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Structure activity relationships of antischistosomal *N*-phenylbenzamides by incorporation of electron-withdrawing functionalities

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Abstract

For the adult *Schistosoma mansoni* flatworm pathogen, we report further structure activity relationships (SAR) of 19 *N*-phenylbenzamide analogs. Our previous SAR studies, designed by selecting representative substituents from the Craig plot, identified **9** and **11** which possessed electron-withdrawing groups that benefited potency. This study sought to enhance the potency of this chemotype by incorporating other electron-withdrawing functionalities not studied previously and to overcome the potential pharmacokinetic liabilities associated with the high lipophilicity of frontrunner compounds. Compared to the most potent compound, **9** (EC_{50} = 80 nM), from our previous work, the most potent compounds in the current study (**32** (EC_{50} = 1.17 μM), **34**

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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.

CRediT authorship contribution statement

Ameera Mohammed Dawoodjee: Writing – review & editing, Writing – original draft, Methodology, Investigation. **John Sichinga:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Harrison Banda:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Steve Mbaya:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Evelyn Funjika:** Writing – review & editing, Supervision, Conceptualization. **Godfrey Mayoka:** Writing – review & editing, Supervision, Conceptualization. **Christabel Hikaambo:** Writing – review & editing, Methodology, Formal analysis. **Karol R. Francisco:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Yujie Uli Sun:** . **Lawrence J. Liu:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Conor R. Caffrey:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Peter Mubanga Cheuka:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2024.101890.

(EC_{50} = 1.64 μM) and **38** (EC_{50} = 1.16 μM)) were less active although they retained single digit micromolar potency. Furthermore, compound **38** generated a CC_{50} value of > 20 μM in counter toxicity screens using HEK 293 cells, translating to a wide selectivity index of > 17.

Keywords

N-phenylbenzamide analogs; *N*-pyridazinylbenzamide analogs; *Schistosoma mansoni*

Introduction

Schistosomiasis which is caused by blood flukes of *Schistosoma* spp. remains a prevalent parasitic infection globally, especially in low-income countries with the largest burden of disease occurring in sub-Saharan Africa [1]. The World Health Organization (WHO) estimates that at least 251.4 million people required preventive treatment for schistosomiasis in 2021 in 78 countries [2]. Transmitted by freshwater snails, the disease thrives in communities that have limited access to clean water and sanitation. Clinical manifestations of the disease can take years to develop, and its often insidious and sub-clinical nature challenges diagnosis and leads to disabling socioeconomic and health consequences among the affected populations. Transmission of schistosomiasis is favoured among agricultural communities and those living around water development schemes. The vulnerable populations affected are usually unable to afford effective prevention measures [3].

Praziquantel has been the only drug for treatment of schistosomiasis for over four decades. At the 40 mg/kg recommended dose, it is safe and reasonably effective [4]. However, it has limitations, key among which is its poor efficacy against juvenile parasites. Further, the drug is prepared as a racemate whereby the *S*-enantiomer is clinically useless and imparts a rancid taste to what is a large tablet, both of which hamper patient compliance, especially by children. In addition, concerns regarding drug resistance remain [5–8]. Therefore, there is a need to discover more effective drugs.

N-phenylbenzamides have demonstrated *in vitro* and *in vivo* antischistosomal properties [9,10]. However, there have been few medicinal chemistry optimization efforts for this chemotype. We have previously reported the antischistosomal activity of compounds **9** and **11** (Fig. 1) [11] arising from an SAR exploration based on compound **1** (Fig. 1) from the Medicines for Malaria Venture's Pathogen Box [7]. The observed biological activities of these two compounds suggested that electron-withdrawing substituents were beneficial in improving *in vitro* antischistosomal potency. Although compound **9** was the most potent of the derivatives described in the earlier work, its high lipophilicity, as reflected by its cLogP value of 5.3, may negatively impact other physicochemical parameters such as solubility. Highly lipophilic compounds (cLogP > 5) are also associated with increased cytotoxicity [12] and greater susceptibility to metabolic degradation [13], which can limit their therapeutic potential. Therefore, in this study, we followed a SAR strategy aimed at attenuating lipophilicity. A less lipophilic but stronger electron-withdrawing substituent, the nitro (NO_2), was incorporated into frontrunner compounds. While compounds bearing aromatic nitro functionalities bear a risk of well described liabilities, literature is replete

with examples of successful drugs containing this moiety [14,15], thus motivating our SAR exploration. Additionally, a pyridazinyl moiety was explored as a replacement of the C ═ C with a N ═ N group. Finally, we investigated the effect of regioisomerism on the previously identified potent compounds.

Materials and methods

Synthesis of target compounds

Scheme 1 was employed to synthesize the target compounds, according to the previously reported methodology, with minor adjustments [11]. The reaction was carried out via carbodiimide-mediated amide-coupling of various substituted benzoic acids and anilines, which resulted in compounds **32** to **43**. Synthesis of pyridazinyl analogs, **44**–**49**, was achieved via EDCI-coupling of substituted benzoic acids with substituted aminopyridazines. The detailed procedures for synthesis and spectroscopic characterization of all target compounds are given in the Supporting Information.

In vitro antischistosomal and cytotoxicity evaluation of target compounds

At a compound concentration of 5 μM, the multiparametric responses in adherence, shape, density and mobility that the schistosome parasite is capable of were visually recorded at 2, 5, 24 and 48 h using a constrained nomenclature of phenotypic descriptors. Each descriptor was given a value, typically 1, and these were tallied to generate a severity score on a scale from zero (no activity) to 4 (maximal activity) [16–19]. The severity scores obtained are presented in Table 1 and the underlying descriptor data are in the Supporting Information. From the severity scores obtained, the most potent compounds (**32**, **34**, and **38**) were selected for measurement of worm motility (as an indicator of parasite viability) using 1:3 serial dilutions over five concentrations (5 – 0.02 μM). Worm motility was measured after 24 h using a WormAssay, a camera-based system that tracks the average motility of worms per well in an assay plate [20]. Concentration-dependent data were used to identify the point at which motility was decreased by 50 % (EC_{50} value; Table 1). Finally, concentration-dependent counter toxicity screens were performed with human embryonic kidney (HEK) 293 cells to derive the CC_{50} value, with which a selectivity index (SI) could be measured relative to the WormAssay EC_{50} values [11]. The detailed protocols for *in vitro* antischistosomal and cytotoxicity testing are given in the Supporting Information.

Results and discussion

In the present study, we investigated the *in vitro* antischistosomal activities of substituted *N*-phenylbenzamides with a primary focus on electronegative and hydrophobic substituents. The substituents explored include CF_3, NO_2, F, and Cl groups, which were appended at *ortho*, *meta* or *para* on the amine and/or acyl portion of the scaffold. Furthermore, for several compounds, electronegative pyridazines were introduced on the amine side. Notable SAR trends from the current work emerge with the general observation that, as earlier described [11], electron-withdrawing substituents at the *meta* and *para* positions on both the anilide and carboxylic portions of the benzamide analogs improve potency.

Compound **32** is an amide-reversed analog of **11** that retains antischistosomal potency suggesting that the orientation of the amide is not a critical feature for activity. On the other hand, *meta* substitution on both phenyl rings of the derivatives is unfavourable to potency as judged from the comparison of **33** and **11**. This substitution pattern completely compromises activity suggesting that *para*-substitution at one of the phenyl groups may be necessary for potency. We also observed that combining *meta* and *para* substitutions is compatible with antischistosomal potency, evidenced by the promising activity of **32**, an amide-reversed analog featuring this substitution pattern on both phenyl rings. However, when the *para* CF_3 in **32** is shifted to the *meta* position, as in **33**, potency is lost. The same *meta* and *para* substitution requirement appears to hold true when **37** and **38** are compared. We also found that di-*para* substituted analogs, such as **34**, were active suggesting that a nitro could replace the CF_3 in di-*para* substituted analogs. This finding is also in agreement with previously published data on this chemotype [11].

As observed from comparing the activities of **34** (*p*-NO_2) and **38** (*m*-NO_2), shifting the NO_2 substituent on the anilide portion enhanced potency suggesting that *meta*-substitution of the nitro functionality on the anilide portion may be necessary for potency. The *ortho*-to-*meta* change in the position of the nitro (**41** to **38**) on the anilide portion increased activity suggesting that *meta*-substitution of the nitro functionality on the anilide portion is important for potency. This *meta* substitution pattern in **38** is also present in **11**. When we compared **35** (*m*-CF_3) to **9** (*p*-CF_3), we discovered that *para*-substitution on the benzoic portion, in the presence of a 3,4-dichlorinated aniline moiety, may be necessary for potency. The replacement of CF_3 in **34** with fluorine to give **39**, and in **36** to give **40**, appears to compromise activity, further supporting our hypothesis that electron-withdrawing substituents are necessary for optimal potency.

The pyridazine-containing compounds, **44** – **49**, were, generally, inactive suggesting that the introduction of this hydrophilic aromatic heterocycle is unfavourable. This result is even more striking when comparing **49** to **11**. In this regard, the subtle replacement of two carbons in **11** with nitrogen atoms to furnish **49** abolished activity.

From the single-concentration severity score assay, the most potent compounds (**32**, **34**, and **38**) were selected for measurement of the EC_{50} value using the worm motility assay. All three compounds exhibited similar potency (EC_{50} = 1.16–1.64 μM; Table 1).

When the antischistosomal activities of the three most potent compounds were compared to the cytotoxicity data generated for the HEK293 cells, **38** appeared to be non-cytotoxic (CC_{50} > 20 μM) with a SI of > 17.2. Nitration on the anilide portion of the derivatives seemed to result in a favourable selective toxicity profile (**11** *vs*. **38**) demonstrating the possibility of modulating the cytotoxic potential for this series. Compound **49**, resulting from an isosteric replacement of C = C in **11** with N = N, was also non-cytotoxic at the highest concentration tested (CC_{50} > 20 μM) although the compound was also inactive against the worm.

Conclusion

In summary, we conducted SAR studies around compounds **9** and **11** using the electron-withdrawing NO_2 group, bio-isosteric replacements of the phenyl with the pyridazinyl ring, and regio-isomerism strategies leading to the design and synthesis of various *N*-phenylbenzamide analogs as antischistosomal compounds. Compounds **32**, **34** and **38** emerged as the most potent derivatives although less potent than the progenitor compounds. Nonetheless, the derivatives feature lower lipophilicity and potentially higher solubility that are desirable for the design of compounds with oral bioavailability. Compound **38** was also apparently non-cytotoxic (CC_{50} >20 μM), offering more than 2- and 1.8-fold improvement in the cytotoxicity profile compared to **9** and **11** respectively. Overall, this study demonstrates how SAR-guided drug design can be used in the development of new antischistosomal drug candidates. Our ongoing and future design strategy includes investigating the amide reversed counterparts of the promising analogs, exploring other electron-withdrawing functionalities, such as CH_3SO_2, $CONH_2$ and SO_2NH_2, and heterocyclic and larger or fused ring systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

No data was used for the research described in the article.

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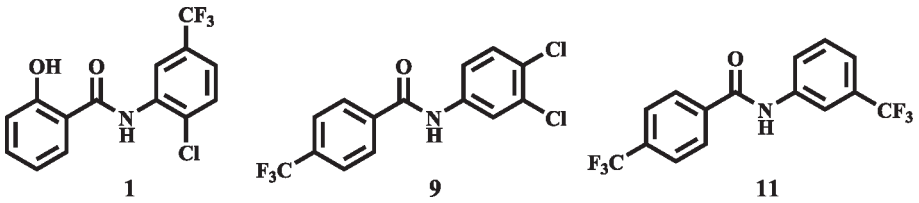
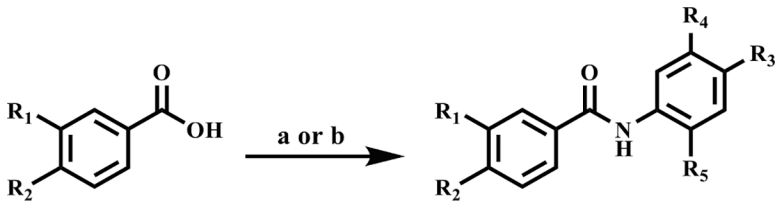


Fig. 1.
Structures of compounds **1**, **9** and **11**.

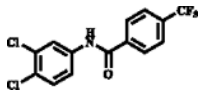
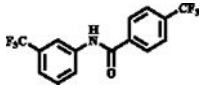
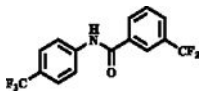
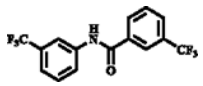
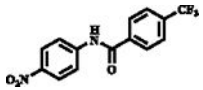
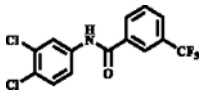
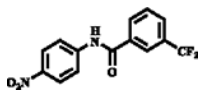
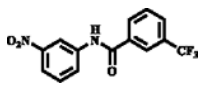


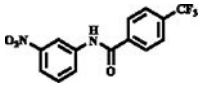
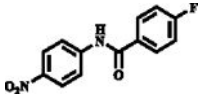
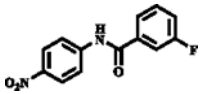
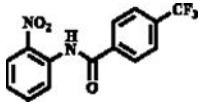
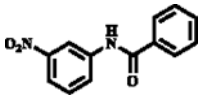
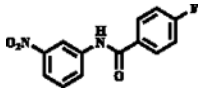
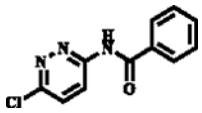
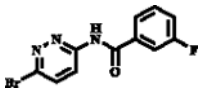
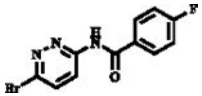
Scheme 1.

Reagents and conditions: (a) (i) EDCI, DMAP, DCM, 0 °C, 30 min; (ii) substituted aniline (or pyridazinamines for **c5** – **c10**), 25–30 °C (or room temperature for **c5** – **c10**), 2–24 h (all analogs except **41**–**43**) or (b) (i) DCC, DMAP, DCM, 0 °C, 20 min; (ii) *m*- or *o*-nitroaniline (**41**–**43**), 30–33 °C, 24–26 h.

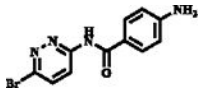
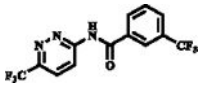
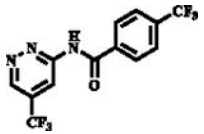
Table 1

In vitro biological activity data of *N*-phenylbenzamide analogs against adult *S. mansoni* and HEK 293 cells.

Code	Chemical structure	Severity scores (5 μM)[a]				Adult *S. mansoni* EC_{50} (μM)[b]	HEK 293 CC50 (μM)[c]	SI[d]	cLogP[e]
		2 h	5 h	24 h	48 h				
9		3	4	4	4	0.08	9.80 ± 1.6	123	5.31
11		3	3	3	3	1.10	11.1 ± 0.2	10.1	4.99
32		1	3	3	3	1.17	9.25 ± 1.51	7.91	4.99
33		0	0	0	0	-	15.6 ± 1.60	-	4.99
34		2	2	3	3	1.64	15.9 ± 3.31	9.70	3.95
35		1	2	1	1	-	10.1 ± 1.50	-	5.31
36		1	2	2	1	-	14.7 ± 1.03	-	3.95
37		1	0	0	0	-	> 20	-	3.95

Code	Chemical structure	Severity scores (5 μM)[a]				Adult *S. mansoni* EC_{50} (μM)[b]	HEK 293 CC50 (μM)[c]	SI[d]	cLogP[e]
		2 h	5 h	24 h	48 h				
38		2	2	3	4	1.16	> 20	> 17.2	3.95
39		0	0	0	2	-	> 20	-	3.16
40		0	1	1	1	-	> 20	-	3.16
41		2	2	0	0	-	> 20	-	3.49
42		1	1	1	0	-	> 20	-	2.94
43		1	0	0	0	-	> 20	-	3.16
44		1	0	0	0	-	> 20	-	1.96
45		1	0	0	0	-	> 20	-	2.28
46		0	0	0	0	-	> 20	-	2.26

Results Chem. Author manuscript; available in PMC 2025 March 24.

Code	Chemical structure	Severity scores (5 μM)[a]				Adult *S. mansoni* EC_{50} (μM)[b]	HEK 293 CC50 (μM)[c]	SI[d]	cLogP[e]
		2 h	5 h	24 h	48 h				
47		0	0	0	0	-	> 20	-	1.20
48		1	1	0	0	-	> 20	-	3.08
49		0	0	0	0	-	> 20	-	3.08

[a] Each compound was screened twice as a singleton and representative data are shown.

[b] Measured with WormAssay; performed once in triplicate over 24 h; otherwise −, not tested.

[c] Assay performed once in triplicate over 48 h.

[d] SI, selectivity index, *i.e.,* HEK 293 CC_{50}/WormAssay EC_{50}.

[e] The logarithm of partition coefficient between *n*-octanol and water [$\log(c_{octanol}/c_{water})$], calculated using ChemDraw Professional 15.0.