



Structure-activity relationship of 4-azaindole-2-piperidine derivatives as agents against *Trypanosoma cruzi*

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ABSTRACT

The structure-activity relationship of a 4-Azaindole-2-piperidine compound selected from GlaxoSmithKline's recently disclosed open-resource "Chagas box" and possessing moderate activity against *Trypanosoma cruzi*, the parasite responsible for Chagas disease, is presented. Despite considerable medicinal chemistry efforts, a suitably potent and metabolically stable compound could not be identified to advance the series into *in vivo* studies. This research should be of interest to those in the area of neglected diseases and in particular anti-kinetoplastid drug discovery.

Chagas disease (American trypanosomiasis) is a neglected disease caused by the kinetoplastid parasite, *Trypanosoma cruzi*, which is estimated to have infected 6–7 million people and causes about 7500 deaths annually, as well as leaving 70 million people at risk.^{1–3} The disease is typically transmitted by the kissing bug, but can also occur by blood transfusion,⁴ through pregnancy,⁵ or via ingestion of contaminated food or drink.^{6,7} The disease is endemic in Latin America but also increasingly present in Europe, Australasia, and North America due to the migration of infected populations. Chagas disease exists in two clinical stages: the initial (or acute) phase, which lasts approximately 4–8 weeks and is often asymptomatic;^{8,9} and the chronic phase, for which a third of patients will develop cardiac or digestive problems and frequently leads to cardiac abnormalities and sudden death.^{10,11} Despite this, the current recommended treatments for Chagas disease have not changed for the past 45 years. Benznidazole (1) and nifurtimox (2) (Fig. 1) are still the only available medicines against the disease in spite of their debilitating side-effects, long treatment regimens (60–90 days),^{12,13} and variable efficacy when used for the treatment of chronic indeterminate patients.¹⁴

In recent years there have been attempts to increase the number of treatment options. However, recent clinical trials of two triazole-containing fungicides: posaconazole (4) and fosravuconazole (E1224, 3), a prodrug of ravuconazole (Fig. 1), were unsuccessful as despite producing short-term reductions in parasitemia at the end of treatment the

infections relapsed during the follow-up period.^{15,16} As such, there remains a large unmet need for new efficacious, safe and affordable medicines against this neglected disease. Furthermore, it is essential to develop new drug candidates with mechanisms of action which are different from the azoles (CYP51 inhibitors) to avoid further unsuccessful clinical trials.

As part of a collaboration with the Drugs for Neglected Diseases initiative (DNDi), our research group has been examining new chemical series with anti-trypanosomal activities as potential leads for drug discovery programs. The azaindole hit 5 (Fig. 2) was first described following a phenotypic high-throughput screening effort led by GlaxoSmithKline, along with approximately a further 600 compounds, as part of a series of anti-kinetoplastid "chemical tool boxes" with activities against the parasites causing either Chagas disease, leishmaniasis, or sleeping sickness.¹⁷ Although the mechanism of action against *T. cruzi* is currently unknown, it does not inhibit CYP51, presenting a promising starting point from a parasitological perspective.

In this article, the SAR of this series is discussed in the hope of aiding other groups considering working with this publicly available hit and to help avoid duplication of efforts by the Chagas disease drug discovery community.

Our previous experience of working with anti-Chagas agents has suggested that sub-micromolar potent starting compounds, which are

Abbreviations: DNDi, Drugs for Neglected Diseases initiative; SAR, structure-activity relationship; ADME, absorption, distribution, metabolism, and excretion; Cl_{int} , intrinsic *in vitro* clearance; POC, proof of concept

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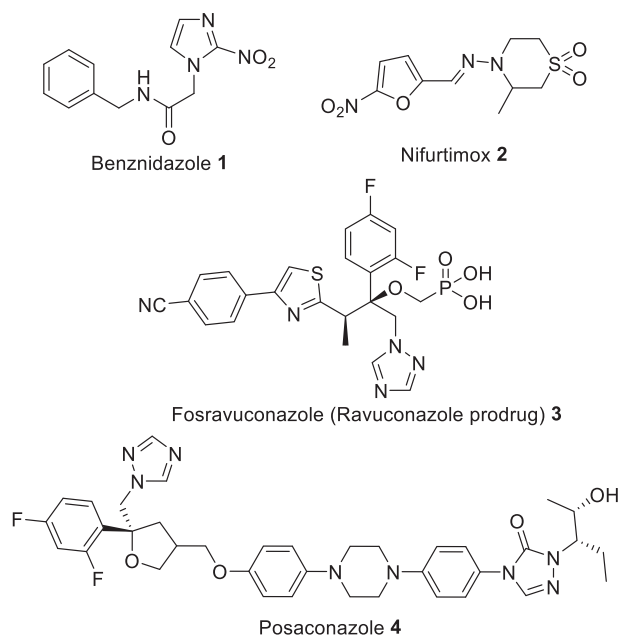


Fig. 1. Currently available treatments (1 and 2) for Chagas disease and recent clinical failures (3 and 4).

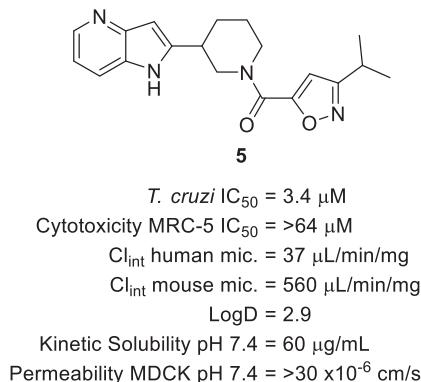


Fig. 2. Original hit from the whole-cell phenotypic screen against *T. cruzi* by GlaxoSmithKline.¹⁷

not CYP51 inhibitors, are difficult to obtain. As such, despite possessing only moderate activity (IC₅₀ 3.4 μ M) against intracellular *T. cruzi* amastigotes and high clearance in mouse liver microsomes, azaindole 5 (Fig. 2) was considered a good starting compound for a hit-to-lead program. Its selectivity over the host mammalian cell line, physicochemical and ADME properties (solubility, permeability, LogD, human microsome *in vitro* clearance) were otherwise good. We next screened approximately 50 closely related analogs from AbbVie's compound collection to obtain more SAR information before proceeding with synthetic studies. Of these, seven further active compounds were found, all with varying aromatic amides (selected examples Table 1, compounds 21–22, see Supplementary Information (SI) for remainder). From these compounds, the 4-methoxyindole analogs possessed the highest potency (1.03 μ M, Table 1, compound 21), whilst the indoles not bearing a 4-methoxy group were less active (4.03 μ M, Table 1, compound 22). However, these indoles suffered from some host cell

cytotoxicity, poor permeability, and high lipophilicity. Other simple aromatic amides were tolerated (see SI for details) so it was felt that improved properties and potency could be obtained by varying this group.

Initially, we approached the synthesis of analogs of 5 following a described route for a similar azaindole compound using a cerium mediated organometallic attack of *N*-protected 3-piperidin-3-one with a lithiated 2-indole species.¹⁸ However, this reaction proved problematic and so two alternative strategies were developed via either a Suzuki coupling/Hydrogenation approach or a Sonogashira coupling/Cyclisation route (Scheme 1). Although not the shortest synthesis to these analogs, these complimentary methods offered a more divergent synthetic strategy. Additionally, use of (*R*)- or (*S*)-14 could readily give enantiopure compounds if required.

To begin with, we focused on the right-hand side of the scaffold, synthesizing a range of aromatic amides (see Table 1 for selected examples, see SI for complete table). Metabolite identification studies had suggested that oxidation of the isopropyl moiety was the major metabolic pathway of 5 in mice (50% turnover in mouse vs. 4% in human after 1 h, using S9 liver fraction). It was hoped that with small modifications at this position we could greatly improve the metabolic stability in mouse liver microsomes whilst retaining activity and quickly advance to *in vivo* proof of concept (POC) studies in a mouse infection model. Using a combination of an in-house metabolic clearance calculator and standard physicochemical cutoffs (e.g. cLogP < 3) to aid design, several amides predicted to have significantly lower clearance, in mouse liver microsomes, than 5 were selected for synthesis (Table 1, compounds 23–33).

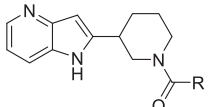
Simple phenyl and *para*-fluoro analogs (Table 1, compounds 23–24) possessed slightly reduced activity against *T. cruzi* (~10 μ M) in addition to the expected increase of metabolic stability in mouse liver microsomes for fluoro analog 24. Electron-rich dimethoxy analog 25 was also moderately active (8.96 μ M, Table 1) and offered a further improvement of metabolic clearance while keeping its selectivity. The electron deficient cyano analog 26 was inactive. This result, along with the inactivity of several pyridyl and pyrimidyl structures (Table 1, compounds 27–30 and SI) is suggestive of a preference for electron-rich aromatics. Interestingly, the metabolic stability of these compounds was much greater, in particular for CF₃-pyridyl 29 (Table 1) confirming that metabolic stability in mice could be sufficiently improved from original hit 5 simply by changing the amide portion of the molecule.


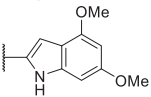
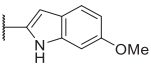



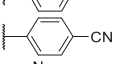












Other small heterocycles such as pyrazoles, furans, thiazoles and oxazoles were also, unfortunately, inactive (Table 1, compounds 31–33 and SI). At this point, we decided to focus specifically on the original isoxazole structure and attempted to make small changes to block or reduce oxidation of the isopropyl group, expecting to retain potency. Five isoxazole amides (Table 1, compounds 34–38) were synthesized and tested against *T. cruzi*. Despite retaining potency and selectivity with simple alkyl groups, the clearance was largely unaffected. This was particularly surprising for CF₃ analog 36 but can be explained by the half-unit increase in eLogD (computer-based calculations predicted a reduction in LogP).

The suspected oxidative metabolite 37 of the original hit 5 was also tested but was found to be inactive against *T. cruzi*. The best combination of potency, selectivity and metabolic stability was obtained with the methoxy substituted analog 38 (Table 1, entry 19), however this was not considered potent and/or stable enough for further investigations *in vivo*.

With limited increase in potency observed through modification of the amide we next looked towards the left-hand side of the scaffold to investigate the effect of modifications in the azaindole group. We hoped that by introducing additional heteroatoms into the ring we could

Table 1
Variation of right-hand side aromatic amide.



Compound	R	<i>T. cruzi</i> IC ₅₀ (μM) ± SD ^a	Cytotoxicity MRC-5 cells CC ₅₀ (μM) ± SD ^b	Human microsome Cl _{int} (μL/min/mg) ^c	Mouse microsome Cl _{int} (μL/min/mg) ^c	eLogD ^d
5		3.35 ± 0.67	> 64.0 ± 0.00	37	560	2.9
21		1.03 ± 0.05	19.5 ± 14.25	n.a.	n.a.	3.8 ^e
22		4.03 ± 0.08	32.0 ± 0.00	n.a.	n.a.	3.8 ^e
23		9.02 ± 0.97	> 64.0 ± 0.00	25	508	2.7
24		10.0 ± 1.09	> 64.0 ± 0.00	38	337	2.9
25		8.96 ± 0.11	> 64.0 ± 0.00	< 23	137	2.6
26		25.6 ± 7.45	> 64.0 ± 0.00	< 23	n.a.	2.4
27		> 64.0 ± 0.00	> 64.0 ± 0.00	< 23	164	1.9
28		14.8 ± 4.67	> 64.0 ± 0.00	114	196	3.0
29		> 64.0 ± 0.00	> 64.0 ± 0.00	28	25	2.7
30		> 64.0 ± 0.00	> 64.0 ± 0.00	< 23	n.a.	1.2
31		> 64.0 ± 0.00	> 64.0 ± 0.00	< 23	79	2.2
32		> 64.0 ± 0.00	> 64.0 ± 0.00	127	128	3.1
33		> 64.0 ± 0.00	> 64.0 ± 0.00	27	32	1.8
34		4.05 ± 0.40	> 64.0 ± 0.00	34	467	2.9
35		2.18 ± 0.00	> 64.0 ± 0.00	177	403	3.4
36		4.08 ± 0.29	> 64.0 ± 0.00	184	449	3.4
37		33.6 ± 1.53	> 64.0 ± 0.00	< 23	< 23	2.4
38		8.10 ± 0.23	> 64.0 ± 0.00	37	129	2.4

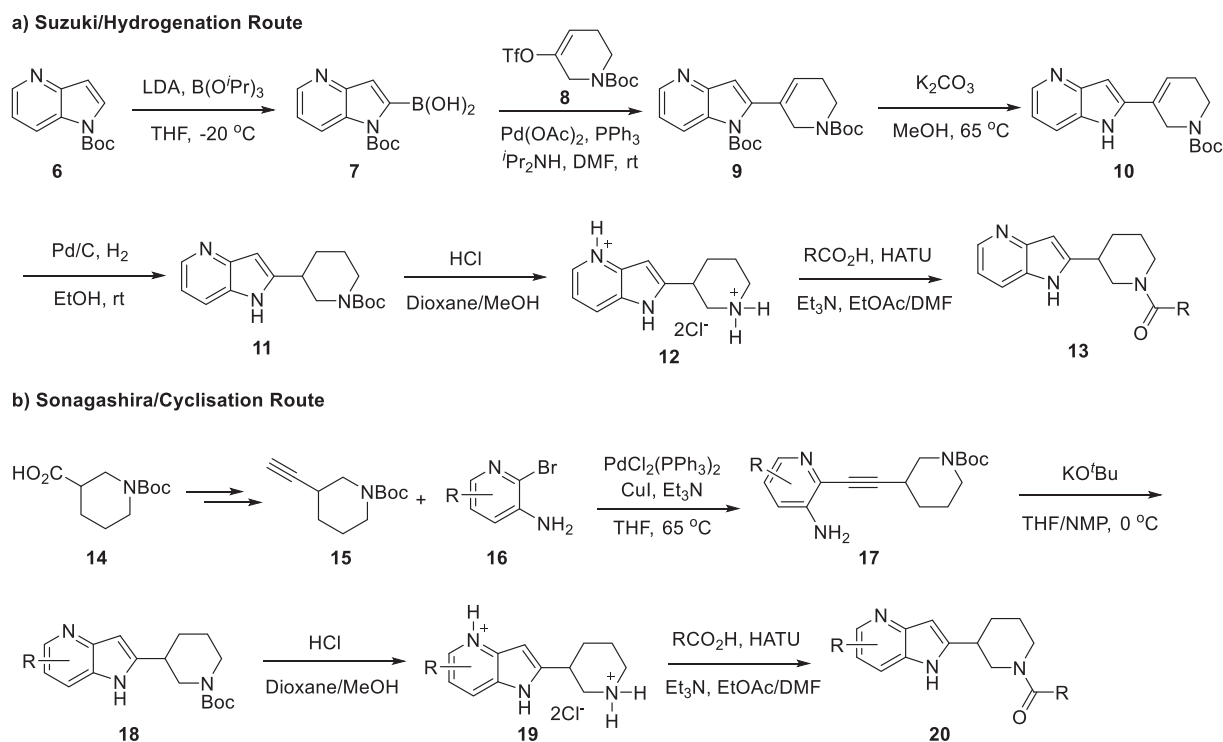
^a Assay was run according to experimental description (see SI) using benznidazole (IC₅₀ 2–5 μM) as a positive control; all values are the geometric mean of at least two experiments.

^b Assay was run according to experimental description (see SI) using vinblastine or paclitaxel (CC₅₀ < 0.01 μM) as positive controls; all values are the geometric mean of at least two experiments.

^c Intrinsic *in vitro* clearance using liver microsomes.

^d HPLC measured LogD.

^e Calculated LogP.



Scheme 1. Synthetic routes for the synthesis of azaindole analogs.

improve the metabolic stability by decreasing lipophilicity. We were also encouraged by a recent publication from a group at Novartis detailing proteasome inhibitors of kinetoplastid parasites.^{19,20} Due to the similarities in both structure and shape of these series', it was thought that they could be operating by a similar mechanism of action. Unfortunately, none of the synthesized alternative aromatic heterocycles 39–42 retained activity against *T. cruzi* (Table 2, compounds 39–42). Whether this is due to the loss of the azaindole hydrogen-bond donor or possibly due to a change in the pK_a of the aromatic nitrogen is unclear. Any substituents added to the azaindole core also led to a loss of activity (Table 2, compounds 43–51), except in the case of 5-aminosubstituted analogs 46–47 (Table 2) but these were also found to be more cytotoxic.

When examining the middle ring of the series, a larger change in potency was observed. A ten-fold increase was observed by introducing unsaturation to the piperidine ring (compare Table 1, compound 23 with Table 3, compound 52). The same improvement in activity was observed between the saturated and unsaturated 4-piperidine analogs 53 and 54 (Table 3). However, changing the phenyl amide of 54 to the isoxazole group (Table 3, compound 55) did not lead to the expected further improvement in activity, instead displaying similar potency as the simple phenyl analog. Despite the increased activity with unsaturated analogs, the addition of a double bond posed a risk of forming a toxic metabolite (through oxidation to the epoxide). As such, these unsaturated analogs were not thought to have offered a sufficient improvement in potency to overcome such a risk and hence were not further explored. However, we remained intrigued by the prospect of other planar systems that could mimic this shape. With this in mind, several aromatic anilides (Table 3, compounds 57–59) were synthesized, in addition to a pyrrolidine ring analog. The pyrrolidine analog 56 was moderately active (8.83 μM) but was also significantly less

metabolically stable (Table 3).

Interestingly, the *meta*-substituted *N*-H aniline 57 (Table 3) was still active against *T. cruzi* (2.08 μM), albeit with increased *in vitro* clearance. The methylated analog 58 (Table 3) was slightly less potent and also less selective over MRC-5 cells, whilst the *para*-substituted analog 59 was inactive (Table 3). Despite possessing activity against *T. cruzi*, these anilides were not pursued further due to their known toxicity risks,²¹ and their structural similarities with previously published anti-Chagas agents.^{19,20} Finally, replacing the piperidine with an acyclic analog 60, resulted in a loss of activity (Table 3) whilst an attempt to improve the clearance by replacing the piperidine with a morpholine ring, although successful, led to an inactive compound (Table 3, compound 61).

In conclusion, a detailed investigation into the SAR of the original azaindole hit 5 was performed. It was determined that the azaindole moiety is crucial for potency and the right-hand side only tolerates limited variation. The middle ring offered significant variation in potency based on the planarity and shape of the molecule. However, promising compounds ($IC_{50} < 2 \mu\text{M}$) were compromised by toxicity risks due to structural alerts and were not investigated further.

Despite extensive efforts, due to a lack of improvement in the balance of potency, selectivity and metabolic stability, a suitable compound could not be found to advance into *in vivo* proof of concept studies. Due to the presence of azaindole 5 in GlaxoSmithKline's "Chagas Box", these results represent an important resource for any group considering further investigation of this series. We are currently looking to undertake mechanistic studies to attempt to discover the true biological target of this series, which might enable a target-based drug discovery campaign and/or further rational optimization of this series.

Table 2
Variation of left-hand side azaindole moiety.

Compound	R ¹	R ²	<i>T. cruzi</i> IC ₅₀ (μM) ± SD ^a	Cytotoxicity MRC-5 CC ₅₀ (μM) ± SD ^b	Human microsome Cl _{int} (μL/min/mg) ^c	Mouse microsome Cl _{int} (μL/min/mg) ^c	eLogD ^d
39			> 64.0 ± 0.00	> 64.0 ± 0.00	< 23	173	2.0
40			> 64.0 ± 0.00	> 64.0 ± 0.00	135	2897	2.4
41			> 64.0 ± 0.00	> 64.0 ± 0.00	91	1269	2.3
42			> 64.0 ± 0.00	> 64.0 ± 0.00	< 23	127	1.7
43			> 64.0 ± 0.00	> 64.0 ± 0.00	26	151	3.4
44			> 64.0 ± 0.00	> 64.0 ± 0.00	78	247	3.9
45			> 64.0 ± 0.00	> 64.0 ± 0.00	78	294	3.5
46			8.33 ± 0.13	48.7 ± 19.0	50	444	3.5
47			2.14 ± 0.01	6.91 ± 1.14	149	517	4.4
48			28.8 ± 7.78	34.9 ± 6.14	45	235	3.8
49			> 64.0 ± 0.00	> 64.0 ± 0.00	33	68	3.5
50			> 64.0 ± 0.00	> 64.0 ± 0.00	88	431	4.3
51			> 64.0 ± 0.00	> 64.0 ± 0.00	196	673	4.6

^a Assay was run according to experimental description (see SI) using benzimidazole (IC₅₀ 2–5 μM) as a positive control; all values are the geometric mean of at least two experiments.

^b Assay was run according to experimental description (see SI) using vinblastine or paclitaxel (CC₅₀ < 0.01 μM) as positive controls; all values are the geometric mean of at least two experiments.

^c Intrinsic *in vitro* clearance using liver microsomes.

^d HPLC measured LogD.

Table 3
Variation of middle ring.

Compound	R	<i>T. cruzi</i> IC ₅₀ (μM) ± SD ^a	Cytotoxicity MRC-5 CC ₅₀ (μM) ± SD ^b	Human microsome Cl _{int} (μL/min/mg) ^c	Mouse microsome Cl _{int} (μL/min/mg) ^c	eLogD ^d
52		0.87 ± 0.60	> 64.0 ± 0.00	238	1039	3.0
53		22.4 ± 5.09	> 64.0 ± 0.00	< 23	1044	2.7
54		2.39 ± 0.34	> 64.0 ± 0.00	< 23	305	2.9
55		2.01 ± 0.08	> 64.0 ± 0.00	59	446	3.2
56		8.83 ± 0.06	> 64.0 ± 0.00	58	1112	3.1
57		2.08 ± 0.11	> 64.0 ± 0.00	363	522	3.9
58		7.25 ± 0.42	26.1 ± 7.18	51	1195	3.3
59		25.2 ± 15.09	> 64.0 ± 0.00	98	187	3.9
60		36.2 ± 1.48	> 64.0 ± 0.00	< 23	382	1.9
61		34.6 ± 0.42	> 64.0 ± 0.00	< 23	78	2.4

^a Assay was run according to experimental description (see SI) using benzimidazole (IC₅₀ 2–5 μM) as a positive control; all values are the geometric mean of at least two experiments.

^b Assay was run according to experimental description (see SI) using vinblastine or paclitaxel (CC₅₀ < 0.01 μM) as positive controls; all values are the geometric mean of at least two experiments.

^c Intrinsic *in vitro* clearance using liver microsomes.

^d HPLC measured LogD.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bmcl.2019.126779>.

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