ANTIMICROBIAL AND CYTOTOXIC EVALUATION OF *(E)*- THIENYL CHALCONES DERIVED FROM THIOPHENE-2-CARBALDEHYDE

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Summary

Background: Chalcones are 1,3 diaryl-2-propene-1-one in which two aromatic rings are linked by a three carbon α, β-unsaturated carbonyl system and have been reported to posses antiviral, antibacterial, antifungal, antitubercular, cytotoxic, antimalarial, analgesic and anti-inflammatory activity.

Methods: The reaction of thiophene-2-carbaldehyde**(1)** with substituted acetophenones**(2a-l)** in the presence of NaOH in ethanol yielded corresponding Chalcones**(3a-l)**. Structures of the compounds synthesized were confirmed by IR, ^{1}H-NMR and MASS spectroscopic analysis. The newly synthesized compounds were screened for anti-bacterial, anti-fungal, antitubercular and cytotoxic activities. Some of the compounds showed remarkable anti-bacterial, anti-fungal, anti-tubercular and cytotoxic activity.

Results: All the newly synthesized compounds resulted in good yields with 50 – 70%. Some of the newly synthesized compounds **3a** and **3d** showed good antibacterial activity against gram positive bacteria *E. Fecalis,* where as none of the compounds have shown good antibacterial activity against gram negative bacteria. Compound **3K** showed promising antifungal activity against the yeast *C. albicans* and **3d** showed promising antifungal activity against the mold *A. fumigatus.* Some of the compounds **3a, 3b, 3d, 3e, 3f , 3g** and **3h** showed good antitubercular activity. Compound **3f,** a fluoro substituted derivative has shown highest cytotoxic activity 56 % at a 200 μg mL^{-1} cytotoxic activity.

Keywords: *(E)*-thienyl chalcones; antibacterial; antifungal; antitubercular; cytotoxic activity.

Introduction

Chalcones are 1,3 diaryl-2-propene-1-one in which two aromatic rings are linked by a three carbon α, β-unsaturated carbonyl system. These are the biogenetic precursors of flavonoids in higher plants.[1] Chalcones constitute an important class of medicinally important small molecules which have been reported to posses antiviral,[2] antibacterial, antifungal, [3] antitubercular, [4] cytotoxic,[5] ant-malarial, [6] analgesic and anti-inflammatory[7] activity. Further the thienyl compounds have been reported to posses antimicrobial,[8] antitubercular,[9] anticancer[10] and anti-inflammatory[11] activity.

A large number of thiophene derivatives occur in plant and in animal metabolism. Thiophene compounds have been found to occur in fungi and higher plants . Example Junipal i.e., 5-1-propynyl-2-thophene carbaldehyde. The vitamin Biotin (Vitamin H) is an essential growth factor for a number of micro-organisms in animals.[12]

Pyrantel, a valuable Anthelmintic used in animal husbandry, one of the old drugs used in chemotherapy which contains the thiophene moiety. Some of the well known Cephalosporin drugs like Cephalothin and Cephaloridine contains the thiophene moiety.[13]

Some of the researchers namely Revanasiddappa et. al[14]., and Rekha Nagavanshi et. al[15]., have reported the synthesis of some substituted thienyl chalcones derived from thiophene-2-carbaldehyde, but none of the researchers have completely explored the diverse biological and pharmacological nature of these molecules.

With the vision of exploring the diverse pharmacological nature of *(E)*-thienyl chalcones, it was contemplated to synthesize some substituted *(E)*-thienyl chalcones, derived from thiophene-2-carbaldehyde. The compounds were screened for anti-bacterial, anti-fungal, anti-tubercular and cytotoxic activities.

Materials and Methods

Melting points were recorded in open capillaries with electrical melting point apparatus and were uncorrected. IR spectra (KBr disks) were recorded using Bruker-α IR spectrophotometer. ^{1}H NMR were recorded in Bruker Avance (400 MHz) Spectrophotometer in $CDCl_3$ solution and chemical shift values are reported as values in ppm relative to TMS ($\delta = 0$) as internal standard, Mass spectra were recorded on a Micromass Q-TOF spectrophotometer, elemental analysis was carried out using Vario Elementar Model CHN analyzer instrument. TLC was performed on silica gel coated plates for monitoring the reactions.

Synthesis of *(E)*-1-(aryl substituted)-3-(thiophen-2-yl)prop-2-en-1-one (3a-l)[16]

To a stirred solution of an equivalent amount of the appropriately substituted acetophenones (0.01 mol) and thiophene-2-carbaldehyde (0.95 ml, 0.01 mol) in ethanol was added aqueous NaOH solution (10% w/v, 10 ml). The resulting solution was stirred at room temperature overnight, poured into water, and acidified to pH 4 with 1N HCl. The resultant precipitate was filtered off, washed with water, and purified by recrystallization using ethanol. **Table 1** summarizes physical data of the above compounds.

***(E)*-1-(4-nitrophenyl)-3-(thiophen-2-yl)prop-2-en-1-one (3a)**

IR (cm^{-1}) : 3097 (Aromatic CH Str), 1653 (C=O Str), 1574 (C=C Str), 972 (trans CH=CH *def*) 1520 (Ar-NO_2 Ass. Str), 1320 (Ar-NO_2 sym Str), 708 (C-S Str).

^{1}H NMR (δ ppm) : 8.34 (d, 2H, *J* = 8.8 Hz, Ar-H), 8.17 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.94(d, 1H, *J*= 15.2 Hz, CH=CH), 7.56 (d, 1H, *J* = 5.0 Hz, thiophene-H), 7.48 (d, 1H, *J*= 3.5 Hz, thiophene-H), 7.32 (d, 1H, *J* = 15.2 Hz, CH=CH), 7.14 (m, 1H, thiophene-H).

Mass: The mass spectrum showed a peak at 259 (M^+)

***(E)*-1-(4-chlorophenyl)-3-(thiophen-2-yl)prop-2-en-1-one (3b)**

IR (cm^{-1}) : 3082 (Aromatic CH Str), 1653 (C=O Str), 1589 (C=C Str), 971 (trans CH=CH *def*) 713 (C-S Str).

^{1}H NMR (δ ppm) : 8.34 (d, 2H, *J* = 8.9 Hz, Ar-H), 8.12 (d, 2H, *J* = 8.9 Hz, Ar-H), 7.98 (d, 1H, *J* = 15.2 Hz, CH=CH), 7.49 (d, 1H, *J*= 5.0 Hz, thiophene-H), 7.42 (d, 1H, *J* = 3.6 Hz, thiophene-H), 7.26 (d, 1H, *J* = 15.1 Hz, CH=CH), 7.12-7.14 (m, 1H, thiophene-H).

Mass: The mass spectrum showed a peak at 249 (M^{+1})

***(E)*-3-(thiophen-2-yl)-1-(p-tolyl)prop-2-en-1-one (3c)**

IR (cm^{-1}) : 3096 (Aromatic CH Str), 2924 (Aliphatic CH Str), 1654 (C=O Str), 1577 (C=C Str), 960 (trans CH=CH *def*) 713 (C-S, Str).

^{1}H NMR (δ ppm) : 7.93 (d, 1H, *J* = 15.1 Hz, CH=CH), 7.91 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.41 (d, 1H, *J* = 5.0 Hz, thiophene-H), 7.35 (d, 1H, *J*=3.4 Hz, thiophene-H), 7.31 (d, 2H, *J*=8.2 Hz, Ar-H), 7.26 (d, 1H, *J* = 15.1 Hz, CH=CH), 7.08-7.10 (m, 1H, thiophene-H), 2.43 (s, 3H, CH_3).

Mass: The mass spectrum showed a peak at 229 (M^{+1})

***(E)*-1-(4-aminophenyl)-3-(thiophen-2-yl)prop-2-en-1-one (3d)**

IR (cm^{-1}) : 3427 (NH_2 Str), 3319 (NH Str), 3101 (Aromatic CH Str), 2924 (Aliphatic CH Str), 1654 (C=O Str), 1598 (C=C Str), 957 (trans CH=CH *def*) 680 (C-S Str).

^{1}H NMR (δ ppm) : 7.89 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.80 (d, 1H, *J*=15.3 Hz, CH=CH), 7.37 (d, 1H, *J* = 5.0 Hz, thiophene-H), 7.32(d, 1H, *J* = 3.3 Hz, thiophene-H), 7.06 (m, 1H, thiophene-H), 6.68 (d, 2H, *J* = 8.6 Hz, Ar-H), 6.64 (d, 1H, *J* = 15.3 Hz, CH=CH), 4.16 (s, 2H, NH_2) .

Mass: The mass spectrum showed a peak at 230 (M^{+1})

Results and Discussion

The main aim of this work was to synthesize various substituted *(E)*-1-(aryl substituted)-3-(thiophen-2-yl)prop-2-en-1-one derivatives **(Scheme 1)** by a base catalyzed Claisen-Schmidt condensation reaction of thiophene-2-carbaldehyde with various substituted acetophenones. All the synthesized chalcones were geometrically pure and with trans-configuration which was confirmed from ^{1}H NMR spectra, (*J* Hα-Hβ = 15.1 – 15.2 Hz) and IR spectra (CH=CH *def* , 957 – 972 cm^{-1}).

All the synthesized compounds resulted in good yields with 50-70%. The formation of title compounds (3a-l) were indicated by the appearance of two doublets due to CH=CH of the chalcones in ^{1}H NMR and appearance of peak due to C=O of chalcones in IR as given above. The mass spectra of the title compounds showed molecular ion peaks corresponding to their molecular weight.
Some of the newly synthesized compounds **3a** and **3d** showed good antibacterial activity against gram positive bacteria *E. Fecalis,* where as none of the compounds have shown good antibacterial activity against gram negative bacteria. Compound **3K** showed promising antifungal activity against the yeast *C. albicans* and **3d** showed promising antifungal activity against mold *A. fumigatus.* Some of the compounds **3a, 3b, 3d, 3e, 3f , 3g** and **3h** showed good antitubercular activity. Compound **3f,** a fluoro substituted derivative has shown highest cytotoxic activity 56 % at a 200 µg mL^{-1} cytotoxic activity.

Biological Evaluation

Antibacterial and Antifungal activity[17]

All the synthesized compounds were screened for their antibacterial activity against *E. Fecalis* (ATCC no 35550)*, S. aureus* (ATCC no 12598)*, K.pneumoneae* (ATCC no 29665) and *E. coli* (ATCC no 25922) using ciprofloxacin as standard drug. All the synthesized compounds were also screened for their antifungal activity against *C. albicans* (ATCC no 2091) and *A. fumigatus* (ATCC no 36607) using fluconazole as the standard drug. Compounds with substituents like $-NO_2$ and $-NH_2$ **(3a and 3d)** showed good antibacterial activity against gram positive bacteria *E. Fecalis*. Compounds with naphthyl substituent **(3k)** showed promising antifungal activity against the yeast *C. albicans* and amino substituted derivative **(3d)** showed promising antifungal activity against the mold *A. fumigatus.* The results are summarized in **table-2.**

Antitubercular activity by Microplate Alamar Blue Assay (MABA)[18]

The invitro antitubercular screening of the synthesized compounds was carried out against *Mycobacterium tuberculosis H37Rv* (MTCC no 300) by Microplate Alamar Blue Assay (MABA).

Microplate Alamar Blue Assay (MABA) is an non-toxic rapid, inexpensive and high throughput assay for antitubercular drug screening and this method gives good correlation with BACTEC radiometric method, a well established antitubercular screening method. The results are summarized in **table-3.**

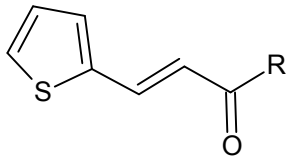
Method

- 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation.
- The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.
- The final drug concentrations tested were 0.2 to 100.0 µg.
- The above said wells were inoculated with *M. tuberculosis* H_{37}Rv Strain.
- Plates were covered and sealed with parafilm and incubated at 37ºC for five days.
- After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.
- A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.
- The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

Cytotoxic activity by tryphan blue dye exclusion method[19]

The synthesized compounds were tested for their cytotoxicity in vitro, in comparision with 5-fluorouracil as reference drug, against Dalton's Lymphoma Ascites Cells (DAC). DAC cells (1 x 10^6) were incubated with synthesized compounds at various concentrations at 25, 50, 100, 200 µg/ml, in 1 ml phosphate buffered saline (incorporated with 10 µl DMSO) at 37^0C for 3 hours. Viable cells were counted in a hemocytometer using the tryphan blue dye exclusion method. The results are summarized in **table-4**.

Table 1: Physical Data of compounds (3a-l)



Comp. code	R	Mol. formula	M.W.	M.P ^{0}C	Elemental analysis Calculated (Found)			% Yield
					C (%)	H (%)	N (%)	
3a	4-NO_2-C_6H_4	$C_{13}H_9NO_3S$	259	155-157	60.22 (60.19)	3.50 (3.48)	5.40 (5.37)	70
3b	4-Cl-C_6H_4	$C_{13}H_9ClOS$	248	98-100	62.78 (62.71)	3.65 (3.62)	-	68
3c	4-CH_3-C_6H_4	$C_{14}H_{12}OS$	228	120-122	73.65 (73.56)	5.30 (5.27)	-	60
3d	4-NH_2-C_6H_4	$C_{13}H_{11}NOS$	229	115-117	68.09 (68.02)	4.84 (4.81)	6.11 (6.09)	73
3e	C_6H_5	$C_{13}H_{10}OS$	214	118-120	72.87 (72.83)	4.70 (4.66)	-	50
3f	4-F-C_6H_4	$C_{13}H_9FOS$	232	95-97	67.22 (67.17)	3.91 (3.86)	-	55
3g	3-NO_2-C_6H_4	$C_{13}H_9NO_3S$	259	80-82	60.22 (60.19)	3.50 (3.47)	5.40 (5.38)	70
3h	4-Br-C_6H_4	$C_{13}H_9BrOS$	293	58-60	53.26 (53.22)	3.09 (3.08)	-	72
3i	4-OCH_3-C_6H_4	$C_{14}H_{12}O_2S$	244	85-86	72.16 (72.07)	3.78 (3.75)	-	70
3j	4-OH-C_6H_4	$C_{13}H_{10}O_2S$	230	122-123	67.80 (67.71)	4.38 (4.35)		66
3k	Naphthyl	$C_{17}H_{12}OS$	264	50-52	77.24 (77.16)	4.58 (4.56)	-	55
3l	Thiophene	$C_{11}H_8OS_2$	220	78-79	59.97 (59.89)	3.66 (3.62)	-	61

M.W: Molecular Weight; M.P: Melting point in ^{0}C

Table 2: Antibacterial and antifungal activity of compounds (3a-l)

Comp. Code	Minimum Inhibitory Concentration in (µg)					
	E.Fecalis	***S. Aureus***	***K. Pneumoniae***	***E. coli***	***C. albicans***	***A. fumigatus***
3a	4.15	62.5	62.5	125	62.5	62.5
3b	62.5	125	62.5	125	62.5	62.5
3c	R	62.5	250	125	125	250
3d	8.3	62.5	62.5	R	125	4.15
3e	R	R	R	R	R	R
3f	16.6	125	31.25	62.5	62.5	62.5
3g	31.25	62.5	62.5	125	125	31.25
3h	125	62.5	31.25	62.5	125	62.5
3i	62.5	125	250	250	31.25	250
3j	125	125	250	250	16.6	250
3k	62.5	31.25	R	500	4.1	500
3l	125	62.5	R	R	62.5	250
Ciprofloxacin[a]	**1µg**	**2µg**	**1µg**	**2µg**	**-**	**-**
Fluconazole [a]	**-**	**-**	**-**	**-**	**16.6µg**	**8.3 µg**

[*] Resistant, [a]Standard drugs

Table 3: antitubercular activity of compounds (3a-l)

Comp. Code	MIC in µg
3a	25
3b	25
3c	50
3d	25
3e	25
3f	25
3g	25
3h	25
3i	50
3j	50
3k	50
3l	50
INH[a]	**0.2**

a: Standard Drug

Table 4: Evaluation of cytotoxic activity of compounds (3a-l)

Comp. Code	Percent cell death at different concentrations (µg) of test drug				
	10	**20**	**50**	**100**	**200**
3a	0	0	2	6	12
3b	0	2	8	10	15
3c	0	0	4	11	18
3d	0	0	3	10	20
3e	0	0	5	8	14
3f	0	5	20	32	56
3g	0	0	3	7	12
3h	0	1	3	8	16
3i	3	5	14	28	42
3j	0	0	5	7	12
3k	0	2	8	12	15
3l	0	0	3	9	17
5-Fluorouracil[a]	**9**	**22**	**36**	**51**	**93**

a: Standard drug

Conclusion

Some of the *(E)*-thienyl Chalcones **3a** and **3d** have exhibited good antimicrobial activity, derivatives **3d** and **3k** have shown promising antifungal activity. Some of the compounds **3a, 3b, 3d, 3e, 3f , 3g** and **3h** have shown good antitubercular activity. Whereas compound **3f,** fluoro substituted derivative has shown moderate cytotoxic activity.

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