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# 1-Toluene-sulfonyl-3-[(3′-hydroxy-5′-substituted)-γ-butyrolactone]-indoles: Synthesis, COX-2 inhibition and anti-cancer activities

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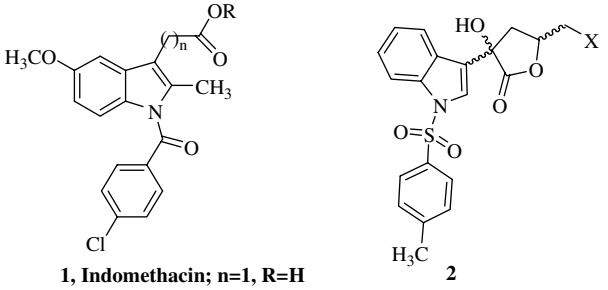
**Abstract**—Indoles carrying a cyclic ester (γ-butyrolactone) at C-3 position have been synthesized by the allylation of 3-indoleglyoxylate followed by iodocyclisation and the nucleophilic replacement of the iodo-group. Screening of these molecules for COX-2 inhibition and anti-cancer activities has identified compounds **10** and **11** as highly potent and selective for COX-2 as well as showing remarkable anti-cancer activities (better than that of indomethacin).


The critical role of indole moiety in indole acetic acid[1] (a plant growth regulator hormone), in tryptophan[1] (an amino acid) and as a part of a number of alkaloids[2] has subjected it to several derivatisations for procuring medicinally important molecules. The development of indomethacin[3] (**1**) as a non-steroidal anti-inflammatory drug (NSAID) (non-selective COX-2 inhibitor) has been followed by the modifications in the different parts of the molecule, especially in the C-3 substituent, for increasing its efficacy[4] as well as for transforming it into selective COX-2 inhibitors (**1**, $n = 2$, 3, branched alkyl groups, R = $NH_2$, alkyl).[5]

In our ongoing research programme for developing COX-2 inhibitors, it has been planned to introduce a γ-butyrolactone at C-3 and a tolylsulfonyl group at N-1 of indole (**2**). The γ-butyrolactone moiety (cyclic ester) mimics the furanone moiety present in rofecoxib[6] (a selective COX-2 inhibitor), while tolylsulfonyl is a key pharmacophore in most of the COX-2 inhibitors. The rationally designed molecules (**2**) have been synthesized and evaluated for their COX-1, COX-2 inhibitory activities and also screened for anti-cancer properties over 59 human tumour cell lines (Fig. 1).

The synthesis of the target molecules has been achieved by the indium mediated diastereoselective allylation of 3-indoleglyoxylate, followed by diastereoselective iodocyclisation[7] and nucleophilic replacement of the iodo-group. Commercially available 3-indoleglyoxylate (**3**) was *N*-alkylated by reaction with *p*-toluene-sulfonyl chloride to get indole substituted α-keto ester **4** (Scheme 1). A solution of **4**, allyl bromide and indium metal (suspension) (1:1.5:1) in THF–$H_2O$ (2:1) on stirring at 0 °C for 6–8 h, after usual workup and chromatography, provided compound **5** (87%) as a thick liquid [$M^+$ *m*/*z* 399] (Scheme 1). The participation of Cram's chelation model in this reaction explains the diastereoselective formation of compound **5**.[7]

Stirring of solution of **5** in $C_2H_5OH$–THF (2:1) with 2% NaOH for 2 h, after acidification and extraction with




**1, Indomethacin; n=1, R=H** **2**

**Figure 1.**

*Keywords*: Indole; Butyrolactone; Synthesis; COX-2 inhibition; Dockings; Anti-cancer activities.
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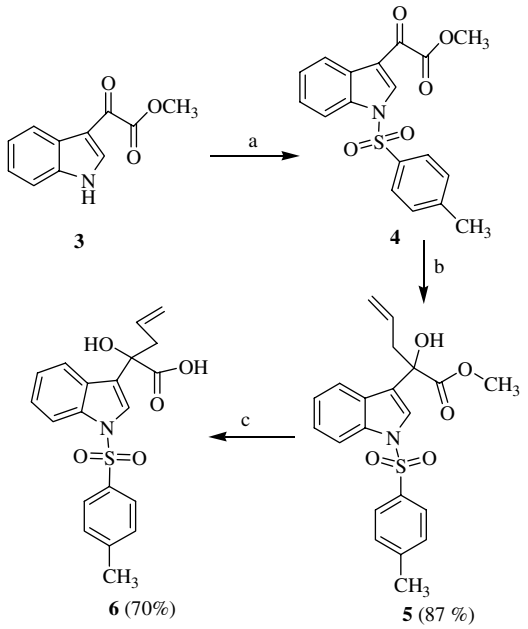
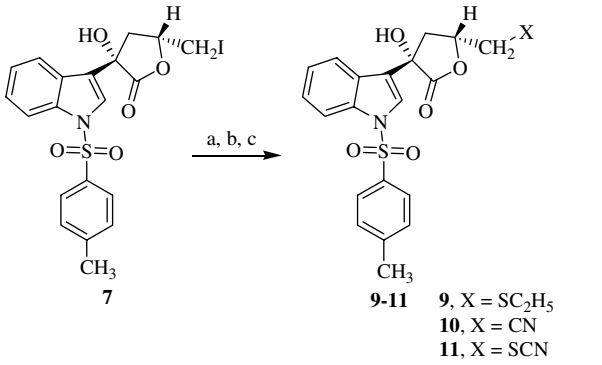


**Scheme 1.** Reagents and conditions: (a) *p*-tolunenesulfonyl chloride, NaH, $CH_3CN$; (b) allyl bromide, In, THF/$H_2O$ (2:1), 0 °C, stir; (c) ethanol–THF, 2% NaOH, stir.

ethyl acetate, gave the corresponding acid **6** (70%) [$M^+-OH$ *m/z* 368] (Scheme 1). Compound **6** on treatment with $I_2$ (3 equiv) and $NaHCO_3$ (3 equiv) in dry $CH_3CN$ for 3–4 h after usual workup gave a mixture of two diastereomers in the ratio 3:1 ($^1H$ NMR integration) (84%) (Scheme 2).[8] In higher $R_f$ component **7**; 61%, mp 130 °C; the observation of NOE between C-5′H and aromatic protons and no NOE between $CH_2I$ unit and aromatic protons indicates that C-5′H and indole moiety are placed on the same face of furanone and helps in assigning stereochemistry 3′S*, 5′R* at the two chiral centers. In case of lower $R_f$ component **8**, 18%, NOE has been observed at ArHs when $CH_2I$ protons were irradiated and accordingly stereochemistry 3′S*, 5′S* has been assigned at the two chiral centres. Compound **6** also underwent iodocyclisation with $I_2$ in the presence of silica gel (for TLC) in ethyl acetate-methanol to give a mixture of **7** and **8** (85%) in the diastereomeric ratio 3:1.

Based upon the previous results,[9] docking studies and the literature reports,[10] it was planned to introduce the

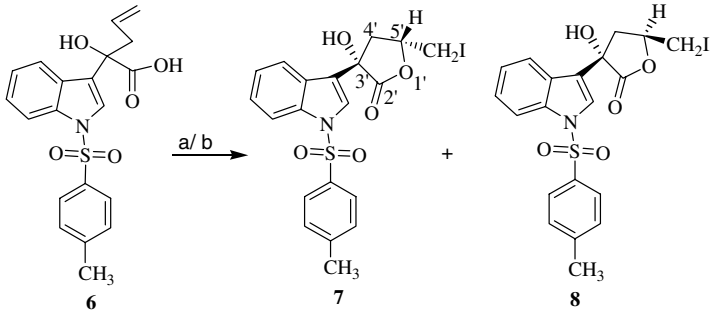


**Scheme 3.** Reagents and conditions: (a) ethanthiol, $CH_3CN$, $K_2CO_3$, stir, rt (for **9**); (b) NaCN, $CH_3CN$, stir, rt (for **10**); (c) KSCN, $CH_3CN$, stir, rt (for **11**).

groups like $CH_2SC_2H_5$, $CH_2SCN$, and $CH_2CN$ at C-5′ of butyrolactone ring. Nucleophilic substitutions of **7** with ethanthiol, NaCN and KSCN in $CH_3CN$ gave compounds **9**, **10** and **11** possessing, respectively, $SC_2H_5$, CN and SCN groups in place of iodine in **7** (Scheme 3).

Compounds **7**, **9**, **10** and **11** have been evaluated for in-vitro COX-1, COX-2 inhibitory activities using COX (ovine) inhibitor screening assay kit (Cayman Chemicals, cat. No. 560101). The results of these investigations are given in Table 1. Out of these four compounds, **10** and **11** with, respectively, $CH_2CN$ and $CH_2SCN$ groups at C-5′ show the best results with 90% and 85% inhibition of COX-2 at $10^{-8}$ M concentration and $IC_{50}$ values <0.01 μM. 60 and 65% COX-1 inhibitions at $10^{-5}$ M concentrations have been observed for compounds **10** and **11**, respectively. Significantly, the results of these two compounds are better than that of indomethacin and rofecoxib. The COX-2 inhibition by compound **9** is also considerable (65% at $10^{-8}$ M) while compound **7** exhibits poor inhibition. The results of these investigations indicate that along with other components, the C-5′ substituent significantly controls the COX-2 inhibitory activities of these compounds.

In order to investigate the interactions of these compounds in the active site of COX-2 and to rationalize the role of C-5′ substituent towards COX-2 inhibition, we carried out the dockings of these molecules in the



**Scheme 2.** Reagents and conditions: (a) $I_2$, $NaHCO_3$, $CH_3CN$, 0 °C, stir; (b) $I_2$, silica, ethyl acetate-methanol, stir, rt.

**Table 1.** In-vitro COX-2 inhibitory activities of indole derivatives

| Compound | % Inhibition | | | | | $IC_{50}$ (μM) | | COX-2 selectivity* |
|---|---|---|---|---|---|---|---|---|
| | COX-2 | | | | COX-1 | COX-2 | COX-1 | |
| | $10^{-5}$ M | $10^{-6}$ M | $10^{-7}$ M | $10^{-8}$ M | $10^{-5}$ M | | | |
| **7** | 30 | 32 | 35 | 44 | 40 | >10 | >10 | |
| **9** | 65 | 62 | 67 | 75 | 35 | <0.001 | >10 | <1000 |
| **10** | 90 | 87 | 88 | 95 | 60 | <0.001 | <10 | >1000 |
| **11** | 85 | 90 | 89 | 92 | 65 | <0.001 | <10 | >1000 |
| Indomethacin[5d] | | | | 97 | 100 | 0.75 | 0.05 | 0.067 |
| Rofecoxib | | | 75 | 100 | 75 | 0.3 | 40 | ~133 |

* COX-2 selectivity = $IC_{50}$ (COX-1)/$IC_{50}$ (COX-2).

active site of COX-2.[11] The crystal structure of COX-2 with indomethacin shows the interactions of the carboxyl group of indomethacin with the guanidine moiety of R120, amino acid active during the turnover phase of the enzyme. Figure 2 validates our programme of docking, where indomethacin docked in the active site of COX-2 nearly overlaps with the one present in the crystal structure of the enzyme (rms deviation 0.88).

Docking of compound **10** in the active site of COX-2 indicates that this molecule fits in the COX-2 active site in the same fashion as indomethacin. In compound **10**, the oxygen of C-3′ OH group is present at a distance of 2.10 Å from the H of guanidine moiety of R120 and interacts through H-bond formation while the CN group present at C-5′ position approaches the guanidine moiety at a distance of 2.43 Å (Fig. 3). The aromatic part of the tosyl group present at N-1 of compound **10** fits in the hydrophobic pocket formed by F518, W387 and Y385 residues. While similar types of interactions are observed during the docking of compound **11** in the active site of COX-2, compounds **7** and **9** are devoid of such interactions.

The role of COX-2 in the propagation of cancer[12] and the evaluations of several COX-2 inhibitors for reducing cancer propagation led us to investigate the present

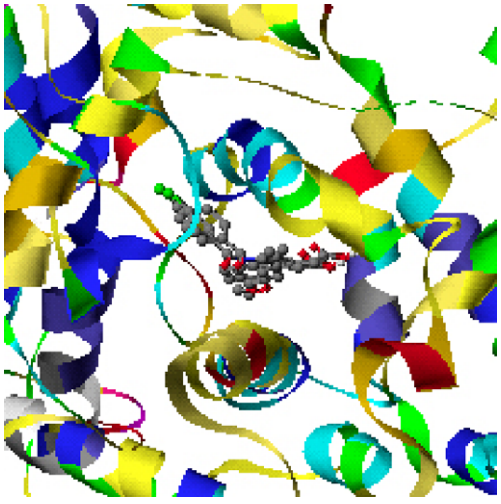


**Figure 2.** Indomethacin docked in the active site of COX-2 exactly overlaps with the one present in the crystal structure of the enzyme.

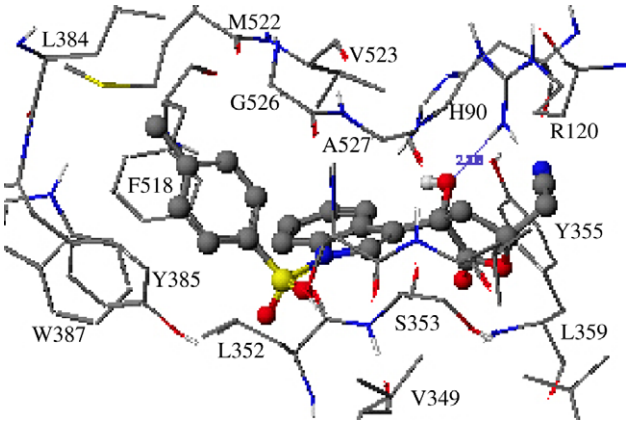



**Figure 3.** Compound **10** docked in the active site of COX-2. OH and CN groups present on the lactone ring approach R120 at a distance of 2.1 and 2.43 Å, respectively. Hs are omitted for clarity.

compounds for their anti-cancer activities. The screening for anti-cancer properties was performed on 59 human tumour cell lines at NIH, Bethesda, USA, using the standard procedure.[13] Out of the four compounds (**7**, **9**–**11**), compounds **10** and **11** with $CH_2CN$ and $CH_2SCN$ group at C-5′ exhibit remarkable anti-cancer activities with average $GI_{50}$ over all the cell lines as 1.9 and 9.1 μM, respectively, which is much better than the average $GI_{50}$ value of indomethacin (64.3 μM)[14] (Table 2). Compound **10** is highly specific for all the cell lines of leukaemia, MALME-3M and M14 of melanoma, RXF393 of renal cancer, PC-3 of prostate cancer and MCF7, MDA-MB-435 of breast cancer where it exhibits $GI_{50}$ in sub-micromolar concentration (0.1–0.8 μM). Moreover, the relatively high $LC_{50}$ values of **10** and **11** show their non-toxicities. However, compounds **7** and **9**, investigated at $10^{-5}$ M concentration only, do not show much anti-cancer activity. Therefore, in parallel with the COX-2 inhibitory activities, compounds **10** and **11** are also highly effective towards various cancer cell lines of human tumour cell panels.

**Table 2.** Anti-cancer activities of compounds **10** and **11** (average $GI_{50}$)

| Compound | $GI_{50}$ (μM) | $LC_{50}$ (μM) |
|---|---|---|
| **10** | 1.9 | 95 |
| **11** | 9.1 | 74 |
| Indomethacin | 64.3 | 100 |

**Table 3.** Lipinski values for compounds **7**, **9**–**11**

| Compound | log *P* | TPSA (Å$^2$) | nON | nOHNH |
|---|---|---|---|---|
| **7** | 2.49 | 85.6 | 6 | 1 |
| **9** | 2.38 | 85.6 | 6 | 1 |
| **10** | 0.94 | 109.4 | 7 | 1 |
| **11** | 1.78 | 109.4 | 7 | 1 |

Calculations of Lipinski values of these compounds (Table 3)[15] indicate a difference in log *P* and Total Polar Surface Area (TPSA) of compounds **10** and **11** from **7** and **9** which might be contributing towards the difference in the bioactivities of these compounds.

In conclusion, we have constructed a γ-lactone moiety at C-3 of indole by the allylation of 3-indoleglyoxylate followed by iodocyclisation. Replacement of iodo-group with nucleophiles like CN, SCN, $SC_2H_5$ and their investigations for COX-2 inhibition have identified compounds **10** and **11** as highly potent and selective for COX-2. Along with COX-2 inhibition, the remarkable anti-cancer activities of **10** and **11** in comparison to indomethacin enable them to be used as leads for further investigations and also support the design of these molecules.

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8. Selected data for compound **7**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 2.36 (s, 3H, $CH_3$), 2.53 (dd, 1H, $^2J$ = 13.2 Hz, $^3J$ = 9 Hz, 1H of 4′-H), 3.04 (dd, 1H, $^2J$ = 13.2 Hz, $^3J$ = 5.4 Hz, 1H of 4′-H), 3.14 (br s 1H, OH, exchanges with $D_2O$), 3.37 (dd, 1H, $^2J$ = 10.2 Hz, $^3J$ = 7.5 Hz, 1H of $CH_2I$), 3.48 (dd, 1H, $^2J$ = 10.2 Hz, $^3J$ = 5.1 Hz, 1H of $CH_2I$), 4.29–4.38 (m, 1H, 5′-H), 7.25–7.32 (m, 3H, ArH), 7.39 (t, 1H, *J* = 7.5 Hz, ArH), 7.53 (s, 1H, ArH), 7.78 (t, 3H, *J* = 8.1 Hz, ArH), 8.01 (d, 1H, *J* = 8.7 Hz, ArH). The multiple signals at $\delta$ 4.29–4.38 on decoupling converted all the four double doublets into doublets. MS (FAB) 511 [$M^+$].
Compound **8**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.60 (br s, 1H, OH, exchanges with $D_2O$), 2.32 (dd, 1H, $^2J$ = 14.1 Hz, $^3J$ = 8.1 Hz, 1H of 4′-H), 2.36 (s, 3H, $CH_3$), 3.04 (dd, 1H, $^2J$ = 14.1 Hz, 1H of 4′-H), 3.26 (dd, 1H, $^2J$ = 10.5 Hz, $^3J$ = 7.5 Hz, 1H of $CH_2I$), 3.41 (dd, 1H, $^2J$ = 10.5 Hz, $^3J$ = 4.5 Hz, 1H of $CH_2I$), 4.77–4.86 (m, 1H, 5′-H), 7.24–7.30 (m, 3H, ArH), 7.38 (d, 1H, *J* = 8.4 Hz), 7.65 (d, 1H, *J* = 7.8 Hz, ArH), 7.76 (s, 1H, ArH), 7.81 (d, 2H, *J* = 8.4 Hz, ArH), 8.02 (d, 1H, *J* = 8.1 Hz, ArH). MS (FAB) 511 ($M^+$).
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11. Crystal structure of COX-2 with indomethacin in the active site was downloaded from protein data bank (pdb

ID 4COX) and refined as per the usual procedure. The dockings were carried out using 'Dock in active site' module of BioMed CaChe 7.0.5.85.
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15. Lipinski values were calculated using molinspiration.