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Synthesis And Characterization of Some New Antimony Compounds With Benzothiazole As Anticancer

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Abstract : The aim of the present work is to synthesize two new antimony compounds containing Benzothiazole (BZT) as a ligand, in the mole ratio 1:1 and 1:2 . Antimony compounds were characterized by FTIR, UV, CHNS analysis, Atomic Absorption and conductivity measurements.
The biological activity of BZT, $SbCl_3$ and the two new compounds was evaluated against Hela and Rhabdomyosarcoma (RD) cell lines. The four compounds were effective, while compound (2) was the best in inhibition.
Keywords : antimony compounds, benzothiazole, cytotoxicity , Hela cell, RD cell.

Introduction:

Antimony has been known since ancient time. It is usually obtained from the ores stibnite (Sb_2S_3) and valentinite (Sb_2O_3). Stibnite has been and to date remains the main source for metallic antimony to be commercially mined [1] .

Antimony as an element is stable, which is not effected by air or moisture , burning generator " luminous flame when heated to the degree of irritation presence of air, when the antimony binds with oxygen under controlled conditions ,form the antimony oxides previously mentioned [2] .

Antimony is a moderately active element. It does not combine with oxygen in the air at room temperature. It also does not react with cold water or with most cold acids. It does dissolve in some hot acids, however, and in aqua regia. It often reacts with materials that do not react with either acid separately [1]. The abundance of antimony is estimated to be about 0.2 parts per million, placing it in the bottom fifth among the chemical elements found in the Earth's crust. It is more abundant than silveror mercury, but less abundant than iodine[3].

It has been known for centuries and is its compounds , in the Babylonian era was used for medical purposes (therapeutic) and some cosmetics as the oldest use of it was eyeliner and treat bacterial infections of the eyes, it has toxicity relatively few [4].

Antimony is a metalloid considered toxic to most organisms at elevated concentrations[5]. Its bioavailability and toxicological effects depend on its chemical form and oxidation state, with the trivalent compounds more toxic than the pentavalent compounds, similar to arsenic[6].

Antimony(III) complexes have recently received considerableattention, as they have been shown to exhibit antitumorproperties [7]. In particular, antimony(III)complexeswith aminopolycarboxy ligands or

organoantimony(III) derivatives have shown a significant antitumor activity[8],[9]**.** Recently we evaluated the biological activity of SbCl3 and MTX compounds as anticancer [10]**.**

The antimony drugs are still the most effective treatment for a number of diseases. The reduction in mortality from 95% to less than 5% in the case of Kala-azar is due to antimony therapy [11]**.** We have also described aseries of antimony(III) halide complexes of thione or thiolateligands with consistent selective antiproliferative activityagainst human cervix carcinoma (HeLa) cells [12]**.**

HeLa cells : are the most widely used cancer cell lines in the world. These cells were taken from a lady called Henrietta Lacks from her cancerous cervical tumor in 1951 which today is known as the HeLa cells. These were the very first cell lines to survive outside the human body and grow [13].

Rhabdomyosarcoma (RMS) : Is a malignancy that arises from skeletal muscle precursors [14]**.** It is the most common type of soft tissue sarcoma in children and adolescents less than 20 years old. There are two major subtypes of RMS, embryonal and alveolar, which differ markedly in their outcomes. Embryonal RMS usually presents in children less than 10 years old and has a 5-year survival of close to 75%. [15]**.**

More than 3000 organic antimony compounds have been described and these can be divided into five groups: Sb(III)/Sb(V) compounds, metallocomplexes, tetracoordinated, stibonium, and hexacoordinated compounds [16].

Benzothiazole:

Benzothiazole, the bicyclic ring system consists of thiazole ring fused with benzene ring. Benzothiazole moiety is very small but is fascinated by scientists because of the different biological activities by benzothiazole and their derivatives. The basic structure of benzothiazole consist of benzene ring fused with 4, 5 position of thiazole [17]**.**

Benzothiazole

A large number of efforts were made to synthesize different benzothiazole compounds and their derivatives in the past decade and were found to numerous pharmacological activities like antitumor[18], anticonvulsant[19]**,** antimicrobial[20]anthelmintic[21] antileishmanial[22]**,** anti-tubercular[23] schictosomicidal[24] antifungal[25], anti-inflammatory[26] antipsychotic[27] and anti diabetic activities[28] .

Experimental

Materials and Instruments

Antimonytrichloride ($SbCl_3$) was supplied from BDH compony, purity 99 % , BZT was supplied from Himedia compony, purity 97 % . The melting points were measured using (Stuart Scientific Co. LTD melting point-SMP1).The C.H.N.S (EuroEA 3000) was used to find the percentages of the components of the prepared complexes. Atomic Absorption Flame Spectrophotometer- Nov AA 350 was used to find the percentage of the antimony in the prepared complexes.

FT-IR spectra were recorded using FT-IR 8000 Shimadzu in the rang of (4000-200) cm^{-1}, samples were measured as (CsI disc). Shimadzu (UV-Vis)-160 was used for characterization, also Elisa Reader-ASYS-Austria was used to evaluate the anticancer activity.

Preparation of the Compounds:

1- Antimony :BZT 1:1 mole ratio reaction

In a round bottom flask, (6.8 gm , 0.03 mole) of antimony(III) chloride dissolved in 15 ml of absolute ethanol was added drop by drop to (3.3 ml, 0.03 mole) of the benzothiazole dissolved in 10 ml of absolute ethanol. The mixture was heated at 30-35 C^0 with stirring for 3hrs. The resulting precipitate was filtered, washed with absolute ethanol, and then dried by using an oven at 50°C for 1 hr. The product was offwhite powder, m.p. 130-132 ^{0}C . Yield 89 % .

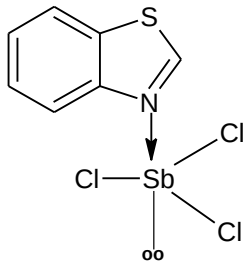
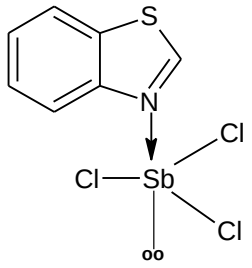
Interaction Equation : $SbCL_3$+ BZT ⟶ BZT $SbCl_3$

2- Antimony :BZT 1:2 mole ratio reaction

The same procedure was used, except the mole ratio was (1:2) Sb : BZT. The product was off-white powder, m.p. 132-134 ^{0}C . Yield 64 % .

Note: The product of 1:2 was also 1:1 reaction as confirmed by the analysis used in characterization.

Table (1): The structure and physical properties of compound 1 and 2

Comp. No.	structure	Color	M.Weight	General formula	M.P. ^{o}C	Yield %
1		Offwhite	363	$C_7H_5N_5SbCl_3$	130-132	89
2		Offwhite	363	$C_7H_5N_5SbCl_3$	132-134	64

Cytotoxicity Assay :

Two cell lines studied (Hela and RD) at one time of exposure 48 hrs, using 6 concentrations (10, 8, 6, 4, 2 and 1 µg/ml) of four stock solutions were prepared of complex 1, 2 , $SbCl_3$ and BZT. Each stock was made by dissolving 0.002 g of the powder of each compound as well as a control negative , and sterilized by filtration using 0.22 µm Millipore filter , then used in the cytotoxic assay on RD and Hela cell line, in order to evaluate their biological activity against Hela and Rhabdomyosarcoma cell lines. Optical density of each well was measured by using **ELISA** reader at a transmitting wave length on 570 nm.

Results and Discussion:

A: Characterization: The prepared compounds were characterized by the following tools :

1- FTIR :

The prepared compounds were characterized by the FTIR technique, the results showed that the appearance of new peaks and the disappearance of another was found in the starting materials, these frequencies are listed in Table (2).

Peaks has appeared for the Sb-N at the frequency (293-285) cm^{-1} , these were not present in the basic materials, some peaks shifted to higher frequencies, this is an evidence of the coordination between Sb and N according to the HSAB theory.

Due to the occurrence of the phenomenon of resonance between the sulfur atom and the nitrogen atom in the thiazole ring led to the emergence of the absorption bands assigned to stretch bond **C-S** in the frequency range (1066) cm^{-1}.

Table (2) : The most diagnostic FTIR bands of the ligand and its metal complexes in (cm^{-1}).

Compd. / **Bands**	**[BZT]**	**[BZT $SbCl_3$] 1:1**	**[BZT $SbCl_3$] 1:2**
v (C-H) arom.	3060, 2844	3093 , 2871	3045 , 2879
v (C=N)	1593	1625	1625
v (C=C)	1471,1456,1423	1506, 1461,1429	1500, 1467, 1421
v (C-N)	1315	1319	1317
v (C-S)	1014-977	1066-958	1066-939
v (C-H) thiol ring	2628	2407	2408
v (Sb-N)	----------	293	285
v (Sb-Cl)	----------	310	304

2-UV :

Also the compounds were characterized by UV spectrophotometry, the results showed electronic transitions of the type Charge Transfere (LMCT) at 272 nm assigned to the $(\pi) \longrightarrow \sigma_{lg}$ LMCT transition which also a characteristic for similar complexes .

2- CHNS Analysis:

Elemental Analysis and Atomic Absorption were performed for target compounds 1and 2 to confirm their basic chemical structure. The results were presented in Table (3), and revealed a good agreement with the calculated percentages. The percent deviation of the observed / calculated was found to be complied with the accurate analysis.

Table (3): The elemental analysis of the compound 1 and 2 .

Compound formula Colour	**Yield %**	**M. P. °C**	**M. Wt. g. mol⁻**	**% Elemental analysis / Found (Calc.)**				**% Metal Found (Calc.)**
				C	**H**	**N**	**S**	
$C_7H_5NS\ SbCl_3$ Offwhite	89	130-132	363	20.4 (21.1)	1.7 (1.9)	3.5 (3.8)	8.6 8.8	32.1 (33.5)
$C_7H_5NS\ SbCl_3$ Offwhite	74	132-134	363	20.7 (33.7)	1.6 (2.0)	3.7 (5.6)	7.9 12.8	32.9 (24.4)

3- Conductivity measurement:

Conductivity measurements were obtained using TRANS-BC3020 Instrument. These measurements were obtained in DMSO solvent as (10^{-3}M) concentration at 25 ^{O}C . The values were (35.0 and 37.2 µs) for compound **1** and **2** respectively.

B: **Biological Activity Evaluation:**

Four samples; two of complexes, BZT, and $SbCl_3$ with concentrations (10, 8, 6, 4, 2 and 1µg/ml)were prepared,the results showed that all these compounds are highly effective in inhibition the tumor cell type (Hela and RD). Results are presented in Tables (4 and 5). The highest values for inhibition were 73, 72.6, 63 and 59.6% for complex 1, 2, BZT and $SbCl_3$ respectively on the Hela cell line, and 78, 88, 75 and 68% for the same complexes and starting materials on the RD cell line**.** Comparing the results of the new compounds (1 and 2) with the starting materials (SbCl3 and BZT), it is very obvious that they are higher in their effectiveness due to the synergestic effect of Sb with BZT since they are themselves were effective against cancer [18]**.**

Cytotoxic Effect of the New Compounds , $SbCl_3$ and BZTAgainst Hela Cell Line

When the cancer cell line (Hela) was treated with the two starting materials and the new complexes, the results showed a significant effects for all of these compounds, in all the concentrations used compared with the control negative which contains only cell line and the culture media.The toxic effect varied between the tested samples showing a significant cytotoxic effect started from 10 µg/ml to 1 µg/ml concentrations**.**

The cytotoxic study was done against Hela cell line (passage number 23) isolated from human as an aggressive cervical adenocarcinoma, exposure time was 48 hrs**.** The inhibition rate percent (I.R.%) was calculated, and the results varied among starting materials and the new complexes as shown in Table (4). Figures (1 and 2)**.**The results showed the cytotoxicity effect of these compounds in all concentrations and the highest inhibition rate (73%) recorded with the high concentration (10µg/ml) comparable to control negative**.** The cytotoxic effect of complex **1**against Hela cell line, Figures (1 and 2) showed that the high concentration (10 µg /ml) gave a significant high inhibition rate (72.6%) against cells, while the low concentration (1 µg /ml) gave the low inhibition rate (33%).

The higher the inhibition rate of the complexes is due to the synergistic effect produced from the coordination of Sb with BZT ; the effectiveness of both antimony and BZT are overlapping, resulting a strengthening of bio-inhibition of the starting materials. The results are concentration dependent.

The inhibition rate follows the order :

Compound2>1>BZT>$SbCl_3$, e.g. I.R.% increased by 10-14% in case of BZT (10 µg /ml) due to the coordination with Sb**.**

Table (4): Initial cytotoxic effect against Hela cell line of compounds 1, 2, $SbCl_3$ and BZT by MTT assay method in time of exposure 48 hrs.

Compound	Concentration µg /ml	Mean	(I.R. %)	Viability %
1	10	0.082	72.6	27.4
	8	0.099	67	33
	6	0.119	60.3	39.7
	4	0.125	58.3	41.
	2	0.193	35.6	64.4
	1	0.201	33	67
2	10	0.081	73	27
	8	0.093	69	31
	6	0.105	65	35
	4	0.132	56	44
	2	0.162	46	54
	1	0.216	28	72

$SbCl_3$	10 8 6 4 2 1	0.121 0.129 0.135 0.156 0.201 0.252	59.6 57 55 48 33 16	40.4 43 45 52 77 84
BZT	10 8 6 4 2 1	0.111 0.120 0.126 0.132 0.15 0.231	63 60 58 56 50 23	37 40 42 44 50 77

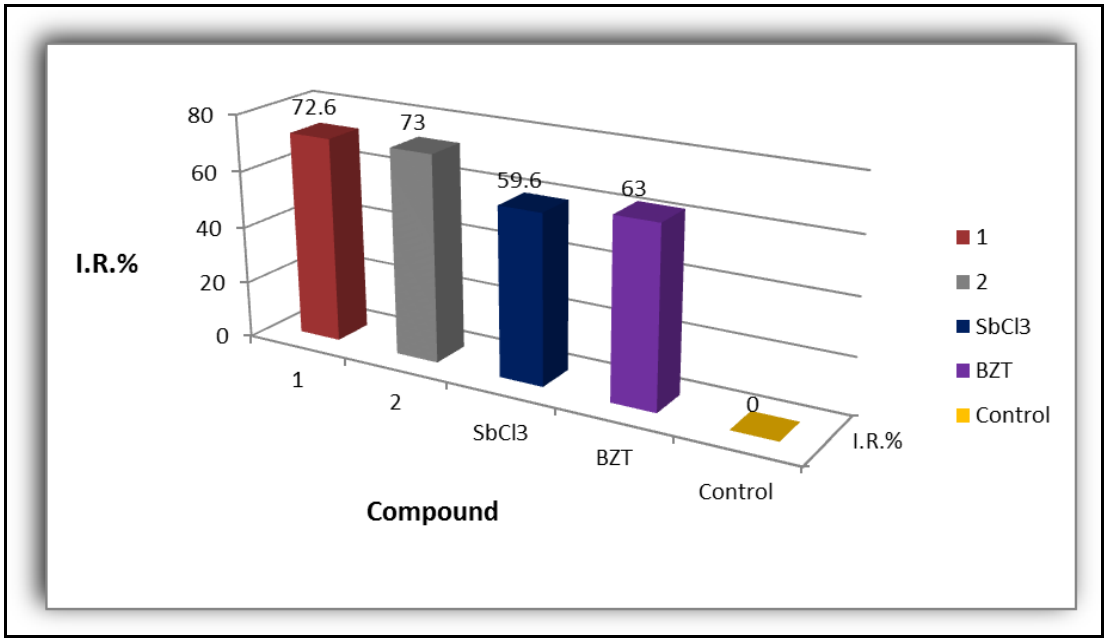


Figure (1): Over all block diagram of cytotoxic effect of compound 1, 2 and the starting materials against Hela cell line.

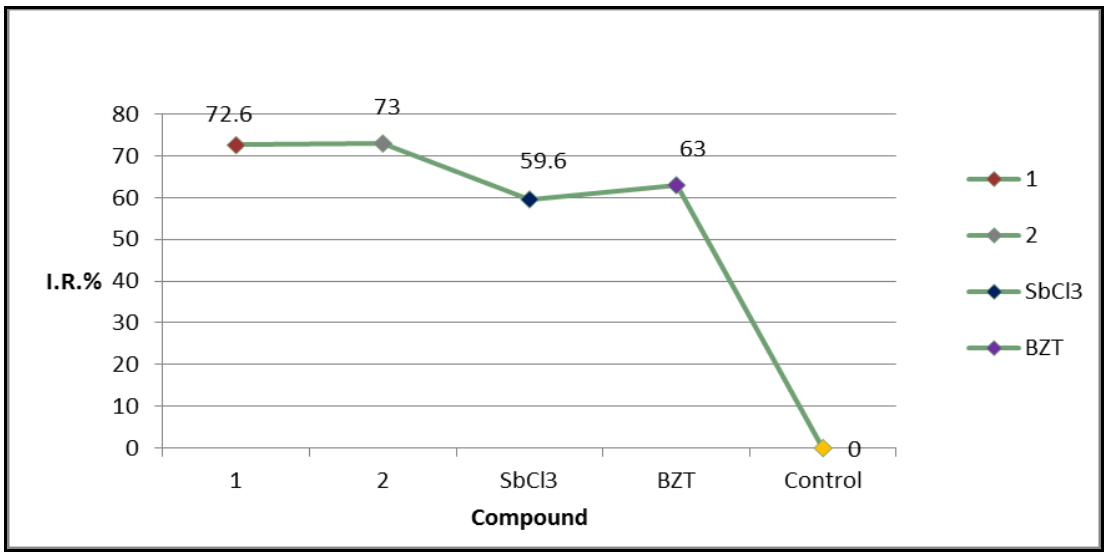


Figure (2): Over all cytotoxic effect of compound 1, 2 and the starting materials against Hela cell line.

Cytotoxic Effect of the New Compounds, $SbCl_3$ and BZT Against RD Cell Line

The results in Table (5) and Figures (3 and 4) showed the inhibition rate percent in 48 hrs exposure time for each cytotoxic effective concentration. The results explained the cytotoxic effect of the complexes 1, 2 and the starting materials $SbCl_3$ and BZT on RD cell line (passage 45), revealed that the high concentration (10 µg/ml) gave the higher inhibition rates of cells (78, 88, 68 and 75 %) respectively **.**

The cytotoxic effect of complex 2 gave the highest inhibition rates (88%) at the high concentration (10 µg/ml) . SbCl3 showed high inhibition rate (68%) at the concentration (10 µg/ml) and decreased as the concentration decreased too**.** The Benzothiazole compound also showed higher inhibition rate (75%) at the concentration (10 µg/ml) and a lower inhibition rate (43%) at the concentration (1 µg/ml) during 48 hrs of exposure , due to proliferation develop during low concentration .

The cytotoxic effect is reduced progressively from concentrations (8, 6, 4, 2 and 1 µg /ml) respectively**.** Unexpected reduction of the inhibition rates (36%) for the complex 1 is seen in the concentration (1 µg /ml)**.**

An explanation for this attitude, that in the cell culture experiment, it was important to be aware of growth state of the cell culture, as well as the quantitative characteristic of cell lines [19]**.**

The inhibition differences in Hela and RD cell lines responding to different treatment might indicate a presence or absence of cellular receptor in both types of cell line. Moreover the metabolic pathways to each treatment differed in response from one cell line to another [20,21]**.** Also the differences are due to the difference between the two cell line types**.**

Table (5): Initial cytotoxic effect against RD cell line of compounds 1, 2, $SbCl_3$ and BZT by MTT assay method in time of exposure 48 hrs.

Compound	Concentration µg/ml	Mean	(I.R. %)	Viability %
1	10	0.066	78	22
	8	0.069	77	23
	6	0.075	75	25
	4	0.105	65	35
	2	0.143	52	48
	1	0.192	36	64
2	10	0.036	88	12
	8	0.051	83	17
	6	0.054	82	18
	4	0.067	77.6	22.4
	2	0.077	74.3	25.7
	1	0.153	49	51
$SbCl_3$	10	0.096	68	32
	8	0.099	67	33
	6	0.114	62	38
	4	0.126	58	42
	2	0.147	51	49
	1	0.159	47	53
BZT	10	0.075	75	25
	8	0.084	72	28
	6	0.092	69.3	30.7
	4	0.115	61.6	38.4
	2	0.138	54	46
	1	0.171	43	57

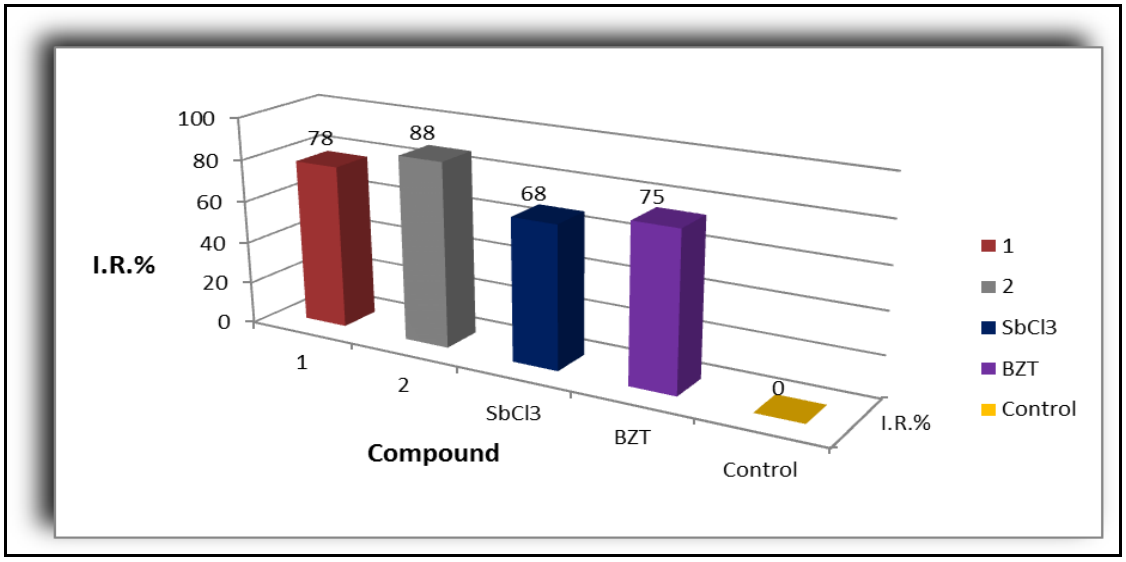


Figure (3): Over all block diagram of cytotoxic effect of compound 1, 2 and the starting materials against RD cell line

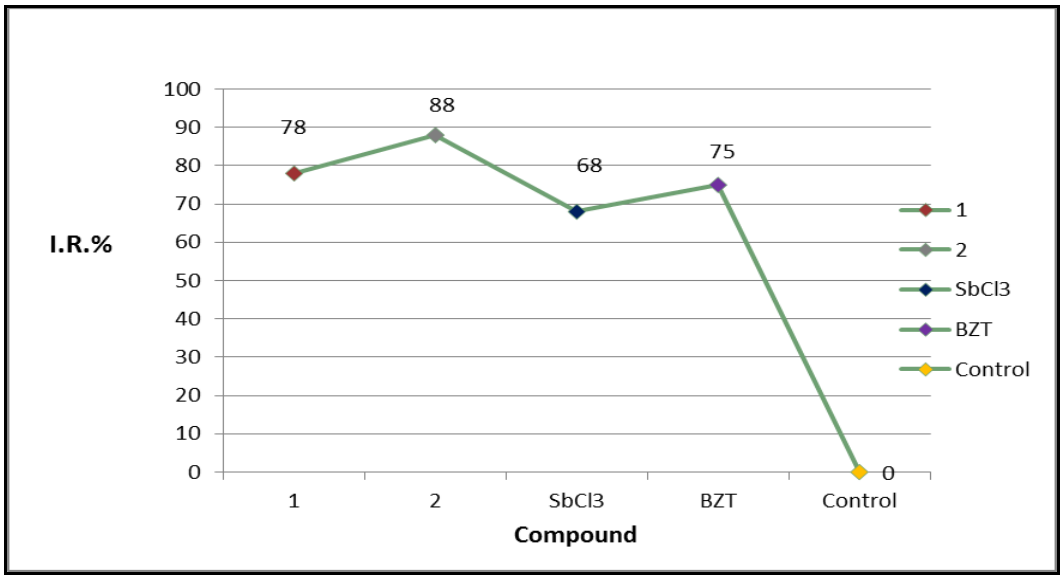


Figure (4): Over all cytotoxic effect of compound 1, 2 and the starting materials against RD cell line.

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