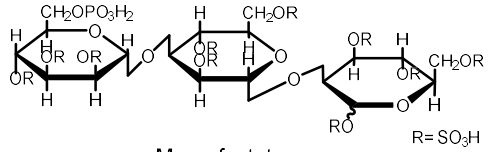
Identification of Novel Potential Heparanase Inhibitors Using Virtual Screening

Alfredo Rus, Victor M. Bolanos-Garcia, Agatha Bastida and Paula Morales

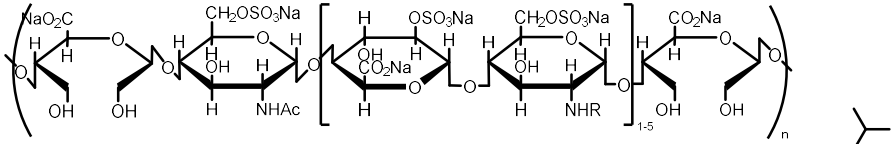
Content

Figure S1. Heparanase inhibitors.

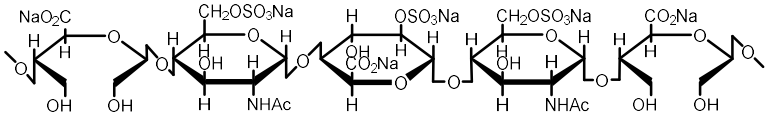
Heparin derivatives or sulfated saccharides



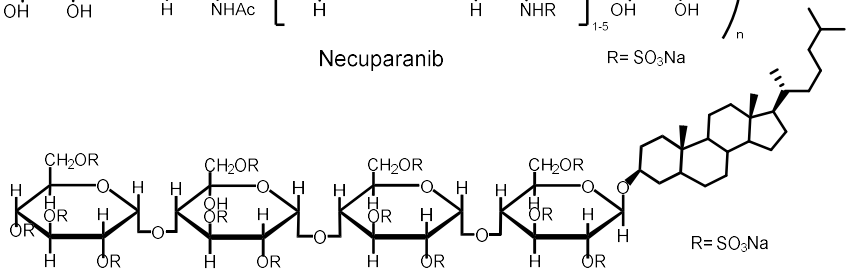
Muparfostat



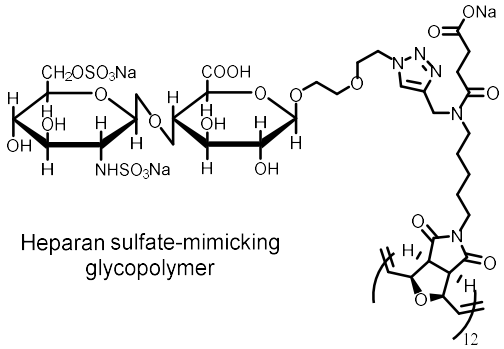
Necuparanib



Roneparstat

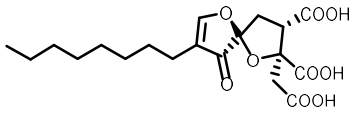


Pixatimod

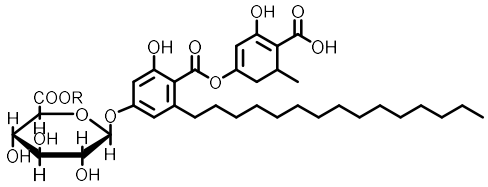


Heparan sulfate-mimicking glycopolymer

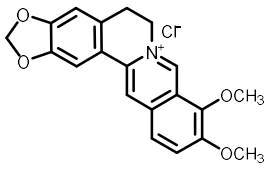
Natural small molecules



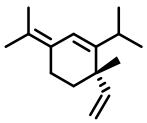
(+)-Trachyspic acid



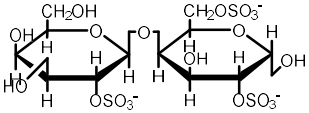
Fungal metabolite from *Acremonium sp.*



Berberine

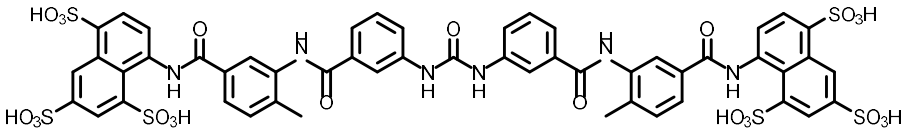


β-Elemene

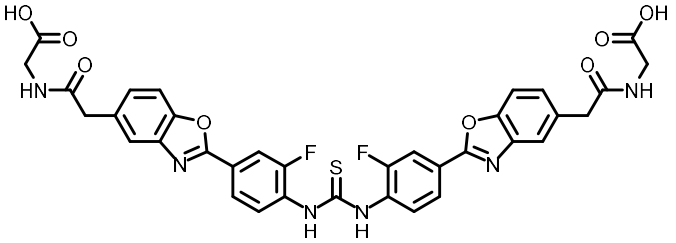


λ-Carrageenan

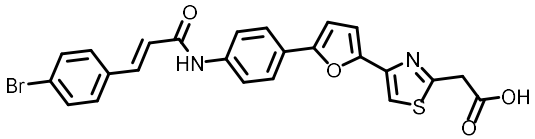
Synthetic small molecules



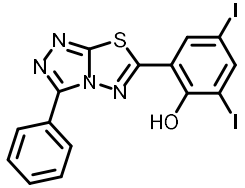
Suramin



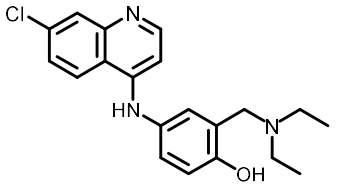
2-phenyl-benzoxazol-5-yl-acetic acid derivative



Furanyl-1,3-thiazole-2-yl-acetic acid derivative



[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivative



Amodiaquine

Figure S2. Workflow used in this study for the identification of novel HPSE inhibitors

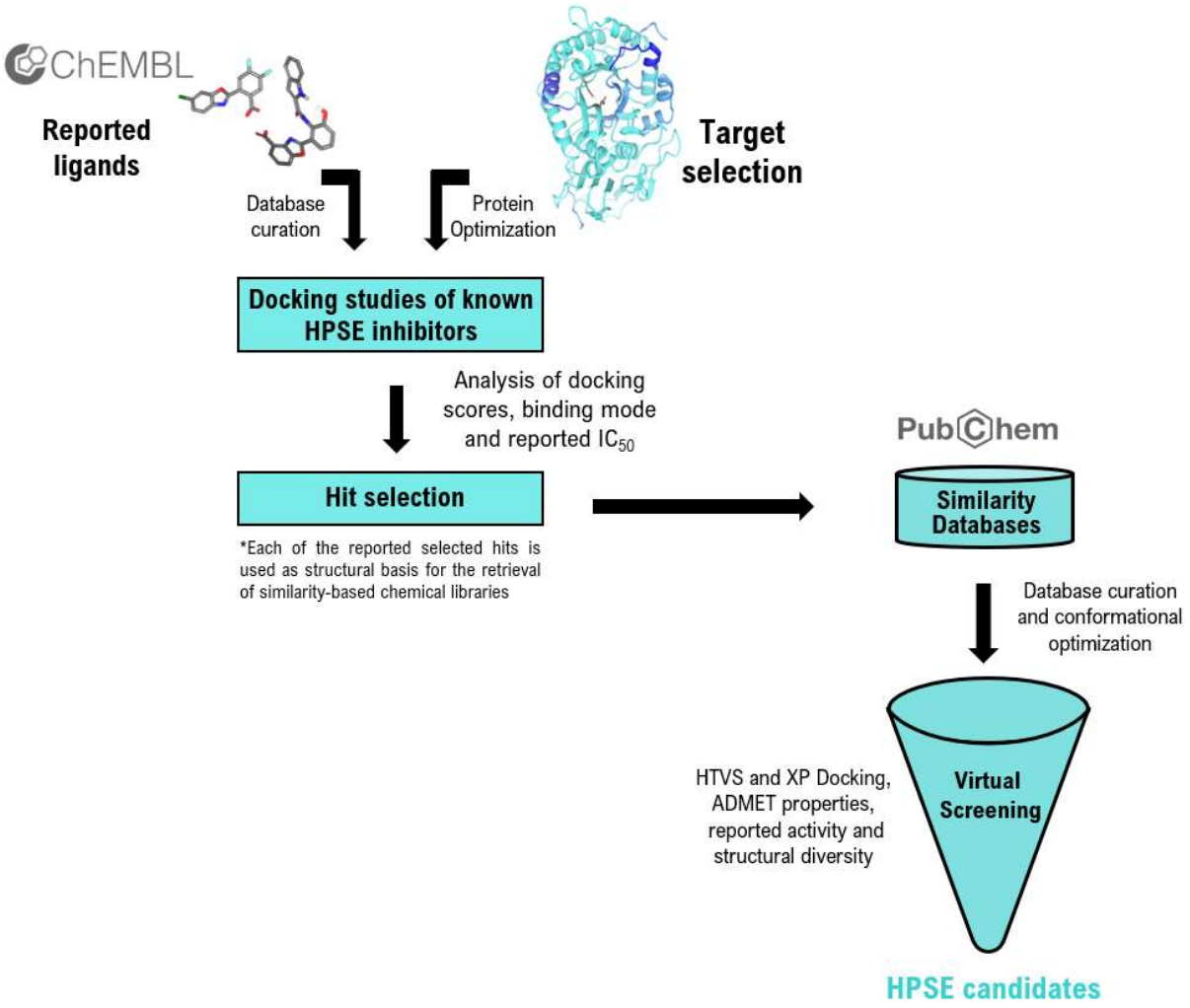
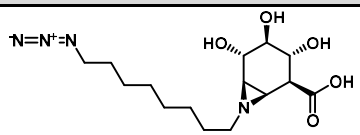
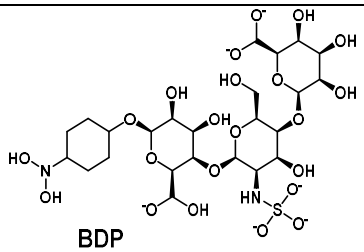
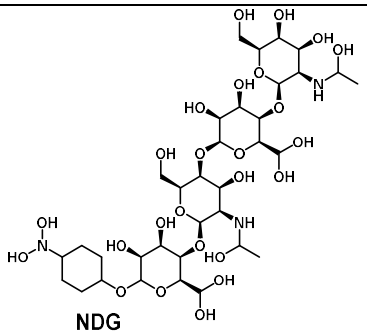
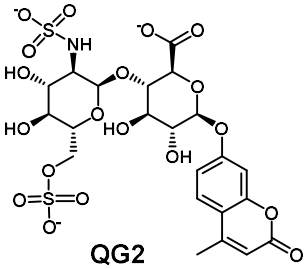
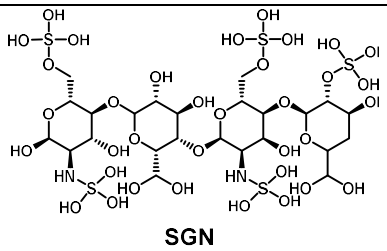
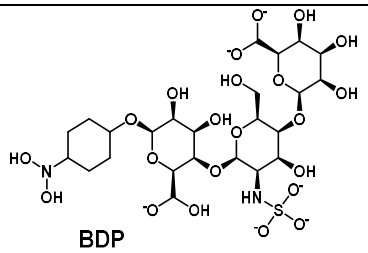
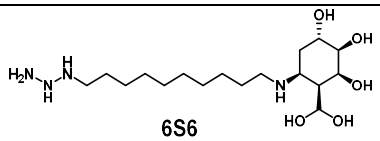
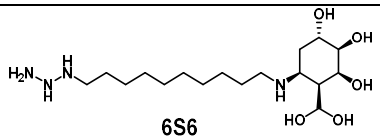


Table S1. Heparanases 3D structures retrieved from the Protein Data Bank (PDB).

PDB ID	Size (kDa)	Resolution (Å)	Mutations	Co-crystallized Ligands	Identity (%)	Organism
5L9Z	54.04	1.57	E343Q and K307R	 GUX	99	*Homo sapiens*
5BWI	91.09	1.60	No	None	23	*Burkholderia pseudomallei*
5E98	53.79	1.63	No	 BDP	99	*Homo sapiens*
5E97	54.22	1.63	No	 NDG	99	*Homo sapiens*
6ZDM	53.71	1.71	No	 QG2	99	*Homo sapiens*
5E9C	54.15	1.73	No	 SGN	99	*Homo sapiens*
5E8M	54.57	1.75	K307R	None	99	*Homo sapiens*

5E9B	53.91	1.88	No	 BDP	99	*Homo sapiens*
5L9Y	53.63	1.88	No	 6S6	99	*Homo sapiens*
5LA4	59.43	1.90	K307R	None	99	*Homo sapiens*
5LA7	59.69	1.94	K307R	 6S6	99	*Homo sapiens*

GUX: (1{R},2{S},3{R},4{S},5{S},6{R})-7-[8-[(azanylidene-{4}-azanylidene)amino]octyl]-3,4,5-tris(oxidanyl)-7-azabicyclo[4.1.0]heptane-2-carboxylic acid; **BDP**: beta-D-glucopyranuronic acid-(1-4)-2-deoxy-2-(sulfoamino)-alpha-D-glucopyranose-(1-4)-beta-D-glucopyranuronic acid; **NDG**: 2-acetamido-2-deoxy-alpha-D-glucopyranose-(1-4)-beta-D-glucopyranuronic acid-(1-4)-2-acetamido-2-deoxy-alpha-D-glucopyranose-(1-4)-beta-D-glucopyranuronic acid; **QG2**: (2~{S},3{S},4{R},5{R},6{S})-3-[(2{R},3{R},4{R},5{S},6{R})-4,5-bis(oxidanyl)-3-(sulfoamino)-6-(sulfooxymethyl)oxan-2-yl]oxy-6-(4-methyl-2-oxidanylidene-chromen-7-yl)oxy-4,5-bis(oxidanyl)oxane-2-carboxylic acid; **SGN**: 4-deoxy-2-O-sulfo-alpha-L-threo-hex-4-enopyranuronic acid-(1-4)-2-deoxy-6-O-sulfo-2-(sulfoamino)-alpha-D-glucopyranose-(1-4)-alpha-L-idopyranuronic acid-(1-4)-2-deoxy-6-O-sulfo-2-(sulfoamino)-beta-D-glucopyranose; **6S6**: 2-acetamido-2-deoxy-beta-D-glucopyranose-(1-4)-[alpha-L-fucopyranose-(1-6)]2-acetamido-2-deoxy-beta-D-glucopyranose.

Figure S3. Ligand interaction diagrams of selected hit HPSE inhibitors ChEMBL2349245, ChEMBL495255, ChEMBL2349247 and ChEMBL4294823.

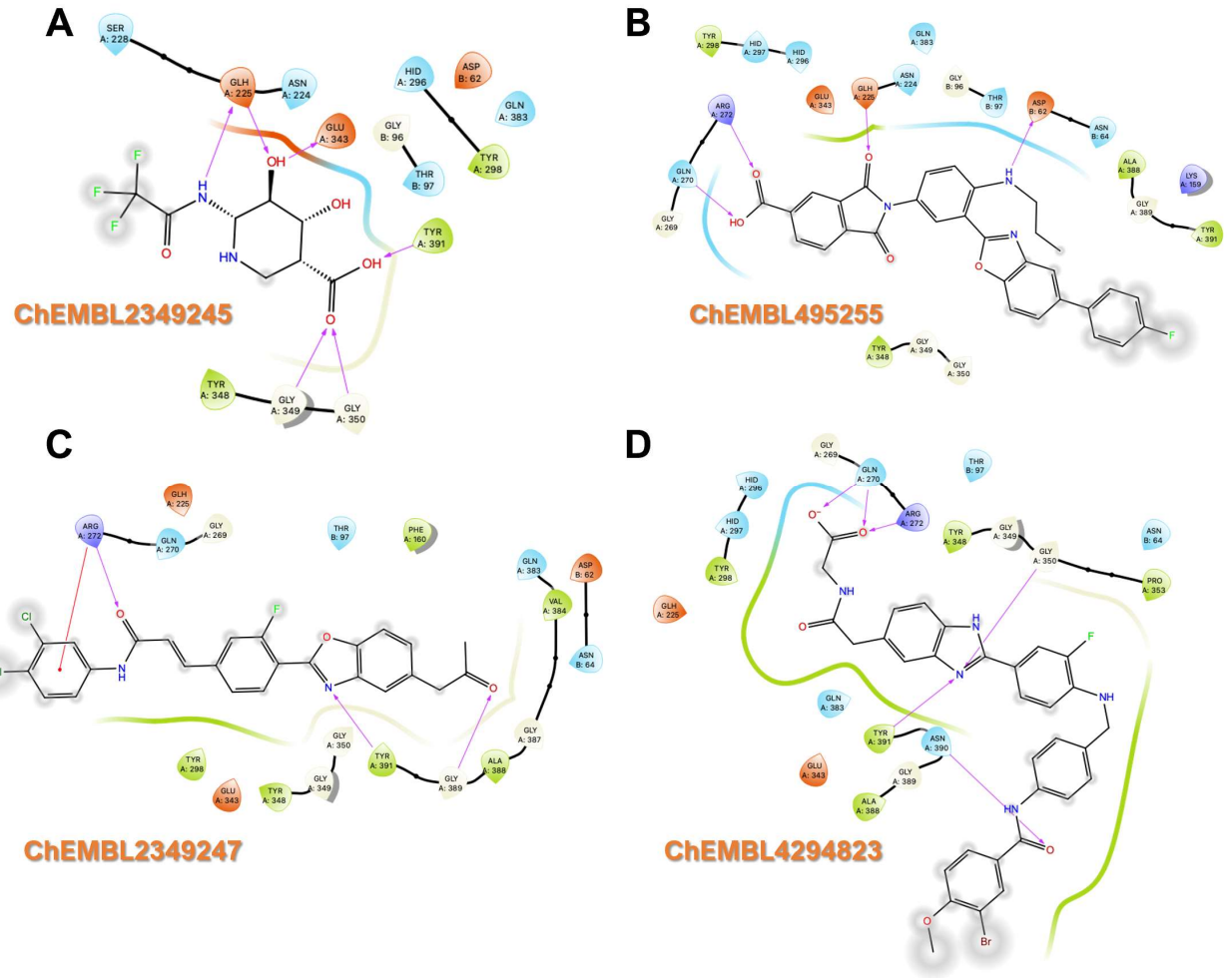
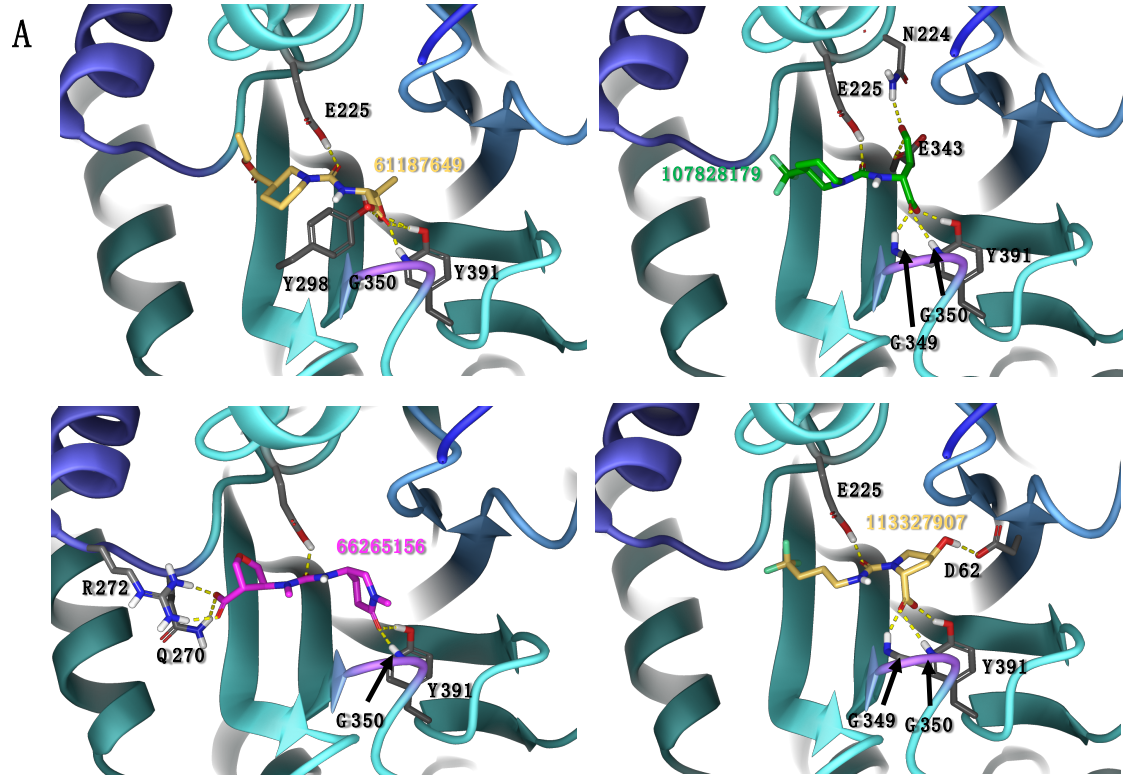


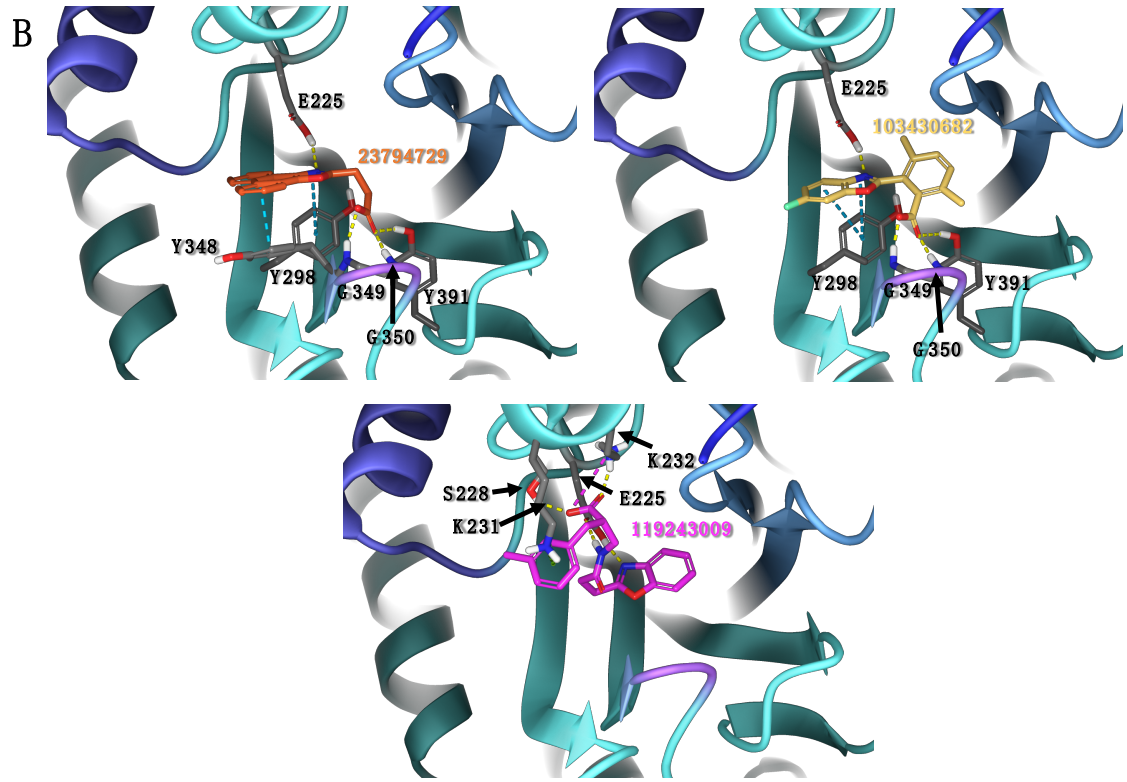
Table S3. Data reported for selected potential HPSE inhibitors.

Compound ID	HPSE IC_{50} (μM)	Reported Targets	References
ChEMBL2349245	1.00	HPSE	[40]
101687126	NR	NR	[44]
61187649	NR	NR	[45]
107828179	NR	NR	[46]
66265156	NR	NR	[47]
113327907	NR	NR	[64]
ChEMBL495255	0.50	HPSE	[36]
25158919	NR	Leishmania donovani	[48]
23794729	NR	NR	[49]
103430682	NR	NR	[50]
119243009	NR	NR	[51]
ChEMBL2349247	0.20	HPSE	[36]
81421830	NR	NR	[52]
58743027	NR	NR	[53]
155906206	NR	NR	[54]
23886486	NR	NR	[55]
6968873	NR	NR	[56]

NR: Not reported

Figure S4. Docking studies of HPSE in complex with selected potential HPSE inhibitors (**A**) ChEMBL2349245-based chemical library: 61187649, 107828179, 66265156 and 113327907; (**B**) ChEMBL495255-based chemical library: 23794729, 103430682 and 119243009; and (**C**) ChEMBL2349247-based chemical library: 58743027, 155906206, 23886486 and 6968873).





C

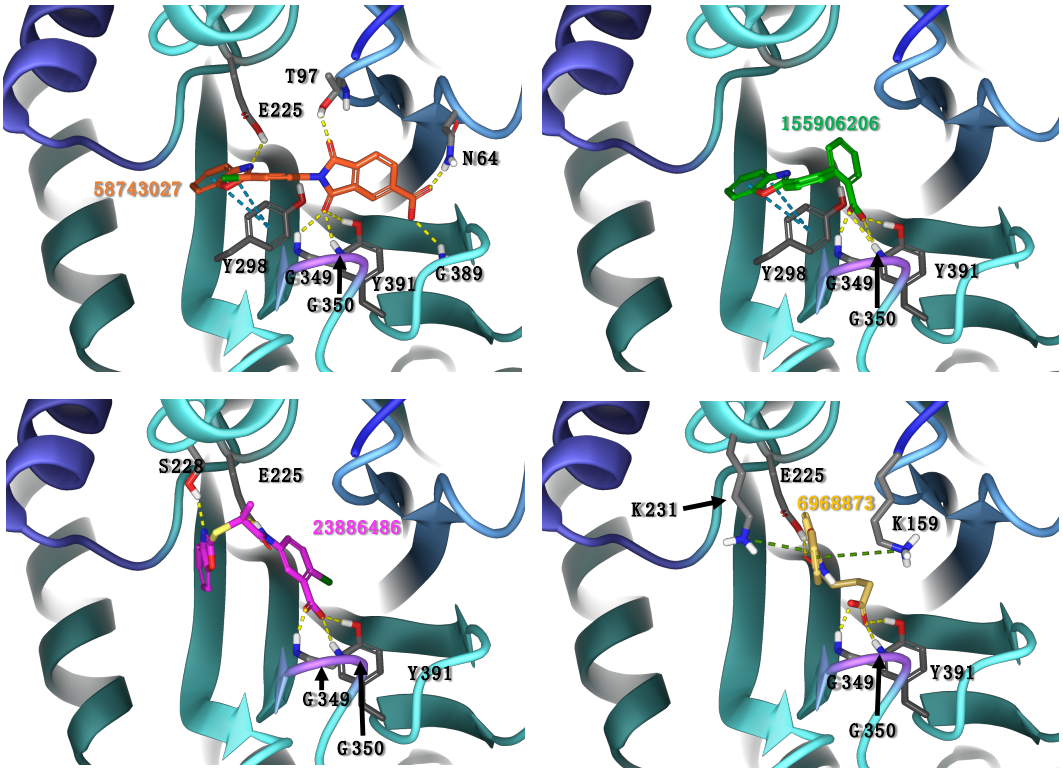
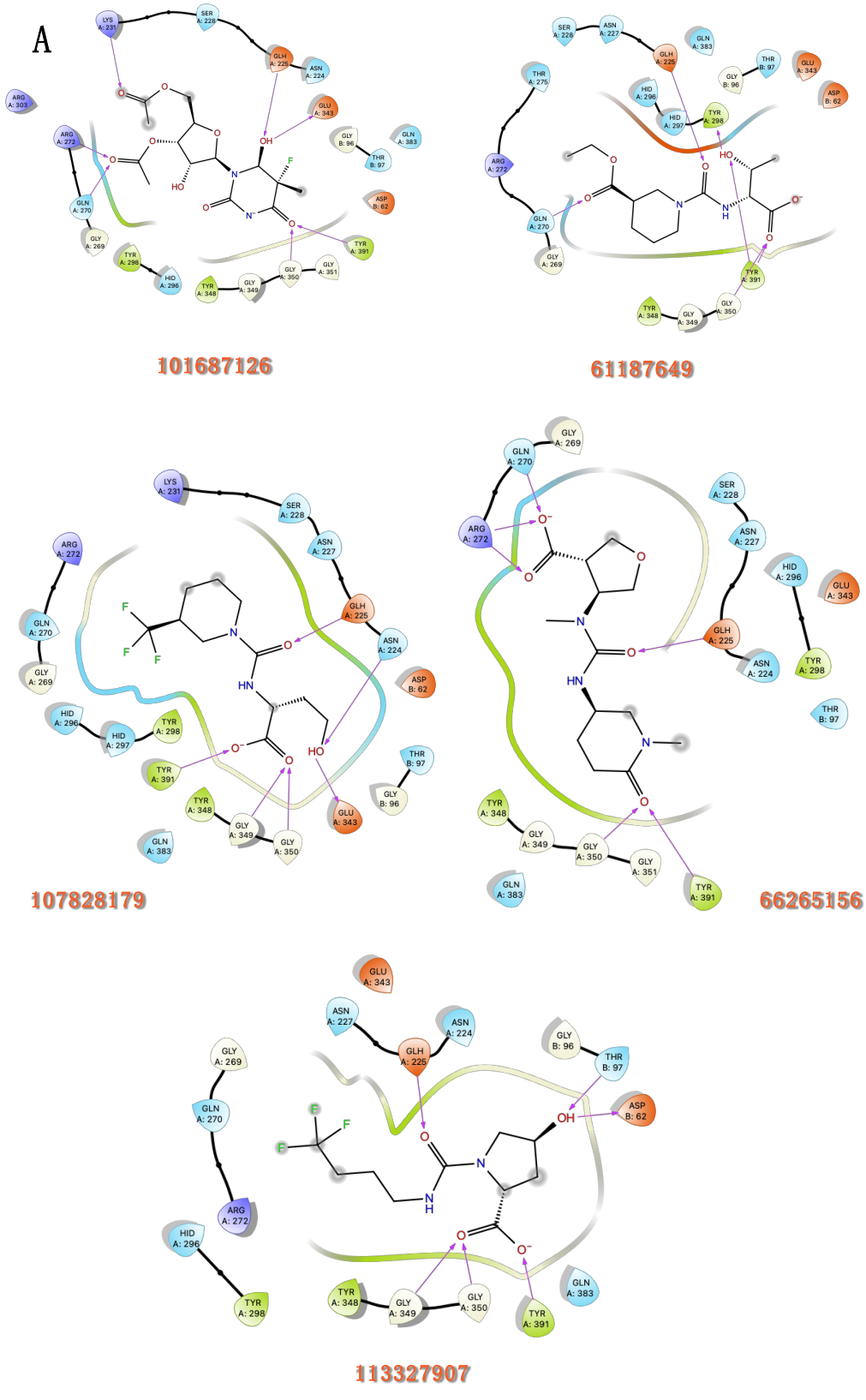

T97
E225
N64
58743027
Y298
G349
Y391
G389
G350
155906206
Y298
G349
Y391
G350
S228
E225
23886486
Y391
G349
G350
K231
E225
6968873
K159
G349
Y391
G350

Figure S5. Ligand interaction diagrams of the 14 selected potential HPSE inhibitors. (**A**) ChEMBL2349245-based chemical library: 101687126, 61187649, 107828179, 66265156 and 113327907; (**B**) ChEMBL495255-based chemical library: 25158919, 23794729, 103430682 and 119243009; and (**C**) ChEMBL2349247-based chemical library: 81428180, 58743027, 155906206, 23886486 and 6968873).



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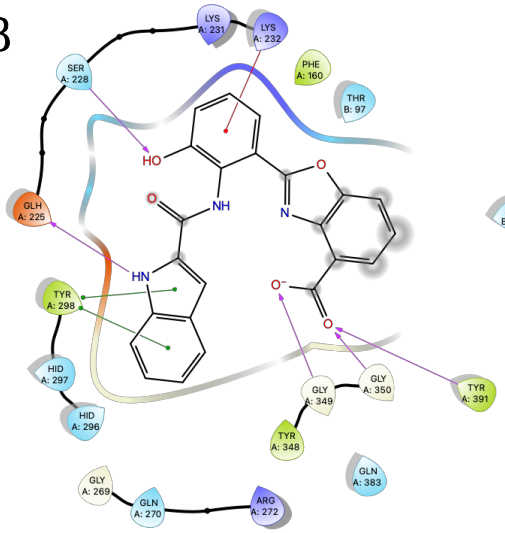
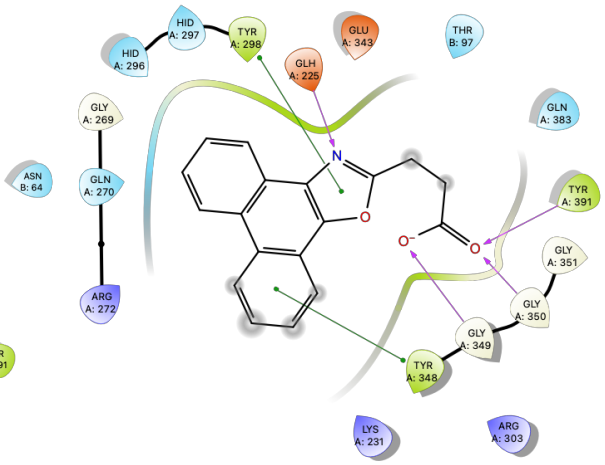


25158919



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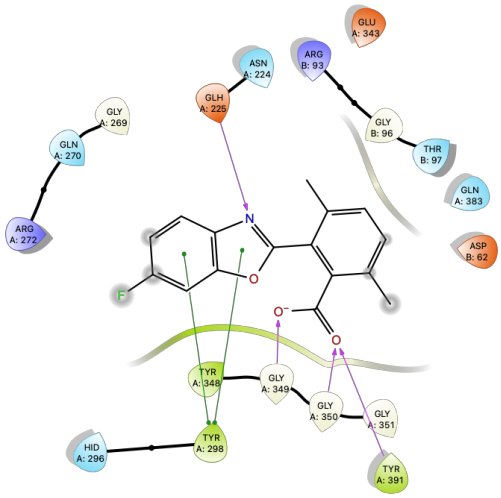

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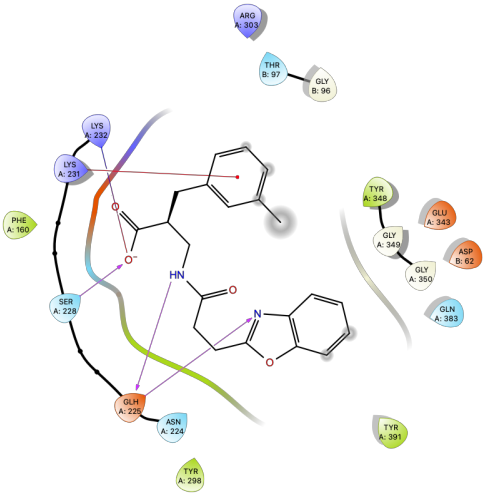
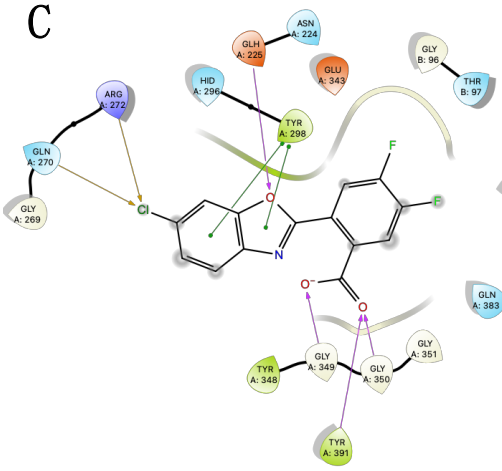
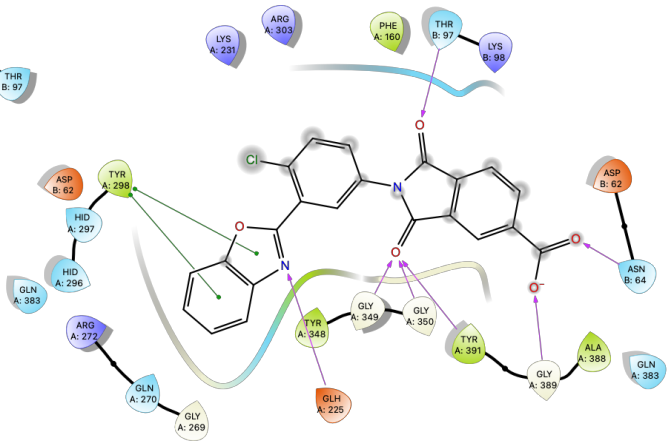


119243009

C



81428180



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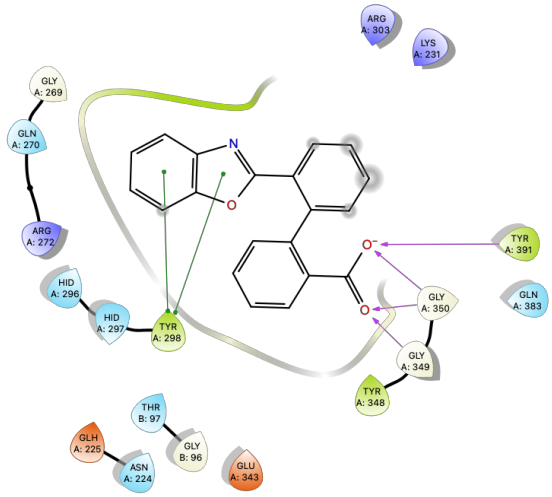

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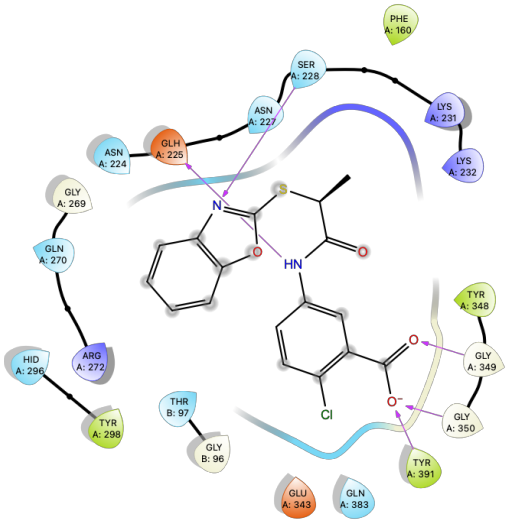
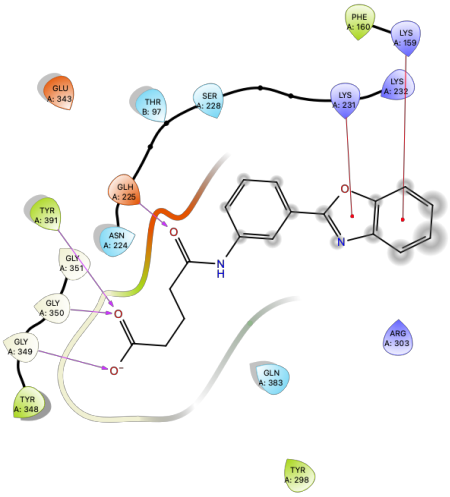


23886486



6968873

References

36 Gozalbes, R.; Mosulén, S.; Ortí, L.; Rodríguez-Díaz, J.; Carbajo, R.J.; Melnyk, P.; Pineda-Lucena, A. Hit identification of novel heparanase inhibitors by structure- and ligand-based approaches. Bioorganic Med. Chem. 2013, 21, 1944–1951.

40 Takahashi, H.; Matsumoto, H.; Smirkin, A.; Itai, T.; Nishimura, Y.; Tanaka, J. Involvement of heparanase in migration of microglial cells. *Biochim. Biophys. Acta—Gen. Subj.* **2008**, *1780*, 709–715, doi:10.1016/j.bbagen.2007.12.014.

44 1-(3-O,5-O-Diacetyl-beta-D-ribofuranosyl)-5-methyl-5-fluoro-6-hydroxydihydropyrimidine-2,4(1H,3H)-dione | $C_{14}H_{19}FN_2O_9$ — PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/101687126 (accessed on 10 March 2022).

45 2-[(3-Ethoxycarbonylpiperidine-1-carbonyl)amino]-3-hydroxybutanoic acid | $C_{13}H_{22}N_2O_6$ — PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/61187649 (accessed on 10 March 2022).

46 (2R)-4-hydroxy-2-[[3-(trifluoromethyl)piperidine-1-carbonyl]amino]butanoic acid | $C_{11}H_{17}F_3N_2O_4$ — PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/107828179 (accessed on 10 March 2022).

47 4-[Methyl-[(1-methyl-6-oxopiperidin-3-yl)carbamoyl]amino]oxolane-3-carboxylic acid | $C_{13}H_{21}N_3O_5$ — PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/66265156 (accessed on 10 March 2022).

48. Tipparaju, S.K.; Joyasawal, S.; Pieroni, M. In Pursuit of Natural Product Leads : Analogues : Discovery of. **2008**, 7344–7347.

49. 3-Phenanthro[9,10-d][1,3]oxazol-2-ylpropanoic acid | $C_{18}H_{13}NO_3$ — PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/23794729 (accessed on 10 March 2022).

50. 2-(6-Fluoro-1,3-benzoxazol-2-yl)-3,6-dimethylbenzoic acid | $C_{16}H_{12}FNO_3$ — PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/103430682 (accessed on 10 March 2022).

51. 2-[[3-(1,3-Benzoxazol-2-yl)propanoylamino]methyl]-3-(3-methylphenyl)propanoic acid | $C_{21}H_{22}N_2O_4$ — PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/119243009 (accessed on 10 March 2022).

52. 2-(6-Chloro-1,3-benzoxazol-2-yl)-4,5-difluorobenzoic acid | $C_{14}H_6ClF_2NO_3$ — PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/81421830 (accessed on 10 March 2022).

53. Stephen, I.; Courtney, M.; Hay, P.A.; Ian, D.; Scopes, C.; Gb, O. PHTHALIMIDE CARBOXYLIC ACID DERVATIVES (US-7138425-B2) 2006.

54. 2′-(Benzo[d]oxazol-2-yl)-[1,1′-biphenyl]-2-carboxylate | $C_{20}H_{12}NO_3^-$ — PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/155906206 (accessed on 10 March 2022).

55. 5-[2-(1,3-Benzoxazol-2-ylsulfanyl)propanoylamino]-2-chlorobenzoic acid | $C_{17}H_{13}ClN_2O_4S$ — PubChem Available online:

https://pubchem.ncbi.nlm.nih.gov/compound/23886486 (accessed on 10 March 2022).

56. 5-[3-(1,3-Benzoxazol-2-yl)anilino]-5-oxopentanoate | $C_{18}H_{15}N_2O_4$- — PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/6968873 (accessed on 10 March 2022).

64 4-Hydroxy-1-(4,4,4-trifluorobutylcarbamoyl)pyrrolidine-2-carboxylic acid | $C_{10}H_{15}F_3N_2O_4$ — PubChem Available online: https://pubchem.ncbi.nlm.nih.gov/compound/113327907 (accessed on 10 March 2022).