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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of *p*-*N*,*N*-dialkyl substituted chalcones as anti-cancer agents

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Abstract Several new *N*,*N*-dialkyl substituted chalcones (chalconoids or benzylideneacetophenones) have been synthesized via the condensation of corresponding *N*,*N*-dialkylbenzaldehyde with various aryl methyl ketones. All the chalcones have been synthesized from readily available and cheap starting materials under environmentally benign conditions in very high yields without work up and column chromatographic purification. Synthesized compounds have been tested for their biological activity against pathogenic microorganisms such as *Escherichia coli*, *Bacillus subtilis*, and *Mycobacterium smegmatis*. Anti-cancer activity of these compounds has also been tested against multiple myeloma (RPMI-8226) and human mammary adenocarcinoma (MCF-7) cell lines. The most hydrophilic molecules **23** and **24** showed very good anti-cancer activity against MCF-7 cell lines at low micro-molar concentrations. All the compounds have also been evaluated for their activity against *Beta-secretase 1* enzyme. One of the synthesized compounds showed *Beta-secretase 1* enzyme inhibition activity at micro-molar concentration.

Keywords Chalcones · Anti-cancer · Anti-microbial · *Beta-secretase 1* enzyme

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Introduction

Chalcones of various classes have been extensively investigated for anti-proteasomal activity (Bazzaro *et al.*, 2011; Achanta *et al.*, 2006), anti-cancer activity (Kim *et al.*, 2010; Dimmock *et al.*, 1999; Echeverria *et al.*, 2009; Go *et al.*, 2005; Zhou *et al.*, 2009), anti-microbial activity (Ahmad *et al.*, 2011; Venkatesan and Maruthavanan, 2011; Choudhary *et al.*, 2011; Liaras *et al.*, 2011; Karamunge *et al.*, 2011), and several other therapeutic uses (Jianzhang *et al.*, 2011; Fei *et al.*, 2011; Umair *et al.*, 2011; Ramesh and Babitha, 2009). Very recently (Chiaradia *et al.*, 2012) reported naphthaline derived chalcones as a potent inhibitor of *Mycobacterium tuberculosis* protein tyrosine phosphatase.

Beta-secretase 1 or beta-site APP cleaving enzyme 1 (BACE1), the main cause of Alzheimer's disease (AD) is an aspartic-acid protease encoded by BACE1 gene. AD is a neurodegenerative disease and the most common type of dementia. AD affects millions of elderly persons worldwide and is a major global social and financial burden. In the US alone, there are 5.4 million people suffering from AD. These people are cared for by 14.9 million unpaid caregivers and AD costs 183 billion annually. Based on mortality data of this decade, death rates have declined for most major diseases while deaths from AD have risen 66 % during the same period (Thies and Bleiler, 2012). Owing to the importance to get an effective therapeutic agent, there are so many groups all over the globe working to treat AD (Malamas *et al.*, 2010). Chalcones have also been explored as BACE1 inhibitors (Ma *et al.*, 2011).

Results and discussions

The ease of synthesis coupled with the wide range of diverse biological applications of chalcones provides

tremendous scope to understand the structure activity relationship and to identify novel chalcones as therapeutic agents. Some of the important chalcone based therapeutic agents are listed in Fig. 1 (Nowakowska, 2007; Keedwell *et al*., 2004).

In this regard, we envisaged the synthesis of piperazinyl, pyrrolidinyl, piperidinyl, and dibenzyl amino substituted chalcones and studied their biological properties against various targets. The synthesis of *N,N*-dibenzylchalcone derivatives was started with *p*-fluorobenzaldehyde. To obtain *N,N*-dibenzylbenzaldehyde **2**, aniline was treated with benzyl bromide, K_2CO_3, and catalytic amount of soap under refluxing conditions, followed by Vilsmeier–Haack reaction of the resulting *N,N*-dibenzylaniline **1** (Scheme 1). The aldehydes upon condensation with various aryl methyl ketones in the presence of KOH in ethanol solvent followed by treatment with 6 M HCl and filtration provided the crude chalcones (Gezegen *et al*., 2010), which were further purified by recrystallization in methanol.

Piperidinylbenzaldehyde derivative **12a** was readily synthesized by treating fluorobenzaldehyde **11** with the piperidine in the presence of K_2CO_3 at refluxing condition in water (Grayson and Charles, 2008). This aminoaldehyde derivative was treated with the corresponding acetophenones to obtain the chalcones (Scheme 2). The products derived from this aldehyde **12a** gave less hydrophobic chalcones (**13**–**20**) than the *N,N*-dibenzyl derivatives (**3**–**10**) described above in Scheme 1. We also synthesized amino aldehyde derivative **12b** by treating fluorobenzaldehyde with pyrrolidine under the same conditions as described in the synthesis of piperidinylbenzaldehyde **12a**. Chalcones (**21**, **22**, **23**, **26**, and **27**) were obtained by treating **12b** with corresponding acetophenone in excellent yields (Scheme 2). Finally, hydrophilic chalcones such as *N*-hydroxyethylpiperazinyl derivatives (**24** and **25**) were synthesized by treating fluorobenzaldehyde with *N*-hydroxyethylpiperazine under the same conditions as discussed above.

To determine the potency of our synthesized chalcones, we tested these molecules against recombinant human BACE1 enzyme. The most hydrophobic chalcone **6** (Fig. 2) showed moderate BACE1 inhibition activity at 50 μM concentration. Due to weak BACE inhibition activity further studies (IC_{50}) were not pursued.

Synthesized molecules were also tested against *Bacillus subtilis, Escherichia coli*, and *Candida albicans*. For anti-microbial studies, the colonies were grown in LB broth at 37 ± 1 °C overnight. The microbial suspension was swabbed on to a LB agar plate. Compound was dissolved in DMSO to a concentration of 0.1 M. Then 5 μL of the 0.1 M stock solution was added to standard paper disks (1 cm) to achieve a final concentration of 100 and 50 μg/disk. The plates were incubated at 37 ± 1 °C for 24 h. The anti-microbial activity was determined based on the zone of inhibition around the disk (which was measured in cm). One of the compounds **9** (Fig. 2) showed weak zone (1.5 cm) of inhibition against *Mycobacterium smegmatis* at 10 mM concentration. Hence, minimal inhibitory concentrations were not determined for these compounds due to the apparent lack of significant anti-mycobacterial activity.

Anti-cancer activity

Finally, the synthetic chalcones were tested for cytotoxicity against multiple myeloma and MCF-7 cell lines. For anti-cancer studies, MCF-7 cells were incubated in 5 % CO_2 atmosphere at 37 °C in IMEM medium containing 10 % Hyclone-III and 1 % Antibiotic (500,000 units pen-strep) in sterile conditions at 100 % humidity. For the present studies, sulforhodamine-B (SRB) assay was utilized. 100 μL of 0.5 % SRB (in 1 % acetic acid) was added in each well and incubated at 37 °C for 45 min. SRB solution

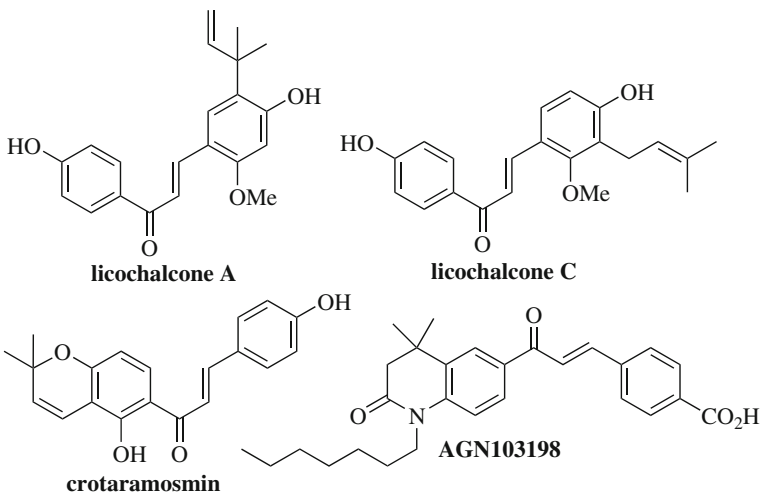
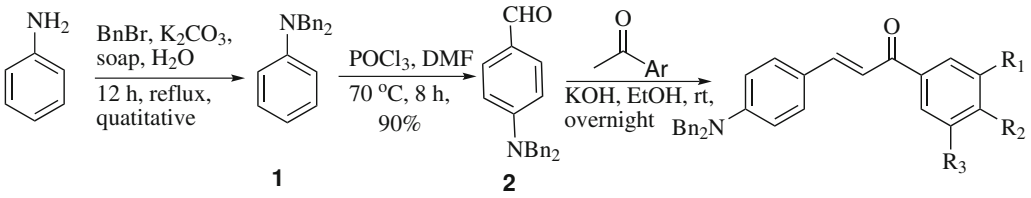
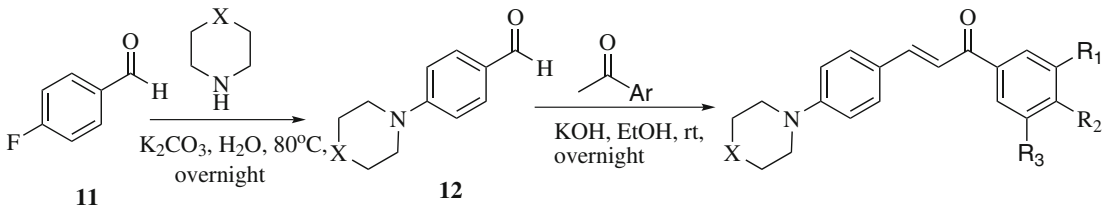


Fig. 1 Biologically active naturally occurring and synthetic chalcones



Chalcones	Substituents
3	$R_1=R_2=R_3=H$
4	$R_1=R_3=H$, $R_2=CH_3$
5	$R_1=R_3=H$, $R_2=OCH_3$
6	$R_1=R_2=R_3=OCH_3$
7	$R_1=R_3=H$, $R_2=Cl$
8	$R_1=R_3=H$, $R_2=Br$
9	Ar = 
10	Ar = 

Scheme 1 Synthesis of *N,N*-dibenzyl chalcone derivatives

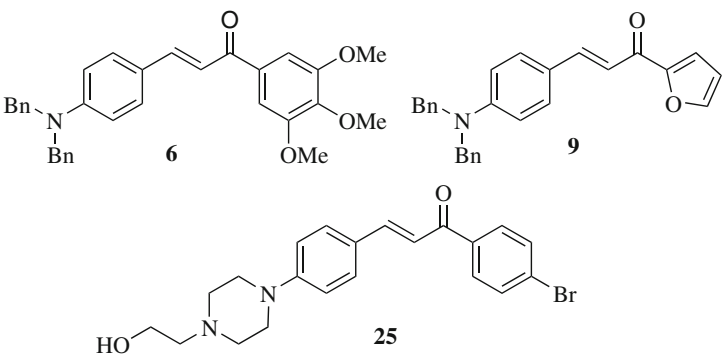


Compounds	X	Substituents
13	CH_2	$R_1=R_2=R_3=H$
14	"	$R_1=R_3=H$, $R_2=CH_3$
15	"	$R_1=R_3=H$, $R_2=OCH_3$
16	"	$R_1=R_2=R_3=OCH_3$
17	"	$R_1=R_3=H$, $R_2=Cl$
18	"	$R_1=R_3=H$, $R_2=Br$
19	"	
20	"	

Compounds	X	Substituents
21	0	$R_1=R_3=H$, $R_2=CH_3$
22	"	$R_1=R_2=R_3=OCH_3$
23	"	$R_1=R_3=H$, $R_2=Cl$
24	NCH_2CH_2OH	$R_1=R_3=H$, $R_2=Cl$
25	"	$R_1=R_3=H$, $R_2=Br$
26	Ar	
27	"	

Scheme 2 Synthesis of pyrrolidine, piperidine, and piperazine Chalcone derivatives

Fig. 2 Active molecules



was removed and the wells were washed 5 times with 1 % acetic acid solution and dried. The cells were dissolved in 400 μL of 10 mM Tris base (pH 10) and absorbance was recorded. The most hydrophilic (hydroxyethylpiperazine) chalcones **24** and **25** showed significant cytotoxicity at 50 μM (IC_{50}) concentration.

BACE1 activity assays

BACE1 activity assays were performed using a FRET peptide substrate Abz-YIWDEIDLMVLD-DNP synthesized by Genscript, Inc (>99 % purity). In kinetic assays, the peptide with that amino acid sequence was initially shown by (Turner *et al*., 2001) to have a higher affinity for BACE1 as well as a larger second order rate constant. Recombinant BACE1 was obtained as described previously (Mallender *et al*., 2001). Final concentrations of substrate and enzyme used for the assay were 25 μM and 0.03 μg/μL, respectively. Assays were performed in Corning half area 96-well plates and read using a Molecular Devices M5 Multifunction Platereader with excitation and emission wavelengths of 320 nm and 420 nm, respectively. All assays were performed in 50 mM sodium acetate, pH 4.5, with 0.25 mg/ml BSA at 23 °C. The final DMSO concentration for all assays was kept at or below 5 %. BACE1 was incubated with each compound in buffer at 23 °C for 30 min prior to the initiation of the assays by the addition of substrate. The reported percent inhibition values are the average of six independent measurements ± SEM relative to the uninhibited assays.

Conclusions

In conclusion, we have synthesized piperidinyl, piperazinyl, and some other amino-based chalcones as potential therapeutic agents. Different class of compounds showed different activity i.e., the most hydrophic chalcone: BACE inhibition, hydrophilic chalcone: cytotoxicity against MCF-7 cell lines and furan derived chalcone: antimycobacterial activity. So, the current study offers preliminary pointers to further modify, design, and investigate the structure of these molecules to expand the utility of these molecules as potential therapeutic agents.

Synthetic procedure (synthesis of **25**)

To a stirred solution of fluorobenzaldehyde (10 mmol) in 10 mL H_2O, *N*-hydroxyethylpiperazine (15 mmol) and potassium carbonate (20 mmol) were added. The reaction mixture was refluxed for 24 h and cooled. The resulting solid was filtered to obtain the crude aldehyde 9.5 mmol (95 % yield), which was utilized for the next step without purification. To a stirred solution of piperazinyl benzaldehyde 1 (10 mmol) in 10 mL (EtOH), 2 mmol KOH and *p*-chloroacetophenone (1.2 mmol) were added. The reaction mixture was stirred overnight and quenched with HCl. The resulting solid was filtered and washed several times with hexane to obtain the chalcone, which was further purified by recrystallization with diethyl ether (78 % yield). Yellow solid, mp. 105–106 °C; IR (KBr pellet) ν 1652, 1584, 1517, 1220, 1009, 812 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.97 (d, $J = 8.5$ Hz, 2H), 7.79 (d, $J = 15.5$ Hz, 1H), 7.58 (d, $J = 8.5$ Hz, 2H), 7.48 (d, $J = 8.5$ Hz, 2H), 7.34 (d, $J = 15.5$ Hz, 1H), 6.92 (d, $J = 8.5$ Hz, 2H), 3.69 (t, $J = 5.0$ Hz, 2H), 3,36 (t, $J = 5.0$ Hz, 4H), 2.63–2.71 (m, 7H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 189.5, 153.0, 145.9, 139.0, 137.3, 130.5, 130.0, 129.0, 125.4, 118.0, 115.0, 59.5, 58.0, 52.8, 48.1; CHN Found: C: 68.15, H: 6.05, N: 7.30; Calculated: C: 68.01, H: 6.25, N: 7.55.

For detailed synthetic procedure and spectral data of all the chalcones see supporting information.

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