Accepted Manuscript

Structural elucidation and bioassays of newly synthesized pentavalent antimony complexes

Tuba Iftikhar, Muhammad Khawar Rauf, Sidra Sarwar, Amin Badshah, Durdana Waseem, Muhammad Nawaz Tahir, Amjad Khan, Khalid Mohammad Khan, Gul Majid Khan

PII: S0022-328X(17)30513-2

DOI: 10.1016/j.jorganchem.2017.09.002

Reference: JOM 20077

To appear in: *Journal of Organometallic Chemistry*

Received Date: 22 March 2017

Revised Date: 24 August 2017

Accepted Date: 2 September 2017

Please cite this article as: T. Iftikhar, M.K. Rauf, S. Sarwar, A. Badshah, D. Waseem, M.N. Tahir, A. Khan, K.M. Khan, G.M. Khan, Structural elucidation and bioassays of newly synthesized pentavalent antimony complexes, *Journal of Organometallic Chemistry* (2017), doi: 10.1016/j.jorganchem.2017.09.002.

Structural elucidation and bioassays of newly synthesized pentavalent antimony complexes

Tuba Iftikhar [1], Muhammad Khawar Rauf [1*], Sidra Sarwar[1], Amin Badshah[1*], Durdana Waseem[2], Muhammad Nawaz Tahir[3], Amjad Khan[2], Khalid Mohammad Khan[4], Gul Majid Khan[2]

[1]*Department of Chemistry, Quaid-i-Azam University, Islamabad-45320, Pakistan*
[2]*Department of Pharmacy, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad-45320, Pakistan*
[3]*Department of Physics, University of Sargodha, Pakistan*
[4] *H.E.J. Research Institute, University of Karachi, Karachi-75270, Pakistan*

[*]Corresponding Authors: Email: aminbadshah@yahoo.com (A. Badshah), Email: mkhawarrauf@yahoo.co.uk (Muhammad Khawar Rauf) orcid.org/0000-0001-9994-4033

Abstract

Antimony (V) organometallics (**1-5**) have been synthesized with general formula $[SbR'_3(O_2CR)_2]$, where R'= phenyl, *p*-tolyl and O_2CR are substituted cinnamates. These complexes have been characterized by FT-IR analysis, multinuclear (^1H, ^{13}C) NMR spectroscopy and single crystal X-ray diffraction analysis. The crystal structures of $[Sb(phenyl)_3(4\text{-}ClC_6H_4C_2H_2O_2C)_2]$ (**1**) and $[Sb(p\text{-}tol)_3(4\text{-}OCH_3C_6H_4C_2H_2O_2C)_2].CHCl_3$ (**5**) displayed distorted geometry between trigonal bipyramidal and square pyramidal with monomeric structure at a five coordinated Sb center. Starting reagents and complexes were evaluated for anticancer, antileishmanial, antibacterial and alpha amylase inhibition potentials. It was observed that complexes **3**, **4** and **5** showed significant ($p<0.05$) antileishmanial and anticancer activities against *Leishmania tropica* KWH23 promastigotes and HepG2 cell lines respectively. Antibacterial activity of compound **3** was also significant against *E. coli* (MIC: 5.55 µg/mL), *K. pneumoniae* (MIC: 16.66 µg/mL), *S. aureus* (MIC: 5.55 µg/mL) and *P. aeruginosa* (MIC: 50 µg/mL). Hence, these new antimony complexes can act as good drug candidates.

Keywords:

Pentavalent antimony complexes, Antileishmanial, Anticancer, Single crystal XRD

1. Introduction

Significance of antimony in modern medicinal chemistry cannot be denied. Although many synthetic drugs were reported in the sixteenth and seventeenth centuries but there was a perpetual debate over antimony compounds than any of the others [1]. A group of researchers had stated the cosmetic use of antimony in prehistoric Egypt [2]. The most substantial medicinal practice of organoantimony (V) complexes in the nineteenth century was surely in the cure of leishmaniasis [3]. Nowadays, the use of antimony compounds in chemotherapy has also gained importance in the treatment of cancer. Derivatives of organoantimony were also investigated for their cytotoxicity against cancer cell lines [4-5]. It was found that organoantimony (III) and (V) complexes are effective against cervical carcinoma and leukemia cell lines. It has been investigated antimony complexes can target protein tyrosine phosphatases, induce apoptosis and increase amount of reactive O_2 species in cancer cells [6]. Organoantimony (V) compounds also exhibited high antitumor activity [7].

About twenty years ago, numerous organoantimony compounds have been successfully synthesized and tested in preclinical assays, which was assumed to be an effort to invent more effective and less toxic antibacterial and antileishmanial medicines. In the last few decades organoantimony (III) complexes were replaced by less toxic organoantimony (V) complexes, like meglumine antimoniate, to cure all types of leishmaniasis [8]**.** Antibacterial and antifungal activities of various antimony complexes have also been reported [9]. Extensive studies have been made on organoantimony (V) complexes on the basis of their various bonding modes [10-12] and biological applications [13-15]. Derivatives of organoantimony also show important properties such as catalyst components, biocides, antioxidants and fungicides [16-17].

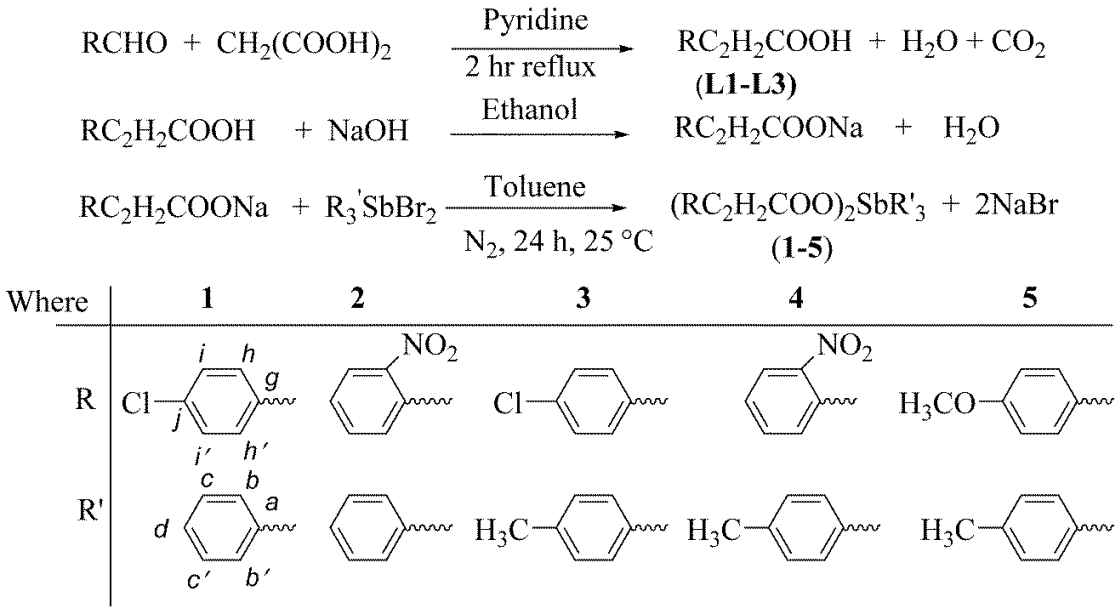
The synthesis of antimony complexes of type $[SbR'_3(O_2CR)_2]$ and to check their leishmanicidal properties for the development of pentavalent antimonial drugs against leishmaniasis is a part of our ongoing project [18-19]. Five organoantimony (V) carboxylates, with variety of cinnamate ligands were synthesized, characterized and screened for their potential as antileishmanial (*Leishmania major* promastigotes), anticancer (hepG2 cell line), antibacterial and alpha amylase enzyme inhibition. Different substituents around the antimony center may lead to more effectiveness as compared to the already present pentavalent antimonials.

2. Material and Methods

Aromatic aldehydes like 4-chlorobenzaldehyde, 2-nitrobenzaldehyde, 4-methoxybenzaldehyde, bromobenzene, magnesium turnings and 4-bromotoluene were obtained from Sigma Aldrich and used without any further purification. Toluene, chloroform, dichloromethane, dimethylsulphoxide and diethyl ether were distilled prior to their use according to standard method [20]. BIO COTE Model SMP10 melting point apparatus was used for determination of melting point and values are reported without correction. The multinuclear NMR spectra i.e. (^{1}H and ^{13}C) were taken on Bruker 300 MHz spectrometer with tetramethylsilane (TMS) as a reference. Elemental analyses were performed using a LECO-932 CHNS analyzer. FT-IR data was recorded on a Bruker TENSOR II FT-IR Spectrometer.

2.1. General procedure for synthesis

Cinnamic acid derivatives (**L1**-**L3**) were synthesized by using reported method [21a]. In two necked round bottom flask aldehyde (0.1 mol) and malonic acid (0.1 mol) were taken in 50 mL pyridine and refluxed for two to three hours. Dilute HCl was added drop wise as reaction mixture was cooled down. Precipitates formed were filtered off and washed with water. Recrystallization was done with ethanol in order to get needle like crystalline material. The acids were converted in to their sodium salts by treating ethanolic solution with aqueous NaOH in equimolar quantities. Sodium salts of cinnamic acid derivatives (**L1-L3**) were separated by slow evaporation at room temperature in crystalline form in good yield. Organoantimony (V) dibromide precursors were synthesized by adapting the methodology as reported in literature [21b]. Organoantimony (V) carboxylates were synthesized according to previously described method with certain changes in solvent and thermal conditions [22]. In a 250 mL two necked round bottom flask, a solution of sodium salt of the respective carboxylic acids (0.01 mol) in 50 ml of dry toluene were added to organoantimony (V) halide (0.005 mol) and stirred overnight. Thin layer chromatography (TLC; *n*-hexane:chloroform in ratio 40:60) was performed in order to check the completion of reaction. Precipitates of the salt were filtered and product was obtained from filtrate by rotary evaporation of toluene. Product was kept for crystallization in ethanol/chloroform mixture. The synthesized complexes are shown in **Scheme 1**.



Scheme 1. Synthesis of acids (**L1-L3**) and complexes (**1-5**).

2.1.1. Bis[3-(4-chlorophenyl)acrylato]tri(phenyl)antimony (V) (1)

0.9 g (4.4 mmol) of sodium salt of 3-(4-chlorophenyl) acrylic acid and 1.126 g (2.2 mmol) of triphenylantimony (V) dibromide were added in toluene.

Yield: 1.29 g, 82%, m.p.: 219-220°, FT-IR (cm^{-1}): C-$H_{aromatic}$ (3056), C-$H_{aliphatic}$ (2927), C=O (1647), Sb-C (563), Sb-O (453), ^{1}H NMR (300 MHz, $CDCl_3$), δ(ppm): 6.29-6.35 (d, 2H, ^{3}J=15.9 Hz, OOC-CH_e=CH-Ph), 7.29-7.45 (m, 10H, Ar-$H_{h, h', i, i', j}$& -CH=CH_f-Ph), 7.51-7.55 (m, 9H, Ar-$H_{c, c', d}$), 8.08-8.12 (m, 6H, Ar-$H_{b, b'}$); ^{13}C NMR (75 MHz, $CDCl_3$), δ(ppm): 121.4 (2C_f), 129.0 (4$C_{h, h'}$), 129.1 (4$C_{i, i'}$), 129.4 (6$C_{c, c'}$), 131.2(3C_d), 133.4 (3C_g), 133.9 (6$C_{b, b'}$), 135.5 (4C_j),138.4 (2$C_{a(ipso\ carbon)}$), 142.0 (4C_e), 170.6 (2C, C=O). Elemental analyses calculated (found) for $C_{36}H_{27}Cl_2O_4Sb$ (%): C, 60.37 (60.35); H, 3.80 (3.79).

2.1.2. Bis[3-(2-nitrophenyl)acrylato]tri(phenyl)antimony (V) (2)

0.9 g (4.1 mmol) of sodium salt of 3-(2-nitroophenyl) acrylic acid and 1.024 g (2 mmol) of triphenylantimony (V) dibromide were added in toluene.

Yield: 1.21 g, 82%, m.p.: 221-222°, FT-IR (cm^{-1}): C-$H_{aromatic}$ (3056), C-$H_{aliphatic}$ (2965), C=O (1643), Sb-C (552), Sb-O (457), ^{1}H NMR (300 MHz, $CDCl_3$), δ(ppm): 6.24-6.30 (d, 2H, ^{3}J=15.6Hz, OOC-CH_e=CH-Ph), 7.48-7.62 (m, 15H, Ar-$H_{c, c', d, h, i, j}$), 7.96-8.02 (m, 4H, Ar-$H_{i'}$& -CH=CH_f-Ph), 8.11-8.15 (m, 6H, Ar-$H_{b, b'}$); ^{13}C NMR (75 MHz, $CDCl_3$), δ(ppm): 124.8 ($3C_d$), 126.5 ($2C_f$, -CH=C_fH-Ph), 128.9 ($2C_i$), 129.6 (6C*c, c'*), 129.7 ($2C_i$), 130.9 ($2C_j$), 131.5 ($3C_a$), 133.3 ($3C_{i'}$), 133.9 ($6C_{b,b'}$), 137.0 ($2C_g$), 138.0 ($2C_e$, -C_eH=CH-Ph), 148.2 ($2C_{h'}$, C-NO_2), 169.1 (2C, C=O). Elemental analyses calculated (found) for $C_{36}H_{27}N_2O_8Sb$ (%):C, 58.64 (58.62); H, 3.69 (3.68); N, 3.80 (3.78).

2.1.3. Bis[3-(4-chlorophenyl)acrylato]tris(*p*-tolyl)antimony (V) (3)

0.9 g (4.4 mmol) of sodium salt of 3-(4-chlorophenyl) acrylic acid and 1.22 g (2.2 mmol) tris(*p*-tolyl)antimony (V) dibromide were added in toluene.

Yield: 1.33 g, 80%, Melting point: 165-168°, FT-IR (cm^{-1}): C-$H_{aromatic}$ (3022), C-$H_{aliphatic}$ (2921), C=O (1644), Sb-C (581), Sb-O (482), ^{1}H NMR (300 MHz, $CDCl_3$), δ(ppm): 2.40 (s, 9H, CH_3), 6.29-6.34 (d, 2H, ^{3}J=15.9Hz, OOC-CH_e=CH-Ph), 7.29-7.42 (m, 16H, Ar-$H_{c, c', h, h', i, i'}$& -CH=CH_f-Ph), 7.95-7.98 (d, 6H, Ar-$H_{b, b'}$, ^{3}J=8.4Hz); ^{13}C NMR (75 MHz, $CDCl_3$), δ(ppm): 21.5 (3C, CH_3), 121.9 ($2C_f$, -CH=C_fH-Ph), 128.9 ($4C_{h, h'}$), 129.0 ($4C_{i, i'}$), 130.1 ($6C_{c, c'}$), 133.5 ($2C_g$), 133.7 ($6C_{b, b'}$), 134.5 ($3C_d$), 135.3 ($2C_j$), 141.4($3C_{a(ipso\text{-}carbon)}$) 141.6 ($2C_e$, -$C_e$H=CH-Ph), 170.2 (2C, C=O). Elemental analyses calculated (found) for $C_{39}H_{33}O_4Sb$ (%): C, 61.77 (61.75); H, 4.39 (4.36).

2.1.4. Bis[3-(2-nitrophenyl)acrylato]tris(*p*-tolyl)antimony (V) (4)

0.9 g (4.1 mmol) of sodium salt of 3-(2-nitrophenyl) acrylic acid and 1.108 g (2 mmol) of tris(*p*-tolyl)antimony (V) dibromide were added in toluene.

Yield=1.29 g(83%). m.p=188-189°. FT-IR (cm^{-1}): C-$H_{aromatic}$ (2998), C-$H_{aliphatic}$ (2858), C=O (1638), Sb-C (583), Sb-O (481). ^{1}H NMR (300 MHz, $CDCl_3$), δ(ppm): 2.42 (s, 9H, CH_3), 6.24-6.29 (d, 2H, ^{3}J=15.9Hz, OOC-CH_e=CH-Ph), 7.25-7.64 (m, 14H, Ar-$H_{c, c', h, i, i', j}$), 7.95-8.02 (m, 8H, Ar-$H_{b, b'}$ & -CH=CH_f-Ph). ^{13}CNMR (75 MHz, $CDCl_3$), δ(ppm): 21.6 (3C, CH_3), 124.8($2C_i$), 126.9 ($2C_f$, -CH=C_fH-Ph), 128.9 ($2C_j$), 130.0 ($2C_h$), 130.3 ($6C_{c, c'}$), 131.0 ($2C_{i'}$), 133.3 ($3C_d$), 133.8 ($6C_{b, b'}$), 135.2 ($2C_g$), 137.7($2C_e$, -C_eH=CH-Ph), 141.7 ($3C_{a(ipso\text{-}carbon)}$), 148.3 ($2C_{h'}$, C-

NO_2), 169.0 (2C. C=O). Elemental analyses calculated (found) for $C_{39}H_{33}N_2O_8Sb$ (%): C, 60.10 (60.07); H, 4.27 (4.26); N, 3.59 (3.57).

2.1.5. Bis[3-(4-methoxyphenyl)acrylato]tris(*p*-tolyl)antimony (V) chloroform solvate (5)

0.9 g (4.4 mmol) of sodium salt of 3-(4-methoxyphenyl) acrylic acid and 1.218 g (2.2 mmol) of tris(*p*-tolyl)antimony (V) dibromide were added in toluene.

Yield=1.23 g(82.5%). m.p=179-180°. FT-IR (cm^{-1}): C-$H_{aromatic}$ (2958), C-$H_{aliphatic}$ (2894), C=O (1657), Sb-C (559), Sb-O (484), ^{1}H NMR (300 MHz, $CDCl_3$), δ(ppm): 2.40 (s, 9H, CH_3), 3.82 (s, 6H, OCH_3), 6.21-6.26 (d, 2H, ^3J=15.9Hz, OOC-CH_e=CH-Ph), 6.85-6.88 (d, 4H, Ar-$H_{h,\ h'}$, ^3J=8.7Hz), 7.31-7.34 (d, 6H, Ar-$H_{c,\ c'}$, ^3J=8.1Hz), 7.38-7.46 (m, 6H, Ar-$H_{i,\ i'}$ & -CH=CH_f-Ph), 7.97-7.99 (d, 6H, Ar-$H_{d,\ d'}$, ^3J=8.1Hz). ^{13}C NMR (75 MHz, $CDCl_3$), δ(ppm): 21.5 (3C, CH_3), 55.3 (2C, OCH_3), 114.1 (4$C_{h,h'}$), 118.9 (2C_f, -CH=C_fH-Ph), 127.7 (2C_g), 129.4 (4$C_{\ i,\ i'}$), 130.0 (6$C_{c,\ c'}$), 133.7 (6$C_{b,\ b'}$), 135.0 (3C_d), 141.1 (3$C_{a(ipso\text{-}carbon)}$), 142.7 (2$C_e$, -$C_e$H=CH-Ph), 160.79 (2C, C-$OCH_3$), 170.9 (2C, C=O). Elemental analyses calculated (found) for $C_{41}H_{39}O_6Sb.CHCl_3$(%): C, 58.06 (58.01); H, 4.64 (4.4.63).

2.2. Single crystal X-ray diffraction (XRD) analysis

Out of all compounds synthesized, complexes **1** and **5** were crystalline and their crystal structure was obtained by Bruker Kappa APEXII CCD diffractometer using monochromatic MoKα (λ = 0.71073Å) radiations. The temperature was maintained at 296(2) K during data collection. Structures of complexes were elucidated using SHELX 97 [23] with structure refinement done by SHELXL 2014 [24].

2.3. Determination of antileishmanial activity

Antileishmanial activity of complexes was investigated by *in vitro* analysis using 2-(4,5-dimethyl-2-thiazolyl)-3,5-diphenyl-bromide (MTT) colorimetric assay according to predefined protocol [25]. Complete growth medium (pH 7.4) containing Medium 199 supplemented with fetal bovine serum (FBS; 10%), streptomycin sulphate (100 µg/mL) and penicillin G (100 IU/mL) was used to grow *Leishmania tropica* kwh 23 promastigotes at 24°C. Refreshed culture of promastigotes (180 µL) at seeding density of 2×10^6 promastigotes/mL was incubated with compounds or amphotericin B (20 µL) at a final concentration of 20 µg/mL in 96 well plate at 24°C for 72 hours. Negative controls wells contained 1% DMSO in PBS. Afterwards, culture in

each well was incubated again for 4 h at 24°C with pre-filter sterilized 4 mg/mL MTT solution (20 µL). Subsequently, supernatant was removed leaving formazan crystals behind, which were dissolved in DMSO (100 µL). Absorbance was measured using microplate reader (Biotech USA, microplate reader Elx 800) at 540 nm. Similar procedure was followed using 3-fold serial dilutions of samples and IC50 was calculated by linear regression.

2.4. Cytotoxicity against HepG2 cancer cell line

Cytotoxicity against human hepatocellular carcinoma cell line HepG2 was determined by *sulforhodamine B* (SRB) assay [26]. Cells that reached to 60-70% confluence in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS, 100 IU/mL penicillin, 0.25 µg/mL amphotericin B and 100 µg/mL streptomycin sulphate were trypsinized and adjusted to seeding density of 1×10^5 cells/mL. About 180 µL of cell culture was incubated with 20 µL of compounds or doxorubicin (20 µg/mL) in CO_2 (5%) incubator (Panasonic, Japan MCO-18AC-PE) at 37ºC. After 72 h of incubation, cells were fixed with 100 µL of 20% trichloroacetic acid, washed with water and air-dried. It was stained with 0.057% SRB (0.4%) 1% in acetic acid for 30 min. Wells were washed with 1% acetic acid, dried and SRB was solubilized in 10 mM Tris base (200 µL; pH10). Absorbance was observed at 515 nm with a microplate reader and linear regression analysis was used to calculate IC50 from data of 3-fold serial dilutions.

2.5. Evaluation of antibacterial activity

Antibacterial activity of compounds was investigated by disc diffusion method [27] against gram positive and gram negative strains. Overnight refreshed cultures of *Staphylococcus aureus* (ATCC-6538), *Escherichia Coli* (ATCC-15224), *Klebsiella pneumonia* (ATCC-1705) and *Pseudomonas aeruginosa* (ATCC-15442) were used. Sterile filter paper discs were impregnated with 5 µL of compounds (10 mg/mL in DMSO) or standards or DMSO. Roxithromycin and cefixime (10 µg/disc) were used as standards. Impregnated discs were placed on sterile agar plates swabbed with bacterial culture. These plates were incubated at 37°C for 24 h and diameter (mm) of zone of inhibition (ZOI) was measured. Compounds showing ZOI ≥ 12 mm were subjected to minimum inhibitory concentration (MIC) determination by microbroth dilution method [28]. Nutrient broth seeded with bacterial inoculum at density of approximately 5×10^4 CFU/mL was incubated with 3-fold dilutions (50 µg/mL to 1.85 µg/mL) of compounds in 96-well plate for 24 h at 37°C. Plate was visually analyzed for presence of turbidity and lowest

concentration of compounds inhibiting the growth of bacterial strain (no turbidity) was considered as MIC.

2.6. Evaluation of alpha amylase inhibition activity

Compounds were tested for their enzyme inhibition capacity against alpha amylase enzyme according to previously described procedure with minor modification [29]. Sample, negative control, positive control and blank wells were prepared in 96 well plate and observed at 540 nm using microplate reader. Sample wells contained 15 µL of phosphate buffer (PB) (pH 6.8), 25 µL of α-amylase enzyme (0.14 U/mL), 10 µL of compounds (4 mg/mL DMSO) and 40 µL of starch solution (2 mg/mL in PB). Acarbose (250 µM) was used as positive control. Negative control was prepared without compounds whereas blank was prepared without compounds and amylase enzyme; each being replaced by equal quantities of buffer. After incubating the plate for 30 min at 50°C, 20 µL of 1 M HCl was added in order to stop the reaction. Then 90 µL of iodine reagent (5 mM iodine, 5 mM potassium iodide) was added to each well and activity was expressed as percent α-amylase inhibition/mg compound and calculated from absorbance values by the following equation:

% α-amylase inhibition = (Os - On) / (Ob - On) × 100

Where On = Absorbance of negative control, Os = Absorbance of sample and Ob = Absorbance of blank well.

2.7. Statistical Analysis

In the present study, data was calculated as mean±SD of respective parameters. Means were compared by ANOVA using GraphPad Prism 5 software.

3. Results and Discussion

The structures of organoantimony (V) complexes were confirmed by FT-IR, NMR spectroscopy and single crystal X-ray diffraction.

3.1. Characterization

In FT-IR spectra of synthesized complexes, a signal at 3500-3300 cm^{-1} due to OH stretching is absent which indicates the formation of Sb-O bond in the organoantimony (V) carboxylates. The carboxylate ligands act as monodentate as the difference of Δν value of ($\nu CO_{2\ asymm}$) and (νCO_2

$_{symm}$) is lower than 200 cm^{-1}. After bonding to antimony, the carbonyl group (C=O) stretching frequency show a valuable shift due to the electron resonance that induces a single bond character to the carbonyl group and hence lowers the frequency. The characteristic IR bands of organoantimony (V) carboxylates includes, 3056-2958 cm^{-1} due to aromatic (C-H), 2965-2858 aliphatic (C-H), 1657-1638 cm^{-1} (C=O), 1587-1580 cm^{-1} (C-N), 735-740 cm^{-1} (C-Cl), 583-552 cm^{-1} (Sb-C), 484-453 cm^{-1} (Sb-O). Signals in the lower infra-red region (583-552 cm^{-1} and 484-453 cm^{-1}) are absent in the spectra of starting carboxylates and are according to reported values [30-31].

^{1}H-NMR spectra of free ligands showed singlet around 12.23 ppm due to carboxylic acid (-COOH) proton, which was not found in the spectra of the synthesized complexes indicating the removal of acid group (-proton from carboxylic COOH) and binding of carboxylate ligand to antimony. Intensity and multiplicity patterns were used to identify all the chemical shifts [38]. Signals due the aromatic protons were visible in the region of 7.19-8.53 ppm. The aromatic signals in the ^{1}H NMR spectra of the complexes varied depending on the type of cinnamic acid used as the starting material. The splitting pattern of the synthesized phenyl and *p*-tolyl derivatives of antimony complexes consisted of singlets, doublets and multiplets, which gave information about neighboring protons. The methoxy proton appeared as a high intensity singlet at 3.82 ppm. The signal of methyl protons observed between 2.40 to 2.68 ppm.

The ^{13}C NMR data showed the signals due to different types of carbon atoms in the compounds. In the spectra of all the complexes (**1-5**), the carbonyl carbon atoms appeared in the range of 169.0-171.3 ppm. Aromatic carbon signals of antimony (V) complexes were assigned on the basis of intensities of signal and comparison of chemical shift with the reported values [18, 30, 31]. Carbon atoms of the aromatic ring were visible in the region 114.1-160.1 ppm. The methyl carbon atoms were appeared between 21.5-21.6 ppm. The methoxy carbon appeared at 55.3 ppm.

3.2. X-ray Crystallography

Crystallization of the products was done in chloroform-toluene mixture. Colorless prism-like crystals of complexes **1** and **5** were obtained, which were analyzed by X-ray diffraction technique. Molecular structures of these complexes are shown in **Fig.** 1 & 3. Crystallographic data summery is shown in **Table 1**. Selected bond lengths and bond angles are given in **Table 2**.

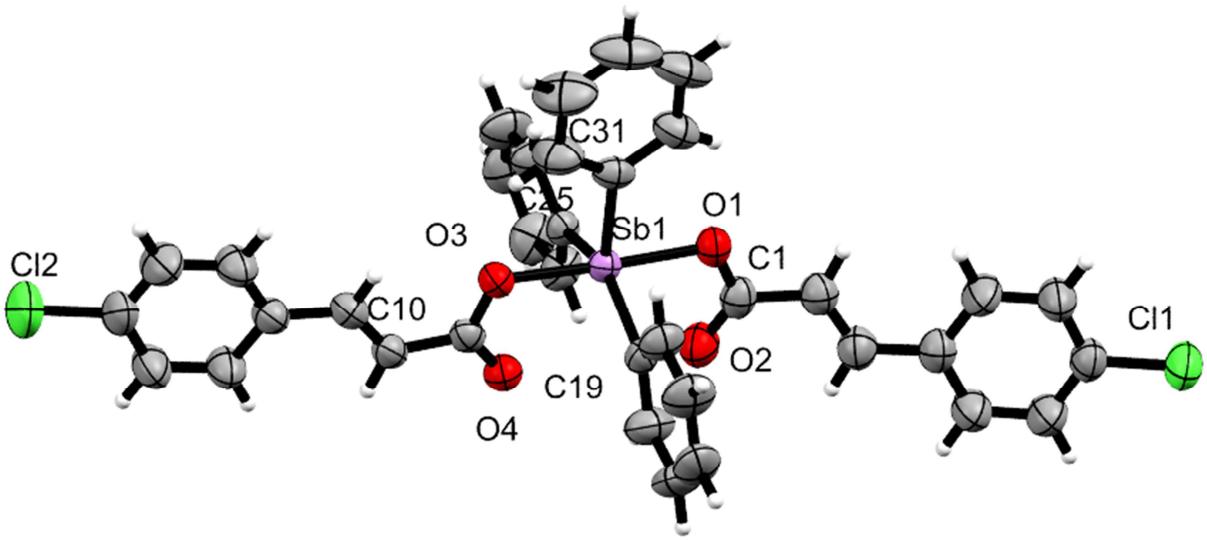


Fig. 1. Molecular structure of **1**. Hydrogen atoms are represented as circles of arbitrary radii.

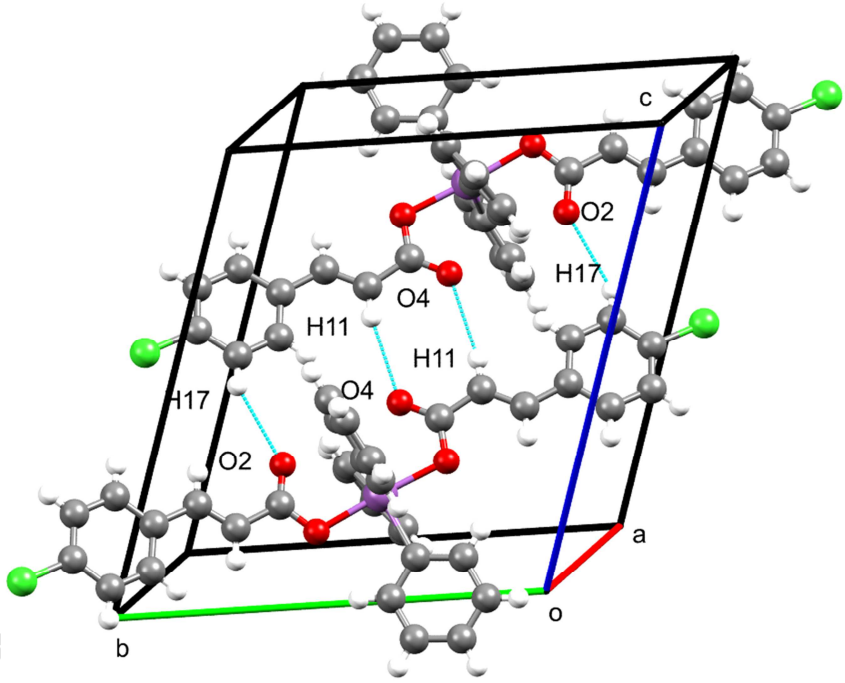


Fig. 2. Packing view of **1** along *c*-axis showing intermolecular (C-H···O) hydrogen bonding.

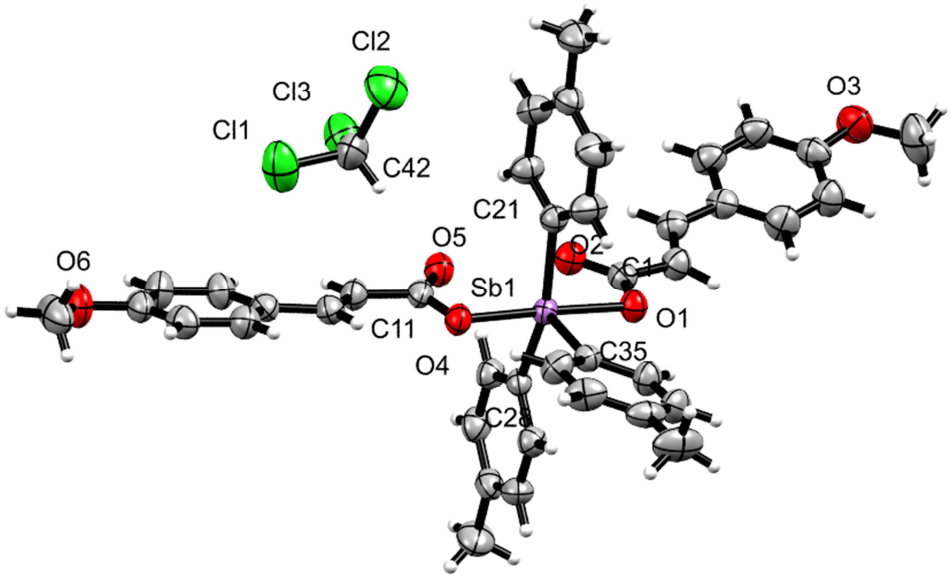


Fig. 3. Molecular structure of **5**. Hydrogen atoms are represented as circles of arbitrary radii. $CHCl_3$ solvent is also shown in lattice.

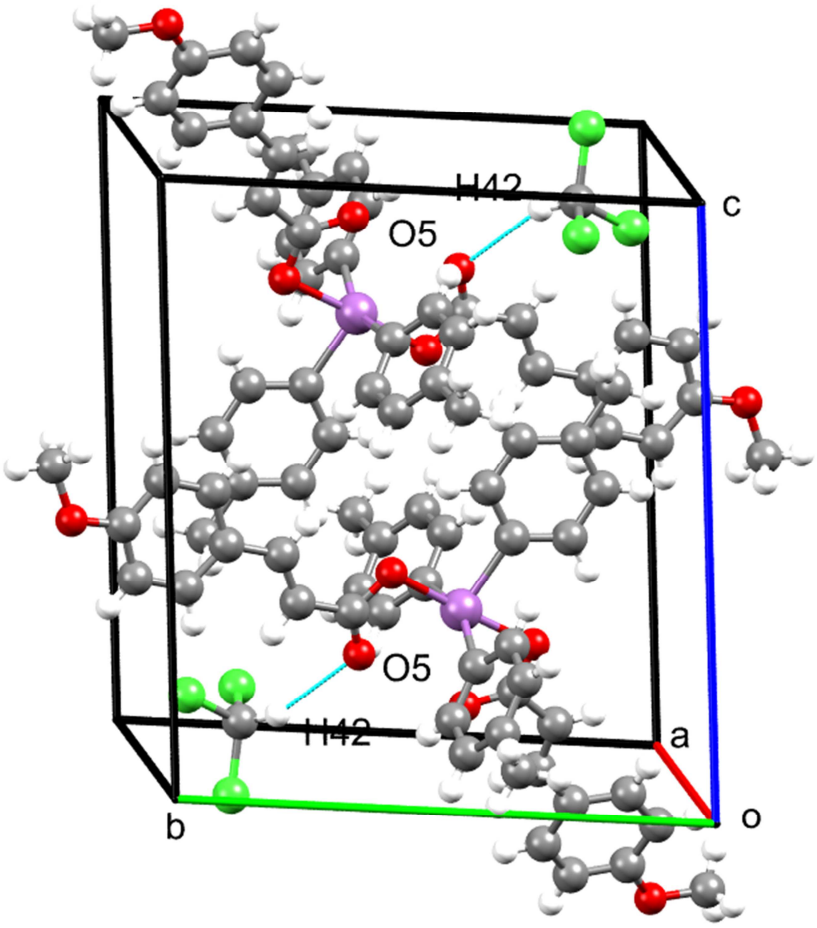


Fig. 4. Packing view of (**5**) along *c*-axis showing intermolecular (C-H···O) hydrogen bonding.

Crystal structures of complexes (**1**) and (**5**) displayed distorted geometry between trigonal bipyramidal and square pyramidal with monomeric structure at a five coordinated Sb center. Two ligand molecules are covalently bonded with antimony through the oxygen atom at axial positions, while three *p*-tolyl/phenyl groups are at equatorial positions. Two of the aromatic rings (phenyl/*p*-tolyl) constituting a wider angle at antimony centre C19-Sb1-C25=146.60° (**1**) and C21-Sb1-C28=155.67° (**5**) are oriented in the same plane while the third one is twisted at somewhat right angle. The two non-bonded oxygen atoms of the carboxylate groups are also bisecting the C-Sb-C angles around the antimony centre and causing a considerable distortion in C-Sb-C angle i.e. 120°, typical for ideal trigonal planar geometries. At the equatorial position of the molecule, summation of all the angles is almost 360.0°. Bond lengths of these complexes are comparable to the respective bond lengths of $[SbPh_3(O_2CCF_3)]_2O$ i.e. [2.100(6)–2.112(6) Å] [18,

32] Sb–O distances are 2.1088(16) Å and 2.1298(16) Å indicating the covalent mode of bonding between them, as their bond lengths are shorter than (3.6 Å) which is the sum total of the antimony and oxygen atoms van der Waals radii [30-31, 33-34].

In the crystal packing of **1** (Fig. **3**), the molecular structures have been stabilized by intermolecular C(11)-H(11)···O(4) 2.640 Å and C(17)-H(17)···O(2) 2.666 Å hydrogen bonds. These molecular structures also fashioned the centrosymmetric dimers $\{\cdots H\text{-}C\text{-}C{=}O\cdots\}_2$ connected by C–H···O (2.640 Å). The crystal packing of **5 (**Fig. **4)** shows only intermolecular interactions between the chloroform C-H and C=O.

Table 1. Crystallographic data and structure refinement for complexes **1** and **5**.

	1	**5**
Empirical formula	$C_{36}H_{27}Cl_2O_4Sb$	$C_{41}H_{39}O_6Sb.CHCl_3$
Formula weight	716.22	868.84
Temperature/K	296(2)	296(2)
Crystal system	Triclinic	Triclinic
Space group	P-1	P-1
a/Å	8.9509(10)	10.8119(12)
b/Å	12.5624(15)	12.6981(15)
c/Å	15.9537 (17)	14.5631(17)
α/°	109.587	85.769(5)
β/°	104.397(4)	82.265(5)
γ/°	95.536(4)	86.316(5)
Volume/$Å^3$	1604.9(3)	1972.8(4)
Z	2	2
$\rho_{calc}g/cm^3$	1.482	1.463
F(000)	720.0	884.0
Crystal size/mm^3	0.40×0.36×0.32	0.38×0.32×0.22
2Θ range for data collection/°	4.784 to 54	5.032 to 54
Reflections collected	24387	30656
Independent reflections	6968 [R_{int} =0.0313,	8501 [R_{int} = 0.0272,

	R_{sigma} = 0.0271]	R_{sigma} = 0.0247]
Data/restraints/parameters	6968/0/338	8501/0/474
Goodness-of-fit on F^2	1.038	1.059
Final R indexes [I>=2σ (I)]	R_1 = 0.0229, wR_2 = 0.0562	R_1 = 0.0258, wR_2 = 0.0617
Final R indexes [all data]	R_1=0.0266, wR_2 = 0.0588	R_1 = 0.0306, wR_2 = 0.0649
Largest diff. peak/hole / e $Å^{-3}$	0.25/-0.40	0.38/-0.44

Table 2. Selected geometric parameters; bond lengths (Å) and bond angles (°) for **1** and **5**.

		1	
Sb1-C19	2.1142(18)	C19-Sb1-C25	146.58(8)
Sb1-C25	2.1187(18)	C19-Sb1-C31	107.18(7)
Sb1-C31	2.1237(18)	C25-Sb1-C31	106.24(7)
Sb1-O3	2.1194(13)	C19-Sb1-O3	91.46(6)
Sb1-O1	2.1257(14)	C25-Sb1-O3	90.02(6)
O1-C1	1.301(3)	O3-Sb1-C31	88.54(7)
O2-C1	1.221(3)	C19-Sb1-O1	89.17(6)
O3-C10	1.318(2)	C25-Sb1-O1	91.25(6)
O4-C10	1.231(2)	C31-Sb1-O1	88.15(7)
		5	
Sb1-C28	2.1129(19)	C28-Sb1-C21	155.67(8)
Sb1-C21	2.119(2)	C28-Sb1-C35	103.53(8)
Sb1-C35	2.1215(19)	C21-Sb1-C35	100.80(8)
Sb1-O1	2.1429(14)	C28-Sb1-O1	90.42(6)
Sb1-O4	2.1517(14)	C21-Sb1-O1	90.27(7)
O1-C1	1.292(2)	C35-Sb1-O1	88.24(7)
O2-C1	1.232(3)	C28-Sb1-O4	89.39(6)

O4-C11	1.304(3)	C21-Sb1-O4	91.01(7)
O5-C11	1.226(3)	C35-Sb1-O4	89.20(7

3.3. Biological Activities

Results of bioassays showed that free acids **(L1-L3)** displayed negligible activities, but pentavalent antimony complexes **(1-5)** of these acids showed considerable activities.

3.3.1. Antileishmanial and antibacterial activities

Promastigotes stage is preferred for *in vitro* evaluation of leishmanicidal properties of compounds, as it is easy to culture and screening is done in a short time period [35]. Results of antileishmanial activity of acids precursors **(L1-L3)** and corresponding antimony (V) complexes **(1–5)** are shown in **Table 3**. In this work, MTT colorimetric assay was performed in order to check the antileishmanial properties of the compounds. The mitochondrial dehydrogenase enzyme of living cells causes the cleavage of the pale yellow tetrazolium ring of MTT into purple-blue formazan crystals, which are impermeable to cell membrane so, the number of viable cells can be reflected in this way. The results of the acid precursors **(L1-L3)** that are not bonded to antimony were poor relative to antimony (V) complexes, which revealed very good activities. The complexes **3**, **4** and **5** showed slightly ($p>0.05$) different results from amphotericin B indicating that their efficacy can be compared to it. The results depicted that the structure of the compound has strong influence on the activity as the complexes containing methyl (CH_3) group at para position exhibited excellent antileishmanial activity than the others. Since, the charge on metal decreases on complexation with donor groups so liphophilicity of compounds increases; thus, causing destruction of lipid membrane of microbe [36]. It is proposed that this activity might be due to inhibition of kinase enzymes or any other pathway, which interfere the growth of promastigotes. Advanced studies are in progress to find out the mechanism of action of these synthesized compounds. Since, these compounds are highly active against leishmaniasis; therefore, there is possibility to develop new and more effective drug for the treatment of leishmaniasis.

Antibacterial activity was performed against four strains of bacteria (*Escherichia coli*, *P. aeruginosa, S. aureus* and *K. pneumoniae*). Our compounds showed variable activity to different bacterial strains with significant antibacterial activity produced by compound **3** against all test

strains. Acid precursors **(L1-L3)** did not display any considerable activity against bacterial strains. Complexes with methyl groups showed better activity that may be due to enhanced membrane permeability. Antibacterial effect of compounds might have been triggered by interaction with cell wall, enzymes or proteins of bacteria.

3.3.2. Cytotoxicity against cancer cell line

Phenyl and tolyl antimony (V) complexes showed significant cytotoxicity against HepG2 cell line. **Table 3** shows the results for anticancer activity of starting acids **(L1-L3)** and synthesized antimony (V) complexes **(1-5).** Free acids depicted no activity, phenyl derivatives of antimony (V) **1** and **2** showed moderate activities, whereas *p*-tolyl derivatives of antimony (V) **3, 4** and **5** exhibited excellent activity. Increased number of methyl groups in complexes **3, 4** and **5** enhanced the cell membrane permeability of the compounds; hence, causing more cytotoxicity. Our results are consistent with previous studies where different derivatives of antimony (V) possess anticancer potential [37]. This indicates significant potential of antimony carboxylates as drug candidates to treat cancer.

Table 3. Anticancer and antileishmanial activities of the ligands (**L1-L3**) and their complexes (**1-5**).

Compounds	Anticancer activity		Antileishmanial activity	
	% inhibition (20 µg/mL)	IC50 µg/mL	% Mortality (20 µg/mL)	IC50 µg/mL
L1	0.0 ± 0.0	-	11.6 ± 0.75	-
L2	0.0 ± 0.0	-	9.6± 0.34	-
L3	0.0 ± 0.0	-	7.5 ± 0.01	-
1	48.1 ± 1.57	-	48.5± 1.20	-
2	35.6 ± 1.15	-	44.9± 1.15	-
3	96.7 ± 1.75*	6.19	99.5 ± 1.82*	1.58
4	98.2 ± 1.83*	4.61	99.6 ± 2.67*	2.05
5	100.00 ± 2.69*	5.36	98.5 ± 0.05*	0.03
Doxorubicin	100 ± 0.01*	0.05	NA	NA
Amphotericin B	NA	NA	100 ± 0.01*	0.01

Data is mean±SD; n=3; * means $p<0.05$, - means no activity; Na = not applied.

3.3.3. Alpha amylase inhibition activity

Results for enzyme inhibition assay are shown in **Table 4.** Maximum inhibition by our compounds was 13.51 % with 200 (µg/mL), which is negligible relative to standard acarbose (94.6%). Alpha amylase is carbohydrate degrading enzyme that plays important role in maintaining plasma glucose concentration [38]. However, our results are not significant in this aspect.

Table 4. Antibacterial and alpha amylase inhibition activities of the ligands (**L1-L3**) and their complexes (**1-5**).

Compound	Antibacterial activity ZOI (mm) 50 µg/disc								α-amylase inhibition (%) 200 µg /ml
	S. aureus	MIC µg/ml	*E. coli*	MIC µgml	*K. pneumoniae*	MIC µg/ml	*P. aeruginosa*	MIC µg/ml	
L1	-	-	-	-	-	-	8 ± 1.24	-	0.72 ± 0.005
L2	-	-	-	-	-	-	11 ± 1.97	-	1.68 ± 0.006
L3	-	-	13 ± 0.15*	1.85	-	-	8 ± 1.24	-	1.97 ± 0.007
1	-	-	-	-	-	-	-	-	4.8 ± 0.015
2	-	-	8 ± 0.07	-	-	-	-	-	7.6 ± 0.079
3	15 ± 0.17*	5.55	19 ± 0.71*	5.55	13± 0.75*	16.66	16 ± 0.75*	50	9.42 ± 0.96
4	-	-	-	-	21 ± 0.93*	50	16 ±0.75*	50	5.63 ± 0.045
5	13 ± 0.5*	5.55	-	-	-	-	15 ± 0.83*	16.66	13.51 ± 0.75
Cefixime (10 µg/disc)	1.24*	1.11	NA	NA	NA	NA	29 ±1.23*	3.33	NA
Roxithromycin (10 µg/disc)	NA	NA	30 ± 0.25*	0.33	32 ± 0.75*	3.33	NA	NA	NA
Acarbose (250 µM)	NA	NA	NA	NA	NA	NA	NA	NA	94.6 ± 1.84 (IC50:4.5 µg/mL)

NA = not applied, (-) indicates no activity, ZOI = zone of inhibition, MIC = minimum inhibitory concentration.

4. Conclusions

Organoantimony (V) complexes of cinnamic acid derivatives were successfully synthesized and confirmed by FT-IR, NMR and XRD analysis. Crystal structures of complexes (**1**) and (**5**) revealed that these complexes exhibited distorted trigonal bipyramidal geometry. In solid state, these complexes are monomeric with Sb (V). Two carboxylate ligands attached to antimony has

unidentate binding mode, which is also confirmed by FT-IR data. Compounds possessed significant antileishmanial and anticancer activities that were comparable with standards amphotericin B and doxorubicin respectively. Result showed high potential para substitution of methyl in tolyl derivatives. Hence, pentavalent antimony complexes, particularly *p*-tolyl derivatives seem to be worthy candidates for further investigations for therapy of leishmaniasis and cancer.

Acknowledgements

Authors are highly thankful to Quaid-i-Azam University, Islamabad and the Higher Education Commission of Pakistan (Research Project no. 20-1826/NRPU/R&D) for financial support to carry out this work.

Supplementary material

The supplementary crystallographic data for this paper can be obtained from The Cambridge Crystallographic Data Centre by www.ccdc.cam.ac.uk/data_request/cif. CCDC numbers of the crystals are 1501847 and 1501848.

Conflict of interest

There is no conflict of interest in this study.

References

[1] C. Warren, Antimony in Medical History: An Account of the Medical Uses of Antimony and Its Compounds Since Early Times to the Present, 2002

[2] C. Hansell, All manner of antimony. Nat. Chem. 7 (2015) 88.

[3] F. Frézard, C. Demicheli, R. Ribeiro, Pentavalent antimonials: new perspectives for old drugs. Molecules. 14 (2009) 2317-2336.

[4] H. Sun, Biological chemistry of arsenic, antimony and bismuth. John Wiley & Sons. 2010.

[5] C. Silvestru, I. Haiduc, E. R. Tiekink, D. de Vos, M. i. Biesemans, R. Willem, M. Gielen, Synthesis, structural characterization and in vitro antitumour properties of

triorganoantimony (V) disalicylates: Crystal and molecular structures of [5-Y-2-(ho)-$C_6H_3COO]_2SbMe_3$ (Y-H, Me, MeO). Appl. Organomet. Chem. 9 (1995) 597-607.

[6] P. Sharma, D. Perez, A. Cabrera, N. Rosas, J. L. Arias, Perspectives of antimony compounds in oncology. Acta. Pharmacol. Sin. 29 (2008) 881-890.

[7] S. Wyllie, A. H. Fairlamb, Differential toxicity of antimonial compounds and their effects on glutathione homeostasis in a human leukaemia monocyte cell line. Biocem. Pharmacol. 71 (2006) 257-267.

[8] S. E. T. Borborema, R. A. Schwendener, J. A. O. Junior, H. F. de Andrade Junior, N. do Nascimento, Uptake and antileishmanial activity of meglumine antimoniate containing liposomes in Leishmania (Leishmania) major-infected macrophages. Int. J. Antimicrobs. Agents. 38 (2011) 341-347.

[9] K. Mahajan, N. Fahmi, R. V. Singh, Synthesis, characterization and antimicrobial studies of Sb (III) complexes of substituted thioimines. 46 (2007) 1221-1225.

[10] K. V. Domasevitch, N. N. Gerasimchuk, A. Mokhir, Organoantimony (V) cyanoximates: synthesis, spectra and crystal structures. Inorg. Chem. 39 (2000) 1227-1237.

[11] H. Mahalakshmi, V. K. Jain, E. R. T. Tiekink, Diphenylphosphinates of tributylantimony (V). Main Group Met. Chem. 23 (2000) 519-524.

[12] M. A. Said, K. Kumara Swamy, D. M. Poojary, A. Clearfield, M. Veith, V. Huch, Dinuclear and tetranuclear cages of oxodiphenylantimony phosphinates: synthesis and structures. Inorg. Chem. 35 (1996) 3235-3241.

[12] Y. Q. Ma, L. Yu, J. S. Li, Synthesis and biological activity of some triarylantimony dipyrazolecarboxylates. Heteroatom. Chem. 13 (2002) 299-301.

[13] J. Duffin, B. G. Campling, Therapy and disease concepts: the history (and future?) of antimony in cancer. J. Hist. Med. Allied Sci. 57 (2002) 61-78.

[14] E. R. Tiekink, Antimony and bismuth compounds in oncology. Crit. Rev. Oncol. Hematol. 42 (2002) 217-224.

[15] M. Barsukova-Stuckart, L. F. Piedra-Garza, B. Gautam, G. Alfaro-Espinoza, N. V. Izarova, A. Banerjee, B. S. Bassil, M. S. Ullrich, H. J. Breunig, C. Silvestru, Synthesis and biological activity of organoantimony (III)-containing heteropolytungstates. Inorg. Chem. 51 (2012) 12015-12022.

[16] S. S. Garje, V. K. Jain, Chemistry of arsenic, antimony and bismuth compounds derived from xanthate, dithiocarbamate and phosphorus based ligands. Coord. Chem. Rev. 236 (2003) 35-56.

[17] M. D. J. Hernandez, E. Luengas, M. Sharma, A. N. Camacho, J. Vazquez, L. R. Castillo, P. Sharma, R. Gutierrez, Antioxidant properties of organostibines against lipid peroxidation in homogenized rat brain. Asian. J. Chem. 25 (2013) 7941.

[18] M. I. Ali, M. K. Rauf, A. Badshah, I. Kumar, C. M. Forsyth, P. C. Junk, L. Kedzierski, P. C. Andrews, Anti-leishmanial activity of heteroleptic organometallic Sb (V) compounds. Dalton. Trans. 42 (2013) 16733-16741.

[19] F. Asghar, A. Badshah, A. Shah, M. K. Rauf, M. I. Ali, M. N. Tahir, , E. Nosheen, , R. Qureshi, Synthesis, characterization and DNA binding studies of organoantimony (V) ferrocenyl benzoates. J. Organomet. Chem. 717 (2012) 1-8.

[20] W. L. Armarego, C. L. L. Chai, Purification of laboratory chemicals, seventh ed., Butterworth-Heinemann: Burlington. 2013

[21] (a) M.Younas, S.A. Faseeh, S.M. Younas, Synthesis of *trans*- and *cis*-diphenyl-4-acrylic acid. J. Chem. Soc. Pak. 5(2) (1983) 89-92.

(b) W. J. Lile, R. C. Menzies, Covalency, co-ordination, and chelation. Part III". J. Chem. Soc. (1950) 617-621.

[22] L. Yu, Y. Q. Ma, R. C. Liu, G.-C. Wang, J. S. Li, G. H. Du, J. J. Hu, Synthesis, characterization and in vitro antitumor activity of some arylantimony ferrocenylcarboxylate derivatives and the crystal structures of $[C_5H_5FeC_5H_4C(CH_3)$-$CHCOO]_2Sb(C_6H_4F_4)_3$ and $[4\text{-}(C_5H_5FeC_5H_4)C_6H_4COO]_2Sb(C_6H_4F_4)_3$, Polyhedron. 23 (2004) 823-829.

[23] G. M. Sheldrick, A short history of SHELX. Acta. Crystallogr. Sect. A 64 (2008) 112-122.

[24] G. M. Sheldrick, Crystal structure refinement with SHELXL. Acta. Crystallogr. Sect. C. 71 (2015) 3-8.

[25] K. Khan, H. Fatima, M. M. Taqi, M. Zia, B. Mirza, Phytochemical and in vitro biological evaluation of Artemisia scoparia Waldst. & Kit for enhanced extraction of commercially significant bioactive compounds. J. Appl. Res. Med. Aromat. Plants. 2 (2015) 77-86.

[26] S. S. Ambrose, P. Solairaj, A. Subramoniam, Hepatoprotective activity of active fractions of Thespesia lampas Dalz and Gibs (Malvaceae). J. Pharmacol. Pharmacother. 3 (2012) 326-328.

[27] P. Kaushik, P. Goyal, In vitro evaluation of Datura innoxia (thorn-apple) for potential antibacterial activity. Indian J. Microbiol. 48 (2008) 353-357.

[28] A. Sharma, S. Chandraker, V. Patel, P. Ramteke, Antibacterial activity of medicinal plants against pathogens causing complicated urinary tract infections. Indian J. Pharm. Sci. 71 (2009) 136-139.

[29] J. S. Kim, C.S. Kwon, K. H. Son, Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. Biosci. Biotechnol. Biochem. 64 (2000) 2458-2461.

[30] R. Mushtaq, M.K. Rauf, M. Bond, A. Badshah, M. I. Ali, A. Nadhman, M.Yasinzai , M.N. Tahir. Appl. Organometal. Chem. 30 (2016) 465–472.

[31] M.K. Rauf, U. Shaheen, F. Asghar, A. Badshah, A. Nadhman, S. Azam, M. I. Ali, G. Shahnaz, M. Yasinzai, Arch. Pharm. Chem. Life Sci. 348 (2015) 1-13.

[32] L. Wen, H. Yin, W. Li, D. Wang, New organoantimony complexes with the isomers of chlorophenylacetic acid: Syntheses, characterizations and crystal structures of 1D polymeric chain, 2D network structure and 3D framework. Inorg. Chim. Acta. 363 (2010) 676-684.

[33] M. N. Gibbons, D. B. Sowerby, Reactions of $[SbR_3X_2]_2O$ with carboxylates and the crystal structures of $[SbPh_3(O_2CCF_3)_2]_2O$ and $[SbMe_3(O_2CCH_3)_2]_2O$. J. Organomet. Chem. 555 (1998) 271-278.

[34] R. Mushtaq, M.K. Rauf, M. Bolte, A. Nadhman, A. Badshah, M.N. Tahir, M. Yasinzai, K.M. Khan, Appl. Organometal. Chem. 31 (2017) 3606.

[35] P. Baiocco, G. Colotti, S. Franceschini, A. Ilari, Molecular basis of antimony treatment in leishmaniasis. J. Med. Chem. 52 (2009) 2603-2612.

[36] Z. H. Chohan, C. T. Supuran, A. Scozzafava, Metalloantibiotics: synthesis and antibacterial activity of cobalt (II), copper (II), nickel (II) and zinc (II) complexes of kefzol. J. Enzyme Inhib. Med. Chem. 19 (2004) 79-84.

[37] R. C. Liu, Y. Q. Ma, L. Yu, J. S. Li, J. R. Cui, R. Q. Wang, Synthesis, characterization and in vitro antitumor activity of some arylantimony ferrocenecarboxylates and crystal structures of $C_5H_5FeC_5H_4CO_2SbPh_4$ and $(C_5H_5FeC_5H_4CO_2)_2Sb$ $(4\text{-}CH_3C_6H_4)_3$. Appl. Organomet. Chem. 17 (2003) 662-668.

[38] R. Subramanian, M. Z. Asmawi, A. Sadikun, In vitro alpha-glucosidase and alpha-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. Acta. Biochim. Pol. 55 (2008) 391-398.

Highlights

►The antimonials were investigated by CHN, FT-IR, NMR & single crystal XRD analysis

►The pentavalent antimonials (**1-5**) exhibit a multitude of medicinal properties

►Leishmanicidal activity assessed against the Promastigotes stage of Leishmania parasite

►The IC_{50} values were comparable with the standard drugs used

►*p*-Tolyl antimonials have shown significant antileishmanial and anticancer activities