Medicinal chemistry

Gaba and Mohan, Med chem 2015, 5:2
http://dx.doi.org/10.4172/2161-0444.1000243

Research Article Open Access

Design, Synthesis and Biological Evaluation of Novel 1, 2, 5-Substituted Benzimidazole Derivatives as Gastroprotective Anti-inflammatory and Analgesic Agents

Monika Gaba[1]* and Chander Mohan[2]

[1]Department of Pharmaceutical Sciences, ASBASJSM College of Pharmacy, Bela, Ropar, Punjab, India
[2]Rayat-Bahra Institute of Pharmacy, Hoshiarpur, Punjab, India

Abstract

In the present investigation, in order to identify GI-safe anti-inflammatory and analgesic agents, a series of novel 1, 2 and 5-substituted benzimidazole derivatives were synthesized and biologically evaluated. The results demonstrated that the compounds 3b, 4b, and 5b could serve as gastroprotective lead compounds for developing a novel class of potent as well as orally active anti-inflammatory and analgesic agents in the future research.

Keywords: Benzimidazole; Inflammation; Drug-likeness; GI-safe; Oxidative stress; Pain

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used as a first choice of drug for the treatment of various inflammatory diseases as well as to relieve aches and pain of everyday life [1]. However, long term use of NSAIDs is associated with side effects like hepatotoxicity, platelet dysfunction and bleeding [2-4]. But the major and potential side effect with chronic use of NSAIDs is gastrointestinal (GI) ulcerations due to inhibition of cyclooxygenase (COX) in tissues where prostaglandins exert their physiological functions, such as gastric mucosal defense and renal homeostasis [5]. The discovery of two COX isoforms, namely COX-1 constitutively expressed in most tissues such as kidney and GI tract, while COX-2 is induced at the sites of inflammation, led to the development of selective COX-2 inhibitors, with the hope of significantly reducing the GI toxicity associated with acute and chronic use of NSAIDs. However, with the increased knowledge of physiological roles of COX-2 enzyme in a variety of tissues, including stomach and kidney, some selective COX-2 inhibitors are withdrawn from the market because of cardiovascular toxicity, have challenged the benefits of selective COX-2 inhibition [6]. As a consequence, the interest for alternative approaches to reduce GI side effects associated with NSAIDs has re-emerged. Different approaches were pursued in the search for GI-sparing NSAIDs like addition of chemical moieties that release gastro protective mediators such as nitric oxide (NO)-releasing or hydrogen sulphide (H_2S)-releasing NSAIDs, dual cyclooxygenase/5-lipoxygenase or microsomal prostaglandin E synthase-1 inhibitors [7,8]. But these approaches are associated with some limitations, [9-14] so a real need exists to develop new anti-inflammatory, analgesic agents with better efficacy, less toxicity and fewer side effects of gastric ulceration. According to reported literature, reactive oxygen species or oxidative stress components are involved in pathophysiology of NSAIDs-induced ulcerations. On synthesizing novel chemical entities or chemical modifications/derivatization of existing molecules could lead to neutral molecules with greatly reduced acidic character and oxidative stress as a useful approach to explore safer and potent anti-inflammatory and analgesic agents [15].

Benzimidazoles and its derivatives substituted at 1, 2, 5 and 6-positions fulfill the minimum structural requirements for anti-inflammatory, analgesic and antioxidant activity [20,23]. Encouraged by the aforementioned findings and in continuation of an ongoing program aiming at finding new gastroprotective leads with potential anti-inflammatory, analgesic activities, it was rationalized to explore novel benzimidazole derivatives. In the present research work, a novel series of 1, 2 and 5-substituted benzimidazole derivatives have been synthesized and bio-evaluated for their anti-inflammatory, analgesic, ulcerogenic as well as antioxidant activity. Moreover, the drug-likeness properties of the resulted compounds are also presented.

Experimental

Materials and methods

All research reagents and solvents used in synthesis were of laboratory grade and were procured from SD Fine Chemicals, Qualigens, Loba Chemie and Sigma-Aldrich, India. All the solvents were dried and distilled before use. Reactions were monitored by thin layer chromatography (TLC) on precoated silica gel plates and compounds were visualized by exposure to iodine vapours. Melting points (m.p.) were determined using open capillary tube method and were uncorrected. Spectral data were obtained as follows: Infra-red (IR) spectra were recorded on Bruker Alpha-FT-IR Spectrophotometer Attenuated total reflection mode from ASBASJSM College of Pharmacy, Bela. The ^{1}H-NMR spectra were recorded on a Bruker Avance DPX-200 (400 MHz) (Panjab University, Chandigarh, India) using tetramethylsilane as an internal standard and the values are expressed in parts per million (ppm). Mass spectra were obtained using electrospray ionization (ESI) technique by Agilent 1100 series LC-MS instrument. Elemental analyses were performed on Leco CHNS-932 (Leco, St. Joseph, MI, USA).

The synthesis of 5-substituted-2-chloromethylbenzimidazoles (1a, 1b and 1c) and intermediate compounds 2a, 2b and 2c was done according to procedures reported in the literature [25,26]. Further, novel benzimidazole derivatives 3a-c, 4a-c and 5a-c were synthesized whose synthesis procedure and spectral data are as follows:

General procedure for synthesis of 3a-c, 4a-c and 5a-c derivatives:

***Corresponding author:** Monika Gaba, Pharmaceutical Chemistry, ASBASJS Memorial College of Pharmacy, Bela-140 111 (Ropar) India, Tel: +919872390321; E-mail: monikagaba12@gmail.com, gaba_monika12@yahoo.co.in

Received January 12, 2015; **Accepted** February 06, 2015; **Published** February 09, 2015

Citation: Gaba M, Mohan C (2015) Design, Synthesis and Biological Evaluation of Novel 1, 2, 5-Substituted Benzimidazole Derivatives as Gastroprotective Anti-inflammatory and Analgesic Agents. Med chem 5: 058-063. doi:10.4172/2161-0444.1000243

For the synthesis of benzimidazole derivatives, different substituted aryl amines were added to the solution of compound 2a-c (2g, 0.006 mol), potassium iodide (2 g, 0.012 mol) and potassium hydroxide (1 g, 0.017 mol) in dry ethanol in a round bottom flask. The mixture was refluxed for 24 h and then poured into ice-cold water. The precipitates formed were filtered at the pump, washed, dried and crystallized by using hot water.

N-((5-Nitro-1-(phenylsulfonyl)-1H-benzo[d]imidazol-2-yl) methyl)benzenamine (3a): Yield: 60%; m.p. 242-24°C; dark yellow solid; R_f: 0.62 [Chloroform:Methanol (92:8)]; IR: 2960 (Aromatic -CH str), 1510 (Aromatic -C=C str), 3425 (-NH str), 1340 (-CN str), 740 (-CCl str), 886, ($S(=O)_2$ str), 1513 (N=O str) cm^{-1}. ^1H NMR (DMSO-*d*6, 400 MHz, δ ppm): 2.19 (s, 2H, $-CH_2$), 4.44 (s, 1H, NH), 7.16-7.39 (m, 5H, Ar-H), 7.87-7.85 (m, 2H, Ar-H), 7.76 (s, 1H, Ar-H), 6.52-6.58 (m, 5H, Ar-H). ^{13}C NMR ($CDCl_3$): δ 147.6, 142.8, 141.5, 139.8, 137.2, 133.8, 129.8, 128.3, 118.0, 117.2, 113.5, 110.5, 36.1. Anal. Calcd for $C_{20}H_{16}N_4O_4S$: C, 58.81; H, 3.95; N, 13.72%. Found: C, 58.70; H, 3.80; N, 13.62%. ESI-MS (m/z): 408 (M^+).

N-((5-Methoxy-1-(phenylsulfonyl)-1H-benzo[d]imidazol-2-yl) methyl)benzenamine (3b): Yield: 60%; m.p. 238-240°C; brown solid, R_f: 0.80 [Chloroform:Methanol (92:8)], IR: 3050 (Aromatic -CH str), 1640 (Aromatic -C=C str), 2767 (-NH str), 990 ($S(=O)_2$ str), 3421 (Alkane $-CH_3$ bending) cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 2.73 (s, 2H, $-CH_2$), 2.13 (s, 3H, $-CH_3$), 4.60 (s, 1H, NH), 7.20-7.30 (m, 5H, Ar-H), 6.60 (m, 3H, Ar-H), 7.46-7.52 (m, 5H, Ar-H). ^{13}C NMR ($CDCl_3$): δ 156.2, 147.6, 141.5, 139.9, 137.9, 133.8, 129.6, 128.3, 123.4, 117.2, 116.3, 113.5, 36.1. Anal. Calcd for $C_{21}H_{19}N_3O_3S$: C, 64.10; H, 4.87; N, 10.68%. Found: C, 64.01; H, 4.77; N, 10.60%. ESI-MS (m/z): 393 (M^+).

N-((5-Chloro-1-(phenylsulfonyl)-1H-benzo[d]imidazol-2-yl) methyl)benzenamine (3c): Yield: 50%; m.p. 230-234°C; dark black solid, R_f: 0.67; [Chloroform:Methanol (92:8)], IR: 3060 (Aromatic -CH str), 1590 (Aromatic -C=C str), 1203 (-CN str), 3300 (-NH str), 790 (-CCl str), 1074 ($S(=O)_2$ str), 1549 (Alkane $-CH_2$ str). ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 2.67 (s, 2H, $-CH_2$), 4.57 (s, 1H, NH), 7.47-7.50 (m, 3H, Ar-H), 7.19-7.31 (m, 5H, Ar-H), 6.49 (m, 3H, Ar-H), 6.67 (s, 2H, Ar-H). ^{13}C NMR ($CDCl_3$): δ 147.6, 141.5, 140.3, 137.9, 133.8, 129.8, 128.3, 124.1, 117.2, 116.7, 115.8, 113.5, 36.1. Anal. Calcd for $C_{20}H_{16}ClN_3O_2S$: C, 60.37; H, 4.05; N, 10.56%. Found: C, 60.30; H, 4.00; N, 10.66%. ESI-MS (m/z): 397 (M^+).

2-Methyl-N-((5-nitro-1-(phenylsulfonyl)-1H-benzo[d] imidazol-2-yl)methyl)benzenamine (4a): Yield: 56%; m.p. 250-252°C; yellow semisolid, R_f: 0.64 [Chloroform:Methanol (92:8)], IR: 3060 (Aromatic -CH str), 1510 (Aromatic -C=C str), 3400 (-NH str), 1340 (-CN str), 880, ($S(=O)_2$ str), 1510 (N=O str) cm^{-1}. ^1H NMR (DMSO-*d*6, 400 MHz, δ ppm): 2.14 (s, 2H, $-CH_2$), 4.61 (s, 1H, NH), 2.04 (s, 3H, $-CH_3$), 8.00-8.07 (m, 3H, Ar-H), 7.19-7.49 (m, 5H, Ar-H), 6.44-6.47 (m, 4H, Ar-H). Anal. Calcd for $C_{21}H_{18}N_4O_4S$: C, 59.70; H, 4.29; N, 13.26%. Found: C, 59.60; H, 4.39; N, 13.36%. ESI-MS (m/z): 422 (M^+).

N-((5-Methoxy-1-(phenylsulfonyl)-1H-benzo[d]imidazol-2-yl) methyl)-2-methylbenzenamine (4b): Yield: 56%; m.p. 262-265°C; yellow color flakes, R_f: 0.60 [Chloroform:Methanol (92:8)], IR: 3058 (Aromatic -CH str), 1517 (Aromatic -C=C str), 1330 (-CN str), 3332 (-NH str), 1070 ($S(=O)_2$ stretching), 2909 (Alkane $-CH_3$ str) cm^{-1}. ^1H NMR (DMSO-*d*6, 400 MHz, δ ppm): 2.30 (*s*, 2H, CH_2), 3.34 (*s*, 3H, $-CH_3$), 2.52 (s, 3H, $-CH_3$), 4.61 (*s*, 1H, NH), 7.94-7.97 (*m*, 5H, Ar–H), 7.29-7.58 (*m*, 3H, Ar–H), 7.21-7.29 (*m*, 1H, Ar–H), 7.61-7.69 (*m*, 3H, Ar–H). ^{13}C NMR (DMSO-*d*6): δ 156.2, 146.5, 141.5, 139.9, 137.9, 133.8, 129.8, 128.3, 126.6, 123.4, 117.1, 116.3, 113.4, 109.8, 55.9, 36.4, 15.5. Anal. Calcd for $C_{22}H_{21}N_3O_3S$: C, 64.85; H, 5.19; N, 10.31%. Found: C, 64.75; H, 5.09; N, 10.40%. ESI-MS (m/z): 407 (M^+).

N-((5-Chloro-1-(phenylsulfonyl)-1H-benzo[d]imidazol-2-yl) methyl)-2-methylbenzenamine (4c): Yield: 52%; m.p. 230-232°C; black semisolid, R_f: 0.88 [Chloroform:Methanol (92:8)], IR: 2850 (Aromatic -CH str), 1300 (-CN str), 3300 (-NH str), 797.04 (-CCl str), 1026 ($S(=O)_2$ str), 1459 (Alkane $-CH_2$ str) cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 2.70 (s, 2H, $-CH_2$), 4.51 (s, 1H, NH), 2.96 (*s*, 3H, CH_3), 7.19-7.31 (m, 5H, Ar-H), 6.67 (m, 3H, Ar-H), 6.49 (m, 1H, Ar-H), 7.47-7.50 (m, 3H, Ar-H). ^{13}C NMR ($CDCl_3$): δ 146.5, 141.5, 140.3, 137.9, 133.8, 129.8, 128.3, 126.6, 124.1, 117.1, 116.7, 115.8, 113.4, 36.4, 15.5. Anal. Calcd for $C_{21}H_{18}ClN_3O_2S$: C, 61.23; H, 4.40; N, 10.20%. Found: C, 61.33; H, 4.50; N, 10.00%. ESI-MS (m/z): 411 (M^+).

2-Nitro-N-((5-nitro-1-(phenylsulfonyl)-1H-benzo[d]imidazol-2-yl)methyl)benzenamine (5a): Yield: 60%; m.p. 240-242°C; orange solid, R_f: 0.80 [Chloroform:Methanol (92:8)], IR: 2964 (Aromatic -CH str), 1500 (Aromatic -C=C str), 3405 (-NH str), 1300 (-CN str), 886, ($S(=O)_2$ str), 1300 (N=O str) cm^{-1}. ^1H NMR (DMSO-*d*6, 400 MHz, δ ppm): 2.10 (s, 2H, $-CH_2$), 4.42 (s, 1H, NH), 7.17-7.29 (m, 5H, Ar-H), 7.85-7.87 (m, 3H, Ar-H), 7.56-7.58 (m, 2H, Ar-H), 6.55-6.58 (m, 2H, Ar-H). Anal. Calcd for $C_{2O}H_{15}N_5O_6S$: C, 52.98; H, 3.33; N, 15.45%. Found: C, 52.88; H, 3.43; N, 15.40; %. ESI-MS (m/z): 453 (M^+).

N-((5-Methoxy-1-(phenylsulfonyl)-1H-benzo[d]imidazol-2-yl)methyl)-2-nitrobenzenamine (5b): Yield: 60%; m.p. 230-235°C; brown semisolid, R_f: 0.87 [Chloroform:Methanol (92:8)], IR: 3003 (Aromatic -CH str), 1640 (Aromatic -C=C str), 3448 (-NH str), 990 ($S(=O)_2$ str) cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 2.40 (s, 2H, $-CH_2$), 2.73 (s, 3H, $-CH_3$), 4.50 (s, 1H, NH), 6.58-6.61 (m, 2H, Ar-H), 7.20-7.30 (m, 5H, Ar-H), 7.46-7.52 (m, 4H, Ar-H), 6.92 (m, 1H, Ar-H). ^{13}C NMR (DMSO-*d*6): δ 156.2, 141.5, 139.9, 138.6, 135.7, 133.8, 132.2, 129.8, 128.3, 123.4, 121.9, 118.1, 116.3, 114.4, 109.8, 100.9, 55.9, 35.1. Anal. Calcd for $C_{21}H_{18}N_4O_5S$: C, 57.53; H, 4.14; N, 12.78%. Found: C, 57.43; H, 4.10; N, 12.88%. ESI-MS (m/z): 438 (M^+).

N-((5-Chloro-1-(phenylsulfonyl)-1H-benzo[d]imidazol-2-yl) methyl)benzenamine (5c): Yield: 58%; m.p. 220-224°C; brown solid, R_f: 0.68 [Chloroform:Methanol (92:8)], IR: 2920 (Aromatic -CH str), 1510 (Aromatic -C=C str), 1317.36 (-CN str), 3333 (-NH str), 1026.97, 880 ($S(=O)_2$ str) cm^{-1}. ^1H NMR (DMSO-*d*6, 400 MHz, δ ppm): 2.16 (s, 2H, $-CH_2$), 4.71 (s, 1H, NH), 6.92-6.96 (m, 5H, Ar-H), 8.00-8.07 (m, 3H, Ar-H), 7.45-7.49 (m, 1H, Ar-H), 7.35-7.39 (m, 3H, Ar-H). ^{13}C NMR (DMSO-*d*6): δ 141.5, 140.3, 138.6, 137.9, 135.7, 133.8, 132.2, 129.8, 128.3, 124.1, 121.9, 118.1, 116.7, 115.8, 114.4, 35.1. Anal. Calcd for $C_{20}H_{15}ClN_4O_4S$: C, 54.24; H, 3.41; N, 12.65%. Found: C, 54.15; H, 3.51; N, 13.00%. ESI-MS (m/z): 397 (M^+).

Biological evaluation

All the experimental procedures used in this study were approved by the Institutional Animal Ethical Committee, registered under Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India. Animals were housed individually in polypropylene cages, maintained under standard conditions of alternating light-and-dark cycles at a constant temperature (25±2°C and 35–60% relative humidity). All the animals were allowed free access to food and water (*ad libitum*).

Carrageenan-induced paw edema model: The newly synthesized test compounds were subjected to *in vivo* anti-inflammatory studies using carrageenan-induced rat paw edema model by employing 1% carrageenan solution as the phlogistic agent [34]. The test compounds were administered *p.o.* as suspensions in 2% sodium carboxy

methylcellulose (CMC), 30 min before the injection of the phlogistic agent, at a dose level of 100 mg/kg body weight. Indomethacin was used as a standard at a dose level of 50 mg/kg body weight. Groups of six SD rats of either sex were used in each experiment. The 2% sodium CMC was served as a control. The paw edema volume was measured with the help of plethysmograph by mercury displacement method at 0 h and 3 h (immediately after injection and 3h post injection of carrageenan). The % edema is shown in Table 1. The percent anti-inflammatory activity was calculated according to the formula as given below:

$$\% \text{ edema} = 100 - [(1 - Vt/Vc) \times 100]$$

$$\% \text{ reduction in edema} = (1 - Vt/Vc) \times 100$$

Vt and Vc is the edema volume in drug treated and control groups, respectively.

Acetic acid-induced writhing assay: The most active compounds (3b, 4b and 5b) emerged in the anti-inflammatory study were further tested for their analgesic potential by employing acetic acid-induced writhing assay [35]. Albino mice of either sex (20-25 g, body weight) were divided into control, standard and different test groups of six mice each. The control group was administered *p.o.*, with 2% sodium CMC, whereas standard and test compound groups were administered with acetyl salicylic acid and different test compounds, respectively at a dose level of 100 mg/kg suspended in 2% sodium CMC, 30 min before the *i.p.* injection of the acetic acid solution (0.6% v/v in distilled water) at a dose level of 1 ml/kg. The number of writhes per animal was recorded for 15 min. The analgesic activity was expressed as percentage of protection and the results are presented in Table 2.

$$\% \text{ Protection} = 100 - [(Vt/Vc) \times 100]$$

Ulcerogenic assay: SD rats of either sex weighing 150-200 g were divided into vehicle control, standard (indomethacin) and different test compound groups (n=6). The test compounds and indomethacin were administered orally in 2% sodium CMC orally at a dose of 100 and 50 mg/kg, respectively. After 6 h later, the rats were sacrificed, and their stomach was removed. Formal-saline (2% v/v) was then injected into the totally ligated stomach for storage overnight. The next day, the stomach were opened along the greater curvature, washed in warm water, and examined under 3-fold magnifier. The lengths of the longest diameters of the lesions were measured and summated to give a total lesion score (in mm) for each animal, the mean count for each group being calculated [36] and results are depicted in Table 3.

Statistical analysis: Statistical analysis of the biological activity of synthesized compounds on animals was performed by one-way variance (ANOVA) followed by Tukey's test; as "*p*" value of less than 0.05 was considered as statistically significant. All values were expressed as mean ± SEM. The SIGMASTAT, version 2.0 by Jandel Corporation was used for statistical analysis.

***In vitro* Antioxidant activity:** The *in vitro* antioxidant activity for all the newly synthesized compounds was performed by FRAP assay based on the reduction of a colorless Fe^{3+}-tripyridyltriazine complex into a blue-colored Fe^{2+}-tripyridyltriazine complex [37]. The working FRAP reagent was prepared by mixing 10 ml of sodium acetate buffer (pH=3.6) with 1 ml of TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) solution and 1 ml $FeCl_3.6H_2O$ in a volume ratio of 10:1:1, respectively. The FRAP reagent was warmed to 37°C before being used and the assay was started by adding 228 µl of FRAP reagent into 96-well microtiter-plate. The 12 µl of methanol as blank, test samples dissolved in methanol and ascorbic acid as standard dissolved in water, were taken into the wells. The reaction was allowed to run for 30 min and absorbance was read at 593 nm. The experiments were performed in triplicate and their mean was calculated for each compound and the absorbance change was translated into FRAP value (in µM) by following formula:

$$\text{FRAP value} = [\text{Sample}_{Abs}\ (30^{th}\text{ min} - 0^{th}\text{ min}) / \text{Standard}_{Abs}\ (30^{th}\text{ min} - 0^{th}\text{ min})] \times \text{conc of standard}$$

Ferrous sulfate was used as standard at conc. 200 µM

0^{th} min=blank methanol at 0^{th} min

Abs: Absorbance

Drug-likeness properties: A promising small molecule inhibitor should have properties similar to existing drugs. These properties are influenced by drug-like properties, i.e. lipophilicity, MW, HBD, hydrogen bond acceptors (HBA), PSA, molar volume (MV), molar refractivity (MR) and flexibility [38]. Drug-likeness properties of all the compounds were calculated by ACD/Labs software.

Results and Discussion

Synthesis of compounds

A novel series of compounds are designed by taking cue from the structures of indomethacin and sulindac with the absence of acidic moiety while retaining the functional groups that are required to bind with cyclooxygenase enzyme. Most of the COX-2 inhibitors contain sulfone moiety and the oxygen of the sulfone group interacts

Compound	% edema at 3 h (Mean ± SEM)	% reduction in edema
Control	100 ± 3.59	0.00
3a	74.63 ± 4.43	25.37
3b[a]	59.70 ± 2.99	40.30[a]
3c	76.12 ± 3.83	23.88
4a	71.64 ± 3.27	28.36
4b[a]	53.73 ± 4.62	46.27[a]
4c	73.13 ± 4.27	26.87
5a	74.63 ± 8.23	25.37
5b[a]	68.66 ± 3.78	31.34[a]
5c	79.10 ± 4.86	20.90
Indomethacin[a]	52.24 ± 4.27	47.76[a]

Values are mean ± standard error of mean (SEM) (n=6)
[a]Statistically significant compared to control group ($p \leq 0.05$)
Data was analyzed by unpaired one-way ANOVA test

Table 1: Anti-inflammatory activity of tested compounds and Indomethacin.

Compound	No. of wriths in 15 min (Mean ± SEM)	% Protection
Control	70.33 ± 3.01	0.00
3b[a]	30.33 ± 3.40	56.87[a]
4b[a]	27.17 ± 5.02	61.37[a]
5b[a]	36.50 ± 2.65	48.10[a]
Acetyl salicylic acid[a]	25.67 ± 1.45	63.51[a]

Values are mean ± SEM (n=6)
[a]Statistically significant compared to control group ($p \leq 0.05$)

Table 2: Analgesic activity of tested compounds and Acetyl salicylic acid.

Compound	Lesion score (mm) (Mean ± SEM)
Control[a]	1.0 0.47[a]
3b[a]	16.33 ± 1.59[a]
4b[a]	14.67 ± 1.68[a]
5b[a]	19.00 ± 0.82[a]
Indomethacin	30.67 ± 1.85

Values are mean ± SEM (n=6).
[a]Statistically significant compared to Indomethacin ($p \leq 0.05$).

Table 3: Ulcerogenic activity of tested compounds and Indomethacin.

with Hist90, Arg513, and Gln192 and forms hydrogen bonds with the enzyme pocket. Moreover, benzimidazole is recently emerged as pharmacophore of choice for anti-inflammatory analgesic action [24]. Inspired from abovementioned findings and with a new hope to develop gastroprotective NSAIDs, we designed novel 1, 2, 5-substitited benzimidazole derivatives (Figure 1). The synthetic strategy employed for the construction of novel benzimidazole derivatives is given in Scheme 1. The compounds 1a, 1b and 1c were prepared according to the reported literature starting from *o*-phenylenediamine i.e. substituted with small activating (CH_3) and deactivating groups (NO_2, Cl) [25]. Sulfonylation of compounds 1a, 1b and 1c was done in the presence of dry pyridine and benzenesulfonyl chloride to give intermediate compound 2a, 2b and 2c [26], which were further reacted with aryl amines of choice to get target compounds 3a-c, 4a-c and 5a-c. The

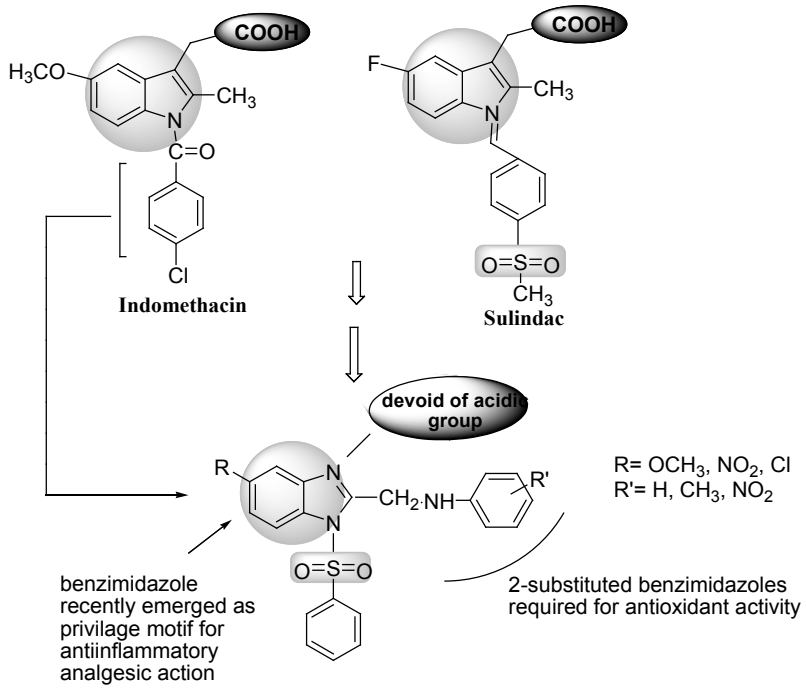
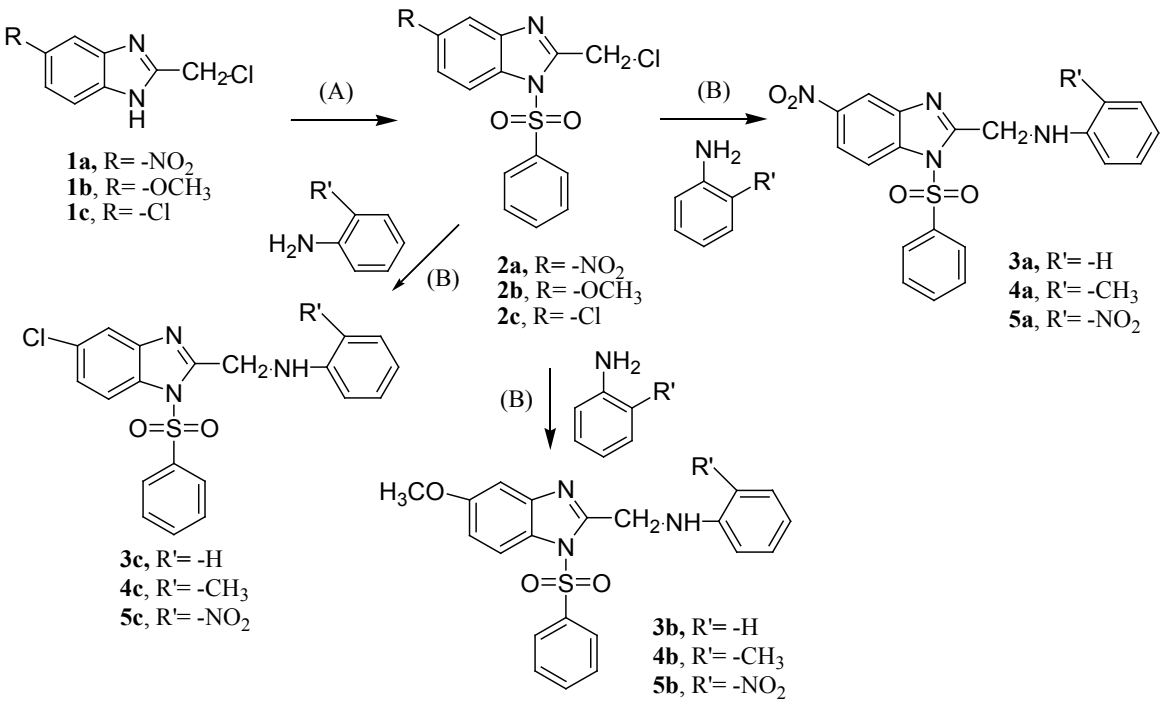


Figure 1: Design strategy for the target compounds.



Scheme 1: Reagents: (A) Benzenesulfonylchloride, Dry pyridine; (B) Ethanol, Potassium iodide, Potassium hydroxide.

purity of the compounds was achieved by column chromatographic separation using silica gel as stationary phase and chloroform: methanol as mobile phase.

Biological assays

The entire series of newly synthesized benzimidazole derivatives were biologically tested in order to evaluate their pharmacological activity. The anti-inflammatory activity of all the test compounds was carried out in rats by the carrageenan-induced rat paw edema model test with interesting activity ranging from 20.90-46.27%. It is observed that the tested compounds 3b, 4b, and 5b with electron donating substituents exhibited significant reduction in edema, 3h post dosing of test compounds, i.e. 40.30, 46.27 and 31.34% comparable to standard drug indomethacin, which exhibited reduction in edema as 47.76% (Table 1). The test compounds 3b, 4b, and 5b that exhibited potent anti-inflammatory activity were further selected to evaluate their analgesic activity. The compounds 3b, 4b, and 5b exhibited percentage protection as 56.87, 61.37 and 48.10% comparable to acetyl salicylic acid which was found to 63.51% against acetic acid-induced writhing test. The ulcerogenic potential of 3b, 4b and 5b was also studied in rats, which was appreciably lower than indomethacin. The mean lesion scores for the test compounds were 16.33, 14.67 and 19.00 mm when compared to indomethacin which was found to 30.67 mm. These findings suggest that the compounds 3b, 4b and 5b can produce less gastric ulceration and may be considered as safer for treating inflammatory conditions. The analgesic and ulcerogenic activity results are illustrated in Table 2 and Table 3, respectively.

It has been well known that local generation of various reactive oxygen species plays a significant role in the formation of gastric ulceration associated with NSAIDs therapy [27,28]. These observations indicate that NSAIDs with antioxidant activity could be a useful approach to prevent gastric ulcers. In present investigation, the antioxidant potential of all the newly synthesized compounds was accessed *in vitro* by Ferric reducing antioxidant power FRAP assay**.** The increase in absorbance is proportional to the antioxidant content. From the results, it is observed the compounds 3b, 4b and 5b were the most efficient compounds in FRAP assay with strongest ferric reductants FRAP values as 272.4, 325.6 and 230.6 µM (Table 4).

Drug-likeness properties

The present study also attempts the calculation of drug-likeness properties of all the newly synthesised derivatives. Lipinski's Rule of five is a rule to predict high probability of success or failure due to drug-likeness for molecules complying with 3 or more of the following [29]:

- High lipophilicity (expressed as log*P* less than 5)
- Molecular weight less than 500 g/mol
- Less than 5 hydrogen bond donors
- Less than 10 hydrogen bond acceptors
- Molar refractivity should be between 40-130

The molecule's hydrophilicity can be reliably evaluated using its log*P* value (logarithm partition coefficient). Molecules with log*P*<5 and molecular weight (MW) of 500 or less are considered to the best candidates for better oral absorption. The newly synthesized derivatives have log*P* in the range of 3.2-4.7 and MW in the range of 393.4-453.4 g/mol indicates good bioavailability with less than 10 freely rotatable bonds [30,31]. The polar surface area (PSA) of all the derivatives is in the range of 72.3-164.0 shows their ability to permeate cell membranes [32]. Similarly, all the derivatives exhibited fewer than 6 hydrogen bond donors (HBD) are viable drug candidates with no violation of drug-likeness [33]. Drug-likeness properties of all newly synthesized compounds were assessed by using ACD/Labs software and the results are disclosed in Table 5.

Compound	Absorbance [Sample (12 µl) + FRAP Reagent (228 µl)]							FRAP Value (µM)
	0 min	5 min	10 min	15 min	20 min	25 min	30 min	
Blank (Methanol)	0.063	0.064	0.065	0.065	0.066	0.066	0.073	3.643
3a	0.063	0.199	0.217	0.230	0.339	0.345	0.497	158.106
3b	0.063	0.284	0.312	0.311	0.328	0.335	0.811	272.495
3c	0.063	0.118	0.129	0.138	0.147	0.155	0.32	93.625
4a	0.063	0.100	0.118	0.133	0.145	0.154	0.452	141.712
4b	0.063	0.074	0.104	0.141	0.247	0.283	0.957	325.683
4c	0.063	0.079	0.087	0.091	0.095	0.098	0.299	85.974
5a	0.063	0.338	0.422	0.844	0.920	1.015	0.15	31.694
5b	0.063	0.102	0.115	0.129	0.137	0.142	0.696	230.601
5c	0.063	0.079	0.087	0.094	0.097	0.101	0.249	67.760
Ascorbic acid	0.063	1.782	1.818	1.861	1.912	1.960	1.009	344.627

Table 4: Ferric reducing antioxidant power of tested compounds and ascorbic acid.

Compound	MW (<500)	LogP (<5)	HBD (<5)	HBA (<10)	MR (40-130)	Number of criteria met (At least 3)	Freely rotatable bonds	PSA	MV
3a	408.4	3.2	1	8	110.0	All	6	118.1	286.1
3b	393.4	3.3	1	6	110.1	All	6	81.6	302.5
3c	397.8	5.7	1	5	108.9	All	5	72.3	290.2
4a	422.4	3.2	1	8	114.4	All	6	118.1	301.3
4b	407.4	3.3	1	6	114.5	All	6	81.6	317.7
4c	411.9	4.1	1	5	113.3	All	5	72.3	305.3
5a	453.4	3.8	1	11	115.6	4	7	164.0	291.4
5b	438.4	3.9	1	9	115.8	All	7	127.4	307.8
5c	442.8	4.7	1	8	114.6	All	6	118.1	295.4
Indomethacin	357.7	4.3	1	5	94.59	All	4	68.5	269.5

Table 5: Drug-likeness properties of benzimidazole derivatives.

Conclusion

In our attempt to discover potent and safer anti-inflammatory, analgesic agents with lower gastric ulceration, a series of 1, 2 and 5-substituted benzimidazole derivatives were synthesized and biologically evaluated. Further, the results suggested that the compounds 3b, 4b and 5b were found to possess encouraging anti-inflammatory and analgesic activity with significant reduction in ulcerogenic side effect. The absence of gastric damage may be attributed to their antioxidant activity. Hopefully, in the future, the compounds 3b, 4b and 5b could serve as lead compounds for developing a novel class of potent as well as orally active GI-safer anti-inflammatory analgesic agents.

References

1. Garavito RM (1996) The cyclooxygenase-2 structure: new drugs for an old target? Nat Struct Biol 3: 897-901.
2. Bergh MS, Budsberg SC (2005) The coxib NSAIDs: potential clinical and pharmacologic importance in veterinary medicine. J Vet Intern Med 19: 633-643.
3. Feldman B, Zinkl J, Jain N (2000) Veterinary hematology. Lippincott Williams and Wilkins, Philadelphia, PP. 213.
4. Trepanier LA (2002) Mechanisms of drug-associated hepatotoxicity in the dog and cat. Proc. 20th Annual Forum of the ACVIM. Dallas, 669-671.
5. Dannhardt G, Kiefer W (2001) Cyclooxygenase inhibitors--current status and future prospects. Eur J Med Chem 36: 109-126.
6. Sun SX, Lee KY, Bertram CT, Goldstein JL (2007) Withdrawal of COX-2 selective inhibitors rofecoxib and valdecoxib: impact on NSAID and gastroprotective drug prescribing and utilization. Curr Med Res Opin 23: 1859-1866.
7. Coruzzi G, Venturi N, Spaggiari S (2007) Gastrointestinal safety of novel nonsteroidal antiinflammatory drugs: selective COX-2 inhibitors and beyond. Acta Biomed 78: 96-110.
8. Wallace JL, Vong L (2008) NSAID-induced gastrointestinal damage and the design of GI-sparing NSAIDs. Curr Opin Investig Drugs 9: 1151-1156.
9. Zhang H, Bhatia M (2008) Hydrogen sulfide: a novel mediator of leukocyte activation. Immunopharmacol Immunotoxicol 30: 631-645.
10. Whiteman M, Winyard PG (2011) Hydrogen sulfide and inflammation: the good, the bad, the ugly and the promising. Expert Rev Clin Pharmacol 4: 13-32.
11. Wagner F, Asfar P, Calzia E, Radermacher P, Szabó C (2009) Bench-to-bedside review: Hydrogen sulfide--the third gaseous transmitter: applications for critical care. Crit Care 13: 213.
12. Kumar V, Abbas A, Fausto N (2001) Robbins and cotran pathologic basis of disease. Elsevier Saunders, Philadelphia, pp. 74.
13. Yedgar S, Krimsky M, Cohen Y, Flower RJ (2007) Treatment of inflammatory diseases by selective eicosanoid inhibition: a double-edged sword? Trends Pharmacol Sci 28: 459-464.
14. Murata T, Ushikubi F, Matsuoka T, Hirata M, Yamasaki A, et al. (1997) Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. Nature 388: 678-682.
15. Kourounakis PN, Tsiakitzis K, Kourounakis AP, Galanakis D (2000) Reduction of gastrointestinal toxicity of NSAIDs via molecular modifications leading to antioxidant anti-inflammatory drugs. Toxicology 144: 205-210.
16. Vicini P, Incerti M, Amoretti L, Ballabeni V, Ogn M, et al. (2002) Synthesis and pharmacological properties of benzisothiazole/benzimidazole derivatives with acidic groups. IL Farmaco 57: 363-367.
17. Naik P, Murumkar P, Giridhar R, Yadav M (2010) Angiotensin II receptor type 1 (AT1) selective non-peptidic antagonists-a perspective review. Bioorg Med Chem 18: 8418-8456.
18. Vyas VK, Ghate M (2010) Substituted benzimidazole derivatives as angiotensin II-AT1 receptor antagonist: a review. Mini Rev Med Chem 10: 1366-1384.
19. Bielory L, Lien KW, Bigelsen S (2005) Efficacy and tolerability of newer antihistamines in the treatment of allergic conjunctivitis. Drugs 65: 215-228.
20. Gaba M, Singh D, Singh S, Sharma V, Gaba P (2010) Synthesis and pharmacological evaluation of novel 5-substituted-1-(phenylsulfonyl)-2-methylbenzimidazole derivatives as anti-inflammatory and analgesic agents. Eur J Med Chem 45: 2245-2249.
21. Hiroshi S, Koji T, Akito K, Yasuhiro I, Isami K, et al. (1995) Preparation of 2-[(1-H benzimidazole-2-yl)sulfonyl methyl] 4-(Substituted amino)5-pyrimidine carboxylic acids as ulcer inhibitors. Chem Pharm Bull 43: 166-168.
22. Chen CJ, Yu JJ, Bi CW, Zhang YN, Xu JQ, et al. (2009) Mutations in a beta-tubulin confer resistance of Gibberella zeae to benzimidazole fungicides. Phytopathology 99: 1403-1411.
23. Bansal Y, Silakari O (2012) The therapeutic journey of benzimidazoles: a review. Bioorg Med Chem 20: 6208-6236.
24. Gaba M, Singh S, Mohan C (2014) Benzimidazole: an emerging scaffold for analgesic and anti-inflammatory agents. Eur J Med Chem 76: 494-505.
25. Amrutkar SV, Bhagat UD, Pargharmol P, Kotgire SS, Ranawat MS (2010) Synthesis and antifungal activity of 1-Alkyl/H-2[4-(Alkyl/Aryl-Piperazin-1-yl)-benzimidazole derivatives. Int J Pharm 2: 84-92.
26. Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR (2007) Vogel's textbook of practical organic chemistry, ELBS, Longman Group, UK, 916-917.
27. Bandyopadhyay U, Das D, Banerjee RK (1999) Reactive oxygen species-oxidative damage and pathogenesis. Curr Sci 77: 658–665.
28. Hassan A, Martin E, Puig-Parellada P (1998) Role of antioxidants in gastric mucosal damage induced by indomethacin in rats. Methods Find Exp Clin Pharmacol 20: 849-854.
29. Sivakumar R, Basha SKN, Kumarnallasivan P, Vijaianand PR, Pradeepchandran R, et al. (2010) A computational design and docking studies on Escherichia coli ß-Ketoacyl-Acyl carrier protein synthase III using auto dock. J Pharm Res 3: 1460-1462.
30. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, et al. (2002) Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem 45: 2615-2623.
31. Tool for molecular property explorer. OSIRIS Property Explorer.
32. Ertl P, Rohde B, Selzer P (2000) Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem 43: 3714-3717.
33. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46: 3-26.
34. Winter CA, Risley EA, Nuss GW (1962) Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med 111: 544-547.
35. Koster R, Anderson M, De Beer EJ (1959) Acetic acid for analgesic screening. Fed Proc 18: 412.
36. Vogel HS (2002) Drug discovery and evaluation (Pharmacological assays). Springer-Verlag, Berlin Heidelberg, New York, pp. 869.
37. Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239: 70-76.
38. Wishart DS, Knox C, Guo AC, Cheng D, Shrivastava S, et al. (2008) Drug Bank: a knowledgebase for drugs, drug actions and drug targets. Nucleic Acids Res 36: D901-906.

Citation: Gaba M, Mohan C (2015) Design, Synthesis and Biological Evaluation of Novel 1, 2, 5-Substituted Benzimidazole Derivatives as Gastroprotective Anti-inflammatory and Analgesic Agents. Med chem 5: 058-063. doi:10.4172/2161-0444.1000243