Supplementary Material

Synthesis and binding affinity of fluorine containing N^G-acyl and -sulfonyl BIBP3226 derivatives: Ligands for the NPY Y\textsubscript{1} receptor

Nigel A. Lengkeek,\textsuperscript{a} Maxine P. Roberts,\textsuperscript{a,*} Lei Zhang,\textsuperscript{b} I-Chieh J Lee,\textsuperscript{b} Christopher J. R. Fookes,\textsuperscript{a} Branko Dikic,\textsuperscript{a} Herbert Herzog,\textsuperscript{b} Andrew Katsifis\textsuperscript{a,§} and Ivan Greguric\textsuperscript{a}

\textsuperscript{a} ANSTO Lifesciences, Australian Nuclear Science and Technology Organization, Locked Bag 2001, Kirrawee DC, NSW, 2232, Sydney, Australia.

\textsuperscript{b} Garvan Institute for Medical Research, 384 Victoria St, Darlinghurst, NSW 2010, Australia.

*Corresponding author contact details: Ph: 61-2-97179894; Fax: 61-2-97173648; E-mail: Maxine.Roberts@ansto.gov.au

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S1. Experimental details and analytical data for compounds 2, 4, 6, 7 and 8

S1.1 Succinimidyl 2,2-diphenylacetate (2).\[^{[1, 2]}\]

The synthesis of (2) was carried out as described in the published literature in 88% yield. The \(^1\)H NMR data obtained was consistent with the published literature.

S1.2 4-tert-Butoxybenzylamine (4).\[^{[3]}\]

The synthesis of (4) was carried out as described in the published literature in 72% yield. The \(^1\)H NMR data obtained was consistent with the published literature.

S1.3. \((R)\)-N-(4-tert-Butoxybenzyl)-N\(^\alpha\)-(2,2-diphenylacetyl)ornithinamide (6).\[^{[4]}\]

The synthesis of (6) was carried out as described in the published literature in 92% yield. The \(^1\)H NMR data obtained was consistent with the published literature.

S1.4. N-Benzylloxycarbonyl-N\(^\omega\)-tert-butoxycarbonyl-1\(^H\)-pyrazole-1-carboxamidine (7).\[^{[5, 6]}\]

The synthesis of (7) was carried out as described in the published literature (via the \(N\)-benzylloxycarbonyl-1\(^H\)-pyrazole-1-carboxamidine intermediate, \[^{[4, 7]}\]) in 41% yield. The \(^1\)H NMR data obtained was consistent with the published literature.

S1.5. \((R)\)-N-(4-tert-Butoxybenzyl)-N\(^\omega\)-tert-butoxycarbonyl-N\(^\alpha\)-(2,2-diphenylacetyl)argininamide (8).\[^{[8]}\]

The synthesis of (8) was carried out as described in the published literature in 81% yield. The \(^1\)H NMR data obtained was consistent with the published literature.

S2. Attempted preparation of N\(^\omega\)-(2-fluoroethylsulfonyl)BIBP3226

Under an atmosphere of \(N_2\), 8 (50.0 mg, 0.079 mmol) was dissolved in dry CH\(_3\)CN (4 mL). After cooling in a salt-ice bath Et\(_3\)N (50 \(\mu\)L, 0.36 mmol) followed by slow addition of 2-fluoroethylsulfonyl chloride (10 \(\mu\)L, 0.11 mmol) was added to the mixture. The mixture was stirred for 20 h and allowed to warm to r.t. The mixture was taken to dryness under high vacuum to give a white solid that was dissolved in a mixture of dry CH\(_3\)CN (3 mL) and TFA (0.5 mL) and heated at reflux for 1 - 3 hrs. The mixture was taken to dryness to give a yellow oil. The crude material was initially separated using a silica gel plug with n-heptane/EtOAc/CH\(_3\)OH, the products eluted with a 0/90/10 (n-heptane/EtOAc/CH\(_3\)OH) mixture of solvents to give a pale yellow oil containing 2 peaks in the HPLC trace. This material was further purified by preparative HPLC employing an isocratic solvent system (28/62/10, CH\(_3\)CN/H\(_2\)O/1\% TFA in H\(_2\)O; n.b. at higher percentages of H\(_2\)O filter blockages were encountered presumably caused by precipitation of the compounds) on a Grace Alltima C\(_{18}\) semi-prep column (10 \(\mu\)m, 250 mm \(\times\) 10 mm ID). Four fractions were collected F1 (14.93 - 16.45 min, 0.8 mg), F2...
S2.1. F1, Product #1 (mixture of 2 compounds)
LR-MS (ES): m/z 384.75, 520.72, 656.73, 676.04 \([C_{31}H_{33}F_3N_5O_7S]^- = [C_{29}H_{33}N_5O_5S] + CF_3CO_2^-\), 792.68; 1H NMR (CD3CN, 400.13 MHz): (2 compounds) 1.39 - 1.65 (m), 1.69 - 1.79 (m), 2.04 - 2.13 (m), 2.99 (m), 3.06 - 3.31 (m), 3.55 (m), 4.21 (m), 4.34 (m), 5.02 (s), 5.3 (s), 5.36 (br s), 6.70 - 6.77 (m), 8.84 (m), 7.00 - 7.09 (m), 7.16 (m) 7.22 - 7.37(m).

S2.2. F2, Product #2
1H NMR (CD3CN, 400.13 MHz): 1.41 (m, 2H), 1.51 - 1.79 (m, 2H), 2.95 (m, 2H), 3.08 (m, 4H), 4.02 (m, 2H), 4.19 (m, 2H), 4.37 (m, 1H), 5.04 (s, 1H), 6.05 (br s, 1H), 6.73 (AB doublet, 2H), 6.83 (m, 1H), 7.03 (AB doublet, 2H), 7.18 - 7.37 (m, 10H); 13C{1H}NMR (CD3CN, 100.61 MHz): δ 14.39, 22.66, 26.45, 29.93, 30.35, 39.29, 40.17, 43.00, 44.74, 54.01, 58.34, 116.13, 116.59, 127.91, 127.96, 129.41, 129.47, 129.70, 129.71, 129.74, 131.12, 131.88, 141.05, 141.17, 156.38, 157.08, 158.81, 172.88, 173.59; LR-MS (ES-): m/z 676.04 \([C_{31}H_{33}F_3N_5O_7S]^- = [C_{29}H_{33}N_5O_5S] + CF_3CO_2^-\); LR-MS (ES+): m/z 585.98 \([C_{29}H_{33}N_5O_5SNa]^+\).

S2.3. F3: Product #3
1H NMR (CD3CN, 400.13 MHz): 1.48 - 1.83 (m, 2H), 2.04 - 2.15 (m, 2H), 3.62 - 3.73 (m, 4H), 3.84 (m, 2H), 4.18 (m, 2H), 5.00 (s, 1H), 6.71 (AB d, 2H), 7.02 (AB d, 2H), 7.18 - 7.35 (m, 10H); LR-MS (ES-): m/z 676.00 \([C_{31}H_{33}F_3N_5O_7S]^- = [C_{29}H_{33}N_5O_5S] + CF_3CO_2^-\); LR-MS (ES+): m/z 563.97 \([C_{29}H_{34}N_5O_5S]^+\), 585.96 \([C_{29}H_{33}N_5O_5SNa]^+\).

S3. Fluoroalkylsulfonyl compounds

S3.1. Thioacetic acid S-(2-fluoroethyl) ester

\[
\begin{align*}
\text{F} & \quad \text{S} \\
& \quad \text{O}
\end{align*}
\]

2-Fluoroethyl methanesulfonate \(^{[9]}\) (7.03 g, 49.45 mmol) and potassium thioacetate (11.63 g, 100.8 mmol) were dissolved in CH3CN (200 mL) and stirred at r.t. for 3 days, the mixture became bright orange over this time. The mixture was poured into DI H2O (600 mL) and extracted with Et2O (3 × 150 mL). The combined organic fractions were dried over MgSO4, filtered and taken to dryness. The viscous brown oil was purified under reduced pressure employing a Kugelrohr apparatus, 70 - 80 °C @ 50 mmHg, giving 2.86 g of a yellow oil. 1H NMR (CDCl3, 400.13 MHz): δ 3.18 (dt, J1 = 21.45 Hz, J2 = 6.2 Hz, 2H), 4.48
(dt $J_1 = 46.93$ Hz, $J_2 = 6.2$ Hz, 2H); $^{13}$C{${}^1$H} NMR (CDCl$_3$, 100.62 MHz): $\delta$ 29.56 (d, $J_1 = 21.97$ Hz, CH$_2$), 30.60 (CH$_3$) 81.83 (d, $J_1 = 170.83$ Hz, CH$_2$), 194.95 (CO).

S3.2. Sodium 2-fluoroethylsulfonate

\[
\text{F} \xrightarrow{\text{SO}_3^-} \text{Na}^+
\]

Thioacetic acid S-(2-fluoroethyl) ester (2.86 g, 23.41 mmol) was dissolved in glacial AR acetic acid (40 mL). After warming the solution to 60 °C, 30% aqueous H$_2$O$_2$ (12 mL) was added dropwise, rapidly discharging the yellow color from solution, the mixture was heated for a further 3 h after addition. After cooling to r.t. the mixture was reduced to dryness to give a thick oil, before being dried under high vacuum overnight. The residue was dissolved in D$_2$O (50 mL) before being carefully neutralized with 1M aqueous Na$_2$CO$_3$. The solution was then taken to dryness to give a fine white powder that was dried under high vacuum overnight. The crude material was used without further purification. $^1$H NMR (D$_2$O - internal Me$_2$CO reference, 400.13 MHz): $\delta$ 3.31 (dt, $J_1 = 25.3$ Hz, $J_2 = 5.4$ Hz, 2H), 4.82 (dt, $J_1 = 46.7$ Hz, $J_2 = 5.4$ Hz, 2H); $^{13}$C{${}^1$H} NMR (D$_2$O - internal Me$_2$CO reference, 100.62 MHz): $\delta$ 51.92 (d, J = 20.1 Hz) 80.11 (d, J = 164.5 Hz).

S3.3. 2-Fluoroethylsulfonyl chloride

\[
\text{F} \xrightarrow{\text{SOCl}_2} \text{O} \xrightarrow{\text{O}} \text{Cl}
\]

Crude sodium 2-fluoroethylsulfonate (3.32 g) was suspended in dry CH$_2$Cl$_2$ (100 mL) to which was carefully added SOCl$_2$ (6 mL) and DMF (0.1 mL). The mixture was heated to reflux under a nitrogen atmosphere for 5 h. The solution was filtered through a pad of oven-dried celite and the eluate taken to dryness under high vacuum to give a yellow liquid. The crude product was purified by distillation under reduced pressure using a Kugelrohr apparatus, 120 - 140 °C at 10 - 15 mmHg to give a yellow liquid, 1.98 g. $^1$H NMR (CDCl$_3$, 400.13 MHz): $\delta$ 4.06 (dt, $J_1 = 21.2$ Hz, $J_2 = 5.2$ Hz, 2H), 4.98 (dt, $J_1 = 46.4$ Hz, $J_2 = 5.2$ Hz, 2H); $^{13}$C{${}^1$H} NMR (CDCl$_3$, 100.61 MHz): $\delta$ 64.88 (d, J = 22.1 Hz, CH$_2$), 76.68 (d, J = 177.9 Hz, CH$_2$); $^{19}$F{${}^1$H} NMR (CD$_3$CN, 376.50 MHz): $\delta$ -222.58 (s, -CH$_2$F).

S3.4. 3-Fluoropropyl methanesulfonate

Prepared in a similar manner to that described for 2-fluoroethyl methanesulfonate.$^{[9]}$
A 2-neck fitted with a dropping funnel and nitrogen inlet was charged with 3-fluoropropanol (5.94 g, 76 mmol) and Et$_3$N (11.57 g, 114 mmol) and anhydrous Et$_2$O (150 mL). Methanesulfonyl chloride (13.11 g, 114 mmol) dissolved in Et$_2$O (50 mL) was added to the dropping funnel. The RB flask was cooled in a salt-ice bath for 30 min before drop-wise addition of the MeSO$_2$Cl/Et$_2$O mixture was commenced. A thick white precipitate rapidly began to form rapidly. After complete addition the mixture was allowed to warm to r.t. The white precipitate (Et$_3$NHCl) was removed by filtration and was washed with Et$_2$O (2 × 150 mL). The volume of the filtrate was reduced to ~100 mL before being washed quickly with H$_2$O (3 × 100 mL), dried over anhydrous MgSO$_4$, filtered and taken to dryness to give a place brown liquid. The crude material was subjected to fractional distillation to remove any residual MeSO$_2$Cl followed by distillation, pressure/temperature, of the desired product as a colorless liquid, 4.30 g. $^1$H NMR (CDCl$_3$, 400.13 MHz): δ 2.13 (m, 2H), 3.02 (s, 3H), 4.36 (t, J =6.11 Hz, 2H), 4.57 (dt, J$_1$ = 46.5 Hz, J$_2$ = 5.7 Hz, 2H).

S3.5. Thioactetic acid S-(3-fluoropropyl) ester

![Thioactetic acid S-(3-fluoropropyl) ester](image)

3-Fluoropropyl methanesulfonate (4.30 g, 27.53 mmol) and potassium thioacetate (6.33 g, 55.42 mmol) were dissolved in CH$_3$CN (150 mL) and stirred at r.t. for 3 days, the mixture became red-brown over this time. The mixture was poured into DeI H$_2$O (600 mL) and extracted with Et$_2$O (3 × 150 mL). The combined organic fractions were dried over anhydrous MgSO$_4$, filtered and taken to dryness. The residue was purified under reduced pressure employing a Kugelrohr apparatus, 85 - 100 °C @ 35 mmHg, giving 2.04g of a pale yellow oil. $^1$H NMR (d$_6$-DMSO, 400.13 MHz): δ 1.89 (tt, J$_1$ = 5.90 Hz, J$_2$ = 7.28 Hz, 2H), 2.33 (s, 3H), 2.91 (t, J$_1$ = 7.28 Hz, 2H), 4.46 (dt, J$_1$ = 47.4 Hz, J$_2$ = 5.90 Hz); $^{13}$C{1H} NMR (d$_6$-DMSO, 100.62 MHz): δ 24.41 (d, J = 5.85 Hz, CH$_2$), 30.00 (d, J = 91.81 Hz, CH$_2$), 30.49 (CH$_3$), 82.38 (d, J = 162.74 Hz, CH$_2$), 195.09 (CO).

S3.6. Sodium 3-fluoropropylsulfonate

![Sodium 3-fluoropropylsulfonate](image)

Thioacetic acid S-(3-fluoropropyl) ester (2.04 g, 14.98 mmol) was dissolved in glacial AR acetic acid (40 mL). After warming the solution to 60 °C, 30% aqueous H$_2$O$_2$ (7.5 mL) was added dropwise, rapidly discharging the yellow color from solution, the mixture was heated for a further 3 h after addition. After cooling to r.t. the mixture was reduced to dryness to give a thick oil, before being dried under high vacuum overnight. The residue was dissolved in DeI H$_2$O (50 mL) before being carefully neutralized
with 1M aqueous Na$_2$CO$_3$. The solution was then taken to dryness to give a fine white powder that was dried under high vacuum overnight. The crude material was used without further purification. $^1$H NMR (D$_2$O - internal Me$_2$CO reference, 400.13 MHz): $\delta$ 2.13 (m, 2H), 3.03 (m, 2H), 4.61 (dt, $J_1$ = 46.9 Hz, $J_2$ = 5.95 Hz); $^{13}$C {$^1$H} NMR (D$_2$O - internal Me$_2$CO reference, 100.62 MHz): $\delta$ 26.11 (d, $J$ = 20.1 Hz, CH$_2$), 47.76 (d, $J$ = 5.92 Hz, CH$_2$), 83.85 (d, $J$ = 160.2 Hz, CH$_2$).

S3.7. 3-Fluoropropylsulfonyl chloride

Crude sodium 3-fluoropropylsulfonate, from the previous reaction, (2.6 g) was suspended in dry CH$_2$Cl$_2$ (100 mL) to which was carefully added SOCl$_2$ (6 mL) and DMF (0.1 mL). The mixture was heated to reflux under a nitrogen atmosphere for 5 h. The solution was filtered through a pad of oven-dried celite and the eluate taken to dryness under high vacuum to give a yellow liquid. The crude product was purified by distillation under reduced pressure using a Kugelrohr apparatus, 120 - 130 °C at 10 - 12 mmHg to give a pale orange oil, 2.00 g. $^1$H NMR (CDCl$_3$, 400.13 MHz): $\delta$ 2.44 (m, 2H), 3.83 (m, 2H), 4.62 (dt, $J_1$ = 46.7 Hz, $J_2$ = 5.5Hz, 2H); $^{13}$C {$^1$H} NMR (CDCl$_3$, 100.62 MHz): $\delta$ 25.91 (d, $J$ = 20.50), 61.63 (d, $J$ = 3.91), 80.55 (d, $J$ = 169.16); $^{19}$F {$^1$H} NMR (CD$_3$CN, 376.50 MHz): $\delta$ -222.28 (s, -CH$_2$F).

S4. Preparation of membranes from mouse brain

To test the Y$_1$R affinity of the synthesized ligands, receptor binding assays (described below) were performed on crude membranes prepared from the brains of Y$_2$R- and Y$_4$R-deficient mice (Y2$^{-/-}$Y4$^{-/-}$), where Y$_1$R accounts for the majority of remaining Y receptors. Membranes were prepared following modified membrane extraction protocol published elsewhere (McCrea KE, Herzog H. Radioligand binding studies. Pharmacological profiles of cloned Y-receptor subtypes. Methods Mol Biol. 2000; 153:231-9). In brief, fresh frozen Y2$^{-/-}$Y4$^{-/-}$ mouse brains were cut into small cubes and homogenized in ice-cold homogenization buffer (50 mM Tris-HCl, 10 mM NaCl, 5 mM MgCl$_2$, 2.5 mM CaCl$_2$, pH7.4, supplemented with 1 mg/mL bacitracin (250,000U; Calbiochem-Novabiochem., La Jolla, CA, USA) prior to use) on ice with a glass homogenizer (Wheaton, USA) using 30 strokes. Subsequently, the homogenates were centrifuged at 32,000g for 15 minutes at 4°C. The resulting pellet was re-suspended in ice-cold homogenization buffer and re-homogenized using 30 strokes on ice, followed by centrifugation at 32,000g for 15 minutes at 4°C to obtain the final pellet. The final pellet was re-suspended in ice-cold homogenization buffer and flash frozen in liquid nitrogen. The protein concentration of the suspension was determined using Bradford protein assay (Quick Start™ Bradford Protein Assay, Bio-Rad Laboratories Pty., Ltd., Hercules, CA, USA).
S4.1. Receptor binding assays

Competition assays were performed on Y2-/-Y4-/- mouse brain membrane preparations following procedures published previously (McCrea KE, Herzog H. Radioligand binding studies. Pharmacological profiles of cloned Y-receptor subtypes. Methods Mol Biol. 2000; 153:231-9). Briefly, equal volumes (25 µL) of non-radioactive ligands and ¹²⁵I-human polypeptide YY (¹²⁵I-hPYY, 2200 Ci/mmol; PerkinElmer Life Science Products, Boston, MA, USA) were added into each assay. The final concentration of ¹²⁵I-hPYY in the assay was 25⁻¹² M. The binding of ¹²⁵I-hPYY was competed by Y1R ligands of interest at increasing concentrations ranging from 10⁻¹² M to 10⁻⁶ M. Non-radioactive human PYY (Auspep, Parkville, VIC, Australia) at 10⁻⁶ M was used as the non-specific binding control. The reaction was initiated by the addition of 50 µL of membrane suspension containing 30 µg of protein into the assay mixture and incubated for 2 hours at r.t. After the incubation, each sample was layered with 200 µL of pre-cooled (4°C) horse serum and centrifuged at 13,000g for 4 minutes to separate of bound from free ¹²⁵I-PYY. The supernatant solution was removed and resultant pellet was harvested and counted for radioactivity using a γ-counter (Wallac 1470 WIZARD® Gamma Counter; PerkinElmer Life Sciences, Turku, Finland). Finally, the data were analyzed with GraphPad Prism Software to determine the IC₅₀ value.

S5. HPLC purity analysis

S5.1. 9b 90.03% purity, tᵣ = 18.57 min.

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S5.2. 9c 96.24% purity, $t_R = 18.73$ min.

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S6. NMR spectra

S6.1. 9b $^1$H NMR
S6.2. 9b $^{13}$C NMR

S6.3. 9c $^1$H NMR
S6.4. 9c $^{13}$C NMR

S6.5. 9d $^1$H NMR
S6.6. 9d $^{13}$C NMR

S7. References


