Synthesis, characterization and antibacterial assessment of 3,4,5-trimethoxy-3',4'-dimethoxychalcone and 2,4,6-trimethoxy-3',4'-dimethoxychalcone

Emmanuel E. Odion, Davy O. Enadeghe and Cyril O. Usifoh.

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City

ABSTRACT

Background: Chalcones are utilised in the synthesis of heterocyclic molecules with antibacterial activity. This study aims to synthesize, characterise and screen 3, 4, 5-trimethoxy-3', 4'-dimethoxychalcone and 2, 4, 6-trimethoxy-3', 4'-dimethoxychalcone for antibacterial property.

Methods: Substituted chalcones were synthesized from condensation reaction between acetophenone and benzaldehyde in 50 % potassium hydroxide at room temperature and subsequently neutralised by 50 % acetic acid. Purity was evaluated by melting point determination and thin layer chromatography (TLC). Characterization was done using IR spectroscopy, ^{1}HNMR, ^{13}CNMR, mass spectroscopy and elemental analysis. The compounds were screened for activity against Gram positive and negative bacteria using well plate method.

Results: 3, 4, 5-Trimethoxy-3', 4'-dimethoxychalcone was obtained as yellow crystals (m. pt. 121-122 ^{o}C, 46 %) and 2, 4, 6-trimethoxy-3', 4'-dimethoxychalcone was also a yellow crystal (m. pt. 150-151 ^{o}C, 41 %). These compounds did not show any zone of inhibition.

Conclusion: Both synthesised and characterised chalcones do not show activity against Gram positive and negative bacteria.

Keywords: Synthesis, Characterization, Aldol Condensation, Antibacterial Property, Chalcones.

1. INTRODUCTION

Chalcones are important moiety that have been used as starting molecule in synthesizing useful polycyclic aromatic compounds [1,2]. One of the basic synthetic procedure occurs via Claisen-Schmidt condensation, which can be illustrated by the reaction between carbonyl compounds, forming aromatic ketone. While a popular and effective method of preparing is the aldol condensation reaction of substituted acetophenone and benzaldehyde in base [4,5]. The malleable structure of chalcones makes them have an array of diverse pharmacological properties [6] which include anti-proliferative, antimicrobial, insecticides, antioxidants and their ability to manage cardiac and vascular diseases, in addition to their risk factors [7,8]. Structural alternation on the A and B-ring at the ortho, meta and para positions by methoxy group have imbibed antioxidant and anti-inflammatory properties, however it full potential is yet to be appreciated in terms of new antibacterial molecules as drugs. Infections are majorly triggered by pathologic microorganisms like bacteria, virus and fungi. Bacterial infections is one of the four major causes of hospital visit yearly [9] and base on the nature of their cell walls, they are classified into Gram positive and Gram negative bacteria [10], which are treated by anti-bacteria or antibiotics. Due to high frequent dependent and usage of antibiotics, bacteria could advance resistance to available drugs [11]. These have occasioned unceasing research for medicines to prevent resistance that may advance, hence the search to synthesize compounds that could be effective against Gram positive and Gram negative bacteria [12]. Thus, this study aims to synthesise, characterise and screen 3, 4, 5-trimethoxy-3', 4'-dimethoxychalcone and 2, 4, 6-trimethoxy-3', 4'-dimethoxychalcone for antibacterial property.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1 Reagents

The following reagents 3, 4-dimethoxyacetophenone, 3, 4, 5-trimethoxybenzaldehyde, 2, 4, 6-trimethoxybenzaldehyde, were obtained from Sigma Aldrich Germany, Acetic acid, potassium hydroxide were obtained from Scharlau, Spain.

* Corresponding author: Email: emmanuel.odion@uniben.edu; Phone: +2349048303020

2.1.2 Equipment

Melting points were determined on a Kofler Electrothermal® melting point apparatus CAT No 1A6304, England. IR spectra were recorded as KBr disc on Buck Infra-Red M500 instrument (Buck Scientific Inc., Norwalk, Connecticut, USA. ^{1}H-NMR spectra were recorded on Varian Germini 200 (250 MHz) (Varian Inc., Palo Alto, California, USA). Chemical shifts are reported in ppm relative to tetramethylsilane as reference standard. ^{13}C-NMR spectra were recorded on Varian Germini 200 (63 MHz, Varian Inc., Palo, California, USA). Chemical Shift were reported in ppm relative to tetramethylsilane as reference standard. The multiplicities are represented as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, J = coupling constant, Hz = hertz. Mass spectra were acquired on a Finnigan MAT44S mass spectrometer (Thermo Finnigan San Jose, California, USA) at 70 eV. Weighing was done using analytical weighing balance (B 15 Matler, Toledo, Switzerland). Analytical Thin Layer chromatography (TLC) on silica gel 60 F_{254} pre-coated plates from Merck Damstardt, Germany.

2.1.3 *Biological Materials*

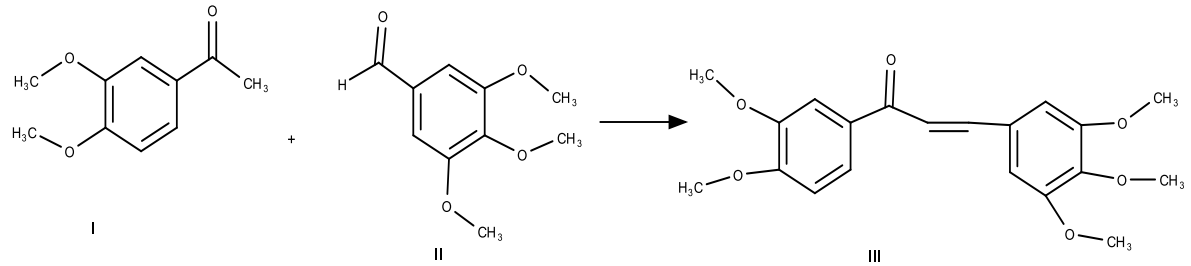
The bacteria *Escherichia coli* (NCTC 10418) and *Staphylococcus aureus* NCTC 6571 used in this study were obtained from University of Benin Teaching Hospital and Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin.

2.2 Methods

2.2.1 ***Synthetic A***. Equal amount of 3,4-dimethoxyacetophenone (0.006 mole) and 3,4,5-trimethoxybenzaldehyde (0.006 mole) was dissolved in 20 ml of methanol and 2 ml of 50 % potassium hydroxide was added and stirred intermittently at room temperature before the mixture was allowed to stand overnight. It was then diluted water with 2.4 ml of distilled water and was brought to pH 7 with sufficient 50 % acetic acid (scheme 1).

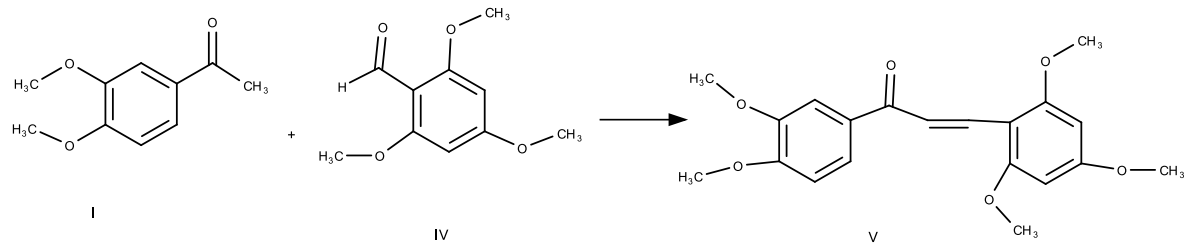
2.2.2 ***Synthetic B***.

Equal amount of 3,4-dimethoxyacetophenone (0.006 mole) and 2,4,6-trimethoxybenzaldehyde (0.006 mole) was dissolved in 20 ml of methanol and 2 ml of 50 % potassium hydroxide was added and refluxed at 70 ^{o}C for 2 hours with intermittent stirring and the mixture was allowed to cool overnight. It was then diluted with 2.4 ml of distilled water and was brought to pH 7 with sufficient 50 % acetic acid (scheme 2).



i: 3,4-Dimethoxyacetophenone, ii:3,4,5-Trimethoxybenzaldehyde, iii: 3,4,5-Trimethoxy-3',4'-dimethoxychalcone

Scheme 1



i: 3,4-Dimethoxyacetophenone, iv: 2,4,6-Trimethoxybenzaldehyde, v: 2,4,6-Trimethoxy-3',4'-dimethoxychalcone

Scheme 2

2.2.3. ***Antibacterial activity***

The synthetic compounds were screened for antibacterial activity by making a concentration of 1 mg/ml in dimethylsulphoxide (DMSO). These solutions were used to evaluate for antibacterial activity by aseptically pouring 25 ml of melted agar into sterile Petri dish and was then allowed to solidify. A 4-hour culture of *E. coli* WCTC10418 and *S. aureus* NCTC6571were flooded on each of the two plates of nutrient agar, then a cork borer (sterile) was used to make four wells on each of the agar plates. Each of the well was sealed by using 0.1 ml of agar and 0.2 ml of the synthetic compounds were aseptically transferred into two of the wells, while DMSO (negative control) and ciprofloxacin (positive control) were placed in the other two wells. These were allowed to stand for 30 minutes before incubation for 18 hours. [13].

2.3 Statistical Analysis

Result of the antibacterial study was presented as Mean±Standard deviation (SD), while statistical analysis was nil.

3.0 RESULTS

3,4,5-Trimethoxy-3',4'-dimethoxychalcone (iii) (1.3203 g; 62.9 %), melting point 121-122 ^{o}C; IR (KBr): υ 3030 (Ar-H), 1646 (C=O), 1573 (C=C). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.65 (s, 3H, CH_3), 3.7 (s, 6H, OCH_3 (C_1, C_2), 3.75 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3), 6.75 (m, 2H, Ar-H), 7.3 (d, J=7.0 Hz, 1H, Ar-H), 7.45 (d, J=7.2 Hz, 2H, Ar-H), 7.55 (s, 1H, Ar-H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 105.7, 110.1, 110.8, 123.2, 130.4, 130.9; 144.3, 149.1, 153.2, 153.4, 189.3. MS: m/z 358 (100), 327 (63) (M^{+}-OCH_3); 298 (5); 283 (8); 255 (6); 165 (5); 115 (1); 77 (2). Elemental analysis $C_{20}H_{22}O_6$ (358), Cal: C.67.09 H.6.19, Found C. 67.07 H. 6.20

2,4,6-Trimethoxy-3',4'-dimethoxychalcone (v) (0.8651 g; 41 %), melting point 150-151 ^{o}C; IR (KBr) 1640 (C=C), 1740 (C=O), 3005 (Ar-H); ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.65 (s, 3H, CH_3), 3.7 (s, 6H, OCH_3 (C_1, C_2), 3.75 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3), 6.75 (m, 2H, Ar-H), 7.3 (d, J =7.0 Hz, 1H, Ar-H), 7.45 (d, J =7.4 Hz, 2H, Ar-H), 7.55 (s, 1H, Ar-H). ^{13}CNMR (50 MHz, $CDCl_3$): δ 105.7, 110.1, 110.8, 123.2, 130.4, 130.9; 144.3, 149.1, 153.2, 153.4, 189.3; MS: m/z 358, 327 (M^{+}-OCH_3), 311 (11), 165 (4), 139 (1), Elemental analysis $C_{20}H_{22}O_6$ (358), Cal: C.67.09 H.6.17, Found C. 67.01 H.6.21.

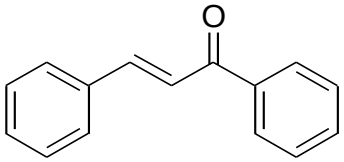
Table 1. Zones of inhibition (mm) of synthesized compounds

S/N	Compounds	*Escherichia coli*	*Staphylococcus aureus*
1.	3, 4, 5-Trimethoxy-3', 4'-dimethoxychalcone	-	-
2.	2, 4, 6-Trimethoxy-3', 4'-dimethoxychalcone	-	-
3.	Ciprofloxacin	18±0.3	22±0.3
4.	DMSO	-	-

\- = negative, + = positive

4. DISCUSSION

Chalcones are 1,3-diphenyl-2-propene-1-ones [14], with two aromatic rings A and B, linked to a three carbon α,β-unsaturated system. The synthetic strategy involved the condensation of benzaldehyde and ketone in the presence of a base as the catalyst. This reaction depends on the reactivity of the carbonyl functional group to form a new carbon-carbon bond, which is an important bond formation process.



1,3-Diphenyl-2-propene-1-ones

The strategy used involve the formation of a conjugate bases of 3, 4, 5-trimethoxybenzaldehyde and 2, 4, 6-trimethoxybenzaldehyde which are then added to individually to 3, 4-dimethoxyacetophenone to give the intermediate product of the cross aldol reaction and subsequent loss of water molecule that result in the double bond seen as a link between the two molecules. Chalcones are able to undergo this reaction due to the presence of hydrogen that are highly replaceable and thus the use in the synthesis of different functional groups or derivative have shown a broad spectrum of antimicrobial property [15,16]. The different pharmacological properties ascribed to chalcones are due to the presence of both α,β-unsaturation [17] and the aryl ring. The yield from this synthesis are moderately low (40-60 %), microwave assisted synthesis can considerably increase the reaction rate with

better yields [18]. Characterization of 3, 4, 5-trimethoxy- 3', 4'-dimethoxychalcone revealed it ^{1}H NMR spectrum with diagnostic chemical shift at 3.7 ppm (6H) belonging to two methoxy groups which appeared as singlet, one methoxy group at 3.75 ppm (3H) which appeared as singlet, one methoxy group at 3.8 ppm (3H) which also appeared as singlet in addition to two methine hydrogens which resonated as doublet at 7.3 ppm and 7.55 ppm. ^{13}C NMR chemical shift of 149.1 ppm for the methine carbon (CH), 153.4 ppm for the methine carbon in conjugation to the carbonyl group and 189 ppm functional group. IR spectrum was able to identify the aromatic stretch at 3030 cm-^{1}, the C=O stretch at 1646 cm-^{1} and C=C vibration at 1573 cm-^{1}. Mass spectrometry showed both the molecular peak and base peaks at 358 m/z which is in agreement with the molecular weight of the compound. Also characterization of 2, 4, 6-trimethoxy-3', 4'-dimethoxychalcone, revealed its proton NMR with two methoxy hydrogen at 3.7 ppm (6H) which appear as singlet, methoxy hydrogen at 3.75 ppm (3H) which appear as singlet and methoxy hydrogen at 3.8 ppm (3H) which appear as singlet. Two methine hydrogen (CH) were also observed at 7.3 ppm and 7.45 ppm as doublet. ^{13}C NMR was done at a frequency of 50 MHz and the following diagnostic chemical shifts were obtained at 149.1 ppm for methine carbon, 153.4 ppm methine carbon close to the carbonyl group, 189.3 ppm for carbonyl carbon. IR spectrum showed bond stretching of C=C at 1640 cm-^{1}, (C=O) at 1740 cm-^{1}, and Ar-H at 3005 cm-^{1}. Mass spectrum showed the molecular weight of the compound to be 358 from the molecular ion peak 358 m/z. Zones of inhibition was assessed via agar well plate experiment against Gram negative and Gram positive bacteria. Ciprofloxacin which is the reference drug showed zones of inhibition of (18±0.3) mm and (25.00 ± 0.30) mm against *E. coli* and *S. aureus*, while 3, 4, 5-Trimethoxy- 3', 4'-dimethoxychalcone and 2, 4, 6-trimethoxy-3', 4'-dimethoxychalcone that did not show any zones of inhibition. This finding is not in agreement with works done by other scholars that have recorded antibacterial activity for chalcones [19,20]. Infections caused by *S. aureus* and *E. coli* can be life threaten when not promptly and properly managed [21]. Chalcones as potent antibacterial agent [22,23], however the inactivity of 3, 4, 5-trimethoxy- 3', 4'-dimethoxychalcone and 2, 4, 6-trimethoxy-3', 4'-dimethoxychalcone could be due to the presence on the Chalcones moiety of methoxy functional groups on ring A or B, since the presence of methoxy in the meta and para positions of Chalcones have been implicated in lack of activity, also the presence of three methoxy group on ring B have been shown to have activity but not as potent as a ring B with one methoxy group [24].

5. CONCLUSION

From this study, 3, 4, 5-trimethoxy- 3', 4'-dimethoxychalcone was synthesized from the reaction between 3, 4-dimethoxyacetophenone and 3, 4, 5-trimethoxybenzaldehyde, while 2, 4, 6-trimethoxy-3', 4'-dimethoxychalcone was the product of the reaction between 3, 4-dimethoxybenzaldehyde and 2, 4, 6-trimethoxybenzaldehyde. These compounds were characterised using various spectroscopic techniques and were shown not to have antimicrobial activity.

Acknowledgement

The authors wish to thank the laboratory staff of the Department of Pharmaceutical Microbiology and declare that the study was self-sponsored.

Conflict of interest

The authors declare no conflict of interest

Contributions of Authors

The concepting and editing of this work was done by Cyril O. Usifoh, Emmanuel E. Odion was involved in writing this work while data collection was handled by David O. Enadeghe.

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