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Introduction of methionine mimics on 3-arylthiophene: influence on protein farnesyltransferase inhibition and on antiparasitic activity†

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In the course of our search for new protein farnesyltransferase inhibitors with antiparasitic activity, we found that addition of a methionine residue at position 2 of 3-arylthiophenes greatly improved enzyme inhibition. To investigate the influence of this methionine residue on FTase and antiparasitic activities, 29 novel tetrasubstituted thiophenes bearing methionine or analogous moieties were synthesised. These new derivatives were evaluated on human and *Trypanosoma brucei* protein farnesyltransferases and on proliferation of 4 protozoan parasites: *Plasmodium falciparum*, *Trypanosoma brucei brucei*, *Trypanosoma cruzi* and *Leishmania donovani*. Some compounds showed promising low micromolar or submicromolar activities on *T. b. brucei* and *L. donovani* confirming the potential of this new class as antiparasitic agents.

Introduction

Parasitic diseases such as malaria, African sleeping sickness, Chagas disease or Leishmaniasis affect each year 1 billion people and are responsible for more than 1 million of deaths according to WHO reports. To control parasite proliferation as well as drug resistance development is crucial to fight against these tropical diseases. The use of new targets to improve the existing therapies could fill this unmet medical need. Among them, protein farnesyltransferase (FTase), first studied in anticancer therapy,[1] is considered nowadays a potential target in the treatment of parasitic diseases.[2,3] This key heterodimeric metalloenzyme belongs to the protein prenyl transferase family. With the involvement of an essential zinc atom,[4] FTase catalyses the transfer of a farnesyl chain (C_{15}) from farnesyl pyrophosphate (FPP) to the cysteine residue of some proteins. This cysteine belongs to the C-terminal CaaX sequence where C is the farnesylated cysteine, a is an aliphatic amino acid and X is a serine, an alanine, a glutamine or a methionine.[5,6] This farnesylation step allows CaaX proteins to be anchored to intracellular membranes and could promote protein–protein interactions.[7,8] Thus, FTase inhibition could lead to cellular disorders and cell death.

In the course of our search for a new class of antiparasitic agents with FTase inhibitory activity, we have previously reported on the discovery of tetrasubstituted 3-arylthiophenes.[9] The first modifications around this original scaffold headed by compound **1** revealed the importance of the presence of a nitrile moiety at position 4 and a carboxylic acid at position 2 for FTase inhibition (Fig. 1). Other structure–activity relationship (SAR) studies on our hit compound **1** were also carried out. Position 5 was modified by S_NAr or Liebeskind–Srogl coupling[10] and position 3 by solid- or liquid-phase Suzuki–Miyaura couplings.[11,12] These modifications confirmed the potency of the thio-isopropyl moiety at position 5 and of bulky or polyoxygenated aromatic groups at position 3 showing low micromolar activities on *T. brucei* FTase (*Tb*FTase).

More interestingly, although ester analogues **7–10** of compounds **1–4** respectively showed micromolar inhibitory activities on *T. b. brucei* proliferation, submicromolar activities were obtained with esters **5** and **6** (Fig. 1). Furthermore, introduction of methionine at position 2 of the thiophene ring (compound **2**) increased the activity on *Tb*FTase inhibition by 2 orders of magnitude displaying submicromolar activities. This activity improvement was not observed on *T. b. brucei* which led us to suspect the cleavage of the amide bond between methionine and thiophene ring in parasites.

In this article we report on two series of new 3-arylthiophenes. In the first series, methionine was introduced on our most active 3-arylthiophenes as mentioned above. The second series deals with compound **2** analogues where the amide bond was modified to be less sensitive to peptidase, or where

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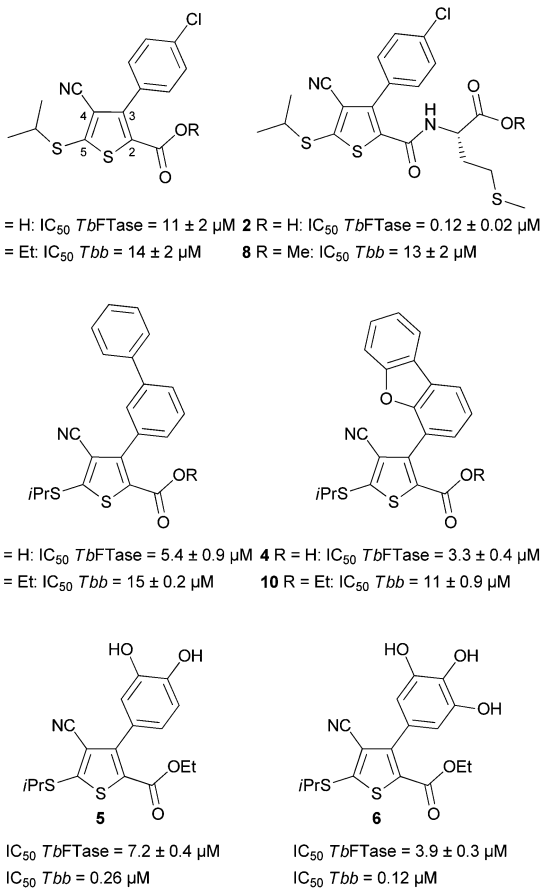


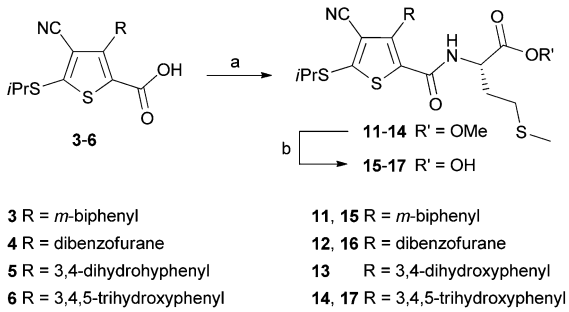
Fig. 1 Structures and inhibitory activities against *Tb*FTase and *T. b. brucei* (*Tbb*) proliferation of our most active thiophenes.

methionine was replaced by other amino derivatives. Synthesis of these new thiophenes and their biological evaluation on FTases and on *P. falciparum*, *T. b. brucei*, *T. cruzi* and *L. donovani* proliferation are described.

Results and discussion

Chemistry

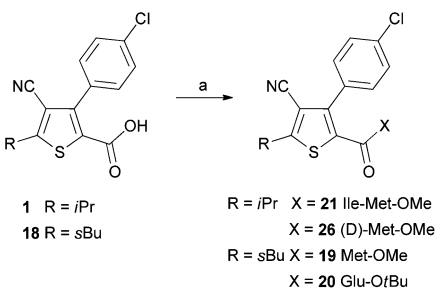
In the first series, methionine was added to compounds **3** and **4** and to the acid derivatives of compounds **5** and **6**. EDCI–HOBt or HBTU–HOBt coupling agents proved to be efficient to afford compounds **11–14** in moderate to good yields (Scheme 1). Because in most cases acids were most potent on FTase inhibition, these compounds were saponified. Because of its low availability compound **13** was not submitted to saponification. Although compounds **11** and **12** were easily hydrolysed with sodium hydroxide giving acids **15** and **16** respectively, ester **14** was resistant to these conditions. This lack of reactivity towards sodium hydroxide has already been described for a few thiophenes bearing a hydroxyphenyl moiety at position 3 and ester at position 2.[12] Therefore, we adapted a hydrolysis procedure using potassium fluoride doped alumina under microwave conditions[13] giving acid **17** in a non-optimized 23% yield (Scheme 1).



Scheme 1 Synthesis of compounds **15–17** by methionine coupling followed by saponification. *Reagents and conditions*: (a) EDCI, HOBt, NMM, CH_2Cl_2, rt, overnight or HBTU, HOBt, DIEA, DMF, 24–36 h, **11**: 64%, **12**: 62%, **13**: 33%, **14**: 48% and (b) NaOH, THF–H_2O–EtOH (2 : 2 : 1), rt, 72 h or KF–Al_2O_3, THF, MW, 300 W, 5 × 30 min, **15**: 62%, **16**: 100%, **17**: 23%.

In the CaaX motifs recognized by FTase, X is mainly a methionine or a glutamine particularly for the parasitic enzymes. Thus, 2-thio-*sec*-butylthiophene **18** having an inhibitory activity close to that of thiophene **1**[9] was coupled efficiently with EDCI–HOBt to these two amino acids giving compounds **19** and **20** respectively (Scheme 2). Furthermore, in order to check whether methionine in compound **2** mimics the X of the Ca_1a_2X box or the a_2 amino acid of this motif, the dipeptide Ile-Met-OMe was classically coupled to acid **1** to afford compound **21** (Scheme 2). This sequence was chosen because it is often observed in the C-terminal part of FTase protein substrates for example in K-Ras4B (CVIM) and B lamine (CAIM). Furthermore, the isoleucine residue is the a_2 part of the Rap2b protein (CVIL), a GGTase I substrate.[14]

Moreover, one of the key interactions for FTI design is with the hydrophobic cavity delimited by Trp102, Trp106 and Tyr361 called the a_2 binding site of Ca_1a_2X box.[15] This hydrophobic pocket was considered essential for the recognition of the inhibitor by FTase. Because of intermediate FTase inhibition observed for **21** (see below) we wondered whether the arylthiophene core interacts in the a_1 or a_2 site. Therefore, methionine was replaced by bulky hydrophobic moieties such as adamantyl and naphthyl known to bind at the a_2 site.[16] These groups were introduced with one or no methylene spacer by formation of an amide bond between thiophene **1** and the corresponding amines. As EDCI–HOBt coupling conditions were ineffective with these groups, acylation reactions were



Scheme 2 Synthesis of compounds **19–21** and **26**. *Reagents and conditions*: (a) EDCI, HOBt, NMM, CH_2Cl_2, rt, 16–36 h, **19**: 68%, **20**: 69%, **21**: 70%, and **26**: 88%.

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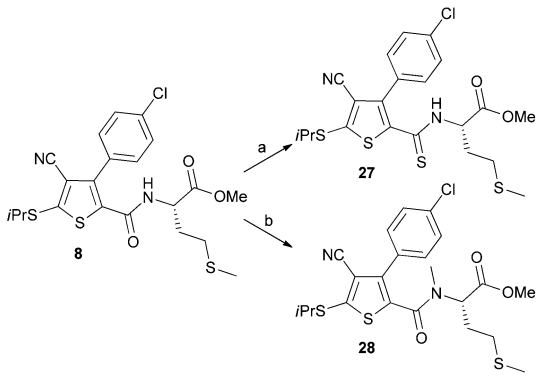
performed between compound **1** acid chloride and the corresponding amines to give analogues **22–25** in moderate to excellent yields (Scheme 3).

To get more active compounds against parasites we designed analogues of compound **2** less prone to peptidase cleavage. The most common modifications to prevent the peptide bond from enzymatic hydrolysis are the inversion of the amino acid absolute configuration, the methylation of the amide nitrogen or its transformation to a thioamide. These moieties provide more stable analogues with sometimes higher affinity towards biological receptors.[17] Configuration of the methionine was first changed by the synthesis of D-methionine analogue **26** (Scheme 2). Thionation of the methionine amide bond was also considered. Lawesson's reagent (LR) is the most used reagent for amino acid thionation, thanks to its high selectivity based on reaction temperatures and to its mild conditions.[18] Choice of the reaction solvent was crucial. In THF compound **8** conversion was very low either at room temperature or at 70 °C. However, in toluene at 110 °C, unselective thionation gave an inseparable mixture of mono- and dithionated compounds whereas at 40 °C selective thioamidation afforded **27** in 55% yield (Scheme 4).

Several methods are available to carry out *N*-methylation of amino acids.[19,20] Benoiton's method, *i.e.* treatment of amide **8** with NaH and methyl iodide, afforded *N*-methylated methionine thiophene **28** in a satisfactory yield (Scheme 4).

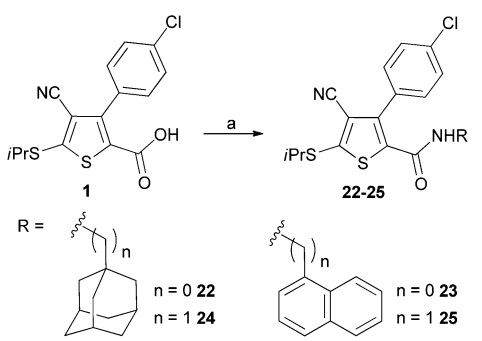
Aza-β3 amino acids are recent promising moieties which could increase metabolic stability, biodisponibility and biological absorption of drugs.[21,22] As far as we know there is only one article mentioning the formation of aza-β3 methionine as a fmoc protected derivative.[23] Therefore, to introduce this aza-β3 methionine bioisostere on the thiophene ring we envisioned two possible pathways. Either (2-(methylthio)ethyl)hydrazine would be added to thiophene **1** and the acetate moiety would further be introduced or the aza-β3 methionine analogue would be first synthesized and then coupled to compound **1**. Both ways were undertaken. 1,1-Dimethoxy-2-(methylsulfanyl)ethane was treated with HCl to afford the corresponding aldehyde which was submitted without any purification to the classical conditions of reductive amination with Boc-hydrazine to give compound **29**. After removal of the Boc group, the hydrazine was coupled with compound **1** to afford compound **30** and



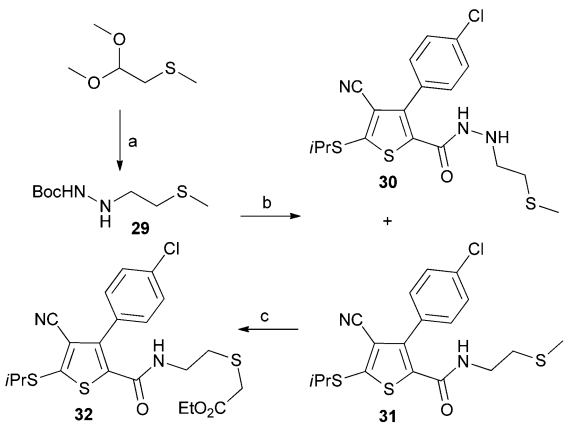
Scheme 4 Thionation and *N*-methylation of compound **8**. *Reagents and conditions*: (a) Lawesson's reagent, toluene, 40 °C, 140 h, 55% and (b) NaH, MeI, DMF, rt, 1 h, 53%.

unpredicted compound **31** both in poor yields, 10% and 17% respectively. It could be hypothesized that **31** came from acylation of the secondary amine followed by N–N bond cleavage as previously reported.[24–26] Addition of the acetate moiety to compound **30** was not possible because of the too small quantity available. However addition of ethyl bromoacetate in the presence of DIEA to compound **31** afforded compound **32** probably through the formation of a sulfonium ion (Scheme 5). Then we carried out the second pathway starting with the synthesis of the aza-β3 methionine analogue. Compound **33** was obtained by nucleophilic substitution between ethyl bromoacetate and Boc-hydrazine.[27] Subsequent alkylation with 1-chloro-2-(methylsulfanyl)ethane proved to be difficult and only high temperature and short time of microwave irradiation allowed the formation of compound **34** though in a low yield. Further Boc removal and coupling with thiophene **1** afforded analogue **35** (Scheme 6).

For FTase inhibition evaluation, all the analogues were hydrolyzed under appropriate conditions in good to excellent yields (Table 1).



Scheme 3 Synthesis of analogues **22–25**. *Reagents and conditions*: (a) (i) $SOCl_2$, reflux, 4.5 h, and (ii) RNH_2, pyridine, CH_2Cl_2, rt, 46 h, **22**: 27%, **23**: 66%, **24**: 87%, and **25**: 91%.



Scheme 5 Synthesis of compound **32**. *Reagents and conditions*: (a) (i) HCl 1%, reflux, 30 min, (ii) BocHN–NH_2, $NaBH(OAc)_3$, CH_3COOH, DCE, rt, 16 h, 29%; (b) (i) 4 M HCl, dioxane, rt, 2 h, (ii) **1**, HBTU, HOBt, DIEA, DMF, rt, 96 h, 10% (**30**), 17% (**31**); (c) $BrCH_2CO_2Et$, DIEA, CH_2Cl_2, 45 °C, 72 h, 52%.

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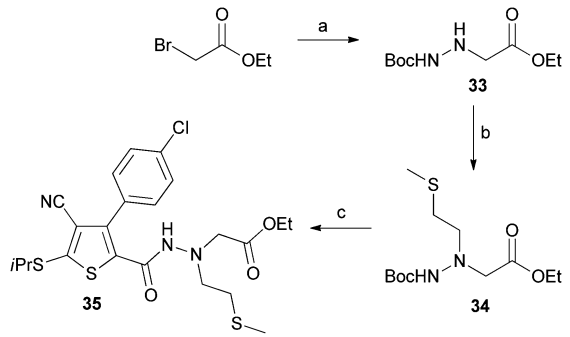


Scheme 6 Synthesis of aza-β3 analogue **35**. *Reagents and conditions*: (a) Boc–NH–NH_2, DIEA, CH_2Cl_2, 45 °C, 16 h, 54%; (b) 1-chloro-2-(methylsulfanyl)ethane, DIEA, 150 °C (MW), 2 × 30 min, 21%; (c) (i) TFA, CH_2Cl_2, rt, 1 h, (ii) **1**, EDCI, HOBt, NMM, CH_2Cl_2, rt, 6 h, 58%.

Biological evaluation

All these analogues of 2-methionine thiophene **2** were evaluated on recombinant human and *T. brucei brucei* FTases using a fluorescence-based assay[28,29] adapted to a 96-well plate format.[30] Results on the first series of compound **2** analogues bearing a methionine residue and variable 3-aryl groups are reported in Table 2.

Introduction of methionine at position 2 of our more active 3-arylthiophenes did not have the same effect as the initial compound (Table 2). This transformation was favourable for compounds bearing bulky *m*-biphenyl and dibenzofuranyl aromatics and improved their activities by 100-fold. Thus, interactions of analogues **15** and **16** in the FTase pocket seem to be similar to compound **2**.[10] In contrast, addition of methionine to 3-polyhydroxyphenylthiophenes had no effect on their enzymatic activity. These results support our former hypothesis[12] that the binding mode or binding site of polyhydroxyphenylthiophenes differs from that of our hit compound **1**. This is also confirmed by the fact that esters **13** and **14** showed micromolar activities whereas ester **8** was inactive.

The second series of compound **2** analogues was also evaluated on FTase inhibition (Table 3). In most cases, acidic derivatives are more active than their ester analogues as previously described.[31] Modification at position 2 of our hit **1** with methionine derivatives improved IC_{50} to a 4 to 240-fold extent on *h*FTase and led to a 1.2 to 240-fold activity enhancement on *Tb*FTase (compound **42** and compound **36** respectively). However none of these derivatives were more active than compound **2** on both enzymes, except compound **36** which displayed slightly improved activity.

Analogues **30–32** and **42** bearing long sulphur chains showed micromolar activities, roughly in the same range as compound **1**. Hydrophobic groups introduced to bind potentially on the hydrophobic cavity of the a_2 residue of the Ca_1a_2 box strongly decreased the inhibition activity (compounds **22–25**). These results may have two reasons: either methionine of compound **2** did not occupy the a_2 binding site, or the other substituents around the thiophene hampered an optimal configuration allowing adamantyl and naphthyl groups to interact with this pocket. Similarly, introduction of another amino acid between methionine and thiophene such as isoleucine which could mimic the a_2 part of the Ca_1a_2X box decreased the activity. Taken together, these results suggest that the 3-arylthiophene motif occupies the a_2 binding site in the same manner as phenylalanine in the complex CVFM-FPP-rat FTase (PDB I.D. code 1JCR).[32]

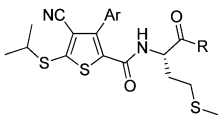
Adding a methionine residue was the optimal transformation affording submicromolar activities. As previously described with analogues bearing a carboxylic acid at position 2,[9] the thioalkyl group at position 5 had little influence on the inhibitory activity (analogues **2** *vs.* **36**). The analogues designed to minimize peptidase sensitivity (acids **26–28** and **35** and esters **39–41** and **43**) were all less active than acid **2** but more efficient than compound **1**. Submicromolar inhibitory activities were obtained for the thioamide derivative **40** and for the aza-β3

Table 1 Hydrolysis of esters[a]

Entry	Ester	Acid	Method	Yield[b] (%)
1	**19**	**36**	A	94
2	**20**	**37**	C	55
3	**21**	**38**	B	100
4	**26**	**39**	A	61
5	**27**	**40**	B	100
6	**28**	**41**	B	95
7	**32**	**42**	B	100
8	**35**	**43**	B	100

[a] Hydrolysis with NaOH 2 M (Method A) or LiOH 2 M (Method B) in THF–EtOH or TFA, Et_3SiH, CH_2Cl_2 (Method C). [b] Isolated yields.

Table 2 Inhibitory activity of thiophenes **2**, **8**, **11–17** against recombinant human FTase (*h*FTase) and *T. brucei* FTase (*Tb*FTase)



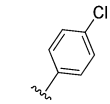
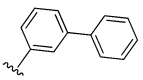
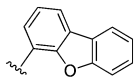
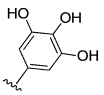
Ar	Cpd	R	IC_{50} (μM) *h*FTase	IC_{50} (μM) *Tb*FTase
	**8**	OMe	Inactive	Inactive
	2	OH	0.077 ± 0.006	0.12 ± 0.02
	**11**	OMe	23 ± 6	19 ± 4
	15	OH	0.042 ± 0.003	0.042 ± 0.001
	**12**	OMe	>50	>50
	16	OH	0.14 ± 0.009	0.19 ± 0.01
	**13**	OMe	4.7 ± 0.4	5.3 ± 0.5
	**14**	OMe	5.6 ± 0.4	3.6 ± 0.2
	17	OH	8.7 ± 1.5	4.8 ± 0.2

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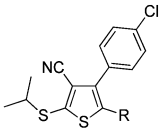
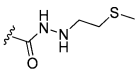
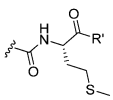
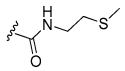
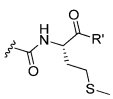
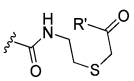
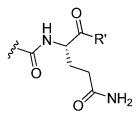
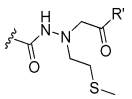
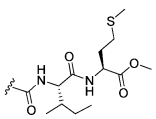
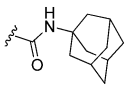
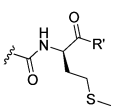
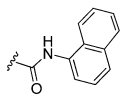
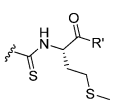
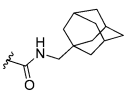
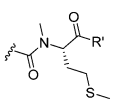
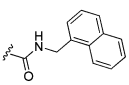
Table 3 Inhibitory activity of novel tetrasubstituted thiophenes against recombinant human FTase (*h*FTase) and *Trypanosoma brucei* FTase (*Tb*FTase)



R	Cpd	R′	IC_{50} (μM) *h*FTase	IC_{50} (μM) *Tb*FTase	R	Cpd	R′	IC_{50} (μM) *h*FTase	IC_{50} (μM) *Tb*FTase
	**7**	OEt	Inactive	Inactive		**30**	—	1.9 ± 0.1	>50
	1	OH	16 ± 2	11 ± 2					
	**8**	OMe	Inactive	Inactive		**31**	—	24 ± 6	>50
	2	OH	0.077 ± 0.006	0.12 ± 0.02					
	**19**[a]	OMe	>50	>50		**32**	OEt	9.3 ± 0.6	22 ± 2
	36[a]	OH	0.067 ± 0.005	0.079 ± 0.003		**42**	OH	3.7 ± 0.3	8.9 ± 0.8
	**20**[a]	O*t*Bu	10.4 ± 0.6	29 ± 13		**35**	OEt	>50	>50
	37[a]	OH	0.076 ± 0.006	1.2 ± 0.07		**43**	OH	1.1 ± 0.1	0.53 ± 0.03
	**21**	OMe	>50	>50		**22**	—	>50	>50
	38	OH	1.9 ± 0.3	2.2 ± 0.60					
	**26**	OMe	>50	2.8 ± 0.5		**23**	—	21.6 ± 1.6	>50
	39	OH	0.45 ± 0.02	1.4 ± 0.1					
	**27**	OMe	0.46 ± 0.08	8.2 ± 1		**24**	—	Inactive	>50
	40	OH	1.7 ± 0.1	0.23 ± 0.02					
	**28**	OMe	Inactive	Inactive		**25**	—	Inactive	>50
	41	OH	1.8 ± 0.05	3.3 ± 1					

[a] S*s*Bu instead of S*i*Pr at position 5.

analogue **43** on *Tb*FTase and for the D-methionine bearing compound **39** on the human enzyme.

This interesting discrepancy between two FTases from different species was also noticed with other derivatives. Though methionine and glutamine are generally described as possible X residues of the CaaX moiety for both human and *T. brucei* FTases, compound **37** displayed a 15-fold lower inhibition of *Tb*FTase than the corresponding methionine analogue **36**. On the other hand, IC_{50} values were very close for both compounds on the human enzyme. In contrast, analogue **40** with thio-methionine was seven times more active on *Tb*FTase. Surprisingly, this selectivity changed with ester analogue **27** displaying a sub-micromolar activity. The spatial disposition of the Ψ[CSNH]Met-OMe chain should be different with the ester and would allow supplementary favourable interactions in the FTase pocket. Moreover, activities of esters **20** and **27** on *Tb*FTase have a similar behaviour to ester analogues bearing polyhydroxyphenyl groups.[12] Thus for these two compounds, the ester moiety should induce a conformational or position change allowing a better interaction with the FTase binding site.

In our aim to develop antiparasitic agents, our ester derivatives were evaluated on the intraerythrocytic stages of *Plasmodium falciparum*,[33,34] responsible for malaria, the bloodstream forms of *Trypanosoma brucei brucei*, the pathogenic agent of African sleeping sickness in cattle, the intracellular development of *Trypanosoma cruzi* responsible for Chagas disease and *Leishmania donovani*, the causative agent of visceral Leishmaniasis (Table 4).[35–37] In most cases, our thiophenes are poorly active on *P. falciparum* and on *T. cruzi*. Our best compound was thiophene **35** with the aza-β3 moiety giving an activity of 10 μM on *P. falciparum*. For most of the compounds evaluated on *T. cruzi*, IC_{50} values were around 15 μM, the best result being observed for compound **12** with IC_{50} = 7.6 μM. Concerning *T. b. brucei* proliferation inhibition by the first series of

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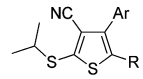
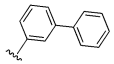
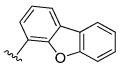
Table 4 *In cellulo* activity of thiophenes on *Plasmodium falciparum* (*Pf*), *Trypanosoma brucei brucei* (*Tbb*), *Trypanosoma cruzi* (*Tc*) and *Leishmania donovani* (*Ld*) proliferation[a,b]



Cpd	Ar	R	IC_{50} (μM) *Pf*	IC_{50} (μM) *Tbb*	IC_{50} (μM) *Tc*	IC_{50} (μM) *Ld*	Cpd	Ar	R	IC_{50} (μM) *Pf*	IC_{50} (μM) *Tbb*	IC_{50} (μM) *Tc*	IC_{50} (μM) *Ld*
7		OEt	>25	14 ± 1	17.5 ± 1	ND[c]	**23**		–NH–CH_2-1-adamantyl	>25	6.4 ± 0.5	—	—
8		Met-OMe	>25	13 ± 2	15 ± 0.2	ND[c]	**24**		–NH-1-naphthyl	>25	>25	—	—
11		Met-OMe	23 ± 4	5.8 ± 2	16 ± 0.4	ND[c]	**25**		–NH–CH_2-1-naphthyl	18 ± 1.6	4.4 ± 0.1	Inactive	>75
12		Met-OMe	20 ± 6	15 ± 0.8	7.6 ± 0.4	0.86 ± 0.02	**26**		(D)-Met-OMe	>25	15 ± 0.3	—	—
13		Met-OMe	11 ± 2.6	1.5 ± 0.1	18 ± 1	ND[c]	**27**		Ψ[CSNH]Met-OMe	>25	11 ± 3.6	15 ± 0.2	ND[c]
14		Met-OMe	>25	14 ± 1.3	—	—	**28**		Ψ[CONMe]Met-OMe	>25	8.1 ± 0.4	13 ± 0.7	>75
19[d]		Met-OMe	20 ± 0.7	11 ± 0.3	14 ± 0.4	ND[c]	**30**		–NH–$(CH_2)_2$–SMe	>25	1.5 ± 0.2	14 ± 1.3	ND[c]
20[d]		Gln-O*t*Bu	>25	8.5 ± 0.2	17 ± 1	ND[c]	**31**		–$(NH)_2$–$(CH_2)_2$–SMe	>25	2.8 ± 0.2	13 ± 3.5	ND[c]
21		Ile-Met-OMe	21 ± 5.5	4.0 ± 0.3	23 ± 1.4	0.95 ± 0.11	**32**		–NH–$(CH_2)_2$–S–CH_2–CO_2Me	23 ± 2	0.8 ± 0.1	Inactive	29.6 ± 2.8
22		–NH-1-adamantyl	>25	8.3 ± 0.5	—	—	**35**		Aza-β3-Met-OMe	10 ± 1.2	3.4 ± 0.3	Inactive	0.89 ± 0.02

[a] IC_{50} values were expressed as the mean values ± standard deviations determined from at least three independent experiments. [b] References: chloroquine (0.072 ± 0.0074 μM) for *Pf* evaluation, pentamidine (0.011 ± 0.0017 μM) for *Tbb* evaluation, benznidazole (1.7 ± 0.2 μM) for *Tc* evaluation and amphotericin B (0.031 ± 0.003 μM) and miltefosine (0.7 ± 0.1 μM) for *Ld* evaluation. [c] Not determined because of toxicity on infected macrophage. [d] S*s*Bu instead of S*i*Pr.

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methionine containing thiophenes, only the biphenyl derivative (**9**) showed a 2-fold better activity (IC_{50} = 5.8 μM). It is noticeable that addition of a methionine on polyhydroxythiophene analogues lowered the inhibition rate. In the second series, analogues designed to be resistant to carboxypeptidases (compounds **39–41**) did not display better activities than compound **7**. Therefore, the hypothesis of a rapid hydrolysis in parasites is not attested. Thus, to explain the difference between *in vitro* enzymatic activity and *in cellulo* proliferation inhibition, problems of cell penetration and/or aqueous solubility could be suspected. It is noteworthy that compounds **22**, **23**, and **25** with adamantyl or naphthyl amide inhibited parasite proliferation in the micromolar range though they were inactive on the isolated enzyme. Thus, for these derivatives, a target other than FTase should be responsible for this activity and would deserve to be identified. The best results were obtained for compounds **30–32** and **35** exhibiting low micromolar or submicromolar inhibitory activities on *T. b. brucei* (IC_{50} from 0.8 to 3.4 μM). This improvement could be due to the higher lipophilicity of these analogues bearing an aliphatic chain that may allow a better cell permeability. Most of the derivatives evaluated on *L. donovani* displayed toxicity against infected macrophage, precluding inhibitory activity measurement. Three derivatives revealed promising submicromolar activities. These compounds were substituted at position 2 of the thiophene ring by one or two amino acids (compounds **12** and **21** respectively) or by an aza-β3 moiety (compound **35**). In this *in vitro* model, the compounds should cross-over three membrane barriers (macrophage, phagolysosome and amastigote). Interestingly, no problem of compound uptake was observed with *L. donovani*. Since the protein farnesyltransferase systems also exist in Leishmania parasites and are sensitive to FTI,[38] it is probable that the compounds act on this target. Anyway, the level of activity monitored justifies further *in vivo* evaluation on the *L. donovani*/Balb/C mice model.

Conclusions

In summary, 29 new tetrasubstituted thiophenes have been synthesized and evaluated for their inhibitory activity on FTase and parasite proliferation. First, to enhance activities, methionine was coupled to promising 3-arylthiophenes. Then, modulations of the methionine at position 2 of the thiophene ring were designed to improve its affinity to the FTase binding pocket and to circumvent hypothetical carboxypeptidases during *in cellulo* assays. To reach our objective, methionine was modified by thionation or *N*-methylation or replaced by several mimics such as the aza-β3 moiety or bulky hydrophobic groups. Combination of the *m*-biphenyl group at position 3 and methionine at position 2 allowed us to obtain a low IC_{50} value of 42 nM for human and *T. brucei* FTase inhibition. Modification of the amide bond between thiophene and methionine did not improve antiparasitic activity, suggesting that no carboxypeptidase was responsible for the moderate activity. These results on parasites showed that further investigations have to be done to better identify the real target of our inhibitors, which is now in progress. It should be noted that the best activities were not observed for the same compounds according to the considered parasite. However compound **35** bearing an aza-β3 moiety was a good *Tb*FTase inhibitor and was among the most active derivatives against *P. falciparum*, *T. b. brucei* and *L. donovani*. Finally, three compounds displayed encouraging submicromolar activities on *L. donovani*. These promising molecules could be the starting point for further modulations, in particular at the not-well-explored position 5 of the thiophene ring, to complete our structure–activity relationship studies on this original class of FTIs and antiparasitic agents.

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Notes and references

1 D. M. Leonard, *J. Med. Chem.*, 1997, **40**, 2971–2990.
2 R. T. Eastman, F. S. Buckner, K. Yokoyama, M. H. Gelb and W. C. Van Voorhis, *J. Lipid Res.*, 2006, **47**, 233–240.
3 J. D. Ochocki and M. D. Distefano, *Med. Chem. Commun.*, 2013, **4**, 476–492.
4 D. A. Tobin, J. S. Pickett, H. L. Hartman, C. A. Fierke and J. E. Penner-Hahn, *J. Am. Chem. Soc.*, 2003, **125**, 9962–9969.
5 H. Zhang, *Handbook of metalloproteins*, 2004, vol. 3, pp. 37–48.
6 H. W. Park and L. S. Beese, *Curr. Opin. Struct. Biol.*, 1997, **7**, 873–880.
7 Y. Reiss, J. L. Goldstein, M. C. Seabra, P. J. Casey and M. S. Brown, *Cell*, 1990, **62**, 81–88.
8 F. L. Zhang and P. J. Casey, *Annu. Rev. Biochem.*, 1996, **65**, 241–269.
9 S. Lethu, M. Ginisty, D. Bosc and J. Dubois, *J. Med. Chem.*, 2009, **52**, 6205–6208.
10 S. Lethu and J. Dubois, *Eur. J. Org. Chem.*, 2011, 3920–3931.
11 S. Lethu, D. Bosc, E. Mouray, P. Grellier and J. Dubois, *J. Enzyme Inhib. Med. Chem.*, 2013, **28**, 163–171.
12 D. Bosc, S. Lethu, E. Mouray, P. Grellier and J. Dubois, *Med. Chem. Commun.*, 2012, **3**, 1512–1517.
13 G. W. Kabalka, L. Wang and R. M. Pagni, *Green Chem.*, 2001, **3**, 261–262.
14 K. T. Lane and L. S. Beese, *J. Lipid Res.*, 2006, **47**, 681–699.
15 A. Ghinet, B. Rigo, J. Dubois, A. Farce, J.-P. Henichart and P. Gautret, *Med. Chem. Commun.*, 2012, **3**, 1147–1154.
16 N. Wlodarczyk, D. Le Broc-Ryckewaert, P. Gilleron, A. Lemoine, A. Farce, P. Chavatte, J. Dubois, N. Pommery, J.-P. Hénichart, C. Furman and R. Millet, *J. Med. Chem.*, 2011, **54**, 1178–1190.
17 J. Vagner, H. Qu and V. J. Hruby, *Curr. Opin. Chem. Biol.*, 2008, **12**, 292–296.
18 M. Jesberger, T. P. Davis and L. Barner, *Synthesis*, 2003, 1929–1958.
19 L. Aurelio, R. T. C. Brownlee and A. B. Hughes, *Chem. Rev.*, 2004, **104**, 5823–5846.

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20 J. R. McDermott and N. L. Benoiton, *Can. J. Chem.*, 1973, **51**, 1915–1919.
21 S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 173–180.
22 M. Laurencin, P. Bauchat and M. Baudy-Floc'h, *Synthesis*, 2009, 1007–1013.
23 O. Busnel, L. Bi and M. Baudy-Floc'h, *Tetrahedron Lett.*, 2005, **46**, 7073–7075.
24 H. Junek and C. Reidlinger, *Monatsh. Chem.*, 1995, **126**, 579–586.
25 A. P. Krapcho and K. L. Avery, *J. Org. Chem.*, 1988, **53**, 5927–5929.
26 P. Magnus, N. Garizi, K. A. Seibert and A. Ornholt, *Org. Lett.*, 2009, **11**, 5646–5648.
27 A. Cheguillaume, A. Salaün, S. Sinbandhit, M. Potel, P. Gall, M. Baudy-Floc'h and P. Le Grel, *J. Org. Chem.*, 2001, **66**, 4923–4929.
28 D. L. Pompliano, R. P. Gomez and N. J. Anthony, *J. Am. Chem. Soc.*, 1992, **114**, 7946.
29 P. B. Cassidy, J. M. Dolence and C. D. Poulter, *Methods Enzymol.*, 1995, **250**, 30–43.
30 L. Coudray, R. Marcia de Figueiredo, S. Duez, S. Cortial and J. Dubois, *J. Enzyme Inhib. Med. Chem.*, 2009, **24**, 972–985.
31 S. Duez, L. Coudray, E. Mouray, P. Grellier and J. Dubois, *Bioorg. Med. Chem.*, 2010, **18**, 543–556.
32 S. B. Long, P. J. Hancock, A. M. Kral, H. W. Hellinga and L. S. Beese, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 12948–12953.
33 J. Guillon, P. Grellier, M. Labaied, P. Sonnet, J.-M. Léger, R. Déprez-Poulain, I. Forfar-Bares, P. Dallemagne, N. Lemaître, F. Péhourcq, J. Rochette, C. Sergheraert and C. Jarry, *J. Med. Chem.*, 2004, **47**, 1997–2009.
34 R. E. Desjardins, C. J. Canfield, J. D. Haynes and J. D. Chulay, *Antimicrob. Agents Chemother.*, 1979, **16**, 710–718.
35 J. O'Brien, I. Wilson, T. Orton and F. Pognan, *Eur. J. Biochem.*, 2000, **267**, 5421–5426.
36 I. M. D. Bastos, F. N. Motta, S. Charneau, J. M. Santana, L. Dubost, K. Augustyns and P. Grellier, *Microbes Infect.*, 2010, **12**, 457–466.
37 P. M. Loiseau, S. Gupta, A. Verma, S. Srivastava, S. K. Puri, F. Sliman, M. Normand-Bayle and D. Desmaele, *Antimicrob. Agents Chemother.*, 2011, **55**, 1777–1780.
38 K. Yokoyama, P. Trobridge, F. S. Buckner, J. Scholten, K. D. Stuart, W. C. Van Voorhis and M. H. Gelb, *Mol. Biochem. Parasitol.*, 1998, **94**, 87–97.

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