

## Research Article



## Synthesis, Characterization, *In-Vitro* Antibacterial and Antifungal Assays of Organotin (IV) Complexes of Nimesulide

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Accepted on: 25-06-2014; Finalized on: 31-08-2014.

### ABSTRACT

Organometallic chemistry is an emerging field combating diseases using unconventional perspectives, encompassing diagnostics and therapeutics, in healthcare. Physicochemical properties of organometallic complexes are quiet unique, which are now being used widely in medicinal inorganic chemistry. Results are quiet impressive as few organometallic complexes have entered clinical trials already. In foreseen future many more are expected to enter as well. Tin complexes of Nimesulide were efficiently synthesized and characterized by spectroscopic measures and biological assays. Infrared (IR), <sup>1</sup>H, <sup>13</sup>C nuclear magnetic resonance (NMR) and elemental analysis indicates the successful formation of the complexes. The point of interest is that some of the organotin complexes of nimesulide are showing antibacterial and antifungal activities on the other hand nimesulide is inert in this regard. However further investigation is needed to assess the other pharmacological and toxicological perspectives of the synthesized complexes which are included in our future research plan.

**Keywords:** Antifungal activity, Antibacterial activity, Metal-Complexes, Nimesulide, Organotin.

### INTRODUCTION

Nimesulide belongs to the group of non-steroidal anti-inflammatory drugs (NSAID).<sup>1</sup> It is widely used for the treatment of inflammation, pain and fever states.<sup>2</sup> It is well tolerated by adults, pediatric and elderly patients in clinical trials and found to at least as effective as other NSAIDs are in short term usage in lowering pain associated with cancer, osteoarthritis, thrombophlebitis, oral surgery and dysmenorrhea.<sup>3</sup> It was discovered in 1985 in Italy<sup>4</sup> but after 1990's it was found to be a selective cyclooxygenase-2 inhibitor (COX-2).<sup>5,6</sup>

Organotin metal complexes are now been widely studied due to their diverse applications as antibacterial, antifungal, antioxidant and cytotoxic agents.<sup>7,8</sup> Tin is linked with mankind from the beginning of the Bronze Age about 3500 BC, with its first use as copper-tin alloy to strength the copper. Since that time tin and its compounds have been utilized in variety of fields including agriculture, catalysis, art, medicine, industry and ecology.<sup>9</sup> After the discovery of cisplatin with its potential anti-tumor activity, several research group jumped into the investigation of possible therapeutic outcome of other metal-based, often organometallic, compounds and at the forefront of those were Organotin compounds.<sup>7,10</sup>

Physicochemical properties of organometallic complexes are quiet unique, which are now being used widely in medicinal inorganic chemistry. Results are quiet impressive as few organometallic complexes have entered clinical trials already.<sup>11</sup> Organotin compounds have gained an edge over other organometallics owing to their bioavailability in ecosystem and entrance into the

food chain, the fact they are less hazardous to the environment and their pharmaceutical applications including antitumor and anticancer uses.<sup>12,13</sup>

Recently novel triphenyltin(IV) complexes of NSAIDs have been synthesized and reported to be potential antitumor compounds.<sup>14</sup> Butyltin(IV) 2-sulfobenzoates and butyltin(IV) 5-sulfosalicylates metal complexes have been synthesized, characterized and found to be very active cytostatic agents against tumor strains as well as active antibacterial agents.<sup>15,16</sup> Present study emphasizes on synthesis and characterization of six Organotin complexes of nimesulide and their results against pathogenic strains of available bacteria and fungi.

### MATERIALS AND METHODS

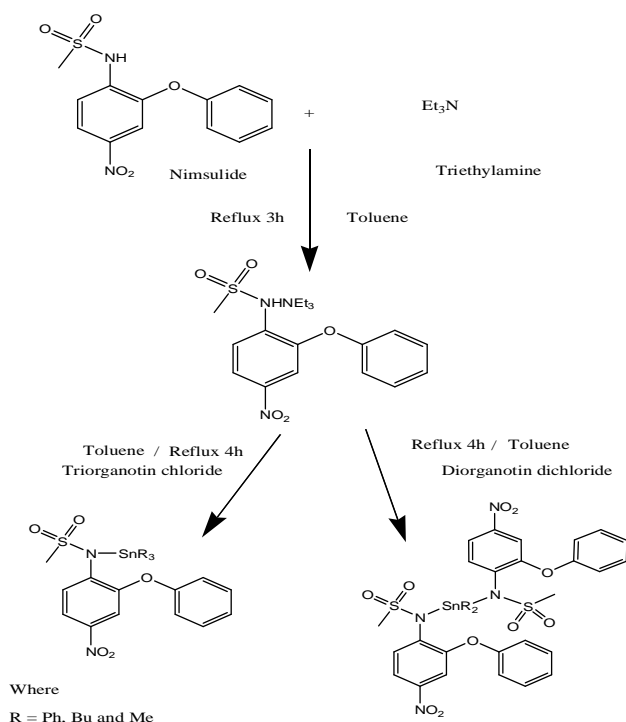
All the chemicals were of analytical grade and were purchased from sigma-Aldrich except Nimesulide, which was a gift from a pharmaceutical industry. All glassware with quick fit joints was used after cleaning and drying at 120C. Melting points were determined using Gallenkamp electrochemical melting point apparatus. Infrared (IR) spectra were measured using Bruker FT-IR spectrophotometer (4000-400cm<sup>-1</sup>). <sup>1</sup>H, and <sup>13</sup>C nuclear magnetic resonance spectra were recorded on a Bruker AV400RG spectrophotometer using CDCl<sub>3</sub> and DMSO as internal solvents and the chemical shifts were given relative to tetramethylsilane (TMS). Elemental analysis was recorded by LECO-183 CHN analyzer.

### General Procedure for Synthesis of Di/Tri Organotin Complexes

Complexes of nimesulide with dimethyltin dichloride, trimethyltin chloride, dibutyltin dichloride, tributyltin chloride, diphenyltin dichloride, triphenyltin chloride



were synthesized with the following scheme. Nimesulide (2mmol) was dissolved in dry toluene (50ml) in a round bottom flask fitted with the condenser for reflux. A stoichiometric quantity of triethylamine was added. This mixture was refluxed for 3-4 hours with constant stirring. Then the reaction mixture was cooled and triorganotin(IV) chloride (2mmol) or diorganotin(IV) (1mmol) was added with constant stirring and the reaction mixture was refluxed again for 6-8 hours. Then so obtained byproduct crystals were removed by filtration of reaction mixture and the synthesized metal complexes were isolated by evaporation. The obtained product was purified by re-crystallization from chloroform/pet.ether mixture (1:1).<sup>17-21</sup> The synthesis of the complexes is given in figure 1.



**Figure 1:** Scheme of synthesis of Organotin complexes

All the complexes were synthesized with the above given method; physical and chemical data of the complexes are given below with the data of Nimesulide (N).

### Nimesulide (N)

Yellow solid, melting point 147°C, IR  $\nu$  (4000-400 $\text{cm}^{-1}$ ): (C-H) 3030, (N-H) 3279, (CH=CH) 1487, (C-N) 1151, (C-O) 1247, (O=S=O) 1334. 1H NMR (CDCl<sub>3</sub>, ppm) 7.18-7.26m (-C<sub>6</sub>H<sub>5</sub>), 7.16-7.54m and 6.76s (-C<sub>6</sub>H<sub>3</sub>NO<sub>2</sub>-), 3.12s (CH<sub>3</sub>SO<sub>2</sub>), 3.5s (-NH-) 13C NMR (CDCl<sub>3</sub>, 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-8/12) 130.8 (C-9/11) 125.34 (C-10) and 41.34 (C-13).

**Complex N1** (Nimesulide complex with Diphenyltin dichloride)

Yield 78%, greenish solid; melting point 158°C, IR  $\nu$  (4000-400 $\text{cm}^{-1}$ ): (CH=CH) 1480, (C-N) 1151, (C-O) 1246, (O=S=O) 1332, (M-N) 477. 1H NMR (CDCl<sub>3</sub>, ppm) 6.82-7.24m

(-C<sub>6</sub>H<sub>5</sub>), 7.5-7.53m and 7.4s (-C<sub>6</sub>H<sub>3</sub>NO<sub>2</sub>-), 3.12s (CH<sub>3</sub>SO<sub>2</sub>), 7.1-7.15m (-C<sub>6</sub>H<sub>5</sub>) 13C NMR (CDCl<sub>3</sub>, ppm) 134.74(C-1), 118.25(C-2), 117.32 (C-3), 138.44 (C-4), 113.64 (C-5), 149.11 (C-6), 156.41(C-7), 123.94 (C-8/12) 129.06 (C-9/11) 124.78 (C-10), 41.34 (C-13) and 130.15 (C<sub>6</sub>H<sub>5</sub>). Elemental analysis for C<sub>38</sub>H<sub>32</sub>N<sub>4</sub>O<sub>10</sub>S<sub>2</sub>Sn: calculated/found C (51.42/51.25), H (3.63/3.65), N (6.31/6.43).

**Complex N2** (Nimesulide complex with Dibutyltin dichloride)

Yield 68%, brown solid, melting point 98°C IR  $\nu$  (4000-400 $\text{cm}^{-1}$ ): (C-H) 2957, 2857, (CH=CH) 1486, (C-N) 1127, (C-O) 1246, (O=S=O) 1339, (M-N) 478. 1H NMR (CDCl<sub>3</sub>, ppm) 7.28-7.45m (-C<sub>6</sub>H<sub>5</sub>), 7.07m, 7.79m and 8.03s (-C<sub>6</sub>H<sub>3</sub>NO<sub>2</sub>-), 1.84s (CH<sub>3</sub>SO<sub>2</sub>), 0.89-1.339m (-C<sub>4</sub>H<sub>9</sub>) 13C NMR (CDCl<sub>3</sub>, ppm) 133.97(C-1), 117.34(C-2), 111.85 (C-3), 143.64 (C-4), 110.61 (C-5), 146.36 (C-6), 154.13(C-7), 119.4 (C-8/12) 130.68 (C-9/11) 125.95 (C-10), 40.54 (C-13) and 13.7-32.85 (C<sub>4</sub>H<sub>9</sub>). Elemental analysis for C<sub>34</sub>H<sub>40</sub>N<sub>4</sub>O<sub>10</sub>S<sub>2</sub>Sn: calculated/found C (48.18/48.1), H (4.76/4.52), N (6.61/6.75).

**Complex N3** (Nimesulide complex with Dimethyltin dichloride)

Yield 80%, yellow solid, melting point 120°C IR  $\nu$  (4000-400 $\text{cm}^{-1}$ ): (C-H) 2927, (CH=CH) 1486, (C-N) 1150, (C-O) 1245, (O=S=O) 1334, (M-N) 476. 1H NMR (CDCl<sub>3</sub>, ppm) 7.48-7.71m (-C<sub>6</sub>H<sub>5</sub>), 7.15m, 8.01m and 7.54s (-C<sub>6</sub>H<sub>3</sub>NO<sub>2</sub>-), 3.19s (CH<sub>3</sub>SO<sub>2</sub>), 0.85s (-CH<sub>3</sub>) 13C NMR (CDCl<sub>3</sub>, ppm) 135.74(C-1), 117.5(C-2), 116.51 (C-3), 137.82 (C-4), 112.99 (C-5), 147.23 (C-6), 153.49 (C-7), 124.57 (C-8/12) 130.26 (C-9/11) 125.61 (C-10), 39.85 (C-13) and 5.01(CH<sub>3</sub>). Elemental analysis for C<sub>28</sub>H<sub>28</sub>N<sub>4</sub>O<sub>10</sub>S<sub>2</sub>Sn: calculated/found C (44.05/44.25), H (3.70/3.59), N (7.34/7.39)

**Complex N4** (Nimesulide complex with Triphenyltin chloride)

Yield 74%, yellowish green solid, melting point 179°C IR  $\nu$  (4000-400 $\text{cm}^{-1}$ ): (C-H) 3066, 2924, (CH=CH) 1481, (C-N) 1151, (C-O) 1247, (O=S=O) 1331, (M-N) 479. 1H NMR (CDCl<sub>3</sub>, ppm) 7.46-7.76m (-C<sub>6</sub>H<sub>5</sub>), 7.42m, 8.0m and 7.89s (-C<sub>6</sub>H<sub>3</sub>NO<sub>2</sub>-), 3.21s (CH<sub>3</sub>SO<sub>2</sub>), 7.50m (-C<sub>6</sub>H<sub>5</sub>) 13C NMR (CDCl<sub>3</sub>, ppm) 136.51(C-1), 118.95(C-2), 119.09 (C-3), 137.34 (C-4), 110.11 (C-5), 146.44 (C-6), 154.79(C-7), 126.47 (C-8/12) 129.76 (C-9/11) 125.56 (C-10), 40.31 (C-13) and 130.85 (C<sub>6</sub>H<sub>5</sub>). Elemental analysis for C<sub>31</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>SSn: calculated/experimental C (56.64/56.55), H (3.99/3.88), N (4.26/4.15)

**Complex N5** (Nimesulide complex with Trimethyltin chloride)

Yield 83%, yellow solid, melting point 127°C IR  $\nu$  (4000-400 $\text{cm}^{-1}$ ): (C-H) 2928, (CH=CH) 1486, (C-N) 1150, (C-O) 1244, (O=S=O) 1334, (M-N) 476. 1H NMR (CDCl<sub>3</sub>, ppm) 7.22-7.53m (-C<sub>6</sub>H<sub>5</sub>), 7.13m, 8.03m and 7.71s (-C<sub>6</sub>H<sub>3</sub>NO<sub>2</sub>-), 3.14s (CH<sub>3</sub>SO<sub>2</sub>), 0.66- 1.09m (-C<sub>4</sub>H<sub>9</sub>) 13C NMR (CDCl<sub>3</sub>, ppm) 137.86(C-1), 120.02(C-2), 119.73(C-3),

142.54 (C-4), 113.19 (C-5), 147.68 (C-6), 155.75(C-7), 121.14 (C-8/12) 130.73 (C-9/11) 125.04 (C-10), 41.24 (C-13) and 8.5-33.25 (C<sub>4</sub>H<sub>9</sub>). Elemental analysis for C<sub>16</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub>SSn: calculated/found C (40.79/40.66), H (4.28/4.45), N (5.95/5.83)

**Complex N6** (Nimesulide complex with Tributyltin chloride)

Yield 75%, light yellow solid, melting point 108°C IRv (4000-400cm<sup>-1</sup>): (CH=CH) 1487, (C-N) 1149, (C-O) 1245, (O=S=O) 1339, (M-N) 475. 1H NMR (CDCl<sub>3</sub>, ppm) 7.24-7.45m (-C<sub>6</sub>H<sub>5</sub>), 7.17m, 8.04 m and 7.74s (-C<sub>6</sub>H<sub>3</sub>NO<sub>2</sub>-), 3.19s (CH<sub>3</sub>SO<sub>2</sub>), 0.89s (-CH<sub>3</sub>) 13C NMR (CDCl<sub>3</sub>, ppm) 136.53(C-1), 119.94(C-2), 119.84 (C-3), 143.27 (C-4), 112.94 (C-5), 147.84 (C-6), 155.55(C-7), 121.43 (C-8/12) 130.79 (C-9/11) 125.28 (C-10), 28.20 (C-13) and 14.11 (CH<sub>3</sub>). Elemental analysis for C<sub>25</sub>H<sub>38</sub>N<sub>2</sub>O<sub>5</sub>SSn: calculated/found C (50.27/50.12), H (6.41/6.52), N (4.69/4.79)

### Antibacterial Assay

The synthesized complexes were screened against the pathogenic strains of bacteria including Gram positive *Staphylococcus Aureus* and Gram negative *Escherichia Coli* using potent antibiotic cefixime 20µg/disc as standard. In vitro, antibacterial assay was carried out by agar well diffusion method<sup>22-25</sup>. Complexes were screened three times and a mean of the observed values were considered to be reported.

Solutions of the metal complexes and the parent drug nimesulide were prepared with a concentration of 15mg/ml using dimethyl sulfoxide (DMSO). The bacterial strains were cultured and maintained at 4°C. Nutrient agar (Merck) was used to perform the activities. It was prepared 2% w/v concentration at around 7.0 pH at 25°C then autoclaved at 121°C for 15 minutes and cooled to 45°C and inoculated with standardized bacterial culture. 11 wells per petri plates (14cm) were made with sterile cork borer (8mm). Taking the help of micropipette 100µl of the solution was poured in the designated wells. The plates were incubated at 37°C for 24 hours and after 24 hours zone of inhibition of the synthesized complexes and the drug was calculated to determine the antibacterial activity.

### Antifungal activity

Antifungal assay of the synthesized complexes was carried out using *Aspergillus Flavous*, *Aspergillus Niger*, *Aspergillus Fumigatus*, *Fusarium Solani* and *Mucor SP*. Antifungal drug Terbinafine HCL was taken as standard and all the activities were performed three times and a mean of three readings was reported. Tube diffusion method<sup>26-28</sup> was used for antifungal assay using sabouraud dextrose agar (Merck) for maintain cultured strains at 27°C. Media was prepared by adding 32.5g sabouraud dextrose agar in 500 ml distilled water, dissolved with the help of steam and 5 ml of it was poured in each tube with screw cap. After labeling and

autoclaving the tubes were allowed to cool a measured amount of the sample solutions prepared in DMSO was added to them before solidification in slanting position. Tubes were inoculated and then incubated for 7 days at 27°C. Growth inhibition was calculated.

## RESULTS AND DISCUSSION

The difference in the range of melting points and physical appearances of the metal complexes and the drug nimesulide supports the formation of the complexes. IR spectra recorded in the range of 400-4000 cm<sup>-1</sup>, expected ranges and specific shapes of bands for different functional groups confirms the formation of the organometallic complexes of the nimesulide. The formation of the new metal nitrogen bond has a peak in the range of 450-550 cm<sup>-1</sup>, which furthermore verifies the formation of metal complexes. The C-N, CH=CH, C-O, S=O and M-N bands of all the synthesized complexes have appeared in predicted range and the disappearance of the N-H peaks in the range 3200-3400 cm<sup>-1</sup> helped us in evaluating the structural assignments.

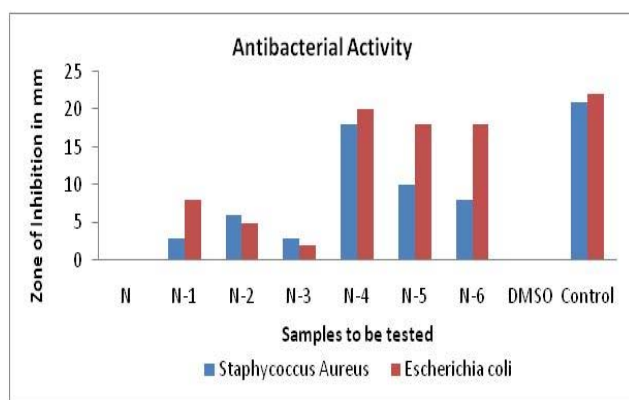
Evaluation of the <sup>1</sup>H and <sup>13</sup>C NMR is done for the structural elucidation of the metal complexes. The presences of the substituent's of Organotins have been justified by the distinguishable new peaks in NMR. In all the complexes -NH and -OH signals disappeared because the molecules undergoes a tautomeric change and coordinates with the Organotin (IV) moiety through aromatic imine nitrogen. Elemental analyses of the synthesized metal complexes are comfortably in margin of the calculated values.

The results of the antibacterial and antifungal activities are given below in table 1 and table 2 respectively. Complex N-4 has shown the highest antibacterial activity against both gram negative and gram positive bacteria within the synthesized products but less than the standard. In antifungal assay N-6 has shown impressive results, which are even better than the antifungal activity of the complex N-4 but again less potent than the efficacy of the standard drug, whereas the parent drug nimesulide has shown no activity in both the assays. Table 1 and table 2 represent the zone of inhibition in mm of the synthesized complexes against the bacterial strains and fungal strains whereas the graphical representations of the results are given in figure 2 and figure 3 respectively.

**Table 1:** Inhibitory effect against bacterial strains in mm

Samples	<i>Staphylococcus Aureus</i>	<i>Escherichia coli</i>
N	0	0
N-1	3	8
N-2	6	5
N-3	3	2
N-4	18	20
N-5	10	18
N-6	8	18
DMSO	0	0
Control	21	22



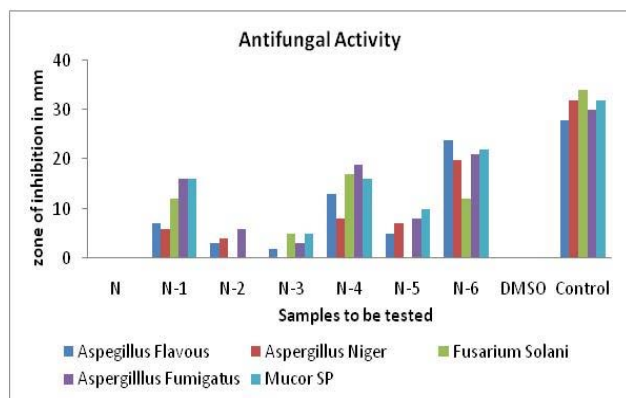


**Figure 2:** graph illustrating the antibacterial results of the parent drug and the synthesized complexes (N1-N6)

### Antifungal Activity

**Table 2:** Inhibitory effect against the fungal strains in mm

Samples	Aspergillus Flavous	Aspergillus Niger	Fusarium Solani	Aspergillus Fumigatus	Mucor SP
N	0	0	0	0	0
N-1	7	6	12	16	16
N-2	3	4	0	6	0
N-3	2	0	5	3	5
N-4	13	8	17	19	16
N-5	5	7	0	8	10
N-6	24	20	12	21	22
DMSO	0	0	0	0	0
Control	28	32	34	30	32



**Figure 3:** Graph representing antifungal results of the parent drug nimesulide and the synthesized complexes (N1-N6).

### CONCLUSION

Organotin Metal complexes of nimesulide were efficiently synthesized and spectroscopically characterized. Antibacterial and antifungal screening of the synthesized complexes revealed that the complexes were more active than the parent drug Nimesulide as Nimesulide showed no antibacterial and antifungal activity. This study has supported our hypothesis and these were only the initial biological screening of the synthesized complexes, our future work includes the antioxidant, protein kinase

assay, DNA binding studies and anticancer activities of these complexes.

**Acknowledgment:** We are highly grateful to Institute of pharmaceutical Sciences, School of biomedical sciences, Kings College London for spectroscopic characterization and Higher education commission of Pakistan for the financial support through International research support initiative program. Assistance in biological screening is highly appreciated from DrihsanUllah, Quaid-e-Azam University Islamabad Pakistan.

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Source of Support: Nil, Conflict of Interest: None.

