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ORIGINAL ARTICLE

# Design, synthesis, and evaluation of anti-inflammatory, analgesic, ulcerogenicity, and nitric oxide releasing studies of novel indomethacin analogs as non-ulcerogenic derivatives

Shashikant V. Bhandari, Jignesh K. Parikh, Kailash G. Bothara, Trupti S. Chitre, Deepak K. Lokwani, Titiksh L. Devale, Nileema S. Modhave, Vidya S. Pawar, and Santosh Panda

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## Abstract

Most non-steroidal anti-inflammatory drugs (NSAIDs) suffer from the deadlier gastrointestinal (GI) toxicities. The free -COOH group is responsible for the GI toxicity associated with all traditional NSAIDs. In the present research work, the main objective was to develop new chemical entities as potential anti-inflammatory agents with no GI toxicities. The results of synthesis and pharmacological screening of a series of hybrid molecules having general formula 2-(5-(substituted phenyl)-2-oxo-ethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl)-2-oxoethyl nitrate are described. These compounds were tested *in vivo* for their anti-inflammatory, analgesic, and ulcerogenic properties, and subjected to histopathological studies. Compound **7c**, 2-(5-(3-hydroxyphenyl)-2-oxo-ethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl)-2-oxoethyl nitrate, was the most potent in this series. The compounds that showed significantly reduced GI ulcerogenicity also showed promising results in histopathological studies, and they were found to cause no mucosal injury. All the synthesized compounds were found to exhibit significant nitric oxide releasing activity in an *in vitro* method. In conclusion, the designed hybrid molecules were found to be significantly promising.

**Keywords:** Hybrid drugs; indomethacin; 1,3,4-oxadiazole; nitric oxide donor; ulcerogenicity

## Introduction

Medical research is constantly seeking to improve the efficiency of drugs. One approach involves the use of so-called hybrid drugs, which comprises the incorporation of two drug pharmacophores in one single molecule with the intention to exert dual drug action<sup>1</sup>. For example, one of the hybrid parts may be incorporated to counterbalance the known side effects associated with the other hybrid part, or to amplify its effect through action on another biotarget.

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most frequently used medications worldwide to treat pain, fever, and inflammation. NSAIDs exert their therapeutic activity by inhibiting cyclooxygenase derived prostaglandin synthesis, but this mechanism of action is inherently responsible for the gastrointestinal (GI)<sup>2–6</sup>, renal<sup>7–9</sup>, and hepatic<sup>10</sup> side effects observed in patients undergoing long term treatment.

The most common side effects associated with NSAID therapy are upper GI irritation, ulceration, dyspepsia, bleeding, and in some cases death<sup>11</sup>. It is clear that NSAID induced toxicity is a serious public health problem, contributing significantly to morbidity and mortality.

Three different strategies have emerged to improve the safety profile of NSAIDs: (1) the development of selective cyclooxygenase-2 (COX-2) inhibitors; (2) the co-administration of a proton pump inhibitor with NSAIDs; and (3) the linkage of a nitrate based nitric oxide (NO) releasing moiety to classical NSAIDs (NO-NSAIDs).

Each strategy presents a different set of advantages and limitations. For example, despite the relatively safe profile of COX-2 inhibitors in the GI tract, their role with respect to the adverse cardiovascular effects reported in some patients undergoing chronic treatment has attracted considerable

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attention<sup>12</sup>. Different NO-NSAIDs have been developed, which are reported in the literature. Among the NO-NSAIDs that have come into clinical trials are nitroaspirin, nitronaproxene, nitroketoprofen, etc. Among the nitric oxide donors adopted to prove the validity of this principle are furoxanes, oximes, hydrazides, and organic nitrates<sup>13–15</sup>. However, the long-term safety profile of such emerging classes of compounds is still to be determined.

Recent strategies adopted to minimize the side effects of NSAIDs include the use of hybrid molecules made up of non-selective or selective COX inhibitors together with a nitric oxide releasing function<sup>16–18</sup>.

Recent data have revealed serious cardiovascular side effects with selective COX-2 inhibitors<sup>17,18</sup>. In addition, such drugs only minimize the development of new gastric ulcers, but do not affect existing ones<sup>19</sup>.

The strategy involving the use of hybrid molecules made up of non-selective COX inhibitors together with a nitric oxide donating moiety constitutes one of the most promising approaches, because nitric oxide supports several endogenous GI tract defense mechanisms, including increase in mucus and bicarbonate secretions, increase in mucosal blood flow, and inhibition of the activation of proinflammatory cells. Moreover, because of the beneficial cardiovascular effects of NO, such drugs are expected to be devoid of the potential adverse cardiovascular effects associated with the use of selective COX-2 inhibitors.

Synthetic approaches based upon chemical modification of NSAIDs have been taken with aim of improving the safety profile and in turn the therapeutic window of NSAIDs. It has been reported in the literature that certain compounds bearing a 1,3,4-oxadiazole nucleus possess significant anti-inflammatory activity<sup>20</sup>. In our attempt to discover new, safer, and potent agents for the treatment of inflammatory diseases, we functionalized the oxadiazole nucleus onto the indomethacin moiety.

In view of the above facts, herein we report the synthesis, anti-inflammatory, analgesic, and ulcerogenic properties, and *in vitro* nitric oxide releasing characteristic of a hybrid molecule which has an indomethacin like structure, oxadiazole moiety, and organic nitrate as the nitric oxide donating group.

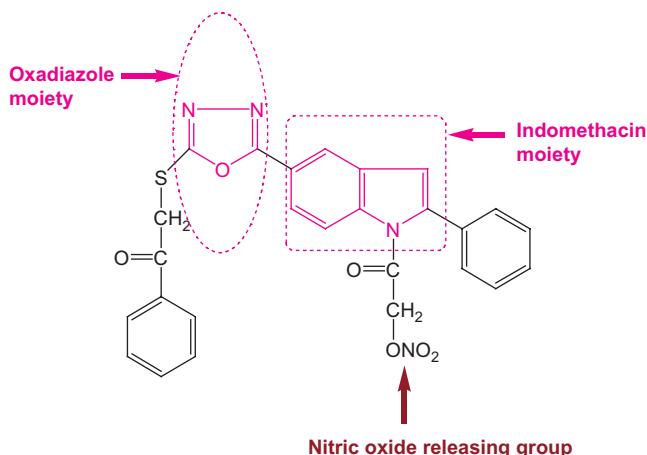
In the present study, we modified indomethacin (non-selective COX-2 inhibitor) in three regions in an attempt both to increase the anti-inflammatory activity and to enhance drug safety by the covalent attachment of an organic nitrate moiety as a nitric oxide donor, as shown in Figure 1.

The compounds designed thus were found to possess a significant analgesic-anti-inflammatory profile with significant reduction in the potential for ulcerogenic toxicities.

## Experimental

### Synthesis studies

All the compounds were synthesized using reported literature procedures, and synthesis procedures were set and optimized as and where required. Melting points were determined by



**Figure 1.** Pharmacophore.

digital melting point apparatus (Veego), model VMP-D, and were uncorrected. Fourier transform infrared (FTIR) spectra of the powdered compounds were recorded using KBr on a Jasco FTIR V 430+ spectrometer, with diffuse reflectance attachment, and are reported in  $\text{cm}^{-1}$ .  $^1\text{H}$  nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury YH300 (300 MHz FT NMR) spectrophotometer using tetramethylsilane (TMS) as an internal reference (chemical shift represented in  $\delta$  ppm). Mass spectra were recorded on a gas chromatography-mass spectrometry (GC-MS) QP5050A system (bench-top quadrupole mass spectrophotometer). NMR and MASS spectral studies were done at Department of Chemistry, University of Pune. Elemental analysis was recorded on a FlashEA112 series machine at the Shimadzu Analytical Center, University of Pune; the values were found to be within  $\pm 0.4\%$  of the theoretical ones. The purity of the compounds was checked on thin layer chromatography (TLC) plates using silica gel G as the stationary phase and iodine vapor as the visualizing agent.

### Synthesis of 4-hydrazino benzoic acid (1)

Compound (1) was synthesized as per the reported procedure<sup>21</sup>. Yield: 82%, M.P. 108–110°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3025 (Ar C-H); 3550 (O-H stretching in acid); 1715 (C=O stretching in acid); 3460 (primary Ar N-H stretching); 1560 (Ar C=C).

### Synthesis of 4-(2-(1-phenylethylidene) hydrazinyl) benzoic acid (2)

Compound (2) was synthesized as per the reported procedure<sup>21</sup>. Yield: 80%, M.P. 120–122°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3030 (Ar C-H); 3550 (O-H stretching in acid); 1715 (C=O stretching in acid); 1546 (C=N); 2941 (aliphatic C-H).

### Synthesis of 2-phenyl-1-H-indole-5-carboxylic acid (3)

Compound (3) was synthesized as per the reported procedure<sup>21</sup>. Yield: 75%, M.P. 192–194°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3030 (Ar C-H); 3540 (O-H

stretching in acid); 1720 (C=O stretching in acid); 1260 (C-N stretching); 3350 (secondary Ar N-H stretching).

#### Synthesis of 2-phenyl-1-H-indole-5-carbohydrazide (4)

A mixture of **3** (4 g, 10 mmol) and excess of thionyl chloride was refluxed for 4 h; excess thionyl chloride was neutralized by using formic acid and the residue was refluxed with excess hydrazine hydrate in ethanol (20 mL) for 6 h. The mixture was left to cool and then evaporated under reduced pressure. The residue obtained was filtered, washed with water, dried, and crystallized from absolute ethanol. Yield: 73.49%, M.P. 200–202°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3020 (Ar C-H); 1650 (C=O stretching); 1275 (C-N stretching); 3520 (primary N-H stretching); 3320 (Ar N-H stretching).

#### Synthesis of 5-(2-phenyl-1-H-indol-5-yl)-1,3,4-oxadiazole-2-thiol (5)

Compound (**5**) was synthesized as per the reported procedure<sup>22</sup>. Yield: 78.50%, M.P. 214–216°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3015 (Ar C-H); 1260 (C-N stretching); 1602 (C-N ring stretch of oxadiazole nucleus); 1055 (C-O stretch of oxadiazole nucleus); 3335 (Ar N-H stretching); 2560 (S-H stretching).

#### General procedure for the preparation of S-substituted phenacyl 1,3,4-oxadiazole-2-thiol derivatives (5a–5f)

S-substituted phenacyl 1,3,4-oxadiazole-2-thiol derivatives (**5a–5f**) were prepared as per the reported procedures<sup>23</sup>.

Synthesis of 2-(5-(2-phenyl-1-H-indol-5-yl)-1,3,4-oxadiazole-2ylthio)-1-(phenyl) ethanone (**5a**) Yield: 72.70%, M.P. 228–230°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3025 (Ar C-H); 1635 (C=O stretching); 1250 (C-N stretching); 3300 (Ar N-H stretching); 1610 (C-N ring stretch of oxadiazole nucleus); 1055 (C-O stretch of oxadiazole nucleus); 2935 (aliphatic C-H).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.5 (s, 2H, -(CH<sub>2</sub>)); 6.9 (s, 1H, -CH-); 7.1–7.3 (m, 5H, -Ar-H); 7.4–7.5 (m, 5H, ArH); 10.1 (s, 1H, indole NH); 7.6–7.8 (m, 3H, 4, 6, 7 ArH). Mass spectra of compound exhibited molecular ion peak at  $m/z$  411 ( $\text{M}^+$ ), other important fragments were observed at 412 ( $\text{M}^+ + 1$ ), 413 ( $\text{M}^+ + 2$ ).

Synthesis of 2-(5-(2-phenyl-1-H-indol-5-yl)-1,3,4-oxadiazole-2ylthio)-1-(4-hydroxyphenyl) ethanone (**5b**) Yield: 75.28%, M.P. 234–236°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3025 (Ar C-H); 1635 (C=O stretching); 1250 (C-N stretching); 3310 (Ar N-H stretching); 1610 (C-N ring stretch of oxadiazole nucleus); 1055 (C-O stretch of oxadiazole nucleus); 2920 (aliphatic C-H); 3293 (O-H stretching).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.7 (s, 2H, -(CH<sub>2</sub>)); 6.4 (s, 1H, -CH-); 7.1–7.2 (m, 5H, -Ar-H); 7.3–7.5 (m, 5H, ArH); 9.6 (s, 1H, indole NH); 7.6–7.8 (m, 3H, 4, 6, 7 ArH); 8.23 (s, 1H, O-H). Mass spectra of compound exhibited molecular ion peak at  $m/z$  427 ( $\text{M}^+$ ), other important fragments were observed at 428 ( $\text{M}^+ + 1$ ), 429 ( $\text{M}^+ + 2$ ).

Synthesis of 2-(5-(2-phenyl-1-H-indol-5-yl)-1,3,4-oxadiazole-2ylthio)-1-(3-hydroxyphenyl) ethanone (**5c**) Yield: 74.88%, M.P. 232–234°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound

showed bands at 3035 (Ar C-H); 1675 (C=O stretching); 1250 (C-N stretching); 3300 (Ar N-H stretching); 1608 (C-N ring stretch of oxadiazole nucleus); 1040 (C-O stretch of oxadiazole nucleus); 2955 (aliphatic C-H); 3320 (O-H stretching).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.7 (s, 2H, -(CH<sub>2</sub>)); 6.6 (s, 1H, -CH-); 7.1–7.21 (m, 5H, -Ar-H); 7.35–7.5 (m, 5H, ArH); 9.5 (s, 1H, indole NH); 7.6–7.82 (m, 3H, 4, 6, 7 ArH), 8.5 (s, 1H, O-H). Mass spectra of compound exhibited molecular ion peak at  $m/z$  427 ( $\text{M}^+$ ), other important fragments were observed at 428 ( $\text{M}^+ + 1$ ), 429 ( $\text{M}^+ + 2$ ).

Synthesis of 2-(5-(2-phenyl-1-H-indol-5-yl)-1,3,4-oxadiazole-2ylthio)-1-(4-nitrophenyl) ethanone (**5d**) Yield: 71.50%, M.P. 238–240°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3025 (Ar C-H); 1675 (C=O stretching); 1230 (C-N stretching); 3320 (Ar N-H stretching); 1602 (C-N ring stretch of oxadiazole nucleus); 1045 (C-O stretch of oxadiazole nucleus); 2941 (aliphatic C-H); 1670 (N=O stretching); 1360 (C-N stretching in C-NO<sub>2</sub>).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 4.2 (s, 2H, -(CH<sub>2</sub>)); 6.8 (s, 1H, -CH-); 7.01–7.1 (m, 5H, -Ar-H); 7.21–7.4 (m, 5H, Ar); 7.5–7.7 (m, 3H, 4, 6, 7 ArH); 8.1 (s, 1H, indole NH). Mass spectra of compound exhibited molecular ion peak at  $m/z$  456 ( $\text{M}^+$ ), other important fragments were observed at 457 ( $\text{M}^+ + 1$ ), 458 ( $\text{M}^+ + 2$ ).

Synthesis of 2-(5-(2-phenyl-1-H-indol-5-yl)-1,3,4-oxadiazole-2ylthio)-1-(3-nitrophenyl) ethanone (**5e**) Compound **5e** was synthesized as per the reported procedure. Yield: 70.60%, M.P. 236–238°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3025 (Ar C-H); 1675 (C=O stretching); 1250 (C-N stretching); 3300 (Ar N-H stretching); 1615 (C-N ring stretch of oxadiazole nucleus); 1025 (C-O stretch of oxadiazole nucleus); 2935 (aliphatic C-H); 1620 (N=O stretching); 1340 (C-N stretching in C-NO<sub>2</sub>).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.5 (s, 2H, -(CH<sub>2</sub>)); 6.9 (s, 1H, -CH-); 7.01–7.1 (m, 5H, -Ar-H); 7.21–7.4 (m, 5H, ArH); 7.5–7.7 (m, 3H, 4, 6, 7 ArH); 8.3 (s, 1H, indole NH). Mass spectra of compound exhibited molecular ion peak at  $m/z$  456 ( $\text{M}^+$ ), other important fragments were observed at 457 ( $\text{M}^+ + 1$ ), 458 ( $\text{M}^+ + 2$ ).

Synthesis of 2-(5-(2-phenyl-1-H-indol-5-yl)-1,3,4-oxadiazole-2ylthio)-1-(3,4-dimethoxyphenyl) ethanone (**5f**) Yield: 76.70%, M.P. 244–246°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3040 (Ar C-H); 1675 (C=O stretching); 1230 (C-N stretching); 3310 (Ar N-H stretching); 1602 (C-N ring stretch of oxadiazole nucleus); 1055 (C-O stretch of oxadiazole nucleus); 2930 (aliphatic C-H); 1150 (O-CH<sub>3</sub> asymmetric stretch).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.4 (s, 2H, -(CH<sub>2</sub>)); 3.73 (s, 6H, (O-CH<sub>3</sub>)<sub>2</sub>); 6.4 (s, 1H, -CH-); 7.1–7.3 (m, 5H, -Ar-H); 7.4–7.6 (m, 5H, ArH); 7.5–7.7 (m, 3H, 4, 6, 7 ArH); 8.7 (s, 1H, indole NH). Mass spectra of compound exhibited molecular ion peak at  $m/z$  471 ( $\text{M}^+$ ), other important fragments were observed at 472 ( $\text{M}^+ + 1$ ), 473 ( $\text{M}^+ + 2$ ).

#### General procedure for the preparation of N-substituted chloro acetyl chloride derivatives (6a–6f)

N-substituted chloro acetyl chloride derivatives (**6a–6f**) were prepared as per the reported procedures<sup>24</sup>.

2-Chloro-1-(5-(5-(2-oxo-2-phenylethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl) ethanone (**6a**) Yield:

70.56%, M.P. 252–254°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3025 (Ar C-H); 1675 (C=O stretching); 1250 (C-N stretching); 1602 (C-N ring stretch of oxadiazole nucleus); 1055 (C-O stretch of oxadiazole nucleus); 2941 (aliphatic C-H); 680 (C-Cl stretching).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.6 (s, 2H, -(CH<sub>2</sub>)); 4.4 (s, 2H, -(CH<sub>2</sub>-Cl)); 6.5 (s, 1H, -CH-); 7.2–7.35 (m, 5H, -Ar-H); 7.4–7.54 (m, 5H, ArH); 7.6–7.8 (m, 3H, 4, 6, 7 ArH). Mass spectra of compound exhibited molecular ion peak at  $m/z$  487 ( $\text{M}^+$ ), other important fragments were observed at 488 ( $\text{M}^+ + 1$ ), 489 ( $\text{M}^+ + 2$ ).

**2-Chloro-1-(5-(5-(2-(4-hydroxyphenyl)-2-oxo-2-phenylethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl) ethanone (6b)** Yield: 69.88%, M.P. 256–258°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3030 (Ar C-H); 1635 (C=O stretching); 1270 (C-N stretching); 1615 (C-N ring stretch of oxadiazole nucleus); 1055 (C-O stretch of oxadiazole nucleus); 2941 (aliphatic C-H); 3280 (O-H stretching); 695 (C-Cl stretching).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.1 (s, 2H, -(CH<sub>2</sub>)); 4.2 (s, 2H, -(CH<sub>2</sub>-Cl)); 6.7 (s, 1H, -CH-); 7.3–7.4 (m, 5H, -Ar-H); 7.5–7.7 (m, 5H, ArH); 7.8–7.92 (m, 3H, 4, 6, 7 ArH); 8.3 (s, 1H, O-H). Mass spectra of compound exhibited molecular ion peak at  $m/z$  503 ( $\text{M}^+$ ), other important fragments were observed at 504 ( $\text{M}^+ + 1$ ), 505 ( $\text{M}^+ + 2$ ).

**2-Chloro-1-(5-(5-(2-(3-hydroxyphenyl)-2-oxo-2-phenylethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl) ethanone (6c)** Yield: 73.74%, M.P. 258–260°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3015 (Ar C-H); 1645 (C=O stretching); 1230 (C-N stretching); 1615 (C-N ring stretch of oxadiazole nucleus); 1035 (C-O stretch of oxadiazole nucleus); 2940 (aliphatic C-H); 3310 (O-H stretching); 670 (C-Cl stretching).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.1 (s, 2H, -(CH<sub>2</sub>)); 4.2 (s, 2H, -(CH<sub>2</sub>-Cl)); 6.8 (s, 1H, -CH-); 7.1–7.32 (m, 5H, -Ar-H); 7.4–7.6 (m, 5H, ArH); 7.7–7.85 (m, 3H, 4, 6, 7 ArH); 8.6 (s, 1H, O-H). Mass spectra of compound exhibited molecular ion peak at  $m/z$  503 ( $\text{M}^+$ ), other important fragments were observed at 504 ( $\text{M}^+ + 1$ ), 505 ( $\text{M}^+ + 2$ ).

**2-Chloro-1-(5-(5-(2-(4-nitrophenyl)-2-oxo-2-phenylethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl) ethanone (6d)** Yield: 75.23%, M.P. 244–246°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3025 (Ar C-H); 1675 (C=O stretching); 1250 (C-N stretching); 1602 (C-N ring stretch of oxadiazole nucleus); 1055 (C-O stretch of oxadiazole nucleus); 2941 (aliphatic C-H); 1670 (N=O stretching); 1360 (C-N stretching in C-NO<sub>2</sub>); 660 (C-Cl stretching).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 4.1 (s, 2H, -(CH<sub>2</sub>)); 4.5 (s, 2H, -(CH<sub>2</sub>-Cl)); 6.3 (s, 1H, -CH-); 7.01–7.1 (m, 5H, -Ar-H); 7.21–7.4 (m, 5H, ArH); 7.5–7.7 (m, 3H, 4, 6, 7 ArH). Mass spectra of compound exhibited molecular ion peak at  $m/z$  532 ( $\text{M}^+$ ), other important fragments were observed at 533 ( $\text{M}^+ + 1$ ), 534 ( $\text{M}^+ + 2$ ).

**2-Chloro-1-(5-(5-(2-(3-nitrophenyl)-2-oxo-2-phenylethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl) ethanone (6e)** Yield: 68.23%, M.P. 242–244°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3025 (Ar C-H); 1675 (C=O stretching); 1250 (C-N stretching); 1602

(C-N ring stretch of oxadiazole nucleus); 1055 (C-O stretch of oxadiazole nucleus); 2941 (aliphatic C-H); 1630 (N=O stretching); 1340 (C-N stretching in C-NO<sub>2</sub>); 675 (C-Cl stretching).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 4.2 (s, 2H, -(CH<sub>2</sub>)); 4.6 (s, 2H, -(CH<sub>2</sub>-Cl)); 6.3 (s, 1H, -CH-); 7.21–7.4 (m, 5H, -Ar-H); 7.4–7.6 (m, 5H, ArH); 7.7–7.85 (m, 3H, 4, 6, 7 ArH). Mass spectra of compound exhibited molecular ion peak at  $m/z$  532 ( $\text{M}^+$ ), other important fragments were observed at 533 ( $\text{M}^+ + 1$ ), 534 ( $\text{M}^+ + 2$ ).

**2-Chloro-1-(5-(5-(2-(3,4-dimethoxyphenyl)-2-oxo-2-phenylethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl) ethanone (6f)** Yield: 74.55%, M.P. 250–252°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3017 (Ar C-H); 1675 (C=O stretching); 1220 (C-N stretching); 1630 (C-N ring stretch of oxadiazole nucleus); 1055 (C-O stretch of oxadiazole nucleus); 2945 (aliphatic C-H); 1140 (O-CH<sub>3</sub> asymmetric stretch); 680 (C-Cl stretching).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.2 (s, 2H, -(CH<sub>2</sub>)); 3.45 (s, 6H, (O-CH<sub>3</sub>)<sub>2</sub>); 4.4 (s, 2H, -(CH<sub>2</sub>-Cl)); 6.4 (s, 1H, -CH-); 7.01–7.1 (m, 5H, -Ar-H); 7.21–7.4 (m, 5H, ArH); 7.5–7.7 (m, 3H, 4, 6, 7 ArH).. Mass spectra of compound exhibited molecular ion peak at  $m/z$  547 ( $\text{M}^+$ ), other important fragments were observed at 548 ( $\text{M}^+ + 1$ ), 549 ( $\text{M}^+ + 2$ ).

#### General procedure for the preparation of nitrate derivatives (7a–7f)

Nitrate derivatives (7a–7f) were prepared as per the reported procedures<sup>25</sup>.

**2-Oxo-2-(5-(5-(2-oxo-2-phenylethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl) ethyl nitrate (7a)** Yield: 75.63%, M.P. 260–262°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3015 (Ar C-H); 1665 (C=O stretching); 1255 (C-N stretching); 1604 (C-N ring stretch of oxadiazole nucleus); 1025 (C-O stretch of oxadiazole nucleus); 2965 (aliphatic C-H); 1444 (CH<sub>2</sub>-O stretching); 1632 (-N=O stretching).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.7 (s, 2H, -(CH<sub>2</sub>)); 4.6 (s, 2H, -(CH<sub>2</sub>-ONO<sub>2</sub>)); 6.4 (s, 1H, -CH-); 6.9–7.1 (m, 5H, -Ar-H); 7.21–7.4 (m, 5H, ArH); 7.5–7.7 (m, 3H, 4, 6, 7 ArH). Mass spectra of compound exhibited molecular ion peak at  $m/z$  514 ( $\text{M}^+$ ), other important fragments were observed at 515 ( $\text{M}^+ + 1$ ), 516 ( $\text{M}^+ + 2$ ). Elemental analysis was observed as C (60.64%), H (3.56%), N (10.92%).

**2-(5-(4-Hydroxyphenyl)-2-oxo-ethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl)-2-oxoethyl nitrate (7b)** Yield: 52.50%, M.P. 266–268°C. FTIR spectra ( $\text{cm}^{-1}$ ) of the compound showed bands at 3035 (Ar C-H); 1675 (C=O stretching); 1240 (C-N stretching); 1608 (C-N ring stretch of oxadiazole nucleus); 1046 (C-O stretch of oxadiazole nucleus); 3010 (aliphatic C-H); 3293 (O-H stretching); 1430 (CH<sub>2</sub>-O stretching); 1635 (-N=O stretching).  $^1\text{H}$  NMR chemical shifts at ( $\text{CDCl}_3$ ,  $\delta$  ppm): 3.6 (s, 2H, -(CH<sub>2</sub>)); 4.3 (s, 2H, -(CH<sub>2</sub>-ONO<sub>2</sub>)); 6.8 (s, 1H, -CH-); 7.1–7.26 (m, 5H, -Ar-H); 7.3–7.4 (m, 5H, ArH); 7.5–7.7 (m, 3H, 4, 6, 7 ArH); 8.12 (s, 1H, O-H). Mass spectra of compound exhibited molecular ion peak at  $m/z$  530 ( $\text{M}^+$ ), other important fragments were observed at 531 ( $\text{M}^+ + 1$ ), 532 ( $\text{M}^+ + 2$ ). Elemental analysis was observed as C (58.90%), H (3.45%), N (10.52%).

**2-(5-(3-Hydroxyphenyl)-2-oxo-ethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl)-2-oxoethyl nitrate (**7c**)** Yield: 64.23%, M.P. 254–256°C. FTIR spectra (cm<sup>-1</sup>) of the compound showed bands at 3025 (Ar C-H); 1675 (C=O stretching); 1270 (C-N stretching); 1630 (C-N ring stretch of oxadiazole nucleus); 1055 (C-O stretch of oxadiazole nucleus); 2920 (aliphatic C-H); 1460 (CH<sub>2</sub>-O stretching); 1650 (-N=O stretching). <sup>1</sup>H NMR chemical shifts at (CDCl<sub>3</sub>, δ ppm): 3.5 (s, 2H, -(CH<sub>2</sub>)); 4.4 (s, 2H, -(CH<sub>2</sub>-ONO<sub>2</sub>)); 6.9 (s, 1H, -CH-); 7.01–7.1 (m, 5H, -Ar-H); 7.21–7.4 (m, 5H, ArH); 7.5–7.7 (m, 3H, 4, 6, 7 ArH); 8.23 (s, 1H, O-H). Mass spectra of compound exhibited molecular ion peak at *m/z* 530 (M<sup>+</sup>), other important fragments were observed at 531 (M<sup>+</sup> + 1), 532 (M<sup>+</sup> + 2). Elemental analysis was observed as C (58.82%), H (3.46%), N (10.52%).

**2-(5-(4-Nitrophenyl)-2-oxo-ethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl)-2-oxoethyl nitrate (**7d**)** Yield: 59.52%, M.P. 274–276°C. FTIR spectra (cm<sup>-1</sup>) of the compound showed bands at 3025 (Ar C-H); 1675 (C=O stretching); 1250 (C-N stretching); 3350 (Ar N-H stretching); 1602 (C-N ring stretch of oxadiazole nucleus); 1055 (C-O stretch of oxadiazole nucleus); 2941 (aliphatic C-H); 1670 (N=O stretching); 1360 (C-N stretching in C-NO<sub>2</sub>); 1450 (CH<sub>2</sub>-O stretching); 1630 (-N=O stretching). <sup>1</sup>H NMR chemical shifts at (CDCl<sub>3</sub>, δ ppm): 3.6 (s, 2H, -(CH<sub>2</sub>)); 4.3 (s, 2H, -(CH<sub>2</sub>-ONO<sub>2</sub>)); 6.4 (s, 1H, -CH-); 6.9–7.1 (m, 5H, -Ar-H); 7.3–7.4 (m, 5H, ArH); 7.6–7.8 (m, 3H, 4, 6, 7 ArH). Mass spectra of compound exhibited molecular ion peak at *m/z* 559 (M<sup>+</sup>), other important fragments were observed at 560 (M<sup>+</sup> + 1), 561 (M<sup>+</sup> + 2). Elemental analysis was observed as C (55.84%), H (3.10%), N (12.56%).

**2-(5-(3-Nitrophenyl)-2-oxo-ethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl)-2-oxoethyl nitrate (**7e**)** Yield: 67.62%, M.P. 286–288°C. FTIR spectra (cm<sup>-1</sup>) of the compound showed bands at 3035 (Ar C-H); 1675 (C=O stretching); 1255 (C-N stretching); 1602 (C-N ring stretch of oxadiazole nucleus); 1040 (C-O stretch of oxadiazole nucleus); 2931 (aliphatic C-H); 1620 (N=O stretching); 1344 (C-N stretching in C-NO<sub>2</sub>); 1430 (CH<sub>2</sub>-O stretching); 1630 (-N=O stretching). <sup>1</sup>H NMR chemical shifts at (CDCl<sub>3</sub>, δ ppm): 3.3 (s, 2H, -(CH<sub>2</sub>)); 4.4 (s, 2H, -(CH<sub>2</sub>-ONO<sub>2</sub>)); 6.9 (s, 1H, -CH-); 7.1–7.2 (m, 5H, -Ar-H); 7.31–7.5 (m, 5H, ArH); 7.6–7.86 (m, 3H, 4, 6, 7 ArH). Mass spectra of compound exhibited molecular ion peak at *m/z* 559 (M<sup>+</sup>), other important fragments were observed at 560 (M<sup>+</sup> + 1), 561 (M<sup>+</sup> + 2). Elemental analysis was observed as C (55.81%), H (3.02%), N (12.56%).

**2-(5-(3,4-Dimethoxyphenyl)-2-oxo-ethylthio)-1,3,4-oxadiazole-2-yl)-2-phenyl-1H-indol-1-yl)-2-oxoethyl nitrate (**7f**)** Yield: 61.34%, M.P. 292–294°C. FTIR spectra (cm<sup>-1</sup>) of the compound showed bands at 3025 (Ar C-H); 1675 (C=O stretching); 1250 (C-N stretching); 1602 (C-N ring stretch of oxadiazole nucleus); 1055 (C-O stretch of oxadiazole nucleus); 2941 (aliphatic C-H); 1150 (O-CH<sub>3</sub> asymmetric stretch); 1450 (CH<sub>2</sub>-O stretching); 1630 (-N=O stretching). <sup>1</sup>H NMR chemical shifts at (CDCl<sub>3</sub>, δ ppm): 3.5 (s, 2H, -(CH<sub>2</sub>)); 3.73 (s, 6H, (O-CH<sub>3</sub>)<sub>2</sub>); 4.4 (s, 2H, -(CH<sub>2</sub>-ONO<sub>2</sub>)); 6.62 (s, 1H, -CH-); 7.1–7.21 (m, 5H, -Ar-H); 7.2–7.38 (m, 5H, ArH); 7.62–7.7 (m, 3H, 4, 6, 7 ArH). Mass spectra of compound exhibited molecular

ion peak at *m/z* 574 (M<sup>+</sup>), other important fragments were observed at 575 (M<sup>+</sup> + 1), 576 (M<sup>+</sup> + 2). Elemental analysis was observed as C (58.51%), H (3.83%), N (9.70%).

## Pharmacology

### Animals

Swiss albino mice of either sex weighing 20–25 g and Wistar rats weighing in the range 100–120 g were obtained from Yash Farm, Pune, India. All the animals were housed under standard ambient conditions of temperature (25 ± 2°C) and relative humidity of 50 ± 5%. A 12:12 h light/dark cycle was maintained. All the animals were allowed to have free access to water, and standard pelletized laboratory animal diet until 24 h prior to pharmacological studies. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of the AISSMS College of Pharmacy, Pune, constituted for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA/257, CPCSEA/300), Government of India.

### Preparation of test compounds

After suspending the test compounds in a 1.0% aqueous solution of sodium carboxymethyl cellulose (CMC), test samples were administered to the test animals orally. The negative and positive control group animals received the same experimental handling as those of the test groups except that the control group animals received only appropriate volumes of vehicle and of the reference drug, indomethacin or diclofenac acid, respectively.

### Anti-inflammatory activity

Anti-inflammatory activity was evaluated using the well known carrageenan induced rat paw edema model of Winter *et al.*<sup>26</sup>, using groups of six animals each. A freshly prepared aqueous suspension of carrageenan (1.0%, w/v, 0.1 mL) was injected in the subplantar region of the right hind paw of each rat. One group was kept as control, and the animals of the other group were pretreated with the test compounds, 1 h before the carrageenan treatment. The volume was measured before and after carrageenan treatment at 60 min intervals with the help of a digital plethysmometer (Panlab LE 7500, USA).

### Analgesic activity

The analgesic activity was evaluated using the acetic acid induced writhing method<sup>27</sup>.

### Acute ulcerogenicity studies

Acute ulcerogenicity screening was done according to method reported by Cioli *et al.*<sup>28</sup>. The mucosal damage was examined by means of an electron microscope. For each stomach specimen, the mucosal damage was assessed according to the following scoring system:

- 0.0, normal (no injury, bleeding, and latent injury);
- 0.5, latent injury or widespread bleeding (>2 mm);

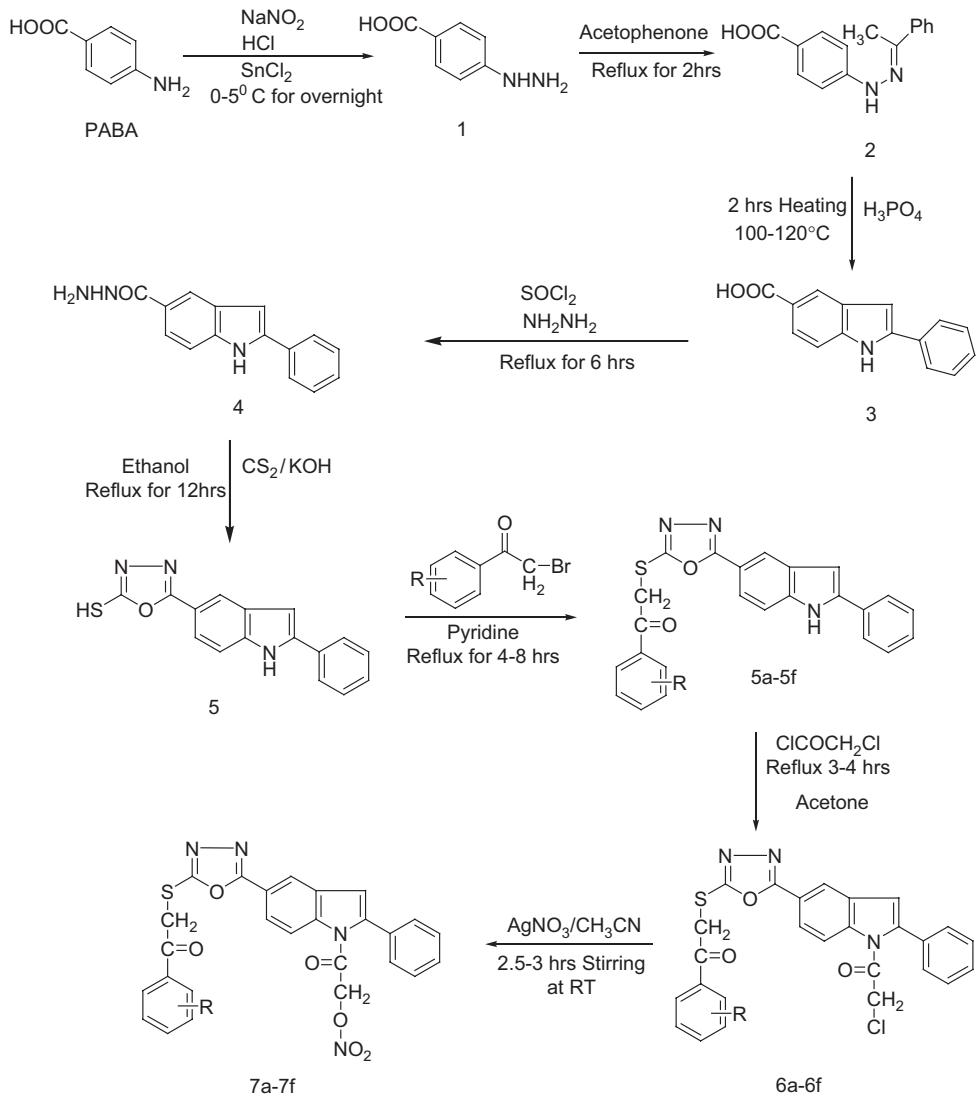
- 1.0, slight injury (2–3 dotted lines);
- 2.0, severe injury (continuous lined injury or 5–6 dotted injuries);
- 3.0, very severe injury (several continuous lined injuries);
- 4.0, widespread lined injury or widened injury.

The mean score for each treated group minus the mean score for the control group was regarded as the severity index of gastric mucosal damage. Data are expressed as mean ulcer score  $\pm$  SEM, data analyzed by one way analysis of variance

(ANOVA) followed by Dunnett's *t* test to determine the significance of the difference between the standard group and rats treated with the test compounds. The differences in results were considered significant when *p* was found to be  $<0.01$ .

#### Histopathological studies<sup>20,29,30</sup>

For the histopathological study, rats were sacrificed 4 h after cold stress and their stomach specimens removed and put into 10% formalin solution. A longitudinal section of the stomach along the greater curvature, which included the ulcer base and both sides of the ulcer margin, was taken and



Compound	-R
7a	-H
7b	-4-OH
7c	-3-OH
7d	-4-NO <sub>2</sub>
7e	-3-NO <sub>2</sub>
7f	-3,4-dimethoxy

**Scheme 1.** Synthesis route for designed NCEs, 7a–7f.

fixed in 10% formalin for 24 h at 4°C and embedded in white solid paraffin. Morphological examination was performed using hematoxylin and eosin staining to analyze histological changes and done under an electron microscope.

#### Detection of nitrite<sup>25</sup>

A solution of the appropriate compound (20 µL) in dimethylsulfoxide (DMSO) was added to a 2 mL of 1:1, v/v, mixture either of 50 mM phosphate buffer (pH 7.4) or of HCl solution (pH 1) with MeOH, containing  $5 \times 10^{-4}$  mM L-cysteine. The final concentration of test compound was  $10^{-4}$  M. After 1 h at 37°C, 1 mL of the reaction mixture was treated with 250 µL of Griess reagent (sulfanilamide (4 g), N-naphthylethylenediamine dihydrochloride (0.2 g), 85% phosphoric acid (10 mL) in distilled water (final volume: 100 mL)). After 10 min at room temperature, the absorbance was measured at 540 nm. Sodium nitrite standard solutions (10–80 µmol/mL) were used to construct the calibration curve. The results are expressed as the percentage of NO released ( $n = 2$ ) relative to a theoretical maximum release of 1 mol NO/mol of test compound.

#### Statistical analysis

Data obtained for each set of anti-inflammatory models are expressed as the mean of change in paw volume  $\pm$  SD and were analyzed by one-way ANOVA followed by Dunnett's *t* test. Data from the acetic acid induced writhing model are expressed as the mean number of writhes  $\pm$  SEM and were analyzed by one-way ANOVA followed by Dunnett's *t* test. The level of significance was set at  $p < 0.05$ . All statistical calculations were performed using the evaluation version of GraphPad Prism 3.0 (USA) statistical software.

## Results and discussion

### Chemistry

The synthetic route used to synthesize the title compounds is outlined in Scheme 1. The compound 4-hydrazinobenzoic acid (**1**) was prepared according to the method reported in the literature, using PABA (*para*-aminobenzoic acid). 4-(2-(1-Phenylethylidene) hydrazinyl) benzoic acid (**2**) was prepared by reacting **1** with acetophenone in ethanol. 2-Phenyl-1-H-indole-5-carboxylic acid (**3**) was prepared by reacting **2** with  $H_3PO_4$ . The reaction of **3** with thionyl chloride followed by hydrazine hydrate yielded 2-phenyl-1-H-indole-5-carbohydrazide (**4**). The reaction of **4** with carbon disulfide in an alkaline medium afforded 5-(2-phenyl-1-H-indol-5-yl)-1,3,4-oxadiazole-2-thiol (**5**). The compounds **5a–f** were prepared by reacting **5** with differently substituted phenacyl bromide in the presence of pyridine. The reaction of these (**5a–f**) with the substituted chloro acetyl chloride gave the corresponding haloalkyl derivatives (**6a–f**). The terminal chloro function was then substituted with nitric oxide releasing moieties ( $AgNO_3$  using acetonitrile as solvent) to get the nitrate derivatives (**7a–f**). The structures of the various synthesized compounds were assigned on the basis of results of different chromatographic and spectral studies. The physical data, FTIR, <sup>1</sup>H-NMR, and mass spectral data, and elemental

analysis data for all the synthesized compounds are given in the above experimental protocols.

The FTIR spectra of the title compounds (**7a–f**) exhibited very similar features and showed the expected bands for the characteristic groups present in the compounds, such as C=O stretching and another specific band for -NO<sub>2</sub> vibrations. In the NMR spectral data, all protons were seen according to the expected chemical shift and integration values. The aromatic protons appeared as multiplet peaks within the range 7.01–7.86 δ ppm.

### Pharmacology

We modified indomethacin (non-selective COX-2 inhibitors) in three regions in an attempt both to increase the anti-inflammatory activity and to enhance drug safety by the covalent attachment of an organic nitrate moiety as a nitric oxide donor. We investigated the anti-inflammatory, analgesic, and ulcerogenic properties as well as the nitric oxide releasing characteristic of the hybrid molecules in which indomethacin was substituted with the oxadiazole moiety and organic nitrate was the nitric oxide donating group. Indomethacin and diclofenac acid were used as reference standards. The experiments were performed using Wistar rats of either sex, weighing 100–120 g, and Swiss albino mice of either sex, weighing 20–25 g. The animals were maintained at 25 ± 2°C, 50 ± 5% relative humidity, and in a 12 h light/dark cycle. The animals were fasted for 24 h prior to the experiments and water was provided *ad libitum*. The test compounds were suspended in 1% aqueous carboxymethyl cellulose (CMC) solution and administered orally to experimental animals for all the studies.

### Anti-inflammatory activity

Anti-inflammatory activity of the synthesized compounds was evaluated by the carrageenan induced rat paw edema model. Equimolar dose, that is, equivalent to indomethacin subplantar injection, of 0.1 mL, 1% carrageenan in the rat paw produced an increase in paw volume (edema) of all the animals of the various groups. The onset of action was evident from 1 h in the various test groups. A significant ( $p < 0.01$ ) reduction of rat paw edema was observed for most of the test compounds at 3 h, compared to the control group; indomethacin was used as standard (Table 1). Compounds with significant anti-inflammatory profile were subjected to GI-ulcerogenicity potential studies at 12 times the therapeutic doses with additional physical (cold) stress. A thorough examination of the results of histopathological studies indicated the absence of disruption of morphology of gastric epithelial cells and absence of ulcers/erosion in test (test compound treated) group animals compared to the reference standard, indomethacin, and control group animals. The results of the ulcerogenicity studies are presented in Table 3 and results of the histopathological studies are depicted in Figure 2. Of the synthesized new chemical entities (NCEs), **7a**, **7b**, and **7c** (55.27%, 62.35%, and 70.65%, respectively) exhibited very significant anti-inflammatory activity, compared to compounds **5** and **6c**. The haloalkyl derivatives were less potent

**Table 1.** Results for anti-inflammatory activity of synthesized compounds against carrageenan induced rat paw edema model in rats.

Compound/dose (mg/kg, p.o.)	Change in paw volume (mL) after drug treatment ( $\pm$ SEM)			Anti-inflammatory activity (% inhibition)		
	1 h	2 h	3 h	1 h	2 h	3 h
Control	1.18 $\pm$ 0.08142	1.052 $\pm$ 0.08958	0.988 $\pm$ 0.0836	—	—	—
Indomethacin (10)	0.798 $\pm$ 0.0315	0.428 $\pm$ 0.699	0.276 $\pm$ 0.0422	32.38**	59.32**	72.93**
<b>5</b> (8.20)	0.8511 $\pm$ 0.0621	0.7370 $\pm$ 0.0902	0.6725 $\pm$ 0.0901	27.87**	29.94**	31.93**
<b>6c</b> (11.96)	0.7223 $\pm$ 0.042	0.6300 $\pm$ 0.031	0.5742 $\pm$ 0.071	38.78*	40.11**	41.88**
<b>7a</b> (14.39)	0.936 $\pm$ 0.026	0.6 $\pm$ 0.02145	0.442 $\pm$ 0.0453	20.388*	42.97**	55.27**
<b>7b</b> (14.84)	0.856 $\pm$ 0.5075	0.506 $\pm$ 0.0463	0.372 $\pm$ 0.0307	20.68**	48.09**	62.35**
<b>7c</b> (14.84)	0.806 $\pm$ 0.0477	0.442 $\pm$ 0.0453	0.29 $\pm$ 0.0283	31.7**	57.98**	70.65**
<b>7d</b> (15.65)	0.926 $\pm$ 0.0668	0.61 $\pm$ 0.0620	0.454 $\pm$ 0.0633	21.53*	42.02**	50.41**
<b>7e</b> (15.65)	0.936 $\pm$ 0.026	0.634 $\pm$ 0.0479	0.444 $\pm$ 0.0646	20.68*	39.74**	47.37**
<b>7f</b> (16.07)	0.972 $\pm$ 0.08218	0.622 $\pm$ 0.0650	0.472 $\pm$ 0.06507	17.63	40.88**	46.36**

Note. Data analyzed by one way ANOVA followed by Dunnett's *t* test ( $n = 6$ ), \* $p < 0.05$ , \*\* $p < 0.01$  significantly different from control.

**Table 2.** Results for analgesic activity of synthesized compounds against acetic acid induced writhing test in mice.

Compound	Dose (mg/kg, p.o.)	No. of writhes in 5–15 min after treatment (mean $\pm$ SE)		% Inhibition
Control	Acetic acid (1%, v/v)	42.4 $\pm$ 2.421	—	—
Indomethacin	10	12.6 $\pm$ 1.72**	70.29	
<b>5</b>	8.20	30.4 $\pm$ 4.363*	28.30	
<b>6c</b>	11.96	29.2 $\pm$ 3.776*	31.13	
<b>7a</b>	14.39	28.2 $\pm$ 4.363*	33.5	
<b>7b</b>	14.84	21 $\pm$ 2.915**	50.48	
<b>7c</b>	14.84	13.4 $\pm$ 2.337**	68.4	
<b>7d</b>	15.65	28.6 $\pm$ 4.308*	32.55	
<b>7e</b>	15.65	28.2 $\pm$ 4.576*	33.5	
<b>7f</b>	16.07	28.6 $\pm$ 3.776*	32.55	

Note. Data analyzed by one way ANOVA followed by Dunnett's *t* test ( $n = 6$ ), \* $p < 0.05$ , \*\* $p < 0.01$  significantly different from control.

**Table 3.** Ulcerogenic effects of synthesized compounds in comparison with diclofenac sodium.

Compound	Dose (mg/kg, p.o.)	Ratio of ulcerated animals	Ulcer index (mean $\pm$ SE)
Diclofenac	10	4/6	1.7 $\pm$ 0.2
	30	6/6	2.3 $\pm$ 0.3
	50	Not tested	—
<b>7a</b>	10	—	—
	30	—	—
	50	—	—
<b>7b</b>	10	—	—
	30	—	—
	50	—	—
<b>7c</b>	10	—	—
	20	—	—
	50	—	—

Note. —, no effect.

than the nitrooxy derivatives. S-substituted phenacyl 1,3,4-oxadaizole-2-thiol derivatives (**6a**–**6f**) were more potent than compound **5**. This indicates that substituted phenacyl bromide may increase the activity. Derivatives **7a**–**7f** were more potent than the corresponding haloalkyl derivatives **6a**–**6f**. This indicates that the nitrate moiety may contribute to the anti-inflammatory action of the final compounds. Based on the findings of these preclinical results, further studies need to be carried out to investigate the other specifications, such as *in vitro* assays, chronic ulcerogenicity studies, and toxicological studies, and the mechanism by which these drugs exhibit potential analgesic and anti-inflammatory activities (Table 1).

### Analgesic activity

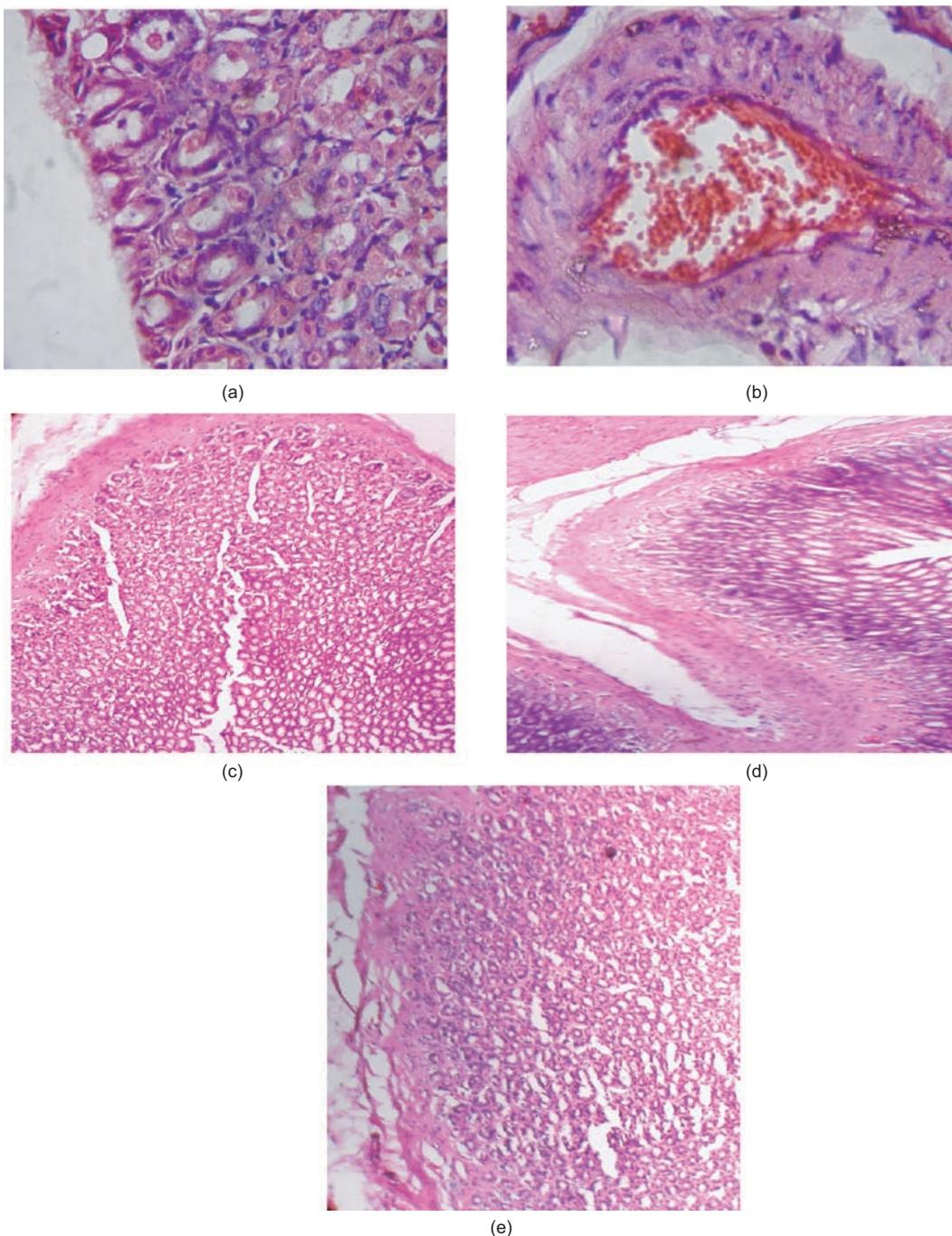
The analgesic activity of the compounds was studied using the acetic acid induced writhing test in mice. The compounds that exhibited significant anti-inflammatory activity, comparable to that of indomethacin, were screened for analgesic activity. The analgesic activity was evaluated at equimolar dose, equivalent to 10 mg/kg (indomethacin) body weight. These compounds exhibited an important analgesic profile measured by the classical acetic acid induced writhing model. From the results, it was noted that **7b** (50.48%) and **7c** (68.4%) possessed significant analgesic activity (Table 2).

### Acute ulcerogenicity studies

The ulcerogenic effect of derivatives **7a**, **7b**, and **7c** with the best overall profile in the animal efficacy model was evaluated for gastric ulcerogenic potential in the rat stress model at 12 times the therapeutic dose of diclofenac acid (Table 3). When compared with diclofenac acid, these three compounds did not cause any gastric ulceration and disruption of gastric epithelial cells at the abovementioned oral dose. The results are shown in Table 3.

### Histopathological studies

Stomach specimens of diclofenac acid treated rats were characterized by complete disruption of the protective mucosal layer (Figure 2b). Histopathological analysis also



**Figure 2.** Hematoxylin and eosin immunohistochemical staining of gastric ulcers after ulcer induction in rats. (a) Intact mucous membrane in control treated rat showing granular tissues composed of macrophages, fibroblasts, and endothelial cells forming microvessels. (b) Congestion of mucosal blood vessels observed in diclofenac treated group. (c–e) No damage to mucosa of rats treated with test compounds **7a**, **7b**, and **7c**, respectively. These specimens were identical to that of the control (a). Original magnification  $\times 200$ .

showed characteristic features of ulceration in the diclofenac acid treated group of animals. The tissue of diclofenac acid treated rats showed that some epithelial cells in the ulcer margin had proliferated and migrated into the ulcer crater, which indicated complete disruption of the gastric epithelial layer. Scanning of stomach specimens using an electron microscope revealed that in the rats treated with synthesized derivatives **7a**, **7b**, and **7c** there was no injury in the stomach

mucosa, as illustrated in Figure 2c–e, which was identical to that of the control (Figure 2a).

#### Nitric oxide release study

The ability of the test compounds to release NO was studied by performing an *in vitro* nitric oxide release study. This study involved evaluation of nitric oxide releasing properties of the synthesized compounds in phosphate buffer, pH 7.4, in the

**Table 4.** Nitric oxide releasing property of compounds 7a-7f.

Compound	NO release <sup>a</sup>
7a	0.35%
7b	0.42%
7c	0.39%
7d	0.48%
7e	0.57%
7f	0.32%

<sup>a</sup>Percentage of NO released ( $n = 2$ ) relative to theoretical maximum release of 1 mol NO/mol test compound; determined by Griess reagent in presence of 5 mM L-cysteine, at pH 7.4.

presence of L-cysteine, relative to nitric oxide released from standard sodium nitrite solution (Table 4).

## Conclusions

Six hybrid derivatives were synthesized and screened for analgesic, anti-inflammatory, and ulcerogenic potential, and subjected to nitric oxide releasing studies. Compounds 7a, 7b, and 7c exhibited significant analgesic and anti-inflammatory activity. Compounds 7b and 7c showed strong analgesia in acetic acid induced writhing tests. Among all the synthesized compounds, compound 7c exhibited the most prominent and consistent anti-inflammatory activity. From detailed analysis of the results of histopathological studies, we conclude that the synthesized compounds have not only retained but show an enhanced anti-inflammatory profile, and are devoid of the deadlier gastrointestinal toxicities; also, all the synthesized derivatives exhibit a significant nitric oxide releasing property. Therefore, it can be concluded that the rationale on which the design of these NCEs was based has proved to be superior, compared to currently used NSAIDs. The outcome of the present research work is very promising. The only concern to be addressed now is the pharmacokinetics profile of the designed NCEs, since the molecular weight of all the designed compounds is more than 500 Da.

The most potent derivatives are in the process of chronic toxicity and pharmacokinetics studies, and, based on those results, a further action plan will be finalized.

## Scope of further research

The most potent compounds will be subjected to following studies:

- Acute and chronic toxicity studies.
- Mechanism of release of NO.
- Pharmacokinetics profile.
- Mechanism of action.

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## Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content of this paper.

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