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Cytotoxic Activities of Thiosemicarbazones and Their Metal Complexes†

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The cytotoxic properties of thiosemicarbazones and their transition metal complexes are reviewed since 2006.

Key Words: Thiosemicarbazones, Cytotoxic, Anti-tumor, Anti-proliferative.

INTRODUCTION

Thiosemicarbazones have more applications in pharmacological field[1]. Regarding the biological activities of thiosemicarbazones and their metal complexes, the literature survey reveals that French *et al.*[2] reported first time anti carcinogenic property of thiosemicarbazones. But not much research was carried out after that, but in 21st century, so many researchers were concentrated on this issue. Due to this reason we found lot of research papers about the cytotoxic ability of thiosemicarbazones from the recent years. This forces us to review the cytotoxic applications of thiosemicarbazones and their metal complexes from the year 2006.

EXPERIMENTAL

Cytotoxicity determination methods: There are number of methods describes the determination of cytotoxicity of human cells. Among these, MTT, WST-8, Tritium-labeled Thymidine uptake method and Calcein-AM method are regarded as more important (Fig. 1). The cell viablility and cytotoxicity assays are used for the drug screening and cytotoxicity test of chemicals including thiosemicarbazones and their metal complexes. These assays were described briefly here under.

MTT method: MTT [(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole], was developed as a quantitative colourimetric assay for mammalian cell survival and proliferation[3].

This method was rapid and precise to assess the cytotoxicity. MTT method can detect the mitochondrial dehydrogenase activities in the living cells. In this method, the MTT has reduced to a purple formazan dye by NADH.

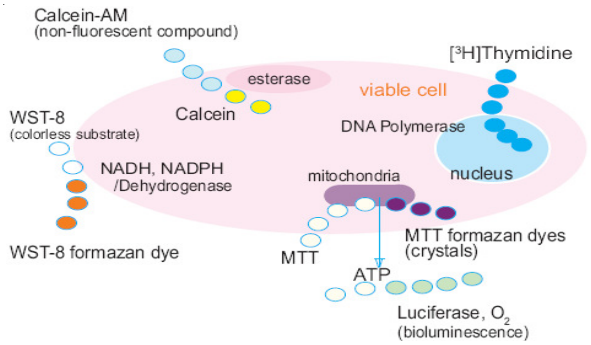

Fig. 1. Different reagents of cytotoxic detection[4]

WST-8 method: Dojindo[4] developed the water soluble tetrazolium salt method to assess the cytotoxicity. Water soluble tetrazolium salt receives two electrons from living cells and convert to yellow, orange or purple formazan dye (Fig. 1).

Tritium-labeled thymidine uptake method: Tritium-labeled thymidine uptake method is useful to quantify DNA synthesis and cell proliferation. This method is based on, tritium labeled thymidine reaction with the nucleus (Fig. 1) of living cells and the amount of tritium in the nucleus is measured by using a scintillation counter.

Calcein- AM: Calcein AM is a cell-permeant dye which can be used to determine cell viability in most eukaryotic cells. In live cells the nonfluorescent calcein AM is converted to a fluorescent calcein by intracellular esterases (Fig. 1).

RESULTS AND DISCUSSION

2-Acetylpyridine thiosemicarbazones and their substituted compounds were tested for cytotoxic activity against RT2

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(expressing p53 protein) and T98 (expressing mutant p53 protein) glioma cells by Lessa *et al.*[5]. They found IC_{50} concentration (required concentration to kill the 50 % of cells) as 24-1.4 nM and 50-1.0 nM for RT2 and T98, respectively. They also evaluated that there was no significant difference between the cytotoxic properties of the studied thiosemicarbazones and their E or Z isomer forms. The anti-proliferative activity *in vitro* against NCI-H460 (non-small cell lung cancer), A2780 and A2780-*cis*R (epithelian ovarian cancer) human cancer cell lines by the palladium complexes of 3,5-diacetyl-1,2,4-triazol bis (^{4}N-substituted thiosemicarbazone) were evaluated by Matesanz *et al.*[6]. These complexes exhibits very low toxicity on normal renal LLC-PK1 cells compared to *cisplatin*.

Halder *et al.*[7] synthesized the Pt(II) complexes of 4-R-benzaldehyde thiosemicarbazones and examined the cytotoxic effect of these complexes on the human leukemia cell line (HL-60) and human lymphoma cell line (U-937). The IC_{50} values of these complexes indicate their potential cytotoxic nature on the tested cells.

The cytotoxic effect of the complex formed by 2-oxo-1,2-dihydroquinoline-3-carbaldehyde 4(N,N)-dimethyl thiosemicarbazone with Cu(II) nitrate in methanol was reported by Senthil Raja *et al.*[8]. They tested the cytotoxic effect of the above said complex on human cervical cancer cell line (HeLa), human laryngeal epithelial carcinoma cells (HEp-2) and human liver carcinoma cells (Hep G2) and concluded that the complex was more effective against HeLa compare to the others. Li *et al.*[9] evaluated the cytotoxic effect of thiosemicarbazones of 2-acetyl -pyridine, 2-acetyl pyrazine-N(4)-methyl, 2-benzoylpyridine and 2-benzoylpyridine N(4)-methyl against leucocy -themia (K562) and liver cancer cell lines (BEL7402). Among the tested thiosemicarbazones, 2-benzoyl pyridine N(4)-methyl thiosemicarbazone was found as active ligand with IC_{50} as 0.02 μM against K562 and 0.138 μM against the BEL7402 cancer lines, respectively.

Diorganotin(IV) complexes of 2-benzoylpyridine-N(4)-phenyl thiosemicarbazone and 2-acetylpyrazine-N(4)-phenyl thiosemicarbazone were synthesized, characterized and evaluated their cytotoxic effect against K562 leukemia cells by Li *et al.*[10]. They synthesized four different organotin complexes with the above mentioned thiosemicarbazones by different substitutions on tin. Among the two ligands, 2-acetylpyrazine-N(4)-phenyl thiosemicarbazone has lower cytotoxic effect with 12.3 μM of IC_{50} value than the 2-benzoylpyridine-N(4)-phenyl thiosemicarbazone which has 1.43 μM of IC_{50} value. All complexes shown greater cytotoxic effect than their parent ligands, among them phenylorganotin complexes with the above ligands were more effective than the methyl organotin complexes. Stringer *et al.*[11] reported the synthesis and cytotoxicity of mono- and dinuclear (η^6-arene) ruthenium(II) benzaldehyde thiosemicarbazones against the oesophageal cancer cell line (WHCO1). But the complexes of Ru(II) with the above said thiosemicarbazone has lower *in vitro* cytotoxic activity than the free ligand.

Au(I) complexes of 2-acetylpyridine thiosemicarbazones (its N(4)-methyl and N(4)-phenyl derivates) and N(4)-phenyl-2-benzoylpyridine thiosemicarbazone were tested for cytotoxic activity against Jurkat (immortalized line of T lymphocyte), HL-60 (acute myeloid leukemia), MCF-7 (human breast adenocarcinoma) and HCT-116 (colourectal carcinoma) tumor cell lines by Lessa *et al.*[12]. One of the Au(I) complexes synthesized with the above ligands was more effective than well known cytotoxic, *i.e.*, auranofin against leukemia cells. Soares *et al.*[13] reported the cytotoxic activity of N^4-phenyl-2-acetylpyridine thiosemicarbazone and its derivatives (N^4-*ortho*-, -*meta*-, -*para*- fluorophenyl, N^4-*ortho*-, -*meta*-, -*para*- chlorophenyl, N^4-*ortho*-, -*meta*-, -*para*-iodo phenyl and N^4-*ortho*-, -*meta*-, -*para*- nitrophenyl) against human malignant breast (MCF-7) and glioma (T98G and U87) cells. They also concluded that all the thiosemicarbazones mentioned above were not inducing haemolytic activity up to 10^{-5} M.

The cytotoxic activity of Ni(II) complexes obtained from salicylaldehyde thiosemicarbazone, 2-hydroxy-acetophenone thiosemicarbazone and 2-hydroxynaphthaldehyde thiosemicarbazone against human breast carcinoma cell line (MCF-7) was reported by Datta *et al.*[14]. Saha *et al.*[15] synthesized Fe(III) complexes of 5-methyl-3-formyl pyrazole-N(4)-dimethyl thiosemicarbazone and 5-methyl-3-formylpyrazole-N(4)-diethyl thiosemicarbazone and tested their cytotoxic activity in culture against cervical carcinoma cells (HeLa). They found that the complexes were shown more activity than their parent ligands. Further the Fe(III) complex of 5-methyl-3-formyl pyrazole-N(4)-dimethyl thiosemicarbazone does not show any role in cell-cycle progression while the other complex shows the cell-cycle progression by arresting the M-phase. Leovac *et al.*[16] synthesized Cu(II) complexes of 3-methyl-5-oxo-1-phenyl-3-pyrazolin-4-carboxaldehyde thiosemicarbazone and 5-oxo-3-phenyl-3-pyrazolin-4-carboxaldehyde thiosemicarbazone and their cytotoxic activities were tested against several cell lines, such as human promyelocytic leukemia (HL60), human acute lymphocytic leukemia (REH), rat glioma (C6), mouse fibrosarcoma (L929) and mouse melanoma (B16). Their flow cytometry results indicates that the reason for the cell death due to apoptosis.

A Ni(II) complex of S-cetronellal thiosemicarbazone was synthesized and its antiproliferative activity tested against human histiocytic lymphoma cell line (U937) at very low concentration of the complex (IC_{50} = 14.4 μM) by Buschini *et al.*[17]. They proposed a hypothetical mechanism for the antiproliferative activity of the complex through DNA damage followed by cell cycle arrest, DNA repair and apoptosis. Belicchi-Ferrari *et al.*[18] synthesized nine aliphatic thiosemicarbazones namely, heptnal, N^1-methyl heptanal, N^1-allyl heptanal, decanal, N^1-methyl decanal, N^1-allyl decanal, undecanal, N^1-methyl undecanal and N^1-allyl undecanal and their Ni(II) complexes to evaluate the cytotoxic activity against human leukemia cell lines (U937). Belicchi-Ferrari *et al.*[19] reported the synthesis and antiproliferative activities of Cu(II) and Ni(II) complexes obtained from citronellal-N^4-ethlyl-morpholine thiosemicarbazone. The antiproliferative activity was tested against human histiocytic lymphoma cell line (U937). The GI_{50} (50 % growth inhibition) values were 2.3 μM and 12.3 μM for Cu(II) and Ni(II) complexes, respectively.

Antiproliferative activity *in vitro* against human breast cancer cell line (MCF-7) and bladder cancer cell line (T24)

with Zn(II) complexes obtained from pyridine-2-carbaldehyde thiosemicarbazone and (1E)-1-pyridin-2-ylethan-1-one thiosemicarbazone was reported by Kovala-Demertzi *et al*.[20]. They also compared their cytotoxic results with the well known cytotoxic drug, *cis-platin*. In particular to T24 cell lines, the cytotoxic activity of the Zn(II) complexes were shown almost 26 times better activity than *cis-platin*. Serda *et al*.[21] synthesized a series of thiosemicarbazones containing a quinoline scaffold. They studied about the iron chelation efficiency and anti-proliferative activity of these thiosemicarbazones. Mo(VI) complexes of 4-(diethyl amino)salicylaldehyde, 2-hydroxy-3-methoxybenzaldeyde and 2-hydorxy-1-naphtaldehyde thiosemicarbazones were synthesized by Vrodljak *et al*.[22]. The antiproliferative activity of free thiosemicarbazone ligands and their Mo(VI) complexes were tested against cervical carcinoma (HeLa), breast carcinoma (MCF-7), colon carcinoma (SW620), pancreatic carcinoma (MiaPaCa-2), laryngeal carcinoma (Hep-2) and diploid fibroblasts (WI 38). Based on their antiproliferative studies, they concluded that there was no significant difference between free ligands and their Mo(VI) complexes. Chelation of Mo(VI) with the thiosemicarbazones studied has not induced any significant cytotoxic property. Dilovic *et al*.[23] synthesized thiosemicarbazones and 4-phenyl-3- thiosemicarbazones of 4-diethyl amino-salicylaldehyde, 3-methoxy-salicylaldehyde, 2-hydroxy-naphthaldehyde and salicylaldehyde. They tested the antiproliferative activity of these thiosemicarbazones and phenyl-thiosemicarbazone against the human cell lines, such as HeLa, MCF-7, SW620, MiaPaCa-2, Hep-2 and WI 38.

Mn(II) complexes with the thiosemicarbazones of 2-acetylpyridine and 2-acetyl-N(4)-methyl, Co(II) complexes with the thiosemicarbazones of 2-benzoylpyridine and 2-benzoyl pyridine-N(4)-methyl and Zn(II) complexes of 2-benzoylpyridine thiosemicarbazone and 2-benzoylpyridine-N(4)-phenyl-thiosemicarbazone were synthesized by Li *et al*.[24]. Antitumor activity of these complexes was tested against human leucocythemia cancer cell line (K562). Their antitumor studies concluded that the bulky group substitutions at N(4) position of the thiosemicarbazone may decrease the antitumor activity. Kovala-Demertzi *et al*.[25] reported the antitumor activity of Pt(II) complexes obtained from 2-formyl and 2-acetyl pyridine and hexamethyleneiminyl ring incorporated at N(4) position of thiosemicarbazones against human cancer cell lines, such as MCF-7, T24 and A-549 (non-small cell lung carcinoma). The cytotoxic activity of Pd(II) and Pt(II) complexes with 5-substituted thiophene -2-carboxalde des against human cervix carcinoma was reported by Karakucuk-Iyidogan *et al*.[26].

Conclusion

From the above discussion, it is concluded that most of the thio- and phenyl thiosemicarbazones have cytotoxic activities. In most cases the cytotoxic activity of the thiosemicarbazones will enhances with the chelation of metal ions[15,10] or some times metal chelation may not change its activity significantly[22]. In future, the researchers have to concentrate on the mechanism of cytotoxicity of thiosemicarbazones due to metal chelation.

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