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Isothiazole Dioxides: Synthesis and Inhibition of *Trypanosoma brucei* Protein Farnesyltransferase

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Abstract—A series of isothiazole dioxides was synthesized and evaluated as inhibitors of protein farnesyltransferase from the parasite that causes African sleeping sickness (*Trypanosoma brucei*). The most potent compound in the series inhibited the parasite enzyme with an IC_{50} of 2 μM and blocked the growth of the bloodstream parasite in vitro with an ED_{50} of 10 μM. The same compound inhibited rat protein farnesyltransferase and protein geranylgeranyltransferase type I only at much higher concentration.

Protein farnesyltransferase (PFT) has been shown to be an ideal drug target for combating infections caused by the protozoan parasite *Trypanosoma brucei*, the causative agent of African sleeping sickness. Not only are inhibitors of *T. brucei* PFT (TB-PFT) able to arrest the growth of cultured parasites,[1,2] but PFT inhibitors are well developed as pharmaceutically active agents that are now in clinical development for the treatment of cancer.[3]

PFT catalyzes the transfer of the farnesyl group from farnesyl pyrophosphate (FPP) to the cysteine SH of the tetrapeptide motif CaaX (C is cysteine, a is usually but not necessarily an aliphatic residue, and X is a variety of different amino acids). The active site of TB-PFT, derived from the homology model using the X-ray structure of mammalian PFT as a template, shows that the residues that contact the farnesyl pyrophosphate (FPP) substrate and the aaX portion of the CaaX substrate are nearly identical to those found in mammalian PFT, but five of nine residues that contact the X portion of CaaX are different in the Trypanosomatid enzyme.[2] Regions of the active site not involved in FPP and CaaX binding also differ between the two PFTs.

This leads to the prediction that some inhibitors of mammalian PFT may also inhibit TB-PFT with similar potency, but that species specific inhibitors should also be obtainable. In our previous studies we have shown that CaaX mimetics including FTI-276 inhibit mammalian PFT and TB-PFT in the low nanomolar range.[1,2] On the other hand, the non-peptide heterocycle SCH-44342, which binds in the CaaX binding region but also extends into a remote enzyme pocket, is highly specific for mammalian PFT.[2] In the present study, we have identified a class of isothiazole dioxides that inhibit TB-PFT in preference to the mammalian enzyme.

Compounds used in this study belong to two different classes in the isothiazole dioxide series. The first class includes 3-diethylamino-4-(4-methoxyphenyl)-isothiazole 1,1-dioxides unsubstituted at C-5 or substituted with different substituents ranging from simple alkyl to aryl, or heteroaryl groups. To the second class belongs a series of 5-substituted 3-diethylamino-4-(4-methoxyphenyl)-isothiazole 1,1-dioxides and the corresponding 4,5-dihydro derivatives whose main feature is an S-atom as a linker between the isothiazole moiety and the substituent.

Compounds **1** and **2** were synthesized through intramolecular cyclization of the corresponding α-ketosulfonylamidines with a known methodology.[4] This methodology is very useful but severely limited by the scarce availability of the sulfonylazides used as the

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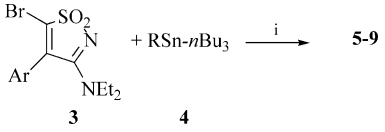
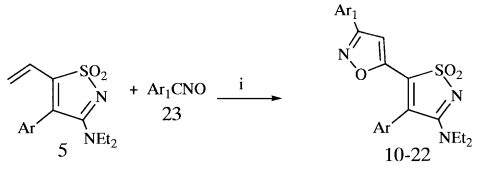
starting material. For this reason, a more general method allowing the preparation of a wide range of C-5 substituted isothiazole dioxides was used. Compounds **5**–**9** were prepared through the Stille coupling of organostannanes **4** with 5-bromo derivative **3**[5] (Scheme 1). Compound **3** is readily accessible by reacting **1** with bromine followed by treatment with TEA. Compounds **10**–**22** were obtained through cycloaddition reaction of **5** with the appropriate nitrile oxides **23** in a very regioselective reaction with satisfactory yield (Scheme 2).[6]

Compounds **24**–**29** and **30**–**32** were obtained from **1** or **3** through nucleophilic addition of the appropriate sulfur nucleophile. Both compounds **1** or **3** have been demonstrated to undergo Michael addition of the nucleophile affording 4,5-dihydro derivatives and, in the case of **3**, the presence of a bromine atom allowed the regeneration of the C–4 C-5 double bond (Schemes 3 and 4).[7]

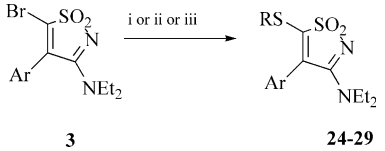
We tested a subset of the compounds (Tables 1 and 2) for in vitro inhibition of recombinant TB-PFT, rat PFT, and rat protein geranylgeranyltransferase type-I (PGGT-I), and results are summarized in Table 3. The most potent inhibitor of TB-PFT is **10**, which inhibits the enzyme by 66% when tested at 5 μM. Retesting at several different inhibitor concentrations gave an IC_{50} of 2 μM. Compound **10** did not inhibit rat PFT when tested at 2 μM, but inhibited this enzyme by 21% at 50 μM, and inhibited rat PGGT-I by 25% at 50 μM. Thus, **10** shows a strong preference for the Trypanosomatid enzyme. Some of the compounds inhibited all 3 prenyltransferases (i.e., **2**), but only partially and at relatively high concentration (100 μM, Table 3). All of the compounds are relatively poor inhibitors ($IC_{50} \geq 100$ μM) of rat PFT and rat PGGT-I, and none of the compounds inhibited rat PFT in preference to Rat PGGT-I.



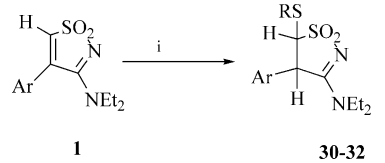
Scheme 1. Reagents and conditions: (i) $(Ph_3P)_2PhCH_2ClPd$, toluene, 110 °C.



Scheme 2. Reagents and conditions: (i) CH_2Cl_2 or benzene.



Scheme 3. Reagents and conditions: (i) MeSNa, CH_2Cl_2/DMF; (ii) RSH, TEA, CH_2Cl_2/DMF; (iii) $NaSH.H_2O$, MeOH then RBr.



Scheme 4. Reagents and conditions: (i) MeSNa or RSH, CH_3CN.

Table 1. Class 1

Compd	R
1[4]	H
2[8]	CH_3
5[5]	Vinyl-
6[5]	C_6H_5
7[5]	2-Pyridyl
8	(*N*-Phenylsulfonyl)-3-indolyl-
9[5]	2-Phenylethynyl-
10[6]	5-[3-(2,4,6-Trimethylphenyl)-4,5-dihydro-isoxazolyl]-
11	5-[3-(4-Methoxyphenyl)-4,5-dihydro-isoxazolyl]-
12[6]	5-[3-(3,5-Dichloro-2,4,6-trimethylphenyl)-4,5-dihydro-isoxazolyl]-
13[6]	5-[3-(2,6-Dichlorophenyl)-4,5-dihydro-isoxazolyl]-
14[6]	5-[3-(4-Chlorophenyl)-4,5-dihydro-isoxazolyl]-
15[6]	5-[3-(4-Nitrophenyl)-4,5-dihydro-isoxazolyl]-
16	5-[3-(2,6-Dimethylphenyl)-4,5-dihydro-isoxazolyl]-
17	5-[3-(2-Methylphenyl)-4,5-dihydro-isoxazolyl]-
18	5-[3-(3,4-Dimethoxyphenyl)-4,5-dihydro-isoxazolyl-
19	5-[3-(4-Methylphenyl)-4,5-dihydro-isoxazolyl]-
20	5-[3-(2,3,4,5,6,-Pentamethylphenyl)-4,5-dihydro-isoxazolyl]-
21[6]	−5-[4-Ethoxy-3-(2,4,6-trimethylphenyl)-4,5-dihydro-isoxazolyl]-
22[6]	5-[3-(2,4,6-trimethylphenyl)-

Table 2. Class 2

Compd	R
24[7]	CH_3
25[7]	C_6H_5
26[7]	2-Pyridyl-
27[7]	4-Methylphenyl-
28	Perillyl-
29[7]	Farnesyl-
30[7]	CH_3
31[7]	C_6H_5
32[7]	Cyclohexyl-

This initial data set showed that Class 2 compounds (**29**–**32**) are poor PFT inhibitors. Among Class 1 compounds, the aryl-isoxazolyl group present in **10** appears to be important for inhibition of TB-PFT. Thus, we prepared and tested a number of analogues of **10** in which the substituents on the phenyl portion were varied, and results are summarized in Table 4. Compounds containing only a single substitution on the 4-position of the phenyl group (4-OMe, 4-Cl, 4-NO_2, and 4-Me present in **11**, **14**, **15**, and **19**, respectively) are much less potent than **10** against TB-PFT. Inhibition is nearly maintained when the remaining hydrogens on the phenyl group of **10** are substituted with chlorines (**12**) or when the three aromatic methyl groups of **10** are replaced with chlorines in the *ortho* positions (**13**). Other compounds are also less potent than **10** (**16**, **17**, and **20**). Compounds containing disruptions to the dihydro-isoxazolyl ring, **21** and **22**, were also less potent TB-PFT inhibitors.

Compound **10** was found to inhibit the growth of the bloodstream form of *T. brucei* in vitro with an ED_{50} of 10 μM.

Table 3. PFT and PGGT-I inhibition

Compd	Concn (μM)	% Inh. TB-PFT	% Inh. rat PFT	% Inh. rat PGGT-I
Class 1				
1	5	0	0	12
2	100	43	27	56
6	100	13	0	26
7	100	12	3	7
8	100	29	5	25
9	2	0	2	7
10	5	66	0	15
Class 2				
29	100	25	0	28
30	100	7	5	36
31	100	62	5	17
32	100	52	6	20

Table 4. PFT and PGGT-I inhibition

Compd	% Inh. at 2 μM TB-PFT	% Inh. at 50 μM rat PFT	% Inh. at 50 μM rat PGGT-I
10	50	21	25
11	8	13	17
12	28	2	7
13	32	14	45
14	0	8	20
15	2	18	8
16	8	22	17
17	2	22	0
20	13	7	No data
21	10	9	12
22	0	8	11

Experimental

3-Diethylamino-4-(4-methoxyphenyl)-5-(*N*-phenyl-sulfonyl-3-indolyl)-isothiazole dioxide 8. Bromoderivative **3** (373 mg, 1 mmol) and *benzylchlorobis* (triphenylphosphine)palladium (0.075 g, 0.1 mmol) were suspended in toluene (15 mL) and refluxed under nitrogen. Tin reagent *N*-sulfonyl-3-tributylstannylindole[9] was added in portions during 30 min. The reaction mixture was refluxed until disappearance of the reactants and purified by chromatography on silica gel (acOEt/cyclohexane 0:100 to 100:0). Yields of all compounds are listed at the end of the experimental synthesis section. Mp 133–135 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.80–1.10 (m, 3H, CH_3), 1.20–1.50 (m, 3H, CH_3), 3.05–3.30 (m, 2H, CH_2), 3.60–3.80 (m, 2H, CH_2), 3.87 (s, 3H, OCH_3), 6.97 (d, AB syst., $J=8.6$ Hz, aryl–H), 7.21 (d, AB syst., $J=8.6$ Hz, aryl–H), 7.15–7.31 (m, 3H, aryl–H), 7.35–7.60 (m, 5H, aryl–H), 7.65–7.90 (m, 3H, aryl–H).

General procedure for the preparation of 3-diethylamino-4-(4-methoxyphenyl)-5-[3-aryl-4,5-dihydro-isoxazolyl]-isothiazole dioxides (11 and 16–20). Equimolecular amount of **5** (1 mmol) and **23** (1 mmol) were dissolved in dichloromethane and stirred until disappearance of the reagents. The solvent was evaporated under reduced pressure and the residue crystallized (ether for **11**, **18**, **19**; CH_2Cl_2/ether for **16**, **17**, **20**) affording pure compounds. **11**: Mp 185 °C; ^{1}H NMR (200 M Hz, $CDCl_3$) 0.90, 1.25 (2t, $J=7$ Hz, 6H, 2 CH_3), 3.10, 3.60 (2q, $J=7$ Hz, 4H, CH_2), 3.50, 3.90 (2 dd, AMX syst., $^3J=8.8$ Hz, $^2J=16.8$ Hz, 2H, CH_2), 3.80 (2s, 6H, OCH_3), 5.25 (t, AMX syst., $^3J=8.8$ Hz), 6.92, 7.53 (2 AB syst., $J=9$ Hz), 6.90–7.40 (m, 4H, aryl–H). **16**: Mp 206 °C; ^{1}H NMR ($CDCl_3$) 0.91, 1.31 (2t, $J=7$ Hz, 6H, 2 CH_3), 2.29 (s, 6H, 2 CH_3); 3.10, 3.65 (2q, $J=7$ Hz, 4H, CH_2), 3.30, 4.00 (2 dd, AMX syst., $^3J=11.7$ Hz, $^2J=17.6$ Hz, 2H, CH_2), 3.88 (s, 3H, OCH_3), 5.25 (t, AMX syst., $^3J=11.7$ Hz), 7.00–7.45 (m, 7H, aryl–H). **17**: Mp 141–142 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.91, 1.31 (2t, $J=7$ Hz, 6H, 2 CH_3), 2.52 (s, 3H, CH_3), 3.10, 3.62 (2q, $J=7$ Hz, 4H, CH_2), 3.52 (dd, AMX syst., $^3J=11.4$ Hz, $^2J=16.8$ Hz, 1H, CH_2), 3.85 (s, 3H, OCH_3), 4.10 (dd, AMX syst., $^3J=8.4$ Hz, $^2J=16.8$ Hz, 1H, CH_2), 5.25 (dd, AMX syst., $^3J=11.4$ Hz, $^3J=8.4$ Hz,), 7.0 (d, AB syst., $J=7.7$ Hz, aryl–H), 7.20–7.37 (m, 6H, aryl–H). **18**: Mp 100–105 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.91, 1.31 (2t, $J=7$ Hz, 6H, 2 CH_3), 3.10, 3.60 (2q, $J=7$ Hz, 4H, CH_2), 3.50 (dd, AMX syst., $^3J=11.3$ Hz, $^2J=16.8$ Hz, 1H, CH_2); 3.85, 3.90, 3.92 (3s, 9H, OCH_3), 4.00 (dd, AMX syst., $^3J=8.8$ Hz, $^2J=16.8$ Hz, 1H, CH_2), 5.26 (dd, AMX syst., $^3J=11.3$ Hz, $^3J=8.8$ Hz), 6.85 (d, AB syst., $J=8$ Hz, aryl-H), 6.98–7.30 (m, 5H, aryl-H). **19**: Mp 175 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.89, 1.30 (2t, $J=7$ Hz, 6H, 2 CH_3), 2.38 (s, 3H, CH_3); 3.00–3.20; 3.45–3.65 (2m, 4H, CH_2), 3.50 (dd, AMX syst., $^3J=11.3$ Hz, $^2J=16.8$ Hz, 1H, CH_2), 3.85 (s, 3H, OCH_3), 3.90 (dd, AMX syst., $^3J=8.8$ Hz, $^2J=16.8$ Hz, 1H, CH_2), 5.25 (dd, AMX syst., $^3J=11.3$ Hz, $^3J=$ 8.8 Hz), 6.90–7.30 (m, 4H, aryl-H). 7.20, 7.45 (2d, AB syst., $J=8.1$ Hz, 4H, aryl-H). **20**: Mp 218 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.91, 1.31 (2t, $J=7$ Hz, 6H, 2 CH_3), 2.18, 2.20, 2.24 (3s, 15H, CH_3),

3.00–3.20, 3.62–3.80 (2m, 4H, CH_2), 3.20, 4.00 (2dd, AMX syst., $^3J = 11.3$ Hz, $^2J = 17.5$ Hz, 2H, CH_2), 3.89 (s, 3H, OCH_3), 5.26 (t, AMX syst., $^3J = 11.3$ Hz), 7.18–7.42 (m, 4H, aryl–H). Anal. $C_{28}H_{39}N_3SO_4$.

3-Diethylamino-4-(4-methoxyphenyl)-5-perillylsulfanyl-isothiazole dioxide 28. Bromoderivative **3** (373 mg, 1 mmol) is suspended in methanol (10 mL) and stirred at room temperature. $NaSH.H_2O$ (148 mg, 2 mmol) is added and stirred until disappearing of **3**. Perillyl bromide (1.4 mmol) is added. After 3 h, the reaction is complete. Solvent is evaporated under reduced pressure; the residue was taken up with water and extracted with dichloromethane. The organic layer was dried with Na_2SO_4 filtered and the solvent evaporated iv. The residue was purified by chromatography (CH_2Cl_2/MeOH 100:0 to 0:100) affording pure **28**. **28**: Mp 119 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.75–1.10 (m, 3H, CH_3), 1.10–2.30 (m, 13H), 3.05–3.30, 3.50–3.75 (2m, 4H, CH_2), 3.84 (s, 3H, OCH_3), 4.08 (s, 2H, SCH_2), 4.65–4.75 (m, 2H), 5.70–5.85 (m, 1H), 6.98, 7.16 (2d, AB syst., $J = 8.9$ Hz, aryl–H).

Compound yields and elemental analyses. Yields are: **1**, 80%; **2**, 60%; **5**, 70%; **6**, 70%; **7**, 60%; **8**, 60%; **9**, 86%; **10**, 89%; **11**, 60%; **12**, 77%; **13**, 78%; **14**, 59%; **15**, 37%; **16**, 69%; **17**, 35%; **18**, 30%; **19**, 33%; **20**, 60%; **21**, 90%; **22**, 65%; **24**, 68%; **25**, 31%; **26**, 73%; **27**, 88%; **28**, 60%; **29**, 50%; **30**, 60%; **31**, 89%; **32**, 90%. Acceptable elemental analysis was obtained for compounds **8**, **11**, **16**–**20**, **28**.

Biological studies. PFT assays were carried out as described previously.[1,2] Assay conditions are as follows: 20 μL reaction mixture containing 30 mM potassium phosphate, pH 7.7, 5 mM dithiothreitol, 0.5 mM $MgCl_2$, 20 μM $ZnCl_2$, 5 μM Ras-CVIM, 0.75 μM (0.3 μCi) [^{3}H]FPP, and 5 ng recombinant PFT,[2] were incubated at 30 °C for 30 min. Experiments with cultured Trypanosomes: Bloodstream forms of *T. brucei* strain 427[10] were cultured in HMI-9 medium as previously described.[10] Cells were incubated in the presence of serial dilutions of test compound for 48 h and growth was measured using the Alamar Blue method.[11]

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