Design, Synthesis and Antitrypanosomal Activities of 2,6-Disubstituted-4,5,7-Trifluorobenzothiophenes

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5 nucleophiles

12 examples

Trypanosoma brucei rhodesiense
IC_{50} 0.53 μM
Design, Synthesis and Antitrypanosomal Activities of 2,6-Disubstituted-4,5,7-Trifluorobenzothiophenes

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Abstract

Current treatments for Human African Trypanosomiasis (HAT) are limited in their application, have undesirable dosing regimens and unsatisfactory toxicities highlighting the need for the development of a safer drug pipeline. Our medicinal chemistry programme in developing rapidly accessible and modifiable heterocyclic scaffolds led to the design and synthesis of novel substituted benzothiophenes, with 6-benzimidazol-1-ylbenzothiophene derivatives demonstrating significant antitrypanosomal activities (IC₅₀ < 1 µM) against Trypanosoma brucei rhodesiense and no toxicity towards mammalian cells.

1. Introduction

Human African Trypanosomiasis, also known as sleeping sickness is caused by infection with Trypanosoma brucei rhodesiense (T.b.r) or Trypanosoma brucei gambiense (T.b.g) parasites. During the haemolymphatic phase, trypomastigotes circulate within the blood and lymphatic system. If not treated sufficiently, the neurological phase ensues as parasites penetrate the blood brain barrier thus infecting the central nervous system from which patient recovery is unlikely. Current treatment for T.b.r is dated and potentially lethal consisting of Suramin for the haemolymphatic phase and arsenic based melarsoprol for the neurological phase. These treatments require complicated dosing regimens and related side-effects such as reactive encephalopathy have proven fatal in up to 9% of patients [1]. Clinical advancements in antitrypanosomal drug development have been limited and resistance is increasing requiring the development of a new drug pipeline to replace existing therapies. Recent literature findings have included the development of 3-nitrotiazole based piperazides exhibiting appreciable in vitro activity against T.b.r parasites as well as series of hybrids of bile acids and Cinchona alkaloids demonstrating high in vitro activities against Trypanosoma brucei brucei [2, 3].

Developing rapidly accessible fluorinated heterocyclic scaffolds [4, 5] with potential for further elaboration is thematic of our medicinal chemistry programme. Benzothiophene ring systems represent privileged scaffolds in medicinal chemistry and have been incorporated into: reverse transcriptase inhibitors; tubulin binders to inhibit microtubule formation; and antiprotozoal agents [6, 7, 8, 9, 10]. Our investigations highlighted in this article yielded diversely substituted fluorinated
benzothiophene scaffolds using aromatic nucleophilic substitution reactions (SNAr) of perfluorinated building blocks [11]. SNAr of perfluorinated arenes occur readily with a wide range of nucleophiles and often proceed under mild conditions without the need for transition metal catalysis [12]. The substitution patterns available by sequential replacement of fluorine differ from those afforded by electrophilic reaction protocols and allow structurally diverse derivatives to be prepared according to drug design strategies [13, 14]. Retaining fluorine is also desirable property in drug design since fluorine can improve metabolic stability due to the strength of the C-F bond (ca. 480 kJmol⁻¹), whilst its small size (van der Waals radius, 147 pm) and high electronegativity impart desirable properties to a molecule which can significantly alter biological response [15, 16].

2. Methods and Results
2.1 Syntheses
Continuing our interest in preparing condensed sulfur containing heterocycles [17] from perfluorooarene precursors, firstly, tetrafluorobenzaldehydes 2a-e suitable for ring annelation reactions to construct the benzothiophene derivatives were prepared through the reaction of pentafluorobenzaldehyde 1 with a range of nucleophiles (Scheme 1) including two diazoles, a phenol and two thiols [18]. Treatment of the aldehydes 2 with α-mercaptocarbonyl compounds 3a or 3b was expected to lead to addition of the thiol to the 2-position of the benzaldehyde and allow condensation of the active methylene group with the adjacent aldehyde to form a fused thiophene ring 5, since thieno[2,3-c]pyridines have been prepared by a related method involving cyclisation onto a nitrile [19].

In practice, pentafluorobenzaldehyde reacted smoothly with selected diazoles, arenethiols and 2-bromophenol to form a series of 4-substituted benzaldehydes 2 in good yields. Addition at the 4-position is in line with the usual orientation of addition observed for perfluorinated arenes [20]. Treatment of the benzaldehydes with either methyl α-mercaptoacetate 3a or 2’-mercaptoacetophenone 3b in the presence of triethylamine as base led to the clean conversion to the 6-substituted-4,5,7-trifluorobenzothiophenes 5aa-5be in moderate to excellent yields. Treatment of pentafluorobenzaldehyde 1 with two equivalents of 3a or 3b led to direct formation of 5af and 5bf in reasonable yields, in which the thiols most likely added consecutively to the 4- and 2-positions of the aldehyde. Subsequently, the intramolecular reaction of the 2-substituent with the adjacent aldehyde group would have resulted in cyclisation forming the benzothiophene scaffold in a one-pot procedure. The intermediate 2-sulfanyl benzaldehydes 4a/b were not isolated. The structures of the new compounds were fully in accord with their analytical and spectroscopic properties. Computational studies to predict oral druglikeness demonstrated no compound within this study violated more than one of the parameters constituting Lipinski’s rule of 5 and all fall within acceptable limits of rotational bond count and polar surface area [21, 22, 23, 24]. The structure of compound 5bb was confirmed by single crystal X-ray diffraction analysis, with the molecular structure shown in Figure 1 [25, 26, 27].

2.2 Antitrypanosomal and Antiproliferative Activity
Synthesised compounds (Table 1) were screened in a phenotypic assay against Trypanosoma brucei rhodesiense (STIB 900) and cytotoxicities determined against MCF7 cells [28].

2.2.1 6-Substituted-benzothiophene-2-carboxylate derivatives
Compound 5ad, synthesised bearing a 6-(2-bromophenylsulfanyl) group demonstrated moderate trypanocidal activity with an IC₅₀ value of 11.9 µM. Compound 5ac was prepared with a 6-(2-bromophenoxy) group and gave an IC₅₀ value of 30 µM, with a similar toxicity value against the MCF7 cell line. The bromophenyl groups in these two compounds were strategically designed to allow further modification of the scaffold by halogen-lithium exchange which would allow ring forming
reactions. The results of such reactions will be reported in due course alongside comparative bioactivity data. Compound 5aa, the 6-imidazolyl substituted analogue did not demonstrate trypanocidal activity, nor did the 6-(4-tert-butylphenylsulfonyl) derivative 5ae. Compound 5af, the 6-benzoylmethylsulfanyl analogue, showed moderate activity but interestingly, the 6-benzimidazolyl analogue 5ab demonstrated respectable trypanocidal activity with an IC\textsubscript{50} value of 0.60 µM, significantly greater than aforementioned benzo thiophene-2-carboxylate derivatives screened. The selectivity ratio of trypanocidal activity against MCF7 cells was greater than 20 fold, making this compound a successful hit within the 2-carboxylate derivatives.

2.2.2 2-Benzoyl-benzothiophene derivatives

In the ketone series, compound 5bd, the 2-benzoylbenzothiophene with a bromophenyl sulfonyl substituent showed comparable antiparasitic and cytotoxic activity to 5ad with an IC\textsubscript{50} value of 9.8 µM. Similarly, the imidazolyl substituted compound 5ba showed an increase in trypanocidal activity with an IC\textsubscript{50} value of 33.0 µM in relation to its carboxylate analogue 5aa, but not to the extent of being classified as a hit compound. Likewise for the pair 5be/5ae the ketone showed 2-fold better antiparasitic activity than the corresponding carboxylate, while for 5bf/5af comparable trypanocidal activity was observed in the region of 10 µM and above. Importantly, the benzimidazole-containing compound 5bb demonstrated a trypanocidal IC\textsubscript{50} value of 0.53 µM, comparable to the 2-carboxylate 5ab and significantly more active than the other ketones evaluated. The selectivity ratio of trypanocidal activity of 5bb against MCF7 cells was almost than 50 fold, making this compound a successful hit within the ketone derivatives.

3. Conclusion

A library of novel and easily accessible fluorinated benzothiophenes were synthesised by employing S\textsubscript{N}Ar substitution reactions of perfluorinated building blocks. Noticeably, 5ab and 5bb were identified as hit compounds with IC\textsubscript{50} values <1 µM against Trypanosoma brucei rhodesiense and no interpreted toxicity against MCF7 cells. Interestingly, both benzothiophene scaffolds were substituted with a benzimidazole moiety. Our hit compounds hold potential for further modification and the benzothiophene scaffold serves as a point of expansion on our current library. Results from further experiments will be published in due course.

4. Experimental

Solvents used were commercially available. Flash column chromatography was carried out on Merck Kiesel 60 silica gel and TLC analysis performed on Merck TLC silica gel 60 F\textsubscript{254} aluminum backed plates. NMR spectra were recorded in CDCl\textsubscript{3} at 400 MHz (\textsuperscript{1}H NMR), 376 MHz (\textsuperscript{19}F NMR) or 100 MHz (\textsuperscript{13}C NMR) on a Bruker Advance 400 MHz instrument or a Joel JNM-ECS400 instrument. IR spectra were recorded on a PerkinElmer Spectrum 65, FT-IR Spectrometer. HRMS spectra were recorded using a Thermofisher Exactive (orbitrap) mass spectrometer with ESI as the ionization mode, or were recorded at the EPSRC UK National Mass Spectrometry Facility at Swansea University. Melting points were recorded using an Electro thermal-IA 9100 melting point instrument. Elemental analyses were determined on a Perkin-Elmer 2400 analyser.

4.1 Synthesis of Compounds 2a-e

A solution of pentafluorobenzaldehyde (2 mmol) in THF (2.5 ml) was added to a solution of the relevant nucleophile (2-4 mmol) in THF (2.5 ml), with Et\textsubscript{3}N (4-10 mmol) used in the reactions of compounds 2c-e. The resulting solution was stirred at room temperature for up to 24 h. The reaction mixture was poured into deionised water and was extracted with ethyl acetate (15 ml x 3). Organic extracts for compounds 2c-e were further washed with 0.2 M HCl (aq) and deionised water, combined and dried over Na\textsubscript{2}SO\textsubscript{4}. The solution was concentrated and the crude residue purified by column chromatography (elution with light petroleum/ethyl acetate).
4-Imidazol-1-yl-tetrafluorobenzoaldehyde (2a) [18]

Yield 68%, red oil. NMR δH (CDCl₃), 10.31 (1H, s, CHO), 7.88 (1H, s), 7.32 (1H, t), 7.29 (1H, s); NMR δF (CDCl₃), 19.4-19.2 (2F, m), 14.9-15.0 (2F, m); NMR δC (CDCl₃), 181.5 (CHO), 147.4 (dd, J = 260, 11, 4 Hz), 140.7 (ddd, J = 255, 12, 5 Hz), 137.5, 130.5 (C=4), 122.0 (t, J = 12 Hz), 119.7, 113.6 (t, J = 10 Hz); IR, νmax /cm⁻¹ 1712 (CHO); MS, m/z found 245.0332, C₁₀H₇F₄N₂O, (M+H⁺) requires 245.0333.

4-Benzimidazol-1-yl-tetrafluorobenzoaldehyde (2b)

Yield 62%, yellow solid, m.p. 160-162 °C. NMR δH (CDCl₃), 10.42 (1H, s, CHO), 8.09 (1H, t, J = 1.8 Hz), 7.96-7.92 (1H, m), 7.37-7.24 (1H, m), 3.33-3.29 (1H, m); NMR δC (CDCl₃), 182.0 (CHO), 153.0, 147.6 (ddd, J = 260, 13, 6 Hz), 140.6 (dd, J = 250, 12 Hz), 134.2, 128.8, 126.3, 125.9, 117.3, 112.6, 111.1 (t, J = 10 Hz); IR, νmax /cm⁻¹ 1705 (CHO); MS, m/z found 348.9489, C₁₄H₁₄F₄N₂O, (M+H⁺) requires 348.9482; elemental analysis, C₁₄H₁₄F₄N₂O requires: C, 57.15; H, 2.06; N, 9.52; found: C, 57.19; H, 2.16; N, 9.37.

4-(2-Bromophenoxy)-2,3,5,6-tetrafluorobenzaldehyde (2c)

Yield 62%, white solid, m.p. 42-44 °C. NMR δH (CDCl₃), 10.30 (1H, s, CHO), 7.67 (1H, dd, J = 8.0, 1.6 Hz), 7.31 (1H, td, J = 8.0, 1.6 Hz), 6.94 (1H, d, J = 8.0 Hz); NMR δC (CDCl₃), 17.1-17.0 (2F, m), 8.4-8.3 (2F, m); NMR δF (CDCl₃), 182.0 (CHO), 153.0, 147.6 (ddd, J = 260, 17, 6 Hz), 140.6 (dd, J = 250, 12 Hz), 134.2, 128.8, 126.3, 125.9, 117.3, 112.6, 111.1 (t, J = 10 Hz); IR, νmax /cm⁻¹ 1705 (CHO); MS, m/z found 348.9478, C₁₃H₆BrF₄O₂, (M+H⁺) requires 348.9482; elemental analysis, C₁₃H₆BrF₄O₂ requires: C, 44.85; H, 1.16; found: C, 44.73; H, 1.16.

4-[(2-Bromophenyl)thio]-2,3,5,6-tetrafluorobenzaldehyde (2d)

Yield 62%, white solid, m.p. 88-90 °C. NMR δH (CDCl₃), 10.33 (1H, s, CHO), 7.65 (1H, dd, J = 8.0, 1.2 Hz), 7.38 (1H, d, J = 7.6 Hz), 7.30 (1H, td, J = 7.6, 1.2 Hz), 7.23 (1H, td, J = 7.6, 1.6 Hz); NMR δC (CDCl₃), 29.7-29.6 (2F, m), 17.4-17.3 (2F, m); NMR δC (CDCl₃), 182.2 (CHO), 146.4 (dd, J = 260, 13 Hz), 146.1 (dd, J = 260, 11 Hz), 133.8, 132.7, 132.0, 130.1, 128.3, 125.9, 125.5 (t, J = 18 Hz), 114.7 (t, J = 11 Hz); IR, νmax /cm⁻¹ 1705 (HC=O); MS, m/z found 364.9251, C₁₃H₆BrF₄OS, (M+H⁺) requires 364.9253; elemental analysis, C₁₃H₆BrF₄OS requires: C, 42.76; H, 1.38; found: C, 42.73; H, 1.35.

4-[(4-[(tert-Butyl)phenyl]thio)-2,3,5,6-tetrafluorobenzaldehyde (2e)

Yield 73%, yellow solid, m.p. 42-44 °C. NMR δH (CDCl₃), 10.30 (1H, s, CHO), 7.44-7.36 (4H, m), 1.33 (9H, s); NMR δF (CDCl₃), 29.1-29.2 (2F, m), 147.0-16.9 (2F, m); NMR δC (CDCl₃), 182.3 (s, CHO), 152.6, 146.6 (dd, J = 260, 16 Hz), 146.1 (dd, J = 260, 10 Hz), 132.3, 127.2, 126.7, 123.4 (t, J = 18 Hz), 114.4 (t, J = 10 Hz), 34.7, 31.2; IR, νmax /cm⁻¹ 1705 (CHO); MS, m/z found 343.0778, C₁₇H₁₂F₄OS, (M+H⁺) requires 343.0774; elemental analysis, C₁₇H₁₂F₄OS requires: C, 59.64; H, 4.12; found: C, 59.61; H, 4.06.

4.2 Synthesis of Compound 3b [29]

2-Bromoacetophenone (2 mmol) was added to a solution of potassium thioacetate (2 mmol) in THF (5 ml). The reaction was stirred at 40 °C for 24 h. The mixture was poured into deionised water and extracted with ethyl acetate (15 ml x 3). The combined extracts were dried with sodium sulfate. The residue was treated with 1M NaOH (1.96 ml) in methanol (4 ml). The solution was stirred at room temperature for 14 h and then poured into deionised water and neutralised with 1 M HCl. The neutralised mixture was extracted with ethyl acetate (15 ml x 3). The combined extracts were dried over sodium sulfate. The crude residue was purified by column chromatography (elution with light petroleum/ethyl acetate).
2-Mercapto-1-phenylethanol (3b)
Yield 37%, yellow oil. NMR δ(H (CDCl3), 8.01-7.98 (2H, m, H-2, H-6), 7.63 (1H, tt, J = 7.6, 1.2 Hz, H-4), 7.52 (2H, t, J = 8 Hz, H-3, H-5), 4.00 (2H, d, J = 7.6 Hz, H-2'), 2.16 (1H, t, J = 7.6 Hz, SH); MS, m/z found 153.0363, C₉H₁₀OS, (M+H⁺) requires 153.0369.

4.3 Syntheses of Compounds 5aa-5ae
Compounds 2a-e (2 mmol) and Et₃N (5 mmol) in THF (6 ml) were treated dropwise with methyl mercaptopropanoic acid (3a, 2 mmol) with ice cooling. The mixtures were stirred at room temperature for up to 16 h and then poured into deionised water and were extracted with ethyl acetate (15 ml x 3). The combined organic extracts were washed with 0.2 M HCl (aq) and deionised water and dried over Na₂SO₄. The crude products were purified by column chromatography (elution with light petroleum/ethyl acetate).

Methyl-4,5,7-trifluoro-6-(1H-imidazol-1-yl)benzo[b]thiophene-2-carboxylate (5aa)
Yield 70%, white solid, m.p. 146-148 °C. NMR δ(H (CDCl3), 8.17 (1H, d, J = 3.2 Hz), 7.81 (1H, s), 7.29 (1H, s), 7.28 (1H, s), 3.99 (3H, s); NMR δ(C (CDCl3), 36.2 (1F, d, J = 19 Hz), 19.8 (1F, t, J =19 Hz), 14.6 (1F, d, J =20 Hz); NMR δ(C (CDCl3), 171.1, 161.5, 146.3 (ddd, J = 248, 4, 3 Hz), 143.5 (ddd, J = 251, 13, 4 Hz), 141.8 (ddd, J = 245, 15, 3 Hz), 137.9, 130.0, 128.6 (ddd, J = 18, 6, 3 Hz), 125.6 (ddd, J = 21, 6, 3 Hz), 125.1 (d, J = 6 Hz), 120.2, 114.6 (t, J = 16 Hz), 53.2; IR, νmax/cm⁻¹ 1728 (C=O), 1265 (C-O); MS, m/z found 313.0264, C₁₃H₁₁F₃N₂O₂S, (M+H⁺) requires 313.0253; elemental analysis, C₁₃H₁₁F₃N₂O₂S requires: C, 50.00; H, 2.26; N, 8.97; found: C, 49.91; H, 2.19; N, 8.88.

Methyl-6-(1H-benzo[d]imidazol-1-yl)-4,5,7-trifluorobenzo[b]thiophene-2-carboxylate (5ab)
Yield 57%, white solid, m.p. 178-179 °C. NMR δ(H (CDCl3), 8.26 (1H, d, J = 3.2 Hz), 8.10 (1H, s), 7.92 (1H, dd, J = 7.6, 2.4 Hz), 7.42-7.37 (2H, m), 7.30 (1H, d, J = 8.0 Hz), 4.02 (3H, s); NMR δ(C (CDCl3), 38.9 (1F, d, J =19 Hz), 20.2 (1F, t, J =19 Hz), 16.8 (1F, d, J =19 Hz); NMR δ(C (CDCl3), 171.1, 161.5, 147.6 (dt, J = 251, 4 Hz), 143.1, 142.8, 142.7 (dt, J = 249, 5 Hz), 142.5 (dt, J = 251, 5 Hz), 138.2, 133.8, 129.5 (ddd, J = 19, 7, 2 Hz), 125.8 (ddd, J = 17, 5, 2 Hz), 125.3 (d, J = 5 Hz), 124.4, 123.5, 120.8, 112.9 (t, J = 6 Hz), 110.4, 53.2; IR, νmax/cm⁻¹ 1720 (C=O), 1249 (C-O); MS, m/z found 363.0402, C₁₃H₁₁F₃N₂O₂S, (M+H⁺) requires 363.0410; elemental analysis, C₁₃H₁₁F₃N₂O₂S requires: C, 56.51; H, 2.21; N, 7.75; found: C, 56.36; H, 2.56; N, 7.62.

Methyl-6-(2-bromophenoxy)-4,5,7-trifluorobenzo[b]thiophene-2-carboxylate (5ac)
Yield 78%, white solid, m.p. 126-128 °C. NMR δ(H (CDCl3), 8.19 (1H, d, J = 3.2 Hz), 7.67 (1H, dd, J = 8, 1.6 Hz, H-3”), 7.24 (1H, td, J = 7.6 Hz, 1.6 Hz, H-5”), 0.93 (1H, td, J = 7.6, 1.6 Hz, H-4”), 6.78 (1H, dd, J = 7.6, 0.8 Hz), 4.01 (3H, s); NMR δ(C (CDCl3), 29.9 (1F, d, J =17, 3 Hz), 19.4 (1F, d, J =19, 17 Hz), 10.3 (1F, d, J = 19 Hz); NMR δ(C (CDCl3), 161.9, 154.1, 146.0 (dt, J = 248, 4 Hz),), 142.8 (ddd, J = 254, 12, 4 Hz), 141.8 (ddd, J = 248, 13, 3 Hz), 136.3, 134.1, 131.5 (t, J = 14 Hz), 128.7, 125.9 (dd, J = 18, 4 Hz), 125.6 (dd, J = 19, 4 Hz), 125.3 (d, J = 6 Hz), 125.0, 115.5, 111.9, 53.1; IR, νmax/cm⁻¹ 1720 (C=O), 1257 (C-O); MS, m/z found 416.9400, C₁₆H₁₂BrF₂O₂S, (M+H⁺) requires 416.9402; elemental analysis, C₁₆H₁₂BrF₂O₂S requires: C, 46.06; H, 1.93; found: C, 46.07; H, 1.93.

Methyl-6-[(2-bromophenylthio)-4,5,7-trifluorobenzo[b]thiophene-2-carboxylate (5ad)
Yield 66%, white solid, m.p. 143-145 °C. NMR δ(H (CDCl3), 8.20 (1H, d, J = 3.2 Hz), 7.60 (1H, dd, J = 8.0 Hz, 1.2 Hz), 7.20 (1H, td, J = 7.2 Hz, 1.2 Hz), 7.10 (1H, td, J = 7.2, 1.2 Hz), 6.94 (1H, d, J = 7.6 Hz), 4.02 (3H, s); NMR δ(C (CDCl3), 53.7 (1F, d, J=17, 3 Hz), 29.7 (1F, d, J=21 Hz), 18.9 (1F, t, J=20 Hz); NMR δ(C (CDCl3), 161.9, 153.2 (d, J = 248 Hz), 143.7 (ddd, J = 250, 16, 5 Hz), 141.3 (d, J = 245, 21, 10 Hz), 138.0, 135.4, 133.4, 130.5 (dd, J = 17, 128), 128.9, 128.0 (d, J = 7 Hz), 125.9-125.4 (m), 125.4 (d, J = 5 Hz), 122.7, 109.2 (t, J = 21 Hz), 53.2; IR, νmax/cm⁻¹ 1720 (C=O), 1265 (C-O); MS, m/z found 432.9129, C₈H₁₄O₂BrF₂S₂, (M+H⁺) requires 432.9174; elemental analysis, C₈H₁₄O₂BrF₂S₂ requires: C, 44.35; H, 1.86; found: C, 44.18; H, 1.74.
Methyl-4,5,7-trifluoro-6-((2-methoxy-2-oxoethyl)thio)benzo[2H, d, Y]

Yield 49%, cream solid, m.p. 132-134 °C. NMR δH (CDCl3), 8.15 (1H, d, J = 3.2 Hz), 7.37-7.31 (4H, m), 4.00 (3H, s), 1.31 (9H, s); NMR δF (CDCl3), 52.6 (1F, d, J = 17 Hz), 29.3 (1F, d, J = 23 Hz), 18.2 (1F, t, J = 20 Hz); NMR δC (CDCl3), 161.9, 152.9 (dt, J = 248, 5Hz), 151.1, 147.0 (dd, J = 245, 13, 4 Hz), 142.5 (dd, J = 253, 18, 4 Hz), 137.4, 130.5, 130.0 (ddd, J = 22, 9, 4 Hz), 130.0, 126.4 (C-3", C-5"), 125.6-125.2 (m), 125.3 (d, J = 5 Hz), 111.8 (t, J = 22 Hz), 53.0, 34.6, 31.2; IR, νmax /cm⁻¹ 1712 (C=O), 1249 (C-O); MS, m/z found 411.0695, C20H13F9O3S2, (M+H⁺) requires 411.0695; elemental analysis, C20H13F9O3S2 requires: C, 55.18; H, 3.20; found: C, 55.07; H, 3.16.

4.4 Syntheses of Compounds 5ba-Sbe

Following the method for compounds 2aa-ae, reaction of compounds 2a-e (2 mmol) and Et3N (5 mmol) in THF (5 ml) with ice cooling. The mixture was stirred at room temperature for 16 h. The reaction was poured into deionised water and was extracted with ethyl acetate (15 ml x 3). The combined extracts were washed with 0.2 M HCl solution and deionised water successively and dried with sodium sulfate. The product was purified by silica column chromatography (elution with light petroleum/ethyl acetate). Yield 62%, white solid, m.p. 118-120 °C. NMR δH (CDCl3), 8.09 (1H, d, J = 3.2 Hz), 3.98 (3H, s), 3.69 (3H, s), 3.65 (2H, s); NMR δF (CDCl3), 52.4 (1F, dd, J = 18, 3 Hz), 28.5 (1F, dd, J = 21, 18 Hz); NMR δC (CDCl3), 161.0, 161.9, 153.1 (d, J = 246 Hz), 147.3 (dd, J = 242, 15, 4 Hz), 142.4 (dd, J = 253, 14, 4 Hz), 137.6, 130.4 (dd, J = 15, 7, 4 Hz), 125.6-125.2 (m), 125.5 (d, J = 7 Hz), 110.1 (t, J = 22 Hz), 53.1, 52.8, 36.1; IR, νmax /cm⁻¹ 1728 (C=O), 1273 (C-O); MS, m/z found 350.9969, C13H9F9O3S2, (M+H⁺) requires 350.9967; elemental analysis, C13H9F9O3S2 requires: C, 44.57; H, 2.59; found: C, 44.40; H, 2.53.

Phenyl-(4,5,7-trifluoro-6-(1H-imidazol-1-yl)benzo[b]thiophen-2-yl)methanone (5ba)

Yield 49%, cream solid, m.p. 132-134 °C. NMR δH (CDCl3), 7.98 (1H, d, J = 3.2 Hz), 7.92 (2H, d, J = 7.2 Hz), 7.87 (1H, s), 7.67 (1H, tt, J = 7.6, 1.6 Hz), 7.57 (2H, t, J = 7.6 Hz), 7.31 (1H, s), 7.28 (1H, s); NMR δF (CDCl3), 36.6 (1F, d, J = 19 Hz), 20.2 (1F, t, J = 17 Hz); NMR δC (CDCl3), 188.1, 147.6, 146.5 (d, J = 255 Hz), 143.1 (dd, J = 255, 16, 5 Hz), 141.8 (dd, J = 250, 14 Hz), 138.0, 136.6, 133.6, 129.9, 129.4, 129.2 (dd, J = 19, 7 Hz), 129.0, 126.2 (dd, J = 19, 7 Hz), 126.5 (d, J = 5 Hz), 120.5, 114.8 (t, J = 15 Hz); IR, νmax /cm⁻¹ 1643 (C=O); MS, m/z found 359.0454, C18H10F3ON7S, (M+H⁺) requires 359.0460; elemental analysis, C18H10F3N7S requires: C, 60.33; H, 2.53; N, 7.82; found: C, 60.08; H, 2.60; N, 7.68.

6-(1H-benzo[d]imidazol-1-yl)-4,5,7-trifluorobenzo[b]thiophen-2-yl(phenyl)methanone (5bb)

Yield 51%, orange solid, m.p. 140-142 °C. NMR δH (CDCl3), 8.09 (1H, s), 8.04 (1H, d, J = 3.2 Hz), 7.94 (2H, d, J = 7.8 Hz), 7.93-7.89 (1H, m) 7.31-7.27 (1H, m); NMR δF (CDCl3), 39.34 (1F, d, J = 17 Hz), 20.6 (1F, t, J = 20 Hz), 16.9 (1F, d, J = 20 Hz); NMR δC (CDCl3), 188.2, 147.8 (d, J = 251 Hz), 147.7, 143.1 (dd, J = 254, 13, 4 Hz), 143.2, 142.8, 142.7 (dd, J = 249, 14 Hz), 136.6, 133.8, 133.6, 129.9 (dd, J = 17, 4 Hz), 129.5, 129.0, 126.2 (dd, J = 18, 4, 3 Hz), 125.7 (d, J = 6 Hz), 124.6, 123.6, 120.9, 113.3 (t, J = 16 Hz), 110.5; IR, νmax /cm⁻¹ 1643 (C=O); MS, m/z found 409.0608, C22H12F5ON4S, (M+H⁺) requires 409.0617; elemental analysis, C22H12F5ON2S requires: C, 64.70; H, 2.69; N, 6.86; found: C, 64.47; H, 2.70; N, 6.96.

6-(2-Bromophenoxy)-4,5,7-trifluorobenzo[b]thiophen-2-yl(phenyl)methanone (5bc)
Yield 48%, yellow solid, m.p. 110-112 °C. NMR δ (CDCl₃), 7.95 (1H, d, J = 2.8 Hz), 7.92 (2H, d, J = 7.6 Hz), 7.71-7.61 (2H, m), 7.56 (2H, t, J = 7.6 Hz), 7.21 (1H, t, J = 7.6 Hz), 7.01 (1H, t, J = 7.2 Hz), 6.76 (1H, d, J = 8.4 Hz); NMR δ (CDCl₃), 146.9, 154.1, 146.1 (dd, J = 249, 4 Hz), 146.0, 143.2 (ddd, J = 251, 14, 3 Hz), 142.1 (ddd, J = 251, 14, 4 Hz), 136.9, 134.1, 133.3, 131.9 (t, J = 14 Hz), 129.4, 128.9, 128.7, 126.4 (dd, J = 17, 4 Hz), 126.3-125.8 (m), 126.1 (d, J = 4 Hz), 125.1, 115.6, 112.0; IR, νmax/cm⁻¹ 1643 (C=O); MS, m/z found 462.9601, C₂₁H₁₃BrF₃O₂S₂ (M+H⁺) requires 462.9610; elemental analysis, C₂₁H₁₃BrF₃O₂S requires: C, 54.44; H, 2.18; found: C, 54.37; H, 2.04.

6-(2-Bromophenyl)thio-4,5,7-trifluorobenzo[b]thiophen-2-yl(phenyl)methanone (5bd)

Yield 46%, brown solid, m.p. 118-120 °C. NMR δ (CDCl₃), 7.96 (1H, d, J = 3.2 Hz), 7.92 (2H, d, J = 7.2 Hz), 7.68 (1H, t, J = 8.0 Hz). 7.59-7.54 (3H, m), 7.17 (1H, td, J = 7.2, 1.1 Hz), 7.08 (1H, td, J = 7.2, 1.5 Hz), 6.94 (1H, d, J = 7.6 Hz); NMR δ (CDCl₃), 54.0 (1F, d, J = 23 Hz), 29.8 (1F, d, J = 17 Hz), 19.1 (1F, t, J = 17 Hz); NMR δC (CDCl₃), 188.5, 153.2 (d, J = 249 Hz), 147.4, 146.9 (dd, J = 249, 17 Hz), 142.9 (dd, J = 256, 12 Hz), 136.7, 135.3, 133.5, 133.4, 131.2 (ddd, J = 18, 8, 4 Hz), 129.4, 129.1, 129.0, 128.2, 128.1, 127.1-126.8 (m), 126.0 (d, J = 5 Hz), 122.9, 109.8 (t, J = 22 Hz); IR, νmax/cm⁻¹ 1635 (C=O); MS, m/z found 478.9370, C₂₂H₁₂BrF₃O₂S₂ (M+H⁺) requires 478.9381; elemental analysis, C₂₂H₁₂BrF₃O₂S requires: C, 52.62; H, 2.10; found: C, 52.45; H, 1.92.

6-[(4-tert-Butylphenyl)thio]-4,5,7-trifluorobenzo[b]thiophen-2-yl(phenyl)methanone (5be)

Yield 49%, red oil. NMR δ (CDCl₃), 7.91 (2H, d, J = 2.0 Hz), 7.90 (2H, d, J = 7.6 Hz), 7.66 (1H, t, J = 7.6 Hz). 7.55 (2H, t, J = 7.6 Hz), 7.31 (4H, AA'BB' m), 1.25 (9H, s, H); NMR δ (CDCl₃), 53.0 (1F, d, J = 17 Hz), 29.4 (1F, d, J = 23 Hz), 18.3 (1F, t, J = 21 Hz); NMR δC (CDCl₃), 188.6, 153.1 (d, J = 250 Hz), 151.2, 147.0 (d, J = 250 Hz), 146.9, 142.7 (d, J = 250 Hz), 136.9, 133.4, 130.7, 136.0-130.4 (m), 130.0, 129.4, 128.9, 126.7-126.3 (m), 126.5, 126.1 (d, J = 9 Hz), 126.3 (t, J = 19 Hz), 34.6, 29.8; IR, νmax/cm⁻¹ 1646 (C=O); MS, m/z found 457.0895, C₂₃H₂₃F₁₂O₁N₂S₂ (M+H⁺) requires 457.0902; elemental analysis, C₂₃H₂₃F₁₂O₁N₂S₂ requires: C, 65.77; H, 4.19; found: C, 65.73; H, 4.46.

2-[[2-Benzoyl-4,5,7-trifluorobenzo[b]thiophen-6-yl]thio]-1-phenylethanone (5bf)

Following the same method for compound 5af, reaction of pentafluorobenzaldehyde (2 mmol) and 3b (4 mmol) afforded compound 5bf after purification by chromatography eluting with light petroleum/ethyl acetate. Yield 21%, orange solid, m.p. 138-139 °C. NMR δ (CDCl₃), 7.93-7.89 (5H, m), 7.67 (1H, t, J = 7.2 Hz), 7.61-7.53 (3H, m), 7.46 (2H, t, J = 7.8 Hz), 4.36 (2H, s); NMR δC (CDCl₃), 53.0 (1F, d, J = 20 Hz), 28.8 (1F, d, J = 20 Hz), 18.2 (1F, t, J = 19 Hz); NMR δC (CDCl₃), 193.2, 188.6, 153.1 (d, J = 246 Hz), 147.3 (ddd, J = 240, 16, 4 Hz), 146.9, 142.5 (ddd, J = 252, 18, 4 Hz), 136.8, 135.1, 133.9, 133.4, 130.7 (ddd, J = 18, 4 Hz), 129.4, 128.9, 128.8, 128.6, 126.0 (d, J = 6 Hz), 125.7 (dd, J = 23, 6 Hz), 110.7 (t, J = 22 Hz), 40.6; IR, νmax/cm⁻¹ 1635 (C=O), 1666 (C=O); MS, m/z found 443.0374, C₂₃H₁₄F₁₂O₂S₂ (M+H⁺) requires 443.0382; elemental analysis, C₂₃H₁₄F₁₂O₂S₂.0.5H₂O requires: C, 61.19; H, 3.12; found: C, 61.5; H, 2.83.

4.5 Antitrypanosomal Activity Assays

Trypanosoma brucei rhodesiense STIB 900, a clone of a population isolated in 1982 from a patient in Tanzania. Stock drug solutions were prepared in DMSO at 20 mg/ml and further diluted to the appropriate concentration using medium. Assays were performed in 96-well microtiter plates with each well containing 100 μl of parasite culture (1 x 10⁵ bloodstream forms) with serial drug dilutions at 37 °C for 72 h in 5% CO₂. Each compound was tested in triplicate with 30 μg/ml the highest concentration of compound used and a 3-fold serial dilution was performed down to a suitable concentration to obtain an IC₅₀ value. Control wells were without drug and blanks were medium only. After 72 h of incubation the plates were inspected to assure growth in control wells and to determine the minimum inhibitory concentration (MIC). Subsequently, 20 μl of Alamar Blue was added to each well and the plates incubated for another 2-4 h. Plates were read on a Gemini Plate
Reader (Molecular Devices) using an excitation wave length of 530 nm and an emission wave length of 580 nm (cut off 550 nm). IC\textsubscript{50} values were calculated using Prism software.

4.6 Antiproliferative Activity Assays
Assays were conducted using the same protocol as described by Krystof et al. [28].

5. Acknowledgments
We thank the EPSRC UK National Mass Spectrometry Facility at Swansea University for recording mass spectra. We are grateful to Mr J. Alastair Daley and Mrs Pauline King for technical support. We thank the Ministry of Education, Youth and Sports of the Czech Republic via the National Program of Sustainability I (grant LO1204), Loughborough University and De Montfort University for financial support.

6. Author Contributions
The manuscript was written through contributions of all authors \textsuperscript{*}. All authors have given approval to the final version of the manuscript. These authors contributed equally.

7. References


[23] www.molinspiration.com


Scheme 1. Overall reaction scheme to generate 2,6-Disubstituted-4,5,7-Trifluorobenzothiophenes

Figures

Figure 1. Crystal structure of 5bb
### Tables

<table>
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<th>Compound*</th>
<th>Structure</th>
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Table 1. Synthesised 2,6-Disubstituted-4,5,7-Trifluorobenzothiophenes

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- perfluorinated building blocks used in synthesis of heterocyclic scaffolds
- $S_N$Ar substitution reactions employed to deliver 6-benzimidazol-1-ylbenzothiophenes
- $IC_{50}$ values of lead compounds were <1 $\mu$M against *Trypanosoma brucei rhodesiense*