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The different modes of docking of a series of benzenesulfonamides and tetrafluorobenzenesulfonamides to the carbonic anhydrase isoform II

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ABSTRACT

It is generally accepted that similar molecules exert their inhibitory activity (or biological activity) at a certain site by binding to it in a similar form. Previous results comparing the docking results with those obtained from formal methods relating electronic structure with activity show that there is some equivalence between them. Here we present the results of the docking of twenty two benzenesulfonamides and tetrafluoro-benzenesulfonamides derivatives to the human carbonic anhydrase isoform II. It is shown for the first time that similar molecules do not necessarily bind in the same form to produce an inhibitory action. The docking results are analyzed from the point of view of the ligand-residue distance, kind of residues involved and kinds of weak and short-range interactions. The results suggest that a formal study of a group of molecules should begin by docking them to their action site. If no structure of the site is available then the results of formal methods using linear multiple regression analysis should be carefully examined to detect and discard any molecule presenting a different mode of binding to the site.

Keywords: Docking, human carbonic anhydrase, isoform II, benzenesulfonamides, tetrafluorobenzenesulfonamides.

INTRODUCTION

The carbonic anhydrases (CAs) are a family of metalloenzymes that catalyze the fast interconversion of CO_2 and H_2O to bicarbonate and protons, a reversible reaction that occurs somewhat slowly in the absence of a catalyst. All human CAs belong to the α-class of this family. Up today, fifteen isoforms have been described. Only twelve of them are catalytically active (I–IV, VA, VB, VI, VII, IX, and XII–XIV). They are widely distributed in many tissues and organs, where they are implicated in a variety of crucial physiological processes [1, 2]. During our research on the relationships between the electronic structure and biological activity we have employed the concept of common skeleton (CS). The CS is defined as a set of atoms common to all molecules analyzed. It is accepted that the variation of the local atomic reactivity indices of the CS accounts for almost all the variation of the activity in a group of molecules. The linear multiple regression analysis is based on the hypothesis that the common skeleton of all molecules is aligned in a similar or very similar mode at the site where they exert their biological activity (see for example [3-27] and references therein). Our recent comparative QSAR and docking studies of the inhibition of microsomal prostaglandin E2 synthase-1 by 2-aryl substituted quinazolin-4(3*H*)-one, pyrido[4,3-*d*] pyrimidin-4(3*H*)-one and pyrido[2,3-*d*]pyrimidin-4(3*H*)-one derivatives [3], the inhibition of tumor necrosis factor by cyclopentenone oximes [7] and the receptor binding affinity of 5-HT_{2A} and 5-HT_{2B} receptors by a family of N-benzylphenethylamines [4, 6] has shown that all the aforementioned hypothesis about the constitution and orientation of the common skeleton are valid. Recently we have focused our attention on the experimental inhibition constants against the human CA isoform II (hCAII, that is involved in glaucoma) of a group of benzenesulfonamides and tetra- fluorobenzenesulfonamides recently published [28]. Here we present the results of a docking study of a group of the above mentioned molecules that show, for the first time, that the inhibitory process could be achieved with the molecules docked in very diverse forms.

METHODS AND CALCULATIONS

The selected molecules are shown in Figure 1 and Table 1.

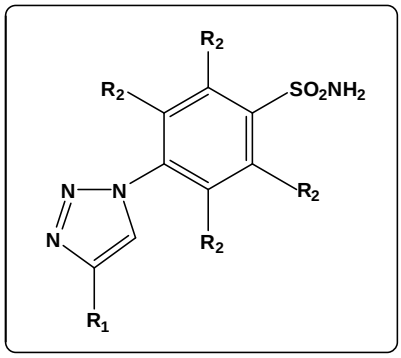

Figure 1. General formula of benzenesulfonamides and tetrafluorobenzenesulfonamides derivatives

Table 2. Benzenesulfonamides and tetrafluorobenzenesulfonamides derivatives [28]

Mol.	R_1	R_2
1	*p*-Me−C_6H_4	H
2	C_6H_5	H
3	*c*C_6H_{11}	H
4	*p*-C_5H_{11}−C_6H_4	H
5	$BrCH_2CH_2$	H
6	$Cl(CH_2)_3$	H
7	$Cl(CH_2)_4$	H
8	MeOOC	H
9	HO−CH_2	H
10	BocNH−CH2	H
11	H_2N−CH_2	H
12	*p*-Me−C_6H_4	F
13	C_6H_5	F
14	*c*C_6H_{11}	F
15	*p*-C_5H_{11}−C_6H_4	F
16	$BrCH_2CH_2$	F
17	$Cl(CH_2)_3$	F
18	$Cl(CH_2)_4$	F
19	MeOOC	F
20	HO−CH_2	F
21	BocNH−CH2	F
22	H_2N−CH_2	F

The common skeleton, supposed to be aligned in a very similar way during the inhibition of hCAII, is shown in Fig. 2.

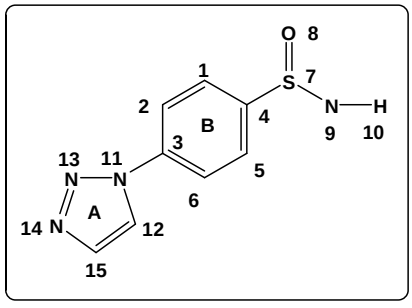

Figure 2. Common skeleton of benzenesulfonamides and tetrafluoro-benzenesulfonamides derivatives

All the molecules of Table 2 were prepared for docking by fully optimizing their geometry within the Density Functional Theory at the B3LYP/6-31g(d,p) level. The Gaussian suite of programs was employed [29]. A model for the carbonic anhydrase II was downloaded from the Protein data Bank (ID: 1BCD) and prepared for use with Autodock Vina [30]. A first docking with the "rigid residues" option was carried out for molecule 1 of Table 2. Then, all the residues inside a 4 Å region around the ligand were considered to be flexible (they can change their

conformation during the docking procedure). A 35x35x35 box was employed. The lowest energy conformer of each molecule was selected for further analysis with Autodock Vina and Discovery Studio Visualizer [31].

RESULTS

Figure 3 shows our results for the docking of molecules 5c (14) and 5h (19) of Ref. [28].

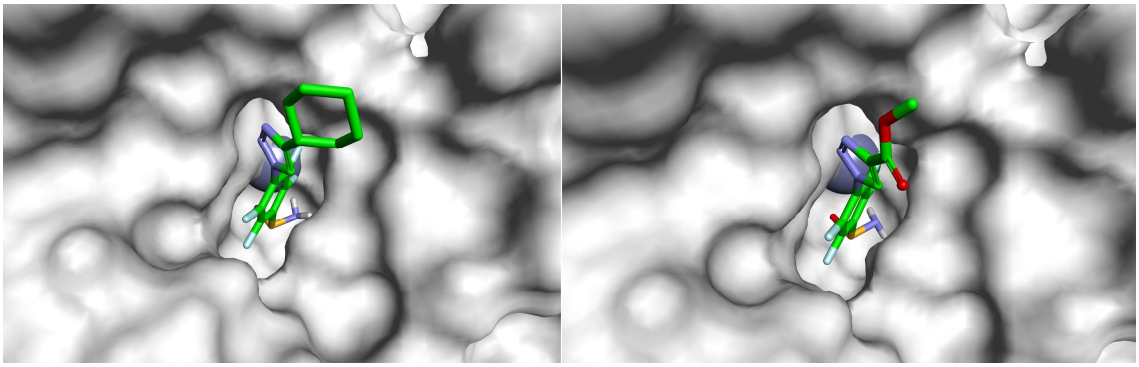

Figure 3. Molecules 14 (left) and 19 (right) docked to hCAII

Figures 4 to 9 show molecules 1-22 docked to hCAII. Table 3 shows the colors associated to each kind of interaction.

Table 3. List of colors for docking figures analysis.

Interaction	Color name	RGB
Pi-alkyl (hydrophobic)	Cotton candy	(255,200,255)
Alkyl (hydrophobic)	Cotton candy	(255,200,255)
Pi-sigma (hydrophobic)	Heliotrope	(200,100,255)
Carbon-hydrogen bond	Honeydew	(220,255,220)
Conventional H-bond	Lime	(0,255,0)
Salt bridge (attractive charge)	Orange peel	(255,150,0)
Pi-anion	Orange peel	(255,150,0)
Pi-Pi stacked	Neon pink	(255,100,200)
Pi-Pi T shaped	Neon pink	(255,100,200)
Halogen	Aqua	(0,255,255)
Attractive charge	Orange peel	(255,150,0)
Carbon-hydrogen bond, halogen	Honeydew	(220,255,220)
Pi-sulphur	Tangerine yellow	(255,200,0)
Unfavorable donor-donor	Red	(255,10,0)
Unfavorable positive-positive	Red	(255,10,0)
Pi-cation	Orange peel	(255,150,0)
Unfavorable acceptor-acceptor	Red	(255,10,0)
π-donor H-bond	Honeydew	(220,255,220),
Metal-acceptor	Very Light Grey	(200,200,200)

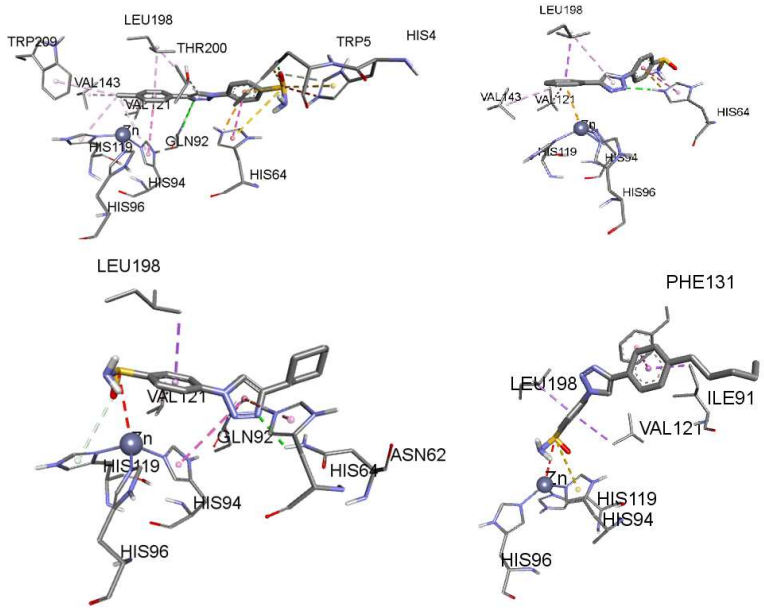

Figure 4. Molecules 1 (upper left), 2 (upper right), 3 (lower left) and 4 (lower right) docked to hCAII

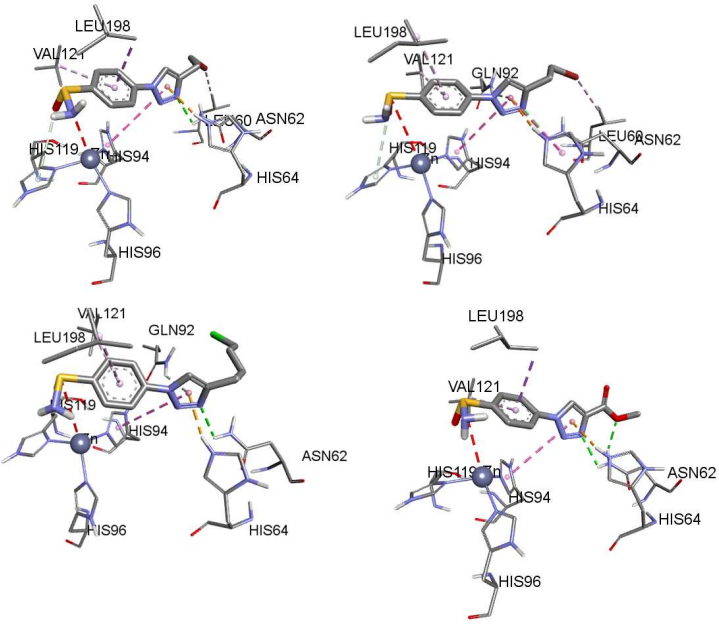

Figure 5. Molecules 5 (upper left), 6 (upper right), 7 (lower left) and 8 (lower right) docked to hCAII

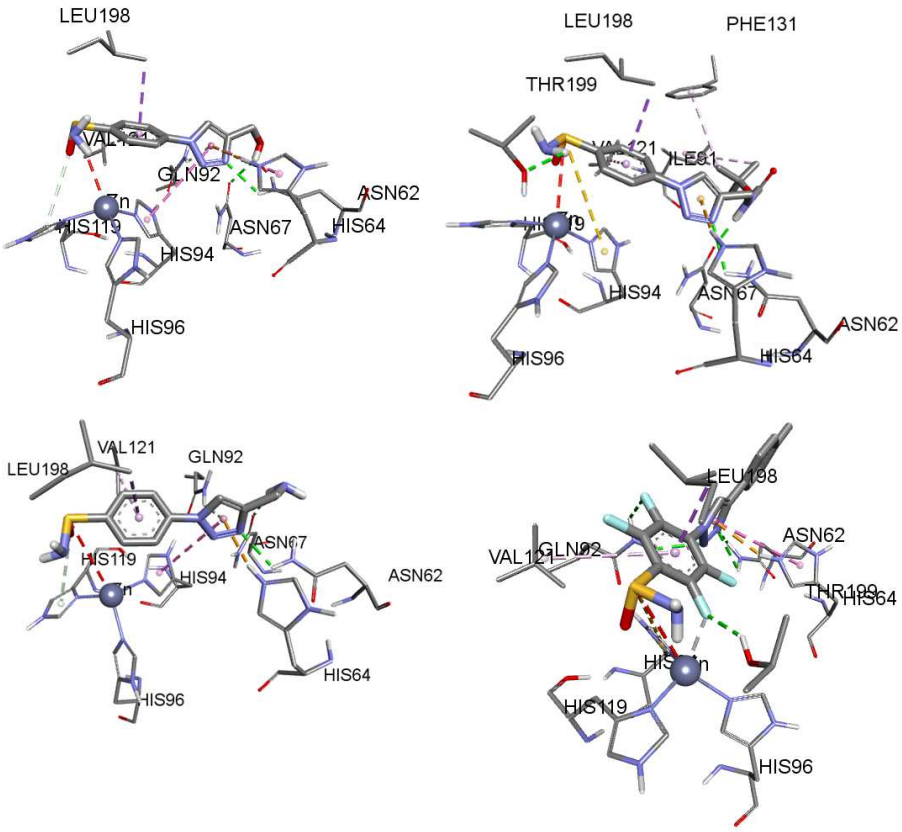

Figure 6. Molecules 9 (upper left), 10 (upper right), 11 (lower left) and 12 (lower right) docked to hCAII

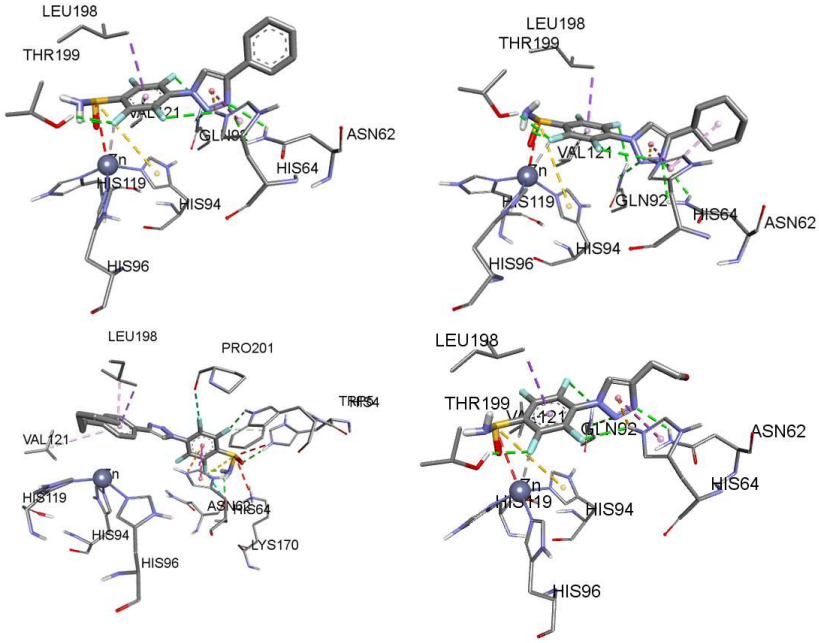

Figure 7. Molecules 13 (upper left), 14 (upper right), 15 (lower left) and 16 (lower right) docked to hCAII

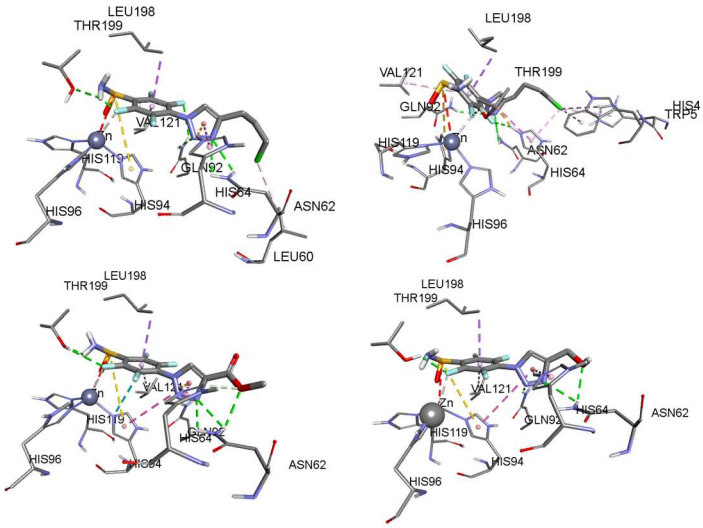

Figure 8. Molecules 17 (upper left), 18 (upper right), 19 (lower left) and 20 (lower right) docked to hCAII

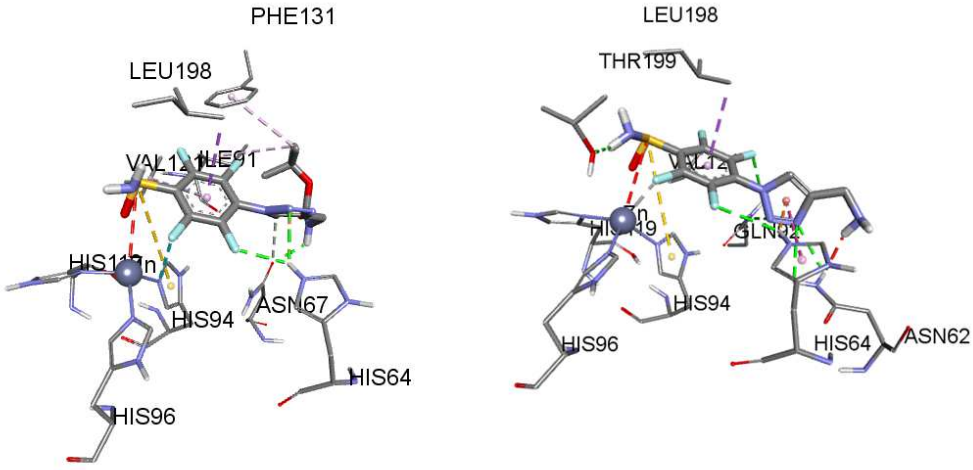

Figure 9. Molecules 21 (upper left) and 22 (upper right) docked to hCAII

Table 4 shows a summary of the ligand-site interactions for each molecule

Table 4. Summary of ligand-site interactions [31]

Mol.	Interactions
1	π-π T-shaped interaction of ring B with His-64 (4.49Å), π-cation interaction of ring B with His-64 (4.03Å), π-sulfur interaction of S7 with His-64 (5.72Å), Trp-5 (5.84Å and 5.49Å) and His-4 (5.08Å), unfavorable positive-positive interaction of S7 with His-4 (4.41Å), carbon H-bond interaction between O8 and His-4 (3.55Å), conventional H-bond between O8 and Trp-5 (2.28), π-alkyl of ring A with Leu-198 (5.45Å), carbon H-bond interaction between C12 and Thr-200 (3.20Å), conventional H-bond between N14 and Gln-92 (2.90Å and 2.90Å), π-alkyl interaction of phenyl substituent with Leu-198 (4.00Å) and Val-121 (4.77Å), π-π T-shaped interaction of phenyl substituent with His-94 (4.84Å), π-alkyl interaction of 4-Me in phenyl substituent with His-94 (5.42Å), His-119 (4.59Å) and Trp-209 (4.73Å), alkyl interaction of 4-Me in phenyl substituent with Val-143 (3.76Å) and Val-121 (4.82Å).
2	π-π T-shaped interaction of ring B with His-64 (4.64Å), π-cation interaction of ring B with His-64 (3.70Å), π-alkyl interaction of ring A with Leu-198 (5.17Å), conventional H-bond between N13 and His-64 (2.39Å), π-alkyl interaction of phenyl substituent with Val-143 (5.04Å) and Val-121 (5.04Å), π-σ interaction of phenyl substituent with Leu-198 (3.59Å), π-cation interaction of phenyl substituent with Zn (3.98Å).
3	π-alkyl interaction of ring B with Val-121 (5.02Å), π-σ interaction of ring B with Leu-198 (3.72Å), unfavorable positive-positive interaction of S7 with Zn (4.40Å), π-donor H-bond between O8 and His-119 (4.11Å), π-π T-shaped interaction of ring A with His-94 (5.18Å) and His-64 (4.74Å), π-cation interaction of ring A with His-64 (2.62Å), π-donor H-bond between ring A and Gln-92 (2.52Å), conventional H-bond between N14 and Asn-62 (2.51Å).
4	π-σ interaction of ring B with Leu-198 (3.61Å) and Val-121 (3.84Å), π-sulfur interaction of S7 with His-94 (5.72Å), unfavorable positive-positive interaction of S7 and Zn (3.92Å), unfavorable metal-donor interaction of H10 and Zn (1.93Å) , π-π T- shaped interaction of phenyl substituent with Phe-131 (5.08Å), π-σ interaction of phenyl substituent with Ile-91 (3.79Å).
5	π-alkyl interaction of ring B with Val-121 (5.05Å), π-σ interaction of ring B with Leu-198 (3.78Å),

	unfavorable positive-positive interaction of S7 with Zn (4.35Å), π-donor H-bond between O8 and His-119 (4.14Å), π-π T-shaped interaction of ring A with His-94 (5.42Å), π-cation interaction of ring A with His-64 (2.57Å), conventional H-bond between N14 and Asn-62 (2.49Å), alkyl interaction between Br and Leu-60 (5.39Å).
6	π-alkyl interaction of ring B with Val-121 (5.02Å) and Leu-198 (4.34Å), unfavorable positive-positive interaction of S7 with Zn (4.38Å), π-donor H-bond between O8 and His-119 (4.08Å), π-π T-shaped interaction of ring A with His-94 (5.21Å) and His-64 (4.78Å), π-cation interaction of ring A with His-64 (2.66Å), π-donor H-bond between ring A and Gln-92 (2.47Å), conventional H-bond between N14 and Asn-62 (2.52Å), alkyl interaction between Br and Leu-60 (5.34Å).
7	π-alkyl interaction of ring B with Val-121 (5.03Å) and Leu-198 (4.33Å), unfavorable positive-positive interaction of S7 with Zn (4.30Å), π-π T-shaped interaction of ring A with His-94 (5.47Å), π-cation interaction of ring A with His-64 (2.61Å), π-donor H-bond between ring A and Gln-92 (2.68Å), conventional H-bond between N14 and Asn-62 (2.53Å).
8	π-alkyl interaction of ring B with Val-121 (5.06Å), π-σ interaction of ring B with Leu-198 (4.00Å), unfavorable positive-positive interaction of S7 with Zn (4.32Å), π-π T- shaped interaction of ring A with His-94 (5.43Å), π-cation interaction of ring A with His-64 (2.54Å), conventional H-bond between N14 and Asn-62 (2.49Å), conventional H-bond between O from the ester substituent and Asn-62 (2.35Å).
9	π-alkyl interaction of ring B with Val-121 (4.97Å), π-σ interaction of ring B with Leu-198 (3.73Å), unfavorable positive-positive interaction of S7 with Zn (4.41Å), π-donor H-bond between O8 and His-119 (4.11Å), π-π T-shaped interaction of ring A with His-94 (5.16Å) and His-64 (4.78Å), π-cation interaction of ring A with His-64 (2.66Å), π-donor H-bond between ring A and Gln-92 (2.46Å), conventional H-bond between N14 and Asn-62 (2.49Å), conventional H-bond between H from 3-MeOH in ring A and Asn-62 (2.38Å), unfavorable donor-donor interaction of H from 4-MeOH in ring A with Asn-62 (1.44Å).
10	π-alkyl interaction of ring B with Val-121 (5.16Å), π-σ interaction of ring B with Leu-198 (4.00Å), π-sulfur interaction of S7 with His-94 (5.94Å), unfavorable positive-positive interaction of S7 and Zn (4.21Å), conventional H-bond between H10 and Thr-199 (2.64Å), π-cation interaction of ring A with His-64 (2.62Å), conventional H-bond between N14 and Asn-62 (2.55Å), conventional H-bond between H from amide nitrogen of the $BocNHCH_2$ moiety and Asn-67 (1.94Å), alkyl interaction of two terminal C in Boc group and Ile-91 (4.33Å and 4.91Å), π-alkyl interaction of a terminal C in Boc group and Phe-131 (4.68Å).
11	π-alkyl interaction of ring B with Val-121 (4.96Å), π-σ interaction of ring B with Leu-198 (3.76Å), unfavorable positive-positive interaction of S7 with Zn (4.40Å), π-donor H-bond between O8 and His-119 (4.10Å), π-π T-shaped interaction of ring A with His-94 (5.16Å), π-cation interaction of ring A with His-64 (2.67Å), π-donor H-bond between ring A and Gln-92 (2.45Å), conventional H-bond between N14 and Asn-62 (2.49Å), conventional H-bond between H from amine moiety of 4-$MeNH_2$ in ring A and Asn-67 (2.21Å).
12	π-alkyl interaction of ring B with Val-121 (5.10Å), π-σ interaction of ring B with Leu-198 (3.77Å), π-sulfur interaction of S7 with His-94 (5.81Å), unfavorable positive-positive interaction of S7 with Zn (4.26Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.46Å), conventional H-bond between 2-F in ring B with Thr-199 (2.50Å), conventional H-bond between 5-F in ring B with Gln-92 (2.95Å), conventional H-bond between N13 and Gln-92 (2.31Å), π-cation interaction of ring A with His-64 (2.64Å), π-π T-shaped interaction of ring A with His-64 (4.67Å), conventional H-bond between N14 and Asn-62 (2.79Å and 2.75Å).
13	π-alkyl interaction of ring B with Val-121 (5.15Å), π-σ interaction of ring B with Leu-198 (3.79Å), π-sulfur interaction of S7 with His-94 (5.78Å), unfavorable positive-positive interaction of S7 with Zn (4.26Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.51Å), conventional H-bond between 2-F in ring B with Thr-199 (2.52Å), conventional H-bond between 5-F in ring B with Gln-92 (2.89Å), conventional H-bond between 3-F and His-64 (2.94Å), conventional H-bond between N13 and Gln-92 (2.44Å), π-cation interaction of ring A with His-64 (2.59Å), π-π T-shaped interaction of ring A with His-64 (4.60Å), conventional H-bond between N14 and Asn-62 (2.72Å and 2.69Å). *Intramolecular H-bond between H10 and 2-F* (2.08Å).
14	π-alkyl interaction of ring B with Val-121 (5.19Å), π-σ interaction of ring B with Leu-198 (3.79Å), π-sulfur interaction of S7 with His-94 (5.67Å), unfavorable positive-positive interaction of S7 with Zn (4.19Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.46Å), conventional H-bond between 2-F in ring B with Thr-199 (2.58Å), conventional H-bond between 5-F in ring B with Gln-92 (2.79Å), conventional H-bond between 3-F and His-64 (2.88Å), conventional H-bond between N13 and Gln-92 (2.59Å), π-cation interaction of ring A with His-64 (2.61Å), π-π T-shaped interaction of ring A with His-64 (4.60Å), conventional H-bond between N14 and Asn-62 (2.79Å and 2.69Å), π-alkyl interaction of cyclohexyl substituent with His-64 (5.05Å) . *Intramolecular H-bond between H10 and 2-F* (1.90Å).
15	π-π T-shaped interaction of ring B with His-64 (4.68Å), π-σ interaction of ring B with His-64 (3.56Å), π-cation interaction of ring B with His-64 (4.61Å), π-sulfur interaction of S7 and His-64 (5.94Å), unfavorable positive-positive interaction of S7 with Lys-170 (4.06Å) and His-4 (3.90Å), conventional H-bond between O8 and His-4 (2.34Å), conventional H-bond between H10 and Asn-62 (2.08Å), carbon H-bond between 2-F and His-4 (3.39Å), conventional H-bond between 2-F and Trp-5 (2.56Å), halogen interaction of 3-F with Pro-201 (3.43Å), halogen interaction of 6-F with Asn-62 (3.58Å), π-alkyl interaction of phenyl substituent with Val-121 (5.24Å), π-σ interaction of phenyl substituent with Leu-198 (3.56Å), alkyl interaction of terminal C from 4-penthylphenyl substituent with Leu-198 (4.02Å). *Intramolecular H-bond between an H from amine in position 9 and 6-F* (1.66Å).
16	π-alkyl interaction of ring B with Val-121 (5.37Å), π-σ interaction of ring B with Leu-198 (3.92Å), π-sulfur interaction of S7 with His-94 (5.48Å), unfavorable positive-positive interaction of S7 with Zn (3.98Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.49Å), conventional H-bond between 2-F in ring B with Thr-199 (2.67Å), conventional H-bond between 5-F in ring B with Gln-92 (2.97Å), conventional H-bond between 3-F and His-64 (2.62Å), conventional H-bond between N13 and Gln-92 (2.59Å), π-cation interaction of ring A with His-64 (2.58Å), π-π T-shaped interaction of ring A with His-64 (4.53Å), conventional H-bond between N14 and Asn-62 (2.52Å).
17	π-alkyl interaction of ring B with Val-121 (5.19Å), π-σ interaction of ring B with Leu-198 (3.82Å), π-sulfur

	interaction of S7 with His-94 (5.73Å), unfavorable positive-positive interaction of S7 with Zn (4.22Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.40Å), conventional H-bond between H10 with Thr-199 (2.60Å), conventional H-bond between 5-F in ring B with Gln-92 (2.81Å), conventional H-bond between N13 and Gln-92 (2.56Å), π-cation interaction of ring A with His-64 (2.54Å), π-π T-shaped interaction of ring A with His-64 (4.55Å), conventional H-bond between N14 and Asn-62 (2.75Å and 2.69Å), alkyl interaction of Cl with Leu-60 (5.01Å). *Intramolecular H-bond between 2-F and H10* (1.62Å).
18	π-alkyl interaction of ring B with Val-121 (5.21Å), π-σ interaction of ring B with Leu-198 (3.92Å), π-sulfur interaction of S7 with His-94 (5.69Å), unfavorable positive-positive interaction of S7 with Zn (4.21Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.40Å), conventional H-bond between 2-F in ring B with Thr-199 (2.58Å), conventional H-bond between 3-F and His-64 (2.95Å), conventional H-bond between N13 and Gln-92 (2.49Å), π-cation interaction of ring A with His-64 (2.55Å), π-π T-shaped interaction of ring A with His-64 (4.55Å), conventional H-bond between N14 and Asn-62 (2.82Å and 2.71Å), π-alkyl interaction of Cl with His-64 (4.55Å), Trp-5 (4.77Å and 4.77Å) and His-4 (4.64Å).
19	π-alkyl interaction of ring B with Val-121 (5.11Å), π-σ interaction of ring B with Leu-198 (3.80Å), π-sulfur interaction of S7 with His-94 (5.98Å), unfavorable positive-positive interaction of S7 with Zn (4.35Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.40Å), conventional H-bond between 2-F in ring B with Thr-199 (2.84Å), conventional H-bond between H10 and Thr-199 (2.62Å), halogen interaction of 3-F with His-94 (3.69Å), conventional H-bond between N13 and Gln-92 (2.14Å), π-cation interaction of ring A with His-64 (2.60Å), π-π T-shaped interaction of ring A with His-64 (4.68Å) and His-94 (5.63Å), conventional H-bond between N14 and Asn-62 (2.75Å and 2.67Å), conventional H-bond between O from ester substituent and Asn-62 (2.62Å), carbon H-bond between O from ester substituent and His-64 (3.54Å).
20	π-alkyl interaction of ring B with Val-121 (5.11Å), π-σ interaction of ring B with Leu-198 (3.80Å), π-sulfur interaction of S7 with His-94 (5.94Å), unfavorable positive-positive interaction of S7 with Zn (4.33Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.40Å), conventional H-bond between 2-F in ring B with Thr-199 (2.39Å), conventional H-bond between N13 and Gln-92 (2.14Å), π-cation interaction of ring A with His-64 (2.61Å), π-π T-shaped interaction of ring A with His-64 (4.38Å) and His-94 (5.71Å), conventional H-bond between N14 and Asn-62 (2.75Å and 2.72Å), conventional H-bond between O from 4-MeOH substituent and Asn-62 (2.53Å).
21	π-alkyl interaction of ring B with Val-121 (5.37Å), π-σ interaction of ring B with Leu-198 (3.97Å), π-sulfur interaction of S7 with His-94 (5.46Å), unfavorable positive-positive interaction of S7 and Zn (3.99Å), halogen interaction of 2-F with His-94 (3.27Å), conventional H-bond between 3-F and His-64 (2.47Å), π-cation interaction of ring A with His-64 (2.85Å), conventional H-bond between N13 and His-64 (2.50Å), carbon H-bond between C12 and Asn-67 (3.56Å), conventional H-bond between H from amide nitrogen in $BocNHCH_2$ moiety and Asn-67 (2.10Å), alkyl interaction of a terminal C in Boc group and Ile-91 (5.04Å), π-alkyl interaction of two terminal C in Boc group and Phe-131 (4.34Å and 4.75Å).
22	π-alkyl interaction of ring B with Val-121 (5.22Å), π-σ interaction of ring B with Leu-198 (3.93Å), unfavorable positive-positive interaction of S7 with Zn (4.24Å), π-sulfur interaction of S7 with His-94 (5.72Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.42Å), conventional H-bond between H10 and Thr-199 (2.71Å), conventional H-bond between 3-F and His-64 (2.96Å), conventional H-bond between 5-F and Gln-92 (2.97Å), conventional H-bond between N13 and Gln-92 (2.50Å), π-cation interaction of ring A with His-64 (2.54Å), π-π T-shaped interaction of ring A with His-64 (4.54Å), conventional H-bond between N14 and Asn-62 (2.75Å and 2.65Å), unfavorable donor-donor interaction of H in 4-$MeNH_2$ substituent and Asn-62 (2.07Å).

DISCUSSION

The comparison of Fig. 3 with the right side of Fig. 2 in Ref. [28] shows that both results are almost identical. The small existing differences probably correspond to the different choice of conformational flexibility of the residues at the binding site. This is a relatively solid basis for the ensuing discussion. Almost all molecules dock to hCAII with the nitrogen atom of the sulfonamide group pointing to the zinc atom. Nevertheless there are exceptions. In molecules 1, 2 and 15 the nitrogen atom of the sulfonamide group does not point to the zinc atom (these three molecules are marked with an asterisk in the Tables). In the case of molecule 15 we were not able to detect any zinc-ligand interaction. Table 5 shows the interactions of the ligands with zinc and/or histidines 94, 96 and 119.

We can see from Table 5 that the zinc atom interacts directly with a partner only in the cases of molecules 2*, 3, 4-14, 16-21. In the case of molecule 2* there is a favorable cation-π interaction of Zn with a phenyl ring. In the remaining cases the interaction is an unfavorable one between the positive net charges of the zinc and sulfur atoms with distances in the interval 3.9-4.4Å. In the case of molecule 4 there is a strong unfavorable metal-donor interaction of H10 and Zn (1.93Å, see Fig. 2). Fluorine substitution produces a relatively strong metal-acceptor and halogen interaction of the 2-F substituent in ring B with the Zn atom in molecules 12 to 20 and 22 in the 2.4-2.5Å distance interval. Concerning the amino acids participating in the ligand-site interaction, Table 6 shows the list of them.

Table 5. Interactions of the ligand with zinc and/or histidines 94, 96 and 119

Mol.	Interactions
1*	π-π T-shaped interaction of the phenyl substituent with His-94 (4.84Å), π-alkyl interactions of the 4-Me moiety in phenyl substituent with His-94 (5.42Å) and His-119 (4.59Å).
2*	π-cation interaction of the phenyl substituent with Zn (3.98Å).
3	Unfavorable positive-positive interaction of S7 with Zn (4.40Å), π-donor H-bond between O8 and His-119 (4.11Å), π-π T-shaped interaction of ring A with His-94 (5.18Å).
4	π-sulfur interaction of S7 with His-94 (5.72Å), unfavorable positive-positive interaction of S7 and Zn (3.92Å), unfavorable metal-donor interaction of H10 and Zn (1.93Å) .
5	Unfavorable positive-positive interaction of S7 with Zn (4.35Å), π-donor H-bond between O8 and His-119 (4.14Å), π-π T-shaped interaction of ring A with His-94 (5.42Å).
6	Unfavorable positive-positive interaction of S7 with Zn (4.38Å), π-donor H-bond between O8 and His-119 (4.08Å), π-π T-shaped interaction of ring A with His-94 (5.21Å).
7	Unfavorable positive-positive interaction of S7 with Zn (4.30Å), π-π T-shaped interaction of ring A with His-94 (5.47Å).
8	Unfavorable positive-positive interaction of S7 with Zn (4.32Å), π-π T-shaped interaction of ring A with His-94 (5.43Å).
9	Unfavorable positive-positive interaction of S7 with Zn (4.41Å), π-donor H-bond between O8 and His-119 (4.11Å), π-π T-shaped interaction of ring A with His-94 (5.16Å).
10	π-sulfur interaction of S7 with His-94 (5.94Å), unfavorable positive-positive interaction of S7 and Zn (4.21Å).
11	Unfavorable positive-positive interaction of S7 with Zn (4.40Å), π-donor H-bond between O8 and His-119 (4.10Å), π-π T-shaped interaction of ring A with His-94 (5.16Å).
12	π-sulfur interaction of S7 with His-94 (5.81Å), unfavorable positive-positive interaction of S7 with Zn (4.26Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.46Å).
13	π-sulfur interaction of S7 with His-94 (5.78Å), unfavorable positive-positive interaction of S7 with Zn (4.26Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.51Å).
14	π-sulfur interaction of S7 with His-94 (5.67Å), unfavorable positive-positive interaction of S7 with Zn (4.19Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.46Å).
15*	None
16	π-sulfur interaction of S7 with His-94 (5.48Å), unfavorable positive-positive interaction of S7 with Zn (3.98Å), metal-acceptor and halogen interactions of 2-F in ring B with Zn (2.49Å).
17	π-sulfur interaction of S7 with His-94 (5.73Å), unfavorable positive-positive interaction of S7 with Zn (4.22Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.40Å).
18	π-sulfur interaction of S7 with His-94 (5.69Å), unfavorable positive-positive interaction of S7 with Zn (4.21Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.40Å).
19	π-sulfur interaction of S7 with His-94 (5.98Å), unfavorable positive-positive interaction of S7 with Zn (4.35Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.40Å).
20	π-sulfur interaction of S7 with His-94 (5.94Å), unfavorable positive-positive interaction of S7 with Zn (4.33Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.40Å).
21	π-sulfur interaction of S7 with His-94 (5.46Å), unfavorable positive-positive interaction of S7 and Zn (3.99Å).
22	Unfavorable positive-positive interaction of S7 with Zn (4.24Å), π-sulfur interaction of S7 with His-94 (5.72Å), metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.42Å).

Table 6. Residues participating in the ligand-site interaction

Mol.	Residues
1*	Val-121, Leu-198, His-64, His-94, Trp-5, His-4, Thr-200, Gln-92, His-119, Trp-209 Val-143.
2*	Val-121, Leu-198, His-64, Val-143.
3	Val-121, Leu-198, His-94, His-64, His-119, Gln-92, Asn-62.
4	Val-121, Leu-198, His-94, Phe-131, Ile-91.
5	Val-121, Leu-198, His-94, His-64, His-119, Asn-62, Leu-60.
6	Val-121, Leu-198, His-94, His-64, His-119, Gln-92, Asn-62, Leu-60.
7	Val-121, Leu-198, His-94, His-64, Gln-92, Asn-62.
8	Val-121, Leu-198, His-94, His-64, Asn-62.
9	Val-121, Leu-198, His-94, His-64, His-119, Gln-92, Asn-62.
10	Val-121, Leu-198, His-94, His-64, Thr-199, Asn-62, Asn-67, Ile-91, Phe-131.
11	Val-121, Leu-198, His-94, His-64, His-119, Gln-92, Asn-62, Asn-67.
12	Val-121, Leu-198, His-94, His-64, Thr-199, Gln-92, Asn-62.
13	Val-121, Leu-198, His-94, His-64, Thr-199, Gln-92, Asn-62.
14	Val-121, Leu-198, His-94, His-64, Thr-199, Gln-92, Asn-62.
15*	Val-121, Leu-198, His-64, Lys-170, His-4, Asn-62, Trp-5, Pro-201.
16	Val-121, Leu-198, His-94, His-64, Thr-199, Gln-92, Asn-62.
17	Val-121, Leu-198, His-94, His-64, Thr-199, Gln-92, Asn-62, Leu-60.
18	Val-121, Leu-198, His-94, His-64, Thr-199, Gln-92, Asn-62, Trp-5, His-4.
19	Val-121, Leu-198, His-94, His-64, Thr-199, Gln-92, His-94, Asn-62.
20	Val-121, Leu-198, His-94, His-64, Thr-199, Gln-92, Asn-62.
21	Val-121, Leu-198, His-94, His-64, Asn-67, Ile-91, Phe-131.
22	Val-121, Leu-198, His-94, His-64, Thr-199, Gln-92, Asn-62.

We can see in Table 6 that the common amino acids involved in the interaction with all the ligands are Val-121 and Leu-198. His-94 appears interacting with molecules 3-14 and 16-22. His-64 and Thr-199 are interaction sites for most but not all molecules. An analysis of Figs. 4-9 and Table 6 suggests that probably some differences in ligand-amino acid interactions are the result of allowing conformational flexibility to the site's residues for the docking process. Now, and for the sake of simplicity, we shall employ the following qualitative classification of interactions: "weak" for interaction distances equal or greater than 5Å, "strong" for distances equal or lesser than 3Å and "intermediate" for distances lesser than 5Å and greater than 3Å. We shall center our discussion on weak and strong interactions. Table 7 shows the weak interactions.

Table 7. Weak interactions (d≥5Å) between the ligand and the site

Mol.	Interactions
1*	π-sulfur interaction of S7 with His-64 (5.72Å), Trp-5 (5.84Å and 5.49Å) and His-4 (5.08Å), π-alkyl of ring A with Leu-198 (5.45Å), π-alkyl interaction of 4-Me in phenyl substituent with His-94 (5.42Å).
2*	π-alkyl interaction of ring A with Leu-198 (5.17Å), π-alkyl interaction of phenyl substituent with Val-143 (5.04Å) and Val-121 (5.04Å).
3	π-alkyl interaction of ring B with Val-121 (5.02Å), π-π T-shaped interaction of ring A with His-94 (5.18Å).
4	π-sulfur interaction of S7 with His-94 (5.72Å), π-π T-shaped interaction of phenyl substituent with Phe-131 (5.08Å).
5	π-alkyl interaction of ring B with Val-121 (5.05Å), π-π T-shaped interaction of ring A with His-94 (5.42Å), alkyl interaction between Br and Leu-60 (5.39Å).
6	π-alkyl interaction of ring B with Val-121 (5.02Å), π-π T-shaped interaction of ring A with His-94 (5.21Å), alkyl interaction between Br and Leu-60 (5.34Å).
7	π-alkyl interaction of ring B with Val-121 (5.03Å), π-π T-shaped interaction of ring A with His-94 (5.47Å).
8	π-alkyl interaction of ring B with Val-121 (5.06Å), π-π T- shaped interaction of ring A with His-94 (5.43Å).
9	π-π T-shaped interaction of ring A with His-94 (5.16Å)
10	π-alkyl interaction of ring B with Val-121 (5.16Å), π-sulfur interaction of S7 with His-94 (5.94Å).
11	π-π T-shaped interaction of ring A with His-94 (5.16Å).
12	π-alkyl interaction of ring B with Val-121 (5.10Å), π-sulfur interaction of S7 with His-94 (5.81Å).
13	π-alkyl interaction of ring B with Val-121 (5.15Å), π-sulfur interaction of S7 with His-94 (5.78Å).
14	π-alkyl interaction of ring B with Val-121 (5.19Å), π-sulfur interaction of S7 with His-94 (5.67Å), π-alkyl interaction of cyclohexyl substituent with His-64 (5.05Å).
15*	π-sulfur interaction of S7 and His-64 (5.94Å).
16	π-alkyl interaction of ring B with Val-121 (5.37Å), π-sulfur interaction of S7 with His-94 (5.48Å).
17	π-alkyl interaction of ring B with Val-121 (5.19Å), π-sulfur interaction of S7 with His-94 (5.73Å), alkyl interaction of Cl with Leu-60 (5.01Å).
18	π-alkyl interaction of ring B with Val-121 (5.21Å), π-sulfur interaction of S7 with His-94 (5.69Å).
19	π-alkyl interaction of ring B with Val-121 (5.11Å), π-sulfur interaction of S7 with His-94 (5.98Å), π-π T-shaped interaction of ring A with His-94 (5.63Å).
20	π-alkyl interaction of ring B with Val-121 (5.11Å), π-sulfur interaction of S7 with His-94 (5.94Å), π-π T-shaped interaction of ring A with His-94 (5.71Å).
21	π-alkyl interaction of ring B with Val-121 (5.37Å), π-sulfur interaction of S7 with His-94 (5.46Å), alkyl interaction of a terminal C in Boc group and Ile-91 (5.04Å).
22	π-alkyl interaction of ring B with Val-121 (5.22Å), π-sulfur interaction of S7 with His-94 (5.72Å).

We can see that these weak ligand-residue interactions are of the π-sulfur, π-alkyl, π-π T-shaped and alkyl kinds. The residues involved in these interactions could be considered as a kind of *external skeleton* involved in the long-range orientation and guiding of the ligand towards its final position. The final position will be achieved through medium and short-range interactions. Table 8 shows the short-range interactions.

We can see that most short-range interactions are classical (conventional) hydrogen bonds. Molecules 4, 9 and 22 show unfavorable interactions that probable are overcome by the rest of the interactions. π-cation interactions exist in almost all the molecules. Fluorination produces halogen interactions of several kinds in several molecules. π-donor H-bond interactions (a XH---π interaction) exist in some molecules [31].

These results seem to be an interesting variety of the "*chicken or the egg*" causality dilemma. The statement "*which came first, the chicken or the egg?*" can be expressed in this specific case as "*which came first, the docking or the linear multiple regression analysis?*". If we give a full validity of the above results then we have a new problem regarding the common skeleton concept. The following way to carry out a formal study of the relationships between the electronic structure and a given biological activity in a group of molecules seems to be the correct path. If we have a structure (i.e., we can find one in the Protein Data Bank for example) then we must first dock all the molecules. If we find one or more with a different mode of binding than the rest, discard them and proceed with the remaining ones. If we have not a known structure of the site, the results of a linear multiple regression analysis must be carefully examined to discard the possible presence of more than one mode of binding.

In summary, we have analyzed the docking of 22 molecules to hCAII. We discovered that few molecules can bind to the site in a very different way than the majority. The docking results were analyzed from the point of view of the

ligand-residue distance, kind of residues involved and kinds of weak and short-range interactions. We suggest a new way to proceed when carrying out formal studies between electronic structure and biological activity.

Table 8. Short-range interactions (d≤3Å) between the ligand and the site

Mol.	Interactions
1*	Conventional H-bond between O8 and Trp-5 (2.28), conventional H-bond between N14 and Gln-92 (2.90Å and 2.90Å).
2*	Conventional H-bond between N13 and His-64 (2.39Å).
3	Conventional H-bond between N14 and Asn-62 (2.51Å).
4	Unfavorable metal-donor interaction of H10 and Zn (1.93Å).
5	π-cation interaction of ring A with His-64 (2.57Å), conventional H-bond between N14 and Asn-62 (2.49Å).
6	π-cation interaction of ring A with His-64 (2.66Å), π-donor H-bond between ring A and Gln-92 (2.47Å), conventional H-bond between N14 and Asn-62 (2.52Å).
7	π-cation interaction of ring A with His-64 (2.61Å), π-donor H-bond between ring A and Gln-92 (2.68Å), conventional H-bond between N14 and Asn-62 (2.53Å).
8	π-cation interaction of ring A with His-64 (2.54Å), conventional H-bond between N14 and Asn-62 (2.49Å), conventional H-bond between O from the ester substituent and Asn-62 (2.35Å).
9	π-cation interaction of ring A with His-64 (2.66Å), π-donor H-bond between ring A and Gln-92 (2.46Å), conventional H-bond between N14 and Asn-62 (2.49Å), conventional H-bond between H from 3-MeOH in ring A and Asn-62 (2.38Å), unfavorable donor-donor interaction of H from 4-MeOH in ring A with Asn-62 (1.44Å).
10	Conventional H-bond between H10 and Thr-199 (2.64Å), π-cation interaction of ring A with His-64 (2.62Å), conventional H-bond between N14 and Asn-62 (2.55Å), conventional H-bond between H from amide nitrogen of the $BocNHCH_2$ moiety and Asn-67 (1.94Å).
11	π-cation interaction of ring A with His-64 (2.67Å), π-donor H-bond between ring A and Gln-92 (2.45Å), conventional H-bond between N14 and Asn-62 (2.49Å), conventional H-bond between H from amine moiety of 4-$MeNH_2$ in ring A and Asn-67 (2.21Å).
12	Metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.46Å), conventional H-bond between 2-F in ring B with Thr-199 (2.50Å), conventional H-bond between 5-F in ring B with Gln-92 (2.95Å), conventional H-bond between N13 and Gln-92 (2.31Å), π-cation interaction of ring A with His-64 (2.64Å), conventional H-bond between N14 and Asn-62 (2.79Å and 2.75Å).
13	Metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.51Å), conventional H-bond between 2-F in ring B with Thr-199 (2.52Å), conventional H-bond between 5-F in ring B with Gln-92 (2.89Å), conventional H-bond between 3-F and His-64 (2.94Å), conventional H-bond between N13 and Gln-92 (2.44Å), π-cation interaction of ring A with His-64 (2.59Å), conventional H-bond between N14 and Asn-62 (2.72Å and 2.69Å).
14	Metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.46Å), conventional H-bond between 2-F in ring B with Thr-199 (2.58Å), conventional H-bond between 5-F in ring B with Gln-92 (2.79Å), conventional H-bond between 3-F and His-64 (2.88Å), conventional H-bond between N13 and Gln-92 (2.59Å), π-cation interaction of ring A with His-64 (2.61Å), conventional H-bond between N14 and Asn-62 (2.79Å and 2.69Å).
15*	π-σ interaction of ring B with His-64 (3.56Å), conventional H-bond between O8 and His-4 (2.34Å), conventional H-bond between H10 and Asn-62 (2.08Å), conventional H-bond between 2-F and Trp-5 (2.56Å).
16	Metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.49Å), conventional H-bond between 2-F in ring B with Thr-199 (2.67Å), conventional H-bond between 5-F in ring B with Gln-92 (2.97Å), conventional H-bond between 3-F and His-64 (2.62Å), conventional H-bond between N13 and Gln-92 (2.59Å), π-cation interaction of ring A with His-64 (2.58Å), conventional H-bond between N14 and Asn-62 (2.52Å).
17	Metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.40Å), conventional H-bond between H10 with Thr-199 (2.60Å), conventional H-bond between 5-F in ring B with Gln-92 (2.81Å), conventional H-bond between N13 and Gln-92 (2.56Å), π-cation interaction of ring A with His-64 (2.54Å), conventional H-bond between N14 and Asn-62 (2.75Å and 2.69Å).
18	Metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.40Å), conventional H-bond between 2-F in ring B with Thr-199 (2.58Å), conventional H-bond between 3-F and His-64 (2.95Å), conventional H-bond between N13 and Gln-92 (2.49Å), π-cation interaction of ring A with His-64 (2.55Å), conventional H-bond between N14 and Asn-62 (2.82Å and 2.71Å).
19	Metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.40Å), conventional H-bond between 2-F in ring B with Thr-199 (2.84Å), conventional H-bond between H10 and Thr-199 (2.62Å), conventional H-bond between N13 and Gln-92 (2.14Å), π-cation interaction of ring A with His-64 (2.60Å), conventional H-bond between N14 and Asn-62 (2.75Å and 2.67Å), conventional H-bond between O from ester substituent and Asn-62 (2.62Å).
20	Metal-acceptor and halogen interaction of 2-F in ring B with Zn (2.40Å), conventional H-bond between 2-F in ring B with Thr-199 (2.39Å), conventional H-bond between N13 and Gln-92 (2.14Å), π-cation interaction of ring A with His-64 (2.61Å), conventional H-bond between N14 and Asn-62 (2.75Å and 2.72Å), conventional H-bond between O from 4-MeOH substituent and Asn-62 (2.53Å).
21	Conventional H-bond between 3-F and His-64 (2.47Å), π-cation interaction of ring A with His-64 (2.85Å), conventional H-bond between N13 and His-64 (2.50Å), conventional H-bond between H from amide nitrogen in $BocNHCH_2$ moiety and Asn-67 (2.10Å).
22	Conventional H-bond between H10 and Thr-199 (2.71Å), conventional H-bond between 3-F and His-64 (2.96Å), conventional H-bond between 5-F and Gln-92 (2.97Å), conventional H-bond between N13 and Gln-92 (2.50Å), π-cation interaction of ring A with His-64 (2.54Å), conventional H-bond between N14 and Asn-62 (2.75Å and 2.65Å), unfavorable donor-donor interaction of H in 4-$MeNH_2$ substituent and Asn-62 (2.07Å).

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