Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for

Halogen bonding for the design of inhibitors by targeting to the S1 pocket of serine proteases

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Table S1. X-ray Data collection and model refinement statistics for three structures.

Data collection			
Crystal	uPA:BrBA at pH4.6	uPA:BrBA at pH7.4	uPA:IBA at pH7.4
Wavelength (Å)	0.979	0.979	0.979
Space group	R3	R3	R3
Resolution (Å)	1.17	2.0	1.45
Unit cell	*a*=120.85 Å, *b*=120.85 Å, *c*=42.63 Å	*a*=121.58 Å, *b*=121.58 Å, *c*=42.21 Å	*a*=120.50 Å, *b*=120.50 Å, *c*=42.72 Å
Unique reflections	73602 (3682)[a]	15624 (776)[a]	40221 (3592)[a]
Completeness (%)	97.8 (97.7)[a]	99.5 (100)[a]	97.9 (87.5)[a]
Redundancy	3.6 (3.0)[a]	3.7 (3.8)[a]	3.7 (3.2)[a]
Average I/σ	29.5 (2.6)[a]	32.6 (2.4)[a]	22.7 (1.4)[a]
R_{merge}[b]	0.083 (0.402)[b]	0.061 (0.503)[b]	0.048 (0.883)[b]
Refinement statistics			
Final *R*-value (%)	19.7	21.5	11.8
Final free *R*-value (%)	22.8	25.7	14.3
R.m.s.d[c] of bond lengths (Å)	0.008	0.007	0.01
R.m.s.d[c] of angle lengths (°)	1.41	1.16	1.34
Mean B-factor of uPA (Å^2)	17.2	56.3	30.0
Mean B-factor of inhibitors (Å^2)	25.1	87.1	31.6
Ramachandran analysis (%)	96.7[d], 2.9[e], 0.4[f]	95.1[d], 4.5[e], 0.4[f]	94.7[d], 4.9[e], 0.4[f]

[a]Numbers in parentheses refer to the highest resolution shells.

[b]Rmerge=$\Sigma_h\Sigma_i|I_i(h)-\langle I(h)\rangle|/\Sigma_h\Sigma_i I_i(h)$, where $\langle I(h)\rangle$ is the mean intensity of the reflections.

[c]R.m.s.d., root mean square deviation.

[d]Percentage of residues in most favored regions.

[e]Percentage of residues in additional allowed regions.

[f]Percentage of residues in generously allowed regions.

Table S2. Binding free energy (KCal mol^{-1}) of uPA in complex with ligands.

Structures	ΔE_{ele}	ΔE_{vdw}	ΔE_{gas}	ΔG_{pb}	$\Delta G_{nonpolar}$	ΔG_{sol}	ΔG_{bind}
uPA:BrBA	-18.97	-22.9	-41.86	7.20	-2.04	24.13	-17.74
uPA:IBA	-4.47	-22.08	-26.55	11.11	-2.04	13.55	-13.00
uPA:APM	-37.5	-18.26	-55.77	13.23	-1.96	48.78	-6.99

Table S3. The length and angle of Halogen bonding between BrBA and uPA in the crystal structure of their complex.

BrBA at pH4.6	uPA	Length (Å)	Angle (°)
Br	Asp189 O1	2.9	144.4
Br	Asp189 O2	2.9	155.4
BrBA at pH7.4	**uPA**	**Length (Å)**	**Angle (°)**
Br	Asp189 O1	3.3	144.3
Br	Asp189 O2	3.0	150.5

Table S4. The hydrogen bonds between BrBA/IBA and uPA in the crystal structure of their complex.

BrBA at pH7.4	uPA	Length (Å)
N1	Ser195 O1	3.5
IBA at pH7.4	**uPA**	**Length (Å)**
N1	Asp189 O1	3.2
N1	Asp189 O2	2.9
N1	Ser190 O	2.7

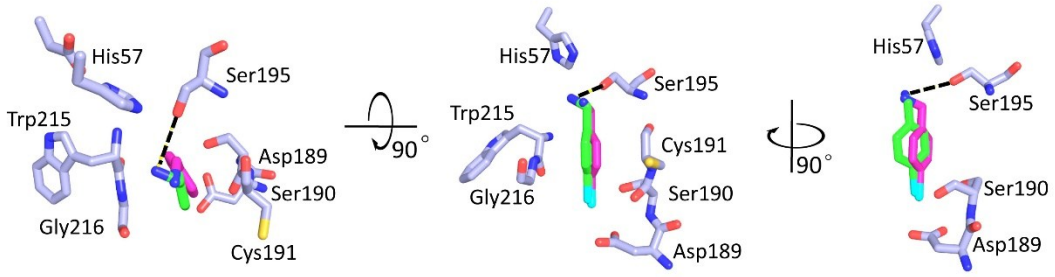

Figure S1. Three different representative views of superposed structures of BrBA:uPA complex at different pH values. BrBA at low pH insets to the S1 pocket deeper (0.4 Å) than it at neutral pH. In addition, BrBA at low pH is slanted (11.7°) and the aromatic ring is nearer to Ser195 of uPA comparing to BrBA at pH7.4, but the amino group of BrBA at low pH did not make any hydrogen bond with uPA, in contrast, the amino group of BrBA at pH7.4 has one hydrogen bond (black dashed lines) with Ser195 of uPA.

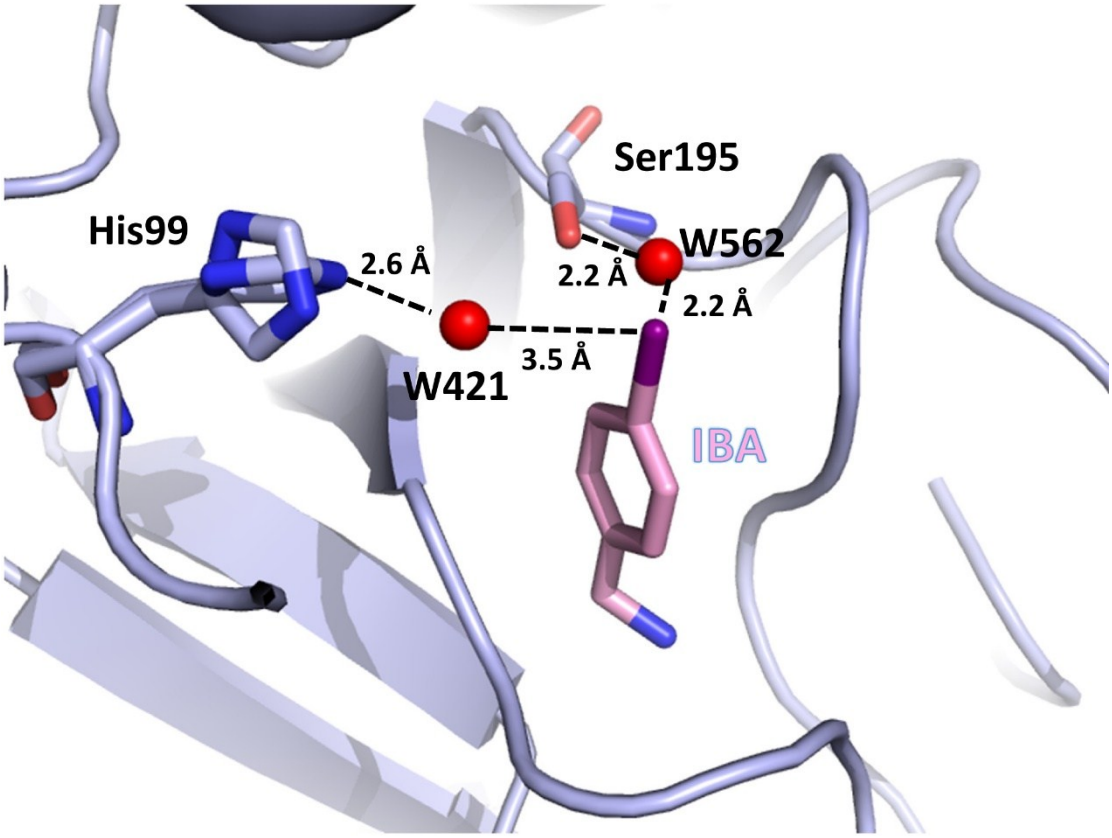

Figure S2. The iodine atom of 4-Iodobenzylamine (IBA) did make two hydrogen bonds with two water molecules (red spheres: W421 and W562), which mediate the interactions between the IBA and residues His99 and Ser195.