**Chimica et Natura Acta**
p-ISSN: 2355-0864 e-ISSN: 2541-2574
Homepage: http://jurnal.unpad.ac.id/jcena

# Synthesis, Characterization, and BSLT Test of Pyrazoline and Pyrazole Compounds from Chalcone using Microwave Irradiation

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DOI: https://doi.org/10.24198/cna.v11.n2.47889

**Abstract:** Pyrazole is an organic compound, classified as an alkaloid, featuring a heterocyclic five-membered ring structure composed of three carbon atoms and two adjacent nitrogen atoms, forming an endocyclic double bond. Pyrazole has various biological activities, such as antibacterial, anticanc er, antiinflammatory, antidepressantion, and antioxidant. Pyrazole 2-(5-(4-chlorophenil)-1-phenil-1H-pyrazole-3il)phenol (TFP-2OH-4Cl) was synthesized via oxidative aromatization reaction of pyrazoline 2-(5-(4-chlorophenil)-1-phenil-4,5-dihydro-1H-pyrazole-3-il)phenol (PF-2OH-4Cl) with glacial acetic acid. Chalcone, pyrazoline, and pyrazole were synthesized using the microwave at 180 W at 85oC. The purity of the compounds was monitored on TLC plates, melting point, and HPLC analysis. The structure of synthesized compounds was confirmed by UV, FTIR, HNMR, and HRMS spectroscopic analyses. The yields obtained from synthesizing 2OHA-4ClBD, PF-2OH-4Cl, and TFP-2OH-4Cl compounds were 63.49%, 64.35 %, and 40.42 %. The cytotoxic activity of chalcone, pyrazoline, and pyrazole was evaluated by Brine Shrimp Lethality Test (BSLT) method with shrimp larvae (Artemia salina Leach). Pyrazoline PF-2OH-4Cl is non-toxic as LC50 260.016 µg/mL, while chalcone 2OHA-4ClBD and pyrazole TFP-2OH-4Cl showed high toxicity with LC50 2.874 and 5.584 µg/mL.

**Keywords:** chalcone, pyrazole, pyrazoline, cytotoxicity

***Abstrak:*** *Pirazol adalah senyawa organik kelompok alkaloid dengan struktur heterosiklik lima cincin dengan tiga karbon dan dua nitrogen yang berdekatan menjadi satu ikatan rangkap endosiklik. Pirazol memiliki berbagai aktivitas biologis, seperti antibakteri, antikanker, antiinflamasi, antidepresi, dan antioksidan. Pirazol 2-(5-(4-klorofenil)-1-fenil-1H-pirazol-3il)fenol(TFP-2OH-4Cl) disintesis melalui reaksi aromatisasi oksidatif pirazolin 2-(5-(4-klorofenil)-1-fenil-4,5-dihidro-1H-pirazol-3-il)fenol (PF-2OH-4Cl) dengan asam asetat glasial. Kalkon, pirazolin, dan pirazol disintesis menggunakan microwave pada 180 W pada suhu 85ºC. Pemantauan kemurnian senyawa dilakukan melalui beberapa metode, termasuk penggunaan kromatografi lapis tipis (KLT), penentuan titik leleh, serta analisis menggunakan kromatografi cair kinerja tinggi (KCKT). Struktur senyawa hasil sintesis dikonfirmasi dengan analisis spektroskopi UV, FTIR, 1HNMR, dan HRMS. Persentase rendemen yang diperoleh dari sintesis senyawa 2OHA-4ClBD, PF-2OH-4Cl, dan TFP-2OH-4Cl masing-masing adalah 63,49%, 64,35%, dan 40,42%. Aktivitas sitotoksik kalkon, pirazolin, dan pirazole dievaluasi dengan metode Brine Shrimp Lethality Test (BSLT) dengan larva udang (Artemia salina Leach). Pirazoline PF-2OH-4Cl tidak beracun dengan* $LC_{50}$ *260.016 µg/mL, sedangkan kalkon 2OHA-4ClBD dan pirazol TFP-2OH-4Cl menunjukkan toksisitas tinggi dengan* $LC_{50}$ *2,874 dan 5,584 µg/mL.*

***Kata kunci:*** *kalkon, pirazol, pirazolin, sitotoksik*

## INTRODUCTION

The development of the chemical and pharmaceutical industries to produce a variety of drug molecules is the target of the reaction of several chemical compounds in a method carried out through the synthesis of organic compounds (Lokesh *et al.* 2018; Coley *et al.* 2019; Struble *et al.* 2020; Kostopoulou *et al.* 2021). One of the interesting organic compounds to observe is pyrazole. Pyrazole compounds have an essential role with high biological activity as a pharmacophore in designing drugs and syntones (a hypothetical target molecule)

to synthesize organic compounds (Ramadan *et al*. 2021). Pyrazoles have garnered significant attention due to their multifaceted role in drug discovery. Their unique structural features make them a cornerstone in medicinal chemistry, enabling the creation of compounds with a wide array of bioactivities. These bioactivities include antibacterial, anti-malarial, anticancer, antiviral, anti-inflammatory, antioxidant, anticonvulsant, antimicrobial, antifungal, anti-tumor, and antipyretic properties. This versatility positions pyrazole-containing compounds as promising candidates for applications across various therapeutic areas. In this context, exploring the potential applications of pyrazoles becomes paramount, as they hold the key to addressing numerous health challenges through innovative drug design (Rani *et al*. 2017; Gomes *et al*. 2020; Mathew *et al*. 2021).

Pyrazole is a pentacyclic heterocyclic organic compound containing a nitrogen atom belonging to the secondary metabolite group of alkaloids. According to Seifinoferest *et al*. (2021), the N-N bonds in the pyrazole ring play an essential role in various biological activities (Tigreros & Portilla 2020; Wang *et al*. 2020). Afriana *et al*. (2020) has made significant strides in the synthesis of methyl group-substituted pyrazole derivatives. This achievement was accomplished through a condensation reaction between phenylhydrazine and ethyl acetoacetate, facilitated by an environmentally friendly catalyst, nano-ZnO. These methyl-substituted pyrazole derivatives have demonstrated remarkable bioactivity, particularly in the medical field, where they have shown promise as potent antimicrobial agents. The synthesis process yielded impressive results, with yields reaching as high as 95% (Zamri *et al*. 2019; Afriana *et al*. 2020; Seifinoferest *et al*. 2021; Tayde & Lande 2021). These findings highlight the potential applications of methyl-substituted pyrazole derivatives in addressing critical healthcare challenges, further underscoring the importance of pyrazoles in medicinal chemistry and drug development.

One of the preparative methods commonly used to synthesize pyrazoles is the cyclization reaction between unsaturated α, β ketones, and hydrazine and its derivatives (Faisal *et al*. 2019; Khatir & Irannejad 2021; Beebany *et al*. 2023). Variations in the structure of pyrazole compounds can be obtained by varying electron-rich functional groups such as fluoro, chloro, bromo, nitro, and methoxy, which will produce compounds with various bioactivities (Lokesh *et al.* 2018; Mathew *et al.* 2021). The introduction of these electron-rich functional groups enables the synthesis of pyrazole derivatives with a wide range of structural variations. By systematically altering the structure of pyrazoles, researchers have harnessed the potential to develop compounds with tailored properties for various therapeutic applications, spanning antibacterial, anti-malarial, anticancer, antiviral, anti-inflammatory, antioxidant, anticonvulsant, antimicrobial, antifungal, anti-tumor, and antipyretic activities. This ability to fine-tune the structural characteristics of pyrazoles underscores their significance in medicinal chemistry and pharmaceutical research, where the pursuit of targeted drug design remains paramount.

This research is interesting because of the modification of pyrazole compounds with chloro and hydroxy halogen groups. According to (Aljamali 2020), adding halogen groups to the ring increases biological activity, which is vital in medicine. This study used microwave irradiation to synthesize pyrazole compounds from the reaction between chalcone and phenylhydrazine to produce pyrazoline, and then the pyrazoline was oxidized using glacial acetic acid (Zamri *et al*. 2019; Afriana *et al*. 2020). This method was chosen because of several advantages, such as being practical for compound synthesis, fast reaction rate, and producing a larger yield than an oxidative aromatization reaction between pyrazoline and glacial acetic acid was carried out using reflux. This not only expedites the production of pyrazole compounds but also aligns with the principles of green chemistry by minimizing resource consumption and waste generation. Microwave irradiation synthesis is a powerful and innovative technique used in chemical and material science research. It involves the use of microwave radiation to heat reaction mixtures and facilitate chemical reactions. This method has gained popularity due to its numerous advantages over traditional reflux methods, which typically involve heating a reaction mixture using a conventional heating source, such as a heating mantle or oil bath.

The synthesized compounds were tested for bioactivity through a cytotoxic activity using the Brine Shrimp Lethality Test (BSLT) method. This examination was conducted as an initial evaluation of substances and served as a foundation for assessing the toxicity of cell cultures and their anti-tumor and anticancer properties. The benefit of this test lies in its swiftness, simplicity, reproducibility, and lack of substantial expenses. However, it's essential to acknowledge that while the BSLT method offers several advantages, it also comes with some limitations. For instance, the results obtained from this test may not always directly correlate with specific biological activities in more complex systems, such as mammalian models. Therefore, while BSLT serves as an efficient initial screening tool, further investigations using more advanced assays and in vivo studies are typically necessary to validate and elucidate the specific bioactivity of compounds. These compounds could serve as valuable candidates for further investigation and development into pharmaceuticals or therapeutic agents. However, it's crucial to approach these findings with caution and recognize the need for additional studies to comprehensively understand the mechanisms and specific applications of these

compounds in medical contexts (Nopitasari *et al.* 2017; Swantara *et al.* 2017; Prasetyaningrum *et al.* 2018; Setiawan *et al*. 2018; Siegel *et al.* 2019; Simorangkir *et al*. 2021).

## MATERIAL AND METHODS

The tools and materials used in this study were a set of distillation apparatus, a set of reflux tools, an analytical balance, a magnetic stirrer, column chromatography, a vacuum pump, a Buchner funnel, a microwave oven (Samsung ME109F), glassware commonly used in the Chemistry Laboratory, FMIPA-UR, a pressure tube using a microwave reactor (Anton-Paar Monowave 50). TLC vessel, Fisher John melting point determination tool (SMP 1-Stuart®), micropipette, UV lamp (Camag® 254 and 366 nm), ultrasonic (Ney®), UV-Visible spectrophotometer (Genesys 10S UV-VIS v4. 002 2L9N175013), HPLC equipment, FTIR spectrophotometer (FTIR Shimadzu), IR Prestige-21, a set of HRMS tools, and NMR spectrometer (Agilent 500 MHz and 125 MHz).

The materials used in this study were 4-chlorobenzaldehyde (Merck), 2-hydroxy acetophenone (Merck), potassium hydroxide (KOH) (Merck), hydrochloric acid (HCl) (Merck), phenylhydrazine (Merck), glacial acetic acid ( Merck), GF254 TLC plates (Merck), methanol, n-hexane, absolute ethanol, ethyl acetate, dimethyl sulfoxide (DMSO) (Merck), universal indicator, dichloromethane (DCM), DM aqua, seawater, aluminum foil, and Artemia larvae Leach's salina obtained from the collection of the Chemical Oceanography Laboratory, Department of Marine Science, FPK, University of Riau.

### Synthesis of chalcone (E)-3-(4-chlorophenyl)-1-(2-hydroxyphenyl)pro*p*-2-en-1-one (2OHA-4ClBD)

The combination of 4-chlorobenzendehyde (0.7031 g; 5 mmol), 2-hydroxy acetophenone (0.6806 g; 5 mmol), and 6 N KOH solution (5 mL) in absolute ethanol (15 mL) was irradiated at microwave 180 W for 1-2 minutes. Reaction control was carried out every 30 seconds using TLC. TLC was used to control the reaction every 30 seconds. Following the reaction, 15 mL of cold DM aqua was added to the reaction mixture, and the pH of the cross was neutralized with 1 N HCl solution. A universal indicator was used to determine the pH of the cross. The resulting reaction mixture was then chilled for 24 hours. The solid was then filtered through a Buchner funnel, rinsed with cold aqua DM and n-hexane, and allowed to dry at room temperature. Methanol was used to recrystallize the unpurified chalcone solid. Furthermore, the resulting solids were TLC tested for purity (Ikhtiarudin *et al*., 2020).

### Synthesis of the pyrazoline compound 2-(5-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)phenol (PF-2OH-4Cl)

An Erlenmeyer flask was filled with the chalcone compound 2OHA-4ClBD (0.2585 g; 1 mmol) and 10 mL of ethanol. The chalcone was finally ultrasonically dissolved until it was homogenous. Following that, phenylhydrazine (0.324 g; 3 mmol) was added and ultrasonically homogenized. Five drops of glacial acetic acid were added to the combination, which was then microwaved at 180 W for 24 hours. TLC was used to regulate a reaction every four hours. After the reaction was completed, the reaction mixture was allowed to stand at ambient temperature for 24 hours and in the refrigerated for 24 hours. The formed solid was rinsed with distilled water and cold methanol before being filtered through a Buchner funnel. Furthermore, the compounds were purified using the TLC test, melting point measurement, and HPLC analysis.

### Synthesis of the pyrazole compound 2-(5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-3-yl) phenol (TFP-2OH-4Cl)

The pyrazoline compound PF-2OH-4Cl (0.209 g; 0.6 mmol) was put into the reflux flask, and glacial acetic acid (5 mL) was added. The mixture was refluxed for 12 hours in an oil bath at 85oC. Reactions were controlled every 6 hours using TLC. After the reaction was complete, the reaction products were transferred to a glass beaker added with DM aqua (20 mL) and then neutralized using NaOH 3 N. Once neutral from the universal indicator was known, then allowed to stand for 24 hours in the refrigerator. The solid formed was filtered using a Buchner funnel, washed with cold methanol, and dried at room temperature. The pyrazole compound that is not pure is columned using hexane: ethyl acetate solvent (eluent) with an elution gradient, then the solvent is evaporated to form the desired solid. Furthermore, the compounds obtained were tested for purity by TLC test, melting point measurement, and HPLC analysis. The scheme of pyrazole synthesis from the initial precursor to the formation of pyrazole can be seen in Figure 1.

### Brine Shrimp Lethality Test

The chalcone, pyrazoline, and pyrazole compounds were measured up to 2 mg and dissolved in 2 mL of methanol (mother liquor, 1000 g/mL concentration), after which dilutions with different concentrations, 100 g/mL, 10 g/mL, 1 g/mL, and 0.1 g/mL, were made by progressive dilution. Different liquid concentrations were pipetted into each vial of up to 0.5 mL three times. After then, the solvent was evaporated until it was entirely dry. After that, each vial was filled with 50 µL DMSO and a small amount of seawater. In the vial, 10 shrimp larvae born from shrimp eggs (Artemia salina Leach) were placed, and saltwater to the calibration limit of 5 mL was added. The number of dead shrimp larvae within 24 hours

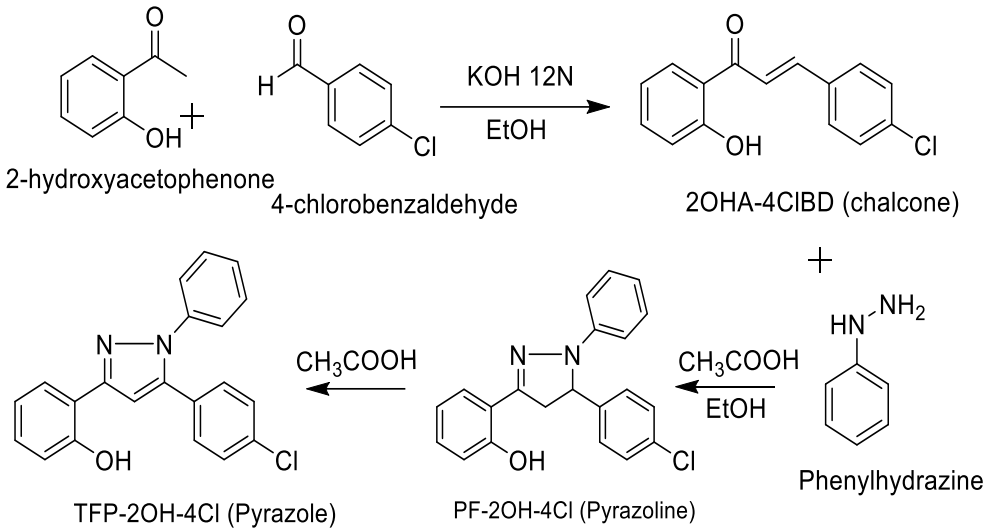
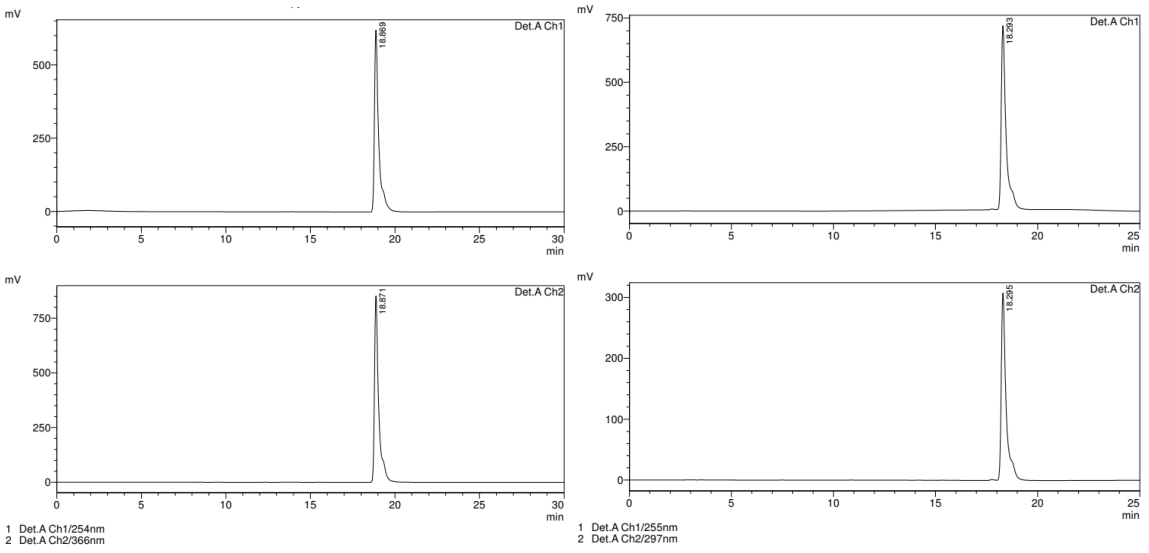



**Figure 1.** The reaction scheme for the synthesis of the pyrazole compound

was used to calculate the cytotoxicity. The acquired data were analyzed using the curve method and the probit analysis table to calculate the LC50 value. This method was based on Meyer *et al*. 1982.

## RESULTS AND DISCUSSION

The pyrazole compound was synthesized from the initial chalcone 2OHA-4ClBD, obtained from the Claisen-Schimidt condensation reaction between 4-chlorobenzaldehyde and 2-hydroxy acetophenone by microwave irradiation at a power of 180 W. The chalcone was then reacted with phenylhydrazine to produce pyrazoline compound PF-2OH- 4Cl, pyrazoline is oxidized with a glacial acetic acid catalyst to produce pyrazole. The PF-2OH-4Cl and TFP-2OH-4Cl synthesis yielded faded yellow crystals (64.35% with 142-143$^{o}$C) and white crystals (40.4% with 128-129$^{o}$C), respectively. A pure compound has a melting point range of 2$^{o}$C from first melting to total melting. The HPLC chromatogram was used to validate the purity of the PF-2OH-4Cl and TFP-2OH-4Cl compounds. The chromatogram displayed a prominent peak at 254 nm and 366 nm, with a retention time of 18.87 minutes, as well as a dominant peak at 255 nm and 297 nm, with a retention time of 18.29 minutes. Since only one dominant peak is observed in the chromatogram, it indicates that the synthesized molecule is of high purity (Figure 2).




(a) (b)

**Figure 2**. HPLC chromatogram of pyrazoline compound PF-2OH-4Cl (a) and pyrazole compound TFP-2OH-4Cl (b).

The PF-2OH-4Cl and TFP-2OH-4Cl compounds were characterized using UV, FTIR, $^1$H-NMR, and HRMS spectroscopy. The UV spectrum of PF-2OH-4Cl showed three maximum absorption peaks at 253 nm, 313 nm, and 353 nm. At a wavelength of 353 nm, it shows the electronic transition π→π* in the *o*-hydroxy substituted phenyl ring conjugated system. Absorption at a wavelength of 253 nm shows an electron transition from the bonding orbital π→π* the conjugated double bond in the phenyl ring attached to the N atom of the pyrazoline ring, while absorption at a wavelength of 313 nm shows an electronic transition π→π* in a substituted phenyl ring *p*-chloro. Meanwhile, the UV spectrum of TFP-2OH-4Cl showed two maximum absorption peaks at wavelengths (λ) 255 and 297 nm.

The maximum absorption at λ 255 nm indicates an electron transition from the π→π* bonding orbital in the o-hydroxy substituted phenyl ring conjugated system, while the absorption at 297 nm shows an electronic transition π→π* in the *p*-chloro substituted phenyl ring. The electronic transition π→π* *p*-chloro-substituted phenyl ring TFP-2OH-4Cl (297 nm) has an absorption maximum at a lower wavelength than that of *p*-chloro-substituted phenyl PF-2OH-4Cl (313 nm) (Figure 3).

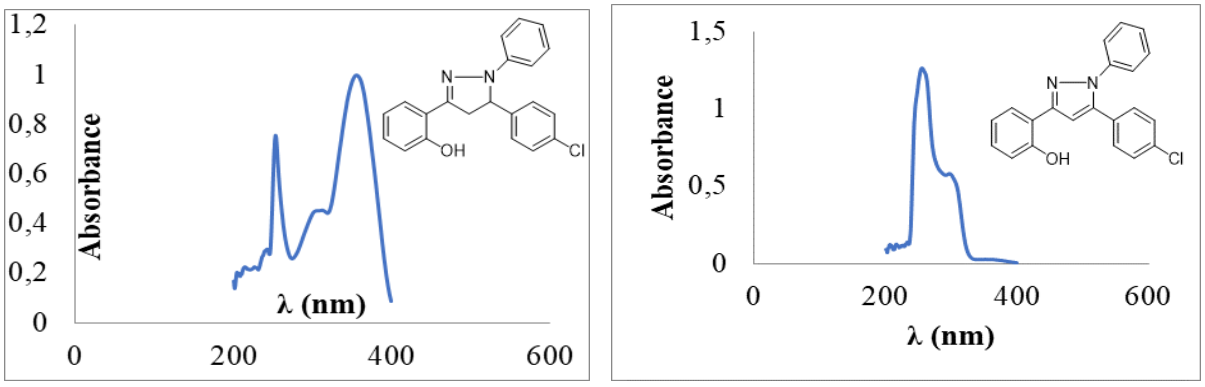
The FTIR spectrum of PF-2OH-4Cl shows absorption at wave numbers (cm$^{-1}$) 3147 (OH), 3046 (C-H Aromatic), 2927 (C-H Aliphatic), 1598 (C=N), 1498 (C=C), 1325 (C-N), 1138 (C-O) and 745 (C-Cl). In the FTIR spectrum of the compounds TFP-2OH-4Cl 3119 (OH), 3068 (C-H Aromatic), 2965 (C-H Aliphatic), 1582 (C=N), 1501 (C=C), 1367 (C-N), 1090 (C-O) and 745 (C-Cl). There is no significant difference in the IR spectra of the two compounds, where the peaks in the wave numbers of the two compounds are not much different. In these compounds, the OH vibrational wave number should be at 3200-3500 cm$^{-1}$. Due to intramolecular hydrogen bonds between the OH and N groups in the pyrazoline and pyrazole frameworks, the wave numbers are smaller (Figure 4).

$^1$H-NMR spectrum of PF-2OH-4Cl compound ($CDCl_3$) $\delta$ (ppm): 10.73 (s, 1H, OH), 7.35 (d, *J* = 8.5 Hz, 2H, H-3”’, H-5”’), 7.32 – 7.26 (m, 3H, H-2”’, H-6”’, H-4”), 7.26 – 7.19 (m, 2H, H-3’, H-5’), 7.13 (dd, *J* = 7.7, 1.6 Hz, 1H, H-6”), 7.08 (dd, *J* = 8.2, 1.2 Hz, 1H, H-3”), 6.95 (d, *J* = 7.6 Hz, 2H, H-2’, H-6’), 6.93 – 6.84 (m, 2H, H-4’, H-5”), 5.22 (dd, $J_{xb}$ = 12.2, $J_{xa}$ = 7.5 Hz, 1H, $H_x$), 3.97 (dd, $J_{ba}$ = 17.2, $J_{bx}$ =12.3 Hz, 1H, $H_b$), 3.23 (dd, $J_{ab}$ = 17.2, $J_{ax}$ = 7.5 Hz, 1H, $H_a$).

$^1$H-NMR spectrum of TFP-2OH-4Cl compound (500 MHz, ($CDCl_3$) $\delta$ (ppm): 10.76 (s, 1H, OH), 7.65 (dd, *J* = 7.8, 1.7 Hz, 1H, H-6”), 7.42 – 7.36 (m, 3H, H-2’, H-6’, H-4”), 7.36 – 7.31 (m, 4H, H-6”’, H-2”’, H-3’, H-5’), 7.31 – 7.21 (m, 3H, H-5”’, H-3”’, H-4’), 7.07 (dd, *J* = 8.3, 1.2 Hz, 1H, H-3”), 7.00 – 6.93 (t, *J* = 7.5 Hz, 1H, H-5”), 6.89 (s, 1H, H-4).

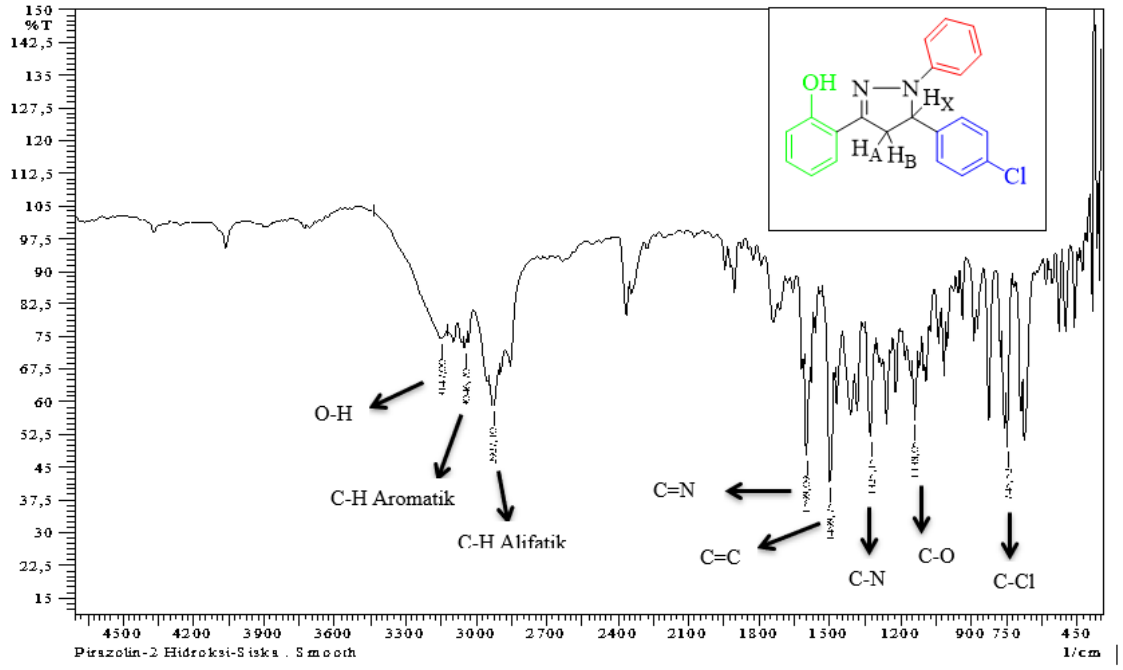
The $^1$H-NMR spectrum shows the number of protons corresponding to the expected structure's protons. The results of $^1$H-NMR analysis of pyrazole compounds do not have the spin pattern of the ABX system like pyrazoline compounds which is the primary identity of pyrazoline compounds. Therefore, this may indicate that the compound being analyzed is a pyrazole and not a pyrazoline compound. The $^1$H-NMR spectra of PF-2OH-4Cl and TFP-2OH-4Cl have the same proton peak from hydroxy substituted in phenyl shown at a chemical shift of 10.73 ppm, and this proton has the farthest chemical change because of π bond conjugation in aromatic compounds so that the proton is not protected (deshielding) (Figure 5).

The $^1$H-NMR spectrum of PF-2OH-4Cl has different proton peaks of Ha, Hb, and Hx compared to that of TFP-2OH-4Cl, which only has one proton peak on C-4. There is a robust conjugation system of two C=C double bonds in the N-N pyrazole group which causes the proton on C-4 to be deshielded compared to the ABX system in pyrazoline so that it has a further chemical shift at 6.89 ppm with an orientation undershirt. The $^1$H-NMR spectrum of PF-2OH-4Cl compound shows a proton peak that is typical for pyrazoline compounds with a doublet of

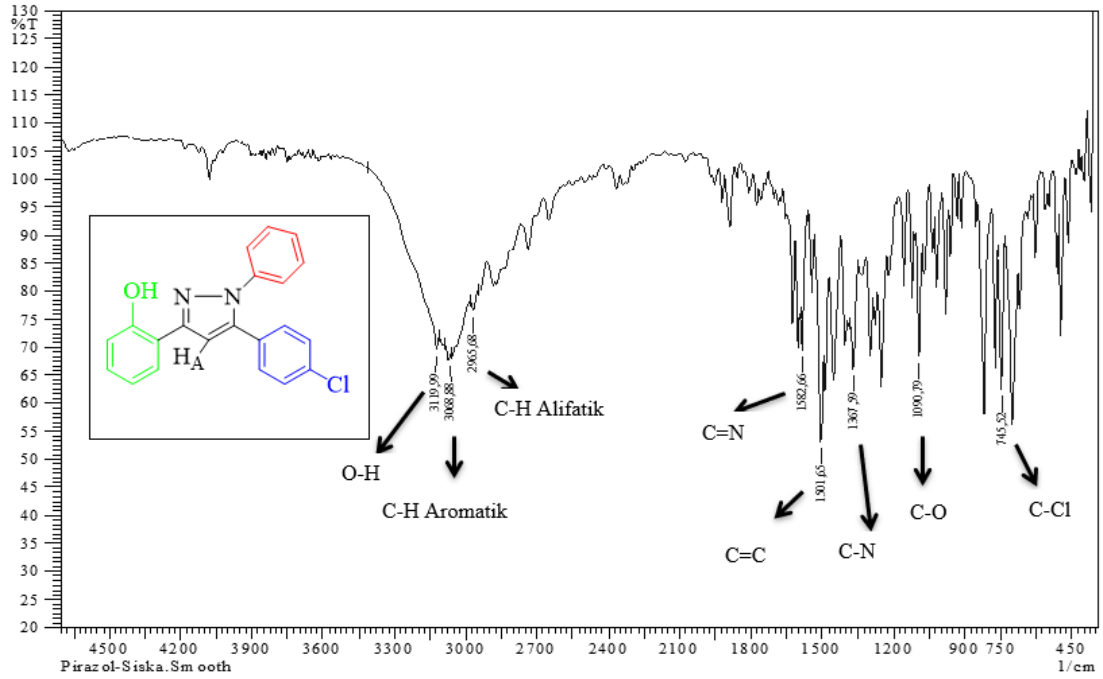



(a) (b)

**Figure 3**. UV spectra of pyrazoline (a) and pyrazole (b).

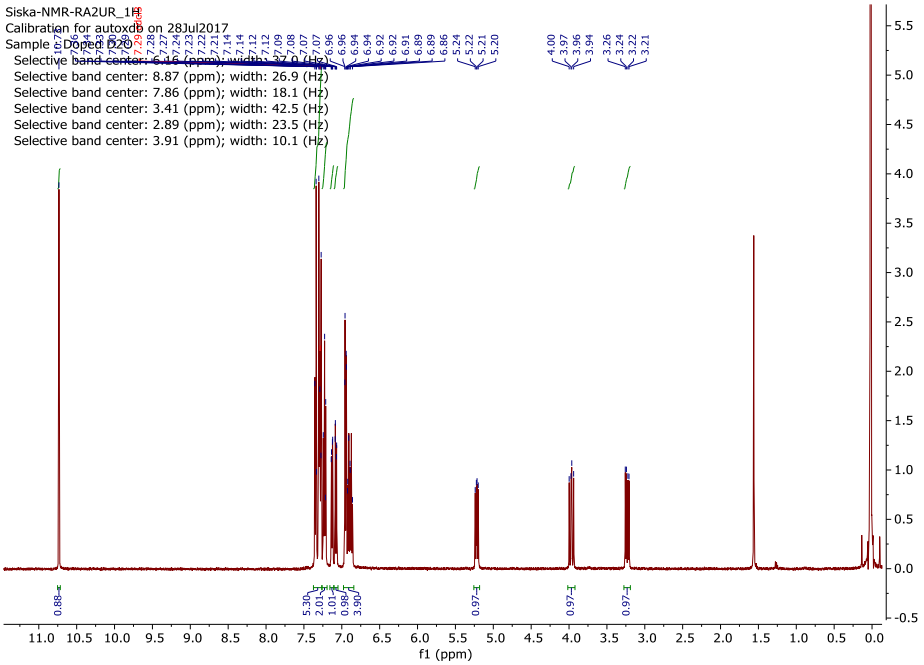



(a)

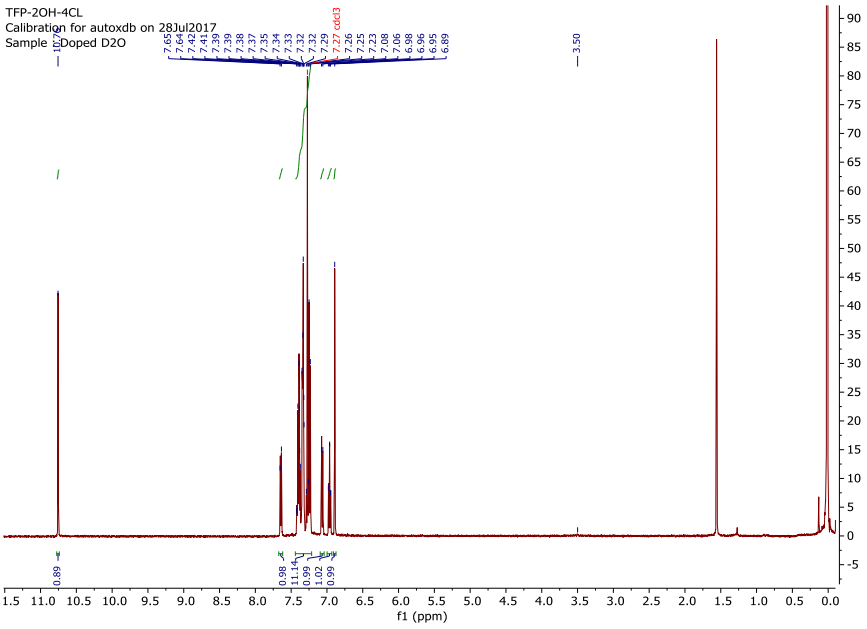



(b)

**Figure 4**. The FTIR of pyrazoline PF-2OH-4Cl (a) and pyrazole TFP-2OH-4Cl (b)




(a)

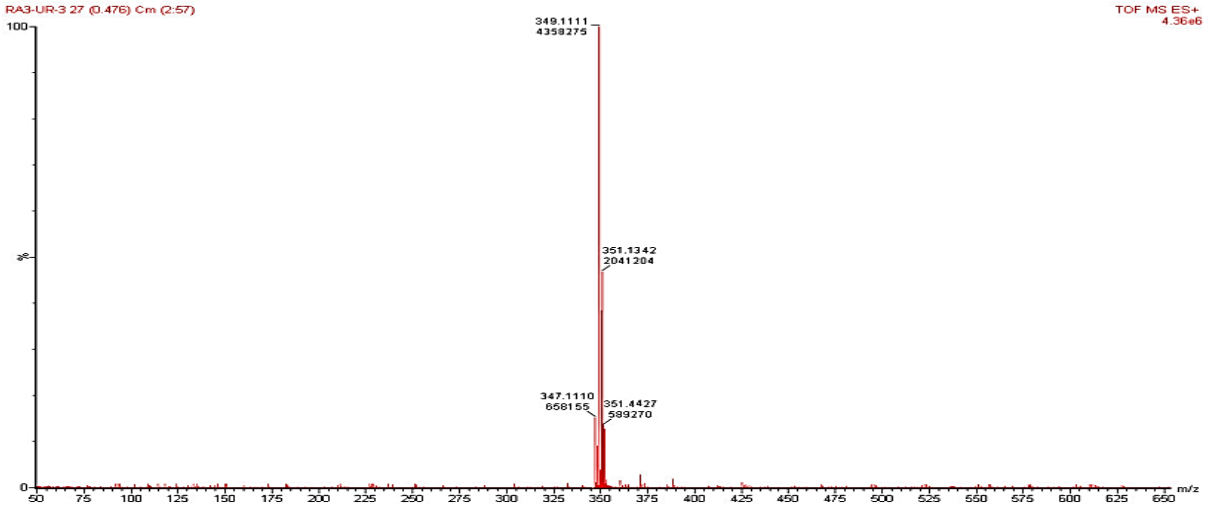



(b)

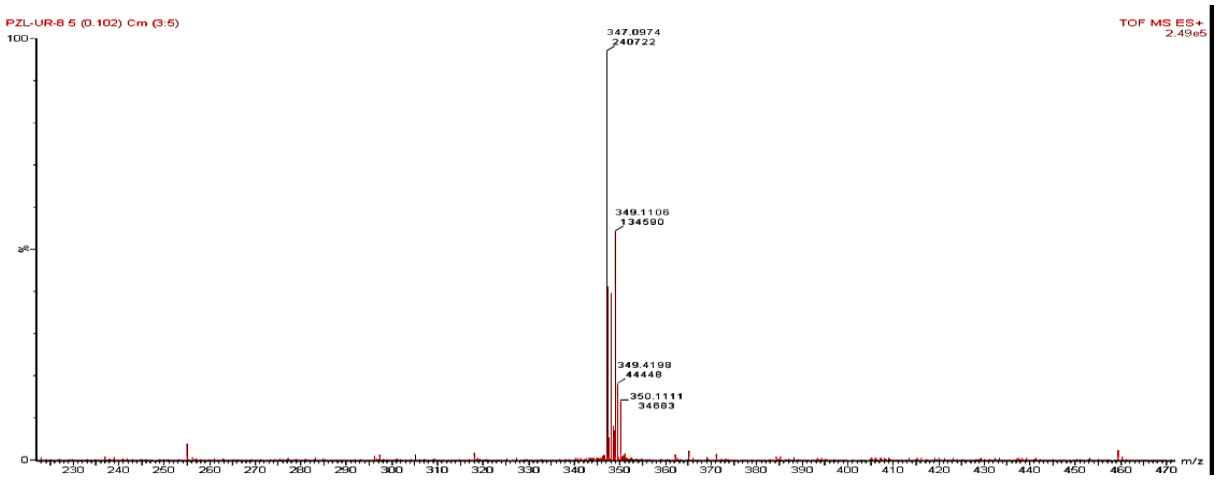
**Figure 5**. The H-NMR spectra of pyrazoline (a) and pyrazole (b)

doublets peak orientation, Ha protons have geminal coupling with Hb protons and vicinal coupling with Hx protons (Jab = 17.2 Hz and Jax = 7.5 Hz). In contrast, Hb protons have geminal coupling with Ha protons and vicinal coupling with Hx protons (Jba = 17.2 Hz and Jbx = 12.2 Hz). The Hx proton has vicinal coupling with the Ha and Hb proton (Jxb = 12.2 and Jxa = 7.5 Hz).

The HRMS spectrum of the pyrazoline compound PF-2OH-4Cl shows the observed molecular ion peak as $[M+H]^+$ with m/z 349.1111. The calculated mass of the target compound with the molecular formula $C_{21}H_{17}ClN_2O$ shows $[M+H]^+$ = 349.1108. A tiny difference of 0.0003 shows the observed molecular ion peak according to the calculated mass. The HRMS of the pyrazole compound TFP-2OH-4Cl showed the observed molecular ion peak as $[M+H]^+$ with m/z 347.0974. The estimated mass of the target compound with the molecular formula $C_{21}H_{15}ClN_2O$ shows $[M+H]^+$ = 347.0951. The slight difference of 0.0023 shows the observed molecular ion peak according to the calculated mass. The HRMS identification results of the pyrazole compound TFP-2OH-4Cl also showed the presence of a molecular ion peak, which was observed as $[M+H]^+$, an isotope




(**a**)




(**b**)

**Figure 6**. H-RMS spectra of pyrazoline PF-2OH-4Cl (**a**) and pyrazole TFP-2OH-4Cl (**b**)

for the formation of pyrazole compounds in the presence of a chloro group substitution (Figure 6). The UV, FTIR, NMR, and HRMS spectra concluded that the structure of the pyrazoline compounds PF-2OH-4Cl and TFP-2OH-4Cl followed the expected structure (Figure 7).

$LC_{50}$ stands for "lethal concentration that kills 50% of the tested organisms." It is a commonly used measurement in toxicology to quantify the concentration of a substance or compound that, when administered to a group of organisms, results in the death of half of the population. This parameter is often used to assess the toxicity of chemicals and compounds, with lower $LC_{50}$ values indicating greater toxicity.

Compounds of chalcone, pyrazoline, and pyrazole were tested for toxicity using the BSLT method using shrimp larvae (*Artemia salina* Leach). In addition to the easy method, the BSLT method can be used to extract compounds with anticancer properties. The results of the toxicity test of the pyrazoline compound showed an $LC_{50}$ value of 260.016 µg/mL, indicating that pyrazoline was not toxic, while the chalcone 2OHA-4ClBD and pyrazole TFP-2OH-4Cl showed $LC_{50}$ values = 2.874 and 5.584 µg/mL indicating that chalcone and pyrazole are toxic. Very toxic because the $LC_{50}$ value is less than 200 µg/mL. A pure compound is said to have toxic properties if it has an $LC_{50}$ value ≤ 200 µg/mL (Afriana *et al*. 2020).

Pyrazoline compound has an $LC_{50}$ > 200 µg/mL which meant less toxic, while chalcone and pyrazole showed an $LC_{50}$<200 µg/mL which indicated that the compound was toxic. The presence of a double bond in chalcone and pyrazole caused the density of pyrazole to be greater than that of pyrazoline so the different results of the toxicity test for the three compounds are obtained. In pyrazoline and pyrazole with the same structural composition, there was a

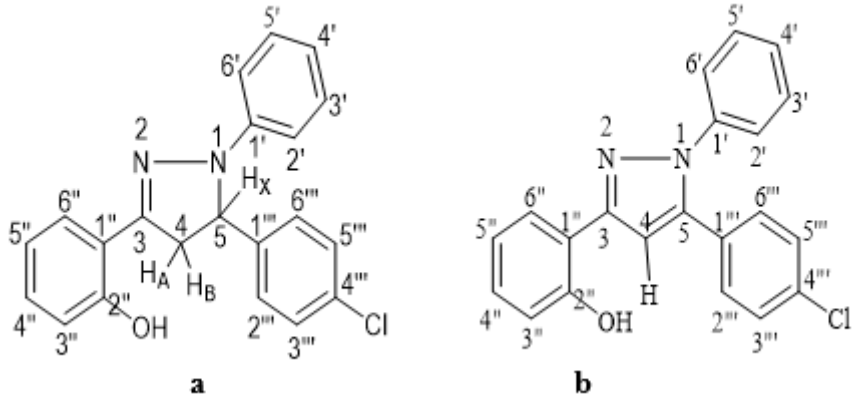



**Figure 7**. The structures of pyrazoline 2-(5-(4- chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole-3-yl)phenol (**PF-2OH-4Cl**) (a) and pyrazole 2-(5-(4-chlorophenyl))-1-phenyl-1*H*-pyrazole-3-yl)phenol (**TFP-2OH-4CL**) (b)

significant difference, where the double bond of the pyrazole caused a resonance pattern to reach the N-N bond, so the resonance pattern leads to a higher electron density around the N-N bond. The higher electron density makes TFP-2OH-4Cl compound more chemically reactive and more likely to interact strongly with other molecules, such as receptors or binding sites. This increased reactivity results in a higher affinity for specific biological targets, potentially leading to toxicity. Consequently, pyrazole exhibits a lower $LC_{50}$ value (<200 µg/mL) compared to pyrazoline, indicating higher toxicity.

While Pyrazoline does not have a double bond within its structure, which means that there are no resonance patterns involving the N-N bond (the bond between two nitrogen atoms). As a result, the distribution of electrons around the molecule is relatively uniform. This uniformity leads to a lower electron density near the N-N bond. In simpler terms, the electrons in pyrazoline are spread out more evenly, which results in lower chemical reactivity and a decreased likelihood of interacting strongly with other molecules, including potential receptors or binding sites. This lower reactivity contributes to a higher $LC_{50}$ value (>200 µg/mL) and indicates lower toxicity (Prasetyaningrum *et al*. 2018; Setiawan *et al*. 2018; Afriana *et al*. 2020).

## CONCLUSION

The pyrazoline compound PF-2OH-4Cl was successfully synthesized by the microwave irradiation method from chalcone 2OHA-4ClBD with a yield of 64.35%. Pyrazole TFP-2OH-4Cl was successfully synthesized by reflux method from pyrazoline PF-2OH-4Cl and acetic acid in a yield of 40.42%. Based on UV, FTIR, NMR, and HRMS spectroscopic characterization results, the synthesized pyrazoline and pyrazole compounds match the expected target compounds. Pyrazoline compounds are non-toxic with $LC_{50}$ values 260.016 µg/mL, and chalcone and pyrazole compounds are toxic with $LC_{50}$ values 2.874 and 5.584 µg/mL.

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