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Original article

Azanonaboranes [$(RNH_2)B_8H_{11}NHR$] as possible new compounds for use in boron neutron capture therapy

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Abstract

The synthesis and biological in vitro and in vivo activities of possible new compounds for Boron Neutron Capture Therapy (BNCT) are reported. The azanonaboranes of the type [$(RNH_2)B_8H_{11}NHR$] are water-soluble when hydrophilic groups are introduced. The reaction of $B_9H_{13}SMe_2$ with primary amines yields azanonaboranes. Five compounds with different numbers of hydroxypropyl groups have been isolated: [$(HO(CH_2)_3NH_2)B_8H_{11}NHCH_3$] (**4**), [$(HO(CH_2)_3NH_2)B_8H_{11}NH(CH_2)_3OH$] (**2**), [$((HO(CH_2)_3)_2NH)B_8H_{11}NHCH_3$] (**6**), [$((HO(CH_2)_3)_2NH)B_8H_{11}NH(CH_2)_3OCH_3$] (**11**) and [$((HO(CH_2)_3)_2NH)B_8H_{11}NH(CH_2)_3OH$] (**8**). In vitro experiments as judged by cloning survival tests showed that two of the synthesised compounds are not toxic. The in vivo experiments were carried out with C3H/He mice bearing SCCVII tumours and C57 mice bearing B16 tumours. Compounds **2** and **6** have no particular affinity to any tissue, but are excluded from the brain. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Azanonaborane; BNCT; Toxicity; Tumour; Boron distribution

1. Introduction

Today two different methods for treatment of local limited tumours are used: surgery and radiotherapy based on external beam radiation. In both cases very often chemotherapy is the following therapy. The disadvantage of radiotherapy is that the normal tissue around the tumour will also be destroyed by irradiation. Therefore, not all locally limited tumours can be treated by radiotherapy.

Boron neutron capture therapy (BNCT) [1] is a binary radiotherapy, which is based on the reaction between thermal neutrons and a boron-10 nucleus. The $^{10}B(n,\alpha)^7Li$ reaction was first observed in 1935 [2] and 1936, the employment of boron-10 in cancer therapy was suggested [3]. The neutron absorption by the ^{10}B nucleus produces an excited ^{11}B nucleus which undergoes a fission reaction.

$$_5{}^{10}B + {}_0{}^1n \rightarrow ({}_5{}^{11}B) \rightarrow {}_2{}^4He + {}_3{}^7Li + \gamma 0.48\ \text{MeV} + 2.31\ \text{MeV}\ (94\%)\ \text{and}\quad {}_2{}^4He + {}_3{}^7Li + 2.79\ \text{MeV}\ (6\%)$$

The fission products of this reaction are high linear energy transfer particles, which are able to destroy the mitotic potential of tumour cells.

The cell-killing effect of the boron neutron capture reaction depends on two preconditions. One is the selective accumulation of boron in the target cells. The other is that the tumour cells are reached by sufficient amount of neutrons for triggering neutron capture reactions.

The advantage of boron compounds is the high capture cross section value (3838 barn) [4] of the boron-10 nucleus for neutrons compared with that for other nuclides in biological material. Two of these other nuclides, nitrogen and hydrogen, can contribute to the radiation dose derived from the tissue exposed to the neutron beam because of their high concentrations in tissue. It becomes essential to reach a boron concentration to 20–35 μg g^{-1} or 10^9 ^{10}B atoms per cell [5,6] to minimise the percentage contribution of nitrogen and hydrogen to the total radiation dose.

To be useful for BNCT, boron compounds must fulfil the following criteria: water solubility, stability under physiological conditions, tumour selectivity (tumour: normal tissue ratio high) and low toxicity. At

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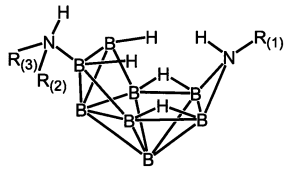

Fig. 1. Azanonaborane with the numbering of the boron atoms with $R_{(1)}$ and $R_{(3)}$ = alkyl and $R_{(2)}$ = H (exo hydrogen atoms are omitted for clarity).

present four principal types of boron carriers (dihydroxyboryl group, dodecaborates, dicarbadodecaboranes and dicarbaundecaborates) are used for BNCT. Compounds with a dihydroxyboryl group (e.g. 4-dihydroxyborylphenylalanine) [7,8] were one of the first ones used for BNCT. The disadvantage of the dihydroxyboryl group is that this boron-delivery agent only contains one single boron atom per molecule. All other carriers contain more boron atoms per molecule. The polyhedral ion ($B_{12}H_{12}^{2-}$) is the boron cluster of one of the most used derivatives for BNCT, mercaptoundecahydrododecarborate(2-) (BSH) and other compounds [9–14]. The *o*-carborane cluster (1,2-dicarbabodecaboranes) compounds generally have a low water solubility; only in one case (BOPP [15–19]) good solubility in water is observed. VCDP [20,21] is an example of a compound containing the nidocarborate cluster instead of the *o*-carborane cluster; it is water-soluble because of the charged cluster.

In the present paper we report on a new type of boron carrier for use in BNCT. The cluster framework contains eight boron atoms and one nitrogen atom and can be described as a nine-vertex azanonaborane (see Fig. 1).

All members of the family $[(RNH_2)B_8H_{11}NHR]$ with R = methyl, ethyl, isopropyl and *t*-butyl [22–25] are air- and water-stable but have limited solubility in water. In order to use this cluster in the synthesis of new boron compounds for BNCT, the azanonaborane $[(RNH_2)B_8H_{11}NHR]$ has to be modified in such a way that its water solubility is increased.

Compared with dodecaborates, dicarbadodecaboranes and dicarbaundecaborates which carry one or two permanent negative charges azanonaboranes are neutral compounds and should have new properties. Examples of water-soluble compounds, their toxicity, and their biodistribution are given.

2. Chemistry

2.1. Synthesis

The azanonaboranes $[(RNH_2)B_8H_{11}NHR]$ are synthesised stepwise by the reaction of $B_9H_{13}SMe_2$ [25] with primary amines. The first step is a ligand exchange reaction. In the second step, an amine is incorporated in the B_9 cluster under loss of one boron atom [22]. The product is an nine vertex cluster with two amine residues (see Fig. 2), one RNH group in the bridging position (part of the cluster framework) derived from the ligand exchange reaction and one amino ligand in exo position derived from the incoming second amine.

To achieve water solubility of the B_8 species, primary amines with additional polar functional groups must be used. Hydrophilic or polar functional groups must be incorporated to achieve water solubility.

There are three possibilities to incorporate polar functional groups as residues R in azanonaboranes of the type $[(RNH_2)B_8H_{11}NHR]$. The first one is to use primary amines with the wanted hydrophilic groups for the synthesis of the azanonaborane. The second one is to use amines with liphophilic residues, which can be modified into hydrophilic residues. A third possibility is to utilise a ligand exchange reaction by refluxing a solution of the unpolar B_8 cluster with an amine [26]. The residues of the amine also could have polar groups or unpolar residues, which can be easily modified. To synthesise an azanonaborane with two hydroxypropyl residues, the first of the described methods would be the easiest. But it is not possible to get a pure compound by reaction of $B_9H_{13}SMe_2$ with propanolamine.

$$B_9H_{13}SMe_2 + H_2N(CH_2)_3OH \rightarrow \text{no pure product}$$

Therefore, it is necessary to choose the second of the described synthetic routes which allows to isolate the wanted compound **2** in pure form. Fig. 3 shows the synthetic route for the new compounds for BNCT. To synthesise **2**, first the reaction of $B_9H_{13}SMe_2$ with allylamine is carried out to yield **1**. The allyl residues are then converted into hydroxypropyl residues by hydroboration and subsequent oxidation (see Fig. 3, route $B_9H_{13}SMe_2$, 1, 2).

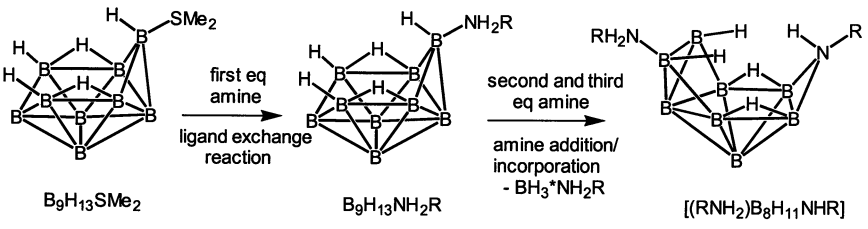

Fig. 2. General synthesis of the azanonaborane (exo hydrogen atoms are omitted for clarity).

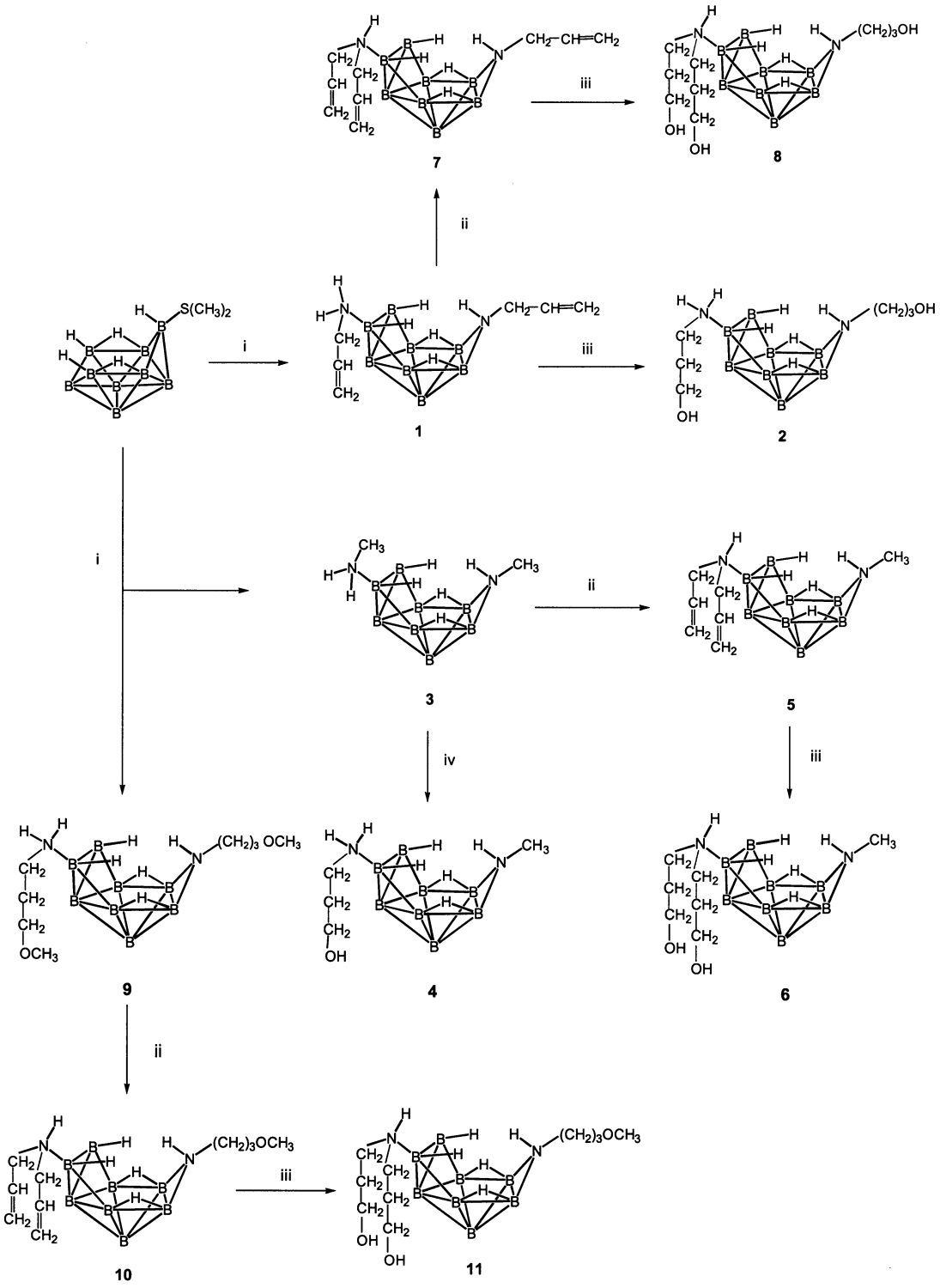

Fig. 3. Synthetic route for the new compounds for BNCT. Conditions: (i) primary amine, benzene, reflux; (ii) diallylamine, benzene, reflux; (iii) diisamylborane, NaOH, H_2O_2, EtOH, 0 °C; (iv) propanolamine, benzene, reflux.

For the synthesis of the other compounds with two hydroxypropyl residues (**6**, **11**) a ligand exchange reaction with diallylamine and subsequent hydroboration and oxidation is necessary (see Fig. 3, route $B_9H_{13}SMe_2$, 3, 5, 6 and $B_9H_{13}SMe_2$, 9, 10, 11).

Compound **4**, an azanonaborane with one hydroxypropyl residue, is synthesised by a ligand exchange reaction of **3** with propanolamine (see Fig. 3, route $B_9H_{13}SMe_2$, 3, 4). To introduce three hydroxypropyl residues in azanonaboranes (see Fig. 3, route

$B_9H_{13}SMe_2$, 1, 7, 8) it is necessary to synthesise **1** at first by using allylamine. The ligand exchange reaction of **1** with diallylamine yields **7** and subsequent hydroboration and oxidation allows to isolate **8**, which contains three hydroxypropyl residues.

3. Pharmacology

After investigation of the toxicity of the three water-soluble compounds by cloning survival tests on V79 Chinese hamster cells in vivo experiments with a tumour model of mice were carried out only with compound **2** and **6** because of the high toxicity of **8**.

The distribution of the water-soluble azanonaboranes containing hydroxypropyl groups in C3H/He mice bearing SCCVII tumours and C57 mice bearing B16 tumours was investigated. Ono et al. studied the combined effect of boronophenylalanine (BPA) and mercaptoundecahydroclosododecaborate (BSH) in BNCT using the SCCVII tumour in C3H/He mice [27]. C57 mice bearing B16 melanoma were used for the in vivo experiments of Morris [28].

4. Results and discussion

With the aim of synthesising azanonaboranes with polar functional residues as possible new compounds for use in BNCT, five new compounds (**2**, **4**, **6**, **8**, **11**) have been prepared. Differences of the water solubility of all five compounds were found. The azanonaborane containing one hydroxypropyl residue bound to the NH_2-ligand and a methyl group bound to the bridging nitrogen (**4**) is not water-soluble. Two hydroxypropyl groups are not sufficient to achieve water solubility when the nitrogen atom in bridging position bears a methoxypropyl group (**11**). To dissolve **11** in water heating to a temperature of 60 °C is necessary. In the case of two hydroxypropyl residues (**2**) as well as in the case of two hydroxypropyl groups and a methyl group bound to the nitrogen bridge (**6**), water solubility is achieved. Solubility in water of **2** is better than of **6**. It is possible to prepare a stock solution of compound **2** with a concentration of 3000 μg boron mL^{-1} (3.48×10^{-2} M), whereas compound **6** can be dissolved to 2000 μg boron mL^{-1} (2.3×10^{-2} M). Both compounds **2** and **6** contain two hydroxypropyl residues in different positions. In the case of compound **6** both hydroxypropyl groups are bound to the nitrogen of the exo ligand. Consequently the hydrophilic part is concentrated on one side of the cluster. In compound **2** one hydroxypropyl residue is bound to the NH_2-ligand while the other hydroxypropyl group is bound to the opposite nitrogen bridge. The water solubility of compound **8** with three hydroxypropyl residues is nearly the same as that of compound **2** (3.46×10^{-2} M). All three compounds have a higher water solubility than BPA (7.66×10^{-3} M; 83 μg boron mL^{-1}) [30]) but a lower water solubility than its derivatives having cascade polyols (monohydroxy derivative BPA(OH): 0.6 M [31], dihydroxy analogue $BPA(OH)_2$: 0.66 M [30] and the tetrahydroxyl analogue $BPA(OH)_4$: 1.2 M [31]). The lower water solubility of the azanonaboranes **2**, **6** and **8** in comparison to the derivatives of BPA is compensated by the higher boron content, so that the same boron concentrations can be easily reached for in vitro experiments.

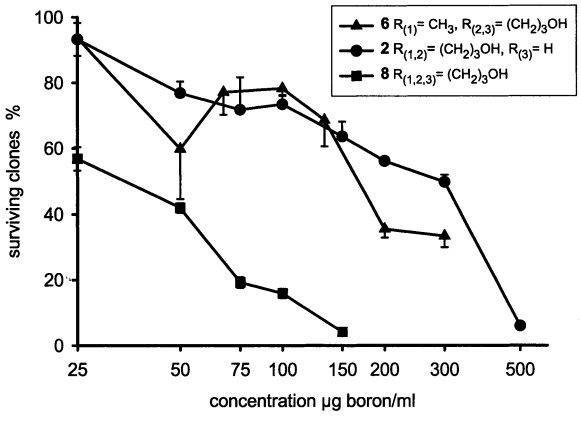

Fig. 4. Investigation of the toxicity of compounds **2**, **6** and **8**. Values are mean ± S.D.

The investigation of the stability of the water-soluble compounds **2**, **6** and **8** which are dissolved in water at room temperature (r.t.) was carried out by ^{11}B-NMR measurements. At different periods of time the ratio of compound to boric acid was determined. The data were interpreted as a first-order kinetics. The rate constant for all three compounds is $k = 0.05$ per day, corresponding to a half-life of 14 days.

Incubation concentrations for all in vitro experiments varied between 25 and 300 μg boron mL^{-1} (see Fig. 4).

For compound **2**, a concentration of 500 μg boron mL^{-1} was also investigated. The results of the clonogenic survival tests show a IC_{50} for compound **2** at 300 μg boron mL^{-1} (3.48×10^{-3} M) and for compound **6**, at 180 μg boron mL^{-1} (2.08×10^{-3} M) (Table 1).

At concentrations higher than 300 μg boron mL^{-1} cell kill is pronounced for compound **2**. The percentage of surviving clones at 500 μg boron mL^{-1} is only 6%. Compound **8** is more toxic than compound **2** and **6** because its IC_{50} is reached at 35 μg boron mL^{-1} (4.05×10^{-4} M) (Table 1). The investigation of the toxicity of compound **2**, **6** and **8** showed that the more CH_2 units the compound contains, the more toxic the compound is.

For comparison the IC_{50} values (defined as the dose that failed to kill 50% of cultured B16 human melanoma cells after 3 days of incubation [32,33]) of

Table 1
IC_{50} values and survival ratios of compound **2**, **6**, **8**, BPA, its derivatives and MACB

Compound	IC_{50} values	Survival ratio (162 μgB mL^{-1}; 1.5×10^{-2} M [31])
2 [a]	300 μgB mL^{-1}; 3.48×10^{-3} M	–
6 [a]	180 μgB mL^{-1}; 2.08×10^{-3} M	–
8 [a]	35 μgB mL^{-1}; 4.05×10^{-4} M	–
BPA [b]	93 μgB mL^{-1}; 8.6×10^{-3} M [32]	$20 \pm 1\%$
BPA(OH) [b]	–	$60 \pm 2\%$
$BPA(OH)_2$ [b]	194 μgB mL^{-1}; 1.8×10^{-2} M [32]	$60 \pm 2\%$
$BPA(OH)_4$ [b]	–	$62 \pm 2\%$
MACB [b]	0.595 μgB mL^{-1}; 5.5×10^{-6} M [33]	–

[a] V79 Chinese hamster cells; incubation time: 20 h.
[b] B16 melanoma cells; incubation time: 3 days.

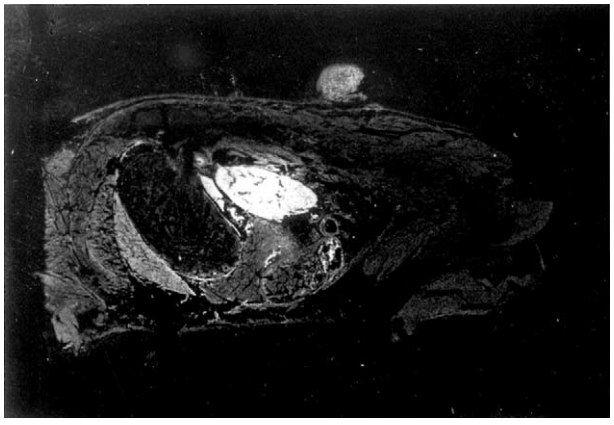

Fig. 5. Whole-body alpha-autoradiogram of a B16 melanoma tumour bearing mouse. The animal was injected intraperitoneally with 1000 μg boron/0.5 mL of compound **2** and sacrificed after 1 h. Structure on top is tumour; bright structure is kidney; structure left of kidney is stomach; structure left of stomach is liver.

BPA, $BPA(OH)_2$ and 1-carboranyl-3-(2-methylaziridino)-2-propanol (MACB) are given in Table 1. Also survival ratios of BPA and its derivatives which were determined after 3 days of incubation at a concentration of 1.5×10^{-2} M (162 μg boron mL^{-1}) are listed [31]. Direct comparison of the toxicity of BPA and its derivatives with azanonaboranes **2**, **6** and **8** is not possible because of the different incubation times and different cell lines. Two of the three water-soluble azanonaboranes (**2**, **6**) are not toxic, so that in vivo studies could be carried out with tumour bearing mice to get information about the biodistribution of these compounds.

By interpretation of the biodistribution of compound **2** and **6** in SCCVII and B16 melanoma tumour bearing mice by quantitative neutron capture radiography (QNCR), boron can be found in tumour tissue (see Fig. 5). Other tissues also contain boron; liver to the same extent as the tumour. Higher boron concentrations can be found in kidney, which suggests that the kidney is involved in the metabolism of both compounds. Virtually no boron can be found in brain.

5. Conclusion

The nine vertex azanonaborane $[(RNH_2)B_8H_{11}NHR]$ is an interesting boron carrier for BNCT because it is a neutral compound compared with presently used boron cluster which are either once or twice negatively charged or very hydrophobic. These azanonaboranes where R is a methyl, ethyl, isopropyl or *tert*-butyl residue are air- and waterstable but have a limited solubility in water. It is possible to introduce one, two or three hydroxypropyl residues by modification of allyl groups (**2**), by ligand exchange reaction (**4**) and by ligand exchange reaction with subsequent modification of the residues (**6**, **8**, **11**). It is necessary to introduce two or more hydroxypropyl groups to the azanonaborane $[(RNH_2)B_8H_{11}NHR]$ to achieve water solubility. Compound **2**, **6** and **8** have a half-life of 14 days in water at r.t. The in vitro experiments by clonogenic survival tests with V79 Chinese hamster cells show that compound **2** and **6** are not toxic while compound **8** is toxic. The azanonaboranes **2** and **6** can be investigated with regard to their biodistribution in tumour bearing mice.

The biodistribution in tumour bearing mice shows no enrichment of **2** and **6** in any special organ except the kidney. Both compounds are found in the same concentration in tumour and in other tissue. To enhance boron uptake in tumour modification of the azanonaboranes are necessary to optimise tumour seeking properties for use in BNCT.

6. Experimental

6.1. Chemistry

Reactions were carried out in dry solvents. Preparative thin-layer chromatography was carried out using 0.75 mm layers of silica gel G (Merck GF_{254}) made from water slurries on glass plates of dimensions of 20×20 cm^2, followed by drying in air at 100 °C. $B_9H_{13}SMe_2$ was prepared by literature method [25]. All other reagents were obtained commercially. NMR spectroscopy was carried out on a Bruker DPX200 instrument operating at 4.7 T. Chemical shifts δ are given in ppm relative to TMS and $BF_3 \cdot OEt$. The NMR data are listed in the following way: NMR (solvens): $\delta(^{11}B)$

[δ(^{1}H)] (position) and (^{13}C) [δ(^{1}H)] (position). Mass spectrometry data were measured at a Finnigan MAT 8222 by fast atom bombardment (FAB) with glycerol or nitrobenzylalcohol (NBA) as matrix and electron impact (EI) at 473 K and 70 eV. Only the signal with the highest intensity of the boron isotopic pattern is listed. The infrared spectroscopy data were carried out on a BIO-RAD SPC 3200 infrared spectrometer. The spectra were measured in KBr. The unit of the absorption values is cm^{-1}. The elemental analyses were measured at a Perkin–Elmer 2400 automatic elemental analyser of compound **2**, **6** and **8**.

*6.1.1. General procedure for compounds (**1**–**11**)*

*6.1.1.1. [(allylNH$_2$)B$_8$H$_{11}$NHallyl] (**1**).* $B_9H_{13}SMe_2$ (945 mg, 5.48 mmol) is dissolved in 5 mL of benzene. After adding 2.25 mL (30 mmol) allylamine, the solution is refluxed for 2 h. Then the solvent is removed by reduced pressure. Recrystallisation from ethanol–water affords **1**. Yield: 36% (415 mg, 1.97 mmol); NMR ($CDCl_3$): −55.1 [−0.62] (B(2)H), −32.1 [+0.78, +0.78, +0.52 (exo), −0.62 endo)] (B(4)H, B(7)H and (B(8)H$_2$), −20.4 [+1.25] (B(3)H), −10.5 [+2.43] (B(5)H and B(6)H), +2.0 [+2.62] (B(1)H), μ(4.5) [−2.09], μ(6.7) [−2.14], +131.7 [+5.72] (NHCH$_2$**CH**), +130.9 [+5.99] (NH$_2$CH$_2$**CH**), +121.4 [+5.36] (NH$_2$CH$_2$CH**CH$_2$**), +118.4 [+5.10] (NHCH$_2$CH**CH$_2$**), +53.8 [+3.18] (NH**CH$_2$**), +52.5 [+3.71] (NH$_2$**CH$_2$**), [+4.22/+4.35] (N**H$_2$**), [−1.20] (N**H**); EIMS: *m*/*z*: 211 ([M + H]$^+$, 32%); IR: 3410 (s, *v*, NH$_2$/NH); 2529, 2433 (s, *v*, BH), 353 (m, *v*, BN), 2850 (w), 1642 (m), 1436 (s), 1363 (s), 1254 (w), 1091 (m), 1026 (m) 924 (s) (*v*, δ, γ of CH$_3$-, CH$_2$-, and CH-groups).

*6.1.1.2. [(HO(CH$_2$)$_3$NH$_2$)B$_8$H$_{11}$NH(CH$_2$)$_3$OH] (**2**).* Compound **1** (300 mg, 1.43 mmol) is dissolved in 5 mL of THF. At 0 °C 6.5 mL of diisamylborane solution (0.5 M in THF) are added. After stirring for 3 h at r.t. the solution is cooled to 0 °C, mixed with 1.4 mL 3 N NaOH, 1.4 mL 30% H_2O_2 and 2.1 mL EtOH, and stirred for 3 h. The solvent is removed by reduced pressure. Purification by thin-layer chromatography on silica gel and CH_2Cl_2–THF (3:1) ($R_f = 0.3$) affords **2**. Yield: 48% (169 mg, 0.686 mmol); NMR (D_2O): −55.6 [−0.84] (B(2)H), −33.0 [+0.64/+0.46/+0.26] (B(4)H, B(7)H and (B(8)H$_{exo}$)), −33.0 [−0.70] (B(8)H$_{endo}$), −20.2 [+1.09] (B(3)H), −10.2 [+2.35] (B(5)H and B(6)H), +1.1 [+2.49] (B(1)H), μ(4.5) [−2.07], μ(6.7) [−2.07], +60.2 [+3.53] (NH(CH$_2$)$_2$-**CH$_2$**OH), +59.7 [+3.66] (NH$_2$(CH$_2$)$_2$**CH$_2$**OH), +50.1 [+2.69] (NH**CH$_2$**), +46.5 [+2.97] (NH$_2$**CH$_2$**), +30.3 [+1.89] (NH$_2$CH$_2$**CH$_2$**), +29.5 [+1.67] (NHCH$_2$-**CH$_2$**); FABMS (Glycerol): *m*/*z*: 246 ([M]$^+$, 18%), *m*/*z*: 246 ([M]$^-$, 91%); Anal. ($B_8C_6H_{28}N_2O_2$) C, H, N; IR: 3392 (s, *v*, OH), 3237 (s, *v*, NH$_2$/NH), 2523, 2428 (s, *v*, BH), 1601 (w, δ, NH$_2$/NH), 1353 (s, *v*, BN), 2955 (m), 2884 (m), 1456 (m), 1430 (m), 1404 (m), 1186 (m), 1114 (s), 1052 (s), 1010 (m) (*v*, δ, γ of CH$_3$-, CH$_2$-, and CH-groups).

*6.1.1.3. [(CH$_3$NH$_2$)B$_8$H$_{11}$NHCH$_3$] (**3**).* Compound **3** was prepared by literature method [25].

*6.1.1.4. [(HO(CH$_2$)$_3$NH$_2$)B$_8$H$_{11}$NHCH$_3$] (**4**).* A mixture of 40 mg (0.252 mmol) of **3** and 23 μL (0.302 mmol) propanolamine in 15 mL benzene is refluxed for 1.5 h. The solvent is removed by reduced pressure. The residue is purified by thin-layer chromatography on silica gel and CH_2Cl_2–THF 1:1 ($R_f = 0.27$). Yield: 30.4% (31 mg, 0.153 mmol); NMR (THF-d_8): −55.2 [−0.71] (B(2)H), −32.4 [+0.67] (B(7)H), −32.4 [+0.67] (B(4)H), −32.4 [−0.71 (endo), +0.43 (exo)] (B(8)H$_2$), −20.4 [+1.11] (B(3)H), −9.7 [+2.37] (B(5)H and B(6)H), +1.7 [+2.49] (B(1)H), μ(4.5) [−2.00], μ(6.7) [−2.11], +63.0 [+3.90] (NH$_2$(CH$_2$)$_2$-**CH$_2$**OH), +50.1 [+3.24] (NH$_2$**CH$_2$**), +38.5 [+2.38] (NH**CH$_3$**), +29.0 [+1.94] (NH$_2$CH$_2$**CH$_2$**), [+4.95/+4.83] (N**H$_2$**(CH$_2$)$_3$OH), [−1.31] (N**H**CH$_3$); FABMS (NBA): *m*/*z*: 202 ([M]$^+$, 45%), *m*/*z*: 202 ([M]$^-$, 52%), 188 ([M − CH$_3$ + H]$^-$, 43%); IR: 3254 (s), 3209 (s) (*v*, NH$_2$/NH), 2541 (s), 2493 (s) (*v*, BH), 1578 (m), 1383 (m) (*v*, BN), 2954 (w), 1463 (m), 1297 (w), 1198 (w), 1143 (s), 1096 (m), 1067 (m), 1043 (m) (*v*, δ, γ of CH$_3$-, CH$_2$-, and CH-groups).

*6.1.1.5. [(allyl)$_2$NH)B$_8$H$_{11}$NHCH$_3$] (**5**).* A mixture of 108 mg (0.681 mmol) of **3** and 110 μL (0.886 mmol) diallylamine is refluxed for 2 h in 15 mL benzene. After refluxing, the solvent is removed by reduced pressure. Thin-layer chromatography on silica gel and CH_2Cl_2 ($R_f = 0.46$) afforded **5**. Yield: 25% (39 mg, 0.174 mmol); NMR ($CDCl_3$): −54.8 [−0.64] (B(2)H), −33.2 [+0.70] (B(7)H), −32.8 [+0.70] (B(4)H), −31.1 [−0.64 (endo), +0.57 (exo)] (B(8)H$_2$), −16.5 [+1.19] (B(3)H), −9.9 [+2.39] (B(5)H and B(6)H), +1.9 [+2.67] (B(1)H), μ(4.5) [−1.94], μ(6.7) [−2.12], +129.0/128.9 [+6.08] (NHCH$_2$**CH**), +123.5 [+5.45] (NHCH$_2$CH**CH$_2$**), +56.0/+55.8 [+3.6 to +4.0] (NH**CH$_2$**), +38.4 [+2.40] (NH**CH$_3$**), [+3.70] (N**H**(CH$_2$CHCH$_2$)$_2$), [−1.29] (N**H**CH$_3$); FABMS (Glycerol): *m*/*z*: 224 ([M]$^+$, 48%), *m*/*z*: 223 ([M]$^-$, 66%); IR: 3292 (s, *v*, NH), 2530 (s, *v*, BH), 1362 (m, *v*, BN), 2925 (s), 1647 (m), 1436 (s), 1253 (s), 1146 (s), 1094 (s), 1028 (m) (*v*, δ, γ of CH$_3$-, CH$_2$-, and CH-groups).

*6.1.1.6. [((HO(CH$_2$)$_3$)$_2$NH)B$_8$H$_{11}$NHCH$_3$] (**6**).* Compound **5** (364 mg, 1.61 mmol) is dissolved at 0 °C in 8 mL diisamylborane solution (0.5 M in THF). After stirring for 1 h at r.t. the solution is cooled to 0 °C, mixed with 1.6 mL 3 N NaOH, 1.6 mL 30% H_2O_2 and

2.4 mL EtOH, and stirred for 1 h. The solvent is removed by reduced pressure. The residue is purified by thin-layer chromatography on silica gel as solid phase and CH_2Cl_2–THF (1:1) ($R_f = 0.32$). Yield: 24% (100 mg, 0.38 mmol); NMR (D_2O): −55.6 [−0.93] (B(2)H), −33.1 [+0.55/+0.27/+0.27] (B(4)H, B(7)H and ($B(8)H_{exo}$)), −33.1 [−0.68] ($B(8)H_{endo}$), −16.2 [+1.07] (B(3)H), −9.8 [+2.27] (B(5)H and B(6)H), +0.6 [+2.50] (B(1)H), μ(4.5)/μ(6.7) [−2.08], +59.9 [+3.09] ($NH(CH_2)_2\mathbf{CH_2}OH$), +51.5/+51.1 [+3.09] ($NH\mathbf{CH_2}$), +27.1/+26.8 [+1.98] ($NH_2CH_2\mathbf{CH_2}$), +38.8 [+2.27] ($NH\mathbf{CH_3}$); FABMS (Glycerol): *m*/*z*: 260 ($[M]^+$, 12%), *m*/*z*: 260 ($[M]^-$, 30%); Anal. ($B_8C_7H_{30}N_2O_2$) C, H, N; IR: 2530 (s, *ν*, BH), 2956 (w), 2884 (w), 1463 (s), 1343 (s), 1145 (s), 1025 (s) (*ν*, *δ*, *γ* of CH_3-, CH_2-, and CH-groups).

*6.1.1.7. [((allyl)$_2$NH)B$_8$H$_{11}$NHallyl] (**7**).* Compound **1** (245 mg, 1.164 mmol) is dissolved in 10 mL of benzene and 186 μL (1.513 mmol) diallylamine are added. After refluxing for 2 h the solvent is removed by reduced pressure. The residue is purified by thin-layer chromatography on silica gel and CH_2Cl_2 ($R_f = 0.64$). Yield: 20.5% (60 mg, 0.239 mmol); NMR ($CDCl_3$): −54.7 [−0.58] (B(2)H), −33.9 [+0.74] (B(7)H), −32.7 [+0.74] (B(4)H), −31.5 [−0.58 (endo), +0.63 (exo)] ($B(8)H_2$)), −16.5 [+1.24] (B(3)H), −10.1 [+2.50/+2.44] (B(5)H and B(6)H), +1.5 [+2.70], (B(1)H); μ(4.5) [−1.90], μ(6.7) [−2.12], +131.6 [+5.74] ($NHCH_2\mathbf{CH}$), +129.0/+128.9 [+6.07] ($NH(CH_2\mathbf{CH}CH_2)_2$), +123.0 [+5.46] ($NH(CH_2CH\mathbf{CH_2})_2$), +118.3 [+5.20] ($NHCH_2CH\mathbf{CH_2}$), +56.0 [+3.85/+3.74] and +55.9 [+3.85/+3.74] ($NH(\mathbf{CH_2}CHCH_2)_2$), +53.7 [+3.19] ($NH\mathbf{CH_2}$), [−1.16] ($N\mathbf{H}CH_2CHCH_2$), [+3.72] ($N\mathbf{H}(CH_2CHCH_2)_2$); FABMS (Glycerol): *m*/*z*: 250 ($[M]^+$, 13%), *m*/*z*: 249 ($[M]^-$, 12%); IR: 3238 (m, *ν*, NH), 2529 (s, *ν*, BH), 2941 (w), 2884 (w), 1643 (w), 1450 (s),1422 (m), 1240 (s), 1082 (s), 995 (s) (*ν*, *δ*, *γ* of CH_3-, CH_2-, and CH-groups).

*6.1.1.8. [((HO(CH$_2$)$_3$)$_2$NH)B$_8$H$_{11}$NH(CH$_2$)$_3$OH] (**8**).* Compound **7** (450 mg, 1.61 mmol) is dissolved at 0 °C in 15 mL diisamylborane solution (0.5 M in THF). After stirring for 1 h at r.t. the solution is cooled to 0 °C, mixed with 3 mL 3 N NaOH, 3 mL 30% H_2O_2 and 4.5 mL EtOH, and stirred for 1 h. The solvent is removed by reduced pressure. The residue is purified by thin-layer chromatography on silica gel and CH_2Cl_2–THF (1:2) ($R_f = 0.42$). Yield: 20% (109 mg, 0.36 mmol); NMR (THF-d_8): −54.9 [−0.56] (B(2)H); −33.5 [−0.62 (endo), +0.48 (exo)] ($B(8)H_2$); −33.5 [+0.59] (B(7)H); −30.5 [+0.59] (B(4)H); −15.9 [+1.15] (B(3)H); −10.9 [+2.31] (B(5)H and B(6)H); +1.1 [+2.65] (B(1)H); μ(4.5) = −2.10; μ(6.7) = −2.23; +60.7 [+3.82] ($NH((CH_2)_2\mathbf{CH_2}OH)_2$); +60.0 [+3.59] ($NH(CH_2)_2\mathbf{CH_2}OH$); +53.3 [+3.32/+3.46] and +52.8 [+3.11/+3.42] ($NH(\mathbf{CH_2}(CH_2)_2OH)_2$); +50.5 [+2.63] ($NH\mathbf{CH_2}(CH_2)_2OH$); +30.3 [+1.59] ($NHCH_2\mathbf{CH_2}CH_2OH$); +27.8/+27.3 [+1.96] ($NH(CH_2\mathbf{CH_2}CH_2OH)_2$); [−0.38] ($N\mathbf{H}((CH_2)_3OH)$; [+5.72] ($N\mathbf{H}((CH_2)_3OH)_2$); OH [+4.11/+3.86]; FABMS (NBA): *m*/*z*: 304 ($[M]^+$, 5%), *m*/*z*: 303 ($[M]^-$, 20%); Anal. ($B_8C_9H_{34}N_2O_3$) C, H, N.

*6.1.1.9. [(H$_3$CO(CH$_2$)$_3$NH$_2$)B$_8$H$_{11}$NH(CH$_2$)$_3$OCH$_3$] (**9**).* $B_9H_{13}SMe_2$ (180 mg, 1.04 mmol) is dissolved in 10 mL of benzene and 450 μL (4.4 mmol) 3-methoxypropylamine are added. After 2 h refluxing the solvent is evaporated by reduce pressure. The residue is purified by thin-layer chromatography on silica gel and CH_2Cl_2 ($R_f = 0.46$). Yield: 48.8% (140 mg, 0.51 mmol); NMR ($CDCl_3$): −55.2 [−0.64] (B(2)H), −32.3 [+0.63, 0.63, +0.41 (exo), −0.64 (endo)] (B(4)H, B(7)H and $B(8)H_2$), −20.4 [+1.41] (B(3)H); −10.5 [+2.32] (B(5)H and B(6)H); +1.9 [+2.50] (B(1)H); μ(4.5) [−2.06], μ(6.7) [−2.20], +73.5 [+3.57] ($NH_2(CH_2)_2\mathbf{CH_2}OCH_3$), +71.7 [+3.35] ($NH(CH_2)_2\mathbf{CH_2}OCH_3$), +59.2 [+3.30] ($NH_2(CH_2)_3O\mathbf{CH_3}$), +58.9 [+3.24] ($NH(CH_2)_3O\mathbf{CH_3}$), +51.4 [+2.66] ($NH\mathbf{CH_2}$), +50.9 [+3.20] ($NH_2\mathbf{CH_2}$), +28.2 [+1.91] ($NH_2CH_2\mathbf{CH_2}CH_2$), +27.7 [+1.72] ($NHCH_2\mathbf{CH_2}CH_2$) [+4.77] ($N\mathbf{H_2}$); [−0.29] ($N\mathbf{H}$); FABMS (NBA): *m*/*z*: 274 ($[M]^+$, 15%), 260 ($[M - CH_3 + H]^+$, 5%), *m*/*z*: 274 ($[M]^-$, 100%), 260 ($[M - CH_3 + H]^-$, 58%); IR: 3224 (m, *ν*, NH_2/NH), 2829 (w, *δ*, OCH_3), 2517, 2414 (s/m, *ν*, BH), 1304 (w, *ν*, BN), 1112 (s, *γ*, COC), 2824 (m), 2879 (m), 1458 (m), 1401 (m), 1185 (m) (*ν*, *δ*, *γ* of CH_3-, CH_2-, and CH-groups).

*6.1.1.10. [((allyl)$_2$NH)B$_8$H$_{11}$NH(CH$_2$)$_3$OCH$_3$] (**10**).* A mixture of 2 g (7.287 mmol) of **9** and 1.2 mL (9.745 mmol) diallylamine in benzene is refluxed for 2 h. After evaporating the solvent, the residue is purified by thin-layer chromatography on silica gel and CH_2Cl_2 (R_f = 0.47). Yield: 20% (411 mg, 1.45 mmol); NMR ($CDCl_3$): −55.2 [−0.64] (B(2)H); −32.3 [+0.73] (B(4)H and B(7)H), −32.6 [+0.58 (exo), −0.53 (endo)] ($B(8)H_2$), −16.4 [+1.27] (B(3)H), −10.3 [+2.46] (B(5)H and B(6)H), +2.3 [+2.46] (B(1)H), μ(4.5) [−1.92], μ(6.7) [−2.16], +129.1 [+6.10] ($NHCH_2\mathbf{CH}CH_2$), +123.4 [+5.47] ($NHCH_2CH\mathbf{CH_2}$), +71.4 [+3.38] ($\mathbf{CH_2}OCH_3$); +58.7 [+3.28] ($O\mathbf{CH_3}$), +55.9 [+3.84] ($NH\mathbf{CH_2}CHCH_2$), +51.1 [+2.74] ($NH\mathbf{CH_2}$-$(CH_2)_2OCH_3$), +27.2 [+1.72] ($NHCH_2\mathbf{CH_2}CH_2$-OCH_3), [+3.78] ($N\mathbf{H}allyl_2$), [−0.14] ($N\mathbf{H}$); EIMS (70 eV, 473 K): *m*/*z*: 283 ($[M]^+$, 15%); IR: 2526 (s) (s, *ν*, BH), 1647 (w), 1426 (m), 1250 (w), 1107 (s), 1027 (m), 925 (m) (*ν*, *δ*, *γ* of CH_3-, CH_2-, and CH-groups).

*6.1.1.11. [((HO(CH$_2$)$_3$)$_2$NH)B$_8$H$_{11}$NH(CH$_2$)$_3$OCH$_3$] (**11**).* Compound **10** (560 mg, 1.982 mmol) is dissolved in 12 mL diisamylborane solution (0.5 M in THF). After 2 h stirring, the solution is cooled to 0 °C and 2.4 mL 3 N NaOH, 2.4 mL 30% H_2O_2 and 3.6 mL ethanol is added. After 2 h, the solvent is removed by reduced pressure. The residue is purified by thin-layer chromatography on silica gel and CH_2Cl_2–THF 1:1 (R_f = 0.45). Yield: 20.9% (132 mg, 0.414 mmol); NMR ($CDCl_3$): −54.1 [−0.82] (B(2)H), −32.2 [+0.38] (B(4)H and (B(7)H), −32.2 [−0.82 (endo), +0.41 (exo)] (B(8)H_2), −14.8 [+1.00] (B(3)H), −9.9 [+2.14] (B(5)H and B(6)H), +2.0 [+2.50] (B(1)H), μ(4.5) [−2.22], μ(6.7) [−2.39], +70.7 [+3.15] (**CH_2**OCH$_3$), +60.6 [+3.54] (**CH_2**OH), +58.1 [+3.06] (O**CH_3**), +53.3/+52.9 [+3.28/+2.95] (NH**CH_2**(CH$_2$)$_2$OH), +50.3 [+2.49] (NH**CH_2**(CH$_2$)$_2$OCH$_3$), +26.9/+26.6 [+1.82/+1.48] (**CH_2**CH$_2$OH), +26.3 [+1.65] (**CH_2**CH$_2$OCH$_3$), [+5.58] (N**H**((CH$_2$)$_3$OH)$_2$), [−0.51] (N**H**(CH$_2$)$_3$OCH$_3$), [+3.87/3.75] (OH); FABMS (NBA): *m/z*: 318 ([M]$^-$, 45%); IR: 3331 (m) (OH), 2538, 2511, 2490 (s, *ν*, BH), 2962 (m), 2926 (m), 2884 (m), 1461 (m), 1409 (m), 1355 (w), 1261 (m), 1188 (w), 1133, 1104, 1053, 801 (s) (*ν*, *δ*, *γ* of CH$_3$-, CH$_2$-, and CH-groups).

6.2. Pharmacology

The toxicity of compound **2**, **6** and **8** was determined in cloning survival test on V79 Chinese hamster cells. The clonogenic assay is based on the ability of the cells to grow and form daughter cells in the fifth generation after exposition to the compound. The in vivo experiments were carried out with compounds **2** and **6** and with C3H/He mice bearing SCCVII tumours and C57 mice bearing B16 tumours.

6.2.1. Cells

V79 Chinese hamster cells, exponentially were grown in 9.89 g l^{-1} HAM'S F-10 (Biochrom KG, Germany) supplemented with 1.2 g $NaHCO_3$ g l^{-1}, 10 mL l^{-1} Penicillin–Streptomycin (10 000 U–10 000 μg mL^{-1}, Biochrom KG, Germany), and 10% foetal calf serum (FCS) (100 mL l^{-1}).

B16 tumour cells were grown in 9.69 g l^{-1} Eagle minimum essential medium (Biochrom KG, Germany) supplemented (MEM) 10 mL l^{-1} Penicillin–Streptomycin (10 000 U–10 000 μg mL^{-1}, Biochrom KG), 2.2 g l^{-1} $NaHCO_3$ and 10% mL FCS (100 mL l^{-1}).

SCCVII tumour cells (mouse squamous cell carcinoma), exponentially were grown in 9.4 g l^{-1} Eagles minimum essential medium (Sigma Aldrich Co., St. Louis, USA) supplemented with 292 mg l^{-1} L-glutamine, 7.5% $NaHCO_3$; 10 mL l^{-1} Penicillin–Streptomycin (10 000 U–10 000 μg mL^{-1}, Biochrom KG) and 12.5% FCS (62.5 mL l^{-1}).

6.2.2. Cloning survival test

For cell toxicity investigation by cloning survival test, cells were incubated overnight (20 h) with the compound. For the toxicity tests of compound **2**, **6** and **8** concentrations of 300, 200, 150, 100, 75 and 50 μg boron mL^{-1} in the incubation medium were used. The cells were incubated for 20 h at 37 °C in a humidified atmosphere containing 5% CO_2. After incubation with the compound the medium were removed from the cell dishes, the cells were washed with 1 mL trypsin, incubated with 0.5 mL trypsin at 37 °C for 15 min to remove them from the substrate and 1.6 mL medium were added. Known amounts of cells were seeded out in 10 cm Petri dishes three times for each amount. After five days, when colonies were formed, the medium was removed and the cells were fixed and stained by GIEMSA–ethanol solution. The stained colonies containing >50 cells were counted and compared with controls where no compound were used. The means and the standard deviations were calculated for each incubation condition.

6.2.3. Mice

The tumour cells SCCVII and B16 were inoculated (1×10^6 cells) into the back of 10–12-week-old female C3H/He mice and female C57 mice, respectively. About 14–21 days later, the tumours reached suitable sizes for experiments (around 150 mg). Compound **2** and **6** were used. The compounds were dissolved in phosphate buffered saline (PBS) at a concentration of 1000 μg boron/0.5 mL (2.86 mg/0.5 mL of **2** and 3.02 mg/0.5 mL of **6**) and 500 μL of that solution was injected intraperitoneally into the mice. The mice were sacrificed after different periods of time (0.5, 1, 2 and 4 h) and frozen rapidly. The frozen mice were embedded in 3% carboxymethylcellulose and 50 μm-thick sections were cut with a microtome [29]. To visualise boron in this tissue sections, track-etch detectors were used. For this, an α-particle-sensitive nitrocellulose film (Kodak LR 115, type 1) was placed in close contact to a freeze-dried tissue section and exposed to a neutron beam at the LFR Petten to a fluence of about 10^{12} N cm^{-2}. After irradiation, tracks were made visible by exposing the track-etch film to 10% NaOH at r.t. for 4 days. By this method, the boron distribution in sections can be investigated quantitatively or qualitatively.

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