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Medicinal Chemistry of Boron-Bearing Compounds for BNCT-Glioma Treatment: Current Challenges and Perspectives

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Abstract

Since its first description, boron neutron capture therapy (BNCT) was a special type of radiotherapy for treatment of cancer and with focus mainly on glioma therapeutic. This procedure requires the selective accumulation of boron into the tumoral cells, and due to this requirement, different boron-enriched compounds have been designed and developed. Efforts to circumvent the selectivity-uptake challenge and other problems, such as solubility, stability, and toxicity, have been to driving force behind the medicinal chemistry field in boron-based compounds. In this regard, a wide diversity of medicinal chemistry hypothesis has been used to obtain new and efficient potential BNCT-glioma drugs. In this chapter, these ideas are analyzed focusing on their medicinal chemistry characteristics.

Keywords: glioma, glioblastoma, drug discovery, boron neutron capture therapy, boron-bearing compounds

1. Introduction

1.1. Boron neutron capture therapy: historical account

In 1932 in the UK, Chadwick discovered neutrons, and for his contribution, he was awarded in 1935 with the Nobel Prize in Physics [1]. One year later in the USA, Gordon Locher introduced the concept of boron neutron capture therapy (BNCT) [2]. He hypothesized that if boron could be selectively concentrated in a tumoral tissue and then exposed to a neutrons

beam, a higher radiation dose to the tumor relative to surrounding normal cells would result. A mere 2 years later, Goldhaber, Hall, and Kruger performed the first radiobiological studies using boric acid and slow neutrons in a murine tumoral model [3]. However, the first clinical trials against human brain tumors (glioblastoma multiforme (GBM)) that used BNCT could not be initiated until 1951 at the Brookhaven National Laboratory in collaboration with the Massachusetts General Hospital and the Massachusetts Institute of Technology (MIT) [4, 5]. In this case, ten patients were treated with borax (disodium tetraborate decahydrate, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$; **Figure 1**) and thermal neutrons without much success. While there were no serious side effects of BNCT in the patients, the large doses of borax (^{10}B -enriched) infused 200 mg/kg, inducing slight toxicity symptoms. In order to improve this, a second approach was developed comprising nine glioma patients but now with a less toxic compound, sodium pentaborate (NaB_5O_8 ; **Figure 1**) in combination with D-glucose. Unlike the first one, a higher dose of ^{10}B was used, and a higher fluency of incident thermal neutron was applied [6]. Unfortunately, again, serious side effects such as radio-dermatoses of the scalp and deep ulcerations were observed [6, 7]. Simultaneously, in 1963, Sweet and co-workers, from the MIT, treated 18 patients using boron-rich disodium decahydrodecaborate ($\text{Na}_2\text{B}_{10}\text{H}_{10}$; **Figure 1**) [8], which was considered to be less toxic and with the ability to deposit more boron atoms per cell. Symptoms of brain necrosis in patients undergoing BNCT were again observed [9]. Due to these disappointing events, the USA halted the progress of research on BNCT in 1961.

On the other hand, in 1968 at Hitachi training reactor, the Japanese neurosurgeon Hiroshi Hatanaka, who in previous years had worked with Sweet in Boston, began treating patients with high-grade malignant gliomas using disodium mercaptoundecahydro-*closo*-dodecaborate ($\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$, named as BSH; **Figure 1**) which originally had been synthesized by Soloway in 1967 [10]. The results reported by Hatanaka and co-workers were extraordinary with a 5-year survival rate of 58% [11–13].

From the 1990s to the present day with the development of new boron compounds and the improvement in radiation source, the boron neutron capture therapy has been expanded to several centers worldwide, among them in the USA at Brookhaven and Cambridge, in the Netherlands at high flux reactor in collaboration with the Department of Radiotherapy of the University of Essen in Germany, in Finland at FiR-1 Otaniemi reactor, in Sweden at R2-R0 reactor, in the Czech Republic at LVR-15 reactor, in Italy at TRIGA reactor, in Japan at Kyoto University Research Reactor Institute, in Argentina at RA-6 and RA-3 reactors, and in Taiwan at THOR reactor, just to name a few [14].

1.2. Boron neutron capture therapy: principles and general requirements

BNCT is considered as a rationale and promising binary therapy modality for treatment of several cancers in particular malignant gliomas. The cell-killing effect of BNCT is based on the nuclear reaction $^{10}\text{B}(n,\alpha)^7\text{Li}$ (**Figure 2**) that occurs when the nuclide ^{10}B , which is a non-radioactive constituent of natural elemental boron (approximately 20% abundance), is irradiated with neutrons of the appropriate energy, thermal neutrons. The nuclear reaction yields excited boron-11 ($^{11}\text{B}^*$) that after instantaneous nuclear fission produces two high-linear energy transfer entities, i.e., α -particle ($^4\text{He}^{2+}$) and recoiling lithium-7 nucleus ($^7\text{Li}^{3+}$). Because of the very short track length of these heavy particles (<10 μm ; roughly one cell diameter), radiation damage is confined to those cells loaded with ^{10}B .

The probability that a nuclide captures a neutron is measured by the neutron capture cross section, σ_{th} , having ^{10}B a value of $\sigma_{th} = 3838$ barns [15]. However, other abundant endogenous nuclei present in the healthy tissue, such as ^1H and ^{14}N , could also capture neutrons yielding after nuclear reactions of a gamma ray in the first case, $^1\text{H}(n,\gamma)^2\text{H}$, and a proton in the second one, $^{14}\text{N}(n,p)^{14}\text{C}$. However, the σ_{th} of these nuclei is smaller than the value for ^{10}B , i.e., $\sigma_{th,1\text{H}} = 0.332$ and $\sigma_{th,14\text{N}} = 1.82$ barns, and the amount of radiation produced by these nuclear reactions is lesser than the produced by the particle and recoiling nucleus in the case of boron [15].

On the other hand, for brain tumor such as GBM, usually higher energy epithermal neutron beams which have a greater depth penetration being thermal neutrons unable to act on tumors located below the tissue surface because of scattering effects have been used. Epithermal neutrons do not suffer from the disadvantages of H-recoil processes and, consequently, allow capture reactions to occur at some distance within the tissue; then, epithermal neutrons are progressively slowed into thermal neutrons through heat-releasing interactions with the hydrogen atom and constituents of biological system, that do not cause damage to the tissue [16].

In order for BNCT to be successful, the ^{10}B -loaded agent must completely fulfill some overriding conditions, namely, (a) selective uptake by tumor tissue relative to normal tissue (preferably accumulating within specific tumoral cell substructure) with ideal tumor:normal tissues and tumor:blood ratios of 3:1 and 5:1, respectively, and (b) appropriate amount of ^{10}B delivered to the tumor tissue, i.e., at least $20 \mu\text{g } ^{10}\text{B/g}$ tumor, corresponding to about 10^9 atoms of $^{10}\text{B}/\text{cell}$.

Figure 1. First and current available boron-based drugs for BNCT.

Figure 2. The two parallel nuclear fission reactions that occur upon capture of a slow (thermal) neutron by a ^{10}B nucleus.

However, this amount could be lower if the boron delivery system is concentrated in or near the cell nucleus; (c) retention of ^{10}B in tumor during the BNCT process; (d) rapid clearance from blood and healthy tissues; (e) and adequate lipophilicity especially for glioma treatment where the drug should be able to cross blood-brain barrier (BBB) [17]. Furthermore, like any drug in medicinal chemistry, the ^{10}B -loaded agent must meet the following requirements: (f) absence of systemic toxicity, (g) chemical and metabolic stability, and (h) appropriate water solubility.

1.3. Boron neutron capture therapy: current therapeutic agents

After the first efforts, during the 1940s and 1950s (see Section 1.1.), the lack of selectivity and low boron tumor accumulation observed for the simplest boron salts (**Figure 1**) used until the moment prevented their application in BNCT clinical trials. However, around the 1960s, the first studies of the two compounds currently in clinical began, both ^{10}B -enriched, the polyhedral borane BSH (**Figure 1**) and L-4-dihydroxyborylphenylalanine, known as L-boronophenylalanine (L-BPA; **Figure 3**) [18], which could be accumulated into desired tissues for its structural analogy to some biomolecules.

Due to BSH is a small hydrophilic molecule (**Figure 1**), it does not cross the intact BBB. It only penetrates into the brain passively when the BBB is disrupted [10], as it is observed in the GBM. Although BSH has been applied for the treatment of GBM in infusions with no toxic effects, the efficacy has been limited due to low observed tumor:brain (3:1) and tumor:blood (0.9–2.5:1) ratios [19]. The main structural advantage of BSH compared to L-BPA is that BSH contains 12 times more B per molecule yielding a higher number of events after neutron capture than in L-BPA. BSH has been studied in different therapeutic schedules, combined or not with other small molecules, like L-BPA, or vehicles looking for the improvement of the

Figure 3. (A) Currently, available boronic acid for treatment of GBM through BNCT. (B) L-BPA-F complex. (C) Esterification of L-BPA with ethylene glycol.

efficacy and the delivery into the glioma [20]. Medicinal chemistry on BSH, structural modifications, has also been done (see below) seeking better biological behavior.

Nowadays, L-BPA (**Figure 3**) is the standard therapeutic drug used in BNCT [21–23]. Since the L-amino acid transport system is highly expressed in tumor cells compared with normal cells in most organs including the brain, some natural amino acid boron derivatives have been studied [24]. L-BPA has very limited water solubility (1.6 g/L), and searching to circumvent this problem, the standard strategy used for clinical BNCT treatment, it is as a soluble fructose complex, known as L-BPA-F (**Figure 3**), which leads to a pharmaceutical product with favorable biodistribution in human GBM, ratios tumor: blood of 3–4:1 [25], low toxicity, and good capability to cross BBB. Other two strategies include (a) the transformation into the corresponding hydrochloride salt and (b) the esterification of the boronic acid moiety with 1,2- and 1,3-diol producing 1,3,2-dioxaborolanes or 1,3,2-dioxaborinanes, respectively, which is then easily hydrolysable in an aqueous environment (**Figure 3**) [26, 27]. On the other hand, L-BPA is actively transported across the tumor cell membrane, by the L-amino acid transporter system. It is highly expressed in tumor cells, including the brain, compared with normal tissues and can be stimulated by the previous accumulation of the L-DOPA resulting in a substrate-coupled antiport (exchange) mechanism [28]. At this point, L-BPA is considered to be a better B delivery agent than BSH.

2. Medicinal chemistry of boron-bearing compounds for BNCT

2.1. General

From a medicinal chemistry point of view, different strategies have been studied in order to identify new and more selective molecules to glioma cells, with adequate ability to cross the BBB, with higher tumor concentration in the path of the neutron beam and drug-like properties. The third-generation products, which potentially may accumulate into glioma for its structural analogy to some biomolecules, could be classified [15] in (a) macromolecular species and (b) low molecular weight molecules. In reference to the first group, we could mention monoclonal and bispecific antibodies, epidermal growth factor, and encapsulating agents such as boron-containing nanovehicles (liposomes). Here, we will discuss compounds belonging to the second group, like polyhedral boron cluster derivatives, boronic acid derivatives, and other boron-containing small molecules (e.g., oxaborolanes, dioxaborolanes, and azaboro-heterocycles, among others).

2.2. Polyhedral boron clusters

2.2.1. Implications for drug discovery: structural features of *closo*-carboranes and metallocarboranes. How do they influence on the drug-like properties?

The most known and commonly used class of polyhedral boron compounds in the medicinal chemistry field are the icosahedral dicarba-*closo*-dodecaborane ($C_2B_{10}H_{12}$) commonly referred to as carboranes which exist in three isomeric forms named with respect to the positioning of the two CH vertices (**Figure 4**): 1,2- or *ortho*- (**1**); 1,7- or *meta*- (**2**); and 1,12- or *para*-carborane (**3**); to a lesser extent, their mono-anionic derivatives resulting from the loss of a B vertex, commonly

known as *nido*-carborane (**4** $[\text{C}_2\text{B}_9\text{H}_{12}]^-$); and their metal complexes known as metallacarborane (**5** $[\text{M}(\text{C}_2\text{B}_9\text{H}_{11})_2]^-$, where M is a metal) that are generated after removal of the bridge hydrogen from the *nido*-carborane (**Figure 4**) [29, 30].

Among the outstanding and widely explored properties of carboranes and metallacarboranes for medicinal chemistry research are (a) the geometry and the electron-deficient nature of the boron atoms, which generate a strong hydride character in the BH shell, which are some of the main features that determine the intermolecular interactions with the biological targets because they make the B-clusters extremely hydrophobic; (b) both the pharmacokinetics and the bioavailability can be modulated according to the chemotype of boron cluster selected, so the hydrophilicity and lipophilicity, or both, could be tuned; (c) the globular architecture and rigid geometry allow for molecular construction in three dimensions improving the docking, or not, with bio-targets; (d) high boron content per molecule and stability to catabolism are important criteria for the development of agent for BNCT; and (e) the well-established chemistry that makes boron clusters attractive synthons to construct novel pharmaceuticals [31].

Nevertheless, some problems persist today that delay the application of boron clusters in the development of new drugs: (a) the relatively high cost of carboranes and their derivatives even more if they will be used in BNCT because ^{10}B -enriched compound will be needed; (b) the difficulty of *in silico* drug design and screening of boron cluster drugs, due to the lack of appropriate descriptors for the interaction potentials of boron and the attached hydrogen atoms; and (c) the lack of libraries of boron cluster compounds for high-throughput screening.

2.2.2. *Closo*-carboranes and metallacarboranes designed and studied for BNCT-glioma treatment

In the past decade, saccharides have been widely studied due to their low toxicities, generally having high water solubility, high expression of specific receptor on the tumor cell surface (specifically in the brain capillary endothelial cells that form the brain barriers, resulting in optimal BBB penetration), and an increased rate of glycolysis in cancer cells. According to this and in order to improve boron uptake, carbohydrates such as ribose, mannose, maltose, and galactose have been chosen to generate carbohydrate-based boron delivery platforms for successful BNCT approach [32, 33]. Since the first description by Hawthorne's group [34], there have been numerous works on the synthesis and biological evaluation of carboranyl-carbohydrate conjugates. Later, Tietze and co-workers developed and evaluated both *in vitro* and *in vivo*, against rat glioma cells (C6) and melanoma cell line (B16), a series of carboranyl glycosides including glucoside, lactoside, and maltoside conjugates (**6–8**; **Figure 5**) [35–37]. Although in the *in vitro* assays significant killing effect was observed because the three derivatives showed an optimum cellular uptake in the C6 glioma cell line, with maltoside **8** being the derivative that exhibited the highest uptake (65.7 ppm at 12 h), the *in vivo* performance in rat model bearing brain tumor revealed that the concentration of all carboranyl-appended carbohydrate in blood was maintained at levels that were unacceptably high for meaningful use in BNCT. In order to overcome this drawback, Tietze and Yamamoto designed a new type of mixed carboranyl bisglycoside derivatives as prodrugs (**9–12**; **Figure 5**) [38]. Initially, selective uptake of these compounds was not expected due to the hydrophilic sugar moieties at both ends of the carborane preventing cell internalization. The authors demonstrated that the activation of the prodrug could be performed using monoclonal antibodies conjugated

Figure 4. (A) Numbering and nomenclature of the *closo*-carborane systems. Synthesis of *meta* and *para*-carborane derivatives from the *ortho*-carborane isomer through thermal isomerization. (B) Partial degradation of *ortho*-, *meta*-, and *para*-carborane and numbering of nido-carborane system according to IUPAC. (C) Synthesis of metallocarborane from nido-carborane.

to glycohydrolases, which bind to tumor-associated antigens, through the glucosidic bond cleavage and concomitant release of carboranyl moiety in the tumor cell surface.

On the other hand, in the last few years, boron-bearing purines, pyrimidines, thymidines, nucleosides, and nucleotides have been widely explored as a novel approach to improve boron uptake in glioma tumor cells. This strategy is based on the fact that tumor cells have higher metabolic activity and an increased requirement for DNA and RNA precursors [39, 40]. Although in recent years several strategies have been addressed, the main focus has been on thymidine analogs substituted with *ortho*-carbonyl cluster at the *N*-3 positions (**13** and **14**, named as 3CTAs; **Figure 6**). Both analogs are potential substrates for human thymidine kinase 1 (hTK1), a cytosolic deoxynucleoside kinase of the DNA synthesis salvage pathway that is predominantly found in proliferating cancer cells. The selective accumulation and retention of 3CTAs in tumor cells via a mechanism known as kinase-mediated trapping (KMT) render these molecules as potential BNCT delivery agents against high-grade brain glioma, such as GBM [41–43]. Another considerable attention has also been to metallocarborane, mainly the bis-(dicarbollyl)-cobalt and bis-(dicarbollyl)-iron derivatives (**15–17**, **Figure 6**). They can also be selectively accumulated in rapidly multiplying neoplastic cells; following their conversion to the corresponding nucleotides, trapped within the cell; or, ideally, incorporated into nuclear DNA of tumors [44]. Despite this, these compounds still have not studied in gliomas.

Barth and co-workers evaluated ¹⁰B-enriched derivative **14**, using the RG2 model as in vivo brain tumor model [45]. First, they demonstrated that derivative **14** efficiently delivers boron atoms in cancer cell, which allowed tumor reduction after BNCT in nude mice bearing tumor induced with TK1 positive cell. In addition, based on these favorable results, BNCT studies carried out in the RG2 rat model lead to an increased in life span (ILS) 2.4× in comparison with L-BPA as control therapy. Nevertheless, the greatest percent ILS (122%) was seen in RG2 glioma-bearing rats that received the combination of derivative **14** and

L-BPA, and this correlated with the fact that the tumor in these animals received the highest physical radiation doses. These biological studies clearly revealed the therapeutic potential of derivative **14** although some problems and limitation appeared. Among these, derivative **14** showed clear solubility problems under physiological conditions, possibly due to the presence of the carboranyl hydrophobic core and the absence of any functional groups that can be ionized.

Since the first description by Haushalter and Rudolph in 1978 (**18** and **19**; **Figure 7**) [46, 47], the potential for medical application of several boron cluster-containing fluorescent dyes, including porphyrins and related macrocyclic, have been highly explored and scrutinized for several reasons, among them: (a) well-known ability to selective accumulate into tumor cells in high amounts, (b) subsequent persistence within tumors, (c) general low dark cytotoxicity, (d) capability to bind to DNA due to their plane aromatic structure, and (e) highly fluorescence, thus enabling tumor diagnosis and facilitating treatment planning. Nonetheless, without any doubt, the most exploited feature of these molecules is the ability to act in photodynamic therapy (PDT). PDT combines a photosensitizer (porphyrin), light, and tissue oxygen, to generate reactive oxygen species, including singlet oxygen, triggering subsequently cell death mechanisms by necrosis and/or apoptosis. The combination of this therapy with BNCT has been of particular interest to control local recurrence of high-grade gliomas such as GBM, because they target different mechanisms of tumor cell death and, thus, increase the

Figure 5. (A) Compound **6** corresponds to carboranyl derivative of D-glucose, and compounds **7** and **8** are derivatives of lactose and maltose, respectively. (B) *Ortho*-carboranyl bisglycosides **9–12** containing lactose, glucose, mannose, and galactose in their structures.

therapeutic effect. Both are bimodal therapies, the individual components of which are non-toxic in isolation, but which are tumoricidal in combination.

In the last few years, a large number of boron-containing natural porphyrins have been synthesized and evaluated both in cellular and animal models (20–23; **Figure 8**) [48]. To date, the tetrakis-carborane carboxylate ester of 2,4-bis(α,β -dihydroxyethyl)deuterioporphyrin IX (22, known as BOPP) was the only compound to be employed in a phase I clinical study against GBM [49, 50]. Pharmacological studies showed its ability to selectively incorporate in tumor cells, in xenograft models of glioma, relative to the surrounding normal brain cells (tumor:brain ratio as high as 400) and situated preferentially in tumor cell mitochondria. Nevertheless, these results could not be replicated in humans.

Figure 6. Chemical structure of the main carborane-bearing nucleoside delivery agents.

Figure 7. First boron-containing porphyrins developed by Haushalter and Rudolph for catalysis application.

The success of derivative **22** was interrupted in a phase I clinical trial for several reasons: (a) it could not deliver to the tumor a therapeutic amount of boron in patients with GBM; (b) thrombocytopenia was observed in patients due to the direct toxic effect of derivative **22** or its metabolites (probably of carboxylic carborane) on platelets; and (c) the pharmacokinetic behavior of derivative **22** in humans was characterized by a prolonged clearance phase, giving rise to potentially toxic metabolites and cutaneous photosensitivity [51].

In order to overcome this drawback, several researchers designed and synthesized a highly boron water-soluble porphyrins as a possible BNCT agents. In this sense, Vicente and co-workers described *nido*-carborane cluster-linked porphyrins via aromatic linkage (e.g., **24**; **Figure 8**). These amphiphilic derivatives showed very low toxicity, were taken up by 9 L and U-373MG cells in both time- and concentration-dependent manners, and were localized preferentially in cell lysosomes [52]. On the other hand, Deen and co-workers developed the polyboronated ionic porphyrin **25**, known as TABP-1, which was administrated by convection-enhanced delivery (CED) to nude rats bearing intracerebral implant of human glioblastoma cell line U-87MG [53]. In contrast to diffusion, CED uses a pressure gradient established at the tip of an infusion catheter to establish bulk flow and distribute drug and solvent throughout the extracellular space. This demonstrated high tumor and low blood boron concentration through intracerebral administration by CED related to systemic administration.

Carborane-containing nucleosides and analogues as the means to concentrate boron within the tumor cell nucleus were described. However, another class of derivatives, which could interact directly to chromosomal DNA, has been developed and widely explored for boron neutron capture therapy. Among this the following could be mentioned (**Figure 9**): (a) alkylating agents (i.e., **26** and **27**), (b) DNA intercalators (i.e., **28** and **29**), (c) minor groove binders (i.e., **30** and **31**), and (d) cationic polyamines (i.e., **32** and **33**) [54]. Regarding the last group of compounds, it should be stated that natural polyamines such as spermidine, spermine, and putrescine, found in both prokaryotic and eukaryotic cell types, are a class of biologically active compounds known to be essential for every cell to support their function such as growth and differentiation. In addition, because they have a specific transportation system, they have been found in high concentration in rapidly proliferating tumor cells. This characteristic was used by Zhuo and co-workers, synthesizing and evaluating derivatives **32** and **33** [55]. These compounds proved to be highly water-soluble, in vitro they have the ability to interact to calf thymus DNA, and they are rapidly taken up by F98 rat glioma cells at levels which match that of clinically used agents. Unfortunately, in vivo biodistribution studies of these derivatives, performed in mice-bearing intracerebral implants of the GL21 glioma and subcutaneous implants of the B16 melanoma, suggested that they were unable to deliver adequate amounts of boron to tumor.

More recently, Vicente's group proposed other interesting strategies that involved the use of porphyrins bearing *p*-carboranylmethylthiol moiety conjugated to polyamines (**34–41**; **Figure 10**) [56]. In vitro, the polyamines displayed low dark cytotoxicity, low phototoxicity, preferential localization in the endoplasmic reticulum, Golgi and the lysosomes, and, except derivative **34**, higher uptake into human glioma T89G cells (up to 12-fold for spermine derivative **39**) than the tri-ethyleneoxy conjugate **41**. All these results suggested that spermine derivative could serve as an effective carrier of boronated porphyrins for the BNCT of tumor.

2.2.3. BSH structural modifications

As it is indicated above, BSH inadequate drug-like properties, i.e., lack tumor selectivity and poor BBB crossing ability, have conducted to develop BSH hybrid compounds containing other pharmacophoric moieties seeking improvement biological behavior.

In this sense, some approaches were based on the particular tumoral amino acid requirement and the special ability of some peptides to interact with receptors that, via endocytosis, are internalized into tumoral cells. In the first strategy, hybrid **42** (**Figure 11**), carrying a fragment derived from an unnatural amino acid that demonstrated a particular uptake by glioblastoma cells [57], was prepared and in vivo evaluated in F98 glioma-bearing animals [58].

Figure 8. (A) Structures of some relevant boron-containing porphyrins. (B) Some relevant polyhedron boron cluster water-soluble porphyrins.

The biodistribution studies showed higher tumor boron concentrations for derivative **42** than for BSH but lower than for L-BPA-F i.v. treatments. The CED intracerebral administration of derivative **42** was assayed combined with BNCT reaching better animals' survival rates than the treatment with L-BPA-F/i.v. On the other hand, in the use of peptides as carrier strategy, the hybrid **43** (**Figure 11**), derived from Tyr³-octreotate peptide that interacts to somatostatin receptor, was synthesized [59]. This BSH octapeptide still has not study biologically.

As it was already mentioned, the biochemical peculiarities of the porphyrin system also allowed the delivery of BSH within the tumor. In this sense, hybrid BSH porphyrin **44** (**Figure 11**) was prepared and evaluated in a 9 L glioma-bearing rat model [60]. Compound **44** displayed higher boron tumor: blood ratio in comparison to BSH 24 h after i.v. treatment and in an in vitro BNCT colony-forming assay higher cytotoxicity than BSH. Another series of BSH porphyrin hybrids consists of compounds like **45** and **46** (**Figure 11**) that still has not been biologically studied on gliomas [61].

GBM contains areas with different oxygenation levels, and consequently different metabolic patterns, which make difficult the therapeutic strategies [62]. Highly oxygenated regions are close to blood vessels, present high proliferation rate and oxidative metabolism and are susceptible to metabolism-active drugs and radiotherapy. While hypoxic regions present low proliferation rate, reductive metabolism and are resistant to chemo- and radiotherapy. The hypoxic condition in GBM tumors, which induces metastasis and promotes angiogenesis and resistance, has been attributed to contribute to tumor regrowth and, therefore, in a relapse. The troublesome characteristics of hypoxic regions have been exploited to generate cancer therapeutics known as hypoxia-selective bioreductive prodrugs which are compounds able

Figure 9. Carborane-containing DNA-interacting agents. (A) Alkylators. (B) Intercalators. (C) Minor-groove binders. (D) Polyamines.

Figure 10. Carborane-containing porphyrins porting polyamine framework.

Figure 11. Some BSH hybrids attempting to improve BSH biological behavior.

to undergo reduction producing cytotoxic events. Some of the well-known bio-reductive pharmacophores, nitroimidazoles, and *N*-oxide containing heterocycles were used to prepare BSH hybrids for potential use in hypoxia. Thereby, 2-nitroimidazoles **47** and **48** or quinoxaline dioxides **49** and **50** (**Figure 12**) were prepared and evaluated against some tumoral models using BNCT, but unfortunately they have not yet been studied on glioma models [63–65].

2.3. Boronic acids and their esters

Boronic acid ($R-B(OH)_2$) has been utilized as a pharmacophore in the searching of new agents to be employed in BNCT. This functional group has some relevant drug-like properties turning it into a useful moiety for biological applications, for example [66–68], (a) the sp^2 -hybridized boron atom possesses a vacant p -orbital which, after biomolecule donor atom attacks, allows the interconversion of boron hybridization, from sp^2 to sp^3 , to generate a new stable anionic compound; (b) the enhanced ability of $-B(OH)_2$ system to interact with biomolecule via (b1) hydrogen bonds, as acceptor and donor through OH groups, and (b2) strong boron Lewis acidity that allows boron-nitrogen, boron-oxygen, or boron-sulfur bonds; (c) the apparent pK_a of $-B(OH)_2$ ranging 4.5–8.8, for arylboronic, that allows large acid-base behavior finding the protonated and deprotonated form of the acid according to physiological pH conditions; and (d) apart from the reactivity mentioned above (section a) the particular ability to react with diol-containing compounds, like sugars and sugar-containing biomolecules, yielding the corresponding stable cyclic ester 1,3,2-dioxaborolane. Additionally, the $-B(OH)_2$ group has weak electronic effects being electron donor for induction and electron withdrawing for mesomerism. In reference to the lipophilicity of the $-B(OH)_2$ moiety, it is as lipophilic as $-CN$ group, lesser lipophilic than $-CO_2CH_3$ and neutral $-CO_2H$ moieties, and more lipophilic than $-CONH_2$, $-CH_2OH$, and $-CHO$ groups (π Hansch constants which are -0.55 , -0.57 , -0.01 , -0.32 , -1.49 , -1.03 , and -0.65 , respectively [69]). In vitro boronic acids degrade leading deboronation via hydrolysis/oxidation yielding boric acid (H_3BO_3). For example, degradation of bortezomib, known as Velcade™ the first boronic acid-containing drug on the market and approved by the US Food and Drug Administration, under acidic and basic conditions seems to be mediated by an initial oxidation producing boric acid and having a plasma half-life of 9–15 h in humans. The end product, boric acid, is not considered especially toxic to humans. For this, it is not expected that boronic acids possess intrinsic toxicity.

The success of the L-BPA in preliminary studies in patients with GBM, followed by PET studies with 1-aminocyclobutane-1- $[^{11}C]$ -carboxylic acid, revealing that cyclic amino acids were located preferentially in this tumor [57], led to the development of series of these cycloalkane-boronic acids (**51–54**; **Figure 13**) [70, 71]. They are water-soluble, cross the BBB, and are not metabolized by tumor cells, and while all accumulate into tumor similarly, the *cis*-isomers (**51** and **53**) biodistributed four times higher in tumor than in blood that of the *trans*-ones (**52** and **54**) and that of L-BPA-F [72–74]. By secondary ion mass spectrometry, it was proven that nearly 70% of the boron pool from derivative **51** was in the nucleus and cytoplasm of T98G GBM cells [75]. Until now there have been no studies of BNCT with these compounds, and further studies should be done prior to clinical trials.

As it is mentioned above, polyamines are essential for differentiation and growth of mammalian cell, and their depletion has growth inhibitory effects on tumors. Furthermore, a polyamine-facilitated transport system is present and allows the uptake by malignant cells [76].

Figure 12. BSH hybrids with potential use in hypoxic glioma.

For that, boronic acid-containing polyamines have been also studied as potential BNCT agents [77]. Compound **55** (Figure 13) with higher hydrophilicity than that of carboranyles **32** and **33** (Figure 9) was designed in order to minimize the carboranyl's nonspecific binding to biolipids that limits the tumor specificity. This boronic acid derivative was able to bind to calf thymus DNA and rapidly was taken up in vitro by F98 rat glioma cells, but the in vivo biodistribution showed unmeasurable levels of boron in tumor, and for this reason, the authors have considered that derivative **55** is not appropriate for BNCT [55].

Thinking about DNA as target to metabolically incorporate boron atoms to the tumoral cell, boronic acid-containing nucleic acids have been studied. One of the first described compounds was the uracil derivative **56** (Figure 13) [78] which demonstrated unfavorable tumor:blood and brain:blood biodistribution ratios being in all studied times in favor to blood. After that some boronic acid-containing nucleosides, such as **57** (Figure 13), were prepared and studied as potential agents for BNCT; however, no studies with glioma were performed [79]. However, recently nucleoside **58**, a boronic cyclic ester (a dioxaborolane derivative; Figure 13), was prepared and evaluated as cytotoxic agent against some tumoral cells [80, 81] including U-118 MG glioblastoma cells [82]. Additionally, the ability of ester **58** to be incorporated into cellular DNA and its selectivity for tumoral cells become its potential usefulness tool in glioma BNCT.

The combination of PDT and BNCT has been also proposed using boronic acid-containing porphyrins, i.e., **59** and **60** (Figure 14) [83]. However, it still has not been conducted studies with these compounds in gliomas.

As previously stated the particular hypoxic condition of some GBM regions was used as strategy to produce boronic acid derivatives that could be selectively accumulated in this tissue. For example, the well-known 2-nitroimidazolyl hypoxia pharmacophore was employed as structural guide to boron-enriched tumoral cells generating the boronic acid ester **61** (Figure 14) [84]. This dioxaborolane was selectively accumulated in D54 glioma when it was injected intratumorally in a xenograft mouse model reaching 9.5 times the levels of boron into the tumor of L-BPA in the same conditions. However, the pharmacokinetic behavior of derivative **61** should be solved in order to improve the use of this agent in BNCT.

Figure 13. Some boronic acid derivatives developed as potential BNCT agents. Amino acids 51–54 were studied as a mixture of enantiomers.

Figure 14. Some boronic acid derivatives developed as two-target drugs.

2.4. Miscellaneous compounds

Other boron-containing moieties have been described for BNCT, for example, in compounds 62–68 (Figure 15) [85–90]. However, in spite of their attractive features as pharmacophores, they have not been applied for glioma treatment. Additionally, they contribute with low number of boron atom per molecule which, as mentioned above, which is a disadvantage respect to boron clusters. However, this could be overcome with a high selective accumulation into the tumor cells. For example, the betaine 68, a boron-containing dipeptide analogue, showed selective accumulation in rat C6 gliosarcomas with ^{10}B tumor: blood and tumor: normal brain ratios of 8.9 and 3.0, respectively [91].

Figure 15. Some other potential boron pharmacophores for glioma BNCT, i.e., boradiazoles, aza-borauracils, 2-boradihydropurines, cyanoboranes, boranophosphates, benzoxaboroles, and trialkylamine-carboxyboranes.

Figure 16. Structure of the well-known PTK inhibitor erlotinib and boron-containing compounds derived from 4-anilinoquinazolinyl pharmacophore.

2.5. Other approaches: site-specific delivery

Beyond the strategies of therapy combination mentioned above, such as phototherapy or hypoxia, another hypothesis has been choosing a bio-system overexpressed in tumoral cells, but not in healthy cells, as a way to selectively accumulate boron drugs in the desired tissue to further BNCT. In this sense, Nakamura and colleagues have described the use of protein tyrosine kinases (PTKs), that its uncontrolled activation is often associated with uncontrolled cell growth and tumor progression, to generate boronic acid and esters hybridized with PTK pharmacophores as new drugs (i.e., **69** and **70**; **Figure 16**) [92]. However, they did not bear

in mind the glioma treatment nor BNCT with this strategy. Nevertheless, we considered the GBM-PTKs [93] as the target to accumulate a boron-enriched drug to further BNCT procedure. Thereby, we hybridized the 4-anilinoquinazolinyI PTKs and the carboranes as boron delivery pharmacophores, i.e., **71** and **72** (**Figure 16**) [94]. Especially, hybrid **71** demonstrated 3.3 times higher activity against C6 glioma cells than the parent drug erlotinib (**Figure 16**), lower cytotoxic effects on normal glia cells, excellent PTK inhibition, capability to accumulate in glioma cells, ability to cross BBB, and stability on simulated biological conditions [94, 95].

3. Conclusion

The development of boron-bearing compound for BNCT has been a vast field of research within medicinal chemistry. New and interesting pharmacophores have been described in order to fulfill the BNCT requirements. Despite this, very few reached the stage of clinical studies, being currently L-BPA and BSH, the only two potential therapeutic agents. However, due to the multidisciplinary approach of the BNCT and the emerging novel structures, we expect with optimism the new developments in the years to come.

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Conflict of interest

The authors declare no conflict of interest.

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