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RESEARCH ARTICLE

Computational docking toward cox-2 and synthesis of 4-formyl-2-methoxyphenyl-4-chlorobenzoate using microwave irradiation

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Keywords
4-Formyl-2-methoxyphenyl-4-chlorobenzoate
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Abstract

Background: Structural modification of organic compounds is needed to increase their bioactivity. Vanillin has been reported to have various therapeutic effects such as antioxidant, antimutagenic, anti-invasive and metastatic suppression potential as well as anti-inflammatory and antinociceptive activity. To increase the activity of organic compounds, their lipophilic properties must be enhanced by modifying their structure. **Objective:** The phenolic hydroxyl group of vanillin can be modified by adding an aromatic ring, carbonyl, and halogen to improve its bioactivity. The 4-formyl-2-methoxyphenyl-4-chlorobenzoate is a vanillin derivative that has been modified at its phenolic hydroxyl. **Method:** In this study, the synthesis of 4-formyl-2-methoxyphenyl-4-chlorobenzoate was carried out by microwave irradiation with various powers of 120, 200 and 400 watts. The characterisations of the synthesised compound were carried out using FTIR, ^{1}H-NMR and ^{13}C-NMR spectrophotometry. The molecular docking study used Autodock software with the COX-2 receptor (PDB ID: 6COX) as the target receptor. **Result:** Microwave powers of 120, 200 and 400 watts were used to synthesise the target compound and produced yields of 89.09%, 72.78% and 34.49%, respectively. Molecular docking at the COX-2 receptors was studied to predict the anti-inflammatory activity of the 4-formyl-2-methoxyphenyl-4-chlorobenzoate. The docking results showed that the binding energy of 4-formyl-2-methoxyphenyl-4-chlorobenzoate was lower on chain A of the receptor (-8.18 kcal/mol) than the starting material, vanillin (-4.96 kcal/mol). It predicted that 4-formyl-2-methoxyphenyl-4-chlorobenzoate had better activity than vanillin. **Conclusion:** 4-formyl-2-methoxyphenyl-4-chlorobenzoate was successfully synthesised indicating that it is essential for this compound to be further developed as an anti-inflammatory agent.

Introduction

Vanillin is one of the main chemical components obtained from the seed pods of Vanilla planifolia, a monocotyledonous orchid, and is widely used in food, medicine, cosmetics and beverages (Srikanth *et al.*, 2012). It is made up of fine and white to slightly yellow crystals, which are usually needle-shaped, and has an odour and taste that is suggestive of vanilla (Brayfield, 2014). It is also believed to have beneficial effects on health due to the high intake of vanillin from food and beverages. Several previous studies have shown that vanillin has various activities such as antioxidant (Tai *et al.*, 2011), antimutagenic (King *et al.*, 2007), anti-invasive and metastatic suppression potential (Lirdprapamongkol *et al.*, 2005). It also had anti-inflammatory and antinociceptive effects caused by carrageenan in rats (Niazi *et al.*, 2014).

Making structural modifications to drugs is very important because the bioactivity of the compound can be increased and compounds which are more selective, convenient to use, less toxic, and economical can be produced. Structural modifications can affect the

physicochemical properties of compounds such as their lipophilic, electronic and steric properties which can increase their biological activity (Siswandono, 2016). In this experiment, structural modification was carried out by adding an aromatic ring, carbonyl, and halogen on the -OH phenolic group of the lead compound, vanillin, to form 4-formyl-2-methoxyphenyl-4-chlorobenzoate. As it has been reported that vanillin has anti-inflammatory activity, it is necessary to determine the anti-inflammatory activity of its derivatives.

To predict the activity of 4-formyl-2-methoxyphenyl-4-chlorobenzoate as an anti-inflammatory agent, an *in silico* study was carried out by studying molecular docking. It was performed at the *cyclooxygenase*-2 (COX-2) receptor and the docking result was presented in the MolDock score (MDS). MDS is the energy released by the ligand to bind to the receptor which is called the binding energy. A lower MDS indicates that a lower energy was required to bind to the receptor, which signifies that a more stable bond has occurred. It can also be considered as the best bond between the ligand and the receptor, so it is predicted to have greater activity (Ekowati *et al.*, 2018).

To carry out an organic chemical reaction, microwave irradiation is a widely used type of energy. A microwave is an electromagnetic wave that travels at the speed of light in a vacuum. Compared to conventional methods, microwaves can accelerate the reaction and give better results and higher purity (Nain *et al.*, 2019). The electromagnetic field in a microwave interacts with polar molecules contained in the mixture leading to rotation and friction between molecules which produces heat with a rapidly increased temperature (Sharma *et al.*, 2018). Synthetic reactions leading to modifications to phenolic hydroxyl were reported by Mumtaza *et al.* (2020) who used a pyridine catalyst and microwave irradiation. The greater the power used, the greater the kinetic energy produced because the high power of microwaves quickly produces high temperatures (Díaz-Ortiz *et al.*, 2019). However, if the microwave power is too high it can also cause damage to the compound. Thus, in this study, the effect of the power of microwave irradiation on the yield of 4-formyl-2-methoxyphenyl-4-chlorobenzoate was examined.

Methods

Synthesis of 4-formyl-2-methoxyphenyl-4-chlorobenzoate

Vanillin (1.3 mmol) was dissolved in 1.0 mL of Tetrahydrofuran (THF) under cold conditions. *p*-Chlorobenzoyl chloride (2.6 mmol) was mixed with 0.5 mL of THF under cold conditions. The *p*-chlorobenzoyl chloride and THF mixture was put into a vanillin solution in THF and then stirred until homogeneous, following which 0.4 mL of pyridine was added. After reaching room temperature, it was put into the microwave which was regulated with various powers of 120, 200 and 400 watts. When the synthesis reaction was running, sampling was carried out every 30 seconds to see the results of the reaction's completion. The reaction completion test was carried out by thin-layer chromatography (TLC). A small amount of the mixture of compounds taken in each sampling was dissolved with 3 drops of chloroform, then the solution was spotted on a TLC plate and eluted with chloroform: ethyl acetate (2:1). Three replications were performed at each power. To obtain the pure 4-formyl-2-methoxyphenyl-4-chlorobenzoate, separation and purification were carried out by adding 5% Na_2CO_3 solution and washing with water. After that, the purity test was carried out by TLC and structure identification was conducted by FTIR spectrophotometry, ^{1}H-NMR spectrometry, and ^{13}C-NMR spectrometry.

Docking study

The structure of the COX-2 receptor (PDB ID: 6COX) was downloaded in PDB file format from the RCSB protein data bank website (https://www.rcsb.org/). The file was then imported into AutoDock 4.2. The molecular docking study was validated by overlaying the reference co-crystallised complex obtained from the PDB website on the re-docked complex by using PyMOL2.5.2. RMSD values were then obtained and its root mean standard deviation value had to be lower than 2.0 Angstrom.

Results

Synthesis of 4-formyl-2-methoxyphenyl-4-chlorobenzoate

The reaction for the formation of 4-formyl-2-methoxyphenyl-4-chloroben-zoate can be seen in Figure 1.

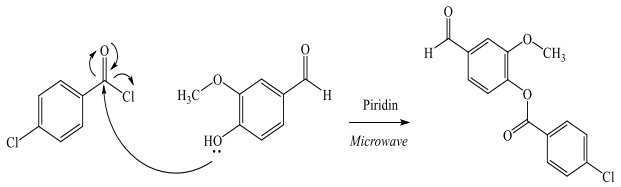

Figure 1: Reaction mechanism of 4-formyl-2-methoxyphenyl-4-chlorobenzoate

During the synthesis of 4-formyl-2-methoxyphenyl-4-chlorobenzoate, evaluation for completeness of the reaction was carried out using TLC with a 2:1 ratio of chloroform to ethyl acetate. Thus, the reaction was considered to be completed if the spot on Rf of the initial compound disappeared. The product was also tested for purity by using TLC and melting point tests. The TLC test used 3 different eluents, chloroform-ethyl acetate (2:1), hexane-ethyl acetate (4:1), and hexane-ethanol (4:1). All combination eluents were indicating the presence of a single spot.

Based on structural identification using the FTIR spectra, the product had an aldehyde C=O functional group (1691.59 cm^{-1}); C=C alkene (1590.39 cm^{-1}); aromatic with meta substitution (742.16 cm^{-1}); aromatic with para substitution (846.78 cm^{-1}); C=O ester (1736.66 cm^{-1}); and C-Cl (674.10 cm^{-1}). The data showed that vanillin had been modified into 4-formyl-2-methoxyphenyl-4-chlorobenzoate as indicated by the loss of phenolic OH spectra of vanillin (around 3300 cm^{-1}) into an ester group (1736.66 cm^{-1}); and the addition of the C-Cl group (674.10 cm^{-1})of 4-formyl-2-methoxyphenyl-4-chlorobenzoate.

Furthermore, structural identification was carried out by NMR spectroscopy with the following results: ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 3.88 (s, 3H); 7.35 (d, 1 H, *J*= 9 Hz); 7.48 (d, 2H, *J* = 2.5 Hz); 7.50-7.54 (m, 2H, *J*= 11; 3 Hz); 8.14 (m, 2H, *J* = 8.5; 2.5 Hz); 9.97 (s, 1H). ^{13}C-NMR (100 MHz, CDCl3, δ) 191.15 (1C), 163.41 (1C), 152.19 (1C), 145.06 (1C), 140.53 (1C), 135.47 (1C), 131.85 (2C), 129.13(2C), 127.35 (1C), 124.88 (1C), 123.59 (1C), 110.97 (1C), 56.24 (1C).

The highest power (400 W) produced the least amount of product. Meanwhile, the lower power (120 W) produced the highest percentage of yield (Table I).

Table I: Percentage of 4-formyl-2-methoxyphenyl-4-chlorobenzoate synthesis

Power (W)	Yields (%)
120	89.09
300	72.78
400	34.49

Docking Study

Validation of the docking method was carried out in chain A and an average RMSD of 0.770 was obtained from the process. Since RMSD < 2.0 Å, the test compound could be docking in this cavity. This step was done in order to ensure that the native ligands would be bound exactly to the active site cleft and to ensure that the method would consistently predict the natural conformation between the ligand and receptor (Shivanika *et al*., 2022).

Table II showed that the free energy of 4-formyl-2-metho-xyphenyl-4-chlorobenzoate at COX-2 receptors was lower than the lead compound, vanilin. The free energy of the synthesized compound was -8.18 kcal/mol.

Table II: Free energy values obtained in the receptor of the tested compounds

Receptor	Chain	Ligand	Free energy (kcal/ mol)	Mean free energy (kcal/mol)
6COX	A	4-formyl-2-methoxyphenyl-4-chlorobenzoate	-8,48	-8,18
			-8,33	
			-7,72	
		Vanillin	-4,96	-4,96
			-4,96	
			-4,96	

From the *in silico* study, the interaction between the tested compound and the receptor could also be examined. It appeared that the 4-formyl-2-methoxyphenyl-4-chlorobenzoate had several interactions with the amino acid residues, Figure 2.

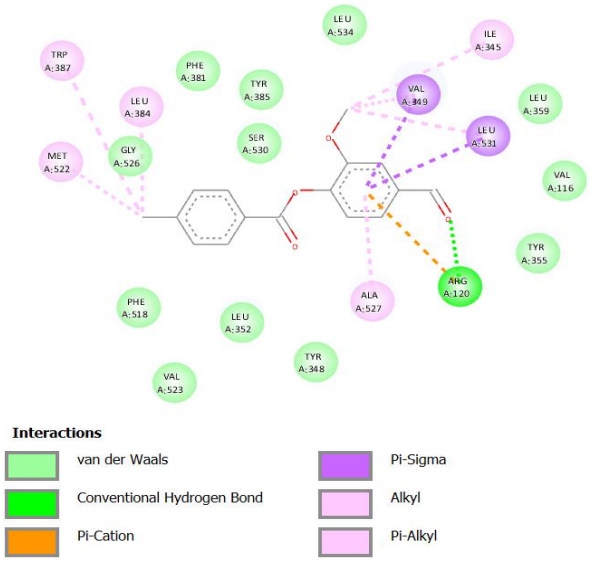

Figure 2: Interactions between 4-formyl-2-methoxyphenyl-4-chlorobenzoate and the COX2 receptor

Discussion

4-formyl-2-methoxyphenyl-4-chloroben-zoate was synthesised via an esterification reaction between acyl halide and vanillin. This reaction used the principle of acyl nucleophilic substitution by adding a nucleophile to the carbonyl atom. The acyl halide used in the synthesis of this vanillin derivative was p-chlorobenzoyl chloride. In this reaction, it was also necessary to add a base catalyst such as pyridine to remove protons from phenol when it attacks the carbonyl group, which accelerated the reaction because phenol became more nucleophilic (Clayden *et al*., 2012).

Based on the completion of the reaction test, the synthesis reaction at 120 watts was completed in 25 x 30 seconds. This was determined because at that time the intensity of the spot with the same Rf as vanillin was reduced, *p*-chlorobenzoyl chloride did not show any spots and the intensity of the spot which was thought to be the target compound was getting higher. When 200 watts of microwave power was used, the reaction also ended in 25 x 30 seconds. This was determined because the spot intensity of the initial compound, namely vanillin, was very thin and the spot intensity of the target compound had increased. When the largest microwave power of 400 watts was used, the reaction ended at 14 x 30 seconds which was determined because the intensity of the vanillin spot was getting thinner. In addition, the physical appearance of the synthesised compound was not brown, indicating that the compound had degraded, and it was therefore determined that the reaction had terminated at those times.

The highest power (400 W) produced the least amount of product. Meanwhile, the lower power (120 W) produced the highest percentage of yield (Table I). This may have occured because the greater the power used, the greater the kinetic energy that was produced. Thus, among the three microwave powers used, 120 watts was the most optimum power to synthesise 4–formyl–2-methoxyphenyl–4-chlorobenzoate. Based on structure identifications, 4-formyl-2-methoxyphenyl-4-chloro-benzoate had been successfully synthesised.

Molecular docking at the COX-2 receptor (PDB ID: 6COX) reported by Agistia *et al.* (2013), showed that the test compound interacted with amino acid residues Ser353, Arg 513, and Ser350 COX-2 receptors. In Figure 2, it appeared that the 4-formyl-2-methoxyphenyl-4-chlorobenzoate interacted with the amino acid residues Leu352, His90, Ser353, Met522, Trp387, and Leu384. There was an interaction equation for the amino acid Ser353.

Conclusion

4-formyl-2-methoxyphenyl-4-chlorobenzoate had been successfully synthesised using microwave irradiation. The optimal percentage yield of 4-formyl-2-methoxyphenyl-4-chlorobenzoate was influenced by the microwave power used, where 120 watts obtained the highest yield (89.09%). Based on the docking study, it was predicted that 4-formyl-2-methoxyphenyl-4-chlorobenzoate has the potential to be an anti-inflammatory with greater activity than vanillin because it has lower free energy. Therefore, the title compound should be further developed to treat inflammatory disease.

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