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Research Article

Metal complexes of Nimesulide; synthesis, characterization and *in-vitro* biological screening

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ABSTRACT

Medicinal importance of metals and their compounds were recognised centuries ago but changed the direction of research of many drug discovery groups after the recognition of promising anticancer ability of Cisplatin. Now many of the metal based drugs have entered the phase of clinical trials. Discovery of new therapeutic moieties require huge funding and efforts, so derivatization of the already existing drugs is the need of the day to investigate their other pharmacological aspects. Keeping the same in mind zinc, copper, iron and antimony complexes of nimesulide were synthesized. These complexes were characterized by elemental analysis, FT-IR, ^{1}H and ^{13}C NMR spectral studies antibacterial and antifungal assays. Interestingly, the results of in-vitro biological screening of the synthesized complexes revealed that the metal complexes are more active than the parent drug nimesulide.

Key words: Metal Complexes, Nimesulide, IR and NMR spectroscopy, *In-vitro* Biological screening

INTRODUCTION

Nimesulide belongs to the sulphonamide class of non-steroidal anti-inflammatory drugs (NSAID)[1]. It is cyclooxygenase-2 inhibitor and clinically advised in the states of fever, pain and inflammation [2,3]. In addition to the above mentioned uses it is also recommended in vascular diseases, arthritic conditions, post-operative and cancer related pains [4]. Recently,syntheses of novel silver complex of nimesulide have been reported with enhanced antibacterial activity against Gram-negative and Gram-positive pathogenic bacterial strains[5]. Metallopharmaceuticals have been able to exhibit unique physical and chemical properties and potential therapeutic outputs. Metals have the capability to form stable complexes with many of the already known pharmacologically active moieties with enhanced properties [6]. Metals and their compounds have shown versatile medicinal properties throughout history and were used as antibacterial, antifungal, antiviral, antioxidant, anti-diabetic, anti-protozoal and anticancer agents [7]. Copper, zinc and iron are considered to be essential part of human body, required in specific amounts for proper growth and performance of all the vital functions of life smoothly. Their deficiency in body causes various disorders and excess may lead to toxic effects [8]. Incorporation of these trace minerals with pharmacologically active molecules have led to the further improvement of the pharmacological profile of the reacting biologically active molecule for example, copper complexes of various non-steroidal anti-inflammatory drugs have been reported as potential anti-inflammatory agents with better efficacy and reduced adverse effects [9]. There is a report in the literature showing synthesis, gastrointestinal toxicity and anti-inflammatory effects of copper and zinc complexes of Indomethacin with less toxicity and unaltered anti-inflammatory action [10]. Zinc upon complexation with drug Kefzol (beta-lactam antibiotic) have improved the potency of the drug [11]. Iron has also exhibited promising effects, when co-ordinated with therapeutic agents for instance its complexes has resulted in elevated antitumor activity of Adriamycin and daunorubicin [12]. Antimony and its compound are known for their anti-leishmanial effects, researchers are also

working to unveil its other medicinal specialities with focus in the discipline of oncology[13]. Recently antimony (III) chloride complexes with sulphonamides and Tosyl sulphonamide derivatives were reported with enhanced antibacterial activity[14]. Keeping in mind the above mentioned hypothesis that metal complexation will produce unique or enhanced biological properties, Cu (II), Zn (II), Fe (III) and Sb (III) complexes of nimesulide were synthesized, spectroscopically characterized and biologically assessed.

MATERIALS AND METHOD

Metal salts, reagents and other required chemicals were purchased from Sigma Aldrich. Glassware was washed and dried at 120°C. Nimesulide was obtained as a gift from pharmaceutical industry. FT-IR was done on using BrukerIR spectrophotometer (4000-400cm^{-1}).^{1}H, and ^{13}C nuclear magnetic resonance spectra were recorded on a Bruker AV400RG NMR spectrophotometer. Elemental analysis was performed using LECO-183 CHN analyser.

Synthesis of Copper complex (N-C)

Copper complex of ribavirin was synthesized using copper (II) acetate 1mmol solution in methanol. The solution of drug (Nimesulide) 1mmol was also made in methanol 15ml. The pH was optimized to 7 with the help of triethylamine. The solution was continuously stirred for four hours and then refrigerated overnight. The product was obtained after filtration, washed with methanol and dried in vacuum [15].

Synthesis of Zinc complex (N-Z)

10ml methanolic solutions of Zinc chloride 2mmol and Nimesulide 2mmol were prepared separately. Then the solution of the metal salt and the drug (nimesulide) were mixed together in a round bottom flask with continuous stirring. The mixture was heated to 60°C for one hour. The product was filtered after cooling down the reaction mixture. Resulting product was washed with ethanol and dried[16]

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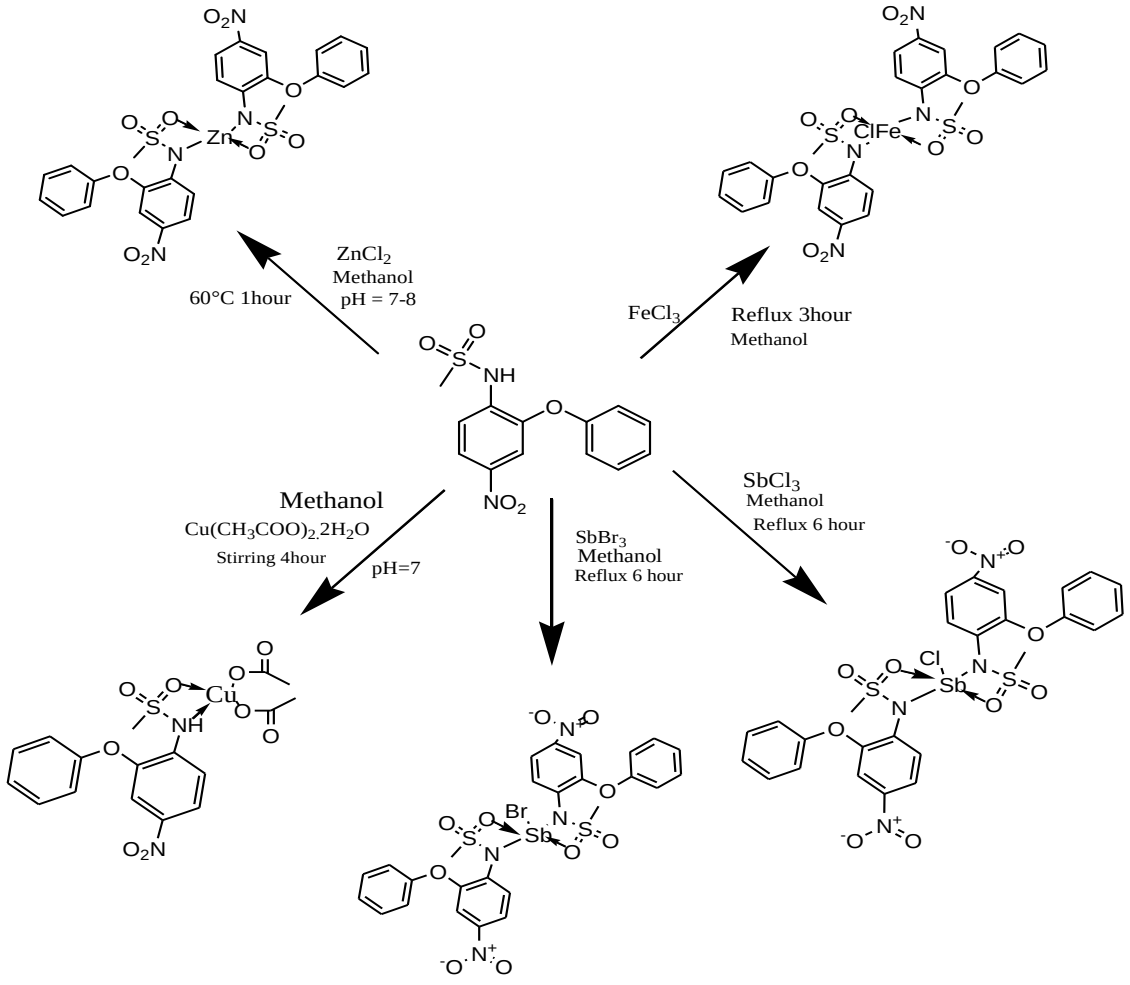

Figure 1:Synthetic scheme of Metal Complexes of Nimesulide.

Metal complexes of nimesulide withZn(II), CU(II), Fe(III) and Sb(III) were synthesized with the given protocols and their physiochemical characteristics are given below.

Nimesulide (N):
Yellow solid, melting point 147°C, IR spectroscopy*ν*(4000-400 cm^{-1}): *ν*(C-H) 3030s, *ν*(N-H) 3279s, *ν*(CH=CH) 1487m,*ν*(O=S=O, stretching) 1334s,*ν*(C-O) 1247s,*ν*(C-N) 1151m. 1H-NMR (400MHz, CDCl3) δ(ppm) 7.26m; 7.18m (−C6H5),7.54m; 7.16m; 6.76s (−C6H3NO2−), 3.12s (CH3SO2), 3.5s (−NH−) 13C NMR (100MHz, CDCl3)δ(ppm) 136.06(C-1), 119.99(C-2), 119.74(C-3), 143.56 (C-4), 112.87 (C-5), 147.92 (C-6), 155.5(C-7), 121.55(C-8, C-12) 130.8 (C-9,C-11) 125.34 (C-10) and 41.34 (C-13).

Cu (II) complex of Nimesulide (N-C)
Yield 70 %, green solid, melting point 180 °C, FT-IR *ν*(4000-400cm^{-1}): ν(CH=CH) 1488m, ν(O=S=O, Stretching), 1339s, ν(C−O) 1247s,ν(C−N) 1151m,ν(M-N) 517m, ^{1}H NMR (400MHz, $CDCl_3$)δ (ppm) 7.57m; 7.39m; 7.28m (−C_6H_5), 7.7d; 6.92d; 7.62s (−$C_6H_3NO_2$−), 3.03s (CH_3SO_2), 1.9s (CH_3CO).^{13}C NMR (100MHz, $CDCl_3$)δ(ppm) 154.58(C-1), 119.36 (C-2/6), 129.85 (C-3, C-5), 124.94 (C-4), 146.28 (C-7), 111.43 (C-8), 137.55 (C-9), 115.73 (C10) 118.33 (C11) 148.11 (C-12), 44.85 (C-13), 25.19 (C-14). Elemental Analysis for $C_{17}H_{18}CuN_2O_9S$: calculated/foundC(41.67/41.83),H(3.70/3.55),N(5.72/5.61)
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Zn (II) complex of Nimesulide (N-Zn)
Yield 77 %, off white solid melting point 230 °C FT-IR *ν*(4000-400cm^{-1}): ν(CH=CH) 1487m,ν(O=S=O,Stretching) 1315s, ν(C−O) 1246s,ν(C−N) 1150m,ν(M−N) 513m. ^{1}H NMR (400MHz, $CDCl_3$)δ (ppm) 7.5m; 7.44m; 7.22m(−C_6H_5), 8.01d;7.14d; 7.7s (−$C_6H_3NO_2$−), 3.19s (CH_3SO_2).^{13}C NMR (100MHz, $CDCl_3$)δ (ppm) 155.44 (C-1), 121.49 (C-2/6), 130.81 (C-3,C-5), 125.36 (C-4), 143.48 (C-7), 112.84 (C-8), 136.07 (C-9), 119.73 (C10) 119.97 (C11) 147.9 (C-12) and 41.30 (C-13). Elemental Analysis for $C_{26}H_{22}N_4O_{10}S_2Zn$: calculated/found C (45.92/4577), H (3.26/3.41), N (8.24/8.11).

Fe (III) complex of Nimesulide (N-Fe)
Yield 65 %, brown solid melting point 105°C, FT-IR *ν*(4000-400cm^{-1}): ν(CH=CH) 1480m,*ν*(O=S=O,Stretching) 1337s,*ν*(C−O) 1216s,*ν*(C−N) 1125m, *ν*(M−N) 526m, ^{1}H NMR (400MHz, $CDCl_3$)δ (ppm) 7.37m; 7.28m; 7.16m (−C_6H_5), 7.9d; 6.98d; 7.65s (−$C_6H_3NO_2$−), 3.1s (CH_3SO_2), ^{13}C NMR (100MHz, $CDCl_3$)δ(ppm) 155.41 (C-1), 121.56 (C-2,C-6), 130.78 (C-3,C-5), 125.31 (C-4), 143.58 (C-7), 112.8 (C-8), 136.1 (C-9), 119.8 (C10),120.05 (C11),147.85 (C-12) and 41.25 (C-13). Elemental Analysisfor $C_{26}H_{22}ClFeN_4O_{10}S_2$: calculated/found C (44.24/44.31), H (3.14/3.00),N(7.94/7.78).

$SbCl_3$ (III) complex of Nimesulide (N-Sb1)
Yield 83 %, white solid, melting point128-132°C, FT-IR (4000-400cm^{-1}): ν(CH=CH) 1483m,ν(O=S=O,Stretching) 1333s,ν(C−O) 1245s,ν(C−N) 1149m, ν(M−N) 514m.^{1}H NMR (400MHz, $CDCl_3$)δ(ppm) 7.26m; 7.41m; 7.46m (−C_6H_5), 8.04d; 7.16d; 7.75s (−$C_6H_3NO_2$−), 3.21s (CH_3SO_2), ^{13}C NMR (100MHz, $CDCl_3$) δ(ppm) 155.49 (C-1), 121.53 (C-2,C-6), 130.81 (C-3, C-5), 125.35 (C-4), 143.54(C-7), 112.86 (C-8), 136.05 (C-9), 119.74 (C10),120.0 (C11),147.91 (C-12) and 41.34 (C-13). Elemental Analysis for $C_{26}H_{22}ClN_4O_{10}S_2Sb$: calculated/found C (40.46/40.26), H (2.87/2.75), N(7.26/7.51).

$SbBr_3$ (III) complex of Nimesulide (N-Sb2)
Yield 78%, light brown solid, melting point 105-109°C. FT-IR (4000-400cm^{-1}) ν(CH=CH) 1487m,ν(O=S=O, Stretching) 1315s, ν(C−O) 1213s, ν(C−N) 1127m, ν(Sb−N) 511m, ^{1}H NMR (400MHz, $CDCl_3$)δ(ppm) 7.5m; 7.43m;7.23m (−C_6H_5), 8.01d; 7.13d; 7.7s (−$C_6H_3NO_2$−), 3.19s (CH_3SO_2), ^{13}C NMR (100MHz, $CDCl_3$)δ(ppm) 155.44 (C-1), 121.53 (C-2, C-6), 130.82 (C-3, C-5), 125.37 (C-4), 143.53 (C-7), 112.83 (C-8), 136.0 (C-9), 119.74 (C10),119.98 (C11), 147.9(C-12),41.38 (C-13). Elemental analysisfor $C_{26}H_{22}BrN_4O_{10}S_2Sb$:calculated/ found C(38.26/38.37), H (2.72/2.89),N(6.86/6.73).

IN-VITROANTIBACTERIAL ACTIVITY
In-vitro Antifungal activity of the synthesized complexes and the parent drug Nimesulide was carried by agar well disc diffusion method [19,20] keeping cefexime as positive control and DMSO as negative control. The activity was done against pathogenic strains of bacteria including Gram negative *Escherichia Coli* and Gram positive *Staphylococcus Aureus* using Nutrient agar medium (Merck).

ANTIFUNGAL ACTIVITY
Antifungal activity of the complexes was performed against the available fungal strains; *Aspergillus Flavous*, *Aspergillus Niger*, *Aspergillus Fumigatus*, *Fusarium Solani* and *MucorSP* using Terbinafine as positive control and DMSO as negative control. Antifungal activity was evaluated byfollowingthe tube diffusion method [21,22] using sabouraud dextrose agar (Merck).

RESULTS AND DISCUSSION

The difference in the physical states and melting points of the synthesized products and the parent drug nimesulidesupports the formation of the metal complexes. Further justification was achieved with the performed elemental analysis.The found values of CHN are well in range of the calculated values. The infrared vibrational spectra of metal, nitrogen bonds of different metal complexes have been studied and reported in the literature, The metal –Nitrogen vibration band is observed in the range 450-550 cm^{-1}. The presence of metal nitrogen bond signal in the expected range justifies the formation of the synthesized metal complexes. The first evidence is the disappearance of the strong*ν*(N-H) band due to the loss of hydrogen atom of parent drug upon coordination and the next is the shift of the vibrational bands to the lower energy values, which ultimately confirms the

complexation. In NMR the change in chemical shift signals of nimesulide and the synthesized complexes indicated the coordination of nimesulide to the metal ions. Observing the chemical shift valves presented above it is also possible to note that all the hydrogens are shifted upfield upon coordination of the metal to the drug. The difference in the IR and NMR spectra of the parent drug and the synthesized complexes confirms the formation of the metal complexes of Nimesulide. Antifungal and antibacterial results of the synthesized complexes are given below in table 1 and table 2 and their graphical illustrations are given in the figure 2 and figure 3 respectively. Interestingly few of the synthesized complexes of nimesulide have shown impressive antibacterial and antifungal activities.

Table 1: Zone of Inhibition in mm against bacterial strains

Samples	*Staphylococcus Aureus*	*Escherichia coli*
N	0	0
N-Zn	13	15
N-Cu	0	9
N-Fe	0	6
N-Sb1	15	12
N-Sb2	7	9
DMSO	0	0
Control	21	22

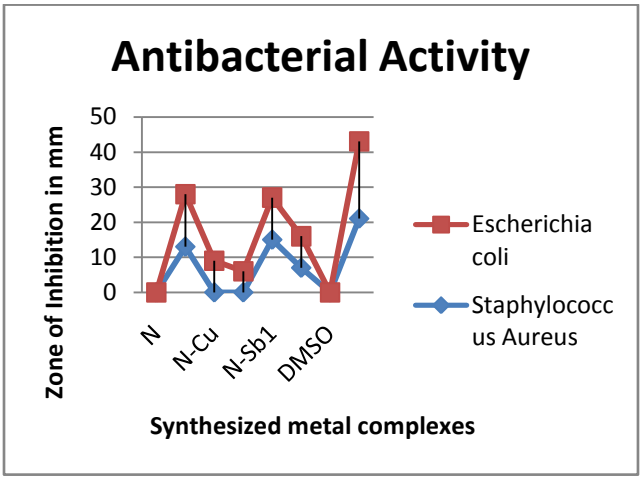

Figure 2: Graphical Illustration of Antibacterial activity of Synthesized Metal Complexes

Table 2: Zone of Inhibition in mm against Fungal Strains

Samples	*A.Flavous*	*A. Niger*	*F. Solani*	*A.Fumigatus*	*Mucor SP*
N	0	0	0	0	0
N-Zn	8	11	10	9	7
N-Cu	0	0	0	0	0
N-Fe	0	0	0	0	0
N-Sb1	0	0	0	0	0
N-Sb2	0	0	0	0	0
DMSO	0	0	0	0	0
Control	28	32	34	30	32

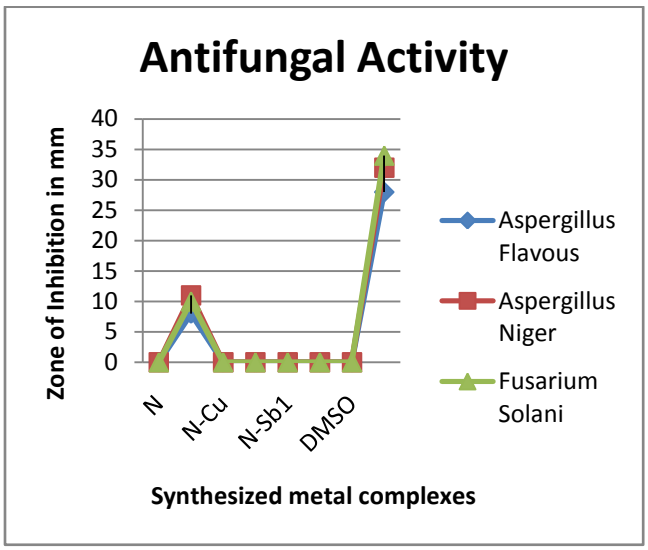

Figure 3: Graphical Illustration of Antifungal activity of Synthesized Metal Complexes

CONCLUSION

Zn(II), Cu(II), Fe(III) and Sb(III) complexes of nimesulide were successfully synthesized and characterized using various spectral techniques. Biological screening of the synthesized complexes proved to be fruitful as it has revealed that some of the complexes are more potent antibacterial and antifungal agents than the drug Nimesulide, which showed inert behaviour. Our future work involves the further investigation of the other pharmacological aspects of the synthesized metal complexes.

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