Synthesis and Anti-inflammatory Activity of Alkyl/Arylidene-2-aminobenzothiazoles and 1-Benzothiazol-2-yl-3-chloro-4-substituted-azetidin-2-ones

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Summary

Ten new derivatives of 1-benzothiazol-2-yl-3-chloro-4-substituted-azetidin-2-ones (3a–j) were synthesized using various Schiff bases (alkyl /arylidene-2-aminobenzothiazoles; 2a–j), which in turn were prepared starting from 2-aminobenzothiazole (1). All the synthesised compounds were characterised by elemental analyses and spectral (IR, 1H-NMR, 13C-NMR and EI-MS) data. The title compounds 2a–j and 3a–j were screened in vivo using carrageenan-induced rat paw edema model. All the test compounds showed anti-inflammatory activity when tested in vivo. In general, compounds 3a–j were found to be more potent compared to compounds 2a–j. Among the compounds tested, compound 2g in the alkyl /arylidene-2-aminobenzothiazoles series and compound 3g in the 1-benzothiazol-2-yl-3-chloro-4-substituted-azetidin-2-ones series were found to be the most potent. All the test compounds were also evaluated to check the gastric ulcer incidence.

In gastric ulceration studies, all the test compounds were generally found to be safe at the 100 mg/kg dose level. Furthermore, the most potent compounds 2g and 3g from each series were screened in vitro for inhibition of both COX-2 and COX-1 catalysed prostaglandin biosynthesis (radiochemical assay).

Like most of the commercially available non-steroidal anti-inflammatory drugs (NSAIDs), in the in vitro study, compounds 2g and 3g showed anti-inflammatory activity by blocking the metabolism of arachidonic acid to prostaglandin via the cyclooxygenase pathways. In general, in the in vitro assay, test compounds 2g and 3g were found to be more active after 15 min pre-incubation with the enzyme. Compound 3g was found to be more COX-2 selective, while compound 2g was found to be equally COX-2 and COX-1 selective.

Keywords
- Aminobenzothiazoles, anti-inflammatory activity, in vitro studies, synthesis, ulcerogenic activity
- Benzothiazol-2-yl-3-chloro-4-substituted-azetidin-2-ones, anti-inflammatory activity, in vitro studies, synthesis, ulcerogenic activity
- Prostaglandin biosynthesis, cyclooxygenase catalysed
- Prostaglandin endoperoxide synthase (COX) inhibitor

Zusammenfassung

Zehn neue Derivate von 1-Benzothiazol-2-yl-3-chloro-4-substituierten Azetidin-2-onen (3a–j) wurden mit Hilfe verschiedener Schiffischer Basen (Alkyl/Arylidene-2-aminobenzothiazol; 2a–j) herge-
1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of a number of inflammatory diseases and are among the most widely used drugs worldwide [1]. The common mechanism of action of this class of drugs can be attributed to the inhibition of cyclooxygenase (prostaglandin endoperoxide H synthase, also called as COX) enzymes, which catalyses fatty acid substrate (arachidonic acid) into prostaglandin (PG) [2]. The main limitation in using NSAIDs consists in their side effects, such as gastrointestinal ulcerogenic activity and bronchospasm [3, 4]. It is now well established that COX exists as two isozymes in mammals, a constitutive form COX-1, and an inducible form COX-2. The isoenzyme COX-1 is expressed in most mammalian cells under physiological conditions [5], whereas COX-2 is induced by pro-inflammatory stimuli such as cytokines, bacterial lipopolysaccharide, growth factors, and tumour-promoting agents [6, 7]. It is currently hypothesised that the undesirable side effects of NSAIDs are due to COX-1 inhibition, whereas the beneficial effects, such as the reduction of swelling and analgesia, are related to COX-2 inhibition [8, 9]. Recent studies revealed that compounds having more COX-1 selectivity show evidence of greater gastrointestinal toxicity [10]. In spite of abundance of NSAIDs in the market, the search continues to develop new drugs that have potent anti-inflammatory activity with minimum side effects. With the discovery of these two isozymes, the current therapeutic approach and chemical design of NSAIDs are targeted towards the development of selective COX-2 inhibitors and as a result, several selective COX-2 inhibitors are now commercially available [11].

Various substituted benzothiazoles are known to possess a wide range of pharmacological activities such as antitumour [12], antimicrobial [13, 14], anthelmintic [15], analgesic [16], anti-inflammatory [17] and anticonvulsant [18] activities. Similarly, the β-lactam nucleus is considered a general lead structure for the design and synthesis of not only new antibacterial products, but also for new inhibitors of enzymes like β-lactamases [19], human leukocyte elastase [20], cholesterol absorption inhibitors [21] and human cytomegalovirus protease [22]. In view of the fact that several benzothiazole derivatives possess useful anti-inflammatory properties and as part of our ongoing studies in the area of aminobenzothiazoles [23–25], we synthesized various derivatives of alkyl / arylidene-2-aminobenzothiazoles (2a–j). Also, with the aim of obtaining the new anti-inflammatory agents with reduced side effects, we found it interesting to combine the well known antimicrobial nucleus (azetidinones) with the benzothiazole molecule.

Thus, in the present investigation, ten different derivatives of alkyl / arylidene-2-amino-benzothiazoles (2a–j) and 1-benzothiazol-2-yl-3-chloro-4-substituted-azetidin-2-ones (3a–j) were synthesised and evaluated for their anti-inflammatory activity.

2. Materials and methods

2.1. Chemistry

2.1.1. Chemicals and apparatus

Melting points were determined in open capillaries using Thermomik Precision Melting point cuim Boiling point apparatus, Model C-PMB-2 (Mumbai, India) and are uncorrected. Purity of the compounds was checked by precoated TLC plates (Kieselgel 60 F254, E. Merck, Mumbai, India). IR spectra were recorded using KBr pellets on a Perkin-Elmer 337 Spectrophotometer from Perkin Elmer, Rotkreuz (Switzerland) (% in cm−1). 1H-NMR and 13C-NMR spectra on a Bruker W.M, 400 Spectrometer (Bruker, Fallanden, Switzerland) at 360 MHz using TMS as internal standard (chemical shifts in δ ppm) and mass spectra (electron impact mass spectra, EI-MS) were recorded at 70 eV on a Jeol D-300 spectrometer (Jeol, Tokyo, Japan). Elemental analyses were carried out using Heraus Carlo Erba 1180 CHN analyser (from Heraeus, Hanau, Germany). All the chemicals used for the synthesis were purchased from Aldrich, Dorset (UK). The compound, 2-aminobenzothiazole 1 was prepared by the reported method [14]. For COX catalysed prostaglandin...
2.1.2. Synthesis

Two series of some novel benzothiazol-2-yl-aryl/arylidene-aminos (2a–j) and 1-benzothiazol-2-yl-3-chloro-4-substituted-azetidin-2-ones (3a–j) were synthesised using an appropriate synthetic route (Scheme 1).

General procedure for the preparation of title compounds using 2-aminobenzothiazoles as starting material along with spectral data of some of the representative compounds of each series has been described as follows:

2.1.2.1. General procedure for preparation of benzothiazol-2-yl-aryl/arylidene-amine 2a–j

To a solution of 2-aminobenzothiazole (0.01 mol) in ethanol (50 ml), sodium acetate (0.01 mol) and various aliphatic/aromatic aldehydes (0.02 mol) were added and the reaction mixture was heated under reflux for 16 h. Excess of the solvent was distilled-off in vacuo. The residue so obtained was washed with diethyl ether and recrystallised from methanol. Using the above procedure ten such compounds (2a–j) were synthesised and characterised. Their physical data are listed in Table 1.

Some representative spectral data for compounds 2a–j.

Benzothiazol-2-yl-benzylidene-amine (2c; R = C6H5): White crystals; Rf: 0.59 (chloroform); IR (KBr): 1680 (N=C stretching) cm⁻¹; 1H-NMR (CDCl3): δ 2.2 (m, 1H, -N=CH-C6H4-CH3), 7.1–8.1 (m, 9H, aromatic) ppm; EI-MS (m/z, %): 238 (M⁺, 100 %).

Benzothiazol-2-yl-(4-methyl-benzylidene)-amine (2d; R = 4-CH3-C6H4): White crystals; Rf: 0.66 (chloroform); IR (KBr): 1670 (N=C stretching) cm⁻¹; 1H-NMR (CDCl3): δ 2.3–2.63 (m, 1H, -N=CH-C6H4-OH), 7.2–8.1 (m, 8H, aromatic), 9.2 (s 1H, -C6H4-OH) ppm; EI-MS (m/z, %): 254 (M⁺, 100 %).

Benzothiazol-2-yl-(2-methoxy-benzylidene)-amine (2g; R = 4-OCH3-C6H4): White crystals; Rf: 0.46 (chloroform); IR (KBr): 1676 (N=C stretching) cm⁻¹; 1H-NMR (CDCl3): δ 2.2–2.3 (m, 1H, -N=CH-C6H4-OH), 7.2–8.0 (m, 8H, aromatic) ppm; EI-MS (m/z, %): 276 (M⁺, 100 %).

2.1.2.2. General procedure for the preparation of 1-benzothiazol-2-yl-3-chloro-4-substituted-azetidin-2-ones 3a–j

To a solution of 2a–j (0.01 mol) and triethylamine (50 µl) in absolute alcohol (100 ml), chloroacetylchloride (0.02 mol) was added. The reaction mixture was heated under reflux for 16 h. Excess of the solvent was distilled-off in vacuo. The residue so obtained was washed with petroleum ether (60–80 °C) and recrystallised from petroleum ether (60–80 °C). The purity of the compounds was confirmed by elemental analyses and根据不同化合物的物理数据 (Table 1) and spectral data (Scheme 1).

Table 1: Physico-chemical data of compounds 2a–j and 3a–j

<table>
<thead>
<tr>
<th>Cpd.</th>
<th>R</th>
<th>M.p. [°C] (yield %)</th>
<th>Mol. formula (Mol.wt.)*</th>
<th>Cpd.</th>
<th>R</th>
<th>M.p. [°C]**</th>
<th>Mol. formula (Mol.wt.)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>CH3</td>
<td>160 (57)</td>
<td>C6H5N4S (176)</td>
<td>3a</td>
<td>CH3</td>
<td>172 (55)</td>
<td>C6H5N4SOCl (252)</td>
</tr>
<tr>
<td>2b</td>
<td>C6H5</td>
<td>164 (59)</td>
<td>C6H5N4S (190)</td>
<td>3b</td>
<td>C6H5</td>
<td>176 (56)</td>
<td>C6H5N4SOCl (206)</td>
</tr>
<tr>
<td>2c</td>
<td>C6H5</td>
<td>102a (63)</td>
<td>C6H5N4S (238)</td>
<td>3c</td>
<td>C6H5</td>
<td>158b (60)</td>
<td>C6H5N4SOCl (314)</td>
</tr>
<tr>
<td>2d</td>
<td>4-NH2-C6H4</td>
<td>194 (66)</td>
<td>C6H5N4SO (253)</td>
<td>3d</td>
<td>4-NH2-C6H4</td>
<td>240 (65)</td>
<td>C6H5N4SOCl (324)</td>
</tr>
<tr>
<td>2e</td>
<td>4-N(CH3)2-C6H4</td>
<td>175a (57)</td>
<td>C6H5N4SO (281)</td>
<td>3e</td>
<td>4-N(CH3)2-C6H4</td>
<td>146b (53)</td>
<td>C6H5N4SOCl (357)</td>
</tr>
<tr>
<td>2f</td>
<td>4-OCH3-C6H4</td>
<td>87az (68)</td>
<td>C6H5N4SO2Cl (268)</td>
<td>3f</td>
<td>4-OCH3-C6H4</td>
<td>124b (65)</td>
<td>C6H5N4SO2Cl (344)</td>
</tr>
<tr>
<td>2g</td>
<td>2-OCH2-C6H4</td>
<td>214 (69)</td>
<td>C6H5N4SO2Cl (268)</td>
<td>3g</td>
<td>2-OCH2-C6H4</td>
<td>276 (60)</td>
<td>C6H5N4SO2Cl (344)</td>
</tr>
<tr>
<td>2h</td>
<td>4-NO2-C6H4</td>
<td>238b (58)</td>
<td>C6H5N4SO2Cl (283)</td>
<td>3h</td>
<td>4-NO2-C6H4</td>
<td>164b (55)</td>
<td>C6H5N4SO2Cl (339)</td>
</tr>
<tr>
<td>2i</td>
<td>4-OC6H4</td>
<td>281b (61)</td>
<td>C6H5N4SO2Cl (254)</td>
<td>3i</td>
<td>4-OC6H4</td>
<td>193b (58)</td>
<td>C6H5N4SO2Cl (330)</td>
</tr>
<tr>
<td>2j</td>
<td>4-CH3-C6H4</td>
<td>206 (60)</td>
<td>C6H5N4SO2Cl (252)</td>
<td>3j</td>
<td>4-CH3-C6H4</td>
<td>214 (55)</td>
<td>C6H5N4SO2Cl (328)</td>
</tr>
</tbody>
</table>

* Recrystallised from methanol. ** Recrystallised from petroleum ether (60–80 °C). a CHN analyses were found to be within the limit of ± 0.4 %. Lit. m.p. [°C]: a1 = 102–103, a2 = 174–175, a2 = 85–86, a3 = 236–237, a4 = 280–281 and b = 157–158, b1 = 145–146, b2 = 120–121, b3 = 160–162, b4 = 190–191 (ref. [14]).
mol) was added dropwise with constant stirring over the period of 1 h. After stirring for 3 h, the reaction mixture was refluxed for 1 h. The reaction mixture was cooled and poured onto ice-cold water. The separated solid was filtered off, dried and recrystallised from petroleum ether (60–80 °C). Using the above procedure ten such compounds (3a–j) were synthesised and characterised.

Some representative spectral data for compounds 3:
1-Benzothiazol-2-yl-3-chloro-4-methyl-azetidin-2-ones (3a–3d): White crystals; Rf: 0.56 (chloroform); m.p.: 172 °C. IR(KBr): 1720 (β-lactam C=O) cm⁻¹; 1H-NMR (CDCl₃): δ 1.3–1.36 (t, 3H, CH₃-), 2.2–2.3 (d, 1H, -CH₂-lactam), 2.36–2.40 (d, 1H, -CH₃-lactam), 7.2–7.5 (m, 4H, aromatic) ppm; 13C-NMR (360 MHz, CDCl₃) δ 20.0 (CH₃-, lactam substitution), 30.2 (CH₃(CH2)-, β-lactam ring), 32.0 (-CH(Cl)-, β-lactam), 120–124 (CH, aromatic), 158.6 (-CH(Cl)-, benzothiazole), 176.3 (-C=O), β-lactam); EI-MS (m/z, %): 254 ([M⁺]+2, 100 %), 168 (46 %), 92 (19 %), 77 (10 %). Anal. found: C, 54.85; H, 3.66; N, 8.50; calc. for C₇H₆N₂SOCl (324): C, 54.87; H, 3.65; N, 8.53 %.

2.2. Pharmacology
Local bread albino mice of either sex weighing between 20 to 25 g, obtained from Biological E. Limited, Hyderabad (India) were used in the present study. Animals were housed in wire-mesh cages under the laboratory conditions (23 ± 2 °C, 12 h light) and maintained on a standard pellet diet (Hindustan Lever Ltd., Mumbai, India) and water ad libitum before the day of the experiment. On the last day food was withdrawn and they were given water only. During the course of experiment, the general behavior of animals was normal. All the experimental protocols were approved by the institutional animal ethical committee and experiments were conducted in accordance with the standard guidelines. The animals were divided into three groups (control, standard and test) and each experimental group consisted of six animals.

2.2.1. Anti-inflammatory activity (in vivo)
The carrageenan induced rat paw edema model according to the method reported by Winter et al [26] was employed for anti-inflammatory activity testing with some modifications. All the test compounds were suspended in 0.5 % carboxymethyl cellulose and administered orally either intraperitoneally (100 mg/kg body weight) 60 min prior the injection of 0.1 ml of freshly prepared solution of carrageenan (0.5 mg /25 ml) in physiological saline (154 mmol/l NaCl) into the sub-plantar tissue of the right hind paw of each mouse. The same volume of saline solution was injected into the left hind paw as an internal control. The difference in footpad thickness between the right and left foot were measured with a pair of dial thickness gauge callipers in a different pattern of intervals. The control groups received appropriate volumes of the vehicle only. Indomethacin (100 mg/kg body weight.) was used as a reference standard. The antiedematous effects of drugs were estimated in terms of percent inhibition. The percent inhibitory effect were calculated by the following equation:

\[
\text{Percent anti-inflammatory activity} = \frac{[(n - n') \times 100]}{n} 
\]

where n was the average difference in thickness between the left and right hind paw of control group and n’ was that of test group of animals.

2.2.2. Gastrointestinal ulceration studies [27]
Mice were fasted for 24 h (with water available ad libitum). The compounds were suspended in a carboxymethylcellulose vehicle and administered orally by gavage at 100 mg/kg/day dose for 5 days in a volume of 0.5 ml/100 g of body weight. The animals were sacrificed with diethyl ether inhalation, their stomachs removed by cutting along the greater curvature, washed under running water and fixed in 5 % formalin solution. The stomachs were then examined for lesions under a dissecting microscope.

2.2.3. COX-1 and COX-2 catalysed prostaglandin biosynthesis assay (in vitro)
The bioassays were performed according to the method of White and Glassman [28], with modification. In brief, in a 96-well microtiter plate with U-shaped wells, 10 µl of COX-1 or
COX-2 enzyme (3.0 units, 0.3 µg) was activated with 50 µl of cofactor solution (l-epinephrine 91.3 mg/ml, reduced glutathione 0.3 mg/ml, and hematin 1.3 mg/ml) in oxygen-free Tris-HCl buffer (0.1 mol/l, pH 7.5). 20 µl solution of the test compounds 2g and 3g (with various dilutions in Tris buffer) or standard (indometacin solution) were added. In order to verify the time-dependent COX-1 and COX-2 inhibitory activity, the assay was carried out with or without preincubating enzymes and inhibitors for 15 min. Then 20 µl radiolabeled \(^{14}C\) arachidonic acid (30 µmol/l, 17 Ci/mmol) was added and the assay mixture incubated at 37 °C for 15 min. The reaction was terminated by adding 10 µl of 1 mol/l HCl, and thereafter the pH 7.5 was balanced with 10 µl 1 mol/l NaOH. Inactivated COX-1 and COX-2 was used to generate background values by transferring 20 µl of each enzyme to a 500-µl microfuge tube and placing it in boiling water for 3 min. 20 µl buffer solution was used as blank. Each sample was assayed in duplicate.

The unmetabolised arachidonic acid was separated from the prostaglandin products by column chromatography and used as blank. Each sample was assayed in duplicate.

2.2.4. Statistical analysis

All the statistical analyses (Student’s t-test, analysis of variance: ANOVA, etc.) were carried out using Origin Data Analysis and Technical Graphics software (version 6), purchased from Microcal Software, Inc. (USA).

3. Results

The synthesis of title compounds 2a–j and 3a–j were carried out as shown in Scheme 1. The 2-aminobenzothiazole 1 was refluxed with various substituted alkyl/aryl-aldehydes in the presence of sodium acetate to obtain various imines or Schiff bases (alkyl /aryldiene-2-aminobenzothiazoles; 2a–j). The reaction takes place by the elimination of water molecules and the excess of water was distilled off. In this reaction sodium acetate acts as base and removes the protons from benzothiazoles. Further, the β-lactam ring (azetidinones) was incorporated in compound 2 by cyclo-condensation with chloroacetyl chloride in the presence of a base. The above reaction takes place by 1,2-cyclocondensation and elimination of hydrogen chloride in the presence of the strong base.

All the synthesized compounds were characterized by their physical, analytical and spectral data (Table 1). The test compounds 2a–j and 3a–j were evaluated in vivo for their anti-inflammatory activity using the carrageenan-induced rat paw edema model. The test compounds were administered either orally or intraperitoneally before carrageenan injection. The paw volume was measured after 2 h and 3.5 h of carrageenan injection. The antiedematous effects of the test compounds were estimated in terms of percent inhibition. All the test compounds were also assessed for their gastric ulcer inducing actions. The results of anti-inflammatory activity and gastric ulcer incidence are presented in Table 2.

The ability to inhibit the cyclooxygenase (COX-1 and COX-2) catalysed prostaglandin biosynthesis assay has been used to evaluate the mode of action of the test compounds. Only the most active compounds of each series, 2g and 3g, were evaluated in vitro. On the basis of previously reported kinetic studies of COX-1 and COX-2 inhibition by various NSAIDs [10], the assay was performed with or without pre-incubation of the enzyme with 50 µg of each test compound for 1 h.

Table 2: The anti-inflammatory activity and gastric ulceration effect of compound 2a–j and 3a–j

<table>
<thead>
<tr>
<th>Cpd.</th>
<th>Ulcer incidence</th>
<th>Swelling in thickness ((\times 10^{-2} \text{ mm}))^a</th>
<th>[% Inhibition]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per oral (p.o.)</td>
<td>Intraperitoneal (i.p.)</td>
<td>2 h</td>
</tr>
<tr>
<td>2a</td>
<td>0/6</td>
<td>49.8 ± 3.8</td>
<td>50.1 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>2b</td>
<td>48.8 ± 2.8</td>
<td>49.3 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>2c</td>
<td>43.8 ± 2.8</td>
<td>46.0 ± 2.6</td>
</tr>
<tr>
<td>2d</td>
<td>0/6</td>
<td>39.8 ± 3.6</td>
<td>40.1 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>2e</td>
<td>39.1 ± 3.4</td>
<td>39.6 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>2f</td>
<td>37.8 ± 3.2</td>
<td>38.1 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>2g</td>
<td>35.0 ± 3.0</td>
<td>35.6 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>2h</td>
<td>40.8 ± 3.1</td>
<td>41.1 ± 1.1</td>
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<tr>
<td></td>
<td>2i</td>
<td>37.7 ± 3.5</td>
<td>38.9 ± 2.0</td>
</tr>
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<td></td>
<td>2j</td>
<td>38.0 ± 3.8</td>
<td>39.1 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>3a</td>
<td>44.8 ± 3.7</td>
<td>45.1 ± 1.1</td>
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<td>3b</td>
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<td>38.8 ± 3.8</td>
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<td></td>
<td>3d</td>
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<td>3e</td>
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<td>34.5 ± 2.8</td>
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<td>3f</td>
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<tr>
<td></td>
<td>3g</td>
<td>30.0 ± 3.0</td>
<td>30.6 ± 2.9</td>
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<tr>
<td></td>
<td>3h</td>
<td>35.8 ± 3.6</td>
<td>36.1 ± 1.7</td>
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<td></td>
<td>3i</td>
<td>32.7 ± 3.5</td>
<td>33.0 ± 5.1</td>
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<tr>
<td></td>
<td>3j</td>
<td>33.0 ± 3.0</td>
<td>34.1 ± 4.0</td>
</tr>
<tr>
<td>Control Standard</td>
<td>6/6</td>
<td>73.5 ± 2.8</td>
<td>73.3 ± 2.5</td>
</tr>
</tbody>
</table>

Number of animals used, n = 6, Dose 100mg / kg body wt, Inhibition % = \(\frac{(n-n_i/n)}{n} \times 100\), where n was the average difference in thickness between the left and right hind paw of control group and \(n_i\) was that of test group of animals. \(^a\) Mean ± SD of four observations.
zyme and inhibitors. The cofactors were added in the radio-assay for the efficient conversion of fatty acid substrate to the prostaglandin products by COX enzymes. A classical non-selective cyclooxygenase inhibitor, indometacin, was chosen as reference standard due to its time-dependent inhibitory action [8].

The inhibition of COX-catalysed prostaglandin biosynthesis was calculated as the relative decrease in radioactivity (disintegration per min [DPM]) of the samples containing test compounds or standard to the blank. The percent inhibition (% I) of cyclooxygenase (COX-1 and COX-2) was calculated as:

\[
% I = \left[1 - \frac{\text{DPM}_{\text{Test/Std}} - \text{DPM}_{\text{Background}}}{\text{DPM}_{\text{Blank}} - \text{DPM}_{\text{Background}}}\right] \times 100.
\]

Table 3: IC50 Values (\(\mu\)mol/l) and COX-2/COX-1 ratios of 2g and 3g with or without 15 min pre-incubation.

<table>
<thead>
<tr>
<th>Cpd.</th>
<th>IC50 ((\mu)mol/l)</th>
<th>COX-2/COX-1 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without preincubation</td>
<td>With 15 min preincubation</td>
</tr>
<tr>
<td></td>
<td>COX-1</td>
<td>COX-2</td>
</tr>
<tr>
<td>2g</td>
<td>&gt; 450 ± 0.14</td>
<td>&gt; 4500 ± 0.2</td>
</tr>
<tr>
<td>3g</td>
<td>205 ± 0.21</td>
<td>1840 ± 0.4</td>
</tr>
<tr>
<td>Standard</td>
<td>3.5 ± 0.23</td>
<td>1706 ± 0.3</td>
</tr>
</tbody>
</table>

Each point represents the mean ± S.D (n = 2). Indometacin was used as standard.

The IC50 values were obtained by plotting (Fig. 1 – 4) the % inhibition (I) against log concentration (log C, \(\mu\)mol 1\(^{-1}\)). The IC50 values (mean ± S.D.) are presented in Table 3.

4. Discussion
4.1. Chemistry

In the IR spectra, compounds 2a–j showed absorption bands at 1660–1680 (N=C stretching) due to conversion of the primary amine into imines. Compounds 3a–j showed strong bands at about 1720–1740 (C=O stretching) due to the monocyclic ß-lactam carbonyl group and at 750 due to C-Cl stretching. The EI-MS, \(^1\)H-NMR,
13C-NMR spectral data of all the synthesised compounds were in conformity with the structure assigned. In the EI-MS spectra, molecular ion (M⁺) peaks, which appeared at different intensities, confirmed the molecular weights of the examined compounds (2a–j and 3a–j). Molecular ion peaks were the base peak for the compounds 2c, 2d, 2g, 2i and 2j. Appearance of an isotope peak (M⁺+2) as intense as the molecular ion peak confirmed the presence of a chlorine atom in compounds 3a–j. Elemental analysis results of the compounds were found within the limit of ± 0.4 % of the theoretical values. Some of the representative IR, 1H-NMR, 13C-NMR and mass spectral data are presented in the experimental section.

4.2. Pharmacological studies
The in vivo anti-inflammatory activity results indicate that all the title compounds possess good anti-inflammatory activity. Moreover, all the test compounds were generally found to be safer from the viewpoint of ulcer induction compared to the reference standard. Almost all the test compounds showed a remarkable gastric tolerance at 100 mg/kg body wt. Although it is difficult to deduce a good correlation between chemical structure and anti-inflammatory activity from the results obtained, some preliminary conclusions can be drawn as follows. In general, compounds 3a–j were found to be more potent compared to compounds 2a–j (Table 2). The comparative anti-inflammatory data showed that all the test compounds have shown higher inhibition when administered intraperitoneally as compared to the oral route. The most likely reason was due to poor absorption of the test compounds when administered by the oral route. Blood levels have to be determined to confirm this statement. Similarly the activity decreases rapidly as inhibition measured after 3.5 h indicated that these compounds were rapidly metabolized in the biological environment. Also in both the series, compounds 2h and 3h, having p-nitro substitution on the aromatic ring (electron withdrawing group) showed less anti-inflammatory activity compared to compounds having electron donating groups. This is due to decreased lipophilicity, which in turn inhibits permeability across the biological membranes. Among the compounds tested in this study, benzothiazol-2-yl-(2-methoxy-benzylidene)-amine (2g) and 1-benzothiazol-2-yl-3-chloro-4-(2-methoxy-phenyl)-azetidin-2-one (3g) showed most prominent anti-inflammatory activity (percent inhibition: 2g = 52.4 [2h] and 51.4 [3.5 h] when administered orally, 53.7 [2 h] and 53.2 [3.5 h] administered intraperitoneally; 3g = 59.2 [2 h] and 58.2 [3.5 h]; when administered orally, 60.3 [2 h] and 59.3 [3.5 h] administered intraperitoneally) with low gastric ulceration incidence compared to the reference standard indometacin.

Furthermore, in the in vitro COX inhibitory assay, the test compounds 2g and 3g showed anti-inflammatory activity by inhibiting both the COX-1 and COX-2 enzymes. From Table 3 and Fig. 5 and 6, it is clear that like indometacin, test compounds 2g and 3g exert time-dependent inhibition of both enzymes and exhibit higher COX-2 selectivity after pre-incubation with the enzymes. Compound 3g showed profound COX-2 selectivity, whereas 2g showed equal COX-1 and COX-2 selectivity even after pre-incubation. From COX-2 / COX-1 ratio values (Fig. 7) it is clear that both test compounds (2g and 3g) showed more COX-2 selectivity.
compared to the reference standard (in vitro), which is one of the possible explanations that these test compounds were found to be less ulcerogenic compared to indomethacin when tested in vivo.

These results certainly reflect the anti-inflammatory potential of the title compounds, however, further studies are still required to explore these molecules as clinically useful anti-inflammatory agents. Thus, for the safe and effective treatment of inflammatory diseases, development of the selective COX-2 inhibitors represents a most promising strategy.

5. References


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