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1 **Organotin(IV) based Anti-HCV drugs: Synthesis, Characterization and**
2 **Biochemical Activity**

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25 Abstract

26 Three new organotin(IV) carboxylates (1–3) of 3,5-dimethylbenzoate, have been synthesized and
27 characterized by elemental analysis, FT-IR, multinuclear NMR (^1H , ^{13}C and ^{119}Sn), mass
28 spectrometry and single crystal X-ray structural analysis. Crystallographic data show that in
29 compound **1** and **2**, the geometry at the central Sn atom is skew-trapezoidal bipyramidal while
30 compound **3** displays distorted trigonal bipyramidal coordination geometry. In case of compound
31 **1** and **2**, the asymmetric chelating mode of the carboxylate groups reflects in the unequal C-O
32 bond distances, those observed for the O1 and O3 oxygen atoms being significantly longer than
33 those found in the O2 and O4 atoms. In case of compound **3**, the carboxylate groups bridge
34 asymmetrically adjacent tin atoms in an anti-syn mode generating polymeric zig zag chains
35 running parallel to the crystallographic *c* axis.

36 The compounds were screened for the anti-HCV (hepatitis C Virus) potency by the Gaussia
37 luciferase Assay using infected Huh 7.5 cells (human hepatocellular cell). Structure-activity
38 relationship studies led to the identification of Dibutyltin(IV)bis(3,5-dimethylbenzoic acid)
39 (compound **1**) as a potent HCV inhibitor, with Log IC_{50} values 0.69 nM in the cell-based assay.
40 The compound **1** was further subjected to quantitative analysis using real-time PCR assays and
41 viral RNA count vs drug concentration confirmed the Gaussia Luciferase Assay results. HCV
42 RNA targeting mode of the compounds (1-3) were confirmed by compound-DNA interaction
43 study. The compounds (1–3)-DNA interaction was investigated by UV–vis spectroscopy and
44 viscometry. The hypochromic effect in spectroscopy evidenced intercalative mode of
45 interaction with the binding affinity in $1 > 3 > 2$ sequence.

46 *Keywords:* Organotin(IV) compounds, HCV, IC_{50} , luciferase assay, DNA, Binding energy
47 constant.

48 **Abstract**

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50 characterized by elemental analysis, FT-IR, multinuclear NMR (^1H , ^{13}C and ^{119}Sn), mass
51 spectrometry and single crystal X-ray structural analysis. Crystallographic data show that in
52 compound **1** and **2**, the geometry at the central Sn atom is skew-trapezoidal bipyramidal while
53 compound **3** displays distorted trigonal bipyramidal coordination geometry. In case of compound
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55 bond distances, those observed for the O1 and O3 oxygen atoms being significantly longer than
56 those found in the O2 and O4 atoms. In case of compound **3**, the carboxylate groups bridge
57 asymmetrically adjacent tin atoms in an anti-syn mode generating polymeric zig zag chains
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59 The compounds were screened for the anti-HCV potency by the Gaussia luciferase Assay using
60 infected Huh 7.5 cells (human hepatocellular cell). Structure-activity relationship studies led to
61 the identification of Dibutyltin(IV)bis(3,5-dimethylbenzoic acid) (compound **1**) as a potent HCV
62 inhibitor, with Log IC_{50} values 0.69 nM in the cell-based assay. The compound **1** was further
63 subjected to quantitative analysis using real-time PCR assays and viral RNA count vs drug
64 concentration confirmed the Gaussia Luciferase Assay results. HCV RNA targeting mode of the
65 compounds (1-3) were confirmed by compound-DNA interaction study. The compounds (1–3)-
66 DNA interaction was investigated by UV–vis spectroscopy and viscometry. The hypochromic
67 effect in spectroscopy evidenced intercalative mode of interaction with the binding affinity in $1 >$
68 $3 > 2$ sequence.

69

70

71 1. Introduction

72 Hepatitis C Virus (HCV) is a major health issue worldwide [1]. According to World Health
73 Organization (WHO), more than 200 million carriers of the virus are present in the world [2].
74 Combination therapy with pegylated interferon (PEG-IFN- α) and ribavirin (RBV) has markedly
75 improved the clinical outcome, but less than half of the patients with chronic hepatitis C can be
76 expected to respond favorably to the currently available agents [3]. Moreover, the present
77 standard therapy (associated pegylated interferon and Ribavirin) [4,5] does not ensure a
78 sustained virological response (SVR) in all genotypes [5]. The reappearance of circulating HCV-
79 RNA after the end of treatment is very frequent and causes frustration in patients undergoing a
80 full schedule of treatment with all related side effects. These problems raise the demand for the
81 development of more efficacious, virus-specific, and better tolerated HCV inhibitors [6]. The
82 advanced research revealed several targets in the HCV life cycle and its protein structural
83 features for the development of potential HCV inhibitors [7-9].

84 The purpose of chronic hepatitis C therapy is the eradication of the infection and prevention of
85 the cirrhosis development which may lead to hepatocellular carcinoma (HCC). Recently,
86 organotin(IV) compounds have been found to be potent HCV inhibitors [10]. Organotin(IV)
87 compounds have the ability to bind with RNA [11] and DNA [12] *via* electrostatic interaction of
88 the Sn(IV)⁺ moiety with the negatively charged oxygen of a phosphate group [11]. The single
89 strand RNA provides greater accessibility to the Sn atom for interaction with nitrogenous bases
90 as compared to double strand DNA and results in a more stable Sn–RNA adducts as compared to
91 Sn–DNA compounds. The greater availability of the RNA basis for interaction with the Sn atom
92 favors the interaction of the compound with viral RNA instead of human cell DNA in cell based
93 assays. The Sn–RNA interaction quenches the RNA and ceases the production of RNA-

94 dependent RNA polymerase NS5B - responsible for the replication of the viral genome [13].
95 Spectroscopic and electrochemical methods have proven to be more reliable and frequently
96 employed methods to study the interactions of metal compounds with DNA/RNA [14].

97 The development of HCV inhibitors requires continuous monitoring of HCV propagation in cell
98 culture (HCVcc). Gaussia luciferase Assay [15] provides the best option for monitoring HCV
99 propagation. Gaussia luciferase Assay has advantages over conventional methods for monitoring
100 gene expression as this technique is more sensitive [16], requires few microliters of blood or
101 urine, is time saving, does not involve any pretreatment and potential threat by exposing tissues
102 to photon [17]. The Gaussia luciferase Assay, also helps to evaluate drug effects on cell
103 proliferation, apoptosis, migration, and provides a higher throughput than time-consuming
104 animal experiments [18,19].

105 In the search of developing potent and more specific anti- HCV agent, organotin(IV) derivatives
106 of 3,5-dimethylbenzoic acid (HL) was prepared and characterized. In the formulation of a new
107 drug, the proliferation of the drug across the cell membrane plays a significant role. In the
108 present study, a small ligand with only polar group “carboxylic acid” is selected to formulate the
109 drug of maximum permeability through cell membranes.

110 2. Experimental

111 3,5-Dimethylbenzoic acid and organotin(IV) chlorides were purchased from Aldrich Chemical
112 (USA). Acetone, toluene, methanol, ethanol, n-hexane, and chloroform were obtained from
113 Merck (Germany) and were purified before use [20]. RiboLockTM Ribonuclease inhibitor and
114 RevertAidTM H Minus M-MuLV were purchased from Fermentas. Melting points of the
115 synthesized compounds were recorded by the electrothermal melting point apparatus MP-D
116 Mitamura Riken Kogyo (Japan) and are uncorrected. Elemental analysis was carried out using a

117 Leco CHNS- 932 analyzer. The IR spectra of the synthesized compounds were obtained using
118 KBr pellets on a Bio-Rad Excaliber FT-IR spectrophotometer. Mass spectral data were collected
119 on a Finnigan MAT-311A spectrometer. The ^1H and ^{13}C NMR spectra were recorded on a
120 Bruker ARX 300 MHz-X Spectrometer at room temperature operating at 300 and 75.3 MHz,
121 respectively. UV-visible spectrophotometric measurements were performed with a PerkinElmer
122 Lambda 20 double-beam spectrophotometer, with the help of UV WinLab software (25 °C).
123 Viscosity experiment was carried out using an Ubbelodhe Viscometer at 25 ± 1 °C. “RoboGene®
124 Hepatitis C virus Quantification Kit” (AJ Roboscreen GmbH, Leipzig Germany) was used for
125 RNA extraction and reverse transcription. QIAquick PCR Purification Kit (Qiagen, USA) was
126 used for the purification of RNA. The Jc1-FLAG2 (p7-nsGluc2A) RNA level inhibition was
127 measured by a Real Time PCR instrument, MyiQ2™ System BIO-RAD Thermal Cycler,
128 equipped with temperature probe (0.2 mL tube size).

129 **2.1 Synthesis of organotin(IV) compounds**

130 **2.1.1 Synthesis of Dibutyltin(IV)bis(3',5'-dimethylbenzoate) (1)**

131 Compound **1** was synthesized by refluxing a mixture of Bu_2SnCl_2 (1.01 g, 3.35 mmol), 3',5'-
132 dimethylbenzoic acid (1 g, 6.7 mmol) and triethylamin (1.8 mL, 6.7 mmol) in dry toluene under
133 reflux for 8 h. The filtrate was concentrated to dryness at reduced pressure and the product was
134 purified by recrystallization from chloroform and n-hexane (4:1v/v) mixture at room
135 temperature. M.p. 131-132 °C: Anal.Calc. C 58.65; H 6.77; Found: C (58.74); H (6.72). IR
136 ($4000\text{-}200\text{ cm}^{-1}$, KBr): 1555 ν (COasym) 1387 ν (COsym) 168 ($\Delta\nu$) 521 ν (Sn-C). ^1H NMR
137 (CDCl_3 , 300MHz) δ (ppm): 7.82 (s, 2H, 2/6); 7.24(s, 1H 4); 2.44(s, 6H 7/8); 0.92 (t, 7.2 terminal
138 methyl protons of n-Butyl); 1.38-1.85 (m, 6H of first 3 carbons of Butyl) ^{13}C NMR ($\text{CDCl}_3\text{-d}_3$,
139 75 MHz) δ ppm: 131(C1); 127(C2/6); 138(C3/5); 127(C4); 40(C7/8); 167(C9); (29.37, 27.07,

140 26.67, 13.53, n-Butyl carbons). EI-MS, m/z (%): [SnL]269 (69), [C₈H₉sn]⁺225 (28) ,
141 [C₉H₉O₂]⁺149 (23), [C₉H₉O]⁺133 (71), [C₈H₉]⁺105 (100), [C₆H₅]⁺77 (52), [C₄H₉]⁺57 (41). ¹¹⁹Sn
142 NMR (CDCl₃): -170 ppm.

143 2.1.2 Synthesis of Dimethyltin(IV)bis(3',5'-dimethylbenzoate) (2)

144 Compound **2** was synthesized in the same way as compound **1** by refluxing a mixture of
145 Me₂SnCl₂ (0.73 g, 3.35 mmol), 3',5'-dimethylbenzoic acid (1 g, 6.7 mmol) and triethylamin (1.8
146 mL, 6.7 mmol) in toluene. The filtrate was concentrated to dryness under reduced pressure and
147 the product was purified by recrystallization from chloroform and n-hexane (4:1v/v) mixture at
148 room temperature. M.p. 183-184 °C: Anal.Calc. C 58.97; H 3.69; Found: C (58.90); H (3.65). IR
149 (4000-200 cm⁻¹, KBr): 1567 v (COasym) 1425 v (COsym) 142 (Δv) 515 v (Sn-C). ¹H NMR
150 (CDCl₃,300 MHz) δ (ppm): 7.58 (s, 2H, 2/6); 7.21(s, 1H 4); 2.50(s, 6H 7/8); 0.83 (t, 6H methyl
151 protons) ²J[¹¹⁹Sn, ¹H] 78Hz. ¹³C NMR (CDCl₃-d₃, 75 MHz) δppm: 131(C1); 127(C2/6);
152 138(C3/5); 127(C4); 40(C7/8); 167(C9); 29.2 (¹J[¹¹⁹Sn, ¹³C] 622Hz methyl carbons). EI-MS,
153 m/z (%): [R₂SnCOOL]433 (17), [C₈H₉sn]⁺225 (23), [Rsn]⁺135 (15), [C₉H₉O₂]⁺149 (18),
154 [C₉H₉O]⁺133 (69), [C₈H₉]⁺105 (100), [C₆H₅]⁺77 (53). ¹¹⁹Sn NMR (CDCl₃): -135 ppm.

155 2.1.3 Synthesis of Trimethyltin(IV)(3',5'-dimethylbenzoate) (3)

156 Compound **3** was synthesized by refluxing an equimolar amount of 3',5'-dimethylbenzoic acid
157 (1 g, 6.7 mmol), Me₃SnCl (1.33 g, 6.7 mmol) and triethylamin (0.93 mL, 6.7 mmol) in dry
158 toluene. The filtrate was concentrated to dryness under reduced pressure and the product was
159 purified by recrystallization from a chloroform methanol (4:1v/v) mixture at room temperature.
160 M.p. 114-115 °C: Anal.Calc. C 45.85; H 5.73; Found: C (45.72); H (5.69). IR (4000-200 cm⁻¹,
161 KBr): 1566 v (COasym) 1424 v (COsym) 142 (Δv) 506 v (Sn-C). ¹H NMR (CDCl₃, 300MHz) δ
162 (ppm): 7.70 (s, 2H, 2/6); 7.25(s, 1H 4); 2.37(s, 6H 7/8); 0.10(s, 9H ²J[^{119/117}Sn, ¹H] 52, 58Hz

163 methyl protons). ^{13}C NMR ($\text{CDCl}_3\text{-d}_3$, 75 MHz) δ ppm: 131(C1); 127(C2/6); 138(C3/5);
164 127(C4); 40(C7/8); 167(C9); -2.27 ($^1J[^{119}\text{Sn}, ^{13}\text{C}]$ 368Hz methyl carbons). EI-MS, m/z
165 (%): $[\text{R}_2\text{SnCOOL}]299$ (32), $[\text{C}_8\text{H}_9\text{Sn}]^+225$ (24), $[\text{R}_2\text{Sn}]^+150$ (22), $[\text{Sn}]^+120$ (38), $[\text{C}_9\text{H}_9\text{O}_2]^+149$
166 (20), $[\text{C}_9\text{H}_9\text{O}]^+133$ (21), $[\text{C}_8\text{H}_9]^+105$ (100), $[\text{C}_6\text{H}_5]^+77$ (91). ^{119}Sn NMR (CDCl_3): 140 ppm.

167

168 2.2 Single-crystal X-ray analysis

169 Good quality single crystals of **1**, **2** and **3** were mounted on a Philips PW 1100, Bruker SMART
170 1000 CCD and Bruker APEX-II CCD diffractometer, respectively, equipped with graphite
171 monochromatized Mo $K\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) fine-focus sealed tubes. For **2** and **3** intensity
172 data were collected using ω scans while for **1** the $\omega/2\theta$ scan technique was used. Crystal data
173 were collected using the Bruker SMART [21] and APEX-II [22] programs for **2** and **3**
174 respectively, while the FEBO [23] system was used for **1**. Data refinement and reduction were
175 performed using the FEBO system for compound **1**, the Bruker SAINT-Plus software [21] for **2**
176 and the SAINT program [22] for **3**. Multi-scan absorption corrections were applied to the
177 intensities of **2** and **3** using SADABS [21,22] while a psi-scan correction [24] was applied to the
178 intensity data of **1**. The structures were solved by direct methods using the programs SHELXS-
179 97 [25] or SIR97 [26], and refined with full-matrix least-squares based on F^2 using program
180 SHELXL-97 [26]. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were
181 placed geometrically and refined using a riding model approximation. In **1** the C21, C22, C25
182 and C26 carbon atoms of the n-butyl chains were found to be disordered over two orientations
183 with a refined occupancy ratio of 0.701(4):0.299(4) for the major and minor components,
184 respectively. During the refinement, the C-C bond distances and the C...C 1-3 separations
185 involving the disordered atoms were restrained to be 1.54(1) and 2.53(1) \AA respectively, and the

186 anisotropic displacement parameters of pairs of atoms in both components were set equal with
187 the command EADP [26]. The molecular graphics and crystallographic illustrations were
188 prepared using the ORTEP-3 for Windows [27] and SCHAKAL-99 [28] programs. All relevant
189 crystallographic data and structure refinement parameters for **1**, **2** and **3** are summarized in Table
190 1. Selected bond lengths and angles for compounds **1**, **2** and **3** are listed in Tables 2, 3 and 4.

191 **2.3 Anti-HCV Activity of organotin(IV) compounds by the Gaussia Luciferase Assay**

192 **System**

193 **2.3.1 Virus stocks**

194 The antiviral activity of compounds is evaluated by the Gaussia luciferase assay system [15]. In
195 this assay, the fully-infectious HCVcc (HCV cell culture) viruses, Jc1FLAG2 (p7-nsGluc2A)
196 was used to infect the Huh 7.5 cells. Jc1FLAG2 (p7-nsGluc2A) is a monocistronic reporter virus
197 encoding the full-length infectious Jc1 genome with a second secreted Gaussia luciferase
198 reporter. This is a highly sensitive HCVcc reporter virus expressing secreted Gaussia luciferase
199 (Gluc), Jc1FLAG2 (p7-nsGluc2A). After inoculation, cultures were washed to remove Gluc
200 carryover, and luciferase second secretion was monitored as an indicator of viral replication.
201 Assay of luciferase activity in infected cell supernatants was used to monitor viral replication.

202 **2.3.2 Cell culture**

203 Huh7.5 cells were maintained in Dulbecco's modified Eagle's medium and incubated at 37 °C,
204 5% CO₂, and 100 % relative humidity. The compounds of 1 mg/mL strength in dimethyl
205 sulfoxide were used. The cells were infected (with or without inhibitors), with Jc1-FLAG2 (p7-
206 nsGluc2A) with MOI-0.1 (1E4 as median tissue culture infective dose [TCID₅₀]/well) in the
207 presence of the compound and the concentration of each compound used was between 1 nM to

208 1000 nM. Huh7.5 cells were incubated at 37 °C for 3 days, and luciferase activity measured
209 using the EnduRen substrate (Promega). Infectious units (TCID₅₀) were quantified by limiting
210 dilution titration on naive Huh7.5 cells.

211 Maximum activity (100% of control) and background were derived from control wells
212 containing DMSO alone or from uninfected wells, respectively. Add 100 µL of Lysis Buffer per
213 well (Renilla Luciferase Assay Lysis Buffer diluted 5:1 in water) and shifting to a 96-Well plate.
214 Store it on -80°C until it becomes ready to read on luminometer. 10 µL of each sample was
215 added to the luciferase plate with Renilla Luciferase Assay, Buffer and Renilla substrate. The
216 secreted Gaussia luciferase (Gluc) was measured on luminometer [29].

217 **2.3.3 Quantitative Assay**

218 The serum samples were collected from 56 patients suffering from HCV. All serum samples were
219 negative for hepatitis B virus surface antigen (HBsAg) but positive for anti-hepatitis C virus antibody
220 (anti-HCV ELISA). RNA was extracted from 140 µl of serum and it was eluted in 20 µL of
221 hybridization solution containing 20U/ml of Ribonuclease inhibitor.

222 A mixture of 7 µL of extracted viral RNA, 1 µL of Reverse NS4A Primer and 3 µL of
223 Diethylpyrocarbonate treated water were mixed gently using Microcentrifuge. Add mixture of 4
224 µL of 5X reaction buffer, 5 µL of RiboLockTM Ribonuclease Inhibitor (20 u/ µL) and 2 µL of
225 Deoxynucleotide Triphosphates (10 mM) in it. The mixture is centrifuged and incubated at 37 °C
226 for 5min. Add 1 µL of RevertAidTM H Minus M-MuLV in the mixture and incubate it at 42 °C
227 for 60 min followed by heating at 70 °C for 10 min. The final mixture is centrifuged in the
228 reaction tube and stored at – 20 °C for RT-PCR. For quantitative anti-HCV activity of the
229 compound **1**, 1000, 667, 444, 198, 132, 26 and 0 nM of compound **1** was added. The RNA level

230 inhibition was measured by the Real Time PCR instrument. The strips were placed in the Real
231 Time PCR instrument with ramping rate of 2.5 °C per search and the process was continued up
232 to 3 hours for complete amplification of cellular RNA. The RT-PCR uses the amplified signal of
233 product and measured as reaction progresses that are in “real time”.

234 **2.3.4 CT-DNA Interaction Study by UV-Visible Spectroscopy**

235 CT-DNA (50 mg) was dissolved by stirring for overnight in double deionized water (pH = 7.0)
236 and kept at 4 °C. The DNA solution in the buffer (20 mM Phosphate buffer “NaH₂PO₄-
237 Na₂HPO₄” pH = 7.2) gave a ratio of 1.8 in UV absorbance at 260 and 280 nm (A_{260}/A_{280}), which
238 indicates protein free DNA [30]. The DNA concentration was determined via absorption
239 spectroscopy using the molar absorption coefficient of 6,600 M⁻¹ cm⁻¹ (260 nm) for the DNA
240 [31] and was found to be 1.4 × 10⁻⁴ M. The solutions of the compounds of 0.2 mM strength were
241 prepared in 80% ethanol. These stock solutions were used to form 9, 18, 27, 36, 45, 54 and 63
242 μM working solutions by dilution method. The UV absorption titrations were performed by
243 keeping the concentration of the compound fixed while varying the DNA concentration.
244 Equivalent solutions of DNA were added to the compound and reference solutions to eliminate
245 the absorbance of DNA itself. Compound -DNA solutions were allowed to incubate for about 10
246 min at room temperature before measurements were made. The stability of the binding properties
247 of the compounds studied towards DNA was examined by taking spectra after 24 and 48 h, and
248 the same results were obtained.

249 **2.3.5 Viscosity Measurements**

250 For viscosity experiment, flow time was measured with a digital stopwatch and each sample was
251 measured three times. Data were presented as $(\eta/\eta_0)^{1/3}$ vs. binding ratio (r) of
252 [compound]/[DNA], where η and η_0 are the relative viscosity of DNA in the presence of

253 compound and without compound. Viscosity values were calculated from the observed flow time
254 of DNA-containing solutions corrected for the flow time (t_0) of 20 mM phosphate buffer solution
255 (pH 7.2) alone. The viscosity for DNA in the presence and absence of the compound was
256 calculated from the following equations [32];

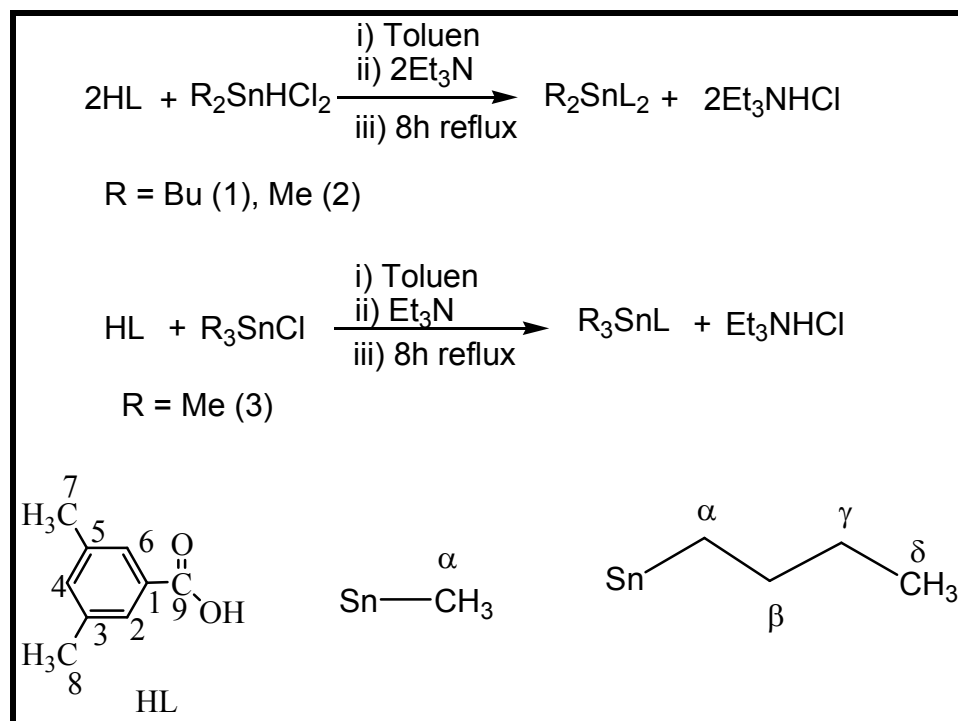
$$257 \quad \eta_{sp} = t - t_0$$

258 and

$$259 \quad \eta_{sp} = \frac{t - t_0}{t_0}$$

260 3. Results and Discussion

261 3,5-Dimethylbenzoic acid (HL), triethylamine and diorganotin(IV) chlorides solution (2:1 molar
262 ratios)/ triorganotin(IV) chloride (1:1 molar ratio) were mixed with anhydrous toluene (100 mL)
263 and the reaction mixture was refluxed for 8h. Triethylamine hydrochloride was removed by
264 filtration and the synthesized organotin(IV) derivatives were obtained in vacuum by removing
265 solvents. The general procedures for the synthesis of organotin(IV) compounds **1**, **2** and **3** are
266 shown in scheme 1.

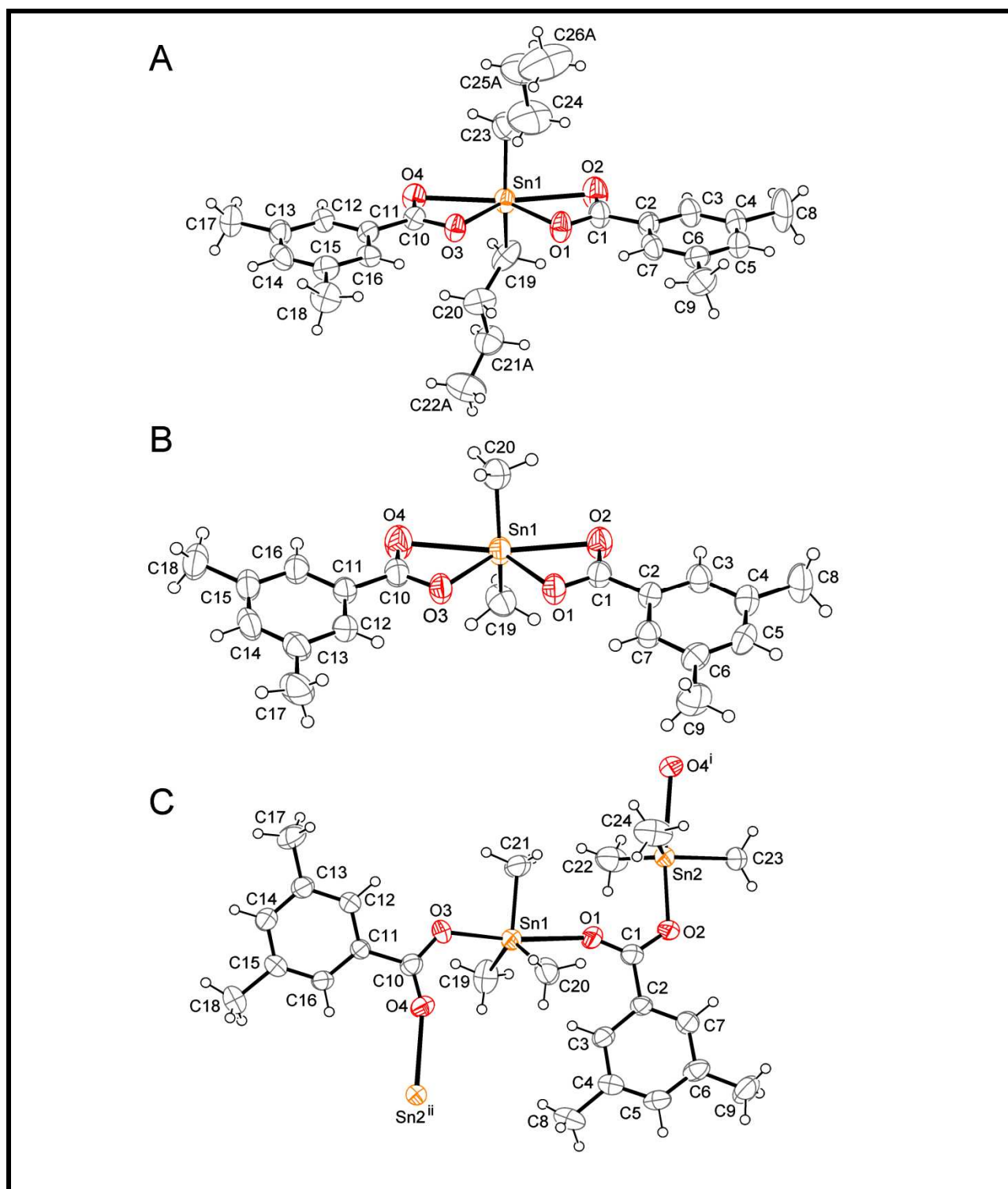


Scheme 1. General procedures for the synthesis of organotin(IV) compounds

3.1 X-ray crystallography

The molecular structure of compound **1**, **2** and **3** is shown in Fig. 1A, 1B and 1C respectively. In compound **1** and **2**, the carboxylate group COO^- of the ligands is bonded in an anisobidentate mode with two shorter bonds (Sn1-O1 and Sn1-O3 , mean values 2.127(7) Å) and two longer bonds (Sn1-O2 and Sn1-O4 , mean value 2.507(18) Å) as reported in Table 2 and 3. The geometry at the central Sn atom is skew-trapezoidal bipyramidal where the equatorial plane is defined by four oxygen atoms of the two chelating carboxylate ligands and the apical positions are occupied by the carbon atoms of two n-butyl (in **1**) or methyl (in **2**) groups. The longer Sn-O distances are remarkably shorter than the sum of the van der Waal's radii (3.68 Å) [33] in both cases. The dialkyltin fragments are arched along the longed edge of the equatorial trapezoid defined by the chelating atoms, the C-Sn-C angles

281 (148.50(16) and 148.67(11)° for compound **1** and **2**, respectively) falling in the range (122.6-
282 156.9°) observed for a skew-trapezoidal bipyramidal geometry [34,35].



283 Fig. 1. ORTEP drawings of the asymmetric units of (A) compound **1**, (B) compound **2** and (C)
284 compound **3** with displacement ellipsoids drawn at the 50% probability level. In (A) only the

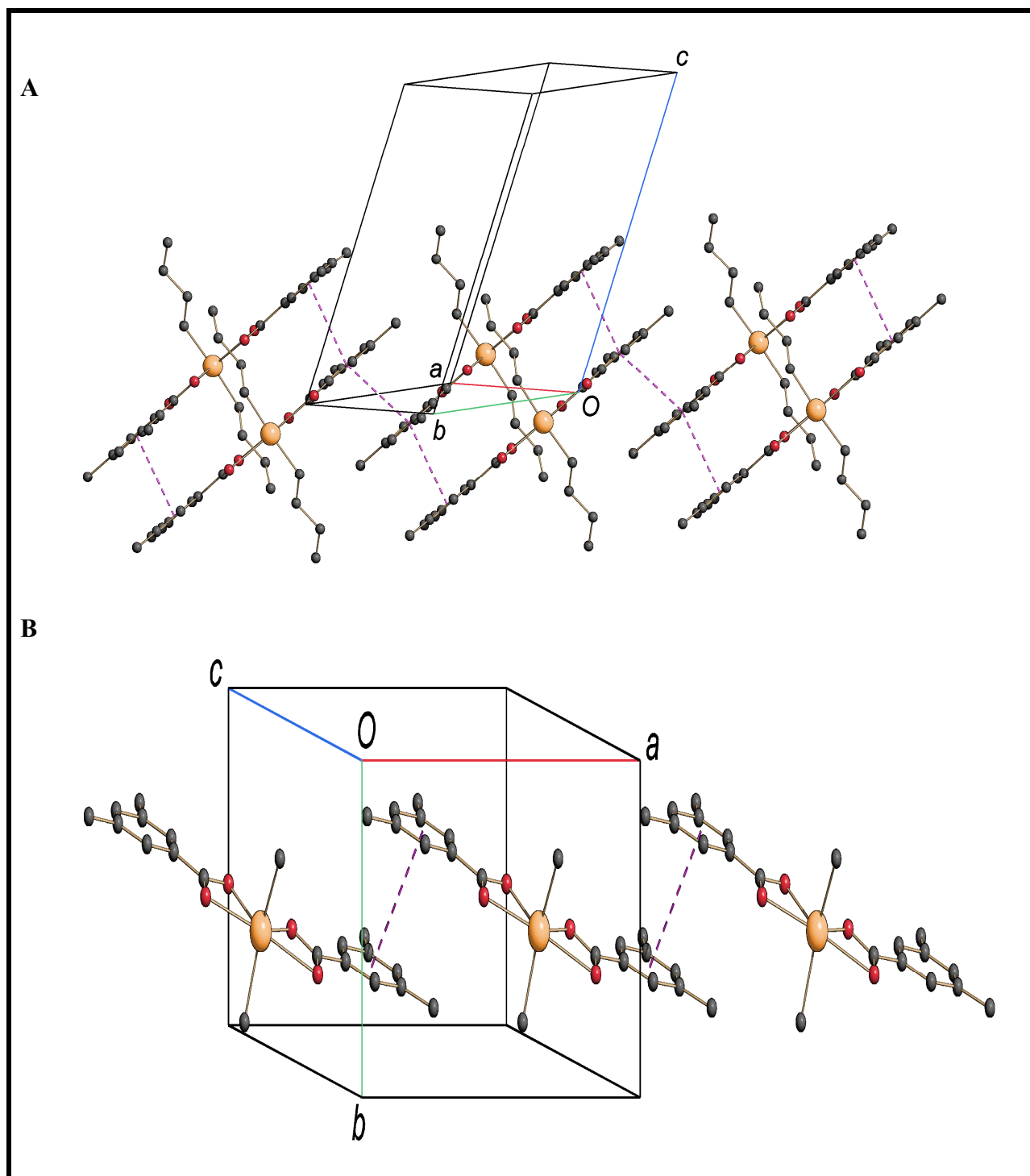
285 major components of the disordered n-butyl groups are shown. Symmetry codes for (C): (i) $x,$
286 $1/2-y, -1/2+z;$ (ii) $x, 1/2-y, 1/2-z.$

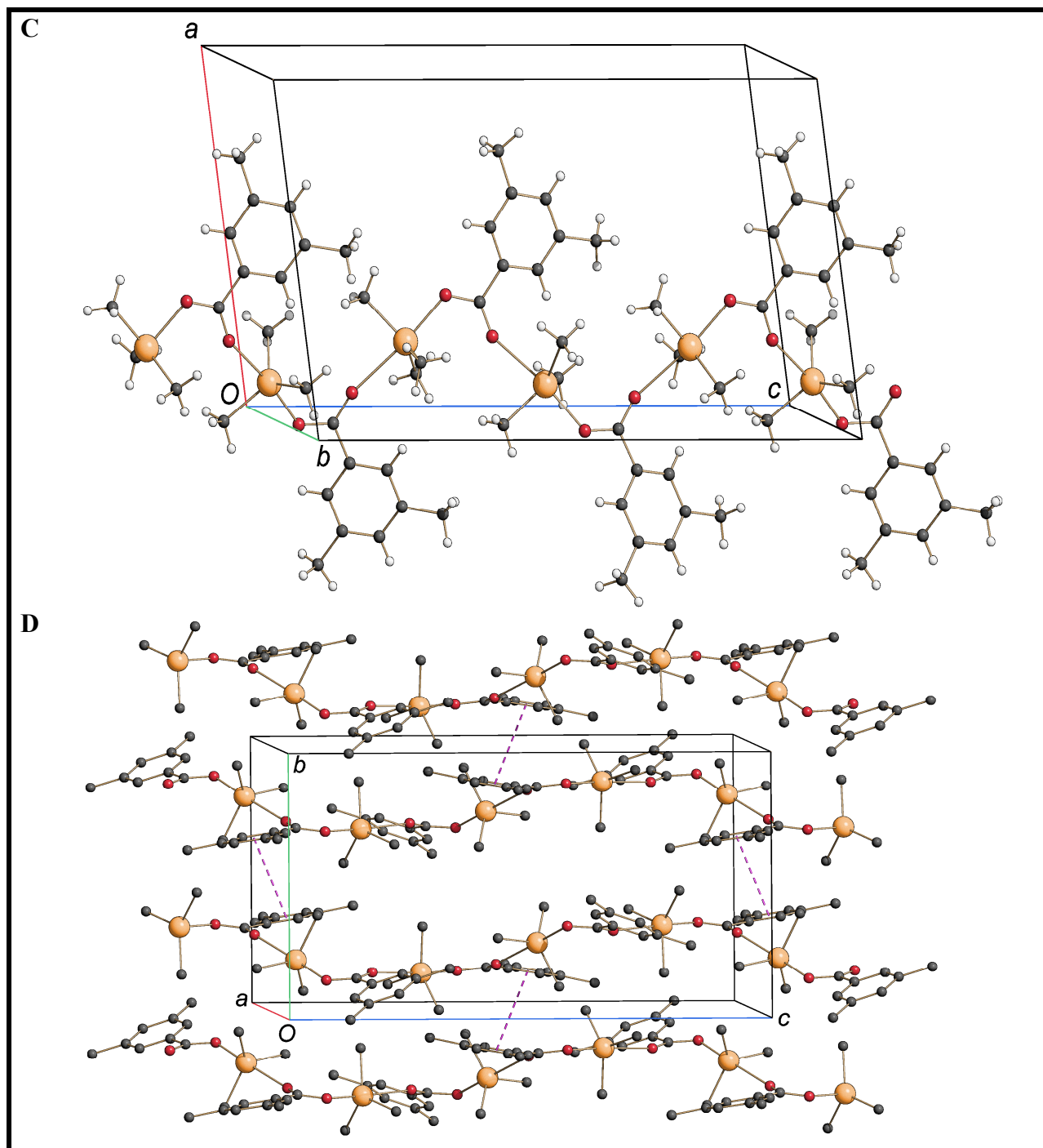
287 The asymmetric chelating mode of the carboxylate groups reflects also in the unequal C-O bond
288 distances, those observed for the O1 and O3 oxygen atoms (mean value 1.287 (3) Å) being
289 significantly longer than those found in the O2 and O4 atoms (mean value 1.244(3) Å) involved
290 in the longer Sn-O interactions.

291 For both compounds **1** and **2** $\pi\cdots\pi$ stacking interactions are effective in stabilizing the crystal
292 structure. In **1**, centrosymmetrically related molecules are linked by pairs of $\pi\cdots\pi$ interactions
293 (centroid-to-centroid distance 3.765(2) Å) into dimers, which are further connected by $\pi\cdots\pi$
294 contacts (centroid-to-centroid distance 3.750(2) Å) to form ribbons parallel to the (110) direction
295 (Fig. 2A) whereas in **2** molecules are linked by $\pi\cdots\pi$ interactions (centroid-to-centroid distance
296 3.8608(15) Å) into chains extending along the (100) direction (Fig. 2B).

297 The asymmetric unit of compound **3** consists of two independent trimethyltin groups and two
298 carboxylate ligands. Each metal atom displays a distorted trigonal bipyramidal coordination
299 geometry, with the methyl carbon atoms forming the equatorial plane and the oxygen atoms of
300 different carboxylate groups occupying the apical positions. The Sn-O bond lengths are
301 remarkably different (Table 4) and in agreement with those reported in the literature for
302 triorganotin(IV) carboxylates [36,37]. The distortion of the coordination polyhedra may be
303 inferred by the deviation from the ideal geometry of the O-Sn-O (171.94(14) and 172.61(13)°)
304 and C-Sn-C angles (115.8(3)-123.9(3)°). The carboxylate groups bridge asymmetrically adjacent
305 tin atoms in an anti-syn mode generating polymeric zig zag chains running parallel to the
306 crystallographic c axis (Fig. 2C). The polymeric bridging behavior is comparable with that
307 observed for related compounds [33]. In the crystal structure (Fig. 2D), the polymeric chains are

308 linked into a three-dimensional network by π ... π stacking interactions (centroid-to-centroid
309 distance 3.708(3) Å).





310 Fig. 2 (A). Partial crystal packing of 1 showing the formation of a molecular ribbon parallel to
311 the (110) direction through π ... π stacking interactions (dashed lines) while Hydrogen atoms are
312 omitted for clarity. Only the major components of the disordered n-butyl groups are shown. (B).
313 Partial crystal packing of 2, showing the formation of a molecular chain parallel to the *a* axis
314 through π ... π stacking interactions (dashed lines). Hydrogen atoms are omitted for clarity. (C).
315 The polymeric chain of 3 extending along the *c* axis. (D). Crystal packing of 3 showing chains
316 connected by π ... π stacking interactions (dashed lines) into a three-dimensional network.
317 Hydrogen atoms are omitted for clarity.

318

Table 1: Crystal data and structure refinement parameters for compounds 1, 2 and 3

	1	2	3
Emp. formula	C ₂₆ H ₃₆ O ₄ Sn	C ₂₀ H ₂₄ O ₄ Sn	C ₂₄ H ₃₆ O ₄ Sn ₂
Formula weight	531.24	447.08	625.91
Crystal system	Triclinic	monoclinic	Monoclinic
Space group	<i>P</i> -1	<i>P</i> 2 ₁ / <i>c</i>	<i>P</i> 2 ₁ / <i>c</i>
<i>a</i> (Å)	9.566(3)	11.540(4)	12.041(3)
<i>b</i> (Å)	11.892(5)	7.298(2)	11.466(3)
<i>c</i> (Å)	13.438(5)	24.778(8)	20.435(5)
α (°)	104.257(6)	90	90
β (°)	99.468(6)	102.918(5)	99.367(4)
γ (°)	109.702(8)	90	90
<i>V</i> (Å ³)	1342.6(9)	2034.0(11)	2783.7(12)
<i>Z</i>	2	4	4
Crystal size (mm)	0.21x0.18x0.06	0.22x 0.16x 0.07	0.18 x 0.10 x 0.07
Crystal habit	Plate	plate	Block
T (K)	295(2)	295(2)	294(2)
μ (mm ⁻¹)	0.977	1.275	1.818
λ(MoK _α) (Å)	0.71073	0.71073	0.71073
Total reflections	7836	23318	25358
Independent reflections	7823	4660	5023
Final R indices [<i>I</i> >2σ (<i>I</i>)]	R1 = 0.0423 wR2 =0.1058	R1 = 0.0267 wR2 =0.0678	R1 = 0.0335 wR2 =0.0666
R indices (all data)	R1 = 0.0618 wR2 =0.1126	R1=0.0312 wR2 =0.0710	R1 = 0.0876 wR2 =0.0817
Goodness-of-fit	0.970	1.097	0.938
θ range for data collections (°)	3.19-30.00	1.69-27.52	1.71- 25.26
Data/restraints/parameters	7823/18/297	4660/0/232	5023/0/ 281

319

320

321

Table 2: Selected bond lengths (Å) and bond angles (°) for 1

Sn1-O1	2.141(2)	Sn1-C23	2.127(4)
Sn1-O2	2.509(3)	O1-C1	1.289(4)
Sn1-O3	2.141(2)	O2-C1	1.251(4)
Sn1-O4	2.480(2)	O3-C10	1.274(4)
Sn1-C19	2.105(4)	O4-C10	1.254(3)
O1-Sn1-O2	55.75(9)	O2-Sn1-C23	86.78(14)
O1-Sn1-O3	84.63(9)	O3-Sn1-O4	55.81(8)
O1-Sn1-O4	140.43(8)	O3-Sn1-C19	103.51(13)
O1-Sn1-C19	99.47(13)	O3-Sn1-C23	102.01(14)
O1-Sn1-C23	101.01(14)	O4-Sn1-C19	89.77(13)
O2-Sn1-O3	140.38(9)	O4-Sn1-C23	89.49(13)
O2-Sn1-O4	163.80(8)	C19-Sn1-C23	148.50(16)
O2-Sn1-C19	85.25(14)		

322

323

Table 3: Selected bond lengths (Å) and bond angles (°) for 2

Sn1-O1	2.1353(15)	Sn1-C20	2.095(3)
Sn1-O2	2.4819(17)	O1-C1	1.287(2)
Sn1-O3	2.1120(17)	O2-C1	1.245(2)
Sn1-O4	2.5483(18)	O3-C10	1.292(3)
Sn1-C19	2.095(3)	O4-C10	1.237(3)
O1-Sn1-O2	55.82(5)	O2-Sn1-C20	89.31(9)
O1-Sn1-O3	83.32(6)	O3-Sn1-O4	55.16(6)
O1-Sn1-O4	138.48(5)	O3-Sn1-C19	99.94(9)
O1-Sn1-C19	102.35(10)	O3-Sn1-C20	100.97(9)
O1-Sn1-C20	103.03(10)	O4-Sn1-C19	87.07(9)
O2-Sn1-O3	139.15(6)	O4-Sn1-C20	86.01(9)
O2-Sn1-O4	165.68(5)	C19-Sn1-C20	148.67(11)
O2-Sn1-C19	89.95(9)		

324

Table 4: Selected bond lengths (Å) and bond angles (°) for 3

325	Sn1-O1	2.517(4)	Sn2-O2	2.130(3)
326	Sn1-O3	2.134(4)	Sn2-O4 ⁱ	2.607(4)
327	Sn1-C19	2.107(7)	Sn2-C22	2.094(6)
328	Sn1-C20	2.096(6)	Sn2-C23	2.106(6)
329	Sn1-C21	2.100(6)	Sn2-C24	2.075(6)
330	O1-C1	1.229(6)	O3-C10	1.278(6)
331	O2-C1	1.280(6)	O4-C10	1.231(6)
332	O1-Sn1-O3	171.94(14)	O2-Sn2- O4 ⁱ	172.61(13)
333	O1-Sn1-C19	87.1(2)	O2-Sn2-C22	98.5(2)
334	O1-Sn1-C20	86.6(2)	O2-Sn2-C23	89.52(19)
335	O1-Sn1-C21	82.42(19)	O2-Sn2-C24	98.1(2)
336	O3-Sn1-C19	93.8(2)	O4 ⁱ -Sn2-C22	88.0(2)
337	O3-Sn1-C20	99.4(2)	O4 ⁱ -Sn2-C23	84.53(19)
338	O3-Sn1-C21	90.1(2)	O4 ⁱ -Sn2-C24	80.8(2)
339	C19-Sn1-C20	123.9(3)	C22-Sn2-C23	115.8(3)
340	C19-Sn1-C21	117.5(3)	C22-Sn2-C24	124.4(3)
	C20-Sn1-C21	116.7(3)	C23-Sn2-C24	116.9(3)

341 3.2 NMR

342 The ¹H and ¹³C spectra were recorded in deuterated chloroform and the data of alkyl-tin species,
343 chemical shift values are deducible from the multiplicity pattern and resonance intensities. The
344 integration values obtained from the resulting spectra are in good agreement with the proposed
345 structures. In the ¹H NMR spectra of the compounds, the complete absence of acidic proton
346 signals suggests the deprotonation of acid and coordination mode to the tin through the oxygen
347 atom of the ligand [38]. ⁿJ(Sn, H) couplings were not observed for the compound **1** due to the
348 complex nature of the n-Bu group protons. The ¹H NMR spectrum of **3** showed a singlet for

349 $\text{CH}_3\text{-Sn}$, having a ${}^2J(^{119/117}\text{Sn-}^1\text{H})$ value (52 and 58 Hz) suggesting a pentacoordinated structure
350 while for compound **2**, ${}^2J(^{119}\text{Sn-}^1\text{H})$ value is found 78 Hz which confirmed the octahedral
351 geometry around tin [38].

352 The ${}^{13}\text{C}$ NMR spectra of the compound support the ${}^1\text{H}$ NMR data. In ${}^{13}\text{C}$ NMR, the Me and n-Bu
353 groups attached to Sn atom signals are found in expected region. The shift is an outcome of an
354 electron density transfer from the ligand to the tin atom. The coupling constants ${}^nJ(^{119}\text{Sn-}^{13}\text{C})$ is
355 one of the important parameters for the structure elucidation of organotin(IV) compounds. The 1J
356 (${}^{119}\text{Sn-}^{13}\text{C}$) coupling constants for the compound **3** shows the pentacoordination number around
357 the tin suggesting trigonal-bipyramidal geometry [38] while ${}^1J(^{119}\text{Sn}, {}^{13}\text{C})$ value for compound **2**
358 suggested octahedral geometry around the tin atom.

359 The ${}^{119}\text{Sn}$ NMR spectra of the compounds are in accordance with proposed one. ${}^{119}\text{Sn}$ chemical
360 shift for compound **1** and **2** are similar as for five or six coordinated environment around the tin
361 atom, while for compound **3** is similar as for four coordinated environment around the tin atom
362 and consistency with literature values [38].

363 **3.3 Anti-HCV Study**

364 **3.3.1 Gaussia Luciferase Assay**

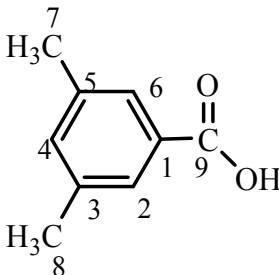
365 The Gaussia Luciferase Assay System was used to study the anti-HCV activity of the
366 organotin(IV) compounds and dose vs response curve are plotted in 2A. In this assay Jc1FLAG2
367 (p7-nsGluc2A) [39] was used to infect the Huh 7.5 cells. The $\log\text{IC}_{50}$ of the tested compounds
368 are summarized in the Table 5 and plotted in Fig. 3B.

369 The dose-response curve in Fig. 3A shows that the viral inhibition depends upon the
370 coordination number of the tin atom, nature and structure of the compound. Among the tested
371 organotin(IV) carboxylates, the n-butyltin(IV) derivative was found more potent against HCV

372 than the methyl derivatives due to its optimal balance among cytotoxicity, solubility, and
373 lypophilicity [40].

374 In organotin(IV) compounds, the role of the ligand and the symmetry of molecules in solution is
375 important in defining the activity of the compound as ligand in a complex is responsible for the
376 transportation of the organotin(IV) species to the site of the action. Previously, we have been
377 working on determining the importance of ligands and substitution on these ligands in anti-HCV
378 activity of the compounds [41]. Optimization of ligand suggests the use of the ligand with the
379 least numbers of polar groups for improvement of activity. In the present case, a symmetrical and
380 small size legend with carboxylate as the only polar group was selected. The small size of the
381 ligand enhanced the activity of the compounds by facilitating an increased cellular uptake of
382 these compounds and the approach of the molecules to the binding site in living systems.

383 **Table 5. Gaussia Luciferase Assay and DNA-organotin(IV) interaction parameters**

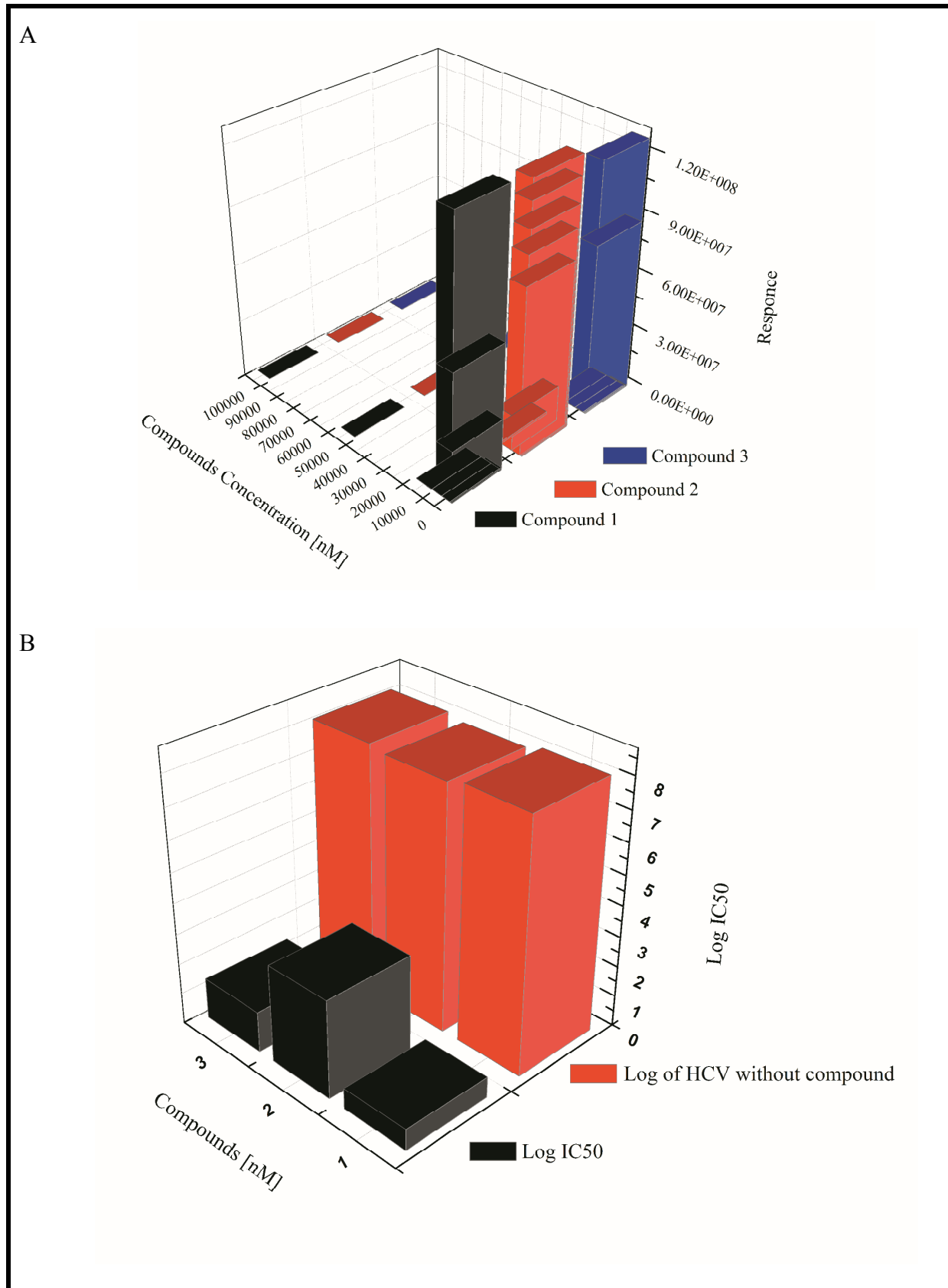
Comp	Ligand	Gaussia Luciferase Assay		Compounds-DNA Interaction Parameters by Spectroscopy	
		R	Log IC ₅₀ (nM)	K/M ⁻¹	-ΔG/kJmol ⁻¹
1		2Bu	0.69	4.35 × 10 ⁴	26.5
2		2Me	3.20	1.04 × 10 ⁴	22.9
3		3Me	1.34	1.34 × 10 ⁴	23.5
4		Telaprevir		2.4	

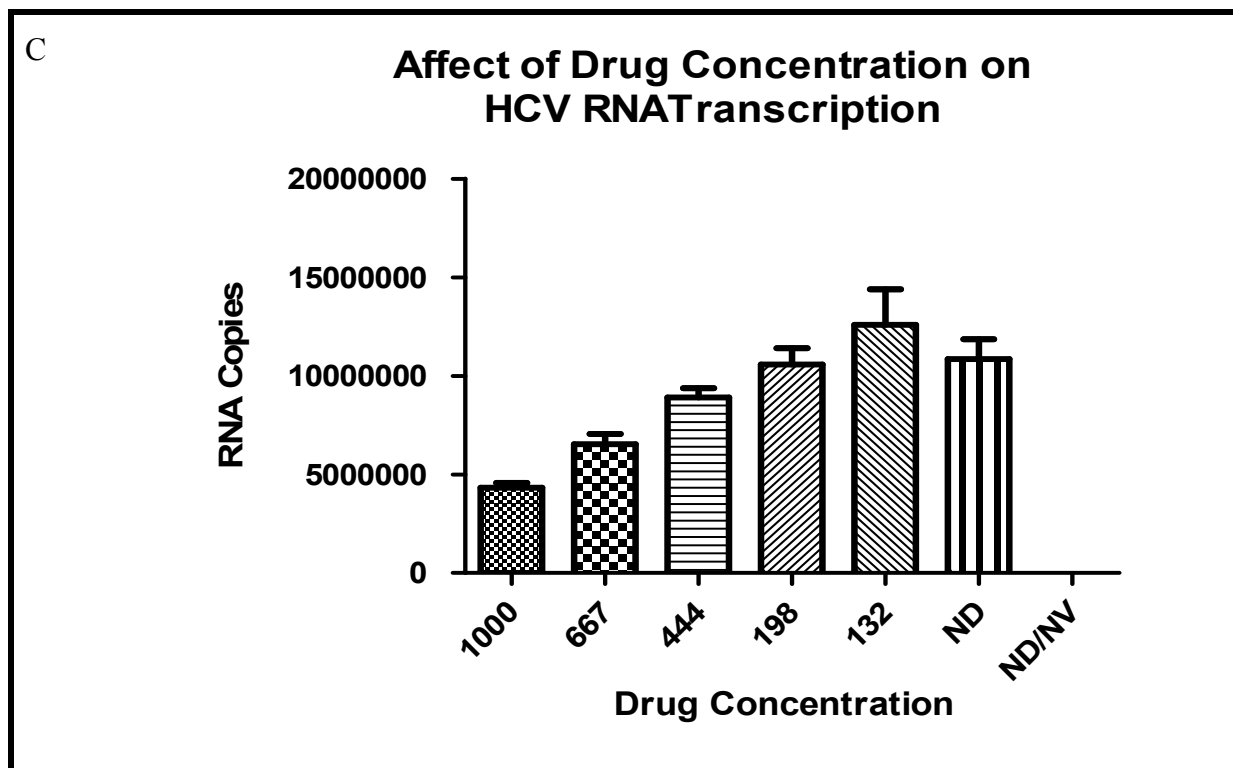
384
385 Organotin(V) moieties are selected for this study due to their RNA binding capabilities. The
386 targeting viral RNA with drug of novel structure in HCV treatment has advantages over targeting

387 proteins as the appearance of drug resistance by point mutations in an RNA motif is slow and the
388 resistance developing capability can be easily overcome.

389 Among the tested organotin(IV) compounds, compound **1** shows the highest anti-HCV activity
390 with a $\log IC_{50}$ value of 0.69. The dibutyltin(IV) moiety was replaced with the dimethyltin(IV)
391 moiety to achieve a more simple and small size molecule, but the butyl group replacement
392 reduced the anti-HCV activity of compound **2** by 4.64 times. The decrease in potency of
393 compound **2** (3.20) may be attributed to the lowered lipophilicity of the resultant compound. The
394 organotin(IV) moiety was further optimized by replacing the dimethyltin(IV) moiety with the
395 trimethyltin(IV) moiety. This replacement provided compound **3** which showed an activity
396 increased by a factor 2.4 with respect to compound **2**. In triorganotin(IV) moieties, five-
397 membered stereochemistry around the tin metal make, it is more easily available to interact
398 with biological systems through the unoccupied sixth position [42]. Compound **1** showed a
399 doubled potency against HCV as compared to compound **3**, which can be attributed to its higher
400 lipophilicity.

401





402 Fig.3. (A) Dose Vs Response curve for Compounds 1, 2 and 3 obtain from Gaussia Luciferase
 403 Assay System (B) LogIC_{50} of compounds 1, 2 and 3 (C) Dose response curve for compound1
 404 from the Real Time PCR instrument

405 3.3.2 Quantitative Analysis of compound 1

406 On the basis of Gaussia Luciferase Assay results, compound 1 was selected for quantitative
 407 analysis by quantitative Real-Time PCR. Initially, the minimum and maximum concentrations
 408 (1000 nM and 10 nM of the compound 1) were used for quantitative analysis to measure viral
 409 titers of compound1 with Jc1FLAG-2. Samples were run through PCR to evaluate the effect of
 410 compound 1 on HCV RNA. The quantitative Real-Time PCR data are plotted in Fig. 3C as RNA
 411 Vs drug concentrations. The RNA Vs drug concentrations authenticated the decrease in viral
 412 replication by compound 1. Compound 1 showed a gradual response between the effective
 413 concentrations and inhibitory activity (Fig. 3C). The RNA Vs drug concentrations showed that
 414 compound 1 is effective even at low concentration and the inhibition of HCV was steady with
 415 time.

416

417

Table 6. IC₅₀ for compound1 by QRT-PCR

IC ₅₀ (nM)	Span	R ²
3.245±0.5668	40862	0.9531

418

419 Different statistical tools were used for determination of the effective drug concentrations using
 420 the QRT-PCR data. The statistical analysis suggested the IC₅₀ value 3.24 nM with standard error
 421 of 0.5 (Table 6). This inhibition concentration is consistent with a previously determined IC₅₀
 422 value from Gaussia Luciferase Assay for compound 1.

423 The cytotoxic effects of compound 1 at different concentrations show that the number
 424 of cells was greatly reduced by compound 1, whereas the numbers of cells were intact in
 425 the controls of ND/NV (p <0.05). Total cell counts were determined by the Trypan blue
 426 exclusion method using a hemocytometer. The effect of 1000nM concentration of compound 1
 427 after 24 and 48h of incubation (Table 7), suggest it a future candidate for lowering viral
 428 replication.

429

Table 7. Cell viability Assay for compound 1

Sample	0 Hrs*	24 Hrs*	48 Hrs*
ND/NV	1000,000	1200,000	1500,000
Compound 1	1000,000	550,000	110,000
No Drug	1000,000	1100,000	1400,000

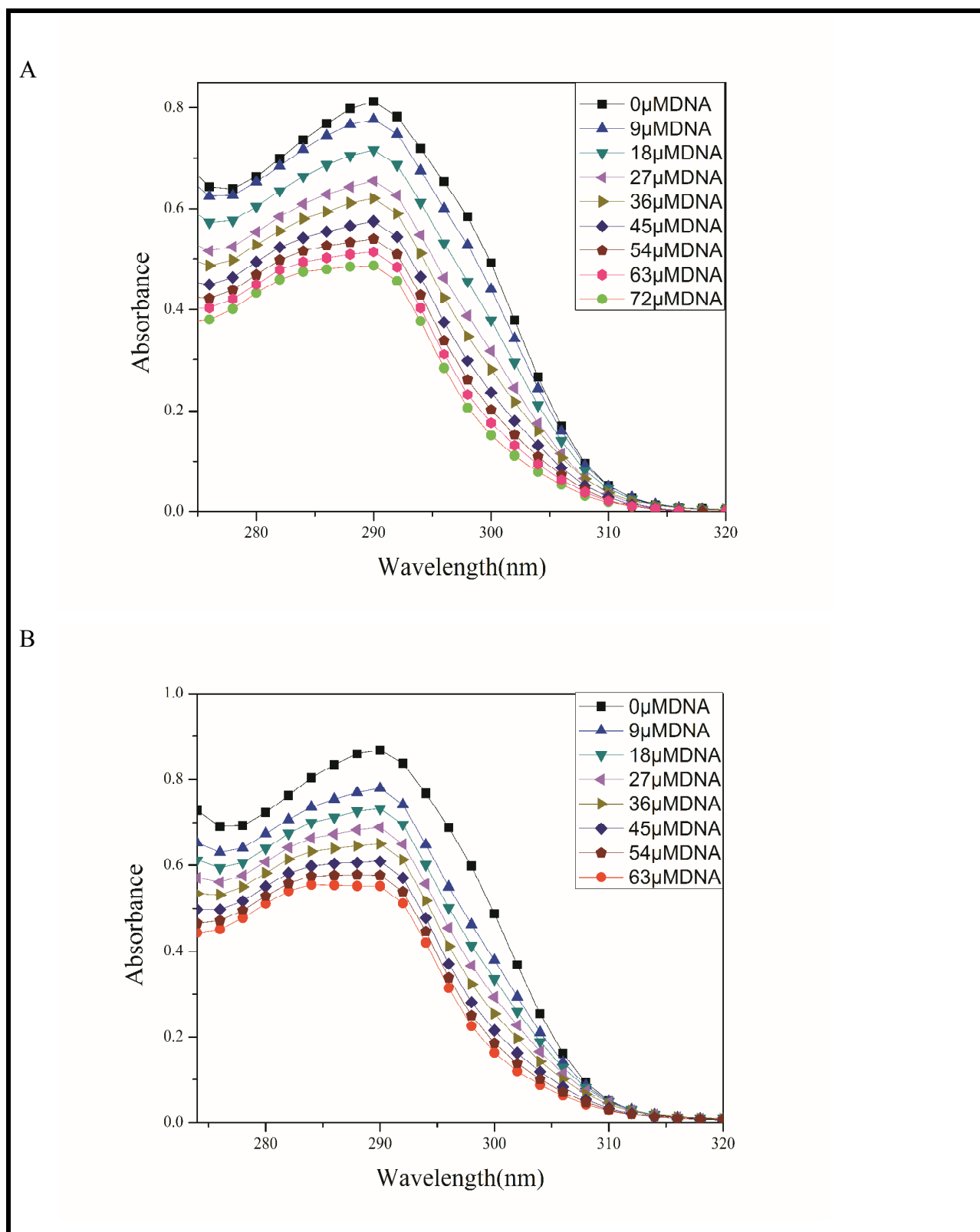
430 **The numbers shown above are mean of three experiments. Variation among the results were*
 431 *less than 6%.*

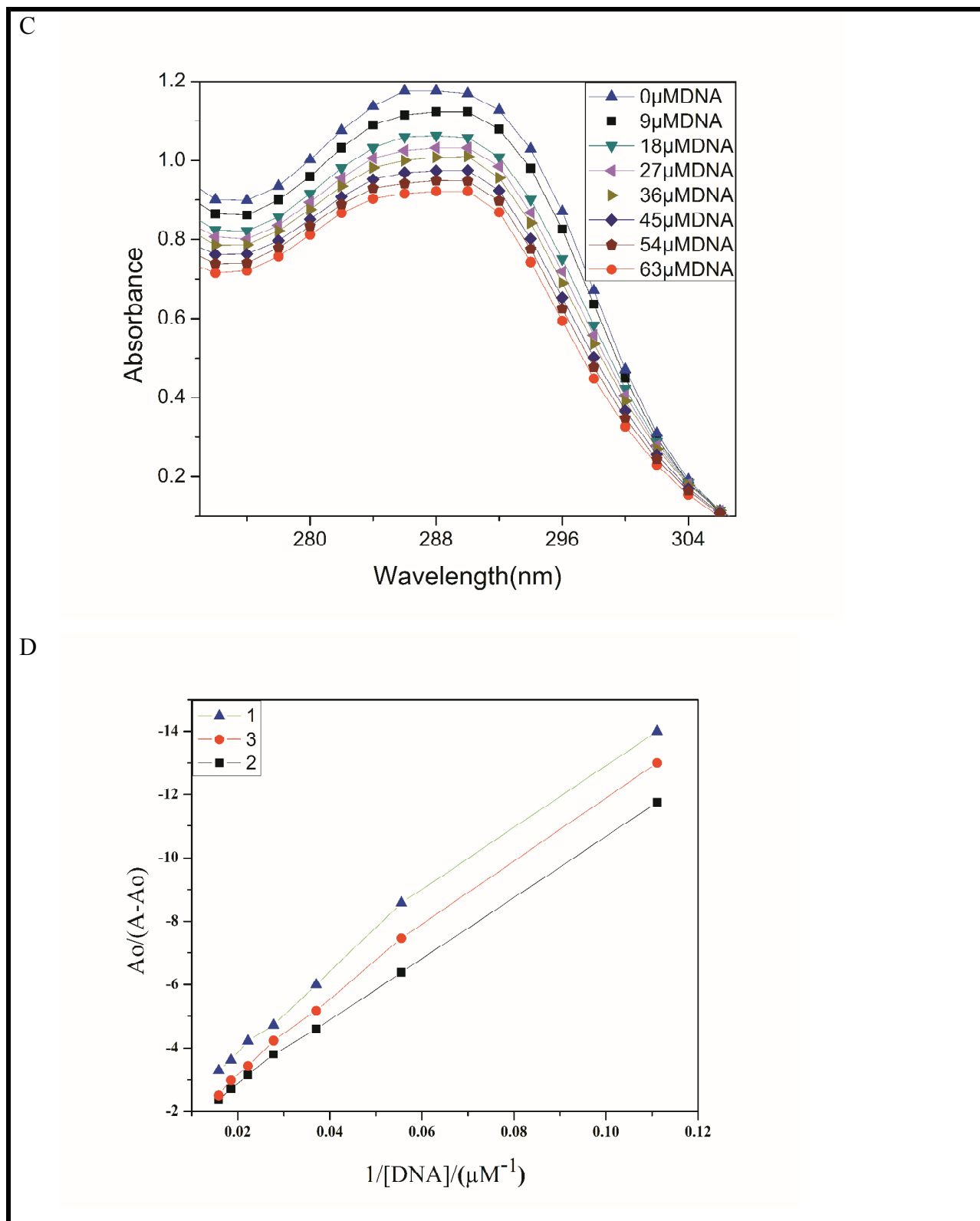
432 3.4 Compound -DNA interaction study

433 3.4.1 UV-Vis absorption study of compound-DNA interaction

434 Thermodynamic parameters of the organotin (IV) compounds - DNA interaction was determined
 435 by UV-Vis spectroscopy, which helped in determining the mode of interaction and binding

436 strength. The effect of varying concentrations of DNA (9-63 μM) on the electronic absorption
437 spectra of 0.2 mM of **1**, **2** and **3** is shown in Fig. 4A-C. The absorption spectra of **1**, **2** and **3**
438 recorded 24, 21 and 27% decrease in peak intensities accompanied with slight blue shift (1~2
439 nm) by the addition of 63 mM DNA. These spectral characteristics are indicative of drug binding
440 to DNA, which results in conformational and structural change of DNA [43,44]. The
441 hypochromism effects observed here is attributed to the intercalation of these compounds into
442 the DNA base pairs. In the intercalation binding mode, the π^* orbital of the binding ligand
443 couple with the π orbital of DNA base pairs. The coupling π^* orbital is partially filled, which
444 decreases the transition probabilities, and results in the hypochromicity [45,46]. The interaction
445 of electronic states of the intercalating chromophore with stacked base pairs of DNA causes the
446 contraction of the DNA helix and change in the conformation of DNA. These results suggest that
447 organotin(IV) compounds interact with DNA via the intercalation mode of interaction, since only
448 a hypochromic effect is observed, without any significant change of shifts in the spectral profiles,
449 which is the indication of a weak interaction with DNA [47].





450 Fig. 4. Absorption spectra of the 2mM compounds (A) Bu_2SnL_2 , (B) Me_2SnL_2 , (C) Me_3SnL and
451 (D) Plot of $A_0/(A-A_0)$ Vs $1/[\text{DNA}]$ for determination of binding constants for compound 1,
452 2 and 3

453 The reason for greater association constant of compound **1** is the additional hydrophobic
454 interaction of the butyl groups with bases of DNA [48]. Based upon the variation in absorbance,
455 the association/binding constants of these compounds with DNA were determined according to
456 the Benesi–Hildebrand equation [49];

$$457 \frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} \times \frac{1}{K[\text{DNA}]}$$

458 Where K is the binding constant, A₀ and A are the absorbances of the free and DNA bound
459 organotin(IV) compounds

460 While ε_G and ε_{H-G} are their absorption coefficients respectively.

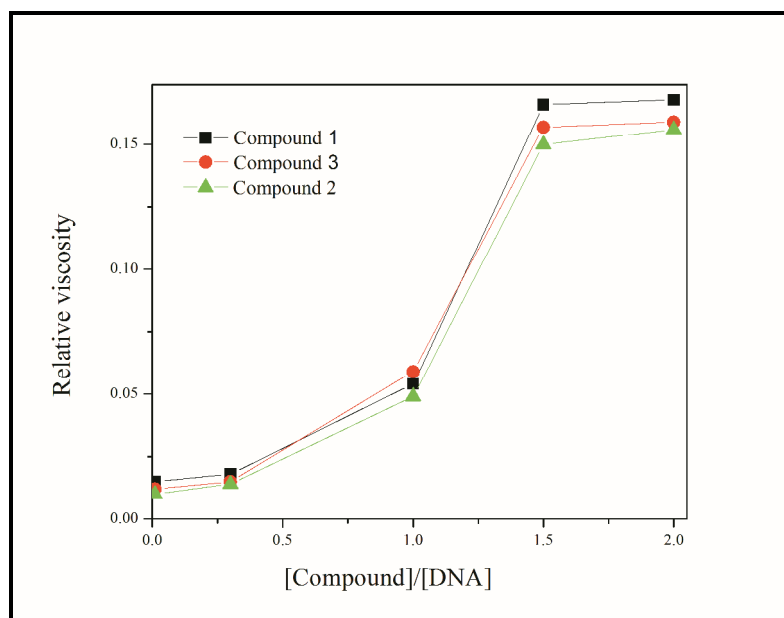
461 3.4.2 Compound-DNA interaction study by Viscometry

462 The change in viscosity of DNA is regulated by the length of DNA, therefore the change in
463 viscosity of DNA upon addition of a compound reflects the intercalative mode of binding. The
464 addition of compound (1-4) to the solution of the DNA results in separation of base pairs to host
465 the binding compound, resulting in the lengthening of the DNA helix and subsequently increased
466 in DNA viscosity as shown Fig.5. On the other hand, the binding of a compound exclusively in
467 DNA grooves by means of partial and/or non-classic intercalation, under the same conditions
468 causes a bend or kink in the DNA helix, reducing its effective length and, as a result, the DNA
469 solution viscosity is decreased or remains unchanged, i.e. groove binders and electrostatic
470 interaction do not increase the lengthen of DNA molecules [50,51]. The present case (Fig.5)
471 suggests an intercalative mode of interaction of the compound (1-4) with DNA.

472 4. Conclusions

473 The organotin(IV) derivatives of 3,5-dimethylbenzoic acid ligand exhibit skew-trapezoidal
474 bipyramidal (**1** and **2**) or distorted trigonal bipyramidal coordination geometry (**3**) geometry both
475 in solution and in solid state.

476 The Gaussia luciferase Assay and real-time PCR assays confirmed the anti-HCV activity of
477 compound **1**. The spectroscopic and viscometry techniques were successfully used for the
478 evaluation of binding parameters of compounds 1–3 with DNA. The UV-titration results agree
479 with the Viscometer data. Based upon the increase in viscosity current and absorption intensity
480 the stability of adduct formation followed the order: 1>3 >2. The results of UV–vis spectra and
481 viscosity indicate that all the compounds 1–3 intercalate into the double helix of DNA. The
482 negative values of ΔG designate the spontaneity of compound–DNA binding. However, further
483 work is required to use compound **1** on a clinical level.



484
485 Fig. 5. The relative viscosity of the DNA with the addition of organotin(IV) compounds

486
487 **5. Supplementary material**

488 CCDC 1003234, 1003233 and 1003235 contain the supplementary crystallographic data for **1**, **2**
489 and **3**, respectively. These data can be obtained free of charge from The Cambridge
490 Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

491 **References**

- 492 1. WHO. Global burden of diseases (GBD) for hepatitis C. *J Clin Pharmacol* 44 (1) (2004)
493 20-9
- 494 2. B.J. Thomson, R.G. Finch, *Clin. Microbiol. Infect.* 11 (2005) 86-94
- 495 3. T. Watanabe, T. Umehera, M. Kohara, *Adv drug Delivery Rev.* 59 (2007) 1263-76
- 496 4. M.G. Ghany, D.B. Strader, D.L. Thomas, L.B. Seeff, *Hepatology* 49(2009) 1335-74
- 497 5. European Association for the Study of the Liver. EASL clinical practice guidelines:
498 management of hepatitis C virus infection. *J Hepatol* 55 (2011) 245-64
- 499 6. L. Delang, L. Coelmont, J. Neyts, *Viruses-Basel.* 2 (2010) 826-866
- 500 7. C.M. Lange, C. Sarrazin, S. Zeuzem, *Aliment. Pharmacol. Ther.* 32 (2010) 14-28
- 501 8. R. Flisiak, A. Parfieniuk. *Expert. Opin. Investig. Drugs* 19 (2010) 63-75
- 502 9. A. Opar, *Nat. Rev. Drug Disc.* 9 (2010) 501-503
- 503 10. F.A. Shah, K. Fatima, S. Sabir, S. Ali, I.Qadri, *Appl. Organometal. Chem.* 28 (2014) 74-
504 80
- 505 11. S. Nafisia, A. Sobhanmanesh, M. Esm-Hosseini, K. Alimoghaddamc, H.A.T. Riahid, J.
506 *Mol. Struct.* 750 (2005) 22-27
- 507 12. F.A. Shah, M. Sirajuddin, S. Ali, S.M. Abbas, M.N. Tahir, C. Rizzoli, *Inorg Chim Acta.*
508 400 (2013) 159-168
- 509 13. D. O'Farrell, R. Trowbridge, D. Rowlands, J. Jager, *J. Mol. Biol.* 326 (2003) 1025-1035
- 510 14. N. Arshad, U. Yunus, S. Razzque, M. Khan, S. Saleem, B. Mirza, N. Rashid, *Eur. J. Med.*
511 *Chem.* 47 (2012) 452-461
- 512 15. D.M. Tscherne, M.J. Evans, T. von, C.T. Jones, Z. Stamataki, J.A. McKeating, B.D.
513 Lindenbach, C.M. Rice, *J. Virol.* 81 (2007) 3693-3703

- 514 16. B.A. Tannous, D. E. Kim, J. L. Fernandez, R. Weissleder, X.O. Breakefield, *Mol. Ther.*
515 11 (2005) 435–443
- 516 17. R. Weissleder, V. Ntziachristos, *Nat Med.* 9 (2003) 123–128
- 517 18. P.S. Dittrich, A. Manz, *Nat. Rev. Drug Discov.* 5 (2006) 210–218
- 518 19. M.H. Wu, S.B. Huang, G.B. Lee, *Lab Chip.* 10 (2010) 939–956
- 519 20. W.L.F. Armaergo, C.L.L. Chai, *Purification of Laboratory Chemicals*, 5th edn.
520 (Butterworth-Heinemann, New York, (2003)
- 521 21. *SMART* (Version 5.059), *SAINT-Plus* (Version 6.01) and *SADABS* (Version 2.01). Bruker
522 AXS Inc., Madison, Wisconsin, USA (1998)
- 523 22. *APEX-II* (Version 2008.1-0), *SAINT* (Version 7.51A) and *SADABS* (Version 2007/4).
524 Bruker AXS Inc., Madison, Wisconsin, USA (2008)
- 525 23. D. Belletti. *FEBO*. Internal Report 1/93, Centro di Studio per la Strutturistica
526 Diffraattometrica del CNR, Parma, Italy (1993)
- 527 24. A. C. T. North, D. C. Phillips, F. S. Mathews. *Acta Cryst.* A24 (1968) 351-359.
- 528 25. G. M. Sheldrick. *Acta Cryst.* A64 (2008) 112
- 529 26. A. Altomare, M. C. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, A. Guagliardi, A.
530 G. G. Moliterni, G. Polidori, R. Spagna. *J. Appl. Cryst.* 32 (1999) 115-119
- 531 27. L. J. Farrugia. *J. Appl. Cryst.* 45 (2012) 849-854
- 532 28. E. Keller. *SCHAKAL99*. University of Freiburg, Germany (1999)
- 533 29. C. T. Jones, M.T. Catanesi, L.M.J. Law, S.R. Khetani, A.J. Syder, A. Ploss, T.S. Oh,
534 J.W. Schoggins, M.R. MacDonald, S.N. Bhatia, C. M. Rice, *Nat Biotechnol.* 28 (2010)
535 167–171

- 536 30. Y. Zhang, X. Wang, L. Ding, *Nucleosides, Nucleotides and Nucleic Acids* 30 (2011) 49-
537 62
- 538 31. C.V. Sastri, D. Eswaramoorthy, L. Giribabu, B.G. Maiya, J. Inorg. Biochem. 94 (2003)
539 138-145
- 540 32. M. Tariq, N. Muhammad, M. Sirajuddin, S. Ali, N.A. Shah, M.R. Khan, M.N. Tahir, J.
541 *Organomet. Chem.*, 723 (1) (2013) 79-89
- 542 33. E.R.T. Tiekink, *Trends Organomet. Chem.* 1 (1994) 71–116
- 543 34. A. Ruzicka, L. Dostal, R. Jambor, V. Buchta, J. Brus, *Appl. Organomet. Chem.* 16 (2002)
544 315–322
- 545 35. M. Parvez, S. Ali, T. Masood, M. Mazhar, M. Danish, *Acta Crystallogr. C* 53 (1997)
546 1211-1213
- 547 36. C.L. Ma, Q.F. Zhang, R.F. Zhang, L.L. Qiu, *J. Organomet. Chem.* 690 (2005) 3033-3043
- 548 37. A.W. Addison, R.T. Nageswara, J. Reedijk, R.J. Van, G.C. Verschoor, *J. Chem. Soc.*
549 *Dalton Trans.* 7 (1984) 1349-1356
- 550 38. W. Rehman, A. Badshah, F. Rahim, M.K. Baloch, H. Ullah, O-U-R. Abid, I. Tauseef,
551 *Inorganica Chimica Acta.* 423 (2014) 177–182
- 552 39. S. Marukian, *Hepatology.* 48 (2008) 1843–1850
- 553 40. (a). M. Gielen, R. Willem, *Anticancer Res.* 12 (1992) 1323-1325 (b). M. Gielen, A. El
554 Khloufi, M. Biesemans, F. Kayser, R. Willem, *Appl. Organomet. Chem.* 7 (1993) 201-
555 206 (c) M. Gielen, R. Willem, *Anticancer Res.* 12 (1992) 257-268 (d) D.-S. Zuo, T.
556 Jiang, H.-S. Guan, X. Qi, K.-Q. Wang, Z. Shi, Z. Chin, *J. Chem.* 19 (2001) 1141-1145
- 557 41. F.A. Shah, K. Fatima, S. Sabir, S. Ali, A. Fischer, M.I. Qadri. *Med. Chem Res.* DOI
558 10.1007/s00044-014-1242-3

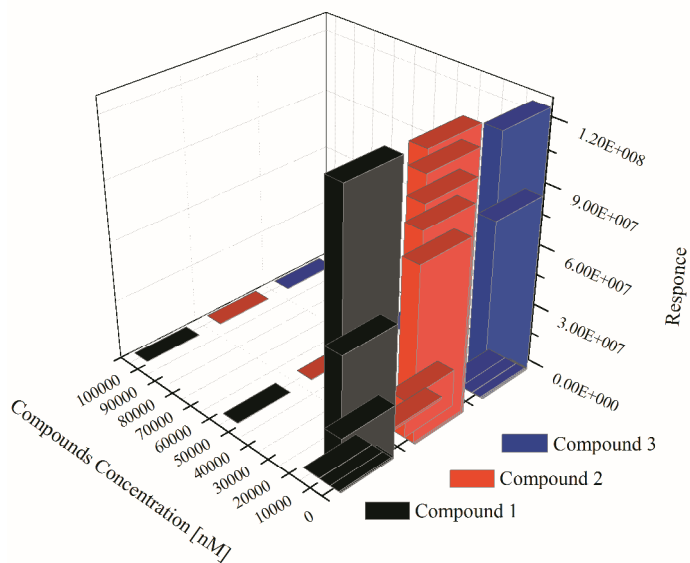
- 559 42. A. Bacchi, A. Bonardi, M. Carcelli, P. Mazza, P. Pelagatti, C. Pelizzi, G. Pelizzi, C.
560 Solinas, F. Zani, *J. Inorg. Biochem.* 69 (1998) 101-112
- 561 43. C.Y. Zhou, J. Zhao, Y.B. Wu, C.X. Yin, P. Yang, *J. Inorg. Biochem.* 101 (2007) 10-18
- 562 44. N. Li, Y. Ma, C. Yang, L. Guo, X. Yang, *Biophys. Chem.* 116 (2005) 199-205
- 563 45. C.C. Ju, A.G. Zhang, C.L. Yuan, X.L. Zhao, K.Z. Wang, *J. Inorg. Biochem.* 105 (2011)
564 435-443
- 565 46. Z. Xu, G. Bai, C. Dong, *Bioorg. Med. Chem.* 13 (2005) 5694-5699
- 566 47. V. Gonzalez-Ruiz, A.I. Olives, M.A. Martin, P. Ribelles, M.T. Ramos, J.C. Menendez,
567 *Biomedical Engineering, Trends, Research and Technologies, InTech, India, (2011) 65*
568 *(ISBN 978-953-307-514-3)*
- 569 48. Q. Lie, P. Yang, H. Wang, M. Guo, *J. Inorg. Biochem.* 64 (1996) 181-195.
- 570 49. M.S. Ibrahim, I.S. Shehatta, A.A. Al-nyeli, *J. Pharm. Biomed. Anal.* 28 (2002) 217-225
- 571 50. D. Li, J. Tian, W. Gu, X. Liu, S. Yan, *J. Inorg. Biochem.* 104 (2010) 171-179
- 572 51. M. Jiang, Y. Li, Z. Wu, Z. Liu, C. Yan, *J. Inorg. Biochem.* 103 (2009) 833-844
- 573

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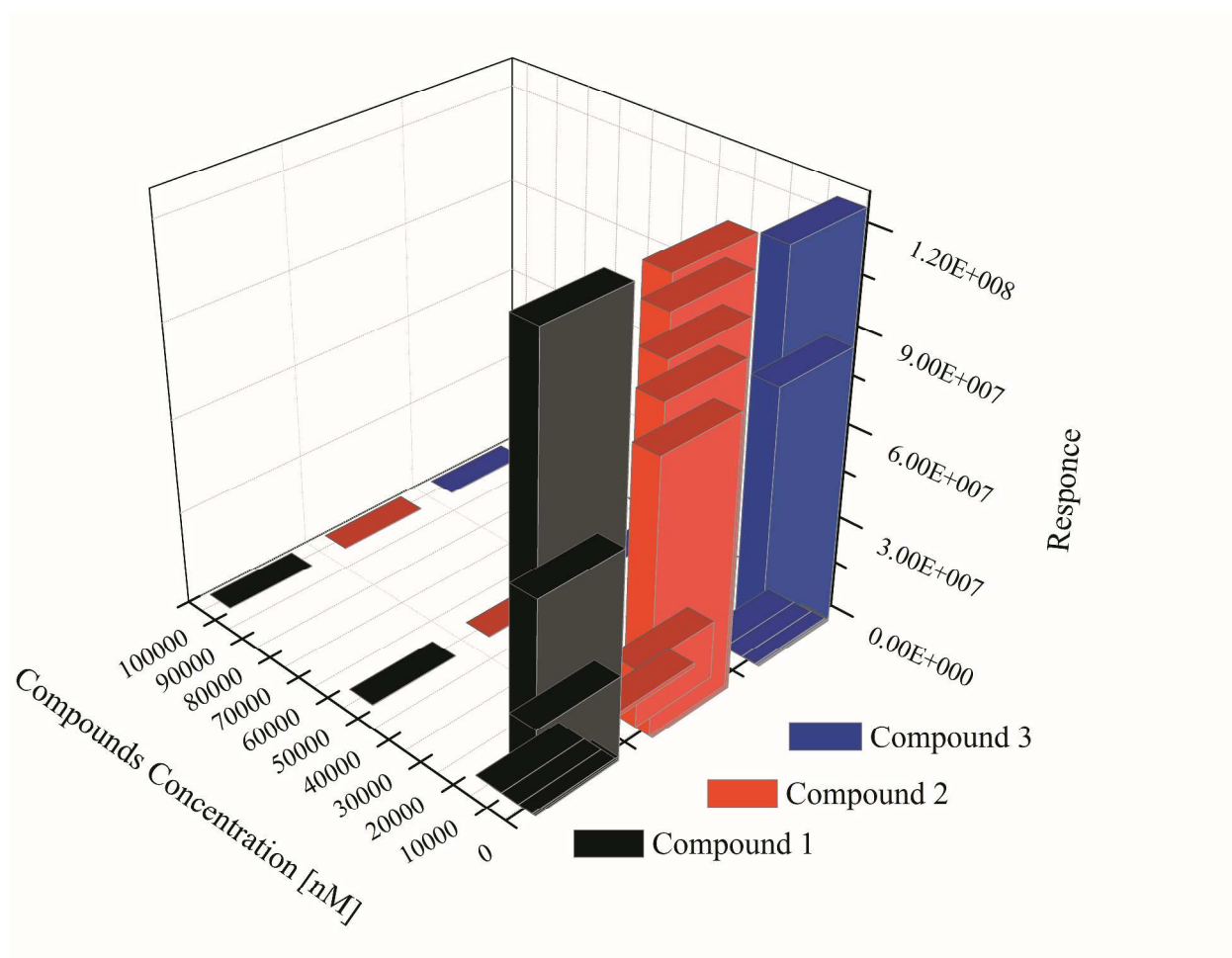
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