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Short communication

Antiproliferative activities of iron and platinum conjugates of salicylaldehyde semi-/thiosemicarbazones against C6 glioma cells

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Abstract

The identification of novel therapeutic agents for the management of malignant gliomas represents an area of active research. Here, we show that among the iron and platinum complexes of 2-hydroxybenzaldehyde semi-/thiosemicarbazones the latter inhibits the growth of C6 glioma cells in vitro with an IC_{50} value of 45 μM. Structural characterization reveals iron complexes to be octahedral having intermediate S = 3/2 spin states while platinum complexes are found to be square planar moieties. Our findings suggest that the platinum compounds of 2-hydroxybenzaldehyde thiosemicarbazone are potential candidates for the treatment of malignant gliomas.

Keywords: Glioma; Thiosemicarbazone; Antitumor effects; Iron and platinum complexes

1. Introduction

Gliomas are the most common primary tumors of central nervous system (CNS) in humans [1]. They are classified into four clinical grades out of which the glioblastoma multiform (GBM) is the most aggressive one [2]. Nearly all GBM patients die of the disease with median survival time of 1 year. Most human gliomas generally affect either signal transduction pathways activated by the receptor tyrosine kinases or cell cycle arrest pathways activated by the receptor tyrosinases, such as CDK 4, CDK 6, cyclin D1, MDM2, p16, p14, RB and p53. Although many genetic alterations have been identified in human gliomas, unequivocal correlation between these genetic alterations and gliomagenesis has been lacking. The current chemotherapeutic treatments are thus often ineffective due to the intrinsic chemoresistance of these tumors [3]. The identification of novel therapeutic agents able to inhibit the growth of these tumors is therefore essential to improve the prognosis of glioma patients.

It has been shown recently that thiosemicarbazone derivatives of 3-amino-pyridine-2-carboxaldehyde are able to cross blood brain barrier exhibiting anticancer activity against L1210 cells in the brain [4]. Similarly platinum complexes of *p*-isopropylbenzaldehyde thiosemicarbazone show growth inhibitory activities against cisplatin-resistant glioma cell line G112 [5]. These reports suggest that thiosemicarbazone is an effective pharmacophore for arresting tumor growth of glioma cells. The potential of thiosemicarbazone ligands and their metal conjugates has been investigated in our laboratory for some time [6] and such compounds have been found to be potent antiproliferative compounds in case of MCF-7 and T47D breast cancer cells [7–9].

In the present work we describe synthesis and characterization of iron and platinum complexes of semi-/thiosemicarbazone derivatives of 2-hydroxybenzaldehyde and evaluation of their antiprolferative activity against C6 glioma cell line, which reveals a very potent activity for the platinum compounds.

2. Chemistry

2-Hydroxybenzaldehyde and semicarbazide hydrochloride were the products of Chemical Drug House (Mumbai). Thiosemicarbazide was obtained from Sisco Research Ltd.

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(Mumbai) while ferric and platinum chlorides were the products of Fluka. All other chemicals and solvents employed for synthesis were of analytical grade and were used as supplied. The ligands were obtained by condensation of semi-/thiosemicarbazide hydrochloride and salicylaldehyde. The purity of compounds was checked by TLC. The iron and platinum complexes were prepared by mixing 1:2 and 1:1 ratio of appropriate ligand and metal chlorides respectively. The structures of all metal complexes were confirmed by elemental analysis and electronic spectral analysis. All the complexes were soluble in DMF, ethanol and DMSO solvents.

3. Results and discussion

The compositional data on the synthesized compounds are in good agreement with their suggested stoichiometries. The non-conducting platinum complexes have 1:1 ligand to metal ratio, while the iron complexes possess 2:l ligand to metal composition and exhibit corresponding conductivities (90 $\Omega cm^2\ mol^{-1}$) in DMF [10].

3.1. IR studies

The IR data on the ligands and their metal complexes show a band due to intramolecularly H-bonded hydroxyl stretching vibration in the region 3433–2475 cm^{-1} which disappears upon metal complexation indicating deprotonation and subsequent involvement of the phenoxyl group in metal coordination [11]. A sharp peak in the range 1613–1622 cm^{-1} is due to the azomethine linkage whose participation in metal coordination is indicated by its downward shift in the metal complexes [12]. The involvement of the carbonyl group in semicarbazide side chain in **1** is indicated by its downward shift to 1670 cm^{-1} [13]. The thiocarbonyl stretching frequency in the thiosemicarbazone side chain in **2** is observed at 855–875 cm^{-1} [14] which undergoes a shift to lower frequency in the region 835–845 cm^{-1} indicating involvement of the thione form in metal coordination. The IR studies thus indicate monoanionic, tridentate nature of the present ligands, which undergo coordination to metal ions with ONO or ONS donor atom sets. Such metal complexation with metal/ligand stoichiometry of 1:2 as indicated above leads to octahedral geometries similar to those observed for iron complexes of thiosemicarbazone derivative of 2-benzoylpyridine [15]. On the other hand platinum complexes having 1:1 metal to ligand compositions tend to be square planar similar to the copper compounds described recently [16].

3.2. Magnetic studies and electronic spectroscopy

The room temperature magnetic moments of the iron complexes (**1a** and **2a**) are in the range 3.40–4.32 BM indicating stabilization of the species having intermediate ferric spin (S = 3/2) state. The occurrence of such intermediate spin states is typical for the octahedral ferric complexes of thiosemicarbazone ligands as shown by Zelenstov [17]; Padhye et al. [18]; Padhye and Kauffman [19].

In case of ferric complexes the d–d bands are laporte forbidden as well as spin-forbidden and are generally obscured by much more intense charge transfer bands [20]. In the present iron(III) complexes the higher energy bands in the region 35,000–29,000 cm^{-1} probably originate from the ligand to metal charge transfer transitions while the lower energy absorption around 16,000 cm^{-1} can be ascribed to $^6A_1 \rightarrow {}^4T_1$ transition and is similar to that observed in analogs octahedral iron complexes [21].

The platinum complexes **1b** and **2b** show ligand-based transitions at 35,000–37,000 cm^{-1} while bands around 22,000 cm^{-1} are assignable to $^1A_{1g} \rightarrow {}^1B_{1g}$ transition in the square planar platinum compounds [22,23].

3.3. Cyclic voltammetric studies of iron complexes

The iron complexes show an irreversible peak around –1.00 V attributable to the reduction of the azomethine linkage [12], while the reversible Fe(III)/Fe(II) redox couple is observed at –0.08 and + 0.05 V for compounds **1a** and **1b** respectively indicating facile reduction in case of the thiosemicarbazone compound which may have relevance to its higher antiproliferative activities against the glioma cells.

3.4. Anticancer activity

The results of antiproliferative activity of iron and platinum complexes against C6 glioma cell lines are depicted in Fig. 1. In general, metal complexes of thiosemicarbazone ligands show dose and time dependant antiproliferative activities while the semicarbazone compounds are found to be inactive. The platinum complex **2b** is the most active compound amongst the present series with IC_{50} value of 45 μM, which is on the lower side than the one reported for the platinum complex of *p*-isopropylbenzaldehyde thiosemicarbazone (IC_{50} = 98 μM) described earlier [24] and may be ascribed to formation of extensive DNA interhelical cross-links by the present compound [25].

4. Conclusions

Our study thus indicates that coordinately unsaturated metal-thiosemicarbazonato compounds with planar structures may offer useful strategies in designing effective antiproliferative compounds against glioma cancers and may have a role in the clinical therapy of gliomas as an adjuvant to conventional chemotherapeutic agents.

5. Experimental protocols

5.1. Instrumentation and measurements

Details of the instrumental measurements have been described earlier [16].

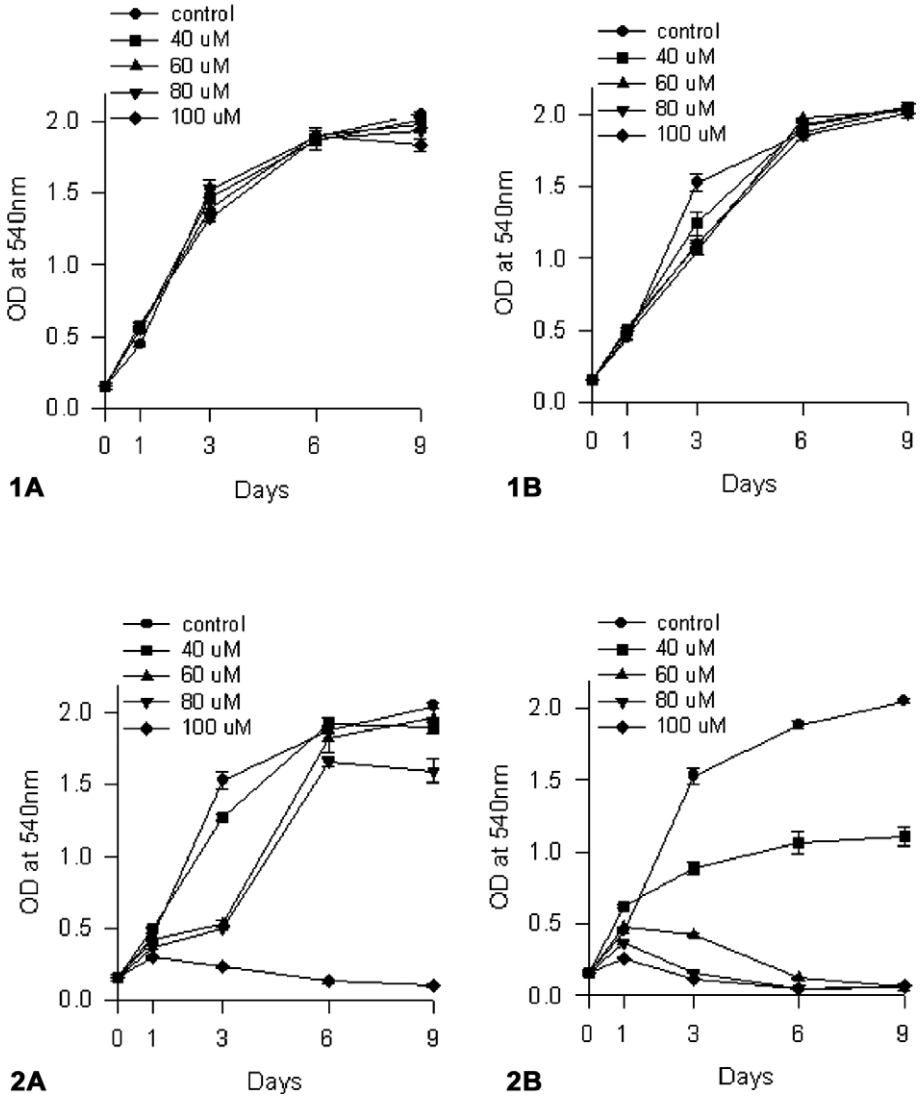
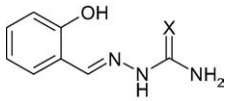


Fig. 1. Antiproliferative activities of iron (**1a**, **2a**) and platinum (**1b**, **2b**) complexes against C6 glioma cells after 9 days exposure.

5.2. Synthesis of ligands

Preparation of semicarbazone (**1**) and thiosemicarbazone (**2**) ligands was carried out according to literature methods [26,27].



X = O; (**1**)
X = S; (**2**)

5.3. Synthesis of complexes: a general method

The metal complexes were prepared by refluxing ethanolic solutions of the ligands in 2:1 stoichiometric ratio iron complexes and 1:1 in case of platinum complexes. On keeping the reaction mixture in refrigerator overnight a green precipitate for each iron complexes were obtained which was filtered and washed with ethanol. In case of platinum complexes 1:1 stoichiometric metal to ligand ratio was used with reaction refluxing for 3 h and stripping off the solvent on rotavapor to obtain solid compounds, which were recrystallized from hot aqueous ethanol. All the complexes obtained were dried in vacuum at room temperature.

5.3.1. $[Fe(\mathbf{1})_2]Cl{\cdot}H_2O$ (**1a**)

Green solid (ethanol). Yield: 75%; Anal. Calc. for ($C_{16}H_{18}N_6O_5ClFe$): C 41.25, H 3.86, N 18.05; Found: C 41.24, H 4.34, N 18.35%; UV/VIS: λ_{max} (cm^{-1}) 35,710, 28,400, 15,200; ν_{max} (cm^{-1}) 1601 (C=N), 1660 (C=O).

5.3.2. $[Fe(\mathbf{2})_2]Cl$ (**2a**)

Green solid (ethanol). Yield: 80%; Anal. Calc. for ($C_{16}H_{14}O_2N_6S_2ClFe$): C 40.22, H 2.93, N 17.59, S 13.40; Found: C 40.05, H 2.73, N 17.52, S 13.35%; UV/VIS: λ_{max} (cm^{-1}) 29,940, 15,340; ν_{max} (cm^{-1}) 1605 (C=N), 835 (C=S).

*5.3.3. [Pt(**1**)Cl] CH_3OH (**1b**)*

Brown solid (ethanol). Yield: 60%; Anal. Calc. for ($C_9H_{12}N_3O_3ClPt$): C 23.97, H 2.66, N 9.32; Found: C 23.21, H 2.49, N 9.03%; UV/VIS: λ_{max} (cm^{-1}) 35,710, 27,030, 21,740; ν_{max} (cm^{-1}) 1602 (C=N), 1654 (C=O).

*5.3.4. [Pt(**2**)Cl] CH_3OH (**2b**)*

Brown solid (ethanol). Yield: 66%; Anal. Calc. for ($C_{16}H_{14}O_2N_6S_2ClFe$): C 22.61, H 1.88, N 9.89, S 7.54; Found: C 22.97, H 2.03, N 9.70, S 7.13%; UV/VIS: λ_{max} (cm^{-1}) 35,710, 27,030, 20,410; ν_{max} (cm^{-1}) 1602 (C=N), 840 (C=S).

5.4. Anticancer activity

The effect of the four test compounds on C6 glioma cells was determined by the MTT viability assay [28]. C6 glioma cells were seeded at a density of 5000 cells/100 μl medium per well of a 96 well microtitre plate. The culture medium used was Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum and antibiotics (100 units/ml penicillin G, 100 μg ml^{-1} streptomycin and 100 units of fungizocin) and the cells were grown at 37 °C in 5% CO_2 and 95% air in a humidified incubator. Twenty-four hours after seeding, the medium was replaced with one containing various concentrations of each of the test compounds dissolved in DMSO. Cell viability was determined over a period of 9 days, with medium containing the agents being replenished every 3 days. MTT was added (20 μl per well of a 5 mg ml^{-1} stock in phosphate buffered saline) every 3 days, and the formazon crystals formed were dissolved with 100 μl of 10% SDS in 0.01 N HCl after an incubation period of 4 h. Optical density was read on an ELISA reader at 540 nm with a reference wavelength of 690 nm.

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