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Original article

Promising carboranylquinazolines for boron neutron capture therapy: Synthesis, characterization, and *in vitro* toxicity evaluation

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Abstract

Novel classes of structurally different boronated quinazolines were designed bearing 22–37% boron by weight for potential application in BNCT of tumors. Firstly, the *o*-carborane cage was linked to quinazoline at C-2 position via thioether linker: 2-*S*-(1,2-dicarba-*closo*-dodecaboran(12)-1-ylmethyl)-3-phenylquinazolin-4(3*H*)-one. Secondly, the *o*-carborane cage connected to quinazoline moiety at C-4 position through an ether linkage: 4-*O*-(*o*-carboran-1-ylmethyl)-2-methylquinazoline. Finally, carborane moieties were also linked to the C-6 position of quinazoline: 6-[*N*-{3-(2-methyl-1,2-dicarba-*closo*-dodecaboran(12)-1-yl)methyl}benzylidinamino]quinazolin-4(3*H*)-one and 6-[*N*-{3,5-di(2-methyl-1,2-dicarba-*closo*-dodecaboran(12)-1-yl)methyl}benzylidinamino]quinazolin-4(3*H*)-one. The water solubility was achieved by the degradative conversion of the *o*-carboranylquinazolines to the corresponding potassium *nido*-carboranyl quinazolines: 2-*S*-(1,2-dicarba-*nido*-undecacarborate-1-ylmethyl)-3-phenylquinazolin-4(3*H*)-one, 4-*O*-(1,2-dicarba-*nido*-undecacarborate-1-ylmethyl)-2-methylquinazoline, 6-[*N*-{3-(2-methyl-1,2-dicarba-*nido*-undecacarborate-1-yl)methyl}benzylidinamino]quinazolin-4(3*H*)-one and 6-[*N*-{3,5-di(2-methyl-1,2-dicarba-*nido*-undecacarborate-1-yl)methyl}benzylidinamino]quinazolin-4(3*H*)-one. The products were confirmed by NMR, elemental analysis, IR, and mass spectrometry. *In vitro* toxicity was performed with B16 melanoma cells and showed that the connection of hydrophilic *nido*-carborane to quinazoline moiety decreases the compound's toxicity. This cytotoxicity effect was not observed in the *nido*-carborane containing two cluster units which was relatively nontoxic and did not inhibit colony formation up to concentrations of 300 μg boron ml^{-1}. The compounds described here can be considered as new candidates for BNCT.
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Keywords: Quinazolines; NMR spectroscopy; BNCT; Carboranes; Anti-tumor agents

1. Introduction

Boron neutron capture therapy (BNCT) is an anti-cancer treatment that involves the irradiation of ^{10}B-rich tumors with low energy neutrons [1,2]. Subsequent productions of high linear energy transfer particles, $^{4}He_2$ (α-particle) and $^{7}Li_3$, cause severe damage to tumor cells through ionization process. The advantage of this binary approach is in the differential dose that can be established between the tumor and its surrounding normal tissue, provided that the compound, which carries the target atom, is avidly taken up in tumor, yielding a high tumor to normal tissue ratio. To achieve that objective, boron-containing analogues of various cellular building blocks have been synthesized [2–6]. Numerous heterocyclic compounds which are analogues of natural substances, may be used as transporters for anti-cancer agents to cancer cells. For example, heterocyclic analogues of neucleo-bases have been used for this goal [7]. The use of pyrimidine bases as a transport vehicle for the delivery of active molecules or their fragments is caused by an easier metabolism of pyrimidines in tumoral cells than in healthy tissues.

Quinazolines as hydrophobic analogues of pyrimidine bases, have great biological significance [8,9]. Many of them showed biological activities such as anti-bacterial, anti-inflammatory, anti-tumor, anti-cancer, and CNS depressant [8–10]. The anti-carcinogenic action of quenazolines is related to their ability to be included in nucleic acids of tumoral cells. The

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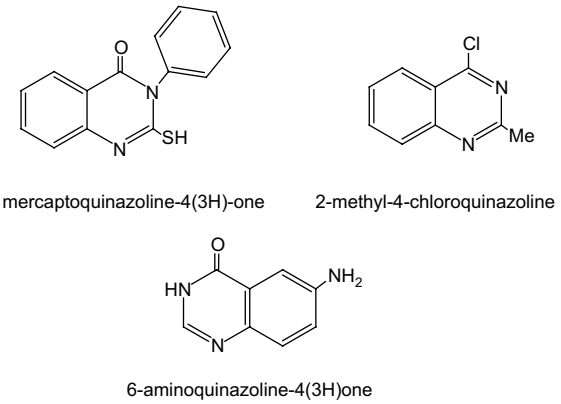


Fig. 1. Building blocks for the synthesis of boronated quinazolines.

first boronated quinazolines were prepared by incorporating the hydroxyboryl group into the pyrimidine ring, but such a ring structure does not possess the needed hydrolytic stability and suffered from the low content of boron atoms per molecule [11]. Additionally, these compounds were found to be biologically unstable, and they failed to become incorporated selectively into tumor cells or into nucleic acids.

The polyhedral *o*-carboranes appear to meet the requirements of possessing high boron percentages, and for this reason, there has been significant effort in the area of compound development directed toward the incorporation of such entities into organic structures [12–16]. Interest persists in such structures because of their inherent stability and their potential for incorporating various organic moieties into these clusters. Carboranes, especially those containing the $C_2B_{10}H_{12}$ nucleus, are very organic in nature, and the method of their incorporation into various organic/biochemical substrates has now become well developed [17]. The advantage of this cage is that it contains 10 boron atoms and that it can be chemically degraded to yield a hydrophilic, open-caged *nido*-carborane moiety [18].

Taken in consideration the last facts, we investigated the synthesis of several classes of carboranylquinazolines starting with 2-mercapto-3-phenylquinazolin-4(*3H*)-one, 4-chloro-2-methylquinazoline, and 6-aminoquinazolin-4(*3H*)-one (Fig. 1). Our objective was to incorporate a carboranyl moiety into quinazolines. The rationale was that such structures would possess: (1) a 10-fold increase in boron content compared with the previously prepared boronated quinazolines [11]; (2) taken up selectively into tumors due to the high mitotic rate of tumor cells vs normal cells; (3) quinazolin-4(3*H*)-ones have different binding modes with DNA and therefore, 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 carboranylquinazolines for cancer treatment by BNCT.

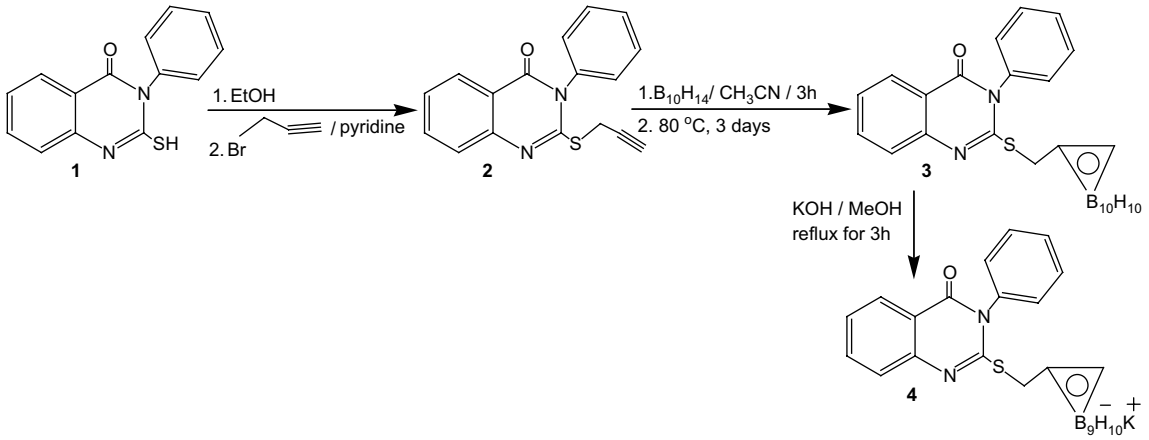
2. Results and discussion

2.1. Chemistry

One problem with the previously used boronated quinazoline is their unstability under hydrolytic conditions. The other, when their stability is enhanced by placing bulky or aromatic groups on boron, the compounds cease to emulate biochemically, the normally occurring substances. To overcome these problems we planned 3-fold strategies: the first strategy aimed to prepare *o*-carborane cage linked to the 2-position of quinazoline via thioester linker. The reaction of 3-phenyl-2-mercaptoquinazolin-4(3*H*)-one **1**, [19] with propargyl bromide in the presence of ethanol and catalytic amount of pyridine gave 2-*S*-propargyl-3-phenylquinazolin-4(3*H*)-one **2** [20] (Scheme 1). In the ^{1}H NMR spectrum, a singlet from CH proton in the ethine moiety of compound **2** is observed at $\delta = 1.76$ ppm, while a doublet of CH_2 group protons is observed at 3.83 ppm. Moreover, compound **2** showed a mass spectrum with an intense molecular ion peak (M^+) with $m/z = 292$. Reaction of **2** with the bis(acetonitrile)decaborane led to the formation of the carboranylquinazoline **3** (Scheme 1). Chromatography performed by column using $CHCl_3$ and acetone (1:5) as eluent gave compound **3** in 55% yield. The ^{1}H NMR spectrum of compound **3** showed two singlets at $\delta = 4.05$ and 4.3 ppm corresponding to carborane CH and the methylene group (CH_2S), respectively. However, another broad singlet appears at 1.5–3.4 ppm due to the B–H protons. Moreover, ^{13}C NMR chemical shifts reflected the connection of quinazoline moiety by the carborane cluster via thioether linker as shown in Fig. 2. Additionally, the assignments of ^{13}C NMR signals were based on DEPT experiments and chemical shift arguments. The carborane cage was then degraded in order to achieve water solubility by using methanolic KOH to produce the *nido*-carboranyl quinazoline **4**. The ^{1}H NMR spectrum of compound **4** in dimethylsulfoxide (DMSO), typically shows the B–H proton on the open face of the *nido*-carborane upfield shifted at −2.30 ppm and the remaining BHs at 1.61 ppm (Fig. 3). The CH-carborane proton adjacent to the open face, appeared shifted at 2.18 ppm and the quinazoline protons remain essentially unchanged ($\Delta\delta$ less than 0.3 ppm).

Secondly, the introduction of *o*-carborane cage takes place through quinazoline moiety by ether linkage: in this case, 4-chloro-2-methylquinazoline **5** was synthesized by treating 2,3-dimethylquinazolin-4(3*H*)-one with PCl_5/PCl_3 according to the literature procedure [21]. The reaction of chloroquinazoline **5** with propargyl alcohol in the presence of alkali gave the corresponding alkynoxy derivative **6**, which was isolated in 67% yield from **5** (Scheme 2). ^{1}H NMR spectrum of **6** confirmed the nucleophilic substitution of chlorine atom of **5** with propynyloxy group, where it showed two singlets at 4.53 and 2.73 ppm due to the methylene and acetylenic protons, respectively. Moreover, the methyl protons attached to the diazine ring resonates at 2.34 ppm. Addition of compound **6** to a solution of decaborane ($B_{10}H_{14}$) in acetonitrile furnished the corresponding carborane **7** as shown by elemental analysis, IR, NMR and mass spectroscopy. Degradation of the carborane cage of boronated quinazoline **7** gave *nido*-carboranyl quinazoline **8**.

The third route, which includes the attachment of *o*-carborane at 6-position of quinazoline, starts with 6-aminoquinazoline-4(3*H*)-one **9**, which was prepared by a multi-step reaction according to the literature [9]. The synthesis of Schiff



Scheme 1.

bases of boronated aldehydes 3-[(1-methyl-*o*-carboran-2-yl)-methyl]benzaldehyde [22] (**10a**) and 3,5-di[(1-methyl-*o*-carboran-2-yl)methyl]benzaldehyde [22] (**10b**) was our strategy to get 6-aminocarboranylquinazolines **11a** and **11b**, respectively (Scheme 3). The reaction of compound **9** with aldehydes **10a** and **10b** in methanol in the presence of catalytic amount of piperdine gave in high yield the suggested compounds **11a** and **11b**, respectively. The ^{1}H NMR spectra of the Schiff bases **11a** and **11b** in DMSO contained singlet signals of the N═CH proton at 8.53 and 8.68 ppm, respectively. The BH protons of carborane appeared in the range 1.48−3.32 ppm, while the aromatic protons resonate at 7.25−7.56 ppm. The ^{13}C NMR spectra of these compounds confirmed the presence of the imine carbon at 166.32 and 164.42 ppm. However, the carborane carbons of the cluster appear at 72.34−73.91 ppm.

Degradation of **11a** and **11b** gave the *nido*-carboranyl analogues **12a** and **12b**, respectively. The proton NMR spectra of the **12a** and **12b**, in d_6-DMSO, showed the B−H protons the open face of the *nido*-carboranes at −2.3 and −2.53 ppm and the remaining BHs absorb at 1.3 and 1.56 ppm, respectively. The methyl protons, adjacent to the open face, appeared upfield shifted at 1.8 and 2.1 ppm, respectively.

The IR spectra of compounds **3**, **4**, **11**, and **12** showed strong absorption bands within the 1662−1682 cm^{-1} region characteristics for CO of carbonyl group. For all compounds, the vibrational frequencies of B−H band ν(B−H) and the B−B band ν(B−B) of *o*-carborane or *nido*-carborane cluster were not found to be sensitive to the connection of quinazoline moieties indicating that the intracluster bonding is not perturbed by its connection with quinazoline ring.

2.2. Biology

We performed a preliminary biological evaluation of a number of structurally different boronated quinazoline derivatives. The usefulness of the carborane containing quinazoline derivatives as potential boron delivery agents for BNCT will ultimately depend upon their *in vivo* 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* behavior by tumor cells. *In vitro* toxicity was evaluated by exposing B16 melanoma cells for 24 h exposure to the test compounds, and comparing the number of surviving

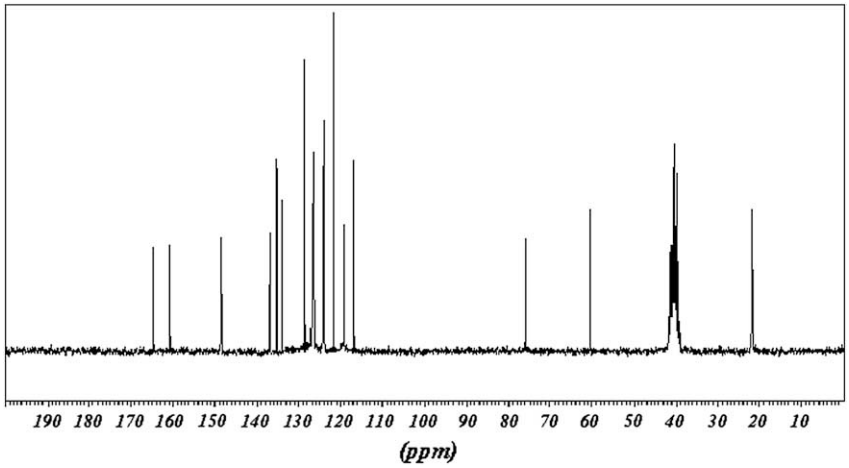


Fig. 2. ^{13}C NMR (200 MHz) spectrum of compound **3** in d_6-DMSO.

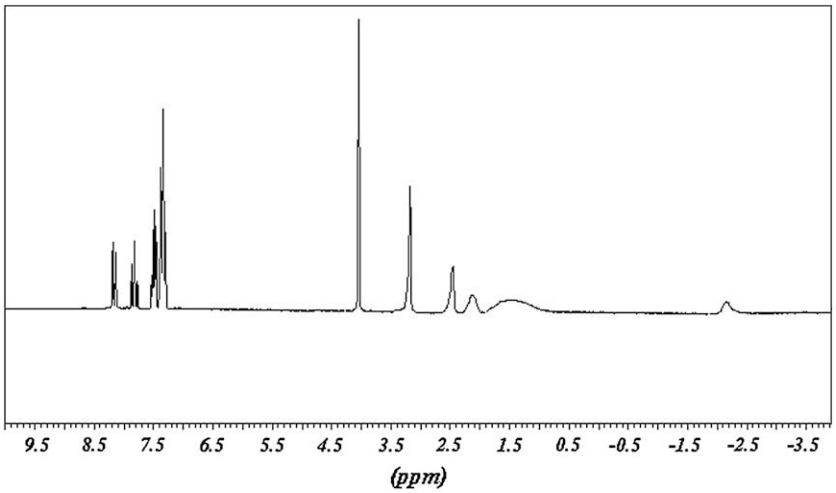


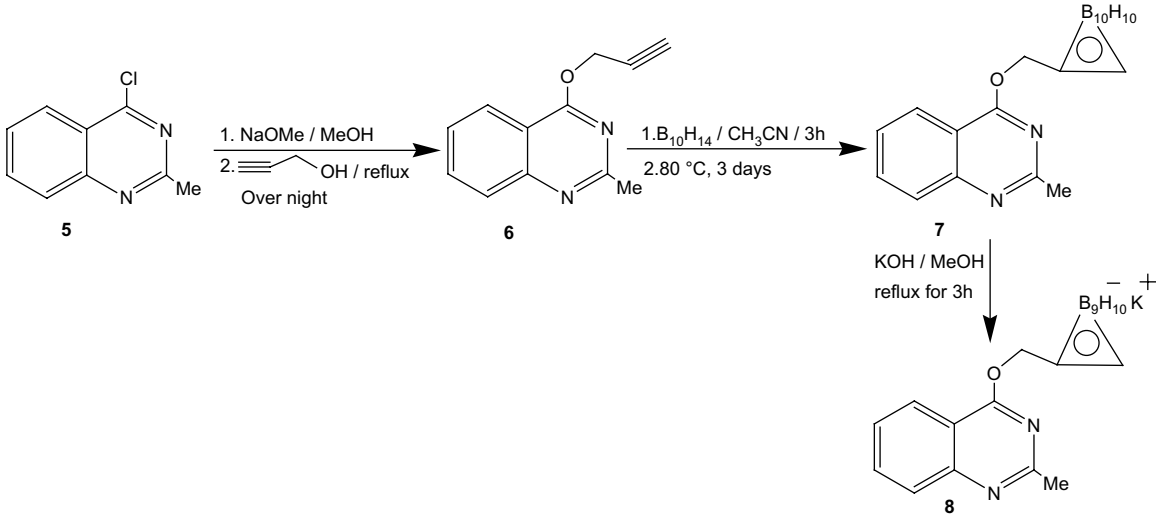
Fig. 3. ^{1}H NMR (200 MHz) spectrum of compound **4** in d_6-DMSO.

cells to the number of surviving cells not exposed to the test compounds.

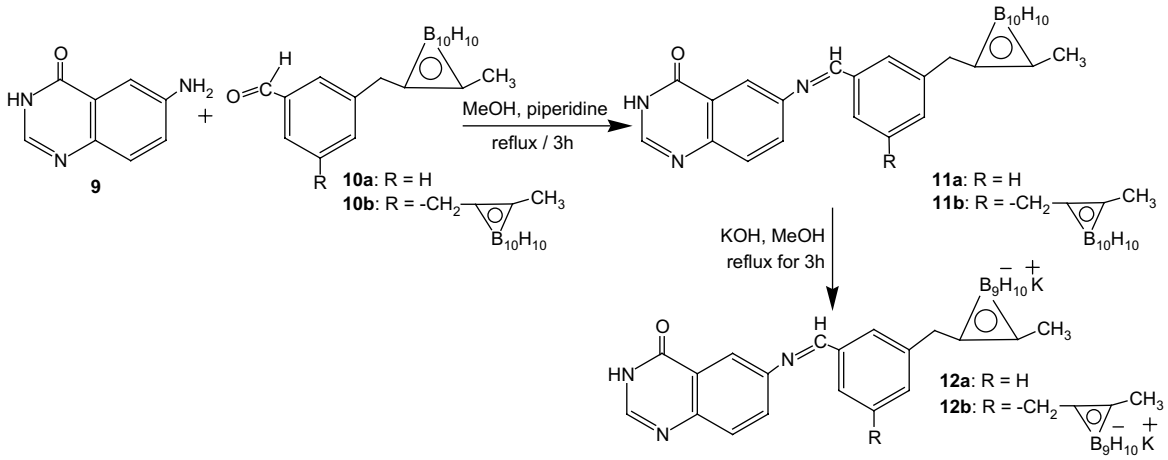
The cytotoxic effects of carborane containing quinazoline derivatives against B16 melanoma cells *in vitro* are shown in Fig. 4. The boronated quinazolines **3**, **4**, **7**, **8**, **11a**, **11b**, **12a**, and **12b** were tested up to a maximum boron concentration of 300 μg boron ml^{-1}. The structure specificity for the cytotoxicity due to the connection of quinazoline moieties to *closo*-carborane of compounds **3**, **7**, **11a**, and **11b** was observed in these compounds were significantly more toxic than *nido*-carboranyl quinazolines **4**, **8**, **12a** and **12b** in inhibiting the colony formation. The cytotoxicity effect was not observed in the case of compound **4** which was relatively nontoxic and did not inhibit the colony formation up to 135 μg boron ml^{-1}. Whereas compound **8** has an LD_{50} value of around 250 μg boron ml^{-1}, compounds **12a** and **12b** have an LD_{50} value of around 150 and 300 μg boron ml^{-1}, respectively (Fig. 4). Toxicity was increased also with increasing the concentration of the compounds. Conversely, compounds **3**, **11a** and **11b** were already toxic at lower boron concentration (50 μg boron ml^{-1}).

3. Conclusion

We have demonstrated a very simple, efficient, and practical method for the synthesis of novel water soluble boronated quinazolines in acceptable yields from readily available starting materials. Three classes of boronated quinazoline were designed bearing 22–37% boron by weight for potential application in BNCT of tumors. The reactions as well as the workup procedures and the purifications for all products were readily feasible. All compounds are highly stable at room temperature compared with previously reported boronated quinazolines [11]. The compounds described in this report are representative of agents which can be prepared by simple coupling reactions to yield a large series of agents for experimental BNCT. The cellular toxicity results showed that *o*-carborane containing quinazolines are more toxic than



Scheme 2.



Scheme 3.

the corresponding *nido*-carboranylquinazolines. Compounds **8** and **12b** appear not to be toxic over a wide range of boron concentrations up to 250 and 300 μg boron ml^{-1}, respectively. We are further exploring the potential of these compounds in view of *in vivo* biodistribution studies using mice bearing tumors.

4. Experimental

4.1. Materials and methods

All reagents, dry solvents, and $B_{10}H_{14}$ were commercially obtained from chemical companies. Quinazolines (e.g. 3-phenyl-2 mercaptoquinazoline-4(*3H*)-one, 2-methyl-4-chloroquinazoline, and 6-aminoquinazoline-4(*3H*)-one), 3-[(1-methyl-*o*-carboran-2-yl)methyl]benzaldehyde (**10a**) and 3,5-di[(1-methyl-*o*-carboran-2-yl)methyl]benzaldehyde (**10b**) were prepared according to the literature methods [9,19,22]. Column chromatography was conducted on silica gel 60 (Fluka). Plate chromatography was conducted on TLC plates, silica gel on aluminum, 20X (Aldrich).

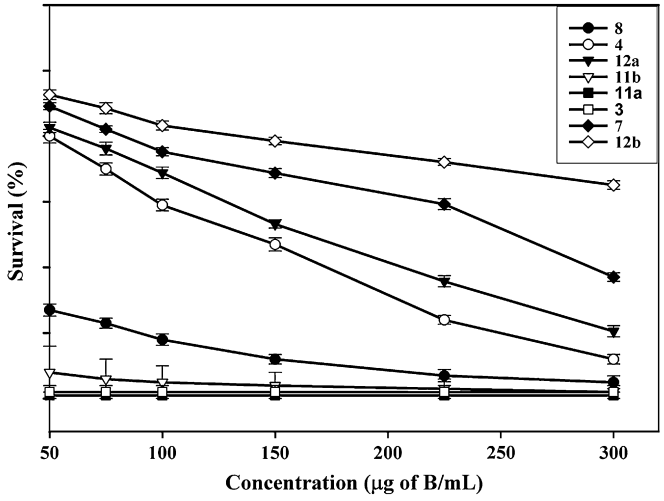


Fig. 4. Percentage (±SD) of *in vitro* survival cells with respect to the concentration of carboranylquinazolines **3**, **4**, **7**, **8**, **12a**, and **12b**.

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, ^{1}H 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 (^{1}H) (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 disc on a Bruker Vector 22 spectrometer. Mass spectrometric data were measured using a Finnigan MAT 8222 instrument, by fast-atom bombardment ionization (FAB) with glycerol or nitrobenzylalcohol (NBA) as matrix. 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 point determinations were performed by the open capillary method using a MEL-TEMP11 melting point apparatus and are reported uncorrected.

4.2. Synthesis of 2-propargylthio-3-phenylquinazolin-4(3H)-one (2)

Propargyl bromide (0.32 ml, 3.6 mmol) and 0.21 ml pyridine were added to a solution of quinazoline **1** (0.5 g, 2.0 mmol) in 10 ml dry ethanol. The reaction mixture was refluxed for 20 min, then the solution was cooled to room temperature and stirred for 2 h. The resulting precipitate was filtered off and recrystallized from ethanol to yield a faint yellow solid substance.

Yield: 72%, 0.42 g, m.p. 186–188 °C; IR ν_{max} (KBr disc)/cm^{-1}: 2122s (C≡C), 1672s (C=O), 1580m (C=N); δ_H (100 MHz; d_6-DMSO; $SiMe_4$) 1.76 (s, 1H, $CH_{acetylenic}$) 3.83 (s, 2H, SCH_2), 7.25–8.02 (m, 9H, H_{arom}); δ_C (50 MHz; d_6-DMSO; $SiMe_4$); 17.6 (CH_2, CH_2S), 69.2 (CH, $CH_{acetylenic}$), 75.8 (C, $C_{acetylenic}$), 118.23 (CH, C_{arom}), 120 (CH, C_{arom}), 122.5 (2CH, C_{arom}), 123.6 (C, C_{arom}), 127.3 (CH, C_{arom}), 128.7 (2CH, C_{arom}), 134 (CH, C_{arom}), 135 (CH, C_{arom}), 136.6 (C, C_{arom}), 148 (C, C_{arom}), 161.3 (C=O), 164.6 (C, C_{arom}); (FAB^+): *m/z* (%) = 294 (26) $[M + 2H]^+$, 293 (55)

$[M+H]^+$, 292 (98) $[M^+]$; elemental analysis calcd (%) for $C_{17}H_{12}N_2OS$: C 69.84, H 4.14, N 9.58; Found: C 69.71, H 4.04, N 9.49.

4.3. Synthesis of 2-S-(1,2-di-closo-dodecaboran(12)-1-ylmethyl)-3-phenylquinazolin-4(3H)-one (3)

A solution of $B_{10}H_{14}$ (1.4 g, 11.7 mmol) in acetonitrile (40 ml) was refluxed for 2 h to obtain bis(acetonitrile)decaborane. Then, quinazolinylacetylene **2** (2.9 g, 10.1 mmol) was added followed by reflux for 4 h. After cooling, the solvent was evaporated and a yellow solid was obtained. Methanol (20 ml) was added and the evolved H_2 was observed from the decomposition of excess bis(acetonitrile)decaborane. After 5 h, MeOH was evaporated in vacuum and the yellow residue washed with hexane (3 × 20 ml) and purified by column chromatography (acetone/$CHCl_3$, 1:5) to give compound **3** as a yellow solid substance.

Yield: 55%, 2.3 g, $R_f = 0.58$, m.p. = 170–172 °C; IR ν_{max} (KBr disc)/cm^{-1}: 2560s (BH), 1682s (C=O), 1586 (C=N); δ_H (100 MHz; d_6-DMSO; $SiMe_4$) 1.5–3.4 (br s, BH), 4.05 (br s, 1H, $CH_{carborane}$), 4.3 (s, 2H, SCH_2), 7.25–7.69 (m, 7H, H_{arom}), 7.79 (t, $J = 2.6$,1H, $H_{arom)}$, 8.16 (d, $J = 2.4$,1H, $H_{arom)}$; δ_C (50 MHz; d_6-DMSO; $SiMe_4$) 16.1 (CH_2, CH_2S), 60.9 (CH, $CH_{carborane}$), 75.4 (C, $C_{carborane}$), 117.8 (CH_{arom}), 119.6 (CH_{arom}), 123.1 ($2CH_{arom}$), 123.3 (C_{arom}), 127.1 (CH_{arom}), 128.6 ($2CH_{arom}$), 134.2 (CH_{arom}), 135.3 (CH_{arom}), 136.9 (C_{arom}), 148.7 (C_{arom}), 161.7 (C=O), 165.1 (C_{arom}); δ_B (33.083 MHz; d_6-DMSO; $SiMe_4$) −2.96 (1B), −4.65 (1B), −9.12 (2B), −11.14 (2B), −12.78 (4B); FAB^+): *m/z* (%) = 411 (59) $[M+H]^+$, 410 (89) $[M^+]$; elemental analysis calcd (%) for $C_{17}H_{22}B_{10}N_2OS$: C 49.73, H 5.4, N 6.82; Found: C 49.52, H 5.12, N 6.62.

4.4. Synthesis of potassium 2-S-(1,2-dicarba-nido-undecacarborate-1-ylmethyl)-3-phenylquinazolin-4(3H)-one (4)

A solution of compound **3** (0.41 g, 1.0 mmol) in methanolic KOH (20 ml, 0.5 M) was refluxed for 3 h. The resulting *nido*-carboranyl quinazoline **4** was purified using preparative TLC (0.1% CH_3CO_2H in 3:1 acetone/$CHCl_3$) as a yellow solid substance.

Yield: 53%, 0.23 g, $R_f = 0.3$, m.p. = 255–256 °C; IR ν_{max} (KBr disc)/cm^{-1}: 2508s (BH), 1673s (C=O), 1589m (C=N); δ_H (100 MHz; d_6-DMSO; $SiMe_4$) −2.3 (br s, 1H, BH), 1.61 (br s, 9H, BH), 2.18 (br s, 1H, $CH_{carboborane}$), 4.05 (s, 2H, SCH_2), 7.41–7.72 (m, 7H, H_{arom}), 7.85 (t, $J = 2.3$, 1H, $H_{arom)}$, 8.23 (d, $J = 2.4$, 1H, H_{arom}); δ_C (50 MHz; d_6-DMSO; $SiMe_4$) 16.2 (SCH_2), 57.3 ($CH_{carborane}$), 69.8 ($C_{carborane}$), 117.2 (CH_{arom}), 119.9 (CH_{arom}), 123.5 ($2CH_{arom}$), 123.4 (C_{arom}), 127.4 (CH_{arom}), 128.5 ($2CH_{arom}$), 134.1 (CH_{arom}), 135.6 (CH_{arom}), 136.7 (C_{arom}), 148.6 (C_{arom}), 161.3 (C=O), 165.8 (C_{arom}); δ_B (33.083 MHz; d_6-DMSO; $SiMe_4$) −9.92 (1B), −10.71 (2B), −11.19 (2B), −14.62 (1B), −22.71 (1B), −32.91 (2B); $(FAB)^-$: *m/z* (%) = 399 (85) $[M^-]$; elemental analysis calcd (%) for $KC_{17}H_{22}B_9N_2OS$: C 46.53, H, 5.05, N 6.38; Found: C 46.22, H 4.89, N 6.14.

4.5. Synthesis of 4-O-propargyl-2-methylquinazoline (6)

To a solution of quinazoline **5** (0.66 g, 3.5 mmol) in 20 ml NaOMe/MeOH, propargyl alcohol (2.23 ml, 4.0 mmol) was added and refluxed for 2 days. After cooling the precipitate formed was filtered and washed with methanol followed by purification with column chromatography using $CHCl_3$ as eluent to give compound **6** as a colorless solid.

Yield: 67%, 0.46 g, $R_f = 0.41$, m.p. = 185–186 °C; IR ν_{max} (KBr disc)/cm^{-1}: 2134s (C≡C), 1563s (C=N); δ_H (100 MHz; d_6-DMSO; $SiMe_4$) 2.34 (s, 3H, CH_3), 2.73 (s, 1H, $CH_{acetylenic}$), 4.53 (s, 2H, OCH_2), 7.73 (t, 1H, H_{arom}), 7.91 (m, 2H, H_{arom}), 8.05 (m, 1H, H_{arom}); δ_C (50 MHz; d_6-DMSO; $SiMe_4$) 27.3 (CH_3), 58.2 (OCH_2), 78.2 ($CH_{acetylenic}$), 79.0 ($C_{acetylenic}$), 114.1 (C_{arom}), 120 (CH_{arom}), 126 (CH_{arom}), 128 (C_{arom}), 135 (CH_{arom}), 167 (C_{arom}), 180 (C_{arom}); (FAB^+): *m/z* (%) = 199 (76) $[M+H]^+$, 198 (92) $[M^+]$; elemental analysis calcd (%) for $C_{12}H_{10}N_2O$: C 72.71, H 5.08, N 14.13; Found: C 72.53, H 4.89, N 13.91.

4.6. Synthesis of 4-O-(1,2-dicarba-closo-dodecaboran(12)-1-ylmethyl)-2-methylquinazoline (7)

Compound **7** was prepared with the same procedure as in compound **3**. The purification was carried out by TLC using $CHCl_3$ as eluent to yield a colorless solid substance.

Yield: (75%, 2.4 g, $R_f = 0.61$, m.p. = 192–193 °C); IR ν_{max} (KBr disc)/cm^{-1}: 2573s (BH),1558s (C=N); δ_H (100 MHz; d_6-DMSO; $SiMe_4$) 1.46–3.51 (br s, BH), 2.38 (s, 3H, CH_3), 4.12 (br s, 1H, carborane CH), 4.2 (s, 2H, OCH_2), 7.38–7.94 (m, 4H, $H_{aromatic}$); δ_C (50 MHz; d_6-DMSO; $SiMe_4$) 26.3 (CH_3), 57.3 (OCH_2), 62.0 ($CH_{carborane}$), 75.8 ($C_{carborane}$), 113.92 (C_{arom}), 121.05 (CH_{arom}), 127.14 (CH_{arom}), 128.52 (C_{arom}), 135.47 (CH_{arom}), 167.09 (C_{arom}), 180.91 (C_{arom}); δ_B (33.083 MHz; d_6-DMSO; $SiMe_4$) −2.92 (1B), −4.58 (1B), −8.99 (2B), −10.97 (2B), −12.59 (4B); (FAB^+): *m/z* (%) = 317 (59) $[M+H]^+$, 316 (78) $[M^+]$; elemental analysis calcd (%) for $C_{12}H_{20}B_{10}N_2O$: C 45.55, H 6.37, N 8.85; Found: C 45.31, H 6.17, N 8.59.

4.7. Synthesis of potassium 4-O-(1,2-dicarba-nido-undecarborate-1-ylmethyl)-2-methylquinazoline (8)

Compound **8** was prepared with the same procedure as in compound **4**. The resulting substance was purified by TLC (0.1% CH_3CO_2H in 1:1 acetone/$CHCl_3$) as a colorless solid substance.

Yield: 56%, 0.19 g, $R_f = 0.27$, m.p. = 233–234 °C; IR ν_{max} (KBr disc)/cm^{-1}: 2520s (BH), 1551s (C=N); δ_H (100 MHz; d_6-DMSO; $SiMe_4$) −1.92 (br s, 2H, BH), 2.13 (br, 8H, BH), 2.24 (s, 3H, CH_3), 2.17 (br s, 1H, $CH_{carborane}$), 4.06 (s, 2H, OCH_2), 7.26–7.74 (m, 4H, H_{arom}); δ_C (50 MHz; d_6-DMSO; $SiMe_4$) 26.3 (CH_3), 56.3 (OCH_2), 56.87 ($CH_{carborane}$), 67.96 ($C_{carborane}$), 113.56 (C_{arom}), 121.24 (CH_{arom}), 127.61 (CH_{arom}),

128.74 (C_{arom}), 135.21 (CH_{arom}), 167.11 (C_{arom}), 180.02 (C_{arom}); δ_B (33.083 MHz; d_6-DMSO; $SiMe_4$) −9.89 (1B), −10.69 (2B), −11.17 (2B), −14.59 (1B), −22.71 (1B), −32.96 (2B); (FAB^-): *m/z* (%) = 305 (87) [M^-]; elemental analysis calcd (%) for $KC_{12}H_{20}B_9N_2O$: C 41.81, H 5.85, N 8.13; Found: C 41.53, H 5.46, N 7.96.

*4.8. Synthesis of 6-[N-{3-(2-methyl-1,2-dicarba-closo-dodecaboran(12)-1-yl)methyl}benzylidine amino]quinazoline-4(3H)-one (**11a**), 6-[N-{3,5-di(2-methyl-1,2-dicarba-closo-dodecaboran(12)-1-yl) methyl}benzylidine amino]quinazoline-4(3H)-one (**11b**)*

To a solution of 6-aminoquinazoline-4(3*H*)-one **9** (1.6 g, 10 mmol) in 20 ml methanol, carboranylbenzaldehyde **10a** or **10b** (11.0 mmol) and piperidine (1 ml) were added. The reaction mixture was heated under reflux for 3 h. After cooling, the deposited solid product was collected by filtration, washed with methanol and purified by column chromatography using acetone-chloroform 1:1 as eluent to give compound **11a** or **11b**.

Compound **11a**. Yield: 84%, 3.5 g, R_f = 0.35, m.p. = 189–191 °C; IR ν_{max} (KBr disc)/cm^{-1}: 2572s (BH), 1668s (C═O), 1628s (C═N), 1585m (C═C); δ_H (100 MHz; d_6-DMSO; $SiMe_4$) 1.51–3.32 (br s, BH), 2.19 (s, 3H, CH_3), 3.4 (s, 2H, CH_2), 7.25–7.56 (m, 6H, H_{arom}), 7.73 (s, 1H, H_{arom}), 8.53 (s, 1H, CH═N), 11.87 (br s, 1H, NH); δ_C (50 MHz; d_6-DMSO; $SiMe_4$) 14.92 (CH_3), 31.21 (CH_2), 72.34, 73.91 ($C_{carborane}$), 105.06, 107.14, 109.07, 121.13, 127.42 (CH_{arom}), 123.61, 140.05 ($3C_{arom}$), 142.43 (CH_{arom}), 147.02, 162.73 ($2C_{arom}$), 166.32 (CH═N); δ_B (33.083 MHz; d_6-DMSO; $SiMe_4$) −2.91 (1B), −4.55 (1B), −9.24 (2B), −11.15 (2B), −12.69 (4B); (FAB^+): *m/z* (%) = (92) 420 $[M+H]^+$, 419 (79) [M^+]; elemental analysis calcd (%) for $C_{19}H_{25}B_{10}N_3O$: C 54.39, H 6.01, N 10.02; Found: C 54.16, H 6.41, N 10.38.

Compound **11b**. Yield: 72%, 4.2 g, R_f = 0.38, m.p. = 205–206 °C; IR ν_{max} (KBr disc)/cm^{-1}: 2575vs (BH), 1662s (C═O), 1632s (C═N), 1579m (C═C); δ_H (100 MHz; d_6-DMSO; $SiMe_4$) 1.48–3.18 (br s, BH), 2.21 (s, 6H, $2CH_3$), 3.56 (s, 4H, $2CH_2$), 7.30–7.48 (m, 6H, H_{arom}), 7.82 (s, 1H, H_{arom}), 8.68 (s, 1H, CH═N), 11.91 (br s, 1H, NH); δ_C (50 MHz; d_6-DMSO; $SiMe_4$) 15.02 ($2CH_3$), 30.82 ($2CH_2$), 72.51, 73.61 ($4C_{carborane}$), 105.25, 108.19, 109.56, 121.74, 123.61, 127.32 ($6CH_{arom}$), 142.51 (C_{arom}), 143.21 (CH_{arom}), 147.82, 161.98 ($2C_{arom}$), 164.42 (CH═N); δ_B (33.083 MHz; d_6-DMSO; $SiMe_4$) −2.85 (2B), −4.55 (2B), −8.94 (4B), −11.06 (4B), −12.54 (8B); (FAB^+): *m/z* (%) = 590 (95) ($[M+H]^+$, 589 (81) [M^+]; elemental analysis calcd (%) for $C_{23}H_{39}B_{20}N_3O$: C 46.84, H 6.66, N 7.12; Found: C 46.53, H 6.31, N 6.91.

*4.9. Synthesis of 6-[N-{3-(2-methyl-nido-carborate-1-yl)methyl}benzylidine amino]quinazoline-4(3H)-one (**12a**), 6-[N-{3,5-di(2-methyl-nido-carborate-1-yl) methyl}benzylidine amino]quinazoline-4(3H)-one (**12b**)*

The conversion of compounds **11a** and **11b** to their *nido*-forms **12a** and **12b** was performed as described for compounds **3**. Pure compounds were obtained after using TLC (0.1% CH_3CO_2H in 2:1 acetone/$CHCl_3$) to yield colorless solid substances.

Compound **12a**. Yield: 78%, 0.35 g, R_f = 0.24, m.p. = 263–264 °C; IR ν_{max} (KBr disc)/cm^{-1}: 2521s (BH), 1673s (C═O), 1619s (C═N), 1584m (C═C); δ_H (100 MHz; d_6-DMSO; $SiMe_4$) −2.3 (br s, 2H, BH), 1.3 (br s, 8H, BH), 1.8 (s, 3H, CH_3), 3.35 (s, 2H, CH_2), 7.29–7.58 (m, 6H, H_{arom}), 7.71 (s, 1H, H_{arom}), 8.56 (s, 1H, CH═N), 11.79 (br s, 1H, NH); δ_C (50 MHz; d_6-DMSO; $SiMe_4$) 14.83 (CH_3), 30.72 (CH_2), 58.21, 57.95 ($C_{carborane}$), 105.15, 106.26, 109.95, 122.01, 126.97 (CH_{arom}), 123.24, 141.27 ($3C_{arom}$), 142.65 (CH_{arom}), 147.12, 162.65 ($2C_{arom}$), 168.11 (CH═N); δ_B (33.083 MHz; d_6-DMSO; $SiMe_4$) −9.91 (1B), −10.57 (2B), −11.21 (2B), −14.65 (1B), −22.79 (1B), −32.91 (2B); (FAB^-): *m/z* (%) = 408 (67) [M^-]; elemental analysis calcd (%) for $KC_{19}H_{25}B_9N_3O$: C 50.96, H 5.63, N 9.38; Found: C 50.64, H 5.14, N 9.02.

Compound **12b**. Yield: 59%, 0.38 g, R_f = 0.15, m.p. = 293–294 °C; IR ν_{max} (KBr disc)/cm^{-1}: 2525vs (BH), 1673s (C═O), 1619s (C═N), 1583m (C═C); δ_H (100 MHz; d_6-DMSO; $SiMe_4$) −2.53 (br s, 2H, BH), 1.56 (br s, 8H, BH), 2.11 (s, 6H, $2CH_3$), 3.62 (s, 4H, $2CH_2$), 7.31–7.47 (m, 6H, H_{arom}), 7.85 (s, 1H, H_{arom}), 8.64 (s, 1H, CH═N), 11.86 (br s, 1H, NH); δ_C (50 MHz; d_6-DMSO; $SiMe_4$) 15.12 ($2CH_3$), 31.65 ($2CH_2$), 59.24, 58.89 ($4C_{carborane}$), 105.16, 108.24, 109.34, 121.69, 123.47, 127.56 ($6CH_{arom}$), 142.59 (C_{arom}), 143.27 (CH_{arom}), 147.83, 161.58 ($2C_{arom}$), 166.92 (CH═N); δ_B (33.083 MHz; d_6-DMSO; $SiMe_4$) −9.94 (2B), −10.71 (4B), −11.09 (4B), −14.64 (2B), −22.86 (2B), −32.01 (4B); (FAB^-) *m/z* (%) = 568 (79) [M^-]; elemental analysis calcd (%) for $K_2C_{23}H_{39}B_{18}N_3O$: C 42.74, H 6.08, N 6.50; Found: C 42.65, H 5.89, N 6.19.

4.10. Biological studies

All tests were repeated 2–3 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–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 to 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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