CHEMICAL REVIEWS

REVIEW

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# Boron in Drug Discovery: Carboranes as Unique Pharmacophores in Biologically Active Compounds

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## 1. INTRODUCTION

Boron, in the form of boric acid, has played a role in medicine as a mild antiseptic and an eye wash since its preparation from borax in the 18th century. However, the use of boron in drug design is only a recent phenomenon,[1] with the pharmaceutical industry and an increasing number of medicinal chemistry researchers now substituting boron for carbon in numerous classes of drug molecules.[2−4] Safety and synthetic concerns have been overcome by significant advancements in the area of boron chemistry and the abundance of safety data for the element.[2] In the area of drug design, boron moieties may be used to target biological receptors that appear to be unaffected by nonboron-containing organic molecules, a property that may be exploited in areas such as bacterial drug resistance. The recent appeal of boron to the pharmaceutical industry also lies in the potential for the design of molecules that are structurally less-complex than their carbon counterparts, which can reduce the cost factors involved in drug research and manufacturing. Recently, some potent boron-containing medicinal agents have either become commercialized or entered human clinical trials. Velcade (Bortezomib) was the first clinically tested and commercially approved boronic acid-containing drug used to treat newly diagnosed multiple myeloma.[1] In late 2010, Anacor Pharmaceuticals commenced the first of two identical phase 3 clinical trials for the boron-containing drug AN2690, which is used to treat onychomychosis, a fungal infection of the nail and nail bed, while several other boronated agents are currently also in advanced clinical development.[2,5] Boron-containing functional groups that have been incorporated into therapeutics to date include diazaborines,[2,6] boronic acids and esters,[3,7] and benzoxaboroles.[2,8]

One important class of boron compounds are the polyhedral borane clusters, and these are also being exploited in drug design, particularly in view of their relationship to the more traditional carbopolycyclic scaffolds[9−11] such as norbornane **1**,[12] cubane **2**,[13] adamantane **3**,[14] pentacyclo[5.4.0.0$^{2,6}$.0$^{3,10}$.0$^{5,9}$]undecane **4**[15−17] (and its more stable and intrinsically chiral isomer pentacyclo[6.3.0.0$^{2,6}$.0$^{3,10}$.0$^{5,9}$]undecane, $D_3$-trishomocubane **5**),[18−20] pentacyclotetradecyl (diamantane) **6**,[21] and fullerene **7**[22−24] (Figure 1). Hydrophobic carbopolycyclics have demonstrated a diverse variety of biological activities in the areas of enzyme inhibition, ion channels, neurological disease, and antiviral research. Medicinal chemists have exploited carbopolycyclic scaffolds in drug design to enhance a drug's physicochemical properties such as lipophilicity, which can drastically enhance transport across cell membranes such as the blood brain barrier (BBB) and central nervous system (CNS). An increase in lipophilicity can also improve the affinity of a drug for the hydrophobic region of a receptor binding site, and the steric bulk and rigidity of a polycyclic skeleton generally improve the stability of

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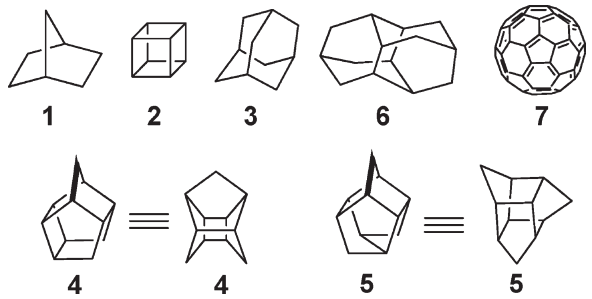


Figure 1

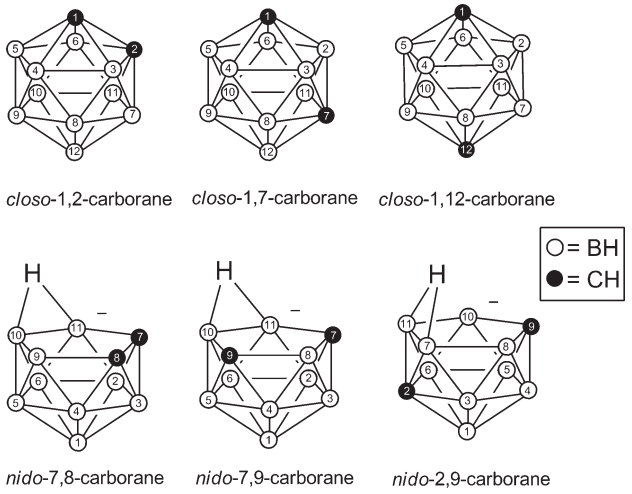


Figure 2

a drug to metabolic degradation. Moreover, modification of the substitution patterns on a polycyclic entity, as a way to control the orientation of functional groups, can alter the biological profile of the polycyclic compound, and it can give rise to new types of drugs for a wide scope of targets. The boron-rich dicarba-*closo*-dodecaborane(12) polycycle (commonly referred to as *closo*-carborane, *closo*-$C_2B_{10}H_{12}$) (Figure 2) is a neutral, hydrophobic heteroanalogue of the hydrophilic, icosahedral dodecahydrododecaborate ion *closo*-$[B_{12}H_{12}]^{2-}$, the largest cluster belonging to the family of polyhedral boranes, *closo*-$[B_nH_n]^{2-}$ (where $n = 6-12$).[25] In contrast to hydrocarbons, simple boron hydrides such as $BH_4^-$ and $B_2H_6$ (and its Lewis base adducts such as $BH_3 \cdot THF$) are highly reactive and are usually air- and moisture-sensitive. Hence, their use in medicinal chemistry, other than as reducing agents or Lewis acids in the preparation of drugs and/or their precursors, is not feasible, but certain polyhedral boranes such as the carboranes are kinetically stable to hydrolysis and can offer many exciting possibilities in medicinal chemistry. It is the three-dimensional delocalization (pseudo-aromaticity) of the $\sigma$-framework electrons in these entities that largely accounts for their remarkably robust properties.

The first in vivo study of polyhedral boranes was reported by Soloway et al.[26] in 1961 when $Na_2[B_{10}H_{10}]$ was evaluated in animals as a potential source of boron for the experimental cancer treatment known as boron neutron capture therapy (BNCT).[27−36] This pioneering research paved the way for the use of boron clusters in BNCT, in particular for the treatment of glioblastoma multiforme, an aggressive and intractable malignant brain tumor. Indeed, one such polyhedral borane known as mercaptoundecahydro-*closo*-dodecaborate (or borocaptate) ion (BSH, $[B_{12}H_{11}SH]^{2-}$) is still used clinically as a BNCT agent.[28,31,35] The syntheses of the first *closo*-carboranes were reported[37] in 1963−4, and their incorporation into bioactive molecules such as amino acids for BNCT followed soon after, with *closo*-L-1,2-carboranylalanine being one of the earliest carborane-based analogues of a biomolecule.[38−40] The preparation of other carborane-based cellular building blocks such as nucleic acids[41−44] and sugars[36,45] was made possible by the optimization of synthetic methods. In the past three decades, the medicinal chemistry of the carboranes has also been exploited in the area of drug design.

The *closo*-carboranes represent a unique type of pharmacophore that can bind to protein sites by hydrophobic interactions that exploit the largely low-polar character of B−H bonds.[46] The use of *closo*-carboranes in medicinal chemistry marries the properties of polycyclic cage skeletons with the unique characteristics of boron to offer a unique and versatile pharmacophore in the field of drug discovery. Carboranes have given researchers the ability to synthesize molecules with highly specific shape and charge distributions, and a considerable amount of research in recent years has shown that some carborane-containing bioactive molecules may prove to be superior to some of their organic counterparts.

In 1977, Schwyzer and co-workers first reported an investigation into the use of a *closo*-carborane group in ligand−receptor interactions with a view of using it as a localized source of high-LET particles in BNCT and also to study carborane-containing ligand−receptor interactions.[47] The use of *closo*-carboranes for probing the aromatic recognition site of the enzyme chymotrypsin pioneered the application of boron clusters as bioisosteres in biologically active molecules. These researchers utilized a peptidic carboranyl derivative that constituted a replacement of the natural amino acid L-phenylalanine with *closo*-L-carboranylalanine[39,48] (Car), and although in vivo studies two decades later showed that Car did not attain an adequate tumor to blood ratio required for BNCT,[49,50] Fischli et al.[47] found that a *closo*-carboranyl group can interact with the phenyl recognition site of chymotrypsin in a competitive, nonagonistic mode. Subsequently, Endo and co-workers were the first researchers to study *closo*-carboranes as hydrophobic bioisosteres in a comprehensive and systematic manner.[51,52] These researchers prepared numerous carborane derivatives in their studies of structure−activity relationships (SARs) and showed that *closo*-carborane can be used as a modifying entity to enhance hydrophobic interactions between potential drugs and their receptors. Additionally, polar and water-soluble entities such the anionic *nido*-dicarbadodecahydroundecaborate(−1) (commonly known as *nido*-carborane)[53] and metallocarboranes[54] can be readily prepared from *closo*-carboranes and thus appeal as a more hydrophilic bioisostere of the phenyl group. The cobalt bis(dicarbollide) ion can be considered as the archetypal metallocarborane as it is the best known and investigated of all metallocarboranes. Its derivatives are promising therapeutic agents,[55] and there exists great opportunity to expand the study of metallocarboranes in the discovery of new therapeutic agents. The ready accessibility of structurally related but more hydrophilic analogues of *closo*-carborane-containing bioactive molecules provides a powerful and unique tool in understanding the intricacies of ligand−receptor interactions, which is vital to drug discovery.

This Review will predominantly examine the use of *closo*-carboranes as components of biologically active molecules for ligand−protein association by hydrophobic interactions. Selected examples of molecules containing *closo*-carborane analogues, such as metallocarboranes and hydrophilic *nido*-carboranes

that can engage in other types of receptor−ligand interactions, are also presented to highlight the versatility of the carborane pharmacophore in drug design. The use of carboranes as pharmacophores in medicinal chemistry has been reviewed previously.[36,38,46,56] This Review provides an up-to-date and a more comprehensive discussion of the topic, and selected examples will be presented in order of the receptor/protein target of the carborane-containing ligands.

### 1.1. Properties of *closo*-Carboranes and Their Hydrophobicity

Structural prefixes associated with carboranes (e.g., *closo*- (closed), *nido*- ("nest"-like), and *arachno*- ("spider web"-like)) describe the structural motif of the carborane cage. Atoms of the *closo*-carborane cage are numbered starting at the apex atom with the lowest number of bonds and proceeding in a clockwise direction such that the carbon atoms are given the lowest possible numbers (Figure 2). Three isomers of *closo*-carborane exist, the 1,2- (*ortho*-), 1,7- (*meta*-), and 1,12- (*para*-), all of which differ in the arrangement of the two carbon atoms in the cage. Deboronation of the *closo*-carborane cluster gives rise to *nido*-carborane anions.[57−59] The three *nido*-carborane isomers 7,8- (*ortho*-), 7,9- (*meta*-), and 2,9- (*para*-) and the numbering of cage atoms are shown in Figure 2. Hydrogen atoms in carborane structures throughout this Review have been omitted for clarity, except in the case of bridging hydrogens.

Different substituents can be introduced onto the carbon or boron vertices in *closo*-carboranes by using established synthetic chemistry to incorporate them into a spatial geometry required for biological activity.[36] The symmetry and rigidity of the carborane cage simplifies conformational considerations in SAR studies. *closo*-Carboranes are characterized by electron-deficient bonding, electron delocalization, and extreme hydrophobicity by virtue of the hydride-like character of the boron cage hydrogen atoms, which prevents them from participating in classical hydrogen-bonding interactions. The volume of a *closo*-carborane cage (70−80 Å$^3$)[36] is comparable to the spherical volume created by the rotational sweep of a phenyl ring. One advantage of a *closo*-carborane over a phenyl group is the increased hydrophobicity and larger surface area, which can potentially increase hydrophobic contacts with nonpolar regions of proteins.[46] In view of the potential application of carboranes in drug design, Endo and co-workers[60] experimentally measured the partition coefficients (log *P*) of various 1,2-, 1,7-, and 1,12-*closo*-carboranyl compounds possessing a phenolic substituent at either a carbon or a boron vertex and determined the Hansch−Fujita hydrophobic parameters ($\pi$) of the various carboranyl groups. The effect of substitution patterns within a carborane cluster on the hydrophobic contributions was compared to 1-adamantyl- and cyclohexyl-phenols. The adamantyl group was chosen on the basis that it resembles a carborane cage in size, shape, and rigidity. The authors found that the contribution toward hydrophobicity from a C-substituted *closo*-carboranyl group exceeded that of an adamantyl group (group 1, Figure 3). The relative positions of the carbon atoms in the *closo*-carborane cage influence its overall dipole moment and hence hydrophobicity. Partition coefficient data support the notion that the absence of a dipole moment in the 1,12-isomer makes it the most hydrophobic entity followed by 1,7- and 1,2-isomers in decreasing order of hydrophobicity. The hydrophobicity of boron-substituted 1,7-carboran-2-yl and 1,12-carboran-2-yl groups (group 2, Figure 3) was found to be comparable to that of the adamantyl group, while the B-substituted 1,2-carboran-9-yl group was found to be the least

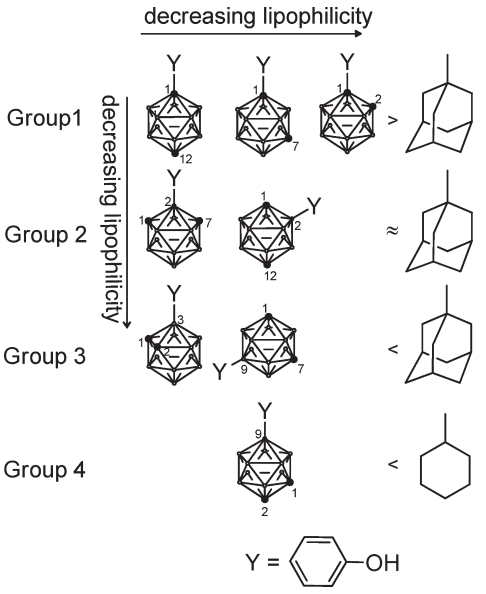


**Figure 3**

hydrophobic of the set and less lipophilic than a cyclohexyl group (group 4, Figure 3). Endo's measurement of Hansch−Fujita hydrophobic parameters ($\pi$) of various *closo*-carboranyl groups can be used as a guide to modulate the hydrophobic properties of a molecule in drug design.

As an example of the application of log *P* and $\pi$ measurements in drug design, a SAR study using estrogen receptor-α (ERα) binding assays was conducted.[61] The study showed that the binding affinity of the various carboranylphenol compounds (Figure 3) for the ER correlated with the hydrophobicity and electronic effects associated with the *closo*-carborane compounds. On the basis of the 3D-structural information of 17$\beta$-estradiol **8** (Figure 4) complexed with ERα,[62] two types of ligand−protein interactions are required: (a) hydrogen bonding from the 4-hydroxyphenyl group to Glu-353 and Arg-394 and from the aliphatic hydroxyl to His-524, and (b) hydrophobic interactions along the body of the skeleton. These interactions delineate the basic structural skeleton required for binding to the ER, with further substitution affecting the biological activity. Protein binding affinity was found to depend upon the specific carborane isomer (i.e., 1,12-, 1,7-, or 1,2-), the position of the para-hydroxyphenyl group on the *closo*-carborane cage, and its relative position to acidic C−H protons.[61] As a general rule, binding affinity was found to increase with hydrophobicity; the para-hydroxyphenyl C-substituted derivatives (group 1, Figure 3) exhibited significantly higher binding affinity for the ERα than did the B-substituted phenol derivatives. Within the C-substituted analogues, the relative binding affinity decreased in the order: 1,12-carboran-1-yl > 1,2-carboran-1-yl > 1,7-carboran-1-yl,[61] with $K_i$ values of 0.4 and 1.1 nM determined for the *para*-hydroxyphenyl-1,12-carboran-1-yl[63] **9a** and *para*-hydroxyphenyl-1,2-carboran-1-yl[64] **10** (Figure 4), respectively. However, the binding affinity of a *closo*-carborane cage for the ERα is also influenced by the acidity of the free C−H proton on the icosahedral cage. Generally within each hydrophobic group (Figure 3), the binding affinity is inversely related to the acidity of the two C−H protons, which decreases in the sequence 1, 2- > 1,7- > 1,12-carborane. An acidic carboranyl C−H proton may be required to interact with the His-524 residue in the ligand


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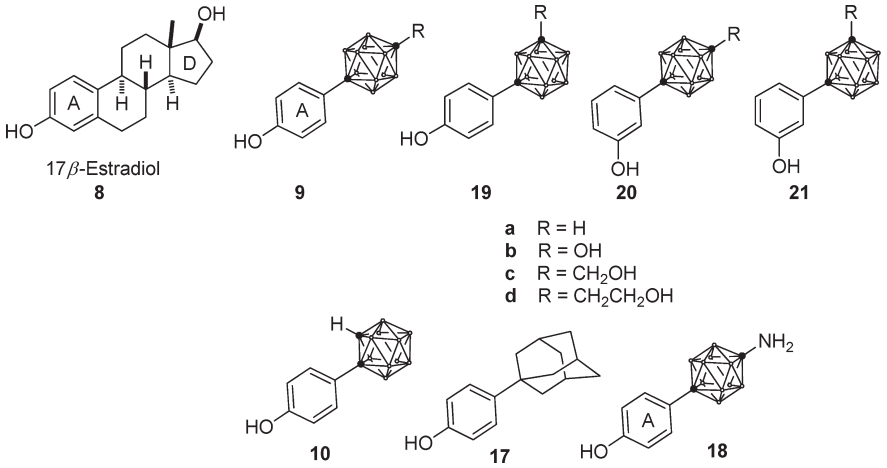


**Figure 4**

binding domain (LBD); however, if it is too acidic it may not enter the hydrophobic pocket of ERα and may engage in a substitution reaction with metal ions or organic bases. Endo and co-workers[65] demonstrated the deleterious effects that acidic *closo*-carborane C−H hydrogen atoms can have on estrogenic activity. However, positive effects have also been demonstrated, such as that seen in the 1,2-carboran-9-yl compound[61] (group 4, Figure 3), which exhibits the fourth highest binding affinity for ERα (after the phenol C-substituted carborane derivatives, group 1, Figure 3) despite being the least hydrophobic species. The position of the *para*-hydroxyphenyl substituent relative to the acidic C−H group more closely mimics the 1,12-carboran-1-yl analogue **9a** (which was found to exhibit the highest binding affinity of all of the analogues studied), as well as the arrangement of functional groups in endogenous ligand **8** required for salient interactions with the ERLBD. The work of Endo and co-workers provides a systematic assessment on the use of various *closo*-carboranes to modulate hydrophobicity in drug design.

## 2. LIGAND−PROTEIN INTERACTIONS

### 2.1. Peptide Ligands for Chymotrypsin, Opioid, Kinin B1 and B2, Angiotensin II, PBAN and Y-Receptors

The use of hydrophobic *closo*-carboranes as probes for investigating the aromatic recognition sites of enzymes and receptors was pioneered approximately 30 years ago.[47] Chymotrypsin is a well-characterized digestive proteolytic enzyme that catalyzes the hydrolysis of peptides at the carboxy end of Tyr, Trp, and Phe residues. The aromatic recognition site of the enzyme is a hydrophobic pocket bound by enzyme peptide bonds 190−191−192 and 215−216.[47,66] A synthetic peptide, N-Cbz-Ala-Ala-Car (Ki = 0.3 mM), containing the Phe derivative *closo*-L-carboranylalanine (Car) was shown to be a better inhibitor of chymotrypsin than was N-Cbz-Ala-Ala-Phe (Ki = 1 mM). The *closo*-carborane cage is accommodated in the binding pocket of the enzyme with deformation of the molecular surroundings of the cavity. Thus, a *closo*-carborane cage was shown to interact with the phenyl recognition site of chymotrypsin in a manner analagous to that of a (rotating) phenyl group.

The replacement of a phenyl group with a *closo*-carboranyl group in aromatic amino acids within other peptide hormones was studied by means of binding assays using enkephalin. Enkephalins are endogenous pentapeptide ligands that bind to opioid receptors and regulate nociception response to potentially harmful stimuli. There are two forms of enkephalins: $Met^5$-enkephalin ($Tyr^1$-$Gly^2$-$Gly^3$-$Phe^4$-$Met^5$) and $Leu^5$-enkephalin ($Tyr^1$-$Gly^2$-$Gly^3$-$Phe^4$-$Leu^5$). As the Phe in position 4 is necessary for opiate receptor affinity and biological activity, an analogue of $Leu^5$-enkephalin possessing a Car residue in position 4 was synthesized. It was found that the binding affinity of $Tyr^1$-$Gly^2$-$Gly^3$-$Car^4$-$Leu^5$ relative to natural $Leu^5$-enkephalin had increased by 3-fold to match the binding affinity of $Met^5$-enkephalin, as determined by the displacement of [$^3$H]-naloxone from rat brain homogenate.[67,68]

On the basis of the suitability of replacing Phe with Car from previous work,[47,67,68] Car was again used to replace the Phe residues present in the kinins bradykinin (BK) nonapeptide and desArg9BK octapeptide, and substance P (4−11)-octapeptides (SP), to evaluate their functional differences.[69,70] The biological data obtained with desArg9BK ($Arg^1$-$Pro^2$-$Pro^3$-$Gly^4$-$Phe^5$-$Ser^6$-$Pro^7$-$Phe^8$) and its [$Car^5$, $Phe^8$]-kinin and [$Phe^5$, $Car^8$]-kinin analogues on the B1 receptor in rabbit aorta showed a 10-fold and >1000-fold reduction in affinity, respectively, as compared to wild-type desArg9BK. Although the [$Car^5$, $Phe^8$] analogue exhibited weaker binding affinity for the B1 receptor than that of desArg9BK, it prolonged the duration of contraction and relaxation in aortic strips suspended in a cascade superfusion system. It was also shown that the replacement of $Phe^8$ with the aliphatic residue $Leu^8$ transformed the agent from an agonist into an antagonist with full binding affinity. This study shows that $Phe^5$ is primarily involved in binding to the B1 receptor and that the phenyl binding site for this group can accommodate a Car. Although the [$Car^5$, $Phe^8$]-kinin peptide maintains only 11% affinity for the B1 receptor, its longer duration of action may be a result of the tighter association of the *closo*-carboranyl group within the peptide, with the phenyl recognition site in the binding pocket of the receptor. The $Phe^8$ residue on the other hand is involved in stimulating activity, and the loss of binding affinity of the [$Phe^5$, $Car^8$]-kinin analogue for the B1 receptor shows that the $Car^8$ residue is not only unable to fit into the receptor binding site but also reduces the propensity of $Phe^5$ to bind strongly.

The biological data obtained with BK ($Arg^1$-$Pro^2$-$Pro^3$-$Gly^4$-$Phe^5$-$Ser^6$-$Pro^7$-$Phe^8$-$Arg^9$) and its [$Car^5$, $Phe^8$]-BK and [$Phe^5$, $Car^8$]-BK analogues in cat ileum demonstrate that the replacement of Phe with Car results in a large decrease in binding affinity for the B2 receptor. Similarly, Car replacements for Phe in


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(4−11)-SP (Pro$^4$-Gln$^5$-Gln$^6$-Phe$^7$-Phe$^8$-Gly$^9$-Leu$^{10}$-Met$^{11}$) also produced decreases in binding affinity in guinea pig ileum and rabbit mesenteric vein. The [Car$^7$, Phe$^8$]-SP analogue displayed one-half the affinity in guinea pig ileum as compared to [Phe$^7$, Car$^8$]-SP. This result may indicate that position 7 is more important than position 8 in the interaction of SP octapeptide with its receptors. Because of the marked decrease in affinities for the receptor, the ability of Car analogues to activate receptors could not be measured. Car appeared to be unsuitable for replacing the Phe residue in the BK and SP polypeptide hormones. These studies indicated that Phe$^5$ and Phe$^8$ of BK and Phe$^7$ and Phe$^8$ of SP were primarily involved in receptor binding as replacement of the aromatic residues with aliphatic ones did not affect their biological activities.[69,71] It appears that the phenyl recognition sites of the receptors for BK and SP are unable to accommodate the larger *closo*-carborane cage.

Car was also used to replace Tyr$^4$ or Phe$^8$ in analogues of the angiotensin II (AT) peptide (Sar$^1$-Arg$^2$-Val$^3$-Tyr$^4$-Val$^5$-His$^6$-Pro$^7$-Phe$^8$) and the effect of these changes studied in the AT smooth muscle receptor in rabbit aorta strips.[70,72] [Sar$^1$, Car$^4$]-AT gave no agonistic or antagonistic response up to a concentration of 150 $\mu$M. The lack of affinity of the [Sar$^1$, Car$^4$]-AT for the AT receptor was postulated to be a result of the highly electron-deficient nature of the *closo*-carboranyl group.[72] On the other hand, the [Sar$^1$, Car$^8$]-AT analogue exhibited weak partial agonistic activity and low binding affinity, but strongly enhanced duration of action. The likely reason for the low affinity is that the Car$^8$ residue in the C-terminus acts as a Phe$^8$ that is locked in free rotation, which is not tolerated by the AT receptor. When the Car$^8$ inserts into the receptor, the subsequent activity depends on the capacity of the side chain to reorientate and to interact with the active site of the receptor. The long duration of action is related to the enhanced lipophilicity of the Car, which is responsible for the slow dissociation of the peptide from the receptor. Therefore, Car is accepted by the angiotensin receptor of rabbit aorta to some extent, but is less suitable for replacing other aromatic residues such as Tyr$^4$ of the same peptide.

The 33-membered pyrokinin/pheromone biosynthesis activating neuropeptides (PBAN) found in insects are peptide modulators, characterized by a 5-amino acid C-terminal active sequence Phe-X-Pro-Arg-Leu-NH$_2$ (X = Gly, Ser, Thr, Val). The pentapeptide is the active component required for diverse physiological function such as stimulation of pheromone biosynthesis in the female moth, muscle contraction, and melanisation. Nachman et al.[73] synthesized a *closo*-carboranylethanoyl containing pentapeptide analogue of PBAN, Cbe-Thr-Pro-Arg-Leu-NH$_2$ (Cbe = *closo*-carboranylethanoyl) **11** (Figure 5). PBAN analogue **11** possessed a 10-fold more potent myotropic activity in cockroach hindgut bioassays than did endogenous neuropeptide leucopyrokinin. It also possessed pheromonotropic activity when applied topically ($ED_{50}$ = 25 pmol/female moth; maximal response at 60 pmol/female) and when injected ($ED_{50}$ = 0.1 pmol/female; maximal response at 2.5 pmol/female). The induction of pheromone production in moths by the carborane PBAN analogue **11** is more potent than that induced by endogenous PBAN ($ED_{50}$ 1.0 pmol/female moth; maximal response at 5.0 pmol/female by injection, and no increase in pheromone production via topical application). In another direct comparison, the pyrokinin pentapeptide Phe-Thr-Pro-Arg-Leu-NH$_2$ **12** elicited a maximal response of 30% of the pheromone titer induced by the endogenous PBAN, at a dose of 70 pmol/female (in a related moth species). The potency of

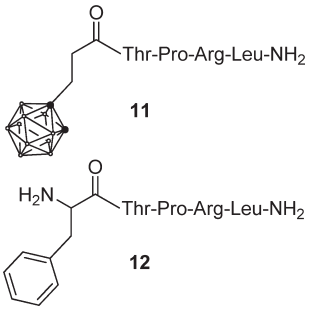


**Figure 5**

*closo*-carborane PBAN analogues has been attributed to strong receptor[74] binding characteristics of the hydrophobic *closo*-carborane cage. Because of the improved potency of the mimetic *closo*-carborane analogue, it offers the potential to modify insect behavior by stimulating continuous pheromone release and thus leads to the depletion of pheromone production. *closo*-Carborane analogues of PBAN can thus be potentially used in insect control.

Recently, Ahrens et al. showed that various neuropeptide Y analogues containing a *closo*-1,2-carborane-modified Lys at position 4 in the peptide sequence retain their strong Y-receptor subtype selectivity, nanomolar binding affinity, and agonistic potency.[75] As demonstrated above, numerous researchers have independently shown that carborane-modified peptides are suitable ligands for receptor binding.

## 2.2. Estrogen Receptor

The estrogen receptor (ER) is a member of the nuclear receptor superfamily, which consists of two highly conserved functional domains: a DNA binding domain that specifically binds to *cis*-acting DNA response elements present in target genes, and a ligand binding domain (LBD) that contains the ligand-dependent transcriptional activation function AF-2.[76] The binding of hormone estrogen to the estrogen receptor ligand binding domain (ERLBD) mediates a variety of nuclear responses. The hormone-bound ER dimerizes and forms complexes with various cofactors, which then bind to promoter DNA to initiate gene transcriptional activity. The act of binding to the ER to either activate (agonist) or inhibit (antagonist) transcriptional activity plays an important role in the CNS, cardiovascular, and reproductive systems, and in bone maintenance. X-ray structures of complexes formed by estrogen agonists 17$\beta$-estradiol **8** and diethylstilbestrol (DES) **13** as well as antagonists 4-hydroxytamoxifen **14** (the active metabolite of tamoxifen **15**) and raloxifene **16** (Figure 6) with the human estrogen receptor-$\alpha$ ligand binding domain (hER$\alpha$LBD) have provided key insights into receptor−ligand interactions.[62,77] These crystal structures have also provided the basis of structure-based design of improved agonists and antagonists for the ER. High binding affinity for ER and estrogenic activity requires a phenolic residue, which hydrogen bonds to both the carboxylate group of Glu-353 and the guanidinium group of Arg-394, a hydrophobic group adjacent to the phenolic group that complements the large hydrophobic core of the LBD, and a hydroxyl group located at a suitable position on the molecule that is able to form a hydrogen bond with the $\delta$-nitrogen of His-524.[62] The molecular volume (450 Å$^3$) of the hydrophobic core of the ER$\alpha$LBD is nearly twice as large as the molecular volume of endogenous estradiol (245 Å$^3$),[62] which allows it to accept a diverse range of structures. The following section provides some examples of the design,


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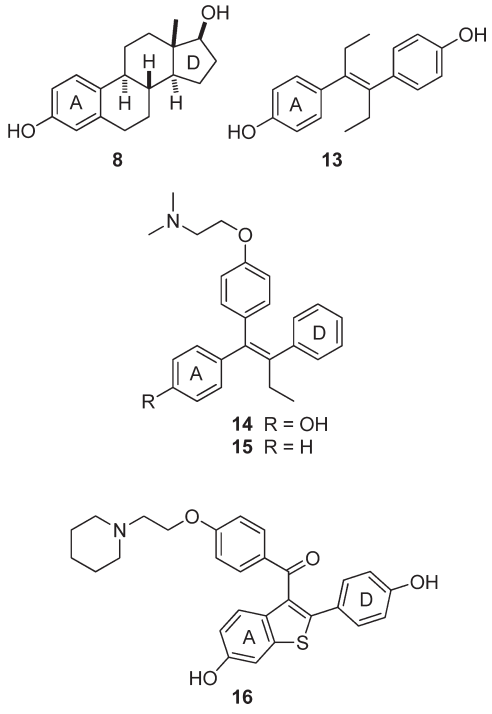


**Figure 6**

synthesis, and biological evaluation of biologically active molecules containing a *closo*-carborane cage as a hydrophobic pharmacophore for the modulation of lipophilicity.

**2.2.1. ER Agonists.** Endo and co-workers first investigated the use of *closo*-carboranes as hydrophobic moieties in ER ligands. The design, synthesis, and biological evaluation of novel carborane-containing estrogenic agonists over the past decade has resulted in the production of estrogen receptor agonists that are more potent than the natural hormone 17$\beta$-estradiol **8**. ER-binding compounds that possess a *closo*-carborane as a hydrophobic skeletal structure can mimic rings C and D in estradiol. Carbon functionalization of 1,12- and 1,7-carborane cages was exploited to fix the direction of the various substituents, allowing for an evaluation of SARs of the functional groups and their spatial orientation to be studied. Carbon functionalization is also required for ligand activity as the presence of acidic C−H hydrogen atoms on the *closo*-carborane potentially reduces its hydrophobicity, thus preventing the cage from entering the hydrophobic pocket of the ERαLBD.[78]

Over 40 phenol-substituted 1,7- and 1,12-carborane analogues mimicking the skeletal structure of endogenous 17$\beta$-estradiol **8** or the clinically used DES **13**, with variations in the position of the A ring hydroxyl group, the *closo*-carborane isomer, and nature and orientation of the substituent on the second *closo*-carborane carbon atom, were synthesized using well-established chemistry. Functionalization of the second carbon atom in the *closo*-carborane cage with hydroxyl,[79,80] amino,[63,79,81] carboxylic,[63,79] benzyl,[64] alkyl alcohols,[63,79,80] pentafluorophenyl,[82] phenol and phenolic alkoxide,[78,82] and phenolic carbamate and thiocarbamate groups[65] provided a good learning set from which SARs could be determined. A competitive binding assay using [6,7-$^3$H]-17$\beta$-estradiol and human recombinant ERα was used to screen the compound list, and the nature of activity (agonist vs antagonist) was determined by using a luciferase transcriptional activation assay, which is dependent upon the expression of ER. In later work, estrogenic activities of lead compounds were evaluated by using cell proliferation-inducing assays.[65,82]

The phenolic hydroxyl group in the A ring was found to be essential for biological activity,[79] with a greater activity observed in the *para*-hydroxyl-substituted series (**9** and **19**) rather than the equivalent *meta*-hydroxyl-substituted series (**20** and **21**) (Figure 4).[80] The activity of compounds bearing a C-substituted 1,12-carborane cage was higher than that of the equivalent 1,7-carborane compounds. As expected, the lowest activities were observed for the compounds bearing both a 1,7-substituent on the carborane group and a *meta*-hydroxyl group on the phenyl group (**21a**−**d**). The most active compound **9c** (Figure 4) in the luciferase transcriptional activation assay also showed the highest affinity ($K_i$ = 0.1 nM) for ERα, which was higher than the inhibition constant for 17$\beta$-estradiol ($K_d$ = 0.4 nM).[63,83] Its potency was at least 10-fold greater than that of 17$\beta$-estradiol. Longer hydroxyalkyl chains on the *closo*-carborane cage gave decreased activities.[79] The *para*-hydroxyphenyl-1,12-carborane **9a**, which lacks the hydroxyl group on the cage, had affinity similar to ($K_i$ = 0.4 nM) and activity comparable to those of the natural 17$\beta$-estradiol **8** (Figure 4), while the *para*-hydroxyphenyl-1,2-carborane **10** also binds strongly to the ERα with a $K_i$ = 1.1 nM.[64] By comparison, the 4-(1-adamantyl)phenol **17** (Figure 4) exhibited an estrogenic activity[63] sequentially lower than its carborane mimetics **19a** and **9a**, which was consistent with the relative Hansch−Fujita hydrophobic contributions from 4-(1-adamantyl) ($\pi$ = 4.04), 4-(1,7-carboran-1-yl) ($\pi$ = 4.26), and 4-(1,12-carboran-1-yl) ($\pi$ = 4.44) groups.[60] The activity of **9a** was increased by the introduction of a hydroxyl group onto the carbon atom of the carborane cage, as seen in **9b**.[80] The amino 1,12-carborane derivative **18** showed slightly reduced affinity ($K_i$ = 0.65 nM) but retained stronger activity than 17$\beta$-estradiol.[63,81] The high affinity suggests that the hydrophobicity of the *closo*-carborane cage matches the hydrophobic surface of the ER cavity, resulting in stronger van der Waals contacts than that of the 17$\beta$-estradiol carbon skeleton and adamantyl group, thus providing further support for the superior lipophilicity of the *closo*-carborane cage.[64] Docking simulations[80] of selected compounds **9b**, **9c**, **19c**, **20c**, and **21c** in the human ERαLBD showed that all compounds assessed in this study were able to fit into the LBD. However, the hydrogen-bond distances of the phenolic and aliphatic hydroxyl groups from the Glu-353, Arg-394, and His-524 amino acid residues of the LBD were found to differ. The data showed that the three hydrogen-bond distances of estradiol **8** were all within 3.2 Å, and it was found that compounds exhibiting the highest biological activity satisfied the hydrogen-bond distance criteria. The reason for the higher activity than that observed with 17$\beta$-estradiol may be the result of stronger hydrophobic interactions involving the *closo*-carborane cage. The lack of one hydrogen bond in the case of compound **9a** was possibly compensated for by an effective hydrophobic interaction resulting in activity equivalent to 17$\beta$-estradiol.[80] The most active compound **9c** was shown to exhibit potent estrogenic action in both uterus and bone tissue in ovariectomised mice by restoring uterine weight and bone mineral density, and its potency in vivo was similar to that of 17$\beta$-estradiol.[79,81]

The bisphenol derivatives bearing a 1,12-carborane **22a** and 1,7-carborane **23a** exhibit potent ER agonistic activity with an affinity that was found to be greater than or similar to that of estradiol, indicating that the bisphenol carborane structure fits into the ERαLBD (Figure 7).[78,82] The position of the hydroxyl

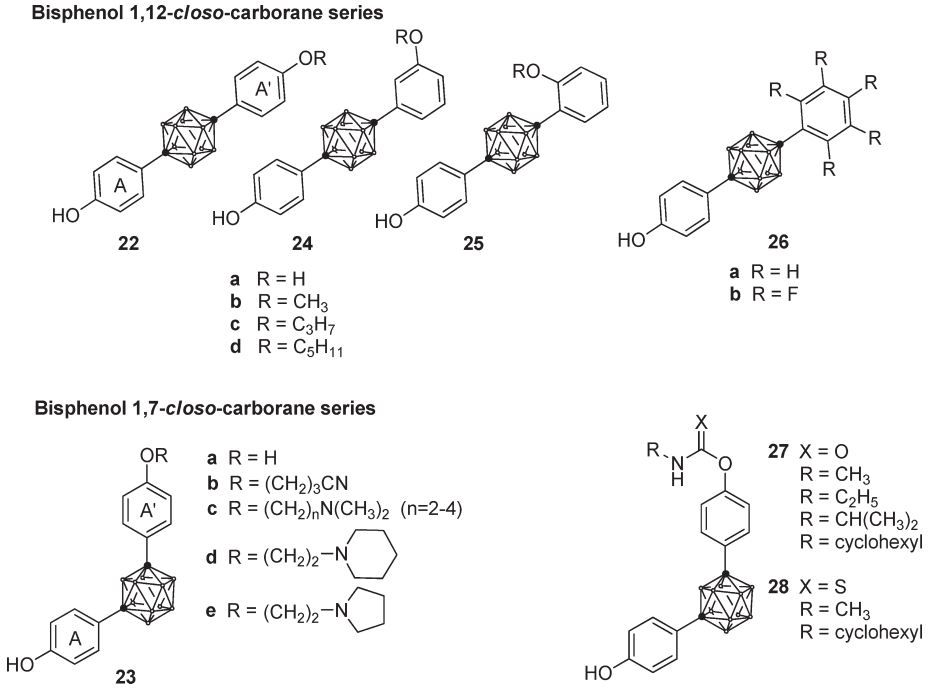


**Figure 7**

group on the A′-ring was found to strongly influence biological activity. The hydroxyl group at the *meta*- and *ortho*-positions on the A′-aryl, as seen in compounds **24a** and **25a**, respectively, decreased the estrogenic activity by up to $10^3$ times relative to the hydroxyl group located at the *para*-position (compound **22a**), with *para*−OH > *meta*−OH > *ortho*−OH, despite all compounds showing a stronger binding affinity than 17$\beta$-estradiol.[82] Conversion of the hydroxyl groups to the corresponding alkoxides reduced the estrogenic activity to a moderate level, and the binding affinity was found to decrease as the alkyl chain length increased through the series **a**−**d**. The absence of a hydroxyl group in the A′-ring **26a** gave an activity similar to that of the *meta*-OH derivative **24a**, while the activity of the pentafluorophenyl derivative **26b** was weaker than the nonfluorinated derivative **26a** on account of the electron-deficient phenyl ring.[82] Within the bisphenol 1,7-carborane series **23a**−**e** (Figure 7), compounds **23a**,**b** and **23c** ($n$ = 3) exhibited only agonistic activity, while the remaining compounds were shown to be partial agonists (vide infra). Although carbamate and thiocarbamate bisphenols **27** and **28**, derived from the 1,7-carborane cage series, were initially designed as potential antagonists to structurally mimic clinically used tamoxifen, they were found to exhibit potent agonistic activity for the ER, albeit with weaker binding affinity than estradiol. The thiocarbamate and carbamate compounds exhibited 3 times and 10 times weaker binding affinity to ER, respectively, as compared to estradiol. The binding affinities correlated with the acidity of the NH proton, which suggests an interaction between the NH group and an amino acid residue different from Asp-351 (which is known to play a role in antagonistic activity) exists.[65]

**2.2.2. ER Antagonists and Selective Estrogen Receptor Modulators.** A clinical ER antagonist is able to counter the cancerous effects of estradiol in the reproductive tissue while maintaining positive effects in other tissue such as the bone. On the basis of X-ray crystallographic studies of ER antagonists raloxifene[62] **16** and 4-hydroxytamoxifen **14** (the active metabolite of tamoxifen **15** used in the treatment of hormone-dependent breast cancer),[77] the region of the molecule corresponding to the phenolic A ring (Figure 6) binds to the ERαLBD in the same way as an agonist to effect hydrogen bonding between the phenolic OH group and residues Glu-353 and Arg-394. However, the binding mode at the D-ring end is different from that of 17$\beta$-estradiol, and the length and steric bulk of the side-chains cause the displacement of helix-12 as they cannot be accommodated in the LBD and thus protrude from the confines of the cavity. The role of Asp-351 within the ERαLBD in antagonistic activity is well established.[62] For example, the side chain of raloxifene **16** makes hydrophobic contacts and is anchored to the protein by a hydrogen bond between Asp-351 and the piperizine ring nitrogen atom, while the side chain in tamoxifen **15** is protonated and electrostatically interacts with the negatively charged carboxylate group of the Asp-351 residue.[62,77]

An optimal spatial relationship is needed for antagonistic activity. It has been established that in the bisphenol series of *closo*-carborane analogues, the 1,12-carborane is suitable for ER agonists and the 1,2-carborane for partial agonists and antagonists.[65] The synthesis of *closo*-carborane-based bisphenol ER antagonists was structurally inspired by known estrogen antagonists such as the clinically used 4-hydroxytamoxifin **14**, raloxifene **16**, and ICI-164,384 **29** (Figure 8). Bisphenol analogues based upon the 1,2-carborane derivative **30** exhibited greater antagonistic activity than their corresponding **1,7**-carborane analogues.[81,84,85] Although the antiestrogenic activity of **30** was moderate, with 70% inhibition of transcriptional response to 17$\beta$-estradiol, its binding affinity of $K_i$ = 60 nM was weaker than that of the corresponding 1,7-carborane (5.0 nM), and all were significantly weaker than 17$\beta$-estradiol (0.4 nM). Computational docking studies[84] using the LBD derived from the X-ray crystal structure of the 4-hydroxytamoxifen **14** and ER complex confirm the lower activity of **30** as compared to that of


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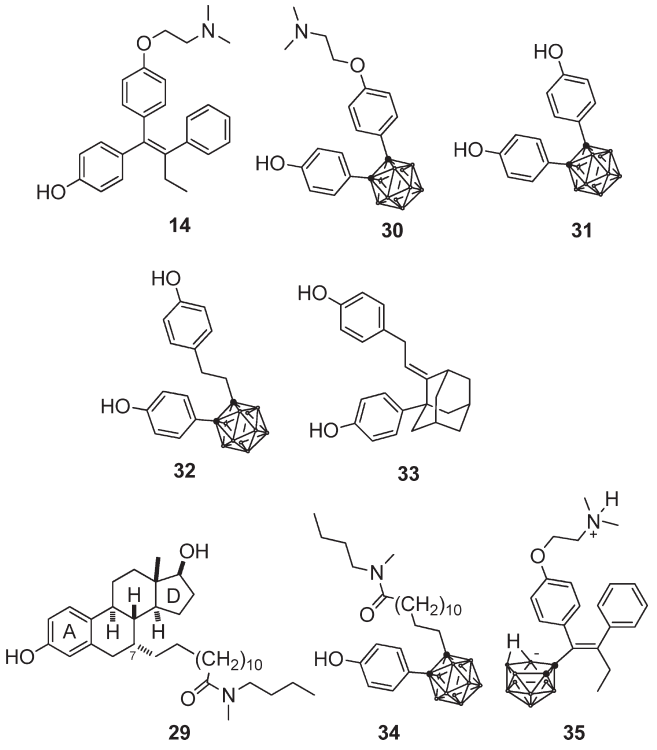


Figure 8

hydroxytamoxifen **14**. The authors demonstrated that the long amino-bearing side chain of *para*-hydroxy-1,2-carborane **30**, although located in the same narrow corridor in the receptor cavity as the side chain of 4-hydroxytamoxifen **14**, does not fit the cavity ideally because of the smaller dihedral angle between the two phenyl groups. Although the simple bisphenol-1,2-carborane analogue **31** showed weaker antagonistic activity than the dimethyaminoethyl analogue **30**, it exhibited a higher binding affinity ($K_i$ = 1.0 nM). Thus, no correlation between binding affinity and potency of a compound is apparent. The fact that the *para*-hydroxyphenyl-1,2-carborane **10** (Figure 4) binds strongly to the ERα ($K_i$ = 1.1 nM) highlights that binding is dominated by interactions with the skeletal A ring and hydrophobic *closo*-carborane core rather than the side chain. As expected, increasing the length of the side chain by inserting methylene tethers between the phenol and 1,2-carborane group, as seen in structure **32** (Figure 8), decreased the binding affinity of the compound for the ER by an order of magnitude relative to estradiol **8** ($K_i$ = 0.4 nM) but generated more potent antagonistic behavior than that of **31**, a partial agonist-antagonist (vide infra).[64] The affinity of **33** bearing an adamantane as a hydrophobic core for the ER was 10-fold weaker than the corresponding 1,2-carborane compound **32**, and, surprisingly, the nature of the interaction resulted in full agonistic activity for the drug.[64] The 1,2-carborane derivative **34** possessing a long alkyl chain and containing an amide functionality to mimic the side chain of the potent steroidal antagonist ICI-164,384 **29** at position 7 was prepared and studied. It exhibited antagonistic activity in a transcriptional assay, albeit to a moderate level as compared to ICI-164,384 **29**.[85] The corresponding 1,7-carborane derivative did not exhibit any antagonistic activity at all. Although the binding mode of 7-substituted steroidal antagonists like **29** was not clarified, the role of the linear alkyl group appears to be similar to that of the 4-hydroxytamoxifen **14**. The orientation of the substituent and length of the side chain appear to play a critical role in antagonistic activity.

Selective estrogen receptor modulators (SERMs) bind to the same ERαLBD but have concurrent agonistic and antagonistic activity depending on the tissue type because of differences in the function of cofactors within tissue. Compound **31** (Figure 8) was found to restore bone loss in ovariectomised mice without estrogenic action in the uterus.[86] This suggests that the compound is a SERM by exhibiting agonistic activity in bone tissue and antiestrogenic activity in the uterus. It was also found that the A′-ring phenoxide side chain of 1,7-carborane bisphenols (Figure 7, compound series **23**) controls the balance between agonistic and antagonistic estrogen receptor activity,[65,78] and thus appears to be suitable for the development of SERMs (Figure 7).[65] Analogues **23c** ($n$ = 2 and 4) were found to be moderate partial agonists with ER agonistic and antagonistic activity. The cyclic amino analogues **23d**,**e**, on the other hand, were found to be potent ER partial agonists with a good balance of agonistic and antagonistic activity and were thus deemed to be the most promising SERMs candidates.[78]

Boroxifen **35** (Figure 8), an analogue of tamoxifen **15** containing a *nido*-carborane cage in place of the A ring, was synthesized for potential antagonistic activity and as a means of concentrating boron in tumor cells for BNCT.[87] To date, there is no report on the biological evaluation of this compound for the ER.

In summary, the *closo*-carborane cage can be used to modulate ER activity. The 1,12-carborane and 1,7-carborane cages are suitable as ER agonists, and the bisphenolic 1,2-carborane system appears to be a suitable choice in the development of partial agonists and antagonists.[65] The bisphenol 1,7-carborane system can produce SERMs candidates with the right choice of phenoxide substituents on the A′-phenyl ring.

### 2.3. Retinoic Acid Receptor and Retinoid X Receptor

Retinoic acid receptors (RARs) and retinoid X receptors (RXRs) are members of the nuclear receptor superfamily. Upon activation with agonists or antagonists, retinoidal receptors (isotypes α, $\beta$, and $\gamma$) are involved in the modulation of biological activity related to cellular differentiation and proliferation in, for example, embryonic development. RARs are activated by both endogenous all-*trans* retinoic acid **36** (an oxidized derivative of vitamin A) and 9-*cis* retinoic acid **37**, albeit with a lower affinity,[88] whereas the RXRs, which are heterodimerization partners, are only activated by the 9-*cis* retinoic acid **37** (Figure 9). RAR forms a heterodimer with RXR, upon which binding of an agonist to the RAR results in an association of the heterodimer with a coactivator protein to form a complex that promotes gene transcription. Agonist bound RXRs are transcriptionally inactive in the RAR−RXR heterodimer unless RAR is also activated by a ligand. RXR agonists (synergists) may also increase the potency of RAR ligands. The X-ray crystal structure of the RXR-9-*cis* retinoic acid **37** complex[76] shows that the RXRαLBD possesses a more pronounced kink than in the RARγLBD complex with all-*trans* retinoic acid,[88] with the binding site of the $\beta$-ionone group of 9-*cis* retinoic acid shifted almost 90° toward the bottom of the LBD relative to RAR.[76] Furthermore, the size of the binding pocket of RXRα (489 Å$^3$) can potentially accommodate bulkier ligands around the methyl-18 and methyl-19 groups of 9-*cis* retinoic acid **37**. A high binding affinity for both the RAR and the RXR requires a carboxylic acid moiety and a hydrophobic group located at a suitable position on the molecule.[89] A relatively

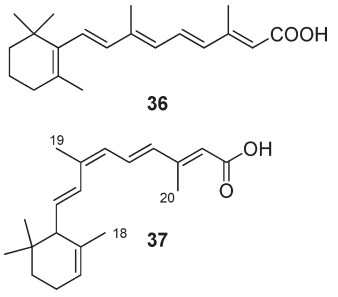


Figure 9

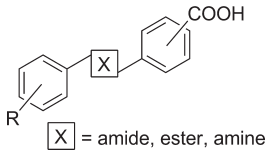


Figure 10

planar conformation along the body of the compound (mimicking all-*trans* retinoic acid) is preferred for a RAR ligand,[51] while a twisted angular conformational structure is preferred for the activation of RXR. Differences in binding selectivity and activity for the RAR and RXR depend not only upon the spatial orientation of the carboxylate group but also on the optimum bulk of the hydrophobic group[90] and optimum distance between the hydrophobic group and carboxylic acid moiety.[91]

The design of carborane-containing derivatives of retinoids is based on the skeletal structure of synthetic retinoids belonging to polyenes, benzanilides, and phenyl esters.[21] Groups necessary for retinoidal activity include an R alkyl chain (e.g., a propyl group) on the linking aryl ring and carboxylic acid functionality at the *meta*- or *para*-position of the second phenyl group (Figure 10). The hydrophobicity of R is important for potent retinoidal activity, and the linking group X (e.g., amide, ester, or amine) has an important role to play in determining the planarity/twist of the molecule and the orientation and distance between the hydrophobic R and carboxylate groups. Another important consideration is the effect of ligand steric bulk on RAR/RXR α, *β*, *γ*-subtype selectivity. The RARα, *β*, *γ* isotypes differ in three amino acid residues, which alter their selectivity for synthetic retinoids. For example, the lower affinity of 9-*cis* retinoic acid **37** for RAR*γ* (as compared to the α or *β* isoform) may be a result of greater steric interactions between the 19-methyl of 9-*cis* retinoic acid and Met-272 than the corresponding smaller Ile residues in RARα and *β*.[88] The work reviewed here summarizes the SARs of carborane-containing retinoid agonists and antagonists consisting of phenylamine and benzamide linker groups. The plethora of work reported in this area further supports the idea that *closo*-carboranes are applicable as hydrophobic pharmacophores in medicinal chemistry.

Retinoidal agonistic activity was demonstrated in terms of the ability for compounds to induce differentiation of human promyelocytic leukemia (HL-60) cells into mature granulocytes, while antagonistic activity was determined by the ability of a compound to inhibit the differentiation-inducing capability of known RARα and *β* activators.[52,90] In the phenylamine linker series of compounds (Figure 11), consisting of a 1,2-carborane cage at the *para*- or *meta*-position on an aromatic group and

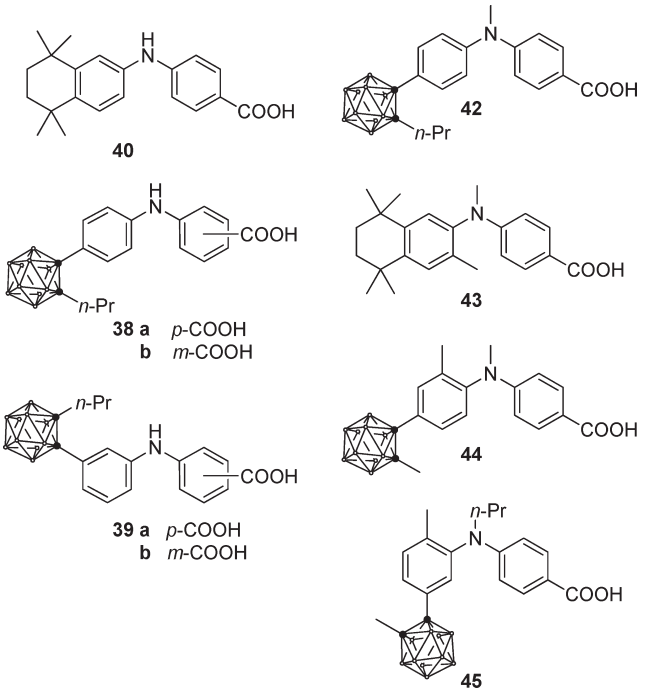


Figure 11

either a *para*- or *meta*-benzoic acid terminus (as seen in compounds **38a**,**b** and **39a**,**b**), all isomeric permutations exhibited similar potent differentiation-inducing activity toward HL-60 cells.[51,89,91] Introduction of the bulky *n*-propyl group on the carborane cage increased the RAR agonistic activity relative to its unsubstituted parent. For example, differentiation activity increased with compound **38a**[51] upon *C*-carborane *n*-propyl substitution to make it comparable in activity to the endogenous *trans*-retinoic acid **36**, and 2 orders of magnitude more active than its structurally related DA010 **40**.[91] This work demonstrates that the bulky carboranyl group on the aryl ring fits into the RAR cavity and that a carboranyl *C*-substituent may influence the hydrophobic interaction between the ligand and receptor. *C*-Carborane substitution with either shorter or longer alkyl groups than *n*-propyl or iso-propyl substituents was found to slightly diminish activity.[51,91] The planar conformation at the phenyl-*N*-phenyl linker is important for RAR agonistic activity. An ether linker possessed good agonistic activity only in the presence of a RXR synergist, while other linkers such as carbonyl, methylene, and 1,1-ethylene possessed no activity even in the presence of a synergist.[91] Docking simulations of **38a** showed that it fit into the hRARα cavity (simulated from the X-ray structure of hRAR*γ*[88]) as well as *trans*-retinoic acid **36** through hydrophobic contacts along the bulky *closo*-carborane and hydrogen bonding at the carboxylate end. However, **38a** remains 1 order of magnitude weaker in retinoidal activity than the potent RAR agonist Am80 **41** (Figure 12). Introduction of an *N*-methyl group and/or alkyl group on the aromatic nucleus (Figure 11) abolished RAR activity due to the twisted conformation of the phenyl-*N*-phenyl moiety.[51,89,91] For example, **38a** exhibited 100 times stronger differentiation inducing ability than did its corresponding *N*-methyl derivative **42**.[91] Unfortunately, no docking simulations in hRARα could be generated for compound **42** under the general docking conditions used.[91] Despite having the twisted conformation required for synergism as well as being a


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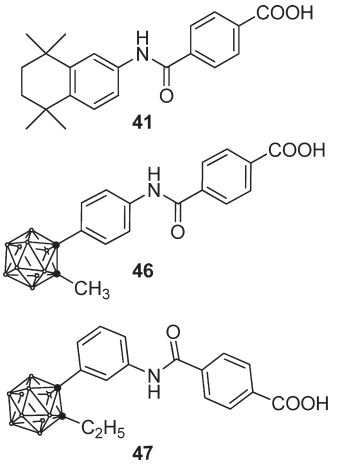


Figure 12

close analogue of DA111 **43**, one of the most potent RXR activators known, compound **44**[91] displayed no synergistic activity. This result suggests that an RXR agonist such as DA111 **43** requires an optimal distance between the hydrophobic group and carboxylic acid moiety as well as less bulk in the hydrophobic region. The design of 1,2-carborane-containing diphenylamines as selective antagonists at the RXR site of the RAR-RXR heterodimer was reported by Ohta et al.[90] Compound **45** and related analogues were found to inhibit differentiation of HL-60 cells induced by a combination of RAR agonist and RXR synergist.[90] This indicates that **45** antagonizes the RXR agonist at the RXR site when both RAR and RXR agonists are bound to the heterodimer.[90] Thus, minor changes in chemical structure result in major changes to the mode and potency of activity.

Although DA010 **40** and AM80 **41** differ only by one atom length in the linker, they behave as agonists for the different receptor subtypes RXR and RAR, respectively. Thus, an amide linker increases the length between the carboxylic acid group and *closo*-carborane as compared to those compounds possessing an amine linker, thereby potentially changing the nature of the activity. For example, a 1,2-carborane located at the *para*-position of the aromatic nucleus linked to a carbamoylbenzoic acid, as in structure **46** (Figure 12), affords potent retinoidal antagonistic activity as demonstrated by the competitive inhibition of Am80 **41**-induced differentiation of HL-60 cells.[52] *C*-Carborane methyl substitution increased the potency of the antagonism, but the introduction of a reversed amide linker, that is, CO−NH instead of NH−CO, did not modify the activity. However, a 1,2-carborane cage located at the *meta*-position of the aromatic ring (e.g., **47**) resulted in a very weak RAR activator of HL-60 differentiation (10%), which was enhanced to 93% by the addition of a retinoidal synergist.[52]

Removal of the aryl ring and direct substitution of 1,2-, 1,7-, and 1,12-carboranes to the amide linker, as found in compounds **48**, **49**, and **50** (Figure 13), resulted in the depletion of all activity (agonistic and antagonistic).[92] However, recovery of retinoidal antagonistic behavior against differentiation inducing Am80 was achieved through the introduction of a bulky decamethylated 1,12-carborane **51**. Elongation of the linker with a urea group, or the introduction of a smaller octamethylcarborane cage, slightly diminished the potency of antagonism. The use of bulkier hydrophobic groups in retinoidal ligands to enhance antagonistic activity has been observed previously. For example, an increase in

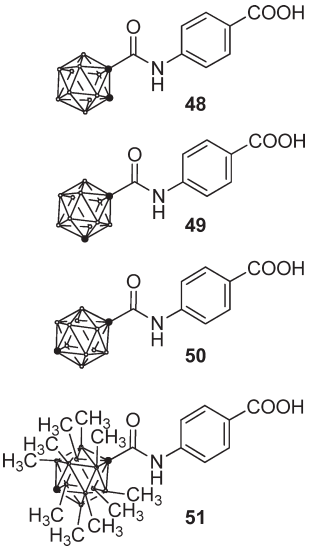


Figure 13

the bulk of the hydrophobic group from adamantyl to diamantyl saw a significant shift from partial agonism to potent antagonism. A *closo*-carborane cage was shown to exhibit effects similar to those of the diamantyl group.[52]

In summary, as a result of the more pronounced bending angle in the RXRα ligand binding domain as compared to RARγ (as determined from X-ray crystallographic studies of LBD-ligand complexes), preferred RXR ligands possess a twisted molecular shape. The distance between the optimally sized hydrophobic group and carboxylic acid group in RXR ligands is relatively short as compared to that found in RAR ligands (Figure 14). The introduction of an alkyl group on the aromatic ring adds to the conformational twist and, coupled with the steric bulk and hydrophobicity of the *closo*-carborane, produces a RXR antagonist, which disturbs the helix-12 folding and subsequently deactivates the RAR−RXR heterodimer from coactivator binding. The structural requirements for a RAR agonist are optimal molecular length, optimal hydrophobic bulk, and a planar molecular shape. In the case of phenylamines, the bulky carboranyl moiety on the aryl ring fits well into the RAR cavity.[91] An alkyl group at the *C*-carborane position in the phenylamine compound increases agonistic potency possibly due to an increase in the number of hydrophobic interactions. A RAR antagonist possesses a planar agonistic core, which buries into the hydrophobic pocket corresponding to the *trans*-retinoic acid binding niche, but possesses a bulky antagonistic extension, which forces helix-12 to adopt an antagonist position.[93] For example, within the benzamide series, *closo*-carborane substitution at the *para*-position of the phenyl ring afforded RAR antagonistic activity. RAR antagonist activity was abolished by shortening the length of the molecule via *meta*-substitution of the *closo*-carborane on the aryl ring.[52]

## 2.4. Androgen Receptor

The suitability of the *closo*-carborane cage as a hydrophobic skeletal structure for binding to the ERα and retinoid receptors has provided the basis for developing novel carborane-containing ligands for other receptors that possess a hydrophobic core. The androgen receptor (AR) is a member of the nuclear receptor superfamily and plays a key role in the development and


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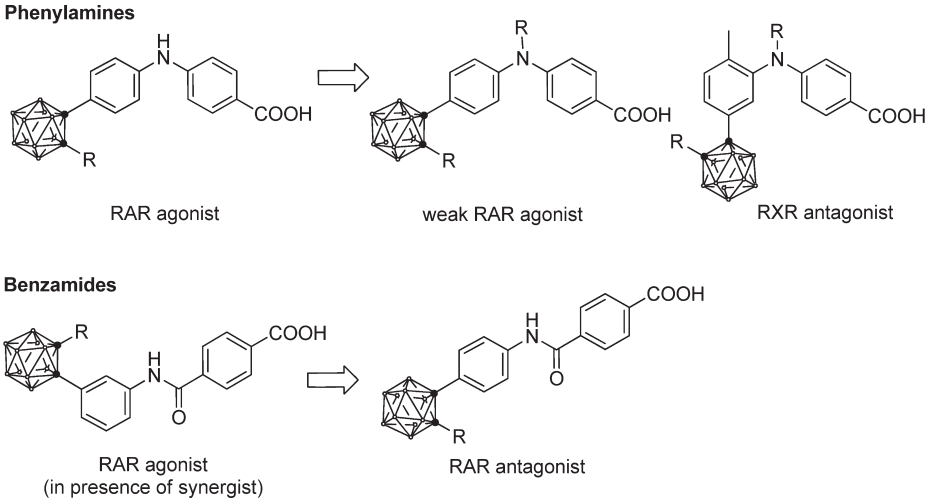


Figure 14

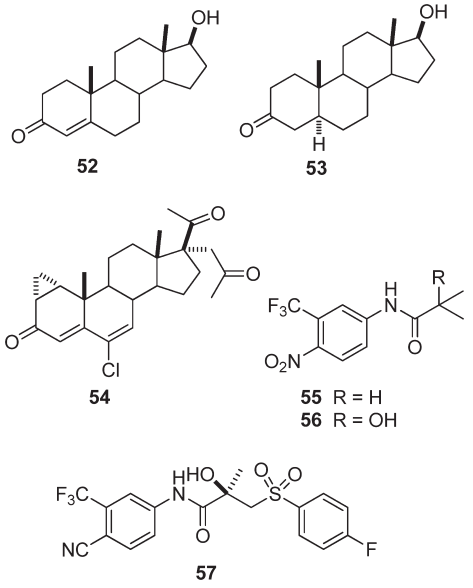


Figure 15

maintenance of the male reproductive system, and muscle and bone mass. Testosterone **52** and 5α-dihydrotestosterone (DHT) **53** (Figure 15) are endogenous androgen agonists. Synthetic androgen antagonists are commonly used to treat androgen-dependent disorders. For example, cyproterone acetate (Cyprostat) **54** is used clinically for the treatment of metastatic prostate cancer. However, because this compound exhibits a number of side-effects due to its cross-activity with other steroid hormone receptors, nonsteroidal AR antagonists such flutamide (Euflex) **55**, hydroxyflutamide **56**, and *R*-bicalutamide (Casodex) **57** are preferred. Although nonsteroidal AR antagonists are already in clinical use for the treatment of metastatic prostate cancer, new nonsteroidal AR ligands are still required for tumors that are (or subsequently become) insensitive to them. Furthermore, despite their antagonistic efficacy, they exhibit lower affinity for the hAR than that of DHT **53**. This means that drug administration doses need to be high, which subsequently leads to toxicity concerns. New drugs that possess high affinities but retain their potency for the hAR are therefore required.[94] Novel biologically active nonsteroidal AR ligands containing *closo*-carborane are examples of potential drugs that could be used to treat AR disorders. The design of AR ligands outlined below is based on the replacement of hydrophobic components in known AR ligands **52**, **55**, and **56** with a *closo*-carborane cage.

The ARLBD consists of a hydrophobic pocket created by several hydrophobic amino acid residues, the identification of which varies depending on the analysis method.[95,96] The LBD is different from the ER in the binding region of the A-ring residue. As seen from the crystal structure of human ARLBD complexed with the agonist DHT **53** or with other steroidal structures, the oxygen of the carbonyl in the A-ring is hydrogen bonded to Arg-752 and Gln-711, and the 17$\beta$-hydroxyl group is hydrogen bonded to the Thr-877 and Asn-705 residues.[96,97] Other polar groups such as nitro in synthetic AR ligands can form similar hydrogen-bond contacts to Arg-752 and Gln-711 to anchor the ligand for binding to the AR. The trifluoromethyl group adjacent to the nitro functionality in flutamide **55** is thought to increase the antagonistic activity of the compound due to its bulk in the closely surrounding hydrophobic pocket. Bulky groups of a ligand, capable of hindering the critical amino acid residues required for agonistic activity, may affect the conformational disposition of functionally important helix-12.[95]

The design of the first carborane-containing AR ligand **58** (Figure 16) was based upon the structures of endogenous testosterone **52** and its biologically active product 5α-dihydrotestosterone (DHT) **53** in an approach similar to that adopted in the design of the potent ER ligand **9c** (Figure 4) (vide supra). Thus, compound **58** contains a *closo*-carborane cage instead of rings C and D and a hydroxymethyl group for hydrogen bonding to polar amino acid residues. This approach was justified on the basis that the AR and ER have similar LBDs. Unexpectedly, rather than exhibiting agonist activity, ligand **58** exhibited potent antagonist activity at a level comparable to that of the clinically used hydroxyflutamide **56** (Figure 15). Strong hydrophobic interactions between the *closo*-carborane cage and the hydrophobic pocket of the ligand binding domain may account for the high affinity, and the bulk of the carborane cage may be responsible for the antagonistic activity. A clash between the *closo*-carborane cage and the Thr-877 residue may lead to displacement of helix-12.[98,99] Subsequent design of new carborane containing antagonists was based on the nonsteroidal ligands flutamide **55** and its


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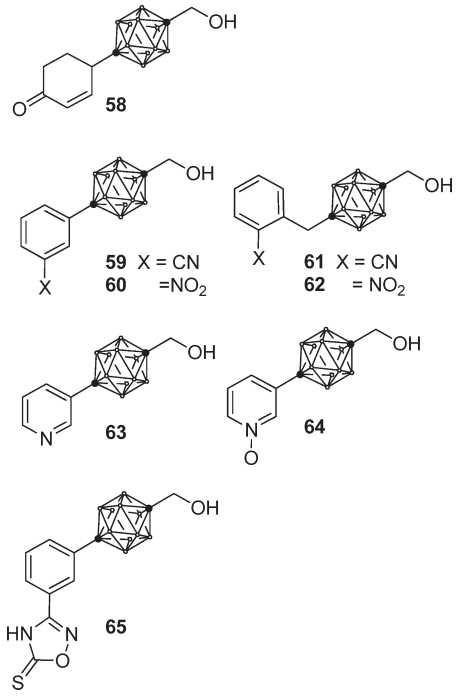


Figure 16

metabolite hydroxyflutamide **56** (Figure 15). Replacement of the cyclohexenone moiety in **58** with a phenyl ring substituted with electron-withdrawing groups (cyano, nitro) at the *meta*-position, and locating the polar groups opposite each other on the C-vertices of the 1,12-carborane cage to afford molecules **59** and **60** resulted in a 10-fold higher binding affinity to the AR and 10-fold higher antagonistic activity than was exhibited by the parent hydroxyflutamide **56**. The cyano and nitro hydrogen-bonding acceptors are assumed to be the anchors for binding to the AR.[99,100] Analogues of **59** and **60** containing a C-disubstituted 1,7-carborane or additional methylene linkers between the hydroxyl and *closo*-carborane cage exhibited a poorer binding affinity toward the AR.[101] Replacement of the *meta*-substituted benzene in **59** and **60** with *ortho*-cyano- and *ortho*-nitrobenzyl groups, as seen in compounds **61** ($IC_{50}$ = 0.96 nM) and **62** ($IC_{50}$ = 0.83 nM), respectively, afforded AR-antagonistic potencies similar to that of flutamide **55** ($IC_{50}$ = 0.63 nM).[99] It is noteworthy that weaker antagonism of the benzyl carborane derivatives was possibly due to the flexibility afforded by the methylene linker.[100] Ohta et al.[100] showed that a rigid pyridine ring is also suitable as an aromatic group, and, as expected, the *meta*-pyridine analogue **63** exhibited the strongest binding affinity, which was comparable to that of flutamide **55**. The importance of the *N*-pyridyl functionality was demonstrated by the inactivity of the pyridine *N*-oxide derivative **64**. However, because the distance between the nitrogen atom and hydroxymethyl group in **63** is shorter than the distance observed in the potent molecules **59** and **60**, the antiandrogenic activity of **63** is expectedly weaker. The reason postulated by the authors is that when the nitrogen atom anchors the molecule to the protein, the hydroxyl group at the other end of molecule **63** is not positioned correctly to interact with Thr-877.[100] Studies by Fujii et al.[102] showed that the use of the acidic oxadiazole-5-thione heterocycle (e.g., **65**), which interacts electrostatically with the positively charged guanidinium group of Arg-752, resulted in a higher AR affinity of the derivatives than hydroxyflutamide **56**, albeit the compounds were inactive as antagonists and agonists. In summary, matching the *closo*-carborane structure to both the hydrophobic region of the LBD and hydrogen bonding to the AR polar residues is required for strong binding affinity and/or potency.

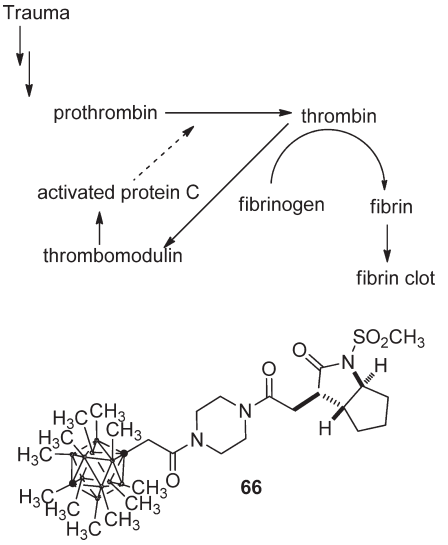


Figure 17

## 2.5. α-Human Thrombin Receptor

α-Human thrombin is a protease inhibitor found in the blood and is involved in a multitude of healing processes in the body following trauma. Thrombin, a serine protease, is an agonist that promotes platelet activation by cleaving the receptor on the platelet. Platelets form a plug at the site of damage as part of the primary hemostasis response. Thrombin is also the final enzyme in the blood coagulation cascade that cleaves soluble fibrinogen into insoluble strands of fibrin to form a hemostatic mesh over wounds, which strengthens the platelet plug as part of the body's secondary hemostasis response. Conversely, thrombin binds to thrombomodulin to aid the anticoagulant process by activation of protein C (Figure 17). Thrombin is associated with many disease states, which makes it an attractive target for anticoagulants.

Thrombin's LBD is comprised of three subsites: (a) active site S1 consists of a catalytic triad and an oxyanion hole formed by Ser-195, His-57, Asp-102, (b) active site S2 is a hydrophobic pocket, which includes Tyr-60A and Trp-60D, and (c) active site S3 is a distal hydrophobic pocket formed by Leu-99, Ile-174, and Trp-215.[103] Known thrombin inhibitors act by acylating the nucleophilic Ser-195 hydroxyl group in the S1 active site. Asp-189 in the S1 recognition site is thought to play a role by anchoring the ligand in the correct orientation for acylation in the active site. Hawthorne and co-workers[103] demonstrated the utility of the *closo*-carborane as a hydrophobic pharmacophore when they prepared a permethylated 1,12-carborane ligand **66** (Figure 17) based on the structural characteristics of known serine protease inhibitors. Docking computational studies indicate that the hydrophobic *closo*-carborane is positioned in the larger S3 distal hydrophobic pocket by van der Waals interactions involving amino residues, which orient the [5,5]-*trans* lactam moiety of the molecule toward the active S1 pocket where it acylates the Ser-195 hydroxyl group. The optimal distance between the two ends of the molecule is maintained by use of

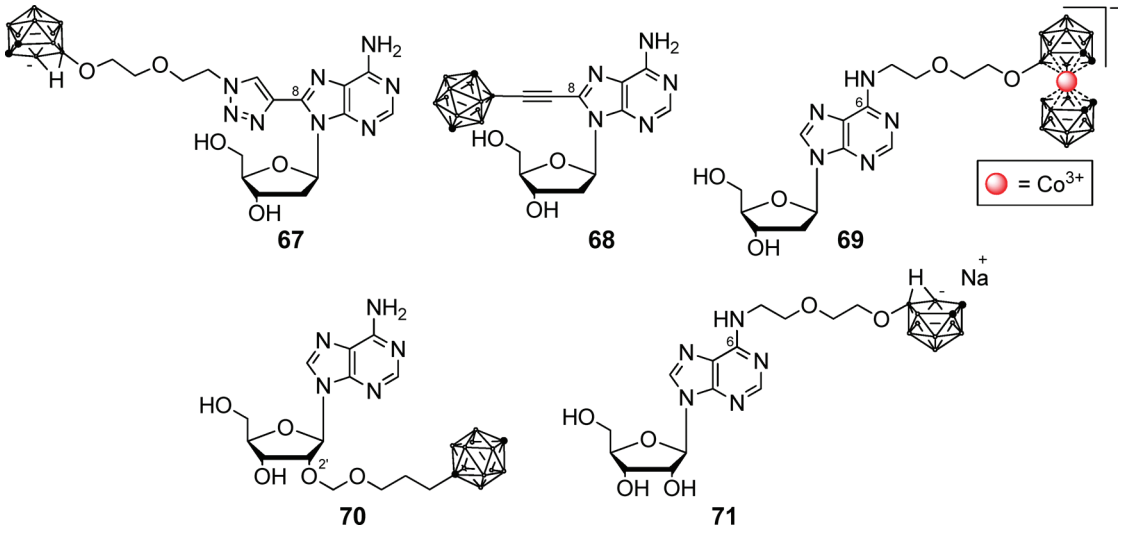


Figure 18

a piperazine ring, which could potentially also interact hydrophobically with the S2 pocket. No biological evaluation of the *closo*-carborane-containing ligand **66** with thrombin has been reported to date.

## 2.6. Purinergic Receptors

Adenosine interacts with the purinergic receptors P1 (with adenosine receptor subtypes $A_1$, $A_{2A}$, $A_{2B}$, $A_3$) and P2, to inhibit platelet aggregation and regulate immune and inflammatory responses. It also acts synergistically with thrombin-inhibiting drugs.[104] Micromolar concentrations of adenosine and 2′-deoxyadenosine physiologically inhibit platelet response to activation by the agonists adenosine diphosphate (ADP) and thrombin. Although adenosine-based drugs are clinically used as cardioprotective, anticancer, and antiviral agents, they are systemically cleared rapidly and have poor selectivity and affinity for receptors. The high metabolic stability of the carborane cage can therefore be used to design compounds that are more stable in vivo and hence more bioavailable than currently used compounds.

Olejniczak et al.[105] first reported the syntheses of 2′-deoxyadenosine analogues substituted at C8 with a *nido*-7,8-carborane connected via an ether linker **67** (Figure 18) or *closo*-1,12-carborane attached via an acetylene linker **68**. Substitution of 2′-deoxyadenosine at N-6 with a metallocarborane derivative afforded compound **69**.[104] Three other carborane-containing adenosines were synthesized, adenosine substituted with *closo*-1,12-carborane at the 2′-position or with *nido*-7,8-carborane at the N-6 position to afford compounds **70** and **71**, respectively. All compounds assessed (i.e., **68**, **69**, **70**, and **71**) were shown to modulate platelet function to activation by ADP and thrombin.[104,105]

Modification of the adenosine at the 2′ position with *closo*-1,12-carborane resulted in the greatest inhibition of platelet activity. The experimental markers for platelet activation are platelet aggregation, protein release, and P-selectin expression.[104] Compound **70** ($IC_{50}$ = 0.05 $\mu$M) was shown to be a much more potent inhibitor of platelet aggregation, induced by ADP, than adenosine ($IC_{50}$ = 1.27 $\mu$M). Compound **70** also showed better thrombine-induced inhibition of platelet aggregation than did adenosine at doses of 10 and 30 $\mu$M. Moreover, in contrast to the other compounds, **70** exhibited a prolonged duration of inhibitory action (2 h). Inhibitory affects of **70** on ADP-induced platelet aggregation and P-selectin expression were achieved at a 100-fold lower concentration (0.1 $\mu$M) as compared to adenosine (10 $\mu$M). Compound **70** was also 100-fold more effective at inhibiting protein release from thrombin-induced platelets than adenosine. In contrast to **70**, compound **71** was not effective at inhibiting ADP-induced platelet aggregation at 1 $\mu$M, nor thrombine-induced platelet aggregation at all tested concentrations (0.05−30 $\mu$M). Compounds **68** and **69** more effectively reduced ADP-induced platelet aggregation than the parent 2′-deoxyadenosine at a dose of 30 $\mu$M, but, like 2′-deoxyadenosine, were ineffective inhibitors of thrombin-induced platelet aggregation. However, the inhibition potency of ADP-induced platelet aggregation by **68** remained lower than **70** at a concentration dose of less than 30 $\mu$M. Surprisingly, compounds **69** and **71** were able to induce platelet activation. In summary, modification at the 2′-position of adenosine with a 1,12-carborane cage resulted in the most potent inhibitory responses against thrombin and ADP-induced aggregation, protein release, and P-selection expression. Substitution at the N-6 positions of adenosine and 2′-deoxyadenosine gave a decrease in inhibitory activity. Indeed, substitution with carboranes at the N-6 position afforded analogues with low-level platelet activation properties.

## 2.7. Dihydrofolate Reductase Receptor

Dihydrofolate reductase (DHFR) is an enzyme that catalyzes the transfer of hydride from NADPH to dihydrofolate to form tetrahydrofolate, a cofactor of numerous enzymes that catalyze one-carbon transfer reactions for the biosynthesis of some amino acids, purines, and thymidylate (a component of DNA) needed for cell proliferation and growth.[106] Because DHFR regulates the amount of tetrahydrofolate in the cell, its inhibition by known antifolates (folate antagonists) such as the anticancer agent methotextrate (MXT) **72**, the antibacterial trimethoprim (TMP) **73**, and the antimalarial pyrimethamine **74** (Figure 19), and consequent depletion of tetrahydrofolate, is exploited clinically. The key interactions between ternary human DHFR-NADPH complexed with MXT-based antifolates, and complexes of ternary *E. coli* DHFR and chicken DHFR with TMP **73**, have been elucidated from X-ray crystallographic studies.[107−110]


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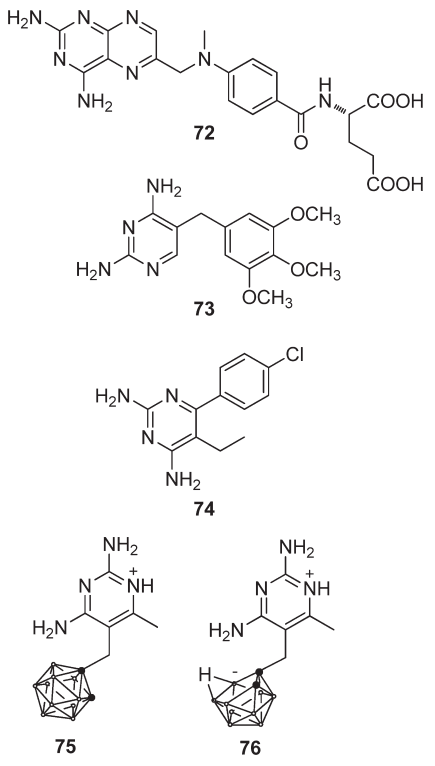


**Figure 19**

Whereas different heterocyclic cores from a variety of DHFR inhibitor molecules access similar spatial regions of the DHFR active sites, regardless of the species of DHFR, the side chains of the different inhibitors occupy different binding pockets.[108−110] Thus binding affinity is modulated by the lipophilic side chains and linker region on the core pteridine ring.[107,109,110] For each species of DHFR, there is an upper and lower binding cleft for inhibitor side chains, and these are larger in the chicken than in the bacterial enzymes. Matthew et al.[109,110] reported that the conformational fits of the TMP **73** inhibitor in the *E. coli* and chicken DHFR complexes are distinctly different, leading to a 3000 times more strongly bound TMP **73** in bacterial than vertebrate DHFR. The greatest difference is observed in the accommodation of the side chain. The trimethoxyphenyl side chain of TMP **73** in the chicken DHFR accesses the upper hydrophobic cavity (as compared to the lower cavity in bacterial DHFR), placing it further away from the nicotinamide ring of NADPH below.

Developed resistance of DHFR to current agents has prompted the search for new agents that inhibit DHFR. Reynolds, Borhani et al.[111] reported the synthesis and biological evaluation of *closo*-1,2-carborane **75** and *nido*-7,8-carborane **76** derivatives of the TMP antifolate **73** in both eukaryotic and prokaryotic DHFRs. The inhibition of DHFRs from human, rat liver, and *T. gondii* prokaryote by *closo*-**75** and *nido*-**76** was found to be more potent than the inhibition by TMP **73**. The potency of *closo*-**75** and *nido*-**76** against the *P. carinii* DHFR was found to be equivalent to that of TMP, while both carboranes exhibited weaker potency against the bacterial DHFRs (*M. avium* and *L. casei*) than did TMP.

The *closo*-**75** analogue was a more potent inhibitor than *nido*-**76** in all DHFRs except in *T. gondii* in which the *nido*-carborane analogue **76** exhibited 2-fold greater activity than the *closo*-carborane and 65 times improved selectivity for *T. gondii* DHFR than for rat liver DHFR. The inhibition activity of *closo*-**75** ($IC_{50}$ = 0.15 $\mu$M) was 100-fold more potent than *nido*-**76** ($IC_{50}$ = 15 $\mu$M) against human DHFR and 1000-fold more active than TMP against rat liver DHFR ($IC_{50}$ = 133 $\mu$M).

The *closo*-**75** compound also exhibited up to 10-fold greater inhibitory growth activity than the *nido* analogue **76** in all seven human tumor cell lines tested and approximately 10-fold greater activity than MXT **72** in four of the human tumor cell lines. The X-ray crystal structures of ternary human DHFR-NADPH-**75** and human DHFR-NADPH-**76** revealed that the carborane cages in both structures were bound in the active DHFR site in a single rotational orientation unique for each carborane. The C2 position in *closo*-**75** adopts a single position centered 4.7 Å above the C3 and C4 atoms of the NADPH nicotinamide ring as a result of electronic factors. In comparison, the open cage position of the chiral *nido* cluster points away from the nicotinamide ring in a single stereoisomeric orientation, thus positioning the C2 distinct from **75**. Both carboranes exhibit very similar binding modes to TMP in the DHFR binding cavities,[109,110] with the carboranes placed in the same cavity as the trimethoxyphenyl ring of TMP and with similar molecular orientations, making hydrophobic interactions with the nicotinamide group as well as DHFR amino acid residues Phe-34, Phe-31, Ile-60, Thr-56, and Leu-22. Despite the presence of a *nido*-carborane group, the resulting zwitterionic biomolecule was able to maintain hydrophobic characteristics for interaction with the hydrophobic cavity of the DHFR receptor.[111]

## 2.8. HIV Protease Receptor

HIV protease (HIV PR) is a homodimeric receptor. The active site lies between two identical subunits and has the highly conserved Asp-25, Thr-26, and Gly-27 sequence. The hydrophobic binding pockets are formed by residues Pro-81, Val-82, and Ile-84 on the base, and Ile-47, Gly-48, and Ile-54 on the flaps. The Asp residues in the active site (one contributed by each monomer) are involved in the cleavage of peptide bonds mediated by water. In addition, each subunit contains a molecular flap, which shifts to close over the active site when a substrate is bound. HIV PR cleaves a polypeptide to create the mature protein of an infectious virus. Thus, inhibition of the HIV PR disrupts the virus replication process and inhibits its ability to infect other cells.

Substituted metallocarboranes are potent, selective, and competitive inhibitors of wild type and mutated HIV PRs that have decreased susceptibility to conventional FDA-approved anti-HIV drugs. The $[Co(1,2\text{-dicarbollide})_2]^-$ **77** (Figure 20) species is a sandwich complex with some degree of hydrophobicity and delocalized negative charge, which enables the unique interaction of the cluster surface with proteins. The metallocarboranes interact with enzyme residues via unconventional proton-hydride (B−H---H−X, X = C, N, and O) bonds with characteristic hydrogen-bond distances of 1.8−2.3 Å and bond strengths of 2.1−5.8 kcal $mol^{-1}$.[112,113] X-ray crystal structures of the HIV PR dimer complex with two cobalt bis(dicarbollide) clusters[114] **77** and HIV PR dimer with complex **78**[115] (Figure 20) show that both cluster compounds have similar binding modes but with key differences in protein−inhibitor interactions as a result of the difference in orientation of the boron clusters relative to one another. In compound **78**, the two clusters bound by the flexible linker are ordered symmetrically and rotated by 37° and 44° relative to the two separate and asymmetrically bound clusters of

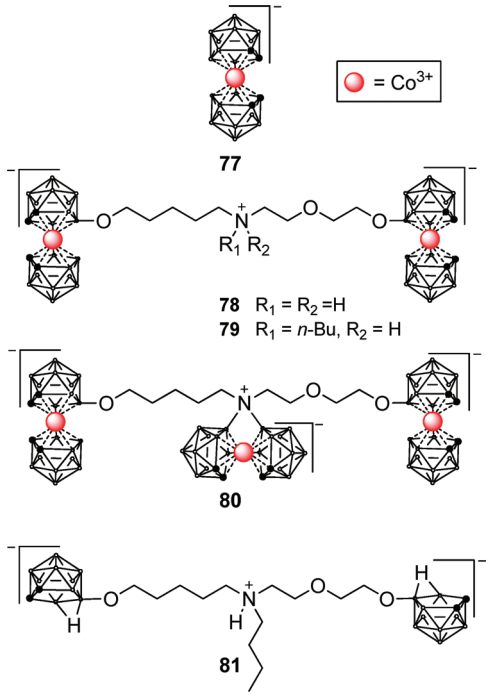


Figure 20

the cobalt bis(dicarbollide) ion **77**. The linker chain of **78**, however, is disordered in the crystal structure, which suggests that it is flexible and able to adopt different conformations. The position of the linker's central secondary amino group was fixed using a water molecule inferred from a $F_o - F_c$ map, which places the central nitrogen atom to within hydrogen-bonding distance of Asp and catalytic water. The nonsymmetrical complexation of bis(dicarbollide) clusters **77** to two identical binding sites of HIV PR is suggestive of the carbollide's ability to potentially adapt to the LBD of mutated HIV PRs.[114] When the two cobalt bis(dicarboliide) clusters **77** bind to the PR active site, they prevent the enzyme flaps from closing over the active site and activating it.[112,115] Inhibition studies were conducted by using enzyme assays that utilized a chromogenic substrate and recombinant wild-type HIV-1 PR or HIV variants that represented drug resistant mutations. Compound **78** and 12 derivatives thereof were synthesized and shown to inhibit wild type HIV-1 PR with varying mechanisms and degrees of potency.[112,114,115] Like **78**, the compounds consist of two cobalt bis(dicarbollide) clusters linked by a flexible ethyleneglycol chain containing a central secondary amine to which substitution of groups of varying size and lipophilicity permitted the mapping of structural requirements to protein binding. The quaternary ammonium species **78**, with a competitive inhibition constant of $K_i$ = 4.9 nM in HIV-1 PR, was a 14-fold more potent inhibitor than the parent cobalt bis(dicarbollide) complex **77**. Substitution of the central amino group with a small hydrophobic group such as *n*-butyl in **79** improved the inhibition potency to $K_i$ = 2.2 nM and gave 10-fold better antiviral activity in tissue culture,[114,115] while substitution with another cobalt−carborane cluster on nitrogen afforded compound **80** with a 20-fold better competitive binding inhibition ($K_i$ = 0.27 nM) as compared to **78**.[114,115] All of the analogues were shown to be specific inhibitors of all seven variants of HIV PR, including three of the most resistant to conventional drug treatments. Surprisingly, however, **80** loses its inhibitory efficacy against four of the tested HIV PR variants, which suggests a steric and/or polarity requirement. Furthermore, compound **81** bearing two *nido*-7,8-carborane groups in place of the cobalt bis(dicarbollide) moiety resulted in a ca. 9-fold less potent inhibitor than compound **79**, thus providing evidence for the requirement of hydrophobic bulky groups in HIV PR ligands.[115]

Computational methods used to model the position of the linker chain in **78** within the HIV PR active site appear to show that the amine moiety of **78** is placed within the symmetric cavity of the enzyme active site, which allows for hydrogen-bonding interactions to Asp. This model also permits small lipophilic substituents on nitrogen such as *n*-butyl to position in the narrow hydrophobic groove of the binding pocket and can thus account for the increase in inhibition potency as compared to unsubstituted **78**. The larger *t*-butyl group can also position itself in the conformationally flexible binding cleft. Polar substituents on nitrogen may replace the catalytic water and occupy the polar pocket with hydrogen-bonding interaction to Asp. However, this model cannot explain the inhibition potency of **80** as the metallocarborane produces a significant steric clash that cannot be accommodated in the PR groove. On the basis of the loss of inhibitory activity of **80** in HIV variants, the authors hypothesized an alternative binding mode. The hypothesis has yet to be confirmed by X-ray structure determinations.[115]

A metallocarborane has degrees of freedom in its binding position not offered to clinical agents, and this position can be adjusted to changes in the binding pocket brought about by mutations. The greater tolerance of metallocarboranes to mutations within the HIV PR binding pocket than clinical agents can be seen from the signficant loss of inhibition potency (up to 1000-fold) of clinical drugs against HIV variants and low loss of inhibitiory activity of metallocarboranes in the same mutated HIV variants. It can be seen again in the smaller range of relative inhibition values of metallocarboranes (0.6−20 nM) as compared to relative inhibition values ranging from 0.8 to 4500 nM of clinical drugs against the same variants.[112,115]

Tetraphenylporphyrin conjugated with one or four cobalt bis(dicarbollide) clusters **82** and **83**, respectively (Figure 21), was also reported to have anti-HIV-1 protease activity, with analogue **83** possessing ca. 4 times better viral inhibition in tissue culture ($IC_{50}$ = 77 ± 13 nM) than **82** ($IC_{50}$ = 290 ± 44 nM) by virtue of the increased number of cobalt bis(1,2-dicarbollide) substituents,[116] and have comparable anti-HIV activity to a porphyrin derivative, which was substituted with *closo*-carborane cages.[117,118] It can be seen from these examples that the hydrophobic contributions from both cobalt bis(dicarbollide) and *closo*-carborane clusters to ligand−receptor complexation are significant.

### 2.9. Other Receptors: Translocator Protein, Tumor Necrosis Factor-α, Protein Kinase C, Transthyretin, Aldo-Keto Reductases, and Thymidine Kinase

Translocator protein (TSPO), formerly known as peripheral benzodiazepine receptor, is found in the outer mitochondrial membrane. It has several functions that include transportation of cholesterol from the outer to the inner mitochondrial membrane for steroidogenesis, modulation of inflammatory response, induction of apoptosis, and stress adaptation. TSPO is overexpressed in tumor cells and lesioned areas of the brain as seen in certain cancers, Alzheimer's disease, and multiple sclerosis, which makes the receptor an appealing target in anticancer and neuropathological therapies.


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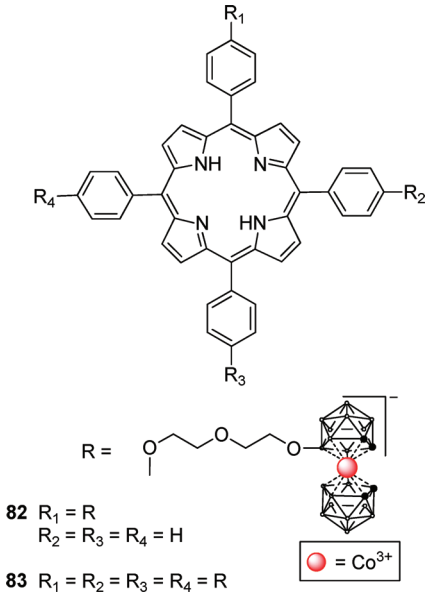


Figure 21

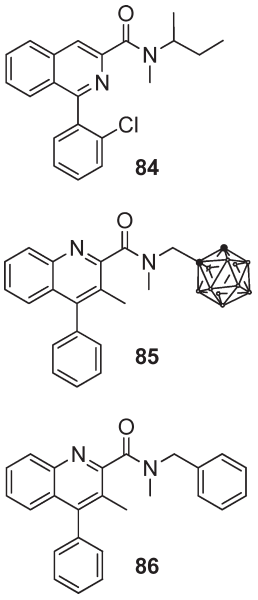


Figure 22

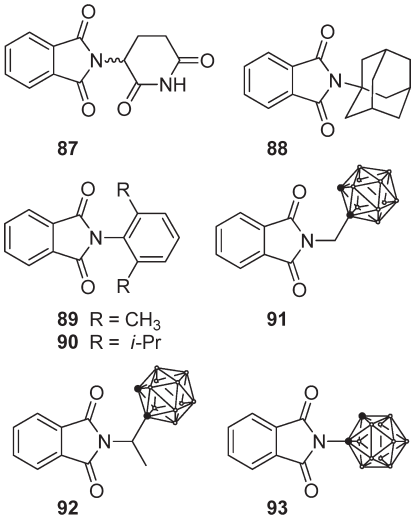


Figure 23

Isoquinolinecarboxamide **84** (Figure 22) is a TSPO ligand, which binds with 8.4 nM affinity and is widely used as a pharmacological tool for the study of TSPO function. Compound **84** is also used as a tumor-selective drug delivery platform when conjugated to anticancer agents by virtue of the selective interaction of **84** with TSPO. Although the carborane-containing derivative **85** was synthesized as a selective binder of TSPO with the aim of using it as a carrier of radionuclides for diagnostic imaging and as a boron carrier for BNCT, it provides yet another example in the use of a carborane cage for ligand−receptor interactions. Compound **85** moderately inhibited [$^3$H]-CB34 binding to rat cortical membrane with an $IC_{50} = 73 \pm 6.1$ nM, as compared to its more potent benzyl-substituted derivative **86**, which inhibits at an $IC_{50} = 4.6 \pm 0.7$ nM, 2-fold more potent than the quinolinecarboxamide **84** ligand.[119]

Tumor necrosis factor-α (TNFα) is a cytokine protein secreted into the bloodstream by cells of the immune system (such as monocytes and macrophages) in response to injury and plays a role in cellular communication and regulation of immune cells. It has various functions in different tissue, but generally TNF is able to induce inflammation and apoptotic cell death, and to inhibit tumorigenesis and viral replication. Whereas low levels of TNFα can lead to wasting syndrome, high levels of TNFα can lead to shock-like symptoms of heat, swelling, and pain in inflammatory response giving rise to clinical problems such as rheumatoid arthritis, psoriasis, and inflammatory bowel syndrome. Therefore, regulators of TNFα production are needed as modulators of biological response.

Although thalidomide **87** (Figure 23) inhibits the production of TNFα,[120] its derivatives show both enhancing and inhibiting activities on TNFα production. Previous SAR studies show that a bulky hydrophobic substituent on the nitrogen atom of the thalidomide ring, as found in compound **88**, and a twisted conformation between the thalidomide ring and 2,6-disubstituted phenyl, as seen in compounds **89** and **90**, are key requirements for potent activity. Thus, the thalidomide derivatives **91** and **92** bearing a *closo*-1,2-carboranylmethyl moiety as a hydrophobic pharmacophore to mimic 2,6-disubstituted phenyl derivatives of **89** and **90**, and derivative **93** containing a *closo*-1,2-carborane cage to mimic the adamantyl group of **88**, were synthesized and evaluated to be bidirectional regulators of TNFα production via TNFα production-regulating activity assays. These derivatives were found to enhance 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced TNFα production and inhibit okadaic acid (OA)-induced TNFα production by HL-60. The efficacy of the carborane-containing compounds **91**, **92**, and **93** as bidirectional regulators was found to be generally comparable to that of 2,6-dimethyl or 2,6-diisopropylphenyl and adamantyl compounds **89**, **90**, and **88**, respectively, and the order of potency was found to be **91** > **90** > **89**.[121]

Protein kinase C (PKC) is a signal transduction protein involved in tumor promotion activity when bound to phorbol esters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and teleocidin **94** (Figure 24); thus, potent modulators of PKC are appealing anticancer targets.

The benzolactam compound **95**, based upon the structure of teleocidin **94**, possesses potent biological activity. Computational


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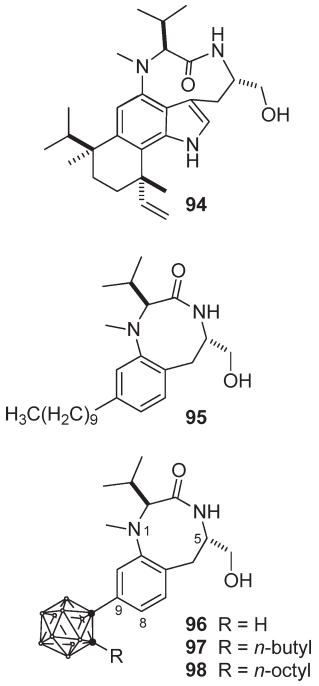


Figure 24

docking studies[122] of modified benzolactams such as **95** to the PKCδ Cys2 domain observed in the X-ray crystal structure of PKCδ with TPA[123] show the structural requirements for binding affinity and PKC activity of benzolactams are defined by the C-2 to C-9 hydrophobic region, a hydrophobic side chain at C-9, and lack of steric bulk at C-8 (Figure 24).[122] Because both a long alkyl chain at C-9 as well as cyclic alkane substituents at C-9 produce effective biological activity, Endo and co-workers suggested that a linear hydrophobic alkyl group is folded when the molecule binds to the receptor.[124] The carbonyl group of the cyclic amide **95** hydrogen bonds to the NH group of Gly-253, the 4-NH of the amide hydrogen bonds to the carbonyl group of Leu-252, and the methylenehydroxyl at C-5 hydrogen bonds to both the carbonyl group of Leu-251 and the NH of Thr-242. The phenyl group sits above the Pro-241 residue, and the isopropyl and amino methyl groups in the hydrophobic region of the molecule engage in hydrophobic interactions with Leu-254 and Leu-251, respectively. The hydrophobic substituent at C-9 evades contact with the protein and points toward the phospholipid membrane.

Endo et al.[124] reported the synthesis and biological evaluation of PKC modulators possessing a benzolactam core, and *closo*-1,2-carborane as a hydrophobic pharmacophore at C-9. Three *closo*-1,2-carborane derivatives **96**, **97**, and **98** with the latter two compounds bearing a *n*-butyl and *n*-octyl group at the C-vertex of the cage, respectively, were synthesized and evaluated for cell growth inhibitory activity and binding affinity. The effective dose ($ED_{50}$) of both **97** and **98** for growth inhibition was found to be 7 nM, comparable to that of the potent benzolactam **95** (5 nM). The growth inhibition activity of **96** was found to be weaker ($ED_{50}$ = 30 nM), probably due to decreased hydrophobicity at C-9 because of the absence of an alkyl group. The binding affinities of benzolactams **96**, **97**, and **98** were measured as inhibition of [$^3$H]-PDBu binding ($K_d$ = 0.76 nM) to PKCδ, and the $K_i$ was found to be 2.0, 1.4, and 1.8 nM, respectively, comparable to the binding potency of **95** (1.8 nM). The conformity of docking simulations

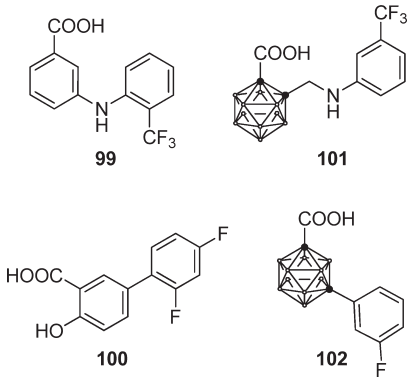


Figure 25

with biological activity indicates that the *closo*-carborane indeed acts as a hydrophobic pharmacophore to maintain the required interactions with PKC for biological activity.

Transthyretin (TTR) is a thyroxine-transport homotetramic protein found in blood. Transthyretin amyloidosis is a genetic disease caused by dissociation of TTR variants to monomeric intermediates, which aggregate in tissue such as heart, peripheral nerve, and intestine to form amyloid fibrils, resulting in tissue degeneration and amyloidosis diseases such as senile systemic amyloidosis (SSA), familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC), and central nervous systems amyloidosis (CNSA). As 95% of TTR is produced by the liver, liver transplantation is the only established treatment to halt amyloid formation, but this method is not without risk and limitations.[125]

As >99% of TTR's T4 binding sites in the blood are not occupied, small molecule inhibitors that stabilize the tetramer without imparting any hormonal activity are desired. Many structurally diverse compounds stabilize the tetramic TTR.[125,126] The use of nonsteroidal anti-inflammatory drugs (NSAID) such as flufenamic acid **99** and diflunisal **100** (currently being evaluated in clinical trials) (Figure 25) and structurally related derivatives to impart kinetic stabilization is limited by their concomitant inhibition of cyclooxygenase (COX), which leads to gastrointestinal irritation and an increased risk in cardiovascular events. On the basis of the typical structure of TTR kinetic stabilizers, that is, two aromatic rings linked either by a spacer[125] group or directly, and choice of acidic functional groups, which enable them to interact with the protonated ε-$NH_2$ group of Lys-15 located at the entrance of the binding channel,[127] Julius et al.[128] used the *closo*-carborane cage as a hydrophobic bioisostere of aromatic rings in the known inhibitors **99** and **100**. Compounds **101** and **102** (Figure 25) were synthesized and biologically evaluated by means of TTR and COX-1/COX-2 assays. Both compounds **101** ($IC_{50}$ = 2.6 μM) and **102** ($IC_{50}$ = 2.1 μM) were found to be potent inhibitors of amyloid formation to the same extent as flufenamic acid **99** ($IC_{50}$ = 2.9 μM). Analogue **102** selectively stabilized TTR almost completely and showed no undesired inhibitory activity for COX at a 10-fold higher concentration than the effective dose for TTR stabilization. The *closo*-1,7-carborane isomer in **102** conformationally positions the carboxylic acid to interact with the protonated ε-$NH_2$ group of Lys-15 in the TTR LBD. Compound **101** exhibited simultaneous COX-1 and COX-2 inhibition, and this difference in the mode of activity as compared to **102** is explained in terms of the increased flexibity of **101** as compared to **102**. The authors postulated that


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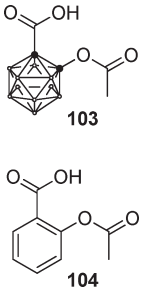


Figure 26

an increase in the degrees of freedom of molecule **101** enables hydrophobic interactions in COX as compared to sterically contrained **102**.[128] This study confirmed that *closo*-carboranes can enter the hydrophobic TTR-binding channel and stabilize the tetramer from dissociation.

Aldo-keto reductases (AKR) are a divergent superfamily of NADPH-dependent oxidoreductase enzymes that metabolize a broad range of endogenous and exogenous carbonyl-containing compounds to their hydroxy metabolites. This metabolic conversion can lead to their bioactivation by subsequent conjugation, for example, or can facilitate their elimination from the body (detoxification).[129] AKR implicated disorders include epilepsy, depressive disorders, cancer development, and diabetic neuropathy.

Asborin **103**, a carborane-containing analogue of aspirin **104** (Figure 26), exhibited weaker COX inhibition than did aspirin (a well-known COX-1 and COX-2 inhibitor, which prevents the conversion of arachidonic acid to prostaglandin $H_2$) because it lacks the ability to acetylate interior active site Ser residues, and instead acetylates Lys residues on the surface of the protein.[130,131] This has been attributed to the readily labile acetyl group of asborin as a result of the electron-withdrawing nature of the *closo*-carborane cage rather than the hydrophobic nature of the cluster itself, which means asborin cannot enter the COX enzyme active site intact. Hey-Hawkins and co-workers[132] however proposed that the hydrophobic *closo*-carborane cluster was well suited to the hydrophobic interior of AKR and used the AKR1A1 as the model enzyme in their studies. Indeed, asborin exhibited far better inhibition of AKR1A1 than aspirin, and this was attributed to the properties of carborane itself as the functional groups on the hydrophobic core in both compounds are identical.[132] Again, surface protein Lys residues proved to be the favored sites for acetylation by asborin. On the other hand, AKR1A1 did not provide a direct activation site for asprin acetylation (aspirin is a much weaker acetylating agent than asborin) like the COX enzyme variants. The above examples demonstrate that the *closo*-carborane cluster is a suitable pharmacophore for fine-tuning activity against different targets.

Thymidine kinase (TK) is part of the pyrimidine salvage pathway and catalyzes the phosphorylation of thymidine and 2′-deoxyuridine. Thymidine kinases have a key function in the synthesis of DNA as they are part of a reaction sequence that introduces deoxythymidine into DNA, and hence the enzymes play an important role in cell proliferation. TK1 activity is present in actively proliferating cells and is present in two forms, TK1 and TK2. TK1 is cell cycle dependent and is present in the cytoplasm of cells ready for division, while TK2 is located in the mitochondria and is cell cycle independent. Soloway and co-workers[133,134] demonstrated that derivatives of thymidine containing 1,2-carboranylalkyl groups at, for example, the N-3 position and possessing

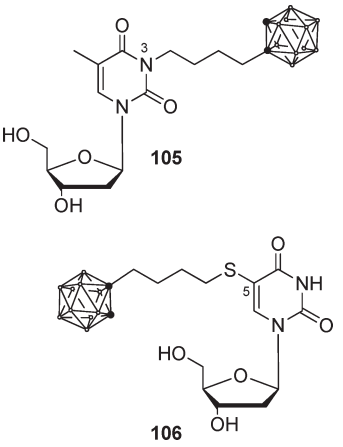


Figure 27

short alkyl spacers such as in compound **105** (Figure 27) were phosphorylated by both recombinant and purified cytosolic TK1. Derivatives of 2′-deoxyuridine containing 1,2-carboranylalkylmercaptan groups at C-5 with short spacer lengths (e.g., **106**) were phosphorylated by TK2, albeit at a low but significant level. TK1 tolerates bulky carboranes at the N-3 position of Thd but does not tolerate bulky carboranes at the C-5 of dUrd. Although the focus of the research was to demonstrate the potential application of these boronated compounds in BNCT, it lends further support to the concept that *closo*-carboranes can be used as bulky hydrophobic moieties in protein binding.

## 3. IN SILICO MOLECULAR MODELING OF CARBORANE-CONTAINING COMPOUNDS IN DRUG DESIGN

Many examples exist where carborane cages have been used as replacement for the hydrophobic portions of molecules and can interact with receptors to produce more efficacious biological activity than the endogenous ligand. Endo and co-workers were the first to describe structure-based drug design of *closo*-carborane derivatives of estradiol,[79,80,84] retinoic acid,[91] and teleocidin[124] using an automatic docking program (ADAM) that docks novel ligands into the binding cavity of proteins based on hydrogen bonding and, finally, by optimization of the position, orientation, and conformation of the molecule in the docked model. The total potential energy including intra- and intermolecular interaction energy, consisting of van der Waals, electrostatic, and hydrogen-bonding energies, is used in all of the screening processes.[135] However, this software package, developed in Japan, is not readily available, and so alternative approaches to pharmacophore modeling and docking studies of carborane-containing ligands are needed. Because of the complexity of bonding within carborane cages, computer-aided molecular design (CAMD) is not commonly applied to carborane-containing ligands. Furthermore, current commercial software packages do not provide built-in default empirical potential energy functions for boron atoms. The following discussion will give an overview of CAMD and computational docking approaches in carborane-based drug design. The general strategy employed involves formatting compounds for study by replacement of boron atoms with carbon atom types, elucidation of active sites from ligand−protein crystal structures for conformational analysis, and their subsequent use in docking studies.

Johnsamuel et al.[136,137] have reported a general strategy for modeling and docking carborane containing derivatives by means of HYPERCHEM, SYBYL, and FLEXX software packages. The *closo*-1,2-, 1,7-, and 1,12-carboranes and their derivatives were constructed by means of the HYPERCHEM platform; energies were minimized by a semiempirical AM1 method and then saved in a SYBIL compatible format. Atomic point charges were calculated using the MOPAC interface of SYBIL. Compounds were formatted for these studies by replacing the boron "B" atoms for carbon atoms "C.3". Docking to the active site was performed using FLEX, and the best-docked conformation was used to generate hydrogen bonds between the ligand and amino acid residues in the active site of the protein. Binding proteins were visualized by superimposing a MOLCAD surface generated from X-ray structures of ligand−protein complexes.

Standard computational 3D-QSAR techniques for carborane-containing antifolates as substrates of human TK1 have been reported.[138] The authors made use of comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) methods derived from a compilation of phosphorylation rates from a training set of 47 molecules to evaluate the 3D-quantitative structure−activity relationship (3D-QSAR) of 27 carborane-containing pyrimidine nucleosides as substrates of human thymidine kinase. The CoMFA and CoMSIA are methods that sample the potential fields surrounding a set of ligands (using SYBYL[139] or HYPERCHEM[138] software package) and correlate these with the biological activities of the ligands interacting with a receptor. Tiwari et al.[140] have reported docking studies of two antifolates containing a *closo*-carboranyl or an anionic *nido*-carboranyl group. These authors computationally determined docking poses of carborane antifolates into the active site of the hDHFR crystal structures[111] by means of the docking programs AUTODOCK, GLIDE, FLEXX, and SURFLEX along with employing the strategy of replacing boron atoms with carbon atom types, and found the first two programs were useful for the docking of *closo*-carborane antifolates, whereas the first three programs were equally efficient at the docking of *nido*-carboranyl antifolates.

## 4. CONCLUSIONS

This Review summarizes key studies in the literature that demonstrate the concept of *closo*-carboranes as hydrophobic pharmacophores in biologically active molecules. Such entities have been used as stable bioisosteres of phenyl rings and other hydrophobic components such as adamantane. *closo*-Carborane derivatives of active compounds have been shown to exhibit improved binding affinity and/or activity as both antagonists and competitive agonists in a range of receptor types containing a hydrophobic ligand binding cavity. *closo*-Carboranes have improved hydrophobic interactions with certain enzymes by virtue of their tighter association and shape complementarity within the enzyme active site, which results in the high affinity between the substrate and enzyme, albeit with loss of activity in some cases. However, this loss of activity can be used to advantage to inhibit protein activity implicated in disease. Another outcome of the strong hydrophobic interactions between a carborane and LBD is the prolonged duration of activity. Because of the well-established synthesis of carboranes and the synthetic flexibility in accessing a range of their analogues, they can be used as a platform to exploit the binding affinity and/or selectivity of agents for biological receptors. The hydrophobicity can be fine-tuned by the judicious choice of carborane isomer, and their ease of chemical derivatization to fix substituents at the appropriate position required for optimized interactions with the protein receptor. The relative ease by which the hydrophilic and anionic *nido*-carborane derivatives can be prepared from *closo*-carboranes allows for direct comparisons between the two classes of pharmacophore. While studies involving the *nido*-carborane pharmacophore are rare, recent comparative X-ray studies confirm that both types of carboranes are able to bind to proteins in a similar manner despite the significant difference in hydrophobicity and charge, a factor that may be exploited in drug transport studies, for example. Metallocarboranes may also offer interesting possibilities in medicinal chemistry as well, but their application as pharmacophores is only just emerging.

It is clear that *closo*-carboranes can now be included in the select list of important hydrophobic polycyclic entities such as adamantane and trishomocubane for use in drug design. The work reported to date is still in its infancy, but it clearly demonstrates the increasing role of carboranes as pharmacophores in medicinal chemistry at a time when boron is beginning to demonstrate its potential as a key element in drug design, particularly in the treatment of diseases for which current therapeutic agents have proved to be inadequate.

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Michael Kassiou is Professor of Medicinal Chemistry at the University of Sydney and Head of the Drug Discovery Research

Unit at the Brain and Mind Research Institute. His main research interests are concerned with the understanding of drug−protein and drug−binding site interactions to obtain structure−activity relationships of bioactive CNS molecules, allowing the rational design of more efficacious treatments for diseases of the brain. He received his Ph.D. in Chemistry from the University of New South Wales in 1992. He has been a Postdoctoral Fellow at Johns Hopkins University and a Fogarty Fellow at the National Institute of Drug Abuse, National Institutes of Health in the U.S. He serves on several journal editorial boards focusing on medicinal chemistry, pharmaceutical design, and drug discovery.

Louis M. Rendina received his B.Sc.(Hons I) and Ph.D. degrees from the Australian National University. He is the recipient of two prestigious national awards from the Royal Australian Chemical Institute (RACI) for his seminal contributions to the areas of Medicinal Chemistry (RACI Biota Medal for Medicinal Chemistry) and Organometallic Chemistry (RACI Organometallic Chemistry Award), the only individual to have ever received both awards. He has also been elected as a Fellow of the RACI (FRACI, *C. Chem.*) and a Fellow of the Royal Society of Chemistry, UK (FRSC). His current research interests lie in the area of bioinorganic medicinal chemistry, in particular the development of new B and Gd agents for Neutron Capture Therapy (NCT) and the use of boron clusters as pharmacophores in medicinal chemistry.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ADAM | automatic docking program |
| ADME | absorption, distribution, metabolism, and excretion |
| ADP | adenosine diphosphate |
| AKR | aldo-keto reductase |
| AR | androgen receptor |
| AT | angiotensin II |
| BBB | blood brain barrier |
| BK | bradykinin |
| BNCT | boron neutron capture therapy |
| Car | L-*closo*-1,2-carboranylalanine |
| Cbz | benzyloxycarbonyl |
| CNS | central nervous system |
| CoMFA | comparative molecular field analysis |
| CoMSIA | comparative molecular similarity indices analysis |
| COX | cyclooxygenase |
| DHFR | dihydrofolate reductase |
| ER | estrogen receptor |
| LBD | ligand binding domain |
| MXT | methotextrate |
| NSAID | nonsteroidal anti-inflammatory drug |
| OA | okadaic acid |
| PBAN | pheromone biosynthesis activating neuropeptide |
| PKC | protein kinase C |
| PR | protease |
| RAR | retinoic acid receptor |
| RXR | retinoid X receptor |
| SAR | structure activity relationship |
| SERM | selective estrogen receptor modulator |
| TK | thymidine kinase |
| TMP | trimethoprim |
| TNF | tumor necrosis factor |
| TPA | 12-*O*-tetradecanoylphorbol-13-acetate |
| TSPO | translocator protein |
| TTR | transthyretin |

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