WILEY

Edited by
Evamarie Hey-Hawkins and Clara Viñas Teixidor

Boron-Based Compounds

Potential and Emerging Applications in Medicine

Boron-Based Compounds

Boron-Based Compounds

Potential and Emerging Applications in Medicine

Edited by

Evamarie Hey-Hawkins
Department of Inorganic Chemistry, Leipzig University
Leipzig, Germany

Clara Viñas Teixidor
Spanish Council for Scientific Research, Institut de Ciència de Materials de Barcelona
Barcelona, Spain

WILEY

This edition first published 2018

Registered Office(s)
John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial Office
9600 Garsington Road, Oxford, OX4 2DQ, UK

For details of our global editorial offices, customer services, and more information about Wiley products visit us at www.wiley.com.

Wiley also publishes its books in a variety of electronic formats and by print-on-demand. Some content that appears in standard print versions of this book may not be available in other formats.

Library of Congress Cataloging-in-Publication Data

Names: Hey-Hawkins, Evamarie, 1957– editor.
Title: Boron-based compounds : potential and emerging applications in medicine / edited by Evamarie Hey-Hawkins, University of Leipzig, Leipzig, Germany, Clara Viñas Teixidor, Institut de Ciencia de Materials de Barcelona, Barcelona, Spain.
Description: First edition. | Hoboken, NJ, USA : Wiley, [2018] | Includes bibliographical references and index. |
Identifiers: LCCN 2017055295 (print) | LCCN 2018009581 (ebook) | ISBN 9781119275589 (pdf) | ISBN 9781119275596 (epub) | ISBN 9781119275558 (cloth)
Subjects: LCSH: Boron compounds. | Boron compounds–Diagnostic use. | Boron compounds–Therapeutic use.
Classification: LCC QD181.B1 (ebook) | LCC QD181.B1 B663 2018 (print) | DDC 615.2/671–dc23
LC record available at https://lccn.loc.gov/2017055295

Cover design: Wiley
Cover image: Designer, MSc Christoph Selg

Set in 10/12pt Warnock by SPi Global, Pondicherry, India

Printed in the UK by Bell & Bain Ltd, Glasgow

10 9 8 7 6 5 4 3 2 1

Contents

List of Contributors

Anna Adamska-Bartłomiejczyk
Laboratory of Molecular Virology and Biological Chemistry, IMB PAS, Łódź, Poland

Diego Alberti
Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy

Khaleel I. Assaf
Department of Life Sciences and Chemistry, Jacobs University Bremen, Germany

Hyun Seung Ban
Biomedical Genomics Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea

Katarzyna Bednarska
Laboratory of Experimental Immunology, IMB PAS, Łódź, Poland

Magdalena Białek-Pietras
Laboratory of Molecular Virology and Biological Chemistry, IMB PAS, Łódź, Poland

Paolo Boggio
Department of Chemistry, University of Torino, Italy

Vladimir I. Bregadze
A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, Russia

Bhaskar C. Das
Departments of Medicine and Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, New York, USA

Sasmita Das
Molecular Bio-nanotechnology, Imaging and Therapeutic Research Unit, Veteran Affairs Medical Center, Kansas City, USA

Annamaria Deagostino
Department of Chemistry, University of Torino, Italy

Yasuyuki Endo
Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, Sendai, Japan

Todd Evans
Department of Surgery, Weill Cornell Medical College of Cornell University, New York, USA

Jindřich Fanfrlík
Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic

Detlef Gabel
Department of Life Sciences and Chemistry, Jacobs University Bremen, Germany

Shanmin Gao
School of Chemistry and Materials Science, Ludong University, China

Marcela A. Garabalino
Department of Radiobiology, National Atomic Energy Commission, San Martín, Buenos Aires Province, Argentina

Simonetta Geninatti Crich
Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy

Vanessa Gómez-Vallejo
Radiochemistry and Nuclear Imaging Group, CIC biomaGUNE, San Sebastian, Spain

Kiran B. Gona
Radiochemistry and Nuclear Imaging Group, CIC biomaGUNE, San Sebastian, Spain Cardiovascular Molecular Imaging Laboratory, Section of Cardiovascular Medicine and Yale Cardiovascular Research Center, Yale University School of Medicine, New Haven, USA Veterans Affairs Connecticut Healthcare System, West Haven, USA

Marta Gozzi
Universität Leipzig, Institut für Anorganische Chemie, Germany

Adrian J. Harwood
School of Biosciences and Neuroscience and Mental Health Research Institute, Cardiff University, Cardiff, UK

Elisa M. Heber
Department of Radiobiology, National Atomic Energy Commission, San Martín, Buenos Aires Province, Argentina

Evamarie Hey-Hawkins
Universität Leipzig, Institut für Anorganische Chemie, Germany

Pavel Hobza
Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic Regional Centre of Advanced Technologies and Materials, Palacký University, Olomouc, Czech Republic

Narayan Hosmane
Department of Chemistry and Biochemistry, Northern Illinois University, DeKalb, USA

Satish S. Jalisatgi
International Institute of Nano and Molecular Medicine, School of Medicine, University of Missouri, Columbia, USA

Zofia M. Kiliańska
Department of Cytobiochemistry, Faculty of Biology and Environmental Protection, University of Łódź, Poland

Jacek Koziorowski
Department of Radiology and Department of Medical and Health Sciences, Linköping University, Sweden

Martin Lepšík
Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic

Zbigniew J. Lesnikowski
Laboratory of Molecular Virology and Biological Chemistry, IMB PAS, Łódź, Poland

Guangzhe Li
School of Pharmaceutical Science and Technology, Dalian University of Technology, China

Jordi Llop
Radiochemistry and Nuclear Imaging Group, CIC biomaGUNE, San Sebastian, Spain

Jonas Malmquist
Department of Radiology and Department of Medical and Health Sciences, Linköping University, Sweden

Irina Manea
Colentina Clinical Hospital, Bucuresti, Romania

Adam Mieczkowski
Department of Biophysics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Andrea Monti Hughes
Department of Radiobiology, National Atomic Energy Commission, Buenos Aires Province, Argentina

Hiroyuki Nakamura
Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Japan

Devi Prasan Ojha
Departments of Medicine and Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, New York, USA

Agnieszka B. Olejniczak
Screening Laboratory, IMB PAS, Łódź, Poland

Valentina A. Ol'shevskaya
A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, Russia

Edyta Paradowska
Laboratory of Molecular Virology and Biological Chemistry, IMB PAS, Łódź, Poland

Adam Pecina
Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic

Emiliano C.C. Pozzi
Department of Radiobiology, National Atomic Energy Commission, San Martín, Buenos Aires Province, Argentina

Nicoletta Protti
Nuclear Physics National Institute (INFN), University of Pavia, Italy

Jan Řezáč
Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic

Benedikt Schwarze
Universität Leipzig, Institut für Anorganische Chemie, Germany

Amanda E. Schwint
Department of Radiobiology, National Atomic Energy Commission, San Martín, Buenos Aires Province, Argentina

Alexander A. Shtil
Blokhin National Medical Research Center of Oncology, Moscow, Russia

Igor B. Sivaev
A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, Russia

Mirosława Studzińska
Laboratory of Molecular Virology and Biological Chemistry, IMB PAS, Łódź, Poland

Zofia Sułowska
Laboratory of Experimental Immunology, IMB PAS, Łódź, Poland

Francesc Teixidor
Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Bellaterra, Spain

Werner Tjarks
Division of Medicinal Chemistry & Pharmacognosy, The Ohio State University, Columbus, USA

Veronica A. Trivillin
Department of Radiobiology, National Atomic Energy Commission, San Martín, Buenos Aires Province, Argentina

Maria da Graça H. Vicente
Department of Chemistry, Louisiana State University, Baton Rouge, USA

Clara Viñas Teixidor
Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Bellaterra, Spain

Joanna Wilińska
Department of Life Sciences and Chemistry, Jacobs University Bremen, Germany

Sunting Xuan
Department of Chemistry, Louisiana State University, Baton Rouge, USA

Andrei V. Zaitsev
A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, Russia

Yinghuai Zhu
School of Pharmacy, Macau University of Science and Technology, Taipa, Macau, China

Jolanta D. Żołnierczyk
Department of Cytobiochemistry, Faculty of Biology and Environmental Protection, University of Łódź, Poland

Preface

Today, medicinal chemistry is still clearly dominated by organic chemistry, and most commercial drugs are purely organic molecules, which, besides carbon and hydrogen, can incorporate nitrogen, oxygen, sulfur, phosphorus, and halogens, all of which are to the right of carbon in the periodic table, whereas boron is located to the left. Boron and carbon are elements that have the ability to build molecules of unlimited size by covalent self-bonding. However, commercial boron-based drugs are still rare. Bortezomib, tavaborole (AN2690), crisaborole (AN2728), epetraborole (AN3365), SCYX-7158 (AN5568), 4-(dihydroxyboryl)phenylalanine (BPA), and sodium mercapto-undecahydro-*closo*-dodecaborate (BSH) are used as drugs, the last two compounds in boron neutron capture therapy (BNCT). All of these boron-containing drugs are derivatives of boronic acids except BSH, which contains an anionic boron cluster. While the pharmacological uses of boron compounds have been known for several decades, recent progress is closely related to the discovery of further boron-containing compounds as prospective drugs. While first developments of the medicinal chemistry of boron were stipulated by applications in BNCT of cancers, knowledge accumulated during the past decades on the chemistry and biology of bioorganic and bioinorganic boron compounds laid the foundation for the emergence of a new area of study and application of boron compounds as skeletal structures and hydrophobic pharmacophores for biologically active molecules. These and other recent findings clearly show that there is still a great, unexplored potential in medicinal applications of boron-containing compounds.

This book summarizes the present status and further promotes the development of new boron-containing drugs and boron-based materials for diagnostics by bringing together renowned experts in the field of medicinal chemistry of boron compounds. It aims to provide a balanced overview of the vibrant and growing field of the emerging and potential applications of boron compounds in medicinal chemistry and chemical biology. The book is aimed at academics and professional researchers in this field, but also at scientists who want to get a better overview on the state of the art of this rapidly advancing area. It contains reviews of important topics, which are divided into three main sections: (1) "Design of New Boron-based Drugs", (2) "Boron Compounds in Drug Delivery and Imaging", and (3) "Boron Compounds for Boron Neutron Capture Therapy".

The first section, "Design of New Boron-Based Drugs", consists of six reviews dealing with the use of carborane derivatives for the development of novel drugs. In his review (Chapter 1.1), Yasuyuki Endo, one of the pioneers in the development of carboranes as

hydrophobic pharmacophores almost 20 years ago, describes the development of a variety of potent nuclear receptor ligands with carborane structures as hydrophobic moieties. Nucleoside drugs have been in clinical use for several decades and have become cornerstones of treatment for patients with cancer or viral infections. One of the new developments in the medicinal chemistry of nucleosides is derivatives comprising a boron component such as a boron cluster, as described in the review by Zbigniew J. Lesnikowski and coworkers (Chapter 1.2), whose group has long-standing expertise in the introduction of boron clusters into molecules with diverse biological activity, where they serve as pharmacophores, building blocks, and modulators of the physicochemical and biological properties. An alternative approach to battling cancer is described by Hiroyuki Nakamura *et al.* in their chapter on the design of carborane-based hypoxia-inducible factor (HIF) inhibitors (Chapter 1.3). Overexpression of HIF1α has been observed in human cancers, including brain, breast, colon, lung, ovary, and prostate cancers; thus, HIF1α is a novel target of cancer therapy, and the Nakamura group has shown carborane-based HIF1 inhibitors to be very promising targets. Another emerging type of boron-based drugs are metallacarboranes. The group of Evamarie Hey-Hawkins has been involved in carborane chemistry for more than 20 years. In Chapter 1.4, they report recent examples of biologically active half- and mixed-sandwich metallacarborane complexes of the dicarbollide ligand, as well as hybrid organic-inorganic compounds containing a *nido*-carborane(−1) as appended moiety. Their potentially beneficial properties, such as stability in aqueous environments and new binding modes due to their lipophilicity, are described. Prospective applications in radio-imaging, radiotherapy, and drug design are envisaged. In Chapter 1.5, Detlef Gabel and coworkers focus on ionic boron clusters that are soluble in water as well as in nonpolar solvents. This highly interesting feature sets them apart from other ionic and nonionic pharmacophores and renders them interesting new entities for drug design. The final review (Chapter 1.6) by Pavel Hobza, Martin Lepšík, and coworkers on the current status of structure-based computer-aided drug design tools for boron-cluster-containing protein ligands concludes this first section.

In the second section, the focus is on "Boron Compounds in Drug Delivery and Imaging". Satish S. Jalisatgi, a collaborator of Frederick Hawthorne, who was the pioneer of boron cluster chemistry almost 60 years ago, gives an overview of closomer drug delivery platforms based on an icosahedral polyhedral borane scaffold (Chapter 2.1). The resulting monodisperse nanostructures are capable of performing a combination of therapeutic, diagnostic, and targeting functions, which is highly useful for emerging applications. A complementary approach is described in the review by Clara Viñas Teixidor (Chapter 2.2), one of the founders of EuroBoron conference, and her colleagues. The anionic boron-based cobaltabis(dicarbollide) can form atypical monolayer membranes with the shape of vesicles and micelles with similar dimensions to those seen in nature, but of a very different chemical composition. These vesicles interact with liposomes and biological membranes to accumulate inside living cells. Their particular properties offer new opportunities for the development of nanoscale platforms to directly introduce new functionality for use in cancer therapy, drug design, and molecular delivery systems.

Diabetes is a chronic disease that has devastating human, social, and economic consequences. A tight control of blood glucose is the most important goal in dealing with diabetes. The majority of blood glucose monitoring tools relies on the glucose

oxidase enzyme (GOx), but they have some drawbacks. A powerful approach for detecting glucose in fluids is the development of boronic acid-based saccharide sensors. The main principles of their design and factors governing their selectivity are discussed by Igor B. Sivaev and Vladimir I. Bregadze in Chapter 2.3.

Drug development is a lengthy process requiring identification of a biological target, validation of the target, and development of pharmacological agents designed and subsequently confirmed by *in vivo* studies. Molecular and functional imaging applied in the initial stages of drug development can provide evidence of biological activity and confirm on-target drug effects. In their contributions, Bhaskar C. Das *et al.* focus on various boron-containing molecular probes used in molecular imaging (Chapter 2.4), and Jordi Llop *et al.* provide an overview of nuclear imaging techniques, as well as the different radiolabeling strategies reported so far for the incorporation of positron and gamma emitters into boron clusters (Chapter 2.5). Finally, some illustrative examples on how radiolabeling and *in vivo* imaging can aid in the process of drug development are described, focusing on BNCT drug candidates containing boron clusters, linking this chapter to the third section dedicated to "Boron Compounds for Boron Neutron Capture Therapy".

Cancer is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015. Treatment typically comprises surgery, radiotherapy, and chemotherapy. BNCT is a unique binary therapy that was developed during the last five to six decades. With the availability of accelerator-based neutron sources at clinics, selective boron compounds for use in BNCT will become very important. In this third section, several novel classes of potential BNCT agents are described. Werner Tjarks critically reviews aspects of the design, synthesis, and biological evaluation of 3-carboranyl thymidine analogs (3CTAs) as boron delivery agents for BNCT over a time span of approximately 20 years (Chapter 3.1). Potential future non-BNCT applications of 3CTAs are also discussed, linking this review to the first section on boron-based drug design. Maria da Graça H. Vicente and Sunting Xuan describe different classes of third-generation boron delivery agents with enhanced tumor-localizing properties, which are under investigation for use in BNCT (Chapter 3.2), and the contribution by Valentina A. Ol'shevskaya and colleagues deals with synthetic approaches leading to tumor-selective boronated porphyrins and chlorins with potential applications in diagnosis, drug delivery, and treatment. This study emphasizes the role of boron in rendering the photoactivatable tetrapyrrolic scaffolds more potent in photodynamic therapy (Chapter 3.3). A highly innovative approach is described in the review by Narayan Hosmane, one of the founders of Boron in the Americas (BORAM), and his coworkers covering the recent developments in the use of nanoparticles as adjuncts to boron-containing compounds in BNCT, involving boron nanotubes (BNTs) and boron nitride nanotubes (BNNTs) (Chapter 3.4). For further implementation of BNCT at the clinical level, new specifically targeted boron carriers for BNCT, conjugated with functional groups detectable by highly sensitive imaging tools, are required. This allows the determination of the local boron concentration, which is crucial to personalize the treatment for each patient. Simonetta Geninatti Crich and coworkers cover this important topic in Chapter 3.5. Furthermore, *in vivo* research in appropriate animal models is important to expand BNCT radiobiology and optimize its therapeutic efficacy for different pathologies. This highly interdisciplinary topic is covered by Amanda E. Schwint and coworkers in their comprehensive contribution in Chapter 3.6.

We are very grateful to all the authors for their contributions and their patience. Last but not least, we would like to thank the Wiley team, especially Sarah Higginbotham and Emma Strickland, for their continuous support in planning and compiling this book, which gives a timely overview of the evolving potential and emerging applications of boron-based compounds in medicine.

Evamarie Hey-Hawkins and
Clara Viñas Teixidor

Part 1

Design of New Boron-based Drugs

1.1

Carboranes as Hydrophobic Pharmacophores: Applications for Design of Nuclear Receptor Ligands

Yasuyuki Endo

Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, Sendai, Japan

1.1.1 Roles of Hydrophobic Pharmacophores in Medicinal Drug Design

A pharmacophore is a partial structure in which important functional groups and hydrophobic structure are arranged in suitable positions for binding to a receptor [1]. Typically, hydrophilic functional groups of the pharmacophore interact with the receptor by hydrogen bonding and/or ionic bonding, and the hydrophobic structure interacts with a hydrophobic surface of the receptor. While hydrogen bonding plays a key role in specific ligand–receptor recognition, the hydrophobic interaction between receptor and drug molecule is especially important in determining the binding affinity. The difference of binding constants between a ligand having a suitable hydrophobic group and a ligand without such a group can be as large as 1000-fold. In medicinal drug design, the hydrophobic structures are often composed of aromatic and heteroaromatic rings, which also play a role in fixing the arrangement of functional groups appropriately for binding to the receptor. On the other hand, three-dimensional hydrophobic structures are not yet widely used in drug design, even though they could be well suited for interaction with the three-dimensional hydrophobic binding pockets of receptors. It is noteworthy that various steroid hormones target distinct steroid hormone receptors owing to differences of functionalization of the hydrophobic steroidal skeleton. The binding of the natural ligand 17β-estradiol to human estrogen receptor-α (ERα) is illustrated in Figure 1.1.1 as an example. The large number of steroid hormones may be a consequence of evolutionary diversification of the functions of the steroidal skeleton. In this context, we aimed to establish a new three-dimensional hydrophobic skeletal structure for medicinal drug design.

Boron-Based Compounds: Potential and Emerging Applications in Medicine, First Edition.
Edited by Evamarie Hey-Hawkins and Clara Viñas Teixidor.

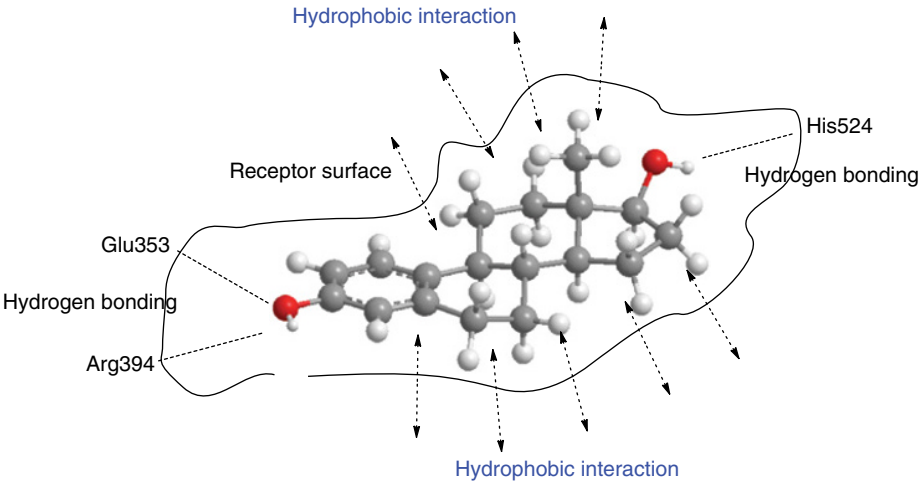

Figure 1.1.1 Interactions of ligand with receptor (example for 17β-estradiol with estrogen receptor-α).

1.1.2 Carboranes as Hydrophobic Structures for Medicinal Drug Design

In the past three decades, there has been increasing interest in globular molecules. In the 1980s, dodecahedrane, which consists of sp^3 carbons, was synthesized [2]; and in the 1990s, the chemistry of fullerene C_{60}, which also consists of sp^2 carbons, was explored [3]. However, the former is not easy to synthesize, while the latter molecule may have limited application because of its large molecular size. On the other hand, icosahedral carboranes [4] are topologically symmetrical, globular molecules, and have been known for more than half a century. The B–B and C–B bonds of 12-vertex carboranes are approximately 1.8 angstroms in length, and the molecular size of carboranes is somewhat larger than adamantane or the volume of a rotated benzene ring. Carboranes have a highly electron-delocalized hydrophobic surface, and are considered to be three-dimensional aromatic compounds [5] or inorganic benzenes. The structures of these compounds are illustrated in Figure 1.1.2. But, although the use of boron derivatives for boron neutron capture therapy (BNCT) of tumors has a long history [6], relatively little attention has yet been paid to the possible use of carboranes as components of biologically active molecules, despite their desirable hydrophobic character, spherical geometry, and convenient molecular size for use in the design and synthesis of medicinal drugs.

Carboranes have three isomers, *ortho*-, *meta*-, and *para*-carboranes (Figure 1.1.2), and their rigid and bulky cage structures hold substituents in well-defined spatial relationships. The two carbon atoms of carboranes have relatively acidic protons, which can readily be substituted with other organic groups [7]. Substituents can also be introduced selectively at certain boron atoms, to construct structures having three or more substituents, as illustrated in Figure 1.1.3 [8]. Carbocyclic skeletons often rearrange under acidic conditions, whereas carborane cage skeletons do not rearrange even in the presence of strong Lewis acids. Adamantane and bicyclo[2,2,2]octane are also available as hydrophobic skeletons, and substituents can readily be introduced at bridgehead carbons of adamantane, but selective introduction at other carbons is difficult, and chirality is also an issue.

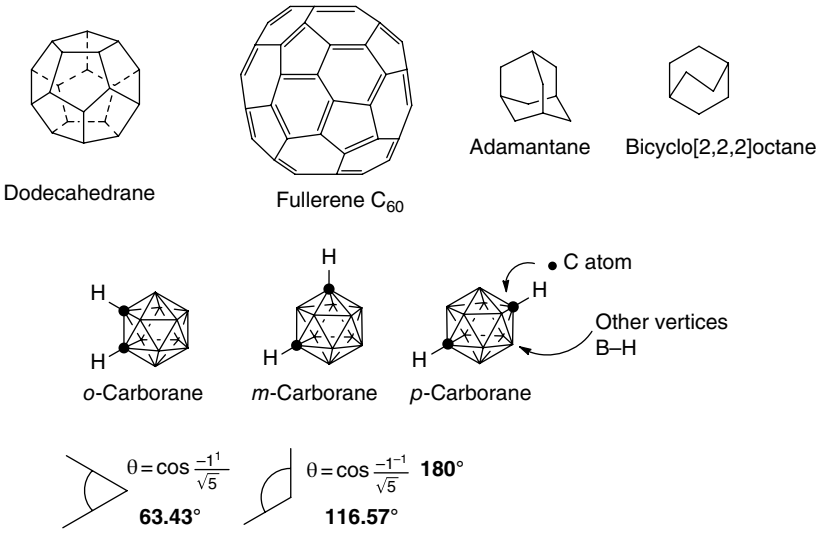

Figure 1.1.2 Structures of globular molecules and characteristics of carboranes.

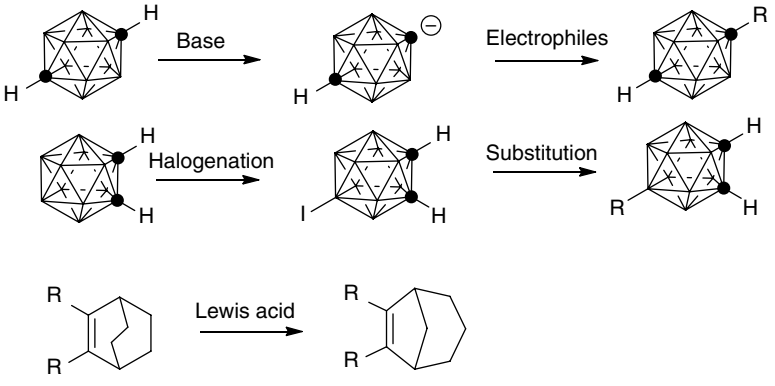

Figure 1.1.3 Advantages of carborane skeleton for synthesis.

1.1.3 Estrogen Receptor Ligands Bearing a Carborane Cage

1.1.3.1 Estrogen Agonists

Estrogen mediates a wide variety of cellular responses through its binding to a specific estrogen receptor (ER). The hormone-bound ER forms an active dimer, which binds to the ER-responsive element of DNA and regulates gene transcription. Endogenous estrogen, such as 17β-estradiol, plays an important role in the female reproductive system, and also in bone maintenance, the central nervous system, and the cardiovascular system. Recent studies on the three-dimensional structure of the complex formed by estradiol and the human ERα ligand-binding domain have identified the structural requirements for estrogenic activity [9]. 17β-Estradiol is oriented in the ligand-binding

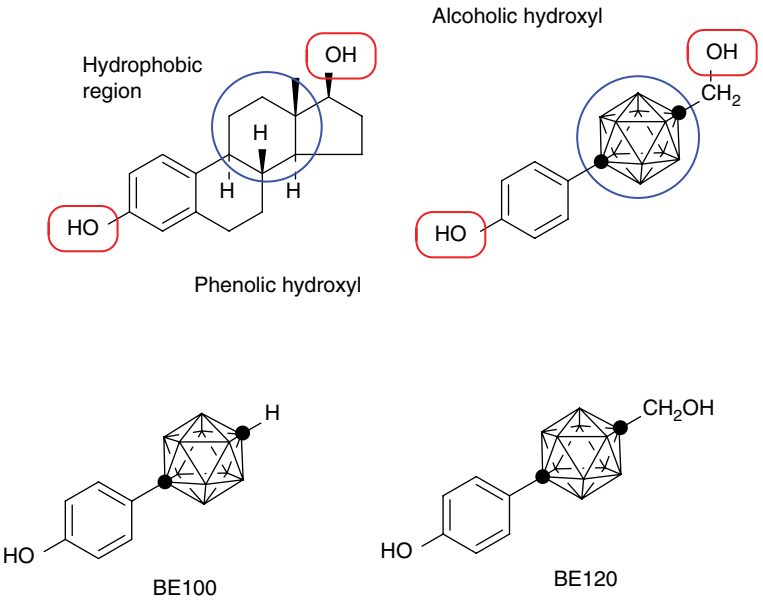

Figure 1.1.4 Structures of β-estradiol and designed molecule bearing *p*-carborane.

pocket by two types of contacts: hydrogen bonding from the phenolic hydroxyl group to Glu353 and Arg394, and from the 17β-hydroxyl group to the nitrogen of His524, and hydrophobic interaction along the body of the skeleton (see Figure 1.1.1). Therefore, we designed a simple compound with a 4-phenolic residue and a hydroxymethylated *p*-carborane, together with some derivatives (Figure 1.1.4) [10].

The estrogenic activities of the synthesized compounds were examined by means of receptor binding assays. Surprisingly, the simple 4-(*p*-carboranyl)phenol BE100 exhibited potent ERα-binding affinity, comparable with that of estradiol, and the most active compound, BE120, was several times more potent than estradiol. In transcriptional assay, the simple 4-(p-carboranyl)phenol BE100 exhibited potent agonistic activity, comparable with that of estradiol. The activity was increased by the introduction of a hydroxylmethyl group onto carbon of the carborane cage, and the resulting compound, BE120, was at least 10-fold more potent than estradiol. In a docking simulation of BE120 with the receptor based on the crystal structure of the estradiol–ERα complex, the phenolic hydroxyl group and hydroxymethyl group of BE120 appeared to play similar roles to those in the case of estradiol. The higher activity of BE120 suggests that the carborane cage binds to the hydrophobic cavity of the receptor more tightly than does the equivalent structure of estradiol [11].

BE120 also showed potent *in vivo* effects. Uterine atrophy due to estrogen deficiency or ovariectomy is blocked by estrogen administration, and this forms the basis of a typical *in vivo* assay for estrogenic activity. Estradiol and BE120 at 100 ng per day both restored the uterine weight, indicating that BE120 reproduces the biological activity of estradiol. Similarly, decrease of the bone mineral density of ovariectomized mice was blocked by administration of either estradiol or BE120, with similar potency [12].

1.1.3.2 Estrogen Antagonists and Selective Estrogen-Receptor Modulators (SERMs)

Since estrogen agonists increase the risk of carcinogenesis in breast and uterus [13], estrogen antagonists can be used as anticancer agents. On the other hand, estrogen agonists may be useful for the control of osteoporosis, if the risk of carcinogenesis can be avoided. Therefore, there is great interest in SERMs that selectively affect different organs, especially agents with agonistic activity in bone, but no effect or antagonistic activity in the reproductive organs. Among SERMs so far developed, tamoxifen is used to treat breast cancer [14], and raloxifene to treat osteoporosis [15].

The balance of activities depends on the precise ligand–receptor complex structure, which influences subsequent binding with co-factors and other proteins, leading to different physiological actions. In the case of tamoxifen [14], the bulky dimethylaminoethoxyphenyl group plays a key role in the antagonistic activity. Taking this into account, we designed compounds containing *o*- and *m*-carborane skeletons, as shown in Figure 1.1.5.

The *o*-carborane derivative BE362 inhibited the activity of estradiol in the concentration range of 10^{-7} M in a transcriptional activity assay, being equipotent with tamoxifen. The *m*-carborane derivative BE262 was somewhat less potent than BE362. In this assay, synthetic intermediate BE360 also exhibited antagonistic activity, although its potency was somewhat weaker than that of BE362 [16]. In spite of its very simple structure, BE360 exhibited strong binding affinity for ER [17]. Therefore, we focused on BE360 as a candidate SERM. Loss of bone mineral density of ovariectomized mice was blocked by administration of BE360 at 1–30 mg/day [18]. BE360 was 1000-fold less potent than estradiol, but was almost equipotent with the osteoporosis drug raloxifene. On the other hand, BE360 did not affect uterine weight at this concentration. Thus, BE360 is a promising lead compound for development of therapeutically useful SERMs.

We next investigated structural development of BE360. Insertion of a methylene group (BE380) changed the partial agonist–antagonist character of BE360 to weak agonist, and insertion of two methylene units generated a potent antagonist (BE381). Replacing the carborane cage with a bicyclo[2,2,2]octane skeleton caused a drastic change of biological activity, affording a potent full agonist (BE1060). It seems clear that altering the three-dimensional hydrophobic core structure is a promising strategy for control of the agonist–antagonist activity balance toward ER [19].

In addition, we have recently reported that BE360 has antidepressant and antidementia effects through enhancement of hippocampal cell proliferation in olfactory bulbectomized mice [20]. Thus, BE360 may have potential for treatment of depression and neurodegenerative diseases, such as Alzheimer's disease.

1.1.4 Androgen Receptor Ligands Bearing a Carborane Cage

1.1.4.1 Androgen Antagonists

Like estrogen, androgen mediates cellular responses through binding to a specific androgen receptor (AR). The hormone-bound AR forms a dimer, which binds to the AR-responsive element of DNA and regulates gene transcription. Endogenous androgen, such as testosterone and dihydrotestosterone, plays an important role in the

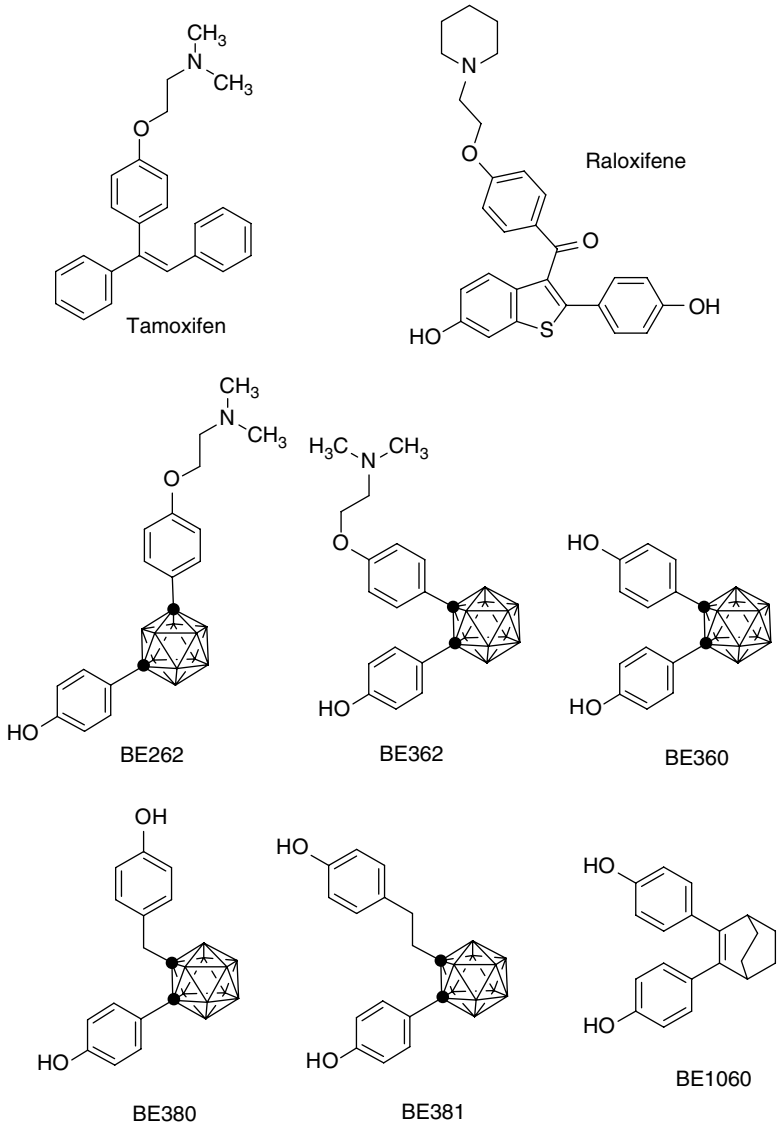

Figure 1.1.5 Structures of selective estrogen receptor modulators: tamoxifen and raloxifene, and designed molecules bearing carborane.

male reproductive system, and also in prostate enlargement, body hair growth, and muscle development. The X-ray structure of the complex of AR ligand-binding domain with an androgen agonist has been reported [21]. The overall structure of the ligand-binding domain is very similar to that of ER, but there are differences in the structures surrounding the ligand-binding pocket. One of the differences between AR and ER ligands is that the aliphatic cyclohexene A-ring of the steroid skeleton bears an 18-methyl group, so that the structure is bulky compared with the flat aromatic A-ring of estrogen. In addition, a ketone is present instead of the phenolic hydroxyl group in