Borneo Journal of Pharmacy
Vol 7 Issue 3 August 2024
Pages 254 – 263

https://journal.umpr.ac.id/index.php/bjop/article/view/6163
DOI: https://doi.org/10.33084/bjop.v7i3.6163
e-ISSN: 2621-4814

Research Article

Structure Modification of Cinnamic Acid to (*E*)-1-(3,4-dihydroisoquinoline-2(1H)-yl)-3-phenylprop-2-en-1-one and Antioxidant Activity Test by DPPH Method

Dian Agung Pangaribowo*

Fathunnisa

Ari Satia Nugraha

Ayik Rosita Puspaningtyas

Indah Purnama Sary

Department of Pharmacy, Universitas Jember, Jember, East Java, Indonesia

*email: agung.farmasi@unej.ac.id; phone: +62331324736

Keywords:
Acylation reaction
Antioxidant
Cinnamic acid
Structure modification

Abstract

Antioxidants can protect cells from free radical damage by stabilizing them. One of the compounds that has antioxidant activity is cinnamic acid. Cinnamic acid and its derivatives have several activities: antibacterial, anticancer, and antioxidant. However, the ability of cinnamic acid to capture free radicals is still relatively low. One of the efforts that can be made to increase the antioxidant activity of cinnamic acid is to modify its structure. Structure modification is an effort to improve the pharmacological activity of a compound through chemical synthesis reactions. The cinnamic acid structure can be modified by changing the carboxylic -OH group into an amine group through an N-atom acylation reaction. This study was conducted by reacting cinnamoyl chloride (**1a**), which is a cinnamic acid derivative with 1,2,3,4-tetrahydroisoquinoline (**2b**) which is a compound of isoquinoline group to produce (*E*)-1-(3,4-dihydroisoquinoline-2(1H)-yl)-3-phenylprop-2-en-1-one (**3b**) and then tested for antioxidant activity using DPPH method. The resulting product compound was yellow crystals with a yield of 81.56%. The antioxidant activity produced by the product is more significant than that of cinnamic acid compounds at the same concentration.

Received: November 23rd, 2023
1st Revised: June 7th, 2024
2nd Revised: August 13th, 2024
Accepted: August 20th, 2024
Published: August 30th, 2024

INTRODUCTION

Free radicals, highly reactive species with unpaired electrons, can cause oxidative stress when produced in excess. The human body possesses defense mechanisms, including enzymes like glutathione peroxidase, catalase, and superoxide dismutase, to counteract free radical damage[1,2]. However, oxidative stress, resulting from an imbalance between free radical production and elimination, is implicated in various serious health conditions, including cancer, atherosclerosis, aging, immunosuppression, inflammation, ischemic heart disease, diabetes, and neurological disorders[3].

Antioxidants, substances capable of neutralizing free radicals by donating electrons, play a crucial role in protecting cells from oxidative damage[3]. Cinnamic acid, a compound commonly used as a food flavoring agent, exhibits antioxidant properties[4-6]. Its unsaturated double bonds facilitate hydrogen atom donation, neutralizing free radicals. However, cinnamic acid's antioxidant activity is relatively limited. Structure modification represents a promising strategy to enhance its antioxidant capacity[7,8].

Structure modification, a key drug development strategy, involves chemically altering existing compounds to enhance their pharmacological activity[9,10]. The cinnamic acid scaffold, characterized by its carboxylic OH group, offers opportunities for modification through N-atom acylation to produce novel derivatives. Tetrahydroisoquinoline compounds hold significant

How to cite: Pangaribowo DA, Fathunnisa, Nugraha AS, Puspaningtyas AR, Sary IP. Structure Modification of Cinnamic Acid to (E)-1-(3,4-dihydroisoquinoline-2(1H)-yl)-3-phenylprop-2-en-1-one and Antioxidant Activity Test by DPPH Method. Borneo J Pharm. 2024;7(3):254-63. doi:10.33084/bjop.v7i3.6163

promise in bioorganic chemistry and medicine. This scaffold has been widely explored in pharmaceutical research, demonstrating diverse biological activities including anti-inflammatory, neurotropic, antibiotic, antipsychotic, antituberculosis, estrogenic receptor blocker, and antioxidant properties[11,12]. The detrimental effects of reactive oxygen species (ROS) on cellular macromolecules have fueled research efforts to develop novel antioxidant compounds[13,14]. To counteract oxidative damage, extensive investigations have been conducted to identify and characterize potential antioxidants.

In this study, we concentrated on the design, synthesis, characterization, and evaluation of the antioxidant activity of the newly cinnamic acid-tetrahydroisoquinoline hybrid compound. The structures of the synthesized compounds were verified based on FTIR and ^{1}H-NMR spectral data. The structural combination of cinnamic acid and tetrahydroisoquinoline is expected to increase the antioxidant activity of cinnamic acid as the lead compound.

MATERIALS AND METHODS

Materials

The synthesis of target compound involved the use of cinnamoyl chloride (Sigma-Aldrich), 1,2,3,4-tetrahydroisoquinoline (Sigma-Aldrich), triethylamine (Merck), tetrahydrofuran (Merck), and ethyl acetate (Merck). Purification was achieved using *n*-hexane and distilled water. Analytical techniques included melting point determination (using a melting point tester), Fourier-Transform Infrared spectroscopy (FTIR; Shimadzu), thin-layer chromatography-densitometry (TLC-densitometry; Camag), and nuclear magnetic resonance spectroscopy (NMR; JEOL Resonance).

Methods

Synthesis of target compound

(*E*)-1-(3,4-dihydroisoquinoline-2(1H)-yl)-3-phenylprop-2-en-1-one (**3b**) was synthesized via a condensation reaction between cinnamoyl chloride (**1a**) and 1,2,3,4-tetrahydroisoquinoline (**2b**) using triethylamine as a catalyst (**Figure 1**). Cinnamoyl chloride (0.004 mol, 0.67 g) and 1,2,3,4-tetrahydroisoquinoline (0.008 mol, 1.07 g) were combined in an ice bath under stirring conditions (220 rpm). Triethylamine (0.008 mol, 0.81 g) was added as a catalyst. The reaction was monitored by TLC and terminated when the cinnamoyl chloride spot disappeared. The reaction mixture was quenched with a saturated sodium bicarbonate solution followed by 50 mL of distilled water. The precipitated product was filtered using a Buchner funnel. The crude product was recrystallized from hot methanol. Recrystallized crystals were filtered using a Buchner funnel and washed with 10 mL of methanol (twice). The final product was dried in an oven at a constant temperature of 50°C. The yield of the synthesized compound was determined by weighing the dried product.

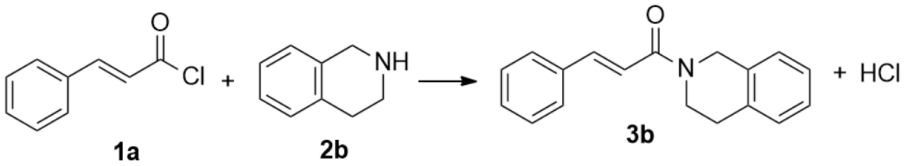


Figure 1. The synthetic route to a target compound (**3b**).

Optimization of reaction time and eluent

Reaction progress was monitored using TLC. Five aliquots were taken from the reaction mixture at specific time points (0, 1, 2, 3, and 4 hours). To optimize the TLC separation, four different eluent systems were evaluated. The comparisons are presented in **Table I**.

Table I. Comparison of eluents for optimization.

Eluent code	Eluent	Comparison
A	*n*-hexane : ethyl acetate	10 : 1
B	*n*-hexane : ethyl acetate	1 : 1
C	*n*-hexane : ethyl acetate : acetone	10 : 1 : 5
D	*n*-hexane : ethyl acetate : acetone	7 : 1 : 5

Data analysis

Organoleptic test

Visual assessment of the product's shape and color was conducted as part of the organoleptic evaluation.

Product compound purity test: melting range test

The melting point of the sample was determined using a capillary tube method. A small amount of the sample was packed into a capillary tube, which was then inserted into the melting point apparatus. The portion of the tube containing the sample was positioned in the center of the apparatus. The temperature was gradually increased, and the melting point was recorded as the temperature at which the sample transitioned from a solid to a liquid state.

Product compound purity test: TLC-densitometry

Thin-layer chromatography was employed to assess the purity of the sample solution. A small amount of the sample was applied to a TLC plate, and the developed chromatogram was visually inspected. The presence of a single spot indicates a pure compound. To further confirm purity, densitometric analysis was performed[15].

Target compound identification: FTIR

The spectra were recorded using the KBr pellet technique in a Shimadzu spectrometer. The pellets were prepared by mixing 1.5-2 mg of 3b with 350 mg of KBr. The 13 mm diameter pellets were prepared in a standard device[16].

Target compound identification: ^{1}H-NMR

Samples were dissolved in $CDCl_3$ within NMR tubes. 1H-NMR spectroscopy was conducted using a JEOL RESONANCE 400 MHz spectrometer. The NMR parameters adhered to the protocols established by Kornberger *et al*.[17] in their previous research.

Antioxidant test using DPPH method

Stock solutions of the target compound (**3b**) and cinnamic acid were prepared at a concentration of 1000 ppm in methanol. Working solutions were then prepared by diluting the stock solutions to concentrations of 100 ppm and 500 ppm using methanol. A 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was prepared in methanol. The DPPH solution was stored in a dark bottle to prevent photodegradation.

The antioxidant activity of **3b** and cinnamic acid solutions was determined using the DPPH free radical scavenging assay. Aliquots (0.2 mL) of each test solution at varying concentrations were mixed with 0.8 mL of DPPH solution. The mixture was then incubated for 30 minutes at room temperature in darkness. Absorbance measurements were recorded at 516 nm using a UV-Vis spectrophotometer[18].

RESULTS AND DISCUSSION

Synthesis of target compound

Compound **3b** was synthesized via a nucleophilic acyl substitution reaction between **1a** and **2b**. The reaction mechanism proceeds through two stages: nucleophilic addition to the carbonyl group and subsequent chloride ion elimination (**Figure 2**)[19]. Triethylamine served as a base to deprotonate intermediate species, facilitate HCl removal, and catalyze the reaction[20]. Tetrahydrofuran was selected as the solvent due to its aprotic nature, which enhances the reaction by minimizing hydrogen bond formation. THF's water solubility facilitates solvent removal through washing[21]. The overall yield of compound **3b** was 81.56%.

Optimization of reaction time and eluent

Reaction optimization revealed that compound **3b** was formed immediately upon mixing of starting materials (**Figure 3**), indicating an instantaneous reaction. Therefore, a reaction time of zero hours was determined to be optimal. The selected eluent system, consisting of *n*-hexane and ethyl acetate in a 1 : 1 ratio (eluent B), demonstrated excellent chromatographic separation, as evidenced by the absence of overlapping spots on the TLC plate (**Figure 4**)[22]. This eluent system effectively

separated compounds with Rf values within the desired range of 0.2-0.8[23,24]. The Rf value of the target compound, **3b**, was determined to be 0.71 using eluent B, confirming its suitability for the separation.

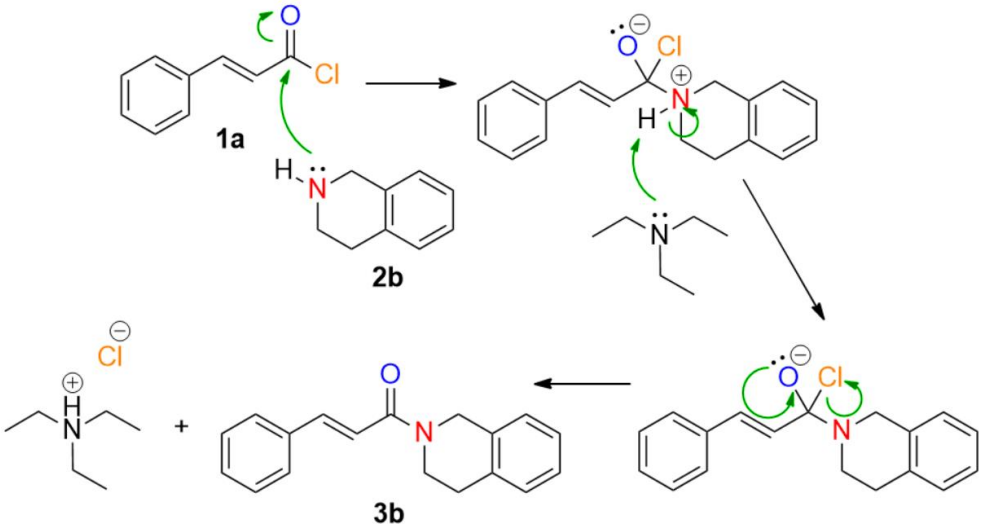


Figure 2. Mechanism for the synthesis of compound **3b**.

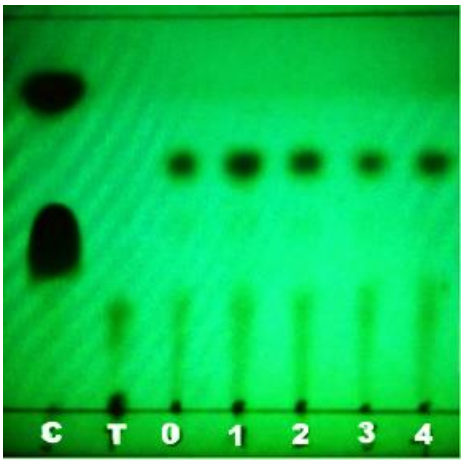
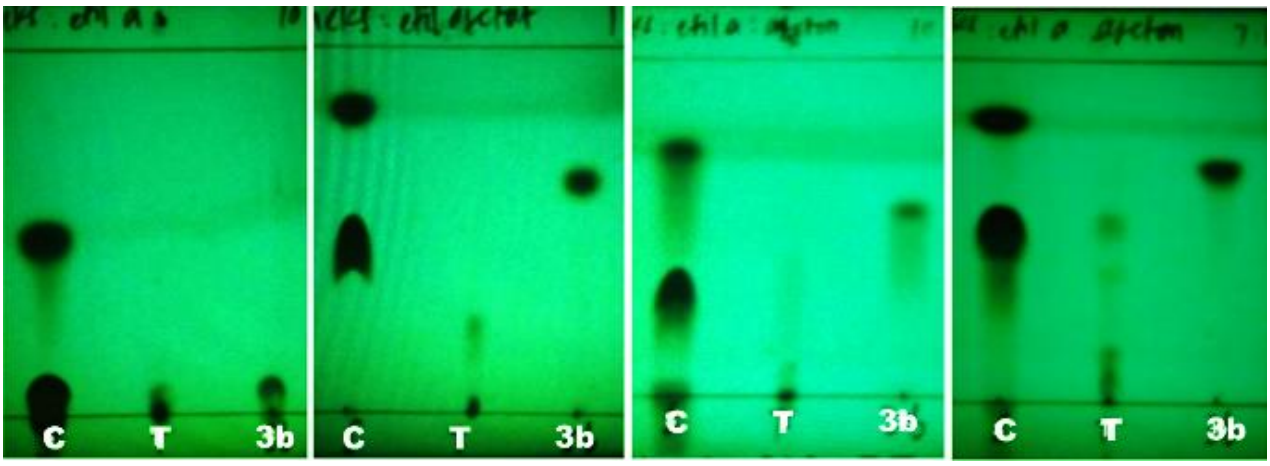


Figure 3. Reaction time optimization results using TLC under UV light 254 nm (**C**: cinnamoyl chloride; **T**: 1,2,3,4-tetrahydroisoquinoline; **0, 1, 2, 3,** and **4**: sampling hours 0, 1, 2, 3, 4).



A **B** **C** **D**

Figure 4. TLC spot from eluent optimization results (eluent **A, B, C**, and **D**); **C**: cinnamoyl chloride; **T**: 1,2,3,4-tetrahydroisoquinoline; **3b**: target compound.

Organoleptic test

The synthesized product exhibited a crystalline form with a distinctive yellow color, as depicted in **Figure 5**.

Figure 5. The crystal of target compound **3b**.

Product compound purity test: melting range test

The purity of a compound can be assessed by its melting range, with a narrow range (typically 1-2°C) indicating high purity[25]. The target compound in this study exhibited a melting range of 72-74°C (**Table II**), confirming its purity.

Table II. Melting range test results of target compounds.

Replication	Melting range (°C)
1	72-74
2	72-73
3	72-74
Average	**72-74**

Product compound purity test: TLC-densitometry

TLC-densitometry analysis revealed three distinct peaks corresponding to the cinnamoyl chloride (**1a**), the tetrahydroisoquinoline (**2b**), and the target compound **3b** (**Figure 6**). The Rf values for these compounds were 0.57, 0.45, and 0.93, respectively. Notably, the chromatogram of the target compound lacked any peaks at the Rf values of **1a** and **2b**, confirming the successful formation of the desired product.

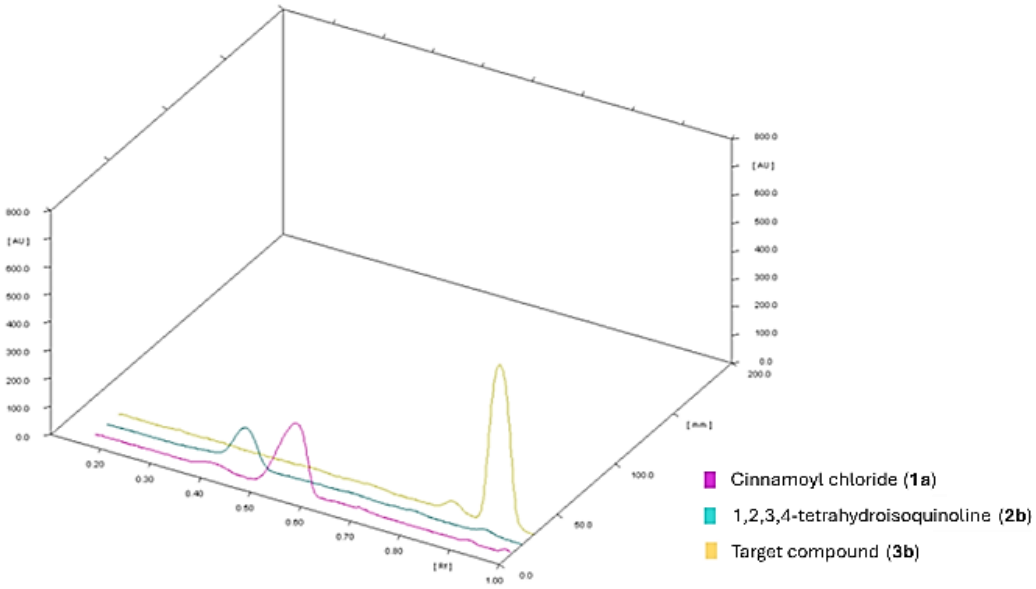


Figure 6. Spectra of compounds **1a**, **2b**, and target compound **3b** at a wavelength of 289 nm.

Target compound identification: FTIR

The synthesized target compound exhibited a distinct functional group compared to the starting compound, an amide group. Amide groups are characterized by their C=O stretching vibration observed at 1680-1630 cm^{-1} in FTIR spectra[26]. The FTIR spectrum of compound **3b** displayed an absorption peak at 1640.35 cm^{-1}, confirming the successful formation of the target product (**Figure 7** and **Table III**).

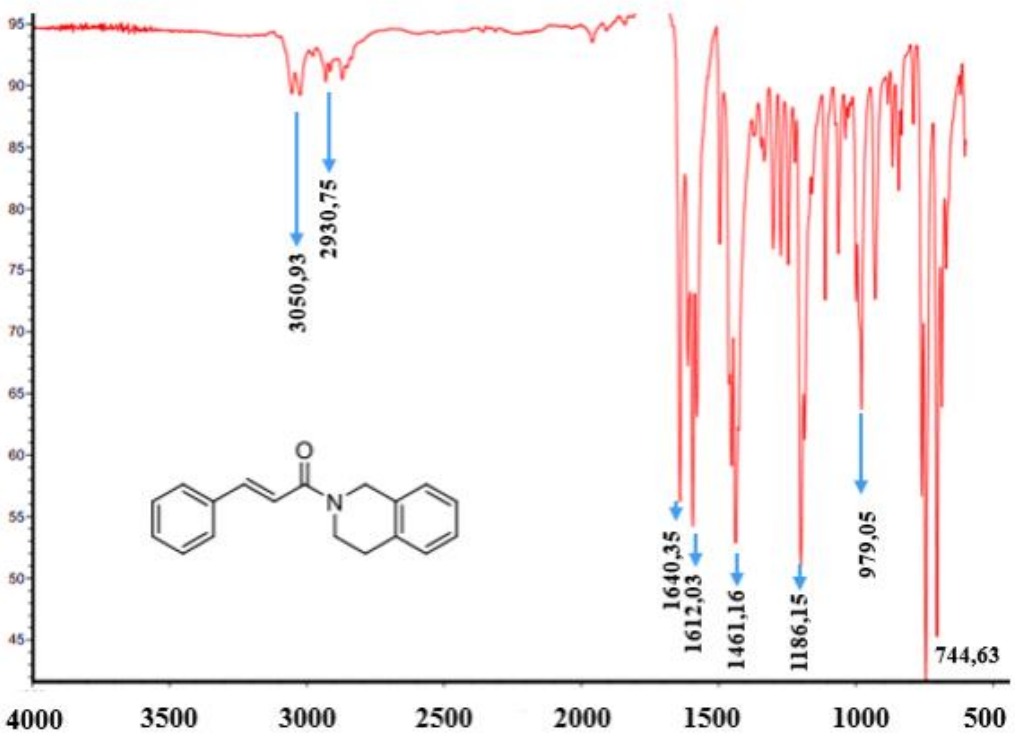


Figure 7. IR spectra of the target compound.

Table III. Interpretation of IR spectra of the target compound.

Functional group	Compound 3b Wavenumber (cm^{-1})	Theoretical Wavenumber (cm^{-1}) (9)
C-H aromatic	744.63	900-690
C-H alkene	979.05	1000-650
C-N	1186.15	1350-1000
C=C aromatic	1461.16	1600 and 1475
C=C	1612.03	1680-1600
C=O amide	1640.35	1680-1630
C-H	2930.75	3000-2850
C-H aromatic	3053.93	3150-3050

Target compound identification: ^{1}H-NMR

^{1}H-NMR spectroscopy provides valuable information regarding the hydrogen atoms present in a molecule[26]. The spectrum of compound **3b**, depicted in **Figure 8**, exhibits eight distinct peaks. Peaks a, b, and c, located at chemical shifts of 2.939 ppm, 3.892 ppm, and 4.832 ppm, respectively, correspond to the -CH_2- group within the piperidine ring. Peaks d and e, appearing at chemical shifts of 6.933 ppm and 7.690 ppm, are attributed to the =CH- group. Peaks f, g, and h, observed within the range of 7.197-7.537 ppm, represent hydrogen atoms from aromatic groups.

The integration ratio in ^{1}H-NMR analysis provides insight into the relative abundance of hydrogen atoms in a compound[27]. For compound **3b**, the integration ratio was determined to be 2 : 2 : 2 : 1 : 1 : 4 : 3 : 2 (**Table IV**). This ratio indicates a total of 17 hydrogen atoms, which aligns with the expected number of hydrogen atoms in compound **3b**. Therefore, the ^{1}H-NMR analysis confirms the identity of the isolated compound as compound **3b**.

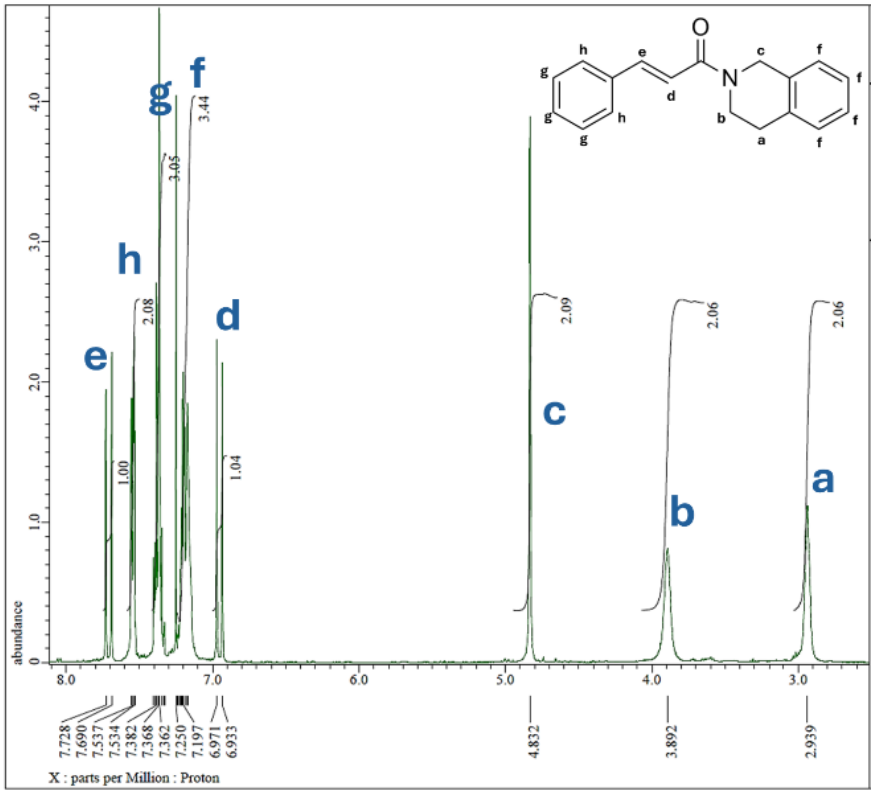


Figure 8. ^{1}H-NMR result of compound **3b**.

Table IV. Interpretation of ^{1}H-NMR spectra of the target compound.

Proton	Type of proton	Chemical shift (ppm)	Integration	Multiplicity
A	-CH_2-	2.94	2.06	Singlet
B	-CH_2-	3.89	2.06	Singlet
C	-CH_2-	4.83	2.09	Singlet
D	=CH-	6.93-6.97	1.04	Doublet
E	=CH-	7.69-7.73	1.00	Doublet
F	H aromatic	7.19-7.25	3.44	Multiplet
G	H aromatic	7.36-7.38	3.05	Multiplet
H	H aromatic	7.53-7.54	2.08	Multiplet

Antioxidant test using DPPH method

The DPPH free radical scavenging assay was employed to evaluate the antioxidant activity of the compounds. This method is based on the principle that antioxidants can donate electrons to neutralize free radicals, leading to a decrease in absorbance at 516 nm[28]. The % inhibition of DPPH serves as an indicator of antioxidant activity. Three concentrations of cinnamic acid (100, 500, and 1000 ppm) and compound **3b** were tested. The results, presented in **Table V**, demonstrate that compound **3b** exhibited significantly higher antioxidant activity compared to cinnamic acid[29], with % inhibition values of 10.61%, 24.24%, and 26.68% at 100, 500, and 1000 ppm, respectively, compared to cinnamic acid's 3.305%, 5.998%, and 6.936%. Statistical analysis using the T-test confirmed a significant difference in antioxidant activities between cinnamic acid and compound **3b**. This suggests that the structural modification involving the addition of the isoquinoline group enhances the antioxidant properties of the compound.

Table V. Compound concentration and antioxidant activity (% inhibition).

Compounds	Concentration (ppm)	% Inhibition ± SD (n=3)
Cinnamic acid	100	3.305 ± 0.245
Cinnamic acid	500	5.998 ± 0.648
Cinnamic acid	1000	6.936 ± 0.187
3b	100	10.61 ± 0.308
3b	500	24.24 ± 1.205
3b	1000	26.68 ± 0.648

CONCLUSION

This study successfully synthesized compound **3b** via a nucleophilic substitution reaction between compounds **1a** and **2b**, yielding an 81.56% yield. Subsequent evaluation of compound **3b's** antioxidant activity at concentrations of 100, 500, and 1000 ppm revealed significant antioxidant potential, with % inhibition values of 11% ± 0.308, 24% ± 1.205, and 27% ± 0.648, respectively. Notably, compound **3b** exhibited superior antioxidant activity compared to cinnamic acid at all tested concentrations. These findings highlight the potential of compound **3b** as a promising antioxidant agent.

ACKNOWLEDGMENT

The authors would like to express their gratitude to the colleagues and students at the Medicinal Chemistry Research Group, Faculty of Pharmacy, Universitas Jember, for their valuable support throughout this research. This research was funded by the Universitas Jember Research and Community Service Grant Program (*Hibah Program Penelitian dan Pengabdian Kepada Masyarakat Universitas Jember*) under Contract Number 3131/UN25.3.1/LT/2023.

AUTHORS' CONTRIBUTION

Conceptualization: Dian Agung Pangaribowo, Ari Satia Nugraha, Ayik Rosita Puspaningtyas, Indah Purnama Sary
Data curation: Dian Agung Pangaribowo, Ari Satia Nugraha, Ayik Rosita Puspaningtyas, Indah Purnama Sary
Formal analysis: Dian Agung Pangaribowo, Ari Satia Nugraha, Ayik Rosita Puspaningtyas, Indah Purnama Sary
Funding acquisition: Dian Agung Pangaribowo, Ari Satia Nugraha, Ayik Rosita Puspaningtyas, Indah Purnama Sary
Investigation: Dian Agung Pangaribowo, Fathunnisa, Ari Satia Nugraha, Ayik Rosita Puspaningtyas, Indah Purnama Sary
Methodology: Dian Agung Pangaribowo, Ari Satia Nugraha, Ayik Rosita Puspaningtyas, Indah Purnama Sary
Project administration: Dian Agung Pangaribowo
Resources: Dian Agung Pangaribowo, Ari Satia Nugraha, Ayik Rosita Puspaningtyas, Indah Purnama Sary
Software: -
Supervision: Dian Agung Pangaribowo, Ari Satia Nugraha, Ayik Rosita Puspaningtyas, Indah Purnama Sary
Validation: Dian Agung Pangaribowo, Ari Satia Nugraha, Ayik Rosita Puspaningtyas, Indah Purnama Sary
Visualization: Dian Agung Pangaribowo, Ari Satia Nugraha, Ayik Rosita Puspaningtyas, Indah Purnama Sary
Writing - original draft: Dian Agung Pangaribowo, Fathunnisa, Ari Satia Nugraha, Ayik Rosita Puspaningtyas, Indah Purnama Sary
Writing - review & editing: Dian Agung Pangaribowo

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Riskianto R, Windi M, Karnelasatri K, Aruan M. Antioxidant Activity of 96% Ethanol Extract of Pepaya Jepang Leaves (Cnidoscolus aconitifolius (Mill.) I. M. Johnst) Using DPPH Method (1,1-diphenyl-2-picrylhydrazyl). Borneo J Pharm. 2022;5(4):315–24. DOI: 10.33084/bjop.v5i4.3511

2. Mortada S, Karrouchi K, Hamza EH, Oulmidi A, Bhat MA, Mamad H, et al. Synthesis, structural characterizations, in vitro biological evaluation and computational investigations of pyrazole derivatives as potential antidiabetic and antioxidant agents. Sci Rep. 2024;14(1):1312. DOI: 10.1038/s41598-024-51290-6; PMCID: PMC10789823; PMID: 38225280

3. Sánchez C. Reactive oxygen species and antioxidant properties from mushrooms. Synth Syst Biotechnol. 2016;2(1):13–22. DOI: 10.1016/j.synbio.2016.12.001; PMCID: PMC5625788; PMID: 29062957

4. Hseu YC, Korivi M, Lin FY, Li ML, Lin RW, Wu JJ, et al. Trans-cinnamic acid attenuates UVA-induced photoaging through inhibition of AP-1 activation and induction of Nrf2-mediated antioxidant genes in human skin fibroblasts. J Dermatol Sci. 2018;90(2):123–34. DOI: 10.1016/j.jdermsci.2018.01.004; PMID: 29395579

5. Drakontaeidi A, Pontiki E. Multi-Target-Directed Cinnamic Acid Hybrids Targeting Alzheimer's Disease. Int J Mol Sci. 2024;25(1):582. DOI: 10.3390/ijms25010582; PMCID: PMC10778916; PMID: 38203753

6. Shollar MM, Merza J, Darwish M, Keshe M. Synthesis, characterization, and biological evaluation of novel cinnamic acid derivatives: cinnamoyl-metronidazole ester and cinnamoyl-memantine amide. Heliyon. 2024;10(9):e29851. DOI: 10.1016/j.heliyon.2024.e29851; PMCID: PMC11058287; PMID: 38694036

7. Natella F, Nardini M, Di Felice M, Scaccini C. Benzoic and cinnamic acid derivatives as antioxidants: Structure- activity relation. J Agric Food Chem. 1999;47(4):1453–9. DOI: 10.1021/jf980737w; PMID: 10563998

8. Pangaribowo DA, Abe M. Photochemical [2 + 2] cycloaddition reaction of carbonyl compounds with Danishefsky diene. Org Biomol Chem. 2020;18(26):4962–70. DOI: 10.1039/d0ob00921k; PMID: 32458869

9. Roussaki M, Zelianaios K, Kavetsou E, Hamilakis S, Hadjipavlou-Litina D, Kontogiorgis C, et al. Structural modifications of coumarin derivatives: Determination of antioxidant and lipoxygenase (LOX) inhibitory activity. Bioorg Med Chem. 2014;22(23):6586–94. DOI: 10.1016/j.bmc.2014.10.008; PMID: 25456384

10. Yang H, Deng M, Jia H, Zhang K, Liu Y, Cheng M, et al. A review of structural modification and biological activities of oleanolic acid. Chin J Nat Med. 2024;22(1):15–30. DOI: 10.1016/s1875-5364(24)60559-5; PMID: 38278556

11. Thangeswaran D, Shamsuddin S, Balakrishnan V. A comprehensive review on the progress and challenges of tetrahydroisoquinoline derivatives as a promising therapeutic agent to treat Alzheimer's disease. Heliyon. 2024;10(10):e30788. DOI: 10.1016/j.heliyon.2024.e30788; PMCID: PMC11128835; PMID: 38803973

12. Jovanović D, Filipović A, Janjić G, Lazarević-Pašti T, Džambaski Z, Bondžić BP, et al. Targeting Alzheimer's Disease: Evaluating the Efficacy of C-1 Functionalized N-Aryl-Tetrahydroisoquinolines as Cholinergic Enzyme Inhibitors and Promising Therapeutic Candidates. Int J Mol Sci. 2024;25(2):1033. DOI: 10.3390/ijms25021033; PMCID: PMC10816625; PMID: 38256107

13. AlNeyadi SS, Amer N, Thomas TG, Al Ajeil R, Breitener P, Munawar N. Synthesis, characterization, and antioxidant activity of some 2-methoxyphenols derivatives. Heterocycl Comm. 2020;26(1):112–22. DOI: 10.1515/hc-2020-0112

14. Kariminezhad Z, Rahimi M, Fernandes J, Maltais R, Sancéau JY, Poirier D, et al. Development of New Resolvin D1 Analogues for Osteoarthritis Therapy: Acellular and Computational Approaches to Study Their Antioxidant Activities. Antioxidants. 2024;13(4):386. DOI: 10.3390/antiox13040386; PMCID: PMC11047542; PMID: 38671833

15. Mukhopadhyay N, Ahmed R, Mishra K, Sandbhor R, Sharma RJ, Kaki VR. A validated, precise TLC-densitometry method for simultaneous quantification of mahanimbine and koenimbine in marketed herbal formulations. Futur J Pharm Sci. 2024;10;23. DOI: 10.1186/s43094-024-00591-8

16. Kim HJ, Liu Y, Zeng L. Fourier Transform Infrared (FT-IR) Spectroscopy and Simple Algorithm Analysis for Rapid and Non-Destructive Assessment of Cotton Fiber Maturity and Crystallinity for Plant Mapping. Sensors. 2024;24(9):2888. DOI: 10.3390/s24092888

17. Kornberger D, Paatsch T, Schmidt M, Salat U. New combined absorption/1H NMR method for qualitative and quantitative analysis of PET degradation products. Environ Sci Pollut Res Int. 2024;31(13):20689–97. DOI: 10.1007/s11356-024-32481-0; PMCID: PMC10927764; PMID: 38393574

18. Yamauchi M, Kitamura Y, Nagano H, Kawatsu J, Gotoh H. DPPH Measurements and Structure—Activity Relationship Studies on the Antioxidant Capacity of Phenols. Antioxidants. 2024;13(3):309. DOI: 10.3390/antiox13030309; PMCID: PMC10967577; PMID: 38539842

19. Munawar S, Zahoor AF, Hussain SM, Ahmad S, Mansha A, Parveen B, et al. Steglich esterification: A versatile synthetic approach toward the synthesis of natural products, their analogues/derivatives. Heliyon. 2024;10(1):e23416. DOI: 10.1016/j.heliyon.2023.e23416; PMCID: PMC10758822; PMID: 38170008

20. Zhang W, Pinna N. Metal Organic Frameworks Synthesis: The Versatility of Triethylamine. Chemistry. 2024;30(23):e202304256. DOI: 10.1002/chem.202304256; PMID: 38300687

21. Lin Y, Zhou Z, Song Z, Shi Q, Hao Y, Fu Y, et al. Insights into the mechanical stability of tetrahydrofuran hydrates from experimental, machine learning, and molecular dynamics perspectives. Nanoscale. 2024;16(12):6296–308. DOI: 10.1039/d3nr04940j; PMID: 38463012

22. Czopek A, Żmudzki P, Dąbrowska M, Starek M, Łątka K, Bajda M, et al. Reversed-phase thin-layer chromatography and ultra-performance liquid chromatography/mass spectrometry to estimate the drug likeness of phosphodiesterase 10A inhibitors with phthalimide core. JPC-J Planar Chromat. 2024;37:299-308. DOI: 10.1007/s00764-024-00298-9

23. Ambarwati N, Nasution NE. Pemurnian Fraksi Ekstrak Etil Asetat Jamur Endofit Aspergillus salwaensis. Farmasis J Sains Farmasi. 2023;4(1):7-12. DOI: 10.36456/farmasis.v4i1.7006

24. Maryam F, Taebe B, Toding DP. Pengukuran Parameter Spesifik dan Non Spesifik Ekstrak Etanol Daun Matoa (Pometia pinnata J.R & G.Forst). J Mandala Pharmacon Indones. 2020;6(1):1–12. DOI: 10.35311/jmpi.v6i01.39

25. Neto BAD, Beck PS, Sorto JEP, Eberlin MN. In Melting Points We Trust: A Review on the Misguiding Characterization of Multicomponent Reactions Adducts and Intermediates. Molecules. 2022;27(21):7552. DOI: 10.3390/molecules27217552; PMCID: PMC9656178; PMID: 36364380

26. Pavia DL, Lampman GM, Kriz GS. Introduction to Spectroscopy: A Guide for Students of Organic Chemistry. Third Edition. Pacific Grove (CA): Thomson Brooks/Cole; 2009. p. 267–96.

27. Emwas AH, Szczepski K, Poulson BG, Chandra K, McKay RT, Dhahri M, et al. NMR as a "Gold Standard" Method in Drug Design and Discovery. Molecules. 2020;25(20):4597. DOI: 10.3390/molecules25204597; PMCID: PMC7594251; PMID: 33050240

28. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. Molecules. 2022;27(4):1326. DOI: 10.3390/molecules27041326; PMCID: PMC8878429; PMID: 35209118

29. Sayed EM, Hassanien R, Farhan N, Aly HF, Mahmoud K, Bakhite EA. Nitrophenyl-Group-Containing Heterocycles. I. Synthesis, Characterization, Crystal Structure, Anticancer Activity, and Antioxidant Properties of Some New 5,6,7,8-Tetrahydroisoquinolines Bearing 3(4)-Nitrophenyl Group. ACS Omega. 2022;7(10):8767-76. DOI: 10.1021/acsomega.1c06994; PMCID: PMC8928486; PMID: 35309417