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*Short Note*

# 5-(4-Fluorophenyl)-3-(naphthalen-1-yl)-1-phenyl-1*H*-pyrazole

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**Abstract:** A new fluorinated pyrazole, 5-(4-fluorophenyl)-3-(naphthalen-1-yl)-1-phenyl-1*H*-pyrazole was successfully synthesized via a two-step reaction. Firstly, the synthesis of pyrazoline was performed via one-pot three-component reaction under microwave irradiation. Secondly, the synthesis of pyrazole was performed via oxidative aromatization of pyrazoline under conventional heating. The structure of the synthesized compound was confirmed by spectroscopic analysis, including FT-IR, HR-MS, 1D and 2D NMR analysis. Then, molecular docking study showed that the binding affinity of the synthesized compound to human estrogen alpha receptor (ERα) was close to 4-OHT as a native ligand.

**Keywords:** fluorinated pyrazole; one-pot reaction; three-component reaction; anti-breast cancer; molecular docking; human estrogen alpha



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## 1. Introduction

Pyrazoles and their derivatives play an important role in some biological activities in medicine. In particular, they are used for their antimicrobial [1], anti-tuberculosis [2], anti-inflammatory [3], antioxidant [4], anti-tumor [5], cytotoxicity activity [6], and analgesic [7] functions. Pyrazole can be synthesized in a variety of ways, such as by Knorr reaction or through the pyrazoline pathway, which is the reaction of α, β-unsaturated ketone with hydrazine derivatives [8] or semicarbazide [9], followed by oxidative aromatization to the corresponding pyrazole molecules [10]. Some studies have also reported that pyrazole derivatives also exhibit anti-cancer activity against breast cancer cell lines [11,12]. Furthermore, many pyrazoles have been patented as hepatic cancer (HePG-2) agents [13], and celecoxib is a commercial drug with cyclooxygenase-2 inhibitory activity [14].

Fluorinated compounds are popular in medicinal chemistry as drug agents. The C-F bond has greater stability than the C-H bond. Moreover, the fluorine substitution can be used to increase the binding affinity of the protein–ligand complex. Celecoxib, which bears a pyrazole core, also has a fluorine substitution in order to increase its metabolic stability [15]. This makes the development of new fluorinated heterocyclic compounds of pyrazole very interesting.

The synthesis of fluorinated pyrazoline has been reported previously by our group in two-step reactions [16]. In this work, we synthesize a new fluorinated pyrazole through the oxidation of pyrazoline under conventional heating. We use irradiation in a microwave oven to synthesize pyrazoline from 1-acetylnaphthalene, 4-fluorobenzaldehyde, and phenyl hydrazine in a one-pot three-component reaction. The structure of the new pyrazole was characterized based on the complete assignments from IR, MS, 1D and 2D NMR analysis. Then, a molecular docking study was performed to investigate its potential as an anti-breast cancer agent by targeting the human estrogen receptor alpha (ERα).

## 2. Results and Discussion

### *2.1. Synthesis*

Compound **4** was synthesized using a one-pot three-component reaction under microwave irradiation with some modifications from the previous method [17–19] in 54.82% yield. The reaction was carried out at 180 watt for 2 min under basic conditions. Then, compound **4** was oxidized using glacial acetic acid under conventional heating at 85 °C for 24 h to obtain compound **5** in 35.16% yield. Compound **4** was converted into **5** through oxidative aromatization reaction (Figure 1).

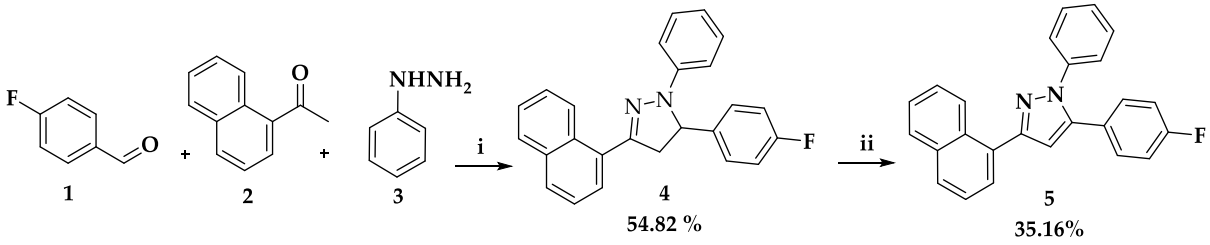



**Figure 1.** Synthesis route of new fluorinated pyrazole; (i) NaOH 12% (*w*/*v*), EtOH, 2 min, 180 W, MW (ii) AcOH, 85 °C, 24 h.

The FT-IR analysis of compound **5** confirmed that there were no vibration bands of aliphatic C-H. The spectrum only showed a vibration of aromatic C-H at 3049 $cm^{-1}$. This showed that the oxidative aromatization was successful. The other vibration bands of compound **5** were similar to compound **4**, including C=N and C-N vibrations appearing at 1593 and 1495 $cm^{-1}$, respectively. Then, the aromatic C=C vibration was identified at 1360 $cm^{-1}$ and the presence of fluoro substituent was confirmed by the vibration band of C-F at 1224 $cm^{-1}$. Mass spectrum, as further analysis, confirmed this to be $[M + H]^+$ $m/z$ 365.1417 (100%).

The structure of compound **5** was characterized by the complete assignments of 1D and 2D NMR analysis. The $^1$H-NMR spectra of compound **5** did not show the presence of ABX aliphatic proton signals. The spectra specifically only showed a singlet signal at δ 6.09 ppm. This data supported the FT-IR interpretation and indicated that the pyrazoline core was successfully converted to a pyrazole core. Furthermore, the fluoro substituent made a specific system on the $^1$H and $^{13}$C-NMR spectra. Based on the reported literature, both the $^1$H and $^{13}$C-NMR spectra showed that the fluoro substituent could be coupled to proton and carbon atoms up to four bonds ($^4J$) [20]. The $^1$H-NMR spectra of compound **5** showed $^2J_{H\text{-}F}$ as a triplet signal and $^3J_{H\text{-}F}$ with doublet of doublets signal, with coupling constants of 8.6, 5.8, and 2.8 Hz, respectively. Upon further analysis, the $^{13}$C-NMR spectra also showed a coupling of $^{13}$C to $^{19}$F with coupling constants of 248.9 Hz ($^1J_{C\text{-}F}$), 21.6 Hz ($^2J_{C\text{-}F}$), 8.2 Hz ($^3J_{C\text{-}F}$), and 3.3 Hz ($^4J_{C\text{-}F}$).

The assignment of each signal in $^1$H and $^{13}$C NMR was performed based on the 2D NMR spectrum, including HSQC and HMBC. The HSQC spectra showed that $C_4$ (δ 108.91 ppm) only had a correlation with $H_4$ at δ 6.83 ppm. This shows that there was a loss of one proton caused by oxidative aromatization of pyrazoline core. Then, the HMBC spectra of compound **5** showed an important correlation between $H_4$ at δ 6.83 ppm with $C_3$ and $C_5$ signals in pyrazole core. The other important correlations were illustrated in Figure 2. The 1D and 2D NMR spectrum were completely attached in Supplementary Materials.

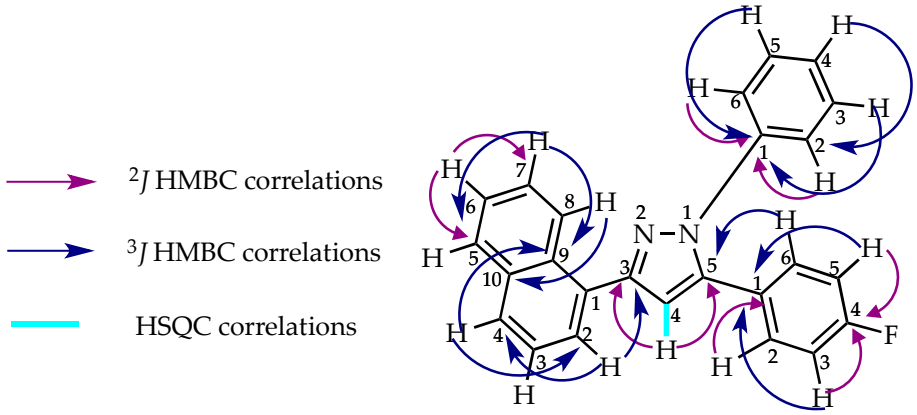



**Figure 2.** 2D NMR interpretation of compound **5**.

### *2.2. Molecular Docking*

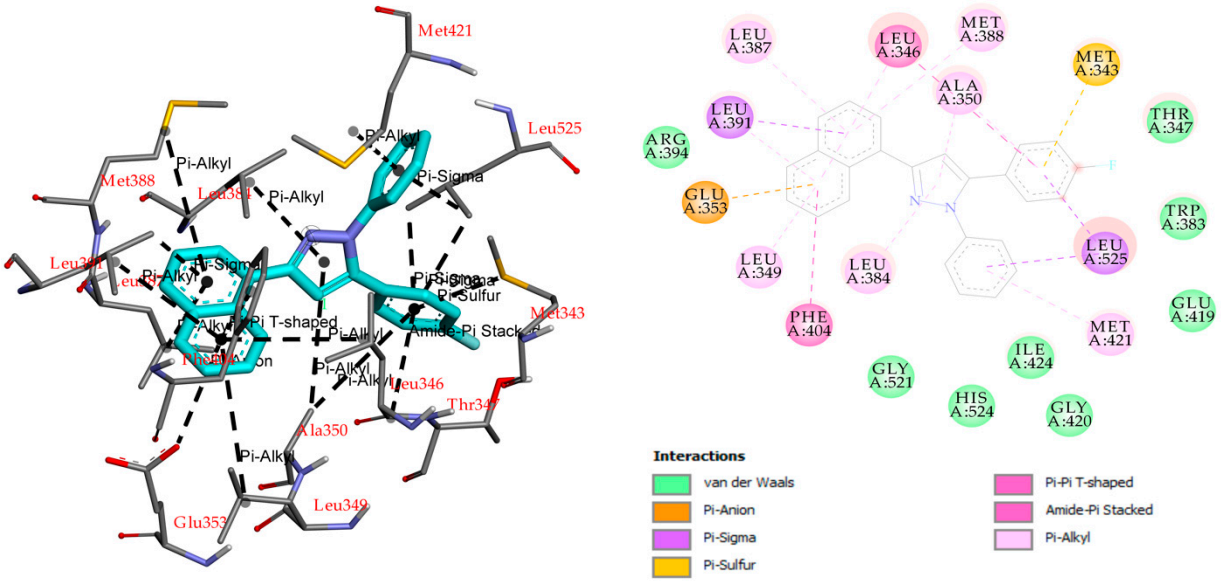
Molecular docking study was performed using the Autodock tools package. Compound **5** was docked into ERα (PDB ID: 3ERT) [21]. The molecular docking study was performed in several steps. First, we validated our docking protocol through redocking the tamoxifen (4-OHT) as the native ligand. The validation results showed an RMSD of 1.1 Å, which indicated that the docking protocol could be used for further investigation. An RMSD value of <2 Å confirms the similarity in binding patterns with 4-OHT in ERα [22,23].

On the basis of Table 1, it can be concluded that compound **5** shows good potential as an ERα inhibitor, because its binding affinity to ERα is close to that of 4-OHT as a native ligand. Their binding affinities are −10.61 and −11.04 Kcal/mol, respectively. Subsequently, the docking results showed that compound **5** also has an inhibition constant (Ki) of 16.71 nM. The docking results showed that compound **5** can interact with amino acid residues in the active side, such as Arg394 and Glu353. Both interactions were also found between 4-OHT and ERα. However, the types of interactions are different. Compound 5 interacted with the two residues through van der Waals and hydrophobic interaction, respectively, while 4-OHT interacted with them through hydrogen bond formation (Figure 3). We assumed that these different kinds of interaction influenced the decreasing Ki value of compound **5**.

**Table 1.** The docking results of compound **5** and 4-OHT to ERα.

| Compounds | ΔG (Kcal/mol) | Ki (nM) | Interaction Compound—Erα | | |
|---|---|---|---|---|---|
| | | | H-Bond | Hydrophobic | Van der Waals |
| **5** | −10.61 | 16.71 | - | Leu391, Leu525, Met343, Glu353, Leu384, Leu349, Ala350, Met388, Met421, Leu387, Phe404, Leu346 | Thr347, Trp383, Glu419, Ile424, His524, Gly420, Gly521, Arg394 |
| 4-OHT [a] | −11.04 | 3.76 | Arg394, Glu353 | Leu391, Leu387, Ala350, Leu346, Ile424, Met388, Leu428, Leu525, Met421 | Leu384, Phe404 Met343, Gly420, His524, Gly521, Leu349, Thr347, Asp351, Leu354, Trp383 |

[a] literature [24].




(**a**) (**b**)

**Figure 3.** The interactions of compound **5** with active sites of ERα; (**a**) 3D visualization; (**b**) 2D visualization.

## 3. Materials and Methods

### *3.1. Materials*

All chemicals and solvents used in this work were purchased from Merck and Sigma Aldrich. The synthesis reaction was performed in an Electrolux EMS3087X microwave oven (PT. Electrolux Indonesia, Jakarta, Indonesia). The melting point was determined on a Fisher-Johns apparatus (Fisher Scientific, Waltham, MA, USA) (uncorr). The Thin Layer Chromatography (TLC) analysis was performed using silica gel $GF_{254}$ TLC plate (Merck Millipore, Darmstadt, Germany) and the spots were observed under UV lamp of 254/366 nm (Camag™, Camag Chemie-Erzeugnisse & Adsorptionstechnik AG, Muttenz, Switzerland). The purity of the compound was analyzed using UFLC Prominence-Shimadzu LC Solution with SPD 20AD as UV detector (Shimadzu Corporation, Kyoto, Japan). The mass spectra were measured by High-Resolution Electrospray Ionization–Time-of-Flight Mass Spectrometry (HR-ESI-TOFMS). Then, the 1D and 2D NMR spectra recorded on an Agilent®(Agilent Technologies, Santa Clara, CA, USA), at 500 and 125 MHz for $^{1}$H and $^{13}$C-NMR, respectively.

### *3.2. Synthesis of 5-(4-Fluorophenyl)-3-(naphthalen-1-yl)-1-phenyl-4,5-dihydro-1H-pyrazole* **4**

The mixture of 4-fluorobenzaldehyde (3 mmol), 1-acetylnaphthalene (3 mmol), 3 equivalent of phenyl hydrazine and 12% sodium hydroxide (10 mL) in absolute ethanol was irradiated in a microwave oven at 180 W for 2 min. The reaction was monitored by TLC every 30 s. Then, after the completion of the reaction, the mixture was left in an ice bath for 24 h. The formed precipitate was filtered in vacuo and rinsed with cold water and *n*-hexane to obtain pure compound 4 in 54.82% yield as a yellow solid (m.p. 152–153 °C). The spectroscopic data of this compound were reported in our previous study [16].

### *3.3. Synthesis of 5-(4-Fluorophenyl)-3-(naphthalen-1-yl)-1-phenyl-1H-pyrazole* **5**

The mixture of compound **4** (1 mmol) and excess glacial acetic acid (5 mL) was heated in an oil bath at 85 °C for 24 h. The reaction was monitored by TLC every 6 h. After the completion of the reaction, the mixture was poured into crushed ice and then neutralized by adding sodium hydroxide solution. The mixture was left in an ice bath for 12 h and the formed precipitate was filtered in vacuo and rinsed by cold distilled water and *n*-hexane. Then, the crude product was purified through column chromatography using *n*-hexane/ethyl acetate with a gradient system.

5-(4-fluorophenyl)-3-(naphthalen-1-yl)-1-phenyl-1*H*-pyrazole (**5**): yield 35.16%; yellow solid (m.p. 48–50 °C), Retention time of HPLC at 17.776 min, FT-IR (KBr) ν ($cm^{-1}$): 3049, 1593, 1495, 1360 and 1224. $^{1}$H NMR (500 MHz, $CDCl_3$) δ 8.71 (d, 1H, *J* = 8.6 Hz); 7.93 (d, 1H, *J* = 6.9 Hz); 7.91 (d, 1H, *J* = 8.9 Hz); 7.85 (d, 1H, *J* = 7.0 Hz); 7.60–7.51 (m, 3H); 7.45 (d, 2H, *J* = 7.8 Hz); 7.40 (t, 2H, *J* = 7.6 Hz); 7.42–7.35 (m, 1H); 7.35 (dd, 2H, *J* = 5.8, *J* = 2.8 Hz); 7.07 (t, 2H, *J* = 8.2 Hz) 6.84 (s, 1H). $^{13}$C-NMR (125 MHz, $CDCl_3$) δ 162.68 (d, $^{1}J_{C\text{-}F}$ = 248.9 Hz), 151.96, 142.62, 139.98, 134.02, 131.41, 130.67 (d, $^{3}J_{C\text{-}F}$ = 8.2 Hz), 129.00, 128.63, 128.37, 127.54, 127.23, 126.73 (d, $^{4}J_{C\text{-}F}$ = 3.3, Hz), 126.71, 126.41, 126.19, 125.82, 125.40, 125.33, 115.68 (d, $^{2}J_{C\text{-}F}$ = 21.6 Hz), 108.92. The molecular ion peak in HRMS spectra $[M + H]^+$ found at *m*/*z* 365.1417, calculated as *m*/*z* 365.1454. The HRMS, 1D and 2D NMR spectrum were attached in Supplementary Materials (Figures S1–S8 and Table S1).

### *3.4. Molecular Docking Study*

The molecular docking study was performed using AutoDock 4.2.6. The ligands and receptor were prepared using AutoDockTools (ADT) 1.5.6 (Scripps Research, San Diego, CA, USA). The PDB file of ERα (3ERT) was taken from the RCSB data bank (https://www.rcsb.org/structure/3ERT, accessed on 6 March 2021). The receptor was prepared using ADT by adding Kollman charges. The ligands were prepared by adding Gasteiger charges, and its hydrogen and minimized energy were determined to be 0.01 Kcal/mol. All of the prepared receptors and ligands were saved in pdbqt format. The docking was performed with a grid box of 60 × 60 × 60, with a spacing of 0.375 Å. The coordinates of the active site were set as x = 30.282, y = −1.913, z = 24.207. Then, the docking results were visualized using BIOVIA Discovery visualizer 2020 (Dassault Systèmes, San Diego, CA, USA).

## 4. Conclusions

In summary, we successfully synthesized a new fluorinated pyrazole, 5-(4-fluorophenyl)-3-(naphthalen-1-yl)-1-phenyl-1*H*-pyrazole (compound **5**) from a fluorinated pyrazoline via oxidative aromatization. Moreover, all spectroscopic data successfully confirmed the structure of the synthesized compound. Based on the molecular docking study, compound **5** showed potential inhibition against Erα, with binding affinity and Ki value of −10.61 Kcal/mol and 16.71 nM, respectively. However, in vitro and in vivo evaluations are required to ensure its anti-breast cancer potential.

**Supplementary Materials:** The following are available online. Figures S1–S8: IR, HRMS, $^{1}$H, $^{13}$C, HSQC and HMBC spectrum; Table S1: NMR spectroscopic data.

**Author Contributions:** J.J., as supervisor, designed and conceived the whole experiments; N.F. reviewed molecular docking method; Y.N. wrote, reviewed and edited the manuscript; A.Z. developed the methodology; I.I. wrote, reviewed and edited the manuscript; G.G. performed the experiments and wrote the original manuscript. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The data presented in this study are available in the Supplementary Materials of this article.

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**Conflicts of Interest:** The authors declare no conflict of interest.

## References


1. Thumar, N.J.; Patel, M.P. Synthesis, characterization, and antimicrobial evaluation of carbostyril derivatives of 1*H*-pyrazole. *Saudi Pharm. J.* **2011**, *19*, 75–83. [CrossRef]
2. Harikrishna, N.; Isloor, A.M.; Ananda, K.; Obaid, A.; Fun, H.-K. Synthesis, and antitubercular and antimicrobial activity of 1′-(4-chlorophenyl)pyrazole containing 3,5-disubstituted pyrazoline derivatives. *New J. Chem.* **2016**, *40*, 73–76. [CrossRef]
3. Kumar, V.; Kaur, K.; Gupta, G.K.; Gupta, A.K.; Kumar, S. Developments in synthesis of the anti-inflammatory drug, celecoxib: A review. *Recent Pat. Inflamm. Allergy. Drug Discov.* **2013**, *7*, 124–134. [CrossRef]
4. Jasril, J.; Teruna, H.Y.; Aisyah, A.; Nurlaili, N.; Hendra, R. Microwave assisted synthesis and evaluation of toxicity and antioxidant activity of pyrazoline derivatives. *Indones. J. Chem.* **2019**, *19*, 583–591. [CrossRef]
5. Zaninetti, R.; Cortese, S.V.; Aprile, S.; Massarotti, A.; Canonico, P.L.; Sorba, G.; Grosa, G.; Genazzani, A.A.; Pirali, T. A concise synthesis of pyrazole analogues of combretastatin A1 as potent anti-tubulin agents. *Chem. Med. Chem.* **2013**, *8*, 633–643. [CrossRef]
6. Jasril, J.; Teruna, H.Y.; Ikhtiarudin, I.; Frimayanti, N. Search for new potential breast cancer inhibitors (MCF7) based on molecular docking and biological assay of pyrazoline analogue compounds. *ASTESJ* **2020**, *5*, 122–126. [CrossRef]
7. Padmini, T.; Kamal, B.R. Synthesis, anti-inflammatory, analgesic and antipyretic activity of novel 1,3,5-trisubstituted pyrazole derivatives. *Asian J. Chem.* **2019**, *31*, 1225–1229. [CrossRef]
8. Jasril, J.; Ikhtiarudin, I.; Zamri, A.; Teruna, H.Y.; Frimayanti, N. New fluorinated chalcone and pyrazoline analogs: Synthesis, docking, and molecular dynamic studies as anticancer agents. *TJPS* **2017**, *41*, 93–98.
9. Palleapati, K.; Kancharlapalli, V.R.; Shaik, A.B. Synthesis, characterization and antitubercular evaluation of some new isoxazole appended 1-carboxamido-4,5-dihydro-1*H*-pyrazoles. *J. Res. Pharm.* **2019**, *23*, 156–163. [CrossRef]
10. Nakamichi, N.; Kawashita, Y.; Hayashi, M. Oxidative aromatization of 1,3,5-trisubstituted pyrazolines and hantzsch 1,4-dihydropyridines by Pd/C in acetic acid. *Org. Lett.* **2002**, *4*, 3955–3957. [CrossRef]
11. Liu, D.C.; Gao, M.J.; Huo, Q.; Ma, T.; Wang, Y.; Wu, C.Z. Design, synthesis, and apoptosis-promoting effect evaluation of novel pyrazole with benzo[d]thiazole derivatives containing aminoguanidine units. *J. Enzym. Inhib. Med. Chem.* **2019**, *34*, 829–837. [CrossRef]
12. Shaik, A.B.; Rao, G.K.; Kumar, G.B.; Patel, N.; Reddy, V.S.; Khan, I.; Routhu, S.R.; Kumar, C.G.; Veena, I.; Shekar, K.C.; et al. Design, synthesis and biological evaluation of novel pyrazolochalcones as potential modulators of PI3K/Akt/mTOR pathway and inducers of apoptosis in breast cancer cells. *Eur. J. Med. Chem.* **2017**, *139*, 305–324. [CrossRef] [PubMed]
13. Khalifa, N.M.H.; Al-Omar, M.A. Substituted Pyrazole Derivatives. US Patent 010039749B1, 7 August 2018.
14. Gao, D.; Hlinak, A.J.; Mazhary, A.M.; Truelove, J.E.; Vaughn, M.B.W. Celecoxib Compositions. WIPO (PCT) 2000032189A1, 8 June 2000.
15. Shah, P.; Westwell, A.D. The role of fluorine in medicinal chemistry. *J. Enzym. Inhib. Med. Chem.* **2007**, *22*, 527–540. [CrossRef]
16. Jasril, J.; Zamri, A.; Ikhtiarudin, I.; Teruna, H.Y. 5-(4-Fluorophenyl)-3-(naphthalen-1-yl)-1-phenyl-4,5-dihydro-1*H*-pyrazole. *Molbank* **2016**, *2016*, M891. [CrossRef]
17. Zamri, A.; Teruna, H.Y.; Wulansari, S.; Herfindo, N.; Frimayanti, N.; Ikhtiarudin, I. 3-(3,4-Dimethoxyphenyl)-5-(2-fluorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole. *Molbank* **2019**, *4*, M1088. [CrossRef]
18. Hawaiz, F.E.; Hussein, A.J.; Samad, M.K. One-pot three-component synthesis of some new azo-pyrazoline derivatives. *Eur. J. C* **2014**, *5*, 233–236. [CrossRef]
19. Movassagh, B.; Bijanzadeh, H.R. A mild and highly efficient one-pot synthesis of 1,3,5-triaryl-2-pyrazolines. *Chem. Heterocycl. Compd.* **2013**, *48*, 1719–1721. [CrossRef]
20. Guo, W.; Wong, T.C. Study of $^{13}$C-$^{19}$F and $^{1}$H-$^{19}$F couplings in some fluorinated aromatic compounds using two-dimensional $^{13}$C-$^{1}$H chemical shift correlation spectroscopy with proton homonuclear decoupling. *Magn. Res. Chem.* **1986**, *24*, 75–79. [CrossRef]
21. Shiau, A.K.; Barstad, D.; Loria, P.M.; Cheng, L.; Kushner, P.J.; Agard, D.A.; Greene, G.L. The Structural Basis of Estrogen Receptor/Coactivator Recognition and the Antagonism of This Interaction by Tamoxifen. *Cell* **1998**, *95*, 927–937. [CrossRef]
22. Smyth, M.S.; Martin, J.H. X ray crystallography. *Mol. Pathol.* **2000**, *53*, 8–14. [CrossRef] [PubMed]
23. Plewczynski, D.; Łaz'niewski, M.; Augustyniak, R.; Ginalski, K. Can we trust docking results? Evaluation of seven commonly used programs on PDBbind database. *J. Comput. Chem.* **2010**, *32*, 742–755. [CrossRef] [PubMed]
24. Mardianingrum, R.; Yusuf, M.; Hariono, M.; Gazzali, A.M.; Muchtaridi, M. Mangostin and its derivatives against estrogen receptor alpha. *J. Biomol. Struct. Dyn.* **2020**. [CrossRef] [PubMed]