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RESEARCH ARTICLE

New protein farnesyltransferase inhibitors in the 3-arylthiophene 2-carboxylic acid series: diversification of the aryl moiety by solid-phase synthesis

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Abstract

A new synthetic pathway was devised to reach tetrasubstituted 3-arylthiophene 2-carboxylic acids in a three-step solid-phase synthesis. This very efficient methodology provided more than 20 new compounds that were evaluated for their ability to inhibit protein farnesyltransferase from different species as well as *Trypanosoma brucei* and *Plasmodium falciparum* proliferation.

Keywords: Suzuki cross-coupling, heterocycles, structure–activity relationships, enzyme inhibition, antiparasitic activity

Introduction

Protein farnesyltransferase (FTase) is a key enzyme in protein post-translational lipidation[1]. Its inhibition prevents correct protein localization in the cell and may cause cellular disorders leading to cell death. These properties were first thought to be of interest to design new anti-cancer agents[2]. Recently, FTase turned out to be a potential target to fight against parasitic tropical diseases[3]. In the course of our search for new protein farnesyltransferase inhibitors (FTIs) with antiparasitic activity, we discovered a new FTI family in the tetrasubstituted 3-arylthiophene series. Our preliminary structure–activity relationship studies have enlightened the importance of a carboxylic acid at position 2 as well as a nitrile function at position 4 on the thiophene ring[4]. To get more insight into this new FTI family, we have modified the isopropyl ether at position 5 by S_NAr reactions[5] and subsequently we intended to study the role of the 3-aryl moiety. Therefore, we elaborated a different synthetic pathway, allowing rapid modification at position 3 of the thiophene ring. In this article, we describe a novel synthesis of 3-arylthiophenes **1**, its application on solid-phase synthesis, the production of a new library of 3-arylthiophene derivatives and their activity on FTase.

Experimental protocols

All solvents and reagents were commercially available and were used without further purification. Wang resin was purchased from Novabiochem (1.3 mmol/g, 100–200 mesh). Solid-phase syntheses were realized on Unimax 2010 (Heidolph) for the binding to Wang resin and on synthesis 1 (Heidolph) for the Suzuki–Miyaura coupling. Analytical thin-layer chromatography was carried out on silica gel $60F_{254}$ aluminium sheets (Merck). Column chromatography was performed with silica gel with prepacked Redisep columns. Preparative LC (PLC) was performed on silica gel $60F_{254}$ glass plates (Merck). ^1H and ^{13}C NMR spectra were recorded at Bruker Avance 300 (300 MHz) and Avance 500 (500 MHz). ^1H chemical shifts (δ) are reported in parts per million (ppm) relative to the singlet at 7.26 ppm for residual $CHCl_3$ and to the central line of residual acetone at 2.05 ppm. ^{13}C chemical shifts (δ are reported in parts per million (ppm) relative to the central line

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either of the triplet at 77.2 ppm for chloroform-d, or of the massif at 29.9 ppm for acetone-d6. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; qi, quintuplet; h: heptuplet; m, multiplet; b, broad and combinations thereof. Coupling constants *J* are reported in hertz (Hz). IR spectra were recorded with an FTIR Perkin-Elmer Spectrum BX spectrometer. Low and high resolutions mass spectra were recorded by navigator LC/MS (source AQA) for electron spray ionization. UHPLC analyses were realized on Waters Acquity UPLC system. Elemental analyses were performed by the ICSN Microanalytical Laboratory.

UHPLC analysis

The purity for all target compounds was measured using reversed-phase UHPLC (HSS C-18 1.8 µm, 2.1 × 50 mm column) with two different solvent systems: compounds were eluted with 95:5 A/B for 0.5 min then with a gradient of 5–100% B/A for 3.5 min followed by 0:100 isocratic for 1 min at a flow rate of 0.6 mL/min, where solvent A was 0.1% formic acid in H_2O and solvent B was 0.1% formic acid in MeCN (system 1) or 0.1% formic acid in MeOH (system 2). Purity was determined on TAC (total absorbance current from 200 to 400 nm).

Chemistry

2-(Bis(isopropylthio)methylene)malononitrile (2)

To a solution (DMF, 350 mL) of malononitrile (10.0 g, 151 mmol, 1.0 equiv.), K_2CO_3 (22.0 g, 159 mmol, 1.05 equiv.) was added. After stirring for 30 min at room temperature, carbon disulfide (9.13 mL, 151 mmol, 1.0 equiv.) was added dropwise and the reaction mixture was stirred at room temperature for 30 min before *i*-propyl iodide (30.3 mL, 302 mmol, 2.0 equiv.) and tetrabutylammonium bromide (9.75 g, 30.2 mmol, 0.2 equiv.) were then added. After being stirred for 70 h at 60°C, the reaction mixture was cooled, diluted with water and extracted four times with diethyl ether. The combined organic layers were washed with sodium thiosulfate 5% and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (heptane/EtOAc 98:2 (v/v)) to afford **2** as a white amorphous solid (24.9 g, 73%). Mp = 68°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.41 (d, J = 6.5 Hz, 12H), 3.99 (h, J = 6.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.2, 42.7, 80.6, 113.1, 180.1. IR (film) 2970, 2928, 2867, 2219, 1479 cm^{-1}. MS (ESI$^+$, MeOH) *m/z* 227 [M+H]$^+$. HRMS (ESI$^+$, MeOH) calcd for $C_{10}H_{15}N_2S_2$ [M+H]$^+$ 227.0677, found 227.0670. Elemental analysis calcd (%) for $C_{10}H_{14}N_2S_2$: C 53.06, H 6.23, N 12.38, S 28.33; found C 53.05, H 6.45, N 12.23, S 28.46.

Ethyl 3-amino-4-cyano-5-(isopropylthio) thiophene-2-carboxylate (3)

To a solution (EtOH, 60 mL) of **2** (4.5 g, 19.9 mmol, 1.0 equiv.), K_2CO_3 (3.07 g, 21.9 mmol, 1.1 equiv.) and ethyl thioglycolate (2.4 mL, 21.9 mmol, 1.1 equiv.) were added. After stirring for 3 h under reflux, water was added to the reaction mixture. The solution was filtered and the precipitate was washed with water before purification by flash chromatography on silica gel (heptane/EtOAc 10:0 to 9:1 (v/v) in 1 h) to afford **3** as a white amorphous solid (4.52 g, 84%). Mp = 114°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.32 (t, J = 7.0 Hz, 3H), 1.39 (d, J = 6.5 Hz, 6H), 3.54 (h, J = 6.5 Hz, 1H), 4.27 (q, J = 7.0 Hz, 2H), 5.74 (bs, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ14.6, 23.3, 42.0, 60.9, 101.5, 103.8, 112.9, 153.2 153.6, 162.9. IR (film) 3424, 3332, 2982, 2928, 2218, 1668, 1622, 1537, 1296 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 293 [M+Na]$^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{11}H_{14}N_2O_2S_2Na$ [M+Na]$^+$ 293.0394, found 293.0401. Elemental analysis calcd (%) for $C_{11}H_{14}N_2O_2S_2$: C 48.97, H 5.22, N 10.36, O 11.84, S 23.72; found C 48.87, H 5.23, N 10.35, O 11.91, S 23.94.

Ethyl 3-bromo-4-cyano-5-(isopropylthio)thiophene-2-carboxylate (4)

To a solution (CH_3CN, 50 mL) of *t*BuONO (2.9 mL, 25 mmol, 1.5 equiv.) and $CuBr_2$ (4.85 g, 21.7 mmol, 1.3 equiv.) a solution (CH_3CN, 50 mL) of **3** (4.5 g, 16.7 mmol, 1 equiv.) was added through cannula. After completion of the addition, the reaction mixture was stirred for 10 min at room temperature, poured on a HCl solution (20%, 100 mL) and extracted four times with diethyl ether. Combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (heptane/EtOAc 10:0 to 7:3 (v/v) in 1 h) to afford **4** as a white amorphous solid (5.25 g, 94%). Mp = 59°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39 (t, J = 7.0 Hz, 3H), 1.43 (d, J = 6.5 Hz, 6H), 3.61 (h, J = 6.5 Hz, 1H), 4.38 (q, J = 7.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ14.4, 23.3, 42.7, 62.4, 112.8, 117.9, 118.2, 129.1, 154.1, 159.2. IR (film) 2983, 2224, 1722, 1226, 1099, 750 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 356 and 358 [M+Na]$^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{11}H_{12}{}^{79}BrNO_2S_2Na$ [M+Na]$^+$ 355.9391, found 355.9384; calcd for $C_{11}H_{12}{}^{81}BrNO_2S_2Na$ [M+Na]$^+$ 357.9370, found 357.9380. Elemental analysis calcd (%) for $C_{11}H_{12}BrNO_2S_2$: C 39.53, H 3.62, N 4.19, O 9.57, S 19.19; found C 39.45, H 3.51, N 4.01, O 9.52, S 18.82.

Ethyl 3-(4-chlorophenyl)-4-cyano-5-(isopropylthio) thiophene-2-carboxylate (5a)

To a solution (toluene/EtOH 9:1 (v/v), 100 mL) of **4** (5.0 g, 15.0 mmol, 1.0 equiv.), K_2CO_3 (2 M in water, 18.8 mL, 37.5 mmol, 2.5 equiv.), $Pd(PPh_3)_4$ (1.74 g, 1.5 mmol, 10% mol) and 4-chlorophenyl boronic acid (4.69 g, 30 mmol, 2.0 equiv.) were added. After stirring for 4 h under reflux, water was added to the reaction mixture which was extracted three times with EtOAc. Combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (heptane/EtOAc 10:0 to 5:5 (v/v) in 40 min) to afford **5a** as a white amorphous solid (4.99 g, 91%). The analytical data were identical to those previously reported[4].

3-(4-Chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carboxylic acid (1a)

This compound was obtained according to previously reported procedure[4].

3-Bromo-4-cyano-5-(isopropylthio)thiophene-2-carboxylic acid (6)

To a solution (THF/EtOH 2:1 (v/v), 20 mL) of **4** (2.58 g, 7.74 mmol, 1.0 equiv.), aqueous NaOH (2 M, 20 mL) was added. After stirring overnight at room temperature, the mixture was neutralized with aqueous HCl (1 M, 40 mL). The precipitated product was filtered, washed with water and dried under vacuum to afford acid **6** as a white powder (2.17 g, quant.). ^{1}H NMR (500 MHz, acetone-d6) δ 1.46 (d, J = 6.5 Hz, 6H), 3.75 (h, J = 6.5 Hz, 1H). ^{13}C NMR (75 MHz, acetone-d6) δ 23.3, 43.3, 114.3, 119.2, 119.3, 131.6, 155.5, 161.0. IR (film) 2969, 2221, 1689, 1650, 1498, 1241 cm^{-1}. MS (ESI$^-$, CH_2Cl_2/MeOH) *m/z* 304 and 306 [M-H]$^-$. HRMS (ESI$^-$, CH_2Cl_2/MeOH) calcd for $C_9H_7{}^{79}BrNO_2S_2$ [M-H]$^-$ 303.9102, found 303.9102; calcd for $C_9H_7{}^{81}BrNO_2S_2$ [M-H]$^-$ 305.9078, found 305.9081. Elemental analysis calcd (%) for $C_9H_8BrNO_2S_2$: C 35.30, H 2.63, N 4.57, O 10.45, S 20.94; found C 35.35, H 2.62, N 4.51, O 10.58, S 20.78.

Ethyl 3-(4-methoxyphenyl)-4-cyano-5-(isopropylthio) thiophene-2-carboxylate (5j)

To a solution (toluene/EtOH 9:1 (v/v), 20 mL) of **4** (0.71 g, 2.12 mmol, 1.0 equiv.), K_2CO_3 (2 M in water, 2.65 mL, 5.3 mmol, 2.5 equiv.), $Pd(PPh_3)_4$ (0.245 g, 0.21 mmol, 10 % mol) and 4-methoxyphenyl boronic acid (0.645 g, 4.2 mmol, 2.0 equiv.) were added. After stirring for 20 h under reflux, water was added to the reaction mixture which was extracted three times with EtOAc. Combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (heptane/EtOAc 100:0 to 85:15 (v/v) in 35 min) to afford **5j** as a white amorphous solid (0.302 g, 50%). Mp = 90°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, J = 7.0 Hz, 3H), 1.46 (d, J = 6.5 Hz, 6H), 3.63 (h, J = 6.5 Hz, 1H), 3.85 (s, 3H), 4.22 (q, J = 7.0 Hz, 2H), 6.97 (d, J = 8.5 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 23.2, 42.1, 55.3, 61.6, 113.5, 113.8, 116.0, 124.2, 128.6, 130.8, 149.1, 152.7, 160.3. IR (film) 2971, 2918, 2837, 2216, 1713, 1610, 1530, 1496, 1458, 1365, 1358, 1264, 1180, 1083, 1024, 828, 759 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 384 [M+Na]$^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{18}H_{19}NO_3S_2Na$ [M+Na]$^+$ 384.0704, found 384.0722.

Solid-phase synthesis

Polymer-bond 3-bromo-4-cyano-5-(isopropylthio)thiophene-2-carboxylate (7) To a solution (THF, 8 mL) of swelled Wang resin (1.3 mmol/g, 1.0 g, 1.3 mmol, 1 equiv.), bromothiophene **6** (793 mg, 2.6 mmol, 2 equiv.), DIAD (1.02 mL, 5.2 mmol, 4 equiv.) and PPh_3 (1.36 g, 5.2 mmol, 4 equiv.) were added. The mixture was stirred on an orbital shaker for 6 h at room temperature. The resin was collected by filtration, washed with THF (3 × 20 mL), THF/MeOH 1:1 (3 × 20 mL), MeOH (3 × 20 mL) and Et_2O (2 × 20 mL), respectively to afford **7** (1.33 g, 97%, 0.94 mmol/g)[6].

Polymer-bond 3-aryl-4-cyano-5-(isopropylthio)thiophene-2-carboxylate (8) Resin **7** (130 mg, 0.75 mmol/g, 1 equiv.) was loaded on a teflon reactor for solid-phase synthesis and suspended in toluene/*t*BuOH (4 mL, 9:1 v/v). The resin was swollen for 6 h at room temperature. Then, $Pd(PPh_3)_4$ (11.3 mg, 9.8 µmol, 10% mol), K_3PO_4 (104 mg, 0.49 mmol, 5 equiv.) and boronic acid (0.49 mmol, 5 equiv.) were added to the solution and the mixture was shaken for 65 h at 110°C. The resin was collected by filtration, washed with THF (2 × 10 mL), THF/H_2O 1:1 (2 × 10 mL), MeOH (2 × 10 mL) CH_2Cl_2 (2 × 10 mL), DMF (2 × 10 mL), THF (2 × 10 mL), MeOH (2 × 10 mL) and CH_2Cl_2 (2 × 10 mL), respectively to afford **8**.

*3-aryl-4-cyano-5-(isopropylthio) thiophene-2-carboxylic acid (**1**)* To the swelled resin **8**, CH_2Cl_2/TFA (1:1 v/v, 5 mL) was added and the mixture was shaken for 5 min. The solution was filtered, then CH_2Cl_2/TFA (1:1 v/v, 5 mL) was added again on the resin that was shaken 40 min. The resin was filtered and washed with CH_2Cl_2. The filtrates were pooled and concentrated under vacuum. The crude residue was diluted to 1 mg/mL and analysed by UHPLC. When needed, the product was purified by PLC (Heptane/EtOAc 5:5) to afford pure desired compounds.

Biological assays

Yeast FTase assay

Assays were realized on 96-well plates, prepared with Biomek NKMC and Biomek 3000 from Beckman Coulter and read on Wallac Victor fluorimeter from Perkin-Elmer. Per well, 20 µL of farnesyl pyrophosphate (10 µM) was added to 180 µL of a solution containing 2 µL of varied concentrations of potential inhibitors (dissolved in DMSO) and 178 µL of a solution composed by 0.1 mL of partially purified recombinant yeast FTase (2.2 mg/mL) and 7.0 mL of dansyl-GCVLS peptide (in the following buffer: 5.8 mM DTT, 6 mM $MgCl_2$, 12 µM $ZnCl_2$ and 0.09% (w/v) CHAPS, 53 mM Tris/HCl, pH 7.5). Then the fluorescence development was recorded for 15 min (0.7 second per well, 20 repeats) at 30°C with an excitation filter at 340 nm and an emission filter at 486 nm. Each measurement was realized twice as duplicate or triplicate.

Human FTase assay

Assays were realized on 96-well plates, as described for yeast FTase but octyl-D-glucopyranoside (0.18%) was used instead of CHAPS and the solution contains 5 µL of partially purified human FTase (1.5 mg/mL)[7] in 1 mL peptide solution.

Cloning and expression of recombinant protein Tb-*FTase*

The cloning of the αβ-*Tb*-FTase from the *Trypanosoma brucei brucei* strain WT 2913 was realized according to

Buckner et al.[8]. Briefly, procyclic forms of *T. brucei brucei* were maintained in semi-defined medium 79 containing 10% (v/v) heat-inactivated fetal calf serum (FCS) at 27°C. Cells were collected by centrifugation from logarithmic cultures and DNA extracted. The α subunit was amplified from genomic DNA by PCR using the sense (5′-CCGGATCCCATGAATAAGAGCGCGGTGCGTAGT GAAGAAAGCCGC-3′) and antisense oligonucleotides (5′-GAAAGCTTCATATGTTAAAACTCCTCAACATAG TCCCGGTTCATAACG-3′), respectively. The 1800 bp PCR fragment was purified (DNA clean up-GEHealthcare) and ligated into a TOPO 2.1 vector (Clontech). The β subunit was similarly amplified using the sense (5′-ATACTTCCATATGTCTTTTGTACCACCTCCAGC-3′) and antisense oligonucleotides (5′-CTATCCTAGGAG ATCTTCAGCACATGAAAGTCTTTGCGC-3′), respectively and also cloned into the TOPO 2.1 vector (Clontech). The α-subunit gene was excised from its vector by BamHI and NdeI digestion, gel purified and cloned into the BamHI/NdeI sites immediately upstream the β-subunit gene into the vector TOPO 2.1-β previously digested with Bam HI and NdeI. The resulting TOPO 2.1-α–β plasmid was digested with BamHI and BglII to release a 3600 bp fragment corresponding to the α and β subunits in tandem of the *Tb*-FTase gene. The α–β tandem was cloned into the BamHI site of the expression plasmid pRSF-1b (Novagen). The recombinant plasmid was used to transform top 10 *E. coli* and the clones with proper orientation were designated pRSF1-*Tb*PFT. For the *Tb*-FTase protein expression, the *E. coli* strain BL21(DE3) was transformed with pRSF1-*Tb*PFT, grown in LB + kanamycine until DO (A_{600}) = 0.6. The culture was then cooled down to 24°C before induction by addition of 0.4 mM IPTG (isopropyl-ß-D-thiogalactopyranoside) and grown 3 h at room temperature. The bacteria are harvested by centrifugation (12 min, 8000 rpm) and the pellet frozen at –20°C. To purify the soluble recombinant protein, bacteria were lysed using Dyno Mill (0.2 mm) and purified according to our reported procedure for recombinant human FTase[7] and stored with 5% glycerol at 4°C.

T. brucei *FTase assay*

Assays were realized on 96-well plates, as described for human FTase with the dansylated peptide Dansyl-GCAIM and the solution contains 15 µL of partially purified *Tb*-FTase (1.0 mg/mL) in 1 mL peptide solution.

Assay for in vitro *inhibition of* Plasmodium falciparum *growth*

The chloroquine-resistant strain FcB1/Colombia of *P. falciparum* was maintained *in vitro* on human erythrocytes in RPMI 1640 medium supplemented by 8% (v/v) heat-inactivated human serum, at 37°C, under an atmosphere of 3% CO_2, 6% O_2, 91% N2. *In vitro* drug susceptibility assays was measured by [^{3}H]-hypoxanthine incorporation as described[9] using a modification of the semi-automated microdilution technique of Desjardins et al.[10]. Drugs were prepared in DMSO at a 10 mM concentration. Compounds were serially diluted two-fold with 100 µL culture medium in 96-well plates. Asynchronous parasite cultures (100 µL, 1% parasitaemia and 1% final hematocrite) were then added to each well and incubated for 24 h at 37°C prior to the addition of 0.5 µ Ci of [^{3}H]-hypoxanthine (GE Healthcare, France, 1 to 5 Ci·mmol/mL) per well. After a further incubation of 24 h, plates were frozen and thawed. Cell lysates were then collected onto glass-fiber filters and counted in a liquid scintillation spectrometer. The growth inhibition for each drug concentration was determined by comparison of the radioactivity incorporated in the treated culture with that in the control culture maintained on the same plate. The concentration causing 50% growth inhibition (IC_{50}) was obtained from the drug concentration-response curve and the results were expressed as the mean values ± standard deviations determined from several independent experiments.

Assay for in vitro inhibition of *T. brucei brucei growth*

Bloodstream forms of *T. brucei brucei* strain 90–13 were cultured in HMI9 medium supplemented with 10% FCS at 37°C under an atmosphere of 5% CO_2[11]. In all experiments, log-phage cell cultures were harvested by centrifugation at 3000*g* and immediately used. Drug assays were based on the conversion of a redox-sensitive dye (resazurin) to a fluorescent product by viable cells[12] and were performed according to the manufacturer recommendations (AlamarBlue® Assay, Invitrogen Corporation). Drug stock solutions were prepared in pure DMSO. *T. brucei brucei* bloodstream forms (3×10^4 cells/mL) were cultured as described above in 24-well plates (1 mL per well) either in the absence or in the presence of different concentrations of inhibitors (0, 0.1, 1, 10 and 100 µM) with a final DMSO concentration of 1%. After a 72-h incubation, AlamarBlue® solution (100 µL) was added in each well and fluorescence was measured at 530 nm excitation and 590 nm emission wavelengths after a further 4-h incubation. Each inhibitor concentration was tested in triplicate and the experiment repeated twice. The percentage of inhibition of parasite growth rate was calculated by comparing the fluorescence of parasites maintained in the presence of drug to that of in the absence of drug.

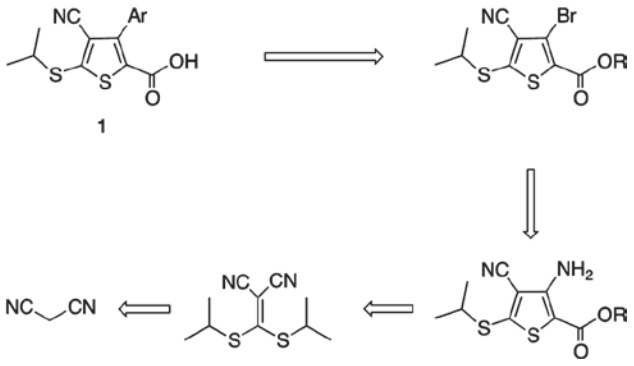
Results and discussion

Novel synthetic pathway to 3-arylthiophene 2-carboxylic acids

According to our strategy to develop new 3-modified derivatives, it seemed reasonable to carry out the introduction of the phenyl ring at the end of the synthesis by a general and efficient method allowing the formation of the biaromatic unit with various substituants. Suzuki–Miyaura cross-coupling[13,14] fulfilled our requirements because of its common use in arylation of halogenothiophene[15–19], though less frequently described in solid phase than in homogenous synthesis[20–22]. This palladium-catalyzed reaction could afford the desired compounds from the corresponding 3-bromothiophene

derivative obtained by a Sandmeyer reaction on a 3-amino thiophene[23] itself synthesized according to previously described Fiesselmann-type reaction on a ketene dithioacetal prepared from commercially available malononitrile (Scheme 1)[24]. This pathway was applied to the synthesis of our hit compound, 3-(4-chlorophenyl)-4--cyano-5-(isopropylthio)thiophene-2-carboxylic acid **1a** and proceeded in a very efficient 48% overall yield from malononitrile (Scheme 2). Ketene dithioacetal **2** was obtained by deprotonation of malononitrile followed by condensation with carbon disulfide and subsequent quenching with isopropyl iodide. Cyclocondensation of **2** with ethyl thioglycolate afforded the amino derivative **3** that was converted to bromothiophene **4** by a Sandmeyer reaction.

The crucial step for our methodology, Suzuki–Miyaura palladium-catalyzed coupling of compound **4** with 4-chlorophenyl boronic acid, had to be optimized. Among many assayed conditions, tetrakis(triphenylphosphine) palladium was found to be the best catalyst for this reaction, potassium carbonate proved to be the most efficient base, the mixture of toluene/ethanol (9:1, v/v) was the best solvent and running the reaction at 110°C afforded the ester **5a** in excellent yield. Compound **1a** was finally obtained after basic hydrolysis. The efficiency of this new pathway where the aryl moiety is introduced at the end of the synthesis allowed us to plan the generation of diverse 3-arylthiophene analogues.

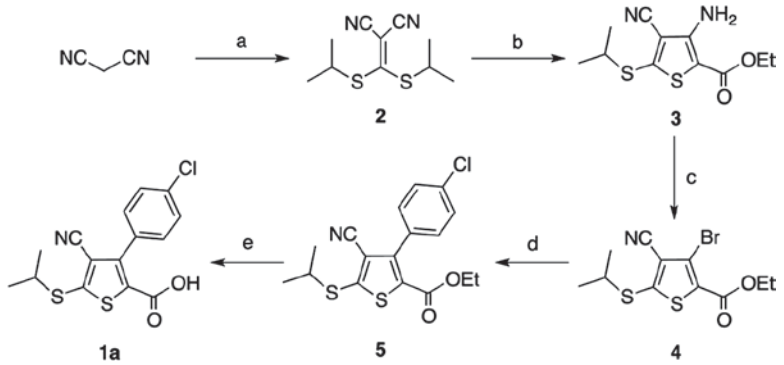


Scheme 1. Retrosynthetic pathway.

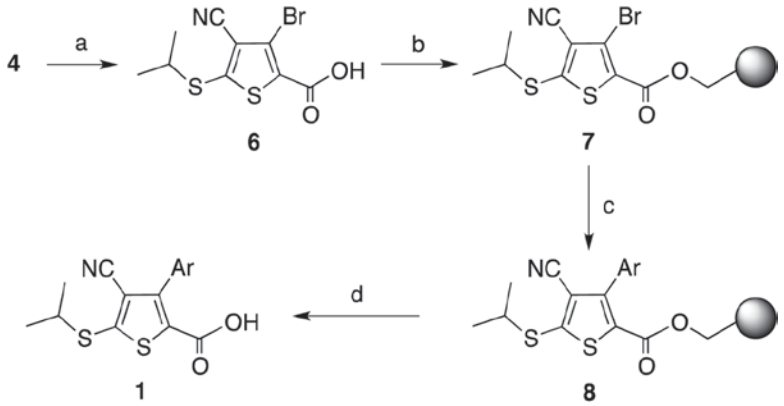
Solid-phase synthesis

To rapidly obtain a 3-arylthiophene-2-carboxylic acid library, we decided to realize the synthesis on solid support. The thiophene could be linked to the resin by an ester function after reaction with the 2-carboxylic moiety. We previously noted that acid derivatives were better FTIs than other 2-carbonyl-containing compounds such as amides or esters[4]. Therefore we chose the Wang resin as solid support that could provide directly the expected acid by cleavage of the resin. Our thiophene derivative was linked to the Wang resin just before the Suzuki–Miyaura cross-coupling step (Scheme 3). Thus, compound **4** had to be saponified to allow subsequent linkage to the resin. Under the conditions used to obtain **1a**, compound **4** hydrolysis afforded the acid derivative **6** in quantitative yield. To attach compound **6** to the polymer, we first investigated the EDC/HOBt method involving the acid activation. Variations in coupling agent, base or acid amounts did not allow to get more than 60% of polymer-bond thiophene. This was observed whatever was the polymer substitution rate we used[25]. Increasing the reaction time did not improve the attachment yield either. Because our acid **6** might be sterically too hindered, we rather thought to activate the polymer-bond hydroxyl function to achieve linkage to the resin. As expected, such coupling was successfully achieved under Mitsunobu conditions[26], leading to a linkage rate up to 97% (calculated by elemental analysis from the sulfur ratio in the polymer). Before performing the Suzuki–Miyaura cross-coupling, we checked whether the classical cleavage method using trifluoroacetic acid (TFA) in CH_2Cl_2 would afford the expected thiophene-2-carboxylic acid. Under these conditions, no degradation or secondary products were observed and the bromo acid **6** was recovered in quantitative yield.

As previously mentioned, the Suzuki–Miyaura cross-coupling has not often been used for thiophene arylation on solid support[20–22] and, to our knowledge, it has never been described for tetrasubstitued resin-bond derivatives. Therefore the reaction of **7** with 4-chlorophenyl boronic acid had to be optimized. $Pd(PPh_3)_4$ was chosen as catalyst according to our previous study in homogenous



Scheme 2. Reagents and conditions: (a) K_2CO_3, DMF, RT, 30 min, then CS_2, RT, 30 min, then iPrI, TBAB, 60°C, 70 h, 73%; (b) $HSCH_2CO_2Et$, K_2CO_3, EtOH, reflux, 3 h, 84%; (c) *t*BuONO, $CuBr_2$, CH_3CN, RT, 10 min, 94%; (d) $Pd(PPh_3)_4$, K_2CO_3, 4-ClPhB$(OH)_2$, toluene/EtOH (9:1), reflux, 4 h, 91%; (e) NaOH 2 M, EtOH, RT, 16 h, 92%.



Scheme 3. Reagents and conditions: (a) NaOH 2 M, EtOH, RT, 16 h, quant.; (b) PPh_3, DIAD, THF, RT, 6 h, 97%; (c) $Pd(PPh_3)_4$, $ArB(OH)_2$, 110°C, 65 h; (d) TFA/CH_2Cl_2 (1:1, v/v), RT, 45 min.

medium and the palladium-catalyzed reaction was carried out with an excess of boronic acid and base. Several bases, solvents and temperatures were assayed for optimization (Table 1). The conversion rate was determined by ^{1}H NMR of the crude filtrate after TFA cleavage.

Temperature was an important factor for the reaction and below 100°C, conversion rate was very low (entries 1–7), whereas increasing temperature to reflux improved it significantly (entries 1 vs 8 and 7 vs 17). Among the solvents we used, the toluene/ethanol mixture as well as dioxane gave the best results. The amount of ethanol was varied to find the best solvent conditions. Toluene alone afforded the desired coupling compound in very low yield (data not shown). Up to 20% EtOH (entry 13), an excellent reaction rate was observed but dropped dramatically as the amount of alcohol increased (entries 14 and 15). The base efficiency was dependant on solvent as experienced with cesium salts that gave good results in dioxane (entry 19) but were ineffective in toluene/ethanol (entries 9 and 10). As well, aqueous potassium carbonate was found to be the best base both in dioxane (entry 17) and in toluene/ethanol (entry 8) but in the latter mixture, aqueous sodium carbonate and potassium phosphate were equally efficient (entries 8, 11 and 12). At that stage of our study, we found difficulties to reproduce our cross-coupling reaction in similar yields whereas conversion was complete. In such basic media, we suspected ethanol to realize transesterification on the polymer-bound compound resulting in loss of substitution rate on the resin. Therefore we changed ethanol to *tert*-butanol less prone to induce transesterification. As expected, excellent and very reproducible yields were observed in such conditions. Potassium phosphate giving the best conversion in toluene/tBuOH, the chosen conditions were $Pd(PPh_3)_4$ (10 mol%), boronic acid (5 equiv.), K_3PO_4 (5 equiv.) in toluene/*t*BuOH (9:1, v/v) at 110°C and the reaction time was increased from 24 h to 65 h to achieve complete conversion in most cases. These conditions were applied on a variety of commercially available arylboronic acids (Table 2). Conversion rates were determined by UHPLC dosing after acidic release from the resin, referring to bromo acid **6** as standard.

Table 1. Optimization of Suzuki–Miyaura cross-coupling conditions for **1a** synthesis*.

Entry	Base	Solvent	Temperature	Conversion
1	K_2CO_3†	Toluene/EtOH 10:1	90°C	26%
2	K_2CO_3†	DME	90°C	8%
3	Na_2CO_3†	THF	90°C	7%
4	Na_2CO_3†	DME	90°C	7%
5	Na_2CO_3†	Xylene/EtOH 25:1	90°C	15%
6	Na_2CO_3†	DMF	90°C	0%
7	Na_2CO_3†	Dioxane	90°C	15%
8	K_2CO_3†	Toluene/EtOH 10:1	110°C	100%
9	CsF	Toluene/EtOH 10:1	110°C	0%
10	Cs_2CO_3	Toluene/EtOH 10:1	110°C	0%
11	Na_2CO_3†	Toluene/EtOH 10:1	110°C	100%
12	K_3PO_4	Toluene/EtOH 10:1	110°C	100%
13	K_2CO_3†	Toluene/EtOH 4:1	110°C	100%
14	K_2CO_3†	Toluene/EtOH 3:1	110°C	85%
15	K_2CO_3†	Toluene/EtOH 2:1	110°C	0%
16	K_2CO_3†	Dioxane	100°C	100%
17	Na_2CO_3†	Dioxane	100°C	73%
18	K_3PO_4	Dioxane	100°C	77%
19	Cs_2CO_3	Dioxane	100°C	75%
20	K_2CO_3†	Toluene/*t*BuOH 9:1	110°C	75%
21	K_3PO_4	Toluene/*t*BuOH 9:1	110°C	100%

*Reaction conditions: $Pd(PPh_3)_4$ 10% mol, 4-ClPhB$(OH)_2$ 5 equiv., base 5 equiv., 24 h, then CH_2Cl_2/TFA (1:1, v/v).
†2 M in water.

Phenylboronic acids bearing *ortho* substituants were less reactive than their *meta* or *para* substituted analogues (entries 3, 6 and 12) or completely unreactive (entries 9 and 18) probably because of steric hindrance. Electron withdrawing groups were also detrimental to cross-coupling (entries 14–17 and 28). Thus, *p*- and *m*-carbomethoxyphenyl were successfully introduced on the thiophene ring but in moderate to low yields. Amino containing phenyl

Table 2. 3-arylthiophenes **1** prepared from Wang resin*.

Entry	Ar	Conversion	Product	Yield†
1	4-chlorophenyl	100%	**1a**	78%
2	3-chlorophenyl	100%	**1b**	69%
3	2-chlorophenyl	66%	**1c**	66%
4	4-fluorophenyl	100%	**1d**	94%
5	3-fluorophenyl	100%	**1e**	94%
6	2-fluorophenyl	36%	**1f**	33%
7	4-(trifluoromethyl)phenyl	100%	**1g**	69%
8	3-(trifluoromethyl)phenyl	99%	**1h**	86%
9	2-(trifluoromethyl)phenyl	3%	**1i**	0%
10	4-methoxyphenyl	78%	**1j**	73%
11	3- methoxyphenyl	70%	**1k**	68%
12	2- methoxyphenyl	72%	**1l**	49%
13	4- hydroxyphenyl	7%	**1m**	0%
14	4-formylphenyl	8%	**1n**	0%
15	4-acetylphenyl	9%	**1o**	0%
16	4-(methoxycarbonyl)phenyl	45%	**1p**	28%
17	3-(methoxycarbonyl)phenyl	54%	**1q**	49%
18	2-(methoxycarbonyl)phenyl	0%	**1r**	0%
19	phenyl	100%	**1s**	87%
20	4-tolyl	90%	**1t**	76%
21	3-tolyl	100%	**1u**	86%
22	2-tolyl	99%	**1v**	86%
23	4-vinylphenyl	5%	**1w**	0%
24	1-naphtyl	94%	**1x**	74%
25	2-naphtyl	100%	**1y**	80%
26	4-biphenyl	100%	**1z**	78%
27	3-biphenyl	100%	**1aa**	76%
28	4-cyanophenyl	13%	**1bb**	0%
29	4-acetamidophenyl	10%	**1cc**	0%
30	4-(dimethylamino)phenyl	6%	**1dd**	0%
31	3-pyridyl	4%	**1ee**	0%
32	3-thienyl	70%	**1ff**	44%
33	1-dibenzofuranyl	66%	**1gg**	64%

*Reaction conditions: $Pd(PPh_3)_4$ (10% mol), $ArB(OH)_2$ (5 equiv.), K_3PO_4 (5 equiv.), in toluene/*t*BuOH (9:1, v/v) at 110°C, 65 h, then CH_2Cl_2/TFA (1:1, v/v).
†Isolated yield.

boronic acids gave no reaction with bromothiophene **7** as well as 4-hydroxyphenyl boronic acid (entries 13, 15, 28-31). We did not succeed in introducing a 4-vinylphenyl group by Suzuki–Miyaura coupling either (entry 23). Such phenylboronic acids bearing electron withdrawing or amino groups have already been coupled on thiophene or benzothiophene rings following this methodology. However, these aromatics were seldom tetrasubstituted or the thiophene ring was not as electrodeficient as our substrate[27–31]. Interestingly, there is no report on palladium-catalyzed thiophene arylation with 4-vinyl phenylboronic acid. Either the vinyl moiety was introduced on the 4-iodo derivative after Suzuki coupling[32] or a rhodium catalyst was used[33].

By this method, 22 new arylthiophenes **1** were obtained in sufficient amount to evaluate their activity on protein farnesyltransferase. We previously observed that the ester forms of our FTIs were more active on parasites than their acidic forms[34]. Therefore, to evaluate the antiparasitic activity of our series, we synthesized the corresponding esters of the selected active thiophene 2-carboxylic acids **1j**, **1aa** and **1gg** (see below), according to Scheme 2. The esters **5j**, **5aa** and **5gg** were obtained from compound **4** and the corresponding boronic acids in 50%, 61% and 74% yields, respectively.

Biological evaluation

Compounds **1a-h**, **1j-l**, **1p-q**, **1s-v**, **1x-aa**, and **1ff-gg** were evaluated for their inhibitory activity against recombinant yeast[35], human[7] and *T. brucei* FTases using a fluorescent-based assay[36,37] adapted to 96-well plate format. Results are reported in Table 3.

The first observation is the difference of inhibitory activity according to FTase species. In general, arylthiophenes are much more active on yeast FTase (IC_{50} = 0.085–10 μM) than on human or trypanosomal enzymes (IC_{50} = 3–>50 μM). This was also observed with the reference compound FTI-276[38], evaluated under our assay conditions, which showed a 20-fold increased inhibitory activity on yeast enzyme relative to the human one. *Ortho* substitution is detrimental for activity in all cases (**1c**, **1f**, **1l** and **1v**) but the presence of a *para*-substituent generally improves the inhibitory activity (compare **1s** with **1a**, **1d**, **1p** and **1t**). The nature of the substituent seems to have little influence on the activity that depends on the FTase species. Bulky aromatic rings like naphtyl (**1x-y**), biphenyl (**1z-aa**) or dibenzofuranyl (**1gg**) moieties increase or at least retain the inhibitory activity against human and *Tb*-FTases with a different exception for each enzyme: the 4-biphenyl derivative **1z** for the trypanosomal protein and the 1-naphtyl analogue **1x** for the human species. On the contrary, there is a 3 to 30-fold drop in activity against the yeast enzyme for these analogues. The most active compound against *y*FTase in this series is the 4-carbomethoxyphenylthiophene (**1p**) which displays a slight increased activity (1.3 fold) compared to parent compound **1a**. For *h*FTase the 3-biphenyl derivative **1aa** exhibits the best activity together with 2-naphtyl **1y** and 4-biphenyl **1z** with a 5 and 3-fold increased activity, respectively. The gain in inhibition is less important for *Tb*-FTase with a 3-fold and 2-fold increase for **1gg** and **1aa**, respectively. However, as far as our search for antiparasitic agents is concerned, compounds **1j** and **1gg** displayed a better selectivity against the parasitic enzyme with a 4.5 ratio compared to the human one. Therefore, we decided to evaluate the antiparasitic activities of the two most active compounds on *Tb*-FTase together with the 4-methoxy derivative which display a good selectivity for the parasitic enzyme. The evaluation was realized on their ester forms **5j**, **5aa** and **5gg** against *P. falciparum* (intraerythrocytic forms) and *T. brucei* (bloodstream form) proliferation. For comparison, we also evaluated our hit compound **1a** and its ester derivative **5a**. Results are reported in Table 4.

Table 3. Inhibition of FTases by 3-arylthiophene-2-carboxylic acids **1**.

Compound	IC_{50} (μM) *y*FTase	IC_{50} (μM) *h*FTase	IC_{50} (μM) *Tb*-FTase
1a	0.11 ± 0.008	16 ± 2	10.6 ± 2
1b	1.3 ± 0.1	48 ± 9	14.5 ± 1.5
1c	1.4 ± 0.3	> 50	37 ± 8
1d	0.27 ± 0.02	20 ± 2	29 ± 8
1e	0.46 ± 0.08	23 ± 4	32 ± 5
1f	1.3 ± 0.2	> 50	46 ± 13
1g	0.58 ± 0.13	nd	15% inhat 10 μM
1h	1.6 ± 0.15	34 ± 5	19 ± 4
1j	0.25 ± 0.03	39 ± 4	8.6 ± 1.1
1k	1.6 ± 0.2	29 ± 4	18 ± 4
1l	9.7 ± 2.8	> 50	37 ± 7
1p	0.085 ± 0.016	12.8 ± 1.4	14 ± 2
1q	0.62 ± 0.07	7.0 ± 0.7	6.9 ± 0.6
1s	0.61 ± 0.04	34 ± 5	42 ± 9
1t	0.19 ± 0.04	21 ± 2	17 ± 4
1u	1.4 ± 0.2	16 ± 3	34 ± 8
1v	3.1 ± 0.8	> 50	> 50
1x	1.1 ± 0.2	32 ± 6	10.5 ± 2
1y	0.32 ± 0.05	4.8 ± 0.15	9.7 ± 1.7
1z	2.7 ± 0.5	5.5 ± 0.6	32 ± 6
1aa	0.64 ± 0.06	3.3 ± 0.2	5.4 ± 0.9
1ff	0.35 ± 0.07	31 ± 5	26 ± 7
1gg	1.0 ± 0.2	15 ± 1.3	3.3 ± 0.4
FTI-276	0.00078 ± 0.00008	0.015 ± 0.004	0.010 ± 0.002

Table 4. Inhibition of *P. falciparum* and *T. brucei* proliferation with selected 3-arylthiophenes*.

Compound	IC_{50} *P. falciparum* (μM)	IC_{50} *T. brucei* (μM)
1a	71†	Inactive at 10 μM
5a	40†	14 ± 0.9
5j	27 ± 0.7	7.0 ± 0.2
5aa	30 ± 0.9	15 ± 0.3
5gg	15 ± 0.9	11 ± 0.9
Pentamidine	–	0.011 ± 0.0017
Chloroquine	0.072 ± 0.0074	–

*Otherwise indicated IC_{50} were expressed as the mean values ± standard deviations determined from at least three independent experiments.
†Determined from two experiments.

As predicted, ester **5a** is more active than the free carboxylic acid **1a**. The improved enzyme inhibition led only to a slight augmentation of the antiparasitic activity. It is to note that these arylthiophenes are more active against *T. brucei* than against *P. falciparum* but their IC_{50} remains in the same micromolar range whatever the aryl moiety may be. However, the methoxy derivative **5j** is the most active compound against *T. brucei* although its *Tb*-FTase inhibition is equivalent to that of our hit compound. It can be assumed that the presence of an additional oxygen atom on these hydrophobic molecules could improve cell penetration and/or aqueous solubility.

Conclusion

We designed an original method to rapidly obtain a small library of 3-aryl-4-cyano-5-(isopropylthio)thiophen-2-carboxylic acids. This method using Suzuki–Miyaura cross-coupling has successfully been employed for the first time on resin-bond tetrasubstitued thiophenes to afford 22 new arylthiophenes **1** in sufficient yield to evaluate their activity on FTase. However it is not applicable to arylthiophene derivatives bearing electron withdrawing groups, hydroxyl or amino moieties or hindered ortho substituants. For these derivatives, the synthesis via arylpropionitrile[4] was more appropriate and will be reported soon. Evaluation of the inhibitory activity of these new 3-arylthiophenes has shown that it is greatly dependent on the FTase species, showing a 2-order of magnitude difference between yeast and human or trypanosomal enzymes. For them all, *para* or *meta* substitution is preferred to nude phenyl group, substitution at the *ortho* position being a less favorable pattern for FTase inhibition. Finally, we succeeded in improving the FTase inhibitory activity of our 3-arylthiophene derivatives by introducing either a carbomethoxy group in the *para* position of the phenyl ring for the yeast enzyme (allowing IC_{50} to go below 100 nM), or a phenyl in the *meta* or *para* position for the human protein and by replacing the *p*-chlorophenyl group by a *m*-biphenyl or benzofuranyl moiety for *Tb*-FTase. As we are aiming to find selective inhibitors of the parasitic enzyme, the *para*-methoxyphenyl and benzofuranyl derivatives seemed promising because of their 4.5 ratio between human and trypanosomal FTase inhibitory activity. These compounds are also more active on *T. brucei* proliferation, though to a small extent. Because of the high arylthiophene hydrophobicity, cell penetration may be a problem for these compounds. Therefore, we thought to add oxygen atoms on these molecules to increase cellular activity what is under current investigation and will be reported soon.

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Declaration of interest

This work was financially supported by ICSN and CNRS. The authors report no conflicts of interest.

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