TRINUCLEAR SILVER(I) COMPLEX OF NON-STEROIDAL ANTI-INFLAMMATORY DRUG NAPROXEN: SYNTHESIS, CHARACTERIZATION, AND *IN VITRO* CYTOTOXICITY

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Herein, a new silver(I) complex with the non-steroidal anti-inflammatory drug naproxen and nitrogen donor 3-picoline ligands was synthesized, characterized, and subsequently tested for its cytotoxicity against different types of cancer cell lines. Elemental analysis, Fourier transform infrared spectroscopy, thermal, and proton nuclear magnetic resonance techniques showed that the molecular formula of the prepared complex is bis(3-picoline)tris(μ-naproxenato)trisilver(I) and naproxen ligands bind to silver ions in a bridging bidentate mode. 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT) results revealed that silver salts and naproxen alone showed quite weak cytotoxic activity against human breast adenocarcinoma (MDA-MB-453), lung adenocarcinoma (A-549), and colorectal adenocarcinoma (HT-29) cell lines ($IC_{50} > 250$ μM), whereas the complex displayed dose dependent cytotoxicity against the aforementioned cell lines. The highest cytotoxicity was observed on MDA-MB-453 cells with an IC_{50} value of 11.73 μM. Moreover, the complex showed higher selectivity against the cancer cell lines compared to fibroblast 3T3-L1 cells. This study provides preliminary scientific data on the complex for further elucidation of its anticancer mechanism of action.

Keywords: silver(I) complex; naproxen; 3-picoline; cytotoxicity; cell culture

ТРИНУКЛЕАРЕН КОМПЛЕКС НА СРЕБРО(I) НА НЕСТЕРОИДНИОТ АНТИИНФЛАМАТОРЕН ЛЕК НАПРОКСЕН: СИНТЕЗА, КАРАКТЕРИЗАЦИЈА И *IN VITRO* ЦИТОТОКСИЧНОСТ

Извршена е синтеза, карактеризација, а потоа и тестирање на цитотоксичноста во однос на различни клетки на рак на нов комплекс на сребро(I) со нестероидниот антиинфламаторен лек напроксен и лиганди на азотен донор 3-пиколин. Елементната анализа, Фуриеовата трансформна инфрацрвена спектроскопија, термички и протонските нуклеарно-магнетни резонатни техники покажаа дека молекулската формула на приготвениот комплекс е бис(3-пиколин)трис(μ-напроксенато)трисребро(I) и дека лигандите на напроксен се сврзани со јоните на сребро на бидентатен начин на премостување. Резултатите со 2,3-бис-(2-метокси-4-нитро-5-сулфофенил)-2*H*-тетразолиум-5-карбосканилид (XTT) покажаа дека солите на сребро и чист напроксен имаат многу слаба цитотоксична активност ($IC_{50} > 250$ μM) спрема клетки на аденокарцином на дојка (MDA-MB-453), на аденокарцином на бели дробови (A-549) и на колоректален аденокарцином (HT-29), додека комплексот покажува цитотоксичност зависна од дозата во однос на наведените клетки. Највисока цитотоксичност е забележана врз клетки на MDA-MB-453 со вредност на IC_{50} од 11,73 μM. Покрај тоа, комплексот покажува повисока селективност во однос на клетките на рак споредено со клетките на фибробласт 3T3-L1. Ова истражување дава прелиминарни научни податоци за понатамошно разјаснување на дејството на антиканцероген механизам на комплексот.

1. INTRODUCTION

Cancer, a very important health problem has significant morbidity, treatment costs, and mortality for patients. In order to treat this disease, cisplatin and platinum-based drugs have been developed and greatly used as anticancer agents in chemotherapy [1,2]. However, the generation of serious side effects, toxicity for non-cancer cells, and high resistance limit their effective and successful applications in chemotherapy [3, 4]. Therefore, many research groups have focused on the synthesis and development of new metal complexes as anti-proliferative agents that are more effective and have fewer side effects for curing tumors [5–10]. Silver-based complexes, which are good anti-proliferative agents against many types of cancer cells, have been widely synthesized for examining their *in vitro* and *in vivo* anti-proliferative activities and comparing them with cisplatin and other platinum-based drugs [11–13].

Non-steroidal anti-inflammatory drugs (NSAIDs), which are commonly used as antipyretic, analgesic, and anti-inflammatory agents as well as for the treatment of neurodegenerative diseases (Alzheimer's) and asthma, possess seven main classes, including anthranilic acids, phenylalkanoic acids, salicylate derivates, oxicams, propanamide, sulphonamides, and furanones [14–19]. NSAIDs have also been stated to strengthen the synergistic effect of the efficiency of specific anticancer drugs, leading to the death of cancer cells via apoptosis or other mechanisms [20, 21]. Furthermore, literature studies revealed that metal-NSAID complexes have various unique biological properties, such as anti-proliferative, antimicrobial, antioxidant, and DNA-albumin binding features, compared to the free NSAIDs and exhibit a significant decrease in gastro-intestinal toxicity and various side effects [22–24].

Naproxen (Hnap), a prominent member of NSAIDs, has been mainly used in the treatment of headaches and fevers based on its analgesic and antipyretic features [25–28]. There are several papers in which silver-naproxen complexes are synthesized and their biological properties evaluated, showing better biological activities than naproxen [29–32]. The synthesis of novel silver(I)-naproxen complexes means designing of a new chemotherapeutic agent, and therefore, significant efforts should be made to synthesize the complexes and investigate their cytotoxic activity against both different types of cancer cell lines and normal cells.

In the present study, a novel silver(I) complex with the non-steroidal anti-inflammatory drug naproxen and nitrogen donor 3-picoline (3-pic) ligands was synthesized, and then its structural and thermal properties were investigated by Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance spectroscopy (^{1}H NMR), elemental, thermogravimetric analysis (TG), derivative thermogravimetric analysis (DTG), and differential thermal analysis (DTA) techniques. Afterwards, the cytotoxic activity of the complex together with the silver salts and naproxen alone was tested against the different types of cancer and normal cell lines.

2. EXPERIMENTAL

2.1. *Materials and methods*

AgNO$_3$, naproxen, 3-picoline, methanol, and acetonitrile were purchased from Sigma Aldrich chemicals and used without further purification. The chemical structures of naproxen and 3-picoline are shown in Fig. 1. The Fourier transform infrared spectrum of the complex was recorded on a Thermo Nicolet 6700 spectrophotometer in the region of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} using the KBr pellet technique. Elemental analysis was performed utilizing a LECO CHNS-932 apparatus. TG/DTG/DTA analyses runs were carried out on a PRIS Diamond TG/DTG apparatus under a dynamic air atmosphere (temperature range: 30–1000 ºC; heating rate: 10 °C min^{-1}). The ^{1}H NMR spectrum of the complex was obtained at room temperature on an Agilent-VNMRS-400 spectrometer operating at 400 MHz utilizing DMSO-d$_6$ as the solvent.
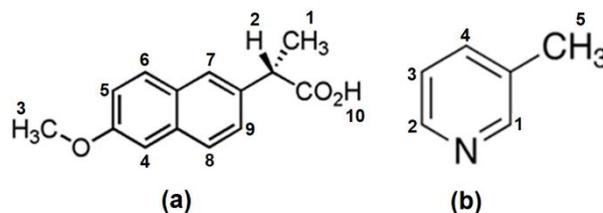
Fig. 1. Molecular structure of (a) naproxen and (b) 3-picoline

2.2. *Procedure for the synthesis of bis(3-picoline)tris(μ-naproxenato)trisilver(I)*

A methanol solution (10 ml) of naproxen (Hnap, 1 mmol) and KOH (1 mmol) was stirred for 1 h at 50 °C. The resulting clear solution and 3-picoline (3-pic, 1 mmol) were added drop by drop to a stirred solution of $AgNO_3$ (1 mmol) in water (10 ml). The addition of 10 ml of acetonitrile to the suspension resulted in a clear solution. The resulting clear solution was kept in the dark at room temperature. After one month, colourless microcrystals of bis(3-picoline)tris(μ-naproxenato)trisilver(I), $[Ag_3(μ-nap)_3(3-pic)_2]$, were acquired and kept for the analyses.

2.3. *Chemical and elemental analyses*

Colourless microcrystal product of $[Ag_3(μ-nap)_3(3-pic)_2]$ (80 %): Analytical data for $[C_{54}H_{56}N_2O_9Ag_3]$:(Found: C, 54.01 %; H, 4.66 %; N, 2.35 %; calcd: C, 54.02 %; H, 4.67 %; N, 2.33 %).

^{1}H NMR (400 MHz, DMSO-d_6) (δ/ppm): 1.45 (9H, d, H^1-nap), 2.28 (6H, s, H^5-3-pic), 3.74 (3H, q, H^2-nap), 3.85 (9H, s, H^3-nap), 7.10 (3H, dd, H^4-nap), 7.25 (3H, d, H^5-nap), 7.31 (2H, t, H^3-3-pic), 7.45 (3H, d, H^9-nap), 7.64 (2H, d, H^4-3-pic), 7.75–7.68 (9H, m, H^6-nap, H^7-nap and H^8-nap), 8.36 (2H, d, H^2-3-pic), 8.41 (2H, s, H^1-3-pic).

2.4. *Cell culture*

The human breast (MDA-MB-453), lung (A-549), colon (HT-29), and mouse healthy (3T3-L1) cell lines were obtained from the American Type Culture Collection (ATCC, USA). MDA-MB-453 cells were grown in the medium Leibovitz's L-15. A-549 cells were grown in F-12K medium. HT-29 cells were grown in McCoy's 5a medium. 3T3-L1 cells were grown in Eagle's Minimum Essential Medium. All mediums were supplemented with fetal bovine serum (10%). The studies were performed in a Class-II biosafety cabinet (Bilser, Turkey), and incubations were done in a CO_2 incubator (5%) at 37 °C (Nuve, Turkey).

2.5. *Cytotoxicity assay*

The cytotoxicity tests were performed with the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT) assay following the manufacturer's instructions (BI, Israil). Accordingly, 5×10^3 cells (for each cell line) were seeded in 96-well plates and incubated in a CO_2 incubator overnight. The next day, all the cancerous cells were treated with the different concentrations (1–100 μM) of the metal complex, ligand (naproxen), and silver alone, whereas healthy cells were treated with only the metal complex (1–250 μM). After incubation for 24 h, the absorbance was measured at 490 nm using an Elisa plate reader (BioTek, USA). Untreated cells were used as a negative control, and carboplatin was evaluated as a positive control. The cytotoxicity results were represented as half-maximal inhibitory concentration (IC_{50}) values, plotted from the dose-response versus percent cell cytotoxicity curve using Graph Pad Prism 6. The Selectivity Index (SI) was determined as the ratio of the IC_{50} value of the complex on the normal cell line to the IC_{50} value on the cancer cell line. The percent cytotoxicity was calculated using the following formula: % Cell cytotoxicity = [1 - Abs(drug)/Abs(control)] × 100.

2.6. *Statistical analysis*

Statistical analyses were carried out by one way analysis of variance (ANOVA) with unpaired *t*-test using the statistical program GraphPad Prism 6 (GraphPad, La Jolla, CA) Software 7.0. All results were represented with their standard error of the mean (SEM) values. Statistical differences between two groups were calculated using Student's *t*-test. $p < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1. *Thermal analyses*

The TG/DTG/DTA curves for the $[Ag_3(μ-nap)_3(3-pic)_2]$ complex demonstrate two weight loss steps (Fig. 2). The first step of extrication occurs within the range of 30–216 °C with the maxima at 95(+) °C, 146(+) °C and corresponds to the loss of two 3-pic molecules with an estimated mass loss of 15.27 % (calc. mass loss of 15.53 %). The DTG curve gives the peaks at 119 °C. The second step can be attributed to the loss of three nap molecules within the range of 216–502 °C, with the maxima at 217(+), 273(−), 231(+), and 414(−) °C and an estimated mass loss of 59.27 % (calc. mass loss of 57.47 %). The DTG curve gives the peaks at 204, 231, 259, and 414 °C. At the end of the thermogram, the metallic silver is the residue, which is in good agreement with the calculated metallic silver (estimated mass loss of 25.46 %; calc. mass loss of 27.00 %).
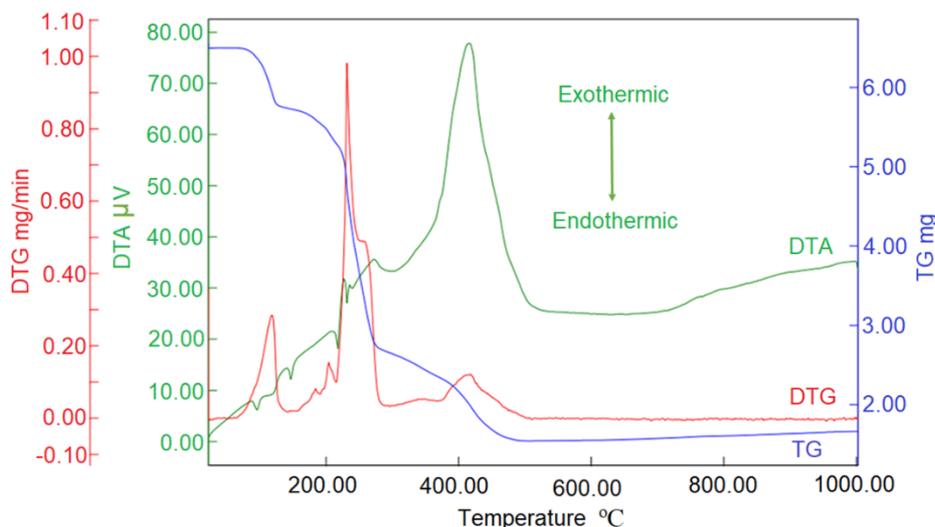
Fig. 2. The thermogravimetric analysis (TG), derivative thermogravimetric analysis (DTG),
and differential thermal analysis (DTA) curves of [Ag$_3$(μ-nap)$_3$(3-pic)$_2$]

3.2. *Fourier transform infrared analyses*

The FTIR spectrum of [Ag$_3$(μ-nap)$_3$(3-pic)$_2$] is shown in Figure 3. The broad functional group region of the FT-IR spectrum of naproxen is due to the carboxylic v(O-H) stretching vibration band at 3250 cm^{-1}, whereas this width disappeared from the functional group region of [Ag$_3$(μ-nap)$_3$(3-pic)$_2$] because of the deprotonation of naproxen. This event indicates that the coordination takes places through the carboxylic group in the complex. The carboxylic v(COOH) stretching vibration observed in naproxen at 1728 cm^{-1} turns into asymmetric and symmetric stretching vibrations with a complex formation at 1537 cm^{-1} and 1378 cm^{-1}, respectively. By making use of the difference between asymmetric (COO$^-$) and symmetric (COO$^-$) stretching vibrations, comments can be made about the bonding of the carboxylic group to the silver(I) ion [33]. The Δv value was determined to be 159 cm^{-1}; this value is consistent with the bridging bidentate mode. The aromatic and aliphatic v(CH) stretching band of naproxen and 3-pic arises between 3062 and 2936 cm^{-1}. The υ(C=C) and υ(C=N) stretching vibrations of rings arises at 1602, 1575, 1479, and 1461 cm^{-1}. The medium intensity band at 1210 cm^{-1} may be appointed to the υ(C-O-C) asymmetric stretching, while the weak bands at around 1029 cm^{-1} are assigned to the υ(C-O-C) symmetric stretching of the naproxen ligand.
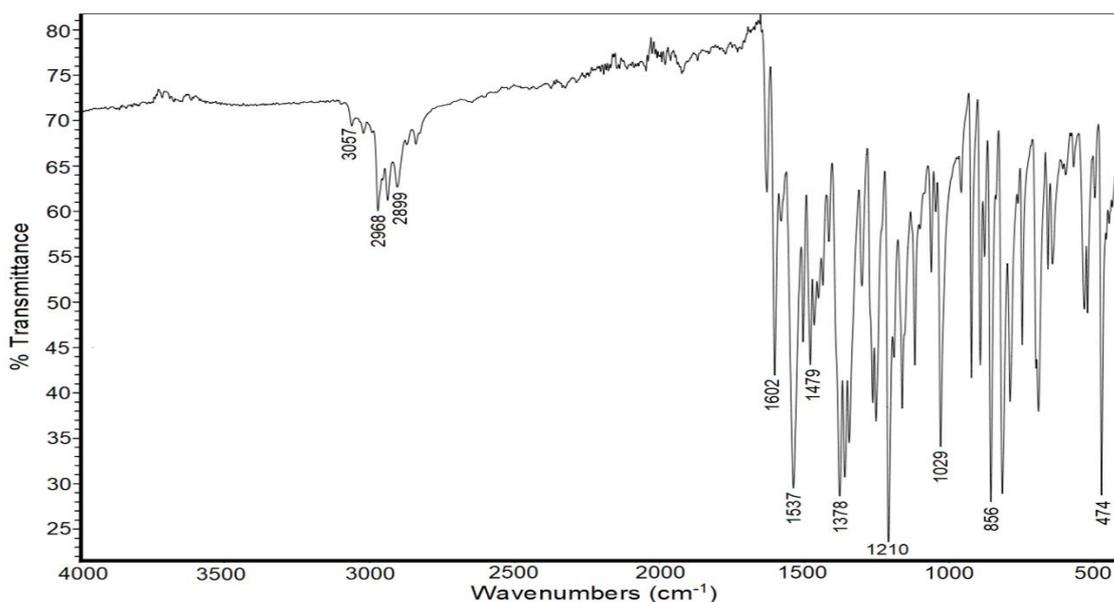
Fig. 3. The Fourier transform infrared spectrum of [Ag$_3$(μ-nap)$_3$(3-pic)$_2$]

3.3. *Proton nuclear magnetic resonance spectroscopy studies*

The ^{1}H NMR spectrum of the silver(I) complex was obtained using DMSO-d$_6$ as a solvent (Fig. 4). The appearance of the ^{1}H NMR peaks belonging to naproxen and 3-picoline in the synthesized silver(I) complex also confirms the formation of the structure. The ^{1}H NMR spectrum of the [Ag$_3$(μ-nap)$_3$(3-pic)$_2$] complex demonstrates singlet signals at 1.45 and 2.28 ppm, which are attributed to the proton of the methyl group of naproxen and 3-picoline, respectively. The methoxy (-OCH$_3$) proton peak of naproxen appears as a singlet at 3.85 ppm. The ^{1}H NMR spectrum of the [Ag$_3$(μ-nap)$_3$(3-pic)$_2$] complex showed all aromatic protons as multiple peaks in the region from 7.10 to 8.41 ppm arising from naproxen and 3-picoline. The spectrum of naproxen demonstrated the signal of the carboxylic –OH proton at 11.00 ppm; this peak vanished in the spectrum of [Ag$_3$(μ-nap)$_3$(3-pic)$_2$], displaying the coordination of naproxen to the silver(I) ion via the carboxylic group. The extinction and slightly shifted downfield or upfield of these proton peaks show the coordination of the ligands to the silver(I) ions. From the number of protons that emerged in the ^{1}H NMR spectrum of [Ag$_3$(μ-nap)$_3$(3-pic)$_2$], we can deduce that the ratio of nap:3-pic is 3:2. The ^{1}H NMR data clearly demonstrate both the purity and presence of the structure.
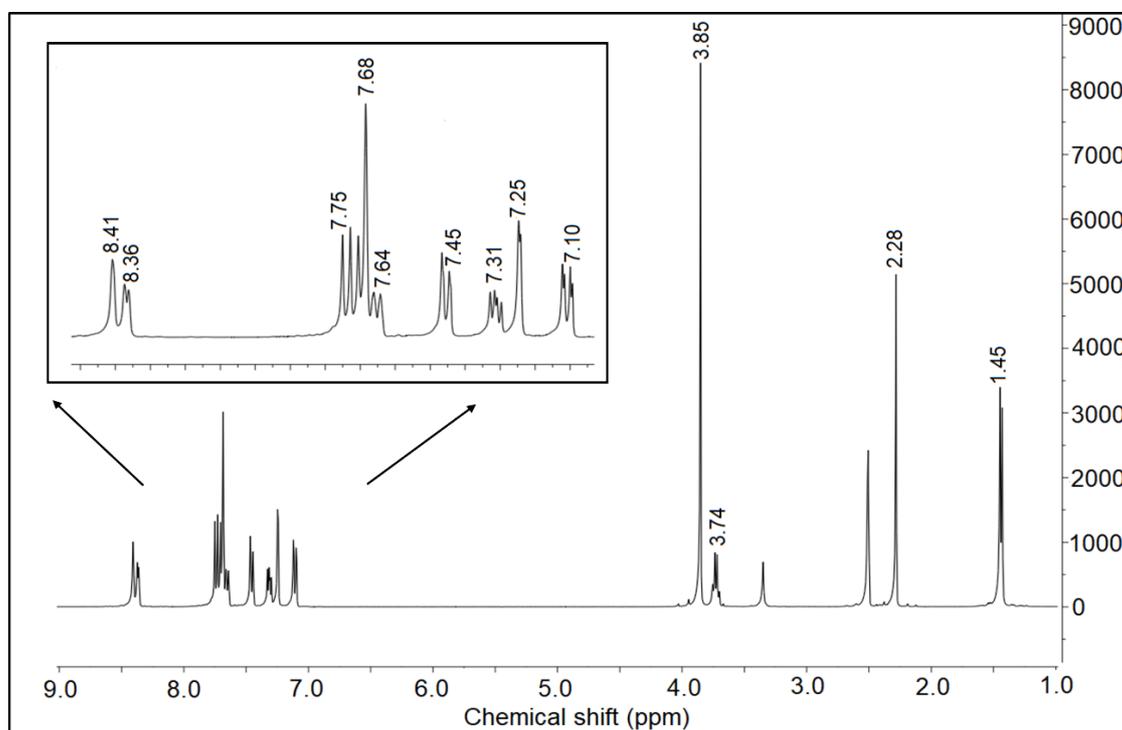
Fig. 4. Proton nuclear magnetic resonance spectrum of [Ag$_3$(μ-nap)$_3$(3-pic)$_2$]

3.4. *Proposed structure*

Based on the thermal and elemental analyses data, [Ag$_3$(μ-nap)$_3$(3-pic)$_2$] is a neutral trinuclear complex. According to the FTIR spectrum data, the naproxenato ligands are deprotonated and anticipated to be bound to silver ions in a bridging bidentate mode through carboxylate oxygen atoms. 3-picoline ligands are bound to silver ions via a nitrogen atom. Additionally, based on the ^{1}H NMR data, [Ag$_3$(μ-nap)$_3$(3-pic)$_2$] complex has a 3:2 (nap:3-pic) stoichiometric ratio around the silver(I) ions.

3.5. *Cytotoxicity*

In the present study, the cytotoxicity of the novel Ag(I) complex with naproxen belonging to NSAIDs was tested against the carcinogenic (MDA-MB-453, A-549, and HT-29) and non-carcinogenic (3T3-L1) cell lines by the XTT assay. In addition, the toxic effects of the silver metals and naproxen alone were also tested on the aforementioned cell lines. In comparison to the non-drug-treated control, the 24-hr treatment of the cancer and non-cancerous cells with the novel Ag(I) complex ranging from 5 to 100 μM resulted

in a significant dose-dependent cytotoxicity in all the cell lines tested (Fig. 5A-D). In addition, the IC_{50} values of the Ag(I) complex together with the naproxen and silver metal alone were calculated, as described in the materials and methods section, and given in Table 1. Considering Table 1, the order of cytotoxic activities of the Ag(I) complex on the cancer and non-cancerous cells from the highest to lowest is as follows; MDA-MB-453 ($IC_{50} = 11.73$ μM) > A-549 ($IC_{50} = 40.38$ μM) > HT-29 ($IC_{50} = 69.33$ μM) > 3T3-L1 (133.36 μM). These results indicate that of the cancer cells tested, MDA-MB-453 cells were the most sensitive to the cytotoxic activity of the Ag(I) complex, whereas HT-29 cells were the most resistant. Moreover, these data reveal the fact that the complex produced lower toxicity on the healthy cell line compared to the cancerous cell lines. As seen in Table 1 and Fig. 5, compared to the chemotherapeutic drug carboplatin commonly used in cancer treatment, the complex exhibited stronger cytotoxicity than this drug against MDA-MB-453 cells (***$p < 0.005$), similar activity on A-549 cells ($p > 0.05$), and moderately lower activity on HT-29 cells (**$p < 0.01$).
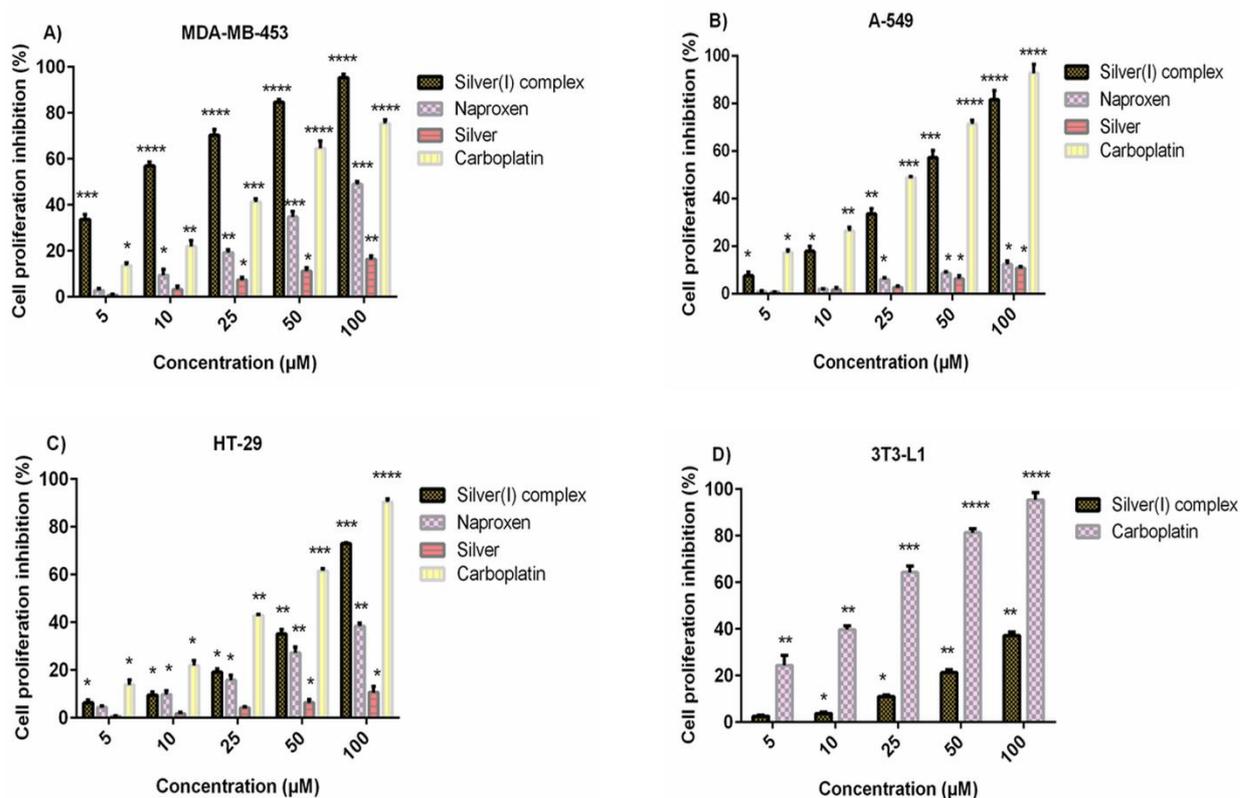
Fig. 5. Dose dependent cytotoxic activities of the silver(I) complex, silver, naproxen, and carboplatin against human breast adenocarcinoma (MDA-MB-453) cells (**a**), lung adenocarcinoma (A-549) cells (**b**), colorectal adenocarcinoma (HT-29) cells (**c**), and non-carcinogenic mouse fibroblast (3T3-L1) cells (**d**). The differences in the data between the control (non-treated) and sample-treated cells were evaluated by Student's *t*-test. *$p < 0.05$; ** $p < 0.01$; ***$p < 0.005$; ****$p < 0.0001$.

One of the most crucial points of this study was the comparison of the selectivity of the Ag(I) complex and carboplatin tested between the cancer and healthy cell lines. As seen in Table 1, the selectivity of the Ag(I) complex on cancer cell lines in order from the highest to lowest is as follows: MDA-MB-453 (SI = 11.36) > A-549 (SI = 3.30) > HT-29 (SI = 1.92). These data suggest that the Ag(I) complex exhibits stronger cytotoxicity with higher selectivity on cancer cells compared to healthy cells. Moreover, the Ag(I) complex exhibits higher selectivity on all 3 cancer cell lines compared to carboplatin, with SI values of 0.47, 0.63, and 0.53 on MDA-MB-453, A-549, and HT-29 cell lines, respectively.

When the effects of the silver in the structure of the complex and naproxen, one of the NSAID groups, are examined, it is seen that silver metals have very low cytotoxic activity on all the cancer cell lines (Fig. 5). It was also determined that the IC_{50} values of the metals alone on the tested cell lines were above 250 μM. On the other hand, naproxen showed moderate cytotoxicity in a dose-dependent manner on the cancer and healthy

cell lines when tested alone (Fig. 5A-D). As seen in Table 1, naproxen exhibited a moderately low level of cytotoxicity on MDA-MB-453 (IC_{50} = 96.22 μM) and HT-29 (IC_{50} = 123.24 μM)

cell lines compared to the Ag(I) complex, while a very low level of cytotoxic activity on A-549 (IC_{50} = 250 μM) and 3T3-L1 (IC_{50} = 250 μM) cell lines was observed.

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The IC_{50} values of the Ag(I) complex, naproxen, silver, and carboplatin against both cancer and normal cell lines

Test samples	Cell lines IC_{50} (μM)[x]			
	MDA-MB-453	A-549	HT-29	3T3-L1
[Ag$_3$(μ-nap)$_3$(3-pic)$_2$] SI(complex) [y]	11.73 ± 0.65 [a] 11.36	40.38 ± 1.25 [a] 3.30	69.33 ± 2.34 [b] 1.92	133.36 ± 2.10 [b]
[Naproxen]	96.22 ± 2.56 [c]	250> [b]	123.24 ± 2.46 [c]	250> [c]
[Silver]	250> [d]	250> [b]	250> [d]	250> [c]
[Carboplatin] SI (carb) [y]	48.47 ± 1.35 [b] 0.47	36.2 ± 1.06 [a] 0.63	42.79 ± 2.12 [a] 0.53	22.87 ± 1.20 [a]

[x]IC_{50}(μM): Different superscripts (a-d) within the same column show the statistical differences.

[y](SI): Selectivity index for the Ag(I) complex and carboplatin.

Toxicity tests have been widely performed as a mandatory step to introduce a new chemical drug to the pharmaceutical industry [34–37]. Many researchers have synthesized numerous novel metal complexes with different metals and ligands belonging to NSAIDs and tested their cytotoxicity against different types of cancer cell lines. For instance, the cytotoxic activities of different metal complexes with mefenamic acid ([Mn(mef)$_2$(H$_2$O)$_2$], [Co(mef)$_2$(H$_2$O)$_2$], [Ni(mef)$_2$(H$_2$O)$_2$], [Cu(mef)$_2$(H$_2$O)]$_2$, [Cd(mef)$_2$(MeOH)], [Bi(mef)$_3$], [Zn(mef)$_2$], and [Cd$_2$(dikl)$_4$1.5(MeOH)$_2$(H$_2$O)]$_n$ were reported against human breast adenocarcinoma (MCF7), lung adenocarcinoma (A-549), and mouse fibroblast (L929) cell lines, and it was determined that their IC_{50} values ranged from 2.5 to 45 μM depending on the type of metal [38, 39]. Giovagnini et al. tested the cytotoxicity of Pd(II) and Pt(II) complexes with picoline derivatives on human squamous cervical adenocarcinoma (HeLa), colorectal adenocarcinoma (LoVo), leukemic promyelocytes (HL60), and human Burkitt lymphoma (Daudi) and determined that their IC_{50} values ranged from 9 to 100 μM based on the cell line [40]. Moreover, in our previous studies, we tested and compared the cytotoxic activities of various Ag(I), Co(II), and Ni(II) complexes with different NSAIDs group, including mefenamic acid, niflumic acid, tolfenamic acid, and diclofenac together with picoline derivatives against different cancer cell lines (MCF-7, MDA-MB-453, HT-29 and HepG2) as well as a healthy cell line (3T3-L1),

showing that their IC_{50} values varied between approximately 20 and 100 μM [41–45]. Furthermore, the IC_{50} values of heteroleptic silver(I) complexes with naproxen on human breast adenocarcinoma (MCF-7), cervical (HeLa), epithelioma (Hep-2), and hepatoma (HepG2) cancerous cells were reported to have different values varying from 8.21 to 9.26 μM [46]. In another study, the IC_{50} values of heteroleptic silver(I) complexes with naproxen against human breast cancer cell lines (MCF-7 and MDA-MB-231) and pancreatic (PANC-1) cell lines were stated to change between the range of 73.43 to 107.09 μM depending on the cell line [47]. When these results are compared with the literature and our previous studies, we can state that the cytotoxic activity of this [Ag$_3$(μ-nap)$_3$(3-pic)$_2$] complex varies considerably depending on the cell line tested. This complex especially exhibits moderate to high cytotoxicity and better selectivity on MDA-MB-453 cells.

Undoubtedly, the cytotoxicity in different cancer cell lines is influenced by the type of NSAIDs in the tested complex. Chang et al. reported the cytotoxic potency of different NSAIDs (indomethacin, diclofenac, ketorolac, and piroxicam) alone against osteoblasts, and the results demonstrated that these NSAIDs at 100 μM cause cytotoxicity on the cells ranging from 30 % to 70 % [48]. Erfani-Moghadam et al. reported the cytotoxic activity of naproxen alone against four different monolayer cell lines (MDA-MDB-231, MCF-7, A549, and HeLa); the IC_{50} values on A549 and

Hela cells are 3300 and 1950 μM, respectively, whereas it is above 4000 μM on MCF-7 and MDA-MB-231 cells [49]. Considering the cytotoxic effects of silver salts alone, the data we obtained in this study are similar to the literature. Ramar *et al*. tested the cytotoxic power of silver nanoparticles against MCF-7 cells and reported the IC_{50} value to be 463 μM [50]. It has been reported that the Ag_2CO_3 compound does not exhibit cytotoxic activity up to 0.5 mg/l on HepG2 cells [51]. Moreover, the IC_{50} value of $AgNO_3$ against HeLa cells was reported to be 158 μM [37]. These results show that soluble silver salts like $AgNO_3$, Ag_2CO_3, as well as silver nanoparticles possess a quite low cytotoxic activity against cancer and healthy cell lines. The data that we have obtained in this study together with the literature show that NSAIDs containing naproxen alone show a very low cytotoxic effect on cancer cell lines. On the other hand, as seen in the present and previous studies, NSAIDs in their complex form exhibit much stronger cytotoxicity with a higher synergistic effect.

4. CONCLUSION

A novel trinuclear Ag(I) complex including the naproxen and 3-picoline ligands was synthesized and characterized. The molecular formula of the complex was determined by ^{1}H NMR, elemental and thermal analysis techniques. The results from all techniques proved the existence of three naproxen ligands that are bound to a silver ion. The Δv value derived from FTIR spectroscopy indicated a bridging bidentate coordination mode of the naproxen ligand. Two major mass loss stages occurred in the decomposition of the complex to metallic silver. As a result of the XTT tests, the newly synthesized Ag(I) complex showed the highest cytotoxic activity against MDA-MB-453 cells among the tested cancer cell lines, while the lowest activity was shown on HT-29 cells. Moreover, the complex exhibited a high selectivity between the healthy and cancer cell lines. Furthermore, despite the drug and silver salts alone that make up the complex exhibiting a very low cytotoxic activity on the cancer cells, the complex possessed a quite robust cytotoxicity with a synergistic effect. These data suggest that the novel silver(I) complex with naproxen could be a unique candidate for future *in vivo* anticancer application studies.

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