Original Article

Synthesis and Comparison of Anti-*Leishmania major* Activity of Antimony and Iron Complexes of 3-Hydroxypyran-4-One and 3-Hydroxypyridine-4-One as Bi-Dentate Ligands

Abstract

Background: Leishmaniasis infection threatens millions of people in under developing and developing countries. Treatment of this neglected disease is very complicated. **Subjects and Methods:** A novel series of antimony (V) complexes using bidentate ligands of hydroxypyranones and hydroxypyridinones have been designed and synthesized. For the synthesis of the complexes, SbCl5 in water was added to the solution of each ligand at 60°C and the pH of mixture was adjusted to 8 using aqueous NaOH. After 24 h stirring, extraction of produced compound into acetone gave the desired complex. The structure of complexes was achieved by using FTIR, 1HNMR, and electron spin ionization mass spectroscopic techniques. All compounds were evaluated for in vitro anti amastogote form of *Leishmania major*. **Results and Conclusion:** The most potent antimony complexes against amastigotes were 5b (after 48 and 72 h) and 5a (after 72 h) with IC50 values of 24.4, 16.3, and 30.1 µg/mL, respectively. Furthermore, antimony and iron complexes were used together for *in vitro* anti amastigote form of *L. major* activity. These compounds were toxic for macrophages and destroyed them.

Keywords: *Anti-leishmania activity, antimony (V) complexes, iron complexes, Leishmania major*

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Introduction

Leishmania – a protozoan parasite in the new and old world – is the reason of different forms of infectious diseases in human including cutaneous, mucosal-cutaneous, and visceral leishmaniasis.[1,2] This infection threatens about 350 million people in 88 countries. The World Health Organization (WHO) denotes the prevalence of this disease as 12 million with 60,000 mortality and annual cases of 1–2 million in the world.[3] The WHO considers leishmaniasis as one of the neglected disease that primarily infects the poor people in underdeveloping and developing countries.[4]

Leishmania genus has more than twenty species and transmits to human's body by the Phlebotomine mosquitoes. The leishmania's life cycle begins phlebotomine swallows the infected human blood. In its body, the promastigote form of the parasite life is developed and transmitted to someone else. Promastigote is taken up by macrophages and converted in amastigote form in the host body. Then, amastigotes multiply by binary fission in macrophages, leading to cutaneous, mucosal-cutaneous and visceral infections (depending on the leishmania species). This form of the parasite is swallowed by the mosquito to start another cycle.[5,6]

Cutaneous leishmaniasis is the most common form of infection worldwide. In visceral leishmaniasis, the parasite spreads in the reticuloendothelial system and could be potentially life threatening. Visceral leishmaniasis is an opportunistic infection that occurs in those suffering from human immunodeficiency virus/AIDS or other cell-mediated immunodeficiency.[7]

Treatment of leishmaniasis is very complicated and dependent on leishmania species, geographic area, and different potencies of different medications.[8] The primary aim of in the treatment of cutaneous and visceral leishmaniasis, is prevention from mortality and morbidity.[7]

A few numbers of medications have emerged for the treatment of leishmaniasis, but none of them can eliminate the parasite completely from the host's body. Despite its high toxicity, amphotericin B is one of the first-line pharmacological agents in the treatment of leishmaniasis.[4] The other medications that act as anti-leishmania agents are pentamidine, paromomycine, and miltefosin. Miltefosin is orally active and was

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tested for cutaneous and mucosal leishmaniasis,[9] but due to its teratogenesity, it has been prohibited in pregnant women.[4] Of all the medications, pentavalent antimonials – sodium stibogluconate and meglumine antimoniate – are used as the main drugs in leishmania treatment[10,11] [Table 1].

The molecular mechanism and cellular function of pentavalent antimonial's is not clear. Some evidences propose the existence of an oxidative system inside the parasite involved in the production of reactive oxygen species, leading to the leishmania cell degradation. It has been proven that pentavalent antimonials have no cytotoxic effect on the parasite in iron deficiency conditions. In addition, it has been shown that iron strengthens the cytotoxic effect of antimony.[12] The use of iron along with pentavalent antimonials can be more effective in destroying the parasite, through the development of the Fenton reaction:

$$(Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^{-})$$

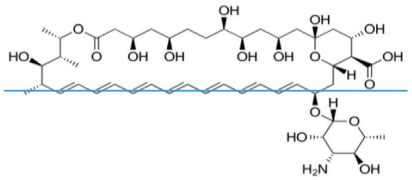
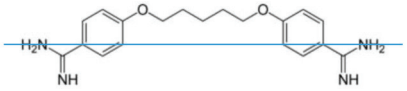
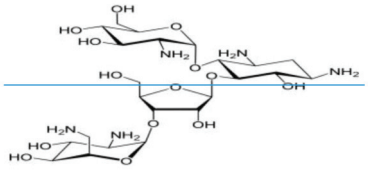
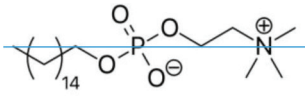
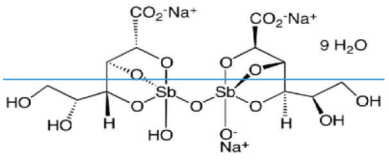
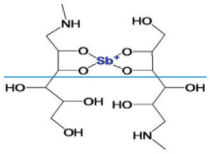
In order to prepare orally active anti-leishmania antimony compounds, a series of complexes with bidentate ligands of 3-hydroxy pyran-4-one and 3-hydroxy pyridine-4-one derivatives were designed and synthesized. It is supposed that the antimony complexes will have anti-leishmania activity. The anti-parasitic potency of these complexes along with the iron complexes is also investigated. The *in vitro* anti-leishmanial activities of the complexes will be assessed against the form of leishmania parasite, amastigote.

Materials and Methods

Chemistry

All chemicals used for this study were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) without more purification. The chemical reactions were evaluated step by step using thin-layer chromatography (TLC) on silica gel plates (Merck, Germany) in different solvent systems using methanol and chloroform. The synthesized products were structurally confirmed by ^{1}HNMR, Fourier-transform infrared spectroscopy (FT-IR), and electron spin ionization mass spectroscopic (ESI-MS) techniques. The FT-IR spectra of all compounds were reported in mid-infrared region (4000-380 cm–1) range with samples in KBr discs using a Perkin–Elmer apparatus. The1HNMR spectra were acquired on a Bruker Ultrashield 400 MHz spectrometer using DMSO-d_6, D_2O, and $CDCl_3$ as the solvents. The chemical shifts (δ) were reported in ppm downfield relative to internal standard

Table 1: The structures of some anti-leishmania agents

Anti-leishmania agent	Structure
Amphotericin B	
Pentamidine	
Paromomycine	
Miltefosin	
Sodium stibogluconate	
Meglumine antimoniate	

tetramethylsilane. The mass spectrometric results were recorded on a liquid chromatography with tandem mass spectrometry "AB SCIEX 3200 QTRAP" spectrometer. Furthermore, melting points were determined.

For the synthesis of antimony complexes of 3a-b and 4a-c, two types of ligands, namely 3-hydroxypyranone (maltol 1a and kojic acid 1b) and 3-hydroxypyridinone derivatives (compounds of 2a-c), were used.

The general method of synthesis of ligands and antimony complexes is shown in Figures 1 and 2.[13]

Experimental procedures

Synthesis of 1, 2-dimethy-3-hydroxypyridone-4-one (L1, 2a)

To a solution of maltol 1 (12.6 g, 0.1 mol) in 100 mL of distilled water, was added methyl-amine (25.8 mL, 0.3 mol) and refluxed for 24 h in 110°C–120°C. When the reaction termination was determined using TLC, 2 g of activated carbon was added and the reaction mixture was heated for 30 min. This mixture was filtered and the filtrate was evaporated to give a brown residue. Recrystallization from water gave a white crystalline solid in a 50% yield (7.0 g), mp 190°C–191°C (lit. value 189°C–190°C[14]); IR: (KBr): 3100–3300 (broad, OH), 2948 (C-H, aliphatic), 1630 (C = O), 1511 (C = C) cm^{-1}; ^{1}HNMR (DMSO-d6): 7.56 (d, J = 3.6 Hz, 1H, C5-H), 6.10 (d, J = 3.6 Hz, 1H, C6-H), 3.63 (s, 3H, N1-CH3), 2.27 (s, 3H, C2-CH3).

A similar procedure was used as previously described in synthesis complex 3a.

Synthesis of 1-ethyl-3-hydroxy-2-methylpyridine-4)-one (2b)

A similar procedure was used as previously described in synthesis ligand 2a except ethylamine (19.8 mL, 0.3 mol) was used instead of methyl amine in the reaction mixture. Recrystallization from methanol gave the desired product as a white powder in a 30% yield (4.6 g), mp 203°C–204°C (lit. value 205°C–206°C[14]).; IR: (KBr): 3100–3300 (broad, OH), 2978 (C-H, aliphatic), 1626 (C = O), 1529 (C = C) cm^{-1}; ^{1}HNMR (D_2O): 7.53 (d, J = 7.2 Hz, 1H, C5-H), 6.39 (d, J = 7.2 Hz, 1H, C6-H), 3.94–3.10 (q, J = 7.2 Hz, 2H, N1-CH_2), 2.29 (s, 3H, C2-CH_3), 1.24 (t, J = 7.2 Hz, 3H, N1-CH_2-CH_3).

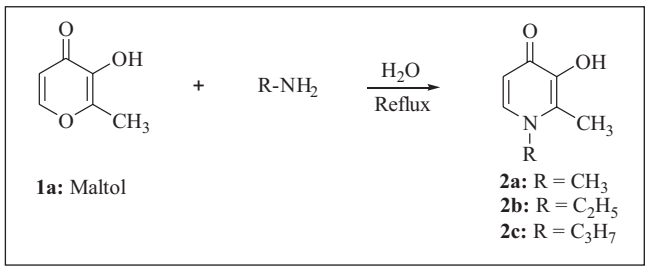


Figure 1: Synthesis of 3-hydroxy-2-methylpyridin-4-one derivatives from maltol

Synthesis of 3-hydroxy-2-methyl-1-propylpyridine-4-one (2c)

A similar procedure was used as previously described in synthesis ligand 2a except propylamine (24.9 mL, 0.3 mol) was used instead of methyl amine in the reaction mixture. Recrystallization from hot acetone gave the white powder in a 20% yield (3.4 g), mp 208°C–209°C (lit. value 206°C–207°C[14]). IR: (KBr): 3100–3300 (broad, OH), 2964 (C-H, aliphatic), 1624 (C = O), 1508 (C = C) cm^{-1}; ^{1}HNMR ($CDCl_3$): 7.23 (d, J = 7.2 Hz, 1H, C5-H), 6.39 (d, J = 7.6 Hz, 1H, C6-H), 3.84 (t, J = 7.6 Hz, 2H, N1-CH_2), 2.41 (s, 3H, C2-CH_3), 1.74–1.83 (m, 2H, N1-CH_2-CH_2), 0.99 (t, J = 7.2 Hz, 3H, N1-CH_2-CH_2-CH_3).

Synthesis of dihydroxo bis (2-methyl-3-hydroxypyranonato) antimony (V) complex (Sb-maltol complex) (3a)

The ligand 2-methyl-3-hydroxypyran-4-one (maltol) 1a (1.26 g, 0.01 mole) was dissolved in 50 mL of distilled water under stirring at 60°C conditions. Freshly precipitated hydrated Sb_2O_5 obtained from hydrolyzing of $SbCl_5$ (1 mL in 10 mL of distilled water) was added to the stirring solution of the ligand. With continued heating (at 60°C)/stirring, the pH of solution was adjusted to 8 by using aqueous NaOH. After 24 h, the mixture was cooling down and filtered. Finally, by adding 50 mL of acetone to filtrate solution a precipitate was produced which was then filtered off and dried to give the desired complex in a 40% yield (1.6 g), Decomposition at 290°C; IR: (KBr): 3100–3400 (broad, OH), 2900 (C-H, aliphatic), 1605(C = O), 1413 (C = C); ^{1}HNMR (D_2O): 7.99 (d, J = 5.2 Hz, 1H, C5-H), 6.52 (d, J = 5.6 Hz, 1H, C6-H), 2.37 (s, 3H, C2-CH_3); ESI − MS (m/z): 445 ($(OH)_2SbL_2$+K).

Synthesis of tris (2-(hydroxymethyl-3-hydroxypyranonato) antimony (V) complex (Sb-(Kojic acid complex) (3b)

A similar procedure was used as previously described in synthesis complex 3a except *Kojic acid* (1.42 g, 0.1 mol) was used instead of maltol in the reaction mixture. Extraction of produced compound into acetone gave the related complex in a 42% yield (1.9 g), Decomposition at 300°C IR: IR: (KBr): 3100–3300 (broad OH), 2923 (C-H, aliphatic), 1624 (C = O), 1546 (C = C); ^{1}HNMR (D_2O): 8.03 (s, 1H, C3-H), 6.53 (s, 1H, C6-H), 4.46 (s, 2H, C2-CH_2OH); ESI-MS (m/z): 585 (SbL_3+K).

Synthesis of tris (1,2-dimethyl-3-hydroxypyridinonato) antimony (V) complex (Sb-(L1 complex) (4a)

A similar procedure was used as previously described in synthesis complex 3a except 1,2-dimethyl-3-hydroxypyridin-4-one 2a (L1) (1.39 g, 0.01 mol) was used instead of maltol in the reaction mixture. Extraction of obtained compound into acetone gave the desired complex in a 45% yield (2.4 g), Decomposition at 295°C; IR: IR: (KBr): 3100–3400 (broad, OH), 2962 (C-H, aliphatic), 1619 (C = O), 1495(C = C); ^{1}HNMR (D_2O): 7.78 (d, J = 6.8 Hz, 1H, C5-H), 6.87 (d, J = 6.8 Hz, 1H, C6-H), 3.87 (s, 3H, N1-CH_3), 2.45 (s, 3H, C2-CH_3); ESI − MS(m/z): 507 (SbL_3-2CH_3), 399 (SbL_2).

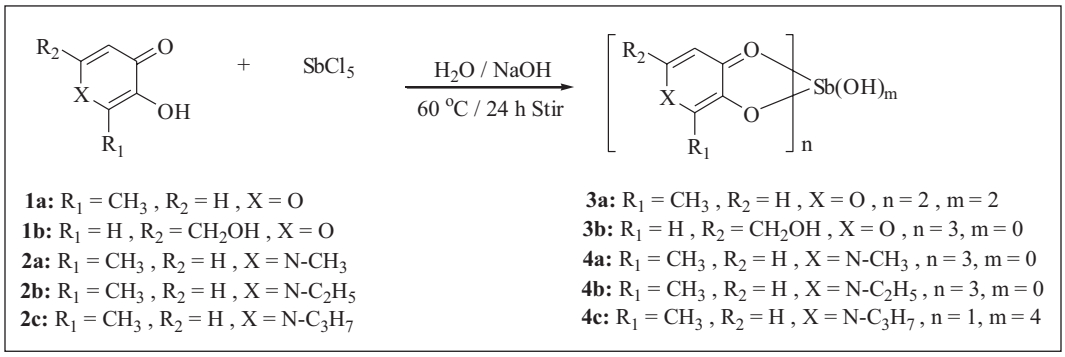


Figure 2: The general procedure for synthesis of antimony complexes

Synthesis of tris (1-ethyl-3-hydroxypyridinonato) antimony (V) complex (4b)

A similar procedure was used as previously described in synthesis complex 3a except 1-ethyl-3-hydroxypyridin-4-one 2b (1.53 g, 0.01 mol) was used instead of maltol in the reaction mixture. Extraction of produced compound into acetone gave the desired complex in a 35% yield (2.0 g), Decomposition at 290°C; IR: (KBr): 3100–3400 (broad, OH), 2900 (C-H, aliphatic), 1619 (C = O), 1493 (C = C) cm^{-1}; ^{1}HNMR (D_2O): 7.86 (d, J = 7.2 Hz,1H, C5-H), 6.93 (d, J = 7.2 Hz,1H, C6-H), 4.21–4.27 (q, J = 7.2 Hz,2H, N1-CH_2), 2.51 (s, 3H, C2-CH_3), 1.37 (t, J = 7.2 Hz, 3H, N1-CH_2-CH_3); ESI − MS (m/z): 578 (SbL_3+H), 425 (SbL_2).

Synthesis of tetrahydroxo-2-methyl-3-hydroxypyranonato antimony (V) complex (4c)

A similar procedure was used as previously described in synthesis complex 3a except 1-propyl-3-hydroxypyridin-4-one 2c (1.67 g, 0.01 mol) was used instead of maltol in the reaction mixture. Extraction of produced compound into acetone gave the desired complex in a 30% yield (1.1 g), decomposition at 285°C; IR: (KBr) cm^{-1}: 3100–3400 (broad, OH), 2965 (C-H, aliphatic), 1617 (C = O), 1508 (C = C) cm^{-1}; ^{1}HNMR (D_2O): 7.81 (d, J = 7.2 Hz, 1H, C5-H), 6.90 (d, J = 6.8 Hz, 1H, C6-H), 4.15 (t, J = 7.2 Hz, 2H, N1-CH_2-CH_2-CH_3), 2.48 (s, 3H, C2-CH_3), 1.72–1.81 (m, 2H, N1-CH_2-CH_2-CH_3), 0.84 (t, J = 7.6 Hz, 3H, N1-CH_2-CH_2-CH_3); ESI – MS (m/z):394 ($(OH)_4$SbL+K).

Biological assay

Leishmania parasite culture

The promastigotes of *Leishmania major* (MRHO/IR/75/ER) were cultured first in NNN (NevyMc Neal N) and then in RPMI-1640 to produce a mass of this parasite. RPMI-1640 medium was enriched with 10% fetal bovine serum (FBS) (Sigma Chemical Co.), Penicillin (100 IU/mL) and Streptomycin (100 µg/mL) at 25°C ± 1°C. Cultures were passaged after 4 days incubation and the growth of promastigotes was monitored daily using an inverted microscope. After this time, the promastigotes reached to infectious or metacyclic phase.

J774 macrophage cell culture

J774 mouse macrophage cell line was grown in a 37°C culture flasks with RPMI-1640 supplemented with 20% FBS, penicillin (100 IU/mL), and streptomycin (100 µg/mL). Cells were passaged when they reached 70% confluence.

Anti-leishmanial activity against Leishmania amastigotes

At first, 24 × 24 sterile cover slips were placed on the bottom of each well. J774 macrophage cells (2 × 106 cells/mL) were seeded in 6-well plates and the plates were incubated at 37°C and 5% CO_2 for 24–48 h to stick macrophages to the bottom of the plate and cover slips. Then, macrophages were infected with metacyclic *L. major* promastigotes at a parasite/macrophage ratio of 7:1 and incubated at 37°C in 5% CO_2 for 24 h. Free promastigotes were washed out of wells with FBS after 6 h and different concentrations of each of the designed compounds (10, 20, 40, and 80 µg/mL). Glucantime with the concentrations of 10, 20, 40, and 80 µg/mL was also added to the container as the positive control. After 24, 48, and 72 h, cover slips were fixed with methanol, stained with 10% Giemsa, and the results were observed using oil-immersion light microscopy. The number of infected macrophages and the average number of parasites per macrophage were determined in 100 cells.

Results

General procedures for synthesis of hydroxypyridinones ligands and their corresponding antimony comolexes

The general procedures for synthesis of the ligands (2a-c) and antimony complexes (3a-b, 4a-c) are summarized in Figures 1 and 2. The hydroxypyridinone derivatives were synthesized by the direct (single step) reaction of maltol with excess proper amines under reflux in aqueous solution. Purification using activated carbon and recrystallization with appropriate solvent resulted in the desired N-alkylsubstituted of 3-hydroxypyridin-4-one derivatives (compounds 2a-c) in the range of 20%–40% yield. These ligands can be synthesized with protection of 3 hydroxyl group in 3 steps. In this method, first maltol is benzylated using benzyl chloride to protect the 3-hydroxyl group. Then, reaction of benzyl maltol with proper amines leads to corresponding ligands, which were subsequently subjected to catalytic hydrogenation under acidic conditions to remove the protecting groups (the average yield of this method is 70%).[14] The single-step method was much easier and less expensive overall but, the yield was lower. In this method, the reaction is done in basic condition; the amines can attach to unprotected hydroxyl group that exist

as an anion in this condition and hence compete to amination of the ring. Because of this reason, the consumption of maltol is high leads to low yield. It should be noticed that, single method limited to small primary amines since larger amines (with more than three carbons) resulted in yields less than 10%.

Five novel antimony complexes of (3a-b and 4a-c) were also synthesized using bidentate ligands of (1a-b and 2a-c) and $SbCl_5$ at 60°C and pH 8 conditions [Figure 2].[13]

All synthesized antimony complexes of 3a-b and 4a-c, are six-coordinate with structure of octahedral (hybrid orbital: Sp3d2) The complexes are all nonvolatile, decomposing above 290°C and are soluble in water at neutral pH. The structures of all the prepared compounds were confirmed by FTIR,1HNMR and mass spectroscopy. The difference between the FTIR and 1HNMR spectra of the ligands and the corresponding complexes confirmed formation of the complexes. The details of the spectral data of the synthetic ligands and all complexes are given above (in experimental section). To confirm the formation of the complexes, the differences between the spectral details of the ligands and the complexes are critical.

The $\nu_{c=o}$, C = O stretching frequencies (cm^{-1}), in complexes are significantly lower than $\nu_{c=o}$ in the corresponding ligands. These variations in $\nu_{c=o}$ ($\Delta\nu_{c=o}$), indicated that all complexes were successfully synthesized [Table 2]. In fact, the formation of complexes are associated with the formation some new metal-oxygen bonds (Sb-O = C). The presence of new bonds causes an increase in the C = O bond order which leads to drive the C = O stretching frequencies down. Below 800 some new bonds appeared, and these were tentatively assigned as metal-oxygen bond formation (ν_{M-O}).

In ^{1}HNMR spectra, during metal chelation, the chemical shifts of the protons in the complexes moved to the lower field due to the change in the electron distribution. Thus, compared to the related hydrogens in the corresponding ligands, these protons have 0.01–0.77 ppm higher chemical shifts. As an example, the chemical shifts for C5-H and C6-H in 4a are 7.78 and 6.90. The chemical shifts for the corresponding hydrogens in the free ligand 2a are 6.10 and 7.56, respectively. These hydrogens are indicative in formation of the complexes. The other hydrogens in the structure of complexes, i.e., N substitution and the methylene hydrogens are less affected by electron distribution due to their far distance from chelating moieties. Thus, their chemical shifts are almost negligible.

The mass spectrometry technique used in this research was ESI-MS. It was shown that the complexes 3b and 4a-b were formed with three bi-dentate ligands (SbL_3), but the complexes of 3a and 4c were composed with one and two ligands in their structures, respectively. It is possible that the steric hindrance of the propyl chain substituted to the ring nitrogen of the complex 4c prevented binding of more than one ligand to antimony.

There were characteristic signals in the acquired spectra for the metal complexes. For example, for the case of complex 4a, the signals of 507 (SbL_3-$2CH_3$) and 399 (SbL_2) m/z for positive mode were the main signals that confirm formation of the complex. Sb has two isotopes of Sb^{121} and Sb^{123} with natural proportions of 57.21 and 42.79. Mass spectrometric signals for these isotopes were obvious in this spectra (4a). The signal of 507 m/z belongs to the complex with 3 ligands in its structure and the signal of 399 m/z belongs to the complex with 2 ligands which is result of instability of the complex leads to being broken of it.

Biological assay

Anti-leishmania activity

In this study, all synthesized antimony complexes were evaluated against amastigote form of *L. major*. Furthermore, the prepared iron complexes of these ligands were used along with antimony complexes for evaluating the anti-leishmania activity.

IC_{50} values (µg/mL) of compounds against amastigote form of leishmania in four concentrations (10, 20, 40 and 80 µg/mL) after 24, 48 and 72 h incubation are shown in Table 3. Compound 4b had the lowest IC_{50} value after 72 h incubation. The most potent compounds were 4b (72 h), 4b (48 h) and 4a (72 h) with IC_{50} values of 16.33 µg/mL, 24.37 µg/mL and 30.1, respectively. The complexes without the chain substituted to the ring nitrogen (3a, 3b) in comparing with chain substituted compounds (4a-c) exhibited less anti-leishmania activity. Most of the compounds destroyed the macrophages after 72 h at the concentration of 80 µg/mL. The antimony complexes (4b, 4c) and iron complex of ligand 1a (which was prepared and reported previously[14]), were also used together in the same concentrations (10, 20, 40 and 80 µg/mL) in the same periods of incubation against amastigote form of Leishmania. Treatment of *L. major* amastigotes with iron complexes along with antimony complexes (5, 6) could not inhibit the amastigotes and they were toxic for macrophages. Thus, it was not possible to determine the anti-leishmania effects of them and these combinations were excluded from the study.

Statistical analysis

ANOVA analysis showed that the anti-leishmania effects of compound 4b at all concentrations and compounds 4a and 4c

Table 2: The $\nu_{c=o}$ values (stretching frequencies, /cm) of hydroxypyranone (1a-b) and hydroxypyridinone (2a-c) ligands and their corresponding antimony (V) complexes (3a-b and 4a-c) ($\Delta\nu_{c=o}$=variations in C = O stretching frequencies)

Ligand	$\nu_{c=o}$ of ligand (/cm)	Sb-complex	$\nu_{c=o}$ of Sb-complex (/cm)	$\Delta\nu_{c=o}$
1a	1620	3a	1605	15
1b	1640	3b	1624	16
2a	1630	4a	1619	11
2b	1626	4b	1619	9
2c	1624	4c	1617	7

Table 3: IC_{50} values for anti-leishmania activity of the studied compounds

Compounds	Anti-amastigote activity (IC_{50} µg/mL)		
	24 h	48 h	72 h
3a	80 <	80 <	80 <
4a	80 <	59.7±0.36	30.1±0.66
4b	80 <	24.37±0.81	16.33±1.5
4c	80 <	59.67±1.5	58.83±1.04
3b	80 <	100 <	100 <
5	_*	_*	_*
6	_*	_*	_*
Glucantime	84.5±1.02	73.2±0.05	50.0±0.1

*These compounds were toxic for the macrophages and destroyed them. Thus, we could not understand the anti-leishmania activity of them

at concentrations of 20, 40 and 80 µg/mL were significant in comparison with the negative control group ($P < 0.05$) and were not significant in comparison with glucantime ($P > 0.05$). The anti-leishmania activities of them are similar to glucantime. The complexes 3a, 4a, 4c and 3b at concentration of 10 µg/mL did not have significant effects compared to negative control group ($P > 0.05$). Compounds 3a and 3b showed significant anti-leishmania activities compared to negative control group and glucantime ($P < 0.05$) at other concentrations (20, 40, and 80 µg/mL). Therefore, these complexes (3a and 3b) had anti-leishmania effects less than glucantime at these concentrations.

Conclusion

In the present study, a novel series of antimony complexes of 3-hydroxypyran-4-one and 3-hydroxypyridine-4-one were synthesized and characterized using FT-IR, ^{1}HNMR and ESI-MS techniques. All compounds were evaluated for anti amastigote form of *L. major*. The potent complexes were 4b (72 h), 4b (48 h) and 4a (72 h) with IC_{50} values of 16.33, 24.37, and 30.1 µg/mL, respectively. Two iron complexes were used in addition to antimony complexes for evaluation of anti-leishmania activities of them. These complexes were toxic for macrophages.

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Conflicts of interest

There are no conflicts of interest.

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