Boron Salicylate Ester Compounds as Boron Therapeutics. Their Synthesis, Structural Characterizations and Anticancer Effects Against MDA-MB-231

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Abstract

The element boron forms a wide range of borate minerals with different properties. Borate minerals make it possible to design boron-containing molecules with new biological properties in terms of their chemical structure and properties. It is known that boron compounds have antioxidant, anti-inflammatory, anti-tumor and anti-cancer properties. This makes boron compounds important for the future development of boron chemotherapeutics, boron supplements and new drugs. Reliable scientific studies on boron compounds will facilitate the clear presentation of their functions in its biological applications and metabolism. In this study, boron monoester and boron diester structures were synthesized with salicylic acid ligand. To stabilize boron ester structures, Na^+, K^+, Mg^{2+}, Ca^{2+} cations were used as counter-ions. Structural properties of the synthesized substances, molecules obtained by crystallization/precipitation from aqueous solutions in solid state, elemental analysis, melting point determination, infrared spectroscopy analysis (FT-IR), thermal analysis (TGA/DTA), mass analysis (GC-MS) and single crystal analysis. Structural properties were tried to be explained by structure analysis (SC-XRD) methods. Additionally, the anticancer potential of boron salicylate esters against the MDA-MB-231 human breast adenocarcinoma cell line was examined. The K-B salicylate diester molecule was found to have the most potential potency with the lowest IC_{50} value against the MDA-MB-231 cell line. The anticancer potential of boron salicylate esters can be further investigated with

other cancer models with the combination of anticancer drugs. It is also thought that the mechanism of action of these molecules may help reveal their further applications.

Keywords: Boron Esters, Boron Therapeutics, Salicylic acid, Borate, Anticancer effect

1. Introduction

Boron, with atomic number 5, is the first member of metalloid or semiconductor elements [1,2]. Boron is necessary for the growth and development of plants [3]. The World Health Organization has reported that boron is "probably essential" for humans [4]. Studies have revealed the necessity of boron in animals as well [5].

Studies reveal that insufficient boron consumption causes growth disorders and attention deficit, and affects the activity of many metabolic enzymes as well as various micronutrients, including calcium, magnesium and vitamin D [6-11]. It is known that boron supplements are useful in preventing/or correcting arthritis, osteoporosis and osteoarthritis, in embryonic development, in wound healing, in alleviating oxidative stress and in detecting and repairing DNA damage [12-19]. It is also known to induce apoptosis of cancer types such as prostate, breast, cervix and lung cancer. It is mentioned as a chemotherapeutic drug for a variety of cancer kinds [18-21]. As a matter of fact, numerous studies on the biological importance of boron show that boron is important in sugar transport, carbohydrate metabolism, RNA metabolism, membrane transport, respiration and regulation of hematological bioprocesses [22-24].

Since boron is recommended as an essential trace element, boric acid (BA) has been added to many dietary supplements. Research shows that studies on boron esters have become very popular in recent years [19]. Due to the popularity of boron food supplements, the European Food Safety Authority (EFSA) expressed its opinion on calcium fructoborate, a boric acid ester, as a result of the requests it received. Accordingly, the Food Safety Agency (EFSA) in Europe, with its decision in 2021, approved the daily use of calcium fructoborate, containing 6.4 mg boron, at a dose of 220 mg, as a food supplement agent in the general adult population (except pregnant and breastfeeding women) [25]. The chemical state of boron in nutrition is very important. In plants, boron is found in the form of sugar esters [26], and there are studies showing that its absorption is highly dependent on the chemistry of boron sugar esters [27,28]. Boron sugar esters are the best chemical form for absorption by cells [29].

Borates, which are boron minerals containing (B-O) bonds, have biological importance and form trigonal and tetrahedral bonds to form complexes with organic functional groups [30-32]. Boric acid gives an esterification reaction with compounds containing more than one hydroxyl group (having a cis-diol group) in its structure and forms tetrahedral anionic ester structures. They enable the synthesis of highly stabilized boron ester derivatives with nucleotides, vitamins and carbohydrates containing cis-diol groups through the esterification reaction [33-46].

Salicylic acid, the main metabolite of aspirin (shown in figure 1), has a broad biopharmaceutical effect [47]. Salicylic acid plays an important role in various processes of plant growth and stress responses. There are also studies on its use as a therapeutic agent in humans [48]. In this sense, it is thought that the combination of the ligand with the boron element has unique properties that we can define as useful compounds and may be more effective in biological applications. Breast cancer is the primary cause of cancer-related deaths among women globally and ranks as the second most common cause of cancer fatalities in women. Breast cancer poses a significant therapeutic challenge, due to its poor response to treatments and its highly invasive characteristics. Moreover, drug resistance hinders effectivity of widely used chemotherapeutics [49]. Boron compounds has been attracting attention for their potential in cancer therapy. A recent study reported potential of polyglycerol functionalized boron carbide nanoparticles for boron neutron capture therapy applications, due to their high dispersibility and low toxicity [50]. Another study stated that boric acid and borax were effective against U-87MG glioblastoma cell line [51]. We have also previously evaluated anticancer potential of boron glycinate esters revealing that the synthesized boron esters were effective against HepG2 hepatocellular carcinoma cell line, by inducing oxidative stress [52].

In this study, salicylate boron esters, which have a chemical structure similar to the natural structure of boron found in daily dietary plants, were synthesized. Na^+, K^+ Mg^{2+}, Ca^{2+} metal cations were used to stabilize these negatively charged ester structures. Boron given orally as a supplement is easily and completely absorbed in the body. It passes into the body without being metabolized, is excreted with a half-life of 21 hours, and is eliminated mostly in bones with a low accumulation level [53].

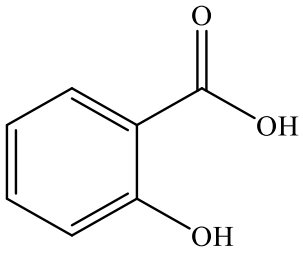


Figure 1. Salicylic acid

2. Materials and Methods

2.1. Materials

MTT reagent was from Thermo Fisher Scientific (USA), whereas dimethyl sulfoxide (DMSO) was from Sigma Aldrich (USA). DMEM high glucose medium, Leibovitz's L-15 medium, fetal bovine serum (FBS) and penicillin/streptomycin (pen/strep) were retrieved from Capricorn Scientific (Germany). Both L929 and MDA-MB-231 cell lines were from ATCC (USA).

2.2. Methods

2.2.1. Synthesis of the Boron Esters

Monoanionic forms of organic bioactive ligand molecules were prepared by reacting salicylic acid solutions with $NaHCO_3$, $KHCO_3$, $MgCO_3$ or $CaCO_3$ at appropriate molar ratios. While preparing monoesters, first H_3BO_3 solution is added into the solution containing the metal salt in a ratio of 1:1. Then, the solvent of the solution is removed under vacuum and cold acetone is added to the prepared solution. After vacuum filtration, the precipitate/crystals are immediately placed in a desiccator to prevent moisture absorption.

Diesters were prepared by adding an equivalent amount of solid biologically active organic acid to the solution containing the boron-monoester of the corresponding biologically active salicylic acid. The next steps continue the same as the preparation of the monoesters mentioned above. The synthesis diagram of boron-salicylate ester compounds based on water elimination is schematized in Figure 2.

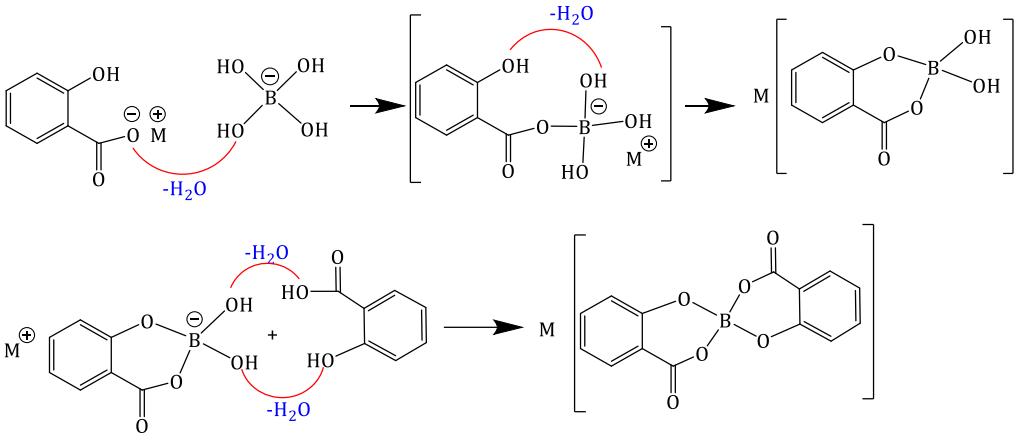


Figure 2. Proposed mechanism for the complexation of boric acid with salicylic acid

2.2.2. Characterization of the Boron Esters

C, H, N contents were determined with a CHNS-932 LECO model analytical device. Melting points were determined with an Electrothermal 9100 model device. Thermal degradation curves (TGA, DTA) were recorded by the Shimadzu DTG-60H system in a dynamic nitrogen atmosphere (100 mL/min), at a heating rate of 10 °C/min, in platinum crucibles as sample containers, using Al_2O_3 as a reference. FT-IR spectra were measured in the range of 450-4000 cm^{-1} using the KBr pellet technique with the Perkin-Elmer Spectrum One device. Bruker APEX3 device was used for single crystal structure analysis of molecules in suitable forms [54].

2.2.3. Cell Viability Assay

Anticancer potential of boron esters was investigated with MTT assay. L929 mouse fibroblast cells were used as healthy control whereas MDA-MB-231 human breast adenocarcinoma cells were used as the cancer cell line. Untreated cells were used as control whereas wells that were not seeded with cells and treated with samples were used as blanks. L929 cells were cultivated with DMEM high glucose medium supplemented with 10% FBS and 0.1% pen/strep whereas MDA-MB-231 cells were cultivated with Leibovitz's L-15 Medium supplemented 10% FBS and 0.1% pen/strep. Media was renewed every other day and cells were passaged when reached 80% confluency. In order to determine cytotoxicity of boron esters, first, cells were seeded to 96-well plates with density of $1x10^4$ cells/well. 24 hours after seeding, cells were treated with 100 µl of varying concentrations (0.25-16 mM) of Na, K, Mg and Ca boron salicylate diesters. In order to be able to make comparison between esters of monovalent and divalent cations, concentration was calculated based on salicylic acid present in the ester structure. After treatment, cells were incubated for 48 hours in 95% CO_2 for L929 cells and 100% air for MDA-MB-231 cells. After 48 h of incubation, media was replaced with 0.5 mg/ml MTT solution in DMEM high glucose

without phenol red and incubated for 4 hours. Then, MTT solution was discarded and formazan crystals formed were dissolved in 100 µl of DMSO. Dissolved formazan crystals were transferred to a new 96-well plate and the absorbance at 570 nm was recorded. Concentration-dependent cell viability was calculated using the Equation 1. Absorbance values obtained from the positive control group were accepted as the group with 100% cell viability and were calculated as percentage viability accordingly. By using the dose dependent cell viability data, IC_{50} values were determined by using the Levenberg-Marquardt algorithm (OriginPro 2017, USA) [55].

$$Cell\ Viability\ (\%) = \frac{A570_{sample} - A570_{blank}}{A570_{control} - A570_{blank}} \times 100\%$$

3. Results and Discussion

3.1. Elemental Analysis

Within the scope of the study, the results of the chemical composition analysis of the boron mono and diester structures of the salicylate anion with sodium, potassium, calcium and magnesium cations and the closed molecular formulas created according to these results are summarized in Table 1.

Table 1. Elemental analysis results of boron salicylate ester compounds

Compound	M.P. (°C)	C(%)	H(%)
		exp.(theo.)	exp.(theo.)
$Na[B(Sal)(OH)_2]\cdot 2H_2O$	274	35.44(35.83)	3.75(4.17)
$Na[B(Sal)_2]\cdot 3H_2O$	370	45.88(46.70)	4.05(3.92)
$K[B(Sal)(OH)_2]\cdot {}^3/_2H_2O$	110	34.45(34.00)	3.45(3.64)
$K[B(Sal)_2]\cdot 2H_2O$	280	47.14(46.90)	3.41(3.35)
$Mg[B(Sal)_2]_2\cdot 7H_2O$	130	46.14(46.94)	3.99(4.22)
$Ca[B(Sal)(OH)_2]_2\cdot H_2O$	115	40.69(40.00)	3.55(3.33)
$Ca[B(Sal)_2]_2.2H_2O$	162	51.77(52.37)	4.21(3.14)

3.2. Infrared Spectroscopy

In the esterification reaction, intermolecular hydrogen bonds formed by the –OH groups of boric acid and salicylic acid take place. Disruption of the hydrogen bonded network appears to cause a narrowing of the O–H stretching band. Some significant peak shifts and intensity changes resulting from boron salicylate complexation are summarized in Table 2. There are changes in the –OH vibrations around 1650 cm^{-1} due to the participation of the salicylic acid ligand in the esterification with boron, but these changes are difficult to clarify due to overlapping vibrations.

The peaks around 2900 cm^{-1} are thought to belong to C-H stretching peaks. It was interpreted that the peaks observed around 1740 cm^{-1} belong to ν(C=O) stretching. The peaks attributed to the groups of carboxylic acid were observed around 1550 cm^{-1} for $\nu_{asym}(COO^{-})$ and 1400 cm^{-1} for $\nu_{sym}(COO^{-})$. As a result of the esterification of boric acid, B-O-C bonds should form in the system and the peaks belonging to the B-O-H bond should disappear. The peaks seen at 1082 cm^{-1} indicate the formation of B-O-C bonds. Since no peak is seen at the 1195 cm^{-1} point of the B-O-H bond, which indicates the presence of boric acid remaining without esterification in the system, it can be said that there is no free boric acid left in the system and all boric acid bonds with salicylate [56]. It is thought that the characteristic vibrations of the B-O bond in the tetrahedral boron complexes in the same region in the 900-1000 cm^{-1} range overlap with the (C-O-) band. The most important evidence supporting the formation of boron ester compounds is the peaks observed in the infrared spectra attributed to $\nu_{asym}(BO)/BO_4$ & ν(C-O) and $\nu_{sym}(BO)/BO_4$ stretching vibrations. These characteristic peaks of the $\nu_{asym}(BO)/BO_4$ & ν(C-O) are expected to be observed in 1220 cm^{-1} region and the $\nu_{sym}(BO)/BO_4$ bidentate peak in the 834 + 815 cm^{-1} region [57]. It has been observed that the characteristic $\nu_{asym}(BO)/BO_4$ peak occurs around 1156 cm^{-1} – 1134 cm^{-1}. It has been observed that the $\nu_{sym}(BO)/BO_4$ bidentate peak shifts to the right in salicylate boron ester complexes. This situation is thought to be due to the metal cations, which are the counter-ions of the anionic complex, and their various interactions. FTIR spectrum of boron salicylate complexes is given in Figure 3.

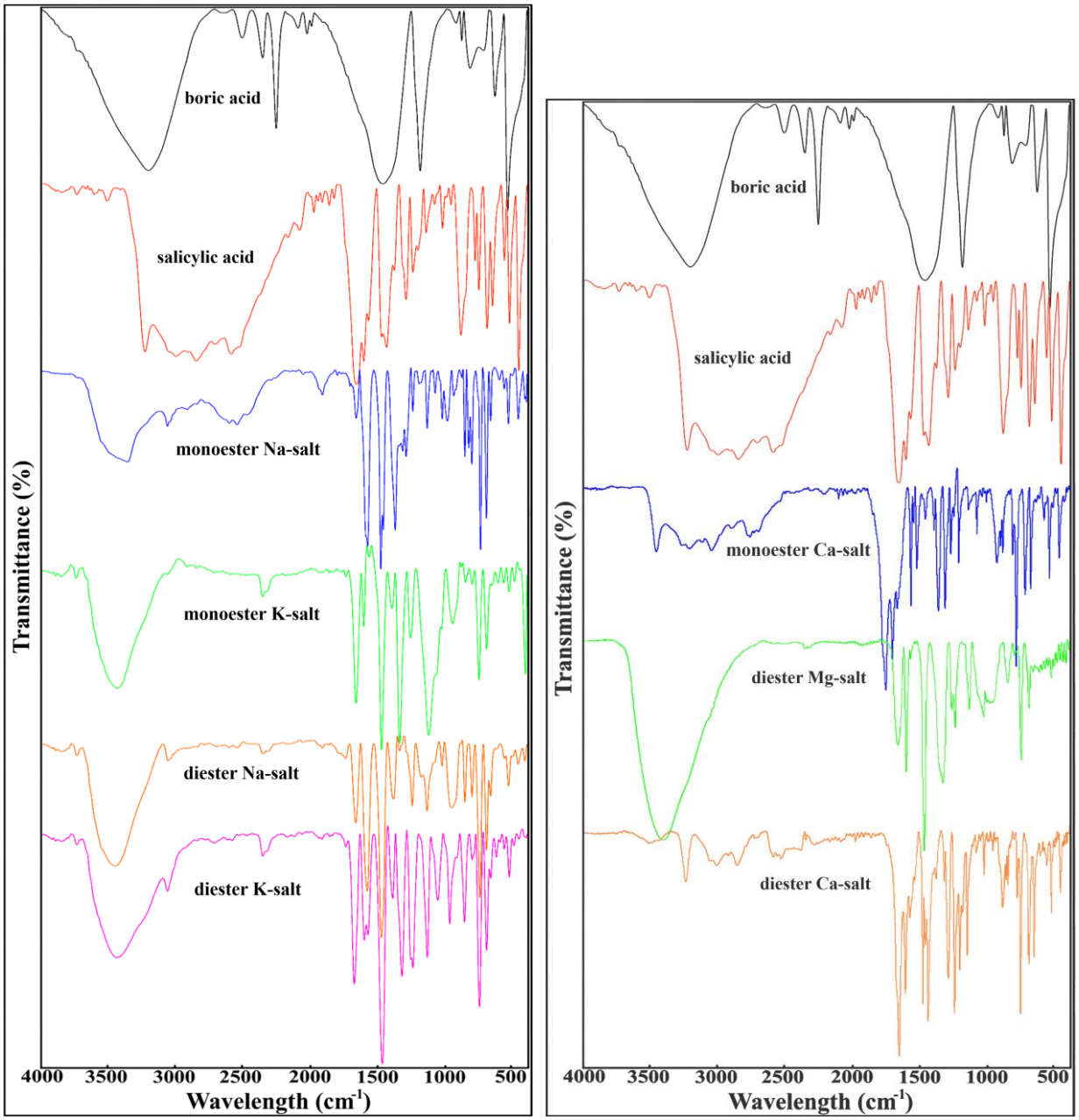


Figure 3. FT-IR spectrum of boron salicylate complexes

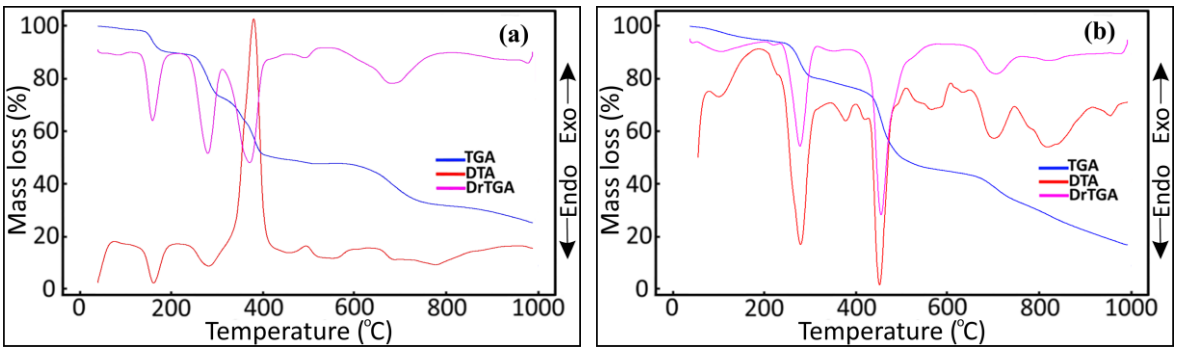
Table 2. Important infrared peaks of boron salicylate ester compounds

Compounds	ν(O-H)	ν(CO) & ν(CC)	ν_a(COO) & ν_s(COO)	ν(C-O-) & ν_a(B-O)/BO_4	ν_s(BO)/BO_4
Salicylic acid [58]	3350-2400	1685s-1613	—	—	—
Boric acid [57,59]	~3300	—	—	1220s	834+815
Sodium boron-salicylate mono-ester	3600-3150	1668	1582-1378	1200-1140	744+700
Sodium boron-salicylate di-ester	3600-3100	1663	1599-1381	1253-1134	860+809
Potassium boron-salicylate mono-ester	3600-3090	1665	1612-1393	1245-1138	755+700

Potassium boron-salicylate di-ester	3600-3100	1683	1609-1394	1210-1156	808+790
Magnesium boron-salicylate di-ester	3600-2900	1673	1610-1337	1247-1140	805-791
Calcium boron-salicylate mono-ester	3300-3150	1658	1578-1384	1210-1155	805+784
Calcium boron-salicylate di-ester	3300-3200	1656	1612-1385	1209-1156	817-797

3.3. Thermal Analysis

Thermal degradation of molecules involves three successive stages; dehydration, endothermic degradation (combustion) of the ligand, and oxidation of the residual organic component (Figure 4). The final decomposition products are expected to be the corresponding metal oxides and boron oxide. Important data on thermal analysis decomposition are summarized in Table 3 (TGA curves of the complexes in which the metal cation was used as a stabilizing cation were carried out in an inert nitrogen gas environment. When the thermal decomposition curves of the synthesized boron esters were examined, it was seen that the thermal decomposition of the ligands showed similar characteristics among themselves). In general, thermal decomposition steps are interpreted as the removal of hydrate waters in the first step. The reason why the water molecules outside the coordination sphere, which we call hydrate water, move away from the structure at different temperature ranges can be interpreted as the fact that the hydrate molecules may be bonded to the sodium or boron atoms with interactions of different strengths. In the following stages of dehydrated boron esters, degradations that can be interpreted as the removal of -OH groups of organic residues in the form of water molecules were detected. The subsequent decomposition steps are generally thought to be in the form of organic residues breaking down by burning in more than one step in a nitrogen environment, and finally various gases such as CO and CO_2 leaving the structure. It was claimed that the relevant metal cation oxides and boron oxide residues remained in the reaction vessel as the final decomposition product in the TGA curves. The harmony between the theoretical and experimental weight losses of the degradation and residue products of the relevant steps supported this result.



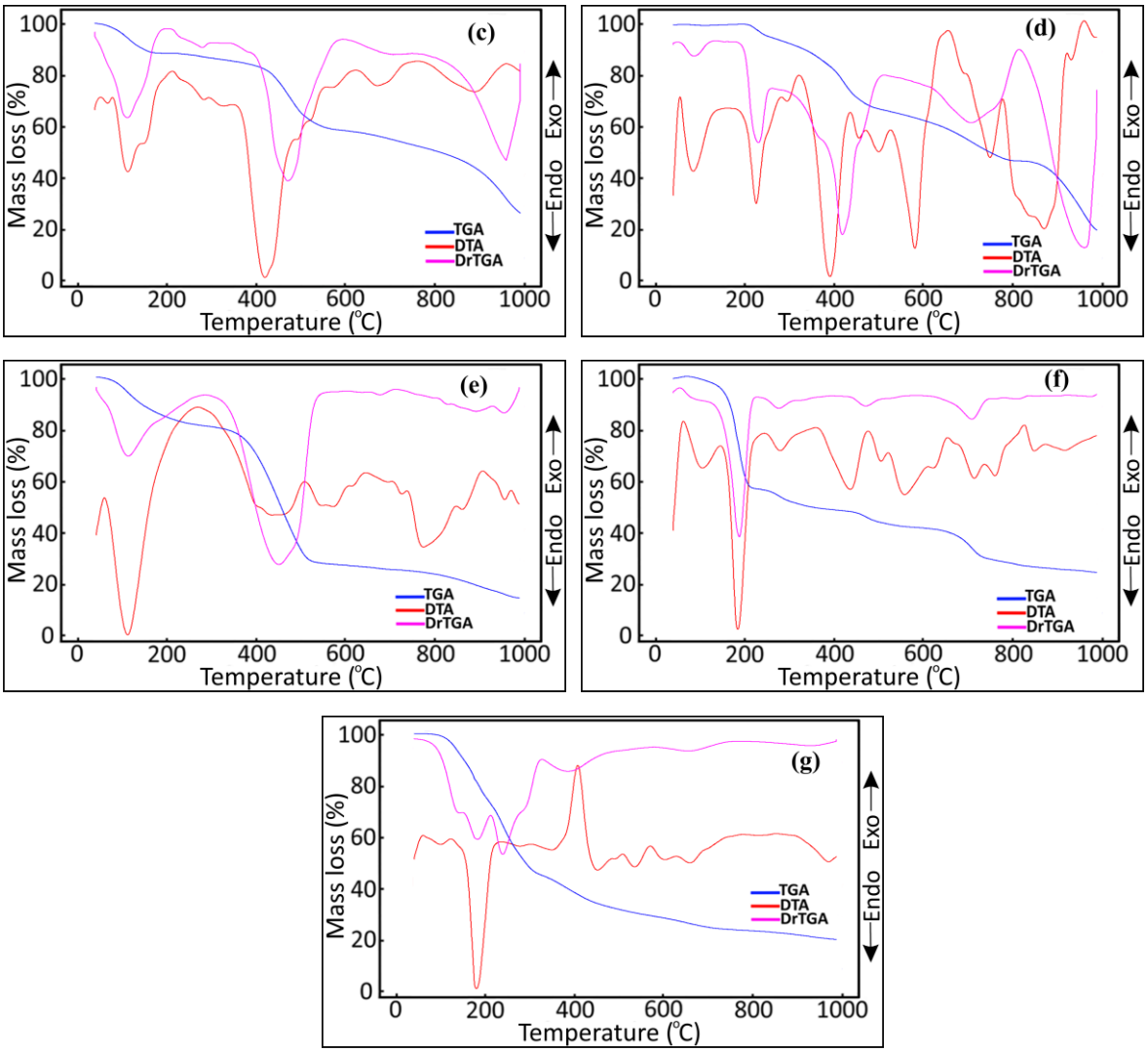


Figure 4. a) $Na[B(OH)_2(Sal)].2H_2O$, **b)** $Na[B(Sal)_2].H_2O$, **c)** $K[B(Sal)(OH)_2]\cdot{}^3/_2H_2O$, **d)** $K[B(Sal)_2]\cdot H_2O$, **e)** $Mg[B(Sal)_2]_2\cdot 7H_2O$, **f)** $Ca[B(OH)_2(Sal)_2]_2\cdot 2H_2O$, **g)** $Ca[B(Sal)_2]_2\cdot 2H_2O$

Table 1. Thermal analysis data of boron-salicylate esters

Molecule		Temp. Range (°C)	DTA_{max} (°C)	Removing Group	Mass Loss (%)		Residue Product (%)		Decomp. Product	Colour
					Den.	Teo.	Den.	Teo.		
$Na[B(OH)_2(Sal)].2H_2O$										Beyaz
$C_7H_{10}BNaO_7$	1	38-125	76	$H_2O_{(nem)}$	1.68	-				
239.95g/mol	2	130-195	154	H_2O	8.20	7.50				
	3	201-298	276	$2H_2O$	15.40	15.00				
	4	300-963	-377; 456; 553;783	$C_7H_4O_2$	48,84	50.06	26.31	27.42	$^1/_2Na_2O.B_2O_3$	Siyah
$Na[B(Sal)_2].H_2O$										Beyaz
$C_{14}H_{14}BNaO_9$	1	59-235	93	H_2O	5.17	5.99				
360.06g/mol	2	236-301	273	$2H_2O$	11.73	11.98				
	3	303-989	373; 449; 565; 704; 822	$2C_7H_4O_2$	64.87	66.71	25.93	18,28	$^1/_2Na_2O.B_2O_3$	Siyah
$K[B(Sal)(OH)_2]\cdot{}^3/_2H_2O$										Beyaz
$C_7H_9BKO_{6,5}$	1	48-158	104,141	$3/2H_2O$	11.15	10.93				
247.04g/mol	2	217-411	277,323	H_2O	6.23	7.29				
	3	414-956	494; 524; 674; 895	$C_7H_4O_2$	48.80	48.62	33.47	33.15	$^1/_2K_2O.B_2O_3$	Siyah
$K[B(Sal)_2]\cdot H_2O$										Beyaz
$C_7H_9BKO_{6,5}$	1	36-114	90	$H_2O_{(nem)}$	0.70	-				
247.04g/mol	2	116-200	166	$2H_2O$	8.50	8.56				
	3	281-892	301; 328; 378; 663; 879	C_9H5O_2; $C_9H_5O_3$	69.12	72.29	21.68	19.21	$^1/_2K_2O.B_2O_3$	Siyah
$Mg[B(Sal)_2]_2\cdot 7H_2O$										Beyaz
$C_{28}H_{30}B_2MgO_{19}$	1	40-257	94	$7H_2O$	17.15	17.59				
716.45g/mol	2	263-519	392,428	$4C_6H_4O$	50.95	51.42				
	3	522-988	570; 727; 780	4CO	16.79	17.59	15.11	15.34	$MgO.B_2O_3$	Siyah
$Ca[B(OH)_2(Sal)_2]_2\cdot 2H_2O$										Yeşil
$C_{14}H_{14}B_2CaO_{11}$	1	47-120	86	H_2O	4.02	4.28				
419.95g/mol	2	122-195	168	$2H_2O;2C_6H_4$	45.15	44.82				
	3	197-790	265; 429; 501; 555; 717; 765	$2CO_2$	20.25	20.96	30.58	29.93	$CaO.B_2O_3$	Siyah
$Ca[B(Sal)_2]_2\cdot 2H_2O$										Yeşil
$C_{28}H_{20}B_2CaO_{14}$	1	69-112	81	$2H_2O$	5.50	5.60				
642.15g/mol	2	114-217	165	$2C_7H_4O$	33.15	32.42				
	3	219-756	400; 446; 532; 660	$2C_7H_4O_3$	41.77	42.39	18.98	19.57	$CaO.B_2O_3$	Siyah

3.4. Single Crystal X-Ray Diffractometer (SC-XRD)

The crystal structure of the magnesium boron salicylate diester complex synthesized in the appropriate crystal form is given in Figure 5, bond lengths (Å) and bond angles (°) are summarized in Table 4. The asymmetric unit of the complex consists of one Mg(II) ion, two $C_{14}H_8BO_6$ molecules, six coordinated water molecules and four free water molecules. The Mg1 atom coordinates with the oxygen atoms coming from six water molecules, creating a distorted octahedral geometry. It forms an aqua complex with six water ligands coordinated around Mg^{2+}, which is in the stabilizing cation position in the structure. Molecular stacking and stability between boron salicylate structures in two diester forms and the Mg-aqua complex are provided by intermolecular O-H···O hydrogen bonds (Table 5). Hydrogen bonds formed between uncoordinated hydrated water molecules and carboxylic oxygen atoms contribute to 2D packing (Figure 6). Crystallographic data for the structures reported in this paper have been deposed with the Cambridge Crystallographic Data Center: CCDC-2374796 for $2(C_{14}H_8BO_6)\cdot H_{12}MgO_6\cdot 4(H_2O)$ contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre, www.ccdc.cam.ac.uk/data request/cif.

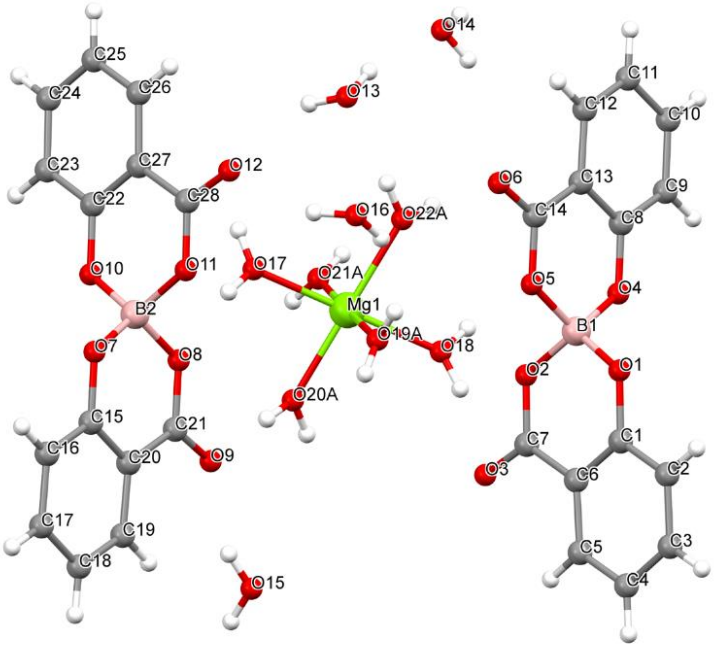


Figure 5. Molecular structure of magnesium salicylic acid boron diester complex

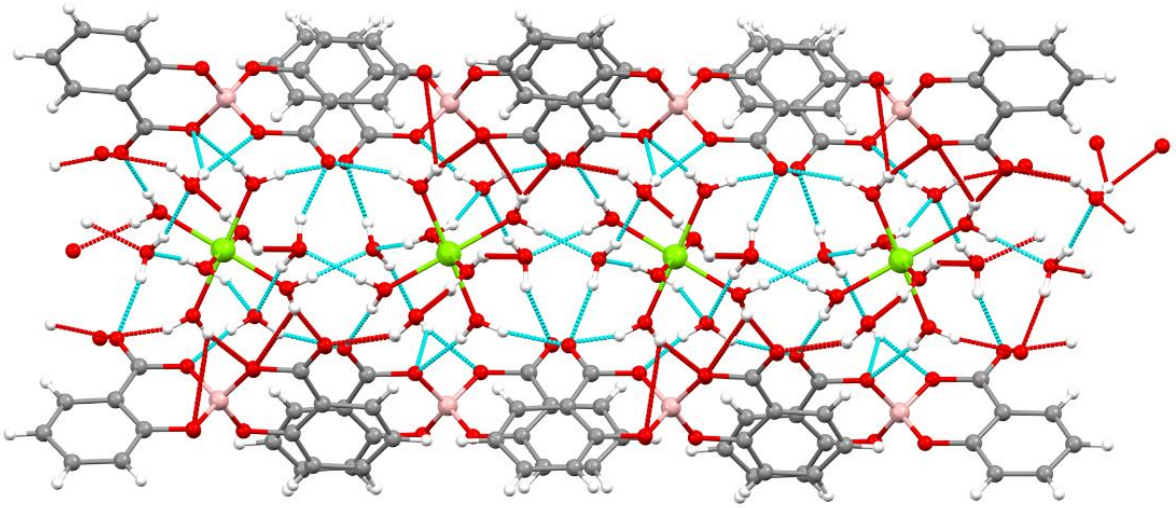


Figure 6. 2D representation of magnesium salicylic acid boron diester complex

Table 2. Magnesium salicylic acid boron diester crystal data

Crystal data	
Chemical formula	$2(C_{14}H_8BO_6)\cdot H_{12}MgO_6\cdot 4(H_2O)$
M_r	770.50
Crystal system, Space group	monoclinic, P21/c
Temperature (K)	296
a, b, c (Å)	9.8984 (13), 12.4168 (14), 28.867 (5)
β (°)	93.592 (5)
V ($Å^3$)	3541.0 (8)
Z	4
Beam source	Mo Kα
μ (mm^{-1})	0.14
Crystal size (mm)	0.15 × 0.13 × 0.12
Data storage	
Diffractometer	Bruker APEX3 CCD
Absorption correction	-
measurable, observable [I > 2σ(I)] and free reflection number	142702, 8804, 4860
R_{int}	0.088
$(\sin \theta/\lambda)_{max}$ (Å−1)	0.667
Refinement	
R[F2 > 2σ(F2)], Wr(F2), S	0.069, 0.161, 1.09
Reflection count	8804
Number of parameters	599
Number of borders	52
H-atom behavior	H atoms examined by a mixture of independent and constant increments
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å−3)	0.22, −0.28

Table 5. Hydrogen bond geometry of magnesium boron-salicylate di-ester molecule (Å,º)

D—H···A	D—H	H···A	D···A	D—H···A
O13—H13A···O3^{i}	0.83 (2)	1.96 (2)	2.787 (3)	172 (4)
O13—H13B···O14	0.85 (2)	2.04 (2)	2.870 (4)	165 (4)
O14—H14B···O11^{i}	0.80 (2))	2.22 (2)	2.964 (3)	156 (3)
O14—H14A···O6^{ii}	0.82 (2)	2.01 (3)	2.749 (3)	149 (4)
O15—H15B···O2^{iii}	0.83 (2)	2.20 (2)	2.984 (3)	158 (4)
O15—H15A···O9	0.84 (2)	1.97 (2)	2.764 (3)	158 (4)
O16—H16A···O12	0.83 (2)	2.00 (3)	2.779 (3)	155 (4)
O16—H16B···O15^{i}	0.85 (2)	2.03 (2)	2.866 (4)	169 (5)
O17—H17A···O3^{i}	0.83 (2)	1.95 (2))	2.770 (3)	170 (5)
O17—H17B···O8	0.81 (2)	2.14 (2)	2.948 (3)	170 (5)
O18—H18A···O5^{ii}	0.82 (2)	2.13 (2)	2.936 (3)	167 (5)
O18—H18B···O12^{iii}	0.83 (2)	1.95 (2)	2.747 (3)	161 (5)
O19A—H19A···O13^{iii}	0.84 (2)	1.89 (3)	2.683 (4)	157 (5)
O19A—H19B···O15i	0.83 (2)	2.03 (2)	2.828 (4)	161 (4)
O20A—H20B···O9	0.83 (2)	1.94 (2)	2.772 (4)	175 (4)
O20A—H20A···O16^{iii}	0.83 (2)	1.91 (3)	2.714 (4)	161 (5)
O21A—H21A···O16ii	0.82 (2)	2.00 (2)	2.706 (4)	145 (4)
O21AH21B···O14iv	0.82 (2)	2.03 (2)	2.833 (4)	164 (4)
O22A—H22B···O13	0.83 (2)	1.87 (2)	2.689 (4)	168 (6)
O22A—H22A···O6ii	0.83 (2)	1.95 (2)	2.761 (4)	165 (5)
O19B—H19D···O9	0.84 (2)	1.96 (5)	2.747 (6)	157 (10)
O20BH20C···O14iv	0.83 (2)	2.05 (3)	2.833 (7)	157 (9)

Table 6. Hydrogen bond geometry of magnesium boron salicylate di-ester molecule (Å,º)

B1—O1	1.433 (4)	C21—O8—B2	125.0 (2)
B1—O4	1.442 (4)	Mg1—O18—H18A	121 (4)
B1—O5	1.488 (4)	Mg1—O18—H18B	130 (4)
B1—O2	1.488 (4)	Mg1—O19A—H19A	131 (4)
B2—O7	1.430 (4)	Mg1—O19A—H19B	123 (2)
B2—O10	1.441 (5)	Mg1—O20A—H20B	127 (2)
B2—O8	1.481 (4)	Mg1—O20A—H20A	124 (2)
B2—O11	1.489 (4)	Mg1—O21A—H21A	125 (2)
C22—O10—B2	122.9 (3)	Mg1—O21A—H21B	125 (2)
O1—B1—O4	111.1 (3)	Mg1—O22A—H22B	125 (3)
O1—B1—O5	107.2 (3)	O1—B1—O2	113.6 (2)
O4—B1—O5	112.5 (2)	O4—B1—O2	107.6 (3)
C28—O11—B2	125.3 (3)	O5—B1—O2	104.8 (3)
O7—B2—O10	110.7 (3)	C7—O2—B1	124.6 (2)
O7—B2—O8	113.4 (3)	C8—O4—B1	122.9 (2)
O10—B2—O8	107.6 (3)	C14—O5—B1	125.4 (2)
O7—B2—O11	107.3 (3)	C15—O7—B2	122.1 (2)
O10—B2—O11	112.8 (3)	C21—O8—B2	125.0 (2)
O8—B2—O11	105.0 (3)	O2—B1—O1—C1	9.0 (4)
O3—C7—O2—B1	175.7 (3)	O9—C21—O8—B2	175.0 (3)
O11—B2—O7—C15	122.9 (3)	C20—C21—O8—B2	5.4 (4)

3.5. Mass Spectroscopy Analysis

Figure 7 shows the mass spectrum pattern of the sodium boron-salicylate monoester compound, one of the boron ester complexes formed with salicylate ligand. The important peak values observed in the mass analysis pattern of the molecule are given in Table 7. The mass peak occurring at 238.77 m/z was evaluated as a molecular ion peak. While the peak at m/z value of 77.10 corresponds to $B(OH)_4^-$ ion, other peak values were evaluated as peaks corresponding to the degradation products of sodium boron-salicylic acid mono-ester salt.

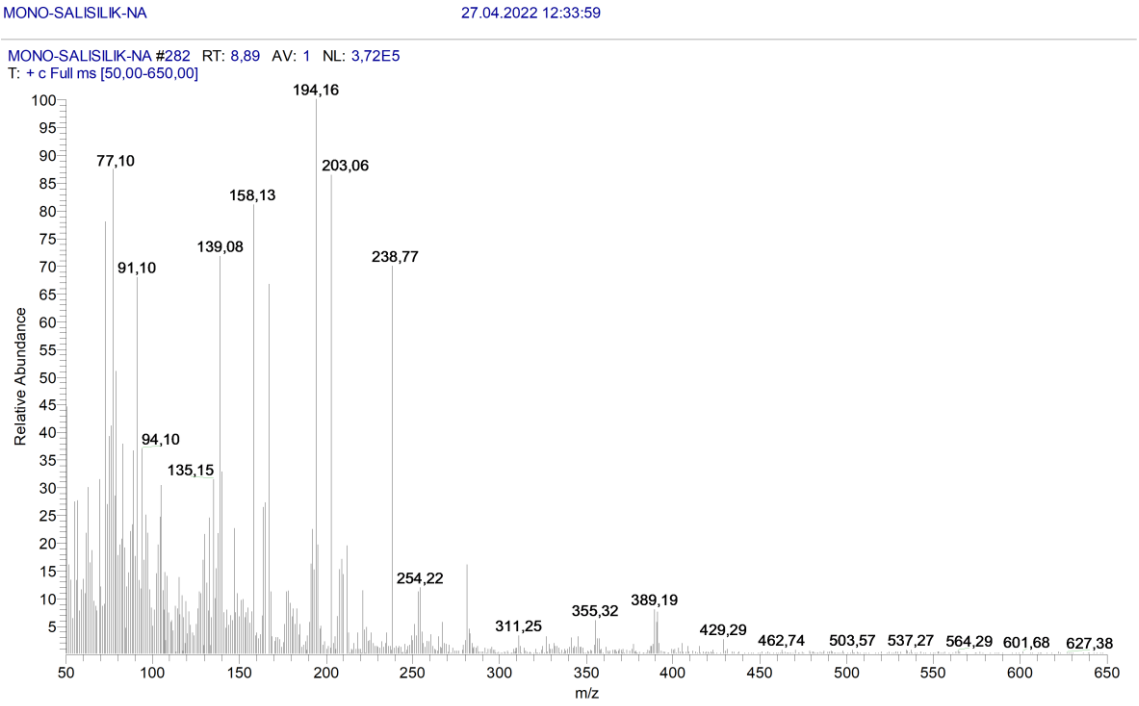


Figure 7. Mass spectrum of sodium boron-salicylate mono ester

Table 3. Mass spectrum peaks of sodium boron-salicylate mono ester

$Na[B(OH)_2(Sal)].2H_2O$ (m/z)
238.77
203.06
158.13
135.15
91.10
77.10

3.6. Anticancer Effect of the Boron Salicylate Esters

Viability of cells treated with different concentrations of esters were determined with MTT assay (Figure 8). It was found that none of the esters were cytotoxic for L929 cells during the time of 48 h incubation. 16 mM was chosen as the maximum dose, due to solubility of the esters. Notably, it was revealed that all esters showed selective toxicity against MDA-MB-231 human breast adenocarcinoma cells, in a dose dependent manner. By using the dose response in terms of percent cell viability, IC_{50} concentrations were calculated (Table 8). It was found that all IC_{50} dose for all boron esters were lower for MDA-MB-231 cell line, indicating that B esters led to selective cytotoxicity for cancer cells, without harming less than 50% of the normal cells. In literature, presence of different B containing compounds with anticancer properties were reported, including boron esters [60]. In a study, B esters of sugars were shown to accumulate more in cancer cells, leading to selective cytotoxicity against prostate cancer cell line. Moreover, mechanism of action was attributed to boron transporters [20]. In another study, boronate ester of phenlyboronic acid and dopamine was stated to be effective against mouse tumor model obtained with A549 lung carcinoma cell line [61]. Salicylic acid was also reported as effective against different cancer cell lines. In a study, salicylic acid was denoted to cause nitric oxide dependent apoptosis, leading to selective cytotoxicity against B16F10 melanoma cell line [62]. In another study, polymerized salicylic acid was reported to act as a dual agent by being effective against CT26 colon carcinoma cell line and being able to carry other cancer drugs [63]. Considering the present literature, potency of B salicylate esters was attributed to both salicylate and B.

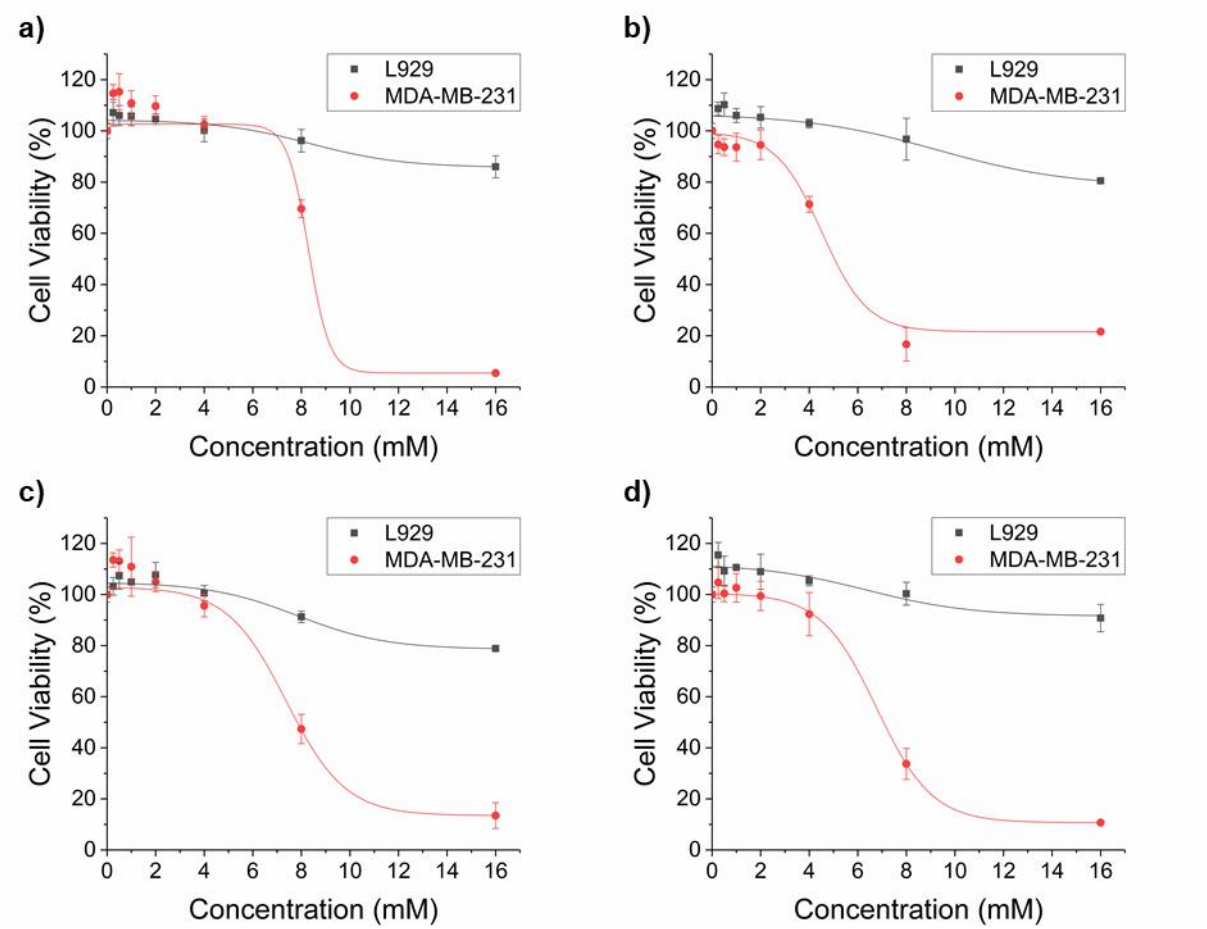


Figure 8. Percent viability of L929 and MDA-MB-231 cells treated with Na-B salicylate diester (a), K-B salicylate diester (b), Mg-B salicylate diester (c) and Ca-B salicylate diester (d) (n=4).

Table 8. Calculated IC_{50} (mM) and values of B salicylate esters for L929 and MDA-MB-231 cell lines.

	L929	**MDA-MB-231**
Na-B Diester	> 16	8.36
K-B Diester	> 16	5.04
Mg-B Diester	> 16	7.83
Ca-B Diester	> 16	7.06

In cancer therapy, drug resistance presents a major problem. A previous study reported IC_{50} value of doxorubicine against non-resistant MDA-MB-231 cell line as around 3mM, whereas the IC_{50} value of doxorubicine against doxorubicine-resistant MDA-MB-231 cell line was around 35.7 mM. In the present study non-resistant MDA-MB-231 cell line was used. Although IC_{50} values of boron salicylate esters were higher than that of doxorubicine against non-resistant MDA-MB-231 cell line, boron esters can still be used against drug resistant cell lines [64].

4. Conclusion

To determine the most beneficial boron derivative, further scientific investigations are required to precisely characterize a stable ester complex containing boron and investigate the function of borate in biological systems (biosystems). The present work involved the synthesis of monoester (1:1) and diester (1:2) compounds of boron ester complexes that incorporate metal cations Na^+, K^+, Mg^{2+}, and Ca^{2+}. The structures of the compounds isolated by crystallization/precipitation from aqueous solutions in solid state were determined by elemental analysis, determination of melting point, infrared spectroscopy analysis (FT-IR), thermal analysis (TGA/DTA), single crystal X-ray diffraction (XRD), and mass analysis (GC-MS). Recommendations for the synthesised boron esters are provided based on the findings of the chemical composition study. Examination of FT-IR analyses revealed the presence of B-O-C bonds in the system and the elimination of B-OH bond peaks due to the esterification of boric acid. The detection of peaks related to $\nu_{asym}(BO)/BO_4$ & ν(C-O) and $\nu_{sym}(BO)/BO_4$ stretching vibrations in the typical infrared spectra is the most crucial evidence confirming the production of boron ester derivatives. Single crystal structure analysis of boron-salicylate ester compounds was carried out on the appropriate crystal obtained for the $Mg[B(Sal)_2]_2.10H_2O$ structure. In the structure, the boron center has completed its coordination with the *cis-diol* –OH groups on the two ligands to the proposed tetrahedral geometry. The presence of metal cations in the coordination sphere, either included or excluded, contributes to the anionic charge of the boron center resulting from the quadruple bond. Consequently, the structures manifest as salt complexes. Spectroscopic, thermal and chemical composition analyses of other ester compounds, which could not be formed into forms suitable for single crystal XRD analysis, are similar to the solved crystal structures. This shows that the structural features suggested by us are also similar. When the mass analysis patterns extracted from the GC-MS chromatograms taken for mass analysis were examined, the molecular ion peaks of the $Na[B(OH)_2(Sal)].2H_2O$ ester structure and the peaks of the $B(OH)_4^-$ ion in the structures were clearly detected. In addition, it was observed that the molecular ion peaks of various decomposition derivatives of the molecules whose mass analysis was performed were also detected. This study demonstrated the anticancer potential of boron salicylate esters against the MDA-MB-231 human breast adenocarcinoma cell line. All of the esters were shown to be non-cytotoxic against the control cell line L929. The K-B salicylate diester molecule was found to have the most potency with the lowest IC_{50} value against the MDA-MB-231 cell line. The anticancer potential of boron salicylate esters can be further investigated with other cancer models in combination with

anticancer drugs. It is also thought that the mechanism of action of these molecules may help reveal their further applications.

5. Conflict of Interest

Authors declare no conflict of interest.

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