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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of α, β-unsaturated ketone as potential antifungal agents

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Abstract With the aim of developing potential antifungals, a series of chalcones incorporating sulfur either as part of a heteroaromatic ring (thiophene) or as a side chain (thiomethyl group) were synthesized and tested for their in vitro activity. Some of the compounds showed appreciable activity against a fluconazole-resistant strain, and could act as new hits for the design of better analogs.

Keywords α, β-unsaturated ketone (chalcones) · Antifungal agents · Fluconazole resistant

Introduction

Fungi are widely distributed in nature and frequently appear as pathogens in the animal and plant kingdoms. The onset of the the acquired immunodeficiency syndrome (AIDS) epidemic (Plattener, 2003) combined with increased use of immunosuppressive drugs for organ transplants and cancer therapy have resulted in increased incidence of life-threatening fungal infections.

Chalcones are reported to have an array of important therapeutic activities such as antihypertensive and cardiovascular activity, antiprotozoal, anti-inflammatory, antidiabetic, nitric oxide inhibitory activity, anticancer activities as well as antifungal and antitubercular activities (Opletalova *et al.,* 2003; Mei *et al.,* 2003; Chun *et al.,* 2001; Satyanarayana *et al.,* 2004; Horng *et al.,* 2003; Ruby *et al.,* 1995;

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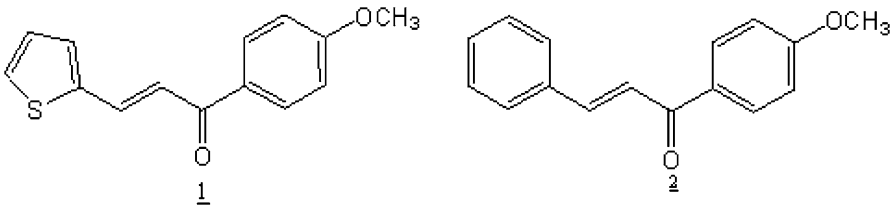
Opletalova and Sedivy, 1999; Lopez *et al.,* 2001; Tsuchiya *et al.,* 1994; El Sohly *et al.,* 2001; Kulkarni 2003; Lin *et al.,* 2002).

Elemental sulfur has long been known to act as an antifungal agent. Also, a well-known antifungal agent, tolnaftate has sulfur in organically combined form. Sulfur is also present in antifungal agents of natural origin, e.g., *Allium sativum* (garlic), which is known to inhibit *Candida albicans* (Lemar *et al.,* 2002).

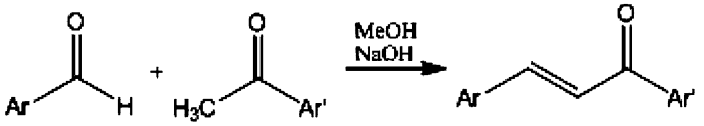
Antifungal activity of chalcones has been investigated by a number of researchers. Recently Nowakowska reviewed the antimicrobial and ant-iinflammatory activity of chalcones (Nowakowska, 2007). Sato *et al.* reported growth inhibitory properties of hydroxyl chalcones to *Candida* (Sato *et al.,* 1994). Tomar *et al.* have reported synthesis and antimicrobial activity of chalcones containing the piperazine or 2,5-dichlorothiophene moiety (Tomar *et al.*, 2007). Lahtchev *et al.* reported a mechanistic study on chalcones using various yeast strains (Lahtchev *et al.*, 2008). Nowakowska *et al.* have reported antibacterial and antifungal activities of chalcones (Nowakowska *et al*., 2008).

Earlier work in our laboratory had indicated that, when a thiophene ring was incorporated into a chalcone structure, the molecule exhibited antifungal activity (Javeri, 2004).



1 was found to have activity against *Candida albicans* at concentration of 25 mg/ml while **2** was inactive at 400 mg/ml.

This led us to explore chalcones incorporating sulfur either as part of a heteroaromatic ring (thiophene) or as a side chain (thiomethyl group). Thus, in this study, various compounds were synthesized and tested for antifungal activity. Some of these chalcones have been reported earlier; however they have not been screened for antimicrobial activity (Hayashi *et al.,* 1989; Tafi *et al.,* 2002; Joo *et al.,* 2003; Sivakumar *et al.,* 2007; Meng *et al.,* 2004; Basaif *et al.,* 2005; Dhar, 1972; Taylor *et al.,* 1980; Goto *et al.,* 1991). The general scheme is shown in Scheme 1.



Ar – Various substituted aromatic aldehydes
Ar' – Various substituted/ unsubstituted aromatic/ heteroaromatic aldehydes

Scheme 1 Synthesis of various chalcones

Results and discussions

Chemistry

A series of α,β-unsaturated ketones incorporating sulfur were synthesized and characterized spectroscopically. Their biological activity against fluconazole-sensitive as well as fluconazole-resistant strains of *Candida albicans* was evaluated.

The typical procedure involved the reaction of various substituted ketones with various aromatic or heteroaromatic aldehydes in equimolar ratio in basic conditions. All the synthesized compounds were characterized spectroscopically. In general, infrared (IR) spectra showed a C=O peak at 1643.9–1655.8 cm^{-1}. The ^{1}H nuclear magnetic resonance (NMR) spectra showed unsaturated and aromatic protons at δ 6.743–7.927. Literature survey revealed that compounds **14**, **16**, and **18** are novel. These were characterized spectroscopically by ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy (MS), and IR spectroscopy. Physicochemical characterizations of the synthesized chalcones are shown in Table 1.

It was observed that compounds with unsubstituted thiophene ring (Table 1b) and thiomethyl substitution at the *para* position of benzaldehyde (Table 1b) exhibited good antifungal activity. High activity was found when both thiomethyl and thiophene ring were present (compound **13**). Bromine substitution on the thiophene ring decreased antifungal activity (Table 1b).

In the first series (Table 1a), maximum activity was obtained with p-fluoro substitution (compound **1**). Activity decreased with increasing halogen size (compounds **1**, **2**, and **3**). Presence of p-methoxy (compound **6**) or hydroxy groups at the *ortho*, *meta* or *para* position also resulted in good activity (compounds **8**, **9**, and **10**) while the p-nitro group as well as the bulky p-phenyl substitution decreased activity (compounds **5** and **11**) as compared with the unsubstituted compound **7**.

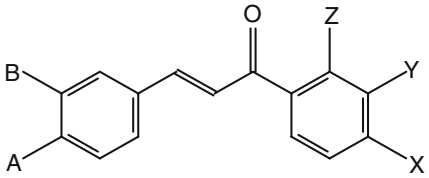
In the second series (Table 1b), *meta* and *para* disubstitution with methoxy (compound **15**) led to increased activity while again the p-phenyl-substituted compounds (**17** and **18**) exhibited considerably decreased activity. All compounds with the bromo thiophene ring (**14**, **16**, **18**, and **20**) exhibited less activity compared with those with the unsubstituted thiophene ring (**13**, **15**, **17**, and **19**).

Experimental

General procedures

Melting points were recorded on Thermomik Compbell electronics with an oil-heating system and were uncorrected. Fourier-transform infrared (FTIR) spectra were recorded on Buck Scientific infrared spectroscopy M500 spectrophotometer using KBr pellets. All NMR spectra were recorded on a FT-NMR JEOL, 60 MHz or JEOL AL 300 MHz spectrometer. The mass spectrum was recorded on Waters

Table 1a Synthesized compounds with their biological activities

S. no	 X, Y, Z	A	B	m.p. (°C)	Biological activity (μg/ml) MIC for *Candida albicans* (fluconazole-resistant strain NCIM 3446)	MIC for *Candida albicans* (fluconazole-sensitive strain ATCC 10231)
F	Fluconazole	–	–	–	100	20
1[a]	X = F, Y = H, Z = H	SCH_3	H	111–113	08	05
2[a]	X = Cl, Y = H, Z = H	SCH_3	H	142–143	20	10
3[a]	X = Br, Y = H, Z = H	SCH_3	H	157–158	100	40
4[b]	X = Cl, Y = H, Z = Cl	SCH_3	H	117–118	200	50
5[a]	X = NO_2, Y = H, Z = H	SCH_3	H	161–163	100	50
6[a]	X = OCH_3, Y = H, Z = H	SCH_3	H	118–110	20	10
7[a]	X = H, Y = H, Z = H	SCH_3	H	132–133	40	20
8[a]	X = OH, Y = H, Z = H	SCH_3	H	150–153	40	20
9[c]	X = H, Y = H, Z = OH	SCH_3	H	84–85	20	20
10[d]	X = H, Y = OH, Z = H	SCH_3	H	120–123	40	20
11[a]	X = phenyl, Y = H, Z = H	SCH_3	H	161–163	250	100
12[e]	X = OCH_3	OCH_3	OCH_3	90–92	10	20

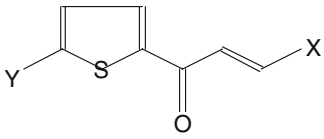
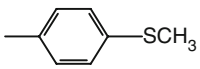
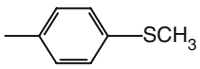
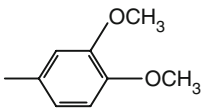
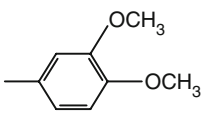
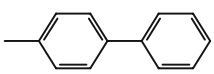
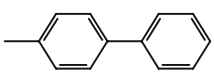
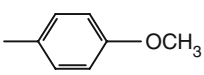
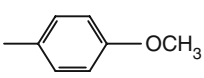
[a] Hayashi *et al.* (1989), [b] Tafi *et al.* (2002), [c] Joo *et al.* (2003), [d] Sivakumar *et al.* (2007), [e] Meng *et al.* (2004)

Micromass spectrometer. Chemical shifts are expressed in δ units relative to tetramethyl silane (TMS) as internal reference using $CDCl_3$ as solvent.

Procedures for the synthesis of 1-(5-bromothien-2-yl)-3-(4-methylthiophenyl) prop-2-en-1-one (**14**)

To a solution of 4-(methylthio) benzaldehyde (0.25 g, 0.00164 mol) in methanol (20 ml) was added 10% aqueous NaOH solution (2.0 ml) in a 50-ml round-bottomed flask immersed in an ice bath. 2-Acetyl 5-bromothiophene (0.337 g, 0.00164 mol) was added dropwise over a period of 15–30 min and the reaction mixture was stirred for 3–4 h. The precipitate so obtained was filtered and washed with distilled water until the filtrate was neutral to litmus. The final washing with cold methanol gave **14** (0.50 g, 89.76 %), m.p. 124–125°C. IR (KBr) 3061.3, 1641.8, 1578.6, 1414.4, 1076, 809 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.13338 – 7.8096 (m, 8 H, Ar–H and HC=CH), δ 2.5139 (s, 3H, SCH_3). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 15.0603, 119.3151, 122.6691, 125.8834, 128.8922, 130.8898, 130.9227, 131.3337, 131.6132, 142.8345, 144.0758, 147.1832, 180.7812. MS (m/z): 339.13 $[M]^+$, 341.14 $[M + 2]^+$.

Table 1b Synthesized compounds with their biological activities

S. no			m.p. (°C)	Biological activity (μg/ml)	
				MIC for *Candida albicans* (fluconazole resistant strain NCIM 3446)	MIC for *Candida albicans* (fluconazole sensitive strain ATCC 10231)
	X	Y			
13[f]		H	80–82	05	05
14		Br	124–125	50	40
15[f]		H	112–114	20	10
16		Br	130–132	100	50
17[g]		H	147–148	250	100
18		Br	176–178	250	100
19[h]		H	78	20	20
20[i]		Br	141–143	100	50

All the compounds synthesized were characterized spectroscopically

Optical density of positive control and negative control was 0.35 and 0.01, respectively

[f] Basaif *et al*. (2005), [g] Dhar (1972), [h] Taylor *et al*. (1980), [i] Goto *et al*. (1991)

Procedures for the synthesis of 1-(5-bromothiophen-2-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**16**)

3,4-Dimethoxybenzaldehyde (0.25 g, 0.0015 mol) was reacted with 2-acetyl-5-bromothiophene (0.189 g, 0.0015 mol) using the general procedure to obtain chalcone **16** (0.456 g, 86.08%), m.p. 130–132°C, IR (KBr) 1655.9, 1607.8, 1076.2, 811.6 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.755–7.806 (d, 1 H, Ar–*H*), 7.580–7.593 (d, 1 H, Ar–*H*), 7.124–7.261 (m, 4H, Ar–H and *HC*=*CH*), 6.874–6.902 (d, 1H, *HC*=*CH*), 3.924–3.943 (s, 6 H, OCH_3). ^{13}C-NMR (300 MHz, $CDCl_3$): δ 55.983, 56.024, 110.165, 111.127, 118.296, 122.439, 123.368, 127.486, 131.293, 131.506, 144.766, 142.274, 149.255, 151.663, 180.847. MS (m/z): 353.18 $[M]^+$, 355.19 $[M + 2]^+$.

Procedures for the synthesis of 3-(biphenyl-4-yl-1-(5-bromothien-2-yl)-)prop-2-en-1-one (**18**)

Biphenyl-4-carboxaldehyde (0.25 g, 0.00137 mol) was reacted with 2-acetyl 5-bromothiophene (0.28 g, 0.00137 mol) using the general procedure to obtain chalcone **18** (0.43 g, 87.22 %), m.p. 176–178°C. IR (KBr): 3050.9, 1643.3, 1581.0, 1412.6, 763.1 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.1436–7.9279 (m, 13 H, Ar–H and HC=CH), ^{13}C-NMR (300 MHz, $CDCl_3$): 120.2358, 120.2687, 122.8335, 127.0590, 127.6262, 127.9797, 128.9415, 129.0730, 131.3748, 131.7612, 133.4464, 140.0230, 143.5579, 144.1580, 147.1421, 180. 8058. MS: MS (m/z): 369.24 $[M]^+$, 371.19 $[M + 2]^+$.

Biological activity

The synthesized compounds were evaluated for their in vitro antifungal activity against fluconazole-sensitive and fluconazole -resistant strains using broth dilution technique. Dimethyl sulfoxide (DMSO) was used for initial dilution. The organisms employed for the in vitro testing were *Candida albicans* ATCC 10231 (fluconazole sensitive) and *Candida albicans* NCIM 3446 (fluconazole resistant). The inoculum was prepared according to the standard procedure (Barry 1976). The medium was prepared using appropriate quantities of Saboraud's dextrose broth. The optical density of the compounds, standard drug, and positive and negative controls of DMSO was also recorded. Different concentrations of the test compound were prepared and added to the inoculated test tubes. The inoculated test tubes containing different concentration of drugs were maintained at room temperature for 48 h in a dark room at 37°C. At the end of the incubation period, the results were interpreted by comparison with negative controls.

Conclusion

A series of α,β-unsaturated ketones (chalcones) were successfully synthesized and characterized spectroscopically by ^{1}H-NMR and IR. Few of the chalcones showed

appreciable antifungal activity against fluconazole-sensitive and fluconazole-resistant organisms, with chalcone **13** exhibiting the highest activity. The information obtained in this study provides a tool for designing better compounds, and for guiding further structural modification and synthesizing potent new antifungal agents.

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