Synthesis and Cytotoxicity of Dinuclear Silver(I)-N-Heterocyclic Carbene Complexes

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Abstract

Synthesis of two new para-xylyl linked bis-benzimidazolium salts (III-IV) and respective dinuclear Ag(I)-N-heterocyclic carbene complexes (V-VI) has been described. The compounds were tested against human colorectal cancer cells (HCT 116) which showed a dose-dependent cytotoxic activity. The IC50 values were found in the range 0.03–65.9 μM. 5-Fluorouracil was used as standard drug (IC50 =5.9 μM).

Keywords: Benzimidazole; Bis-benzimidazolium salts; N-heterocyclic carbenes (NHCs); Ag(I)-NHC Complexes; human colon cancer (HCT 116).

Introduction

Human colon cancer is the third most common and the fourth most frequent cause of cancer deaths worldwide [1]. The marketing chemotherapeutic drugs for the treatment of colon related cancer are Carboplatin, Levamisole, Miltefosine, Semustine and Floxuridine (Chart 1). The chart displays that most of the drugs are organic compounds. Among these, only Carboplatin is a metal based anticancer drug that has been recently approved to treat human colon cancer.

The incorporation of Carboplatin against cancer has recently attracted researchers to further explore the area of metal based anticancer drugs [2,3]. Except transition metals, the organometallic compounds based on coagin metals (Ag, Au, Cu) have now attracted the researchers for medicinal applications [4,5]. Recently, Au(I)-NHC complexes have been tested against HCT 116 cell line (Chart 2), showed significant in vitro anticancer results [6]. However, according to the published studies, Ag(I)-N-Heterocyclic Carbene (NHC) complexes have been rarely studied against this specific cancer. The current study is an effort in this area.

Silver(I)-NHC complexes have shown excellent anticancer results against various types of cancerous cell lines [7-23]. Silver containing organometallic compounds, other than silver(I)-NHC complexes, have also shown significant biological activities [23,24]. Due to aforementioned reasons, two new bis-benzimidazolium salts (organic salts, III-IV) were synthesized and bonded with silver metal ions through carbene carbon (metal complexes, V-VI) to further assess their medicinal efficacy against HCT 116. This work is a consistency of our slogs [14,15,24,25].

Experimental

Reagents and instruments: NMR spectra were recorded on Bruker 500 MHz UltrashieldTM spectrometer at ambient temperature.

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FT-IR spectra were recorded on a Perkin Elmer-2000. Elemental analyses were carried out on a Perkin Elmer series II. 2400 microanalyzer. The melting points were assessed by using a Stuart Scientific SMP-1 (UK) instrument.

RPMI 1640 was purchased from ScienCell, USA. Trypsin and heat inactivated foetal bovine serum (HIFBS) were obtained from Gibco, UK. Phosphate buffered saline (PBS), penicillin/streptomycin (PS) solution, MTt reagent and 5-fluorouracil were purchased from Sigma-Aldrich, Germany. All other chemicals used in this study were analytical grade or better.

Human colorectal tumor (HCT 116) cell line was purchased from American type culture collection (Rockville, MD, USA). HCT 116 cell line has been derived from colonic epithelial carcinoma. The cells were maintained in RPMI 1640 containing 10% HIFBS and 1% PS. Cells were cultured in 5% CO2-humidified atmosphere at 37°C. Preparation of cell culture and MTT Assay was performed according to the reported procedure [15,24].

Syntheses: Syntheses of N-substituted benzimidazoles (I-II), bis-benzimidazolium salts (III-IV) and respective Ag(I)-NHC complexes (V-VI) were carried out according to the our previously reported procedure [9,13,14,15,24].

3,3’-(1,4-phenylenebis(methylene))bis(1-pentyl-benzimidazolium) dibromide (III.2Br).

Following the reported procedure [15,24], N-pentylbenzimidazole (1), 20 mM (3.76 g) and 1,4-bis(bromomethylene)benzene 10 mM (2.63 g). The product appeared as white precipitates, filtered, washed with fresh 1.4-dioxane (3×5 ml) and dried at ambient temperature. Beige colored powder; yield: 88% (5.62 g); mp 283-285°C. FT-IR (KBr, Ѽmax, cm-1): 3108, 3056, 3031 (C-H arom); 2952, 2930, 2873 (C-H aliph); 1620, 1584 (C=C stretch); 1436, 1476 (Ag-C arom-Nbenzimi). 1H NMR (500 MHz, DMSO-d6): δ=7.0 Hz, 8H, Ar-H, 7.68 (d, J=8.5 Hz, 4H, Ar-H). 13C NMR (125.7 MHz, DMSO-d6): δ=13.5 (CH2), 21.6, 26.1, 28.5, 30.0, 31.5 (alkyl chain 5CH2), 48.5 (N-Ch2-Ar), 110.2, 124.2, 127.2, 133.5, 136.3 (Ar-C), Ag-C was not observed. Anal. Calc. for C36H48F12N4P2: C, 61.01; H, 6.32; N, 8.31. (qnt=quintet; br.m=broad multiplet).

3,3’-(1,4-phenylenebis(methylene))bis(1-heptyl-benzimidazolium) dibromide (IV.2Br).

Following the reported procedure [15,24], IV.2Br, 40 mM (2.8 g) and silver oxide, 7.0 mM (1.6 g). Beige colored powder; yield: 71% (2.2 g); mp 283-285°C. FT-IR (KBr, Ѽmax, cm-1): 3108, 3036, 3004 (C-H arom); 2968, 2932, 2873 (C-H aliph); 1625, 1570 (C=C stretch); 1352, 1421, 1452, 1476 (Ag-C arom-Nbenzimi). 1H NMR (500 MHz, DMSO-d6): δ=7.0 Hz, 8H, 4×N-CH2-Ar, 7.13 (s, 8H, Ar-H), 7.41 (br.m, 8H, Ar-H), 7.74 (d, J=8.0 Hz, 4H, Ar-H), 7.84 (d, J=8.0 Hz, 4H, Ar-H). 13C NMR (125.7 MHz, DMSO-d6): δ=13.5 (CH2), 21.6, 26.1, 28.5, 30.0, 31.5 (alkyl chain 5CH2), 48.5 (N-Ch2-Ar), 110.2, 124.2, 127.2, 133.5, 136.3 (Ar-C), Ag-C was not observed. Anal. Calc. for C36H48F12N4P2: C, 61.01; H, 6.42; N, 8.35. Found: C, 58.57; H, 6.53; N, 7.81.

Results and Discussion

Synthesis: The reaction of two equivalents of N-alkylated benzimidazole with 1,4-bis(bromomethylene)benzene in 1,4-dioxane at 100°C for 24 h afforded the para-xylly linked bis-benzimidazolium salts (III-IV) with good yields. The salts (III-IV) appeared as white precipitates in 1,4-dioxane, filtered and washed with fresh 1,4-dioxane (3×5 ml). The precipitates so obtained were dried at ambient temperature for 24 h. This is in accordance with the previously reported para-xylly linked bis-benzimidazolium salts having different terminal N-substitutions [14,15]. The so salts so obtained were found to be soluble in polar solvents like methanol, ethanol, DMSO, DMF but remained insoluble in non-polar solvents like diethyl ether, dichloromethane and chloroform. Also, the reported salts were found insoluble in water. However, on changing counter ions from halide to hexafluorophosphat (PF6), the solubility of salts affected. For example, with PF6 as counter anions the salts became insoluble in methanol, ethanol but soluble in acetonitrile. However, the salts remained soluble in DMSO and DMF with both types of anions. The change of counter anions changes the polarity of compound. With the halide as counter anion polarity of these salts increased.

Synthesis of dinuclear Ag(I)-NHC complexes using bis-benzimidazolium halides (III-IV) as starting material is of significance importance, because using this method pure form of desired complexes can be obtained with ease. Scheme 1 shows three simple steps for the synthesis, starting from N-alkylated benzimidazoles to PF6 salts of dinuclear Ag(I)-NHC complexes. As a preliminary confirmation of successful synthesis, it was noticeable that the melting points of

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Ag(I)-NHC complexes were significantly higher than compared to respective ligands. This is in accordance with the previous reports [14,15,26,27].

**FT-IR spectra of the compounds:** The synthesized bis-benzimidazolium salts do not have many functional groups to be distinguished by FT-IR spectroscopic technique but it is possible to

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**Scheme 1:** Synthesis of N-alkyl benzimidazoles (I-II), 3,3’-(1,4-phenylenebis(methylene))bis(1-alkyl-Benzimidazolium) salts (III-IV), and Ag-NHC complexes (V-VI). complexes.
signals for methylene (N-CH2-Ar) carbon appeared at 49.2 δ ppm and salts displayed a distinguished peak in the most downfield region at could be observed as primary indicators of a successful synthesis [15]. The methylene (N-CH2-Ar) group, which connects of observable changes in aromatic (Ar-H) peaks (Supplementary I). Furthermore, the resonance of -substituted alkyl chain protons with benzimidazolium units, exhibited a sharp singlet at 5.7 δ ppm. The ring vibrations, other than –HC=N, are intense bands at around 1050 and 1220 cm⁻¹.

Some interesting FT-IR features to confirm a successful synthesis of Ag(I)-NHC complexes were observed [15]. It was observed that bonding of NHC carbon with silver ion strengthened vibrations in the range 1350 to 1500 cm⁻¹ and a typical “four fingers (f.f.s)” pattern appeared for the synthesized Ag(I)-NHC complexes [13,14,24,29]. This region is specific for –C=N (Carom-Nbenzimidazoles (I-II) and -benzimidazolium salts (III-IV), appeared in the range 0.8-4.5 δ ppm. Similarly, the structural features of the salts were confirmed by the 13C NMR data. The spectra of all the shift regions 51 and 13-49 δ ppm, respectively. These signals are 2-3 δ ppm downfield compared to respective ligands. All these observations indicated the formation of desired complexes (IV-VI).

In vitro anticancer activity: Effect of benzimidazolium salts (ligands) and respective silver(I)-NHC complexes on proliferation of HCT 116.

In this work, antiproliferative potential of NHC pro-ligands and respective dinuclear Ag(I)-NHC complexes was evaluated against HCT 116 cancerous cells. The results have been compiled as mean percentage inhibition of cell proliferation (±S.D). The compounds (III-VI) were tested against HCT 116, exhibited a strong antiproliferative activity with IC₅₀ values in the range 0.03–65.9 μg/ml (Table 1). The results were more pronounced than the standard drug 5-fluorouracil (IC₅₀=5.9 μM). Recently, we reported anticancer activity of meta-α, para-xyllyl linked bis-benzimidazolium salts and respective dinuclear Ag(I)-NHC complexes. We found that in general the complexes are more active against human colon cancer compared to respective ligands. The current results support our previous findings. Furthermore, Benzimidazole, the starting material, was also tested against HCT 116 cell lines and was found almost inactive (IC₅₀=>200 μM). Hence the bis-benzimidazolium salts (IC₅₀=0.3–65.9 μM) were found to be active against human colon cancer compared to benzimidazole moiety whereas the Ag(I)-NHC complexes of these bis-benzimidazolium salts were further found to be relatively active (IC₅₀=0.03–5.6 μM).

The Figure 1 illustrates the anti-proliferative effect of the test compounds. The graphs clearly show that, all the compounds inhibited the proliferation of HCT 116 cells in a dose dependent manner. Figure 2 shows the cell images of cell line with 48 hours control without and with drugs.

A) Cancer cells from the control group exhibited a thoroughly confluent growth with densely proliferating HCT 116 cells. B) Treatment with the ligand IV exhibited noteworthy inhibitory effect on cancer cell proliferation with IC₅₀=0.3 μM. The picture divulged

| Table 1: IC₅₀ values of tested samples against HCT 116 cancer cell line. |
|------------------------|--------|----------|---------|
| Ligands                | Compounds | HCT 116 |
| III                   | IV     | 65.9     |
| Ag(I)-NHC Complexes   | V      | 5.6      |
| Starting Material      | benzimidazole | >200  |
| Standard Drug          | 5-Fluorouracil | 5.9   |

Figure 1: Dose dependent anti-proliferative effect of synthetic ligands and their metal complexes on human colorectal tumor cells (HCT 116).
and their Ag(I)-NHC complexes were synthesized. The compounds were characterized for their possible cytotoxicity on human colorectal cancer (HCT 116). All tested compounds showed dose-dependent cytotoxicity against human colon cancer. The IC₅₀ values for HCT 116 cells were between 0.03 and 65.9 μM. Some of the tested compounds showed IC₅₀ values significantly better than commercially available drug (5-FU, IC₅₀=5.9 μM). Among all, the complex VI showed a strongest cytotoxic effect of the complex (IC₅₀=0.03 μM). It can be visualized clearly that the compound affected the proliferation of all most all the cells of the group which rendered the cells to lose their pseudopodial like extensions. D) Treatment with the ligand III showed a moderate cytotoxicity (IC₅₀=65.9 μM) than compared to the respective Ag(I) N-heterocyclic carbene complexes. Applied Organometallic Chemistry 27: 465-473.

Conclusions

In conclusion, two new para-xylyl linked bis-benzimidazolium salts and their Ag(I)-NHC complexes were synthesized. The compounds were characterized for their possible cytotoxicity on human colorectal cancer (HCT 116). All tested compounds showed dose-dependent cytotoxicity against human colon cancer. The IC₅₀ values for HCT 116 cells were between 0.03 and 65.9 μM. Some of the tested compounds showed IC₅₀ values significantly better than commercially available drug (5-FU, IC₅₀=5.9 μM). Among all, the complex VI was found to be the most active against human cancer cell line (IC₅₀=0.03 μM). Based on the results it can be concluded that incorporation of silver ions with organic moieties enhances their anticancer potential.

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References


