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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and Gaussia luciferase Assay of triorganotin(IV)-based HCV inhibitors

Farooq Ali Shah · Kaneez Fatima · Shaista Sabir · Saqib Ali · Andreas Fischer · Mohammad Ishtiaq Qadri

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Abstract The discovery and optimization of a novel triorganotin(IV) complexes with anti-HCV properties are presented. Organotin(IV) moiety has the ability to bind phosphate group of RNA backbone. The organotin(IV) moiety is bonded with ligands and groups, which are known for inhibiting HCV enzymes. Triorganotin(IV) complexes were synthesized and evaluated for their potency against HCV by using luciferase assay. Structure–activity relationship studies led to the identification of Tributyltannic[3-(3′,4′-dichlorophenylamido)propanoate] (compound **1**) as a potent HCV inhibitor, with log IC_{50} values 0.79 nM in the cell-based assay. Triorganotin(IV) complexes containing chlorine and nitro group display higher potency. Gaussia luciferase Assay shows that among triorganotin(IV) derivatives, butyl substituted triorganotin(IV) complexes are more active than methyl- and phenyl-substituted complexes.

Keywords Organotin(IV) complexes · HCV · IC_{50} · Viruses · Luciferase assay · RNA · SAR

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Introduction

Hepatitis C virus is vicious. HCV infection can lead to liver disorders such as fibrosis, cirrhosis, hepatocellular carcinoma, and is a major reason for liver transplantation. The worldwide prevalence of chronic HCV infection is estimated to be approaching 270–300 million people. HCV virus is a member of Flaviviridae family, and its genome is a single-stranded linear RNA of positive sense (Purcell, 1997). HCV displays an extensive genetic heterogeneity: at least 6 genotypes and more than 50 subtypes have been identified. When HCV was discovered in 1989, drugs specifically targeting the viral proteins were expected to reach clinics rapidly. But more than two decades on, patients and physicians are still waiting. The current standard treatment for HCV infection involves weekly injections of pegylated interferon-α, along with twice-daily oral ribavirin. These drugs, both of which are generally inhibitors of viral infection, often have serious side effects, including depression and flu-like symptoms. Furthermore, completion of the year-long course of treatment does not always cure the HCV patient, with success depending on characteristics of the virus, such as strain (the viral genotype), as well as host attributes, such as genetic variations (Ge *et al.*, 2009; Suppiah *et al.*, 2009; Tanaka *et al.*, 2009). Gao *et al.* (2010) have identified a compound through a relatively long synthetic sequence that inhibits HCV replication and come up with a few surprises. This agent is the

most potent HCV inhibitor reported so far, and it does not attack the virus's usual weak spots. Traditionally, viral enzymes are the prime targets for drug development. Several HCV enzymes are required for the virus replication, including two proteases (NS2-3 and NS3-4A), a helicase (NS3) and a polymerase (NS5B). Of these, NS3-4A and NS5B have gathered the most attention as drug targets, with several candidates showing encouraging results in clinical trials (De Francesco and Migliaccio, 2005; Kwong *et al.*, 2008). But the rapid and error-prone replication of HCV makes the emergence of drug resistance mutant with reduced susceptibility to one or more compounds during in vitro and in vivo testing's (De Francesco and Migliaccio, 2005; Kwong *et al.*, 2008). Clearly, new strategies to prevent and treat infections caused by this virus are urgently needed. Presently, there is a need of antiviral agents that attack different targets along the viral life cycle and, perhaps, the hosts themselves to ensure the control of infection.

In the present study, triorganotin(IV) complexes are synthesized and investigated as potential HCV inhibitors using HCV-infected Huh-7.5 cells. The organotin(IV) moieties are promising in vitro activities against human tumor cell lines owing to their DNA binding capability (Pizarro *et al.*, 2010; Li *et al.*, 2004a, b). The tin metal ion has an appropriate hard-soft character and abundant valence shell orbital for interaction with oxygen sites (electrovalent bonding) and nitrogen sites of DNA (covalent bonds) (Casas *et al.*, 2004; Li *et al.*, 1996). In case of RNA, binding is mainly through the backbone of phosphate group, but in the presence of high concentration of drug, minor interaction with RNA bases is also observed (Nafisi *et al.*, 2005). The RNA controls the RNA-dependent RNA polymerase NS5B which plays a key role in the life cycle of the virus since it is responsible for the replication of the viral genome (Butcher *et al.*, 2001). These results of the organotin(IV) complex interaction with RNA encourage their application as anti-HCV drug. So the triorganotin (IV) with different legends was used, and the selection of ligands was made on the basis of substitutions, which are known for effecting the HCV or host enzymes.

Anti-HCV activity of triorganotin(IV) complexes was determined by the Gaussia luciferase Assay (Tannous, 2009) using real-time imaging of hepatitis C virus-infected human hepatoma (Huh-7.5) cells (Jones *et al.*, 2010). In Gaussia luciferase Assay, secreted reporters are used as a tool for monitoring of different biological processes in the conditioned medium of cultured cells, blood, and urine of experimental animals (Wurdinger, 2008). The reporter's levels in the conditioned medium are linear with respect to cell number, growth, and proliferation (Tannous *et al.*, 2005, Badr *et al.*, 2007). Gaussia luciferase Assay has advantages over conventional methods for monitoring gene expression as this technique is more sensitive, requires small sample size, fast 20,000-fold (in vitro), 1000-fold (in vivo) and does not involve any prior treatment of the samples.

HCV-dependent fluorescence relocalization (HDFR) reporter system, a high level of HCV-specificity combined with genotype independence, is used as a marker of viral replication. HDFR is independent of cell fixation, signal amplification and any processed to detect infection. These advantages allow real-time visualization of HCV infection in live cells. Real-time imaging of hepatitis C virus infection using a fluorescent cell-based reporter system is used for sensitive distinction of individual HCV-infected cells. Cell-based fluorescent reporter system is a reliable technology for live-cell imaging of viral propagation and host response, as well as visualizing infection of primary hepatocyte cultures.

Materials and methods

Materials

All the chemicals were purchased from Aldrich and used as it is while solvents were purified (Armarego and Chai, 2003). Melting points were determined on MP-D Mitamura Riken Kogyo (Japan) while IR spectra of the triorganotin(IV) complexes were recorded in the range of 4000–200 cm^{-1} on a Bio-Rad Excaliber FT-IR spectrophotometer by using KBr pellets. EI mass spectra were recorded on a Kratos MS 25RFA (70 eV) spectrometer and data are given as *m/z*. The ^{1}H, ^{13}C and ^{119}Sn NMR data were recorded on a Bruker ARX 300 MHz spectrophotometer. In Gaussia luciferase Assay, a Zeiss Axiovert 200 inverted microscope equipped with an Ultra View spinning disk confocal head (Perkin-Elmer), an Orca ER-cooled CCD camera (Hamamatsu), a 20 ×/ 0.75 N.A. Plan-Apochromat objective, and an environmental chamber (Solent Scientific) was used for imaging. Quantitative measurements of RNA level Jc1-FLAG2(p7-nsGluc2A) were performed by Real-time PCR (MyiQ2TM System BIO-RAD Thermal Cycler) using "RoboGene® Hepatitis C virus Quantification Kit" (AJ Roboscreen GmbH, Leipzig Germany) for RNA extraction and reverse transcription.

Methods

Synthesis of triorganotin(IV) complexes

Chemistry of complexes **10** (Shah *et al.*, 2010); **3**, **11** (Shah *et al.*, 2010); **6**, **9** (Shah *et al.*, 2009); **5** (Shah *et al.*, 2013) and single crystal structure of complexes **5** (Shah *et al.*, 2012) and **6** (Shah *et al.*, 2009) are reported in literature.

*Synthesis of tributyltannic[3-(3′,4′-dichlorophenylamido)propanoate] (**1**) white crystals (chloroform methanol, 4:1 v/v)* Complex **1** was synthesized by refluxing equimolar amount of ligand HL (1 g, 3.80 mmol), Bu_3SnCl (1.25 g, 3.80 mmol), and triethylamin (0.53 mL, 3.80 mmol) in dry toluene (100 mL). The filtrate was concentrated to dryness under reduced pressure, and the product was Purified by recrystallization from a chloroform methanol (4:1 v/v) mixture at room temperature. M.p. 66–68 °C. IR (4000–200 cm^{-1}, KBr): 3327 *v* (NH), 1705 *v* (amide C=O), 1548 *v* (COasym), 1406 *v* (COsym), 142 (Δ*v*), 520 *v* (Sn–C), 416 *v* (Sn–O). ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.70 (*s*, 1H, 2); 7.53 (*d*, *J* (^1H, ^1H) = 7.6 Hz, 1H 5); 7.69 (*d*, *J* (^1H, ^1H) = 7.6 Hz, 1H, 6); 2.57 (*t*, *J* (^1H, ^1H) = 6.0 Hz, 2H, 8); 2.69 (*t*, *J* (^1H, ^1H) = 6.0 Hz, 2H 9); 8.5 (*s*, 1H NH); 0.889 (*t*, 7.2 terminal methyl protons of butyl); 1.23–1.37 (*m*, 6H of first 3 carbons of butyl). ^{13}C NMR ($CDCl_3$-d3, 75 MHz) δ (ppm): 137.5 (C1); 121.5 (C2); 132 (C3); 126 (C4); 130.2 (C5); 119.5 (C6); 170.9 (C7); 30.3 (C8); 29.7 (C9); 178 (C10); (29.7^1 *J* [349,332], 27.9^2*J* [21], 26.8^3*J* [64], 14.4, *n*-butyl carbons). EI-MS, *m/z* (%): $[C14H13N2O6Cl2Sn]^+$ 496 (100), $[C6H4Cl2Sn]^+$ 269 (12), $[C7H3ClSn]^+$ 242 (20), $[SnCOO]^+$ 164 (18), [SnL] 341 (8), $[R_3Sn]^+$ 291 (8), $[R_2Sn]^+$ 234 (5), $[RSn]^+$ 177 (30), $[Sn]^+$ 120 (7), $[C_6H_4NCl_2]^+$ 161 (19), $[C_4H_9]^+$ 57 (25). Anal. Calcd for $C_{22}H_{35}NO_4Cl_2Sn$ (566.82): C 46.61; H 6.17; N 2.47; Found: C (47.63); H (6.53); N (2.54).

*Synthesis of tributylstannic[3-(3,5-dimethylphenylamido)propionate] (**2**) white crystals (chloroform methanol, 4:1 v/v)* Complex **2** was synthesized in the same way as **1**, by refluxing equimolar amount of ligand HL (1 g, 4.6 mmol), Bu_3SnCl (1.48 g, 4.6 mmol), and triethylamin (0.64 mL, 4.6 mmol) in dry toluene (100 mL). The filtrate was concentrated to dryness under reduced pressure, and the product was purified by recrystallization from a chloroform methanol (4:1 v/v) mixture at room temperature. M. p. 152–154 °C. IR (4000–200 cm^{-1}, KBr): 3300 *v* (NH), 1713 *v* (amide C=O), 1556 *v* (COasym), 1412 *v* (COsym), 144 (Δ*v*), 535 *v* (Sn–C), 431 *v* (Sn–O). ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.70 (*s*, 1H, 2); 7.53 (*s*, 1H, 4); 7.69 (*d*, 1H, 6); 2.57 (*d*, *J*(^1H, ^1H) = 6.0 Hz, 1H, 10); 2.69 (*d*, *J*(^1H, ^1H) = 6.0 Hz, 1H 11); 11.02 (*s*, 1H NH); 0.85 (*t*, 7.5 terminal methyl protons of butyl); 1.22–1.34 (*m*, 6H of first 3 carbons of butyl). ^{13}C NMR ($CDCl_3$-d3, 75 MHz) δ (ppm): 133.2 (C1); 124.9 (C2/6); 138.6 (C3/5); 126 (C4); 21 (C7/8); 163.7 (C9); 139.1 (C10); 132.1 (C11); 168.4 (C12); (29.5^1*J* [342], 27.9^2*J* [27], 26.9^3*J* [77], 14.0, *n*-butyl carbons). EI-MS, *m/z* (%): $[R_2SnCOOL]$ 451 (22), $[R_3Sn]^+$ 297 (38), $[R_2Sn]^+$ 234 (16), $[RSn]^+$ 177 (39), $[Sn]^+$ 120 (49), $[C_{12}H_{12}NO_2]^+$ 202 (78), $[C_{11}H_{12}NO]^+$ 174 (37), $[C_4H_9]^+$ 57 (100). Anal. Calcd for $C_{24}H_{39}NO_4Sn$ (523.96): C 55.01; H 7.44; N 2.67; Found: C (53.8); H (7.51); N (2.61).

*Synthesis of tributylstannic(2′,4′,6′-trichlorobenzoate) (**4**) white needles (chloroform methanol, 4:1 v/v)* Complex **4** was synthesized in the same way as **1**, by refluxing equimolar amount of 2′,4′,6′-trichlorobenzoic acid (1 g, 4.4 mmol), Bu_3SnCl (1.3 g, 4.4 mmol), and triethylamin (0.62 mL, 4.4 mmol) in dry toluene (100 mL). The filtrate was concentrated to dryness under reduced pressure, and the product was purified by recrystallization from a chloroform methanol (4:1 v/v) mixture at room temperature. M. p. 79–80 °C. IR (4000–200 cm^{-1}, KBr): 1547 *v* (COasym), 1417 *v* (COsym), 130 (Δ*v*), 552 *v* (Sn–C), 465 *v* (Sn–O). ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.79 (*s*, 2H 3/5); 0.81 (*t*, *J* (^1H, ^1H) = 6.9 terminal methyl protons of butyl); 1.0–1.51 (*m*, 6H of first 3 carbons of butyl). ^{13}C NMR ($CDCl_3$-d3, 75 MHz) δ (ppm): 131.7 (C1); 135.6 (C2/6); 129.3 (C3/5); 135.3 (C4); 170.8 (C7); (28.1 ^1J [355], 27.8 ^2J [21], 26.1 ^3J [60], 14.0, *n*-butyl carbons). EI-MS, *m/z* (%): $[R_2SnCOOL]$ 459 (82), $[SnCOOL]^+$ 345 (12), [SnL] 301 (44), $[R_2Sn]^+$ 234 (12), $[RSn]^+$ 177 (32), $[Sn]^+$ 120 (33), $[C_7H_2OCl_3]^+$ 208 (18), $[C_4H_9]^+$ 57 (100). Anal. Calcd for $C_{19}H_{29}O_2Cl_3Sn$ (514.24): C 44.37; H 5.64; Found: C (44.41); H (5.71).

*Synthesis of trimethylstannic[3-(2-fluorophenylamido)propanoate] (**7**) white crystals (chloroform methanol, 4:1 v/v)* Complex **7** was synthesized in the same way as **1**, by refluxing equimolar amount of ligand HL (1 g, 4.74 mmol), Me_3SnCl (0.94 g, 4.74 mmol), and triethylamin (0.65 mL, 4.74 mmol) in dry toluene (100 mL). The filtrate was concentrated to dryness under reduced pressure, and the product was purified by recrystallization from a chloroform methanol (4:1 v/v) mixture at room temperature. M. p. 158–161 °C. IR (4000–200 cm^{-1}, KBr): 3385 *v* (NH), 1699 *v* (amide C=O), 1578 *v* (COasym), 1419 *v* (COsym), 156 (Δ*v*), 530 *v* (Sn–C), 462 *v* (Sn–O). ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.20–7.10 (*m*, 4H, 3–6); 2.68 (*t*, *J* (^1H, ^1H) = 9.0 Hz, 2H, 8); 2.75 (*t*, *J* (^1H, ^1H) = 9.0 Hz, 2H, 9); 9.48 (*s*, 1H NH); 0.084 (*s*, 9H methyl); ^2J [$^{119/117}Sn$, ^1H] 52, 58.8 Hz. ^{13}C NMR ($CDCl_3$-d3, 75 MHz) δ (ppm): 123.2 (C1); 154.8 (C2) ^1J [^{13}C–^{19}F] 243 Hz; 114.8 (C3) ^2J [^{13}C–^{19}F] 18.75 Hz; 124.67 (C4) ^3J [^{13}C–^{19}F] 11.25 Hz; 127.2 (C5); 127 (C6); 170.8 (C7); 30.6 (C8); 33.5 (C9); 175.1 (C10); −2.3 (^1J [378] methyl carbons).EI-MS, *m/z* (%): $[R_2SnCOOL]$ 360 (78), $[C_8H_{10}NOFSn]^+$ 270 (30), $[C_5H_4O_2Sn]^+$ 193 (52), $[R_3Sn]^+$ 165 (100), $[RSn]^+$ 135 (36), $[C_6H_5NF]^+$ 110 (58), $[C_3H_3O]^+$ 55 (22). Anal. Calcd for $C_{13}H_{18}NO_4FSn$ (389.83): C 40.05; H 4.62; N 3.59; Found: C (40.90); H (4.92); N (3.69).

*Synthesis of trimethylstannic(3′,4′-dimethoxybenzoate) (**8**) white crystals (chloroform methanol, 4:1 v/v)* Complex **8** was synthesized in the same way as **1**, by refluxing equimolar amount of 3′,4′-dimethoxybenzoic acid (1 g, 5.5 mmol),

Me_3SnCl (1.09 g, 5.5 mmol) and triethylamin (0.77 mL, 5. 5 mmol) in dry toluene (100 mL). The filtrate was concentrated to dryness under reduced pressure and the product was purified by recrystallization from a chloroform methanol (4: 1 v/v) mixture at room temperature. M.p. 141–142 °C. IR (4000–200 cm^{-1}, KBr): 1605 *v* (COasym), 1465 *v* (COsym), 140 (Δv), 510 *v* (Sn–C), 449 *v* (Sn–O). ^1H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.57 (*s*, 1H 2); 7.02 (*s*, 1H 5); 7.6 (*s*, 1H 6); 3.81 (*s*, 6H 7/8); 0.112 (*s*, 9H methyl) ^2J [$^{119/117}Sn$, ^1H] 54.2, 59.1 Hz. ^{13}C NMR ($CDCl_3$-d3, 75 MHz) δ (ppm): 124. 0 (C1); 112.5 (C2); 148.6 (C3); 153.9 (C4); 111.3 (C5); 123.5 (C6); 56.1 (C7/C8); 168 (C9); −2.15 (^1J [329] methyl carbons). EI-MS, *m/z* (%): $[R_2SnCOOL]$ 331 (8), $[R_3Sn]^+$ 165 (24), $[RSn]^+$ 135 (4), $[Sn]^+$ 120 (28), $[C_9H_9O_4]^+$181 (73), $[C_9H_9O_4]^+$ 165 (22), $[C_6H_5]^+$77 (41). Anal. Calcd for $C_{12}H_{18}O_4Sn$ (344.82): C 41.80; H 5.22; Found: C (41.71); H (5.31).

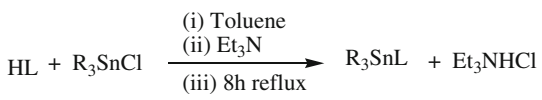
Anti-HCV activity

Virus stocks

The anti-HCV potency of the complexes is evaluated by the Gaussia luciferase assay system (Jones *et al.*, 2010). In this assay, fully infectious HCVcc (HCV cell culture) viruses, Jc1FLAG2 (p7-nsGluc2A) was used as a tool for infecting Huh 7.5 cells. Jc1FLAG2 (p7-nsGluc2A) is a monocistronic reporter virus encoding the full-length infectious Jc1 genome with a second secreted Gaussia luciferase reporter. HCVcc reporter virus expresses secreted Gaussia luciferase (Gluc), Jc1FLAG2 (p7-nsGluc2A). After inoculation, cultures were washed to remove Gluc carryover, and luciferase second secretion was monitored as an indicator of viral replication. Assay of luciferase activity in infected cell supernatants was used to monitor viral replication.

Cell culture

Huh7.5 cells were used in this experiment, maintained in Dulbecco's modified Eagle's medium and incubated at specific climatic condition (37 °C, 5 % CO_2, and 100 % relative humidity). The complexes of 1 mg mL^{-1} strength in dimethylsulfoxide were used. The cells were infected (with or without inhibitors), with Jc1-FLAG2 (p7-nsGluc2A) with MOI-0.1 [1E4 as median tissue culture infective dose (TCID50)/well] in the presence of the complex, and the concentration of each complex used was between 1–1000 nM. Huh7.5 cells were incubated at 37 °C for 3 days, and measured luciferase activity using the EnduRen substrate (Promega). Infectious units (TCID50) were quantified by limiting dilution titration on naive Huh7.5 cells.



Scheme 1 General procedures for synthesis of triorganotin(IV) complexes

Maximum activity (100 % of control) and background were derived from control wells containing DMSO alone or from uninfected wells, respectively. 100 uL of Lysis Buffer was added per well (Renilla Luciferase Assay Lysis Buffer diluted 5:1 in water) and transferring to a 96-Well plate. Store it at −80 °C until it gets ready to read on luminometer. 10 µL of each sample was added into the luciferase plate with Renilla Luciferase Assay, Buffer and Renilla substrate. The secreted Gaussia luciferase (Gluc) was measured on luminometer.

Results and discussion

Ligand, triorganotin(IV) chloride, and triethylamine were mixed in dry toluene, and the reaction mixture was refluxed for 8 h as shown in scheme 1. Crystals of Et_3NHCl were removed by the filtration, and the synthesized organotin(IV) derivative was obtained in vacuum by removing solvents. The products were purified by crystallization with chloroform: pet. ether (1:1).

Crystal structure

The structure of compound **7** is shown in Fig. 1. The crystal data of compound **7** are listed in Table 1 while the selecting the bond length and bond angles are given in Table 2. The compound **7** showed the polymeric structure with trigonal bipyramidal geometry around the tin atom. The geometry around the tin atom can be characterized by the value of $\tau = (\beta - \alpha)/60$ (Addison *et al.*, 1984), where β is the largest angle and α is the second largest angle around the tin atom.

The value of τ is unity in the case of perfect trigonal bipyramidal geometry and zero in case of perfect square pyramidal structure. In the present case, the value of τ is 0.79, which indicates distorted trigonal bipyramidal geometry around the tin atom. The carboxylate oxygen makes zig zag chain with anti-syn configuration. The axial positions are occupied by the two oxygen atoms of bridging carboxylate ligands (O–Sn–O angle 173.3°), while equatorial angle is occupied by C–Sn–C angles (sum of equatorial angle is 358°). Thus, carboxylate ligand bridges the two symmetry-related Sn atoms and gives rise to the unequal Sn–O bond distances which are reflected in the

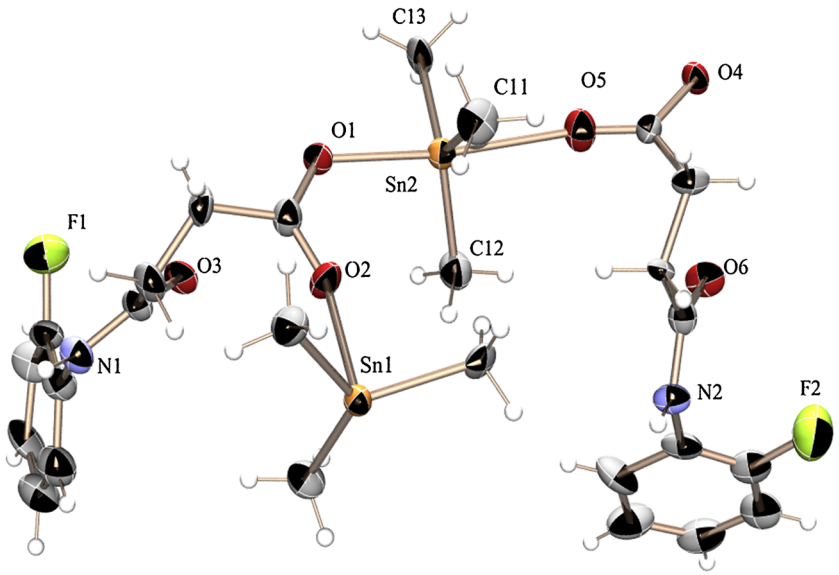


Fig. 1 ORTEP drawing of Me3SnL (**7**)

Table 1 Crystal data and structure refinement parameters for compound **7**

Parameter	Value
Emp. formula	$C_{13}H_{18}O_3NFSn$
Formula weight	373.979
Color	White
Crystal habit	Plate
Crystal system	Triclinic
Space group	P−1
T	299 K
a (Å)	9.1411(11)
b (Å)	9.974(2)
c (Å)	16.834(5)
α (°)	87.12(2)
β (°)	86.448(12)
γ (°)	89.948(12)
V (Å^3)	2783.7(12)
Z (*Z*′)	4
Crystal size (mm)	0.02 × 0.24 × 0.33
Cell volume	1530.0(6)
μ (MoKα̅) (cm^{-1})	0.71073
Total reflections	11060
Independent reflections All	2332
Final R indices [I > 2σ (I)]	R1 = 0.1025
	wR2 = 0.1978
R indices (all data)	R1 = 0.0791
	wR2 = 0.1792
Goodness-of-fit	1.048
θ range for data collections (°)	4.55–26.50
Data/restraints/parameters	3113/1.048/299

Table 2 Selected bond lengths (Å) and bond angles (°) for compound **7**

C12–Sn2	2.136(18)	C13–Sn2	2.122(17)
C11–Sn2	2.108(17)	O5–Sn2	2.512(13)
O1–Sn2	2.168(10)	C1–O1	1.277(19)
C1–O2	1.24(2)	C4–O3	1.242(17)
C6–F1	1.38(2)		
C11–Sn2–C13	116.3(9)	C11–Sn2–C12	125.4(8)
C13–Sn2–C12	116.3(8)	O2–C1–O1	122.7(17)
O5–C14–O4	121.7(17)	O1–Sn2–O5	173.3(4)
C11–Sn2–O1	94.6(7)	C12–Sn2–O5	85.7(6)
C7–C6–F1	118.5(19)	C13–Sn2–O1	89.2(5)
C12–Sn2–O1	99.8(6)	C11–Sn2–O5	85.2(7)
C13–Sn2–O5	85.0(6)		

associated C–O bond lengths; the longer C–O bond is involved in the shorter Sn–O interaction and vice versa. These bond lengths and the polymeric bridging behavior are in accordance with the reported triorganotin(IV) carboxylates (Ma *et al.*, 2005).

Anti-HCV activity

Anti-HCV potency of the triorganotin(IV) complexes was measured by Gaussia luciferase Assay system, and Jc1FLAG2 (p7-nsGluc2A) (Marukian, 2008) was used to infect the Huh 7.5 cells. The second secretion was monitored as anti-HCV activity. In order to have HCV inhibitors for targeting different stages of viral life cycle, triorganotin(IV) complexes with different ligands were synthesized,

Table 3 Activities of triorganotin(IV) complexes against HCV

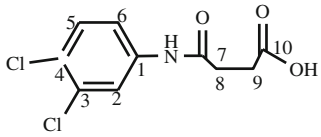
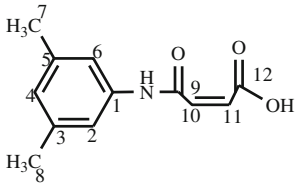
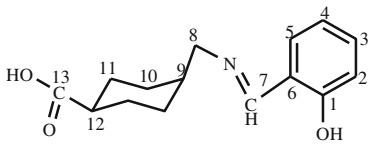
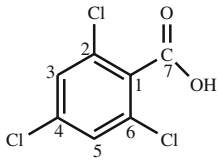
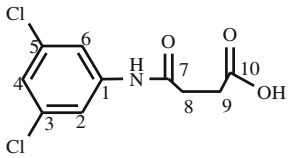
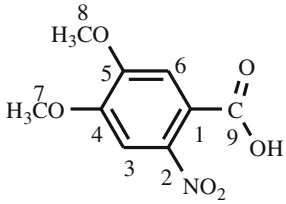
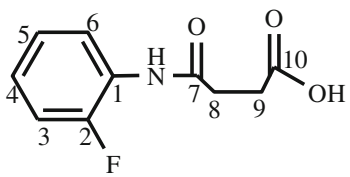
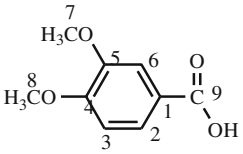
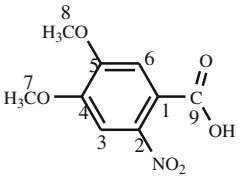
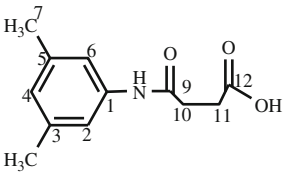
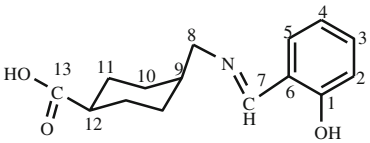
Complex	L	R	Log IC_{50} (nM)
1		Bu	0.79
2		Bu	1.20
3		Bu	1.30
4		Bu	0.81
5		Bu	3.67
6		Bu	0.97
7		Me	2.49

Table 3 continued

Complex	L	R	Log IC_{50} (nM)
8		Me	1.14
9		Me	1.69
10		Ph	4.04
11		Ph	5.71

and selection of ligands was made on the basis of substitutions, which are known for effecting the HCV or host enzymes. Triorganotin(IV) moieties are selected as they are relatively more active than diorganotin(IV) moieties due to their ability to bind proteins (Barbieri *et al.*, 2001; Nath *et al.*, 2003). Among the tested triorganotin(IV) carboxylates, the *n*-butyltin(IV) derivatives were founded more potent against HCV than phenyl and methyl group due to their optimal balance between the bioactivity, solubility, and lypophilicity (Zuo *et al.*, 2001). The anti-HCV activity of the triorganotin(IV)-based inhibitors is summarized in Table 3. The obtained data show that the viral inhibition depends upon the nature, structure of the complex, and coordination number of the central tin atoms of the complex used.

Initial SAR studies were focused on determining the importance of ligands and substitution on these ligands. To probe the importance of the ligand, several tributyltin(IV) complexes with different ligands were tested as shown in Fig. 2. Optimization of ligand in the tributyltin(IV) carboxylates along with their log IC_{50} is summarized in Table 3.

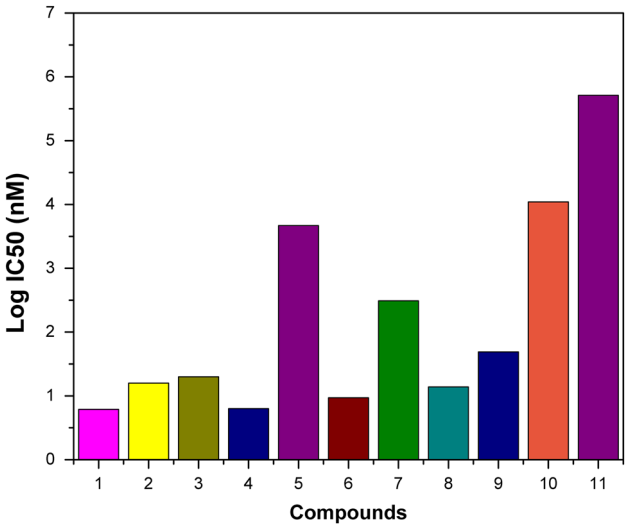


Fig. 2 Triorganotin(IV) complexes vs their logIC50

The activity of same organotin(IV) moieties varies with different ligands (Garrod *et al.*, 1981) to be mainly dependent on the polarity of the complexes and their permeability across cell membranes.

In tested organotin(IV) complexes, complex **1** shows highest activity against HCV, which may be attributed to cholera substitution at the para position of the legend. In the cell-based assays, chlorine-substituted compounds are known for anti-HCV activity by retarding NS5B polymerase enzyme (Li *et al.*, 2007), and the activity is more pronounced when the chlorine atom is substituted at the para position in compound (Li *et al.*, 2004a, b). Different positions of the same group in the compound change the anti-viral activity of compounds because the compounds with same substituted group at different positions change the polarity of compound and their approaching capability to target area (Tonelli *et al.*, 2009).

In the chlorine-substituted complexes (**1**, **4**, **5**), the para position of the chlorine group increased the potency of the complex **1** 4.6 folds and complex **4** 4.5 folds as compared to complex **5**. However, complex **4** showed lower potency against HCV than complex **1** in cell-based assay. The higher activity of complex **1** is attributed to amide and keto group substituted on ligand. Diketo and amide group are the active structure part of the anti-HCV inhibitors, presently used at the clinical level (Wai *et al.*, 2000; Hazuda *et al.*, 2000; Shaw-Reid *et al.*, 2003).

Derivatives containing α-ketoamide electrophilic trap have been reported in the literature to be potent inhibitors of HCV NS3 serine protease (Victor *et al.*, 2004), while diketo groups interact directly with Mg ions present in the enzyme active site of HCV NS5B polymerase (De Francesco *et al.*, 2003) to stop the replication of HCV. Chelators of the ions in the enzyme active site are therefore regarded as potential inhibitors for RNase-H function. Diketo acid derivatives are also considering interfering in the process of binding of phosphoryl groups of the nucleotide substrates at the active site of the viral polymerase and inhibiting the formation of phosphordiester bonds catalyzed by the enzyme. Owing to chelating capability, diketo acids served as starting points for the design and optimization of most of the inhibitors (Wai *et al.*, 2000; Hazuda *et al.*, 2000; Shaw-Reid *et al.*, 2003). In complex **6**, the nitro-substituted ligand is used as Nitro group which is found to intensify the pharmacological activity of a compound (Da Silva Junior *et al.*, 2007) by chelating metals in the target binding site or to its ability to act as hydrogen bond acceptor (Mansoor *et al.*, 2011).

Anti-HCV activity of complex **6** is almost comparable to complex **4.** The log IC_{50} values of the complex **2** and **3** suggested them least active among tributyltin(IV) complexes but more potent than complex **5**. The methyl group substitution at C5 and C6 and double bond insertion at position C10 lowers the polarity of complex **2** and enhanced its potency 3.1 folds as compared to complex **5.** Lower polarity enhanced the permeability of the complex molecules through cell membranes. These results suggested that the ligands' nature and structure are critical for anti-HCV activity.

In case of trimethyltin(IV) complexes, the anti-HCV activity of the complexes is found to be regulated by the nature and size of the legend. Gaussia luciferase Assay shows that complex **8** is a more active agent against HCV among the tested trimethyltin(IV) complexes due to the small size of the ligand. In the case of complex **9**, the legend is comparable in size, but the nitro group increases the polarity of the complex and reduces its permeability through the cell membrane as compared to complex **8.** Similarly, the reduced HCV inhibition activity of complex **7** is due to its high polarity caused by fluorine at the ligand.

Gaussia luciferase Assay data for triphenyltin(IV) complexes are given in Table 1, and the data revealed that the nature of ligand played a critical role in anti-HCV activity of the complexes. In case of triphenyltin(IV) complexes, complex **10** is more active than complex **11**. The lower potency of complex **11** (1.4folds) may be attributed to the bulky size legend and lack of ketoamide group. The higher potency of complex **10** is attributed to increasing accessibility of tin metal ion to phosphate groups in RNA and chelating ability of ketoamide groups at ligand.

Among the studied triorganotin(IV) complexes, butyl substituted complexes are more active than methyl and phenyl substituted. Complex **3** and **11** have the same nature of ligands, but complex **3** is 4.4 folds more potent against HCV than complex **11** due to butyl-substituted groups. Similarly, among complex **6** and **9**, ligands are same, but

former one is 1.7 folds more active than later one due to butyl-substituted groups. Lower lipophilicity of methyl and phenyl groups as compared to butyl significantly reduces the passage of complex **3** and **6** through cell membranes, which results in lower activity of these complexes (Nath *et al.*, 2010; Zhang *et al.*, 2007).

Conclusion

Triorganotin(IV) complexes were synthesized and characterized both in solution and solid state by elemental analysis, IR, NMR spectroscopy, Mass spectrometry, and single-crystal analysis. The triorganotin(IV) complexes were screened for Anti-HCV activity using the Gaussia luciferase assay. The results showed that tributyltin(IV) complexes are more active as compared to trimethyl and triphenyltin(IV) complexes. Among tributyltin(IV) complexes, the complex **1** exhibited the more promising performance due to the chloro group substitution at para position and ketoamide groups on the ligand.

Supplementary materials

Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 727660 for compound **7**. Copies of the information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB21EZ [Fax +44(1223) 336-033], e-mail deposit@ccdc.cam,ac.uk or http://WWW.ccdc.cam.ac.uk.

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