Supporting Information

Fragment evolution for GPCRs: the role of secondary binding sites in optimization

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Results

The $\beta_1$AR and $\beta_2$AR binding sites

Figure S1: Comparison of the human $\beta_1$AR (residues in magenta, model downloaded from the GPCRdb) and the human $\beta_2$AR (residues in light grey, PDB code: 2RH1) binding sites. Carazolol, which is bound to the $\beta_2$AR structure, is shown with white sticks.
Pharmacological analysis of the 21 core fragments

Figure S2: Inhibition of $^3$H-CGP12177 binding to CHO-β₁ and CHO-β₂ cells by core compounds A₅, A₉, A₁₂ and A₁₅. Bars represent total $^3$H-CGP12177 binding and non-specific binding (determined in the presence of 10 µM propranolol). The concentration of $^3$H-CGP12177 was 0.94 nM. Data points are mean ± s.e.m. of triplicate determinations.
Table S1: Affinity (log $K_D$ values) of core compounds 1-21 obtained from $^3$H-CGP 12177 whole cell binding in CHO cells stably expressing the human $\beta_1$AR or $\beta_2$AR. Values represent mean + or - s.e. mean of n separate experiments. Selectivity ratios are also given where a ratio of 1 demonstrates no selectivity for a given receptor subtype over another. When the competing ligand caused > 50% inhibition of specific binding, an IC$_{50}$ value was determined by extrapolating the curve to non-specific levels and assuming that a greater concentration would have resulted in 100% inhibition. These values are given as apparent K$_D$ values in the data table.

| Article ID | $\beta_1$AR Log $K_D$ | n   | $\beta_2$AR Log $K_D$ | n   | Selectivity ratio |
|------------|-----------------------|-----|-----------------------|-----|-------------------|
| A1         | -5.44 ± 0.03          | 11  | -5.62 ± 0.02          | 11  | 1.5               |
| A2         | -5.49 ± 0.04          | 10  | -5.59 ± 0.04          | 10  | 1.3               |
| A3         | -5.62 ± 0.02          | 10  | -5.93 ± 0.03          | 10  | 2.0               |
| A4         | -6.77 ± 0.03          | 12  | -7.45 ± 0.04          | 12  | 4.8               |
| A5         | -6.39 ± 0.03          | 10  | -6.87 ± 0.04          | 9   | 3                 |
| A6         | No binding            | 6   | No binding            | 6   |                   |
| A7         | No binding            | 6   | No binding            | 6   |                   |
| A8         | -4.01 ± 0.03          | 6   | -4.78 ± 0.02          | 6   | 5.9               |
| A9         | -3.71 ± 0.05          | 5   | -4.34 ± 0.05          | 6   | 4.3               |
| A10        | -3.98 ± 0.03          | 6   | -4.48 ± 0.03          | 6   | 3.2               |
| A11        | -4.70 ± 0.05          | 6   | -5.69 ± 0.03          | 6   | 9.8               |
| A12        | -5.70 ± 0.05          | 6   | -6.67 ± 0.05          | 6   | 9.3               |
| A13        | -4.26 ± 0.04          | 6   | -4.64 ± 0.05          | 6   | 2.4               |
| A14        | -4.15 ± 0.03          | 6   | -4.84 ± 0.04          | 6   | 4.9               |
| A15        | No binding            | 6   | No binding            | 6   |                   |
| A16        | -3.71 ± 0.02          | 5   | -4.23 ± 0.05          | 6   | 3.3               |
| A17        | -4.24 ± 0.04          | 6   | -4.73 ± 0.03          | 6   | 3.1               |
| A18        | No binding            | 6   | No binding            | 6   |                   |
| A19        | -4.22 ± 0.02          | 6   | -4.63 ± 0.03          | 6   | 2.6               |
| A20        | -3.73 ± 0.04          | 6   | -4.38 ± 0.05          | 6   | 4.5               |
| A21        | No binding            | 6   | No binding            | 6   |                   |

Figure S3: The five core fragments selected for further growing.
Figure S4: 2D depiction of the 21 OBP fragments.
Figure S5: 2D depiction of the A1 derivative products.
Figure S6: 2D depiction of the A2 derivative products.
Figure S7: 2D depiction of the A₃ derivative products.
Figure S8: 2D depiction of the A4 derivative products.
Figure S9: 2D depiction of the A$_5$ derivative products.
Figure S10: Matrix of the designed bitopic compounds for β₂AR.
Figure S11: Matrix of the designed bitopic compounds for the β₁AR.
**Success rate of the library synthesis**

|    | A1 | A2 | A3 | A4 | A5 |
|----|----|----|----|----|----|
| B1 | *  | *  | #  | *  | *  |
| B2 | *  | *  | *  | #  | *  |
| B3 | *  | *  | *  | #  | *  |
| B4 | *  | *  | *  | #  | *  |
| B5 | *  | *  | *  | #  | *  |
| B6 | *  | *  | #  | *  | #  |
| B7 | *  | *  | *  | #  | *  |
| B8 | *  | *  | *  | #  | *  |
| B9 | *  | *  | #  | *  | #  |
| B10| *  | *  | *  | #  | *  |
| B11| *  | *  | *  | #  | *  |
| B12| *  | *  | *  | #  | *  |
| B13| *  | *  | *  | #  | *  |

Figure S12: A. Success rate of the $\beta_1$ matrix; * 5 eq. AcOH added; # only dialkylated product formed. B. Success rate of the $\beta_2$ matrix; * 5 eq. AcOH added; # only dialkylated product formed, grey cells: not reacted in the sparse matrix.
**Pharmacological analysis of the optimization matrices**

Figure S13: Scatter plot distribution of the affinity gain for the $\beta_1$-SBP bitopic compounds (blue dots) and the $\beta_2$-SBP bitopic compounds (orange dots) when compared to their initial OBP fragments. The vertical blue line separates the compounds which exhibit an improved affinity against the $\beta_1$ AR versus the ones which do not. Thus any blue point (i.e. designed to bind to the $\beta_1$ AR) located to the right of the blue line can be regarded as a positively designed compounds. The same logic applies for the $\beta_2$AR-SBP bitopic compounds and the horizontal orange line.
Figure S14: Box plot representation of the LLE of the (a) core OBP fragments and (b) the SBP fragments in both optimization matrices.
Methods

Chemical synthesis
All chemical reagents used were purchased from commercial chemical suppliers. Flash chromatography was performed using Teledyne ISCO CombiFlash Lumen+ Rf. Purifications by preparative-HPLC were performed with Hanbon NS4205 Binary high pressure semi-preparative HPLC.

Figure S15: Example of a reductive alkylation. Primary amine (A5) and aromatic aldehyde (B19), the imine intermediate (imine A5B19) and secondary amine product A5B19.

Figure S16: General procedure for reductive alkylation library synthesis.
Characterisation of the library members

The LC-MS measurements were performed on Shimadzu LC-MS2020 LC/MS system. The purity of all compounds was over 90% based on LC-MS measurements. High resolution mass spectrometric measurements were performed using a Q-TOF Premier mass spectrometer (Waters Corporation, Milford, MA, USA) in positive electrospray ionization mode. The NMR experiments were performed at 500 MHz (\(^1\)H) on a Varian VNMR SYSTEM spectrometer. Chemical shifts are referenced to the residual solvent signals, 2.50 ppm for \(^1\)H in DMSO-d6 and 7.28 ppm for 1H in CDCl\(_3\)).
### HR-MS results of the β1-receptor matrix

| Compound | Mass (Da) | Calculated Mass (Da) | Measurement Error (ppm) | Formula | Measured Ion |
|----------|-----------|-----------------------|-------------------------|---------|--------------|
| A1B1     | 371.1416  | 371.1429              | -3.5                   | C20H22N2O3S  | M+H         |
| A1B2     | 456.1030  | 456.1036              | -1.3                   | C24H22ClN4O4S | M+H         |
| A1B3     | 450.1100  | 450.1110              | -2.2                   | C24H20F3N3O2S  | M+H         |
| A1B4     | 364.1482  | 364.1484              | -0.5                   | C21H21N3OS  | M+H         |
| A1B5     | 426.1639  | 426.1640              | -0.2                   | C26H23N3OS  | M+H         |
| A1B7     | 362.1240  | 362.1226              | 3.9                    | C19H20F3N3O3S | M+H         |
| A1B8     | 328.1368  | 328.1371              | -0.9                   | C19H21NO2S  | M+H         |
| A1B10    | 377.1321  | 377.1324              | -0.8                   | C22H20NO2S  | M+H         |
| A1B11    | 412.1492  | 412.1484              | 1.9                    | C25H21N3OS  | M+H         |
| A1B12    | 337.1581  | 337.1586              | -1.5                   | C17H24N2O3S  | M+H         |
| A1B13    | 504.0729  | 504.0745              | -3.2                   | C26H22BrN3OS  | M+H         |
| A2B3     | 444.1559  | 444.1546              | 2.9                    | C26H22FN3OS  | M+H         |
| A2B4     | 356.1753  | 356.1763              | -2.8                   | C23H23N3O  | M+H         |
| A2B5     | 420.2081  | 420.2076              | 1.2                    | C28H25N3O  | M+H         |
| A2B7     | 356.1659  | 356.1662              | -0.8                   | C21H22F3N03  | M+H         |
| A2B8     | 322.1793  | 322.1807              | -4.3                   | C21H23N02  | M+H         |
| A2B10    | 371.1763  | 371.1760              | 0.8                    | C24H22N2O2  | M+H         |
| A2B11    | 406.1918  | 406.1919              | -0.2                   | C27H23N3O  | M+H         |
| A2B13    | 498.1186  | 498.1181              | 1.0                    | C28H24BrN3O  | M+H         |
| A3B2     | 458.1374  | 458.1370              | 0.9                    | C24H24CINO6  | M+H         |
| A3B3     | 452.1436  | 452.1444              | -1.8                   | C24H22FN3O3S  | M+H         |
| A3B4     | 366.1829  | 366.1818              | 3.0                    | C21H23N03   | M+H         |
| A3B5     | 428.1965  | 428.1974              | -2.1                   | C26H25N3O3  | M+H         |
| A3B7     | 364.1557  | 364.1560              | -0.8                   | C19H22F3N05  | M+H         |
| A3B8     | 330.1696  | 330.1705              | -2.7                   | C19H23N04  | M+H         |
| A3B10    | 379.1653  | 379.1658              | -1.3                   | C22H22N2O4  | M+H         |
| A3B11    | 414.1817  | 414.1818              | -0.2                   | C25H23N3O3  | M+H         |
| A5B5     | 450.2184  | 450.2182              | 0.4                    | C29H27N3O2  | M+H         |
| A5B9     | 351.2084  | 351.2073              | 3.1                    | C22H26N2O2  | M+H         |
| A5B10    | 401.1862  | 401.1865              | -0.7                   | C25H24N2O3  | M+H         |
| A5B11    | 436.2023  | 436.2025              | -0.5                   | C28H25N3O2  | M+H         |
| A5B13    | 528.1279  | 528.1287              | -1.5                   | C29H26BrN3O2  | M+H         |
| A6B1     | 359.1972  | 359.1971              | 0.3                    | C20H26N2O4  | M+H         |
| A6B2     | 444.1560  | 444.1578              | -4.1                   | C24H26CINO5  | M+H         |
| A6B3     | 438.1646  | 438.1652              | -1.4                   | C24H24F3N02S  | M+H         |
| A6B4     | 352.2025  | 352.2025              | 0.0                    | C21H25N3O2  | M+H         |
| A6B7     | 350.1765  | 350.1768              | -0.9                   | C19H24F3N04  | M+H         |
| A6B8     | 316.1913  | 316.1913              | 0.0                    | C19H25N03  | M+H         |
| A6B11    | 400.2016  | 400.2025              | -2.2                   | C25H25N3O2  | M+H         |
| A6B13    | 492.1276  | 492.1287              | -2.2                   | C26H26BrN3O2  | M+H         |
NMR-spectra of representative β1-receptor matrix compounds

B1-A1B3, (S)-benzyl (2-((2-(benzo[b]thiophen-2-yl)-2-hydroxyethyl)amino)ethyl)carbamate

1H NMR (500 MHz, DMSO-d6) δ 8.86 (s; 1H), 7.94 (d; J=7.9 Hz; 1H), 7.83-7.78 (m; 3H), 7.62 (d; J=5.2 Hz; 1H), 7.55 (d; J=3.6 Hz; 1H), 7.41-7.30 (m; 5H), 7.19 (t; J=8.7 Hz; 1H), 5.45 (dd; J=12.9 Hz; 1H), 4.72-4.66 (m; 1H), 4.43-4.35 (m; 2H), 3.46-3.41 (m; 1H), 3.32-3.26 (m; 1H)

B1-A1B7, (S)-1-(benzo[b]thiophen-2-yl)-2-((3-fluoro-4-(2-hydroxyethoxy)benzyl)amino)ethanol

1H NMR (500 MHz, DMSO-d6) δ 7.87 (d; J=7.9 Hz; 1H), 7.74 (d; J=7.8 Hz; 1H), 7.32-7.24 (m; 3H), 7.18 (d; J=12.4 Hz; 1H), 7.08-7.03 (m; 2H), 5.84 (s; 1H), 4.96 (t; J=12.6 Hz; 1H), 4.87 (t; J=11.0 Hz; 2H), 4.02 (t; J=10.1 Hz; 2H), 3.72-3.66 (m; 4H), 2.77 (d; J=6.3 Hz; 2H), 2.06 (s; 1H)

B1-A1B10, (S)-1-(benzo[b]thiophen-2-yl)-2-(((6-phenoxy-pyridin-3-yl)methyl)amino)ethanol

1H NMR (500 MHz, DMSO-d6) δ 8.07 (d; J=2.6 Hz; 1H), 7.88 (d; J=7.9 Hz; 1H), 7.81 (dd; J=10.8 Hz; 1H), 7.49-7.45 (m; 1H), 7.42 (t; J=15.0 Hz; 2H), 7.22 (t; J=14.8 Hz; 1H), 7.15 (d; J=8.5 Hz; 2H), 6.88 (d; J=8.4 Hz; 1H), 3.65 (t; J=12.6 Hz; 2H), 2.78 (d; J=4 Hz; 1H), 2.72 (d; J=6.9 Hz; 1H), 2.61 (s; 2H), 1.26 (s; 1H)

B1-A2B10, (R)-1-(naphthalen-2-yl)-2-(((6-phenoxy-pyridin-3-yl)methyl)amino)ethanol

1H NMR (500 MHz, CDCl3) δ 8.09 (d; J=2.4 Hz; 1H), 7.85-7.78 (m; 5H), 7.62 (dd; J=10.9 Hz; 1H), 7.49-7.45 (m; 1H), 7.42 (t; J=15.0 Hz; 2H), 7.22 (t; J=14.8 Hz; 1H), 7.15 (d; J=8.5 Hz; 2H), 6.88 (d; J=8.4 Hz; 1H), 3.65 (t; J=12.6 Hz; 2H), 2.78 (d; J=4 Hz; 1H), 2.72 (d; J=6.9 Hz; 1H), 2.61 (s; 2H), 1.26 (s; 1H)

B1-A2B11, (R)-2-(((2-(naphthalen-1-yl)pyrimidin-5-yl)methyl)amino)-1-(naphthalen-2-yl)ethanol

1H NMR (500 MHz, DMSO-d6) δ 8.91 (s; 1H), 8.82 (s; 1H), 8.78 (s; 1H), 8.56-8.52 (m; 1H), 8.04-7.80 (m; 7H), 7.64-7.43 (m; 64.64H), 4.91-4.87 (m; 1H), 4.64 (s; 2H), 3.92 (d; J=14.3 Hz; 2H), 1.32 (s; 1H)
B1-A3B3, (S)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-(((1-(4-fluorophenyl)-3-(thiophen-2-yl)-1H-pyrazol-4-yl)methyl)amino)ethanol

\[ \text{H NMR (500 MHz, CDCl}_3 \delta 8.12 (s; 1H), 7.68-7.74 (m; 2H), 7.35 (d; J=5.0; 2H), 7.13-7.10 (m; 3H), 6.84 (s; 1H), 6.79-6.75 (m; 2H), 4.85 (s; 1H), 4.25-4.16 (m; 7H), 3.04 (d; J=44.7; 2H), 1.26 (s; 1H)} \]

\[ \text{1H NMR (500 MHz, CDCl}_3 \delta 8.12 (s; 1H), 7.68-7.74 (m; 2H), 7.35 (d; J=5.0; 2H), 7.13-7.10 (m; 3H), 6.84 (s; 1H), 6.79-6.75 (m; 2H), 4.85 (s; 1H), 4.25-4.16 (m; 7H), 3.04 (d; J=44.7; 2H), 1.26 (s; 1H)} \]

B1-A3B7, (S)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-((3-fluoro-4-(2-hydroxyethoxy)benzyl)amino)ethanol

\[ \text{1H NMR (500 MHz, DMSO-}d_6 \delta 7.18 (d; J=13.2 Hz; 1H), 7.19-7.05 (m; 2H), 6.85 (s; 1H), 6.81-6.74 (m; 2H), 4.58 (t; J=12.7 Hz; 1H), 4.20 (s; 4H), 4.18 (s; 4H), 4.03-4.01 (m; 3H), 3.71-3.69 (m; 4H), 2.59 (d; J=7.3 Hz; 2H), 2.16 (s; 1H)} \]

B1-A3B8, (S)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-((4-(2-hydroxyethyl)benzyl)amino)ethanol

\[ \text{1H NMR (500 MHz, CDCl}_3 \delta 7.26-7.16 (m; 4H), 6.97 (s; 1H), 6.87-6.71 (m; 2H), 4.71-4.65 (m; 1H) 4.52-4.49 (m; 1H), 4.24-4.19 (m; 5H), 3.84-3.77 (m; 3H), 2.87-2.81 (m; 4H), 2.56 (d; J=7.2; 2H), 2.16 (s; 1H)} \]

B1-A3B10, (S)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-((6-phenoxy pyridin-3-yl)methyl)amino)ethanol

\[ \text{1H NMR (500 MHz, DMSO-}d_6 \delta 8.04 (d; J=2.4 Hz; 1H), 7.78 (dd; J=11.0 Hz; 1H), 7.40 (t; J= 15.9; 2H), 7.18 (t; J= 13.6; 1H), 7.08 (d; J=8.8 Hz; 2H), 6.95 (d; J=8.4 Hz; 1H), 6.78 (s; 1H), 6.74 (s; 2H), 4.52 (t; J=12.4 Hz; 1H), 4.20-4.16 (m; 5H), 3.68 (s; 2H), 2.56 (d; J=7.2; 2H), 2.16 (s; 1H), 2.27 (s; 1H)} \]

B1-A3B11, (S)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-(((2-(naphthalen-1-yl)pyrimidin-5-yl)methyl)amino)ethanol

\[ \text{1H NMR (500 MHz, DMSO-}d_6 \delta 8.87 (s; 1H), 8.84 (s; 1H), 8.60 (d; J=9.6 Hz; 1H), 8.03 (d; J=7.1 Hz; 1H), 7.96 (d; J=8.1 Hz; 1H), 7.91 (d; J=7.5 Hz; 1H), 7.85-7.49 (m; 3H), 6.98 (s; 1H), 6.89 (s; 1H), 6.81 (s; 1H), 4.74 (dd; J=12.2 Hz; 1H), 4.69 (s; 1H), 4.21 (s; 4H), 3.90 (s; 2H), 2.93 (dd; J=16.0 Hz; 1H), 2.87-2.83 (m; 1H), 2.27 (s; 1H)} \]
B1-A5B2, (R)-ethyl-5-(4-chlorophenyl)-2-(((2-hydroxy-3-(naphthalen-1-yloxy)propyl)amino)methyl)furan-3-carboxylate

\[
\begin{align*}
\text{H NMR (500 MHz, DMSO-}d_6\text{) } & \delta 8.08 \text{ (d; } J = 8.7 \text{ Hz; 1H), 7.86-7.77 \text{ (m; 2H), 7.52-7.25 \text{ (m; 7H),}} \\
& \text{6.97 \text{ (d; } J = 7.6 \text{ Hz; 1H), 4.41 \text{ (t; } J = 10.2 \text{ Hz; 1H),}} \\
& \text{4.30-4.23 \text{ (m; 2H), 2.10-2.03 \text{ (m; 2H), 1.90 \text{ (d;}} \\
& \text{ } J = 11.8 \text{ Hz; 1H), 1.84 \text{ (d; } J = 7.0 \text{ Hz; 1H), 1.34-1.23 \text{ (m; 3H)}}
\end{align*}
\]

B1-A5B9, (2R)-1-((1-(4-(aminomethyl)phenyl)ethyl)amino)-3-(naphthalen-1-yloxy)propan-2-ol

\[
\begin{align*}
\text{H NMR (500 MHz, DMSO-}d_6\text{) } & \delta 8.12-8.10 \text{ (m; 2H), 8.08 \text{ (t; } J = 12.0 \text{ Hz; 2H), 7.90-7.86 \text{ (m; 2H),}} \\
& \text{7.55-7.49 \text{ (m; 5H), 7.44 \text{ (t; } J = 15.8 \text{ Hz; 1H), 6.96 \text{ (d; } J = 6.7 \text{ Hz; 1H), 5.32-5.28 \text{ (m; 1H),}} \\
& \text{4.31 \text{ (dd.}} \\
& \text{ } J = 14.4 \text{; 2H), 4.23-4.20 \text{ (m; 3H), 3.59-3.54 \text{ (m; 1H),}} \\
& \text{3.41-3.36 \text{ (m; 1H), 2.05 \text{ (s; 4H) 1.84 \text{ (s; 3H), 1.24 \text{ (s; 1H)}}}}
\end{align*}
\]
| Compound | Mass (Da) | Calculated Mass (Da) | Measurement Error (ppm) | Formula       | Measured Ion |
|----------|-----------|----------------------|-------------------------|---------------|--------------|
| A1B1     | 333.1018  | 333.1021             | -0.9                    | C15H16N4O3S  | M+H          |
| A1B2     | 346.1029  | 346.1032             | -0.9                    | C19H20CINOS   | M+H          |
| A1B4     | 334.0573  | 334.0572             | 0.3                     | C16H15N4O3S2 | M+H          |
| A1B5     | 344.0951  | 344.0957             | -1.7                    | C18H17N4O3S  | M+H          |
| A1B6     | 356.0893  | 356.0891             | 0.6                     | C18H17N4O3S2 | M+H          |
| A1B7     | 346.0916  | 346.0913             | 0.9                     | C18H16FNO3S  | M+H          |
| A1B8     | 342.1172  | 342.1164             | 2.3                     | C19H19N4O3S  | M+H          |
| A1B9     | 370.1037  | 370.1048             | -3.0                    | C19H19N4O3S2 | M+H          |
| A1B10    | 357.0823  | 357.0828             | -1.4                    | C19H17CINO2S | M+H          |
| A1B11    | 386.1422  | 386.1426             | -1.0                    | C21H23N4O4S  | M+H          |
| A1B12    | 388.1218  | 388.1219             | -0.3                    | C20H21N4O5S  | M+H          |
| A2B1     | 327.1443  | 327.1457             | -4.3                    | C20H19N4O4   | M+H          |
| A2B2     | 340.1472  | 340.1468             | 1.2                     | C20H19N4O3S  | M+H          |
| A2B3     | 388.1538  | 388.1549             | -2.8                    | C20H18FNO3S  | M+H          |
| A2B4     | 328.1011  | 328.1007             | 1.2                     | C21H21N4O3S  | M+H          |
| A2B5     | 338.1383  | 338.1392             | -2.7                    | C21H21N4O3S  | M+H          |
| A2B6     | 350.1319  | 350.1327             | -2.3                    | C21H19CIN2O  | M+H          |
| A2B8     | 336.1595  | 336.1600             | -1.5                    | C22H23N4O5S  | M+H          |
| A2B10    | 351.1253  | 351.1264             | -3.1                    | C19H22CIN2O  | M+H          |
| A2B11    | 380.1863  | 380.1862             | 0.3                     | C22H21N4O6S  | M+H          |
| A2B12    | 382.1649  | 382.1654             | -1.3                    | C16H17N4O5S  | M+H          |
| A3B1     | 335.1356  | 335.1355             | 0.3                     | C19H21N4O3S  | M+H          |
| A3B2     | 348.1371  | 348.1366             | 1.4                     | C19H19CIN2O3 | M+H          |
| A3B3     | 396.1451  | 396.1447             | 1.0                     | C21H25N4O6S  | M+H          |
| A3B4     | 336.0912  | 336.0906             | 1.8                     | C20H23N4O7S  | M+H          |
| A3B5     | 346.1296  | 346.1291             | 1.4                     | C18H20N4O4S  | M+H          |
| A3B6     | 358.1225  | 358.1225             | 0.0                     | C22H24CIN2O2 | M+H          |
| A3B7     | 348.1245  | 348.1247             | -0.6                    | C25H23N4O5S  | M+H          |
| A3B8     | 344.1489  | 344.1498             | -2.6                    | C19H19N4O4S  | M+H          |
| A3B9     | 372.1393  | 372.1382             | 3.0                     | C21H21N4O5S  | M+H          |
| A3B10    | 359.1152  | 359.1162             | -2.8                    | C21H21N4O3S  | M+H          |
| A3B12    | 390.1546  | 390.1553             | -1.8                    | C22H23N4O4S  | M+H          |
| A5B1     | 357.1555  | 357.1563             | -2.2                    | C15H20N4O4S  | M+H          |
| A5B2     | 370.1570  | 370.1574             | -1.1                    | C19H24CIN2O2 | M+H          |
| A5B3     | 418.1655  | 418.1654             | 0.2                     | C22H23N4O5S  | M+H          |
| A5B4     | 358.1112  | 358.1113             | -0.3                    | C16H19N4O4S  | M+H          |
| A5B5     | 368.1496  | 368.1498             | -0.5                    | C18H21N4O5S  | M+H          |
| A5B6     | 380.1438  | 380.1433             | 1.3                     | C18H21N4O3S  | M+H          |
| A5B7     | 370.1451  | 370.1455             | -1.1                    | C18H20FNO4S  | M+H          |
| A5B8     | 366.1700  | 366.1705             | -1.4                    | C19H23N4O4S  | M+H          |
| A5B13    | 460.0758  | 460.0760             | -0.4                    | C22H22BrNO5S | M+H          |
| A5B14    | 378.1703  | 378.1705             | -0.5                    | C23H23N4O4S  | M+H          |
| A5B15    | 425.0857  | 425.0865             | -1.9                    | C22H21BrN2O2 | M+H          |
| A5B16    | 381.1268  | 381.1273             | -1.3                    | C21H20N4O3S  | M+H          |
| A6B1     | 321.1559  | 321.1563             | -1.2                    | C15H20N4O4S  | M+H          |
| A6B2     | 334.1564  | 334.1574             | -3.0                    | C19H24CIN2O2 | M+H          |
| A6B3     | 382.1646  | 382.1654             | -2.1                    | C22H23N4O5S  | M+H          |
| A6B4     | 322.1111  | 322.1113             | -0.6                    | C16H19N4O4S  | M+H          |
| A6B5     | 332.1496  | 332.1498             | -0.6                    | C18H21N4O5S  | M+H          |
|     |       |       |     |       |       |
|-----|-------|-------|-----|-------|-------|
| A6B6 | 344.1440 | 344.1433 | 2.0 | C18H21N3O2S | M+H  |
| A6B7 | 334.1451 | 334.1455 | -1.2 | C18H20FNO4 | M+H  |
| A6B8 | 330.1708 | 330.1705 | 0.9  | C19H23NO4   | M+H  |
| A6B13| 424.0757 | 424.0760 | -0.7 | C19H22BrNO5 | M+H  |
| A6B14| 342.1705 | 342.1705 | 0.0  | C20H23NO4   | M+H  |
| A6B15| 389.0861 | 389.0865 | -1.0 | C19H21BrN2O2| M+H  |
| A6B16| 345.1272 | 345.1273 | -0.3 | C18H20N2O3S | M+H  |

**NMR-spectra of representative β₂-receptor matrix compounds**

B2-A1B6, (S)-2-(((5-(1H-pyrazol-5-yl)thiophen-2-yl)methyl)amino)-1-(benzo[b]thiophen-2-yl)ethanol

\[ \text{H NMR (500 MHz, DMSO-d}_6\] \( \delta \): 12.73 (s; 1H), 7.88 (d; J=8.0 Hz; 1H), 7.74-7.71 (m; 2H), 7.32-7.24 (m; 2H), 7.19-7.16 (m; 1H), 6.91-6.87 (m; 2H), 6.51 (s; 1H), 5.81 (s; 1H), 4.98-4.94 (m; 1H), 3.92 (s; 2H), 2.83 (d; J=6.3 Hz; 2H), 1.22 (s; 1H)

B2-A1B8, (S)-2-(4-(((2-(benzo[b]thiophen-2-yl)-2-hydroxyethyl)amino)methyl)phenyl)acetic acid

\[ \text{H NMR (500 MHz, DMSO-d}_6\] \( \delta \): 7.89 (d; J=7.9 Hz; 1H), 7.75 (d; J=7.4 Hz; 1H), 7.45 (d; J=8.0 Hz; 1H), 7.34-7.11 (m; 6H), 5.72 (s; 1H), 5.05-5.03 (m; 1H), 3.83 (s; 1H), 3.58-3.49 (m; 4H), 2.88 (d; J=4.1 Hz; 1H), 2.73-2.71 (m; 1H), 2.06 (s; 1H)

B2-A3B9, (S)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-(((5-(1-methyl-1H-pyrazol-4-yl)thiophen-2-yl)methyl)amino)ethanol

\[ \text{H NMR (500 MHz, DMSO-d}_6\] \( \delta \): 7.91 (s; 1H), 7.61 (s; 1H), 6.93 (d; J=3.3 Hz; 1H), 6.81 (d; J=3.3 Hz; 1H), 6.77 (s; 1H), 6.74 (s; 2H), 4.52 (t; J=12.9 Hz; 1H), 4.19 (s; 1H), 4.17 (s; 1H), 3.84 (s; 2H), 3.81 (s; 3H), 2.61 (t; J=13.2 Hz; 2H), 1.22 (s; 1H)

B2-A5B1, (R)-2-(4-(((2-hydroxy-3-(naphthalen-1-yloxy)propyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetic acid

\[ \text{H NMR (500 MHz, DMSO-d}_6\] \( \delta \): 8.21 (d; J=7.3 Hz; 1H), 7.84 (d; J=9.4 Hz; 1H), 7.74 (s; 1H), 7.51-7.36 (m; 4H), 6.94 (d; J=8.1 Hz; 1H), 5.15 (br.s; 1H), 4.58 (s; 2H), 4.13-4.10 (m; 1H), 4.05 (d; J=6.4 Hz; 2H), 3.76 (s; 2H), 2.83 (dd; J=16.2 Hz; 1H), 2.75 (dd; J=18.4 Hz; 1H), 2.06 (s; 1H)
B2-A5B14, (R,E)-3-(4-(((2-hydroxy-3-(naphthalen-1-yloxy)propyl)amino)methyl)phenyl)acrylic acid

\[ \text{H NMR (500 MHz, DMSO-}\delta) \delta 8.15 \ (d; J=8.3 Hz; 1H), 7.84 \ (d; J=8.0 Hz; 1H), 7.53-7.33 \ (m; 9H), 6.94 \ (d; J=7.5 Hz; 1H), 6.43 \ (d; J=15.9 Hz; 1H), 5.06 \ (br.s; 1H), 4.14-4.11 \ (m; 1H), 4.06 \ (d; J=7.0 Hz; 2H), 3.76 \ (s; 2H), 2.77 \ (dd; J=16.3 Hz; 1H), 2.68 \ (dd; J=18.0 Hz; 1H), 1.22 \ (s; 1H) \]

B2-A6B1, (R)-2-(4-(((2-hydroxy-3-(o-tolyloxy)propyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetic acid

\[ \text{H NMR (500 MHz, DMSO-}\delta) \delta 7.91 \ (s; 1H), 7.11 \ (t; J=14.9 Hz; 2H), 6.89-6.78 \ (m; 2H), 4.73 \ (s; 2H), 4.48 \ (s; 1H), 4.09-4.04 \ (m; 1H), 3.98 \ (s; 1H), 3.90 \ (d; J=4.7 Hz; 2H), 2.98 \ (dd; J=16.0 Hz; 1H), 2.85-2.80 \ (m; 1H), 2.11 \ (s; 3H); 2.06 \ (s; 1H) \]

B2-A6B5, (R)-3-hydroxy-4-(((2-hydroxy-3-(o-tolyloxy)propyl)amino)methyl)benzoic acid

\[ \text{H NMR (500 MHz, DMSO-}\delta) \delta 7.37 \ (s; 1H), 7.35-7.32 \ (m; 2H), 7.29 \ (d; J=8.3 Hz; 1H), 7.13-7.08 \ (m; 1H), 6.88 \ (d; J=8.1 Hz; 1H), 6.82 \ (t; J=14.6 Hz; 1H), 4.49 \ (s; 1H), 4.03-4.01 \ (m; 1H), 3.95 \ (s; 1H), 3.91 \ (t; J=11.3 Hz; 2H), 3.15 \ (s; 2H), 2.87 \ (dd; J=15.9 Hz; 1H), 2.75 \ (dd; J=19.8; 1H), 2.08 \ (s; 3H) \]

B2-A6B6, (R)-1-(((5-(1H-pyrazol-5-yl)thiophen-2-yl)methyl)amino)-3-(o-tolyloxy)propan-2-ol

\[ \text{H NMR (500 MHz, DMSO-}\delta) \delta 12.76 \ (d; J=12.7 Hz; 1H), 7.71 \ (s; 1H), 7.17-7.03 \ (m; 3H), 6.91-6.74 \ (m; 3H), 6.52 \ (d; J=12.3 Hz; 1H), 4.03-3.98 \ (m; 1H), 3.94-3.79 \ (m; 5H), 2.76 \ (dd; J=15.9 Hz; 1H), 2.65-2.61 \ (m; 1H), 2.10 \ (s; 3H), 2.03 \ (s; 1H) \]

B2-A6B7, (R)-2-fluoro-4-(((2-hydroxy-3-(o-tolyloxy)propyl)amino)methyl)benzoic acid

\[ \text{H NMR (500 MHz, DMSO-}\delta) \delta 7.76 \ (t; J=15.5 Hz; 1H), 7.25 \ (d; J=20.2 Hz; 1H), 7.15-7.08 \ (m; 3H), 6.88 \ (d; J=8.0 Hz; 1H), 6.81 \ (t; J=14.3 Hz; 1H), 5.31 \ (d; J=10.0 Hz; 1H), 3.92-3.88 \ (m; 4H), 3.80 \ (a; 1H), 2.71 \ (d; J=11.3 Hz; 2H), 2.08 \ (s; 3H), 2.06 \ (s; 1H) \]

B2-A6B14, (R,E)-3-(4-(((2-hydroxy-3-(o-tolyloxy)propyl)amino)methyl)phenyl)acrylic acid

\[ \text{H NMR (500 MHz, DMSO-}\delta) \delta 7.62-7.51 \ (m; 3H), 7.36 \ (d; J=7.9 Hz; 1H), 7.24 \ (t; J=17.2 Hz; 1H), 7.10 \ (d; 6.2 Hz; 2H), 6.88 \ (d; J=7.9 Hz; 1H), 6.81 \ (t; J=14.8 Hz; 1H), 6.47 \ (d; J=15.9 Hz; 1H), 3.96-3.86 \ (m; 3H), 3.76 \ (s; 2H), 2.70-2.68 \ (m; 1H), 2.61-2.57 \ (m; 1H), 2.07 \ (s; 3H), 2.06 \ (s; 1H) \]
Computational details

Receptor X-ray structure preparation

Docking calculations were performed using FRED with the basal conformation of the human β2AR in complex with carazolol (PDB: 2RH1) and a model of the human β1AR also in a basal state, which was downloaded from the GPCRdb (gpcrdb.org). For 2RH1, all ligands, solvent, lipid molecules as well as the T4-lysozyme insertion were removed. Hydrogen atoms were placed and minimized using the HBUILD module in CHARMM (B. R. Brooks, R. E. Bruccoleri, D. J. States, S. Swaminathan and M. Karplus, J. Comput. Chem., 1983, 4, 187–217.). CHARMM22 atom types and MPEOE partial charges were assigned using the program Witnotp [Novartis Pharma AG, unpublished].

Selection of the primary-amine-containing OBP fragments

The fragment-like subset of ZINC 23 (www.zinc-docking.org) containing 1’611’889 fragments was used and all fragments featuring a primary amine were extracted using the PINGUI 'Filter your Library’ module, leaving 387’707 fragments. We focused on primary-amine-containing OBP fragments, as most adrenergic receptor ligands feature such a protonable moiety and it is also ideally suited for reductive alkylation in order to grow the potential hits. This reaction was already successfully applied in our previous works.

Selection of the surrogate SBP fragments and enumeration of the virtual library of bitopic (OBP→SBP) compounds

The MolPort building-blocks dataset was downloaded from the MolPort website and contained 305’838 building blocks at that time (January 2017). All aldehyde-containing building blocks were extracted using the PINGUI Filter your Library’ module. This procedure yielded 12’454 SBP fragments compatible with reductive alkylation, and thus our growing strategy. Ketones were initially discarded, as they would introduce a chiral center in the derivative.
products, but a small set of 1394 ketones with one H-bond donor group was added at a later stage of the project in order to increase diversity of the resulting products, thus giving rise to a total of 13’848 SBP fragments. This dataset was further pruned using our chemist-in-the-loop filtering procedure (Figure S17) in order to improve our chances for a high synthesis success rate. In our experience, frequent interactions with the chemist when designing the molecular matrices cannot be given enough importance. The constraints imposed by our chemist were (i) no building blocks containing two functional groups, i.e. two aldehydes or ketones, such that the occurrence of unwanted side products is minimized, (ii) electron-withdrawing groups (EWG) present on the ring close to the aldehyde are preferred, as they should lead to increased reactivity of the aldehyde, (iii) no ortho decoration on the ring close to the aldehyde in order to avoid sterical hindrance that might hamper the reaction and (iv) no building blocks with more than one chiral center. Every compatible building block was then converted to the corresponding surrogate by means of a python script written using the rdkit library (www.rdkit.org) as reported in our previous work. For each of the five core fragments to grow, the derivative products based on reductive alkylation were generated with the aforementioned aldehyde library and the PINGUI ‘Create your Virtual Library’ module. Those procedures yielded exactly as many surrogates and virtual products as the initial count of compatible reactants (7’893).

Figure S17: Workflow depicting the CitL (Chemist-in-the-loop) filter aimed at improving the synthesis rate of our matrices.
Discussion

Figure S18: Binding mode prediction of A1B14 (left) and A1B17 (right) in the β₁AR (residues in magenta) and the β₂AR (residues in light grey) binding sites. Ligands are shown with green sticks and polar contacts are represented with cyan dashed lines.

Figure S19: Binding mode prediction of A1B18 (left) and A1B21 (right) in the β₁AR (residues in magenta) and the β₂AR (residues in light grey) binding sites. Ligands are shown with green sticks and polar contacts are represented with cyan dashed lines.