Erzincan Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
2022, 15(3), 736-748
ISSN: 1307-9085, e-ISSN: 2149-4584
Araştırma Makalesi

Erzincan University
Journal of Science and Technology
2022, 15(3), 736-748
DOI: 10.18185/erzifbed.1130634
Research Article

In Vitro Cytotoxic Evaluation of a Silver(I) Complex Including Non-Steroidal Anti-Inflammatory Drug Niflumic Acid and 3-Picoline on Human-Derived Cancer Cell Lines

Sema CAGLAR [1*], Ahmet ALTAY [1], Bulent CAGLAR [1], Esma YENİCERİ [2], Betül HARURLUOGLU [2]

[1] Department of Chemistry, Faculty of Arts and Sciences, Erzincan Binali Yıldırım University, 24100 Erzincan, Turkey
[2] Department of Chemistry, Institute of Science and Technology, Erzincan Binali Yıldırım University, 24030, Erzincan, Turkey

Received: 14/06/2022, **Revised:** 02/10/2022, **Accepted:** 02/10/2022, **Published:** 30/12/2022

Abstract

Here, a novel silver(I) complex including the non-steroidal anti-inflammatory drug niflumic acid and 3-picoline was synthesized and characterized by FT-IR, 1H NMR, elemental and thermal analysis techniques. These techniques demonstrated that the formula of the synthesized complex is [Ag(nif)(3-pic)]. The cytotoxic ability of the complex, ligand (niflumic acid) and silver ions alone were tested against human breast adenocarcinoma (MDA-MB-453), lung adenocarcinoma (A-549), colorectal adenocarcinoma (HT-29), and mouse fibroblast (3T3-L1) cell lines. The XTT results indicated that although AgNO3 and niflumic acid alone exhibited modest cytotoxicity on the cancer and healthy cell lines, the complex indicated strong cytotoxic activity on the cancer cells in dose-dependent manner. The strongest cytotoxicity and the highest selectivity by the complex were determined on HT-29 cells. These findings provide fundamental outputs for the evaluation of the novel silver(I) complex in advanced anticancer studies.

Keywords: Silver(I) complex, niflumic acid, cytotoxicity, cell culture

Steroid Olmayan Anti-İnflamatuvar İlaç Niflumik Asit ve 3-Pikolin İçeren Gümüş(I) Kompleksinin İnsan Kaynaklı Kanser Hücre Hatları Üzerinde İn Vitro Sitotoksik Değerlendirmesi

Öz

Burada, steroidal olmayan anti-enflamatuar ilaç niflumik asit ve 3-pikolin içeren yeni bir gümüş(I) kompleksi sentezlendi ve FT-IR, 1H NMR, elemental ve termik analiz teknikleri ile karakterize edildi. Bu teknikler, sentezlenen kompleksin formülünün [Ag(nif)(3-pic)] olduğunu gösterdi. Kompleks, ligand (niflumik asit) ve gümüş iyonlarının tek başına sitotoksik yeteneği, insan meme adenokarsinomu (MDA-MB-453), akciğer adenokarsinomu (A-549), kolorektal adenokarsinom (HT-29) ve fare fibroblast hücre hatlarına (3T3-L1) karşı test edildi. XTT sonuçları, AgNO3 ve niflumik asidin tek başına kanser ve sağlıklı hücre hatları üzerinde orta düzeyde sitotoksisite sergilemesine rağmen, kompleksin kanser hücreleri üzerinde doza bağlı bir şekilde güçlü sitotoksik aktivite sergiledi. Kompleksin en güçlü sitotoksisitesi ve en yüksek seçiciliği HT-29 hücrelerinde olduğu belirlendi. Bu bulgular, gelişmiş antikanser çalışmalarında yeni gümüş(I) kompleksinin değerlendirilmesi için temel oluşturmaktadır.

Anahtar Kelimeler: Gümüş(I) kompleks, niflumik asit, sitotoksisite, hücre kültürü.

Corresponding Author: scaglar@erzincan.edu.tr
Sema CAGLAR, https://orcid.org/0000-0002-7424-2315
Ahmet ALTAY, https://orcid.org/0000-0001-8120-8900
Bülent CAGLAR, https://orcid.org/0000-0002-6087-3685
Esma YENİCERİ, https://orcid.org/0000-0002-6860-9758
Betül HARURLUOGLU, https://orcid.org/0000-0003-2521-9677

1. Introduction

Cancer is a very common type of disease that starts with the uncontrollable growth of abnormal cells and finally spreads to other parts of the body. World health organization (WHO) has declared that cancer is the second leading reason of death [1]. Therefore, scientists are making great efforts to develop new drugs and treatment methods to struggle with cancer. Especially, with the exploration of the anti-proliferative effect of cisplatin in cancer treatment in 1844, interest in medicinal inorganic chemistry has raised [2,3]. After that, a large number of metal-including complexes such as platinum, palladium, gold, copper and ruthenium have been designed and synthesized for using in preclinical and clinical trials [4–9]. Researches have also demonstrated that coordination complexes indicate high biological activity than the starting metal salts.

Silver compounds have been used in the treatment of burns, wounds infection, and especially antimicrobial diseases for thousands of years [10,11]. Despite silver compounds for the cure of infections have been utilized for a long time, the design of anticancer drugs on silver has been a comparatively new area of medicinal chemistry. The studies of silver compounds are increasing day by day due to their robust cytotoxic activity against cancer cells and low toxic effect towards healthy cells when compared to cisplatin [12–14].

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs in patient. They have been shown to display a variety of biological properties such as antipyretic, analgesic, anti-inflammatory agents. Additionally, NSAIDs display good anti-proliferative activity against various cancer cell lines [15–18]. NSAIDs compounds have been also utilized as ligand because they contain a nitrogen atom and oxygen atoms of carboxyl group that can form donor acceptor bonds with metal ions. Moreover, studies have demonstrated that coordination complexes including NSAIDs can improve the biological activity than free NSAIDs. The therapeutic effect of NSAIDs has encouraged researchers to synthesize new metal complexes and investigate their pharmacological applications [19–21].

Therefore, in the current study, a novel silver(I) complex of niflumic acid with auxiliary ligand 3-picoline was synthesized and characterized by Fourier transform infrared spectroscopy (FT-IR), proton nuclear magnetic resonance (^{1}H NMR), elemental and thermal analysis techniques. Afterwards, its potential cytotoxic ability and selectivity were determined over three different cancer cell lines and one normal cell line.

2. Material and Methods

2.1. Physical measurements

$AgNO_3$, niflumic acid, 3-picoline, methanol and acetonitrile were purchased from Sigma Aldrich and used without further purification. The molecular structures of niflumic acid and 3-picoline are shown in Fig. 1. The LECO CHNS-932 device was used for elemental analysis. The FT-IR spectra were recorded with The Thermo Nicolet 6700 spectrophotometer (4000-400

cm^{-1}). The PRIS Diamond device was utized for thermal analysis (TG/DTA/DTG) curves. The Agilent-VNMRS-400 spectrometer (400 MHz) was used for ^1H NMR spectra.

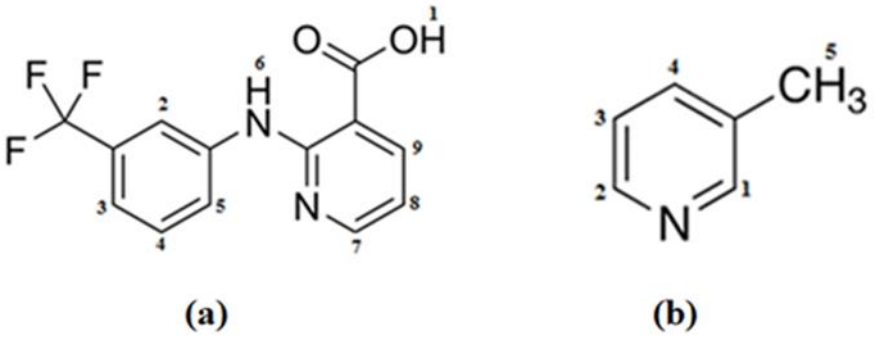

Figure 1. The molecular structures of (a) niflumic acid (b) 3-picoline

2.2. Synthesis of [Ag(nif)(3-pic)]

Niflumic acid (Hnif, 1 mmol) and KOH (1 mmol) were dissolved in 10 mL of methanol under stirring for 1 h at 50°C. Silver(I) nitrate (1 mmol) was dissolved in 10 mL of distilled water in the other beaker. After stirring 1 h, 3-picoline (3-pic, 1 mmol) and niflumic acid solution were added to silver(I) nitrate solution and white suspension occurred immediately. The addition of 10 mL of acetonitrile changed the suspension to colourless solution. The obtained clear solution was kept in the dark at room temperature. After two months, colourless microcrystals of complex were acquired. The molecular structure of [Ag(nif)(3-pic)] was given in Fig.2.

Colourless crystal product of **[Ag(nif)(3-pic)]** (81%): Analytical data for [$C_{19}H_{15}N_3O_2F_3Ag$] Found: C, 47.29; H, 3.12; N, 8.70%; calcd: C, 47.28; H, 3.11; N, 8.71%).

^1H NMR (400 MHz, DMSO-d_6) (δ/ppm): 2.31 (3H, s, H^5-3-pic), 6.85 (1H, dd, H^8-nif), 7.21 (1H, d, H^5-nif), 7.34 (1H, dd, H^3-3-pic), 7.49 (1H, t, H^4-nif), 7.64 (1H, d, H^4-3-pic), 7.78 (1H, d, H^3-nif), 8.30-8.26 (2H, m, H^2-nif and H^9-nif), 8.36 (1H, s, H^7-nif), 8.39 (1H, d, H^2-3-pic), 8.44 (1H, s, H^1-3-pic), 12.73 (1H, s, H^6-nif).

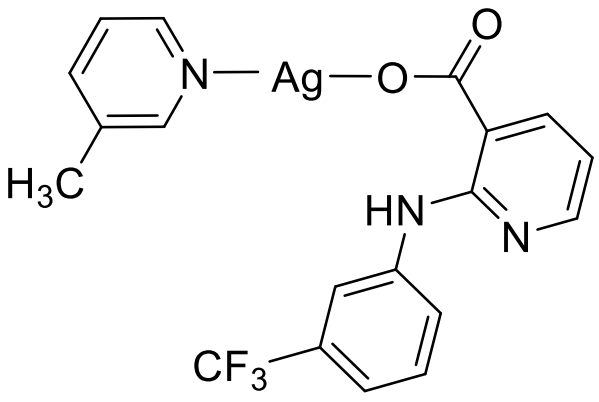

Figure 2. The molecular structure of [Ag(nif)(3-pic)]

2.3. Biological activity

2.3.1. Cell culture

Human breast adenocarcinoma (MDA-MB-453), lung carcinoma (A-549), colorectal adenocarcinoma (HT-29), and normal mouse fibroblast (3T3-L1) cell lines were purchased from the American Type Culture Collection (ATCC, USA). Passaging and culturing of the cells were performed according to the company's instructions. Accordingly, culturing of MDA-MB-453, A-549, HT-29, and 3T3-L1 cells were performed with the mediums of Leibovitz's L-15, F-12K, McCoy's 5a, and Eagle's Minimum Essential Medium, respectively. The studies were done using biosafety cabinet (Bilser, Turkey) and incubations were performed using 5% CO_2 incubator at 37°C (Nuve, Turkey).

2.3.2. Cell proliferation assay

2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H tetrazolium-5-carboxanilide (XTT) assay was carried out to investigate the potential anti-proliferative activity of the test compounds [22,23]. Accordingly, 7500 cells were planted into 96-well plate and incubated for 24 h to obtain their morphological shapes. After that, each cancer cell line was treated with varying concentrations of the Ag(I) complex (1-100 μM) and also ligand (niflumic acid) and silver(I) nitrate alone. The dose range applied for 3T3-L1 cells was from 1 to 250 μM. Following the exact incubation time, the absorbance of each well was measured spectrophotometrically at 490 nm (BioTek, USA). Carboplatin was evaluated as positive control. The IC_{50} concentration of the compounds was calculated by plotting the graph between the different concentration of the compounds and the % inhibition of cell proliferation. Selectivity Index (SI) was calculated from the ratio of IC_{50} concentration of the complex over the normal cell line (3T3-L1) to the IC_{50} concentration over the cancer cell line tested. The percent cell proliferation inhibition was calculated using the following formula;

% Cell proliferation inhibition = 100-[Abs(drug)/Abs(control)]×100.

2.3.3. Statistical analysis

Student's t-test was performed for statistical analysis using GraphPad Prism 6 (GraphPad, La Jolla, CA) Software 7.0) and $p < 0.05$ was considered significant.

3. Results and Disscussion

3.1. FT-IR Spectra

The FT-IR spectra of niflumic acid and [Ag(nif)(3-pic)] complex are demonstrated in Figure 3 and Figure 4, respectively. Niflumic acid displayed band at 3315 cm^{-1} which is attributed to stretching vibration of ν(NH) group and this peak is seen in almost the same region in the spectrum of [Ag(nif)(3-pic)] complex. This position showed that there is no interplay between the NH group and Ag(I) ion. This band at 1661 cm^{-1} for niflumic acid is assigned to the carboxylate group. The spectrum of the complex indicated that this band turn into asymmetric

(COO^-) and symmetric (COO^-) stretching vibrations of carboxylate group of niflumato ligand at 1601 and 1389 cm^{-1}. $\Delta\nu$ value (difference between $\nu_{asym}(COO^-)$ and $\nu_{sym}(COO^-)$ stretching vibrations) is 212 cm^{-1}, representing a monodentate coordination mode between the silver(I) ion since the characteristic $\Delta\nu$ value for monodentate coordination mode is larger than for ionic compounds of the ligand ($\Delta\nu$(nifNa): 206 cm^{-1}). The bands between 3062 and 2936 cm^{-1} are related to the aromatic and aliphatic ν(C-H) stretching vibrations. The characteristic C=N and C=C stretching vibrations of benzene and pyridine rings are observed at 1605, 1597, 1514 and 1450 cm^{-1}. Also, the bands at 1330 and 775 cm^{-1} probably correlate with CF_3 stretching vibration and CF_3 deformation, respectively.

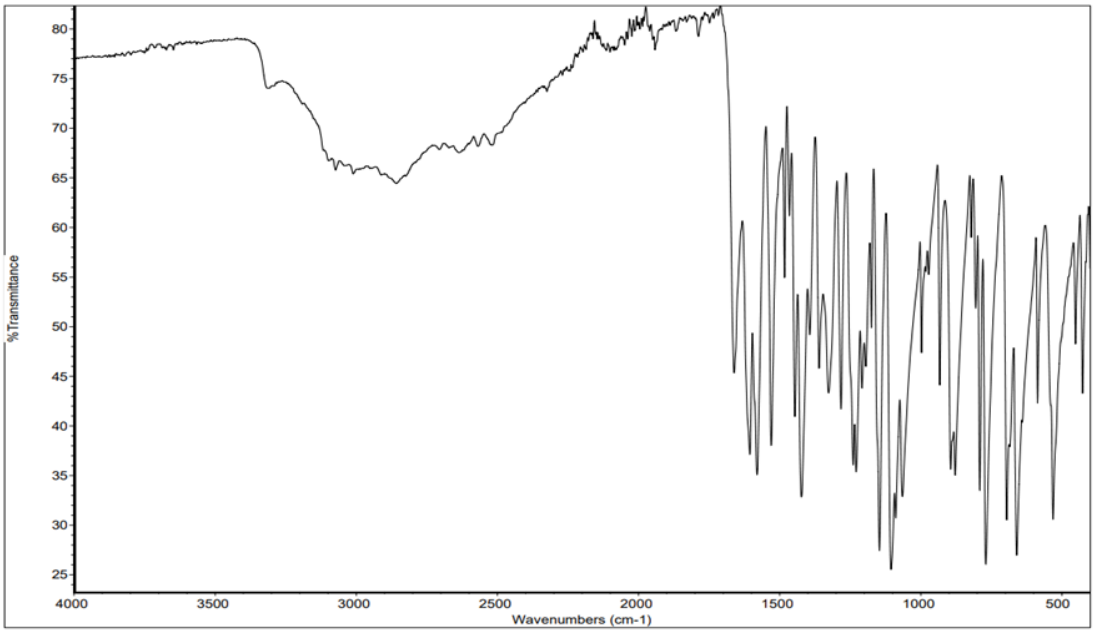

Figure 3. FT-IR spectrum of niflumic acid

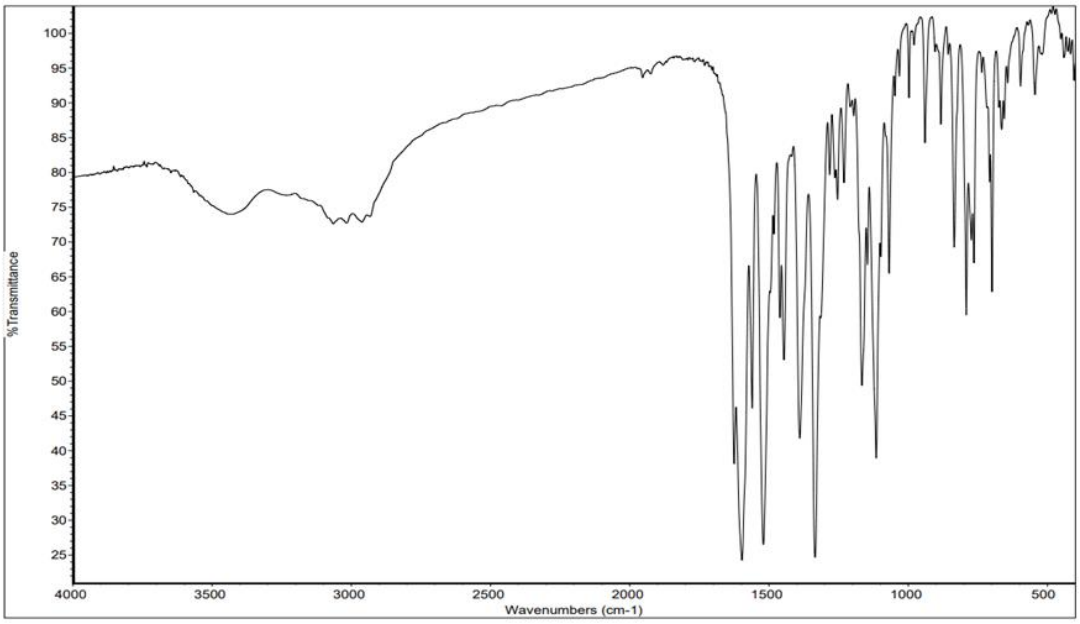

Figure 3. FT-IR spectrum of [Ag(nif)(3-pic)]

3.2. Thermal Analysis of [Ag(nif)(3-pic)]

[Ag(nif)(3-pic)] complex undergoes decomposition in two steps is showed in Fig. 5. The first decomposition step was occurred in the temperature range between 30 and 205 ºC, showing the loss of 3-pic molecule. The percentage experimental mass loss (18.25%) is consistent with the calculated mass loss (19.28%) (DTA: 119(+), 178(-) ºC, DTG: 114, 151, 178 ºC). The second decomposition step was accompanied by weight loss (exper. mass loss of 60.22%; calc. mass loss 58.31%), appointed to the loss of nif ligand. This step was appeared to compose of three stages as indicated in DTG curve (at 213, 244 and 433 ºC) whereas the DTA curve gave the peaks at 231(-), 259(-) and 414(-) ºC. The residual thermal product was described as metallic silver according to experimental mass loss (21.53%) and calculated mass loss (22.36%).

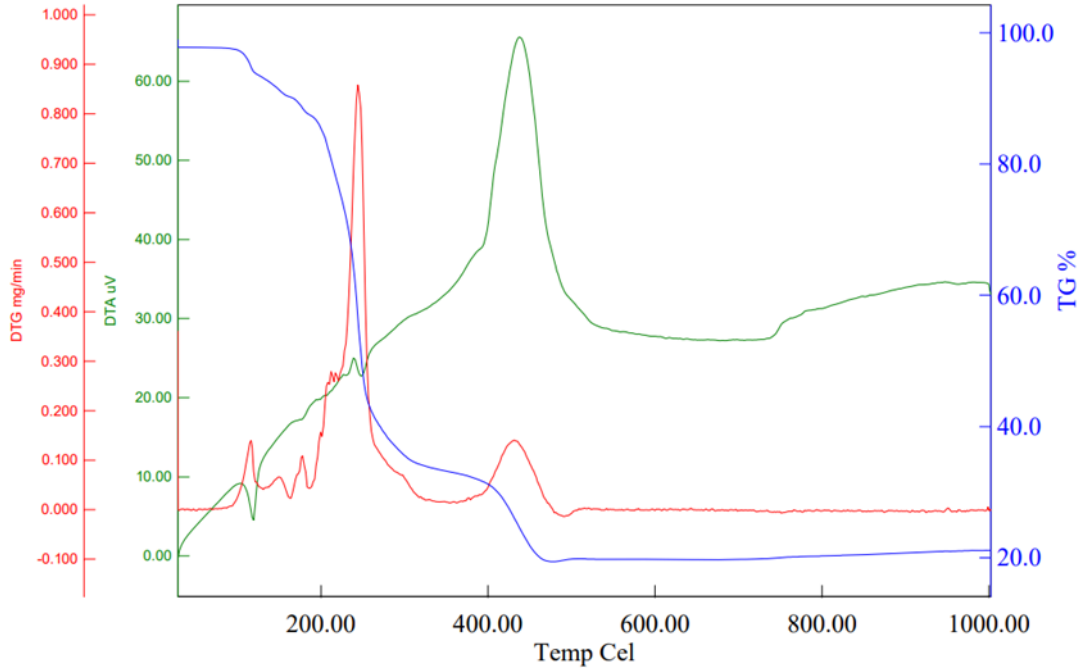

Figure 5. The TG/DTG/DTA curves of [Ag(nif)(3-pic)].

3.3. ^{1}H NMR studies

^{1}H NMR technique is used to determine the presence and ratio of ligands in complexes. ^{1}H NMR spectrum of [Ag(nif)(3-pic)] complex is demonstrated in Fig. 6. In the synthesized complex, all the expected signals of ligands were observed and also their multiplicities and integrals were shown to compatible with the structures. The number of protons showed that the ratio of nif: 3-pic is 1:1. All proton signals are observed to a little change binding to Ag(I) ion. The ^{1}H NMR spectrum of [Ag(nif)(3-pic)] complex showed a singlet signal at 2.31 ppm, which is ascribed to the methyl proton of 3-picoline ligands. The singlet peak at 12.73 ppm is attirubuted to NH proton of nif ligand. The spectrum was determined to be multiple resonance peaks for the aromatic protons at 6.85, 721, 7.49, 7.78, 8.26-8.30 and 8.36 ppm (niflumic acid) [24] and 7.34, 7.64, 8.39 and 8.44 ppm (3-picoline) [25]. The vanished of carboxylate hydrogen peak of the complex shows the connection of the nif ligand to Ag(I) ion via carboxylate group.

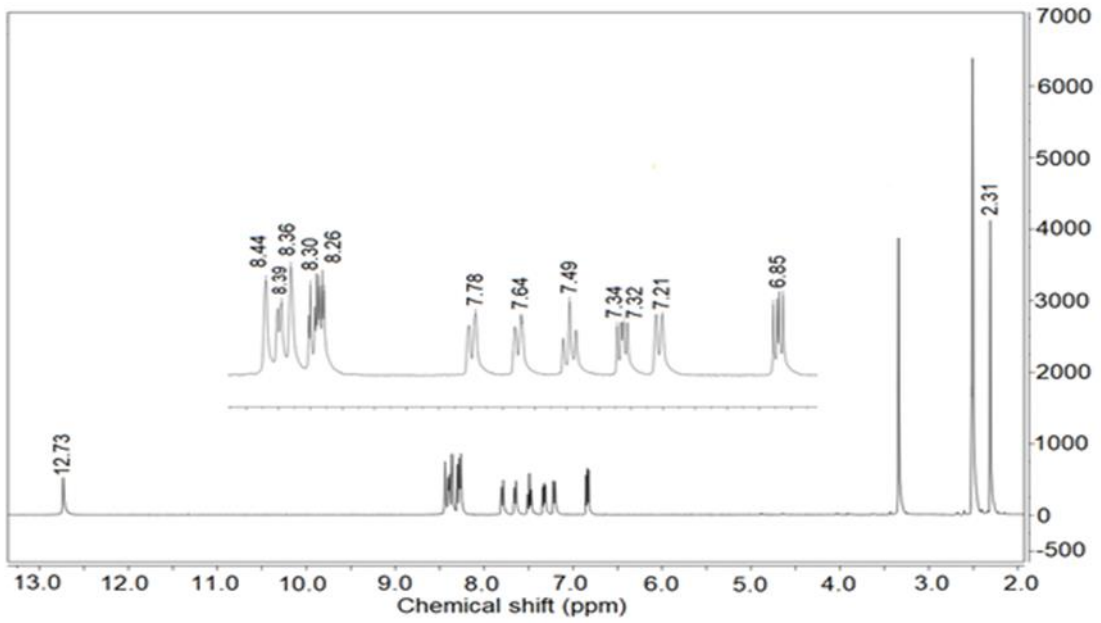

Figure 6. ^{1}H NMR spectrum of [Ag(nif)(3-pic)]

3.4. Cytotoxicity

The cytotoxic effects of the novel [Ag(nif)(3-pic)] complex and also ligand (niflumic acid) and silver(I) nitrate alone were tested on three different cancer and one healthy cell line. XTT results indicated that although niflumic acid and $AgNO_3$ alone showed very low cytotoxic activity against the human breast adenocarcinoma (MDA-MB-453), lung adenocarcinoma (A-549), colorectal adenocarcinoma (HT-29), and mouse fibroblast (3T3-L1) cell lines up to 100 μM, the complex exhibited dose-dependent cytotoxicity against three cancer cell lines (Fig. 7A-D). The half-maximum inhibitory concentrations (IC_{50}) of the complex, ligand, and silver salts were calculated from the standard curve using correlation–regression analysis and expressed with their mean ± standard deviations in Table 1. To investigate the anticancer feature of a new chemical agent isolated from a natural source or synthesized by organic and inorganic ways and also to determine its selectivity between normal and cancer cells is of a great importance. Therefore, we determined the IC_{50} values of the agents on the tested cell lines.

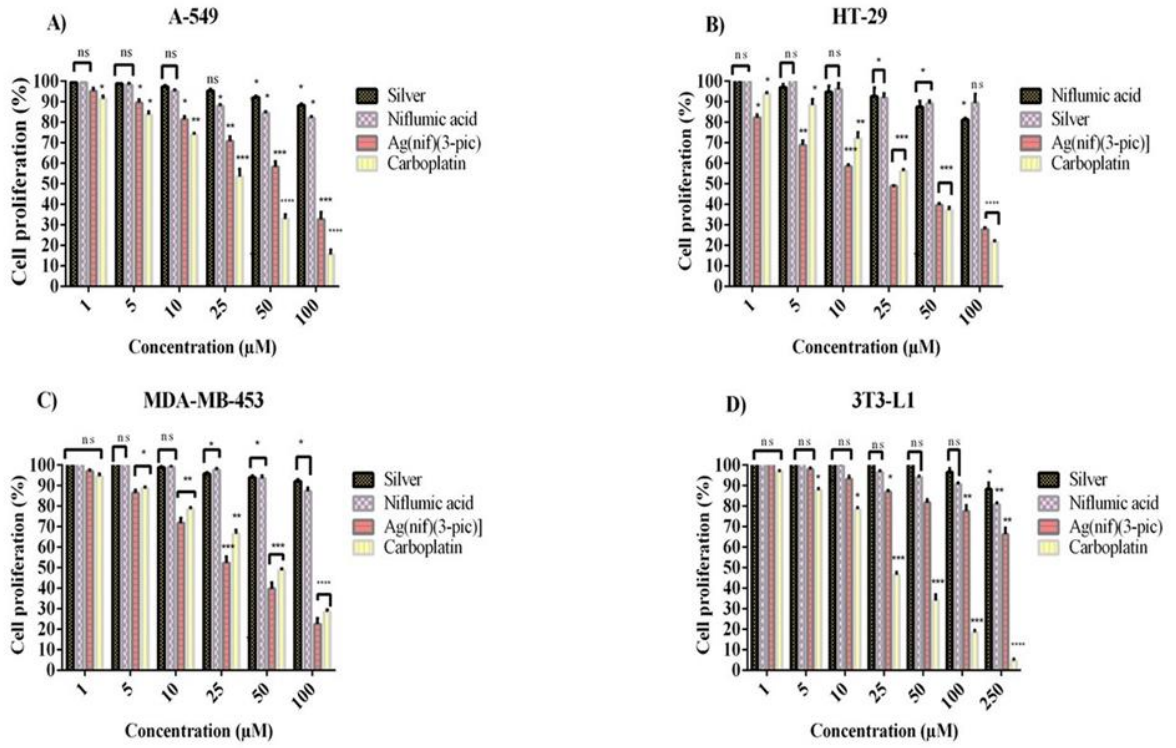

Figure 7. Dose-dependent anti-proliferative effects of [Ag(nif)(3-pic)], silver, niflumic acid, and carboplatin on human lung carcinoma (A-549), colorectal adenocarcinoma (HT-29) (B), breast adenocarcinoma (MDA-MB-453) (C), and normal mouse fibroblast (3T3-L1) (D) cell lines.

Table 1. The IC_{50} values of the test compounds on cancer and fibroblast cells.

	Cell lines IC_{50} (µM)[x]			
Test samples	**A-549**	**HT-29**	**MDA-MB-453**	**3T3-L1**
[Ag(nif)(3-pic)]	66.45±1.52[b]	39.05± 0.85[a]	47.71±1.34[a]	182.62 ± 2.40[b]
SI(complex)[y]	2.74	4.67	3.82	
[Niflumic acid]	>100[c]	>100[c]	>100[c]	>250[c]
[Silver(I) nitrate]	>100[c]	>100[c]	>100[c]	>250[c]
[Carboplatin]	40.42±1.5[a]	47.15±2.02[b]	56,73±1.12[b]	43.16±1.45[a]
SI (carb)[y]	1.06	0.91	0.76	

[x]IC_{50} (µM): a-c superscripts in the same column displays the differences at $p < 0.05$.
[y](SI): Selectivity index

As given in Table 1, the IC_{50} concentrations of the ligands and silver(I) nitrate alone on the cancer and normal cell lines were determined as greater than 100 µM and 250 µM, respectively. These results were consistent with the literature. For instance, the cytotoxic activity of niflumic acid against human osteosarcoma MG63 cells was reported to be between the range of 200 and 700 µM [26]. As for the silver salts, the IC_{50} concentration of silver nanoparticles against human breast cancer (MCF-7) cells was reported to be 463 µM [27]. In another study, the IC_{50} value

of $AgNO_3$ against human cervix adenocarcinoma (HeLa) was recorded as 158 μM [28]. These data confirm that niflumic acid and $AgNO_3$ alone exhibit a highly low cytotoxicity against cancer cell lines and also their IC_{50} concentration change depending on the cell line.

Considering the cytotoxic activity of the [Ag(nif)(3-pic)] complex formed by niflumic acid belonging to NSAIDs, auxiliary ligand picolin, and silver salts against the cancer and normal cell line, the complex showed a good dose-dependent cytotoxicity on all of the cancer cell lines while exhibiting no considerable cytotoxicity on normal 3T3-L1 cells. As seen in Table 1, the Ag(I) complex exhibited the highest cytotoxic effect on HT-29 cells with the lowest IC_{50} value (39.05 μM), followed by MDA-MB-453 (47.71 μM) and A549 (66.45 μM) cells, respectively. Whereas, the complex showed a very low cytotoxicity against normal 3T3-L1 cells with a very high IC_{50} value (182.62 μM). Upon compared to carboplatin used as a chemotherapeutic drug, the complex was determined to show statistically higher cytotoxic activity against MDA-MB-453 and HT-29 cells, but comparable cytotoxicity on A-549 cells (Table 1). In the literature, the various cytotoxic activities of the metal complexes formed by niflumic acid with different metal ions against various cancer cells were examined. For instance, the cytotoxic activity of $[Co(bcp)(nif)_2]$ was scrunized on various cancer cell types, namely HeLa, HT-29, PC-3 (human prostate cancer), and MCF-7 cells, and the IC_{50} values belonging these cell lines were reported to be 38.75, 43.67, 27.06, and 41.53 μM, respectively [29]. In another study, the IC_{50} value of the $[Zn(neo)(nif)_2]$ complex on two endometrial (hTERT and 12Z) cell lines was reported to be 4.2 and 0.9 μM, respectively [30]. Moreover, in our previous study we tested the cytotoxicity of $AgH(nif)_2$ complex against MCF-7, HT-29, and human hepatoma cells (HepG2) cancer cell lines, and the results indicated that the IC_{50} values of this complex on each cell line was 39.24, 71.02, and 58.38 μM, respectively [31]. When HT-29 cells tested in both studies was considered, the cytotoxic activity was observed to be much stronger upon 3-picoline was introduced into the structure of the previous $[AgH(nif)_2]$ complex. These data show that the type of NSAIDs, auxiliary ligand, and metal ion in the structure of the complex significantly affect the cytotoxicity, and also the cytotoxic effect varies significantly according to the cell type.

One of the necessary parameters to be considered in cytotoxicity studies is the selectivity of the test agents between cancer and healthy cells [32–34]. In this context, as seen in Table 1, the [Ag(nif)(3-pic)] complex displayed much higher selectivity on cancer cells when compared to normal cells, and the highest selectivity was determined against HT-29 cells with an SI value of 4.67, followed by MDA-MB-453 (SI: 3.82) and A-549 (SI: 2.74) cells, respectively. Besides, the complex exposed more selectivity against all the cancer cell lines compared to carboplatin. Many studies have shown that the synergistic effect contributes greatly to the stronger anticancer effect of various complexes of NSAIDs with transition metals compared to their individual effects [35]. In the synergistic action, both transition metals and NSAIDs contribute to cytotoxicity individually via exhibiting numerous characteristics including redox activity, variable coordination modes, stability, and reactivity towards the targeted-organic substrates involved in different anticancer mechanism pathways [36]. Herein, the findings in the present study indicated that [Ag(nif)(3-pic)] formed by silver(I) nitrate with niflumic acid and picoline ligand showed much higher cytotoxicity and selectivity against the aforementioned cancer cell

lines with higher synergistic effect, which still needs to be clarified with further studies of anticancer mechanisms of action.

4. Conclusion

This study defined the synthesis, characterization, and anti-proliferative potency of a new Ag(I) complex of NSAIDs. The structure of the synthesized complex was identified by FT-IR, elemental, ^{1}H NMR, and thermal analysis techniques and subsequently subjected to the cytotoxicity treatments by XTT assay. Biological activity studies revealed that the novel Ag(I) complex exhibited the strongest cytotoxic activity against HT-29 cells with the lowest IC_{50} value and the weakest activity against A-549 cells with the highest IC_{50} value. The results also confirmed that the complex exhibited higher selectivity towards to the tested cancer cells compared to normal cells. To conclude, these findings provide an important basis for the evaluation of the [Ag(nif)(3-pic)] complex in *in vivo* anticancer activity studies.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Acknowledgements

This work was financially supported by grants from Erzincan Binali Yıldırım University Scientific Research Projects Coordination Commission (EU-BAP) (Project No: FBA-2020-683).

Author Contributions

Conceived and designed the experiments: SC, AA. Performed the experiments: SC, AA, BC, EY, BH Analysed the data: SC, AA, and BC. Contributed reagents/materials/analysis tools: AA, SC, and BC. Wrote the article: AA, SC, and BC. All authors read and approved the final manuscript.

References

[1] Petersen, PE.(2009) Oral cancer prevention and control - The approach of the World Health Organization. Oral Oncol, 45(4–5): 454–60.

[2] Rosenberg B.(1980) Cisplatin: Its History and Possible Mechanisms of Action, Current Status and New Developments. Academic Press.

[3] Benedetti BT, Peterson EJ, Kabolizadeh P, Martínez A, Kipping R, Farrell NP.(2011) Effects of noncovalent platinum drug-protein interactions on drug efficacy: Use of fluorescent conjugates as probes for drug metabolism. Mol. Pharm, 8(3): 940–48.

[4] Karlenius TC, Tonissen KF.(2010) Thioredoxin and cancer: A role for thioredoxin in all states of tumor oxygenation. Cancers, 2:209–232.

[5] Bindoli A, Pia M, Scutari G, Gabbiani C, Casini A, Messori L.(2009) Thioredoxin

reductase : A target for gold compounds acting as potential anticancer drugs. Coord. Chem. Rev., 253: 1692–707.

[6] Gupte A, Mumper RJ.(2009) Elevated copper and oxidative stress in cancer cells as a target for cancer treatment. Cancer Treat. Rev, 35(1): 32–46.

[7] Monneret, C.(2011) Platinum anticancer drugs. From serendipity to rational design. Ann. Pharm. Fr., 69(6): 286–295.

[8] Johnstone TC, Suntharalingam K, Lippard SJ.(2016) The Next Generation of Platinum Drugs: Targeted Pt (II) Agents , Nanoparticle Delivery, and Pt (IV) Prodrugs. Chem. Rev, 116:3436–3486.

[9] Mi Q, Ma Y, Gao X, Liu R, Liu P, Mi Y, Fu X, Gao Q.(2016) 2-Deoxyglucose conjugated platinum (II) complexes for targeted therapy: design, synthesis, and antitumor activity. J. Biomol. Struct. Dyn, 34(11): 2339–50.

[10] Hindi KM, Panzner MJ, Tessier CA, Cannon CL, Youngs WJ.(2009) The Medicinal Applications of Imidazolium Carbene−Metal Complexes. Chem. Rev, 109(8): 3859–3884.

[11] Gasser G, Ott I, Metzler-Nolte N.(2011) Organometallic anticancer compounds. J. Med. Chem, 54:3–25.

[12] Pedro M, Mara M, Lidia C.(2014) Organometallic Compounds in Cancer Therapy: Past Lessons and Future Directions. Bentham Sci. Publ, 14:1199–1214.

[13] Kunkely H, Vogler A.(2011) Absorption and luminescence spectra of cochineal. Inorg. Chem. Commun, 14:1153–1155.

[14] Li S, Zhang S, Jin X, Tan X, Lou J, Zhang X, Zhao Y.(2014) Singly protonated dehydronorcantharidin silver coordination polymer induces apoptosis of lung cancer cells via reactive oxygen species-mediated mitochondrial pathway. Eur. J. Med. Chem, 86: 1–11.

[15] Mahendiran D, Kumar RS, Rahiman AK.(2017) Heteroleptic silver(I) complexes with 2,2′:6′,2″-terpyridines and naproxen: DNA interaction, EGFR/VEGFR2 kinase, growth inhibition and cell cycle arrest studies. Mater. Sci. Eng. C, 76:601–615.

[16] Johnsen, JI, Lindskog M, Ponthan F, Pettersen I.(2005) NSAIDs in neuroblastoma therapy. Cancer Lett, 228(1–2): 195–201.

[17] Banti CN, Giannoulis AD, Kourkoumelis N, Owczarzak AM, Kubickic M, Hadjikakou SK.(2014) Novel metallo-therapeutics of the NSAID naproxen. Interaction with intracellular components that leads the cells to apoptosis. Dalt. Trans, 18: 6848–63.

[18] Ho Woo D, Han IS, Jung G.(2004) Mefenamic acid-induced apoptosis in human liver

cancer cell-lines through caspase-3 pathway. Life Sci, 75:2439–2449.

[19] Kyros L, Banti CN, Kourkoumelis N, Kubicki M, Sainis I, Hadjikakou SK.(2014) Synthesis, characterization, and binding properties towards CT-DNA and lipoxygenase of mixed-ligand silver (I) complexes with 2-mercaptothiazole and its derivatives and triphenylphosphine. J. Biol. Inorg. Chem, 6: 449–464.

[20] Núñez C, Fernández-Lodeiro A, Fernández-Lodeiro J, Carballo J, Capelo JL, Lodeiro C.(2014) Synthesis, spectroscopic studies and in vitro antibacterial activity of Ibuprofen and its derived metal complexes. Inorg. Chem. Commun, 45: 61–65.

[21] Pereira IM, De Moraes Profirio D, De Paiva REF, Lancellotti M, Barboza Formiga AL, Corbi PP.(2013) A silver complex with ibuprofen: Synthesis, solid state characterization, DFT calculations and antibacterial assays. J. Mol. Struct, 1049: 1–6.

[22] Altay A, Bozoğlu F.(2017) *Salvia fruticosa* Modulates mRNA Expressions and Activity Levels of Xenobiotic Metabolizing CYP1A2, CYP2E1, NQO1, GPx, and GST Enzymes in Human Colorectal Adenocarcinoma HT-29 Cells. Nutr. Cancer,0:1–12.

[23] Harurluoglu B, Altay A, Caglar S, Kubra E, Yeniceri K, Caglar B, Sahin ZS.(2021) Binuclear silver (I) complexes with the non-steroidal anti-inflammatory drug tolfenamic acid: Synthesis, characterization, cytotoxic activity and evaluation of cellular mechanism of action. Polyhedron,202:115189.

[24] Tarushi A, Raptopoulou CP, Psycharis V, Kessissoglou DP, Papadopoulos AN, Psomas G. (2017) Interaction of zinc(II) with the non-steroidal anti-inflammatory drug niflumic acid. J Inorg Biochem, 176:100-112.

[25] Yu X, Ji D, Liu R, Yang F, Xie J, Zhou J, Li X, Yi P. (2010) NMR and theoretical study on the interaction between diperoxovanadate and 3-picoline derivatives. J. Coord. Chem., 63:1555-1562.

[26] Liao WC, Chou CT, Liang WZ, Hao LJ, Kuo CC, Lin KL, Wang JL, Jan, CR.(2018) Exploration of niflumic acid's action on Ca^{2+} movement and cell viability in human osteosarcoma cells. Chin. J. Physiol, 61(6): 341–48.

[27] Ramar M, Manikandan B, Marimuthu PN, Raman T, Mahalingam A, Subramanian P, Karthick S, Munusamy A.(2015) Synthesis of silver nanoparticles using Solanum trilobatum fruits extract and its antibacterial, cytotoxic activity against human breast cancer cell line MCF 7. Spectrochim. Acta - Part A Mol. Biomol. Spectrosc.,140: 223–228.

[28] Miura N, Shinohara Y.(2009) Cytotoxic effect and apoptosis induction by silver nanoparticles in HeLa cells. Biochem. Biophys. Res. Commun ,390:733–737.

[29] Smolko L, Smolková R, Samoľová E, Morgan I, Saoud M, Kaluđerović GN.(2020) Two

isostructural Co (II) flufenamato and niflumato complexes with bathocuproine : Analogues with a different cytotoxic activity. J. Inorg. Biochem, 111160.

[30] Smolko L, Špaková I, Klepcová Z, Dubayová K, Samoľová E, Rabajdová M, Mareková M.(2021) Zinc(II) niflumato complex with neocuproine: Synthesis, crystal structure, characterization and cytotoxic effects on human endometrial cell lines. J. Mol. Struct, 1237.

[31] Altay A, Caglar S, Caglar B.(2022) Silver(I) complexes containing diclofenac and niflumic acid induce apoptosis in human-derived cancer cell lines. Arch. Physiol. Biochem,128:69-79.

[32] Altay A, Caglar S, Caglar B, Sahin ZS.(2019) Novel silver(I) complexes bearing mefenamic acid and pyridine derivatives: Synthesis, chemical characterization and in vitro anticancer evaluation, Inorganica Chim. Acta, 493: 61–71.

[33] Caglar S, Altay A. Harurluoğlu, B. and Caglar, B.(2021) Trinuclear silver(I) complex of non-steroidal anti-inflammatory drug naproxen: Synthesis, characterization, and in vitro cytotoxicity, Maced. J. Chem. Chem. Eng, 40(2): 171-80.

[34] Caglar S, Altay A.(2021) In Vitro Anticancer Activity of Novel Co (II) and Ni (II) Complexes of Non-steroidal Anti-in fl ammatory Drug Niflumic Acid Against Human Breast Adenocarcinoma MCF-7 Cells, Cell Biochem. Biophys, 79(4): 729-46.

[35] Adolfo R, Marta C, Pedro L, Otaciro N, Daniel D, Joaquín L, Ignácio L, Lucas B, Luis L, Diógenes I.(2021) Synergy of DNA intercalation and catalytic activity of a copper complex towards improved polymerase inhibition and cancer cell cytotoxicity. Dalt. Trans, 34.

[36] Umar N, Ndumiso M, Mahmoud ES.(2017) Metal complexes in cancer therapy – an update from drug design perspective. Drug Des Devel Ther,11: 599–616.