Supporting Information

Discovery of a new class of protein farnesyltransferase inhibitors in the arylthiophene series

Sébastien Lethu, Maryon Ginisty, Damien Bosc and Joëlle Dubois*

Institut de Chimie des Substances Naturelles, UPR2301 CNRS, Centre de Recherche de Gif-sur-Yvette, Avenue de la Terrasse, 91198 Gif-sur-Yvette cedex, France.

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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 (CH_2Cl_2), triethylamine (Et_3N), diisopropylamine and toluene were distilled over calcium hydride. *N,N'*-dimethylformamide (DMF) was dried over $MgSO_4$ followed by distillation under reduced pressure. Pyridine was stored over KOH and other solvents over 4Å molecular sieves. Analytical thin-layer chromatography was carried out on precoated silica gel aluminium plates (SDS TLC plates, silica gel $60F_{254}$). Column chromatography was performed with silica gel SDS 60 A CC (40-63 µm) or with prepacked Redisep columns. Preparative TLC (PLC) was performed on Merck TLC with silica gel $60F_{254}$.
NMR spectra (^1H and ^{13}C) were recorded on a Brucker Avance 300 (300 MHz) and Avance 500 (500 MHz). Chemical shifts (δ) are given in ppm relative to $CDCl_3$ (7.26 ppm; 77.2 ppm), CD_3OD (3.34 ppm; 49.9 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 Thermofinningan 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.

2-(4-chlorobenzoyl)-3,3-bis(isopropylthio)acrylonitrile (4)

To a solution (DMF, 20 mL) of **3** (1.93 g, 10.8 mmol, 1.0 equiv.) was added NaH (60% in oil, 1.08 g, 27.0 mmol, 2.5 equiv.). After stirring for 45 min at room temperature carbon disulfide (0.65 mL, 10.8 mmol, 1.0 equiv.) was added dropwise. The reaction mixture was stirred at room temperature for 2 h before *i*-propyl iodide (2.56 mL, 22.6 mmol, 2.1 equiv.) was added dropwise. After being stirred for 19 h at 60°C, the reaction mixture was cooled and concentrated under reduced pressure. The residue was dissolved with water and extracted four times with diethyl ether. The organic layers were pooled, 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 1/0 to 3/1 (v/v) in 25 min) to afford **4** as a yellow oil (3.0 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.32 (d, J = 4.0 Hz, 6H), 1.46 (d, J = 4.0 Hz, 6H), 3.77 (h, J = 4.0 Hz, 1H), 3.96 (h, J = 4.0 Hz, 1H), 7.47 (d, J = 8.5 Hz, 2H), 7.86 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.9, 23.0, 40.9, 42.5, 110.8, 117.1, 129.0, 130.7, 134.6, 140.1, 172.4, 186.1. IR (film) 3089, 2966, 2925, 2864, 2203, 1675, 1585 cm^{-1}. MS (ESI$^+$, MeOH) *m/z* 340.0 $[M+H]^+$, 362.0 $[M+Na]^+$. HRMS (ESI$^+$, MeOH) calcd for $C_{16}H_{18}{}^{35}ClNOS_2Na$ $[M+Na]^+$ 362.0416, found 362.0414. Elemental analysis calcd (%) for $C_{16}H_{18}ClNOS_2$: C 56.54, H 5.34, N 4.12; found C 56.52, H 5.43, N 4.19.

Ethyl 3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carboxylate (5)
To a solution (EtOH, 11.5 mL) of **4** (0.9 g, 2.66 mmol, 1.0 equiv.) were added dropwise ethyl thioglycolate (0.26 mL, 2.66 mmol, 1.0 equiv.) and Et_3N (0.41 mL, 2.92 mmol, 1.1 equiv.). After stirring for 3 h at room temperature, water was added to the reaction mixture which was extracted three times with EtOAc. The organic layers were pooled, 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 1/0 to 3/1 (v/v) in 25 min) to afford **5** as a yellowish amorphous solid (0.9 g, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22 (t, J = 7.0 Hz, 3H), 1.47 (d, J = 6.5 Hz, 6H), 3.63 (h, J = 6.5 Hz, 1H), 4.22 (q, J = 7.0 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.43 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 23.4, 42.5, 62.0, 113.7, 115.7, 128.6, 129.5, 130.8, 130.9, 135.6, 148.0, 153.6, 160.2. IR (film) 2983, 2225, 1689, 1371, 1298 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 388 $[M+Na]^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{17}H_{16}{}^{35}ClNO_2S_2Na$ $[M+Na]^+$ 388.0209, found 388.0202.

Ethyl 3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)furan-2-carboxylate (6)
To a solution of LiHMDS (1M in THF, 10.7 ml, 10.7 mmol, 2.7 equiv.) at -78°C was added a solution (THF, 6.5 mL) of ethyl bromoacetate (1.1 mL, 9.8 mmol, 2.5 equiv.). After stirring for 10 min at -78°C, a solution (THF, 13 mL) of **4** (1.34 g, 3.95 mmol, 1.0 equiv.) was added dropwise. After stirring for 45 min at -78°C then 6 h at room temperature, a saturated aqueous NH_4Cl solution was added to the reaction mixture which was extracted three times with EtOAc. The organic layers were pooled, washed with water, 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 8/2 (v/v) in 40 min) to afford **6** as a yellowish oil (0.47 g, 34%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.20 (t, J = 7.0 Hz, 3H) , 1.39 (d, J = 7.0 Hz, 6H), 3.78 (h, J = 7.0 Hz, 1H), 4.22 (q, J = 7.0 Hz, 2H) , 7.37 (d, J = 7.5 Hz, 2H), 7.40 (d, J = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 23.7, 40.3, 61.6, 103.0, 112.0, 126.5, 128.6, 130.9, 134.0, 135.8, 141.2, 157.4, 160.5. IR (film) 2963, 2239, 1726, 1604, 1270 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 372.0 $[M+Na]^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{17}H_{16}{}^{35}ClNO_3SNa$ $[M+Na]^+$ 372.0437, found 372.0435. Elemental analysis calcd (%) for $C_{17}H_{16}ClNO_3S$: C 58.37, H 4.61, N 4.00; found C 58.13, H 4.78, N 3.95.

Ethyl 3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)-1-methyl-1H-pyrrole-2-carboxylate (7)
To a solution (EtOH, 21 mL) of **4** (1.23 g, 3.6 mmol, 1.0 equiv.) were added ethyl sarcosinate (0.61 g, 3.96 mmol, 1.1 equiv.) and Et_3N (1.5 mL, 10.8 mmol, 3.0 equiv.). After stirring under reflux for 5 h, water was added to the cooled reaction mixture that was extracted three times with EtOAc. The organic layers were pooled, washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (heptane/EtOAc 10/0 to 8/2 (v/v) in 40 min) to afford **7** as a yellowish oil (0.61 g, 46%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.07 (t, J = 7.0 Hz, 3H) , 1.34 (d, J = 6.5 Hz, 6H), 3.45 (h, J = 6.5 Hz, 1H), 4.06 (s, 3H), 4.15 (q, J = 7.0 Hz, 2H) , 7.32 (d, J = 8.5 Hz, 2H),

7.86 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 23.3, 35.0, 41.8, 60.9, 103.2, 115.0, 123.2, 128.1, 131.0, 133.8, 134.1, 136.1, 160.3. IR (film) 2966, 2224, 1701, 1531 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 385.1 $[M+Na]^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{18}H_{19}{}^{35}ClN_2O_2SNa$ $[M+Na]^+$ 385.0753, found 385.0753. Elemental analysis calcd (%) for $C_{18}H_{19}ClN_2O_2S$: C 59.58, H 5.28, N 7.72; found C 59.52, H 5.24, N 7.70.

3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carboxylic acid (1)
To a solution (THF/EtOH 2/1, 2 mL) of **5** (71.4 mg, 0.195 mmol, 1.0 equiv.) was added aqueous NaOH (2M, 1 mL). After stirring overnight at room temperature, the mixture was neutralized with aqueous HCl (1M, 2 mL). The precipitated product was filtered, washed with water and dried under vacuum to afford acid **1** as a white powder (61.3 mg, 93%). Mp = 179°C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.48 (d, J = 6.7 Hz, 6H), 3.65 (h, J = 6.7 Hz, 1H), 7.34 (d, J = 8.5 Hz, 1H), 7.42 (d, J = 8.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 23.4, 42.4, 113.4, 115.2, 127.2, 128.8, 130.3, 130.9, 135.9, 149.8, 156.1, 164.5. IR (film) 2960, 2923, 2545, 2234, 1694, 1667, 1484, 1284, 1200, 1084, 870, 826 cm^{-1}. MS (ESI$^-$, CH_2Cl_2/MeOH) *m/z* 336.0 $[M-H]^-$. HRMS (ESI$^-$, CH_2Cl_2/MeOH) calcd for $C_{15}H_{11}{}^{35}ClNO_2S_2$ $[M-H]^-$ 335.9920, found 335.9920. Elemental analysis calcd (%) for $C_{15}H_{12}ClNO_2S_2$: C 55.33, H 3.58, N 4.15; found C 53.47, H 3.75, N 3.98.

3-(4-Chlorophenyl)-4-cyano-5-(isopropylthio)furan-2-carboxylic acid (8)
To a solution (EtOH, 1.6 mL) of **6** (227 mg, 0.65 mmol, 1.0 equiv.) was added aqueous NaOH (2M, 1.6 mL). After stirring for 15 h at room temperature, aqueous HCl (1M, 3.4 mL) was added to the reaction mixture which was extracted twice with CH_2Cl_2. The organic layers were pooled, dried over $MgSO_4$ and concentrated under reduced pressure to afford **8** as a slightly pink amorphous solid (181 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40 (d, J = 7.0 Hz, 6H), 3.80 (h, J = 7.0 Hz, 1H), 7.30 (d, J = 6.5 Hz, 2H), 7.37 (d, J = 6.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.7, 40.2, 102.2, 111.6, 125.9, 128.8, 130.9, 136.1, 136.5, 139.7, 161.8, 162.3. IR (film) 2970, 2925, 2868, 2585, 2228, 1672, 1604 cm^{-1}. MS (ESI$^-$, CH_2Cl_2/MeOH) *m/z* 320.0 $[M-H]^-$. HRMS (ESI$^-$, CH_2Cl_2/MeOH) calcd for $C_{15}H_{11}{}^{35}ClNO_3S$ $[M-H]^-$ 320.0148, found 320.0152.

3-(4-Chlorophenyl)-4-cyano-5-(isopropylthio)-1-methyl-1H-pyrrole-2-carboxylic acid (9)
To a solution (THF/MeOH/H_2O 2/1/1 (v/v), 1.9 mL) of **7** (370 mg, 1.02 mmol, 1.0 equiv.) was added LiOH (73 mg, 3.06 mmol, 3.0 equiv.). After stirring for 3 h at 60°C, aqueous HCl (1M, 3 mL) was added to the cooled reaction mixture which was extracted three times with CH_2Cl_2. The organic layers were pooled, dried over $MgSO_4$ and concentrated under reduced pressure to afford **9** as a white amorphous solid (302 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35 (d, J = 7.0 Hz, 6H), 3.50 (h, J = 7.0 Hz, 1H), 4.06 (s, 3H), 7.36 (d, J = 6.5 Hz, 2H), 7.41 (d, J = 6.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.3, 35.5, 41.8, 103.8, 114.6, 121.4, 128.4, 131.0, 131.1, 134.5, 136.2, 137.9, 164.3. IR (film) 2966, 2929, 2859, 2619, 2545, 2495, 2225, 1659 cm^{-1}. MS (ESI$^-$, CH_2Cl_2/MeOH) *m/z* 333.0 $[M-H]^-$. HRMS (ESI$^-$, CH_2Cl_2/MeOH) calcd for $C_{16}H_{14}{}^{35}ClN_2O_2S$ $[M-H]^-$ 333.0465, found 333.0464. Elemental analysis calcd (%) for $C_{16}H_{15}ClN_2O_2S$: C 57.40, H 4.52, N 8.37; found C 57.46, H 4.74, N 8.25.

Ethyl 3-(4-chlorophenyl)-3-oxopropanoate (10)
To a solution (Et_2O, 88 mL) of LDA prepared in situ ((iPr)$_2$NH: 8.79 mL, 62.4 mmol, 2.0 equiv., and BuLi 1.6M in hexanes: 37.1 mL, 59.3 mmol, 1.9 equiv.) under argon at -78°C was added dropwise a solution (Et_2O, 16 mL) of EtOAc (3.05 mL, 31.2 mmol, 1.0 equiv.). After stirring for 45 min at -78°C a solution (Et_2O, 16 mL) of 4-chlorobenzoyl chloride (4.0 mL,

31.2 mmol, 1.0 equiv.) was added dropwise. The reaction mixture was stirred for 15 h at room temperature and poured onto iced sulfuric acid (260 g of ice and 18.8 mL of concentrated sulfuric acid). The reaction mixture was stirred for 15 min, extracted three times with Et_2O. The organic layers were pooled, dried over $MgSO_4$ and concentrated under reduced pressure to afford **10** as a slight orange oil (5.99 g, 85%). This compound is a mixture of ketone and enol forms (2/1). ^{1}H NMR (500 MHz, $CDCl_3$) (ketone) δ 1.24 (t, J = 7.0 Hz, 3H), 3.95 (s, 2H), 4.20 (q, J = 7.0 Hz, 2H), 7.43 (d, J = 7.0 Hz, 2H), 7.87 (d, J = 7.0 Hz, 2H) ; (enol) δ 1.32 (t, J = 7.5 Hz, 3H), 4.20 (q, J = 7.5 Hz, 2H), 5.62 (s, 1H), 7.37 (d, J = 7.0 Hz, 2H), 7.69 (d, J = 7.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) (ketone) δ 13.0, 44.8, 60.4, 128.0, 128.9, 133.3, 139.1, 166.2, 190.3; (enol) δ 13.2, 59.4, 86.6, 126.3, 127.7, 130.8, 136.2, 168.9, 172.0. IR (film) 2981, 2941, 2908, 1737, 1686, 1642, 1621 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 249.0 $[M+Na]^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{11}H_{11}{}^{35}ClO_3Na$ $[M+Na]^+$ 249.0294, found 249.0300. Elemental analysis calcd (%) for $C_{11}H_{11}ClO_3$: C 58.29, H 4.48, O 21.18; found C 58.43, H 5.0, O 21.06.

Ethyl 2-chloro-3-(4-chlorophenyl)-3-oxopropanoate (11)

To a solution (toluene, 40 mL) of **10** (5.86 g, 25.8 mmol, 1.0 equiv) was added dropwise sulfuryl chloride (2.08 mL, 25.8 mmol, 1.0 equiv.). After stirring for 4 h at room temperature, the reaction was quenched by water and neutralized with a saturated aqueous $NaHCO_3$ solution. The reaction mixture was extracted three times with EtOAc, the organic layers were pooled, washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (heptane/CH_2Cl_2 100/0 to 50/50 in 100 min) to afford **11** as a colorless oil (5.72 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (t, J = 7.0 Hz, 3H), 4.30 (q, J = 7.0 Hz, 2H), 5.56 (s, 1H), 7.49 (d, J = 7.0 Hz, 2H), 7.95 (d, J = 7.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 58.0, 63.3, 129.3, 130.7, 131.3, 141.0, 165.0, 187.2. IR (film) 2983, 1760, 1741, 1688, 1588, 1488, 1177, 1091 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 283.0 $[M+Na]^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{11}H_{10}{}^{35}Cl_2O_3Na$ $[M+Na]^+$ 282.9905, found 282.9875. Elemental analysis calcd (%) for $C_{11}H_{10}Cl_2O_3$: C 50.60, H 3.86, O 18.38; found C 50.56, H 4.09, O 18.18.

Ethyl 2-amino-4-(4-chlorophenyl)thiazole-5-carboxylate (12)

To a solution (EtOH, 50 mL) of **11** (4.41 g, 16.9 mmol, 1.0 equiv) was added thiourea (1.29 g, 16.9 mmol, 1.0 equiv.). After stirring under reflux for 15 h, the reaction mixture was cooled and neutralized with a 28% ammonia solution. The reaction mixture was extracted four times with CH_2Cl_2, the organic layers were pooled, washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to afford **12** as a white amorphous solid (4.69 g, 98%). ^{1}H NMR (300 MHz, DMSO) δ 1.15 (t, J = 7.0 Hz, 3H), 4.09 (q, J = 7.0 Hz, 2H), 7.43 (d, J = 8.5 Hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 7.89 (s, 2H). ^{13}C NMR (75 MHz, DMSO) δ 14.0, 60.1, 108.7, 127.3, 131.4, 161.0, 169.9. IR (film) 3406, 3282, 3094, 2989, 2943, 2713, 1660, 1638, 1597, 1518, 1474, 1085 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 283.0 $[M+H]^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{12}H_{12}{}^{35}ClN_2O_2S$ $[M+H]^+$ 283.0308, found 283.0306. Elemental analysis calcd (%) for $C_{12}H_{11}ClN_2O_2S$: C 50.97, H 3.92, N 9.91; found C 50.86, H 3.88, N 9.96.

Ethyl 2-bromo-4-(4-chlorophenyl)thiazole-5-carboxylate (13)

To a solution (MeCN, 28 mL) of $CuBr_2$ (4.74 g, 21.2 mmol, 3.0 equiv.) was added slowly *t*-butylnitrite (1.26 mL, 10.6 mmol, 1.5 equiv.) at room temperature. A solution (MeCN, 48ml) of **12** (2.0 g, 7.1 mmol, 1.0 equiv) was added dropwise at 60°C to the reaction mixture. After stirring at 60°C for 4 h, the reaction mixture was poured onto aqueous NaOH (1M, 110 mL) and then was extracted three times with EtOAc. The organic layers were pooled, dried over

$MgSO_4$ and concentrated under reduced pressure to afford **13** as a white amorphous solid (2.18 g, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.33 (t, J = 7.0 Hz, 3H), 4.31 (q, J = 7.0 Hz, 2H), 7.43 (d, J = 8.5 Hz, 2H), 7.75 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, DMSO) δ 14.1, 62.1, 126.5, 128.1, 131.0, 131.3, 135.8, 140.1, 158.6, 160.1. IR (film) 2977, 1904, 1718, 1594, 1570, 1519, 1474, 1075, 1015 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 348.0 $[M+H]^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{12}H_{10}{}^{81}Br^{35}ClNO_2S$ $[M+H]^+$ 347.9284, found 347.9290. Elemental analysis calcd (%) for $C_{12}H_9BrClNO_2S$: C 41.58, H 2.62, N 4.04; found C 41.59, H 2.59, N 4.02.

Ethyl 4-(4-chlorophenyl)-2-(isopropylthio)thiazole-5-carboxylate (14)
To a solution (THF, 18 mL) of **13** (0.6 g, 1.74 mmol, 1.0 equiv) was added at 10°C propane-2-thiol (1.77 mL, 1.89 mmol, 1.1 equiv.) and *t*BuOK (0.213 g, 1.89 mmol, 1.1 equiv.). The reaction mixture was stirred at room temperature for 4 h, diluted with water and EtOAc and extracted three times with EtOAc. The organic layers were pooled, 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 70/30 in 25 min) to afford **14** as a colorless oil (0.45 g, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.31 (t, J = 7.0 Hz, 3H), 1.50 (d, J = 7.0 Hz, 6H), 3.97 (h, J = 7.0 Hz, 1H), 4.28 (q, J = 7.0 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H), 7.76 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, DMSO) δ 14.2, 23.1, 39.8, 61.5, 121.8, 127.9, 131.4, 132.2, 135.3, 158.7, 161.0, 169.4. IR (film) 2998, 2974, 1912, 1711, 1593, 1570, 1514, 1477, 1073 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 342.0 $[M+H]^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{15}H_{17}{}^{35}ClNO_2S_2$ $[M+H]^+$ 342.0389, found 342.0389. Elemental analysis calcd (%) for $C_{15}H_{16}ClNO_2S_2$: C 52.70, H 4.72, N 4.10; found C 52.64, H 4.77, N 4.12.

4-(4-Chlorophenyl)-2-(isopropylthio)thiazole-5-carboxylic acid (15)
To a solution (EtOH, 1.1 mL) of **14** (150 mg, 0.44 mmol, 1.0 equiv.) was added aqueous NaOH (2M, 1.1 mL). After stirring for 48 h at room temperature, aqueous HCl (1M, 2.2 mL) was added to the reaction mixture which was stirred for additional 1.5 h at room temperature. The white precipitate was filtered, washed with water and dissolved in CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to afford **15** as a white amorphous solid (131 mg, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (d, J = 7.0 Hz, 6H), 3.88 (h, J = 7.0 Hz, 1H), 7.30 (d, J = 8.5 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.1, 39.9, 120.7, 128.1, 131.5, 131.8, 135.6, 160.2, 166.2, 171.4. IR (film) 2970, 2921, 2861, 2779, 2525, 1672 cm^{-1}. MS (ESI$^-$, CH_2Cl_2/MeOH) *m/z* 312.0 $[M-H]^-$. HRMS (ESI$^-$, CH_2Cl_2/MeOH) calcd for $C_{13}H_{11}{}^{35}ClNO_2S_2$ $[M-H]^-$ 311.9920, found 311.9928. Elemental analysis calcd (%) for $C_{13}H_{12}ClN_2O_2S_2$: C 49.75, H 3.85, N 4.46; found C 49.82, H 4.00, N 4.39.

3-(4-chlorophenyl)-5-(isopropylthio)thiophene-2-carboxylic acid (16)

1-(4-chlorophenyl)-3,3-bis(isopropylthio)prop-2-en-1-one
To a solution (DMF, 2 mL) of 4-chloroacetophenone (175 mg, 1.132 mmol, 1.0 equiv.) was added NaH (60% in oil, 135.8 mg, 3.396 mmol, 3.0 equiv.). The resulting mixture was stirred at room temperature for 1 h before addition of CS_2 (75 µL, 1.245 mmol, 1.1 equiv.) and stirred additionally at room temperature for 4 h. After addition of *i*-propyl iodide (339 µL, 3.39 mmol, 3 equiv.) and tetrabutylammonium bromide (73 mg, 0.226 mmol, 0.2 equiv.), the mixture was stirred overnight at 60°C and concentrated under reduced pressure. The residue was diluted in EtOAc/water 1/1 (20 mL) and the aqueous layer was extracted three times by EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced

pressure. The crude product was purified by flash chromatography on silica gel (heptane 100% for 10 min) to afford the expected ketene dithioacetal as an orange oil (282 mg, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (d, J = 6.7 Hz, 6H), 1.45 (d, J = 6.7 Hz, 6H), 3.62 (h, J = 6.7 Hz, 1H), 3.83 (h, J = 6.7 Hz, 1H), 6.84 (s, 1H), 7.39 (d, J = 8.5 Hz, 2H), 7.82 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.4, 23.9, 36.2, 38.8, 112.4, 128.9, 129.4, 137.9, 138.2, 163.9, 184.6. IR (film) 2962, 2923, 2864, 1469, 1219, 1089, 1010, 962, 785 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 337.1 $[M+Na]^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{15}H_{19}{}^{35}ClOS_2Na$ $[M+Na]^+$ 337.0464, found 337.0466.

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

To a solution (EtOH, 1.5 mL) of the previous ketene dithioacetal (76.8 mg, 0.244 mmol, 1.0 equiv.) were added K_2CO_3 (37.1 mg, 0.268 mmol, 1.1 equiv.) and ethyl thioglycolate (28 µL, 0.256 mmol, 1.05 equiv.) and the reaction mixture was stirred under reflux for 4 h. After cooling to room temperature, the solution was diluted with water (5 mL) and extracted three times with EtOAc. The combined organic layers were 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 6/4 (v/v) in 20 min) to afford the expected ester as a yellow oil (58.9 mg, 71%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25 (t, J = 7.1 Hz, 3H), 1.36 (d, J = 6.7 Hz, 6H), 3.33 (h, J = 6.7 Hz, 1H), 4.28 (q, J = 7.1 Hz, 2H), 6.99 (s, 1H), 7.37 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 23.5, 41.9, 61.4, 128.2, 129.6, 130.8, 134.0, 134.3, 136.3, 141.0, 147.4, 161.4. IR (film) 2962, 2926, 2865, 1713, 1485, 1414, 1242, 1200, 1088, 1072, 1015, 821, 761 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 363.0 $[M+Na]^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{16}H_{17}{}^{35}ClO_2S_2Na$ [M+Na] 363.0256, found 363.0267.

3-(4-chlorophenyl)-5-(isopropylthio)thiophene-2-carboxylic acid (16)

To a solution (THF/EtOH 2/1, 1.5 mL) of the above-mentioned ester (71 mg, 0.227 mmol, 1.0 equiv.) was added aqueous NaOH (2M, 0.5 mL) and the mixture was stirred overnight at room temperature. After neutralization with aqueous HCl (1M, 1 mL), the aqueous layer was extracted three times by EtOAc and the combined organic layers were 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 20 min) to afford **16** as a white solid (41.7 mg, 64%). Mp = 149°C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.38 (d, J = 6.7 Hz, 6H), 3.38 (h, J = 6.7 Hz, 1H), 6.98 (s, 1H), 7.36 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 23.4, 41.9, 127.7, 128.4, 130.8, 133.6, 134.5, 135.8, 143.8, 149.0, 166.6. IR (film) 2961, 2924, 2863, 2621, 2525, 1667, 1485, 1416, 1273, 1090, 824. MS (ESI$^-$, CH_2Cl_2/MeOH) *m/z* 311.0 $[M-H]^-$. HRMS (ESI$^-$, CH_2Cl_2/MeOH) calcd for $C_{14}H_{12}{}^{35}ClO_2S_2$ $[M-H]^-$ 310.9967, found 310.9978.

4-(4-Chlorophenyl)-2- isopropylthio)thiophene-3-carbonitrile (23)

To a solution (quinoline, 2 mL) of **1** (1 g, 2.96 mmol, 1.0 equiv.) was added copper powder (132 mg, 2.07 mmol, 0.7 equiv.). The reaction was stirred at 250°C in a metallic bath for 3 h. The mixture was cooled to room temperature and quenched by HCl (1M, 2 mL) and diluted in CH_2Cl_2. The organic solution was then extracted by CH_2Cl_2, dried over $MgSO_4$ and concentrated under reduced pressure to afford **23** as a white amorphous solid (704 mg, 81 %). Mp = 78°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39 (d, J = 6.5 Hz, 6H), 3.49 (h, J = 6.5 Hz, 1H), 7.35 (s, 1H), 7.43 (d, J = 8.5 Hz, 2H), 7.54 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.4, 43.0, 114.9, 116.0, 125.7, 129.1, 129.4, 132.0, 135.0, 143.2, 148.1. IR (film) 3090, 2965, 2923, 2224, 1481, 1087, 1008, 831, 766 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 348.0

$[M+Na+MeOH]^+$. HRMS (ESI^+, CH_2Cl_2/MeOH) calcd for $C_{15}H_{16}{}^{35}ClNOS_2Na$ $[M+Na+MeOH]^+$ 348,0260, found 348,0264.

3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carboxamide (24)

To a solution (CH_2Cl_2, 1 mL) of **1** (50 mg, 0.148 mmol, 1.0 equiv.) at 0°C was added thionyle chloride (108 µL, 1.48 mmol, 10 equiv.). The reaction mixture was stirred at room temperature for 1 h and concentrated under reduced pressure. The crude product was solubilized in THF (2 mL) and a 28% ammonia solution (10.8 µL, 0.178 mmol, 1.2 equiv.) was added. This solution was stirred at room temperature for 3 h and then diluted by water (2 mL). The aqueous layer was extracted three times by EtOAc and the combined organic layers were washed by brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by flash chromatography on silica gel (heptane/EtOAc 10/0 to 2/8 (v/v) in 20 min) to afford **24** as a yellow powder (42.7 mg, 86%). Mp = 186°C. ^1H NMR (500 MHz, $CDCl_3$) δ 1.46 (d, J = 6.5 Hz, 6H), 3.61 (hept, J = 6.5 Hz, 1H), 5.34 (sb, 1H), 5.64 (sb, 1H), 7.41 (d, J = 8.5 Hz, 2H), 7,54 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.8, 42.5, 113.5, 115.1, 130.1, 130.2, 130.8, 135.9, 136.9, 142.5, 153.6, 161.9. IR (film) 3406, 3286, 3233, 3183, 2972, 2922, 2228, 1650, 1603, 1484, 1392, 1090, 1013, 831, 638 cm^{-1}. MS (ESI^+, CH_2Cl_2/MeOH) *m/z* 359.0 $[M+Na]^+$. HRMS (ESI^+, CH_2Cl_2/MeOH) calcd for $C_{15}H_{13}{}^{35}ClN_2OS_2Na$ $[M+Na]^+$ 359.0056, found 359.0073.

(R)-methyl 2-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carboxamido)-4-(methylthio)butanoate (25)

To a solution (CH_2Cl_2, 3 mL) of methionine methyl ester (49.0 mg, 0.30 mmol, 1.0 equiv.) and **1** (100 mg, 0.30 mmol, 1.0 equiv.) were added HOBt (81.1 mg, 0.6 mmol, 2.0 equiv.) and Et_3N (41.7 µL, 0.30 mmol, 1.0 equiv.) at 0°C. After 5 min, EDCI was added and the reaction was stirred overnight. The reaction was diluted by CH_2Cl_2 and washed by a saturated aqueous Na_2CO_3 solution and 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 15 min) to afford **25** as an amorphous solid (130 mg, 90%). ^1H NMR (300 MHz, $CDCl_3$) δ 1.36 (s, 3H), 1.39 (s, 3H), 1.78 (qi, J = 7.0 Hz, 1H), 1.93 (m, 4H), 2.18 (t, J = 7.5 Hz, 2H), 3.52 (h, J = 6.5 Hz, 1H), 3.61 (s, 3H), 4.59 (q, J = 5.5 Hz, 1H), 6.05 (d, J = 7.5 Hz, 1H), 7.36 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.4, 23.1, 29.6, 31.0, 42.4, 50.0, 52.6, 113.3, 115.3, 129.8, 129.9, 130.7, 136.1, 136.5, 141.8, 152.4, 159.6, 171.1. IR (film) 3401, 2920, 2224, 1740, 1646, 1508, 1483, 1436, 1400, 1366, 1204 cm^{-1}. MS (ESI^+, CH_2Cl_2/MeOH) *m/z* 505.0 $[M+Na]^+$. HRMS (ESI^+, CH_2Cl_2/MeOH) calcd for $C_{21}H_{23}{}^{35}ClN_2O_3S_3Na$ $[M+Na]^+$ 505.0457, found 505.0440.

(R)-2-(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophene-2-carboxamido)-4-(methylthio)butanoic acid (26)

To a solution (MeOH/THF 2/1 (v/v), 12 mL) of **25** (130 mg, 0.27 mmol, 1.0 equiv.) was added aqueous LiOH (1M, 3.8 mL, 3.8 mmol, 14.0 equiv.). After two days at room temperature, the reaction was concentrated under reduced pressure. To the residue were added water (3 mL) and aqueous HCl (1M, 2 mL). The organic phase was extracted three times by EtOAc, 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 3/7 in 20 min) to afford **26** as a pale-orange oil (63 mg, 50 %). ^1H NMR (300 MHz, $CDCl_3$) δ 1.36 (s, 3H), 1.39 (s, 3H), 1.79 (m, 1H), 1.95 (s, 3H), 2.20 (m, 2H), 2.58 (dd, J = 8.5 Hz, J = 3.0 Hz, 1H), 3.52 (m, J = 3.5 Hz, J = 6.5 Hz, 1H), 4.55 (m, 1H), 6.11 (d, J = 7.5 Hz, 1H), 7.48-7.18 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.4, 23.2, 29.7, 30.6, 42.4, 52.2, 113.3,

115.2, 129.8, 129.9, 130.8, 135.6, 136.5, 142.1, 152.8, 160.1, 174.8. IR (film) 3066, 2920, 2849, 2223, 1728, 1633, 1530, 1510 cm^{-1}. MS (ESI^-, CH_2Cl_2/MeOH) *m/z* 467.0 $[M-H]^-$. HRMS (ESI^-, CH_2Cl_2/MeOH) calcd for $C_{20}H_{21}{}^{35}ClN_2O_3S_3$ $[M-H]^-$ 467.0325, found 467.0330.

4-(4-chlorophenyl)-5-(hydroxymethyl)-2-(isopropylthio)thiophene-3-carbonitrile (28)
To a solution (EtOH/THF 2/1, 1.5 mL) of **5** (120 mg, 0.328 mmol, 1.0 equiv.) were added lithium chloride (30.6 mg, 0.722 mmol, 2.2 equiv.) and sodium borohydride (27.3 mg, 0.722 mmol, 2.2 equiv.). After stirring at room temperature for 25 h, the reaction mixture was cooled to 0°C and hydrolyzed by a 10% aqueous citric acid solution. The aqueous layer was extracted three times by CH_2Cl_2 and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by flash chromatography on silica gel (heptane/EtOAc 10/0 to 0/10 (v/v) in 15 min) to provide **28** as a colorless oil (85 mg, 78%). ^1H NMR (500 MHz, $CDCl_3$) δ 1.39 (d, J = 6.5 Hz, 6H), 3.48 (h, J = 6.5 Hz, 1H), 4.72 (s, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.45 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.4, 42.9, 58.5, 114.6, 116.9, 129.3, 130.7, 130.9, 135.2, 139.5, 143.8, 146.0. IR (film) 3411, 2963, 2924, 2864, 2225, 1487, 1090 cm^{-1}. MS (ESI^-, CH_2Cl_2/MeOH) *m/z* 322.0 $[M-H]^-$. HRMS (ESI^-, CH_2Cl_2/MeOH) calcd for $C_{15}H_{13}{}^{35}ClNOS_2$ $[M-H]^-$ 322,0127, found 322,0138. Elemental analysis calcd (%) for $C_{15}H_{13}ClNOS_2$: C 55.63, H 4.36, N 4.32; found C 55.47, H 4.43, N 4.44.

(3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)thiophen-2-yl)methyl acetate (29)
To a solution (CH_2Cl_2, 2 mL) of **28** (100 mg, 0.31 mmol, 1.0 equiv.) were added DMAP (114 mg, 0.93 mmol, 3.0 equiv.) and acetic anhydride (88 µL, 0.93 mmol, 3.0 equiv.). The reaction was stirred at room temperature for 36 h. The mixture was quenched by a saturated aqueous NH_4Cl solution and diluted in CH_2Cl_2. After extraction by CH_2Cl_2, the organic solution was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was then purified by flash chromatography on silica gel (heptane/CH_2Cl_2 10/0 to 15/85 in 20 min) to afford **29** as a white amorphous solid (86 mg, 76%).^1H NMR (300 MHz, $CDCl_3$) δ 1.32 (d, J = 6.7 Hz, 6H), 2.03 (s, 3H), 3.43 (h, J = 6.7 Hz, 1H), 4.97 (s, 2H), 7.29 (d, J = 8.5 Hz, 2H), 7.39 (d, J = 8.5 Hz, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 20.8, 23.2, 42.6, 58.6, 114.2, 116.0, 129.2, 130.3, 130.6, 135.2, 136.8, 142.2, 147.6, 170.5. IR (film) 2964, 2925, 2358, 2225, 1741, 1488, 1368, 1216 cm^{-1}. MS (ESI^-, CH_2Cl_2/MeOH) *m/z* 388.0 $[M+Na]^+$. HRMS (ESI^-, CH_2Cl_2/MeOH) calcd for $C_{17}H_{16}{}^{35}ClNO_2S_2Na$ $[M+Na]^+$ 388.0209, found 388.0225.

3-(4-chlorophenyl)-4-cyano-5-(isopropylthio)-N-methoxy-N-methylthiophene-2-carboxamide (30)
To a solution (THF, 1.5 mL) of ester **5** (56.3 mg, 0.154 mmol, 1.0 equiv.) and N,O-dimethylhydroxylamine (45 mg, 0.462 mmol, 3.0 equiv.) at -15°C was added *i*-propylmagnesium chloride (1.8M in THF, 0.513 mL, 0.923 mmol, 6.0 equiv.). The reaction mixture was stirred at -15°C for 1.5 h and quenched by a saturated aqueous NH_4Cl solution. The aqueous layer was extracted three times by EtOAc and the 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 6/4 (v/v) in 15 min) to afford **30** as a white oil (46.8 mg, 80%). ^1H NMR (500 MHz, $CDCl_3$) δ 1.42 (d, J = 6.5 Hz, 6H), 3.18 (s, 3H), 3.58 (h, J = 6.5 Hz, 1H), 3.60 (s, 3H), 7.35 (d, J = 8.5 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.4, 33.5, 42.7, 61.8, 114.0, 116.1, 129.1, 130.2, 131.0, 131.9, 135.3, 145.7, 150.3, 161.2. IR (film) 2964, 2925, 2864, 2225, 1643, 1484, 1367, 1089, 1014, 981, 829 cm^{-1}. MS (ESI^+, CH_2Cl_2/MeOH) *m/z* 403.0 $[M+Na]^+$. HRMS (ESI^+, CH_2Cl_2/MeOH) calcd for $C_{17}H_{17}{}^{35}ClN_2O_2S_2Na$ $[M+Na]^+$ 403.0318, found 403.0317 and calcd for $C_{17}H_{17}{}^{37}ClN_2O_2S_2Na$ $[M+Na]^+$ 405.0288, found 405.0297.

5-acetyl-4-(4-chlorophenyl)-2-(isopropylthio)thiophene-3-carbonitrile (31)
To a solution (THF, 1 mL) of **30** (38.6 mg, 0.101 mmol, 1.0 equiv.) at -78°C was added methyllithium (1.2 M in THF, 0.127 mL, 0.152 mmol, 1.5 equiv.). The mixture was stirred at -78°C for 30 min and quenched with a saturated solution of ammonium chloride. The aqueous layer was extracted three times with EtOAc and the combined organic layers were 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 20 min) to afford **31** as an off-white solid (24 mg, 74%). Mp = 61°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.48 (d, J = 6.7 Hz, 6H), 2.03 (s, 3H), 3.65 (h, J = 6.7 Hz, 1H), 7.33 (d, J = 8.5 Hz, 2H), 7.50 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.4, 29.1, 42.3, 113.4, 114.7, 129.5, 130.7, 131.3, 136.4, 141.1, 146.1, 156.2, 190.2. IR (film) 2966, 2920, 2862, 2228, 1650, 1483, 1364, 1271, 1087, 1014, 860, 824 cm^{-1}. MS (ESI$^+$, CH_2Cl_2/MeOH) *m/z* 358.0 $[M+Na]^+$. HRMS (ESI$^+$, CH_2Cl_2/MeOH) calcd for $C_{16}H_{14}{}^{35}ClNOS_2Na$ $[M+Na]^+$ 358.0103, found 358.0105.

PURITY OF TESTED COMPOUNDS

The purity for tested compounds were measured using reversed-phase UHPLC (HSS C-18, 2.1 x 50 mm s-1.8 µM) with two diverse 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).

Table listing the degree of purity for tested compounds (retention time and percent).

Compound	System 1 (MeCN)	System 2 (MeOH)
1	2.97 min (95.5%)	3.53 min (95.5%)
2	2.83 min (95%)	3.40 min (94.5%)
8	2.87 min (90.5%)	3.46 min (92%)
9	2.88 min (95.5%)	3.43 min (96.5%)
15	3.14 min (96.5%)	3.75 min (97%)
16	2.97 min (95%)	3.53 min (95.5%)
17	2.66 min (97%)	3.23 min (96%)
18	3.16 min (>98%)	3.69 min (>98%)
19	3.14 min (95%)	3.67 min (95%)
20	3.12 min (>99%)	3.66 min (>99%)
21	3.32 min (93.5%)	3.82 min (93.5%)
22	2.45 min (>99%)	3.07 min (>99%)
23	3.51 min (97%)	3.75 min (96%)
24	2.80 min (95%)	3.25 min (95.5%)
25	3.33 min (>98%)	3.60 min (>98%)
26	3.00 min (95%)	3.49 min (>98%)
27	2.83 min (>98%)	3.24 min (>98%)
28	3.03 min (95.5%)	3.43 min (95.5%)
29	3.42 min (97.5%)	3.69 min (97.5%)
30	3.17 min (95.5%)	3.47 min (95%)
31	3.33 min (95%)	3.60 min (95%)

BIOLOGICAL ASSAYS

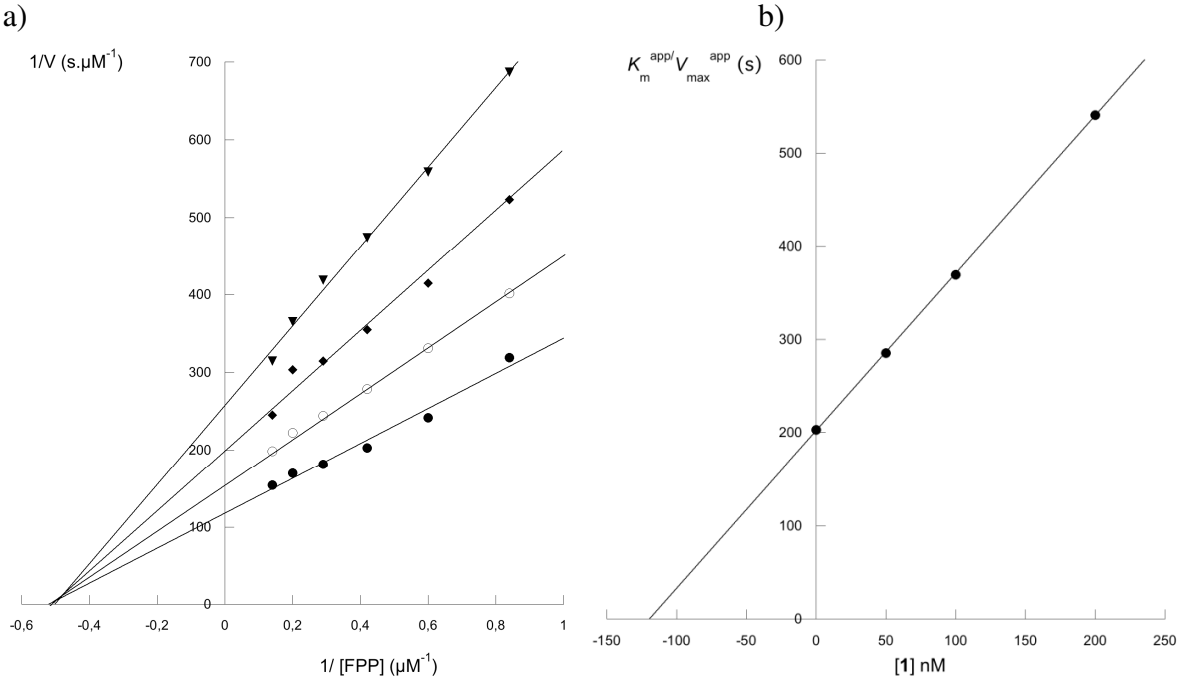
FTase assay

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 seconds 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.

The kinetic experiments were realized under the same conditions either with FPP as varied substrate with constant concentration of Dns-GCVLS of 2.5 µM or with Dns-GCVLS as varied substrate with constant concentration of FPP of 10 µM. Non linear regressions were made by KaleidaGraph 4.03 software. K_m^{app} and V_{max}^{app} were obtained from Michaelis-Menten fits of fluorescent data and K_i was deduced from the linear regression of K_m^{app}/V_{max}^{app} as a function of the inhibitor concentration respectively.

Double reciprocal graphs

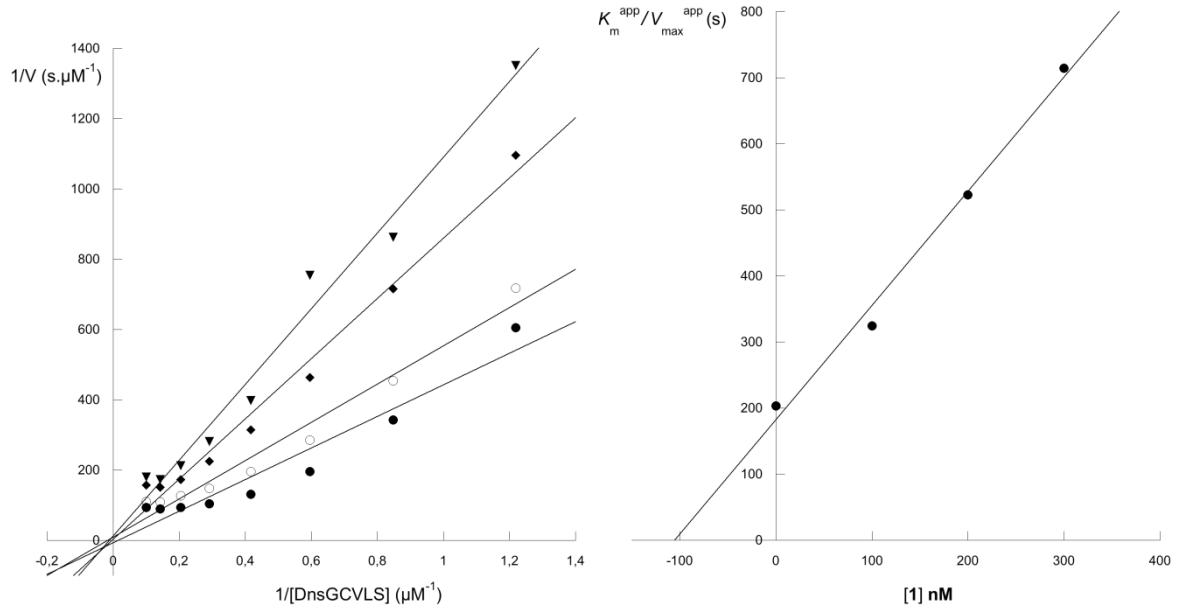
Competition with FPP : Double reciprocal with FPP as the varied substrate and fixed concentration of DnsGCVLS.



a) Concentrations of **1** were: 200 nM (▾), 100 nM (◆), 50 nM (○), 0 nM (●); b) K_i^{app} measured from competition with FPP.

Competition with DnsGCVLS : Double reciprocal with DnsGCVLS as the varied substrate and fixed concentration of FPP.

a) b)



a) Concentrations of **1** were: 300 nM (▾), 200 nM (◆), 100 nM (○), 0 nM (●); b) K_i^{app} measured from competition with DnsGCVLS.