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Lead Selection of Antiparasitic compounds from a focused library of benzenesulfonyl derivatives of heterocycles

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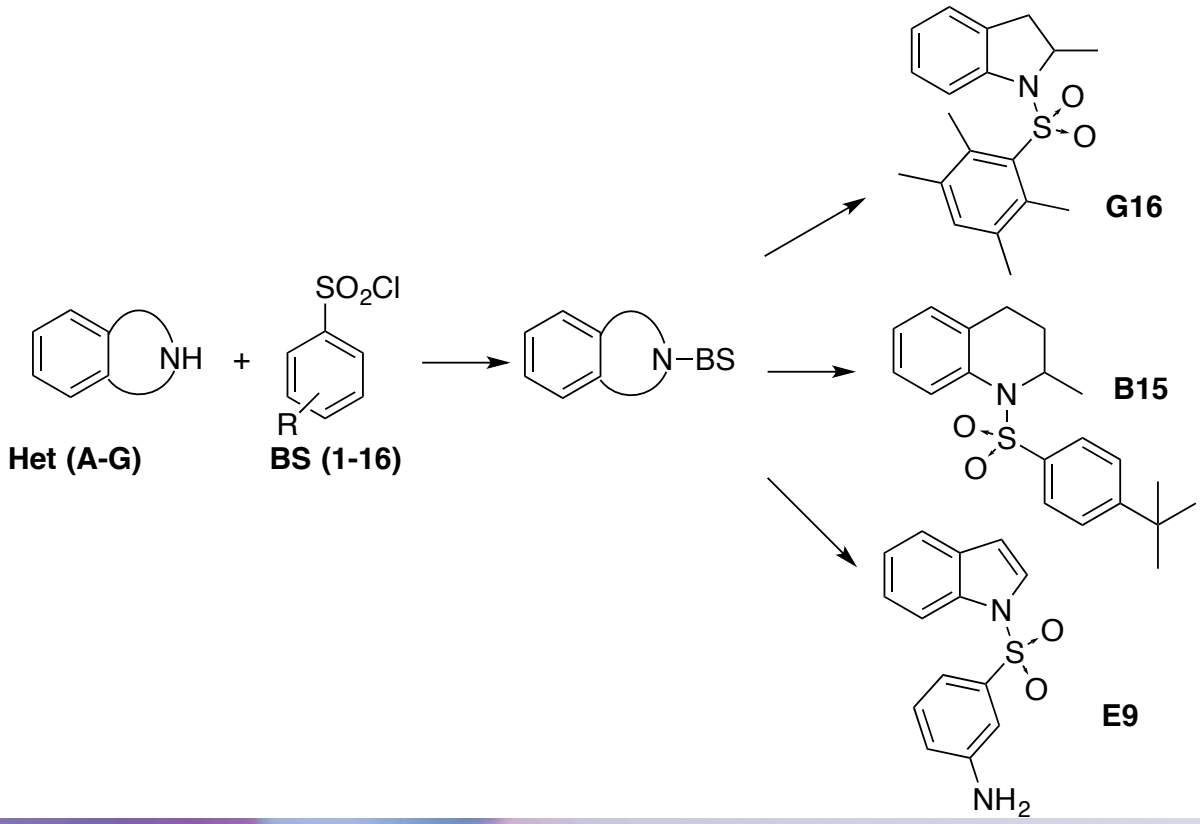
Lead Selection of Antiparasitic compounds from a focused library of benzenesulfonyl derivatives of heterocycles

Graphical Abstract



Abstract:
A library of 69 synthetic benzenesulfonyl derivatives of heterocycles, with drug-like properties, was assayed for in vitro antiparasitic activity and the results were added to previously reported by us for a comprehensive SAR discussion. Seven compounds showed an IC_{50} between 0.25-3µM against *L. donovani* and low cytotoxicity. Compound 1-(2,3,5,6-tetramethylphenylsulfonyl)-2-methylindoline (**G16**), was particularly interesting with an IC_{50} similar to the reference drug miltefosine. In addition, seven compounds showed an IC_{50} below 6µM against *T. cruzi*, and three of them (**E3**, **E9** and **G3**) were identified as lead scaffolds for further optimization based on their activity-toxicity profile. Furthermore, two promising structures (**B15** and **G15**) have shown moderate inhibitory activity against *P. falciparum*. In general, the presence of a benzenesulfonyl moiety improves the antiparasitic activity of the heterocycles included in this study (with exception of *T. b. rhodesiense*) validating the criteria used in the selection of the fragment-based drug design approach by using privileged structures. Finally, from the SAR analysis it could be concluded that the presence of electron withdrawing and lipophilic groups were favorable for the antiparasitic activity.
Keywords: antiparasitic activity, benzenesulfonyl, heterocycles, SAR, library compounds.

Introduction

Many of the parasitic diseases are considered Neglected Tropical Diseases (NTDs) as established by World Health Organization (WHO):

- more than 1 billon people is currently affected by one or more of these NTDs.[1]
- There is a strong correlation between NTDs and poverty.
- Among the classical NTDs we can mention the African trypanosomiasis (sleeping sickness) caused by *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) and *T. b. gambiense*, the Chagas disease caused by *Trypanosoma cruzi* (*T. cruzi*), and the visceral leishmaniasis by *Leishmania donovani* (*L. donovani*) parasites.[2,3]
- Malaria (caused by *Plasmodium* species; particularly *P. falciparum* and *P. vivax)*, is the most prevalent protozoan parasitic disease in humans with almost half of the world's population being at risk.[4]
- Currently available therapies for NTDs and malaria have not been successful due to their high cost, low safety and efficacy and specially by the development of resistance.[4,5]
- There is a urgent need for a continuous development of new drugs, which was recognized by WHO, through the endorsement of various funding programs.

Introduction

The “fragment-based drug design” is a well-accepted strategy used for the generation of libraries during the early stages of lead discovery:

•This approach makes the assumption that the combination of active privileged structures would lead to molecules with better or new pharmacological profiles.

•We have previously reported the design and synthesis of a focused library of N-benzenesulfonyl derivatives of active heterocycles (BS-Het, compounds **A,B and D {1-10}**, Figure 1).

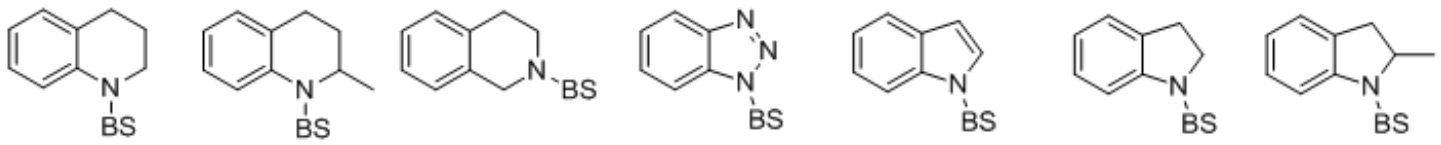
•Several of these compounds showed promising activity in vitro when screened against *T. cruzi*, *L. donovani*, and *P. falciparum* as well as low cytotoxicity.[6,7,8]

•We have expanded the number of members of the initial library by including new heterocycles and BS moieties (Figure 1).

•The actual focused library consists of series of 7 different heterocycles: 1,2,3,4-tetrahydroquinoline (A), 2-methyl-1,2,3,4-tetrahydroquinoline (B), 1,2,3,4-tetrahydroisoquinoline (C), benzotriazole (D), indole (E), indoline (F) and 2-methyl-indoline (G). Every series includes 16 analogs with different substitutions on the BS moiety (**1-16**, Figure 1).

•The substituents were chosen applying the Craig strategy, to obtain an appropriate range of physicochemical properties.

Introduction



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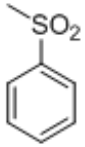	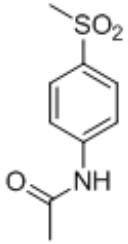	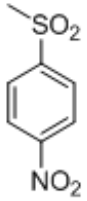	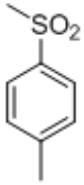	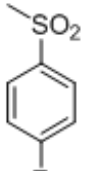	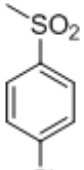	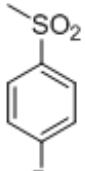	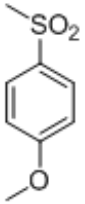
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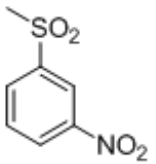
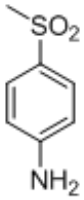
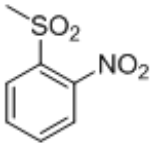
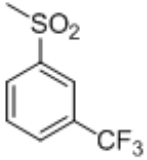
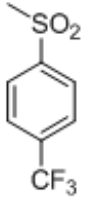
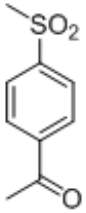
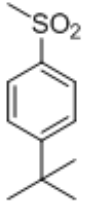
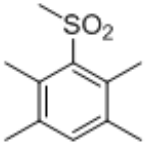
							

Figure 1. Library of BS-Het.

Results

Table 1. Percentage of inhibition at 0.8 and 4.8 ug/mL of the new BS-Het library members against *L.donovani, T. cruzi, P. falciparum,* and *T.b.rhodesiense.*

Compd[a]	L. d.		T. c.		P. f.		Tbr	
	0.8µg/mL	4.8µg/mL	0.8µg/mL	4.8µg/ml	0.8µg/ml	4.8µg/ml	0.8µg/ml	4.8µg/ml
A15	49.8	99.1	0	55.6	23.5	91.6	6.7	5.5
A16	94.2	100	15.5	60.3	3.1	56.5	2.7	3.7
B12	25.8	82.3	5.4	95.6	5.8	81.3	1.8	0
B13	29	96	5.3	98.2	26.4	98.8	5.5	8.1
B14	6	45.7	0	91.9	4	29.8	0	1.5
B15	62	100	28	100	79.9	100	8.2	0
B16	73.1	100	9.7	69.3	6.7	78	0	0
C15	31.6	100	0	26.7	11.6	86	10.8	8.7
C16	63.3	100	1.8	23.1	4.5	18.8	2	1.7
E3	37.6	100	34.7	100	0	69.7	27.4	98
E7	17.9	77.7	4.2	28.1	9.8	63.5	5.3	0
E9	15.9	95.4	36.4	100	14.7	79.2	0	4.3
E15	49.4	100	5.2	54.1	29.5	99.1	1.9	5.8
E16	61.3	100	10.3	31.4	3.3	41.8	5.5	5.7
F3	24.2	77.6	18.3	75	2.5	60.4	8.6	29.3
F15	27.3	92.2	0	0	13.9	92.3	0	0
F16	76.4	100	0.2	24.7	3.2	41.4	3.1	2.7
G3	41.7	79.5	19.4	94.4	18	72.6	6.9	68.2
G6	9	83.3	0	100	17.7	87.9	6.5	4
G7	17.6	81.4	0	100	9.1	76	0	0.9
G8	2.9	35	1.8	100	5.3	49.3	1.2	4.4
G15	53.3	100	7.7	100	52.8	99.9	0	6.4
G16	77.2	100	0	75.2	5.5	76.7	0	0
MLT[e]	60.1	93.3						
BNZ			63.3	98.5				
ART[e]					80	100		
MLR							44.4	98.3

[a] Numbers as in Figure 1. [b] Percentage of growth inhibition against *L.donovani* (MHOM-ET-67/L82, axenic amastigotes). [c] Activity of **A-D{1-10}** in Reference.[9, 10] [d] Activity was measured at 0.8µg/ml and 4.8µg/ml which correspond to approximately 6µM and 40µM, respectively. [e] Reference drug for *L. donovani* activity.

Results

Table 2. Antiparasitic IC_{50} values and cytotoxicity of selected compounds within BS-Het library.

Compd[a]	*T.cruzi* IC_{50} (μM)[b]	*L. donovani* IC_{50} (μM)[c]	*P. falciparum* IC^{50} (μM)[d]	Cytotoxicity (μM)[e]	SI[f] (*T.cruzi*)	SI[f] (*L.donivani*)	SI[f] (*P.falciparum*)
References Drugs	BNZ	MLT	CHQ	PDP			
	2.13	0.25	0.25	0.01			
A3	11.44	>271	8.31	248.3			
A6	15.98	18.87	8.43	20.46			
A7	13.78	21.35	6.65	7.36			
A16		0.36		2.71		7.53	
B12	6.6			13.1	1.98		
B13	5.89			12.25	2		
B15	6.06	2.16	1.5	6.81	1.12	3.15	4.57
B16		0.62		12.38		19.96	
C16		0.76		3.6		4.8	
E3	1.15			9.1	7.9		
E9	1.08			15.1	13.98		
E16		0.73		12.55		17.19	
F16		0.64		8.97		13.39	
G3	2.8			69.4	24.78		
G6	4.79			11.1	2.31		
G7	5.76			13.2	2.29		
G8	9.46			0.44	0.04		
G15	6.98	1.53	1.5	12.16	1.74	7.95	8.1
G16		0.23		6.62		28.78	

[a] Numbers as in Figure 1. [b] *T.cruzi Tulahuen C4* (intracellular amastigotes). [c] *L.donovani* MHOM-ET-67/L82 (axenic amastigotes). [d] *P.falciparum* K1 resistant to chloroquine and pyrimethamine (intra-erythrocytic forms). [e] Cytotoxicity in mouse myoblasts (L6). [f] Selectivity Index SI = IC50 L6/IC50 parasite. [g] Activity for derivatives **A-D{1-10}** have been informed previously elsewhere

Discussion

SAR against *L. donovani*:

- The presence of *p-tert*-butyl (**A-G{16}**) or 2,3,5,6-tetramethyl (**A-G{15}**) substituents on the BS generate the most active compounds (Table 1).
- These substituents led to the most lipophilic compounds in each series, suggesting that the antiparasitic activity might be influenced by lipophilicity.
- Other compounds with lipophilic substituents (*p*-Cl (**E-G{6}**), *p*-Br (**E-G{7}**) and *p*-CF_3 (**A-G{13}**) showed moderate inhibitory activity, while the less lipophilic (*p*-$COCH_3$ (**A-G{14}**), *p*-$NHCOCH_3$ (**F-G{2}**) or *p*-NH_2 (**F-G{10}**) were clearly less active.
- The presence of an electron-withdrawing group in the BS moiety led to compounds with moderate activity, i.e. *p*-NO_2 substituent (**A-G{3} vs A-G{1}**).
- Whether the electron-withdrawing group was in *para*, *meta* or *ortho* position had no major effect in the activity (**E-G3** (%inh 100, 77, 79%), **E-G9** (95, 59, 66%) and **E-G11** (81, 38, 52%)), being the order of relative activity p>m>o.
- Other compounds with electron-withdrawing groups did not show strong inhibitory activity (*p*-$COCH_3$). This peculiar behavior can be explained considering the lipophilic properties, suggesting that the potency against *L. donovani* depends on a combination of both, electronic and lipophilic effects. Similar considerations may explain the activity of derivatives with lipophilic and electron donating group such as *p-tert*-butyl, 2,3,5,6-tetramethyl and *p*-CH_3 (**E-G{4}**).

Discussion

SAR against *T. cruzi*: In regard to the heterocycle moiety, series **B** and **G** demonstrated to be the most active against this protozoan strain (Table 1). Furthermore, these series showed a better antitrypanosomal profile than their demethylated analogs **A** and **F**, respectively, suggesting a positive influence of a 2-methyl group on the heterocyclic moiety.

•When considering the BS moiety, it is clear that the electron-withdrawing properties are favorable for the antitrypanosomal activity, also observed with the previously reported series A and B.[6,7] Substitutions on the BS with a strong electron-withdrawing group, such as *p*-NO_2, resulted in one of the most active compounds of the library (**E-G{13}**).

•When comparing the inhibitory activity of analogs with different NO_2 substituent pattern (i.e. **F3**/**F9**/**F11** or **G3**/**G9**/**G11**, Table 1) the order was *p*≥*m*>*o*. This is the same trend previously observed for series **A** and **B** .[6,7]

•The inhibitory activity of the more lipophilic compounds showed no clear trend within the series (i.e., in series **G**, one of the most active series, the relative order of activity was: *p*-*tert*-butyl derivatives (**G15**) > *p*-Br (**G7**) > *p*-Cl (**G6**) > *p*-CH_3 (**G4**) > 2,3,5,6-tetramethyl (**G16**) > *p*-CF_3 (**G13**)> *m*-CF_3 (**G12**)(Table 1). The same trend was observed in series B.

Discussion

SAR against *P. falciparum:*

- We observed an increase in the in vitro inhibitory activity against *P. falciparum* of the compounds with the presence of electron-withdrawing substituents like *p*-NO_2 (**E3**, **G3**), *p*-Cl (**E6**, **G6**), *p*-Br (**E7**, **G7**) or *p*-CF_3 (**A-B{13}**, **E-G{13}**) with the lipophilicity apparently playing a minor role.
- The activity was not affected by moving the substituents from *para* to *meta* position on the BS moiety. In fact, *p*- and *m*-NO_2 derivatives (i.e. **E3**/**E9**, **F3**/**F9** or **G3**/**G9**) showed almost the same activity in all series as well as the *p*- and *m*-CF_3 analogs (i.e. **F13**/**F12** and **G13**/**G12**).
- This is consistent with our previous observations on series **A** and **B**. However, it appears that moving the substituent to the *ortho* position may be detrimental for the activity, since all *o*-NO_2 derivatives had lower potency (**A-G{11}**) than the *m*- and *p*-NO_2, and had almost equal potency to the un-substituent derivative.

Discussion

Cytotoxicity and Selectivity Index

Table 2 summarizes the IC_{50} values for the 16 members of the library that were chosen, following the WHO/TDR Hits-activity selection criteria, for the determination of the IC_{50} against *P. falciparum*, *T. cruzi*, and *L. donovani*. These compounds were also subject of cytotoxicity studies against mammalian cells, which allows us to determinate the selectivity index as (SI = IC_{50} L6/IC_{50} parasite):

- All the selected compounds showed lower cytotoxicity to cells than the reference drug, podophyllotoxin.
- From the selected drugs, 13 were at least 650-fold less toxic than the reference.
- Against *P. falciparum*, the new members proved to have a poor antiplasmodial activity with a moderate selectivity index. The compounds with the better antiplasmodial profile were **B15** and **G15,** both with an IC_{50} of 1.5µM, which is 6-fold higher than CHQ (IC_{50} = 0.25µM), and the selectivity indexes (SI) were 8.1 and 4.6, respectively.

Discussion

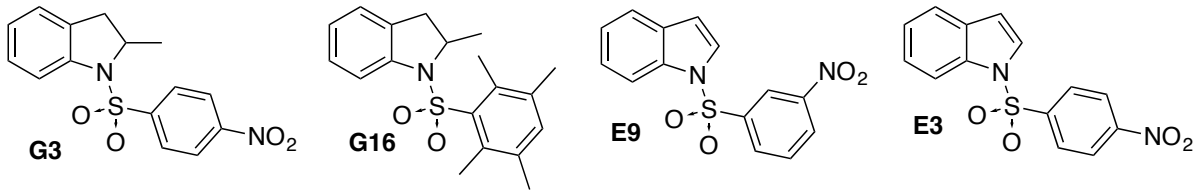
Cytotoxicity and Selectivity Index cont.

•Against T. cruzi, ten of the new members showed an IC_{50} below 10µM (Table 2), the threshold that defines a lead structure in the lead-generation stage of drug discovery.[9] Three compounds were particularly interesting, **G3** with an IC_{50} of 2.80µM (SI = 24), similar to the reference (benznidazole, IC_{50} = 2.13µM); **E3** and **E9**, that showed better activity than the reference with IC_{50}'s of 1.15µM and 1.08µM, with a SI values of 7.9 and 14, respectively. Due to this interesting anti-trypanosomal activity and good selectivity index, we identified compounds **G3**, **E3** and **E9** as lead scaffolds.[9]

•Against *L. donovani*, eight compounds showed IC_{50}'s between 0.23 and 2.16µM, six of those were the highly lipophilic 2,3,5,6-tetramethyl derivatives of different heterocycles. The most active compound was **G16** (IC_{50} = 0.23µM), with an in vitro activity comparable to the reference (MLT IC_{50} = 0.25µM) and a good SI of 28.8. Due to their promising profile **G16**, **B16** (IC_{50} = 0.62µM), **E16** (IC_{50} = 0.73µM) and **F16** (IC_{50} = 0.64µM) were selected as lead scaffold. Compounds **A16** (IC_{50} = 0.35µM) and **C16** (IC_{50} = 0.76µM) showed good activity but their SI values were less than 7, and were therefore discarded. A similar inhibitory profile was observed for the *p-tert*-butyl derivatives **G15** (IC_{50} = 1.53µM, SI = 7.95) and **A15** (IC_{50} = 2.16µM, SI = 7.52) (Table 2).

Conclusions

- Sixty-nine new N-benzenesulfonyl derivatives of heterocycles were tested against representative parasites.
- *T. cruzi* and *L. donovani* appeared to be the most sensitive microorganisms to the BS-Het library, while some compounds showed moderately activity against *P. falciparum*.
- The results demonstrated that the combination of the BS moiety with nitrogenated heterocycles have a positive effect on antiparasitic activity, validating the criteria used in the fragment-based drug design approach.
- The SAR analysis against *L. donovani* and *T. cruzi* reveal that no unique parameter can describe the bioactivity of this series, rather a combination of lipophilic and electronic characteristics would play an important role on the antiparasitic activity of this series.
- Based on these results we have been able to identify a lead structure (**G16**) against *L. donovani,* and select three (**G3**, **E3** and **E9**) as lead against *T. cruzi*.



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Acknowledgments

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