Boronated DNA-Binding Compounds as Potential Agents for Boron Neutron Capture Therapy

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Abstract: Boron Neutron Capture Therapy (BNCT) is a binary cancer treatment that exploits the short range particles released from a nuclear fission reaction involving the non-radioactive ^{10}B nucleus and low-energy (thermal) neutrons for the destruction of tumour cells. If boronated agents are targeted towards chromosomal DNA, the efficiency of BNCT is greatly enhanced. This article presents a concise review of DNA-binding compounds that have been functionalised with boron.

Key Words: Boron neutron capture therapy, boronated agent, carborane, DNA-targeting, boronic acid.

INTRODUCTION

In the field of oncology, a therapeutic treatment that is able to destroy malignant cells without damaging the surrounding normal tissue is the ultimate objective. Vast budgets and considerable research endeavours are spent every year in the pursuit of this goal, most of which focus on identifying and exploiting the differences between normal tissue and malignant growth. At present, there are three main treatments available for cancer: surgery, chemotherapy and radiotherapy [1]. Combinations of two or all three of these therapies are also commonly used in the clinic. Surgery is most commonly used to remove as much of the malignant growth as possible; this is most effective in the early stages of cancer when the tumour is contained and can safely be removed from the patient. However, it is not always possible to remove all the cancerous cells, especially if the tumour has already begun to metastasise. Radiotherapy exploits subtle differences in the ability to repair damage between tumour cells and normal tissues. However, the sensitivity of the surrounding normal tissues in the irradiation field is always the limiting factor in the dose that can be delivered to the tumour. Chemotherapy relies on the premise that rapidly-proliferating tumor cells have a greater cellular uptake of nutrients from the blood. As with radiotherapy, the dose used in chemotherapy is limited by the damage to non-cancerous cells, especially those cells in rapidly dividing normal tissues such as the bone marrow or the intestinal epithelium.

The toxicity problems associated with conventional therapies have led the researchers to examine other possibilities for the treatment of cancer. One such approach is commonly referred to as a "binary therapy". Classic binary systems typically consist of two non-toxic components. Although they are innocuous in themselves, when combined in the tumour they result in a cytotoxic effect. The advantages of a binary therapy for cancer are that each component can be manipulated independently and only one needs to be confined to the tumour cell [2, 3]. Additionally, a binary therapy is believed to have greater clinical safety and efficacy than the unitary methods currently employed in radiotherapy and chemotherapy. In order to optimise the effect of binary therapy in cancer treatment, the cytotoxic products should remain confined to the tumour cell and be extremely effective in promoting cell death. This will minimise the damage to surrounding healthy tissue, as well as maximise damage to the tumour.

There currently exist three potentially-useful binary systems in cancer therapy, *viz:* photodynamic therapy (PDT) [4, 5], photon activation therapy (PAT) [6, 7] and neutron capture therapy (NCT). This review is primarily concerned with the chemistry of compounds used for NCT and, as this binary system has already been reviewed extensively elsewhere [2, 3, 8-10], only the salient aspects are presented here.

NEUTRON CAPTURE THERAPY (NCT)

Locher first suggested the potential medical applications of NCT in the mid-1930s [11]. NCT is based on the ability of certain atomic nuclei to absorb neutrons and produce a short-lived excited nucleus, which then undergoes fission to produce lethal, high energy particles. It has been known for many years that the ability of atomic nuclei to capture thermal neutrons varies widely over the Periodic Table. The neutron capture cross section of a nucleus is expressed in barns (1 barn = 10^{-24} cm^2) and is dependent on the structure rather than the mass of the nucleus [3].

Importantly, the capture cross sections of carbon, hydrogen, oxygen and nitrogen (which together make up 96% of tissue) are relatively low, the highest being nitrogen, which has a value of only 1.82 barns (for the ^{14}N isotope) (Table **1**). This ensures that damage to healthy cells due to the capture reactions of the neutrons is minimised, but nevertheless these nuclei can still make a significant contribution to the total radiation dose due to their high concentrations in tissue. Although many nuclei have the high neutron capture cross section required for NCT, this is only one of the many factors that must be considered. Few of the nuclides are suitable

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due to their toxicity, instability or radioactivity. For example, ^{135}Xe has one of the highest known capture cross sections (2.6×10^6 barns) but is not appropriate for use in NCT due to its significant radioactivity and absence of any tumour selectivity.

Table 1. Thermal Neutron Capture Cross Sections of Selected Nuclides [12]

Nuclide	Nuclear Capture Cross Section (barns)
^{1}H	0.332
^{10}B	3 838
^{12}C	3.4×10^{-3}
^{14}N	1.82
^{16}O	1.8×10^{-4}
^{135}Xe	2.6×10^{6}
^{157}Gd	2.55×10^{5}

The products of the fission reaction must also be taken into consideration. ^{157}Gd has a neutron capture cross section of 2.55×10^5 barns. However, the fission products are γ-rays that are able to damage surrounding cells and Auger and Coster-Kronig electrons of an energy too low to cause sufficient damage unless closely associated with the DNA macromolecule [13, 14]. In this review, the use of boron for NCT will be considered due to its dominance in the field and its long history in the treatment of cancer.

BORON NEUTRON CAPTURE THERAPY (BNCT)

Almost all research into the clinical applications of NCT has focused on the ^{10}B isotope, which is non-radioactive and makes up approximately 20% of naturally-occurring boron. Its large neutron capture cross-section (3838 barns) makes it ideal for use in NCT. The fission products of the neutron capture reaction are $^{4}He^{2+}$ (α-particles) and recoiling $^{7}Li^{3+}$ nuclei, known collectively as high linear energy transfer (LET) particles. Due to their size and energy, they are largely confined within the cell they arise from; the range of the $^{7}Li^{3+}$ and $^{4}He^{2+}$ nuclei is typically 5 μm and 9 μm, respectively [3].

OPTIMISATION OF THE NEUTRON CAPTURE REACTION

The theoretical basis for BNCT was developed well before an appropriate neutron source had become feasible [11] and it was only with the development of nuclear technology in the 1940s that the first clinical trials became possible. The neutrons of choice are thermal (slow) neutrons, which have an energy of only 0.025 eV, which is suitable for immediate neutron capture, but not high enough to cause ionisation damage to tissues. Nevertheless, neutrons experience considerable scattering from hydrogen atoms. The thermal energy neutrons required for the neutron capture reaction are attenuated exponentially in tissue, thus their ability to treat more deep-seated tumours is restricted. Epithermal beams, with an energy in the 0.5 – 10 keV range are used to address this limitation as the kinetic energy of the neutrons decreases sufficiently due to scattering, such that they undergo neutron capture at depths sufficient to reach the tumour site.

The status of reactor-based neutron beams used for NCT has been recently reviewed [15]. Epithermal neutron irradiation facilities are now available, which combine near theoretically optimum beam purity with intensities which are well suited to clinical studies. Further incremental improvements in beam quality are possible but the major challenge facing BNCT is the optimisation of the boron compounds and compound delivery. The development of alternative neutron sources (e.g. a low energy accelerator-based neutron source [16]) is also an ongoing area of research and it would greatly alleviate the financial and political issues associated with current BNCT modalities that rely upon nuclear reactors as the neutron source.

OPTIMISATION OF BNCT

For clinical applications of BNCT, it is critical that a sufficiently high concentration of boron within the tumour cell is obtained in order to maximise tumour cell destruction and minimise the total radiation dose to the patient, i.e. maximise the therapeutic index. This has been calculated to be approximately 10^9 ^{10}B atoms per cell (a figure corresponding to *ca.* 10-30 μg of ^{10}B g^{-1} tumor) when the boronated agent is evenly distributed throughout the cytoplasm [17-19], although it must be emphasised that the experimental data to date have been limited and confined largely to simple borate salts [20] and the clinically-approved compounds **1** and **2** (see below) [21]. A number of other requirements must also be met for any compound to be developed into a potential BNCT agent, including:

a) The compound should possess some degree of selectivity towards the tumour, so that boron concentrations within the tumour are at least 3 - 4 times greater than the concentrations found within the normal tissue.

b) The toxicity of the compound must be low enough for the patient to tolerate a therapeutic dose. This is a particularly important requirement for global boronated agents that display only marginal tumour selectivity.

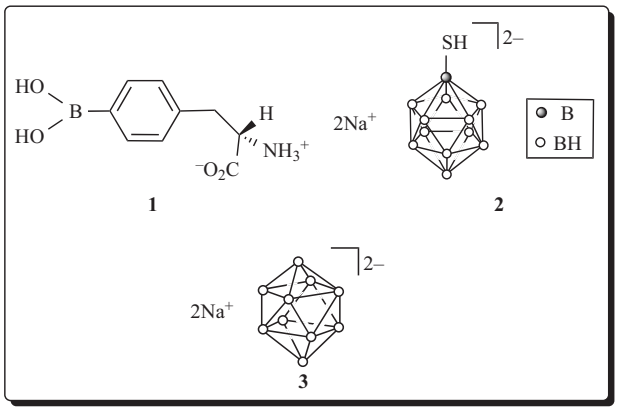
c) The compound must persist in the tumour during the neutron irradiation period in order to ensure that nearby healthy cells are not greatly affected, with rapid clearance from the blood and normal tissues.

d) The compound should have as many ^{10}B atoms as possible per molecule in order to maximise the intracellular ^{10}B concentration within target cells thereby minimising (i) the dose of agent administered to the patient and (ii) the neutron beam irradiation time, which contributes to the total radiation dose.

e) A close proximity of the boronated agent to the chromosomal DNA of tumour cells is highly desirable [18, 22], as this decreases the required therapeutic ^{10}B concentration and increases the potency of the neutron capture reaction leading to an increase in cell kill.

As boron can readily be incorporated into organic structures, an immense variety of boronated compounds have

been synthesised to date. These have included boronic acid analogues such as *p*-boronophenylalanine (BPA, **1**), usually administered to the patient as the water-soluble fructose complex, and the polyhedral borane borocaptate ion (BSH, **2**), both of which are undergoing clinical trials for BNCT in several countries [9, 23]. The polyhedral borane GB-10 (**3**) is approved for use in humans as well [24, 25].



Despite its reasonable tumour selectivity and very low toxicity, one of the most significant limitations to the use of **1** in BNCT is that very high doses (typically 250 - 900 mg/kg body weight) of agent are required to be therapeutically useful [9]. Furthermore, observations of regions of frank tumour necrosis but also evidence of tumour progression in the high dose target volume was interpreted as a non-uniform distribution of boron to all tumour cells [26]. Longer infusions of **1** seemed to increase the median survival. The dose escalation BNCT trials with **1** have reached the point where the normal brain tolerance becomes limiting; the incidence of a somnolence syndrome in patients treated with the higher doses was reported. This indicates that dose escalation trials with **1** as the single boron delivery agent have reached the limit both in terms of the amount of **1** administered and in terms of the escalation of the beam dose. With **1**, there is a significant amount of boron in the normal brain and this undoubtedly contributes to the brain dose and the incidence of the somnolence syndrome.

Those agents which contain more than one boron atom per molecule not only increase the efficiency of the neutron capture reaction but can also be administered at much lower doses to the patient. Hence, the incorporation of functionalities containing multiple boron atoms has become a focus of BNCT research. These include polyhedral borane anions such as **2** and **3**, as well as the carboranes. Carboranes, in particular the dicarba-*closo*-dodecaborane(12) family of isomers, offer excellent kinetic stability to hydrolysis, ease of functionalisation, low toxicity and high boron content, which makes them ideal candidates for development into novel BNCT agents [3].

THE CELL NUCLEUS AS A TARGET

Many approaches have been established for the selective delivery of boron to the tumour site, in particular the cell nucleus, and one such approach involves the targeting of chromosomal DNA which offers an incredible number of potential binding sites to small molecules containing boron and, furthermore, theoretical studies demonstrate the clear benefit of boron delivery to the cell nucleus rather than the cell surface or cytoplasm [18]. In principle, boron can be delivered in at least two ways to chromosomal DNA, including (i) incorporation within DNA using boron-containing derivatives of nucleotides, nucleosides and oligonucleotides or (ii) direct delivery to DNA using boron analogues of well-known DNA binders. Hawthorne and co-workers have also described the nuclear accretion of oligomeric phosphodiesters (OPDs) containing multiple *nido*-carborane units [27]. These entities offer the prospect of incorporating significant levels of boron into the tumour cell nucleus, but at this stage only direct microinjection studies of the OPDs into mammalian TC7 cells have been reported. Potential tumour targeting by the use of a porphyrin-OPD conjugate is also feasible, but biological experiments involving this system have yet to be reported.

BORONATED NUCLEOTIDE, NUCLEOSIDE AND OLIGONUCLEOTIDE DERIVATIVES

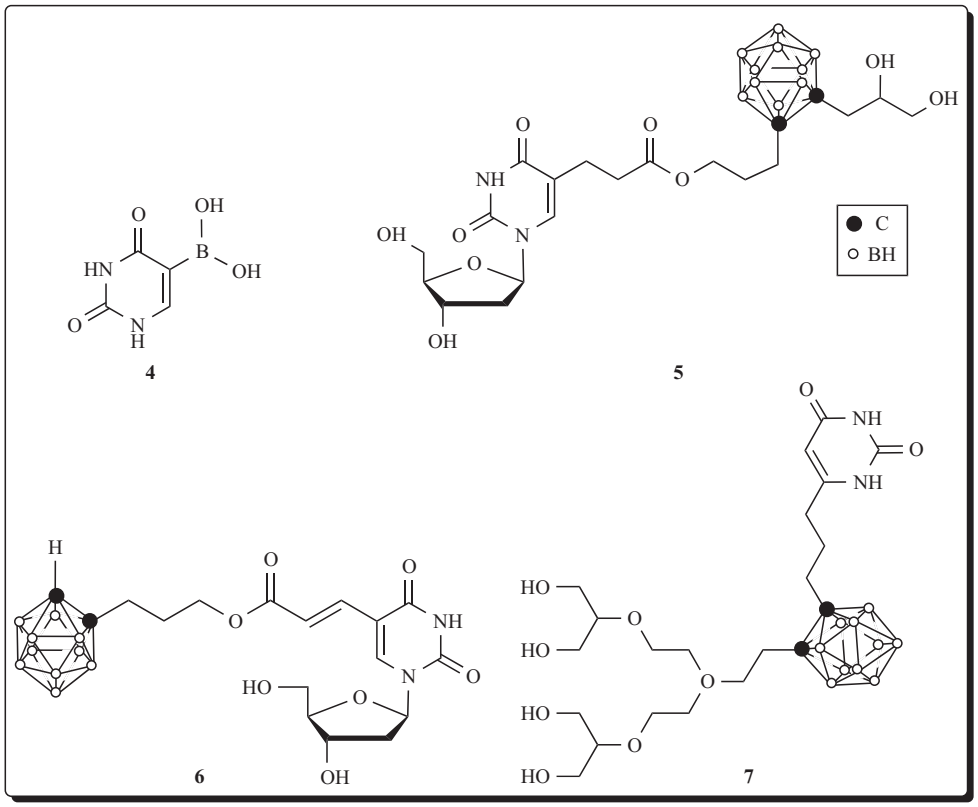
Molecular mimicry as a means of delivering boron to the tumour cells is an important area of contemporary BNCT research. As malignant cells undergo mitosis more rapidly than normal cells, such cells have a greater requirement for the substrates required for cell growth and division [1]. In general, traditional cancer chemotherapy exploits this metabolic difference. In BNCT, the opportunity exists to prepare boron-containing analogues of the biomolecules needed for cell growth and division which might ultimately lead to an increase in the boron concentration within the rapidly-dividing tumour cells, thus resulting in an improvement in the selectivity of the drug. The most widely-studied cellular building blocks for BNCT are boronated nucleic acid precursors such as **4** [28]. The rationale behind their development is straightforward [29]; if the biological analogues were successfully incorporated into DNA, they would be ideally placed to maximise damage upon neutron irradiation, ultimately leading to cell death. As mentioned previously, the low boron content of **4** is not ideal for BNCT and various carborane nucleotide and nucleoside derivatives have been synthesised, including **5** [30], **6** [31] and **7** [32]. The chemistry and biology of boronated oligonucleotides as potential anti-sense molecules and BNCT agents have recently been reviewed by Lesnikowski *et al*. [29, 33, 34].

BORONATED DNA-BINDING AGENTS

An alternative to use boronated nucleic acid precursors or oligonucleotides as a means to concentrate boron within the tumour cell nucleus is to develop boronated compounds which bind directly to chromosomal DNA. The classes of compounds include alkylating agents, intercalators, minor-groove binders, cationic polyamines and biologically-active metal complexes. Each of these classes will be dealt with in turn.

Alkylating Agents

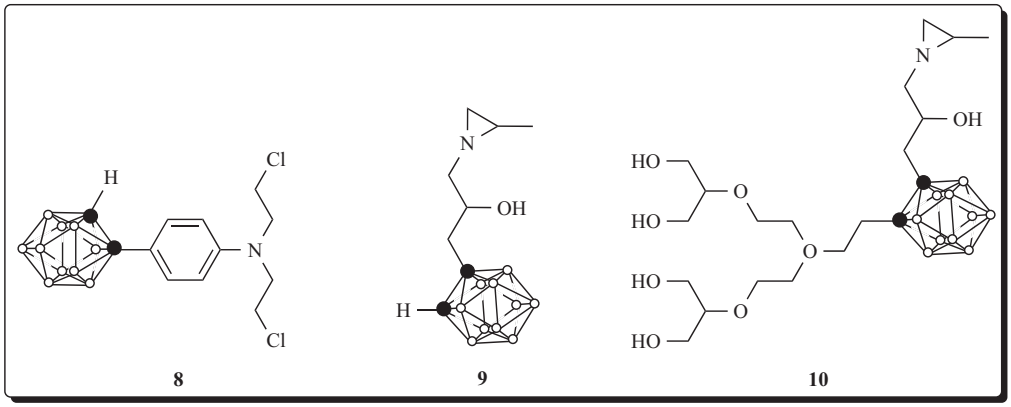
Alkylating agents are highly electrophilic compounds which are able to form irreversible, strong covalent bonds with nucleophilic groups on DNA. Soloway and Butler prepared and evaluated the biological activity of the 1,2-

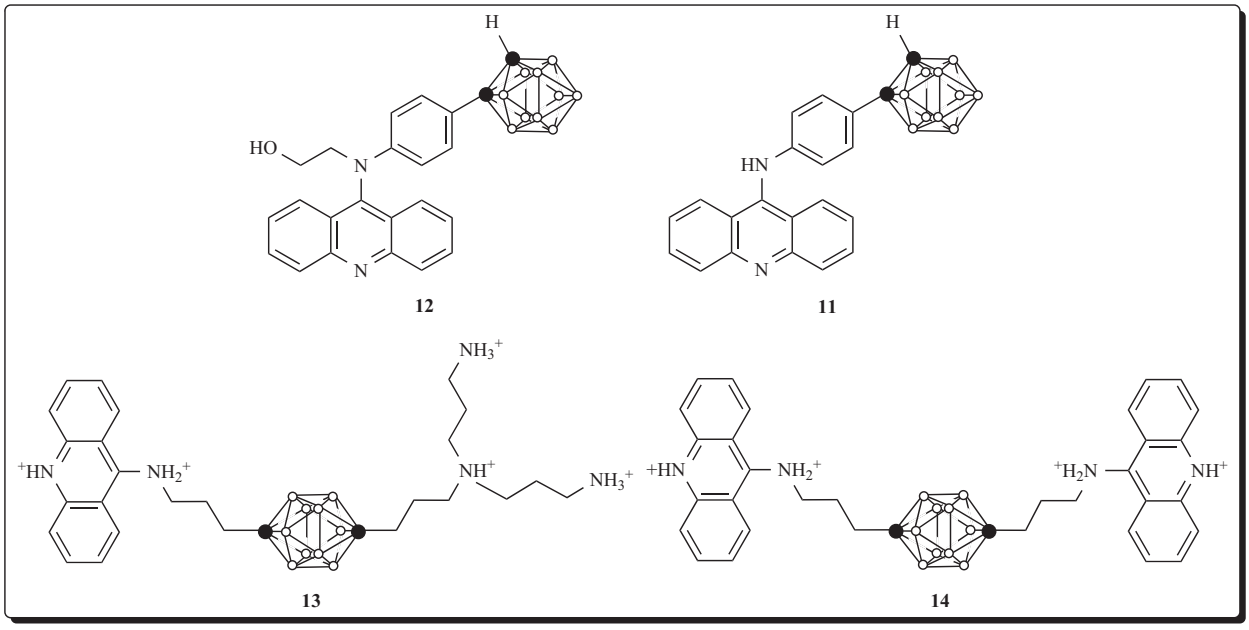


carborane-containing nitrogen mustard **8** for the delivery of boron to chromosomal DNA, the first example of a DNA-binding agent containing boron which was developed 40 years ago. The boronated alkylating agent was administered to C3H mice bearing subcutaneous ependymomas and the biodistribution of boron was analysed. Although the levels of boron in the mice 48 hr after administration showed that the compound was retained in the body, the compound did not show any significant tumour selectivity [35]. Current research into carborane-containing DNA alkylating agents has focused on reducing their lipophilicity in order to improve their biodistribution and perhaps decrease their toxicity. Yamamoto and Nakamura synthesised a carborane-containing aziridine **9** and have evaluated its cytotoxicity and uptake in cancer cells. The aziridine derivative was found to exhibit a selective cytotoxicity for B16 melanoma and Hep G2 liver tumor cells, with significantly lower toxicity towards normal human foetal lung cells. Selective uptake of the drug was observed in B16 malignant melanoma cells, showing a tumour: normal cell ratio of 1.32 [36, 37]. Yamamoto and Sadayori have increased the water solubility of **9** by incorporating a cascade-type polyol into the carborane to afford compounds such as **10**. The addition of the water-solubilising element decreased the cytotoxicity of the aziridine derivatives towards B16 cells, however, the cellular uptake was higher for the cascade-polyol modified aziridines [38].

DNA Intercalators

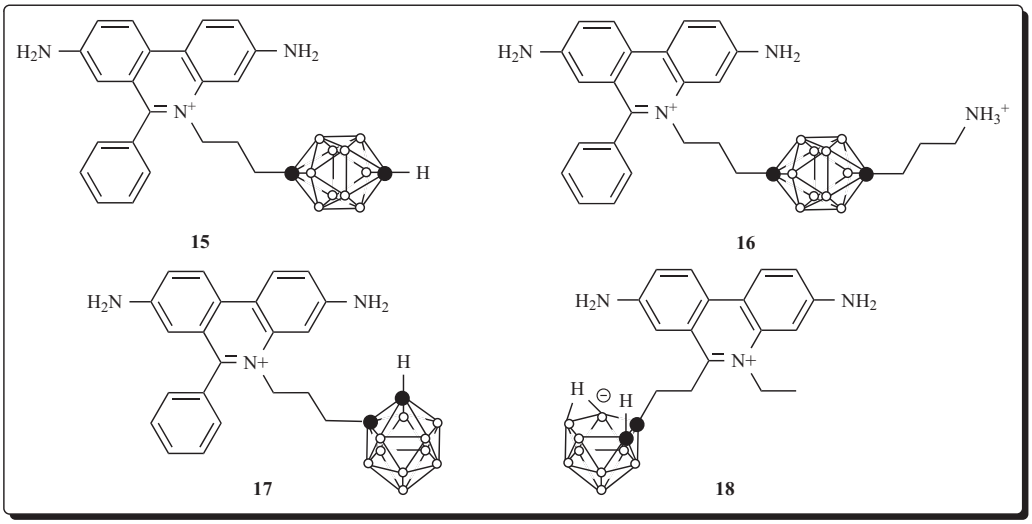
DNA intercalators are polyaromatic compounds that are capable of inserting non-covalently between the base-pairs, disrupting the helix sufficiently to interfere with transcription. Several boronated acridine dyes have been synthesised

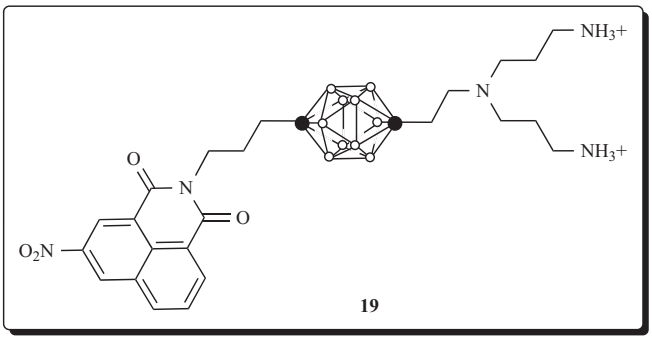




to date and current research has focused on reducing the toxicity of these compounds towards the liver, kidney and spleen. Carborane-containing acridines **11** and **12** were first prepared by Davis and Soloway [39] and their *in vitro* activity was investigated. The high LD_{50} values for both these compounds confirmed their low toxicities, a factor that would make them suitable for BNCT. The boron-rich acridine derivatives were administered to C3H mice bearing subcutaneous ependymomas in order to evaluate localisation of the agent within the tumour. Although the tumour: blood and tumour: brain ratios were found to be high, the compounds were also found at high levels in muscle, liver, kidney and spleen tissue. Recent studies involving two DNA-affinic agents, acridine and spermidine (see below), have shown successful DNA intercalating properties, particularly with the 9-aminoacridine derivative. Ghaneolhosseini and coworkers have synthesised the boronated acridine-spermidine **13** and boronated diacridine **14** as potential candidates for BNCT [40]. The EGF targeting of tumours using synthetic liposomes loaded with boronated acridine **13** has recently been reported [41].

Tjarks and co-workers have investigated the synthesis of boronated ethidium derivatives. A series of novel *para*- and *nido*-carboranyl phenanthridinium compounds, e.g. **15**, was successfully synthesised but they exhibited only slight water-solubility [42]. A new phenanthridinium derivative **16** containing an aminoalkyl group attached to C-12 of the *para*-carborane was prepared in order to increase its hydrophilicity and decrease non-specific cell-binding [43]. Gedda and coworkers have also studied the DNA binding and cytotoxicity of a family of boronated phenanthridinium analogues, e.g. **17** and **18**, which contain *closo*- and *nido*-carborane respectively [44]. The compounds were all shown to intercalate DNA and the 5-*para* compound was found to be the most cytotoxic, causing complete cell death at 5 μg mL^{-1}. No cy-





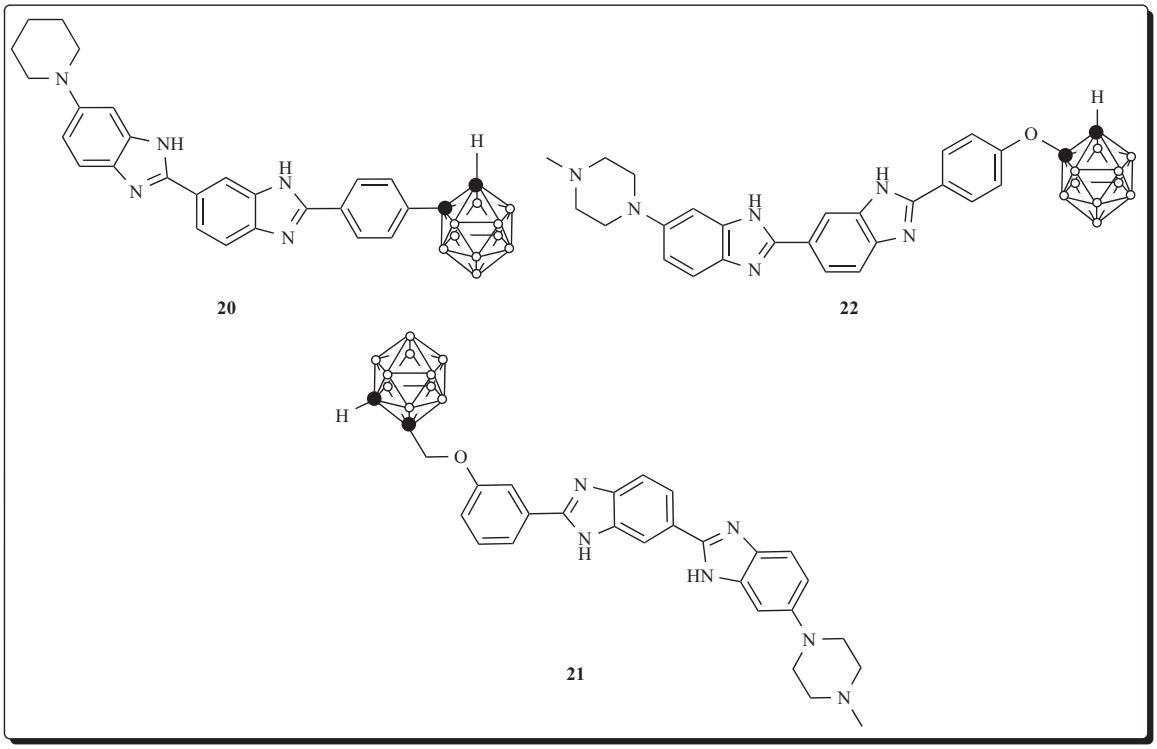
19

totoxic effects were observed for any compound at 1 μg mL^{-1}. Fluorescence studies demonstrated a comparatively low uptake of the compounds into the nuclei of the viable cells. Therefore, the authors suggested that these compounds were unsuitable for the selective two-step targeting of tumour cell DNA. A series of phenanthridinium and acridine derivatives containing 1,2- and 1,12-carborane has been reported by Gedda and colleagues [45] and their accumulation in cultured human malignant glioma spheroids was investigated. It was found that the more lipophilic compounds had an extremely high accumulation in the spheroids, giving a boron concentration greater than two orders of magnitude over the concentration found in the culture medium. In contrast, the less lipophilic compounds were less toxic and had a lower accumulation but greater penetration into the inner region of the spheroids.

Ghaneolhosseini and Sjöberg have studied boron-containing analogues of selected naphthalimides [46] based on mitonafide and DMP 840, two well-known DNA intercalators that have GC-rich sequence specificity. The synthesis of a water-soluble boronated naphthalimide derivative **19** was carried out knowing that the nitro or amino substituents on the chromophore rings are essential for anti-tumour activity and DNA-binding, but the DNA binding capacity of **19** was not reported.

DNA Minor-Groove Binders

Kelly and co-workers have developed four boron-containing bibenzimidazoles which were designed to target the DNA minor-groove [47], as has been observed for the archetypal minor-groove binder Hoechst 33258 (4-[5-(4-methyl-1-piperazine)-2,5'-bi-1*H*-benzimidazol-2'-yl]phenol). The boronated derivatives were prepared in order to examine the effect of position, type of functional group and number of boron atoms per molecule. Cell uptake and neutron capture experiments with **20** appeared promising but its toxicity still required further evaluation. A related series of 1,2-carborane analogues of Hoechst 33258, e.g. **21**, was also prepared and their DNA-binding characteristics investigated by Bateman and co-workers [48]. The carborane cage was bound to the



20 **22**

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bibenzimidazole system by an ether linkage at either the 2- or 3-position. The DNA-binding capacity of the compounds was assessed by UV-visible spectroscopy and circular dichroism (CD), however, they were shown to bind only weakly to DNA, possibly due to the close proximity of the carborane cage to the minor groove. Future studies are aimed at increasing the length of the tether between the carborane cage and the bibenzimidazole, but these results have yet to be reported. A related 1,2-carborane derivative of Hoechst 33258 (**22**) has also been synthesised by Argentini and co-workers but no biological experiments were reported [49].

Yamamoto and co-workers have prepared a series of analogues of the minor-groove binders netropsin and distamycin which contain a 1,2-carborane moiety, e.g. **23** [50]. In order to improve the aqueous-solubility of the compounds, di- and tetra-ols were attached to the carborane cage. It was found that the overall DNA-binding capacity of these derivatives was reduced due to steric hindrance associated with the inclusion of the carborane cage when compared to the parent compounds netropsin and distamycin A. Conversely, as well as improving the water solubility of the analogues, increasing the number of hydroxyl groups improved DNA-binding selectivity and non-covalent interactions (e.g. H-bonding) of these groups with the macromolecule are inferred.

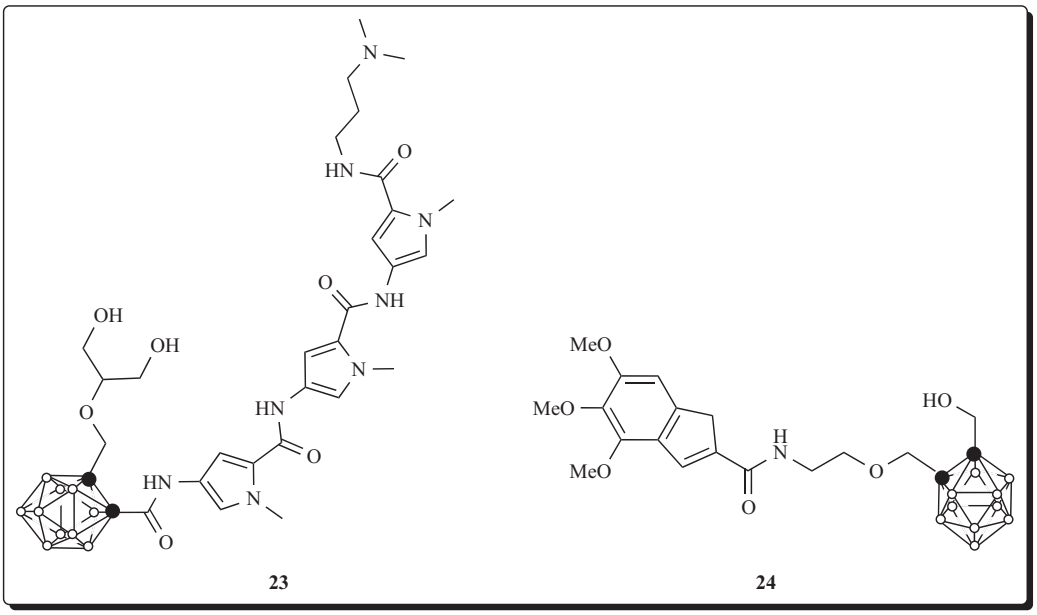
Tietze and colleagues have prepared 1,2-carborane containing derivatives of trimethoxyindole [51], a fragment known to contribute to the DNA minor-groove binding of the potent anticancer antibiotic duocarmycin A. The derivatives, especially **24**, were found to possess high levels of uptake in B-16 melanoma cells after only a 3 hr incubation.

Cationic Polyamines

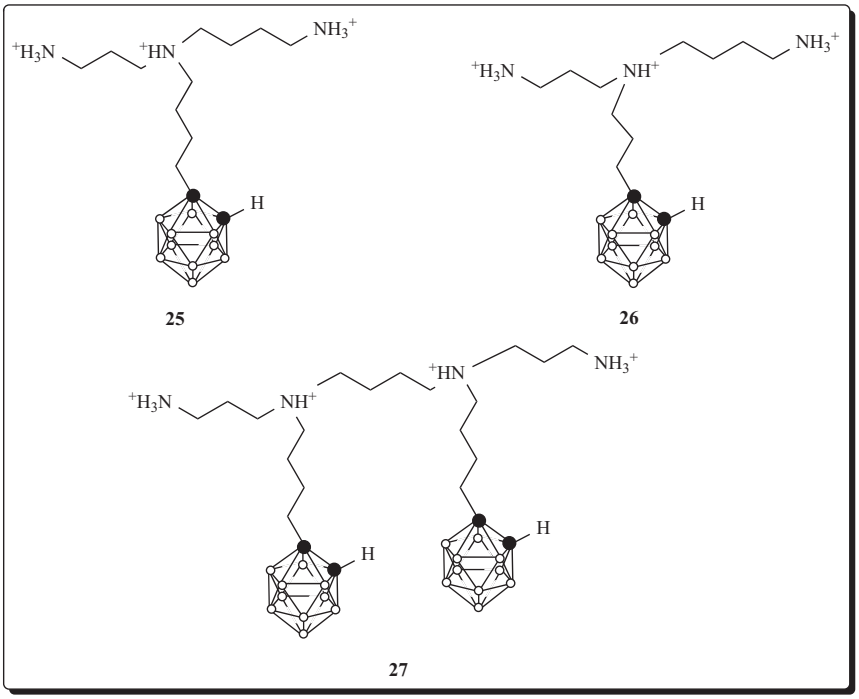
Polyamines are a class of biologically-active compounds known to be essential for cell growth and differentiation, and have been found in high concentrations in rapidly proliferating tumour cells largely due to the presence of a specific transport system. They include two naturally-occurring compounds, spermidine and spermine. Soloway and co-workers have described the synthesis of the first boron-containing polyamines for DNA-targeting in BNCT [52]. Beginning with the naturally-occurring polyamine spermidine, two novel compounds were prepared: 1,8-diamino-4-(4-o-carboranyl-butyl)-4-azaoctane **25** and 1,8-diamino-4-(3-o-carboranylpropyl)-4-azaoctane **26** as their hydrochloride salts. Soloway and co-workers have also investigated three series of boron-containing spermidine/spermine analogues such as **27** [53]. In these derivatives, the internal or terminal nitrogen atoms were boronated and for each compound, a 1,2-carborane moiety was tethered to the polyamines by a tetramethylene chain. Their results demonstrated that the polyamine analogues have DNA-binding properties comparable to those of the naturally-occurring compounds and also have the capacity to displace ethidium bromide from calf-thymus DNA. The compounds display rapid uptake into F98 glioma cells, but a high cellular toxicity has limited their potential application as potential BNCT agents.

A series of novel azanonaborane compounds containing an 8-atom boron cluster diamine, e.g. **28**, has been synthesised by El-Zaria and colleagues [54]. Preliminary *in vitro* cytotoxicity tests using V79 Chinese hamster fibroblasts have shown promising results, which suggest that two of the prepared azanonaborane compounds are non-toxic at levels required to achieve a sufficiently high boron concentration within the cell for BNCT studies.

Delcros and associates have synthesised boronic acid derivatives of the benzyl polyamines putrescine and spermidine [55]. The boron-containing polyamines **29** and **30** displayed a greater affinity for DNA than the parent compounds, as well as a reduction in cytotoxicity. The polyamine-uptake transport system has been shown to be responsible for the efficient accumulation of the polyamine derivatives into cells, thus accounting for the observed higher accumulation of intracellular boron compared to the clinically-used BNCT agents **1** and **2**.



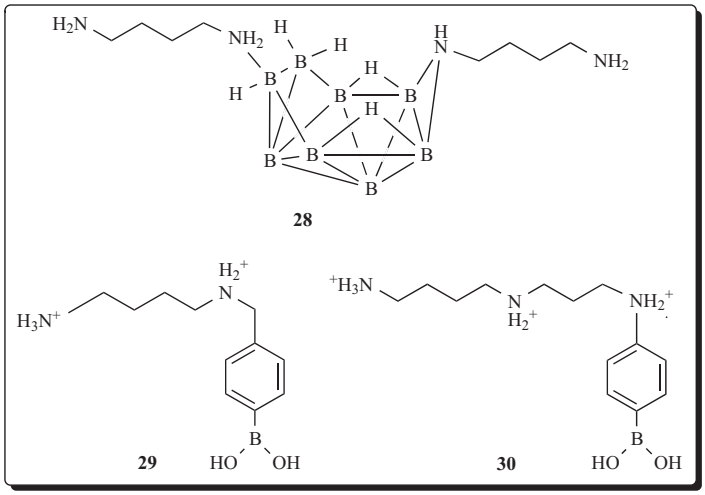
23 **24**

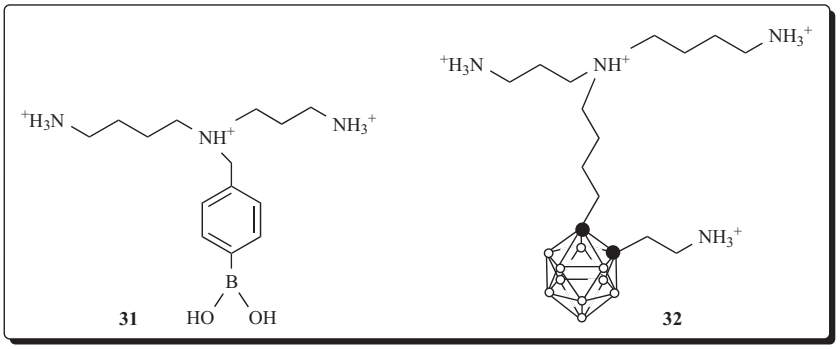


Zhou and co-workers have also prepared and evaluated a series of boronated spermidine and spermine analogues, bearing either a boronic acid group (e.g. **31**) or 1,2-carborane (e.g. **32**) [56]. It was found that these compounds were highly water-soluble, as well as stable in solution, when the amine hydrochloride salts were isolated. *In vitro* cell studies demonstrated that these compounds were less toxic than those described earlier [53] and also showed adequate uptake into the cells. However, the *in vivo* results were less promising as tumour cells were found to have insufficient levels of boron for BNCT and the compounds tended to accumulate in the liver, spleen and kidneys instead of the tumour.

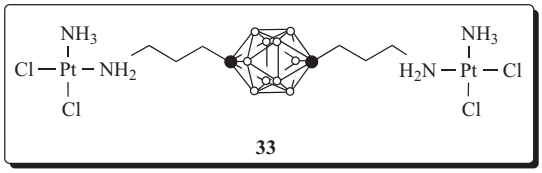
Biologically-Active Metal Complexes

The ability of certain metal complexes to bind to DNA has been known since the discovery of cisplatin as a potent anti-cancer agent, but this class of drug had not been exploited for potential use in BNCT until recently when Rendina and co-workers reported a new class of boronated DNA-binding agents incorporating both platinum and a boron-rich carborane entity, the first examples of platinum-amine complexes containing boron [57, 58]. A number of di- and tri-nuclear platinum(II)-amine species were prepared and the DNA-binding and preliminary cell uptake characteristics were also determined for selected complexes including **33**. Besides the potential for additive or perhaps synergistic biological effects associated with the DNA-binding reactions of the Pt-B agent coupled with the neutron capture reactions associated with the ^{10}B nuclei, there exists an additional advantage that such agents can be radiolabelled by the use of isotopes such as ^{195m}Pt which would allow their tumour uptake and biodistribution characteristics to be monitored by





means of gamma imaging following administration into the body ($t_{1/2}$ = 4 d).



It has been known since 1974 that some metal complexes containing a planar aromatic ligand are able to intercalate DNA and these are known as metallointercalators [59]. They bind DNA in a similar way to the many types of organic intercalators by inserting between the base pairs in a non-covalent manner, unwinding and lengthening the double-helix. Recent work by Rendina and co-workers has resulted in the synthesis of a series of mono- and di-nuclear platinum(II)-2,2':6',2"-terpyridine complexes with thioalkylcarborane ligands that are able to target DNA and exhibit *in vitro* anti-cancer properties [60-62]. In particular, **34** binds to calf-thymus DNA and has shown promising *in vitro* anti-cancer activity in both wild-type and cisplatin-resistant human ovarian cancer cells. However, unfavourable steric interactions between the carborane moiety and DNA, as well as its poor aqueous-solubility, have greatly limited the therapeutic potential of the complex. There are several approaches to the problem of high lipophilicity, including the addition of hydrophilic groups to the carborane cage such as cascade polyols [63]. This approach led to the preparation of the first example of a water-soluble metal-carborane complex **35** [64]. At the time of writing, biological evaluation of the complex had not been completed.

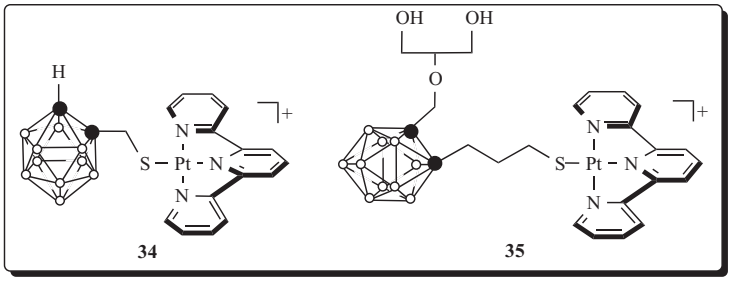
Boronated Porphyrins

Numerous studies of boronated porphyrins and related compounds, e.g. phthalocyanines, have been reported [9, 65-67] and their propensity to accrete within tumour cells at very high levels is very well known. A few boronated porphyrins are known to interact with DNA *in vitro* [68-70] and indeed can damage the macromolecule upon irradiation with light, but many boronated porphyrins appear to target tumour cell mitochondria [71] and/or lysosomes [72] rather than chromosomal DNA and are, therefore, beyond the scope of this review.

CONCLUSION AND FUTURE OUTLOOK

The promise of BNCT as a potential cancer treatment now reaches the crossroads whereby critical decisions will need to be made on its future viability [8, 9, 25]. As discussed earlier, the only boronated compounds approved for use in humans are **1**, **2** and more recently, **3**. Phase I/II clinical trials of BNCT for the treatment of high-grade gliomas have been carried out with **1** alone and with **2** alone, many of which are still ongoing. The results have been reported and discussed in several recent reviews [9, 23].

Historically, only malignant brain tumours, particularly glioblastoma multiforme and malignant melanoma have been treated by means of BNCT in the clinic with the aforementioned boronated agents, which despite the refractory nature of these tumours and marginal specificity of **2** and **3** for tumour cells, the reality is that clinical trials can only make use of the approved agents at the present time. There is little doubt that this factor alone has seriously impeded new compound development [25], its associated research funding and consequently, the progress of BNCT. Good pre-clinical data are required for any new potential BNCT agent that displays promising preliminary biological effects. The three clinically-approved BNCT agents do not act by DNA-binding and there exists a great opportunity to explore the biological effects of some of the classes of agents described in this review. DNA-binding compounds also offer the distinct possibility of greatly reducing the amount of agent administered to the patient, thus reducing its toxic side effects compared



to global agents, which display little tumour specificity. The potential for an exploration of any additive and/or synergistic effects associated with the DNA-binding of the agent in addition to the neutron capture reaction involving the ^{10}B nuclei is another advantage. Furthermore, tumour types other than those treated currently could also become available for treatment, bringing BNCT out of the "experimental" or "esoteric" status and into the domain of more conventional cancer treatments, but perhaps with a greater efficacy than is observed with conventional radiotherapy, for example, or even in combination with, or as an adjunct to, conventional therapies. Indeed, it is possible that for some of the promising compounds described in this review, the most effective use may be in combination with other boron compounds with a different cell targeting profile. This is the approach taken in chemotherapy and it is to be expected that the same path may be necessary for the optimisation of BNCT.

Further progress in clinical BNCT for high-grade gliomas will require significant alterations in the boron delivery methodology. One approach is the use of mixtures of boron delivery agents, which is based on the premise that the two boronated agents would target the boron to different populations of tumour cells. Barth and co-workers have shown that the use of **1** and **2** in combination is more effective than either agent alone in BNCT of the rat F98 glioma [73]. Ono and co-workers have presented data in the SCCVII rat squamous cell carcinoma that this is also the case with combinations of **1** and **2**. This group used *in vivo* BrdU labelling to identify proliferating tumour cells and a micronucleus assay for DNA damage in tumour cells isolated from tumours following BNCT [74]. The results indicated that **1** targeted the proliferating tumour cells but was less effective at targeting the quiescent tumour cells *in vivo* and **2** was more effective at inducing DNA damage in the quiescent cells (no BrdU uptake *in vivo*) than was **1**. These authors concluded that the combination of **1** and **2** would be more effective than either compound alone. Based on these data, this group has initiated clinical trials for both head and neck tumours [75] and gliomas [76, 77].

With cautious optimism, BNCT can indeed provide a novel means of treating certain cancers but the issues associated with the advancement of new boronated agents into the clinic, especially those which have the capacity to target intracellular biomolecules such as DNA or cellular structures, need to be addressed by the BNCT community if significant developments in this exciting area are to be made in the future.

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