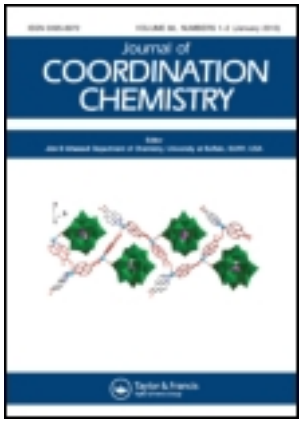

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Synthesis, crystal structure, enzyme inhibition, DNA protection, and antimicrobial studies of di- and triorganotin(IV) derivatives of 2-thiopheneacetic acid

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Synthesis, crystal structure, enzyme inhibition, DNA protection, and antimicrobial studies of di- and triorganotin(IV) derivatives of 2-thiopheneacetic acid

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A series of organotin(IV) complexes, $[Bu_2Sn(C_6H_5O_2S)_2]$ (**1**), $[Bu_3Sn(C_6H_5O_2S)]$ (**2**), $[Oct_2Sn(C_6H_5O_2S)_2]$ (**3**) of 2-thiopheneacetic acid (**HL**) have been prepared and characterized through FT-IR and NMR spectroscopy. The crystal structure of **2** has been confirmed by X-ray single crystal analysis, in which tin adopts a trigonal bipyramidal geometry. The synthesized complexes have been screened for antibacterial, DNA protection, and enzyme inhibition activities against acetylcholinesterase as well as butylcholenesterase.

Keywords: 2-Thiopheneacetic acid; Organotin(IV); Spectroscopy; X-ray crystallography; Enzyme inhibition; DNA protection; Antibacterial assay

1. Introduction

Organotin(IV) carboxylates have found much attention owing to their versatility in morphology and applications in the field of agriculture and medicines. Organotin(IV) compounds can adopt different geometries based on binding modes of the carboxylate. Thus, these organotin(IV) complexes can have monomeric, dimeric, cyclic, hexameric, and polymeric structures [1–3]. Steric and electronic factors of the organic substituents on tin play an important role in stabilizing these complexes. Organotin(IV) compounds have a wide range of applications as ionophores in sensors [4], stabilizers for PVC, catalysts [5], and ion carriers in electrochemical membrane design [6].

The biological activity of organotins is highly dependent on the nature of the carboxylate, the organic substituents attached to tin, and the coordination number of tin [7, 8]. Tin compounds have been used as antibacterial [9], antitumor [10, 11], anticancer [12], anti-tuberculosis agents [13], antifungal and cytotoxic [14], anti-proliferative [15], anti-inflammatory [16], and anti-microbial activities [17]. Similarly, organotins are promising urease inhibitors [18].

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In this work, we have synthesized three organotin(IV) complexes and characterized them by FT-IR and NMR spectroscopy. The structure of **2** has been confirmed by X-ray single-crystal analysis. All of the complexes have prominent antibacterial, DNA protection, and enzyme inhibition activities. 2-Thiopheneacetic acid has been selected for this work as it has sulfur in the ring whose lone pair may contribute in the biological application of these complexes.

2. Experimental

2.1. *Materials*

The ligand and organotin(IV) compounds were purchased from Aldrich, USA, and used without purification. The solvents were from E. Merck, Germany, and dried before use according to the reported procedures [19]. Acetylcholinesterase (AChE) (EC 3.1.1.7, type VI-S from Electric Eel), butyrylcholinesterase (BuChE) (EC 3.1.1.8, from horse serum), acetylthiocholine iodide (ATCI), *S*-butyrylthiocholine chloride (BTCCl), dimethylsulfoxide (DMSO), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), neostigmine-methylsulfate, and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. *Physical measurements*

Melting points were confirmed on a Gallen Kamp apparatus and are uncorrected. Infrared measurements (4000–400 cm^{-1}) were taken on a Thermoscientific NICOLET 6700 FT-IR spectrophotometer. Multinuclear (^1H and ^{13}C NMR) spectra in solution were recorded on a Bruker ARX 300 MHz using solvents as internal reference.

A colorless prismatic crystal of **2** was coated with Paratone 8277 oil (Exxon) and mounted on a glass fiber**.** All measurements were made on a Bruker APEX2 CCD installed on a Nonius Kappa Goniometer diffractometer with graphite monochromated Mo-Kα radiation. The data were collected using ω and φ scans and corrected for Lorentz polarization and absorption effects using multi-scan methods [20]. The structure was solved by direct methods using SHELXS [21]. The non-hydrogen atoms were refined anisotropically. Hydrogens were included at geometrically idealized positions and were not refined. The final cycle of the full-matrix least-squares refinement using SHELXL [21] converged with unweighted and weighted agreement factors, goodness of fit, *etc.*, listed in table 1. The weighting schemes were based on counting statistics and the final difference Fourier maps were essentially featureless. The figure was plotted with the aid of ORTEP-3 for Windows [22].

2.3. *Synthesis and spectroscopic data*

2.3.1. 2-Thiopheneacetic acid (HL). The ligand 2-thiopheneacetic acid (HL) was purchased from Aldrich, USA. ^1H NMR δ (ppm): 11.60 {s, OH, 1H}, 3.92{s, H_2, 2H}, 7.03–7.00 {m, (H_4, H_5, 2H}, 7.28–7.26 {d, H_6, 1H}; ^{13}C NMR δ (ppm): 177.1 (C_1), 35.4 (C_2), 134.0 (C_3), 127.0 (C_4), 127.3 (C_5), 125.4 (C_6).

Table 1. Crystal data and structure refinement details for tri-n-butylstannyl(-2-thiopheneacetate).

CCDC no.	916,577
Empirical formula	C_{18} H_{32} O_2 S Sn
Formula weight	431.19
Temperature	173(2) K
Wavelength	0.71073 Å
Unit cell dimensions	$a = 12.3920(4)$ Å $\alpha = 90.000(2)°$ $b = 11.0670(4)$ Å $\beta = 103.680(2)°$ $c = 15.1450(5)$ Å $\gamma = 90.0000(17)°$
Volume	2018.10(12) Å^3
Z	4
Density (calculated)	1.419 Mg/m^3
Absorption coefficient	1.374 mm^{-1}
Crystal size	0.16 × 0.14 × 0.08 mm^3
Goodness-of-fit on F^2	1.090
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0308$, $wR2 = 0.0718$
R indices (all data)	$R1 = 0.0352$, $wR2 = 0.0746$

2.3.2. Di-*n*-butylstannyl bis[2-thiophene acetate] (1). Di-*n*-butyltin(IV) oxide (0.50 g, 2.0 mM) and HL (0.57 g, 4.0 mM) were mixed in 1 : 2 ratio in toluene and refluxed for 2–3 h. $C_{20}H_{28}O_4S_2Sn$, m.p. 122–123 °C, Yield, 80%. Recrystallization $CHCl_3$/*n*-hexane 4 : 1. IR (KBr) (cm^{-1}): $\nu_{asym(OCO)}$ 1574, $\nu_{sym(OCO)}$ 1389, $\Delta\nu_{asym-sym}$ 185, ν_{Sn-C} 577, ν_{Sn-O} 466. ^{1}H NMR δ (ppm): 3.92{s, H_2, 4H}, 6.95 {d, H_4, 2H}, 7.02 {dd, H_5, 2H}, 7.30–7.22 {m, H_6, 2H}, 1.66–1.25 {m, H_α, H_β, H_γ, 12H}, 0.90 {t, H_δ, 6H}. ^{13}C NMR δ (ppm): 180.6 (C_1), 35.3 (C2), 135.3 (C_3), 127.1 (C_4), 127.33 (C_5), 125.0 (C_6), 25.2 (C_α), $^1J(^{119}Sn–^{13}C)$ [564], 25.6 (C_β), $^2J(^{119}Sn–^{13}C)$ [23 Hz], 26.5 (C_γ) $^3J(^{119}Sn–^{13}C)$ [98], 13.6 (C_δ).

2.3.3. Tri-*n*-butylstannyl [2-thiophene acetate] (2). Bis(tri-*n*-butyltin(IV)) oxide (1.04 mL, 2.0 mM) and HL (0.57 g, 4.0 mM) were mixed in 1 : 2 ratio in toluene and refluxed for 2–3 h. $C_{18}H_{32}O_2SSn$, m.p. 62 °C, Yield, 79%. Recrystallization $CHCl_3$/*n*-hexane 4:1. IR (KBr) (cm^{-1}): $\nu_{asym(OCO)}$ 1575, $\nu_{sym(OCO)}$ 1384, $\Delta\nu_{asym-sym}$ 191, ν_{Sn-C} 553, ν_{Sn-O} 452. ^{1}H NMR δ (ppm): 3.85{s, H_2, 4H}, 6.94 {d, H_4, 2H}, 6.95 {dd, H_5, 2H}, 7.20–7.18 {d, H_6, 2H}, 1.70–1.21 {m, H_α, H_β, H_γ, 18H}, 0.91 {t, H_δ, 9H}. ^{13}C NMR δ (ppm): 175.6 (C_1), 36.3 (C_2), 137.3 (C_3), 126.2 (C_4), 126.5 (C_5), 124.5 (C_6), 16.5 (C_α), $^1J(^{119/117}Sn–^{13}C)$ [352/337 Hz], 27.8 (C_β), $^2J(^{119}Sn–^{13}C)$ [20.3 Hz], 27.0 (C_γ), $^3J(^{119/117}Sn–^{13}C)$ [64/50 Hz], 13.7 (C_δ).

2.3.4. Di-*n*-octylstannyl bis[2-thiophene acetate] (3). Di-*n*-octyltin(IV) oxide (0.72 g, 2.0 mM) and HL (0.57 g, 4.0 mM) were mixed in 1 : 2 ratio in toluene and refluxed for 2–3 h. $C_{28}H_{44}O_4S_2Sn$, m.p. 49–51 °C, Yield, 74%. Recrystallization $CHCl_3$/*n*-hexane 4:1. IR (KBr) (cm^{-1}): $\nu_{asym(OCO)}$ 1547, $\nu_{sym(OCO)}$ 1422, $\Delta\nu_{asym-sym}$ 125, ν_{Sn-C} 540, ν_{Sn-O} 485. ^{1}H NMR δ (ppm): 3.92 {s, H_2, 4H}, 6.95 {d, H_4, 2H), 7.01 {dd, H_5, 2H}, 7.28–7.21 {d, H_6, 2H}, 1.72–1.25 {b, H_α, $H_{\alpha'}$, H_β, $H_{\beta'}$, H_γ, $H_{\gamma'}$, H_δ, 28H}, 0.91 {t, $H_{\delta'}$, 6H}. ^{13}C NMR δ (ppm): 180.4 (C_1), 35.6 (C_2), 135.5 (C_3), 125.7 (C_4), 126.8 (C_5), 124.9 (C_6), 25.5 (C_α), $^1J(^{119/117}Sn–^{13}C)$, [537/522 Hz], 24.4 (C_β), $^2J(^{119}Sn-^{13}C)$ [36 Hz], 33.2 (C_γ), $^3J(^{119/117}Sn-^{13}C)$ [93/78 Hz], 31.8 (C_δ), 29.3 ($C_{\alpha'}$), 29.0 ($C_{\beta'}$), 22.7 ($C_{\gamma'}$), 14.0 ($C_{\delta'}$).

2.4. *Biological assay*

2.4.1. Antibacterial activity. The organotin(IV) complexes were evaluated against different strains of both Gram-negative bacteria (*Escherichia coli* and *Shigella flexneri*) and Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). Ciprofloxacin was used as a standard for assessment of activity. The minimum inhibitory concentrations (MICs) were determined using the broth micro-dilution method, as recommended by the National Committee for Clinical Laboratory Standards. Bacterial strains stored in Muller-Hinton broth (Merck) were subcultured for assay in similar medium and allowed to grow at 37 °C. Then the cells were suspended in saline solution to produce a suspension of about 10^{-5} CFU mL^{-1} (colony-forming units per mL). Serial dilutions of organotin derivatives were prepared in tubes to final concentrations of 2.5, 1.25, 0.625, 0.313, and 0.156 μg/mL. 100 μL of a 24 h old inoculum was mixed in each test tube. The MIC value, defined as the lowest concentration of the test compound, which inhibited visible growth after 18 h, was determined visually after incubation for 24 h at 37 °C. Test tubes using DMF as negative control were carried out in parallel to compare the effect of the solvent.

2.4.2. *In vitro* inhibition studies of AChE and BuChE. The enzyme inhibition assays of acetyl- and butylcholinesterases were performed following Ellman's method [23]. These assays were carried out with some modifications as in Ingkaninan *et al.* method [24], by using 96 μL well plates. A series of newly synthesized organotin derivatives were tested as AChE and BuChE inhibitors. Initially, each compound was dissolved in DMSO (end concentration of DMSO was less than 1% in the assay) and tested at a final concentration of 1 mM and 10 μL of DMSO (as a negative control) in wells for initial screening. Organotin(IV) derivatives with considerable inhibition (more than 50%) were subjected to further analysis by preparing six to seven serial dilutions in an assay buffer (50 mM Tris–HCl, 0.1 M NaCl, and 0.02 M $MgCl_2 \cdot 6H_2O$ at pH 8.0). Reaction mixture comprised 50 μL of 3 mM DTNB (5,5′-dithiobis(2-nitrobenzoic acid)) in assay buffer, 10 μL of AChE (0.031 IU/ml) or BuChE (0.5 IU/mL) and 10 μL of test compound. The mixture was pre-incubated for 10 min at 25 °C. After pre-incubation, the enzymatic reaction was initiated by the addition of 10 μL of 10 mM BTCCl or ATCI according to the respective enzyme and the mixture was further incubated for 15 min more. The amount of enzymatic product formed was measured by using a micro-plate reader (Bio-TekELx 800^{TM}, Instruments, Inc. USA) by the change in absorbance at 405 nm. Enzyme dilution buffer was comprised of 50 mM Tris–HCl containing 0.1% (w/v) BSA (pH 8). The effect of DMSO on the activity of the enzyme was deduced by a negative control containing DMSO only, instead of inhibitor. Each concentration was assayed in triplicate and K_i values were calculated from IC_{50} values by using a non-linear curve fitting program PRISM 5.0 (GraphPad, San Diego, California, USA).

2.4.3. Antioxidant and DNA protection activity. Antioxidant and DNA protection activities of test comounds were determined by following standard procedures [25]. To the Eppendorf tube, 5 μl of stock solution of the test compound at 3000 ppm was poured; 3 μl of diluted plasmid DNA was then transferred to the Eppendorf tubes. Stock solutions gave 1000 ppm concentration in the final reaction mixture. Then 3 μl of 2 mM $FeSO_4$ and 4 μl of 30% H_2O_2 were added successively to each tube.

Treatment of pBR322 DNA with $FeSO_4$ and H_2O_2 was used as a positive control. The reaction mixture was incubated for one hour in the dark at 37 °C and plasmid DNA was run on 0.9% agarose gel. Agarose (0.9 g) was taken in 250 mL flask and then dissolved in 100 mL of 1X-TAE buffer and heated in a microwave oven to get a homogeneous mixture. After slight cooling to bearable temperature, 20 µl of staining agent ethidium bromide was added to agarose. After gentle shaking, the agarose was poured in the gel tray and allowed to solidify for 30 min. After solidification, the 1X TAE (gel running buffer) was poured to such a level that both of the electrodes submerged in the buffer. Bromophenol blue (loading dye) (3 µl) was added to each reaction mixture after incubation and then samples were loaded on 0.9% agarose gel wells containing TAE buffer. Each reaction mixture with controls was run horizontally in TAE buffer at 50 V and 30 mA for one hour in an electrophoresis apparatus. The gels were photographed under UV light. For each run, a molecular marker, a negative control, H_2O_2 treatment, $FeSO_4$ treatment, and test concentrations of CME were loaded.

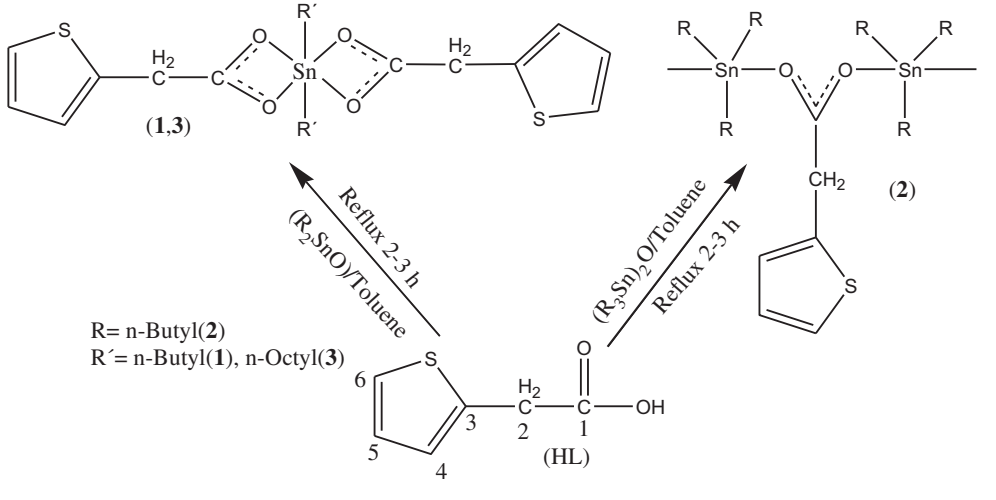
3. Results and discussion

3.1. *Synthesis of the complexes*

Di-*n*-butyltin oxide, bis(tributyltin oxide), and di-*n*-octyltin oxide were reacted with 2-thiopheneacetic acid (HL) in dry toluene in a molar ratio of 1 : 2, producing organotin(IV) complexes by azeotropic removal of H_2O from the reaction mixture (scheme 1).

3.2. *Infrared spectroscopy*

Complexes **1–3** have been assigned characteristic IR peaks according to previously reported values. The most important vibrational frequencies include $\nu_{asym(COO)}$, 1547–1574 cm^{-1}; $\nu_{sym(COO)}$, 1384–1422 cm^{-1}; ν(Sn–C), 540–577 cm^{-1}; and ν(Sn–O) 466–485 cm^{-1}. The carboxylate binding can be recognized by the parameter, $\Delta\nu$ [$\nu_{asym(COO)}$–$\nu_{sym(COO)}$]. The Δν



Scheme 1. Schematic diagram showing reaction between ligand acid and organotin(IV) oxides.

values for **1–3** come in the range $< 200\,cm^{-1}$, indicating bidentate carboxylate. The bidentate bonding can be evidenced by X-ray crystallographic study of **2** [26, 27].

3.3. *NMR spectroscopy*

Non-coordinating solvent ($CDCl_3$) was used to record ^1H and ^{13}C NMR spectra of the free ligand acid and **1–3** at room temperature. The probable molecular compositions of the organotin(IV) derivatives were confirmed by assigning the chemical shifts to protons by their multiplicity and integration values. Ligand protons showed a slightly different pattern than tin(IV) complexes. In spectra of the complexes, a multiplet and a clear triplet were observed for *R* that is, C_4H_9 and C_8H_{17} groups. The coupling constant values were not observed due to the complex pattern which is due to $(–CH_2–)_n$ framework [28]. However, a clear triplet due to terminal methyl appeared at 0.90–0.91 ppm.

In ^{13}C NMR, the coordination behavior of Sn in solution can be identified by calculating the coupling constant values which are important indicators for the structural elucidation of organotin(IV) carboxylates. The carbons attached to Sn have satellites due to ^nJ [$^{119}Sn/^{117}Sn–^{13}C$] coupling. Tributyltin(IV) derivative **2** revealed coupling constants ^1J[352/337 Hz], ^2J[20 Hz], and ^3J[64/50 Hz], which indicates a tetrahedral arrangement around tin due to dissociation of the polymeric tributyltin(IV) complex in solution [29]. In diorganotin(IV) derivatives, coordination around Sn can be predicted as skew trapezoidal as suggested from the coupling constant values ^1J[564 Hz], ^2J[23 Hz], ^3J[98 Hz] for **1** and ^1J[536/522 Hz], ^2J[36 Hz], ^3J[93/78 Hz] for **3**; these values are in accord with literature [30, 31].

3.4. *X-ray crystal structure of 2*

The crystal structure of **2** is shown in figure 1 with details of crystal data and structure refinement provided in tables 1 and 2. The crystal structure shows that Sn is coordinated

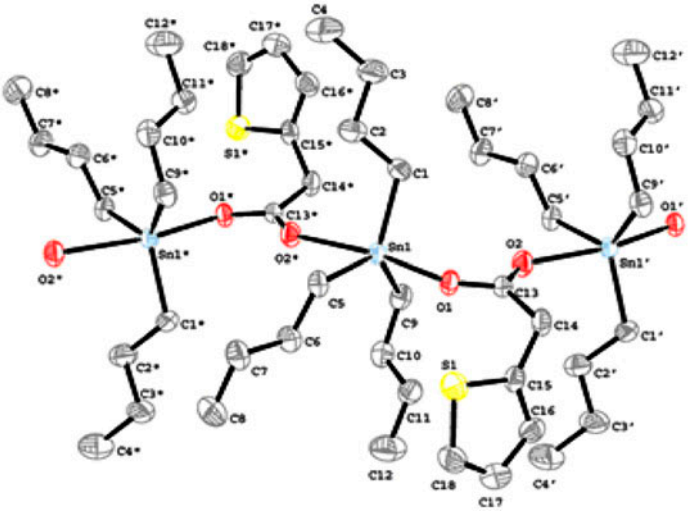


Figure 1. Crystal structure of $[Bu_3Sn(C_6H_5O_2S)]$ (**2**).

Table 2. Selected bond lengths (Å) and angles (°) of tri-*n*-butylstannyl(-2-thiopheneacetate).

Bond lengths (Å)	
Sn1–C1	2.138(3)
Sn1–C5	2.128(3)
Sn1–C9	2.143(3)
Sn1–O1	2.199(2)
Sn1–O2*	2.415(2)
Bond angles (°)	
C1–Sn1–C5	115.57(4)
C5–Sn1–C9	124.64(13)
C1–Sn1–C9	118.98(14)
C1–Sn1–O1	94.44(11)
C1–Sn1–O2*	92.86(10)
C5–Sn1–O1	88.40(10)
C5–Sn1–O2*	81.28(10)
C9–Sn1–O1	96.13(11)
C9–Sn1–O2*	87.25(10)
O2*–Sn1–O1	169.20((7)

with two oxygens of 2-thiophene acetate and it acquires a polymeric chain structure (figure 1). The Sn(1)–O(1) bond distance of 2.199(2) Å is significantly different from the Sn(1)–O(2)* bond distance of 2.415(2) Å, indicating that the ligand coordinates in anisobidentate manner. The Sn–C bond distances are almost identical within experimental error [2.138(3), 2.128(3), 2.143(3) å] and consistent with those reported for other triorganotin derivatives [32, 33]. The angles C(5)–Sn(1)–C(9), C(1)–Sn(1)–C(9), and C(1)–Sn(1)–C(5) with values of 124.64(13), 118.98(4) Å, and 115.57(4) Å, respectively, are in close agreement with the angle of 120° of a regular trigonal plane. Similarly, the C–Sn–O angles lie in the range of 81.28(10) to 96.13(11)° and the O(2)*–Sn(1)–O(1) angle is 169.20(5)°. This evidence suggests a description of the Sn environment as a trigonal bipyramid with O (1) and O(2) in the apical positions and the three butyl groups in the equatorial positions. The sum of the equatorial angles is 359.19° instead of the ideal 360° which indicates a slightly distorted bipyramidal geometry, as reported earlier [31]. The geometry around Sn can be characterized by $\tau = (\beta - \alpha)/60$ [31], where β is the largest, and α the second largest basal angle around tin. For complex (**2**) the angle (β = O(2)*–Sn(1)–O(1) is 169.20(5)o) and α = C(5)–Sn(1)–C(9) is 124.64(13)°. The τ value (0.74) indicates a distorted square-pyramidal geometry. The Sn–O(1) and Sn–O(2) bond lengths (2.199(2) and 2.415(2) Å) are less than the sum of van der Waals radii of Sn and O (3.68 Å).

3.5. *Biological screening*

3.5.1. Antibacterial activity. All tested organotin(IV) derivatives showed antibacterial activity against the tested strains except ligand acid and its salt. The activities are listed in figure 2. Against *E. coli*, **3** was the most efficient anti-bacterial agent with (MIC: 0.156 μg/mL) as potent *in vitro* as Ciprofloxacin (MIC 0.156 μg/mL). In the case of *B. subtilus*, **2** showed highest antibacterial activity with MIC value of 0.156 μg/mL, which is half of that of Ciproflaxacin (MIC: 0.313 μg/mL). Complexes **2** and **3** proved to be more potent than **1** for *S. aureus*, both having MIC values of 0.625 μg/mL. The activity of *S. flexneri*

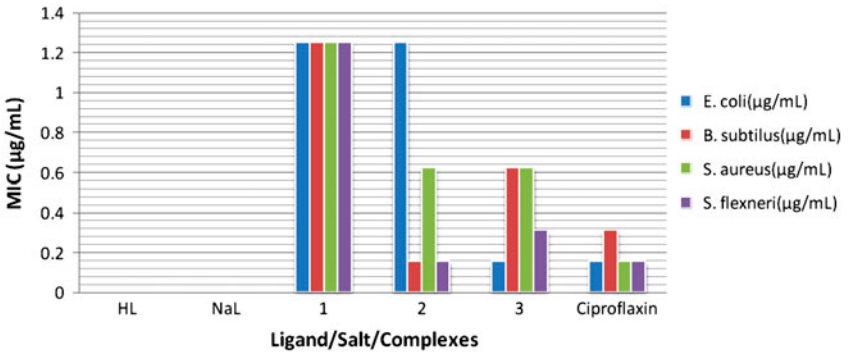


Figure 2. The antibacterial activity of tri- and diorganotin(IV) derivatives.

bacterial stain was reduced quite efficiently by **2** having MIC value of 0.156 µg/mL, which is exactly the same as that of the standard drug, i.e., 0.156 µg/mL.

3.5.2. *In vitro* inhibition studies of AChE and BuChE. The ligand acid and all of the three dimeric and polymeric complexes of **HL** have been evaluated against AChE and BuChE using spectrophotometric methods with neostigmine methylsulphate as a reference compound (table 3).

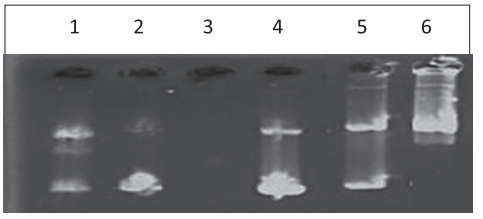
It is clear from the K_i values that the designed organotin(IV) derivatives have potent and particular inhibitory actions in the nanomolar range towards cholinesterases. Complex **3** containing two bulky octyl groups is the most potent compound against rabbit AChE with K_i of 0.07 ± 0.01 µM. The value of K_i depicts that **3** showed similar potency to that of the reference compound. A decrease in activity was observed for **1** having two butyl groups attached to a central tin. It showed a K_i of 0.13 ± 0.03 µM. However, a decrease in potency was observed for **2** having three butyl groups with K_i of 0.4 ± 0.2 µM. These complexes were also evaluated for their enzyme inhibition activity against BuChE and exhibited excellent potencies.

Complex **3** was the most potent inhibitor of BuChE with K_i of 0.05 ± 0.02 µM followed by **1** with K_i of 0.05 ± 0.005 µM, even more potent than the standard inhibitor used in the

Table 3. *In vitro* inhibition studies of AChE and BuChE.

No.	Comp. code	K_i value ± SEM (µM) or % inhibition[a] on acetylcholinesterase	K_i value ± SEM (µM) or % inhibition[a] on butyrylcholinesterase
1	HL	inactive	inactive
2	NaL	inactive	inactive
3	**1**	0.13 ± 0.03	0.05 ± 0.005
4	**2**	0.4 ± 0.2	0.05 ± 0.02
5	**3**	0.07 ± 0.01	2 ± 3
	Neostigmine methylsulfate (Standard)	0.056 ± 0.006	0.06 ± 0.005

[a]Values are expressed as the mean ± standard error of the mean of three experiments. K_i inhibitory concentration (µM) of AChE from Electrophorus Electricus (EeAChE) and BuChE from Horse serum (hBuChE).



Lane # 1 = control 1 (2 mM $FeSO_4$); Lane # 2 = control 2 (30% H_2O_2);

Lane # 3 = control 3 (2 mM $FeSO_4$ + 30% H_2O_2);

Lane # 4 = **2**; Lane # 5 = **3**; Lane # 6 = **1**

Figure 3. Effect of test compounds (100 ppm in DMSO) on plasmid DNA.

assay. Decrease in potency was observed for **3** having two dioctyl groups attached to tin with a K_i of 2 ± 3 μM.

3.5.3. Antioxidant and DNA protection activity. The test compounds were evaluated for DNA protection activity by using the DNA damage assay. The DNA protection activity was carried out using pBR322. A concentration of 1000 ppm, in DMSO, of the test compounds was used. Results are presented in figure 3. In this assay, the antioxidant activity of the test compounds was evaluated on DNA by observing an increase or loss of supercoiled monomer DNA. The experiment was carried out in the dark in order to avoid the effects of photoexcitation of the samples. Supercoiled DNA treated with 2 mM $FeSO_4$ + 30% H_2O_2 and untreated supercoiled DNA were used as controls along with tested samples. For control 2 mM $FeSO_4$ (Lane 1), 30% H_2O_2 (Lane 2), and both 2 mM $FeSO_4$ and 30% H_2O_2 (Lane 3) were used. Lane 4 to lane 6 indicate the test compounds. All of the test compounds have a certain level of DNA protection (Lanes 4–6). The H_2O_2 and $FeSO_4$ produce free radicals that can damage DNA, while organotin(IV) compounds prevent DNA damage and act as scavenger or antioxidants. Compound **1** (Lane 4) has maximum DNA protection as most of the plasmid DNA is retained in its supercoiled form. The second highest protection activity is shown by 3 (Lane 5) followed by 2 (Lane 6).

4. Conclusion

Three organotin(IV) complexes were synthesized by reacting 2-thiopheneacetic acid with di-*n*-butyltin oxide, bis(tributyltin oxide), and di-*n*-octyltin oxide. All of the newly synthesized complexes were characterized by FT-IR and NMR spectroscopy while the structure of **2** was confirmed by single crystal X-ray spectroscopy. Complex **3** has shown the most efficient anti-bacterial and enzyme inhibition activity against AChE and BuChE, while **1** (Lane 4) proved to be the most prominent scavenger or antioxidant.

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