Full Paper

# Trimethyl-4-oxo-4,5,6,7-tetrahydroindazole-1-acetic Acid: A New Lead Compound with Selective COX-2 Inhibitory Activity

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A novel series of 3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazole-1-acetic acid derivatives was designed and synthesized by a new one-step pathway. Structure elucidation of the synthesized compounds was confirmed by various spectral and elemental analyses. The prepared compounds were evaluated for their ability to inhibit cyclooxygenase-2 (COX-2) and cyclooxygenase-1 (COX-1) enzymes *in vitro*. Among the synthesized compounds, the 2-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)acetic acid **4** emerged as the most potent COX-2 inhibitor ($IC_{50}$ value: 150 nM) with the highest selectivity index (COX-1/COX-2 inhibition ratio: 570.6). Docking studies of compound **4** in the active site of COX-2 recognized its potential binding mode to the enzyme. Based on the preliminary results, compound **4** was considered as a lead compound for further optimization.

**Keywords:** Selective COX-2 inhibitors / Structure elucidation / Tetrahydroindazoles

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## Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed therapeutics worldwide, primarily for the treatment of pain and inflammation. They exert their pharmacological effect by inhibiting the cyclooxygenase (COX) enzymes, which catalyze the transformation of arachidonic acid to prostaglandins. It is well established that there are at least two COX isozymes (COX-1 and COX-2). COX-1 is a housekeeping enzyme responsible for providing the physiological maintenance actions (vascular and renal homeostasis, gastroprotection) [1]. On the other hand, COX-2 isozyme is induced by proinflammatory stimuli resulting in inflammatory actions [2]. Thus, selective inhibition of COX-2 is useful for the treatment of inflammations with reduced gastrointestinal toxicities [3, 4].

The pyrazole unit is one of the core structures in a number of anti-inflammatory drugs including selective COX-2 inhibitors such as celecoxib and SC-558 (Fig. 1) [5–7]. Additionally, aryl and heteroaryl acetic acid derivatives [8] have made a significant impact in NSAIDs research including pyrazole acetic acid derivatives that have been previously reported [9].

Tetrahydroindazoles are a member of the fused-pyrazole system that have recently attracted attention in the wide field of medicinal chemistry due to their wide range of biological actions such as anti-inflammatory [10], HSP-90 inhibitory [11], and anticancer [12]. Despite their pharmacological potential, they have not been investigated as COX inhibitors so far.

Herein, we reported the synthesis of novel tetrahydroindazole-1-acetic acid derivatives and the findings of their COX-1/COX-2 inhibitory activities.

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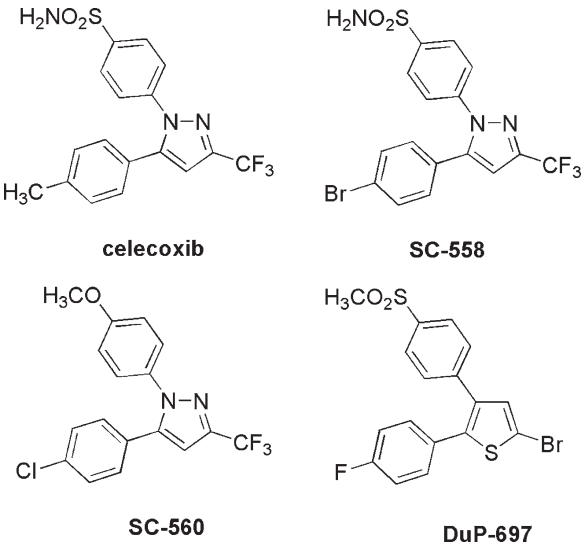



**Figure 1.** Examples of selective COX-2 and COX-1 inhibitors.

## Results and discussion

### Chemistry

Condensation of 2-acyl dimedone with hydrazines is a general method used for the preparation of tetrahydroindazolones [12–14]. In the present investigation, the starting compound, ethyl 2-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)acetate **3** was prepared by reaction of 2-acetyl-dimedone **2** with ethyl hydrazinoacetate (Scheme 1).

From a literature review, the reaction of 2-acetyl-dimedone with the hydrazine derivative is expected to proceed straightforward to give only one product [15]. However some recent reports showed that two possible isomeric structures (Fig. 2, structures A and B) can be formed [16, 17]. To resolve this disagreement, the structure elucidation of compound **3** was studied in detail by NMR analysis. The chemical shift of each proton and carbon was assigned by $^{1}H$, $^{13}C$, COSY, and HMQC.

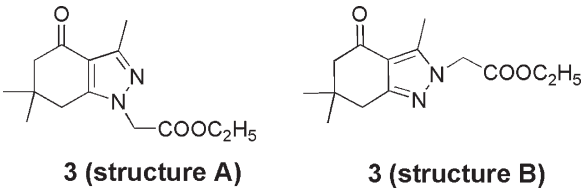


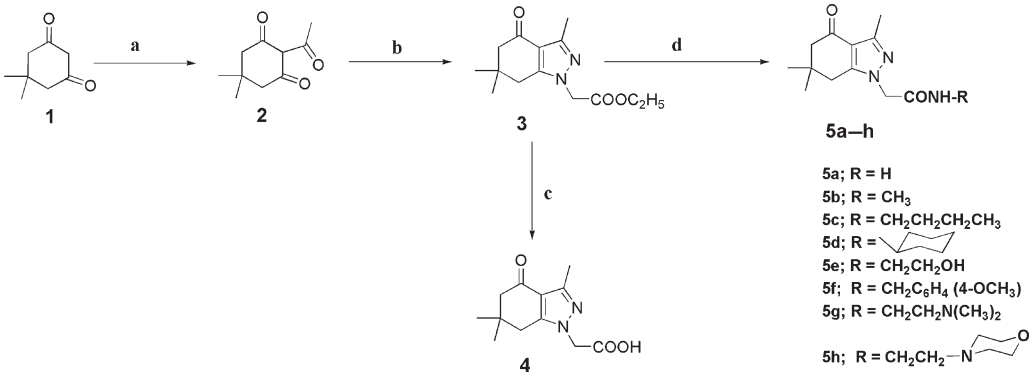

**Figure 2.** Expected isomeric structures of the ester **3**.

The connectivity of key protons and carbons were determined by HMBC (Fig. 3). In the HMBC spectrum, a correlation between the $CH_2$ protons at the acetic acid side chain and the carbon atoms at C-7a and C-7 was found, with no correlations between these $CH_2$ and the carbon at C-3. Other correlations were found in the HMBC spectrum (see Fig. 3 and supplementary data). Based on the above information, the structure of the product was conclusively identified as ethyl 2-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)acetate **3** (structure A, Fig. 2). Upon hydrolysis of the ester **3**, the free acetic acid derivative **4** was obtained, Scheme 1.

Reactions of the ester **3** with appropriate amines at room temperature afforded the *N*-substituted amide derivatives **5a–h** (Scheme 1). The structure elucidation of **5a–h** was confirmed by spectroscopic (IR, $^{1}H$-NMR, and $^{13}C$-NMR) as well as elemental analyses data.

### Cyclooxygenase inhibitory activity

Compounds **3**, **4**, and **5a–h** were tested for their ability to inhibit COX-1 and COX-2 using ovine COX-1 and recombinant human COX-2 by a COX fluorescence inhibitor assay *in vitro* (catalog number 700100, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's assay protocol. SC-560 and DuP-697 (Fig. 1) were used as reference drugs for selective COX-1 and COX-2 inhibition, respectively. Test compounds and reference drugs were tested at different concentrations and the $IC_{50}$'s were then determined.




**Scheme 1.** Synthesis of the target compounds **3**, **4** and **5a–h**. Reagents and conditions: (a) $(CH_3CO)_2O$, $Et_3N$, DCM (b) $NH_2NHCH_2COOC_2H_5 \cdot HCl$, $Et_3N$, EtOH; (c) NaOH solution (10% w/v), reflux 30 min; (d) $NH_2$–R, EtOH.

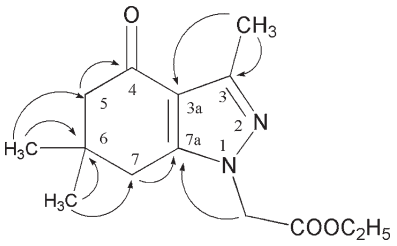
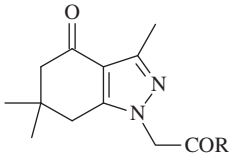



**Figure 3.** HMBC correlations of the ester **3** (structure A).

The results (Table 1) show that the presence of the acetic acid side chain is critically important. Having a free carboxyl group as the side chain, compound **4** showed strong COX-2 inhibition ($IC_{50}$ value: 0.15 μM) with the highest selectivity index (COX-1/COX-2 inhibition ratio: 570.6). Interestingly, esterification of the free carboxyl group resulted in complete loss of both COX-1 and COX-2 activity.

Replacement of the free carboxyl group by the amides generally resulted in slightly reduced COX-1 inhibitory activity and noticeably reduced COX-2 activity. The primary amide (compound **5a**) showed the best COX-2 inhibitory activity among this series ($IC_{50}$ value: 0.86 μM) with good selectivity index (COX-1/COX-2 inhibition ratio: 80.5). Increasing the bulkiness of the *N*-substituent or elongation of the side chain of the amides (**5b**–**h**) generally reduce COX-2 inhibitory activity.

**Table 1.** Primary *in vitro* COX-1 and COX-2 inhibition assay results.




| Compd. no. | R | $IC_{50}$ (μM) | | SI[a)] |
|---|---|---|---|---|
| | | COX-1 | COX-2 | |
| **3** | $OC_2H_5$ | >100 | >100 | – |
| **4** | OH | 85.6 | 0.15 | 570.6 |
| **5a** | $NH_2$ | 69.2 | 0.86 | 80.5 |
| **5b** | $NHCH_3$ | 74.1 | 32.4 | 2.3 |
| **5c** | $NH(CH_2)_3CH_3$ | >100 | >100 | – |
| **5d** | $NH–C_6H_{11}$ | >100 | 76.0 | >1.3 |
| **5e** | $NH(CH_2)_2OH$ | >100 | 52.7 | >1.9 |
| **5f** | $NHCH_2C_6H_4(4\text{-}OCH_3)$ | >100 | >100 | – |
| **5g** | $NH(CH_2)_2N(CH_3)_2$ | >100 | 85.6 | >1.2 |
| **5h** | $NH(CH_2)_2$morpholine | >100 | >100 | – |
| SC-560 | – | 0.0085 | Nd[b)] | – |
| DuP-697 | – | Nd | 0.0416 | – |

[a)] SI, selectivity index calculated as the $IC_{50}$ COX-1/$IC_{50}$ COX-2.
[b)] Nd, not determined.
SC-560 was used as selective COX-1 inhibitor while DuP-697 was used as a selective COX-2 inhibitor.

From the biological activity studies, several conclusions can be deduced: (i) Derivatization of the acetic acid side chain is critically important for activity. (ii) Compounds carrying the free carboxyl group (compound **4**) or the primary amide group (compound **5a**) showed superior COX-2 inhibitory activity than the *N*-substituted amide derivatives **5b**–**e**. (iii) Amides having smaller *N*-substituents showed better activities than bulky groups. This short SAR study supports that compound **4** is highly potent with good selective COX-2 inhibitory activity deserving further biological evaluation.

## Docking studies

Molecular docking studies of compound **4** were performed in order to rationalize the obtained biological results and understand its likely interactions with the COX-2 enzyme active site (Fig. 4).

Docking studies were performed by MOE (Molecular Operating Environment) [18] using murine COX-2 co-crystallized with SC-558 (PDB ID: 1CX2) as a template. We performed 100 docking iterations for the ligand in the enzyme active site and the top scoring configuration of the ligand–enzyme complexes was selected on energetic grounds. This molecular modeling of compound **4** showed that the acetic acid side chain ($CH_2COOH$) was oriented within the COX-2 secondary pocket in a region comprising His90, Gln192, Arg513, Val523, and Leu352. The N-2 atom of the compound **4** formed a strong hydrogen bond interaction with the NH group of His90 (distance = 1.97 Å). Furthermore, the carbonyl group at C-4 formed a hydrogen bond interaction with the Arg513 (distance = 2.69 Å). Finally a hydrogen bond interaction between the carboxyl OH and the carbonyl group of the backbone Phe518 was also observed (distance = 3.58 Å). The C-6 dimethyl moiety was located near Val523 forming hydrophobic interactions (distance = 2.33 Å).

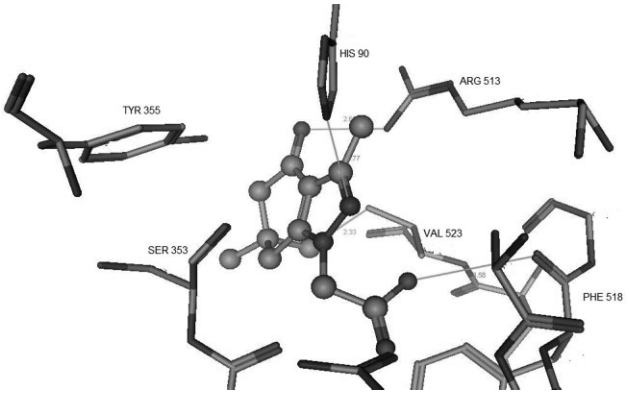



**Figure 4.** A view of the energy-minimized structure of the complex of **4** bound to COX-2, viewed using the Molecular Operating Environment (MOE) program.

The above observations show that compound **4** is strongly bound to the COX-2 active site, the same as that resulting from the selective COX-2 inhibitor, SC-558 [19]. Consequently, it provides guidelines to establish the SAR of this class of compounds and to correlate COX-inhibitory activities. Thus, short and hydrogen donor substituents at N-1 are essential for activity while increasing steric bulkiness may reduce the activity.

Therefore, molecular docking experiments will guide the design and synthesis of new analogues with anticipated good COX-2 inhibitory activities such as (i) the hydroxamate and sulfonate analogues of the acid **4**, (ii) acetic acid analogues with modifications in the tetrahydroindazole C-3, (iii) other derivatives with short chain at the N-1 nitrogen.

## Conclusions

In conclusion, based on this preliminary results, we identified **4** as a lead compound for the novel class of trimethyl-4-oxo-tetrahydroindazole-1-derivatives as selective COX-2 inhibitors. A docking pose of the energy-minimized structure of the complex of **4** bound to the COX-2 showed a tight binding mode and provided a guide for further intensive SAR modifications to be performed and comprehensively evaluated as COX-2 inhibitors.

## Experimental

Melting points were determined using an electrothermal apparatus (Stuart Scientific, England) and were uncorrected. IR spectra were recorded as KBr disks using a Shimadzu IR 200-91527 spectrophotometer (Shimadzu Corp., Kyoto, Japan) and the data are given in $\upsilon_{max}$ ($cm^{-1}$). $^1$H-NMR (400 MHz) spectra were carried out on a Bruker 400 MHz, (Varian, Palo Alto, CA, USA) and the chemical shifts are given in $\delta$ (ppm). Mass spectra were performed on a Jeol, JMS-600 spectrometer at an ionization voltage of 70 eV (Jeol, Tokyo, Japan). Elemental analyses were performed on an ''Analytischer Funktionstest vario EL Fab.-Nr. 11982027'' (Germany). All reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 $GF_{245}$ precoated sheets $20 \times 20$ $cm^2$, layer thickness 0.2 mm (E-Merck, Germany) and were visualized by UV-lamp at a wavelength ($\lambda$) of 254 nm. All chemicals and solvents were of reagent grade, and the latter were distilled and dried before use.

### *Ethyl 2-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)acetate **3***

Equimolar amounts of ethyl hydrazinoacetate HCl, 2-acetyldimedone **2** and triethylamine were mixed in ethanol; then the mixture was refluxed for 4 h. The reaction mixture was cooled and the solvent was evaporated under reduced pressure. The residue was dissolved in ether, washed with water and brine, dried over anhydrous $MgSO_4$, then filtered off. The filtrate was concentrated under reduced pressure to an oily material that was purified with a small silica column using 6% $CH_3OH/CH_2Cl_2$ to give a yellow oily product that solidified on standing. Yield (80%), mp 64–65°C. IR (KBr, $cm^{-1}$): 3530, 3410, 2950, 1740, 1647, 1180. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.05 (s, 6H, $2CH_3$), 1.21 (t, $J = 7.2$ Hz, 3H, $OCH_2CH_3$), 2.26 (s, 2H, $CH_2$), 2.37 (s, 3H, $CH_3$), 2.52 (s, 2H, $CH_2$), 4.16 (q, $J = 7.2$ Hz, 2H, $OCH_2CH_3$), 4.71 (s, 2H, $-NCH_2CO$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 13.28 ($CH_3$ ester), 14.08 ($CH_3$ on C-3), 28.44 $(CH_3)_2$, 35.18 (C-7), 50.24 (C-5), 52.25 (C-6), 53.48 ($NCH_2-$), 62.13 ($CH_2$ ester), 116.05 (C-3), 149.08 (C-3a), 150.63 (C-7a), 167.09 (CO ester), 193.12 (C-4). Anal. calcd for $C_{14}H_{20}N_2O_3$; C, 63.62; H, 7.63; N, 10.60. Found: C, 63.70; H, 8.00; N, 10.62.

### *2-(3,6,6-Trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)-acetic acid **4***

A mixture of the ester **3** (4 mmol) and NaOH solution (10% w/v, 5 mL) was refluxed for 30 min. The reaction mixture was cooled to room temperature, and washed with ether, acidified with dilute HCl solution, extracted with ether (20 mL × 2), dried over anhydrous $MgSO_4$, and filtered. The solvent was evaporated under reduced pressure, and the residue was crystallized from ether. Yield 25% as white powder, mp 191–193°C. IR (KBr, $cm^{-1}$): 3430, 2910, 1730, 1654. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.05 (s, 6H, $2CH_3$), 2.29 (s, 2H, $CH_2$), 2.38 (s, 3H, $CH_3$), 2.54 (s, 2H, $CH_2$), 4.82 (s, 2H, $-NCH_2CO$), 8.29 (bs. 1H, COOH). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 12.85, 28.46, 34.97, 35.62, 49.47, 52.17, 115.81, 149.38, 150.93, 169.06, 193.38. MS (EI): $m/z$ 235.96 [74.9%, $M^+$], ESI positive ion mode $m/z$ 237 [$M^+$+1], $m/z$ 259 [$M^+$+Na].

### *General procedure for synthesis of 2-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)acetamides **5a–h***

The appropriate amine (2 mL) was added to a solution of the ester **3** (0.05 mmol) in ethanol (2 mL) with constant stirring at room temperature. The mixture was stirred at this temperature until completion of reaction (about 24–48 h as monitored by TLC). The mixture was then evaporated under vacuum, extracted by dichloromethane, washed with water and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, evaporated till dryness. The solid materials obtained after drying in a vacuum desiccator was crystallized from ethanol.

### *2-(3,6,6-Trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)-acetamide **5a***

As reported in Ref. [17].

### *N-Methyl-2-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)acetamide **5b***

Yield 78% as pale yellow powder; mp 130–132°C. IR (KBr, $cm^{-1}$): 3270, 2925, 1665, 1645. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.05 (s, 6H, $2CH_3$), 2.27 (s, 2H, $CH_2$), 2.41 (s, 3H, $CH_3$), 2.56 (s, 2H, $CH_2$), 2.75 (d, $J = 4.8$ Hz, 3H, $-NHCH_3$), 4.58 (s, 2H, $-NCH_2CO$), 6.21 (bs, 1H, CONH). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 13.38, 26.35, 28.48, 35.21, 35.59, 51.87, 52.30, 116.11, 150.26, 150.61, 166.96, 193.08. Anal. calcd for $C_{13}H_{19}N_3O_2$; C, 62.63; H, 7.68; N, 16.85. Found: C, 62.59; H, 8.00; N, 16.76.

### *N-Butyl-2-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)acetamide **5c***

Yield 90% as yellow powder; mp 80–82°C. IR (KBr, $cm^{-1}$): 3240, 2925, 1667, 1645. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.82 (t, $J = 7.3$ Hz, 3H, $-CH_2CH_2CH_2CH_3$), 1.05 (s, 6H, $2CH_3$), 1.20 (m, 2H, $CH_2$), 1.35 (m, 2H, $CH_2$), 2.28 (s, 2H, $CH_2$ of indazole), 2.41 (s, 3H, $CH_3$), 2.56

(s, 2H, $CH_2$ of indazole), 3.17 (q, $J = 7.1$ Hz, 2H, −NH*CH*$_2$), 4.57 (s, 2H, −N*CH*$_2$CO), 6.08 (s, 1H, CONH). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 12.29, 12.62, 18.90, 27.43, 30.31, 34.17, 34.55, 38.31, 51.00, 51.27, 115.05, 149.11, 149.56, 165.19, 192.07. Anal. calcd for $C_{16}H_{25}N_3O_2$; C, 65.95; H, 8.65; N, 14.42. Found: C, 65.75; H, 8.91; N, 14.37.

### *N-Cyclohexyl-2-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)acetamide* ***5d***

Yield 50% as yellow powder; mp 118–120°C. IR (KBr, cm$^{-1}$): 3265, 2910, 1658, 1644. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.05 (s, 6H, 2$CH_3$), 1.28 (m, 3H, cyclohexyl), 1.55 (m, 4H, cyclohexyl), 1.75 (m, 3H, cyclohexyl), 2.28 (s, 2H, $CH_2$ of indazole), 2.41 (s, 3H, $CH_3$), 2.56 (s, 2H, $CH_2$ of indazole), 3.70 (m, 1H, −NH*CH* of cyclohexyl), 4.55 (s, 2H, −*NCH*$_2$CO), 5.91 (d, $J = 7.4$ Hz, 1H, CONH). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 13.33, 15.30, 24.52, 24.98, 25.32, 28.48, 32.70, 33.15, 35.24, 35.63, 48.40, 52.33, 65.88, 116.13, 150.08, 150.58, 165.35, 193.14. Anal. calcd for $C_{18}H_{27}N_3O_2$; C, 68.11; H, 8.57; N, 13.24. Found: C, 67.91; H, 8.80; N, 12.99.

### *N-(2-Hydroxyethyl)-2-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)acetamide* ***5e***

Yield 60% as pale yellow powder; mp 110–111°C. IR (KBr, cm$^{-1}$): 3350, 3260, 2920, 1655. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.06 (s, 6H, 2$CH_3$), 2.29 (s, 2H, $CH_2$), 2.42 (s, 3H, $CH_3$), 2.60 (s, 2H, $CH_2$), 2.75 (br s, 1H, OH) 3.37 (q, $J = 5.6$ Hz, 2H, −NH*CH*$_2$), 3.64 (t, $J = 5.1$ Hz, 2H, −*CH*$_2$OH), 4.66 (s, 2H, −N*CH*$_2$CO), 6.82 (s, 1H, CONH). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 12.16, 27.43, 34.13, 34.56, 41.31, 50.77, 51.25, 60.47, 116.00, 149.00, 149.99, 165.89, 191.99. Anal. calcd for $C_{14}H_{21}N_3O_3$; C, 60.20; H, 7.58; N, 15.04. Found: C, 59.90; H, 7.92; N, 14.74.

### *N-(4-Methoxybenzyl)-2-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)acetamide* ***5f***

Yield 70% as yellow powder; mp 114–116°C. IR (KBr, cm$^{-1}$): 3230, 2920, 1673, 1649, 1250. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.00 (s, 6H, 2$CH_3$), 2.25 (s, 2H, $CH_2$), 2.37 (s, 3H, $CH_3$), 2.54 (s, 2H, $CH_2$), 3.71 (s, 3H, −$OCH_3$), 4.29 (d, $J = 5.8$ Hz, 2H, −NH*CH*$_2$), 4.61 (s, 2H, −N*CH*$_2$CO), 6.41 (s, 1H, CONH), 6.61 (d, $J = 8.7$ Hz, 2H, Ar–H), 7.05 (d, $J = 8.6$ Hz, 2H, Ar–H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 13.18, 28.42, 35.19, 35.54, 43.07, 52.05, 52.29, 55.30, 114.13, 116.14, 128.93, 129.45, 149.98, 150.71, 159.16, 165.97, 192.93. Anal. calcd for $C_{20}H_{25}N_3O_3$; C, 67.58; H, 7.09; N, 11.82. Found: C, 67.32; H, 7.16; N, 11.54.

### *N-(2-(Dimethylamino)ethyl)-2-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)acetamide* ***5g***

Yield 92% as yellow powder; mp 88–90°C. IR (KBr, cm$^{-1}$): 3390, 3266, 2930, 1678, 1646. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.05 (s, 6H, 2$CH_3$), 2.14 (s, 6H, N($CH_3$)$_2$), 2.27 (s, 2H, $CH_2$), 2.34 (t, $J = 6.0$ Hz, 2H, −*CH*$_2$N($CH_3$)$_2$) 2.40 (s, 3H, $CH_3$), 2.57 (s, 2H, $CH_2$), 3.26 (q, $J = 5.6$ Hz, 2H, −CONH*CH*$_2$), 4.60 (s, 2H, −N*CH*$_2$CO), 6.64 (s, 1H, CONH). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 13.75, 28.91, 35.71, 36.08, 37.16, 45.37, 52.48, 52.80, 57.64, 116.51, 150.35, 151.15, 166.78, 193.65. Anal. calcd for $C_{16}H_{26}N_4O_2$; N, 18.29. Found: N, 17.95.

### *N-(2-Morpholinoethyl)-2-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydroindazol-1-yl)acetamide* ***5h***

Yield 92% as yellow powder; mp 130–131°C. IR (KBr, cm$^{-1}$): 3405, 3240, 2920, 1669, 1637. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.05 (s, 6H, 2$CH_3$), 2.28 (s, 2H, $CH_2$), 2.35 (t, $J = 4.0$ Hz, 4H, −N($CH_2$)$_2$ of morpholine), 2.39 (t, $J = 6.0$ Hz, 2H, −*CH*$_2$N of morpholine) 2.43 (s, 3H, $CH_3$), 2.57 (s, 2H, $CH_2$), 3.29 (q, $J = 5.7$ Hz, 2H, −CONH*CH*$_2$), 3.58 (t, $J = 4.4$ Hz, 4H, −O($CH_2$)$_2$ of morpholine), 4.60 (s, 2H, −N*CH*$_2$CO), 6.64 (s, 1H, CONH). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 13.49, 28.43, 35.24, 35.53, 35.64, 51.99, 52.31, 53.09, 56.30, 66.71, 116.08, 149.95, 150.55, 166.39, 192.98. Anal. calcd for $C_{18}H_{28}N_4O_3$; C, 62.05; H, 8.10; N, 16.08. Found: C, 61.67; H, 7.92; N, 15.81.

## Cyclooxygenase inhibition assay

*In vitro* cyclooxygenase (COX) inhibition assay: The ability of compounds **3**, **4**, and **5a–h** to inhibit ovine COX-1 and recombinant human COX-2 was determined using a COX fluorescence inhibitor assay (catalog number 700100, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's assay protocol. SC-560 and DuP-697 were used as reference drugs for selective COX-1 and COX-2 inhibitors, respectively. Compounds were assayed in concentrations ranging from 100 to 0.01 μM; then the $IC_{50}$ values were calculated.

## Docking studies

All the molecular modeling studies were carried out on an Intel Core i3 processor, 3 GB memory with Windows 7 operating system using Molecular Operating Environment (MOE 2008, Chemical Computing Group, Canada) as the computational software. All the minimizations were performed with MOE until a RMSD gradient of 0.05 kcal mol$^{-1}$ Å$^{-1}$ with MMFF94X force-field and the partial charges were automatically calculated.

The X-ray crystallographic structure of murine COX-2 complexed with SC-558 (PDB ID: 1CX2) was obtained from the protein data bank. The enzyme was prepared for docking studies where: (i) Ligand molecule was removed from the enzyme active site. (ii) Hydrogen atoms were added to the structure with their standard geometry. (iii) MOE Alpha Site Finder was used for the active sites search in the enzyme structure and dummy atoms were created from the obtained alpha spheres. (iv) The obtained model was then used in predicting the ligand–enzyme interactions at the active site.

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