Full Paper

Synthesis and Anti-Inflammatory Activity of Novel (4-Hydroxyphenyl)(2,4-dimethoxyphenyl) Methanone Derivatives

Kambappa Vinaya[1], Raja Naika[2], Channappillekoppal S. Ananda Kumar[2], Somasagara R. Ranganath[1], Salekoppal B. Benaka Prasad[1], Venkatarangaiah Krishna[1], and Kanchugarakoppal S. Rangappa[1]

[1] Department of Studies in Chemistry, University of Mysore, Mysore, India
[2] Phytochemistry and Pharmacology Laboratory, Post Graduate Department of Studies and Research in Biotechnology and Bioinformatics, School of Biological Sciences, Kuvempu University, Shankaraghatta, India

In the scope of the research program aiming to perform the synthesis and pharmacological evaluation of novel possible anti-inflammatory compounds, in this manuscript, we report the synthesis of novel carboxamide **9a-d** and thioamide **10a-d** derivatives from the benzophenone and piperidine nucleus. Variation in the functional group at the *N*-terminal of piperidine led to two sets of compounds, bearing the carboxamide and thioamide, respectively. The characterization of this new class of compounds was performed with ^{1}H-NMR, LC-MS, IR, and elemental analysis. The newly synthesized compounds were screened for their anti-inflammatory activity by carrageenan-induced foot pad oedema assay and were compared with a standard drug. All the compounds exhibited anti-inflammatory activity at the dose of 30 mg/kg *p.o.* with varying degree from 52 to 67% inhibition of oedema. The compounds **9d** and **10d** with dichloro and fluoro substitution showed more potent activity at 30 mg/kg *p.o.* than the standard drug.

Keywords: Anti-inflammatory activity / Isocyanates / Isothiocyanates / Piperidine

Received: January 29, 2009; accepted: March 16, 2009

DOI 10.1002/ardp.200900021

Introduction

Inflammatory diseases are widely prevalent throughout the world and a major health problem of mankind. Drugs used for the treatment of acute and chronic inflammatory disorders suppress natural processes that contribute to the onset and symptoms of inflammation. Drugs used for the treatment of simple forms of arthritis such a gout and osteoarthritis relieve pain and swelling and improve mobility. These agents are often referred to as non steroidal anti-inflammatory drugs (NSAIDS) [1]. Many attempts have been made to find a cure for inflammatory diseases and, as a result, many non steroidal anti-inflammatory drugs such as phenylbutazone, oxyphenbutazone, diclofenac, ibuprofen, fenoprofen, indomethacin, benoxaprofen, benorylate, caprofen, tiopinac, ketoprofen, sulindac, etc. are available in the market. Though, many anti-inflammatory drugs are available, they cannot be used continuously for a long time as they have major ulcerogenic side effects [2].

In several instances in the literature, the proficiency of benzophenone analogues as chemotherapeutic agents, especially as anti-inflammatory, is well documented [3]. Benzophenone analogues synthesized by several scientists have been reported as effective anti-inflammatory agents [4–6]. Recently, the synthesis and structure-activity relationship of benzophenones as a novel class of p38-MAP kinase inhibitors with high anti-inflammatory activity have been reported [7]. We are interested to incorpo-

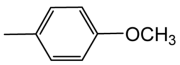
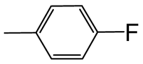
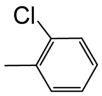
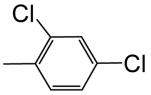
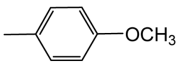
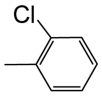
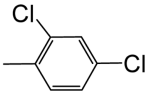
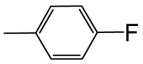
Correspondence: Prof. K. S. Rangappa, Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore, 570-006, India.
E-mail: rangappaks@gmail.com;
rangappaks@chemistry.uni-mysore.ac.in
Fax: +91 821 241-2191

Abbreviation: *o*-benzotriazol-1-yl-*N,N,N′,N′*-tetramethyl uranium tetrafluoroborate (TBTU)

rate a piperidine group in one of the phenyl rings, since the piperidine scaffold is wide-ranging in its therapeutic uses as it is ubiquitously found in drugs. Piperidine heterocycles play an important role in the field of medicinal chemistry. Several derivatives of this class have been found to possess useful biological activities such as herbicidal, insecticidal, fungicidal, bactericidal, anti-inflammatory, antihistaminic, hypotensive, anticancer, CNS stimulant, and are relevant in case of depressant and nerve activities [8 – 15].

It is well documented in the literature that the incorporation of two of the structural features required for activity in a single molecule has given rise to a significant enhancement in activity [16]. Due to a broad spectrum of activities reported so far, we have synthesized a system which combines these two biolabile components. We are targeting for potent molecules possessing anti-inflammatory activity. In this paper, we discuss the synthesis of such molecules and we screened the anti-inflammatory activity of all the compounds *in vivo*.

Table 1. Chemical structures and yields of synthesized compounds **9a – d** and **10a – d**.

Compound	R_1 and R_2	Yield (%)
9a		78
9b		77
9c		75
9d		80
10a		80
10b		74
10c		78
10d		78

Results and discussion

All the newly synthesized compounds were tested *in vivo* in order to evaluate their anti-inflammatory activity. In the present study, an acute inflammatory condition in the animals was produced by adopting the of Carrageenan method – inducing pedal inflammation. Pretreatment of the animals with compounds **9a – d** and **10a – d** (Table 1) with 10, 20, and 30 mg/kg, *p.o.* reduced paw oedema induced by carrageenan in a dose- and time-dependent manner. The percentage inhibition of paw oedema was found to be very significant at the end of a 3-h period. The percentage inhibition of paw oedema in the animals treated with compounds **10a – d** at a dose of 20 and 30 mg/kg body weight, was found to be significant compared to control group of animals (Table 2). However, a dose-dependent activity was observed. Whereas, the animals treated with compounds **9a – d** exhibited moderate anti-inflammatory activity as the percentage of inhibition of acute paw oedema induced by carrageenan was much less even after 3 h of carrageenan injection (Table 3).

The animals treated with **9a-d** also showed a significantly reduced acute paw-oedema volume as compared to the controls. Compound **9d**, at 20 and 30 mg/kg body weight, resulted in (59 and 66%, respectively) a dose-dependent reduction of carrageenan-induced oedema. The percentage inhibition of paw oedema of animals treated with compounds **9a**, **9b**, and **9c** at a dose of 20 and 30 mg/kg body weight was found to be a moderate anti-inflammatory activity as compare with their control group of animals. The potent inhibitory activity of the compound **9d** may be due to the presence of the electron-withdrawing dichloro group at 2nd and 4th position on the substituted carboxamide moiety.

Among the series **10a – d**, compound **10d** exhibited the highest anti-inflammatory activity of the tested animal models. Pretreatment of the animals with **10d** at 20 and 30 mg/kg body weight resulted in (56 and 67%, respectively) a dose-dependent reduction of carrageenan-induced oedema and the results were comparable to that of the standard reference drug Diclofenac sodium (72%). Compound **10c** showed moderate anti-inflammatory activity at 20 and 30 mg/kg body weight resulting in a 44 and 58% reduction, respectively. Similarly, compound **10a** showed moderate activity by reducing oedema by 49% and 54%. Compound **10b** showed poor anti-inflammatory activity. The potency of the compound **10d** is attributed to the presence of the electron-withdrawing fluoro at 4th position of the substituted thioamide moiety.

Inflammation, a dynamic process considered as a protective mechanism, leads to chronic inflammatory state when deregulated [17]. Indigenous drug systems can be

Table 2. Effect of compounds **9a–d** on carrageenan-induced oedema in rats.

Treatment	Dose (mg/kg)	Oedema (mL) (Mean ± SEM)			%-Inhibition after 3 h
		60 min	120 min	180 min	
Control		0.92 ± 0.03	1.15 ± 0.04	0.78 ± 0.03	–
	10	0.70 ± 0.02	0.90 ± 0.02	0.68 ± 0.04	13
9a	20	0.69 ± 0.03	0.70 ± 0.03	0.36 ± 0.03	54*
	30	0.64 ± 0.02	0.65 ± 0.03	0.35 ± 0.01	56**
	10	0.70 ± 0.01	0.91 ± 0.02	0.71 ± 0.01	9
9b	20	0.70 ± 0.01	0.70 ± 0.03	0.38 ± 0.04	52*
	30	0.65 ± 0.01	0.66 ± 0.03	0.36 ± 0.02	54*
	10	0.70 ± 0.02	0.89 ± 0.02	0.69 ± 0.01	12
9c	20	0.70 ± 0.01	0.68 ± 0.03	0.38 ± 0.03	52*
	30	0.65 ± 0.01	0.63 ± 0.02	0.34 ± 0.01	57**
	10	0.70 ± 0.02	0.89 ± 0.02	0.66 ± 0.01	16
9d	20	0.70 ± 0.01	0.70 ± 0.03	0.32 ± 0.03	59**
	30	0.65 ± 0.01	0.66 ± 0.03	0.27 ± 0.01	66**
Diclofenac sodium	20	0.40 ± 0.01	0.37 ± 0.01	0.22 ± 0.02	72**
F-Value		**289**	**121.17**	**240**	

Each value represents mean ± SE of six animals; * $P < 0.01$ when compared to control; ** $P < 0.001$ when compared to control.

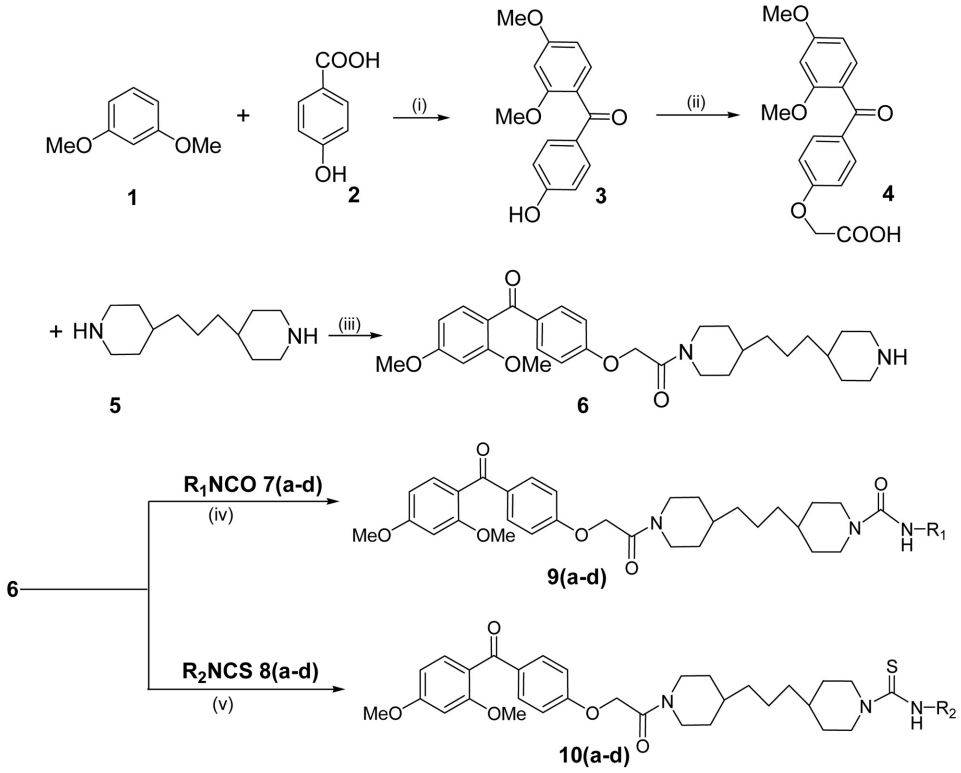
Table 3. Effect of compounds **10a–d** on carrageenan-induced oedema in rats.

Treatment	Dose (mg/kg)	Oedema (mL) (Mean ± SEM)			%-Inhibition after 3 h
		60 min	120 min	180 min	
Control		0.92 ± 0.03	1.19 ± 0.04	0.78 ± 0.03	–
	10	0.70 ± 0.02	0.90 ± 0.06	0.72 ± 0.01	8.0
10a	20	0.70 ± 0.03	0.70 ± 0.01	0.40 ± 0.03	49*
	30	0.65 ± 0.01	0.66 ± 0.01	0.36 ± 0.03	54*
	10	0.99 ± 0.01	1.10 ± 0.08	0.68 ± 0.01	13
10b	20	0.92 ± 0.01	1.109 ± 0.08	0.63 ± 0.08	20
	30	0.89 ± 0.01	1.103 ± 0.05	0.56 ± 0.04	29
	10	0.63 ± 0.01	0.66 ± 0.01	0.53 ± 0.01	33
10c	20	0.52 ± 0.01	0.50 ± 0.01	0.44 ± 0.01	44*
	30	0.51 ± 0.01	0.45 ± 0.01	0.33 ± 0.01	58**
	10	0.55 ± 0.01	0.54 ± 0.01	0.49 ± 0.01	40
10d	20	0.46 ± 0.01	0.42 ± 0.01	0.35 ± 0.01	56**
	30	0.43 ± 0.01	0.40 ± 0.01	0.26 ± 0.03	67**
Diclofenac sodium	20	0.39 ± 0.01	0.37 ± 0.01	0.22 ± 0.02	72**
F-Value		**18.18**	**243**	**454**	

Each value represents mean ± SE of four animals; * $P < 0.01$, when compared to control; ** $P < 0.001$, when compared to control.

the source of a variety of new drugs which can provide relief in inflammation. The most widely used primary test to screen new anti-inflammatory agent's measures the ability of a compound to reduce local oedema induced in the rat paw by injection of an irritant agent [18]. This oedema depends on the participation of kinins and polymorphonuclear leukocytes with their pro-inflammatory factors including prostaglandins [19]. Carrageenan is biochemically a sulphated polysaccharide obtained from seaweed (Rhodophyceae) and carrageenan-induced oedema has been commonly used as an experimental animal model for acute inflammation. The development of oedema in the paw of the rat after the injection of carrageenan has been described as a biphasic event. The early phase (1–2 h) of the carrageenan model is mainly attributed to the release of histamine, serotonin, and increased synthesis of prostaglandins into the damaged tissue surroundings. The late phase, which is accelerating the phase of swelling, is sustained by the release of prostaglandin-like substances and mediated by bradykinin, protease, leukotrienes, lysosomes, polymorphonuclear cells, and prostaglandins produced by tissue macrophages [20].

From the results obtained, electron-donating or electron-withdrawing groups attached to phenyl ring as substituents linked to the carboxamide / thiocarboxamide



Reaction and reagent condition: (i) $ZnCl_2$, $POCl_3$, 60-70°C, 2 h; (ii) $ClCH_2COOH$, K_2CO_3, reflux, 10 h; (iii) *N*-methylmorpholine, TBTU, *N,N*-dimethylformamide, 5 h; (iv) R_1NCO **7a – d**, triethylamine, dichloromethane, 5-6 h; (v) R_2NCS **8a-d**, triethylamine, dichloromethane, 5-6 h; (**7a**: 4-methoxyphenyl isocyanate; **8a**: 4-methoxyphenyl isothiocyanate; **7b**: 4-fluorophenyl isocyanate; **8b**: 2-chlorophenyl isothiocyanate; **7c**: 2-chlorophenyl isocyanate; **8c**: 2,4-dichlorophenyl isothiocyanate; **7d**: 2,4-dichlorophenyl isocyanate; **8d**: 4-fluorophenyl isothiocyanate).

Scheme 1. Synthesis of compounds **9a – d** and **10a – d**.

group are studied for anti-inflammatory efficacy. Upon introduction of electron-withdrawing chloro, fluoro, dichloro groups on the phenyl ring of **9b – d** and **10b – d** enhanced activity was observed, whereas the compounds containing an electron-donating methoxy group, **9a** and **10a**, showed a decrease in activity. On the other hand, compound **9d**, **10c** with two electron-withdrawing chloro groups at positions 2 and 4 showed superior activity when compared to **9c**, **10b** having only one chloro group at the 4-position. Another structural correlation of the synthesized compounds reveals that keeping the same substituents at the same position on the phenyl ring of both the carboxamide and thioamide series **9b**, **10d**, **9c**, **10d**, and **9d**, **10c**, both linkages are responsible for potent anti-inflammatory activity. This emphasizes that the nature of the functional linkage (-CO-NH- or -CS-NH-) influences the anti-inflammatory activity. The results of the present pharmacological investigation showed that compounds **9d** and **10d** possess a potential anti-inflammatory effect, which was evidenced by the significant reduction in paw oedema.

Conclusion

In conclusion, a series of novel compounds **9a – d** and **10a – d** were synthesized in good yield and their anti-inflammatory activities were evaluated (Scheme 1). Compounds **9d** and **10d** demonstrated potent anti-inflammatory activity in the tested experimental animal models. The two structural correlation studies reveal that both linkage and substituents on phenyl ring are responsible for the anti-inflammatory activity of these classes of agents. Further, an extensive research of these derivatives may lead to therapeutically very important anti-inflammatory compounds.

One of the authors (K. Vinaya) is grateful to the Council of Scientific and Industrial Research (CSIR) for financial assistance under CSIR-SRF order No. 09/119(0172)2K8 EMR-I. Elemental Analysis and IR spectroscopic data were obtained from instruments funded by DST – FIST and UGC-SAP (phase I) No.F.540/10/DRS/2004-05 (SAP-I) is greatly acknowledged.

The authors have declared no conflict of interest.

Experimental

Infrared (IR) spectra were recorded using a Jasco FTIR-4100 series (Jasco, Japan). Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Bruker AM-400 (Bruker Bioscience, USA), and chemical shifts are expressed in parts per million (ppm, for δ) relative to tetra methyl silane as an internal standard and DMSO-d_6 as solvent. Spin multiplets are given as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass and purity were recorded on a LC-MSD-Trap-XCT. Elemental (CHNS) analysis was obtained on Vario EL III Elementar (Elementar, Hanau, Germany). Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck made TLC plates (Merck, Germany). All of the reagents and chemicals were purchased from Sigma Aldrich Chemicals Pvt Ltd., Bengalooru, India.

Chemistry

For the synthesis of the target key intermediate compound **6**, the reaction sequences outlined in Scheme 1 were followed. (4-Hydroxyphenyl)(2,4-dimethoxyphenyl)methanone **3** was synthesized by Friedel–Crafts reaction with 1,3-dimethoxy benzene **1** (1 eq) and *p*-hydroxy benzoic acid **2** (1.2 eq) in the presence of phosphorus oxychloride (7.0 eq) and zinc chloride (2.5 eq) at 60 to 70°C for 2 h. The absence of –COOH and the presence of a phenolic-proton peak in ^{1}H-NMR and IR spectra confirmed the formation of compound **3**. Treatment of (4-hydroxyphenyl)(2,4-dimethoxyphenyl) methanone **3** with chloroacetic acid (4.5 eq) in potassium carbonate solution (7.0 eq), and refluxing for 10 h gave the *O*-alkylated product. The absence of Ar–OH and the presence of the -COOH proton peak in **4** confirmed the formation of the product. [4-(2,4-Dimethoxybenzoyl)-phenoxy]-acetic acid **4** (1.0 eq) and 4-(3-(piperidin-4-yl)propyl)piperidine **5** (1.0 eq) in *N*,*N*-dimethyl formamide (DMF) were taken, to which *N*-methylmorpholine (NMP) (3.0 eq) and 10% of *o*-benzotriazol-1-yl-*N*,*N*,*N*^{1},*N*^{1}-tetramethyl uranium tetrafluoroborate (TBTU) catalyst were added. The reaction mixture was stirred for 5 h at room temperature; this yielded the target key intermediate **6**. The absence of the –COOH proton peak and the presence of the -NH proton peak confirmed the formation of compound **6**. The nucleophilic substitution reaction of 2-[4-(2,4-dimethoxybenzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone **6** with differently substituted aromatic isocyanates (R-N=C=O) / isothiocyanates (R-N=C=S) was carried out in the presence of triethylamine and dichloromethane as solvent with a good yield of 74–80%. The absence of -NH and the presence of -CO-NH, -CS-NH proton peaks (respectively) in the synthesized derivatives **9a–d** and **10a–d** in ^{1}H-NMR and IR spectra confirmed the identity of the products. This was also confirmed by IR data for the carboxamide series **9a–d** and thioamide series **10a–d**; IR data showed stretching frequencies at 3350–3360 cm^{-1} for the -NH and 1640–1660 cm^{-1} for the -C=O group. The chemical structures and yields of all the synthesized compounds are given in Table 1.

*Procedure for the synthesis of (4-hydroxyphenyl)(2,4-dimethoxyphenyl)methanone **3***

A solution of *p*-hydroxy benzoic acid **2** (23.99 g, 17.39 mmol) was taken, phosphorus oxychloride (92.9 mL, 101.43 mmol) was added slowly under stirring condition. Then, dimethoxybenzene **1** (20 g, 14.49 mmol) was added, and finally zinc chloride was added (48.96 g, 36.22 mmol). The reaction mixture was heated at 60–70°C for 2 h. Progress of the reaction was monitored by TLC. Upon completion, the reaction mass was slowly poured into the ice cold water with stirring. The compound was extracted with ethyl acetate, the organic layer was washed with water and brine solution. Evaporation of the organic layer was carried out under reduced pressure followed by recrystallisation using methanol and water; pure compound was obtained with 90% yield. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.97 (s, 1H, Ar-H), 7.80 (d, 1H, Ar-H), 7.36 (d, 1H, Ar-H), 7.02 (d, 1H, Ar-H), 6.78 (d, 1H, Ar-H), 6.62 (dd, 2H, Ar-H), 8.18 (s, 1H, -OH). IR (KBr, cm^{-1}): 1669, 1042, 1258. Anal. calcd. for $C_{15}H_{14}O_4$ (%): C,69.76; H, 5.46. Found: C, 69.69; H, 5.41.

*Procedure for the synthesis of [4-(2,4-dimethoxybenzoyl)phenoxy]acetic acid **4***

Solutions of (4-hydroxyphenyl)(2,4-dimethoxyphenyl)methanone **3** (15 g, 5.81 mmol), and potassium carbonate (56.21 g, 40.67 mmol) were taken up in water (125 mL). Then, chloroacetic acid (24.7 g, 26.14 mmol) was slowly added in portions (1 eq / 1 h time gap) to the reaction mixture and adjusted to pH 9–10 by adding potassium carbonate solution. The reaction mixture was refluxed for 10 h. Progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was cooled to room temperature, diluted with water, acidified using 3 M HCl solution (pH = 4–5), and extracted twice with ethyl acetate. The organic layer was washed with water and brine solution until the bottom impurities were removed. The compound was extracted into the sodium carbonate solution from the organic layer and the aqueous layer was washed by using ethyl acetate to remove remaining initial compound. The aqueous layer was acidified using conc. HCl (pH = 1). The pure compound precipitated into the aqueous solution. The compound was filterd and dried. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.92 (s, 1H, Ar-H), 7.83 (d, 1H, Ar-H), 7.35 (d, 1H, Ar-H), 7.00 (d, 1H, Ar-H), 6.73 (d, 1H, Ar-H), 6.60 (dd, 2H, Ar-H), 5.18 (s, 2H, -OCH_2), 9.75 (s, 1H, -COOH). IR (KBr, cm^{-1}): 1674, 1045, 1248. Anal. calcd. for $C_{17}H_{16}O_6$ (%): C, 64.55; H, 5.10. Found: C, 64.50; H, 5.04.

*Procedure for the synthesis of 2-[4-(2,4-dimethoxybenzoyl)phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]ethanone **6***

A solution of [4-(2,4-dimethoxybenzoyl)-phenoxy]-acetic acid **4** (5 g, 1.57 mmol) in dry *N*,*N*-dimethyl formamide was taken, 4-(3-(piperidin-4-yl)propyl)piperidine **5** (3.32 g, 1.57 mmol) was added to the solution, and then *N*-methylmorpholine (4.78 g, 4.73 mmol) and 10% of the TBTU catalyst were added. The reaction mixture was stirred at room temperature for 5 h and progress of the reaction was monitored by TLC. Upon completion of the reaction, water was added and the reaction mixture was filtered, washed with ether, and dried under vacuum. A pink amorphous solid compound was obtained with 88% yield. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.02 (s, 1H, Ar-H), 7.78 (d, 1H, Ar-H), 7.34 (d, 1H, Ar-H), 6.97 (d, 1H, Ar-H), 6.78 (s, 1H, Ar-H), 6.60 (dd, 2H, Ar-H), 5.31 (s, 2H, -OCH_2), 3.84 (s, 3H, -OCH_3), 3.72 (s, 3H, -OCH_3), 2.87-3.03 (m, 8H, -N-CH_2-), 2.05 (s, 1H, -NH), 2.02 (br s, 2H, -CH_2-), 1.22–1.32 (m, 14H, -CH_2-). IR (KBr, cm^{-1}): 1687, 1040, 1249. Anal. calcd. for $C_{30}H_{40}N_2O_5$ (%): C, 70.84; H, 7.93; N, 5.51. Found: C, 70.79; H, 7.89; N, 5.46.

*General procedure for synthesis of 2-[4-(2,4-dimethoxybenzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone derivatives **9a-d** and **10a – d***

A solution of 2-[4-(2,4-dimethoxybenzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone **6** (1.0 eq) in dry dichloromethane was taken and cooled to 0 – 5°C in an ice bath. Triethylamine (3.0 eq) was added to this cold reaction mixture and stirred for 10 min, then different isocyanates (1.0 eq) or isothiocyanates (1.0 eq) were added and allowed to stir at room temperature for 5 – 6 h. Progress of the reaction mixture was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and the residue was taken up in water and extracted with ethyl acetate. The organic layer was washed with 10% ammonium chloride solution and finally the organic layer was washed with water and dried with anhydrous sodium sulphate. The solvent was evaporated to get the crude product which was purified by column chromatography over silica gel (60 – 120 mesh) using hexane / ethyl acetate (8 : 2) as eluent.

*Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxybenzoyl)phenoxy]acetyl]-4-piperidyl]propyl]-N-(4-methoxyphenyl)piperidine-1-carboxamide **9a***

The general synthetic method described above afforded **9a** and the product obtained was a pale yellow oil made from 2-[4-(2,4-dimethoxybenzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone **6** (0.25 g, 0.491 mmol), 4-methoxyphenyl isocyanate **7a** (0.328 g, 2.27 mmol), and triethylamine (0.667 g, 6.81 mmol). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.23 (s, 1H, -NH), 7.81 (d, 2H, Ar-H), 7.33 (m, 2H, Ar-H), 6.95 (m, 4H, Ar-H), 6.84 (d, 1H, Ar-H), 6.59 (d, 2H, Ar-H), 5.29 (s, 2H, -OCH_2), 3.89 (s, 3H, -OCH_3), 3.83 (s, 3H, -OCH_3), 3.73 (s, 3H, -OCH_3), 2.85 – 2.98 (m, 8H), 2.05 (bs, 2H), 1.09 – 1.28 (m, 14H). IR (KBr, cm^{-1}): 3356, 2889, 1716, 1648, 1293, 1250, 1124, 1105. MS (ESI) *m/z*: 658.34 [M + H$^+$]. Anal. calcd. for $C_{38}H_{47}N_3O_7$ (%): C, 69.38; H, 7.20; N, 6.39. Found: C, 69.44; H, 7.26; N, 6.36.

*Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxybenzoyl)phenoxy]acetyl]-4-piperidyl]propyl]-N-(4-fluorophenyl)piperidine-1-carboxamide **9b***

The general synthetic method described above afforded **9b** and the product obtained was a pale yellow oil made from 2-[4-(2,4-dimethoxybenzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone **6** (0.25 g, 0.491 mmol), 4-fluorophenyl isocyanate **7b** (0.301 g, 2.27 mmol), and triethylamine (0.667 g, 6.81 mmol). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.19 (s, 1H, -CONH), 7.79 (d, 2H, Ar-H), 7.27 – 7.33 (m, 2H, Ar-H), 6.94 (m, 4H, Ar-H), 6.86 (d, 1H, Ar-H), 6.54 (d, 2H, Ar-H), 5.25 (s, 2H, -OCH_2), 3.87 (s, 3H, -OCH_3), 3.72 (s, 3H, -OCH_3), 2.89 – 2.98 (m, 8H), 2.81 (bs, 2H), 1.74 (m, 2H), 1.09 – 1.26 (m, 12H). IR (KBr, cm^{-1}): 3346, 2885, 1710, 1639, 1287, 1252, 1121, 1039. MS (ESI) *m/z*: 646.32 [M + H$^+$]. Anal. calcd. for $C_{37}H_{44}FN_3O_6$ (%): C, 68.82; H, 6.87; N, 6.71. Found: C, 68.88; H, 6.82; N, 6.73.

*Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxybenzoyl)phenoxy]acetyl]-4-piperidyl]propyl]-N-(2-chlorophenyl)piperidine-1-carboxamide **9c***

The general synthetic method described above afforded **9c** and the product obtained was a pale yellow oil made from 2-[4-(2,4-dimethoxybenzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone **6** (0.25 g, 0.491 mmol), 2-chlorophenyl isocyanate **7c** (0.337 g, 2.27 mmol), and triethylamine (0.667 g, 6.81 mmol). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.20 (s, 1H, -NH), 7.80 (d, 2H, Ar-H), 7.35 (m, 2H, Ar-H), 7.12 (m, 4H, Ar-H), 6.89 (d, 1H, Ar-H), 6.62 (d, 2H, Ar-H), 5.27 (s, 2H, -OCH_2), 3.84 (s, 3H, -OCH_3), 3.72 (s, 3H, -OCH_3), 2.83 – 3.0 (m, 8H), 2.03 (bs, 2H), 1.12 – 1.28 (m, 14H). IR (KBr, cm^{-1}): 3361, 2920, 2862, 1728, 1674, 1278, 1256, 1123, 1045, 725. MS (ESI) *m/z*: 662.30 [M + H$^+$]. Anal. calcd. for $C_{37}H_{44}ClN_3O_6$ (%): C, 67.11; H, 6.70; N, 6.35. Found: C, 67.16; H, 6.77; N, 6.41.

*Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxybenzoyl)phenoxy]acetyl]-4-piperidyl]propyl]-N-(2,4-dichlorophenyl)piperidine-1-carboxamide **9d***

The general synthetic method described above afforded **9d** and the product obtained was a pale yellow oil made from 2-[4-(2,4-dimethoxybenzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone **6** (0.25 g, 0.491 mmol), 2,4-dichlorophenyl isocyanate **7d** (0.413 g, 2.27 mmol), and triethylamine (0.667 g, 6.81 mmol). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.22 (s, 1H, -NH), 7.76 (d, 1H, Ar-H), 7.33 (d, 1H, Ar-H), 7.25 (d, 2H, Ar-H), 7.02 (m, 2H, Ar-H), 6.94 (d, 1H, Ar-H), 6.68 (d, 2H, Ar-H), 6.51 (bs, 1H, Ar-H), 5.31 (s, 2H, -OCH_2), 3.84 (s, 3H, -OCH_3), 3.73 (s, 3H, -OCH_3), 2.87 – 3.02 (m, 8H), 2.02 (bs, 2H), 1.12 – 1.22 (m, 14H). IR (KBr, cm^{-1}): 3356, 2935, 2874, 1731, 1640, 1270, 1248, 1120, 1041, 723. MS (ESI) *m/z*: 697.25 [M + H$^+$]. Anal. calcd. for $C_{37}H_{43}Cl_2N_3O_6$ (%): C, 63.79; H, 6.22; N, 6.03. Found: C, 63.84; H, 6.18; N, 6.09.

*Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxybenzoyl)phenoxy]acetyl]-4-piperidyl]propyl]-N-(4-methoxyphenyl)piperidine-1-carbothioamide **10a***

The general synthetic method described above afforded **10a** and the product obtained was a pale yellow oil from 2-[4-(2,4-dimethoxybenzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone **6** (0.25 g, 0.491 mmol), 4-methoxyphenyl isothiocyanate **8a** (0.292 g, 1.77 mmol), and triethylamine (0.667 g, 6.81 mmol). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.25 (s, 1H, -NH), 7.79 (d, 2H, Ar-H), 7.35 (m, 2H, Ar-H), 6.96 (m, 4H, Ar-H), 6.82 (d, 1H, Ar-H), 6.56 (d, 2H, Ar-H), 5.25 (s, 2H, -OCH_2), 3.85 (s, 3H, -OCH_3), 3.80 (s, 3H, -OCH_3), 3.72 (s, 3H, -OCH_3), 2.02 (bs, 8H), 1.58 – 1.75 (bs, 4H), 1.09 – 1.22 (m, 12H), 1.0 (m, 4H). IR (KBr, cm^{-1}): 2939, 2881, 1730, 1643, 1274, 1245, 1128, 1117, 1041. MS (ESI) *m/z*: 674.32 [M + H$^+$]. Anal. calcd. for $C_{38}H_{47}N_3O_6S$ (%): C, 67.73; H, 7.03; N, 6.24. Found: C, 67.80; H, 6.98; N, 6.28.

*Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxybenzoyl)phenoxy]acetyl]-4-piperidyl]propyl]-N-(2-chlorophenyl)piperidine-1-carbothioamide **10b***

The general synthetic method described above afforded **10b** and the product obtained was a pale yellow oil from 2-[4-(2,4-dimethoxybenzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone **6** (0.25 g, 0.491 mmol), 2-chlorophenyl isothiocyanate **8b** (0.30 g, 1.77 mmol), and triethylamine (0.667 g, 6.81 mmol). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.19 (s, 1H, -NH), 7.81 (d, 2H, Ar-H), 7.33 (m, 2H, Ar-H), 7.21 (m, 4H, Ar-H), 6.91 (d, 1H, Ar-H), 6.60 (d, 2H, Ar-H), 5.29 (s, 2H, -OCH_2), 3.83 (s, 3H, -OCH_3), 3.70 (s, 3H, -OCH_3), 2.85 – 2.98 (m, 8H), 2.05 (bs, 2H), 1.12 – 1.2 (m, 14H). IR (KBr, cm^{-1}): 2952, 2876, 1741, 1648, 1270, 1241, 1129, 1097,

722. MS (ESI) *m/z*: 678.27 [M + H$^+$]. Anal. calcd. for $C_{37}H_{44}ClN_3O_5S$ (%): C, 65.52; H, 6.54; N, 6.20. Found: C, 65.58; H, 6.59; N, 6.26.

*Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxybenzoyl)phenoxy]acetyl]-4-piperidyl]propyl]-N-(2,4-dichlorophenyl)piperidine-1-carbothioamide **10c***

The general synthetic method described above afforded **10c** and the product obtained was a pale yellow oil from 2-[4-(2,4-dimethoxybenzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone **6** (0.25 g, 0.491 mmol), 2,4-dichlorophenyl isothiocyanate **8c** (0.361 g, 1.77 mmol), and triethylamine (0.667 g, 6.81 mmol). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.17 (s, 1H, -NH), 7.75 (d, 1H, Ar-H), 7.35 (d, 1H, Ar-H), 7.25 (d, 2H, Ar-H), 7.05 (m, 2H, Ar-H), 6.95 (d, 1H, Ar-H), 6.70 (d, 2H, Ar-H), 6.54 (bs, 1H, Ar-H), 5.34 (s, 2H, -OCH_2), 3.82 (s, 3H, -OCH_3), 3.73 (s, 3H, -OCH_3), 2.85 – 2.98 (m, 8H), 2.05 (bs, 2H), 1.09 – 1.22 (m, 14H), 1.0 (m, 4H). IR (KBr, cm^{-1}): 2959, 2880, 1735, 1653, 1276, 1240, 1124, 1058, 727. MS (ESI) *m/z*: 713.23 [M + H$^+$]. Anal. calcd. for $C_{37}H_{43}Cl_2N_3O_5S$ (%): C, 62.35; H, 6.08; N, 5.90. Found: C, 62.41; H, 6.13; N, 5.96.

*Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxybenzoyl)phenoxy]acetyl]-4-piperidyl]propyl]-N-(4-fluorophenyl)piperidine-1-carbothioamide **10d***

The general synthetic method described above afforded **10d** and the product obtained was a pale yellow oil from 2-[4-(2,4-dimethoxybenzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone **6** (0.25 g, 0.491 mmol), 4-fluorophenyl isothiocyanate **8d** (0.271 g, 1.77 mmol), and triethylamine (0.667 g, 6.81 mmol). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.23 (s, 1H, -NH), 7.80 (d, 2H, Ar-H), 7.28 (m, 2H, Ar-H), 6.96 (m, 4H, Ar-H), 6.53 (d, 2H, Ar-H), 5.27 (s, 2H, -OCH_2), 3.92 (s, 3H, -OCH_3), 3.83 (s, 3H, -OCH_3), 2.85 – 2.92 (m, 8H), 2.08 (bs, 2H), 1.09 – 1.19 (m, 14H), 1.0 (m, 4H). IR (KBr, cm^{-1}): 2892, 1717, 1632, 1280, 1252, 1125, 1041. MS (ESI) *m/z*: 662.30 [M + H$^+$]. Anal. calcd. for $C_{37}H_{44}FN_3O_5S$ (%): C, 67.15; H, 6.70; N, 6.35. Found: C, 67.21; H, 6.65; N, 6.36.

Pharmacological activity

The experiments were performed with male albino rats of the Charles-Foster strain of weighing between 150 – 200 g. Acute toxicity was tested in albino mice (15 – 25 g). The animals were procured from the Central animal house, National College of Pharmacy, Shimoga, Karnataka, India. Food (chaw pallet) and water were given to the animals *ad libitum*. All the compounds were dissolved in propylene glycol. Diclofenac sodium drug was used as reference drug.

Anti-inflammatory activity

The method of Winter *et al.* [21] was used to study anti-inflammatory activity using a plethysmograph apparatus to measure the paw volume. The rats were divided into three groups (control, drug-treated, and standard drug) of six animals each. A mark was made on both the hind paws just below the tibio-tarsal junction so that every time the paw could be dipped in the mercury column of the plethysmograph up to the mark to ensure constant paw volume. After 30 min of above treatment, an inflammatory oedema was induced in the left hind paw by injecting 0.1 mL of 1% carrageenin solution in the plantar tissue of the paw of all the animals. The right paw served as non-inflamed paw reference for comparison. The initial paw volume was measured plethysmographically with in 30 s of the injection. The relative increase in paw volume was measured in control, standard, and treated group at 60, 120, and 180 min after carrageenin injection. The percentage increase in the paw volume over the initial reading was calculated. This increase in the paw volume in the animals treated with the standard drug and the different doses of the compounds were compared with the increase in paw volume of untreated control animals after 60, 120, and 180 min. The percentage anti-inflammatory activity was calculated according to the formula as given below:

$$\text{Percentage of inhibition of oedema} = (1 - V_t/V_c)\,100 \quad (1)$$

Where V_t and V_c are the volume of oedema in paw of rats in the drug treated and control group, respectively.

Preparation of carrageenin suspension

1% w/v suspension of carrageenin was prepared by sprinkling 100 mg of carrageenin powder in 10 mL of saline solution (0.9% w/v NaCl) and set aside to soak for 1 h. A homogenous suspension was then obtained by thorough mixing with a magnetic stirrer.

Acute toxicity

The approximate lethal doses (ALD_{50}) of all the compounds were investigated according to the method of Smith [22]. All the compounds were studied for acute toxicity; ALD_{50} value were found to be >1000 mg/kg *p.o.*.

Statistical analysis

Results are expressed as mean ± SEM. Statistical Analysis was carried out using One-Way ANOVA followed by Dunnets't' tests. Results were considered highly significant if $P < 0.01$ and less significant if $P < 0.05$.

References

[1] J. W. Hadden, R. G. Coffey, *Immunopharmacol. Rev*. **1990**, *1*, 273 – 376.

[2] M. E. Wolff. (Ed.), *Burger's Medicinal Chemistry: Part III, 4th edn*. John Wiley and Sons, New York **1981**, pp. 1205 – 1273.

[3] A. Palomer, J. J. Perez, S. Navea, O. Llorens, *et al.*, *J. Med. Chem.* **2000**, *43*, 2280 – 2284; A. Palomer, J. Pascual, M. Cabre, L. Borras, *et al.*, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 533 – 537.

[4] J. Jiri, P. Miroslav, P. Josef, W. Stanislav, *Csech CS.* **1991**, *271*, 185 – 191, [Chem. Abstr. **1992**, *117*, 170994d].

[5] M. Williams, E. A. Kowaluk, S. P. Arneric, *J. Med. Chem.* **1999**, *42*, 1481 – 1500.

[6] I. Yoshiyuki, K. Miwako, K. Shusuke, *Jpn. Kokai Tokkya Koho JP.* **1991**, *03*, 209, 318 [Chem. Abstr. **1992**, *116*, 99311a].

[7] E. R. Ottosen, M. D. Sorensen, F. Bjorkling, T. Skak-Nielsen, *et al.*, *J. Med. Chem.* **2003**, *46*, 5651 – 5662.

[8] I. G. Mobia, A. T. Soldatendkov, V. O. Fedorov, E. A. Ageev, *et al.*, *Khim. Farm. Zh.* **1989**, *23*, 421 – 427.

[9] C. N. Nalini, M. Pharm Dissertation, Dr. M.G.R. Medical University, India, 1997.

[10] V. Georgiev, B. Petkova, *Acta Physiol. Pharmacol. Bulg.* **1974**, *2*, 76 – 81.

[11] S. Vankov, J. Nachnoizsled, *Khim. Farm. Inst.* **1974**, *9*, 231 – 235.

[12] W. B. Severs, W. J. Kinnard, J. P. Buckley, *J. Pharm. Sci.* **1965**, *54*, 1025 – 1031.

[13] N. A. Iskarev, K. S. Shadurshkii, *Farmakol. Toksikol.* **1965**, *28*, 184 – 188.

[14] B. Ileana, V. Dobre, N. J. Duaz, *J. Prakt. Chem.* **1985**, *327*, 667 – 674.

[15] C. R. Ganellin, R. G. W. Spickett, *J. Med. Chem.* **1965**, *8*, 619 – 625.

[16] D. C. Reeves, M. Congreve, C. W. Murray, R. Carr, *Nat. Rev. Drug Discovery* **2004**, *3*, 660 – 673.

[17] M. Ahmed, H. A. Shikha, S. K. Sadhu, M. T. Rahman, B. K. Datta, *Pharmazie* **2001**, *56*, 657 – 660.

[18] C. A. Winter, E. A. Risely, G. W. Nuss, *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544 – 547.

[19] J. Damas, G. Remacle-Volon, E. Deflandre, *Arch. Pharmacol.* **1986**, *332*, 196 – 200.

[20] A. R. M. S. Brito, M. A. Antonio, *J. Ethnopharmacol.* **1998**, *61*, 215 – 228.

[21] C. A. Winter, E. A. Risley, G. M. Nuss, *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544 – 550.

[22] Q. E. Smith (Ed.), *Pharmacological Screening Test Progress in Medicinal Chemistry-I.* Butterworth, London **1960**, pp. 1 – 33.

