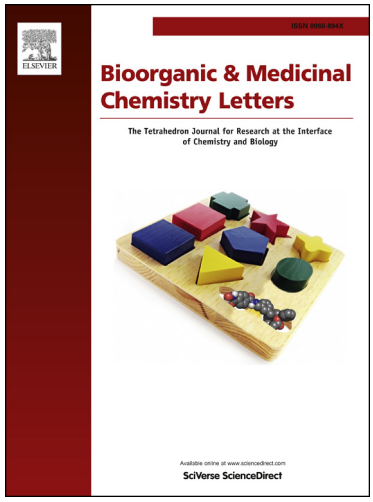

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Synthesis, anti-inflammatory, ulcerogenic and cyclooxygenase activities of indenopyrimidine derivatives

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Abstract

Objective of the present work was to evaluate the anti-inflammatory, ulcerogenicity and cyclooxygenase activity of indenopyrimidine derivatives. Anti-inflammatory activity of the tested compounds is investigated by carrageenan-induced rat paw edema assay. Compounds A1, A6, A7 and A12 exhibit the comparable anti-inflammatory activity (79.33-81.33 %) to the standard drug diclofenac sodium (85.33%), while A6, A7, A9, A12 and A14 show better ulcer index than the reference standard diclofenac sodium. To rationalize the anti-inflammatory activity, docking experiments are performed to study the ability of these compounds to bind into the active site of COX-2 enzyme.

Keywords Indenopyrimidine; anti-inflammatory; anti-ulcerogenic; COX-2; molecular docking

Non-steroidal anti-inflammatory drugs (NSAIDs) are used for the treatment of pain and inflammation and arthritis.[1] Prostaglandins are produced within the body by the enzyme cyclooxygenase which can exist in cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) forms. COX-1 and COX-2 catalyze arachidonic acid to its own prostaglandins but they differ in their physiological functions.[2-5] COX-1 plays an important role in physiological functions while pro-inflammatory COX-2 present at the site of inflammation and also in central nervous system which is mainly responsible for inflammation.[6]

Most of the presently used NSAIDs exert their anti-inflammatory activity by inhibition of both isoforms of cyclooxygenase enzymes.[7-9] Some of the reported nonselective NSAIDs allied with side effects such as gastric ulcer, gastrointestinal bleeding and cardiovascular effects.[10-13] The selective COX-2 inhibitors such as Rofecoxib and Valdecoxib are withdrawn from market because of their cardiovascular side effects.[14] Therefore, there is need to develop safer and selective COX-2 inhibitors to overcome the problems associated with current drugs.

Pyrimidine heterocycles plays an important role in diversity oriented synthesis, combinatorial synthesis and medicinal chemistry because of their noteworthy various pharmacological and biological properties[15-26] and are highly studied for their anti-inflammatory activities.[27-28]

In view of the facts mentioned above and in continuation of our ongoing efforts towards the development of new bioactive molecule,[29] we evaluated in *vivo* assessment of anti-inflammatory, ulcerogenicity and in *vitro* COX-2/COX-1 selectivity study of indenopyrimidine derivatives for the development of non-ulcerogenic anti-inflammatory candidates. On the basis of the docking score, seven compounds were selected and screened for their anti-inflammatory, ulcerogenicity and cyclooxygenase activity.

Recently, we have successfully reported the synthesis and anti-cancer activity against breast cancer cell lines of indenopyrimidine derivatives. Based on the docking study of the said compounds, we have decided to explore the anti-inflammatory activity of the same compounds. The compounds used here are synthesized by our own method[30] and fully characterized by spectroscopic techniques. In fact, sixteen compounds have been synthesized, including six compounds are novel one as shown in **Scheme 1** and given in **table 1.**

<Scheme 1>

< Table 1>

In the Pharmacological study, all the synthesized compounds were docked into the active site of cyclooxygenase enzyme for the indication of credible anti-inflammatory activity. We have investigated anti-inflammatory, ulcerogenicity and cyclooxygenase activity of some selected derivatives of 2-amino-4-phenyl-5*H*-indeno [1, 2-*d*] pyrimidine-5-one.

Anti-inflammatory activities of selected seven compounds were evaluated by carrageenan- induced rat paw edema assay.[31-32] The compounds were tested at 25 and 50 mg/kg in albino Wistar rats and compared with reference drug diclofenac at 30 mg/kg. The tested compounds showed anti-inflammatory activity ranging from 78.00 to 81.33% inhibition as compared to diclofenac (85.33%) and presented in **Table 2.** Among the tested compounds of 2-amino-4-phenyl-5*H*-indeno [1, 2-*d*] pyrimidine-5-one derivatives, A1, A7 and A12 showed best activity (79.33-80.66 %). The compound A6 showed highest activity (81.33%). The results also showed that there is no significant effect of the substituent present on the phenyl ring at fourth position. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC) of SCAN research Laboratory, Bhopal, (MP) constituted for the purpose of control and

supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

< Table 2>

After evaluating the anti-inflammatory activity, ulcerogenic activity was screened by reported method.[33-34] The tested compounds and Diclofenac sodium were given orally to rats and they were sacrificed for the evolution of ulcerogenic activity. The stomach was removed, opened along the greater curvature, washed with distilled water and finally rinsed gently with normal saline. The number of mucosal damage for each stomach was examined using magnifying lens for the macroscopically visible lesions. It was counted and their ulcerogenic severity was assessed according to the grading system. The ulcer index of A1, A6, A7, A9, A11, A12 and A14 compounds was calculated and presented in **Table 3** and **figure 1.**

All the tested compounds showed reduction in ulcerogenic activity ranging from 2.80 ± 0.14 to 3.00 ± 0.16 as compared to standard drug Diclofenac 5.70 ± 0.17. Ulcerogenic effect was compared to a classical NSAID, diclofenac sodium. Results revealed that compounds A6, A7, A12 and A14 shows good ulcerogenic activity than diclofenac sodium. Compound A9 having three methoxy substituent showed maximum reduction in ulcerogenic activity (2.80 ± 0.14).

< Table 3>

< Figure 1>

The ability of the test compounds A1, A6, A7, A9, A11, A12 and A14 to inhibit COX-1 and COX-2 was assayed using cyclooxygenase ovine inhibitor screening kit (catalogue number 560131, Cayman chemical, Ann Arbor, MI, USA) by the method of Gierse et al.[35] The ratio of IC_{50} of COX-2 to IC_{50} of COX-1 (COX-2/COX-1) of the above compounds showed that

compounds A7, A9, A11, A12 and A14 are selective COX-2 inhibitor with a ratio of 0.70, 0.69, 0.70, 0.69 and 0.68 respectively **Table 4.**

< Table 4>

Significant anti-inflammatory activity of synthesized compounds prompted us to perform molecular docking studies of compounds A1, A6, A7, A9, A11, A12 and A14 to understand the ligand–protein interactions and cyclooxygenase-2 (COX-2) selectivity in detail. Automated docking analysis of the synthesized derivatives was carried out using biopredicta module of the V Life MDS 4.3. The crystal structure of COX-2 (PDB: 1CX2) and COX-1 (PDB: 2OYE) were used for docking study. As per the docking study with 1CX2 the following results can be drawn. A6 is the active compound in the series showed five hydrogen bond interactions with ALA 527 ($1.8A^0$), VAL 523 ($2.2A^0$), HIS 90 ($1.8A^0$, 2.2A0, $2.0A^0$) and two aromatic interactions TYR 348 ($5A^0$) and PHE 518 ($5.2A^0$). All the active molecules A1, A6, A7, A9, A11, A12, and A14 showed one common interaction with the hydrogen of the NH_2 of Arg 513 with a bond distance of 1.9, 2.2, 2.4, 1.7, 2.4, 1.9 and 1.8 A^0 respectively, while A12 showed hydrogen bond interactions with LEU 352 (2.4 A^0), ARG 513 (1.9 A^0) and aromatic interactions with HIS 90 (4.4 A^0). Docking results are a clear indication that substitution on the phenyl ring does not contribute significantly towards the binding of the molecules with COX-II. Derivatives with the free amino groups at R^1 are showing significant interaction with the COX-II which also helps to two phenyl ring to accommodate parallel conformation with respect to the heterocyclic amino acids like HIS 90 which are located on the upper half of the receptor to form aromatic interactions. Pharmacophoric identification of the synthesized derivatives indicated four point pharmacophore consisting of three hydrogen bond accepter and two aromatic features which

show synthesized derivatives, are binding with the COX-II receptor *via* the creation of hydrogen bonds and aromatic interaction.

< Figure 2>

< Figure 3>

Synthesized molecules were screened for the drug likeness using Lipinski 'Rule of Five' and oral absorption of the lead molecules was also calculated using Molinspiration Chemoinformatics server. All the seven molecules screened are having acceptable drug like properties and none of them is violating the Lipinski rule. A6, A12 and A14 molecules are having good % oral absorption greater than 85 %, which shows these molecules can be orally active. **Table 5**

<Table 5>

The tested indenopyrimidine derivatives showed better anti-inflammatory activity with minimum ulcerogenic activity than the Diclofenac sodium. Also, the ratio of COX-2/COX-1 suggests the selectivity of compounds towards COX-2. The compound A14 was a potent, selective inhibitor to COX-2 than COX-1.

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Index of Tables and Figures

Table 1 Synthesis of indenopyrimidine derivatives

Entry	R	R^1	Time (hrs) (hrs)	Yield (%)	mp ^{0}C Obs.	Lit
A1	H	NH_2	7	80	89-91	90
A2	3,4-$(OCH_3)_2$	C_6H_5	8	75	202-204	
A3	3,4,5-$(OCH_3)_3$	C_6H_5	8.10	78	>300	
A4	4-$N(CH_3)_2$	C_6H_5	8	80	210-212	
A5	4-NO_2	C_6H_5	7.5	83	260-262	
A6	4-NO_2	NH_2	8	84	>300	>300
A7	3-NO_2	NH_2	7.4	86	271-273	272
A8	3,4-$(OCH_3)_2$	NH_2	8.2	82	177-179	178
A9	3,4,5-$(OCH_3)_3$	NH_2	8.3	80	238-240	240
A10	4-*N*$(CH_3)_2$	NH_2	8.4	81	260	
A11	4-Cl	NH_2	7.5	86	242-244	244
A12	4-Br	NH_2	8.2	83	207-209	210
A13	4-OCH_3	NH_2	8.3	80	99-101	100
A14	3-OCH_3	NH_2	8.2	79	168-171	
A15	Pyridine-3-yl	NH_2	8.4	81	185-187	186
A16	Thiophen-2-yl	NH_2	8.1	78	193-195	194

Table 2 Effect of compounds on paw edema induced by carrageen in rats

Treatment	Dose (mg/kg)	Mean differences in Paw Volume (ml)	Percentage of Inhibition (%)
Control	0.1 ml of 1% (w/v)	1.50 ± 0.05	--
Diclofenac	30	0.97 ± 0.05*	85.33
A1	25	1.10 ± 0.05	76.66
	50	1.04 ± 0.06*	80.66
A6	25	1.09 ± 0.05	77.33
	50	1.03 ± 0.04*	81.33
A7	25	1.11 ± 0.06	76.00
	50	1.06 ± 0.05*	79.33
A9	25	1.12 ± 0.07	75.33
	50	1.07 ± 0.07*	78.66
A11	25	1.11 ± 0.06	76.00
	50	1.08 ± 0.07*	78.00
A12	25	1.10 ± 0.08	76.66
	50	1.05 ± 0.06*	80.00
A14	25	1.12 ± 0.06	75.33
	50	1.08 ± 0.04*	78.00

Each data suggests Mean ± SEM (n=6). One-way ANOVA using Dunnett's test is applied for statistical analysis, treatment groups compared with Control group.

Significant at* $p < 0.01$, compared to control group.

Table 3 Ulcer index of tested compounds of the most active compounds and diclofenac sodium

Treatment	Dose (mg/kg)	No. of animals with ulcer	Ulcer Index
Diclofenac	100	5/5	5.70 ± 0.17
A1	50	4/5	3.00 ± 0.16
A6	50	4/5	2.90 ± 0.15
A7	50	4/5	2.90 ± 0.16
A9	50	4/5	2.80 ± 0.14
A11	50	4/5	3.00 ± 0.17
A12	50	4/5	2.90 ± 0.15
A14	50	4/5	2.90 ± 0.15

Each data suggests Mean ± SEM (n=6). One-way ANOVA using Dunnett's test is applied for statistical analysis, Treatment groups compared with Control group.

Significant at* $p < 0.01$, compared to control group.

Figure1 Determination of ulcer index- diclofenac-induced gastric ulceration in rats

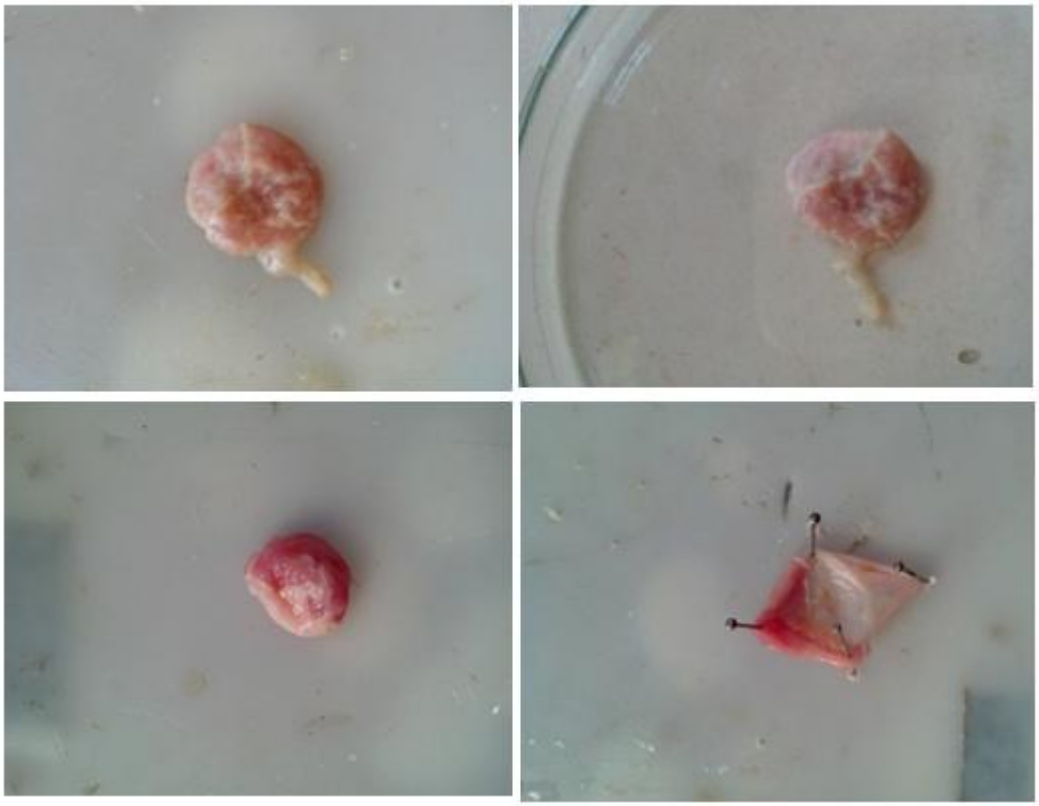


Table 4 COX-2/COX-1 ratio of tested compounds and Celecoxib

Treatment	Dose (mg/kg)	COX-2/COX-1
Celecoxib	100	0.04
A1	25	0.44
	50	0.60
A6	25	0.40
	50	0.66
A7	25	0.45
	50	0.70
A9	25	0.47
	50	0.69
A11	25	0.48
	50	0.70
A12	25	0.50
	50	0.69
A14	25	0.41

50	0.68

Figure 2 Docking of compounds into the active site of COX-2

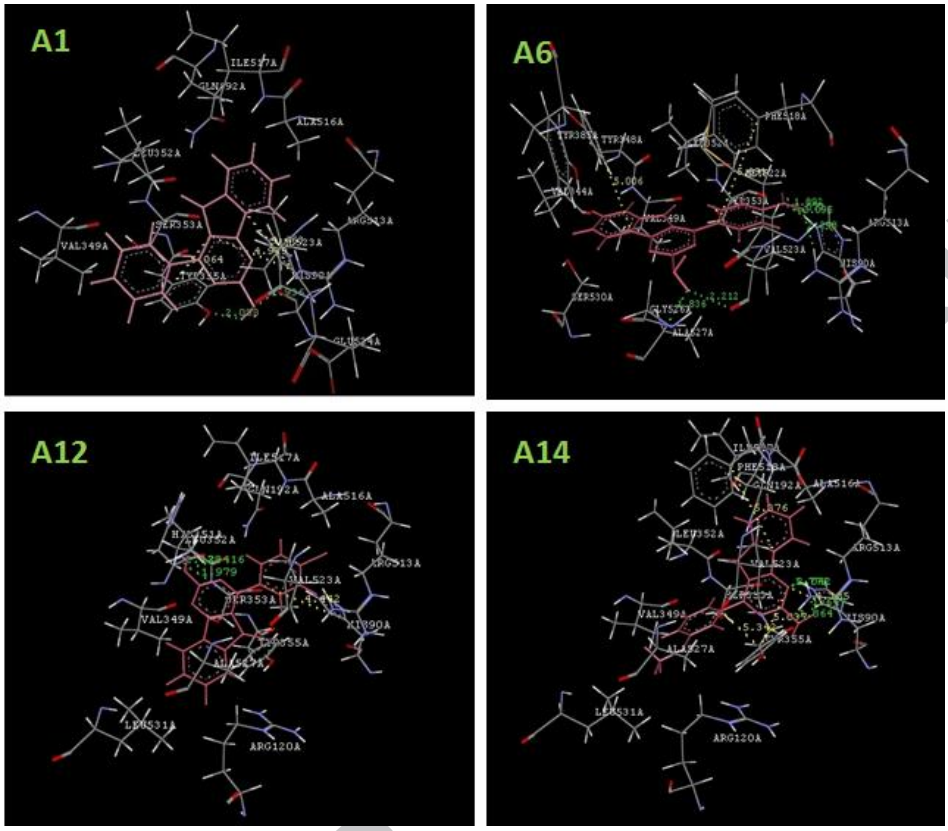


Figure 3 Pharmacophoric hypothesis of the synthesized compounds

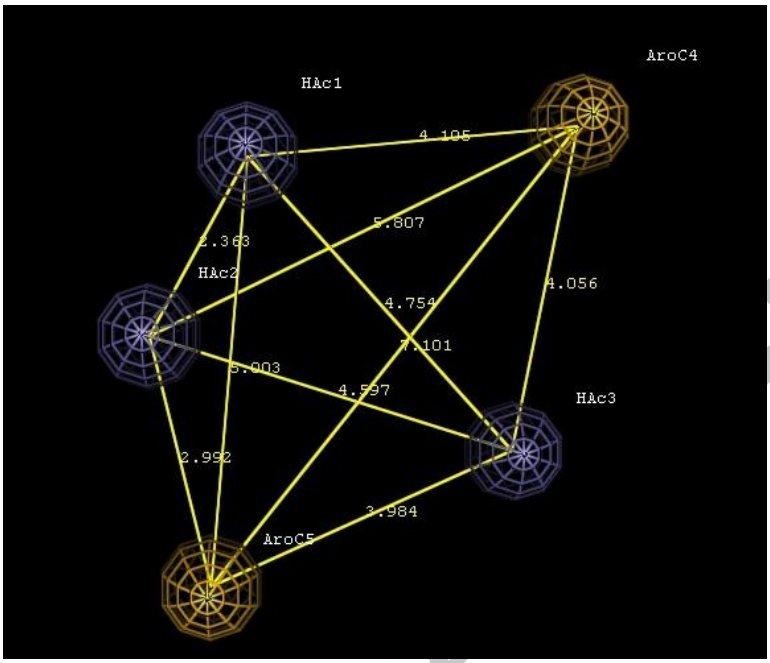
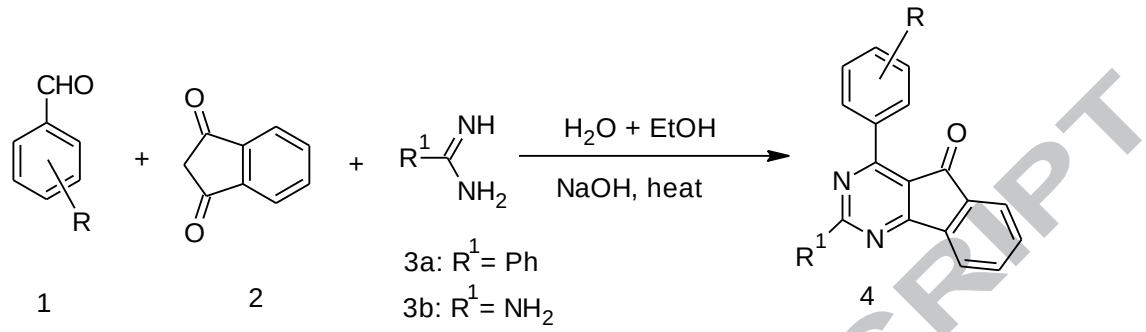


Table 5 Pharmacokinetic properties for good oral bioavailability of selected compounds

Entry	MW	H-acceptor count	H-Donor count	Rotatable bond count	XlogP	% oral absorption
Diclofenac sodium*	318.13	3	1	4	1.45	--
A1	273.29	2	1	2	2.721	82.0520
A6	318.29	5	1	3	2.593	87.8446
A7	318.29	5	1	3	2.593	69.4285
A9	363.37	5	1	8	2.61	69.4285
A11	377.44	2	0	5	5.125	75.6799
A12	352.19	2	1	2	3.517	85.2364
A14	303.32	3	1	4	2.684	85.2364

*Reference no: 38-39.

Scheme 1 General reaction for the synthesis of indenopyrimidine derivatives



Grapical Abstract

The synthesized indenopyrimidine derivatives were screened for their anti-inflammatory, ulcerogenic and cyclooxygenase activities.