Asian Journal of Chemistry; Vol. 27, No. 3 (2015), 961-968

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.17788

Design, Synthesis of Some Novel Thiazolidin-4-one Derivatives Bearing Benzimidazole Nucleus and Biological Evaluation of their Possible *in vitro* Antiinflammatory as Cyclooxygenase Inhibitors and Antioxidant Activity

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Received: 9 April 2014; *Accepted*: 17 June 2014; *Published online*: 19 January 2015; AJC-16705

A series of 2-[2-(4-cyanophenyl)-6-substituted-1*H*-benzimidazol-1-yl]-N-[2-(substituted)-4-oxo-1,3-thiazolidin-3-yl]acetamide [**9(I-XXXI)**] were synthesized. Substituted *o*-phenylenediamine was reacted with substituted 4-cyanobenzaldehydes in the presence of sodium metabisulfite to furnish substituted 2-(4-cyanophenyl)-1*H*-benzimidazoles (**1**). When these substituted 2-(4-cyanophenyl)-1*H*-benzimidazoles were further treated with ethyl chloroacetate in KOH/DMSO, N-alkylated product (2-(4-cyanophenyl)-benzimidazol-1-yl)-acetic acid ethyl esters (**2**) was formed. To synthesize 2-(4-cyanophenyl)-benzimidazol-1-yl-acetic acid hydrazides (**3**) chemical reactions were conducted between hydrazine hydrate and the esters (**2**). When a mixture of 2-(4-cyanophenyl)-benzimidazol-1-yl)-acetic acid hydrazide (**3**) react with substituted aldehydes in ethanol was reflux, imines intermediates [**4(I-XXXI)**] was formed. To synthesize **9(I-XXXI)** reactions were occurred between a mixture of imine intermediate and thioglycollic acid in dioxane. The structures of newly synthesized compounds **9(I-XXXI)** was confirmed by spectroscopic techniques. All the synthesized compounds were screened for its *in vitro* antioxidant and antiinflammatory activity. The *in vitro* antioxidant and antiinflammatory activity might be attributed due to the presence of more electrons withdrawing group and moiety having more lipophilicity also more electronegativity in nature.

Keywords: *o*-Phenylenediamine, 4-Cyanobenzaldehyde, Thiazolidinone, Antioxidant, Antiinflammatory.

INTRODUCTION

The structural and therapeutic diversity coupled with commercial viability of small molecules has fascinated organic and medicinal chemists. There has been considerable interest in the chemistry of thiazolidin-4-one ring systems, which is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activity[1] such as anti-mycobacterial[2], anti-fungal[3], anti-cancers[4], anti-convulsant[5], antiinflammatory and analgesic[6] activities. Therefore, a general, simple and efficient method for rapid synthesis of thiazolidine-4-ones would be greatly advantageous and warrants further investigations in drug discovery. Consequently, many different protocols have been developed that allow the synthesis of thiazolidin-4-one skeletons. Their long-term clinical employment is associated with significant side effects and the steady use determines the onset of gastrointestinal lesions, bleeding and nephrotoxicity[7,8]. When the coxibs were marketed, evidence for a new side effect appeared and rofecoxib was banned in 2004. Subsequently, some of the other coxibs have been voluntarily withdrawn from the market[9]. Some of the studies have suggested that rofecoxib's adverse cardiovascular events may not be a class effect but rather an intrinsic chemical property related to its metabolism[10]. Literature survey make known that the thiazolidinone moiety as it was an important scaffold for **COX-2** inhibition[11-13]. In the present study whatever thiazolidinone-4-one derivatives was synthesized which may not have the irritation to the gastric mucosa also which may not cause additional damage to the gastrointestinal tract. Thiazolidin-4-ones are an interesting backbone unit in medicinal chemistry and responsible for numerous pharmacological properties and biological activity[14-19] which gives the considerable research interest in this area has been done towards the synthesis of thiazolidinone unit. In addition, several interesting investigations have been made through thiazolidin-4-ones based heterocyclic compounds and exhibited numerous biological properties such as antibacterial, antifungal, antitumor, antiarrhythmic, antithrombic, calcium antagonist, hypotensive and neuroleptic activities. An essential component of the search for new leads in drug designing program is the synthesis of molecules, which are novel still resemble known biologically active molecule by virtue of the presence of some

pharmacophoric groups. Certain small heterocyclic molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and useful molecules. Benzimidazole derivatives are well known for their anti-inflammatory activity and more recently have been discovered to have anticancer effect[20,21]. Therefore, in the present paper we planned to incorporate the benzimidazole moiety with thiazolidin-4-ones to have better antioxidant and antiinflammatory activity.

EXPERIMENTAL

Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr) were recorded on a Shimadzu IR Affinity-1 spectrophotometer. ^{1}H NMR and ^{13}CNMR spectra were recorded on a Perkin-Elmer EM 300 MHz spectrometer using TMS as internal standard. The mass spectra were recorded on a JEOL JMS-D 300 spectrometer operating at 70 eV. Purity of the compounds was checked by TLC silica coated plates obtained from Merck.

General procedure: For the preparation of 2-[2-(4-cyanophenyl)-6-substituted-1*H*-benzimidazol-1-yl]-*N*-[2-(substituted)-4-oxo-1,3-thiazolidin-3-yl] acetamide [**9(I-XXXI)**]

A mixture of imine intermediate [**4(I-XXXI)**] (0.01 mol) and thioglycollic acid (0.01 mol) in 15 mL dioxane was refluxed at 115 °C for 24 h. The reaction mixture was triturated with 10 % sodium bicarbonate solution. The neutral solution was poured into crushed ice. The solid formed was collected by filtration, washed with water and dried. The product was crystallized from methanol to give **9(I-XXXI)**. The purity of the compounds was established by single spot on TLC plates. The physico-chemical characteristic data is given in Table-1.

DPPH assay: This experimental procedure followed the method of Wang *et al.*[22]. In an ethanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, test compounds at different concentrations were added. The reaction mixtures were shaken vigorously and then kept in the dark for 0.5 h. The absorbance of the resulting solutions was measured in 1 cm cuvettes, using a UV/visible spectrophotometer at 226 nm against blank without DPPH. Decreasing of DPPH solution absorbance indicates an increase of DPPH radical scavenging activity. This activity is given as % DPPH radical scavenging that is calculated in the equation:

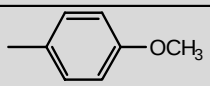
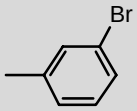
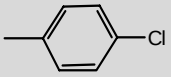
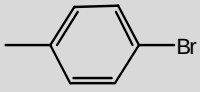
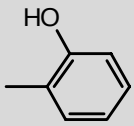
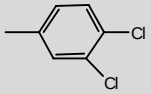
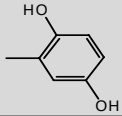
$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

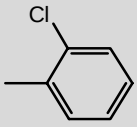
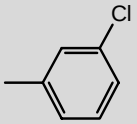
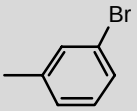
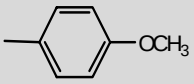
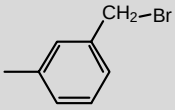
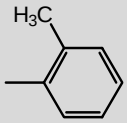
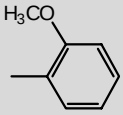
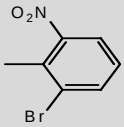
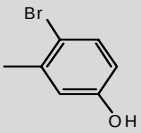
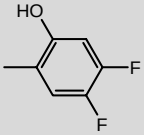
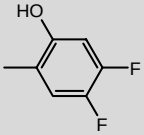
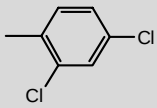
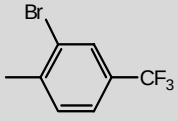
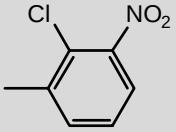
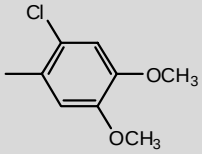
The DPPH solution without sample solution was used as control. All tests were run in triplicate and averaged. Ascorbic acid was used as positive control. The results were compared with ascorbic acid (Table-2).

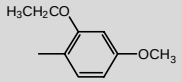
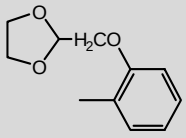
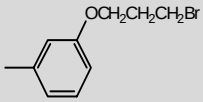
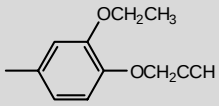
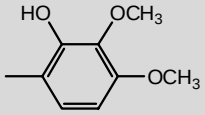
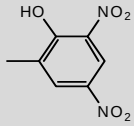
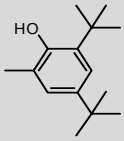
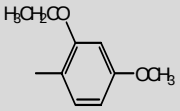
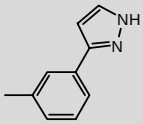
in vitro Anti-inflammatory activity

Inhibition of protein denaturation method: Test solution (0.5 mL) consists of 0.45 mL of BSA (5 % w/v aqueous solution) and 0.05 mL of different concentration of test solutions (50, 100, 150, 200 µg/mL). Test control solution (0.5 mL) consists of 0.45 mL of BSA (5 % w/v aqueous solution) and 0.05 mL of distilled water. Product control solution (0.5 mL) consists of 0.45 mL of distilled water and 0.05 mL of different concentration of test solutions (50, 100, 150, 200 µg/mL). Standard solution (0.5 mL) consists of 0.45 mL of BSA (5 % w/v aqueous solution) and 0.05 mL different concentration of diclofenac sodium's (50, 100, 150, 200 µg/mL). All the above solutions were adjusted to pH 6.3 using 1N hydrochloric acid.

TABLE-1
CHARACTERIZATION DATA SCHEME 1

Compound code	R_1	R_2	m.f.	m.w.	Yield (%)	m.p. (°C)	R_f Value
9(I)	-Cl		$C_{26}H_{20}ClN_5O_3S$	517.98	21	271-273	0.7121
9(II)	-Cl		$C_{25}H_{17}BrClN_5O_2S$	566.85	31	269-271	0.7251
9(III)	$-NO_2$		$C_{25}H_{17}ClN_6O_4S$	532.95	32	214-216	0.6972
9(IV)	$-NO_2$		$C_{25}H_{17}BrN_6O_4S$	577.4	31	209-211	0.7211
9(V)	$-NO_2$		$C_{25}H_{18}N_6O_5S$	514.51	33	271-273	0.7011
9(VI)	-Cl		$C_{25}H_{16}Cl_2N_6O_4S$	567.4	21	269-271	0.6859
9(VII)	$-NO_2$		$C_{25}H_{18}N_6O_6S$	530.51	22	240-242	0.7121

9(VIII)	-NO_2		$C_{25}H_{17}ClN_6O_4S$	532.95	29	275-277	0.7251
9(IX)	-NO_2		$C_{25}H_{17}ClN_6O_4S$	532.95	33	211-213	0.6972
9(X)	-NO_2		$C_{25}H_{17}BrN_6O_4S$	577.4	33	270-272	0.7211
9(XI)	-NO_2		$C_{26}H_{20}N_6O_5S$	528.53	31	213-215	0.7011
9(XII)	-NO_2		$C_{26}H_{19}BrN_6O_4S$	591.43	27	209-211	0.6859
9(XIII)	-NO_2		$C_{26}H_{20}N_6O_4S$	512.53	22	181-183	0.7121
9(XIV)	-Cl		$C_{26}H_{20}ClN_5O_3S$	517.98	25	210-212	0.7251
9(XV)	-Cl		$C_{25}H_{16}BrClN_6O_4S$	611.85	31	196-198	0.6972
9(XVI)	-Cl		$C_{25}H_{17}BrClN_5O_3S$	582.85	31	224-226	0.7211
9(XVII)	-Cl		$C_{25}H_{16}ClF_2N_5O_3S$	539.94	22	251-253	0.7011
9(XVIII)	-NO_2		$C_{25}H_{16}F_2N_6O_5S$	550.49	32	241-243	0.6859
9(XIX)	-Cl		$C_{25}H_{16}Cl_3N_5O_2S$	556.85	23	249-251	0.7121
9(XX)	-Cl		$C_{26}H_{16}BrClF_3N_5O_2S$	634.85	31	281-283	0.7251
9(XXI)	-Cl		$C_{25}H_{16}Cl_2N_6O_4S$	567.4	32	270-272	0.6972
9(XXII)	-Cl		$C_{27}H_{21}Cl_2N_5O_4S$	582.45	22	249-251	0.7211

9(XXIII)	-Cl		$C_{28}H_{24}ClN_5O_4S$	562.03	33	259-261	0.7011
9(XXIV)	$-NO_2$		$C_{29}H_{24}N_6O_7S$	600.6	31	219-221	0.6859
9(XXV)	-Cl		$C_{28}H_{23}BrClN_5O_3S$	624.93	18	210-212	0.7121
9(XXVI)	-Cl		$C_{30}H_{24}ClN_5O_4S$	586.06	22	220-222	0.7251
9(XXVII)	-Cl		$C_{27}H_{22}ClN_5O_5S$	564.01	22	221-223	0.6972
9(XXVIII)	-Cl		$C_{25}H_{16}ClN_7O_7S$	593.95	21	223-225	0.7211
9(XXIX)	$-NO_2$		$C_{33}H_{34}N_6O_5S$	626.72	18	209-211	0.7011
9(XXX)	-Cl		$C_{28}H_{24}ClN_5O_4S$	562.03	31	259-261	0.6859
9(XXXI)	-Cl		$C_{28}H_{20}ClN_7O_2S$	554.02	18	219-221	0.7121

The samples were incubated at 37 °C for 20 min and the temperature was increased to keep the samples at 57 °C for 3 min. After cooling, 2.5 mL of phosphate buffer saline was added to the above solutions. The absorbance was measured using UV-visible spectrophotometer at 416 nm[23]. The percentage inhibition of protein denaturation was calculated as,

Inhibition (%) =

$$\frac{\text{O. D of test solution} - \text{O. D of product control}}{\text{O. D of test control}} \times 100$$

The results were compared with diclofenac sodium (Table-3).

***in vitro* Anti-inflammatory activity**

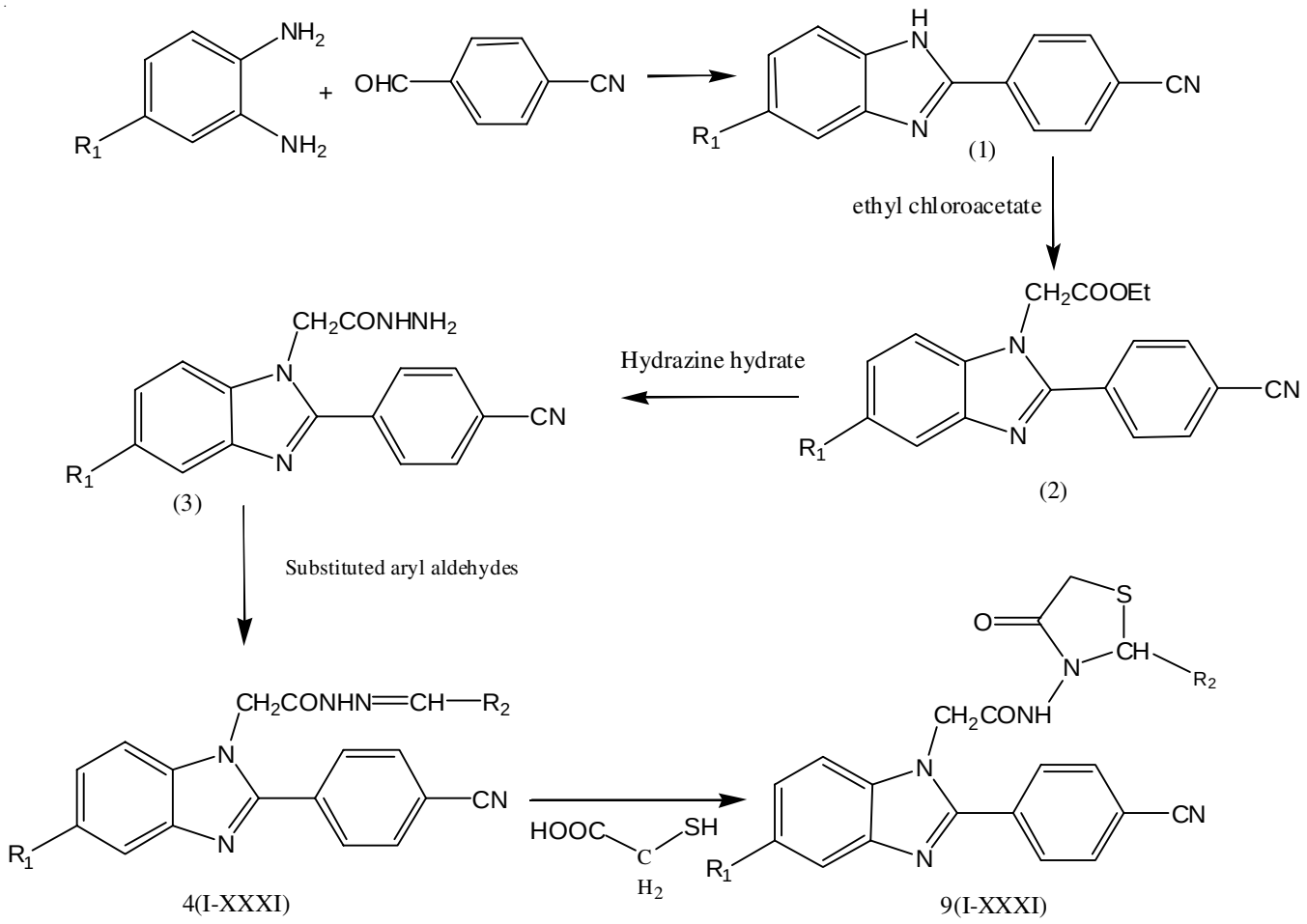
Cyclooxygenase (COX) inhibitor screening assay: The inhibitory activities against COX-1 and COX-2 were determined using a colourimetric COX (ovine) inhibitor assay kit (Cayman Chemical Co., Cat. No. 760111) according to the manufacturer's protocol[24,25]. The inhibitory activities of the compounds were measured by monitoring the production of oxidized N,N,N',N'-tetramethyl-*p*-phenylenediamine (TMPD) at 405 nm followed by incubation of either ovine COX-1 or COX-2 with arachidonic acid. The enzymes were preincubated for 5 min at 25 °C with the test compounds prior to addition of arachidonic acid (final concentration 1.1 mM) and TMPD and incubation for 5 min at 25 °C. The COX-inhibiting activity was calculated according to the equation.

COX inhibiting activity (%) = $[1-(A_1-A_2)/A_0] \times 100$

where A_0 was the absorbance of the control (without the test compound), A_1 was the absorbance in the presence of the test compound and A_2 was the absorbance sample blank (without TMPD). The IC_{50} is the concentration of tested compounds reducing 50 % of ovine COX-1 or COX-2 under the given experimental conditions and was calculated using a calibration curve with different concentrations of samples.

RESULTS AND DISCUSSION

For the synthesis of target compounds the reaction sequences outlined in **Scheme-I** were followed. 2-[2-(4-cyanophenyl)-6-substituted-1*H*-benzimidazol-1-yl]-*N*-[2-(substituted)-4-oxo-1,3-thiazolidin-3-yl]acetamide [**9(I-XXXI)**] were synthesized employing conventional techniques. Substituted *o*-phenylenediamine was reacted with appropriately 4-cyanobenzaldehyde in the presence of sodium metabisulfite to furnish substituted 2-(4-cyanophenyl)-1*H*-benzimidazoles (**1**). These substituted 2-(4-cyanophenyl)-1*H*-benzimidazoles were further treated with ethyl chloroacetate in KOH/DMSO gave



Scheme-I: Synthetic route for the preparation of the compounds

the N-alkylated product, [2-(4-cyanophenyl)-benzimidazol-1-yl]-acetic acid ethyl esters (**2**). To endow 2-(4-cyanophenyl)-benzimidazol-1-yl)-acetic acid hydrazide (**3**) reaction were occurred between hydrazine hydrate and the esters (**2**). A mixture of 2-(4-cyanophenyl)-benzimidazol-1-yl)-acetic acid hydrazides and respective aldehydes were react to generate imines intermediates [**4(I-XXXI)**]. The imine intermediate and thioglycollic acid in 15 ml dioxane react to produce the products **9(I-XXXI)** in Fig. 1.

Compound 9(VI): 2-[2-(4-Cyanophenyl)-6-nitro-1*H*-benzimidazol-1-yl]-*N*-[2-(3,4-dichlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide yield: 21 %; m.p.: 269-271 °C; IR (KBr, ν_{max}, cm^{-1}): 3265 (N-H), 3065 (Ar-C-H), 2845 (C-H), 2214 (C=N), 1678 (C=O), 1650 (C=N), 1576, 1443, 1435 (C=C), 1355 (N=O), 1335 (C-N), 795 (C-Cl).

^{1}H NMR ($CDCl_3$) δ (ppm) and ^{13}C NMR δ (ppm): 5.007 (s, 2H, -CH_2), 7.16-8.04 (m, 10 H, aromatic protons), 3.67-6.802 (m, 3H, thiazolidin-4-one protons), 8.098 (s, 1H, -NH), ^{13}C: 32, 55, 70, 106, 114, 118, 119, 126, 127, 128, 129, 130, 131, 133, 137, 138, 142, 145, 164, 167; MS: *m/z* 567 (M^+).

Compound 9(XVII): 2-[2-(4-Cyanophenyl)-6-chloro-1*H*-benzimi-dazol-1-yl]-*N*-[2-(4,5-difluoro-2-hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide: yield: 22 %; m.p.: 251-253 °C; IR (KBr, ν_{max}, cm^{-1}): 3456 (O-H), 3265 (N-H), 3065 (Ar-C-H), 2845 (C-H), 2214 (C=N), 1678 (C=O), 1650 (C=N), 1576, 1443, 1435 (C=C), 1335 (C-N), 1223 (C-F), 795 (C-Cl).

^{1}H NMR ($CDCl_3$) δ (ppm) and ^{13}C NMR δ (ppm): 5.007 (s, 2H, -CH_2), 6.48-8.04 (m, 9H, aromatic protons), 3.67-6.48 (m, 3H, thiazolidin-4-one protons), 8.098 (s, 1H, -NH), 6.52 (s, 1H, OH), ^{13}C: 32, 55, 59, 104, 112, 114, 119, 123, 124, 126, 129, 131, 133, 136, 137, 140, 143, 148, 150, 164, 167; MS: *m/z* 540 (M^+).

Compound 9(XVIII): 2-[2-(4-Cyanophenyl)-6-nitro-1*H*-benzimi-dazol-1-yl]-*N*-[2-(4,5-difluoro-2-hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide yield: 32 %; m.p.: 241-243 °C; IR (KBr, ν_{max}, cm^{-1}): 3456 (O-H), 3265 (N-H), 3065 (Ar-C-H), 2845 (C-H), 2214 (C=N), 1678 (C=O), 1650 (C=N), 1576, 1443, 1435 (C=C), 1355 (N=O), 1335 (C-N), 1223 (C-F).

^{1}H NMR ($CDCl_3$) δ (ppm) and ^{13}C NMR δ (ppm): 5.007 (s, 2H, -CH_2), 6.48-8.43 (m, 9H, aromatic protons), 3.67-6.69 (m, 3H, thiazolidin-4-one protons), 8.098 (s, 1H, -NH), 6.52 (s, 1H, OH), ^{13}C: 32, 55, 59, 104, 112, 114, 119, 124, 131, 133, 137, 140, 142, 148, 150, 164, 167; MS: *m/z* 550 (M^+).

Compound 9(XIX): 2-[2-(4-Cyanophenyl)-6-chloro-1*H*-benzimidazol-1-yl]-*N*-[2-(2,4-dichlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide yield: 23 %; m.p.: 249-251 °C; IR (KBr, ν_{max}, cm^{-1}): 3265 (N-H), 3065 (Ar-C-H), 2845 (C-H), 2214 (C=N), 1678 (C=O), 1650 (C=N), 1576, 1443, 1435 (C=C), 1335 (C-N), 795 (C-Cl).

^{1}H NMR ($CDCl_3$) δ (ppm) and ^{13}C NMR δ (ppm): 5.007 (s, 2H, -CH_2), 6.99-8.04 (m, 10H, aromatic protons), 3.67-6.96

(m, 3H, thiazolidin-4-one protons), 8.098 (s, 1H, -NH), ^{13}C: 32, 55, 65, 114, 119, 123, 125, 126, 128, 129, 130, 131, 132, 133, 134, 136, 137, 143, 164, 167; MS: *m/z* 557 (M^+).

Compound 9(XX): 2-[2-(4-Cyanophenyl)-6-chloro-1*H*-benzimi-dazol-1-yl]-*N*-{2-[2-bromo-4-(trifluoromethyl)phenyl]-4-oxo-1,3-thiazolidin-3-yl}acetamide yield: 31 %; m.p.: 281-283 °C; IR (KBr, ν_{max}, cm^{-1}): 3265 (N-H), 3065 (Ar-C-H), 2845 (C-H), 2214 (C=N), 1678 (C=O), 1650 (C=N), 1576, 1443, 1435 (C=C), 1335 (C-N), 1223 (C-F), 795 (C-Cl), 546 (C-Br).

^{1}H NMR ($CDCl_3$) δ (ppm) and ^{13}C NMR δ (ppm): 5.007 (s, 2H, -CH_2), 7.11-8.04 (m, 10H, aromatic protons), 3.67-6.61 (m, 3H, thiazolidin-4-one protons), 8.098 (s, 1H, -NH), ^{13}C: 32, 55, 69, 114, 119, 120, 123, 124, 125, 126, 127, 129, 130, 131, 133, 136, 137, 142, 143, 164, 167; MS: *m/z* 635, 637 (M^+).

Compound 9(XXII): 2-[2-(4-Cyanophenyl)-6-chloro-1*H*-benzimi-dazol-1-yl]-*N*-[2-(2-chloro-4,5-dimethoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide yield: 22 %; m.p.: 249-251 °C; IR (KBr, ν_{max}, cm^{-1}): 3265 (N-H), 3065 (Ar-C-H), 2845 (C-H), 2214 (C=N), 1678 (C=O), 1650 (C=N), 1576, 1443, 1435 (C=C), 1335 (C-N), 1189 (C-O-C), 795 (C-Cl).

^{1}H NMR ($CDCl_3$) δ (ppm) and ^{13}C NMR δ (ppm): 5.007 (s, 2H, -CH_2), 3.77 (s, 6H, -CH_3), 6.70-8.04 (m, 9H, aromatic protons), 3.67-6.96 (m, 3H, thiazolidin-4-one protons), 8.098 (s, 1H, -NH), ^{13}C: 32, 55, 56, 66, 111, 113, 114, 119, 123, 126, 127, 129, 131, 132, 133, 136, 137, 143, 148, 149, 164, 167; MS: *m/z* 582 (M^+).

Compound 9(XXIII): 2-[2-(4-Cyanophenyl)-6-chloro-1*H*-benzimi-dazol-1-yl]-*N*-[2-(2-ethoxy-4-methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide yield: 33 %; m.p.: 259-261 °C; IR (KBr, ν_{max}, cm^{-1}): 3265 (N-H), 3065 (Ar-C-H), 2845 (C-H), 2214 (C=N), 1678 (C=O), 1650 (C=N), 1576, 1443, 1435 (C=C), 1368 (C-C), 1335 (C-N), 1189 (C-O-C), 795 (C-Cl).

^{1}H NMR ($CDCl_3$) δ (ppm) and ^{13}C NMR δ (ppm): 3.86-5.007 (s, 4H, -CH_2), 1.45-3.82 (s, 6H, -CH_3), 6.308-8.04 (m, 10H, aromatic protons), 3.67-6.67 (m, 3H, thiazolidin-4-one protons), 8.098 (s, 1H, -NH), ^{13}C: 15, 32, 55, 62, 65, 97, 102, 114, 119, 123, 126, 128, 129, 131, 133, 136, 137, 143, 156, 157, 164, 167; MS: *m/z* 562 (M^+).

Compound 9(XXVI): 2-[2-(4-Cyanophenyl)-6-chloro-1*H*-benzimi-dazol-1-yl]-*N*-{2-[3-ethoxy-4-(prop-2-yn-1-yloxy) phenyl]-4-oxo-1,3-thiazolidin-3-yl}acetamide yield: 22 %; m.p.: 220-222 °C; IR (KBr, ν_{max}, cm^{-1}): 3265 (N-H), 3065 (Ar-C-H), 2845 (C-H), 2214 (C=N), 2117 (C=C), 1678 (C=O), 1650 (C=N), 1576, 1443, 1435 (C=C), 1368 (C-C), 1335 (C-N), 1189 (C-O-C), 795 (C-Cl).

^{1}H NMR ($CDCl_3$) δ (ppm) and ^{13}C NMR δ (ppm): 2.41 (s, 1H, -CH), 4.09-5.007 (s, 6H, -CH_2), 1.488 (s, 3H, -CH_3), 6.66-8.04 (m, 10H, aromatic protons), 3.67-6.55 (m, 3H, thiazolidin-4-one protons), 8.098 (s, 1H, -NH), ^{13}C: 15, 32, 55, 57, 65, 70, 76, 79, 110, 111, 114, 119, 121, 123, 126, 129, 131, 133, 136, 137, 143, 148, 151, 164, 167; MS: *m/z* 586 (M^+).

Compound 9(XXX): 2-[2-(4-Cyanophenyl)-6-chloro-1*H*-benzimi-dazol-1-yl]-*N*-[2-(2-ethoxy-4 methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide yield: 31 %; m.p.: 259-261 °C; IR (KBr, ν_{max}, cm^{-1}): 3265 (N-H), 3065 (Ar-C-H), 2845 (C-H), 2214 (C=N), 1678 (C=O), 1650 (C=N), 1576, 1443, 1435 (C=C), 1368 (C-C), 1335 (C-N), 1189 (C-O-C), 795 (C-Cl).

^{1}H NMR ($CDCl_3$) δ (ppm) and ^{13}C NMR δ (ppm): 5.007 (s, 2H, -CH_2), 1.45-3.82 (s, 6H, -CH_3), 6.30-8.04 (m, 10H, aromatic protons), 3.67-6.67 (m, 3H, thiazolidin-4-one protons), 8.098 (s, 1H, -NH), ^{13}C: 15, 32, 55, 62, 64, 97, 102, 114, 119, 123, 126, 128, 129, 131, 133, 136, 137, 143, 157, 164, 167; MS: *m/z* 562 (M^+).

Compound 9(XXXI): 2-[2-(4-Cyanophenyl)-6-chloro-1*H*-benzimidazol-1-yl]-*N*-{4-oxo-2-[3-(1H-pyrazol-3-yl) phenyl]-1,3-thiazolidin-3-yl}acetamide yield: 18 %; m.p.: 219-221 °C; IR (KBr, ν_{max}, cm^{-1}): 3265 (N-H), 3065 (Ar-C-H), 2845 (C-H), 2214 (C=N), 1678 (C=O), 1650 (C=N), 1576, 1443, 1435 (C=C), 1368 (C-C), 1335 (C-N), 795 (C-Cl).

^{1}H NMR data ($CDCl_3$) δ (ppm) and ^{13}C NMR data (ppm): 5.007 (s, 2H, -CH_2), 6.53-8.04 (m, 11H, aromatic protons), 3.67-6.58 (m, 3H, thiazolidin-4-one protons), 6.85-7.49 (m, 2H, pyrazole protons), 8.098-13.8 (s, 2H, NH), ^{13}C: 32, 55, 70, 102, 114, 119, 123, 125, 126, 127, 129, 130, 131, 132, 133, 136, 137, 139, 143, 149, 164, 167; MS: *m/z* 554 (M^+).

Compounds **9(XIX)** (160 ± 0.881) μg/mL, **9(XX)** (108 ± 1.04) μg/mL and **9(XXVI)** (165 ± 0.884) μg/mL were highly active at low concentration and compounds **9(VI)** (264 ± 0.975) μg/mL, **9(XVII)** (396 ± 0.949) μg/mL, **9(XVIII)** (283 ± 1.029) μg/mL, **9(XXII)** (186 ± 1.04) μg/mL, **9(XXIII)** (325 ± 0.934) μg/mL and **8(XXX)** (181 ± 1.18) μg/mL were moderately active at higher concentration as compared to ascorbic acid (109 ± 0.7296) μg/mL in free radical scavenging activity by 2,2-diphenyl-1-picryl hydrazide assay method (Table-2).

TABLE-2
IC_{50} ± SEM (STANDARD ERROR OF MEAN) VALUES OF ASCORBIC ACID AND COMPOUNDS **9(I-XXXI)**

Compound code	IC_{50} Value (μg/mL)	IC_{50} Value ± SEM (μg/mL)
Ascorbic acid	109	109 ± 0.7296
9(VI)	264	264 ± 0.975
9(XVII)	396	396 ± 0.949
9(XVIII)	283	283 ± 1.029
9(XIX)	160	160 ± 0.881
9(XX)	108	108 ± 1.04
9(XXII)	186	186 ± 1.04
9(XXIII)	325	325 ± 0.934
9(XXVI)	165	165 ± 0.884
9(XXX)	181	181 ± 1.18

Values represent the mean of triplicates

in vitro Antiinflammatory by inhibition of protein denaturation method the compounds **9(VI)** (68.26 ± 0.9514) μg/mL, **9(XXII)** (72.76 ± 0.97) μg/mL and **9(XXX)** (75.67 ± 0.92) μg/mL were found to be highly active in low concentration and compounds **9(XVII)** (110.1 ± 0.9765) μg/mL, **9(XVIII)** (86.7 ± 1.0225) μg/mL, **9(XIX)** (108.4 ± 0.87) μg/mL, **9(XX)** (93 ± 0.725) μg/mL, **9(XXIII)** (92.03 ± 1.022) μg/mL, **9(XXVI)** (92.12 ± 1.03) μg/mL and **9(XXXI)** (87.35 ± 0.786) μg/mL were found to be moderately active at higher concentration as compared to diclofenac sodium (57.08 ± 0.7296) μg/mL (Table-3). The results may be attributes due to the presence of more electrons withdrawing group and moiety having more lipophilicity also more electro negativity in nature, good antioxidant and antiinflammatory properties.

All the newly synthesized compounds **9(I-XXXI)** were screening for inhibitory effect on ovine COX-1 activity *in vitro*.

TABLE-3
IC_{50} ± SEM (STANDARD ERROR OF MEAN) VALUES OF DICLOFENAC SODIUM AND COMPOUNDS **9(I-XXXI)**

Compound code	IC_{50} Value (μg/mL)	IC_{50} Value ± SEM (μg/mL)
Diclofenac sodium	57.08	57.08 ± 0.7296
9(VI)	68.26	68.26 ± 0.9514
9(XVII)	110.1	110.1 ± 0.9765
9(XVIII)	86.7	86.7 ± 1.0225
9(XIX)	108.4	108.4 ± 0.87
9(XX)	93	93 ± 0.725
9(XXII)	72.76	72.76 ± 0.97
9(XXIII)	92.03	92.03 ± 1.022
9(XXVI)	92.12	92.12 ± 1.03
9(XXX)	75.67	75.67 ± 0.92
9(XXXI)	87.35	87.35±0.786

Values represent the mean of triplicates

It was observed that among the thiazolidinone derivatives fluro, chloro and methoxy substitution is more favourable. 2-[2-(4-cyanophenyl)-6-chloro-1*H*-benzimidazol-1-yl]-*N*-[2-(2,4-dichlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide **[9(XIX)]** exhibited the highest COX-1 inhibition at low concentration (4.82 ± 0.15 μg/mL), followed by 2-[2-(4-cyanophenyl)-6-chloro-1*H*-benzimidazol-1-yl]-*N*-[2-(2-chloro-4,5-dimethoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide **[9(XXII)]** 4.89 ± 0.12 μg/mL, 2-[2-(4-cyanophenyl)-6-nitro-1*H*-benzimidazol-1-yl]-*N*-[2-(4-methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide **[9(XI)]** 4.93 ± 0.12 μg/mL, respectively and 2-[2-(4-cyanophenyl)-6-chloro-1*H*-benzimidazol-1-yl]-*N*-[2-(4-methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide **[9(I)]** 5.12 ± 0.13 μg/mL, respectively. The replacement of the more lipophilic chloro substituent by the less lipophilic nitro group, leads to a decrease of COX-1 inhibitory activity in the case of compounds **9(III)** and **9(XXI)** substituted derivatives (8.67 ± 0.17 μg/mL and 18.99 ± 0.15 μg/mL, respectively). Replacement of the 2-Br, 5-OH benzaldehyde derivative and 2,5-dihydroxy benzaldehyde derivative with 4,5-dichloro benzaldehyde derivative, 3-chloro benzaldehyde derivative and 2-methody benzaldehyde derivative resulted in obtaining compounds with increased activity at low concentration (compounds **9(XVI)**, **9(VII)**, **9(VI)**, **9(IX)** and **9(XIV)**, 23.6 ± 0.12, 17.65 ± 0.13, 8.65 ± 0.16, 8.06 ± 0.18 and 8.39 ± 0.12 μg/mL, respectively), as compared to indomethacin (1.44 ± 0.17 μg/mL) (Table-4). Other compounds were considered to be less active or inactive.

All the newly synthesized compounds **9(I-XXXI)** were screening for inhibitory effect on ovine COX-2 activity *in vitro*. It was observed that among the thiazolidinone derivatives fluro, chloro and methoxy substitution is more favourable. 2-[2-(4-cyanophenyl)-6-chloro-1*H*-benzimidazol-1-yl]-*N*-[2-(2,4-dichlorophenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide **9(XIX)** exhibited the highest COX-2 inhibition at low concentration (3.597 ± 0.14 μg/mL), followed by 2-[2-(4-cyanophenyl)-6-chloro-1*H*-benzimidazol-1-yl]-*N*-[2-(2-chloro-4,5-dimethoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide **9(XXII)** 3.454 ± 0.11 μg/mL, 2-[2-(4-cyanophenyl)-6-nitro-1*H*-benzimidazol-1-yl]-*N*-[2-(4-methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl] acetamide **9(XI)** 4.24 ± 0.11 μg/mL and 2-[2-(4-cyanophenyl)-6-chloro-1*H*-benzimidazol-1-yl]-*N*-[2-(4-methoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]acetamide **9(I)** 4.835 ± 0.12 μg/mL, respectively. The replacement of the more lipophilic chloro substituent by the less lipophilic nitro group, leads to a decrease of COX-1 inhibitory activity in the case of **9(III)** and **9(XXI)** substituted derivatives (7.821 ± 0.16 and 18.17 ± 0.14 μg/mL, respectively). Replacement of the 2-Br, 5-OH benzaldehyde derivative and 2,5-dihydroxy benzaldehyde derivative with 4,5-dichloro benzaldehyde derivative, 3-chloro

TABLE-4
INHIBITORY EFFECT OF SYNTHESIZED COMPOUNDS **9(I-XXXI)** ON OVINE COX-1 AND COX-2 ACTIVITY *in vitro*[a]

Compound code	COX-1 IC_{50}[b] (μg/mL)	COX-2 IC_{50}[b] (μg/mL)	COX-2 Selectivity[c]
9(I)	5.12 ± 0.13	4.835 ± 0.12	1.05
9(II)	10.84 ± 0.11	9.982 ± 0.1	1.08
9(III)	8.67 ± 0.17	7.821 ± 0.16	1.1
9(IV)	11.79 ± 0.13	10.82 ± 0.12	1.08
9(V)	>100	16.77 ± 0.12	>5.96
9(VI)	8.65 ± 0.16	7.736 ± 0.15	1.11
9(VII)	17.65 ± 0.13	16.64 ± 0.12	1.06
9(VIII)	>100	17.08 ± 0.1	>5.85
9(IX)	8.06 ± 0.18	7.792 ± 0.17	1.03
9(X)	10.42 ± 0.12	9.604 ± 0.11	1.08
9(XI)	4.93 ± 0.12	4.24 ± 0.11	1.16
9(XIV)	8.39 ± 0.12	7.38 ± 0.11	1.13
9(XV)	>100	18.45 ± 0.13	>5.42
9(XVI)	23.6 ± 0.12	22.4 ± 0.11	1.05
9(XVII)	11.33 ± 0.11	10.82 ± 0.1	1.04
9(XVIII)	10.87 ± 0.15	9.78 ± 0.14	1.11
9(XIX)	4.82 ± 0.15	3.597 ± 0.14	1.34
9(XX)	11.77 ± 0.11	10.88 ± 0.1	1.08
9(XXI)	18.99 ± 0.15	18.17 ± 0.14	1.04
9(XXII)	4.89 ± 0.12	3.454 ± 0.11	1.41
Indomethacin	1.44 ± 0.17	1.291 ± 0.16	1.11

[a]Other compounds were considered to be inactive (IC_{50} >100 μg/mL) in both COX-1 and COX-2
[b]Each value is expressed as the mean of triplicate experiments
[c]COX-2 selectivity was determined by $IC_{50\ COX\text{-}1}/IC_{50\ COX\text{-}2}$

benzaldehyde derivative and 2-methoxy benzaldehyde derivative resulted in obtaining compounds with increased activity at low concentration (compounds **9(XVI)**, **9(VII)**, **9(IX)**, **9(VI)** and **9(XIV)**, 22.4 ± 0.11, 16.64 ± 0.12, 7.792 ± 0.17, 7.736 ± 0.15 and 7.38 ± 0.11 µg/mL, respectively), as compared to indomethacin (1.291 ± 0.16 µg/mL) (Table-4). Other compounds were considered to be less active or inactive.

ACKNOWLEDGEMENTS

The authors are thankful to the authority of Sumandeep Vidyapeeth University, Department of Pharmacy and Century Pharmaceuticals Ltd., India for providing the necessary research facilities.

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