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

Avninder S. Bhambra [a]*, Mark Edgar [b], Mark R.J. Elsegood [b]*, Yuqi Li [b], George W. Weaver [b]*, Randolph R. J. Arroo [c], Vanessa Yardley [d], Hollie Burrell-Saward [d], Vladimir Krystof [e].

[a] School of Allied Health Sciences, De Montfort University, The Gateway, Leicester, LE1 9BH, UK.
[b] Department of Chemistry, Loughborough University, Loughborough, LE11 3TU, UK.
[c] Leicester School of Pharmacy, De Montfort University, The Gateway, Leicester, LE1 9BH, UK.
[d] London School of Hygiene and Tropical Medicine, London, WC1E 7HT, UK.
[e] Palacky University & Institute of Experimental Botany, 11 783 71 Olomouc, CZ.

Corresponding Authors:
*[a] Avninder S. Bhambra, abhambra@dmu.ac.uk
*[b] George W. Weaver, g.w.weaver@lboro.ac.uk
*[b] *Mark R.J. Elsegood, m.r.j.elsegood@lboro.ac.uk (author for X-ray crystallography)*

KEYWORDS Benzothiophenes, Fluorinated drugs, Antitrypanosomal activity, Sleeping sickness, Human African Trypanosomiasis

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 (IC_{50} <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-nitrotriazole 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].

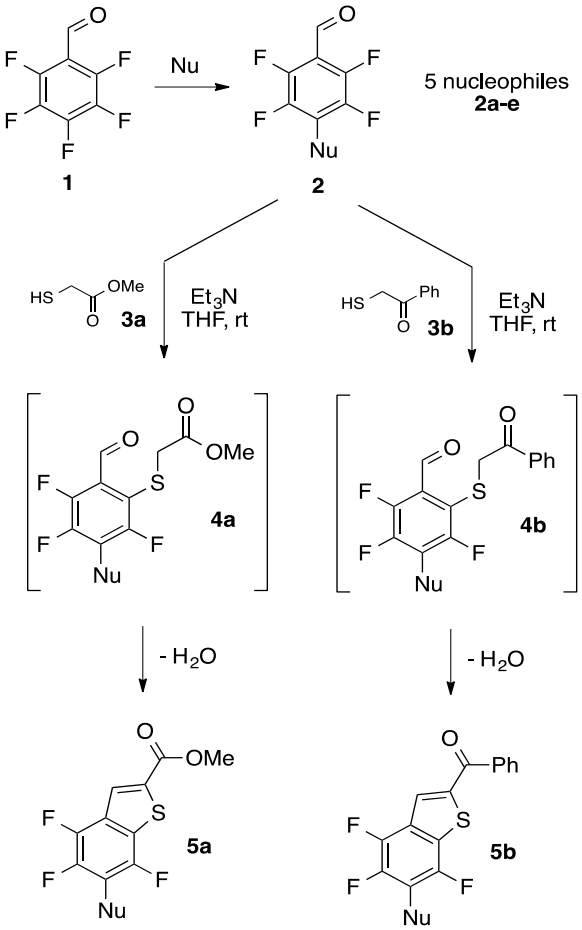
Developing rapidly accessible fluorinated heterocyclic scaffolds [4, 5] with potential for further elaboration is thematic of our medicinal chemistry programme. Benzothiophene ring systems represent privileged scaffolds in medicinal chemistry and have been incorporated into: reverse transcriptase inhibitors; 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 (S_NAr) of perfluorinated building blocks [11]. S_NAr 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 perfluoroarene 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].



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

In practice, pentafluorobenzaldehyde reacted smoothly with selected diazoles, arenethiols and 2-bromophenol to the 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].

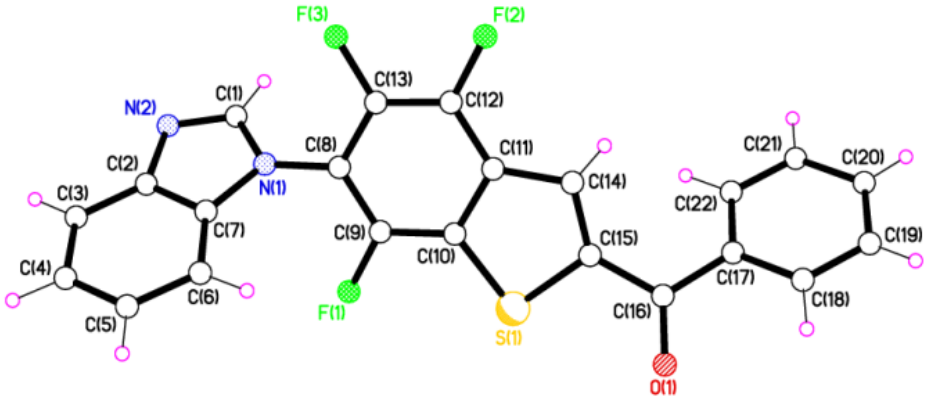
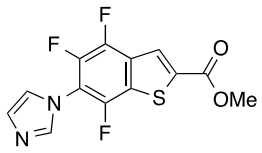
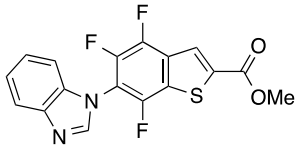
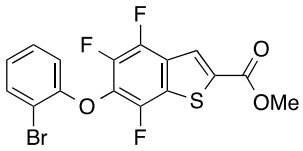
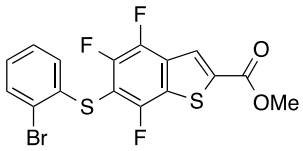
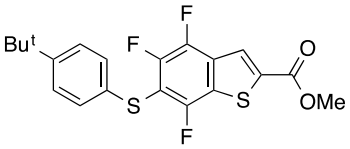
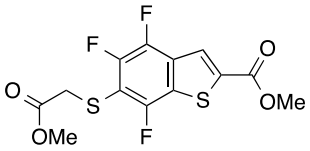


Figure 1. Crystal structure of 5bb

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].

		Activity (IC50 µM)	
Compound*	**Structure**	***T.b.r***	**MCF7**
5aa		**129.9**	**>100**
5ab		**0.60**	**>12**
5ac		**30.0**	**>25**
5ad		**11.9**	**>25**
5ae		**67.2**	**>50**
5af		**>10**	**>25**

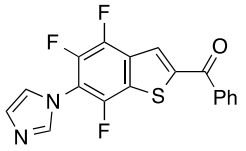
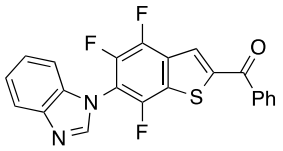
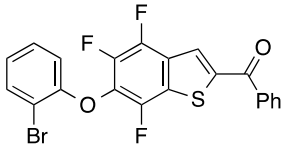
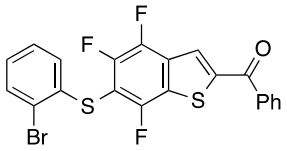
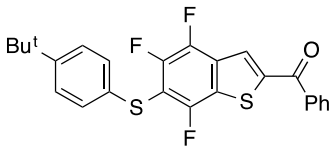
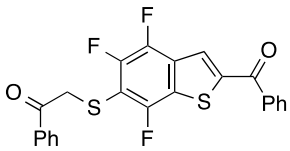
5ba		**33.0**	**>60**
5bb		**0.53**	**>25**
5bc		**15.9**	**>25**
5bd		**9.8**	**>25**
5be		**31.4**	**>25**
5bf		**>10**	**>50**
Melarsoprol		**0.051**	**-**
Roscovitine			**11**

Table 1. Synthesised 2,6-Disubstituted-4,5,7-Trifluorobenzothiophenes

2.2.1 6-Substituted-benzothiophene-2-carboxylate derivatives

Compound **5ad**, synthesised bearing a 6-(2-bromophenylsufanyl) group demonstrated moderate trypanocidal activity with an IC_{50} value of 11.9 µM. Compound **5ac** was prepared with a 6-(2-bromophenoxy) group and gave an IC_{50} 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-butylphenylsulfanyl) 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_{50} value of 0.60 µM, significantly greater than aforementioned benzothiophene-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 sulfanyl substituent showed comparable antiparasitic and cytotoxic activity to **5ad** with an IC_{50} value of 9.8 μM. Similarly, the imidazolyl substituted compound **5ba** showed an increase in trypanocidal activity with an IC_{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_{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_NAr substitution reactions of perfluorinated building blocks. Noticeably, **5ab** and **5bb** were identified as hit compounds with IC_{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_{254} aluminum backed plates. NMR spectra were recorded in $CDCl_3$ at 400 MHz (^{1}H NMR), 376 MHz (^{19}F NMR) or 100 MHz (^{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_3N (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 $NaSO_4$. 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 δ_H ($CDCl_3$), 10.31 (1H, s, CHO), 7.88 (1H, s), 7.32 (1H, s,), 7.29 (1H, s); NMR δ_F ($CDCl_3$), 19.4-19.2 (2F, m), 14.9-15.0 (2F, m); NMR δ_c ($CDCl_3$), 181.5 (CHO), 147.4 (ddd, *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, ν_{max} /cm^{-1} 1712 (CHO); MS, m/z found 245.0332, $C_{10}H_5F_4N_2O$, ($M+H^+$) requires 245.0333.

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

Yield 62%, yellow solid, m.p. 160-162 °C. NMR δ_H ($CDCl_3$), 10.42 (1H, s, CHO), 8.09 (1H, t, *J* = 1.8 Hz), 7.96-7.92 (1H, m), 7.47-7.42 (2H, m), 7.33-7.29 (1H, m); NMR δ_F ($CDCl_3$), 19.5-19.4 (2F, m), 18.2-18.1 (2F, m); NMR δ_c ($CDCl_3$), 181.6 (CHO), 147.3 (ddd, *J* = 260, 12, 5 Hz), 143.2, 142.0 (ddd, *J* = 250, 14, 6 Hz), 141.9, 132.7, 125.0, 124.1, 121.0, 120.8 (d, *J* = 10 Hz), 114.6 (d, *J* = 10 Hz), 110.7 (t, *J* = 3 Hz); IR, ν_{max} /cm^{-1} 1705 (CHO); MS, m/z found 295.0488, $C_{14}H_7F_4N_2O$, ($M+H^+$) requires 295.0489; elemental analysis, $C_{14}H_6F_4N_2O$ 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. 48-50 °C. NMR δ_H ($CDCl_3$), 10.31 (1H, s, CHO), 7.67 (1H, dd, *J* = 8.0, 1.6 Hz), 7.31 (1H, td, *J* = 8.0, 1.6 Hz), 7.11 (1H, td, *J* = 8.0, 1.2 Hz,), 6.94 (1H, d, *J* = 8.0 Hz); NMR δ_F ($CDCl_3$), 17.1-17.0 (2F, m), 8.4-8.3 (2F, m); NMR δ_c ($CDCl_3$), 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, ν_{max} /cm^{-1} 1705 (CHO); MS, m/z found 348.9478, $C_{13}H_6{}^{79}BrF_4O_2$, ($M+H^+$) requires 348.9482; elemental analysis, $C_{13}H_4BrF_4O_2$ 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 δ_H ($CDCl_3$), 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 δ_F ($CDCl_3$), 29.7-29.6 (2F, m), 17.4-17.3 (2F, m); NMR δ_c ($CDCl_3$), 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, ν_{max} /cm^{-1} 1705 (HC=O); MS, m/z found 364.9251, $C_{13}H_6{}^{79}BrF_4OS$, ($M+H^+$) requires 364.9253; elemental analysis, $C_{13}H_5BrF_4OS$ 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 δ_H ($CDCl_3$), 10.30 (1H, s, CHO), 7.44-7.36 (4H, m), 1.33 (9H, s); NMR δ_F ($CDCl_3$), 29.1-29.2 (2F, m), 147.0-16.9 (2F, m); NMR δ_c ($CDCl_3$), 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, ν_{max} /cm^{-1} 1705 (CHO); MS, m/z found 343.0778, $C_{17}H_{15}F_4OS$, ($M+H^+$) requires 343.0774; elemental analysis, $C_{17}H_{14}F_4OS$ 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-phenylethanone (3b)
Yield 37%, yellow oil. NMR δ_H ($CDCl_3$), 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, C_8H_9OS, ($M+H^+$) requires 153.0369.

4.3 Syntheses of Compounds 5aa-5ae
Compounds **2a-e** (2 mmol) and Et_3N (5 mmol) in THF (6 ml) were treated dropwise with methyl mercaptoacetate (**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

$NaSO_4$. The crude products were purified by column chromatography (elution with light petroleum/ethyl acetate).

Methyl-4,5,7-trifluoro-6-(1*H*-imidazol-1-yl)benzo[*b*]thiophene-2-carboxylate (5aa)
Yield 70%, white solid, m.p. 146-148 °C. NMR δ_H ($CDCl_3$), 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_F ($CDCl_3$), 36.2 (1F, d, *J* = 19 Hz), 19.8 (1F, t, *J* =19 Hz), 14.6 (1F, d, *J*= 20 Hz); NMR δ_C ($CDCl_3$), 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), 120.4, 114.6 (t, *J* = 16 Hz), 53.2; IR, ν_{max} /cm^{-1} 1728 (C=O), 1265 (C-O); MS, m/z found 313.0264, $C_{13}H_7F_3N_2OS$, ($M+H^+$) requires 313.0253; elemental analysis, $C_{13}H_7F_3N_2O_2S$ 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 ($CDCl_3$), 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_F ($CDCl_3$), 38.9 (1F, d, *J*=19 Hz), 20.2 (1F, t, *J*=19 Hz), 16.8 (1F, d, *J*=19 Hz); NMR δ_c ($CDCl_3$), 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, ν_{max} /cm^{-1} 1720 (C=O), 1249 (C-O); MS, m/z found 363.0402, $C_{17}H_9F_3N_2O_2S$, ($M+H^+$) requires 363.0410; elemental analysis, $C_{17}H_9F_3N_2O_2S$ 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 ($CDCl_3$), 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"), 7.03 (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_F ($CDCl_3$), 29.9 (1F, dd, *J* =17, 3 Hz), 19.4 (1F, dd, *J* =19, 17 Hz), 10.3 (1F, d, *J* = 19 Hz); NMR δ_C ($CDCl_3$), 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, ν_{max} /cm^{-1} 1720 (C=O), 1257 (C-O); MS, m/z found 416.9400, $C_{16}H_9BrF_3O_3S$, ($M+H^+$) requires 416.9402; elemental analysis, $C_{16}H_8BrF_3O_3S$ requires: C, 46.06; H, 1.93; found: C, 46.07; H, 1.93.

Methyl-6-[(2-bromophenyl)thio]-4,5,7-trifluorobenzo[*b*]thiophene-2-carboxylate 5ad
Yield 66%, white solid, m.p. 143-145 °C. NMR δ_H ($CDCl_3$), 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_F ($CDCl_3$), 53.7 (1F, dd, *J*=17, 3 Hz), 29.7 (1F, d, *J*=21 Hz), 18.9 (1F, t, *J*=20 Hz); NMR δ_C ($CDCl_3$), 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, 7 Hz), 128.9, 128.0, 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, ν_{max} /cm^{-1} 1720 (C=O), 1265 (C-O); MS, m/z found 432.9129, $C_{16}H_9O_2$ BrF_3S_2, ($M+H^+$) requires 432.9174; elemental analysis, $C_{16}H_8BrF_3O_2S_2$ requires: C, 44.35; H, 1.86; found: C, 44.18; H, 1.74.

Methyl-6-((4-(tert-butyl)phenyl)thio)-4,5,7-trifluorobenzo[*b*]thiophene-2-carboxylate (5ae)
Yield 76%, white solid, m.p. 127-129 °C. NMR δ_H ($CDCl_3$), 8.15 (1H, d, *J* = 3.2 Hz), 7.37-7.31 (4H, m), 4.00 (3H, s), 1.31 (9H, s); NMR δ_F ($CDCl_3$), 52.6 (1F, d, *J* = 17 Hz), 29.3 (1F, d, *J* = 23 Hz), 18.2 (1F, t, *J* = 20 Hz); NMR δ_c ($CDCl_3$), 161.9, 152.9 (dt, *J* = 248, 5Hz), 151.1, 147.0 (ddd, *J* = 245, 13, 4 Hz), 142.5 (ddd, *J* = 253, 18, 4 Hz), 137.4, 130.5, 130.2 (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, ν_{max} /cm^{-1} 1712 (C=O), 1249 (C-O); MS, m/z found 411.0696, $C_{20}H_{18}F_3O_2S_2$, ($M+H^+$) requires 411.0695; elemental analysis, $C_{20}H_{17}F_3O_2S_2$ requires: C, 58.52; H, 4.17; found: C, 58.40; H, 4.12.

Methyl-4,5,7-trifluoro-6-((2-methoxy-2-oxoethyl)thio)benzo[*b*]thiophene-2-carboxylate (5af)

Methyl mercaptoacetate (4 mmol) was added dropwise to a solution of pentafluorobenzaldehyde (2 mmol) and Et_3N (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 δ_H ($CDCl_3$), 8.09 (1H, d, *J* = 3.2 Hz), 3.98 (3H, s), 3.69 (3H, s), 3.65 (2H, s); NMR δ_F ($CDCl_3$), 52.4 (1F, dd, *J* = 18, 3 Hz), 28.5 (1F, d, *J* = 21 Hz), 18.3 (1F, dd, *J* = 21, 18 Hz); NMR δ_c ($CDCl_3$), 169.2, 161.9, 153.1 (d, *J* = 246 Hz), 147.3 (ddd, *J* = 242, 15, 4 Hz), 142.4 (ddd, *J* = 253, 14, 4 Hz), 137.6, 130.4 (ddd, *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, ν_{max} /cm^{-1} 1728 (C=O), 1273 (C-O); MS, m/z found 350.9969, $C_{13}H_{10}F_3O_4S_2$, ($M+H^+$) requires 350.9967; elemental analysis, $C_{13}H_9F_3O_4S_2$ requires: C, 44.57; H, 2.59; found: C, 44.40; H, 2.53.

4.4 Syntheses of Compounds 5ba-5be

Following the method for compounds **5aa-ae**, reaction of compounds **2a-e** (2 mmol) and **3b** (0.304 g, 2 mmol) provided compounds **5ba-be**, purified by flash column chromatography eluting with light petroleum/ethyl acetate.

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

Yield 49%, cream solid, m.p. 132-134 °C. NMR δ_H ($CDCl_3$), 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 δ_F ($CDCl_3$), 36.6 (1F, d, *J* = 19 Hz), 20.2 (1F, t, *J* = 17 Hz), 14.7 (1F, d, *J* = 17 Hz); NMR δ_C ($CDCl_3$), 188.1, 147.6, 146.5 (d, *J* = 255 Hz), 143.1 (ddd, *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), 125.6 (d, *J* = 5 Hz), 120.5, 114.8 (t, *J* = 15 Hz); IR, ν_{max} /cm^{-1} 1643 (C=O); MS, m/z found 359.0454, $C_{18}H_{10}F_3ON_2S$, ($M+H^+$) requires 359.0460; elemental analysis, $C_{18}H_9F_3N_2OS$ requires: C, 60.33; H, 2.53; N, 7.82; found: C, 60.08; H, 2.60; N, 7.68.

6-(1*H*-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 δ_H ($CDCl_3$), 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) 7.70 (1H, t, *J* = 7.2 Hz), 7.59 (2H, t, *J* = 7.6 Hz), 7.42-7.34 (2H, m), 7.31-7.27 (1H, m); NMR δ_F ($CDCl_3$), 39.34 (1F, d, *J* = 17 Hz), 20.6 (1F, t, *J* = 20 Hz), 16.9 (1F, d, *J* = 20 Hz); NMR δ_c ($CDCl_3$), 188.2, 147.8 (d, *J* = 251 Hz), 147.7, 143.1 (ddd, *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 (ddd, *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, ν_{max} /cm^{-1} 1643 (C=O); MS, m/z found 409.0608, $C_{22}H_{12}F_3ON_2S$, ($M+H^+$) requires 409.0617; elemental analysis, $C_{22}H_{11}F_3ON_2S$ 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_H ($CDCl_3$), 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_F ($CDCl_3$), 30.3 (1F, d, *J* = 15 Hz), 19.6 (1F, t, *J* = 20 Hz), 10.5 (1F, d, *J* = 20 Hz); NMR δ_C ($CDCl_3$), 188.4, 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, ν_{max} /cm^{-1} 1643 (C=O); MS, m/z found 462.9601, $C_{21}H_{11}BrF_3O_2S$, ($M+H^+$) requires 462.9610; elemental analysis, $C_{21}H_{10}BrF_3O_2S$ 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_H ($CDCl_3$), 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_F ($CDCl_3$), 54.0 (1F, d, *J* = 23 Hz), 29.8 (1F, d, *J* = 17 Hz), 19.1 (1F, t, *J* = 17 Hz); NMR δ_C ($CDCl_3$), 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, ν_{max} /cm^{-1} 1635 (C=O); MS, m/z found 478.9370, $C_{21}H_{11}BrF_3OS_2$, ($M+H^+$) requires 478.9381; elemental analysis, $C_{21}H_{10}BrF_3OS_2$ 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_H ($CDCl_3$), 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_F ($CDCl_3$), 53.0 (1F, d, *J* = 17 Hz), 29.4 (1F, d, *J* = 23 Hz), 18.3 (1F, t, *J* = 21 Hz); NMR δ_C ($CDCl_3$), 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, 130.6-130.4 (m), 130.0, 129.4, 128.9, 126.7-126.3 (m), 126.5, 126.1 (d, *J* = 9 Hz), 112.3 (t, *J* = 19 Hz), 34.6, 29.8; IR, ν_{max} /cm^{-1} 1646 (C=O); MS, m/z found 457.0895, $C_{25}H_{20}F_3ON_2S_2$, ($M+H^+$) requires 457.0902; elemental analysis, $C_{25}H_{19}F_3OS_2$ 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_H ($CDCl_3$), 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 δ_F ($CDCl_3$), 53.0 (1F, d, *J* = 20 Hz), 28.8 (1F, d, *J* = 20 Hz), 18.2 (1F, t, *J* = 19 Hz); NMR δ_C ($CDCl_3$), 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 (dd, *J* = 18, 4 Hz), 129.4, 128.91, 128.88, 128.6, 126.0 (d, *J* = 6 Hz), 125.7 (dd, *J* = 23, 6 Hz), 110.7 (t, *J* = 22 Hz), 40.6; IR, ν_{max} /cm^{-1} 1635 (C=O), 1666 (C=O); MS, m/z found 443.0374, $C_{23}H_{14}F_3O_2S_2$, ($M+H^+$) requires 443.0382; elemental analysis, $C_{23}H_{13}F_3O_2S_2.0.5H_2O$ 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 10^3 bloodstream forms) with serial drug dilutions at 37 °C for 72 h in 5% CO_2. 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 IC_{50} 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_{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 [a-e]. All authors have given approval to the final version of the manuscript. These authors contributed equally.

7. References

[1] Tarral, A., Blesson, S., Mordt, O.V., Torreele, E., Sassella, D., Bray, M.A., Hovsepian, L., Evene, E., Gualano, V., Felices, M., Strub-Wourgaft, N., Determination of an Optimal Dosing Regimen for Fexinidazole, a Novel Oral Drug for the Treatment of Human African Trypanosomiasis: First-in-Human Studies. Clin Pharmacokinet. 53 (2014) 565-580.

[2] Papadopoulou, M.V., Bloomer, W.D., Rosenzweig, H.S., O'Shea, I.P., Wilkinson, S.R., Kaiser, M., 3-Nitrotriazole-based piperazides as potent antitrypanosomal agents. Euro. J. Med. Chem. 103 (2015) 325-334.

[3] Leverrier, A., Bero, J., Cabrera, J., Frederich, M., Quetin-Leclercq, J., Palermo, J.A., Structure-activity relationship of hybrids of Cinchona alkaloids and bile acids with in vitro antiplasmodial and antitrypanosomal activities. Eur. J. Med. Chem, 100 (2015) 10-17.

[4] Zhang, K.Y.J., Milburn, M.V., Artis, D.R., Scaffold-Based Drug Discovery in Structure-Based Drug Discovery, Springer (2007) pp. 129-153.

[5] Sandford, G., Slater, R., Yufit, D.S., Howard, J.A.K., Tetrahydropyrido[3,4-*b*]pyrazine Scaffolds from Pentafluoropyridine, J. Org. Chem. 70 (2005) 7208-7216.

[6] Hou, C., He, Q. and Yang, C., Direct Synthesis of Diverse 2-Aminobenzo[b]thiophenes via Palladium-Catalyzed Carbon–Sulfur Bond Formation Using $Na_2S_2O_3$ as the Sulfur Source, Org. Lett. 16 (2014) 5040-5043.

[7] Krajewski, K., Zhang, Y., Parrish, D., Deschamps, J., Roller, P.P., Pathak, V.K., New hiv-1 reverse transcriptase inhibitors based on a tricyclic benzothiophene scaffold: synthesis, resolution, and inhibitory activity. Bioorg. Med. Chem. 16 (2006) 3034-3038.

[8] Romagnoli, R., Baraldi, P.G., Carrion, M.D., Cara, C.L., Preti, D., Fruttarolo, F., Pavani, M.G., Tabrizi, M.A., Tolomeo, M., Grimaudo, S., Christina, A., Balzarini, J., Hadfield, J.A., Brancale, A., Hamel, E., Synthesis and biological evaluation of 2- and 3-aminobenzo[b]thiophene derivatives as antimitotic agents and inhibitors of tubulin polymerization. J. Med. Chem. 50 (2007) 2273-2277.

[9] Papadopoulou, M.V., Bloomer, W.D., Rosenzweig, H.S., Wilkinson, S.R., Kaiser, M., Novel nitro(triazole/imidazole)-based heteroarylamides/sulfonamides as potential antitrypanosomal agents, Eur. J. Med. Chem. 87 (2014) 79-88.

[10] Lam, C.F.C., Pearce, A.N., Tan, S.H., Kaiser, M., Copp, B.R., Discovery and evaluation of thiazinoquinones as anti-protozoal agents, Mar. Drugs. 11 (2013) 3472-3499.

[11] Brooke, G.M., The preparation and properties of polyfluoro aromatic and heteroaromatic compounds. Journal of Fluorine Chemistry, 86 (1997) 1-76.

[12] Petrov, V.A., Fluorinated Heterocyclic Compounds, V.A Wiley, 2009.

[13] Chambers, R.D. and Sargent, C.R. Polyfluoroheteroaromatic compounds. Adv. Heterocycl. Chem. 28 (1982) 1-71.

[14] Chambers, R.D., Fluorine in Organic Chemistry, Blackwells, 2004.

[15] Gourveneur, V., Müller, K., Fluorine in Pharmaceutical and Medicinal Chemistry, Imperial College Press, 2012.

[16] Ojima, I., Fluorine in Medicinal Chemistry, Imperial College Press, 2012.

[17] Ponce González, J., Edgar, M., Elsegood, M.R.J., Weaver, G.W., Synthesis of fluorinated fused benzofurans and benzothiophenes: smiles-type rearrangement and cyclisation of perfluoro(het)aryl ethers and sulfides. Org. Biomol. Chem. 9 (2011) 2294-2305.

[18] Fujii, S. Maki, Y., Kimoto, H., Nucleophilic substitution of pentafluoropyridines with imidazole, J. Fluorine Chem. 43 (1989) 131-144.

[19] Fox, M.A., Sandford, G., Slater, R., Yufit, D.S., Howard, J.A.K., Vong, A., Reactions of 4-substituted tetrafluoropyridine derivatives with sulfur nucleophiles: S_NAr and annelation processes, J. Fluorine Chem. 143 (2012) 148-154.

[20] Pažitný, A., Solčán, T., Végh, D., Pentafluorobenzaldehyde and its utilizing in organic synthesis, J. Fluorine Chem. 130 (2009) 267-294.

[21] Veber, D. F, Johnson, S. R, Cheng, H. Y, Smith, B. R, Ward, K. W, Kopple, K.D., Molecular properties that influence the oral bioavailability of drug candidates, J. Med. Chem. 45 (2002) 2615-2623.

[22] Lipinski, C. A., Lead and drug-like compounds: the rule-of-five revolution, Drug Discovery Today: Technologies, 4 (2004) 337–341.

[23] www.molinspiration.com

[24] Ertle, P., Rohde, B., Selzer, P., Fast Calculation of Molecular Polar Surface Area as a Sum of Fragment-Based Contributions and Its Application to the Prediction of Drug Transport Properties, J. Med. Chem. 43 (2000) 3714-3717.

[25] APEX 2 and SAINT software for CCD diffractometers, Bruker AXS Inc., Madison, USA, 2013.

[26] Sheldrick, G.M., A short history of *SHELX*, Acta Crystallogr. A64 2008, 112-122.

[27] Parsons, S., Flack, H.D., Wagner, T., Use of intensity quotients and differences in absolute structure refinement, Acta Crystallogr, B69 (2013) 249-259.

[28] Krystof, V., McNae, I.W, Walkinshaw, M. D, Fischer, P.M., Müller, P, Vojtesek, B, Orság, M., Havlícek, L., Strnad, M., Antiproliferative activity of olomoucine ii, a novel 2,6,9-trisubstituted purine cyclin-dependent kinase inhibitor, Cell. Mol. Life Sci. 62 (2005) 1763–1771.

[29] Ramazani, A., Nasrabadi, F. Z., The reaction of thioacids with α-haloketones in water: an environmentally green synthesis of thioester derivatives. Phosphorus, Sulfur Silicon Relat. Elem. 188 (2013) 1214-1219.