Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 1, 2013

Research Article

NOVEL 4-METHOXY-2-ACETYL BENZOFURAN BASED CHALCONES: A NEW PERCEPTIVITY INTO THEIR ANTIOXIDANT POTENTIALS

NAGARAJA NAIK*[1], H. VIJAY KUMAR[2], SANDRA MARIA DIAS[1], J. RANGA SWAMY[1]

[1]Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India, [2]Department of Organic Chemistry, Indian Institute of Science, Bangalore–560 012, Karnataka, India. Email: drnaikchem@gmail.com

Received: 20 Oct 2012, Revised and Accepted: 02 Dec 2012

ABSTRACT

A new series of 4-methoxy-2-acetyl benzofuran based chalcones (**2a-i**) were synthesized by aldol base condensation reaction. The synthesized compounds were characterized by IR, ^{1}H NMR, [13]C NMR, mass and elemental analysis and were evaluated for their antioxidant potential using various *in vitro* assays like 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, microsomal lipid peroxidation inhibition (LPO) assay and ferric reducing power assay. Butylated hydroxy anisole (BHA) was used as a reference compound and the comparative study with the newly synthesized compounds was also performed. All the newly synthesized analogues showed certain degree of antioxidant activity, among the entire synthesized analogues compound (**2h**) bearing electron donating hydroxy and methoxy group on the phenyl moiety showed predominant activity and exhibits even more activity than the standard.

Keywords: 4-methoxy-2-acetyl benzofuran, Chalcones, Free radicals, Antioxidant activity.

INTRODUCTION

A wide range of human diseases are associated with a disturbed cellular redox balance known as oxidative stress (OS)[1]. OS results from an increase in intracellular concentrations of oxidizing species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to the oxidation of membranes, proteins, DNA and ultimately to cell death. There is increasing evidence of the implication of free radicals and reactive oxygen species in a variety of diseases and pathophysiological events including inflammation, cancer, myocardial infraction, arthritis and neurodegenerative disorders[2-4]. Stoichiometric antioxidant strategies that focus on inhibiting oxidative damage early in the progression of OS related diseases have recently been investigated *in vitro* and *in vivo*[5-9]. Efforts to counteract the damage caused by these species are gaining acceptance as a basis for novel therapeutic approach and the field of preventive medicine is experiencing an upsurge of interest in medically useful antioxidants. Benzofuran are the important group of heterocyclic compound, several derivatives of which have been marked as biologically and pharmacologically active product[10]. Widespread interest in the chemistry of benzofuran in a large number of natural products has attracted due to their biological activities and their potential applications as pharmacological agents. Several benzofuran ring systems bearing various substituents at the C-2 position are widely distributed in nature, e.g., ailanthoidol, is a neolignan derivative, has been reported to have antiviral, antioxidant and antifungal activities[11]. Furthermore, most of compounds prepared from 2-acetyl benzofurans have antimicrobial, antitumor, anti-inflammatory, fungicidal, weed-killing activity and used for treatment of cardiac arrhythmias[12-17]. In the interest of the above findings, and in continuation of research work on the synthesis of biologically active heterocycles[18-21]herein, we reported the synthesis and evaluation of antioxidant properties of 4-methoxy-2-acetyl benzofuran based chalcones.

MATERIALS AND METHODS

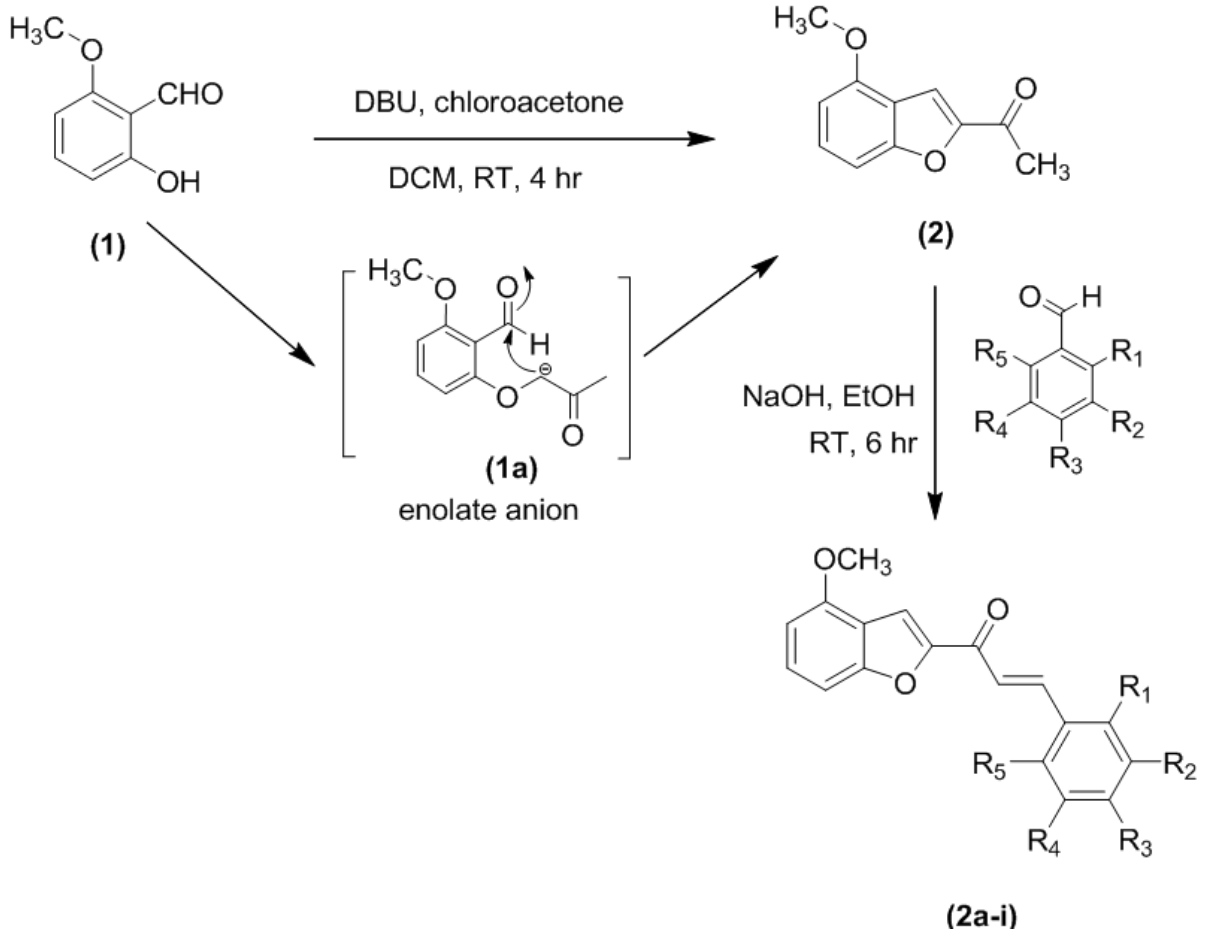
Chemical & Reagents

All chemicals used were of analytical grade (Qualigen, Merck). The melting points were determined by open capillary method on a Campbel electronic apparatus and are uncorrected. The ultraviolet absorption spectra were determined in methanol by using a Shimadzu 1601 UV-Visible double beam spectrophotometer. The IR spectra of synthesized compounds were recorded on a Shimadzu 8400S FT-IR in potassium bromide disks. The 1H NMR and [13]C was recorded in CDCl3 using a NMR Varian-Mercury 400 MHz and 100 MHz spectrometer and chemical shifts are given in units as δ ppm, downfield from tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained by Water-Q-TOF ultima spectrometer. The progress of reactions was monitored by thin layer chromatography using chloroform-methanol and hexane-ethyl acetate as the solvent systems and spots were visualized after exposure to iodine vapours or under ultraviolet (UV) light.

Chemistry

A typical synthetic strategy employed to obtain the title compounds (**2a-i**) in excellent yields is depicted in **Scheme 1**. Initially, the core compound 4-methoxy-2-acetyl benzofuran (**2**) was obtained by reaction between *o*-vanillin with chloroacetone through cyclization via intramolecular displacement by the enolate anion (**a**) in the presence of 1,8-diaza bicycle [5.4.0] undec-7-ene (DBU) as base. Further, coupling of substituted aryl aldehydes by aldol condensation reaction in the presence of sodium hydroxide (NaOH) afforded the 4-methoxy-2-acetyl benzofuran chalcones (**2a-i**).

Compounds	R_1	R_2	R_3	R_4	R_5
2a	H	H	H	H	H
2b	H	H	CH_3	H	H
2c	H	H	Cl	H	H
2d	H	H	NO_2	H	H
2e	H	H	OH	H	H
2f	H	OH	H	H	H
2g	H	H	OCH_3	H	H
2h	OH	OCH_3	H	H	H
2i	H	OCH_3	OCH_3	H	H



Scheme 1: Synthetic protocol for the synthesis of 4-methoxy-2-acetyl benzofuran based chalcones (2a-i).

Procedure for the synthesis of 4-methoxy-2-acetyl benzofuran (2)

To the well stirred solution of *o*-vanillin (4 mmol, 0.60 g) and 1,8-diaza bicycle [5.4.0] undec-7-ene (2 mmol, 0.4 ml) in dichloromethane, chloroacetone (4.2 mmol, 0.33 ml) was added in the syringe and the mixture was stirred for 4 hrs. Progress of the reaction was monitored by TLC using hexane: ethyl acetate (8:2) mixture as mobile phase. After the completion of the reaction, the reaction mixture was washed with 10% HCl solution followed by water. The organics were dried over anhydrous sodium sulphate. The yellow liquid product was obtained by desolventisation in a rotary evaporator at room temperature. The compound was separated and purified by column chromatography by using mixture of hexane: ethyl acetate (8:2) as eluent.

4-methoxy-2-acetyl benzofuran (**2**): Yellow liquid, yield 80%; b.p, 300-302 ^{o}C; IR (KBr)λ_{max}(cm^{-1}): 3200-3350 (Ar–H), 1650 (C=O); ^{1}H NMR (400 MHz) ($CDCl_3$) δ (ppm): δ 6.63–7.71 (m, 3H, Ar–H), 7.59 (d, 1H, furan H), 3.6 (s, 3H, –OCH_3), 2.33 (s, 3H, $COCH_3$); MS (ESI) m/z: 190.06 (M^+); Anal. Calc. for $C_{11}H_{10}O_3$ C, 69.46; H, 5.30; O, 25.24, Found: 69.43; H, 5.31; O, 25.21%

General procedure for the synthesis of 4-methoxy-2-acetyl benzofuran based chalcones (2a-i)

A mixture of 4-methoxy-2-acetyl benzofuran **(2)** (0.5 mmol 0.06 ml), aryl aldehydes (1 mmol) and NaOH (0.5 mmol, 0.02g), in absolute ethanol (5 ml) was stirred for 6 hrs. The progress of the reaction was monitored by TLC using methanol:chloroform (2:8) mixture as mobile phase. After the completion of the reaction a dark yellow precipitate was formed and this served as indicator for monitoring the reaction. Visually EtOH was removed under reduced pressure. The residue was diluted with water (5 ml) neutralized with 2% aqueous HCl and extracted with ethyl acetate (3x5 ml). The combined ethyl acetate extract were washed with brine solution (5 ml) dried over anhydrous Na_2SO_4 and concentrated under vacuum at 50 ^{o}C afforded the respective products. The analogues were separated and purified by column chromatography by using mixture of methanol: chloroform (2:8) as eluent. The products were characterized by IR, mass,^{1}H NMR, [13]C NMR and elemental analysis.

(E)-1-(4-methoxybenzofuran-2-yl)-3-phenylprop-2-en-1-one (2a)

Brown solid, Yield 77%; m.p, 332-334 ^{o}C; IR (KBr)λ_{max}(cm^{-1}) 3400-3428 (Ar-H), 1676 (C=O); ^{1}H NMR (400 MHz) ($CDCl_3$) δ (ppm): 7.7 (s, 1H, α-CH of chalcone), 6.9-7.6 (m, 8H, Ar-H), 7.5 (s, 1H, CH of benzofuran), 6.5 (s, 1H, β-CH of chalcone), 3.8 (s, 3H, OCH_3); [13]C NMR ($CDCl_3$, 100 MHz) δ ppm: 56.4, 151.1, 113.4, 124.9, 103.8, 155.4, 101.0, 116.4, 155.7, 177.0, 121.5, 144.8, 135.0, 128.5, 128.6, 127.9; MS (ESI) m/z: 278.30 (M^+).; Anal. Calc. for, $C_{18}H_{14}O_3$; C, 77.68; H, 5.07; O, 17.25, Found: C, 77.65; H, 5.06; O, 17.27%

(E)-1-(4-methoxybenzofuran-2-yl)-3-p-tolylprop-2-en-1-one (2b)

Brown solid, Yield 81%; m.p, 381-383 ^{o}C; IR (KBr)λ_{max}(cm^{-1}) 3401-3430 (Ar-H), 1630-1710 (C=O); ^{1}H NMR (400 MHz) ($CDCl_3$) δ (ppm): 6.7-7.8 (m, 7H, Ar-H), 7.5 (s, 1H, α-CH of chalcone), 7.4 (s, 1H, CH of benzofuran), 6.5 (s, 1H, β-CH of chalcone), 3.82 (s, 3H, OCH_3), 2.29 (s, 3H, CH_3);[13]C NMR ($CDCl_3$, 100 MHz) δ ppm: 56.2, 149.9, 113.5, 125.4, 103.5, 155.2, 101.2, 116.6, 155.2, 177.8, 132.3, 145.1, 135.2, 128.1, 129.0, 21.3; MS (ESI) m/z: 292.33 (M^+); Anal. Calc. for, $C_{19}H_{16}O_3$; C, 78.06; H, 5.52; O, 16.42, Found: C, 78.04; H, 5.50; O, 16.41%

(E)-3-(4-chlorophenyl)-1-(4-methoxybenzofuran-2-yl)prop-2-en-1-one (2c)

Yellow solid, Yield 74%; m.p, 214-216 ^{o}C; IR (KBr)λ_{max}(cm^{-1}) 3405-3432 (Ar-H), 1660-1712 (C=O); ^{1}H NMR (400 MHz) ($CDCl_3$) δ (ppm):

7.0-7.7 (m, 7H, Ar-H), 7.6 (s, 1H, CH of benzofuran), 7.4 (s, 1H, α-CH of chalcone), 6.7 (s, 1H, β-CH of chalcone), 3.79 (s, 3H, OCH_3);[13]C NMR ($CDCl_3$, 100 MHz) δ ppm: 56.0, 149.5, 113.2, 126.0, 103.8, 155.0, 101.7, 116.4, 155.1, 177.4, 122.0, 145.6, 133.4, 128.6, 128.1, 130.1, 133.1; MS (ESI) m/z: 312.75 (M^+); Anal. Calc. for, $C_{18}H_{13}ClO_3$; C, 69.13; H, 4.19; O, 15.35, Found: C, 69.10; H, 4.15; Cl, 11.30; O, 15.31%

(E)-1-(4-methoxybenzofuran-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (2d)

Brown semi solid, Yield 80%; IR (KBr)λ_{max}(cm^{-1}) 3408-3433 (Ar-H), 1653-1713 (C=O), 1500 (NO_2); ^{1}H NMR (400 MHz) ($CDCl_3$) δ (ppm): 6.8-7.5 (m, 7H, Ar-H), 7.2 (s, 1H, CH of benzofuran), 7.6 (s, 1H, α-CH of chalcone), 6.4 (s, 1H, β-CH of chalcone), 3.82 (s, 3H, OCH_3);[13]C NMR ($CDCl_3$, 100 MHz) δ ppm: 46.7, 129.4, 122.9, 129.4, 117.2, 157.7, 117.2, 117.8, 166.6, 187.0, 123.3, 142.2, 141.3, 129.0, 123.8, 147.1; MS (ESI) m/z: 323.30 (M^+); Anal. Calc. for, $C_{18}H_{13}NO_5$; C, 66.87; H, 4.05; N, 4.33; O, 24.74, Found: C, 63.17; H, 5.28; O, 31.52%

(E)-3-(4-hydroxyphenyl)-1-(4-methoxybenzofuran-2-yl)prop-2-en-1-one (2e)

Light yellow solid, Yield 83%; m.p, 380-382 ^{o}C; IR (KBr)λ_{max}(cm^{-1}) 3410-3435 (Ar-H), 1630-1715 (C=O); ^{1}H NMR (400 MHz) ($CDCl_3$) δ (ppm): 6.9-7.6 (m, 7H, Ar-H), 7.5 (s, 1H, CH of benzofuran), 7.7 (s, 1H, α-CH of chalcone), 6.7 (s, 1H, β-CH of chalcone), 3.80 (s, 3H, OCH_3), 5.3 (s, 1H, OH);[13]C NMR ($CDCl_3$, 100 MHz) δ ppm: 56.3, 149.8, 113.4, 125.6, 103.8, 156.2, 101.2, 116.6, 155.2, 177.8, 121.3, 145.1, 127.2, 130.6, 115.8, 157.7; MS (ESI) m/z: 294.30(M^+); Anal. Calc. for $C_{18}H_{14}O_4$; C, 73.46; H, 4.79; O, 21.75, Found: C, 73.43; H, 4.78; O, 21.72%

(E)-3-(2-hydroxyphenyl)-1-(4-methoxybenzofuran-2-yl)prop-2-en-1-one (2f)

Brown solid, Yield 81%; m.p, 272-274 ^{o}C; IR (KBr)λ_{max}(cm^{-1}) 3411.2-3436 (Ar-H),1640-1718 (C=O); ^{1}H NMR (400 MHz) ($CDCl_3$) δ (ppm): 6.9-7.6 (m, 7H, Ar-H), 7.5 (s, 1H, CH of benzofuran), 7.3 (s, 1H, α-CH of chalcone), 6.8 (s, 1H, β-CH of chalcone), 3.78 (s, 3H, OCH_3), 5.3 (s, 1H, OH); [13]C NMR ($CDCl_3$, 100 MHz) δ ppm: 55.9, 150.2, 113.3, 125.4, 103.6, 156.5, 101.0, 116.8, 155.0, 177.6, 121.8, 145.5, 135.0, 117.4, 121.1, 130.0, 115.1, 158.4; MS (ESI) m/z: 294.09 (M^+); Anal. Calc. for $C_{18}H_{14}O_4$; C, 73.46; H, 4.79; O, 21.75, Found: C, 73.44; H, 4.75; O, 21.76%

(E)-1-(4-methoxybenzofuran-2-yl)-3-(2-methoxyphenyl)prop-2-en-1-one (2g)

Yellow semi solid, Yield 81%; IR (KBr)λ_{max}(cm^{-1}) 3412-3439 (Ar-H),1640-1718 (C=O); ^{1}H NMR (400 MHz) ($CDCl_3$) δ (ppm): 6.1-7.6 (m, 7H, Ar-H), 7.8 (s, 1H, CH of benzofuran), 7.5 (s, 1H, α-CH of chalcone), 6.5 (s, 1H, β-CH of chalcone), 3.82 (s, 6H, OCH_3); [13]C NMR ($CDCl_3$, 100 MHz) δ ppm: 55.9, 149.7, 113.8, 125.9, 103.2, 156.4, 101.6, 116.4, 155.2, 177.3, 121.5, 145.3, 127.5, 130.2, 114.2, 159.8, 55.8; MS (ESI) m/z: 308.10 (M^+); Anal. Calc. for $C_{19}H_{16}O_4$; C, 74.01; H, 5.23; O, 20.76, Found: C, 74.03; H, 5.21; O, 20.75%

(E)-3-(2-hydroxy-3-methoxyphenyl)-1-(4-methoxybenzofuran-2-yl)prop-2-en-1-one (2h): Light brown solid, Yield 73%; m.p, 385-387 ^{o}C; IR (KBr)λ_{max}(cm^{-1}) 3414-3440 (Ar-H),1640-1718 (C=O); ^{1}H NMR (400 MHz) ($CDCl_3$) δ (ppm): 6.6-7.4 (m, 6H, Ar-H), 7.65 (s, 1H, CH of benzofuran), 7.4(s, 1H, α-CH of chalcone), 6.6 (s, 1H, β-CH of chalcone), 3.81 (s, 6H, OCH_3), 5.35 (s, 1H, OH); [13]C NMR ($CDCl_3$, 100 MHz) δ ppm: 56.2, 150.0, 113.2, 125.6, 103.5, 156.3, 101.4, 116.5, 155.4, 177.7, 121.2, 141.0, 117.5, 151.5, 121.2, 122.2, 111.9, 148.8, 56.1; MS (ESI) m/z: 324.10 (M^+); Anal. Calc. for $C_{19}H_{16}O_5$; C, 70.36; H, 4.97; O, 24.67 Found: C, 70.35; H, 4.98; O, 24.65%

(E)-3-(3,4-dimethoxyphenyl)-1-(4-methoxybenzofuran-2-yl)prop-2-en-1-one (2i)

Yellow solid, yield 76%; m.p, 255-257 ^{o}C; IR (KBr)λ_{max}(cm^{-1}): 3416 - 3444 (Ar-H), 1657-1719 (C=O); ^{1}H NMR (400 MHz) ($CDCl_3$) δ (ppm): 6.7-7.5 (m, 7H, Ar-H), 7.35 (s, 1H, CH of benzofuran), 7.8 (s, 1H, α-CH of chalcone), 6.4 (s, 1H, β-CH of chalcone), 3.84 (s, 9H, OCH_3); [13]C NMR ($CDCl_3$, 100 MHz) δ ppm: 56.3, 149.4, 113.1, 125.2, 103.4, 156.6, 101.5, 116.5, 155.4, 177.5, 121.0, 145.0, 127.3, 122.5, 111.7, 111.5, 149.7, 149.0, 56.1; MS (ESI) m/z: 338.12 (M^+); Anal. Calc. for $C_{20}H_{18}O_5$; C, 70.99; H, 5.36; O, 23.64, Found: C, 70.97; H, 5.35; O, 23.63%

Antioxidant activity

The newly synthesized compounds were screened for their antioxidant potentials by using three well documented assays like 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, microsomal lipid peroxidation (LPO) assay and ferric reducing power assay. The compounds under studies were dissolved in distilled ethanol (50 mL) to prepare 1000 µM solution. Solutions of different concentrations (10, 25, 50, 100, 200 and 500 µM) were prepared by serial dilution and the antioxidant activity was studied.

DPPH radical scavenging activity

The DPPH radical scavenging effect was carried out according to the method first employed by Blois[22]. Compounds of different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions having different concentrations (10, 25, 50, 100, 200 and 500 µM) were taken in different test tubes, 4 mL of a 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer and the remaining DPPH was calculated. The percent decrease in the absorbance was recorded for each concentration and percent quenching of DPPH was calculated on the basis of the observed decreased in absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_o - A_1 / A_o) \times 100]$$

Where A_o is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound. The radical scavenging activity of BHA was also measured and compared with that of the newly synthesized compound.

Inhibition of microsomal LPO assay

Liver excised from adult male Wister rats, was homogenized (20 g/100 mL Tris buffer) in 0.02 mol/L, tris buffer (pH=7.4). Microsomes were isolated by the calcium aggregation method[23]. 100 µL of liver microsomal suspension (0.5 mg protein) was incubated with 1 mmol/L each of $FeSO_4$ and ascorbic acid with or without compounds in a total volume of 1 mL in 0.1 mol/L phosphate buffer (pH=7.4). After incubation at 37^{o}C for 60 min, the reaction mixture was boiled with TBA (0.67 g/ 100 mL water) for 15 min. Formation of TBA reactive substances (TBARS) was calculated from the absorbance at 535 nm[24].

Reducing power assay (Iron reducing activity)

The reducing power of synthesized compounds was determined according to the method of Oyaizu[25]. The compounds having different concentration were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferric cyanide and then incubated at 50 ^{o}C for 20 min. To this mixture 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 20 min. The upper layer (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride and the absorbance was taken at 700 nm. Increased absorbance of the reaction mixture indicates an increased reducing power.

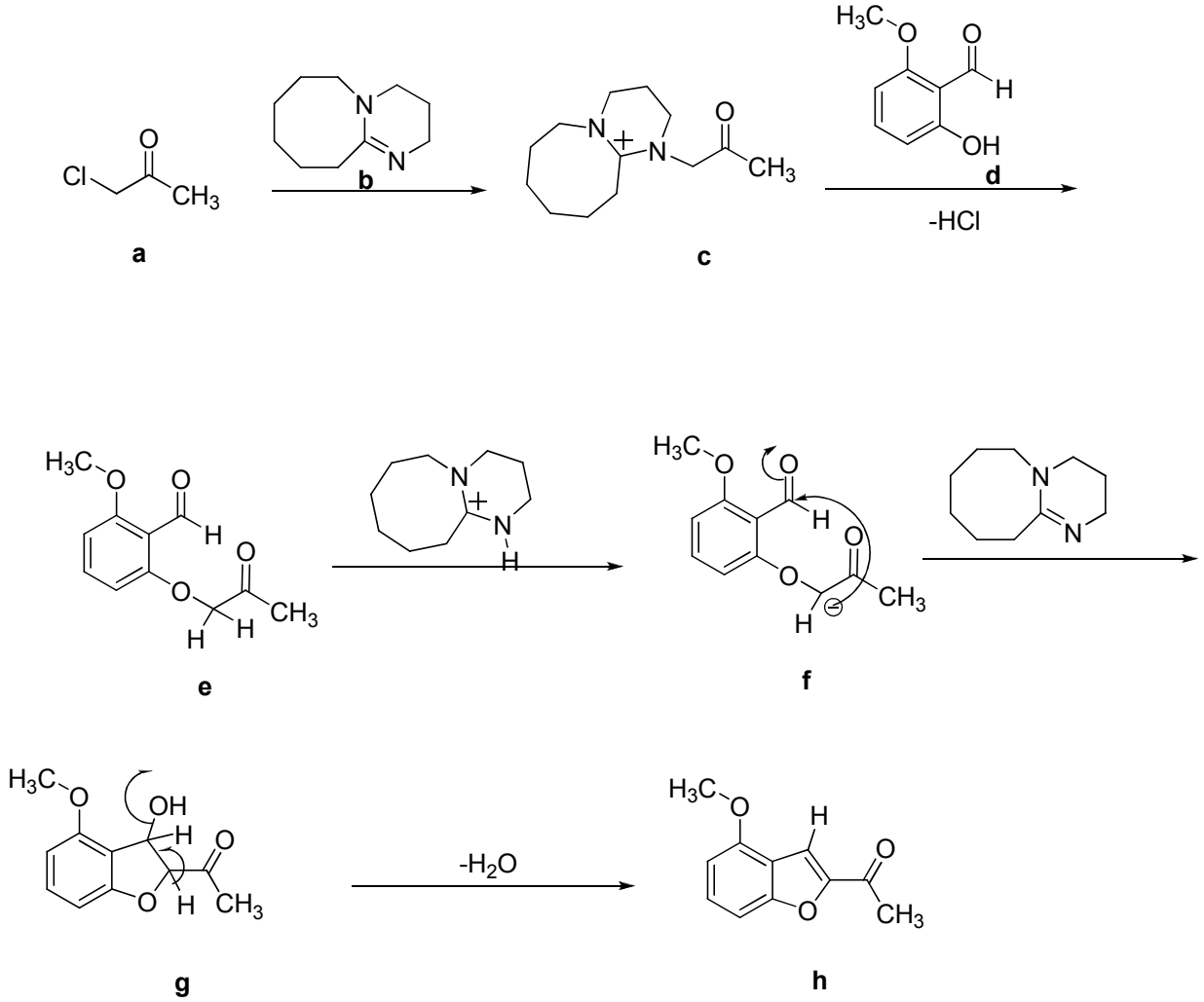
Statistical analysis

Tests were carried out in triplicate for 3-5 separate experiments. The amount of compound needed to inhibit DPPH free radicals and Lipid peroxyl radical concentration by 50% inhibition (IC_{50}) was estimated using a linear regression algorithm.

RESULTS AND DISCUSSION

A series of novel 4-methoxy-2-acetyl benzofuran based chalcones (**2a-i**) were synthesized in good yields **Scheme 1**, characterized by different spectral studies and their antioxidant activities were

determined. Initially, a novel method approaching towards synthesis of 4-methoxy-2-acetyl benzofuran was achieved. The mechanism involves, *o*-alkylation of *o*-vanillin **(d)** with chloro acetic acid **(a)** in presence of DBU **(b)** as organic base furnished *o*-alkylated salicyaldehyde **(e)** which subsequently generates enolate anion **(f)** undergo intramolecular cyclocondensation reaction afforded 4-methoxy-2-acetyl benzofuran **(h)** in excellent yield **(Scheme 2)**. IR spectrum revealed that the absence of phenolic-OH stretching at 3500 cm^{-1} and aldehyde C=O stretching at 1760 cm^{-1}. The appearance of carbonyl stretching at 1650 cm^{-1}confirmed the cyclization of *o*-vanillin to 4-methoxy-2-acetyl benzofuran (**2**). ^1H NMR spectra also revealed the absence of aldehyde proton at 11 ppm. Further, coupling of aryl aldehydes by aldol condensation reactions in the presence of NaOH affords 4-methoxy-2-acetyl benzofuran based chalcones (**2a-i**). The IR spectra of all the target compounds showed C=O stretching at 1650-1720 $cm^{-1}$and the presence of aromatic peaks (Ar-H) at the respective region (3400-3450 cm^{-1}). ^1H NMR spectra of all conjugated analogues **(2a-i)** showed OCH_3 protons as singlet at 3.6 ppm, aromatic proton as multiplet at 6.48-8.31 ppm, proton of furan ring observed as doublet at 7.54 ppm, allylic proton appears as doublet at 6.09-7.18 ppm, phenolic -OH 5.35. All the analogues showed mass according to their M^+ ions.



Scheme 2: Mechanism involves towards the synthesis of 4-methoxy-2-acetyl benzofuran in presence of DBU.

Antioxidant activity

The obtained novel compounds were subjected for the evaluation of antioxidant properties by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, microsomal lipid peroxidation (LPO) assay and ferric reducing power assay. In DPPH assay, compound (**2**) showed considerable activity. This may be due to the presence of electron donating -OCH_3 group on the benzofuran moiety. Further, coupling of aryl aldehydes gave the significant change in the activity. Compound **2h** bearing methoxy group (electron donating group) and as well as hydroxyl group on the phenyl ring showed dominant DPPH activity compare to BHA an internal standard. Whereas compounds **2e**, **2f** possessing hydroxy substituent on the phenyl ring and compounds **2g** and **2i** having electron donating group such methoxy at different position on the phenyl ring exhibited moderate to good radical scavenging activity. The other analogues of 4-methoxy-2-acetyl benzofuran showed less activity. 50% Inhibitory concentration (IC_{50}) for all the compounds were calculated and depicted in the Table 1.

The increasing order of DPPH activity of newly synthesized analogues are as follows **2h>BHA>2e>2f>2i>2g>2b>2d>2a>2c>2**

LPO has been broadly defined as the oxidative deterioration of polyunsaturated lipids[26]. Initiation of a peroxidation sequence in a membrane or polyunsaturated fatty acid is due to abstraction of a hydrogen atom from the double bond in the fatty acid. The free radical tends to stabilize by a molecular rearrangement to produce a conjugated diene, which then readily reacts with oxygen molecule to give a peroxy radical[27]. Peroxy radicals can abstract a hydrogen atom from another molecule to give lipid hydro peroxide R-OOH. A probable alternative fate of peroxy radicals is to form cyclic endoperoxides fragment to aldehydes such as malondialdehyde (MDA) and polymerization products. MDA and 4-hydroxynonenal are the major break down products of LPO. MDA is usually taken as a marker of LPO and oxidative stress[28]. IC_{50} values of LPO inhibition for the newly synthesized analogues were depicted in **Table 1**. From LPO studies initially, scaffold **2** showed considerable activity further coupling of aryl aldehyde which is accounted for the enhanced

activity. Among the synthesized compounds **2b, 2c** and **2d** having methyl and electron withdrawing substituents (NO_2, Cl) on the phenyl ring, inhibit the lipid peroxidation moderately and showed considerable activities. The presence of electron releasing substituent (-OCH_3) and hydroxy substituent on the phenyl ring at different position in compound **2e, 2f, 2g** and **2i** fairly inhibits the peroxyl radicals generated in the system showed good activities. Whereas, compound **2h** bearing both methoxy group and phenolic moiety demonstrate dominant LPO inhibition effect.

Reducing power of 4-methoxy-2-acetyl benzofuran based chalcones at different concentrations (10 µM, 25 µM, 50 µM, 100 µM, 200 µM and 500 µM) were determined (**fig. 1**). In this assay, depending on the reducing power of antioxidant compounds the test solutions changes into various shades of green and blue colours. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reduction of ferricyanide (Fe^{3+}) complex to the ferrous (Fe^{2+}) form can be monitored by measuring the formation of Perl's Prussian blue at 700 nm which occurs in the presence of reductants (antioxidant compounds).

Table 1: 50% Inhibition of DPPH· radical and microsomal LPO· inhibition by 4-methoxy-2-acetyl benzofuran based chalcones (2a-i). Each value represents mean ± SD (n=3)

Compound No	DPPH activity IC_{50}(µM/mL)[a]	LPO inhibition IC_{50}(µM/mL)[b]
2	126±0.10	187±0.26
2a	64±0.21	61±0.53
2b	25±0.52	30±0.33
2c	121±0.41	113±0.62
2d	61±0.24	56±0.41
2e	13±0.93	10±0.84
2f	15±0.11	19±0.14
2g	18±0.72	24±0.31
2h	9±0.14	5±0.47
2i	17±048	20±0.75
BHA	12±0.10	7±0.58

[a]*IC_{50} = the concentration (µM) exhibiting 50% inhibition of DPPH radical.*

[b]*IC_{50} = the concentration (µM) exhibiting 50% inhibition of LPO oxidation.*

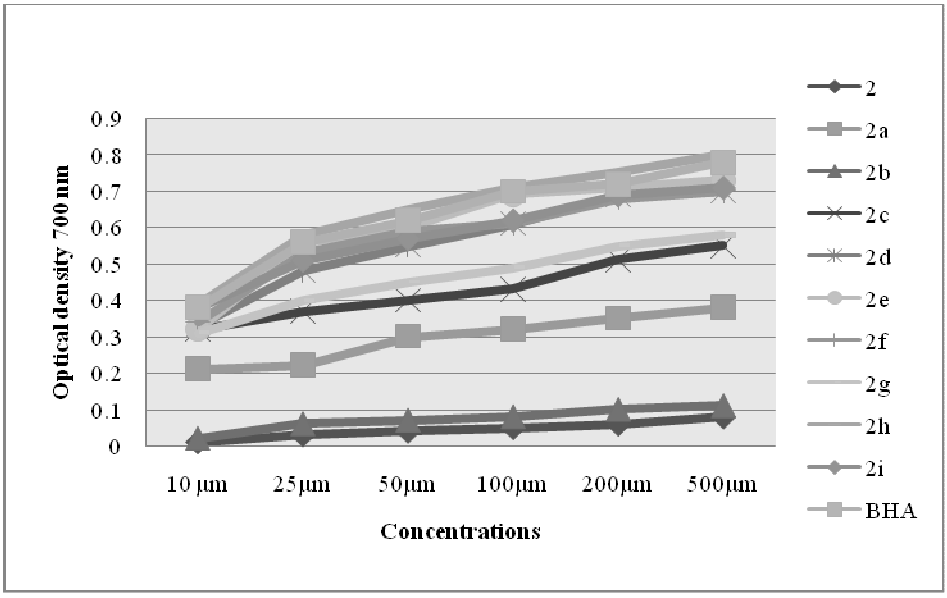


Fig. 1: Reducing power ability of 4-methoxy-2-acetyl benzofuran based chalcones (2a-i) at different concentrations. Higher absorbance indicates the higher reducing ability. Each value represents mean ± SD (n=3).

Compound **2e, 2f, 2g** and **2i** posses good reducing power ability, this could be due to presence of hydroxyl and methoxy substituent's on the phenyl ring at different position. Whereas, compound **2h** predominantly reduces Fe^{3+} to Fe^{2+} showed significant activity even higher than that of standard, this may be presence of electron releasing group like methoxy addition to phenolic moiety. All the synthesised compounds exhibited same order of antioxidant activity in all the antioxidant assays performed.

CONCLUSION

A novel methodology was described for synthesis of 4-methoxy-2-acetyl benzofuran **(2)**. Further, 4-methoxy-2-acetyl benzofuran based chalcones **(2a-i)** were synthesized by aldol condensation and investigated their antioxidant property. Initially, the core compound 4-methoxy-2-acetyl benzofuran (**2**) showed considerable activity. Auxiliary, coupling of different substituted aryl aldehydes to 4-methoxy-2-acetyl benzofuran enhanced the antioxidant activity. Among the analogues, 4-methoxy-2-acetyl benzofuran conjugated with 2-hydroxy-3-methoxy benzaldehyde **2h** revealed high antioxidant activity and also more than the standard (BHA). Our study may provides the sound evidence that the coupling of aryl aldehyde to 4-methoxy-2-acetyl benzofuran had significant influence for enhanced antioxidant activity. This investigation may be useful in the treatment of pathologies in which free radical oxidation plays a fundamental role.

ACKNOWLEDGEMENT

The authors are also thankful to NMR Research Center, Indian Institute of Science, Bangalore for providing spectral data.

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