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Synthesis, characterization and antimicrobial evaluation of some chalcones derivatives

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ABSTRACT: Through Claisen-Schmidt reactions, several chalcones having antibacterial properties have been created and engineered. According to docking tests, the amino group of the amino chalcone derivatives of chlorine plays a crucial role in the inhibitory activity by electrostatic contact through salt bridges and it produces more stable and better affinity complexes. Several aromatic aldehydes and substituted acetophenones were physically crushed in an alkaline solution using a mortar and pestle. The synthesised compounds were characterised using IR, ^{1}H-NMR, and $_{13}$C-NMR spectroscopic techniques, and their potential antibacterial activity was then investigated. TLC was used to keep track of the response.
KEYWORDS: Methoxychloro chalcones, Claisen-Schmidt Reaction, anti malarial, antiulcer, cytotoxic etc.

INTRODUCTION: Malaria, cancer, diabetes, and other ailments have all been documented in dangerous situations in recent years. Chalcone have the ability to prevent such circumstances. Chalcones are naturally occurring substances that can also be produced synthetically utilising a straightforward synthesis method. The synthesis of various heterocyclic compounds with pharmacological potential is becoming more and more popular [1–5]. They represent a significant class of naturally occurring bioactive substances [6–8]. Chalcones play a key role in the production of a number of these heterocyclic compounds [9–11]. They are made up of open chain flavonoids, which are compounds with two aromatic rings connected by a three carbon - unsaturated carbonyl system. It has been discovered that the antibacterial action of chalcones is caused by the presence of reactive - unsaturated keto function [12–14]. A number of chalcones have been examined recently for their cytotoxic, anticancer, antiviral, insecticidal, and enzyme inhibitory activities [15, 16]. Numerous chalcones with amino and alkoxy groups in various positions have been found to have antiulcer [17], antifungal [18], antimalarial [19, 20], antidiabetic [21], antioxidant [22], and antibacterial [23–26] properties. We synthesised several chalcone derivatives because of the many significant, beneficial characteristics and biological activities of chalcone derivatives.

MATERIALS AND METHODS: The uncorrected melting points were established using an open capillary tube melting point device. On a Nicolet 400D spectrometer, the infrared spectra of KBr pellets were captured. FT-IR, ^{1}H-NMR, and $_{13}$C-NMR spectrum data were used to corroborate the molecular structures of the produced compounds. All of the hydrogen atoms in the olefinic carbon-carbon bond were found to be in a trans conformation, according to 1H-NMR coupling constant measurements. As the carbonyl peak was seen at a lower wave number than a typical carbonyl peak (about 1650–1660 cm1) and from the $_{13}$C–NMR spectra, it was clear that a carbonyl group was conjugated with the olefinic carbon–carbon bond. On a Bruker spectrometer operating at 400 MHz, ^{1}H NMR spectra in $CDCl_3$ with TMS as the internal standard was captured. Chemical shifts were measured in ppm (parts per million). Using n-hexane and ethyl acetate as the solvent system, TLC was used to determine the purity of the compounds on silica-Gel plates with a 2 mm thickness. In an iodine chamber, spot visualisation was performed.
Schematic procedure for synthesis of compounds: Chalcones were created using a base-catalyzed Claisen-Schmidt condensation reaction using substituted aromatic aldehydes and substituted acetophenones. In a clean mortar, 1 mmol each of aromatic aldehyde and acetophenone were added. The aforementioned combination was combined with a small pellet of sodium hydroxide, and the reaction mixture was mechanically ground for about 30 minutes at room temperature with the use of a pestle. TLC kept track of the reaction's development. The reaction mixture was then covered with filter paper and left in the mortar for the night. The finished product was then put into a beaker of water while the mortar's washings were collected in a separate beaker. By using 1:1 HCl to neutralise the excess alkali, the solid was produced, which was then filtered, water-washed, dried, and recrystallized from ethanol.

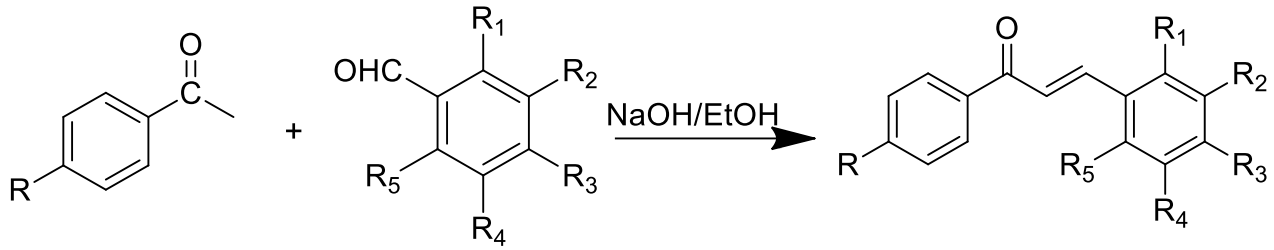
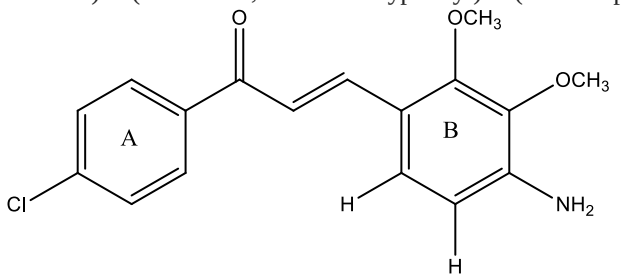


Table -1: Some useful substitutents of Chalcones

Compounds	R	R_1	R_2	R_3	R_4	R_5
1a	Cl	$COCH_3$	OCH_3	NH_2	H	H
1b	Cl	H	$COCH_3$	OCH_3	H	NH_2
1c	Cl	H	OCH_3	H	NH_2	$COCH_3$
1d	Cl	H	OCH_3	NH_2	H	H
1e	Cl	OCH_3	NH_2	H	H	H

Synthesis of E)-3-(4-amino-2, 3-dimethoxyphenyl)-1-(4-chlorophenyl) prop-2-en-1- one **[1a]**

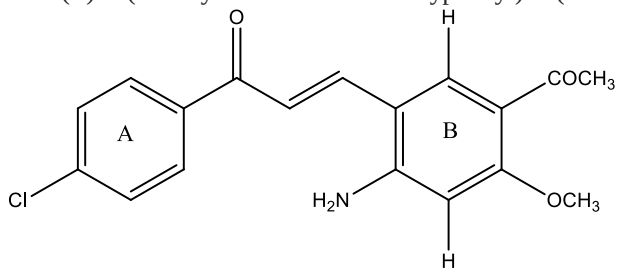


(*E*)-3-(4-amino-2,3-dimethoxyphenyl)-1-(4-chlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl) ethanone 1.50 g (1 mmol) and 4-amino-2,3-dimethoxybenzaldehyde 2.82 g (1 mmol) were grinded along with sodium hydroxide as in general procedure to give 1a. The excess of alkali was neuralised by 1:1 HCl and the liquid separated by separated funnel from ethanol to get yellow crystals (0.89 g, 41%); 1.28g, Yield 86% of [1a].

Mol. Formula: $C_{17}H_{16}ClNO_3$, Mol.Wt: 317.77, M.P. 117-120^{0}C, Rf = 0.42 (*n*-hexane/ethyl acetate: 3/2); IR (KBr): 3445 and 3344 cm^{-1} (-NH2); 1040 cm^{-1} (C-O), 1210 cm^{-1} (O-CH_3), 712 cm^{-1} (Cl)., 1412 cm^{-1} (CH=CH), 1513 cm^{-1} (C-C), 1673 cm^{-1} (C=O), 3030 cm^{-1} (Ar-CH). 1173(C-O-Caryl alkyl ether); 1H-NMR (CDCl3) δ 8.13 (*d*, 1H, *J* = 15.1 Hz); 7.65 (*d*, 1H, *J* = 15.3 Hz); 7.02 (*t*,1H); 7.40 (*s*, 1H); 6.95 (*s*, 1H,); 6.93 (*s*, 1H); 7.98 (*d*, 1H, *J* = 8.5 Hz); 6.70 (*d*, 1H, *J* = 8.9 Hz); 3.91 (*s*, 3H); 4.21 (*s*, br, 2H); 13C-NMR (CDCl3) δ 140.0; 123.1; 126.0; 159.0; 114.5; 129.1; 121.09; 135.3; 128.1;132.3; 115.1; 155.1; 115.1; 130.10; 66.2; 189.80.

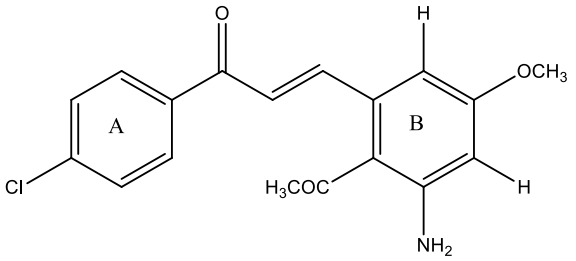
Synthesis of (E)-3-(5-acetyl-2-amino-4-methoxyphenyl)-1-(4-chlorophenyl) prop-2-en-1-one (**[1b]**



(*E*)-3-(5-acetyl-2-amino-4-methoxyphenyl)-1-(4-chlorophenyl)prop-2-en-1-one

By the same method 1-(4-chlorophenyl) ethanone and 5-acetyl-2-amino-4-methoxybenzaldehyde was grinded with sodium hydroxide to get 1b with recrystalization from ethanol gives yellow crystals (1.21 g; 78% yield). Mol. Formula: $C_{18}H_{16}ClNO_3$ Mol. Wt. 329.78, M.P. 158-161^{0}C, Rf = 0.49 (*n*-hexane/ethyl acetate: 3/2); IR (KBr): 3441 and 3340 (-NH2), 1645; 3021 cm^{-1} (Ar-CH), 1669 cm^{-1} (C=O), 1518 cm^{-1} (C-C), 1413 cm^{-1} (CH=CH), 1039 cm^{-1} (-OCH_3), 778 cm^{-1} (Cl).. 1170 (C-O-C aryl alkyl ether); 1H-NMR (CDCl3) δ 7.87 (*d*, 1H, *J* = 14.9 Hz); 7.51 (*d*, 1H, *J* = 15.8 Hz); 7.20 (*t*, 1H,*J* = 2 Hz); 7.31 (*d*, 1H, *J* = 7.6 Hz); 7.38 (*t*, 1H, *J* = 8 Hz); 7.98 (*d*, 1 H, *J* = 8.7 Hz); 7.12 (*d*, 1H,*J* = 8.5 Hz); 8.11 (*d*, 1 H, *J* = 8.8 Hz); 4.01 (*s*, 3H), 3.98 (*s*, br, 2 H). 13C-NMR (CDCl3) δ 143.14; 123.14;137.11; 116.23; 160.14; 113.38; 130.35; 121.10; 128.66; 130.08; 119.90; 152.10; 119.93; 61.12; 187.68..

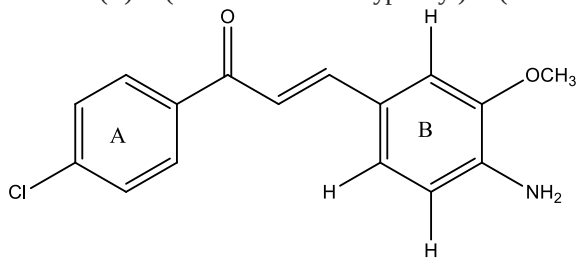
Synthesis of (E)-3-(2-acetyl-3-amino-5-methoxyphenyl)-1-(4-chlorophenyl) prop-2-en-1-one **[1c]**



(*E*)-3-(2-acetyl-3-amino-5-methoxyphenyl)-1-(4-chlorophenyl)prop-2-en-1-one

By Using the same method 1-(4-chlorophenyl) ethanone and 2-acetyl-3-amino-5-methoxybenzldehyde were grinded with sodium hydroxide to get (1c) with recrystalization from ethanol gives yellow crystals (1.10 g, 70% yield.. Mol. Formula: $C_{18}H_{16}ClNO_3$ Mol. Wt. 329.78, M.P. 175-177^{0}C, Rf = 0.42 (*n*-hexane/ethyl acetate: 3/2); IR (KBr): 3450 and 3320 (-NH_2), 2983 cm^{-1} (Ar-CH), 1620 cm^{-1} (C=O), 1510 cm^{-1} (C-C), 1600, 1510 cm^{-1} (CH=CH), 1020 cm^{-1} (-OCH_3), 782 cm^{-1} (Cl)., 1625 (C=O), 1250 (C-O-$C_{aryl\ alkyl\ ether}$); 1H-NMR ($CDCl_3$) δ 7.80 (*d*, 1H, *J* = 15.5 Hz); 7.51 (*d*, 1H, *J* = 15.3 Hz); 7.68 (*d*,1H, *J* = 8.7 Hz); 6.82 (*d*, 1H, *J* = 8.1 Hz); 7.56 (*d*, 1H, *J* = 8.2 Hz); 7.95 (*d*, 1H, *J* = 8.9 Hz); 7.24 (*d*, 1H,*J* = 8.9 Hz); 8.12 (*d*, 1H, *J* = 8.7 Hz); 3.97 (*s*, 3H); 4.56 (*s*, br, 2H). 13C-NMR (107.21 MHz, $CDCl_3$) δ140.99; 120.83; 129.13; 131.12; 113.96; 162.83; 128.40; 131.64 114.14; 151.13; 132.11; 56.44; 188.28.

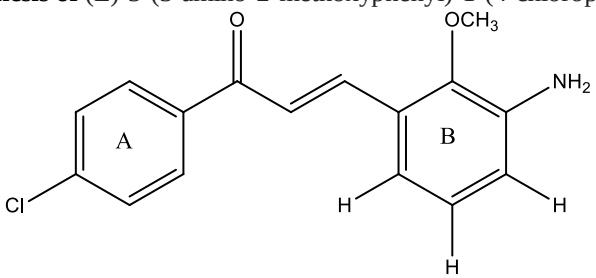
Synthesis of (E)-3-(4-amino-3-methoxyphenyl)-1-(4-chlorophenylprop-2-en-1-one **[1d]**



(*E*)-3-(4-amino-3-methoxyphenyl)-1-(4-chlorophenyl)prop-2-en-1-one

By Using the same method 1-(4-chlorophenyl) ethanone and 4-amino-3-methoxybenzaldehyde were grinded with sodium hydroxide to get (1d) with recrystalization from ethanol gives yellow crystals of 0.84g, 54% yield. Mol. Formula: $C_{16}H_{14}ClNO_2$ Mol. Wt. 287.74, M.P. 241-246^{0}C, IR (KBr, cm^{-1}): 3520 and 3487 (-NH_2), 1638 cm^{-1} (C=O), 1623, 1531 cm^{-1} (>C=C<), 1052 cm^{-1} (OCH_3), 772 cm^{-1} (Cl).. 1169 (C-O-C $_{aryl\ alkyl\ ether}$); ^{1}H-NMR ($CDCl_3$) δ 8.05 (*d*, 1H, *J* = 15.9 Hz); 7.68 (*d*, 1H,*J* = 13.6 Hz); 7.23 (*d*, J_1 = 1.2 Hz; J_2 = 8.2 Hz); 7.17 (*t*, 1H, *J* = 8.4 Hz); 6.80 (*d*, 1H, J_1 = 1.4 Hz;J_2 = 8.5 Hz); 8.32 (*d*, 1H, *J* = 9.2 Hz); 6.78 (*d*, 1H, *J* = 8.9 Hz); 8.16 (*d*, 1H, *J* = 8.7 Hz); 4.11 (*s*, 3H);4.19 (*s*, 3H); 4.56 (*s*, br, 2H). ^{13}C-NMR ($CDCl_3$) δ 133.6; 130.1; 141.9; 154.5; 155.4; 121.7; 132.6; 120.6; 130.4; 133.1; 121.7; 152.1; 62.1; 56.5; 179.8.

Synthesis of (E)-3-(3-amino-2-methoxyphenyl)-1-(4-chlorophenyl) prop-2-en-1-one **[1e]**



(*E*)-3-(3-amino-2-methoxyphenyl)-1-(4-chlorophenyl)prop-2-en-1-one

By Using the same 1-(4-chlorophenyl) ethanone and 3-amino-2-methoxybenzaldehyde were grinded with sodium hydroxide to get (1e) with recrystalization from ethanol gives yellow crystals of 0.98g, 65% yield. Mol. Formula: $C_{16}H_{14}ClNO_2$ Mol. Wt. 287.74, M.P. 196-198^{0}C, IR (KBr, cm^{-1}): 3450 and 3387 (-NH_2), 1658 cm^{-1} (C=O), 1587, 1526 cm^{-1} (>C=C<), 1034 cm^{-1} (OCH_3), 712 cm^{-1} (Cl).. 1187 (C-O-C $_{aryl\ alkyl\ ether}$); ^{1}H-NMR ($CDCl_3$) δ 8.05 (*d*, 1H, *J* = 15.6 Hz); 7.58 (*d*, 1H,*J* = 1.2 Hz); 6.43 (dd, J_1 = 1.2 Hz; J_2 = 8.7 Hz); 7.57 (d, 1H, *J* = 8.7 Hz); 7.40 (*d*, 1H, J_1 = 1.2 Hz;J_2 = 8.4 Hz); 8.22 (*d*, 1H, *J* = 8.2 Hz); 6.88 (*d*, 1H, *J* = 9.1 Hz); 8.36 (*d*, 1H, *J* = 8.9 Hz); 4.16 (*s*, 3H);4.19 (*s*, 3H); 4.56 (*s*, br, 2H). ^{13}C-NMR ($CDCl_3$) δ 133.6; 130.1; 141.9; 154.5; 155.4; 121.7; 132.6; 122.4; 132.5; 136.1; 127.4; 152.71; 60.31; 55.7; 189.7.

RESULTS AND DISCUSSION: By using IR, ^{1}H-NMR, and $_{13}$C-NMR spectrum analyses, the structures of the produced compounds were verified. Titled compounds were verified by IR spectral data revealing strong bands in the region between 1030 and 1660 cm^{-1}that showed the existence of the C=O group. The compounds (1a-1e) were also verified by ^{1}H-NMR spectrum spectroscopy. The ^{1}H-NMR spectra revealed that the chalcones were geometrically pure and trans-configured. The outcomes demonstrated that most of the produced compounds inhibited Gram positive bacteria to varied degrees, as shown in **Table 2**. At both concentrations, 500 g/ml and 1000 g/ml, the **(1a)** shown excellent efficacy against Staphylococcus aureus. At both concentrations, i.e. 500 g/ ml and 1000 g/ ml, the compounds 1b and 1c have demonstrated good to moderate efficacy against Staphylococcus aureus. At both doses, 500 g/ml and 1000 g/ml, the compounds 1b and 1c demonstrated good to moderate efficacy. Due to their limited agar media diffusion potential, two of the chalcones with anti-staphylococcal activity (1d and 1e) failed to produce any inhibitory zones. Finally, drugs against the Gram negative bacteria Pseudomonas aeruginosa showed no

efficacy. The selectivity of the current drugs against Gram positive Staphylococcus aureus can be attributed to the well-known fact that Gram positive and negative organisms differ greatly in their membrane composition and architecture.

ANTIBACTERIAL ACTIVITY: The disc diffusion method was used to assess the antimicrobial activity of all produced compounds [27–31]. For the purpose of determining activity, all human pathogenic bacteria, including Staphylococcus aurenus (737) and Pseudomonas aeruginosa (1688), were employed. The conventional protocol was followed in the preparation of nutritional broth, Subculture, base layer medium, agar medium, and peptone water. Whatman no.1 filter paper was used to punch discs with a 6.25 mm diameter. Dimethylsulfoxide (1% DMSO) was used to dilute a stock solution of synthetic chemicals to provide final concentrations of 500 and 1000 g/ml. An appropriately weighted quantity of chloramphenicol (500 and 1000 g/ml, respectively) was dissolved in sterile distilled water to create a reference standard for both gramme positive and gramme negative microorganisms. The incubation was place for 24 hours at 37°C. Three copies of each experiment were run throughout. 0.1 mL of dimethyl sulfoxide was used to maintain controls at the same time, however this showed no inhibition. Each compound's zones of inhibition were quantified in millimetres (mm). **Table 2** lists the findings of investigations on antibacterial agents.

Table 2: Antimicrobial activity of the synthesized compounds

S.No.	**Antimicrobial activity (% inhibition)**			
	Staphylococcus aureus (737)		**Pseudomonas aeruginosa (1688)**	
	500 µg/ml	1000 µg/ml	500 µg/ml	1000 µg/ml
1a	26.0	35.7	08	03
1b	23.4	33.5	11	09
1c	19	21	10	07
1d	----	----	---	----
1e	11	10	07	08
Chloramphenicol	43.4	56.3	60.8	80.3
DMSO	1.4	----	1.2	----

CONCLUSION: The intended products might be obtained with greater yields using the grinding procedure. In tests against Staphylococcus aureus and Pseudomonas aeruginosa, the produced compounds demonstrated moderate to good antibacterial properties.

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