Synthesis and evaluation of antibacterial properties of chalcones derived from thiophene-2-carbaldehyde

Running title: Antibacterial studies of thiophene substituted chalcones

Abstract

Chalcone is a simple chemical structure which is present in most of naturally occurring substances. Many chalcone derivatives are synthesized by Claisen-schmidt condensation reaction. It is a subject of great research opportunity due to numerous biological activities and convenient synthesis of chalcones. This study aims on synthesizing different α, β unsaturated ketones (chalcones) containing thiophene ring from thiophene-2-carboxldehyde and different substituted acetophenones, further evaluating antibacterial activity of synthesized compounds. The synthesized compounds are characterized for their spectral study. From the antibacterial study it was observed that the compounds bearing electron withdrawing group, electron releasing group exhibited excellent to moderate antibacterial activity. These results showed that chalcones incorporated with thiophenes have better scopes for further development for the antimicrobial agents.

Key words: Chalcones, Claisen-schmidt condensation, antibacterial.

Introduction

The field of medicinal chemistry is well advanced with the discovery of variety of drug molecules with different organic and heterocyclic moieties with various substitutions for the various diseases caused by pathogens by suitably focusing on the target site. Currently we have number of antimicrobial agents to treat the different infectious diseases.[1] There are challenges associated with anti-microbial agents, as most of the micro-organisms develop resistance against current antibiotics/antibacterial agents; hence there is a scarcity of new antimicrobial agents globally. As we know, infectious disease is the reason for one-half of death especially in the developing countries. Hence there is a need for effective, novel and inexpensive antimicrobial agents.[2]

During the study for such alternatives, compounds having broad spectrum biological activities like chalcones and its derivatives were highly considered. This led to the research and development of chalcones as it is a multifunctional molecule since it possesses various biological activities in a single structure like anti-inflammatory, antimicrobial, antiviral, antioxidant, anticancer, immunomodulatory, antitubercular, analgesic, antihyperglycemic, antiplatelet etc., [2]

Chalcones are also known by the terms benzalacetophenone and benzylideneacetophenone. Chalcone or chalconoids describes compounds with aromatic ketone which form the central core for a variety of important biological compounds, which are known collectively as chalcones. In chalcones 2 aromatic rings are joined by a three-carbon aliphatic alpha beta unsaturated carbonyl chain. The interconnected chain has highly electrophilic alpha beta unsaturation and conjugated double bonds.[3] Molecules possessing such system have relatively low redox potentials and have a greater probability of undergoing electron transfer reactions. They mainly obtained by the condensation reaction between the aromatic aldehyde and acetophenone in the presence of acid /base catalyst (claisen smith condensation).[4]

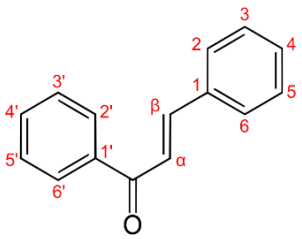
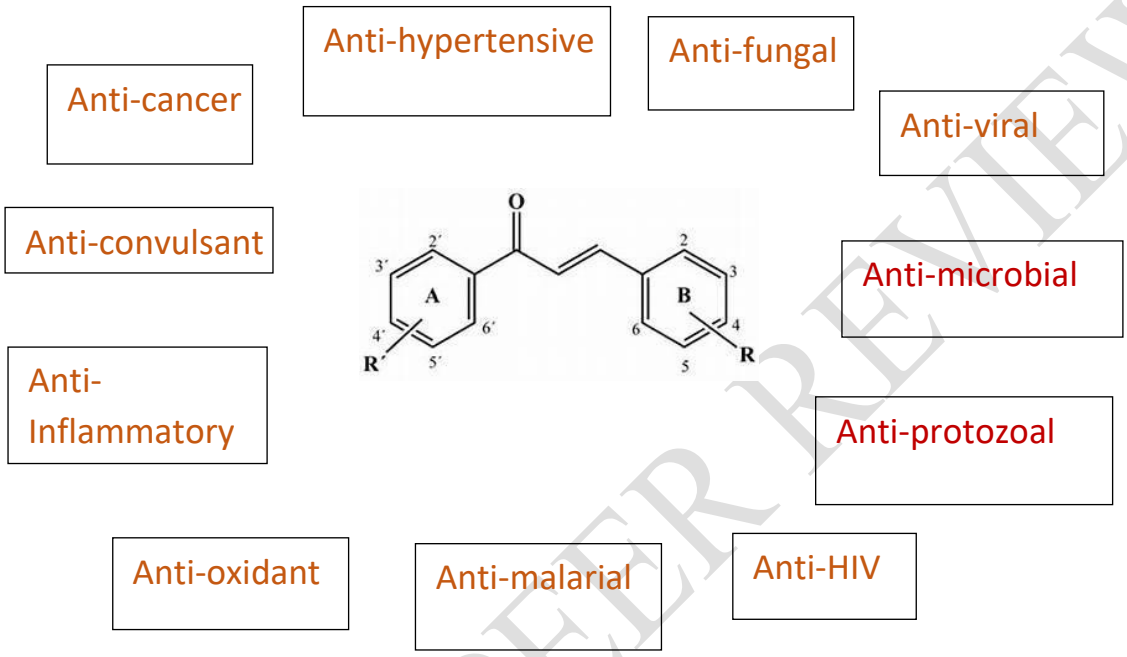


Fig. 1. Chemical structure

Chalcones and its analogues have found its significant role in the development of new medicinal formulations. Thus, it has become an object of interest in synthesis and research.[5] Recent studies on chalcones documented its use for the treatment of microbial diseases, stomach cancer, food additives, CVS disorders and cosmetic formulation preparation.[6,7]

Fig. 2. Biological importance of chalcones:



So in the present study we have considered thiophene incorporated chalcones containing different substitution to find a better advantageous anti-bacterial molecule with improved activity with lower side effect as thiophene is an active, potential 5-membered, 'S' containing hetero cyclic ring commonly present as building block in the most of the drugs.

Materials and Methods:

All the reactions were carried out under specified laboratory conditions. All the synthetic work was done by procuring laboratory grade reagents and analytical grade solvents. All the aromatic substituted acetophenones were obtained commercially. The products were purified by recrystallisation using suitable solvents. Melting points were determined by Digital melting point apparatus and were uncorrected.

Procedure for the synthesis of thiophene incorporated chalcones:

An equimolar mixture of substituted acetophenone (0.01mol) and thiophene-2- carbaldehyde (0.01 mol) was taken in iodine flask with 30 ml ethanol as solvent and aqueous solution of KOH was added to reaction mixture. Further it is kept in magnetic stirrer and reaction mixture is stirred for 6-8 hours. The mixture is then poured into crushed ice and acidified with conc. HCl. The chalcone derivatives precipitates out which was then filtered using whatman filter paper. Further purification of compounds was done by recrystallization using ethanol. [8,9]

Scheme:

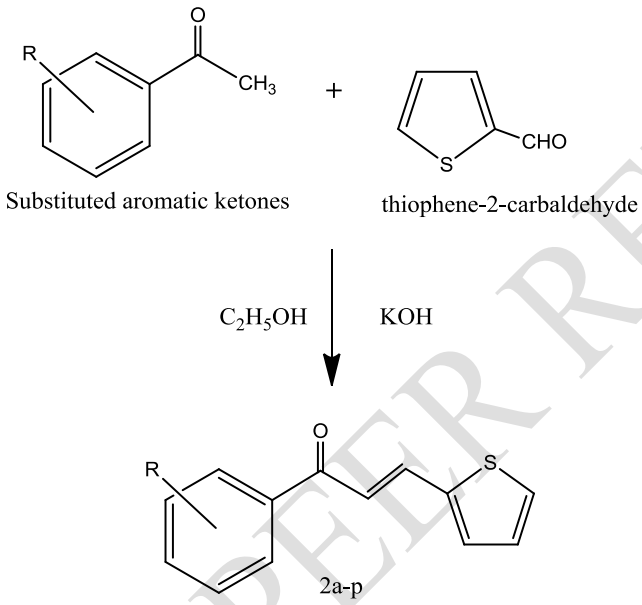


Fig. 3: Synthesis of substituted α-β unsaturated ketones (2a-p)

R= H, p-F, p-NO2, p-NH2, m-NO2, m-CH3

***In-vitro* Antibacterial studies:**

The *in-vitro* antibacterial study was performed by tube dilution and cup plate method. In this study we have taken gram negative organism Escherichia coli to evaluate antibacterial properties of novel synthesized compounds (2a-f). Ciprofloxacin was used as standard reference drug. Fresh 24h old bacterial culture is used for the activity.

Tube Dilution method:

This method is mainly used to determine MIC, it is the minimum or lowest concentration of a compound which prevents the growth of a bacteria. Two- fold dilutions of the compounds and standard drugs were prepared using DMSO ranging from 15.625-500µg/ml (A1-A6). Both the positive control (Nutrient broth+ solvent inoculated with culture, to determine the effect of solvent) and negative control (Nutrient broth+ solvent, to ensure that there is no growth and media is sterile) (A7 and A8). All the tubes were kept for incubation for 24h in incubators at 37^0C. The inhibitory concentration was noted by visual observation after 24h. [10,11,12,13]

Cup plate method:

We have selected two concentrations of the compounds based on the minimum inhibitory concentration from the tube dilution method (40µg/ml and 100µg/ml). Solution of the compounds and standard drug was prepared by dissolving 1mg each in 1ml of DMSO (concentration= 1000 µg/ml). Volumes of 40 µL and 100µL (0.04ml and 0.1ml) is used for the testing. Same volume of the solvent is used as control. A liquid agar medium was prepared by mixing the nutrient agar with water and sterilizing it in an autoclave for 15 min at 121^0C. The freshly prepared liquid agar medium was immediately poured into each of the Petri plate and allowed to solidify. The plates were inoculated with fresh 24h culture using and L-shaped spreader. The sterile borer was used to make wells in the medium. The sample solution of particular concentration was added into the wells. Procedure is repeated for the standard drug. The plates were then incubated for 24 hours at 37^0C. After incubation, the zone of inhibition was observed and measured in mm.[14, 15, 16, 17]

RESULTS:

Physico-chemical data of synthesized compounds:

Name	R	Molecular formula	Molecular weight	Melting point (^{0}C)	Percentage Yield
2a	4-F	$C_{13}H_9OSF$	232	76-78	68
2b	4-NH_2	$C_{13}H_{11}NOS$	229	80-82	72
2c	4-CH_3	$C_{14}H_{12}SO$	228	84-86	85
2d	3-NO_2	$C_{13}H_9SNO_3$	259	132-134	81
2e	H	$C_{13}H_{10}SO$	214	64-66	58
2f	4-NO_2	$C_{13}H_9SNO_3$	259	146-148	89

Table 1: physical data of synthesized compounds 2a-f

Characterization of synthesized compounds:

(2*E*)-1-(4-fluorophenyl)-3-(thiophen-2-yl)prop-2-en-1-one(2a): yellow powder, yield:68%, M.P: 76-78^{0}C. IR (KBr,cm^{-1}) : 1580.47(C=C, aliphatic), 1655.31(C=O), 1190.52(C-F), 3459.92(C-H stretch, aromatic), 1500.54(C=C aromatic), 1025.8(C-H bend), 703(C-S-C). ^{1}H NMR(DMSO, δ ppm): 7.47(d, 1H, CH of olefin), 7.32(d, 1H, CH of olefin), 6.98(m, 3H), 7.85 (d, 1H, Ar-H), 7.54 (d, 1H, Ar-H), 6.86 (d, 1H, Ar-H), 6.39 (d, 1H, Ar-H). Mass (LC-MS, m/z): 232.31 (M^+).

(2*E*)-1-(4-aminophenyl)-3-(thiophen-2-yl)prop-2-en-1-one(2b):IR (KBr,cm^{-1}): yellow powder, yield:72%, M.P: 80-82^{0}C**.** 3214.92(N-H stretch), 1573.52(C=C), 1644.01(C=O), 3746.49(C-H stretch, aromatic), 1044.51(C-H bend), 1409(C=C, aromatic), 701(C-S-C). ^{1}H NMR(DMSO, δ ppm): 7.38(d, 1H, CH of olefin), 7.23(d, 1H, CH of olefin), 6.92(m, 3H), 7.88 (d, 1H, Ar-H), 7.59 (d, 1H, Ar-H), 6.88 (d, 1H, Ar-H), 6.41 (d, 1H, Ar-H), 4.94(s, 2H, NH_2). Mass (LC-MS, m/z): 229.24 (M^+).

(2*E*)-1-(4-methylphenyl)-3-(thiophen-2-yl)prop-2-en-1-one(2c): IR (KBr, cm^{-1}): creamish white powder, yield:85%, M.P: 84-86^{0}C**.** 1584.37(C=C aliphatic), 1655.88(C=O), 1361(C=C aromatic), 3819.46(C-H stretch, aromatic), 1013.23(C-H bend),714.62(C-S-C). ^{1}H NMR(DMSO, δ ppm): 7.38(d, 1H, CH of olefin), 7.23(d, 1H, CH of olefin), 6.92(m, 3H), 7.88 (d, 1H, Ar-H), 7.59 (d, 1H, Ar-H), 6.88 (d, 1H, Ar-H), 6.41 (d, 1H, Ar-H), 3.789(s, 3H, CH_3). Mass (LC-MS, m/z): 228.12 (M^+).

(2*E*)-1-(3-nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one(2d): IR (KBr,cm^{-1}): yellow powder, yield:81%, M.P: 132-134^{0}C**.** 152.4(C=C aliphatic), 1656.28(C=O), 1346.08(C=C aromatic), 3649.59(C-H stretch), 1084.55(C4 bend), 716.01(C-S-C), 1569.86 and 1420.52(NO_2). ^{1}H NMR(DMSO, δ ppm): 7.43(d, 1H, CH of olefin), 7.32(d, 1H, CH of olefin), 6.89(m, 3H), 7.82 (d, 1H, Ar-H), 7.55 (d, 1H, Ar-H), 6.85 (d, 1H, Ar-H), 6.44 (d, 1H, Ar-H).Mass (LC-MS, m/z): 259.34

(M^+).

(2*E*)-1-phenyl-3-(thiophen-2-yl)prop-2-en-1-one(2e): white powder, yield: 58%, M.P: 64-66^{0}C**.** IR (KBr,cm^{-1}): 1585.04(C=C aliphatic), 1654.86(C=O), 1445.64(C=C aromatic), 3368.42(C- H stretch), 1032.57(C-H bend), 685.21(C-S-C). ^{1}H NMR(DMSO, δ ppm): 7.59(d, 1H, CH of olefin), 7.45(d, 1H, CH of olefin), 6.74(m, 3H), 7.86-7.51 (m, 5H, Ar-H). Mass (LC-MS, m/z): 214.09 (M^+)

(2*E*)-1-(4-nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one (2f): yellow powder, yield:81%, M.P: 146-148^{0}C**.** 152.4(C=C aliphatic), 1656.28(C=O), 1346.08(C=C aromatic), 3649.59(C-H stretch), 1084.55(C4 bend), 716.01(C-S-C), 1569.86 and 1420.52(NO_2). ^{1}H NMR(DMSO, δ ppm): 7.48(d, 1H, CH of olefin), 7.36(d, 1H, CH of olefin), 6.84(m, 3H), 7.80 (d, 1H, Ar-H), 7.15 (d, 1H, Ar-H), 6.65 (d, 1H, Ar-H), 6.49 (d,1H, Ar-H).Mass (LC-MS, m/z): 259.41(M^+).

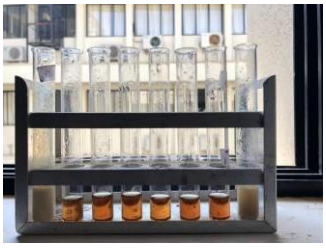
Anti-microbial studies:

Tube dilution method:

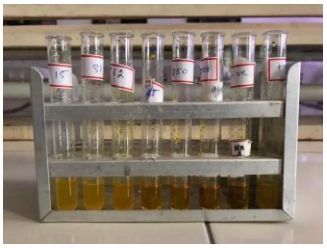
Compound	**Minimum inhibitory concentration(µg/ml) against E.coli**
2a	62.5
2b	31.25
2c	125
2d	62.5
2e	125
2f	31.25
Standard (Ciprofloxacin)	15.625
Positive control	-
Negative control	-

Table 2. Minimum inhibitory concentration of 2a-f and Ciprofloxacin

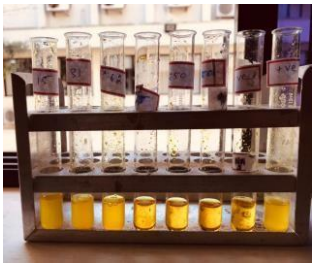
2a



2b



2c



2d **2e** **2f**

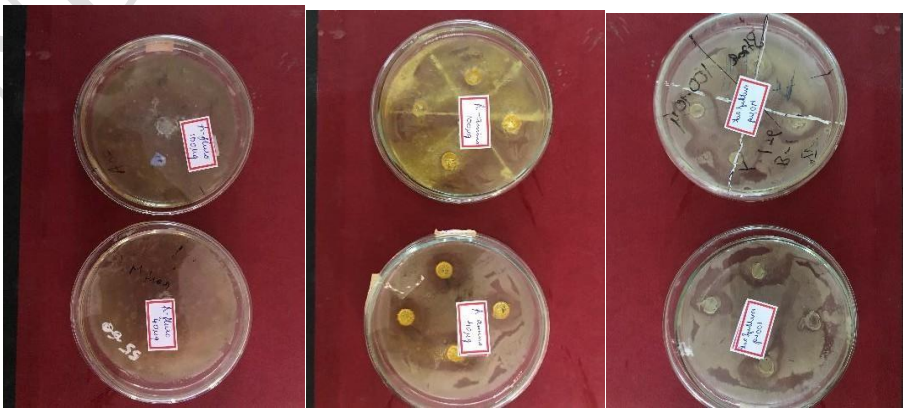
Standard

Fig. 4. Tube dilution method

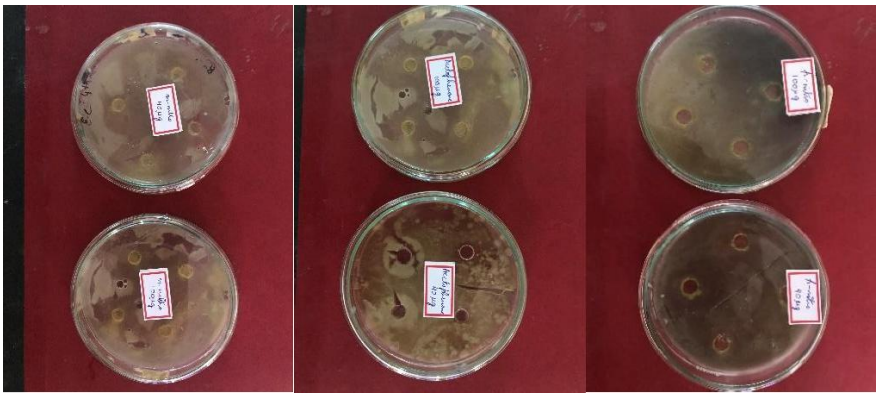
Cup plate method:

Compound	**Zone of inhibition**	
Concentration	**40µg**	**100 µg**
2a	9mm	15mm
2b	13mm	19mm
2c	7mm	11mm
2d	**15mm**	**21mm**
2e	5mm	9mm
2f	**14mm**	**24mm**
Standard (ciprofloxacin)	25mm	36mm
Control	2mm	2mm

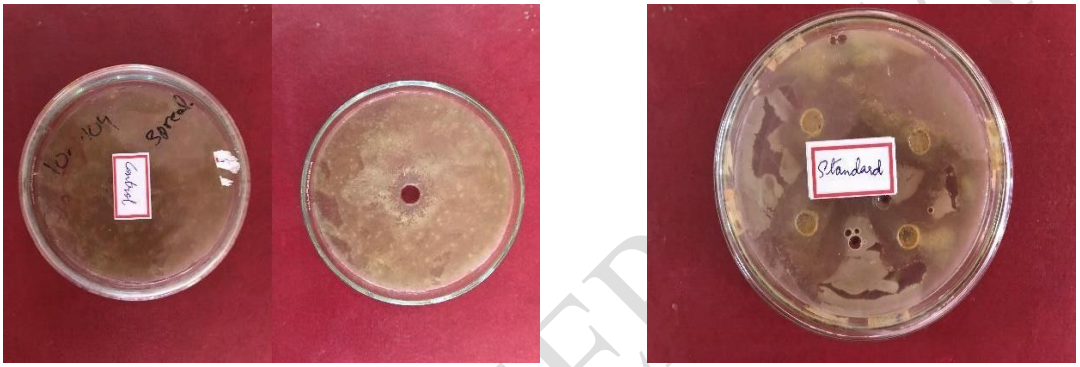
Table 3. Zone of inhibition produced by compound 2a-f and ciprofloxacin



2a **2b** **2c**



2d **2e** **2f**



Control **Standard**

Fig. 5. Cup plate method

DISCUSSION

The synthesis of thiophene incorporated chalcones were synthesised by scheme 1 using conventional method of synthesis of chalcones i.e., Claisen Schmidt condensation using substituted acetophenones and thiophene-2-carboxaldehyde. All the synthesised compound were purified by recrystallization using ethanol and characterised by IR, NMR and Mass spectroscopy. We have observed the important peaks for different functional groups in IR like C=C (aliphatic, aromatic) C=O, CH stretch and bend, C-S-C and for important functional groups like F, NO_2, NH_2 and CH_3. In NMR spectra, hydrogen's attached to the carbon atoms of the olefin linkage gave prominent peaks, peaks for hydrogen's attached to aromatic ring were also observed. In mass spectrum, M^+ peak was observed for all the compounds. The physico-chemical properties of all the newly synthesized compounds are given in Table 1.

All the synthesized chalcones were evaluated for *in-vitro* anti-bacterial potency against gram negative bacteria i.e., E. coli by tube dilution and cup plate method using ciprofloxacin as the reference standard.

All the tested compounds showed minimum inhibitory concentration of 62.5, 31.25, 125, 62.5, 125, 31.25 and15.625 µg/ml respectively for 2a, 2b, 2c, 2d, 2e, 2f and ciprofloxacin. Whereas in the positive control there was no inhibition and in the negative control the growth was not observed which ensures the sterility of the media. Out of all six compounds 2b, 2f, 2a, 2d exhibited comparatively better inhibition with lower MIC, where the inhibition of compound 2b and 2f are comparable with standard. Based on MIC, two fixed doses of the compounds were selected i.e., 40 µg /ml and 100 µg/ml for further evaluation by cup plate method.

All the six test compounds along with standard were evaluated further by cup plate method. After 24hours of incubation, we have measured the zone of inhibition produced by the compounds. Out of all the six tested compounds 2d and 2f exhibited excellent, 2a and 2b exhibited moderate and 2c and 2e exhibited mild anti-bacterial activity. Out of all the compounds 2f has exhibited excellent antibacterial activity which was considered as promising molecule was further evaluation.

CONCLUSION

The main objective of this particular project was to synthesize the novel thiophene incorporated chalcones and evaluation of their antimicrobial properties. Out of the synthesized compounds, molecules bearing electron withdrawing group, electron releasing group and electronegative atom exhibited excellent to moderate antibacterial activity. These results showed that these synthesized chalcones incorporated with thiophene especially 2f, 2d, 2a and 2b are the promising molecules with antibacterial properties have better scope for further developmentod the antibacterial; agents and potency of these compounds are required to confirm further by *in-vivo* screening.

Consent: It is not applicable.

Ethical Approval: It is not application

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