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Original Article

Synthesis and antileishmanial activity of antimony (V) complexes of hydroxypyranone and hydroxypyridinone ligands

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Abstract

A novel series of antimony (V) complexes with the hydroxypyranone and hydroxypyridinone ligands were synthesized and characterized by ^{1}HNMR, FT-IR and electron spin ionization mass spectroscopic (ESI-MS) techniques. The synthesis process involved protection of hydroxyl group followed by the reaction of the intermediate with primary amines and finally deprotection. All compounds were evaluated for *in vitro* activities against the amastigote and promastigote forms of *Leishmania major*. Most of the synthesized compounds exhibited good antileishmanial activity against both forms of *L. major*. IC_{50} values of the most active compounds; **9d**, **9d** and **9e**, after 24, 48 and 72 h against amastigote model were 15, 12.5 and 5.5 µg/mL, respectively. **9e**, **11** and **9e** inhibited the promastigote form of parasite after 24, 48 and 72 h with IC_{50} values of 10, 2 and 1 µg/mL, respectively.

Keywords: Antileishmania; Sb (V) complexes; Hydroxypyranone; Hydroxypyridinone

INTRODUCTION

Leishmaniasis is a disease caused by the protozoan parasite of the *Leishmania* species transmitted to human by the bite of infected female sand fly of the *Phlebotomus* and *Lutzomyia* genus. Leishmaniasis affects 12 million people around the world, mainly in underdeveloped countries with 2 million new cases each year. The disease is usually observed in four main subtypes: cutaneous, diffuse cutaneous, mucocutaneous and the fatal visceral form which is also known as Kala-azar, the reason for 60,000 deaths each year (1,2).

Since *Leishmania* parasite is eukaryotic, it shares many common features with its mammalian host which makes the development of selective drugs a hard work (3). Antimony (Sb) compounds including N-methylglucamine Sb (V), pentavalent antimonial sodium stibogluconate, meglumas antimonite, sodium Sb (III) gluconate and potassium Sb (III) tartrate have been widely used for the treatment of leishmaniasis (4). Despite their toxic side effects and the emergence of drug resistance that has reached epidemic proportion, particularly in parts of India, pentavalent antimonials are still the first-line drugs against all forms of leishmaniasis in several countries (5,6).

The mechanism of action of these drugs is not completely understood. Two major models have been proposed to describe this mechanism.

In the first model, Sb^{5+} is considered as a prodrug converted to a more toxic and active trivalent state (Sb^{3+}) by non-enzymatic and enzymatic reduction. Glutathione (GSH), trypanothione ($T(SH)_2$) and thiol-dependent reductase 1 (TDR1) are involved in this process.

Trivalent Sb binds the trypanothione reductase (TR) active site and strongly inhibits enzyme activity leading to the death of the parasite.

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According to the second model, sodium stibogluconate inhibits particularly type I DNA topoisomerase by binding the enzyme, therefore inhibiting unwinding and cleavage which are necessary for the parasitic DNA replication (7-9).

Attempts have been made to synthesize safer and more effective Sb complexes as antileishmanial drugs in the last decades.

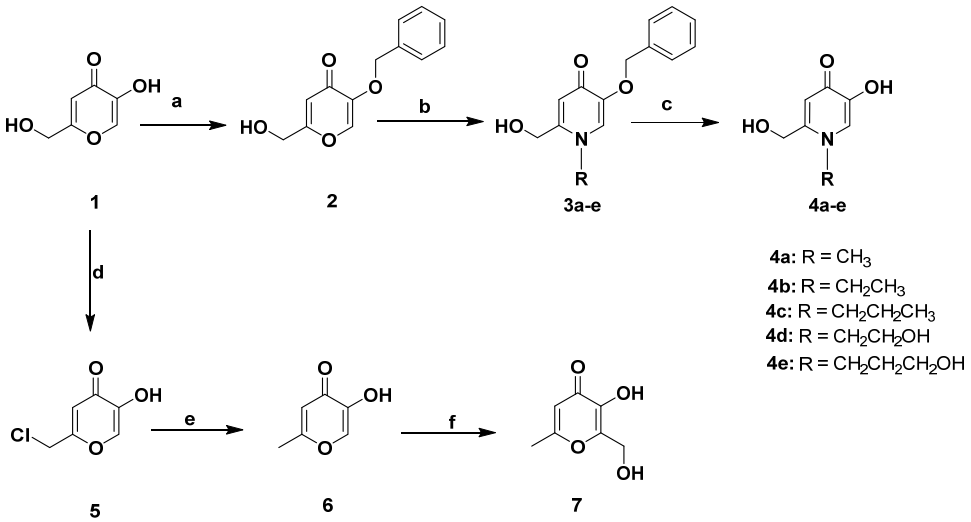
In the present study, we report the synthesis and antileishmanial activity of ten novel complexes of Sb (V) with hydroxypyranone (HP) and hydroxypyridinone (HPO) ligands. α-Hydroxyketones such as HP- and HPO-based compounds are considered hard acids able to coordinate metal cations like Sb and iron. These ligands may transfer Sb to the macrophages and release it, then make complex with the iron cations which are essential for the conversion of promastigote to amastigote inside the macrophage. The *in vitro* antileishmanial activities of the complexes were assessed against two different forms of leishmania parasite, amastigote and promastigote.

MATERIALS AND METHODS

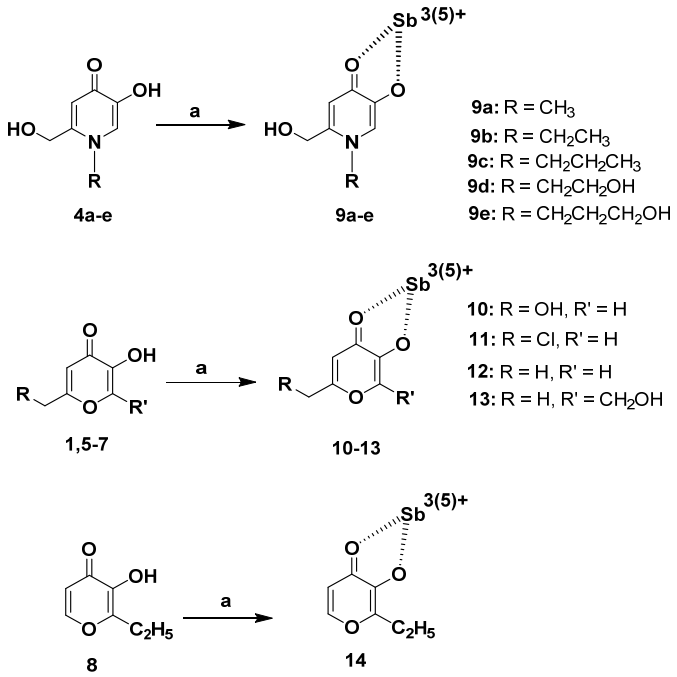
Chemistry

All chemicals used in this study were purchased from Sigma–Aldrich chemical Co. (USA) as synthesis grade and used without further purification. Chemical reactions were monitored by analytical thin-layer chromatography (TLC) using several solvent systems with different polarities on pre-coated silica gel 60 F254 aluminum plates (Merck, Germany). The synthesized compounds were structurally confirmed using spectroscopic methods of Fourier transform-infrared spectroscopy (FT-IR), ^{1}H nuclear magnetic resonance (^{1}HNMR) and electron spin ionization mass spectroscopy (ESI-MS) as well as determination of melting point as a physical constant. FT-IR spectra of all the compounds were recorded in the 4000–380 cm^{-1} range with samples in KBr discs using a Perkin-Elmer apparatus. ^{1}HNMR spectra were scanned on a Bruker Ultrashield 400 MHz spectrometer using DMSO-d_6 as the solvent. Chemical shifts (δ) were reported in ppm relative to internal standard tetramethylsilane (TMS). Mass spectrometry of all compounds was recorded on an LC-MS/MS Quattro Micro API micromass Waters 2695 spectrometer. Proper names for new complexes were given with the help of ChemOffice 2013 software.

The ligands needed for the preparation of the complexes were HPs including kojic acid (**1**), its derivatives (**5-7**), and ethyl maltol (**8**). Pyridinone analogues prepared from kojic acid (**4a-e**) were other ligands applied in this study. General procedure for the preparation of the ligands is provided in Scheme 1. All ligands were employed in Sb complex formation as it is shown in Scheme 2.



Scheme 1. General procedure for the synthesis of hydroxypyranone and hydroxypyridinone based ligands. (a) BnBr, NaOH, MeOH, 70 °C, 12 h reflux; (b) RNH_2, EtOH/H_2O (1:1), NaOH, 12 h reflux; (c) H_2 (30 psi), Pd/C, EtOH, room temperature, 2 h vigorous shaking; (d) $SOCl_2$; (e) Zn powder, HCl, H_2O, 4 h; (f) HCHO, NaOH, H_2O, 12 h.



Scheme 2. General procedure for the preparation of the antimony complexes, $SbCl_5$ in H_2O, NaOH, 4 h.

Preparation of the ligands and their intermediates and the final Sb (V) complexes were prepared as follows:

*Synthesis of 5-(benzyloxy)-2-(hydroxymethyl)-4H-pyran-4-one (**2**)*

This compound was prepared as previously reported (10-12). To a solution of kojic acid (6.4 g, 0.045 mol) in methanol (70 mL) was added sodium hydroxide (2 g, 0.05 mmol) dissolved in water (5 mL). The mixture was heated up to reflux. Benzyl bromide (6.06 mL, 0.05 mol) was added dropwise over 30 min, and the resulting mixture was refluxed for 12 h. After removal of the solvent by vacuum evaporation, the residue was dissolved in water (50 mL) and extracted with dichloromethane (3 × 50 mL). The combined extracts were washed with 5% aqueous NaOH (50 mL) then the organic fraction was dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo* to yield a crude product as a yellow solid. Recrystallization from ethanol gave 5-(benzyloxy)-2-(hydroxymethyl)-4*H*-pyran-4-one as a white crystalline solid.

*Synthesis of 5-(benzyloxy)-2-(hydroxymethyl)-1-(hydroxy) alkyl pyridin-4(1H)-one derivatives (**3a-e**)*

To a solution of compound **2** (0.0181 mol) in 100 mL solution of ethanol:water (1:1) was added the proper amine (0.054 mol). pH was adjusted to 13 using 2 N sodium hydroxide solution and the reaction mixture was refluxed for 12 h. The progress of the reaction was monitored by TLC until the reaction was complete. After adjustment of pH to 7 with HCl, volume of the reaction mixture was reduced to about half, followed by extraction of the product into dichloromethane (4 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered off and evaporated *in vacuo* to give a light brown solid. Recrystallization from ethanol gave **3a-d** as white crystalline solids except for **3e** which was an oily compound (10-12).

*Synthesis of (**4a-e**)*

Compounds **3a-e** were dissolved in ethanol (60 mL) and palladium/charcoal catalyst (5% w/w) was added. The solution was stirred for 2 h under a constant stream of hydrogen. The residual catalyst was cautiously removed by filtration, followed by evaporation of the solvent under vacuum to yield the crude solid product. Recrystallization from ethanol gave **4a-e** as white or yellow crystalline solids (10-12).

*Synthesis of 2-(chloromethyl)-5-hydroxy-4H-pyran-4-one (chlorokojic acid) (**5**)*

Chlorokojic acid was prepared according to the procedure provided by Ellis, *et al* (11). In

brief, kojic acid **1** (5 g, 0.035 mol) was dissolved in $SOCl_2$ (5 mL). The reaction mixture was stirred for 30 min at 25 °C until a yellow solid mass was formed. The solid product was filtered and washed with n-hexane. Recrystallization from water gave chlorokojic acid as a white crystalline solid.

*Synthesis of 5-hydroxy-2-methyl-4H-pyran-4-one (**6**)*

To a solution of **5** (4 g, 0.025 mol) in distilled water (50 mL) was added zinc dust (3.25 g, 0.05 mol), then concentrated HCl (7.45 mL) was added dropwise while stirring at a temperature between 70-80 °C. The solution was stirred for 4 h at 70 °C. The residual zinc was removed by filtration and the filtrate was extracted into dichloromethane (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo* to yield the crude product. Recrystallization from *iso*-propanol gave compound **6** as a colorless crystalline solid (13).

*Synthesis of 3-hydroxy-2-(hydroxymethyl)-6-methyl-4H-pyran-4-one (**7**)*

To a solution of NaOH (0.56 g, 0.016 mol) in distilled water (50 mL) was added 6-methyl-3-hydroxy pyran-4(1*H*)-one (1.75 g, 0.015 mol) and stirred for 10 min at 25 °C. Then a 35% solution of formaldehyde (1.27 mL) was added dropwise over 15 min. The resulting mixture was stirred for 12 h at room temperature. The solution was acidified by concentrated hydrochloric acid to pH 1 and cooled down to 2-4 °C for 13 h to give a crystalline deposit (13).

*Synthesis of **9a-e**, and **10-14***

The ligand (each of **1**, **4a-e** and **5-8**) was dissolved in water (25 mL) under stirring at 55 °C. Freshly precipitated hydrated Sb_2O_5 obtained from hydrolyzing of $SbCl_5$ in water was added to the stirring solution of ligand. This solution was stirred for 3 h at 55 °C, pH **7** adjusted by aqueous NaOH. At the end of this time after cooling down the solution, a precipitate was produced by adding 75 mL of acetone which was then filtered off and dried to give **9a-e**, and **10-14** as the desired complexes (14,15).

Biological assays

Leishmania parasite culture

The promastigotes of *L. major* (MRHO/IR/75/ER) were cultured first in Nevy Mcneal N and then in RPMI1640 to produce a mass of this parasite. RPMI-1640 medium was enriched with 10% fetal bovine serum (FBS) (Sigma Chemical Co.), Gentamicin (80 µg/mL), penicillin (100 IU/mL) and streptomycin (100 µg/mL) at 25 ± 1 ºC. Cultures were passaged after 4 days incubation. The growth of promastigotes was monitored daily using an inverted microscope.

Promastigote assay

To assess inhibitory concentration of each of 10 synthesized compounds on *L. major*, 96- well plates were used. In each well was poured 100 µL of medium containing 10^6 parasites in stationary phase. Then parasites were treated with each of the 10 compounds at 11, 22, 44 and 88 µg/mL and glucantime with the concentrations of 11, 22, 44 and 88 µg/mL as the positive control. A well devoid of the studied compounds as a negative control was included in the study. Concentrations were tested in triplicates, incubated at 24 ºC and evaluated after 24, 48 and 72 h. Afterwards, hemocytometer lamella and 0.4% tripan blue dye were used for observing results and counting the number of promastigotes (16-18).

J774 macrophage cell culture

J774 mouse macrophage cell line was grown in a culture flasks at 37 ºC with RPMI supplemented with 10% FBS, penicillin (100 IU/mL) and streptomycin (100 µg/mL). Cells were passaged when they reached 70% of confluence (19).

Antileishmanial activity against leishmania amastigotes

At first, 24 × 24 sterile cover slips were placed on the bottom of each well. J774 macrophage cells (2 × 10^6 cells/mL) were seeded in 6-well plates and the plates were incubated at 37 ºC and 5% CO_2 for 24 to 48 h for macrophages to stick 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 34 ºC in 5% CO_2 for 24 h. Free promastigotes were washed out of wells with phosphate buffered saline (PBS) after 6 h and different concentrations of each of the 10 designed compounds (11, 22, 44 and 88 µg/mL) were prepared. Glucantime with the concentration 11-88 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 (20).

RESULTS

Chemistry

Ten novel complexes of Sb (V) with HP and HPO described here were synthesized following the synthetic routes outlined in Scheme 1. Reaction of kojic acid (5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one, (**1)**) with benzyl chloride resulted in the protected form of this molecule (**2**) which was then reacted with amines to produce the corresponding pyridin-4(1*H*)-one derivatives (**3a-e**). 2-(Chloromethyl)-5- hydroxy-4*H*- pyran-4 -one (**5**) was prepared by stirring kojic acid with $SOCl_2$ at room temperature. Elimination of chlorine atom using zinc dust in concentrated HCl gave 5-hydroxy-2-methyl-4*H*-pyran -4 -one (**6**) which then reacted with formaldehyde to give 3-hydroxy-2-(hydroxymethyl)- 6-methyl-4*H*-pyran-4-one (**7**). After debenzylation of the 5-benzyloxy group of the **3a-e**, the resulting compounds were used as ligands in the formation of Sb (V) complexes (**9a-e**). Kojic acid (**1**) and its derivatives (**5-7**) and also ethyl maltol (**8**) were also used for the preparation of some more Sb complexes (**10-14**). The structures of all compounds were confirmed by FTIR, ^{1}HNMR and mass spectroscopy. The details of the spectral data are given in the following. The differences between the spectral details of the ligands and the complexes confirm the formation of the complexes.

*5- Hydroxy-2-(hydroxymethyl)-1- methylpyridin-4(1H)-one sb (v) complex (**9a**)*
Decomposition at 231 °C; IR: (KBr Disc) v/cm^{-1}: 3100–3400 (broad, OH), 1628 (C=O), 1560 (C=C); ^{1}HNMR (DMSO-d_6): 7.89 (s, 1H, C6-H), 6.65 (s, 1H, C3-H), 4.60 (s, 2H, C$\underline{H}_2$OH), 3.92 (s, 3H, CH_3); ESI−MS (m/z): 156 (L+H), 463($(OH)_2SbL_2$-2H), 465 ($(OH)_2SbL_2$).

*1-Ethyl-5-hydroxy-2-(hydroxymethyl) pyridin-4(1H)-one Sb (V) complex (**9b**)*
Decomposition at 238 °C; IR: (KBr Disc) v/cm^{-1}: 3100–3300 (broad, OH), 1624 (C=O), 1568 (C=C); ^{1}HNMR (DMSO-d_6): 7.84 (s, 1H, C6-H), 6.51(s, 1H, C3-H), 4.51 (s, 2H, CH_2OH), 3.91 (q, J = 7.2 Hz, 2H, NC$\underline{H}_2$$CH_3$), 1.34 (t, J = 7.2 Hz, 3H, $NCH_2C\underline{H}_3$); ESI−MS (m/z): 170 (L+H), 340 ($(OH)_3SbL$-2H), 342 ($(OH)_2SbL$), 491 ($(OH)_2SbL_2$-2H), 493 ($(OH)_2SbL_2$).

*5-Hydroxy-2-(hydroxymethyl)-1-propylpyridin-4(1H)-one Sb (V) Complex (**9c**)*
Decomposition at 260 °C; IR: (KBr Disc) v/cm^{-1}: 3100–3400 (broad, OH), 1640 (C=O), 1564 (C=C) ; ^{1}HNMR (DMSO-d_6): 7.75 (s, 1H, C6-H), 6.32 (s, 1H, C3-H), 4.44 (s, 2H, C$\underline{H}_2$OH), 3.91 (t, J = 7.2 Hz, 2H, NC$\underline{H}_2CH_2CH_3$), 1.75 (m, 2H, $NCH_2C\underline{H}_2CH_3$), 0.923 (t, J = 7.2 Hz, 3H, $NCH_2CH_2C\underline{H}_3$); ESI−MS(m/z): 185 (L+2H), 356 ($(OH)_3SbL$+H), 521 ($(OH)_2SbL_2$), 519 ($(OH)_2SbL_2$-2H), 670 (SbL_3), 671 (SbL_3 + H), 672 (SbL_3 +2H), 673 (SbL_3 +3H).

*5- Hydroxy-1-(2- hydroxyethyl)-2- (hydroxyl-methyl)pyridin-4(1H)-one Sb (V) Complex (**9d**)*
Decomposition at >300 °C; IR: (KBr Disc) v/cm^{-1}: 3100–3400 (broad, OH), 1624 (C=O), 1566 (C=C); ^{1}HNMR (DMSO-d_6): 7.53 (s, 1H, C6-H), 6.57 (s, 1H, C3-H), 4.55 (s, 2H, $C_2\underline{H}$OH), 4.08 (t, J = 5.2 Hz, 2H, NC$\underline{H}_2CH_2$OH), 3.79 (t, J = 5.2 Hz, 2H, $NCH_2C\underline{H}_2$OH); ESI−MS (m/z): 186 (L+H), 525 ($(OH)_2SbL_2$), 523 ($(OH)_2SbL_2$-2H).

*5- Hydroxy-2- (hydroxymethyl)-1-(3-hydroxy-propyl)pyridin-4(1H)-one Sb (V) Complex (**9e**)*
Decomposition at >300 °C; IR: (KBr Disc) v/cm^{-1}: 3100–3400 (broad, OH), 1624 (C=O), 1560 (C=C) ; ^{1}HNMR (DMSO-d_6): 7.66 (s, 1H, C6-H), 6.68 (s, 1H, C3-H), 4.47 (s, 2H, C$\underline{H}_2$OH), 3.91 (t, J=7.2 Hz, 2H, NC$\underline{H}_2CH_2CH_2$OH), 3.45 (t, J = 5.6 Hz, 2H,

$NCH_2CH_2C\underline{H}_2OH$), 1.75 (m, 2H, $NCH_2C\underline{H}_2CH_2OH$); ESI−MS (m/z): 200 (L+H), 553 ($(OH)_2SbL2$), 551 ($(OH)_2SbL2$-2H).

*5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one Sb (V) complex (**10**)*

Decomposition at 225 °C; IR: (KBr Disc) v/cm^{-1}: 3100–3400 (broad, OH), 1635 (C=O), 1577 (C=C); ^{1}HNMR (DMSO-d_6): 8.03 (s, 1H, C6-H), 6.49 (s, 1H, C3-H), 4.51 (s, 2H, $C\underline{H}_2OH$); ESI−MS (m/z): 439 ($(OH)_2SbL_2$), 440 ($(OH)_2SbL_2$ +H).

*2-(Chloromethyl)-5-hydroxy-4H-pyran-4-one Sb (V) Complex (**11**)*

Decomposition at: 259 °C; IR: (KBr Disc) v/cm^{-1}: 1637 (C=O), 1590 (C=C; ^{1}HNMR (DMSO-d_6): 8.21 (s, 1H, C6-H), 6.67 (s, 1H, C3-H), 4.76 (s, 2H, $C\underline{H}_2CL$); ESI−MS (m/z): 160 (L), 296 ($(OH)_3SbL$-2H),475 ($(OH)_2SbL_2$), 600 (SbL_3-H), 601 (SbL_3).

*5-Hydroxy-2-methyl-4H-pyran-4-one Sb (V) complex (**12**)*

Decomposition at: 230 °C; IR: (KBr Disc) v/cm^{-1}: 1621 (C=O), 1575 (C=C) ; ^{1}HNMR (DMSO-d_6): 8.07 (s, 1H, C6-H), 6.32 (s, 1H, C3-H), 2.29 (s, 3H, CH_3); ESI−MS (m/z): 127 (L+H), 391 ($(OH)SbL_2$+H), 407 ($(OH)_2SbL2$), 408 ($(OH)_2SbL_2$+H).

*3-Hydroxy-2-(hydroxymethyl)-6-methyl-4H-pyran-4-one Sb (V) complex (**13**)*

Decomposition at: 251 °C; IR: (KBr Disc) v/cm^{-1}: 3200–3400 (broad, OH), 1632 (C=O), 1578 (C=C); ^{1}HNMR (DMSO-d_6); 6.32 (s, 1H, C5-H), 4.42 (s, 2H, $C\underline{H}_2OH$), 2.31 (s, 3H, CH_3); ESI−MS (m/z):157 (L), 469 ($(OH)_2SbL_2$), 470 ($(OH)_2SbL_2$+H), 468 ($(OH)_2SbL_2$-H).

*2-Ethyl-3-hydroxy-4H-pyran-4-one Sb (V) complex (**14**)*

Decomposition at: 198 °C; IR: (KBr Disc) v/cm^{-1}: 1637 (C=O), 1606 (C=C); ^{1}HNMR (DMSO-d_6): 8.18 (d, J=5.6 Hz, 1H, C6-H), 6.63 (d, J = 5.6 Hz, 1H,C5-H), 2.64 (q, J = 7.6 Hz, 2H, $C\underline{H}_2CH_3$), 1.14 (t, J = 7.6 Hz, 3H, $CH_2C\underline{H}_3$); ESI−MS(m/z): 279 ((OH)SbL), 435 ($(OH)_2SbL_2$), 436 ($(OH)_2SbL_2$+H).

Biologyical assay

Antileishmanial activity

IC_{50} values (μg/mL) against promastigotes after 24, 48 and 72 h incubation are provided in Table 1. The lowest IC_{50} values in 24, 48 and 72 h incubation were belong to the compounds **9e** and **14** (24 h), **11** (48 h), **9e** and **13** (72 h). The antiamastigote activity of the studied compounds after 24, 48 and 72 h in terms of IC_{50} values are listed in Table 1. The most potent compounds were found to be **9d** (24 h), **9d** (48 h) and **9e** (72 h), respectively.

Statistical analysis

The average number of amastigotes in 100 macrophages and infected macrophages in three different treatments with studied compound and with glucantime and without any antileishmanial agent are shown graphically in Fig. 1.

Table 1. IC_{50} values for antileishmanial activity of the studied compounds**.**

Compounds	Anti-promastigote activity, IC_{50} (μg/mL)			Anti-amastigote activity, IC_{50} (μg/mL)		
	24 h	48 h	72 h	24 h	48 h	72 h
9a	61 ± 1.60	45 ± 0.85	9 ± 1.00	93 ± 0.45	47 ± 0.80	25 ± 0.75
9b	57 ± 1.75	44 ± 1.00	6 ± 0.75	75 ± 0.30	37.5 ± 0.95	28 ± 1.00
9c	23 ± 0.90	12.5 ± 0.25	2.5 ± 0.30	65 ± 0.80	17.5 ± 0.40	10 ± 0.95
9d	12.5 ± 0.75	5.5 ± 1.50	2 ± 0.25	15 ± 1.00	12.5 ± 1.00	7.5 ± 0.75
9e	10 ± 0.50	4 ± 0.70	1 ± 0.15	45 ± 0.45	15 ± 0.25	5.5 ± 0.80
10	50 ± 1.00	39 ± 0.30	13.5 ± 0.25	84 ± 1.00	50 ± 0.45	17.5 ± 0.45
11	10.5 ± 0.50	2 ± 0.45	1.5 ± 0.02	78 ± 0.30	25 ± 0.95	7.5 ± 1.00
12	22 ± 1.20	12.5 ± 0.45	3 ± 0.15	75 ± 0.25	20 ± 0.45	18 ± 0.25
13	13.5 ± 0.35	5.5 ± 0.25	1 ± 0.08	89 ± 0.40	31 ± 1.00	17 ± 0.40
14	10 ± 0.65	7.5 ± 0.50	3 ± 0.20	65 ± 1.00	25 ± 1.00	10.5 ± 0.30
Glucantime	32 ± 0.25	31 ± 0.35	30 ± 0.20	80 ± 0.85	72 ± 1.00	60 ± 0.25

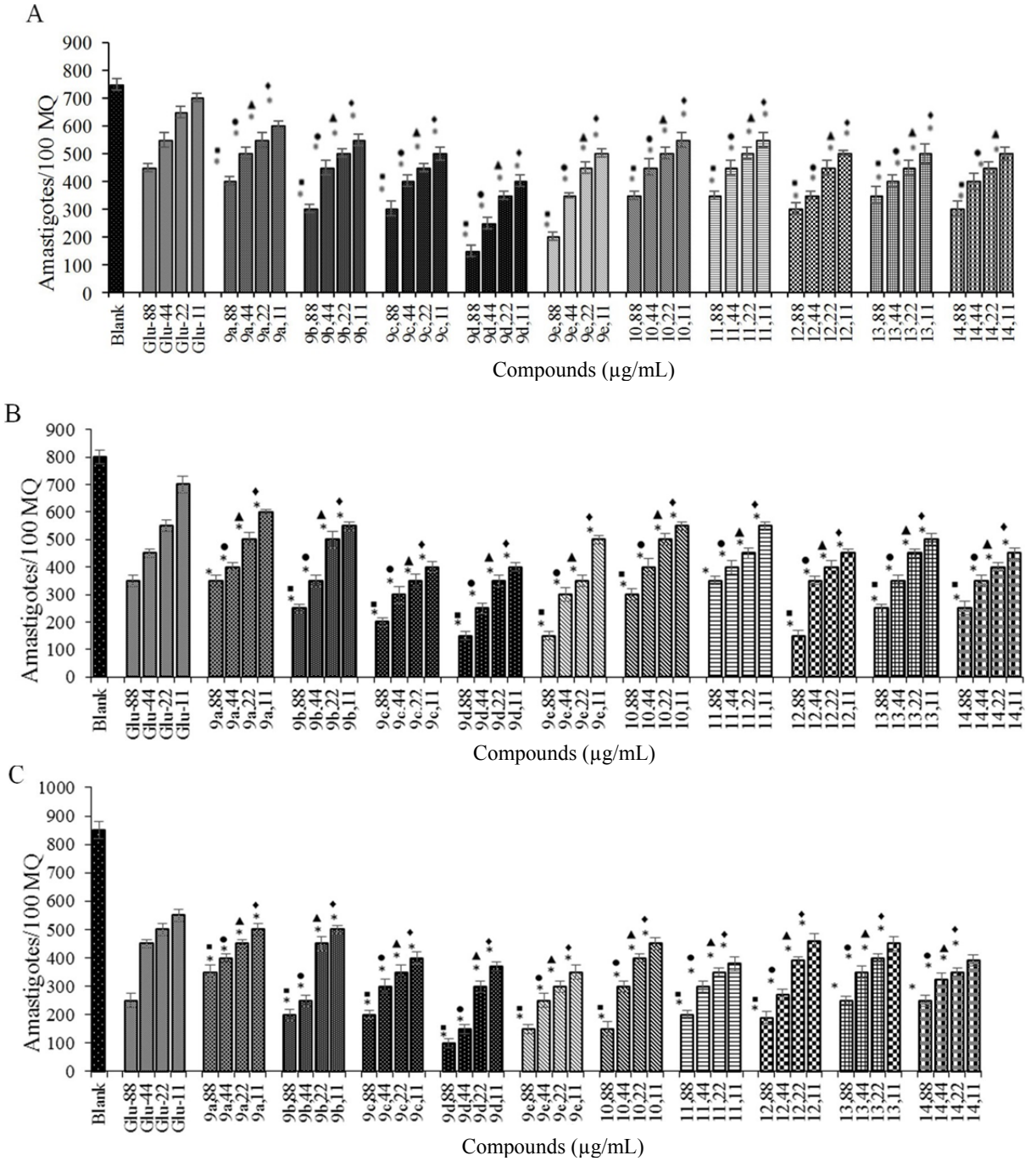


Fig. 1. *In vitro* activity of selected compounds against intramacrophage amastigotes of *Leishmania major*. (A) The mean number of amastigotes per 100 macrophage after treatment with selected compounds for 24 h. (B) The mean number of amastigotes per 100 macrophage after treatment with selected compounds for 48 h. (C) The mean number of amastigotes per 100 macrophage after treatment with the studied compounds for 72 h. Statistical analyses for all experiments were performed by one-way ANOVA with SPSS.24 software. (*$P < 0.05$ *vs.* blank, compounds; ▪$P < 0.05$ *vs.* Glu-88, compounds-88; •$P < 0.05$ *vs.* Glu-44, compounds-44; ▲$P < 0.05$ *vs.* Glu-22, compounds-22; ♦$P < 0.05$ *vs.* Glu-11, compounds-11).

ANOVA analysis showed that the cytotoxic effects of all prepared complexes at all concentrations were significant in comparison with the negative control ($P < 0.05$). All concentrations of the complexes with few exceptions indicated significant inhibitory effects compared with glunatime ($P < 0.05$). The inhibitory effects of compounds **11** and **9a** during 48 h at concentration of 88 µg/mL and compounds **13** and **14** during 72 h at concentration of 88 µg/mL were not significant.

DISCUSSION

All complexes showed characteristic peaks in FT-IR spectra of which the peaks belonging to the stretching vibrations of carbonyl group between 1621-1637 cm^{-1} and the double bond inside the heterocycle between 1560 and 1606 cm^{-1} were the most important ones for confirming the formation of the complexes. The corresponding peaks for the ligands appeared between 1660-1635 cm^{-1} and 1645-1570 cm^{-1}, respectively.

Thus, comparing the FT-IR spectra of the complexes and the corresponding ligands, it is obvious that a shift of 9 to 29 cm^{-1} for the carbonyl group and 2 to 34 cm^{-1} for the double bond takes place after the formation of the Sb (V) complexes. When the chelating moieties, i.e. carbonyl and hydroxyl groups take part in the reaction of the complex formation, bond stretching constant decreases leading to the observed shift in the frequencies. In fact, the change in the electron distribution which happens during metal chelation by the α-hydroxyketone moiety is the reason for the bond stretching constant. As an example, the shifts for **9c** which is a complex of ligand **4c** were from 1640 to 1622 cm^{-1} for the carbonyl group and from 1574 to 1566 cm^{-1} for the double bond.

In ^{1}HNMR spectra, the chemical shifts of the protons belonging to the complex structure moved to the lower field due to a change in the electron distribution during metal chelation. Thus, these protons have a 0.02-0.4 ppm higher chemical shift compared to the same hydrogens in the corresponding free ligand. For instance, chemical shifts of C6-H and C3-H in **9c** appeared in 7.75 and 6.32, respectively, while the corresponding shifts were 7.47 and 6.27 in ligand **4c** which is an indicative of complex formation. The chemical shifts of the protons on N1 propyl substitution and the methylene hydrogens on the C2-CH_2OH (0.05-0.06 ppm) were almost negligible since they were much farther from the chelating moiety, α-hydroxyketone, which experiences the electron distribution.

The mass spectrometry technique used in this research project was ESI-MS. The characteristic signals for the prepared complexes were detectable in the obtained spectra. In the case of complex **9c** as an example, ions of 183, 356, 521 and 670 m/z were observed. The 183 m/z belongs to the signal of the **4c** ligand ($C_9H_{13}NO_3$, exact mass: 183.09). This observation can be explained by instability of some complexes breaking down to the free ligand and Sb. Sb has two isotopes of Sb^{121} and Sb^{123} with natural proportions of 57.21/42.79 for Sb^{121} to Sb^{123}. Mass spectrometric signals for these isotopes are observed in the mass spectra of **9c**. The signals of 356, 521 and 670 m/z are indicatives of complexes with 1, 2 and 3 ligands in their structures, respectively.

In this research the antileishmania activities of Sb (V) complexes of HP and HPO ligands (**9a-e** and **10-14**) were evaluated on the leishmania parasite. Glucantime was used as a positive control in these experiments. Compounds **9e**, **13**, **11** and **9d** were the most potent ones against promastigotes after 72 h of incubation with the IC_{50} values of 1, 1, 1.5 and 2 μg/mL, respectively. There was an increase in the antileishmania activity of compounds **9a**, **9b** and **9c** parallel with the increase in the lipophilicity of the compounds in all time intervals. This can be explained with the increase in the penetration in parasite cell membrane. **9b** and **9c** exhibited lower potency (higher IC_{50} values) compared to **9d** and **9e**. Also activities of all compounds against the amastigote form of the parasite were evaluated.

The most potent compounds after 72 h incubation were **9e**, **9d**, **11** and **9c** with IC_{50} values of 5.5, .5, 7.5 and 10, respectively. For the remaining compounds, IC_{50} range after the same periods of incubation time were 15-93, 12.5-50 and 5.5-49 μg/mL, respectively. The anti-leishmania activity of **9a**, **9b** and **9c** against the amastigote form increased in an accordance with the length of the chain substituted to the ring nitrogen. The same trend was observed for the anti-promastigote activity, **9b** and **9c** proved to be less potent compared with **9d** and **9e**. All compounds were weaker anti-amastigotes than anti-promastigotes. **9d** and **9e** indicated greater antileishmanial activities against both forms of the parasite.

CONCLUSION

In the present study, a novel series of Sb (V) complexes with the HP and HPO ligands were synthesized and characterized by ^{1}HNMR, FT-IR and ESI-MS spectroscopic techniques. All compounds were evaluated for *in vitro* activity against the amastigote and promastigote forms of *L. major*. Most of the synthesized compounds exhibited good antileishmanial activity against both forms of *L. major*. IC_{50} values of the most active compounds; **9d**, **9d** and **9e**, after 24, 48 and 72 h against amastigote model were 15, 12.5 and 5.5 µg/mL, respectively. **9e**, **11** and **9e** inhibited the promastigote form of parasite after 24, 48 and 72 h with IC_{50} values of 10, 2 and 1 µg/mL, respectively.

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