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Cytotoxic and apoptotic effects of 1,2-diborolanes with strong donor substitutes on human cancer cells

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ABSTRACT

In recent years, boron compounds have become more common as chemotherapy agents against certain types of cancers. Along with the development of boron-based therapeutic agents have come investigations into the various cancers and biochemical and molecular mechanisms affected by boron compounds and the relationships between boron compounds and chemical protection against cancer. In this preliminary study, the effects of new 1,2-*N*-substituted-1,2-diborolane derivatives on types of breast and liver cancers were examined for the first time. Four were found to significantly affect the cell viabilities and mitochondrial membrane potential changes in MCF-7, HepG2 and Hep3B cancer cells. Each was prepared in *n*-hexane at various concentrations (5, 10, 25, 50, 75 and 100 μg/mL). Human peripheral blood lymphocytes were used as control cells. Compounds 1, 2, 3a, and 3b 1,2-diborolane derivatives selectively killed cancer cells, but compound 1 was cytotoxic in a concentration-dependent manner on HepG2 and Hep3B and only at concentrations of at least 75 μg/mL on MCF-7 cells. Compound 3a exhibited cytotoxic effect on lymphocytes at 75 and 100 μgmL^{-1} concentrations, but compounds 1, 2 and 3b, 3c and 3d have not possessed significant cytotoxic effect on lymphocytes. Compounds 3c and 3d have not possessed significant cytotoxic effects. Mitochondrial membrane potential assay results supported these findings. Our results reveal that 1,2-diborolane derivates have high cytotoxic and apoptotic activities on human hepatocarcinoma cells and are therefore potential candidates in the development of new drugs against liver cancer.

1. Introduction

No cancer treatment is completely safe and secure, and many drugs commonly used as chemotherapic agents are not effective enough or lose effectiveness due to drug resistance [1]. Some researchers have attempted to induce apoptosis using natural and synthetic compounds, seeking adequate efficacy and sensitivity. It has recently been determined that boron molecules and their complexes can be biologically active [2–6]. A wide variety of boron compounds are currently being investigated as therapeutic against cancer. Studies focus primarily on boron, boric acid and their derivatives. Boron-based compounds are increasingly common as chemotherapy agents against certain types of cancers that are costly to treat or inoperable [7–11].

In our efforts to produce bioactive organoboron compounds [12], we developed a new 1,2-diborolane derivatives, finding efficacy against some of these types of cancers. The literature currently does not provide reports of the anticancer activities of 1,2-*N*-substituted-1,2-diborolane derivatives. In this study, 1,2-diborolane derivatives containing strong donor groups connected to the boron atoms were shown to have effective cytotoxic and apoptotic activities against certain types of cancer cells (MCF-7, HepG2 and Hep3B). How these activities change based on the structures of these groups was also determined. 1,2-diborolane derivatives have particular anticancer effects against human liver cell lines (HepG2 and Hep3B cells). The anticancer activities of 1,2-diborolane compounds and their potential to induce apoptosis in cancer cells suggest that they have potential in medical applications and bioorganic chemistry.

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2. Results and discussion

2.1. Spectroscopic data and crystal structure

While diborolane derivatives 1, 2, 3b and 3d were synthesized according to the literature [12], 3a and 3c ware similarly recently prepared from the reaction of 1,2-dichloro-1,2-diborolane [13,14] with MesNHLi and p-BrPhNHLi in THF/*n*-Pentane mixture. Its structures have been confirmed by ^{1}H, ^{11}B and ^{13}C NMR spectra in one and two dimensions, Elemental analysis, GC–MS as well as a single crystal X-ray diffraction study. Because of the partial back bonds between the N and B atoms ($sp^2 + \pi$), the o-Me protons of the mesitylene group give a very broad peak in the ^{1}H NMR at 1.75 to 2.23 ppm. Also, the integration of peak corresponds to about fifteen methyl protons (see supporting information).

Considering the NMR spectra and X-ray structure of 3a and 3c, the substituents are not symmetrically attached to the boron atoms as shown in Fig. 1.

To determine the geometric positions of phenyl rings, twist and fold angles between the rings (R denotes as the five membered ring, R1 and R2 are shown in Fig. 1) are determined as follows: In 3a, twist angles of R/R1, R/R2, and R1/R2 are 51.0°, 84.7°, 88.8°; fold angles are 129.0°, 139.3°, 69.0°, respectively. In 3c, in the same order twist angles are 30.8°, 148.7°, 151.4°; fold angles are 106.0°, 86.2°, 166.7°.

In 3a, the unsymmetrical conformation resulted in C—H…π and N—H…π type intra-molecular interactions between C5—H5…R1 and N1—H1…R2 with the H…Cg distances of 2.87 Å and 2.90 Å, respectively. In 3c, intramolecular π… π interaction, as shown in Fig. 2, between the phenyl rings is observed. As a consequence of the fold angle of R1/R2, that is not far from the value of 180°, this π… πinteraction is considerable. In both 3a and 3c, as in the literature [12], bulky substituents (steric effects) twisted the five-membered rings on C1—C2 bond with the B1—B2—C3—C2 and B2—B1—C1—C2 torsion angles of 11.6° and −4.16° for 3a, and −27.2° and −3.2° for 3c, respectively.

Although the substituents bonded to the ring boron atom had an asymmetric position with respect to each other, a single broad peak was observed at 55 ppm for both boron atoms in the ^{11}B NMR spectra. This value is consistent with the chemical shifts of diborolane derivatives (δ = 57–61 ppm) given in the literature [12]. The characteristic ^{13}C chemical shifts of the five-membered ring 3a are 25.2 ppm (CSi), 28.1 ppm (CB) and the ^{1}H chemical shifts are 1.73 ppm a triplet (HCSi), 2.10 ppm a doublet (HCB) ppm. (For detailed experimental results, see supporting information).

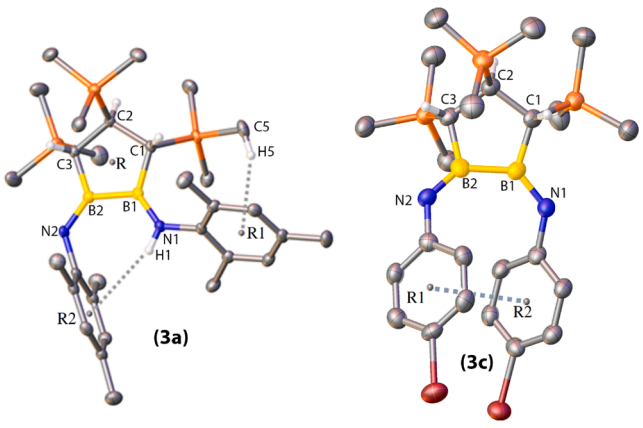


Fig. 1. Molecular structure of the compound **3a** and **3c**. H atoms are omitted for clarity. R, R1 and R2 are ring centroids. Intra-molecular H…π and π … π interactions are shown as dashed lines. Selected distances of **3a**: B1-B2:1.701(3) Å, H1…R2:2.905 Å and H5…R1: 2.868 Å; and **3c**: B1-B2: 1.726(8) Å and R1-R2 3.590(3) Å.

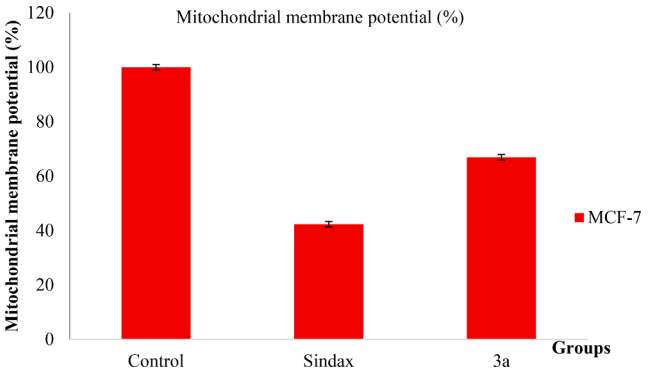


Fig. 2. Changes (%) in mitochondrial membrane potential of MCF-7 cells incubated with CC_{50} concentration of 3a for 72 h. Fluorescence ratio of JC-1 dimers/JC^{-1} monomers of control was assumed as 100%. *p < 0.05.

2.2. Cell viability assay

In our research, we used MTT assay, which is a colorimetric method, to determine viable cell rates after treatment with the newly synthesized 1,2-diborolane compounds (1, 2, 3a, 3b, 3c and 3d) on cancer cell lines (MCF-7, HepG2 and Hep3B cells) and lymphocytes. 1,2-diborolane treatments were done at various concentrations (5, 10, 25, 50, 75, and 100 μgmL^{-1}) for 72 h (Table.1). The results of MTT assay are shown in Table 1 and CC_{50} values of the compounds are present in Table 2. 1,2-diborolane compounds were cytotoxic and induced significant cell death in MCF-7, HepG2 and Hep3B cancer cells in a concentration-dependent manner except 3c and 3d (Table 1). The highest cytotoxic effect on MCF-7 cells was observed after treatment of cells with compound 3a for 75 h and 100 μgmL^{-1} concentrations for 72 h. The maximum percentages of cell death were 85% and 91%, respectively. However, treatment of the other compounds (1, 2, 3a, 3b, 3c and 3d) did not show cytotoxic effect even after 72 h incubation. The cytotoxic effect of the anticancer drug sindaxel 40 μgmL^{-1} concentrations on MCF-7 cells was determined as 89.91%.

Compound 3a showed cytotoxic effect at 75 and 100 μgmL^{-1} concentrations on lymphocytes. However, treatment of the other compounds (1, 2, 3a, 3b, 3c and 3d) did not show significant cytotoxic effect after 72 h incubation on lymphocytes.

Table 2 presents the CC_{50} values of 1,2-diborolane compounds on MCF-7, HepG2 and Hep3B cells and lymphocytes. For MCF-7 cells, except for compound 3a, the CC_{50} concentration value of other compounds could be calculated.

HepG2 and Hep3B cancer cells showed significantly different susceptibility to only four of the six 1,2-diborolan compounds (1, 2, 3a and 3b). Compounds 3c and 3b did not show cytotoxic effects on these cells. Interestingly, it was observed that HepG2 and Hep3B human liver cancer cells were more sensitive towards four 1,2-diborolane derivatives (1, 2, 3a and 3b) than MCF-7 cells (Table 1). The cytotoxic effect of 40 μgmL^{-1} anticancer drug sindaxel on HepG2 liver cancer cells was determined as 94.08%. On HepG2 cells, 2 and 3a compounds showed very high cytotoxic effects at 50 μgmL^{-1} concentration and above. 1 compound also showed its cytotoxic effect on HepG2 cells from 75 μgmL^{-1}. The 3b also showed its cytotoxic effect on HepG2 cells from 75 μgmL^{-1}.

1, 2, 3a and 3b derivatives showed high cytotoxic effects on Hep3B cells (Table 1), it is seen that the 1, 2 and 3a are effective from 50 μgmL^{-1} concentration, while the 3b is significantly effective at all concentrations starting from the lowest concentration (5 μgmL^{-1}). 40 μgmL^{-1} sindaxel showed 94.60% cytotoxic effect on Hep3B cells. 75 μgmL^{-1} and 100 μgmL^{-1} concentrations of compounds 1 and 3a, 50 μgmL^{-1}, 75 μgmL^{-1} and 100 μgmL^{-1} of 2, and 100 μgmL^{-1} of 3b produced clearly higher cytotoxic effect on He3B cells than sindaxel (Table 1). Calculated CC_{50} values of the derivatives for HepG2 and

Table 1
Cytotoxic effect of six 1,2-diborolane compounds on MCF-7, HepG2 and Hep3B cells and lymphocytes after 72 h of treatment.

Groups	Concentrations	Cytotoxicity (%) ± SD			
		MCF-7	HepG2	Hep3B	Lymphocytes
Control	—	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
n-Hexane (solvent control)	0.1%	2.76 ± 0.132	1.82 ± 0.053	1.98 ± 0.071	0.67 ± 0.030
Sindaxel	40 μgmL^{-1}	89.91 ± 0.002*	94.08 ± 0.114*	94.60 ± 0.011*	95.27 ± 0.031*
1	5 μgmL^{-1}	0.40 ± 0.176	−3.11 ± 0.736	18.44 ± 0.307	0.00 ± 0.00
	10 μgmL^{-1}	6.85 ± 0.380	3.57 ± 0.034	28.61 ± 0.151*	4.72 ± 0.034
	25 μgmL^{-1}	17.04 ± 0.003	5.16 ± 0.226	48.93 ± 0.008*	7.63 ± 0.224
	50 μgmL^{-1}	22.68 ± 0.226*	28.32 ± 0.441*	67.44 ± 0.174*	9.46 ± 0.119
	75 μgmL^{-1}	27.02 ± 0.069*	80.49 ± 0.091*	95.42 ± 0.004*	11.48 ± 0.425
	100 μgmL^{-1}	36.90 ± 0.041*	94.68 ± 0.003*	95.73 ± 0.005*	29.05 ± 0.057*
2	5 μgmL^{-1}	9.77 ± 0.128	−3.04 ± 0.070	14.81 ± 0.509	0.00 ± 0.00
	10 μgmL^{-1}	10.08 ± 0.069	−7.37 ± 0.034	19.20 ± 0.122	0.00 ± 0.00
	25 μgmL^{-1}	15.83 ± 0.025	34.93 ± 0.126*	45.73 ± 0.130*	6.08 ± 0.380
	50 μgmL^{-1}	16.53 ± 0.058	94.46 ± 0.001*	95.17 ± 0.002*	14.19 ± 0.070
	75 μgmL^{-1}	24.19 ± 0.033*	95.06 ± 0.001*	95.55 ± 0.004*	17.57 ± 0.115
	100 μgmL^{-1}	31.55 ± 0.050*	95.14 ± 0.009*	95.67 ± 0.007*	18.92 ± 0.023
3a	5 μgmL^{-1}	4.33 ± 0.023	18.22 ± 0.080	13.68 ± 0.146	0.00 ± 0.00
	10 μgmL^{-1}	12.20 ± 0.092	22.63 ± 0.037*	17.87 ± 0.119	6.75 ± 0.005
	25 μgmL^{-1}	25.20 ± 0.054*	37.51 ± 0.299*	36.64 ± 0.115*	21.62 ± 0.122*
	50 μgmL^{-1}	37.20 ± 0.122*	89.52 ± 0.123*	53.07 ± 0.355*	21.62 ± 0.107*
	75 μgmL^{-1}	85.88 ± 0.047*	90.96 ± 0.048*	94.67 ± 0.008*	35.81 ± 0.013*
	100 μgmL^{-1}	91.23 ± 0.009*	94.91 ± 0.001*	95.61 ± 0.001*	49.32 ± 0.054*
3b	5 μgmL^{-1}	5.24 ± 0.107	7.13 ± 0.062	52.51 ± 0.111*	2.70 ± 0.092
	10 μgmL^{-1}	8.47 ± 0.049	12.22 ± 0.261	55.46 ± 0.174*	11.48 ± 0.073
	25 μgmL^{-1}	12.10 ± 0.064	36.52 ± 0.426*	63.43 ± 0.273*	16.89 ± 0.213
	50 μgmL^{-1}	13.21 ± 0.150	49.58 ± 0.074*	68.70 ± 0.051*	17.57 ± 0.012
	75 μgmL^{-1}	14.01 ± 0.117	61.80 ± 0.091*	76.22 ± 0.106*	18.92 ± 0.058
	100 μgmL^{-1}	15.42 ± 0.233	91.42 ± 0.029*	94.67 ± 0.002*	19.59 ± 0.050
3c	5 μgmL^{-1}	9.33 ± 0.047	0.52 ± 0.075	−10.97 ± 0.167	−21.10 ± 0.132*
	10 μgmL^{-1}	10.77 ± 0.025	7.31 ± 0.034	−5.89 ± 0.174	−8.26 ± 0.430
	25 μgmL^{-1}	16.51 ± 0.032	−1.27 ± 0.048	−1.64 ± 0.380	18.35 ± 0.292
	50 μgmL^{-1}	17.13 ± 0.049	7.01 ± 0.056	2.45 ± 0.227	20.18 ± 0.122
	75 μgmL^{-1}	23.08 ± 0.112*	19.31 ± 0.093	17.02 ± 0.130	24.77 ± 0.013*
	100 μgmL^{-1}	29.13 ± 0.067*	21.40 ± 0.112*	18.33 ± 0.070	32.21 ± 0.005*
3d	5 μgmL^{-1}	1.53 ± 0.142	−0.22 ± 0.084	−61.05 ± 0.119*	−34.86 ± 0.320*
	10 μgmL^{-1}	5.03 ± 0.210	0.52 ± 0.107	−111.40 ± 0.123*	−43.12 ± 0.144*
	25 μgmL^{-1}	0.82 ± 0.050	8.50 ± 0.099	−112.77 ± 0.058*	−44.95 ± 0.092*
	50 μgmL^{-1}	−2.77 ± 0.037	10.66 ± 0.082	−158.10 ± 0.033*	−47.70 ± 0.041*
	75 μgmL^{-1}	−14.15 ± 0.089	11.86 ± 0.115	−135.35 ± 0.015*	−49.54 ± 0.056*
	100 μgmL^{-1}	−3.59 ± 0.092	22.30 ± 0.051*	−16.04 ± 0.018	−52.29 ± 0.022*

*Statistically significant differences as compared to the control groups (− and +) ($p < 0.05$). Values are means ± S.D. Values are presented of three separate experiments ($n = 3$).

Table 2
CC_{50} values of 1,2-diborolanes compounds for MCF-7, HepG2, Hep3B cells and lymphocytes.

Cells	CC_{50} values of the compounds (μgmL^{-1})					
	1	2	3a	3b	3c	3d
MCF-7	ND	ND	51.79	ND	ND	ND
HepG2	58.03	42.88	34.16	52.54	ND	ND
Hep3B	33,37	32,42	42,11	2,06	ND	ND
Lymphocytes	ND	ND	ND	ND	ND	ND

Values are presented of three separate experiments ($n = 3$); CC_{50} value for 1, 2, 3a, 3b, 3c and 3d compounds cannot be determined (ND).

Hep3B liver cancer cells also reveal cytotoxic effects of the derivatives (Table 2). Although the HepG2 and Hep3B of the four of 1,2-diborolanes derivatives showed a high level of cytotoxic effect, only the 3a compound was effective on MCF-7 cells. For lymphocytes, which have been used as healthy control cells, the CC_{50} concentration values of compounds could not be calculated.

Tetrazolium-based analyzes (i.e., MTT, MTS, XTT, WST-1 and, WST-8 assays) measuring cytotoxicity via mitochondrial activity are commonly used to evaluate cell proliferation, cell [15] viability, and drug cytotoxicity, especially in cancer cells studies [16]. This suggests that the compounds act selectively in cancer cells. In experimental cytotoxicity studies on cancer cells, it was revealed that boron could have antiproliferative effect on cells depending on the dosage and the duration of exposure [17]. In a study which boric acid was administered to HeLa cancer cells in a dose of 100–500 mg/L, it was shown that boric acid increased cell proliferation to a high level by demonstrating mitogenic effects, but when it was applied at a dose of 1000 mg/L or higher, it inhibited cell proliferation. Boron-based inhibition of cell growth and reproduction suggested that boron could have an effect on cell growth-related on signaling pathways [18,19]. High dosage (12.5–50 mM) of boric acid slows cell replication and caused induction of apoptosis in breast cancer cells (MDA-MB 231) and melanoma cells [7]. Boric acid inhibits the proliferation of the human prostate cancer cell lines DU-145 and LNCaP *in vitro* in a dose-dependent manner [17]. The results of our study are also similar to those of other boron compounds.

2.3. *Effect of the compounds on mitochondrial membrane potentials in cancer cells*

Apoptosis is a cellular process which involves genetically events causing the death of the cells. During this process, several important events happen in mitochondria. Among these events, the most important is the loss of mitochondrial transmembrane potential [20,21]. Throughout the life of a cell, the mitochondria use oxidizable substrates to generate an electrochemical proton gradient across the mitochondrial membrane which is used to produce ATP [22]. The mitochondrial membrane electrochemical gradient decreases during apoptosis.

Mitochondrial membrane potential is, thus, an important mitochondrial activity parameter that can be used as measure of cell health [21]. JC^{-1} is a lipophilic, cationic dye displaying naturally green fluorescence. It is used for monitor mitochondrial potential. Since it may be reach the mitochondria, it has accumulated there and begins to build to form reversible complexes and aggregates of JC^{-1}. In non-apoptotic cells, JC^{-1} is present as dimer and accumulates in mitochondrial aggregates that stain red and in apoptotic and necrotic cells, JC^{-1} exist in monomeric form in cytosol and stains it green [21].

Cell death also happens in various cancers following cytotoxic drug therapy, and the primary strategy of many anticancer medications is apoptosis induction. The development of anticancer drugs to date has concentrated on inducing apoptosis in tumor cells. Moreover, significant evidence suggests that the susceptibility or tolerance to anticancer agents may be altered by apoptosis [23].

In many cells, morphological and molecular changes in mitochondria are a crucial stage in apoptosis induced by different compounds. Decrease or collapse in mitochondrial membrane potentials indicates that cells go to apoptosis. In this study, the MCF-7, HepG2 and Hep3B cells were stained with a fluorogenic probe JC^{-1}. A reduction in the mitochondrial membrane potential is indicated by a decrease in red/green fluorescence intensity ratio. Figs. 1 and 2 shows that cancer cells non-significantly depolarize the mitochondrial membrane after 72 h of incubation. In the MMP assay, calculated CC_{50} concentrations of compounds with high cytotoxic effects were used for each cell. CC_{50} values of 1, 2, 3b, 3c and 3d compounds were not calculated for the MCF-7 cell line. Furthermore, CC_{50} values of 3c and 3d were not calculated for theHepG2 and Hep3 B cell lines. Therefore, these compounds were not used in mitochondrial membrane potential assay for MCF-7 cells. CC_{50} concentrations of the compounds significantly decreased the mitochondrial membrane potentials of MCF-7, HepG2 and Hep3B cells compared to the control groups. In MCF-7 cells, 51.79 μgmL^{-1} concentration of 3a compound decreased the membrane potential to 64.94% (Fig. 1). This decrease is significant in comparison with the control. Anticancer drug sindaxel also decreased the membrane potential of MCF-7 cells to 42.34%.

In HepG2 cells, CC_{50} values of the compounds also decreased the mitochondrial membrane potential of the cells, significantly. 58.03 μgmL^{-1} 1 decreased the mitochondrial membrane potential to 69.43%, 42.88 μgmL^{-1} 1 to 62.76%, 34.16 μgmL^{-1} 3a to 62.40%, and 52.54 3b to 64.92%, respectively. Anticancer drug sindaxel decreased the membrane potential of HepG2 cells to 43.19% (Fig. 2). In Hep3B cells, 33,37 μgmL^{-1} 1 decreased the mitochondrial membrane potential to 75.51%, 32,42 μgmL^{-1} 2 to 59.12%, 42,11 μgmL^{-1} 3a to 54.61%, and 2,06 μgmL^{-1} 3b to 57.45% and these decreases were also found statistically significant in comparison with the control. Anticancer drug sindaxel decreased the membrane potential of Hep3B cells to 35.88% (Fig. 3).

Although the results of the mitochondrial membrane potential assay support the results of the MTT test, the results in cytotoxicity were not exactly same and some differences occurred. For example, the CC_{50} concentration of compound 1 reduced the mitochondrial membrane potential to 75.51% in Hep3B cells. The reason for this difference may be that some cells die in a way other than apoptosis (see Scheme 1).

Chemotherapy drugs block the development of DNA, mRNA or proteins, specifically damaging DNA or impairing the mechanism required for growth or proliferation. Cells treated in this way can suffer mitotic catastrophe, lose the ability to sustain the activity of plasma membrane or become senescent, even if the reaction to their apoptotic stress response has been compromised [24,25].

Mitochondria play a central role in the apoptosis induction process. Differences between cancerous and non-cancerous mitochondria can be targeted to allow for the release of pro-apoptotic factors to induce apoptosis selectively in cancer cells [26]. Mitochondrial dysfunctions have been associated with various disorders such as cancer, cardiovascular diseases, diabetes, and neurodegenerative diseases [27]. The xenobiotic compounds reduce mitochodrial membranpotential (MMP)

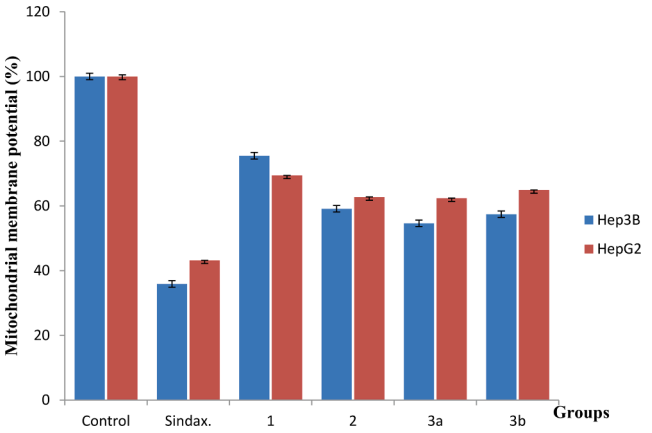


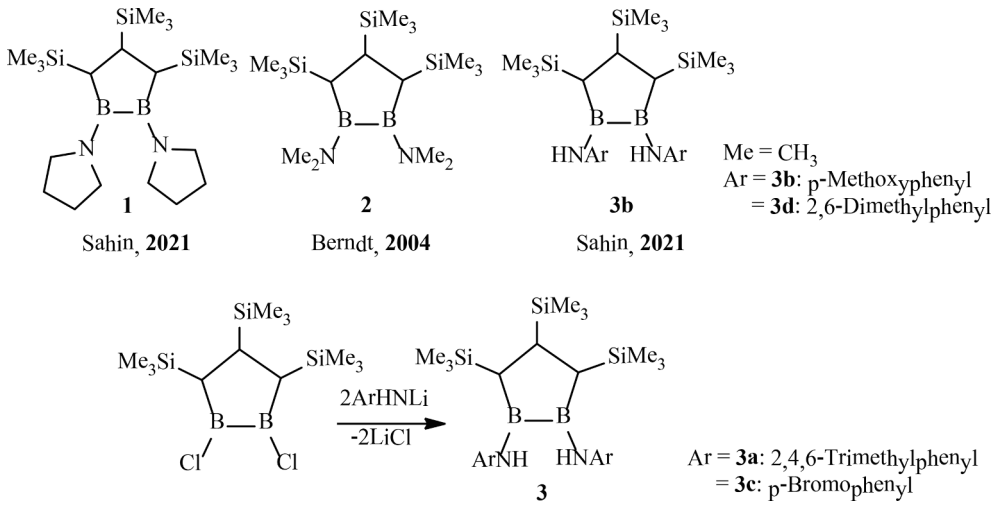
Fig. 3. Changes (%) in mitochondrial membrane potential of HepG2 and Hep3B cells incubated with CC_{50} concentrations of 1, 2, 3a and 3b for 72 h. Fluorescence ratio of JC^{-1} dimers/JC^{-1} monomers of control was assumed as 100%. *$p < 0.05$.

by perturbing a variety of macromolecules in the mitochondria, and therefore affecting different mitochondrial functions. A decrease in the MMP may also be linked to apoptosis [28]. Thus, these organelles are an ideal target for *in vitro* toxicity studies. Apoptotic cells are stained green by JC-1 dye as it accumulates in the cytoplasm due to the collapse of the mitochondrial membrane integrity. Changes in the MMP have been reported as an early event in the apoptotic signaling pathway [29]. Decrease in the MMP is reported to be associated with apoptosis where the mitochondrial membrane becomes permeable due to the translocation of Bax protein and the release of cytochrome *c* into the cytoplasm [30].

The ability of a cell to undergo mitochondrial apoptosis is governed by pro- and anti-apoptotic members of the BCL-2 protein family. The equilibrium of pro- versus anti-apoptotic BCL-2 proteins ensures appropriate regulation of programmed cell death during development and maintains organismal health. When unbalanced, the BCL-2 family can act as a barrier to apoptosis and facilitate tumor development and resistance to cancer therapy [31]. Whether stressed cells live or die is largely determined by interplay between opposing members of the Bcl-2 protein family. Bcl-2 and its closest homologs promote cell survival, but two other factions promote apoptosis. The BH3-only proteins sense and relay stress signals, but commitment to apoptosis requires Bax or Bak. The BH3-only proteins appear to activate Bax and Bak indirectly, by engaging and neutralizing their pro-survival relatives, which otherwise constrain Bax and Bak from permeabilizing mitochondria. The Bcl-2 family may also regulate autophagy and mitochondrial fission/fusion. Its pro-survival members are attractive therapeutic targets in cancer and perhaps autoimmunity and viral infections [32].

Wei et al. (2016) [33] attempted to elucidate a borax-induced apoptotic pathway in HepG2 cells that involves p53 and Bax upregulation and Bcl-2 down-regulation. According to this research, borax inhibited *in vitro* time- and dose-dependent proliferation of HepG2 cells, and the apoptotic mechanism activated by borax involved up-regulation of p53 and Bax and down-regulation of Bcl-2, confirmed by a change in the ability of the mitochondrial membrane potential. The cytotoxic effect of borax pentahydrate (BPH) on glioblastoma multiform (GBM) model, which represents fourth stage brain cancer, on the U-87MG cell line was investigated in terms of apoptosis and autophagy induction. As a result of the study, while determining the cytotoxic effect of BPH on U-87 MG cells, which form a model for GBM, it was determined that the form of cell death occurred through apoptosis rather than autophagy [34].

As a result, 1,2-diborolane compounds caused a significantly higher cytotoxic effect on liver cancer cells than MCF-7 cells. This low cytotoxic effect observed in MCF-7 cells may be due to the resistance of breast



Scheme 1. Synthesis of 3 derivatives.

cancer cells to 1,2-diborolane compounds. Cell membrane, membrane glycoproteins, enzyme system, amount and affinity of hormone receptors in breast cancer, cancer-related genes, DNA repair and tumor microenvironment can lead to the development of resistance to drugs used [35–38] 1,2-diborolane compounds may also have induced resistance in MCF-7 cells by one of these mechanisms. There is no study in the literature regarding cytotoxic and apoptotic effects of 1,2-diborolane compounds on the selected cancer cell lines. This study is important in that it is the first report on this subject.

3. Conclusion

In this study was presented that a new 1,2-*N*-substituted-1,2-diborolane derivative (3a) was prepared with high yields. The structure of 3a was characterized by one- and two-dimensional NMR spectroscopy and X-ray crystallography. Also, antitumor activities of 1, 2, 3a, 3b, 3c and 3d were examined. The results showed that 1, 2, 3a and 3b 1,2-diborolane compounds reduced the proliferation of the MCF-7, HepG2 and Hep3B cells line in a concentration dependent manner *in vitro*. Our findings also support the potential of the novel 1,2-*N*-substituted-1,2-diborolan derivative for the prevention and treatment of selected types of cancer, since at high concentrations other compounds than compound 3a did not cause a significant cytotoxic effect in lymphocytes. Further in vivo and human studies are needed to evaluate molecular mechanisms of 1,2-diborolane induced apoptosis in cancer cells and safety and clinical utility of this finding.

4. Experimental

4.1. Synthesis

General considerations: All reactions were carried out under argon, using standard schlenk techniques. Solvents were dried, distilled, and saturated with argon. Glassware was dried using a heat gun under high vacuum. NMR spectra were measured on a Varian 400 spectrometer. The chemical shifts are given in ppm, and are referenced against residual solvent signals. Elemental analysis war done on a Leco-932 and GC–MS war done on a Thermo Scientific Trace ULTRA-DSQ II GC–MS. 1,2-Dichloro-3,4,5-tris(trimethylsilyl)-1,2-diborolane [13] were synthesized according to literatures.

4.1.1. Synthesis of 1,2-dimesitylamino-3,4,5-tris(trimethylsilyl)-1,2-diborolane (3a)

An equivalent amount of *n*-BuLi (2.2 ml, 3.3 mmol, 1.6 M solution in hexane) was added dropwise to a THF/pentane mixture (approx. 1:3) (approx. 50 ml) from 2,4,6-trimethylphenylamin (approx. 3.2 mmol) at 0 °C. The mixture was warmed to room temperature and stirred overnight. The resulting suspension of MesNHLi was cooled to −10 °C and 1,2-dichloro-1,2-borolane [13] (2.24 g, 6.4 mmol) was added dropwise. All volatile components were removed in vacuum after the mixture was slowly warmed to room temperature. The residue was extracted 1:1 (ca. 60 ml) in hexane CH_2Cl_2 mixture. The solution was concentrated to a volume of about ca. 30 ml. The concentrated solution was kept at −25 °C and the crystals were obtained.

Yielding 80%, m.p.: 136 °C.

^{1}H NMR (400 MHz, $CDCl_3$, 300 K): δ = -0.36, 0.01, 0.18 (je s, Me_3Si), 1.02, 1.07 (je d, je H, $^2J_{H\text{-}H}$ = 4 Hz, HCB), 1.63 (t, H, $^3J_{H\text{-}H}$ = 4 Hz, HCSi), 1.75 to 2.23 (br., insg. 15H, Me_Mes), 2.37 (insg. 3H, Me_Mes), 4.86, 5.58 (je s, je H, NH), 6.79, 6.84, 6.95 (je s, insg. 4H, m-H, Mes); ^{13}C NMR (100 MHz, $CDCl_3$, 300 K): δ = -2.6, 0.8, 0.9 (je s, Me_3Si), 19.1, 19.9, 20.6, 20.8 (insg. 6C, o-, und m-Me, Mes), 25.3 (C, CSi), 28.2 (br. 2C, CB), 128.9, 129.1 (je 2C, m-C, Mes), 133.5, 134.9,135.2 (je 2C, o-, und p-C, Mes), 139.5, 140.3 (je C, i-C, Mes); ^{11}B NMR (128.32 MHz, $CDCl_3$, 300 K): δ = 56 (2B). Anal. Calcd. For $C_{30}H_{54}B_2N_2Si_3$ (548.38): C, 65.68; H, 9.92; N, 5.11. Found: C, 65.68; H, 10.24; N, 5.53. GC–MS, calculated for $C_{30}H_{54}B_2N_2Si_3$: 548.38, founded (M^+) 548.

4.1.2. Synthesis of 1,2-bis(p-bromophenyl)amino-3,4,5-tris(trimethylsilyl)-1,2-diborolane (3c)

Yielding 54%, m.p.: 135 °C

Compound 3c was prepared similarly to compound 3a. For the synthesis of 3c, a solution *n*-BuLi (6.0 ml, 9.6 mmol, 1.6 M solution in hexane) was added dropwise to a THF/pentane mixture (approx. 1:3) (approx. 50 ml) from p-bromophenylamin (approx. 9.6 mmol) at 0 °C. The mixture was warmed to room temperature and stirred overnight. The resulting suspension of p-Br-PhNHLi was cooled to −10 °C and 1,2-dichloro-1,2-borolane in pentane [13] (1.68 g, 4.8 mmol) was added dropwise. From *n*-hexane/CH_2Cl_2 mixture at −30 °C furnished 3c in the form of colorless crystals.

^{1}H NMR (400 MHz, $CDCl_3$, 300 K): δ = −0.11, 0.08, 0.12 (je s, Me_3Si), 1.04 (d, 2H, $^2J_{H\text{-}H}$ = 4 Hz, HCB), 1.51 (t, H, $^3J_{H\text{-}H}$ = 4 Hz, HCSi), 6.19 (s, H, NH), 6.42 (d, $^2J_{H\text{-}H}$ = 8 Hz, H, Ph), 6.49 (s, H, NH), 6.83, 6.99, 7.33, 7.45 (je d, 7H, $^2J_{H\text{-}H}$ = 8 Hz, H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$, 300 K): δ = −3.1, 0.7, 1.1 (9C, Me_3Si), 24.9 (C, CSi), 30.3 (br., 2C, CB), 115.8, 116.3 (je C, p-C, PhBr), 121.8, 123.5, 123.7, 130.8, 131.9 (8C, m- and o-C, Ph), 143.4, 143.7 (je C, i-C, Ph); ^{11}B NMR (128.32 MHz, $CDCl_3$, 300 K): δ = 61 (2B). Anal. Calcd. For $C_{24}H_{40}B_2Br_2\ N_2Si_3$ (620.11): C, 46.32; H, 6.48; B, 3.47; Br, 25.68; N, 4.50; Si, 13.54. Found: C, 46.34; H, 6.64; N, 4.20.

4.2. Cell culture and cell viability assay

The chemicals dimethyl sulfoxide (DMSO), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and phosphate-buffered saline (PBS) were purchased from Sigma Aldrich (St Louis, MO, USA). JC-1 was purchased from Biotium (California, USA).

MCF-7 (human breast adenocarcinoma), HepG2 and Hep3B (human hepatoma cellular carcinoma) cells are often used as a model for studying responses to drug therapy both *in vitro* and in vivo [29–31]. HepG2 and Hep3B are the two most widely used liver cancer cell lines as cellular models for *in vitro* liver cancer and toxicity research. "There are major variations between these two lines of cells, considering their similarity, and HepG2 and Hep3B cells are from different ethnic backgrounds. In reaction to the same pharmacological therapy under the same laboratory circumstances, they frequently demonstrate distinct and even opposite effects. Such differential findings include cytotoxicity divergences in chemo sensitivity, activation of gene expression, cell cycle response and biochemical impact [39]. Considering these differences between the two cells, we chose to use both of these cell lines in our study. Female breast cancer has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases and globally, breast cancer is the most commonly diagnosed cancer and the major cause of cancer-related death among females [40]. Therefore, we also used MCF-7 breast cancer cells in our study. Human peripheral lymphocytes were used as healthy control cells in experiments.

MCF-7, Hep3B, and HepG2 cells were cultured in DMEM, under specific culture conditions as specified in American Type Cell Culture (ATCC) (Details of culture conditions are given in supplementary data S3). Cell viabilities were determined by the MTT assay [41] (Details of MTT assay are given in supplementary data S3).

4.3. Measurement of mitochondria membrane potential (MMP)

1,2-diborolane derivatives induced changes of mitochondrial membrane potential in the cells were detected with the sensitive fluorescent dye JC^{-1} (5,5′,6,6′-tetrachloro1,1′,3,3′ tetramethylbenzimidazolylcarbocyanineiodide; Biotium, USA) according to the manufacturer's protocol. JC^{-1} is a fluorescent carbocyanine dye which accumulates in two forms (monomers or dimers) in the mitochondrial membrane, depending on the ability of the mitochondrial membrane [42]. At 485 and 538 nm wavelengths, JC^{-1} monomers display maximal fluorescence excitation and emission respectively. The inner mitochondrial membrane's negative potential promotes the development of dye aggregates, resulting in a transition in JC^{-1} monomer Florence to red light (from ex = 530 nm to em = 590 nm) [43]. The JC^{-1} dimer-to-monomer fluorescence ratio calculation is therefore a simple and accurate tool for estimating improvements in the capacity of mitochondrial membrane potential (The details of MMP assay are given in supplementary data S4).

4.4. Statistical analysis

All statistical analyses were performed using SPSS 20.00 software (SPSS 20.00, NY, USA). All experimental data are shown as the mean ± standard deviation (SD) of three independent experiments and analyzed using Onaway analysis of variance (ANOVA) and Dunnett's post hoc test. The criterion for statistical significance was set at $p < 0.05$. Value of $p < 0.05$ was considered statistically significant difference.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Crystallographic data and refinement parameter of **3a** and **3c** have been deposited at the Cambridge Crystallographic Data Centre with CCDC numbers 1988961 and 1988960 for **3a** and **3c**, respectively. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2021.105443.

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