Antimicrobial and antiurease potential of Diorganotin(IV) Schiff bases

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Abstract: Six new diorganotin (IV) Schiff bases, $[(CH_3)_2SnL]$ (I), $[(C_2H_5)_2SnL]$ (II), $[(n\text{-}C_4H_9)_2SnL]$ (III), $[(C_6H_5)_2SnL]$ (IV), $[(CH_2C_6H_5)_2SnL]$ (V) and $[(n\text{-}C_8H_{17})_2SnL]$ (VI) where L = N-(5-bromo-2-oxidobenzylidene)phenylacetohydrazide, were synthesized and characterized by elemental analysis, FT-IR, multinuclear NMR (^1H, ^{13}C) and UV-visible spectroscopy. Spectroscopic studies indicate coordination of ligand to the diorganotin (IV) moieties via ONO donor sites generating a pentacoordinated tin center. The synthesized compounds were tested *in vitro* for antibacterial activity against *Klebsiella pneumonia, Salmonella typhimurium, Escherichia coli, Aeromonas, Staphylococcus aureus, Vibrio cholerae, Pseudomonas aeruginosa, Shigella flexneri*, and *in vitro* antifungal activity against *Aspergillus nigar*, *Fusarium solani, Aspergillus fumigatus* and *Alternaria species*. All the compounds were also screened for antiurease activity.

Keywords: Organ tin (IV) Schiff base, antibacterial activity, antifungal activity, antiurease assay.

INTRODUCTION

The emergence of multi drug resistant bacterial and fungal strains and the limited treatment options available for the infected patients has increased the need to design and synthesize potent drugs which can be safely used as pharmaceuticals (Freire-Moran *et al.*, 2011; Perlin *et al.*, 2015). Organotin (IV) compounds has been highlighted for their structural diversity and wide applications in biotechnology, catalysis, medicinal and analytical chemistry (Zamudio-Rivera *et al.*, 2005; Yin, *et al.*, 2004; Rehman, *et al.*, 2011; Pellerito *et al.*, 2002; Bregadze *et al.*, 2003). A large number of organotin compounds are used as pesticides, stabilizers, fire retardants, miticides, molluscicides, additives in marine antifouling paints, surface disinfectants and wood preservatives (Omae *et al.*, 2003). Among the main group metal complexes organotin compounds exhibit pronounced biological and pharmaceutical activities as antitumor, antimicrobial, antituberculosis, antimalarial and antiurease agents (Baul *et al.*, 2007; Abbas *et al.*, 2013; Terzioglu *et al.*, 2003; Cocco *et al.*, 2006; Easmon *et al.*, 2001; Patole *et al.*, 2003; Walcourt *et al.*, 2004; Rehman *et al.*, 2011). The variation in coordination number, geometries, redox states, thermodynamic and kinetic characteristics effects the biological activities of these complexes. Furthermore, the nature of groups directly bonded with tin center not only change the fat solubility of complexes but also play an important role in the transportation of these compounds to active sites and impart special pharmaceutical features to these compounds (Tariq *et al.*, 2014; Casas *et al.*, 2003). Schiff bases also exhibit a broad range of activities, including antibacterial, antifungal, antimalarial etc. (Przybylski *et al.*, 2009), therefore, the two types of compounds are good synthons for preparation of metallo-organic hybrid systems that may exhibit interesting biological properties.

In continuation with our previous studies on diorganotin(IV) Schiff bases (Shujah *et al.*, 2011; Shujah *et al.*, 2013), and to explore biocidal potential of metallo-organic hybrid systems, we report herein the synthesis and spectroscopic characterization of six new diorganotin (IV) compounds $[R_2SnL]$ (R= $-CH_3$ (I), $-C_2H_5$ (II), $-C_4H_9$ (III), $-C_6H_5$ (IV), $-CH_2C_6H_5$ (V), $-C_8H_{17}$ (VI) where L = N-(5-bromo-2-oxidobenzylidene) phenylacetohydrazide. All the synthesized compounds were evaluated for antibacterial, antifungal and antiurease activities.

MATERIALS AND METHODS

Diorganotin (IV) dichlorides, di-*n*-octyltin (IV) oxide, phenylacetichydrazide and 5-bromo-2-hydroxybenzaldehyde were purchased from Aldrich. Dibenzyltin (IV) dichloride was synthesized by a method already reported in literature (Sisido *et al.*, 1961). All the chemicals were used as supplied, however, the analytical grade solvents were freshly dried before use adopting standard procedures (Perrin *et al.*, 2003). The melting points were recorded on an electrothermal melting point apparatus, model MP-D Mitamura Rieken Kogyo (Japan) and were uncorrected. Element analyses were performed with a Leco CHNS 932 apparatus. The infrared (IR) spectra were recorded on a Bio-Rad Excaliber FT-IR, model FTS

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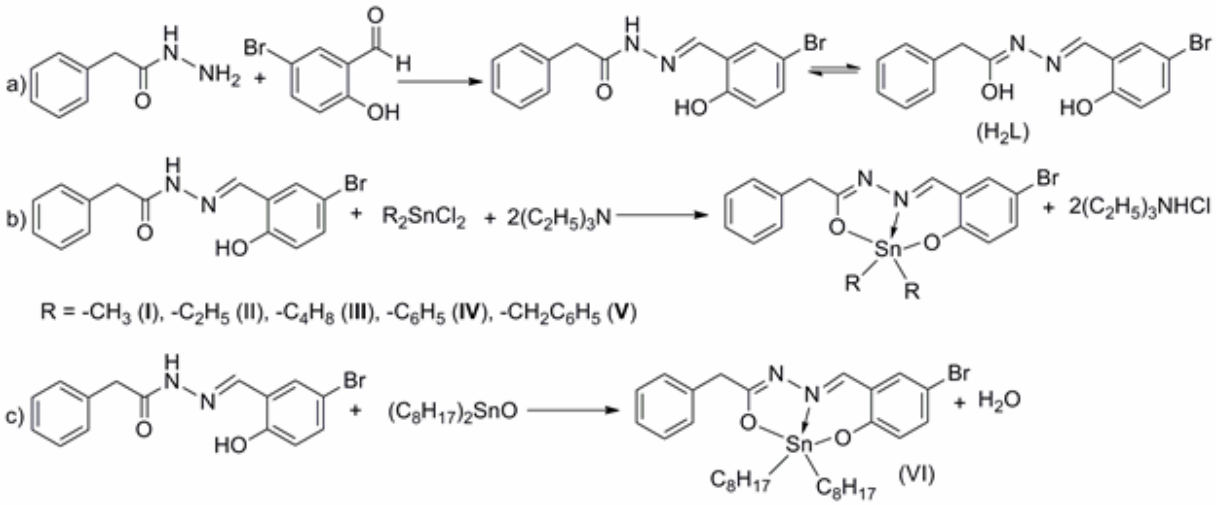

Scheme 1: Synthesis of ligand and its diorganotin(IV) complexes

Table 1: Antibacterial activity of ligand and its diorganotin(IV) complexes[a-c]

Bacterial strains	Average zone of Inhibition (mm)							
	H_2L	I	II	III	IV	V	VI	Standard drug
K. pneumoniae	12.5	11.0	13.5	16.2	20.3	12.7	12.0	21.2
S. typhimurium	9.9	-	12.1	17.7	18.1	14.4	10.5	22.3
E. coli	10.1	10.6	11.7	16.9	15.1	-	12.3	18.7
Aeromonas	11.3	9.8	10.1	19.3	17.5	-	9.7	21.4
S. aureus	-	-	9.7	16.4	16.1	12.3	-	24.2
V. cholera	10.1	10.2	-	19.3	15.8	13.1	12.6	23.3
P. aeruginosa	-	-	-	12.6	11.9	15.2	-	17.5
S. flexneri	-	9.5	10.3	18.3	17.2	-	13.3	21.6

a. - Shows no activity; b. Concentration: 1mg/mL; c. standard drug: Amoxycillin

300 MX spectrophotometer (USA) in the frequency range of 4000-400 cm^{-1} using KBr discs. Multinuclear NMR (^1H, ^{13}C) spectra were recorded in DMSO on a Bruker ARX 600 MHz-FT-NMR spectrometer, Switzerland. The Chemical shifts (δ) are expressed in ppm and coupling constants (*J*) are given in Hz.

Synthesis of ligand and diorganotin complexes

Synthesis of N-(5-bromo-2-hydroxybenzylidene) phenylacetohydrazide (H_2L) The ligand N-(5-bromo-2-hydroxybenzylidene) phenylacetohydrazide (H_2L) was synthesized by reacting 2-phenylacetichydrazide (0.8 g, 5.4mmol) with 5-bromo-2-hydroxybenzaldehyde (1.1 g, 5.4mmol) in ethanol (25mL). The reaction mixture was stirred and refluxed for 2 hours. The solution was cooled and solid product recovered (Scheme1a).

Yield: 78%; colour: white; m.p. 176–178 °C; elemental analysis calculated for ($C_{15}H_{13}BrN_2O_2$): C 54.07%, H 3.93%, N 8.41% Found: C, 53.98%, H 4.01%, N 8.29%. IR (KBr, cm^{-1}): 3282 ν(O-H), 3178 ν(N-H), 1654 ν(C=O), 1621 ν(C=N), 1033 ν(N-N). UV(DMF) λ_{max} 292, 334. ^{1}HNMR (DMSO-d_6, ppm): δ 11.13 (s, 1H, Ar-OH), δ 6.85 (d, 1H, Ar-H), δ 7.40 (d, 1H, Ar-H), δ 7.78 (s, 1H, Ar-H), δ 8.36 (s, 1H, C=NH), δ 3.36 (s, 2H, CH_2), δ 7.20-7.36 (m, 5H, Ar-H). ^{13}C-NMR (DMSO-d_6, ppm): δ 166.6, 118.6, 135.3, 110.4, 133.1, 121.2 (Ar-C), δ 144.4 (C=N), δ 172.2 (C=O), δ 41.0 (CH_2), δ 135.7, 129.3, 128.3, 127.8 (Ar-C).

Dimethyltin (IV) N-(5-bromo-2-oxidobenzylidene) phenylacetohydrazide (I)

The ligand N-(5-bromo-2-hydroxybenzylidene) phenylacetohydrazide (0.33g, 1.0mmol) and triethylamine (0.3mL, 2.0mmol) were mixed in dry chloroform. The reaction mixture was stirred for 15min. Then a chloroform solution of dimethyltin (IV) dichloride (0.22 g, 1.0 mmol) was added. The mixture was stirred and refluxed for additional 2 hour. The solution was cooled and filtered to remove the Et_3NHCl salt. The solvent was evaporated using rotary evaporator under reduced pressure. The yellow product obtained was recrystallized from $CHCl_3$ and *n*-hexane mixture (1:1) (Scheme 1b). Numbering scheme of ligand and organotin(IV) complexes is provided in fig. 1.

Yield: 70%; colour: light yellow; m.p. 137–139 °C; elemental analysis calculated for ($C_{17}H_{17}BrN_2O_2Sn$): C, 42.54%, H 3.57%, N 5.84%; Found: C 42.58%, H 3.60%, N 5.90%. IR (KBr, cm^{-1}): 1607 ν(C=N), 1072 ν(N-N),

Table 2: Antifungal activity (% inhibition) of ligand and its diorganotin (IV) complexes[a-e]

Fungal Strains	Mean value of growth percentage inhibition							
	H_2L	I	II	III	IV	V	VI	Standard drug
A. niger	41.48	23.21	28.6	10.1	59.4	-	15.3	100
F. solani	35.2	-	15.8	42.2	97.2	44.5	20.1	100
A. fumigates	54.0	32.5	25.6	9.66	80.5	35.7	18.0	100
Alternaria sp	41.48	15.5	20.4	34.87	78.7	54.7	30.5	100

a.- Show no activity. b. *In vitro* agar tube dilution method, c. concentration: 200 µg/mL of DMSO. d. % inhibition of fungal growth = 100 – gt/gc ×100. gt = linear growth in test (mm) and gc = linear growth in vehicle control (mm) e. Reference: Fluconazole

Table 3: Antiurease activity of ligand and its diorganotin (IV) complexes[a,b]

Compound No.	H_2L	I	II	III	IV	V	VI
% Inhibition	55.7	14.2	-	85.0	80.1	-	-
IC_{50}± S.D[µM]	248.5±0.07	-	-	92.4±1.18	172.7±0.78	-	-

a Sample Concentration [mM]: 0.5; b Standard: thiourea, IC_{50} ± S.D [mM]: 21.2 ± 1.3.

582 ν(Sn-O), 462 ν(Sn-N). UV(DMF) λ_{max} 315, 324, 416. ^{1}HNMR (DMSO-d_6, ppm): δ 6.56 (d, 1H, Ar-H), δ 7.41 (d, 1H, Ar-H), δ 7.52 (s, 1H, Ar-H), δ 8.61 (s, 1H, C=NH, ^3J = [37 Hz]), δ 3.51 (s, 2H, CH_2), δ 7.26-7.32 (m, 5H, Ar-H), δ 0.55 (s, 6H, Sn(CH_3) $^2J(^{119}$Sn–1H) =88 Hz). ^{13}C-NMR (DMSO-d_6, ppm): δ 164.7, 119.1, 136.1, 106.0, 135.6, 123.3 (Ar-C), δ 157.9 (C=N), δ 173.5 (C=O), δ 45.4 (CH_2), δ 136.9, 128.8, 128.2, 126.3 (Ar-C), δ 1.6 (Sn-CH_3). *Diethyltin (IV) N-(5-bromo-2-oxidobenzylidene) phenylacetohydrazide (II)* Complex (II) was synthesized in the same way as (I), using N-(5-bromo-2-hydroxybenzylidene) phenylacetohydrazide (0.33g, 1.0 mmol), triethylamine (0.3mL, 2.0mmol) and diethyltin(IV) dichloride (0.25g, 1.0 mmol) in molar ratio of 1:2:1. The product was recrystallized from chloroform and n-hexane (4:1) mixture.

Yield: 72%; colour: light yellow; m.p. 120–122 °C; elemental analysis calculated for ($C_{19}H_{21}BrN_2O_2Sn$): C 44.92%, H 4.17%, N 5.51 %; Found: C 44.88%, H 4.21%, N 5.47%. IR (KBr, cm^{-1}): 1615 ν(C=N), 1074 ν(N-N), 550 ν(Sn-O), 465 ν(Sn-N). UV(DMF) λ_{max} 312, 333, 402. ^{1}HNMR (DMSO-d_6, ppm): δ 6.53 (d, 1H, Ar-H), δ 7.40 (d, 1H, Ar-H), δ 7.50 (s, 1H, Ar-H), δ 8.68 (s, 1H, C=NH, ^3J = [34 Hz]), δ 3.52 (s, 2H, CH_2), δ 7.24-7.33 (m, 5H, Ar-H), δ 1.47, 1.26 (q, t, 4H, 6H, Sn-C_2H_5). ^{13}C-NMR (DMSO-d_6, ppm): δ 165.4, 118.7, 136.3, 105.6, 135.7, 123.1 (Ar-C), δ 158.6 (C=N), δ 174.1 (C=O), 45.4 (CH_2), δ 136.9, 128.8, 128.4, 126.1 (Ar-C), δ 14.6, 9.4 (Sn-C_2H_5).

*Di-n-butyltin (IV) N-(5-bromo-2-oxidobenzylidene) phenylacetohydrazide (III)*Complex (III) was prepared in the same way as (I), using N-(5-bromo-2-hydroxybenzylidene) phenylacetohydrazide (0.33 g, 1.0 mmol), triethylamine (0.3 mL, 2.0 mmol) and dibuthyltin (IV) dichloride (0.30 g, 1.0 mmol) in molar ratio of 1:2:1. The product was recrystallized from chloroform and n-hexane (4:1) mixture.

Yield: 70%; colour: light yellow; m.p. 105–109 °C. elemental analysis calculated for ($C_{23}H_{29}BrN_2O_2Sn$): C 48.97%, H 5.18%, N 4.97%; Found: C 49.01%, H 5.25%, N 5.02%. IR (KBr, cm^{-1}): 1613 ν(C=N), 1071 ν(N-N), 553 ν(Sn-O), 467 ν(Sn-N). UV (DMF) λ_{max} 318, 330, 406. ^{1}HNMR (DMSO-d_6, ppm): δ 6.54 (d, 1H, Ar-H), δ 7.39 (d, 1H, Ar-H), δ 7.51 (s, 1H, Ar-H), δ 8.69 (s, 1H, C=NH, ^3J = [34 Hz]), δ 3.50 (s, 2H, CH_2), δ 7.25-7.33 (m, 5H, Ar-H), δ 1.24-1.33, 1.15-1.18, 1.10-1.12, 0.69 (m, m, m, t, 4H, 4H, 4H, 6H, Sn-C_4H_9), ^{13}C-NMR (DMSO-d_6, ppm): δ 165.5, 118.8, 136.3, 105.7, 135.6, 123.2 (Ar-C), δ 158.6 (C=N), δ 174.1 (C=O), δ 45.4 (CH_2), 136.9, 128.8, 128.4, 126.1 (Ar-C), δ 25.5, 26.5, 25.6, 13.4 (Sn-C_4H_9).

Diphenyltin(IV)N-(5-bromo-2-oxidobenzylidene) phenylacetohydrazide (IV) Complex (IV) was prepared in the same way as (I), using N-(5-bromo-2-hydroxybenzylidene) henylacetohydrazide (0.33 g, 1.0 mmol), triethylamine (0.3mL, 2.0mmol) and diphenyltin(IV) dichloride (0.34g, 1.0mmol) in molar ratio of 1:2:1. The product was recrystallized from chloroform and n-hexane (4:1) mixture. *Yield*: 72%. colour: light yellow; m.p. 128-125°C. elemental analysis calculated for ($C_{27}H_{21}BrN_2O_2Sn$): C 53.68%, H 3.50%, N 4.64%; Found: C 53.53%, H 3.55%, N 4.70%.

IR (KBr, cm^{-1}): 1612 ν(C=N), 1068 ν(N-N), 576 ν(Sn-O), 478 ν(Sn-N). UV(DMF) λ_{max} 317, 329, 410. ^{1}HNMR (DMSO-d_6, ppm): δ 6.77 (d, 1H, Ar-H), δ 7.41 (d, 1H, Ar-H), δ 7.50 (s, 1H, Ar-H), δ 8.60 (s, 1H, C=NH, ^3J = [36 Hz]), δ 3.57 (s, 2H, CH_2), δ 7.26-7.39 (m, 5H, Ar-H), δ 7.26-7.39 (m, 6H, Sn-C_6H_5). ^{13}C-NMR (DMSO-d_6, ppm): δ 165.1, 118.6, 135.6, 110.3, 133.4, 121.3 (Ar-C), δ 156.2 (C=N), δ 173.2 (C=O), δ 45.3 (CH_2), δ 135.9,

129.3, 128.4, 127.6 (Ar-C), δ 144.2, 134.5, 123.9, 127.9 (Sn-C_6H_5).

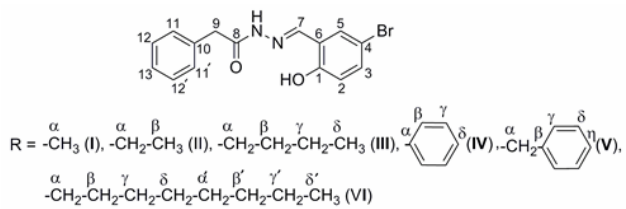

Fig. 1: Numbering scheme of ligand and alkyl group

Dibenzyltin (IV) N-(5-bromo-2-oxidobenzylidene) phenylacetohydrazide (V) Complex (V) was synthesized in the same way as (I), using N-(5-bromo-2-hydroxybenzylidene) phenylacetohydrazide (0.33g, 1.0 mmol), triethylamine (0.3mL, 2.0 mmol) and dibenzyltin (IV) dichloride (0.37g, 1.0mmol) in molar ratio of 1:2:1. The product was recrystallized from chloroform and *n*-hexane (4:1) mixture.

Yield: 68%. colour: light yellow; m.p. 140–142°C. elemental analysis calculated for ($C_{29}H_{25}BrN_2O_2Sn$): C 55.10%, H 3.99%, N 4.43%; Found: C 55.16%, H 4.09%, N 4.50%. IR (KBr, cm^{-1}): 1610 ν(C=N), 1060 ν(N-N), 570 ν (Sn-O), 468 ν(Sn-N). UV(DMF) λ_{max} 314, 327, 408. ^{1}HNMR (DMSO-d_6, ppm): δ 6.64 (d, 1H, Ar-H), δ 7.40 (d, 1H, Ar-H), δ 7.52 (s, 1H, Ar-H), δ 8.50 (s, 1H, C=NH, ^3J = [38 Hz]), δ 3.54 (s, 2H, CH_2), δ 7.23-7.38 (m, 5H, Ar-H), δ 2.50, 7.23-7.38 (s, m, 4H, 10H, Sn-$CH_2C_6H_5$). ^{13}C-NMR (DMSO-d_6, ppm): δ 164.9, 119.7, 136.8, 106.5, 135.9, 123.8 (Ar-C), δ 157.5 (C=N), δ 173.7 (C=O), δ 45.1 (CH_2), δ 136.3, 128.2, 128.0, 126.7 (Ar-C), δ 36.4, 139.2, 135.5, 125.9, 128.7 (Sn-$CH_2C_6H_5$).

Di-n-octyltin (IV) N-(5-bromo-2-oxidobenzylidene) phenylacetohydrazide (VI) Complex (VI) was synthesized by suspending N′-(5-bromo-2-hydroxybenzylidene) phenylaceto-hydrazide (0.33g, 1.0mmol) and di-*n*-octyltin (IV) oxide (0.36g, 1.0mmol) in dry toluene (100 mL). The mixture was refluxed for 3 h and water formed during the reaction was removed by the Dean and Stark apparatus. Solid yellow product was obtained by evaporating solvent with rotary evaporator. The product was recrystallized from chloroform and *n*-hexane (4:1) mixture (Scheme 1c).

Yield: 68%. colour: light yellow; m.p. 98–101 °C. elemental analysis calculated for ($C_{31}H_{47}BrN_2O_2Sn$): C 54.89%, H 6.89%, N 4.13; Found: C 54.91%, H 6.92%, N 4.18%.

IR (KBr, cm^{-1}): 1615 ν(C=N), 1074 ν(N-N), 550 ν(Sn-O), 464 ν(Sn-N). UV (DMF) λ_{max} 316, 320, 405. ^{1}HNMR (DMSO-d_6, ppm): δ 6.52 (d, 1H, Ar-H), δ 7.42 (d, 1H, Ar-H), δ 7.50 (s, 1H, Ar-H), δ 8.68 (s, 1H, C=NH, ^3J = [38 Hz]), δ 3.51 (s, 2H, CH_2), δ 7.26-7.31 (m, 5H, Ar-H), δ 1.64-1.73, 1.48-1.57, 1.22-1.41, 0.89 (m, m, bs, t, 4H, 4H, 20H, 6H, Sn-C_8H_{17}). ^{13}C-NMR (DMSO-d_6, ppm): δ 165.5, 118.8, 136.3, 105.7, 135.6, 123.2 (Ar-C), δ 158.6 (C=N), δ 174.1 (C=O), δ 45.4 (CH_2), δ 136.9, 128.8, 128.4, 126.1 (Ar-C), δ 22.2, 24.5, 33.3, 29.1, 29.2, 31.7, 22.6, 14.2 (Sn-C_8H_{17}).

Antimicrobial activities

The ligand H_2L and its diorganotin(IV) complexes (I-VI) were screened for antibacterial activity against eight pathogenic bacterial strains *Klebsiella pneumonia, Salmonella typhimurium, Escherichia coli, Aeromonas, Staphylococcus aureus, Vibrio cholerae, Pseudomonas aeruginosa* and *Shigella flexneri*, using agar well diffusion method. The antifungal activity was determined against four fungal strains (*Aspergillus nigar*, *Fusarium solani, Aspergillus fumigatus* and *Alternaria species*) using agar tube dilution method (Rahman *et al.*, 2001).

Antiurease activity

The activity was done by spectrophotometric continuous rate determination microtiter-plate urease inhibition assay by indophenol method (Weatherburn, 1967). Absorptions were measured at 630 nm using Spectra Max 340 Micro plate reader (Molecular devices, USA). Thiourea was used as standard and % inhibition was calculated from the formula 100-(A_{630} nm_{Test}/A_{630} $nm_{Controle}$ ×100)

RESULTS

The ONO donor Schiff base and its diorganotin(IV) complexes were synthesized in good yield by reacting N-(5-bromo-2-hydroxybenzylidene)phenylacetohydrazide with triethylamine and diorganotin(IV) dichloride in mole ratio 1:2:1 and di-*n*-octyltin(IV) oxide with ligand in mole ratio 1:1 as shown in Scheme 1. The Schiff base ligand is white whereas all the complexes are yellow to orange in color. All the synthesized compounds are solids with sharp melting points, stable in air and soluble in common organic solvents. The results of elemental analysis are in close agreement with the proposed structures of synthesized compounds. All the compounds were also characterized by IR and multinuclear NMR (^{1}H, ^{13}C). All the compounds have been screened for their antibacterial (table 1), antifungal (table 2) activities, and antiurease potential (table 3).

DISCUSSION

The IR spectra of the synthesized ligand (H_2L) and its diorganotin(IV) derivatives (I - VI) were recorded as KBr pellets in the range of 4000-400 cm^{-1}. Absorption bands observed in the IR spectrum of ligand around 3282, 3178 and 1654 cm^{-1} suggest the presence of –OH, -NH and C=O groups, respectively. Furthermore, the band found in the region of 1621 cm^{-1} indicates the presence of C=N group. The absence of bands due to υ(OH), υ(NH) and υ(C=O) stretching vibrations in the IR spectra of

orgnaotin (IV) complexes indicates enolization, deprotonation and formation of complexes. The ν(C=N) bands is shifted to lower frequency suggesting the coordination of azomethine nitrogen to diorganotin (IV) moiety (Pettinari *et al.*, 2001). The band due to ν(N-N) in the IR spectra of organotin (IV) complexes is shifted to higher frequencies in the range of 1060-1072 cm^{-1}. An increase in the frequency of this band is attributed to an increase in bond length and decrease in repulsion of the lone pairs of electrons on the nitrogen atoms. The appearance of new bands in the regions of 553-582 cm^{-1} and 462-478 cm^{-1} due to the formation of Sn-O and Sn-N bonds, respectively, further support the complex formation (Sirajuddin *et al.*, 2012).

^{1}H NMR spectra of synthesized compounds have been recorded on 600 MHz NMR spectrometer in DMSO and the spectral data is provided in experimental section. The expected resonances are assigned by their peak multiplicity, intensity pattern, integration and tin satellites.

In the ligand H_2L the protons of hydroxyl group resonate at 11.13 ppm. The azomethine protons appear as a singlet at 8.36 ppm. The aromatic protons appear in the range of 6.85 – 7.78 ppm while the methylene protons give a singlet at 3.36 ppm. Some extra peaks are also observed in the NMR spectra of ligand which support the existence of amido and iminol tautomeric forms of the ligands. In the ^{1}H NMR spectra of complexes signal for hydroxyl proton are absent which suggests deprotonation of the ligand during complex formation and replacement of hydroxyl proton by organotin(IV) moiety. The appearance of tin satellites and downfield shift of azomethine proton signal to 8.50 – 8.69 ppm indicate coordination of azomethine nitrogen to tin atom. The calculated $^3J[^{119}Sn\text{-}^1H]$ coupling constant are in the range of 34 - 38 Hz. The values are consistent with the reported values. The protons of *n*-butyltin, di-*n*-octyltin and diphenyl moieties of the diphenyltin(IV) derivatives show a complex pattern in the range 1.10 – 1.33 ppm, 1.22 – 1.73 and 7.26 – 7.39 ppm, respectively. Despite the complex pattern of ^{1}H NMR spectra of di-*n*-butyltin(IV) and di-*n*-octyltin derivatives, a clear triplet due to terminal methyl group appear at 0.69 and 0.89 ppm, respectively. The ethyl protons in complex (II) resonate at 1.47 and 1.26 ppm as quintet and triplet. The methyl protons of dimethyltin(IV) derivative (I) appear as sharp singlet at 0.55 ppm with well-defined satellites and $^2J[^{119}Sn\text{-}^1H]$ coupling constant 88 Hz. The tin proton coupling may be used to find out the Me-Sn-Me bond angle in solution using Lockhart's equation $\theta = 0.0105\ [^2J]^2 - 0.799[^2J] + 122.4$. On substitution the value of ^2J we get θ the Me-Sn-Me angle in solution equal to 133° indicating a pentacoordinated tin atom (Lockhart *et al.*, 1986).

The ^{13}C NMR spectral data for the alkyl groups (R) attached to the tin atom where R = Me, Et, *n*-Bu, Ph, Bez and *n*-Oct were assigned by comparison with related analogues as model compounds. The positions of the dialkyl carbon signals undergo a minor variation in the complexes as compared to those observed in free ligand. The azomethinic carbon shifts to a lower field region in all the complexes, indicating participation of the azomethinic nitrogen in coordination to tin (IV). All the R groups give signals in the expected range. $^1J\ [^{119}Sn\text{-}^{13}C]$ can be calculated by using the Lockhart's equation $^1J\ [^{119}Sn', ^{13}C] = 11.4\ \theta - 875$ [25]. Substitution of θ gives us ^1J equal to 641 Hz suggesting pentacoordination around tin center with distorted trigonal bipyramidal geometry (Lockhart et al., 1986; Lockhart, Davidson, 1987).

Electronic spectra of the ONO donor ligands and their diorganotin (IV) derivatives were recorded on Shimadzu 1800 spectrophotometer using 1 x 10^{-4} M solution in DMF as solvent. The absorption bands found in the region of 292-318 nm in both the ligands and their organotin (IV) derivatives are assigned to π- π^* transition while bands found in the regions of 324-334 are due to $n \rightarrow \pi^*$ transitions. The observed hyperchromic shift suggests the coordination of C=N-N=C nitrogen to tin atom (Nath *et al.*, 2006). In all the complexes, new bands are found in the region of 406-416 nm. These bands are assigned to ligand to metal charge transfer transition (LCMT) (Mukhopadhyay *et al.*, 2003; Maurya *et al.*, 1997)

Antibacterial activity

The ligand and its diorganotin(IV) complexes (I - VI) were tested for their antibacterial activity using the agar well diffusion method against *Staphylococcus aureus* (Gram-positive) and *Klebsiella pneumonia, Salmonella typhimurium, Escherichia coli, Aeromonas, Vibrio cholerae, Pseudomonas aeruginosa* and *Shigella flexneri* (Gram-negative). The results in terms of zone of inhibition are provided in table 1. Amoxycillin was used as standard drug. All the complexes (I-VI) show higher activity than the ligand (H_2L). The high activity of complexes can be explained on the basis of Overtone's concept and Tweedy's chelation theory (Anjaneyula *et al.*, 1986; Nagashri *et al.,* 2011; Dharamaraj *et al.*, 2001), which demands high lipophilic character for better antibacterial activity. The lipophilic nature of diorganotin (IV) moiety increases on complexation, which facilitate the permeation of the complexes through the cell membrane (Rehman *et al.*, 2009). The complex (III) and (IV) demonstrate high activities against *Vibrio cholerae, Aeromonas, Shigella flexneri,* and *Salmonella typhimurium*. The highest activity among all the synthesized compounds was shown by the diphenyltin (IV) derivative (IV) against *Klebsiella pneumonia* which is almost comparable to the standard drug.

Antifungal activity

The ligand and its diorganotin (IV) complexes (I-VI) were also evaluated for their antifungal activity against four

fungal strains (*Aspergillus nigar*, *Fusarium solani, Aspergillus fumigatus* and *Alternaria species*) using fluconazole as standard drug. The results shown in Table 2 indicate that complex (IV) has the highest activity against *Fusarium solani* which is almost close to the standard drug.

Antiurease activity

The *in vitro* antiurease activity of synthesized ligands and its diorganotin (IV) complexes was done by spectrophotometric continuous rate determination microtiter-plate urease inhibition assay by indophenols method using 0.5 mM concentration. Thiourea was used as the standard inhibitor having an $IC_{50} \pm$ S.D [μM] value of 21.2±1.3 (table 3). The antiurease activity of organotin compounds can be explained on the basis of their ability to establish secondary interactions with the active site of enzyme. Complex (IV) exhibit significant urease inhibition. The highest activity was shown by complex (III) with an IC_{50} value of 92.4 μM. However, none of the compound is more active than the standard drug.

CONCLUSIONS

Six new diorganotin (IV) derivatives of an ONO donor Schiff base were synthesized and characterized by elemental analysis, FT-IR and NMR (^{1}H and ^{13}C) and UV-Visible spectroscopy.

The spectroscopic data authenticate the pentacoordination around tin center in all the complexes. The biological screening of synthesized compounds revealed that dibutyltin (IV) (III) and diphenyltin (IV) (IV) complexes are biologically active and exhibit significant antibacterial, antifungal and antiurease activities.

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