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Synthesis and Evaluation of Biological Activity of New Antimony Compounds With Methotrexate and Benzothiazole

Jalil R. Ugal[1]*, Farooq I. Mohammad[2], Zainab K. Ali[3]

[1,3]Department of Chemistry, College of Science for Women, University of Baghdad, Iraq

[2] Department of Biotechnology Research Center, AL- Nahrain University, Iraq

Abstract : The aim of the present work is to synthesize four new antimony compounds containing Methotrexate (MTX) and Benzothiazole (BZT) as ligands, in the mole ratio 1:1 and 1:2 .Antimony complexes were characterized by FTIR, UV, CHNS analysis, Atomic Absorption and conductivity measurements.
The biological activity of MTX, BZT, $SbCl_3$ and the new compounds was evaluated against Leishmania parasite. All compounds were effective, while compound (4) was the best in inhibition.
Keywords : antimony complexes, methotrexate, benzothiazole, cytotoxicity , leishmania parasite.

Introduction:

The chemistry of antimony with arsenic and bismuth has been an active area of research for more than four decades. Sustained interest in compounds of this group (V) elements is mainly due to their wide range of applications. Especially antimony (Sb) which plays an important role in both, fundamental research and in applications owing to its unique physical properties [1].

The major use for antimony is now as a trioxide for flame-retardants[2] .It is a metalloid considered toxic to most organisms at elevated concentrations [3]. 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 [4].

It is physically and chemically very similar to arsenic, but with a lower toxicity. It is a nonessential metal and was formerly used for a number of medicinal purposes, such as inducing sweating and vomiting or in the treatment of leishmaniasis[5].

Biological Activity:

Group V elements, As, Sb, and Bi are used clinically in the treatment of Syphilis, Leishmaniasis and in acute hepatic and peptic ulcers. These metals/metalloids containing organic compounds (metal-carbon bond) may offer certain advantages in drug therapy, e.g., the coordination of an organic molecule to a metal centre may alter the normal metabolic pathway of the body and may lead to a slow release mechanism for delivery of organic molecules. Organoantimony compounds proved potent not only against infection caused by Trypanasomes, but also active against Leishmanial organisms [6,7].

As therapeutic agents, antimony and its compounds have been mostly used for the treatment of two parasitic diseases (leishmaniasis and schistosomiasis) since their prescription by the alchemist John of Rupescissa in the 14th century **[8].**Also antimony compounds were used as anticancer for their high activity ; e.g. with Hela and RD cell lines **[9].**

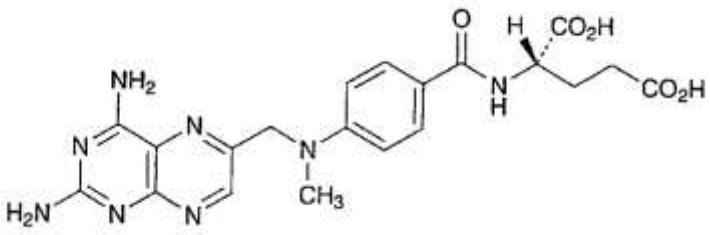
Leishmaniasis: is a disease spread by the bite of the female sandfly,caused by an intracellular protozoan parasite of the Leishmania gender**[10]**.

More than 12 million people in the world are affected by Leishmaniasis, which are diseases caused by an intracellular protozoan parasite of the Leishmania gender **[11]**. **Cutaneous leishmaniasis (CL)**: is the most prevalent clinical manifestation of leishmaniasis causing simple or multiple, localized or disseminated ulcers in the skin, but also in the mucous of mouth, nose and throat cavities. The most affected countries are Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica and Peru that account for at least 70% of global estimated incidence for CL **[12]**.

Treatments chiefly involving antimony has been called antimonials. Potassium antimony tartrate has been used to treat cough for reducing the excretion of sputum as an effective ingredient in Compound Liquorice**[13].**Antimony potassium tartrate, also known as potassium antimonyltartrate, potassium antimontarterate, or emetic tartar**[14]**,has the formula $K_2Sb_2(C_4H_2O_6)_2$ is the double salt of potassium and antimony of tartaric acid. The compound has long been known as a powerful emetic, and was used in the treatment of schistosomiasis and leishmaniasis**[15]**.

The survey of literature related to benzothiazoles reveals the presence of this bicyclic ring system in various amine or terrestrial natural compounds, which have useful biological properties**[16]**. In recent years heterocyclic compounds analogues and derivatives have attracted strong interest due to their biological and pharmacological properties. Benzothiazole derivatives possess a wide spectrum of biological applications such as antitumor**[17]**, antimicrobial**[18]**,schictosomicidal**[19]**, anti-inflammatory**[20]**, anticonvulsants**[21]**, antidiabetic**[22]**, antipsychotic and diuretic etc**[23]**.

Methotrexate:$C_{20}H_{22}N_8O_5$2-[4-[(2,4-diaminopteridin -6-ylmethyl)methylamino] benzoylamido] pentanedioic acid. **[24].**



It is one of the most widely used anticancer agents, with indications and established protocols in a range of childhood and adult cancers. Unlike other chemotherapeutic agents, MTX is used in a wide variety of doses. For example, MTX can be given in low doses (eg, 20 mg/Kg) in maintenance chemotherapy and in the treatment of psoriasis and rheumatoid arthritis, but it can also be given in much higher doses (eg, 1000 mg/Kg) over prolonged intravenous (IV) infusion for the treatment of certain cancers **[25,26].**

Experimental

Materials and Instruments

Antimony trichloride ($SbCl_3$) was supplied from BDH, purity 99% ,Methotrexate was supplied from China, purity %98 , Benzothiazole was supplied from India, purity 97%. The melting points were measured using (Stuart Scientific Co. LTD melting point-SMP1).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 spectro was used to record spectra of complexes. Also Elisa Reader- ASYS-Austria, was used in the biological activity evaluation.

Preparation of the Complexes:

1- Antimony Complex [SbL1] 1:1 mole ratio

In a round bottom flask (1.0 gm, 0.004 mole) of antimony(III) chloride dissolved in 5 ml of absolute ethanol was added drop wise to (2.0 gm, 0.004 mole) of the methotrexate dissolved in 15 ml of absolute ethanol. The mixture was heated to 30-35 ^oC with stirring for 3hrs. The resulting precipitate was filtered, washed with absolute ethanol , and then dried by using an oven at 50°C for 1h. The product was an orange powder, m.p. 170-172 ^oC . Yield 72 % .

Interaction Equation: $SbCl_3$+ MTX ⟶ MTX $SbCl_3$

2- Antimony Complex [SbL1] 1:2 mole ratio

The same procedure was used, except the mole ratio was (1:2) Sb : MTX. The product was an orange powder, m.p. 172-174 ^oC . Yield 54 % .

Interaction Equation:

$SbCl_3$+ 2 MTX ⟶ MTX $SbCl_3$ + MTX

3- Antimony Complex [SbL2] 1:1 mole ratio

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 wise to (3.3 ml, 0.03 mole) of the benzothiazole dissolved in 10 ml of absolute ethanol. The mixture was heated to 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 1h. The product was off white powder, m.p. 130-132 ^0C . Yield 89 % .

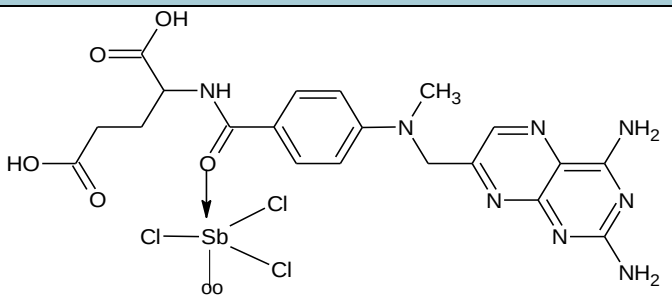
Interaction Equation: $SbCl_3$+BZT ⟶ BZT $SbCl_3$

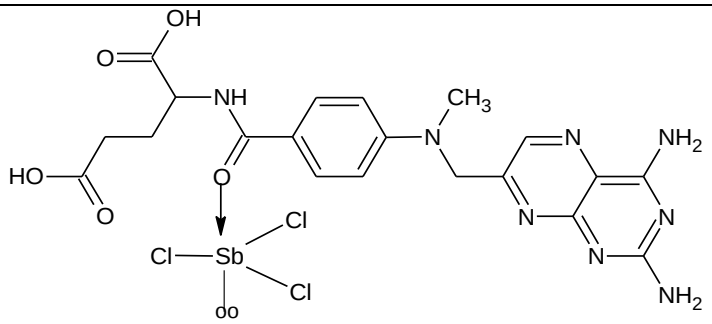
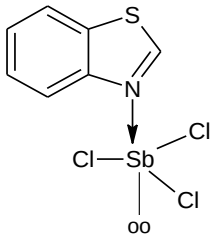
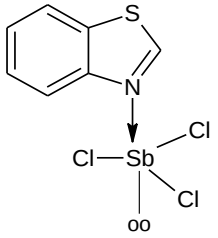
4- Antimony Complex [SbL2] 1:2 mole ratio

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

Interaction Equation: $SbCl_3$ +2 BZTBZT $SbCl_3$ + BZT

Table (1): The structure and physical properties of compounds 1, 2, 3 and 4

Comp. No.	Structure	M.P. ^oC	color	Mole ratio	Yield %
1		170-172	orange	1:1	72

2		172-174	orange	1:2	54
3		130-132	Off-White	1:1	89
4		132-134	Off-White	1:2	64

Cytotoxicity Assay :

Seven samples of compounds 1, 2, 3, 4, $SbCl_3$, BZT and MTX with six concentrations (10, 8, 6, 4, 2 and 1µg/ml) for each compound were prepared to evaluate their biological activity against Leishmania parasite. The results showed that all these compounds are highly effective in the inhibition of Leishmania parasite. Results are presented in Table (8).

Results and Discussion:

A:Characterization

The prepared compounds were characterized by the following techniques :

1- FTIR :

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

Peaks appeared at 428 cm^{-1}, are attributed to Sb-O bond which is not present in the basic materials, some peaks shifted tohigher frequencies,this is an evidence of coordination correlation between **Sb** and **O**inMTX according to the HSAB theory, and new bands appeared in the spectra of these complexes corresponding to stretching frequency of ν(Sb-N) band in the range (293-285 cm^{-1})confirming the coordination of Sb via the nitrogen atom in the thiol ring ,This can be explained according to HSAB theory , nitrogen is in the borderline and Sb also in the borderline , so it is easy to bond with antimony **[jx]** .

2- UV :

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

3- CHNS Analysis:

Elemental Analysis was performed for compounds 1, 2, 3 and 4. The results listed in Tables (3 and 4), confirming their basic chemical structure. 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 (2) : The most diagnostic FTIR bands of the ligand L1 and its metal complexes in (cm^{-1}).

Compd. / **Bands**	**[MTX]**	**[MTX $SbCl_3$] 1:1**	**[MTX $SbCl_3$] 1:2**
ν(O-H)	3479 - 3458	3450 - 3380	3425 - 3388
ν(N-H) of NH_2	3415 - 3307	3363 - 3299	3309 - 3236
ν(N-H) of amide	3348	3346	3344
ν (C-H) arom.	3265, 3203 , 3163	3265, 3203, 3163	3265, 3203, 3163
ν(C-H) aliph.	2389- 2343	2389- 2343	2395-2360
ν(C=N)	1508	1512	1508
ν(C-N)	1367	1367	1367
ν(C=O) of amide	1672	1724	1720
ν(C=O) of COOH	1639, 1604	1638, 1604	1643, 1606
ν(Sb-Cl)	----------	318	320
ν(C=C)	1446-1404	1446-1402	1450-1404
ν(C-O) of COOH	1247- 1209	1247 -1205	1251-1207
ν(C-H) of CH_3	2636	2636	2636
ν(Sb-O)	----------	428	428
δ (C-H) arom.	833	833	833

The differences in the values between the two compounds is due to the presence of very little amount of MTX with compound (2) **.**

Table (3) : The most diagnostic FTIR bands of the ligand L2 and its metal complexes in(cm^{-1}).

Compd. / Bands	[BZT]	[BZT $SbCl_3$] 1:1	[BZT $SbCl_3$] 1:2
ν(C-H) arom.	3060, 2844	3093 , 2871	3045 , 2879
ν(C=N)	1593	1625	1625
ν(C=C)	1471,1456,1423	1506, 1461,1429	1500, 1467, 1421
ν (C-N)	1315	1319	1317

ν(C-S)	1014-977	1066-958	1066-939
ν(C-H) thiol ring	2628	2407	2408
ν(Sb-N)	----------	293	285
ν(Sb-Cl)	----------	310	304

The differences in the values between the two compounds is due to the presence of very little amount of BZT with compound (4) **.**

4- Atomic Absorption:

The results are listed in Tables (4 and 5), confirm their basic chemical structure.

Table (4): Some physical and analytical data of the antimony complexes with methotrexate ligand (L1) .

Compound formula Colour	**Yield %**	**M. P. °C**	**M. Wt. g. mol⁻**	**% Elemental analysis / Found (Calc.)**				**% Metal Found (Calc.)**
				C	**H**	**N**	**S**	
C20H22N8O5SbCl3 Orange	72	170-172	682	36.1 (35.2)	3.3 (3.2)	16.1 (16.4)	----------	17.6 (17.8)
C20H22N8O5SbCl3 Orange	54	172-174	682	36.5 (42.2)	3.4 (3.8)	16.7 (19.7)	----------	17.8 (10.7)

Table (5): Some physical and analytical data of the antimony complexes with benzothiazole ligand (L2) .

Compound formula Colour	**Yield %**	**M. P. °C**	**M. Wt. g. mol⁻**	**% Elemental analysis / Found (Calc.)**				**% Metal Found (Calc.)**
				C	**H**	**N**	**S**	
C7H5NS SbCl3 Off-White	89	130-132	363	20.4 (21.1)	1.7 (1.9)	3.5 (3.8)	8.6 8.8	32.1 (33.5)
C7H5NS SbCl3 Off-White	74	132-134	363	20.7 (33.7)	1.6 (2.0)	3.7 (5.6)	7.9 12.8	32.9 (24.4)

The found values of CHNS analysis confirm that the product is (1:1) not (1:2) compared with the results obtained in 1:1 mole ratio reaction. The suggested molecular formula, Table (6) was supported by spectroscopic studies and molar conductivity measurements .

Table (6) : Molecular formula and nomenclature of antimony complexes with ligands (L1) and (L2).

Molecular Formula	**Nomenculture**
$C_{20}H_{22}N_8O_5SbCl_3$	Antimony(III)methotrexate
$C_{20}H_{22}N_8O_5SbCl_3$	Antimony(III)methotrexate
$C_7H_5NS\ SbCl_3$	Antimony(III)benzothiazole
$C_7H_5NS\ SbCl_3$	Antimony(III)benzothiazole

5-Conductivity measurement:

The molar conductance values of all the complexes were measured in DMSO as a solvent in a concentration of 10^{-3} M at room temperature to determin the ionic or non-ionic nature of the complexes. Results are listed in Table (7) showing all of them to be of ionic nature .

Table (7): The conductivity measurement of metal complexes

Comp.No.	Measurements / µs
1	34.3
2	36.5
3	35.0
4	37.2

B- Biological Activity Evaluation:

The resultspresented in Table (8) showed that all these compounds are highly effective in inhibition of leishmania parasite. The highest values, were 46, 58, 52, 62, 44, 43 and 40% for the compounds 1, 2, 3, 4, $SbCl_3$, BZT and MTXrespectively, Table (7).Comparing the results of the new compounds (1, 2, 3 and 4) with the starting materials ($SbCl_3$, BZTand MTX), it is very obvious that the inhibition rates are higher referring to their effectiveness due to the synergestic effect of Sb with MTX and BZT since they are themselves were effective agains tleishmania parasite**[dt,aq]** .

Cytotoxic Effect of the New Complexes, $SbCl_3$, BZT and MTX onLeishmania parasite

When the leishmania parasite was treated with the three 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 parasiteand 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 onleishmania parasite, 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 (8). Figures (1 and 2)**.**

The results showed the cytotoxicity effect of these compounds in all concentrations and the highest inhibition rate (62%) recorded with the higher concentration (10µg/ml) comparable to control negative**.** A decrease in inhibition rates (25% and 14%) happened in the lower concentrations (2 and 1 µg /ml), respectively**.**

The cytotoxic effect of complex **2** onleishmania parasite, Figures (1 and 2) showed that the high concentration (10 µg /ml) gave a significant high inhibition rate (58%) on parasite, while the low concentration (1 µg /ml) gave the low inhibition rate (31%).

The higher the inhibition rate of the complexes is due to the synergistic effect produced from the coordination of Sb with MTX or BZT ; the effectiveness of antimony, MTX 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 :

Compound **4>2>3>1> $SbCl_3$> BZT >MTX,** e.g.I.R increased by 6-18% in case of MTX (10µg/ml) , and by 9-19% in case of BZTdue to the coordination with Sb.

Table (8): Initial cytotoxic effect on leishmania parasite of compounds 1, 2, 3, 4, $SbCl_3$, BZT and MTX by MTT assay method in time of exposure 48 hrs.

NO. of Compound	Concentration µg/ml	Mean	(I.R %)	Viability %
1	10	0.120	46	54
	8	0.121	45	55
	6	0.125	43	57
	4	0.126	40	60
	2	0.134	39	61
	1	0.141	36	64
2	10	0.105	58	42
	8	0.110	52	48
	6	0.125	48	52
	4	0.126	44	56
	2	o.148	35	65
	1	0.154	31	69
3	10	0.093	52	48
	8	0.106	50	50
	6	0.114	43	57
	4	0.123	40	60
	2	0.143	33	67
	1	0.151	30	70
4	10	0.083	62	38
	8	0.093	58	42
	6	0.103	53	47
	4	0.116	47	53
	2	0.134	39	61
	1	0.174	21	79
$SbCl_3$	10	0.123	44	56
	8	0.127	42	58
	6	0.130	41	59
	4	0.126	40	60
	2	0.136	38	62
	1	0.161	27	73
BZT	10	0.125	43	57
	8	0.125	43	57
	6	0.129	41	59
	4	0.126	40	60
	2	0.143	35	65
	1	0.161	27	73
MTX	10	0.130	40	60
	8	0.136	38	62
	6	0.145	34	66
	4	0.152	31	69
	2	0.165	25	75
	1	0.189	14	86

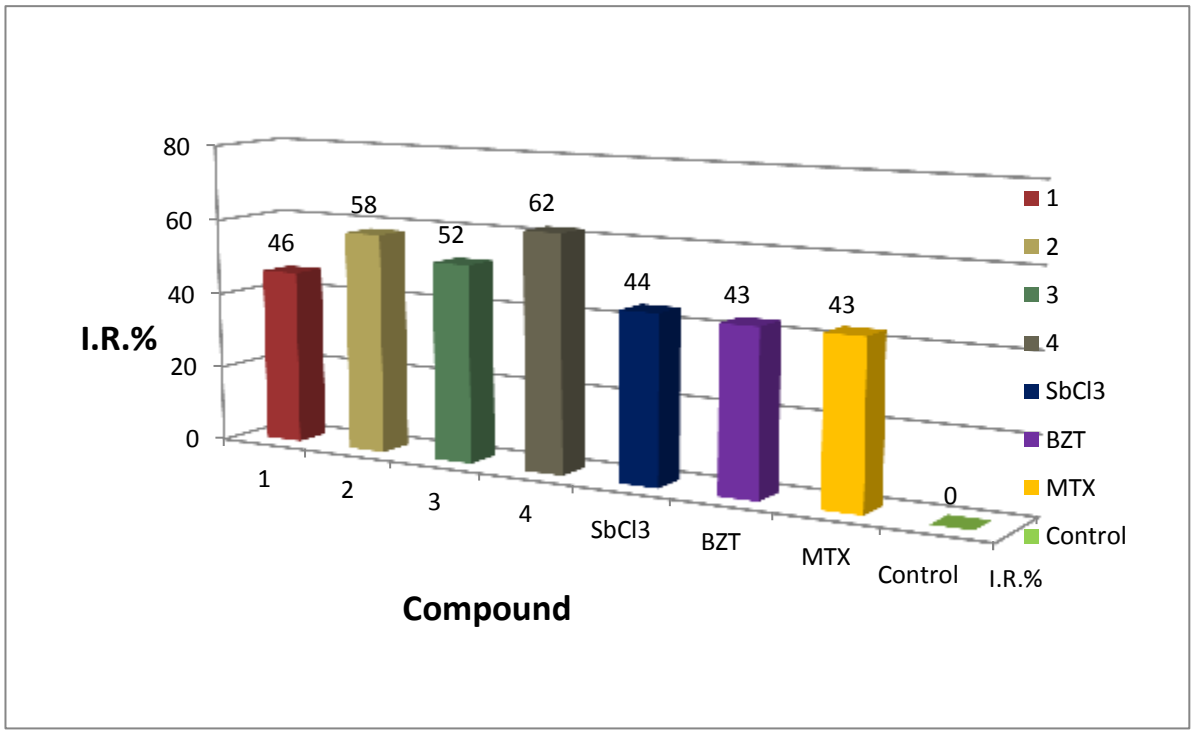


Figure (1): Over all block diagram of Cytotoxic Activity of Compounds 1, 2, 3, 4, $SbCl_3$, BZT and MTXon leishmania parasite

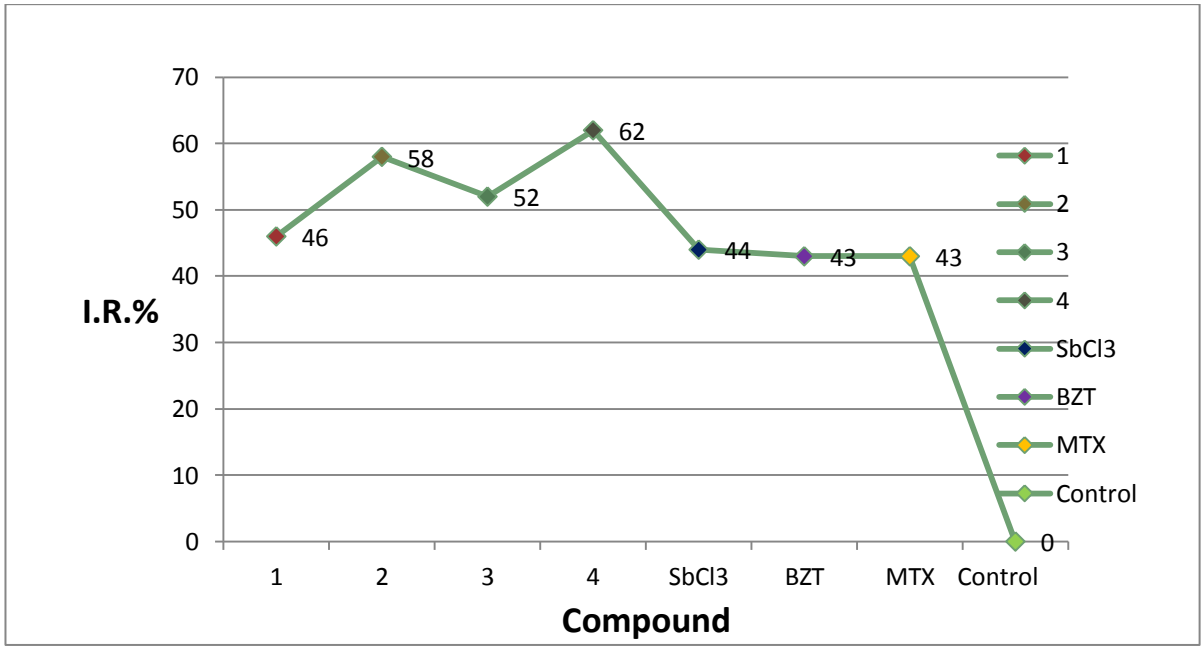


Figure (2): Over all Cytotoxic Activity of Compounds 1, 2, 3, 4, $SbCl_3$, BZT and MTX on leishmania parasite

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