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Synthesis, characterization and cytotoxicity of novel mixed-ligand butyl-hexyltin(IV) complexes with N-hydroxybenzamides

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ABSTRACT

Five new mononuclear butyl-hexyltin(IV) complexes with para-position substituents of N-hydroxybenzamide anions [para-position substituent = H (**1**), F (**2**), Cl (**3**), Br (**4**), I (**5**)], formulated as the [^{n}Bu^{n}HexSnL$_2$] (Bu = butyl, Hex = hexyl, HL = substituted N-hydroxybenzamide), were prepared and characterized by elemental analyses, FT-IR, ^{1}H, ^{13}C, ^{119}Sn NMR spectroscopies. Crystal structure of complex **2** was determined by X-ray diffraction analysis. The cytotoxicity was tested by SRB assay. The results indicate that the complex **5** with iodine atoms has the best cytotoxicity against KB cells, even being better than that of cisplatin. It suggests that both halogens and alkyl groups around tin have important effects on cytotoxicity.

1. Introduction

Organotin(IV) compounds are a widely studied class of metal-based antitumour drugs [1–10]. In the structure–activity relationships for organotin(IV) derivatives RnSn(L)m, it can be concluded that dialkyltin(IV) compounds generally exhibit higher antitumour activity than those of the corresponding mono-, tri- and tetraorganotin(IV) or the inorganic tin derivatives, and within the diorganotin(IV) class (e.g. dimethyltin(IV), diethyltin(IV), di-*n*-butyltin(IV) and diphenyltin(IV) complexes), neither dimethyltin(IV) nor diethyltin(IV) complexes exhibit good antitumour activity [2,11,16,18]. The highest activity is exerted by the di-*n*-butyltin(IV) complexes, and the nature of the organic group R is the primary factor.

According to the literature, those dialkyltin(IV) complexes having been investigated their antitumour activity almost contained the homoleptic di-*n*-butyltin(IV) varieties. Furthermore, changing the organic radicals in the homoleptic species di-*n*-butyltin(IV) may result in altering the target species in terms of antitumour activity. So far, only a limited number of heteroleptic mixed methyl-heptyltin(IV) [12] or methyl-aryltin(IV) systems [13–15] are known, and most of which are triorganotin(IV) and tetraorganotin(IV) compounds. As an extension of the research as above, we are interested in the activities about the diorganotin(IV) complexes with different carbon chain lengths. We are wondering if much longer carbon chain can increase the antitumour activity and if two butyl groups in butyltin(IV) complexes are really necessary for the good antitumour activity.

No more information has been given on this type of mixed alkyl-alkyltin(IV) complexes with N-hydroxybenzamides, and their antitumour activities have not yet been investigated. As part of our ongoing work, in this paper, five new butyl-hexyltin(IV) complexes, [^{n}Bu^{n}HexSnL$_2$] (Bu = butyl, Hex = hexyl, HL = substituted N-hydroxybenzamide), have been prepared (Scheme 1) and characterized by elemental analyses, FT-IR, ^{1}H, ^{13}C, ^{119}Sn NMR spectroscopies and X-ray diffraction analysis. Here, we also report their *in vitro* cytotoxicity in human nasopharyngeal carcinoma (KB) cell lines. The IC$_{50}$ values for the butyl-hexyltin(IV) complexes are comparable to both dibutyltin(IV) complexes and cisplatin, and some preliminary structure–activity relationships are discussed.

2. Results and discussion

2.1. Syntheses

Butyl-hexyltin(IV) compounds of the type [^{n}Bu^{n}HexSnL$_2$] can be prepared by reacting butyl-hexyltin(IV) oxide [^{n}Bu^{n}HexSnO] with 4-fluoro-N-hydroxybenzamide (4-chloro-N-hydroxybenzamide, 4-bromo-N-hydroxybenzamide or 4-iodo-N-hydroxybenzamide) under reflux condition. The detailed methodologies for the preparation of [^{n}Bu^{n}HexSnO] and **1**–**5** are reported in Sections 4.1.2–4.1.7. These complexes are all white crystalline solids and stable for months. Complexes **1**–**5** are not soluble in water, but soluble in chloroform, dichloromethane, ethanol, benzene, toluene and DMSO, and only partly soluble in methanol.

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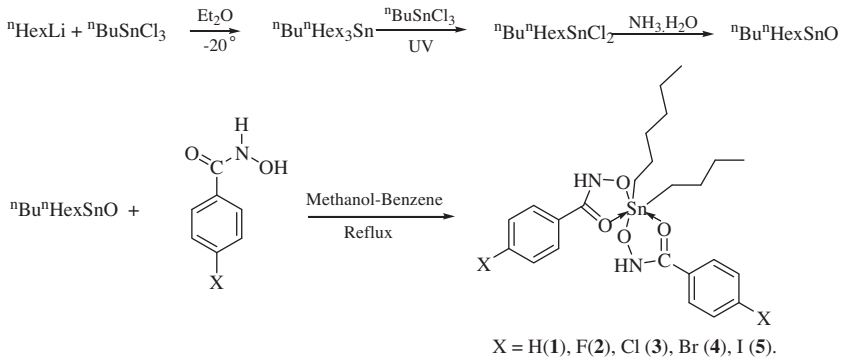

Scheme 1. Synthesis of the butyl-hexyltin(IV) complexes of substituted N-hydroxybenzamides (**1**–**5**).

2.2. Spectroscopy

In the infrared spectra, the important absorption bands are strong ν(O–H) in the region 3230–3020 cm^{-1} in 4-halo-N-hydroxybenzamides (4-fluoro-N-hydroxybenzamide, 4-chloro-N-hydroxybenzamide, 4-bromo-N-hydroxybenzamide and 4-iodo-N-hydroxybenzamide) [9]. These peaks disappear for the complexes (**1**–**5**), indicating that the reaction had taken place through the replacement of the N–OH hydrogen by the organotin moiety. The IR spectra of all the five compounds show evidence for the coordination of the 4-halo-N-hydroxybenzamide anions by both oxygen atoms of the CONHO– group. In fact, the ligation through the carbonyl oxygen is indicated by the ν(C=O) shift to a lower frequency, i.e. from ca. 1630 cm^{-1} in the free species to 1600 cm^{-1} in the chelated one. The bands in the range 493–437 cm^{-1} and 596–503 cm^{-1} are assigned as ν_{sym}(Sn–C) and ν_{asym}(Sn–C), respectively. These absorption bands indicate the presence of Sn–C bonds and are compatible with literature values [16]. Similarly, the existence of ν(C–H) bending frequencies in CH_2 and CH_3, confirming the presence of *n*-alkyl groups of all the synthesized compounds.

The proton chemical shift assignments of the butyl-hexyltin(IV) moiety are readily deducible from the multiplicity patterns and resonance intensities. The assignments of the signals for compounds **1**–**5** are reported in Sections 4.1.3–4.1.7. The N–H signals in **1**–**5** appeared as two separate singlets in the region of 14.83–9.93 ppm, indicating the different surroundings of two N-hydroxybenzamide anions. The integration values are consistent with the structures. In the ^{1}H NMR spectra of complex **1**–**5**, the complication of peak shape in the region of 1.9–0.9 ppm is different from those di-*n*-butyltin(IV) derivatives [2–5,16–18], which is consistent with the presence of hexyl group. Due to the *n*-butyl protons overlap with the signal of *n*-hexyl groups ligated to the tin atom, what hampers the identification of the individual protons of the ligand moieties and the measurement of the J_{Sn-H} coupling constants.

In the range of alkyl carbon (13.8–30.4 ppm), ^{13}C NMR spectra of complexes **1**–**5** give ten peaks distinctly, which well correspond to every carbon atom (butyl and hexyl groups). Besides this, another fourteen carbons also can find every peak value in the aromatic carbon part and carbonyl part. Similarly, due to the *n*-butyl carbons overlap with the signal of *n*-hexyl groups ligated to the tin atom, what hampers the identification of the individual carbons of the ligand moieties and the measurement of the J_{Sn-C} coupling constants.

^{119}Sn NMR spectra in $CDCl_3$ solution of **1**–**5** display only one resonance (the chemical shifts are from −350 to −400 ppm), indicating one type of tin site. The chemical shifts are agreement with the hexa-coordinated tin atoms [19–21], which shows that the structure and the type of tin atom in the solid state are still retained in solution. One of them will be further confirmed by single-crystal X-ray analysis as below.

2.3. X-ray crystallography

A view of the molecular structure and atomic numbering scheme for **2** is shown in Fig. 1. The selected bond lengths and angles of the complex are given in Table 2. A summary of these data by single-crystal X-ray diffraction analysis and selected experimental information are given in Table 1.

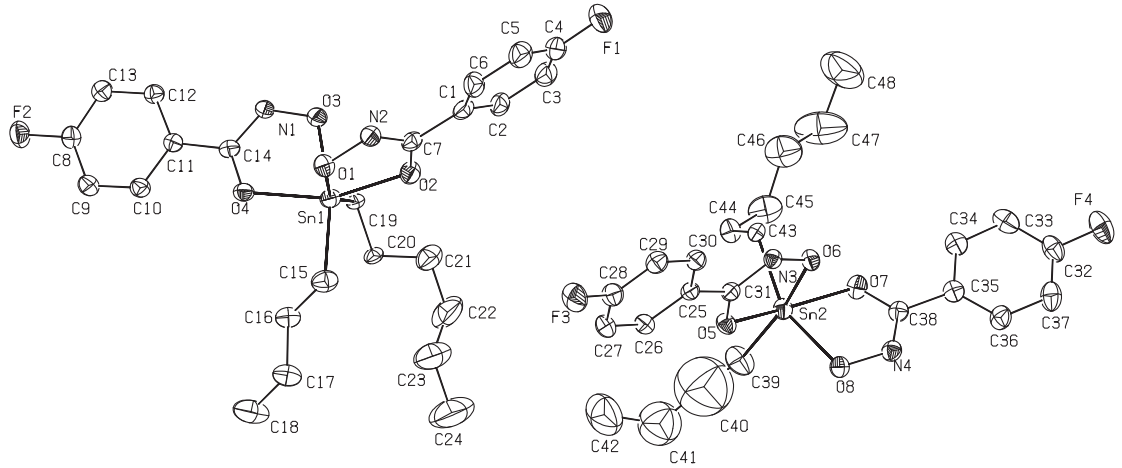

Fig. 1. Molecular structure and atomic numbering scheme for complex **2** (hydrogen atoms are omitted for clarity).

Table 1
Experimental data for crystallographic analysis for complex **2**.

	2
Chemical formula	$C_{24}H_{32}F_2N_2O_4Sn$
M (g mol^{-1})	569.21
Crystal system	tetragonal
Space group	$I4(1)/a$
a (Å)	27.6757(10)
b (Å)	27.676
c (Å)	26.5129(10)
α (°)	90
β (°)	90
γ (°)	90
V (Å^3)	20307.4(11)
Z	32
ρ_{calc} (g cm^{-3})	1.489
μ (mm^{-1})	1.052
Reflections collected	116992
Individual reflections	12027
R_{int}	0.0368
θ completeness [%]	27.49° [99.2]
Data/restraints/parameters	12027/118/595
GOF on F^2	1.085
Final indices [$I > \sigma(I)$]	R_1 = 0.0642
	wR_2 = 0.1872
Final indices (all data)	R_1 = 0.0975
	wR_2 = 0.2463

Table 2
Summary of the most relevant bond distances (Å) and angles (°) of **2**.

Sn(1)–O(2)	2.116(3)	Sn(2)–O(8)	2.126(3)
Sn(1)–O(1)	2.125(3)	Sn(2)–O(7)	2.125(3)
Sn(1)–O(4)	2.174(3)	Sn(2)–O(6)	2.153(3)
Sn(1)–O(3)	2.136(3)	Sn(2)–O(5)	2.172(3)
Sn(1)–C(15)	2.129(6)	Sn(2)–C(43)	2.060(4)
Sn(1)–C(19)	2.049(4)	Sn(2)–C(39)	2.126(6)
C(7)–O(2)	1.285(5)	C(38)–O(7)	1.287(5)
C(7)–N(2)	1.279(6)	C(38)–N(4)	1.293(6)
C(14)–O(4)	1.271(5)	C(31)–O(5)	1.270(5)
C(14)–N(1)	1.318(6)	C(31)–N(3)	1.316(6)
C(19)–Sn(1)–C(15)	104.07(19)	C(43)–Sn(2)–C(39)	104.3(3)
C(19)–Sn(1)–O(2)	86.41(15)	C(43)–Sn(2)–O(8)	155.04(14)
C(19)–Sn(1)–O(1)	156.15(15)	C(43)–Sn(2)–O(7)	86.14(15)
O(2)–Sn(1)–O(1)	75.77(13)	O(7)–Sn(2)–O(8)	76.05(13)
O(2)–Sn(1)–O(3)	85.73(13)	O(7)–Sn(2)–O(6)	85.76(12)

The crystal structure of **2** consists of two independent complexes in the asymmetric unit with closely comparable geometries. The coordination polyhedron consists of one butyl group, one hexyl group and two oxygen atoms (one oxygen derived from C=O, the other oxygen stemmed from –OH) from asymmetric N-hydroxybenzamide moieties, leading to a highly distorted octahedral geometry [22–24]. Each tin centre is hexa-coordinated in *cis* coordination geometry [25], with angles of 104.07(19)° for C19–Sn1–C15 and 104.3(3)° for C43–Sn2–C39, being much smaller than those reported dialkyltin(IV) complexes with N-hydroxybenzamides [26,27]. For example, the tin centers of [$Me_2Sn\{4\text{-}ClC_6H_4C(O)NHO\}_2$] with angles of 142.7(2)° C–Sn–C and [$Me_2Sn\{4\text{-}OCH_3C_6H_4C(O)NHO\}_2$] with angles of 140.8(2)° [16].

In the structure the two N-hydroxybenzamide residues are functioning as bidentate ligands, but not equivalent. One group of Sn–O bond lengths in **2** [Sn1–O2, 2.116(3) Å and Sn1–O1, 2.125(3) Å] are very close to that in [$Me_2Sn\{4\text{-}ClC_6H_4C(O)NHO\}_2$] and [$Me_2Sn\{4\text{-}OCH_3C_6H_4C(O)NHO\}_2$] [16], forming one short covalent bond and one longer coordinate oxygen–tin bond (Table 2). However, the other group of Sn–O bond lengths in **2** [Sn1–O3, 2.136(3) Å and Sn1–O4, 2.174(3) Å] are shorter than those in [$Me_2Sn\{4\text{-}ClC_6H_4C(O)NHO\}_2$] and [$Me_2Sn\{4\text{-}OCH_3C_6H_4C(O)NHO\}_2$]

Table 3
Hydrogen bond geometry of complex **2**.

D–H...A	D–H (Å)	H...A (Å)	D...A (Å)	∠D–H...A (°)
N(1)–H(1)...O(6)#1	0.86	1.92	2.729(5)	156.8
N(3)–H(3A)...O(3)#2	0.86	1.95	2.748(5)	154.8
C(10)–H(10)...F(2)#3	0.93	2.51	3.292(6)	141.7
C(12)–H(12)...O(6)#1	0.93	2.45	3.276(6)	148.1
C(19)–H(19A)...N(4)#1	0.97	2.03	2.780(5)	132.2
C(30)–H(30)...O(3)#2	0.93	2.51	3.369(6)	154.5
C(43)–H(43A)...N(2)#2	0.97	2.07	2.731(5)	123.8
C(40)–H(40B)...O(5)	0.97	2.94	3.409(16)	110.6

Symmetry codes for **2**: (i) $x + 1/2$, y, $-z + 1/2$; (ii) $-x + 3/2$, $-y + 3/2$, $-z + 1/2$; (iii) $y + 1/4$, $-x + 7/4$, $-z - 1/4$.

and much shorter than those found in five-coordinated diorganotin(IV) complexes [18,28,29], e.g. 2.321(4) [18]. Meanwhile, the bond length of Sn–C19 (tin-hexyl) [2.049(4) Å] is slightly shorter than that of Sn–C15 (tin-butyl) [2.129(6) Å], showing better stabilization of Sn–hexyl bond than Sn–butyl bond.

In the molecular structure, the two five-member rings around Sn are oriented at big dihedral angle degrees of 85.32° (between the planes of Sn1/O1/O2 and Sn1/O3/O4). While in the structures of other mononuclear organotin(IV) N-hydroxybenzamides [17,18], such as [$Bu_2Sn\{4\text{-}ClC_6H_4C(O)NHO\}(4\text{-}ClC_6H_4COO)$] and [$Me_2Sn\{3,4\text{-}F_2C_6H_3C(O)NHO\}_2$], the values of the corresponding dihedral angle are 6.64° and 8.99°, respectively, being much less than that of complex **2**. So, the environment of the tin atoms reported here for [$^nBu^nHexSnL_2$] (**2**) is different from those mononuclear diorganotin(IV) N-hydroxybenzamides found in previous derivatives published [17,18,22].

In complex **2**, there are four sets of hydrogen bonds [N–H···O, C–H···F, C–H···O and C–H···N] (see Table 3), which stabilize the complex **2** into a three-dimensional structure as illustrated in Fig. 2. Moreover, π···π stacking interaction between two associate benzene rings plays an important role in constructing the supramolecular network.

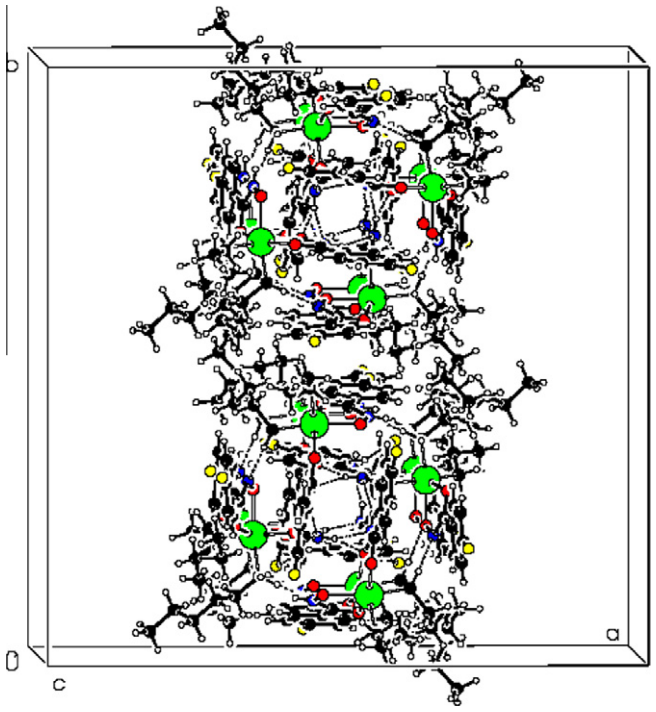

Fig. 2. Packing diagram of the complex **2** viewed along *c*-axis.

Table 4
The cytotoxicity of compounds **1**–**5** against KB human tumor cells.

Compounds	IC_{50} (μM)
1	24.12 ± 1.27
2	38.40 ± 2.92
3	21.03 ± 1.02
4	2.54 ± 0.30
5	2.02 ± 0.37
$[Bu_2Sn\{C_6H_5C(O)NHO\}_2]$	1.30 ± 0.10
$[Bu_2Sn\{4\text{-}FC_6H_4C(O)NHO\}_2]$	0.85 ± 0.05
$[Bu_2Sn\{4\text{-}ClC_6H_4C(O)NHO\}_2]$	0.01 ± 0.00
$[Bu_2Sn\{4\text{-}BrC_6H_4C(O)NHO\}_2]$	1.25 ± 0.12
$[Bu_2Sn\{4\text{-}IC_6H_4C(O)NHO\}_2]$	0.78 ± 0.05
Cisplatin	2.65 ± 0.33

2.4. Cytotoxicity of compounds

According to the previous results [29], diorganotin(IV) complexes is very sensitive to human cancer KB cell lines, so in this experiment we select KB cell as model to check cytotoxicity. In order to examine whether the introduced chemical modifications improve their *in vitro* antitumour properties, the five butyl-hexyltin(IV) complexes with N-hydroxybenzamides, the five dibutyltin(IV) analogs and cisplatin have been screened at identical conditions for the preliminary cytotoxicity against human nasopharyngeal carcinoma (KB) cell lines at five different concentrations. The IC_{50} values obtained are listed in Table 4.

The following structure–activity relationships could be recognized: (i) Among the complexes **1**–**5**, the complexes **1**, **2** and **3** exhibited lower *in vitro* cytotoxic activities towards the KB tumor cell lines, while the complexes **4** and **5** exhibited stronger activities against the same cell lines with lower IC_{50} values (<3 μM). (ii) For the series of butyl-hexyltin(IV) derivatives, the data show gradually increased cytotoxicity against human tumor KB cell lines with a decrease of electron-withdrawing properties of X substituents, following the orders: I > Br > Cl > F (**5** > **4** > **3** > **2**). The trend suggests these X substituents of the substituted N-hydroxybenzamide anions have important effects on cytotoxicity. (iii) Among **1**–**5** compounds, the complex **5** containing iodine atoms exhibit the greatest cytotoxic properties, being even more active than "cisplatin", the clinically used drug. (iv) Comparison of the IC_{50} values suggests that dibutyltin(IV) complexes [11,16] of substituted N-hydroxybenzamides with two butyl groups are better than the butyl-hexyltin(IV) analogs with one hexyl and one butyl groups, indicating that the hexyl group led to a huge decrease in biological activity.

3. Conclusions

In conclusion, for the first time, we have successfully prepared five new butyl-hexyltin(IV) complexes with N-hydroxybenzamides, which were fully characterized by elemental analyses, IR, ^1H, ^{13}C, ^{119}Sn NMR and, for **2**, X-ray single-crystal diffraction analysis. These compounds exhibit *in vitro* antitumor activity towards human tumor KB cell lines to some extent. According to our experimental results, organic group R plays an important role indeed, and butyl groups in di-*n*-butyltin(IV) complexes are necessary for the good antitumour activity. The contribution of much longer carbon chain (e.g. hexyl group ligated to the tin atom) to antitumour activity might be limited. On the other hand, the electronic effect of oxygen-ligands (N-hydroxybenzamides) also has an important effect on cytotoxic activity. The case of complex **5** containing I groups is the most active one and the type of the X substituents in the 4-halo-N-hydroxybenzamide affects the cytotoxic activity, following the orders: I > Br > Cl > F (**5** > **4** > **3** > **2**). Therefore, for the butyl-hexyltin(IV) complexes, the order of antitumour activity suggests a close correlation between activity and electronic properties of halogens, which may be beneficial in the design of new metal-based antitumour agents.

4. Experimental

4.1. Chemistry

4.1.1. Materials and methods

Methyl 4-fluorobenzoate, methyl 4-chlorobenzoate, methyl 4-bromobenzoate, methyl 4-iodobenzoate, N-hydroxybenzamide, *n*-hexyllithium and $^nBuSnCl_3$ were purchased from Aldrich and used as received. (^{n}Butyl)(^{n}hexyl)tin(IV) oxide was prepared as below according to the literature methods (see Section 4.1.2) [30–32]. The other reagents were of analytical grade. 4-Fluoro-N-hydroxybenzamide, 4-chloro-N-hydroxybenzamide, 4-bromo-N-hydroxybenzamide and 4-iodo-N-hydroxybenzamide were prepared according to a known general procedure [10]. Five di-*n*-butyltin(IV) analogs $[Bu_2Sn\{C_6H_5C(O)NHO\}_2]$, $[Bu_2Sn\{4\text{-}FC_6H_4C(O)NHO\}_2]$, $[Bu_2Sn\{4\text{-}ClC_6H_4C(O)NHO\}_2]$, $[Bu_2Sn\{4\text{-}BrC_6H_4C(O)NHO\}_2]$ and $[Bu_2Sn\{4\text{-}IC_6H_4C(O)NHO\}_2]$ were synthesized by reported methods [18].

Elemental analyses were performed on a PE-2400-II elemental analyzer. IR spectra in the range 4000–400 cm^{-1} were recorded on a Perkin Elmer FT-IR spectrophotometer in KBr discs. ^1H, ^{13}C, ^{119}Sn NMR spectra were recorded on a Varian INOVA 600 spectrometer (600.0 MHz for ^1H, 150.8 MHz for ^{13}C and 223.6 MHz for ^{119}Sn) at ambient temperature [δ values in ppm relative to Me_4Si (^1H, ^{13}C) or Me_4Sn (^{119}Sn)].

Suitable single crystal of the complex **2** was mounted in glass capillaries for X-ray structural analysis. Diffraction data were collected on a Bruker SMART CCD diffractometer with Mo Kα (λ = 0.71073 Å) radiation at room temperature. During the intensity data collection, no significant decay was observed. The intensities were collected for Lorentz-polarization effects and empirical absorption with the SADABS program. The structure was solved by direct methods using the SHELXL-97 program. All non-hydrogen atoms were found from the difference Fourier syntheses. The H atoms were included in calculated positions with isotropic thermal parameters related to those of the supporting carbon atoms but were not included in the refinement. All calculations were performed using the Bruker Smart program [33].

4.1.2. Synthesis of (^{n}Bu)(^{n}Hex)SnO

Reaction of *n*-hexyllithium (^{n}HexLi) with $^nBuSnCl_3$ in Et_2O at −20 °C gave $^nBu^nHex_3Sn$. Keeping 2 mol $^nBuSnCl_3$ and 1 mol $^nBu^nHex_3Sn$ in UV light 3 h gave 90% $^nBu^nHexSnCl_2$. $^nBu^nHexSnO$ was precipitated from an aqueous solution of $^nBu^nHexSnCl_2$ by the addition of aqueous ammonia. The precipitate was washed with water until it was free of chloride ion and dried overnight in a vacuum oven at 70 °C. Yield: 85%, m.p. > 300 °C. Elemental *Anal.* Calc. for $C_{10}H_{22}OSn$: C, 43.36; H, 8.01. Found: C, 42.59; H, 7.87%.

4.1.3. Synthesis of [(^{n}Bu)(^{n}Hex)Sn{$C_6H_5C(O)NHO$}$_2$] (**1**)

A solution of 0.249 g (1.0 mmol) butyl-hexyltin(IV) oxide was added to a solution of 2.0 mmol N-hydroxybenzamide in dry ethanol–toluene (1:3 v/v, 120 mL). The mixture was refluxed under nitrogen atmosphere for 6 h, and the solvent was evaporated to 5 mL under reduced pressure. The precipitate thus formed was filtered off, recrystallized from *n*-hexane and dried to constant weight. Yield: 59%. Elemental *Anal.* Calc. for $C_{24}H_{34}N_2O_4Sn$: C, 54.06; H, 6.43; N, 5.25. Found: C, 54.26; H, 6.49; N, 5.28%. IR: 3427m (N–H), 3130, 1595s (C=O)/(N–C), 990s, 930w (N–O), 576m (Sn–C), 480s (Sn–O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 10.30 (s, 1H, N–H), 9.72 (s, 1H, N–H), 7.38 [t, $^3J_{HH}$ = 8.1, 4H]; 7.75 [dd,

$^3J_{HH}$ = 8.1, 4H]; 1.83–1.03 [m, 16H, 8CH_2]; 0.83 [t, 3H, $^3J_{HH}$ = 5.4, CH_3], 0.81 [t, 3H, $^3J_{HH}$ = 6.0, C′H_3] ppm; ^{13}C NMR ($CDCl_3$): δ = 163.4, 162.9 (C=O), 116.3, 128.7, 114.4, 28.0–13.3 [Sn–R] ppm. ^{119}Sn ($CDCl_3$): δ = − 356.9 ppm.

4.1.4. Synthesis of [(^{n}Bu)(^{n}Hex)Sn{4-FC_6H_4C(O)NHO}$_2$] (**2**)

A solution of 0.249 g (1.0 mmol) butyl-hexyltin(IV) oxide was added to a solution of 2.0 mmol 4-fluoro-N-hydroxybenzamide in dry ethanol–toluene (1:3 v/v, 120 mL). The mixture was refluxed under nitrogen atmosphere for 6 h, and the solvent was evaporated to 5 mL under reduced pressure, the residue drying slowly by exposure to the air. 15 mL *n*-hexane was then added to soak the solid at room temperature for 24 h, filtrating. The filtrate lay in the air for 1 week, and many long sheer prismy crystals thus formed. Yield: 56%. Elemental *Anal.* Calc. for $C_{24}H_{32}F_2N_2O_4Sn$: C, 50.64; H, 5.67; N, 4.92. Found: C, 50.68; H, 5.65; N, 4.89%. IR: 3423m (N–H), 3163, 1606s (C=O)/(N–C), 917s (N–O), 509m (Sn–C), 418s (Sn–O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 14.83 (s, 1H, N–H), 13.38 (s, 1H, N–H), 7.26 [t, $^3J_{HH}$ = $^3J_{HF}$ = 8.4, 4H]; 7.55 [dd, $^3J_{HH}$ = 9.0, $^4J_{HF}$ = 4.8, 4H]; 1.90–1.88, 1.77–1.61, 1.50–1.37 [m, 16H, 8CH_2]; 0.97 [t, 3H, $^3J_{HH}$ = 5.4, CH_3], 0.94 [t, 3H, $^3J_{HH}$ = 7.8, C′H_3] ppm; ^{13}C NMR ($CDCl_3$): δ = 165.6, 165.0 (C=O), 163.9, 163.3 (C–F), 161.0, 160.5 (C1, C1′); 129.1, 129.0, 128.2, 128.1, 122.8, 122.1, 114.7, 114.6 [C2, C2′, C3, C3′, C5, C5′, C6, C6′]; 30.4, 29.6, 27.4, 26.2, 21.5, 19.5, 19.1, 14.1 [(CH_2)$_3$ and (CH_2)$_5$], 13.9 (CH_3), 13.8 (CH_3) ppm. ^{119}Sn ($CDCl_3$): δ = −390.6 ppm.

4.1.5. Synthesis of [(^{n}Bu)(^{n}Hex)Sn{4-ClC_6H_4C(O)NHO}$_2$] (**3**)

Similarly prepared employing 2.0 mmol 4-chloro-N-hydroxybenzamide. Yield: 45%. Elemental *Anal.* Calc. for $C_{24}H_{32}Cl_2N_2O_4Sn$: C, 47,87; H, 5.36; N, 4.65. Found: C, 47.92; H, 5.45; N, 4.57%. IR: 3431m (N–H), 3189, 1598 (C=O)/(N–C), 914s (N–O), 571m (Sn–C), 498s (Sn–O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 12.17 (s, 1H, N–H), 11.02 (s, 1H, N–H), 7.38 (m, 8H, 2C_6H_4), 1.68–1.61, 1.44–1.28 (m, br, 16H, 8CH_2), 0.959 (t, 3H, $^3J_{HH}$ = 7.2, C′H_3), 0.907 (t, 3H, $^3J_{HH}$ = 7.2, CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 161.6 (C=O), 145.9, 128.9, 128.3, 127.8 (aryl-C), 31.7, 29.6, 28.3, 27.3, 26.6, 25.1, 22.7, 19.1 [(CH_2)$_3$ and (CH_2)$_5$], 13.9 (CH_3), 13.8 (CH_3) ppm. ^{119}Sn ($CDCl_3$): δ = −389.3 ppm.

4.1.6. Synthesis of [(^{n}Bu)(^{n}Hex)Sn{4-BrC_6H_4C(O)NHO}$_2$] (**4**)

Prepared accordingly using 2.0 mmol 4-bromo-N-hydroxybenzamide. Yield: 41%. Elemental *Anal.* Calc. for $C_{24}H_{32}Br_2N_2O_4Sn$: C, 47,71; H, 4.67; N, 4.05. Found: C, 47.88; H, 4.72; N, 3.99%. IR: 3429m (N–H), 3182, 1593 (C=O)/(N–C), 912s (N–O), 565m (Sn–C), 481s (Sn–O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 11.98 (s, 1H, N–H), 10.16 (s, 1H, N–H), 7.85–7.43 (m, 8H, 2C_6H_4), 1.78–1.28 (m, 16H, 8CH_2), 0.945 (t, H_3, $^3J_{HH}$ = 7.2, CH_3), 0.848 (t, H_3, $^3J_{HH}$ = 7.2, C′H_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 164.9 (C=O), 161.0 (C–Br), 132.8, 131.9, 131.2, 128.3, 127.9, 123.4, 31.7, 29.7, 28.3, 27.3, 26.9, 26.8, 26.5, 23.3, 19.4, 13.9, 13.6 [butyl-C and hexyl-C] ppm. ^{119}Sn ($CDCl_3$): δ = −391.7 ppm.

4.1.7. Synthesis of [(^{n}Bu)(^{n}Hex)Sn{4-IC_6H_4C(O)NHO}$_2$] (**5**)

Prepared accordingly using 2.0 mmol 4-iodo-N-hydroxybenzamide. Yield: 46%. Elemental *Anal.* Calc. for $C_{24}H_{32}I_2N_2O_4Sn$: C, 36.72; H, 4.11; N, 3.57. Found: C, 36.88; H, 4.25; N, 3.51%. IR: 3429m (N–H), 3182, 1565 (C=O)/(N–C), 988s, 918w (N–O), 585m (Sn–C), 480s (Sn–O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 10.55 (s, 1H, N–H), 9.93 (s, 1H, N–H), 7.66–7.13 (m, 8H, 2C_6H_4), 1.69–1.06 (m, 16H, 8CH_2), 0.95 (t, H_3, $^3J_{HH}$ = 7.2, CH_3), 0.85 (t, H_3, $^3J_{HH}$ = 7.2, C′H_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 162.9 (C=O), 151.7 (C–I), 137.3, 132.2, 124.5 (aryl-C), 27.5–13.9 [butyl-C and hexyl-C] ppm. ^{119}Sn ($CDCl_3$): δ = −380.4 ppm.

4.2. Pharmacology

In order to compare with our previous experimental results, the human nasopharyngeal carcinoma (KB) cell lines were used for screening. They were grown and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37 °C in humidified incubators in an atmosphere of 5% CO_2.

The complexes were dissolved in DMSO at a concentration of 5 mM as stock solution, and diluted in culture medium at concentrations of 1.0, 10, 100, and 500 μM as working-solution. To avoid DMSO toxicity, the concentration of DMSO was less than 0.1% (v/v) in all experiments.

The cells harvested from the exponential phase were seeded equivalently into a 96-well plate, and then the complexes were added to the wells to achieve final concentrations. Control wells were prepared by addition of culture medium. Wells containing culture medium without cells were used as blanks. All experiments were performed in quintuplicate. The SRB assay was performed as previously described in KB cells [34]. Upon completion of the incubation for 48 h, the cells were fixed in 10% trichloroacetic acid (100 μl) for 30 min at 4 °C, washed five times and stained with 0.1% SRB in 1% acetic acid (100 μl) for 15 min. The cells were washed four times in 1% acetic acid and air-dried. The stain was solubilized in 10 mM unbuffered Tris base (100 μl) and the OD was measured at 540 nm as above. The IC_{50} value was determined from plots of % viability against doses of compounds added.

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Appendix A. Supplementary data

CCDC 661105 contains the supplementary crystallographic data for **2**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

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