The Discovery of Pyridone and Pyridazone Heterocycles as Gamma Secretase Modulators

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Experimental Procedure

Analytical methods: All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Flash chromatography was carried out with EM science Silica gel 60 (neutral, 230-400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 500 NMR Spectrometer with chemical shifts reported in ppm relative to the residual deuterated solvent. HRMS was recorded on a Micromass QT of Ultima API US mass spectrometer by FAB. LCMS was recorded on Applied Biosystem API-150.

***In vitro* assay:**

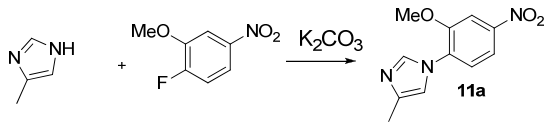
Cell bassed assay. Human embryonic kidney (HEK) 293 cells stably transfected with APPsw-lon in pcDNA3.1 vector (Invitrogen) were treated with GSM compounds for 5 hrs. Aβ in conditioned media was measured using MesoScale Discovery (MSD) technology based sandwich immunoassays. Aβ42 was measured using a pair of labeled antibodies TAG-G2-11 and biotin-4G8; Aβ40 was measured using antibody pair of TAG-G2-10 and biotin-4G8; total Aβ was measured using TAG-W02 and biotin-4G8.

***In vivo* assay:**

CSF and cortex Aβ assays. Rat CSF Aβ40 and Aβ42 were analyzed using AlphaLISA Amyloid Assay kits (PerkinElmer) according to manufacture's instruction. For brain cortex Ab42 analysis, half cortex was homogenized and extracted in 5 M guanidine-HCl/50 mM Tris-HCl, pH 8. The extracts were sonicated and partially purified using a solid phase extraction matrix in 96-well format, the HLB plate (Waters). The samples eluted from HLB plate were dried and resuspended in freshly prepared PBS/0.5% Tween 20. Aβ40 and Aβ were measured using AlphaLISA amyloid assay kits.

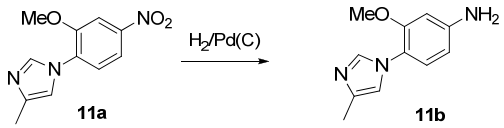
General procedure for the amine linker formation is illustrated by the preparation of compound 11.

Step A:



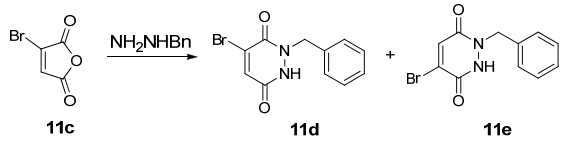
Two equivalent of 4-methylimidazole, 1 equivalent of 3-methoxy-4-fluoro-nitrobenzene and 5 eq. of K_2CO_3 were stirred in CH_3CN at room temperature over night. The reaction mixture was filtered and concentrated under reduced pressure. The crude product was recrystalized with EtOAc to give desired product **11a**.

Step B:



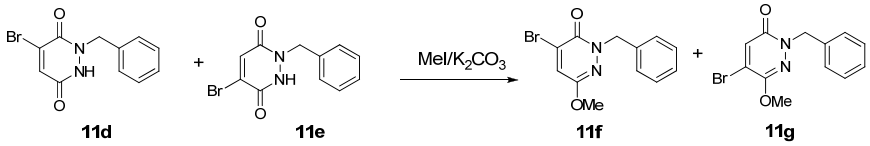
Compound **11a** was hydrogenated with hydrogen balloon in the presence of Pd(C) as the catalyst (10 wt%) in MeOH over night. The mixture was filtered and concentrated under reduced pressure to give crude product as a mixture of 5/1 mixture of regioisomers which was recrystalized from EtOAc to give **11b**.

Step C:



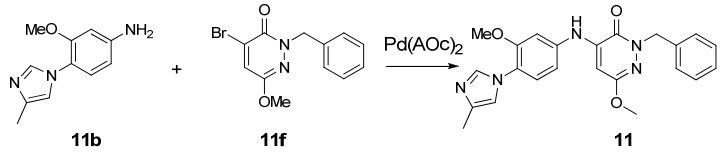
Compound **11c** (3.0 g, 16.95 mmol) and benzylhydrazine (3.47 g, 17.8 mmol) in water (20 mL) was stirred and heated at 100 ^{o}C overnight. The mixture was cooled to room temperature and filtered to collect the solid as a mixture of compound **11d** and compound **11e** (4.0 g).

Step D:



A mixture of compound **11d** and compound **11e** (1.5 g, 5.34 mmol), MeI (0.646 mL, 10.68 mmol) and K_2CO_3 (2.21 g, 16.0 mmol) in DMF (6.0 mL) was stirred at room temperature overnight. The mixture was diluted with EtOAc (150 mL) and HCl solution (30 mL, 0.5 M). The organic layer was washed with water (3x), brine, dried over $MgSO_4$, and concentrated to give the crude product. The crude residue was purified by column chromatography eluting with EtOAc/hexanes to yield compound **11f** (0.75 g) and compound **11g** (0.7 g).

Step e:



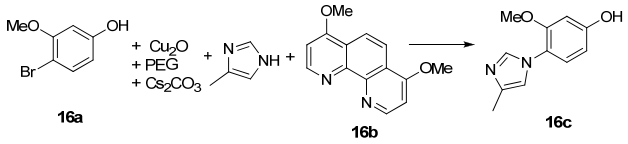
To the mixture of **11b** (0.192 mmol) in toluene (10 mL) was added compound **11f** (0.192 mmol) and solid K_2CO_3 (132 mg, 0.960 mmol), followed by the addition of BINAP (4.8 mg, 4%mol) and $Pd(OAc)_2$ (1.7 mg, 4%mol), the resultant mixture was kept stirring at 120 ^{o}C for 48 h. The mixture was cooled to RT, EtOAc (10 mL) and NH_4Cl (6 mL) were added, the insoluble material was filtered off through Celite. The filtrate was separated, the aqueous was extracted once more with EtOAc (6 mL). The combined organic was

dried over anhydrous $MgSO_4$, and concentrated. The residue was purified via Gilson to obtain **11** as yellow syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 8.65 (br. s, 1 H), 7.86 (br. s, 1 H), 7.47 (d, *J* = 6.5 Hz, 2 H), 7.36 (t, *J* = 7.0 Hz, 2 H), 7.32 (m, 2 H), 7.01 (br. s, 1 H), 6.95 (dd, *J* = 8.5, 2.5 Hz, 1 H), 6.88 (d, *J* = 2.5 Hz, 1 H), 6.48 (s, 1 H), 5.27 (s, 2 H), 3.89 (s, 3 H), 3.83 (s, 3 H), 2.48 (s, 3 H). ES-LCMS: *m/z* = 418.2 $[M+H]^+$.

Other aniline compounds were prepared using the same procedure by coupling the imidazole aniline and corresponding bromide with $Pd(OAc)_2$.

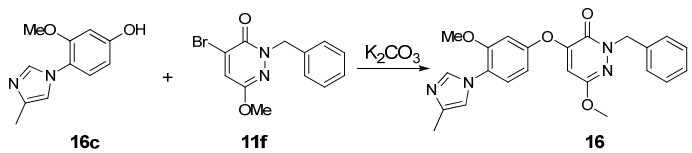
General procedure for the oxygen linker formation is illustrated by the preparation of compound 16.

Step A:



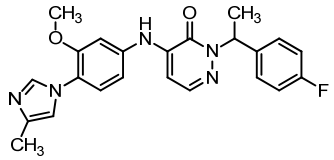
A mixture of compound **16a** (2.03 g, 10 mmol), Cu_2O (0.288 g, 2 mmol), PEG (4.0), Cs_2CO_3 (9.77 g, 30 mmol), 4-methylimidazole (0.98 g, 12 mmol) and **16b** (0.72 g, 3 mmol) in NMP (15 mL) was vacuum-nitrogen exchange degassed and stirred in a sealed tube at 120 ^{o}C for 48 hours. The mixture was cooled to room temperature and diluted with CH_2Cl_2 followed with addition of silica gel. The mixture was stirred for 20 minutes and filtered. The organic layer was washed with water (3x), brine, dried over $MgSO_4$, and concentrated to give the crude product. The crude residue was purified by column chromatography eluting with CH_2Cl_2/MeOH to yield compound **16c** (0.2 g).

Step B:



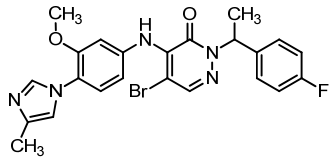
A mixture of compound **16c** (0.104 mmol) and compound **11f** (0.104 mmol), and K_2CO_3 (71.8 mg, 0.52 mmol) in DMF (1.5 mL) was stirred at 80 ^{o}C overnight. The mixture was

diluted with EtOAc (50 mL) and NH_4Cl solution (10 mL, saturated). The organic layer was washed with water (3x), brine, dried over $MgSO_4$, and concentrated to give the crude product. The crude residue was purified by Gilson reverse phase HPLC to yield compound **16** (25 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 8.73 (br. s, 1 H), 7.59 (d, *J* = 8.0 Hz, 2 H), 7.35 (m, 4 H), 7.05 (s, 1 H), 6.87 (d, *J* = 2.5 Hz, 1 H), 6.81 (dd, *J* = 8.5, 2.5 Hz, 1 H), 6.29 (s, 1 H), 5.28 (s, 2 H), 3.85 (s, 3 H), 3.83 (s, 3 H), 2.48 (s, 3 H). ES-LCMS: *m/z* = 419.2 $[M+H]^+$.



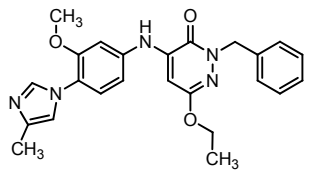
8

^{1}H NMR (500 MHz, METHANOL-d4) δ ppm 9.11 (d, *J* = 1.26 Hz, 1H), 7.80 (d, *J* = 5.04 Hz, 1H), 7.52 - 7.58 (m, 4H), 7.47 (dd, *J* = 5.36, 8.51 Hz, 1H), 7.23 (d, *J* = 1.89 Hz, 1H), 7.14 (dd, *J* = 2.21, 8.51 Hz, 2H), 7.07 (t, *J* = 8.67 Hz, 1H), 6.97 (d, *J* = 5.04 Hz, 1H), 6.37 (q, *J* = 6.94 Hz, 1H), 3.94 (s, 3H), 2.45 (s, 3H), 1.75 - 1.83 (m, 3H); ES-LCMS: *m/z* = 420.2 $[M+H]^+$.



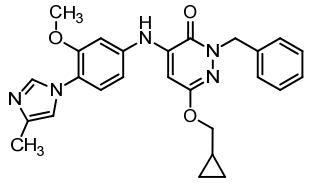
10

^{1}H NMR (500 MHz, METHANOL-d4) δ ppm 9.09 (s, 1H), 7.99 (s, 1H), 7.54 (s, 1H), 7.40 - 7.50 (m, 4H), 7.09 (d, *J* = 8.51 Hz, 2H), 6.84 (d, *J* = 1.89 Hz, 1H), 6.70 (m, 1H), 6.28 (q, *J* = 6.94 Hz, 1H), 3.86 (s, 3H), 2.44 (s, 3H), 1.81 (d, *J* = 6.94 Hz, 3H); ES-LCMS: *m/z* = 500.3 $[M+H]^+$.



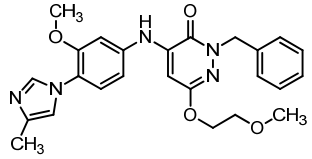
12

^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.73 (s, 1H), 7.94 (s, 1H), 7.51 (d, *J* = 7.25 Hz, 2H), 7.28 - 7.42 (m, 3H), 7.19 - 7.25 (m, 1H), 6.98 - 7.11 (m, 2H), 6.91 - 6.95 (m, 1H), 6.52 - 6.58 (m, 1H), 5.31 (s, 2H), 4.26 (q, *J* = 6.94 Hz, 2H), 3.94 (s, 3H), 2.53 (s, 3H), 1.52 (t, *J* = 6.94 Hz, 3H); ES-LCMS: *m/z* = 432.2 $[M+H]^+$.



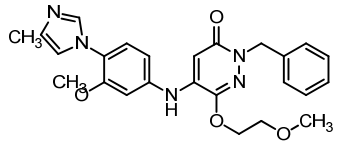
13

^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.73 (br. s., 1H), 7.93 (s, 1H), 7.50 (d, *J* = 6.94 Hz, 2H), 7.28 - 7.42 (m, 4H), 7.02 - 7.11 (m, 2H), 6.93 (s, 1H), 6.58 (s, 1H), 5.30 (s, 2H), 4.05 (br. S., 1H), 3.93 (br. S., 1H), 3.90 (s, 3H), 2.53 (s, 3H), 0.90 - 1.02 (m, 1H), 0.64 - 0.71 (m, 2H), 0.35 - 0.42 (m, 2H); ES-LCMS: *m/z* = 458.3 $[M+H]^+$.



14

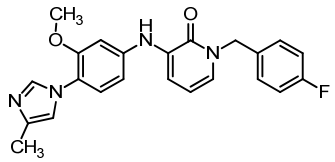
^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.70 (br. s., 1H), 7.87 (s, 1H), 7.49 (d, J = 6.94 Hz, 2H), 7.28 - 7.42 (m, 3H), 6.99 - 7.10 (m, 2H), 6.91 (s, 1H), 6.58 (s, 1H), 5.30 (s, 2H), 4.34 - 4.39 (m, 2H), 3.95 (s, 3H), 3.72 - 3.79 (m, 2H), 3.47 (s, 3H), 2.53 (s, 3H); ES-LCMS: *m/z* = 462.3 $[M+H]^+$.



15

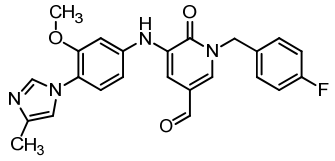
^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.70 (br. s., 1H), 7.89 (s, 1H), 7.49 (d, J = 7.25 Hz, 2H), 7.28 - 7.42 (m, 4H), 7.00 - 7.10 (m, 2H), 6.91 (s, 1H), 6.58 (s, 1H), 5.30

(s, 2H), 4.35 - 4.40 (m, 2H), 3.94 (s, 3H), 3.73 - 3.78 (m, 2H), 3.48 (s, 3H), 2.53 (s, 3H); ES-LCMS: *m/z* = 462.3 $[M+H]^+$.



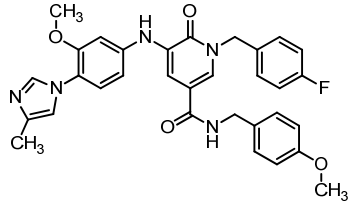
19

^{1}H NMR (500 MHz, CHLORODORM-d) δ ppm 8.66 (br. s., 1H), 7.44 (br. s., 1H), 7.34((m, 2H), 7.20-7.23(m, 2H), 7.04 - 7.14 (m, 3H), 6.91 - 7.02 (m, 2H), 6.86 (d, J = 1.89 Hz, 1H), 6.32 (t, J = 7.25 Hz, 1H), 5.23 (s, 2H), 3.87 (s, 3H), 2.46 (s, 3H); ES-LCMS: *m/z* = 405.2 $[M+H]^+$.



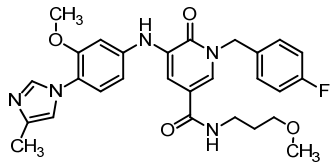
21

^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 9.61 (s, 1H), 7.70 (s, 1H), 7.51 (dd, J = 1.89, 9.77 Hz, 2H), 7.43 (dd, J = 5.36, 8.51 Hz, 2H), 7.25 - 7.36 (m, 2H), 7.23 (s, 1H), 7.09 - 7.19 (m, 2H), 6.83 - 6.97 (m, 3H), 5.32 (s, 2H), 3.90 (s, 3H), 2.36 (s, 3H); ES-LCMS: *m/z* = 433.2 $[M+H]^+$.



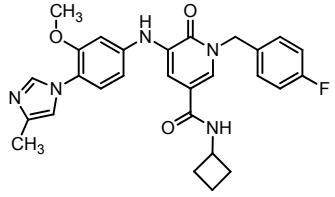
22

^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.63 (br. s., 1H), 7.68 (s, 1H), 7.51 (s, 1H), 7.34 - 7.41 (m, 2H), 7.23 - 7.34 (m, 4H), 7.04 - 7.12 (m, 2H), 6.88 - 6.96 (m, 2H), 6.84 (s, 1H), 6.48 (br. s., 1H), 5.25 (s, 2), 4.55 (d, J = 5.36 Hz, 2H), 3.84 (s, 3H), 3.87 (s, 3H), 2.51 (s, 3H); ES-LCMS: *m/z* = 468.3 $[M+H]^+$.



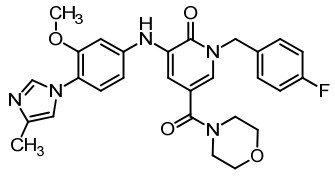
23

^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.67 (br. s., 1H), 7.67 (m, 1H), 7.48 (d, J = 1.58 Hz, 1H), 7.34 - 7.43 (m, 2H), 7.25 - 7.34 (m, 2H), 7.04 - 7.13 (m, 3H), 6.97 - 7.03 (m, 1H), 6.88 - 6.93 (m, 1H), 5.27 (s, 2H), 3.93 (s, 3H), 3.55 - 3.65 (m, 4H), 3.34 (s, 3H), 2.52 (s, 3H), 1.90 (m, 2H); ES-LCMS: *m/z* = 520.3 $[M+H]^+$.



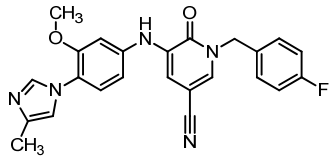
24

^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.73 (br. s., 1H), 7.68 (br. s., 1H), 7.52 (br. s., 1H), 7.34 - 7.43 (m, 2H), 7.26 - 7.34 (m, 1H), 7.07 - 7.14 (m, 2H), 6.97 - 7.01 (m, 1H), 6.88 (s, 1H), 6.35 (br. s., 1H), 5.27 (s, 2H), 4.55 (d, J = 7.88 Hz, 1H), 3.92 (s, 3H), 2.52 (s, 3H) 2.40 - 2.48 (m, 2H), 2.00 – 2.04 (m, 2H), 1.78 - 1.86 (m, 2H); ES-LCMS: *m/z* = 502.3 $[M+H]^+$.



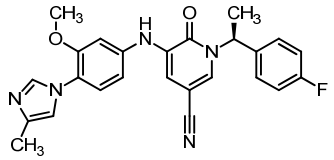
25

^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.71 (s, 1H), 7.38 - 7.45 (m, 3H), 7.20 (d, J = 1.89 Hz, 1H), 7.22 (d, J = 1.89 Hz, 1H), 7.09 - 7.17 (m, 2H), 7.06 (s, 1H), 6.97 (dd, J = 2.21, 8.51 Hz, 1H), 6.86 (d, J = 2.21 Hz, 1H), 5.25 (s, 2H), 3.93 (s, 3H), 3.72 (br. s., 4H), 3.67 (br. s., 4H), 2.53 (s, 3H); ES-LCMS: *m/z* = 518.3 $[M+H]^+$.



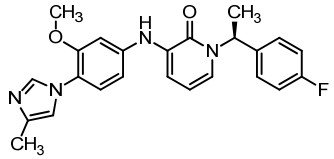
26

^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.69 (s, 1H), 7.28 - 7.43 (m, 6H), 7.10 - 7.18 (m, 3H), 7.06 (s, 1H), 6.98 (dd, J = 2.21, 8.51 Hz, 1H), 6.86 (d, J = 1.89 Hz, 1H), 5.25 (s, 2H), 3.94 (s, 3H), 2.53 (s, 3H); ES-LCMS: *m/z* = 430.2 $[M+H]^+$.



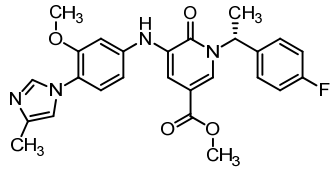
27

^{1}H NMR (500 MHz, METHANOL-d4) δ ppm 9.09 (s, 1H), 7.79 (d, J = 1.89 Hz, 1H), 7.54 (s, 1H), 7.44 - 7.50 (m, 3H), 7.25 (d, J = 1.89 Hz, 1H), 7.12 - 7.18 (m, 3H), 7.08 (dd, J = 2.21, 8.51 Hz, 1H), 6.37 (q, J = 7.04 Hz, 1H), 3.93 (s, 3H), 2.45 (s, 3H), 1.84 (d, J = 7.25 Hz, 3H); ES-LCMS: *m/z* = 444.2 $[M+H]^+$.



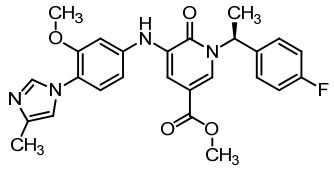
28

^{1}H NMR (500 MHz, METHANOL-d4) δ ppm 9.06 (s, 1H), 7.50 - 7.53 (m, 4H), 7.35 - 7.44 (m, 18H), 7.09 - 7.16 (m, 14H), 7.05 (s, 5H), 6.98 (dd, J = 2.21, 8.51 Hz, 5H), 6.36 - 6.44 (m, 2H), 3.91 (s, 3H), 2.44 (s, 3H), 1.81 (d, J = 7.25 Hz, 3H); ES-LCMS: *m/z* = 419.2 $[M+H]^+$.



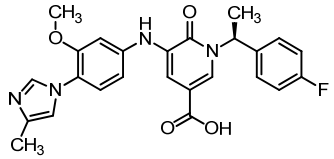
30

^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.69 (br. s., 1H), 7.75 (d, J = 1.89 Hz, 1H), 7.70 (d, J = 1.89 Hz, 1H), 7.28 - 7.41 (m, 2H), 7.14 (t, J = 8.67 Hz, 2H), 7.07 (s, 1H), 6.98 (dd, J = 1.89, 8.51 Hz, 1H), 6.92 (d, J = 1.89 Hz, 1H), 6.47 (q, J = 6.94 Hz, 1H), 3.95 (s, 3H), 3.85 (s, 3H), 2.53 (s, 3H), 1.87 (d, J = 7.25 Hz, 3H); ES-LCMS: *m/z* = 477.3 $[M+H]^+$.



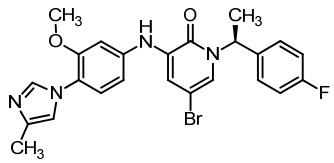
31

^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.71 (br. s., 1H), 7.70 - 7.76 (m, 2H), 7.30 - 7.42 (m, 3H), 7.14 (t, J = 8.51 Hz, 3H), 6.97 - 7.01 (m, 1H), 6.93 (s, 1H), 6.47 (q, J = 6.83 Hz, 1H), 3.93 (s, 3H), 3.89 (s, 3H), 2.53 (s, 3H), 1.87 (d, J = 7.25 Hz, 3H); ES-LCMS: *m/z* = 477.3 $[M+H]^+$.



32

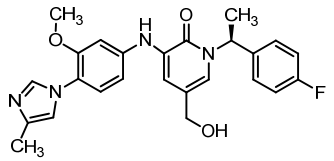
^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 7.78 (s, 1H), 7.72 (s, 1H), 7.41 (d, J = 8.20 Hz, 2H), 7.36 (s, 2H), 7.32 (s, 31H), 7.10 - 7.16 (m, 3H), 6.95 (s, 2H), 6.23-6.28 (m, 1H) 3.87 (s, 3H), 2.38 (s, 3H), 1.88 (d, J = 7.25 Hz, 3H); ES-LCMS: *m/z* = 463.3 $[M+H]^+$.



33

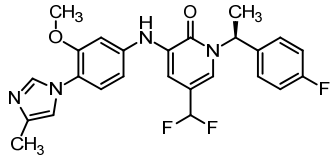
^{1}H NMR (500 MHz, METHANOL-d4) δ ppm 9.07 (m, 1H), 7.54 (s, 1H), 7.42 - 7.50 (m, 2H), 7.26 (m, 1H), 7.11 - 7.18 (m, 3H), 7.05 (dd, J = 2.21, 8.51 Hz, 1H), 6.37 (q, J = 7.15

Hz, 1H), 4.12 (q, J = 7.04 Hz, 1H), 3.92 (s, 3H), 2.45 (s, 3H), 2.34 (s, 1H), 1.81 (d, J = 6.94 Hz, 3H); ES-LCMS: *m/z* = 497.3 $[M+H]^+$.



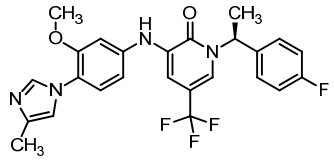
34

^{1}H NMR (500 MHz, METHANOL-d4) δ ppm 9.06 (s, 1H), 7.52 (s, 1H), 7.38 - 7.46 (m, 4H), 7.01 - 7.15 (m, 5H), 6.39 - 6.44 (m, 1H), 4.36 (s, 2H), 3.92 (s, 3H), 2.44 (s, 3H), 1.82 (d, J = 7.25 Hz, 3H); ES-LCMS: *m/z* = 449.2 $[M+H]^+$.



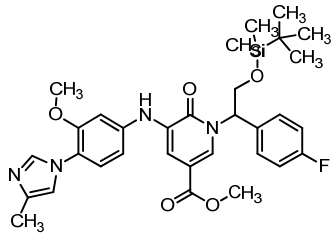
35

^{1}H NMR (500 MHz, METHANOL-d4) δ ppm 9.08 (d, J = 1.58 Hz, 1H), 7.54 (s, 1H), 7.42 - 7.50 (m, 3H), 7.38 (d, J = 1.89 Hz, 1H), 7.34 (d, J = 1.89 Hz, 1H), 7.12 - 7.17 (m, 2H), 7.04 (dd, J = 2.21, 8.51 Hz, 1H), 6.62 (t, J = 55Hz, 1H), 6.40 (q, J = 6.94 Hz, 1H), 3.92 (s, 3H), 2.45 (s, 3H), 1.83 (d, J = 7.25 Hz, 3H); ES-LCMS: *m/z* = 469.3 $[M+H]^+$.



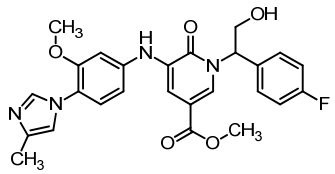
36

^{1}H NMR (500 MHz, METHANOL-d4) δ ppm 8.26 (br. s., 1H), 8.11 (s, 1H), 7.42 - 7.49 (m, 2H), 7.33 - 7.42 (m, 2H), 7.12 - 7.22 (m, 4H), 7.04 - 7.12 (m, 1H), 7.00 (dd, J = 2.21, 8.51 Hz, 1H), 6.36 - 6.43 (m, 1H), 3.84 - 3.93 (m, 3H), 2.31 (s, 3H), 1.84 (d, J = 7.25 Hz, 3H); ES-LCMS: *m/z* = 487.3 $[M+H]^+$.



37

^{1}H NMR (500 MHz, CHLOROFORM-d) δ ppm 7.98 (d, J = 2.21 Hz, 1H), 7.66 - 7.71 (m, 2H), 7.43 (dd, J = 5.36, 8.51 Hz, 2H), 7.25 - 7.28 (m, 1H), 7.10 - 7.16 (m, 3H), 6.91 - 6.94 (m, 2H), 6.87 (d, J = 1.89 Hz, 1H), 6.28 (t, J = 4.57 Hz, 1H), 4.38 (dd, J = 5.52, 11.51 Hz, 1H), 4.28 (dd, J = 4.10, 11.35 Hz, 1H), 3.88 (d, J = 8.20 Hz, 6H), 2.36 (s, 3H), 0.86 - 0.91 (m, 9H); ES-LCMS: *m/z* = 607.3 $[M+H]^+$.



38

^{1}H NMR (500 MHz, METHANOL-d4) δ ppm 9.07 (s, 1H), 8.07 (d, J = 1.89 Hz, 1H), 7.73 (d, J = 1.89 Hz, 1H), 7.54 (s, 1H), 7.44 - 7.49 (m, 3H), 7.10 - 7.18 (m, 3H), 7.04 (dd, J = 2.05, 8.67 Hz, 1H), 6.23 - 6.28 (m, 1H), 4.34 (d, J = 7.88 Hz, 1H), 4.25 (d, J = 4.73 Hz, 1H), 3.89 - 3.93 (m, 3H), 3.83 - 3.87 (m, 3H), 2.43 - 2.46 (m, 3H); ES-LCMS: *m/z* = 493.3 $[M+H]^+$.