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Research Article

Synthesis, characterization and biological assay of Organotin derivatives of Sulphanilamide

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ABSTRACT

Metals and its compound had played a major role as therapeutic agents in history of medicine and in modern pharmacology as well. A family of Organotin complexes (Sn^1 - Sn^6) of sulphanilamidewere synthesized by the reaction of triorganotin or diorganotin chloride with the sulphanilamide by adding triethylamine in dry toluene. All the synthesized Complexes were characterized by combination of different techniques using Fourier Transform-Infrared spectroscopy, ^1H and ^{13}C Nuclear magnetic resonance spectroscopy and elemental analysis.*In-vitro* antibacterial and antifungal activities were investigated. Biological screening showed that most of the derived complexes have significant activity against different tested pathogenic strains of bacteria and fungi.

Key words: Sulphanilamide, Organotin, FT-IR, *In-vitro* Biological assay

INTRODUCTION

Organotin compounds have several applications in many fields from many years. Organotin(IV) complexes have been the subject of interest form the last few decades because of their biomedical and commercial applications [1]. It has been observed that several organotin complexes are effective antifouling, antimicrobial [2], and antiviral agents. The interesting application of metal complexes in the treatment of numerous human diseases is a vigorously expanding area in biomedical and inorganic chemistry [3]. The variation in coordination number, geometries, accessible redox states, thermodynamic, and kinetic characteristics and the intrinsic properties of the metal ion are some special characteristics of organometallic complexes that offer the medicinal chemists to employ different strategies for their exploitation[4]. Their use in cancer chemotherapy is gaining mounting importance after the discovery of metal based drug Cisplatin [5]. Sulphanilamideis a potent organic antibacterial agent consisting of an aniline derivitized with a sulphonamide group. Sulphanilamide competitively inhibits enzymatic reactions involving para-amino benzoic acid (PABA)[6]. PABA is involved in enzymatic reactions that produce folic acid which acts as a coenzyme in the synthesis of purine, pyrimidine and other amino acids. Sulphanilamide was used in World War 2 as a first-aid treatment to reduce infection rates and contributed to a dramatic reduction in mortality rates compared to previous wars[7,8]. Modern antibiotics have supplanted sulfanilamide on the battlefield; however, sulfanilamide remains in use for treatment of vaginal yeast infections[9]. Present studyinvolves the synthesis, spectroscopic characterization, elemental analysis and biological screening of the newly synthesized organotin complexes of sulphanilamide.

MATERIALS AND METHODS

All the chemicals, metal salts, reagents were purchased from Sigma Aldrich laboratories and were used as purchased except toluene which was dried by using sodium wire prior to use. All the glassware were properly dried at 120°C. Synthesis was done at Riphah Institute of Pharmaceutical Sciences. Biological screening was done at Quaid-e-Azam University Pakistan. Spectroscopic characterization was done at Institute of Pharmaceutical Sciences, Kings Collage London.

General Procedure for the Synthesis of Organotin Derivatives of Sulphanilamide

Sulphanilamide was suspended in dry toluene (50 ml) and treated with triethylamine Et_3N. The mixture was refluxed for 3 hours. To a solution triorganotin chloride or Diorganotin dichloride was added as solid to a reaction flask with constant stirring and the reaction mixture refluxed 3 hours. The reaction mixture contains Et_3NHCl is filtered off such that filtrate had the organotin derivative. The solvent is removed through rotary apparatus. The mass left behind will be recrystallization from $CHCl_3$[10-14]. [9-13].

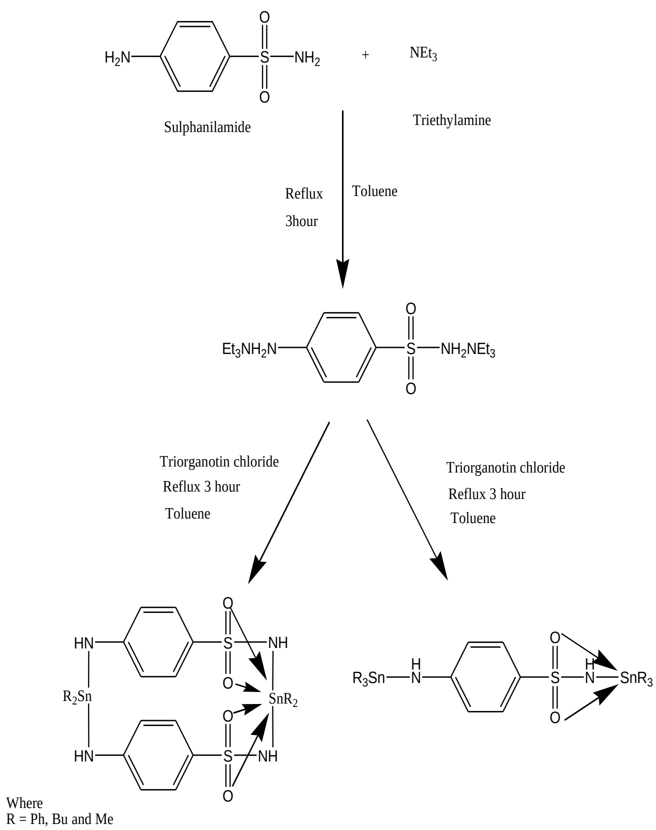

Figure 1: Scheme for Synthesis of Organotin Derivatives.

The physiochemical properties of the synthesized organotin metal complexes are described below

Sn (Sulphanilamide)

White powder, m. p. 165°C, FT-IR (cm^{-1}) 3476s, 3372s, 3264s ν(NH), 1593 ν (CH=CH), 1143 ν(C−N), 1303 ν(S=O) ^1H NMR ($DMSO_{4-D6,}$ ppm), 7.43-7.46d; 6.56-6.0d ($-C_6H_{4-}$), 6.9s ($-NH_2$), 5.81s(H_2N-SO_2), ^{13}C NMR ($DMSO_4$−D6, ppm), 152.38 (C-1), 112.88 (C-2/6), 127.88 (C-3/5), 130.45 (C-4).

Sn[1] (Triphenyltin derivative of sulphanilamide) [($Ph_3Sn)_2$-sulphanilamide]

Yield (81%), m. p. 150 °C, Elemental Analysis: calculated for $C_{42}H_{36}N_2O_2SSn_2$: C, 57.97; H, 4.17; N, 3.22; found: C, 58.13; H, 3.99; N, 3.35; FT-IR (cm^{-1}) 3373s ν(NH), 3067 ν(CH),1594 ν (CH=CH), 1145 ν(C−N), 1304 ν(S=O), 438 ν(Sn−N), ^1H NMR ($DMSO_{4-D6,}$ ppm), 7.83-7.84d; 6.57-6.59d; ($-C_6H_{4-}$), 7.41-7.45m; ($-C_6H_5$), , 6.89s (−NH−), 5.81s ($-NHSO_2$) ^{13}C NMR ($DMSO_4$−D6, ppm), 151.89 (C-1), 112.38 (C-2/6), 128.37 (C-3/5), 129.0 (C-4), 129.97 (C-7), 136.19 (C-8/12), 128.03(C-9/11), 127.38 (C-10).

Sn[2] (Diphenyltin derivative of sulphanilamide) [($Ph_2Sn)_2$-(sulphanilamide)$_2$]

Yield (73%), semisolid,Elemental Analysis : calculated for $C_{36}H_{32}N_4O_4S_2Sn_2$: C, 48.79; H, 3.64; N, 6.32; found: C, 48.68; H, 3.71; N, 6.22; FT-IR (cm^{-1}) 3369s ν(NH), 3069 ν(CH), 1595 ν (CH=CH), 1147 ν(C−N), 1310 ν(S=O), 452 ν(Sn−N), ^1H NMR ($DMSO_{4-D6,}$ ppm), 7.91-7.95d; 6.55-6.57d ($-C_6H_{4-}$), 6.88s (-NH-), 6.80s ($-NH-SO_2$), 7.42m, 7.44m; ($-C_6H_5$), ^{13}C NMR ($DMSO_4$−D6, ppm), 151.9 (C-1), 112.37 (C-2/6), 134.75 (C-3/5), 129.94 (C-4), 127.06 (C-7), 136.33 (C-8/12), 127.75(C-9/11), 127.38 (C-10).

Sn[3] (Tributyltin derivative of sulphanilamide) [($Bu_3Sn)_2$-sulphanilamide]

Yield (65%), m. p. 180°C, ElementalAnalysis:calculated for $C_{30}H_{60}N_2O_2SSn_2$: C, 48.02; H, 8.06; N, 3.73; found: C, 47.95; H, 8.15; N, 3.61; FT-IR (cm^{-1}) 3372s ν(NH),2956 ν(CH), 1594 ν (CH=CH), 1144 ν(C−N), 1304 ν(S=O), 477 ν(Sn−N), ^1H NMR ($DMSO_{4-D6,}$ ppm), 7.45-7.47d; 6.58-6.60d; ($-C_6H_{4-}$), 6.90s (−NH−), 5.82s ($-NH-SO_2$), 0.90d (CH_3-), 1.31-1.63m;($-CH_2-CH_2-$), 1.29m;($Sn-CH_2$)^{13}C NMR ($DMSO_4$−D6, ppm), 151.88 (C-1), 112.37 (C-2/6), 129.96 (C-3/5), 127.38 (C-4), 13.62 (C-7), 26.22 (C-8), 27.71 (C-9), 21.02 (C-10).

Sn[4] (Dibutyltin derivative of sulphanilamide) [($Bu_2Sn)_2$-(sulphanilamide)$_2$]

Yield (59%), semisolid, ElementalAnalysis: calculated for $C_{28}H_{48}N_4O_4S_2Sn_2$: C, 41.71; H, 6.00; N, 6.95; found: C, 41.85; H, 5.87; N, 7.11; FT-IR (cm^{-1}) 3372s ν(NH), 2959 ν(CH), 1596 ν (CH=CH), 1148 ν(C−N), 1338 ν(S=O), 411 ν(Sn−N), ^1H NMR ($DMSO_{4-D6,}$ ppm), 7.42-7.47d; 6.56-6.11d; ($-C_6H_{4-}$), 6.88s; (−NH−), 5.80s; ($-NH-SO_2$), 0.87-0.88m; (CH_3-), 1.65m; ($-CH_2-CH_2-$) 1.16-1.29m; ($-CH_2-$), 13C NMR ($DMSO_4$−D6, ppm), 152.02 (C-1), 112.35 (C-2/6), 129.94 (C-3/5), 127.36 (C-4), 8.68 (C-7), 27.31 (C-8), 25.65 (C-9), 13.48 (C-10).

Sn[5] (Trimethyltin derivative of sulphanilamide) [($Me_3Sn)_2$-sulphanilamide]

Yield (67%), m. p. 155°C, ElementalAnalysis: calculated for $C_{12}H_{24}N_2O_2SSn_2$: C, 28.95; H, 4.86; N, 5.63; found: C, 29.09; H, 5.00; N, 5.42; FT-IR (cm^{-1}) 3373s ν(NH),2958 ν(CH), 1595 ν (CH=CH), 1146 ν(C−N), 1311 ν(S=O), 411 ν(Sn−N), ^1H NMR ($DMSO_{4-D6,}$ ppm), 7.45-7.48d; 6.59-6.61d;($-C_6H_{4-}$), 6.92s (−NH−), 5.83s ($-NH-SO_2$), 0.87s (CH_3-) ^{13}C NMR ($DMSO_4$−D6, ppm), 151.88 (C-1), 112.4 (C-2/6), 129.96 (C-3/5), 127.39 (C-4), 5.2 (C-7).

Sn[6] (Dimethyltin derivative of sulphanilamide) [($Me_2Sn)_2$-(sulphanilamide)$_2$]

Yield (55%), m. p. 140°C, ElementalAnalysis: calculated for $C_{16}H_{24}N_4O_4S_2Sn_2$: C, 30.12; H, 3.79; N, 8.78; found: C, 29.98; H, 3.68; N, 8.87; FT-IR (cm^{-1}) 3344s ν(NH),3066,2984 ν(CH), 1593 ν (CH=CH), 1144 ν(C−N), 1335 ν(S=O), 447 ν(Sn−N), ^{1}H NMR ($DMSO_{4-D6}$, ppm), 7.44-7.46d; 6.58-6.60d; ($-C_6H_{4-}$), 6.9s (−NH−), 5.83s ($-NH-SO_2$), 1.17s (CH_3-), ^{13}C NMR ($DMSO_4$−D6, ppm), 151.89 (C-1), 112.38 (C-2/6), 129.93 (C-3/5), 127.37 (C-4), 8.69 (C-7).

Antibacterial activity

The antibacterial activity of organotin derivatives of sulphanilamide was tested against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) using the agar well diffusion method [15,16]. Cefexime and DMSO were used as positive and negative controls respectively. The wells were dug in the media by using a sterile metallic borer with the centre at least 24 mm apart. The recommended concentration of the test sample (2 mg /ml in DMSO) was introduced into the respective wells. Other wells were supplemented with DMSO and reference antibacterial drugs serving as negative and positive controls, respectively. The plates were incubated immediately at 37 °C for 20 h. The activity was determined by measuring the diameter of zones showing complete inhibition in millimetres. Growth inhibition was calculated with reference to positive control.

Antifungal activity

The antifungal activity of synthesized organotin derivatives of sulphanilamide were tested against *Aspergillus Flavus*, *Aspergillus Niger*, *Rhizoctonia Solani*, *Aspergillus Fumigatus* and *Mucor* by using the tube diffusion test [17,18]. Terbinafine (200 mg/ ml) was used as standard drug, positive control and DMSO as negative control. The amount of growth inhibition was calculated as:

Inhibition (%) = [(A-B)/B] × 100

A = Diameter of fungal colony in control plate

B = Diameter of fungal colony in test plate

RESULTS AND DISSCUSSION

The organotin derivatives of sulphanilamide were mostly solid except diphenyltin and dibutyltin these were semisolid and all derivatives were physically stable. FT-IR spectra rang 4000-400cm^{-1} were measure and the three sharps peaks of NH_2, and NH_2-SO_2between the range of 3350 and 3250cm^{-1} (stretching) become single peak around 3300-3400cm^{-1} symmetric The peak of CH aliphatic 2850-2960cm^{-1} (stretching *sp*^{3}) and CH aromatic at 3000cm^{-1}(stretching *sp*^{2}) appeared and were more profound in butyltin, methyltin and phenyltin derivatives respectively. 1HNMR and 13CNMR shows the no. of proton and carbon according to structure in the expected ranges. Elemental analysis also corresponds well with the calculated values. Antibacterial and antifungal activities were done and the results showed that sulphanilamide were inactive against the microbes but interestingly the synthesized organotin derivatives were active to some extent. The highest activity was exhibited by Sn^3 (Tributyltin derivative of sulphanilamide) [$(Bu_3Sn)_2$-sulphanilamide] against the pathogenic bacterial as well as fungal strains. The results of the biological screening are given below in table 1 and table 2, whereas in figure 1 and figure 2 graphical illustrations of the results are shown.

Table 1: Antibacterial activity of Organotin Derivatives of Sulphanilamide

Samples	***Staph. Aureus***	***E.coli***
Sulphanilamide	0	0
Sn^1	14	0
Sn^2	7	0
Sn^3	30	30
Sn^4	8	0
Sn^5	15	16
Sn^6	10	0
Cefixime	21	21
DMSO	0	0

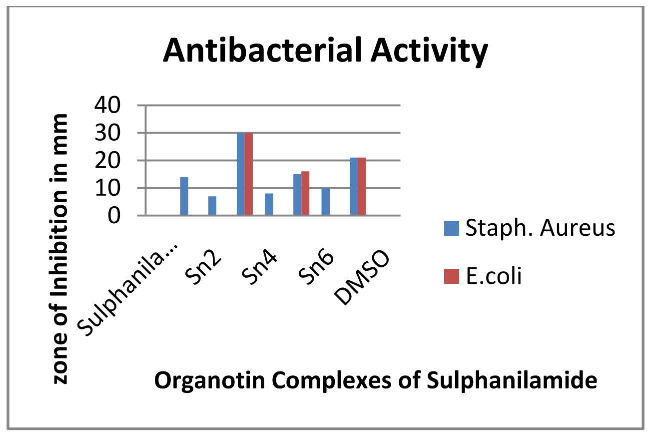

Figure 2: Graph representing Antibacterial activity of Organotin Derivatives of Sulphanilamide

Table2. Antifungal activity of the organotin derivatives of Sulphanilamide

Sample	*A. Flavous*	*A. Niger*	*A. Solani*	*A. Fumigatus*	Mucor SP
Sulphanilamide	0	0	0	0	15
Sn^1	11	14	15	19	18
Sn^2	11	14	15	20	17
Sn^3	26	25	14	29	15
Sn^4	0	0	0	0	0
Sn^5	0	0	6	0	0
Sn^6	0	0	0	0	0
Terbinafine	28	33	35	30	33
DMSO	0	0	0	0	0

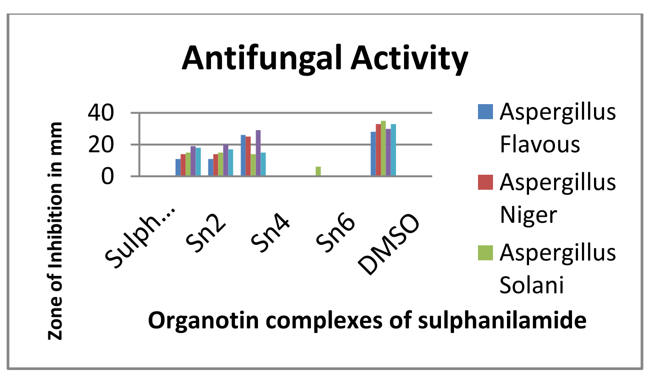

Figure 3: Graph Representing Antifungal activity of the organotin derivatives of Sulphanilamide.

CONCLUSION

Organotin derivatives of sulphanilamide were synthesized in appreciable yeild and characterized spectroscopically**.** Newly synthesized Organotin derivatives of sulphanilamide were observed to be more active than their parent drug sulphanilamide against bacterial and fungal strains. Hence we conclude that metal complexation enhances the pharmacological potential of the drug.

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