

COORDINATION-DRIVEN BIOACTIVITY OF AN OXIME-BASED COBALT (II) CHELATE: SYNTHESIS, STRUCTURAL CHARACTERIZATION, AND IN VITRO ANTIOXIDANT AND ANTICOAGULANT EVALUATION

Mayur S. Tekade^{1*}, Pallavi M. Patil², Rupali A. Jinturkar³

^{1*}Department of Pharmaceutical Chemistry, PES's Modern College of Pharmacy, Nigdi, Pune, Affiliated to Savitribai Phule Pune University Pune, Maharashtra, India. Email: mayurtekade99@gmail.com, (<http://orcid.org/0000-0003-2109-8329>)

²Department of Pharmaceutical Chemistry, PES's Modern College of Pharmacy, Nigdi, Pune, Affiliated to Savitribai Phule Pune University Pune, Maharashtra, India. (<http://orcid.org/0000-0002-2089-0288>)

³Department of Pharmaceutical Chemistry, PES's Modern College of Pharmacy, Nigdi, Pune, Affiliated to Savitribai Phule Pune University Pune, Maharashtra, India.

ABSTRACT:

A ligand with sulfur and nitrogen atom functionality, 2-prop-2-en-1-yl-N-hydroxyprop-2-ene-1-sulfinimidothioate oxime (L1), was utilized to synthesize a cobalt (II) complex (CoL1) and test its in vitro scavenging ability as well as plasma clotting-time response. Ligand L1 was synthesized through the reaction of S-(prop-2-en-1-yl) prop-2-ene-1-sulfinothioate with hydroxylamine hydrochloride in aqueous alkaline medium. Further reaction between the synthesized ligand and a cobalt (II) salt in methanol, while heating under mildly acidic to nearly neutral pH condition yielded CoL1. The synthesized compound was tested for its DPPH radical inhibition and plasma clotting activity from 100 to 500 µg/mL. Inhibition of DPPH increased from 20.39% at 100 µg/mL to 70.82% at 500 µg/mL, while sample absorbance decreased from 0.0652 to 0.0239 in comparison to the control absorbance of 0.0819. In the plasma assay, clotting time increased from 44 s (ratio 1.38) to 102 s (ratio 3.19) compared to the control of 32 s (ratio 1.00). These responses in terms of concentrations suggest that CoL1 is a candidate compound for further study. These data represent descriptive screening results. Replicate numbers, variances, statistical analysis and interference controls were unavailable. Therefore, no mechanism of action, safety or efficacy could be determined from these observations.

KEYWORDS: Cobalt (II) chelate, oxime ligand, sulfur-nitrogen donor, synthesis, DPPH, radical scavenging, aPTT, in vitro screening

INTRODUCTION:

Coordinated compounds incorporating transition metal ions with multifunctional organic ligands represent a valuable family of molecular substances. The coordinating environment has a strong effect on the geometry, electronic distribution, solubility, stability and redox properties.[1] The presence of a multi-donor group is beneficial for coordinating chemistry since the ligand is able to coordinate with the metal center by means of various donor groups.[2] S and N coordinated systems provide the possibility to create complexes with various metal ions due to their combined donor properties. The oxime moiety, which is normally written as C=N-OH, offers nitrogen and oxygen donor atoms whose coordination behaviour is dictated by the structure of the ligand, its protonation, metal used, and reaction conditions. Additionally, the presence of sulfur in the ligand may introduce other forms of coordination and changes the electronic properties.[3] It is, thus, a suitable compound for the study of metal-ligand coordination and properties of the formed chelate. In this case, the work focuses on the ligand L1, 2-prop-2-en-1-yl-N-hydroxyprop-2-ene-1-sulfinimidothioate oxime, and its metal complex CoL1. [3-5] Cobalt is one of the transitional metals that have available oxidation states and coordination geometries; thus, research on cobalt compounds is undertaken in various fields, including material chemistry and bioinorganic chemistry.[6] Nonetheless, the biological significance of metal complexes cannot be deduced from the presence of metal or structural studies alone. Assays in vitro may give information about the reactivity or interaction of a substance with the biological system; however, this requires proper assay design and control experiments. [7]

DPPH test is a non-cellular test which relies on the detection of the reduction of a stable-colored radical through a change in absorbance. It is commonly used for the primary screening but optical interference, solubility, and kinetics can influence the results. Activated Partial Thromboplastin Time (APTT) is a clotting test based on plasma and gives a functional assessment of the intrinsic and common pathways of coagulation. Extended clotting time in an in vitro system does not necessarily indicate a particular target of coagulation factor. [8-10]

This article discusses the preparation of CoL1, the procedure used to prepare the ligand and its complex with cobalt, and the findings of the DPPH and aPTT assays, all of which are discussed within the same report focused on cobalt. This chemical preparation is provided in order to authenticate the materials used, but the primary focus of this paper is the pharmacological activity profile [11-12]

2. MATERIALS AND METHODS:

2.1. Chemicals and reagents

S-(prop-2-en-1-yl) prop-2-ene-1-sulfinothioate, sodium hydroxide, hydroxylamine hydrochloride, hydrochloric acid, methanol, and ammonia solution were listed as the reagents for the synthesis process. The salt of cobalt (II) was selected for the chelate compound. Distilled water was used for the aqueous solutions. The reagents for the biological assay include the DPPH reagent, physiological saline, trisodium citrate, CK-PREST aPTT reagent, and calcium chloride. Reagent grades and lot numbers are to be added to the form along with the specific cobalt salt hydrate.

2.2. Instruments and general analytical approach

The characterization study carried out on the concerned ligand and metal complexes was conducted using FT-IR, UV-visible, elemental analysis, mass spectrometry, ICP-MS, TGA, nuclear magnetic resonance spectroscopy and XRD. In regard to the current CoL1 study, only those results which were derived from the concerned CoL1 complex would be filled in. The model used, acquisition parameters, spectral positions, percent of elements, mass-to-charge ratio and thermal or diffraction details have not been provided in the current cobalt specific database and are thus not fabricated.

2.3. Synthesis of ligand L1

Dissolve sodium hydroxide (5.0 g) in 40 mL water in a suitable beaker, and allow the solution to cool using an ice bath. Add S-(prop-2-en-1-yl) prop-2-ene-1-sulfinothioate (4.0 g; roughly 2×10^{-2} mol) to the above solution while vigorously stirring the mixture with the aid of a magnetic stirrer to give Solution A. In another flask, dissolve hydroxylamine hydrochloride (2.0 g) in 40 mL distilled water to give Solution B. Slowly add Solution A to Solution B with stirring. Heat the reaction mixture in a water bath at 50–60 °C for 1 hour.

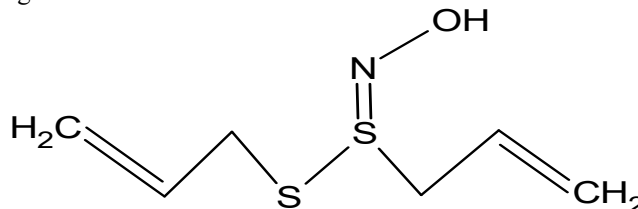


Fig. 1: IUPAC name – 2-prop-2-en-1-yl-N-hydroxyprop-2-ene-1-sulfinimidothioate oxime

The chilled solution was then processed using dilute hydrochloric acid in order to control the pH level and precipitate the substance. The obtained solid was filtered through vacuum filtration, and was then washed with cold water and collected in filter paper. The substance was dried under heating as per the preparation protocol. The actual values of the pH level, drying temperature, drying time, the mass and the percent yield should be determined from the laboratory notebook. The ligand is referred to as L1 in this case.

2.4. Synthesis of cobalt (II) chelate CoL1

L1 (0.346 g; roughly 2×10^{-3} mol based on the observed amount) was dissolved in 20 mL methanol using a two-necked round bottom flask. The mixture was refluxed for about 15–20 min to produce a clear ligand solution. Another solution containing the selected Co (II) salt was prepared in methanol in accordance with the stoichiometric requirement. The Co solution was slowly added into the boiling ligand solution while maintaining the temperature around 60 °C.

Thereafter, the reaction mixture was refluxed for a certain amount of time. According to the experiment record, the time taken for the heating process is about 30 minutes, while the total time of reflux in the case of the related chelate process is relatively longer than this. Ammonia solution was employed for pH adjustment to be about 5.5–6.5. The mixture was heated, cooled to room temperature, and then filtered.

2.5. Synthetic scheme

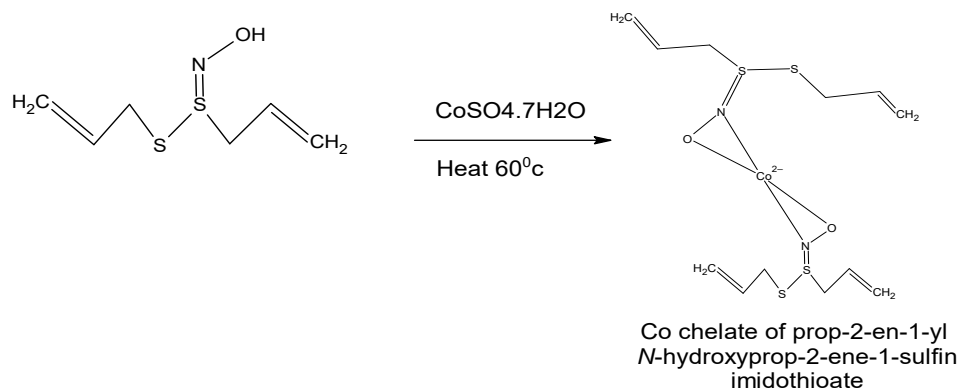


Figure 2: Reaction flow chart for synthesis of cobalt metal chelate with 2-prop-2-en-1-yl-N-hydroxyprop-2-en-1-sulfinimidothioate oxime (CoL1).

2.6. Preparation of test solutions

Concentration of CoL1 was determined for 100, 200, 300, 400 and 500 µg/mL at nominal levels. The stock concentration, the composition of solvent, and percentage of vehicle in the test solutions were not mentioned in the provided information. It is necessary to double-check and report the above information, as well as include a vehicle control in future work.

2.7. DPPH radical-scavenging assay

DPPH stock was made by dissolving 25 mg of DPPH in 100 mL of methanol to get 0.25 mg/mL. Reference preparation was taken with 3 mL of DPPH stock solution and 100 µL of methanol. Test preparation was carried out by mixing 3 mL of DPPH solution with the specific amount of CoL1 solutions. Test and control preparations were kept in dark for 30 minutes at room temperature. The absorbance was taken at 517 nm wavelength. The percentage inhibition was calculated as:

% inhibition = $[(Ac - As)/Ac] \times 100$, where

Ac = control absorbance

As = sample absorbance

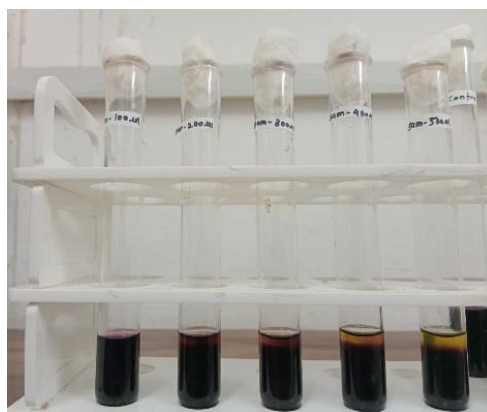


Figure 3: After incubation of (CoL1)

2.8. In vitro clotting-time assay

Blood from rat was taken in 3.2% trisodium citrate in the ratio of 9:1. The mixture was centrifuged for 20 min at $3756 \times g$, and the obtained supernatant was centrifuged using the same parameters for getting platelet-poor plasma (PPP). To each sample, 45 µL PPP and 5 µL CoL1 were taken and incubated for 3 min at 37 °C. Afterward, CK-PREST aPTT reagent (50 µL) was added, and incubated for another 3 min at 37 °C. The process of clotting was triggered by the addition of 100 µL of 25 mM $CaCl_2$, and the clotting time in seconds was determined. The control was performed with physiological saline instead of the test solution [13]

2.9. Calculation and presentation of aPTT ratio

Ratio of aPTT was determined as the ratio of observed clotting time in the sample to the control clotting time (32 sec). Data are reported as observed. The data set available does not contain information on number of independent experiments, number of replicates, standard deviation, standard error or statistical technique used. Therefore, no significance was assigned to observed differences. [14-16]

3. RESULTS AND DISCUSSION:

3.1. DPPH radical-scavenging response

CoL1 produced a concentration-associated increase in DPPH inhibition across the tested range. Sample absorbance decreased progressively from 0.0652 at 100 µg/mL to 0.0239 at 500 µg/mL, while the control absorbance remained 0.0819. The calculated inhibition increased from 20.39% to 70.82%. [17-21]

Table 1. DPPH radical-scavenging response

Sample	Concentration (µg/mL)	Sample absorbance (As)	Control absorbance (Ac)	Inhibition (%)
CoL1	100	0.0652	0.0819	20.39
CoL1	200	0.0538	0.0819	34.31
CoL1	300	0.0415	0.0819	49.33
CoL1	400	0.0313	0.0819	61.78
CoL1	500	0.0239	0.0819	70.82

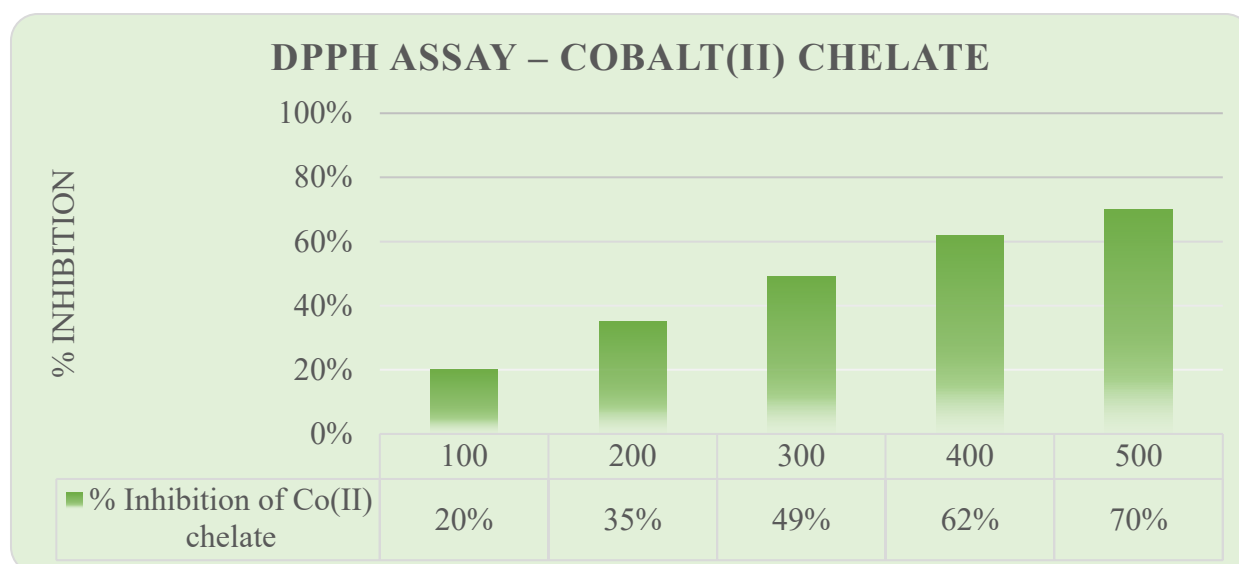


Figure 4. DPPH Assay of Cobalt (II)

The response rose at each successive concentration in the supplied series. The available record does not provide a validated concentration-response fit, uncertainty interval or an independently verified IC_{50} for CoL1; therefore, an IC_{50} value is not assigned in this manuscript.

3.2. aPTT clotting-time response

CoL1 increased the measured clotting time at every tested concentration relative to the 32 s control. The clotting time reached 102 s at 500 µg/mL, corresponding to an aPTT ratio of 3.19. [18,22-25]

Table 2. aPTT clotting-time response

Sample	Concentration (µg/mL)	Clotting time (s)	aPTT ratio	Recorded observation
Control	0	32	1.00	Normal clotting
CoL1	100	44	1.38	Moderate prolongation
CoL1	200	54	1.69	Increased clotting time
CoL1	300	68	2.13	Significant prolongation
CoL1	400	85	2.66	Very strong prolongation
CoL1	500	102	3.19	Maximum effect

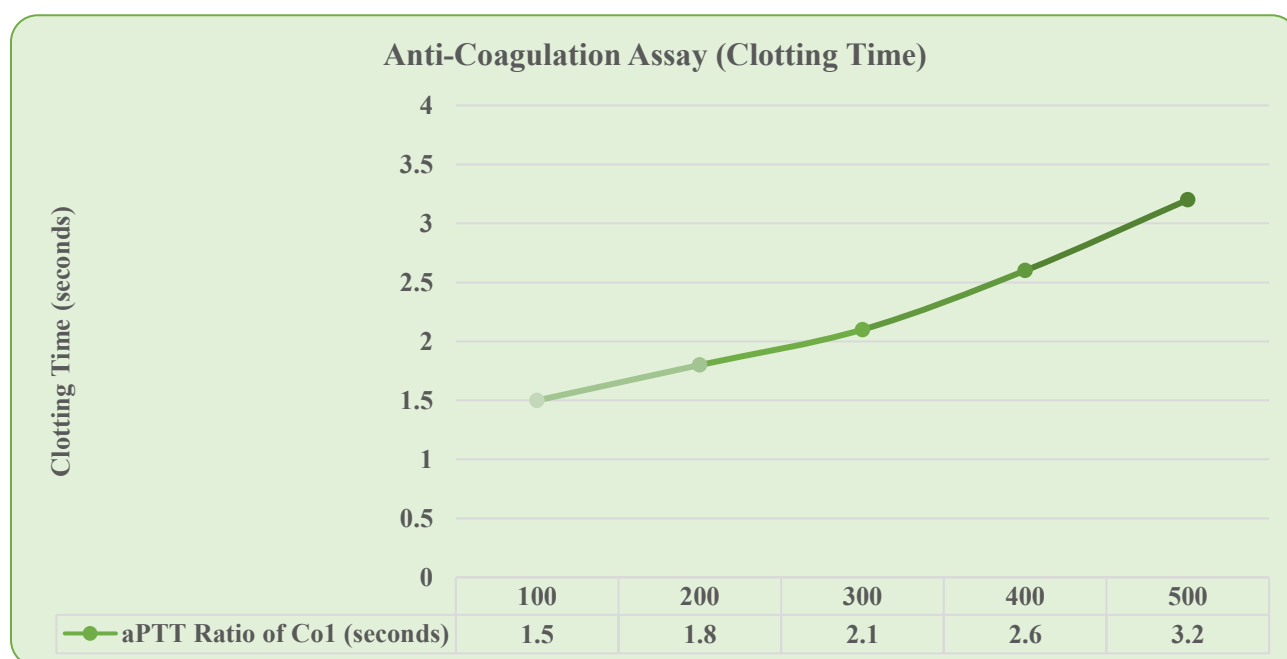


Figure 5. Anti-Coagulation Assay of Cobalt (II)

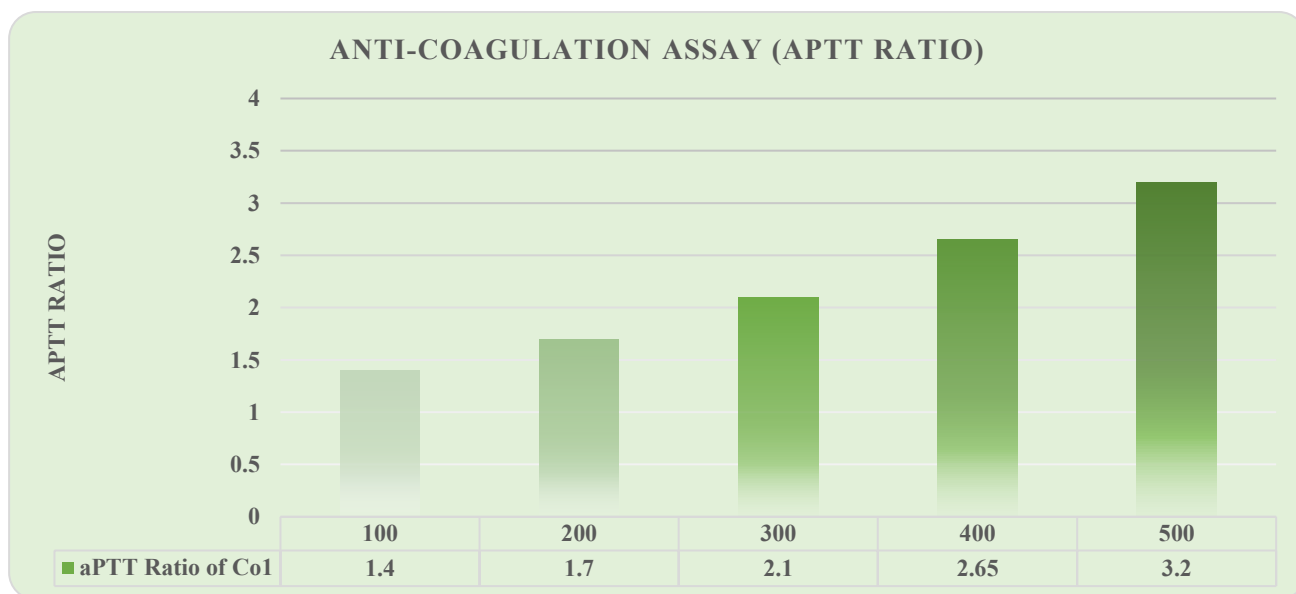


Figure 6. Anti-Coagulation Assay (aPTT Ratio)

Table 3. Consolidated pharmacological profile

Assay	Endpoint	Range observed	Pattern
DPPH	Radical inhibition	20.39–70.82%	Increase across 100–500 µg/mL
aPTT	Clotting time	44–102 s	Increase across 100–500 µg/mL
aPTT	Ratio to control	1.38–3.19	Increase across 100–500 µg/mL

When we tested the antioxidant and anticoagulant properties of a new compound called CoL1, we got some interesting results. This compound is made up of a special molecule called an oxime-based cobalt(II) chelate. We used a test called the DPPH radical-scavenging assay to see how well it could neutralize free radicals. What we found was that as we increased the concentration of CoL1, its antioxidant activity also increased. At a concentration of 100 µg/mL, it was able to inhibit 20.39% of the free radicals, but at 500 µg/mL, this number jumped to 70.82%. This suggests that CoL1 is really good at interacting with free radicals, especially at higher concentrations. But what's not entirely clear is exactly how it's doing this - is it the special properties of the oxime molecule, or the way it's binding to the cobalt(II) centre? We also tested CoL1's anticoagulant properties, and found that it was able to slow down blood clotting in a concentration-dependent way. At 100 µg/mL, it took 44 seconds for the blood to clot, but at 500 µg/mL, this time increased to 102 seconds. This is a pretty significant delay, and it suggests that CoL1 is having some kind of impact on the blood's ability to clot. However, we're not entirely sure which part of the clotting process is being affected - is it a specific protein, or a more general mechanism? One thing that's clear is that CoL1 has some interesting biological properties, and we'd like to learn more about how it works. To do this, we'll need to do some more experiments, including comparing CoL1 to other similar compounds, and getting a better sense of how it's interacting with the body's systems. We'll also need to collect more data and do some statistical analysis to make sure our findings are reliable. But for now, these initial results are a promising start.

4. CONCLUSION:

The present investigation describes the synthesis. In vitro evaluation of an oxime-based cobalt (II) chelates, CoL1, with particular emphasis on its antioxidant and anticoagulant activities. The compound exhibited a concentration- increase in DPPH scavenging activity reaching 70.82% inhibition at 500 µg/mL. It also demonstrated a progressive prolongation of plasma clotting time reaching 102 seconds with an aPTT ratio of 3.19 at the highest tested concentration. These findings indicate that CoL1 possesses measurable in vitro scavenging and clotting-time-modifying properties under the conditions investigated. The results provide a basis for exploration of oxime-based cobalt (II) coordination compounds, as bioactive chemical systems. However additional studies involving confirmation, comparative ligand–chelate evaluation, statistical validation, coagulation-pathway investigations and cytotoxicity assessment are essential to establish the reproducibility, mechanism of action and biological safety of CoL1.

5. ACKNOWLEDGMENTS:

Authors are sincerely thanks PES's Modern college of Pharmacy, Nigdi, Pune and express their heartfelt gratitude to the principal for his continuous encouragement and support throughout this study

6. Conflict of interest:

The authors declare no conflict of interest

7. Ethics statement:

All animal experiments were performed in accordance with institutional guidelines and approved by the Animal Care and Use Committee of Invitox R&D Institute and Proposal No. IRDI/IEAC/M05/03/2025-26

8. REFERENCES:

1. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958;181:1199–1200. <https://doi.org/10.1038/1811199a0>
2. Ramadan AM, Shaban SY, Ibrahim MM. Synthesis, characterization, and ascorbic acid oxidase biomimetic catalytic activity of cobalt(III) oxime complexes. *J Coord Chem*. 2011;64(19):3376–3392. <https://doi.org/10.1080/00958972.2011.621166>
3. Kukushkin VY, Tudela D, Pombeiro AJL. Metal-ion assisted reactions of oximes and reactivity of oxime-containing metal complexes. *Coord Chem Rev*. 1996;156:333–362. [https://doi.org/10.1016/0010-8545\(95\)01234-6](https://doi.org/10.1016/0010-8545(95)01234-6)
4. Renfrew AK, O'Neill ES, Hambley TW, New EJ. Harnessing the properties of cobalt coordination complexes for biological application. *Coord Chem Rev*. 2018;375:221–233. <https://doi.org/10.1016/j.ccr.2017.11.027>
5. Kukushkin VY, Pombeiro AJL. Oxime and oximate metal complexes: unconventional synthesis and reactivity. *Coord Chem Rev*. 1999;181(1):147–175. [https://doi.org/10.1016/S0010-8545\(98\)00215-X](https://doi.org/10.1016/S0010-8545(98)00215-X)
6. Singh BK, Jetley UK, Sharma RK, Garg BS. Synthesis, characterization and biological activity of complexes of 2-hydroxy-3,5-dimethylacetophenoneoxime with Cu(II), Co(II), Ni(II) and Pd(II). *Spectrochim Acta A Mol Biomol Spectrosc*. 2007;68(1):63–73. <https://doi.org/10.1016/j.saa.2006.11.001>
7. Abd El-Lateef HM, El-Dabea T, Khalaf MM, Abu-Dief AM. Recent overview of potent antioxidant activity of coordination compounds. *Antioxidants*. 2023;12(2):213. <https://doi.org/10.3390/antiox12020213>
8. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol*. 1995;28(1):25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
9. Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. *Lebensm Wiss Technol*. 1997;30(6):609–615. <https://doi.org/10.1006/fstl.1997.0240>
10. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Technol*. 2004;26(2):211–219.
11. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. *J Food Sci Technol*. 2011;48:412–422. <https://doi.org/10.1007/s13197-011-0251-1>
12. Mishra K, Ojha H, Chaudhury NK. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food Chem*. 2012;130(4):1036–1043. <https://doi.org/10.1016/j.foodchem.2011.07.127>
13. Pyrzynska K, Pełkal A. Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. *Anal Methods*. 2013;5:4288–4295. <https://doi.org/10.1039/C3AY40367J>
14. Gülçin İ, Alwasel SH. DPPH radical scavenging assay. *Processes*. 2023;11(8):2248. <https://doi.org/10.3390/pr11082248>
15. Apak R, Özyürek M, Güçlü K, Çapanoğlu E. Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer-based assays. *J Agric Food Chem*. 2016;64(5):997–1027. <https://doi.org/10.1021/acs.jafc.5b04739>
16. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem*. 2005;53(10):4290–4302. <https://doi.org/10.1021/jf0502698>
17. Fritsma GA, Dembitzer FR, Randhawa A, Marques MB, Van Cott EM, Adcock-Funk D, Peerschke EIB. Recommendations for appropriate activated partial thromboplastin time reagent selection and utilization. *Am J Clin Pathol*. 2012;137(6):904–908. <https://doi.org/10.1309/AJCP3J1ZKYBFQXJM>
18. Favaloro EJ, et al. How to optimize activated partial thromboplastin time (APTT) testing: solutions to establishing and verifying normal reference intervals and assessing APTT reagents for sensitivity to heparin, lupus anticoagulant, and clotting factors. *Semin Thromb Hemost*. 2019. <https://doi.org/10.1055/s-0038-1677018>
19. Rasmussen KL, Philips M, Tripodi A, Goetze JP. Unexpected, isolated activated partial thromboplastin time prolongation: a practical mini-review. *Eur J Haematol*. 2020;104(6):519–525. <https://doi.org/10.1111/ejh.13394>
20. Levy JH, Szlam F, Wolberg AS, Winkler A. Clinical use of the activated partial thromboplastin time and prothrombin time for screening: a review of the literature and current guidelines for testing. *Clin Lab Med*. 2014;34(3):453–477. <https://doi.org/10.1016/j.cll.2014.06.005>
21. Adcock DM, et al. ICSH recommendations for the performance and interpretation of activated partial thromboplastin time and prothrombin time mixing tests. *Int J Lab Hematol*. 2024;46(5):777–788. <https://doi.org/10.1111/ijlh.14344>
22. Tripodi A. Review: the laboratory and clinical consequences of a prolonged activated partial thromboplastin time. *Thromb Res*. 2004;114(5–6):377–382. <https://doi.org/10.1016/j.thromres.2004.06.003>
23. Tarai A, Nath B. A review on oxime functionality: an ordinary functional group with significant impacts in supramolecular chemistry. *Chem Commun*. 2024;60:7266–7287. <https://doi.org/10.1039/D4CC01397B>

24. Perontsis S, Hatzidimitriou AG, Psomas G. Coordination compounds of cobalt(II) with carboxylate non-steroidal anti-inflammatory drugs: structure and biological profile. *Dalton Trans.* 2024;53:15215–15235. <https://doi.org/10.1039/D4DT01846J>
25. Gülçin İ, et al. The versatility of antioxidant assays in food science and safety—chemistry, applications, strengths, and limitations. *Antioxidants.* 2020;9(8):709. <https://doi.org/10.3390/antiox9080709>

s