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New keys for old locks: carborane-containing drugs as platforms for mechanism-based therapies

Philipp Stockmann,  Marta Gozzi,  Robert Kuhnert,  Menyhárt B. Sárosi * and Evamarie Hey-Hawkins *

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Icosahedral carboranes in medicine are still an emerging class of compounds with potential beneficial applications in drug design. These highly hydrophobic clusters are potential “new keys for old locks” which open up an exciting field of research for well-known, but challenging important therapeutic substrates, as demonstrated by the numerous examples discussed in this review.

Key learning points

1. Highlight important receptors (“old locks”) as targets for efficient therapeutic treatments and propose carboranes as new class of drugs (“new keys”).
2. Present the benefits of carboranes as building block in drug design and outline novel carborane-based receptor ligands.
3. Guideline for readers through the versatility of potential medical applications of carborane-containing agents.
4. Current challenges of this novel strategy and the possibilities for structural modifications for enhancing drug properties and effects.
5. Molecular docking strategies available for carborane-based receptor ligands.

1. Introduction

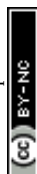
The first time the term “chemotherapy” appeared in the scientific community was at the beginning of the 20th century, when Paul Ehrlich introduced the concept of therapeutic treatments based on chemical substances (“magic bullets”, in German *Zauberkegel*) with selective affinity, or toxicity, for specific biological targets, such as pathogens. For his discoveries, he received the Nobel Prize in Physiology and Medicine in 1908, and is nowadays considered to be the father of chemotherapy. Over the last 100 years, chemicals-based therapies have considerably evolved, and are world-wide approved for treatment of tumours, viral, parasitic, or bacterial infections, inflammation, pain, and combinations thereof, although the underlying principle is, for all of them, Ehrlich’s heritage of mechanism-based therapies.¹ Structurally, most of the drugs on the market are small molecules ($M_w < 1000$ Da), usually organic compounds, like doxorubicin, a topoisomerase II inhibitor used for the treatment of leukaemia, Hodgkin’s lymphoma, breast, bladder and other cancers, the anti-malarial drug chloroquine, or the non-steroidal anti-inflammatory (NSAID) drug ibuprofen.²

Among metal-based drugs, a few platinum-containing complexes are approved for treatment of ovarian, testicular, cervical, breast and other cancers: examples are cisplatin, oxaliplatin and carboplatin.³ Another big class of drugs are therapeutic peptides (M_w up to 2000 Da), such as insulin and adrenocorticotrophic hormone (ACTH), which are mostly used as “replacement therapies” to supply hormone deficiency.⁴

In the last 20 years, great interest has arisen for hybrid organic–inorganic compounds as novel small molecule drugs, which contain boron–carbon–hydrogen clusters, known as dicarba-*closo*-dodecaboranes (also known as carbaboranes or carboranes, Fig. 1). Although carborane-based drugs are yet to be approved for therapeutic treatments, many compounds were shown to be very promising drug candidates for applications in several fields: for boron neutron capture therapy (BNCT), *e.g.* carboranyl nucleosides and sugars,⁵ or enzyme inhibitors, *e.g.* cobalt bis(dicarbollide) complexes ($[\text{commo-3,3'}\text{-Co(1,2-C}_2\text{B}_9\text{H}_{11})_2]^-$, known as COSAN species) for potent and selective inhibition of HIV-1 protease,⁶ or combinations thereof, as in the case of carborane-based epidermal growth factor receptor (EGFR) inhibitors, recently reported by Couto *et al.*⁷

Similar to purely organic and organometallic drugs, the medicinal chemistry of carboranes typically follows target-vector recognition approaches.⁸ There is in fact a rich literature on carborane-containing compounds, which were designed for

Universität Leipzig, Fakultät für Chemie und Mineralogie, Institut für Anorganische Chemie, Johannisallee 29, 04103 Leipzig, Germany. E-mail: menyhart.sarosi@uni-leipzig.de, hey@uni-leipzig.de; Fax: +49-341-9739319; Tel: +49-341-9736151



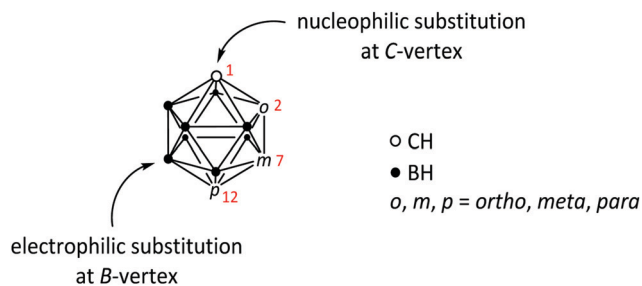


Fig. 1 General structure of icosahedral carboranes of type *closo*-C₂B₁₀H₁₂. Numbers in red indicate the numbering of the C-vertices of the cluster in the respective isomer: 1,2 (*ortho*, o), 1,7 (*meta*, m) and 1,12 (*para*, p). For detailed explanation of the numbering system in carborane clusters see ref. 1. The two most common types of substitution reactions (nucleophilic at C- and electrophilic at B-vertices) are indicated.

interacting with a specific target, such as an enzyme receptor, thereby deserving the classification as “new keys”, in the sense of novel lead structures, for “old locks”, *i.e.* long- and well-known useful therapeutic targets, such as the EGF receptor.⁷

To understand the great potential of using these inorganic clusters for drug design, one must consider two main perspectives: on the one side, the physico-chemical and biophysical properties of carboranes, and on the other side, the type and strength of non-covalent binding interactions of a potential drug structure with the chosen biological target.

Carboranes are an extensive class of compounds, ranging from 4-vertex (sub-icosahedral) to 12-vertex (icosahedral) and 14-vertex (supra-icosahedral) structures.⁹ In the present review, we focus our attention solely on the 12-vertex clusters,



Philipp Stockmann

Philipp Stockmann received his MSc from Leipzig University (Germany) in 2017. He worked as a Research Assistant in computational chemistry investigating in silico design of carborane-containing drug molecules. Currently, he holds a position as a doctoral student at Leipzig University under the supervision of Prof. Evamarie Hey-Hawkins. His research focuses on carborane chemistry with medicinal applications.



Marta Gozzi

Marta Gozzi received her BSc degree from the University of Bologna (Italy). She joined the Erasmus Mundus program Advanced Spectroscopy in Chemistry and received a double MSc degree (University of Lille1 and Leipzig University). She carried out her doctoral studies at Leipzig University (with Prof. E. Hey-Hawkins), and received her doctoral degree in early 2019. Her research interests are in the field of bioorganometallic chemistry, particularly on metallocarborane complexes for application in medicine. She contributed book chapters to Boron-based Compounds: Potential and Emerging Applications in Medicine, and the Handbook of Boron Chemistry in Organometallics, Catalysis, Material Science and Medicine.



Robert Kuhnert

Robert Kuhnert studied chemistry at TU Bergakademie Freiberg (Germany) and Leipzig University (Germany). He received his masters degree 2013 and started his doctoral degree in the group of Prof. Evamarie Hey-Hawkins at Leipzig University investigating carboranes as phenyl mimetics for lipoxygenase inhibitors and boron carriers for boron neutron capture therapy (BNCT). After he received his doctoral degree in 2017, he was scientific manager

of the European network on Smart Inorganic Polymers (SIPs). Since October 2018, he is laboratory head of a medium-sized chemical company in Bitterfeld (Germany).



Menyhárt B. Sárosi

Menyhárt B. Sárosi received his doctoral degree in chemistry from Babeş-Bolyai University (2011) funded by the Sectoral Operational Programme Human Resources Development, Contract POSDRU 6/1.5/S/3 – “Doctoral studies: through science towards society” (Romania). Afterwards, he was a postdoctoral researcher financed by the European Union and the Free State of Saxony (ESF-NFG 100148835) at the Faculty of Chemistry and Mineralogy, Leipzig University. Since 2015, he is a junior research group leader supported by the German Research Foundation (DFG, SA 2902/2-1) mainly interested in the molecular modelling of carborane-containing drugs and their interactions with biomolecules.



dicarba-*closo*-dodecaboranes, of the general formula *closo*-C₂B₁₀H₁₂.[†] Icosahedral carboranes are clusters of ten BH units and two CH vertices (Fig. 1).⁹ The carbon atoms in the cluster can be organised in a 1,2-, 1,7- and 1,12-fashion, giving rise to three isomers, namely *ortho*-, *meta*-, or *para*-carborane. All three isomers are commercially available; the *ortho* isomer is synthesised from decaborane (B₁₀H₁₄) and acetylene, in the presence of a Lewis base; thermal isomerisation to the *meta* and *para* isomers occurs at 450–500 °C and 700 °C, respectively.⁹ Of the three isomers, the *ortho* one is the most reactive, due to higher inductive electron attraction (–I) than in the *meta* and *para* isomers.⁹ This means that *ortho*-carboranes are generally easier to functionalise than the other two isomers, which require harsher reaction conditions. Also, *ortho*-carborane is more prone to undergo deboronation (*i.e.* loss of a BH unit) upon nucleophilic attack, to give the corresponding 11-vertex *nido*-[7,8-C₂B₉H₁₂][–] species.^{8,9} Due to the different nature of the cluster-bound hydrogen atoms, *i.e.* acidic (δ⁺) C–H, *vs.* hydridic (δ[–]) B–H, substitution reactions at either C- or B-vertices follow different mechanisms, which means that substituents can be attached to selected cluster positions (regioselectivity).⁸ The carborane cluster occupies a volume similar to that of adamantane, and is a little larger than a rotating benzene ring. Therefore, carboranes are often described as three-dimensional benzene analogues, in which the 26 skeletal electrons occupy 13 bonding molecular orbitals (MOs). The analogy with the benzene ring is indeed a strong motivation for the use of carborane clusters in drug design, following the well-known concept of bioisosteric replacement, inherited from the medicinal organic and organometallic chemistry.¹⁰ However, in contrast

to aromatic hydrocarbons, which feature delocalised carbon–carbon π bonding, the aromaticity in polyhedral boron clusters utilises σ bonding interactions, involving a set of orbitals tangential to the polyhedral surface, and another set directed towards the centre.¹¹ This means that the physico-chemical properties of the carborane cluster are substantially different from those of their organic counterparts, which largely substantiates the “benzene-inspired” medicinal chemistry of carboranes. There is in fact a rich literature on hybrid organic–inorganic compounds, which have been rationally designed to interact with a specific enzyme, based on well-known purely organic inhibitors, where the carborane cluster replaces either a phenyl ring or an adamantyl group.^{7,12–14} Following this approach, one might address common problems associated with the biological stability of organic drugs, *e.g.* fast metabolism and rapid clearance from body fluids, while preserving, or modulating, the target-vector recognition capabilities of the drug.¹⁵ Properties of carborane clusters, which are particularly relevant for application in medicine, are (i) their high hydrophobicity, due to the low polarity of the hydridic B–H groups, which proved to be beneficial for enhancing transport across cellular membranes and the blood–brain barrier (BBB),¹⁶ (ii) their inorganic nature, which prevents them from undergoing conventional enzymatic degradation, thereby strongly promoting drug stability under biological conditions, (iii) the possibility of three-dimensional orthogonal functionalisation of the cluster, superior to benzene systems, which are limited to two dimensions, leading to higher structural flexibility and, therefore, possibilities of fine-tuning the structure of the final compound, and (iv) the multitude of possible non-covalent interactions with biological targets: B–H···H–X (X = N, C, and S) dihydrogen bonds, B–H···Na⁺ bridging interactions, and C–H···X (X = O, N, S, F, π system) hydrogen bonds.⁸ Another favourable property of carborane clusters for drug design is their general tendency to self-assemble in aqueous solution, spontaneously forming nano- to micrometre sized particles.¹⁷ Nanometre sized particles potentially represent a very efficient way for the selective delivery of chemotherapeutics to cancer cells, *via* exploitation of the so-called “enhanced permeability and retention” (EPR) effect.¹⁸ The self-assembly behaviour is relatively broadly investigated and well characterised for anionic boron cluster compounds (ABCCs), *e.g.* COSAN species,¹⁹ and only recently are studies appearing on neutral carborane-containing compounds, such as the carboranyl-cysteine published by He *et al.*²⁰ However, studies that specifically relate this spontaneous behaviour with the biological activity of carborane-containing drug candidates are still very rare in the literature.

Nowadays it is fairly routine to predict the binding mode and binding affinity of promising drug candidates to their target receptors with the help of computational tools. Molecular docking is one of the most often used methods in structure-based drug design for ranking potential ligands and for selecting the most promising ones. First, the possible conformations of a potential ligand within the binding site of a receptor are sampled, then the conformations are ranked using a scoring function.



Evamarie Hey-Hawkins

Evamarie Hey-Hawkins has been Professor of Inorganic Chemistry at Leipzig University, Germany, since 1993. She received her diploma (1982) and doctoral degree (1983) at Philipps University Marburg, Germany. After stays in the UK (1984/85) and Australia (1985/87), she completed her habilitation in Marburg (1988). Her scientific interests are organophosphorus chemistry, biologically active boron compounds, and hetero-

metallic transition metal complexes with applications in catalysis and materials science. She has published over 480 papers, edited a book on Boron-Based Compounds: Potential and Emerging Applications in Medicine (with Clara Viñas Teixidor), is a member of the European Academy of Sciences, and has received several prestigious awards.

[†] For nomenclature and numbering of the carborane clusters refer to the IUPAC project 2012-045-1-800 by Beckett *et al.*, nomenclature for boranes and related species, 2018.



An ideal molecular docking protocol should be able to reproduce the experimental binding mode and select it as the preferred conformation. Molecular docking of carboranes or even boron-containing compounds is not straightforward, since the corresponding atom types, bonding and non-covalent interactions are not commonly available. Nevertheless, the number of publications containing molecular docking of carborane-derivatives is increasing.^{7,12,14,15,21–25}

In recent years, several reviews have appeared in the literature on the topic of carboranes in medicine (see for example Scholz *et al.*,⁸ Issa *et al.*,¹⁶ Leśnikowski,⁶ and Schwarze *et al.*²⁶). Therefore, in this review we focus our attention on the most recent developments on hybrid organic–inorganic compounds containing a *closo*-carborane cluster, which were designed as “new keys” for target-vector recognition mechanisms. Specifically, we review those compounds which have appeared in the literature since the extensive review work of Scholz *et al.* and Issa *et al.* (2011),^{8,16} and which were designed to trigger inhibition or activation of a specific enzyme. Moreover, the related molecular docking studies are also mentioned.^{7,12,14,15,21–25}

The cannabinoid receptor type 1 (CB1) agonists designed by Vázquez *et al.* in 2012 are not discussed here, since after the rationally designed synthesis of the potential drug candidates no evaluation of their biological activity has been published so far.²⁷

2. Carborane-based receptor ligands

2.1 Estrogen receptor ligands

Endo and co-workers pioneered in the early 2000s the structure-based drug design of carborane derivatives, supported by molecular docking studies.²⁸ Their idea was to make use of the high hydrophobicity of the *closo*-carborane cluster, to modulate the binding affinity of receptor–ligand complexes. They focused their attention on potential novel ligands for the nuclear (intracellular) estrogen receptors (ERs). Particularly, they designed carborane-containing analogues of 17 β -estradiol (Fig. 2), the major female sex hormone, which plays a central role in growth, differentiation and physiology of both male and female reproductive systems, as well as in bone marrow maintenance.²⁹ More recently, the same group worked on the synthesis and biological evaluation of carboranyl-based compounds with selective affinity for specific ER subtypes.³⁰ Nuclear ERs exist in fact as two major subtypes, α and β , which are composed of several domains: DNA binding, hormone binding and activation of

transcription domains. A variety of nuclear responses are mediated through the binding of estrogens (17 β -estradiol, estrone, estriol, estetrol), or estrogen analogues, to the ligand binding domain (LBD), which transactivates gene expression upon ligand binding. LBDs of ER α and ER β are rather similar; therefore, many ligands bind unselectively to both receptor subtypes. However, ER β selectivity for novel ER modulators has emerged as attractive therapeutic approach for the regulation of immune responses associated with chronic inflammation. Following this trend, Endo and co-workers synthesised a small series of tetrahydrofluorenone analogues, varying the length and the position of alkyl substituents at the cluster vertices, with *n*-propyl derivative **2** exhibiting up to 7 times higher affinity toward ER β (Fig. 2). The rational design idea was that the aliphatic group should disfavour binding to the ER α , due to steric hindrance with the Met421 residue in the LBD, which is replaced in the ER β subtype with Ile373. Besides this, the ER α Leu384 is replaced by Met336 in ER β . Therefore, a wide range of ER β -selective ligands obtain their ER subtype selectivity through steric, electronic or hydrophobic interactions with these essential amino acid residues. Based on the theory to specifically decrease the affinity for ER α by introducing ligand substituents that interfere sterically with Met421 of ER α , various ER β -selective derivatives have been reported. With emulating, for example, structural properties of non-carborane receptor subtype-selective agents, such as androstane (a C19 steroid with a gonane core) skeleton analogues **3** or the modification of the carborane cage by halogenation **4** (Fig. 3), binding affinities to ER α were reduced with up to nearly 60 times higher affinity towards ER β .²⁹ Although the selectivity of the cyclohexanol derivative **3** is significantly higher than **4**, the still low binding affinity toward ER β does not allow for an adequate application as a potential drug. Thus, recent studies held the idea of modification through iodinated carborane cages, resulting in further possible ER β -selective ligands, such as the 9,10-diiodo-*m*-carborane **5** (Fig. 3) with a 14 times higher affinity towards ER β than ER α .³¹

The first successful agent BE120 (1-hydroxymethyl-12-(4-hydroxy-phenyl)-1,12-dicarba-*closo*-dodecaborane) **6** (Fig. 3), an estradiol analogue bearing a *para*-carborane for mimicking the C and D ring of the steroid skeleton, exhibited a higher binding affinity ($K_i = 0.1$ nM) than estradiol ($K_D = 0.4$ nM) and acted as an ER α super agonist.²⁸ Revisiting BE120, Endo and co-workers recently presented different derivatives of 1-(4-methoxyphenyl)-12-hydroxy-methyl-*p*-carborane **7** (Fig. 3), a precursor for BE120, and their anti-proliferative activities. Studies revealed that with an increasing number of phenolic methoxy groups the cell growth inhibitory activity is enhanced. Moreover, the trimethoxy derivative **8** (Fig. 3) provided the most potent activity for cell growth inhibition within an assay of 39 human cancer cell lines (the concentration for 50% of maximal inhibition of cell proliferation – GI_{50} value: 5.8 μ M) and additionally inducing apoptosis for a certain breast cancer cell line, making this concept interesting for further research.³²

On the basis of the known selective ER modulators (SERMs) raloxifene ([6-hydroxy-2-(4-hydroxyphenyl)-1-benzothiophen-3-yl]-[4-(2-piperidin-1-ylethoxy)phenyl]methanone) and tamoxifen ((*Z*)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-*N,N*-dimethylethylamine)

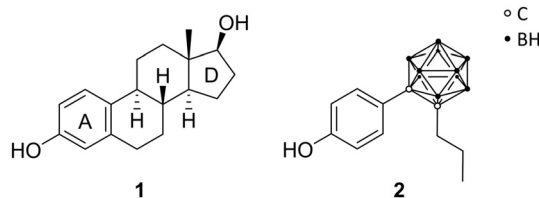
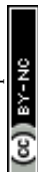


Fig. 2 Structures of 17 β -estradiol **1** and the *n*-propyl *ortho*-carborane derivative **2**.



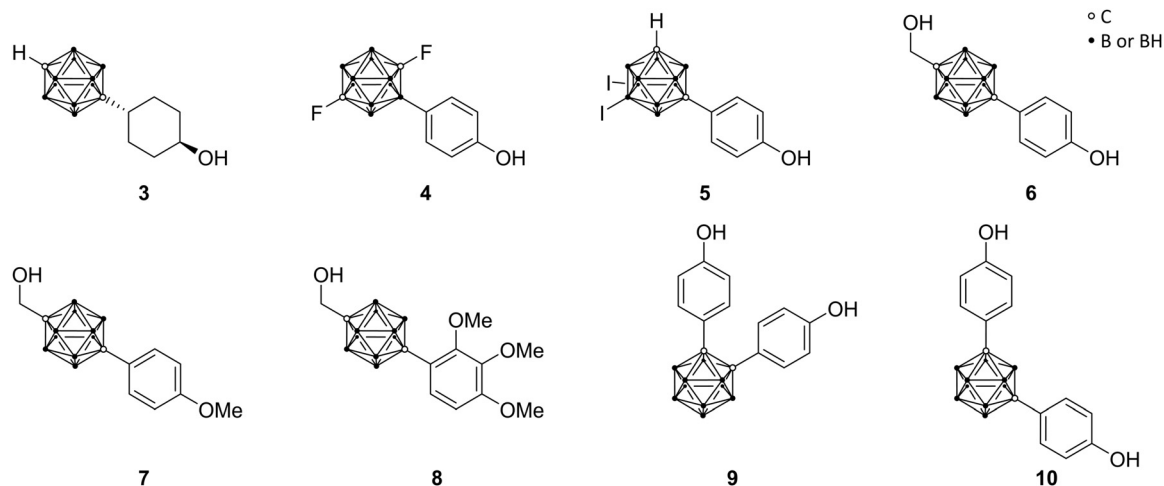


Fig. 3 Molecular structures of estrogen receptor ligands; androstane analogue **3**, halogenated analogues **4** and **5**, BE120 **6** and BE120-precursor **7**, trimethoxy derivative of BE120 **8**, and bisphenolic carborane derivatives **9** (BE360) and **10**.

and their structural features, various bisphenolic and related carborane derivatives were designed. Ligands based on *meta*- and *ortho*-carborane proved to be favoured as modulators. SERMs act as antagonists or agonists for targeting tissue-specific expressed ER subtypes. Tissue-specific expression of co-regulatory proteins and the variation of ligand-induced ER conformational changes are reasons for SERM specificity. Simple bis(4-hydroxyphenyl)-carborane derivatives were synthesised, such as the *ortho*-carborane-based BE360 **9** (Fig. 3), exhibiting strong ER binding affinity and modulating properties *in vivo* with the significant prevention of bone loss without any estrogenic activity in the uterus, as revealed by studies on ovariectomised mice. Being 1000 times less potent than estradiol, BE360 exhibited an almost equal agonistic potency in bone tissue as the osteoporosis drug raloxifene. Then again, the *m*-carborane-based analogue **10** (Fig. 3) showed potent ER α agonistic activity and represented therefore a versatile scaffold for further research.²⁹

2.2 Androgen receptor ligands

Prompted by the encouraging results of ER agents, similar targets were investigated by the same authors, particularly the androgen receptor, as member of the nuclear steroid hormone receptor family. Specific for the androgens testosterone and dihydrotestosterone (DHT), the androgen receptor (AR) regulates, similar to ER, various physiological functions in male reproductive, bone and muscle tissues. Prostate cancer, one of the major causes of death in adult males, is promoted through agonists of the androgen receptor, including the natural hormones, based on irregular behaviour of AR-mediated functions. This leads to a demand for AR ligands as antagonistic agents for prostate cancer treatments.

Clinically used non-steroidal AR antagonists, as compounds without a steroid scaffold, such as flutamide (**11**) and bicalutamide (**12**) (Fig. 4), are used as models for further research. Adapted from this, Endo and co-workers concentrated on *para*-carborane derivatives bearing an electron-withdrawing group at

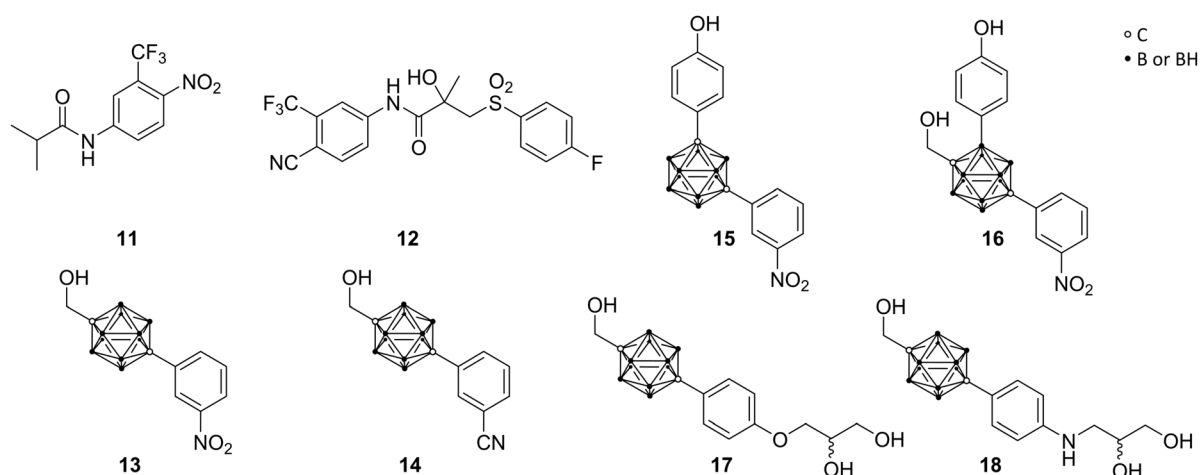


Fig. 4 Structures of androgen receptor (AR) ligands flutamide **11**, bicalutamide **12**, BA321 **13**, BA341 **14**, the phenolic derivatives **15** and **16**, and the glycerol and aminoglycerol carborane derivatives **17** and **18**.



the carborane-bound benzene ring (BA 321 **13**, BA341 **14**, Fig. 4). Studies revealed up to ten times higher binding activity to AR than hydroxyflutamide, the major active metabolite of flutamide, and both carborane derivatives **13** and **14** exhibited a dose-dependent inhibition of DHT activity and potent antagonistic activities.²⁹ However, recent *in vivo* studies revealed that the carborane **13** acts as an androgen in bone tissues, but not in the reproductive system. Therefore, **13** occurs to be a potential AR modulator for bone homeostasis in elderly men with age-related bone weakness.²⁸

Relying on the idea of opposing effects of estrogen agonists toward agonistic actions of androgens, Endo and co-workers attempted to design AR-ER dual ligands. Based on *meta*- and *para*-carborane structures, compounds **15** and **16** were evaluated (Fig. 4). **15** exhibited higher ER-agonistic activity at lower concentration than AR-antagonistic activity, and was therefore classified as ER agonist. On the contrary, **16** showed opposite behaviour, being closer to an AR antagonist.²⁸

In turn, prostate cancer frequently progresses to advanced stages through the occurrence of hormone-independent cancer cells or endogenous androgen metabolites, promoting tumour growth. Since the mechanism of operation for androgen receptor is yet not entirely understood, studies report that mutations can occur. A point mutation of Thr877 to alanine (T877A) within the AR-LBD was found to be strictly related to aggressive advance of the disease. **13** and **14** exhibited high inhibitory activity toward wild-type prostate cancer cells, whereas they show agonistic activity towards human T877A-mutated prostate cancer cell lines (LNCap cells). The aforementioned organic drug bicalutamide **12** acts as AR antagonist in both types of prostate cancer cells, wild and T877A, whereby resistances also have been reported. Endo and co-workers followed the idea of the design of unaffected antagonistic activity of full antagonists for wild-type and mutant cells.²²

Based on crystallographic data of the complex structure of bicalutamide bound to the mutant AR-LBD, they were able to show that the cyano group of the ligand binds to Gln711 and Arg752 through a water molecule. Therefore, following the approach of mimicking the binding mode of bicalutamide, glycerol and amino glycerol groups were introduced at the phenolic *para* position of the *para*-carborane derivative. The novel compounds **17** and **18** (Fig. 4) exhibited anti-androgenic activity by inhibiting the mutant androgen receptor in LNCaP cell lines. **17** and **18** exhibited antagonistic activities in LNCaP-proliferation assay (IC_{50} = 0.39 μ M and 0.42 μ M, respectively), similar to that of the (*R*)-bicalutamide, the active enantiomer (IC_{50} = 0.43 μ M). Furthermore, none of the carborane derivatives showed inhibition of cell proliferation in PC3 cell lines (AR-independent prostate cancer cell line), confirming that inhibition of cell proliferation in AR-dependent cell lines is caused by inhibition of AR activation.²² Docking simulations of the two enantiomers of **17** helped to elucidate the observed isomer-dependent AR antagonistic activity. The chirality of the secondary hydroxy group of **17** resulted in different orientation of the *para*-carborane cages of the two enantiomers and the steric repulsion between (*R*)-**17** and helix-12

of the AR-LBD was proposed as the cause of the potent anti-androgenic activity.²²

2.3 Vitamin D receptor ligands

As a ligand-inducible nuclear receptor specific for vitamin D (formally known as calcitriol **19** or 1,25(OH)₂D₃, the metabolically activated form of vitamin D₃), the vitamin D receptor (VDR) is expressed in most tissues of the human body. The receptor is involved in many essential physiological processes, by regulating the expression of specific target genes involved in, for example, cell proliferation and differentiation, immune regulation, bone metabolism, and homeostasis of calcium and phosphate. Playing, thus, a pivotal role in the pathogenesis and therapy of diseases, VDR ligands have been evaluated as potential therapeutic agents for the treatment of arthritis, osteoporosis, psoriasis, and cancers.²⁹

Reported by Fujii *et al.*, the first VDR ligands bearing a carborane cage are non-secosteroidal ligands imitating calcitriol.²⁹ Following the pattern of increasing the hydrophobicity of nuclear receptor ligands for more effective hydrophobic interactions, the C and D rings of calcitriol, similar to ER receptor ligands, were replaced with the carborane moiety. Furthermore, the three necessary hydroxyl groups should be preserved (Fig. 5). The carborane analogues exhibited, compared to the natural hormone, a moderate activity.²⁸ Crystallographic data of the complexed LBD with the carborane-based 1,25(OH)₂D₃ analogue **20** confirmed hydrophobic interactions with the hydrophobic region of the VD-LBD (protein data bank code: 3VJS).²⁹ Otero *et al.* investigated the first secosteroidal vitamin D analogue with a carborane unit (**21**), which was found to efficiently bind to the VDR-LBD (protein data bank code: 5E7V).²⁵ The binding mechanism could be observed by co-crystallisation of the carborane analogue and VDR with **21** mimicking the 1,25(OH)₂D₃ hydroxyl interactions through boron-mediated dihydrogen bonds. Further, biological properties were analysed on different cell lines, such as MCF-7, HaCaT

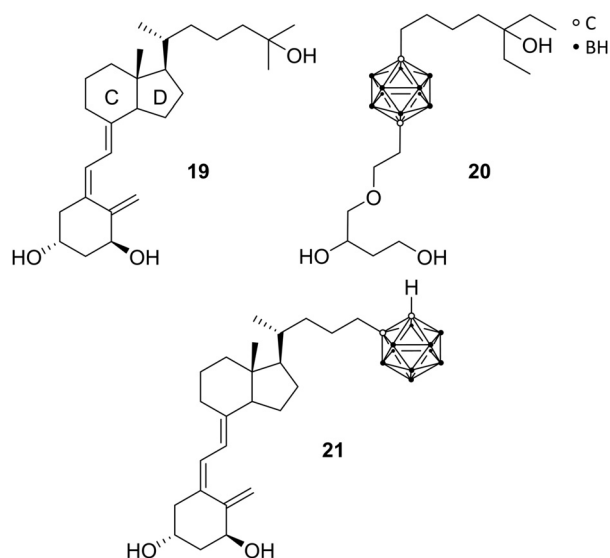


Fig. 5 Structures of calcitriol **19** and vitamin D receptor ligands **20** and **21**.



keratinocytes and HEK 293, showing decreased cell proliferation and differentiation-inducing activity compared to 1,25(OH)₂D₃. **21** has been designed with the help of molecular docking simulations.²⁵ The docked pose of the carborane analogue corresponded well with the subsequently obtained crystal structure. Unfortunately, the source of the boron parameters used for docking has not been specified.

2.4 Purinergic receptor ligands

The P1 receptors, also called adenosine receptors (AdRs), are purinergic G-protein coupled receptors. AdRs are preferentially activated by adenosine and are implicated in various pathological states and physiological processes, making them therapeutic targets. Over the last decades, selective and potent exogenous AdR agonists and antagonists as well as inhibitors for extracellular adenosine metabolism were designed.³³ Keeping in mind that AdR ligands bear a promising approach for several clinical applications, the problem of the omnipresence of purinergic receptors in the human body is difficult to overcome. The approach of modulating the cell function by purine-based derivatives yielded numerous compounds but gave rise to only a few agents as approved drug or still in clinical trials. Therefore, there is an urgent need for new agents, which target tissue-specific AdRs.³⁴

Bednarska *et al.* focused on modified adenosine analogues, carrying a *para*-carborane moiety, such as **22** and **23** (Fig. 6). These compounds were able to induce inhibitory activity for production of reactive oxygen species (ROS) in human neutrophils (specialised immune cells) stimulated with fMLP (*N*-formyl-met-leu-phe; termed as chemotactic peptide) in the low micromolar range, while adenosine as well as the appropriate *nido* derivatives are inefficient inhibitors at these low concentrations. Interestingly, the inhibitory effect of **22**, a *para*-carborane bearing adenosine derivative, was considerably higher than that of adenosine, with IC₅₀ values of 0.72 and 1.81 μM, respectively. Furthermore, **22** still showed inhibition of ROS production in low nanomolar ranges (up to 10 nM), with adenosine exhibiting no

effect at these concentrations.³³ Indeed, these compounds opened up new possibilities for the design of novel AdR modulators and agents affecting inflammatory mechanisms, as recent *in silico* studies on AdRs A_{2A} and A₃ have demonstrated.³⁵

In the wide field of human purinergic receptors, different individual subtype receptors are gaining interest as a potential biological target, through their ubiquity and variety of functions. Wilkinson *et al.* focused on the P2X purinergic receptor 7, the P2X₇R. P2X₇R is a protein being composed of ion channels, which are cation selective and ligand gated, opening in response to ATP binding.³⁴ Upon iterative and persistent exposure of P2X₇ to agonistic agents, non-selective pore formation is induced, leading to permeability for cations (*M_w* (cation) < 900 Da).³⁶ The latter induce apoptosis and the secretion of cytokines (interleukin 1β), which are correlated to neuronal disorders, such as hyperalgesia, depression and neurodegeneration. P2X₇R antagonistic ligands are, therefore, feasible anti-analgesic, anti-depressant or neuroprotective agents. Furthermore, as a potent stimulant of immunity and inflammation, and a promoter of cancer cell growth, P2X₇R might be the target for, *e.g.*, anti-inflammatory and anti-cancer therapies. Based on previous studies,³⁷ Wilkinson *et al.* replaced the adamantyl moieties of **24** in order to increase metabolic stability.³⁸ They synthesised a mono-functionalised *ortho*-carboranyl benzamide (**25**, Fig. 6) as potential anti-depressant targeting the central nervous system (CNS). **25** showed slight enhancement of the inhibitory activity towards human P2Y₇R (hP2Y₇R) in comparison with its adamantyl analogue **24** (Fig. 6), with IC₅₀ values of 8.7 and 10.5 nM, respectively. Subsequently, animal behaviour tests were performed on mice, assessing and approving CNS activity and the ability of crossing the blood-brain barrier (BBB). Despite their inhibitory potency *in vitro* against hP2X₇R, **24** and **25** showed no anti-depressant efficacy *in vivo*. Not crossing the BBB and/or interspecific distinctions and species-specific differences between organisms may be possible reasons for failure. Interestingly, when **25** is converted to its *nido*-carborane derivative, significant anti-depressant activity is observed with increased efficacy by two orders of magnitude, despite decreased lipophilicity by its anionic charge. Thus, the *nido* analogue of **25** is representing the first carborane-containing agent showing the ability to modify CNS activity. Nevertheless, **25** exhibited significantly higher values of lg *P* (partition coefficient), *i.e.* higher lipophilicity than the reported CNS ligands which are effective *in vivo*, suggesting a potential inhibition of the BBB penetration despite the fact that normally higher lipophilicity enhances crossing the BBB.³⁹ Thus, presented *closo*-carborane derivatives can be considered as starting point for prospective CNS agents or, furthermore, as potential precursor for their corresponding *nido* analogues.³⁴

2.5 Hypoxia-inducible factor ligands

Angiogenesis, the formation of new blood vessels from pre-existing vessels, is a vital process in the human body and, therefore, similarly essential for tumour cells in growth and metastasis. Erythropoietin (EPO) and the vascular endothelial growth factor (VEGF), as major angiogenesis factors in tumours,

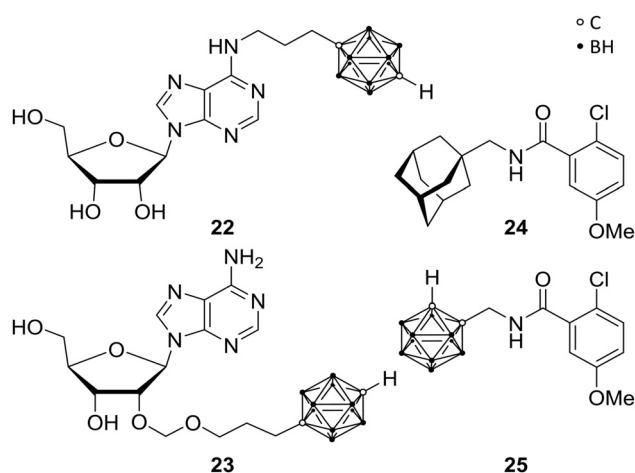
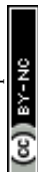


Fig. 6 Adenosine receptor ligands **22** and **23** and P2X₇R ligands **24** and **25**.



are activated gene hormones and are physiologically expressed in response to the stimulation by the hypoxia-inducible factor 1 (HIF-1). The heterodimeric transcription factor HIF-1, consisting of two subunits (α and β), is formed under hypoxic conditions. Under normal, aerobic conditions (normoxia) no dimer is formed, due to hydroxylation of the proline residues in the HIF-1 α protein, which is recognised by tumour suppressor proteins for further degradation through the ubiquitin-proteasome system. It was found that HIF-1 α is overexpressed in various cancers and, thus, represents a promising target for a new type of approach in cancer therapy.⁴⁰

Nakamura and co-workers used LW6, a phenoxyacetanilide derivative (LW6, **26**, Fig. 7), as structural model for boron- and carborane-based analogues. Particularly, the adamantyl moiety of LW6 was replaced by an *ortho*-carborane cage to obtain derivatives such as **27** and **28** (Fig. 7), which showed a more potent HIF-1 inhibitory activity (IC_{50} = 0.7 and 1.7 μ M, respectively) than LW6 (IC_{50} = 3.1 μ M), using a reporter assay in HRE($\times 5$)-Luc transfected HeLa cells (HRE, hypoxia response element). **27** showed the ability to suppress the accumulation of HIF-1 α under hypoxic conditions. Further studies revealed a similar inhibitory activity on heat shock protein 60 (HSP60) chaperone activity through the higher binding affinity evoked by the carborane cage, resulting in a potential mechanistic connection between HIF-1 and HSP60.⁴⁰ Further modifications of the LW6 model led to structures such as **29** and **30** (Fig. 7), containing either an *ortho*- or a *meta*-carborane cluster, which feature different phenylamino moieties and substituted phenyl or alkyl groups at the two C-vertices.^{40,42} Generally, it was found that increasing the steric demand of the alkyl substituent attached to the *meta*-carborane moiety enhanced the inhibitory activity. Particularly, **29**, with an isobutyl group, showed substantial inhibitory activity for HIF-1 transcription (IC_{50} = 0.55 μ M). Phenoxyacetanilides with *meta*-carborane backbone showed strong inhibitory potential with IC_{50} values in the low-micromolar range.

The *meta*-carborane derivatives are generally more potent than their *ortho*-carboranyl counterparts, except for one *ortho* analogue, **30**. The derivative **30**, containing an ethylene linker for a rigid conformation, exhibited the highest inhibitory activity with an IC_{50} of 0.53 μ M (compound **27** was used as reference: IC_{50} = 2.2 μ M). The authors stated that sterically bulky groups are necessary for an efficient inhibitory potential. Furthermore, these compounds inhibited cell growth and showed cytotoxic effects for HeLa cells in the low micromolar range.⁴⁰

Additionally, recent studies examined analogues of combretastatin A-4, a tubulin-binding *cis*-stilbene which induces apoptosis of cancer cells by disrupting microtubule dynamics. By replacing the *cis*-stilbene scaffold with an *ortho*-carborane, various compounds were synthesised, among which **31a–d** (Fig. 7) exhibited a significant inhibition of HIF-1 transcriptional activity (IC_{50} of 0.6, 1.2, 0.8 and 0.4 μ M, respectively), in hypoxic cancer cell line assay, with slightly higher potency than the known HIF inhibitor YC-1, used as positive control (IC_{50} = 1.5 μ M). Additional investigations were able to indicate tubulin, a cell protein, as primary target for carboranyl combretastatin A-4 analogues, and the inhibitory activity toward HIF-1 is, therefore, mediated *via* the tubulin pathway, setting a novel concept for prospective anti-cancer therapy.¹³

2.6 Epidermal growth factor receptor ligands

Couto *et al.* recently reported on carborane-based epidermal growth factor receptor (EGFR) inhibitors. The EGFR is a transmembrane glycoprotein, which acts as receptor for epidermal growth factors (EGFs) of extracellular protein ligands, and is composed of a hydrophobic transmembrane section, an intracellular tyrosine kinase domain and an extracellular binding domain. Upon binding of EGFs, downstream signals are activated, which stimulate physiological processes, such as cell proliferation, differentiation and cell growth, and are, thus, part of the regulatory functions in cells. Overexpression of

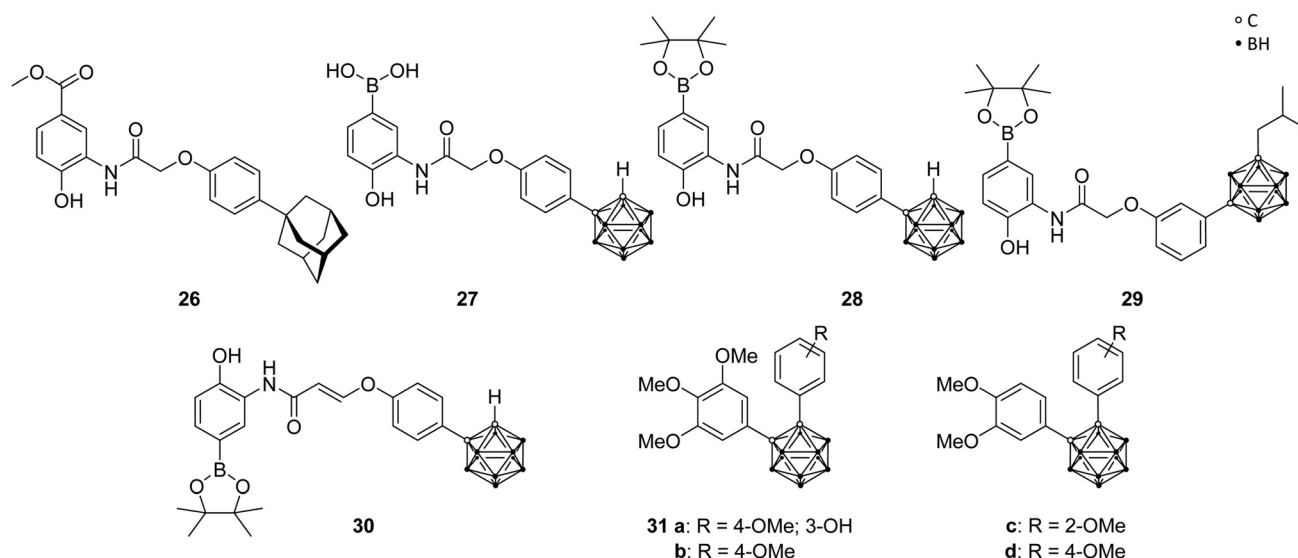
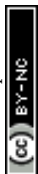


Fig. 7 Hypoxia-inducible factor ligand structures of LW6 **26**, the carborane analogues **27–30** and the carboranyl combretastatin analogues **31**.



EGFR was found in various solid tumours, *e.g.* breast, colon, lung and glioma. Thus, perturbing the EGFR signalling through inhibition of the tyrosine kinase activity or blocking of the EGFR binding site is a promising therapeutic approach for EGFR-related tumour growth.⁷

Previous investigations had shown that carboranyl anilinoquinazoline derivatives of the known EGFR inhibitor erlotinib (**32**, Fig. 8) exhibited selective cytotoxic effects against EGFR-overexpressing C6 rat glioma cells.⁴¹ Couto *et al.* evaluated, with further help of molecular docking studies, the mechanism of action by investigating the inhibitory activity on wild-type EGFR and T790M, an erlotinib-resistant mutational form of EGFR. T790M-mutation involves the replacement of threonine 790 with methionine. As a result, the larger methionine residue prevents binding of a tyrosine kinase inhibitor, such as erlotinib **32**. EGFR resistance to tyrosine kinase inhibitors has been described for numerous cancers. The activities for the wild-type EGFR (wt-EGFR) are similar for C6 glioma cell lines. Among the tested ligands, the carborane derivative **33** (Fig. 8) exhibited the strongest inhibitory effect on wt-EGFR ($IC_{50} = 2.3$ nM), almost ten times more potent than erlotinib ($IC_{50} = 22.9$ nM). Docking of **33** into wt-EGFR, using non-covalent interaction parameters derived from quantum chemical calculations for the carborane cluster, revealed the perfect fit of **33** into the catalytic cleft.⁷ The inhibitory activity for EGFR was measured using a cell-free kinase inhibition assay. Ligand **33** showed the ability to cross the brain-blood barrier ($\Delta \lg P = 1.49$; $\Delta \lg P = \lg P(\text{octanol/PBS}) - \lg P(\text{cyclohexane/PBS})$) and sufficient inhibitory activity for the T790M-mutated EGFR, with erlotinib showing no effect on the mutated cells. Thus, **33** is a promising lead structure for the design of dual agents, which target the EGFR and for potential BNCT applications.⁷

2.7 Thymidine kinase ligands

Recently, the impact of the human thymidine kinase 1 (hTK1) in cancer research intensified, as increased levels of hTK1 in blood serum were found when malignant tumours were present and, interestingly, even before the occurrence of physical symptoms. The enzyme hTK1 is embedded as vital member of the synthetic recycling pathway in the nucleotide synthesis

and, despite its normal activity during the S-phase of the cell cycle, high hTK1 activity can occur in cancer cells during other cell cycle phases.⁴²

Since thymidine is a major endogenous hTK1 substrate, Agarwal *et al.* prepared numerous derivatives, bearing a carborane cage attached to N-3 of the thymidine skeleton (Fig. 9) *via* various linkers, such as amidinyl, guanidyl, tetrazolylmethyl, and tetrazolyl groups, to improve hTK1 substrate activities and water solubility compared with previously presented carboranyl thymidine derivatives, such as N5-2OH **34**. Unfortunately, the introduction of carboranes led to problematic solubility and the compounds showed no stability in aqueous solution, limiting biological investigations. The guanidyl- (**35**) and amidinyl-substituted carboranyl thymidine analogues (**36**) exhibited low phosphorylation rates (<30% relative to thymidine), in phosphoryl transfer assays. **36a** and **36b** showed >1900 and >1500 times better solubility in phosphate-buffered saline solutions than **34**.⁴³ However, further improvement of the reported ligands on phosphorylation rates, solubility and metabolic stability were needed.⁴³

In recent studies, Agarwal *et al.* presented further carborane-based thymidine kinase 1 inhibitors, including derivative YB18A **37** reported earlier.^{44,45} Phosphorylation rates (rPr) relative to that of thymidine were determined, exhibiting low values for most of the new ligands. The rPr values of amino *meta*-carborane

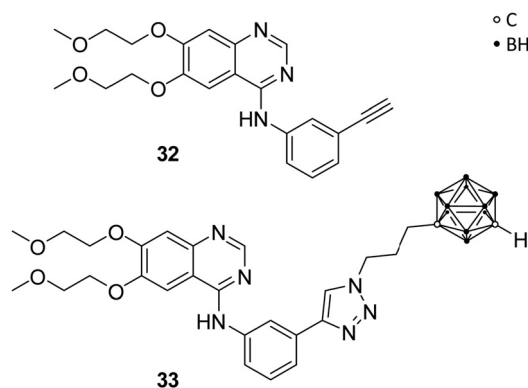


Fig. 8 Structures of EGFR inhibitors erlotinib **32** and the carborane derivative **33**.

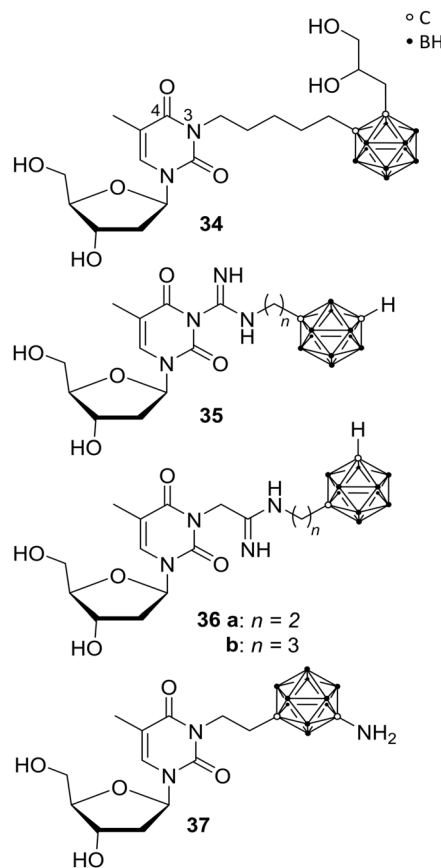
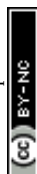


Fig. 9 Thymidine kinase inhibitors N5-2OH **34**, the guanidyl **35** and amidinyl derivatives **36** and YB18A **37**.



derivative YB18A **37** were twice as high as for N5-2OH **34** (78.7 and 40.0%, respectively; with thymidine set as 100%). Biodistribution studies of these compounds in mice bearing intracerebral RG2 gliomas showed very good tumour-to-blood ratios of up to 62.5 and tumour-to-brain ratios of up to 4.9. The boron concentration of **37** in the tumour tissue was determined by ICP-OES (inductively coupled plasma – optical emission spectrometry) to be $32.5 \mu\text{g g}^{-1}$ tumour.⁴⁵ Thus, these compounds might also be suitable candidates for BNCT.

In addition, Agarwal *et al.* were able to show that a substitution at the 4-position of the pyrimidine ring eliminated any activity. It was presumed that, based on the examinations, the linker length between pyrimidine scaffold and carborane is defining the location of the cluster inside or outside of the binding pocket and, further, the carborane-attached amino group of **37** interacts with amino acid residues of the receptor, resulting in improved hTK1 binding affinity and, subsequently, enhanced substrate features and antagonistic effects toward thymidine.⁴⁵

2.8 Nicotinamide phosphoribosyltransferase ligands

The nicotinamide phosphoribosyltransferase enzyme (NAMPT) has a pivotal role in cellular proliferation and maintenance, metabolism and inflammatory response and, thus, holds a promising opportunity as target in treatments for several diseases, such as diabetes, arthritis and cancer. Overexpression of this enzyme leads to severe symptoms, such as sepsis and pulmonary inflammation. NAMPT is a rate-limiting enzyme, which catalyses the synthesis of nicotinamide mononucleotide (NMN), within the nicotinamide adenine dinucleotide (NAD⁺) biosynthesis and salvage pathway. NAMPT is able to regulate immune response and, furthermore, has been reported to be a cytokine that promotes B-cell maturation and inhibits neutrophil apoptosis.

Lee *et al.* reported novel NAMPT ligands based on FK866 (**38**, Fig. 10), comparing all three carborane isomers.¹⁴ The organic NAMPT inhibitor **38** had been previously identified through ligand library screening, showing induction of apoptosis by lowering of NAD⁺ levels, the oxidised form of nicotinamide adenine dinucleotide, without affecting the cell energy metabolism.⁴⁶ Through co-crystallisation of the enzyme-drug complex, narrow tunnels at each of the two active sites of the protein dimeric surface were identified as fundamental for binding affinity. Hence, molecular docking studies were carried out, showing hydrophobic interactions with several hydrophobic residues, including tyrosine, valine, and isoleucine. However, the introduction of carborane led to 100-fold increased inhibitory potency towards NAMPT and a 10-fold increase in anti-proliferative activity against cancer cells over their purely organic counterparts. Despite the fact that *para*-carboranes are the most hydrophobic isomers, therefore presumably interacting more strongly with hydrophobic sites of NAMPT, the *meta*-derivative MC4 *m*-**39** (Fig. 10) demonstrated by far the most potent effect compared to FK866 against human cell lines for lung (A549), colon (DLD1), and breast tumours (T47D) in the low nanomolar range ($<0.3 \text{ nM}$).¹⁴

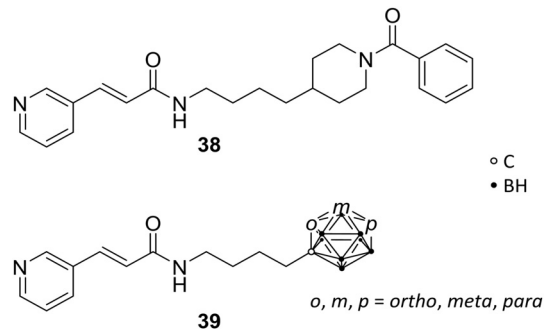


Fig. 10 Molecular structures of nicotinamide phosphoribosyltransferase inhibitor FK866 **38** and the carborane analogues **39**.

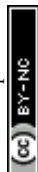
Further *in vitro* and *in vivo* studies of MC4 revealed stronger inhibitory activity than FK866 on CLP-induced (cecal ligation and puncture) mortality, TNF α levels, pulmonary myeloperoxidase activity, alveolar injury, and interleukin 6 and interleukin 1 beta messenger RNA levels. **39** proved to be a potential novel agent for sepsis and inflammation treatments in prospective clinical applications.⁴⁷

2.9 Carbonic anhydrase ligands

Human carbonic anhydrases (CA) are metalloenzymes, containing a Zn²⁺ ion at the catalytic centre, which exhibits an important role in numerous physiological and pathological functions (*e.g.* in epilepsy and tumourigenicity). Therefore, CA inhibitors represent attractive scaffolds for drug design. CA exists as several isozymes, which differ in some amino acid residues at the entrance of the binding site and, therefore, allow for selective targeting of CA subtypes. Brynda *et al.* reported rational drug design, based on manual molecular docking studies of CA subtype II (CAII). The introduction of carboranes led to highly efficient inhibitors, with IC₅₀ values in the low micro- to higher nanomolar range, and selectivity indices (SI) of up to 18 for specific CA subtypes. Through manual molecular docking into the binding site of CAII, lead structure **40** was designed (Fig. 11), bearing a methylene-bridged sulfamide moiety attached to the *ortho*-carborane cluster. **40** exhibited inhibitory activities towards CAII ($K_i = 0.70 \mu\text{M}$) and CAIX ($K_i = 0.38 \mu\text{M}$), with SI of 2 for CAIX.¹² CAIX is a trans-membrane protein, with an extracellular catalytic site, and is over-expressed in hypoxic solid tumours.⁴⁸ Further, co-crystallisation of the ligand-protein complex confirmed the predicted interactions and binding at the active site (PDB code 4MDG), leading to the evaluation of further carborane-based derivatives of **40**. A second sulfamide group (**41**, Fig. 11) increased inhibitory activity towards both tested isozymes, with the same SI as for **40**. Higher SIs were only achieved with *nido* derivatives.¹²

2.10 Glutamate carboxypeptidase II ligands

The prostate-specific membrane antigen (PSMA), also referred to as the glutamate carboxypeptidase II (GCPII), is highly expressed in nervous and prostatic tissues. It is a membrane-residing zinc metallopeptidase responsible for neuronal signalling through hydrolysis of *N*-acetylaspartylglutamate (NAAG), a major peptide neurotransmitter. Also, GCPII is highly overexpressed in



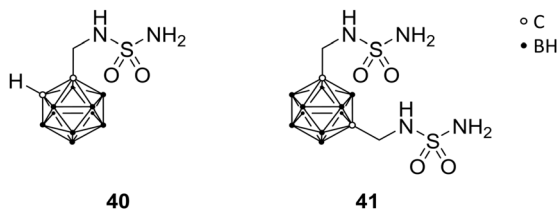


Fig. 11 Carbonyl anhydrase inhibitors **40** and **41**.

androgen-independent and metastatic tumours, like prostate carcinoma, and therefore a biomarker for prostate cancers.⁴⁹

Ligand-enzyme interactions in the two major binding pockets of GPCII were determined by X-ray structures of ligand-GPCII complexes. The S1 pocket, situated at the entrance funnel of the active site of GPCII, shows a size-limited hydrophobic cavity, bearing a positively charged arginine residue. The S1' pocket features a glutamate-recognition site. Among the numerous reported inhibitors, Youn *et al.* designed carboranyl analogues of the urea-based (*S*)-2-{3-[(*S*)-1-carboxy-5-(4-iodobenzamido)pentyl]ureido}pentanedioic acid (DCIBzL) **42** (Fig. 12), replacing the iodophenyl group with *ortho*- or *meta*-carborane. DCIBzL shows a singular and very specific binding mode in GPCII's active site, whereas the iodophenyl moiety fits into the S1 binding pocket, thus promoting high binding affinity. The carborane analogues **43** (*ortho*) and **44** (*meta*) were found to be potent inhibitors with IC₅₀ values in the low nanomolar range (15.6 nM and 20.5 nM, respectively, Fig. 12) in fluorescence polarisation assays, although less potent than DCIBzL, used as control (IC₅₀ < 1 nM). The compounds were co-crystallised and crystal structure analysis revealed the carborane being situated at the entrance funnel and outside the S1 binding pocket, due to its sterically demanding bulky structure. Consequently, the location of the carborane moiety leads to the assumption that the cluster can be further functionalised in order to provide even more potent inhibitors or, also, introducing cluster-halogen substituents for imaging and anti-tumour therapy.⁵⁰

2.11 Cyclooxygenase ligands

Cyclooxygenase (COX) is an enzyme that catalyses the key steps in the biosynthesis of prostaglandins, and exists as two different isoforms, COX-1 and COX-2. COX-1, the so-called 'housekeeping' enzyme, is constitutively expressed, whereas COX-2 is highly inducible at sites of inflammation. COX inhibitors are among the most used medications, primarily for the treatment of inflammation. The well-known non-steroidal anti-inflammatory drugs (NSAIDs; *e.g.*, aspirin, ibuprofen, and indomethacin) are generally non-selective inhibitors of both COX isozymes. COX-2 is upregulated in various tumours and tumour microenvironment, leading to tumour-promoted inflammation. Therefore, the COX-2 variant emerged as promising drug target. Since the active site of COX-2 is approximately 25% larger than that of COX-1, a size-extension of classical NSAIDs successfully yielded inhibitors with COX-2 selectivity. Indomethacin (**45**, Fig. 13) offers two possible positions, the chlorophenyl ring or the carboxylic acid moiety, in the periphery of the indole core for

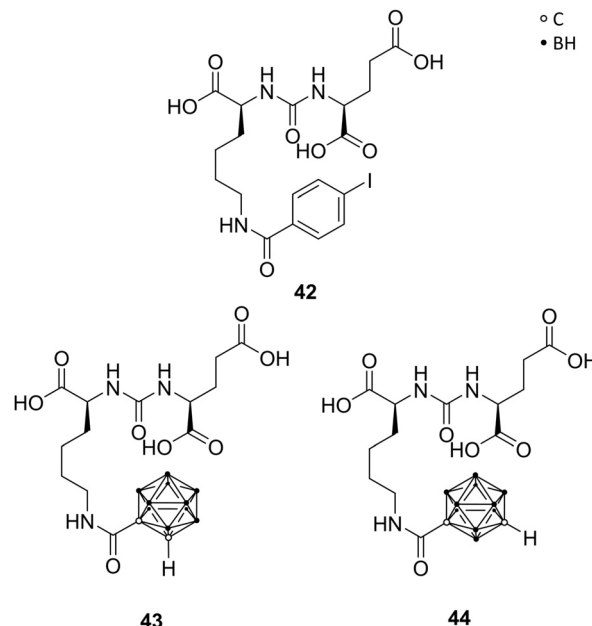


Fig. 12 Structures of the GPCII inhibitors DCIBzL **42** and the carborane derivatives **43** and **44**.

the introduction of bulky substituents, in order to induce the desired selectivity.

Scholz *et al.* described carborane-based cyclooxygenase inhibitors derived from indomethacin **45**, *via* replacement of the chlorophenyl moiety by a carborane cage. Previous studies had shown that modifications of the chlorophenyl ring are synthetically more challenging but revealed also very potent inhibitors. The presence of two additional chlorine substituents in *meta* position to the chlorine atom of **45** in *para* position created a highly selective COX-2 inhibitor. The chlorine residues increased both size and electron deficiency of the benzoyl group. These two effects are probably responsible for the introduction of COX-2 selectivity and, thus, suitable for a replacement by a carborane moiety. The *ortho*-carborane indomethacin methyl ester derivative **46** (Fig. 13) showed high selectivity towards the cancer-specific isoform COX-2 in radioactive assays (conversion of [¹⁴C]-arachidonic acid) with IC₅₀ values in the mid nanomolar range (84 nM). The *meta* and *para* isomers of **46** exhibited practically no inhibitory activity.⁵¹ Following studies by Neumann *et al.* revealed that the electron-withdrawing effect of the carbonyl group directly attached to the carborane promotes rapid deboronation of **46** in aqueous solution. Tailored synthesis and evaluation of the deboronated *nido* species of **46** revealed increased COX-2 inhibition *in vitro* (IC₅₀[*closo*-**46**] = 72 nM; IC₅₀[*nido*-**46**] = 51 nM) and higher water solubility.²⁴ Docking into the COX-2 structure (PDB code 3LN1) using boron parameters predicted a binding pose fairly similar to the subsequently determined crystal structure (PDB code 4ZOL), placing the carborane cluster in the vicinity of Leu531. Leu531 rotates during crystallisation and opens up a hydrophobic sub-pocket to accommodate the *nido*-carborate. Furthermore, the COX-2 membrane-binding domain shifted considerably.



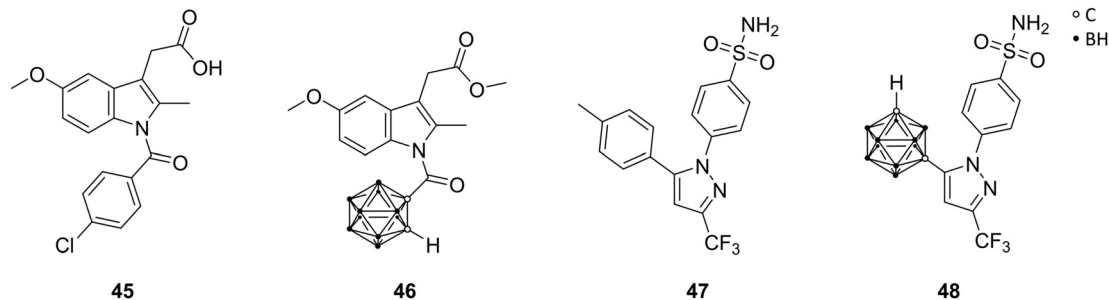


Fig. 13 Structures of indomethacin **45**, the methyl ester derivative **46** and celecoxib **47** and its carborane analogue **48**.

This large shift in the protein structure could not be accounted for in the utilised docking protocol, giving similar predictions for both enantiomers of the deboronated *nido* species of **46**.²⁴ However, docking into the 4ZOL structure predicted a higher binding probability for the *S* enantiomer of the *nido* species of **46**, probably due to the planar chirality of the cluster.

Recent studies by Buzharevski *et al.* presented celecoxib analogues, bearing carboranes as pharmacophores. Celecoxib (**47**, Fig. 13) belongs to the family of COX-2-selective inhibitors (COXIBs), which were developed to address the side effects (bleeding, ulcers in the gastrointestinal tract) of the non-selective NSAIDs, due to inhibition of constitutive COX-1. The carborane derivatives reported by Buzharevski *et al.* showed promising cytostatic activities against various melanoma and colorectal adenocarcinoma cell lines. Inhibited cell proliferation accompanied by caspase-independent apoptotic cell death

was found to be the main cause of decreased cell viability upon treatment with the most efficient celecoxib analogue (**48**, Fig. 13).⁵²

2.12 Lipoygenase ligands

5-Lipoxygenase (5-LO) catalyses the conversion of arachidonic acid into leukotrienes. These fatty acid metabolites are involved in normal host defence, inflammatory responses, and cellular signalling, including apoptosis, but they are also associated with the development of numerous pathologies, *e.g.* asthma, several types of cancer, atherosclerosis, and cardiovascular disorders. The activity of 5-LO is regulated by the 5-lipoxygenase activating protein (FLAP). An early inhibitor of FLAP-mediated activation of 5-LO is Rev-5901 **49** (Fig. 14), a rapidly metabolised drug. To overcome metabolic instability, Kuhnert *et al.* used carboranes as sterically demanding bioisosteres for replacement of phenylene moieties, resulting in analogues CarbORev-5901 **50** and CarbSRev-5901 **51** (Fig. 14). The binding of **50** and **51** to FLAP was investigated by means of docking simulations.¹⁵ The inhibitory activity of 5-LO was decreased relative to Rev-5901, but cytotoxicity toward the melanoma A375 cell line was strongly increased. A375 cells are nitric oxide (NO)-producing cells: **50** and **51** inhibited the generation of NO and reactive oxygen and nitrogen species (ROS/RNS); Rev-5901 acted oppositely, indicating that the structural modification of Rev-5901 with *meta*-carborane leads to a different mechanism of action. Thus, presented 5-LO ligands are considered as promising candidates for the treatment of NO-dependent tumours, known to be highly metastatic.¹⁵

Kuhnert *et al.* were inspired by another inhibitor of the 5-LO pathway, ZD-2138 **52** (Fig. 14).²³ The hydroxyalkyl substituent of **52** was introduced into further carborane derivatives (Fig. 14) in order to improve aqueous solubility as well as its inhibitory and cytotoxic potential through increased receptor binding affinity. Docking studies of the envisioned derivatives into the FLAP-MK591 crystal structure, co-crystallised complex of FLAP and the 5-LO specific inhibitor MK591, identified the isosteric replacement of the phenylene moiety in ZD-2138 by *meta*-carborane functionalised with 2-methylquinoline as the most promising approach, similar to CarbORev-5901. IC₅₀ values for 5-LO product formation in intact polymorphonuclear leukocytes (granulocytes, PMNL) were determined, and the monofunctionalised *meta*-carborane analogue of 2-(phenoxy)methyl)quinoline **53**

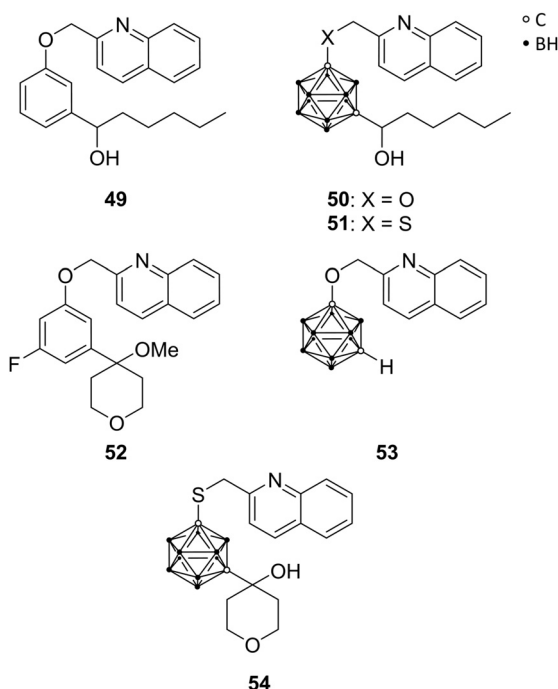
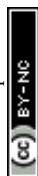


Fig. 14 Structures of 5-lipoxygenase inhibitors Rev-5901 **49**, CarbORev-5901 **50**, CarbSRev-5901 **51**, ZD-2138 **52**, *meta*-carborane analogue of 2-(phenoxy)methyl)quinoline **53** and the 4-hydroxytetrahydropyranyl carborane derivative **54**.



exhibited the highest inhibitory activity of all tested compounds toward the 5-LO pathway with a value of 2.30 μM (Rev-5901: IC_{50} = 0.58 μM). Screening for anti-tumour activity on various tumour cell lines, selected based on 5-LO expression and the well-known inflammatory background in melanoma (A375, B16 and its metastatic clone B16F10) and colon cancer pathogenesis (CT26CL25 and HCT116), surprisingly revealed that the most potent 5-LO inhibitor (**53**) was completely inactive (all tumour activity values > 50 μM), indicating that 5-LO inhibitory activity could not be correlated with the anti-tumour activity. Compound **54** (Fig. 14) functionalised with a 4-hydroxytetrahydropyranyl moiety, revealed remarkably higher cytotoxic activity against melanoma and colon cancer cells, being more potent than **50** and **51**, despite exhibiting lower 5-LO inhibitory activity. This effect could be partly explained by the co-inhibition of another cellular target of compound **54**, namely, heat shock protein 90 (HSP90). Docking simulations were useful for identifying the off-target receptors of **54**. The multichaperone protein HSP90 facilitates cell growth, metastasis and angiogenesis and has anti-apoptotic effects.²³

2.13 ATP-Binding cassette transporter G2 ligands

Neoplastic cells often develop resistance against chemotherapeutic treatments, leading to insufficiency of anti-cancer therapies. Specific transmembrane transporter proteins are involved in the efflux of chemotherapeutic agents from the intracellular space and can cause multidrug resistances (MDRs). Several ATP-binding cassette transporters (ABCs) are known ATP-dependent efflux-pump proteins for xenobiotics, and were found to be overexpressed in various cancer cell lines. By upregulation of those ABCs, chemotherapeutics can be excreted from the cell, decreasing the concentration of the drug to a non-therapeutic level. Multidrug resistance is associated with three transport proteins, namely the permeability glycoprotein (Pgp, also referred to as multidrug resistance protein 1, MDR1, and ABCB1), the ABCC1, and the ABC transporter G2 (ABCG2, also known as the breast cancer resistance protein BCRP), which gained attention within the last years as targets for efficient and promising strategies of cancer chemotherapy. With the focus on selective inhibition of ABCG2-mediated excretion of xenobiotics, the efficacy of anti-cancer treatments can be enhanced.⁵³

Köhler *et al.* prepared ABCG2-selective inhibitors, with further regards to their selectivity against the MDR-involved MDR1. Based on a known heteroaryl-phenyl lead structure **55** (Fig. 15), a series of analogues was designed, including two *meta*-carborane derivatives, **56a** and **b** (Fig. 15), as phenyl or adamantyl mimetics. Both carborane derivatives **56a** and **56b** exhibited enhanced activity compared to **55** and their adamantyl model towards ABCG2-overexpressed MDCK II BCRP cells (BCRP transfected canine kidney cell lines) using the Hoechst 33342 assay. The functionalisation with carboranes led to efficient inhibition of ABCG2 at low micromolar concentrations. Both derivatives, **56a/b**, showed lower inhibitory potency (IC_{50} = 0.508 and 0.807 μM , respectively) than **55** (IC_{50} = 0.067 μM) for inhibition of ABCG2 activity and, interestingly, targeted MDR1 less significantly than their adamantyl analogue.⁵⁴ However,

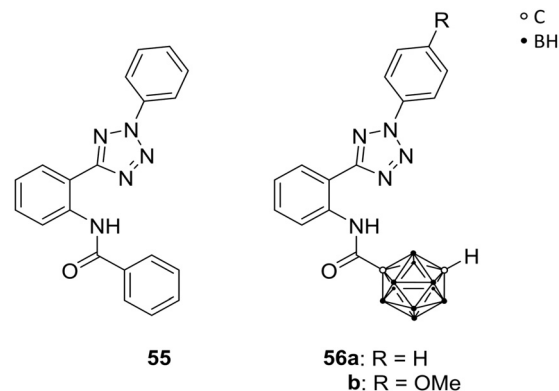


Fig. 15 ABCG2 inhibitor **55** and the carborane analogues **56**.

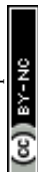
for selective ABCG2 inhibition the phenyl-heteroaryl-phenylamide framework of **55** and **56** is essential, as SAR studies revealed.

2.14 Indoleamine-2,3-dioxygenase-1 ligands

Austin *et al.* selected the indoleamine-2,3-dioxygenase-1 (IDO1) for ligand-enzyme interaction studies, which contains two hydrophobic binding pockets and, thus, the authors designed highly hydrophobic carborane-based pharmacophores.⁵⁵ IDO1 is a haem-containing enzyme that catalyses the first and rate-limiting step in the major tryptophan metabolic pathway during inflammation and disease, the kynurenine pathway. Involved in immune response modulation, IDO1 shows the ability to limit the functions of T cells and can engage immune tolerance mechanisms. Thus, it is believed that IDO1 is activated in tumour cell progression for downregulating the immune system-response against malignant cells.⁵⁶ Thus, IDO1 inhibitors possess the potential as immunoregulatory chemotherapeutics, restoring regular physiological immune reactions. Moreover, IDO1 can be downregulated by COX-2 inhibitors, as IDO1 expression in tumour cells is driven by COX-2 expression.⁵⁷

Determination of the crystal structure of IDO1 resulted in better insight into the enzyme structure and, thus, of the active site, which features a small hydrophobic cavity near the active site's entrance and a larger hydrophobic, haem catalytic site-containing pocket. The pyranonaphthoquinone derivatives dehydro- α -lapachone (**57**, Fig. 16) and the diastereomeric *cis*-hydroxy-benzylamino analogues **58** (Fig. 16) were evaluated recently as potent inhibitors for IDO1. Austin *et al.* replaced the phenylene moiety of **58** with *ortho*-carborane. The carborane derivatives **59** and **60** (Fig. 16) displayed inhibitory activity towards human recombinant enzyme in the micromolar range, with IC_{50} values of 0.78 and 1.77 μM , respectively, and are thus half as potent as the racemic parental analogues **58** (IC_{50} = 0.35–0.50 μM).⁵⁵

Furthermore, Austin *et al.* designed two novel carborane-based ligands on the basis of smaller sized hydroxyamides, which presented high inhibitory potency toward IDO1. Hydroxyamide analogue **61** (Fig. 16), bearing a methylene spacer between the cluster and the hydroxyamide group, showed inhibition activity in the low micromolar level (IC_{50} = 1.90 μM), whereas **62** (Fig. 16), the boron-substituted *meta*-carborane



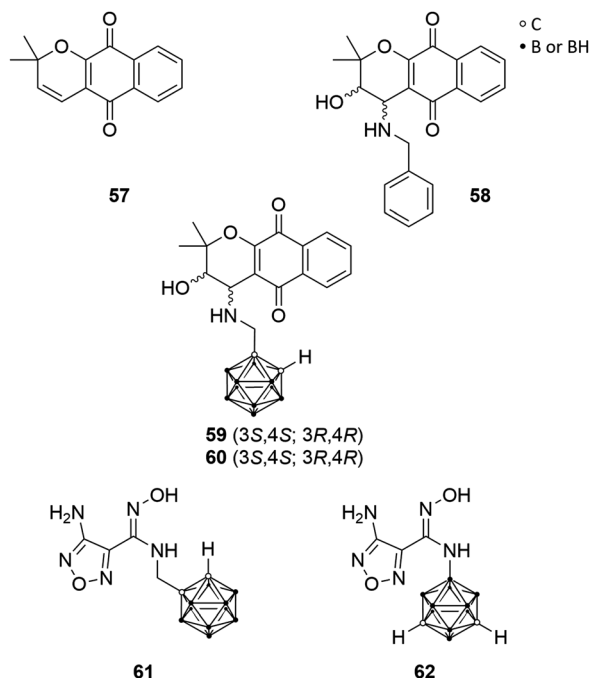


Fig. 16 Indoleamine 2,3-dioxygenase-1 ligands: structures of dehydro- α -lapachone **57**, the benzylamino derivative **58**, the carborane derivatives **59/60** and carborane-based hydroxyamidines derivatives **61** and **62**.

derivative, was inactive. This may be attributed to a different binding mode of **62** due to minor conformational freedom and enhanced steric hindrance, due to the missing methylene linker, as revealed by docking simulations. Nevertheless, these novel carborane-based IDO1 ligands may be feasible as prospective IDO1 inhibitors and lead structure.²¹

2.15 *N*-Alkyl-deoxynojirimycins as glycoprocessing enzyme ligands

Deoxynojirimycin analogues (DNMs), in particular *N*-alkylated deoxynojirimycin derivatives, represent a major class of glycoprocessing enzyme inhibitors. A variety of glycosidase and glycosyltransferase inhibitory agents are approved drugs for different applications, *e.g.* for the treatment of lysosomal storage disorder through glucosylceramide synthase inhibition (*N*-butyl-DNM) or anti-diabetics (hydroxyethyl-DNM) as intestinal glycosidase inhibitor.⁵⁸

Hoogendoorn *et al.* prepared *ortho*-carboranyl *N*-alkyl-deoxynojirimycins that are suitable for the simultaneous inhibition of several glycoprocessing enzymes: glucosylceramide synthase (GCS), lysosomal glucosyl-ceramidase (GBA) and neutral glucosylceramidase (GBA2). Based on previous results, the authors evaluated *N*-substituted DNMs with different hydrophobic moieties, including an adamantyl group. The adamantane-based *N*-alkylated DNM (AMP-DNM **63**, Fig. 17) showed higher potency as GCS inhibitor than the clinically used *N*-butyl-DNM, with also good activities toward GBA, GBA2 and intestinal glycosidase. The C5-epimer of **63**, L-AMP-DNM **64** (Fig. 17), differs in its action compared to AMP-DNM, enhancing the inhibition of GCS, but lowering the GBA inhibitory activity.

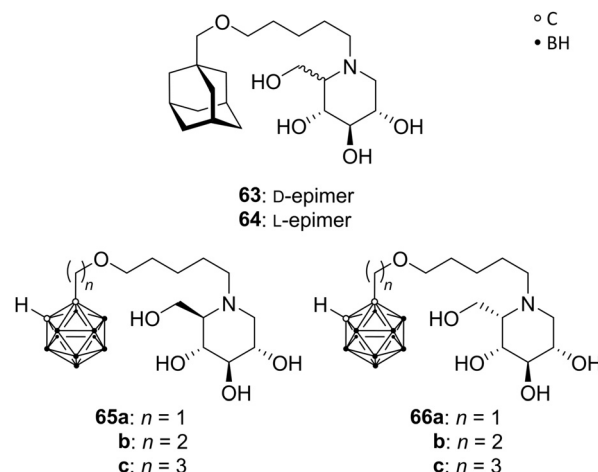


Fig. 17 Structures of AMP-DNMs **63** and **64** and carborane-based analogues **65** and **66**.

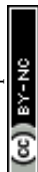
Thus, a hydrophobic and sterically demanding carborane cluster was introduced in both epimeric forms to improve the inhibitory potency. A series of carboranyl derivatives of D-DNM (**65a–c**) and L-DNM (**66a–c**) were designed (Fig. 17), varying the length of the alkyl spacer. Despite the fact that no improvement in the inhibitory activity on GCS, GBA and GBA2 was accomplished, the most efficient compound **66c** inhibits all three enzymes in low micromolar ranges, below 0.5 μ M, similar to **63** and **64**.⁵⁹

3. Conclusions

With respect to medicinal organic chemistry, carboranes in medicine are still an emerging class of compounds. Researchers have just begun to scratch the surface of the potential beneficial applications of these highly hydrophobic clusters in drug design. Mechanisms of action in the cells, as well as the cause-effect relationship of the biological activities with respect to the specific biological targets, is still largely underexplored. Often, introduction of a *closo*-carborane cluster into the skeleton of a purely organic agonist, antagonist or inhibitor, completely changes the activity profile of the drug, and also modulates its effects on cell viability in several types of tumours. Therefore, icosahedral carboranes deserve the classification as “new keys for old locks” and open up an exciting field of research for well-known, but challenging, important therapeutic substrates, as demonstrated by the various examples discussed in this review. For the efficient treatment of several pathologies (neuronal or immune disorders, cancer, pain, inflammation, *etc.*), there is in fact urgent need for novel chemotherapeutics to overcome the problems associated with the traditional organic-based drugs. In this scenario, carboranes are definitely very promising, robust and chemically stable keys.

Conflicts of interest

There are no conflicts to declare.

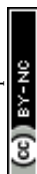


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Notes and references

- 1 P. Imming, *The Practice of Medicinal Chemistry*, Elsevier, 2015, pp. 3–13.
- 2 C. O. Wilson, J. M. Beale and J. H. Block, *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*, Lippincott Williams & Wilkins, Baltimore MD, 12th edn, 2011.
- 3 T. C. Johnstone, K. Suntharalingam and S. J. Lippard, *Chem. Rev.*, 2016, **116**, 3436–3486.
- 4 J. L. Lau and M. K. Dunn, *Bioorg. Med. Chem.*, 2018, **26**, 2700–2707.
- 5 R. F. Barth, P. Mi and W. Yang, *Cancer Commun.*, 2018, **38**, 35.
- 6 Z. J. Leśnikowski, *J. Med. Chem.*, 2016, **59**, 7738–7758.
- 7 M. Couto, M. F. García, C. Alamón, M. Cabrera, P. Cabral, A. Merlino, F. Teixidor, H. Cerecetto and C. Viñas, *Chem. – Eur. J.*, 2018, **24**, 3122–3126, and references therein.
- 8 M. Scholz and E. Hey-Hawkins, *Chem. Rev.*, 2011, **111**, 7035–7062, and references therein.
- 9 R. N. Grimes, *Carboranes*, Academic Press, Amsterdam, 2016, and references therein.
- 10 N. A. Meanwell, *J. Med. Chem.*, 2011, **54**, 2529–2591.
- 11 W. H. Eberhardt, B. Crawford and W. N. Lipscomb, *J. Chem. Phys.*, 1954, **22**, 989–1001.
- 12 J. Brynda, P. Mader, V. Šícha, M. Fábry, K. Poncová, M. Bakardiev, B. Grüner, P. Cígler and P. Řezáčová, *Angew. Chem., Int. Ed.*, 2013, **52**, 13760–13763.
- 13 H. Nakamura, L. Tazaki, D. Kanoh and S. Sato, *Pure Appl. Chem.*, 2015, **87**, 143–154.
- 14 M. W. Lee, Y. V. Sevrugina, A. Khan and S. Q. Ye, *J. Med. Chem.*, 2012, **55**, 7290–7294.
- 15 R. Kuhnert, M.-B. Sárosi, S. George, P. Lönnecke, B. Hofmann, D. Steinhilber, B. Murganić, S. Mijatović, D. Maksimović-Ivanić and E. Hey-Hawkins, *ChemMedChem*, 2017, **12**, 1081–1086.
- 16 F. Issa, M. Kassiou and L. M. Rendina, *Chem. Rev.*, 2011, **111**, 5701–5722, and references therein.
- 17 R. Fernandez-Alvarez, V. Đorđević, M. Uchman and P. Matějček, *Langmuir*, 2018, **34**, 3541–3554, and references therein.
- 18 Y. Nakamura, A. Mochida, P. L. Choyke and H. Kobayashi, *Bioconjugate Chem.*, 2016, **27**, 2225–2238.
- 19 M. Tarrés, E. Canetta, E. Paul, J. Forbes, K. Azzouni, C. Viñas, F. Teixidor and A. J. Harwood, *Sci. Rep.*, 2015, **5**, 7804–7811.
- 20 T. He, J. C. Misuraca and R. A. Musah, *Sci. Rep.*, 2017, **7**, 16995–17005.
- 21 C. J. D. Austin, M. Moir, J. Kahlert, J. R. Smith, J. F. Jamie, M. Kassiou and L. M. Rendina, *Aust. J. Chem.*, 2015, **68**, 1866–1870.
- 22 A. Kaise, K. Ohta, S. Fujii, A. Oda, T. Goto and Y. Endo, *Bioorg. Med. Chem.*, 2018, **26**, 3805–3811.
- 23 R. Kuhnert, M.-B. Sárosi, S. George, P. Lönnecke, B. Hofmann, D. Steinhilber, S. Steinmann, R. Schneider-Stock, B. Murganic, S. Mijatovic, D. Maksimovic-Ivanic and E. Hey-Hawkins, *ChemMedChem*, 2018, **14**, 255–261.
- 24 W. Neumann, S. Xu, M. B. Sárosi, M. S. Scholz, B. C. Crews, K. Ghebreselasie, S. Banerjee, L. J. Marnett and E. Hey-Hawkins, *ChemMedChem*, 2016, **11**, 175–178, and references therein.
- 25 R. Otero, S. Seoane, R. Sigüeiro, A. Y. Belorusova, M. A. Maestro, R. Pérez-Fernández, N. Rochel and A. Mouriño, *Chem. Sci.*, 2016, **7**, 1033–1037.
- 26 B. Schwarze, M. Gozzi and E. Hey-Hawkins, in *Boron-Based Compounds*, ed. E. Hey-Hawkins and C. Viñas Teixidor, John Wiley & Sons, Ltd, Chichester, UK, 2018, pp. 60–108 and references therein.
- 27 N. Vázquez, V. Gómez-Vallejo and J. Llop, *Tetrahedron Lett.*, 2012, **53**, 4743–4746.
- 28 Y. Endo, in *Boron-Based Compounds*, ed. E. Hey-Hawkins and C. Viñas Teixidor, John Wiley & Sons, Ltd, Chichester, UK, 2018, pp. 1–19 and references therein.
- 29 S. Fujii, *Med. Chem. Commun.*, 2016, **7**, 1082–1092, and references therein.
- 30 K. Ohta, T. Ogawa, A. Kaise, A. Oda and Y. Endo, *Chem. Pharm. Bull.*, 2014, **62**, 386–391.
- 31 K. Ohta, T. Ogawa and Y. Endo, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 4030–4033.
- 32 A. Kaise, K. Ohta and Y. Endo, *Eur. J. Med. Chem.*, 2016, **122**, 257–263.
- 33 K. Bednarska, A. B. Olejniczak, A. Piskala, M. Klink, Z. Sulowska and Z. J. Lesnikowski, *Bioorg. Med. Chem.*, 2012, **20**, 6621–6629.
- 34 A. Adamska-Bartłomiejczyk, K. Bednarska, M. Białek-Pietras, Z. M. Kiliańska, A. Mieczkowski, A. B. Olejniczak, E. Paradowska, M. Studzińska, Z. Sulowska, J. D. Żońmierczyk and Z. J. Lesnikowski, in *Boron-Based Compounds*, ed. E. Hey-Hawkins and C. Viñas Teixidor, John Wiley & Sons, Ltd, Chichester, UK, 2018, pp. 20–34.
- 35 M. Vincenzi, K. Bednarska and Z. J. Leśnikowski, *Molecules*, 2018, **23**, 1846–1866.
- 36 C. Volonté, S. Apolloni, S. D. Skaper and G. Burnstock, *CNS Neurol. Disord.: Drug Targets*, 2012, **11**, 705–721.
- 37 A. Baxter, J. Bent, K. Bowers, M. Braddock, S. Brough, M. Fagura, M. Lawson, T. McNally, M. Mortimore, M. Robertson, R. Weaver and P. Webborn, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 4047–4050.
- 38 S. M. Wilkinson, H. Gunosewoyo, M. L. Barron, A. Boucher, M. McDonnell, P. Turner, D. E. Morrison, M. R. Bennett, I. S. McGregor, L. M. Rendina and M. Kassiou, *ACS Chem. Neurosci.*, 2014, **5**, 335–339, and references therein.
- 39 F. Di Virgilio, D. Dal Ben, A. C. Sarti, A. L. Giuliani and S. Falzoni, *Immunity*, 2017, **47**, 15–31.
- 40 G. Li, H. S. Ban and H. Nakamura, in *Boron-Based Compounds*, ed. E. Hey-Hawkins and C. Viñas Teixidor, John Wiley & Sons, Ltd, Chichester, UK, 2018, pp. 35–59 and references therein.



- 41 M. Couto, I. Mastandrea, M. Cabrera, P. Cabral, F. Teixidor, H. Cerecetto and C. Viñas, *Chemistry*, 2017, **23**, 9233–9238.
- 42 M. M. Alegre, R. A. Robison and K. L. O'Neill, *J. Oncol.*, 2012, **2012**, 1–5.
- 43 H. K. Agarwal, C. A. McElroy, E. Sjuvarsson, S. Eriksson, M. V. Darby and W. Tjarks, *Eur. J. Med. Chem.*, 2013, **60**, 456–468.
- 44 Y. Byun, J. Yan, A. S. Al-Madhoun, J. Johnsamuel, W. Yang, R. F. Barth, S. Eriksson and W. Tjarks, *J. Med. Chem.*, 2005, **48**, 1188–1198.
- 45 H. K. Agarwal, A. Khalil, K. Ishita, W. Yang, R. J. Nakkula, L.-C. Wu, T. Ali, R. Tiwari, Y. Byun, R. F. Barth and W. Tjarks, *Eur. J. Med. Chem.*, 2015, **100**, 197–209.
- 46 M. Hasmann and I. Schemainda, *Cancer Res.*, 2003, **63**, 7436–7442.
- 47 P. Huang, M. W. Lee, K. Sadrerafi, D. P. Heruth, L. Q. Zhang, D. Maulik and S. Q. Ye, *Drug Des., Dev. Ther.*, 2017, **11**, 629–641.
- 48 P. C. McDonald and S. Dedhar, *Subcell. Biochem.*, 2014, **75**, 255–269.
- 49 P. Sácha, J. Zámecník, C. Barinka, K. Hlouchová, A. Vícha, P. Mlcochová, I. Hilgert, T. Eckschlager and J. Konvalinka, *Neuroscience*, 2007, **144**, 1361–1372.
- 50 S. Youn, K. Im Kim, J. Ptacek, K. Ok, Z. Novakova, Y. Kim, J. Koo, C. Barinka and Y. Byun, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 5232–5236.
- 51 M. Scholz, A. L. Blobaum, L. J. Marnett and E. Hey-Hawkins, *Bioorg. Med. Chem.*, 2012, **20**, 4830–4837.
- 52 A. Buzharevski, S. Paskas, M.-B. Sárosi, M. Laube, P. Lönnecke, W. Neumann, S. Mijatovic, D. Maksimovic-Ivanic, J. Pietzsch and E. Hey-Hawkins, *ChemMedChem*, 2019, **14**, 315–321.
- 53 S. C. Köhler, K. Silbermann and M. Wiese, *Eur. J. Med. Chem.*, 2016, **124**, 881–895.
- 54 S. C. Köhler, S. Vahdati, M. S. Scholz and M. Wiese, *Eur. J. Med. Chem.*, 2018, **146**, 483–500.
- 55 C. J. D. Austin, J. Kahlert, F. Issa, J. H. Reed, J. R. Smith, J. A. Ioppolo, J. A. Ong, J. F. Jamie, D. Hibbs and L. M. Rendina, *Dalton Trans.*, 2014, **43**, 10719–10724.
- 56 D. H. Munn and A. L. Mellor, *Trends Immunol.*, 2016, **37**, 193–207.
- 57 M. Hennequart, L. Pilotte, S. Cane, D. Hoffmann, V. Stroobant, E. D. Plaen and B. van den Eynde, *Cancer Immunol. Res.*, 2017, **5**, 695–709.
- 58 T. Wennekes, R. J. B. H. N. van den Berg, W. Donker, G. A. van der Marel, A. Strijland, J. M. F. G. Aerts and H. S. Overkleeft, *J. Org. Chem.*, 2007, **72**, 1088–1097.
- 59 S. Hoogendoorn, E. D. Mock, A. Strijland, W. E. Donker-Koopman, H. van den Elst, R. J. B. H. N. van den Berg, J. M. F. G. Aerts, G. A. van der Marel and H. S. Overkleeft, *Eur. J. Org. Chem.*, 2015, 4437–4446.

