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Synthesis and antimicrobial evaluation of new chalcones containing piperazine or 2,5-dichlorothiophene moiety

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Abstract—Two new series of chalcones have been synthesized by reacting 1-(4-piperazin-1-yl-phenyl)ethanone and 1-(2,5-dichloro-3-thienyl)-1-ethanone with different substituted benzaldehydes in turn by Claisen–Schmidt condensation. The compounds have been characterized by IR, ^{1}H NMR spectral and microanalysis data. All the synthesized compounds have been evaluated for antimicrobial activity. Some of these derivatives are potentially active against Gram-positive bacteria, *Staphylococcus aureus* and *Escherichia coli* while the most potent compound (**1**) in this study showed MIC_{50} value of 2.22 μg/mL against *Candida albicans*.

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite tremendous progress in medicinal chemistry. The impact is more acute in developing countries due to nonavailability of desired medicines and emergence of widespread drug resistance.[1] Antimicrobial resistance settings have failed to address this essential aspect of drug usage.[2] Specifically, multi-drug-resistant Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* (MRSE) and vancomycin resistant *enterococci* (VRE) are of major concern.[3] There are a number of clinically efficacious antibiotics becoming less effective due to development of resistance. The bacterial resistance to antibiotics is a major health problem and resulted in emergence of an endless area for study and debate. Chalcones, considered as the precursor of flavonoids and isoflavonoids, are abundant in edible plants. Chemically they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system. Studies revealed that compounds with chalcone-based structure have shown an array of pharmacological activities, such as antiprotozoal,[4–6] antifungal,[7] anti-inflammatory,[8–10] antileishmanial,[11–14] nitric oxide inhibition,[15] inhibition of the production of interleukin-1[16] and anticancer[17] activities. Licochalcone A (Fig. 1), an oxygenated chalcone isolated from the root of Chinese liquorice,[18] was shown to possess activity against Gram-positive strains of bacteria.[19,20]

Piperazinyl linked Ciprofloxacin dimers are reported to be potent antibacterial agents against resistant strains, a novel class of mixed D2/D4 receptor antagonists, dual calcium antagonist, antimalarial agents and potential antipsychotic agents.[21] Recently, piperazine derivatives containing tetrazole nucleus have been reported as antifungal agents.[22]

The requirement is to synthesize novel molecules having good potential with high therapeutic index. Keeping in view the diverse therapeutic activities of chalcones for the preparation of bioactive heterocycles, it was contemplated to synthesize a novel series of chalcones. Atten-

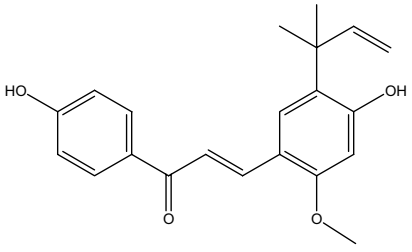

Figure 1. Licochalcone A.

Keywords: Chalcones; Claisen–Schmidt reaction; Antibacterial; Antifungal.
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tion has been focused on the modification of the acetophenone moiety of chalcones to achieve a new antimicrobial profile. The present study describes synthesis of the title compounds and screening them for their in vitro microbial activity.

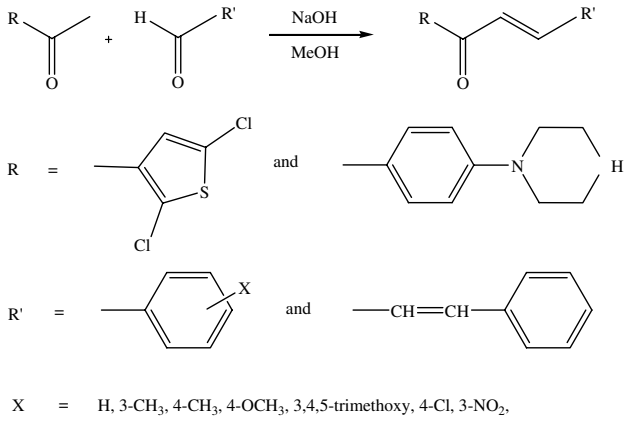
A series of substituted chalcones has been prepared by introducing new functionality 4′-piperazinoacetophenone or 3-acetyl-2,5-dichlorothiophene in place of acetophenone. All the chalcone derivatives have been prepared by the Claisen–Schmidt condensation using catalytic amount of sodium hydroxide in ethanol.[23] The residues were treated with 10% HCl in methanol to obtain the corresponding chalcones in good yield (Table 1). All spectral data (IR, ^{1}H NMR and MS) obtained were consistent with the structures assigned.

The spectral data confirm the presence of —C=O and —C=C— and benzene ring (IR at 1670–1643, 1583–1510, 815 and 840 cm^{-1}). Similarly NMR multiplets in the range of (6.7–8) ppm also confirm the presence of aromatic rings. Coupling constant values in NMR show that trans isomer has formed. The synthesis of A (**1**–**8**) and B (**9**–**16**) is depicted in Scheme 1.

The synthesized compounds were tested for their antibacterial activity by adopting (Cup-plate method)[24] agar well diffusion technique. The following bacterial cultures were used for anti-bacterial activity studies.

(i) *S. aureus* 209 p, (ii) *E. coli* ESS 2231, (iii) *Proteus vulgaris*, (iv) *Klebsiella pneumoniae* and (v) *Aspergillus fumigatus*.

Ciprofloxacin was used as the standard drug. Nutrient agar was poured onto the sterilized petri dishes (20–25 mL: each petri dish). The poured material was allowed to set (1–1.5 h) and thereafter the 'CUPS' (10 mm diameter) was made by punching into the agar surface with a sterile cork borer and scooping out the punched part of the agar. Into these cups the test compound solution was added with the help of a sterile syringe. The plates were incubated at 37 °C for 48 h and the results were noted. A solvent control (10% DMSO in methanol) was also run to note the activity of the blank (solvent). The above-mentioned standard drug was also screened under similar conditions for comparison. All the compounds were also screened for their in vitro antifungal activity against *Candida albicans*, *C. albicans* ATCC 10231 and *Candida glabrata*. The antifungal activities of test compounds were compared to that of standard fluconazole. The results of these experiments are summarized in Tables 2 and 3. Biological screening results were mentioned in mm, showing diameter of inhibition zone (i.e.) and these are categorised, as 0–6 mm for mild, 7–13 mm for moderate and 14–26 mm for efficacy, respectively.



Scheme 1. Synthesis of chalcones.

From Table 2 it is evident that compounds **7**, **9**, **10**, **12** and **15** indicated good activity against *S. aureus* (24–26), **9** and **15** for *E. coli* while the remaining compounds are statistically equivalent in antibacterial activity against their microorganisms with moderate activity (14–24). Most of the tested compounds possess potent in vitro activity against *P. vulgaris*, *K. pneumoniae* and *A. fumigatus* but were less active than the reference drugs.

MIC_{50}s were recorded as the minimum concentration of a compound that inhibits the 50% growth of the tested microorganisms. All of the compounds tested

Table 1. Physical data of the synthesized compounds

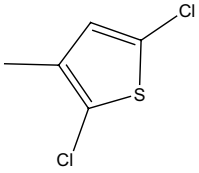
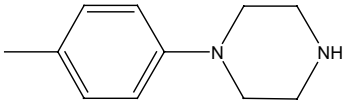
Compound	R	R′ (benzaldehyde)	Yield (%)	Mp (°C)
1		H	83	53–54
2		3-CH_3	76	45–46
3		4-CH_3	81	188–9
4		4-OCH_3	86	58
5		3,4,5-Trimethoxy	72	189–92
6		4-Cl	86	98–99
7		3-NO_2	84	148–52
8		Cinnamaldehyde	88	152–3
9		H	83	48–9
10		3-CH_3	78	94–96
11		4-CH_3	73	146–7
12		4-OCH_3	77	135–6
13		3,4,5-Trimethoxy	69	161–3
14		4-Cl	79	167–8
15		3-NO_2	63	158–9
16		Cinnamaldehyde	74	108–110

Table 2. Antibacterial and antifungal data of chalcones

Compound	A	B	C	D	E	F	G	H
1	18	20	22	18	24	26	26	26
2	11	20	06	10	20	10	22	14
3	12	16	09	08	06	16	10	12
4	14	18	14	16	22	20	18	18
5	12	14	12	06	14	06	22	20
6	14	16	14	08	18	24	22	14
7	24	22	18	20	16	18	12	14
8	09	16	11	07	20	08	16	16
9	22	24	20	18	22	24	24	24
10	24	22	20	16	20	16	24	22
11	16	14	14	14	18	22	22	24
12	24	22	14	16	18	22	14	18
13	06	08	06	09	12	09	12	09
14	18	22	16	14	12	18	20	14
15	24	26	18	14	20	16	18	18
16	10	16	08	05	14	09	14	06
Ciprofloxacin	22	24	24	24	24	Fluconazole 29	Fluconazole 29	Fluconazole 29

Zone of inhibition is expressed in mm at concentration level of 250 μg/mL of chalcones.
A, *Staphylococcus aureus* 209 p; B, *Escherichia coli* ESS 2231; C, *Proteus vulgaris*; D, *Klebsiella pneumoniae*; E, *Aspergillus fumigatus*; F, *Candida albicans*; G, *Candida krusei* GO_3; H, *Candida glabrata* HO_3.

Table 3. MIC_{50} values (μg/mL) of chalcones

Compound	A	B	C	D	E	F	G	H
1	80	20	12.70	14.15	4.13	2.22	3.17	4.65
2	90	22.76	100	28.02	32.13	51.13	42.34	65
3	75	25.65	40	30	70	70	70	80
4	60	9.13	40	65	9.26	5	18.16	16.37
5	60	60	50	50	55	78	40.13	45.13
6	50	60	40	30	40	4.65	70	40
7	3.25	6.27	65	7.17	16.75	5.97	50	50
8	100	40	60	60	60	75	55.65	60
9	100	3.58	3.95	12.75	6.85	4.56	5.38	7.57
10	3.13	5.62	3.85	18.64	5.56	6.89	5.69	9.94
11	70	50	45	40	18.35	8.75	7.02	7.66
12	5.25	10.28	45	55	35	9.12	50	16.25
13	70	80	45	65	75	65	75	60
14	75	75	70	50	50	55	60	60
15	4.75	2.75	60	50	7.17	6.75	18.56	16.8
16	100	35	200	70	50	90	60	75
Ciprofloxacin	100	100	100	100	100			
Carbencillin	200	200	200	200	200	Fluconazole 50	Fluconazole 50	Fluconazole 50
Amoxycillin	100	100	100	100	100			

A, *Staphylococcus aureus* 209 p; B, *Escherichia coli* ESS 2231; C, *Proteus vulgaris*; D, *Klebsiella pneumoniae*; E, *Aspergillus fumigatus*; F, *Candida albicans*; G, *Candida krusei* GO_3; H, *Candida glabrata* HO_3.

illustrated significant antibacterial and antifungal activity compared to reference drugs. The MIC_{50} values are generally within the range of 2.22–100 μg/mL against all evaluated strains. While comparing MIC_{50} values with that of Ciprofloxacin, all compounds were found to be effective against *S. aureus*. Compounds **7**, **10**, **12** and **15** especially showed very high activity. All the compounds were effective against *E. coli*. Compounds **9**, **10** and **15** showed very high activity. Compounds **4**, **7** and **12** especially showed strong activity while compounds **2** and **3** showed moderate activity.

In comparing their MIC values with that of Ciprofloxacin, it is apparent that all compounds were effective against *P. vulgaris*. Compounds **9** and **10** especially showed high activity and compound **1** showed good activity. On the other hand the compounds exhibited comparable activities against *K. pneumoniae*. Compound **7** showed high activity and compounds **1**, **2**, **9** and **10** showed moderate activity. From the results obtained with *A. fumigatus*, out of all the 16 compounds, **1**, **4**, **9**, **10** and **15** showed moderate activity, whereas compounds **2**, **7** and **11** showed less activity compared to Ciprofloxacin.

The antifungal activity of the compounds was studied with three pathogenic fungi. The results are summarized in Tables 2 and 3. Fluconazole has been used as reference for inhibitory activity against fungi. All the compounds showed good antifungal activity. Compounds **1**, **9**, **10** and **11** showed activity against all the three fungi. In addition compounds **4**, **6**, **7** and **12** also exhibited good activity against *C. albicans* while **4** and **15** showed

moderate activity against fungi *Candida krusei* GO_3. Compounds **4**, **12** and **15** also showed moderate activity against *Candida glabrata* HO_3.

To conclude, two series of compounds incorporating piperazine and 2,5-dichlorothiophene heterocyclic nuclei have been synthesized as potent antimicrobial agents. These modifications resulted in change in the potency and antibacterial activity profile of the chalcones and indicated that introduction of nitro group at *m*-position in benzaldehyde ring is well tolerated in terms of activity against bacteria (*S. aureus*). Furthermore, studies have revealed that the presence of electron-attracting groups encourages the antibacterial activity. Substitution of a bulky group with high polarizability probably enhances the potency of these compounds as antibacterial agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.08.021.

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