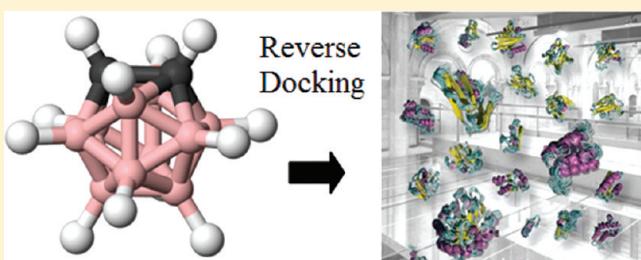


In Silico Carborane Docking to Proteins and Potential Drug Targets

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ABSTRACT: The presence of boron atoms has made carboranes, $C_2B_{10}H_{12}$, attractive candidates for boron neutron capture therapy. Because of their chemistry and possible conjugation with proteins, they can also be used to enhance interactions between pharmaceuticals and their targets and to increase the *in vivo* stability and bioavailability of compounds that are normally metabolized rapidly. Carboranes are isosteric to a rotating phenyl group, which they can substitute successfully in biologically active systems. A reverse ligand–protein docking approach was used in this work to identify binding proteins for carboranes. The screening was carried out on the drug target database PDTD that contains 1207 entries covering 841 known potential drug targets with structures taken from the Protein Data Bank. First, for validation, the protocol was applied to three crystal structures of proteins in which carborane derivatives are present. Then, the model was applied to systems for which the protein structure is available, but the binding site of carborane has not been reported. These systems were used for further validation of the protocol, while simultaneously providing new insight into the interactions between cage and protein. Finally, the screening was carried out on the database to reveal potential carborane binding targets of interest for biological and pharmacological activity. Carboranes are predicted to bind well to protease and metalloprotease enzymes. Other carborane pharmaceutical targets are also discussed, together with possible protein carriers.



■ INTRODUCTION

The dicarba-*closو*-dodecaboranes (carboranes)¹ are icosahedral carbon-containing boron clusters with noteworthy characteristics and properties such as resistance to catabolism, kinetic inertness to reagents, and strong hydrophobicity. They are now recognized as offering an important opportunity in medicinal chemistry^{2–5} and representing a potentially rich but underutilized class of pharmacophores.⁶ The regioselectivity and ease of derivatization of carborane allows facile syntheses of a wide variety of structures⁷ that can be exploited to enhance the selectivity of bioactive molecules.^{2–7}

The medicinal chemistry of carboranes has traditionally centered on their use in boron neutron capture therapy (BNCT),⁸ a binary method for the treatment of cancer^{9,10} in which a nuclear reaction takes place only inside those cells that have accumulated boron compounds and does not damage the surrounding tissue. Carboranes are promising candidates for BNCT because of the large number of boron atoms in the molecule, the extremely high stability of the clusters, and their rather low toxicity. Recent developments have extended the boron carrier approach for BNCT, and carboranes have been utilized as “prosthetic groups” for radiohalogens in the design and synthesis of radiotherapeutics and imaging agents.^{5,11} In fact, the cages are readily halogenated, and the boron–halogen bonds of these structures appear to be less susceptible to *in vivo* cleavage than carbon–halogen bonds.^{12,13}

Carboranes can also be used to enhance hydrophobic interactions between pharmaceuticals and their targets and to increase the *in vivo* stability, and hence bioavailability, of compounds that

are normally metabolized rapidly. The carborane cage has a diameter of about 5.5 Å, which is just a little larger than a rotating phenyl group (ca. 4.7 Å). In addition to direct conjugation to a pendent functionality, carboranes can therefore be introduced instead of specific aryl groups. For this reason, carboranes have been used as bioisosteric replacements for (hetero)aromatic and (hetero)aliphatic rings and other bulky entities in the design and synthesis of carboranyl derivatives of various amino acids and peptides;¹⁴ estrogen receptor modulators;^{15–18} radiopharmaceutical targets;¹⁹ androgen receptor antagonists;^{20–23} retinoids;²⁴ benzolactamic protein kinase C inhibitors;²⁵ and substrates for thymidine kinases,^{26,27} thalidomide,²⁸ flufenamic acid,²⁹ diflunisal,²⁹ aspirin,³⁰ thrombin inhibitors,³¹ trimethoprim,³² and tamoxifen.³³ Many of these boronated derivatives display biological activities comparable, or even superior, to those of their nonboronated counterparts. In addition, carboranes and carborane derivatives have been found to be effective inhibitors of HIV-1 protease,^{34–36} inducers of 20S proteasome activity,³⁷ and modulators of human blood platelet function.³⁸

In an effort to expand the medicinal chemistry of carboranes, we have attempted to identify further biological targets for which they might prove beneficial. In this systematic computational work, we lay the ground for an approach that is able to account for the interactions between a carborane molecule and proteins.

Identification of proteins that are able to carry carboranes can reveal further possible applications of carboranes that range from

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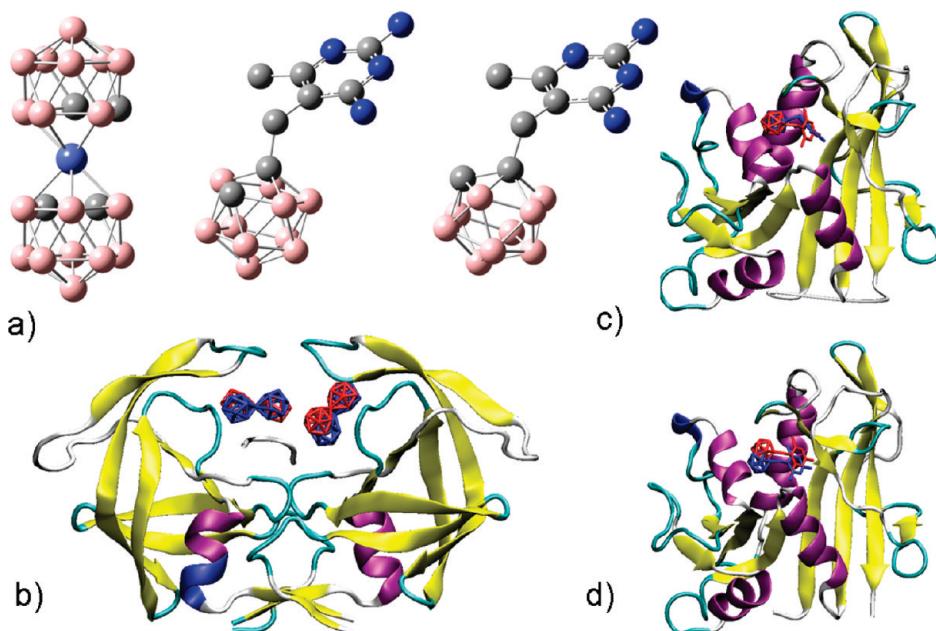


Figure 1. Carborane derivatives present in the PDB: (a) from left to right, cobalt bis(1,2-dicarbollide), [5-(1,2-closo-dicarbadodecarboran-1-yl)methyl]-2,4-diamino-6-methylpyrimidine, and [5-(7,8-nido-dicarbaundecaboran-7-yl)methyl]-2,4-diamino-6-methylpyrimidine; (b) crystal structure of HIV-1 PR (PDB code 1ZTZ) with cobalt bis(dicarbollide) (red molecules) superimposed with the best carborane docking poses (blue molecules); (c) crystal structure of human DHFR (PDB code 2C2S) complexed with [5-(1,2-closo-dicarbadodecarboran-1-yl)methyl]-2,4-diamino-6-methylpyrimidine (red molecule) superimposed with the best carborane docking pose (blue molecule); (d) crystal structure of human DHFR (PDB code 2C2T) complexed with [5-(7,8-nido-dicarbaundecaboran-7-yl)methyl]-2,4-diamino-6-methylpyrimidine (red molecule) superimposed with the best carborane docking pose (blue molecule).

the development of improved carborane bioconjugates for therapy (BNCT) and imaging to the direct use of carboranes as drugs that inhibit specific protein functions.

■ RESULTS AND DISCUSSION

Typical techniques to investigate biosystems are database screening and docking.^{39–42} These techniques have been of help in drug design. The docking of carboranes with proteins is potentially simple to describe, compared to the docking of other molecules, because of the structural rigidity of carboranes. A reverse ligand–protein docking approach^{43–47} can identify possible binding proteins for a specific molecule, such as the carborane. This is the opposite of a “direct” docking approach that identifies binding molecules for a specific protein. Very recently, the predictive ability of this protocol was demonstrated in a search for fullerene-binding proteins.^{46,47}

An important database of protein structures is the drug target database PDTD.⁴⁸ PDTD contains 1207 entries that cover 841 known and potential drug targets with structures taken from the Protein Data Bank (PDB) and is representative of the entire PDB.

After validation, the reverse docking protocol was applied to PDTD.

Phase 1, Carborane@Protein Structures. In the Protein Data Bank (PDB) are three crystal structures containing carborane derivatives. They can be used for validation of the protocol.

The first carborane–protein complex structure determined by protein crystallography was the structure of the cobalt bis(1,2-dicarbollide) complexed with HIV protease (PDB code 1ZTZ).³⁴ Two molecules of the compound bind to the hydrophobic pockets in the flap-proximal region of the S3 and S3' subsites. The compounds block the flap closure in addition to filling the corresponding binding pockets.³⁴

The other two carborane–protein structures were the complexes of human dihydrofolate reductase (DHFR) with lipophilic 2,4-diamino-6-methylpyrimidine antifolates derivative containing closo-o- and nido-o- icosahedral carborane.³²

Figure 1 shows the results of the calculations. The protocol identifies the carborane binding pocket in the proteins with great accuracy. In red are the molecules in the crystallographic positions. In blue are the best poses calculated by the protocol, which is able to recognize the two binding sites of cobalt bis(1,2-dicarbollide) in the hydrophobic pockets of the flap-proximal region of HIV PR³⁴ (Figure 1b) and the unique orientations of the icosahedral carborane derivatives when they bind to DHFR.³² All three crystallographic complexes investigated here appear in the top-ranked poses of our protocol. The performance of this docking procedure is better than that found for other docking protocols⁴⁹ and attests to the accuracy of the procedure.

Importantly, the search space is usually defined by the collection of amino acids within a certain distance from the bound ligand. This definition strongly reduces the conformational space for docking. In the present protocol, the whole structure of the protein was used, the search was carried out on the global surface of the protein (blind test), and the automatic identification of the carborane binding pocket was required to be considered as highly significant. Reproduction of the experimental binding pose is an important confirmation of the reliability of the procedure.

Phase 2, Carborane@Protein Interactions. A few examples of experimental characterization of proteins interacting with carborane derivatives have been published. In these systems, the protein structure is available, but the site of binding of carborane has not been reported with certainty, and only biochemical and SAR (structure–activity relationship) data relative to carborane derivatives are available. These systems can be used

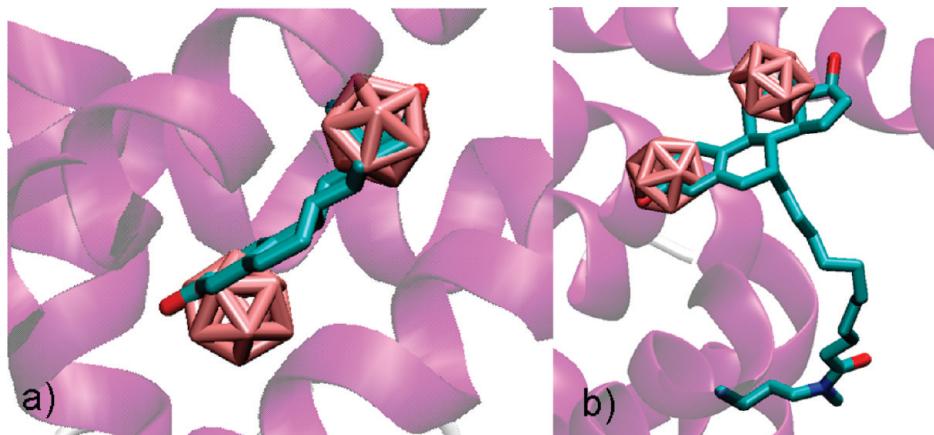


Figure 2. (a) Crystal structure of ER- α (PDB code 1PCG) with estradiol superimposed with the best carborane docking. (b) Crystal structure of ER- β (PDB code 1HJ1) with *N*-butyl-11-[(7*R*,8*R*,9*S*,13*S*,14*S*,17*S*)-3,17-dihydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-deahydro-6*H*-cyclopenta[*a*]phenanthren-7-yl]-*N*-methyldodecanamide (pure antagonist ICI 164,384) superimposed with the best carborane docking poses.

for further validation of the protocol, while simultaneously providing new insight into the interactions between cage and protein.

Estrogen Receptor. The estrogen receptor (ER) is a member of the superfamily of ligand-dependent transcriptional factors.⁵⁰ Endogenous estrogen, 17 β -estradiol, plays an important role in the reproductive system, in bone maintenance, in the central nervous system, and in the cardiovascular system.⁵¹ The first step in the appearance of estrogenic activity is the binding of agonist ligands to ER- α and - β , resulting in a conformational change. The resulting ligand-bound ER then dimerizes, forms complexes with various cofactors, and binds to specific promoter elements of DNA to initiate gene transcription. Compounds that either induce or inhibit cellular estrogen responses have potential value as biochemical tools and candidates for drug development.⁵¹ Because the discovery of nonsteroidal estrogens, many estrogen agonists and antagonists have been developed as agents for regulating fertility, preventing and controlling hormone-responsive breast cancer, and postmenopausal hormone replacement.⁵¹

Binding of ligands to the ER ligand-binding domain (LBD) primarily requires a phenolic ring, which hydrogen bonds to Glu353 and Arg394 of the human ER (hER) LBD.¹⁸ It is also well-known that the secondary alcohol group of E2 interacts with His524 of hER.¹⁸ Binding of ligands to the ER ligand-binding domain also requires an appropriate hydrophobic group adjacent to the phenolic ring. The hydrophobic group must closely match the hydrophobic surface of the ER and serves to increase the binding affinity. The hydrophobic structure also plays a role as a scaffold, fixing the spatial positions of hydrogen-bonding functional groups.¹⁸ The C and D rings of the structure of the natural hormone, 17 β -estradiol, play an important role in stabilizing the ligand/receptor complex by hydrophobic interactions. The size of the carborane cage is appropriate for the hydrophobic skeletal structure in place of the C, D ring structure. Substitution of the two carbon atoms of the carborane isomers should allow for suitable fixation of the direction of the functional groups. Endo and co-workers carried out intensive work to characterize the interaction of ER with carborane derivatives.^{15–18}

The docking protocol fits the carborane cage into the estradiol binding pocket, in both ER- α ⁵² (Figure 2a) and ER- β ⁵³ (Figure 2b). On top of the well-known isosteric replacement of the C, D rings

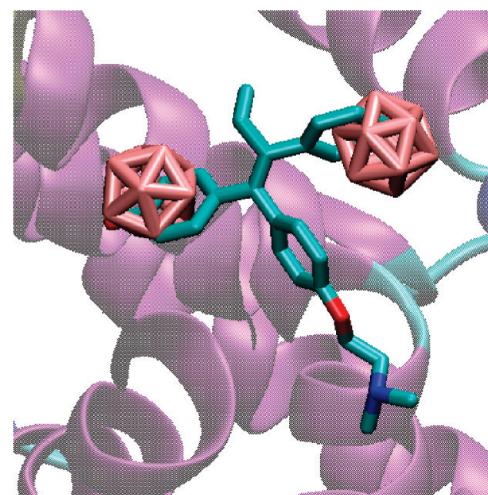


Figure 3. Crystal structure of ER- α (PDB code 3ERT) with 4-hydroxytamoxifen superimposed with the best carborane docking poses.

(best ranking score), our protocol suggests the possibility that carborane can be used to replace the A ring as well.

Very recently, Valliant and co-workers synthesized carborane analogues of tamoxifen, in which the A ring in the backbone of tamoxifen was replaced with an ortho carborane cluster.³³ The crystal structure of the human estrogen receptor hER- α bound to tamoxifen is known.⁵⁴ Tamoxifen is currently used as a frontline treatment in cases of hormone-dependent breast cancer. The agent is described by the World Health Organization (WHO) as an essential drug for the treatment of breast cancer.⁵⁵ The carborane tamoxifen analogue has greater stability toward degradation than tamoxifen itself and the results of a cell proliferation assay indicated that it shows similar inhibition.³³

If we compare the crystal structure of the complex of tamoxifen and ER- α ⁵⁴ with our docking results (Figure 3), the predictive ability of the protocol is strongly supported. In fact, in the best poses, carborane recognizes the A- and C-ring binding pockets, explaining the activity of the A-ring carborane analogue of tamoxifen and suggesting other possible improvements in the design of C-ring analogues.

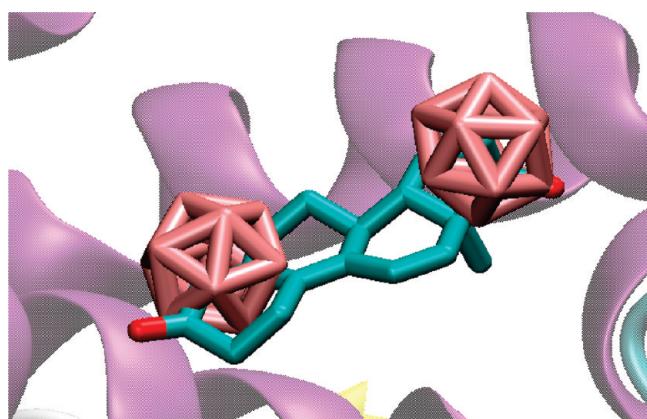


Figure 4. Crystal structure of human AR (PDB code 1E3G) with metribolone superimposed with the best carborane docking poses.

Androgen Receptor. The androgen receptor (AR) is a member of the nuclear receptor superfamily of ligand-regulated transcription factors⁵⁶ and plays a key role in the development and maintenance of the male reproductive system.⁵⁷ Physiological actions, such as prostate enlargement, body hair growth, acne, and muscle development, are initiated by the binding of the steroid hormones testosterone and/or 5 α -dihydrotestosterone to the AR, and an intricate machinery, involving translocation of AR into the nucleus, binding to specific DNA sites, formation of transcriptional complex, and activation of the expression of specific genes, begins to work.⁵⁷ AR ligands have been applied clinically; for instance, AR agonists are used for the treatment of aplastic anemia and AR antagonists for prostate cancer.⁵⁸ Recently, Endo and co-workers presented a new class of nonsteroidal androgen antagonists with a carborane cage in place of the steroid C, D rings of endogenous AR ligands.^{20–23} It was suggested that hydrophobic interaction of the carborane structure with the hydrophobic region of the AR ligand-binding pocket might account for the high binding affinity to AR.^{20–23}

As in the estrogen receptor, in the best poses, the carborane position (Figure 4) matches the crystallographic A and C rings of bound steroid, confirming the possibility of exploiting carborane analogues in the design of androgen receptor agonists or antagonists.

Retinoic Acid Receptors (RARs) and Retinoid X Receptors (RXRs). Retinoids modulate various biological functions, such as cell differentiation, cell proliferation, and embryonic development in vertebrates,^{59,60} by binding to and activating two types of specific nuclear receptors, namely, retinoic acid receptors (RARs)⁶¹ and retinoid X receptors (RXRs).⁶² RARs and RXRs each have three subtypes, α , β , and γ , and their endogenous ligands are all-trans retinoic acid (t-RA) and 9-cis-retinoic acid (9-cis RA). The availability of three-dimensional structural information has revealed the structural requirements for the appearance of retinoidal activity. High binding affinity for RAR and RXR requires a carboxylic acid moiety and an appropriate hydrophobic group. In addition, the role of the linking group between the hydrophobic and hydrophilic pharmacophores at the ends of the molecule is critical for the appearance of biological activity, especially for subtype selectivity of RARs and selectivity between RARs and RXRs.

Endo and co-workers exploited the hydrophobic characteristics of carborane to synthesize retinoid agonists and antagonists with a substituted carborane.²⁴

Our procedure recognizes that the primary site of docking to RAR and RXR (Figure 5) is that recognized by the cyclohexene ring of the retinoic system, more specifically, close to the position of the C1 atom that is characterized by a double methyl substitution to form the most hydrophobic part of the molecule.

The second site of interaction of carborane is different for RARs and RXRs. Figure 10 (below) shows that the carborane can match the C12 atom region of RARs (Figure 10a), whereas in RXRs, it fits the region of the C=C double bond in the cis configuration (Figure 10b). Only 9-cis RA is the cognate ligand of RXRs, whereas both 9-cis RA and t-RA compete for binding to the RARs in a common binding site. Selective binding is important to activate differential retinoic-responsive gene pathways. Our docking study suggests that carborane derivatives can be used in the development of specific agonists or antagonists of RARs and RXRs.

Transthyretin. Transthyretin (TTR) is a 55-kDa homotetrameric protein comprising 127 amino acids with an extended β -sheet conformation. TTR is found in human plasma (0.2 mg/mL, 3.6 μ M tetramer), where it binds and transports thyroxine (T4) in two funnel-shaped binding sites defined by the dimer–dimer interface.^{63,64} TTR has been implicated in a variety of amyloid-related diseases. Studies have indicated that the mechanism of TTR amyloid fibril formation proceeds through tetramer dissociation to a monomeric intermediate that subsequently aggregates to form the pathogenic amyloid fibrils.⁶⁵ Investigations have focused on small-molecule inhibitors that can stabilize the tetrameric structure.⁶⁶ A variety of nonsteroidal antiinflammatory drugs (NSAIDs) and structurally related derivatives kinetically stabilize TTR, thus inhibiting its dissociative fragmentation and subsequent aggregation to form putative toxic amyloid fibrils.⁶⁶ Hawthorne and co-workers synthesized and assessed transthyretin amyloidosis inhibitors that contained carborane pharmacophores.²⁹ These molecules are analogues of diflunisal and flufenamic acid, two of the most promising compounds in this field. The hydrophobic binding channels of TTR seem ideally suited for the utilization of carboranes as a skeletal core. Crystal structures of TTR indicate that the funnel-shaped T4-binding site can form a spacious outer binding pocket, large enough to bind sterically bulky substituents, and a smaller inner pocket. Hawthorne hypothesized that the carborane could fill the outer pocket, while maximizing hydrophobic interactions.

Structure–activity relationship studies of these carborane-containing systems indicated that the carborane is able to enter the TTR-binding channel and inhibit dissociation. The binding is governed by carboxylate interaction of inhibitors with the ammonium groups of Lys15, and the best performance was shown by a carborane derivative in which the carboxylic acid functionality was α with respect to the carborane cage. The substitution of a carborane moiety with a phenyl ring in NSAIDs with known TTR activity retains the TTR potency of the compound.

Our docking studies (Figure 6) showed that multiple favorable pockets are able to accommodate the icosahedral cage of carborane. They are located in the vicinity of the aromatic ring, in the entrance of the inner pocket, or even at the end of the funnel. This finding implies the possibility of further optimizing the binding of these important inhibitors that contain carborane pharmacophores.

Phase 3, Breaking New Ground. Screening of a protein database with the docking procedure can reveal potential carborane binding targets of interest for biological and pharmacological activity. A reasonable threshold for the reliable prediction

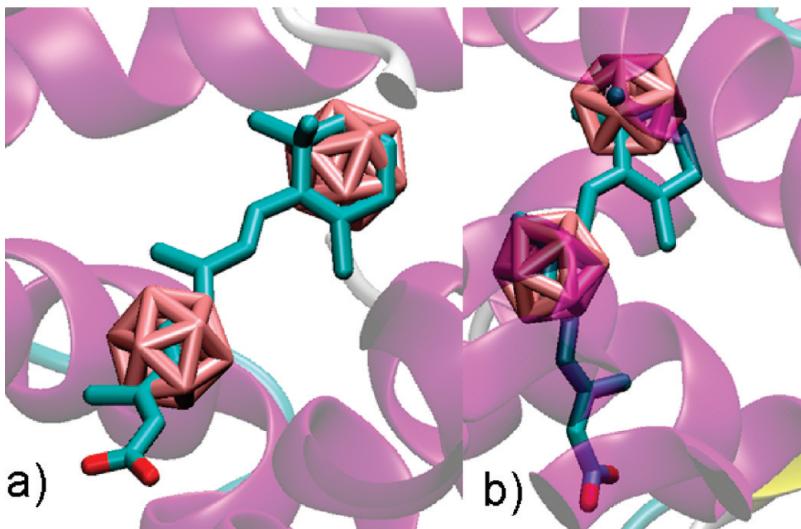


Figure 5. (a) Crystal structure of RAR (PDB code 2LBD) with all-trans retinoic acid superimposed with thecarborane best docking poses. (b) Crystal structure of RXR (PDB code 1K74) with 9-cis-retinoic acid superimposed with the best carborane docking poses.

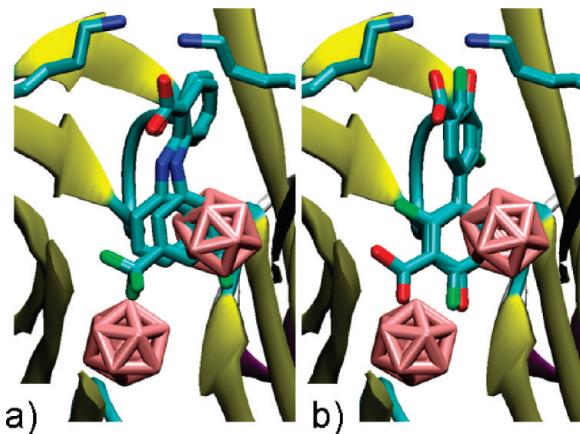


Figure 6. (a) Crystal structure of TTR (PDB code 1BM7) with flufenamic acid superimposed with the best carborane docking poses. (b) Crystal structure of TTR (PDB code 3D2T) with diflunisal superimposed with the best carborane docking poses.

of good targets in the drug target database (PDTD) can be set by selecting the top 10% of the most binding proteins,^{46,66} which forms a set of about 120 proteins. Because the ranking of the target protein is based on a scoring function that takes into account the binding energy between the carborane and the protein, proteins in the top positions should show a higher affinity toward the carborane than proteins in lower positions.

Table 1 presents the 10% most binding proteins as they were ranked by the reverse docking program employed in this work.

In the following subsections, the top five scorers are analyzed in detail.

Plasma Kallikrein. Rank 1 is occupied by plasma kallikrein.⁶⁷ Plasma kallikrein is a serine protease that has many important functions, including modulation of blood pressure and mediation and maintenance of inflammatory responses. Only the catalytic domain of plasma kallikrein has been determined.⁶⁷ The protease domain adopts a typical chymotrypsin-like serine protease conformation that consists of two juxtaposed β -barrels and two α -helices, with the active-site catalytic triad bridging the barrels.⁶⁸

The docking protocol identified two possible binding pockets. The first pocket is located between the β -barrel domains, on the opposite side of the active site (docked complex 1, Figure 7a,c). Considering the residues within a radius of 5 Å from the carborane cage, this site appears to be highly hydrophobic (Trp24, Trp27, Pro28, Trp29, Ile70, Leu71, Phe117, Leu155), and the icosahedral structure of the carborane fits perfectly in the pocket, without leaving empty spaces. The pocket consists of loop Ser23/Gln30 and residues Gly69, Ile70, Leu71, Phe117, and Leu115.

The second binding pocket is located in the S₁ site (docked complex 2, Figure 7b,e,f).⁶⁸ The S₁ site (Figure 7f) is adjacent to the catalytic Ser195 and is primarily responsible for substrate peptide recognition. It is a deep hydrophobic pocket formed by residues 189–192, 214–216, and 224–228. The carborane matches perfectly the crystallographic position of the phenyl ring of benzamidine in complex with kallikrein (Figure 7e). In addition to confirming the ability of the carborane as a possible bioisosteric replacement for aromatic rings, this result suggests the possibility of using carborane derivatives as kallikrein inhibitors. The possible use of carborane and carborane derivatives as specific protease inhibitors is discussed again below.

Dihydropyrimidine Dehydrogenase. Dihydropyrimidine dehydrogenase (DPD) catalyzes the first step in pyrimidine degradation, namely, the NADPH-dependent reduction of uracil and thymine to the corresponding 5,6-dihydropyrimidines.⁶⁹ Its controlled inhibition has become an adjunct target for cancer therapy, because the enzyme is also responsible for the rapid breakdown of the chemotherapeutic drug 5-fluorouracil. The crystal structure of the homodimeric pig liver enzyme reveals a highly modular subunit organization, consisting of five domains with different folds.

DPD homodimer contains two flavin adenine nucleotides (FADs), two flavin mononucleotides (FMNs), and eight [4Fe–4S] clusters, arranged in two electron-transfer chains that pass the dimer interface twice.

The four best poses of the carborane cage docked in DPD show the ability of carborane to be isosteric to the [4Fe–4S] cluster. The carborane recognizes and fits into the four [4Fe–4S]

Table 1. Top 10% of the Protein Target Candidates for Carborane Binding Identified by the Reverse Docking Procedure

rank	target details	therapeutic area	biochemical function	ID (PDB)
1	plasma kallikrein	—	enzyme	2ANY
2	dihydropyrimidine dehydrogenase	—	enzyme	1H7X
3	γ -chymotrypsin	gastrointestinal functions	enzyme	1GHB
4	hyperpolarization-activated, cyclic nucleotide-modulated (HCN) channels	—	transport proteins	1Q3E
5	mannose-binding lectin-associated serine protease-2 (MASP-2)	—	enzyme	1ZJK
6	actin	—	—	1NWK
7	phosphoinositide 3-kinase	—	—	1E8W
8	<i>p</i> -hydroxybenzoate hydroxylase/cyclooxygenase 1,2(COX-1,COX-1)	inflammation	enzyme	2PHH
9	glutamate mutase	blood and blood-forming organs	enzyme	1I9C
10	thiamin pyrophosphokinase	vitamins	enzyme	1IG3
11	triose phosphate isomerase	—	enzyme	8TIM
12	HIV-1 reverse transcriptase	viral infections	enzyme	1EET
13	ubiquitin thiolesterase-6	—	enzyme	2AYO
14	carboxypeptidase A	neoplasit diseases	enzyme	5CPA
15	matrix metalloproteinase (MMP-3) stromelysin-1	—	enzyme	1UEA
16	protoporphyrinogen IX oxidase	—	enzyme	1SEZ
17	pyruvate decarboxylase	vitamins	enzyme	1QPB
18	histone acetyltransferase Hat1	—	enzyme	1BOB
19	S-methyltetrahydrofolate-homocysteine methyltransferase, methionine synthase	blood and blood-forming organs	enzyme	1BMT
20	bone morphogenetic protein-7	—	factor, regulator, and hormones	1M4U
21	trypsin	hormones and hormone antagonists	enzyme	3TPI
22	prostaglandin H synthase-1 (PGHS-1)	—	—	1DIY
23	neurophysin 2	—	factor, regulator, and hormones	1JK4
24	thrombin heparinase	—	enzyme	1XMN
25	myosin light chain kinase	—	structural proteins	2BKH
26	dihydrofolate reductase	immunomodulation, neoplastic diseases	enzyme	1DR1
27	glucose-6-phosphate dehydrogenase	—	enzyme	2BH9
28	matrix metalloprotease 8	—	enzyme	1JAP
29	DXP reductoisomerase	—	enzyme	1JVS
30	tubulin	—	enzyme	1TUB
31	casein kinase-I γ -2	—	enzyme	2C47
32	cyclophilin 40/peptidyl-prolyl cis-trans isomerase	immunomodulation	enzyme	1IHG
33	polyamine oxidase	—	enzyme	1H82
34	caspase-7	—	enzyme	1K86
35	heat shock cognate 71 Kda	—	factor, regulator, and hormones	1HX1
36	methylthioadenosine phosphorylase	—	enzyme	1JP7
37	CDK2/cyclin A	—	enzyme	1O19
38	glycerol-3-phosphate dehydrogenase	—	enzyme	1EVZ
39	Fab (Igg2A)	immunomodulation	monoclonal antibodies	1IGJ
40	neutrophil collagenase, MMP8, neutrophil collagenase, MMP	neoplastic diseases	enzyme	1BZS
41	GDP fucose synthetase	—	enzyme	1E6U
42	carboxyl acceptor phosphotransferase, phosphoglycerate kinase	—	enzyme	3PGK
43	interleukin-1 β converting enzyme (ICE)	—	enzyme	1BMQ

Table 1. Continued

rank	target details	therapeutic area	biochemical function	ID (PDB)
44	human class I histocompatibility antigen	viral infections	enzyme	1HHJ
45	mitochondrial creatine kinase	—	enzyme	1CRK
46	type I inosine monophosphate dehydrogenase	—	enzyme	1JCN
47	L-isoaspartyl D-aspartyl O-methyltransferase	—	enzyme	1JG1
48	histone deacetylase-8	—	enzyme	1VKG
49	serine proteinase α-thrombin	blood and blood-forming organs	enzyme	1BMM
50	MHC class I H-2KB heavy chain	—	monoclonal antibodies	1OSZ
51	porcine pancreatic elastase	gastrointestinal functions	enzyme	1JIM
52	α-hydroxysteroid dehydrogenase	—	enzyme	1QSM
53	1-aminocyclopropane-1-carboxylate synthase 2	—	enzyme	1IAY
54	oxidosqualene cyclase	—	enzyme	1W6K
55	DNA-directed RNA Polymerase II 19 KDa polypeptide	neoplastic diseases	enzyme	1I6V
56	methionine γ-lyase	fungal infections	enzyme	1ESF
57	mycolic acid cyclopropane synthase	—	enzyme	1L1E
58	glucokinase	—	enzyme	1SZ2
59	β-galactosidase	—	enzyme	1TG7
60	monoamine oxidase B	synaptic and neuroeffector junctional sites and central nervous system	enzyme	1GOS
61	copper-containing amine oxidase	—	enzyme	1W6G
62	peptide N-myristoyltransferase	—	enzyme	1IIC
63	endonuclease III	—	enzyme	2ABK
64	type III chloramphenicol acetyltransferase	bacterial infections	enzyme	3CLA
65	acetolactate synthase	vitamins	enzyme	1OZF
66	stromelysin	—	enzyme	1QIA
67	aldehyde dehydrogenase	—	enzyme	1AG8
68	β-glucosidase	hormones and hormone antagonist	enzyme	1GNX
69	cytochrome C peroxidase	hormones and hormone antagonist	enzyme	1AEB
70	lipoxygenase-1	—	enzyme	1F8N
71	prophospholipase A2	—	enzyme	1HN4
72	prostaglandin G/H synthase 2, COX-2	inflammation	enzyme	1CX2
73	aconitate hydratase	neoplastic diseases	enzyme	1ACO
74	glucosylceramidase	—	enzyme	1OGS
75	anthocyanidin synthase	—	enzyme	1GP6
76	human rhinovirus 14	viral infections	enzyme	1HRI
77	pentosyltransferase	—	enzyme	1QE5
78	protein tyrosine phosphatase 1b	hormones and hormone antagonist	enzyme	1GFY
79	ATP binding cassette	—	structural proteins	1F3O
80	MAP KAP kinase 2	—	enzyme	1NY3
81	farnesoid X receptor	gastrointestinal functions	receptor	1OSH
82	aspartate aminotransferase, cytoplasmic, aspartate transaminase	synaptic and neuroeffector junctional sites and central nervous system	enzyme	1O4S
83	riboflavin synthase	—	enzyme	1I8D
84	human class I histocompatibility antigen	immunomodulation	monoclonal antibodies	1HHG
85	4–4–20 (IgG2A) Fab fragment	immunomodulation	monoclonal antibodies	4FAB
86	succinate dehydrogenase	—	enzyme	1NEK
87	peptidase T	—	enzyme	1FNO
88	3-methyl-2-oxobutanoate dehydrogenase (lipoamide)	hormones and hormone antagonist	enzyme	1OLS
89	carnitine acyltransferase	—	enzyme	1NM8
90	nitric oxide synthase	—	enzyme	1ED5
91	alcohol sulfotransferase	hormones and hormone antagonist	enzyme	1J99
92	3-dehydroquinate synthase	—	enzyme	1NRS
93	alanine racemase	bacterial infections	enzyme	1SFT
94	bovine mitochondrial F1-ATPase	—	enzyme	1EFR

Table 1. Continued

rank	target details	therapeutic area	biochemical function	ID (PDB)
95	adenosine phosphate deaminase	—	enzyme	2A3L
96	rho-associated kinase	blood and blood-forming organs	enzyme	1ETR
97	methionine aminopeptidase	—	enzyme	2B3K
98	DNA gyrase B subunit	bacterial infections	enzyme	1EI1
99	cytochrome P450BM-3	blood and blood-forming organs	enzyme	1BVY
100	pregnane X receptor	—	nuclear receptor	1ILH
101	human serum albumin	—	transport proteins	1E7B
102	caspase-1	—	enzyme	1M72
103	methionine adenosyltransferase	—	enzyme	1QM4
104	pyruvate dehydrogenase kinase-2	—	enzyme	2BUS
105	histone lysine methyltransferase	—	enzyme	1ZKK
106	cytochrome P ₄₅₀ 2C8	—	enzyme	1PQ2
107	5'-nucleotidase	—	enzyme	1USH
108	glycogen phosphorylase B	hormones and hormone antagonist	enzyme	1P4G
109	arginase II	renal and cardiovascular	enzyme	1PQ3
110	A-G adenine DNA glycosylase	—	enzyme	1WEI
111	fucosidase α	—	enzyme	1HL9
112	xanthine dehydrogenase	—	enzyme	1FO4
113	aromatic-L-amino-acid decarboxylase	hormones and hormone antagonist	enzyme	1JS3
114	dihydroorotate dehydrogenase	immunomodulation	enzyme	1OVD
115	poly(ADP-ribose) polymerase PARP	—	enzyme	2PAW
116	immunoglobulin lambda light chain dimer (Mcg)	immunomodulation	monoclonal antibodies	1MCJ
117	D-amino acid oxidase	—	enzyme	1VE9
118	aldose reductase	—	enzyme	2ACS
119	indole-3-pyruvate decarboxylase	vitamins	enzyme	1OVM
120	methyltransferase	—	enzyme	1R18

cluster binding pockets of the DPD monomer (Figure 8). Iron–sulfur [4Fe–4S] clusters are ubiquitous prosthetic groups that are required to sustain fundamental life processes such as electron transfer, substrate binding/activation, iron/sulfur storage, regulation of gene expression, and enzyme activity.⁷⁰ The effect of replacement of [4Fe–4S] clusters with carborane or carborane derivatives could be of great consequence.

γ-Chymotrypsin. Chymotrypsin is a digestive enzyme that catalyzes the hydrolysis of peptide bonds of proteins in the mammalian gut. It is secreted in the pancreas as chymotrypsinogen, a single-chain protein of 245 amino acids, and is activated to chymotrypsin by the hydrolysis of a single peptide bond, catalyzed by trypsin.⁷¹ Chymotrypsin preferentially cleaves peptide amide bonds in which the carboxyl side of the amide bond (the P₁ position) is a tyrosine, tryptophan, or phenylalanine. These amino acids contain an aromatic ring in their side chain that fits into a hydrophobic pocket (S₁ position) of the enzyme. The hydrophobicity and shape complementarity between the peptide substrate P₁ side chain and the enzyme S₁ binding cavity account for the substrate specificity of this enzyme.⁶⁸

Carborane replaces the Trp crystallographic position of the inhibitor N-acetyl-D-tryptophan in complex with γ-chymotrypsin (Figure 9) fitting in the S₁ hydrophobic pocket of the protein.⁷² As in kallikrein, carborane fits in the hydrophobic binding pocket of the protein more snugly than aromatic rings, which, in turn, suggests the possibility of exploiting the regioselectivity and ease of derivatization of carboranes to synthesize a wide variety of novel selective protease inhibitors.

Hyperpolarization-Activated, Cyclic Nucleotide-Modulated (HCN) Channels. The family of hyperpolarization-activated, cyclic nucleotide-modulated (HCN) channels is crucial for a variety of electrical signals, including cardiac and neuronal pacemaker activity, setting the electrical properties of resting membranes and dendritic integration.⁷³ These nonselective cation channels, underlying the I_b, I_h, and I_q currents of heart and nerve cells, are activated by membrane hyperpolarization and modulated by the binding of cyclic nucleotides such as cAMP and cGMP. The binding of cAMP to the cyclic nucleotide-binding domain (CNBD) accelerates the activation kinetics and shifts the voltage dependence of activation to more positive voltages. This modulation causes the channels to open faster and more completely in the presence of cAMP. The HCN2 C-terminal region is composed of two domains. The C-linker domain consists of six α-helices, designated A'–F', that are separated by short loops. The CNBD follows the C-linker domain and includes four α-helices (A, P, B, C) with a β-roll between the A- and B-helices. The β-roll comprises eight β-strands in a jelly-roll-like topology. Cyclic nucleotides are bound inside the jelly roll and interact with the β-roll and the C-helix.

The carborane fits in the cyclic nucleotide binding pocket (Figure 10), in particular, in the cyclical phosphate region, which interacts exclusively with the β-roll. The presence of the interaction between carborane and Arg591 is interesting. In the crystallographic structure, Arg591 is identified as the most important residue in the recognition of the phosphate of cAMP, and mutations of Arg591 have been shown to cause a large decrease

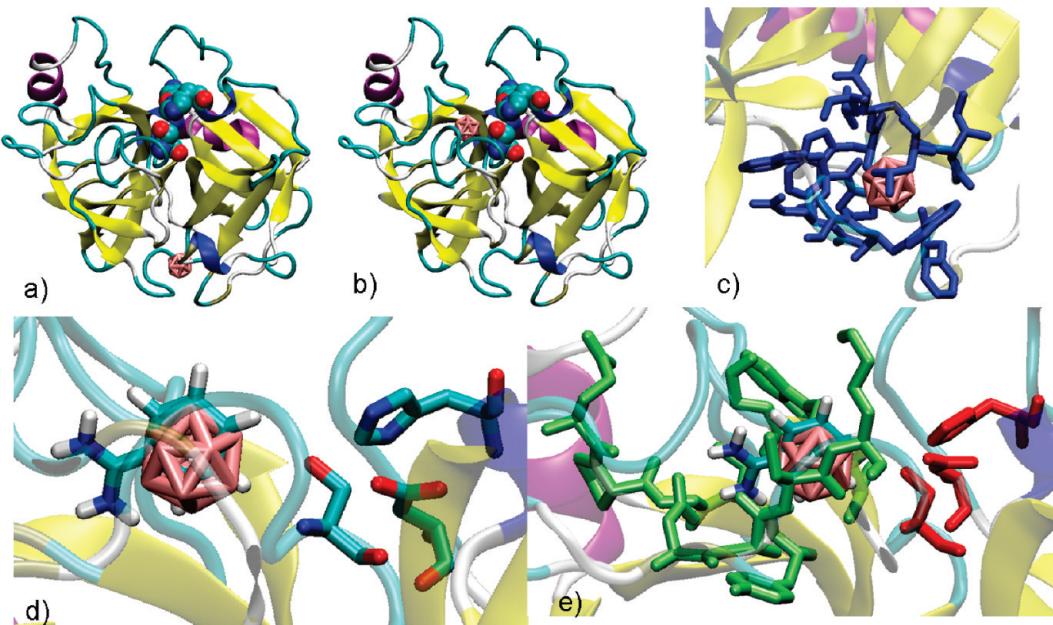


Figure 7. (a) Docked complex 1 of kallikrein (PDB code 2ANY) and carborane. (b) Docked complex 2 of kallikrein (PDB code 2ANY) and carborane (pink) in the licorice representation, with the catalytic triad of kallikrein in the WdW representation. (c) Closeup of binding pocket 1 (blue). (d) Crystal structure of kallikrein (PDB code 2ANY) with benzamidine superimposed with docking pose 2 of carborane. (e) Closeup of binding pocket 2. S₁ site in green; catalytic triad in red.

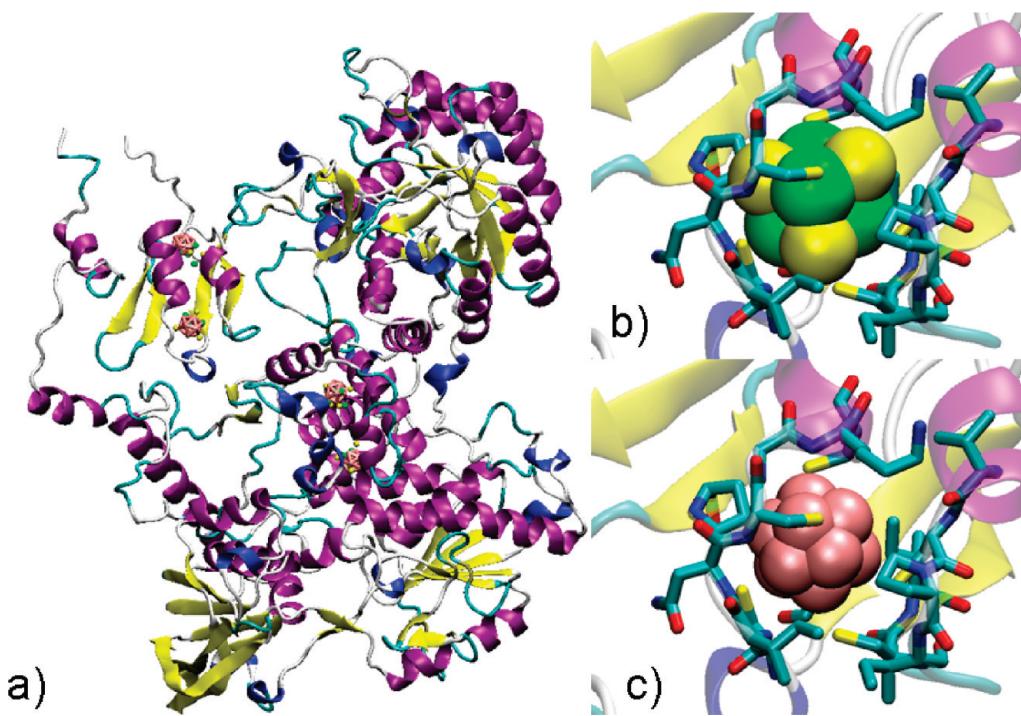


Figure 8. (a) Docked complex of carborane (four best poses) and DPD (PDB code 2ANY), with carborane (pink) in the licorice representation, and the [4Fe-4S] clusters in the CPK representation. (b) Closeup of a [4Fe-4S] cluster binding pocket. (c) Closeup of the same [4Fe-4S] cluster binding pocket occupied by carborane (best docking pose).

in HCN2 affinity for cAMP.⁷⁴ Because the synthesis of adenosine-containing carborane is available,⁷⁵ it might be possible to use this derivative to exploit the cAMP binding pocket region of the protein, making this family of compounds novel channel modulators for HCN channels an important pharmaceutical target.⁷⁶

Mannose-Binding Lectin-Associated Serine Protease-2 (MASP-2). Mannose-binding lectin (MBL) is the recognition subunit of the lectin pathway,⁷⁷ a group of blood proteins that mediates the specific antibody response. It provides the first line of defense against invading pathogens.⁷⁸ MBL binds to carbohydrate arrays

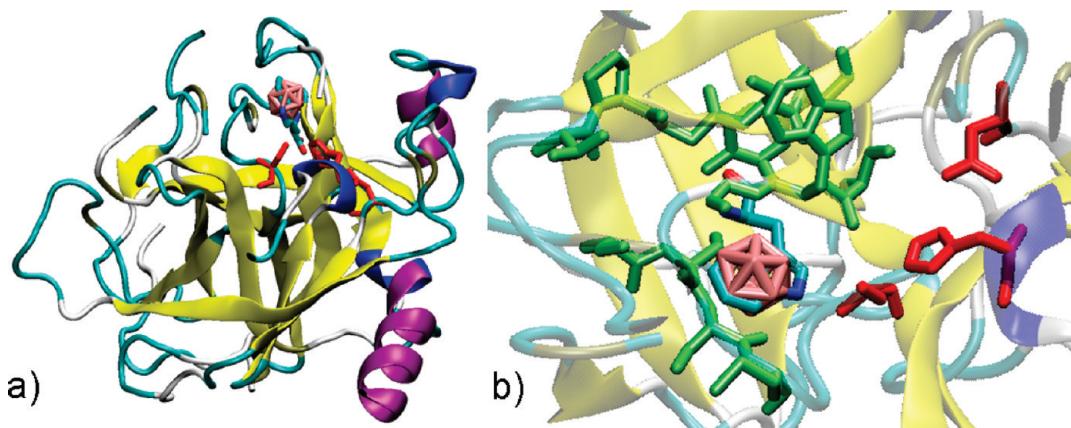


Figure 9. (a) Docked complex of carborane and chymotrypsin. (b) Closeup of the binding pocket. Crystal structure of chymotrypsin (PDB code 1GHB) with N-acetyl-D-tryptophan superimposed with the best carborane docking pose. S₁ site in green; catalytic triad in red.

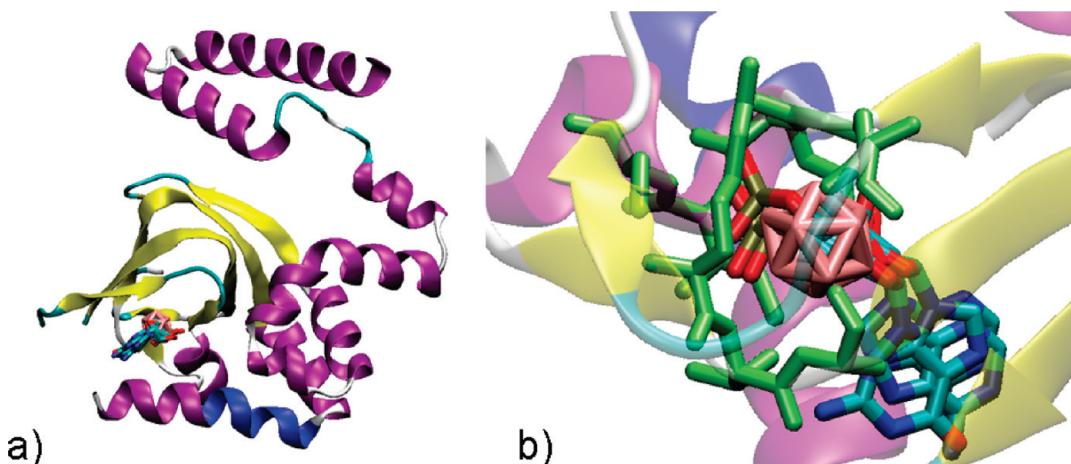


Figure 10. (a) Docked complex of carborane and the HCN channel. (b) Closeup of the binding pocket. Crystal structure of HCN channel with cGMP (cyclic guanine monophosphate) (PDB code 1Q3E) and cAMP (adenosine-3',5'-cyclic-monophosphate) (PDB codes 1Q5O, 1Q43) superimposed with the best carborane docking pose. Cyclical phosphate region binding pocket in green.

on the surface of pathogens, which results in the self-activation of MBL-associated serine protease-2 (MASP-2). MASP-2 is the only known MBL-associated protease that can directly initiate the complement cascade, playing a key enzymatic role in the lectin pathway. The C-terminus of MASP-2 has a structural domain typical of trypsin-like serine protease (SP), preceded by five noncatalytic modules.⁷⁷

Carborane is able to recognize and bind to the serine protease domain in proximity of the S₁ site. In the MASP-2 structure, the S₁ pocket is blocked by the basic side chain of Arg630 (a form of control of the autoactivation). In an enzyme with trypsin-like substrate specificity of the chymotrypsin family, a basic residue in this position is unusual. Arg630 in the zymogen structure inhibits the binding or adequate positioning of the P₁ basic residue of the substrate. Carborane binds in the proximity of this residue (Figure 11) in a hydrophobic pocket that can control the activation of the zymolytic activity of the enzyme.

Phase 4, Where Carboranes Can Be Most Effective, or the Case of Protease and Metalloprotease Enzymes. The analysis of Table 1 shows that carborane can bind to protease proteins. In the first five positions, three of the proteins are proteases: Plasma kallikrein ranks 1, γ -chymotrypsin ranks 3, and MASP-2 ranks 5.

Moreover, carboxypeptidase A ranks 14, matrix metalloproteinase (MMP-3) stromelysin-1 ranks 15, trypsin ranks 21, thrombin heparinase ranks 24, matrix metalloprotease 8 ranks 28, caspase-7 ranks 34, serine proteinase α -thrombin ranks 49, porcine pancreatic elastase ranks 51, peptidase T ranks 87, methionine aminopeptidase ranks 97, and caspase-1 ranks 102.

Protease enzymes selectively catalyze the hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein. Proteases are currently classified into six broad groups: aspartic, serine, cysteine, threonin, glutamic acid, and metallo protease.⁷⁹

Their control over protein synthesis, turnover, and function enables them to regulate physiological processes such as digestion, fertilization, growth, differentiation, cell signaling/migration, immunological defense, wound healing, and apoptosis.⁸⁰ Proteases are also crucial for disease propagation, and their inhibitors are emerging for promising therapeutic uses in the treatment of diseases such as cancers; parasitic, fungal, and viral infections (e.g., schistosomiasis, malaria, *C. albicans*, HIV, hepatitis, herpes); and inflammatory, immunological, respiratory, cardiovascular, and neurodegenerative disorders including Alzheimer's disease.⁸¹

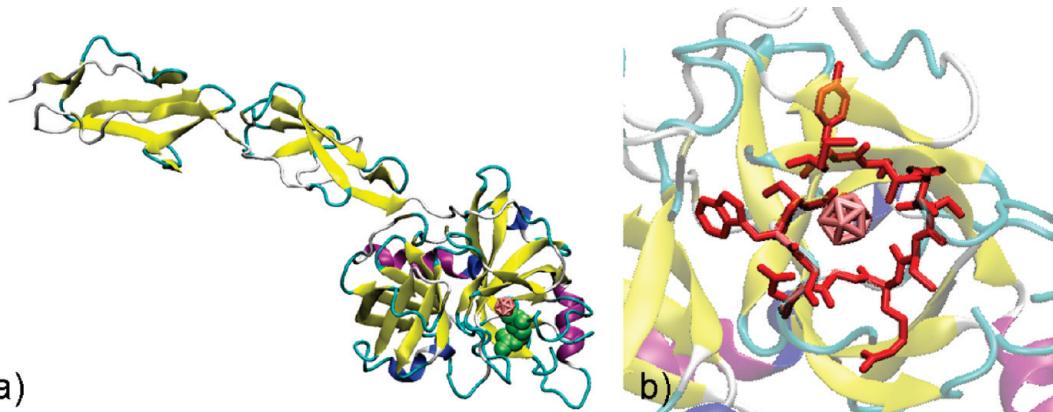


Figure 11. (a) Docked complex of carborane and MASP-2. (b) Closeup of the carborane binding pocket. S₁ site in red.

There are now many potent and selective protease inhibitors that slow or halt disease progression. To be effective as biological tools, protease inhibitors must not only be potent, but must also be highly selective in binding to a particular protease. As potential drugs, protease inhibitors must also have appropriate pharmacokinetic and pharmacodynamic properties.⁸¹

Carborane is an ideal target to achieve a new generation of inhibitors thanks to its peculiar properties: (i) Its higher hydrophobic pharmacophore performance, compared to that of aromatic rings, can improve the strength of the binding with hydrophobic regions of the enzyme. (ii) The regioselectivity and ease of derivatization of carboranes allows facile syntheses of inhibitors selective toward the different recognition sites of the various peptidases. (iii) The chemical stability and metabolic inertness of the carborane cage could overcome the common problem of the metabolic instability typical of protease inhibitors.

These results can extend the suggestions of Hawthorne and co-workers about the use of a substituted icosahedral carborane as a hydrophobic pharmacophore in an irreversible thrombin inhibitor³¹ and the work already in an advanced status of the development of carborane derivatives as inhibitors of HIV protease.^{34–36}

Phase 5, Carboranes As New Lead Compounds for Pharmaceutical Targets. From the analysis of Table 1, other interesting targets appear to be the following:

i. Kinases. Phosphoinositide 3-kinase ranks 7, thiamin pyrophosphokinase ranks 10, myosin light chain kinase ranks 25, casein kinase-I γ -2 ranks 31, CDK2/cyclin A ranks 37, phosphoglycerate kinase ranks 42, mitochondrial creatine kinase ranks 45, glucokinase ranks 58, MAP KAP kinase 2 ranks 80, rho-associated kinase ranks 96, and pyruvate dehydrogenase kinase-2 ranks 104. Endo and co-workers proposed the use of the carborane cage as a hydrophobic pharmacophore in the synthesis of protein kinase C modulators.²⁵ A number of diseases, including cancer, diabetes, and inflammation, are linked to perturbation of protein kinase-mediated cell signaling pathways.⁸² The human genome encodes some 518 kinases that share a catalytic domain conserved in sequence and structure but that are notably different in how their catalysis is regulated. The ATP-binding pocket is between the two lobes of the kinase fold. This site, together with less conserved surrounding pockets, has been the focus of inhibitor design that has exploited differences in kinase structure and pliability in order to achieve selectivity. Carborane might be the ideal target for a new generation of

specific inhibitors because of its hydrophobicity, ease of derivation, and chemical and metabolic stability.

*ii. Enzymes Involved in Prostaglandin Synthesis.*⁸³ p-hydroxybenzoate hydroxylase/cyclooxygenase 1,2 (COX-1, COX-1) ranks 8, prostaglandin H synthase-1 (PGHS-1) ranks 22, and prostaglandin G/H synthase 2 (COX-2) ranks 72. These enzymes are involved in relief from pain and inflammation, could prove useful in the chemo-prevention of cancer, and are also being investigated in a range of neurological diseases.⁸³ Preliminary studies showed that asborin, the carbaborane analogue of aspirin, inhibits both enzyme variants, COX-1 and COX-2.³⁰

iii. HIV-1 Reverse Transcriptase Ranks 12. Carborane compounds can be effective inhibitors at the same time of HIV-1 protease^{34–36} and HIV-1 reverse transcriptase.

iv. Histone Acetyltransferase, Hat1, Ranks 18. HATs are implicated in a number of diseases.⁸⁴ The impact of the various HATs on cellular physiology and diseases would greatly benefit from the identification of specific pharmacological inhibitors, but very few have been described to date.

v. Dihydrofolate Reductase Ranks 26. Inhibitors of this protein have found clinical use as antitumor, antimicrobial, and antiprotozoal agents.^{85,86} The high hydrophobicity of carborane can overcome a disadvantage of classical antifolates antitumor agents that require an active transport mechanism to enter cells, which, when impaired, causes tumor resistance.^{87,88} The hydrophobicity of carborane is an advantage for passing the cellular membrane, a further benefit in the development of a drug.^{87,88} The use of carborane derivative as dihydrofolate reductase was discussed in the past.³²

vi. Antibodies. Several antibodies seem to be able to recognize carborane. They are the antidigoxin monoclonal antibody 26-10 (rank 39), MHC class I H-2KB heavy chain (rank 50), human class I histocompatibility antigen (rank 84), 4-4-20 (IgG2A) Fab fragment (rank 85), and immunoglobulin lambda light chain dimer (Mcg) (rank 116). In principle, monoclonal antibody (mAB) conjugates can provide a general class of exquisitely selective boron carriers. mABs show enormous potential as biological tools in the diagnosis and treatment of human diseases.⁸⁹ The use of mABs to deliver drugs, radionuclides, and toxins has rapidly developed,⁸⁹ and the delivery of boron to specific sites through mAB conjugates is presently the subject of intensive investigation.^{90,91} Potentially, mABs could also be used for quantitative measurement of carborane during carborane treatment.

Last but Not Least. Position 101 is occupied by human serum albumin. Human serum albumin (HSA) is the most abundant protein in human blood plasma. It acts as a plasma carrier by nonspecifically binding several hydrophobic compounds. It was demonstrated that carborane derivatives can bind to serum albumin noncovalently.⁹² This implies that HSA can be used as carrier of highly hydrophobic carborane.

Table 1 can be a source for searches for other carborane-binding proteins. As with all predictions, a word of caution to the reader is that some false positive intruders might be present.

CONCLUSIONS

Investigation of the interactions of carboranes with proteins can reveal candidates for carborane binding proteins that can be exploited in several ways. Carboranes can be used in boron neutron capture therapy or for nuclide therapy and imaging. The bioconjugates of proteins and carboranes can be used to deliver boron to tumor cells. The bioconjugates can also act as molecular Trojan horses and ferry carboranes, for instance, across the blood–brain barrier. Carboranes can be used as pharmacophores, lead compounds, and drugs upon identification of proteic pockets and active sites where the cage can bind because they are isosteric to a rotating phenyl group, which they can substitute. The reverse ligand–protein docking approach was used in this work to identify binding proteins for carborane. The protocol was first validated against crystal structures of proteins containing carborane derivatives. The model was further validated with proteins whose structure is available but whose site of binding of carborane has not been reported with certainty and for which only biochemical and SAR (structure–activity relationship) data relative to carborane derivatives are available. Finally, the screening was carried out on a protein drug target database to reveal potential carborane binding targets of interest for biological and pharmacological activity. Possible applications of carboranes, from the development of improved carborane bioconjugates for BNCT and radiohalogens to the direct use of carboranes as drugs that inhibit specific protein functions, were discussed.

EXPERIMENTAL SECTION

Proteins were screened for their potential binding to carborane. A feature of carborane crucial for this work is that the most important energy term that describes carborane binding to proteins is the van der Waals interactions between the cage and the protein surface. The database selected was the drug target database (PDTD).⁴⁸ PDTD is a comprehensive, web-accessible database of drug targets and focuses on those drug targets with known three-dimensional structures. PDTD contains 1207 entries covering 841 known and potential drug targets with structures from the Protein Data Bank (PDB). The coordinates of the proteins were isolated from the PDB. Because not all PDB structures are of equal quality, when several redundant records were available in the PDB, a protein structure was selected according to the following criteria: (i) Select the structure without mutation and missing residues around the active site. (ii) Select the structure with high resolution. (iii) Select the structure complexed with ligand.⁴⁸ Each selected drug target of PDTD was categorized into 15 and 13 types according to two criteria: therapeutic areas and biochemical criteria, respectively. To take protein flexibility into account, PDTD includes redundant entries for proteins known to be flexible. Docking models were obtained using the PatchDock algorithm.⁹³ PatchDock takes as

input two molecules and computes three-dimensional transformations of one of the molecules with respect to the other with the aim of maximizing surface shape complementarity while minimizing the number of steric clashes. Given a protein and a molecule, PatchDock first divides their surfaces into patches according to the surface shape (concave, convex, or flat). Then, it applies the geometric hashing algorithm to match concave patches with convex patches and flat patches with flat patches and generates a set of candidate transformations. Each candidate transformation is further evaluated by a set of scoring functions that estimate both the shape complementarity and the atomic desolvation energy⁹⁴ of the complex. These terms are the most important for the binding of hydrophobic carborane with proteins, and the algorithm has been demonstrated to work perfectly in recognizing these interactions.^{46,47} Redundant solutions were discarded by use of rmsd (root-mean-square deviation) clustering. PatchDock is highly efficient, because it utilizes advanced data structures and spatial pattern detection techniques based on the matching of local patches. The local shape information is then extended and integrated to achieve global solutions. The algorithm implicitly addresses surface flexibility by allowing minor penetrations. Accurate rescoring of the complexes was then carried out using the FireDock program.⁹⁵ This method simultaneously targets the problem of flexibility and scoring of solutions produced by fast rigid-body docking algorithms.

Possible readjustments of the protein structure in the presence of the solvent were taken into account. Redundant entries were present in the database for proteins known to be flexible, with side-chain flexibility modeled by rotamers and Monte Carlo minimization.⁹⁶ Following rearrangement of the side chains, the relative position of the docking partners was refined by Monte Carlo minimization of the binding score function.

Desolvation free energy in the binding process was taken into account by a solvation model using estimated effective atomic contact energies (ACEs).⁹⁴

All candidates were ranked with a binding score.⁹⁵ This score included, in addition to atomic contact energy,⁹⁵ van der Waals interactions, partial electrostatics, explicit hydrogen and disulfide bonds contributions, and π -stacking and cation– π interactions. The electrostatic contribution for protein–carborane binding energy is zero because all charges of carbon atoms modeling carborane are zero. The most important contribution derives from the van der Waals energy (E_{vdW}). E_{vdW} between two atoms a_i and a_j is defined as the modified Lennard-Jones 6–12 potential with linear short-range repulsive score,^{95,97} that is

$$E_{vdW}(a_i, a_j) = \begin{cases} \varepsilon_{ij} \left(\frac{\sigma_{ij}^{12}}{r_{ij}^{12}} \right)^2 - 2 \frac{\sigma_{ij}^6}{r_{ij}^6}, & r_{ij} > 0.6\sigma_{ij} \\ \varepsilon_{ij}[A + B(r_{ij} - 0.6\sigma_{ij})], & \text{otherwise} \end{cases} \quad (1)$$

where

$$\begin{aligned} A &= \frac{\sigma_{ij}^{12}}{(0.6\sigma_{ij})^{12}} - 2 \frac{\sigma_{ij}^6}{(0.6\sigma_{ij})^6}, \quad B \\ &= -12 \frac{\sigma_{ij}^{12}}{(0.6\sigma_{ij})^{13}} + 12 \frac{\sigma_{ij}^6}{(0.6\sigma_{ij})^7} \end{aligned} \quad (2)$$

The parameter σ_{ij} is the sum of the atomic radii, and the parameter ε_{ij} is the energy well depth, derived from the CHARMM19 force field.⁹⁸ The hydrogen atomic radii were reduced by 40%

because of their uncertain positions. All energy terms were calculated inside a 6-Å radius of interatomic distances. To describe boron atoms, the simplest and most efficient strategy is based on the replacement of boron atom types with carbon atom types. This strategy has been extensively tested and validated in recent years.^{99–105} The scoring function used here was extensively and successfully tested on protein–protein docking benchmark of ~80 complexes and on all of the CAPRI targets.¹⁰⁶

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