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Synthesis, Characterization and Antiamoebic Activity of Benzimidazole Derivatives and Their Vanadium and Molybdenum Complexes

Neelam Bharti,[a] Shailendra,[a] M. T. Gonzalez Garza,[b] Delia E. Cruz-Vega,[b] J. Castro-Garza,[b] Kishwar Saleem,[a] Fehmida Naqvi,[a] Mannar R. Maurya[c] and Amir Azam[a,*]

[a]*Department of Chemistry, Jamia Millia Islamia, Jamia Nagar, New Delhi-110025, India*
[b]*Division de Biologia Celular of Molecular, Centro de Investigacion, Biomedica sdel Noreste, IMSS, Monetary, NL, Mexico*
[c]*Department of Chemistry, University of Roorkee, Roorkee-247667, India*

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Abstract—Reaction of $[MoO_2(acac)_2]$ (where, acac = acetyl acetone) and KVO_3 with 2-(salicylidieneimine) benzimidazole lead to form new complexes $[MoO_2(sal\text{-}BMZ)_2]$ and K $[VO_2(sal\text{-}BMZ)_2]$ [where, sal-BMZ = 2-(salicylidieneimine) benzimidazole], which showed the monobasic bidentate nature of the ligand in which the phenolic oxygen and the imine nitrogen of the ligand are coordinated to the metal ion. These complexes were characterized along with nine other complexes of oxoperoxovanadium (V), molybdenum (Vl) and tungsten (Vl) with benzimidazole derivatives and screened in vitro by micro dilution technique for their amoebicidal activity with a view to search for a more effective agent against *Entamoeba histolytica* suggests that compound **2** and **3** might be endowed with important antiamoebic properties since they showed IC_{50} values in a μM range. © 2002 Published by Elsevier Science Ltd.

Amoebiasis is the infection of the human gastrointestinal tract by *Entamoeba histolytica,* a protozoan parasite that is capable of invading the intestinal mucosa and can infect almost every organ of the body, the most frequent form of extra intestinal amoebiasis is the amoebic liver abscess. Being responsible for approximately 100,000 deaths annually, placing it second only to malaria in mortality due to protozoan parasite.[1] The ideal treatment for amoebiasis does not yet exist, mainly due to the toxicity of current antiamoebic drugs.[2] Hydroxy substituted benzimidazoles showed the most promising activity against enteroviruses, which have been attributed to their coordinating ability with metal ions.[3] 2-Aminobenzimidazole derivatives have been tested on some parasite models and showed protozoicidal and antihelmintic activities.[4] Vanadium (V) complexes are of interest in biochemistry because the heteroligand can shifts the redox potential of V(V)/V (IV) ($E_{298}{}^0 = 1.00$ v) towards the point where an intramolecular electron transfer could occur which reduces vanadium (V) to (IV) and oxidizes peroxo group to the superoxide radical. This superoxide ion generated in the process could trigger further biological events.[5,6] Although metals have been used in medicine for centuries, the success of *cis*-$PtCl_2(NH_3)_2$ (cisplatin) as an anticancer drug[7–9] has stimulated a renewed interest in metal based chemotherapy. It is desirable to continue the search for new, more specific antiamoebic drugs, which could provide a new dimension in therapy. The significant potentials of transition metal complexes as antiamoebic agents have so far been very little explored.[10–12] Approaches using vanadium, molybdenum and tungsten as antiparasitic agents has not yet been reported. The biological importance[6,13–16] of these metals led us to study, the synthesis and characterization of two new vanadium and molybdenum complexes of 2-(salicylideneimine) benzimidazole (**1**) along with nine previously reported compounds of vanadium, molybdenum and tungsten[3] with ligands (**II**) and (**III**) (Fig. 1) and they were tested in vitro against HM-1/1MSS strain of *E. histolytica*. To the best of our knowledge this is the first report of screening using these

*Corresponding author. Tel.: +91-11-683-1717-338; fax: +91-11-684-0229; e-mail: amir_jmi@rediff.com

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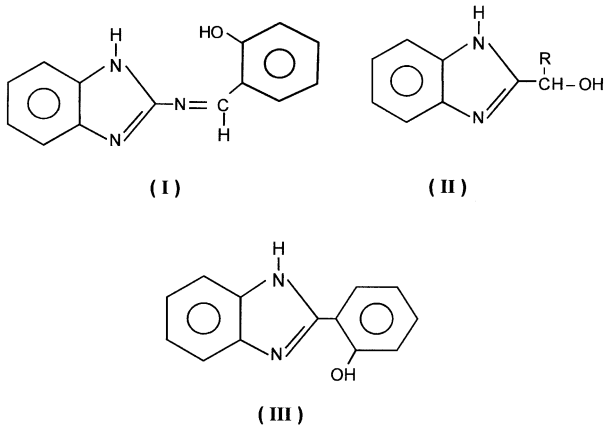

Figure 1. Structure of benzimidazole derivatives (where R = H, CH_3).

transition metal complexes with bezimidazole derivatives against amoebiasis.

Chemistry

2-(Salicylideneimine) benzimidazole was prepared by mixing salicyladehyde and 2-aminobenzimidazole in equimolar ratio in methanol and refluxed for 4 h. 2-(α-hydroxyalkyl) benzimidazoles and 2-(α-hydroxyphenyl) benzimidazoles were prepared by literature methods.[17,18] All these ligands were characterized by recording their mp, spectral studies and elemental analysis. Aqueous solution of KVO_3 reacts with potassium salt of Hsal–BMZ (prepared in-situ by the reaction of KOH) in 1:2 molar ratio at pH 7.5 to give dioxovanadium (V) complex, $K[VO_2\ (sal–BMZ)_2]$.[19] The dioxomolybdenum (VI) complex, $[MoO_2\ (sal–BMZ)_2]$.[20] was isolated according to eq. 1.

$$\left[MoO_2\ (acac)_2\right] + 2\ H\ sal-BMZ \rightarrow MoO_2\ (sal-BMZ)_2 + 2acacH \quad (1)$$

All the complexes are soluble in DMF and DMSO, sparingly soluble in methanol, ethanol and insoluble in water. Analytical and spectral data (IR, electronic and ^1H NMR spectra)[19,20] are in good agreement with the proposed structure. In IR spectra both complexes exhibit two IR active spectral bands in the 880–982 cm^{-1} region due to *cis*-MO_2 structure arising due to antisymmetric and symmetric stretching mode. A considerable higher frequency shift of ν(C = N) indicates the involvement of azomethine nitrogen in coordination. The retention of broad feature at 2650–2800 cm^{-1} and only slight shift of ν(C=N) (ring) from free schiff base (1620 cm^{-1}) in these complexes suggests the involvement of −NH nitrogen in hydrogen bonding and non-involvement of tertiary nitrogen (ring) in coordination. Coordination of phenolic oxygen is authenticated by the absence of any band in the 3400 cm^{-1} region. Thus, IR data indicates the monobasic bidentate nature of the ligand. Both complexes exhibit two bands in the electronic spectra, one in the UV region at ca. 285 nm and another at 389 nm (vanadium complex) or 337 nm (molybdenum complex) in the visible region. These are arising due to ligand and ligand to metal charge transfer band (LMCT), respectively. On the basis of spectral data an octahedral structure has been proposed for these complexes (Fig. 2). Other complexes (**5**–**7**, **9**, **10**, **12**) were synthesized and characterized by IR, electronic, ^1H NMR spectra and thermogravimetric analysis. Spectral and other physicochemical data compare well with the data reported in the literature.[6,18]

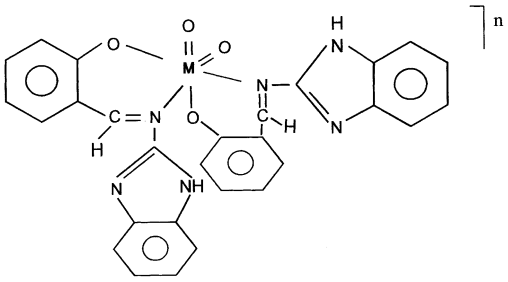

Figure 2. Proposed structure of dioxovanadium (V) ($n = -1$) and dioxomolybdenum (VI) ($n = 0$) complexes (M = V, Mo).

In vitro anti-amoebic activity

The anti-amoebic activity was assessed against HM-1/1MSS strain of *E. histolytica*. The trophozoites were maintained axenically in PEHPS medium[21] by serial culture. Trophozoites were used from 3-day axenic culture, grown in 16×125 mm screw capped tubes with 11 mL of PEHPS medium. The medium was sterilized for 15 min at 121 °C. Culture tubes containing 11 mL of PEHPS were inoculated with 1000 trophozoites mL^{-1} and incubated at 36 °C, the sub cultivation frequency was every 4 days. The drug potency test was performed as described earlier.[22] In brief, Borosilicate screw capped culture tubes containing 5.5 mL of fresh sterile PEHPS medium were added with 50 μL of different concentration of samples of metronidazole. In all determinations the metronidazole and sample concentrations were maintained in the ranges 0.0–3.0 and 0.0–50.0 μM/mL, respectively. Metronidazole was dissolved with phosphate buffer saline (PBS). All the compounds were dissolved in dimethyl sulphoxide (DMSO). The tubes were incubated for 72 h at 36 °C, cooled on ice water for 10 min and the number of trophozoites mL^{-1} in each tube determined with a haemocytometer. The results were estimated as the percentage of growth inhibition compared with the untreated controls and plotted as probit values as a function of the drug concentration. The IC_{50} and 95% confidence limits were interpolated in the corresponding dose–response curve. At least three experiments were performed for each compound tested.

The in vitro inhibitory effect of the compound (**1**–**12**) screened against *E. histolytica* upon the growth of axenic culture of HM1/1MSS strain are summarized in Table 1. The antiamoebic effect was compared with the most widely used antiamoebic medication metronidazole. Metronidazole had an 50% inhibition concentration (IC_{50}) of 2.10 μM in our experiment, which lies within the range of IC_{50} value of metronidazole (2.92

Table 1. In-vitro antiamoebic activity of benzimidazole derivatives, metal complexes and metronidazole against HM-1/1MSS strain of *E. histolytica*

S. No.	Compd	IC_{50} μM/mL	SD
1	2-(Salicylideneimine) benzimidazole	9.20	1.45
2	$K[VO_2\{$(salicylideneimine) benzimidazole$\}_2]$	2.35	0.72
3	$[MoO_2\{$(salicylideneimine) benzimidazole$\}_2]$	2.99	0.87
4	2-(2-Hydroxymethyl) benzimidazole	9.59	1.81
5	$[MoO\ (O_2)\{$(2-hydroxymethyl) benzimidazole$\}_2]$	4.19	0.54
6	$K\ [VO\ (O_2)\{$(2-hydroxymethyl) benzimidazole$\}_2]$	5.14	1.12
7	$[WO\ (O_2)\{$(2-hydroxymethyl) benzimidazole$\}_2]$	9.13	1.75
8	2-(2-Hydroxyethyl)benzimidazole	10.12	1.63
9	$[MoO(O_2)\{$(2-hydroxyethyl)benzimidazole$\}_2]$	5.16	0.94
10	$K[VO(O_2)\{$(2-hydroxyethyl)benzimidazole$\}_2]$	9.60	1.69
11	2-(2-Hydroxyphenyl) benzimidazole	11.52	1.97
12	$[MoO\ (O_2)\{$(2-hydroxyphenyl) benzimidazole$\}_2]$	8.55	1.37
13	Metronidazole	2.10	0.34

SD, standard deviation.

μM) as reported previously.[23,24] Table 1 shows that all the compounds screened for antiamoebic activity in vitro, 10 compounds had IC_{50} of less than 10 μM and two of these had IC_{50} less than 3 μM. As reported by the other researcher, the compounds, which showed IC_{50}, value less than 20 μM were considered as significant amoebicidal agents.[25] The activity of compound **2** and **3** showed that by introducing metal in organic moiety enhances the activity of the compound as they show the IC_{50} 2.35 and 2.99 μM, respectively. All the complexes are more active than their respective ligands, this indicates that the complexation to metal enhances the activity of the ligand. It is therapeutically more relevant to test potential new drugs on *E. histolyti*ca.

Acknowledgements

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19. Synthesis of $K[VO_2(sal–ABMZ)_2]$: V_2O_5(0.50 g, 5 mmol) was dissolved in aqueous KOH (0.30 g, 5 mmol in 10 mL) and stirred for 2 h. Hsal–ABMZ (2.37 g, 10 mmol) was also dissolved in aqueous KOH (0.6 g, 10 mmol in 20 mL) and added after filtering to the above solution with stirring. The pH value of the reaction mixture was adjusted to 7.5 with 4 M HCl. After 2 h of stirring, the light yellow solid was separated, filtered, washed with water and dried in vacuo. Yield 45% (found: C, 56.3; H, 3.6; N, 14.0. $C_{28}H_{20}KN_6O_4V$ requires C, 56.6; H, 3.4; N, 14.1%); λ_{max}/nm [$HOCN(CH_3)_2$] 285, 389; ν_{max}/cm^{-1} 1686, 1628 (C=N), 982 (VO_2 asym), 927 (VO_2 sym); ^1H NMR($(CD_3)SO$)/ppm 7.26 (16H, m, aryl); 11.32 (2H, s, NH); 8.10 (2H, s, HC=N).
20. Synthesis of $[MoO_2(sal–BMZ)_2]$: $[MoO_2(acac)_2]$ (0.33 g, 1 mmol) was added to a hot solution of Hsal-BMZ (0.48 g; 2 mmol) in 20 mL MeOH while stirring and the resulting reaction mixture was refluxed for 1 h. The light yellow solid, which separated, was filtered, washed with methanol and dried in vacuo. Yield 65% (found: C, 56.2; H, 3.2; N, 13.9. $C_{28}H_{20}MoN_6O_4$ requires C, 56.0; H, 3.3; N, 14.0%); λ_{max}/nm [$HOCN(CH_3)_2$] 283, 337; ν_{max}/cm^{-1} 1688, 1628 (C=N), 900 (MoO_2 asym), 880 (MoO_2 sym); ^1H NMR($(CD_3)SO$)/ppm 7.37 (16H, m, aryl); 11.59 (2H, s, NH); 8.27 (2H, s, HC=N).
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