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DESIGN AND SYNTHESIS OF NOVEL BENZIMIDAZOLE THIOSEMICARBAZIDE AS POTENT ANALGESIC AND ANTI-INFLAMMATORY AGENTS

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ABSTRACT
With the aim of developing potent analgesic, and anti-inflammatory agents a series of novel benzimidazole semicarbazide derivatives were synthesized and characterized by FT-IR, ^{1}H-NMR, Mass spectroscopy and bases of elemental analysis. Tail-flick technique, and carrageenan-induced foot paw edema test were performed for screening analgesic, and anti-inflammatory, respectively. Moreover all potent compounds were examined for its ulcerogenicity. Results revealed that entire series of compounds exhibited mild to good analgesic, and anti-inflammatory activity. The potent analgesic and anti-inflammatory active compounds displayed low to moderate ulcer index. From the study it was found that, in this series nature of the substituent played a major role in analgesic and anti-inflammatory activity than its position. Compound 1-(4-chlorobenzylidene)-4-(4-(1-((dimethyl amino)methyl)-1*H*-benzimidazol-2-yl)phenyl)thiosemicarbazide **VIc** was determined to be the most active compound.

KEYWORDS: Benzimidazole, Thiosemicarbazide, Analgesic, Anti-inflammatory, Ulcerogenicity.

INTRODUCTION
Non-steroidal anti-inflammatory drugs (NSAIDs) represent a heterogeneous family of pharmacologically active compounds used to alleviate acute and chronic inflammation, pain and fever. In the past decade, numerous advances have taken place in the understanding of pathogenesis and as a result, significant progress has been made and is still being made in the development of novel NSAIDs.[1] The most important mechanism of NSAIDs is closely related to their ability to inhibit both isoforms of the enzyme cyclooxygenase (COX) also known as prostaglandin H_2 (PGH_2) synthase *i.e.* COX-1 and COX-2 because it catalyzes the conversion of arachidonic acid to PGH_2.[2] The constitutive COX-1 isoform is mainly responsible for the synthesis of prostaglandins which exert cytoprotective effect in the gastrointestinal tract and control renal function in the kidneys; while the inducible COX-2 is selectively activated by pro-inflammatory stimuli and facilitates the release of prostaglandins involved in the inflammatory process.[3] However, long term clinical usages of NSAIDs are associated with significant side effects such as severe gastrointestinal ulceration, bleeding, in tolerance and nephrotoxicity.[4-5] Therefore, investigation of new NSAIDs is still a major challenge and production of safer and more active NSAIDs are needed.

In addition, from pharmacoeconomic viewpoint, and seeking better patient compliance, the discovery of an analgesic and anti-inflammatory agent with potential activity with fewer adverse effects is a priority of the current global research, and consequently has occupied prime interest in recent years. Therefore, our aim is to find a compound having analgesic, and anti-inflammatory activities with less ulcer index. While searching for such a compound, we have found that benzimidazole ring is one of the moieties on which studies have been concentrated.

Benzimidazoles and their analogs are well known biologically active *N*-containing heterocycles, reported to poses various biological activities.[6-21] On the other hand, pharmacologically, semicarbazide and its derivatives represent one of the most important class of organic compounds, possessing various biological activities.[22-24] Some of its derivatives have been reported to exhibit significant analgesic and anti-inflammatory activities.[25-26]

Based on these findings, and in continuation of our drug research program concerning synthesis of new, safer and more biologically active compounds, it was of interest to synthesize a new series of benzimidazole thiosemicarbazide derivatives with the hope to obtain

more active and less toxic analgesic, and anti-inflammatory agents. The ulcerogenic activity of the compounds was also determined.

MATERIALS AND METHODS

General

The chemicals and reagents used were obtained from various chemical units Qualigens, E. Merck India Ltd., CDH, and SD Fine Chem. These solvents used were of LR grade and purified before their use. The silica gel G used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. All the melting points were taken in open glass capillary and are uncorrected. ^{1}H-NMR spectra were recorded at 500 MHz on Bruker Avance-500 NMR spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. The chemical shifts are reported in ppm scale. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. All the IR spectra were recorded in KBr pellets on a Jasco FT-IR 410 spectrometer. Elemental analyses were performed on a Perkine Elmer model 2400C analyzer and were within ± 0.4 % of the theoretical values.

Synthesis of 4-(1H-benzimidazol-2-yl)benzenamine (I)

A mixture of *o*-phenylenediamine (1.08 g, 0.01 mol) and *p*-aminobenzoic acid (1.37 g, 0.01 mol) were stirred in a syrupy *o*-phosphoric acid (15 ml) at 200° C for 2 h. The reaction mixture was cooled and poured on crushed ice. The bulky white precipitate obtained was stirred in cold water (150 ml) and sodium hydroxide solution (5 M) was added until the pH reaches 7. The resulting solid obtained **I** was filtered and recrystallized from methanol. Yield = 70 %, m.p. 246-248 °C. IR (KBr) cm^{-1}: 3357 (NH), 2985 (Ar-CH), 1675 (C=N), 1586 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.09-8.24 (m, 8H, Ar-CH), 5.78 (s, 1H, NH of benzimidazole), 4.30 (s, 2H, Ar-NH_2). EI-MS *m/z*: 209 (M^+). *Anal*. Calcd for $C_{13}H_{11}N_3$: C, 74.62; H, 5.30; N, 20.08. Found: C, 74.86; H, 5.28; N, 20.01.

Synthesis of 4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)benzenamine (II)

A mixture of 4-(1*H*-benzimidazol-2-yl)benzenamine **I** (2.09 g; 0.01 mol), formaldehyde (0.45 g; 0.015 mol) and dimethylamine (0.67 g; 0.015 mol) in ethanol (15 ml) were stirred mechanically for the period of 1 h. The obtained mixture was then refluxed in a water bath for 4 h. The resulting reaction mixture was cooled and poured in ice cold water with vigorous stirring. The obtained product 4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)benzenamine **II** was filtered and recrystallised using methanol. Yield = 74 %, m.p. 221-222 °C. IR (KBr) cm^{-1}: 3335 (NH), 3041 (Ar-CH), 2956 (CH_3-CH), 1649 (C=N), 1614 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 6.95-8.18 (m, 8H, Ar-CH), 5.22 (s, 2H, CH_2 linkage), 4.27 (s, 2H, Ar-NH_2), 2.73 (s, 6H, $N(CH_3)_2$). EI-MS *m/z*: 266 (M^+). *Anal*. Calcd for $C_{16}H_{18}N_4$: C, 72.15; H, 6.81; N, 21.04. Found: C, 72.39; H, 6.79; N, 20.98.

Synthesis of methyl 4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenylcarbamo dithioate (IV)

A solution of 4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)benzenamine **II** (2.66 g; 0.01 mol) in dimethyl sulfoxide (10 ml) was stirred vigorously in magnetic stirrer. To this solution, carbon disulphide (0.76 g; 0.01 mol) and aqueous sodium hydroxide (0.6 ml; 20 M) was added drop wise during 30 min with stirring. Then the reaction mixture was immediately kept in freezing mixture and dimethyl sulphate (1.26 g; 0.01 mol) was added gradually to the formed intermediate **III** [sodium 4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl) phenylcarbamodithioate] with stirring and the stirring was continued for further 2 h. Finally, the above obtained reaction mixture was poured into ice cold water and stirred well. The solid obtained **IV** was filtered, washed with water, dried and recrystallized from ethanol. Yield = 77 %, m.p. 269-271 °C. IR (KBr) cm^{-1}: 3340 (NH), 2998 (Ar-CH), 2945 (CH_3-CH), 1642 (C=N), 1106 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.29-8.25 (m, 8H, Ar-CH), 5.06 (s, 2H, CH_2 linkage), 4.30 (s, 1H, Ar-NH), 2.98 (s, 6H, $N(CH_3)_2$), 2.34 (s, 3H, CH_3). EI-MS *m/z*: 356 (M^+). *Anal*. Calcd for $C_{18}H_{20}N_4S_2$: C, 60.64; H, 5.65; N, 15.72. Found: C, 60.45; H, 5.67; N, 15.77.

Synthesis of 4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl) thiosemicarbazide (V)

Hydrazine hydrate (0.5 g; 0.01 mol) and the above prepared 4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)phenylcarbamodithioate **IV** (3.56 gm; 0.01 mol), were dissolved in ethanol (20 mL). To this anhydrous potassium carbonate (100 mg) was added and refluxed in water bath for 18 h. The reaction mixture was cooled in ice and the solid separated was filtered and purified by dissolving in 10 % alcoholic sodium hydroxide solution and re-precipitated by treating with dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried, and recrystallized from ethanol to afford compound **V**. Yield = 71 %, m.p. 255-256 °C. IR (KBr) cm^{-1}: 3378 (NH), 3032 (Ar-CH), 2920 (CH_3-CH), 1645 (C=N), 1603 (C=C), 1154 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.12-8.04 (m, 8H, Ar-CH), 4.93 (s, 2H, CH_2 linkage), 4.18 (s, 1H, Ar-NH), 2.64 (s, 6H, $N(CH_3)_2$), 2.31 (s, 1H, NH of $NHNH_2$), 2.19 (s, 2H, NH_2). EI-MS *m/z*: 340 (M^+). *Anal*. Calcd for $C_{17}H_{20}N_6S$: C, 59.97; H, 5.92; N, 24.69. Found: C, 60.18; H, 5.94; N, 24.60.

Synthesis of 1-(substitutedbenzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl)thiosemi carbazide (VIa-VIm)

To a well stirred mixture of different aromatic aldehydes (0.01 mol) in ethanol (30 ml) and glacial acetic acid (0.5 ml), 4-(4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)phenyl) thiosemicarbazide **V** (3.40 g; 0.01 mol) was added in fraction with stirring. The resulting mixture was refluxed on water bath over night and kept aside for some time. Then the mixture was poured in ice cold water with stirring and the product that separated out

VIa-VIm was filtered, dried and recrystallized from ethanol. The method used for the preparation and isolation of the compounds gave materials of good purity, as evidenced by their spectral analyses.

1-Benzylidene-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl) thiosemicarbazide (VIa)

Yield = 79 %, m.p. 172-174 °C. IR (KBr) cm^{-1}: 3323 (NH), 3097 (Ar-CH), 2930 (CH_3-CH), 1671 (C=N), 1605 (C=C), 1169 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.42 (s, 1H, CH=N), 7.14-8.20 (m, 13H, Ar-CH), 4.95 (s, 2H, CH_2 linkage), 4.31 (s, 1H, Ar-NH), 2.63 (s, 6H, $N(CH_3)_2$), 2.19 (s, 1H, NHN=). EI-MS *m/z*: 428 (M^+). *Anal*. Calcd for $C_{24}H_{24}N_6S$: C, 67.26; H, 5.64; N, 19.61. Found: C, 67.50; H, 5.63; N, 19.54.

1-(4-Nitrobenzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl) thiosemicarbazide (VIb)

Yield = 73 %, m.p. 158-159 °C. IR (KBr) cm^{-1}: 3346 (NH), 3081 (Ar-CH), 2955 (CH_3-CH), 1643 (C=N), 1620 (C=C), 1548 & 1319 (NO_2), 1142 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.65 (s, 1H, CH=N), 7.31-8.18 (m, 12H, Ar-CH), 4.82 (s, 2H, CH_2 linkage), 4.06 (s, 1H, Ar-NH), 2.99 (s, 6H, $N(CH_3)_2$), 1.94 (s, 1H, NHN=). EI-MS *m/z*: 473 (M^+). *Anal*. Calcd for $C_{24}H_{23}N_7O_2S$: C, 60.87; H, 4.90; N, 20.70. Found: C, 61.06; H, 4.89; N, 20.64.

1-(4-Chlorobenzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl) thiosemicarbazide (VIc)

Yield = 76 %, m.p. 199-202 °C. IR (KBr) cm^{-1}: 3307 (NH), 3087 (Ar-CH), 2939 (CH_3-CH), 1662 (C=N), 1615 (C=C), 1150 (C=S), 763 (C-Cl). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.81 (s, 1H, CH=N), 7.06-8.35 (m, 12H, Ar-CH), 5.08 (s, 2H, CH_2 linkage), 4.23 (s, 1H, Ar-NH), 2.77 (s, 6H, $N(CH_3)_2$), 2.02 (s, 1H, NHN=). EI-MS *m/z*: 465 (M^{+2}), 463 (M^+). *Anal*. Calcd for $C_{24}H_{23}ClN_6S$: C, 62.26; H, 5.01; N, 18.15. Found: C, 62.05; H, 5.03; N, 18.21.

1-(4-Methylbenzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl) thiosemicarbazide (VId)

Yield = 70 %, m.p. 145-147 °C. IR (KBr) cm^{-1}: 3359 (NH), 3092 (Ar-CH), 2948 (CH_3-CH), 1655 (C=N), 1613 (C=C), 1156 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.39 (s, 1H, CH=N), 7.13-8.16 (m, 12H, Ar-CH), 5.01 (s, 2H, CH_2 linkage), 4.15 (s, 1H, Ar-NH), 2.82 (s, 3H, CH_3), 2.58 (s, 6H, $N(CH_3)_2$), 2.24 (s, 1H, NHN=). EI-MS *m/z*: 442(M^+). *Anal*. Calcd for $C_{25}H_{26}N_6S$: C, 67.84; H, 5.92; N, 18.99. Found: C, 67.60; H, 5.91; N, 19.06.

1-(4-Methoxybenzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl) thiosemicarbazide (VIe)

Yield = 75 %, m.p. 160-161 °C. IR (KBr) cm^{-1}: 3331 (NH), 3076 (Ar-CH), 2943 (CH_3-CH), 1640 (C=N), 1625 (C=C), 1168 (C=S), 1052 (C-O-C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.52 (s, 1H, CH=N), 7.08-8.30 (m, 12H, Ar-CH), 4.96 (s, 2H, CH_2 linkage), 4.35 (s, 1H, Ar-NH), 3.96 (s, 3H, OCH_3), 2.81 (s, 6H, $N(CH_3)_2$), 2.03 (s, 1H, NHN=). EI-MS *m/z*: 458 (M^+). *Anal*. Calcd for $C_{25}H_{26}N_6OS$: C, 65.48; H, 5.71; N, 18.33. Found: C, 65.73; H, 5.70; N, 18.27.

1-(4-Hydroxybenzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl) thiosemicarbazide (VIf)

Yield = 78 %, m.p. 183-186 °C. IR (KBr) cm^{-1}: 3519 (OH), 3315 (NH), 3090 (Ar-CH), 2958 (CH_3-CH), 1676 (C=N), 1622 (C=C), 1163 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.07 (s, 1H, CH=N), 6.85-7.82 (m, 12H, Ar-CH), 5.43 (s, 1H, OH), 5.19 (s, 2H, CH_2 linkage), 4.26 (s, 1H, Ar-NH), 2.70 (s, 6H, $N(CH_3)_2$), 1.88 (s, 1H, NHN=). EI-MS *m/z*: 444 (M^+). *Anal*. Calcd for $C_{24}H_{24}N_6OS$: C, 64.84; H, 5.44; N, 18.90. Found: C, 64.61; H, 5.43; N, 18.96.

1-(4-(Dimethylamino)benzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl) phenyl)thiosemicarbazide (VIg)

Yield = 74 %, m.p. 151-153 °C. IR (KBr) cm^{-1}: 3351 (NH), 3098 (Ar-CH), 2935 (CH_3-CH), 1650 (C=N), 1612 (C=C), 1147 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.51 (s, 1H, CH=N), 7.29-8.24 (m, 12H, Ar-CH), 4.87 (s, 2H, CH_2 linkage), 4.00 (s, 1H, Ar-NH), 2.98 (s, 6H, $N(CH_3)_2$), 2.52 (s, 6H, $N(CH_3)_2$), 1.95 (s, 1H, NHN=). EI-MS *m/z*: 471 (M^+). *Anal*. Calcd for $C_{26}H_{29}N_7S$: C, 66.21; H, 6.20; N, 20.79. Found: C, 66.43; H, 6.18; N, 20.74.

1-(3-Nitrobenzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl) thiosemicarbazide (VIh)

Yield = 71 %, m.p. 213-215 °C. IR (KBr) cm^{-1}: 3362 (NH), 3085 (Ar-CH), 2956 (CH_3-CH), 1664 (C=N), 1601 (C=C), 1543& 1328 (NO_2), 1150 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.36 (s, 1H, CH=N), 7.12-8.07 (m, 12H, Ar-CH), 4.90 (s, 2H, CH_2 linkage), 4.34 (s, 1H, Ar-NH), 2.91 (s, 6H, $N(CH_3)_2$), 2.13 (s, 1H, NHN=). EI-MS *m/z*: 473 (M^+). *Anal*. Calcd for $C_{24}H_{23}N_7O_2S$: C, 60.87; H, 4.90; N, 20.70. Found: C, 61.02; H, 4.88; N, 20.66.

1-(3-Chlorobenzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl) thiosemicarbazide (VIi)

Yield = 77 %, m.p. 164-166 °C. IR (KBr) cm^{-1}: 3338 (NH), 3073 (Ar-CH), 2959 (CH_3-CH), 1667 (C=N), 1602 (C=C), 1146 (C=S), 781 (C-Cl). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.63 (s, 1H, CH=N), 7.35-8.39 (m, 12H, Ar-CH), 5.12 (s, 2H, CH_2 linkage), 4.37 (s, 1H, Ar-NH), 2.56 (s, 6H, $N(CH_3)_2$), 1.80 (s, 1H, NHN=). EI-MS *m/z*: 465 (M^{+2}), 463 (M^+). *Anal*. Calcd for $C_{24}H_{23}ClN_6S$: C, 62.26; H, 5.01; N, 18.15. Found: C, 62.43; H, 5.02; N, 18.09.

1-(3-Methylbenzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl) thiosemicarbazide (VIj)

Yield = 79 %, m.p. 177-178 °C. IR (KBr) cm^{-1}: 3340 (NH), 3082 (Ar-CH), 2948 (CH_3-CH), 1675 (C=N), 1619 (C=C), 1141 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.26 (s, 1H, CH=N), 7.08-8.11 (m, 12H, Ar-CH), 4.93 (s, 2H, CH_2 linkage), 4.12 (s, 1H, Ar-NH), 3.05 (s, 3H, CH_3), 2.84 (s, 6H, $N(CH_3)_2$), 2.07 (s, 1H, NHN=). EI-MS *m/z*: 442(M^+). *Anal*. Calcd for $C_{25}H_{26}N_6S$: C, 67.84; H, 5.92; N, 18.99. Found: C, 67.65; H, 5.94; N, 19.03.

1-(3-Methoxybenzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl) thiosemicarbazide (VIk)

Yield = 72 %, m.p. 149-151 °C. IR (KBr) cm^{-1}: 3317 (NH), 3080 (Ar-CH), 2932 (CH_3-CH), 1648 (C=N), 1621 (C=C), 1165 (C=S), 1059 (C-O-C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.74 (s, 1H, CH=N), 7.25-8.13 (m, 12H, Ar-CH), 5.06 (s, 2H, CH_2 linkage), 4.08 (s, 1H, Ar-NH), 3.80 (s, 3H, OCH_3), 2.72 (s, 6H, $N(CH_3)_2$), 1.79 (s, 1H, NHN=). EI-MS *m/z*: 458 (M^+). *Anal*. Calcd for $C_{25}H_{26}N_6OS$: C, 65.48; H, 5.71; N, 18.33. Found: C, 65.29; H, 5.73; N, 18.36.

1-(3-Hydroxybenzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl)phenyl) thiosemicarbazide (VIl)

Yield = 75 %, m.p. 190-192 °C. IR (KBr) cm^{-1}: 3520 (OH), 3384 (NH), 3091 (Ar-CH), 2956 (CH_3-CH), 1652 (C=N), 1608 (C=C), 1159 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.10 (s, 1H, CH=N), 6.99-7.97 (m, 12H, Ar-CH), 5.28 (s, 1H, OH), 4.91 (s, 2H, CH_2 linkage), 4.34 (s, 1H, Ar-NH), 2.65 (s, 6H, $N(CH_3)_2$), 1.92 (s, 1H, NHN=). EI-MS *m/z*: 444 (M^+). *Anal*. Calcd for $C_{24}H_{24}N_6OS$: C, 64.84; H, 5.44; N, 18.90. Found: C, 64.70; H, 5.45; N, 18.95.

1-(3-(Dimethylamino)benzylidene)-4-(4-(1-((dimethylamino)methyl)-1H-benzimidazol-2-yl) phenyl)thiosemicarbazide (VIm)

Yield = 78 %, m.p. 206-208 °C. IR (KBr) cm^{-1}: 3326 (NH), 3075 (Ar-CH), 2940 (CH_3-CH), 1672 (C=N), 1629 (C=C), 1158 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 8.48 (s, 1H, CH=N), 7.17-8.05 (m, 12H, Ar-CH), 5.04 (s, 2H, CH_2 linkage), 4.29 (s, 1H, Ar-NH), 2.76 (s, 6H, $N(CH_3)_2$), 2.51 (s, 6H, $N(CH_3)_2$), 1.86 (s, 1H, NHN=). EI-MS *m/z*: 471 (M^+). *Anal*. Calcd for $C_{26}H_{29}N_7S$: C, 66.21; H, 6.20; N, 20.79. Found: C, 66.40; H, 6.21; N, 20.71.

Biological activities

Pharmacology

The synthesized compounds were evaluated for analgesic, anti-inflammatory and ulcerogenic activities. One-way analysis of variance (ANOVA) was performed to certain the significance of all the exhibited activities. The test compounds and the standard drugs were administered in the form of a suspension (1% carboxy methyl cellulose as a vehicle) by oral route of administration for analgesic and anti-inflammatory but for ulcerogenicity studies by intraperitoneally as suspension in 10% v/v Tween-80. Each group consisted of six animals. The animals were maintained in colony cages at 25 ± 2°C, relative humidity of 45–55%, under a 12 h light and dark cycle; were fed standard animal feed.[27] All the animals were acclimatized for a week before use.

Analgesic activity

The analgesic activity was performed by tail flick technique using Wistar albino mice (25-35 g) of either sex selected by random sampling technique.[28,29] Diclofenac sodium at a dose level of 10 and 20 mg/kg was administered orally as reference drug for comparison. The test compounds at two dose levels *i.e.* 10 and 20 mg/kg were administered orally. The reaction times were recorded immediately before and 30 min, 1, 2 and 3 h after the treatment and cut-off time was 10 s. The percent analgesic activity (PAA) was calculated by the following formula. PAA = $[T_2\text{-}T_1/10\text{-}T_1] \times 100$; where T_1 is the reaction time (s) before treatment, and T_2 is the reaction time (s) after treatment. The observed results are presented in Table 1.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan induced paw oedema test in rats.[30] Diclofenac sodium 10 and 20 mg/kg was administered as standard drug for comparison. The test compounds were administered at two dose levels of 10 and 20 mg/kg. The paw volumes were measured using the mercury displacement technique with the help of plethysmograph immediately before and 30 min, 1, 2 and 3 h after carrageenan injection. The percent inhibition of paw oedema was calculated according to the following formula, percent inhibition I = 100[1-(a - x)/(b - y)] where x is the mean paw volume of rats before the administration of carrageenan and test compounds or reference compound (test group), a is the mean paw volume of rats after the administration of carrageenan in the test group (drug treated), b is the mean paw volume of rats after the administration of carrageenan in the control group, y is the mean paw volume of rats before the administration of carrageenan in the control group. All the percent inhibition results are shown in Table 2.

Ulcerogenicity

Ulceration in rats was induced as reported method.[31] Albino rats of Wistar strain weighing 150-200 g of either sex were divided into various groups each of six animals. Control group of animals were administered only with 10% v/v Tween-80 suspension intraperitoneally. One group was administered with Aspirin intraperitoneally in a dose of 200 mg/kg once daily for three days. Diclofenac was also administered as standard drug at 20 mg/kg once daily for three days to another group of animals in the same route. The remaining group of animals was administered with test compounds intraperitoneally in a dose of 20 mg/kg. On fourth day,

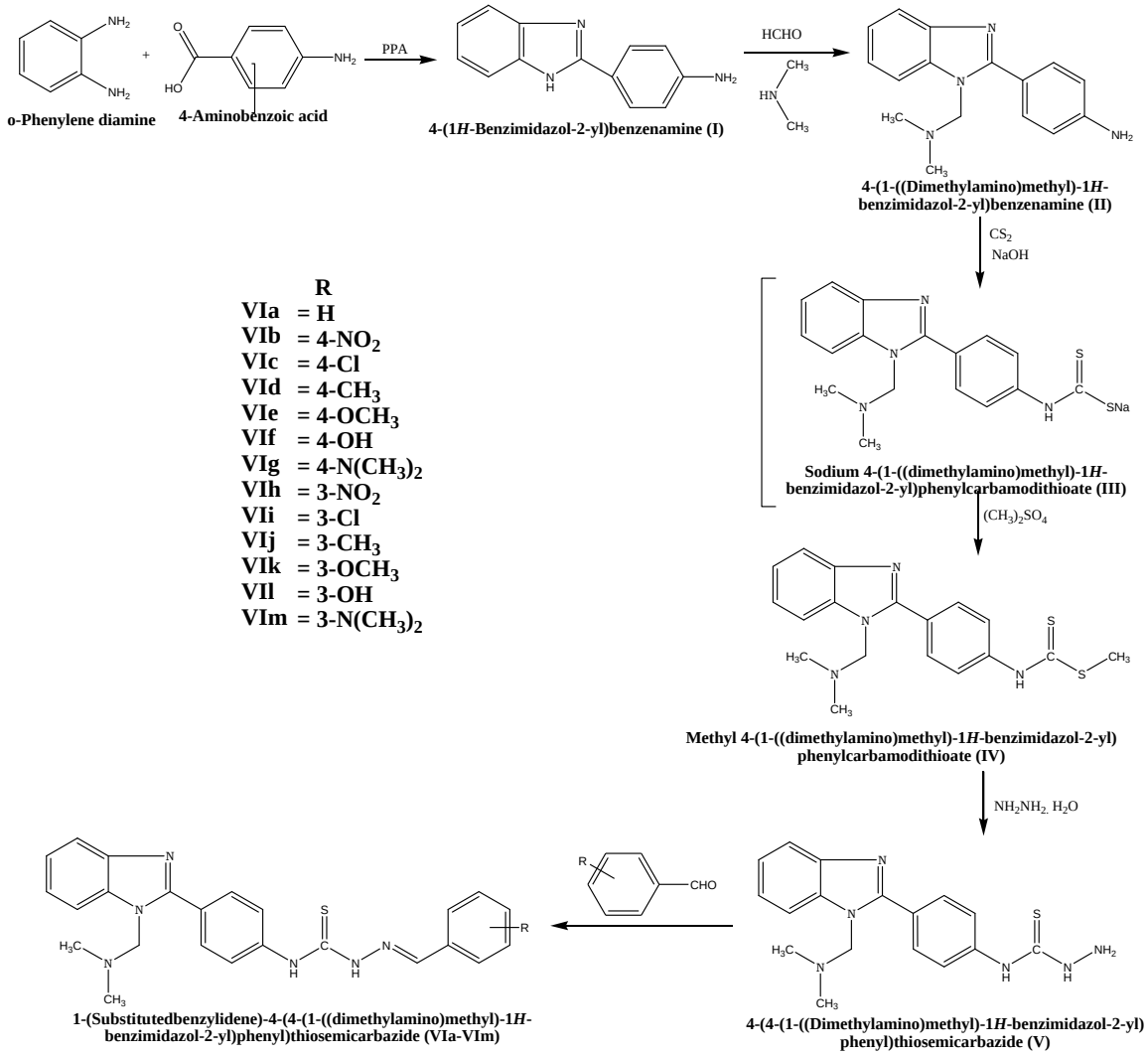
pylorus was ligated as per previous reported method.[32] Animals were fasted for 36 h before the pylorus ligation procedure. Four hours after the ligation, animals were sacrificed. The stomach was removed and opened along with the greater curvature. Ulcer index was determined by earlier reported method and presented in Table 2.[33]

RESULTS AND DISCUSSION

Chemistry

The title compounds **VIa-VIm** was synthesized as per the protocol shown in Scheme 1. In the present work, by substituting different benzylidene moiety at N-1 of 4-(4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)phenyl)thiosemicarbazide, a sequence of novel benzimidazole derivatives **VIa-VIm** were synthesized. By a multistep synthesis, various novel 1-(substitutedbenzylidene)-4-(4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)phenyl)thiosemicarbazide **VIa-VIm** were synthesized from *o*-phenylenediamine & 4-amino benzoic acid. Initially *o*-phenylenediamine was treated with 4-amino benzoic acid in presence of polyphosphoric acid (PPA) to obtain 4-(1*H*-benzimidazol-2-yl)benzenamine **I** by cyclisation reaction. In the second step, obtained amino derivative **3** was treated with formaldehyde and dimethylamine to produce 4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)benzenamine **II** through Mannich reaction. In the next step compound **II** was treated with carbon di sulphide in presence of sodium hydroxide to get the corresponding sodiumcarbamodithioate **III**; subsequently the obtained sodiumcarbamodithioate **III** was treated with dimethylsulpahte to synthesize methyl 4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)phenylcarbamodithioate **IV**. In the pre final step compound **IV**, was treated with hydrazine hydrate to obtain corresponding thiosemicarbazide derivatives **V**. In the final step, the obtained 4-(4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)phenyl)thiosemicarbazide **V** undergoes Schiff base reaction with various aromatic aldehydes and produces corresponding benzylidene substituted 4-(4-(1-((dimethylamino) methyl)-1*H*-benzimidazol-2-yl)phenyl)thiosemicarbazide analogs **VIa-VIm**. To optimize the reactions for purity and completion, TLC was performed throughout the reactions.

Assigned structures of the test compound were confirmed using various spectral studies (IR, NMR, mass spectra, and elemental analyses). Presence of particular groups was identified from IR spectra by means of some characteristic absorption peaks. Disappearance of a broad absorption peak between 3000-3500 cm^{-1} corresponds to COOH and presence of absorption peak at 3357 cm^{-1} in IR due to presence of NH stretching, confirms the formation of 4-(1*H*-benzimidazol-2-yl)benzenamine **I.** It is further supported by appearance of singlet in its ^{1}H-NMR spectra at δ 5.78 and 4.30 ppm for one and two proton which might be assigned to NH and NH_2 proton, respectively. The formations of Mannich base **II** was confirmed from the appearance of three singlet peaks at δ 5.22, 4.27 and 2.73 ppm for two, two and six protons which might be assigned to CH_2 linkage, NH_2 and $N(CH_3)_2$ proton, respectively. Formation of compound **IV** was confirmed by the appearance of absorption peak at 1106 cm^{-1} in IR due to presence of C=S. This is further confirmed from NMR spectroscopy by the appearance of singlet peak for one proton of NH at δ 4.30 ppm and singlet peak at δ 2.34 ppm for three protons which might be assigned to CH_3. The structure of compound **V** was confirmed from its ^{1}H-NMR spectra by the absence of singlet peak for three protons of methyl group around δ 2.00-2.50 ppm and appearance of two new singlet peaks at δ 2.31 and 2.19 ppm for one and two protons which might be assigned to NH of $NHNH_2$ and NH_2 of $NHNH_2$ proton, respectively. The formation of novel 1-(substitutedbenzylidene)-4-(4-(1-((dimethyl amino)methyl)-1*H*-benzimidazol-2-yl)phenyl)thiosemicarbazide analogs **VIa-VIm** from 4-(4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)phenyl)thiosemicarbazide **V** can be recognized from the disappearance of singlet peaks around δ 2.00 ppm corresponds to NH_2 proton. The structure of title compounds **VIa-VIm** were further confirmed by appearance of various other peaks in NMR spectroscopy corresponds to assigned structure. Mass spectrum further confirmed their molecular weight and purity.



R
VIa = H
VIb = 4-NO_2
VIc = 4-Cl
VId = 4-CH_3
VIe = 4-OCH_3
VIf = 4-OH
VIg = 4-$N(CH_3)_2$
VIh = 3-NO_2
VIi = 3-Cl
VIj = 3-CH_3
VIk = 3-OCH_3
VIl = 3-OH
VIm = 3-$N(CH_3)_2$

Scheme 1. Synthesis of **1-(Substitutedbenzylidene)-4-(4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)phenyl)thiosemicarbazide (VIa-VIm)**

Table 1 Percent analgesic activity of the synthesized compounds 3-16 (Tail flick method)

Compound	Dose (mg/kg)	Percent analgesic activity			
		30 min	1 h	2 h	3 h
VIa	10	29±0.71**	36±1.23*	41±0.56*	28±2.14*
	20	34±1.83*	45±0.51**	49±1.36**	30±0.75*
VIb	10	15±0.47 NS	20±0.95*	22±2.30*	13±1.33**
	20	19±1.39*	25±2.10*	30±1.31 NS	17±0.74*
VIc	10	41±1.34**	49±0.46***	57±0.95*	36±0.75**
	20	49±2.03*	64±1.62**	73±1.10**	46±0.56*
VId	10	38±0.40*	47±1.15**	53±1.61*	35±1.49***
	20	45±1.25**	60±0.78*	72±0.59**	41±2.14*
VIe	10	21±0.61*	27±1.35*	30±0.53*	19±0.78 **
	20	25±0.86*	32±2.29*	34±2.37*	24±1.17 NS
VIf	10	18±1.24*	23±0.60**	27±1.52*	15±1.08*
	20	22±0.90 NS	29±0.86*	33±0.71*	20±0.45*
VIg	10	33±2.12*	41±0.91*	49±1.05**	32±1.68*
	20	41±0.68*	56±1.04***	67±1.47*	39±0.90**
VIh	10	14±0.91*	19±1.12**	22±0.86*	11±2.48*
	20	17±1.15**	24±2.24 NS	26±0.68*	16±1.82*
VIi	10	40±2.31*	46±1.34**	57±1.26***	36±0.42*
	20	48±1.20**	62±0.86*	72±0.69*	44±0.44**
VIj	10	36±1.65**	47±0.93*	52±1.17**	35±1.40*

	20	44±0.89*	58±2.05**	70±0.51*	41±1.38***
VIk	10	20±1.36*	25±2.27 ^{NS}	29±2.10*	19±0.69*
	20	23±0.98***	31±1.13*	34±0.79 *	22±2.04*
VIl	10	18±0.57*	24±0.51*	25±0.53**	14±0.97 ^{NS}
	20	20±0.71*	28±2.17**	31±1.20*	19±0.82*
VIm	10	33±1.15**	40±0.80*	47±1.02**	31±1.11**
	20	40±0.45*	54±0.58**	63±0.63*	38±0.91*
Control	NA	04±0.52	05±0.47	07±0.65	03±0.70
Diclofenac	10	39±0.87**	47±1.32*	54±2.13***	36±0.64**
	20	46±2.49*	59±0.76***	71±1.48*	43±0.92*

Each value represents the mean ± SEM (n=6); NA: Not applicable; ^{NS}: Non significant.
*Significance levels *p < 0.5, **p < 0.01, ***p < 0.001 as compared with the respective control.*

Table 2 Percent anti-inflammatory activity (Carrageenan induced paw oedema test in rats) and ulcer index of the synthesized compounds 3-16

Compound	Dose (mg/kg)	Percent protection				Ulcer index
		30 min	1 h	2 h	3 h	
VIa	10	25±1.06 *	29±0.91**	35±1.22*	22±0.48*	0.92±0.56
	20	30±0.50*	42±1.14*	48±0.86**	32±1.93**	
VIb	10	10±1.27**	16±0.53 ^{NS}	19±1.89*	09±0.51*	ND
	20	17±0.72*	20±0.99*	26±1.47*	18±2.50**	
VIc	10	37±0.47*	42±1.25**	49±0.71**	31±0.94*	0.57±0.43
	20	45±1.12*	57±1.38*	72±1.25**	47±1.26***	
VId	10	33±2.05**	40±0.51*	43±0.83*	29±1.42**	0.65±0.31
	20	43±0.33*	55±2.05*	67±1.64**	44±2.39*	
VIe	10	15±2.11 ^{NS}	22±1.55**	24±2.20*	14±1.67*	ND
	20	23±1.38*	27±0.91*	38±1.77*	22±0.40**	
VIf	10	12±0.69*	19±1.72*	22±1.31 ^{NS}	13±0.45*	ND
	20	21±0.94*	23±0.75**	35±1.91*	20±2.12*	
VIg	10	29±1.23*	35±2.46*	41±0.48**	27±1.05*	0.69±0.22
	20	38±1.05*	50±0.74**	64±1.10*	41±1.39**	
VIh	10	08±0.86 ^{NS}	13±1.30*	18±2.11**	09±0.64*	ND
	20	15±0.51**	17±0.78*	24±0.87*	14±1.97 ^{NS}	
VIi	10	35±0.96**	41±0.59*	48±1.43***	31±0.81*	0.60±0.29
	20	45±1.45*	56±0.82**	70±2.24*	46±0.97**	
VIj	10	32±0.52*	39±1.18**	43±1.17**	28±1.34*	0.66±0.38
	20	41±1.22*	54±1.60**	67±0.86*	43±1.76**	
VIk	10	14±0.58**	20±0.83*	24±2.14*	14±0.17*	ND
	20	22±0.70*	25±1.39 ^{NS}	38±1.63**	22±1.57*	
VIl	10	12±2.15*	18±1.61**	20±0.70*	12±0.43 ^{NS}	ND
	20	20±1.68*	22±0.93*	30±1.15**	18±1.84*	
VIm	10	28±0.72**	33±1.35*	41±0.62*	25±2.20*	0.71±0.45
	20	37±0.46*	48±1.59*	60±2.13**	39±0.71*	
Control	NA	04±0.43	07±0.75	05±0.36	04±0.91	0.24±0.11
Diclofenac	10	34±1.39***	40±2.13**	45±1.31**	29±0.86*	1.72±0.49
	20	43±0.64*	56±1.19***	69±0.81*	44±1.39**	

Each value represents the mean ± SEM (n=6); NA: Not applicable; ND: Not determined; ^{NS}: Non significant.
*Significance levels: *p < 0.5, **p < 0.01, ***p < 0.001 as compared with the respective control.*

Biological activities

Analgesic activity

Entire test compounds **VIa-VIm** was tested for their analgesic activity by tail-flick technique using Wistar albino mice. The results of analgesic study are summarized in Table 1. The reports indicate that almost all test compounds exhibited significant activity and graded dose response. Moreover this study revealed that test compounds showed moderate analgesic activity at 30 min of reaction time; the activity increased at 1 h, further it reached to peak level at 2 h and past its best in activity was observed at 3 h. Compound **VIa** with unsubstituted phenyl derivative showed moderate analgesic activity compared to standard drug Diclofenac sodium. With the increased lipophilicity dimethylamino derivative **VIg** & **VIm** showed an increase in activity. Replacement of

dimethylamine moiety with methyl **VId** & **VIj** further increases the lipophilicity results in enhanced activity which was found to be almost equipotent with reference standard Diclofenac. Exchange of methyl with chlorine **VIc** & **VIi** further increases the lipophilicity led to moderate increase in activity which was found to be more potent than standard drug tested. Exchange of chlorine by nitro or methoxy or hydroxyl results in sharp fall in activity may be due to decreased lipophilicity. Compound 1-(4-chlorobenzylidene)-4-(4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)phenyl) thiosemicarbazide **VIc** & 1-(3-chlorobenzylidene)-4-(4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)phenyl)thiosemicarbazide **VIi** was found to be the most active analgesic agent and it is moderately more potent when compared to the reference standard Diclofenac sodium.

Anti-inflammatory activity

Carrageenan-induced paw edema test was performed to assess the anti-inflammatory activity of test compounds using Wistar rats. The anti-inflammatory activity results (Table 2) showed that all the test compounds protected rats from carrageenan-induced inflammation reasonably at 30 min of reaction time; the activity increased at 1 h and it reached to maximum level at 2 h. Declining in activity was observed at 3 h. The compounds possessing unsubstituted phenyl ring **VIa** exhibited moderate anti-inflammatory activity when compared to the reference standard Diclofenac sodium. With increased lipophilicity the compound with dimethylamino substituent **VIg** & **VIm** showed moderately more activity than **5a**. Methyl derivative **VId** & **VIj** showed equipotent activity with reference standard Diclofenac sodium. Among all tested compounds chloro analog **VIc** & **VIi** exhibited better activity which is more potent than Diclofenac. A deep fall in activity was observed when chloro group was replaced with nitro or methoxy or hydroxyl group **VIb, Vie, VIf, VIh, VIk** & **VIl**. Like analgesic activity compounds **VIc** & **VIi** was found to be the most active agent which is moderately more potent when compared to the reference standard Diclofenac sodium.

Ulcerogenicity

Further more potent test compounds were examined for its ulcerogenicity and the results are summarized in Table 2. Entire tested compounds exhibited less ulcer index compared to standard Diclofenac. Results of ulcer index revealed that the compounds bearing chloro substituent **VIc** & **VIi** showed negligible ulcer index, whereas replacement of chlorine by methy/dimethylamino moiety leads to slight increases in ulcer index. Over other test compounds, unsubstituted derivative exhibited higher ulcer index. The test compounds exhibited 33-53% of the ulcer index when compared to the reference drug Diclofenac (1.72±0.49). Among the tested compounds, 1-(4-chlorobenzylidene)-4-(4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)phenyl)thiosemicarbazide **VIc** exhibited least ulcer index (0.57±0.43) which is about one-third of the ulcer index of reference standards. Out of entire tested compounds, 1-benzylidene-4-(4-(1-((dimethylamino)methyl)-1*H*-benzimidazol-2-yl)phenyl) thiosemicarbazide **VIa** was found to possess highest ulcer index (0.92±0.56) which is about 53% of the ulcer index of Diclofenac and Aspirin.

CONCLUSION

In summary, a series of novel benzimidazole thiosemicarbazide derivatives **VIa-VIm** were synthesized and characterized by FT-IR, ^{1}H-NMR, Mass spectroscopy and elemental analysis. These derivatives were evaluated for their analgesic, anti-inflammatory, and ulcerogenicity activity. In general, chlorine substituted compounds exhibited potent analgesic and anti-inflammatory activity with negligible ulcer index. From the study it was found that position of the substituent doesn't affected the analgesic and anti-inflammatory activity greatly. Meta substituted derivative exhibited almost same activity like that of corresponding ortho substituted analogs. Hence from the study it was concluded that, in this series nature of the substituent played a major role in analgesic and anti-inflammatory activity than its position. Among several tested compounds, 1-(4-chlorobenzylidene)-4-(4-(1-((dimethyl amino)methyl)-1*H*-benzimidazol-2-yl)phenyl)thiosemicarbazide **VIc** showed better analgesic and anti-inflammatory activity which is more potent than reference standard Diclofenac. Interestingly this derivative possessed about 33% of the ulcer index of reference standards. Hence, this analog could be developed as a new class of analgesic, and anti-inflammatory agents. However, further structural modification is planned to enhance these activities with the low ulcerogenic index.

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REFERENCES

1. Bhandari SV, Dangre SC, Bothara KG, Patil AA, Sarkate AP, Lokwani DK. Design, synthesis and pharmacological screening of novel nitric oxide donors containing 1,5-diarylpyrazolin-3-one as nontoxic NSAIDs. Eur J Med Chem, 2009; 44: 4622-36.
2. Dannhardt G, Kiefer W. Cyclooxygenase inhibitors-current status and future prospects. Eur J Med Chem, 2001; 36: 109-26.
3. Sondhi SM, Singhal N, Johar M, Reddy BSN, Lown JW. Heterocyclic compounds as inflammation inhibitors. Curr Med Chem, 2002; 9: 1045-74.
4. Sherif AFR, Ibrahim MElA, Heba AAElR, Mona HB, Hayam MAA. Design and synthesis of some thiazolyl and thiadiazolyl derivatives of antipyrine as potential non-acidic anti-inflammatory, analgesic

and antimicrobial agents. Eur J Med Chem, 2009; 17: 882-95.
5. Bertolini A, Ottani A, Sandrini M. Selective COX-2 inhibitors and dual acting anti-inflammatory drugs: critical remarks. Curr Med Chem, 2002; 9: 1033-43.
6. Sri Krishnanjaneyulu I, Saravanan G, Vamsi J, Supriya P, Udaya Bhavana J, Venkata Sunil Kumar M. Synthesis, characterization and antimicrobial activity of some novel benzimidazole derivatives. J Adv Pharm Tech Res, 2014; 5: 21-7.
7. Monika G, Chander M. Design, synthesis and biological evaluation of novel 1,2,5-substituted benzimidazole derivatives as gastroprotective anti-inflammatory and analgesic agents. Med Chem, 2015; 5: 58-63.
8. Kaur G, Kaur M, Silakari O. Benzimidazoles: An ideal privileged drug scaffold for the design of multitargeted anti-inflammatory ligands. Mini Rev Med Chem, 2014; 14: 747-67.
9. Jun C, Jiangtao X, Xianjin L. Synthesis and antiviral activity against Coxsackie virus B3 of some novel benzimidazole derivatives. Bioorg Med Chem Lett, 2005; 15: 267-69.
10. Castro AR, Rivera IL, Rojas LC, Vazquez GN, Rodríguez AN. Synthesis and preliminary evaluation of selected 2-aryl-5(6)-nitro-1*H*-benzimidazole derivatives as potential anticancer agents. Arch Pharm Res, 2011; 34: 181-189.
11. Yoon YK, Ali MS, Wei AC, Choon TS, Ismail R. Synthesis and evaluation of antimycobacterial activity of new benzimidazole aminoesters. Eur J Med Chem, 2015; 93: 614-24.
12. Bhrigu B, Siddiqui N, Pathak D, Alam MS, Ali R, Azad B. Anticonvulsant evaluation of some newer benzimidazole derivatives: Design and synthesis. Acta Pol Pharm, 2012; 69: 53-62.
13. Siddiqui N, Alam MS, Ali R, Yar SM, Alam O. Synthesis of new benzimidazole and phenylhydrazinecarbothiomide hybrids and their anticonvulsant activity. Med Chem Res, 2016; 25: 1390-1402.
14. Mayank RM, Satish B. Synthesis of benzothiazole-benzimidazole thiazolidin derivatives as anti-inflammatory and analgesic. J Chem Biol Phys Sci, 2016; 6: 410-8.
15. Gurusamy M, Rajib H, Faruk A, Rashmi K, Uddhav P, Shyamalendu N. Synthesis and biological evaluation of 2-substitutedbenzimidazole derivatives. Arabian J Chem, 2015; 8: 715-9.
16. Akbar M, Behvar A, Mahsa J. Design and synthesis of new benzimidazole and pyrimidine derivatives as α-glucosidase inhibitor. Iranian J Pharm Res, 2015; 14: 723-31.
17. Faruk A, Biplab KD, Kamal S, Arpita C, Pallab K. Synthesis, antimicrobial and anthelmintic activity of some novel benzimidazole derivatives. Int J Drug Res Tech, 2014; 4: 31-8.
18. Katarzyna BS, Paulina O, Elzbieta MO. Biological approach of anticancer activity of new benzimidazole derivatives. Pharmacol Rep. 2014; 66: 100-6.
19. Sarika S, Dhiman N, Aman M, Gaurav K. Synthesis and antioxidant activity of the 2-methyl benzimidazole. J Drug Delivery Ther, 2016; 6: 100-2.
20. Maste MM, Jeyarani P, Kalekar MC, Bhat AR. Synthesis and evaluation of benzimidazole derivatives for antitubercular and antimicrobial activities. Asian J Res Chem, 2011; 4: 1055-8.
21. Muthu Bhupathi G, Padma Latha K, Deepthi K, Anusha O, Chamundeswari B. Synthesis and screening for antimicrobial activity of novel benzimidazole chalcone. Eur J Biomed Pharm Sci, 2016; 3: 276-87.
22. Borgave SS, Barhate VD. Synthesis, characterization and study of microbiological activity of rhodium (III) complex with, 2-(5-bromo-2-oxoindolin-3-ylidene) hydrazinecarbothioamide. Eur J Biomed Pharm Sci, 2016; 3: 410-5.
23. Chinnasamy RP, Sundararajan R, Govindaraj S. Anticonvulsant activity of novel 1-(substituted benzylidene)-4-(1-(morpholino/piperidinomethyl)-2,3-dioxoindolin-5-yl)-semicarbazide derivatives in mice and rats acute seizure models. Chem Bio Drug Des, 2012; 80: 524-32.
24. Saravanan G, Alagarsamy V, Prakash CR. Design, synthesis and anticonvulsant activities of novel 1-(substituted/unsubstituted benzylidene)-4-(4-(6,8-dibromo-2-(methyl/phenyl)-4-oxoquinazolin-3(4*H*)-yl)phenyl)semicarbazide derivatives. Bioorg Med Chem Lett, 2012; 22: 3072-8.
25. Prakash CR, Raja S, Saravanan G. Design and synthesis of 4-(1-(4-chlorobenzyl)-2,3-dioxoindolin-5-yl)-1-(4-substituted/ unsubstituted benzylidene)semicarbazide: Novel agents with analgesic, anti-inflammatory and ulcerogenic properties. Chin Chem Lett, 2012; 23: 541-4.
26. Saravanan G, Alagarsamy V. Pharmacological evaluation of some 1-(substitutedbenzylidene)-4-(4-(2-(methyl/phenyl)-4-oxoquinazolin-3(4*H*)-yl)phenyl) semicarbazide derivatives. Int J Therap Appli, 2012; 5: 14-20.
27. Olfert ED, Cross BM, McWilliam AA. Canadian council of animal care guide to the care and use of experimental animals. 1993; Vol 1: 2nd edn.
28. Kulkarni SK. Heat and other physiological stress-induced analgesia: Catecholamine mediated and naloxone reversible response. Life Sci, 1980; 27: 185-8.
29. D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther, 1941; 72: 74-9.
30. Winter CA, Risley EA, Nuss GW. Carrageenin induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Exp Biol Med, 1962; 111: 544-7.
31. Goyal RK, Chakrabarti A, Sanyal AK. The effect of biological variables on the anti ulcerogenic effect of

vegetable plantain banana. Planta Med, 1985; 29: 85-8.
32. Shay M, Komarov SA, Fels D, Meranze D, Grunstein H, Siplet H. A simple method for the uniform production of gastric ulceration in the rats. Gastroenterology, 1945; 5: 43-61.
33. Ganguly AK, Bhatnagar OP. Effect of bilateral adrenalectomy on production of restraint ulcers in the stomach of albino rats. Can J Physiol Pharmacol, 1973; 51: 748-50.