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Novel glycosylated carboranylquinazolines for boron neutron capture therapy of tumors: synthesis, characterization, and *in vitro* toxicity studies

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The synthesis of a series of *N*-glycosyl caboranylquinazolines is described. The condensation reaction of nitro-acetylanthranilic acid with aminophenylcarborane gave 3-[(*o*-carboran-1-yl)phenyl]-2-methyl-6-nitroquinazolin-4(3*H*)-one 1 followed by reduction with Na_2S to the corresponding 6-amino-3-[(*o*-carboran-1-yl)phenyl]-2-methylquinazolin-4(3*H*)-one 2. Reaction of compound 2 with D-glucose or D-ribose in methanol in the presence of a catalytic amount of acetic acid affords boronated *N*-glycosylaminoquinazolines namely: 2-methyl-3-[4-(*o*-carboran-1-yl)phenyl]-6-[*N*-β-D-glucopyranosyl)]aminoquinazolin-4(3*H*)-one 3 or 2-methyl-3-[4-(*o*-carboran-1-yl)phenyl]-6-[*N*-β-D-ribofuranosyl)]aminoquinazolin-4(3H)-one 4, respectively. Degradation of the *o*-caborane cage of compounds 3 and 4 yielded highly water-soluble compounds of sodium 2-methyl-3-[4-(*nido*-undecarborate-1-yl)phenyl]-6-[*N*-β-D-glucopyranosyl]aminoquinazolin-4(3H)-one 5 and sodium 2-methyl-3-[4-(*nido*-undecarborate-1-yl)phenyl]-6-[*N*-β-D-ribofuranosyl)]aminoquinazolin-4(3H)-one 6, respectively. The structures were established on the basis of elemental analysis, NMR, IR and mass spectrometry. The *in vitro* toxicity test using B16 melanoma cells showed that *N*-glycosyl of *nido*-undecaboranylquinazolines (5 and 6), with higher water solubility, is not toxic at boron concentration of 3000 μg boron ml^{-1}, whereas, *N*-glycosyl of *closo*-carboranylquinazolines (3 and 4) has $LD_{50} > 200$ μg boron ml^{-1}. The compounds described here may be considered as potential agents for BNCT.

Keywords: *N*-glycosides; BNCT; quinazolines; carboranes, antitumor agents

Introduction

The development of new boronated compounds is essential to the continued evaluation of cancer treatment by boron neutron capture therapy (BNCT).[1–3] This requires synthesis and evaluation of nontoxic agents that selectively target malignant cells in contrast with adjacent normal tissue and are retained intracellulary. The potential use of boronated compounds is based upon the unique nuclear properties of the nonradioactive ^{10}B nucleus and its propensity to absorb thermal neutrons.[4–6] The resulting activated ^{11}B nucleus undergoes prompt fission, generating highly destructive $^{4}He^{2+}$ and $^{7}Li^{3+}$ ions by a neutron capture reaction. These ions have path lengths <10 μm in biological tissue and the lethal damage caused is largely restricted to the tumor.[3–5] Recently, the development of nanomaterial-based BNCT agents was described.[7]

The considerable biological importance of the group of compounds incorporating the quinazoline ring has stimulated much work on this heterocycle.[8–10] The presence of a pyrimidine nucleus in many heterocyclic compounds, for example the quinazolines, often leads to very interesting biological and pharmaceutical activities.[10] Several quinazoline derivatives showed biological activities, such as antifungal, antibacterial, anticancer, anti-inflammatory, antitumor and antiproliferative activities.[11–16] The anticarcinogenic action of quenazolines is related to their ability to be included in nucleic acids of tumoral cells. Initial approaches for its use in BNCT were concentrated on the synthesis of benzo-fused boronated pyrimidine derivatives.[17,18] In this case only one boron atom was placed within the pyrimidine nucleus and flanked by two nitrogen atoms. However, these compounds were found to be hydrolytically and biologically unstable, and they failed to become incorporated selectively into tumor cells or into nucleic acids. Moreover, the standard glycosylation procedures on these compounds failed to produce the required nucleosides.[18]

The rotational for choosing a carborane cage as the boron containing moiety was the 10-fold increase in boron content compared with above mentioned groups which contain just one boron atom. In addition, there is an increased lipophilicity due to the carborane moiety, which could aid cellular penetration.[19–23] All these properties have led many researchers to design different compounds containing carboranes for tumor targeting.[24–28] Moreover, there are significant differences in the carbohydrate composition of the cell membrane surface of malignant cells by comparison with the normal cells from which they are derived.[3,29] Such compositional changes in these various carbohydrate-containing compounds can be used as the basis for the selective targeting of tumor cells and to achieve the needed differential concentrations between tumor and normal tissues. The purpose of the carbohydrate functionality was solely to increase

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the compound's aqueous solubility. These observations caused interest in the synthesis of carborane-containing sugars through flexible spacers of various types and lengths in order to find compounds which can be used as BNCT agents.[30–41] The *in vitro* toxicity of some of these compounds is low and governed mostly by its linker between carborane and sugar moiety.

For this purpose, we planned to synthesize a new series of carborane containing biologically active *N*-glycosylaminoquinazolines (Fig. 1). Our objective was to incorporate a carboranyl moiety into *N*-glycosylaminoquinazolines. The rationale was that such structures would possess: (1) a 10-fold increase in boron content compared with the previously prepared boronated quinazolines;[17,18] and (2) enhanced membrane penetration due to the presence of hydrophilic (sugar) and hydrophobic (carborane) parts in the compounds; additionally, (3) quinazolin-4(3*H*)-ones have different binding modes with DNA and therefore, *N*-glycosyl carboranylquinazolines may be able to target DNA directly once they penetrate the cell membrane. Additionally, the results of *in vitro* studies are presented to evaluate the preliminary application of the new *N*-glycosyl carboranylquinazolines for cancer treatment by BNCT.

Results and Discussion

Chemistry

A relatively large set of carborane–quinazoline conjugates needs to be prepared in order to ensure the success of the quinazoline-mediated targeting. The high boron content of organic compounds substituted with polyhedral boron clusters makes them interesting for use in BNCT and has attracted much attention in recent years. For this reason, we synthesized *N*-glycosylaminoquinazolines containing carborane cluster in acceptable yields. The syntheses started by the condensation of 2-acetamido-5-nitrobenzoic acid[42] and 1-(*p*-aminophenyl)-*o*-caborane[43] to produce 3-[(*o*-carboran-1-yl)phenyl]-2-methyl-6-nitroquinazolin-4(3*H*)-one **1** in 59% yield (Scheme 1). This boronated nitroquinazoline was converted into the corresponding amino-analog by reduction with sodium sulfide (Na_2S) and HCl. The resulting 6-amino-3-[(*o*-carboran-1-yl)phenyl]-2-methylquinazolin-4(3*H*)-one **2** was easily purified by column chromatography on silica gel, using dichloromethane : petroleum ether (5 : 1). The ^{1}H NMR spectrum of compound **2** showed the amino protons to resonate at 5.61 ppm, whereas the carborane protons appeared as broad singlet bands at 4.07 and 1.5–3.41 ppm for CH and BHs protons, respectively. The ^{1}H and ^{13}C NMR chemical shifts reflected the reduction of NO_2 group of compound **1** to NH_2 group in compound **2**. Additionally the assignments of ^{13}C NMR signals were based on DEPT experiments and chemical shift arguments.

N-Glycosylation of boronated aminoquinazoline **2** accomplished by treatment with D-glucose or D-ribose in boiling methanol in the presence of a catalytic amount of glacial acetic acid. The corresponding stereoselective *N*-β-D-glucosylaminoquinazoline **3** and *N*-β-D-ribosylaminoquinazoline **4**, respectively, were obtained in good yields (Scheme 2). The constitution and purity of each of the compounds (**1–4**) were established by NMR, IR, mass spectroscopy and elemental analysis. Also, ^{1}H and ^{13}C NMR spectra indicate the appearance of new signals corresponding to the sugar moiety of each carboranylquinazolines **3** and **4** (see the Experimental section for details). ^{1}H NMR spectra of the compounds **3** and **4** indicated that the anomeric proton appeared as broad signals at 5.21 and 5.03 ppm, respectively. These bands converted to doublet signals with $J = 8.6$ and 7.8 Hz after D_2O addition corresponding to the diaxial orientation of the H-1′ and H-2′ protons which favor the β-configuration with $^4C_1$ (D) conformation.[44,45] The β-configuration was also confirmed by the ^{13}C NMR spectroscopic data which showed that the anomeric carbon of **3** and **4** resonates at 86.02 and 86.82 ppm, respectively. The introduction of sugar moieties into the boronated quinazolines only slightly affected the aromatic and caborane protons. The IR spectra of these compounds showed absorption bands in the 3232–3224 cm^{-1} region which may be attributed to the glycosidic νNH and νOH of the sugar moiety. However, the BH band appeared as a strong signal at 2562 cm^{-1} and the C=O band at 1732 cm^{-1}.

Compounds **3** and **4**, however, were not highly soluble in water. A 10% DMSO aqueous solutions of these boronated compounds were used in biological studies. More hydrophilic *nido*-analogues were synthesized to assess the potential of these N-glycosyl boronated quinazolines in neutron capture therapy (NCT). The *closo*-carboranyl cage was degraded to the corresponding *nido*-cages using pyrrolidine as reported previously,[46] followed by treatment with acidic ion exchange resin (Na^+ form) to effort the negatively-charged water soluble sodium salt of 6-N-(β-D-glucopyranosyl)amino-3-[4(*nido*-undecarborate)phenyl]-2-methylquinazolin-4(3*H*)-one **5** and 6-N-(β-D-ribofuranosyl)amino-3-[4(*nido*-undecarborate)phenyl]-2-methylquinazolin-4(3*H*)-one **6**. The conversion of the *nido* cages was established by elemental analysis, IR, NMR and mass spectroscopy. In the case of compound **5**, the ^{1}H NMR spectrum showed board signals at −2.05, 2.09 and 2.19 ppm corresponding to the *nido* carborane protons. The FAB-MS spectra for the anions of compounds **5** and **6** showed the most intense molecular ion

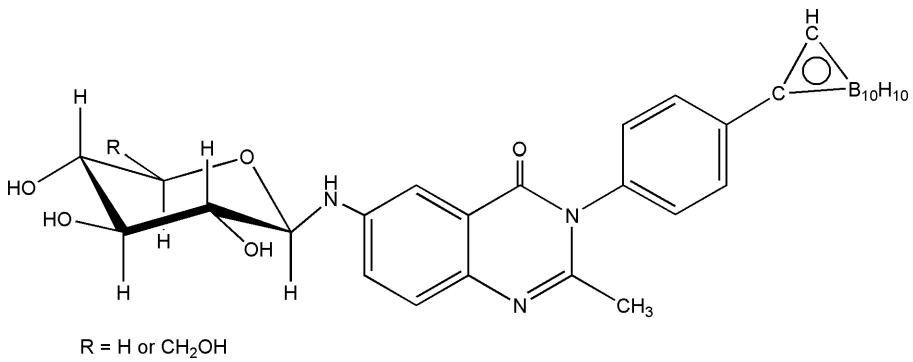
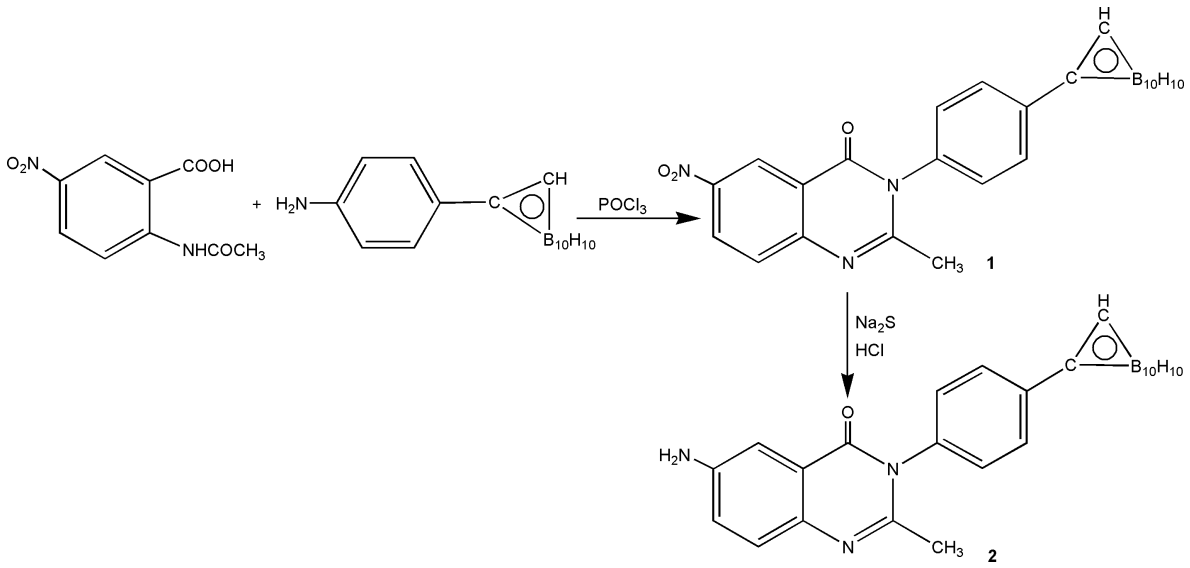
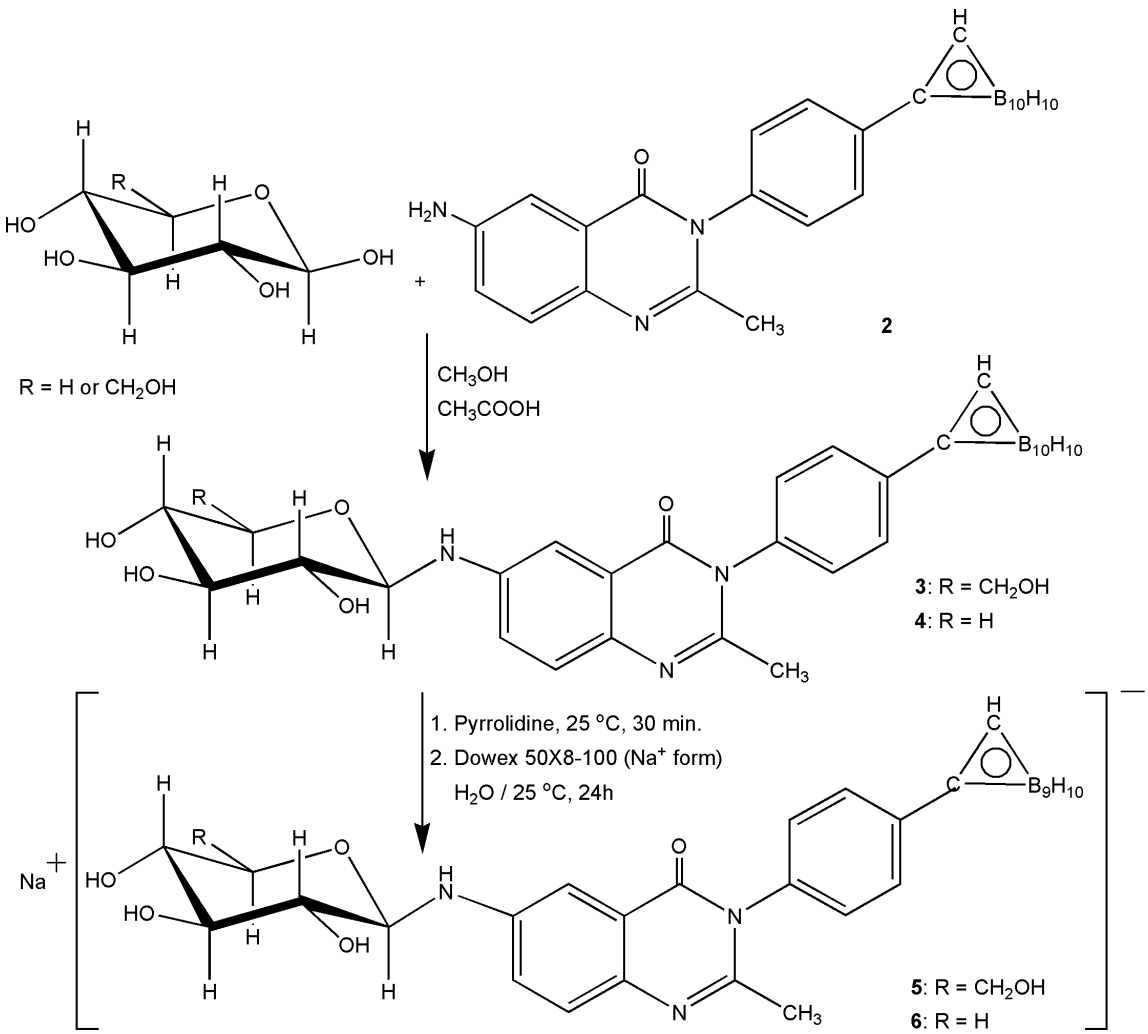


Figure 1. Schematic structure of carboranyl-*N*-glycosylaminoquinazolines.



Scheme 1. Synthesis of a carboranylquinazolines **1** and **2** .



Scheme 2. Synthetic route of N-glycosyl carboranylquinazolines **3–6** .

peak [M^-] at $m/z = 544$ and 514, respectively, corresponding to the ^{11}B isotope. However, FAB^+-MS spectra of these compounds showed no evidence for the formation of a cation resulting from the protonation of the glycosidic NH group. Data for mass spectrometry and elemental analysis were in full accord with the proposed structures of compounds **5** and **6**.

Biology

The most interesting, and potentially useful, compounds for BNCT would be those that attain a high concentration in tumor cells and are minimally toxic to the host and normal cells. This means that the boron-containing delivery agent should selectively tumor cells, and, ideally, be localized within the nucleus. The usefulness of the *N*-glycosylcaboranyl quinazoline derivatives as potential boron delivery agents for BNCT will ultimately depend upon their *in vitro* tumor localizing properties and their ability to selectively deliver the requisite amounts of boron to tumors. The first step in evaluating this potential is the *in vitro* uptake by tumor cells. Previous experience with the assay has shown it to be a useful *in vitro* test for identifying nontoxic compounds that subsequently could be evaluated *in vivo*.[47] *In vitro* toxicity tests of compounds **1**–**6** against B16 melanoma cells are summarized in Table 1 and shown in Fig. 2. All compounds were tested up to a maximum concentration of boron (300 μg boron ml^{-1}). *In vitro* toxicity was evaluated by exposing B16 melanoma cells for 24 h to the test compounds, and comparing the number of surviving cells to the number of surviving cells not exposed to the test compounds. The *in vitro* toxicity of compound **1** indicates that the compound was already toxic at lower boron concentration; however, the toxicity of compound **2** increased when its boron concentrations in the medium increased from 100 μg boron ml^{-1} to 3000 μg boron ml^{-1} with $LD_{50} > 75$ μg boron ml^{-1}. Moreover, the survival ratio of the compounds **3** and **4** decreased when its concentration in the medium increased from 50 to 3000 μg boron ml^{-1} with $LD_{50} > 200$ μg boron ml^{-1} (Table 1, Fig. 2). Even at the higher levels of boron concentration, no toxicity from the *N*-glycosyl boronated quinazolines (**5** and **6**) was observed.

In conclusion, the compounds described here represent a novel class of *N*-glycosyl boronated quinazolines as potential boron delivery agents for BNCT. These species offer the advantages of high boron content (17–27% boron by weight), water solubility and very low cellular toxicity compared to the previously boronated quinazolines.[18] The compounds were synthesized in acceptable yields from readily available starting materials. The reactions as well as workup procedures and the purifications for all products were readily feasible. The results from our preliminary studies indicate that compounds **5** and **6** are not toxic over wide range of boron concentration, even at higher concentrations (3000 μg boron ml^{-1}). As a result, further work is now underway to investigate biodistribution studies of these compounds using mice bearing tumors.

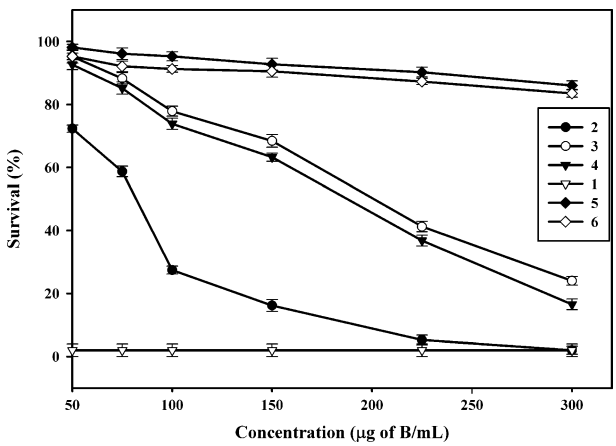


Figure 2. Percentage (± SD) of *in vitro* survival cell with respect to the concentration of *N*-glycosyl caboranylquinazolines **1**–**6**.

Experimental

Materials and methods

2-Acetamido-5-nitrobenzoic acid and 1-(*p*-aminophenyl)-*o*-carborane were prepared according to the literature methods.[42,43] The other reagents, dry solvents and sugars were commercially obtained from chemical companies. Column chromatography was conducted on silica gel 60 (Fluka). Elemental analyses were performed by a Perkin-Elmer 2400 automatic elemental analyzer. All compounds gave elemental analysis within ±0.4%. The measurements for NMR (^{11}B, ^1H and ^{13}C) were carried out on a Bruker DPX 200 spectrometer. The chemical shifts δ are given in ppm relative to $\Xi = 100$ MHz for δ (^1H) (nominally $SiMe_4$), $\Xi = 50$ MHz for δ (^{13}C) (nominally $SiMe_4$), and $\Xi = 32.083$ MHz for δ (^{11}B) (nominally F_3BOEt_2) in d_6-DMSO. IR (cm^{-1}) spectra were determined as KBr disks on a Bruker Vector 22 spectrometer. Mass spectrometric data were measured using a Finnigan MAT 8222 instrument, either (a) by fast-atom bombardment ionization (FAB) with glycerol or nitrobenzylalcohol (NBA) as matrix, or (b) by 70 eV electron impact ionization (EI) at 473 K. Only the signal with the highest intensity of the boron isotopic pattern is listed and compared with distribution of isotopes calculated by ISOFORM program. Melting points determination were performed by the open capillary method using a MEL-TEMP11 melting point apparatus and are reported uncorrected.

Synthesis of 2-methyl-3-[(o-carboran-1-yl)phenyl]-6-nitroquinazolin-4(3H)-one (1)

A mixture of 2-acetamido-5-nitrobenzoic acid (0.76 g, 4.0 mmol) and 1-(4-aminophenyl)-*o*-carborane (1.2 g, 5.0 mmol) was heated under reflux in phosphorus oxychloride (20 ml) for 30 min. Excess phosphorus oxychloride was distilled off under reduced pressure. The residue was triturated with 0.1 M NaOH solution and the solid residue, washed with methanol and dried to give faint yellow solid substance.

Yield: 59%, 0.99 g, m.p. = 278–280 °C; ν_{max}(KBr disk)/cm^{-1}: 2562s (BH), 1772s (C═O), 1650m (C═N), 1270s (C–N); ^1H NMR (100 MHz; d_6-DMSO; $SiMe_4$) 1.45–3.34 (bs, BH), 2.81 (s, 3H, CH_3), 4.02 (bs, 1H, $CH_{carborane}$), 7.38 (d, $J = 3.4$ Hz, 1H, H_{arom}), 7.69–7.73 (m, 4H, H_{arom}), 8.31 (m, 2H, H_{arom}); ^{13}C NMR (50 MHz; d_6-DMSO; $SiMe_4$); 21.12 (CH_3), 59.96 ($CH_{carborane}$), 73.67 ($C_{carborane}$), 105.25 (C-7), 112.42 (C-phenyl), 122.19 (C-5), 123.09 (C-4a), 127.85 (C-8), 128.32, 128.93, 133.81 (3C-phenyl), 139.98 (C-8a), 141.02 (C-2), 147.23 (C-6), 161.39 (C═O); *m/z* (EI) = 423 (M^+, 96%), 422 ($[M-H]^+$, 87%); anal. found: C, 47.89; H, 4.76; N 9.67; $B_{10}C_{17}H_{21}N_3O_3$ requires: C, 48.22; H, 5.00; N, 9.92%.

Synthesis of 6-amino-3-[(o-carboran-1-yl)phenyl]-2-methylquinazolin-4(3H)-one (2)

A mixture of compound **1** (3.78g, 10.0 mmol), sodium sulfide(2.35 g, 30.0 mmol), water(50 ml) and concentrated HCl (5 ml)

Table 1. *In vitro* toxicity of compounds **1–6** by B16 melanoma cells[a]

C_{media} (μg of B ml^{-1})	Percentage of survival (%)					
	1	**2**	**3**	**4**	**5**	**6**
50	<1	72.32 ± 1.14	95.24 ± 2.10	89.64 ± 1.57	98.13 ± 0.97	95.29 ± 1.02
75	–	58.71 ± 1.68	88.26 ± 1.79	78.21 ± 1.95	96.14 ± 1.85	92.12 ± 1.67
100	–	27.42 ± 1.24	77.87 ± 1.56	65.84 ± 1.74	95.27 ± 1.45	91.26 ± 1.02
150	–	16.19 ± 1.89	68.43 ± 1.99	52.25 ± 1.26	92.76 ± 1.89	90.56 ± 1.89
225	–	5.24 ± 1.57	41.25 ± 1.67	16.78 ± 1.72	90.27 ± 1.56	87.29 ± 0.99
300	–	<1	24.0 ± 1.36	5.52 ± 1.69	86.06 ± 1.42	83.52 ± 1.24

[a] B16 cells were incubated with boronated compounds for 24 h at compound concentrations corresponding to the boron amounts indicated. Cells were washed (PBS), trypsinized and seeded out for colony formation. After one week, colonies were washed, stained, washed again (ethanol) and counted.

was heated under reflux for 30 min. The mixture was filtered off and the filtrate was neutralized with aqueous Na_2CO_3 solution. The solid thus obtained was filtered, washed with water, dried and purified by column chromatography using CH_2Cl_2 as eluent to give the titled compound as a white solid substance.

Yield: 33%, 2.99 g, R_f = 0.52, m.p. = 243–245 °C; ν_{max}(KBr disk)/cm^{-1}: 3320s (NH_2), 2557s (BH), 1754s (C═O), 1648 (C═N), 1272s (C–N); ^{1}H NMR (100 MHz; d_6-DMSO; $SiMe_4$) 1.5–3.41 (bs, BH), 4.07 (bs, 1H, $CH_{carborane}$), 2.65 (s, 2H, CH_3), 5.61 (s, 2H, NH_2), 7.23 (d, J = 3.0, 1H, H_{arom}), 7.56–7.63 (m, 4H, H_{arom}), 8.1 (m, 2H, H_{arom}); ^{13}C NMR (50 MHz; d_6-DMSO; $SiMe_4$) 21.57 (CH_3), 60.15 ($CH_{carborane}$), 74.69 ($C_{carborane}$), 106.12 (C-7), 113.15 (C-phenyl), 121.46 (C-5), 123.72 (C-4a), 127.65 (C-8), 128.62, 129.01, 133.56 (3C-phenyl), 139.32 (C-8a), 141.64 (C-2), 146.56 (C-6), 161.87 (C═O); m/z (EI) = 393 (M^+, 89%), 392 ([M − H]$^+$, 73%); anal. found: C, 51.63; H, 5.71; N 10.59; $B_{10}C_{17}H_{23}N_3O$ requires: C, 51.89; H, 5.89; N, 10.68%.

Synthesis of 2-methyl-3-[4-(*o*-carboran-1-yl)phenyl]-6-[*N*-β-D-glucopyranosyl)]aminoquinazolin-4(3H)-one (3) and 2-methyl-3-[4-(*o*-carboran-1-yl)phenyl]-6-[*N*-β-D-ribofuranosyl)]aminoquinazolin-4(3H)-one (4)

To a solution of compound **2** (0.39g, 1.0 mmol) in methanol (100 ml), D-glucose or D-ribose (1.2 mmol) and glacial acetic acid (0.5 ml) were added. The reaction mixture was refluxed for 2 h. After cooling down, the formed precipitate filtered off, washed with water and purified by column chromatography (CH_2Cl_2 : MeOH, 5 : 1) to give **3** or **4** as a white solid substance.

Compound **3**

Yield: 62%, 0.34 g R_f = 0.34, m.p. = 186–188 °C; decomp.; ν_{max}(KBr disk)/cm^{-1}: 2927s (OH), 3232s (NH), 2552s (BH), 1732s (C═O), 1632s (C═N), 1273s (C–N); ^{1}H NMR (100 MHz; d_6-DMSO; $SiMe_4$) 1.48–3.36 (bs, BH), 4.02 (bs, 1H, $CH_{carborane}$), 2.72 (s, 2H, CH_3), 3.35–5.05 (m, 10H, glucose protons), 5.21 (bs, 1H, H-1′), 6.71 (d, J = 8 Hz, 1H, NH), 7.28 (d, J = 3.6, 1H, H_{arom}), 7.52–7.71 (m, 4H, H_{arom}), 8.21 (m, 2H, H_{arom}); ^{13}C NMR (50 MHz; d_6-DMSO; $SiMe_4$) 22.39 (CH_3), 61.22 ($CH_{carborane}$), 61.3 (C-6′), 68.02 (C-5′), 69.81 (C-4′), 73.79 ($C_{carborane}$), 73.91 (C-3′), 75.12 (C-2′), 86.02 (C-1′), 104.65 (C-7), 113.95 (C-phenyl), 121.47 (C-5), 123.65 (C-4a), 127.37 (C-8) 128.61, 128.99, 133.81 (3C-phenyl), 138.85 (C-8a), 141.65 (C-2), 147.45 (C-6), 162.25 (C═O); ^{11}B NMR (33.083 MHz; d_6-DMSO; $SiMe_4$) −2.86 (1B), −4.75 (1B), −9.25 (2B), −11.21 (2B), −12.81 (4B); m/z (FAB$^+$) = 556 ([M + H]$^+$, 55%), 555 (M^+, 100%); anal. found: C, 49.43; H, 5.84; N, 7.51; $B_{10}C_{23}H_{33}N_3O_6$ requires: C, 49.72; H, 5.99; N, 7.56%.

Compound **4**

Yield: 69%, R_f = 0.28, 0.36 g, m.p. = 177–179 °C; decomp.; ν_{max}(KBr disk)/cm^{-1}: 2936s (OH), 3224s (NH), 2560s (BH), 1728s (C═O), 1641s (C═N), 1264s (C–N); ^{1}H NMR (100 MHz; d_6-DMSO; $SiMe_4$) 1.45–3.39 (bs, BH), 4.05 (bs, 1H, $CH_{carborane}$), 2.69 (s, 2H, CH_3), 3.36–4.49 (m, 8H, ribose protons), 5.03 (bs, 1H, H-1′), 6.85 (d, J = 7.3 Hz, 1H, NH), 7.21 (d, J = 4.2, 1H, H_{arom}), 7.48–7.65 (m, 4H, H_{arom}), 7.99 (m, 2H, H_{arom}); ^{13}C NMR (50 MHz; d_6-DMSO; $SiMe_4$) 21.87 (CH_3), 60.31 ($CH_{carborane}$), 67.1 (C-5′), 68.54 (C-4′), 70.34 (C-3′), 72.83 ($C_{carborane}$, C-2′), 86.82 (C-1′), 103.95 (C-7), 113.21 (C-phenyl), 121.59 (C-5), 123.72 (C-4a), 127.41 (C-8), 128.72, 129.17, 134.04 (3C-phenyl), 139.12 (C-8a), 141.12 (C-2), 146.98 (C-6), 161.97 (C═O); m/z (FAB$^+$) = 526 ([M + H]$^+$, 67%), 525 (M^+, 86%); anal. found: C, 49.96; H, 5.87; N, 7.89; $B_{10}C_{22}H_{31}N_3O_5$ requires: C, 50.27; H, 5.94; N, 7.99%.

Synthesis of sodium 2-methyl-3-[4-(nido-undecarborate-1-yl) phenyl]-6-[*N*-β-D-glucopyranosyl)]aminoquinazolin-4(3H)-one (5) and sodium 2-methyl-3-[4-(nido-undecarborate-1-yl) phenyl]-6-[*N*-β-D-ribofuranosyl)]aminoquinazolin-4(3H)-one (6)

A 1.25 mmol quantity of compound **5** or **6** was dissolved in 10 ml of pyrrolidine and stirred for 30 min at room temperature. The pyrrolidine was evaporated and the remaining residue was added to 80 g of Dowax 50X8-100 ion-exchange resin (Na^+ form) in 150 ml of distilled H_2O. After stirring for 24 h at room temperature, the resin was filtered off and the filtrate was evaporated to dryness the resulting residue was purified by column chromatography (CH_2Cl_2 : CH_3OH, 1 : 1).

Compound **5**

Yield: 63%, 0.45 g; ν_{max}(KBr disk)/cm^{-1}: 2927s (OH), 3232s (NH), 2518s (BH), 1735s (C═O), 1625s (C═N), 1252s (C–N); ^{1}H NMR (100 MHz; d_6-DMSO; $SiMe_4$) −2.05 (bs, 1H, BH), 2.09 (br, 9H, BH), 2.23 (bs, 1H, $CH_{carborane}$), 2.68 (s, 2H, CH_3), 3.28–4.97 (m, 10H, glucose protons), 5.45 (bs, 1H, H-1′), 6.80 (d, J = 7.6 Hz, 1H, NH), 7.31 (d, J = 3.6, 1H, H_{arom}), 7.63–7.75 (m, 4H, H_{arom}), 8.32 (m, 2H, H_{arom}); ^{13}C NMR (50 MHz; d_6-DMSO; $SiMe_4$) 22.39 (CH_3), 58.3 ($CH_{carborane}$), 61.3 (C-6′), 67.02 (C-5′), 70.12 (C-4′,$C_{carborane}$), 74.86 (C-3′), 75.24 (C-2′), 86.65 (C-1′), 103.99 (C-7), 112.75 (C-phenyl), 121.16 (C-5), 123.31 (C-4a), 127.38 (C-8), 128.97, 129.35,

134.01, (3C-phenyl), 138.76 (C-8a), 141.24 (C-2), 147.46 (C-6), 161.64 (C═O); ^{11}B NMR (33.083 MHz; d_6-DMSO; $SiMe_4$) −9.96 (1B), −10.78 (2B), −11.24 (2B), −14.67 (1B), −22.78 (1B), −33.01 (2B); *m/z* (FAB^-) = 544 (M^-, 88%); anal. found: C, 48.29; H, 5.59; N, 7.19; $B_9C_{23}H_{33}N_3O_6Na$ requires: C, 48.65; H, 5.86; N, 7.40%.

Compound **6**

Yield: 72%, 0.47 g; ν_{max}(KBr disk)/cm^{-1}: 2936s (OH), 3224s (NH), 2522s (BH), 1730s (C═O), 1636s (C═N), 1243s (C–N); ^{1}H NMR (100 MHz; d_6-DMSO; $SiMe_4$) −2.12 (bs, 1H, BH), 2.20 (br, 9H, BH), 2.19 (bs, 1H, $CH_{carborane}$), 2.67 (s, 2H, CH_3), 3.28–4.97 (m, 8H, ribose protons), 5.32 (bs, 1H, H-1′), 6.78 (d, J = 7.6 Hz, 1H, NH), 7.40 (d, J = 4.2, 1H, H_{arom}), 7.59–7.81 (m, 4H, H_{arom}), 8.91 (m, 2H, H_{arom}); ^{13}C NMR (50 MHz; d_6-DMSO; $SiMe_4$) 22.39 (CH_3), 57.98 ($CH_{carborane}$), 67.53 (C-5′), 79.69 (C-4′,$C_{carborane}$), 74.62 (C-3′), 75.80 (C-2′), 86.37 (C-1′), 104.55 (C-7), 113.13 (C-phenyl), 121.88 (C-5), 123.69 (C-4a), 127.72 (C-8), 128.29, 128.87, 133.63 (3C-phenyl), 139.56 (C-8a), 141.32 (C-2), 147.57 (C-6), 162.02 (C═O); *m/z* (FAB^-) = 514 (M^-, 84%); anal. found: C, 49.02; H, 5.64; N, 7.71; $B_9C_{22}H_{31}N_3O_5Na$ requires: C, 49.13; H, 5.81; N, 7.81%.

Biological studies

All tests were repeated two or three times. For each compound Petri dishes were seeded with B16 melanoma cells grown in 9.69 g l^{-1} Eagle minimum essential medium (Biochrom KG) supplemented (EMEM) 10 ml l^{-1} penicillin–streptomycin (10 000 U to 10 000 µg ml^{-1}, Biochrom KG), 2.2 g l^{-1} $NaHCO_3$ and 10% FCS. Dishes were incubated overnight at 37 °C in a humidified atmosphere containing 5% CO_2. The medium was replaced with medium containing varying concentrations of the boron compounds and incubated for an additional 24 h at 37 °C. The medium was removed from the dishes. The cells were suspended by trypsinization, counted and seeded out into new dishes at different dilutions. The numbers of colonies formed after one week were compared with the numbers of colonies formed in the control without boron. The medium was removed, washed with PBS, dyed with GIEMSA for 10–15 min and washed again with ethanol.

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