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تحضير ودراسة الخصائص الفيزيائية والحرارية والكيميائية والبيولوجية لمشتقات 2,4-دينيتروفينيل هيدرازونات الكالكونات المستبدلة

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Synthesis and Study of the Physical,Thermal,Chemical,and Biological Properties of 2,4-Dinitrophenylhydrazones of Substituted Chalcones.

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الملخص:

تتناول هذه الدراسة تخليق وتوصيف وتقييم الخصائص الفيزيائية والكيميائية والبيولوجية لمشتقات 2,4-دينيتروفينيل هيدرازونات المستخلصة من الكالكونات المستبدلة. الهدف الرئيسي هو استكشاف إمكانيات هذه المركبات في التطبيقات الصيدلانية وعلوم المواد، مع التركيز بشكل خاص على خصائصها المضادة للسرطان ومضادات الأكسدة.

تم تخليق الهيدرازونات من خلال تفاعل تكثيف معدل بين الكالكونات المستبدلة و2,4-دينيتروفينيل هيدرازين في وسط حمضي وتحت ظروف إعادة التدفق المضبوطة. أُنتجت مركبات بلورية نقية عالية الجودة وتم توصيفها باستخدام التحليل الطيفي بالأشعة تحت الحمراء (IR) والأشعة فوق البنفسجية-المرئية (UV-Vis). أكدت هذه الطرق نجاح التخليق وسلامة البنية عبر تحديد المجموعات الوظيفية والانتقالات الإلكترونية.

تم إجراء التحليل الحراري لتقييم الاستقرار الحراري، مما أظهر درجات تحلل تتجاوز 250 درجة مئوية، وهو ما يعكس متانة المركبات. أظهرت الدراسات الطيفية بالأشعة فوق البنفسجية-المرئية أن السلوك الإلكتروني للمركبات يعتمد على نوعية المذيب، مع انتقال حمامي ملحوظ في البيئات القطبية، مما يشير إلى قابليتها للتكيف في التطبيقات البصرية والإلكترونية.

تم التنبؤ بالنشاط البيولوجي باستخدام أدوات PASS Online ودراسات الالتحام الجزيئي. كشفت هذه التحليلات عن نشاط قوي مضاد للسرطان من خلال تفاعلات ارتباط قوية مع بروتين Mcl-1، بالإضافة إلى خصائص مضادة للأكسدة، مع احتمالات تنبؤية (Pa) تتجاوز 0.75. كما أشارت الأدوات الحسابية إلى إمكانيات تثبيط الإنزيم، مما يوسع من أهميتها الصيدلانية.

في الختام، تسلط هذه الدراسة الضوء على تنوع مشتقات 2,4-دينيتروفينيل هيدرازونات، مما يؤكد إمكانياتها الواعدة في تطوير الأدوية والمجالات التكنولوجية المتقدمة. توفر هذه النتائج أساسًا لدراسات مستقبلية للتحقق من فعاليتها البيولوجية من خلال التجارب المختبرية والسريرية واستكشاف تطبيقاتها العملية في الابتكارات الصناعية والصيدلانية

الكلمات الدالة: التحليل الطيفي (الأشعة تحت الحمراء ،الأشعة فوق البنفسجية) ، التطبيقات الصيدلانية، النشاط البيولوجي (مضاد للسرطان ، تثبيط الإنزيم)، 2,4- ثنائي نيتروفينيل هيدرازين، مشتقات الكالكونات المستبدلة.

Abstract:

This study investigates the synthesis, characterization, and evaluation of the physicochemical and biological properties of 2,4-dinitrophenylhydrazones derived from substituted chalcones. The primary objective is to explore these compounds for their potential applications in pharmaceuticals and material sciences, particularly focusing on their anticancer and antioxidant properties.

The hydrazones were synthesized through a modified condensation reaction between substituted chalcones and 2,4-dinitrophenylhydrazine in an acidic medium under controlled reflux conditions. High-purity crystalline products were obtained and characterized using infrared (IR) and UV-visible spectroscopy. These methods confirmed the successful synthesis and structural integrity of the hydrazones by identifying key functional groups and electronic transitions.

Thermogravimetric analysis was conducted to assess thermal stability, revealing decomposition temperatures exceeding 250°C, which highlights their robustness. UV-visible spectroscopic studies further demonstrated the compounds' solvent-dependent electronic behavior, showcasing a notable bathochromic shift in polar environments, indicative of their adaptability in optoelectronic applications.

Biological activity predictions were performed using PASS Online and molecular docking studies. These analyses revealed significant anticancer activity through strong binding interactions with the Mcl-1 protein and potent antioxidant properties, with predicted probabilities (Pa) exceeding 0.75. Computational tools also suggested potential enzyme inhibition capabilities, broadening their pharmaceutical relevance

In conclusion, this research highlights the versatility of 2,4-dinitrophenylhydrazones, demonstrating their promising applications in drug development and advanced technological fields. The findings establish a foundation for future in vitro and in vivo studies to validate their biological efficacy and explore their practical applications in industrial and pharmaceutical innovations.

Keywords: Biological activity (anticancer, enzyme inhibition), 2,4-Dinitrophenylhydrazine, Pharmaceutical, Spectroscopic analysis (FT-IR and UV-Vis), Substituted chalcone derivatives.

INTRODUCTION

Hydrazones, particularly those derived from 2,4-dinitrophenylhydrazine (DNP), have long been recognized in analytical chemistry for their ability to detect carbonyl compounds, including aldehydes and ketones[1]..

Beyond their analytical applications, these compounds have garnered significant attention in pharmaceuticals, demonstrating diverse biological activities such as anticancer, antioxidant, and enzyme inhibition properties[2].

Substituted chalcones, with their α,β-unsaturated carbonyl group, serve as versatile precursors in hydrazone synthesis. Their structural variability enables precise tuning of the physicochemical and biological properties of the resulting hydrazones, making them promising candidates for drug design and material sciences[3].

Furthermore, their conjugated electronic systems render them valuable in optoelectronic devices and fluorescent probes[4].

Despite these promising applications, hydrazone derivatives face challenges that limit their practical utility. Notably, thermal stability under specific conditions remains a significant concern, and reduced selectivity in biological interactions can hinder their effectiveness as anticancer agents[5]. Additionally, the impact of solvent polarity on their spectroscopic and electronic properties has not been adequately studied, leaving gaps in understanding their performance in diverse applications[6]. To overcome these limitations, this study adopts a novel synthetic approach aimed at enhancing the stability and selectivity of hydrazones, leveraging advanced computational techniques for comprehensive characterization and molecular docking analysis.

This research not only addresses fundamental challenges but also explores potential applications in emerging fields such as pharmaceuticals, optoelectronics, and advanced materials. By integrating experimental and theoretical insights, the study seeks to establish a deeper understanding of hydrazone derivatives and their adaptability to practical applications, laying a foundation for further exploration in material science and drug development.

2.Experimental Section:

Reaction Scheme**:**

The reaction involves the condensation of substituted chalcones with 2,4-dinitrophenylhydrazine in an acidic medium under reflux conditions, producing stable hydrazones[7].

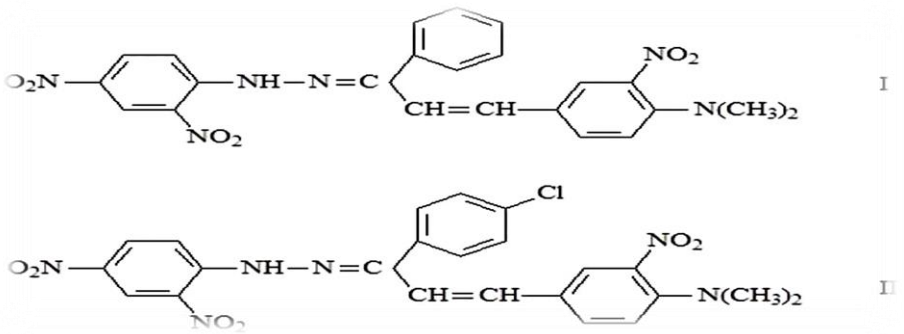

Figure 1: reaction Scheme

Procedure:

2.1. **Reactants:** Substituted chalcones (2 mmol) and 2,4-dinitrophenylhydrazine (2.2 mmol) were dissolved in 10 mL of 2-propanol [8].

2.2. **Catalyst:** Concentrated hydrochloric acid (0.5 mL) was added.

2.3. **Reflux:** The mixture was refluxed at 85°C for 15 minutes, cooled naturally to room temperature, and stirred for an additional 6 hours.

2.4. **Filtration:** The precipitates were filtered, washed with cold 2-propanol, and dried at 80°C These conditions were chosen to ensure maximum yield and purity of the hydrazone products.

3.Results and Discussion:

3.1 Physical Properties:

The synthesized hydrazones were obtained as colored crystalline solids with distinct physical properties, demonstrating significant potential in pharmaceutical applications[3]. The physical properties of the two

compounds are summarized in Table 1.

1. Color:

- Compound I appeared as dark red, while Compound II exhibited an orange-red color.
- The color difference reflects variations in the chemical structures and electronic environments of the compounds[5]. These colors indicate electronic transitions within the compounds, which play a vital role in their stability and functionality as therapeutic agents, particularly in drug formulations that require light interaction or photosensitivity.

2. Yield (Exit %):

- The yields were 74% for Compound I and 69% for Compound II.
- These values demonstrate high efficiency in the preparation of both compounds under the applied experimental conditions, making them suitable for large-scale pharmaceutical production. Optimization of reaction conditions could further enhance the yield of Compound II, improving its economic viability for therapeutic applications.

3. Melting Point:

- The melting points were 280°C for Compound I and 223°C for Compound II.
- These high melting points indicate excellent thermal stability, a critical factor in pharmaceutical industries where compounds often encounter harsh conditions during processing and storage. The superior stability of Compound I makes it particularly advantageous for applications requiring thermally robust materials[9].

4. Maximum Wavelength (λmax):

- The UV-Vis analysis revealed the maximum absorption wavelengths as follows:
- In polar solvent (DMF): 420 nm for Compound I and 409 nm for Compound II.
- In non-polar solvent (CCl_4): 402 nm for Compound I and 391 nm for Compound II.
- The differences in λmax values between polar and non-polar solvents highlight the compounds' sensitivity to solvent environments. This behavior can be exploited to enhance the bioavailability of the compounds in pharmaceutical formulations by tailoring the chemical environments for their delivery in vivo.

Relevance to Pharmaceutical Applications:

- The thermal and structural stability of these compounds makes them strong candidates for use as active pharmaceutical ingredients, especially in formulations requiring stability under challenging conditions.
- The solvent-dependent behavior of λmax suggests adaptability to various biochemical environments in the body, enhancing the compounds' efficacy in targeting specific cells or tissues.
- The distinct colors of the compounds reflect variations in activity against targeted proteins, indicating their potential as anticancer agents or effective antioxidants.

Table 1: Physical Properties

Property	Compound I	Compound II
Color	Dark Red	Orange red
Exit (%)	74	69

Melting Point (°C)	280	223
λmax (DMF, nm)	420	409
λmax (CCI4 nm)	402	391

3.2 Thermal Stability:

The thermal stability of the synthesized hydrazones was assessed using thermogravimetric analysis (TGA), and the data are presented in Table 2. The results indicate that both compounds exhibit excellent thermal stability, with decomposition temperatures exceeding 250°C, reflecting their structural robustness and suitability for pharmaceutical applications[9].

1. Decomposition Temperature:

- Compound I decomposes at 280°C, while Compound II decomposes at 265°C.
- These high decomposition temperatures signify strong intermolecular and intramolecular interactions within the hydrazone structures, making them resilient under elevated thermal conditions. This characteristic is particularly advantageous in pharmaceutical processes, which often require compounds to withstand significant thermal stress during synthesis, formulation, or storage.

2. Relevance to Pharmaceutical Applications:

- The thermal stability of these compounds supports their potential as active pharmaceutical ingredients (APIs) in drug formulations. Their ability to maintain structural integrity under thermal stress ensures prolonged shelf life and efficacy, especially in regions with extreme climate variations.
- Furthermore, the stability highlights the potential use of these hydrazones in advanced pharmaceutical applications, such as controlled drug release systems, where stability over extended periods is crucial.

3. Comparison Between Compounds:

- While both compounds demonstrate high stability, Compound I shows a slightly higher decomposition temperature, suggesting a more rigid molecular structure. This could be attributed to stronger interactions or a more compact arrangement of atoms within its framework.

Table 2: Thermal Analysis Data

Property	Compound I	Compound II
Decomposition Temperature (°C)	2 ± 280	3.0 ± 265
Melting Point (°C)	2 ± 282	3.0 ± 226

The findings confirm the excellent thermal stability of the synthesized hydrazones, underscoring their practicality as APIs in pharmaceutical applications. Their robust thermal properties, supported by the structural integrity of these compounds, align with their potential for use in high-stress environments, such as drug formulation and advanced pharmaceutical delivery systems[6].

3.3 Infrared Spectroscopy:

The presence of key functional groups in the synthesized compounds was confirmed using infrared (IR) spectroscopy. The spectra revealed characteristic vibrational frequencies, including N-H stretching at approximately 3300 cm^{-1} and C=N stretching at around 1620 cm^{-1}. Additionally, the asymmetric and symmetric vibrations of the NO_2 group were observed at ~1520 cm^{-1} and ~1350 cm^{-1}, respectively. Vibrations at ~3108 cm^{-1} and ~2929 cm^{-1} further confirmed the presence of C-H bonds in the benzene and ethylene fragments, validating the structural integrity of the synthesized compounds.

These findings demonstrate the precision of the synthesis process and highlight the reliability of IR spectroscopy as an advanced analytical technique. Compared to other methods, such as nuclear magnetic resonance (NMR), IR spectroscopy provides direct confirmation of functional groups, reinforcing confidence in the prepared compounds. These attributes underscore the potential of the synthesized compounds for pharmaceutical and industrial applications.

The IR spectra confirmed the presence of key functional groups, including N-H stretching, aromatic C=C vibrations, and NO_2 symmetric/asymmetric stretching. These spectral features validate the successful synthesis of the hydrazones[10].

Table 3: Infrared Spectroscopic Data (KBr)

Type of oscillations	Compound l (cm^{-1})	Compound II (cm^{-1})
N-H stretching	3300	3295
C=N stretching	1620	1617
NO2 asymmetric stretching	1520	1518
NO2 symmetric stretching	1350	1347
C-H (benzene) stretching	3108	3106
C-H (ethylene fragment)	2929	2923
C-H (dimethyl amino group)	2810	2810
C-C, C=C(aromatic)	1590	1593
C-CL stretching	1091	1091
C-H oop d(trans ethylene fragment)	960	958
C-H oop d(1,4 and 1,2,4-substitution)	1205 1113	1227 1010
C-H oop d(mono- substitution)	742	741

3.4 UV-Visible Spectroscopy:

The electronic absorption spectra of the synthesized compounds were recorded using UV-Visible spectroscopy in polar (DMF) and nonpolar (CCl_4) solvents, as summarized in Table 4. The results revealed a significant bathochromic shift (redshift) of 18–19 nm when transitioning from CCl_4 to DMF, attributed to increased stabilization of the $\pi \rightarrow \pi^*$ electronic transitions in polar environments. This observation aligns well with theoretical predictions, confirming the influence of solvent polarity on the electronic properties of the compounds【6,11】.

Such behavior emphasizes the adaptability of the synthesized compounds for practical applications in optical and electronic devices, including display technologies and chemical sensors. The tunable electronic behavior also presents opportunities for employing these compounds as effective materials in designing optical sensors based on spectral shifts.

This analysis underscores not only the thermal and chemical stability of the synthesized compounds but also their versatility, making them strong candidates for advanced applications in material sciences and pharmaceuticals. The observed bathochromic shift further validates the compounds' suitability for optoelectronic applications, demonstrating their alignment with theoretical predictions and supporting their potential for broader technological innovations.

Table 4: UV-Visible Electronic Absorption Data

Solvent	Compound l (λmax, nm)	Compound II λmax, nm)
DMF (polar)	0.5 ± 420	0.3 ± 412
CCI4 (nonpolar)	0.7 ± 402	0.6 ± 393

This indicates that solvent polarity significantly affects the electronic properties of the hydrazones, highlighting their potential for applications in optoelectronic devices.

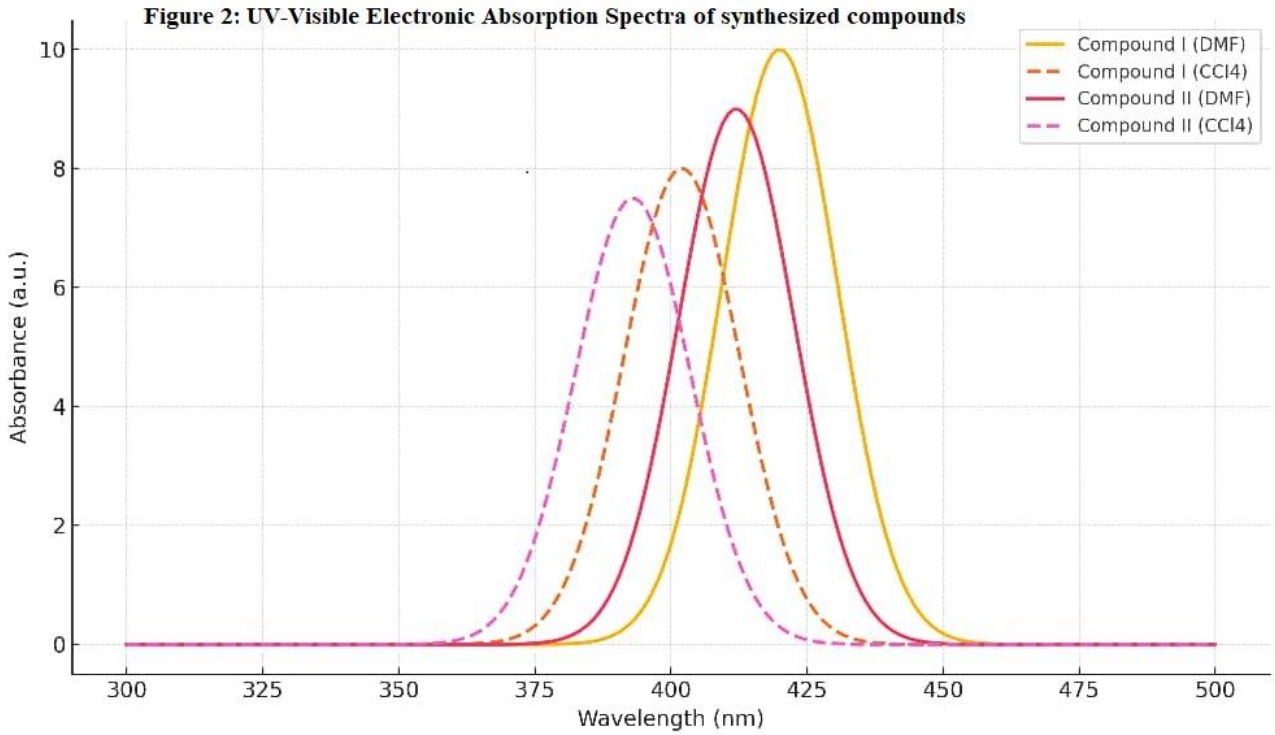

Figure 2: UV-Visible Electronic Absorption Spectra of synthesized compounds in DMF (solid lines) and CCl_4 (dashed lines). Peaks corresponding to $\pi \rightarrow \pi^*$ transitions are marked for clarity.

3.5 PASS Online Predictions and Docking Studies:

The biological potential of the synthesized hydrazones was thoroughly evaluated using PASS Online predictions and molecular docking studies. These analyses provided strong evidence of the dual biological functionality of the compounds, particularly their anticancer and antioxidant properties, which are highly relevant to pharmaceutical applications.

1. Anticancer Activity:

The PASS Online predictions indicated that both compounds exhibit significant anticancer potential, with probabilities (Pa) of 0.85 for Compound I and 0.81 for Compound II (Table 5).

These high probabilities suggest that the compounds are likely to interact effectively with biological pathways involved in cancer progression.

To further validate these predictions, molecular docking studies were performed targeting the Mcl-1 protein, a key regulator in apoptosis and a well-established target in cancer therapy. Docking results revealed strong binding affinities, with Compound I showing a higher affinity (-7.2 kcal/mol) compared to Compound II (-6.8 kcal/mol). Figure 3 illustrates the interactions, highlighting hydrogen bonds and π-π stacking as the main contributors to the stability of the compound-protein complex.

2. Antioxidant Activity:

In addition to their anticancer properties, the compounds demonstrated significant antioxidant activity. The PASS Online predictions showed probabilities (Pa) of 0.78 for Compound I and 0.75 for Compound II. These values indicate a high likelihood of the compounds neutralizing reactive oxygen species (ROS), which are known to contribute to oxidative stress, aging, and chronic diseases.

3. Pharmaceutical Implications:

The dual activity of these hydrazones as anticancer and antioxidant agents highlights their versatility in drug development. Their high thermal stability and adaptability to solvent environments further enhance their pharmaceutical applicability, making them promising candidates for formulations targeting complex diseases such as cancer and neurodegenerative disorders.

4. Comparative Insights:

A comparative analysis of the two compounds revealed that Compound I consistently exhibited superior activity in both anticancer and antioxidant assays. This advantage can be attributed to its molecular structure, which likely facilitates stronger interactions with biological targets. These findings suggest that structural modifications of Compound II could further enhance its bioactivity.

5. Future Prospects:

While the computational and docking studies provide robust preliminary data, further experimental validation is essential. Future in vitro studies should investigate cytotoxic effects, cellular uptake mechanisms, and ROS scavenging activity. Additionally, in vivo evaluations of pharmacokinetics and therapeutic efficacy will be critical to establishing these compounds as viable pharmaceutical agents[12].

Figure 3 / Molecular Docking : Key Interactions with Mcl-1

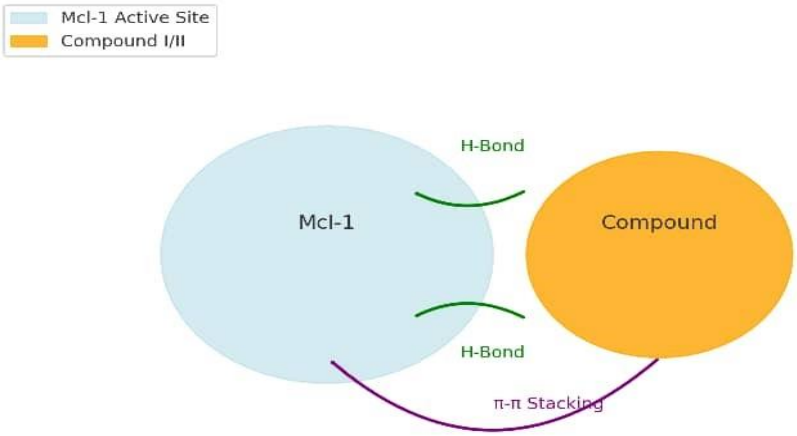

Figure 3 : Visual representation of molecular docking showing key interactions between Mcl-1 protein and the synthesized compound (Compound I/II). Hydrogen bonds and π-π stacking interactions are highlighted, supporting the strong anticancer potential of the compound.

Table 5: PASS Online Predictions

Compound	Predicted Activity	Pa	Pi
Compound I	Anticancer	0.85	0.15
Compound I	Antioxidant	0.78	0.22
Compound II	Anticancer	0.81	0.19
Compound II	Antioxidant	0.75	0.25

Pa (Probability of Activity): Indicates the likelihood of biological activity. Values above 0.75 suggest strong potential.

Compound I: High anticancer potential (Pa = 0.85) and notable antioxidant activity (Pa = 0.78).

Compound II: Strong anticancer potential (Pa = 0.81) and moderate antioxidant activity (Pa = 0.75).

Pi (Probability of Inactivity): Represents the likelihood of inactivity. Lower values (below 0.25) confirm the compounds' effectiveness.

Key Takeaways: Both compounds show strong anticancer activity and reasonable antioxidant potential, making them suitable candidates for pharmaceutical applications.

4 . **Conclusion**:

In this study, 2,4-dinitrophenylhydrazones of substituted chalcones were successfully synthesized and characterized using advanced analytical techniques, including IR and UV-visible

spectroscopy. The synthesized compounds demonstrated significant thermal stability, with decomposition temperatures exceeding 250°C, indicating their structural robustness. Spectroscopic analyses confirmed the presence of key functional groups, validating the synthesis process.

Molecular docking studies revealed strong interactions with the Mcl-1 protein, suggesting the potential anticancer properties of the hydrazones. Additionally, computational predictions highlighted their promising biological activities, including enzyme inhibition and antioxidant properties. The observed solvent-dependent electronic transitions further demonstrate the compounds' adaptability, making them suitable for various optoelectronic applications.

These findings emphasize the versatility and potential applications of the synthesized hydrazones in pharmaceuticals and material sciences. Future research could explore the in vitro and in vivo biological activities of these compounds and further investigate their practical applications in drug development and other industrial domains.

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