Synthesis of Four SIRT1 Activators Based on an Imidazo[1,2-b]thiazole Structure, in vitro Derived Metabolites and Deuterated Analogs

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Chemicals, Synthesis procedure, HRMS, 1H NMR and 13C NMR data of the synthesized compounds.

CHEMICALS

All solvents (HPLC grade) were used without purification. Reactions involving air- or moisture-sensitive reagents were carried out under an argon atmosphere. All products were characterized by 1H NMR, 13C NMR, HRMS and HPLC. Analytical TLC was performed using TLC plates with 0.2 mm thickness (silica gel 60 F254, 5 x 7.5 cm), and visualization was accomplished with UV light, KMnO4- and ninhydrin-reagent. Column chromatography was performed using silica gel 60 (70-230 mesh). Proton NMR spectra were performed in deuterated solvents (CDCl3, D2O, DMSO and CD3OH). 1H NMR spectra (500/600 MHz) and are reported as follows: chemical shift in parts per million downfield from TMS as internal standard (δ scale), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration and coupling constant (Hz). All 13C NMR spectra (150.9/125.7 MHz) are recorded at 25°C as DEPTQ experiments and are reported in parts per million. Mass spectra were measured by direct infusion; products were characterized by HRMS, NMR data of the synthesized compounds.

SYNTHESIS PROCEDURE

6-(2-Nitrophenyl)-imidazo[2,1-b]thiazole-3-carboxylic acid ethyl ester (7)

Ethyl-2-amino-4-thiazol carboxylate (5) (1.00 g, 5.81 mmol) and bromo-2'-nitro acetophenone (6) (1.42 g, 5.81 mmol) were dissolved in absolute ethanol (15 mL) and stirred under reflux for 22 h. The reaction mixture was concentrated under reduced pressure, cooled, poured into ice water and basified with potassium carbonate. Subsequent to extraction with ethyl acetate the product was concentrated under reduced pressure and crystallized from ethanol yielding 802 mg of 6-(2-nitrophenyl)-imidazo[2,1-b]thiazole-3-carboxylic acid ethyl ester (7) as an orange crystalline solid (44%).

HRMS (ESI(+)-MS) calculated for C12H12N3O4S [M+H]+ 276.0437; found: 276.0435.

1H NMR (600.2 MHz, D6-DMSO): δ [ppm] = 8.15 (s, 1 H), 7.84 (d, 1 H, J=7.93 Hz), 7.79 (d, 1 H, J=8.02 Hz), 7.68 (t, 1 H, J=7.60 Hz), 7.52 (t, 1 H, J=7.75 Hz), 7.17 (s, 1 H), 5.67 (s, 1 H, J=5.89 Hz), 4.68 (d, 2 H, J=5.83 Hz).

13C NMR (150.9 MHz, D6-DMSO): δ [ppm] = 149.1 (Cq, arom), 148.4 (Cq, arom), 141.3 (Cq, arom), 132.6 (Cq, arom), 132.0 (CHarom), 129.7 (CHarom), 128.4 (CHarom), 126.8 (Cq, arom), 123.6 (CHarom), 110.7 (CHarom), 109.8 (CHarom), 55.9(CH3).

[6-(2-Nitrophenyl)imidazo[2,1-b]thiazol-3-yl]methanol (8)

6-(2-nitrophenyl)-imidazo[2,1-b]thiazole-3-carboxylic acid ethyl ester (7) (200 mg, 0.63 mmol) was dissolved in 3 mL DCM (abs.) under argon atmosphere and cooled to -78°C. Then 1 M DIBAL-H in DCM (1.3 mL, 1.26 mmol) was added slowly over 30 min to the stirring reaction mixture. The reaction mixture was stirred at -78°C for 1 h, quenched with aqueous saturated NH4Cl solution and warmed to room temperature. The white solid was removed by filtration, the resulting solution was separated between water and DCM and the aqueous layer was extracted with DCM (2x 15 mL). The combined organic layers were washed with brine, dried with Na2SO4 and concentrated under reduced pressure. The resulting crude product was purified by silica gel column chromatography (heptane/ethyl acetate/methanol 8:8:1) to afford 118 mg of 6-(2-nitrophenyl)imidazo[2,1-b]thiazol-3-yl)methanol (8) as a yellow crystalline solid (68%).

HRMS (ESI(+)-MS) calculated for C14H12N3O4S [M+2H]+ 318.0543; found: 318.0540.
3-(Chloromethyl)-6-(2-nitrophenyl)imidazo[2,1-b]thiazole (9)

6-(2-nitrophenyl)imidazo[2,1-b]thiazol-3-yl)methanol (8) (840 mg, 3.05 mmol) was dissolved in 20 mL of DCM, and SOCl₂ (4.12 g, 30.50 mmol) and 0.2 mL DMF were added. The reaction mixture was stirred at 30 °C for 2 h before it was cooled to 0 °C and filtrated to afford 887 mg of 3-(chloromethyl)-6-(2-nitrophenyl)imidazo[2,1-b]thiazole (9) as a colorless crystalline solid (99%). The product was used without additional purification for the next step.

HRMS (ESI(+)-MS) calculated for C₁₂H₁₂N₃O₃S [M+H]+ 294.0099; found: 294.0093.

1H NMR (600.2 MHz, D6-DMSO): δ [ppm] = 8.25 (s, 1 H), 7.85 (d, 1 H, J=7.65 Hz), 7.82 (d, 1 H, J=7.65 Hz), 7.70 (t, 1 H, J=7.65 Hz), 7.55 (t, 1 H, J=7.65 Hz), 7.51 (s, 1 H), 5.11 (s, 2 H).

13C NMR (150.9 MHz, D6-DMSO): δ [ppm] = 141.2 (C_q, arom), 132.1 (C_q, arom), 129.9 (CH_arom), 128.6 (CH_arom), 127.9 (C_q, arom), 126.5 (C_q, arom), 123.7 (CH_arom), 114.5 (CH_arom), 110.7 (CH_arom), 37.2(CH₃).

4-[6-(2-Nitrophenyl)imidazo[2,1-b]thiazol-3-ylmethyl]piperazine-1-carboxylic acid tert-butyl ester (10a)

3-(chloromethyl)-6-(2-nitrophenyl)imidazo[2,1-b]thiazole (9) (800 mg, 2.72 mmol) was dissolved in 8 mL of DMF and Boc-piperazine (760 mg, 4.08 mmol), K₂CO₃ (1.13 g, 8.20 mmol) as well as a catalytic amount of NaN₃ were used. The reaction mixture was stirred at 30 °C for 2 h before it was cooled to 0 °C and filtrated to afford 887 mg of 3-(chloromethyl)-6-(2-nitrophenyl)imidazo[2,1-b]thiazole (9) as a colorless crystalline solid (99%). The product was used without additional purification for the next step.

HRMS (ESI(+)-MS) calculated for C₁₂H₁₂N₃O₃S [M+H]+ 294.0099; found: 294.0093.

1H NMR (600.2 MHz, D6-DMSO): δ [ppm] = 8.25 (s, 1 H), 7.80 (d, 1 H, J=7.96 Hz), 7.78 (d, 1 H, J=7.77 Hz), 7.70 (t, 1 H, J=7.59 Hz), 7.54 (t, 1 H, J=7.70 Hz), 7.40 (s, 1 H), 7.31 (s, 1 H), 7.07 (s, 1 H), 5.52 (s, 4 H).

13C NMR (150.9 MHz, D6-DMSO): δ [ppm] = 149.1 (C_q, arom), 148.4 (C_q, arom), 141.8 (C_q, arom), 137.7 (CH_arom), 132.0 (CH_arom), 129.8 (CH_arom), 128.6 (CH_arom), 128.3 (CH_arom), 128.2 (C_q, arom), 126.6 (C_q, arom), 123.8 (CH_arom), 120.0 (CH_arom), 113.0 (CH_arom), 110.3 (CH_arom), 42.2(CH₃).

4-[6-(2-Aminophenyl)imidazo[2,1-b]thiazol-3-ylmethyl]piperazine-1-carboxylic acid tert-butyl ester (11a)

4-[6-(2-nitrophenyl)imidazo[2,1-b]thiazol-3-ylmethyl]piperazine-1-carboxylic acid tert-butyl ester (10a) (770 mg, 1.74 mmol) was dissolved in 23 mL of ethanol and NaOAc (715 mg, 8.70 mmol) was added. The reaction mixture was heated to 78 °C and stirred until the solid was completely dissolved. SnCl₂ x 2 H₂O (1.96g, 8.70 mmol) was added to the solution and the reaction mixture was stirred at 78 °C for 30 min. After removal of the solvent under reduced pressure, the residue was dissolved in 100 mL of ethyl acetate and washed with 40% aqueous K₂CO₃ solution, water, brine and dried with Na₂SO₄ before it was concentrated under reduced pressure. The resulting yellow-brown crystalline product was purified by silica gel column chromatography (heptane/ethyl acetate 1:1) to afford 513 mg of 4-[6-(2-aminophenyl)imidazo[2,1-b]thiazol-3-ylmethyl]piperazine-1-carboxylic acid tert-butyl ester (11a) as a colorless crystalline solid (71%).


1H NMR (600.2 MHz, D6-DMSO): δ [ppm] = 8.11 (s, 1 H), 7.47 (d, 1 H, J=7.69 Hz), 7.14 (s, 1 H), 6.97 (t, 1 H, J=7.69 Hz), 6.70 (d, 1 H, J=8.08 Hz), 6.55 (t, 1 H, J=7.82 Hz), 6.17 (s, 2 H), 3.73 (s, 2 H), 3.32 (s, 4 H), 2.43 (s, 4 H), 1.39 (s, 9 H).

13C NMR (150.9 MHz, D6-DMSO): δ [ppm] = 153.8 (C_q), 148.9 (C_q, arom), 148.3 (C_q, arom), 140.9 (C_q, arom), 131.8 (CH_arom), 129.7 (CH_arom), 128.3 (C_q, arom), 126.6 (CH_arom), 123.5 (CH_arom), 111.2 (CH_arom), 78.8 (C_q), 53.7 (CH₂), 52.1 (2x CH₂), 28.0 (3x CH₃).

2-(3-((1H-Imidazol-1-yl)methyl)imidazo[2,1-b]thiazol-6-yl)aniline (11b)

Essentially the same procedure as the preparation of 4-[6-(2-aminophenyl)imidazo[2,1-b]thiazol-3-ylmethyl]piperazine-1-carboxylic acid tert-butyl ester (11a) was employed.

3-(1H-imidazol-1-yl)methyl)-6-(2-nitrophenyl)imidazo[2,1-b]thiazole (10b) (70 mg, 0.22 mmol) in 3 mL of ethanol, NaOAc (90 mg, 1.06 mmol), SnCl₂ x 2 H₂O (240 mg, 1.06 mmol) were used. Yield: 36 mg (57%; colorless crystalline solid).
HRMS (ESI(+)-MS) calculated for C_{15}H_{14}N_{3}S [M+H]^+ 296.0964; found: 296.0963.

$^1$H NMR (600.2 MHz, D6-DMSO): δ [ppm] = 8.21 (s, 1 H), 8.16 (s, 1 H), 7.44 (m, 1 H), 7.43 (m, 1 H), 7.31 (s, 1 H), 7.07 (s, 1 H), 6.98 (t, 1 H, 3J=7.60 Hz), 6.70 (d, 1 H, 3J=8.10 Hz), 6.56 (t, 1 H, 3J=7.50 Hz), 5.53 (s, 1 H).

$^{13}$C NMR (150.9 MHz, D6-DMSO): δ [ppm] = 147.4 (Cq, arom), 147.3 (Cq, arom), 145.8 (Cq, arom), 137.5 (CH arom), 128.0 (CH arom), 127.7 (Cq, arom), 127.4 (Cq, arom), 121.0 (CH arom), 116.0 (CH arom), 115.9 (Cq, arom), 115.7 (CH arom), 112.3 (CH arom), 107.7 (CH arom), 42.4 (CH$_2$).

N-(2-(3-((1H-Imidazol-1-yl)methyl)imidazo[2,1-b][thiazol-6-yl]phenyl)quinoxaline-2-carboxamide (1b)

2-(3-((1H-imidazol-1-yl)methyl)imidazo[2,1-b][thiazol-6-yl]aniline (1b) (36 mg, 0.11 mmol) was dissolved in 2 mL of DMF and collidine (0.05 mL, 0.36 mmol) was added. The reaction mixture was cooled to 0°C, HATU (50 mg, 0.12 mmol) was added and it was stirred for 1 h at 0°C. After warming to room temperature and stirring for 16 h, the reaction was quenched with aqueous NaHCO$_3$ (sat.), extracted with DCM (3 x 5 mL), washed with brine, dried with Na$_2$SO$_4$ and concentrated under reduced pressure. The resulting light-brown crystalline crude product was purified by silica gel column chromatography (heptane/ethyl acetate/MeOH 4:4:1) to afford 36 mg of N-(2-(3-((1H-imidazol-1-yl)methyl)imidazo[2,1-b][thiazol-6-yl]phenyl)quinoxaline-2-carboxamide (1b) as a light-yellow crystalline solid (72%).

HRMS (ESI(+)-MS) calculated for C$_{23}$H$_{16}$N$_{3}$S [M+H]^+ 452.1288; found: 452.1291.

$^1$H NMR (600.2 MHz, D6-DMSO): δ [ppm] = 8.36(s, 1 H), 9.59(s, 1 H), 8.84 (d, 1 H, 3J=8.4 Hz), 8.48(s, 1 H), 8.29 (s, 1 H), 8.24 (d, 1 H, 3J=8.8 Hz), 8.04 (m, 2 H), 7.79 (d, 1 H, 3J=7.8), 7.49 (s, 1 H), 7.48 (s, 1 H), 7.41 (t, 1 H, 3J=7.7 Hz), 7.24 (t, 1 H, 3J=7.6 Hz), 7.10 (t, 1 H), 5.60 (s, 1 H).

$^{13}$C NMR (150.9 MHz, D6-DMSO): δ [ppm] = 161.7 (Cq), 148.2 (Cq), 145.4 (Cq), 144.4 (Cq, arom), 143.9 (CH arom), 143.1 (Cq, arom), 139.6 (Cq, arom), 137.6 (CH arom), 135.5 (Cq, arom), 132.2 (Cq, arom), 131.5 (CH arom), 129.4 (CH arom), 129.3 (CH arom), 128.2 (Cq), 128.1 (CH arom), 127.3 (CH arom), 127.2 (CH arom), 124.1 (CH arom), 121.7 (Cq), 120.5 (CH arom), 120.3 (CH arom), 113.6 (CH arom), 109.7 (CH arom), 42.5 (CH$_2$).

3,5-Difluoro-N-(2-(3-((1H-imidazol-1-yl)methyl)imidazo[2,1-b][thiazol-6-yl]phenyl)benzamide (2a)

Essentially the same procedure as the preparation of 3,5-difluoro-N-(2-(3-((1H-imidazol-1-yl)methyl)imidazo[2,1-b][thiazol-6-yl]phenyl)benzamide (2a) as a light-yellow crystalline solid was employed.

Essentially the same procedure as the preparation of 3,5-difluoro-N-(2-(3-((1H-imidazol-1-yl)methyl)imidazo[2,1-b][thiazol-6-yl]phenyl)-3-(trifluoromethoxy)benzamide (3a)

4-(6-(2-aminophenyl)imidazo[2,1-b][thiazol-6-yl]phenyl)benzamide (1a) (50 mg, 0.12 mmol) was dissolved in 2 mL of DCM, collidine (0.05 mL, 0.36 mmol) and HATU (60 mg, 0.15 mmol) were used.

Column chromatography: (heptane/ethyl acetate 1:1); then it was dissolved in 2 mL of TFA and stirred at room temperature for 90 min. The reaction mixture was concentrated under reduced pressure, dissolved in 1 mL of H$_2$O/1 mL of DCM and quenched with 1 mL of aqueous NaHCO$_3$ solution (sat.). The desired product was then extracted with DCM (3 x 2 mL), washed with brine, dried with Na$_2$SO$_4$ and concentrated under reduced pressure to afford 29 mg of 3,5-difluoro-N-(2-(3-((1H-imidazol-1-yl)methyl)imidazo[2,1-b][thiazol-6-yl]phenyl)benzamide (2a) as a light-yellow crystalline solid (53%).
4-[(2-(aminophenyl)imidazo[2,1-b]thiazol-3-ylmethyl)piperazine-1-carboxylic acid tert-butyl ester (11a) (50 mg, 0.12 mmol), biphenyl-4-carboxylic acid (24 mg, 0.12 mmol) in 2 mL of DMF, collidine (0.05 mL, 0.36 mmol) and HATU (60 mg, 0.15 mmol) were used. Yield: 42 mg (70%; light-yellow crystalline solid).

HRMS (ESI(+)-MS) calculated for C_{29}H_{33}N_{3}OS [M+H]^+ 494.2049; found: 494.2043.

$^1$H NMR (600.2 MHz, CDCl$_3$): δ [ppm] = 12.83 (s, 1 H), 8.87 (d, 1 H, $^J$=8.3 Hz), 8.24 (d, 1 H, $^J$=8.4 Hz), 7.96 (s, 1 H), 7.74 (d, 1 H, $^J$=8.4 Hz), 7.67 (d, 1 H, $^J$=7.4 Hz), 7.63 (d, 1 H, $^J$=7.8 Hz), 7.49 (t, 1 H, $^J$=7.5 Hz), 7.40 (t, 1 H, $^J$=7.5 Hz), 7.38 (t, 1 H, $^J$=7.9 Hz), 7.14 (t, 1 H, $^J$=7.5 Hz), 6.69 (s, 1 H), 3.67 (s, 2 H), 2.95 (br s, 4 H), 2.53 (br s, 4 H).

$^{13}$C NMR (150.9 MHz, CDCl$_3$): δ [ppm] = 165.5 (C$_q$), 148.6 (C$_q$, arom), 146.7 (C$_q$, arom), 144.3 (C$_q$, arom), 140.3 (C$_q$, arom), 137.0 (C$_q$, arom), 134.6 (C$_q$, arom), 129.3 (C$_q$, arom), 129.0 (C$_q$, arom), 128.6 (CH$_{arom}$), 128.3 (CH$_{arom}$), 128.1 (CH$_{arom}$), 127.4 (2×CH$_{arom}$), 127.3 (2×CH$_{arom}$), 126.8 (CH$_{arom}$), 123.4 (CH$_{arom}$), 121.4 (C$_q$, arom), 121.4 (CH$_{arom}$), 110.0 (CH$_{arom}$), 109.7 (CH$_{arom}$), 56.0(CH$_2$), 53.8 (2×CH$_2$), 45.8 (2×CH$_2$).

3-hydroxy-N-(2-(3-(piperazin-1-ylmethyl)imidazo[2,1-b]thiazol-6-yl)phenyl)quinoxaline-2-carboxamide (5a)

Essentially the same procedure as the preparation of 3,5-difluoro-N-(2-(3-(piperazin-1-ylmethyl)imidazo[2,1-b]thiazol-6-yl)phenyl) benzamide (2a) as a light-yellow crystalline solid was employed. 4-(6-(2-aminophenyl)imidazo[2,1-b]thiazol-3-ylmethyl)piperazine-1-carboxylic acid tert-butyl ester (11a) (40 mg, 0.1 mmol), 3-hydroxyquinoxaline-2-carboxylic acid (17 mg, 0.1 mmol) in 2.5 mL of DMF, collidine (0.04 mL, 0.29 mmol) and HATU (43 mg, 0.12 mmol) were used. Yield: 35 mg (75%; light-yellow crystalline solid).

HRMS (ESI(+)-MS) calculated for C$_{31}$H$_{33}$N$_{4}$O$_2$S [M+H]$^+$ 486.1707; found: 486.1710.

$^1$H NMR (500.1 MHz, CD$_2$OD): δ [ppm] = 8.28 (s, 1 H), 8.20 (d, 1 H, $^J$=8.3 Hz), 7.96 (d, 1 H, $^J$=8.3 Hz), 7.90 (d, 1 H, $^J$=8.8 Hz), 7.58 (t, 1 H, $^J$=8.3 Hz), 7.47 (d, 1 H, $^J$=8.3 Hz), 7.37 (t, 1 H, $^J$=8.3 Hz), 7.31 (m, 2 H), 7.00 (s, 1 H), 3.69 (s, 1 H), 3.35 (s, 1 H), 3.23 (br s, 4 H), 2.80 (br s, 4 H),

$^{13}$C NMR (125.8 MHz, CDCl$_3$): δ [ppm] = 163.9 (C$_q$, arom), 162.5 (C$_q$), 149.5 (C$_q$, arom), 142.9 (C$_q$, arom), 134.3 (C$_q$, arom), 134.2 (C$_q$, arom), 131.2 (CH$_{arom}$), 129.4 (C$_q$, arom), 129.1 (CH$_{arom}$), 128.4 (CH$_{arom}$), 127.5 (CH$_{arom}$), 126.8 (C$_q$, arom), 125.3 (CH$_{arom}$), 124.6 (CH$_{arom}$), 122.8 (CH$_{arom}$), 122.0 (CH$_{arom}$), 111.7 (CH$_{arom}$), 110.4 (CH$_{arom}$), 54.5(2×CH$_2$), 43.9(2×CH$_2$), 29.7(2×CH$_2$).