

# *Supporting Information*

## **Combining the Fragment Molecular Orbital and GRID Approaches for the Prediction of Ligand–Metalloenzyme Binding Affinity: The Case Study of hCA II Inhibitors**

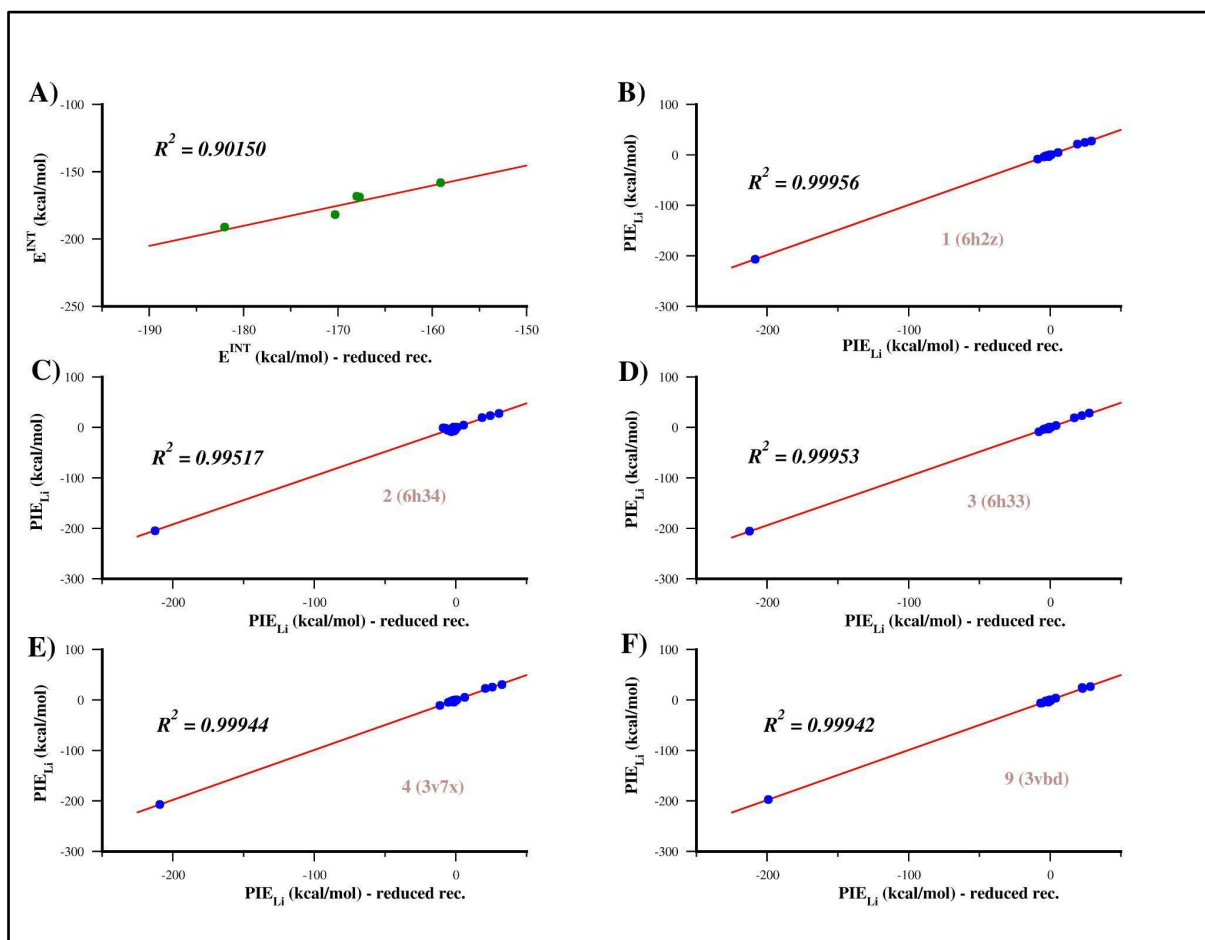
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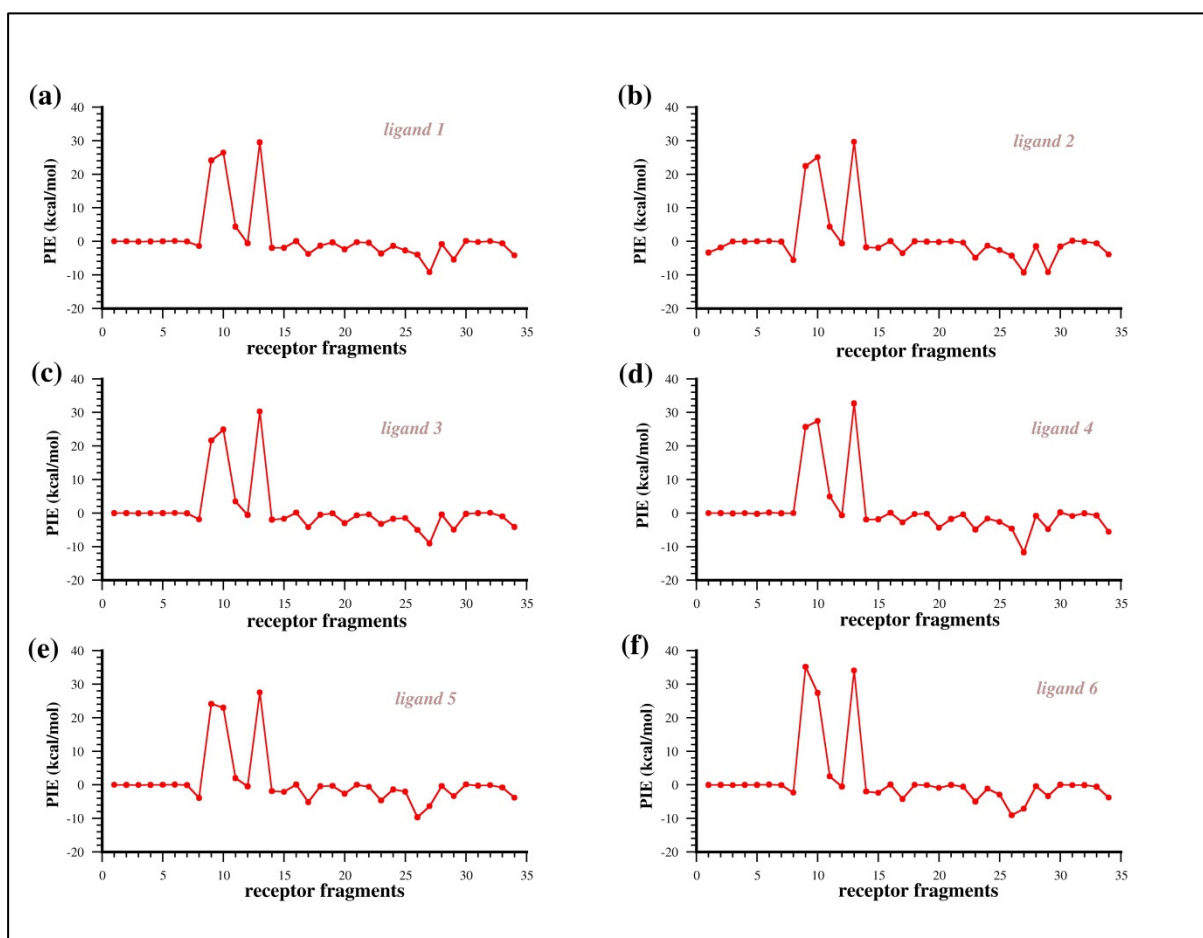
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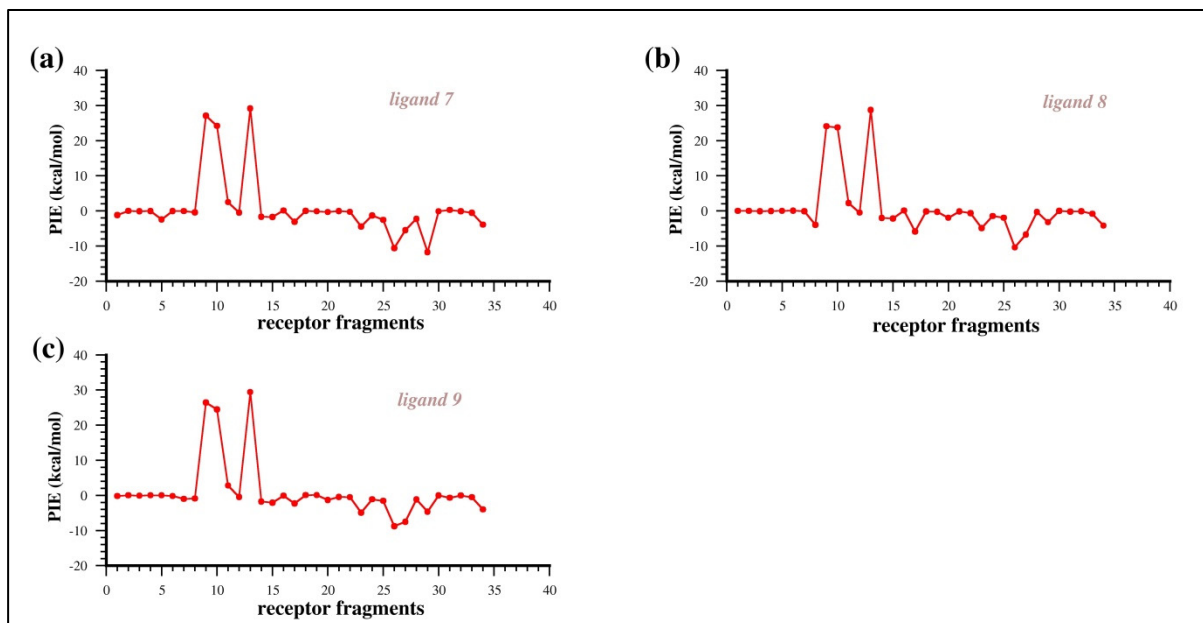
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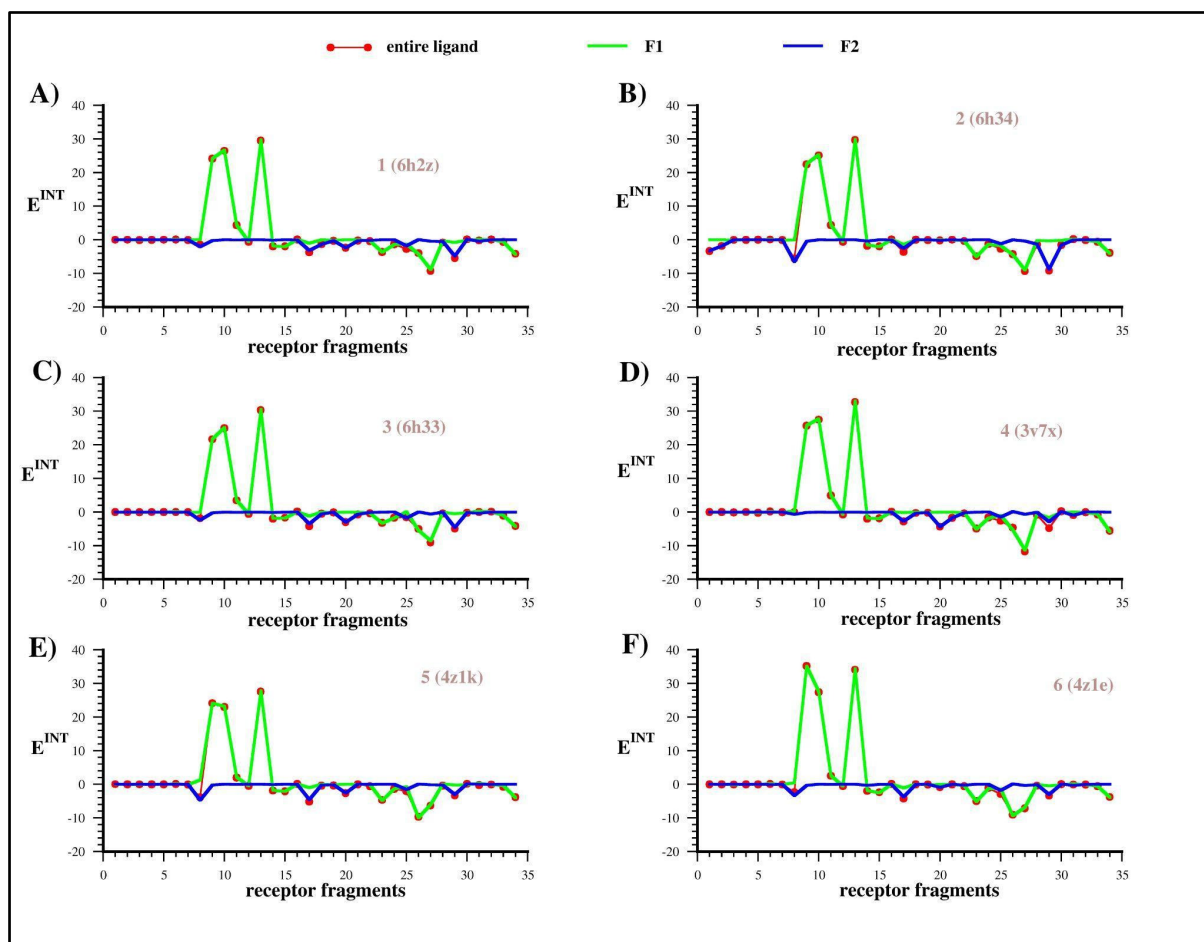
**Figure S1.** A) Scatter plot of  $E^{\text{INT}}$  computed considering the reduced and the entire receptor for ligand 1, 2, 3, 4 and 9. B-F) Correlation between the PIE values computed for the ligand-residues interaction using the reduced receptor (x axis) and the same residues in the entire receptor (y axis) computed for ligand 1 (B), 2, (C), 3 (D), 4 (E) and 9 (F), respectively. The correlation coefficient ( $R^2$ ) and line (in red) are also shown.



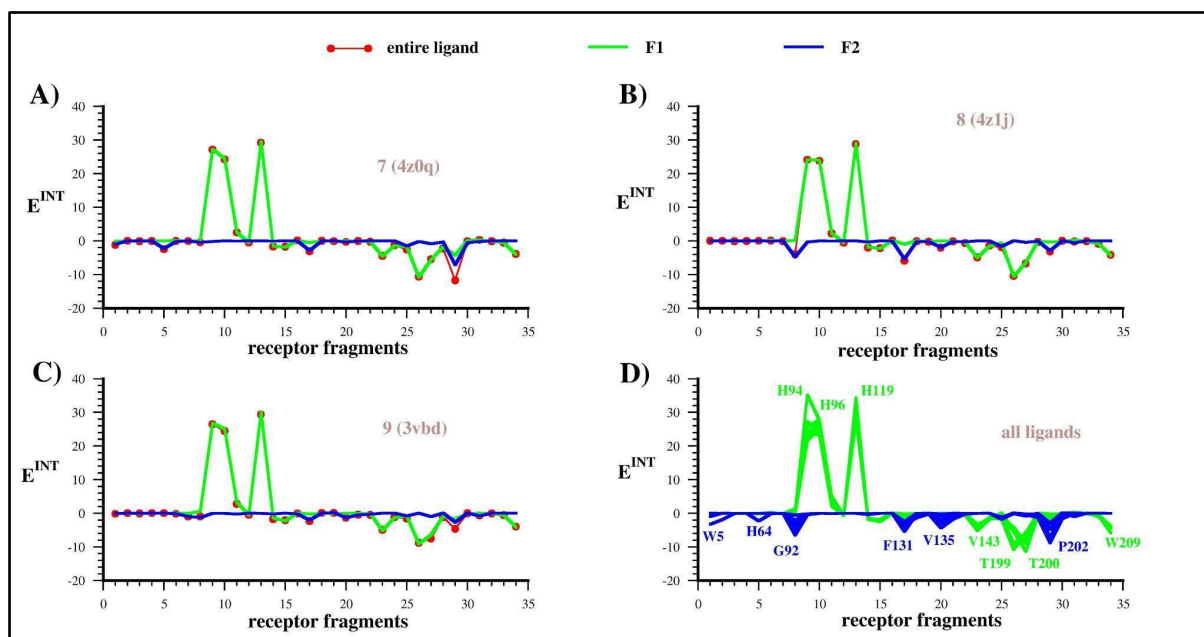
**Figure S2.** PIE graphs of interactions between ligands 1–6 and residues of the reduced hCA II structure (figures a, b, c, d, e and f, respectively).



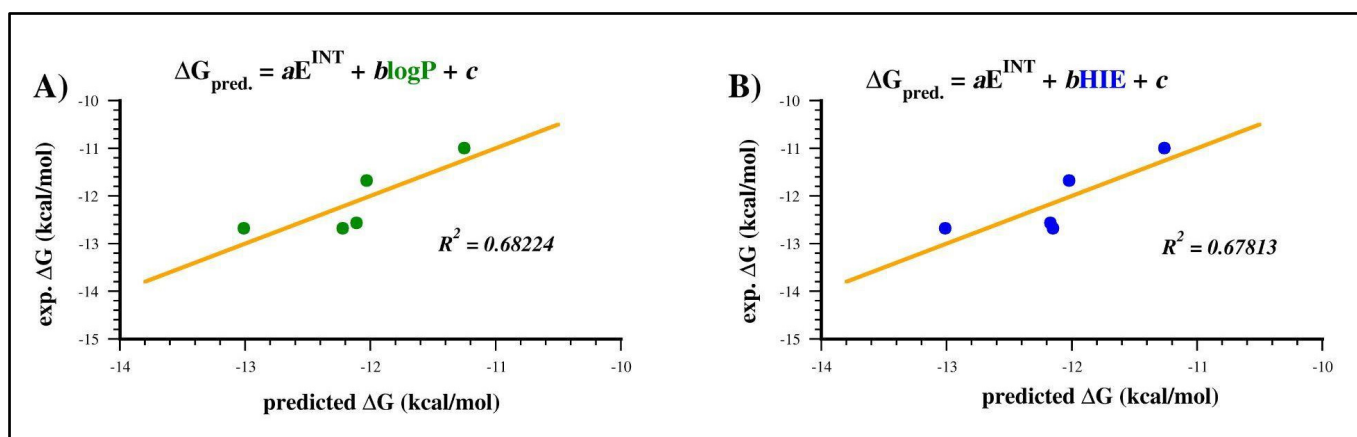
**Figure S3.** PIE graphs of interactions between ligands 7–9 and residues of the reduced hCA II structure (figures a, b, and c, respectively).



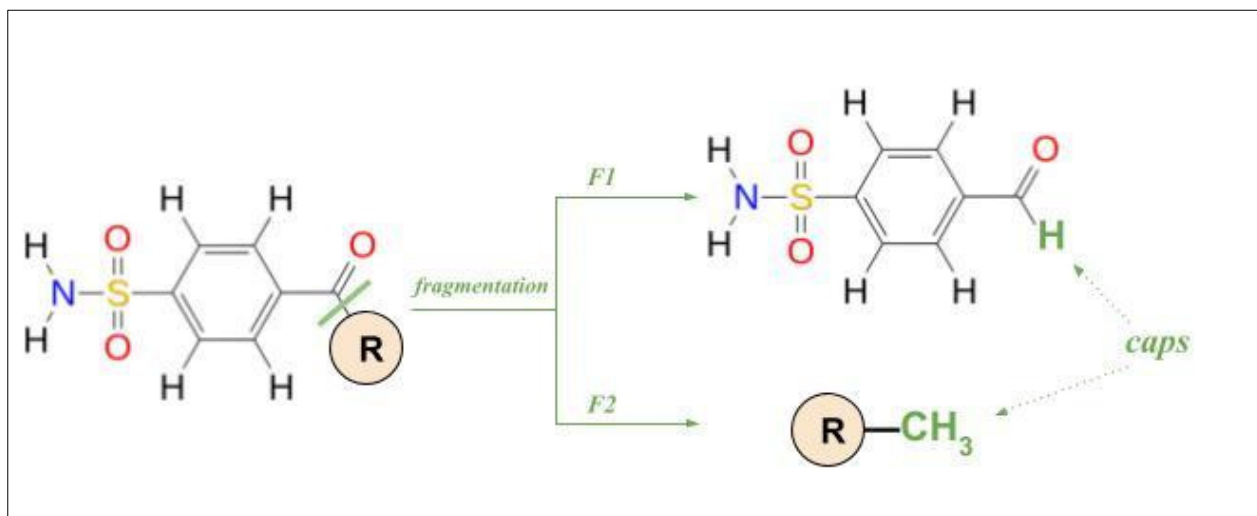
**Figure S4.** PIE graphs of the interactions of F1 (green) and F2 (blue) fragments (ligands 1–6 ) with the residues in the reduced hCA II structure (figures A-F, respectively). The PIE values computed adopting the entire ligand are shown with red dots.



**Figure S5.** PIE graphs of interactions between F1 (green) and F2 (blue) fragments of ligands 7–9 and residues of the reduced hCA II structure (figures A, B, and C, respectively). The PIE values computed adopting the entire ligand are shown with red dots; D) superposition of PIE graphs computed for F1 (green) and F2 (blue) fragments all ligands, 1-9, with the most important receptor residues reported by using the one letter code.



**Figure S6.** Scoring functions obtained using the MLR approach combining  $E^{\text{INT}}$  with A)  $\log P$  ( $a=0.0808$ ,  $b = -0.1066$ ,  $c = 2.0297$ ) and with B) HIE ( $a = 0.1$ ,  $b = 0.0003$ ,  $c = 1.5854$ ) using a reduced data set (complexes 1, 2, 3, 4 and 9).



**Figure S7.** Ligands' fragmentation scheme of adopted for the second run of FMO calculations. The F1 fragment, including the benzene-sulphonamide group, and the hydrophobic tail, F2, are capped by using -H and -CH<sub>3</sub>, respectively.

**Table S1.** List of the FMO fragments composing the reduced LR complex.

Fragment	Description	Fragments	Description
<i>1</i>	Trp5	<i>19</i>	Lys133
<i>2</i>	Phe20	<i>20</i>	Val135
<i>3</i>	Ser29	<i>21</i>	Gln136
<i>4</i>	Asn62	<i>22</i>	Leu141
<i>5</i>	His64	<i>23</i>	Val143
<i>6</i>	Asn67	<i>24</i>	Ser197
<i>7</i>	Ile91	<i>25</i>	Leu198
<i>8</i>	Gln92	<i>26</i>	Thr199
<i>9</i>	His94	<i>27</i>	Thr200
<i>10</i>	His96	<i>28</i>	Pro201
<i>11</i>	Glu106	<i>29</i>	Pro202
<i>12</i>	His107	<i>30</i>	Leu203
<i>13</i>	His119	<i>31</i>	Leu204
<i>14</i>	Val121	<i>32</i>	Cys206
<i>15</i>	His122	<i>33</i>	Val207
<i>16</i>	Asp130	<i>34</i>	Trp209
<i>17</i>	Phe131	<i>35</i>	Zn <sup>2+</sup>
<i>18</i>	Gly132	<i>36</i>	ligand

**Table S2.** E<sup>INT</sup> values computed at RI-MP2/6-31G//PCM[1] level of theory for ligands **1**, **2**, **3**, **4** and **9** using the entire and reduced LR complex.

Ligand	E <sup>INT</sup> ( <i>entire LR complex</i> )	E <sup>INT</sup> ( <i>reduced LR complex</i> )
1	-168.4	-168.0
2	-191.2	-182.0
3	-182.0	-170.3
4	-168.9	-167.7
9	-158.3	-159.1

**Table S3.** EDA of the ligand-Zn<sup>2+</sup> interaction energy computed using the reduced LR model. All energy values are in kcal/mol.

<b>Ligand</b>	<b>E<sup>INT</sup> §</b>	<b>E<sup>es</sup></b>	<b>E<sup>ex</sup></b>	<b>E<sup>ct</sup></b>	<b>E<sup>disp</sup></b>	<b>E<sup>sol</sup></b>
<i>1</i>	-210.9	-416.3	106.9	-34.6	-13.1	146.1
<i>2</i>	-208.9	-411.6	109.6	-35.2	-13.2	141.4
<i>3</i>	-209.2	-419.0	115.8	-36.7	-13.3	144.0
<i>4</i>	-211.2	-432.7	139.2	-41.8	-13.7	137.9
<i>5</i>	-207.2	-419.0	117.2	-36.7	-13.4	144.7
<i>6</i>	-215.4	-461.6	155.2	-42.9	-13.4	147.3
<i>7</i>	-208.2	-419.4	109.0	-33.7	-12.9	148.8
<i>8</i>	-205.9	-410.8	107.4	-34.1	-13.1	144.7
<i>9</i>	-200.7	-352.6	139.6	-40.6	-13.2	66.1

$$§ E^{\text{INT}} = E^{\text{es}} + E^{\text{ex}} + E^{\text{ct}} + E^{\text{disp}} + E^{\text{sol}}$$

**Table S4.** EDA of the ligand-residues interaction energy computed using the reduced LR complex. All energy values are in kcal/mol.

<b>Ligand</b>	<b>E<sup>INT</sup> §</b>	<b>E<sup>es</sup></b>	<b>E<sup>ex</sup></b>	<b>E<sup>ct</sup></b>	<b>E<sup>disp</sup></b>	<b>E<sup>sol</sup></b>
<i>1</i>	37.7	121.8	25.1	-15.5	-43.9	-49.7
<i>2</i>	22.7	100.6	31.6	-19.1	-51.2	-39.1
<i>3</i>	33.7	119.2	24.3	-15.6	-45.1	-49.0
<i>4</i>	37.6	118.8	29.0	-15.3	-47.7	-47.3
<i>5</i>	26.1	103.8	25.3	-16.2	-44.7	-42.1
<i>6</i>	52.2	121.6	26.8	-9.9	-42.1	-44.3
<i>7</i>	28.1	120.0	24.5	-16.3	-43.7	-56.5
<i>8</i>	26.5	104.0	25.9	-15.6	-45.0	-42.8
<i>9</i>	36.9	104.0	25.2	-15.6	-41.9	-34.7

$$§ E^{\text{INT}} = E^{\text{es}} + E^{\text{ex}} + E^{\text{ct}} + E^{\text{disp}} + E^{\text{sol}}$$

**Table S5.** Experimental binding free energy values ( $\Delta G_{\text{exp}}$ ) and all the basic properties values used to build the SF. All energy terms are in kcal/mol.

Ligand	$\Delta G_{\text{exp}}$	$\Delta E^{\text{FMO}}$	F2LE	$E^{\text{INT}}$	FE	HIE	HIE-E	logP
<i>1</i>	-12.68	-37.6	-1.6	-173.2	-7.2	-38.9	-1.6	0.92
<i>2</i>	-12.68	-53.7	-2.1	-186.2	-7.2	-37.9	-1.5	-0.01
<i>3</i>	-12.57	-37.4	-1.5	-175.5	-7.0	-28.1	-1.1	-0.36
<i>4</i>	-11.68	-42.7	-1.7	-173.6	-6.9	-30.6	-1.2	0.41
<i>5</i>	-11.36	-61.1	-2.5	-181.1	-7.5	-35.0	-1.5	-0.28
<i>6</i>	-11.24	-36.7	-1.5	-163.2	-6.8	-24.3	-1.0	0.68
<i>7</i>	-11.14	-67.6	-3.1	-180.1	-8.2	-32.0	-1.5	0.6
<i>8</i>	-11.06	-70.5	-3.2	-179.3	-8.2	-30.2	-1.4	0.32
<i>9</i>	-11.00	-38.6	-1.8	-163.8	-7.4	-34.3	-1.6	0.46

**Table S6.** Binding free energy values derived from experimental  $K_i$ , the number of ligand heavy atoms for each hCA II inhibitors investigated in this work, and the PDB IDs of the corresponding LR complexes.

Ligand	$\Delta G_{\text{exp}}$ (kcal/mol)	# heavy atoms	PDB ID
<i>1</i>	-12.682	24	6h2z
<i>2</i>	-12.682	26	6h34
<i>3</i>	-12.574	25	6h33
<i>4</i>	-11.684	25	3v7x
<i>5</i>	-11.355	24	4z1k
<i>6</i>	-11.241	24	4z1e
<i>7</i>	-11.137	22	4z0q
<i>8</i>	-11.063	22	4z1j
<i>9</i>	-10.997	22	3vbd