WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 9, 1657-1673. Research Article ISSN 2277– 7105

AN EFFICIENT ONE-POT SYNTHESIS OF *N*-(3-(BENZOFURAN-3-YL)-3-OXO-1-PHENYLPROPYL) ACETAMIDE SCAFFOLDS VIA MULTICOMPONENT REACTION: AS A NOVEL CLUSTER OF FREE RADICAL, OXIDATION AND BACTERIAL INHIBITORS

Javarappa Rangaswamy[1], Honnaiah Vijay Kumar[2] and Nagaraja Naik*[1]

[1]Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore – 570006, Karnataka, India.

[2] Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, USA.

Article Received on
22 July 2016,

Revised on 11 August 2016,
Accepted on 31 August 2016

DOI: 10.20959/wjpps20169-7033

***Corresponding Author**
Nagaraja Naik
Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore –570006, Karnataka, India.

ABSTRACT

A series of *N*-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds **(4a-j)** were synthesized by multicomponent one pot reaction. The structures of newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass, elemental analysis and further screened for their *in vitro* antioxidant and antibacterial activities. All the synthesized scaffolds in this series showed comparably high, equal or less activity to the corresponding standard. Among them interestingly, compound **4g** was observed to be an effective antioxidant. However, compounds **4i** and **4e** exhibited moderate antioxidant potency, while compounds **4d** and **4f** exhibited maximum bacterial inhibition effect. Resulting in the finding that, these classes of derivatives have displayed the significant antioxidant and antibatcterial activity.

KEYWORDS: *N*-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds, substituted benzaldehyde, antioxidant activity, antibacterial activity.

INTRODUCTION

Multi-component reactions (MCRs) have emerged as an efficient and powerful tool in modern synthetic organic chemistry allowing the facile creation of several new bonds in a one-pot reaction.[1] The development of multi-component reactions for the synthesis of heterocyclic compounds has attracted significant interest in recent years.[2] Clearly, for multi-

step synthetic procedures a number of reactions and purification steps are among the most important criteria for the efficiency and practicability of the process and should be as low as possible. The strategy of MCRs especially for the preparation of heterocyclic compounds is a particularly attractive field in light of the paramount role of these targets in pharmaceutical chemistry.[3,4]

Heterocyclic synthesis has emerged as powerful technique for generating new molecules useful for drug discovery.[5] Heterocyclic compounds provide scaffolds on which pharmacophores can arrange to yield potent and selective drugs.[6] Benzofurans are highly valuable molecular motifs often found in various natural products. An intense effort has been made directed towards the synthesis of benzofuran derivatives[7] due to their biological activity as potential pharmacological agents[8-11] and to their occurrence in nature. Also, such compounds are widely distributed in nature, *e.g*., ailanthoidol and have been reported to have antiviral, antioxidant and antifungal activities.[12] Many compounds that synthesized from 2-acetylbenzofurans have shown antitumor, anti-flammatory and fungicidal activities.[13-15] Furthermore, compounds containing benzofuran moiety also have *in-vitro* antibacterial activities. Examples include bacterial enzymes involved in the methionine cycle (e.g. methionine aminopeptidase and deformylase), enzymes involved in peptidoglycan synthesis (e.g. UDP-*N*-acetylmuramyl-*L*-alanine ligase) and chorismate synthesis.[16] In addition, benzofurans are used in cosmetic formulations and have application as synthetic precursors for optical brighteners.[17]

Hence, we are interested to build a library of small molecules incorporating the 2-acetyl benzofuran moiety for assessing their antioxidant and antibacterial properties by employing *in vitro* methods. To the best of our knowledge, there was no report on the synthesis, antioxidant and antibacterial activity of *N*-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds. In continuation of our research work on the synthesis and biological activity of novel classes heterocyclic derivatives[18-21] herein, we wish to synthesis *N*-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds and evaluated their free biological potentials.

EXPERIMENTAL

MATERIALS AND METHODS

All reagents and solvents were purchased from Merck (Darmstadt, Germany) chemical AR grade and were used as provided. DPPH was purchased from Sigma-Aldrich chemical Co.

(St. Louis, MO, USA). TLC analysis was performed on alumina sheets precoated with silica gel 60F-254 and SiO_2, 200-400 mesh (Merck) was used for column chromatography. ^1H NMR (300 MHz) ^{13}C NMR (100 MHz) were obtained AC Bruker spectrometer in the appropriate (DMSO) solvent. Melting points were obtained on a reichert thermopan melting point apparatus, equipped with a microscope and are uncorrected. Mass spectra were obtained on an Electron Impact mass spectrometer at 70 ev ionizing beam and using a direct insertion probe. Micro analytical data were obtained by elemental-Vario EL-III.

General procedure for synthesis of N-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds (4a-j)

A solution of the substituted benzaldehyde **(2a-j)** (2 mmol), 2-acetyl benzofuran **(1)** (2 mmol), acetyl chloride (4 mmol), acetonitrile (4 ml) and Zinc oxide (ZnO) (1 mmol) taken in a 100ml round bottomed flask, the reaction mixture was stirred for 6 hrs at room temperature, the progress of reaction was monitored by TLC. After completion of the reaction, the mixture was poured into 50 ml of ice water. The solid residue was purified by column chromatography n-hexane:ethylacetate (80:20) used as eluent to furnish *N*-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds **(4a-j)**.

N-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide (4a): Yellow solid; IR (KBr)λmax(cm^{-1}): 2857 (Ar-H), 3240 (NH), 1751 (amide C=O); ^1H NMR (300 MHz) (DMSO-d_6) δ (ppm): 8.93 (s, 1H, furan CH), 8.05 (d, 1H, NH), 7.27-7.85 (m, 9H, Ar-H), 5.18 (q, 1H, β-acetamide proton), 3.10 (d, 2H, CH_2), 1.83 (s, 3H, CH_3-CO); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 194.3, 170.7, 160.0, 156.3, 143.4, 128.0, 126.8, 125.4, 124.5, 123.0, 120.7, 11.5, 71.6, 51.4, 23.5; Mass (m/z%): M^+ 307.12; Anal.calcd. for $C_{19}H_{17}NO_3$: C, 74.25; H, 5.58; N, 4.56; Found: C, 74.20; H, 5.62; N, 4.50%

N-(3-(benzofuran-3-yl)-3-oxo-1-p-tolylpropyl)acetamide (4b): Off white solid; IR (KBr)λmax(cm^{-1}): 2857 (Ar-H), 3240 (NH), 1751 (amide C=O); ^1H NMR (300 MHz) (DMSO-d_6) δ (ppm): 8.90 (s, 1H, furan CH), 8.08 (d, 1H, NH), 7.17-7.86 (m, 8H, Ar-H), 5.18 (q, 1H, β-acetamide proton), 3.10 (d, 2H, CH_2), 2.34 (s, 3H, Ar-CH_3), 1.83 (s, 3H, CH_3-CO); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 194.6, 170.7, 160.1, 156.3, 140.4, 136.2, 128.8, 125.8, 125.3, 124.7, 123.2, 120.9, 111.4, 71.7, 51.4, 23.6, 21.3; Mass (m/z%): M^+ 321.14; Anal.calcd. for $C_{20}H_{19}NO_3$: C, 74.75; H, 5.96; N, 4.36; Found: C, 74.73; H, 5.89; N, 4.39% .

N-(3-(benzofuran-3-yl)-1-(4-methoxyphenyl)-3-oxopropyl)acetamide (4c): Light brown solid; IR (KBr)λmax(cm^{-1}): 2855 (Ar-H), 3243 (NH), 1774 (amide C=O); ^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 8.92 (s, 1H, furan CH), 8.03 (d, 1H, NH), 6.94-7.92 (m, 8H, Ar-H), 5.20 (q, 1H, β-acetamide proton), 3.83 (s, 3H, OCH_3), 3.13 (d, 2H, CH_2), 1.84 (s, 3H, CH_3-CO); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 190.0, 170.5, 160.0, 158.6, 156.5, 135.8, 126.6, 125.8, 124.8, 123.3, 120.9, 114.1, 111.4, 71.5, 55.8, 51.5, 23.6; Mass (m/z%): M^+ 337.13; Anal.calcd. for $C_{20}H_{19}NO_4$: C, 71.20; H, 5.68; N, 4.15; Found: C, 71.18; H, 5.35; N, 4.22%

N-(3-(benzofuran-3-yl)-1-(4-nitrophenyl)-3-oxopropyl)acetamide (4d): Blackish red solid; IR (KBr)λmax(cm^{-1}): 2856 (Ar-H), 3238 (NH), 1768 (amide C=O); ^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 8.93 (s, 1H, furan CH), 7.32-8.21 (m, 8H, Ar-H), 8.05 (d, 1H, NH), 5.15 (q, 1H, β-acetamide proton), 3.08 (d, 2H, CH_2), 1.85 (s, 3H, CH_3-CO); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 190.5, 170.7, 160.0, 156.3, 149.6, 145.9, 125.8, 124.7, 123.7, 123.4, 123.1, 120.8, 111.2, 71.7, 51.5, 23.5; Mass (m/z%): M^+ 352.11; Anal.calcd. for $C_{19}H_{16}N_2O_5$: C, 64.77; H, 4.58; N, 7.95; Found: C, 64.79; H, 4.62; N, 8.05%

N-(3-(benzofuran-3-yl)-1-(2-hydroxyphenyl)-3-oxopropyl)acetamide (4e): Yellow solid; IR (KBr)λmax(cm^{-1}): 2862 (Ar-H), 3263 (NH), 1751 (amide C=O); ^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 8.95 (s, 1H, furan CH), 8.05 (d, 1H, NH), 6.90-7.85 (m, 8H, Ar-H), 5.33 (s, 1H, OH), 5.15 (q, 1H, β-acetamide proton), 3.10 (d, 2H, CH_2), 1.83 (s, 3H, CH_3-CO); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 194.2, 170.5, 160.1, 156.2, 154.0, 130.9, 128.1, 126.5, 125.8, 124.7, 123.2, 121.1, 120.5, 115.3, 111.4, 72.0, 45.2, 23.3; Mass (m/z%): M^+ 323.12; Anal.calcd. for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33; Found: C, 70.58; H, 5.30; N, 4.33%

N-(3-(benzofuran-3-yl)-1-(4-chlorophenyl)-3-oxopropyl)acetamide (4f): Light yellow solid; IR (KBr)λmax(cm^{-1}): 2854(Ar-H), 3235 (NH), 1762 (amide C=O); ^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 8.93 (s, 1H, furan CH), 8.10 (d, 1H, NH), 7.44-7.90 (m, 8H, Ar-H), 5.18 (q, 1H, β-acetamide proton), 3.10 (d, 2H, CH_2), 1.84 (s, 3H, CH_3-CO); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 194.4, 170.7, 160.3, 156.2, 141.6, 132.3, 128.6, 127.2, 125.8, 124.7, 123.1, 111.2, 71.5, 51.4, 23.6; Mass (m/z%): M^+ 341.08; Anal.calcd. for $C_{19}H_{16}ClNO_3$: C, 66.77; H, 4.72; Cl, 10.37; N, 4.10; Found: C, 66.75; H, 4.81; Cl, 10.32; N, 4.08%.

N-(3-(benzofuran-3-yl)-1-(4-hydroxy-3-methoxyphenyl)-3-oxopropyl)acetamide (4g): Dark brown solid; IR (KBr)λmax(cm^{-1}): 2860 (Ar-H), 3248 (NH), 1751 (amide C=O); ^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 8.88 (s, 1H, furan CH), 8.05 (d, 1H, NH), 6.68-7.87 (m, 7H, Ar-H), 5.35 (s, 1H, OH), 5.15 (q, 1H, β-acetamide proton), 3.82 (s, 3H, OCH_3), 3.12 (d, 2H, CH_2), 1.85 (s, 3H, CH_3-CO); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 194.5, 170.3, 160.3, 156.3, 147.3, 146.7, 137.1, 125.7, 124.6, 123.3, 123.1, 120.9, 119.3, 115.4, 111.4, 110.2, 71.5, 56.1, 51.5, 23.5; Mass (m/z%): M^+ 353.13; Anal.calcd. for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96; Found: C, 68.01; H, 5.38; N, 4.03%

N-(3-(benzofuran-3-yl)-1-(3,4-dimethoxyphenyl)-3-oxopropyl)acetamide (4h): Light yellow solid; IR (KBr)λmax(cm^{-1}): 2858 (Ar-H), 3252 (NH), 1748 (amide C=O); ^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 8.93 (s, 1H, furan CH), 8.03 (d, 1H, NH), 6.74-7.89 (m, 7H, Ar-H), 5.16 (q, 1H, β-acetamide proton), 3.83 (s, 6H, OCH_3), 3.08 (d, 2H, CH_2), 1.84 (s, 3H, CH_3-CO); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 194.3, 170.7, 160.1, 156.0, 149.6, 147.8, 136.8, 125.7, 124.6, 123.2, 121.9, 120.7, 118.9, 111.8, 109.8, 71.3, 56.1, 51.5, 23.2; Mass (m/z%): M^+ 367.14; Anal.calcd. for $C_{21}H_{21}NO_5$: C, 68.65; H, 5.76; N, 3.81; Found: C, 68.65; H, 5.76; N, 3.81%

N-(3-(benzofuran-3-yl)-1-(4-hydroxyphenyl)-3-oxopropyl)acetamide (4i): Yellow solid; IR (KBr)λmax(cm^{-1}): 2853 (Ar-H), 3245 (NH), 1755 (amide C=O); ^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 8.93 (s, 1H, furan CH), 8.03 (d, 1H, NH), 6.70-7.92 (m, 8H, Ar-H), 5.35 (s, 1H, OH), 5.15 (q, 1H, β-acetamide proton), 3.07 (d, 2H, CH_2), 1.84 (s, 3H, CH_3-CO); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 194.5, 170.7, 160.1, 156.5, 156.3, 136.1, 127.0, 125.6, 124.5, 123.3, 120.9, 115.5, 111.5, 71.7, 51.4, 23.6; Mass (m/z%): M^+ 323.12; Anal.calcd. for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33; Found: C, 70.61; H, 5.35; N, 4.30%

N-(3-(benzofuran-3-yl)-3-oxo-1-(3,4,5-trimethoxyphenyl)propyl)acetamide (4j): Light yellow solid; IR (KBr)λmax(cm^{-1}): 2855 (Ar-H), 3238 (NH), 1750 (amide C=O); ^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 8.90 (s, 1H, furan CH), 8.07 (d, 1H, NH), 6.52-7.87 (m, 6H, Ar-H), 5.15 (q, 1H, β-acetamide proton), 3.80 (s, 9H, OCH_3), 3.05 (d, 2H, CH_2), 1.88 (s, 3H, CH_3-CO); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 193.8, 170.7, 160.1, 156.3, 152.7, 137.5, 137.0, 125.4, 124.8, 123.2, 120.5, 111.5, 102.0, 70.5, 60.7, 56.0, 51.8, 23.4; Mass (m/z%): M^+ 397.15; Anal.calcd. for $C_{22}H_{23}NO_6$: C, 66.49; H, 5.83; N, 3.52; Found: C, 66.45; H, 5.79; N, 3.58%.

Antioxidant activity

The newly synthesized compounds were screened for their radical scavenging activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and inhibition of human low-density lipoproteins (LDL) oxidation .

DPPH radical scavenging assay

The DPPH radical scavenging effect was carried out according to the method first employed by Blois.[22] Compounds at different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions having different concentrations (10 µM, 50 µM, 100 µM, 200 µM 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:

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. IC_{50} values were calculated by liner regression algorithm.

Human LDL oxidation assay

Fresh blood was obtained from fasting adult human volunteers and plasma was immediately separated by centrifugation at 1500 rpm for 10 min at 4 ^oC. LDL (0.1 mg LDL protein/mL) was isolated from freshly separated plasma by preparative ultra centrifugation using a Beckman L8-55 ultra centrifuge. The LDL was prepared from the plasma according literature method.[23] The isolated LDL was extensively dialyzed against phosphate buffered saline (PBS) pH 7.4 and sterilized by filtration (0.2 µm Millipore membrane system, USA) and stored at 4 ^oC under nitrogen. 1 mL of various concentrations (10 and 25 µM) of compounds were taken in test tubes, 40 µL of copper sulphate (2 mM) was added and the volume was made up to 1.5 mL with phosphate buffer (50 mM, pH 7.4). A tube without compound and

with copper sulphate served as a negative control, and another tube without copper sulphate with compound served as a positive control. All of the tubes were incubated at 37 ^{o}C for 45 min. To the aliquots of 0.5mL drawn at 2, 4 and 6 hr intervals from each tube, 0.25 mL of thiobarbutaric acid (TBA, 1% in 50 mM NaOH) and 0.25 mL of trichloro acetic acid (TCA, 2.8%) were added. The tubes were incubated again at 95 ^{o}C for 45 min and cooled to room temperature and centrifuged at 2500 rpm for 15 min. A pink chromogen was extracted after the mixture was cooled to room temperature by further centrifugation at 2000 rpm for 10 min. Thiobarbituric acid reactive species in the pink chromogen were detected at 532 nm by a spectrophotometer against an appropriate blank. Data were expressed in terms of malondialdehyde (MDA) equivalent, estimated by comparison with standard graph drawn for 1,1,3,3-tetramethoxy-propane (Which was used as standard) which give the amount of oxidation and the results were expressed as protection per unit of protein concentration (0.1 mg LDL protein/mL). Using the amount of MDA, the percentage protection was calculated using the formula: % inhibition of LDL oxidation = (Oxidation in control – oxidation in experimental / oxidation in control) x 100.

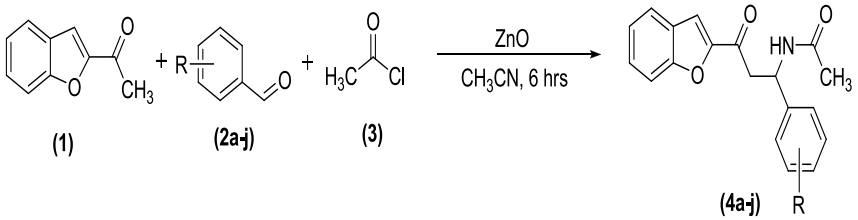
Antibacterial activity

The antibacterial activities of newly synthesized compounds were determined by well plate method in Mueller-Hinton Agar.[24] The antibacterial activity was carried out against 24 hr old cultures of bacterial strains. In this work, *E. coli*, *S. aureus*, *B. subtilis*, and *S. typhimurium* were used to investigate the activity. The test compounds were dissolved in dimethyl sulfoxide (DMSO) at concentration of 1000 mg/mL. 20 mL of sterilized agar media was poured into each pre-sterilized Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 ºC for an hour. About 60 mL of 24 hr old culture suspension were poured and neatly swabbed with the pre-sterilized cotton swabs. Six millimeter diameter well were then punched carefully using a sterile cork borer and 30 mL of test solutions of different concentrations were added into each labeled well. The plates were incubated for 24 hr at 37 ºC. The inhibition zone that appeared after 24 hr, around the well in each plate were measured as zone of inhibition in mm. Experiments were triplicates and standard deviation was calculated.

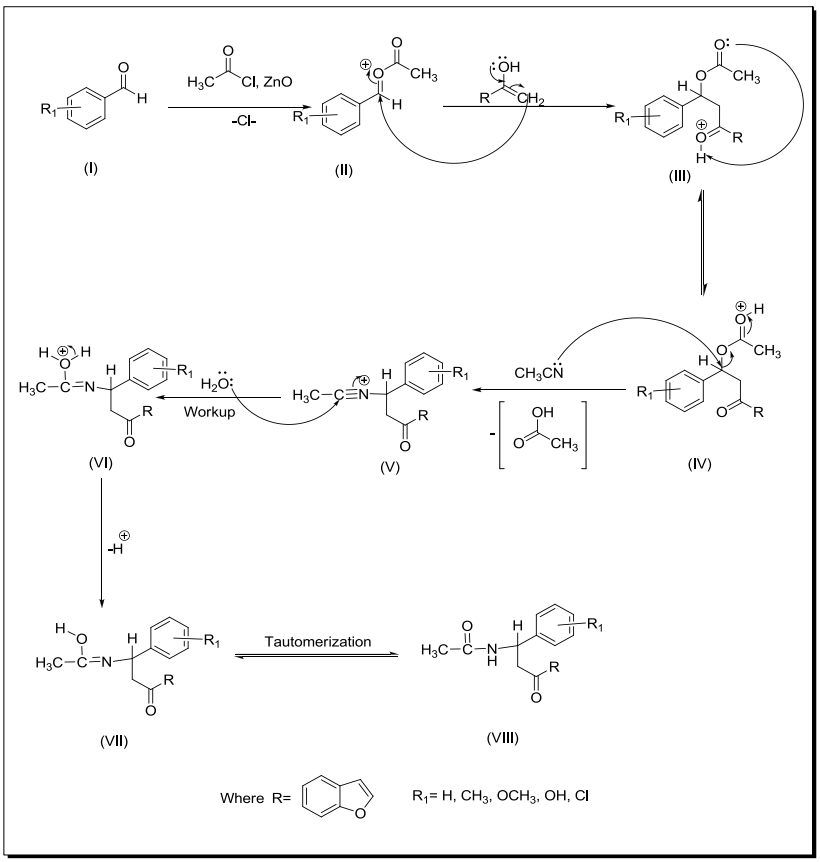
RESULTS AND DISCUSSION

Chemistry

The 2-acetyl benzofuran was synthesized by literature method.[25] An efficient one pot, multicomponent synthesis of *N*-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds **(4a-j)** was done by condensing of 2-acetyl benzofuran **(1)**, substituted benzaldehyde **(2a-j)**, acetyl chloride **(3)** and acetonitrile in the presense of Zinc oxide (ZnO) as catalyst **Scheme 1**. The reaction was carried on the basis of previously reported mechanism,[26] it is suggested that the aldehyde was first acylated to an intermediate **I** which then reacts with the enol form of acetophenone derivative to produce **III** after exchange of H^+ from **II.** Next, acetonitrile attacks **III,** with elimination of acetate to give **IV**. Generated HCl could be trapped by ZnO which is converted to $ZnCl_2$. Hydrolysis of **IV** accompanied by tautomerization gave the desired *N*-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds (**Scheme 2**) in good yields. The synthesized compounds was characterized by various physico-chemical and spectroscopic techniques.

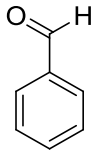
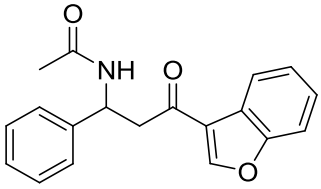
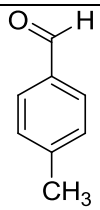
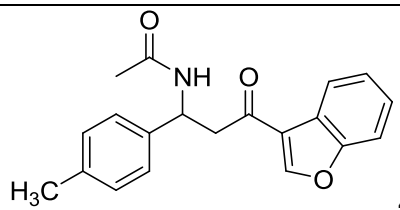
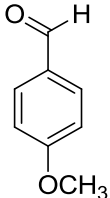
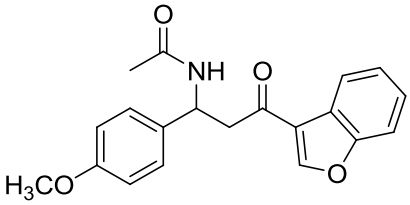


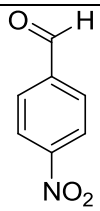
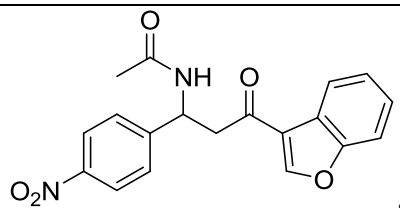
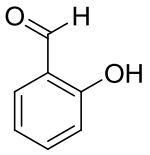
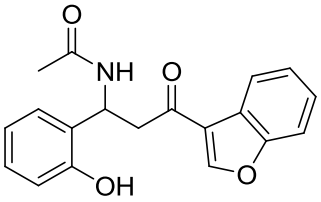
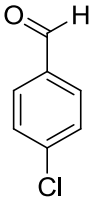
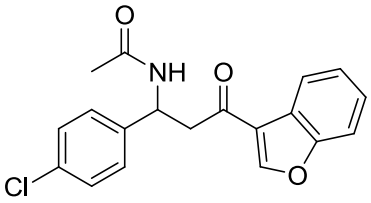
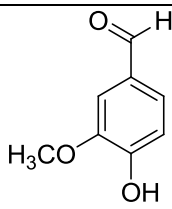
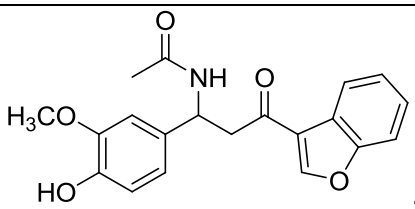
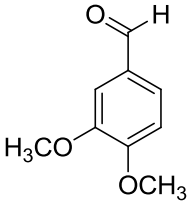
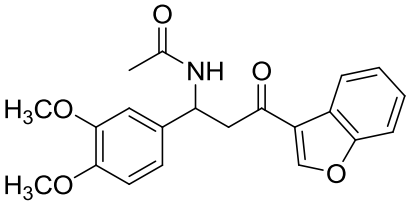
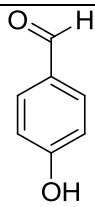
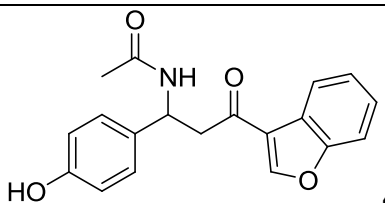
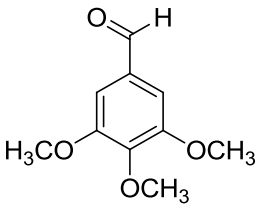
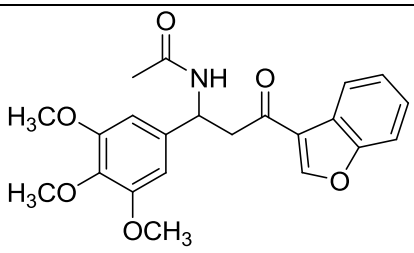
Scheme1.Protocol for the synthesis of *N*-(3-(benzofuran-2-yl)-3-oxo-1-phenylpropyl) acetamide scaffolds (4a-j)



Scheme 2. General synthetic mechanism for title compounds

Table 1. Synthesis of *N*-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds (4a-j)

Entry	Aldehydes	Products	Yield (%)	Melting points (°C)
1	 **2a**	 **4a**	78.10	120-123
2	 **2b**	 **4b**	66.20	183-186
3	 **2c**	 **4c**	58.00	170-173

4	 **2d**	 **4d**	88.25	105-108
5	 **2e**	 **4e**	69.00	188-191
6	 **2f**	 **4f**	78.30	160˜163
7	 **2g**	 **4g**	77.00	127˜130
8	 **2h**	 **4h**	89.75	165-168
9	 **2i**	 **4i**	87.30	175-178
10	 **2j**	 **4j**	85.40	168-171

Antioxidant activity

DPPH radical scavenging assay

DPPH radical scavenging activity (RSA) is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical inhibition effect of specific compounds. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption

maximum at 517 nm. This purple colour generally fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecule can quench DPPH free radical (i.e., by providing hydrogen atoms or by electron donation, conceivable) and convert them to a colourless/ bleached product (i.e., 2,2-diphenyl-1-picrylhydrazine, or a substituted analogues hydrazine), resulting in a decrease in absorbance. Hence, more rapidly the absorbance decrease, the more potent the antioxidant activity of the compound. Initially, 2-accetyl benzofuran **(1)** showed insignificant radical inhibition activity, further reaction with substituted benzaldehyde enhanced the antioxidant activity IC_{50} for all the compounds were calculated and depicted in **Table 2**. Majority of compounds (**4a-j**) in this series showed comparably equal or less activity to the standards. The compound **4g** bearing electron donating methoxy group addition to hydroxyl moiety at *para* position exhibited dominate DPPH radical inhibition effect than the standard. The electron withdrawing (Cl, NO_2) substituents containing compounds **4d** and **4f** was inadequate for the enhanced activity. Whereas, the compounds **4b**, **4c**, **4e**, **4i** and **4h** contains electron releasing methyl, hydroxy and methoxy groups at different positions of phenyl ring exhibited six to seven folds more RSA inhibition effect than that of starting material. On the other hand compound **4j** also having methoxy group on phenyl ring at 3,4,5 positions but showed less activity may due to the presence of steric hindrance between methoxy groups. Since, compound **4a** does not have any substituent on the ring showed least activity compared to other compounds. The presence of electron-donating groups on the phenyl ring at positions 3,4,5 might be favor the DPPH radical scavenging activity.

Table 2. 50% Inhibition of DPPH radical by compounds (4a-j). Each value represents mean ± SD (n=3)

Compounds	IC_{50}(µM)/mL
	DPPH[a]
1	230±0.11
4a	201±0.01
4b	138±0.24
4c	98±0.33
4d	157±0.45
4e	17±0.62
4f	176±0.71
4g	11±0.25
4h	57±0.43
4i	15±0.22
4j	190±0.78
BHA	12±0.05

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

Human LDL oxidation assay

Oxidation modification is known to play an important role in the pathogenesis of atherosclerosis and coronary heart diseases[27] and the dietary antioxidants that protect LDL from oxidation may therefore reduce atherogenesis and coronary heart diseases.[28] In general, oxidation of LDL follows a radical chain reaction that generates conjugated diene hydroperoxides as its initial products. It has been reported that inhibition of human LDL oxidation may arise due to free-radical scavenging.[29] The antioxidant activity of *N*-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds (**4a-j)** against human LDL oxidation with different concentrations (10 µM and 25 µM) is depicted in the figure 1 and 2.

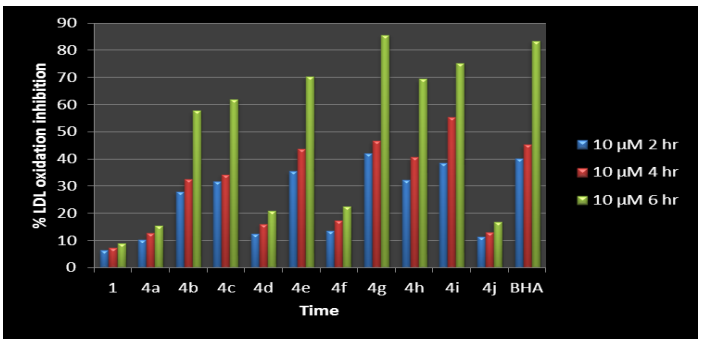


Figure 1. Percentage LDL oxidation inhibition by novel *N*-(3-(benzofuran-2-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds (4a-j) at 10 µM concentration.

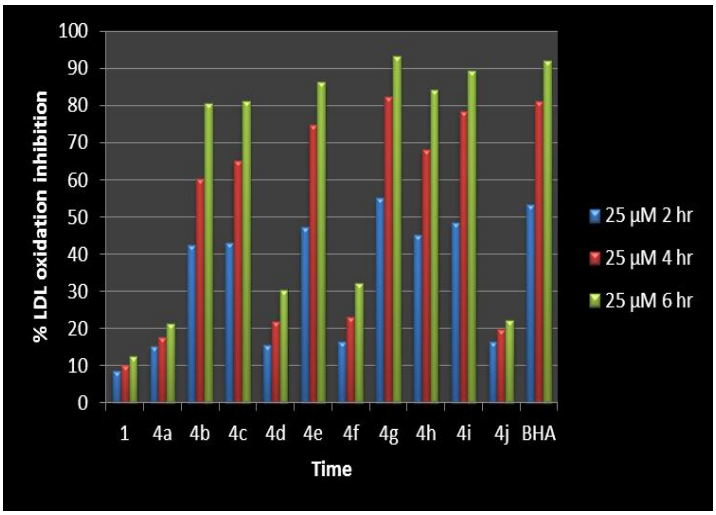


Figure 2. Percentage LDL oxidation inhibition by novel *N*-(3-(benzofuran-2-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds (4a-j) at 25 µM concentration.

The poly unsaturated fatty acid (PUFA) of human LDL were oxidized and the malonoldehyde (MDA) formed have been estimated by using thiobarbituric acid (TBA)

method. Initially, the formation of conjugated dienes due to copper-induced LDL oxidation was unaffected by compound (**1**), showed less activity but *N*-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds **(4a-j)** effectively inhibited LDL oxidation showed huge-good activity. The average induction time for copper mediated LDL oxidation was around 4 min without the addition of compounds. The compounds protect LDL from oxidation was measured by the prolongation of the induction time for the formation of conjugated dienes. Among the synthesized compounds, at the end of 2 hr after the induction of oxidation, compound **4g** exhibited 42.70 and 55.20% protection at 10 and 25 μM levels. Whereas, it was 85.65 and 93.25% protection at the end of 6 hr showing dominant inhibition over LDL oxidation and exhibits more activity than the standard BHA. Compound (**1**) exhibited 6.34 and 8.43% protection at the 10 and 25 μM levels at the end of 2 hr after the induction of oxidation. Whereas, it showed 8.87 and 12.43% protection at the end of 6 hr showed less activity. The results indicate a dose-dependent inhibition effect of compounds against LDL oxidation. The compounds **4b**, **4c**, **4e**, **4i** and **4h** contains electron releasing methyl, hydroxy and methoxy groups at different positions of phenyl ring after the completed 6 hr exhibited 57.60-75.32 and 80.45-89.35%. However, compounds **4d** and **4f** gathering with electron withdrawing nitro and chloro groups demonstrated considerable protection at the end of 6 hr after the induction of oxidation.

In short, we made an attempt to enhance the free radical inhibition effect by substituents attaching to the phenyl ring led to various potential radical inhibitors, depending on the nature, position and number of functional attached, whereas, high potency has been observed in the scaffolds **4g** in the both assays this may be due to the presence of electron donating methoxy group on phenyl ring. This has thrown open a new era for exploring suitably designed, new scaffolds as potential radical inhibitors.

Antibacterial activity

The synthesized compounds **(4a-j)** were also evaluated for their *in vitro* antimicrobial activity against various bacterial strains using well plate method in Mueller-Hinton Agar. As shown in our results, some analogues of this series were found to have near equal potency to the standard drug ciprofloxacin while some of them have least potency. Among the synthesized compounds **4f** exhibited maximum bacterial inhibition power against all the tested microorganisms at a concentration of 1000 mg/mL similar to that of the standard ciprofloxacin. Whereas, compounds **4c** and **4h** displayed good antibacterial activity. The activity is considerably effected by substituents present at the *para* position of phenyl ring.

The high potency of **4c**, **4f** and **4h** may be attributed to the presence of lipophilic or H-bond acceptor type group's placement such as Cl and OCH_3 at 4-positions, respectively.[30] This might be the reason compounds **4c, 4f** and **4h** are highly active than other compounds. Rest of the compounds **(4a-b)**, **(4d-e)**, **4g** and **(4i-j)** bearing substituent such as OH, NO_2, CH_3, and also having OCH_3 groups at different position showed considerable or least activity with respect to standard drug against the all test strains.

It is clear from our results (**Table 3**) that the SAR of *N*-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds **(4a-j)** for bacterial inhibition effect strongly correlates with the position *C*-2 and *C*-4 terminal of phenyl ring which favorable site for high inhibition effect.

Table 3. Antibacterial activity of the compounds (4a-j). Inhibitory zone (diameter) mm of the synthesized compounds against tested bacterial strains by well plate method.

Compound	Gram positive		Gram negative	
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Salmonella typhimurium*
Control	00	00	00	00
1	01±0.12	NA	NA	02±0.21
4a	02±0.33	01±0.17	02±0.17	01±0.34
4b	03±0.24	02±0.12	01±0.14	02±0.25
4c	16±0.30	13±0.11	17±0.19	16±0.26
4d	05±0.32	03±0.18	07±0.16	03±0.32
4e	NA	01±0.13	01±0.13	02±0.21
4f	19±0.21	15±0.16	20±0.11	17±0.10
4g	01±0.17	NA	NA	02±0.24
4h	18±0.23	15±0.20	19±0.27	15±0.37
4i	01±0.34	NA	02±0.11	NA
4j	03±0.10	04±0.21	03±0.23	05±0.14
Ciprofloxacin	22±0.32	16±0.24	22±0.24	18±0.10

The concentration of test compounds was 1000g/mL. Solvent which used DMSO. NA = no active. The data represent mean value (SEM).

CONCLUSION

In conclusion, we described an ZnO assisted one pot, multicomponent synthesis of *N*-(3-(benzofuran-3-yl)-3-oxo-1-phenylpropyl)acetamide scaffolds **(4a-j)** in reasonably good yields. The newly synthesized analogues were evaluated for their *in vitro* antioxidant, antibacterial and anti-inflammatory potential. Among the analogues, **4g** demonstrated powerful antioxidant. While, the compounds **4i** and **4e** exhibited good inhibition effect in

comparison with their standard BHA. Whereas, compound **4d** and **4f** revealed the highest bacterial inhibition power against all tested bacterial strains. This study extends the knowledge of different substituents at phenyl ring which might be of interest for the identification of novel class biological agents. Further, detailed studies are required to understand the mechanism of action of these compounds.

ACKNOWLEDGEMENTS

The authors are grateful to the University Grants Commission, Government of India, for granting UGC-Post Doctoral Fellowship-PDFSS and UGC SAP Phase III.

REFERENCES

1. Nakamura I, Yamamoto Y. Transition-metal-catalyzed reactions in heterocyclic synthesis. Chem Rev, 2004; 104: 2127–2198.
2. Abbas R, Neda P. Synthesis of various heterocyclic compounds via multi‐component reactions in water J Iran Chem Soc (2015)12: 993–1036.
3. Negwar M. In organic-chemical drugs and their synonyms, 7 ed. Akademie, Berlin. 1994.
4. Safaei-Ghomi J, Ghasemzadeh MA. An efficient multicomponent synthesis of 14-aryl-14H-dibenzo [a,j]xanthene derivatives by AgI nanoparticles. J Saudi Chem Soc, 2012, http://dx.doi.org/ 10.1016/j.jscs.2012; 05: 007.
5. Hermakens PH, Ottenheijm HC. Solid-phase organic reactions II: A review of the literature Nov 95–Nov 96. Tetrahedron, 1997; 53: 5643-5678.
6. Gordon E, Barrett RW, Dower WJ, Foder SP. Applications of combinatorialtechnologies to drug discovery. 2.Combinatorial organic synthesis, library screening strategies, and future directions. J Med Chem, 1994; 37:1485.
7. Cagniant P, Cagniant D. Recent Advances in the Chemistry of Benzo[b]furan and Its Derivatives. Part I: Occurrence and Synthesis. Adv Heterocycl Chem, 1975; 18: 337–482.
8. Huang HC, Chamberlain TS, Seibert K, Koboldt CM, Isakson PC, Reitz DB. Diaryl indenes and benzofurans: Novel classes of potent and selective cyclooxygenase-2 inhibitors. Bioorg Med Chem Lett, 1995; 5: 2377–2380.
9. Malamas MS, Sredy J, Moxham C, Katz A, Xu W, McDevitt R, Adebayo FO, Sawicki DR, Seestaller L, Sullivan D, Taylor JR. Novel Benzofuran and Benzothiophene Biphenyls as inhibitors of Protein Tyrosine Phosphatase 1B with Antihyperglycemics properties. J Med Chem, 2000; 43: 1293–1310.

10. Wyatt PG, Allen MJ, Chilcott J, Gardner CJ, Livermore DG, Mordaunt JE, Nerozzi F, Patel M, Perren MJ, Weingarten GG, Shabbir S, Woollard PM, Zhou P. Identification of potent and selective oxytocin antagonists. Part 2: further investigation of benzofuran derivatives. Bioorg Med Chem Lett, 2002; 12: 1405–1411.
11. Smith RA, Chen J, Mader MM, Muegge I, Moehler U, Katti S, Marrero D, StirtanWG, Weaver DR, Xiao H, Carley W. Solid-Phase synthesis and investigation of benzofurans as selective estrogen receptor modulators. Bioorg Med Chem Lett, 2002; 12: 2875–2878.
12. Fuganti C, Serra S. A new approach to 2-aryl-7-alkoxy-benzofurans: synthesis of ailanthoidol, a natural neolignan. Tetrahedron Lett, 1998; 39: 5609-5610.
13. Basawaraj R, Yadav B, Sangapure SS. Synthesis of some 1*H*-pyrazolines bearing benzofuran as biologically active agents. Ind J Heterocycl Chem, 2001; 11: 31-34.
14. Singh DV, Mishra AR, Mishra RM, Pandey AK, Singh, CR, Dwivedi AK. Synthesis and fungicidal activity of benzofuran incorporated substituted pyrimidines. Ind J Heterocycl Chem, 2005; 14: 319-322.
15. Basavaraja KM, Vaidya VP, Chandrashekhar C. Synthesis of benzofuro[3,2-*e*]-1,4-diazepines of pharmacological interest. E-J Chem, 2008; 5: 567-571.
16. Abdel-Wahab BF, Abdel-Aziz HA, Ahmed EM. Synthesis and antimicrobial evaluation of some 1,3-thiazole, 1,3,4-thiadiazole, 1,2,4-triazole, and 1,2,4-triazolo[3,4-*b*][1,3,4]-thiadiazine derivatives including a 5-(benzofuran-2-yl)-1-phenylpyrazole moiety. Monatsh Chem, 2009; 140: 601-605.
17. Safaei-Ghomi J, Ghasemzadeh MA, Ahmad Kakavand Q. CuI-nanoparticles-catalyzed one-pot synthesis of benzo[b]furans via three-component coupling of aldehydes, amines and alkyne. J Saudi Chem Soc, 2012; http://dx.doi.org/10.1016/j.jscs.2012; 07: 010.
18. Rangaswamy J, Kumar HV, Harini ST, Naik N. An easy eccess to benzofurans via DBU Induced condensation reaction of active 2-hydroxy acetophenones with phenacyl chlorides: A novel class of antioxidant agents. J Heterocycl Chem, 2015; 52: 938-943.
19. Rangaswamy J, Kumar HV, Harini ST, Naik N. Synthesis of novel benzofuran-gathered C-2,4,6-substituted pyrimidine derivatives conjugated by sulfonyl chlorides: orally bioavailable, selective, effective antioxidants and antimicrobials drug candidates. J Heterocycl Chem, 2015; 52: 1349-1360.
20. Rangaswamy J, Kumar HV, Harini ST, Naik N. Synthesis of benzofuran based 1,3,5-substituted pyrazole derivatives: As a new class of potent antioxidants and antimicrobials-A novel accost to amend biocompatibility. Bioorg Med Chem Lett, 2012; 22: 4773-4777.

21. Ashok B, Kumar Naik KH, Avinash P, Naik N. Efficient synthesis of novel diorganyl selenides via cleavage of Se–Se bond of diselenides by $RuCl_3$/zn and screened for their biological efficacy. World J Pharm Res, 2016; 5: 608-618.
22. Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. Nature, 1958; 26: 1199.
23. Princen HMG, Poppel GV, Vogelezang C, Butytenhek R, Kok F. Supplementation with Vitamin E but not beta-carotene *in vivo* protects low density lipoprotein from lipid peroxidation *in vitro*: effect of cigarette smoking. Arteriosc Thromb, 1992; 12: 554-562.
24. Lavanya, A.; Sribalan, R.; Padmini, V. Synthesis and biological evaluation of new benzofuran carboxamide derivatives. J Saudi Chem Soc, doi:10.1016/j.jscs.2015; 06: 008.
25. Rangaswamy J, Kumar HV, Harini ST, Naik N. Functionalized 3-(benzofuran-2-yl)-5-(4 methoxyphenyl)-4,5-dihydro-1H-pyrazole scaffolds: A new class of antimicrobials and antioxidants Ara JChem, 2013; doi:10.1016/j.arabjc.2013;10: 012.
26. Malek TM, Asadollah H, Hamid RS, Sayyed MHK, Elaheh M. Zinc oxide as an economical and efficient catalyst for the one-pot preparation of b-acetamido ketones via a four-component condensation reaction. Tetrahedron Lett, 2007; 48: 1729–1734.
27. Teinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med, 1989; 320: 915-924.
28. Kinsella JE, Frankel E, German B, Kanner J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. Food Technol, 1993; 47: 85-89.
29. Decker EA, Ivanov V, Zhu BZ, Frei B. Inhibition of low-density lipoprotein oxidation by carnosine histidine. J Agric Food Chem., 2001; 49: 511-516.
30. Rajesh HT, Atish HR, Girish DH, Ashish PK. The novel 3,4-dihydropyrimidin-2(1H)-one urea derivatives of N-aryl urea: synthesis, anti-inflammatory, antibacterial and antifungal activity evaluation. Bioorg Med Chem Lett, 2011; 21: 4648-4651.