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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, analgesic and anti-inflammatory activities of chalconyl-incorporated hydrazone derivatives of mefenamic acid

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Abstract A series of chalconyl-incorporated hydrazone derivatives of mefenamic acid was synthesized in order to obtain new compounds with potential analgesic and anti-inflammatory activity having lesser side effects. The structures of all synthesized compounds were confirmed by means of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectra. All compounds were evaluated for their analgesic and anti-inflammatory activities by tail-flick method and carrageenan-induced rat paw edema test, respectively. Among all the synthesized compounds, compounds (**4a**) and (**4j**) exhibited the most prominent and consistent anti-inflammatory activity. In acute ulcerogenicity study, it can be concluded that compounds (**4a**) and (**4j**) are devoid of the deadlier gastrointestinal toxicities.

Keywords Mefenamic acid · Hydrazones · Chalcone · Analgesic · Anti-inflammatory

Introduction

Pain is a problem not solved by medicine and is the most common reason that patients seek advice from pharmacists and other professionals and represent important therapeutic and economic cost for the community. According to the World Health Organization, 90 % of diseases are associated with pain (Gokçe *et al.*, 2009). Humans have been using nonsteroidal anti-inflammatory drugs (NSAIDs) in various forms for more than 3,500 years and have been among the most widely used drugs for the treatment of pain and inflammation (Vane, 2000). NSAIDs develop their mode of action by blocking the cyclooxygenase (COX) enzyme and thus the biosynthesis of Prostaglandins (PGs). Cyclooxygenase (COX) is the key enzyme in the rate-limiting step that converts arachidonic acid to prostaglandins and thromboxane (Vane, 1971; O'Banion *et al.*, 1991). Three isoforms of the COX enzyme have been characterized: cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and cyclooxygenase-3 (COX-3) (Willoughby *et al.*, 2000).

COX-1 is the constitutional isoenzyme that is important in maintaining renal function, platelet function and the gastrointestinal epithelium (Mitchell and Warner, 1999). The isoenzyme COX-2 is inducible isoenzyme and primarily associated with inflammation. Cytokines and growth factors increase the expression of COX-2, mainly at inflammatory sites, producing prostaglandins that mediate inflammation, pain and fever (Crofford, 1997). A splice variant of COX-1 mRNA isolated that was found in high concentrations in the heart and cerebral cortices of the dog, which they reported to be COX-3 (Chandrasekharan *et al.*, 2002). Expression of COX-3 mRNA is particularly marked in the hypothalamus, pituitary and choroid plexus, sites which are targets of antipyretic action of paracetamol. COX-3 mRNA accounts for 30 % of COX-1 mRNA levels. Inducible COX-3 may have a role in remission periods in chronic inflammatory disease (Kam and So, 2009).

The major adverse effects of NSAIDs are gastrotoxic (e.g., damage of gastric mucosa may provoke gastric

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bleeding and gastroduodenal ulcers), increased bleeding tendency and delay of the birth process. Selective COX-2 inhibitors fail to inhibit constitutive COX-1 isotherm and have no gastrointestinal adverse effects. However, it has been suggested that COX-2 inhibitors, like rofecoxib and celecoxib, may be prothrombic and increase the risk of myocardial infarction (Savić *et al.*, 2011). Therefore, a synthesis of novel NSAIDs, with potent anti-inflammatory, analgesic and antipyretic action, with no adverse effects is highly desired.

Chalcones belong to the flavonoid family from plant origin and some of them possess anti-inflammatory activity (Kim *et al.*, 2007; Bano *et al.*, 2013; Bonifait *et al.*, 2014). A new pharmacophore 'Chalconesemicarbazone' was designed by pharmacophore hybridization approach of drug design. A series of novel 'Chalconylsemicarbazide' derivatives was synthesized and evaluated for their analgesic and anti-inflammatory activity. Most of the compounds were found to be more or comparable potent than the reference standard drugs (Singh *et al.*, 2010).

Mefenamic acid is a well-known nonsteroidal anti-inflammatory drug. 1,3,4-oxadiazole derivatives of mefenamic acid were synthesized and evaluated for analgesic and anti-inflammatory activity. The oxadiazole ring being weak acidic in nature reduces the ulcerogenicity of mefenamic acid and retains its anti-inflammatory potential (Somani and Bhanushali, 2011). A series of *N*-arylhydrazone derivatives of mefenamic acid was synthesized and evaluated for analgesic and anti-inflammatory activity. From the results, it was concluded that replacement of the acidic moiety of mefenamic acid with *N*-arylhydrazone moiety can create potent analgesic anti-inflammatory compounds (Almasirad *et al.*, 2005).

In the present work, we planned to incorporate the substituted chalcones to the hydrazide derivative of mefenamic acid moiety as powerful and novel non-ulcerogenic analgesic, anti-inflammatory lead-candidates.

Results and discussion

Chemistry

The synthetic route used to synthesize title compounds is outlined in Scheme 1. Methyl-2-(2,3-dimethylphenylamino)benzoate (**1**), the starting material, was prepared according to the method reported in the literature, using 2-(2,3-dimethylphenylamino)benzoic acid (mefenamic acid) (Chandrasekhar *et al.*, 2012). The 2-(2,3-dimethylphenylamino)benzohydrazide (**2**) was prepared by esterification of 2-(2,3-dimethylphenylamino)benzoic acid followed by treatment with hydrazine hydrate in absolute ethanol.

Various acetophenones were reacted with substituted aromatic aldehydes and gave chalcones by via Claisen Schmidt reaction (**3a–3j**). The proposed structures of the compounds were confirmed on the basis of elemental analysis and spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR). The IR spectra showed C=C absorption bands at 1,613–1,576 cm^{-1}. The ^{1}H NMR spectra of the compounds **3a–3j** showed the two doublets of CH=CH in the region $\delta = 7.69$–7.83 and 8.06–8.26 ppm. The remaining protons appeared at the expected chemical shifts.

In the last step, chalcones (**3a–3j**) were reacted with 2-(2,3-dimethylphenylamino)benzohydrazide (**2**) and gave chalconyl-incorporated hydrazone derivatives of mefenamic acid (**4a–4j**). The proposed structures of **4a–4j** were confirmed by elemental analysis and spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR). The IR spectra showed C=N absorption bands at 1,639–1,611 cm^{-1}. The ^{1}H NMR spectra of the compounds **4a–4j** showed the one singlet of –NH–N=C– at the region $\delta = 8.97$–9.23 ppm. For the compounds (**4a–4j**), the signals belonging to benzylidene group were observed at aromatic region, while the signals belonging to $-NHNH_2$ disappeared, indicating functionalization of hydrazide to hydrazone with substituted chalcones. The remaining protons appeared at the expected chemical shifts. The physical data, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data for all the synthesized compounds are reported in experimental protocols.

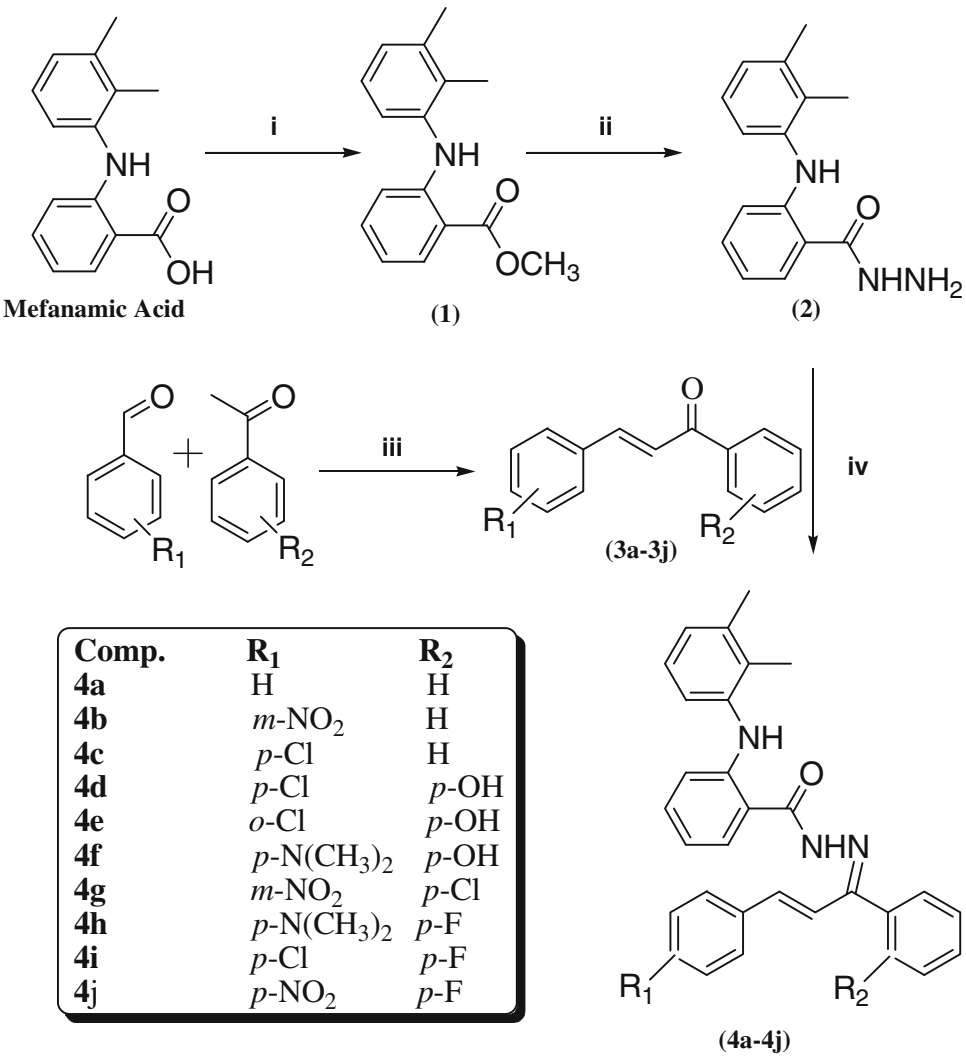
Pharmacology

In the pharmacological study, we have investigated in vivo analgesic and anti-inflammatory activity as well as the acute ulcerogenicity of chalconyl-incorporated hydrazone derivatives of mefenamic acid (**4a–4j**). Mefenamic acid, the parent compound, was used as a reference standard. The experiments were performed on albino rats of Wistar strain of either sex, weighing 200–250 g (from animal house of BN College of Pharmacy, Udaipur). The animals were maintained at 25 ± 2 °C, 50 ± 5 % relative humidity and 12 h light/dark cycles. The animals were fasted for 24 h prior to the experiments, and water provided ad libitum. The test compounds were suspended in 1 % aqueous carboxy methyl cellulose (CMC) solution and administered orally to experimental animals (Table 1).

Analgesic activity

The analgesic activity was determined in vivo by the abdominal constriction test induced by tail-flick method. All the compounds (**4a–4j**) were screened for analgesic activity. The analgesic activity was evaluated at equimolar doses equivalent to 25 mg/kg (mefenamic acid) body weight (Khan and Akhter, 2005). From the results, it was

Scheme 1 Synthesis of chalconyl-incorporated hydrazone derivatives of mefenamic acid: (*i*) CH_3OH, Conc. HCl; (*ii*) $NH_2NH_2.H_2O$; (*iii*) EtOH, NaOH; (*iv*) $CHCl_3$, CH_3COOH



Comp.	R_1	R_2
4a	H	H
4b	*m*-NO_2	H
4c	*p*-Cl	H
4d	*p*-Cl	*p*-OH
4e	*o*-Cl	*p*-OH
4f	*p*-$N(CH_3)_2$	*p*-OH
4g	*m*-NO_2	*p*-Cl
4h	*p*-$N(CH_3)_2$	*p*-F
4i	*p*-Cl	*p*-F
4j	*p*-NO_2	*p*-F

Table 1 Results of anti-inflammatory activity of entitled compounds (**4a–4j**) against carrageenan-induced rat paw edema model in rats

Compounds	Volume of edema (mean) ± SEM (ml)				Anti-inflammatory activity (% inhibition)			
	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
Control	0.030 ± 0.001	0.035 ± 0.001	0.050 ± 0.006	0.033 ± 0.005	–	–	–	–
Mefanamic acid	0.021 ± 0.003^{ns}	0.013 ± 0.001**	0.007 ± 0.001**	0.003 ± 0.000**	29.16	60.71	85.00	90.56
4a	0.023 ± 0.003^{ns}	0.015 ± 0.002**	0.006 ± 0.006**	0.004 ± 0.001**	20.83	55.34	87.50	86.79
4b	0.012 ± 0.001**	0.026 ± 0.003^{ns}	0.028 ± 0.003*	0.026 ± 0.002^{ns}	58.33	23.20	42.50	18.86
4c	0.024 ± 0.002^{ns}	0.016 ± 0.002**	0.007 ± 0.002**	0.005 ± 0.001**	18.73	51.77	85.00	84.90
4d	0.025 ± 0.002^{ns}	0.015 ± 0.001**	0.005 ± 0.001**	0.002 ± 0.001**	14.56	57.14	90.00	92.45
4e	0.019 ± 0.004*	0.026 ± 0.002^{ns}	0.030 ± 0.001^{ns}	0.028 ± 0.002^{ns}	35.40	23.20	40.00	60.36
4f	0.019 ± 0.001*	0.023 ± 0.004**	0.013 ± 0.013**	0.009 ± 0.004**	35.40	32.14	72.50	71.70
4g	0.024 ± 0.002^{ns}	0.026 ± 0.002^{ns}	0.020 ± 0.006**	0.013 ± 0.004**	18.70	23.70	58.70	60.36
4h	0.028 ± 0.004^{ns}	0.021 ± 0.003**	0.014 ± 0.003**	0.008 ± 0.003**	06.20	39.28	71.20	75.47
4i	0.015 ± 0.003**	0.021 ± 0.004**	0.016 ± 0.003**	0.009 ± 0.002**	47.90	39.28	67.50	71.70
4j	0.026 ± 0.003^{ns}	0.020 ± 0.002**	0.010 ± 0.001**	0.005 ± 0.000**	12.50	40.05	78.70	83.02

Data analyzed by one-way ANOVA followed by Dunnett's '*t*' test, ($n = 6$)

ns not significant

* $P < 0.05$; ** $P < 0.01$ significant from control

Table 2 Results of analgesic activity of entitled compounds (**4a–4j**) against tail-flick method

Compounds	Reaction time (mean ± SEM) s				Relative analgesic activity (%)			
	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
Control	2.23 ± 0.110	2.41 ± 0.081	2.79 ± 0.143	3.50 ± 0.091	–	–	–	–
Mefanamic acid	3.99 ± 0.208**	5.28 ± 0.153**	5.94 ± 0.118**	6.81 ± 0.183**	100	100	100	100
4a	3.36 ± 0.163**	4.30 ± 0.128**	5.11 ± 0.249**	6.15 ± 0.144**	63.93	65.95	73.53	80.13
4b	2.71 ± 0.087^{ns}	3.51 ± 0.143**	3.45 ± 0.407^{ns}	4.30 ± 0.362^{ns}	27.06	38.50	20.96	24.10
4c	2.87 ± 0.204^{ns}	4.04 ± 0.046**	4.74 ± 0.142**	5.86 ± 0.224**	36.29	56.89	62.03	71.36
4d	3.60 ± 0.242**	4.72 ± 0.232**	5.01 ± 0.334**	5.71 ± 0.377**	77.89	80.76	70.36	66.88
4e	2.62 ± 0.182^{ns}	3.49 ± 0.128**	3.81 ± 0.329^{ns}	4.80 ± 0.348**	21.76	37.80	32.40	39.21
4f	3.44 ± 0.283*	4.71 ± 0.162**	5.33 ± 0.258**	5.69 ± 0.257**	68.94	80.24	80.74	66.13
4g	2.89 ± 0.206**	3.07 ± 0.292*	3.53 ± 0.234^{ns}	4.26 ± 0.314^{ns}	37.43	23.27	23.44	22.89
4h	3.14 ± 0.243^{ns}	4.21 ± 0.120**	4.45 ± 0.106**	5.84 ± 0.238**	51.39	62.82	52.57	70.91
4i	2.79 ± 0.090*	3.00 ± 0.065^{ns}	3.40 ± 0.358^{ns}	4.24 ± 0.102^{ns}	31.62	20.55	19.31	22.28
4j	3.40 ± 0.322**	4.18 ± 0.088**	5.33 ± 0.185**	6.38 ± 0.151**	66.66	61.67	80.78	86.99

Data analyzed by one-way ANOVA followed by Dunnett's '*t*' test, ($n = 6$)

ns not significant

* $P < 0.05$, ** $P < 0.01$ significant from control

Table 3 Ulcerogenic effects of compounds **4a** and **4j** in comparison with mefanamic acid

Compounds	Ulcer score (mean ± SEM)	Ulcer index
Control	0.333 ± 0.105	–
Mefanamic acid	2.167 ± 0.401**	1.834
4a	0.750 ± 0.112^{ns}	0.417
4j	1.167 ± 0.279^{ns}	0.834

Data analyzed by one-way ANOVA followed by Dunnett's '*t*' test, ($n = 6$)

ns not significant

* $P < 0.05$; ** $P < 0.01$ significant from control

noticed that most of the compounds possess significant analgesic activity (Table 2). Compounds **4a** and **4j** showed comparable analgesic activity to standard mefenamic acid.

Anti-inflammatory activity

Anti-inflammatory activity of the synthesized compounds was evaluated by carrageenan-induced rat paw edema model, that is, equivalent to 25 mg/kg, of mefenamic acid subplanter injection of 0.1 ml, 1 % carrageenan produced an increase in paw volume (edema) of all the animals of various groups. The onset of action was evident from 1 h in various test groups. The significant ($P < 0.01$) reduction in rat paw edema was observed by most of the test compounds at 4 h compared to control group (Table 1).

From close inspection of the results of in vivo experiments, we can conclude that substituted chalconyl hydrazone derivatives of mefenamic acid yielded compounds with different therapeutic efficacy. In this series, compounds **4a**, **4c**, **4d** and **4j** exhibited very significant anti-inflammatory activity compared to standard drug mefenamic acid, among them **4d** (*p*-chloro and *p*-hydroxy substituted) showed 92.45 % inhibition, whereas standard mefenamic acid showed 90.56 % inhibition.

Based on the findings of these preclinical results, further studies need to be carried out to investigate the other specifications, such as in vitro assays, chronic ulcerogenicity studies and toxicological studies.

From the analgesic and anti-inflammatory activity profile, compounds **4a** and **4j** were subjected to ulcerogenicity potential test at four times the therapeutic doses with additional physical (cold) stress.

Acute ulcerogenicity studies

Ulcerogenic effect of most active chalconyl-incorporated hydrazone derivatives of mefenamic acid (**4a** and **4j**) was evaluated for gastric ulcerogenic potential in the rat stress model at four times the therapeutic doses. When compared with standard, these compounds showed less ulceration than the standard drugs. The results are shown in Table 3.

Computational studies

The TPSA and molecular volume of synthesized compounds (**4a–4j**) were calculated as shown in Table 4. Topological polar surface area (TPSA), i.e., surface belonging to polar atoms, is a descriptor that was shown to correlate with passive molecular transport through

Table 4 Molecular properties calculated by Molinspiration important for good oral bioavailability

Compounds	MV	TPSA	%ABS
Mefanamic acid	227.97	49.32	91.98
4a	425.85	90.54	77.76
4b	449.19	74.73	83.21
4c	439.39	90.54	77.76
4d	447.41	83.56	80.17
4e	447.41	83.56	80.17
4f	479.78	82.44	80.55
4g	462.72	83.56	80.17
4h	476.69	89.42	78.15
4i	444.32	90.54	77.76
4j	454.12	74.73	83.21

MV molecular volume, *TPSA* topological polar surface area, *%ABS* percentage of absorption

membranes and, therefore, allows prediction of transport properties of drugs in the intestines and blood–brain barrier crossing (Ertl *et al.*, 2000). The percentage of absorption (%ABS) was calculated using TPSA. From all these parameters, it can be observed that all synthesized compounds exhibited a great %ABS ranging from 77.76 to 83.21 % (Table 4).

Conclusion

In conclusion, we described analog-based design of chalconyl-incorporated hydrazone derivatives of mefenamic acid for in vivo analgesic and anti-inflammatory activity as COX inhibitor. Most of the entitled compounds exhibited promising analgesic and anti-inflammatory activity. Among all the synthesized compounds, compounds (**4a**) and (**4j**) exhibited most prominent and consistent anti-inflammatory activity. From acute ulcerogenicity studies, it can be concluded that compounds (**4a**) and (**4j**) are devoid of the deadlier gastrointestinal toxicities.

Topological polar surface area (TPSA) and molecular volume were calculated using the web-based program Molinspiration. The percentage of absorption (%ABS) was calculated using TPSA. From all these parameters, it can be observed that all synthesized compounds exhibited a great %ABS ranging from 77.76 to 83.21 %.

The present investigation suggests that chalcone-incorporated hydrazone derivatives of conventional NSAIDs are promising template for the design of new gastric safe anti-inflammatory agents. Further, QSAR investigations are in progress to examine possible descriptors influences on various classes of COX inhibitors.

Experimental protocols

Synthetic procedures

Melting points of the synthesized compounds were determined in open capillary tubes and are uncorrected. Elemental analysis (C, H and N) was undertaken with a Perkin-Elmer model 240C analyzer, and all analyses were consistent with theoretical values (within 0.4 %) unless indicated.

IR absorption spectra were recorded on Bruker alpha, KBr diffuse reflectance. ^{1}H NMR and ^{13}C NMR spectra were recorded on the Bruker DPX-400 instrument at 400 and 100 MHz, respectively. The ^{1}H and ^{13}C chemical shifts are reported as parts per million (ppm) downfield from TMS (Me_4Si). ^{1}H NMR and IR spectra were consistent with the assigned structures. The LC mass spectra of the compounds were recorded on Shimadzu 8201PC spectrometer. The homogeneity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silica gel G (Merck)-coated aluminum plates, visualized by iodine vapor.

*Methyl 2-(2,3-dimethylphenylamino)benzoate (**1**)*

A solution of the appropriate mefenamic acid (10 mmol), absolute methanol (10 ml) and concentrated sulfuric acid (1 ml) was heated under reflux for the appropriate time 35–42 h. The solvent was evaporated under reduced pressure, the remaining contents cooled to room temperature and neutralized with a concentrated solution of sodium carbonate, and then the aqueous solution extracted with ether. The combined ether extracts were dried, and the solvent is removed under reduced pressure to yield the corresponding ester. Yield, 82 %; mp 176–180 °C; IR (KBr) υ_{max} 3,260 (–OH), 1,692 (C=O) 1,589 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 8.89$ (s, 1H, NH), 6.89–7.44 (m, 7H, Ar–H), 3.86 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.19 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 169.7$ (C=O), 148.3 (C_1–Ar_2), 148.2 (C_2–Ar_1), 139.9 (C_3–Ar_2), 134.7 (C_4–Ar_1), 133.8 (C_6–Ar_1), 127.5 (C_2–Ar_2), 124.8 (C_5–Ar_2), 124.4 (C_4–Ar_2), 121.7 (C_5–Ar_1), 121.2 (C_3–Ar_1), 120.1 (C_1–Ar_1), 119.5 (C_6–Ar_2), 54.8 (–OCH_3), 18.5 (–3–CH_3), 15.7(–2–CH_3); LCMS m/z $[M]^+$ 255.1; Anal. Calcd. for $C_{16}H_{17}NO_2$ (255.31): C, 75.27; H, 6.71; N, 5.49. Found: C, 75.36; H, 6.61; N, 5.44.

*2-(2,3-Dimethylphenylamino)benzohydrazide (**2**)*

A solution of hydrazine hydrate (99.9 %, 5 mmol) and the appropriate ethyl ester (1 mmol) was brought to a gentle reflux for the appropriate time 22 h and then cooled to

room temperature. The solid formed was filtered (ice/water mixture was added in some cases to complete precipitation) and washed with several portions of water. The filtrate was dried by suction. Crystallization of crude product from EtOH afforded the corresponding mefenamic acid hydrazides. Yield, 84 %; mp 202–204 °C; IR (KBr) υ_{max} 3,306 (NH), 2,909, 2,857 (CH Str.), 1,645 (C=O), 1,573 (CH=CH), 1,443, 1,423 (Ar. C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 8.73$ (s, 1H, NH), 8.27 (s, 1H, NH), 6.93–7.57 (m, 7H, Ar–H), 3.14 (s, 2H, NH_2), 2.35 (s, 3H, CH_3), 2.23 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,) $\delta = 174.7$ (C=O), 147.8 (C_1–Ar_2), 147.3 (C_2–Ar_1), 138.9 (C_3–Ar_2), 134.5 (C_4–Ar_1), 128.4 (C_6–Ar_1), 127.8 (C_2–Ar_2), 125.6 (C_5–Ar_2), 124.9 (C_5–Ar_2), 122.3 (C_4–Ar_2), 121.6 (C_3–Ar_1), 120.2 (C_1–Ar_1), 119.3 (C_6–Ar_2), 18.9 (–CH_3), 13.7 (–CH_3); LCMS m/z $[M]^+$ 255.1; Anal. Calcd. for $C_{15}H_{17}N_3O$ (255.31): C, 70.56; H, 6.71; N, 16.46. Found: C, 70.43; H, 6.63; N, 16.54.

*1,3-Diphenyl-2-propen-1-one (**3a**)*

A solution of benzaldehyde (12 g, 11.7 ml, 0.1 mol) in ethanol was mixed with acetophenone (10.60 g, 10.2 ml, 0.1 mol), and an aqueous solution of sodium hydroxide (10 %) was added to it till no turbidity occurs. The reaction mixture was stirred for 20 h and kept overnight at room temperature. The mixture was poured into crushed ice and acidified with dilute hydrochloric acid. The crude product so obtained was filtered and recrystallized from methanol and dried at room temperature. The completion of the reaction was monitored by running TLC (Singh *et al.*, 2010). Yield, 78 %; mp 58–60 °C; IR (KBr) υ_{max} 1,671 (C=O), 1,598 (CH=CH), 1,493 (Ar. C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 8.06$ (d, 1H, CH), 7.79 (d, 1H, CH), 6.96–7.33 (m, 10H, Ar–H); ^{13}C NMR (DMSO-d_6, 100 MHz,) $\delta = 193.9$ (C=O), 147.3 (C_3), 141.2 (C_1–Ar_1), 137.8 (C_1–Ar_3), 136.0 (C_4–Ar_1), 132.4 (C_4–Ar_2), 131.2 ($C_{3,5}$–Ar_3), 130.6 ($C_{3,5}$–Ar_1), 129.5 ($C_{2,6}$–Ar_1), 128.0 ($C_{2,6}$–Ar_3), 124.9 (C_1); LCMS m/z $[M]^+$ 208.1; Anal. Calcd. for $C_{15}H_{12}O$ (208.25): C, 86.51; H, 5.81. Found: C, 86.48; H, 5.88.

The other compounds **3b** to **3j** were prepared by the same procedure using the corresponding aldehydes and ketones.

*3-(3-Nitrophenyl)-1-phenylprop-2-en-1-one (**3b**)*

Yield, 81 %; mp 134–136 °C; IR (KBr) υ_{max} 1,661 (C=O), 1,608 (CH=CH), 1,349 (–NO_2), 1,349 (Ar. C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 8.12$ (d, 1H, CH), 7.72 (d, 1H, CH), 6.98–7.52 (m, 9H, Ar–H); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 192.8$ (C=O), 146.1 (C_3–Ar_3), 145.3 (C_3), 140.7 (C_1–Ar_1), 139.9 (C_1–Ar_3), 136.4 (C_6–Ar_3), 135.0 (C_4–Ar_1), 130.3 (C_5–Ar_3), 129.6 ($C_{2,6}$–Ar_1), 128.7 ($C_{3,5}$–Ar_1), 126.5 (C_4–Ar_3), 124.3 (C_2–Ar_3), 123.2 (C_2); LCMS m/z $[M]^+$ 253.1; Anal. Calcd. for $C_{15}H_{11}NO_3$ (253.25): C, 71.14; H, 4.38; N, 5.53. Found: C, 71.23; H, 4.31; N, 5.49.

*3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (**3c**)*

Yield, 64 %; mp 100–102 °C; IR (KBr) υ_{max} 2,898 (CH Str.), 1,672 (C=O), 1,591 (CH=CH), 1,445 (Ar. C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 8.17$ (d, 1H, CH), 7.83 (d, 1H, CH), 7.04–7.39 (m, 9H, Ar–H); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 189.3$ (C=O), 144.3 (C_1), 138.2 (C_1–Ar_1), 134.2 (C_4–Ar_3), 133.1 (C_1–Ar_3), 132.1 (C_4–Ar_1), 130.3 ($C_{3,5}$–Ar_3), 129.1 ($C_{2,6}$–Ar_3), 128.6 ($C_{2,6}$–Ar_1), 127.7 ($C_{3,5}$–Ar_1), 122.9 (C_2); LCMS m/z $[M]^+$ 242.0; Anal. Calcd. for $C_{15}H_{11}ClO$ (242.70): C, 74.23; H, 4.57. Found: C, 74.26; H, 4.63.

*3-(4-Chlorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**3d**)*

Yield, 61 %; mp 142–146 °C; IR (KBr) υ_{max} 3,627 (OH Str), 2,922 (CH Str.), 1,644 (C=O), 1,576 (CH=CH), 1,519 (Ar·C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 8.39$ (s, 1H, OH), 8.08 (d, 1H, CH), 7.78 (d, 1H, CH), 7.03–7.32 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 191.8$ (C=O), 164.2 (C_4–Ar_1), 145.4 (C_3), 138.2 (C_1–Ar_3), 134.1 (C_4–Ar_3), 133.3 ($C_{2,6}$–Ar_1), 131.2 ($C_{2,6}$–Ar_3), 129.3 ($C_{3,5}$–Ar_3), 128.1 (C_1–Ar_1), 123.9 (C_2), 116.8 ($C_{3,5}$–Ar_1); LCMS m/z $[M]^+$ 258.0; Anal. Calcd. for $C_{15}H_{11}ClO_2$ (258.70): C, 69.64; H, 4.29. Found: C, 69.69; H, 4.21.

*3-(2-Chlorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**3e**)*

Yield, 65 %; mp 166–168 °C; IR (KBr) υ_{max} 3,219 (OH), 1,650 (C=O), 1,588 (CH=CH), 1,517 (Ar. C=C), 755 (C–Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 8.32$ (s, 1H, OH), 8.19 (d, 1H, CH), 7.69 (d, 1H, CH), 6.91–7.39 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 191.3$ (C=O), 163.8 (C_4–Ar_1), 144.1 (C_3), 133.2 (C_2–Ar_3), 132.7 (C_1–Ar_3), 131.4 ($C_{2,6}$–Ar_1), 131.0 (C_3–Ar_3), 130.1 (C_4–Ar_3), 129.4 (C_1–Ar_1), 128.6 (C_6–Ar_3), 127.3 (C_5–Ar_3), 126.1 (C_2), 115.5 ($C_{3,5}$–Ar_1); LCMS m/z $[M]^+$ 258.0; Anal. Calcd. for $C_{15}H_{11}ClO_2$ (258.70): C, 69.64; H, 4.29. Found: C, 69.68; H, 4.19.

*3-(4-(Dimethylamino)phenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**3f**)*

Yield, 74 %; mp 84–88 °C; IR (KBr) υ_{max} 3,648 (OH Str.), 2,933, 2,899 (CH Str.), 1,612 (CH=CH), 1,653 (C=O), 1,507 (Ar·C=C), 1,362 (C–N) cm^{-1}; ^{1}H NMR (DMSO-d_6,

400 MHz,): $\delta = 8.53$ (s, 1H, OH), 8.16 (d, 1H, CH), 7.59 (d, 1H, CH), 6.84–7.46 (m, 8H, Ar–H), 2.63 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 192.1$ (C=O), 163.4 (C_4–Ar_1), 151.1 (C_4–Ar_3), 144.3 (C_3), 131.2 ($C_{2,6}$–Ar_1), 129.9 ($C_{2,6}$–Ar_3), 129.3 (C_1–Ar_1), 123.8 (C_1–Ar_3), 122.9 (C_2), 115.9 ($C_{3,5}$–Ar_1), 112.7 ($C_{3,5}$–Ar_3), 41.9 (–$N(CH_3)_2$); LCMS m/z $[M]^+$ 267.1; Anal. Calcd. for $C_{17}H_{17}NO_2$ (267.32): C, 76.38; H, 6.41; N, 5.24. Found: C, 76.32; H, 6.46; N, 5.31.

*1-(4-Chlorophenyl)-3-(3-nitrophenyl)prop-2-en-1-one (**3g**)*

Yield, 76 %; mp 132–134 °C; IR (KBr) υ_{max} 1,667 (C=O), 1,588 (CH=CH), 1,522 (Ar. C=C), 1,350 (–NO_2), 741 (C–Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 8.23$ (d, 1H, CH), 7.79 (d, 1H, CH), 6.74–7.59 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 191.7$ (C=O), 148.1 (C_3–Ar_3), 145.3 (C_3), 139.6 (C_4–Ar_1), 138.9 (C_1–Ar_3), 137.3 (C_1–Ar_1), 135.2 (C_6–Ar_3), 130.6 ($C_{2,6}$–Ar_1), 129.2 (C_5–Ar_3), 128.8 ($C_{3,5}$–Ar_1), 125.5 (C_4–Ar_3), 123.9 (C_2–Ar_3), 121.2 (C_2); LCMS m/z $[M]^+$ 287.0; Anal. Calcd. for $C_{15}H_{10}ClNO_3$ (287.69): C, 62.62; H, 3.50; N, 4.87. Found: C, 62.73; H, 3.44; N, 4.92.

*3-(4-(Dimethylamino)phenyl)-1-(4-fluorophenyl)prop-2-en-1-one (**3h**)*

Yield, 70 %; mp 96–98 °C; IR (KBr) υ_{max} 2,899 (CH Str.), 1,648 (C=O), 1,592 (CH=CH), 1,519 (Ar·C=C), 1,027 (C–F) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 8.21$ (d, 1H, CH), 7.73 (d, 1H, CH), 6.91–7.56 (m, 9H, Ar–H). 2.71 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 190.9$ (C=O), 164.4 (C_4–Ar_1), 152.1 (C_4–Ar_3), 147.6 (C_3), 136.8 (C_1–Ar_1), 134.0 ($C_{2,6}$–Ar_1), 130.9 ($C_{2,6}$–Ar_3), 124.8 (C_1–Ar_3), 122.3 (C_2), 115.8 ($C_{3,5}$–Ar_1), 112.7 ($C_{3,5}$–Ar_3), 42.9 (-$N(CH_3)_2$); LCMS m/z $[M]^+$ 269.1; Anal. Calcd. for $C_{17}H_{16}FNO$ (269.31): C, 75.82; H, 5.99; N, 5.20. Found: C, 75.69; H, 6.12; N, 5.28.

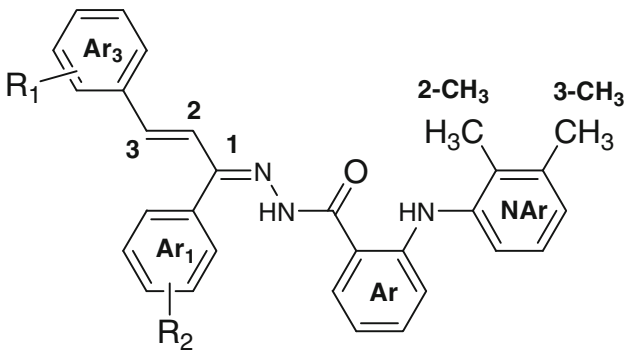


Fig. 1 Entitled compounds labeled for ^{13}C NMR

*3-(4-Chlorophenyl)-1-(4-fluorophenyl)prop-2-en-1-one (**3i**)*

Yield, 68 %; mp 136–138 °C; IR (KBr) υ_{max} 1,662 (C=O), 1,602 (CH=CH), 1,488 (Ar. C=C), 1,012 (C–F) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 8.26$ (d, 1H, CH), 7.76 (d, 1H, CH), 6.85–7.48 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 192.4$ (C=O), 165.3 (C_4–Ar_1), 145.5 (C_3), 136.8 (C_4–Ar_3), 135.1 (C_1–Ar_3), 134.8 (C_1–Ar_1), 133.1 ($C_{2,6}$–Ar_1), 130.3 ($C_{3,5}$–Ar_3), 129.5 ($C_{2,6}$–Ar_3), 122.9 (C_2), 115.4 ($C_{3,5}$–Ar_1); LCMS m/z $[M]^+$ 260.0; Anal. Calcd. for $C_{15}H_{10}ClFO$ (260.69): C, 69.11; H, 3.87. Found: C, 69.24; H, 3.78.

*1-(4-Fluorophenyl)-3-(3-nitrophenyl)prop-2-en-1-one (**3j**)*

Yield, 72 %; mp 164–166 °C; IR (KBr) υ_{max} 1,669 (C=O), 1,613 (CH=CH), 1,507 (Ar·C=C), 1,339 (–NO_2), 1,031 (C–F) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 8.19$ (d, 1H, CH), 7.83 (d, 1H, CH), 6.79–7.53 (m, 8H, Ar–H); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 188.7$ (C=O), 166. 4 (C_4–Ar_1), 151.1 (C_3–Ar_3), 147.3 (C_3), 137.9 (C_1–Ar_3), 136.4 (C_6–Ar_3), 135.7 (C_1–Ar_1), 133.01 ($C_{2,6}$–Ar_1), 130.2 (C_5–Ar_3), 127.5 (C_4–Ar_3), 124.9(C_2–Ar_3), 121.4 (C_2), 114.9 ($C_{3,5}$–Ar_1); LCMS m/z $[M]^+$ 271.0; Anal. Calcd. for $C_{15}H_{10}FNO_3$ (271.24): C, 66.42; H, 3.72; N, 5.16. Found: C, 66.57; H, 3.64; N, 5.10 (Fig. 1).

*2-(2,3-Dimethylphenylamino)-N′-(1,3-diphenylallylidene)benzohydrazide (**4a**)*

A mixture of 2-(2,3-dimethylphenylamino)benzohydrazide (2.55 g, 0.01 mol) and chalcone (2.08 g, 0.01 mol) in chloroform was stirred at 60–70 °C for 6 h in the presence of 2–3 ml of glacial acetic acid. The reaction mixture was poured into a beaker containing crushed ice and allowed to stand for 2 h. The precipitate so formed was filtered and washed with ice-cold water. The crude product was dried and recrystallized from chloroform. The completion of the reaction was monitored by running TLC. Yield, 65 %; mp 176–180 °C; IR (KBr) υ_{max} 3,312 (NH), 3,033 (NH), 1,686 (C=O), 1,639 (C=N), 1,574 (CH=CH), 14 61 (Ar·C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.48$ (bs, 1H, NH), 9.08 (bs, 1H, NH), 8.11 (d, 1H, CH), 7.68 (d, 1H, CH), 6.65–7.53 (m, 17H, Ar–H), 2.34 (s, 3H, CH_3), 2.21 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 171.3$ (C=O), 151.2 (C=N), 148.4 (C_2–Ar), 146.3 (C_1–NAr), 138.9 (C_1–Ar_1), 137.9 (C_3–NAr), 136.6 (C_3, CH), 135.8 (C_1–Ar_3), 134.7 (C_4–Ar), 129.8 (C_4–Ar_3), 129.0 ($C_{3,5}$–Ar_3), 128.5 ($C_{3,5}$–Ar_1), 128.4 (C_6–Ar), 128.1 (C_4–Ar_1), 127.6 ($C_{2,6}$–Ar_3), 127.3 (C_2–NAr), 127.2 ($C_{2,6}$–Ar_1), 126.8 (C_2), 124.9 (C_4–NAr), 123.6 (C_5–NAr), 122.0 (C_5–Ar), 120.8 (C_3–Ar), 120.1 (C_1–Ar), 118.8 (C_6–NAr), 19.7 (–3–CH_3), 14.1 (–2–CH_3); LCMS m/z $[M]^+$ 445.2; Anal. Calcd.

for $C_{30}H_{27}N_3O$ (445.55): C, 80.87; H, 6.11; N, 9.43. Found: C, 80.74; H, 6.19; N, 9.51.

The other compounds **4b** to **4j** were prepared by the same procedure using the corresponding chalcones.

*2-(2,3-Dimethylphenylamino)-N′-(-3-(3-nitrophenyl)-1-phenylallylidene)benzohydrazide (**4b**)*

Yield, 68 %; mp 140–142 °C; IR (KBr) υ_{max} 3,326 (NH), 3,063 (NH) 2,974 (CH Ar.), 1,660 (C=O), 1,616 (C=O), 1,576 (CH=CH), 1,495 (Ar·C=C), 1,346 (–NO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.62$ (bs, 1H, NH), 8.97 (bs, 1H, NH), 8.03 (d, 1H, CH), 7.71 (d, 1H, CH), 6.71–7.64 (m, 16H, Ar–H), 2.38 (s, 3H, CH_3), 2.18 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 170.2$ (C=O), 151.5 (C=N), 149.1 (C_3–Ar_3), 148.9 (C_2–Ar), 146.5 (C_1–NAr), 137.7 (C_1–Ar_3, C_3–NAr), 136.4 (C_1–Ar_1), 135.4 (C_6–Ar_3), 134.2 (C_3), 134.0 (C_4–Ar), 131.2 (C_5–Ar_3), 129.5 ($C_{3,5}$–Ar_1), 128.6 (C_6–Ar), 128.2 (C_4–Ar_1), 127.6 (C_2–NAr), 127.5 ($C_{2,6}$–Ar_1), 126.4 (C_2), 125.5 (C_4–Ar_3), 124.6 (C_4–NAr), 124.2 (C_5–NAr), 123.9 (C_2–Ar_3), 122.7 (C_5–Ar), 121.6 (C_3–Ar), 120.1 (C_1–Ar), 119.0 (C_6–NAr), 21.6 (–3–CH_3), 15.8 (–2–CH_3); LCMS m/z $[M]^+$ 490.2; Anal. Calcd. for $C_{30}H_{26}N_4O_3$ (490.55): C, 73.45; H, 5.34; N, 11.42. Found: C, 73.28; H, 5.46; N, 11.38.

*N′-(3-(4-Chlorophenyl)-1-phenylallylidene)-2-(2,3-dimethylphenylamino)benzohydrazide (**4c**)*

Yield, 59 %; mp 184–186 °C; IR (KBr) υ_{max} 3,308 (NH), 2,914 (CH Ar.), 1,645 (C=O), 1,624 (C=N), 1,574 (CH=CH), 1,436 (Ar. C=C), 751 (C–Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.57$ (bs, 1H, NH), 9.05 (bs, 1H, NH), 8.13 (d, 1H, CH), 7.76 (d, 1H, CH), 6.78–7.58 (m, 16H, Ar–H), 2.29 (s, 3H, CH_3), 2.22 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 168.1$ (C=O), 152.5 (C=N), 149.6 (C_2–Ar), 147.4 (C_1–NAr), 139.6 (C_3–NAr), 138.8 (C_1–Ar_1), 137.7 (C_3), 136.4 (C_4–Ar_3), 135.2 (C_1–Ar_3), 134.1 (C_4–Ar), 132.3 ($C_{3,5}$–Ar_3), 130.1 ($C_{2,6}$–Ar_3), 129.4 ($C_{3,5}$–Ar_1), 128.6 (C_6–Ar), 128.3 (C_4–Ar_1), 127.6 (C_2–NAr), 127.5 ($C_{2,6}$–Ar_1), 126.6 (C_2), 125.6 (C_4–NAr), 124.9 (C_5–NAr), 122.6 (C_5–Ar), 121.5 (C_3–Ar), 120.3 (C_1–Ar), 119.4 (C_6–NAr), 20.4 (–3–CH_3), 14.5 (–2–CH_3); LCMS m/z $[M]^+$ 479.2; Anal. Calcd. for $C_{30}H_{26}ClN_3O$ (479.99): C, 75.07; H, 5.46; N, 8.75. Found: C, 75.17; H, 5.53; N, 8.69.

*N′-(3-(4-Chlorophenyl)-1-(4-hydroxyphenyl)allylidene)-2-(2,3-dimethylphenylamino)benzohydrazide (**4d**)*

Yield, 62 %; mp 162–166 °C; IR (KBr) υ_{max} 3,145 (OH), 3,023 (NH), 1,648 (C=O), 1,621 (C=N), 1,557 (CH=CH), 1,522 (Ar·C=C), 1,354 (C–N), 725 (C–Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.72$ (bs, 1H, NH), 9.13 (bs, 1H, NH), 8.48 (s, 1H, OH), 8.18 (d, 1H, CH), 7.81 (d, 1H, CH), 6.82–7.62 (m, 15H, Ar–H), 2.37 (s, 3H, CH_3), 2.26 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 172.6$ (C=O), 159.6 (C_4–Ar_1), 153.2 (C=N), 147.4 (C_2–Ar), 145.3 (C_1–NAr), 139.2 (C_3–NAr), 136.5 (C_3), 135.2 (C_4–Ar_3), 134.9 (C_1–Ar_3), 134.1 (C_4–Ar), 132.2 ($C_{2,6}$–Ar_1), 130.4 ($C_{3,5}$–Ar_3), 129.8 ($C_{2,6}$–Ar_3), 129.2 (C_6–Ar), 128.6 (C_1–Ar_1), 127.5 (C_2–NAr), 126.6 (C_2), 124.9 (C_4–NAr), 124.3 (C_5–NAr), 123.2 (C_5–Ar), 121.3 (C_3–Ar), 120.6 (C_1–Ar), 119.5 (C_6–NAr), 117.3 ($C_{2,5}$–Ar_1), 18.6 (–3–CH_3), 13.2 (–2–CH_3); LCMS m/z $[M]^+$ 495.2; Anal. Calcd. for $C_{30}H_{26}ClN_3O_2$ (495.99): C, 72.65; H, 5.28; N, 8.47. Found: C, 72.54; H, 5.23; N, 8.59.

*N′-(3-(2-Chlorophenyl)-1-(4-hydroxyphenyl)allylidene)-2-(2,3-dimethylphenylamino)benzohydrazide (**4e**)*

Yield, 67 %; mp 194–196 °C; IR (KBr) υ_{max} 3,309 (NH), 2,923 (OH), 1,646 (C=O), 1,633 (C=N), 1,575 (CH=CH), 1,436 (Ar·C=C), 763 (C–Cl) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.69$ (bs, 1H, NH), 9.18 (bs, 1H, NH), 8.54 (s, 1H, OH), 8.08 (d, 1H, CH), 7.79 (d, 1H, CH), 6.78–7.53 (m, 15H, Ar–H), 2.32 (s, 3H, CH_3), 2.28 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 170.1$ (C=O), 160.2 (C_4–Ar_1), 152.5 (C=N), 148.9 (C_2–Ar), 148.3 (C_1–NAr), 139.9 (C_3–NAr), 137.10 (C_3), 136.10 (C_4–Ar), 134.2 (C_2–Ar_3), 133.7 (C_1–Ar_3), 132.06 (C_3–Ar_3), 131.3 ($C_{2,6}$–Ar_1), 130.1 (C_4–Ar_3), 129.6 (C_6–Ar_3), 128.4 (C_6–Ar), 127.6 (C_1–Ar_1), 127.5 (C_1–NAr), 127.3 (C_2), 127.2 (C_5–Ar_3), 125.6 (C_4–NAr), 124.8 (C_5–NAr), 123.4 (C_5–Ar), 122.8 (C_3–Ar), 121.9 (C_1–Ar), 119.0 (C_6–NAr), 116.3 ($C_{3,5}$–Ar_1), 20.2 (-3–CH_3), 14.5 (–2–CH_3); LCMS m/z $[M]^+$ 495.2; Anal. Calcd. for $C_{30}H_{26}ClN_3O_2$ (495.99): C, 72.65; H, 5.28; N, 8.47. Found: C, 72.57; H, 5.19; N, 8.54.

*N′-(3-(4-(Dimethylamino)phenyl)-1-(4-hydroxyphenyl)allylidene)-2-(2,3-dimethylphenylamino)benzohydrazide (**4f**)*

Yield, 56 %; mp 144–146 °C; IR (KBr) υ_{max} 3,396 (NH), 3,308 (NH), 3,063 (CH Ar.), 1,652 (C=O), 1,618 (C=N), 1,575 (CH=CH), 1,436 (Ar·C=C), 1,362 (C–N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.64$ (bs, 1H, NH), 9.21 (bs, 1H, NH), 8.47 (s, 1H, OH), 8.09 (d, 1H, CH), 7.83 (d, 1H, CH), 6.79–7.58 (m, 15H, Ar–H), 2.87 (s, 6H, CH_3), 2.32 (s, 3H, CH_3), 2.19 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 169.3$ (C=O), 159.8 (C_4–Ar_1), 153.1 (C=N), 152.4 (C_4–Ar_3), 148.4 (C_2–Ar), 146.5 (C_1–NAr), 138.9 (C_3–NAr), 137.6 (C_3), 135.1 (C_4–Ar), 133.4 ($C_{2,6}$–Ar_1), 131.9 ($C_{2,6}$–Ar_3), 129.4 (C_6–Ar), 128.6 (C_1–Ar_1),

127.8 (C_2–NAr), 126.9 (C_2), 125.6 (C_4–NAr), 124.7 (C_5–NAr), 123.7 (C_1–Ar_3), 122.0 (C_5–Ar), 121.5 (C_3–Ar), 120.5 (C_1–Ar), 119.3 (C_6–NAr), 115.3 ($C_{3,5}$–Ar_1), 113.7 ($C_{3,5}$–Ar_3), 42.9 (–$N(CH_3)_2$), 19.5 (–3–CH_3), 15.3 (–2–CH_3); LCMS m/z $[M]^+$ 504.3; Anal. Calcd. for $C_{32}H_{32}N_4O_2$ (504.62): C, 76.16; H, 6.39; N, 11.10. Found: C, 76.28; H, 6.31; N, 11.02.

*2-(2,3-Dimethylphenylamino)-N′-(1-(4-fluorophenyl)-3-(4-hydroxyphenyl)allylidene)benzohydrazide (**4g**)*

Yield, 63 %; mp 156–158 °C; IR (KBr) υ_{max} 3,312 (NH), 3,033 (NH), 1,686 (C=O), 1,626 (C=N), 1,574 (CH=CH), 1,461 (Ar·C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.76$ (bs, 1H, NH), 9.18 (bs, 1H, NH), 8.62 (s, 1H, OH), 8.23 (d, 1H, CH), 7.84 (d, 1H, CH), 6.75–7.58 (m, 15H, Ar–H), 2.33 (s, 3H, CH_3), 2.18 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 171.8$ (C=O), 161.0 (C_4–Ar_1), 160.3 (C_4–Ar_3), 154.2 (C=N), 149.9 (C_2–Ar), 145.3 (C_1–NAr), 139.8 (C_3–NAr), 138.6 (C_3), 135.1 (C_1–Ar_1), 134.1 (C_4–Ar), 132.3 ($C_{2,6}$–Ar_1), 130.9 ($C_{2,6}$–Ar_3), 129.6 (C_6–Ar), 128.6 (C_1–Ar_3), 128.5 (C_2–NAr), 126.9 (C_2), 125.3 (C_4–NAr), 124.2 (C_5–NAr), 122.0 (C_5–Ar), 121.3 (C_3–Ar), 120.6 (C_1–Ar), 119.9 (C_6–NAr), 117.7 ($C_{3,5}$–Ar_3), 116.3 ($C_{3,5}$–Ar_1), 21.4 (–3–CH_3), 13.8 (–2–CH_3); LCMS m/z $[M]^+$ 469.2; Anal. Calcd. for $C_{30}H_{26}FN_3O_2$ (479.54): C, 75.14; H, 5.46; N, 8.76. Found: C, 75.03; H, 5.57; N, 8.69.

*N′-(3-(4-(Dimethylamino)phenyl)-1-(4-fluorophenyl) allylidene)-2-(2,3-dimethylphenylamino)benzohydrazide (**4h**)*

Yield, 62 %; mp 152–156 °C; IR (KBr) υ_{max} 3,308 (NH), 3,063 (CH Ar.), 1,645 (C=O), 1,611 (C=N), 1,573 (CH=CH), 1,504 (Ar·C=C), 1,328 (C–N) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.76$ (bs, 1H, NH), 9.23 (bs, 1H, NH), 8.57 (s, 1H, OH), 8.13 (d, 1H, CH), 7.79 (d, 1H, CH), 6.82–7.65 (m, 15H, Ar–H), 2.82 (s, 6H, CH_3), 2.39 (s, 3H, CH_3), 2.21 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 170.3$ (C=O), 160.8 (C_4–Ar_1), 153.1 (C_4–Ar_3), 152.5(C=N), 146.4 (C_2–Ar), 146.1 (C_1–NAr), 139.2 (C_3–NAr), 138.5 (C_3), 135.4 (C_1–Ar_1), 134.8 (C_4–Ar), 131.3 ($C_{2,6}$–Ar_1), 130.2 ($C_{3,5}$–Ar_3), 129.4 (C_6–Ar), 127.5 (C_2–NAr), 126.6 (C_2), 125.9 (C_4–NAr), 124.6 (C_5–NAr), 123.9 (C_1–Ar_3), 122.5 (C_5–Ar), 120.9 (C_3–Ar), 120.2 (C_1–Ar), 119.3 (C_6–NAr), 115.8 ($C_{3,5}$–Ar_1), 112.6 ($C_{3,5}$–Ar_3), 42.9 (–$N(CH_3)_2$), 19.77 (–3–CH_3), 14.2 (–2–CH_3); LCMS m/z $[M]^+$ 506.2; Anal. Calcd. for $C_{32}H_{31}FN_4O$ (506.61): C, 75.87; H, 6.17; N, 11.06. Found: C, 75.73; H, 6.24; N, 11.14.

*N′-(3-(4-Chlorophenyl)-1-(4-fluorophenyl)allylidene)-2-(2,3-dimethylphenylamino)benzohydrazide (**4i**)*

Yield, 58 %; mp 134–136 °C; IR (KBr) υ_{max} 3,343 (NH), 3,064 (CH Ar.), 1,652 (C=O), 1,623 (C=N), 1,567 (CH=CH), 1,489 (Ar·C=C), 1,011 (C–F) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.63$ (bs, 1H, NH), 9.18 (bs, 1H, NH), 8.49 (s, 1H, OH), 8.22 (d, 1H, CH), 7.83 (d, 1H, CH), 6.67–7.59 (m, 15H, Ar–H), 2.34 (s, 3H, CH_3), 2.17 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 169.8$ (C=O), 160.0 (C_4–Ar_1), 150.5 (C=N), 146.4 (C_2–Ar), 145.3 (C_1–NAr), 137.9 (C_3–NAr), 136.6 (C_3), 135.8 (C_4–Ar_3), 135.1 (C_1–Ar_3), 134.2 (C_1–Ar_1), 134.1 (C_4–Ar), 131.0 ($C_{2,6}$–Ar_1), 129.3 ($C_{3,5}$–Ar_1), 129.1 ($C_{2,6}$–Ar_1), 128.4 (C_6–Ar), 127.5 (C_2–NAr), 126.6 (C_2), 124.9 (C_4–NAr), 124.6 (C_5–NAr), 122.0 (C_5–Ar), 121.6 (C_3–Ar), 120.1 (C_1–Ar), 118.7 (C_6–NAr), 115.2 ($C_{3,5}$–Ar_1), 19.9 (–3–CH_3), 16.2 (–2–CH_3); LCMS m/z $[M]^+$ 497.2; Anal. Calcd. for $C_{30}H_{25}ClFN_3O$ (497.16): C, 72.36; H, 5.06; N, 8.44. Found: C, 72.51; H, 4.97; N, 8.37.

*2-(2,3-Dimethylphenylamino)-N′-(1-(4-fluorophenyl)-3-(4-nitrophenyl)allylidene)benzohydrazide (**4j**)*

Yield, 66 %; mp 126–128 °C; IR (KBr) υ_{max} 3,308 (NH), 1,628 (C=N), 1,660 (C=O), 1,575 (CH=CH), 1,506 (–NO_2), 1,436 (Ar·C=C) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz,): $\delta = 9.82$ (bs, 1H, NH), 9.23 (bs, 1H, NH), 8.22 (d, 1H, CH), 7.78 (d, 1H, CH), 6.69–7.54 (m, 15H, Ar–H), 2.38 (s, 3H, CH_3), 2.23 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz,): $\delta = 170.4$ (C=O), 159.6 (C_4–Ar_1), 151.2 (C=N), 148.8 (C_4–Ar_3), 146.9 (C_2–Ar), 146.2 (C_1–NAr), 143.4 (C_1–Ar_3), 138.8 (C_3–NAr), 137.5 (C_3), 135.1 (C_1–Ar_1), 134.9 (C_4–Ar), 130.8 ($C_{2,6}$–Ar_1), 129.7 ($C_{2,6}$–Ar_3), 128.8 (C_6–Ar), 127.6 (C_2–NAr), 126.8 (C_2), 125.3 (C_4–NAr), 124.9 (C_5–NAr), 124.4 ($C_{3,5}$–Ar_3), 123.7 (C_5–Ar), 121.3 (C_3–Ar), 120.8 (C_1–Ar), 119.1 (C_6–NAr), 116.2 ($C_{3,5}$–Ar_1), 20.6 (–3–CH_3), 15.6 (–2–CH_3); LCMS m/z $[M]^+$ 508.2; Anal. Calcd. for $C_{30}H_{25}ClFN_4O_3$ (508.54): C, 70.85; H, 4.96; N, 11.02. Found: C, 70.93; H, 4.89; N, 10.89.

Pharmacology

Animals

Wistar rats weighing in the range 200–250 g were obtained from BN College of Pharmacy, Udaipur, India. All the animals were housed under standard ambient conditions of temperature (25 ± 2 °C) and relative humidity of 50 ± 5 %. A 12:12 h light/dark cycle was maintained. All the animals were allowed to have free access to water and

standard palletized laboratory animal diet 24 h prior to pharmacological studies. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of College, constituted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The protocol of the study was approved by the Institutional Animal Ethical Committee (Ref. No. 99/LSC/BNCP-012/IAEC).

Preparation of test compounds

After suspending the test compounds in 1.0 % aqueous solution of sodium carboxymethyl cellulose (CMC), test samples were administered to test animals orally. The positive and negative control group animals received the same experimental handling as those of the test groups except that the drug treatment, and control group animals received only appropriate volumes of the vehicle and of the reference drug, and mefenamic acid, respectively.

Analgesic activity

Prescreened animals (reaction time: 3–4 s) of either sex were assigned to seven groups of six each. Mefenamic acid was used as a standard; 1 % CMC was used as a control. Tail-flick latency was assessed with an analgesiometer. The strength of the current passing through the naked nichrome wire was kept constant at 6 amps. The reaction time was recorded at 30 min, 1 and 2 h after the treatment, and the cutoff time was set at 10 s to avoid tissue damage. The difference in reaction time (sec) was calculated by comparing the test compounds/standard drug and the normal controls. The % relative analgesic activity was calculated by using the following formula:

$$\% \text{ Relative analgesic activity } = (\mathrm{DRT_{test}}/\mathrm{DRT_{std}}) \times 100$$

where $\mathrm{DRT_{test}}$ is the difference in reaction time of the test compound with respect to (w.r.t.) the control, and $\mathrm{DRT_{std}}$ is the difference in reaction time of the standard drug used w.r.t control (Sharma *et al.*, 2009).

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using the well-known carrageenan-induced rat paw edema model of Winter et al. 30 using groups of six animals each. A freshly prepared aqueous suspension of carrageenan (1.0 % w/v, 0.1 ml) was injected in the subplanter region of right hind paw of each rat. One group was kept as control, and the animals of the other group were pretreated with the test drugs, 1 h before the carrageenan treatment. The volume was measured before and after carrageenan treatment at the 1, 2, 3 and 4 h intervals with the help of plethysmometer.

Acute ulcerogenesis

The acute ulcerogenesis test was performed according to the literature method (Cioli *et al.*, 1979). Albino rats have been divided into different groups consisting of six animals in each group. Ulcerogenic activity was evaluated after *p.o.* administration of test compounds or standard at the dose of 30 mg/kg. Control rats received *p.o.* administration of vehicle (suspension of 1 % carboxy methyl cellulose). Food but not water was removed 24 h before administration of the test compounds. After the drug treatment, the rats were fed a normal diet for 17 h and then killed. The stomach was removed and opened along the greater curvature, washed with distilled water, and cleaned gently by dipping in saline. The mucosal damage was examined by means of a magnifying glass. For each stomach, the mucosal damage was assessed according to the following scoring system:

0.0 scores were given to normal stomach (no injury, bleeding and latent injury).
0.5 scores were to latent injury or widespread bleeding (>2 mm).
1.0 was to slight injury (2–3 dotted lines).
2.0 for severe injury (continuous lined injury or 5–6 dotted injuries).
3.0 for very severe injury (several continuous lined injuries) and
4.0 for widespread lined injury or widened injury.

The mean score of each treated group minus the mean score of the control group was regarded as severity index of gastric mucosal damage.

Data are expressed as mean ± SEM; Data analyzed by one-way ANOVA followed by Dunnett's test, the significance of the difference between the control group and rats treated with the test compounds. The difference in results was considered significant when $P < 0.01$.

Statistical analysis

The results are expressed as the mean ± SEM per group, and the data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test as a post hoc test. P value <0.05 was considered statistically significant. All statistical calculations were performed using the evaluation version of Graph Pad® Prism 3.0 (USA) statistical software.

Computational studies

Calculation of physicochemical parameters

In-silico study of synthesized compounds (**4a–4j**) was performed for prediction of ADME properties. Polar surface area (TPSA) and molecular volume were calculated online using Molinspiration. Absorption (%ABS) was calculated by: $\%ABS = 109 - (0.345 \times TPSA)$ (Zhao *et al.*, 2002).

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