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In vitro anti-leishmanial activities and structure-activity relationship analysis of new antimony(III) complexes

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New fourteen antimony(III) complexes of general formula [SbL_nCl_3] (where n= 1 or 2, L =2-aminopyridine, 5-methyl-2-aminopyridine, 2-aminopyrimidine, 4,6-dimethoxy-2-aminopyrimidine, 2-benzyl-2-thiopseudeourea,2-guanidinobenzimidazole, 2-amino-5-thiol-4,6-dimethoxypyrimidine, 2-amino-5-(1H-tetrazol-5-ylthio)-4,6-dimethoxypyrimidine, N-2-pyrimidine, 2-piperidinecarboxamide, N-2-pyrimidine, 2-pyrrolidinecarboxamide, 2-amino-5-(1H-tetrazol-5-iltiyo)-4,6-dimethoxypyrimidine-2-pyrrolidinecarboxamide and N-2-pyrimidine, 5-chloro-2-thiophenecarboxamide**,** N-2-benzothiazol-2-pyrrolidinecarboxaamide, N,N-(1,2-phenyl)dipyrrolidine-2-carboxamide) have been synthesized and their anti-leishmanial activity have been assessed in vitro against *Leishmania tropica* promastigotes. The best complex, Sb(2-guanidinobenzimidazole)Cl_3 is demostrated 3.16% growth inhibition at a concentration of 31.25 µg/mL. In general, antimony(III) complexes containing pyrimidine ligands has showed higher anti-leishmanial activity than antimony(III) complexes bearing pyridine ligands, and electron-donating substituents decrease the anti-leishmanial activity. All complexes have been optimised with DFT/B3LYP/LANL2DZ method in the gas phase. Several descriptors are tested to find a quantitative correlation between anti-leishmanial activity and structural properties of the complexes by best multiple linear regression method. Good correlations are obtained with minimum net atomic charge for a C atom and maximum bond order of a Cl atom. The developed QSAR equation is internally validated.

Keywords: Antimony(III) complexes, Anti-leishmanial activity, Structure-activity relationship

Leishmaniasis is a deadly disease caused by parasitic protozoa of the genus Leishmania. The fatal version of the disease, Visceral leishmaniasis, causes about 1.5–2 million new cases per year[1]. According to the World Health Organization (WHO), leishmaniasis is the second most important neglected tropical disease, and can have a fatality rate as high as 100% within two years[2]. Despite the growing importance of this disease, very few studies have been carried out on the development of new antileishmanial drugs. In fact, antimony(IV) compounds, meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam) have been used in the treatment of leishmaniasis for over 50 years, but clinical resistance to these drugs has emerged.[3,4] Therefore, more efforts in leishmanial drug discovery are urgently needed todevelop cheap, safe and effective new drugs. Pentavalent antimonial are known to be prodrugs and they are reduced to active Sb(III) form inside the macrophage and parasite cell.[5-7] Additionally, antimony complexes have various potential usage in the field of health and pharmacy[8,9] such as anthelminthic,[10,11] antitrypanosomal,[12] antimicrobial[13] and anticancer[14-17] as well as antileishmanial[18-20] agents. Furthermore, antimony compounds are used popularly as catalysts in organic synthesis.[21-23]

Quantitative structure–activity relationship (QSAR) methods have been widely used for drug discovery, lead optimization and toxicity prediction vs. traditional QSAR studies pioneered by Hansch et al. is a useful approach to correlate the bioactivity of compounds with structural and physicochemical parameters.[24,25] In the literature, anti-leishmanial activity of organic compounds and Bi, Fe, Mn like metal complexes have been previously reported.[26-34] However, no QSAR equation was reported for anti-leishmanial activity of antimony(III) compounds.

Recently, we synthesized and characterized new fourteen antimony(III) complexes, [Sb(2-aminopyridine)$_2Cl_3$] (**1**), [Sb(5-methyl-2-

aminopyridine)$_2$Cl$_3$] (**2**), [Sb(2-aminopyrimidine)$_2$Cl$_3$] (**3**), [Sb(4-6-dimethoxy-2-aminopyrimidine)$_2$Cl$_3$] (**4**), [Sb(2-benzyl-2-thiopseudeourea)$_2$Cl$_3$] (**5**), [Sb(2-guanidinobenzimidazole)Cl$_3$] (**6**), **[**Sb**(**2-amino-5-thiol-4,6-dimethoxypyrimidine)$_2$Cl$_3$] (**7**), **[**Sb**(**2-amino-5-(1H-tetrazol-5-ylthio)-4,6-dimethoxypyrimidine)$_2$ Cl$_3$] (**8**), [Sb(N-2-pyrimidine, 2-piperidinecarboxamide) Cl$_3$] (**9**), [Sb(N-2-pyrimidine, 2-pyrrolidinecarboxamide) Cl$_3$] (**10**), [Sb(2-amino-5-(1H-tetrazol-5-iltiyo)-4, 6-dimethoxypyrimidine-2-pyrrolidinecarboxamide)Cl$_3$] (**11**), **[**Sb**(**N-2-pyrimidine, 5-chloro-2-thiophenecarboxamide)$_2$Cl$_3$] (**12**), [Sb(N-2-benzothiazol-2-pyrrolidinecarboxaamide)Cl$_3$] (**13**), [Sb(N,N-(1,2-phenyl)dipyrrolidine-2-carboxamide)Cl$_3$] (**14**).[35-37] In this study, all complexes were evaluated for their in vitro anti-leishmanial activity against promastigote forms of *Leishmania tropica*. All complexes were optimized at DFT/B3LYP/ LANL2DZ level in gas phase. Then, approximately 370 molecular descriptors (constitutional, topological, geometrical, electrostatic, and quantum-chemical) of the complexes were calculated. A quantitative structure activity relationship (QSAR) study was carried out to get more insights into parameter responsible for the anti-leishmanial activity.

Materials and Methods

All chemicals were purchased from Sigma-Aldrich, and used without further purification. The NMR spectra were recorded in d$_6$-DMSO in a Bruker Ultra shield 300 MHz spectrometer. Elemental analyses were determined on a LECO CHNS–932 auto elemental analysis apparatus. The molecular conductivities were measured with WTW Cond 330i. Infrared spectra were recorded using a Mattson 1000 FTIR Spectrometer, from 4000–400 cm^{-1} in KBr pellet. Liquid Chromatography Mass spectra were obtained by using a Paltform LC-MS with methanol-acetonitrile mixture as the solvent.

Synthesis and characterization of the complexes

All complexes were synthesized as described in literatüre.[35-37] Thus, methanolic solution of antimony(III) chloride was slowly added to methanolic solution of relate ligand in the mole ratio of 2:1 in hydrochloric acid, and the resulting solution was refluxed for 2 days at 60 °C. Then the mixture was concentrated to 1/3 of its initial volume and allowed to stand for crystallization at room temperature.

[Sb(2-aminopyridine)$_2$Cl$_3$] (1): Anal. Calcd. for $C_{10}H_{12}Cl_3N_4Sb$: C, 28.85; H, 2.91; N, 13.46. Found: C, 28.17; H, 2.75; N, 13.97 IR (KBr, ν/cm^{-1}): 3442 (ν NH$_2$), 1661(ν CC), 1621 (ν CN), 994(ν CH). LC-MS (MeOH): *m/z* [found (calcd)]: 418.34 (416.35) (M+2): ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 2.50 (2H, NH$_2$), 7.95 (H$_1$, Ar), 7.90 (H$_3$, Ar), 6.98 (H$_4$, Ar), 6.86 (H$_3$, Ar), m.p. 174-175 °C, yield: 58.82%.

[Sb(5-methyl-2-aminopyridine)$_2$Cl$_3$] (2): Anal. Calcd. for $C_{12}H_{16}Cl_3N_4Sb$: C, 32.43; H, 3.63; N, 12.61. Found: C, 33.02; H, 3.82; N, 12.10. IR (KBr, ν/cm^{-1}): 3429 (ν NH$_2$), 3040 (ν CH), 1668 (ν CC), 1625 (ν CN). LC-MS: m/z (calcd) = 444.40 (444.86) (M+). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 2.2 (3H, CH$_3$), 3.50 (2H, NH$_2$), 6.95 (H$_6$, Ar), 7.8 (H$_5$, Ar), 7.90 (H$_1$, Ar), m.p. 174-176 °C, yield: 87.12 %.

[Sb(2-aminopyrimidine)$_2$Cl$_3$] (3): Anal. Calcd. For $C_8H_{10}Cl_3N_6Sb$: C, 22.97; H, 2.41; N, 20.09. Found: C, 23.39; H, 2.71; N, 20.74. IR (KBr, ν/cm^{-1}): 3346 (ν NH$_2$), 1658 (νCC$_{ring}$), 1619 (νCN$_{ring}$), 990 (νCH$_{ring}$). LC-MS: m/z (calcd) = 420.32 (419.35) (M+1). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 5.40 (2H, NH$_2$), 8.40 (H$_1$, H$_3$, Ar), 6.75 (H$_2$, Ar), m.p. 170-171 °C, yield: 62.50 %.

[Sb(4-6-dimethoxy-2-aminopyrimidine)$_2$Cl$_3$] (4): Anal. Calcd. for $C_{14}H_{20}Cl_3N_4O_4Sb$: C, 26.77; H, 3.37; N, 15.61. Found: C, 27.11; H, 3.67; N, 15.96. IR (KBr, ν/cm^{-1}): 3391 (ν NH$_2$), 2940 (ν CH), 2909 (ν CH), 1684 (ν CC), 1656 (ν CN), 939 (ν CH). LC-MS: m/z (calcd) = 540.43 (539.15) (M+1). : ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 3.50 (2H, NH$_2$), 4.90 (3H, OCH$_3$), 5.70 (1H, Ar), m.p. > 400 °C, yield: 30.24 %.

[Sb(2-benzyl-2-thiopseudeourea)$_2$Cl$_3$] (5): Anal. Calcd. for $C_{16}H_{18}Cl_3N_4S_2Sb$: C, 34.40; H, 3.25; N, 10.03; S, 11.48. Found: C, 34.77; H, 3.18; N, 10.43; S, 11.97. IR (KBr, ν/cm^{-1}): 3305 (ν NH$_2$), 3194 (ν NH), 3085 (ν CH), 1637 (νCC$_{ar}$), 1385 (ν CH). LC-MS: m/z (calcd) = 560.59 (559.51) (M+1). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 4.50 (2H, CH$_2$S), 7.40 (5H, Ar), 9.30 (2H, NH$_2$) and 9.10 (1H, NH), m.p. > 400 °C, yield: 54.27 %.

[Sb(2-guanidinobenzimidazole)Cl$_3$] (6): Anal. Calcd.for $C_8H_9Cl_3N_5Sb$**:** C, 23.82; H, 2.25; N, 17.36. Found: C, 23.37; H, 2.02; N 17.13. IR (KBr, ν/cm^{-1}): 3430 (ν NH$_2$), 3333 (ν NH), 3063 (ν CH), 1689 (ν CC), 1629 (ν CN). LC-MS: m/z (calcd) = 404.08 (403.31) (M+1): ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 11.90 (1H, NH), 8.20 (1H, NH), 7.40 and 7.20

(4H, Ar), 6.40 (3H, NH_2 and NH), m.p. 217-218 °C, yield: 53.22 %.

[Sb(2-amino-5-thiol-4,6-dimethoxypyrimidine)$_2$Cl$_3$] (7): Anal. Calcd.for$C_{12}H_{18}Cl_3N_6O_4S_2Sb$: C, 25.28; H, 3.43; N, 13.61; S, 10.38. Found: C, 24.37; H, 3.74; N, 13.82; S, 11.03. IR (KBr, ν/cm^{-1}): 3466 (ν NH_2), 2941 (ν CH), 1653 (ν CN). LC-MS: m/z (calcd) = 603.09 (602.66) (M+1). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.65 (1H, SH), 7.00 (2H, NH_2), 3.66 (6H, CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6): 171.67, 170.44, 163.47, 71.83, 55.27, m.p. > 400 °C, yield: 35%.

[Sb(2-amino-5-(1H-tetrazol-5-ylthio)-4,6-dimethoxypyrimidine)$_2$Cl$_3$] (8): Anal. Calcd. for $C_{14}H_{18}Cl_3N_{14}O_4S_2Sb$:C, 22.77; H, 2.46; N, 26.55; S, 8.68. Found: C, 23.07; H, 2.79; N, 26.11; S, 9.04. IR (KBr, ν/cm^{-1}): 3431 (ν NH_2), 3332 (ν NH), 2922 (ν CH), 1701 (ν CC), 1649 (ν CN). LC-MS: m/z (calcd) = 779.03 (738.63) (M+ CH_3CN). ^{1}H NMR (300 MHz, DMSO-d_6): 15.60 (1H, NH), 7.20 (2H, NH_2), 3.80 (6H, CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 172.24, 171.11, 162.84, 156.33, 75.37, 54.92, m.p. > 400 °C, yield: 46%.

[Sb(N-2-pyrimidine-2-piperidinecarboxamide)Cl$_3$] (9): Anal. Calcd. for$C_{10}H_{14}Cl_3N_4OSb$: C, 27.65; H, 3.25; N, 12.90. Found: C, 28.11; H, 3.44; N, 13.18. IR (KBr, ν/cm^{-1}):3422 (ν NH), 1662 (ν CO), 1592 (ν CN), 1109 (ν CH). LC-MS: m/z (calcd) = 435.15 (434.36) (M+1). ^{1}H NMR (300 MHz, DMSO-d_6): 8.50 (1H, NH), 7.60 (2H, H), 6.80 (1H, H), 3.80 (1H, CH), 3.50 (2H, -CH_2), 2.00 (2H, CH_2) 2.90 (1H, -NH), 1.75 (2H, -CH_2), 1.60 (2H, -CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.03, 169.68, 158.86, 157.98, 110.83, 58.55, 43.94, 28.05, 21.61, 19.01, m.p. > 400 °C, yield: 84%.

[Sb(N-2-pyrimidine-2-pyrolincarboxamide)Cl$_3$] (10): Anal. Calcd. for$C_9H_{12}Cl_3N_4OSb$: C, 25.72; H, 2.88; N, 13.33.Found: C, 25.19; H, 2.97; N, 13.21. IR (KBr, ν/cm^{-1}):3368 (ν NH), 1649 (ν CO), 1637 (ν CN), 987 (ν CH). LC-MS: m/z (calcd) = 453.19 (420.33) (M+CH_3OH). ^{1}H NMR (300 MHz, DMSO-d_6): 9,40 (1H,-NH), 8,10 – 6.10 (3H, H_{ar}), 4,40 (1H, CH), 3.70 (2H, CH_2), 3.30 (2H, CH_2), 2.25 (1H, NH),1.90 (2H, -CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.70, 164.64, 160.33, 158.15, 110.55, 59.09, 46.08, 28.18, 23.56, m.p. > 400 °C, yield: 43%.

[Sb(2-amino-5-(1H-tetrazole-5-ylthio)-4,6-dimethoxypyrimidine-2-pyrrolinecarboxamide)Cl$_3$] (11): Anal. Calcd. for$C_{12}H_{16}Cl_3N_8O_3SSb$:C, 24.83; H, 2.78; N, 19.30; S, 5.52. Found: C, 24.12; H, 2.83; N, 19.78; S, 5.76. IR (KBr, ν/cm^{-1}):3462.90 (ν NH), 2955.31 (ν NH), 1639.91 (ν CO), 1547.55 (ν CN), 1450 (ν CC). LC-MS: m/z (calcd) = 580.29 (580.48) (M^+). ^{1}H NMR (300 MHz, DMSO-d_6): δ 15.80 (1H, NH), 11.02 (1H, NH), 4,20 (1H, CH), 4.00 (2H, CH_2), 3.70 (6H, OCH_3), 1.95 (1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.63, 162.57, 162.53, 50.48, 63.87, 45.55, 33.13, 28.34, m.p. > 400 °C, yield: 35%.

[Sb(5-chloro-N-(2-pyrimidine)-2-thiophencarboxamide)$_2$Cl$_3$] (12): Anal. Calcd. for $C_{18}H_{12}Cl_5N_6O_2S_2Sb$: C, 30.56; H, 1.71; N, 11.88; S, 9.06. Found: C, 30.79; H, 1.87; N, 11.26; S, 8.89. IR (KBr, ν/cm^{-1}): 3373.90(ν NH), 1667.84 (ν CO), 1627.92 (ν CN), 1536.33 (ν CC). LC-MS: m/z (calcd) = 708.08 (707.46) (M+1). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.58 (1H, NH), 8.30 (1H, H), 7.60 (1H, H), 7.25 (1H, H), 6.80 (2H, H), ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.28, 157.67, 157.18, 135.29, 133.88, 133.83, 128.11, 110.32, m.p. > 400 °C, yield: 80%.

[Sb(N-2-benzothiazole-2-pyrrolidinecarboxamide)Cl$_3$] (13):Anal. Calcd. For $C_{12}H_{13}Cl_3N_3OSSb$: C, 30.32; H, 2.76; N, 8.84; S, 6.74. Found: C, 30.26; H, 2.68; N, 8.62; S, 6.47. IR (KBr, ν/cm^{-1}): 3374 (ν NH), 3114 (ν NH), 1634 (ν CO), 1572 (ν CN). LC-MS: m/z (calcd) = 476.17 (475.43) (M+1).^{1}H NMR (300 MHz, DMSO-d_6): 9.60 (1H, NH), 7.80 (1H, H), 7.45 (1H, H), 7.35 (1H, H), 7.20 (1H, H), 4.40 (1H, CH), 3.75 (2H, CH_2), 3.20 (2H, -CH_2), 2.25 (1H, NH), 1.90 (2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d6): δ 169.64, 168.66, 143.32, 127.34, 126.44, 123.69, 122.91, 115.70, 59.20, 45.89, 28.15, 23.54, m.p. > 400 °C, yield: 78%.

[Sb(N,N-(1,2-phenyl)dipyrrolidine-2-carboxamide)Cl$_3$] (14): Anal. Calcd. For $C_{16}H_{22}Cl_3N_4O_2Sb$: C, 36.23; H, 4.18; N, 10.56. Found: C, 36.48; H, 4.07; N, 10.87. IR (KBr, ν/cm^{-1}): 2955 (ν NH), 1734 (ν CO), 1383 (ν CC), 1037 (ν CH). LC-MS: m/z (calcd) = 530.97 (530.49) (M^+).^{1}H NMR (300 MHz, DMSO-d_6): 10.10 (1H, NH), 9.00 (1H, NH), 7.20 (2H, -H), 7.00 (1H, H), 6.85 (1H, H),4.40 (1H, H), 3.70 (2H, H), 3,20 (2H, H), 2,30 (2H, NH), 1,90 (2H, H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.76, 169.59, 150.22, 149.83, 139.23, 132.88, 130.57, 124.61, 59.10, 53.48, 45.76, 28.37, 23.51, m.p. > 400 °C, yield: 68%.

***In vitro* leishmanial activity assay**

The method described by Östan et al. was used for determination of antileishmanial activity of the compounds.[38] Promastigotes of *Leishmania tropica*

were isolated from a patient from Aydın, Turkey using NNN medium. Promastigotes transferred to RPMI 1640 medium supplemented with 15% Fetal calf serum for mass cultivation. Compounds were diluted with DMSO transferred to the culture plates to obtain final concentrations of 250, 125, 62.5, 31.2, 15.6, 7.8 μg/mL. After 1 h at 37 °C, 150 μL of culture medium complemented with 1×10^6 parasites/mL, from a logarithmic phase culture, were added. DMSO and Glucantime were used as drug carrier control and positive control (20 μg/mL), respectively. All plates were incubated at 26 °C. After 24, 48 and 72 h; viable *L. tropica* promastigotes were identified and counted microscopically with a hemocytometer on the basis of their aspect and motility. After incubation samples of each well were subcultured in fresh medium for another 48 h without drugs. All tests were carried out in triplicate. All microscopic examinations were performed blindly by two investigators. MLC (The minimum lethal concentration) was determined to be the lowest concentration of drugs at which no motile cells were found. Growth inhibition (GI) % as calculated with respect to the growth control is as follows: $\%GI = (1- GR_{extract}/GR_{control}) X 100$.

Computational details

Quantum calculations were carried out using the B3LYP/ LANL2DZ (method/basis) by Gaussian 03W program.[36] All the structures were fully optimized. The absence of imaginary frequencies verified that all structures were true minima. The output files from Gaussian were transferred to the program CODESSA to calculate approximately 350 molecular descriptors (constitutional, topological, geometrical, electrostatic, quantum-chemical). For regression analysis, anti-leishmanial activity values were converted to negative logarithm scale ($pIC_{50} = - logIC_{50}$) and used as dependent variables. Calculated descriptor values were used as independent variables. Best Multiple Linear Regression method in CODESSA software was used to select of the descriptors, and to build the model equation. The "breaking point" rule was applied to determine the optimal number of descriptors in the model equations. This rule is based on the significant improvement of R^2 ($\Delta R^2 < 0.02–0.04$) with respect to the number of descriptors in the model. Consequently, two descriptors were used as independent variables in our models. The squared correlation coefficient (R^2), leave-one-out cross-validated squared correlation coefficient (R^2cv), the Fisher criteria (F), and standard error (s^2) were used as criteria for the stability and the robustness of the models. The obtained model was also validated with an internal validation method.

Results and Discussion

Structure of the complexes

Antimony(III) complexes given in Fig. 1, reported our previous studies,[35-37]were characterized using elemental analyses, LC-MS, FTIR, ^1H NMR spectroscopy and molar conductivity measurements. Their geometries optimized using DFT/B3LYP/ LANL2DZ level in gas phase were given in Fig. 2. It was found that all these complexes have square-pyramidal geometry.

In vitro anti-leishmanial activity

All complexes were tested in vitro for their antileishmanial activitiy against*Leishmania tropica* promastigotes with one reference drug, Glucantime (20 μg/mL), and the resultswere pictorized at Fig. 3. In general, all complexes showed weaker activity compared to reference drug Glucantime (2.76% growth inhibition at 20 μg/mL). Sb(2-guanidinobenzimidazole)Cl_3 (**6)** exhibited the best anti-leismanial activity with 3.16% growth inhibition at 31.25 μg/mL. Following this, complex **5** has second highest anti-leishmanial activity with 7.24 % growth inhibition at 31.25 μg/mL, and complex **3** has the third highest anti-leishmanial activity with 3.42 % growth inhibition at 62.50 μg/mL. Generally, carboxamide Sb(III) complexes **(9-14)** showed moderate antileishmanial activity, and they have lower activity than pyrimidine-Sb(III) complexes **(3, 4, 7, 8)**. Among the carboxamide complexes, Sb(N-2-pyrimidine,5-chloro-2-thiophenecarboxamide)$_2Cl_3$ (**12)** has the highest anti-leishmanial activity, and carboxamide complex bearing pyroline group (**9)** has more activity than that of bearing piperidine group (**10).** In addition, pyrimidine-Sb(III) complexes have more antileishmanial activity than pyridine-Sb(III) complexes, and electron-donating substituents decrease the activity.

QSAR analysis

We also performed a QSAR (quantitative structure activity relationship) analysis to find the common parameters correlating with antileishmanial activities of fourteen complexes. For this reason, LC_{50} values were determined by linear regression, relating the inhibition percentage. Obtaining molar antileishmanial activity values were converted to

negative logarithm scale (pLC_{50} = $-\log LC_{50}$) and used as dependent variables in regression analysis (Table 1). The most stable structure of all complexes calculated using DFT/B3LYP/LanL2DZ method was used to obtain average 370 descriptors with CODESSA (version 2.7.10) software.[40] BMLR (Best Multi Linear Regression) method embedded in CODESSA software was used for multilinear regression analysis.

Two-parameter equation calculated with BMLR method for 14 complexes is given in Table 1. In this table, X and ΔX are regression coefficients of the model equation and their standard errors, respectively. In this equation, r^2 is a measure of the fit of the

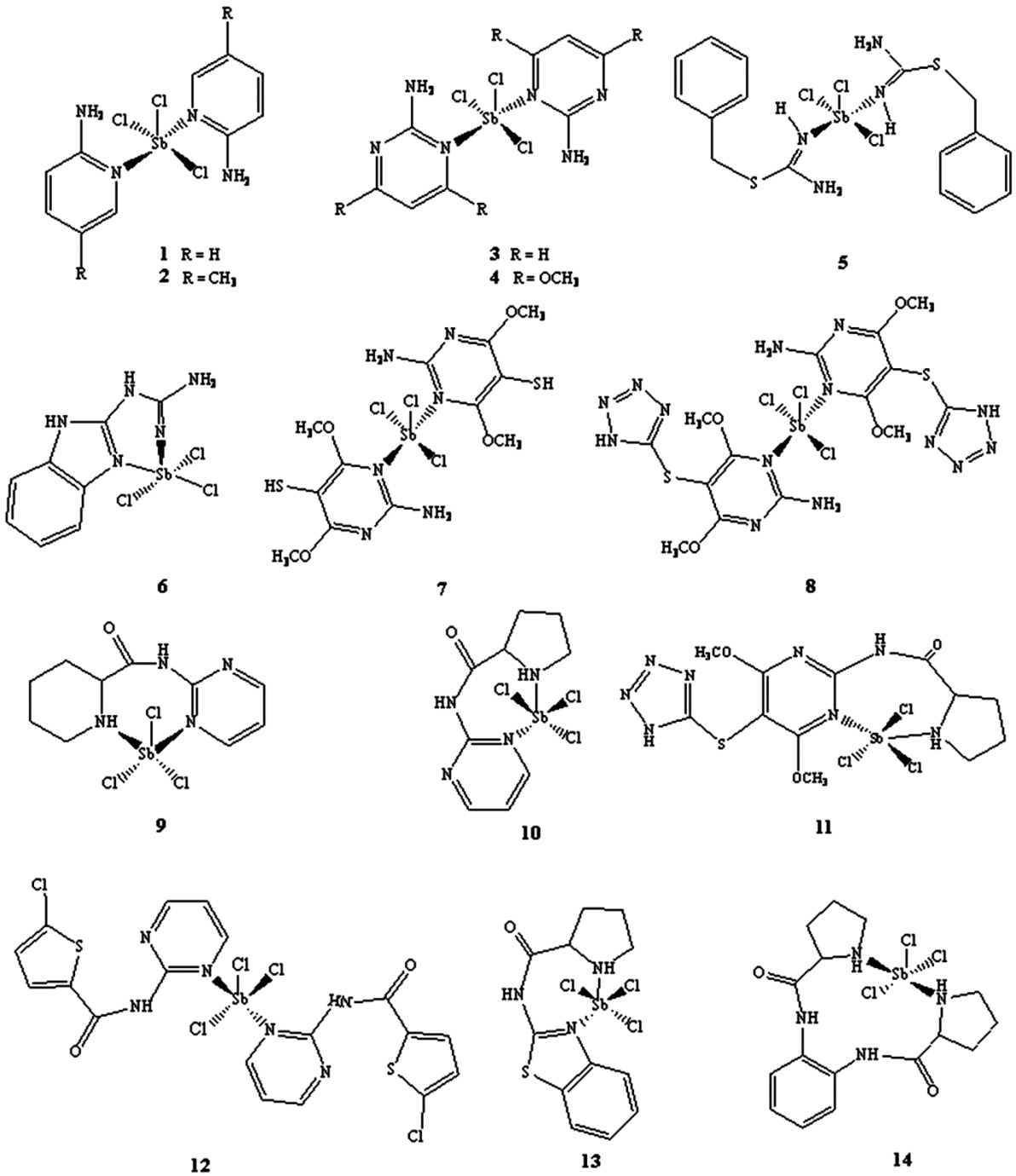

Fig. 1 — Structures of the synthesized antimony(III) complexes

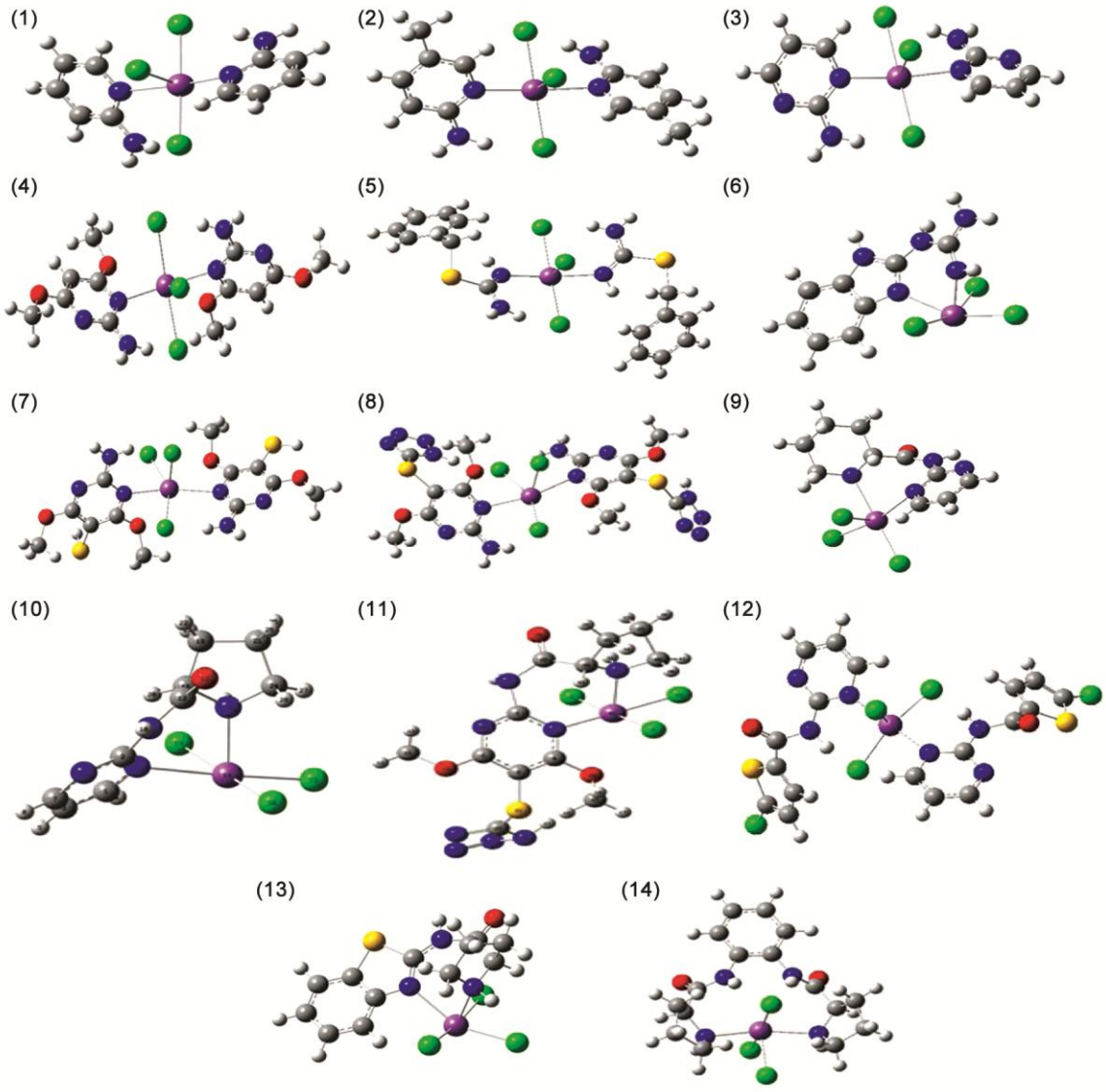

Fig. 2 — Ground state optimized structures of the complexes (1-14) calculated with B3LYP/LANL2DZ method

regression equation. s^2 is the standard deviation of the regression. Fisher test (F) corresponds to the ratio of the variance of the equation and the variance due to the error in the equation. Higher values of the F-test indicate the significance of the equation. r^2_{CV} is "leave-one-out" (LOO) cross-validated coefficient; it is a practical method for testing the predictive performance and stability of the regression equation.

In the model equation, the first descriptor is the "minimum net atomic charge for a C atom" (or the maximum negative atomic charge) obtained from the Mulliken charge distribution scheme of quantum chemical calculations.[41,42] It is related with electrostatic and Coulombic interactions. The positive regression coefficient of this descriptor indicates that their higher values is favorable for the antileishmanial activity of the compounds. Second descriptor is "maximum bond order of a Cl atom". This is a valency-related descriptor describing the strength of intramolecular bonding or multipole interactions of Cl atom.[43] It is inversely proportional to antileishmanial activity due to the negative signs of regression coefficient.

A graphical presentation of the relationship between the experimental and the predicted pLC_{50} values of the equation is given in Fig. 4. Observed and predicted pLC_{50} values, their difference and molecular parameters in the equation is

listed in Table 2. Correlation matrix of the descriptors involved in the equation is shown in Table 3. No significant correlation was found between two descriptors (-0.0045).

In addition to LOO method, we also used "internal validation" method given in Table 4. In this method, firstly, 14 compounds were categorized in rising order according to the pLC_{50} value, secondly, they were divided into three subsets A, B and C: the first, fourth, seventh structure, etc., formed subset (A); the second, fifth, eight, etc. formed subset (B); and the thirdly, sixth, ninth structure, etc. formed subset (C). Thirdly, three sets were prepared as the combination of two training subsets (A + B), (A + C) and (B + C). The remaining subsets (C, B and A, respectively) become the corresponding test sets. As seen in Table 4, R^2

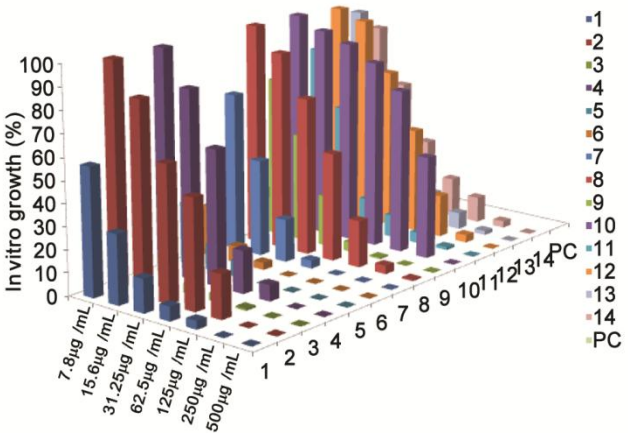

Fig. 3 — İn vitro antileishmanial activitiy of the complexes

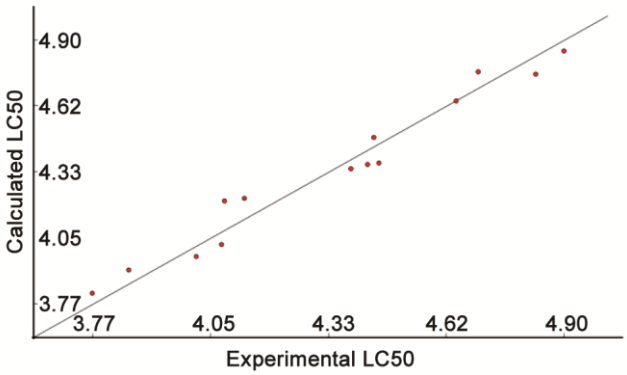

Fig. 4 — Correlation of observed and estimated pIC50

Table 1 — DFT-based two parameters QSAR model equation by BMLR method

	X	X	t-test	Descriptor
0	−1.5589	0.84300	−1.0659	Intercept
1	2.1132	0.33822	3.6944	Min net atomic charge for a C atom
2	−1.6275	0.32459	−2.4448	Max bond order of a Cl atom
n=14	R^2=0.9315	F=21.76	s^2=0.014	R^2_{cv}= 0.9315

Table 2 — The inhibitor activity and molecular parameters of the complexes

Compounds No.	Antileishmanial activity				Descriptors	
	LC50 (M)	p_{LC50} (calc)	p_{LC50} (exp)	$\Delta_{calc-exp}$	D1*	D2**
1	$2.19x10^{-5}$	4,7890	4.660	0,1290	−0.2897	0.7350
2	$1.40x10^{-4}$	3,9121	3.854	0,0581	−0.6477	0.7292
3	$1.47x10^{-5}$	4,7509	4.833	−0,0821	−0.3214	0.7377
4	$7.38x10^{-5}$	4,2187	4.132	0,0867	−0.4284	0.7165
5	$9.63x10^{-5}$	3,9679	4.016	−0,0481	−0.5089	0.7055
6	$1.26x10^{-5}$	4,8485	4.900	−0,0515	−0.2447	0.5959
7	$2.28x10^{-5}$	4,6336	4.642	−0,0084	−0.3832	0.6698
8	$8.24x10^{-5}$	4,2069	4.084	0,1229	−0.3524	0.7928
9	$3.50x10^{-5}$	4.3358	4.456	−0,1202	−0.4313	0.7243
10	$1.71x10^{-4}$	3,8129	3.767	0,0459	−0.4331	0.6976
11	$4.08x10^{-5}$	4,3440	4.389	−0,0450	−0.4276	0.7772
12	$8.39x10^{-5}$	4,0182	4.076	−0,0578	−0.2938	1.0550
13	$3.72x10^{-5}$	4,3636	4.429	−0,0654	−0.4405	0.6309
14	$3.60x10^{-5}$	4,4799	4.444	0,0359	−0.4263	0.7184

D1: minimum net atomic charge for a C atom
D2: maximum bond order of a Cl atom

Table 3 — Internal validation of the QSAR model

Training Set	N	R^2	R^2cv	F	s^2	Test Set	N	R^2
A+B	10	0.9733	0.9475	255.17	0.0068	C	4	0.9791
A+C	9	0.9825	0.9524	283.14	0.0079	B	5	0.9632
B+C	9	0.9864	0.9885	261.61	0.0236	A	5	0.9653

Table 4 — Correlation matrix of descriptors used in equation

Descriptors	D1	D2
D1	1.0000	−0.0041
D2	−0.0041	1.0000

values training set and predicting set demonstrate that the obtained equation has a satisfactory statistical stability and validity.

Conclusions

New fourteen antimony(III) complexes of general formula $[SbL_nCl_3]$ have been synthesized and their anti-leishmanial activities have been assessed in vitro against *Leishmania tropica* promastigotes. The best complex, Sb(2-guanidinobenzimidazole)Cl_3 isdemostrated 3.16% growth inhibition ata concentration of 31.25 μg/mL. All complexes have been optimized with DFT/B3LYP/LANL2DZ method in the gas phase.

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