DOI 10.5012/bkcs.2010.31.5.1407

Design of Human FIH-1 Inhibitors through Virtual Screening

Jihoon Kang,[a] Kwang-su Park,[a] Youhoon Chong,[*] and Jungwoo Choe[†,*]

Department of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Korea
[†]Department of Life Sciences, University of Seoul, Seoul 130-743, Korea. [*]E-mail: jchoe@uos.ac.kr
Received January 27, 2010, Accepted March 17, 2010

Key Words: FIH-1, Virtual screening, Inhibitor

Hypoxia-inducible factor (HIF-1), a heterodimeric transactivator composed of α and β subunits, is an oxygen-dependent transcriptional activator, which acts as a master regulator of oxygen-regulated gene expression. Oxygen levels can affect the protein stability, subcellular localization and transcriptional potency of the HIF-α subunits, whereas the β-subunit is constitutively expressed with its activity unaffected by hypoxia (reduced O_2 availability).[1] The target genes of HIF-1 encode angiogenic factors as well as proliferation/survival factors and are particularly relevant to cardiovascular diseases and cancer.[2,3]

For full HIF-1 activity, HIF-1α must interact with coactivator proteins such as p300 (300 KDa co-activator protein)/CBP (CREB binding protein) through its C-terminal transactivation domain (TAD-C),[4] and the interaction between TAD-C and p300/CBP is regulated by hydroxylation-dependent switch: under normoxic conditions, factor inhibiting HIF-1 (FIH-1) hydroxylates Asn803 of HIF-1α within the TAD-C, which prevents the interaction of HIF-1α with coactivators.[4,5] Therefore, inhibition of the hydroxylation activity of FIH-1 can lead to the activation of HIF-1α and its target genes, such as EPO and VEGF. As a result, FIH-1 has emerged as a key molecular target for developing new therapeutic agents for conditions like cerebral and myocardial infarctions.[6]

FIH-1 belongs to 2OG (oxoglutarate)- and Fe(II)- dependent dioxygenase superfamily,[7] which has common structural features of a jellyroll-like-barrel embracing the conserved active site with bound Fe(II).[7] Several structures of FIH-1 were already determined[8-10] but, recently, we have determined the structure of human FIH-1 in complex with more drug-like neutral inhibitors such as 5-chloro-7-iodo-8-hydroxyquinoline (CQ, Clio quinol) and 8-hydroxyquinoline (HQ) (Fig. 1) bound to the active site.[11]

Analysis of the complex structure of FIH-1 with CQ shows that coordination of Fe(II) with 8-hydroxyl group and 1-nitrogen atom of CQ as well as hydrophobic interactions between halogenated quinoline ring and surrounding residues are two critical binding interactions.[11] This structure also indicates that more potent and selective FIH-1 inhibitors can be designed by adequate modifications on the CQ structure. More specifically, CQ binds deep inside the binding pocket with its C6 and C7 positions pointing to two different open spaces (OS-1 & OS-2, Fig. 2), and therefore it was proposed that introduction of functional groups at the C6 or C7 positions of CQ would result in enhanced binding affinity to FIH-1 and thereby increased inhibitory activity.[11]

In this study, a pharmacophore-guided virtual screening study was performed to see if the open spaces close to the CQ binding site can serve as additional inhibitor binding sites, which can provide valuable information for the design of more potent FIH-1 inhibitors.

For virtual screening study, a pharmacophore was determined first. From the analysis of the crystal structure of FIH-1 in complex with CQ, metal chelation by N1 and 8-OH of CQ as well

Figure 1. Structures of clioquinol (CQ) and 8-hydroxyquinoline (HQ).

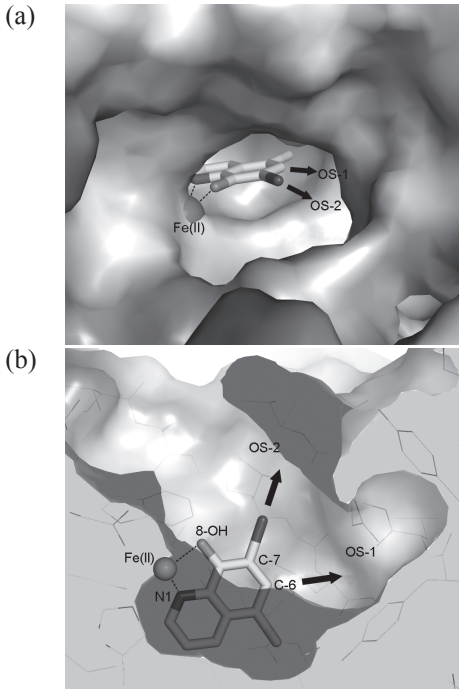

Figure 2. Binding mode of CQ to the active site of FIH-1. (a) CQ coordinates Fe(II) deep inside the binding pocket. C6 and C7 are exposed from the binding pocket facing outward. Two open spaces around C6 and C7 (OS-1 and OS-2) are indicated as filled arrows. (b) the view is rotated about 90° from (a) to show the open spaces (OS-1 and OS-2).

[a]These two authors contributed equally to this work

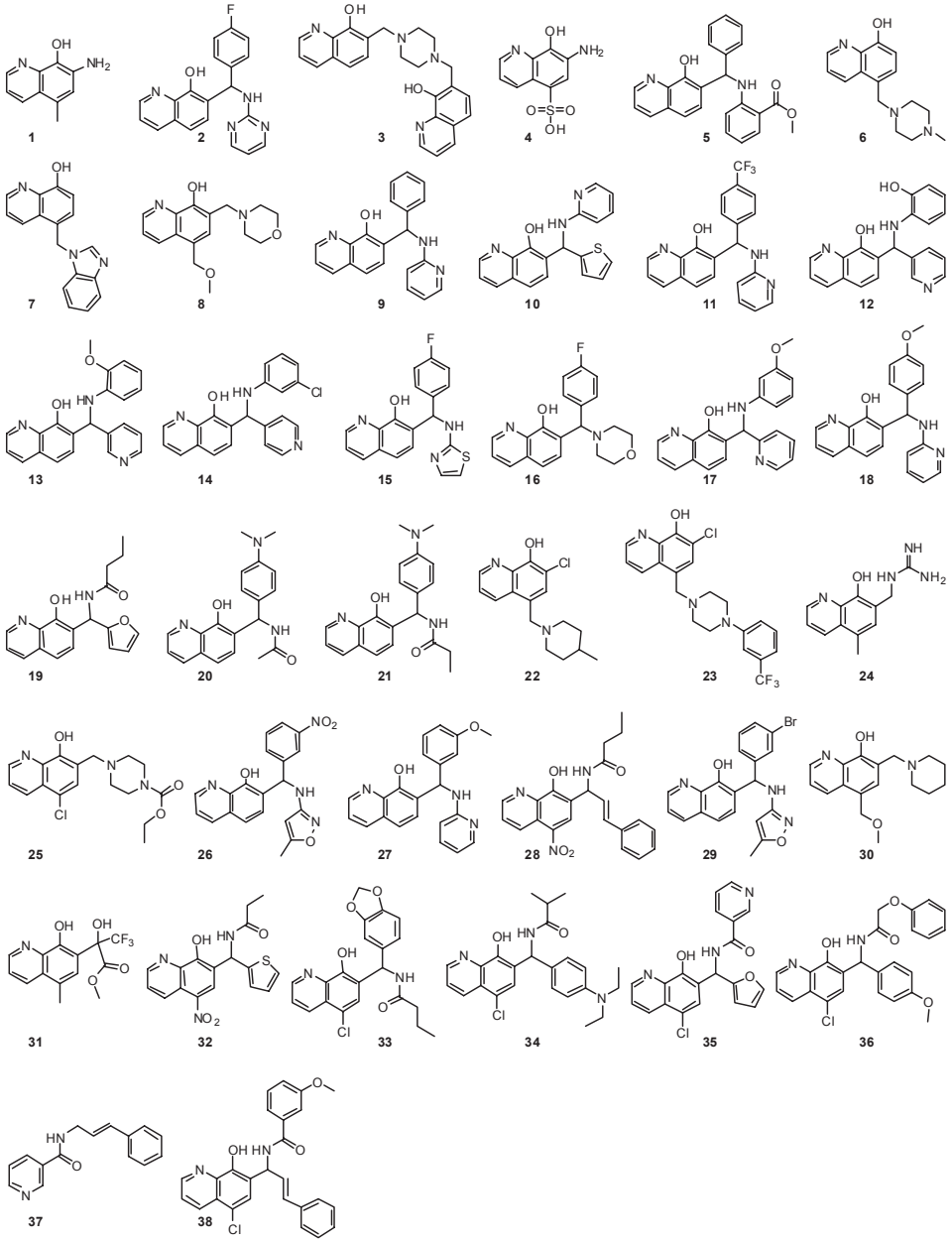

Figure 3. Structures of 38 hit compounds selected from UNITY search.

as hydrophobic fused aromatic ring system located inside the binding pocket was found to be the most relevant pharmacophore. Therefore, a molecular fragment composed of N1-C2-C3-C4–C10-C9-C8-O8 of CQ structure (Fig. 1, thick lines) was used as a pharmacophore query for virtual screening. A compound library commercialized by ChemDiv, Inc. with a total of 693,042 compounds was used as a screening library, and UNITY's conformationally flexible 3D searching was then executed. Total of 38 molecules with various functional groups at C5, C6, or C7 positions were found after flexible search (Fig. 3). The relative binding affinities of these molecules were then compared by docking scores obtained after docking individual molecules at the active site using the FlexX module in Sybyl.[12] These superimposed docking poses of the 38 hit compounds and docking scores are summarized in Fig. 4 and Table 1, res-

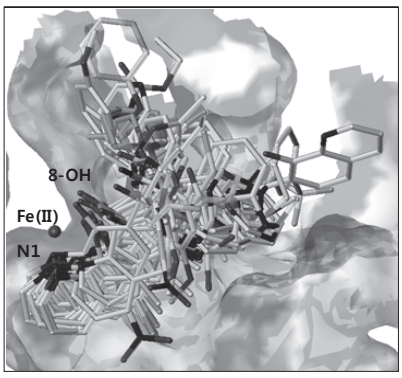

Figure 4. Superposition of docking poses of 38 compounds selected from the virtual screening.

Table 1. Docking scores of 38 hit compounds and CQ

Compd	FlexX_SCORE	D_SCORE	PMF_SCORE	G_SCORE	CHEMSCORE	CSCORE
38	−37.73	−86.76	−142.14	−138.80	−47.65	3
26	−34.82	−86.51	−123.35	−182.52	−40.43	5
28	−34.29	−71.52	−146.47	−152.19	−46.49	3
17	−28.59	−70.97	−124.97	−146.32	−40.08	2
21	−28.04	−70.33	−111.86	−143.62	−33.02	2
35	−27.68	−76.36	−133.67	−169.28	−42.61	5
32	−27.57	−58.88	−113.89	−163.71	−42.63	3
20	−26.33	−59.50	−105.54	−150.93	−35.96	4
33	−26.26	−87.03	−128.43	−178.82	−39.29	4
13	−25.04	−69.91	−130.46	−129.81	−38.89	3
27	−25.04	−68.34	−133.68	−155.18	−39.62	4
2	−25.01	−79.37	−86.53	−104.03	−26.34	2
29	−24.55	−97.32	−114.89	−186.86	−40.38	5
9	−24.40	−79.10	−123.11	−126.35	−35.59	5
5	−24.40	−70.65	−109.01	−103.22	−22.40	2
18	−24.33	−70.97	−98.30	−130.95	−34.63	3
12	−24.03	−61.68	−94.83	−89.33	−25.48	1
14	−23.93	−99.61	−97.18	−167.33	−35.39	5
10	−23.50	−87.88	−121.20	−159.27	−38.66	5
36	−23.49	−91.72	−139.82	−134.06	−40.66	3
24	−23.39	−43.62	−102.39	−145.97	−27.79	4
19	−22.97	−52.57	−114.15	−120.83	−40.38	3
15	−21.92	−59.67	−73.67	−48.26	−20.22	1
34	−21.41	−62.04	−42.28	−38.63	−21.87	1
7	−21.11	−53.74	−79.47	−100.51	−35.42	2
25	−20.92	−61.30	−79.36	−103.82	−31.65	1
37	−20.65	−105.42	−91.59	−189.41	−31.01	3
11	−20.46	−87.33	−100.60	−187.81	−39.78	4
31	−20.45	−58.88	−108.65	−78.18	−34.07	3
3	−19.56	−83.32	−100.04	−186.40	−41.05	5
4	−19.30	−48.43	−77.26	−77.21	−24.96	3
30	−18.52	−42.94	−77.53	−145.60	−28.49	3
1	−18.04	−45.93	−29.26	−56.44	−19.18	2
23	−16.85	−73.41	−52.81	−111.01	−36.22	4
22	−16.20	−66.19	−20.99	−120.01	−12.88	3
8	−16.02	−59.34	−57.46	−84.65	−24.30	2
6	−15.99	−36.29	−65.17	−108.88	−16.96	3
16	−15.24	−55.08	−88.70	−114.69	−26.80	3
CQ	−16.56	−41.27	−66.33	−89.02	−29.59	4

pectively.

The hit molecules are shown to have at least one substituent at C5, C6, or C7 positions, and they showed significantly higher docking scores compared with CQ (Table 1). Analysis of the docking poses and scores indicates that the additional substituents of the CQ core structure are successfully accommodated by either or both of the two open spaces (OS-1 and OS-2) around the ligand binding site, which can be ascribed to the stabilizing interaction of the CQ-substituents with the enzyme residues constituting the open spaces. In particular, molecule **38** with a branched chain at C7 position shows a full coverage of both OS-1 and OS-2 with its substituent leading to the best docking score (Table 1, Fig. 5). The binding mode of compound **38** (Fig. 5) shows that both of the OS-1 and OS-2 can accommodate aromatic rings two carbons away from the branch point of the CQ-substituent, which might be the optimum size of each branch. Also, in comparison with OS-1, OS-2 interacts with the upper branch of **38** through Arg238 and Glu202. Thus, for optimum interaction, the CQ analogue looks to be required to have two branches located in the same distance from the branch point. Particularly, one of the branches needs to be equipped with hydrogen bond acceptors.

In summary, the crystal structure of FIH-1: CQ complex showed that the 7- and 6-positions of CQ are facing outward to the surface or open space available for additional ligand binding (OS-2 and OS-1), which can be modified for increased binding affinity. Pharmacophore-guided virtual screening using a pharmacophore that maintains the moiety for Fe(II) chelation (N1 and 8-OH of CQ) found 38 hit compounds that have better docking scores than CQ. Interestingly, most of the hit compounds contained the original hydroxyquinoline moiety of CQ, modified with various functional groups at the 7-position. Also, the high scoring hits included compounds with branched chain substituents at the 7-position including the top scorer, compound **38**. These branched chain substituents can increase the binding affinity by filling both of the open spaces close to

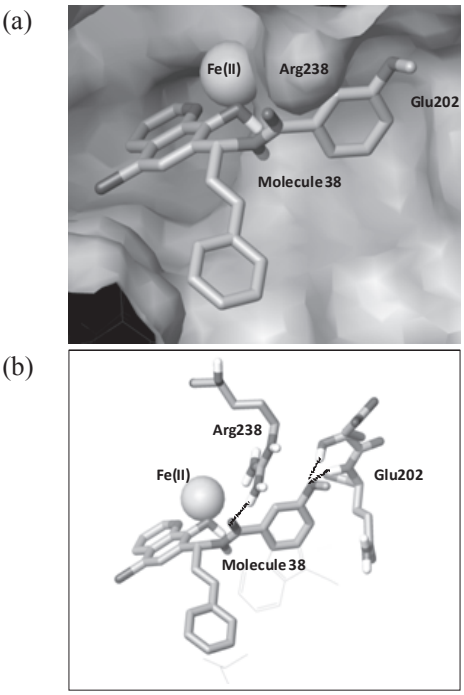

Figure 5. (a) Surface representation of the docking pose of compound **38**. (b) Hydrogen bonding interactions of compound **38** with Arg238 and Glu202.

the active sites (OS-1 and OS-2). From these modeling results, we propose that improved FIH-1 inhibitors can be designed to exploit open spaces close to the CQ binding site and thereby improve the affinity and specificity of CQ derivatives.

Experimental Section

Pharmacophore-guided virtual screening. Pharmacophore-guided virtual screening study was performed using UNITY module implemented in SYBYL 7.2 software package installed on a Linux Enterprise OS. The pharmacophore composed of N1-C2-C3-C4–C10-C9-C8-O8 of CQ structure was used as a pharmacophore query. ChemDiv compound library with 693,042 compounds was converted to 3D-UNITY database by means of Concord™ and used as a screening library. UNITY's conformationally flexible 3D searching was then executed on the ChemDiv database which was restricted with modified Lipinski's rule and for further screening the number of rotatable bonds was set to 8.

Molecular docking. The molecular docking studies were performed on FIH-1 protein (PDB code: 3KCX) employing the FlexX docking procedure using SYBYL 7.2 software package installed on a Linux Enterprise OS. FlexX is a fast automated program based on incremental construction procedure.[15] The active site was assigned at a radius of 8 Å around the reference ligand, CQ. Docking produced 30 possible docked conformations for each of the ligands and different scoring functions were used for scoring the docked conformations: FlexX,[13] C-Score,[14] PMF-Score,[15] D-Score,[16] G-Score,[16] and ChemScore.[17] Among the 30 conformational solutions of ligands, the ones with the best total_score (rank 1) were chosen as the optimal conformational poses in all docking experiment. The rank 1 conformations showed better binding interactions compared to other solutions.

Acknowledgments. This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093824) and a grant from Biogreen 21 (Korea Ministry of Agriculture and Forestry) and by the Korean Research Foundation Grant, funded by the Korean Government (Ministry of Education & Human Resources Development, Basic Research Promotion Fund, KRF-2007-331-C00212).

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