Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2013, 5(4):87-98
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis and biological evaluation of some new oxadiazole and pyrazole derivatives incorporating benzofuran moiety

Hanumanagoud H. and K. M. Basavaraja*

Department of Industrial Chemistry, Vijayanagara Shri Krishnadevaraya University, Vinayak Nagara, Bellary (Cant), Karnataka, India

---

ABSTRACT

*The reaction of 7-methoxy-benzofuran-2-carboxylic acid hydrazide **(2)** with acetic anhydride gave the acetyl derivative **3**, which underwent cyclisation by treatment with $POCl_3$ yielded **4,** with ethylchloroformate in methanol yielded **5** and with ethylchloroformate in dry benzene followed by $POCl_3$ cyclisation resulted into **7** via the compound **6.** On the other hand, the reaction of **2** with acetyl acetone, benzoyl acetone, and ethylacetoacetate resulted into pyrazoles **8, 9** and **10** respectively. All the new heterocycles have been characterized by elemental analysis, IR, ^{1}H-NMR and GCMS spectral data. Further, these have been screened for antimicrobial, antioxidant, analgesic, anti-inflammatory and antipyretic activities. Compounds **5** and **7** exhibited encouraging results and remaining exhibited moderate activity.*

Keywords: Benzofuran, Oxadiazole, Pyrazole, Antimicrobial, Antioxidant, Antiinflammatory, Analgesic, Antipyretic.

INTRODUCTION

Benzofuran derivatives have attracted much interest due to their wide range of biological and pharmacological properties [1,2], such as anti-inflammatory [3], antihistaminic [4], antitumor [5], antifungal [6], antihyper-glycemic [7] and anthelmintic [8] activities. In addition, the benzofuran derivative Amiodarone is one of the most important benzofuran based synthetic pharmaceutics, it is a highly effective antiarrhythmic agent and used in the treatment of both ventricular and supraventricular arrhythmias [9].

It has been reported in the literature that compounds bearing oxadiazole ring possess significant biological properties such as antibacterial [10,11], antifungal, anti-inflammatory [12], analgesic [13], antitubercular [14], anticancer [15], antiviral [16] activities. Some of the oxadiazole derivatives act as hypnotic, sedatives [17], antimitotic [18] and show muscle relaxant activities [19]. Some material applications of oxadiazole derivatives in the fields of liquid crystals [20] and photo sensitizers have also been reported [21].

The compounds including pyrazole nucleus are known to possess antibacterial, anti-inflammatory, analgesic, antipyretic, anticonvulsant, antidiabetic, antiarrhythmic, muscle relaxant, psycho analeptic, hypertensive and monoamine oxidase inhibitor activities [22–29]. Pyrazole derivative, Celebrex is a potential anti-inflammatory drug [30]. Recently, some of 3-substituted-5-(benzofuran-2-yl)-pyrazole derivatives showed significant antimicrobial activities towards various microorganisms [31]. In the interest of the above facts, and in continuation of our previous

work in the synthesis of biologically active heterocycles [32,33], we are reporting the synthesis, antimicrobial, antioxidant, analgesic, anti-inflammatory and antipyretic activities of some new oxadiazole and pyrazole derivatives incorporating benzofuran moiety.

MATERIALS AND METHODS

Meting points were determined in open capillary tubes and are uncorrected. IR spectra (KBr disk) were recorded using a Perkin–Elmer 237 spectrophotometer, ^{1}H NMR spectra were recorded on a Bruker Avance Spectrometer (at 400 MHz) using TMS as an internal standard. $CDCl_3$ and DMSO-d_6 as solvent, chemical shifts (δ) are given in parts per million (ppm). The mass spectra (MS) were recorded on a Jeol GCmate GC-MS. Elemental analysis (C, H, N) was performed on Perkin Elmer 240 analyzer. The purity of the compounds were checked on silica gel G coated plates by using ethyl acetate and petroleum ether (1:1) as the eluent and observed in UV light. All the chemicals used were of analytical grade.

Preparation of 7-methoxy-benzofuran-2-carboxylic acid ethyl ester (1)
A solution of o--vanillin (0.01 mol) and diethyl bromomalonate (0.013 mol) in ethyl methyl ketone (40 mL) was treated with anhydrous potassium carbonate (10 g). The reaction mixture was heated under reflux for 10 h on steam bath. Solvent was distilled off under reduced pressure and the residual salts were dissolved in about 200 mL of water and cooled in an ice bath and carefully acidified with dilute hydrochloric acid. The product (1) was extracted with ether and ethereal extract was washed with saturated sodium bicarbonate solution and dried over anhydrous calcium chloride. Removal of the solvent gave the colorless solid. IR (KBr): 1714 cm^{-1} due to carbonyl group of ester. ^{1}H NMR ($CDCl_3$) ppm: 1.39 (t, 3H, CH_3), 4.01 (s, 3H, OCH_3), 4.41 (quartet, 2H, OCH_2), 6.90-7.52 (m, 4H, Ar-H). GCMS *m/z:* 220.

Preparation of 7-methoxy-benzofuran-2-carboxylic acid hydrazide (2).
To a solution of 7-methoxy-benzofuran-2-carboxylic acid ethyl ester **1** (0.01 mol) in ethanol (30 mL), hydrazine hydrate (25 mL) was added and the mixture was heated under reflux for 4 h on a water bath. Excess of ethanol was removed under reduced pressure and then diluted with water. The separated carbohydrazide **2** was collected and crystallized from ethanol as colourless needles. IR (KBr): 3400 and 3275 cm^{-1} due to NH.NH_2 group, 1670 cm^{-1} due to carbonyl group. ^{1}H NMR (DMSO-d_6) ppm: 3.95 (s, 3H, OCH_3), 4.58 (s, 2H, NH_2, D_2O exchangeable), 7.01-7.29 (m, 3H, Ar-H), 7.49 (s, 1H,C3-H), 10.01 (s, 1H, NH, D_2O exchangeable). GCMS *m/z:* 206.

Preparation of 7-methoxy-benzofuran-2-carboxylic acid N'-acetyl-hydrazide (3)
The 7-methoxy-benzofuran-2-carboxylic acid hydrazide **2** (0.003 mol) was warmed in acetic anhydride (15 mL) for 30 min. The reaction mixture was cooled and poured into ice water with stirring. The product **3** which was separated as colorless solid recrystallised from ethanol.

IR(KBr, cm^{-1}): 1696, 1605 (CO), 3393, 3216 (NH). ^{1}H NMR ($CDCl_3$, ppm): 2.17 (*s*, 3H, CH_3), 4.01 (*s*, 3H, OCH_3), 6.93-7.28 (*m*, 3H, Ar-H), 7.55(*s*, 1H, C3-H), 8.65 (s, br, 1H, NH, D_2O exchangeable), 9.26 (s, br, 1H, NH, D_2O exchangeable). GCMS (*m/z*): 248

Preparation of 2-(7-methoxy-benzofuran-2-yl)-5-methyl-[1,3,4]oxadiazole (4)
A mixture of 7-methoxy-benzofuran-2-carboxylic acid N'-acetyl-hydrazide **3** (0.002 mol) and $POCl_3$(2 mL) was refluxed gently for 2 h. The reaction mixture was cooled and poured into ice water. The aqueous solution was treated with sodium carbonate solution (10%) until alkaline. The resulting solid **4** was collected washed with water and crystallized from aqueous ethanol as microscopic needles.

IR(KBr, cm^{-1}): 1636 (C=N), 1274 (C-O-C). ^{1}H NMR (DMSO-d_6, ppm): 2.62 (*s*, 3H, CH_3), 3.98 (*s*, 3H, OCH_3), 7.10-7.36 (*m*, 3H, Ar-H), 7.76 (*s*, 1H, C3-H). GCMS (*m/z*): 230.

Preparation of 2-chloro-5-(7-methoxy-benzofuran-2-yl)-[1,3,4]oxadiazole (5)
A mixture of 7-methoxy-benzofuran-2-carboxylic acid hydrazide **2** (0.001 mol) and ethylchloroformate (0.001 mol) in methanol (20 mL) was refluxed on a steam bath for 5 h and the solvent was removed under reduced pressure. The solid **5** obtained was recrystallised from dioxane.

IR(KBr, cm^{-1}): 1640 (C=N), 1276 (C-O-C). ^1H NMR ($CDCl_3$, ppm): 3.96 (*s*, 3H, OCH_3), 6.91-7.26 (*m*, 3H, Ar-H), 7.52 (*s*, 1H, C3-H). GCMS (*m/z*): 250.

Preparation of N-(7-methoxy-benzofuran-2-carbonyl)-hydrazinecarboxylic acid ethyl ester (6)
A mixture of 7-methoxy-benzofuran-2-carboxylic acid hydrazide **2** (0.0015 mol) ethylchloroformate (0.0015 mol) and anhydrous potassium carbonate (5g) were refluxed in dry benzene (15 mL) for 15 h. The reaction mixture was filtered from the potassium salts and the filtrate was concentrated under reduced pressure. The residual resinous mass solidified after standing over night. Analytically pure product was obtained from ethanol as colorless needles.

IR(KBr, cm^{-1}): 1731, 1673 (C=O), 3239 (NH). ^1H NMR ($CDCl_3$, ppm): 1.28 (*t*, 3H, CH_3), 4.01 (*s*, 3H, OCH_3), 4.21 (*quartet*, 2H, OCH_2), 6.72 (*s*, br, 1H, NH, D_2O exchangeable), 6.91-7.27 (*m*, 3H, Ar-H), 7.55 (*s*, 1H, C3-H), 8.40 (*s*, br, 1H, NH, D_2O exchangeable). GCMS (*m/z*): 279.

Preparation of 5-(7-methoxy-benzofuran-2-yl)-[1,3,4]oxadiazol-2-ol (7)
A mixture of N'-(7-Methoxy-benzofuran-2-carbonyl)-hydrazinecarboxylic acid ethyl ester **6** (0.001 mol) and $POCl_3$ (3 mL) was refluxed gently for 2 h. The reaction mixture was cooled and poured into ice water. The aqueous solution was treated with sodium carbonate solution (10%) until alkaline. The resulting solid **7** was collected washed with water and crystallized from aqueous ethanol as microscopic needles.

IR(KBr, cm^{-1}): 1639 (C=N), 1271 (C-O-C), 3412 (OH). ^1H NMR ($CDCl_3$, ppm): 3.96 (*s*, 3H, OCH_3), 6.90-7.26 (*m*, 3H, Ar-H), 7.52 (*s*, 1H, C3-H), 9.00 (*s*,1H, OH, D_2O exchangeable). GCMS (*m/z*): 232.

Preparation of (3,5-dimethyl-pyrazol-1-yl)-(7-methoxy-benzofuran-2-yl)-methanone (8)
A mixture of 7-methoxy-benzofuran-2-carboxylic acid hydrazide **2** (0.001 mol) and acetyl acetone (0.001 mol) was refluxed in methanol (20 mL) containing 4-5 drops of acetic acid for 4 h. The reaction mixture was cooled to room temperature and the separated product **8** was collected by filtration and washed with methanol. It was crystallized from ethanol as colourless needles.

IR(KBr, cm^{-1}): 1665 (C=O), 1580 (C=N), 1525 (C=C). ^1H NMR ($CDCl_3$, ppm): 2.32 (*s*, 3H, CH_3), 2.64 (*s*, 3H, CH_3), 4.01 (*s*, 3H, OCH_3), 6.05 (*s*, 1H, =CH), 6.90-7.32 (m, 3H, Ar-H), 7.51 (*s*, 1H, C3-H). GCMS (*m/z*): 271.

Preparation of (7-methoxy-benzofuran-2-yl)-(5-methyl-3-phenyl-pyrazol-1-yl)-methanone (9)
A mixture of 7-methoxy-benzofuran-2-carboxylic acid hydrazide **2** (0.001 mol) and benzoyl acetone (0.001 mol) was refluxed in methanol (20 mL) containing 4-5 drops of acetic acid for 4 h. The reaction mixture was cooled to room temperature and the separated product **9** was collected by filtration and washed with methanol. It was crystallized from ethanol as colorless needles.

IR(KBr, cm^{-1}): 1668 (C=O), 1582 (C=N), 1521 (C=C). ^1H NMR ($CDCl_3$, ppm): 2.34 (*s*, 3H, CH_3), 3.91 (*s*, 3H, OCH_3), 6.31-7.42 (m, 9H, Ar-H), 7.52 (*s*, 1H, C3-H). GCMS (*m/z*): 332.

Preparation of 2-(7-methoxy-benzofuran-2-carbonyl)-5-methyl-2,4-dihydro-pyrazol-3-one (10)
To a solution of 7-methoxy-benzofuran-2-carboxylic acid hydrazide **2** (0.001 mol) in methanol was added ethylacetoacetate (0.001 mol) containing 4-5 drops of acetic acid. The reaction mixture was refluxed for 4 h. The reaction mixture was cooled to room temperature and the product separated **10** was collected by filtration and washed with little methanol, dried and crystallized with aqueous ethanol.

IR(KBr, cm^{-1}): 1665 (C=O), 1595 (C=N). ^1H NMR ($CDCl_3$, ppm): 1.3 (*s*, 3H, CH_3), 2.2 (*s*, 2H, CH_2), 3.96 (*s*, 3H, OCH_3), 6.90-7.26 (m, 3H, Ar-H), 7.52 (*s*, 1H, C3-H). GCMS (*m/z*): 273.

PHARMACOLOGICAL STUDIES

ANTIMICROBIAL STUDIES

All the synthesized compounds **2–10** were screened for their antibacterial and antifungal activity at 50 µg/disc by the disc diffusion method [34]. Antibacterial activity was carried out against three Gram-negative bacteria, viz., *Escherichia coli, Psuedomonus aeruginosa, Klebsiella pneumoniae,* and two Gram-positive bacteria, viz., *Staphylococcus aureus, and Streptococcus faecalis*. Ciprofloxacin was used as standard (Table 2). Antifungal

activity was carried out against five fungi, viz., *Aspergilus flavus, Aspergilus fumigatus, Candida albicans, Penicillium notatum and Rhizopus*. Fluconazole was used as standard (Table 3).

ANTIOXIDANT STUDIES

In vitro antioxidant activity (table 4) of the synthesised compounds performed by ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Method [35]. ABTS solution I (2 mM of ABTS solution) and solution II (17 mM of potassium persulfate) were prepared using distilled water. Solution II (0.3 mL) was added to 50 mL of solution I and the reaction mixture was left to stand at room temperature overnight in dark before use. Test solutions were prepared by dissolving drug samples and the standard (ascorbic acid) were accurately weighed (10 mg) separately and dissolved in 1 mL of DMSO. These solutions were serially diluted with DMSO to obtain the lower dilutions. Distilled DMSO (1 mL) was added to 0.2 mL of various concentrations of the drug samples or standard, and 0.16 mL of ABTS solution was added to make a final volume of 1.36 mL. After 20 min, the absorbance was measured spectrophotometrically at 734 nm using ELISA reader. Blank was maintained without ABTS. IC_{50} value obtained was the concentration of the sample required to inhibit 50% ABTS radical mono cation. The statistical analysis was performed by One way ANOVA followed by Tukey'spost-hoc test was employed to analyze the results (Graph Pad Prism Software). The difference below the probability level of 0.05 was considered as statistically significant.

ANIMALS

Swiss Albino mice (weighing 20-25 g) and Wistar rats (weighing 150-200 g) were used for studying *in vivo* analgesic, anti-inflammatory and anti-pyretic activities. Animals were maintained under standard laboratory conditions (24±2 ^{o}C; relative humidity 60-70%). Study protocol was approved by the Institutional Animal Ethics Committee before conducting the experiments. The mice and rats were used in the study. The animals were kept in polypropylene cages and maintained on balanced diet with free access to drinking water. All the experimental procedures were conducted in accordance with the guide for Care and Use of Laboratory Animals and in accordance with the Local Animal Care and Use Committee.

ACUTE TOXICITY STUDIES

For testing the acute toxicity potential of the test compounds, Swiss Albino mice of either sex weighing 20-25 g were selected, separated into groups each containing six mice. The dosage was varied from 100 up to 3000 mg kg^{-1} body weight. The mice were continuously observed for 8 h for any signs of increased-decreased motor activity, ataxia, tremors, convulsions, sedation, lacrimation, etc. After 24 h, the mice were sacrificed and stomach, intestine and liver were inspected under the magnifying lenses for any ulcer-haemorrhagic spots.

ANALGESIC STUDIES

The analgesic activity was carried out by Tail-flick and writhing methods.

Tail-flick method

After over-night fasting, the rats were divided into different groups (n=6) as shown in Table 5. The reaction time was measured at the end of 0, 30, 60 and 90 min after the administration of the compounds. The drugs were dispersed in 0.5% w/v of sodium carboxy methyl cellulose (sodium CMC) and administered orally. The control group (no drug) was administered with 0.5 ml of 0.5% w/v of sodium CMC. The tail-flick latency was assessed by considering the time taken by the rat to withdraw its tail from the hot water bath (55 ± 0.5 ^{o}C) [36]. . The tail-flick latency of treated animals was compared with control animals.

Writhing method

After an over-night fast, the mice were distributed into different groups and treated as shown in Table 6. The drugs were dispersed in 0.5% w/v of sodium CMC and administered orally. The control group (no drug) was administered with 0.5 ml of 0.5% w/v of sodium CMC. The standard drug used was nimesulide (12.5 mg/ kg). One hour after the treatment, the mice were given an intraperitoneal injection of 0.7% v/v acetic acid solution (volume of injection was 0.1 ml/ 10 g body weight). The number of writhes produced in these animals was counted for 30 min. The analgesic activity was evaluated in terms of the percentage of writhe inhibitions [37].

ANTI-INFLAMMATORY STUDIES

Carrageenan-induced rat paw edema model was used to evaluate the anti-inflammatory activity of the synthesized compounds [38]. The overnight fasted rats were divided into different groups (n=6) and treated as shown in Table 7.

The drugs were administered orally by dispersing in 0.5% w/v of sodium CMC. The control group (no drug) was administered with 0.5 ml of 0.5% w/v of sodium CMC. Nimesulide (9 mg/kg) was used as standard drug. After 30 min of drug administration, all the rats were challenged by a subcutaneous injection of 0.1 ml of 1% solution of carrageenan in saline into the plantar site of the right hind paw. The paw volumes were measured with a plethysmometer, prior to administration of carrageenan and after 1, 2 and 3 h of administration. The difference in the volume was the amount of oedema developed and the percent inhibition of edema between control group and the compound treated groups was calculated.

ANTI-PYRETIC STUDIES

Prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature were selected for the study. The anti-pyretic activity was evaluated using Brewer's yeast induced pyrexia in rats [39]. Fever was induced by injecting 20 mL/kg (s.c.) of 20% w/v aqueous suspension of Brewer's yeast in normal saline intramuscularly. After 18 h, the animals developed 0.5 °C or more rise in the rectal temperature (about 60% of the total animals injected). The rats were divided into different groups (n=6) and treated orally as shown in Table 8. The compounds were dispersed in 0.5% w/v of sodium CMC for administration. The control group was fed with 0.5 ml of 0.5% w/v of sodium CMC orally. Standard drug group was administered orally with nimesulide (9 mg/ kg; p.o.). At different time intervals the rectal temperature was recorded using clinical thermometer. Percentage reduction in rectal temperature was calculated by considering the total fall in temperature to normal level as 100%.

STATISTICAL ANALYSIS

One way ANOVA followed by Dunnet's (to compare the values with control) and Tukey's (to compare all values) post-hoc tests was employed to analyze the results (Graph Pad Prism Software). The difference below the probability level of 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

CHEMISTRY

In the present work we are reporting the synthesis of compound 7-methoxy-benzofuran-2-carboxylic acid ethyl ester **(1)** and the various oxadiazoles **4**, **5** and **7,** and various pyrazoles **8**, **9** and **10**. The key intermediate **1** was obtained by refluxing *o*-vanillin and diethylbromomalonate in alkaline medium using dry potassium carbonate in dry methylethylketone. The condensation and cyclisation took place simultaneously by refluxing the reaction mixture for prolonged time. The ethanolic solution of the compound **1** was refluxed with hydrazine hydrate to get 7-methoxy-benzofuran-2-carboxylic acid hydrazide **2** [33]. The acid hydrazide **2** on reacting with acetic anhydride yielded acetyl derivative **3** in good yield which was further cyclised in presence of $POCl_3$ to form 2-(7-methoxy-benzofuran-2-yl)-5-methyl-[1,3,4]oxadiazole **(4).** Further, the acid hydrazide **2** was condensed with ethyl chloroformate to get N'-(7-methoxy-benzofuran-2-carbonyl)-hydrazine carboxylic acid ethyl ester **(6).** The compound **6** on cyclisation with $POCl_3$ to yielded 5-(7-methoxy-benzofuran-2-yl)-[1',3',4']oxadiazol-2-ol **(7).** Finally the acid hydrazide **2** underwent direct cyclisation with ethyl chloroformate in methanol to yield 2-chloro-5-(7-methoxy-benzofuran-2-yl)-[1',3',4']oxadiazole **(5)** (Scheme 1).

The acid hydrazide **2** in other route**,** was refluxed with acetyl acetone, benzoyl acetone and ethyl acetoacetate in methanol in presence of catalytic amount of acetic acid resulted in the formation of pyrazoles **8, 9** and **10** respectively (Scheme 2).

The structures of all the synthesized compounds in the present investigation were confirmed by the support of analytical data (table 1) and spectral data given in the experimental section.

ANTIMICROBIAL STUDIES

The investigation of antibacterial screening data (table 2 and 3) revealed that all the tested compounds showed moderate to good microbial inhibition. In the series, the compounds bearing chlorine **5** and hydroxyl group **7** exhibited potent activities compared to others. The compound **5** is potent antibacterial than **7**, whereas vice-versa is true for antifungal activity.

ANTIOXIDANT STUDIES

The *in vitro* antioxidant activity of synthesized compounds was assessed by ABTS method. In this method, the pre-formed radical monocation of ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] is generated by oxidation of ABTS with potassium persulfate (a blue chromogen) and is reduced in the presence of such hydrogen donating antioxidants. The results (Table 4) indicated that all the synthesized compounds exhibited moderate to good antioxidant activity with ABTS method. Among the series, oxadiazole compound bearing chlorine (**5**) and bearing hydroxyl group (**7**) showed maximum activity with IC_{50} value of 61.97±1.09 and 62.49±1.54 micromolar concentrations, respectively. Although the synthesized compounds exhibited moderate antioxidant activity, the IC_{50} values were significantly different ($p<0.05$) from that of ascorbic acid (standard drug). Ascorbic acid showed potent ability to inhibit free radicals with IC_{50} values of 12.10±0.51 micromolar concentration.

ACUTE TOXICITY STUDIES

The mice did not show any signs of increased or decreased motor activity, ataxia, tremors, convulsions, sedation, lacrimation, etc during 8 h after administration (p.o.) of varying doses (100 - 3000 mg/kg) of all synthesized compounds. The stomach, intestine and liver of dissected mice after 24 h of drug administration did not exhibit any ulcer-haemorrhagic spots, indicating the greater safety margin of the compounds when administered orally.

ANALGESIC STUDIES

The synthesized compounds **2-10** were screened for analgesic activity by tail-flick method (in rats) and acetic acid induced writhing method (in mice). Tail-flick test was employed to assess centrally mediated analgesia by synthesized compounds. Tail flick responses to thermal stimuli are mediated via supra-spinal centres. The results of analgesic activity by tail flick method are shown in Table 5. The results indicated that there was a little increase in the reaction time to flick the tail in all the treated groups. However the increase in reaction and hence analgesic activity was not appreciable, although the time required to flick the tail at 90 min was significantly different ($p<0.05$) than that of control. The compound with chloro **5** and hydroxy **7** showed comparatively better analgesic activity than others.

The results of analgesic activity by writhing method are shown in Table 6. The acetic acid-induced writhing method is generally used for the evaluation of peripheral antinociceptive activity because of its sensitivity in detecting a antinociceptive potential of the compounds which may appear inactive in other models. Writhing is demonstrated as acute pain due to tissue damage and sensitization of nociceptors by inflammatory mediators. Synthesized compounds inhibited painful writhes suggesting its inhibitory action on these mediators of inflammation and pain. Control group showed 72±3 writhes. The standard drug (nimesulide) and all the compounds showed significantly ($p<0.05$) less number of writhes in comparison with control. Among the series, the compounds **5** and **7** showed highest analgesic activity (Compound 5 - No. of writhes: 43±4 and % Reduction: 39.41%; Compound 7 - No. of writhes: 42±4 and % Reduction: 42.92). Although, the reduction in number of writhes by compounds were significantly less compared to control, the values were not significantly different ($p>0.05$) than that of nimesulide, which exhibited highest reduction in number of writhes. The extent of analgesia produced by compounds in writhe method was considerably better than that in tail flick method. This indicates that the compounds may act as analgesic agents by acting peripherally instead of centrally. However more experiments with different models are necessary to precisely know the mechanism by which these metal complexes exhibit their activity.

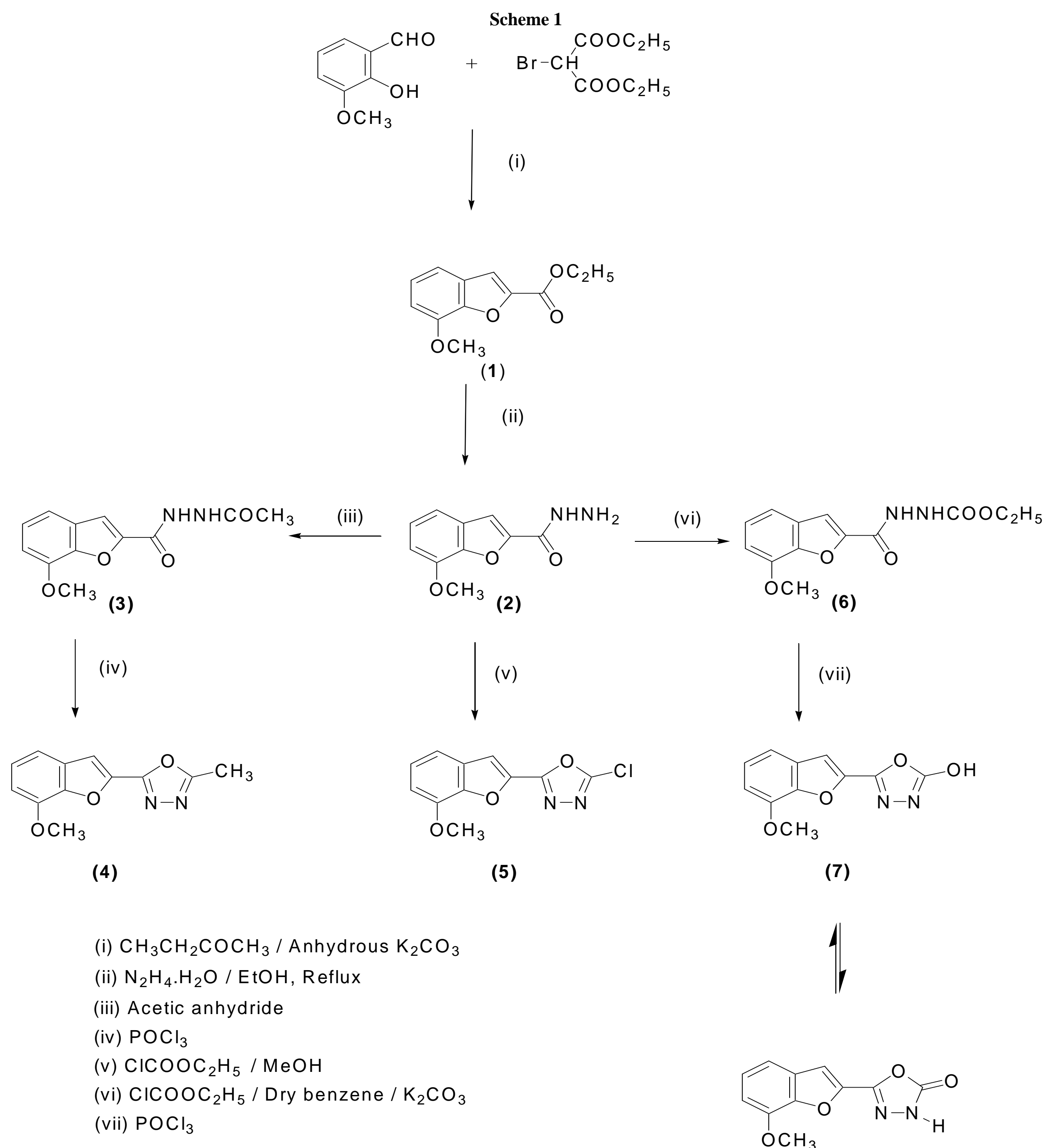
ANTI-INFLAMMATORY STUDIES

The results of anti-inflammatory activity of compounds as well as a standard drug (nimesulide) against carrageenan-induced edema are shown in Table 7 [38]. After the administration of carrageenan, inflammation was induced in rats and the effect was measured at the intervals of 1, 2, 3 and 5h. The percent oedema inhibition was calculated with respect to control group. All the values of % reduction at different time intervals of all the tested compounds were significantly ($p<0.05$) different from those of the control group. The compounds **5** and **7** exhibited highest activity among synthesized compounds (compound **7**: 15.69±1.25, 24.27±1.24, 33.86±1.81and 25.00±1.25% reduction in oedema at 1, 2, 3 and 5 h). On the contrary, all the % reduction values were significantly ($p<0.05$) lower than those of nimesulide (standard drug) which showed highest activity.

ANTI-PYRETIC STUDIES

All the synthesized compounds were screened for anti-pyretic activity by using the Brewer's yeast-induced pyrexia method in rats [39]. The results of anti-pyretic activity of synthesized compounds are shown in Table 8. All the synthesized compounds showed significant ($p<0.05$) reduction in the rectal temperature at all the time intervals

compared to control. The compound **7** showed better percentage of reduction in pyrexia (44.11±5.12, 51.47±5.42 and 57.35±6.12% at 1, 2 and 3 h, respectively) among all the synthesized compounds. Nimesulide (standard drug) showed highest antipyretic and all the % reduction values from all the tested compounds were significantly ($p<0.05$) lower than those of nimesulide.



The results of pharmacological studies demonstrate the significant analgesic, anti-inflammatory and anti-pyretic effect of synthesized copounds in comparison with respective control groups. Although the compounds **5** and **7** showed appreciable pharmacological activities, the effect was significantly lower than that of standard drug. The results indicate the need to carry out the similar studies at different dose levels of synthesized compounds in different preclinical experimental models to precisely check the extent and mechanism of pharmacological effects.

Scheme 2

(8) (2) (10) (9)

(i) $CH_3COCH_2COCH_3$ / MeOH / AcOH

(ii) $CH_3COCH_2COC_6H_5$ / MeOH / AcOH

(iii) $CH_3COCH_2COOC_2H_5$ / MeOH / AcOH

Table 1. Analytical data of compounds (2-10)

Compound	Solvent for crystallization	Melting point (°C)	% Yield	Molecular formula	% Elemental analysis Found (cacld.)		
					C	H	N
1	Ethanol	85-86	80	$C_{12}H_{12}O_4$	65.42 (65.45)	5.46 (5.49)	-
2	Ethanol	180-181	85	$C_{10}H_{10}N_2O_3$	58.30 (58.25)	4.90 (4.89)	13.60 (13.59)
3	Ethanol	175-176	86	$C_{12}H_{12}N_2O_4$	58.05 (58.06)	4.84 (4.87)	11.21 (11.29)
4	Aq. ethanol	190-191	65	$C_{12}H_{10}N_2O_3$	62.56 (62.60)	4.37 (4.38)	12.19 (12.17)
5	Dioxane	189-190	80	$C_{11}H_7ClN_2O_3$	52.66 (52.71)	2.78 (2.82)	11.14 (11.18)
6	Ethanol	156-157	68	$C_{13}H_{14}N_2O_5$	56.06 (56.11)	5.08 (5.07)	10.04 (10.07)
7	Aq. ethanol	173-174	75	$C_{11}H_8N_2O_4$	56.86 (56.90)	3.44 (3.47)	12.01 (12.06)
8	Ethanol	210-211	70	$C_{15}H_{14}N_2O_3$	66.62 (66.66)	5.18 (5.22)	10.31 (10.36)
9	Ethanol	210-211	65	$C_{21}H_{20}N_2O_3$	72.36 (72.40)	5.74 (5.79)	8.01 (8.04)
10	Aq. ethanol	145-146	67	$C_{14}H_{12}N_2O_4$	61.72 (61.76)	4.40 (4.44)	10.21 (10.29)

Table 2. Results of antibacterial activity of the compounds (2-10) at 50 µg/mL

Compound	Diameter of the zone of inhibition in mm (Relative inhibition %)				
	Gram negative			Gram positive	
	E. coli	*P. aeruginosa*	*K. pneumoniae*	*S. aureus*	*S. faecalis*
2	8 (44.4)	12 (48)	11 (55)	9 (47.3)	11 (55)
3	10 (55.6)	13 (52)	12 (60)	10 (52.6)	12 (60)
4	12 (66.7)	17 (68)	16 (80)	15 (78.9)	16 (80)
5	15 (83.3)	20 (80)	18 (90)	17 (89.5)	19 (95)
6	11 (61.1)	14 (56)	13 (65)	11 (57.9)	12 (60)
7	14 (77.8)	18 (72)	17 (85)	16 (84.2)	18 (90)
8	13 (72.2)	16 (64)	16 (80)	14 (73.7)	13 (65)
9	13 (72.2)	15 (60)	15 (75)	13 (68.4)	14 (70)
10	12 (66.7)	14 (56)	14 (70)	12 (63.1)	15 (75)
Ciprofloxacin	18 (100)	25 (100)	20 (100)	19 (100)	20 (100)

Table 3. Results of antifungal activity of the compounds (2-10) at 50 µg/mL

Compound	Diameter of the zone of inhibition in mm (Relative inhibition %)				
	A. flavus	*A. fumigates*	*C. albicans*	*P. notatum*	*Rhizopus*
2	12 (40)	12 (50)	13 (54.1)	12 (46.1)	13 (60)
3	13 (43.3)	14 (58.3)	14 (58.3)	13 (50)	14 (53.8)
4	26 (86.7)	20 (83.3)	19 (79.1)	21 (80.7)	22 (84.6)
5	28 (93.3)	22 (91.7)	20 (83.3)	22 (84.6)	24 (92.3)
6	14 (46.7)	16 (66.7)	15 (62.5)	14 (53.84)	15 (57.7)
7	30 (100)	23(95.83)	22 (91.66)	24 (92.3)	25 (96.1)
8	21 (70)	19 (79.1)	18 (75)	19 (73.1)	18 (69.2)
9	20 (66.7)	18 (75)	16 (66.7)	17 (65.38)	17 (65.38)
10	19 (63.3)	16 (66.7)	15 (62.5)	15 (57.7)	16 (61.5)
Fluconazole	30 (100)	24 (100)	24 (100)	26 (100)	26 (100)

Table 4. Results of antioxidant activity of compounds (2–10) by ABTS method

Compound	2	3	4	5	6	7	8	9	10	Standard (Ascorbic acid)
IC_{50} Value* Micromolar	103.10 ±1.95	98.71 ±1.23	64.73 ±1.54	61.97 ±1.09	97.82 ±1.87	62.49 ±1.54	95.51 ±1.62	89.94 ±1.59	70.83 ±1.32	12.10 ±0.51

**The results are presented as Mean±SEM, n=6; IC_{50} values of all the synthesized compounds are significantly different ($p<0.05$) from that of the standard (ascorbic acid).*

Table 5. Results of analgesic activity of the compounds (2-10) by tail flick method in rats

Compounds	Average reaction time (sec) at different time intervals[b,c]			
	0 min	30 min	60 min	90 min
Control[a]	3.70±0.36	3.76±0.28	4.07±0.36	4.01±0.36
2	3.86±0.32	4.08±0.29	4.15±0.21	4.25±0.21
3	3.92±0.42	4.28±0.48	4.11±0.20	4.38±0.36
4	3.85±0.24	4.15±0.28	4.69±0.37*	5.01±0.33*
5	3.77±0.26	4.45±0.52*	5.05±0.46*	5.35±0.45*
6	3.91±0.38	4.18±0.31	4.57±0.35	4.55±0.35
7	3.95±0.36	4.55±0.50*	5.12±0.35*	5.46±0.35*
8	3.82±0.22	4.11±0.29	4.15±0.21	4.42±0.30
9	3.88±0.37	4.27±0.58	4.46±0.47	4.65±0.25
10	3.67±0.29	4.28±0.59	4.62±0.45	4.98±0.45*

[a]Control group was administered (p.o.) with 0.5 mL of 0.5% w/v CMC;
[b]Test drugs were administered (p.o.) at a dose level of 100 mg/ kg;
*[c]All the values are expressed as Mean±SD, n=6; * Significant ($p<0.05$) compared to control.*

Table 6. The results of analgesic activity of compounds (2-10) by Writhe method on mice

Compound[c]	No. of Writhes	Inhibition (%)
Control[a]	72±3	---
Standard drug[b]	32±3*	54.92
2	67±4[#]	6.84
3	68±4[#]	5.59
4	46±4*[#]	36.36
5	43±4*[#]	39.41
6	53±4*[#]	26.54
7	42±4*[#]	42.92
8	66±3[#]	8.42
9	42±4*[#]	40.67
10	49±3*[#]	32.15

[a]*Control group was administered (p.o.) with 0.5 mL of 0.5% w/v CMC;*
[b]*Standard drug group was administered (p.o.) with 12.5 mg/ kg of Nimesulide;*
[c]*Test drugs were administered (p.o.) at a dose level of 100 mg/ kg; All the values are expressed as Mean±SD, n=6;*
**Significant (p<0.05) compared to control;*
[#]*Significant (p<0.05) compared to standard drug*

Table 7. The results of anti- inflammatory activity of compounds (2-10) by Carrageenan induced Paw Edema method in rats

Compounds[c]	Paw volume (mL) (Percentage of edema reduction)				
	0 h	**1 h**	**2 h**	**3 h**	**5 h**
Control[a]	0.850±0.031	0.870±0.035 (-2.35± 0.35)	0.876±0.041 (-3.06±0.38)	0.865±0.032 (-1.76±0.41)	0.855±0.043 (-0.58± 0.15)
Standard drug[b] (Nimesulide)	0.856±0.035	0.664±0.042 (22.42±1.66)*	0.600±0.032 (29.90±1.78)*	0.451±0.031 (47.31± 2.25)*	0.490±0.021 (42.75±2.15)*
2	1.150±0.075	1.120±0.052 (2.60±0.26)*[#]	1.122±0.055 (2.43± 0.31)*[#]	1.110±0.065 (3.47± 0.35)*[#]	1.101±0.063 (4.26±0.45)*[#]
3	0.825±0.048	0.812±0.033 (1.57±0.12)*[#]	0.805±0.035 (2.42±0.29)*[#]	0.800±0.032 (3.03±0.34)*[#]	0.792±0.041 (4.00±0.31)*[#]
4	0.650±0.031	0.577±0.042 (11.23±0.51)*[#]	0.515±0.031 (20.76±1.25)*[#]	0.464±0.024 (28.61±1.24)*[#]	0.501±0.027 (22.92±1.32)*[#]
5	0.658±0.035	0.575±0.025 (12.61±1.01)*[#]	0.505±0.032 (23.25±1.21)*[#]	0.451±0.034 (31.46±1.51)*[#]	0.496±0.023 (24.62±1.65)*[#]
6	0.752±0.044	0.670±0.045 (9.57±0.85)*[#]	0.600±0.043 (20.21±1.12)*[#]	0.552±0.031 (26.59±1.02)*[#]	0.572±0.026 (23.93±1.55)*[#]
7	0.688±0.043	0.580±0.035 (15.69±1.25)*[#]	0.521±0.022 (24.27±1.24)*[#]	0.455±0.042 (33.86±1.81)*[#]	0.516±0.032 (25.00±1.25)*[#]
8	0.845±0.048	0.821±0.024 (2.92±0.34)*[#]	0.801±0.033 (5.35±0.32)*[#]	0.795±0.046 (6.09±0.41)*[#]	0.805±0.041 (4.87±0.35)*[#]
9	0.848±0.044	0.764±0.035 (9.90±1.21)*[#]	0.701±0.033 (17.33±1.21)*[#]	0.635±0.051 (25.11±1.08)*[#]	0.675±0.044 (20.40±1.08)*[#]
10	0.901±0.065	0.800±0.045 (11.20±0.99)*[#]	0.705±0.030 (21.75±1.35)*[#]	0.631±0.042 (29.96±1.52)*[#]	0.686±0.035 (23.86±1.14)*[#]

[a]*Control group was administered (p.o.) with 0.5 mL of 0.5% w/v CMC;*
[b]*Standard drug group was administered (p.o.) with 9 mg/ kg of Nimesulide;*
[c]*Test drugs were administered (p.o.) at a dose level of 100 mg/ kg; All the values are expressed as Mean±SD, n=6;*
** Significant (p<0.05) compared to control;*
[#] *Significant (p<0.05) compared to standard drug.*

Table 8. The results of anti-pyretic activity of compounds (2-10) on Brewer's yeast induced pyrexia in rats

Compounds[c]	Rectal temperature (°C)		Rectal temperature after administration of drugs (°C)[d]		
	Normal (A)	18 h after administration of yeast (B)	1 h (C_1)	2 h (C_2)	3 h (C_3)
Control[a]	37.85±0.41	38.50±0.32	38.48±0.15 (3.07±0.61)	38.48±0.15 (3.07±0.61)	38.47±0.20 (4.61±0.81)
Standard drug[b]	37.31±0.48	38.00±0.31	37.60±0.21 (57.97±4.11)*	37.50±0.25 (72.46±4.56)*	37.47±0.28 (76.81±5.12)*
2	37.65±0.41	38.36±0.28	38.34±0.31 (2.81±0.51) #	38.33±0.29 (4.22±0.62) #	38.32±0.25 (5.63±0.68) #
3	37.65±0.50	38.35±0.29	38.32±0.31 (4.28±0.52) #	38.32±0.31 (4.28±0.52) #	38.30±0.29 (7.14±0.69) #
4	37.62±0.48	38.21±0.32	38.06±0.35 (25.42±3.01) #*	37.98±0.29 (39.98±3.88)* #	37.96±0.32 (42.37±4.55)* #
5	37.82±0.52	38.47±0.28	38.20±0.31 (41.53±4.26)* #	38.17±0.32 (46.15±5.15)* #	38.15±0.41 (49.23±5.21)* #
6	37.32±0.51	38.00±0.36	37.83±0.38 (25.00±3.21)* #	37.75±0.40 (36.76±4.52)* #	37.72±0.32 (41.17±3.68)* #
7	37.61±0.49	38.29±0.31	37.99±0.39 (44.11±5.12)* #	37.94±0.37 (51.47±5.42)* #	37.90±0.42 (57.35±6.12)* #
8	37.62±0.61	38.21±0.21	38.16±0.25 (8.47±0.92) #	38.15±0.29 (10.16±1.26) #	38.14±0.35 (11.86±1.51) #
9	37.65±0.42	38.30±0.29	38.15±0.31 (23.07±3.12)* #	38.08±0.32 (33.84±4.01)* #	38.05±0.40 (38.46±4.55)* #
10	37.55±0.55	38.25±0.32	38.06±0.40 (27.14±3.12)* #	37.98±0.42 (38.57±4.82)* #	37.45±4.12 (42.85±5.32)* #

[a]*Control group was administered (p.o.) with 0.5 mL of 0.5% w/v CMC;*
[b]*Standard drug group was administered (p.o.) with 9 mg/ kg of Nimesulide;*
[c]*Test drugs were administered (p.o.) at a dose level of 100 mg/ kg; All the values are expressed as Mean±SD, n=6;*
[d]*Percentage reduction in rectal temperature after administration of drugs is given in parenthesis; Statistics was applied to percentage reduction values;*
**Significant (p<0.05) compared to control;*
 # Significant (p<0.05) compared to standard drug.
Percentage reduction = (B-Cn)/(B-A) × 100, where n = 1, 2 or 3.

CONCLUSION

A series of novel benzofuranyl-oxadiazoles and pyrazoles **2-10** have been synthesized in good yield and screened for their antimicrobial, antioxidant, analgesic, antipyretic and anti-inflammatory activities. In the series, the compounds bearing chlorine **5** and hydroxyl group **7** exhibited potent activities compared remaining. However these *in vivo* evaluations in different experimental models and detailed toxicological studies are necessary to further support these results. The detailed metabolic stability assay is still in progress.

REFERENCES

[1] V Kumar, R Aggarwal, P Tyagi, S P Singh, *Eur. J. Med. Chem.,* **2005**, 40, 922-927.
[2] P D Sauzem, P Machado, M A Rubin, G S Sant'Anna, H B Faber, A H Souza,C F Mello, P Beck, R A Burrow, H G Bonacorso, N Zanatta, M A P Martins, *Eur.J. Med. Chem.* **2008**, 43, 1237-1247.
[3] K M Dawood, H Abdel-Gawad, E A Ragab, M Ellithey, H A Mohamed, *Bioorg.Med. Chem.* **2006**, 14, 3672-3680.
[4] M Sun, C Zhao, G A Gfesser, C Thiffault, T R Miller, K Marsh, J Wetter, M Curtis, R Faghih, T A Esbenshade, A A Hancock, M Cowart, *J. Med. Chem.* **2005**, 48, 6482-6490.
[5] I Hayakawa, R Shioya, T Agatsuma, H Furukawa, Y Sugano, *Bioorg. Med.Chem. Lett.* **2004**, 14, 3411-3414.
[6] N Gundog du-Karaburun, K Benkli, Y Tunali, U Ucucu, S Demirayak, *Eur. J. Med. Chem.* **2006**, 41, 651-656.
[7] M S Malamas, J Sredy, C Moxham, A Katz, W Xu, R McDevitt, F O Adebayo, D R Sawicki, L Seestaller, D Sullivan, J R Taylor, *J. Med. Chem.* **2000**, 43, 1293-1310.
[8] M Masubuchi, H Ebiike, K Kawasaki, S Sogabe, K Morikami, Y Shiratori, S Tsujii, T Fujii, K Sakata, M Hayase, H Shindoh, Y Aoki, T Ohtsuka, N Shimma, *Bioorg. Med. Chem.* **2003**, 11, 4463-4478.
[9] B Carlsson, B N Singh, M Temciuc, S Nilsson, Y Li, C Mellin, J Malm, *J. Med.Chem.* **2002**, 45, 623-630.
[10] S A Khanum, S Shashikanth, S Umesh, R Kavitha, *Eur. J. Med. Chem.* **2005**, 40, 1156-1162.
[11] K Obi, A Kojima, H Fukuda, K Hirai, *Bioorg. Med. Chem. Lett*. **1995**, 5, 2777-2782.
[12] L V G Nargund, G R N Reddy, V Hariprasad, *J. Pharm. Sci.* **1994**, 83, 246-248.
[13] S Bhandari, K Bothara, M Raut, A Patil, A Sarkate, V Mokale, *Bioorg. Med.Chem.* **2008**, 16, 1822-1831.
[14] M G Mamola, D Zampieri, L Voi, M Fermeglia, M Ferrone, S Pricl, G Scialinoc, E Banfi, *Bioorg. Med. Chem.* **2005**, 13, 3797-3809.
[15] A Aboraia, H M A Rahman, N Mahfuz, A Mohmoud, E L Gendy, *Bioorg. Med.Chem.* **2006**, 14, 1236-1246.
[16] S G Kucukguzel, I K Kucukguzel, E Tatar, S Rollas, F Sahin, M Gulluce, E D Clercq, L Kabasakal, *Eur. J. Med. Chem*. **2007**, 42, 893-901.
[17] G W Adelstein, C H Yen, E Z Dajani, R G Bianchi, *J. Med. Chem.* **1976**, 19, 1221-1225.

[18] D Ghiram, I Schwartz, I Simiti, *Farmaco* **1974**, 22, 141-149.
[19] H L Yale, K Losee, *J. Med. Chem.* **1966**, 9, 478-483.
[20] N K Chudgar, S N Shah, R A Voral, *Mol. Liq. Cryst.* **1989** 51, 172-177.
[21] E Schinzel, T Martini, W Spatzeir, H D Probst, A G Hoeshst, *Chem. Abst*. **1983**, 98, 199850.
[22] P D Sauzem, P Machado, M A Rubin, G S Sant'Anna, H B Faber, A H Souza, C F Mello, P Beck, R A Burrow, H G Bonacorso, N Zanatta, M A P Martins, *Eur.J. Med. Chem.* **2008**, 43, 1237-1247.
[23] A Hall, A Billinton, S H Brown, N M Clayton, A Chowdhury, G M P Giblin, P Goldsmith, T G Hayhow, D N Hurst, I R Kilford, A Naylor, B Passingham, L Winyard, *Bioorg. Med. Chem. Lett.* **2008**, 18, 3392-3399.
[24] F R Souza, V T Souza, V Ratzlaff, L P Borges, M R Oliveira, H G Bonacorso, N Zanatta, M A P Martins, C F Mello, *Eur. J. Pharm.* **2002**, 451, 141-147.
[25] M Iovu, C Zalaru, F Dumitrascu, C Draghici, M Moraru, E Cristea, *Il Farmaco* **2003**, 58, 301-307.
[26] F Manna, F Chimenti, A Bolasco, D Secci, B Bizzarri, O Befani, P Turini, B Mondovı, S Alcaro, A Tafi, *Bioorg. Med. Chem. Lett.* **2002**, 12, 3629-3633.
[27] M Abid, A Azam, *Bioorg. Med. Chem.* 2005, 13, 2213-2220.
[28] G Turan-Zitouni, P Chevallet, F S Kilic, K Erol, *Eur. J. Med. Chem.* **2000**, 35, 635-641.
[29] S Samshuddin, B Narayana, B K Sarojini, R Srinivasan, Vinayachandra, K R Chandrashekar, *Der Pharma Chemica,* **2012**, 4, 587-592.
[30] K R Gans,W Galbraith, R J Roman, S B Haber, J S Kerr, W K Schmidt, C Smith,
W C Hewes, N R Ackerman, *J. Pharm. Exp. Ther.* **1990**, 254, 180-187.
[31] B F Abdel-Wahab, H A Abdel-Aziz, E M Ahmed, *Arch. Pharm. Chem. Life Sci*. **2008**, 341, 734-739.
[32] S K Giri, H Hanumanagoud, K M Basavaraja, *J. Chem. Pharm. Res.,* **2010**, 2, 387-392.
[33] H Hanumanagoud, K M Basavaraja, *J. Chem. Pharm. Res.,* **2012**, 4, 5165-5171.
[34] E W Koneman, S D Allen, W M Janda, P C Schreckenberger, C W Washington Jr, Colour atlas and textbook of diagnostic microbiology (Philadelphia: Lippincott-Raven Publishers) Chapter 15, Antibacterial susceptibility testing, 5th edn, pp. **1997**, 785–844.
[35] P Sithisarn, R Supabphol, W Gritsanapan, *J Ethnopharmacol,* **2005**, 99, 109-112.
[36] R A Turner, Screening Methods in Pharmacology. Academic Press, New York and London, **1965**, p. 223-251.
[37] A Mehrotra, R Shanbhag, C M Rao, V P Singh, J Mudgal, *Eur. J. Pharmac.* **2011**, 666, 80–86.
[38] C A Winter, E A Risley, G W Nuss, *Proc. Soc. Exp. Biol. Med.* **1962**, 111, 544–547.
[39] S Mutalik, K Paridhavi, C M Rao, N Udupa, *Indian J. Pharmacology*, **2003**, 35, 312-315.