Using esterase selectivity to determine the *in vivo* duration of systemic availability and abolish systemic side-effects of topical β-blockers.

Jillian G Baker[1,2,*], Christophe Fromont[3], Marjorie Bruder[2], Kevin SJ Thompson[1], Barrie Kellam[2,3], Stephen J Hill[1,2], Sheila M Gardiner[1], Peter M Fischer[2,3]

[1]Cell Signalling Research Group, Division of Physiology, Pharmacology and Neuroscience, School of Life Sciences, University of Nottingham, Nottingham, UK.

[2]Centre of Membrane Proteins and Receptors, University of Birmingham and University of Nottingham, The Midlands, UK.

[3] School of Pharmacy and Centre for Biomedical Sciences, University of Nottingham, UK

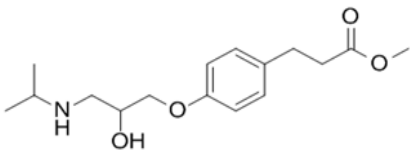
*Corresponding author jillian.baker@nottingham.ac.uk, School of Life Sciences, Queen's Medical Centre, University of Nottingham, NG7 2UH, UK, tel: +44 115 8230085;

Supporting information

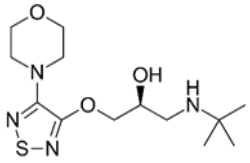
Table of Contents

Figure S1 - Structure of commercially available ligands

Esmolol



Timolol



Betaxolol

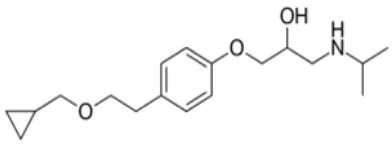


Figure S2 – liver esterase activity is concentration dependent (Sigma E3019)

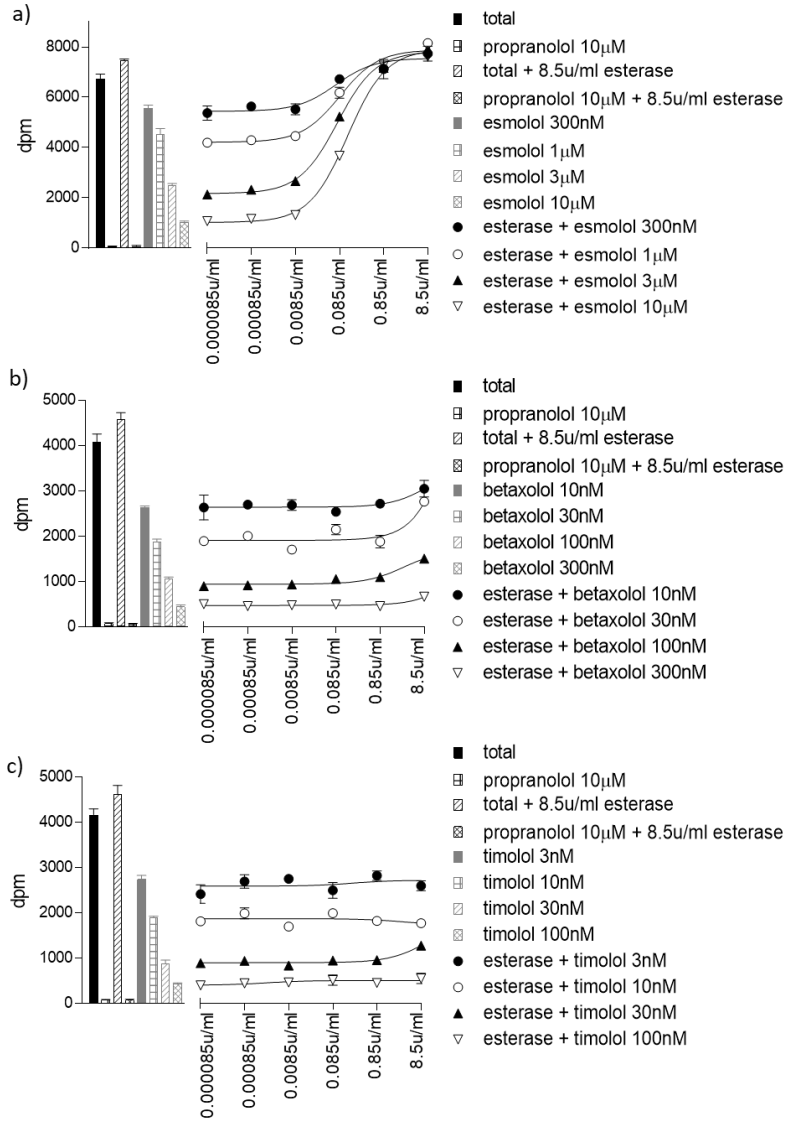


Figure S2

Inhibition of ^{3}H-CGP12177 whole cell binding in CHO-β1 cells by increasing concentrations of liver esterase (Sigma E3019) in the presence of fixed concentrations of a) esmolol, b) betaxolol and c) timolol. Bars represent total binding and non-specific binding (determined by the presence of 10μM propranolol) in the absence and presence of the maximum concentration of esterase obtainable (8.5u/ml). The fixed concentrations of esmolol, betaxolol and timolol were chosen to represent different points on their competition-binding curves, thus 300nM esmolol caused only a little inhibition of specific binding whereas 10μM inhibited most of the specific binding. Concentrations of esterase of 0.085u/ml and above hydrolysed esmolol such that it was no longer able to inhibit specific binding whereas betaxolol and timolol were not affected. Maximum concentrations of esterase (8.5u/ml) caused a small increase in total binding over the 6hr incubation which may be partly responsible for the increase in binding seen in the betaxolol and timolol at this concentration of esterase. The concentrations of ^{3}H-CGP12177 were a) 1.09nM, b) 0.84nM and c) 0.84nM. Data points are mean ± s.e.mean of triplicate determinations and these single experiments are representative of 7 separate experiments in each case.

Figure S3 – liver esterase activity is concentration dependent (Sigma E2884)

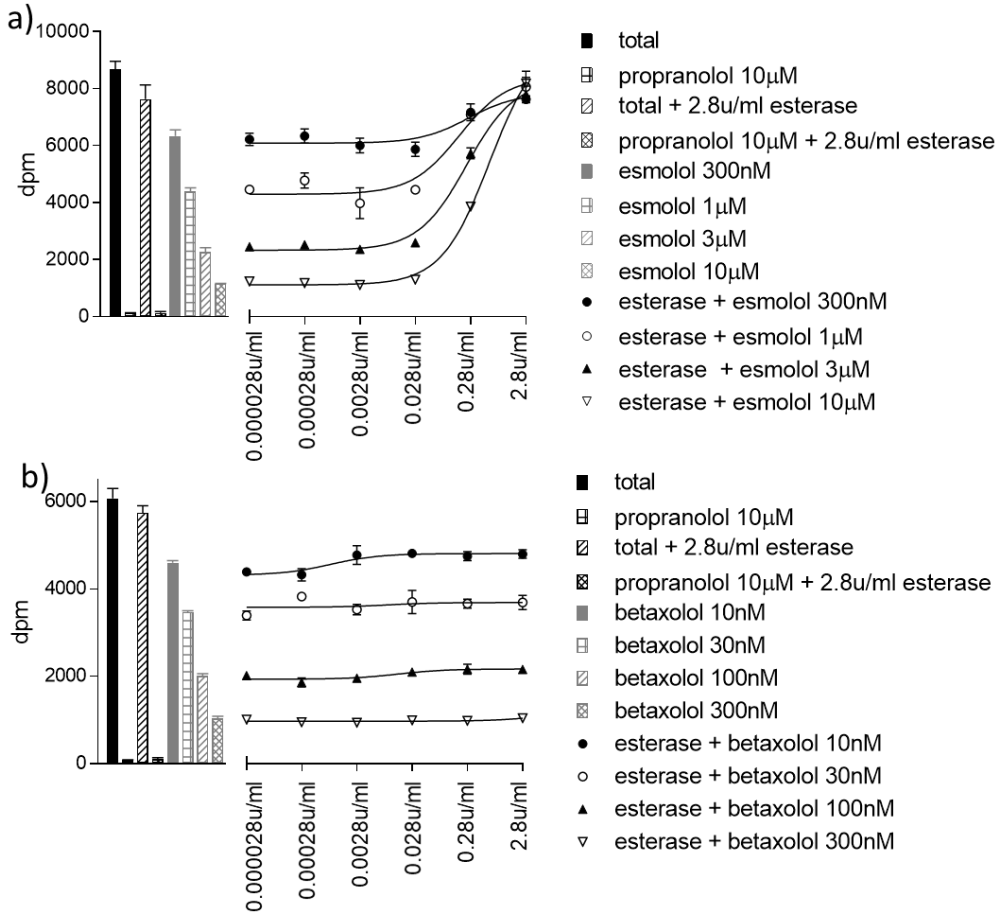


Figure S3
Inhibition of ^{3}H-CGP12177 whole cell binding in CHO-β1 cells in response to increasing concentrations of porcine liver carboxyl esterase (Sigma E2884) in the presence of fixed concentrations of a) esmolol and b) betaxolol. Bars represent total binding and non-specific binding (determined by the presence of 10μM propranolol) in the absence and presence of the maximum concentration of esterase obtainable (2.8u/ml). The fixed concentrations of esmolol and betaxolol were chosen to represent different points on their concentration response, thus 300nM esmolol caused only a little inhibition of specific binding whereas 10μM inhibited most of the specific binding. Concentrations of esterase of 0.28u/ml and above hydrolysed esmolol such that it was no longer able to inhibit specific binding whereas betaxolol and timolol were not affected. The concentrations of ^{3}H-CGP12177 was 1.09nM.

Figure S4 – liver esterase activity is time dependent (Sigma E2884)

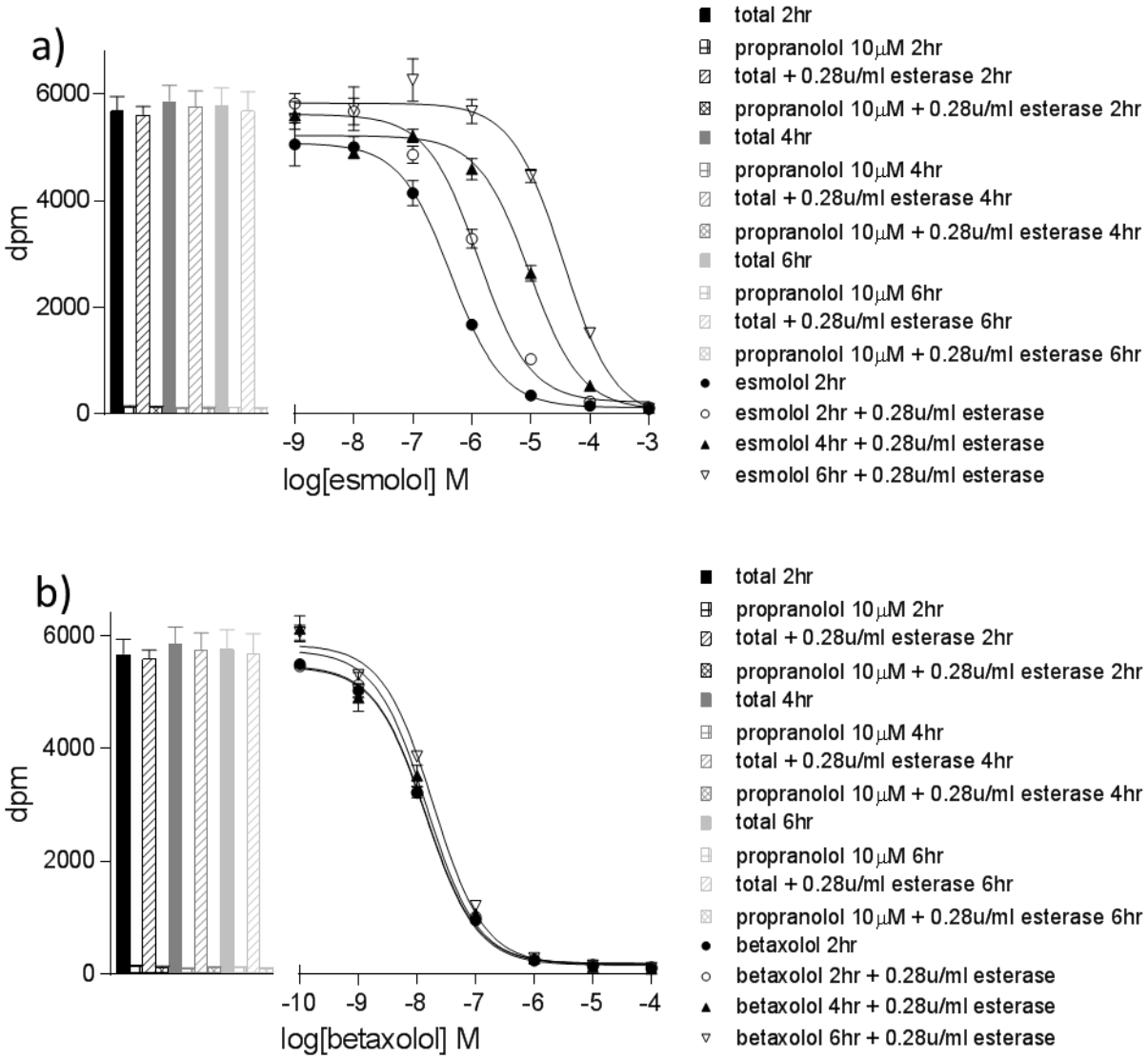


Figure S4
Inhibition of ^{3}H-CGP12177 whole cell binding in CHO-β1 cells in response to a) esmolol and b) betaxolol. Cells were incubated for 2hr with ^{3}H-CGP 12177 or for 2hr, 4hr or 6hr with ligand, 0.28u/ml porcine liver carboxyl esterase (Sigma E2884). Bars represent total and non-specific binding (determined by 10μM propranolol) at each time point in the absence and presence of 0.28u/ml esterase. Longer incubation caused more hydrolysis of esmolol and thus increasing rightward shifts of the esmolol concentration response curve whereas the response to betaxolol were unaffected. The concentrations of ^{3}H-CGP12177 was 1.00nM in each experiment.

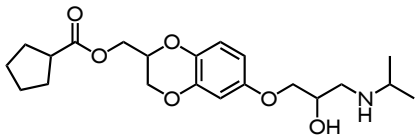
Table S1 – affinity of ester ligand hydrolysis products

parent	Hydrolysis product	CHO-β1 Log K_D	n		CHO-β2 Log K_D	n
Ligands sensitive to liver esterase only						
Esmolol	**31** = ASL8123	-4.06 ± 0.04	5		No binding	5
2	**32**	-6.88 ± 0.06	5		-6.49 ± 0.03	5
3	**33**	-6.56 ±0.08	7		-6.16 ± 0.04	5
Ligands sensitive to liver and serum esterase						
10	**34**	-6.10 ± 0.02	4		>-4	4
18	**35**	-5.27 ± 0.07	6		-5.74 ± 0.07	6
Ligands with rbc esterase activity						
24, 26, 28	**36**	-6.47 ± 0.03	5		-5.01 ± 0.05	5

Affinity (log K_D values) esterase hydrolysis products. Values are mean ± s.e.mean for n separate experiments. Thus, the affinity (log K_D) for esmolol at the β1-AR was -6.56 (Table 2), whereas the affinity of its hydrolysis product was very poor (-4.06).

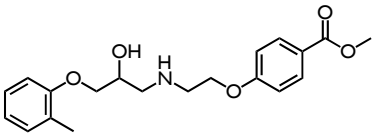
Analytical Data for all novel compounds and General Experimental Chemistry Details

Chemicals and solvents utilised in compound synthesis were purchased from standard suppliers and used without further purification. Melting points (mp) were recorded using a MP50 Melting point System (Mettler Toledo) and are uncorrected. High-resolution mass spectra (HRMS) time-of-flight electrospray (TOF ES ±) were recorded on a Waters 2795 separation module/micromass LCT platform or a Bruker microTOFII instrument with flow injection sample introduction and using electrospray ionisation (ESI). ^{1}H-NMR spectra were recorded on a Bruker-AV 400 at 400.13 MHz. ^{13}C-NMR spectra were recorded at 101.62 MHz. Chemical shifts (δ) are recorded in parts per million (ppm) with reference to the chemical shift of the deuterated solvent or an internal tetramethylsilane (TMS) standard. Coupling constants (J) and carbon−fluorine coupling constants (JCF) are recorded in Hz and the significant multiplicities described by singlet (s), doublet (d), triplet (t), quadruplet (q), broad (br), multiplet (m), doublet of doublets (dd), and doublet of triplets (dt). Liquid chromatography–mass spectrometry (LC-MS) analysis was performed using a Shimadzu UFLCXR chromatography system coupled to an Applied Biosystems API2000 mass spectrometer. Column elution was at 40 ^{o}C with a flow rate of 0.5 mL/min using the following programme: linear gradient from 5% to 98% solvent B in solvent A over 2 min, followed by isocratic elution at 98% B for 2 min, where solvent A was 0.1% HCOOH in H_2O and solvent B was 0.1% HCOOH in MeCN. Eluents were monitored using UV detection (λ 220 and 254 nm) and MS. The following HPLC columns were used: Phenomenex Gemini-NX, 3 μm particle size, 110 Å pore size, C18 stationary phase, 50 × 2mm column length and diameter (system 1) and Phenomenex Luna, 3 μm particle size, 110 Å pore size, pentafluorophenyl propyl ligand (PFP2) stationary phase, 50 × 2mm column length and diameter (system 2).



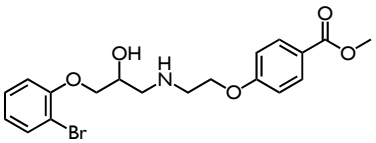
***6-(2-Hydroxy-3-(isopropylamino)propoxy)-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methyl cyclopentanecarboxylate* (1)**

^{1}H NMR (DMSO-d_6) : δ 0.97 (dd, *J* = 2.0/ 4.2 Hz , 6H), δ 1.47-1.89 (m, 9H), δ 2.62 (d, *J* = 4.2 Hz, 1H), δ 2.64-2.73 (m, 2H), δ 2.74-2.83 (m, 1H), δ 3.73-3.81 (m, 2H), δ 3.82-3.88 (m, 1H), δ 4.00 (dd, *J* = 7.0/ 4.6 Hz, 1H), δ 4.26 (d, *J* = 5.1 Hz, 2H), δ 4.28-4.38 (m, 2H), δ 4.92 (s, 1H), δ 6.43 (dd, *J* = 2.9/ 5.9 Hz, 1H), δ 6.47 (d, *J* = 2.7 Hz, 1H), δ 6.77 (d, *J* = 8.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) : δ 175.50, 153.23, 142.93, 136.42, 117.23, 108.00, 103.07, 71.28, 70.52, 68.18, 64.67, 62.10, 49.84, 48.26, 42.86, 29.44, 25.30, 22.70. mp 70 – 71 °C; LCMS R_t: 2.26 min, $[MH]^+$ 394.3, Purity 95%; HRMS (TOF ES^+) $C_{21}H_{32}NO_6$ $[MH]^+$ calculated 394.2225; found 394.2235.



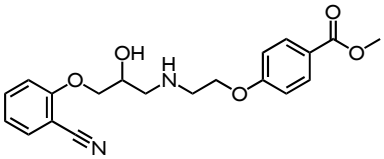
***Methyl 4-(2-(2-hydroxy-3-(o-tolyloxy)propylamino)ethoxy)benzoate* (2)**

^{1}H NMR ($CDCl_3$) δ: 7.96-8.00 (m, 2H), 7.12-7.16 (m, 2H), 6.89-6.92 (m, 2H), 6.87 (ddd, *J* = 7.5/7.5/0.9 Hz), 6.80 (d, broad, *J* = 8.3 Hz), 4.09-4.14 (m, 3H), 3.96-4.03 (m, 2H), 3.88 (s, 3H), 3.08 (t, *J* = 5.1 Hz, 2H), 2.87-3.00 (m, 2H), 2.71 (s, broad, 2H), 2.22 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 166.90, 162.58, 156.74, 131.71, 130.84, 126.96, 126.80, 122.94, 120.90, 114.17, 111.23, 70.53, 68.59, 67.65, 52.04, 51.97, 48.74, 16.36; mp: 92-95 °C; LCMS R_t: 2.21 min $[MH]^+$ 361.3, HRMS *m/z* $C_{20}H_{26}NO_5^+$ $[MH]^+$ calculated 360.1805, found 360.1817



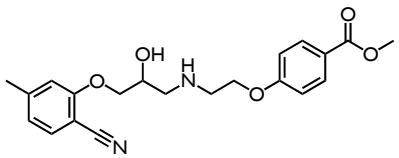
***Methyl 4-(2-(3-(2-bromophenoxy)-2-hydroxypropylamino)ethoxy)-benzoate* (3)**

^{1}H NMR ($CDCl_3$) δ: 7.95-7.99 (m, 2H), 7.52 (dd, *J* = 7.9/1.5 Hz, 1H), 7.24 (ddd, *J* = 8.3/7.5/1.5 Hz, 1H), 6.83-6.93 (m, 4H), 4.11-4.16 (m, 3H), 4.02-4.09 (m, 2H), 3.88 (s, 3H), 3.07-3.13 (m, 2H), 2.94-3.03 (m, 2H), 2.87 (s, broad, 2H); ^{13}C NMR ($CDCl_3$) δ: 166.94, 162.60, 155.05, 133.44, 131.74, 128.69, 122.96, 122.53, 114.22, 113.73, 112.47, 71.89, 68.25, 67.59, 52.00, 51.75, 48.78; mp: 84-87 °C; LCMS R_t: 2.23 min $[MH]^+$ 424.1/426.2; HRMS *m/z* $C_{19}H_{23}{}^{79}BrNO_5^+$ $[MH]^+$ calculated 424.0754, found 424.0766.



***Methyl 4-(2-(3-(2-cyanophenoxy)-2-hydroxypropylamino)ethoxy)benzoate* (4)**

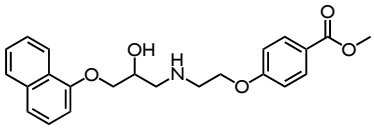
^{1}H NMR ($CDCl_3$) δ: 7.92-7.97 (m, 2H), 7.46-7.53 (m, 2H), 6.99 (ddd, *J* = 7.6/7.6/0.8 Hz, 1H), 6.94 (d, broad, *J* = 8.5 Hz, 1H), 6.87-6.91 (m, 2H), 4.02-4.16 (m, 5H), 3.86 (s, 3H), 3.08 (t, *J* = 5.2 Hz), 2.89-3.05 (m, 4H); ^{13}C NMR ($CDCl_3$) δ: 166.89, 162.57, 160.45, 134.51, 133.73, 131.68, 122.85, 121.28, 116.53, 114.18, 112.57, 102.15, 71.53, 67.99, 67.53, 51.94, 51.62, 48.64; mp: 104-107 °C; LCMS R_t: 2.13 min $[MH]^+$ 371.3; HRMS *m/z* $C_{20}H_{23}N_2O_5^+$ $[MH]^+$ calculated 371.1601, found 371.1613.



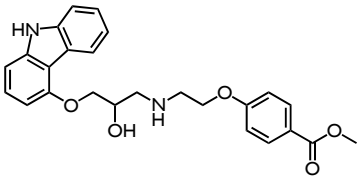
***Methyl 4-(2-(3-(2-cyano-5-methylphenoxy)-2-hydroxypropylamino)-ethoxy)benzoate* (5)**

^{1}H NMR ($CDCl_3$) δ: 7.95-7.98 (m, 2H), 7.41 (d, *J* = 7.8 Hz, 1H), 6.90-6.93 (m, 2H), 6.82 (d, broad, *J* = 7.9 Hz, 1H), 6.77 (s, broad, 1H), 4.06-4.16 (m, 5H), 3.87 (s, 3H), 3.11 (t, *J* = 3.1 Hz,

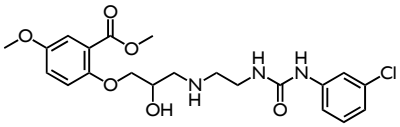
2H), 2.93-3.06 (m, 2H), 2.86 (s, broad, 2H), 2.38 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 166.92, 162.59, 160.46, 145.94, 133.45, 131.73, 122.96, 122.30, 116.84, 114.24, 113.40, 99.31, 71.40, 67.95, 67.52, 51.98, 51.56, 48.71, 22.38; mp: 126-128 °C; LCMS R_t: 2.18 min $[MH]^+$ 385.2; HRMS *m/z* $C_{21}H_{25}N_2O_5^+$ $[MH]^+$ calculated 385.1758, found 385.1771.



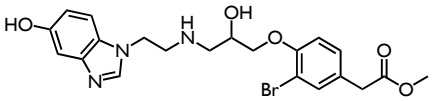
Methyl 4-(2-(2-hydroxy-3-(naphthalen-1-yloxy)propylamino)ethoxy)-benzoate **(6)**
^{1}H NMR ($CDCl_3$) δ: 8.23 (d, broad, J = 8.2 Hz, 1H), 7.95-7.98 (m, 2H), 7.80 (d, broad, *J* = 8.0 Hz, 1H), 7.40-7.51 (m, 3H), 8.23 (dd, *J* = 7.9/7.9 Hz, 1H), 6.87-6.90 (m, 2H), 6.80 (d, broad, *J* = 7.6 Hz, 1H), 4.24-4.29 (m, 1H), 4.11-4.20 (m, 4H), 3.88 (s, 3H), 3.20 (s, broad, 2H), 3.09 (t, *J* = 5.0 Hz, 2H), 2.94-3.07 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 166.93, 162.52, 154.31, 134.60, 131.71, 127.66, 126.56, 125.91, 125.60, 125.41, 122.95, 121.83, 120.83, 114.18, 105.05, 70.64, 68.55, 67.45, 52.07, 51.98, 48.66; mp: 83-86 °C; LCMS R_t: 2.29 min $[MH]^+$ 396.2; HRMS *m/z* $C_{23}H_{26}NO_5^+$ $[MH]^+$ calculated 396.1805, found 396.1814.



Methyl 4-(2-(3-(9H-carbazol-4-yloxy)-2-hydroxypropylamino)ethoxy)-benzoate **(7)**
^{1}H NMR ($CDCl_3$) δ: 9.71 (s, broad, carbazole N-H), 8.13 (d, broad, *J* = 7.8 Hz, 1H), 7.79-7.82 (m, 2H), 7.29 (d, broad, *J* = 8.0 Hz, 1H), 7.20 (ddd, *J* = 8.1/7.1/1.1 Hz, 1H), 7.15 (dd, *J* = 8.0/8.0 Hz, 1H), 6.99-7.03 (m, 1H), 6.94 (d, broad, *J* = 8.0 Hz, 1H), 6.73-6.77 (m, 2H), 6.51 (d, broad, *J* = 7.9 Hz, 1H), 4.08-4.23 (m, 3H), 4.02 (t, *J* = 5.2 Hz, 2H), 3.74 (s, 3H), 2.98-3.03 (m, 3H), 2.86-2.91 (m, 1H), 2.71 (s, broad, 2H); ^{13}C NMR ($CDCl_3$) δ: 166.54, 162.33, 154.88, 141.18, 138.94, 131.31, 126.17, 124.49, 122.62, 122.44, 122.11, 118.85, 113.93, 112.24, 110.06, 103.92, 100.48, 70.19, 68.31, 67.28, 52.30, 51.62, 48.39; mp: 105-109 °C; LCMS R_t: 2.26 min $[MH]^+$ 435.3; HRMS *m/z* $C_{25}H_{27}N_2O_5^+$ $[MH]^+$ calculated 435.1914, found 435.1918.

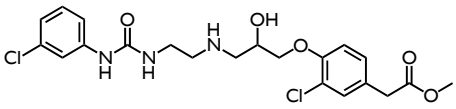


Methyl 2-(3-((2-(3-(3-chlorophenyl)ureido)ethyl)amino)-2-hydroxypropoxy)-5-methoxybenzoate **(8)**
^{1}H NMR (CD_3OD) δ: 7.51 (t, *J* = 2.0 Hz, 1H), 7.26 (dd, *J* = 2.0/1.6 Hz, 1H), 7.20-7.12 (m, 3H), 7.06-7.05 (m, 2H), 6.93 (ddd, *J* = 7.3/2.0/1.6 Hz, 1H), 4.11-4.04 (m, 3H), 3.87 (s, 3H), 3.75 (s, 2H), 3.40-3.37 (m, 2H), 2.97-2.84 (m, 4H); ^{13}C NMR (CD_3OD) δ: 168.13, 158.01, 155.01, 154.00, 142.56, 135.38, 130.92, 122.91, 121.83, 120.70, 119.55, 117.81, 117.12, 116.97, 74.25, 69.28, 56.21, 52.91, 52.6650.38, 39.91; LCMS R_t: 2.22 min $[MH]^+$ 452.2/454.2; HRMS *m/z* $C_{21}H_{27}ClN_3O_6^+$ $[MH]^+$ calculated 452.1583, found 452.1600



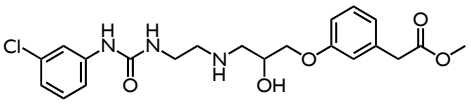
***Methyl 2-(3-bromo-4-(2-hydroxy-3-((2-(5-hydroxy-1H-benzo[d]imidazol-1-yl)ethyl)amino)propoxy)phenyl)acetate* (9)**

^{1}H NMR (D_6 DMSO) δ: 8.95 (s, 1H), 8.02 (s, 1H), 7.47 (d, *J* = 2.1 Hz, 1H), 7.36 (d, *J* = 8.6 Hz, 1H), 7.19 (dd, *J* = 8.5/2.1 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.93 (d, *J* = 2.2 Hz, 1H), 6.72 (d, *J* = 8.6/2.2 Hz, 1H), 4.94 (d, *J* = 5.0 Hz, 1H), 4.21 (t, *J* = 6.2.0 Hz, 2H), 3.93 (d, *J* = 5.3 Hz, 2H), 3.88-3.81 (m, 1H), 3.63 (s, 2H), 3.61 (s, 3H); 2.92 (t, *J* = 6.3 Hz, 2H), 2.74 (dd, *J* = 12.0/4.7 Hz, 1H), 2.64 (d, *J* = 12.0 /6.5 Hz, 1H); LCMS R_t: 1.83 min $[MH]^+$ 478.1/480.2; HRMS *m/z* $C_{21}H_{25}{}^{79}BrN_3O_5{}^+$ $[MH]^+$ calculated 478.0972, found 478.0971.



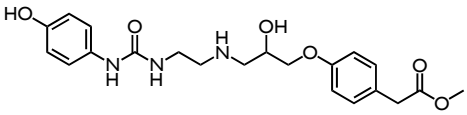
***Methyl 2-(3-chloro-4-(3-((2-(3-(3-chlorophenyl)ureido)ethyl)amino)-2-hydroxypropoxy)phenyl)acetate* (10)**

^{1}H NMR (D_6 DMSO) δ: 7.66 (s, 1H), 7.66 (t, *J* = 2.0 Hz, 1H), 7.32 (d, *J* = 2.0 Hz, 1H), 7.24-7.14 (m, 3H), 7.09 (d, *J* = 8.6 Hz, 1H), 6.93-6.90 (m, 1H), 6.28 (t, *J* = 5.4 Hz, 1H), 4.04-3.90 (m, 3H), 3.62 (s, 2H), 3.61 (s, 3H), 3.18 (q, *J* = 5.9 Hz, 2H), 2.80-2.63 (m, 4H); ^{13}C NMR (D_6 DMSO) δ: 171.51, 155.04, 152.86, 142.12, 133.05, 130.70, 130.16, 129.18, 127.60, 121.02, 120.46116.87, 115.88, 113.80, 71.52, 67.68, 51.91, 51.68, 48.98, 38.60; LCMS R_t: 2.27 min $[MH]^+$ 470.1/472.2; HRMS *m/z* $C_{21}H_{26}{}^{35}Cl_2N_3O_5{}^+$ $[MH]^+$ calculated 470.1244, found 470.1260.



***Methyl 2-(3-(3-((2-(3-(3-chlorophenyl)ureido)ethyl)amino)-2-hydroxypropoxy)phenyl)acetate* (11)**

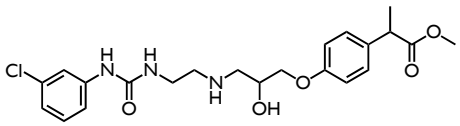
^{1}H NMR (CD_3OD) δ: 7.57 (br s, 1H), 7.22-7.17 (m, 3H), 6.98-6.93 (m, 1H), 6.88-6.84 (m, 3H), 4.17-4.11 (m, 1H), 3.99 (d, *J* = 5.2 Hz, 2H), 3.67 (s, 3H), 3.60 (s, 2H), 3.06-2.90 (m, 4H); ^{13}C NMR (CD_3OD) δ: 173.88, 160.25, 158.28, 142.45, 137.10, 135.42, 131.00, 130.58, 123.10, 123.08, 119.71, 117.97, 116.74, 114.20, 71.49, 68.69, 52.48, 52.45, 50.40, 49.28, 41.65, 39.47; LCMS R_t: 2.25min $[MH]^+$ 436.2; HRMS *m/z* $C_{21}H_{27}{}^{35}ClN_3O_5{}^+$ $[MH]^+$ calculated 436.1634, found 436.1642.



***Methyl 2-(4-(2-hydroxy-3-((2-(3-(4-hydroxyphenyl)ureido)ethyl)amino)propoxy)-phenyl)acetate* (12)**

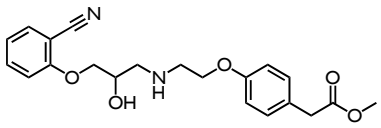
^{1}H NMR (D_6 DMSO) δ: 8.92 (br s, 1H), 8.24 (s, 1H), 7.18-7.12 (m, 4H), 6.89 (d, *J* = 8.5 Hz, 2H), 6.63 (d, *J* = 9.3 Hz, 2H), 6.06 (t, *J* = 5.5 Hz, 1H), 3.96-3.86 (m, 3H), 3.60 (s, 3H), 3.59 (s, 2H),

3.17 (q, *J* = 5.9 Hz, H), 2.79-2.63 (m, 4H); ^{13}C NMR (D_6 DMSO) δ: 171.86, 157.54, 155.71, 151.89, 132.04, 130.32, 126.26, 119.79, 115.03, 114.38, 70.60, 67.63, 51.86, 51.57, 49.22, 39.22, 38.79; LCMS R_t: 1.98min $[MH]^+$ 418.4; HRMS *m/z* $C_{21}H_{27}{}^{35}ClN_3O_5^+$ $[MH]^+$ calculated 418.1973, found 418.1966.



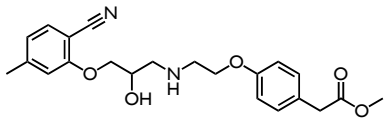
Methyl 2-(4-(3-((2-(3-(3-chlorophenyl)ureido)ethyl)amino)-2-hydroxypropoxy)phenyl)propanoate **(13)**

^{1}H NMR (D_6 DMSO) δ: 8.87 (s, 1H), 7.67 (t, *J* = 1.9 Hz, 1H), 7.24-7.15 (m, 4H), 6.91 (ddd, *J* = 7.7/1.9/1.3 Hz, 1H), 6.88 (d, *J* = 8.6 Hz, 2H), 6.31 (t, *J* = 5.4 Hz, 1H), 3.94-3.85 (m, 3H), 3.71 (q, *J* = 7.2 Hz, 1H), 3.56 (s, 3H), 3.19 (q, *J* = 5.8 Hz, 2H), 2.76-2.61 (m, 4H), 1.35 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (D_6 DMSO) δ: 174.50, 157.62, 155.09, 142.14, 133.07, 132.57, 130.18, 128.31120.47, 116.87, 115.88, 114.54, 70.65, 67.78, 51.94, 51.65, 49.00, 43.49, 18.56; LCMS R_t: 2.28min $[MH]^+$ 450.2/452.0; HRMS *m/z* $C_{22}H_{29}{}^{35}ClN_3O_5^+$ $[MH]^+$ calculated 450.1790, found 450.1801.



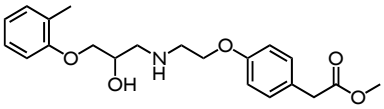
Methyl 2-(4-(2-(3-(2-cyanophenoxy)-2-hydroxypropylamino)ethoxy)phenyl)acetate **(14)**

^{1}H NMR ($CDCl_3$) δ: 7.47-7.54 (m, 2H), 7.14-7.17 (m, 2H), 6.95-7.01 (m, 2H), 6.83-6.86 (m, 2H), 4.08-4.18 (m, 3H), 4.06 (t, *J* = 5.1 Hz, 2H), 3.66 (s, 3H), 3.54 (s, 2H), 3.04-3.06 (m, 3H,), 2.88-3.01 (m, 3H); ^{13}C NMR ($CDCl_3$) δ: 172.42, 160.46, 157.92, 134.49, 133.71, 130.38, 126.37, 121.20, 116.52, 114.68, 112.58, 102.12, 71.45, 67.93, 67.26, 52.07, 51.57, 48.78, 40.32; LCMS R_t: 2.14 min [M+1] 385.3; HRMS *m/z* $C_{21}H_{25}N_2O_5^+$ $[MH]^+$ calculated 385.1758, found 385.1770

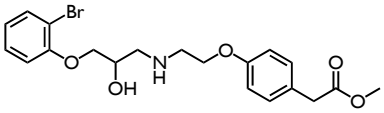


Methyl 2-(4-(2-(3-(2-cyano-5-methylphenoxy)-2-hydroxypropylamino)-ethoxy)phenyl)acetate **(15)**

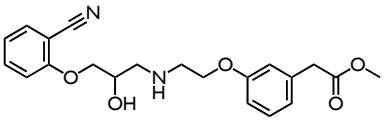
^{1}H NMR ($CDCl_3$) δ: 7.41 (d, 1H), 7.15-7.19 (m, 2H), 6.84-6.88 (m, 2H), 6.79-6.83 (m, 2H), 4.08-4.13 (m, 3H), 4.06 (t, *J* = 5.1 Hz, 2H), 3.67 (s, 3H), 3.55 (s, 2H), 3.04-3.07 (m, 2H), 2.89-3.02 (m, 2H), 2.63 (s, broad, 2H), 2.38 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 172.44, 160.50, 158.02, 145.88, 133.44, 130.42, 126.43, 122.21, 116.83, 114.76, 113.41, 99.29, 71.42, 68.05, 67.47, 52.10, 51.54, 48.90, 40.40, 22.37; mp: 69-72 °C; LCMS R_t: 2.22 min $[MH]^+$ 399.3; HRMS *m/z* $C_{22}H_{27}N_2O_5^+$ $[MH]^+$ calculated 399.1914, found 399.1924.



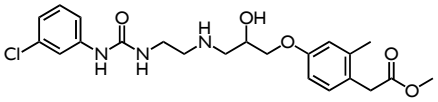
Methyl 2-(4-(2-(2-hydroxy-3-(o-tolyloxy)propylamino)ethoxy)phenyl)-acetate **(16)**
^{1}H NMR ($CDCl_3$) δ: 7.12-7.20 (m, 4H), 6.80-6.89 (m, 4H), 4.11-4.17 (m, 1H), 4.09 (t, *J* = 5.08 Hz, 2H), 3.95-4.03 (m, 2H), 3.68 (s, 3H), 3.56 (s, 2H), 3.04-3.12 (m, 4H), 2.89-3.03 (m, 2H), 2.22 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 172.43, 157.91, 156.77, 130.84, 130.46, 126.97, 126.86, 126.57, 120.90, 114.76, 111.27, 70.48, 68.33, 67.15, 52.12, 51.95, 48.84, 40.39, 16.40; LCMS R_t: 2.23 min $[MH]^+$ 374.3; HRMS *m/z* $C_{21}H_{28}NO_5^+$ $[MH]^+$ calculated 374.1962, found 374.1967.



Methyl 2-(4-(2-(3-(2-bromophenoxy)-2-hydroxypropylamino)ethoxy)-phenyl)acetate **(17)**
^{1}H NMR ($CDCl_3$) δ: 7.52 (dd, *J* = 7.9/1.6 Hz, 1H), 7.24 (ddd, *J* = 8.2/7.5/1.6 Hz, 1H), 7.15-7.19 (m, 2H), 6.79-6.93 (m, 4H), 4.10-4.15 (m, 1H), 4.04-4.08 (m, 4H), 3.68 (s, 3H), 3.55 (s, 2H), 3.06 (t, *J* = 5.1 Hz, 2H), 2.91-3.01 (m, 4H); ^{13}C NMR ($CDCl_3$) δ: 172.43, 157.96, 155.05, 133.37, 130.41, 128.63, 126.41, 12.38, 114.71, 113.65, 112.41, 71.83, 68.16, 67.34, 52.10, 51.74, 48.91, 40.36; LCMS R_t: 2.23 min [M+1] 438.1/440.1; HRMS *m/z* $C_{20}H_{25}{}^{79}BrNO_5^+$ $[MH]^+$ calculated 438.0911, found 438.0914.

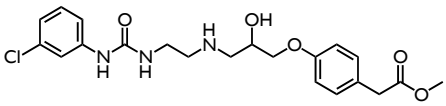


Methyl 2-(3-(2-((3-(2-cyanophenoxy)-2-hydroxypropyl)amino)ethoxy)-phenyl)acetate **(18)**
^{1}H NMR ($CDCl_3$) δ: 7.45-7.58 (m, 2H), 7.20-7.24 (m, 1H), 6.97-7.05 (m, 2H), 6.80-6.87 (m, 3H), 4.16-4.21 (m, 1H), 4.10-4.13 (m, 4H), 3.68 (s, 3H), 3.59 (s, 2H), 3.10-3.13 (m, 2H), 2.96-3.10 (m, 2H), 2.82 (s, broad, 2H); ^{13}C NMR ($CDCl_3$) δ: 172.05, 160.50, 158.88, 135.60, 134.55, 133.80, 129.75, 122.12, 121.37, 116.51, 115.79, 113.35, 112.73, 102.34,71.37, 67.71, 66.87, 52.21, 51.51, 48.79, 41.28; LCMS R_t: 2.15 min $[MH]^+$ 385.2; HRMS *m/z* $C_{21}H_{25}N_2O_5^+$ $[MH]^+$ calculated 385.1758, found 385.1765.



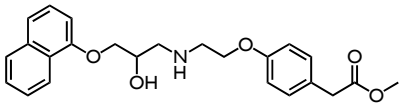
Methyl 2-(4-(3-((2-(3-(3-chlorophenyl)ureido)ethyl)amino)-2-hydroxypropoxy)-2-methylphenyl)acetate **(19)**
^{1}H NMR (D_6 DMSO) δ: 8.78 (s, 1H), 7.66 (t, *J* = 1.9 Hz, 1H), 7.24-7.17 (m, 1H), 7.16 (ddd, *J* = 8.3/1.9/1.1 Hz, 1H), 7.05 (d, *J* = 8.3 Hz, 1H), 6.91 (ddd, *J* = 7.7/2.0/1.1 Hz, 1H), 6.76 (d, *J* = 2.3 Hz, 1H), 6.70 (dd, *J* = 8.3/2.5 Hz, 1H), 6.23 (t, *J* = 5.3 Hz, 1H), 4.94 (br s, 1H), 3.95-3.83 (m, 3H), 3.59 (s, 3H), 3.58 (s, 2H), 3.16 (q, *J* = 5.8 Hz, 2H), 2.71-2.56 (m, 4H), 2.17 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 171.73, 157.64, 154.99, 142.14, 137.90, 133.06, 131.16, 130.17, 125.14, 120.44, 116.84, 116.14, 115.86, 111.62, 70.61, 68.12, 52.23, 51.56, 49.13, 39.21, 37.40, 19.26. LCMS R_t:

2.27 min $[MH]^+$ 450.2/452.0; HRMS *m/z* $C_{22}H_{29}{}^{35}ClN_3O_5^+$ $[MH]^+$ calculated 450.1790, found: 450.1792.



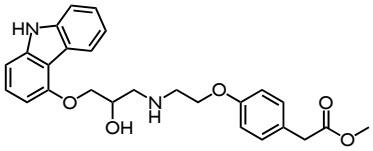
***Methyl 2-(4-(3-((2-(3-(3-chlorophenyl)ureido)ethyl)amino)-2-hydroxypropoxy)-phenyl)acetate* (20)**

^1H NMR (D_6 DMSO) δ: 8.79 (s, 1H), 7.67 (t, *J* = 2.0 Hz, 1H), 7.21 (d, *J* = 7.8 Hz, 1H), 7.18 (dd, *J* = 1.9/1.3 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 1H), 6.91 (ddd, *J* = 7.6/2.1/1.3 Hz, 1H), 6.88 (d, *J* = 8.6 Hz, 1H), 6.24 (t, *J* = 5.4 Hz, 1H), 3.95-3.84 (m, 3H), 3.59 (s, 3H), 3.58 (s, 2H), 3.17 (app q, *J* = 5.8 Hz, 2H), 2.72-2.57 (m, 4H); ^{13}C NMR (D_6 DMSO) δ: 171.85, 157.60, 155.02, 142.15, 133.07, 130.30, 130.17, 126.20, 120.45, 116.87, 115.87, 114.37, 70.73, 68.06, 52.16, 51.56, 49.11, 39.22, 39.15. LCMS R_t: 2.24 min $[MH]^+$ 436.2/438.2; HRMS *m/z* $C_{21}H_{29}{}^{35}ClN_3O_5^+$ $[MH]^+$ calculated 436.1634, found: 436.1640.



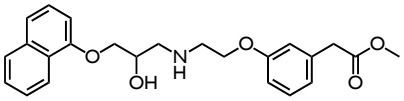
***Methyl 2-(4-(2-(2-hydroxy-3-(naphthalen-1-yloxy)propylamino)ethoxy)-phenyl)acetate* (21)**

^1H NMR ($CDCl_3$) δ: 8.24-8.26 (m, 1H), 7.78-7.82 (m, 1H), 7.41-7.50 (m, 3H), 7.34 (dd, *J* = 7.9/7.9 Hz, 1H), 7.13-7.17 (m, 2H), 6.82-6.86 (m, 2H), 6.77 (dd, *J* = 7.6/0.7 Hz, 1H), 4.27-4.33 (m, 1H), 4.10-4.19 (m, 2H), 4.07-4.09 (t, *J* = 5.1 Hz, 2H), 3.75 (s, broad, 2H), 3.68 (s, 3H, CH_3), 3.54 (s, 2H, CH_2-C=O), 3.06-3.11 (m, 3H, N-C**H**$_2$-CH_2, N-C**H**$_2$-CH-OH), 2.96-3.01 (m, 1H); ^{13}C NMR ($CDCl_3$) δ: 172.40, 157.75, 154.28, 134.53, 130.40, 127.57, 126.51, 125.89, 125.55, 125.36, 121.90, 120.69, 114.68, 104.99, 70.53, 68.14, 66.82, 52.06, 52.01, 48.66, 40.28; LCMS R_t: 2.29 min $[MH]^+$ 410.3; HRMS *m/z* $C_{24}H_{28}NO_5^+$ $[MH]^+$ calculated 410.1962, found 410.1965.



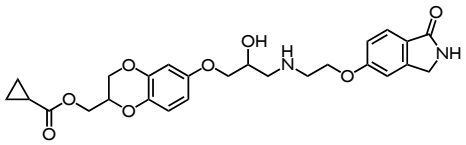
***Methyl 2-(4-(2-(3-(9H-carbazol-4-yloxy)-2-hydroxypropylamino)ethoxy)-phenyl)acetate* (22)**

^1H NMR ($CDCl_3$) δ: 8.26 (d, broad, J = 7.8 Hz, 1H), 8.19 (s, broad, 1H), 7.34-7.40 (m, 2H), 7.30 (dd, *J* = 8.0/8.0 Hz, 1H), 7.19-7.23 (m, 1H), 7.13-7.17 (m, 2H), 7.04 (dd, *J* = 8.1/0.5 Hz, 1H), 6.79-6.83 (m, 2H), 6.66 (d, broad, *J* = 8.0 Hz, 1H), 4.20-4.34 (m, 3H), 4.05 (t, *J* = 5.1 Hz, 2H), 3.69 (s, 3H,), 3.55 (s, 2H), 2.98-3.13 (m, 4H), 2.93 (s, broad, 2H); ^{13}C NMR ($CDCl_3$) δ: 172.55, 157.94, 155.19, 141.11, 138.89, 130.43, 126.78, 126.46, 125.14, 123.01, 122.60, 119.81, 114.75, 112.83, 110.20, 104.07, 101.38, 70.40, 68.58, 67.22, 52.14, 52.07, 48.88, 40.40; mp: 120-123 °C; LCMS R_t: 2.27 min $[MH]^+$ 449.2; HRMS *m/z* $C_{26}H_{29}N_2O_5^+$ $[MH]^+$ calculated 449.2071, found 449.2078.



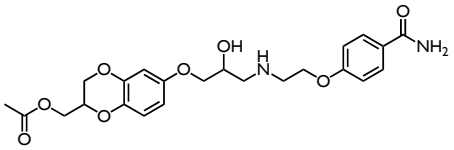
***Methyl 2-(3-(2-((2-hydroxy-3-(naphthalen-1-yloxy)propyl)amino)ethoxy)-phenyl)acetate* (23)**

^{1}H NMR ($CDCl_3$) δ: 8.23-8.26 (m, 1H), 7.79-7.81 (m, 1H), 7.42-7.50 (m, 3H), 7.36 (dd, *J* = 7.8/7.8 Hz, 1H), 7.22 (dd, *J* = 7.8/7.8 Hz, 1H), 6.80-6.87 (m, 4H), 4.38-4.42 (m, 1H), 4.13-4.28 (m, 3H), 4.10 (t, *J* = 5.1 Hz, 2H), 3.68 (s, 3H), 3.57-3.58 (m, 2H), 3.05-3.10 (m, 3H), 2.95-3.00 (m, 1H), 2.84 (s, broad, 2H); ^{13}C NMR ($CDCl_3$) δ: 172.01, 158.97, 154.42, 135.56, 134.61, 129.72, 127.62, 126.55, 125.94, 125.66, 125.40, 122.04, 121.95, 120.76, 115.74, 113.29, 105.07, 70.67, 68.49, 67.29, 52.18, 52.06, 48.90, 41.26; LCMS R_t: 2.32 min $[MH]^+$ 410.2; HRMS *m/z* $C_{24}H_{28}NO_5^+$ $[MH]^+$ calculated 410.1962, found 410.1961.



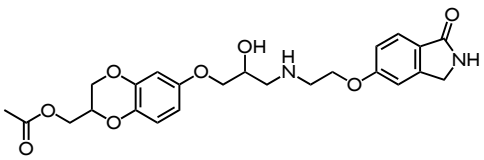
***(6-(2-Hydroxy-3-((2-((1-oxoisoindolin-5-yl)oxy)ethyl)amino)propoxy)-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methyl cyclopropanecarboxylate* (24)**

^{1}H NMR (DMSO-d_6) : δ 0.82-0.95 (m, 4H), δ 1.64-1.72 (m, 1H), δ 1.92 (s, 1H), δ 2.59-2.75 (m, 2H), δ 2.92 (t, *J* = 5.4 Hz, 2H), δ 3.74-3.89 (m, 3H), δ 4.00 (dd, *J* = 7.0/4.3 Hz, 1H), δ 4.09 (t, *J* 5.5 Hz, 2H), δ 4.17-4.40 (m, 6H), δ 4.97 (d, *J* = 4.97 Hz, 1H), δ 6.40-6.49 (m, 2H), δ 6.79 (d, *J* = 8.8 Hz, 1H), δ 7.00 (d, *J* = 8.1 Hz, 1H), δ 7.11 (s, 1H), δ 7.54 (d, *J* = 8.5 Hz, 1H), δ 8.29 (s, 1H); ^{13}C NMR (DMSO-d_6) : δ 173.86, 169.89, 161.54, 153.25, 146.48, 142.95, 136.41, 125.10, 124.03, 117.29, 115.13, 108.62, 107.99, 103.09, 71.20, 70.52, 68.23, 68.05, 64.67, 62.45, 52.37, 48.25, 44.75, 12.35, 8.41, 8.39; mp 166 – 168 °C, LCMS R_t: 2.12 min $[MH]^+$ 499.3; HRMS (TOF ES^+) *m/z* $C_{26}H_{31}N_2O_8$ $[MH]^+$ calculated 499.2075; found 499.2083.



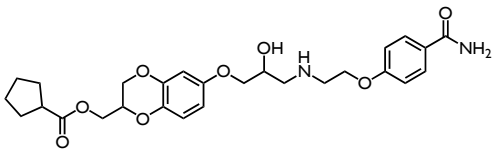
***(6-(3-((2-(4-Carbamoylphenoxy)ethyl)amino)-2-hydroxypropoxy)-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methyl acetate* (25)**

^{1}H NMR (DMSO-d_6) : δ 0.82-0.96 (m, 3H), δ 1.64-1.72 (m, 1H), δ 1.92 (s, 1H), δ 2.59-2.76 (m, 2H), δ 2.85-3.00 (m, 2H), δ 3.73-3.92 (m, 3H), δ 4.01 (dd, *J* = 7.0/ 4.3 Hz, 1H), δ 4.09 (t, *J* = 5.4 Hz, 2H), δ 4.19-4.42 (m, 5H), δ 4.97 (d, *J* = 4.6 Hz, 1H), δ 6.43 (dd, *J* = 2.8/ 5.9 Hz, 1H), δ 6.47 (d, *J* = 2.8 Hz, 1H), δ 6.79 (d, *J* = 8.7 Hz, 1H), δ 7.01 (dd, *J* = 2.1/ 6.2 Hz, 1H), δ 7.11 (s, 1H), δ 7.55 (d, *J* = 8.4 Hz, 1H), δ 8.29 (s, 1H); ^{13}C NMR (DMSO-d_6) : δ 170.21, 167.40, 160.95, 153.23, 142.95, 136.40, 129.34, 126.47, 117.29, 113.85, 107.98, 103.08, 71.19, 70.50, 68.18, 67.72, 64.66, 62.21, 52.37, 48.25, 20.55; mp 133 – 135 °C, LCMS R_t: 1.99 min $[MH]^+$ 461.2; HRMS (TOF ES^+) *m/z* $C_{23}H_{29}N_2O_8$ $[MH]^+$ calculated 461.1918; found 461.1919.



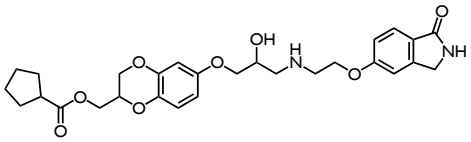
***(6-(2-Hydroxy-3-((2-((1-oxoisoindolin-5-yl)oxy)ethyl)amino)propoxy)-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methyl acetate* (26)**

^{1}H NMR (DMSO-d_6) : δ 1.23 (s, 1H), δ 2.06 (s, 3H), δ 2.62-2.83 (m, 2H), δ 2.98 (t, *J* = 5.3 Hz, 2H), δ 3.73-3.91 (m, 3H), δ 4.00 (dd, *J* = 7.2/ 4.2 Hz, 1H), δ 4.12 (t, *J* = 5.1 Hz, 2H), δ 4.18-4.26 (m, 2H), δ 4.27-4.39 (m, 4H), δ 5.08 (s, 1H), δ 6.43 (dd, *J* = 2.8/ 5.9 Hz, 1H), δ 6.48 (d, *J* = 2.8 Hz, 1H), δ 6.79 (d, *J* = 8.9 Hz, 1H), δ 7.01 (dd, *J* = 2.1/ 6.2 Hz, 1H), δ 7.11 (s, 1H), δ 7.55 (d, *J* = 8.4 Hz, 1H), δ 8.30 (s, 1H); mp 159 -160 °C; LCMS R_t: 2.04 min $[MH]^+$ 473.2; HRMS (TOF ES^+) *m/z* $C_{23}H_{29}N_2O_8$ $[MH]^+$ calculated 461.1918; found 461.1919.



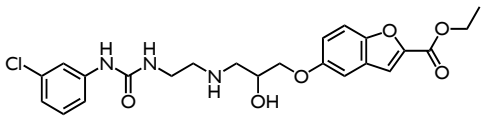
***(6-(3-((2-(4-Carbamoylphenoxy)ethyl)amino)-2-hydroxypropoxy)-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methyl cyclopentanecarboxylate* (27)**

^{1}H NMR (DMSO-d_6) : δ 1.49-1.89 (m, 9H), δ 2.32-2.34 (m, 1H), δ 2.59-2.74 (m, 3H), δ 2.76-2.82 (m, 1H), δ 2.91 (t, *J* = 5.7 Hz, 2H), δ 3.76-3.89 (m, 3H), δ 4.00 (dd, *J* = 7.0/ 4.5 Hz, 1H), δ 4.07 (t, *J* = 5.5 Hz, 2H), δ 4.24-4.38 (m, 4H), δ 4.98 (d, *J* = 4.1 Hz, 1H), δ 6.43 (dd, *J* = 2.8/ 6.0 Hz, 1H), δ 6.48 (d, *J* = 2.8 Hz, 1H), δ 6.77 (d, *J* = 8.9 Hz, 1H), δ 6.97 (d, *J* = 8.8 Hz, 2H), δ 7.83 (d, *J* = 9.0 Hz, 2H); ^{13}C NMR (DMSO-d_6) : δ 175.52, 167.38, 160.94, 153.21, 142.94, 136.44, 129.33, 126.47, 117.24, 113.84, 107.98, 103.08, 71.19, 70.52, 68.17, 67.71, 64.67, 62.11, 52.36, 48.24, 42.86, 29.45, 25.31; mp 145 – 146 °C; LCMS R_t: 2.25 min $[MH]^+$ 515.2; HRMS (TOF ES^+) *m/z* $C_{27}H_{35}N_2O_8$ $[MH]^+$ calculated 515.2388; found 515.2403.



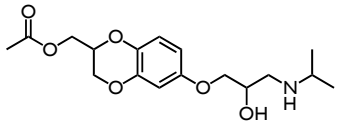
***(6-(2-Hydroxy-3-((2-((1-oxoisoindolin-5-yl)oxy)ethyl)amino)propoxy)-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methyl cyclopentanecarboxylate* (28)**

^{1}H NMR (DMSO-d_6) : δ 1.47-1.89 (m, 9H), δ 2.59-2.84 (m, 4H), δ 2.92 (t, *J* = 5.4 Hz, 2H), δ 3.75-3.88 (m, 3H), δ 3.99 (dd, *J* = 7.1/ 4.5 Hz, 1H), δ 4.09 (t, *J* = 5.5 Hz, 2H), δ 4.23-4.34 (m, 5H), δ 4.97 (d, *J* = 4.6 Hz, 1H), δ 6.43 (dd, *J* = 2.8/ 5.8 Hz, 1H), δ 6.47 (d, *J* = 2.8 Hz, 1H), δ 6.77 (d, *J* = 8.8 Hz, 1H), δ 7.00 (dd, *J* = 2.1/ 6.1 Hz, 1H), δ 7.11 (s, 1H), δ 7.55 (d, *J* = 8.0 Hz, 1H), δ 8.29 (s, 1H); ^{13}C NMR (DMSO-d_6) : δ 175.52, 169.85, 161.53, 153.22, 146.47, 142.94, 136.44, 125.10, 124.01, 117.24, 115.12, 108.62, 107.98, 103.07, 71.19, 70.53, 68.20, 68.03, 64.67, 62.11, 52.36, 48.24, 44.73, 42.86, 29.45, 25.31; mp 171 – 174 °C; LCMS R_t: 2.24 min $[MH]^+$ 527.2; HRMS (TOF ES^+) $C_{28}H_{35}N_2O_8$ $[MH]^+$ calculated 527.2388; found 527.2388.



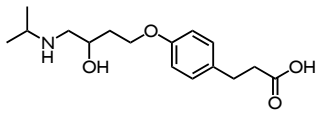
***Ethyl 5-(3-((2-(3-(3-chlorophenyl)ureido)ethyl)amino)-2-hydroxypropoxy)benzofuran-2-carboxylate* (29)**

^{1}H NMR (D_6 DMSO) δ: 8.78 (s, 1H), 7.65 (t, *J* = 1.9 Hz, 1H), 7.62 (t, *J* = 1.0 Hz, 1H), 7.61 (d, *J* = 9.4 Hz, 1H), 7.26 (d, *J* = 2.5 Hz, 1H), 7.20 (d, *J* = 7.9 Hz, 1H), 7.16 (ddd, *J* = 7.8/1.8/1.2 Hz, 1H), 7.13 (d, *J* = 9.0/2.6 Hz, 1H), 6.91 (ddd, *J* = 7.7/2.0/1.1 Hz, 1H), 6.24 (t, *J* = 5.4 Hz, 1H), 5.01 (br s, 1H), 4.35 (q, *J* = 7.1 Hz, 2H), 4.04-3.99 (m, 1H), 3.95-3.90 (m, 2H), 3.17 (q, *J* = 5.8 Hz, 2H), 2.74-2.61 (m, 4H), 1.33 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (D_6 DMSO) δ: 158.61, 155.58, 155.01, 150.03, 145.63, 142.13, 133.06, 130.16, 127.22, 120.45, 117.98, 116.84, 115.85, 114.12, 112.79, 105.09, 71.46, 68.10, 61.10, 52.11, 49.11, 14.11; LCMS R_t: 2.32 min $[MH]^+$ 476.1/478.0; HRMS (TOF ES^+) *m/z* $C_{23}H_{27}{}^{35}ClN_3O_6$ $[MH]^+$ calculated 476.1583; found 476.1585.



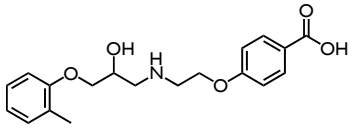
***(6-(2-Hydroxy-3-(isopropylamino)propoxy)-2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methyl acetate* (30)**

^{1}H NMR (DMSO-d_6) : δ 0.98 (dd, *J* = 1.3/ 3.1 Hz, 6H), δ 2.05 (s, 3H), δ 2.61-2.72 (m, 2H), δ 3.73-3.83 (m, 3H), δ 3.85 (dd, *J* = 2.7/ 6.4 Hz, 1H), δ 4.00 (dd, *J* = 7.2/ 6.1 Hz, 1H), δ 4.20-4.30 (m, 2H), δ 4.30 (dd, *J* = 1.3/ 5.8 Hz, 2H), δ 4.98 (s, 1H), δ 6.43 (dd, *J* = 2.9/ 4.1 Hz, 1H), δ 6.48 (d, *J* = 2.7 Hz, 1H), δ 6.79 (d, *J* = 8.9 Hz, 1H). LCMS R_t: 1.97 min $[MH]^+$ 340.2; HRMS (TOF ES^+) *m/z* $C_{17}H_{26}NO_6$ [MH+] calculated 340.1755; found 340.1760.



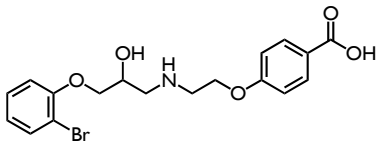
***3-(4-(2-hydroxy-3-(isopropylamino)propoxy)phenyl)propanoic acid* (31 = ASL8123)**

LC/MS Rt: 0.56min) $[MH]^+$ 282.4, *m/z* $C_{15}H_{23}NO_4$ calculated 281.35.



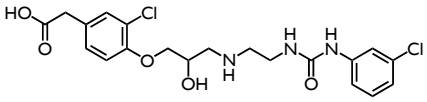
***4-(2-((2-Hydroxy-3-(o-tolyloxy)propyl)amino)ethoxy)benzoic acid* (32)**

^{1}H (400 MHz, MeOH-d_4) δ 7.99 (d, *J* 8.9 Hz, 2H), 7.17-7.03 (m, 4H), 6.89 (d, *J* 8.1 Hz, 1H), 6.84 (at, *J* 7.4 Hz, 1H), 4.42 (t, *J* 5.2 Hz, 3H), 4.42-4.33 (m, 1H), 4.09 (dd, *J* 9.9, 4.8 Hz, 1H), 4.01 (dd, *J* 9.9, 5.7 Hz, 1H), 3.61 (t, *J* 5.0 Hz, 2H), 3.48 (dd, *J* 12.8, 3.0 Hz, 1H), 3.29 (d, *J* 2.7 Hz, 1H), 2.18 (s, 3H); ^{13}C (100 MHz, MeOH-d_4) δ 169.5, 163.0, 157.8, 132.9, 132.6, 131.6, 128.0, 127.7, 122.0, 115.4, 112.4, 71.0, 66.6, 64.5, 51.6, 48.0, 16.4; Mp 211-213 °C; HRMS (ESI+): m/z calcd for $C_{19}H_{24}NO_5$ $[M+H]^+$: 346.1649; found: 346.1646



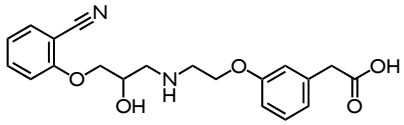
4-(2-((3-(2-Bromophenoxy)-2-hydroxypropyl)amino)ethoxy)benzoic acid **(33)**

^{1}H (400 MHz, DMSO-d_6) δ 12.66 (bs, 1H), 9.46 (bs, 2H), 7.91 (d, *J* = 8.8 Hz, 2H), 7.56 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.40-7.26 (m, 1H), 7.15 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.07 (d, *J* = 8.9 Hz, 2H), 6.90 (td, *J* = 7.8, 1.3 Hz, 1H), 6.02 (bs, 1H), 4.42 (t, *J* = 5.2 Hz, 2H), 4.39-4.29 (m, 2H), 4.13 (dd, *J* = 10.1, 4.7 Hz, 1H), 4.07 (dd, *J* = 10.1, 5.5 Hz, 1H), 3.44 (d, *J* = 10.2 Hz, 2H), 3.32 (dd, *J* = 12.8, 2.8 Hz, 2H), 3.17 (dd, *J* = 12.7, 9.3 Hz); ^{13}C (100 MHz, DMSO-d_6) δ 166.8, 161.2, 154.5, 132.9, 131.3, 129.0, 123.6, 122.3, 114.5, 114.0, 111.1, 70.7, 64.7, 63.5, 50.0, 46.0; mp 238-239 °C (HCl salt); HRMS (ESI+): m/z calcd for $C_{18}H_{21}BrNO_5$ $[M+H]^+$: 410.0598; found: 410.0596.



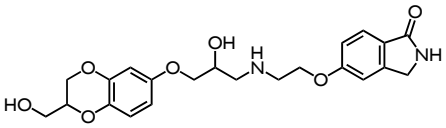
2-(3-chloro-4-(3-((2-(3-(3-chlorophenyl)ureido)ethyl)amino)-2-hydroxypropoxy)-phenyl)acetic acid **(34)**

^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.25 (s, 1H), 7.67 (br s, 1H), 7.30 (d, *J* = 2.2 Hz, 1H), 7.21 - 7.18 (m, 2H), 7.14 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.05 (d, *J* = 8.5 Hz, 1H), 6.90 (ddd, *J* = 6.5, 2.2, 2.2 Hz, 1H), 6.79 (br t , *J* = 5.5 Hz, 1H), 3.98 (br s, 3H), 3.43 (s, 2H), 3.25 (br q, *J* = 5.8 Hz, 2H), 2.87 - 2.83 (m, 1H), 2.76 - 2.71 (m, 3H); ^{13}C NMR (DMSO-d_6) δ: 173.50, 155.25, 152.37, 142.30, 133.03, 130.63, 130.13, 129.76, 129.06, 120.88, 120.37, 116.85, 115.87, 113.70, 71.42, 67.14, 51.52, 48.71, 40.63, 38.29; LCMS Rt: 2.01 min $[MH]^+$ 456.1/458.1; HRMS (ESI+) calcd for $C_{20}H_{24}{}^{35}Cl_2N_3O_5$ [M+H+]: 456.1088; found: 456.1093



2-(3-(2-((3-(2-cyanophenoxy)-2-hydroxypropyl)amino)ethoxy)phenyl)acetic acid **(35)**

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.7 – 7.6 (m, 2H), 7.2 (t, *J* = 8.1 Hz, 2H), 7.1 (td, *J* = 7.7, 0.9 Hz, 1H), 7.0 (s, 1H), 7.0 (d, *J* = 7.6 Hz, 1H), 6.9 (dd, *J* = 8.2, 2.4 Hz, 1H), 4.4 (dq, *J* = 9.4, 4.4 Hz, 1H), 4.3 (t, *J* = 5.0 Hz, 2H), 4.3 (dd, *J* = 9.9, 4.7 Hz, 1H), 4.2 (dd, *J* = 9.9, 5.4 Hz, 1H), 3.6 – 3.5 (m, 4H), 3.5 (dd, *J* = 12.9, 3.2 Hz, 1H), 3.4 – 3.4 (m, 1H); ^{13}C NMR (101 MHz, DMSO) δ 160.9, 160.8, 158.9, 135.5, 134.1, 129.5, 121.5, 121.4, 116.9, 116.1, 113.7, 113.6, 112.6, 101.1, 71.9, 70.9, 70.1, 62.9, 52.5, 48.8. LCMS R_t: 2.07 min $[MH]^+$; HRMS (ESI^+) calcd for $C_{20}H_{22}N_2O_5$ $[M+H^+]$: 371.1611; found: 371.1601.



5-(2-((2-Hydroxy-3-((2-(hydroxymethyl)-2,3-dihydrobenzo[b][1,4]dioxin-6-yl)oxy)-propyl)amino)ethoxy)isoindolin-1-one **(36)**

^{1}H (400 MHz, MeOH-d_4/D_2O (1:1)) δ 7.68 (1 H, d, *J* 8.5 Hz), 7.16 (1 H, d, *J* 1.7 Hz), 7.10 (1 H, dd, *J* 8.5, 2.2 Hz), 6.80 (1 H, d, *J* 8.8 Hz), 6.48 (1 H, dd, *J* 8.8, 2.9 Hz), 6.45 (1 H, d, *J* 2.8 Hz), 4.42 (4 H, d, *J* 6.0 Hz), 4.33 (2 H, dt, *J* 8.5, 4.2 Hz), 4.29 (1 H, dd, *J* 11.5, 2.2 Hz), 4.19-4.10 (1 H, m), 4.06-3.95 (3 H, m), 3.75 (2 H, dd, *J* 5.1, 1.6 Hz), 3.61 (1 H, q, *J* 4.2 Hz), 3.43 (1 H, dd, *J* 13.0, 3.5 Hz) , 3.34 (1 H, dd, *J* 13.0, 8.6 Hz); ^{13}C (125 MHz, MeOH-d_4/D_2O (1:1)) δ 173.8, 162.3, 153.6, 148.0, 144.0, 138.4, 125.6, 125.5, 118.5, 116.5, 109.7, 109.2, 104.5, 74.5, 71.3, 66.3, 66.1, 64.2, 61.3, 50.6, 47.5, 46.8; Mp 226-228 °C (HCl salt); HRMS (ESI+): m/z calcd for $C_{22}H_{27}N_2O_7$ $[M+H]^+$: 431.1813; found: 431.1818.