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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, anti-inflammatory and antioxidant activity of ring-A-monosubstituted chalcone derivatives

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Abstract A library of ring-A-monosubstituted chalcone derivatives (**4a–4i**, **5a** and **5b**) was designed and synthesised. The structures as well as the identities of these compounds were established on the basis of spectral (^{1}H NMR, ^{13}C NMR, FT-IR and Mass) and elemental (C, H, N) analyses. All the derivatives were evaluated for their anti-inflammatory and antioxidant activities in vitro using the inhibition of protein denaturation and 2,2-diphenyl-1-picrylhydrazyl radical scavenging assays, respectively. The results indicated a promising anti-inflammatory activity for most of the synthesised compounds with many derivatives showing activities similar to or greater than that of the standard. The sulphonamide-substituted chalcones **4h**, **4i**, **5a** and **5b** were found to be the most active derivatives across the concentration range tested. However, all the derivatives exhibited rather mild antioxidant activity compared to the ascorbic acid standard. Interestingly, it was observed that the unsubstituted parent chalcone was one of the optimal compounds with only the trifluoromethyl analogue **4a** showing better activity as an antioxidant. The two regioisomeric aminochalcones and 4′-cyanochalcone **4b** also seemed to possess decent antioxidant potential.

Keywords Chalcone · Antioxidant · Anti-inflammatory · Sulphonamide · Protein denaturation · DPPH

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Introduction

The chalcone moiety remains a popular scaffold amongst medicinal chemists owing to its structural simplicity. Not only are chalcones abundantly found in nature, but also they are key intermediates for many other medicinally important natural products like flavonoids, isoflavonoids and aurones (Detsi *et al*., 2009; Venkatachalam *et al*., 2012). The therapeutic activities of chalcones are attributed to the presence of the key pharmacophoric α,β-unsaturated keto group, more specifically a 2-propen-1-one chain (Orlikova *et al*., 2011). It is proved that the inherent electrophilicity of this α,β-unsaturated carbonyl functionality is involved in the antioxidant and anti-inflammatory properties of chalcones (Kumar *et al*., 2011; Maydt *et al*., 2013). Both the terminal carbons of the 2-propen-1-one fragment are attached to a phenyl ring, conventionally referred to as ring A and ring B on each of which a multitude of functional groups may be appended. Besides such substituted phenyl groups, the synthetically derived chalcones may

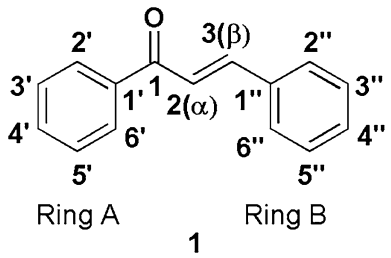


Fig. 1 Prototypical structure of chalcone

also carry heterocyclic and condensed ring systems as their aryl substituents (Meng *et al*., 2007; Solomon and Lee, 2012; Tran *et al*., 2012). While the presence of a double bond allows these molecules to exist as *cis* or *trans* geometric isomers, the latter configuration has been proven to be thermodynamically as well as biologically favourable (Cheng *et al*., 2000). The structure of the simplest unsubstituted chalcone (**1**) is illustrated in Fig. 1.

The diverse pharmacological applications of chalcones include their potential utility as antioxidant, antibacterial, antileishmanial, antiangiogenic, analgesic, antifungal, antiprotozoal, gastric protectant, antimutagenic, antitumorigenic and anti-inflammatory agents amongst others (Gacche *et al*., 2008; Wu *et al*., 2011; Yadav *et al*., 2011; Reddy *et al*., 2012). Some of the well-known natural anti-inflammatory chalcones also possessing good antioxidant potential are butein, xanthohumol, isoliquiritigenin, cardamonin, licochalcone A, flavokawain A and B, all of which represent predominantly hydroxylated, methoxylated and alkylated versions of the basic chalcone scaffold (Nowakowska, 2007; Srinivasan *et al*., 2009; Vogel *et al*., 2010). Even though many chalcones found in nature are known to be traditional folk remedies for inflammatory conditions, the effects of substituting various functional groups at different positions on the rings can be studied systematically only if the target molecules are synthesised chemically. The availability of a simple method to prepare chalcones via Claisen–Schmidt reaction, involving the condensation of substituted acetophenone precursors with substituted benzaldehydes in the presence of a base, has made it easier to assemble large numbers of derivatives within a short amount of time (Bandgar *et al*., 2010; Sing *et al*., 2012).

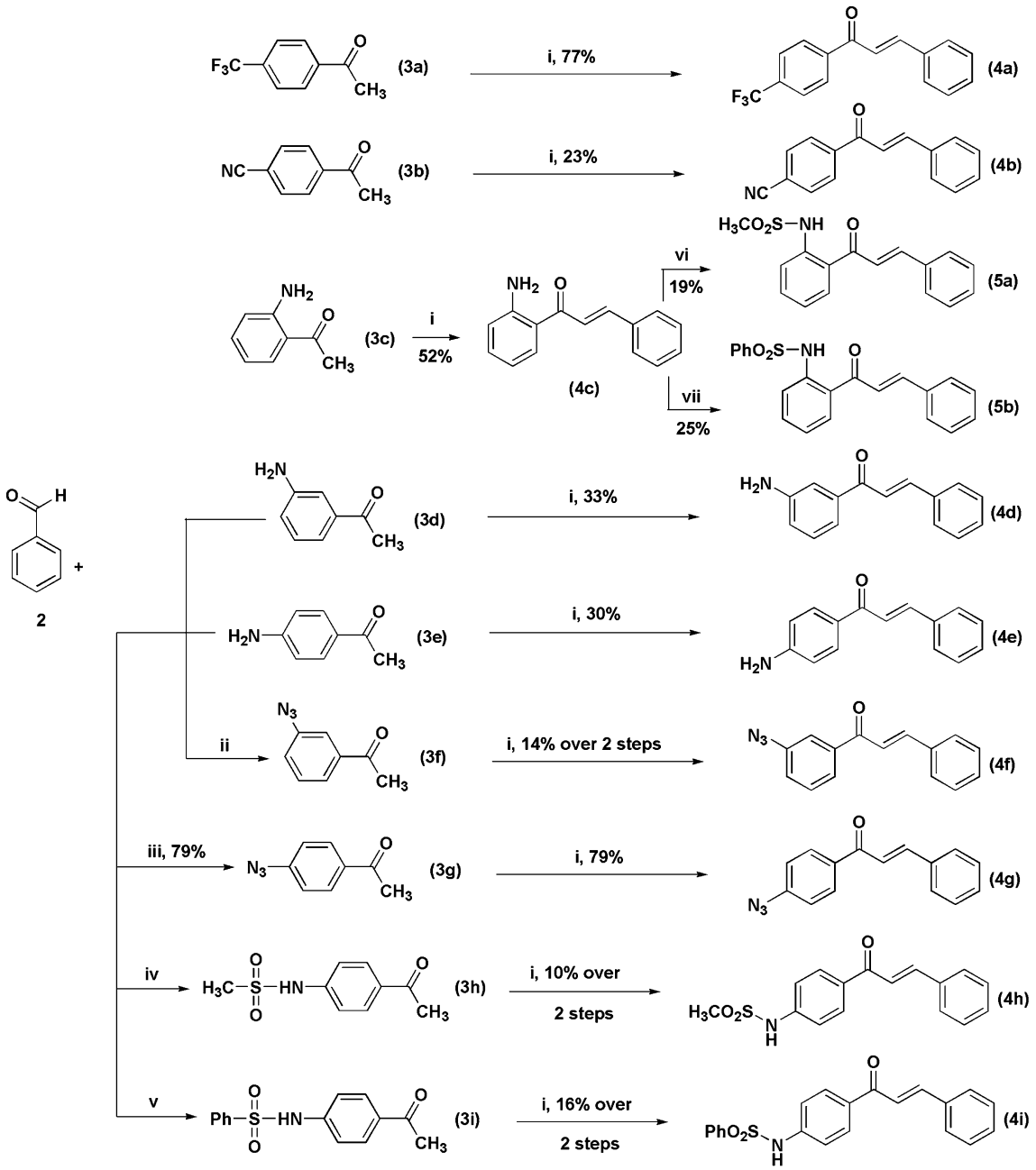
Many of the reports pertaining to synthetic chalcones are limited in their ability to establish a detailed structure activity relationship (SAR) footprint with respect to anti-inflammatory properties of these molecules. The crucial aspect in our ongoing search for a potent yet safe anti-inflammatory compound is to bridge such a wide information gap that currently exists between structure and anti-inflammatory potential of this scaffold. A closer look at the various mechanisms of inflammatory responses points out that the presence of antioxidants capable of protecting oxidative stress-related injury and inflammatory disease will improve the anti-inflammatory activity (Isa *et al*., 2012; Schinella *et al*., 2002). The notion of this correlation between antioxidant and anti-inflammatory activity is strengthened by the findings of research groups that attribute the importance of free radicals in the progression of oxidative as well as inflammatory damage (Roome *et al*., 2008; Saldanha *et al*., 1990). It is, therefore, felt that the estimation of antioxidant potential is a logical extension of studying a compound's anti-inflammatory activity and accordingly, the study presented herein deals with the rational design of a focused library of ring-A-monosubstituted chalcones, their synthesis and subsequent in vitro evaluation for anti-inflammatory and antioxidant activities. Apart from helping elucidate SAR, estimation of the antioxidant activity of these chalcones also represents our attempt to probe structural attributes that would confer dual pharmacological activity to the hits identified.

Experimental

Materials and methods

All commercial reagents were used as provided unless otherwise indicated. All reactions were performed in oven-dried glassware. TLC was performed on silica gel G 40 μm particle size-coated glass plates, and spots were visualized by UV and/or I_2 chamber. Melting points were determined using a Roy capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 or Bruker AV III 500 MHz spectrometer. ^{19}F NMR was recorded on Bruker AV III 500 MHz spectrometer. Proton, carbon and fluorine chemical shifts are reported in ppm. The internal standard for proton and carbon was residual $CHCl_3$ (7.26 and 77.16 ppm, respectively). Proton chemical data are reported as follows: chemical shift, multiplicity (ovlp. = overlapping, s = single, d = doublet, t = triplet, dt = doublet of triplet, tt = triplet of triplet and m = multiplet), coupling constant and integration. IR spectra were recorded on a Shimadzu FT-IR spectrophotometer or Perkin Elmer spectrum RXI FT-IR spectrophotometer. LC–MS chromatograms and mass spectra were obtained on a Waters e 2695-Waters 3100 instrument with ESI-PMT arrangement as the mode of ionisation and type of detector, respectively. Elemental analyses were determined on an Elementar Vario EL III elemental analyser.

For studying the anti-inflammatory activity, ibuprofen standard was obtained as a gift from Porus Labs Pvt. Ltd., Hyderabad. Protein used in this assay was bovine serum albumin fraction V (98 %, Nice Chemicals). Analytical



Scheme 1 Synthesis of relevant acetophenone precursors and target chalcones

grade DMF (99.5 %, Otto Chemei, India) was used to solubilise the test compounds. Absorbance readings were taken from Schimadzu UV–Vis spectrophotometer. With regard to the antioxidant assay using DPPH, all chemicals were purchased from SDFCL, Mumbai unless specified otherwise. DPPH (90 %) was procured from Sigma Aldrich (Germany). A Shimadzu UV-1800 Spectrophotometer was used for measuring the optical density of the samples.

Design and in silico analysis of target chalcones

The design of ring-A-monosubstituted chalcones was primarily based on the electronic properties of the substituents. The selected functional groups are placed at 2′-(*ortho*), 3′-(*meta*) or 4′-(*para*) position of ring A of the scaffold while ring B is left intact. Significant groups like amino and cyano groups were selected for their mesomeric (+*M*/−*M*) effect; similarly groups like trifluoromethyl and azido were selected for their inductive (+*I*/−*I*) effect. To test the effect of lipophilicity on the pharmacological activities of chalcones, sulphonamide functional groups including methanesulphonamide and benzenesulphonamide at *ortho* and *para* (**5a**, **4h**, **5b** and **4i**; Scheme 1) positions were employed (Balasubramanian and Vijayagopal, 2012). The proposed molecules were then subjected to in silico Lipinski Rule of Five (Ro5) analysis for assessing drug-likeness using Molsoft (molsoft.com/mprop/), an online academic software wherein properties like molecular weight, hydrogen bond donors as well as acceptors and calculated partition coefficient of the molecules were determined.

Synthesis

A total of eleven monosubstituted chalcones were sought out in this study of which ten were successfully prepared. While the synthetic route(s) used to access them are presented in Scheme 1, experimental procedures and characterisation data of the respective compounds are detailed herein. Typically, the classic base-catalysed aldol-type condensation was employed to assemble the target compounds **4a**, **4b**, **4d**–**4i** as well as the congener **4c** that acted as a precursor to the two other target sulphonamides **5a** and **5b**. Spectral characterisation following the synthesis unambiguously confirmed the structure of the compounds.

General procedure for synthesis of 4a–4e

To a solution of substituted acetophenone (10 mmol) in rectified spirit (30 mL), benzaldehyde (1.01 mL, 10 mmol) was added followed by an aqueous solution of 10 % KOH (10 mL). The mixture was stirred and kept overnight at room temperature. The contents of the reaction mixture were poured into crushed ice and acidified with dil. HCl (0.1–0.2 N). The precipitated chalcone derivative was filtered off and recrystallised from rectified spirit. Column chromatography (10–20 % EtOAc/cyclohexane) was performed whenever recrystallisation failed to sufficiently purify the target compound.

4′-Trifluoromethylchalcone ***4a*** *(Wilhelm et al., 2012)*

Off-white crystals; yield: 77 %; $R_f = 0.50$ (10 % EtOAc/cyclohexane); mp 94–98 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.10 (d, $J = 8$ Hz, 2H), 7.83 (d, $J = 15.5$ Hz, 1H), 7.77 (d, $J = 8$ Hz, 2H), 7.64–7.66 (m, 2H), 7.49 (d, $J = 15.5$ Hz, 1H), 7.42–7.45 (m, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.7, 146.2, 141.1, 134.5, 134.2, 133.9, 131.0, 129.1, 128.9, 128.7, 125.8, 125.7, 125.6, 124.8, 122.6, 121.6; ^{19}F NMR ($CDCl_3$, 470 MHz) δ −63.00 (s, 3F); IR (KBr) 3060 (arom. –CH str.), 1943 (C–F_3 str.), 1666 (α,β-unsaturated keto group, –C=O str.), 1608, 1574 (C=C arom. str., C=C olefinic str.), 1321 (ip arom. C–H bend.), 985 (oop –CH bend. vibration of alkene), 840 (1,4 disubstitution), 772 (arom. bend.) cm^{-1}; Anal. calcd. for $C_{16}H_{11}F_3O$: C 69.56, H 4.01. Found: C 69.42, H 3.99; ESI MS (*m/z*, relative abundance) 277 [(M+H)$^+$, 100], 298 [(M+Na)$^+$, 44], 320 [(M+2Na)$^+$, 25], [$C_{32}H_{20}O_2^{2+}$, 7].

4′-Cyanochalcone ***4b*** *(Kumar et al., 1985)*

White crystals; yield: 23 %; $R_f = 0.57$ (20 % EtOAc/cyclohexane); mp 92–96 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.08 (d, $J = 8.5$ Hz, 2H), 7.83 (d, $J = 15.5$ Hz, 1H), 7.80 (d, $J = 8.5$ Hz, 2H), 7.64–7.66 (m, 2H), 7.47 (d, $J = 15.5$ Hz, 1H), 7.43–7.46 (m, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.2, 146.6, 141.5, 134.4, 132.5, 131.2, 129.1, 128.9, 128.7, 121.2, 118.0, 116.0; IR (KBr) 3100 (arom. –CH str.), 2230 (arom. C≡N), 1662 (α,β-unsaturated keto group, –C=O str.), 1600, 1574 (C=C arom. str., C = C olefinic str.), 984 (oop –CH bend. vibration of alkene), 833 (1,4 disubstitution), 766 (arom. bend.) cm^{-1}; Anal. calcd. for $C_{16}H_{11}NO$: C 82.38, H 4.75, N 6.00. Found: C 82.17, H 4.88, N 6.02; ESI MS (*m/z*, relative abundance) 234 [(M+H)$^+$, 100].

2′-Aminochalcone ***4c*** *(Mannich and Dannehl, 1938)*

Yellow flakes; yield: 52 %; $R_f = 0.68$ (20 % EtOAc/cyclohexane); mp 50–54 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.85 (dd, $J = 1.5$, 8.5 Hz, 1H), 7.73 (d, $J = 15.5$ Hz, 1H), 7.62 (dd, $J = 1.5$, 8.5 Hz, 1H), 7.61 (d, $J = 8.5$ Hz, 1H), 7.60 (d, $J = 15.5$ Hz, 1H), 7.41 (ovlp. d, $J = 8.5$ Hz, 1H), 7.36–7.42 (m, 2H), 7.26–7.30 (m, 1H), 6.66–6.71 (m, 2H), 6.30 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.7, 151.0, 142.9, 135.3, 134.3, 131.0, 130.1, 128.9, 128.2, 123.2, 119.1, 117.3, 115.9; IR (KBr) 3444, 3325 (–NH str., primary amine), 1641 (α,β-unsaturated keto group, –C=O str.), 1605, 1573 (C=C arom. str., C=C olefinic str.), 1336 (arom. amino group, C–N str.), 738 (1,2 disubstitution) cm^{-1}; ESI MS (*m/z*, relative abundance) 224 [(M+H)$^+$, 100].

3′-Aminochalcone ***4d*** *(Karaman et al., 2010)*

Yellow crystals obtained by column chromatography; yield: 33 %; $R_f = 0.35$ (20 % EtOAc/cyclohexane); mp 90–94 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.79 (d, $J = 16$ Hz, 1H), 7.63 (d, $J = 5.5$ Hz, 1H), 7.62 (d, $J = 5.5$ Hz, 1H), 7.48 (d, $J = 16$ Hz, 1H), 7.41 (ovlp. d, $J = 5.5$ Hz, 1H), 7.40 (ovlp. d, $J = 5.5$ Hz, 1H), 7.38–7.42 (m, 2H), 7.26–7.32 (m, 2H), 6.88–6.90 (m, 1H), 3.85 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 190.7, 146.9, 144.5, 139.4, 135.0, 130.5, 129.5, 128.9, 128.4, 122.4, 119.4, 118.9, 114.4; IR (KBr) 3367, 3203 (–NH str., primary amine), 1660 (α,β-unsaturated keto group, –C=O str.), 1650, 1587 (C=C arom. str., C=C olefinic str.), 1336 (arom. amino group, C–N str.), 759 (1,3 disubstitution) cm^{-1}; Anal. calcd. for $C_{15}H_{13}NO$: C 80.69, H 5.87, N 6.27. Found: C 80.90, H 5.79, N 6.41; ESI MS (*m/z*, relative abundance) 224 $[(M+H)^+$,100].

4′-Aminochalcone ***4e*** *(Applequist and Gdanski, 1981)*

Yellow crystalline needles; yield: 30 %; $R_f = 0.42$ (20 % EtOAc/cyclohexane); mp 130–136 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.93 (d, $J = 8.5$ Hz, 2H), 7.78 (d, $J = 15.5$ Hz, 1H), 7.62 (d, $J = 8.5$ Hz, 2H), 7.54 (d, $J = 15.5$ Hz, 1H), 7.36–7.41 (m, 3H), 6.69 (d, $J = 9$ Hz, 2H), 4.22 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 188.1, 151.2, 143.1, 135.3, 131.0, 130.0, 128.8, 128.4, 128.2, 122.0, 113.9; IR (KBr) 3338, 3200 (–NH str., primary amine), 1628 (α,β-unsaturated keto group, –C=O str.), 1603, 1577 (C=C arom. str., C=C olefinic str.), 1341 (arom. amino group, C–N str.), 830 (1,4 disubstitution), 766 (arom. bend.) cm^{-1}; Anal. calcd. for $C_{15}H_{13}NO$: C 80.69, H 5.87, N 6.27. Found: C 80.81, H 5.95, N 6.22; ESI MS (*m/z*, relative abundance) 224 $[(M+H)^+$, 100], 249 $[(M+Na)^+$, 10].

Synthesis of 3′-azidochalcone **4f**

To a solution of 3-amino acetophenone (1.0 g, 7 mmol) in THF (10 mL), 20 mL of 10 % aqueous HCl was added. Sodium nitrite (1.0 g, 14.7 mmol) was added as a solid to this solution at 0 °C. After 45 min of stirring at 0–5 °C, sodium azide (4.81 g, 74 mmol) was added in small portions over 10 min, so that the temperature would not exceed 5 °C. The reaction was allowed to stir for a further 1–2 h at room temperature. The reaction mixture was extracted with Et_2O (2 × 15 mL); the organic layers were washed with distilled water (2 × 20 mL) and saturated sodium chloride solution (20 mL). The washings were re-extracted with Et_2O (15 mL), the combined Et_2O layers were dried over Na_2SO_4 and evaporated to get the crude product **3f**. To the solution of this crude 3-azido acetophenone in rectified spirit (30 mL), benzaldehyde (1.01 mL, 10 mmol) was added followed by an aqueous solution of 10 % KOH (10 mL). The mixture was stirred and kept overnight at room temperature. The contents of the reaction mixture were poured into crushed ice and acidified with dil. HCl (0.1–0.2 N). The precipitated 3′-azidochalcone was filtered off and recrystallised from rectified spirit. Further purification by column chromatography (20 % EtOAc/cyclohexane) afforded cream-coloured crystals. Yield: 14 % over two steps; $R_f = 0.68$ (20 % EtOAc/cyclohexane); mp 46–50 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (d, $J = 15.6$ Hz, 1H), 7.69 (d, $J = 7.6$ Hz, 1H), 7.56–7.59 (m, 3H), 7.41 (d, $J = 7.6$ Hz, 1H), 7.39 (d, $J = 15.6$ Hz, 1H), 7.34–7.43 (m, 3H), 7.14–7.17 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 188.5, 144.6, 139.9, 138.9, 133.7, 129.8, 129.0, 128.0, 127.5, 123.9, 122.1, 120.7, 117.8; IR (thin film) 3062 (arom. –CH str.), 2113 (asym. NNN str.), 1583 (α,β-unsaturated keto group, –C=O str.), 985 (oop –CH bend. vibration of alkene), 759 (1,3 disubstitution) cm^{-1}; Anal. calcd. for $C_{15}H_{11}N_3O$: C 72.28, H 4.45, N 16.86. Found: C 72.08, H 4.49, N 16.77; ESI MS (*m/z*, relative abundance) 222 $[(M-N_2)^+$, 100], 250 $[(M+H)^+$, 30].

Synthesis of 4′-azidochalcone **4g** (Zarghi *et al*., 2006)

Sodium nitrite (1.0 g, 14.7 mmol) was added as a solid to a 30 mL solution of 4-amino acetophenone (1.0 g, 7.4 mmol) in trifluoroacetic acid at 0 °C. After 45 min of stirring at 0–5 °C, sodium azide (4.81 g, 74 mmol) was added in small portions over 10 min, so that the temperature would not exceed 5 °C. An aliquot of Et_2O (20 mL) was added, and the reaction was allowed to stir in the dark for 2 h. The reaction mixture was washed with distilled H_2O (2 × 15 mL) and saturated sodium chloride solution (20 mL). The washings were re-extracted with Et_2O (15 mL); the Et_2O layers combined, dried over Na_2SO_4 and evaporated to get a dark brown residue from which the desired azido acetophenone **3g** was extracted by boiling with hexane (3 × 20 mL). Pure 4-azido acetophenone (Kym *et al*., 1993) eventually precipitated as light yellow crystals after keeping the hexane extract in refrigerator overnight. Yield: 79 %; $R_f = 0.60$ (20 % EtOAc/cyclohexane); mp 40–44 °C; IR (KBr) 3000 (arom. –CH str.), 2128 (asym. NNN str.), 1685 (C=O str.), 1654, 1596 (C=C arom. str.), 831 (1,4 disubstitution), 721 (arom. bend.) cm^{-1}.

To the solution of 4-azido acetophenone (0.15 g, 0.9 mmol) in rectified spirit (30 mL), benzaldehyde (0.15 mL, 0.9 mmol) was added followed by an aqueous solution of 10 % KOH (5 mL). The mixture was stirred and kept overnight at room temperature. The contents of the reaction mixture were poured into crushed ice and acidified with dil. HCl (0.1–0.2 N). The precipitated 4′-azidochalcone was

filtered off and recrystallised from rectified spirit to afford a light brown solid. Yield 79 %; $R_f = 0.72$ (20 % EtOAc/cyclohexane); mp 80–84 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.05 (d, $J = 9$ Hz, 2H), 7.82 (d, $J = 15.5$ Hz, 1H), 7.64 (d, $J = 9$ Hz, 2H), 7.51 (d, $J = 15.5$ Hz, 1H), 7.42 (ovlp. d, $J = 8.5$ Hz, 1H),7.41–7.43 (m, 2H), 7.13 (d, $J = 8.5$ Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 188.6, 144.9, 144.7, 134.8, 134.7, 130.6, 130.4, 129.0, 128.4, 121.5, 119.0; IR (KBr) 3050 (arom. –CH str.), 2150 (asym. NNN str.), 1645 (α,β-unsaturated keto group, –C=O str.), 1600, 1570 (C=C arom. str., C=C olefinic str.), 985 (oop –CH bend. vibration of alkene), 830 (1,4 disubstitution), 775 (arom. bend.) cm^{-1}; Anal. calcd. for $C_{15}H_{11}N_3O$: C 72.28, H 4.45, N 16.86. Found: C 72.15, H 4.32, N 16.91; ESI MS (*m/z*, relative abundance) 222 [$(M-N_2)^+$, 100], 250 [$(M+H)^+$, 40].

Synthesis of 4′-Methanesulphonamide chalcone **4h** (Zarghi *et al.*, 2006)

To a solution of 4-aminoacetophenone (0.5 g, 3.7 mmol) in dry CH_2Cl_2 (10 mL), Et_3N (1.18 mL, 8 mmol) was added followed by methanesulphonyl chloride (0.32 mL, 4 mmol) at 0 °C. After maintaining the temperature between 0 and 5 °C for 3–4 h, the reaction mixture was gradually warmed to room temperature and the contents were stirred overnight. Once TLC indicated the completion of the reaction, the mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (2 × 15 mL) followed by saturated sodium chloride solution (15 mL). Drying (Na_2SO_4) and then distillation of the organic extracts afforded the crude intermediate **3h** as a light yellow solid. To the crude 4-methanesulphonamide acetophenone dissolved in rectified spirit (30 mL), benzaldehyde (1.01 mL, 10 mmol) was added followed by an aqueous solution of 10 % KOH (10 mL). The mixture was stirred and kept overnight at room temperature. The contents of the reaction mixture were poured into crushed ice and acidified with dilute HCl (0.1–0.2 N). The precipitated chalcone derivative was filtered off, recrystallised from rectified spirit and further purified by column chromatography (20 % EtOAc/cyclohexane) to get yellow crystals. Yield: 10 % over two steps; $R_f = 0.24$ (20 % EtOAc/cyclohexane); mp 112–116 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.06 (d, $J = 7.25$ Hz, 1H), 8.05 (d, $J = 7.25$ Hz, 1H), 7.83 (d, $J = 16$ Hz, 1H), 7.65 (d, $J = 7.25$ Hz, 1H), 7.64 (d, $J = 7.25$ Hz, 1H), 7.51 (d, $J = 16$ Hz, 1H), 7.44 (ovlp. d, $J = 6.25$ Hz, 1H), 7.43 (ovlp. d, $J = 6.25$ Hz, 1H), 7.42–7.44 (m, 1H), 7.33 (d, $J = 6.25$ Hz, 1H), 7.32 (d, $J = 6.25$ Hz, 1H), 7.15 (s, 1H), 3.12 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 188.9, 145.1, 141.1, 134.8, 134.4, 131.1, 130.7, 130.6, 129.4, 129.0, 128.5, 128.3, 121.5, 118.4, 118.2, 40.1; IR (thin film) 3248 (–NH str.), 3035 (arom. –CH str.), 1653 (α,β-unsaturated keto group, –C=O str.), 1601, 1500 (C=C arom. str., C=C olefinic str.), 1338 (asym. SO_2 str.), 1178 (sym. SO_2 str.), 934 (SN str.), 837 (1,4 disubstitution) cm^{-1}; Anal. calcd. for $C_{16}H_{15}NO_3S$: C 63.77, H 5.02, N 4.65, S 10.64. Found: C 63.79, H 4.98, N 4.49, S 10.78; ESI MS (*m/z*, relative abundance) 300 [$(M-H)^+$, 100].

Synthesis of 4′-benzenesulphonamide chalcone **4i** (Moustafa and Ahmad, 2003)

To a solution of 4-aminoacetophenone (0.5 g, 3.7 mmol) in dry CH_2Cl_2 (10 mL), Et_3N (1.18 mL, 8 mmol) was added followed by benzenesulphonyl chloride (0.51 mL, 4 mmol) at 0 °C. After maintaining the reaction mixture between 0 and 5 °C for 3–4 h, the contents were gradually brought up to room temperature and stirred overnight. After TLC indicated the completion of the reaction, the mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (2 × 15 mL) followed by saturated sodium chloride solution (15 mL). Drying (Na_2SO_4) and then distillation of the organic layer afforded the crude acetophenone intermediate **3i** as a yellow solid. This crude 4-benzenesulphonamide acetophenone was dissolved in rectified spirit (30 mL). Benzaldehyde (1.01 mL, 10 mmol) was added to it followed by an aqueous solution of 10 % KOH (10 mL). The mixture was stirred and kept overnight at room temperature. The contents of the reaction mixture were poured into crushed ice and acidified with dilute HCl (0.1–0.2 N). The precipitated product was filtered off and recrystallised from rectified spirit affording the title compound as light yellow crystals. Yield: 16 % over two steps; $R_f = 0.44$ (20 % EtOAc/cyclohexane); mp 126–130 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.99 (d, $J = 8.5$ Hz, 2H), 7.95 (d, $J = 8.5$ Hz, 1H), 7.94 (d, $J = 8.5$ Hz, 1H), 7.84 (d, $J = 16$ Hz, 1H) 7.70 (tt, $J = 2$, 8 Hz, 2H), 7.64–7.66 (m, 2H), 7.57 (s, 1H), 7.56–7.59 (ovlp. m, 2H), 7.49 (d, $J = 16$ Hz, 1H), 7.42–7.45 (m, 2H), 7.17 (dt, $J = 2$, 8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.4, 145.8, 139.5, 139.3, 137.9, 134.6, 134.2, 131.9, 130.9, 129.3, 129.2, 129.1, 128.6, 121.5; IR (thin film) 3344 (–NH str.), 3066 (arom. –CH str.), 1662 (α,β-unsaturated keto group, –C=O str.), 1595, 1489 (C=C arom. str., C=C olefinic str.), 1340 (asym. SO_2 str.), 1166 (sym. SO_2 str.), 920 (SN str.), 867 (1,4 disubstitution) cm^{-1}; Anal. calcd. for $C_{21}H_{17}NO_3S$: C 69.40, H 4.71, N 3.85, S 8.82. Found: C 69.29, H 4.64, N 3.78, S 8.99; ESI MS (*m/z*, relative abundance) 362 [$(M-H)^+$, 100].

Synthesis of 2′-methanesulphonamide chalcone **5a** (Batt *et al.*, 1993)

To a solution of 2′-aminochalcone (0.18 g, 0.81 mmol) in dry CH_2Cl_2 (5 mL), pyridine (0.66 mL, 0.83 mmol) was

added followed by methanesulphonyl chloride (0.07 mL, 0.9 mmol) at 0 °C under N_2 atmosphere. The contents were stirred overnight at room temperature by gradually warming the reaction mixture from 0 °C. After completion of the reaction as indicated by TLC, the reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with water (2 × 15 mL) and saturated sodium chloride solution (15 mL), dried over Na_2SO_4 and distilled off. The crude compound thus obtained was purified using column chromatography (10 % EtOAc/cyclohexane). Bright yellow crystals; yield: 19 %; $R_f = 0.35$ (20 % EtOAc/cyclohexane); mp 74–76 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.23 (s, 1H), 8.04 (dd, $J = 1.5$, 8.5 Hz, 1H), 7.85 (d, $J = 15.5$ Hz, 1H), 7.80 (dd, $J = 1.5$, 8.5 Hz, 1H), 7.66 (d, $J = 6.75$ Hz, 1H), 7.65 (d, $J = 6.75$ Hz, 1H), 7.59 (d, $J = 15.5$ Hz, 1H), 7.58 (t, $J = 1$ Hz, 1H), 7.43–7.46 (m, 3H), 7.20–7.23 (m, 1H), 3.08 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 192.7, 146.3, 140.7, 134.9, 134.5, 131.1, 131.0, 129.1, 128.7, 123.4, 122.8, 121.8, 118.9, 40.2; IR (thin film) 3200 (–NH str.), 3053 (arom. –CH str.), 1643 (α,β-unsaturated keto group, –C=O str.), 1593, 1500 (C=C arom. str., C=C olefinic str.), 1342 (asym. SO_2 str.), 1170 (sym. SO_2 str.), 981 (SN str.), 767 (1,2 disubstitution) cm^{-1}; Anal. calcd. for $C_{16}H_{15}NO_3S$: C 63.77, H 5.02, N 4.65, S 10.64. Found: C 63.66, H 5.10, N 4.76, S 10.63; ESI MS (*m/z*, relative abundance) 300 [$(M-H)^+$, 100].

Synthesis of 2′-benzenesulphonamide chalcone **5b** (Donnelly and Farrell, 1989)

To a solution of 2′-aminochalcone (0.4 g, 3 mmol) in dry CH_2Cl_2 (10 mL), Et_3N (0.7 mL, 5 mmol) was added followed by benzenesulphonyl chloride (0.51 mL, 4 mmol) at 0 °C. The contents were stirred overnight at room temperature by gradually warming the reaction mixture from 0 °C. After the completion of the reaction as indicated by TLC, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (2 × 15 mL) and saturated sodium chloride solution (15 mL). The organic extracts were then dried over Na_2SO_4 and evaporated under reduced pressure to give the crude compound which was subsequently purified by column chromatography (10 % EtOAc/cyclohexane) to afford yellow crystals of the title compound. Yield: 25 %; $R_f = 0.47$ (20 % EtOAc/cyclohexane); mp 76–78 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.19 (s, 1H), 7.85 (d, $J = 8.5$ Hz, 1H), 7.79–7.83 (m, 2H), 7.75 (d, $J = 8.5$ Hz, 1H), 7.66 (d, $J = 15.5$ Hz, 1H), 7.58–7.60 (m, 2H), 7.49 (t, $J = 7.5$ Hz, 1H), 7.38–7.44 (m, 6H), 7.34 (d, $J = 15.5$ Hz, 1H), 7.14 (t, $J = 7.5$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 192.8, 146.1, 139.9, 139.4, 134.4, 134.3, 132.9, 131.0, 130.6, 129.1, 129.0, 128.6, 127.3, 124.9, 123.2, 122.1, 120.8; IR (thin film) 3144 (–NH str.), 3070 (arom. –CH str.), 1641 (α,β-unsaturated keto group, –C=O str.), 1593, 1494 (C=C arom. str., C=C olefinic str.), 1336 (asym. SO_2 str.), 1163 (sym. SO_2 str.), 931 (SN str.), 746 (1,2 disubstitution) cm^{-1}; Anal. calcd. for $C_{21}H_{17}NO_3S$: C 69.40, H 4.71, N 3.85, S 8.82. Found: C 69.43, H 4.88, N 3.97, S 8.81; ESI MS (*m/z*, relative abundance) 362 [$(M-H)^+$, 100].

Determination of biological activity by in vitro methods

Anti-inflammatory activity

A well-established literature protocol that involved inhibition of protein denaturation was followed with minor modifications for the estimation of in vitro anti-inflammatory activity (Mizushima and Kobayashi, 1968). The standard drug ibuprofen and test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer saline (0.2 M, pH 7.4) in such a way that concentration of DMF in all solutions was less than 2.5 %. Each of the test solutions was mixed with bovine serum albumin (BSA) solution (2 mL, 2 mmol) in phosphate buffer saline and incubated at 27 ± 1 °C in an incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ± 1 °C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm with a UV visible spectrophotometer and the percentage of inhibition of denaturation calculated from control where no drug is added. The percentage of inhibition was calculated using the formula: percentage inhibition $= A_C - A_S/A_C \times 100$, where A_C = absorbance of control and A_S = absorbance of test sample.

Antioxidant activity

The method of Doble and co-workers was adopted with minor modifications for carrying out the DPPH assay (Sivakumar *et al*., 2011). Stock solutions of the compounds were prepared by solubilising 2 mg of the compound in 200 µL of DMF and then making up the volume to 10 ml with methanol. Corresponding dilutions of 10, 20, 30, 40 and 50 µg mL^{-1} were prepared with methanol. 0.1 mM DPPH solution and test samples were taken in a ratio of 1:2, and the mixture was vortexed and kept for equilibration by incubating in dark at 25 °C for 20 min. The optical density was measured at 517 nm in a UV spectrophotometer with ascorbic acid being used as the positive control. Pure methanol was used as the blank, and DPPH solution and methanol in a ratio of 1:2 were used as the control. The actual decrease in the absorption induced by the test compound was estimated by subtracting the absorbance of the test from that of the control. The percentage of scavenging of DPPH was calculated using the formula:

Table 1 The Lipinski parameters and TPSA of the selected compounds and ibuprofen

Compound	Molecular weight (g mol^{-1})	Number of hydrogen bond acceptor(s)	Number of hydrogen bond donor(s)	Mol logP	TPSA ($Å^2$)
4a	276.08	1	2	3.27	17.07
4b	233.08	2	0	3.71	40.86
4d	223.10	1	2	3.59	43.09
4e	223.10	1	2	3.59	43.09
4f	246.09	3	0	4.07	66.82
4g	246.09	3	0	3.99	66.82
4h	301.08	3	1	3.08	55.24
4i	363.09	3	1	4.66	54.10
5a	301.08	3	1	3.20	54.48
5b	363.09	3	1	5.03	53.34
1	208.09	1	0	3.95	17.07
Ibuprofen	206.13	2	1	3.38	37.30

$$\%\,\text{scavenging of DPPH} = A_C - A_S/A_C \times 100,$$

where A_C = absorbance of control and AS = absorbance of test sample

Statistical analysis

All assays were carried out in triplicate, and results were expressed as mean ± SD. All statistical analysis was performed using Graph Pad (Version-6) Prism software. ANOVA was used to test the differences between the percentage inhibition values of the different derivatives in both the anti-inflammatory and antioxidant assays followed by Tukey's multiple comparison tests, and $p < 0.05$ was considered statistically significant.

Results and discussion

Drug-likeness assessment

The drug-likeness of compounds was evaluated by calculating the Lipinski parameters using the high-speed molecular properties calculator, a free module in the Molsoft software package. All chalcone derivatives have less than or equal to two hydrogen bond donors and possess no more than three hydrogen bond acceptors, the total maximum permissible count being 10. Besides, none of their molecular weights exceeded 500 daltons. The octanol–water partition coefficient (logP) is not more than 5 for any of the ligands except compound **5b**. These results that are shown in Table 1 strongly suggest drug-likeness, and the target compounds can be said to have properties that would make them amenable to oral administration in humans. Topological polar surface area (TPSA) of the target compounds and reference standard was below 70 $Å^2$ implying easy permeability through cell membrane and ready penetrability across the blood brain barrier (BBB).

Chemistry

Out of the ten target compounds obtained, eight were synthesised by direct Claisen-Schmidt condensation of substituted acetophenones and benzaldehyde while sulphonamides **5a** and **5b** were prepared from 2′-aminochalcone (**4c**) as clearly illustrated in Scheme 1. The structures of all the synthesised derivatives were assigned on the basis of IR, ^1H & ^{13}C NMR, mass spectral data as well as elemental analysis and comparison with published data. It was observed that the results are in agreement with the proposed structures.

The IR spectra of **4a** and **4b** showed characteristic peaks at 1,943 cm^{-1} corresponding to C–F stretch of the $–CF_3$ group and 2,230 cm^{-1} that is attributable to C≡N stretching of the –CN group, respectively. The ^{19}F NMR of 3′-trifluoromethylchalcone gives a singlet at −63.00 ppm corresponding to the three equivalent fluorine atoms of the aromatic $–CF_3$ group. With regard to the amino-substituted chalcones, it was difficult to purify the *meta* and *para* analogues due to the interference of side products. While 3′-aminochalcone (**4d**) was purified by repeated column chromatography of the crude product, precipitation of pure **4e** occurred upon dissolving it in boiling ethanol and keeping overnight at room temperature. A broad singlet around 4 ppm in the ^1H NMR represents the two protons of the $–NH_2$ group. Deuterated water (D_2O) exchange spectrum of **4d** clearly demonstrates the exchange of $–NH_2$ protons with deuterium atom(s). As seen in Fig. 2, the $–NH_2$ peak disappears and a singlet corresponding to HOD appears around 4.7 ppm post equilibration of the sample.

The *meta* and *para* azidochalcones (**4f** and **4g**, respectively) were synthesised in two steps involving the preparation of intermediate azido acetophenones and their subsequent condensation with benzaldehyde to afford the target compounds. The intermediate 4-azido acetophenone (**3g**) was isolated and characterised, whereas **3f** could not be purified and was consequently coupled with benzaldehyde as such. The crude **4f** was then purified by column chromatography. It is pertinent to note that **4f** gradually darkens from off-white to brownish colour when kept at room temperature. The characteristic NNN stretching of azides was displayed by both the azidochalcones in their IR spectra at around 2,200–2,100 cm^{-1}. Although the diagnostic molecular ion peaks were seen in the mass spectra of azidochalcones, the characteristic and most intense peak was at m/z 222 representing the fragment corresponding to

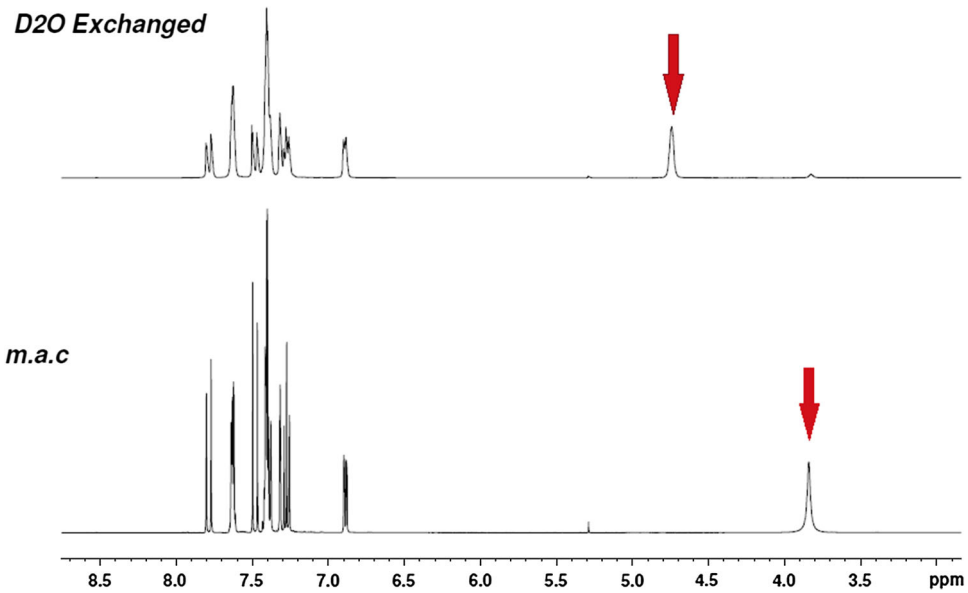


Fig. 2 D_2O exchange observed in 3′-aminochalcone

the loss of nitrogen. The chalcone bearing the azide group at the 2′-(*ortho*) position was also targeted, but the efforts for its isolation failed. The formation of this product was confirmed by the mass spectrum showing an intense peak at 222 as well as deduced from IR spectrum which displayed the characteristic peak of azide group between 2,200 and 2,100 cm^{-1}. The presence of a stubborn impurity was, however, seen in the compound's ^{1}H and ^{13}C NMR spectra which led to its eventual disqualification from further in vitro study.

Two benzenesulphonamides and two methanesulphonamides, each appended to the 2′- or 4′- position of the unsubstituted chalcone, comprise the sulphonamides designed for the study. The 2′-sulphonamide chalcones **5a** and **5b** were synthesised by direct sulphonylation of **4c,** whereas the 4′-sulphonamides **4h** and **4i** were accessed from 4-amino acetophenone (**3e**) via the intermediary 4-sulphonamide acetophenones. Pyridine or Et_3N was employed as a base in the sulphonamide synthesis. The reaction, though slower when carried out using Et_3N compared to pyridine, was, however, driven to completion under the former conditions. Three out of the four sulphonamides were purified by column chromatography which was difficult to be performed due to the similarity in the R_f values of the impurities to that of the desired products. The remaining analogue, 4′-benzenesulphonamide chalcone (**4i**), was purified by stirring in boiling ethanol for 5–10 min and filtering the solution. The pure product being insoluble in hot ethanol is obtained as the filter cake and the impurities go along with the hot ethanol. Analogous to the aminochalcones, the presence of an exchangeable amide proton was tested by recording the D_2O exchange spectra of all the sulphonamides (data not shown). As expected, the exchange phenomenon resulted in the disappearance of amide proton singlet in the range of 11–11.5 ppm and the concomitant appearance of a singlet corresponding to HOD in the range of 4.5–5 ppm.

Pharmacological evaluation

In our study, the in vitro anti-inflammatory potential of chalcone derivatives was evaluated against denaturation of BSA. The results indicated a concentration-dependent inhibition of protein denaturation by the entire chalcone library throughout the concentration range of 10–30 μg mL^{-1}. Ibuprofen that was used as reference drug also exhibited a similar concentration-dependent profile of inhibition. It is clear from the data (Table 2) that the substitution pattern on the phenyl ring of the acetophenic group of chalcone moiety plays an important role in modulating inflammation. Inspection of the tabulated values easily sets apart the four sulphonamides as the better anti-inflammatory analogues with the 2′-benzenesulphonamide chalcone **5b** emerging as the best compound within this subset. The *para* azido- and aminochalcones also fared better than ibuprofen, while the rest of the target compounds exhibited activity similar to that of the reference. Significantly, none of the library members were inactive in this assay.

From a SAR perspective, electron-withdrawing substituents in the form of a trifluoromethyl group (via inductive effect) in **4a** and a cyano group (via mesomeric effect) in **4b** had no effect on the anti-inflammatory activity. With respect to the azide group, the *para* regioisomer was preferred over the *meta* congener. This particular finding is in conformation with reports published by Knaus and co-workers revealing the structural as well as functional basis for the incorporation of an azide as a key bioisosteric pharmacophore in containing inflammation (Habeeb *et al.*, 2001; Rao *et al.*, 2004).

Table 2 The anti-inflammatory and antioxidant profiles of the synthesised derivatives (**4a–4i**, **5a**, **5b** and **1**)

Compound	Concentration (μg mL^{-1})							
	Anti-inflammatory activity (% inhibition)			Antioxidant activity (% inhibition)				
	10	20	30	10	20	30	40	50
4a	80.69 ± 0.33^{a}	84.21 ± 0.55^{a}	85.66 ± 0.12^{a}	15.01 ± 0.30^{a}	17.83 ± 0.18^{a}	20.45 ± 0.26^{a}	22.12 ± 0.26^{a}	24.39 ± 0.18^{a}
4b	81.06 ± 0.33^{a}	84.76 ± 0.16^{a}	85.39 ± 0.16^{a}	14.69 ± 0.63^{a}	16.57 ± 0.59^{b}	17.53 ± 0.52^{b}	18.67 ± 0.19^{b}	19.89 ± 0.25^{b}
4d	80.69 ± 0.33^{a}	84.21 ± 0.55^{a}	85.66 ± 0.12^{a}	8.69 ± 0.30^{b}	10.98 ± 0.32^{c}	12.24 ± 0.32^{c}	13.9 ± 0.37^{c}	15.18 ± 0.24^{c}
4e	84.11 ± 0.36^{b}	84.82 ± 0.41^{a}	85.73 ± 0.62^{a}	9.24 ± 0.24^{b}	10.95 ± 0.14^{c}	13.61 ± 0.37^{d}	15.73 ± 0.20^{d}	17.41 ± 0.15^{d}
4f	81.01 ± 0.51^{a}	82.50 ± 0.36^{b}	82.59 ± 0.48^{b}	1.95 ± 0.35^{c}	3.08 ± 0.33^{d}	4.73 ± 0.14^{e}	6.80 ± 0.35^{e}	9.30 ± 0.23^{e}
4g	83.30 ± 0.54^{b}	85.48 ± 0.73^{a}	86.01 ± 0.33^{a}	2.19 ± 0.31^{d}	3.62 ± 0.23^{d}	5.57 ± 0.28^{e}	7.45 ± 0.10^{e}	9.54 ± 0.33^{e}
4h	85.11 ± 0.18^{b}	87.15 ± 0.18^{c}	88.76 ± 0.09^{c}	7.85 ± 0.36^{b}	8.67 ± 0.21^{e}	9.23 ± 0.23^{f}	10.02 ± 0.17^{f}	11.34 ± 0.44^{f}
4i	84.15 ± 0.43^{b}	85.26 ± 0.04^{a}	86.04 ± 0.20^{a}	8.97 ± 0.55^{b}	9.50 ± 0.23^{e}	10.21 ± 0.28^{g}	11.00 ± 0.12^{f}	11.55 ± 0.14^{f}
5a	84.94 ± 0.33^{b}	86.69 ± 0.79^{a}	88.65 ± 0.24^{c}	8.06 ± 0.36^{b}	8.87 ± 0.16^{e}	9.76 ± 0.19^{g}	10.35 ± 0.14^{f}	11.40 ± 0.18^{f}
5b	85.81 ± 0.48^{b}	87.95 ± 0.24^{c}	91.07 ± 0.09^{d}	2.54 ± 0.18^{e}	3.60 ± 0.22^{d}	4.55 ± 0.39^{e}	5.87 ± 0.18^{g}	7.84 ± 0.28^{g}
1	81.43 ± 0.27^{a}	81.96 ± 0.16^{b}	85.71 ± 0.15^{a}	14.15 ± 0.39^{a}	16.65 ± 0.36^{b}	17.63 ± 0.29^{b}	19.92 ± 0.40^{h}	22.21 ± 0.45^{h}
Std[i]	80.37 ± 0.51^{a}	80.79 ± 0.41^{b}	84.29 ± 0.72^{e}	93.62 ± 0.17^{f}	94.45 ± 0.30^{f}	95.04 ± 0.18^{h}	95.52 ± 0.23^{i}	96.43 ± 0.21^{i}

Data presented as Mean ± SD of triplicate observations; different letters on the same column show significant differences from each other at $p < 0.05$

[i] Reference drugs for anti-inflammatory and antioxidant activity were ibuprofen and ascorbic acid, respectively

A similar and subtler positional effect was mirrored in the case of the amino substituent as well. Previous studies in our group have led to the finding that electron-donating substituents, especially of the mesomeric type like hydroxy- and amino-, placed at the 2′-position potentiate the anti-inflammatory activity (Balasubramanian *et al.*, 2013). Since free radicals have been definitively implicated in the progression of inflammatory damage, a regiochemical preference for the *ortho* position can be unambiguously explained by the superior ability of such a substituent to form an intramolecular hydrogen bond (iHB) and consequently, the relative ease of free radical formation. More specifically, the unique hydrogen bonding ability in **4c** allows for the formation of an oxygen-centred radical rather than a nitrogen-centred radical, the former being a more favourable process owing to the lower bond dissociation energy (BDE) of O–H versus N–H bond (Bendary *et al.*, 2013). Taken together with the results of this work, a useful activity rank order presents itself in the context of regioisomeric aminochalcones: *ortho* > *para* > *meta*.

A marked increase in the percent inhibition of the sulphonamides over the parent chalcone underscores the importance of this functional group as a ring A substituent. Sulphur-based functional groups have been successfully employed in blockbuster anti-inflammatory drugs. While sulphonamides are present in coxibs (celecoxib, valdecoxib and parecoxib) and oxicams (meloxicam, piroxicam and tenoxicam), reverse sulphonamides i.e. sulphonanilides are seen in drugs such as nimesulide and flosulide. The role of methylsulphonyl group in potentiating anti-inflammatory activity has been highlighted in depth (Gans *et al.*, 1990; Talley, 1999). A detailed investigation has provided further valuable insight into the binding mode and consequently, the selectivity imparted by a *p*-$NHSO_2CH_3$ substituent in cyclooxygenase-2 (COX-2) inhibition (Garavito and DeWitt, 1999; Zarghi *et al.*, 2006). Therefore, it is quite natural that the sulphonamides have emerged superior in this library. Even though target-specific in silico analysis was not a part of our study, it is not difficult to envision the benzenesulphonamide **5b** as the most optimal compound because the phenyl group therein would likely participate in hydrophobic interactions with the relevant residues in the COX-2-binding pocket similar to those established in the case of a *p*-$NHSO_2CH_3$ group.

Recently, a report published by Menichini and co-workers (Conforti *et al.*, 2009) as well as findings in our laboratory (manuscript accepted for publication in *Free Rad and Antiox*) have further corroborated the correlation between antioxidant and anti-inflammatory activity. Moreover, literature suggests that antioxidant and anti-inflammatory activities can be expected to co-exist owing to the involvement of reactive oxygen species (ROS) in cyclooxygenase and lipoxygenase-mediated inflammatory pathways from arachidonic acid (Chebil *et al.*, 2007; Melagraki *et al.*, 2009). The focused library under consideration in this study was, therefore, felt suitable for carrying out the antioxidant assay as it would help satisfy the twin objectives of SAR exploration and an extension of anti-inflammatory:antioxidant activity correlation. However, the results displayed in Table 2 indicate that the derivatives did not fare well in their antioxidant activity, with many showing

negligible percentage inhibition compared to the reference compound. In fact, identification of the unsubstituted chalcone as one of the active compounds renders the members of this focused library ineffective for their ability to probe antioxidant SAR. Interestingly enough, one of the suboptimal anti-inflammatory compounds bearing the trifluoromethyl group was the only library member that exhibited better antioxidant potential than the parent. On a similar note, the potential anti-inflammatory sulphonamides had very poor antioxidant ability. These observations preclude the possibility of extending any meaningful correlation between the two activities. The azide-bearing chalcones **4f** and **4g** turned out to be the least active analogues in the DPPH radical scavenging assay.

Conclusions

The study reveals the design, synthesis and evaluation of anti-inflammatory and antioxidant activity of a series of monosubstituted chalcone derivatives. Most of the target compounds were found to have significant anti-inflammatory activity with many showing activity profile similar to that of the standard drug. Some key structural features that are beneficial for anti-inflammatory activity have been identified. The utility of sulphonamides in containing inflammation has been further corroborated in this work. We have shown that a shift of the azide group from the *para* to the *meta* position is detrimental to the activity. A clear regiochemical preference for primary amino groups being placed in the *ortho* position of ring A has emerged reinforcing our earlier observations. 2′-Benzenesulphonamide derivative (**5b**) possessed the best anti-inflammatory potential in this series. These results can act as an impetus for further research in designing potent anti-inflammatory synthetic chalcone derivatives. However, we realise that these compounds are not the right probes for studying antioxidant activity and consequently, a possible correlation between the two activities could not be extended.

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Conflict of interest The authors declare no conflict of interest.

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