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Human Topoisomerase I mediated cytotoxicity profile of L-valine-quercetin diorganotin(IV) antitumor drug entities

Sabiha Parveen, Sartaj Tabassum, Farukh Arjmand*

Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India

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ABSTRACT

New chiral L-/D-valine-quercetin diorganotin(IV) complexes $[(CH_3)_2Sn(Q)(val)]$ $\mathbf{1_{L/D}}$, $[(C_6H_5)_2Sn(Q)(val)]$ $\mathbf{2_{L/D}}$, were synthesized and thoroughly characterized by elemental analysis, mass spectrometry, IR, ^1H NMR, ^{119}Sn NMR spectroscopy. Preliminary comparative DNA binding studies on enantiomeric complexes $\mathbf{1_{L/D}}$ and $\mathbf{2_{L/D}}$ were carried out by UV–vis, fluorescence titrations, thermal denaturation and circular dichroic techniques to ascertain their DNA binding propensity. Thermal denaturation studies of complexes in the absence and presence of CT–DNA have been carried out and ΔT_m was calculated to be 2 –3 °C depicting electrostatic mode of binding corroborated well with the results of UV–vis and fluorescence studies. The intrinsic binding constant, K_b and binding constant, K values revealed that both L-enantiomers of complexes **1** and **2** exhibited exceptionally high binding propensity as compared to their D-enantiomers and between L-enantiomers $\mathbf{2_L}$ exhibited greatest binding affinity and followed the trend: $\mathbf{2_L} > \mathbf{1_L} > \mathbf{2_D} > \mathbf{1_D}$. The cytotoxicity profile of $\mathbf{1_L}$ and $\mathbf{2_L}$ was studied on four different human cancer cell lines; HeLa, MCF7, Hep-G2, MIA-Pa-Ca-2 by SRB assay which revealed remarkably good cytotoxic activity (with GI_{50} values of <10 μg mL^{-1}) and $\mathbf{2_L}$ exhibited better cytotoxic activity than $\mathbf{1_L}$. Furthermore, the chemotherapeutic action of drug entities was found to be mediated by human Topoisomerase I enzymatic inhibition.

1. Introduction

Quercetin (3,5,7,3′,4′-pentahydroxyflavone), a natural bioflavonoid pharmacophore has attracted considerable interest due to its diverse biological properties *viz.* antioxidant, antibacterial, antimicrobial and antitumor *via* apoptosis [1,2]. The chemical structure of quercetin possesses conjugated electron rich aromatic rings and a number of ionizable hydroxyl groups which make them very good hydrogen and electron donors [3]. Quercetin as a pharmacophore is therefore, highly desirable motif for the robust drug design strategy particularly, in combination with other recognition element domains [4]. In the recent past, many quercetin metal complexes were synthesized and thoroughly characterized. It has been observed that incorporating metal ions in quercetin scaffold dictates the geometric spatial orientation at the active site which is responsible for better pharmacokinetic responses both *in vitro* and *in vivo* [5,6].

Tin and organotin(IV) derivatives have gained much attention recently as a notable class of antitumor chemotherapeutics [7,8]. Organotin compounds are coordinatively unsaturated and can expand their coordination number from four to seven upon addition of neutral organic donor ligands, therefore they could exhibit wider possibilities of hybridisation beyond sp, sp^2 and sp^3 [9]. Functionalizing organotin(IV) complexes with tailored ligand scaffolds could yield drug entities with altered pharmacological properties, such as improved biocompatibility, reduced systemic toxicity, target specificity and selectivity [10,11]. A plethora of organotin derivatives have been prepared and tested *in vitro* and *in vivo*, firstly against murine leukaemia cell lines and after that, against different panels of human cancer cell lines [12]. Organotin(IV) complexes have been identified as effective drug candidates in organometallic oncology with remarkably good ID_{50} values (63 ng mL^{-1} against MCF-7 and 121 ng mL^{-1} against WiDr cell line as compared to 600 and 976 ng mL^{-1}, respectively for cisplatin) [13]. Several diorganotin and triorganotin(IV) compounds which were conjugated to bioactive donor ligands have also shown high

Abbreviations: CD, Circular dichroism; CT–DNA, Calf thymus DNA; Q, Quercetin; Topo I, Topoisomerase I; EB, Ethidium bromide; SRB, Sulphorhodamine-B; Q, Quercetin; CT–DNA, Calf thymus DNA; EB, Ethidium bromide; CD, Circular dichroism; Topo I, Topoisomerase I; SRB, Sulphorhodamine-B.

* Corresponding author.
E-mail address: farukh_arjmand@yahoo.co.in (F. Arjmand).

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antiproliferative activity *in vitro* against a panel of solid and hematologic cancers [14]. In this work, we describe the synthesis of quercetin organotin compounds with chiral auxiliary L-/D-enantiomers of valine which could be responsible for regio/stereoselective recognition with target biomolecules [15]. The preferential interaction of one enantiomer of a racemate with chiral macromolecules of the body leads to expressed pharmacokinetic and pharmacodynamic effect. Furthermore, disease states, route of administration, genetic variability and drug-interactions may be stereospecific. The synergistic conjugation of quercetin bioactive pharmacophore, chiral amino acid ligand recognition domain and organotin(IV) as apoptotic director could result in enhanced DNA inhibition, ultimately better cell-killing effect with reduced systemic toxicity [16,17].

Nuclear enzymes Topo I and II are over-expressed in cancerous cells therefore, they are considered one of the major targets for anticancer drugs [18]. Topoisomerses are thought to be responsible to trigger a series of cellular events *viz*. DNA replication, recombination, chromosome segregation and gene transcription inducing apoptosis. Although topoisomerases are most active during S-phase, Topo I is not cell cycle specific and does not require energy co-factor such as adenosine triphosphate for its activity. Topo I is up-regulated in tumor cells as compared to normal cells suggesting a therapeutic advantage of Topo I targeting drugs for both slow growing and rapidly proliferating tumors [19]. Quercetin inhibits both Topo I and Topo II by poisoning them in the ternary 'DNA-Topo-quercetin' complex, the 'cleavable complex' which leads to the disentangling of double-stranded DNA by generation of either single or double strand breaks resulting in cell cycle arrest [20]. Nevertheless, the drugs that target Topoisomerase I *viz*. camptothecin, indenoisoquinoline damage DNA by trapping the covalent reaction intermediate called Topo I cleavage complex [21].

Herein, we describe the synthesis, characterization of L-/D-valine-quercetin diorganotin(IV) complexes $\mathbf{1_{L/D}}$ and $\mathbf{2_{L/D}}$ which is the first demonstration in literature to best of our knowledge. Various spectroscopic methods such as absorption, fluorescence, thermal denaturation, circular dichroism and electrophoresis techniques have been employed to probe the interaction of L-/D-enantiomeric complexes with DNA. The L-enantiomeric complexes $\mathbf{1_L}$ and $\mathbf{2_L}$ have shown exceptionally high DNA binding affinity (of the order 10^5), significant Topo I inhibition and potent cytotoxic activity against four tested human cancer cell lines.

2. Experimental section

2.1. Materials

All reagent grade chemicals were used without further purification for all syntheses and experiments. Dimethyltin(IV) dichloride, diphenyltin(IV) dichloride, Quercetin (Sigma-Aldrich), Valine(Merck), were used as received. Disodium salt of Calf thymus DNA (CT–DNA) was purchased from Sigma Chemical Co. and was stored at 4 °C. All reagents were of the best commercial grade and were used without further purification.

2.2. Methods and instrumentation

Elemental analyses (C, H and N) were performed on Elementar Vario EL III. Fourier-transform infrared (FTIR) spectra were recorded on an Interspec2020 FTIR spectrometer in KBr pellets from 400 to 4000 cm^{-1}. Electronic spectra were recorded on UV–vis spectrophotometer Lambda 20(Perkin Elmer), data were reported in λ_{max}(nm). The ^1H and ^{13}C and ^{119}Sn NMR spectra were obtained on a BrukerDRX-400 spectrometer operating at 400, 100 and 150 MHz, respectively. ESI-MS spectra were recorded on a Micromass Quattro II triple quadrupole mass spectrometer. Molar conductivity measurements were done at room temperature on Eutech con 510 electronic conductivity bridge. Circular dichroism (CD) spectra were recorded on a Jasco J-815CD spectrometer operating at room temperature, 25 °C. The region of wavelength between 200 and 700 nm was scanned for each sample using a 1 cm path quartz cell. The thermal denaturation experiments were carried out by monitoring the absorbance of CT–DNA (1×10^{-4} M) at 260 nm with varying temperature in the absence and presence of complexes ($\mathbf{1_L}$, $\mathbf{1_D}$) and ($\mathbf{2_L}$, $\mathbf{2_D}$) in 1:2 ratio of complex to DNA, the samples were gradually heated at the rate of 2 °C min^{-1} temperature increase in Tris buffer (pH 7.2) using a Peltier system attached to the UV–vis spectrophotometer.

2.3. Syntheses

2.3.1. Synthesis of $[(CH_3)_2Sn(Q)(val)]$ $\mathbf{1_{L/D}}$

To a stirred solution of quercetin ($Q.2H_2O$) (0.604 g, 2 mmol) in MeOH was added a methanolic solution of L-/D-valine (0.234 g, 2 mmol) and dimethyltin dichloride (0.439 g, 2 mmol). Few drops of triethylamine were also added. The reaction mixture was kept under reflux for 5 h. Then, the reaction mixture was filtered, and the filtrate was evaporated to half the volume. The resulting orange colored product was washed with diethyl ether and dried in *vacuo*.

Complex $\mathbf{1_L}$: Yield, 73%. m.p. > 300 °C; Anal. Calc. for $[C_{22}H_{29}NSnO_{11}]$ (%) C, 43.88; H, 4.85; N, 2.33. Found (%) C, 44.13; H, 4.83; N, 2.22. FT–IR data (KBr, ν, cm^{-1}): 3393 ν(O–H), 3083 ν(NH); 2978 ν(C–H); 1648 ν(C=O), 1274 ν(C–O–C), 1170 ν(CH_2); 422 ν(Sn–O). ^1H NMR δ_H (400 MHz; DMSO-d_6 ppm): 12.47 (5-OH), 10.59 (7-OH), 7.72 (2′-H), 7.58(6′-H), 6.92 (5′-H), 6.47 (8-H), 6.23 (6-H), 3.78 (NH), 3.66 (CH, 1H), 2.55 (CH_2) 0.86 (CH_3,6H). ^{13}C NMR δ_C (100 MHz; DMSO-d_6 ppm): 175.6 (C-1″), 170.8 (C=O), 163.9 (C-7), 160.6 (C-5), 156.02 (C-3′), 160.6 (C-4′), 156.02 (C-9), 146.6 (C-2), 135.6 (C-3), 133.1 (C-1′), 121.9 (C-5′), 119.87 (C-2′), 115.4 (C-6′), 102.9 (C-6), 98.1 (C-10), 93.3 (C-8), 57.74 (C-2″), 29.0 (C-3″), 18.0 (C-4″).^{119}Sn NMR δsn (150 MHz; DMSO–d6, ppm): −539. ESI–MS (*m/z*): 603 $[C_{22}H_{24}NSnO_9+2H_2O + H]^+$. Molar Conductance, Λ_M (10^{-3} M, DMSO): 19.0 Ω^{-1} cm^2 mol^{-1} (non-electrolyte). UV–vis absorption: λ_{max} (DMSO, 10^{-3} M), 272 and 456 nm.

Complex $\mathbf{1_D}$: Yield, 71%. m.p. > 300 °C; Anal. Calc. for $[C_{22}H_{29}NSnO_{11}]$ (%) C, 43.88; H, 4.85; N, 2.33. Found (%) C, 44.06; H, 4.82; N, 2.26. FT–IR data (KBr, ν, cm^{-1}): 3391 ν(O–H), 3084 ν(NH); 2978 ν(C–H); 1649 ν(C=O), 1277 ν(C–O–C), 1169 ν(CH_2); 422 ν(Sn–O). ^1H NMR δ_H (400 MHz; DMSO-d_6 ppm): 12.43 (5-OH), 10.76 (7-OH), 7.84 (2′-H), 7.56 (6′-H), 6.95 (5′-H), 6.43 (8-H), 6.21 (6-H), 3.78 (NH), 3.65 (CH, 1H), 2.54 (CH_2) 0.87 (CH_3,6H). ^{13}C NMR δ_C (100 MHz; DMSO-d_6 ppm): 175.6 (C-1″), 170.8 (C=O), 163.9 (C-7), 160.6 (C-5), 156.02 (C-3′), 160.6 (C-4′), 156.02 (C-9), 146.6 (C-2), 135.6 (C-3), 133.1 (C-1′), 121.9 (C-5′), 119.87 (C-2′), 115.4 (C-6′), 106.7 (C-6), 98.1 (C-10), 93.2 (C-8), 57.75 (C-2″), 29.0 (C-3″), 17.9 (C-4″). ^{119}Sn NMR δsn (150 MHz; DMSO–d6, ppm): −558. ESI–MS (*m/z*): 603 $[C_{22}H_{24}NSnO_9+2H_2O + H]^+$. Molar Conductance, Λ_M (10^{-3} M, DMSO): 16.0 Ω^{-1} cm^2 mol^{-1} (non-electrolyte). UV–vis absorption: λ_{max} (DMSO, 10^{-3} M), 273 and 455 nm.

2.3.2. Synthesis of $[(C_6H_5)_2Sn(Q)(val)]$ $\mathbf{2_{L/D}}$

These complexes were synthesized according to the procedure described above for ($\mathbf{1_L}$, $\mathbf{1_D}$) except with diphenyltin dichloride (0.687 g, 2 mmol).

Complex $\mathbf{2_L}$: Yield, 74%. m.p. > 300 °C; Anal. Calc. for $[C_{32}H_{33}NSnO_9]$ (%) C, 55.36; H, 4.79; N, 2.02 Found (%) C, 55.79; H, 4.43; N, 1.91. FT–IR data (KBr, ν, cm^{-1}): 3349 ν(O–H), 3071 ν(NH); 2968 ν(C–H); 1647 ν(C=O), 1267 ν(C–O–C), 1168 ν(CH_2); 439 ν(Sn–O). ^1H NMR δ_H (400 MHz; DMSO-d_6 ppm): 12.47 (5-OH), 10.59 (7-OH), 7.72 (2′-H), 7.58 (6′-H), 6.92 (5′-H), 6.59 (8-H), 6.15 (6-