On the discovery of new potent human farnesyltransferase inhibitors: emerging pyroglutamic derivatives

Supplementary information

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Materials and methods for synthesis and characterizations

Starting materials are commercially available and were used without further purification (suppliers: Carlo Erba Reagents S.A.S., Thermo Fisher Scientific Inc., and Sigma-Aldrich Co.). Intermediates were synthesized according to the methods described in the literature (references are given below, in figure S1). Melting points were measured on a MPA 100 OptiMelt[®] apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were acquired at 400 MHz for ¹H NMR and at 100 MHz for ¹³C NMR on a Varian 400-MR spectrometer with tetramethylsilane (TMS) as internal standard, at room temperature (RT). Chemical shifts (δ) are expressed in ppm relative to TMS. Splitting patterns are designed: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet; sym m, symmetric multiplet; br s, broaden singlet; br t, broaden triplet. Coupling constants (J) are reported in Hertz (Hz). Thin layer chromatographies (TLC) were realized on Macherey Nagel silica gel plates with fluorescent indicator and were visualized under a UV-lamp at 254 nm and 365 nm. Column chromatographies were performed with a CombiFlash Rf Companion (Teledyne-Isco System) using RediSep packed columns. IR spectra were recorded on a Varian 640-IR FT-IR Spectrometer. UV spectra were recorded on a Jenway 6800 UV/Vis spectrometer. Elemental analyses (C, H, N) of new compounds were determined on a Thermo Electron apparatus by 'Pôle Chimie Moléculaire-Welience', Faculté de Sciences Mirande, Université de Bourgogne, Dijon, France. LC-MS was accomplished using an HPLC combined with a Surveyor MSQ (Thermo Electron) equipped with APCI source. Mass spectra were acquired on a LTQ Orbitrap XL (Thermo Scientific) apparatus equipped with electrospray ionization source in positive and negative mode. Mass spectra samples were first dissolved in DMSO then diluted in methanol to obtain a final concentration of approximatively 10⁻⁵ M.

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7d(ref 5)7e(ref 2)7g (ref 6)7h (ref 5)6 (ref 7)%8d(ref 1)Figure S1. Reaction intermediates, synthesized as described in the literature (references are given at the end of this document).

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Synthesis of compound 1f and 1h using Bredereck's reagent.

General procedure A for the synthesis of tetrahydro-1H-pyrrolo[1,2-c]imidazole-1,5(6H)-dione derivatives 1f and 1h using Bredereck's reagent:

Pyroglutamide derivative (1 equiv) was added to Bredereck's reagent (5 equiv). The mixture was stirred for 24 hours at 120°C under nitrogen atmosphere. Volatiles were then evaporated under low pressure and the resulting mixture was purified by direct crystallization in diethyl ether or purified by column chromatography on silica gel to afford pure compound.



Figure S2. Synthesis and yields of tetrahydro-1*H*-pyrrolo[1,2-*c*]imidazole-1,5(6*H*)-dione derivatives **1f** and **1h** using Bredereck's reagent.



(3R, 6E, 8S)-3-(Dimethylamino)-6-[(dimethylamino)methylene]-2-[(1R)-1-phenylethyl]tetrahydro-1H-pyrrolo[1,2c]imidazole-1,5(6H)-dione (**1f**). The general procedure *A* was followed using *N*-[(1R)-1-phenylethyl]-*L*-pyroglutamide **7f** (0.20 g, 0.86 mmol). Et₂O/EtOH (95/5) was then added and a precipitate was formed, which was filtered to afford pure **1f** as an orange powder in 61% yield (0.18 g, 0.52 mmol). mp (Et₂O) 172-183 °C; TLC R*f* (CH₂Cl₂/MeOH: 95/5) 0.4; IR (neat): v cm⁻¹ 3271, 1681, 1657, 1614, 1537, 696; ¹H NMR (CDCl₃, 400 MHz): δ ppm 1.65 (d, *J* = 7.4 Hz, 3H, CHC*H*₃), 2.20 (s, 6H, NCHN(C*H*₃)₂), 3.00 (s, 6H, CCHN(C*H*₃)₂), 3.24 (m, 2H, CC*H*₂CH), 4.13 (dd, *J* = 10.0, 4.8 Hz, 1H, CCH₂C*H*), 5.11 (s, 1H, NC*H*N(CH₃)₂), 5.23 (q, *J* = 6.9 Hz, 1H, CHCH₃), 6.97 (br s, 1H, CCHN(CH₃)₂), 7.32 (m, 5H, Ar*H*); ¹³C NMR (CDCl₃, 100 MHz): δ ppm 17.6 (CH₃), 26.9 (CH₂), 38.2 (2 CH₃), 41.8 (2 CH₃), 51.5 (CH), 58.6 (CH), 89.1 (CH), 91.7 (C), 127.9 (CH), 128.2 (2 CH), 128.5 (2 CH), 138.7 (C), 145.6 (CH), 172.7 (C), 178.8 (C). Anal. Calcd for C₁₉H₂₆N₄O₂: C, 66.64; H, 7.65; N, 16.36. Found: C, 66.66; H, 7.66; N, 16.37%.





(*6E*,*8S*)-*3*-(*Dimethylamino*)-*6*-[(*dimethylamino*)*methylene*]-*2*-[*2*-(*dimethylamino*)-*1*-*pyridin*-*4*-*ylvinyl*]*tetrahydro*-1*Hpyrrolo*[1,2-*c*]*imidazole*-1,*5*(*6H*)-*dione* (**1h**). The general procedure *A* was followed using *N*-(4-pyridylmethyl)-*L*pyroglutamide **7h** (2.00 g, 9.12 mmol). The desired product crystallizes in Et₂O/CH₂Cl₂ (9/1) as a yellow powder, which was filtered to afford pure **1h** in 47% yield (1.37 g, 12.43 mmol). mp (Et₂O) 163-164 °C; TLC R*f* (CH₂Cl₂/MeOH: 95/5) 0.05; IR (neat): v cm⁻¹ 3468, 2942, 2892, 1703, 1586, 1033; ¹H NMR (DMSO-*d*₆, 400 MHz): δ ppm 2.18 (s, 6H, NCHN(CH₃)₂), 2.72 (s, 6H, ArCCHN(CH₃)₂), 3.00 (s, 6H, CH₂CCHN(CH₃)₂), 3.05 (m, 1H, CCH₂CH), 3.37 (m, 1H, CCH₂CH), 4.46 (m, 1H, CCH₂CH), 5.43 (s, 1H, NCHN), 6.94 (br s, 1H, CH₂CCHN(CH₃)₂), 7.04 (s, 1H, ArCCHN(CH₃)₂), 7.09 (d, *J* = 5.3 Hz, 1H, Ar*H*), 8.22 (d, *J* = 5.3 Hz, 1H, Ar*H*); ¹³C NMR (DMSO-*d*₆, 100 MHz): δ ppm 25.9 (CH₂), 40.1 (2 CH₃), 41.3 (2 CH₃), 41.6 (2 CH₃), 56.3 (CH), 90.7 (C), 90.8 (CH), 101.5 (C), 116.8 (2 CH), 140.8 (CH), 145.1 (CH), 146.6 (C), 148.6 (2 CH), 174.3 (C), 177.5 (C). Anal. Calcd for C₂₀H₂₈N₆O₂: C, 62.48; H, 7.34; N, 21.86. Found: C, 62.45; H, 7.34; N, 21.84%.



Ring opening - Synthesis of compounds 2c, 2e, and 2f

General procedure **B** for the ring opening of pyrroloimidazoldiones 1c, 1e, and 1f:

Pyrroloimidazoledione was stirred in MeOH (20 mL) at RT overnight. Et_2O was added and the resulting solid was filtered to afford pure compound.



Figure S3. Synthesis and yields of compounds **2c**, **2e**, and **2f** by ring opening of pyrroloimidazolediones **1c**, **1e**, and **1f**.



(*4E*)-*4*-[(*Dimethylamino*)*methylene*]-*N*-(2-*methoxybenzy*])-*L*-*pyroglutamide* (2c). The general procedure *B* was followed using (3*R*,6*E*,8*S*)-3-(dimethylamino)-6-[(dimethylamino)methylene]-2-(2-methoxybenzyl)tetrahydro-1*H*-pyrrolo[1,2-*c*]imidazole-1,5(6*H*)-dione **1c** (0.30 g, 0.84 mmol). The desired product crystallizes in Et₂O as a white powder, which was filtered to afford pure **2c** in 59% yield (0.15 g, 0.49 mmol). TLC *Rf* (CH₂Cl₂/MeOH: 95/5) 0.6; IR (neat): v cm⁻¹ 3265, 2924, 1655, 1600, 1237; ¹H NMR (CDCl₃, 400 MHz): δ ppm 2.97 (s, 6H, N(*CH*₃)₂), 3.01 (dd, *J* = 15.4, 5.5 Hz, 1H, CCH₂CH), 3.50 (dd, *J* = 15.4, 10.4 Hz, 1H, CCH₂CH), 3.85 (s, 3H, OCH₃), 4.11 (ddd, *J* = 10.4, 5.5, 2.1 Hz, 1H, CCH₂CH), 4.40 (dd, *J* = 14.5, 5.6 Hz, 1H, NHCH₂), 4.49 (dd, *J* = 14.5, 6.0 Hz, 1H, NHCH₂), 5.23 (br s, 1H, NHCH₂), 6.88 (d, *J* = 7.9 Hz, 1H, ArH), 6.89 (br s, 1H, CCHN(CH₃)₂), 6.92 (dd, *J* = 7.4, 0.8 Hz, 1H, ArH), 6.95 (t, *J* = 1.8 Hz, 1H, NHCH₂), 7.25 (dd, *J* = 5.9, 1.5 Hz, 1H, ArH), 7.28 (dd, *J* = 7.7, 1.7 Hz, 1H, ArH); ¹³C NMR (CDCl₃, 100 MHz): δ ppm 30.6 (CH₂), 39.3 (CH₂), 41.7 (2 CH₃), 54.5 (CH), 55.4 (CH₃), 90.7 (C), 110.4 (CH), 120.7 (CH), 125.7 (C), 129.1 (CH), 129.9 (CH), 144.1 (CH), 157.6 (C), 172.9 (C), 176.2 (C). Anal. Calcd for C₁₆H₂₁N₃O₃: C, 63.35; H, 6.98; N, 13.85. Found: C, 63.36; H, 7.01; N, 13.84%.





(*4E*)-*4*-[(*Dimethylamino*)*methylene*]-*N*-[(*1S*)-1-*phenylethyl*]-*L*-*pyroglutamide* (*2e*). The general procedure *B* was followed using (3*R*,6*E*,8*S*)-3-(dimethylamino)-6-[(dimethylamino)methylene]-2-[(1*S*)-1-phenylethyl]tetrahydro-1*H*-pyrrolo[1,2-*c*]imidazole-1,5(6*H*)-dione **1e** (0.32 g, 0.93 mmol). The desired product crystallizes in Et₂O as a white powder, which was filtered to afford pure **2e** in 67% yield (0.18 g, 0.63 mmol). mp (Et₂O) 99-100 °C; TLC R*f* (CH₂Cl₂/MeOH: 95/5) 0.2; IR (neat): v cm⁻¹ 3238, 2920, 1660, 1553; ¹H NMR (CDCl₃, 400 MHz): δ ppm 1.47 (d, *J* = 7.1 Hz, 3H, CHCH₃), 2.95 (m, 1H, CCH₂CH), 2.94 (s, 6H, N(CH₃)₂), 3.41 (m, 1H, CCH₂CH), 4.13 (m, 1H, CCH₂CH), 5.09 (q, *J* = 7.1 Hz, 1H, CHCH₃), 6.12 (br s, 1H, NHCHCH₂), 6.88 (br s, 1H, CCHN(CH₃)₂), 6.91 (t, *J* = 1.7 Hz, 1H, NHCHCH₃), 7.23 (m, 5H, Ar*H*); ¹³C NMR (CDCl₃, 100 MHz): δ ppm 22.1 (CH₃), 30.0 (CH₂), 42.9 (2 CH₃), 48.6 (CH), 54.6 (CH), 86.9 (C), 126.5 (2 CH), 127.4 (CH), 128.6 (2 CH), 139.8 (CH), 143.9 (C), 170.6 (C), 171.1 (C). Anal. Calcd for C₁₆H₂₁N₃O₂: C, 66.88; H, 7.37; N, 14.62. Found: C, 66.88; H, 7.36; N, 14.65%.





(*4E*)-*4*-[(*Dimethylamino*)*methylene*]-*N*-[(*1R*)-*1*-*phenylethyl*]-*L*-*pyroglutamide* (*2f*). The general procedure *B* was followed using (3*R*,6*E*,8*S*)-3-(dimethylamino)-6-[(dimethylamino)methylene]-2-[(1*R*)-1-phenylethyl]tetrahydro-1*H*-pyrrolo[1,2-*c*]imidazole-1,5(6*H*)-dione **1f** (0.25 g, 0.73 mmol). The desired product crystallizes in Et₂O as a white powder, which was filtered to afford pure **2f** in 71% yield (0.15 g, 0.52 mmol). mp (Et₂O) 100-101 °C; TLC *Rf* (CH₂Cl₂/MeOH: 95/5) 0.2; IR (neat): v cm⁻¹ 3236, 2923, 1659, 1546; ¹H NMR (CDCl₃, 400 MHz): δ ppm 1.74 (d, *J* = 6.6 Hz, 3H, CHCH₃), 2.90 (m, 1H, CCH₂CH), 2.94 (s, 6H, N(CH₃)₂), 3.46 (m, 1H, CCH₂CH), 4.13 (m, 1H, CCH₂CH), 5.09 (dd, *J* = 8.3, 7.0 Hz, 1H, CHCH₃), 5.49 (br s, 1H, NHCHCH₂), 5.74 (s, 1H, CCHN(CH₃)₂), 6.69 (br s, 1H, NHCHCH₃), 6.94 (t, *J* = 1.6 Hz, 1H, ArH), 7.29 (m, 4H, ArH); ¹³C NMR (CDCl₃, 100 MHz): δ ppm 22.1 (CH₃), 30.0 (CH₂), 42.9 (2 CH₃), 48.4 (CH), 54.6 (CH), 86.9 (C), 126.5 (2 CH), 127.3 (CH), 128.6 (2 CH), 138.7 (CH), 143.9 (C), 170.6 (C), 171.1 (C). Anal. Calcd for C₁₆H₂₁N₃O₂: C, 66.88; H, 7.37; N, 14.62. Found: C, 66.88; H, 7.36; N, 14.65%.



Aminolysis - Synthesis of compound 7f.



Figure S2. Synthesis and yield of pyroglutamide 7f by aminolysis.

N-[(1*R*)-1-*Phenylethyl*]-*L*-*pyroglutamide* (7*f*). A mixture of (*S*)-methyl pyroglutamate 7 (5.91 g, 41.26 mmol), (1*R*)-1-phenylethylamine (5.00 g, 41.26 mmol) and PTSA (0.39 g, 2.06 mmol) was stirred for 24 hours at 120°C under nitrogen atmosphere. The desired product crystallizes in CH₃CN as a white powder, which was filtered to afford pure **7f** in 67% yield (6.43 g, 27.68 mmol). mp (EtOH) 149-151 °C; TLC R*f* (CH₂Cl₂/MeOH: 95/5) 0.2; IR (neat): v cm⁻¹ 3293, 3245, 1680, 1650, 1563, 1260, 697; ¹H NMR (CDCl₃, 400 MHz): δ ppm 1.51 (d, *J* = 6.8 Hz, 3H, CHC*H*₃), 2.12 (m, 1H, CH₂CH₂CH), 2.31 (br t, *J* = 8.25 Hz, 2H, CH₂CH₂CH), 2.51 (m, 1H, CH₂CH₂CH), 4.16 (dd, *J* = 8.7, 5.3 Hz, 1H, CH₂CH₂CH), 5.13 (m, 1H, CHCH₃), 6.39 (br s, 1H, NHCHCH₂), 6.45 (br d, *J* = 8.1 Hz, 1H, NHCHCH₃), 7.30 (m, 5H, Ar*H*); ¹³C NMR (CDCl₃ 100 MHz): δ ppm 21.6 (CH₃), 26.0 (CH₂), 29.2 (CH₂), 48.9 (CH), 57.0 (CH), 126.0 (2 CH), 127.6 (CH), 128.8 (2 CH), 142.6 (C), 170.9 (C), 179.1 (C). Anal. Calcd for C₁₃H₁₆N₂O₂: C, 67.22; H, 6.94; N, 12.06. Found: C, 67.20; H, 6.99; N, 12.05%.



Chiral chromatography

Materiel and Methods

Chiral Supercritical Fluid Chromatography Apparatus:

Stationary phases:

The chiral analytical column used for this study, was a Chiralpak AD-H, purchased from Chiral Technologies Europe (Illkirch, France). This column had dimensions 250 mm x 4.6 mm i.d. with 5 μ m particle size and was coated on a silica-gel support.

Chromatographic system and conditions:

The chromatographic system used was an SFC-PICLAB hybrid 10-20 apparatus (PIC Solution, Avignon, France) equipped with an autosampler comprised a 48-vial plate and a 24-vial plate (model Alias, Emmen, Netherlands), three model 40P pumps: two for CO₂ and a third for the modifier (Knauer, Berlin, Germany). a column oven with a Valco ten-position column selection valve, and a Valco six-position solvent switching valve. The proportion of the modifier in the mobile phase was adjusted by a piston pump. It was then directly added in the CO_2 feeding, and the mixture of the both (modifier and CO_2) was pumped by another piston pump at the total flow rate. The pump head used for pumping the CO_2 was cooled to $-8^{\circ}C$ by a cryostat (model Minichiller, Huber, Offenburg, Germany). The injection valve was supplied with 50 or 100 µL sample loops. The system was also composed of a Smartline 2600 diode array detector (DAD) (Knauer, Berlin, Germany). Detection wavelength was set at 210 nm. After the detector, the outlet pressure was controlled by a back-pressure regulator (BPR). The outlet regulator tube was heated to 55°C to avoid ice formation during the CO_2 depressurization. The system was controlled and the data were acquired with the SFC PicLab Analytic Online v.3.1.2 software and the data were processed with the Analytic Offline v.3.2.0 software (PIC Solution, Avignon, France). During the separation optimization, the mobile phase was CO₂modifier mixtures with the proportion of methanol of 20%, flow rate was 4 mL/min. All analyses were run in isocratic mode. The column oven temperature was 40°C and the outlet pressure was maintained at 120 bar for all experiments.

<u>Results</u>

Chiral chromatography of compound **1e** resulted in one isomer at initial time (t=0 min). The sample was reintroduced after 30 minutes and 2 hours, respectively, and showed the degradation of compound **1e**.



Chelation assay⁸

The interaction of pyroglutamide derivatives with divalent cations was studied in methanolic solution by mixing solutions of ligand and cation.

Samples were prepared from a 10^{-4} mol.L⁻¹ solution of ligands and a 10^{-4} mol.L⁻¹ solution of Zn(OAc)₂ or Mg(OAc)₂. Spectra of pure ligand, and spectra of pure Zn(OAc)₂ and Mg(OAc)₂ were recorded with methanol as the reference. Spectra of the mixtures of n% ligands and (100-n)% of Zn(OAc)₂ or Mg(OAc)₂ were recorded with n% ligands and (100-n)% of NaOAc as the reference.

















References:

¹ Homerin, G.; Lipka, E.; Rigo, B.; Millet, R.; Dezitter, X.; Furman, C.; Ghinet, A. *Tetrahedron*, 2017, in press, accepted manuscript, <u>https://doi.org/10.1016/j.tet.2017.07.036</u>.

² Ellis, T. K.; Ueki, H.; Tiwari, R.; Soloshonok, V. A. Tetrahedron: Asymmetry 2009, 20, 2629.

³ Brunel, S.; Brumas, V.; Fiallo, M. M. L. Inorg. Chim. Acta 2009, 342, 4043.

⁴ Koehn, U.; Schramm, A.; Kloss, F.; Goerls, H.; Arnold, E.; Anders, E. *Tetrahedron: Asymmetry* **2007**, *18*, 1735.

⁵ Homerin, G.; Baudelet, D.; Dufrenoy, P.; Rigo, B.; Lipka, E.; Dezitter, X.; Furman, C.; Millet, R.; Ghinet, A. *Tetrahedron Lett.* **2016**, *57*, 1165.

⁶ Baudelet, D.; Daich, A.; Rigo, B.; Lipka, E.; Gautret, P.; Homerin, G.; Claverie, C.; Rousseau, J.; Abuhaie, C.-M.; Ghinet, A. *Synthesis* **2016**, *48*, 2226.

⁷ Caulier, P.; Rigo, B.; Fasseur, D.; Couturier, D. J. Heterocycl. Chem. 1991, 28, 1143.

⁸ Billamboz, M.; Bailly, F.; Lion, C.; Touati, N.; Vezin, H.; Calmels, C.; Andréola, M.-L.; Christ, F.; Debyser, Z.; Cotelle, P. *J. Med. Chem.* **2011**, *54*, 1812.