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Synthesis, Characterization and Biological Evaluation of Carboranlymethylbenzo[\textit{b}]acridones as Novel Agents for Boron Neutron Capture Therapy

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Herein we present the synthesis and characterization of benzo[\textit{b}]acridin-12(7\textit{H})-ones bearing carboranyl moieties and test their biological effectiveness as boron neutron capture therapy (BNCT) agents in cancer treatment. The cellular uptake of these novel compounds into the U87 human glioblastoma cells was evaluated by boron analysis (ICP-MS) and by fluorescence imaging (confocal microscopy). The compounds enter the U87 cells exhibiting a similar profile \textit{i.e.}, preferential accumulation in the cytoskeleton and membranes and low cytotoxic activity (IC\textsubscript{50} values higher than 200 µM). The cytotoxic activity and cellular morphological alterations after neutron irradiation in the Portuguese Research Reactor (6.6 \times 10^7 neutrons cm\textsuperscript{-2} s\textsuperscript{-1}, 1 MW) were evaluated by the MTT assay and by electronic microscopy (TEM). Post-neutron irradiation revealed that BNCT has a higher cytotoxic effect on the cells. Accumulation of membranous whorls in the cytoplasm of cells treated with one of the compounds correlates well with the cytotoxic effect induced by radiation. Results provide a strong rationale for considering one of these compounds as a lead candidate for a new generation of BNCT agents.

Keywords: DNA intercalators; fluorescent probes; Benzo[\textit{b}]acridones; dodecarboranes; Boron Neutron Capture Therapy

Introduction

Malignant gliomas are the most common type of primary malignant brain tumour and most frequently found brain tumour diagnosed in up to 49% of cases. According to the World Health Organization (WHO) glioblastoma or grade IV glioma is the most common form of gliomas and represent one of the most aggressive and treatment resistant type of human cancer.\textsuperscript{1,2} Glioblastoma remain an incurable disease despite technological and therapeutic improvements in surgery, radiotherapy and chemotherapy or combined therapeutic modalities. The outlook has improved only modestly, and the survival rate of patients stays less than a few percent.\textsuperscript{3,4} The therapeutic challenge is to develop strategies that can selectively target malignant cells, with little
or no effect on normal cells and tissues adjacent to the
tumour. One such approach is the so called “binary therapy”
in which two non-toxic components can be combined in order
to produce a cytotoxic effect.\textsuperscript{5,6} The advantage is that each
component can be modulated and manipulated independently
to maximize the therapeutic efficacy.

A potential useful binary system is the boron neutron capture
therapy (BNCT). As a binary treatment modality, BNCT
combines a radiosensitizer, \(^{10}\text{B}\) (19.8\% natural abundance)
with thermal neutrons (n), an indirectly ionizing radiation.
The subsequent \((n,\alpha)\) reaction yields 1.47 MeV \(\alpha\) particles
and 0.84 MeV \(^{7}\text{Li}\) ions, both having high linear energy
transfer (LET) causing radiotoxic effects in the range of a cell
diameter (ca. 10 \(\mu\)m). The efficient energy transfer produces
selective damage in the cells containing \(^{10}\text{B}\) so that boron
content and distribution in tumour are pivotal to the
therapeutic efficacy of this treatment modality.\textsuperscript{5-7}

Boron atoms have to be targeted to tumour cells using a
suitable boron carrier in order to maximize tumour cell
damage and minimize total radiation dose to the patient. The
boron drugs sodium borocaptate (BSH) and
boronophenylalanine (BPA) have been extensively studied
and are currently approved to treat glioma, melanoma, head
and neck tumours and hepatocarcinoma.\textsuperscript{8-10} These compounds
are however far from ideal, exhibiting insufficient selectivity
and efficiency, besides other limitations such as reduced
capability to cross the blood brain barrier (BBB) and poor
water solubility (BPA).\textsuperscript{11-13} The subcellular distribution of
these compounds in a glioma cell model showed that most of
the boron was localized in the cytoplasm of the cells, but only
minimal concentrations of boron in the nuclei.\textsuperscript{14}

The major challenge in the development of boron delivery
agents is to deliver a sufficient amount of boron into the
tumour cells to maximize the doses of radiation to the tumour
with minimal normal tissue toxicity.\textsuperscript{15} The use of boron
clusters such as a dodecarborane instead of single boron
atoms, allows increasing loading ability. Moreover,
dodecarborane contain 10 boron atoms and possess a rather
low cytotoxicity and is compatible and extremely stable in
biological media.\textsuperscript{16,17} To increase the selectivity and efficacy
of dodecarboranes towards cancer cells a large variety of
derivatives have been designed and evaluated, taking into
account specific features of cancer cells, such as enhanced
metabolism and over-expression of target receptors.\textsuperscript{18-22} DNA
binding agents and tumour seeking molecules like porphyrins
and porphyrinic macrocyclic compounds, such as
phthalocyanines, are also alternatives for selective delivery of
boron moiety into tumour cells.\textsuperscript{23-27} However, until now there
is no new boron compound that has reached the stage for
phase I clinical trials. Both BSH and BPA still remain today
as the only two drugs in clinical trial for BNCT.\textsuperscript{28}

Research in BNCT involves the design, synthesis and
evaluation of more selective tumour targeting agents. The
close proximity of the boron agent to DNA of tumour cells is
also highly desirable. DNA intercalators such as acridine and
acridone derivatives are excellent candidates since they target
the DNA and, at the same time, act as fluorescent probes to
follow them inside the cells.\textsuperscript{29-33} In addition, these compounds
have attracted considerable attention from scientific community due to their wide range of biological activities.\textsuperscript{34-36} Benzo[b]acridones are much less studied than acridones. The most common derivatives of this type of compounds are benzo[b]acyronycines which present potent broad spectra of antitumour activities.\textsuperscript{37,38} The application of these compounds as fluorescent probes was also described.\textsuperscript{39} Due to our interest in the search for novel antitumour agents, here we present the synthesis, and preliminary biological evaluation of a series of acridone and benzo[b]acridone derivatives bearing carboranyl moieties and the evaluation of its potential as BNCT agents. These results may offer new insights for consideration in malignant glioma treatment strategies.

Results and discussion

Synthesis

The main goal of this study was to prepare benzo[b]acridin-12(7H)-ones bearing carboranyl methyl moieties attached to the nitrogen atom as suitable agents for BNCT. Benzo[b]acridin-12(7H)-ones 1a-c were synthesized through a two-steps method developed by our group.\textsuperscript{40} Propargylation of benzo[b]acridin-12(7H)-ones 1a-c was first attempted by refluxing benzo[b]acridin-12(7H)-one (1a) with an excess of propargyl bromide in acetone using potassium carbonate as base, although even after a long reaction time (20 h) there was still remaining 39% of starting material. Beside this, two new compounds were isolated, one corresponding to the expected propargylated benzo[b]acridin-12(7H)-one 2a (30%) and the other to 7-(propa-1,2-dien-1-yl)benzo[b]acridin-12(7H)-one 3 (15%) (Fig. 1).

Since the obtained yields were not satisfactory and the reaction time was too long it was decided to replace potassium carbonate for sodium hydride (2 equiv) and the reaction was carried out in dry THF. After refluxing the reaction mixture for 4 h it was observed the complete disappearance of benzo[b]acridin-12(7H)-one (1a) and the formation of the desired 7-(prop-2-yn-1-yl)benzo[b]acridin-12(7H)-one (2a) in good yield (72%), being the corresponding allene 3 isolated in 22% yield. After several attempts using different amounts of base and temperatures, the optimal conditions were found and considered the use of
1.8 equiv of sodium hydride in dry THF at 40 °C during 2 h and allowed to obtain only the propargylated benzo[\(b\)]acridin-12(\(7H\))-one \(2a\) in good yield (86%). It was observed that the formation of the allene \(3\) is promoted by increasing the amount of sodium hydride. The reaction of \(1b,c\) with propargyl bromide under the conditions described above gave the corresponding propargylated derivatives \(2b,c\) in good yields (75% and 76%, respectively). These conditions were also used for the propargylation of the commercial acridin-9(\(10H\))-one (\(6\)) and allowed the synthesis of 10-(\(prop-2-yn-1-yl\))acridin-9(\(10H\))-one (\(7\) in very good yield (92%) although, as observed for the case of benzo[\(b\)]acridin-12(\(7H\))-one (\(1a\)), the use of 2 equiv of sodium hydride promoted the formation of 10-(\(propa-1,2-dien-1-yl\))acridin-9(\(10H\))-one (\(8\)).

The cycloaddition reaction of the propargyl triple bond with decaborane were performed following a linear procedure based on the reaction of acetylenic moiety with a bis(acetonitrile)decaborane complex.\(^{41}\) There are some methodologies concerning this type of reaction. Some authors described that bis(acetonitrile)decaborane complex must be formed, by reacting decaborane with acetonitrile in toluene for 1 h, before the reaction with the acetylenic compound.\(^ {42}\) In the first attempt to prepare carboranyl methylbenzo[\(b\)]acridin-12(\(7H\))-one (\(4a\)), \(2a\) was left reacted with 1.5 equiv of decaborane at 80 °C for 24 h, then a new main product was isolated and after characterization revealed to be the corresponding cycloadduct bearing the acridone carbonyl group hydrogenated \(5a\) (45%). Similar results were obtained in the reaction of 7-(\(prop-2-yn-1-yl\))benzo[\(b\)]acridin-12(\(7H\))-ones \(2b,c\) with decaborane (for \(2b\) the reaction proceed in 30 h and afforded 37% of the dihydroacridine \(5b\) and for \(2c\) the reaction proceed in 24 h and afforded 30% of the dihydroacridine \(5c\)).

Trying to understand the reaction mechanism it was decided to perform some studies with commercial available acridin-9(\(10H\))-one (\(6\)), since the synthetic pathway of benzo[\(b\)]acridones involves many steps. Cycloaddition reaction of 10-(\(prop-2-yn-1-yl\))acridin-9(\(10H\))-one (\(7\)) with decaborane afforded after 20 h, under the reaction conditions described above, the 10-carboranyl methyl-9,10-dihydroacridine (\(10\)) in moderate yield (30%). This result led us to reduce the reaction time trying to get the desired adduct \(9\); after 1 h the reaction was stopped since product \(10\) began to be observe. After reaction mixture purification, dihydroacridine \(10\) was isolated in 15% yield and a second product with a similar \(R_f\) value to acridin-9(\(10H\))-one \(7\), that after characterization was identified as the 10-carboranyl methylacridin-9(\(10H\))-one (\(9\)) (63%) (Fig. 1). Thus, it was proved that 10-carboranyl methylacridin-9(\(10H\))-one (\(9\)) is formed in the reaction and over time is completely reduced to the corresponding dihydro derivative \(10\).

Therefore, the cycloaddition reaction of 7-(\(prop-2-yn-1-yl\))benzo[\(b\)]acridin-12(\(7H\))-ones \(2a-c\) with decaborane were performed and finished when the appearance of the dihydroacridine derivative \(5a-c\) was saw, since the \(R_f\) value of 7-carboranyl methylbenzo[\(b\)]acridin-12(\(7H\))-ones \(4a-c\) were very similar to the corresponding starting materials \(2a-c\).

After 2 h for derivatives \(2a\) and \(2b\) and 4 h for derivative \(2c\)
7-carboranylmethylbenzo[b]acridin-12(7H)-ones 4a-c (4a: 53%; 4b: 46%; 4c: 45%) were obtained as major products, although the corresponding 7-carboranylmethyl-7,12-dihydrobenzo[b]acridines 5a-c (5a: 22%; 5b: 15%; 5c: 19%) were always isolated as by-products.

**Cytotoxicity**

In order to evaluate the cytotoxicity of compounds and also to select a dose for irradiation, the U87 human glioma cells, a clinically relevant tumour cell line for BNCT, were treated with compounds 9, 4a-4c in the concentration range 5-200 µM for 6 h continuous incubation at 37°C. A low cytotoxicity for a boronated agent is an important parameter in BNCT so that boron concentrations within tumours can be maximised.

The cytotoxic activity of the boron compounds was determined using the MTT assay which evaluates the reduction of the tetrazolium salt by a mitochondrial dehydrogenase in metabolic active cells to insoluble formazan crystals. The cellular viability in the presence of the tested compounds was compared to that observed in controls (no additions) and the cellular viability (%) was calculated. The corresponding inhibition curves (effect on cellular viability vs. compound concentration) are depicted in Fig. 2. As can be observed the boron compounds 4a-4c did not show activity against the U87 cells in the range 5-200 µM. However, compound 9, the 7-carboranylmethylacridin-9(10H)-one seemed to be more active presenting an IC_{50} value of 225.0 ± 61.3 µM.

**In vitro BNCT**

To evaluate the potential of the compounds 9, 4a-4c as BNCT agents their cytotoxic activities towards U87 cells were tested after neutron irradiation for 5 h at room temperature. Compounds were added at equimolar concentration of 200 µM. The cellular viability was determined by the MTT assay after 24 h incubation in compound free medium. The viability of cells after neutron irradiation in the presence or absence of the tested compounds was compared to that observed in controls (cells only). As can be seen in Fig. 3, neutron irradiation induced an increase in the cytotoxic effect in particular for compound 4a.
Fig. 3 Cellular viability (%) of U87 cells after neutron irradiation. Cells with or without compounds were irradiated at room temperature for $5\text{ h}$ at nominal thermal neutron fluence rate $6.6 \times 10^7 \text{ neutrons cm}^{-2} \text{s}^{-1}$, $1 \text{ MW}$. After irradiation cellular viability was evaluated by the MTT assay. Results as mean $\pm$ SD of $2$ independent experiments show a significant cytotoxic effect induced by neutron irradiation in particular for compound $4a$.

**Cellular distribution by ICP-MS**

As can be observed from Fig. 4, comparative cellular distribution studies of compounds $9$, $4a-4c$ with the U87 cells were conducted in order to determine the boron content delivered by the compounds. The boron content was determined by inductively-coupled plasma mass spectroscopy (ICP-MS) in the cytosol, membrane/particulate, nucleus and cytoskeletal fractions isolated from cells after $6\text{ h}$ exposure to the compounds at $200 \mu\text{M}$. Thus, treatment of cells with compounds showed that total boron content is similar for all compounds with exception of $4c$. Moreover, the amount of boron localized in the membrane and cytoskeletal fractions represent about $80\%$ ($4a$) and more than $90\%$ ($9$, $4b$ and $4c$) of total boron taken up by cells. The uptake in the nucleus was small; the highest value found was $4.6\%$ for $4a$. In addition compound $4a$ present higher uptake in the cytosol.

This result indicates that compounds can target cellular components present mainly at the membrane or cytoskeleton, however without a cytotoxic effect, a factor that are critical in the design of new agents for BNCT. Moreover the concentration of boron deposited into the cell is adequate for this therapeutic modality. We calculated an uptake of approx. $1.4 \times 10^{11}$ B atoms and consequently $2.8 \times 10^{10}$ B atoms per cell, an amount superior to the recommended concentration of $10^8-10^9$ B atoms.$^{25}$

**Fig. 4** The subcellular distribution (boron content) of the carboranylmethylacridones into the U87 cells. Cells were incubated with the compounds at $200 \mu\text{M}$ for $6\text{ h}$ challenge. The cytosol, membrane/particulate, nuclear and cytoskeletal fractions were extracted and their boron content was determined by ICP-MS.
determined by ICP-MS. Results show the boron content expressed as: a) μg/10^6 cells and b) percentage of total uptake (mean ± SD of 2 independent experiments).

**Cellular trafficking by fluorescence microscopy**

The cellular trafficking of compounds was studied by time-lapse confocal fluorescence microscopy in live cells, by taking advantage of their fluorescent properties. Cells were previously incubated with dihydroethidium (DHE) or Hoechst 33342 for whole-cell or nuclei co-localization and imaged every minute for 30 min after addition of compounds in the medium (200 µM, final concentration). As depicted in Fig. 5 4a rapidly accumulates in the U87 cells but not significantly in the nucleus. Considerable accumulation of fluorescence was already evident after 15 min incubation. The accumulation of 4c is slower but present identical features as 4a, i.e., accumulation in the cells but no visible uptake in the nucleus. The cellular trafficking of compounds 9 (absorption/emission: 387/400 nm) and 4b (absorption/emission: 450/560 nm) was not possible to follow. The former, due to the particular excitation and emission characteristics of the compound in the UV spectra, the later, probably due to a lower fluorescence intensity. In fact, in compound 4b there is a conjugation between the methoxyl group oxygen atoms and the benzoacridone nucleus, which means an increase on the electronic density of the benzoacridone nucleus, but the same does not occur in the case of derivative 4c due to the steric hindrance of the bromo substituents. This effect can be observed by the chemical shift of carbon methoxyls, 4b at 55 ppm and 4c at 61 ppm. The presence of the dimethoxy-hydroquinone system could induce fluorescence quenching and the presence of the halogens could counteract this effect.

**Ultrastructural Analysis**

Results from the ultrastructural study of the cells treated with compounds 9, 4a-4c and after neutron irradiation are presented in Fig. 6. Micrographs showed for cells treated with compound 4a a clear accumulation of membranous whorls in the delimiting areas of cytoplasm devoid of organelles, which correlates with the higher cytotoxic effect of this compound combined with radiation. Cells treated with the other compounds did not show significant ultrastructural alterations in comparison with the control.
Ultrastructural studies. Morphological alterations induced by compounds when combined with neutron irradiation.

a) Control cells showing normal organelles in the cytoplasm; b) Cells treated with compound 9; c) Cells treated with compound 4a showing whorls of membranes sequestering areas of cytoplasm devoid of organelles (arrows); d) Cells treated with compound 4b; e) Cells treated with compound 4c. All cells except those treated with compound 4a display normal ultrastructural features. N – Nucleus; M – Mitochondria; V – cytoplasmic vacuoles; G – Golgi apparatus.

Experimental

Materials

Most of the reagents used for synthesis were obtained from Sigma-Aldrich and were used without further purification. Decaborane was obtained from Alfa Aesar. Melting points were determined on a Büchi Melting Point B-540 apparatus and are uncorrected. NMR spectra were recorded on Bruker Avance 300 (300.13 MHz for $^1$H and 75.47 MHz for $^{13}$C) and Bruker Avance 500 (500.13 MHz for $^1$H and 125.76 MHz for $^{13}$C) spectrometers, using CDCl$_3$ as solvent. Chemical shifts ($\delta$) are reported in ppm values and the coupling constants ($J$) in Hz. The internal standard was TMS. $^{13}$C assignments were made using 2D gHSQC and gHMBC (long-range C/H coupling constants were optimised to 7 Hz) experiments. Positive-ion ESI mass spectra were acquired using a Q-TOF 2 instrument [diluting 1 µL of the sample chloroform solution ($\sim$10$^{-5}$ M) in 200 µL of 0.1% formic acid/methanol solution. Nitrogen was used as nebuliser gas and argon as collision gas. The needle voltage was set at 3000 V, with the ion source at 80°C and desolvation temperature at 150°C. Cone voltage was 35 V]. High resolution mass spectra (HRMS-ESI$^+$) were performed on a microTOF (focus) mass spectrometer. Ions were generated using an Apollo II (ESI) source. Ionization was achieved by electrospray, using a voltage of 4500 V applied to the needle, and a counter voltage between 100 and 150 V applied to the capillary. Preparative thin layer chromatography was carried out with Riedel silica gel 60 DGF254, and column chromatography using Acros silica gel 60, 35-70 µm.

Synthesis and characterization

7-(Prop-2-yn-1-yl)benzo[b]acridin-12(7H)-ones 2a-c and 10-(prop-2-yn-1-yl)acridin-9(10H)-one (7)

A solution of the appropriate acridone 1a-c or 6 (0.102 mmol), sodium hydride (4.4 mg, 0.183 mmol) and propargyl bromide (22 µL, 0.204 mmol) in dry THF (3 mL) was refluxed for 2 h (derivative 1a), 2.5 h (derivative 1b) and 4 h
(derivative 1c and 6). After this period light petroleum (5 mL) was added and the reaction mixture was refluxed for more 30 minutes.

The mixture was extracted with AcOEt (2 x 30 mL), and dried over Na$_2$SO$_4$. The residue obtained after concentration of the solvent was purified by flash column chromatography over silica gel using light petroleum:AcOEt (1:1) as eluent.

7-(Prop-2-yn-1-yl)benzo[b]acridin-12(7H)-one (2a)

Yellow solid (24.8 mg, 86%); m.p. 261-262 °C; $^1$H NMR (300.13 MHz, CDCl$_3$): $\delta$ = 2.45 (t, 1H, J 2.4 Hz, H-3'), 5.13 (d, 2H, J 2.4 Hz, H-1'), 7.32 (dd, 1H, J 7.2, 7.7 Hz, H-10), 7.47 (ddd, 1H, J 1.6, 7.2, 8.3 Hz, H-3), 7.58 (d, 1H, J 8.4 Hz, H-8), 7.61 (ddd, 1H, J 1.1, 7.2, 8.3 Hz, H-4), 7.80 (ddd, 1H, J 1.6, 7.2, 8.4 Hz, H-9), 7.91 (s, 1H, H-6), 8.08 (d, 1H, J 8.3 Hz, H-5), 8.08 (d, 1H, J 8.3 Hz, H-2), 8.57 (dd, 1H, J 1.6, 7.7 Hz, H-11), 9.14 (s, 1H, H-1); $^{13}$C NMR (75.47 MHz, CDCl$_3$): $\delta$ = 37.1 (C-1'), 73.8 (C-3'), 77.6 (C-2'), 110.4 (C-6), 114.3 (C-8), 121.2 (C-10), 121.6 (C-11a), 122.7 (C-12a), 124.8 (C-3), 127.1 (C-5), 128.2 (C-11), 128.3 (C-1a), 128.9 (C-4), 129.3 (C-1), 129.6 (C-2), 134.8 (C-9), 136.5 (C-5a), 138.8 (C-6a), 142.7 (C-7a), 179.4 (C-12); ESI$^-$-MS m/z (%) = 284 (100) [M+H]$^-$, 306 (21) [M+Na]$^-$, 589 (68) [2M+Na]$^-$, 872 (4) [3M+Na]$^-$; HRMS-ESI$^-$ m/z for C$_{20}$H$_{14}$NO calcd 284.1070; found 284.1066.

2,5-Dimethoxy-7-(prop-2-yn-1-yl)benzo[b]acridin-12(7H)-one (2b)

Yellow solid (26.3 mg, 75%); m.p. 285-286 °C; $^1$H NMR (300.13 MHz, DMSO-$d_6$): $\delta$ = 2.46 (t, 1H, J 2.2 Hz, H-3'), 4.00 and 4.01 (s, 6H, 2,5-OCH$_3$), 5.36 (d, 2H, J 2.2 Hz, H-1'), 6.81 (d, 1H, J 8.4 Hz, H-3), 7.01 (d, 1H, J 8.4 Hz, H-4), 7.35 (ddd, 1H, J 0.7, 6.7, 7.9 Hz, H-10), 7.82 (d, 1H, J 8.5 Hz, H-8), 7.90 (ddd, 1H, J 1.7, 6.7, 8.5 Hz, H-9), 8.28 (s, 1H, H-6), 8.36 (dd, 1H, J 1.7, 7.9 Hz, H-11), 9.20 (s, 1H, H-1); $^{13}$C NMR (75.47 MHz, DMSO-$d_6$): $\delta$ = 36.2 (C-1'), 55.7 and 55.9 (2,5-OCH$_3$), 75.9 (C-3'), 78.6 (C-2'), 101.8 (C-3), 105.7 (C-6), 106.5 (C-4), 115.6 (C-8), 120.7, 120.8 and 121.3 (C-1a, C-11a and C-12a), 121.4 (C-10), 122.4 (C-1), 127.1 (C-11), 129.0 (C-5a), 135.1 (C-9), 138.3 (C-6a), 142.2 (C-7a), 147.7 (C-5), 149.7 (C-2), 177.8 (C-12); ESI$^-$-MS m/z (%) = 344 (100) [M+H]$^-$, 366 (6) [M+Na]$^-$, 709 (38) [2M+Na]$^-$; HRMS-ESI$^-$ m/z for C$_{22}$H$_{18}$NO$_3$ calcd 344.1281; found 344.1277.
7-(Propa-2-dien-1-yl)benzo[b]acridin-12(7H)-one (3)
Yellow solid (4.3 mg, 15%); m.p. 262-263°C; 1H NMR (300.13 MHz, CDCl₃): δ = 5.47 (d, 2H, J 6.3 Hz, H-3'), 6.68 (t, 1H, J 6.3 Hz, H-1'), 7.27-7.31 (m, 1H, H-10), 7.45 (ddd, 1H, J 1.3, 6.7, 8.4 Hz, H-3), 7.58 (ddd, 1H, J 1.3, 6.7, 8.3 Hz, H-4), 7.71-7.73 (m, 2H, H-8 and H-9), 7.92 (d, 1H, J 8.3 Hz, H-5), 8.03 (s, 1H, H-6), 8.07 (d, 1H, J 8.4 Hz, H-2), 8.56 (dd, 1H, J 0.8, 1.4, 8.1 Hz, H-1); 13C NMR (75.47 MHz, CDCl₃): δ = 83.4 (C-3'), 95.5 (C-1'), 112.1 (C-6), 115.9 (C-8), 121.0 (C-11a), 121.1 (C-10), 122.2 (C-12a), 124.6 (C-3), 127.0 (C-5), 127.9 (C-11), 128.4 (C-1a), 128.6 (C-4), 129.0 (C-1), 129.6 (C-2), 134.2 (C-9), 136.2 (C-5a), 138.9 (C-6a), 142.8 (C-7a), 178.0 (C-9), 209.9 (C-2'); ESI-MS m/z (%) = 284 (100) [M+H]⁺, 306 (22) [M+Na]⁺, 589 (66) [2M+Na]⁻; HRMS-ESI⁺ m/z for C₂₂H₁₆BrNO calcd 499.9485; found 499.9471; for C₂₂H₁₆Br₂NO calcd 501.9471; found 501.9465; for C₂₂H₁₆Br₃NO calcd 503.9450; found 503.9445.

10-(Propa-1,2-dien-1-yl)acridin-9(10H)-one (7)
White solid (6.4 mg, 27%); m.p. 221-222 °C; 1H NMR (300.13 MHz, CDCl₃): δ = 5.42 (d, 2H, J 6.3 Hz, H-3'), 6.60 (t, 1H, J 6.3 Hz, H-1'), 7.28-7.34 (m, 2H, H-2, H-7), 7.69-7.71 (m, 4H, H-3, H-4, H-5, H-6), 8.55 (dd, 2H, J 0.8, 1.4, 8.1 Hz, H-1, H-8); 13C NMR (75.47 MHz, CDCl₃): δ = 83.4 (C-3'), 95.3 (C-1'), 116.2 (C-4 and C-5), 121.8 (C-2 and C-7), 122.2 (C-8a and C-9a), 127.6 (C-1 and C-8), 133.5 (C-3 and C-6), 141.9 (C-4a and C-4b), 178.0 (C-9), 209.9 (C-2'); ESI-MS m/z (%) = 234 (100) [M+H]⁺, 256 (7) [M+Na]⁺, 272 (2) [M+K]⁺; HRMS-ESI⁺ m/z for C₁₆H₁₂NO calcd 234.0913; found 234.0910.

7-Carboranylmethylbenzo[b]acridin-12(7H)-ones 4a-c and 7-carboranylmethylacridin-9(10H)-one (9)
A solution of decaborane (32.2 mg, 0.264 mmol) in acetonitrile (345 µL, 0.660 mmol) and toluene (700 µL for derivatives 2a, 2b and 7 or 1000 µL for derivative 2c) was refluxed for 1 h. Then the appropriated acridone 2a-c or 7 (0.176 mmol) was added and the reaction mixture refluxed for 2 h (derivative 2a, 2b) or 4 h (derivative 2c and 7). The reaction was finished by adding 10 mL of methanol to destroy the excess of decaborane. After cooling, the solvent was evaporated and the residue purified by preparative thin layer chromatography using a mixture of light petroleum:AcOEt (4:1) as eluent.
7-Carboranymethylbenzo[b]acridin-12(7H)-one (4a)

Yellow solid (37.4 mg, 53%); m.p. 304-305 °C; 1H NMR (300.13 MHz, CDCl₃): δ = 0.95-3.32 (m, 10H, BH-carb), 3.68 (br-s, 1H, H-3'), 5.25 (AB, 1H, J 17.3 Hz, H-1', 2'), 5.35 (AB, 1H, J 17.3 Hz, H-1'), 7.38 (dd, 1H, J 7.2, 7.6 Hz, H-10), 7.50 (d, 1H, J 8.7 Hz, H-8), 7.53 (ddd, 1H, J 1.0, 6.8, 8.2 Hz, H-3), 7.67 (ddd, 1H, J 1.1, 6.8, 8.3 Hz, H-4), 7.82 (ddd, 1H, J 1.8, 7.2, 8.7 Hz, H-9), 7.86 (s, 1H, H-6), 7.94 (d, 1H, J 8.3 Hz, H-5), 8.10 (d, 1H, J 8.2 Hz, H-2), 8.63 (dd, 1H, J 1.8, 7.6 Hz, H-11), 9.18 (s, 1H, H-1); 13C NMR (75.47 MHz, CDCl₃): δ = 48.7 (C-1'), 57.9 (C-3'), 73.0 (C-2'), 101.5 (C-3), 104.8 (C-6), 106.2 (C-4), 113.9 (C-8), 121.5 (C-11a or C-12a), 121.6 (C-11a or C-12a), 122.06 (C-1a), 122.08 (C-10), 124.9 (C-1), 128.9 (C-11), 129.5 (C-5a), 134.8 (C-9), 139.0 (C-6a), 142.6 (C-7a), 148.1 (C-5), 150.7 (C-2), 178.3 (C-12); ESI-MS m/z (%) = 459, 460, 461, 462 (100) ([M+H]+, 10B2, 11B3), 463, 464; HRMS-ESI+ m/z for C22H2810B211B3NO3 caleled 462.3067; found 462.3062.

3,4-Dibromo-2,5-dimethoxy-7-carboranymethylbenzo[b]acridin-12(7H)-one (4c)

Yellow solid (49.0 mg, 45%); m.p. 277-278 °C; 1H NMR (300.13 MHz, CDCl₃): δ = 1.03-3.44 (m, 10H, BH-carb), 3.76 (br-s, 1H, H-3'), 4.09 and 4.10 (s, 6H, 2,5-OCH3), 5.23 (AB, 1H, J 17.3 Hz, H-1'), 5.31 (AB, 1H, J 17.3 Hz, H-1'), 7.39 (dd, 1H, J 7.2, 7.6 Hz, H-10), 7.50 (d, 1H, J 8.7 Hz, H-8), 7.84 (dd, 1H, J 1.7, 7.2, 8.7 Hz, H-9), 8.15 (s, 1H, H-6), 8.59 (dd, 1H, J 1.7, 7.6 Hz, H-11), 9.33 (s, 1H, H-1); 13C NMR (75.47 MHz, CDCl₃): δ = 49.8 (C-1'), 58.3 (C-3'), 82.2 (C-2'), 106.2 (C-3), 104.8 (C-6), 106.2 (C-4), 113.9 (C-8), 121.5 (C-11a or C-12a), 121.6 (C-11a or C-12a), 122.06 (C-1a), 122.08 (C-10), 124.9 (C-1), 128.9 (C-11), 129.5 (C-5a), 134.8 (C-9), 139.0 (C-6a), 142.6 (C-7a), 148.1 (C-5), 150.7 (C-2), 178.3 (C-12); ESI-MS m/z (%) = 459, 460, 461, 462 (100) ([M+H]+, 10B2, 11B3), 463, 464; HRMS-ESI+ m/z for C22H2810B211B3NO3 caleled 462.3067; found 462.3062.

2,5-Dimethoxy-7-carboranymethylbenzo[b]acridin-12(7H)-one (4b)

Yellow solid (37.4 mg, 46%); m.p. 288-289 °C; 1H NMR (300.13 MHz, CDCl₃): δ = 1.01-3.41 (m, 10H, BH-carb), 3.72 (br-s, 1H, H-3'), 4.02 and 4.05 (s, 6H, 2,5-OCH3), 5.19 (AB, 1H, J 17.5 Hz, H-1'), 5.35 (AB, 1H, J 17.5 Hz, H-1'), 6.64 (d, 1H, J 8.3 Hz, H-3), 6.82 (d, 1H, J 8.3 Hz, H-4), 7.34 (dd, 1H, J 7.3, 7.7 Hz, H-10), 7.50 (d, 1H, J 8.7 Hz, H-8), 7.79 (ddd, 1H, J 1.7, 7.3, 8.7 Hz, H-9), 8.19 (s, 1H, H-6), 8.59 (dd, 1H, J 1.7, 7.7 Hz, H-11), 9.48 (s, 1H, H-1); 13C NMR (75.47 MHz, CDCl₃): δ = 49.2 (C-1'), 55.7 and 56.0 (2,5-OCH3), 58.2 (C-3'), 73.2 (C-2'), 101.5 (C-3), 104.8 (C-6), 106.2 (C-4), 113.9 (C-8), 121.5 (C-11a or C-12a), 121.6 (C-11a or C-12a), 122.06 (C-1a), 122.08 (C-10), 124.9 (C-1), 128.9 (C-11), 129.5 (C-5a), 134.8 (C-9), 139.0 (C-6a), 142.6 (C-7a), 148.1 (C-5), 150.7 (C-2), 178.3 (C-12); ESI-MS m/z (%) = 459, 460, 461, 462 (100) ([M+H]+, 10B2, 11B3), 463, 464; HRMS-ESI+ m/z for C22H2810B211B3NO3 caleled 462.3067; found 462.3062.

7-Carboranymethyl-7,12-dihydrobenzo[b]acridine (5a)

White solid, (15.0 mg, 22%); m.p. 298-299 °C; 1H NMR (300.13 MHz, CDCl₃): δ = 1.02-3.40 (m, 10H, BH-carb), 3.58
J NMR (75.47 MHz, CDCl\(_2\)), 7.65 (s, 1H, H-6), 8.01 (s, 1H, H-1); compound), 387, 388; 13C NMR (75.47 MHz, CDCl\(_2\)): \(\delta = 33.5\) (C-12), 47.7 (C-1’), 58.2 (C-3’), 75.0 (C-2’), 108.5 (C-6), 112.8 (C-8), 122.5 (C-10), 124.4 (C-3), 125.4 (C-11a), 126.2 (C-4), 126.8 (C-5), 126.9 (C-2), 127.0 (C-1 and C-12a), 127.4 (C-9), 128.8 (C-11), 130.0 (C-1a), 133.0 (C-5a), 140.2 (C-6a), 141.4 (C-7a); ESI-MS \(m/z = 383, 384, 385, 386\) (79) ([M-H]+, \(^{10}\)B\(_2\), \(^{11}\)B\(_3\), acridinium type compound), 387, 388; \(m/z = 385, 386, 387, 388\) (100) ([M+H]+, \(^{10}\)B\(_2\), \(^{11}\)B\(_3\)), 389, 390; HRMS-ESI+ \(m/z\) for C\(_{20}\)H\(_{26}\)\(^{10}\)B\(_2\)\(^{11}\)B\(_3\)N calculated 388.0363; found 388.0361; \(m/z\) for C\(_{20}\)H\(_{26}\)\(^{10}\)B\(_2\)\(^{11}\)B\(_3\)N (acridinium type compound) calculated 386.2906; found 386.2904.

2,5-Dimethoxy-7-carboranyl methyl-7,12-dihydrobenzo[b]acridine (5b)

White solid (20.2 mg, 19%); m.p. 262-263 °C; \(^1^H\) NMR (500.13 MHz, CDCl\(_3\)): \(\delta = 1.38-3.17\) (m, 10H, BH-carb), 3.61 (br-s, 1H, H-3’), 3.98 and 3.99 (s, 6H, 2,5-OCH\(_3\)), 4.06-4.17 (m, 2H, H-12), 4.93 (AB, 1H, J 16.8 Hz, H-1’a), 5.02 (AB, 1H, J 16.8 Hz, H-1’b), 7.01 (d, 1H, J 8.2 Hz, H-8), 7.06 (t, 1H, J 7.4 Hz, H-10), 7.24-7.27 (m, 2H, H-9, H-11), 7.50 (s, 1H, H-6), 7.89 (s, 1H, H-1); \(^1^3^C\) NMR (75.47 MHz, CDCl\(_3\)): \(\delta = 33.5\) (C-12), 47.8 (C-1’), 55.7 and 55.8 (2,5-OCH\(_3\)), 58.1 (C-3’), 75.0 (C-2’), 101.5 (C-3), 102.8 (C-6), 103.4 (C-4), 112.6 (C-8), 121.6 (C-1), 122.2 (C-10), 122.4 (C-1a), 125.0 (C-11a), 125.8 (C-5a), 126.0 (C-12a), 127.3 (C-9), 128.8 (C-11), 140.1 (C-6a), 141.3 (C-7a), 148.6 (C-5), 149.1 (C-2); ESI-MS \(m/z\) = 443, 444, 445, 446 (100) ([M-H]+, \(^{10}\)B\(_2\), \(^{11}\)B\(_3\), acridinium type compound), 447, 448; HRMS-ESI+ \(m/z\) for C\(_{20}\)H\(_{26}\)\(^{10}\)B\(_2\)\(^{11}\)B\(_3\)NO\(_2\) (acridinium type compound) calculated 446.3118; found 446.3112.

10-Carboranyl methylacridin-9(10H)-one (9)

White solid (39.0 mg, 63%); m.p. 278-279 °C; \(^1^H\) NMR (300.13 MHz, CDCl\(_3\)): \(\delta = 1.12-3.45\) (m, 10H, BH-carb), 3.62 (br-s, 1H, H-3’), 5.22 (s, 2H, H-1’), 7.40 (dd, 2H, J 7.3, 7.8...
Hz, H-2, H-7), 7.51 (d, 2H, J 8.7 Hz, H-4, H-5), 7.80 (ddd, 2H, J 1.7, 7.3, 8.7 Hz, H-3, H-6), 8.61 (dd, 2H, J 7.1, 7.8 Hz, H-1, H-8); 13C NMR (75.47 MHz, CDCl₃): δ = 48.4 (C-1'), 58.1 (C-3'), 72.9 (C-2'), 114.0 (C-4 and C-5), 122.6 (C-8a and C-9a), 122.8 (C-2 and C-7), 128.8 (C-1 and C-8), 134.5 (C-3 and C-6), 141.7 (C-4a and C-4b), 177.4 (C-9); ESI⁻-MS m/z (%) = 349, 350, 351, 352 (100) ([M+H⁻], 10B², 11B₈), 353, 354; HRMS-ESI⁺-MS m/z for C₁₆H₂₂¹⁰B₂¹¹B₈N calcd 352.2699; found 352.2695.

10-Carboranylmethyl-9,10-dihydroacridine (10)
White solid (8.9 mg, 15%); m.p. 257-258 ºC; 1H NMR (300.13 MHz, CDCl₃): δ = 1.00-3.30 (m, 10H, BH-carb), 3.55 (br-s, 1H, H-3'), 3.91 (s, 2H, H-9), 4.83 (s, 2H, H-1'), 6.93-7.01 (m, 4H, H-2, H-4, H-5, H-7), 7.17-7.27 (m, 4H, H-1, H-3, H-6, H-8); 13C NMR (75.47 MHz, CDCl₃): δ = 33.0 (C-9), 47.4 (C-1'), 57.9 (C-3'), 75.0 (C-2'), 112.5 (C-4 and C-5), 122.4 (C-2 and C-7), 125.0 (C-8a and C-9a), 127.2 (C-3 and C-6), 128.8 (C-1 and C-8), 141.3 (C-4a and C-4b); ESI⁻-MS m/z (%) = 333, 334, 335, 336 (100) ([M-H⁻], 10B₂, 11B₈, acridinium type compound), 337, 338; HRMS-ESI⁺-MS m/z for C₁₆H₂₂¹⁰B₂¹¹B₈N (acridinium type compound) calcd 336.2750; found 336.2745.

Cell studies

Cell culture and cellular viability
U87 human glioblastoma multiforme cell lines (American Type Culture Collection, ATCC) were cultured in DMEM with GlutaMax I (Gibco) containing 10% fetal bovine serum and 1% antibiotics in a humidified atmosphere (95% air/5% CO₂) at 37°C. The cytotoxic activity of the boron compounds was determined using the MTT assay {MTT = [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]}. For this purpose, cells (10⁴ cells/well) were seeded in growth media in 96-well plates. After 24 h, the media was replaced and cells incubated with the compounds in aliquots of 200 µL/well. Compounds were first solubilized in DMSO and then in medium, and added to final concentrations from 5 µM to 200 µM. The final concentration of DMSO in cell culture medium did not exceed 1%. After continuous exposure for 6 h at 37°C, the medium was discarded and cells incubated with 200 µL of MTT solution in PBS (0.5 mg/mL) for further 3–4 h at 37°C/5% CO₂. Then, the solution was removed and the formazan crystals formed inside the cells dissolved in 200 µL DMSO. The cellular viability was evaluated by comparing the absorbance of the resulting solutions at 570 nm for the treated cells with the absorbance for non-treated cells in a multi-well plate spectrophotometer (PowerWave Xs, Bio-Tek Instruments, USA). The cytotoxic activity of the compounds was quantified by calculating the drug concentration inhibiting tumour cell growth by 50% (IC₅₀), based on non-linear regression analysis of dose response data (GraphPad Prism software).

All compounds were tested in, at least, two independent experiments, each comprising eight replicates per concentration.

Dosimetry
The Portuguese Research Reactor (RPI) was used as neutron source for in vitro cell irradiations. Neutron irradiation was carried out at the vertical access of the thermal column of the
RPI, consisting of a graphite stacking that moderates the fission neutrons. Cell irradiations are performed on top of the graphite using an experimental design similar to that described by others. The radiation field at the irradiation facility was calculated using a general-purpose Monte Carlo code for radiation transport simulation and a detailed model which included a validated model of the reactor core. The calculated neutron energy distribution was further adjusted via the multiple-foil activation method and standard unfolding codes. The intensity of the neutron beam varies with the distance from the core, i.e., is constant (within ±3%) in the parallel direction and decreases with increasing distance with a larger relative contribution of thermal neutrons as more graphite is traversed. The conventional thermal neutron fluence rate (\(\phi_0\)) at the highest intensity position is 6.6 \(\times\) 10\(^7\) neutron cm\(^{-2}\) s\(^{-1}\) at 1 MW reactor power; the ratio between the epithermal neutron fluence rate per unit lethargy (\(\theta\)) and \(\phi_0\) varies between 0.03% and 0.05% in the cell flasks location. The contribution of epithermal neutrons to the dose received by the boron loaded cells is therefore negligible.

Regarding photon doses, low neutron sensitivity thermoluminescent dosimeters of Al\(_2\)O\(_3\):Mg,Y were used to evaluate the accuracy of the photon simulations and measure the photon dose itself. It was concluded that the major (>99%) contribution to the photon dose are neutron interactions with the facility materials, therefore a direct relation between photon dose and thermal neutron fluence can be derived. Details on the procedures may be found in.\(^\text{47}\)

The neutron fluence and photon dose received by the cells in the actual irradiation was monitored using pure gold foils underneath the cell flasks. The ratio of the foil response to the thermal and epithermal neutron fluence rates and to the photon dose rate was previously determined in a calibration run.

**In vitro cell neutron irradiation**

Prior to irradiation, the cells in culture flasks at a density of approximately \(10^6\) cells/5mL medium were pre-incubated for 1 h with the compounds at a concentration of 200 µM in medium. Thereafter the cells were irradiated at room temperature for 5 h at 1 MW reactor power. The conventional thermal neutron fluence and the photon dose imparted to the cells during the irradiation were 6.6\(\times\)10\(^7\) n cm\(^{-2}\) s\(^{-1}\) and 720 mGy, respectively. The maximum discrepancy between the measurements and this reported value is 17% due to the neutron fluence gradient within the irradiation facility.

Exponentially growing U87 cells were distributed in the following groups: 1) untreated cells without neutrons; 2) cells + neutrons; 3) cells with the compounds at 200 µM without neutrons; 4) cells with the compounds at 200 µM + neutrons. Cells that have not been treated with the boron compounds were used as controls. Following irradiation, the cells were placed in boron free medium in a humidified atmosphere containing 5% CO\(_2\) at 37°C. After 24 h the cellular viability was evaluated by the MTT assay. The absorbance of the resulting solutions was measured at 570 nm with a Varian DMS 80 UV-Vis spectrophotometer.

**Boron analysis by ICP-MS**
The total cellular boron content was analysed by a Thermo X-Series Quadrupole inductively coupled plasma mass spectrometer (ICP-MS). U87 cells (approx. $10^6$ cells/5 mL medium) were exposed to the compounds at 200 µM for 6 h at 37°C, then washed with ice-cold PBS and centrifuged to obtain a cellular pellet. The cytosol, membrane/particulate, cytoskeletal and nuclear fractions were extracted using a FractionPREP™, cell fractionation system (BioVision, USA) and performed according to the manufacturer’s protocol. The boron content in the different fractions were measured after digestion of the samples in a closed pressurized microwave digestion unit (Mars5, CEM) with medium pressure HP500 vessels and then diluted in ultrapure water to obtain 2.0% (v/v) nitric acid. The instrument was tuned using a multielement ICP-MS 71 C standard solution (Inorganic Venture). Indium ($^{115}$In) at 10 mM was used as internal standard.

**Uptake by fluorescence microscopy**

Cellular uptake of compounds 4a and 4c was visualized by performing time-lapse confocal microscopy imaging of live U87 cells. Briefly, cells in medium (ca. $10^5$ cells/mL) were seeded on sterile 35 mm Petri dishes (MatTek, Ashland, MA, USA). After 24 h incubation at 37°C, cells were labelled with two different fluorescent dyes (Molecular Probes, Eugene, OR, USA) dehydroethidium (DHE) ($\lambda_{ex}$ 500; $\lambda_{em}$ 600) for compound 4a ($\lambda_{ex}$ 453; $\lambda_{em}$ 507) or Hoechst 33342 ($\lambda_{ex}$ 350; $\lambda_{em}$ 480) for compound 4c ($\lambda_{ex}$ 464; $\lambda_{em}$ 518) at 1 µg/mL for 5 min at 37°C. DHE freely permeates cell membranes and inside the cell is oxidized to ethidium bromide (red fluorescence). After labelling, the cells were washed and maintained in DMEM/F12 without phenol red for live imaging experiments. Cells were imaged with a Zeiss LSM 510 META inverted laser scanning confocal microscope (Carl Zeiss, Germany) fitted with a large incubator for 37°C (Pecon, Germany) with use of a PlanApochromat 63/1.4 oil-immersion objective. Ethidium bromide fluorescence was detected by use of the 514 nm laser line of an argon laser (45 mW nominal output) and a 530-600 nm band-pass filter. Hoechst fluorescence was detected using a Diode 405 nm laser (50 mW nominal output) and a 420-480 nm band-pass filter. The fluorescence of 4a was detected using the 420-480 nm band-pass filter. A transmission detector (PMT) was used to provide a transmission light image of the sample by detecting the scanning 488 nm laser. The pinhole aperture was adjusted in both channels to achieve the same optical slice thickness (1 µm). After addition of the compounds to the cells (200 µm, final concentration) sequential images in both blue (4a) or green (4c) and red (ethidium bromide) or blue (Hoechst) channels were acquired every minute over a 30 min time period.

**Ultrastructural analysis**

After irradiation (cells +/- compounds + neutrons) the culture media was replaced by primary fixative (5 mL) consisting of glutaraldehyde (3%) in sodium cacodylate buffer (pH 7.3, 0.1 M). After primary fixation for 2 h at 4°C, the glutaraldehyde was replaced by sodium cacodylate buffer. Cells were scraped, pelleted, and embedded in agar (2%) for further processing. The samples were washed in cacodylate buffer
and secondarily fixed for 3 h in osmium tetroxide (1%) in sodium cacodylate buffer (pH 7.3, 0.1 M). Then samples were washed in acetate buffer (pH 5.0, 0.1 M) and further fixed in uranyl acetate (0.5%) in the same buffer for 1 h. Dehydration was carried out with increasing concentrations of ethanol. After passage through propylene oxide, the samples were embedded in Epon-Araldite, with use of SPI-Pon as an Epon 812 substitute. Thin sections were made with glass or diamond knives and stained with aqueous uranyl acetate (2%) and Reynold’s lead citrate. The stained sections were studied and photographed with a JEOL 100-SX electron microscope.

Conclusions

Glioblastoma has long been the focus of BNCT research due to its radioresistance and infiltrative growth pattern. Clinical and experimental BNCT using BSH and BPA has shown potential but no proven therapeutic advantages. Research is therefore justified to further evaluate this experimental treatment modality. Recent literature discussed some of more recent results and progress in BNCT providing hope for this unique and exciting mode of clinical therapy.\(^\text{48}\)

Two criteria must be fulfilled for clinical application of BNCT: an adequate source of high flux thermal neutrons along with a boron carrier that is able to concentrate in glioma cells and tissues. High concentration of boron within tumour cell should be obtained in order to maximize the therapeutic effect.

In this study a series of four acridone and benzo[b]acridone derivatives bearing carboranyl moieties have been synthesized, characterized and evaluated for new BNCT agents using the U87 human glioblastoma cells. The compounds enters the cells and deposited an adequate amount of B atoms \((2.8\times10^{10} \text{ B atoms per cell})\) superior to the recommended concentration of \(10^8-10^9\) B atoms.\(^\text{24}\) The compounds also fulfilled the requirement of a low cytotoxicity. Remarkable was the fact that compound 4a, carboranyl methylbenzo[b]acridin-12(7H)-one presented considerably high activity in the U87 cells if combined with neutron irradiation. Therefore, although further studies need to be done, this set of results suggests that compound 4a may be considered as lead compound for a new generation of BNCT agents although implications for therapy applications are at present obviously only exploratory. We found the RPI an excellent facility to conduct in vitro/in vivo experiments and we consider that a standard protocol for further trials had been created.

**Graphical abstract** Acridone derivatives bearing carboranyl moieties as fluorescent probes for boron neutron capture therapy (BNCT) of the glioblastoma.
Acknowledgements

Thanks are due to the University of Aveiro, Portuguese Foundation for Science and Technology (FCT), European Union, QREN, FEDER and COMPETE for funding the QOPNA research unit (project PEst-C/QUI/UI0062/2013) and the Portuguese NMR Network. A.F.F. Silva and R.S.G.R. Seixas thank project PEst-C/QUI/UI0062/2013, QOPNA and FCT for their grants (BI/UI51/5615/2011 and SFRH/BPD/74282/2010, respectively.

Notes

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