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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Ulcerogenicity devoid novel non-steroidal anti-inflammatory agents (NSAIDS): syntheses, computational studies, and activity of 5-aryliden-2-imino-4-thiazolidinones

Neha Singh · Avinash C. Tripathi · Aseem Tewari · Ravi Kumar · Shailendra K. Saraf

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Abstract A series of new 5-aryliden-2-imino-4-thiazolidinones (**5a–e** and **6a–e**) were synthesized via a three-step reaction and characterized by physicochemical and spectral data. The uniqueness of the derivatives lies in the fact that none of them had an acidic group, like conventional NSAIDS, but exhibited significant in vivo activity in acute inflammation models. In particular, 5-(3-chlorobenzyliden)-2-(pyridin-2-yl-imino)-4-thiazolidinone(**5a**) and 5-(3-chlorobenzyliden)-2-(5-methylisoxazol-3-yl-imino)-4-thiazolidinone (**6a**) showed remarkable paw oedema inhibition (67.76 and 74.47 % oedema inhibition, respectively, after 3 h) comparable to that of Ibuprofen (74.56 % oedema inhibition, after 3 h) at half of the dose of the standard drug. Also, compounds **5a** (72.86 %) and **6a** (80.20 %) were found to possess significant inhibition of albumin denaturation when screened for in vitro anti-inflammatory activity. In addition, these compounds were docked into the known active site of COX-2 protein using Glide XP and QPLD algorithms, and the binding-free energy was calculated using Prime MM/GBSA simulation methods. The combined use of molecular docking and MM/GBSA methods gave a good correlation between the predicted binding-free energy and experimentally determined biological activities. It was also evident from the docking results that 2-methylisoxazolylimino or 2-(pyridin-2-yl-imino substitution and 3-chloro moiety on 5-benzylidin nucleus of these 4-thiazolidinone derivatives can easily occupy the COX-2 binding pocket, considered as the critical interaction for COX-2 inhibition. Moreover, pharmacokinetic properties of all the synthesized compounds were predicted, with good results. Further, the synthesized derivatives showed neither acute toxicity nor symptoms of gastric ulceration, at extended doses, owing to the absence of an acidic group.

Keywords NSAIDS · 4-Thiazolidinones · COX-2 inhibitors · Glide-XP docking · MM/GBSA · In silico ADME prediction

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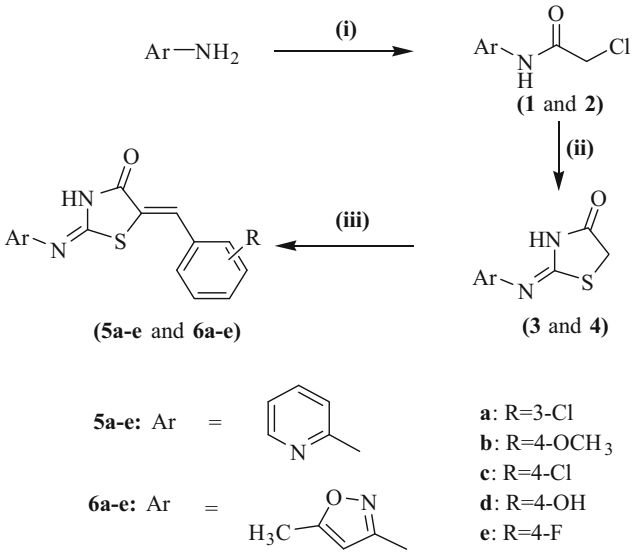
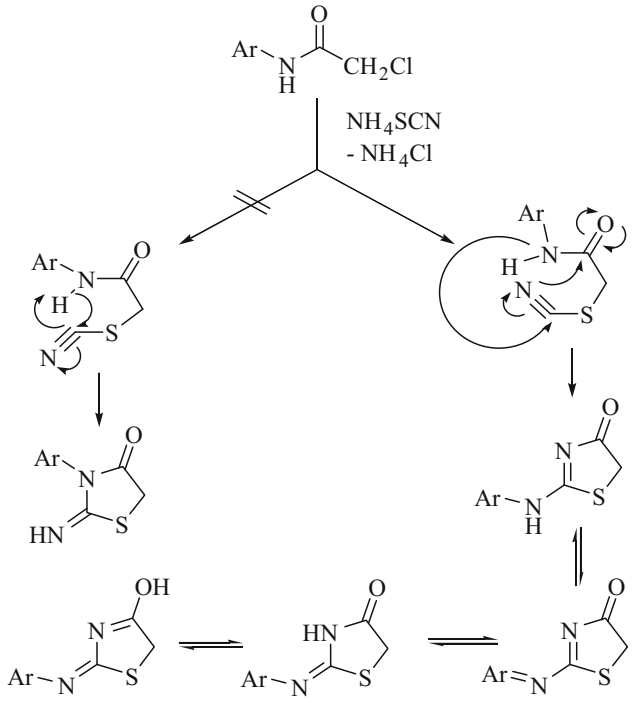
Introduction

Inflammation is a multi-factorial process. It reflects the response of an organism to various stimuli and is related to many disorders such as arthritis, asthma, and psoriasis, which require prolonged or repeated treatment. Although several mediators support the inflammatory processes, the main target of nonsteroidal anti-inflammatory drugs (NSAIDs) is cyclooxygenase (COX) enzyme. Traditional NSAIDs act via the inhibition of the COX-1 isoenzyme or the combined inhibition of COX-1 and COX-2 isoenzymes (Geronikaki *et al.*, 2008; Leval *et al.*, 2002; Vane and Botting 1987). Because COX-2 isoenzyme was found to be over expressed during inflammation, drug investigation was focused on selective COX-2 inhibition, hoping to prevent inflammation by sidestepping the undesired side

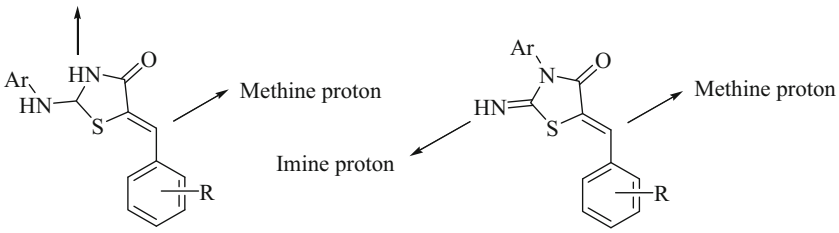


Scheme 1 Synthesis of 5-aryliden-2-imino-4-thiazolidinones. Reagents and conditions *i*$ClCOCH_2Cl$, DMF, RT, 4h; *ii* NH_4SCN, EtOH, reflux, 1h;*iii* RC_6H_4CHO, CH_3COOH, CH_3COONa, reflux.



Scheme 2 Mechanistic pathway for 2-iminothiazolidin-4-ones (**3** and **4**) and its tautomers

Scheme 3 Different protons of 5-arylidene-2-(heteroarylimino)-4-thiazolidinones observed in ^1H NMR spectra



effect of COX-1 or non-selective inhibitors. Recently, highly selective COX-2 inhibitors belonging to the classes of diarylheterocycles and methanesulfonanilides have been developed and marketed (de Leval *et al*., 2000; Graul *et al*., 1997). Such kind of compounds have shown interesting stereoselective anti-inflammatory/analgesic activities, together with better gastrointestinal safety profile than known NSAIDs, suggesting they might preferentially interact with inducible COX-2 isoform. Suitable selectivity assays as well as molecular modeling studies had also supported this hypothesis (Ottana *et al*., 2002). Thiazolidinone derivatives possessing anti-inflammatory and analgesic activities have been reported in the literature (Goel *et al*., 1999; Kumar *et al*., 2003, 2007; Ottana *et al*., 2005; Previtera *et al*., 1987; Vazzana *et al*., 2004; Vigorita *et al*., 2003). In addition to the above mentioned activities, thiazolidinone derivatives possessing antitumour, antimicrobial, anticancer, anti-HIV, and various other activities have also been reported (Agrawal *et al*., 2013; Chawla *et al*., 2011a, b; Chen *et al*., 2009; Gouveia *et al*., 2009; Havrylyuk *et al*., 2010; Omar *et al*., 2010; Rawal *et al*., 2007; Sadashiva *et al*., 2009; Tripathi *et al*., 2014; Verma and Saraf 2008; Zhang *et al*., 2009; Zhou *et al*., 2008). Moreover, pyridine and isoxazole are other important pharmacodynamic heterocyclic nuclei which, when incorporated in different heterocyclic templates, have been reported to possess potent anti-inflammatory activity (Balsamo *et al*., 2003; Haviv *et al*., 1983; Hosni and Abdulla 2008; Sondhi *et al*., 2008; Thirumurugan *et al*., 2010). Thus, it was decided to synthesize a new series of structurally novel 4-thiazolidinone derivatives by incorporating pyridine and isoxazole moieties at 2-position of the thiazolidinone nucleus. The structural variations were selected by introducing different arylidene substituents at the 5-position of the thiazolidinone nucleus. The effect of chloro, methoxy, hydroxy, and fluoro substituents of the arylidene moiety on the anti-inflammatory profile was considered. The groups were selected on the basis of previous SAR studies on thiazolidinone nucleus (Geronikaki *et al*., 2008; Ottana *et al*., 2005), and also with a view at the most frequent substituents in coxibs structure. The proposed 5-arylidene-2-imino-thiazolidin-4-one derivatives can be considered as diarylheterocyclic coxib like compounds, with promising anti-inflammtory potential.

Table 1 Percent oedema inhibition, percent inhibition of denaturation and percent hydrolysis data of the synthesized compounds **5a–e** and **6a–e**

Comps.	Dose (mg/kg b.w.)	% Oedema inhibition				Ulcerogenicity (at tested dose)	ALD_{50}	[a]Mean absorbance ± SD	% Inhibition of denaturation	% Hydrolysis	
		1 h	3 h	5 h	7 h					SGF	SIF
5a	50	−92.12	**67.76**	59.16	43.08	NO	>500	0.1694 ± 0.003	**72.86**	08.21	67.76
5b	50	−80.00	60.24	52.92	33.76	NO	>500	0.1449 ± 0.009	47.86	21.05	60.24
5c	50	−62.24	56.32	51.28	30.48	NO	>500	0.1628 ± 0.012	66.12	40.60	56.32
5d	50	−34.64	47.40	32.24	23.40	NO	>500	0.1598 ± 0.002	63.02	19.54	47.40
5e	50	−82.84	56.68	53.20	43.36	NO	>500	0.1559 ± 0.003	59.08	09.35	56.68
6a	50	−90.28	**74.47**	64.76	35.36	NO	>500	0.1864 ± 0.012	**80.20**	11.24	74.47
6b	50	−49.04	67.20	40.40	28.08	NO	>500	0.1272 ± 0.001	29.80	03.69	67.20
6c	50	−79.20	61.32	46.08	42.92	NO	>500	0.1672 ± 0.003	70.61	14.64	61.32
6d	50	−57.12	50.80	44.12	37.96	NO	>500	0.1472 ± 0.001	50.20	06.57	50.80
6e	50	−44.23	55.40	44.20	16.92	NO	>500	0.1330 ± 0.002	35.71	09.04	55.40
Control	1 % NaCMC	–	–	–	–	NO	>500	0.0980 ± 0.009	–	–	–
Ibuprofen	100	−75.00	74.56	65.24	42.04	Observed	>500	0.1901 ± 0.001	93.98	–	–

Most active derivatives have been represented in bold

NO not observed

[a] Values were recorded in triplicates (i.e., Mean ± SD)

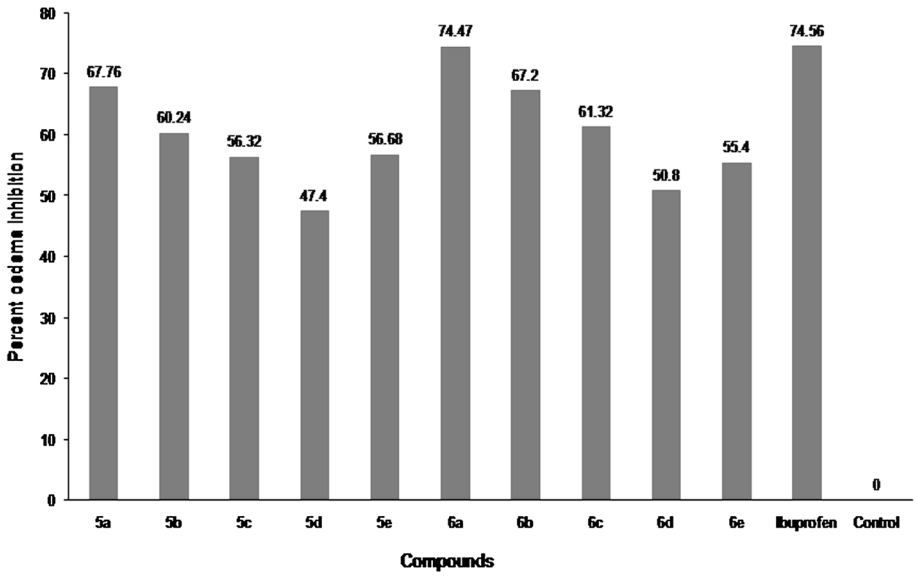


Fig. 1 Percent oedema inhibition by synthesized compounds **5a–e** and **6a–e** at 3 h

Results and discussion

Chemistry

2-Chloro-*N*-(etheroaryl) acetamides (1 and 2) were synthesized using a reported procedure (Geronikaki and Theofilidis 1992), and these 2-chloro-*N*-(etheroaryl) acetamides were then reacted with heteroaromatic amines, in the presence of ammonium thiocyanate and refluxing ethanol, to efficiently produce 2-(heteroarylimino) thiazolidin-4-ones **(3** and **4)**. The 5-aryliden-2-imino-4-thiazolidinones **(5a–e** and **6a–e)** were obtained by refluxing 2-(heteroarylimino) thiazolidin-4-ones with appropriate aldehydes in buffered glacial acetic acid (Scheme 1).

All the synthesized compounds **5a–e** and **6a–e** were characterized by melting point, elemental analysis, and spectral data (IR, MS, ^{1}H NMR, and ^{13}C NMR). The suggested mechanisms of the heterocyclization step and the theoretically tautomeric forms of key intermediate 2-iminothiazoldin-4-ones (3 and 4) have been shown in Scheme 2. The features of a γ-lactam heterocycle for the compounds of series **3** and **4** were supported by the IR

Table 2 XP Glide scores, QPLD, and MM-GBSA scores of the synthesized compounds **5a–e** and **6a–e**

Comps.	XP Glide scores	QPLD scores	MM–GSBA scores
5a	**−9.04672**	**−8.841**	**−128.673**
5b	−7.65001	−7.913	−120.124
5c	−8.95744	−8.236	−125.441
5d	−8.36668	−7.926	−114.232
5e	−8.56640	−8.675	−120.988
6a	**−8.96656**	**−8.982**	**−127.303**
6b	−7.85296	−7.457	−119.08
6c	−8.45790	−8.467	−126.297
6d	−7.80472	−8.180	−118.248
6e	−7.90542	−7.084	−113.081

XP Glide score obtained after redocking of native co-crystallized ligand Celicoxib in COX−2 binding site was −11.2541(RMSD between two poses = 0.4382)

Most active derivatives have been represented in bold

XP (*Extra Precision*) Glide score, *MM-GBSA* molecular mechanics generalized born surface area, *QPLD* quantum polarized ligand docking

spectral data. Characteristic peaks of γ-lactam heterocyclic compounds were determined to be 3,174–3,072 cm^{-1} (multiple band) for the –NH group, and a strong band of the –C=O group in the 1,725–1,687 cm^{-1} region (Ottana *et al*., 2005; Pavia *et al*., 2007). IR spectra of all the compounds were in accordance with the reported values for the characteristic groups.

All the compounds (**5a–e** and **6a–e**) can exist as potential E and Z geometrical isomers; the Z conformation of the 5 exocyclic C=C double bond was assigned on the basis of ^{1}H NMR spectroscopy and on the basis of tha literature data for analogous 4-thiazolidinones and 2,4-thiazolidinediones (Bruno *et al*., 2002; Ottana *et al*., 2005). The 1H NMR spectra of the compounds **5a–e** and **6a–e** showed only one kind of methine proton that, deshielded by the adjacent C=O group, was detected at 6.172–8.525 ppm, at higher chemical shift values than the expected ones for *E* isomers that have a methane proton with a lesser deshielding effect. The –NH proton observed at 12.322–12.729 ppm showed the substitution on 2-position rather than on 3-position, in agreement with a lactam proton since an imine proton appears at a much higher field (about 9.70) (Scheme 3).

Anti-inflammatory activity

The novel 4-thiazolidinones (**5a–e** and **6a–e**) were explored for their in vivo anti-inflammatory activity by carrageenan-induced rat paw oedema model and in vitro screening by inhibition of albumin denaturation. For in vivo screening, injection of carrageenan into the rat paw produced a marked increase of volume from the first hour, reaching its maximal effect after 3–4 h. Pretreatment of rats with synthesized compounds, administered (50 mg/kg body weight, p.o) 1 h before the carrageenan injection, significantly attenuated inflammatory response.

Table 3 Comparison of performance of docking scoring functions with biological data

Biological data	In correlation with (correlation coefficients)		
	XP Glide scores	QPLD scores	MM–GSBA scores
In vivo data (i.e., percent oedema inhibition)	−0.372495321	−0.407489121	−0.682696804
In vitro data (i.e., percent inhibition of denaturation)	−0.844114526	−0.882300416	−0.730207507

The percent inhibition of inflammation was calculated for each compound at different intervals of time and compared with that of the standard. All the synthesized compounds showed maximum percent of oedema inhibition at third hour after carrageenan administration (Table 1). The 3-chloroarylidene derivative (**6a**) proved to be the most effective compound of the series, achieving an inhibition level (74.47 % oedema inhibition, after 3 h) similar to that of the standard drug, ibuprofen (74.56 % oedema inhibition, after 3 h) at half the dose of the standard. Moreover, the comparison between the two series of 5-arylidene-2-imino-thiazolidin-4-ones, i.e., 5-arylidene-2-(pyridin-2-yl-imino)-4-thiazolidinones (**5a–e**) and 5-arylidene-2-(5-methylisoxazole-3-yl-imino)-4-thiazolidinones (**6a–e**), indicated that the compounds substituted with 5-methyl-3-amino isoxazole moiety at the 2-position were more active as compared to those having substitution of 2-aminopyridine at the 2-position. The compounds substituted at para position showed less activity than the meta-substituted compounds at the same interval of the time. Compounds having 4-OCH_3 substituents (**5b** and **6b**) also showed very good oedema inhibition, (60.24 and 67.20 %, respectively), at the third hour. Addition of a hydroxyl group at the para position resulted in less active compounds (47.40 %, **5d** and 50.80 %, **6d** oedema inhibition, respectively) compared to the other compounds of the series. The replacement of the 4-hydroxy substituent with an electron withdrawing group, such as chlorine and fluorine, increased the activity of the compounds (*p*-Cl (**5c**); 56.32 %, *p*-F (**5e**); 56.68 %, *p*-Cl (**6c**); 61.32 %, *p*-F (**6e**); 55.40 %, oedema inhibition, respectively). The maximum percent inhibition found after 3 h was plotted for each compound and is shown in Fig. 1.

All the synthesized compounds (**5a–e** and **6a–e**) were also screened in vitro, for their anti-inflammatory potential,

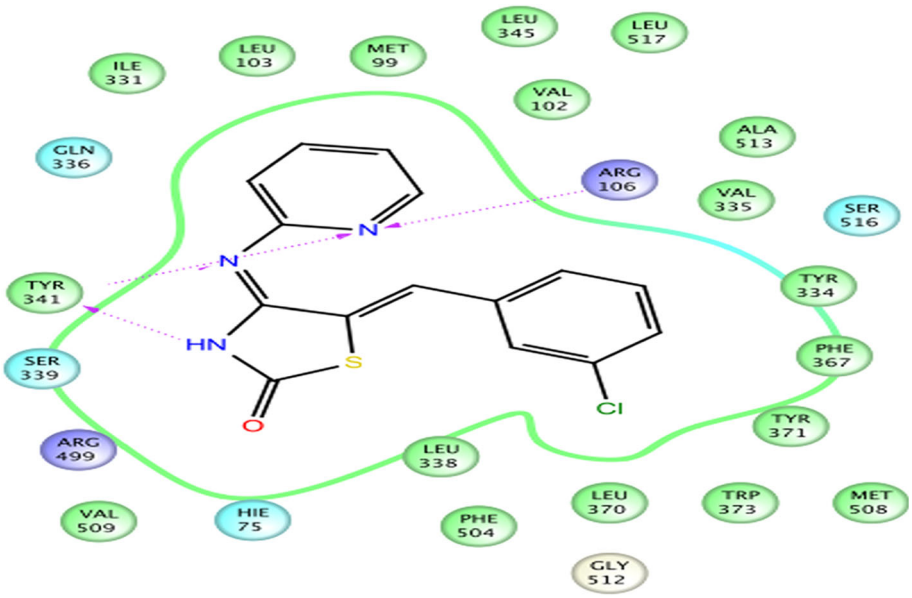


Fig. 2 2D plot of ligand–receptor interaction of compound **5a**

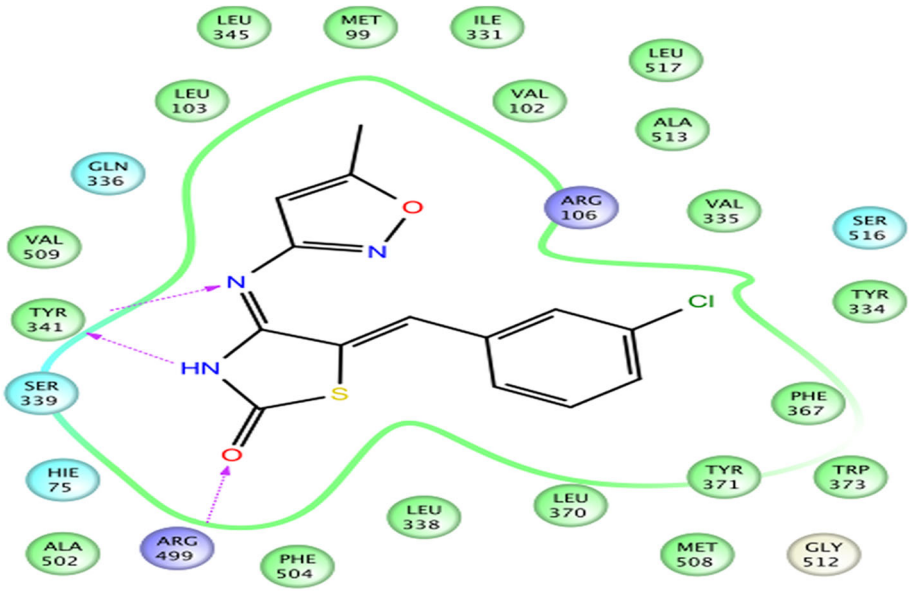


Fig. 3 2D plot of ligand–receptor interaction of compound **6a**

using inhibition of albumin denaturation, and the results are summarized in Table 1. The compounds **5a** and **6a** emerged as the most potent compounds of the series, showing almost similar trend as observed for in vivo activity.

None of the synthesized compounds showed evidence of acute toxicity up to a dose level 500 mg/kg b.w., suggesting their good safety margin. Also, these derivatives were found to possess no ulcerogenicity, in comparison to that exhibited by the standard drug Ibuprofen, when tested.

5-Aryliden-2-imino-4-thiazolidinone derivatives were evaluated for the influence of log *P* value on the anti-inflammatory effect. 3-Chloroarylidene derivative, **5a** was the most lipophilic (log *P*; 2.85) as well as one of the most active (74.47 % oedema inhibition) compounds of the series. Introduction of the hydroxyl group at para position reduced both lipophilicity and the potency (**5d**: log *P*; 1.95, 47.40 %, and **6d**: log *P*; 1.38, 50.80 % oedema inhibition). Hence, it was found that the activity of a compound was in direct relation with its lipophilicity (Table 2).

All the synthesized compounds were hydrolyzed more in SIF than the SGF, thereby indicating their stability in the gastro-intestinal tract. In SGF, the maximum hydrolyzed compound of the series was **5c** (40.67 %) while the least

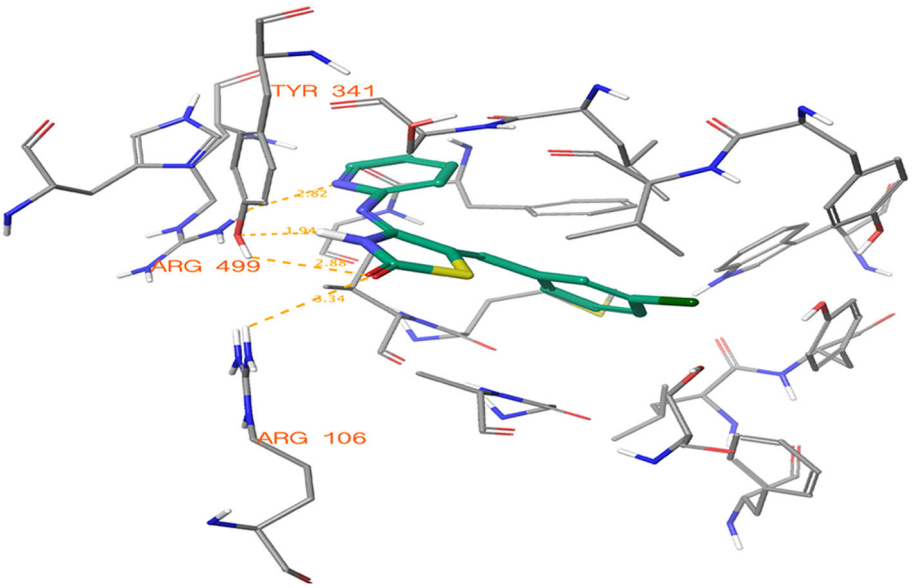


Fig. 4 3D binding interactions of compound **5a** to the COX-2 receptor site (PDB ID-3LN1)

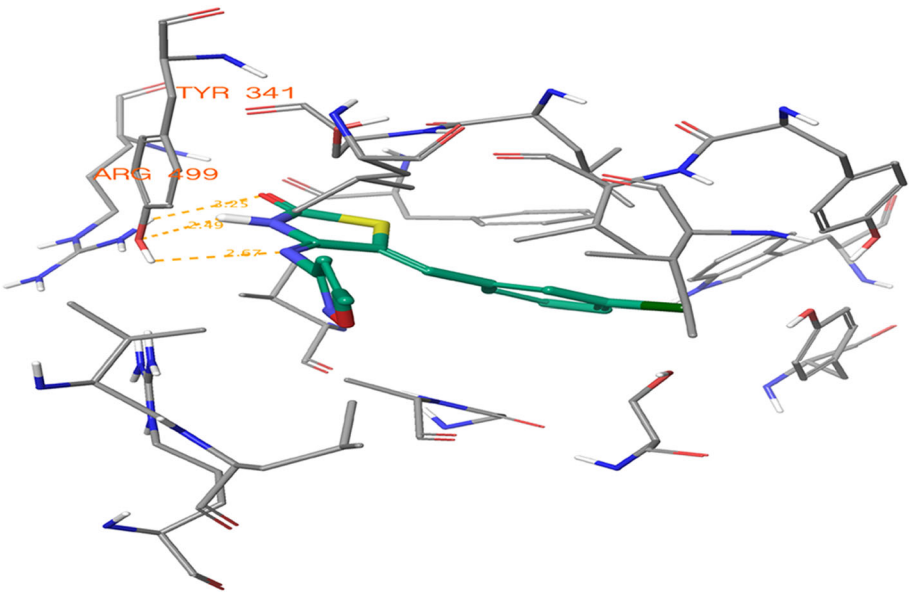


Fig. 5 3D binding interactions of compound **6a** to the COX-2 receptor site (PDB ID-3LN1)

hydrolyzed compound was **6b** (3.69 %). In SIF, compound **5c** (96.42 %) was the maximum hydrolyzed while **6e** (13.79 %) was the least hydrolyzed.

The inflammation induced by carrageenan is believed to be biphasic. The early phase (up to 1 h) involves the release of histamine, serotonin, and bradykinin, and the late phase (over 1 h) is due to the release of prostaglandin like substances. Therefore, the decrease of inflammation in the second phase indicates the inhibition of cyclooxygenase enzyme (Salvemini *et al*., 1996; Sauzem *et al*., 2008). Carrageenan-induced paw oedema was significantly prevented in a time-dependent manner by previous treatment with compounds **5a–e** and **6a–e** (Table 1). None of the tested compounds exhibited inhibitory effect up to 1 h after the carrageenan injection. However, all the compounds inhibited increase in paw volume from the third to the fifth hour after carrageenan administration. This inhibition of paw oedema, only in the late phase of inflammation, suggests that the 5-arylidine-2-imino-thiazolidin-4-ones (**5a–e** and **6a–e**) may act on the prostaglandin biosynthesis by inhibition of COX-2 enzyme, which is involved in the late phase of carrageenan-induced inflammation.

In order to ascertain COX-2 enzyme inhibition and nature of ligand–receptor interaction, docking experiments were carried out into the active site of COX-2 enzyme using Glide-Extra Precision (XP) mode from Schrödinger

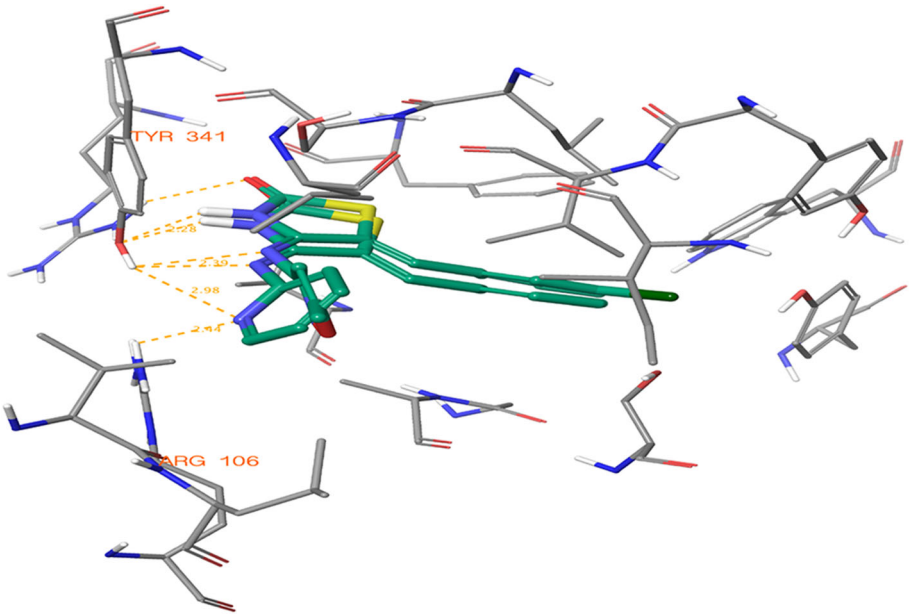


Fig. 6 3D binding interactions of the superimposed compounds **5a** and **6a** to the COX-2 receptor site (PDB ID-3LN1)

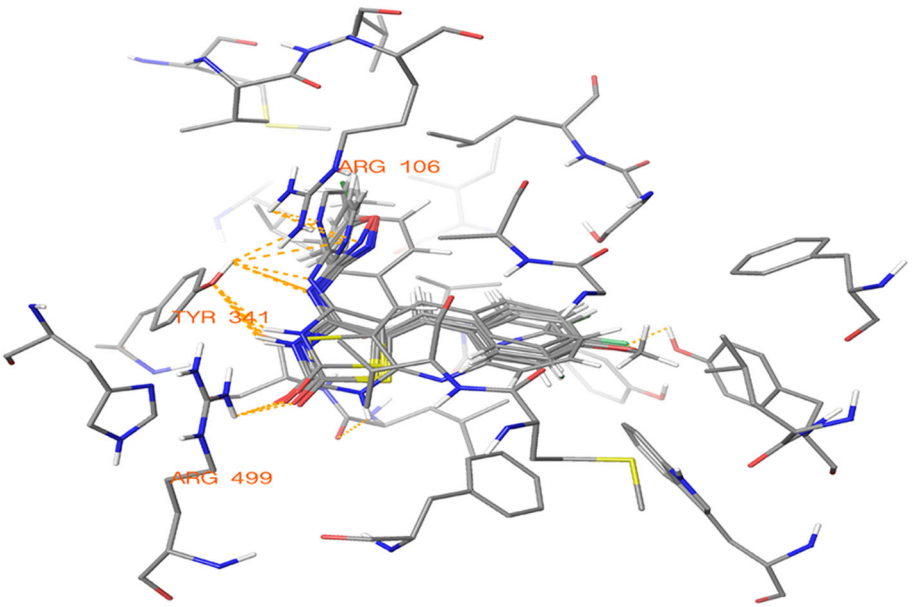


Fig. 7 3D binding interactions of all the superimposed derivatives (**5a–e** and **6a–e**) to the COX-2 receptor site (PDB ID-3LN1)

Inc. Results of the study showed good comparative scores (Table 2) and a remarkable correlation (−0.844114526) with in vitro data (Table 3). To validate the docking protocol, re-docking experiment was performed in which the ligand conformation of Celicoxib was extracted from the crystal structure of the corresponding COX-2/ligand complex and later docked back into the binding pocket. Glide-XP was able to perfectly reproduce the experimental position (Fig. 2) of the ligand (with a Glide score of −11.2541 and RMSD of 0.4382 from native co-crystallized structure), confirming the ability of the method to accurately predict the binding conformation. Also, to study the accurate role of electrostatic charges in determining the ligand bound conformation, QPLD has been performed which combines the docking power of Glide with the accuracy of Q Site. QPLD scores were found to be in good correlation with the results of in vitro studies and evidenced a noteworthy correlation coefficient of −0.882300416 with QPLD scores (Table 3). Moreover, MM/GBSA approach was employed to study the energetics of biomolecular systems and especially to determine the binding-free energies in bimolecular complex (i.e., protein-drug complex). A reasonable correlation of MM/GBSA scores with in vivo (−0.682696804) as well as in vitro (−

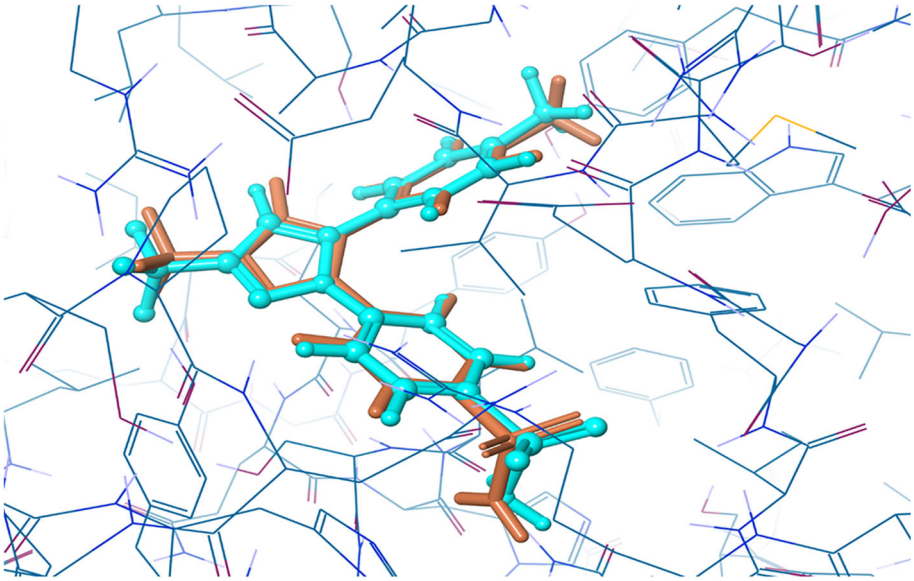


Fig. 8 Superimposition of co-crystallized (*Brown color*) and re-docked pose (*Aqua color*) of Celicoxib into the COX-2 receptor site (PDB ID-3LN1) (Color figure online)

Table 4 In silico predicted pharmacokinetic properties of synthesized compounds **5a–e** and **6a–e** using QikProp program from Schrodinger Inc

Comps.	MW	donarHB	accptHB	QlogPo/w predicted (observed)	metab	QPlog Khsa	% Human oral absorption	Volume	PSA	Violations of rule of five
5a	315.776	1	4.50	3.095 (2.85)	1	0.119	94.454	930.550	69.058	0
5b	311.358	1	5.25	2.705 (2.52)	2	0.017	92.161	961.948	77.356	0
5c	315.776	1	4.50	3.100 (2.68)	1	0.120	94.482	931.158	69.062	0
5d	297.331	2	5.25	1.872 (1.95)	2	−0.124	78.000	909.755	91.615	0
5e	299.322	1	4.50	2.845 (2.69)	1	0.049	92.988	903.124	69.062	0
6a	319.765	1	5.00	2.620 (2.48)	1	0.042	86.947	939.184	83.379	0
6b	315.346	1	5.75	1.666 (1.54)	2	−0.063	81.364	970.235	91.671	0
6c	319.765	1	5.00	2.621 (2.43)	1	0.042	86.962	939.306	83.368	0
6d	301.319	2	5.75	1.433 (1.38)	2	−0.191	70.715	917.875	105.93	0
6e	303.310	1	5.00	2.141 (2.27)	1	−0.032	83.231	909.362	84.735	0
Standard values/ range	130–725	0–6	2–20	−2.0 to 6.5	1–8	−1.5 to 1.5	>80 % is high; <20 % is poor	500–2,000	7–200	Maximum is 4

donorHB Hydrogen bond donor, *accptHB* Hydrogen bond acceptor, *metab* Number of likely metabolic reactions, *QPlogKhsa* Prediction of binding to human serum albumin; *Volume* Total solvent accessible volume in cubic angstrom using a probe with 1.4 Å radius, *PSA* Van der Waals polar surface area of nitrogen and oxygen atoms, Number of violations of Lipinski's rule of five

0.730207507) was observed (Table 3). Finally, results of the docking studies showed that compounds **5a** and **6a** were the most promising compounds in the series, with good scoring functions (Table 2).

The most stable ligand–receptor complex configurations and the interactions of the most active compounds **5a**, and **6a** with amino acid residues have been depicted in Figs. 2, 3, 4, 5, 6, 7 and 8. Compounds **5a** and **6a** clearly preferred a single binding position in the typical binding pocket of COX-2, which were the best predictions since these had the lowest energy among the others. In such an orientation, the bound conformation of compound **6a** was engaged in hydrogen bonding with Tyr341 and Arg499; polar interaction with Gln178, Ser339, Ser516, and His75; charged (positive) interaction with Arg106 and Arg499 and was involved in hydrophobic contacts with the residues of the binding pocket Val509, Tyr341, Ala513, Leu517, Val335, Tyr334, Trp373, Phe367, Tyr371, Leu370, Met508, Leu338, Ile503, Ala502, and Phe504 (Figs. 4, 6). However, that of compound **5a** involved hydrogen bonding with Tyr341 and Arg106; polar interaction with Ser339, Ser516, and His75, charged (positive) interaction with Arg106 and

Arg499, hydrophobic interaction with Val509, Tyr341, Ala513, Leu345, Met99, Val102, Leu517, Val335, Tyr334, Phe367, Tyr371, Trp373, Met508, Leu370, Leu338, and Phe504 (Figs. 3, 5).

Previous studies had shown that the COX-2 binding site was 25 % larger than that of COX-1, due to the substitution of a single amino acid (valine for isoleucine at position 523), thus creating a secondary internal pocket not accessible in COX-1(Gierse *et al*., 1996). It was suggested that the selectivity of Celecoxib for COX-2 over COX-1 might result from additional contacts with residues along this deeper binding region. In fact, the structural analysis of Celecoxib/COX-2 complex indicated that the selective inhibitor molecule interacted with amino acid residues located at the bottom of the COX-2 gorge. It is noteworthy that compounds **5a** and **6a** were able to reach such a gorge, rationalizing the COX- 2 selectivity.

Also, in silico pharmacokinetic properties of the synthesized compounds **5a–e** and **6a–e** were predicted using Qik Prop program from Schrodinger Inc., which showed promising results (Table 4). Therefore, all the synthesized derivatives can be good drug candidates as they fulfill the ADME prerequisites.

Conclusion

The synthetic strategy adopted here is a versatile approach for the preparation of thiazolidinone derivatives. The results obtained expound that the different synthesized derivatives can be utilized as promising anti-inflammatory agents when a differently substituted arylidene moiety is inserted at position-5 (**5a–e** and **6a–e**). However, compounds substituted with isoxazole moiety at position-2 (**6a–e)** were found to be more potent anti-inflammatory agents than the compounds substituted with aminopyridine moiety (**5a–e**). In particular, on the substitution with meta-chloroarylidene (**6a**), a good in vivo (74.47 % oedema inhibition, 3 h), in vitro (80.20 % inhibition of denaturation) anti-inflammatory profile was achieved reaching Ibuprofen level but half at the dose. The compounds substituted at para position showed less potency than the meta-substituted compounds at the same intervals of time. Also, a straight relationship was observed between log P and anti-inflammatory activity, i.e., more lipophilic derivatives were found to possess better activity. The synthesized thiazolidin-4-one derivatives neither possess any acidic functional group such as carboxyl nor a functional group susceptible to hydrolysis. Moreover, the results of the stability studies in SGF indicated that the compounds were stable in SGF, thus indicating their stability in the gastro-intestinal tract. ADME characteristics of the synthesized compounds were also approximated by in silico methods and found to be within the range. All the compounds were found to inhibit paw oedema only in the late phase (3 h) of inflammation, suggesting that they were involved in the inhibition of the prostaglandin biosynthesis, similar to coxib-like compounds, which was also proven by different docking algorithms (such as XP-Glide, MM/GBSA, and QPLD) with good docking scores. Their interaction to COX-2 enzyme binding site showed excellent correlation with the anti-inflammatory activity. None of the compound in the synthesized series was found to possess ulcerogenicity or acute toxicity at extended dose levels (upto 500 mg/kg b.w.) suggesting their safety in terms of undesired side effects, such as ulceration, associated with most of the NSAIDs.

Experimental

Chemistry

Synthetic starting material, reagents and solvents were of reagent grade and procured from S.D. Fine Chemicals, Mumbai and were used without further purification. Melting range was determined using melting point apparatus by open capillary method and is uncorrected.

The progress of the reactions was monitored by performing thin layer chromatography (TLC) on silica gel G plates, using iodine vapors and UV light as the visualizing agents. UV spectra were recorded on Shimadzu UV-1700 spectrophotometer. IR spectra were recorded, using KBr pellet technique, on a FTIR-8400S spectrophotometer (Shimadzu) and the reported values are expressed in cm^{-1}. Mass spectra were recorded on a JEOL-AccuTOF JMS-T100LC spectrometer having a DART (Direct Analysis in Real Time) source. ^{1}H-NMR spectra were recorded on a Bruker DRX-300 (at 300 MHz) spectrometer and ^{13}C-NMR data were recorded on Advance-400 MHz, Bruker (Switzerland) spectrometer. Chemical shifts are reported as δ (ppm) relative to TMS as the internal standard. Elemental analysis was performed on an Elemental Vario EL III analyzer, for C, H and N. The found values were within the range of the theoretical ones. The spectral and elemental analysis was carried out at the Sophisticated Analytical Instrument Facility (SAIF), Central Drug Research Institute (CDRI), Lucknow.

*General procedure for synthesis of 2-chloro-N-(etheroaryl) acetamide (**1** and **2**)*

To a solution of appropriate amine (0.02 mol) in 6.5 mL dimethylformamide (DMF), a cooled solution of chloroacetylchloride (2.5 mL, 0.03 mol) in 6.5 mL DMF was added drop wise. The reaction mixture was refluxed on a

water bath at 50 °C for 4 h. The completion of the reaction was monitored by TLC. The residue was washed with 10 % aqueous sodium bicarbonate solution followed by cold water. The crude product was dried and crystallized from ethanol (Geronikaki and Theofilidis 1992).

*2-Chloro-N-(pyridin-2-yl) acetamide (**1**)* Yield: 15.28 %. mp: 180–182 °C, TLC: methanol/diethylether (0.5:9.5). IR (KBr) ν_{max}/cm^{-1}: 3064.75 (N–H), 1770.53 (C=O), 1649.02 (C=N), 773.40 (C–Cl). $C_7H_7ClN_2O$ (170.02); Observed molecular ion peak $[M+1]^+$: 171.03.

*2-Chloro-N-(5-methylisoxazol-3-yl) acetamide (**2**)* Yield: 25.97 %. mp: 192–194 °C, TLC: methanol/diethylether (0.5:9.5). IR (KBr) ν_{max}/cm^{-1}: 3222.83 (N–H), 1712.67 (C=O), 1639.38 (C=N), 1205.43 (C–O), 756.04 (C–Cl). $C_6H_7ClN_2O_2$ (174.02); Observed molecular ion peak $[M+1]^+$: 175.0.

*General procedure for synthesis of 2-(heteroarylimino) thiazolidin-4-ones (**3** and **4**)* A solution of 2-chloro-*N*-(etheroaryl) acetamide (5 mmol) and ammonium thiocyante (0.759 g, 10 mmol) in 20 mL of absolute ethanol was refluxed for 1 h and allowed to stand overnight. The completion of the reaction was monitored by TLC. The precipitate was filtered, washed with water, and recrystallized from dioxane (Vicini *et al*., 2008).

*2-(Pyridin-2-yl-imino) thiazolidin-4-ones (**3**)* Yield: 19.25 %. mp: 194–198 °C, TLC: methanol/diethylether (0.5:9.5). IR (KBr) ν_{max}/cm^{-1}: 3058.89 (N–H stretch), 1687.60 (C=O lactam), 1623.95 (C=N stretch), 1556.45 (N–H bend). $C_8H_7N_3OS$ (193.03); Observed molecular ion peak $[M+1]^+$: 194.08.

*2-(5-Methylisoxazole-3-yl-imino) thiazolidin-4-ones (**4**)* Yield: 55.26 %. mp: 204–206 °C, TLC: methanol/diethylether (0.5:9.5). IR (KBr) ν_{max}/cm^{-1}: 3415.70 (N–H stretch), 1724.24 (C=N stretch), 1635.52 (N–H bend), 1600.81 (C=O lactam), 1197.71 (C–O stretch). $C_7H_7N_3O_2S$ (195.03); Observed molecular ion peak $[M+1]^+$: 196.04.

*General procedure for synthesis of 5-arylidene-2-(heteroarylimino)-4-thiazolidinones (**5a–e** and **6a–e**)*

A well-stirred solution of 2-(heteroarylimino) thiazolidin-4-one (4 mmol) in 35 mL of acetic acid was buffered with sodium acetate (8 mmol) and added to the appropriate arylaldehyde (6 mmol). The solution was refluxed over different periods till the completion of the reaction. The reaction mixture was then cooled to room temperature and the solid precipitate was filtered, washed with water and then recrystallized from dioxane. The completion of the reaction was monitored by TLC using the solvent system hexane:ethylacetate (2:1) (Vicini *et al*., 2008).

*5-(3-Chlorobenzyliden)-2-(pyridin-2-yl-imino)-4-thiazolidinone (**5a**)* Reaction time: 18 h. Yield: 50.75 %, mp: 192–194 °C. IR (KBr) ν_{max}/cm^{-1}: 3060.82 (N–H stretch), 1710.74 (C=O lactam), 1625.88 (C=N stretch), 1556.45 (N–H bend). ^{1}H NMR ($CDCl_3$–DMSO; δ ppm): 12.531 (s, 1H), 8.508–8.521 (d, 1H), δ 7.848–7.895 (t, 1H), 7.735 (s, 1H), 7.438–7.641 (m, 4H), 7.193–7.233 (t, 2H). ^{13}C NMR ($CDCl_3$–DMSO; δ ppm): 175.21 (CONH, amide), 117.04 (C=C, ethylene), 165.07 (C=N, imino), 127.5–130.04 (4CH, benzene), 133.05 (2C, benzene), 159.26 (C=N, pyridine), 150.30 (CH, pyridine), 116.25 (2CH, pyridine), 135.96 (CH, pyridine). MS $[M+1]^+$: 316. Anal. Calcd. for $C_{15}H_{10}ClN_3OS$ (315.02): C, 57.05; H, 3.19; N, 13.31. Found C, 57.45; H, 3.16; N, 13.46.

*5-(4-Methoxybenzyliden)-2-(pyridin-2-yl-imino)-4-thiazolidinone (**5b**)* Reaction time: 17 h. Yield: 23.33 %; mp: 237–240 °C. IR (KBr) ν_{max}/cm^{-1}: 3467.77 (N–H stretch), 1706.88 (C=O lactam), 1627.81 (C=N stretch), 1560.30 (N–H bend). ^{1}H NMR ($CDCl_3$–DMSO; δ ppm): 12.363 (s, 1H), 8.509–8.521 (d, 1H), 7.834–7.882 (t, 1H), 70619–70649 (d, 3H), 7.179–7.217 (m, 2H), 7.117–7.147 (d, 2H). ^{13}C NMR ($CDCl_3$–DMSO; δ ppm): 174.71 (CONH, amide), 115.46 (C=C, ethylene), 164.23 (C=N, imino), 114.05 (2CH, benzene), 130.41 (2CH, benzene), 126.01 (C, benzene), 160.63 (2CH, benzene), 56.12 (CH_3, methyl) 160.33 (C=N, pyridine), 151.02 (CH, pyridine), 118.3 (2CH, pyridine), 136.11 (CH, pyridine). MS $[M+1]^+$: 312.1. Anal. Calcd. for $C_{16}H_{13}N_3O_2S$ (311.07): C, 61.72; H, 4.21; N, 13.50. Found C, 61.97; H, 4.16; N, 13.46.

*5-(4-Chlorobenzyliden)-2-(pyridine-2-yl-imino)-4-thiazolidinone (**5c**)* Reaction time: 10 h. Yield: 68.99 %; mp: 216–218 °C. IR (KBr) ν_{max}/cm^{-1}: 3475.49 (N–H stretch), 1710.74 (C=O lactam), 1685.67 (C=N stretch), 1625.88 (N–H bend). ^{1}H NMR ($CDCl_3$–DMSO; δ ppm): 8.503–8.516 (d, 1H), 7.845–7.894 (t, 1H), 7.613–7.703 (m, 5H), 7.196–7.233 (m, 2H). ^{13}C NMR ($CDCl_3$–DMSO; δ ppm): 175.69 (CONH, amide), 118.04 (C=C, ethylene), 166.13 (C=N, imino), 129.35–136.11 (6CH, benzene), 161.37 (C=N, pyridine), 151.02 (CH, pyridine), 117.04 (2CH, pyridine), 134.22 (CH, pyridine). MS $[M+1]^+$: 316. Anal. Calcd. for $C_{15}H_{10}ClN_3OS$ (315.02): C, 57.05; H, 3.19; N, 13.31. Found C, 56.21; H, 3.36; N, 13.04.

*5-(4-Hydroxybenzyliden)-2-(pyridine-2-yl-imino)-4-thiazolidinone (**5d**) (Wang et al., 2008)* Reaction time: 22 h. Yield: 33.69 %; mp: 220–223 °C. IR (KBr) ν_{max}/cm^{-1}: 3404.13 (N–H stretch), 1600.81 (C=O lactam), 1581.52(C=

N stretch), 1552.59 (N–H bend). ^{1}H NMR (CDCl3-DMSO; δ ppm): 12.322 (s, 1H), 10.239 (s, 1H), 8.512-8.525 (d, 1H), 7.827–7.874 (t, 1H), 7.510–7.562 (t, 3H), 7.169–7.208 (m, 2H), 6.922–6.951 (d, 2H). ^{13}C NMR ($CDCl_3$-DMSO; δ ppm): 175.88 (CONH, amide), 118.32 (C=C, ethylene), 165.07 (C=N, imino), 115.77 (2CH, benzene), 131.17 (2CH, benzene), 159.84 (C, benzene), 156.89 (C=N, pyridine), 148.98 (CH, pyridine), 113.75 (2CH, pyridine), 137.18 (CH, pyridine). MS $[M+1]^+$: 298.08. Anal. Calcd. for $C_{15}H_{11}N_3O_2S$ (297.06): C, 57.05; H, 3.19; N, 13.31. Found C, 57.93; H, 3.33; N, 13.39.

*5-(4-Fluorobenzyliden)-2-(pyridine-2-yl-imino)-4-thiazolodinone (**5e**)* Reaction time: 15 h. Yield: 29.69 %; mp: 198-200 °C. IR (KBr) ν_{max}/cm^{-1}: 3064.68 (N–H stretch), 1710.74 (C=O lactam), 1683.74 (C=N stretch), 1558.38 (N–H bend), 1234.36 (C–F stretch). ^{1}H NMR (CDCl3–DMSO; δ ppm): 12.473 (s, 1H), 8.507–8.520 (d, 1H), 7.844–7.893 (t, 1H), 7.665–7.760 (m, 3H), 7.388–7.446 (t, 2H), 7.205–7.230 (d, 2H). ^{13}C NMR ($CDCl_3$–DMSO; δ ppm): 171.21 (CONH, amide), 121.04 (C=C, ethylene), 162.07 (C=N, imino), 130.04 (C, benzene), 115.77 (2CH, benzene), 131.17 (2CH, benzene), 159.84 (C, benzene), 159.32 (C=N, pyridine), 150.48 (CH, pyridine), 116.25 (2CH, pyridine), 135.96 (CH, pyridine). MS $[M+1]^+$: 300.08. Anal. Calcd. for $C_{15}H_{10}FN_3OS$ (299.05): C, 60.19; H, 3.37; N, 14.04.

*5-(3-Chlorobenzyliden)-2-(5-methylisoxazol-3-yl-imino)-4-thiazolidinone (**6a**)* Reaction time: 17 h. Yield: 93.70 %; mp: 198–200 °C. IR (KBr) ν_{max}/cm^{-1}: 3060.82 (N–H stretch), 1710.74 (C=O lactam), 1625.88 (C=N stretch), 1556.45 (N–H bend), 1184.21 (C–O strech). ^{1}H NMR ($CDCl_3$–DMSO; δ ppm): 12.729 (s, 1H), 7.698 (s, 2H), 7.521–7.518 (m, 3H), 6.233 (s, 3H), 2.405 (d, 3H). ^{13}C NMR ($CDCl_3$–DMSO; δ ppm): 175.21 (CONH, amide), 117.91 (C=C, ethylene), 166.54 (C=N, imino), 127.29–130.64 (4CH, benzene), 133.77 (2C, benzene), 173.46 (C, isoxazole), 168.63 (C, isoxazole), 101.27 (CH, isoxazole), 13.86 (CH_3, methyl). MS $[M+1]^+$: 320.02. Anal. Calcd. for $C_{14}H_{10}N_3O_2S$ (319.06): C, 52.59; H, 3.15; N, 13.14. Found C, 52.93; H, 3.07; N, 13.26.

*5-(4-Methoxybenzyliden)-2-(5-methylisoxazol-3-yl-imino)-4-thiazolidinone (**6b**)* Reaction time: 16 h. Yield: 46.00 %; mp: 230–232 °C. IR (KBr) ν_{max}/cm^{-1}: 3120.61 (N–H stretch), 1714.60 (C=O lactam), 1639.38 (C=N stretch), 1595.02 (N–H bend), 1174.57 (C–O strech). ^{1}H NMR ($CDCl_3$–DMSO; δ ppm): 7.625 (s, 1H), 7.555–7.583 (d, 2H), 7.098–7.127 (d, 2H), 6.172 (s, 1H), 2.500 (s, 3H). ^{13}C NMR ($CDCl_3$–DMSO; δ ppm): 175.21 (CONH, amide), 117.91 (C=C, ethylene), 166.54 (C=N, imino), 114.05 (2CH, benzene), 131.07 (2CH, benzene), 125.23 (C, benzene), 161.25 (2CH, benzene), 57.01 (CH_3, methyl), 173.72 (C, isoxazole), 167.44 (C, isoxazole), 102.09 (CH, isoxazole), 14.52 (CH_3, methyl). MS $[M+1]^+$: 316.10. Anal. Calcd. for $C_{15}H_{13}N_3O_3S$ (315.06): C, 57.13; H, 4.16; N, 13.33. Found C, 56.01; H, 4.07; N, 13.41.

*5-(4-Chlorobenzyliden)-2-(5-methylisoxazol-3-yl-imino)-4-thiazolidinone (**6c**)* Reaction time: 8 h. Yield: 43.13 %; mp: 224–26 °C. IR (KBr) ν_{max}/cm^{-1}: 2925.81 (N–H stretch), 1724.24 (C=O lactam), 1637.45 (C=N stretch), 1600.81 (N–H bend), 1093.56 (C–O strech). ^{1}H NMR ($CDCl_3$-DMSO; δ ppm): 7.695 (s, 1H), 7.625 (s, 4H), 6.223 (s, 1H), 2.402 (s, 3H). ^{13}C NMR ($CDCl_3$–DMSO; δ ppm): 178.02 (CONH, amide), 119.23 (C=C, ethylene), 165.66 (C=N, imino), 129.35–130.39 (4CH, benzene), 133.48 (2C, benzene), 171.28 (C, isoxazole), 168.11 (C, isoxazole), 101.55 (CH, isoxazole), 16.10 (CH_3, methyl). MS $[M+1]^+$: 321.01. Anal. Calcd. for $C_{14}H_{10}ClN_3O_3S$ (320.07): C, 52.59; H, 3.15; N, 13.14. Found C, 53.04; H, 3.12; N, 13.07.

*5-(4-Hydroxybenzyliden)-2-(5-methylisoxazol-3-yl-imino)-4-thiazolidinone (**6d**)* Reaction time: 18 h. Yield: 55.41 %; mp: 196–198 °C. IR (KBr) ν_{max}/cm^{-1}: 3253.69, (N–H stretch), 1679.88, (C=O lactam), 1631.67, (C=N Stretch), 1577.66 (N–H bend), 1174.57, (C–O stretch). ^{1}H NMR ($CDCl_3$–DMSO; δ ppm): 12.501 (s, 1H), 10.287 (s, 1H), 7.612 (s, 1H), 7.458–7.486 (d, 2H), 6.918–6.946 (d, 2H), 6.221 (s, 1H), 2.403 (s, 3H). ^{13}C NMR ($CDCl_3$–DMSO; δ ppm): 175.67 (CONH, amide), 117.14 (C=C, ethylene), 165.69 (C=N, imino), 131.17 (2CH, benzene), 115.76 (2CH, benzene), 124.69 (C, benzene), 159.84 (C, benzene), 177.04 (C, isoxazole), 167.51 (C, isoxazole), 100.16 (CH, isoxazole), 14.33 (CH_3, methyl). MS $[M+1]^+$: 302.16. Anal. Calcd. for $C_{14}H_{10}ClN_3O_3S$ (301.05): C, 55.89; H, 3.68; N, 13.95. Found C, 55.66; H, 3.52; N, 13.87.

*5-(4-Fluorobenzyliden)-2-(5-methylisoxazol-3-yl-imino)-4-thiazolidinone (**6e**)* Reaction time: 11 h. Yield: 38.38 %; mp: 174–176 °C. IR (KBr) ν_{max}/cm^{-1}: 3124.47, (N–H stretch), 1618.46, (C=O lactam), 1600.81 (N–H bend), 1641.31, (C=N Stretch), 1193.85, (C–O stretch), 1240.14 (C–F, stretch). ^{1}H NMR ($CDCl_3$-DMSO; δ ppm): 12.671 (s, 1H), 7.664–7.719 (m, 3H), 7.373–7.19 (t, 2H), 6.227 (s, 1H), 2.405 (s, 3H). ^{13}C NMR ($CDCl_3$–DMSO; δ ppm): 173.21 (CONH, amide), 119.04 (C=C, ethylene), 168.07 (C=N, imino), 131.22 (C, benzene), 116.08 (2CH, benzene), 131.77 (2CH, benzene), 160.12 (C, benzene), 172.58 (C, isoxazole), 165.44 (C, isoxazole), 101.92 (CH, isoxazole), 18.26 (CH_3, methyl). MS $[M+1]^+$: 304.10. Anal. Calcd. for $C_{14}H_{10}FN_3O_2S$ (303.05): C, 55.44; H, 3.32; N, 13.85.

Pharmacology

In vivo anti-inflammatory screening

Male Wistar albino rats, weighing 175–300 g, were housed in polypropylene cages with steel net, under standard living conditions of temperature 25 ± 2 °C and relative humidity of 55 ± 5, with regular 12 h light and 12 h dark cycles and allowed free access to standard laboratory food and water. All animals were treated humanely in accordance with the guidelines laid down by the Institutional Animal Ethics Committee (IAEC) (Raghvan 2000). The experimental protocols were approved by the IAEC (BBDGEI/IAEC/13/2011).

Group of five animals were used for control and treated rats. The test compounds (at a dose of 50 mg/kg) and the standard drug, ibuprofen (at the dose 100 mg/kg), were used for the study by suspending in 1.0 % sodium carboxymethyl cellulose (NaCMC) and administered orally (p.o.). The paw of the same animal that is not induced for the oedema was used as control and the control group received 1 ml of 1.0 % NaCMC, orally. After 1 h of treatment, 0.1 ml of 1 % carrageenan solution in normal saline was injected subcutaneously into the plantar side of left hind paw to induce inflammation in tested animals. The paw volume was measured, by plethysmometer, immediately after injection and at the time intervals of 1, 3, 5, and 7 h, respectively. The mean paw volume of each group at different time intervals was statically evaluated and compared with that of the control group at the same time intervals. The percent inhibition of each compound was calculated by the following equation; (Ottana *et al*., 2005)

$$\% \text{ Oedema inhibition } = [V_c - V_t)/V_c] \times 100$$

where V_t = volume of paw of tested group, and V_c = volume of paw of control group.

In vitro anti-inflammatory screening

The synthesized compounds were screened for their anti-inflammatory activity using inhibition of albumin denaturation technique, which was studied according to Mizushima and Kobayashi (Mizushima and Kobayashi 1968) with a slight modification. The standard drug and test compounds were dissolved in a minimum quantity of DMF and diluted with phosphate buffer (pH 7.4). Final concentration of DMF in all solutions was less than 2.5 %. Test solution (1 mL), containing different concentrations of drug, was mixed with 1 mL of 1 % albumin solution in phosphate buffer and incubated at 27 ± 1 °C in a water bath for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ± 1 °C in the water bath for 10 min. After cooling, the turbidity was measured at 660 nm using a UV–Visible Spectrophotometer UV-1700 (Shimadzu). Percent inhibition of denaturation was calculated from control, where no drug was added. Each experiment was done in triplicate and the average was taken. Ibuprofen was used as the standard drug.

The percent inhibition of albumin denaturation was calculated using following formula;

$$\% \text{ Inhibition of denaturation } = 100 \text{ X } [V_t / V_c - 1]$$

where V_t = Mean absorbance of test sample; V_c = Mean absorbance of control

Ulcerogenicity

Ulcerogenicity newly synthesized compounds was evaluated by the method of (Verma *et al*., 1981). For this, Wistar albino rats were fasted for 24 h prior to drug administration, and then all the animals were sacrificed 8 h after drug treatment. Their stomachs and small intestines were microscopically examined to assess the incidence of hyperemia, shedding of epithelium, petechial and frank hemorrhages and erosion or discrete ulceration, with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity.

Acute toxicity study

Acute toxicity of synthesized compounds was investigated, in BALB/c albino mice, according to the method of Smith (Smith 1960). The test compounds were administered orally in separate groups of animals and after 24 h of drug administration, mortality in each group was observed.

Partition coefficient

Partition coefficient of the synthesized compounds was determined by "Shake Flask Method" (Aulton 2002; Harrold and Yee 2005). Absorbance of each compound was plotted against respective concentrations to draw a standard curve. Regression analysis was performed and equations for a straight line were obtained for each compound. Concentration of the compounds in organic phase (octanol) and aqueous phase was calculated by straight line equation.

In vitro hydrolysis

To determine the stability of compounds in the GI tract, all the compounds were subjected to simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) in vitro. Both SGF and SIF were prepared as per the USP method (The United State Pharmacopoeia. USP-NF-XXV 2002). For

this, 10 mg of the synthesized compound was dispersed/dissolved in 100 ml of SGF/SIF, and then the resulting mixture was shaken in an orbital shaker at a temperature of 37 ± 2 °C. Sampling was done at time intervals of 15 min for 1.5 h (SGF)/and 2 h (SIF) and the volume was replaced after each withdrawal. The withdrawn samples were suitably diluted, filtered and analyzed spectrophotometrically at the designed absorption maxima of each compound. Percent hydrolysis by subtracting the per cent remaining from 100 % was calculated.

Docking studies

The crystal structure of COX-2 (PDB ID: 3LN1), retrieved from the Protein Data Bank (PDB), (Wang *et al*., 2010) was used for molecular docking. The crystal structure of the COX-2 protein was subsequently optimized and minimized with the "protein preparation wizard" workflow. The ligands were built using Maestro 9.3 build panel and prepared by LigPrep 2.5 version v25111(Schrödinger, LLC, USA) application that uses optimized potential liquid simulations (OPLS) 2005 force field, and it gave the corresponding energy minima 3D conformers of the ligands. The default settings were used for all other parameters. All ligand atoms, but no protein atoms, were allowed to move during the calculations. In order to gain some structural insights into the binding mode of the ligand and establish a correlation with biological activity, all the synthesized compounds were computationally docked into the X-ray crystal structure of macromolecule target, COX-2 enzyme active site using Glide-XP and QPLD algorithms, present in Maestro user interface of Schrödinger Inc (Maestro, version 9.3, Schrödinger, LLC, New York, NY, 2012).

Post docking calculations

The post docking calculations for the docked complexes were performed by Prime MM/GBSA module of Schrodinger suite, which predicts the free energy of binding for a given receptor and a set of given ligands. As the docking algorithms provide good quality binding sites, an energy function with a well-defined description of binding contributions was utilized to re-score the docking results. The total free energy of binding is then expressed by equation given below:

$$\Delta G_{\text{bind}} = G_{\text{complex}} - \left(G_{\text{protein}} + G_{\text{ligand}}\right), \quad \text{where } \Delta G_{\text{bind}} \text{is ligand binding} \tag{1}$$

$$\Delta G_{\text{bind}} = \Delta E_{\text{MM}} + \Delta G_{\text{solv}} - T\Delta S \tag{2}$$

where ΔE_{MM} is the change of the gas phase MM energy upon binding, and includes $\Delta E_{\text{internal}}$ (bond, angle, and dihedral energies), ΔE_{Elect}, (electrostatic), and ΔE_{VDW} (van der Waals) energies. ΔG_{solv} is the change of the solvation free energy upon binding, and includes the electrostatic solvation free energy ΔG_{solvGB} (polar contribution calculated using generalized Born model), and the nonelectrostatic salvation component ΔG_{solvSA} (nonpolar contribution estimated by solvent accessible surface area). Finally, $T\Delta S$ is the change of the conformational entropy upon binding (Lyne *et al*., 2006; Munoz *et al*., 2012).

In silico prediction of pharmacokinetic properties

Nearly 40 % of drug candidates fail in clinical trials due to poor ADME (absorption, distribution, metabolism, and excretion) properties. These late-stage failures contribute significantly to the rapidly escalating cost of new drug development. The ability to detect problematic candidates early can dramatically reduce the amount of wasted time and resources, and streamline the overall development process. QikProp, version 3.5, Schrödinger, LLC, New York, NY, 2012, program was used for in silico prediction of pharmacokinetic properties of the synthesized compounds.

Statistical analysis

All the values of the experimental results are expressed as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's test for the possible significant ($P < 0.01$) identification between various groups. Statistical analysis was carried out using Graph pad prism 3.0 (Graph pad software, San Diego, CA).

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