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Pentafluorosulfanyl as a Novel Building Block for Enzyme Inhibitors: Trypanothione Reductase Inhibition and Antiprotozoal Activities of Diarylamines

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The pentafluorosulfanyl (SF_5) group is a perfectly stable building block under physiological conditions. Pioneering work on the synthesis and properties of SF_5-containing aromatic compounds was carried out by Sheppard half a century ago.[1–3] Thereafter, this novel substituent had been largely ignored by the chemical community for several decades. Due to the increased availability of SF_5-substituted derivatives,[4–10] the organic chemistry of SF_5-containing derivatives has come recently under more widespread investigation and has lead to multiple applications in material sciences.[11–13] Fueled by the knowledge of the pronounced influence of fluorine on the physical and chemical properties[14,15] and therefore on the bioactivity of molecules, the interest of the life science industry in novel fluorine containing substituents is nowadays prevalent. Accordingly, the SF_5 group has gained the attraction of crop science in the recent years, as underlined by the growing number of patents[16] and research articles[5,17,18] for mainly agrochemicals containing this functional group.

The SF_5 group bears much similarity to the CF_3 substituent, but it is even more electronegative (Hammett substituent constants: SF_5: $\sigma_p = +0.68$; CF_3: $\sigma_p = +0.54$)[3] and has a higher lipophilicity (Hansch hydrophobicity constants: SF_5: $\pi = 1.51$, CF_3: $\pi = 1.09$).[19] In addition, the SF_5 group is distinguished by a higher thermal and chemical stability than its carbon relative[1,2] and has been characterized as a "super-trifluoromethyl" group in the literature.[4] Its strong polarity in combination with high lipophilicity and thermal stability renders the SF_5 group a highly interesting structural motif in medicinal chemistry.

To the best of our knowledge, no structure–activity relationship (SAR) data on the target level have been reported for SF_5-containing derivatives to date. We selected the flavoenzyme trypanothione reductase (TR, EC 1.6.4.8), found in parasites of the trypanosomatid family, as a target for the design of SF_5-bearing inhibitors. Trypanosomatid parasites possess trypanothione [N^1,N^8-bis(glutathionyl)spermidine] and the enzyme TR instead of the nearly ubiquitous glutathione system composed of glutathione and the flavoenzyme glutathione reductase (GR, EC 1.6.4.2).[20] TR, which is the key enzyme of the trypanothione-based antioxidant defence systems of parasitic trypanosomes and *Leishmania*, has been shown to be essential; this renders the enzyme a promising target for the development of new drugs against antiparasitic drugs. *Trypanosoma brucei* is the causative agent of human African trypanosomiasis (African sleeping sickness), which threatens millions of people in ~36 countries of sub-Saharan Africa with an estimated number of current cases between 50000 and 70000.[21] *Trypanosoma cruzi* is the pathogen of Chagas' disease (American trypanosomiasis), an infection widespread in central and southern America and is responsible for 14000 deaths each year.[22] Another trypanosomatid-caused disease, leishmaniasis, is provoked by various species of *Leishmania*. The different forms of the disease range from cutaneous to visceral infections with millions of people infected. Around 50000 deaths per year are quoted mainly due to *L. donovani*.[22] New and improved drugs to fight these diseases are urgently needed, as current therapy for all forms of trypanosomiases and leishmaniases is problematic due to the severe adverse effects of the drugs in use, the long duration and high costs of treatment, and an increasing number of drug resistant pathogens.[23,24]

In the search for new drugs against trypanosomatid-induced diseases, TR has become an increasingly popular target. Various compounds have been discovered over the last decade that moderately inhibit TR.[24] Noticeably, several of them feature a basic or quaternary nitrogen connected through a flexible alkyl chain to a hydrophobic core. One of the prototypes of this inhibitor class are diaryl sulfide-based compounds, first reported by Sergheraert et al.[25] and further explored by Douglas et al.[26] In order to explore the eligibility of SF_5 as a building block for TR inhibitors and to compare it with the corresponding CF_3 or $C(CH_3)_3$ analogues, we designed and synthesized diarylamine derivatives **1**–**6**, which are structurally related to the known class of diphenyl sulfide inhibitors (Scheme 1).

In order to synthesize the diphenylamine core of the inhibitor scaffold, *para*-CF_3-substituted aniline **7** was reacted with 2,5-dichloronitrobenzene, which delivered the desired diphenylamine **8** together with a considerable amount of triphenylamine **9**. This twofold coupled side product of the nucleophilic

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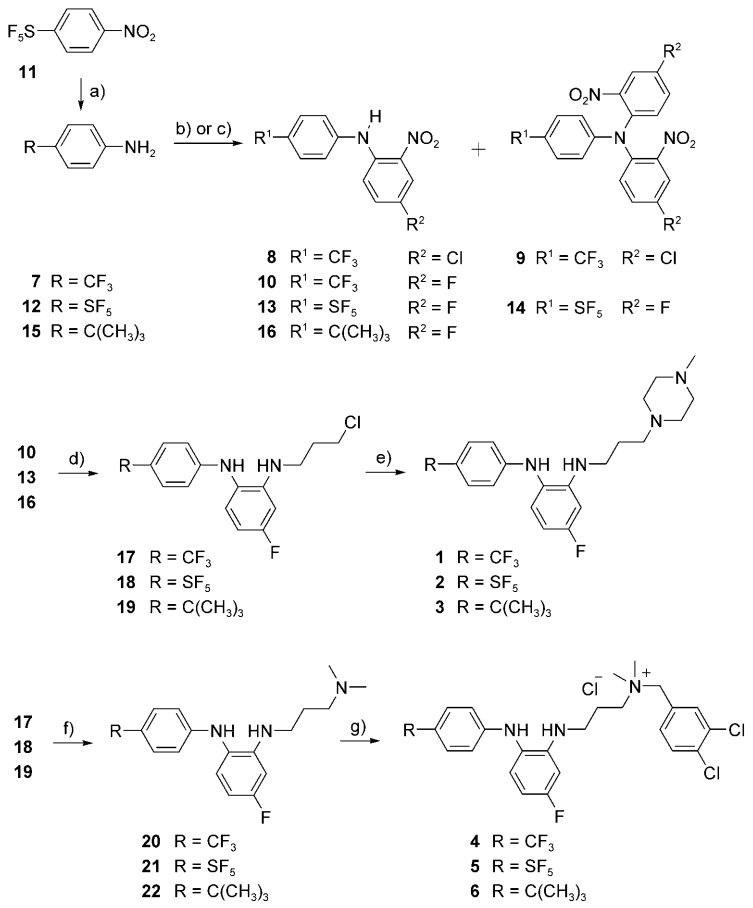
Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.



Scheme 1. Synthesis of diarylamine-based TR inhibitors. a) H_2 (5 bar), Raney-Ni, MeOH, 65 °C, **12** (97%); b) 2,5-dichloronitrobenzene, *t*BuOK, Me_2SO, 14 h, **8** (20%), **9** (9%); c) 2,5-difluoronitrobenzene, *t*BuOK, Me_2SO, 14 h, **10** (35%), **13** (25%), **14** (16%), **16** (38%); d) 1. Zn, NH_4Cl, MeOH, 65 °C, 1–5 h; 2. 3-chloropropionyl chloride, THF, 1 h; 3. BH_3·THF, THF, 66 °C, 4–16 h, **17** (55%), **18** (74%), **19** (62%); e) *N*-methylpiperazine, MeCN, 60–70 °C, 12–14 h, **1** (92%), **2** (86%), **3** (93%); f) $NHMe_2$ (40%, in H_2O), DMF, 60 °C, 14–16 h, **20** (91%), **21** (91%), **22** (92%); g) 3,4-dichlorobenzyl chloride, Et_2O, acetone, 50 °C, 16 h, **4** (55%), **5** (67%), **6** (60%). DMF = *N*,*N*-dimethylformamide; THF = tetrahydrofuran.

aromatic substitution (S_NAr) reaction could largely be avoided by using 2,5-difluoronitrobenzene instead of 2,5-dichloronitrobenzene as an electrophile, yielding the desired diphenylamine **10**. To obtain the SF_5-containing diarylamine core, *p*-nitrophenylsulfur pentafluoride (**11**), which in the meantime is commercially available, was hydrogenated as described in the literature.[5] The Supporting Information contains the first crystal structure of this valuable precursor (**11**) that can be used for the introduction of aryl-SF_5 residues. The obtained aniline (**12**) was reacted with 2,5-difluoronitrobenzene, which delivered the desired diphenylamine **13** as well as the corresponding triphenylamine **14** in low yields. The structures of triarylamines **9** and **14** were confirmed by X-ray analysis (see the Supporting Information). To generate the $C(CH_3)_3$ derivative, *p*-(*tert*-butyl)-aniline (**15**) was converted as described above to yield diphenylamine **16**.

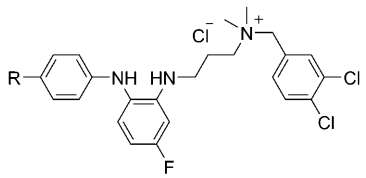
Reduction of the nitro group of diphenylamines **10**, **13**, and **16**, followed by acylation and subsequent reduction of the obtained amide functionality, delivered alkyl chlorides **17**–**19**, which were transformed into the potential TR inhibitors **1**–**3** by treatment with *N*-methylpiperazine.

In order to access the quaternary analogues, chlorides **17**–**19** were treated with dimethylamine. Quaternization of the obtained tertiary amines **20**–**22** by using 3,4-dichlorobenzyl chloride yielded the desired benzylammonium cations **4**–**6**.

The ability of these compounds to inhibit *T. cruzi* TR was investigated using a photometric assay (Supporting Information). Low solubility of the *N*-methylpiperazine derivatives **1**–**3** in the assay buffer prevented an accurate kinetic analysis (data not shown). Enhanced solubility of derivatives **4**–**6**, which feature a quaternary ammonium ion-terminated alkyl chain, allowed the determination of the TR inhibition constants. The kinetic analysis demonstrated fully competitive inhibition towards trypanothione disulfide (TS_2) for the CF_3 substituted diarylamine **4** with a K_{ic} (competitive inhibition constant) of 24 μM (Table 1). Exchange of the CF_3 with the SF_5 substituent resulted in a very similar inhibition potency but changed the mode of inhibition from purely competitive for **4** to mixed competitive-uncompetitive for **5** (K_{ic} = 28 μM; K_{iu} [uncompetitive inhibition constant] = 72 μM). The same kinetic pattern was observed for **6**, while the affinity for the parasite enzyme was significantly reduced upon the incorporation of the sterically demanding *tert*-butyl substituent into the diarylamine core (K_{ic} = 84 μM; K_{iu} = 158 μM). In comparison to structurally related diaryl sulfide-based inhibitors, the replacement of the diaryl sulfide by a diarylamine core led to a decrease in affinity. For the sulfur analog of the CF_3-substituted diarylamine **4**, a K_{ic} of 6 μM was previously reported.[27]

Potential inhibitors were manually docked in the empty active site structure of *T. cruzi* TR cocrystallized with trypanothione (PDB code: 1BZL).[28] The enzyme structure was fixed (except for the Glu18 side chain), and the energy of the system was minimized using the MAB force field as implemented in the computer program

Table 1. Inhibition of *T. cruzi* TR by the quaternary diarylamine-based derivatives **4**–**6**.



	R	K_{ic} [μM][a]	K_{iu} [μM][a]	Mode of inhibition
4	CF_3	24 ± 5	–	competitive
5	SF_5	28 ± 4	72 ± 16	mixed
6	$C(CH_3)_3$	84 ± 15	158 ± 34	mixed

[a] 5–10 mU cm^{-3} *T. cruzi* TR, 25 °C. Mixed: mixed competitive-uncompetitive. The assay was run in triplicate.

MOLOC.[29] Evaluation of different binding conformations of the inhibitors was based on 1) avoidance of unfavorable steric contacts, 2) forming of H-bonding contacts, and 3) optimal filling of the space within binding pockets by use of maximal capacity for hydrophobic contacts between enzyme and ligand.

Adopting the binding mode proposed for structurally closely related diaryl sulfide inhibitors,[27] the phenylamine moiety bearing a CF_3, SF_5, or *t*Bu substituent occupies the hydrophobic "mepacrine binding site" roughly framed by Trp21, Tyr110, and Met113. Furthermore, the 1,2-diaminophenyl motif should act as a ligand for Glu18 whereas the cationic nitrogen may interact electrostatically with Glu465′/Glu466′. The 3,4-dichlorobenzyl unit points into the Z-site, as proposed by Douglas et al. for phenothiazine derivatives[30] (Figure 1).

The observed changes in binding affinities in this series can be rationalized by the steric as well as electronic properties of the CF_3, SF_5, and $C(CH_3)_3$ substituents. The mixed competitive-uncompetitive inhibition pattern hints at the undefined binding mode of inhibitor **6**, as the bulky $C(CH_3)_3$ groups may prevent the efficient occupation of the mepacrine binding site. According to the modeling study, the introduction of the $C(CH_3)_3$ substituent pushes the phenylamine ring away from the surface of the protein, which results in a loss of favorable edge-to-face contacts of this aromatic inhibitor moiety with Trp21 and also weakens the sulfur-π interaction with Met113 (Figure 2). The CF_3 substituent is considerably smaller and allows for better accommodation of the phenylamine moiety in this region of the TR active site. The size of the SF_5 group lies between those of the CF_3 and the $C(CH_3)_3$ group. However, the strong electron-withdrawing nature of the SF_5 substituent might strengthen the T-shaped interaction of inhibitor **5** with the electron-rich Trp21, Tyr110, and Met113 residues, which explains the comparable binding affinity of the bulkier inhibitor **5** compared to its CF_3 analogue **4**. The loss of affinity of the diarylamine compared to the diaryl sulfide inhibitors might arise from the less favorable conformation of the diarylamine core for binding to the TR active site.

The newly synthesized dimethylamine-, piperazine-, and benzylammonium derivatives were tested in vitro for their ability to inhibit the growth of trypanosomatids *L. donovani*, *T. cruzi*, and *Trypanosoma brucei rhodesiense* as well as against the malarial parasite *Plasmodium falciparum*. While none of the diarylamines showed a significant effect on the growth of axenic *L. donovani*, the nonquaternized piperazine and dimethylamine derivatives **1**, **2**, **3**, **21**, and **22** moderately inhibited the growth of *T. cruzi*. The most active compound against this intracellular parasite is the *tert*-butyl derivative **3**, which has an IC_{50} (concentration of an inhibitor that results in 50% growth inhibition) value of 42.3 μM. Much stronger activities were observed for the nonquaternized derivatives against *T. b. rhodesiense*, with IC_{50} values between 2.4 and 3.7 μM for piperazines **1**, **2**, and **3** and 2.4 μM for diphenylamine **22** (Table 2). Any activity against *T. b. rhodesiense* is abolished upon introduction of the quaternary 3,4-dichlorobenzylammonium substituent as headpiece of the flexible alkyl chain, as earlier shown for diphenyl sulfide-based TR inhibitors,[31] and might be due to inefficient uptake of the permanently charged scaffolds.

Although *P. falciparum* does not possess the unique trypanothione metabolism, simultaneous activities against *T. b. rhodesiense* and *P. falciparum* have been observed recently for diaryl sulfide-based TR inhibitors.[26,27] The same pattern emerged with the series of diphenylamines presented here. The nonquaternary derivatives, with the exception of the trifluoromethyl compound **20**, exhibit decent activities with IC_{50} values between 1.2 and 2.8 μM. Incorporation of a quaternary ammonium center does not affect the activity against the malaria parasite, as underlined by the IC_{50} values for the diphenylamines **4** and **5**. The observed activity against *P. falciparum* shows that the diarylamines must have a different mode of action, other than TR inhibition, for these parasites. Therefore, the activity against *T. b. rhodesiense* may also be due to other cellular targets.

The cytotoxicity IC_{50} values on myoblast cells are in most cases at least ten-fold higher compared to the IC_{50} values against the protozoan parasites. Interestingly, in the series of piperazine derivatives, replacement of the CF_3 or *tert*-butyl substituent (as in **1** or **3**) by SF_5 (as in **2**) increased the selectivity

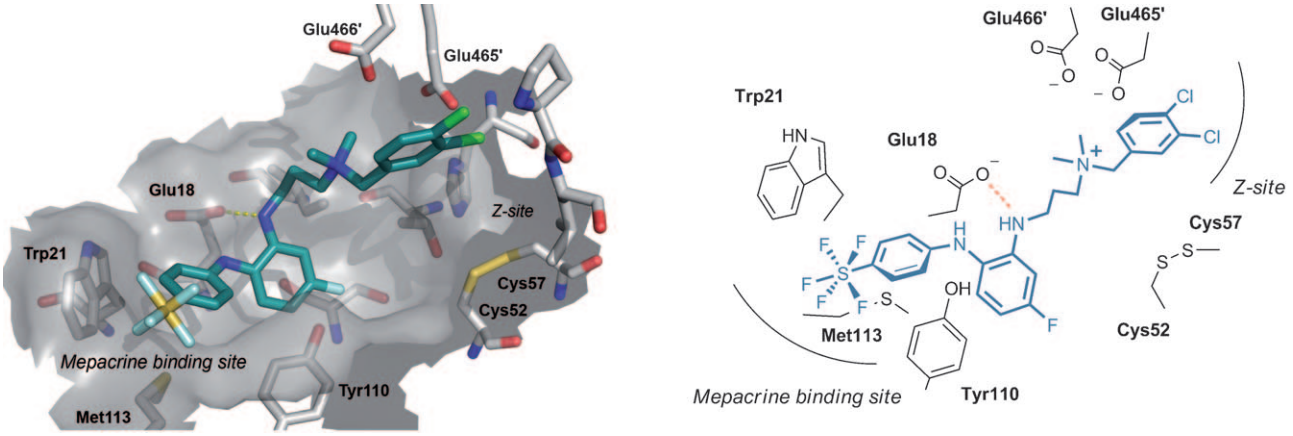


Figure 1. Inhibitor **5** is shown docked into the trypanothione disulfide binding site of *T. cruzi* TR (PDB ID: 1BZL) using the modeling package MOLOC.[29] Cys52 and Cys57 form the redox active dithiol/disulfide of the enzyme that is involved in catalysis together with the FAD cofactor (not shown). Glu465′ and Glu466′ are residues provided by the second subunit of the homodimer.

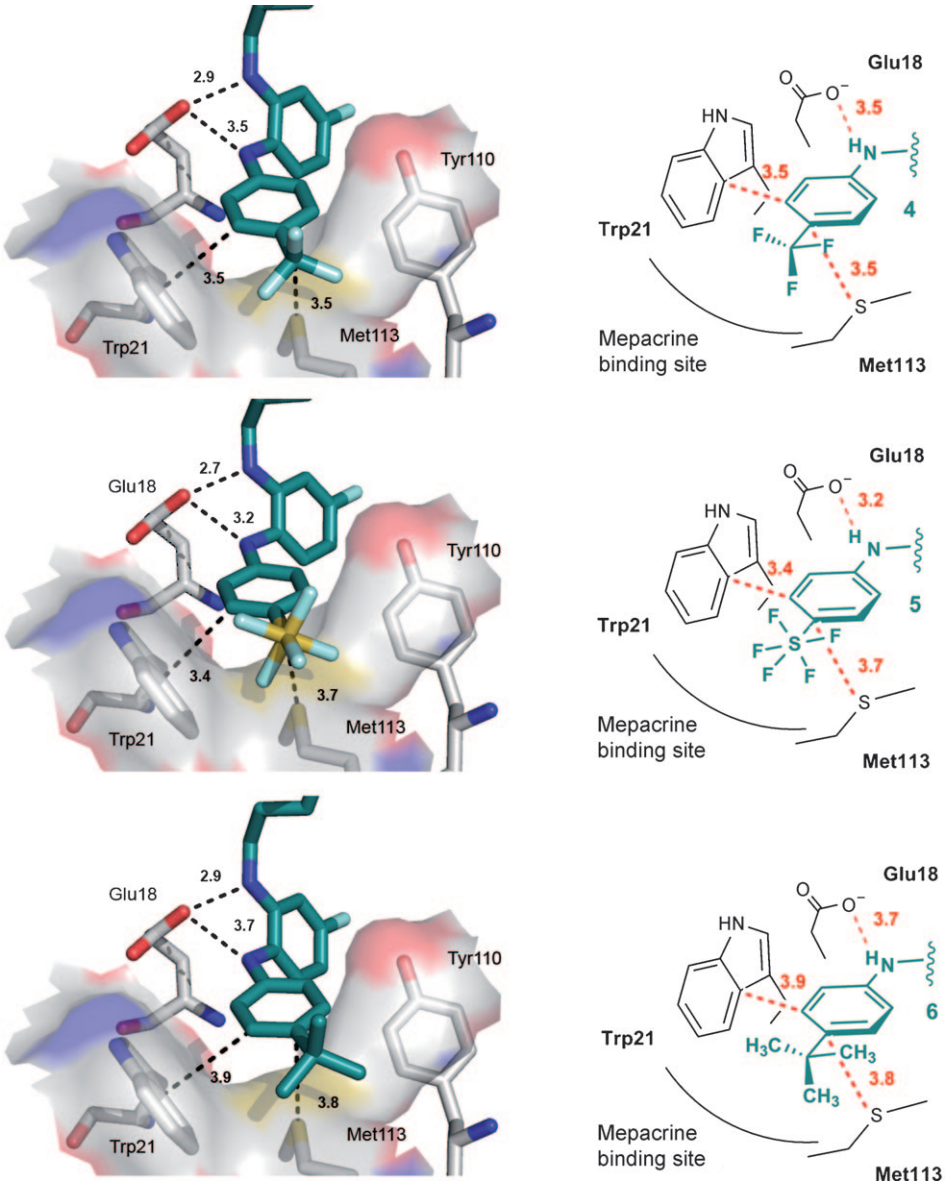


Figure 2. Modeled binding of diphenylamine inhibitors **4**–**6** to *T. cruzi* TR (PDB ID: 1BZL).

Table 2. In vitro activity against *T. b. rhodesiense* and *P. falciparum* as well as cytotoxicity of diphenylamine derivatives.

	T. b. rhodesiense IC_{50}[a] [μM]	*P. falciparum* IC_{50}[b] [μM]	Cytotoxicity IC_{50}[c] [μM]
1	2.4	n.d.	27.9
2	4.4	n.d.	> 192.1
3	2.5	1.6	31.9
4	n.d.	1.5	22.1
5	n.d.	1.2	53.2
21	154.4	3.2	9.7
22	2.4	1.3	11.7

Assays were run in duplicate and repeated once. [a] STIB900 *T. b. rhodesiense* strain, trypomastigote stage [b] at K1 *P. falciparum* strain, intra-erythrocytic form (IEF). [c] Rat myoblast cells to assess cytotoxicity. n.d.: not determined: less than 50% growth inhibition at 0.8 μg inhibitor per mL.

against the parasites considerably. Both SF_5 derivatives **2** and **5** exhibit the lowest cytotoxicity of all compounds tested.

Finally, we measured the membrane permeability of piperazine **2** using a parallel artificial membrane permeation assay (PAMPA).[32] The partition of the SF_5 derivative between a donor well and acceptor well separated by a egg lecithin mimic phospholipid layer at pH 6.5 was measured by UV absorption. The SF_5-derivative **2** displays good passive membrane permeability (0.22×10^{-6} cm s^{-1}). Its logarithmic distribution coefficient at pH 7.4 (log *D* value) of 3.56 lies in the desirable range, and its pK_a values of 7.31 and 3.72 suggest that the piperazine moiety is partially protonated under physiological conditions.

In summary, we report here that SF_5 is a suitable substituent for novel diarylamine-based TR inhibitors with micromolar affinities for the parasite's enzyme. A preference of the mepacrine binding site for CF_3 and SF_5 substituents over the bulkier *t*Bu residue, can be rationalized by molecular modeling. In vitro studies on different trypanosomatides as well as with the apicomplexan *P. falciparum* revealed micromolar activities for the piperazine- and dimethylamine-bearing ligands against *T. b. rhodesiense* as well as against *P. falciparum*. An exclusive activity against the malaria parasite was found for diarylamine derivatives with quaternary ammonium-terminated alkyl chains. Finally, it should be noted that the ligands with SF_5 substituents display the lowest cytotoxicity among all compounds tested and show good membrane permeability.

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