http://dx.doi.org/10.5012/bkcs.2013.34.4.1051

Facile Docking and Scoring Studies of Carborane Ligands with Estrogen Receptor

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Received December 24, 2012, Accepted January 8, 2013

Closo-carborane has been considered as an efficient boron-carrier for boron neutron capture therapy (BNCT) and an attractive surrogate of lipophilic phenyl or cyclohexyl ring in drug design. Despite a great number of carborane-containing ligands have been synthesized and evaluated, molecular modeling studies of carborane ligands with macromolecules have been rarely reported. We herein describe a facile docking and scoring-function strategy of 16 carborane ligands with an estrogen receptor by using the commercial Gaussian, Chem3D Pro and Discovery Studio (DS) computational programs. Docked poses of the carborane ligands *in silico* exhibited similar binding modes to that of the crystal ligand in the active site of estrogen receptor. Score analysis of the best docked pose for each ligand indicated that the Ligscore1 and the Dockscore have a moderate correlation with *in vitro* biological activity. This is the first report on the scoring-correlation studies of carborane ligands with macromolecules. The integrated Gaussian-DS approach has a potential application for virtual screening, *De novo* design, and optimization of carborane ligands in medicinal chemistry.

Key Words : Carborane, *In silico* docking, Scoring functions, Estrogen receptor, Drug design

Introduction

Carboranes ($C_2B_{10}H_{12}$), polyhedral boron clusters, have been exploited as pharmacophore scaffolds in drug design because they can enhance the lipophilicity of the parent drugs to cross the cell membrane and the blood-brain barrier (BBB) by passive diffusion, modulate their physicochemical properties by converting neutral and hydrophobic *closo*-carboranes into anionic and hydrophilic *nido*-carboranes, increase their metabolic stability, undergo a number of organic reactions such as nucleophilic and electrophilic substitutions, and improve their binding affinities for target proteins *via* hydrophobic interactions.[1,2] In terms of size and lipophilicity, carboranes occupy the 3-D space generated by a hypothetically rotating benzene ring and are more lipophilic than the benzene ring.[1,2] Carborane clusters were evaluated initially as boron-delivery moieties for boron-neutron capture therapy (BNCT) because the high boron content of carborane clusters can efficiently achieve the necessary boron concentrations for BNCT.[3,4]

Recently, *closo*-carborane clusters have also been applied as hydrophobic pharmacophore moieties in medicinal chemistry to replace lipophilic benzene or cyclohexane ring. Indeed, carborane-containing ligands exhibited the enhanced binding affinities to a number of receptors/enzymes proteins (*e.g.*, estrogen receptor, androgen receptor, HIV protease) compared to non-carborane ligands.[5-11] Despite the utilization of *closo*-carboranes as hydrophobic scaffolds in drug design and as boron-delivery moieties for BNCT has expanded, *in silico* molecular modeling of carborane-containing molecules with macromolecules including docking studies, scoring functions and virtual screening have been rarely reported.[12-14] The difficulty of carborane ligands for molecular modeling is that the forcefields of hexavalent carbon and boron atoms in the carborane cluster are not compatible with most commercially-available docking programs. Although there have been a few of reports on docking results of carborane ligands with proteins, those approaches simply modified forcefield of boron atoms by assigning them into sp^3-carbon atom or by using the closed-share modeling program such as ADAM.[12-14]

As an effort to develop facile and convenient docking strategy of carborane ligands with macromolecules, we integrated two common modeling softwares: 1) Gaussian03 (Gaussian Inc.) and ChemDraw 3D Pro program (ChemBridgeSoft) to generate and optimize carborane ligands and 2) Discovery Studio program (Accelrys Inc.) to perform docking studies and analyze the scoring functions of the docked poses. The LigandFit module in Discovery Studio (DS) recognizes hexavalent carbon and boron atoms of carborane clusters, thus, can calculates the scoring functions including Dock score, Ligscore1, Ligscore2, -PLP1 score, -PMF score, and Jain score.[15,16] The integrated method to combine ChemDraw 3D Pro with LigandFit is relatively simple while the method to combine Gaussian03 and LigandFit are highly sophisticated. We aimed at evaluating that the integrated methods can predict the binding modes of carborane-containing ligands in the active site of the target proteins, calculate the binding energies of protein-ligand complexes, and get plausible relationships of docking scores

with *in vitro* biological activities.

Experimental

Ligand Preparation and Optimization. The ligands containing carborane cluster were generated by ChemBioDraw (version: 11.0.1) and were transferred to Gaussian 03 program and Chem3D Pro. All ligands were optimized with Gaussian 03 program (Gaussian Inc., Wallingford, CT) and Chem3D Pro (version: 11.0.1). Energy minimization calculations were performed at the B3LYP 6-31* level at the Gaussian Program and at the "Minimize Energy" option of MM2 calculation at the Chem3D Pro. In case that one or two hydrogens ("H") of carborane are flipped into the cavity of the cluster in the Chem3D Pro, the hydrogens were deleted and re-added to generate proper icosahedral carborane structures. The most stable conformer of carborane-containing ligands was obtained by "Molecular Dynamics", followed by "Minimize Energy" option. The energy-minimized structures were saved as the mol file and were transferred to Discovery Studio 3.0 for docking studies.

Protein Preparation. For docking studies, the protein structures in PDB format were downloaded from RCSB protein data bank. The original crystal ligand and water molecules were removed from the protein-ligand complexes and side chains of amino acid residues were fixed. Hydrogen atoms were added by application of CHARMm Force field and Momany-Rone charge as a default setting in DS 3.0. The active sites by using "Receptor Cavity" option were defined by selecting amino acids nearby the original ligands.

Docking and Scoring Function Studies. The docking studies of the prepared carborane ligands were performed by LigandFit module in Discovery Studio 3.0. The number of generated poses was set as 100 for each ligand with default settings for the other parameters. Scoring function scores were obtained with Dock score, Ligscore1, Ligscore2, -PLP1 score, -PMF score, and Jain score.

Results and Discussion

Prior to investigating the scoring functions of carborane-based ligands with estrogen receptor, we evaluated the compatibility of the new approach by performing docking studies of the crystallized carborane ligands and comparing the RMSD values of the docked pose with the crystal ligand. Up to date, three protein X-ray crystal structures in complex with carborane-containing ligands have been reported.[17-19] Three complexes are (a) dihydrofolate reductase (DHFR) in complex with 2,4-diamino-5-(1-*o*-carboranylmethyl)-6-methylpyrimidine (PDB ID: 2C2S),[17] (b) HIV protease (PDB ID: 1ZTZ), an aspartyl protease to be essential for the cell cycle of HIV, in complex with the cobalt bis(1,2-dicarbollide),[18] and (c) vitamin D receptor (VDR) with (*R*)- and (*S*)-stereoisomers of carborane-containing ligand 3 (PDB ID: 3VJT and 3VJS) in Figures 1 and 2.[19]

Two approaches, the Gaussian03/LigandFit and the Chem3D/LigandFit approach were evaluated for its accuracy on the

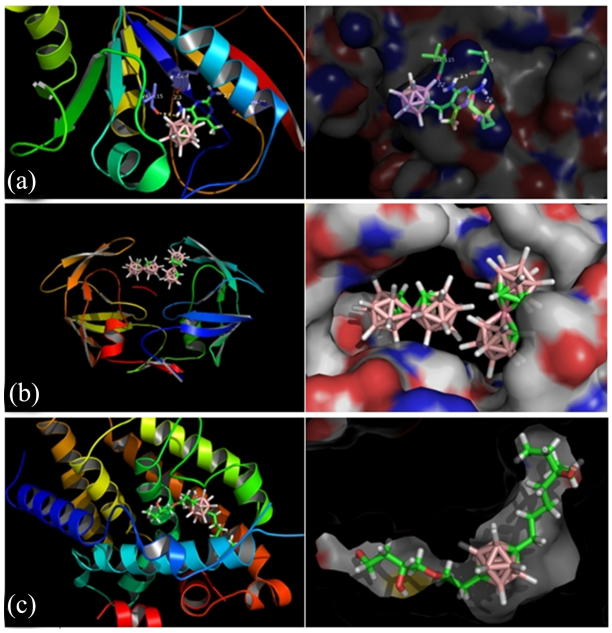

Figure 1. (a) The best docked pose of 2,4-diamino-5-(1-*o*-carboranylmethyl)-6-methylpyrimidine with DHFR, (b) The docked pose of cobalt bis(1,2-dicarbollide) with HIV protease, (c) The best docked pose of (*R*)-stereoisomer of the ligand **3** with VDR.

regeneration of the binding mode of the crystal ligands, respectively. We minimized carborane-containing ligands by Gaussian03 or Chem3D Pro. Both programs recognize the atom types of hexavalent carbons and borons in the carborane cluster. The coordinates of the energy-minimized ligands were transferred into Discovery Studio 3.0 and used for *in silico* docking studies without further modification. LigandFit module in DS 3.0 recognizes the hexavalent carbon and boron atoms. Previous reports on molecular

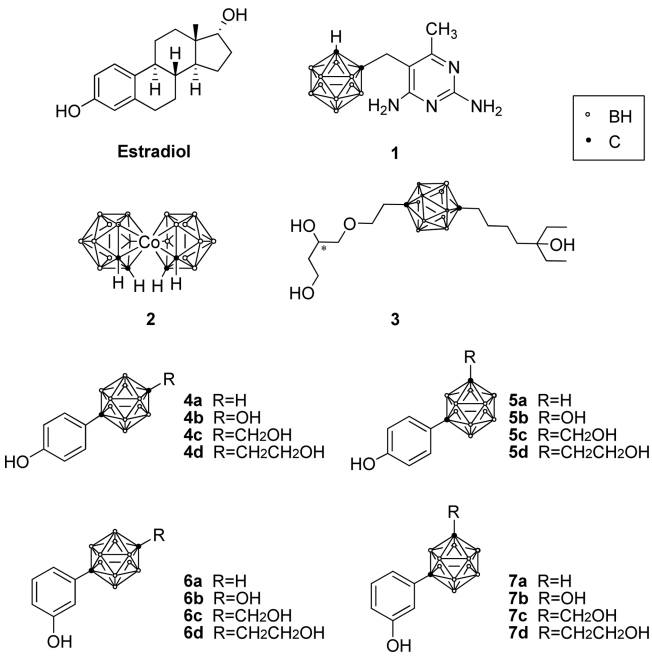

Figure 2. Chemical structures of carborane ligands for this study.

modeling of carborane ligands applied manipulation of boron atoms by changing hexavalent boron atom into C.3 atom. The best docked pose for the three carborane-containing ligands successfully regenerated the binding modes of X-ray crystal ligands. Figure 1 shows the best docked pose of 2,4-diamino-5-(1-*o*-carboranylmethyl)-6-methylpyrimidine with DHFR, cobalt bis(1,2-dicarbollide) with HIV protease, and (*R*)-stereoisomer of the ligand 3 with VDR. RMSD values between the best docked pose and the crystal ligand were 0.464 Å, 0.882 Å and 1.387 Å, respectively. Chem3D/LigandFit also showed the results similar to Gaussian03/LigandFit. The results implied that both Gaussian03/LigandFit and Chem3D/LigandFit approaches regenerate the binding modes of the carborane crystal ligands and can be further applied for predicting binding modes of carborane-containing ligands with the other proteins.

Encouraged by the preliminary result, we carried out the docking studies and investigated the relationships of *in silico* binding scores with *in vitro* binding affinities of carborane-containing ligands. To the best of our knowledge, there has been no report on co-relationship studies between *in silico* binding energies and *in vitro* biological activities of carborane ligands. We selected estrogen receptor alpha (ER_α) as a target protein for *in silico* docking studies because the X-ray crystal structure of ER_α (PDB ID: 1ERE) with the natural ligand (*e.g.*, estradiol) was determined and *in vitro* ER_α binding activities of the carborane-ligand library were reported.[8,20]

Estrogen receptors (ER), one of nuclear receptors to modulate the transcriptions of target genes, are activated by the binding of estrogens such as estradiol (E2), estrone, and estriol. The two hydroxyl groups at C-3 on the aromatic A ring and at C-17 on the D ring in steroidal estrogen ligands are the essential features for selective high-affinity binding to the estrogen receptors.[20] Endo and his co-workers successfully synthesized carborane-based ER_α modulators by replacing the B and C rings with carborane clusters.[5-8] Carborane-based estrogen ligands exhibited strong transcriptional activities *in vitro* in a similar manner to the natural ligand E2.[8]

For docking studies, 3-D structures of 16 carborane ligands and the natural ligand E2 were constructed and minimized by Gaussian03 and Chem3D Pro. Mol files of the ligands were transferred to DS3.0 and used for molecular modeling without further modification. Ligand docking was performed by the LigandFit module, in which the ligands and the ER_α active site are rigid. The best one among 100 docked poses for each ligand was selected for *in silico* studies. Each pose of the tested 16 ligands exhibited similar binding modes in the active site of estrogen receptor to that of the natural ligand E2. As shown in Figure 3, the best docked pose of carborane ligand **4c** with the highest DOCK score and Ligscore1 as well as strong binding affinity to ER_α, exhibited strong hydrogen bonding interactions with Arg 394, Glu 353 and His 524. The phenol group, which corresponds to the 3'-OH of E2, makes hydrogen bonding with Glu 353 and Arg 394 in the range of 2.034 Å and 1.745 Å, respectively. The hydrogen bonding length between carboranyl alcohol and His 524 is 2.145 Å. Table 1 summarizes the hydrogen bonding interactions between key amino acids of ER_α active site and the representative best docked pose for each compound set **4**, **5**, **6**, and **7**.

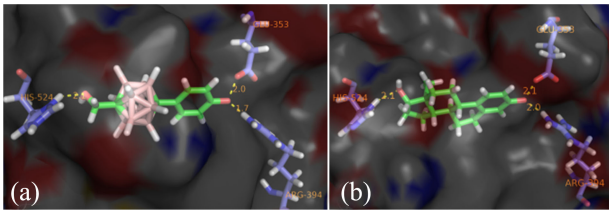

Figure 3. (a) The ER_α active site with the best docked pose of ligand **4c** and (b) The ER_α active site with the crystal ligand **E2**.

Table 1. Key interactions of ER_α active site with the carborane ligands **4c**, **5b**, **6c**, **7d** and **E2**

Ligand	Glu 353	Arg 394	His 524
4c	2.034	1.745	2.145
5b	1.994	1.722	2.855
6c	2.164	3.874	2.309
7d	2.120	3.179	2.383
E2	2.089	2.037	2.145

The best pose of all ligands reflected the binding mode of E2 to the ER_α. Scores of the best docked pose for each ligand were calculated. The correlations of each score with *in vitro* biological activity were calculated. Table 2 shows the best scoring functions, Dockscore from Gaussian03/LigandFit and Ligscore1 from Chem3D/LigandFit. Ligscore1 in Chem3D/LigandFit method performed much better than the other scoring functions with R^2 values of 0.604 (Figure 4) while Dockscore in Gaussian 03/LigandFit method works

Table 2. Scoring function results of 16 carborane ligands after performing docking studies with ER_α

Entry	Dockscore (Gaussian)	Ligscore1 (MM2)	Relative Luciferase Response[a]
4a	66.629	3.46	9
4b	68.766	4.36	13
4c	73.831	4.37	14
4d	71.965	3.70	11
5a	68.506	3.50	9
5b	69.429	4.16	9
5c	68.584	3.82	13
5d	63.934	3.51	9
6a	65.878	2.64	7
6b	70.189	3.66	9
6c	68.961	3.76	16
6d	64.478	3.60	10
7a	65.890	2.67	5
7b	68.805	2.89	5
7c	66.766	2.91	7
7d	66.629	3.20	4
E2	68.766	4.49	11

[a]Data was adapted from reference 8.

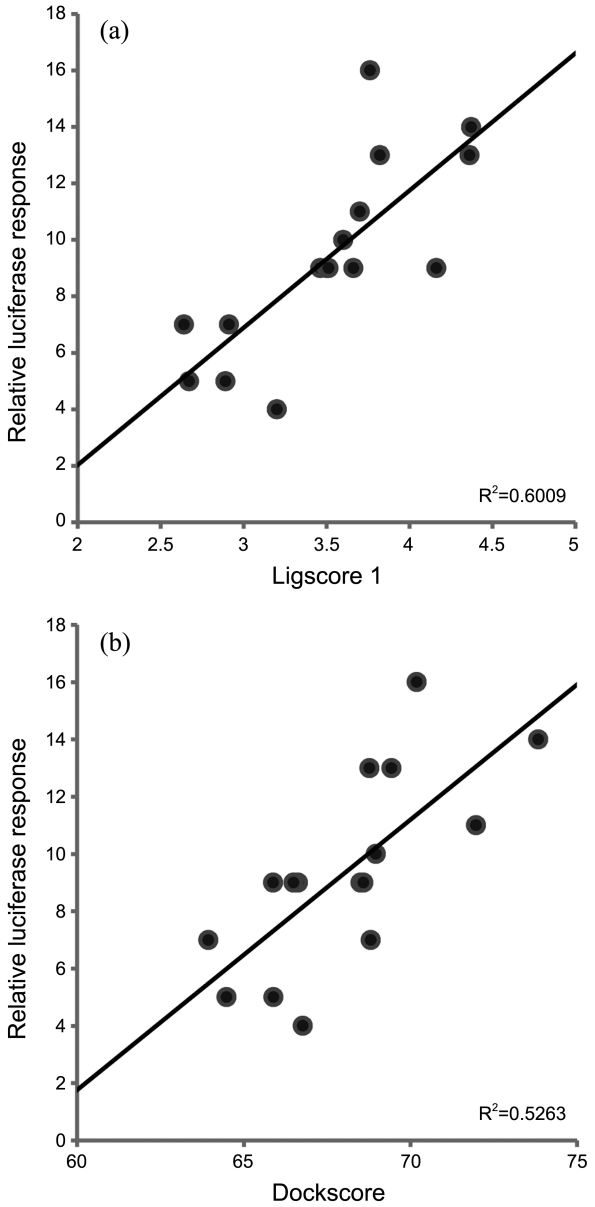

Figure 4. Correlation plots of (a) LigScore1 and (b) Dockscore of 16 carborane ligands with their relative luciferase response of ER_α *in vitro*.

best. Although R^2 values are moderate, the combined methods were accurate enough to predict the binding modes of carborane ligands and their affinities to estrogen receptor.

Conclusion

In summary, docking and scoring function studies of carborane ligands with estrogen receptor were successfully performed. This is the first report on the *in silico* scoring function relationship studies of carborane-containing molecules with protein. Although computer-aided drug design has rapidly become a valuable tool to identify and design new chemical scaffolds, carborane-containing molecules have been a challenge to the research field of carborane molecular modeling. Although the Gaussian03/LigandFit and Chem3D/LigandFit methods need being developed and fine-tuned, they appear to be a facile and predictive docking methods of carborane ligands with a variety of proteins. They can be applied for designing novel carborane ligand structures specific to target protein as well as predicting their binding modes in the active site.

Acknowledgments. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2012R1A1A1010000 and No. 20110018933).

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