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Antimicrobial activity of some isatin-3--thiosemicarbazone complexes

SANDRA S. KONSTANTINOVIĆ[1]*, BLAGA C. RADOVANOVIĆ[2], SOFIJA P. SOVILJ[3#] and SVETLANA STANOJEVIĆ[4]

[1]*Faculty of Technology, Bulevar Oslobođenja 124, 16000 Leskovac,* [2]*Department of Chemistry, Faculty of Science, Višegradska 33, 18000 Niš,* [3]*Faculty of Chemistry, University of Belgrade, P.O. Box 158, 11001 Belgrade and* [4]*Health Center, Biochemistry Laboratory, Svetozara Markovića 166, 16000 Leskovac, Serbia*

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Abstract: Isatin-3-thiosemicarbazone complexes with Co(II), Ni(II), Cu(II), Zn(II), Hg(II) and Pd(II) were synthesized and evaluated for their antimicrobial activity against 7 pathogenic bacteria and 4 fungi. The complexes have an enhanced activity compared to the ligand due to transition metal involved in coordination. The anti-amoebic activity *in vitro* was evaluated against the HM1:IMSS strain of *Entamoeba histolytica* and the results were compared with the standard drug, metronidazole. The preliminary test results showed that the complexes had better anti-amoebic activity than their respective ligands. Moreover, the complexes showed better inhibition of the test organism.

Keywords: isatin-3-thiosemicarbazone complex; antibacterial activity; antifungal activity; anti-inflammatory activity; anti-amoebic activity.

INTRODUCTION

Thiosemicarbazones are of considerable interest because of their chemistry and potentially beneficial biological activities, such as antitumor, antibacterial, antiviral and antimalarial activities.[1,2] Although thiosemicarbazones as ligands and their complexes are not unknown, there is not enough data about their antibacterial, antifungal and anti-amoebic activity. In the present study, the aim was to achieve a better biological profile at lower concentrations, by preparing and evaluating isatin-3-thiosemicarbazone and the corresponding complexes with Co(II), Ni(II), Cu(II), Zn(II), Hg(II) and Pd(II).

EXPERIMENTAL

Materials, methods and instruments

All chemicals used in the present work, *viz.*, isatin, thiosemicarbazide, metal chlorides and solvents were of analytical reagent (A.R.) grade (E. Merck).

* Corresponding author. E-mail: andjelkovic_s@yahoo.com
# Serbian Chemical Society member.
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General procedure for the preparation of isatin-3-thiosemicarbazone (ITC) and the complexes

Isatin-3-thiosemicarbazone (ITC) was prepared using a standard method.[3] The complexes were synthesized using the direct method between the ligand and the required metal(II) chloride (2:1 for Ni(II) Co(II)) and 1:1 molar ratio for Cu(II), Zn(II), Pd(II) and Hg(II).[3] The solutions were heated under reflux for 3–5 h and the products were filtered, washed with ethanol and dried *in vacuo* over $CaCl_2$ (Fig. 1).

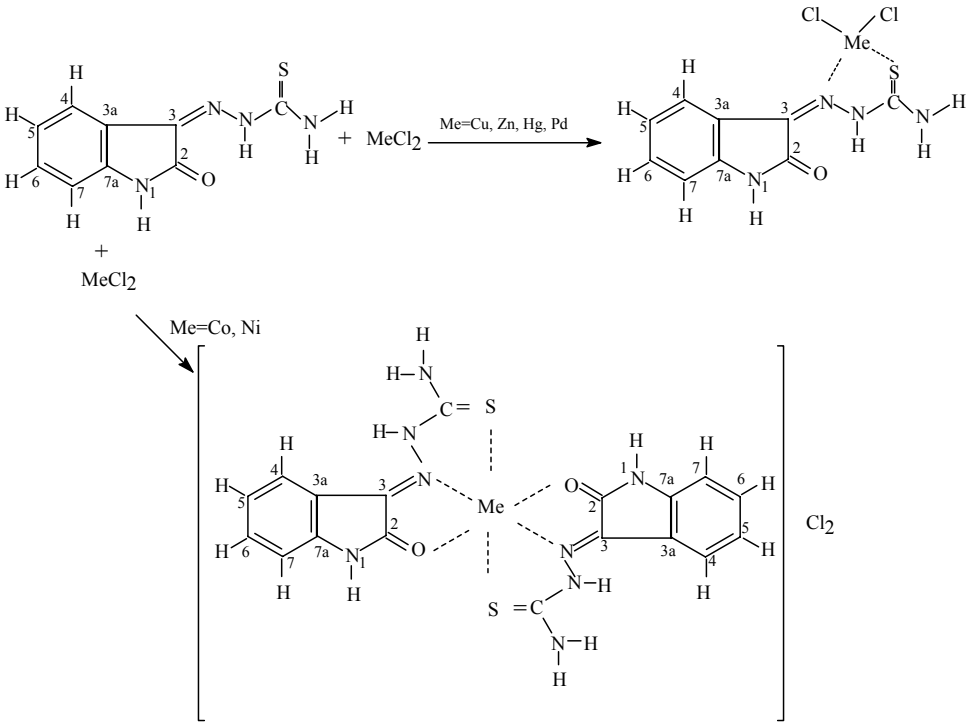

Fig. 1. Reaction scheme of the formation of the complexes.

Analytical measurements

Microanalysis for carbon, hydrogen and nitrogen was carried with a Carlo Erba 1106 microanalyzer. The chloride content was determined potentiometrically. The metal contents were determined using a Virial AA-457 double beam spectrometer. The FTIR spectra were recorded on a Michaelson Bomen MB-series spectrophotometer, using the KBr pellet technique (1 mg/100 mg). The electronic spectra were recorded on a Perkin/Elmer Lambda 15 UV/Vis spectrophotometer using 10^{-3} mol dm^{-3} solutions in DMF. The ^{1}H-NMR spectra were obtained in DMSO solution using a Gemini-200 "HF NMR" spectrometer. The magnetic susceptibility measurements were made at room temperature using an MSB-MKI magnetic balance (Sherwood Scientific Ltd.). The data were corrected for diamagnetism.

In vitro *antibacterial, antifungal and anti-amoebic activity*

The compounds were evaluated for their *in vitro* antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter* sp., *Proteus mirabilis*, *Bacillus anthracis*, *Pseudomonas aeruginosa* and *Streptococcus faecalis*. The minimum inhibitory concentration (MIC) was assessed by the agar dilution method according to guidelines established by the NCCLS

standard M7-A5.[4] A series of different concentrations of the compounds with Mueller Hinton broth was inoculated and incubated at 37 °C for 24 h. The minimum inhibitory concentration (MIC, in μg cm^{-3}) was considered to be the lowest concentration, which exhibited the same turbidity as the blank tube. The compounds were evaluated for their *in vitro* antifungal activity against *Microsporum gypsum*, *Epidermophyton floccosum*, *Histoplasma capsulatum*, *Candida albicans* and *Aspergillus niger* following the guidelines in the NCCLS document M27-A using the microdilution broth method.[5] The antifungal activities of the yeast were performed in RPMI 1640 medium as outlined in the document M27-A in DMF as solvent. The chemical compounds–broth medium serial tube dilutions inoculated with each yeast were incubated at 37 °C for 48–72 h. The anti-amoebic activities of the in vitro culture against the HM1:IMSS strain of *Entamoeba histolytica* was performed by the standard method.[6-9] The *E. histolytica* strain HM1:IMSS was cultured using Diamond TYIS-33 medium.[8] All the compounds were dissolved in DMF whereby the maximum concentration of DMF did not exceed 0.1 %, at which level no inhibition of amoebae growth occurred.[9,10] All the experiments were carried out in triplicate at each concentration level and repeated twice. The optical density of the resulting solution in each well was determined at 490 nm with a microplate reader. The per cent inhibition of amoebae growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the tested drug. Linear regression analysis was used to determine the best fitting straight line, from which the IC_{50} value was found.

RESULTS AND DISCUSSION

The analytical data for the prepared ligand and complexes are given below.

ITC. Yield: 91.1 %; yellow crystalline; m.p. 239–241 °C. Anal. calcd.: C, 49.08; H, 3.70; N, 25.32; S 14.56. Found: C, 49.05; H, 3.75; N, 25.30; S 14.51; FTIR (KBr, cm^{-1}): 1710 (C=O stretching), 1585 (C=N stretching), 1250, 854 (C=S); ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 6.91–7.64 (*m*, 4H, Ar), 8.69, 9.04 (*s*, 2H, NH_2), 11.21 (*s*, 1H, NH), 12.47 (*s*, 1H, NH); UV/Vis (λ (cm^{-1})/$\varepsilon_{max}\times10^{-3}$ (mol^{-1} dm^3 cm^{-1})): 28.6/0.946 ($\pi\rightarrow\pi^*$); 27.3/1.325 ($\pi\rightarrow\pi^*$).

$Co(ITC)_2Cl_2$. Yield: 54.52 %, dark red microcrystalline, m.p. 264 °C. Anal. calcd.: C, 42.30; H, 3.00; N, 21.70; Cl, 12.16; Co, 10.44. Found: C, 42.28; H, 2.95; N, 21.65; Cl, 12.12; Co, 10.45; FTIR (KBr, cm^{-1}): 1650 (C=O stretching), 1575 (C=N stretching), 1228, 838 (C=S). UV/Vis (λ (cm^{-1})/$\varepsilon_{max}\times10^{-3}$ (mol^{-1} dm^3 cm^{-1})): 19.5/0.817 ($^4T_{1g}(F)\rightarrow{}^4T_{1g}(P)$); 16.7/0.013 ($^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$); 14.7/0.005 ($^4T_{1g}\rightarrow{}^4A_{2g}$). μ_{eff} = 4.82 μ_B.

$Ni(ITC)_2Cl_2$. Yield: 47.89 %, brown microcrystalline, m.p. 298 °C. Anal. calcd.: C, 38.90; H, 2.54; N, 21.80; Cl, 12.16; Ni, 10.03. Found: C, 39.01; H, 2.50; N, 21.81; Cl, 12.15; Ni, 10.06. FTIR 1659 (C=O stretching), 1554 (C=N stretching), 1227, 814 (C=S). UV/Vis (λ (cm^{-1})/$\varepsilon_{max}\times10^{-3}$ (mol^{-1} dm^3 cm^{-1})): 22.2/1.223 ($^3A_{2g}\rightarrow{}^3T_{1g}(P)$); 18.7/0.226 ($^3A_{2g}\rightarrow{}^3T_{1g}(F)$); 13.5/0.02 ($^3A_{2g}\rightarrow{}^3T_{2g}(F)$). μ_{eff} = 3.39 μ_B.

$Cu(ITC)Cl_2$. Yield: 45.56 %, brown microcrystalline, m.p. 279 °C. Anal. calcd.: C, 36.90; H, 2.50; N, 18.50; Cl, 11.63; Cu, 19.53. Found: C, 36.87; H, 2.60; N, 18.39; Cl, 11.65; Cu, 19.56. FTIR (KBr, cm^{-1}): 1639 (C=O stretching); 554 (C=N stretching), 1237, 833 (C=S). UV/Vis (λ (cm^{-1})/$\varepsilon_{max}\times10^{-3}$ (mol^{-1} dm^3 cm^{-1})): 25.3/0.451 ($^2B_{1g}\rightarrow{}^2E_g$); 19.8/0.176 ($^2B_{1g}\rightarrow{}^2A_{1g}$); 13.4/0.02 ($^2B_{1g}\rightarrow{}^2B_{2g}$). μ_{eff} = 1.85 μ_B.

Zn(ITC)Cl$_2$. Yield: 55.56 %, yellow microcrystalline, m.p. 259 °C. Anal. calcd.: C, 30.30; H, 2.65; N, 15.17; Cl, 19.12; Zn, 18.28. Found: C, 30.35; H, 2.70; N, 15.13; Cl, 19.15; Zn, 18.25. FTIR (KBr, cm^{-1}): 1638 (C=O stretching); 1575 (C=N stretching); 1233, 838 (C=S). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 6.91––7.64 (*m*, 4H, Ar), 8.75, 9.11 (*s*, 2H, NH$_2$), 11.28 (*s*, 1H, NH), 12.41 (*s*, 1H, NH). UV/Vis (λ (cm^{-1})/$\varepsilon_{max}\times10^{-3}$ (mol^{-1} dm^3 cm^{-1})): 18.2/0.058 (CT), 17.1/0.055 (d→d*).

Hg(ITC)Cl$_2$. Yield: 68.89 %, orange powder, m.p. 292 °C. Anal. calcd.: C, 22.31; H, 1.59; N, 11.09; Cl, 14.83. Found: C, 22.32; H, 1.55; N, 11.05; Cl, 14.85. FTIR (KBr, cm^{-1}): 1638 (C=O stretching), 1570 (C=N stretching), 1228, 838 (C=S). ^{1}H-NMR (200 MHz, DMSO-d_6, δ, ppm): 6.91–7.65 (*m*, 4H, Ar); 8.77, 9.11 (*s*, 2H, NH$_2$); 11.28 (*s*, 1H, NH); 12.41 (*s*, 1H, NH). UV/Vis (λ (cm^{-1})/$\varepsilon_{max}\times10^{-3}$ (mol^{-1} dm^3 cm^{-1})): 24.5/3.435 (CT), 17.0/0.029 (d→d*).

Pd(ITC)Cl$_2$. Yield: 59.69 %, orange powder, m.p. 286 °C. Anal. calcd.: C, 28.71; H, 2.09; N, 13.39; Cl, 18.54. Found: C, 28.75; H, 2.06; N, 13.35; Cl, 18.56. FTIR (KBr, cm^{-1}): 1705 (C=O stretching), 1575 (C=N stretching), 1231, 834 (C=S). UV/Vis (λ (cm^{-1})/$\varepsilon_{max}\times10^{-3}$ (mol^{-1} dm^3 cm^{-1})): 19.5/0.817 (CT), 16.7/0.01 (d→d*).

The complexes were evaluated for their *in vitro* antimicrobial activity against 7 pathogenic bacteria and 4 fungi. The data for metal(II) chlorides, sulfamethoxazole, trimethoprim and clotrimazole are included for comparison. All compounds have different antibacterial activity *in vitro* against the tested gram-positive and gram-negative bacteria as well as the fungi. The obtained results of the coordination compounds show enhanced activity compared to the ligand, which indicates that the coordinated metal have an influence on the anitimicrobial effects. Since all complexes are soluble in DMF, the different activities cannot be correlated with different solubility. However, the higher activity of the complexes, as compared to the free ligand, can be understood in terms of the chelation theory. This theory explains that a decrease in the polarizability of the metal could enhance the lipophilicity of the complexes.[10] The complexes with Hg(II), Pd(II) and Zn(II) were the most active against all the tested bacteria and fungi (Tables I and II).

All the complexes showed a higher activity than sulfamethoxazole, but the ligand had the same activity against *B. anthracis* and *E. coli* and less activity against *Enterobacter* sp. All the compounds showed higher activity than trimethoprim against *S. aureus*, *B. anthracis* (except the ligand) and *P. aeruginosa* and lower activity against *E. coli*. The complex with Hg(II) showed better activity than trimethoprim against *P. mirabilis* (Zn(II) and Pd(II) complexes exhibited the same activity), while against *S. faecalis*, the Hg(II) and Pd(II) complexes exhibited higher activity, while the Zn(II) complex had the same activity as trimethoprim.

The antifungal activity of the compounds was studied against four pathogenic fungi (Table II). Clortrimazole was used as the reference for inhibitory activity against fungi.

All the compounds showed a significant antifungal activity, especially the Hg(II) complex, against all the tested fungi. When compared to clotrimazole,

Pd(II) exhibited higher activity against *M. gypsum*, while Zn(II) and Ni(II) complexes were equipotent against *M. gypsum*. The complexes with Co(II), Cu(II) and Pd(II) were equipotent against *A. niger*.

TABLE I. MIC Values (μg cm^{-3}) of the investigated compounds against the tested bacteria

Compound	*S. aureus*	*B. anthracis*	*Enterobacter* sp.	*E. coli*	*P. aeruginosa*	*P. mirabilis*	*S. faecalis*
ITC	1250	2500	1250	1250	2500	1250	1250
$Co(ITC)_2Cl_2$	78.12	39.06	312.5	312.5	156.25	312.5	156.25
$Ni(ITC)_2Cl_2$	78.12	312.5	156.25	156.25	625	312.5	156.25
$Cu(ITC)Cl_2$	156.25	156.25	625	625	156.25	312.5	312.5
$Zn(ITC)Cl_2$	39.06	39.06	156.25	156.25	156.25	156.25	78.12
$Hg(ITC)Cl_2$	<5	<5	<5	<5	<5	<5	<5
$Pd(ITC)Cl_2$	9.76	39.06	156.25	156.25	312.5	39.06	9.76
Sulfamethoxazole	2500	2500	625	1250	>5000	2500	2500
Trimethoprim	2500	2500	156.25	19.53	>5000	156.25	78.12
$CoCl_2 \cdot 6H_2O$	>5000	>5000	>5000	>5000	>5000	>5000	>5000
$NiCl_2 \cdot 6H_2O$	>5000	>5000	>5000	>5000	>5000	>5000	>5000
$CuCl_2 \cdot 2H_2O$	>5000	>5000	>5000	>5000	>5000	>5000	>5000
$ZnCl_2$	>5000	>5000	>5000	>5000	>5000	>5000	>5000
$HgCl_2$	156.25	625	156.25	625	625	625	625
$PdCl_2$	2500	5000	5000	2500	5000	2500	2500

TABLE II. MIC Values (μg cm^{-3}) of the investigated compounds against the tested fungi

Compound	*M. gypsum*	*E. floccosum*	*H. capsulatum*	*C. albicans*	*A. niger*
ITC	39.06	39.06	156.25	78.12	78.12
$Co(ITC)_2Cl_2$	9.76	19.53	39.06	9.76	2.44
$Ni(ITC)_2Cl_2$	4.88	1953	39.06	4.88	4.88
$Cu(ITC)Cl_2$	39.08	9.76	78.12	9.76	2.44
$Zn(ITC)Cl_2$	4.88	9.76	78.12	9.76	4.88
$Hg(ITC)Cl_2$	<0.3	<0.3	<0.3	<0.3	<0.3
$Pd(ITC)Cl_2$	2.44	4.88	78.12	2.44	2.44
Clotrimazole	4.88	2.44	19.53	0.3	2.44

The *in vitro* anti-amoebic activities of isatin-3-thiosemicarbazone and its complexes were tested using the HM1:IMSS strain of *E. histolytica* to ascertain the effectiveness of the metal complexes in comparison with their ligand. Metronidazole was used as the reference drug with an IC_{50} 1.8 μM and the results are given in Table III.

The results were estimated as the percentage of growth inhibition compared with the inhibited controls and plotted as probate values as a function of the drug

concentration. IC_{50} and 95 % confidence limits were interpolated in the corresponding dose response curve. Complexing enhances the activity of the ligand, which may be due to chelation, which reduces the polarity of the central metal atom because of partial sharing of its positive charge with the ligand. The Co(II), Zn(II) and Pd(II) complexes were more active than metronidazole with smaller IC_{50} values (1.44, 0.90 and 0.70 μM, respectively). The results were statistically evaluated by analysis of the variance. The null hypothesis was tested using the *t*-test. The significance of the difference between the IC_{50} values of metronidazole and the complexes with Co(II), Zn(II) and Pd(II) was evaluated by the *t*-test. The calculated *t* values were found to be higher than the table value of *t* at the 5 % level. Thus it can be concluded that the character under study was significantly influenced by the treatment. Detailed studies of the toxicity of these compounds, mechanism of action as well as *in vivo* studies are in progress.

TABLE III. *In vitro* anti-amoebic activities of isatin-3-thiosemicarbazone and its complexes against the (HM1:IMSS) strain of *E. histolytica*

Compound	IC_{50} / μM	*S.D.*[a]
ITC	3.90	0.14
$Co(ITC)_2Cl_2$	1.44	0.06
$Ni(ITC)_2Cl_2$	2.08	0.06
$Cu(ITC)Cl_2$	2.23	0.05
$Zn(ITC)Cl_2$	0.90	0.05
$Pd(ITC)Cl_2$	0.70	0.15
Metronidazole	1.80	0.10

[a]Standard deviation

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И З В О Д

АНТИМИКРОБНА АКТИВНОСТ НЕКИХ КОМПЛЕКСА СА ИЗАТИН-3-ТИОСЕМИКАРБАЗОНОМ

САНДРА С. КОНСТАНТИНОВИЋ[1], БЛАГА Ц. РАДОВАНОВИЋ[2], СОФИЈА П. СОВИЉ[3] и СВЕТЛАНА СТАНОЈЕВИЋ[4]

[1]Tehnolo{ki fakultet, Univerzitet u Ni{u, Bulevar Oslobo|ewa 124, 16000 Leskovac, [2]Prirodno-matemati~ki fakultet, Odsek za hemiju, Univerzitet u Ni{u, Vi{egradska 33, 18000 Ni{, [3]Hemijski fakultet, Univerzitet u Beogradu, p. pr. 158, 11001 Beograd i [4]Dom zdravqa, Biohemijska laboratorija, Svetozara Markovi}a 166, 16000 Leskovac

Синтетисана су координациона једињења Co(II), Ni(II), Cu(II), Zn(II), Hg(II) и Pd(II) са изатин-3-тиосемикарбазоном и испитивана је њихова антимикробна активност у односу на 7 патогених бактерија и 4 гљиве. Такође, *in vitro* је испитана активност једињења у односу на HM1:IMSS сој *Entamoeba histolytica*. Резултати показују да комплекси поседују бољу активност у односу на лиганд у свим изведеним тестовима.

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