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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of (substituted benzamidostyryl) 1*H*-benzimidazoles and their screening for anti-inflammatory activity

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Abstract A series of substituted (benzamidostyryl) benzimidazole (**3a–r**) were synthesized and evaluated for their possible anti-inflammatory and ulcerogenicity. The structures of the synthesized compounds were confirmed on the basis of their spectral data and elemental analysis. Majority of the compounds were active in carrageenan-induced hind paw edema method test and compounds **3b**, **3k** had shown high potency after 3 and 4 h time intervals ($P < 0.001$) almost equipotent to the standard drug indomethacin and showed less severity index than it.

Keywords 1*H*-Benzimidazole · Carrageenan-induced hind paw edema · Severity index

Introduction

Benzimidazole is one of the most promising heteroaryl moiety that yielded many successful drugs (Preston 1980). Its derivatives are of wide interest because of their diverse biological activity and clinical applications (Kazimierczuk *et al*., 2002). Benzimidazole moiety fulfills the minimum structural requirements that are common for anti-inflammatory compounds (Nicholson *et al*., 1982); (Gund and Jensen, 1983). It had been reported that many compounds containing benzimidazole moiety possess significant analgesic as well as anti-inflammatory activity (Mertens *et al*., 1987). 1*H* Benzimidazole and simple derivatives of it had also been claimed to prevent stomach damage caused by inflammation inhibitors (Evans *et al*., 1996). Literature survey also shows that among the benzimidazole derivatives, 2-substituted ones are found to be pharmacologically more potent and hence the design and synthesis of 2-substituted benzimidazoles are the potential area of research (Foks *et al*., 2006). Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibit the immense potential and varied bioactivities; therefore efforts have been made from time to time to generate libraries of these compounds and screened them for potential biological activities. We have reported (Khan *et al*., 2009) earlier different heterocyclic compounds, which have shown considerable anti-inflammatory activities (Mullick *et al*., 2009). Now looking at the importance of 1*H* benzimidazole nucleus, it was thought that it would be worthwhile to design and synthesize some new 2-substituted 1*H* benzimidazole derivatives with the objective of discovering novel and potent anti-inflammatory agents that might be devoid of gastrointestinal side effects.

Chemistry

The titled compounds were synthesized as presented in Scheme 1 by hydrolysing 4-(substituted benzylidene)-2-phenyl-4*H*-oxazol-5-one, the azalactone precursor in 50% acetic acid medium and treating the product with substituted *o*-phenylenediamine (OPDA) in situ. Derivatives of former were synthesized from the hippuric acid and substituted aromatic aldehyde by Erlenmeyer-Plochl azalactone synthesis while hippuric acid was synthesized from the glycine by Schotten–Baumann benzoylation reaction. Thin layer chromatography (TLC) was run throughout the reactions to optimize the reactions for purity and completion. The physical data for the newly synthesized compounds are presented in Table 1.

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Scheme 1 Synthetic pathway of the targeted compounds **3a**–**r**

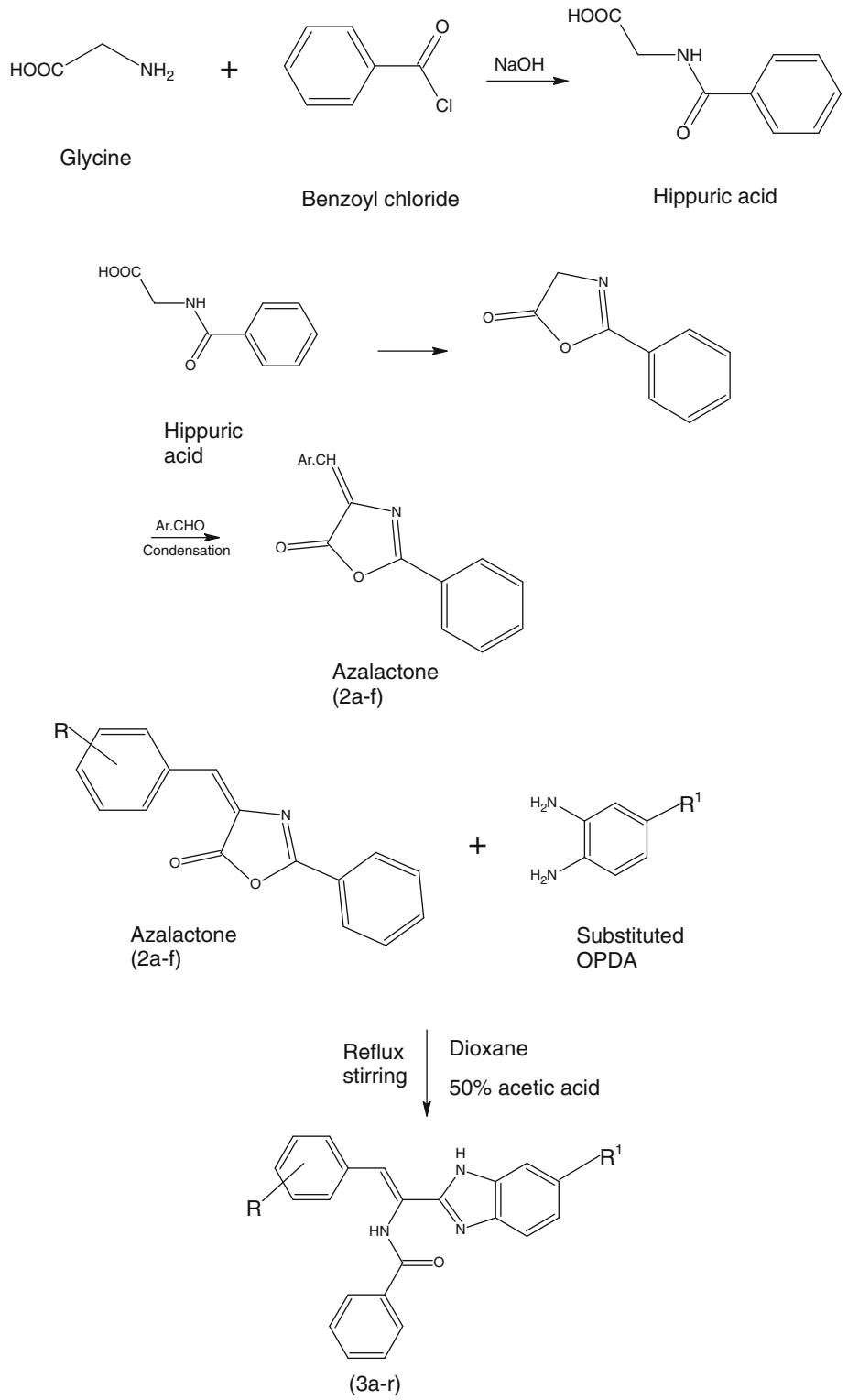


Table 1 Physicochemical data of *N*-[(E)-2-(2/4-substituted phenyl)-1-(6 substituted1*H*-benzimidazol-2-yl) vinyl] benzamide

Compounds	R	R^1	Yield (%)	M.P[a] (°C)	Mol. formula[b]
3a	H	–H	43	235–238	$C_{22}H_{17}N_3O$ (339.40)
3b	*p*-CH_3	–H	32	266–268	$C_{23}H_{19}N_3O$ (353.43)
3c	*p*-OH	–H	26	187–190	$C_{22}H_{17}N_3O_2$ (355.40)
3d	*p*-OCH_3	–H	23	278	$C_{23}H_{19}N_3O_2$ (369.43)
3e	*o*-Cl	–H	36	236–238	$C_{22}H_{16}ClN_3O$ (373.85)
3f	*p*-$N(CH_3)_2$	–H	23	234–235	$C_{24}H_{22}N_4O$ (382.47)
3g	H	–NO_2	24	255–259	$C_{22}H_{16}N_4O_3$ (384.40)
3h	*p*-CH_3	–NO_2	33	220–221	$C_{23}H_{18}N_4O_3$ (398.42)
3i	*p*-OH	–NO_2	32	187–189	$C_{22}H_{16}N_4O_4$ (400.40)
3j	*p*-OCH_3	–NO_2	28	198–200	$C_{23}H_{18}N_4O_4$ (414.42)
3k	*o*-Cl	–NO_2	29	280	$C_{22}H_{15}ClN_4O_3$ (418.84)
3l	*p*-$N(CH_3)_2$	–NO_2	27	248–251	$C_{24}H_{21}N_5O_3$ (427.47)
3m	H	–CH_3	39	281	$C_{23}H_{19}N_3O$ (353.43)
3n	*p*-CH_3	–CH_3	33	206–209	$C_{24}H_{21}N_3O$ (367.45)
3o	*p*-OH	–CH_3	43	165–168	$C_{23}H_{19}N_3O_2$ (369.43)
3p	*p*-OCH_3	–CH_3	28	233–235	$C_{24}H_{21}N_3O_2$ (383.45)
3q	*o*-Cl	–CH_3	37	279	$C_{23}H_{18}ClN_3O$ (387.87)
3r	*p*-$N(CH_3)_2$	–CH_3	23	236–238	$C_{25}H_{24}N_4O$ (396.50)

[a] Melting point of the compounds at their decomposition

[b] Elemental analyses for C, N were within ±0.4% of the theoretical values

Pharmacology

All the newly synthesized (benzamidostyryl) benzimidazole derivatives were tested in vivo to evaluate their pharmacological activity. Carrageenan-induced rat paw edema model was used as initial screen. These derivatives exhibited anti-inflammatory activity of varying degree from 18.88 to 42.42% after 3 h and 34.61 to 58.97% after 4 h interval. The results are summarized in Table 3 and Fig. 1. It was observed that the compounds **3g**, **3j**, **3k**, **3p**, and **3q** when administered at 20 mg/kg/i.p. exhibits significant reduction in edema 4 h post-dosing of the test compounds, that is, 49.99, 51.28,58.97, 53.84, and 50.00%, respectively, comparable to standard drug indomethacin which was administered at 10 mg/kg/i.p. These highly active compounds were further evaluated for their ulcerogenic activity which showed lower ulcerogenic potential. The results are shown in Table 4.

Results and discussion

The anti-inflammatory activity of *N*-[(*E*)-2-(2/4-substitutedphenyl)-1-(5/6 substituted1*H*-benzimidazol-2-yl) vinyl] benzamide was carried out using carrageenan-induced hind paw edema method. Indomethacin was used as a standard drug at the dose level of 10 mg/kg. Results are presented as percentage inhibition ± SEM (Table 3 and Fig. 1) and comparison was done with the control using ANOVA's Bonferron's multiple comparison test at the time intervals of 3 and 4 h. Compounds **3b** and **3k** had shown high potency after 3 and 4 h time intervals ($P < 0.001$) almost equipotent to the standard drug indomethacin. Compounds **3c**, **3j**, **3m** had also shown to be potent and results are statistically significant after 3 and 4 h interval. Thus, all above mentioned compounds of this series i.e., **3b–c**, **3j–k**, **3m** found to have quick onset and long duration of action. Compounds **3a**, **3d–g**, **3p–q** found to have good potency

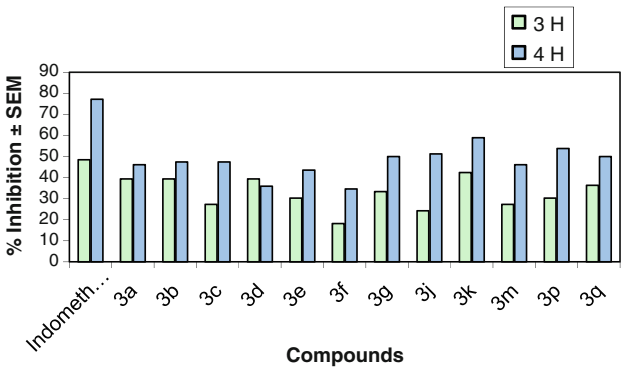


Fig. 1 Bar diagram of anti-inflammatory activity of compounds **3a–r**

after 4 h interval and were not statistically significant at 3 h indicating late onset of action of these compounds, while those compounds which seems to be potent but statistically insignificant even at ($P < 0.05$) after the 3 h were not considered. Thus, results indicate that the some of the compounds have very good potency along with quick onset and long duration of action.

Carrageenan-induced edema is a non-specific inflammation maintained by the release of histamine, 5-hydroxytryptamine, kinins, and later by prostaglandins (Tsurumi *et al*., 1986). The inhibitory effect of acid NSAIDs, such as indomethacin, is usually weak in the first phase (1–2 h), in contrast with their strong inhibition in the second phase (3–4 h) (Gavalas *et al*., 1991). Good inhibition of the second phase of carrageenan-induced edema was observed for the compounds tested, suggesting that they interfere with prostaglandin synthesis (Table 3).

From structure analysis, it seems that both electron-donating and withdrawing group played role in the potent compounds i.e., **3b–c**, **3j–k**, **3m**. Thus, this lead can further be exploited for the future research for the development of potent anti-inflammatory agent, thus keeping this in mind further toxicity studies (ulcerogenic index) was calculated for some of the potent compounds of the series. Results are presented in form of severity index in Table 4. Results showed that the entire synthesized compounds found to have very less index value compared to standard drug indomethacin. Thus, this further strengthens our results and assumption that this lead can be further exploited to develop into more potent and safest anti-inflammatory agent.

Conclusion

In summary, we have identified novel series of substituted (benzamidostyryl) benzimidazole, which may develop into the potential anti-inflammatory agents with lower ulcerogenic activity. Since GI problems due to NSAID continue to be the major impediment to their use in therapeutics, these properties of the new anti-inflammatory derivatives prove them useful lead molecules for the development of better NSAID with greatly improved therapeutic index. Further studies are required to confirm this suggestion.

Experimental protocols

Chemistry

Melting points were determined in an open end capillary tubes on Hicon digital melting point apparatus and are uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on FT-IR Rez Bio Rad Win-IR (KBr) and Brucker DRX-300 instruments, respectively. Chemical shifts were expressed in parts per million (ppm) relative to tetramethyl silane as an internal standard. The elemental analysis (C/N) were performed on Vario EL III CHNS analyzer using sulphanilic acid as a standard, and all the values were within ±0.4% of the theoretical compositions. The Fast atom bombardment (FAB) spectra were recorded on Jeol SX 102/DA-600 mass spectra system using Argon/Xenon (6 kV 10mA) as the FAB gas. The homogeneity of the compounds was monitored by ascending TLC, visualized by iodine vapor.

*Synthesis of hippuric acid (**1**)*

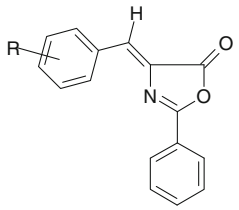
Glycine was dissolved in 10% sodium hydroxide solution contained in a conical flask to which was added equimolar quantity of benzoyl chloride in several divided portions. After each addition, stoppered the flask and it was shaken vigorously until all the chloride reacted, then placed a few grams of crushed ice in the solution and later concentrated hydrochloric acid was added slowly to it with continuous stirring until mixture became acidic, which resulted into the precipitation of crude product, which might had contamination of benzoic acid which was removed by gently boiling the crude product in carbon tetrachloride. Then mixture was allowed to cool slightly, filtered under gentle suction and washed the product on filter with 10–20 ml of carbon tetrachloride. Recrystallized the obtained dried product from boiling water. Collected the benzoylglycine and dried it. The practical yield of product is 80% and its m.p. 186°C.

*General procedure for the synthesis of 4-benzylidene-2-phenyl-4H-oxazol-5-one derivatives (**2a–f**)*

A mixture of aromatic aldehyde (0.25 mol), hippuric acid (0.25 mol), acetic anhydride (0.75 mol), and anhydrous sodium acetate was taken in a 500 ml conical flask and heated on an electric hotplate with constant shaking. As soon as the mixture liquefied completely, transferred the flask to water bath and heated for 2 h. Then added 100 ml of ethanol slowly to the contents of the flask and allowed the mixture to stand overnight. The crystallized product filtered with suction, washed with two 25 ml portions of ice-cold ethanol and then washed with two 25 ml portions of boiling water, dried at 100°C. The physicochemical data are mentioned in Table 2.

These synthesized intermediates show characteristic absorption bands in IR spectra: 1795-1785 (–C=O stretching of lactone), 1657-1648 (–C=N stretching) cyclic, 1606-1588, 1553-1498 (–C–C–(skeletal) stretching of benzene ring),

Table 2 Physicochemical data of 4-(substituted benzylidene)-2-phenyl-4*H*-oxazol-5-one



S. no.	R	M.P[a] °C	Percentage yield	Color	Mol. formula[b] (M.W.)	^{1}H-NMR (δ ppm, DMSO-d6)
1	H	158	60	Yellow needle	$C_{16}H_{11}NO_2$ (249.27)	8.31-8.29 (d, 2H, 2′, 6′ Ar–H), 8.14-8.12 (d, 2H, 2, 6 Ar–H), 7.74-7.52 (m, 6H, Ar–H), 7.35 (s, 1H, CH=C)
2	*p*-CH_3	134	58	Parrot green	$C_{17}H_{13}NO_2$ (263.20)	8.38-8.35 (d, 2H, 2′, 6′ Ar–H), 8.14-8.12 (d, 2H, 2, 6 Ar–H), 7.74-7.49 (m, 3H, Ar–H), 7.45 (s, 1H, CH=C), 7.28-7.26 (d, 2H, 3, 5 Ar–H), 2.37(s, 3H, CH_3)
3	*p*-OH	172–173	62	Yellow	$C_{16}H_{11}NO_3$ (265.27)	10.62 (s, 1H, OH), 8.38-8.35 (d, 2H, 2′, 6′ Ar–H), 8.19-8.16 (d, 2H, 2, 6 Ar–H), 7.70-7.45 (m, 3H, Ar–H), 7.39 (s, 1H, CH=C), 6.94-6.91 (d, 2H, 3,5 Ar–H)
4	*p*-OCH_3	156	52	Yellow fluffy	$C_{17}H_{13}NO_3$ (279.30)	8.20-8.17 (d, 2H, 2′,6′ Ar–H), 8.14-8.12 (d, 2H, 2,6 Ar–H), 7.63-7.43 (m, 3H, Ar–H), 7.39 (s, 1H, CH=C), 6.85-6.83 (d, 2H, 3, 5 Ar–H), 3.82 (s, 3H, OCH_3)
5	*o*-Cl	156–158	61	Yellow	$C_{16}H_{10}ClNO_2$ (283.72)	8.17-8.15 (d, 2H, 2′, 6′ Ar–H), 7.92-7.90 (d, 1H, 6 Ar–H), 7.66-7.56 (m, 5H, Ar–H), 7.48 (s,1H, CH=C), 7.18-7.13 (d, 1H, Ar–H)
6	*p*-$N(CH_3)_2$	217–219	55	Dark brown	$C_{18}H_{16}N_2O_2$ (292.34)	8.20-8.17 (d, 2H, 2′, 6′ Ar–H), 8.08-8.06 (d, 2H, 2, 6 Ar–H), 7.67-7.61 (t, 3H, 3′,4′, 5′ Ar–H), 7.24 (CH=C), 6.85-6.83 (d, 2H, 3, 5 Ar–H), 3.08 (s, 6H, –$N(CH_3)_2$)

[a] Melting point of the compounds at their decomposition

[b] Elemental analyses for C, N were within ±0.4% of the theoretical values

1039-1030 (–C–O stretching of ether), 881-851, 832-813 (Ar–H bending). The spectral data of all the synthesized compounds are presented in Table 2.

*General procedure for the synthesis of substituted (benzamidostyryl) benzimidazole (**3a–r**)*

To equimolar quantity of 2-phenyl-4-substituted benzylidine-5-oxazolone (**2a–f**) and OPDA was added 20 ml of 50% acetic acid, and then 30 ml of dioxane until all the solids were dissolved. The solution was refluxed at 135–140°C for 20 h with stirring. The reaction mixture was cooled and evaporated to near dryness. The brownish material obtained was redissolved in methanol, then activated charcoal was added to it, and again the mixture was refluxed for 20 min, cooled, and the solid which was separated by filtration was then recrystallized from ethanol, colored crystals were then obtained. The physicochemical data of all the synthesized compounds are given in Table 1.

*N-[(E)-1-(1H-Benzimidazol-2-yl)-2-(phenyl)vinyl]benzamide (**3a**)* IR: ν_{max} (cm^{-1}); 3278 (NH), 1656 (C=O), 1602 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 12.01 (bs, 1H, CONH, D_2O exchangeable), 9.6 (s, 1H, NH, D_2O exchangeable), 8.14-8.11 (d, 2H, 2′,6′ Ar–H), 7.76-7.42 (m, 12H, Ar–H), 7.35 (s, 1H, CH=C), Mass spectra: M^+ (339), M-77 (262), M-105 (234).

*N-[(E)-1-(1H-Benzimidazol-2-yl)-2-(4-methylphenyl)vinyl] benzamide (**3b**)* IR: ν_{max} (cm^{-1}); 3269 (NH), 1640 (C=O), 1597 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 12.08 (bs, 1H, CONH), 9.54 (s, 1H, NH), 8.03-8.01 (d, 2H, Ar–H), 7.70-7.34 (m, 9H, Ar–H), 7.33 (CH=C), 7.12-7.10 (d, 2H, Ar–H), 2.37 (s, 3H, CH_3).

*N-[(E)-1-(1H-Benzimidazol-2-yl)-2-(4-hydroxyphenyl)vinyl] benzamide (**3c**)* IR: ν_{max} (cm^{-1}); 3274 (NH), 1648 (C=O), 1604 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 12.03 (bs, 1H, CONH), 10.41 (s, 1H, OH), 9.40 (s, 1H, NH), 8.06-8.03 (d, 2H, Ar–H), 7.77-7.38 (m, 9H, Ar–H), 7.33 (s, 1H, CH=C), 6.94-6.91 (d, 2H, Ar–H).

*N-[(E)-1-(1H-Benzimidazol-2-yl)-2-(4-methoxyphenyl)vinyl] benzamide (**3d**)* IR: ν_{max} (cm^{-1}); 3265 (NH), 1643 (C=O), 1604 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 12.01 (bs, 1H, CONH), 9.54 (s, 1H, NH), 812-8.10 (d, 2H, 2′, 6′ Ar–H),

7.66-7.41 (m, 9H, Ar–H), 7.19 (s,1H, CH=C), 6.85-6.82 (d, 2H, Ar–H), 3.79 (s, 3H, OCH_3), Mass spectra: M^+ (369), M-77 (292), M-105 (264).

*N-[(E)-1-(1H-Benzimidazol-2-yl)-2-(2-chlorophenyl)vinyl] benzamide (**3e**)* IR: ν_{max} (cm^{-1}); 3271 (NH), 1654 (C=O), 1602 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 12.04 (bs, 1H, CONH), 9.41 (s, 1H, NH), 8.05-8.03 (d, 2H, Ar–H), 7.76-7.43 (m, 7H, Ar–H), 7.42 (s, 1H, CH=C), 7.22-7.04 (m, 4H, Ar–H).

*N-[(E)-1-(1H-Benzimidazol-2-yl)-2-(4-dimethylaminophenyl) vinyl]benzamide (**3f**)* IR: ν_{max} (cm^{-1}); 3273 (NH), 1656 (C=O), 1603 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 12.01 (bs, 1H, CONH), 9.42 (s, 1H, NH), 8.05-8.03 (d, 2H, 2′,6′ Ar–H), 7.78-7.41 (m, 9H, Ar–H), 7.28 (s, 1H, CH=C), 6.85-6.83 (d, 2H, Ar–H), 3.04 (s, 6H, CH_3).

*N-[(E)-1-(6-Nitro-1H-benzimidazol-2-yl)-2-phenylvinyl]benzamide (**3g**)* IR: ν_{max} (cm^{-1}); 3298 (NH), 1644 (C=O), 1605 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 13.21 (bs, 1H, CONH), 10.38 (s, 1H, NH), 8.49 (s, 1H, Ar–H), 8.18-8.16 (d, 1H, Ar–H), 8.03-8.01 (d, 2H, Ar–H), 7.73-7.70 (d, 2H, Ar–H), 7.64-7.58 (m, 5H, Ar–H), 7.42 (s, 1H, CH=C), 7.37-7.32 (t, 2H, Ar–H).

*N-[(E)-2-(4-Methylphenyl)-1-(6-nitro-1H-benzimidazol-2-yl)vinyl]benzamide (**3h**)* IR: ν_{max} (cm^{-1}); 3312 (NH), 1658 (C=O), 1607 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 13.37 (bs, 1H, CONH), 10.37 (s, 1H, NH), 8.51 (s, 1H, Ar–H), 8.14-8.12 (d, 1H, Ar–H), 8.03-8.01 (d, 2H, Ar–H), 7.66-7.38 (m, 6H, Ar–H), 7.34 (s, 1H, CH=C), 7.12-7.10 (d, 2H, Ar–H), 2.38 (s, 3H, CH_3).

*N-[(E)-2-(4-Hydroxyphenyl)-1-(6-nitro-1H-benzimidazol-2-yl)vinyl]benzamide (**3i**)* IR: ν_{max} (cm^{-1}); 3296 (NH), 1647 (C=O), 1606 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 13.32 (bs, 1H, CONH), 10.49 (s, 1H, OH), 10.38 (s, 1H, NH), 8.49 (s, 1H, Ar–H), 8.14-8.12 (d, 1H, Ar–H), 8.03-8.01 (d, 2H, Ar–H), 7.76-7.32 (m, 6H, Ar–H), 7.29 (s, 1H, CH=C), 6.91-6.89 (d, 2H, Ar–H).

*N-[(E)-2-(4-Methoxyphenyl)-1-(6-nitro-1H-benzimidazol-2-yl)vinyl]benzamide (**3j**)* IR: ν_{max} (cm^{-1}); 3308 (NH), 1650 (C=O), 1604 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 13.34 (bs, 1H, CONH), 10.38 (s, 1H, NH), 8.49 (s, 1H, Ar–H), 8.14-8.12 (d, 1H, Ar–H), 8.03-8.01 (d, 2H, Ar–H), 7.76-7.41 (m, 6H, Ar–H), 7.34 (s, 1H, CH=C), 6.86-6.84 (d, 2H, Ar–H), 3.84 (s, 3H, OCH_3).

*N-[(E)-2-(2-Chlorophenyl)-1-(6-nitro-1H-benzimidazol-2-yl)vinyl]benzamide (**3k**)* IR: ν_{max} (cm^{-1}); 3306 (NH), 1654 (C=O), 1602 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 13.30 (bs, 1H, CONH), 10.33 (s, 1H, NH), 8.52 (s, 1H, Ar–H), 8.14-8.11 (d, 1H, Ar–H), 7.99-7.97 (d, 2H, Ar–H), 7.77(s, 1H, CH=C), 7.73-7.70 (d, 2H, Ar–H), 7.64-7.58 (m, 4H, Ar–H), 7.37-7.32 (t, 2H, Ar–H).

*N-[(E)-2-(4-Dimethylaminophenyl)-1-(6-nitro-1H-benzimidazol-2-yl)vinyl]benzamide (**3l**)* IR: ν_{max} (cm^{-1}); 3310 (NH), 1645 (C=O), 1611 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 13.38 (bs, 1H, CONH), 10.24 (s, 1H, NH), 8.50 (s, 1H, Ar–H), 8.24-8.22 (d, 1H, Ar–H), 8.13-8.11 (d, 2H, Ar–H), 7.71-7.68 (d, 2H, Ar–H), 7.64-7.58 (m, 4H, Ar–H), 7.26 (s, 1H, CH=C), 6.86-6.83 (d, 2H, Ar–H), 3.07 (s, 6H, $N(CH_3)_2$).

*N-[(E)-1-(6-Methyl-1H-benzimidazol-2-yl)-2-phenylvinyl]-benzamide (**3m**)* IR: ν_{max} (cm^{-1}); 3305 (NH), 1648 (C=O), 1602 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 12.41 (bs, 1H, CONH), 10.15 (s, 1H, NH), 8.03-8.01 (d, 2H, Ar–H), 7.81 (s, 1H, Ar–H), 7.68-7.44 (m, 8H, Ar–H), 7.34 (s, 1H, CH=C), 7.14-7.12 (d, 2H, Ar–H), 2.37 (s, 3H, CH_3).

*N-[(E)-2-(4-Methylphenyl)-1-(6-methyl-1H-benzimidazol-2-yl)vinyl]benzamide (**3n**)* IR: ν_{max} (cm^{-1}); 3310 (NH), 1647 (C=O), 1602 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 12.33 (bs,1H, CONH), 10.13 (s,1H, NH), 8.06-8.04 (d, 2H, Ar–H), 7.82 (s, 1H, Ar–H), 7.76-7.37 (m, 6H, Ar–H), 7.36 (s, 1H, CH=C), 7.20-7.13 (m, 3H, Ar–H), 2.39 (s, 3H, CH_3), 2.26 (s, 3H, CH_3).

*N-[(E)-2-(4-Hydroxyphenyl)-1-(6-methyl-1H-benzimidazol-2-yl)vinyl]benzamide (**3o**)* IR: ν_{max} (cm^{-1}); 3296 (NH), 1656 (C=O), 1604 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 12.30 (bs, 1H, CONH, D_2O exchangeable), 11.05 (s, 1H, OH, D_2O exchangeable), 10.11 (s, 1H, NH, D_2O exchangeable), 8.03-8.01 (d, 2H, Ar–H), 7.79 (s,1H, Ar–H), 7.68-7.42 (m, 6H, Ar–H), 7.34 (s, 1H, CH=C), 7.14-7.12 (d, 1H, Ar–H), 7.03-7.01 (d, 2H, Ar–H), 2.37 (s, 3H, CH_3).

*N-[(E)-2-(4-Methoxyphenyl)-1-(6-methyl-1H-benzimidazol-2-yl)vinyl]benzamide (**3p**)* IR: ν_{max} (cm^{-1}); 3312 (NH), 1648 (C=O), 1605 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 12.44 (bs, 1H, CONH), 10.12 (s, 1H, NH), 8.03-8.01 (d, 2H, Ar–H), 7.80 (s, 1H, Ar–H), 7.62-7.37 (m, 6H, Ar–H), 7.34 (s, 1H, CH=C), 7.14-7.12 (d, 1H, Ar–H), 6.85-6.82 (d, 2H, Ar–H), 3.79 (s, 3H, OCH_3), 2.38 (s, 3H, CH_3).

*N-[(E)-2-(2-Chlorophenyl)-1-(6-methyl-1H-benzimidazol-2-yl)vinyl]benzamide (**3q**)* IR: ν_{max} (cm^{-1}); 3308 (NH), 1654 (C=O), 1602 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 12.54 (bs, 1H, CONH), 10.14 (s, 1H, NH), 7.98-7.95

Table 3 Anti-inflammatory activity of tested compounds (**3a–r**)

Compd. no.	Initial paw volume	Final paw volume		Difference after		Percentage inhibition ± SEM	
		3 h	4 h	3 h	4 h	3 h	4 h
Control	0.53 ± 0.03	0.64 ± 0.04	0.80 ± 0.04	0.11 ± 0.010	0.26 ± 0.020		
Indomethacin	0.54 ± 0.04	0.59 ± 0.04	0.60 ± 0.05	0.05 ± 0.008	0.06 ± 0.012	48.48 ± 3.03***	77.21 ± 1.96***
3a	0.52 ± 0.02	0.59 ± 0.02	0.66 ± 0.02	0.06 ± 0.010	0.14 ± 0.010	39.39 ± 3.83	46.15 ± 2.81**
3b	0.50 ± 0.03	0.57 ± 0.02	0.64 ± 0.02	0.06 ± 0.010	0.13 ± 0.010	39.39 ± 3.83***	47.43 ± 2.36***
3c	0.52 ± 0.02	0.59 ± 0.02	0.65 ± 0.01	0.08 ± 0.010	0.13 ± 0.010	27.27 ± 4.69*	47.43 ± 3.67***
3d	0.52 ± 0.02	0.59 ± 0.02	0.69 ± 0.02	0.06 ± 0.010	0.16 ± 0.010	39.39 ± 3.83	35.89 ± 2.56**
3e	0.54 ± 0.02	0.62 ± 0.02	0.68 ± 0.02	0.07 ± 0.008	0.14 ± 0.010	30.30 ± 3.03	43.56 ± 1.62***
3f	0.52 ± 0.02	0.61 ± 0.02	0.69 ± 0.02	0.09 ± 0.010	0.17 ± 0.010	18.18 ± 4.06***	34.61 ± 1.72**
3g	0.52 ± 0.02	0.59 ± 0.02	0.65 ± 0.02	0.07 ± 0.010	0.13 ± 0.010	33.33 ± 6.06	49.99 ± 2.62***
3j	0.54 ± 0.03	0.62 ± 0.03	0.66 ± 0.03	0.08 ± 0.008	0.12 ± 0.010	24.24 ± 3.03**	51.28 ± 1.62***
3k	0.55 ± 0.03	0.61 ± 0.03	0.66 ± 0.03	0.06 ± 0.008	0.10 ± 0.010	42.42 ± 3.03**	58.97 ± 1.62***
3m	0.54 ± 0.03	0.62 ± 0.03	0.68 ± 0.02	0.08 ± 0.010	0.14 ± 0.010	27.27 ± 6.63*	46.15 ± 1.98**
3p	0.51 ± 0.03	0.58 ± 0.03	0.63 ± 0.03	0.07 ± 0.008	0.12 ± 0.010	30.30 ± 3.03	53.84 ± 1.98***
3q	0.54 ± 0.02	0.61 ± 0.02	0.67 ± 0.02	0.07 ± 0.010	0.13 ± 0.010	36.36 ± 4.06	50.00 ± 1.72***

$N = 6$; Dose = 10 mg/kg; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (compared to standard). Data was analyzed by ANOVA's Bonferron's multiple comparison test

(d, 2H, Ar–H), 7.78 (s, 1H, Ar–H), 7.67-7.48 (m, 6H, Ar–H), 7.41 (s, 1H, CH=C), 7.34-7.23 (m, 2H, Ar–H), 7.04-6.90 (t, 1H, Ar–H), 2.40 (s, 3H, CH_3).

*N-[(E)-2-(4-Dimethylaminophenyl)-1-(6-methyl-1H-benzimidazol-2-yl)vinyl]benzamide (**3r**)* IR: v_{max} (cm^{-1}); 3310 (NH), 1650 (C=O), 1603 (C=N), ^{1}H NMR(DMSO-d_6) (δ, ppm): 12.21 (bs, 1H, CONH), 10.14 (s, 1H, NH), 8.03-8.01 (d, 2H, Ar–H), 7.79 (s, 1H, Ar–H), 7.62-7.37 (m, 6H, Ar–H), 7.33 (s, 1H, CH=C), 7.14-7.12 (d,1H, Ar–H), 6.85-6.83 (d, 2H, Ar–H), 3.08 (s, 6H, $N(CH_3)_2$), 2.38 (s, 3H, CH_3).

Biological assays

Wistar albino rats of either sex (150–180 g) were used as experimental animals. The Institutional Animal Ethics committee (IAEC) reviewed and approved all the animal procedures adopted. The animals were housed at an ambient temperature of 25 ± 2°C, in groups as required per metabolic cages and allowed free access to chow pellets and water. The light/dark cycle of 12 h: 12 h was maintained. All the synthesized test compounds were suspended in 30% polyethylene glycol (PEG 200).

Anti-inflammatory activity

The anti-inflammatory activity of the test compounds was carried out using carrageenan-induced rat paw edema model according to Winter *et al*. (1962) by employing 1.0% carrageenan solution as the phlogistic agent. The test compounds were administered i.p. in 30% PEG-200, 30 min before the injection of the phlogistic agent, at dose level of 20 mg/kg body weight. Indomethacin was used as a standard at a dose level of 10 mg/kg body weight. Groups of six albino rats of either sex were used in each experiment. The PEG-200 (30%) served as a control. The paw edema volume was measured with the help of plethysmometer at 3 and 4 h interval post injection of carrageenan. The percentage edema is shown in Table 3 (Fig. 1). The percent anti-inflammatory activity was calculated according to the formula as given below:

$$\% \text{ Anti - inflammatory activity } = [1 - V_t / V_c] \times 100,$$

whereas, V_t represents the mean increase in paw volume in rats treated with test compounds and V_c represents the mean increase in paw volume in control group of rats.

Gastric ulcerogenic activity

Nonsteroidal anti-inflammatory agents like indomethacin induce gastric lesions in experimental animals by inhibition of gastric cyclooxygenase resulting in less formation of prostacyclin, the predominant prostanoid produced in the gastricmucosa (Vogel, 2002). Wistar rats of either sex weighing 150–200 g were divided into vehicle control, standard (indomethacin), and different test compound groups (n¼ 6). The test compounds and indomethacin were administered orally in 30% PEG-200 solution orally at a dose of 30 and 20 mg/kg b.w., respectively. After 17 h they were sacrificed and dissected for the estimation of

Table 4 Ulcerogenic index of the potent anti-inflammatory compounds

Compd. code	Observation	Score	Severity index
Control	Normal	0	0.0
	Normal	0	
	Normal	0	
	Normal	0	
	Normal	0	
	Normal	0	
Indomethacin	6 Ulcers + H.S. + redness	5.0	4.50 ± 0.316
	7 Ulcers + spot ulcers + redness	4.5	
	H.S. + redness + spot ulcer	3.0	
	4 Ulcers + H.S. + redness	5.0	
	5 Ulcers + H.S.	4.5	
	5 Ulcers + H.S. + redness	5.0	
Total score		27	
3b	Redness + H.S.	2.0	1.00 ± 0.250
	Redness	0.5	
	H.S.	1.5	
	Redness	0.5	
	Spot ulcer	1.0	
	Redness	0.5	
Total score		6.0	
3j	Redness + H.S	2.0	1.41 ± 0.560
	Redness	0.5	
	Spot ulcer	1.0	
	Redness	0.5	
	2 Ulcer + H.S. + redness	4.0	
	Redness	0.5	
Total score		8.5	
3k	Redness	0.5	0.75 ± 0.170
	H.S.	1.5	
	Redness	0.5	
	Spot ulcer	1.0	
	Redness	0.5	
	Redness	0.5	
Total score		4.5	

ulcerogenic activity. The stomach was dissected out and washed with running water and opened along the greater curvature, washed gently with cotton swab, wetted with saline, and pinned on the wax tray. The glandular portion of the stomach was cleaned with saline and carefully observed with magnifying glass for the presence of any redness, hemorrhage, streak, spot ulcers, and big ulcers (Cioli *et al*., 1979). For grading and incidence of ulcers of each sample of stomach for mucosal damage was assessed according to (Wilheme and Gdynia, 1972), the mean count for each group being calculated as depicted in Table 4. The mean score of each treated group minus the mean score of control was regarded as severity index of the gastric mucosal damage.

Statistical analysis

Data were presented as mean ± SEM. Statistical analysis was performed by one way variance (ANOVA) Bonferron's multiple comparison test; a "*P*" value of less than 0.05 was considered as statistically significant.

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