Carborane-containing urea-based inhibitors of glutamate carboxypeptidase II: Synthesis and structural characterization

Sihyun Youn a,1, Kyung Im Kim a,1, Jakub Ptacek b, Kiwon Ok a, Zora Novakova b, YunHye Kim a, JaeHyung Koo c, Cyril Barinka b,*, Youngjoo Byun a,d,*

a College of Pharmacy, Korea University, 2511 Sejong-ro, Jochiwon-eup, Sejong 339-700, South Korea
b Department of Brain Science, Daegu Gyeongbuk Institute of Science & Technology, 50-1 Sang-Ri, Hyeonpung-Myeon, Dalseong-Gun, Daegu 711-873, South Korea
c Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Laboratory of Structural Biology, Vídeňská 1083, 14220 Prague 4, Czech Republic
d Biomedical Research Center, Korea University Guro Hospital, Korea University, Seoul, South Korea

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Glutamate carboxypeptidase II (GCPII) is a zinc metalloprotease on the surface of astrocytes which cleaves N-acetylaspartylglutamate to release N-acetylaspartate and glutamate. GCPII inhibitors can decrease glutamate concentration and play a protective role against apoptosis or degradation of brain neurons. Herein, we report the synthesis and structural analysis of novel carborane-based GCPII inhibitors. We determined the X-ray crystal structure of GCPII in complex with a carborane-containing inhibitor at 1.79 Å resolution. The X-ray analysis revealed that the bulky closo-carborane cluster is located in the spacious entrance funnel region of GCPII, indicating that the carborane cluster can be further structurally modified to identify promising lead structures of novel GCPII inhibitors.

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Carboranes are icosahedral clusters consisting of 2 carbon atoms, 10 boron atoms and 12 hydrogen atoms. Due to their unique physical and chemical properties such as high lipophilicity, thermal stability and boron content, carboranes have been widely applied in medical science, material science and physical science.1–6 In the field of medical science, carboranes have been long considered to be attractive boron-carriers for boron neutron capture therapy (BNCT) in the treatment of devastating cancers such as glioblastoma, melanoma, and head and neck cancer.7–9 Due to high boron content, resistance to metabolism, and diverse chemical transformations, carboranes have been conjugated with targeting agents such as carbohydrates and nucleosides for BNCT.10–16 More recently, closo-carboranes have been utilized as surrogates of lipophilic scaffolds for the identification and discovery of novel bioactive molecules.17–21 The hydrophobic closo-carboranes have successfully replaced the cyclohexyl or phenyl ring of various nuclear receptor ligands.22 These include the vitamin D receptor, retinoid receptor, estrogen receptor, and androgen receptor ligands.23–27 The binding affinities of carborane-containing ligands for their respective target proteins were maintained or enhanced when the carborane cluster was introduced in place of the lipophilic moiety.23–27

Glutamate carboxypeptidase II (GCPII) is a type II zinc-dependent metalloprotease which catalyzes the cleavage of N-acetylaspartylglutamate (NAAG) to produce N-acetylaspartate (NAA) and glutamate (Glu) in the brain.28 It is also highly expressed in androgen-independent metastatic prostate cancer and is referred to as prostate-specific membrane antigen (PSMA). PSMA is now considered to be an excellent biomarker in the diagnosis of advanced prostate cancer. For the last two decades, a number of PSMA-targeted inhibitors have been successfully discovered and translated into clinical studies for the imaging and therapy of prostate cancer.29–32 However, the obstacles present in the imaging and therapy of CNS diseases targeting GCPII in the brain due to low penetration of hydrophilic GCPII inhibitors across the blood–brain barrier (BBB), still need to be overcome.

To date, there have been very few reports on the X-ray crystal structure of carborane-containing ligands in complex with the cognate proteins, although the carborane cluster has been widely introduced into pharmacologically active molecules in drug design and medicinal chemistry.33–37 The determined X-ray crystal structures of protein–carborane complexes are dihydrofolate reductase (DHFR) with 2,4-diamino-5-(1-o-carboranylmethyl)-6-methylpyrimidine (PDB ID: 2C2S),33 HIV protease with cobalt bis(1,2-dicarbollide) (PDB ID: 1Z7Z),34 vitamin D receptor (VDR) with (R)- and (S)-stereoisomers of carborane-based ligand (PDB ID: 3VJT),35 and...
carbonic anhydrase with 1-(sulfamido)methyl-1,2-dicarba-closo-dodecaborane analogs (PDB ID: 4MDG).\textsuperscript{36,37} X-ray structures of GCPII–ligand complexes identified two principal specificity pockets that contribute prominently to interactions between the enzyme and its inhibitors/substrates. The S1’ (or pharmacophore) pocket serves as a glutamate-recognition site ensuring high specificity as well as affinity for glutamate-containing small-molecules that serve as GCPII-specific inhibitors.\textsuperscript{38,39} The most prominent feature of the S1 pocket, which is located in the entrance funnel of GCPII, is the presence of a positively charged arginine patch and electrostatic interactions between arginine guanidinium groups and negatively charged P1 functionalities of inhibitors contribute markedly to their affinity towards GCPII.\textsuperscript{38,40} In addition, the ‘S1 hydrophobic accessory pocket’ and the arene-binding site were identified as important structural features that could be used in the design of high affinity GCPII-specific probes.\textsuperscript{41} Here, we determine and analyze the X-ray crystal structure of GCPII in complex with a carborane-containing ligand, and further discuss the interaction between the carborane-based inhibitor and the GCPII protein.

Since 2-(phosphonomethyl)pentanedioic acid (2-PMPA) was published as a GCPII inhibitor for the first time in 1996, a great number of GCPII inhibitors have been reported.\textsuperscript{42} Based on functional groups that interact with zinc ions in the active site, GCPII inhibitors are largely classified into phosphonate-, urea-, and thiol-based subclasses. Among urea-based GCPII inhibitors, DCIBzL (Fig. 1) exhibited unique binding mode in the active site of GCPII. The additional S1 accessory pocket with its maximum observed size of 7 Å × 8.5 Å × 9 Å could accommodate the 4-iodophenyl group of DCIBzL, thus contributing to its extremely high affinity for GCPII.\textsuperscript{43} Structural studies suggested that this accessory pocket can have variable size, that is, defined by the concerted repositioning of the Arg536, Arg534 and Arg463 side chains. At the same time, it is not clear, whether an even bigger opening of this pocket is possible, allowing for the accommodation of even larger hydrophobic moieties. To test this hypothesis as well as to synthesize more lipophilic GCPII inhibitors than DCIBzL, we designed and synthesized two novel carborane-based GCPII inhibitors, where the p-iodobenzene ring of DCIBzL was replaced with a closo α- or m-carborane cluster.

Figure 1. Chemical structure of DCIBzL and carborane-containing GCPII inhibitors 1 and 2.

Scheme 1. Synthesis of carborane-containing GCPII inhibitors. Reagents and conditions: (a) 1,2-dicarba-closo-dodecaborane-1-carboxylic acid, HATU, TEA, DMF, rt, 12 h, 30%; (b) 1,7-dicarba-closo-dodecaborane-1-carboxylic acid, HATU, TEA, DMF, rt, 12 h, 25%; (c) TFA/CH₂Cl₂, rt, 4 h, 44% for 1 and 32% for 2.
The synthetic scheme for the preparation of the carborane-containing GCPII inhibitors is outlined in Scheme 1. Lys-urea-Glu scaffold 3 was synthesized from commercial protected \( \text{L-lysine} \) and \( \text{L-glutamic acid} \) in 3 steps by applying a previously published method.\(^4\) Another starting materials, the carboxylic acids of closo \( \text{o-carborane} \) and \( m \)-carborane were synthesized in 1 step by using the reported synthetic procedure (see Supplementary data).\(^4\) Briefly, treatment of closo \( \text{carborane} \) with \( n \)-BuLi, followed by the addition of dry ice, afforded \( o \)-, \( m \)-carboranyl acid in 70% and 72% yield, respectively. Reaction of 3 with \( o \)- and \( m \)-carboranyl acids under the traditional peptide coupling conditions (HATU and DIPEA in DMF) afforded 4 and 5 in moderate yield, respectively. The low coupling yield in both cases was due to the bulkiness of closo-carboranyl acid which prevented the carboxylic acid group being activated by HATU. Deprotection of the \( t \)-butyl groups of 4 and 5 was achieved by the treatment of TFA at room temperature for 4 h to give the corresponding ligands 1 and 2, respectively. The compounds 1 and 2 were fully analyzed by \( ^1 \text{H} \) NMR, \( ^{13} \text{C} \) NMR, HPLC and ESI-MS. The ESI-MS spectra for 1 and 2 showed a \([\text{M}--\text{H}]^-\) peak at 488.3 in the negative mode with the typical isotope distribution pattern of the carborane cluster (see Supplementary data).

To compare the relative lipophilicity of 1 and 2 with DCIBzL, the retention time of the compounds were determined by reversed-phase HPLC with an isocratic mode (65% water/35% acetonitrile, 0.1% formic acid). Compounds 1 and 2 were eluted at 11.37 and 11.38 min, respectively, while DCIBzL was collected at 4.02 min (see Supplementary data). This result indicated that the closo-carborane cluster is more lipophilic than the 4-iodobenzene ring, resulting in

<table>
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<th>Amino acids</th>
<th>DCIBzL</th>
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<tr>
<td>Arg210</td>
<td>2.72</td>
<td>2.66</td>
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<tr>
<td>Asn257</td>
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<td>2.96</td>
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<tr>
<td>Tyr700</td>
<td>2.62</td>
<td>2.55</td>
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Figure 2. In vitro GCPII inhibition of DCIBzL and compounds 1–2.

Figure 3. Comparison of the binding modes of DCIBzL and 1 in the internal pocket of human GCPII. Complexes of the GCPII/inhibitor were superimposed on to corresponding Cα atoms of the enzyme. Inhibitors are in stick representation, with atoms colored red (oxygen), blue (nitrogen), violet (iodine), and pink (boron). Carbon atoms are colored green (compound 1) and yellow (DCIBzL). The zinc ions are shown as orange spheres and selected parts of GCPII are shaded gray.

Figure 4. Interactions between compound 1 and key amino acid residues in the active site of GCPII.
the delayed retention time of 1 and 2 as compared with DCIBzL. With the aim of characterizing compounds 1 and 2 as surrogates of DCIBzL, the interaction pattern between the closo-carborane cage and the residues lining the entrance funnel of GCPII was investigated. This data could in turn be used for the rational design of carborane-based bioactive molecules. To this end, we first determined inhibition constants for the carborane-containing inhibitors using a fluorescence polarization assay and the acquired data are shown in Figure 2. The IC\textsubscript{50} values for compounds 1 and 2 were 20.5 nM and 15.6 nM, respectively, while the IC\textsubscript{50} for DCIBzL as a control was determined to be below 1 nM which is the detection limit of the assay. In fact, earlier studies using a fluorescence- or radiometry-based assay determined inhibition constants for the carborane-containing inhibitors of GCPII. The X-ray crystal structure of GCPII in complex with the carborane-based inhibitor was determined at 1.79 Å resolution. As the bulky closo-carborane cluster is located in the spacious entrance funnel region of GCPII, the current carborane-containing compounds can be further structurally modified to identify promising lead structures of novel GCPII inhibitors.

In summary, we designed and synthesized novel carborane-containing urea-based inhibitors of GCPII. The X-ray crystal structure of GCPII in complex with the carborane-based inhibitor was determined at 1.79 Å resolution. As the bulky closo-carborane cluster is located in the spacious entrance funnel region of GCPII, the current carborane-containing compounds can be further structurally modified to identify promising lead structures of novel GCPII inhibitors.

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Supplementary data

Supplementary data (experimental procedures and analytical data of final compounds 1–2 and intermediates 4–5) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.09.062.

References and notes