a clear oil (12 mg, 0.4%). The reported analysis is for the free base. 1H NMR (500 MHz, CD3OD): δ = 1.44–1.51 (4H, m, cyclohexyl CHCH2 & 2 × CH2), 1.73–1.79 (2H, m, 2 × CH2), 2.01–2.14 (4H, m, 2 × CH2), 2.27 (2H, s, CH2), 2.45–2.67 (8H, 4 × piperazine CH2), 7.09 (1H, s, benzo[b]thiophene H3), 7.25–7.32 (2H, m, benzothiophene H5 & H6), 7.70 (1H, d, J = 7.5, 1H, benzothiophene H7), 7.75–7.76 ppm (1H, m, benzothiophene H8). 13C NMR (125 MHz, CDCl3): δ = 22.4 (CH2), 25.9 (CH3), 35.1 (CH2), 44.9 (NCH3), 45.6 (CH2), 55.9 (NCH3), 60.7 (C1), 121.4 (benzo[b]thiophene CH), 121.9 (benzo[b]thiophene CH), 123.7 (benzo[b]thiophene CH), 129.9 (benzo[b]thiophene CH), 130.9 (benzo[b]thiophene CH), 139.6 (benzo[b]thiophene CH), 147.7 ppm (piperazine CH). MS (LCMS ES+): m/z (%) 215 (62) [M–piperazine]+, 315 (100) [M+H]+. HRMS (ES+): calc for C19H27N2S1 [M+H]+ 315.1889, found 315.1882 (2.46 ppm).

1-(Benzo[b]thieno-2-yl)cyclopropenyl)piperidine 17: Prepared by method A from benzo[b]thiophene (4 mmol, 537 mg) and 1-(1-piperidino)cyclopropene (1 mmol, 151 mg). The product was obtained as a yellow semisolid (28 mg, 10%). The reported analysis is for the HCl salt. 1H NMR (500 MHz, CD3OD): δ = 1.15–1.24 (1H, m, CH(C=C)), 1.55–1.68 (3H, m, 2 × CH2 & 2 × CH(N)), 1.80–1.93 (6H, m, 2 × CH2 & 2 × CH(N)), 2.18–2.24 (2H, m, 2 × CH2), 2.74–2.85 (4H, m, 2 × CH2 & 2 × CH(N)), 3.59–3.64 (2H, m, 2 × CH2), 3.73–3.77 (2H, m, 2 × benzothiophene H), 7.69 (1H, s, benzo[b]thiophene H3), 7.80–7.85 ppm (2H, m, 2 × benzothiophene H). 13C NMR (125 MHz, CD3OD): δ = 22.7 (CH2), 22.9 (CH2), 24.6 (CH3), 38.2 (CCH2), 52.3 (NCH3), 77.3 (C), 123.3 (benzo[b]thiophene CH), 125.7 (benzo[b]thiophene CH), 126.2 (benzo[b]thiophene CH), 127.1 (benzo[b]thiophene CH), 130.1 (benzo[b]thiophene CH3), 138.0 (benzo[b]thiophene CH), 140.8 (benzo[b]thiophene CH), 141.6 ppm (benzo[b]thiophene CH). MS (LCMS ES+): m/z (%) 86 (100) [piperidine + H]+, 201 (82) [M–piperidine]+. HRMS (ES+): calc for C19H27N2S1 [M+H]+ 286.1624, found 286.1619 (1.81 ppm).

4-Benzothiophen-2-yl-1’-methyl-1,4’bi(piperidine) 18: Prepared by method B2 from benzothiophene (27.5 mmol, 3.69 g) and nitrile 22 (10 mmol, 2.07 g). The product was obtained as a white solid (87 mg, 3%). The reported analysis is for the free base. 1H NMR (500 MHz, CDCl3): δ = 1.30–1.35 (2H, m, CH2CH2), 1.52–1.57 (4H, m, CH2CH2), 2.23–2.27 (4H, m, 2 × CH2), 2.29 (2H, s, CH2), 2.36–2.46 (6H, m, 2 × CH2 & 2 × CH(N)), 2.72–2.76 (2H, m, 2 × CH2 & 2 × CH(N)), 7.04 (1H, s, benzo[b]thiophene H3), 7.28–7.35 (2H, m, benzo[b]thiophene H5 & H6), 7.74 (1H, d, J = 7.5 Hz, benzo[b]thiophene H7), 7.80 ppm (1H, d, J = 8.0, benzo[b]thiophene H4).
13C NMR (125 MHz, CDCl3): δ = 24.9 (CH2CH2CH2), 27.0 (CH2CH2CH2), 35.0 (CCH), 45.8 (C), 46.6 (NCH), 51.9 (CHNCH), 58.8 (C), 120.9 (benzof[2,1-b]thiophene C), 122.0 (benzof[2,1-b]thiophene C), 132.2 (benzof[2,1-b]thiophene C7), 138.3 (benzof[2,1-b]thiophene C), 139.6 (benzof[2,1-b]thiophene C), 147.0 ppm (benzof[2,1-b]thiophene C). MS (LCMS ES+): m/z (%) 230 (100) [M – piperidine]4+, 315 (9) [M + H]4+. HRMS (ES+): calcd for C17H11N5S6 [M + H]4+: 315.1889, found 315.1882 (2.35 ppm).

1-(2-Benzof[2,1-b]thiophen-2-yl)propan-2-ylpiperidine 19: Prepared by method A from benzof[2,1-b]thiophene (8 mmol, 1.07 g) and 1-(prop-1-en-2-y)lpropyldine (3.9 mmol, 250 mg). The product was obtained as a yellow oil (79 mg, 15%). The reported analysis is for the free base. 1H NMR (500 MHz, CDCl3): δ = 1.41–1.46 (2H, CH2CH2), 1.47 (6H, s, 2xCH3), 1.55–1.59 (4H, m, CH2CH2CH2), 2.48–2.55 (4H, m, 2xCH2), 7.04 (1H, s, benzof[2,1-b]thiophene H3), 7.23–7.31 (2H, m, benzof[2,1-b]thiophene H5 & H6), 7.66 (1H, d, J = 7.5 Hz, benzof[2,1-b]thiophene H7), 7.70 ppm (1H, d, J = 8.0 Hz, benzof[2,1-b]thiophene H4), 11C NMR (125 MHz, CDCl3): δ = 25.0 (CH3CH2CH2), 25.2 (CH2), 26.8 (CH2CH2), 47.7 (NCH3), 59.7 (C), 118.4 (benzof[2,1-b]thiophene C3), 122.2 (benzof[2,1-b]thiophene C4), 122.8 (benzof[2,1-b]thiophene C7), 123.5 (benzof[2,1-b]thiophene CH), 123.7 (benzof[2,1-b]thiophene C19), 139.8 (benzof[2,1-b]thiophene C), 139.9 (benzof[2,1-b]thiophene C), 185.9 ppm (benzof[2,1-b]thiophene C). MS (LCMS ES+): m/z (%) 175 [M – piperidine]4+, 260 [M + H]4+. HRMS (ES+): calcd for C17H11N5S6 [M + H]4+: 260.1467, found 260.1458 (3.77 ppm).

α-Amino nitriles (20, 21 & 22): Prepared following the α-amino nitrile synthesis described in reference [15] and used without further purification.

Phenylin-1-(piperidin-1-yl)cyclohexylmethanone 23: To a solution of nitrite 20 (5 mmol, 960 mg) in anhyd EtO (25 mL) at 78 °C was slowly added phenylthiiranium (6 mmol, 1.8 M solution in dibutylether, 3.33 mL) over 30 min. The reaction was then allowed to warm to 4 °C and stirred for 16 h. Aq HCl (10%, 20 mL) was then added to the reaction and the reaction further stirred for 30 min at 0 °C. The reaction was then diluted with EtOAc (50 mL), the layers separated and the organic layer extracted with 10% (NH4)2CO3 and extracted with CH2Cl2 (3 x 25 mL). The combined aqueous layers were basified to pH 10 (solid KOH) and extracted with CH2Cl2 (4 x 50 mL). The combined CH2Cl2 layers were dried (MgSO4), filtered and concentrated in vacuo. The resultant crude product was purified by flash column chromatography (EtOAc/Hexane, 1:0.1–1:50) to give a white solid (239 mg, 68%). The reported analysis is for the free base. 1H NMR (500 MHz, CDCl3): δ = 1.28–1.36 (1H, m, CH2CH2CH2), 1.43–1.93 (11H, m, 5xCH3 & CH2CH2), 2.21–2.29 (2H, m, 2xCHCH), 2.52–2.58 (2H, m, 2xCH), 3.07–3.19 (4H, m, 2xCH2N), 7.41 (2H, t, J = 7.5 Hz, 2xPhH), 7.49 (4H, dd, J = 7.5 Hz, 4xPh-H). 11C NMR (125 MHz, CDCl3): δ = 22.7 (CH3), 23.5 (CH2), 27.0 (CH2), 29.7 (CCH2), 54.7 (NCH3), 80.6 (C), 83.0 (C), 128.8 (o-Ph CH), 129.5 (p-Ph CH), 129.7 (m-Ph CH), 143.5 ppm (Ph CH). MS (LCMS ES+): m/z (%) 350 (100) [M + H]4+. HRMS (ES+): calcd for C17H14N2O5 [M + H]4+: 352.2478, found 352.2481 (0.83 ppm).

Diphenyl(1-(piperidin-1-yl)cyclohexyl)methanone 25: To a solution of ketone 20 (1 mmol, 271 mg) in anhyd EtO (10 mL) at 0 °C was added phenyllithium (1 mmol, 1.8 M solution in dibutylether, 556 µL) and the reaction was allowed to warm to 25 °C and stirred for 2.5 h. Workup was initiated by the addition of saturated aq NaHCO3 (10 mL), the layers were separated and the aqueous phase further extracted with EtO (3 x 10 mL), the combined organics were dried (MgSO4), filtered and concentrated in vacuo. The resultant crude product was purified by flash column chromatography (EtOAc/Hexane, 1:0.1–1:50) to give a white solid (239 mg, 68%). The reported analysis is for the free base. 1H NMR (500 MHz, CDCl3): δ = 1.47 (9H, s, bBu), 1.49–1.53 (2H, m, CH2CH2CH2), 1.61–1.72 (6H, m, CH2CH2CH2 & 2xCH2N), 2.11–2.16 (2H, m, 2xCH2), 2.56–2.64 (4H, m, 2xCH2), 3.12–3.21 (2H, m, 2xNbcOCH2), 3.89–4.06 ppm (2H, m, 2xNbcOCH2). 11C NMR (125 MHz, CDCl3): δ = 24.1 (CH2CH2CH2), 26.1 (CH2CH2CH2), 28.4 (bBuCH), 33.6 (CCH2) [broad peak due to restricted flexibility of the ring system], 39.6 & 40.4 (BocCH2) [two peaks due to restricted flexibility of the ring system], 47.7 (NCH3), 60.5 (C), 80.0 (bBuC), 118.3 (CN), 154.4 ppm (CO). MS (LCMS ES+): m/z (%) 211 (20) [M – bBu – CN + H]4+, 238 (64) [M – bBu + H]4+, 267 (18) [M – CN]4+, 294 (100) [M + H]4+. HRMS (ES+): calcd for C25H22N2O5 [M + H]4+: 354.1674, found 354.1672 (0.83 ppm).
tert-Butyl 4-[(benzo[thiophen-2-yl]-1,4'-bipiperidin-1'-carboxylic acid) 27: Prepared by method B2 from benzo[b]thiophene (55 mg, 0.25 mmol) and 1-benzylmorpholine hydrochloride (0.5 mmol, 93 mg) and the reaction mixture stirred at 0 °C for 2 h before the reaction was concentrated in vacuo. The crude secondary amine 28 was redissolved in anhyd pyridine (5 mL), before the addition of cat DMAP (1 mg) and the relevant acid chloride (4 eq) and the reaction mixture stirred at RT for 16 h. The reaction was concentrated in vacuo and the crude mixture partitioned between CH2Cl2 (5 mL) and aq NaOH (2 mL, 5 mL) and further worked up and purified as described for 28 above.

4-[(Benzo[b]thiophen-2-yl]-1,4'-bipiperidin-1'-yl)ethanol 29: Prepared following the general acylation procedure using AcCl (1 mmol, 78.5 mg) to give a brown glass (58 mg, 68%). The reported analysis is for the HCl salt. 1H NMR (500 MHz, CDCl3): δ = 1.29–1.39 (1H, m, CH2CH2CH2), 1.75–1.90 (3H, m, CH2CH2CH2), 1.97–2.03 (2H, m, CH2CH2CH2), 2.05–2.12 (1H, m, CH2), 2.15 (3H, s, CH3), 2.17–2.24 (1H, m, CH2), 2.63–2.70 (1H, m, CH2H), 2.73–2.82 (2H, m, 2×CH2), 2.97–3.03 (2H, m, 2×CH2), 3.15–3.22 (1H, m, CH2), 3.81–3.87 (2H, m, 2×CH2), 4.13–4.18 (1H, m, CH2H), 4.72–4.78 (1H, m, CH2H), 7.49–7.54 (2H, m, 2×benzo[b]thiophene H), 7.88 (1H, s, benzo[b]thiophene H3), 7.97–8.02 ppm (2H, m, 2×benzo[b]thiophene H). MS (LCMS ES+): m/z (%) 258 (44) [M–piperidine]+, 343 (100) [M+H]+. HRMS (ES+): calcd for C25H26N2O1S1 [M+H]+ 405.1995, found 405.1981 (3.44 ppm).

4-[(Benzo[b]thiophen-2-yl]-1,4'-bipiperidin-1'-yl)phenyl)methane 30: Prepared following the general acylation procedure using benzyl chloride (1 mmol, 141 mg) to give a brown glass (77 mg, 76%). The reported analysis is for the HCl salt. Note, peaks are broad and poorly defined, possibly due to rotamers, or restricted flexibility in the aliphatic ring systems. 1H NMR (500 MHz, CDCl3): δ = 1.27–1.37 (1H, m, CH2CH2CH2), 1.72–1.94 (7H, m, 4×CH2), 2.13–2.32 (2H, m, 2×CH2), 2.65–3.17 (6H, m, 2×CH2 & 2×CH3), 3.67–4.04 (3H, m, 3×CH3), 4.77–4.89 (1H, m, CH), 7.45–7.56 (7H, m, 5×Ph & 2×benzo[b]thiophene H), 7.87 (1H, s, benzo[b]thiophene H3), 7.97–8.02 ppm (2H, m, 2×benzo[b]thiophene H). MS (LCMS ES+): m/z (%) 320 (70) [M–piperidine]+, 405 (100) [M+H]+. HRMS (ES+): calcd for C22H20N2O1S1 [M+H]+ 405.1995, found 405.1981 (3.44 ppm).

4-[(Benzo[b]thiophen-2-yl]-1,4'-bipiperidin-1'-yl)-2-phenylethenone 31: Prepared following the general acylation procedure using phenylacetyl chloride (0.5 mmol, 77 mg) to give a clear glass (40 mg, 76%). The reported analysis is for the HCl salt. 1H NMR (500 MHz, CDCl3): δ = 1.27–1.37 (1H, m, CH2CH2CH2), 1.72–1.94 (7H, m, 4×CH2), 2.13–2.32 (2H, m, 2×CH2), 2.65–3.17 (6H, m, 2×CH2 & 2×CH3), 3.67–4.04 (3H, m, 3×CH3), 4.77–4.89 (1H, m, CH), 7.45–7.56 (7H, m, 5×Ph & 2×benzo[b]thiophene H), 7.87 (1H, s, benzo[b]thiophene H3), 7.97–8.02 ppm (2H, m, 2×benzo[b]thiophene H). MS (LCMS ES+): m/z (%) 258 (44) [M–piperidine]+, 405 (100) [M+H]+. HRMS (ES+): calcd for C22H20N2O1S1 [M+H]+ 405.1995, found 405.1981 (3.44 ppm).
mixture stirred at 82 °C for 4 d. The reaction was then filtered and the reaction mixture adsorbed directly onto silica and purified as described for 2f to give a clear glass. The final isolated product is for the HCl salt. Note, peaks are broad and poorly defined making assignment of the spectra difficult. 1H NMR (500 MHz, CD3OD): δ = 1.32–1.41 (1 H, m, CH4), 1.76–1.82 (1 H, m, CH4), 1.89–2.05 (4H, m), 2.77–2.88 (4H, m), 2.77–2.88 (4H, m), 2.99–3.11 (2H, m), 3.21–3.68 (10H, m). [Note, overlaps solvent peak, 3.83–4.03 (8H, m), 7.51–7.57 (2H, m, 2 x benzol/thiophene H), 7.94 (1H, s, benzol/thiophene H3), 7.98–8.04 ppm (2H, m, 2 x benzol/thiophene H). MS (LCMS ES+): m/z (%) = 207 (68) [M+2H]2+, 329 (26) [M–piperidine]+, 414 (100) [M+H]+. HRMS (ES+): calcd for C32H33N3S1+ 414.2574, found 414.2579 (1.11 ppm).

4-‘(Benzo[thiophen-2-yl]-1)-1’-benzyl,1,4’-bipiperidine 34: LiAlH4 (0.22 mmol, 2.0 mL in THF, 143 mL) was added to a solution of 30 (0.095 mmol, 39 mg) in anhyd THF (3 mL) and the reaction heated at 40 °C for 3 h before the reaction was quenched by the careful addition ofaq HCl (10 %, 5 mL). The aqueous phase was then added to the arylcerium solution and the reaction allowed to react for 16 h. The workup was initiated by pouring the reaction into water (200 mL) followed by adjusting the aqueous solution to pH 7 (aq NH4OH), before the layers were separated and the aqueous phase extracted with CH2Cl2 (3 x 100 mL). The combined organics were dried (MgSO4), filtered and concentrated in vacuo. The resultant crude solid was purified by flash column chromatography (EtOAc/Hexane, 1:0.16–0.95) to give a mixture of cis and trans isomers as a white solid (7.56 g, 73 %). Note, a small aliquot of the product was further purified to separate the isomers for analytical purposes. Note, the assignment of the isomers as cis, or trans is made by comparison of the shifts of the tert-butyl peaks in the 1H NMR spectra as compared to those published for 1-phenyl-4-tert-butyl-cyclohexanol.[22]

For cis-36: Rf = 0.34 (EtOAc/hexanes, 1:9). 1H NMR (500 MHz, CDCl3): δ = 0.93 (9H, s, tBu), 1.10–1.16 (1H, m, CH1), 1.51–1.60 (2H, m, 2 x CH2CH(CH3)2), 1.72–1.77 (2H, m, 2 x CH2CH2), 1.86–1.94 (2H, m, 2 x CH2CH), 2.10–2.15 (2H, m, 2 x CH2CH2), 1.97 (1H, s, benzol/thiophene H3), 7.27–7.35 (2H, m, benzol/thiophene H6 & H6’), 7.71 (1H, d, J = 8.0 Hz, benzol/thiophene H-7), 7.81 ppm (1H, d, J = 8.0 Hz, benzol/thiophene H-4). 13C NMR (125 MHz, CDCl3): δ = –22.8 (CH2CH3), 27.6 (CH2), 32.5 (tBu), 40.2 (CCH2), 47.4 (CH), 72.1 (COH), 117.9 (benzol/thiophene C3), 122.4 (benzol/thiophene C4), 123.3 (benzol/thiophene C7), 123.9 (benzol/thiophene CH), 124.2 (benzol/thiophene CH), 139.0 (benzol/thiophene C1), 139.9 (benzol/thiophene C), 155.9 ppm (benzol/thiophene CH). MS (LCMS ES+): m/z (%) = 271 (100) [M+–H]0, 599 (12) [2M+Na]+.

For trans-36: Rf = 0.16 (EtOAc/hexanes, 1:9). 1H NMR (500 MHz, CDCl3): δ = 0.81 (9H, s, tBu), 1.15–1.25 (3H, m, CH3 & 2 x CH2CH3), 1.82–1.91 (4H, m, 2 x CH2CH2 & 2 x CH2CH), 2.49–2.53 (2H, m, 2 x CH2CH), 7.32–7.39 (3H, m, benzol/thiophene H-3, H-5 & H-6), 7.76–7.77 (1H, m, benzol/thiophene H-7), 7.85 ppm (1H, d, J = 7.5 Hz, benzol/thiophene H-4). 13C NMR (125 MHz, CDCl3): δ = –25.0 (CH2CH3), 27.6 (CH2), 32.5 (tBu), 40.0 (CCH2), 47.6 (CH), 72.7 (COH), 121.0 (benzol/thiophene C3), 122.4 (benzol/thiophene C4), 123.6 (benzol/thiophene C7), 124.2 (benzol/thiophene CH), 124.4 (benzol/thiophene CH), 139.5 (benzol/thiophene C1), 139.9 (benzol/thiophene C), 151.2 ppm (benzol/thiophene CH). MS (LCMS ES+): m/z (%) = 271 (100) [M+–H]0.
1-(1-(Benz[a][b]thiophen-2-yl)-4-tert-butylcyclohexyl)piperidine 38: To a suspension of amine 37 (0.3 mmol, 84 mg) and K₂CO₃ (1.35 mmol, 186 mg) in dimethylformamide (10 mL) was added 1,5-dibromopentane (0.66 mmol, 152 mg). The subsequent reaction mixture was heated at reflux for 84 h, filtered and concentrated in vacuo. The resultant crude product was partitioned between H₂O and Et₂O (1:2, 75 mL), the layers separated and the aqeous phase extracted with Et₂O (2×50 mL). The combined Et₂O layers were then basified to pH 10 (aq NaOH) and subsequently extracted with EtOAc (3×100 mL). The combined EtOAc layers were dried (MgSO₄), filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (EtOAc/Hexane, 1:0:10—50:50) to give cis- and trans-38, the latter of which was further purified by trituration of the HCl salt from EtOAc.

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