CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200501580

Azanonaboranes Containing Imidazole Derivatives for Boron Neutron Capture Therapy: Synthesis, Characterization, and In Vitro Toxicity Evaluation

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Abstract: A number of azanonaboranes containing imidazole derivatives have been synthesized by a ligand-exchange reaction. The *exo*-NH_2R group of the azanonaborane of the type $[(RH_2N)B_8H_{11}NHR]$ can be exchanged by one hetero-nitrogen atom of the imidazole ring. In the case of histamine, the exchange takes place on the aliphatic amino group, the hetero-nitrogen atom of the imidazole ring or both of them. The products were confirmed by NMR, IR spectroscopy, elemental analysis, and mass spectrometry. The electron-withdrawing effect of the nitro group in 2-nitroimidazole is the main hindrance to achieve the exchange reaction. In vitro experiments were performed with B16 melanoma cells. A comparison of the biological properties of the products in which the B_8N cluster is connected to the hetero-nitrogen atom of imidazole ring or the aliphatic NH_2 group showed that incorporation of B_8N cluster unit into primary amino group increases the compound's toxicity. In contrast, this specificity for cytotoxicity effect was not observed in the case of histamine containing two B_8N clusters which was relatively nontoxic and did not inhibit colony formation up to concentrations of 2 mM.

Keywords: BNCT · boranes · cluster compounds · imidazoles

Introduction

The improvement of binary radiotherapy of cancer depends upon two agents, a radiation source and a target atom. The potential use of boron-containing compounds in cancer therapy is based on the nuclear property of the ^{10}B isotope as target atom which interacts with thermal neutrons, releasing an α-particle and Li ion. These particles represent high linear energy transfer radiations which result in lethal damage of tumor cells. This radiation has a tissue range of less than 10 μm, therefore cellular damage is restricted just to those cells in which the reaction occurs.[1] Numerous chemical approaches have been suggested including the conjugation of boron to carbon nanotubes.[1c]

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The considerable biological importance of the group of compounds incorporating the imidazole ring has stimulated much work on this heterocycle.[2] Nitroimidazoles have been widely used as antimicrobial chemotherapies[3] and as radiosensitizers for photon therapy of hypoxic tumors.[4] They also have properties that make them attractive potential candidates as boron carriers, especially for hypoxic solid tumors: 1) nitroimidazoles readily penetrate tumors and can produce blood and intratumor concentrations approaching 1 mM;[5] 2) they can undergo nitroreduction under hypoxic conditions to yield electrophilic substances which can damage protein and nucleic acids;[6] 3) the metabolism and toxicology of nitroimidazoles, particularly metronidazole, has been characterized.[7] While interest in nitroimidazoles as antiprotozoal agents is currently evident,[8] it is noteworthy that the nitro derivatives of the essential amino acid histidine and its congener histamine, both containing the imidazole moiety, have thus far been overlooked.[9]

All these properties have led many researchers to design boron-containing nitroimidazole derivatives for tumor targeting. Carborane cages attached to nitroimidazole derivatives have been designed.[10] The initial focus was to prepare O-carboranyl linked to 2-nitroimidazoles.[10a] The hydrophobic nature of these carborane-containing imidazoles and their low water solubility led to the tethering of the carbor-

ane through a series of oxyethylene units[10b,c] which improve the aqueous solubility and preliminary studies in tumor bearing mice.[10d] Misonidazoles attached to *closo*-carborane or *nido*-carborane have also been designed.[11a] These compounds were less lipophilic than misonidazole itself but still possessed selective toxicity for hypoxic cells.[11a] Another approach in the development of a boron-containing radiation sensitizer is described by Swenson et al.[11b,c] Their research involved a displacement reaction and incorporation of the BSH moiety into 1-(2-bromoethyl)-2-methyl-5-nitroimidazole. The product from this reaction is a negatively charged species and in contrast to the carboranylmisonidazoles is not highly lipophilic. Recently, the usefulness of 2-nitroimidazole sodium borocaptate–^{10}B conjugates as ^{10}B carrier in BNCT was evaluated and considered as promising for use in actual BNCT.[12]

The hydrophilic properties of the azanonaborane cluster distinguishes the $(RH_2N)B_8H_{11}NHR$ cluster (Figure 1) from *O*-carboranes (which are also neutral, but extremely hydrophobic). The electric neutrality distinguishes it from nido-*O*-carborane, $B_{12}H_{12}^{2-}$ and $SnB_{11}H_{11}^{2-}$, which are charged.[13] It offers therefore an additional approach to the synthesis of uncharged, water soluble compounds for BNCT. We therefore report the synthesis and preliminary biological evaluation of a number of azanonaborane clusters containing imidazole derivatives as new boron carriers.

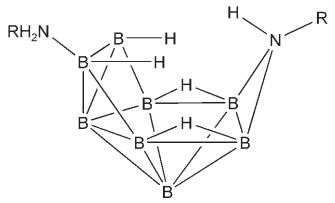


Figure 1. Schematic structure of azanonaborane cluster (*exo*-H atoms are omitted for clarity).

Abstract in German: *Eine Reihe von Azanonaboran-haltigen Imidazolderivaten wurde durch Ligandenaustauschreaktion hergestellt. Die exo-NH_2R Gruppe des Azanonaborans vom Typ [$(RH_2N)B_8H_{11}NHR$] kann durch ein Hetero-Stickstoffatom des Imidazolrings ausgetauscht werden. Die Produkte wurden durch NMR, IR Spektroskopie, Elementaranalyse, und Massenspektrometrie identifiziert. Der elektronenziehende Effekt der Nitrogruppe in 2-Nitroimidazol verhindert eine Austauschreaktion. In vitro Experimente wurden mit B16 Melanomzellen durchgeführt. Ein Vergleich der biologischen Eigenschaften der Produkte, in denen der B_8N-Cluster mit dem Stickstoffatom des Imidazolrings oder der aliphatischen NH_2-Gruppe verknüpft ist, zeigt, dass der Einbau des B_8N-Clusters in primäre Amine die Toxizität der Verbindungen erhöht. Im Gegensatz dazu wurde dieser spezifische Cytotoxizitätseffekt im Fall des Histamins, das zwei B_8N-Cluster enthält, nicht gefunden; diese Verbindung war relativ nichttoxisch und hemmte die Bildung von Zellkolonien bis zu Konzentrationen von 2 mM nicht.*

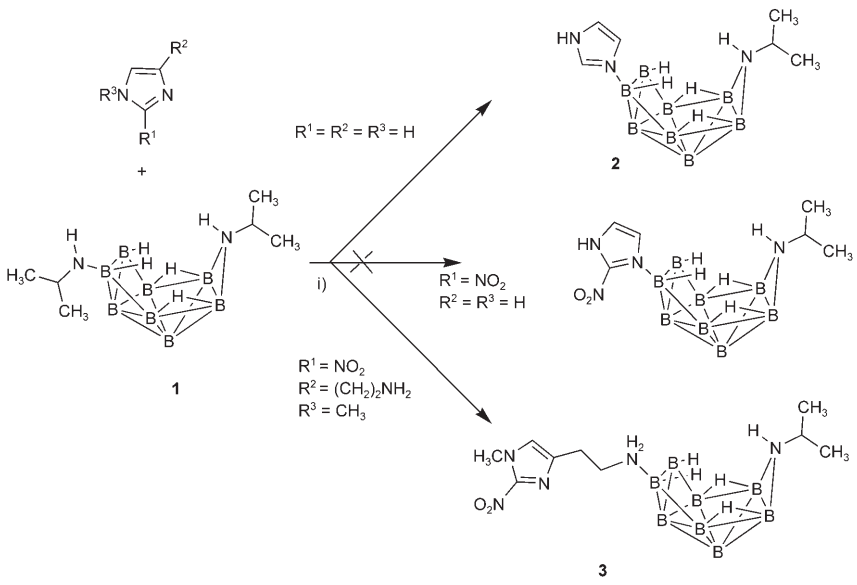
Results and Discussion

Preparation: Azanonaboranes $[(RH_2N)B_8H_{11}NHR]$ are easily synthesized by the reaction of 1 mol of dimethylsulfide-*arachno*-nonaborane with 3 mol of primary amino ligand in refluxing benzene.[13] The molecular structure of *hypho*-type is based on a $[B_8]$ cluster with one nitrogen-bridge and one *exo*-amino ligand (Figure 1). One of the most interesting reactions of azanonaboranes is the ligand-exchange reaction in which the *exo* (NH_2R) group is replaced by other ligands.[14] The exciting results with the ligand-exchange reaction stimulated us to explore new species of azanonaborane containing imidazole, histamine, 2-nitroimidazole, and *N*-methyl-2-nitrohistamine.

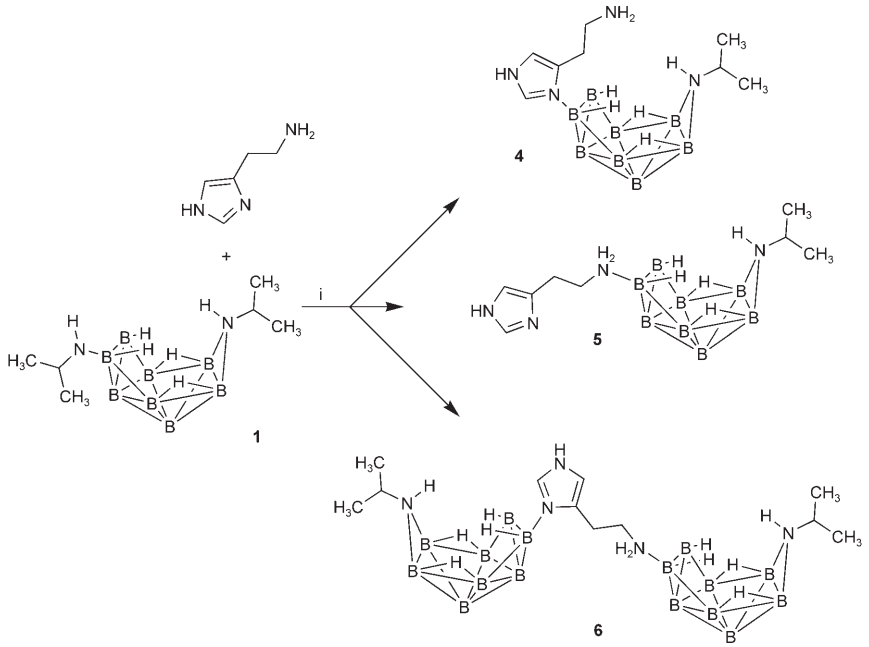
We have found that the exchange of the *exo*-amino ligand by imidazole derivatives is an equally convenient route to prepare new azanonaboranes containing the imidazole moiety. For example, imidazole reacts with $[(i\text{PrH}_2\text{N})B_8H_{11}NHi\text{Pr}]$ **1** in 1:1 ratio in refluxing benzene for 2 h to give $[(C_3H_4N_2)B_8H_{11}NHi\text{Pr}]$ **2** in 52% yield. In contrast, when compound **1** was treated under the same reaction conditions with 2-nitroimidazole, no exchange reaction was observed. Only unreacted starting compound **1** was recovered, even after prolonged heating. Presumably the mesomeric effect of the nitro group hinders the substitution of the isopropyl amine unit by the more electron deficiency 2-nitroimidazole unit. In contrast, the use of *N*-methyl-2-nitrohistamine[9] under the same reaction conditions in place of 2-nitroimidazole leads to the formation of compound **3** in reasonable yield 45% (Scheme 1).

Three compounds (**4**, 39%), (**5**, 42%), and (**6**, 19%) were obtained by the ligand-exchange reaction of histamine with compound **1** in 1:2 ratio in refluxing benzene for 2 h (Scheme 2). In compound **4** the *exo*-isopropylamine was exchanged by the nitrogen atom of imidazole ring. This was expected because it contains two hetero nitrogen atoms and only one of nitrogen electron pair is needed for the aromatic π-electron system. In the case of compound **2** the exchange takes place on the aliphatic primary amino group. The formation of compound **6** containing two clusters was attributed to the exchange reaction on both sites. These results seem interesting, because the exchange took place on both hetero-nitrogen atom and aliphatic amino group which gives the chance to obtain compound containing two B_8N units. The structures of the new compounds were confirmed by elemental analysis, IR, NMR apectroscopy and mass spectrometry.

Spectroscopic data: NMR data of compounds **2**–**6** are given in Table 1. Assignments are readily made by comparison with data reported previously. Azanonaboranes of the type $[(i\text{PrH}_2\text{N})B_8H_{11}NHi\text{Pr}]$ are easily identified by NMR spectroscopy, as their ^{11}B NMR spectra present a characteristic shielding pattern over the quite large range of about −55 to +2 ppm, showing only minor differences in their overall ^{11}B cluster shielding patterns. A change of ligands to imidazole and imidazole derivatives, however, shows significant sub-



Scheme 1. i) Reflux in benzene for 2 h.



Scheme 2. i) Reflux in benzene for 2 h.

stituent effects at the substituted B_3 site, although the shielding at other sites remain similar to the primary amine models. When the *exo*-NH_2*i*Pr group in compound **1** is successively replaced by imidazole or its derivatives, $\delta(^{11}B)$ for B_3 is shifted progressively to lower shielding, from -20.0 ppm in **1** to -19.06 ppm in **2** and hence to -18.79 ppm in **4**, respectively. Interestingly, replacement of the *exo*-NH_2*i*Pr group by histamine, in compounds **3** and **5** has effect on the B_3 chemical shift similar to aliphatic primary amine. This atom gives a signal at -20.04 ppm, comparable to that in compounds **4**. The dimeric compound **6** cause a shift to low-field of about -20.14 ppm, which is within the range of established substituent effects for aliphatic primary amine and imidazole substitution. In the ^{11}B NMR spectrum of compound **6**, the bands were broad and not well resolved.

In the ^1H NMR spectra, the N-bound H atoms of the *exo*-NH_2R group diagnostically resonate at about $+4$ ppm. Large deviations in these positions are observed in compounds **4–6** as expected. In compounds **5** and **6** the resonances of the exo-NH_2 group are shifted by about 1.7 ppm to low field relative to **1** when the *exo*-NH_2*i*Pr group is substituted by histamine. However the H atoms of the *exo*-NH_2 group of compounds **4** resonates at δ 4.63 ppm. In the case of compounds **2**, the aromatic protons exhibited three singlets at δ 6.94, 7.21, and 7.95 ppm whereas the NH proton resonates at 10.41 ppm. Moreover, ESI-MS spectra of all compounds showed the molecular ion peak $[M^+]$ attributed to the typical pattern of boron isotopes (^{10}B and ^{11}B).

The IR spectra of compounds **3–6** proved the presence of the NH_2 group. For all compounds, only slight differences were found in the vibrational frequency of B-H band [ν(B-H), lies in range 2539 to 2535 cm^{-1}] or the B-B band [ν(B-B) varies from 1059 to 1052 cm^{-1}], indicating that the intracluster bonding is not perturbed by the type substitution on B_3 atom of the cluster.

Biology: A series of imidazole derivatives has been shown to selectively sensitize hypoxic cells, present in solid tumors, toward the lethal effect of ionizing radiation. We therefore performed a preliminary biological evaluation of a number of boronated imidazole derivatives. The usefulness of the azanonaboranes containing imidazole or imidazole 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

Table 1. 200 MHz (^{11}B, ^{1}H) NMR data of B_8N clusters **2–6** in CD_3Cl at 20 °C.

Compound	B1	B2	B3	B4	B5	B6	B7	B8	μH(4,5)[a] μH(6,7)	NH
	$\delta(^{11}B)$ [$\delta(^{1}H)$]	$\delta(^{11}B)$[$\delta(^{1}H)$]	$\delta(^{11}B)$ [$\delta(^{1}H)$]	$\delta(^{11}B)$ [$\delta(^{1}H)$]	$\delta(^{11}B)$ [$\delta(^{1}H)$]	$\delta(^{11}B)$ [$\delta(^{1}H)$]	$\delta(^{11}B)$ [$\delta(^{1}H)$]	$\delta(^{11}B)$ [$\delta(^{1}H)$]	[$\delta(^{1}H)$]	[$\delta(^{1}H)$]
2	1.72	−55.23	−19.06	−33.64	−11.34]	−11.34	−32.57	−30.85		
	[2.56]	[−0.61]	[1.27]	[0.85]	[2.77	[2.57]	[0.85]	[0.58]	[−1.86]	[−1.49]
								[−0.35]	[−1.86]	
3	1.86	−55.06	−20.02	−34.36	−10.97	−10.97	−32.54	−30.85		
	[2.58]	[−0.71]	[1.23]	[0.76]	[2.56]	[2.56]	[0.76]	[0.51]	[−2.19]	[−1.36]
								[−0.69]	[−2.18]	
4	1.69	−55.34	−18.79	−34.25	−11.07	−11.07	−31.29	−30.54		
	[2.51]	[−0.68]	[1.19]	[0.71]	[2.56]	[2.55]	[0.71]	[0.52]	[−1.89]	[−1.43]
								[−0.64]	[−1.89]	
5	1.83	−55.12	−20.04	−34.23	−11.24	−11.24	−32.65	−30.49		
	[2.55]	[−0.66]	[1.21]	[0.82]	[2.53]	[2.56]	[0.82]	[0.62]	[−2.21]	[−1.51]
								[−0.51]	[−2.21]	
6[b]	1.06	−55.61	−20.14	−33.93	−11.25	−11.25	−32.23	−30.14		
	[2.5]	[−0.69]	[1.22]	[0.79]	[2.59]	[2.59]	[0.79]	[0.68]	[−2.19]	[−1.43]
								[−0.59]	[−2.19]	[−1.61]

[a] μH = bridging hydrogen. [b] All bands were broad.

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

The cytotoxic effects of azanonaboranes containing imidazole derivatives against B16 melanoma cells in vitro are shown in Figure 2 and summarized in Table 2. The azanonaboranes **3–6** were tested to a maximum boron concentration of 300 μg boron mL^{-1}. The position specificity for the cytotoxicity due to the connection of B_8N cluster to hetero-nitrogen atom of the imidazole ring (**4**) or the aliphatic NH_2 group (**5**) was observed in that compound **5** was significantly more toxic than compound **4** in inhibiting the colony formation. However, this specificity for cytotoxicity effect was not observed in the case of compound **6** which was relatively nontoxic and did not inhibit the colony formation up to 150 μg boron mL^{-1}. Whereas compound **6** has an LD_{50} value of around 160 μg boron mL^{-1}, compound **4** has an LD_{50} value of around 280 μg boron mL^{-1} (Figure 2, Table 2). Toxicity also increased with increasing the concentration of compound **6**. Conversely, azanonaborane **3** was already toxic at lower boron concentration (50 μg boron mL^{-1}). The in vitro toxicities of the compounds were not tested at lower concentrations because the achievable concentration of boron would not be effective for BNCT. According to these results, as well as those reported by others,[15] incorporation of a hydrophobic group into the chain of primary amine increases the compound's toxicity.

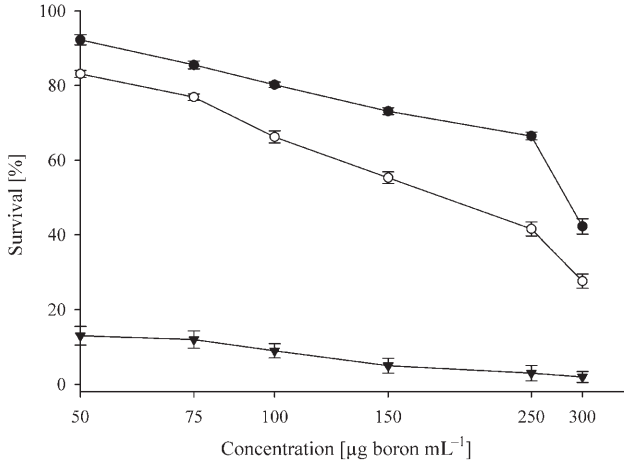


Figure 2. Percentage (± SD) of in vitro survival cell with respect to the concentration of the B_8N cluster compounds **3** (▼), **4** (●) and **6** ○. Data for **5** are identical to those of **3**.

Table 2. In vitro toxicity of azanonaboranes (**3–6**) in B16 melanoma cells.[a]

c_{media} (μg of B mL^{-1})	Percentage of survival (%) **3**	**4**	**5**	**6**
50	13 ± 2.47	92.24 ± 1.36	13 ± 2.27	83.12 ± 0.92
75	12 ± 2.32	85.54 ± 1.05	11 ± 2.51	76.89 ± 0.87
100	9 ± 1.89	80.21 ± 0.75	8 ± 1.76	66.25 ± 1.63
150	5 ± 1.99	73.13 ± 0.89	5 ± 1.81	55.29 ± 1.59
250	3 ± 2.05	66.47 ± 1.05	3 ± 1.96	41.58 ± 1.88
300	2 ± 1.46	42.26 ± 2.03	2 ± 1.51	27.64 ± 1.87

[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.

Conclusion

The ligand-exchange reaction of B_8N cluster with imidazole derivatives proved to be one of the best methods to prepare azanonaboranes containing imidazole derivatives. The compounds described herein represent a novel class of boron-containing imidazole derivatives which were synthesized in acceptable yields from readily available starting materials. The reactions as well as the workup procedures and the pu-

rifications for all products were readily feasible. It seems to be of no importance for the cellular toxicity of azanonaboranes containing imidazole if the B_8N unit is linked to the aliphatic NH_2 of the histamine. The B_8N clusters **5** appear not to be toxic over a wide range of boron concentrations up to 250 μg boron mL^{-1}. These results therefore encourage further continued investigation in the development of azanonaboranes containing imidazole derivatives as potential agents for BNCT.

Experimental Section

Materials and methods: Reactions were carried out in dry solvents and dry nitrogen but subsequent manipulatory and separatory procedures were carried out under air. $B_9H_{13}(SMe_2)$,[13a] [(*i*PrH$_2$N)B_8H_{11}NH*i*Pr][13c] and *N*-methyl-2-nitrohistamine[9] were prepared by literature methods and all other reagents were obtained commercially. Preparative thin layer chromatography (TLC) was carried out using 0.75 mm layers of silica gel G (Merck, GF_{254}) made from water slurries on glass plates of dimensions 20×20 cm^2, followed by drying in air at 100 °C. 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 Ξ= 100 MHz for δ (^1H) (nominally $SiMe_4$), Ξ=50 MHz for δ (^{13}C) (nominally $SiMe_4$), and Ξ=32.083 MHz for δ (^{11}B) (nominally F_3BOEt_2) in CD_3Cl. IR (cm^{-1}) spectra were determined as KBr disc on a Biorad FTS-7 spectrometer. Electron spray ionization (ESI) mass spectra were recorded on a Bruker Esquire in CH_3OH. Only the signal with the highest intensity of the boron isotopic pattern is listed.

General procedure for the synthesis of compounds 2–6: A solution of B_8N cluster **1** (1 mmol) was dissolved in anhydrous benzene (10 mL), and the appropriate imidazole or imidazole derivatives for ligand-exchange reaction were added (1 mmol). After heating the solution under reflux for 2 h the solvent was evaporated under vacuum. The residue, consisting of the crude azanonaborane product, was then purified by repeated thin layer chromatography on silica gel using dichloromethane and THF 1:1 as liquid phase. Criteria of purity and identity for this well-recognized molecular type **2–6** were multinuclear NMR spectra consistent with the molecular formation, along with the corresponding molecular ions in the mass spectrum, and also microanalysis of all resulting compounds.

Compound 2: Yield: 0.2 g, 52%, colorless solid; R_f=0.75 (THF/CH_2Cl_2 1:1); ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ = 10.41 (br s, 1H, aromatic HN), 8.09, 7.31, 7.03 (s, 3H, aromatic CH), 2.56 (hept, 1H, CH), 1.09 ppm (d, 6H, $(CH_3)_2$); ^{13}C NMR (50 MHz, CD_3Cl, Me_4Si): δ = 135.68, 127.12, 117.69 (3C, aromatic CH), 53.54 (C, HC-NB_8), 21.75, 21.67 ppm (2C, $(CH_3)_2$); IR (KBr disc): $\tilde{\nu}_{max}$=2985w (CH), 2525s (BH), 1623s (C=C), 1596s (NH), 1438s (BN), 1397s (CH_3), 1145s cm^{-1} (CN); ESI-MS: *m*/*z* (%): 225 (95) [M^+]; elemental analysis calcd (%) for $C_6H_{23}B_8N_3$ (223.4): C 32.22, H 10.29, N 18.8; found: C 32.16, H 10.19, N 18.75.

Compound 3: Yield: 0.11 g, 45%, colorless solid; R_f=0.31 (THF/CH_2Cl_2 1:1); ^1H NMR (200 MHz, $CDCl_3$ Me_4Si): δ = 7.96 (s, 1H, aromatic CH), 3.12 (s, 3H, CH_3N), 3.21 (t, 2H, CH_2-NH_2), +.89 (t, 2H, CH_2), 2.52 (hept, 1H, CH), +.04 ppm (d, 6H, $(CH_3)_2$); ^{13}C NMR (50 MHz, CD_3Cl, Me_4Si); δ = 155.72, 128.15, 118.21 (3C, aromatic CH), 53.31 (C, HC-NB_8), 25.54 (C, CH_2), 33.89 (2C, CH_2-NH_2), 21.15, 21.24 ppm (2C, $(CH_3)_2$); IR (KBr disc): $\tilde{\nu}_{max}$ = 2985w (CH), 2528s (BH), 1629s (C=C), 1586s (NH), 1448s (BN), 1389s (CH_3), 1146s (CN), 1463m, 1446m cm^{-1} (CH_2 groups); ESI-MS: *m*/*z* (%): 327 (46) [M^+]; elemental analysis calcd (%) for $C_9H_{29}B_8N_5O_2$ (325.4): C 33.18, H 8.91, N 21.51; found: C 33.02, H 8.89, N 21.23.

Compound 4: Yield: 0.058 g, 39%, colorless solid; R_f=0.32 (THF/CH_2Cl_2 1:1); ^1H NMR (200 MHz, CD_3Cl, Me_4Si): δ = 7.65 (s, 1H, aromatic HN), 6.97, +6.91 (s, 2H, aromatic CH), 3.27 (t, 2H, CH_2-NH_2), 2.91 (t, 2H, CH_2), 2.56 (hept, 1H, CH), 1.09 ppm (d, 6H, $(CH_3)_2$); ^{13}C NMR (50 MHz, CD_3Cl, Me_4Si): δ = 135.72, 128.15, 118.21 (3C, aromatic CH), 53.31 (C, HC-NB_8), 34.47 (C, CH_2-NH_2), 25.68 (C, CH_2), 21.25, 21.13 ppm (2C, $(CH_3)_2$); IR (KBr disc): $\tilde{\nu}_{max}$ = 2988w (CH), 2521s (BH), 1632s (C=C), 1596s (NH), 1452s (BN), 1393s (CH_3), 1149s (CN), 1466m, 1445m cm^{-1} (CH_2 groups); ESI-MS: *m*/*z* (%): 268 (52) [M^+]; elemental analysis calcd (%) for $C_8H_{28}B_8N_4$ (266.4): C 36.03, H 10.51, N 21.02; found: C 35.87, H 10.29, N 20.91.

Compound 5: Yield: 0.062 g, 42%, colorless solid; R_f=0.51 (THF/CH_2Cl_2 1:1); ^1H NMR (200 MHz, CD_3Cl, Me_4Si): δ = 7.54 (s, 1H, aromatic HN), 6.97, 6.86 (s, 2H, aromatic CH), 3.14 (t, 2H, CH_2-NH_2), 2.83 (t, 2H, CH_2), 2.5 (hept, 1H, CH), 1.03 ppm (d, 6H, $(CH_3)_2$); ^{13}C NMR (50 MHz, CD_3Cl, Me_4Si): δ = 135.46, 125.8, 117.89 (3C, aromatic CH), 53.32 (C, HC-NB_8), 34.57 (C, CH_2-NH_2), 25.71 (C, CH_2), 20.57, 20.49 ppm (2C, $(CH_3)_2$); IR (KBr disc): $\tilde{\nu}_{max}$ = 2992w (CH), 2529s (BH), 1635s (C=C), 1598s (NH), 1462s (BN), 1395s (CH_3), 1151s (CN), 1469m, 1441m cm^{-1} (CH_2 groups); ESI-MS: *m*/*z* (%): 268 (60) [M^+]; elemental analysis calcd (%) for $C_8H_{28}B_8N_4$ (266.4): C 36.03, H 10.51, N 21.02; found: C 35.92, H 10.32, N 20.89.

Compound 6: Yield: 0.029 g, 19%, colorless solid; R_f=0.75 (THF/CH_2Cl_2 1:1); ^1H NMR (200 MHz; CD_3Cl; Me_4Si): δ = 7.7 (br s, 1H, aromatic HN), 6.98, 6.86 (s, 2H, aromatic CH), 3.15 (t, 2H, CH_2-NH_2), 2.86 (,2H, t CH_2), 2.51 (hept, 1H, CH), +1.04 ppm (d, 6H, $(CH_3)_2$); ^{13}C NMR (50 MHz, CD_3Cl, Me_4Si): δ = 135.4, 125.8, 122.1 (3C, aromatic CH), 53.3 (C, HC-NB_8), 40.1 (C, CH_2-NH_2), 25.0 (C, CH_2), 21.7, 21.3 ppm (2C, $(CH_3)_2$); IR (KBr disc): $\tilde{\nu}_{max}$ = 2986w (CH), 2532s (BH), 1636s (C=C), 1596s (NH), 1459s (BN), 1396s (CH_3), 1152s (CN), 1467m, 1449m cm^{-1} (CH_2 groups); ESI-MS: *m*/*z* (%): 425 (48) [M^+]; elemental analysis calcd (%) for $C_{11}H_{47}B_{16}N_5$ (421.8): C 31.29, H 11.14, N 16.59; found: C 31.06, H 10.93, N 16.27.

Biological studies: All tests were repeated 2–3 times. For each compound Petri dishes were seeded with B16 melanoma cells grown in 9.69 gL^{-1} Eagle minimum essential medium (EMEM) (Biochrom KG) supplemented with 10 mLL^{-1} penicillin/streptomycin (10000 U, 10000 μgmL^{-1}, Biochrom KG), 2.2 gL^{-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 medium was removed, washed with PBS, dyed with GIEMSA for 10–15 minutes and washed again with ethanol. The number of colonies formed after one week was counted and compared to the number of colonies formed in the control without boron.

Acknowledgement

The financial support from the University of Bremen, Germany, to M.E. and A.G. is gratefully acknowledged.

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Received: December 16, 2005
Revised: March 28, 2006
Published online: July 25, 2006

