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Design, spectral characterization, anti-tumor and anti-inflammatory activity of triorganotin(IV) hydroxycarboxylates, apoptosis inducers: *In vitro* assessment of induction of apoptosis by enzyme, DNA-fragmentation, acridine orange and comet assays

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ABSTRACT

Interaction of triorganotin(IV) chlorides with sodium salt of hydroxycarboxylic acids results in the formation of triorganotin(IV) hydroxycarboxylates, $R_3Sn(L)$ [R = Me, *n*-Bu and Ph; L = anion of glucuronic (HGlu), gallic (HGal) and mandelic (HMal) acid]. They exhibit trigonal–bipyramidal geometry which is well supported by elemental analysis, IR, ^{1}H, ^{13}C, ^{119}Sn NMR and ESI-MS spectral data. Triorganotin(IV) hydroxycarboxylates have been screened *in vitro* against five cancer cell lines of human origin *viz.* MCF-7, HEK-293, PC-3, HCT-15 and HepG-2. Title complexes are found to be cytotoxic to mildly cytotoxic, and exhibited IC_{50} values in the range 4–30 μg/mL. The results of enzyme assays *viz.* glutathione reductase, glutathione peroxidase, total glutathione content and lipid peroxidase assay on MCF-7 cells indicate that the reactive oxygen species generated in the cancer cells by triorganotin(IV) hydroxycarboxylates is responsible for cell death. Marginal increase of lactate dehydrogenase suggests that necrosis is also occurring to a small extent. DNA (deoxyribonucleic acid) fragmentation assay, acridine orange assay and comet assay clearly support that the cell death is mainly due to apoptosis. The results obtained for the *in vivo* anti-inflammatory activity (% inhibition) and toxicity (LD_{50} in mg/kg) suggested that the complexes have low toxicity and good anti-inflammatory activity.

1. Introduction

Globally cancer among the various deadly diseases is one of the leading health concerns for humans, accounting for more than 15% of human deaths. Recently, it has been reported by WHO that about 27 million new cancer cases and 11.5 million deaths due to cancer are expected in the next 2 decades [1]. Cancers, caused by abnormal and uncontrolled cell division, are usually derived from numerous tissues with multiple etiologies and endless combination of genetic and/or epigenetic alterations; therapies for cancers are as diverse as the disease itself. The treatments for cancer include surgery, radiotherapy, photodynamic therapy and chemotherapy, and chemotherapy is based on the inhibition of the rapid proliferation of cancer cells for which the replication of DNA is to be arrested. A number of cancer chemotherapeutic drugs are commercially available for treatment of different types of cancers, but each of which has one or other severe side effects [2]. That is why; one of the most rapidly developing areas of pharmaceutical research is the discovery, design and synthesis of robust, effective and selective cancer chemotherapeutic drugs which should have minimum side effects.

Further, cancer chemotherapy based on metallotherapeutic drugs has gained momentum after the serendipitous discovery of *cis*-platin (*cis*-diamminedichloroplatinum(II)) [3,4]. However, despite its remarkable success, there are well-known drawbacks associated with Pt drugs, *viz.* nephrotoxicity or neurotoxicity, intrinsic acquired drug resistance and patient compliance [5,6]. Therefore, this discovery stimulated the search for other metal-based chemotherapeutics with potential antitumor activity having fewer side effects [7,8]. Among these, organotins have emerged as potential biologically active metallopharmaceuticals,

Abbreviations: Bz-d_6, deteuratedbenzene; HGlu, glucuronic acid; HGal, gallic acid; HMal, mandelic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DNA, deoxyribonucleic acid.

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and several research groups are actively engaged in the field [9–23]. Many organotin compounds have been established as potential cancer chemotherapeutic agents because of their apoptotic inducing character [22–26], and they occupy an important place in cancer chemotherapy reports [11,12,17b,24]. Furthermore, the National Cancer Institute has tested about 2000 tin-based compounds, the largest number ever tested among metal-based drug candidates [27], and recently it has been reported that four out of thirty interesting inorganic pharmaceuticals are tin compounds [28]. Despite this, the exact mechanism of mode of action of organotin compounds remains unestablished.

Organotin(IV) carboxylates are among the most widely studied class of compounds owing to their rich structural chemistry ranging from monomeric, dimeric, tetrameric, oligomeric to polymeric motifs [13,22,23,29–31]. The structures can be easily controlled by adjusting the carboxylato ligands, tin–R groups and metal-to-ligand molar ratio [29,32,33]. In addition to their structural diversity, the organotin(IV) carboxylates also present very promising antitumor activities [11,12,22,23,27,34–42]. Among these, several di-*n*-butyltin(IV) carboxylates have been found to be the most active, and in some cases they exhibit much higher activity than clinically used reference compounds such as *cis*-platin, doxorubicine and methotrexate [11,12,27,37].

The systemic toxicity of chemotherapeutic drugs, their lack of tumor localization and an even distribution throughout the body including tumor tissues are the most significant challenges facing effective cancer chemotherapy. Therefore, a rational drug design is essentially required to optimize specific targeting features and to control toxicity (side effects), by controlling thermodynamic and kinetic processes of metal complexes *viz.*, choice of metal ion and its oxidation state [43] or tailoring of ligand scaffold. It is also well known that the cytotoxicity is related to some extent to lipophilicity of the drugs *i.e.*, the most lipophilic compounds are the most cytotoxic [44]. Further, the ligands having biologically active pharmacophore with biocompatible properties are tethered to others having multifunctional N and O donors to obtain modulated ligand scaffold which can mute the potential toxicity of metallopharmaceuticals drugs [8].

Anti-tumor activity of several organotin compounds has been reported by various workers, but their solubility has always remained a problem. This can be solved by choosing ligands having polar groups such as hydroxy groups, or other oxygen or nitrogen containing groups or substituted fluorine which may enhance their solubility. Glucuronic acid (HGlu), mandelic acid (HMal) and gallic acid (HGal) are such hydroxycarboxylic acids which are highly soluble in water, methanol, ethanol and other organic solvents and possess various medicinal properties. Glucuronic acid (uronic acid) exhibits anti-oxidant activity [45]. Mandelic acid (α-hydroxyl carboxylic acid) has been used for the treatment of skin problems, and also possess anti-bacterial activity [46]. Gallic acid is a naturally occurring plant phenol obtained by the hydrolysis of tannins and is known to display some pharmacological activities such as anticancer, fungicidal/fungi-static, antiviral, anti-inflammatory and anti-oxidant properties [47].

Interaction of glucuronic acid with bis(tributyltin) oxide (studied via NMR and mass spectroscopy) in solution [48], the solution studies of tris(1-butyl)stannyl-D-glucuronate through NMR spectral analysis [49], di-*n*-butyltin(IV) and diethyltin(IV) complexes of methoxy analog of gallic acid [50], reaction of ethyltrichlorostannane with *N*,*N*-dimethylamide of mandelic acid [51] and synthesis of trimethyltin derivative of mandelic acid [52] are few reports available in literature. Moreover, the functional groups attached to carboxylic acid may have pronounced influence (increase/decrease) on the biological properties of organotin(IV) carobxylates [22,34–37]. Therefore, it becomes indispensable to synthesize organotin(IV) derivatives of β-D-glucuronic acid (HGlu), (±)-mandelic acid (HMal) and gallic acid (HGal) (Fig. 1), and investigate their biological activities, *viz. in vitro* anti-tumor activity (against MCF-7, HEK-293, PC-3, HCT-15 and HepG-2), *in vivo* anti-inflammatory activity and toxicity studies. Furthermore, to view insight the mode of action of the studied complexes various assays, such as lactate dehydrogenase, glutathione reductase, glutathione peroxidase, total glutathione content, lipid peroxidase, DNA fragmentation, acridine orange and comet assay have also been conducted and discussed in this manuscript.

2. Material and methods

2.1. Materials

The specifications and makes of triorganotin(IV) compounds, and methods to obtain various dried solvents are same as reported previously [22]. Gallic acid, β-D-glucuronic acid and (±)-mandelic acid were procured from Alfa Aesar and used as received. Further, a large number of chemicals used for biological activities are also same as reported in our previous manuscript [22]. Five cancer cell lines of human origin, *viz.* MCF-7 mammary cancer, HEK-293 kidney cancer, PC-3 prostate cancer, HCT-15 colon cancer and HepG-2 liver cancer were purchased from National Center for Cell Science (NCCS) Pune, India. For anti-inflammatory activity and acute toxicity Swiss albino mice were procured from All India Institute of Medical Science (AIIMS) Delhi, India.

2.2. Physical measurements

The melting points, microanalyses (C, H and N) and tin content were determined as described previously [22]. Molar conductance measurements, infrared and far-infrared, nuclear magnetic resonance (^{1}H, ^{13}C and ^{119}Sn) spectra were recorded on the same instruments as reported previously [22]. ESI-MS spectra were recorded on Bruker MicroTOF-Q II mass spectrometer at a flow rate of 180 μL/h on positive mode in water–acetonitrile mixture. Optical density (OD) in MTT assay, gel electrophoresis, enzymes assays, *in vivo* anti-inflammatory activity and toxicity were done according to the reported procedures with slight modifications as described in our previous manuscript [22].

2.3. Synthesis

2.3.1. Synthesis of sodium salt of hydroxycarboxylic acids

Hydroxycarboxylic acid (HGlu/HMal/HGal, 2.0 mmol) was dissolved in 10 mL of specially dried ethanol under dry nitrogen and added to sodium ethoxide, prepared by reacting sodium (0.058 g, 2.5 mmol) with dry ethanol (25 mL). The resulting mixture was refluxed giving a clear solution of sodium salt of the acid within half an hour. Refluxing was continued for another 1–2 h with constant stirring. The solution was concentrated and solid was dried in vacuum.

2.3.1.1. Na(Glu). IR (cm^{-1}): ν_{as}(OCO) 1616s, ν_{s}(OCO) 1429m, $\Delta\nu$ 187, ν_{as}(COC) 1245m, ν_{s}(COC) 1110m, ν(OH-1) 3533s, ν(OH-2) 3400s, ν(OH-3) 3488s, ν(OH-4) 3509s. ^{1}H NMR (500.13 MHz, Bz-d_6, ppm): δ 4.63 (d, 1H, H-1, $J_{1,2}$ = 8.0 Hz), 3.25 (dd, 1H, H-2, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.1 Hz), 3.50 (dd, 1H, H-3, $J_{2,3}$ = 9.1 Hz, $J_{3,4}$ = 9.2 Hz), 3.96 (dd, 1H, H-4, $J_{3,4}$ = 9.2 Hz, $J_{4,5}$ = 9.6 Hz), 3.93 (d, 1H, H-5, $J_{4,5}$ = 9.6 Hz), 4.81 (s, 1H, OH-1), 3.55 (s, 1H, OH-2), 3.72 (s, 1H, OH-3), 3.86 (s, 1H, OH-4). ^{13}C NMR (125.75 MHz, Bz-d_6, ppm): δ 95.86 (C-1), 74.53 (C-2), 72.43 (C-3), 72.74 (C-4), 76.48 (C-5), 175.31 (C-6).