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N-1 and *C*-3 substituted indole Schiff bases as selective COX-2 inhibitors: Synthesis and biological evaluation

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ABSTRACT

A group of *N*-1 and *C*-3 disubstituted-indole Schiff bases bearing an indole *N*-1 (R′ = H, CH_2Ph, COPh) substituent in conjunction with a *C*-3 –CH=N–C_6H_4–4-X (X = F, Me, CF_3, Cl) substituent were synthesized and evaluated as inhibitors of cyclooxygenase (COX) isozymes (COX-1/COX-2). Within this group of Schiff bases, compounds **15** ($R^1 = CH_2Ph$, X = F), **17** ($R^1 = CH_2Ph$, X = CF_3), **18** (R^1 = COPh, X = F) and **20** (R^1 = COPh, X = CF_3) were identified as effective and selective COX-2 inhibitors (COX-2 IC_{50}'s = 0.32–0.84 μM range; COX-2 selectivity index (SI) = 113 to >312 range). 1-Benzoyl-3-[(4-trifluoromethylphenylimino)methyl]indole (**20**) emerged as the most potent (COX-1 IC_{50} >100 μM; COX-2 IC_{50} = 0.32 μM) and selective (SI >312) COX-2 inhibitor. Furthermore, compound **20** is a selective COX-2 inhibitor in contrast to the reference drug indomethacin that is a potent and selective COX-1 inhibitor (COX-1 IC_{50} = 0.13 μM; COX-2 IC_{50} = 6.9 μM, COX-2 SI = 0.02). Molecular modeling studies employing compound **20** showed that the phenyl CF_3 substituent attached to the C=N spacer is positioned near the secondary pocket of the COX-2 active site, the C=N nitrogen atom is hydrogen bonded (N···NH = 2.85 Å) to the H90 residue, and the indole *N*-1 benzoyl is positioned in a hydrophobic pocket of the COX-2 active site near W387.

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely used for the relief of pain, fever and inflammation. NSAIDs act by inhibiting the cyclooxygenase (COX) catalyzed biotransformation of arachidonic acid (AA) to prostaglandins (PGs), prostacyclin (PGI_2), and thromboxane A_2 (TXA_2).[1–3] Traditional NSAIDs (aspirin, ibuprofen, naproxen and indomethacin), and selective inhibitors of COX-2 (celecoxib, rofecoxib and valdecoxib), exert their desirable therapeutic and unwanted side effects by suppressing the functions of COX-1 and/or COX-2 isozymes.[4–9] The COX-1 isoform, that is expressed constitutively in most tissues, provides important physiological maintenance actions such as synthesis of cytoprotective prostaglandins in the gastrointestinal tract, vascular homeostasis,[10] induction of labor pain, and the biosynthesis of proaggregatory TXA_2 in blood platelets.[11] The use of non-selective NSAIDs (aspirin, ibuprofen and indomethacin), based on their cytoprotective and associated actions of the COX-1 isoform, is limited due to contraindicated side effects that include gastrointestinal (GI), ulcerogenic, hepatic and renal toxicity.[12–22] Unlike COX-1, the COX-2 isoform is induced by mitogenic and proinflammatory stimuli[23] resulting in peripheral inflammatory actions.[24] Selective inhibition of COX-2 was found to be a safer way to reduce gastrointestinal toxicity. Accordingly, selective COX-2 inhibitors provide effective treatment of inflammatory disease states such as rheumatoid arthritis and osteoarthritis. A number of selective COX-2 inhibitors such as celecoxib, rofecoxib and valdecoxib (see structures **1**–**3** in Fig. 1),[25–28] have been developed and were approved for clinical use.

Unfortunately, the cardiovascular side effects of selective COX-2 inhibitors soon became evident since it was found that celecoxib[29] and rofecoxib[30] cause a significant decline in the urinary excretion of the metabolite prostacyclin (PGI_2 is a potent vasodilator, antithrombotic), but not thromboxane A_2 (TxA_2 is prothrombotic). This biochemical imbalance in PGI_2/TxA_2 production is considered to be the major cause of adverse cardiovascular thrombotic events associated with the use of highly selective COX-2 inhibitors.[31–35] Ongoing safety concerns pertaining to the use of non-selective NSAIDs has provided a stimulus for the development of new COX-2 inhibitors with a greater safety profile.

Numerous investigations exploring the design of indole ring based NSAIDs have been described that were targeted at improving their COX-2 selectivity and safety profile. These studies encompassed modifications of the indole ring and side chains of the highly ulcerogenic and selective COX-1 inhibitor indomethacin (**4**).[36–42] In continuation of our ongoing research work directed towards the development of selective COX-2 inhibitors,[43–45] we have selected the indole ring as the template. A group of indole based Schiff bases (**9–11**, **15–21**; Fig. 1), employing focused substitution at the *N*-1 and *C*-3 positions of the indole ring, were synthesized. The COX-1/COX-2 inhibitory activities of this new class of compounds were determined, and plausible binding interactions

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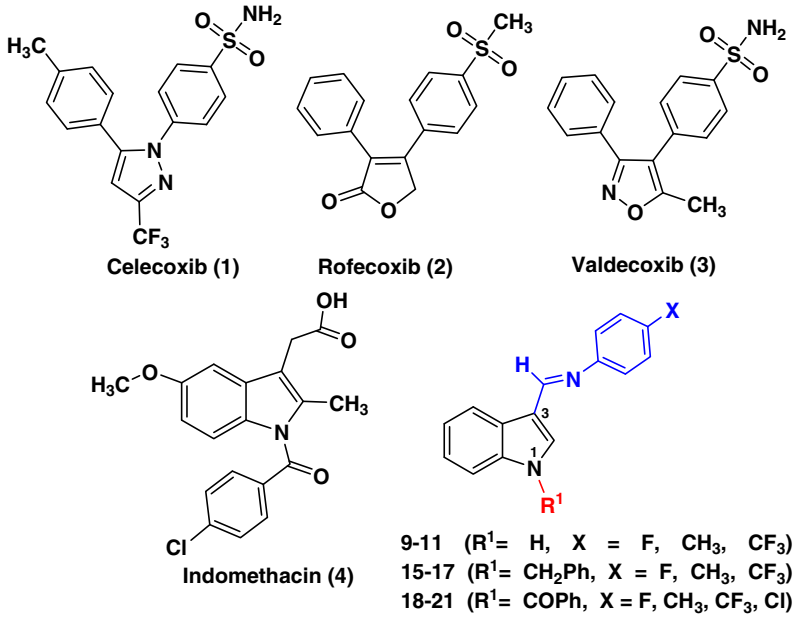
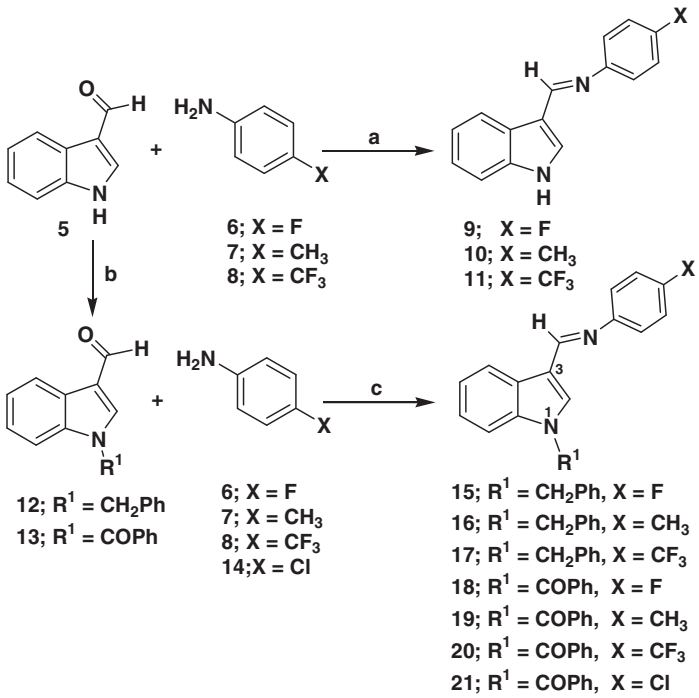


Figure 1. Chemical structures of some selective COX-2 inhibitors (**1**–**3**), a non-selective COX-2 inhibitor (**4**), and the target Schiff base derivatives of indole (**9**–**11** and **15**–**21**).



Scheme 1. Reagents and conditions: (a) *p*-toluenesulfonic acid, absolute ethanol, reflux, 8 h for compound **9**; 4 h for compound **10**; 12 h for compound **11**; (b) benzyl bromide, sodium hydride (NaH), dry THF, 25 °C, 10 min for compound **12**; benzoyl chloride, sodium hydride (NaH), dry THF, 25 °C, 20 min for compound **13**, (c) *p*-toluenesulfonic acid, absolute ethanol, reflux, 20 min for compound **15**; 10 min for compound **16**; 3 h for compound **17**; 30 min for compound **18**; 10 min for compound **19**; 1 h for compound **20**; 40 min for compound **21**.

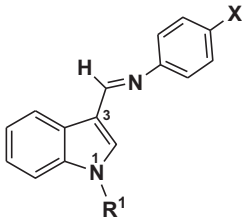
of these compounds within the COX-1/COX-2 active sites were explored using molecular modeling studies.

The synthetic methodologies used to synthesize the target compounds **9**–**11** and **15**–**21** are shown in Scheme 1. Reaction of indole-3-carboxaldehyde (**5**) with a *para*-substituted-aniline **6**–**8** (H_2N–C_6H_4–4-X; X = F, Me, CF_3) in the presence of *p*-toluenesulfonic acid in absolute ethanol at 70–80 °C with reaction times of 4–12 h furnished compounds **9**–**11** (**9**, 80%; **10**, 78%; **11**, 72%; isolated yield).

The *N*-benzyl (**12**, 90%) and *N*-benzoyl (**13**, 87%) derivatives of indole-3-carboxaldehyde were prepared by reaction of **5** with benzyl bromide and benzoyl chloride in the presence of sodium hydride (NaH) in dry THF. The subsequent reaction of these *N*-substituted-indole-3-carboxaldehydes (**12**–**13**) with a *para*-substituted-aniline (H_2N–C_6H_4–4-X; X = F, Me, CF_3, Cl) in the presence of *p*-toluenesulfonic acid with absolute ethanol as solvent at 70–80 °C with a reaction time from 10 min to 3 h furnished the target products **15**–**21** (**18**, 82%; **19**, 85%; **20**, 64%; **21**, 89%; **22**, 86%; **23**, 83%; **24**, 88%; isolated yield).

In vitro COX-1/COX-2 isozyme inhibition studies (Table 1) showed that the compounds (**9–11** and **15–21**) are more potent

Table 1
In vitro COX-1 and COX-2 inhibition, COX-2 selectivity index (SI), and $E_{intermolecular}$ data



Compound	R^1	X	IC_{50}[a] (μM)		COX-2 SI[b]	$E_{intermolecular}$[c]	
			COX-1	COX-2		COX-1	COX-2
9	H	F	68.4	17.2	4.0	−8.77	−9.64
10	H	CH_3	62.3	30.4	2.0	−9.01	−10.01
11	H	CF_3	78.2	11.6	6.7	−8.01	−10.12
15	CH_2Ph	F	91.1	0.71	128.3	−8.09	−13.52
16	CH_2Ph	CH_3	81.5	17.5	4.7	−7.99	−11.13
17	CH_2Ph	CF_3	95.5	0.84	113.6	−7.80	−12.21
18	COPh	F	>100	0.43	>232	−7.90	−13.10
19	COPh	CH_3	80.2	12.4	6.5	−8.10	−12.01
20	COPh	CF_3	>100	0.32	>312	−8.12	−12.50
21	COPh	Cl	86.1	6.53	13.2	−7.61	−13.01
Indomethacin			0.13	6.9	0.02	—	—
Aspirin			0.35	2.4	0.14	—	—
Celecoxib			7.7	0.07	110	—	—

[a] The in vitro test compound concentration required to produce 50% inhibition of ovine COX-1 or human recombinant COX-2. The result (IC_{50}, μM) is the mean of two determinations acquired using the enzyme immuno assay kit (Catalogue No. 560131, Cayman Chemicals Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.
[b] In vitro COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).
[c] Calculated energy of intermolecular interactions during docking in the COX-1/COX-2 active site.

inhibitors of COX-2 (IC_{50} = 0.32–30.4 μM range) than COX-1 (IC_{50} = 62.3 to >100 μM range). Structure–activity data acquired showed that the indole *N*-substituent (R^1) was a determinant of COX-2 inhibitory potency and the COX-2 selectivity index where the relative profile was –COPh > CH_2Ph > H, irrespective of whether the X-substituent was F, Me or CF_3. The anilino X-substituent was also a determinant of COX-2 potency and COX-2 selectivity index where the relative profile was CF_3 > F > Me when the R^1-substituent was –COPh (**18**–**21**) or H (**9**–**11**). In comparison, for compounds **15**–**17** possessing a R^1 = benzyl substituent, the relative profile with respect to the X-substituent was F > CF_3 > Me. Within the N^1-benzoyl group of compounds **18**–**21**, the halogeno X-substituent was a determinant of COX-2 inhibitory potency and selectivity where the relative profile was F (**18**) > Cl (**21**). Among the ten compounds tested, compound **15** (R^1 = CH_2Ph, X = F, COX-2 IC_{50} = 0.71 μM and SI = 128.3), **17** (R^1 = CH_2Ph and X = CF_3, COX-2 IC_{50} = 0.84 μM and SI = 113.6), **18** (R^1 = COPh, X = F, COX-2 IC_{50} = 0.43 μM and SI = >232), **20** (R^1 = COPh and X = CF_3, COX-2 IC_{50} = 0.32 μM and SI = >312), were identified as potent and selective COX-2 inhibitors. In contrast, indomethacin and aspirin are selective COX-1 inhibitors. Accordingly, replacement of the C-3 acetic acid moiety in indomethacin by a Schiff base –CH=N–C_6H_4–4-X moiety (X = F, Me, CF_3, Cl) constitutes a drug design strategy that confers COX-2 inhibitory activity and selectivity. In this regard, these Schiff base analogs of indomethacin possess a profile that is more similar to that of the selective COX-2 inhibitor celecoxib.

To gain insight into the plausible mode of interaction(s) of the compounds within the COX-1 and COX-2 isozymes, molecular modeling (docking) experiments were performed using X-ray crystal structure data for COX-1 and COX-2 obtained from the protein data bank.[46,47] Docking results for two of the lead compounds **15** and **20** are provided. Compound **15** assumes a favorable orientation within the COX-2 binding site (Fig. 2), wherein the C=N nitrogen atom shows hydrogen bonding with S530 (N···HO = 2.80 Å), and the indole nucleus is positioned near the COX-2 hydrophobic pocket in the vicinity of Y385, W387 and F518 residues. The phenyl ring of the benzyl group is orientated towards the entrance of the COX-2 active site secondary pocket lined by H90, R513, Q192 and A516 residues. The F-atom is positioned near V116 where the measured distance between the F-atom and the –C=O group of V116 is 3.11 Å.

A docking study for the compound **20** indicates that the phenyl ring bearing the CF_3 substituent, that is attached to the indole ring via a C=N linker, is oriented toward the secondary pocket of the COX-2 active site (Fig. 3) with the CF_3 group in close proximity of the Q192 residue (3.24 Å). The C=N nitrogen is hydrogen bonded to the H90 residue (N···NH = 2.85 Å), the benzoyl group is positioned in the vicinity of the W387 residue that is part of the COX-2 active site hydrophobic pocket, and the carbonyl oxygen atom assumes a position close to V523 (3.11 Å). The energy associated with intermolecular interactions ($E_{intermolecular}$) obtained upon computational analysis (docking) for all of the compounds within the COX-1 and COX-2 active site is summarized in Table 1. The comparison of $E_{intermolecular}$ for compound **9**, **11** (R^1 = H) with **15**, **17** (R^1 = CH_2Ph) and **18**, **20** (R^1 = COPh) supports the appreciable interaction of compounds **15**, **17**, **18** and **20** within the COX-2 active site. These favorable entry and binding interactions of compound **15**, **17**, **18** and **20** within the COX-2 active site are consistent with their experimentally observed potent COX-2 inhibition. Compounds **15** and **20**, which show only partial entry into the COX-1 active site, did not show any significant interactions with the COX-1 active site residues (data not shown).

In conclusion, a group of new *N*-1, *C*-3 substituted indole Schiff bases were synthesized[48] for evaluation[53] as COX-1/COX-2 inhibitors. Molecular modeling (docking) studies[54] indicate appreciable binding interactions within the COX-2, but not the COX-1, active site that is enhanced by substituents at the indole N^1-position ($PhCH_2$–, PhCO–). Compounds **15**, **17**, **18** and **20** were identified as the most potent and selective COX-2 inhibitors. Within this

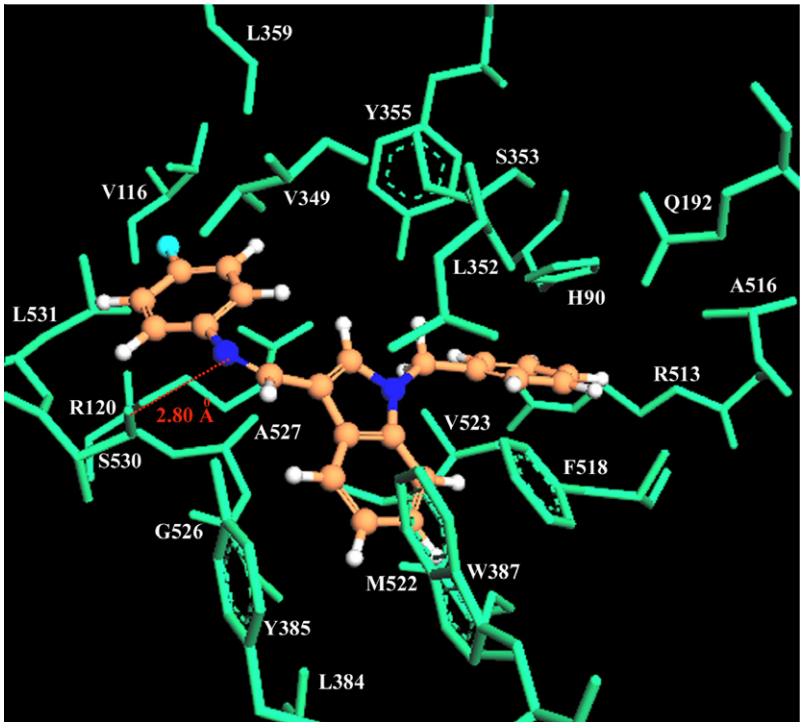


Figure 2. Molecular modeling (docking) of compound **15** (carbon atoms in orange) in the binding site of COX-2 (pdb ID 6COX; $E_{intermolecular}$ = −13.52 kcal/mol). Hydrogen atoms of amino acid residues have been removed to improve clarity.

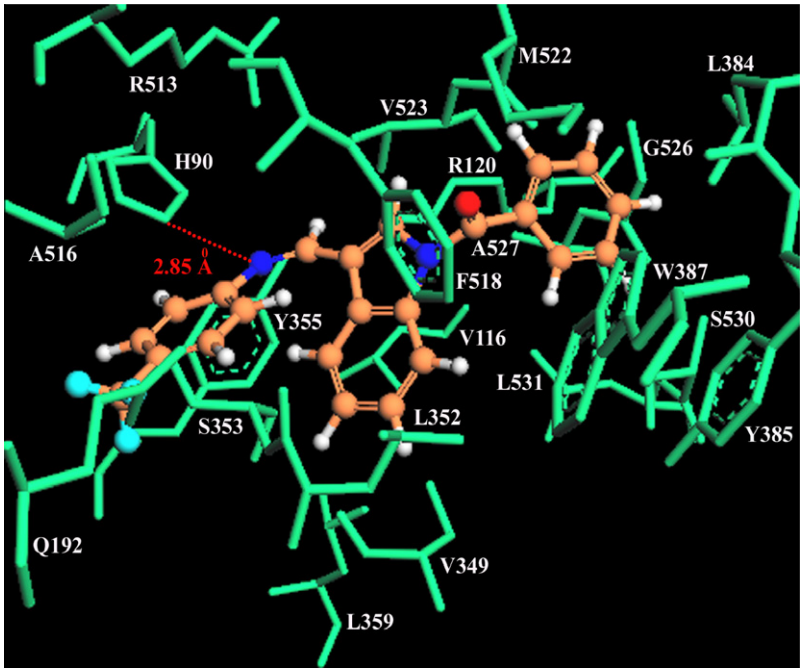


Figure 3. Molecular modeling (docking) of compound **20** (carbon atoms in orange) in the binding site of COX-2 (pdb ID 6COX; $E_{intermolecular}$ = −12.50 kcal/mol). Hydrogen atoms of amino acid residues have been removed to improve clarity.

group of compounds, a 'lead-compound' 1-benzoyl-3-[(4-trifluoromethylphenylimino)methyl]indole (**20**) was identified that exhibited appreciable COX-2 inhibitory activity and selectivity (COX-1 IC_{50} >100 μM; COX-2 IC_{50} = 0.32 μM; COX-2 SI >312). Replacement of the C-3 acetic acid moiety in the potent, highly ulcerogenic, and very selective COX-1 inhibitor indomethacin by a Schiff base –CH=N–C_6H_4–4-X moiety (X = F, Me, CF_3, Cl) constitutes a drug design strategy that confers COX-2 inhibitory activity and selectivity. The suitability of the imino compounds **9**–**11**, **15**–**21** (–CH=N–R^1; R^1 = aryl) for oral administration has not been determined. Their hydrolysis to the respective aldehyde and amine, which would be more difficult compared to when R^1 is H, requires acid or base catalysis. The Schiff base compounds described in this study, unlike indomethacin having a pK_a of 4.5, are not acidic.

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48. *General*: Melting points were measured in capillaries using a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 300 NMR spectrometer using $CDCl_3$/DMSO-d_6 as solvent. ^{13}C NMR spectra were acquired using the *J* modulated spin echo technique where methyl and methine carbons appear as positive peaks and methylene and quaternary carbon resonances appear as negative peaks. Mass spectra (MS) were recorded on a Water's Micromass ZQ 4000 mass spectrometer using the ESI ionization mode. The purity of the compounds was established using elemental analyses which were performed for C, H, N by the Microanalytical Service Laboratory, Department of Chemistry, University of Alberta. Compounds showed a single spot on Macherey-Nagel Polygram Sil G/UV254 silica gel plates (0.2 mm) using a low, medium, and highly polar solvent system, and no residue remained after combustion, indicating a purity >98%. Column chromatography was performed on a Combiflash R_f system using a gold silica column. All other reagents, purchased from the Aldrich Chemical Co. (Milwaukee, WI), were used without further purification.
3-[(4-Fluorophenylimino)methyl]indole (***9***): A solution of indole-3-carboxaldehyde (300 mg, 1 mmol) and *p*-toluenesulfonic acid (3 mg) in absolute ethanol (10 mL) was stirred at 70–80 °C for 15 min, a solution of *p*-fluoroaniline (0.179 ml, 1.2 mmol) in absolute ethanol (2 mL) was added slowly, and the reaction mixture was refluxed for 8 h. Removal of the solvent in vacuo furnished a thick liquid which produced a brown precipitate on addition of water. The precipitate was filtered off, washed with water, and dried to afford **9** as a brown solid; 80% yield; mp 143–145 °C (lit[49] mp 142–144 °C); ^{1}H NMR (DMSO-d_6): δ 7.14–7.27 (m, 6H, phenyl hydrogens, indole H-5, H-6), 7.47 (d, *J* = 8.5 Hz, 1H, indole H-7), 7.99 (d, *J* = 2.4 Hz, 1H, C*H*=N), 8.35 (d, *J* = 7.3 Hz, 1H, indole H-4), 8.68 (s, 1H, indole H-2), 11.77 (br s, 1H, N*H*).
3-[(4-Methylphenylimino)methyl]indole (***10***): A solution of indole-3-carboxaldehyde (300 mg, 1 mmol) and *p*-toluenesulfonic acid (3 mg) in absolute ethanol (10 mL) was stirred at 70–80 °C for 15 min, a solution of *p*-methylaniline (264 mg, 1.2 mmol) in absolute ethanol (2 mL) was added slowly, and the reaction mixture was refluxed for 4 h. Removal of the solvent in vacuo furnished a thick liquid which afforded a yellow precipitate on addition of ether. The precipitate was filtered off, washed with hexane and dried to furnish **10** as a yellow solid[50]; 78% yield; mp 170–172 °C; ^{1}H NMR (DMSO-d_6): δ 2.30 (s, 3H, CH_3), 7.09–7.23 (m, 6H, phenyl hydrogens, indole H-5, H-6), 7.46 (d, *J* = 7.3 Hz, 1H, indole H-7), 7.96 (d, *J* = 2.2 Hz, 1H, C*H*=N), 8.35 (d, *J* = 7.3 Hz, 1H, indole H-4), 8.68 (s, 1H, indole H-2), 11.72 (br s, 1H, N*H*).
3-[(4-Trifluoromethylphenylimino)methyl]indole (***11***): A solution of indole-3-carboxaldehyde (300 mg, 1 mmol) and *p*-toluenesulfonic acid (3 mg) in absolute ethanol (10 mL) was stirred at 70–80 °C for 15 min, a solution of 4-(trifluoromethyl)aniline (0.311 ml, 1.2 mmol) in absolute ethanol (2 mL) was added slowly, and the reaction mixture was refluxed for 12 h. Removal of the solvent in vacuo furnished a thick liquid which gave a light greenish precipitate on washing with ether. The precipitate was filtered off and dried to give **11** as a pale green solid; 72% yield; mp 148–150 °C; ^{1}H NMR (DMSO-d_6): δ 7.14–7.27 (m, 6H, phenyl hydrogens, indole H-5, H-6), 7.47 (d, *J* = 6.1 Hz, 1H, indole H-7), 7.98 (s, 1H, C*H*=N), 8.35 (d, *J* = 7.3 Hz, 1H, indole H-4), 8.68 (s, 1H, indole H-2), 11.77 (br s, 1H, N*H*); ^{13}C NMR (DMSO-d_6): δ 111.3, 112.9, 119.2 (q, $^{1}J_{CF}$ = 274.0 Hz, CF_3), 121.5, 123.5, 125.3, 126.2, 127.2, 128.6, 128.7, 136.9 (q, $^{2}J_{CCF}$ = 14.7 Hz, CCF_3), 137.1, 156.4, 156.7 (*C*H=N); ESI-MS: 289 $[M-H]^+$; Anal. Calcd for $C_{16}H_{11}F_3N_2$: C, 66.66; H, 3.85; N, 9.72. Found: C, 66.62; H, 3.81; N, 9.74.
1-Benzylindole-3-carboxyaldehyde (***12***) *and 1-Benzoylindole-3-carboxyaldehyde* (***13***): A solution of indole-3-carboxaldehyde (1 g, 1 mmol) was added the solution of sodium hydride (1.5 mmol) in dry THF (10 mL) and the reaction mixture was stirred for 5 min at 0 °C. Either benzyl bromide (1.76 g, 1.5 mmol), or benzoyl chloride (1.45 g, 1.5 mmol)), was added, and the reaction was stirred at 0 °C for 10–20 min to furnish the title compound **12** or **13**. The ^{1}H NMR spectra for **12** and **13** were identical to that reported.[51,52]
1-Benzyl-3-[(4-fluorophenylimino)methyl]indole (***15***): A solution of 1-benzylindole-3-carboxaldehyde (300 mg, 1 mmol) and *p*-toluenesulfonic acid (3 mg) in absolute ethanol (10 mL) was stirred at 70–80 °C for 15 min, a solution of *p*-fluoroaniline (0.145 mL, 1.2 mmol) in absolute ethanol (2 mL) was added slowly, and the reaction mixture was refluxed for 20 min. Removal of the solvent in vacuo furnished a viscous liquid which formed a brown precipitate on addition of water. The precipitate were filtered off, washed with water and dried to afford **15** as a brown solid; 82% yield; mp 98–100 °C; ^{1}H NMR (DMSO-d_6): δ 5.51 (s, 2H, Ph-CH_2), 7.16–7.35 (m, 11H, phenyl and fluorophenyl hydrogens, indole H-5, H-6), 7.54 (d, *J* = 6.1 Hz, 1H, indole H-7), 8.15 (s, 1H, C*H*=N), 8.36 (d, *J* = 7.3 Hz, 1H, indole H-4), 8.69 (s, 1H, indole H-2); ^{13}C NMR (DMSO-d_6): δ 49.4, 110.8, 114.3, 115.6 (d, $^{2}J_{CCF}$ = 21.8 Hz, ArCH),

121.2, 121.9, 122.2 (d, $^{3}J_{CCCF}$ = 7.6 Hz, Ar-CH), 122.9, 125.3, 127.1, 127.5, 128.5, 136.1, 136.9, 137.2, 149.3 (d, $^{4}J_{CCCCF}$ = 2.1 Hz), 154.9 (CH=N), 159.6 (d, $^{1}J_{CF}$ = 239.1 Hz, ArC-F); ESI-MS: 329 $[M-H]^{+}$; Anal. Calcd for $C_{22}H_{17}FN_2$: C, 80.47; H, 5.22; N, 8.53. Found: C, 80.45; H, 5.20; N, 8.50.

1-Benzyl-3-[(4-methylphenylimino)methyl]indole (***16***): A solution of 1-benzylindole-3-carboxaldehyde (300 mg, 1 mmol) and *p*-toluenesulfonic acid (3 mg) in absolute ethanol (10 mL) was stirred at 70–80 °C for 15 min, a solution of *p*-methylaniline (0.163 mg, 1.2 mmol) in absolute ethanol (2 mL) was added slowly, and the reaction mixture was refluxed for 10 min. Removal of the solvent in vacuo furnished a viscous liquid which produced a yellow precipitate on trituration with ether. The precipitate was filtered off, washed with hexane and dried to afford **16** as a yellow solid; 85% yield; mp 108–110 °C; ^{1}H NMR (DMSO-d_6): δ 2.30 (s, 3H, CH_3), δ 5.51 (s, 2H, PhCH_2), 7.11–7.35 (m, 11H, indole H-5, H-6, phenyl hydrogens), 7.55 (d, *J* = 7.3 Hz, 1H, indole H-7), 8.17 (s, 1H, C*H*=N), 8.36–8.39 (d, *J* = 6.1 Hz, 1H, indole H-4), 8.72 (s, 1H, indole H-2); ^{13}C NMR (DMSO-d_6): δ 20.5, 49.4, 110.8, 114.2, 114.7, 120.5, 121.2, 121.9, 123.0, 125.4, 127.1, 127.5, 128.6, 129.5, 133.8, 136.9, 137.2, 142.3, 154.0 (*C*H=N); ESI-MS: 325 $[M-H]^{+}$; Anal. Calcd for $C_{23}H_{20}N_2$: C, 85.15; H, 6.21; N, 8.63. Found: C, 85.12; H, 6.20; N, 8.61.

1-Benzyl-3-[(4-trifluoromethylphenylimino)methyl]indole (***17***): A solution of 12 (300 mg, 1 mmol) and *p*-toluenesulfonic acid (3 mg) in absolute ethanol (10 mL) was stirred at 70–80 °C for 15 min, a solution of 4-(trifluoromethyl)aniline (0.191 ml, 1.2 mmol) in absolute ethanol (2 mL) was added slowly, and the reaction mixture was refluxed for 3 h. Removal of the solvent in vacuo furnished a thick liquid which gave a light brown precipitate on addition of water. The precipitate was filtered off, washed with water and dried to yield **17** as a pale brown solid; 64% yield; mp 110–112 °C; ^{1}H NMR (DMSO-d_6): δ 5.53 (s, 2H, PhCH_2), 7.19–7.39 (m, 9H, 4-trifluoromethylphenyl H-2, H-6, benzyl $C_6H_5CH_2$, indole H-5, H-6), 7.57 (d, *J* = 6.1 Hz, 1H, indole H-7), 7.71 (d, *J* = 7.9 Hz, 2H, 4-trifluoromethylphenyl H-3, H-5), 8.23 (s, 1H, C*H*=N), 8.37 (d, *J* = 6.7 Hz, 1H, indole H-4), 8.72 (s, 1H, indole H-2); ^{13}C NMR (DMSO-d_6): δ 49.8, 110.9, 111.3, 112.9, 114.4, 119.1 (q, $^{1}J_{C,F}$ = 269.2 Hz, CF_3), 121.0, 123.5, 125.3, 126.1, 127.2, 127.6, 127.7, 128.5, 129.0, 136.9 (q, $^{2}J_{CCF}$ = 15.2 Hz), 137.1, 152.1, 156.7 (*C*H=N); ESI-MS: 379 $[M-H]^{+}$; Anal. Calcd for $C_{23}H_{17}F_3N_2$: C, 73.01; H, 4.53; N, 7.40. Found: C, 73.00; H, 4.54; N, 7.41.

1-Benzoyl-3-[(4-fluorophenylimino)methyl]indole (***18***): A solution of **13** (300 mg, 1 mmol) and *p*-toluenesulfonic acid (3 mg) in absolute ethanol (10 mL) was stirred at 70–80 °C for 15 min, a solution of 4-(fluoro)aniline (0.136 ml, 1.2 mmol) in absolute ethanol (2 mL) was added slowly, the reaction mixture was refluxed for 30 min. On cooling, a cream colored solid separated, the solid was filtered off, washed with water, and dried to furnish **18** as a pale yellow solid; 89% yield; mp 120–121 °C; ^{1}H NMR (DMSO-d_6): δ 7.20–7.51 (m, 6H, Ar-H), 7.61–7.85 (m, 5H, Ar-H), 8.08 (s, 1H, C*H*=N), 8.33 (d, *J* = 7.32 Hz, 1H, indole H-7), 8.53 (d, *J* = 7.32 Hz, 1H, indole H-4), 8.78 (s, 1H, indole H-2); ^{13}C NMR (DMSO-d_6): δ 115.6, 115.8 (d, $^{2}J_{CCF}$ = 22.2 Hz, ArCH), 119.3, 122.4, 122.5 (d, $^{3}J_{CCCF}$ = 7.6 Hz, ArCH), 124.6, 125.7, 127.1, 128.7, 129.2, 132.3, 133.3, 134.8, 136.3, 148.2 (d, $^{4}J_{CCCCF}$ = 3.2 Hz, ArCH), 155.1 (CH=N), 160.2 (d, $^{1}J_{CF}$ = 240.1 Hz, ArC-F), 168.2 (C=O); ESI-MS: 343 $[M-H]^{+}$; Anal. Calcd for $C_{22}H_{15}FN_2O$: C, 77.18; H, 4.42; N, 8.18. Found: C, 77.17; H, 4.45; N, 8.15.

1-Benzoyl-3-[(4-methylphenylimino)methyl]indole (***19***): A solution of the aldehyde **13** (300 mg, 1 mmol) and *p*-toluenesulfonic acid (3 mg) in absolute ethanol (10 mL) was stirred at 70–80 °C for 15 min, a solution of *p*-methylaniline (0.154 mg, 1.2 mmol) in absolute ethanol (2 mL) was added slowly, and the reaction mixture was refluxed for 10 min. On cooling, a light yellow solid separated, the solid was filtered off, washed with hexane, and dried to provide **19** as a pale yellow solid; 86% yield; mp 132–133 °C; ^{1}H NMR (DMSO-d_6): δ 2.30 (s, 3H, CH_3), 7.15–7.19 (m, 4H, Ar-H), 7.22–7.85 (m, 7H, Ar-H), 8.07 (s, 1H, C*H*=N), 8.31 (d, *J* = 8.55 Hz, 1H, indole H-7), 8.53 (d, *J* = 9.15 Hz, 1H, indole H-4), 8.77 (s, 1H, indole H-2); ^{13}C NMR (DMSO-d_6): δ 20.5, 115.7, 119.5, 120.7, 122.5, 124.6, 125.7, 127.3, 128.7, 129.2, 129.6, 132.3, 133.4, 134.4, 134.9, 136.3, 149.3, 154.1 (*C*H=N), 168.2 (C=O); ESI-MS: 339 $[M-H]^{+}$; Anal. Calcd for $C_{23}H_{18}N_2O$: C, 81.63; H, 5.36; N, 8.28. Found: C, 81.61; H, 5.39; N, 8.25.

1-Benzoyl-3-[(4-trifluoromethylphenylimino)methyl]indole (***20***): A solution of the aldehyde **13** (300 mg, 1 mmol) and *p*-toluenesulfonic acid (3 mg) in absolute ethanol (10 mL) was stirred at 70–80 °C for 15 min, a solution of 4-(trifluoromethyl)aniline (0.232 ml, 1.2 mmol) in absolute ethanol (2 mL) was added slowly, and the reaction mixture was refluxed for 1 h. On cooling, a cream colored solid separated, the solid was filtered off, washed with water, and dried to give **20** as a pale yellow solid; 83% yield; mp 150–152 °C; ^{1}H NMR (DMSO-d_6): δ 7.39–7.53 (m, 4H, Ar-H), 7.61–7.77 (m, 7H, Ar-H), 8.18 (s, 1H, C*H*=N), 8.34 (d, *J* = 8.52 Hz, 1H, indole H-7), 8.50 (d, *J* = 9.15 Hz, 1H, indole H-4), 8.81 (s, 1H, indole H-2); ^{13}C NMR (DMSO-d_6): ^{13}C NMR: δ 112.7, 114.8, 115.8, 120.0 (q, $^{1}J_{C,F}$ = 238.0 Hz, CF_3), 121.3, 121.4, 122.4, 124.8, 126.0, 127.0, 128.8, 129.3, 129.7, 132.7, 135.9, 136.3 (q, $^{2}J_{CCF}$ = 14.2 Hz, *C*CF_3), 152.1, 157.4 (*C*H=N), 168.4 (C=O); ESI-MS: 393 $[M-H]^{+}$; Anal. Calcd for $C_{23}H_{15}F_3N_2O$: C, 70.40; H, 3.85; N, 14.53. Found: C, 70.42; H, 3.84; N, 14.51.

1-Benzoyl-3-[(4-chlorophenylimino)methyl]indole (***21***): A solution of the aldehyde **13** (300 mg, 1 mmol) and *p*-toluenesulfonic acid (3 mg) in absolute ethanol (10 mL) was stirred at 70–80 °C for 15 min, a solution of *p*-chloroaniline (0.184 mg, 1.2 mmol) in absolute ethanol (2 mL) was added slowly, and the reaction mixture was refluxed for 40 min. On cooling, the cream coloured solid that separated was filtered off, washed with hexane, and dried to afford **21** as a pale yellow solid; 88% yield; mp 161–163 °C; ^{1}H NMR (DMSO-d_6): δ 7.26–7.29 (m, 2H, Ar-H), 7.43–7.51 (m, 4H, Ar-H), 7.61–7.85 (m, 5H, Ar-H), 8.11 (s, 1H, C*H*=N), 8.34 (d, *J* = 8.55 Hz, 1H, indole H-7), 8.50 (d, *J* = 8.52 Hz, 1H, indole H-4), 8.79 (s, 1H, indole H-2); ^{13}C NMR (DMSO-d_6): δ 115.7, 119.2, 122.4, 122.6, 124.7, 125.8, 127.1, 128.7, 129.0, 129.2, 129.8, 132.4, 133.3, 135.2, 136.3, 150.7, 155.9 (*C*H=N), 168.2 (C=O); ESI-MS: 359 $[M-H]^{+}$, 361 $[M-H]^{+}$; Anal. Calcd for $C_{22}H_{15}ClN_2O$: C, 73.64; H, 4.21; N, 7.81. Found: C, 73.61; H, 4.24; N, 7.80.

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53. The ability of the test compounds **9**–**11** and **15**–**21** listed in Table 1 to inhibit ovine COX-1 and human recombinant COX-2 (IC_{50} value, μM) was determined using an enzyme immuno assay (EIA) kit (Catalog No. 560131, Cayman Chemical, Ann Arbor, MI, USA) according to a previously reported method (Uddin, M. J.; Rao, P. N. P.; McDonald, R.; Knaus, E. E. *J. Med. Chem.* **2004**, *47*, 6108).
54. Crystal coordinates from the X-ray crystal structure of COX-1 (ovine, 1EQG, ibuprofen bound in the active site) and COX-2 (murine, 6COX, SC558 bound in the active site) were obtained from the protein data bank. Compounds were constructed using the builder toolkit of the software package ArgusLab 4.0.1 (Mark, A. ArgusLab, Version 4.0.1; Thompson Planaria Software LLC: Seattle, WA) and energy minimized using the semi-empirical quantum mechanical method PM3. The monomeric structure of the enzyme was chosen and the active site was defined around the ligand. The molecule to be docked in the active site of the enzyme was inserted in the work space carrying the structure of the enzyme. The docking program implements an efficient grid based docking algorithm which approximates an exhaustive search within the free volume of the binding site cavity. The conformational space was surveyed by the geometry optimization of the flexible ligand (rings are treated as rigid) in combination with the incremental construction of the ligand torsions. Thus, docking occurred between the flexible ligand parts of the compound and enzyme. The ligand orientation was determined by a shape scoring function based on Ascore and the final positions were ranked by lowest interaction energy values. The $E_{interaction}$ is the sum of the energies involved in H-bond interactions, hydrophobic interactions and van der Waal's interactions. H-bond and hydrophobic interactions between the compound and enzyme were explored by distance measurements.