Technologies and Materials for Renewable Energy, Environment & Sustainability

Synthesis, Identification, and Cytotoxic Assay of Some Complexes with Naproxen

AIPCP25-CF-TMREES2025-00060 | Article

Synthesis, Identification, and Cytotoxic Assay of Some Complexes with Naproxen

Zinah N. Mahmood[1, a)]

[1]*Ministry of Education, Baghdad, Iraq.*

[a)] *Corresponding author: zinahnazeh@gmail.com*

Abstract. The naproxen ligand was employed to build several complexes via its interaction with metal ions (Co(II) and Zn(II)). The structures of these novel compounds were examined in both solid and solution phases utilizing several spectroscopic techniques, including UV-Vis, FTIR, and elemental studies, all performed at room temperature. The naproxen complexes coordinate with metal ions through the carbonyl oxygen of their carboxyl groups. The chemical formulae of the produced complexes reveal that the CoL complex has octahedral geometry, whereas the ZnL complex indicates tetrahedral geometry. The cytotoxicity and viability of MDA231 cell lines results demonstrated substantial inhibition rates for all ligands and their metal complexes at different dosages, in comparison to cisplatin, which acted as the positive control.

Keywords: Spectroscopic, Bidentate, Complexes, Schiff bases, and Transition metal.

INTRODUCTION

Naproxen is a nonsteroidal anti-inflammatory analgesic commonly used in the clinical management of arthritis and for treating both chronic and acute pain conditions, including osteoarthritis [1-3]. However, a significant occurrence of gastrointestinal ulcers limits its efficacy. When taken orally, naproxen crystallizes and adheres to the digestive mucus due to its acidic properties and limited solubility. The gradual breakdown process may cause damage to the gastric walls [4]. Compounds containing naproxen amide demonstrate reliable anti-inflammatory effects [5]. Additionally, amide derivatives of naproxen, such as naproxen glycolamide, have shown anti-inflammatory properties with significantly reduced gastrointestinal injury compared to the parent compound [6]. The interaction of a medication with a metal element can either enhance or diminish its efficacy; in some instances, the resulting complex may exhibit effects that are not present in the original substance [7]. The use of metal complexes alongside conventional pharmaceuticals as therapeutic agents for various disorders has been extensively studied in the field of therapeutics [8]. The carboxylate functional group contains four lone pairs of electrons on its two oxygen atoms, which are available for metal-ligand chelation. This chelation enhances the inhibitory efficacy for specific substrates [9]. Additionally, the transition metal complexes formed from these pharmaceuticals exhibit greater efficacy compared to their individual ligands [10]. The distinct modes of action of metal complexes in drug development provide an alternative strategy for innovative drug delivery methods [5]. The interaction between a medication and a metalloelement can either enhance or diminish its efficacy; in some instances, the resulting complex may display activity that the original substance lacks [9]. This research intends to create transition metal complexes of Naproxen Fig. (1), investigate the synthesis and characteristics of Co(II) and Zn(II), and determine their geometric structures using various physical methods.

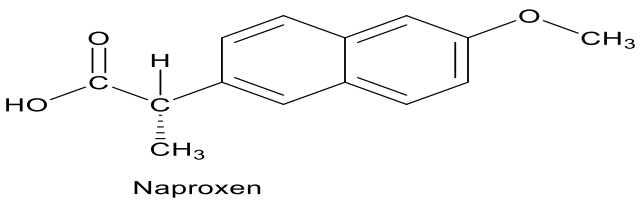

FIGURE 1. The Illustrating of Naproxen

MATERIAL AND METHODS

Naproxen, analytical-grade metal chlorides, and Solvents were obtained from Merck (Schnelldorf, Germany). All chemicals included in this investigation exhibit the highest analytical purity. The products were analyzed using CHNS elemental analysis, ultraviolet-visible (UV-Vis) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and melting point assessment. CHNS microanalytical data were obtained using the Euro EA3000 and AA-680 Shimadzu Atomic Absorption Spectroscopy instruments. The FTIR spectra of the synthesized compounds were obtained using a CsI disc with Shimadzu and Perkin Elmer FTIR spectrometers. The electronic spectra of the ligands and their complexes in solution were obtained using a UV-Vis 1800 PC Shimadzu Spectrophotometer.

SYNTHESIS OF SODIUM SALT OF NAPROXEN

0.82 g (1 mmol) of naproxen (ligand) was dissolved in anhydrous ethanol while continuously stirring until the solid was completely dissolved. An incremental addition of 0.183 g (1 mmol) of $NaHCO_3$, which was dissolved in deionized water, was made to the solution. The reaction mixture was stirred at ambient temperature for one hour, then placed in a water bath to evaporate until a crystalline layer formed. Upon cooling, a white precipitate appeared[8].

SYNTHESIS OF METAL COMPLEXES

A solution of sodium salt of naproxen (1 mmol) was prepared in ethanol in a round-bottom flask and stirred for 15 minutes. Equimolar amounts of metal chlorides, $CoCl_2 \cdot 2H_2O$ (0.237 g) and $ZnCl_2$ (0.137 g) (1.0 mmol each), were then added to 10 mL of ethanol. The mixture underwent reflux with continuous agitation for three hours [11]. The complexes that resulted were isolated from the solution, washed with ethanol, and dried in a desiccator.

$$Nap + NaHCO_3 \rightarrow NaNap + H_2O + CO_2 \quad (1)$$

$$NaNap + MCl_x . nH_2O \rightarrow (Nap)Cl_x . nH_2O + NaCl \quad (2)$$

CYTOTOXIC ASSAY

A colorimetric method was employed to evaluate the cytotoxicity of the compounds via the 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTT) assay [12,13]. The MDA-231 cell lines utilized in this work were acquired from the Biotechnology Center at Al-Nahrain University. The cells were cultured in Minimum Essential Medium (MEM) that was supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, and 100 mg/ml of streptomycin. This was done in a humidified incubator set at 37°C with 5% CO_2. The cells were relocated to 96-well microtiter plates. After exposure to multiple concentrations of the compounds and a positive control (cis-Pt) at doses of 400, 200, 100, 50, and 25 µg/ml for 48 hours, the cells were rinsed once with phosphate-buffered saline (PBS). Subsequently, 100 µl of serum-free media containing 5 mg/ml of MTT was added to each well. The absorbance of the resulting mixture was measured at a wavelength of 620 nm. The Statistical Analysis System (SAS, 2012) Version 9.1 was utilized to assess the impact of varying dosages on the inhibition rate. The Least Significant Difference (LSD) test, originating from One-Way Analysis of Variance (ANOVA), was employed to discern significant differences among means.

RESULT AND DISCUSSION

The resulting compounds were stable, colorful powders that maintained their properties for an extended period in the open atmosphere. Table (1) summarizes the analytical data for the ligands and their complexes, as well as some physical characteristics.

TABLE 1. Physical Properties for Ligand and Metal Complexes

Comp.	Color	M.Wt g/mol	m.p^{0}C	Yield %	Molar ratio (M:L)	Analysis (%) calculated (found)			Compound
						C%	H%	M%	
L	White	230.263	153-155	---	---	66.98	5.18	-----	$C_{14}H_{13}O_3$

						(66.70)	(5.06)		
CoL	Light red	553.450	245-247	66.8	1:2	51.60 (52.65)	3.66 (3.82)	16.33 (16.06)	$C_{24}H_{30}O_8Co$
ZnL	White	559.900	226-228	77.6	1:2	51.03 (51.2)	4.34 (4.79)	19.77 (19.85)	$C_{24}H_{30}O_8Zn$

FT-IR SPECTRA

The type of functional group attached to the metal ion can be inferred from the FTIR spectra. When compared to literature values, the distinctive frequencies of free ligands and their metal complexes have been readily assigned [9]. The infrared bands of L exhibited sharp and medium distinctive absorption bands at 1545 and 1409 cm^{-1}, representing the asymmetric and symmetric COO groups' respective stretching. The frequency change, $\Delta\boldsymbol{\nu}$, was measured at 136 cm^{-1}. When discussing the structure of the produced compounds, the infrared spectra of the complexes revealing $\Delta\boldsymbol{\nu}$(COO-) are particularly informative. The difference Δν (COO-) indicates bidentate coordination, with the Δν value being lower than that of the ionic compound [14], as noted in Table (2). The νasym (COO-) stretching appeared in the range of 1520 to 1525 cm^{-1}, while the vibrations in the range of 1389 to 1399 cm^{-1} correspond to νsym (COO-). Additional indications of weak or new medium bands that appeared in the produced complexes were attributed to the ν (M–O) in the range of 470 to 439 cm^{-1}. The presence of ethanol or water molecules in the vicinity, whether coordinated or not, is suggested by a broad peak that arises in the range of 3633 to 3625 cm^{-1} [13–15]. Additional bands are listed in Table (2).

TABLE 2. FTIR Absorption for Ligands and Metal Complexes

Formal	ν OH	νCOO^- (asym.)	νCOO^- (sym.)	ν C-H
L	3648	1545	1409	3177
CoL	3633	1520	1389	3156
NiL	3625	1525	1399	3180

MAGNETIC MOMENTS, ELECTRONIC SPECTRAL DATA AND CONDUCTIVITY MEASUREMENTS

The electronic spectrum of the free ligand (L) displayed two primary absorption bands. The first band, arising from an interligand transition (π→π*) within the pi-system, was observed at 253 nm. The second band, associated with the (n→π*) electronic transition, occurred at 312 nm.

CoL: The electronic spectra of the light red Co(II) complex exhibit bands at 10256, 23777, and 18654 cm^{-1}. These bands are attributed to the transitions 4T1g→4T2g, 4T1g→4A2g(F), and 4T1g→4T1g, respectively [12]. This complex is characterized by an octahedral geometry [16].

The UV-Vis spectrum of the white Zn(II) complex, ZnL, indicates that it has a d10 electronic configuration [17, 18] and does not show any d-d transitions. The observed bands are attributed to internal ligand charge transfer [13, 14]. Conductivity studies of these synthesized compounds in DMF solvent at room temperature revealed that they are non-ionic. Based on spectroscopy methods and data analysis, a tetrahedral structure can be proposed for these compounds [19].

CYTOTOXIC ASSAY

Breast cancer has emerged as a significant healthcare concern in recent decades due to its high death rate, substantial treatment costs, and widespread prevalence. Treatment responses remain inadequate, despite the addition of numerous supplementary medications. Therefore, the development of more effective anticancer medications is essential. The primary goal of most research teams is to find an effective anticancer medication suitable for treating human cancers [20].

In this study, the MTT test was utilized to evaluate the cytotoxicity and viability of MDA231 cell lines after 48 hours of exposure to different doses of chemicals (400, 200, 100, 50, and 25 µg/ml) (Table 3). The MTT assay results indicated significant inhibition rates for all ligands and their metal complexes at various dosages when compared to cisplatin, the positive control. A statistical analysis comparing the three ligands to the positive control (cisplatin)

revealed a significant impact. To create dose-response curves, we employed linear regression analysis with a 95% confidence interval [21,22]. These curves demonstrated that inhibition rates decreased as concentrations diminished from high to low, with significant differences ($P<0.05$) observed across all concentrations when compared to the ligand and positive control related to cisplatin.

The mechanism of action for the generated complexes remains unclear. The results of this investigation suggest that several factors, including metal size, charge distribution, geometric arrangement, bond type, polarity, metal oxidation state, and the arrangement of organic compound substitutions, may influence the pharmacological activity of these complexes.

TABLE 3. Effect of Concentration in Inhibition Rate

Conc.	Cis pt	L	CoL	ZnL	LSD value
400	73.78 ± 5.78 a A	60.00 ± 2.85 a B	79.99 ± 4.52 a A	75.66 ± 5.16 a A	12.08 *
200	61.91 ± 11.07 ab A	56.00 ± 3.36 ab A	67.25 ± 4.70 b A	64.01 ± 3.52 b A	9.461 NS
100	51.00 ± 9.03 bc A	50.78 ± 4.76 ab A	46.99 ± 2.69 c A	47.99 ± 5.47 c A	8.397 NS
50	44.09 ± 8.44 cd A	47.90 ± 7.92 b A	30.47 ± 4.75 d B	38.33 ± 4.03 d B	8.941 *
25	35.00 ± 8.57 d AB	36.13 ± 5.84 c A	23.87 ± 2.73 d C	25.98 ± 3.06 e BC	8.026 *
LSD value	15.913 *	9.595 *	7.275 *	7.922 *	---

Means having with the different small letters in same column and big letters in row differed significantly , * ($P<0.05$).

CONCLUSION

We created and characterized Nap complexes. The non-electrolytic nature of these complexes was demonstrated by their molar conductance in DMF solvent. The nap employs the carboxylate group to function as a bidentate ligand. Predicted geometries include octahedral for the Co (II) complex, tetrahedral for the Zn (II) complex, and a monomer structure for all complexes. Furthermore, The complexes demonstrated high selectivity between malignant and healthy cell lines. The complexes also displayed significant cytotoxicity with a synergistic effect, despite both the drug and the complexes showing cytotoxic action on cancer cells. These findings suggest that the naproxen-containing complexes may represent a promising candidate for future in vivo anticancer application research.

REFERENCES

1. M. S. Hasan, R. Kayesh, F. Begum, and S. M. Rahman, "Transition metal complexes of naproxen: synthesis, characterization, forced degradation studies, and analytical method verification," J. Anal. Methods Chem. 2016, 3560695 (2016). https://doi.org/10.1155/2016/3560695
2. Z. Mirzaei-Kalar, A. A. Khandar, J. M. White, H. Abolhasani, T. K. Movahhed, S. P. Best, and A. Jouyban, "Investigation of biological activity of nickel (II) complex with naproxen and 1,10-phenanthroline ligands," J. Biomol. Struct. Dyn. 39, 6939 (2021). https://doi.org/10.1080/07391102.2021.1897189
3. K. Xie, X. Y. Lu, H. Zhu, L. Y. Zhu, R. T. Li, and R. R. Ye, "Iridium (III) complexes conjugated with naproxen exhibit potent anti-tumor activities by inducing mitochondrial damage, modulating inflammation, and enhancing immunity," Dalton Trans. 53, 8772–8780 (2024). https://doi.org/10.1039/D4DT01073K
4. J. Sharma, A. K. Singla, and S. Dhawan, "Zinc–naproxen complex: synthesis, physicochemical and biological evaluation," Int. J. Pharm. 260, 217–227 (2003). https://doi.org/10.1016/S0378-5173(03)00251-5
5. N. Nedeljković, et al., "Synthesis and investigation of anti-inflammatory activity of new thiourea derivatives of naproxen," Pharmaceutics 16, 666 (2023). https://doi.org/10.3390/ph16050666
6. H. Y. Khan, S. Parveen, I. Yousuf, S. Tabassum, and F. Arjmand, "Metal complexes of NSAIDs as potent anti-tumor chemotherapeutics," Coord. Chem. Rev. 453, 214316 (2022). https://doi.org/10.1016/j.ccr.2021.214316
7. R. Sohail, et al., "Effects of non-steroidal anti-inflammatory drugs (NSAIDs) and gastroprotective NSAIDs on the gastrointestinal tract: a narrative review," Cureus 15, e37080 (2023). https://doi.org/10.7759/cureus.37080
8. M. S. Hasan and N. Das, "A detailed in vitro study of naproxen metal complexes in quest of new therapeutic possibilities," Alexandria J. Med. 53, 157–165 (2017). https://doi.org/10.1016/j.ajme.2016.06.003
9. C. N. Banti, A. A. Piperoudi, C. P. Raptopoulou, V. Psycharis, C. M. Athanassopoulos, and S. K. Hadjikakou, "Mitochondriotropic agents conjugated with NSAIDs through metal ions against breast cancer cells," J. Inorg. Biochem. 250, 112420 (2024). https://doi.org/10.1016/j.jinorgbio.2024.112420

10. S. M. El-Megharbel, R. Z. Hamza, and M. S. Refat, "Synthesis, chemical identification, antioxidant capacities and immunological evaluation studies of a novel silver(I) carbocysteine complex," Chem. Biol. Interact. 220, 169–180 (2014). https://doi.org/10.1016/j.cbi.2014.06.024
11. S. Yaqub, I.-U. Haq, S. Ali, B. Mirza, F. Ahmed, and S. Shahzadi, "Interaction of Naproxen with transition metals," J. Coord. Chem. 62, 3463–3470 (2009). https://doi.org/10.1080/00958970903100408
12. P. Kumari, N. Agrawal, and S. Mujwar, "Pyrazole paradigms: unveiling synthetic pathways and unraveling anti-cancer potential," Med. Chem. 20, 799–817 (2024). https://doi.org/10.2174/1573406419666231231102444
13. S. K. Rastogi, V. C. Ciliberto, M. Z. Trevino, B. A. Campbell, and W. J. Brittain, "Green approach toward triazole forming reactions for developing anticancer drugs," Curr. Org. Synth. 21, 380–420 (2024). https://doi.org/10.2174/1570179419666230517142040
14. K. Nakamoto, Infrared and Raman Spectra of Inorganic and Coordination Compounds, 6th ed. (Wiley, New York, 2009). https://doi.org/10.1002/9780470405840
15. Q.-Q. Xiao, D. Liu, Y.-L. Wei, and G.-H. Cui, "Two new ternary Mn(II) coordination polymers," J. Solid State Chem. 273, 67–74 (2019). https://doi.org/10.1016/j.jssc.2019.02.027
16. G. B. Deacon and R. J. Phillips, "Relationships between carboxylato complexes and IR stretching frequencies," Coord. Chem. Rev. 33, 227–250 (1980). https://doi.org/10.1016/S0010-8545(00)80455-5
17. A. Abebe, et al., "Mono and binuclear cobalt(II) mixed ligand complexes," Future J. Pharm. Sci. 6, 1–9 (2020). https://doi.org/10.1186/s43094-020-00030-4
18. F. S. Jaafar and M. F. Alias, "Chemistry of metalloguanines: an overview of their synthesis routes and their implementations for the period 2000–2024," Karbala Int. J. Mod. Sci. 11, 12 (2025). https://doi.org/10.33640/2405-609X.3392
19. Z. N. Mahmood, M. Alias, E. Yousif, S. Baqer, M. Kadhom, D. Ahmed, A. Ahmed, A. Husain, M. Yusop, and A. Jawad, "Organo-metallic palladium complexes used for CO_2 storage and environmental remediation," Pollution 9, 693–701 (2023). https://doi.org/10.22059/POLL.2022.348855.1632I
20. J. H. Abdulameer and M. F. Alias, "Heavy metal complexes of 1,2,3-triazole derivative: synthesis, characterization, and cytotoxicity appraisal against breast cancer cell lines (MDA-MB-231)," Baghdad Sci. J. 19, 35 (2022). https://doi.org/10.21123/bsj.2022.7178
21. I. H. Ibraheem, A. S. Sadiq, M. Al-Tameemi, and M. F. Alias, "Synthesis, spectral identification, antibacterial evaluation and theoretical study of Co, Fe, Rh and Pd complexes for 2-benzoylthiobenzimidazol," Baghdad Sci. J. 19, 27 (2022). https://doi.org/10.21123/bsj.2022.6704
22. A. S. Abu-Surrah, et al., "New palladium(II) complexes bearing pyrazole-based Schiff base ligands," Eur. J. Med. Chem. 45, 471–475 (2010). https://doi.org/10.1016/j.ejmech.2009.10.029