Target identification and mode of action of four chemically divergent drugs against Ebola virus infection

Jingshan Ren, Yuguang Zhao, Elizabeth E. Fry, David I. Stuart

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Supporting Information Tables 1

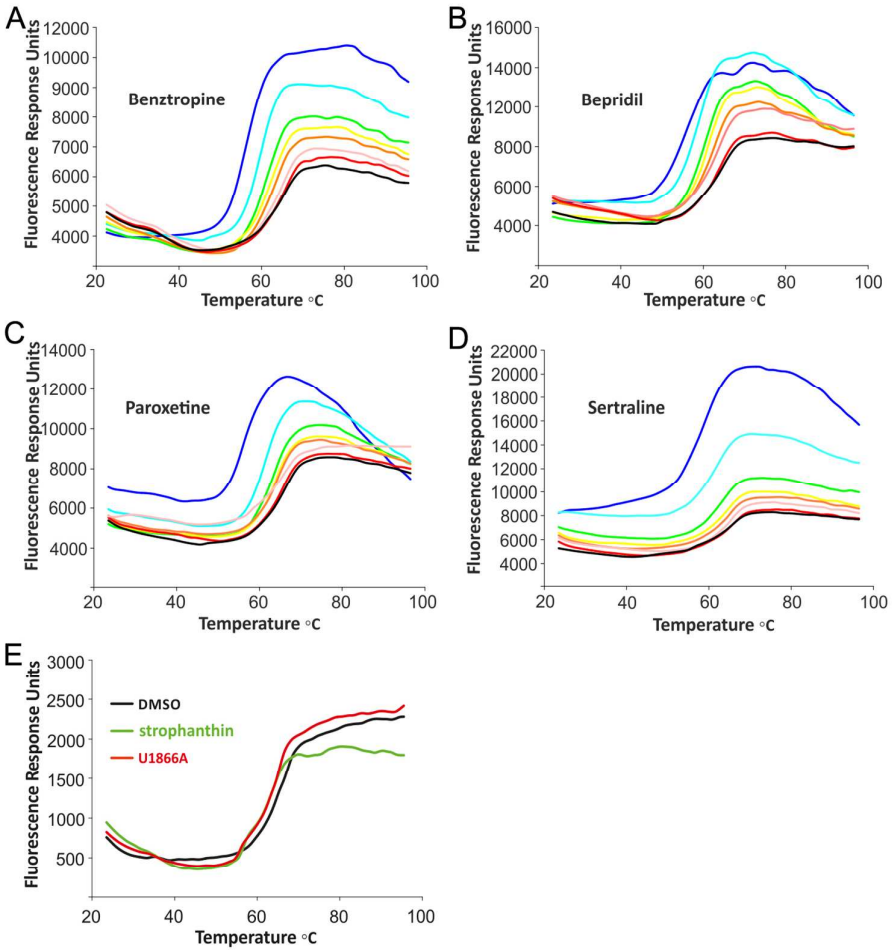

Figure S1, Raw data of thermal-shift assay. Representative thermal melt curves of EBOV GP treated with 2-fold serial dilution of compounds. The lines are rainbow colored from highest concentration in blue to the lowest concentration in red, the black line is protein only. (A) benztropine from a starting concentration of 1 mM, (B) bepridil from a starting concentration of 1.4 mM, (C) paroxetine and (D) sertraline from starting concentrations of 2.5 mM. (E) Fluorescence traces of strophanthin and compound U1866A that were used as negative controls at concentration of 0.5 mM.

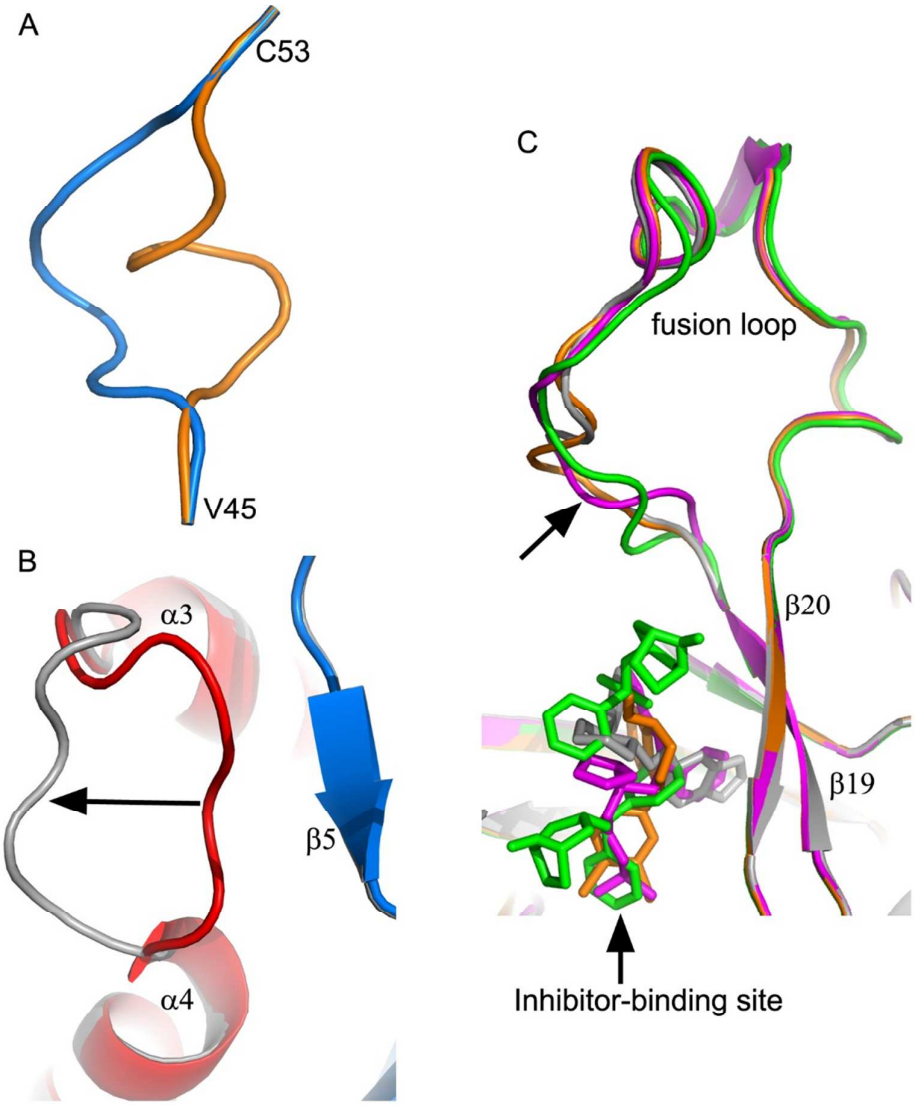

Figure S2, Structural differences. (A) The loop (residues 46-52) preceeding the GP1-GP2 disulphide (C53-C609) in the GP-paroxetine complex has two conformations, one (blue) is similar to that observed in apo GP, GP-toremifene and GP-bepridil, and the other (orange) to that in GP-ibuprofen, GP-benztropine and GP-sertraline. (B) The conformation of α3-α4 loop in GP-benztropine (grey) compared to that in other GP structures (blue and red). (C) The N-terminal end of the fusion loop (indicated by a black arrow) is flexible, and its conformation can be affected by the binding of inhibitors such as benztropine. The colour scheme is GP-benztropine, green; GP-bepridil, magenta; GP-paroxetine, grey; and GP-sertroline, orange.

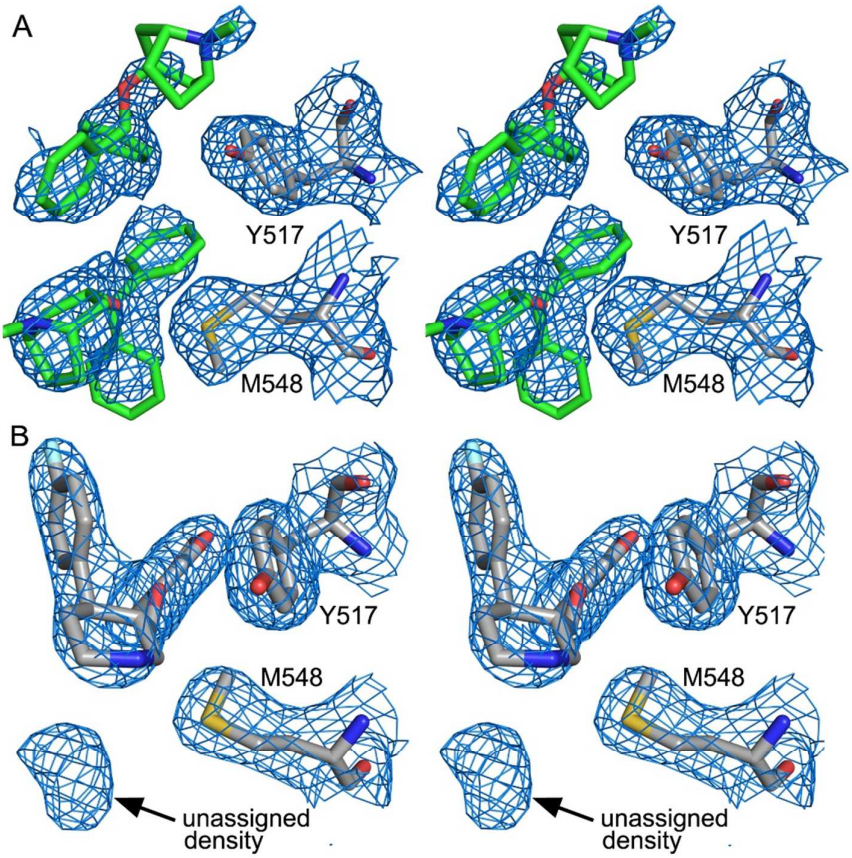

Figure S3, Electron density maps. Stereo diagrams of |*2Fo-Fc*| electron density maps contoured at 1σ showing the density for the bound benztropines (A) and paroxetine (B). Residues Y517 and M548 are also shown in both panels. An arrow indicates the electron density for an unknown molecule in the GP-paroxetine.

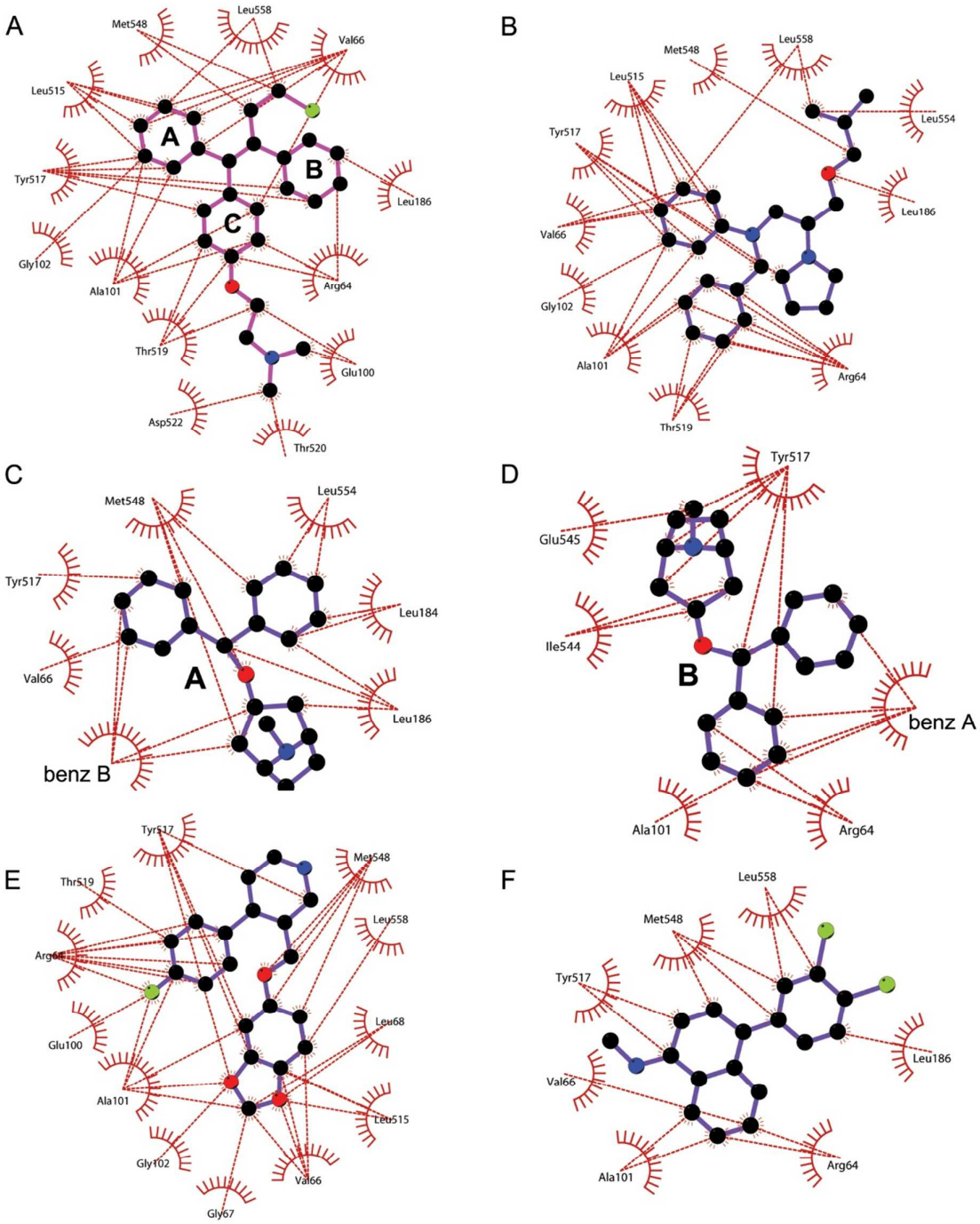

Figure S4, Ligplots of protein inhibitor interactions. (A) Toremifene, (B) bepridil, (C) benztropine A, (D) benztropine B, (E) paroxetine and (F) sertraline. The interactions are defined as distances between protein and inhibitor of less than 3.9 Å.

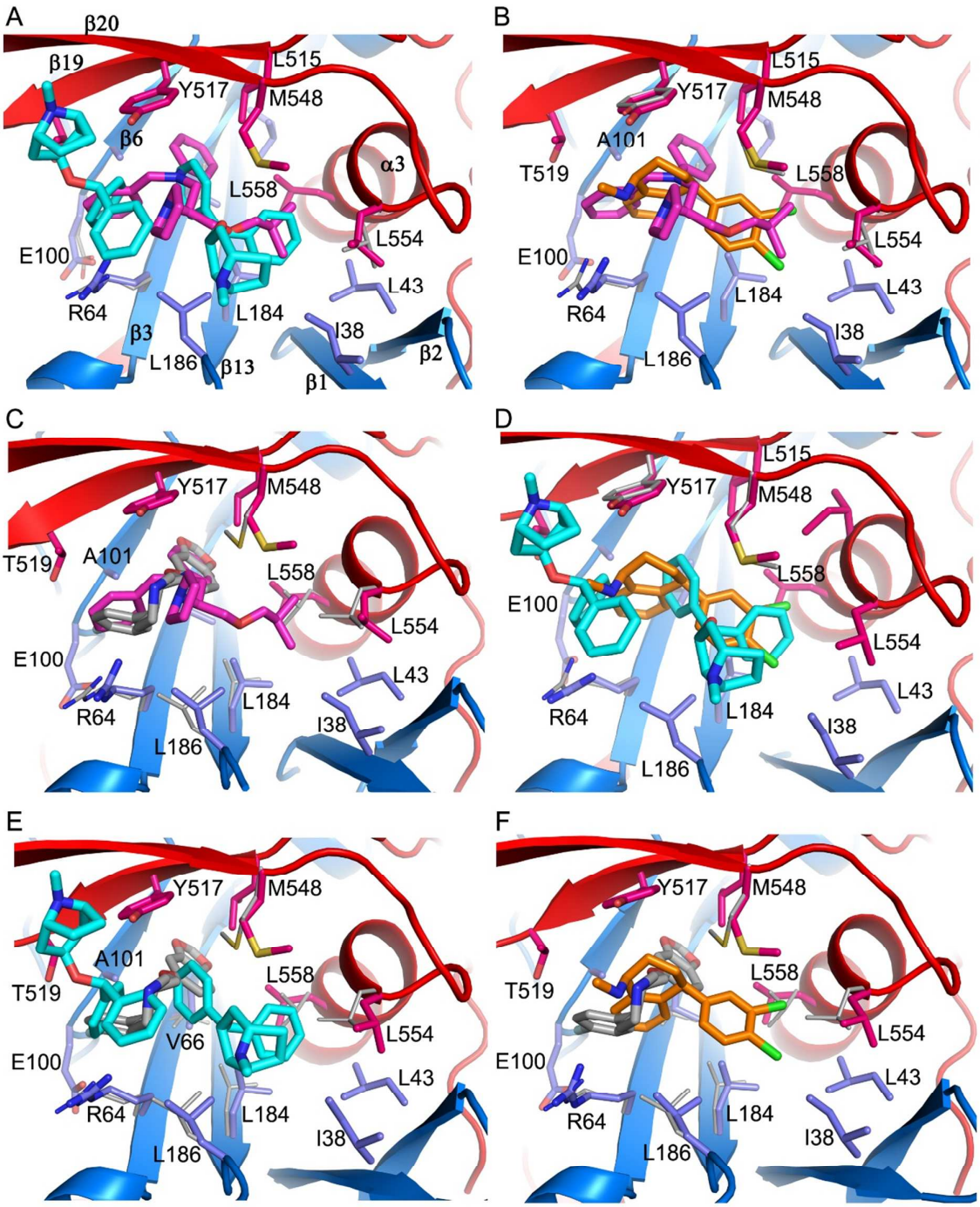

Figure S5, Comparing protein-inhibitor interactions. The structure of GP-bepridil complex is overlapped with (A) GP-benztropine, (B) GP-sertraline and (C) GP-paroxetine complexes. The protein main-chains and side-chains are shown as ribbons and sticks, respectively, with GP1 coloured in blue and GP2 in red. Bepridil, benztropine, sertraline and paroxetine are

drawn as magenta, green, orange and grey sticks respectively. Side-chains of GP-benztropine,GP-sertraline and GP-paroxetine having large conformational differences to those of GP-bepridil are shown as grey sticks. (D, E) GP-benztropine is overlapped with GP-sertraline and GP-paroxetine complexes, respectively. The protein backbone and side-chains of GP-benztropine are shown as coloured ribbons and sticks, side-chains of GP-sertraline and GP-paroxetine as grey sticks. (F) Overlapped GP-sertraline and GP-paroxetine complexes. The protein backbone and side-chains of GP-sertraline are shown as coloured ribbons and sticks, side-chains of GP-paroxetine as grey sticks.

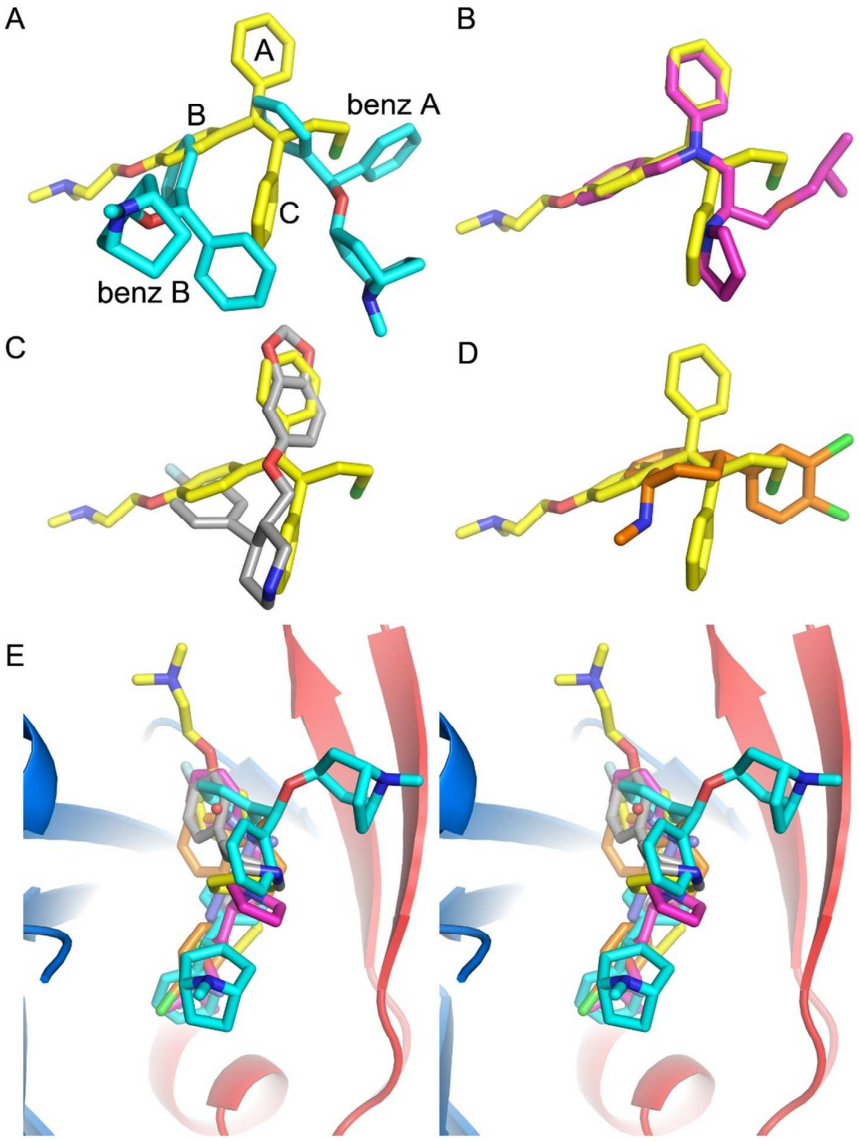

Figure S6, Comparison of binding modes. (A-D) Positions and orientations of benztropine (green sticks), bepridil (magenta sticks), paroxetine (grey sticks) and sertraline (orange sticks) relative to toremifene (yellow sticks), after superimposition of the protein structures upon GP-toremifene. The three phenyl rings of toremifene are labeled in (A). (E) Stereo diagram showing the spatial volume of the binding cavity occupied by the drugs including ibuprofen (blue sticks), the protein backbone of GP-toremifene is shown as ribbons (GP1, blue; GP2 red).

Table S1, Data collection and refinement statistics

	GP-benztropine	GP-bepridil	GP-paroxetine	GP-sertraline
Data collection				
Space group		*R*32		
Cell dimensions				
a, *b*, *c* (Å)	114.4, 114.4, 305.7	114.1, 114.1, 307.2	113.7, 113.7, 306.3	114.3, 114.3, 306.2
α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120
Resolution (Å)	60.5–2.29 (2.19–2.15)*	83.1–2.07 (2.11–2.07)	60.4–2.40 (2.44–2.40)	60.5–2.15 (2.33–2.29)
R_{merge}	0.061 (---)	0.103 (---)	0.094 (---)	0.110 (---)
$I / \sigma I$	21.9 (1.2)	21.2 (1.1)	28.9 (1.6)	34.2 (1.4)
Completeness (%)	100(100)	99.9 (100)	99.8 (96.9)	100 (100)
Redundancy	18.4 (10.8)	33.6 (20.4)	37.2 (13.6)	66.0 (14.2)
$CC_{1/2}$	1.0 (0.80)	1.0 (0.53)	1.0 (0.70)	1.0 (0.71)
Refinement				
Resolution (Å)	60.5–2.29	52.2–2.07	52.0-2.40	60.5–2.15
No. reflections	32976/1758	44883/2350	28653/1519	40257/1997
R_{work} / R_{free}	0.207/0.233	0.187/0.200	0.192/0.212	0.184/0.204
No. atoms				
Protein	2979	2974	3059	3007
Ligand/glycan/ion	107	94	58	76
Water	61	146	105	115
B-factors				
Protein	87	76	84	72
Ligand/glycan/ion	121	83	94	94
Water	61	63	69	61
r.m.s. deviations				
Bond lengths (Å)	0.002	0.003	0.003	0.004
Bond angles (°)	0.6	0.7	0.7	0.7

*Values in parentheses are for highest-resolution shell.