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Synthesis, Characterization, Antimicrobial and Anti-Inflammatory Properties of 4-Methoxy, 4, 6-Dipheny L-2-Thiopyrimidine and Epoxide Derivatives of Chalcones

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Abstract **Review Article**

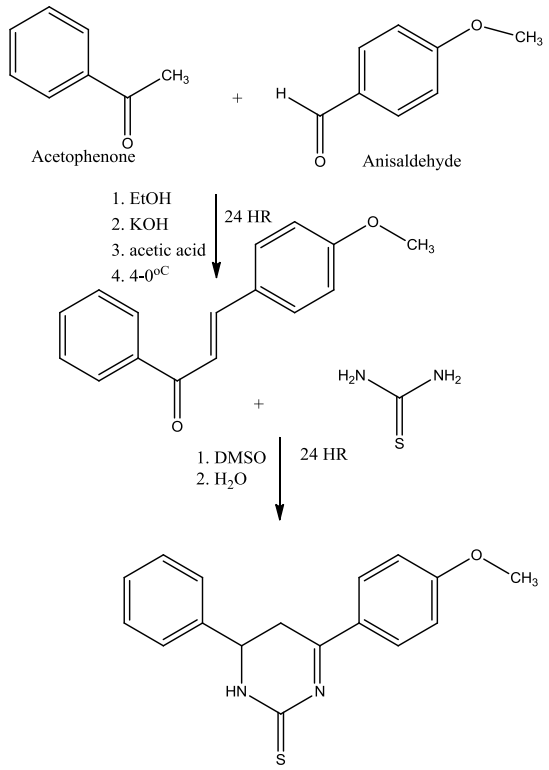
Chalcone, its 4-methoxy, 4-methoxy-4,6-diphenyl-2-thiopyrimidine derivatives and chalcone epoxides were synthesized using the Claisen-Schmidt condensation between benzaldehyde derivatives and acetophenone at very low temperature in the presence of KOH and ethanol. Further cyclization reaction with thiourea gave the heterocyclic derivative. The melting points, actual yield, percentage yields and R_F values of all the synthesized compounds were determined and their physical appearances recorded. The structures of the compounds were determined using FTIR, ^1H, ^{13}C NMR experiments. The compounds were screened for *in-vivo* anti-inflammatory activity at the dose of 1mg/kg, 2mg/kg and 4mg/kg using egg albumin-induced paw oedema in rat. Diclofenac, 2mg/kg was used as the reference drug. The synthesized compounds were also subjected to *in-vitro* antimicrobial screening against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albican*. Ciprofloxacin, gentamycin and ketoconazole were used as reference standards. The compounds showed no antimicrobial activity but the 4-methoxy-4, 6-diphenyl-2-thiopyrimidne displayed remarkable anti-inflammatory activity.

Keywords: Chalcones, Claisen-Schmidt condensation, Anti-inflammatory, Antifungal, Cnalcone epoxides, Heterocycles.

INTRODUTION

The increasing morbidity and mortality due to the upsurge of resistant microbes has made research in this field a continuous burden for the researchers and donor agencies. Also, inflammatory diseases pose a serious concern as it has been known to be the root cause of so many terminal ailments even cancer [1, 2]. The available treatment options, though effective are not without serious side effects [2]. As a result, effort towards the addition of new therapeutic options for the treatment of infectious and inflammatory diseases cannot be a wasted one. Chalcones derivatives had been reported [4] to have some antimicrobial activity, against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa.* Also, some derivatives showed appreciable activity against fluconazole-sensitive and fluconazole-resistant strain of fungi infections [8]. Moreover, chalcones and their derivatives demonstrate a wide range of potential biological activities including anti-inflammation and 2-amino-chalcones have been studied as potential antitumor agents [1-3]. It has been found that the Quantitative Structure Activity Relationship equation of chalcones revealed that selected electronic, steric and lipophilic parameters had good correlation with antibacterial activity [10]. The finding suggests that the chalcone basic moiety is an attractive template for structure modification to achieve higher potency, lower toxicity, and wider spectrum of antibacterial activity. Chalcones can be prepared by Claisen-Schmidt condensation reaction between benzaldehyde and acetophenone in the presence of sodium hydroxide or potassium hydroxide as catalyst. Substituted (3,5-disubtituted 1*H*)-pyrazole can be prepared from a suitably substituted chalcone by reaction of hydrazine hydrate in the presence of elemental sulfur or sodium persulfate, or by using a hydrazone, where an azine is formed as a by-product [6, 7]. As part of our search for bioactive chemical compounds, we prepared chalcone and its substituted derivates; which were tested for both anti-microbial and anti-inflammatory activity.

Reaction scheme



4-(4-Methoxyphenyl)-6-phenylpyrimidine-2(1*H*)-thione

EXPERIMENTAL MATERIALS

Anisaldehyde, benzaldehyde, acetophenone, dimethyl sulfoxide (DMSO), ethanol, thiourea, Sodium bicarbonate, KOH, methanol, acetic acid, petroleum spirit and ethyl acetate were purchased from BDH chemicals, England and used without further purification. Other materials were sourced locally from reliable dealers. The microbial cultures (*E. coli*, *S. aureus*, *Ps. aeruginosa*, *C. albican*) were obtained from the Department of Pharmaceutical microbiology and Biotechnology, Niger Delta University, Wilberforce Island. Nuclear Magnetic Resonance (NMR) experiment was performed on a 400 MHz instrument, obtained from Varian Inc. Palo Alto, California, USA. The IR data was acquired on FTIR-8400S instrument obtained from Shimadzu global links, North America. The melting point was done with a Gallen-Kamp melting point apparatus and were uncorrected.

Preparation of Chalcone (1,3-diphenylprop-2-en-1-one) Sample E1

Benzaldehyde, 10.6 g and acetophenone, 12.0 g were weighed, transferred to a round bottom flask containing ethanol, 25 mL and stirred while immersed in ice. Twenty percent (20%) KOH was added to the mixture drop-wise from a burette with continuous stirring, until 20 mL was completely added. The stirring continued for 15-20 minutes; after which, it was allowed to stand for 24 hours. Some ice chips were added to the mixture and titrated with 25 mL of very cold 20% acetic acid until it was acidified, resulting in the formation of precipitate which was filtered under suction and recrystallized in ethanol, air dried, weighed and melting point determined. This same procedure was repeated for the preparation of 4-methoxy-chalcone. The difference was in the reagents used.

Preparation of Chalcone epoxide (Sample E2)

Chalcone, 5.16 g was weighed and transferred to a beaker. 60ml of methanol and 10ml of 10% NaOH were separately measured and added. The mixture was dissolved by stirring on heating mantle. H_2O_2, 20ml was added and stirred continuously for 30 minutes and acidified with 10% acetic acid. The product was collected, recrystallized with methanol, filtered and air dried. The actual yield and melting point were respectively determined.

Preparation of 4-methoxy-chalcone epoxide (Sample E4)

Anisaldehyde, 13.6g and acetophenone, 12.0g were weighed, transferred to Erlenmeyer flask containing a magnetic bar and the flask was immersed in ice. The same procedure was repeated as for chalcone, giving 4-methoxy-chalone. 2.38 g of the 4-methoxychalcone was weighed and transferred to a beaker. 60 mL of methanol and 10ml of 10% NaOH were separately measured and added. The procedure in chalcone epoxide above was repeated, leading to 4-methoxychalcone epoxide. The yield and melting point were then determined.

Preparation of 4-dimethylamino-chalcone epoxide (Sample E6)

Para-dimethylamino-benzaldehyde, 7.45 g and acetophenone, 6.0 g were weighed, transferred to Erlenmeyer flask containing a magnetic bar and the flask was immersed in ice. The same procedure was repeated as for chalcone, giving rise to 4-dimethylaminochalone. 2.51 g of the 4-dimethylaminochalone was weighed and transferred to a beaker. 60 mL of methanol and 10 mL of 10% NaOH were separately measured and added. The procedure in chalcone epoxide above was repeated, leading to 4-dimethylaminochalone epoxide. The yield and melting point were then determined.

Preparation of 4-chloro-chalcone epoxide (Sample E8)

Para-chlorobenzaldehyde, 7.025 g and acetophenone 6.0 g were weighed and transferred to Erlenmeyer flask containing a magnetic bar and the flask was immersed in ice. The same procedure was repeated as for chalcone, giving rise to 4-chlorochalcone. 4.8g of the 4-chlorochalcone was weighed and transferred to a beaker. Methanol 60 mL and 10% NaOH, 10 mL were separately measured and added. The procedure in chalcone epoxide above was repeated, leading to 4-chlorochalcone epoxide. The yield and melting point were then determined.

Preparation of 4 –Methoxy-4,6-diphenyl-2-thiopyrimidine (Sample E9)

4-methoxy-chalone, 2.38 g, sodium bicarbonate 2.12 g and thiourea, 1.52 g were weighed and transferred to a round bottom flask containing DMSO, 30 mL. The mixture was refluxed under Nitrogen for 2 hours. The reaction was monitored with Thin Layer Chromatography (TLC). At the end of the reaction, water was added to the mixture and allowed to stand for 24 hours. The precipitate formed was filtered under suction and the residue was recrystallized with diethyl ether and petroleum spirit. The crystals were dried, the yield and melting point were then determined.

Antimicrobial Assay

The agar-well diffusion method was used to determine the antimicrobial activity of the synthesized compounds. Four clinical microbial isolates (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albican*), were obtained from the microbial bank of the Department of Pharmaceutical Microbiology and Biotechnology, Niger Delta University, Wilberforce Island Bayelsa state, Nigeria, for the antimicrobial work. The bacterial isolates were standardized using colony suspension method and matching the strain's suspension with 0.5 McFarland standard to give a resultant concentration of 1.5 x 10^8cfu/mL. The antibiotic susceptibility testing was determined using the modified Kirby-Bauer diffusion technique by swabbing the Mueller–Hinton agar (MHA) (Oxoids U.K) plates with the resultant saline suspension of each strain and six wells were bored on the agar surfaces seeded with the test organisms using size 6 mm cork borer. The wells were sealed at the bottom with molten sterilized agar. Stock solutions of the compounds were prepared by dissolving 500 mg of each in 5 mL DMSO to give concentration of 100 mg/mL. 0.1, 0.05, 0.025 and 0.0125 mL of the stock corresponding to 10, 5, 2.5 and 1.25 mg/mL respectively, of the synthesized compounds were aseptically introduced into the wells. Also, 10 and 5 mg/mL of the ketoconazole were equally transferred to the wells. While gentamicin and ciprofloxacin discs were placed on the plates. The plates were left undisturbed on the bench for 30 minutes to enable the compounds absorb adequately. The plates were then incubated at 37^{o}C for 24 hours and were observed for confluent growth of the microorganisms and clear zones of inhibition around the samples in the wells. The zones of inhibition were measured in millimeters and compared with the control group using the method of Bonev *et al.*, [15]. The result is shown in Table-2.

Anti-Inflammatory Assay

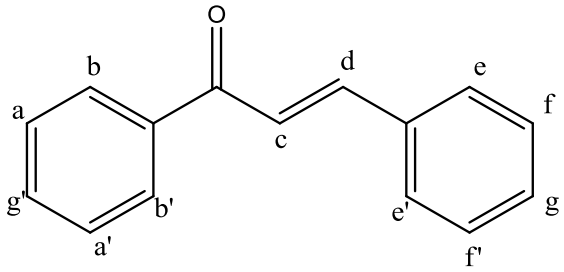
Wister rats (123 – 224 g) of both sexes were purchased from Olabisi Onabanjo University, animal house, Sagamu, Ogun state, Nigeria. The animals were housed in the central animal facility of the Niger Delta University College of Health Sciences (NDUCHS) under the supervision of trained personnel; with 12hour dark/12h light cycles and were fed with grower feeds. The animals were fasted overnight, with free access to water, prior to the experiments. The test was performed according to the procedure described by Sugishita *et al*., [16] and Lewis *et al*., [17].

Anti-inflammatory activity of the compounds was evaluated using egg albumin-induced rat paw oedema assay model [16]. The animals were divided into five groups, A - E (four per group) of both sexes. The test sample was orally administered (1, 2 and 4 mg/kg doses to groups A -C), while the animals in group D were administered diclofenac (2 mg/kg). Tween 80 (10%) used to solubilize the synthesized compounds was administered to group E, which served as negative control. After an hour 0.05ml of egg albumin was injected into the sub-plantar area of the right hind paw. The paw thickness was measured hourly over a period of 6 hours with the aid of veneer caliper. The anti-inflammatory activity was evaluated by the method of Duffy *et al.*, [18] and the percentage inhibition of oedema level by drugs were compared to control as shown in Table-1.

RESULTS

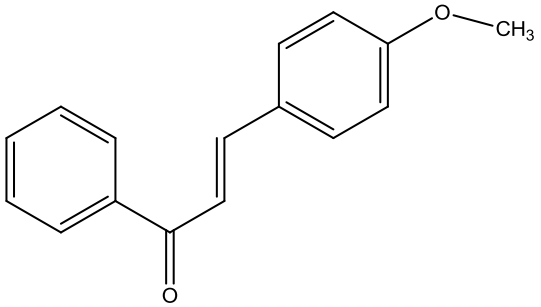
The compounds were obtained in good yield (56.33 – 89.10%) and high purity as shown by the melting point and TLC.

Chalcone (1,3-diphenylprop-2-en-1-one)



Yield: 18.533g (89.10%), Melting point: 45-47^{o}C. FTIR (KBr):3055. 35(HC=CH, alkene), 1654.95 (C=O), 1589.40 (C=C, aromatic),1437.02,1329.00, 1211.34,993.37,742.62, 680.89 cm^{-1}. ^{1}HNMR (DMSO-d_6) δppm: 7.45(t, J = 2H, Ar- H, gg´), 7.57(t, J = 2H, Ar-H, ff´), 7.65 – 7.69 (m, 2H, Ar-H, ee´), 7.74 – 7.78(d, J = 15.6, 1H, =CH, d), 7.88 – 7.90(m, 2H, Ar-H, aa´), 7.93 – 7.97(d, J = 15.6, 1H, =CH, c), 8.15 – 8.17(m, 2H, Ar-H, bb´). ^{13}C NMR **(**DMSO-d_6) ppm: 123 (C=C, c), 129-138 (Ar-C), 144(C=C, d), 190 (C=O).

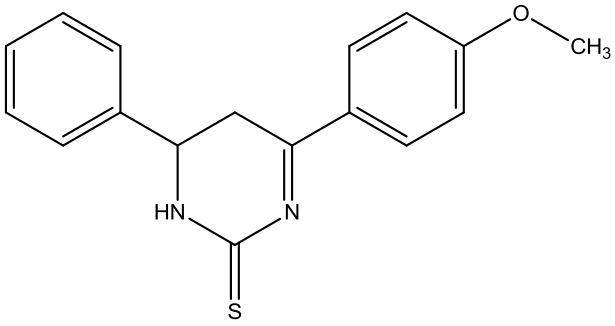
4-Methoxychalcone



(2E)- 3-(4-Methoxyphenyl)-1-phenyl-prop-2-en-1-one.

Yield: 16.202 g (68.09%), Melting point: 58-59^{o}C. FTIR (KBr): 3506 (HC=CH, alkene), 1654.98(C=O), 1593.25(C=C), 1018.45(C-O), 821.70, 773.48, 652.82, 518.67 cm^{-1}. ^{1}HNMR (DMSO-d_6) δppm: 3.82 (s, H_3C-O), 7.01-7.03 (HC=CH), 7.55-7.87 (Ar-H). ^{13}C NMR **(**DMSO-d_6) (ppm): 55 (OCH_3), 122(C=C), 126-138 (Ar-C), 144 (C=C), 189 (C=O).

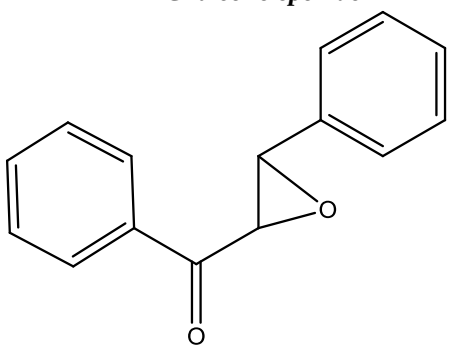
4-Methoxy-4,6-diphenyl-2-thiopyrimidine



4-(4-Methoxyphenyl)-6-phenylpyrimidine-2(1*H*)-thione

Yield 1.656g (56.33%), Melting point: 52-54^{o}C. FTIR (KBr): 3034 (N-H), 1654.98(C=S), 1587.47 (C=N), 1319.35 (C-N), 1004.98 cm^{-1}. ^{1}HNMR (DMSO-d_6) δppm: 3.34 (s, N-H), 3.82 (s, OCH_3) 7.01-7.03-8.15 (Ar-H). ^{13}CNMR **(**DMSO-d_6) (ppm): 55 (OCH_3), 144 (N-C), 120 (C=N), 127-144 (Ar-C), 189 (C=S).

Chalcone epoxide

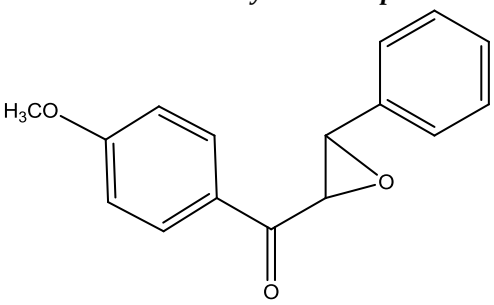


Phenyl (3-phenyloxiran)-2-yl methanone

(Chalcone epoxide)

Yield 3.466 g (76.01%), Melting point: 78-80^{o}C. FTIR (KBr): 3045.70 (H-C=CH) 1681.98, (C=O), 1429.30, 1236.41, 684.75 cm^{-1}. ^{1}HNMR (DMSO-d_6) δppm: 4.15(d, J = 4 Hz, H-CO), 4.83(d, J = 4 Hz, H-CO), 7.39-8.03 (Ar-H). ^{13}C NMR (DMSO-d_6) δppm: 58-60 (C-O), 126-137 (Ar-C), 193 (C=O).

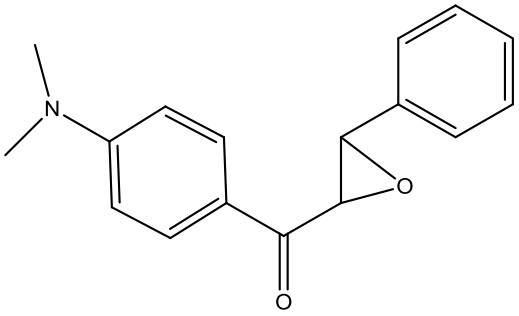
4-Methoxychalcone epoxide



4-Methoxyphenyl (3-phenyloxiran)-2-yl methanone
(4-methoxychalcone epoxide)

Yield 2.151g (84.69%), Melting point: 48-50^{o}C. FTIR (KBr): 3441.12 (H-CO), 3045.7 (H-C=C), 1658.84 (C=O), 1587.47(C-O), 1244.13, 1008.8, 827.49 cm^{-1}. ^{1}HNMR (DMSO-d_6) δppm: 2.08(H_3C-O), 3.68(H-C-O), 3.81 (H-C-O), 6.56-8.15(Ar-H). ^{13}C NMR (DMSO-d_6) δppm: 55 (OCH_3), 127-144 (Ar-C), 189 (C=O).

4-Dimethylaminochalcone epoxide

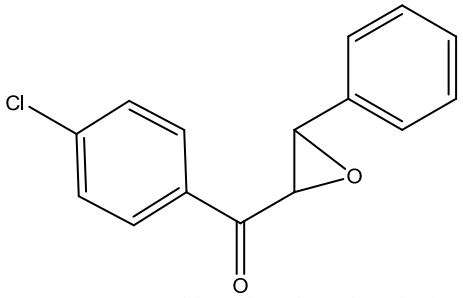


[4-(Dimethylamino)phenyl]
(3-phenyloxiran)-2-yl
methanone

(4-Dimethylamino chalcone
epoxide)

Yield 0.739g (27.68%), Melting point: 94-97^{o}C. FTIR (KBr): 3757.46 (N-H), 3055.35 (H-C-O), 1645.33(C=O), 1543.10 (C=C), 1163.11(C-O), 1010.73cm^{-1}. ^{1}HNMR (DMSO-d_6) δppm: 3.00 (s, H_3C-N-CH_3), 3.35 (s, HC-O), 6.75 - 8.11 (Ar-H). ^{13}C NMR (DMSO-d_6) δppm: 112 (H_3C-N-CH_3), 116 (C-O), 122 (C-O), 128-152(Ar-C), 189 (C=O).

4-Chlorochalcone epoxide



4-Chlorophenyl (3-phenyloxiran)-2-yl methanone
(4-Chlorochalcone epoxide

Yield 3.045g (58.9%), Melting point: 56-58^{o}C. FTIR (KBr): 1656.91 (C=O), 1579.75 (C=C) 1093.67 (C-O), 698.25 cm^{-1}. ^{1}HNMR (DMSO-d_6) δppm: 3.34 (s, HC-O), 3.82 (s, HC-O), 7.4-8.03 (Ar-H). ^{13}C NMR (DMSO-d_6) δppm: 58 (C-O), 60 (C-O), 128-135 (Ar-C), 198 (C=O).

Anti-Inflammatory and Antimicrobial Activities

The results of the anti-inflammatory and antimicrobial activities are shown in Table 1 and 2 respectively. The percentage oedema inhibition was calculated using the formula stated under methods. It was observed that of the heterocyclic derivative of Chalcone displayed good anti-inflammatory activity but void of antimicrobial activity.

Table-1: Percentage inhibition of paw size oedema of *4-methoxy-4,6-diphenyl-2-thiopyrimidine* (%I = Dc – Dt/Dc x 100)

Time		**1 hr**	**2hrs**	**3hrs**	**4hrs**	**5hrs**	**6hrs**
Water (H_2O)		-	-	-	-	-	-
DCF std 4mg/kg		11.06	9.00	15.40	23.93	26.98	19.70
Test Sample							
4-Chloro chalcone	1 mg/kg	39.23	20.39	28.20	30.06	40.05	44.55
	2 mg/kg	24.61	12.30	29.65	15.95	60.49	19.27
	4 mg/kg	0.45	19.42	7.27	15.95	12.81	5.51
4-methoxy-4,6-diphenyl-2-thiopyrimidine	1mg/kg	32.73	16.83	32.27	22.39	51.23	41.74
	2mg/kg	8.35	16.83	13.37	22.70	31.61	15.60
	4mg/kg	17.16	4.53	16.86	7.36	43.32	11.47

Table-2: Effects of Chalcone and its derivatives on microbial growth

Sample	**Conc (mg/mL)**	**Zone of inhibition (mm)**			
		E. coli	***C. albican***	***Ps. Aeruginosa***	***S. aureus***
Chalcone	10	-	-	-	-
	5	-	-	-	-
	2.5	-	-	-	-
4-Methoxychalcone	10	-	-	-	-
	5	-	-	-	-
	2.5	-	-	-	-
4-Methoxy-4,6-diphenyl-2-thiopyrimidine	10	-	-	-	-
	5	-	-	-	-
	2.5	-	-	-	-
Chalcone epoxide	10	-	-	-	-
	5	-	-	-	-
	2.5	-	-	-	-
4 – Methoxy chalcone epoxide	10	-	-	-	-
	5	-	-	-	-
	2.5	-	-	-	-
4 – Dimethylamino chalcone	10	-	-	-	-
	5	-	-	-	-
	2.5	-	-	-	-
4 – Chloro chalcone	10	-	-	-	-
	5	-	-	-	-
	2.5	-	-	-	-
Ciproflox std	10mcg	41mm	-	38mm	24mm
Gentamycin std	10cmg	20mm	-	-	12mm
Ketoconazole std	10mg/ml	-	-	-	-
	5mg/ml	-	-	-	-

Dc = change in control, Dt = change in treatment, %I = percentage inhibition, DCF = Diclofenac, std = standard, mm =millimeter.

DISCUSSION

The test compounds showed no antibacterial and antifungal activity but 4-methoxy-4,6-diphenyl-2-thiopyrimidine and 4-chloro chalcone displayed marked anti-inflammatory activity. From the result (Table-1), percentage inhibition of the rat paw size oedema was observed remarkably at 4th hr after the oedema induction. The *in vivo* anti-inflammatory activity of the compounds was carried out using egg albumin-induced oedema assay, which is a working model of inflammation in the search for new anti-inflammatory agents that could possibly be used as therapeutic agents [19]. The oedema which develops in rat paw after the injection of egg albumin in the sub-planar area is a biphasic event [20]. The initial phase is attributed to the release of histamine and the second phase (from the third hour) is attributed to prostaglandin [21]. The percentage inhibition of the rat paw size at the 5 hr following induction of oedema showed that the heterocyclic compound possesses promising anti-inflammatory activity when compared to the standard anti-inflammatory drug (Diclofenac) used for the study. There is need to evaluate and establish the safety of these molecules for use against inflammatory disorders.

The compounds had no antimicrobial activity with respect to the test organisms. This result is in line with the study by D. Ere *et al.*, [22], on the antibacterial activity of 4-methoxy derivatives of chalcones. The fact that *Candida albican* was resistant to Ketoconazole (standard drug) in this study, demonstrates reason for the growing concern of resistance to antifungal agents by microorganisms which further indicates the need for new and more effective agents for which the microbes have no resistance.

CONCLUSION

The finding of the above study demonstrates that, the synthesized compounds, chalcone and its derivatives, though showed no antimicrobial activity, the heterocyclic derivatives, 4-methoxy-4,6-diphenyl-2-thiopyrimidine and 4 -chlorochalcone epoxide showed promising anti-inflammatory activity.

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