CHAPTER 5

ADVANCEMENT TOWARDS TIN-BASED ANTICANCER CHEMOTHERAPEUTICS: STRUCTURAL MODIFICATION AND COMPUTER MODELING APPROACH TO DRUG-ENZYME INTERACTIONS
Chapter 5

5.1. Introduction

Metal complexes and organometallic compounds have been gaining growing importance in oncology due to significant side effects and the emergence of drug resistance of cisplatin in cancer therapy [1-14]. A recent study involving platinum and other metal-containing antitumor drugs in preclinical development is also documented [15].

In order to minimize the side effects associated with the use of platinum compounds as anticancer drugs, metals such as Ti, Ga, Ge, Pd, Au, Co, Ru and Sn have been explored [1-14]. Consequently, a large number of organotin(IV) compounds has been investigated in this direction, showing promising in vivo and in vitro activity [16-23]. One of the main drawbacks of tin(IV) compounds as anticancer drugs is the poor water solubility; however, this can be surmounted by a rational design of the complex structures. In this context, a great deal of work has been performed with organotin(IV) carboxylates. Among these, triorganotin(IV) carboxylates have remarkably high in vitro anti-tumor activities, e.g., triphenyltin(IV) benzoates, salicylates [24], 3,6-dioxahexanoate, 3,6,9-trioxadecanoate [25], 4-carboxybenzo-15-crown-5, 4-carboxybenzo-18-crown-6 [25,26], steroidcarboxylate [27], terebates [28-30] and aminoacetates (Schiff bases) [31,32]. From these examples it becomes apparent that tin compounds with high in vitro antitumor activity and sufficient water solubility can be developed. A general procedure is that the organotin(IV) compounds are dissolved in DMSO and then diluted with test medium prior to the in vitro testing. Nevertheless, the limited solubility needs to be further enhanced in a way comparable to cisplatin. In view of the activity of triphenyltin(IV) carboxylates, triphenyltin(IV) 2/4-[(E)-2-(aryl)-1-diazenyl]benzoates and related systems have been recently investigated [33,34]. These organotin(IV) compounds bear a diazenyl group which shows not only high in vitro antitumor activity across a panel of cell lines, but also demonstrates interesting interactions with various enzymes [33,34]. Following our research on the synthesis, characterization and cytotoxic potential of organotin complexes, we now report on mononuclear and dinuclear organotin(IV) carboxylates of the composition $\text{Ph}_3\text{SnL}^{16-17} \text{H} (21-22)$ and $(\text{Ph}_3\text{Sn})_2\text{L}^{18} \text{H} (23)$, in which the ligand skeletal framework has been modified in an attempt to improve the dissolution properties without compromising
the cytotoxicity (Scheme 5.1). The carboxylate ligands selected herein show variations in the position of the carboxylate functionality and all three ligand molecules contain diazo and imino- groups. Compounds 21-23 were characterized by elemental analysis and spectroscopic ($^1$H, $^{119}$Sn NMR, IR) techniques. The characterization was accomplished by a single crystal X-ray diffraction analysis of a representative compound ($\text{Ph}_3\text{Sn})_2\text{L}^{18}\text{H}$ (23). All triphenyltin(IV) compounds (21-23) were tested across a panel of human tumor cell lines consisting of A498 (renal cancer), EVSA-T (mammary cancer), H226 (non-small-cell lung cancer), IGROV (ovarian cancer), M19 MEL (melanoma), MCF-7 (mammary cancer) and WIDR (colon cancer). Their activities were compared with that of the parent triphenyltin(IV) compound $\text{Ph}_3\text{SnL}^\text{P}\text{H.OH}_2$ (C1), which contains only a diazenyl group (see Scheme 5.1 for description of the compound). The mechanistic role of the cytotoxic activity of 21-23 was examined by docking studies with enzymes, e.g., ribonucleotide reductase (pdb ID: 4R1R), thymidylate synthase (pdb ID: 2G8D), thymidylate phosphorylase (pdb ID: 1BRW) and topoisomerase II (pdb ID: 1QZR). These enzymes take part in the synthesis of raw materials for DNA and its replication [35].

\[ \text{C1} \]

\[ \text{A} \]

\[ \text{B} \]

\[ \text{C} \]

\[ \text{R} = \text{H}; \text{R}^\prime = \text{CH}_3 \text{(L}^{18}\text{H}); \text{R} = \text{SnPh}_3; \text{R}^\prime = \text{CH}_3 \text{(21)} \]

\[ \text{R} = \text{CH}_3; \text{R}^\prime = \text{H} \text{(L}^{18}\text{H}); \text{R} = \text{SnPh}_3; \text{R}^\prime = \text{SnPh}_3 \text{(22)} \]

\[ \text{R} = \text{H}; \text{R}^\prime = \text{H} \text{(L}^{18}\text{H})_2; \text{R} = \text{SnPh}_3; \text{R}^\prime = \text{SnPh}_3 \text{(23)} \]

**Scheme 5.1** Chemical structures of the ligands (H and H' refer to phenolic and carboxylic protons, respectively), and triphenyltin(IV) compounds (C1 and 21-23).
5.2. Synthesis of triorganotin(IV) compounds

A general method was followed for the synthesis of compounds 21-23. A mixture of Ph₃SnOH and the appropriate ligands (L¹⁶HH’, L¹⁷HH’ or L¹⁸HH’₂) in a suitable stoichiometric ratio was heated to reflux in anhydrous toluene (50 mL) for 5 h in a round bottom flask equipped with a Dean-Stark apparatus and a water cooled condenser. The reaction mixture was filtered while hot and the solvent was removed using a rotary evaporator. The residue was washed, boiled with hexane, filtered and dried in vacuo. The residue upon crystallization using appropriate solvent(s) yielded the desired product.

5.3. Spectroscopic characterization of triorganotin(IV) compounds

The compounds (21-23) have been characterized by IR, ¹H, and ¹¹⁹Sn NMR spectroscopic techniques. The crystal structure of dinuclear compound, viz., (Ph₃Sn)₂L¹⁸H (23) is reported.

5.3.1. Ph₃SnL¹⁶H (21)

IR (cm⁻¹): 1725 ν_asym(OCO)COOMe, 1633 ν_asym(OCO)COOSn. ¹H NMR (CDCl₃); δ_H: 13.4 [s, 1H, OH(B)], 8.31 [s, 1H, C(H)N], 8.13 [d, 8.5 Hz, 2H, H-3'” and H-5'”], 7.90-7.21 [m, 23H, remaining ligand and Sn-Ph protons], 7.0 [d, 8.5 Hz, 1H, H-5’], 3.95 [s, 3H, CO₂CH₃ (C)] ppm. ¹¹⁹Sn NMR (CDCl₃): δ_Sn: – 104.6 ppm.

5.3.2. Ph₃SnL¹⁷H (22)

IR (cm⁻¹): 1709 ν_asym(OCO)COOMe, 1621 ν_asym(OCO)COOSn. ¹H NMR (CDCl₃); δ_H: 13.6 [s, 1H, OH(B)], 8.71 [s, 1H, C(H)N], 8.17 [d, 8.5 Hz, 2H, H-3” and H-5”], 8.0-7.05 [m, 23H, remaining ligand and Sn-Ph protons], 6.57 [d, 8.5 Hz, 1H, H-5’], 3.87 [s, 3H, CO₂CH₃ (C)] ppm. ¹¹⁹Sn NMR (CDCl₃): δ_Sn: – 104.6 ppm.

5.3.3. (Ph₃Sn)₂L¹⁸H (23)

IR (cm⁻¹): 1618, 1597 ν_asym(OCO)COOSn. ¹H NMR (CDCl₃); δ_H: 13.5 [s, 1H, OH(B)], 8.24 [s, 1H, C(H)N], 8.55 [d, 8.5 Hz, 2H, H-3’” and H-5’”], 8.0-7.0 [m, 38H, remaining ligand and Sn-Ph protons], 6.42 [d, 8.5 Hz, 1H, H-5’] ppm. ¹¹⁹Sn NMR (CDCl₃): δ_Sn: – 108.5 and – 109.0 ppm.
Compounds 21-23 are colored crystalline solids with well defined melting points, which are stable in air and soluble in common organic solvents. The analytical purity of the compounds was judged by elemental analyses and NMR (\(^{1}\text{H}\) and \(^{119}\text{Sn}\)) spectroscopy. Pertinent infrared absorption frequencies for the \(\nu(\text{C}=\text{O})\) stretching vibrations of the ligands (\(\text{L}^{16}\text{HH}', \text{L}^{17}\text{HH}'\) and \(\text{L}^{18}\text{HH}_2'\)) and the corresponding antisymmetric \(\nu_{\text{asym}}(\text{OCO})\) stretching vibration of the carboxylate groups in the triphenyltin(IV) compounds (21-23) are given in this section. The assignment of the symmetric \(\nu_{\text{sym}}(\text{OCO})\) stretching vibration band could not be made owing to the complex pattern of the spectra. The \(\nu(\text{C}=\text{O})\) stretching vibrations for the \(\text{C}=\text{O}\) groups in \(\text{L}^{16}\text{HH}'\) have been detected at 1732 cm\(^{-1}\) and 1710 cm\(^{-1}\), and are assigned to \(\nu(\text{C}=\text{O})_{\text{COOH}}\) and \(\nu(\text{C}=\text{O})_{\text{COOMe}}\), respectively. This assignment is in line with the values reported for uncomplexed 2-[(\(E\))-2-(3-formyl-4-hydroxyphenyl)-1-diazenyl] benzoic acid (1733 cm\(^{-1}\)) [36] and 4-(carbox methoxy) aniline (1682 cm\(^{-1}\)), respectively. For \(\text{L}^{17}\text{HH}'\), the bands for the \(\nu(\text{C}=\text{O})_{\text{COOMe}}\) and \(\nu(\text{C}=\text{O})_{\text{COOH}}\) stretching vibrations were detected at 1732 cm\(^{-1}\) and 1700 cm\(^{-1}\), respectively, and are in good agreement with those reported for methyl-2-[(\(E\))-3-formyl-4-hydroxyphenyl]diazenyl]benzoate [37]. On the other hand, for \(\text{L}^{18}\text{HH}_2'\) a strong broad band centered at 1719 cm\(^{-1}\) was observed, indicating that the vibrations for the two COOH groups are possibly overlapped. In the IR spectrum of 21, a band typical for the asymmetric stretching vibration of a metal-coordinated carboxylate group, \(\nu_{\text{asym}}(\text{OCO})\) was found at 1633 cm\(^{-1}\), indicating a bonding interaction to the tin atom [36]. The shift of the \(\nu(\text{C}=\text{O})_{\text{COOMe}}\) vibration was observed at 1725 cm\(^{-1}\), as expected. In 22, the \(\nu_{\text{asym}}(\text{OCO})\) band was found at 1621 cm\(^{-1}\), while the band for \(\nu(\text{C}=\text{O})_{\text{COOMe}}\) was detected at 1709 cm\(^{-1}\). For 23, the \(\nu_{\text{asym}}(\text{OCO})\) band was bifurcated with values of 1618 cm\(^{-1}\) and 1597 cm\(^{-1}\). The former one can be assigned to the tin ester function of ring A, since a similar value (1619 cm\(^{-1}\)) was reported for \(\text{C}1\), while the latter matches closely with that reported for triphenyltin(IV) \(p\)-aminobenzoate (1600 cm\(^{-1}\)) and corresponds to the tin ester in ring B. The shift of the \(\nu(\text{C}=\text{O})\) stretching vibration is indicative of coordination of the carboxylate oxygen to the tin atom [33,34,38-40]. However, the assignment of the symmetric \(\nu_{\text{sym}}(\text{OCO})\) stretching vibration band could not be ascertained owing to the complex pattern of the spectra, and hence the criterion of shift difference of the two bands (\(\Delta \nu = \nu_{\text{asym}}(\text{OCO})-\nu_{\text{sym}}(\text{OCO})\)) could not be utilized to infer the mode of coordination of
carboxylate O atom(s) (monodentate, bidentate or bridging). The $^1$H NMR integration values were fully consistent with the formulation of the products. The numbering protocols for the assignment of the $^1$H NMR signals are shown in Scheme 5.1. Efforts to assign $^{13}$C NMR signals and $^nJ(^{13}$C-$^{119/117}$Sn) coupling constants [41,42] were not made owing to signal overlap. In general, the $^1$H NMR spectra of the compounds 21-23 displayed only one set of NMR signals for all three phenyl groups (Sn-Ph), which indicates magnetic equivalence on the NMR time scale in solution. In CDCl$_3$, compounds 21 and 22 exhibited a single sharp $^{119}$Sn NMR resonance at approximately $-104$ ppm, while 23 gave two signals at $-108$ and $-109$ ppm. The shift displacements suggest that the Sn-atom(s) have four-coordinate coordination environments in the compounds [41,42].

5.4. Molecular structures of triorganotin(IV) compounds

A perspective view of the molecular structure of 23 is given in (Fig. 5.1). Selected geometric parameters are summarized in (Table 5.1). Compound 23 is a discrete monomeric dinuclear Sn-complex in which two triphenyltin groups are bridged by a L$^{18}$H ligand. The two Sn(IV) centers have similar distorted tetrahedral coordination geometries, in which the metal atoms are covalently bonded to three phenyl carbon atoms and one oxygen atom from a carboxylate group. The average bond angles around Sn(1) and Sn(2) are $113.5$ and $112.2^\circ$, respectively. The covalent Sn-O bond distances are 2.053(6) Å for Sn(1)-O(1) and 2.055(6) Å for Sn(2)-O(4). Additionally, the carboxylate carbonyl O-atoms, i.e. O(2) and O(5), interact weakly with the Sn-atoms, Sn(1)···O(2): 2.771(6) Å and Sn(2)···O(5): 2.706(6) Å. The resulting anisobidentate coordination modes become apparent also from the differences in the C-O bond lengths, i.e. C(1)-O(1): 1.319(10) Å versus C(1)-O(2): 1.215(10) Å, and C(21)-O(4): 1.331(10) Å versus C(21)-O(5): 1.221(10) Å for Sn(1) and Sn(2), respectively. Similar monocapped tetrahedral geometries have been reported previously for related triphenyltin(IV) carboxylates such as bis(triphenylstannyl) phenylmaleate [43] and bis(triphenylstannyl)-2-aminoterephthalate [44].
**Fig. 5.1** Perspective view of the molecular structure of (Ph$_3$Sn)$_2$L$^{18}$H (23). Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radii.

### 5.5. Quantum Chemical Calculations

The geometries of the triphenyltin(IV) compounds (21-23) were fully optimized using the quantum mechanical method PM3. Harmonic frequency calculations were performed for all stationary points to characterize their nature and to ensure that the optimized structures of 21-23 corresponded to global minima. The molecular structures of 21-23 obtained after full geometry optimization are shown in (Figs. 5.2-5.4), respectively, while selected geometric parameters are collected in (Table 5.1). In general, the experimental geometrical parameters for 23 obtained from single-crystal X-ray diffraction analysis match with that of the calculated values with exception of the Sn···O bond distances, as expected. Compounds 21 and 22 have a four-coordinate structure (Figs. 5.2 and 5.3). The carboxylate groups on the ligands act as monodentate coordinating functions, giving a distorted tetrahedral geometry around the tin atom. Such a configuration is commonly encountered for triphenyltin(IV) carboxylates [33,34,38-40]. The basic structures and coordination geometries of the triphenyltin(IV) compounds 21 and 22 are similar for the differently substituted ligands (L$^{16}$HH' and L$^{17}$HH'), and it can be expected that the ligand properties may directly influence the stability of the corresponding triphenyltin(IV) compounds and/or the binding to enzymes, as well as the cytotoxic activity. On the other hand, a similar tetrahedral geometry around both Sn(1) and Sn(2) is observed in compound...
23 (Fig. 5.4) and this is further corroborated by the data obtained from the single-crystal X-ray diffraction analysis (Fig. 5.1, Table 5.1).

**Fig. 5.2** The structure of Ph₃SnL¹⁶H (21) obtained after full geometry optimization.

**Fig. 5.3** The structure of Ph₃SnL¹⁷H (22) obtained after full geometry optimization.

**Fig. 5.4** The structure of (Ph₃Sn)₂L¹⁸H (23) obtained after full geometry optimization.
Table 5.1 Selected bond lengths (Å) and angles (°) for energy minimized structures of triphenyltin(IV) compounds 21-23.

<table>
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<th></th>
<th>23</th>
<th>21</th>
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<td>Sn-O(1)</td>
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<td>2.771(6)</td>
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<td>2.706(6)</td>
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<td>2.108(9)</td>
<td>Sn-C(23)</td>
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<td>2.109(10)</td>
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<td>95.1(4)</td>
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<td>C(52)-Sn(2)-C(40)</td>
<td>108.5</td>
<td>112.8(4)</td>
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5.6. Cytotoxicity Studies

Most recently, our research has shown that certain triphenyltin(IV) carboxylates bearing an imino framework (C8-C13; Table 5.2) have potential cytotoxicity in vitro, and among them, compound C10 was found to be the best performer, with the disadvantage of having low stability [31,32]. Consequently, some organotin(IV) carboxylates with a diazenyl framework such as triphenyltin(IV) 2-[(E)-2-(aryl)-1-diazenyl]benzoates (C1, C2-C7) were developed, which also exhibited promising cytotoxic activity in vitro and were found to be stable for days and among the conditions of the testing protocols [33,34]. In addition, these compounds have shown effective docking to a range of enzymes, particularly due to hydrogen bonding interactions through the diazenyl nitrogen atoms and the oxygen atoms of the formyl, carbonyl and ester functions. In line with these achievements, the triphenyltin(IV) carboxylates, Ph₃SnL¹⁶H (21), Ph₃SnL¹⁷H (22) and (Ph₃Sn)₂L¹⁸H (23), provide a considerable scope for optimizing the design, both in terms of their biological
activity and the binding properties to enzymes. Compounds 21-23 contain simultaneously a diazenyl- and an imino-framework. In the case of 21, the triphenyltin(IV) ester is bonded close to the diazenyl group and the imino component is connected in the form of an aromatic methyl ester, while in 22, the skeletal arrangement is reverse such that the triphenyltin(IV) ester is located close to the imino group. The purpose of the incorporation of diazenyl- and imino-groups is to attain the molecular flexibility, and the methyl ester components in 21 and 22 are to increase the solubility. On the other hand, in 23 the methyl ester of 21 has been replaced by a triphenyltin(IV) ester group, which gives rise to a dinuclear triphenyltin(IV) compound (Fig. 5.1). The in vitro cytotoxic properties of 21-23 across a panel of human tumor cell lines, viz., A498, EVSA-T, H226, IGROV, M19 MEL, MCF-7, and WIDR (Table 5.2), were compared with the standard drugs used clinically. The cytotoxic results of 21-23 were also compared with the results of other triphenyltin(IV) 2-[(E)-2-(aryl)-1-diazenyl]benzoates (C1, C2-C7), Schiff base carboxylates (C8-C13), among others (C14-C16) (Table 5.2), showing that the cytotoxicity (ID50) of compounds 21 and 22 is of the same order of magnitude which indicates that the change of the Ph3Sn group does not influence the cytotoxic activity. Under identical conditions, C1 displayed better activity than 21 and 22, across a panel of cell lines. Compound 23 was found to be the superior candidate showing the best activity among the investigated set of compounds (21-23) including other triphenyltin(IV) 2-[(E)-2-(aryl)-1-diazenyl]benzoates (C1, C2-C7) (Table 5.2). The superior activity of 23 was noted particularly for the EVSA-T and MCF-7 cell lines. Further, 23 scored better activity than cisplatin (2-15 folds), 5-fluorouracil and etoposide across a panel of cell lines.
Table 5.2 *In vitro* ID$_{50}$ values (ng/mL) of test compounds 21-23, previously reported triphenyltin(IV) compounds (C1,C2-C16) and standard drugs using cell viability tests in seven human tumor cell lines $^a$.

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<th>EVSA-T</th>
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<td>$&lt;$3.2</td>
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</tbody>
</table>

$^a$Abbreviation: DOX, doxorubicin; CDDP, cisplatin; 5-FU, 5-fluorouracil; MTX, methotrexate; ETO, etoposide and TAX, paclitaxel. Standard drug reference values have been obtained under identical conditions and are cited immediately after the test compounds.
Additional triphenyltin compounds (C2-C16) have been included for comparison and the ligand descriptions are as follows: 2-[(E)-(5-tert-butyl-2-hydroxyphenyl)diazenyl]benzoate (L^bH) [45,46], 2-[(E)-2-(2-hydroxy-5-methylphenyl)diazen-1-yl]benzoate (L^bH) [41], 2-[(E)-2-(4-hydroxy-5-methylphenyl)diazen-1-yl]benzoate (L^bH) [33], 4-[(E)-2-(2-hydroxy-5-methylphenyl)-1-diazenyl]benzoate (L^dH) [34], 4-[(E)-2-(4-hydroxy-3-methylphenyl)-1-diazenyl]benzoate (L^dH) [45] and 4-[(E)-(5-tert-butyl-2-hydroxyphenyl)diazenyl]benzoate (L^dH) [45,47], 2-[(2Z)-(3-hydroxy-1-methyl-2-butenyldiene)amino]-4-methyl-pentanoate (L^gH) [32], 2-[(E)-1-(2-hydroxyphenyl)methylidene]amino]-4-methyl-pentanoate (L^hH) [32], 2-[(E)-1-(2-hydroxyphenyl)-ethylidene]amino]-4-methyl-pentanoate (L^iH) [32], 2-[(2Z)-(3-hydroxy-1-methyl-2-butenyldiene)amino]phenylpropionate (L^jH) [31], 2-[(E)-1-(2-hydroxyphenyl)methylidene]amino]phenylpropionate (L^kH) [31], 2-[(E)-1-(2-hydroxyphenyl)ethylidene]amino]phenylpropionate (L^lH) [31], carboxylate residues such as terebate (L^m), -steroid-carboxylate (L^n), -benzocrown-carboxylate (L^o) [29].
This encouraging cytotoxic effect may be predictive of *in vivo* antitumor activity. The higher activities of 23 when compared to 21-22 may be attributed to the presence of two tin centers since all three compounds bear both diazenyl- and imino-skeletons. However, the variations in the *in vitro* cytotoxicity among 21-23 across tumor cell lines may also be due to a different kinetic and mechanistic behavior. Generally, it is difficult to define the overall spectrum of compound activity and possible selectivity owing to the limited numbers of compounds studied. However, the results strongly support the view that triphenyltin(IV) compounds are potent cytotoxic agents and deserve attention as potential anticancer agents.

### 5.7. Molecular docking Studies

The encouraging cytotoxic activity for the test compounds 21-23, across a panel of cell lines, *viz.*, A498, EVSA-T, H226 IGROV, M19 MEL, MCF-7 and WIDR, prompted us to execute molecular docking studies to comprehend the complex-protein interactions. Earlier, such interactions were noted for some triphenyltin(IV) 2-[(E)-2-(aryl)-1-diazenyl]benzoates which indicated that the diazenyl group nitrogen atoms and formyl, carbonyl and ester oxygen atoms of the triphenyltin compounds are responsible for interacting with enzymes [33]. The triphenyltin(IV) compounds 21-23 of the present investigation contain a diazenyl- and an imino-framework within the molecular structure and the dinuclear tin compound 23 contains an additional tetrahedral triphenyltin moiety. As a consequence, the molecular dimensions of 21-23 are much larger compared to that of triphenyltin(IV) 2-[(E)-2-(aryl)-1-diazenyl]benzoates, and hence the molecular structures can influence the binding ability with proteins in a different manner, as expected. Prior to the docking studies of 21-23 with enzymes, the docking programme was validated by docking GDP (Guanosine-5’-diphosphate), UMP (2’-Deoxyuridine-5’-monophosphate), URA (Uracil) and CDX ((S)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione) to the active site of enzymes RNR, TS, TP and Topo II (pdb ID 4R1R, 2G8D, 1BRW and 1QZR), respectively and a close overlapping between the docked ligand and the one present in the crystal structures of the enzymes is observed (Figs. 5.5-5.8).
**Fig. 5.5** A 3D view in the form of a CPK model, showing the result of docking GDP to the active sites of enzyme RNR. The ligand (green) docked to the active site of the enzyme overlaps with the native GDP ligand (pink).

**Fig. 5.6** A 3D view in the form of a CPK model, showing the result of docking UMP to the active sites of enzyme TS. The ligand (green) docked to the active site of the enzyme overlaps with the native UMP ligand (yellow).

**Fig. 5.7** A 3D view in the form of a CPK model, showing the result of docking URA to the active sites of enzyme TP. The ligand (green) docked to the active site of the enzyme overlaps with the native URA ligand (yellow).
Fig. 5.8 A 3D view in the form of a CPK model, showing the result of docking CDX to the active sites of enzyme Topo II. The ligand (green) docked to the active site of the enzyme overlaps with the native CDX ligand (yellow).

The docking studies of 21-23 were performed with enzymes ribonucleotide reductase, thymidylate synthase, thymidylate phosphorylase and topoisomerase II with pdb ID 4R1R, 2G8D, 1BRW and 1QZR, respectively, and their interactions are shown in (Figs. 5.9-5.11) (Tables 5.3-5.5), respectively. The docking of compound 21 to the active sites of TP reveals H-bonding interactions with oxygen O1 in the Sn-ester function, the phenolic oxygen atom O3, atoms O4 and O5 of the methyl ester and the imino nitrogen N3, besides further interactions (Fig. 5.9c). Interactions with atoms O4 and O5 of the methyl ester and the imino nitrogen N3 were also noted for enzyme RNR (Fig. 5.9a). When docked to the active site of TS, compound 21 showed also H-bonding interactions with the amino acid residues E84 and R23 of the enzyme through atoms O3 and O5, respectively (Fig. 5.9b). Compound 21 showed also interactions with the amino acid residue K417 of Topo II; however, in this case it did not enter to the active site of enzyme. Compound 21 demonstrated many interactions (Table 5.4) and a common aspect was that all enzymes interacted with the phenolic oxygen atom O3 and atom O4 of the methyl ester fragment. The diazenyl- and imino-nitrogen atoms (N2 and N3) of 21 showed hydrogen bonding interactions with amino acid residues of TS, TP and Topo II; however, no such bonding was found in the case of RNR (Fig. 5.10a-5.10d). It should be pointed out here that although the change of the Ph3Sn bonding site does not alter the cytotoxicity of 21 and 22, such a variation leads to remarkable changes in the hydrogen bonding ability. This is evidenced by the interactions of 22, which is now masked inside the active site of Topo II (Fig. 5.10d). This further indicates a dissimilar mode of action of 21 and 22. The docking
studies of the dinuclear triphenyltin(IV) compound 23 revealed strong interactions with enzymes TP and Topo II, both through atom O1 of the Sn(1)-ester, and atoms O4 and O5 of the Sn(2)-ester, and atoms N1 and N3 of the diazenyl- and imino-functions, besides other interactions (Table 5.5). No hydrogen bonding interactions with the oxygen atoms of the Sn(1)- and Sn(2)-esters (O1, O2 and O4, O5), were found with RNR; nevertheless, hydrogen bonding was detected between atoms N1 and N2 and the amino acid residue R251.

Fig. 5.9a Ph₃SnL¹⁶H (21) docked to the binding site of enzyme RNR (pdb ID 4R1R). Hydrogen bonding interactions between various groups of 21 and amino acid residues are shown. Hydrogen atoms are omitted for clarity.
**Fig. 5.9b** Ph$_3$SnL$^{16}$H (21) docked to the binding site of enzyme TS (pdb ID 2G8D). Hydrogen bonding interactions between various groups of 21 and amino acid residues are shown. Hydrogen atoms are omitted for clarity.

**Fig. 5.9c** Ph$_3$SnL$^{16}$H (21) docked to the binding site of enzyme TP (pdb ID 1BRW). Hydrogen bonding interactions between various groups of 21 and amino acid residues are shown. Hydrogen atoms are omitted for clarity.
**Fig. 5.9d** $\text{Ph}_3\text{SnL}^{16}\text{H}$ (21) docked to the binding site of enzyme TopoII (pdb ID 1QZR). Hydrogen bonding interactions between various groups of 21 and amino acid residues are shown. Hydrogen atoms are omitted for clarity.

**Fig. 5.10a** $\text{Ph}_3\text{SnL}^{17}\text{H}$ (22) docked to the binding site of enzyme RNR (pdb ID 4R1R). Hydrogen bonding interactions between various groups of 22 and amino acid residues are shown. Hydrogen atoms are omitted for clarity.
Fig. 5.10b Ph₃SnL¹⁷H (22) docked to the binding site of enzyme TS (pdb ID 2G8D). Hydrogen bonding interactions between various groups of 22 and amino acid residues are shown. Hydrogen atoms are omitted for clarity.

Fig. 5.10c Ph₃SnL¹⁷H (22) docked to the binding site of enzyme TP (pdb ID 1BRW). Hydrogen bonding interactions between various groups of 22 and amino acid residues are shown. Hydrogen atoms are omitted for clarity.
Fig. 5.10d $\text{Ph}_3\text{SnL}^{1\text{H}} \text{H} \,(22)$ docked to the binding site of enzyme Topo II (pdb ID 1QZR). Hydrogen bonding interactions between various groups of 22 and amino acid residues are shown. Hydrogen atoms are omitted for clarity.

Fig. 5.11a $(\text{Ph}_3\text{Sn})_2\text{L}^{1\text{H}} \text{H} \,(23)$ docked to the binding site of enzyme RNR (pdb ID 4R1R). Hydrogen bonding interactions between various groups of 23 and amino acid residues are shown. Hydrogen atoms are omitted for clarity.
Fig. 5.11b (Ph₃Sn)L¹⁸H (23) docked to the binding site of enzyme TS (pdb ID 2G8D). Hydrogen bonding interactions between various groups of 23 and amino acid residues are shown. Hydrogen atoms are omitted for clarity.

Fig. 5.11c (Ph₃Sn)L¹⁸H (23) docked to the binding site of enzyme TP (pdb ID 1BRW). Hydrogen bonding interactions between various groups of 23 and amino acid residues are shown. Hydrogen atoms are omitted for clarity.
Fig. 5.11d (Ph₃Sn)₂L¹⁸H (23) docked to the binding site of enzymeTopo II (pdb ID 1QZR). Hydrogen bonding interactions between various groups of 23 and amino acid residues are shown. Hydrogen atoms are omitted for clarity.

**Table 5.3** H-bonding interactions between functional groups of 21 and amino acid residues in the active sites of RNR, TS, TP and Topo II.

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<th>Enzyme</th>
<th>Hydrogen bonding atom*</th>
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*H-bond distances in Å are given in parentheses.
Table 5.4 H-bonding interactions between functional groups of 22 and amino acid residues in the active sites of RNR, TS, TP and Topo II.

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<th>O4</th>
<th>O5</th>
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*aH-bond distances in Å are given in parentheses.

Table 5.5 H-bonding interactions between functional groups of 23 and amino acid residues in the active sites of RNR, TS, TP and Topo II.

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<td>(2.93)</td>
<td>(2.85)</td>
<td>(2.65)</td>
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*aH-bond distances in Å are given in parentheses.

The docking studies indicate that the diazenyl- and and imino- nitrogen atoms, as well as the oxygen atoms of the triphenyltin ester, methyl ester and phenolic groups play an important role for the complexation of organotin compounds to the active sites of enzymes. Replacement of the methyl ester in 21 by a triphenyltin ester in 23 afforded more hydrogen bonding interactions with the enzymes. Nevertheless, the possibility of coordination through tin beyond the active site of the enzymes cannot be ruled out completely, since it is difficult to predict the role of such atoms in binding proteins.
Although the overall molecular dimensions of **21-22** had little influence on the cytotoxic activity, the molecular dimensions together with potential donor atoms revealed an effect on the binding ability with enzymes. The superior activity of **23** compared to **C1** and **21-22** may be related to the presence of two triphenyltin(IV) units which possibly provide the proper dimension of the molecular framework to penetrate better into the active site. This observation would suggest that there is an optimum dimension needed for maximum toxicity. This finding may give investigators a basis for designing new toxicants against cancer cell lines.

In line with our ongoing work on the synthesis and evaluation of the cytotoxic potential of organotin(IV) carboxylates, three new triphenyltin(IV) carboxylates of formulations \( \text{Ph}_3\text{SnL}^{16}\text{H} \) (21), \( \text{Ph}_3\text{SnL}^{17}\text{H} \) (22) and \( (\text{Ph}_3\text{Sn})_2\text{L}^{18}\text{H} \) (23) were investigated. All complexes were screened *in vitro* for the evaluation of their cytotoxic potential against seven human tumor cell lines. Among the triphenyltin(IV) compounds examined herein, the dinuclear compound **23**, demonstrated superior cytotoxicity across a panel of cell lines. It should be noted that the application of such dinuclear triphenyltin(IV) carboxylates might be a new approach in tin-based cancer chemotherapy. The cytotoxicity of the triphenyltin(IV) compounds can be rationalized in terms of the nature of molecular constitution, dimensions and binding ability with enzymes. Nevertheless, the diazenyl- and imino- nitrogen atoms, as well as the oxygen atoms of the triphenyltin ester, methyl ester and phenolic moieties play an important role for docking to the active sites of enzymes. The data from the current study suggest that compound **23** merits further investigation as potential drug, and the molecular framework provides considerable scope for optimizing the design, both in terms of the cytotoxic activity and dissolution properties. We would like to point out that docking and quantum chemical calculations were performed in our study in order to gain more knowledge about the structural patterns responsible for the development of anticancer activity in triphenyltin(IV) compounds. However, other computational methodologies such as QSAR (Quantitative Structure-Activity Relationships) [48-100], and related chemo-bioinformatic approaches [101-106], could serve as useful complements for *in silico* selection of triphenyltin(IV) compounds as potent anticancer agents, with posterior generalization to other organometallic compounds. Further work in this area is in progress.
5.8. Experimental

5.8.1. Synthesis of pro-ligands

5.8.1.1. Synthesis of 2-((E)-(4-hydroxy-3-((E)-(4(methoxycarbonyl)phenyl)imino)methyl)phenyldiazenyl))benzoic acid (L^{16}HH')

An equimolar amount of 4-(carbomethoxyl)aniline (1.0 g, 6.61 mmol) in hot absolute ethanol solution (10 mL) was added to a hot solution of absolute ethanol (30 mL) containing 2-[(E)-2-(3-formyl-4-hydroxyphenyl)-1-diazenyl]benzoic acid (1.17 g, 6.61 mmol). The reaction mixture was refluxed for 3 h followed by stirring for 2 h at room temperature. The brick-red precipitate was filtered, washed with methanol and diethyl ether (3 x 5 mL), and dried in vacuo. The residue was washed with hexane and then extracted with chloroform (80 mL). The chloroform extract was concentrated to about half of the initial solvent volume, cooled to room temperature and kept overnight, whereupon an orange-red crystalline product of L^{16}HH' is obtained in 59% yield. M. p.: 222-225 °C. Anal. Found. C, 65.20; H, 3.95; N, 10.13%. Calc. for C_{22}H_{17}N_{3}O_{5}: C, 65.50; H, 4.25; N, 10.42%. IR (cm⁻¹): 1732 ν_{asym}(OCO)COOH, 1710 v_{asym}(OCO)COOMe. ¹H NMR (DMSO-d₆, 400.13 MHz); δH: 13.2 [br s, 2H, OH(A,B)], 9.13 [s, 1H, C(H)N], 8.30 [s, 1H, H-2'], 8.03 [d, 8.5 Hz, 2H, H-3'' and H-5''], 7.95 [d, 8.5 Hz, 1H, H-6'], 7.77 [d, 8.5 Hz, 1H, H-3], 7.65 [d, 8.5 Hz, 1H, H-5], 7.55 [m, 4H, H-4, H-6, H-2'' and H-6''], 7.16 [d, 8.5 Hz, 1H, H-5'], 3.85 [s, 3H, CO_{2}CH_{3}(C)] ppm.

5.8.1.2. Synthesis of 4-((E)-(2-hydroxy-5-((E)-(2-(methoxycarbonyl)phenyl)diazenyl)benzylidene amino))benzoic acid (L^{17}HH')

A similar synthetic procedure as for L^{16}HH' was used, except that 4-(carbomethoxyl)aniline and 2-[(E)-2-(3-formyl-4-hydroxyphenyl)-1-diazenyl]benzoic acid were replaced by methyl 2-[(E)-(3-formyl-4-hydroxyphenyl)diazenyl]benzoate and 4-aminobenzoic acid, giving a yellow precipitate. The precipitate was filtered, washed with ethanol, and dried in vacuo. The residue was thoroughly washed with hexane and dried in vacuo to afford crude L^{17}HH', which was dissolved in a hot aqueous solution containing two equivalents of NaHCO₃ and filtered while hot. The product was then precipitated with dilute acetic acid, filtered and was washed with water until the filtrate was neutral. The
yellow residue was washed with methanol (3 x 5 mL), diethyl ether and dried in vacuo. Yield 66%. M. p.: 242-243 °C. Anal. Found. C, 65.60; H, 4.18; N, 10.40%. Calc. for C$_{22}$H$_{17}$N$_3$O$_5$: C, 65.50; H, 4.25; N, 10.42%. IR (cm$^{-1}$): 1732 $v_{\text{asym}}$(OCO)$_{\text{COOMe}}$, 1700 $v_{\text{asym}}$(OCO)$_{\text{COOH}}$. $^1$H NMR (DMSO-d$_6$, 400.13 MHz); $\delta$H: 13.0 [br s, 2H, OH(B,C)], 9.17 [s, 1H, C(H)N], 8.30 [s, 1H, H-2'], 8.04 [d, 8.5 Hz, 2H, H-3'' and H-5''], 7.97 [d, 8.5 Hz, 1H, H-6'], 7.77 [d, 8.5 Hz, 1H, H-3], 7.67 [m, 2H, H-5 and H-6], 7.58 [d, 8.5 Hz, 1H, H-4], 7.54 [d, 6.0 Hz, 2H, H-2'' and H-6''], 7.17 [d, 8.5 Hz, 1H, H-5'], 3.83 [s, 3H, CO$_2$CH$_3$ (A)] ppm.

5.8.1.3. Synthesis of 2-((E)-(3-((E)-((4-carboxyphenyl)imino)methyl)-4-hydroxyphenyl)diazenyl)benzoic acid (L$^{18}$HH$_2$')

A similar synthetic procedure as for L$^{16}$HH' was used, except that 4-(carbomethoxyl)aniline was replaced by 4-aminobenzoic acid, giving a brick-red precipitate. The precipitate was washed with ethanol and dried in vacuo. The residue was refluxed in anhydrous toluene and filtered. The residue (M. p.: 270-275 °C) was dissolved in a hot aqueous solution containing three equivalents of NaHCO$_3$ and filtered while hot. The product was then precipitated with dilute acetic acid, filtered and washed with water until the filtrate was neutral. The brick-red residue was washed with methanol (3 x 5 mL), diethyl ether and dried in vacuo. Yield 65%. M. p.: 277-278 °C. Anal. Found. C, 65.10; H, 3.90; N, 10.90%. Calc. for C$_{21}$H$_{15}$N$_3$O$_3$: C, 64.78; H, 3.88; N, 10.79%. IR (cm$^{-1}$): 1719 $v_{\text{asym}}$(OCO)$_{\text{COOH}}$. $^1$H NMR (DMSO-d$_6$, 400.13 MHz); $\delta$H: 13.0 [br s, 3H, OH (A,B,C)], 9.13 [s, 1H, C(H)N], 8.30 [s, 1H, H-2'], 8.05 [d, 8.5 Hz, 2H, H-3'' and H-5''], 7.95 [d, 8.5 Hz, 1H, H-6'], 7.78 [d, 8.5 Hz, 1H, H-3], 7.60 [d, 8.5 Hz, 1H, H-5], 7.55 [m, 4H, H-4, H-6, H-2'' and H-6''], 7.18 [d, 8.5 Hz, 1H, H-5'] ppm.

5.8.2. Synthesis of triorganotin(IV) compounds

A typical method for the synthesis of triphenyltin(IV) compounds is described in section 5.2 while the characterization data are given below.
5.8.2.1. Synthesis of \( \text{Ph}_3\text{SnL}^{16}\text{H} \) (21)

\( \text{Ph}_3\text{SnOH} \) (0.91 g, 2.47 mmol); \( \text{L}^{16}\text{HH}' \) (1.0 g, 2.47 mmol). Recrystallized from chloroform and toluene (3:1) to give maroon microcrystalline product in 56% yield. M.p.: 132-135 °C. Anal. Found: C, 64.08; H, 3.95; N, 5.30%. Calc. for \( \text{C}_{40}\text{H}_{31}\text{N}_3\text{O}_5\text{Sn} \): C, 63.85; H, 4.15; N, 5.58%.

5.8.2.2. Synthesis of \( \text{Ph}_3\text{SnL}^{17}\text{H} \) (22)

\( \text{Ph}_3\text{SnOH} \) (0.91 g, 2.47 mmol); \( \text{L}^{17}\text{HH}' \) (1.0 g, 2.47 mmol). Recrystallized from ethanol to give orange microcrystalline product in 52% yield. M.p.: 168-170 °C. Anal. Found: C, 63.65; H, 4.05; N, 5.38%. Calc. for \( \text{C}_{40}\text{H}_{31}\text{N}_3\text{O}_5\text{Sn} \): C, 63.85; H, 4.15; N, 5.58%.

5.8.2.3. Synthesis of \((\text{Ph}_3\text{Sn})_2\text{L}^{18}\text{H} \) (23)

\( \text{Ph}_3\text{SnOH} \) (0.91 g, 2.47 mmol); \( \text{L}^{18}\text{HH}_2' \) (0.50 g, 1.23 mmol). Recrystallized from benzene and petroleum ether (1:1) to give orange microcrystalline product in 56% yield. M.p.: 180-182 °C. Anal. Found: C, 63.05; H, 4.25; N, 3.78%. Calc. for \( \text{C}_{40}\text{H}_{31}\text{N}_3\text{O}_5\text{Sn} \): C, 62.96; H, 3.99; N, 3.86%.

5.8.3. Chemicals used for the preparations

\( \text{Ph}_3\text{SnOH} \) was prepared from \( \text{Ph}_3\text{SnCl} \) (Fluka) by following the literature method [107]. \((\text{Ph}_3\text{Sn})_2\text{O} \) (Fluka), anthranilic acid (Spectrochem) and 4-aminobenzoic acid (Hi Media) were used without further purification. Methyl 2-[(E)-3-formyl-4-hydroxyphenyl]diazrenyl]benzoate was prepared by reacting 2-[(E)-2-(3-formyl-4-hydroxyphenyl)-1-diazenyl]benzoic acid [36] with \( \text{SOCl}_2 \) in anhydrous methanol [37]. 4-(Carbomethoxyl)aniline (M.p. 113-115 °C) was obtained by reacting 4-aminobenzoic acid in methanol with \( \text{SOCl}_2 \) at -10 °C after a suitable work-up procedure [37]. Compound \( \text{C1} \) was prepared by the method described earlier [36]. The solvents used in the reactions were of AR grade and dried using standard procedures. Toluene was distilled from sodium benzophenone ketyl.
5.8.4. Physical measurements

Carbon, hydrogen and nitrogen analyses were performed with a Perkin Elmer 2400 series II instrument. IR spectra in the range 4000-400 cm\(^{-1}\) were obtained on a Perkin Elmer Spectrum BX series FT-IR spectrophotometer with samples investigated as KBr discs. \(^1\)H NMR spectra of the ligands were recorded on a Bruker Avance 250 spectrometer and measured at 250.53 MHz. The \(^1\)H NMR spectra of compounds 21-23 were recorded on a Bruker AMX 400 spectrometer and measured at 400.13 MHz. \(^{119}\)Sn NMR spectra were recorded either on a Jeol GX 270 or Mercury 400 spectrometer and measured at 100.75 MHz or 149.32 MHz, respectively. The \(^1\)H and \(^{119}\)Sn NMR chemical shifts were referenced to Me\(_4\)Si and Me\(_4\)Sn, both set at 0.00 ppm, respectively.

5.8.5. X-ray crystallography

Single crystals suitable for X-ray diffraction analysis were grown by slow evaporation at room temperature from a solution of the compound (Ph\(_3\)Sn)\(_2\)L\(^{18}\)H (23) in benzene and petroleum ether (v/v 1:1). Crystal data for C\(_{57}\)H\(_{43}\)N\(_3\)O\(_5\)Sn\(_2\) (M = 1087.32 g.mol\(^{-1}\)): Triclinic, \(P\)-\(I\), \(a = 9.1958(7)\), \(b = 10.8465(9)\), \(c = 25.886(2)\) Å, \(\alpha (°) = 82.420(2)\), \(β (°) = 85.954(2)\), \(γ (°) = 73.237(2)\), \(V = 2449.2(3)\) Å\(^3\), \(Z = 2\), \(D = 1.474\) g cm\(^{-3}\), \(F(000) = 1092, \mu = 1.072\) mm\(^{-1}\). Intensity data were collected at \(T = 293(2)\) K for an orange prism (0.43 x 0.22 x 0.06 mm) on a Bruker-APEX diffractometer equipped with a CCD area detector using MoK\(α\) radiation with \(λ = 0.71073\) Å via \(ω/ϕ\)-rotation at 10 s per frame in the range of \(2 < θ < 25°\) [108]. The measured intensities were reduced to \(F^2\) and corrected for absorption with SADABS [109]. Corrections were made for Lorentz and polarization effects. Structure solution, refinement and data output were carried out with the SHELXTL-NT program package [110,111]. Non hydrogen atoms were refined anisotropically. C-H hydrogen atoms were placed in geometrically calculated positions using the riding model. The O-H hydrogen atom has been located from iterative examination of difference Fourier maps following least squares refinements of the previous models with \(d_{O-H} = 0.84\) Å and \(U_{iso}(H) = 1.5U_{eq}(O)\). There were 8595 unique reflections (\(R_{int} = 0.086\)) and 5322 data with \(I ≥ 2σ(I)\). The final \(R\) (obs. data) and \(wR\) (all data) values were 0.0833 and 0.1718, respectively.
5.8.6. Experimental protocol for cytotoxicity tests

The *in vitro* cytotoxicity testing of triphenyltin(IV) compounds 21-23 was performed using the SRB test for estimation of cell viability. The experimental protocol for C1 has been reported previously [33]. The results are included for convenience of discussion. The cell lines WIDR, M19 MEL, A498, IGROV and H226 belong to the currently used anticancer screening panel of the NCI, USA [112]. The MCF7 cell line is an estrogen receptor (ER)+ and progesterone receptor (PgR)+, and EVSA-T is (ER)-/(PgR)-. Prior to the experiments, a mycoplasma test was carried out on all cell lines and found to be negative. All cell lines were maintained in a continuous logarithmic culture in RPMI 1640 medium with HEPES and phenol red. The medium was supplemented with 10% FCS, 100 μg/mL of penicillin and 100 μg/mL of streptomycin. The cells were mildly trypsinized for passage and for the use in the experiments. RPMI and FCS were obtained from Gibco (Paisley, Scotland). SRB, DMSO, Penicillin and streptomycin were obtained from Sigma (St. Louis MO, USA), TCA and acetic acid from Baker BV (Deventer, NL), and PBS from NPBI BV (Emmer-Compascuum, NL).

The test compounds C1, 21-23 and the reference compounds were dissolved to a concentration of 2.5x10^5 ng/mL in the complete medium, by dilution of a stock solution which contained 5 mg/mL of compounds 21-23 in DMSO. The cytotoxicity was estimated by the microculture sulforhodamine B (SRB) test [113].

The experiment was started on day 0. On day 0, 1500-2000 cells per well were seeded into flat-bottomed micro titer plates containing 96 wells (Cellstar, Greiner Bio-one). The plates were pre-incubated overnight at 37 °C with 5% CO₂ to allow the cells to adhere to the bottom. On day 2, a three-fold dilution sequence of ten steps was realized in the complete medium, starting with the 2.5x10^5 ng/mL stock solution. Every dilution was used in quadruplicate by adding 50 μL to a column of four wells. This procedure results in the highest concentration of 6.25x10^4 ng/mL being present in column 12. Column 2 was used for the blank.

To column 1 medium was added to diminish interfering evaporation. The incubation was terminated on day 7. Subsequently, the cells were fixed with 10% trichloroacetic acid in PBS and placed at 4°C for an hour. After three washings with tap water, the cells were stained for at least 15 min with 0.4% SRB dissolved in 1% acetic
acid. After staining, the cells were washed with 1% acetic acid to remove the unbound stain. The plates were air-dried and the bound stain was dissolved in 150 μL of 10 mM Tris-base. The absorbance was measured at 540 nm using an automated microplate reader (Labsystems Multiskan MS). The data were used for the construction of concentration-response curves and the determination of the ID50 values by use of the Deltasoft 3 software. ID50 is the dose in ng/mL that causes 50% inhibition of the tumor cells.

The variability of the in vitro cytotoxicity test depends on the cell lines used and the serum applied. With the same batch of cell lines and the same batch of serum the inter-experimental CV (coefficient of variation) is 1-11% depending on the cell line and the intra-experimental CV is 2-4%. These values may be higher with other batches of cell lines and/or serum.

5.8.7. Computational methods

The molecular structures and geometries of the triphenyltin(IV) compounds (21-23) were fully optimized using the semi-empirical quantum chemistry method (PM3) [114-117]. Docking studies of compounds 21-23 to the active sites of various enzymes were performed using ArgusLab 4.0.1. [118-120]. This program was also applied for visualization and molecular modeling of the molecular structures. The 3D coordinates of the key enzymes ribonucleotide reductase (RNR), thymidylate synthase (TS), thymidylate phosphorylase (TP) and topoisomerase II (Topo II) with pdb ID 4R1R, 2G8D, 1BRW and 1QZR, respectively, were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank. The docking program implements an efficient grid-based docking algorithm, which approximates an exhaustive search of possible binding modes within the free volume of the binding site cavity. The conformational space was explored by geometry optimization of the flexible ligand (rings are treated as rigid) in combination with incremental construction of ligand torsions. The ligand orientation was determined by a shape scoring function based on Ascore and the final positions were ranked according to the interaction energy values. Prior to docking, the ground state of molecule was optimized using the PM3QM basis set implemented in the geometry optimization module of the program package to confirm that no significant divergence in the conformations of the complexes due to crystal packing effects had occurred.
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