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Synthesis and Antibacterial Activities of 2-Phenyl-4h-Chromen-One (Flavone)

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Abstract: The development of new drug-resistant is needed than ever as the rapid emergence of new infections and diseases. As such flavones and their derivatives have attracted a tremendous amount of attention due to their significant antibacterial and anti-cancer activities. Hence, this research was aimed to synthesize flavone via. oxidative cyclization of chalcone and determine its antibacterial and anti-cancer activities which its information may help the development of medicine and health research. The synthesis of chalcone was performed by Claisen-Schmidt condensation. Significant reactivity of chalcone was due to conjugated double bonds and completely delocalized π-electron system on both benzene rings. The 2-hydroxy-4, 6-dimethoxyacetophenone was reacted with 4-bromobenzaldehyde in aqueous KOH which act as a catalyst in ethanol to form 3-(4-bromophenyl)-1-(2-hydroxy-4, 6-dimethoxyphenyl)-propan-1-one. It further underwent oxidative cyclization by reaction with iodine (I_2) in DMSO as a catalyst to form 2-(4-bromophenyl)-5, 7-dimethoxy-4H-chromen-4-one. Meanwhile, 2-hydroxy-4, 6-dimethoxychalcone (CP1) was synthesized from 2-methoxy-4,6-dimethoxyacetphenone and benzaldehyde. The synthesis of these compounds was characterized using Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography-Mass Spectroscopy (GCMS), ^{13}C nuclear magnetic resonance and ^{1}H Nuclear Magnetic Resonance (NMR). The antibacterial activities against *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* (SA) and *Streptococcus pyogenes* (SP) were carried out by a serial dilution method for determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The antibacterial assays indicated 2-hydroxy-4, 6-dimethoxy-4-bromobenzaldehyde is a potent inhibitor against all four types of bacteria with MBC values of 450 μg/mL. However, 2-(4-bromophenyl)-5,7-dimethoxy-4H-chromen-4-one is not a strong inhibitor to all kinds of bacteria in both of antibacterial activities such as MIC and MBC. Besides, CP1 is a potent inhibitor against all four types of bacteria with MBC values of 225 μg/mL. Nevertheless, CP1 demonstrated weak antiproliferation activity against three cancer cell lines namely, breast cancer (MCF-7), cervical cancer (HeLa) and liver cancer (HepG2) which determined using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay.

Key words: Flavone, chalcone, antibacterial, anti-cancer, bacteria, inhibitor

INTRODUCTION

As the bacterial and fungal infection cases and cancer death rate increase, the presence of drug-resistant bacteria also increase to fulfill the demand for the consumers. The synthesize of flavone is one of the promising alternative way other than the flavone extracts from the plants. Flavone (2-phenyl-4H-chromen-4-one) is one of the families in flavonoids. Generally, flavonoids are derived from the secondary metabolism of plants and fungus, thus, become the essential plant pigments that capable of processing the colouration in flowers (Qing-Hui *et al*., 2017). Flavonoids are known as plant metabolites that constitute several natural products. Plant pigmentation, UV filtration, symbiotic fixation and various physiological regulations are examples of the important role of nature flavonoids (Stanek and Stodulski, 2016). According to Valdameri *et al*. (2010), flavonoids play an essential role in biological activities such as selective anticancer activity, anti-inflammatory and cell growth inhibition.

Flavones may be synthesized from chalcone as chalcones are outstanding precursors to flavones and other compounds due to its simple chemistry, easily to

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synthesis and have a broad range of varieties of promising biological activities such as anticancer and antibacterial (Mahapatra *et al*., 2015).

MATERIALS AND METHODS

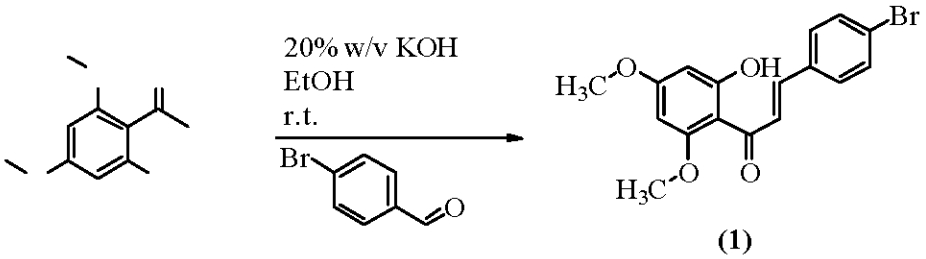
The synthesis of chalcone and flavone was performed based on chromatographic and spectroscopic methods. The biological activities such as antibacterial activity and anti-cancer activity were assayed based on the standard protocols.

3-(4-bromophenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) propan-1: IR ν_{max} (ATR) cm^{-1}: 1525 (C=O), 1563 and 1439 (C = C aromatic), 1212 (C-O), 757 (C-Br); ^{1}H NMR (400 MHz; $CDCl_3$): δ_H3.86 (3H, s,-OCH_3), 3.93 (3H, s, -OCH_3), 5.99 (1H, d, J = 4.0 Hz, H-3), 6.13 (1H, d, J = 4.0 Hz, H-50, 7.48 (2H, d, J = 8.0 Hz, H-3', 5'), 7.55 (2H, d, J = 8.0 Hz, H-2', 6'), 7.71 (1H, d, J = 16.0 Hz, H-α), 7.89 (1H, d, J = 16.0 Hz, H-β); ^{13}C NMR (100 MHz; $CDCl_3$) ppm: δ_C55.7 (-OCH_3, CH_3), 55.9 (-OCH_3, CH_3), 91.4 (C-5, C-H), 93.8 (C-3, C-H), 106.4 (C-1, C-4°), 124.2 (C-α, C-H), 128.2 (C-4', C-4°), 129.7 (C-4', C-4°), 132.1 (C-2' C-6', C-H), 134.5 (C-3', C-5' C-H), 140.8 (C-1', C-4°), 162.4 (C-β, C-H), 166.4 (C-2, C-4°), 168.5 (C-6, C-4°) and 192.1 (C = O, C-4°); M/S: M^+ 363.1781, $C_{17}H_{15}BrO_4$, m/z 207.1, 361.2, 181.1, 102.1.

2-(4-bromophenyl)-5,7-dimethoxy-4H-chromen-4-one: Pale yellow crystals; IR ν_{max} (ATR) cm^{-1}: 1525 (C=O), 1563 and 1439 (C = C aromatic), 1212 (C-O), 757 (C-Br); ^{1}H NMR (400 MHz; $CDCl_3$): δ_H3.86 (3H, s, -OCH_3), 3.93 (3H, s-OCH_3), 6.12 (1H, d, J = 4.0 Hz, H-3), 6.13 (1H, d, J = 4.0 Hz, H-50, 7.47 (2H, d, J = 8.0 Hz, H-3', 5'), 7.55 (2H, d, J = 8.0 Hz, H-2', 6'), 7.71 (1H, d, J = 16.0 Hz, H-α), 7.89 (1H, d, J = 16.0 Hz, H-β); ^{13}C NMR (100 MHz; $CDCl_3$) ppm: δ_C55.7 (-OCH_3, CH_3), 55.9 (-OCH_3, CH_3), 91.3(C-5, C-H), 93.8 (C-3, C-H), 106.3 (C-1, C-4°), 107.6 (C-α, C-H), 124.2 (C-4', C-4°), 128.1 (C-4', C-4°), 129.7 (C-2' C-6', C-H), 132.1 (C-3', C-5' C-H), 134.5 (C-1', C-4°), 162.5 (C-β, C-H), 166.4 (C-2, C-4°), 168.5 (C-6, C-4°) and 192.3 (C = O, C-4°); M/S: M^+ 363.1781, $C_{17}H_{15}BrO_4$, m/z 207.1, 361.2, 181.1, 102.1.

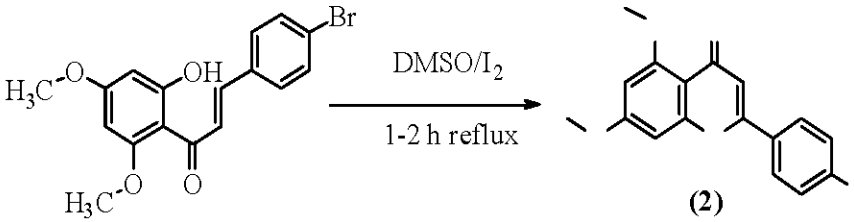
2-hydroxy-4,6-dimethoxychalcone: Yellow-needle crystals; IR ν_{max} (ATR) cm^{-1}: 1614 (C=O), 1565 and 1437 (C=C aromatic), 1209 (C-O); ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 3.82 (3H, s, -OCH_3), 3.90 (3H, m, J = 1.2 Hz, H-3', 4', 5'), 7.59 (2H, dd, J = 4.0 and 2.0 Hz. H-2'. 6'), 7.77 (1H, d, J = 15.6 Hz, H-α), 7.89 (1H, d, J = 15.6 Hz, H-β); ^{13}C NMR (100 MHz; $CDCl_3$): δc 55.7, (-OCH_3, CH_3), 55.9 (-OCH_3, CH_3), 91.4 (C-5, C-H), 93.9 (C-3, C-H), 106.4, (C-1, C-4°), 127.6 (C-α, C-H), 128.5 (C-2', C-6', C-H), 128.9 (C-3', C-5', C-H), 130.2 (C-4', C-H), 135.6 (C-1', C-4°), 142.4 (C-β, C-H), 162.6 (C-2, C-4°), 168.5 (C-4, C-4°) and 192.7 (C = O, C-4°); M/S: M^+ 284.2310, $C_{17}H_{16}O_4$, m/z 207.2, 283.3, 181.1, 103.1.

Synthesis of chalcone via. Claisen-Schmidt condensation: About 20% of aqueous potassium hydroxide, KOH (3.0 mL) was added to a solution of 2-hydroxy-4, 6-dimethoxyacetophenone (1.0 g) and benzaldehyde (3.0 mL) in ethanol, C_2H_5OH (25 mL). The reaction was stirred for 24 h at room temperature until the reaction has completed. Completion of the reaction was monitored by TLC. After that the reaction was acidified with aqueous 10% HCl (pH lower than 7) and was poured into ice water. The precipitate was filtered, washed with an excess of water, dried and purified using column chromatography (Hexane: Ethyl acetate, 4:1) to obtain pure 3-(4-bromophenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) propan-1-one as bright as yellow crystals (1.8479 g, 95.33%). All structures were confirmed by mass and NMR (Shenvi *et al*., 2013).



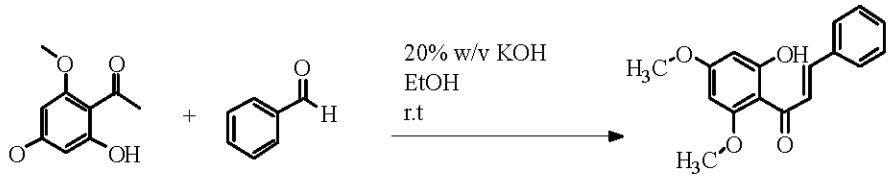
(1)

Synthesis of flavone via. oxidative cyclization: I_2 (catalytical amount) was added to a compound 3-(4-bromophenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) propan-1-one (0.5011 g) in 10.0 mL of DMSO. The reaction mixture was heated to reflux for 2 h. Next, the reaction was cooled and poured into water. The reaction mixture was extracted into ethyl acetate, EtOAc (3x25.0 mL). The organic layer was washed with brine and will be dried over $MgSO_4$. The solvent was evaporated to get 2-(4-bromophenyl)-5,7-dimethoxy-4H-chromen-4-one (0.2268 g, 50.22%). All structures were confirmed by mass and NMR (Shenvi *et al*., 2013).



(2)

Synthesis of 2-hydroxy-4,6-dimethoxychalcone (CP1): 1.0 mL of benzaldehyde was weighed and placed into Erlenmeyer flask 50.0 mL. Then, 1.0012 g of 2-methoxy-4, 6-dimethoxyacetophenone was added into the flask followed with 4.0 mL 95% ethanol. The flask was swirled to mix all the reagents. After that, 1.0 mL of potassium hydroxide was added into the flask then the flask was swirled until all the reagents solidified or the colour of the mixture becomes cloudy. 10.0 mL of ice water was added into the flask to ensure a solid formed from the mixture. Then, the solid was transferred into a beaker with 15.0 mL of ice water. The solid was collected by using vacuum filtration followed by cold water that used to wash the solid. The solid was dried for 30 min to get the 1-(2-hydroxyphenyl) ethan-1-one (0.6937 g, 80.27%). All structures were confirmed by mass and NMR (Shenvi *et al*., 2013).



(3)

Thin layer chromatography: The 1 mg of sample was dissolved in $CHCl_3$ and then spotted on to the TLC plate (Merck, Kieselgel 60 F_{254}, 0.25 mm) using a capillary glass tube with its end left dipping into the solvent in the chromatography tank. A trial and error method on various combinations of different ratios of the solvent system was used to find the most suitable solvent. After the chromatogram had been developed, the TLC plate was left to dry. Colourless substances were detected by using a UV light spectrometer or detector UVP CC-10 (254 nm wavelengths). Other reagents such as sulfuric acid and Dragendorff were also used for the non-colour component under UV. Most of the reagents were specific to a certain functional group only.

Antibacterial activity: The assay was carried out by a micro-dilution method for determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Both MIC and MBC tests are according to Gulluce *et al.* From the MIC values, the proposed classifications for antibacterial activity can be classified as strong inhibitors (<500 μg/mL), moderate inhibitors (600-1500 μg/mL and weak inhibitors (>1600 μg/mL). Streptomycin sulphate was used as a positive control.

Anti-cancer activity: Compound (3) was chosen as a sample for anti-cancer activity. The sample was tested for its antiproliferative activity on three cancer cell lines which are breast cancer (MCF-7), cervical cancer (HeLa) and liver cancer (HepG2). All cell lines were cultured and kept in an incubator at 37°C in 5% CO_2 atmosphere. The antiproliferation activity for this sample was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Boonpisuttinant *et al*., 2016).

RESULTS AND DISCUSSION

Structure elucidation of chalcone and flavone: Compound (1) was obtained as bright yellow crystals with a melting point of 358-359°C. The mass spectrum showed the molecular ion peak at m/z = 363.5764, indicated the formal formula of $C_{17}H_{15}BrO_4$. The IR spectrum showed the presence of carbonyl group (C=O) of ketone at 1738.1 cm^{-1}, C=C alkene at 1635.98 cm^{-1}, C=C aromatic at 1570.04 and 1440.4 cm^{-1}, C-O ester functionalities at 1217.82 cm^{-1} and C-Br bending at 758.47 cm^{-1}. The ^{1}H NMR spectrum showed a proton singlet at δ 14.26 due to hydroxyl group where H was attached to O. The other peaks were 7.89 (d, J= 16.0 Hz, 1H), 7.70 (d, J= 16.0 Hz, 1H), 7.55 (d, J= 8.0 Hz, 2H), 7.46 (d, J= 8.0 Hz, 2H), 6.15 (d, J = 6.14 Hz, 2H), 3.93 (s, 3H) and 3.86 (s, 3H). The ^{13}C NMR assignment of 3-(4-bromophenyl)-1-(2-hydroxy-4,6-dimethoxyphenyl) propan-1-one was performed using the attached proton test APT, which is showing the presence

of 15 signals attributed to 17 different carbons. The signals for methyl carbon were observed at 55.65 and 55.92. The spectrum also confirmed the presence of two methyl carbons, seven quaternary carbons including carbon 4' where bromine atom attached to and eight methane carbons in this compound.

Compound (2) was obtained as pale-yellow crystals with a melting point of 341-342°C. The mass spectrum was showed the molecular ion peak at m/z = 361.8436, indicated the formal formula of $C_{17}H_{13}BrO_4$. The IR spectrum showed the presence of C-H stretching of the aromatic ring at range 3000-3150 cm^{-1}, a weak carbonyl group (C=O) of ketone at 1715 cm^{-1}, C=C alkene at 1624.77 cm^{-1}, C=C aromatic at 1586.03 and 1560.15 cm^{-1}, C-O ester functionalities at 1213.51 cm^{-1} and C-H bending of the aromatic ring at 818.15 cm^{-1}. The ^{1}H NMR spectrum showed a proton doublet at δ 7.89 due to present of H on carbon 3'' and carbon 5''. Besides that, the other proton peak detected were 7.69 (d, J = 16.0 Hz, 1H), 7.51 (dd, J = 32.0 Hz, 2H), 6.11 (d, J = 4.0 Hz, 1H), 3.93 (s, 3H) and 3.86 (s, 3H). The ^{13}C NMR assignment was performed using ^{13}C APT which showed the presence of 15 signals attributed to 15 different carbons. The presence of seven quaternary carbons and five methane carbons in the compound were confirmed by the spectrum.

Compound (3) was obtained as yellow-needle crystals with melting a point of 286-287°C. The mass spectrum was showed the molecular ion peak at m/z = 207.1666, indicated the formal formula of $C_{17}H_{16}O_4$. The IR absorptions characteristics of carbonyl (1614 cm^{-1}), aromatic C=C (1565 and 1437 cm^{-1}) and C-O (1209 cm^{-1}) functionalities. It gives a yellow colour with potassium permanganate solution followed by heating but no IR absorption band for hydroxyl (-OH) group at the C-2 position. The ^{1}H NMR spectrum displayed two singlets at δ 3.82 (3H) and δ 3.90 (3H) due to the two methoxyl groups at C-4 and C-6, respectively. The meta coupled protons of the A-ring appeared at δ 5.95 (1H, d, J= 2.4 Hz, H-3) and δ 6.10 (1H, d, J= 2.4 Hz, H-5). The five aromatic protons of the B-ring were observed at δ 7.39 (3H, m, J= 1.2 Hz, H-3', 4', 5') and 7.59 (2H, dd, J= 4.4 and 2.0 Hz) assigned to H-2' and H-6', respectively. The characteristics signals for a chalcone moiety appeared as two doublets at δ 7.77 (1H, d, J= 15.6 Hz, H-α) and δ 7.89 (1H, d, J = 15.6 Hz, H-β). The APT ^{13}C NMR spectrum showed the presence of 15 signals attributed to 17 different carbons. The signals for methyl carbons were observed at δ 55.7 and 55.9. The spectrum also confirmed the presence of two methyl carbons, six quaternary carbons and nine methine carbons in this compound.

Antibacterial activity: Compound (1-3) were tested against four bacteria such as *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* (SA) and *Streptococcus pyogenes* (SP). According to Zeidan *et al*. (2013), all these types of bacterial pathogens included in this analysis capable of causing various types of infections such as skin infections, food poisoning, urinary tract infection and strep throat. From the findings, compound (1) was strong inhibitor towards all four bacteria with MBC and MIC values of 450 µg/mL for *Eschericha coli* (EC), *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* (SA) and *Streptococcus pyogenes* (SP). Meanwhile, compound (3) was a strong inhibitor towards *Eschericha coli* (EC), *Staphylococcus aureus* (SA) and *Streptococcus pyogenes* (SP) bacteria with MIC and MBC values of 450 and 225 µg/mL for *Pseudomonas aeruginosa* (PA). However, compound (2) was not able to stop the inhibition of all types of bacteria in all concentration for MIC and MBC. The result was summarized in Table 1 which revealed that compound (1) and (3) have prominent potential to develop as pharmaceutical products that cure skin infection.

Table 1: Inhibitory concentration of MIC and MBC assays for synthesized compounds

	SA		SP		PA		EC	
Compounds	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Chalcone(1)	450	450	450	450	450	450	450	450
CP1(3)	450	450	450	450	225	225	450	450
Flavone(2)	na.	na.	na.	na.	na.	na.	na.	na.
Streptomycin sulphate	14.1	14.1	14.1	14.1	14.1	14.1	14.1	14.1

SA-*Staphylococcus aureus*, SP-*Streptococcus pyogenes*, PA-*Pseudomonas aeruginosa*, EC-*Escherichia coli* (unit in µg/mL); Less than 500 µg/mL: strong, 501 µg/mL to 1500 µg/mL: moderate, more than 1500 µg/mL: weak; na: >1800 µg/mL (not within the tested concentration range)

Anti-cancer activity: MTT assay is widely used for screening collection of compounds to determine if the test molecules have effects on cell proliferation or show direct cytotoxic effects which lead to cell death. In this study, the compound (3) was evaluated for its cytotoxicity against human breast cell line (MCF-7), human cervix cell line (HeLa) and human liver cell line (HepG2). Furthermore, three types of drugs such as cisplatin, doxorubicin and fluorouracil were used as the positive control. These drugs are widely used in chemotherapy session to treat those cancer cells. From the observation Table 2, compound (3) showed weak inhibition against all three cancer cell lines compared to cisplatin, doxorubicin and fluorouracil. However, the IC_{50} result in fluorouracil was quite lower than the compound (3). Thus, it can be concluded that compound (3) also able to exhibit selectivity cytotoxic yet weaker compared to these three drugs.

Table 2: Anti-cancer activity of compound (3)

	IC_{50} (mg/mL) of the extracts on cancer cell lines		
Sample	MCF-7	HeLa	HepG2
CP1	4.1413	4.0776	4.2205
Cisplatin	0.0311	0.0369	0.0363
Doxorubicin	0.2778	0.2464	0.1942
Fluorouracil	3.8744	3.8880	4.0026

CONCLUSION

The synthesis of 2-phenyl-4H-chromen-one from chalcone was successfully done. The identification of compounds was carried out using spectroscopic methods: Infrared (IR) spectroscopy, Nuclear Magnetic Resonance (NMR) spectroscopy and Gas Chromatography Mass Spectroscopy (GC-MS) and a comparison with the literature data of compound was expected. The antibacterial activity showed where the compound (1) and (3) could act as strong inhibitors against four investigated pathogenic bacteria. The anti-cancer activity showed where the compound (3) weak against all three cancer cell lines.

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