

Supporting Information

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

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SYNTHESIS

General methods

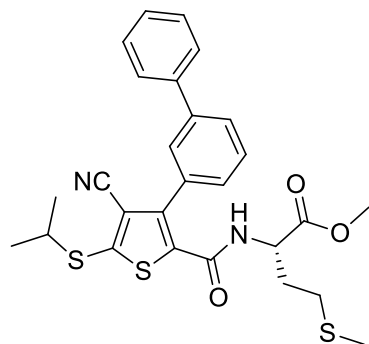
Unless otherwise indicated, all reactions were carried out with magnetic stirring and in case of air-sensitive compounds reactions were carried out in oven-dried glassware under argon. Commercial compounds were used without any further purification. Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone. Dichloromethane (CH₂Cl₂), triethylamine (Et₃N), diisopropylamine and toluene were distilled over calcium hydride. N,N'-dimethylformamide (DMF) was dried over MgSO₄ followed by distillation under reduced pressure.

Analytical thin-layer chromatography was carried out on precoated silica gel aluminium plates (SDS TLC plates, silica gel 60F₂₅₄). Column chromatography was performed prepacked Redisep columns. Preparative TLC (PLC) was performed on Merck TLC with silica gel 60F₂₅₄.

NMR spectra (¹H and ¹³C) were recorded on a Bruker Avance 300 (300 MHz) and Avance 500 (500 MHz). Chemical shifts (δ) are given in ppm relative to CDCl₃ (7.26 ppm; 77.2 ppm), CD₃OD (3.34 ppm; 49.9 ppm), acetone-d₆ (2.05 ppm; 30.5 ppm) or DMSO-d₆ (2.50 ppm; 39.5 ppm). Splitting patterns are designed 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 on a Perkin-Elmer Spectrum BX. Mass spectra were recorded on ThermoFinnigan Automass with a quadripole detection (IE) and on Thermoquest AQA Navigator with a TOF detection (ESI-HRMS). UHPLC analyses were realized on Waters Acquity UPLC. Elemental analyses were performed by the Microanalytical Laboratory of the ICSN-Gif-sur-Yvette. Melting points were measured on Büchi b-450 and are uncorrected.

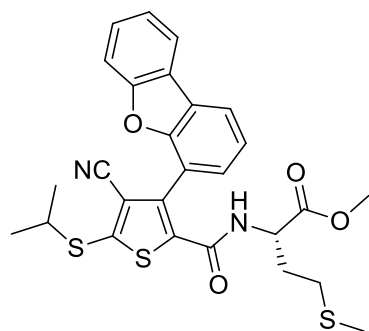
The purity of all target compounds was measured using reversed-phase UHPLC (HSS C-18, 2.1×50 mm s-1.8 μ m): compounds were eluted with 95:5 A/B for 0.5 min then with a gradient of 5-100% B/A for 3.5 min followed by 0:100 isocratic for 1 min at a flow rate of 0.6 mL/min, where solvent A was 0.1% formic acid in H₂O and solvent B was 0.1% formic acid in CH₃CN. Purity was determined on TAC (total absorbance current from 200 to 400 nm).

Chemistry



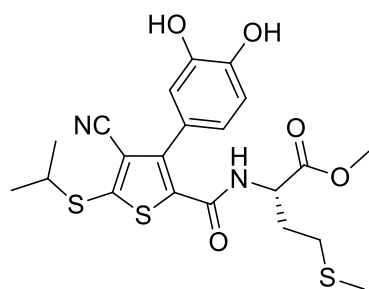
(S)-methyl 2-(3-(biphenyl-3-yl)-4-cyano-5-(isopropylthio)thiophene-2-carboxamido)-4-(methylthio)butanoate (**11**)

To a solution (DMF, 0.3 mL) of methionine methyl ester (0.022 g, 0.11 mmol, 1.0 equiv.) and thiophene **3** (0.042 g, 0.11 mmol, 1.0 equiv.) were added HBTU (0.042 g, 0.11 mmol, 1.2 equiv.), HOBt (0.017 g, 0.11 mmol, 1.0 equiv.) and N,N-diisopropylethylamine (38.0 μ L, 0.22 mmol, 2.0 equiv.). The reaction was stirred at room temperature overnight. DMF was evaporated under reduced pressure. The residue was diluted in CH₂Cl₂ and washed once with a 5% citric acid solution, once with water, once with 5% Na₂CO₃ solution, once with water and once with brine. The organic phase was dried over MgSO₄ and concentrated under reduced pressure. The purification of the crude product was performed by flash chromatography on silica gel (heptane/EtOAc 10/0 to 40/60 in 25 min) to afford **11** as a white amorphous solid (0.037 g, 64%). IR (film, ν , cm⁻¹) 3399, 2966, 2922, 2223, 1741, 1645, 1530, 1506, 1436, 1368, 1199, 1170, 1058, 760, 719, 698. ¹H NMR (300 MHz, CDCl₃) δ 1.49 (d, 6H, J = 6.6 Hz), 1.77 (m, 1H), 1.92 (s, 3H), 1.95 (m, 1H), 2.15 (m, 2H), 3.62 (s, 3H), 3.63 (h, 1H, J = 6.6 Hz), 4.66 (m, 1H), 6.18 (d, 1H, J = 7.8 Hz), 7.40-7.82 (m, 9H). ¹³C NMR (75 MHz, CDCl₃) δ 15.3, 23.2, 29.4, 31.9, 42.4, 51.9, 52.5, 113.5, 115.6, 127.3, 127.9, 128.0, 128.0, 128.9, 130.0, 130.1, 132.1, 136.1, 139.7, 142.8, 143.1, 152.4, 159.8, 171.2. MS (ESI⁺, MeOH/CH₂Cl₂) m/z 525.1 [M+H]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) m/z calcd for C₂₇H₂₉N₂O₃S₃⁺ [M+H]⁺ 525.1340, found 525.1348. UHPLC 6.33 min, 90%. $[\alpha]_D^{20}$ = -7.4° (MeOH, 0.68).



(S)-methyl 2-(4-cyano-3-(dibenzo[b,d]furan-4-yl)-5-(isopropylthio)thiophene-2-carboxamido)-4-(methylthio)butanoate (12)

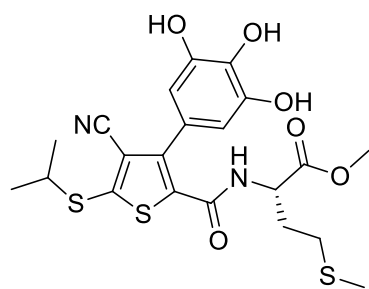
To a solution (CH_2Cl_2 , 1.1 mL) of methionine methyl ester (0.083 g, 0.41 mmol, 1.0 equiv.) and thiophene **4** (0.163 g, 0.41 mmol, 1.0 equiv.) were added EDCI (0.095 g, 0.50 mmol, 1.2 equiv.), HOBt (0.127 g, 0.83 mmol, 2.0 equiv.) and N-methylmorpholine (45.0 μL , 0.41 mmol, 1.0 equiv.). The reaction was stirred at room temperature during 29 h. The reaction mixture was washed once with a 5% citric acid solution, once with water, once with 5% Na_2CO_3 solution, once with water and once with brine. The organic phase was dried over MgSO_4 and concentrated under reduced pressure. The purification of the crude product was performed by flash chromatography on silica gel (heptane/EtOAc 10/0 to 5/5 in 25 min) to afford **12** as a white amorphous solid (0.138 g, 62%). IR (film, ν , cm^{-1}) 3358, 2962, 2921, 2225, 1740, 1645, 1528, 1511, 1450, 1415, 1369, 1188, 1052, 846, 754. ^1H NMR (300 MHz, MeOD) δ 1.45 (d, 6H, $J = 6.6$ Hz), 1.60 (m, 2H), 1.78 (s, 3H), 1.92 (m, 2H), 3.49 (s, 3H), 3.66 (h, 1H, $J = 6.6$ Hz), 4.41 (m, 1H), 7.40-7.65 (m, 5H), 8.08-8.21 (m, 2H). ^{13}C NMR (75 MHz, MeOD) δ 15.2, 23.2, 29.2, 31.3, 42.5, 51.8, 52.4, 112.0, 113.4, 115.6, 116.0, 121.0, 122.9, 123.5, 123.6, 123.8, 125.7, 127.9, 128.1, 136.9, 137.6, 152.2, 153.1, 156.2, 159.8, 170.9. MS (ESI^+ , MeOH/ CH_2Cl_2) m/z 561.1 $[\text{M}+\text{Na}]^+$. HRMS (ESI^+ , MeOH/ CH_2Cl_2) m/z calcd for $\text{C}_{27}\text{H}_{26}\text{N}_2\text{O}_4\text{S}_3\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 561.0952, found 561.0949. UHPLC 6.19 min, 93%. $[\alpha]_D^{20} = -25.0^\circ$ (MeOH, 0.20).



(S)-methyl 2-(4-cyano-3-(3,4-dihydroxyphenyl)-5-(isopropylthio)thiophene-2-carboxamido)-4-(methylthio)butanoate (13)

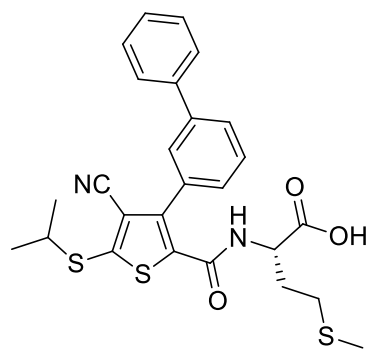
To a solution (CH_2Cl_2 , 0.3 mL) of methionine methyl ester (0.023 g, 0.12 mmol, 1.0 equiv.) and thiophene **5** (0.039 g, 0.12 mmol, 1.0 equiv.) were added EDCI (0.027 g, 0.14 mmol, 1.2 equiv.), HOBt (0.035 g, 0.23 mmol, 2.0 equiv.) and N-methylmorpholine (13.0 μL , 0.12 mmol, 1.0 equiv.). The reaction was stirred at room temperature during 36 h. The reaction mixture was extracted three times with CH_2Cl_2 . The organic phases were pooled, dried over MgSO_4 and concentrated under reduced pressure. The purification of the crude product was performed by flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 10/0 to 98/2 in 20 min) to

afford **13** as an off-white oil (0.018 g, 33%). IR (film, ν , cm^{-1}) 3370, 2966, 2913, 2226, 1740, 1626, 1602, 1538, 1496, 1437, 1281, 1174, 1114, 1041, 734. ^1H NMR (300 MHz, CDCl_3) δ 1.37 (d, 6H, $J = 6.6$ Hz), 1.75 (m, 1H), 1.94 (s, 3H), 2.19 (t, 2H, $J = 7.2$ Hz), 3.52 (h, 1H, $J = 6.6$ Hz), 3.63 (s, 3H), 4.63 (m, 1H), 6.30 (d, 1H, $J = 7.8$ Hz), 6.84-6.97 (m, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ 15.4, 23.2, 29.6, 31.2, 42.2, 51.9, 52.8, 113.7, 116.3, 116.5, 116.6, 120.2, 122.1, 123.1, 143.3, 144.5, 146.2, 152.6, 160.3, 171.8. MS (ESI^+ , $\text{MeOH}/\text{CH}_2\text{Cl}_2$) m/z 481.1 $[\text{M}+\text{H}]^+$. HRMS (ESI^+ , $\text{MeOH}/\text{CH}_2\text{Cl}_2$) m/z calcd for $\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_5\text{S}_3^+$ $[\text{M}+\text{H}]^+$ 481.0926, found 481.0919. UHPLC 4.88 min, 90%. $[\alpha]_D^{20} = -11.9^\circ$ (MeOH, 0.42).



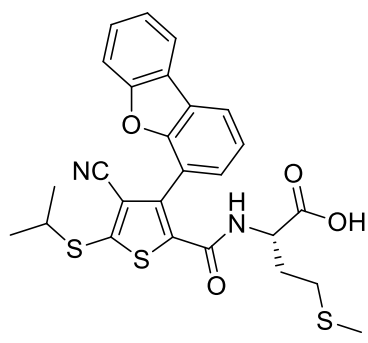
(S)-methyl 2-(4-cyano-5-(isopropylthio)-3-(3,4,5-trihydroxyphenyl)thiophene-2-carboxamido)-4-(methylthio)butanoate (14)

To a solution (DMF, 0.7 mL) of methionine methyl ester (0.049 g, 0.25 mmol, 1.0 equiv.) and thiophene **6** (0.087 g, 0.25 mmol, 1.0 equiv.) were added HBTU (0.094 g, 0.25 mmol, 1 equiv.), HOBt (0.038 g, 0.25 mmol, 1.0 equiv.) and diisopropylamine (85.0 μL , 0.50 mmol, 2.0 equiv.). The reaction was stirred at room temperature during 24 h. DMF was evaporated under reduced pressure. The residue was diluted in CH_2Cl_2 and extracted three times with CH_2Cl_2 . The organic phases were pooled, dried over MgSO_4 and concentrated under reduced pressure. The purification of the crude product was performed by flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 10/0 to 98/2 in 20 min) to afford **14** as a yellow oil (0.059 g, 48%). IR (film, ν , cm^{-1}) 3365, 2952, 2231, 1739, 1602, 1529, 1438, 1309, 1189, 1036, 732. ^1H NMR (300 MHz, CDCl_3) δ 1.36 (d, 6H, $J = 6.6$ Hz), 1.77 (m, 1H), 1.95 (s, 3H), 1.98 (m, 1H), 2.22 (t, 2H, $J = 7.2$ Hz), 3.51 (h, 1H, $J = 6.6$ Hz), 3.63 (s, 3H), 4.64 (m, 1H), 6.44 (d, 1H, $J = 7.8$ Hz), 6.54 (s, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 15.4, 23.2, 29.6, 31.1, 42.3, 52.0, 52.9, 109.3, 113.6, 115.0, 122.6, 133.8, 135.1, 143.3, 145.1, 152.8, 160.3, 171.9. MS (ESI^+ , $\text{MeOH}/\text{CH}_2\text{Cl}_2$) m/z 497.1 $[\text{M}+\text{H}]^+$. HRMS (ESI^+ , $\text{MeOH}/\text{CH}_2\text{Cl}_2$) m/z calcd for $\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_6\text{S}_3^+$ $[\text{M}+\text{H}]^+$ 497.0875, found 497.0862. UHPLC 4.50 min, 93%. $[\alpha]_D^{20} = -20.8^\circ$ (MeOH, 0.30).



(S)-2-(3-(biphenyl-3-yl)-4-cyano-5-(isopropylthio)thiophene-2-carboxamido)-4-(methylthio)butanoic acid (15)

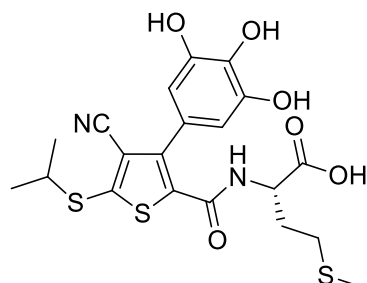
To a solution (THF/EtOH 2/1, 0.37 mL) of **11** (0.027 g, 0.05 mmol, 1.0 equiv.) was added aqueous NaOH (2M, 0.25 mL). After stirring 72 h at room temperature, the mixture was neutralized with aqueous HCl (1M, 0.6 mL) and extracted two times with CH₂Cl₂. The organic layers were pooled, dried over MgSO₄ and concentrated under reduced pressure. The purification of the crude product was performed by chromatography on preparative plate (CH₂Cl₂/MeOH/HCOOH 95/5/0.4) to afford **15** as a white amorphous solid (0.016 g, 62%). IR (film, ν , cm⁻¹) 3393, 2971, 2918, 2223, 1598, 1532, 1400, 1362, 1242, 1021, 759, 697. ¹H NMR (300 MHz, MeOD) δ 1.33 (d, 6H, J = 6.6 Hz), 1.66 (m, 1H), 1.79 (s, 3H), 1.88 (m, 1H), 1.99 (m, 2H), 3.51 (h, 1H, J = 6.6 Hz), 4.36 (m, 1H), 7.25-7.65 (m, 9H). ¹³C NMR (75 MHz, MeOD) δ 15.2, 23.5, 30.8, 32.1, 43.8, 54.0, 114.7, 117.4, 128.3, 128.9, 129.0, 129.3, 129.5, 130.0, 130.9, 133.8, 137.7, 141.5, 143.6, 144.8, 151.3, 162.5, 171.3. MS (ESI⁺, MeOH/CH₂Cl₂) m/z 511.1 [M+H]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) m/z calcd for C₂₆H₂₇N₂O₃S₃⁺ [M+H]⁺ 511.1184, found 511.1195. UHPLC 5.73 min, 60%. [α]_D²⁰ = -29.4° (MeOH, 0.68).



(S)-2-(4-cyano-3-(dibenzo[b,d]furan-4-yl)-5-(isopropylthio)thiophene-2-carboxamido)-4-(methylthio)butanoic acid (16)

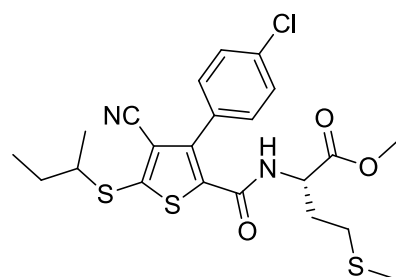
To a solution (THF/EtOH 2/1, 0.41 mL) of **12** (0.029 g, 0.05 mmol, 1.0 equiv.) was added aqueous NaOH (2M, 0.27 mL). After stirring 72 h at room temperature, the mixture was neutralized with aqueous HCl (1M, 0.6 mL) and extracted two times with CH₂Cl₂. The organic layers were pooled, dried over MgSO₄ and concentrated under reduced pressure. The purification of the crude product was performed by chromatography on preparative plate (CH₂Cl₂/MeOH/HCOOH 95/5/0.4) to afford **16** as a white amorphous solid (0.028 g, quantitative yield). IR (film, ν , cm⁻¹) 2957, 2920, 2226, 1732, 1631, 1533, 1446, 1415, 1188, 1052, 846, 753. ¹H NMR (300 MHz, MeOD) δ 1.35 (d, 6H, J = 6.6 Hz), 1.50 (m, 2H), 1.66 (s, 3H), 1.84 (m, 2H), 3.55 (h, 1H, J = 6.6 Hz), 4.26 (m, 1H), 7.29-7.54 (m, 5H), 7.96-8.10 (m,

2H). ^{13}C NMR (75 MHz, MeOD) δ 15.0, 23.5, 30.7, 31.8, 43.8, 53.4, 112.9, 114.3, 117.4, 117.6, 122.2, 123.6, 124.6, 124.7, 125.1, 126.6, 129.1, 129.3, 138.9, 138.9, 151.7, 154.5, 157.6, 162.5, 173.8. MS (ESI⁺, MeOH/CH₂Cl₂) m/z 525.1 [M+H]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) m/z calcd for C₂₆H₂₅N₂O₄S₃⁺ [M+H]⁺ 525.0976, found 525.0990. UHPLC 5.58 min, 90%. $[\alpha]_D^{20} = -31.9^\circ$ (MeOH, 0.94).



(S)-2-(4-cyano-5-(isopropylthio)-3-(3,4,5-trihydroxyphenyl)thiophene-2-carboxamido)-4-(methylthio)butanoic acid (17)

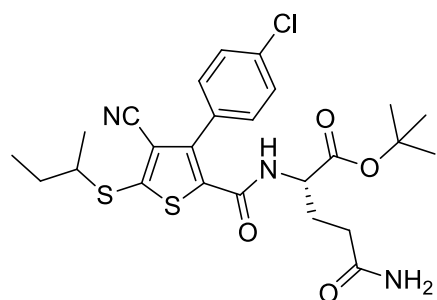
Thiophene **14** (0.013 g, 0.003 mmol, 1.0 equiv.) was added to a solution of KF/Al₂O₃ (0.039 g, 40 wt% KF) in THF (0.3 mL). The reaction mixture was irradiated five times under microwaves condition at 300 W during 30 minutes. After cooling, water was added to the solid and stirred for 20 min. The mixture was filtered and washed with water. The filtrate was neutralized by addition of aqueous HCl. The organic phases were pooled, dried over MgSO₄ and concentrated under reduced pressure. The purification of the crude product was performed by chromatography on preparative plate (CH₂Cl₂/MeOH/HCOOH 95/5/0.4) to afford **17** as a brown oil (0.003 g, 23%). IR (film, ν , cm⁻¹) 3345, 2971, 2918, 2226, 1604, 1537, 1441, 1377, 1240, 1197, 1045, 873. ^1H NMR (500 MHz, acetone-d₆) δ 1.44 (d, 6H, $J = 6.5$ Hz), 1.87 (m, 1H), 2.05 (s, 3H), 2.08 (m, 1H), 2.35 (t, 2H, $J = 7.0$ Hz), 3.41 (h, 1H, $J = 6.5$ Hz), 4.53 (m, 1H), 6.56 (s, 2H), 6.90 (m, 1H). MS (ESI⁺, MeOH/CH₂Cl₂) m/z 481.1 [M-H]⁻. HRMS (ESI⁻, MeOH/CH₂Cl₂) m/z calcd for C₂₀H₂₁N₂O₆S₃⁻ [M-H]⁻ 481.0562, found 481.0544. UHPLC 4.04 min, 95%. $[\alpha]_D^{20} = -6.3^\circ$ (MeOH, 0.80).



(S)-methyl 2-(5-(sec-butylthio)-3-(4-chlorophenyl)-4-cyanothiophene-2-carboxamido)-4-(methylthio)butanoate (19)

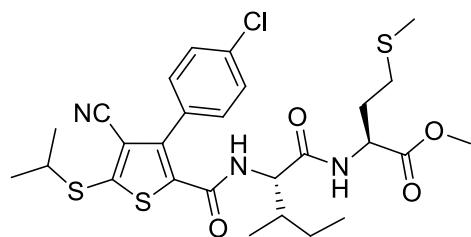
To a solution (CH₂Cl₂, 2.0 mL) of methionine methyl ester (0.144 g, 0.72 mmol, 1.0 equiv.) and **18** (0.253 g, 0.72 mmol, 1.0 equiv.) were added EDCI (0.165 g, 0.86 mmol, 1.2 equiv.), HOBT (0.220 g, 1.44 mmol, 2.0 equiv.) and N-methylmorpholine (79.0 μL , 0.72 mmol, 1.0 equiv.). The reaction was stirred at room temperature overnight. The reaction mixture was diluted in CH₂Cl₂ and washed once with a 5% citric acid solution, once with water, once with 5% Na₂CO₃ solution, once with water and once with brine. The organic phase was dried over MgSO₄ and concentrated under reduced pressure. The purification of the crude product was

performed by flash chromatography on silica gel (heptane/EtOAc 10/0 to 5/5 in 25 min) to afford **19** as a white amorphous solid (0.243 g, 68%). IR (film, ν , cm^{-1}) 3300, 2964, 2922, 2219, 1739, 1681, 1663, 1527, 1484, 1435, 1356, 1267, 1202, 1174, 1090, 1014, 865, 828, 761, 672. ^1H NMR (300 MHz, CDCl_3) δ 1.10 (t, 3H, $J = 7.5$ Hz), 1.47 (d, 3H, $J = 6.6$ Hz), 1.80 (qd, 2H, $J = 7.2, 7.5$ Hz), 1.90 (m, 1H), 2.05 (s, 3H), 2.06 (m, 1H), 2.28 (t, 2H, $J = 7.2$ Hz), 3.42 (tq, 1H, $J = 6.6, 7.2$ Hz), 3.71 (s, 3H), 4.70 (m, 1H), 6.10 (d, 1H, $J = 7.8$ Hz), 7.46 (d, 2H, $J = 8.4$ Hz), 7.58 (d, 2H, $J = 8.4$ Hz). ^{13}C NMR (75 MHz, CDCl_3) δ 11.3, 15.5, 20.6, 29.6, 29.7, 31.1, 49.1, 52.0, 52.6, 113.4, 115.1, 129.9, 130.7, 130.8, 135.8, 136.6, 141.8, 153.0, 159.7, 171.2. MS (ESI⁺, MeOH/ CH_2Cl_2) m/z 519.1 $[\text{M}+\text{Na}]^+$. HRMS (ESI⁺, MeOH/ CH_2Cl_2) m/z calcd for $\text{C}_{22}\text{H}_{25}^{35}\text{ClN}_2\text{O}_3\text{S}_3\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 519.0614, found 519.0612. UHPLC 6.21 min, 96%. $[\alpha]_D^{20} = -8.9^\circ$ (MeOH, 0.56).



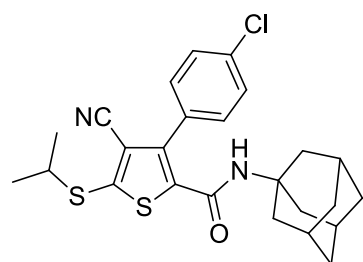
(S)-tert-butyl 5-amino-2-(5-(sec-butylthio)-3-(4-chlorophenyl)-4-cyanothiophene-2-carboxamido)-5-oxopentanoate (20)

To a solution (CH_2Cl_2 , 0.8 mL) of glutamine *tert*-butyl ester (0.063 g, 0.31 mmol, 1.0 equiv.) and **18** (0.109 g, 0.31 mmol, 1.0 equiv.) were added EDCI (0.071 g, 0.37 mmol, 1.2 equiv.), HOBt (0.095 g, 0.62 mmol, 2.0 equiv.) and *N*-methylmorpholine (34.0 μL , 0.31 mmol, 1.0 equiv.). The reaction was stirred at room temperature overnight. The reaction mixture was diluted in CH_2Cl_2 and washed once with a 5% citric acid solution, once with water, once with 5% Na_2CO_3 solution, once with water and once with brine. The organic phase was dried over MgSO_4 and concentrated under reduced pressure. The purification of the crude product was performed by flash chromatography on silica gel (heptane/EtOAc 10/0 to 5/5 in 20 min) to afford **20** as a white amorphous solid (0.114 g, 69%). IR (film, ν , cm^{-1}) 3361, 3180, 2972, 2928, 2226, 1729, 1651, 1532, 1485, 1451, 1368, 1285, 1248, 1152, 1091, 1015, 829, 738. ^1H NMR (300 MHz, CDCl_3) δ 1.08 (t, 3H, $J = 7.5$ Hz), 1.42 (s, 9H), 1.44 (d, 3H, $J = 6.6$ Hz), 1.74 (qd, 2H, $J = 7.2, 7.5$ Hz), 2.12 (m, 2H), 2.16 (m, 2H), 3.40 (tq, 1H, $J = 6.6, 7.2$ Hz), 4.43 (m, 1H), 5.58 (br s, 1H), 6.01 (br s, 1H), 6.36 (d, 1H, $J = 7.8$ Hz), 7.43 (d, 2H, $J = 8.4$ Hz), 7.53 (d, 2H, $J = 8.4$ Hz). ^{13}C NMR (75 MHz, CDCl_3) δ 11.3, 20.6, 27.9, 28.3, 29.7, 31.4, 49.2, 53.0, 83.0, 113.5, 115.3, 129.8, 130.7, 135.8, 136.3, 142.0, 152.5, 160.2, 169.8, 174.0. MS (ESI⁺, MeOH/ CH_2Cl_2) m/z 558.1 $[\text{M}+\text{Na}]^+$. HRMS (ESI⁺, MeOH/ CH_2Cl_2) m/z calcd for $\text{C}_{25}\text{H}_{30}^{35}\text{ClN}_3\text{O}_4\text{S}_2\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 558.1264, found 558.1259. UHPLC 5.65 min, 91%. $[\alpha]_D^{20} = -5.0^\circ$ (MeOH, 1.00).



(S,S)-methyl 8-benzyl-11-isopropyl-15,15-dimethyl-7,10,13-trioxo-14-oxa-2-thia-6,9,12-triazahexadecane-5-carboxylate-11-oxa-2-thia-6,9-diazatridecane-5-carboxylate (21)

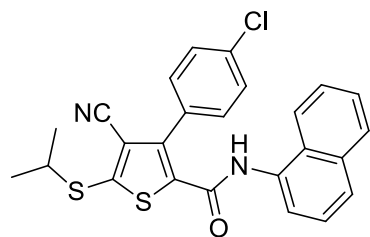
The dipeptide Ile-Met-OMe¹ (0.084 g, 0.22 mmol, 1.0 equiv.) was solubilized in trifluoroacetic acid (0.6 mL) and CH₂Cl₂ (0.6 mL). The reaction mixture was stirred during 40 minutes at room temperature. Et₂O was added to the reaction mixture and concentrated under reduced pressure until to obtain a solid. To a solution (CH₂Cl₂, 0.59 mL) of the deprotected dipeptide and thiophene **1** (0.075 g, 0.22 mmol, 1.0 equiv.) were added EDCI (0.051 g, 0.27 mmol, 1.2 equiv.), HOBT (0.068 g, 0.44 mmol, 2.0 equiv.) and N-methylmorpholine (24.0 μL, 0.22 mmol, 1.0 equiv.). The reaction was stirred at room temperature overnight. The reaction mixture was diluted in CH₂Cl₂ and washed once with a 5% citric acid solution, once with water, once with 5% Na₂CO₃ solution, once with water and once with brine. The organic phase was dried over MgSO₄ and concentrated under reduced pressure to afford **21** as a yellow amorphous solid (0.092 g, 70%). IR (film, ν, cm⁻¹) 3293, 3221, 2965, 2918, 2227, 1743, 1667, 1627, 1538, 1488, 1436, 1209, 1171, 1090, 1015, 877, 833. ¹H NMR (500 MHz, CDCl₃) δ 0.78 (d, 3H, *J* = 6.5 Hz), 0.81 (t, 3H, *J* = 7.5 Hz), 1.47 (d, 6H, *J* = 6.5 Hz), 1.64 (m, 2H), 2.00 (m, 1H), 2.08 (s, 3H), 2.15 (m, 1H), 2.50 (t, 2H, *J* = 7.5 Hz), 3.62 (m, 1H), 3.77 (s, 3H), 4.25 (m, 1H), 4.67 (m, 1H), 5.93 (d, 1H, *J* = 8.5 Hz), 7.44 (d, 2H, *J* = 8.0 Hz), 7.57 (d, 2H, *J* = 8.0 Hz). ¹³C NMR (75 MHz, CDCl₃) δ 11.3, 15.3, 15.5, 23.1, 24.6, 29.9, 31.3, 37.3, 42.4, 51.5, 52.6, 58.3, 113.3, 115.5, 130.0, 130.1, 130.6, 136.5, 136.6, 141.7, 152.2, 160.0, 169.9, 172.0. MS (ESI⁺, MeOH/CH₂Cl₂) *m/z* 618.1 [M+Na]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) *m/z* calcd for C₂₇H₃₄³⁵CIN₃O₄S₃Na⁺ [M+Na]⁺ 618.1298, found 618.1310. UHPLC 6.15 min, 93%. [α]_D²⁰ = -10.4° (MeOH, 0.96).



N-(adamantan-1-yl)-3-(4-chlorophényl)-4-cyano-5-(isopropylthio)thiophène-2-carboxamide (22)

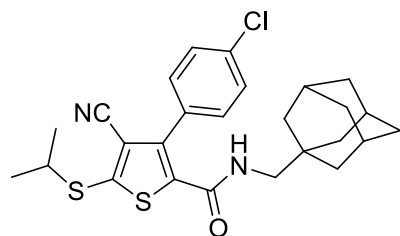
Thiophene **1** (0.118 g, 0.35 mmol, 1.0 equiv.) was solubilized in thionyle chloride (0.25 mL, 3.49 mmol, 10 equiv.). One drop of DMF was added to the solution which was stirred 5 h at reflux. Solvents are evaporated and residue was solubilized in dry CH₂Cl₂ (0.4 mL) under argon. 1-adamantanamine (0.063 g, 0.42 mmol, 1.2 equiv.) and pyridine (0.071 mL, 0.87 mmol, 2.5 equiv.) were added to the solution. The reaction mixture was stirred 46 h at room temperature and then evaporated under reduced pressure. The residue was purified by flash

chromatography on silica gel (heptane/CH₂Cl₂ 10/0 to 5/5 in 20 min) to afford **22** as a yellow amorphous solid (0.045 g, 27%). IR (film, ν , cm⁻¹) 3404, 2962, 2905, 2856, 2227, 1653, 1535, 1518, 1487, 1357, 1301, 1090, 1038, 1013, 880, 836, 744. ¹H NMR (500 MHz, CDCl₃) δ 1.45 (d, 6H, J = 7.0 Hz), 1.63 (m, 6H), 1.76 (s, 6H), 2.03 (s, 3H), 3.59 (h, 1H, J = 7.0 Hz), 5.12 (br s, 1H), 7.41 (d, 2H, J = 8.5 Hz), 7.56 (d, 2H, J = 8.5 Hz). ¹³C NMR (75 MHz, CDCl₃) δ 23.2, 29.3, 36.1, 41.2, 42.4, 52.9, 113.6, 115.5, 129.7, 130.2, 130.8, 136.4, 139.5, 140.3, 151.0, 158.8. MS (ESI⁺, MeOH/CH₂Cl₂) m/z 493.1 [M+Na]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) m/z calcd for C₂₅H₂₇³⁵ClN₂OS₂Na⁺ [M+Na]⁺ 493.1151, found 493.1154. UHPLC 7.21 min, 100%.



3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)-N-(naphthalen-1-yl)thiophene-2-carboxamide (23)

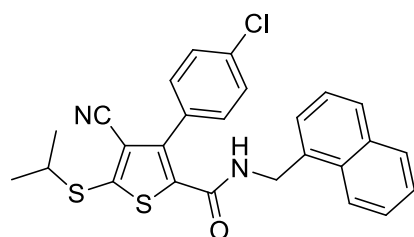
Thiophene **1** (0.129 g, 0.38 mmol, 1.0 equiv.) was solubilized in thionyle chloride (0.28 mL, 3.83 mmol, 10 equiv.). One drop of DMF was added to the solution which was stirred 5 h at reflux. Solvents are evaporated and residue was solubilized in dry CH₂Cl₂ (0.5 mL) under argon. 1-naphthalenamine (0.066 g, 0.46 mmol, 1.2 equiv.) and pyridine (0.077 mL, 0.96 mmol, 2.5 equiv.) were added to the solution. The reaction mixture was stirred 46 h at room temperature and then evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (heptane/CH₂Cl₂ 10/0 to 5/5 in 20 min) to afford **23** as a yellow amorphous solid (0.117 g, 66%). IR (film, ν , cm⁻¹) 3414, 2968, 2918, 2221, 1650, 1535, 1499, 1482, 1399, 1366, 1347, 1263, 1089, 1052, 1015, 901, 832, 804, 775, 736. ¹H NMR (300 MHz, CDCl₃) δ 1.42 (d, 6H, J = 6.6 Hz), 3.59 (h, 1H, J = 6.6 Hz), 6.22 (d, 1H, J = 8.7 Hz), 7.24-8.20 (m, 11H). ¹³C NMR (75 MHz, CDCl₃) δ 23.2, 42.4, 113.3, 115.2, 118.4, 118.6, 125.1, 125.5, 125.8, 126.1, 126.2, 128.9, 130.1, 130.6, 131.1, 131.5, 133.8, 137.2, 137.8, 140.9, 153.3, 158.2. MS (ESI⁺, MeOH/CH₂Cl₂) m/z 485.0 [M+Na]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) m/z calcd for C₂₅H₁₉³⁵ClN₂OS₂Na⁺ [M+Na]⁺ 485.0525, found 485.0549. UHPLC 6.58 min, 87%.



N-(adamantan-1-ylmethyl)-3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carboxamide (24)

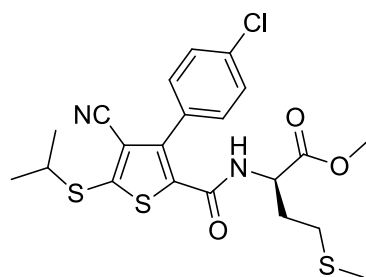
Thiophene **1** (0.148 g, 0.44 mmol, 1.0 equiv.) was solubilized in thionyle chloride (0.32 mL, 4.37 mmol, 10 equiv.). One drop of DMF was added to the solution that was stirred 5 h at reflux. Solvents are evaporated and residue was solubilized in dry CH₂Cl₂ (0.3 mL) under argon. Methyl-1-adamantanamine (0.087 g, 0.52 mmol, 1.2 equiv.) and pyridine (0.088 mL,

1.09 mmol, 2.5 equiv.) were added to the solution. The reaction mixture was stirred 20 h at room temperature and then evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (heptane/EtOAc 10/0 to 3/1 in 20 min) to afford **24** as an orange amorphous solid (0.184 g, 87%). IR (film, ν , cm^{-1}) 3418, 2897, 2848, 2232, 1639, 1530, 1509, 1484, 1364, 1270, 1088, 1012, 878, 744. ^1H NMR (300 MHz, acetone- d_6) δ 1.09 (d, 6H, $J = 3.0$ Hz), 1.29 (d, 6H, $J = 6.6$ Hz), 1.50 (m, 6H), 1.75 (s, 3H), 2.73 (d, 2H, $J = 6.0$ Hz), 3.51 (h, 1H, $J = 6.6$ Hz), 6.04 (d, 1H, $J = 6.0$ Hz), 7.47 (d, 2H, $J = 8.4$ Hz), 7.54 (d, 2H, $J = 8.4$ Hz). ^{13}C NMR (75 MHz, acetone- d_6) δ 23.4, 29.2, 34.2, 37.5, 40.6, 43.2, 52.1, 114.1, 117.2, 130.5, 132.3, 136.4, 139.9, 141.5, 149.7, 160.7. MS (ESI $^+$, MeOH/ CH_2Cl_2) m/z 507.1 $[\text{M}+\text{Na}]^+$. HRMS (ESI $^+$, MeOH/ CH_2Cl_2) m/z calcd for $\text{C}_{26}\text{H}_{29}^{35}\text{ClN}_2\text{OS}_2\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 507.1308, found 507.1321. UHPLC 7.24 min, 94%.



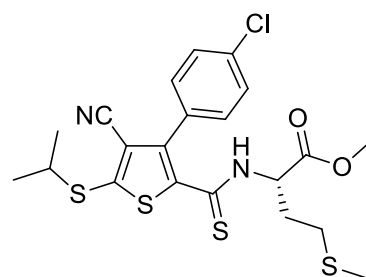
3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)-N-(naphthalen-1-ylmethyl)thiophene-2-carboxamide (25)

Thiophene **1** (0.145 g, 0.43 mmol, 1.0 equiv.) was solubilized in thionyl chloride (0.31 mL, 4.29 mmol, 10 equiv.). One drop of DMF was added to the solution which was stirred 5 h at reflux. Solvents are evaporated and residue was solubilized in dry CH_2Cl_2 (0.5 mL) under argon. Methyl-1-naphthalenamine (0.076 g, 0.52 mmol, 1.2 equiv.) and pyridine (0.087 mL, 1.07 mmol, 2.5 equiv.) were added to the solution. The reaction mixture was stirred 20 h at room temperature and then evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (heptane/AcOEt 10/0 to 40/60 in 25 min) to afford **25** as a yellow amorphous solid (0.186 g, 91%). IR (film, ν , cm^{-1}) 3413, 2966, 2918, 2223, 1640, 1531, 1504, 1484, 1366, 1264, 1208, 1090, 1014, 829, 776. ^1H NMR (300 MHz, acetone- d_6) δ 1.43 (d, 6H, $J = 6.6$ Hz), 3.64 (h, 1H, $J = 6.6$ Hz), 4.87 (d, 2H, $J = 5.7$ Hz), 6.98 (br s, 1H), 7.21 (d, 2H, $J = 8.4$ Hz), 7.35 (d, 2H, $J = 8.4$ Hz), 7.30-7.99 (m, 7H). ^{13}C NMR (75 MHz, acetone- d_6) δ 23.4, 42.3, 43.3, 114.2, 116.9, 124.3, 126.2, 126.8, 127.3, 127.4, 129.2, 129.6, 129.7, 131.4, 131.9, 132.2, 134.3, 134.8, 135.8, 138.7, 142.1, 149.9, 160.5. MS (ESI $^+$, MeOH/ CH_2Cl_2) m/z 499.1 $[\text{M}+\text{Na}]^+$. HRMS (ESI $^+$, MeOH/ CH_2Cl_2) m/z calcd for $\text{C}_{26}\text{H}_{21}^{35}\text{ClN}_2\text{OS}_2\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 499.0682, found 499.0688. UHPLC 6.62 min, 100%.



(R)-methyl 2-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carboxamido)-4-(methylthio)butanoate (26)

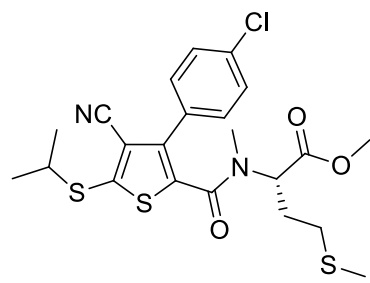
To a solution (CH_2Cl_2 , 1.2 mL) of D-methionine methyl ester (0.086 g, 0.43 mmol, 1.0 equiv.) and thiophene **1** (0.146 g, 0.43 mmol, 1.0 equiv.) were added EDCI (0.099 g, 0.52 mmol, 1.2 equiv.), HOBT (0.132 g, 0.86 mmol, 2.0 equiv.) and N-methylmorpholine (48.0 μL , 0.043 mmol, 1.0 equiv.). The reaction was stirred at room temperature overnight. The reaction mixture was diluted in CH_2Cl_2 and washed once with a 5% citric acid solution, once with water, once with 5% Na_2CO_3 solution, once with water and once with brine. The organic phase was dried over MgSO_4 and concentrated under reduced pressure. The purification of the crude product was performed by flash chromatography on silica gel (heptane/EtOAc 10/0 to 5/5 in 25 min) to afford **26** as a yellow oil (0.331 g, 88%). IR (film, ν , cm^{-1}) 3301, 2966, 2918, 2226, 1750, 1647, 1537, 1516, 1488, 1437, 1366, 1308, 1227, 1208, 1171. ^1H NMR (300 MHz, CDCl_3) δ 1.38 (d, 6H, $J = 6.6$ Hz), 1.78 (m, 1H), 1.95 (s, 3H), 2.00 (m, 1H), 2.19 (t, 2H, $J = 7.2$ Hz), 3.53 (h, 1H, $J = 6.6$ Hz), 3.62 (s, 3H), 4.60 (m, 1H), 6.03 (d, 1H, $J = 7.8$ Hz), 7.37 (d, 2H, $J = 8.4$ Hz), 7.48 (d, 2H, $J = 8.4$ Hz). ^{13}C NMR (75 MHz, CDCl_3) δ 15.5, 23.2, 29.6, 31.0, 42.4, 50.0, 52.7, 113.4, 115.2, 129.9, 129.9, 130.7, 136.1, 136.6, 141.8, 152.6, 159.7, 171.2. MS (ESI^+ , $\text{MeOH}/\text{CH}_2\text{Cl}_2$) m/z 505.1 $[\text{M}+\text{Na}]^+$. HRMS (ESI^+ , $\text{MeOH}/\text{CH}_2\text{Cl}_2$) m/z calcd for $\text{C}_{21}\text{H}_{23}^{35}\text{ClN}_2\text{O}_3\text{S}_3\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 505.0457, found 505.0450. UHPLC 5.98 min, 98%. $[\alpha]_D^{20} = +18.5^\circ$ (MeOH, 1.08).



(S)-methyl 2-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carbothioamido)-4-(methylthio)butanoate (27)

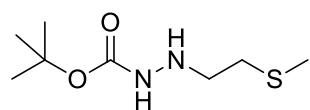
In a dried round-bottom flask under argon, Lawesson's reagent (0.121 g, 0.30 mmol, 1.0 equiv.) was added to a solution of **8** (0.144 g, 0.30 mmol, 1.0 equiv.) solubilized in toluene (3 mL). The reaction mixture was stirred during 6 days at 40°C . Solvent was evaporated under reduced pressure. The purification of the crude product was performed by flash chromatography on silica gel (heptane/EtOAc 10/0 to 3/1 in 25 min) to afford **27** as a yellow oil (0.082 g, 55%). IR (film, ν , cm^{-1}) 3346, 2957, 2917, 2224, 1740, 1521, 1481, 1436, 1378, 1247, 1171, 1090, 1014, 834, 739. ^1H NMR (300 MHz, CDCl_3) δ 1.49 (d, 6H, $J = 6.9$ Hz), 1.96 (m, 1H), 2.03 (s, 3H), 2.14 (m, 1H), 2.22 (m, 2H), 3.66 (h, 1H, $J = 6.9$ Hz), 3.71 (s, 3H), 5.10 (m, 1H), 7.45 (d, 2H, $J = 8.7$ Hz), 7.56 (d, 2H, $J = 8.7$ Hz), 7.64 (d, 1H, $J = 6.9$ Hz). ^{13}C

NMR (75 MHz, CDCl₃) δ 15.1, 23.4, 30.2, 30.9, 43.1, 52.8, 58.7, 100.9, 114.2, 130.2, 131.6, 132.1, 136.1, 138.3, 144.7, 151.2, 170.8, 188.7. MS (ESI⁺, MeOH/CH₂Cl₂) m/z 521.0 [M+Na]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) m/z calcd for C₂₁H₂₃³⁵ClN₂O₂S₄Na⁺ [M+Na]⁺ 521.0229, found 521.0232. UHPLC 6.52 min, 100%. [α]_D²⁰ = -90.9° (MeOH, 0.44).



(S) methyl 2-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)-N-methylthiophene-2-carboxamido)-4-(methylthio)butanoate (28)

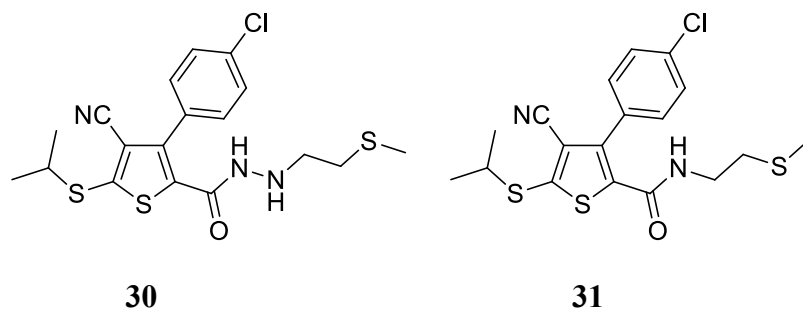
In a dried round-bottom flask under argon, to a solution (DMF, 10 mL) of **8** (0.144 g, 0.30 mmol, 1.0 equiv.) was added sodium hydride (60% in oil, 0.036 g, 0.89 mmol, 3.0 equiv.). The reaction was stirred at room temperature during 15 minutes. Methyl iodide (19 μ L, 0.30 mmol, 1.0 equiv.) is added to the reaction mixture. After 50 minutes of stirring at room temperature, brine (23 mL) was added. The reaction mixture was extracted twice by CH₂Cl₂. The organic phases were pooled, dried over MgSO₄ and concentrated under reduced pressure. The purification of the crude product was performed by flash chromatography on silica gel (heptane/EtOAc 10/0 to 5/5 in 15 min) to afford **28** as a yellow oil (0.078 g, 53%). IR (film, ν , cm⁻¹) 2957, 2921, 2226, 1740, 1634, 1488, 1436, 1397, 1235, 1203, 1156, 1092, 1053, 1014, 831, 735. ¹H NMR (300 MHz, acetone-d₆) δ 1.45 (d, 6H, J = 6.6 Hz), 2.03 (m, 1H), 2.08 (s, 3H), 2.20 (m, 1H), 2.28 (m, 2H), 2.72 (s, 3H), 3.66 (h, 1H, J = 6.6 Hz), 3.70 (s, 3H), 5.00 (m, 1H), 7.60 (m, 4H). ¹³C NMR (75 MHz, acetone-d₆) δ 15.2, 23.5, 28.5, 31.1, 34.7, 43.6, 52.6, 57.7, 114.5, 116.6, 130.0, 130.1, 131.5, 135.8, 145.9, 158.1, 164.0, 171.1. MS (ESI⁺, MeOH/CH₂Cl₂) m/z 519.0 [M+Na]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) m/z calcd for C₂₂H₂₅³⁵ClN₂O₃S₃Na⁺ [M+Na]⁺ 519.0614, found 519.0610. UHPLC 6.09 min, 95%. [α]_D²⁰ = -45.5° (MeOH, 1.10).



tert-butyl 2-(2-(methylthio)ethyl)hydrazinecarboxylate (29)

To a solution (water, 9.4 mL) of 1% w/v HCl (0.26 mL) was added (2,2-diméthoxyéthyl)(méthyl)sulfane (3.0 mL, 22.5 mmol, 1.0 equiv.). The reaction mixture was stirred at reflux during 30 minutes and then neutralized with a saturated NaHCO₃ solution. The reaction mixture was extracted twice with CH₂Cl₂. The organic layers were pooled, dried over MgSO₄ and concentrated under reduced pressure to afford the aldehyde as a brown oil. Under argon, the obtained aldehyde (1.10 g, 12.2 mmol, 1.0 equiv.) was solubilized in CH₂Cl₂ (61 mL). Boc hydrazine (1.77 g, 13.4 mmol, 1.1 equiv.), glacial acetic acid (2.79 mL, 48.8 mmol, 4.0 equiv.) and sodium triacetoxyborohydride (5.17 g, 24.4 mmol, 1.0 equiv.) were added to the reaction mixture. After 1 h of stirring at room temperature, a saturated NaHCO₃ solution was added. The reaction mixture was extracted three times by EtOAc. The organic

phases were pooled, washed with saturated NaHCO₃ solution, dried over MgSO₄ and concentrated under reduced pressure. The obtained oil was purified by flash chromatography on silica gel (heptane/EtOAc 10/0 to 5/5 in 45 min) to afford **29** as an off-white oil (1.35 g, 29%). ¹H NMR (300 MHz, CDCl₃) δ 1.40 (s, 9H), 2.06 (s, 3H), 2.56 (t, 2H, *J* = 6.6 Hz), 3.00 (t, 2H, *J* = 6.6 Hz), 6.02 (br s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 15.5, 28.3, 32.4, 50.0, 80.6, 159.2. MS (ESI⁻, MeOH/CH₂Cl₂) *m/z* 205.1 [M-H]⁻. HRMS (ESI⁻, MeOH/CH₂Cl₂) *m/z* calcd for C₈H₁₇N₂O₂S⁻ [M-H]⁻ 205.1011, found 205.1009.



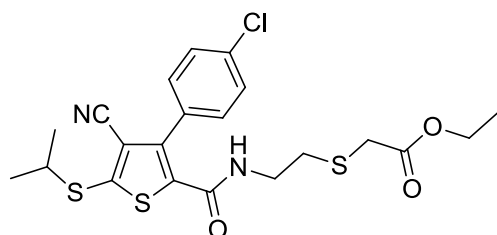
3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)-N'-(2-(methylthio)ethyl)thiophene-2-carbohydrazide (30) and 3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)-N-(2-(methylthio)ethyl)thiophene-2-carboxamide (31)

To a solution (dioxane, 0.8 mL) of concentrated HCl solution was added **29** (0.075 g, 0.36 mmol, 1.0 equiv.). After 3 h of stirring at room temperature, the reaction mixture was evaporated under reduced pressure. To the obtained yellow oil solubilized in DMF (1.0 mL) were added **1** (0.122 g, 0.36 mmol, 1.0 equiv.), HBTU (0.137 g, 0.36 mmol, 1.0 equiv.), HOBT (0.055 g, 0.36 mmol, 1.0 equiv.) and diisopropylethylamine (0.124 mL, 0.72 mmol, 2.0 equiv.). The reaction was stirred at room temperature overnight. The reaction mixture was diluted in saturated NaHCO₃ solution and extracted three times by EtOAc. The organic phases were pooled, washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The obtained oil was purified by flash chromatography on silica gel (heptane/EtOAc 10/0 to 5/5 in 35 min) to afford **30** (0.015 g, 10%) and **31** (0.026 g, 17%) as yellow oils.

Compound **30**: IR (film, ν , cm⁻¹) 3269, 2966, 2919, 2223, 1644, 1526, 1485, 1441, 1398, 1366, 1285, 1243, 1089, 1050, 1014, 829, 738. ¹H NMR (300 MHz, CDCl₃) δ 1.47 (d, 6H, *J* = 6.9 Hz), 2.10 (s, 3H), 2.52 (t, 2H, *J* = 6.6 Hz), 2.98 (t, 2H, *J* = 6.6 Hz), 3.62 (h, 1H, *J* = 6.9 Hz), 6.88 (br s, 1H), 7.42 (d, 2H, *J* = 8.4 Hz), 7.55 (d, 2H, *J* = 8.4 Hz). ¹³C NMR (75 MHz, MeOD) δ 14.9, 23.6, 32.6, 43.9, 50.9, 109.3, 114.6, 129.6, 130.0, 132.2, 144.5, 153.5, 159.2. MS (ESI⁺, MeOH/CH₂Cl₂) *m/z* 426.1 [M+H]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) *m/z* calcd for C₁₈H₂₁³⁵ClN₃OS₃⁺ [M+H]⁺ 426.0535, found 426.0544. UHPLC 5.50 min, 85%.

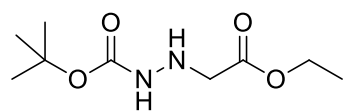
Compound **31**: IR (film, ν , cm⁻¹) 3375, 2962, 2919, 2225, 1642, 1531, 1506, 1484, 1366, 1281, 1210, 1090, 1050, 1014, 829, 735. ¹H NMR (300 MHz, CDCl₃) δ 1.37 (d, 6H, *J* = 6.9 Hz), 1.88 (s, 3H), 2.40 (t, 2H, *J* = 6.6 Hz), 3.36 (td, 2H, *J* = 6.0, 6.6 Hz), 3.52 (h, 1H, *J* = 6.9 Hz), 5.80 (t, 1H, *J* = 6.0 Hz), 7.34 (d, 2H, *J* = 8.4 Hz), 7.45 (d, 2H, *J* = 8.4 Hz). ¹³C NMR (75 MHz, MeOD) δ 14.5, 23.2, 33.3, 37.5, 42.4, 113.5, 115.4, 129.9, 130.0, 130.7, 136.5, 137.0, 141.2, 152.0, 159.9. MS (ESI⁺, MeOH/CH₂Cl₂) *m/z* 411.0 [M+H]⁺. HRMS (ESI⁺,

MeOH/CH₂Cl₂) m/z calcd for C₁₈H₂₀³⁵ClN₂O₃⁺ [M+H]⁺ 411.0426, found 411.0423. UHPLC 5.87 min, 87%.



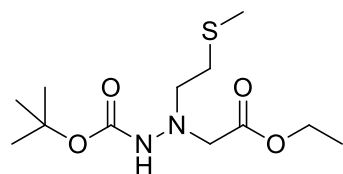
ethyl 2-((2-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carboxamido)ethyl)thio)acetate (32)

Under argon, to a solution (CH₂Cl₂, 2.6 mL) of **31** (0.084 g, 0.20 mmol, 1.0 equiv.) was added ethyl bromoacetate (22 μ L, 0.20 mmol, 1.0 equiv.). The reaction was stirred at 45°C during 60 h. The reaction mixture was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (heptane/EtOAc 95/5 to 70/30 in 20 min) to afford **32** as an off-white oil (0.053 g, 52%). IR (film, ν , cm⁻¹) 3389, 2968, 2928, 2225, 1732, 1644, 1532, 1511, 1485, 1366, 1274, 1154, 1127, 1090, 1015, 830, 735. ¹H NMR (300 MHz, CDCl₃) δ 1.20 (t, 3H, J = 6.9 Hz), 1.37 (d, 6H, J = 6.6 Hz), 2.58 (t, 2H, J = 6.6 Hz), 3.02 (s, 2H), 3.37 (td, 2H, J = 6.0, 6.6 Hz), 3.52 (h, 1H, J = 6.6 Hz), 4.08 (q, 2H, J = 6.9 Hz), 5.91 (t, 1H, J = 6.0 Hz), 7.35 (d, 2H, J = 8.4 Hz), 7.45 (d, 2H, J = 8.4 Hz). ¹³C NMR (75 MHz, CDCl₃) δ 14.2, 23.3, 32.3, 33.1, 38.0, 42.5, 61.7, 113.5, 115.5, 129.9, 130.0, 130.8, 136.4, 136.9, 141.3, 151.9, 159.9, 170.2. MS (ESI⁺, MeOH/CH₂Cl₂) m/z 483.1 [M+H]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) m/z calcd for C₂₁H₂₄³⁵ClN₂O₃S₃⁺ [M+H]⁺ 483.0638, found 483.0637. UHPLC 5.91 min, 83%.



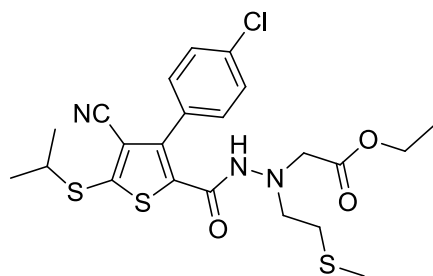
tert-butyl 2-(2-ethoxy-2-oxoethyl)hydrazinecarboxylate (33)

Under argon, to a solution (CH₂Cl₂, 113.0 mL) of *tert*-butyl hydrazinecarboxylate (1.14 g, 8.64 mmol, 1.0 equiv.) were added ethyl bromoacetate (0.959 mL, 8.64 mmol, 1.0 equiv.) and diisopropylethylamine (1.51 mL, 8.64 mmol, 1.0 equiv.). The reaction mixture was stirred at 45°C overnight and then evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (heptane/EtOAc 10/0 to 3/1 in 40 min) to afford **33** as an off-white oil (1.01 g, 54%). IR (film, ν , cm⁻¹) 3371, 3245, 2985, 2933, 1738, 1703, 1547, 1482, 1367, 1247, 1155, 1026, 937, 833, 782, 755. ¹H NMR (300 MHz, CDCl₃) δ 1.21 (t, 3H, J = 6.9 Hz), 1.38 (s, 9H), 3.57 (s, 2H), 4.13 (q, 2H, J = 6.9 Hz), 6.30 (br s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 14.2, 28.4, 53.0, 61.2, 81.0, 156.2, 171.3. MS (ESI⁺, MeOH/CH₂Cl₂) m/z 241.1 [M+Na]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) m/z calcd for C₉H₁₈N₂O₄Na⁺ [M+Na]⁺ 241.1164, found 241.1160.



tert-butyl 2-(2-ethoxy-2-oxoethyl)-2-(2-(methylthio)ethyl)hydrazinecarboxylate (34)

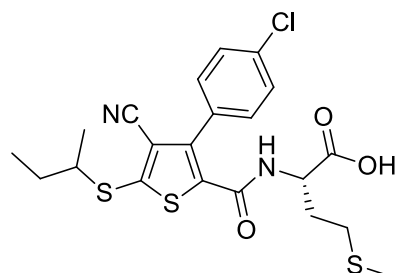
In a dried microwaves tube under argon, to **33** (1.36 g, 6.25 mmol, 1.0 equiv.) were added (2-chloroethyl)(methyl)sulfane (0.622 mL, 6.25 mmol, 1.0 equiv.) and diisopropylethylamine (1.09 mL, 6.25 mmol, 1.0 equiv.). The reaction mixture was stirred twice at 150°C during 30 minutes under microwaves irradiation and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (heptane/EtOAc 10/0 to 7/3 in 70 min) to afford **34** as an off-white oil (0.211 g, 12%). IR (film, ν , cm^{-1}) 2978, 2918, 1728, 1482, 1453, 1366, 1241, 1197, 1147, 1024, 848, 772. ^1H NMR (300 MHz, CDCl_3) δ 1.19 (t, 3H, $J = 7.5$ Hz), 1.35 (s, 9H), 2.03 (s, 3H), 2.55 (t, 2H, $J = 7.0$ Hz), 3.03 (t, 2H, $J = 7.0$ Hz), 3.64 (s, 2H), 4.10 (q, 2H, $J = 7.5$ Hz), 6.54 (br s, 1H). ^{13}C NMR (75 MHz, CDCl_3) δ 14.4, 15.8, 28.2, 31.8, 56.4, 57.7, 60.8, 80.2, 155.2, 170.7. MS (ESI⁺, MeOH/ CH_2Cl_2) m/z 315.1 $[\text{M}+\text{Na}]^+$. HRMS (ESI⁺, MeOH/ CH_2Cl_2) m/z calcd for $\text{C}_{12}\text{H}_{24}\text{N}_2\text{O}_4\text{SNa}^+$ $[\text{M}+\text{Na}]^+$ 315.1354, found 315.1366. UHPLC 4.47 min, 100%.



ethyl 2-(2-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carbonyl)-1-(2-(methylthio)ethyl)hydrazinyl)acetate (35)

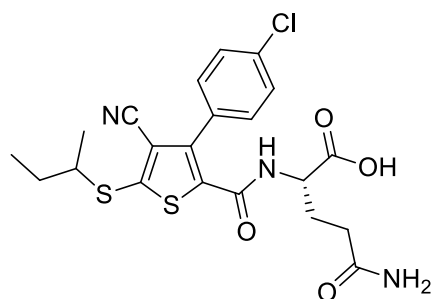
Compound **34** (0.086 g, 0.29 mmol, 1.0 equiv.) was solubilized in trifluoroacetic acid (0.6 mL) and CH_2Cl_2 (0.6 mL). The reaction mixture was stirred during 1 h at room temperature. Et_2O was added to the reaction mixture and concentrated under reduced pressure until to obtain a solid. To a solution (CH_2Cl_2 , 0.80 mL) of the deprotected hydrazine and **1** (0.100 g, 0.29 mmol, 1.0 equiv.) were added EDCI (0.068 g, 0.35 mmol, 1.2 equiv.), HOBt (0.090 g, 0.59 mmol, 2.0 equiv.) and N-methylmorpholine (32.0 μL , 0.29 mmol, 1.0 equiv.). The reaction was stirred 6 h at room temperature. The reaction mixture was diluted in CH_2Cl_2 and washed once with a 5% citric acid solution, once with water, once with 5% Na_2CO_3 solution, once with water and once with brine. The organic phase was dried over MgSO_4 and concentrated under reduced pressure to afford a residue which was purified by flash chromatography on silica gel (heptane/EtOAc 10/0 to 7/3 in 70 min) to afford **35** as an off-white oil (0.087 g, 58%). IR (film, ν , cm^{-1}) 3329, 2982, 2909, 2219, 1729, 1660, 1536, 1499, 1485, 1417, 1374, 1197, 1088, 1029, 1014, 828, 745. ^1H NMR (500 MHz, CDCl_3) δ 1.28 (t, 3H, $J = 7.2$ Hz), 1.48 (d, 6H, $J = 6.6$ Hz), 2.11 (s, 3H), 2.57 (t, 2H, $J = 7.5$ Hz), 2.99 (t, 2H, $J = 7.5$ Hz), 3.62 (h, 1H, $J = 6.6$ Hz), 3.63 (s, 2H), 4.10 (q, 2H, $J = 7.2$ Hz), 7.40 (d, 2H, $J = 8.4$ Hz), 7.57 (d, 2H, $J = 8.4$ Hz), 7.58 (s, 1H). ^{13}C NMR (75 MHz, CDCl_3) δ 14.1, 15.8, 23.1,

31.7, 42.4, 56.1, 56.6, 61.1, 113.4, 115.2, 129.8, 130.5, 135.6, 136.4, 141.6, 152.2, 159.3, 169.9. MS (ESI⁺, MeOH/CH₂Cl₂) *m/z* 512.1 [M+H]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) *m/z* calcd for C₂₂H₂₇³⁵ClN₃O₃S₃⁺ [M+H]⁺ 512.0903, found 512.0884. UHPLC 5.96 min, 100%.



(S)-2-(5-(sec-butylthio)-3-(4-chlorophenyl)-4-cyanothiophene-2-carboxamido)-4-(methylthio)butanoic acid (36)

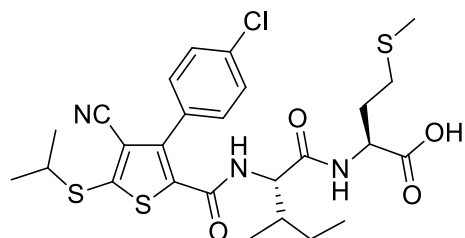
To a solution (THF/EtOH 2/1, 1.9 mL) of **19** (0.123 g, 0.25 mmol, 1.0 equiv.) was added aqueous NaOH (2M, 1.2 mL). After stirring 36 h at room temperature, the mixture was neutralized with aqueous HCl (1M, 2.4 mL) and extracted two times with CH₂Cl₂. The organic layers were pooled, dried over MgSO₄ and concentrated under reduced pressure to afford acid **36** as a white amorphous solid (0.113 g, 94%). IR (film, ν , cm⁻¹) 2966, 2920, 2225, 1729, 1634, 1532, 1508, 1484, 1401, 1364, 1208, 1091, 1014, 828, 738. ¹H NMR (300 MHz, CDCl₃) δ 1.09 (t, 3H, *J* = 7.5 Hz), 1.46 (d, 3H, *J* = 6.6 Hz), 1.77 (qd, 2H, *J* = 7.2, 7.5 Hz), 1.91 (m, 1H), 2.04 (s, 3H), 2.05 (m, 1H), 2.33 (t, 2H, *J* = 7.2 Hz), 3.42 (tq, 1H, *J* = 6.6, 7.2 Hz), 4.70 (m, 1H), 6.13 (d, 1H, *J* = 7.8 Hz), 7.45 (d, 2H, *J* = 8.4 Hz), 7.55 (d, 2H, *J* = 8.4 Hz). ¹³C NMR (75 MHz, CDCl₃) δ 10.3, 14.4, 19.6, 28.6, 28.7, 29.6, 48.1, 51.0, 112.3, 113.9, 128.1, 128.9, 129.7, 134.3, 135.6, 141.1, 152.4, 159.0, 174.4. MS (ESI⁺, MeOH/CH₂Cl₂) *m/z* 505.1 [M+Na]⁺. HRMS (ESI⁺, MeOH/CH₂Cl₂) *m/z* calcd for C₂₁H₂₃³⁵ClN₂O₃S₃Na⁺ [M+Na]⁺ 505.0457, found 505.0452. UHPLC 5.58 min, 93%. [α]_D²⁰ = -11.5° (MeOH, 1.30).



(S)-(2S)-5-amino-2-(5-(sec-butylthio)-3-(4-chlorophenyl)-4-cyanothiophene-2-carboxamido)-5-oxopentanoic acid (37)

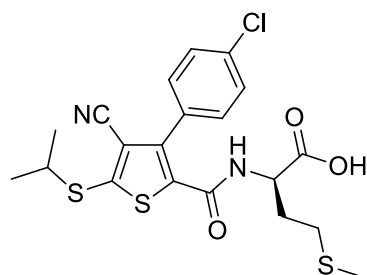
To a solution (CH₂Cl₂, 0.2 mL) of **20** (0.059 g, 0.11 mmol, 1.0 equiv.) was added trifluoroacetic acid (0.22 mL, 2.88 mmol, 26.0 equiv.) and triethylsilane (40 μ L, 0.28 mmol, 2.5 equiv.). After stirring 16 h at room temperature, Et₂O was added to the reaction mixture which was then evaporated under reduced pressure. The residue was extracted twice with CH₂Cl₂. The organic layers were pooled, dried over MgSO₄ and concentrated under reduced pressure. The purification of the crude product was performed by chromatography on preparative plate (CH₂Cl₂/MeOH 9/1 with formic acid 1%) to afford **37** as an off-white oil (0.029 g, 55%). IR (film, ν , cm⁻¹) 3353, 2971, 2913, 2221, 1597, 1533, 1482, 1399, 1207, 1091, 1015. ¹H NMR (300 MHz, CDCl₃) δ 1.00 (t, 3H, *J* = 7.5 Hz), 1.36 (d, 3H, *J* = 6.6 Hz),

1.65 (qd, 2H, $J = 7.2, 7.5$ Hz), 1.86-2.15 (m, 4H), 3.34 (tq, 1H, $J = 6.6, 7.2$ Hz), 4.38 (m, 1H), 6.44 (d, 1H, $J = 7.8$ Hz), 6.67 (br s, 2H), 7.34 (d, 2H, $J = 8.4$ Hz), 7.44 (d, 2H, $J = 8.4$ Hz). ^{13}C NMR (75 MHz, CDCl_3) δ 11.3, 20.6, 20.7, 29.7, 31.2, 49.2, 52.6, 113.3, 115.2, 129.8, 129.9, 130.8, 135.1, 136.3, 142.4, 153.0, 160.2, 171.1, 176.6. MS (ESI^+ , $\text{MeOH}/\text{CH}_2\text{Cl}_2$) m/z 502.1 $[\text{M}+\text{Na}]^+$. HRMS (ESI^+ , $\text{MeOH}/\text{CH}_2\text{Cl}_2$) m/z calcd for $\text{C}_{21}\text{H}_{22}^{35}\text{ClN}_3\text{O}_4\text{S}_2\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 502.0638, found 502.0645. UHPLC 4.50 min, 91%. $[\alpha]_D^{20} = -8.3^\circ$ (MeOH, 0.60).



(S,S)-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carboxamido)-3-methylpentanamido)-4-(methylthio)butanoic acid (38)

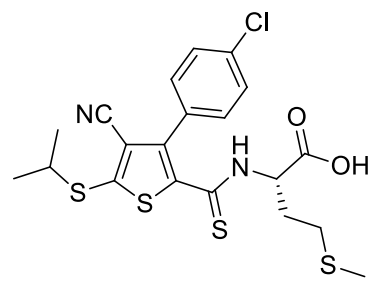
To a solution (THF/EtOH 2/1, 0.6 mL) of **21** (0.050 g, 0.08 mmol, 1.0 equiv.) was added aqueous LiOH (2M, 0.42 mL). After stirring 64 h at room temperature, the mixture was neutralized with aqueous HCl (1M, 0.8 mL) and extracted twice with CH_2Cl_2 . The organic layers were pooled, dried over MgSO_4 and concentrated under reduced pressure to afford acid **38** as a white amorphous solid (0.049 g, quantitative yield). IR (film, ν , cm^{-1}) 3293, 2966, 2928, 2865, 2227, 1718, 1627, 1531, 1485, 1436, 1210, 1151, 1090, 1015, 829. ^1H NMR (500 MHz, CDCl_3) δ 0.66 (d, 3H, $J = 6.5$ Hz), 0.71 (t, 3H, $J = 7.5$ Hz), 1.32 (d, 6H, $J = 6.5$ Hz), 1.52 (m, 2H), 1.88 (m, 1H), 1.97 (s, 3H), 2.02 (m, 1H), 2.47 (t, 2H, $J = 7.5$ Hz), 3.50 (m, 1H), 4.10 (d, 1H, $J = 7.5$ Hz), 4.43 (m, 1H), 7.38 (d, 2H, $J = 8.0$ Hz), 7.48 (d, 2H, $J = 8.0$ Hz). ^{13}C NMR (75 MHz, CDCl_3) δ 11.4, 15.4, 15.9, 23.6, 25.6, 31.2, 32.3, 38.2, 43.8, 52.6, 59.9, 114.4, 117.3, 130.7, 132.1, 132.2, 137.1, 138.0, 143.4, 151.4, 162.4, 173.1, 174.7. MS (ESI^+ , $\text{MeOH}/\text{CH}_2\text{Cl}_2$) m/z 604.1 $[\text{M}+\text{Na}]^+$. HRMS (ESI^+ , $\text{MeOH}/\text{CH}_2\text{Cl}_2$) m/z calcd for $\text{C}_{26}\text{H}_{32}^{35}\text{ClN}_3\text{O}_4\text{S}_3\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 604.1141, found 604.1161. UHPLC 5.61 min, 90%. $[\alpha]_D^{20} = -10.9^\circ$ (MeOH, 0.46).



(R)-2-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carboxamido)-4-(methylthio)butanoic acid (39)

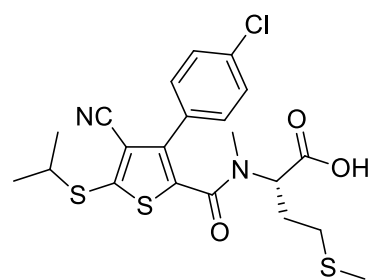
To a solution (THF/EtOH 2/1, 1.7 mL) of **26** (0.081 g, 0.17 mmol, 1.0 equiv.) was added aqueous NaOH (2M, 1.1 mL). After stirring 36 h at room temperature, the mixture was neutralized with aqueous HCl (1M, 2.3 mL) and extracted twice with CH_2Cl_2 . The organic layers were pooled, dried over MgSO_4 and concentrated under reduced pressure to afford acid **39** as an orange amorphous solid (0.048 g, 62%). IR (film, ν , cm^{-1}) 3293, 2967, 2918, 2226,

1748, 1646, 1537, 1486, 1439, 1305, 1266, 1208, 1170, 1093, 1015, 827, 794. ^1H NMR (300 MHz, acetone- d_6) δ 1.29 (d, 6H, $J = 6.6$ Hz), 1.79 (m, 1H), 1.90 (s, 3H), 1.93 (m, 1H), 2.20 (t, 2H, $J = 7.2$ Hz), 3.52 (h, 1H, $J = 6.6$ Hz), 4.45 (m, 1H), 6.76 (d, 1H, $J = 7.8$ Hz), 7.47 (m, 4H). ^{13}C NMR (75 MHz, acetone- d_6) δ 15.2, 23.4, 30.5, 31.6, 43.2, 52.8, 114.2, 117.0, 130.2, 131.6, 132.2, 136.2, 138.0, 142.6, 150.4, 160.7, 172.4. MS (ESI $^-$, MeOH/ CH_2Cl_2) m/z 467.0 [M-H] $^-$. HRMS (ESI $^-$, MeOH/ CH_2Cl_2) m/z calcd for $\text{C}_{20}\text{H}_{20}^{35}\text{ClN}_2\text{O}_3\text{S}_3^-$ [M-H] $^-$ 467.0325, found 467.0317. UHPLC 5.34 min, 92%. $[\alpha]_D^{20} = +14.3^\circ$ (MeOH, 1.40).



(S)-2-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carbothioamido)-4-(methylthio)butanoic acid (40)

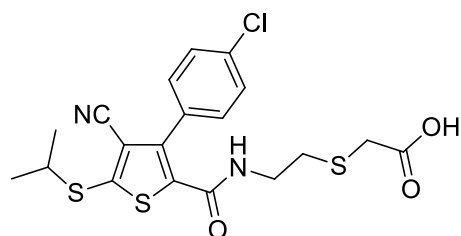
To a solution (THF/EtOH 2/1, 0.9 mL) of **27** (0.059 g, 0.12 mmol, 1.0 equiv.) was added aqueous LiOH (2M, 0.6 mL). After stirring 16 h at room temperature, the mixture was neutralized with aqueous HCl (1M, 1.2 mL) and extracted twice with CH_2Cl_2 . The organic layers were pooled, dried over MgSO_4 and concentrated under reduced pressure to afford acid **40** as a white amorphous solid (0.058 g, quantitative yield). IR (film, ν , cm^{-1}) 3327, 2962, 2918, 2223, 1713, 1596, 1521, 1504, 1480, 1368, 1243, 1175, 1139, 1090, 1048, 1014, 834, 734. ^1H NMR (300 MHz, CDCl_3) δ 1.40 (d, 6H, $J = 6.6$ Hz), 1.90 (m, 1H), 1.96 (s, 3H), 2.13 (m, 1H), 2.20 (m, 2H), 3.58 (h, 1H, $J = 6.6$ Hz), 5.06 (m, 1H), 7.44 (d, 2H, $J = 8.4$ Hz), 7.45 (d, 2H, $J = 8.4$ Hz), 7.55 (d, 1H, $J = 7.2$ Hz). ^{13}C NMR (75 MHz, CDCl_3) δ 15.5, 23.2, 29.5, 29.8, 42.2, 57.3, 113.3, 114.7, 130.0, 130.2, 130.6, 136.6, 137.2, 142.5, 155.7, 174.6, 186.2. MS (ESI $^+$, MeOH/ CH_2Cl_2) m/z 485.0 [M+H] $^+$. HRMS (ESI $^+$, MeOH/ CH_2Cl_2) m/z calcd for $\text{C}_{20}\text{H}_{22}^{35}\text{ClN}_2\text{O}_2\text{S}_4^+$ [M+Na] $^+$ 485.0253, found 485.0241. UHPLC 5.86 min, 100%. $[\alpha]_D^{20} = -102^\circ$ (MeOH, 0.54).



(S)-2-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)-N-methylthiophene-2-carboxamido)-4-(methylthio)butanoic acid (41)

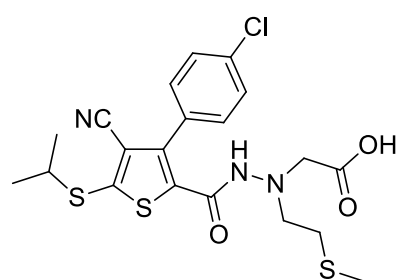
To a solution (THF/EtOH 2/1, 0.65 mL) of **28** (0.043 g, 0.09 mmol, 1.0 equiv.) was added aqueous LiOH (2M, 0.45 mL). After stirring 16 h at room temperature, the mixture was neutralized with aqueous HCl (1M, 0.9 mL) and extracted twice with CH_2Cl_2 . The organic layers were pooled, dried over MgSO_4 and concentrated under reduced pressure to afford acid **41** as a white amorphous solid (0.040 g, quantitative yield). IR (film, ν , cm^{-1}) 2966, 2921,

2226, 1732, 1597, 1489, 1448, 1402, 1155, 1091, 1053, 1014, 830, 733. ^1H NMR (300 MHz, acetone- d_6) δ 1.29 (d, 6H, $J = 6.9$ Hz), 1.87 (m, 1H), 1.92 (s, 3H), 2.09 (m, 1H), 2.12 (m, 2H), 2.58 (s, 3H), 3.49 (h, 1H, $J = 6.9$ Hz), 4.87 (m, 1H), 7.45 (m, 4H). ^{13}C NMR (75 MHz, acetone- d_6) δ 15.1, 23.4, 28.5, 31.1, 34.6, 43.6, 57.4, 114.5, 116.7, 130.0, 131.0, 131.5, 132.0, 135.8, 157.3, 162.6, 171.6. MS (ESI $^+$, MeOH/CH $_2$ Cl $_2$) m/z 483.1 [M+H] $^+$. HRMS (ESI $^+$, MeOH/CH $_2$ Cl $_2$) m/z calcd for C $_{21}$ H $_{24}$ ^{35}Cl N $_2$ O $_3$ S $_3$ $^+$ [M+H] $^+$ 483.0638, found 483.0632. UHPLC 5.40 min, 91%. $[\alpha]_D^{20} = -16.7^\circ$ (MeOH, 1.02).



2-((2-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carboxamido)ethyl)thio)acetic acid (42)

To a solution (THF/EtOH 2/1, 0.45 mL) of **32** (0.030 g, 0.06 mmol, 1.0 equiv.) was added aqueous LiOH (2M, 0.30 mL). After stirring 48 h at room temperature, the mixture was neutralized with aqueous HCl (1M, 0.7 mL) and extracted twice with CH $_2$ Cl $_2$. The organic layers were pooled, dried over MgSO $_4$ and concentrated under reduced pressure to afford acid **42** as a white amorphous solid (0.029 g, quantitative yield). IR (film, ν , cm $^{-1}$) 2965, 2928, 2224, 1712, 1634, 1533, 1484, 1367, 1243, 1090, 1014, 829, 734. ^1H NMR (300 MHz, CDCl $_3$) δ 1.39 (d, 6H, $J = 6.6$ Hz), 2.59 (t, 2H, $J = 6.6$ Hz), 3.07 (s, 2H), 3.39 (td, 2H, $J = 6.0, 6.6$ Hz), 3.52 (h, 1H, $J = 6.6$ Hz), 5.83 (t, 1H, $J = 6.0$ Hz), 7.34 (d, 2H, $J = 8.4$ Hz), 7.45 (d, 2H, $J = 8.4$ Hz). ^{13}C NMR (75 MHz, CDCl $_3$) δ 23.3, 32.3, 32.8, 38.0, 42.4, 113.4, 115.3, 128.5, 129.9, 130.0, 130.8, 136.5, 141.5, 152.3, 160.2, 174.5. MS (ESI $^+$, MeOH/CH $_2$ Cl $_2$) m/z 455.0 [M+H] $^+$. HRMS (ESI $^+$, MeOH/CH $_2$ Cl $_2$) m/z calcd for C $_{19}$ H $_{20}$ ^{35}Cl N $_2$ O $_3$ S $_3$ $^+$ [M+H] $^+$ 455.0325, found 455.0322. UHPLC 5.02 min, 90%.



2-(2-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carbonyl)-1-(2-(methylthio)ethyl)hydrazinyl)acetic acid (43)

To a solution (THF/EtOH 2/1, 0.6 mL) of **35** (0.039 g, 0.08 mmol, 1.0 equiv.) was added aqueous LiOH (2M, 0.40 mL). After stirring 48 h at room temperature, the mixture was neutralized with aqueous HCl (1M, 1.0 mL) and extracted twice with CH $_2$ Cl $_2$. The organic layers were pooled, dried over MgSO $_4$ and concentrated under reduced pressure to afford acid **43** as a white amorphous solid (0.038 g, quantitative yield). IR (film, ν , cm $^{-1}$) 3327, 2976, 2921, 2225, 1724, 1646, 1525, 1484, 1401, 1366, 1206, 1091, 1046, 1015, 828, 733. ^1H NMR (500 MHz, CDCl $_3$) δ 1.48 (d, 6H, $J = 6.6$ Hz), 2.09 (s, 3H), 2.53 (t, 2H, $J = 7.5$ Hz), 3.00 (t,

2H, $J = 7.5$ Hz), 3.62 (h, 1H, $J = 6.6$ Hz), 3.67 (s, 2H), 7.35 (br s, 1H), 7.40 (d, 2H, $J = 8.4$ Hz), 7.53 (d, 2H, $J = 8.4$ Hz). ^{13}C NMR (75 MHz, CDCl_3) δ 15.9, 23.4, 31.7, 42.5, 56.0, 57.3, 113.3, 114.9, 130.0, 130.6, 134.3, 136.6, 142.0, 153.5, 158.2, 160.0, 173.1. MS (ESI⁺, MeOH/ CH_2Cl_2) m/z 484.1 $[\text{M}+\text{H}]^+$. HRMS (ESI⁺, MeOH/ CH_2Cl_2) m/z calcd for $\text{C}_{20}\text{H}_{23}^{35}\text{ClN}_3\text{O}_3\text{S}_3^+$ $[\text{M}+\text{H}]^+$ 484.0590, found 484.0580. UHPLC 5.15 min, 100%.

BIOLOGICAL ASSAYS

Human 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 5 μL of partially purified human FTase (1.5 mg/mL) and 1.0 mL of Dansyl-GCVLS peptide (in the following buffer: 5.8 mM DTT, 6 mM MgCl_2 , 12 μM ZnCl_2 and 0.18% (w/v) Octyl-D-glucopyranoside, 53 mM Tris/HCl, pH 7.5).² Then the fluorescence development was recorded for 15 min (0.7 seconds per well, 15 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.

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.³

Assay for *in vitro* inhibition of *P. falciparum* growth

The chloroquine-resistant strain FcB1/Colombia of *Plasmodium 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% N_2 . *In vitro* drug susceptibility assays was measured by [^3H]-hypoxanthine incorporation as described.^{4,5} 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 μCi of [^3H]-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 \pm standard deviations determined from several independent experiments. Chloroquine was used as antimalarial drug control.

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

Bloodstream forms of *Trypanosoma brucei brucei* strain 93 were cultured in HMI9 medium supplemented with 10% FCS at 37°C under an atmosphere of 5% CO₂.⁶ In all experiments, log-phase cell cultures were harvested by centrifugation at 3,000 x *g* and immediately used. Drug assays were based on the conversion of a redox-sensitive dye (resazurin) to a fluorescent product by viable cells.⁷ Drug stock solutions were prepared in pure DMSO. *T. b. brucei* bloodstream forms (3x10⁴ cells/ml) were cultured as described above in 96-well plates (200 µL per well) either in the absence or in the presence of different concentrations of inhibitors and with a final DMSO concentration that did not exceed 1%. After a 72-h incubation, resazurin solution was added in each well at the final concentration of 45 µM. 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. DMSO was used as a control. IC₅₀s were determined from the dose-response curves with drug concentrations ranging from 100 µM to 50 nM. IC₅₀ value is the mean +/- the standard deviation of three independent experiments. Pentamidine was used as anti-trypanosomal drug control.

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

The β-galactosidase-expressing *T. cruzi* trypomastigotes of the Tulahuen strain (*lacZ* clone 4)⁸ were maintained in monolayers of L-6 cells grown in RPMI medium supplemented with 100 units/ml penicillin, 100 µg/ml streptomycin, and 5% (v/v) fetal calf serum at 37°C in 5% CO₂. Drug stock solutions were prepared in pure DMSO. Inhibition assays of intracellular parasite multiplication were performed in 96-well plates as previously described.⁹ Briefly, trypomastigotes were incubated to L-6 cell cultures plated 24 h before (5·10³ cells/well) with a trypomastigotes/cell ratio of 20:1 and with different concentrations of drugs. Cells were maintained for 96 h at 37°C in 5% CO₂. Cells were then lysed with 1% Nonidet P-40 and β-galactosidase activity was quantified using the chromogenic substrate red β-D-galactopyranoside (100 µM final concentration) by measuring the absorbance at 570 nm in an automated microplate reader. Wells with β-galactosidase activity turned from yellow to red. The growth inhibition for each drug concentration was determined by comparison of the absorbance of the control cultures processed in the same way but receiving equivalent amount of DMSO instead of drug. The concentration causing 50% growth inhibition (IC₅₀) was obtained from the drug concentration-response curve and the results were expressed as the mean values ± standard deviations determined from several independent experiments. Benznidazole was used as anti-trypanosomal drug control.

Assay for *in vitro* inhibition of *L. donovani* growth

Promastigote forms of *L. donovani* (MHOM/ET/67/HU3) were grown in M-199 medium supplemented with 40 mM HEPES, 100 μ M adenosine, 0.5 mg/L haemin, 10% heat inactivated foetal bovine serum (FBS) and 50 μ g/mL gentamycin at 26 °C in a dark environment under an atmosphere of 5% CO₂. All the experiments were performed with parasites in their logarithmic phase of growth. Differentiation of promastigotes into axenic amastigotes was achieved by dilution of 1×10^6 promastigotes in 5 mL of axenic amastigote media (15 mM KCl; 8 mM glucose; 5 mM glutamine 1 X M-199, 2.5% BBL trypticase peptone, 4 mM haemin, and 20% FBS). The pH was adjusted to pH 5.5. Axenic amastigotes were grown at 37 °C in 5% CO₂.

The mouse monocyte/macrophage cell line RAW 264.7 was maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum. RAW 264.7 cells were seeded into a 96-well microtiter plate at a density of 5×10^3 cells/well in 100 μ L of DMEM. After incubation in a 5% CO₂ incubator at 37 °C for 24 h, the culture medium was replaced with 100 μ L of fresh DMEM containing a suspension of *L. donovani* amastigote forms of 10^6 cells/mL. After incubation in a 5% CO₂ incubator at 37 °C for 24 h the culture medium was replaced with 100 μ L of fresh DMEM containing the test compounds for a new incubation of 48 hours. The viability of the amastigotes into macrophages was then assessed using the SYBR[®] green I (Invitrogen, France) incorporation method. Parasite growth is determined by using SYBR[®] Green I, a dye with marked fluorescence enhancement upon contact with parasite DNA. The cells were lysed following direct PCR-Cell genotyping without DNA isolation protocol (Euromedex, France). 10 μ L of lysed parasite solution of each well was added to 40 μ L of PCR-Cell reagent containing the SYBR[®] green I in a qPCR plate of 96 wells, and the contents were mixed. Fluorescence was measured with Mastercycler[®] ep realplex (Eppendorf, France). Fluorescence obtained was compared to those from the range obtained with parasite, infected cell and non-infected cell densities. Miltefosine and amphotericin B were used as reference compounds. The results are expressed as the concentrations inhibiting parasite growth by 50% (IC₅₀) \pm SD after a 48-h incubation period.

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