SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SPIRO- OXINDOLE-CHROMENE DERIVATIVE COMPOUNDS BASED CURCUMINOID AND CHALCONE

S. H. Oktavia¹, A. H. Cahyana¹, , M. Hapsari¹, Rika Tri Yunarti¹
and Agus Rimus Liandi²

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia,
Depok 16424, Indonesia.

²Department of Chemistry, Faculty of Science and Technology, Syarif Hidayatullah State
Islamic University Jakarta, South Tangerang 15412, Indonesia.

Corresponding Author: herrykim@ui.ac.id

ABSTRACT

Multicomponent reactions (MCRs) are an efficient strategy to synthesize various heterocyclic compounds in one pot, and a simple step by combining the reactants in the same flask. In this study, a series of spiro-oxindole substituted chromene rings were synthesized via Knoevenagel and Micheal addition reaction using curcuminoid and chalcone as precursors. The reaction was assisted by Nickel ferrite (NiFe₂O₄) nanocatalyst in ethanol solvent at 70°C. NiFe₂O₄ was synthesized by the co-precipitation method and characterized by FTIR, XRD, and SEM-EDX. The two spiro-oxindole-chromene derivatives synthesized with the NiFe₂O₄ nanocatalyst were characterized their functional group vibrations and molecular mass by FTIR and GCMS, respectively. Under conditions with the addition of 5% (w/w) catalyst, the yields of compound 1 and compound 2 were 95% and 90%. Only compound 2 responded to antibacterial activity test against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*).

Keywords: Spiro-oxindole, Chromene, Knoevenagel Reaction, NiFe₂O₄, Antibacterial Activity.

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INTRODUCTION

Multicomponent reaction (MCR) is a reaction that is carried out using more than two reactants to make a product in the same flask. MCRs allow reactions to build new bonds in one step.¹ One of the reactions that use MCR is in the synthesis of spiro-oxindole that can be widely distributed for drugs and natural material products. Spirooxindole derivative compounds have a variety of antibacterial², anti-cancer³, and significant activity in the heart and Alzheimer's disease.⁴ Based on the research of Ballini et al., the presence of different heterocyclic parts (two or more) in one molecule will increase biocidal activity⁵, such as heterocyclic spiro-oxindole rings, with 4H-chromene substitution. The division of C-3 in spiro-oxindol with pyran/chromene rings (spiropyrans or spiro-oxindole-chromene)⁶ has received attention because of spasmolytic, anticoagulant, diuretic, anticancer, and antinafalic activities.⁷

The biological potential of spiro-oxindole-chromene always encourages chemists to develop efficiency to synthesize it.⁶ The classic spiro-oxindole-chromene synthesis involves one-pot three-components of condensation between isatin, malononitrile, and C-H acid which can be evolved such as dimedone, barbituric acid, naphthols, 4-hydroxycoumarin, resorcinol, 8-hydroxyquinoline^{7,8}, but there are no reports of synthesis spiro-oxindole-chromene using curcuminoid and chalcone as precursors.

Currently, to make the reaction more efficient and maximize the product in a short time many researchers have synthesized spiro-oxindole-chromene compounds through the MCR approach using different catalysts such as urea, potassium phthalimide, inorganic salts, CaCl₂, NH₄Cl, tungstate acid, cellulose - HClO₄, organic bases (DBU, TEA, DMAP, DABCO), cysteine, L-proline and melamine as donor-acceptor pairs, p-TSA, [TBD] [TFA].⁹⁻²⁰ In this study, NiFe₂O₄ nanoparticles which have magnetic properties would be used as catalysts in synthesizing spiro-oxindol-chromene derivatives.

Nickel ferrite (NiFe_2O_4) nanoparticles have been used for catalytic purposes. In recent years there have been many studies that synthesize organic compounds using NiFe_2O_4 catalysts, such as synthesis of 1,4-dihydropyran [2,3-c] pyrazole derivatives²¹, cyanation reactions of various types of aryl halides and heteroaryl halides²², synthesis spiro [indole-3,2'-pyrrole]-2.5'(1H, 1'H)-diones via multi-component condensation reaction²³, oxidation of thiol disulfides and sulfides to sulfoxide at room temperature using $\text{NiFe}_2\text{O}_4\text{-H}_2\text{O}_2$ ²⁴, formation of substituted 4H-chromene reactions²⁵, and synthesis of 1,4-dihydropyridine derivative compound.²⁶ Therefore, this study reports the use of NiFe_2O_4 nanocatalysts for the synthesis of spiro-oxindole-chromene compounds through multicomponent reactions between isatin, malononitrile, curcuminoid, and hydroxy chalcone compounds. The synthesized compounds were characterized and tested for their bioactivity as antibacterial.

EXPERIMENTAL

Materials

All chemicals used were analytical grade not subjected to further purification. These included, $\text{Fe}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$, $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, NaOH, acetophenone, 2-hydroxybenzaldehyde, isatin, malononitrile, curcuminoid, 2-hydroxychalcone, gram-positive bacteria *Bacillus Subtilis* and *Staphylococcus aureus*, gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*, DMSO, ethanol, ethyl acetate.

Synthesis of NiFe_2O_4 Nanoparticles

The NiFe_2O_4 were synthesized via the co-precipitation method reported by Reza was modified. As much as 3 mmol of $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 6 mmol of $\text{Fe}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ were added into 25 mL of deionized water and stirring at room temperature for 30 min. Thereafter, 0.5 M NaOH solution was added dropwise until the pH reaches 10 and a brown precipitate is formed then the precipitate was filtered. The precipitate was washed using deionized water to normal pH (pH=7) and dried at 60°C for 12 h. Then the metal hydroxide composites formed were calcined at 660°C for 2 h.

Synthesis of 2-Hydroxychalcone

The 2-hydroxychalcone synthesis procedure refers to the previous method by replacing vanillin with 2-hydroxybenzaldehyde. 15 mmol acetophenone and 2.5 grams NaOH (in 20 mL distilled water) were added to 3.36 grams 2-hydroxybenzaldehyde (15 mmol) in 9 mL ethanol. The mixture was refluxed for 3 hours at 70°C and monitored by TLC. HCl 10% was added until pH=1 to neutralize the mixture. Then the reaction results were separated by extraction method using ethyl acetate and the product was characterized by its functional group vibration and maximum wavelength. The general reaction of 2-hydroxychalcone formation is shown in Scheme-1.

Scheme-1: Claisen-Schmidt Reaction of 2-hydroxychalcone

Synthesis of Spiro-oxindole-chromene Derivative Compounds

Compound 1 was synthesized by reacting 0.4 mmol isatin, 0.4 mmol malononitrile, and 0.2 mmol curcuminoid. Compound 2 was synthesized by reacting 0.1 mmol isatin, 0.1 mmol malononitrile, and 0.1 mmol 2-hydroxychalcone. Each reaction was assisted by NiFe_2O_4 nanocatalyst under reflux condition in ethanol solvent at 70°C. The reaction was carried out for 4 hours and monitored with TLC. After completion, the mixture was separated from the catalyst using an external magnet and the product was purified by recrystallization with hot ethanol.

Scheme-2: General Reaction of Compounds 1 and 2

Characterization

Functional group vibration is measured by Fourier Transform Infrared Spectroscopy (FTIR, Shimadzu series infrared spectrophotometer in KBr disc). The phase formation was analyzed by X-ray Diffraction (XRD, Malvern Analytical operating at 40 mA, 40 kV). The surface morphologies were scanned by Scanning Electron Microscope (SEM, Sigma Zeiss microscope) and Energy Dispersive X-ray (EDX, Amatek). Mass spectrum was determined by Liquid Chromatography-Mass Spectrometer (LC-MS/MS, UPLCTMH-Class and combined with a Bruker Daltonics Esquire 2000TM ion trap).

Spectral Data of The Representative Compounds

(E)-3-(2-hydroxyphenyl)-1-phenylprop-2-en-1-one. LC-MS/MS $[M+H]^+$: 225.08 (Rt 10.62). FTIR: 3305.16 cm^{-1} (O-H); 3065.02 cm^{-1} (C-H sp^2); 1684.89 cm^{-1} (C=O); 1504.54 cm^{-1} (C=C aromatic).

Compound 1: LC-MS/MS $[M+H]^+$: 759.2220 (Rt 9.325). FTIR: 3732.41 cm^{-1} (N-H amide); 3521.50 cm^{-1} (N-H primary); 3585.85 cm^{-1} (N-H primary); 3194.25 cm^{-1} (C-H sp^2); 2201.83 cm^{-1} (C≡N); 1732.15 cm^{-1} (C=C aromatic); 1619.31 cm^{-1} (C=O amide aromatic); 1271.14 cm^{-1} (C-N stretching); 1034.85 cm^{-1} (C-N); 754.19 cm^{-1} (N-H wag); 685.72 cm^{-1} (C-H aromatic).

Compound 2: LC-MS/MS $[M+H]^+$: 420.13 (Rt 7.808). FTIR: 3640.79 cm^{-1} (N-H amide); 3368.82 cm^{-1} (N-H primary); 3351.46 cm^{-1} (N-H primary); 3091.06 cm^{-1} (C-H sp^2); 2213.41 cm^{-1} (C≡N); 1723.47 cm^{-1} (C=C aromatic); 1654.03 cm^{-1} (C=O amide aromatic); 1227.74 cm^{-1} (C-N stretching); 1019.42 cm^{-1} (C-N); 758.05 cm^{-1} (N-H wag); 689.58 cm^{-1} (C-H aromatic).

Antibacterial Activities

All of the synthesized products were examined for their antibacterial activities against gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and gram-positive bacteria (*Bacillus Subtilis* and *Staphylococcus aureus*) using the disc diffusion method.²⁷ The compound to be tested was made in a concentration of 1000 ppm, which is 0.005 g of the synthesized compound dissolved in 5 mL DMSO. The 0.5 MacFarland standard bacterial cells were spread on the surface agar plate. Impregnate each disc into 50

μL of the synthesized compounds and placed it on the agar media. Then, all plate was incubated at 37°C for 24 h. After an incubation period of observation and measurement of the clear zone that occurs.

RESULTS AND DISCUSSION

The bonds formed in the NiFe_2O_4 nanoparticles are confirmed with Fourier Transform Infrared Spectroscopy (FTIR). Each bond of a functional group will have a different vibration when measured by FTIR. The NiFe_2O_4 FTIR spectrum can be seen in Fig.-1.

Fig.-1: FTIR Spectrum of NiFe_2O_4 Nanoparticles

Figure-1. shows the FTIR absorption spectrum of NiFe_2O_4 functional group vibration ($400\text{--}4000\text{ cm}^{-1}$). Based on the test results, the absorption peaks appear at wavenumbers 3445.97 ; 1617.38 ; 591.20 and 403.13 cm^{-1} . Wavenumber 3445.97 cm^{-1} is vibration stretching between O and H atoms. The broad peak indicates the O-H groups participating in H-bonding interactions to a certain degree. The peak at 1617.38 cm^{-1} is O-H bending group. The two main peaks of metal and oxygen ion absorption groups related to the ferrite spinel structure in NiFe_2O_4 synthesis are in the range of $400\text{--}600\text{ cm}^{-1}$. The first peak is at 591.20 cm^{-1} and the second at 403.13 cm^{-1} , each related to the octahedral and tetrahedral sites of the position of the metal ions in the ferrite spinel.²⁸ The ferrite spinel structure of NiFe_2O_4 , all Ni^{2+} will be on the octahedral side while the Fe^{3+} ions will be distributed evenly on the octahedral and tetrahedral parts. Therefore, the absorption peak that appears at the wave number 403.13 cm^{-1} is the absorption group of atoms at the octahedral site (vibrations from Ni, Fe and O), while the absorption peak that appears at the wave number 591.20 cm^{-1} is the absorption group of atoms at the tetrahedral site (vibrations from Fe and O).²⁹ Open octahedral sites can provide empty orbitals that allow for reactions.³⁰

Fig.-2. illustrate the X-ray diffraction of NiFe_2O_4 nanoparticles. From the results of XRD analysis on nanoparticles, it can be seen that there is a typical peak of NiFe_2O_4 at 2θ 30.34° ; 35.76° ; 37.24° ; 43.31° ; 54.20° ; 57.42° ; 63.06° . These peaks indicate the formation of NiFe_2O_4 (JCPDS: 01-074-2081) and the crystal structure was cubic spinel.³¹ However, there was another peak at 2θ 33.21° which is a typical peak of the $\alpha\text{-Fe}_2\text{O}_3$ (hematite) phase is antiferromagnetic. While NiFe_2O_4 is ferrimagnetic, therefore the presence of hematite in the NiFe_2O_4 can contribute to the magnetic properties of NiFe_2O_4 nanoparticles. Based on XRD data, the crystal size of the NiFe_2O_4 nanoparticles was calculated using the Debye-Scherrer equation.

$$D = \frac{0,9 \lambda}{\beta \cos \theta} \quad (1)$$

Where, λ is the electromagnetic wavelength (nm), while β is the peak width of the half-peak (FWHM) in radians, and θ is the Bragg angle position. From calculations using these equations, the crystal size of NiFe_2O_4 is 23.15 nm .

Fig.-2: XRD Patterns of NiFe_2O_4

Morphology and surface of NiFe_2O_4 nanoparticles were investigated by scanning electron microscopy (SEM). SEM analysis shows that NiFe_2O_4 nanoparticles do not have a uniform size.³² The white granules seen on the surface are due to the induction of Fe in Ni. The average particle size of NiFe_2O_4 nanoparticles based on SEM results is 71.65 nm. SEM micrographs illustrate that the sample contains a micrometric aggregation of small particles. The agglomeration shows that crystallites have pores on the surface. The SEM image shows the agglomerated form of NiFe_2O_4 nanoparticles. Because nanoparticles have high surface energy, they usually agglomerate and grow into larger aggregate.³³

Fig.-3: Morphology of NiFe_2O_4 Nanoparticles with SEM

The scanning electron microscope (SEM) can also be used to find out information on the composition of elements contained in a sample by detecting characteristic X-rays. The EDX analysis is used to evaluate the elemental composition of NiFe_2O_4 nanoparticles. Based on Fig.-4, the existence of Ni, Fe, and O elements is already approaching the NiFe_2O_4 atomic ratio of 1: 2: 4.

Fig.-4: The EDX Patterns of NiFe_2O_4 Nanoparticles

The various amount of catalyst in the reaction was applied to optimize the reaction condition for the synthesis of spiro-oxindole-chromene on the reaction of isatin, malononitrile and curcuminoid/ 2-hydroxychalcone is examined (Table-1). The two of spiro-oxindole-chromene derivatives synthesized namely 2-amino-6-((1E,6E)-7-(2-amino-3-cyano-8-methoxy-2'-oxospiro[chromene-4,3'-indolin]-5-yl)-3,5-dioxohepta-1,6-dien-1-yl)-8-methoxy-2'-oxospiro[chromene-4,3'-indoline]-3-carbonitrile (compound 1), and (Z)-2-amino-2'-oxo-8-(3-oxo-3-phenylprop-1-en-1-yl)spiro[chromene-4,3'-indoline]-3-carbonitrile (compound 2). From Table-1, it was showed that the addition of 5% NiFe₂O₄ nanoparticles was sufficient to complete the reaction in 4 hours and produced good yields in 10 mL ethanol as a solvent. Increasing the amount of catalysts by more than 5% could not increase the yield in the reaction, it proves that NiFe₂O₄ is an efficient catalyst and can be used in organic reactions.

Table-1: Optimization of the Catalyst Concentration in the Reaction

Entry	NiFe ₂ O ₄	Compound 1	Compound 2
1	0 %	trace	trace
2	5 %	95 %	90 %
3	10 %	90 %	86 %

Evaluation of the biological activity of spiro-oxindole-chromene derivatives was tested as antibacterial agents against various types of gram-negative and gram-positive bacteria using the disk diffusion method. From the test, the antibacterial activity of the spiro-oxindole-chromene compounds is shown in Table 2. Only compound 2 responded to antibacterial activity test against gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*).

Table-2: Antibacterial Activity of Spiro-oxindole-chromene

Code	Parameter	Inhibition Zone in mm			
		<i>B. subtilis</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
AB	Antibiotic Gentamicin	11.57	13.6	10.28	11.74
968	Compound 1	-	-	-	-
969	Compound 2	7.5	-	9.4	-
DMSO	DMSO	-	-	-	-

Fig.-5: Antibacterial Activity (a) *B.subtilis* (b) *S.aureus*

CONCLUSION

An efficient and simple procedure for synthesis of spiro-oxindole-chromene has been found by multicomponent reaction of isatin, malononitrile, and curcuminoid/ 2-hydroxychalcone in 10 mL ethanol at 70°C in the presence of NiFe₂O₄ as an efficient catalyst. The two of spiro-oxindole-chromene derivatives synthesized namely 2-amino-6-((1E,6E)-7-(2-amino-3-cyano-8-methoxy-2'-oxospiro[chromene-4,3'-indolin]-5-yl)-3,5-dioxohepta-1,6-dien-1-yl)-8-methoxy-2'-oxospiro[chromene-4,3'-indoline]-3-carbonitrile (compound 1), and (Z)-2-amino-2'-oxo-8-(3-oxo-3-phenylprop-1-en-1-yl)spiro[chromene-4,3'-indoline]-3-carbonitrile (compound 2) Therefore, based on the screened spiro-oxindole-chromene

derivates, compound 2 was considerably promising as an antibacterial agent with inhibition zone 7.5 mm for *B. subtilis* and 9.4 mm for *P. aureginosa*, respectively.

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