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Development of energetic pharmacophore for the designing of 1,2,3,4-tetrahydropyrimidine derivatives as selective cyclooxygenase-2 inhibitors

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Abstract We present here the Energetic pharmacophore model representing complementary features of the 1,2,3,4-tetrahydropyrimidine for selective cyclooxygenase-2 (COX-2) inhibition. For the development of pharmacophore hypothesis, a total of 43 previously reported compounds were docked on active site of COX-2 enzyme. The generated pharmacophore features were ranked using energetic terms of Glide XP docking for 1,2,3,4-tetrahydropyrimidine scaffold to optimize its structure requirement for COX-2 inhibition. The thirty new 4,5,6-triphenyl-1,2,3,4-tetrahydropyrimidine derivatives were synthesized and assessed for selective COX-2 inhibitory activity. Two compounds **4B1** and **4B11** were found to be potent and selective COX-2 inhibitors. The molecular docking studies revealed that the newly synthesized compounds can be docked into COX-2 binding site and also provide the molecular basis for their activity.

Keywords E-pharmacophore · Docking · 1,2,3,4-tetrahydropyrimidine · COX-2 · Glide XP

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, fever, and inflammatory diseases such as rheumatoid arthritis and osteoarthritis. Cyclooxygenase (COX) is the key enzyme of inflammatory process and an important target of most of the currently used NSAIDs. The discovery of two distinct isozymes (COX-1 and COX-2) in 1990s led to the development of a new class of NSAIDs known as selective COX-2 inhibitors [1]. COX-2 is primarily responsible for proinflammatory conditions, while COX-1 is constitutive and responsible for the maintenance of physiological homeostasis, such as gastrointestinal integrity and renal function [2]. Thus, selective COX-2 inhibitors such as celecoxib, rofecoxib, valdecoxib and etoricoxib (Fig. 1) provide a potential class of anti-inflammatory agents and exhibit enhanced gastrointestinal safety comparable to that of nonselective NSAIDs [3, 4]. Unfortunately, withdrawal of rofecoxib from market in the fall of year 2004 due to its association with increased risk of cardiovascular toxicity has widely affected the safety of coxib [5, 6]. In 2006, analysis of non-selective NSAIDs showed that they may also be associated with cardiovascular risk [7]. However, a recent study on 923 patients with inflammatory polyarthritis suggests that in certain situations, NSAIDs are not associated with increased risk and could be cardio-protective due to the synthesis of prostaglandins [8]. It was also argued that adverse effect of rofecoxib may be medicated by a maleic anhydride metabolite rather than the inhibition of prostacyclin [9]. The incidence of acute myocardial infarction is not significantly associated with lumiracoxib, a newer member of coxib family which poses a challenge on regulatory agencies to allow the use of coxib [10, 11]. However, lumiracoxib was also withdrawn from market in some countries in 2007 due to its liver toxicity [12]. Thus, it still remains

dubious whether the observed cardiovascular toxicity of rofecoxib is a class effect or individually drug specific. Hence, the conflict associated with coxib can only be reduced by development of new generation of COX-2 inhibitors to avoid the unwanted cardiac effects.

3, 4-Dihydropyrimidine scaffold is one of the frequently found pharmacophore in a wide variety of NSAIDs [13, 14], antihypertensive [15] and antifungal agents [16]. Several analogues of 2-thioxo/amino-1,2,3,4-tetrahydropyrimidine were synthesized by our group and reported for anti-inflammatory activity [17–19] (Fig. 1). Computational studies have been shown as an important tool for structure optimization and virtual screening of compounds using pharmacophore development method [20, 21]. The present study involves the docking of our previously reported 1,2,3,4-tetrahydropyrimidine analogues and development of pharmacophore hypothesis based on values of energetic terms generated by Glide XP docking. Using these Energetic (E) pharmacophoric features, we further explored the structural requirements of 1,2,3,4-tetrahydropyrimidine scaffold for COX-2 inhibitory activity. Finally, the new 1,2,3,4-tetrahydropyrimidine analogues were designed (Fig. 1), synthesized and evaluated for their COX-2 inhibitory activity.

Experimental studies

Molecular docking studies

Molecular Docking Studies were performed in Maestro 9.1 using Glide v5.6 (Schrödinger, LLC, New York, NY, 2010). All compounds were built using Maestro build panel and optimized to lower energy conformers using Ligprep v2.4 which uses OPLS_2005 force field. Epik v2.1was used to generate ionized state of all compounds at target pH 7.0 ± 2.0. The coordinate for COX-2 enzyme (PDB ID 1CX2) were taken from RCSB Protein Data Bank and prepared for docking using 'protein preparation wizard' in Maestro v9.1. Water molecules in the structures were removed and termini were capped by adding ACE and NMA residue. The bond orders and formal charges were added for heterogroups and hydrogens were added to all atoms in the structure. Side chains that are not close to the binding cavity and do not participate in salt bridges were neutralized. After preparation, the structure was refined to optimize the hydrogen bond network using OPLS_2005 force field. This helps in reorientation of side chain hydroxyl group. The minimization was terminated when the energy converged or the RMSD reached a maximum cutoff of 0.30 Å. Grids were then defined around refined structure by centering on ligand using default box size. The extra precision (XP) docking mode for all compounds was performed on generated grid of protein structure [22]. The final evaluation of ligand–protein binding was done with Glide score (docking score).

$$\begin{aligned} \text{G(GLIDE) score} = {} & a \times \text{vdw} + b \times \text{Coul} + \text{Lipo} \\ & + \text{H bond} + \text{Metal} + \text{BuryP} + \text{Rot B} \\ & + \text{Site} \end{aligned}$$

where, vdW: van der Waal energy; Coul: Coulomb energy; Lipo: Lipophilic contact term; HBond: Hydrogen-bonding term; Metal: Metal-binding term; BuryP: Penalty for buried

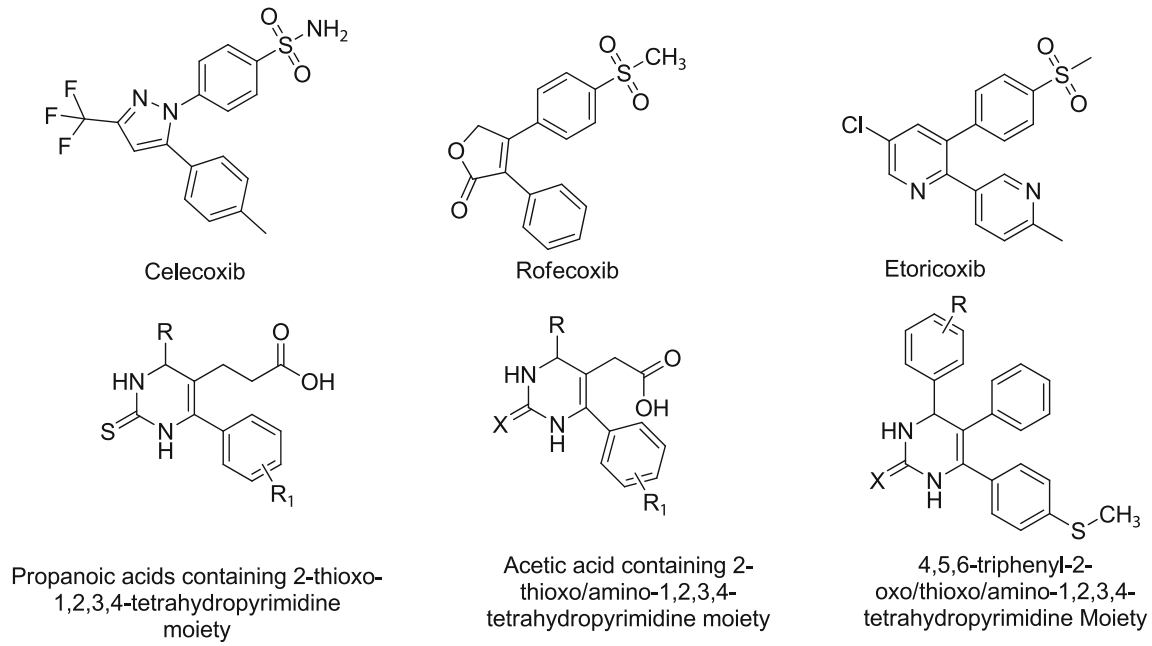


Fig. 1 Chemical structures of compounds

Table 1 Docking score and energetic terms generated by glide XP docking

Ligand	GScore[a]	Lipo[b]	Phob[c]	HBond[d]	Electro[e]	Site[f]	MW[g]	Penal[h]	RotPenal[i]
Celecoxib	−13.18	−6.51	−1.87	−1.11	−0.75	−0.83	−0.23	0	0.11
1	−11.16	−4.81	−1.25	−1.91	−0.69	−0.54	−0.18	0	0.21
2	−10.34	−4.02	−1.52	−1.24	−0.5	−0.97	−0.35	0	0.27
3	−9.75	−4.63	−2.24	−0.01	0.12	−0.76	−0.48	0	0.25
4	−9.73	−4.65	−1.58	−1.45	−0.32	−0.66	−0.36	0	0.28
5	−9.69	−5.24	−1.83	−0.05	0.04	−0.4	−0.46	0	0.24
6	−9.62	−4.14	−0.98	−1.69	−0.73	0	−0.25	0	0.18
7	−9.42	−4.61	−1.95	−0.01	0.14	−0.76	−0.5	0	0.27
8	−9.4	−4.64	−1.71	0	0.14	−1.02	−0.4	0	0.22
9	−9.29	−4.61	−2.52	−0.01	−0.14	−0.8	−0.46	0	0.25
10	−9.29	−4.55	−2.56	−0.01	−0.17	−0.8	−0.43	0	0.23
11	−9.22	−4.38	−2.7	−0.01	−0.15	−0.8	−0.41	0	0.23
12	−9.11	−4	−2.44	−0.02	0.18	−0.76	−0.26	0	0.18
13	−8.91	−4.04	−2.06	0	0.06	−0.71	−0.36	0	0.21
14	−8.86	−4.29	−1.44	−0.42	−0.16	−0.4	−0.36	0	0.21
15	−8.79	−4.64	−1.94	−0.01	−0.17	−0.8	−0.47	0	0.25
16	−8.76	−4.4	−0.9	−0.53	−0.09	−0.69	−0.36	0	0.21
17	−8.73	−4.41	−1.18	−0.16	0.09	−0.89	−0.41	0	0.23
18	−8.69	−4.5	−0.85	−0.71	−0.45	0	−0.4	0	0.22
19	−8.65	−4.36	−1.08	−1.26	−0.27	−0.57	−0.41	0	0.3
20	−8.56	−3.83	−1.31	−0.56	−0.34	−0.54	−0.21	0	0.22
21	−8.5	−4.09	−1.14	−0.21	−0.03	−0.88	−0.34	0	0.2
22	−8.45	−4.64	−0.98	0	−0.04	−0.75	−0.29	0	0.25
23	−8.43	−3.62	−1.62	−0.07	−0.11	−0.82	−0.42	0	0.23
24	−8.26	−3.67	−1.07	−0.56	−0.26	−0.5	−0.42	0	0.23
25	−8.24	−4.44	−0.95	−0.35	−0.36	0	−0.34	0	0.2
26	−8.23	−3.63	−1.15	−0.56	−0.23	−0.5	−0.38	0	0.21
27	−8.06	−4.81	−1.63	−0.25	0.16	−0.91	−0.32	1.5	0.2
28	−7.99	−5.23	−1.58	−0.24	0.1	−0.4	−0.33	1.5	0.2
29	−7.94	−5.11	−1.69	−0.23	0.15	−0.4	−0.38	1.5	0.21
30	−7.86	−4.78	−1.78	−0.15	0.19	−0.75	−0.28	1.5	0.18
31	−7.71	−3.56	−1.12	−1.6	−0.24	0	−0.43	0	0.23
32	−7.7	−4.11	−0.91	−0.94	−0.14	−0.38	−0.46	0	0.24
33	−7.69	−4.11	−0.84	0	0.13	−0.77	−0.3	0	0.19
34	−7.63	−4.14	−1.03	−0.57	−0.35	−0.51	−0.19	1	0.16
35	−7.51	−4.35	−0.7	−0.77	−0.16	−0.42	−0.29	0	0.19
36	−7.48	−5.08	−1.56	0	0.19	−0.37	−0.36	1.52	0.21
37	−7.33	−4.14	−0.78	−0.56	−0.28	−0.46	−0.31	1	0.19
38	−7.21	−2.96	−1.12	0	−0.13	−0.84	−0.37	0	0.21
39	−7.2	−3.63	−0.83	−0.9	−0.2	−0.52	−0.31	0	0.19
40	−7.06	−4.97	−0.15	−0.47	−0.14	−0.48	−0.03	1	0.18
41	−6.97	−4.23	−0.8	−1.53	−0.29	0	−0.32	1	0.2
42	−6.47	−3.93	−1.6	0	−0.01	−0.88	−0.32	2	0.26
43	−6.41	−4.17	−0.62	−0.85	−0.21	−0.51	−0.21	1	0.17

[a] *GScore* Total GlideScore; sum of XP terms

[b] *Lipo* Lipophilic term derived from hydrophobic grid potential and fraction of the total protein ligand vdW energy

[c] *Phob* Hydrophobic enclosure reward

[d] *HBond* ChemScore H-bond pair term

[e] *Electro* Electrostatic rewards; includes Coulomb and metal terms

[f] *Site* SiteMap ligand-receptor non-H bonding polar-hydrophobic terms

[g] *MW* Reward for ligands with low molecular weight

[h] *Penal* Polar atom burial and desolvation penalties, and penalty for intra-ligand contacts

[i] *RotPenal* Rotatable bond penalty

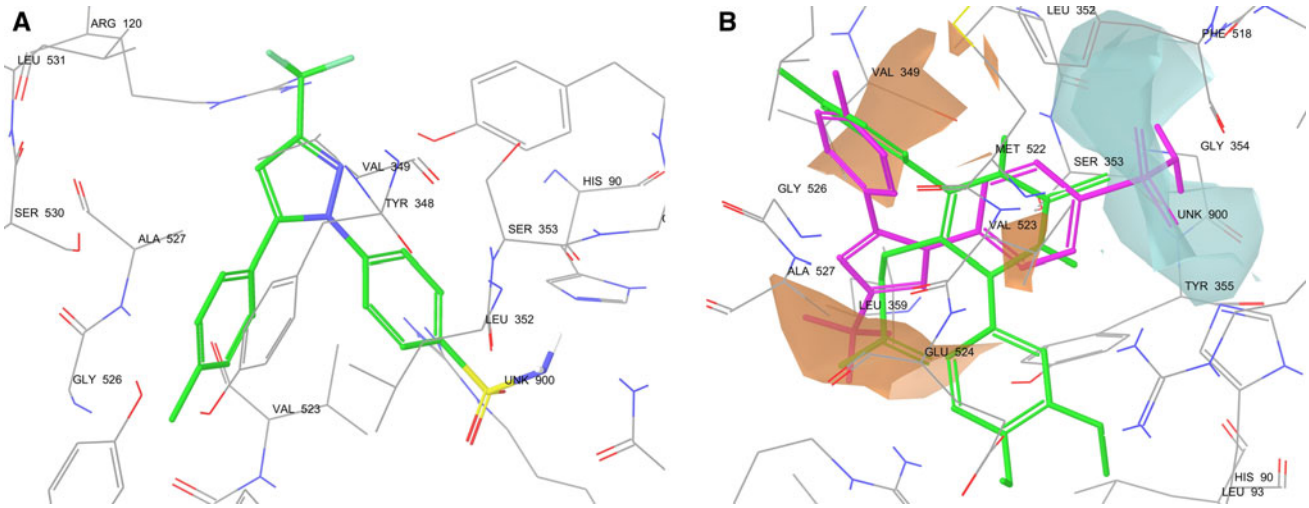


Fig. 2 The docked pose of the compounds **a** Celecoxib **b** overlap of binding pose of celecoxib (*purple color*) and compound **1** (*green color*) in the active region of COX-2 enzyme

polar groups; RotB: Penalty for freezing rotatable bonds; Site: Polar interactions at the active site; and The Coefficients of vdW and Coul are: a = 0.065, b = 0.130.

E-pharmacophore hypothesis generation

The energetic pharmacophoric hypothesis was generated using E-pharmacophores script of docking post-processing script center in Maestro [23, 24]. This script uses Phase v3.2 to initially generate the pharmacophoric sites using default set of six chemical features: hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H), negatively charged group (N), positively charged group (P), and aromatic ring (R). Hydrogen bond acceptor sites were represented as vectors along the hydrogen bond axis in accordance with the hybridization of the acceptor atom. Hydrogen bond donors were represented as projected points, located at the binding site. The E-pharmacophore script then assigned the energetic value to each pharmacophoric feature which is equal to the sum of Glide XP contributions of the atoms comprising the site. This allows sites to be ranked on the basis of these energetic terms [23]. The Glide XP descriptors include terms: lipophilic derived from hydrophobic grid potential and fraction of the total protein ligand vdW energy, hydrophobic enclosure reward, hydrophobic packed hydrogen bonds, ChemScore H-bond pair term, electrostatic rewards, π–π stacking, π-cation, SiteMap ligand-receptor non-H bonding polar-hydrophobic terms, reward for ligands with low molecular weight, polar atom burial and desolvation penalties, and penalty for intra-ligand contacts, rotatable bond penalty and other interactions. The values generated for all these terms after docking of our previously reported compounds are depicted in Table 1 and the terms that show invariable contribution for the all compounds were deleted.

Synthesis of 1-(4-(methylthio)phenyl)-2-phenylethanone **(3)** [25]

Thionyl chloride (0.2 mol) was added to phenyl acetic acid **(1)** (0.1 mol), drop wise with stirring. The solution was refluxed in fume hood chamber for 2.5 h until no further fumes were evolved. The reaction mixture was cooled to room temperature and excess of thionyl chloride was removed under reduced pressure. The produced phenylacetyl chloride **(2)** was added to a solution of thioanisole (0.1 mol) in dichloromethane (100 ml). Anhydrous $AlCl_3$ (0.12 mol) was then added portion wise and the solution was stirred at room temperature for 24 h. The reaction mixture was poured onto ice-water mixture. The organic layer was separated, washed with saturated solution of Na_2CO_3, then with distilled water, dried over anhydrous sodium sulfate and filtered. Dichloromethane was recovered and product was recrystallized from ethanol.

Yield (solid): 70%; M.P.:101.2 °C.

General procedure for synthesis of compounds 4A1-4C9 [17–19]

A mixture of 1-(4-(methylthio)phenyl)-2-phenylethanone **(3)** (0.06 mol), urea/thiourea/guanidine hydrochloride (0.06 mol), aldehyde (0.06 mol) and K_2CO_3 (0.06 mol) in 100 ml ethanol was refluxed in oil bath for 8–12 h. The completion of reaction was monitored on TLC. The reaction mixture was then poured onto ice-water mixture and the solid obtained was filtered. The solid was dissolved in

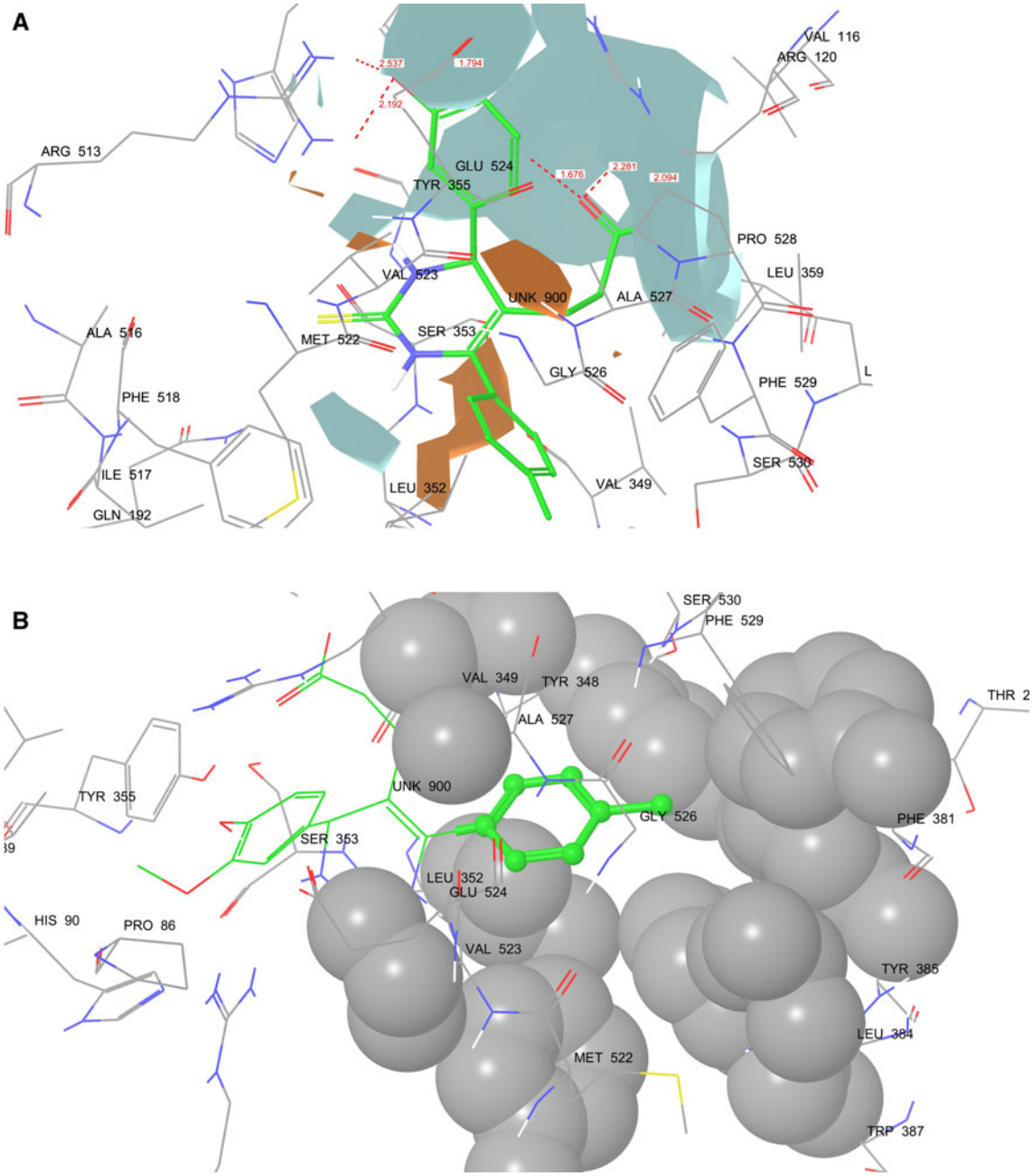


Fig. 3 The docked pose of the compound **1 a** showing H-bonding with amino acid (*red dotted lines*). Turquoise color in surface indicate hydrophobic volume and orange color indicates hydrophilic space of the COX-2 enzyme **b** The fitness of phenyl ring of compound 1 in hydrophobic pocket of COX-2 enzyme

hot water and filtered. The filtrate was neutralized with acetic acid. The product obtained was filtered, dried and recrystallized from ethyl acetate. Spectral analytical data of all these compounds is shown in supporting material.

In vitro cyclooxygenase (COX) inhibition assays:

The assay was performed using colorimetric COX (ovine) inhibitor screening assay kit (Cayman chemical, MI, USA). The assay utilizes the peroxidase activity of ovine cyclooxygenase to oxidize the colorimetric substrate, N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD). Enzyme assays were performed with total volume of 220 µL. The mixture in background wells, 100% initial activity wells and inhibitor wells were prepared according to the instructions provided by the manufacturers and preincubated for five min at 25 °C. The reaction was initiated by addition of 20 µL of TMPD solution followed by 20 µL of arachidonic acid in all

Table 2 Scoring of pharmacophoric features based on energetic terms of glide XP docking

Celecoxib			Compound 1		
Rank	Feature label	Score	Rank	Feature label	Score
1	R9	−1.51	1	N9	−1.35
2	R10	−1.20	2	R10	−1.18
3	D4	−0.65	3	R11	−1.01
4	D5	−0.46	4	D3	−0.48
5	H6	−0.33	5	H8	−0.21
6	H7	−0.06	6	A1	−0.08

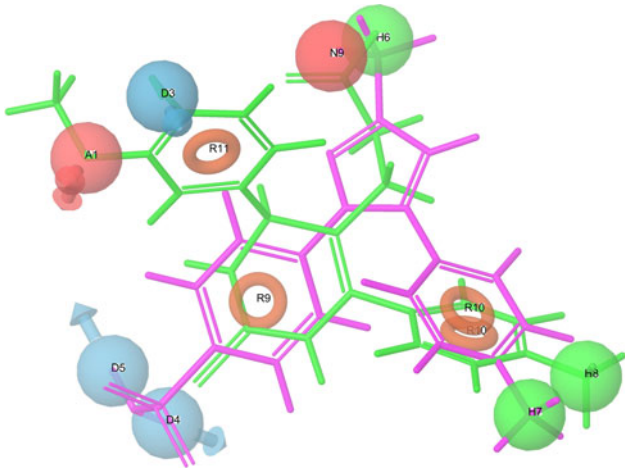


Fig. 4 The overlap of pharmacophoric features of celecoxib and top scoring compound (**1**)

the wells. The assay mixture was mixed thoroughly and incubated at 25 °C for 5 min. The enzyme activity was determined by measuring absorbance at 590 nm in a microplate reader (Molecular Devices, USA).

Result and discussion

Docking study

Docking studies of all 1,2,3,4-tetrahydropyrimidine analogues, reported earlier along with standard celecoxib on COX-2 enzyme were performed for the development of pharmacophoric features. The structure of all these compounds is shown in supporting material (Table S1). The developed pharmacophoric features were helpful for extension of our previous work and designing of new compounds.

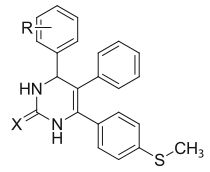
The crystal structure of COX-1 and COX-2 provide useful guidelines that can be used to set H-bond constraints for specific binding of ligand into COX-2 active site. It has been reported that replacement of His 513 in COX-1 by Arg 513 in COX-2 alters the chemical environment of side pocket and can interact with polar moieties [26, 27]. COX-2 isozyme has a larger active site than COX-1, which preferentially reduces steric and ionic crowding by the charged Arg 120 in COX-2 and thus enhances the binding of non-acidic NSAIDs [26, 27]. Further, ligand receptor interaction tool of Maestro and LIGPLOT [28] detected the H-bonding of co-crystalized ligand (SC58) with amino acids Arg 120, Arg 513 and His 90 in COX-2 enzyme (PDB ID 1CX2). Thus while performing the docking studies, hydrogen bonding with amino acid Arg 120, Arg 513 and His 90 were selected as constraints for the specificity of binding. The enzyme was checked during protein preparation for H-bonding conflict and steric clashes between ligand and receptor. The best docking pose obtained from co-crystalized inhibitor (SC58) was compared with its bound conformation for further confirming the reliability of docking studies. This was done by removing co-crystallized inhibitor (SC58) from their active site and subjecting again to docking into the binding pocket in the conformation found in the crystal structure. As a result, a root mean square deviation (RMSD) of 1.065 Å, and 0.163Å was found for flexible and rigid docking respectively. The complete validation study suggested that the prepared protein could be further used to dock and predict the binding mode of compounds and thus could be used for the pharmacophoric feature development based on docking results.

The docking study showed that the standard celecoxib acquired the 'V' or 'butterfly' like binding pose showing body (pyrazole ring) attached to two wings (aromatic rings), shown in Fig. 2a. The overlapping of docking pose of compound **1** and celecoxib is depicted in Fig. 2b. The overlapping showed that the binding space of pyrazole ring in celecoxib intersects by aliphatic chain attached to acid group in compound **1**. Similarly, aromatic ring substituted by sulphonyl group in celecoxib is overlapped by pyrimidine ring of compound 1 and second wing (aromatic ring attached to methyl group) of celecoxib is occupied by aromatic ring of compound **1**. However, our compounds contain one extra wing (aromatic ring) which covers the hydrophobic space in the enzyme and helps to support the binding. As defined in constraints, most of compounds showed hydrogen bonding with amino acid Arg 120 and Arg 513 (Fig. 3a). The binding score (Gscore) and Glide XP terms of all the compounds are depicted in Table 1. The outcome of docking studies confirmed that the results can be used for development of pharmacophoric features based on Glide XP energetic terms.

E-pharmacophore development

The values in Table 1 for energetic terms gave an overview of the features of active site region of COX-2 enzyme and

Table 3 Structure of designed compounds along with their fitness score on E-pharmacophore hypotheses



A, X=O; **B,** X=S; **C,** X=NH

Sr. no.	Comp. code	*R*	E-pharmacophore hypotheses							
			Celecoxib				Compound 1			
			Align score[a]	Vector score[b]	Volume score[c]	Fitness[d]	Align score[a]	Vector score[b]	Volume score[c]	Fitness[d]
1	4A1	-H	0.876	0.885	0.459	1.614	0.892	0.707	0.372	1.336
2	4A2	-4-CH_3	0.855	0.803	0.357	1.447	0.852	0.617	0.542	1.449
3	4A3	-4-OH	1.121	0.559	0.287	0.912	0.927	0.887	0.375	1.489
4	4A4	-4-Cl	0.854	0.802	0.367	1.457	0.856	0.618	0.548	1.453
5	4A5	4-OCH_3	0.854	0.808	0.326	1.422	0.932	0.862	0.377	1.462
6	4A6	-2-NO_2	0.876	0.886	0.458	1.614	0.885	0.694	0.408	1.364
7	4A7	-3- NO_2	0.876	0.886	0.420	1.577	0.888	0.685	0.389	1.334
8	4A8	-4-$N(CH_3)_2$	0.873	0.921	0.402	1.595	0.902	0.827	0.383	1.458
9	4A9	3,4-$(OCH_3)_2$	0.886	0.751	0.426	1.439	0.855	0.690	0.340	1.318
10	4A10	2,4-$(OCH_3)_2$	1.091	0.475	0.309	0.874	0.934	0.883	0.368	1.473
11	4B1	-H	0.876	0.889	0.457	1.617	0.892	0.714	0.377	1.347
12	4B2	-4-CH_3	0.853	0.814	0.356	1.460	0.852	0.604	0.536	1.430
13	4B3	-4-OH	1.120	0.575	0.287	0.929	0.923	0.883	0.374	1.487
14	4B4	-4-Cl	0.853	0.812	0.373	1.475	0.855	0.605	0.560	1.452
15	4B5	4-OCH_3	0.874	0.924	0.407	1.603	1.018	0.632	0.516	1.300
16	4B6	-2-NO_2	0.876	0.888	0.455	1.613	0.893	0.801	0.350	1.407
17	4B7	-3-NO_2	0.876	0.889	0.419	1.578	0.887	0.676	0.393	1.330
18	4B8	-4-$N(CH_3)_2$	0.874	0.925	0.394	1.591	0.755	0.705	0.545	1.620
19	4B9	3,4-$(OCH_3)_2$	0.885	0.759	0.422	1.444	0.901	0.795	0.373	1.417
20	4B10	2,5-$(OCH_3)_2$	1.073	0.452	0.309	0.867	0.930	0.883	0.366	1.474
21	4B11	2,4-$(OCH_3)_2$	0.969	0.414	0.345	0.951	1.158	0.755	0.375	1.165
22	4C1	-H	0.875	0.348	0.373	0.992	0.932	0.763	0.407	1.393
23	4C2	-4-CH_3	0.781	0.592	0.333	1.274	0.852	0.637	0.542	1.469
24	4C3	-4-OH	0.829	0.435	0.375	1.119	0.939	0.889	0.370	1.477
25	4C4	-4-Cl	0.769	0.592	0.333	1.285	0.857	0.639	0.540	1.466
26	4C5	4-OCH_3	0.759	0.578	0.353	1.298	1.040	0.702	0.507	1.342
27	4C6	-2-NO_2	0.873	0.468	0.351	1.092	0.889	0.767	0.345	1.371
28	4C7	-3- NO_2	0.855	0.545	0.351	1.184	1.088	0.456	0.310	0.859
29	4C8	3,4-$(OCH_3)_2$	0.799	0.649	0.339	1.323	0.919	0.916	0.538	1.688
30	4C9	2,5-$(OCH_3)_2$	0.855	0.546	0.340	1.174	0.763	0.686	0.540	1.590

[a] *Align Score* Root-Mean-Squared Deviation (RMSD) in the site-point positions

[b] *Vector Score* Average cosine of the angles formed by corresponding pairs of vector features (acceptors, donors, and aromatic rings) in the aligned structures

[c] *Volume Scor*e Overlap of van der Waals models of the non-hydrogen atoms in each pair of structures

[d] *Fitness* Linear combination of the site and vector alignment scores and the volume score

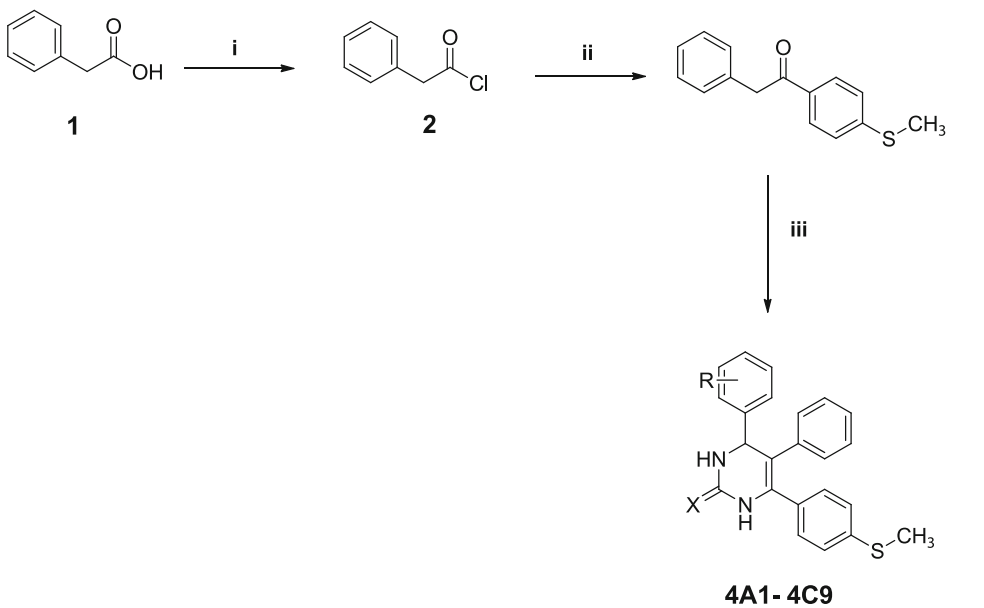
also helped to explore the features of 1,2,3,4-tetrahydropyrimidine required for COX-2 binding. Figure 3b shows the importance of phenyl ring in hydrophobic pocket of COX-2 enzyme. This indicates the requirement of aromatic rings to fit into hydrophobic pocket of COX-2 enzyme. Similarly, all the energetic terms affect the Gscore and thus

suggest the binding affinity of compounds in the active region of the enzyme. The E-pharmacophoric features can be developed for docked single ligand or fragments of all the ligands that bind to the enzyme. Here, we report the E-pharmacophoric features for celecoxib and compound **1** docked on COX-2 enzyme. Generally the common pharmacophore is generated based on the chemical knowledge of active ligands or ligand-receptor complex and can be used for further structure optimization, designing and screening of novel compounds. However, the E-pharmacophore method uses the same concept, but has added Glide XP energetic terms to score or rank the importance of pharmacophoric features. The score and ranking of all the features generated is depicted in Table 2. The overlap of pharmacophoric features of celecoxib and top scoring compound **1** required for binding to the enzyme is shown in Fig. 4. It was observed from the score of pharmacophoric features that diaryl ring ($\mathbf{R_9}$ and $\mathbf{R_{10}}$) attached to pyrazole moiety, along with SO_2NH_2 is necessary for COX-2 activity of celecoxib rather than the hydrophobic substituents. Similarly, aliphatic chain containing acid group (**N9**) and diaryl ring ($\mathbf{R_{10}}$ and $\mathbf{R_{11}}$) attached to pyrimidine ring in compound **1** shows importance for binding to COX-2 enzyme. Thus, it is revealed that the aromatic ring ($\mathbf{R_{11}}$) in compound **1** has acquired the free hydrophobic space in enzyme and hence shows good score.

The overall study helped us to design new 1,2,3,4-tetrahydropyrimidine analogues by modification of our previously reported compounds. It was found from docking study and E-pharmacophore features score that acid group in compound **1** is important for binding to COX-2 enzyme. However overlap of acid group by pyrazole moiety in celecoxib shows higher G-score, good values for all energetic descriptor and thus perfect binding to the hydrophobic pocket of the enzyme. Thus in this study, we have tried to add new wing (aromatic ring) in place of acid group to body (pyrimidine; Fig. 1), so that it covers the complete space occupied by pyrazole ring of celecoxib while docking to COX-2 enzyme. All the designed compounds were aligned over both the E-pharmacophoric hypothesis generated by docking of celecoxib and compound 1 and their fitness score are shown in Table 3.

Chemistry

The synthetic route for 4,5,6-triphenyl-1,2,3,4-tetrahydropyrimidine derivatives is outlined in Scheme 1. The overall synthesis involves major two-step reaction. The starting material, 1-(4-(methylthio)phenyl)-2-phenylethanone was synthesized in first step using intermediate phenylacetyl chloride which was prepared by chlorination of phenyl acetic acid using thionyl chloride. The synthesized phenylacetyl chloride was made to react with thioanisole in dichloromethane in the presence of anhydrous $AlCl_3$ to undergo Friedel–Crafts acylation which gave 1-(4-(methylthio)phenyl)-2-phenylethanone. The final compounds were synthesized using one pot Biginelli reaction of 1-(4-(methylthio)phenyl)-2-phenylethanone, substituted aldehyde and urea/thiourea/guanidine hydrochloride in the presence of potassium carbonate and ethanol as solvent. The completion of all the reactions was checked on TLC plates. The physical data, ^{1}HNMR and mass spectral data for all the synthesized compounds are reported in supporting material.



Scheme 1 Synthesis of series of 4,5,6-triphenyl-1,2,3,4-tetrahydropyrimidine derivatives

(i) $SOCl_2$ **(ii)** thioanisole in CH_2Cl_2, $AlCl_3$ **(iii)** aromatic aldehydes, urea/thiourea/guanidine HCl in ethanol, K_2CO_3

COX enzyme assay

All the synthesized compounds were tested for their ability to inhibit COX-2 and/or COX-1 enzymes using colorimetric COX (Ovine) inhibitor screening assay kit. The in vitro activity of these compounds was reported as percent inhibition of the enzyme activity at 50 μM concentration (Table 4).

In this preliminary study towards development of new safer COX-2 selective compounds, we describe here, the modification of 1,2,3,4-tetrahydropyrimidine analogues reported earlier as anti-inflammatory agent containing acid moiety. It is well established that 1,2,3,4-tetrahydropyrimidine is a good template for selective COX-2 inhibition. Analysis of the newly synthesized derivatives revealed that of the 30 compounds screened for COX-2 inhibitory activity, two compounds (Compound **4B1** and **4B11**) inhibited at least 50% of the COX-2 isoform. The compound **4B1** possesses un-substituted phenyl ring and compound **4B11** is substituted by methoxy group at C_2 and C_3 position of phenyl ring at C_6 position of 1,2,3,4-tetrahydropyrimidine ring. Both compounds have sulfur moiety at C_2 position of 1,2,3,4-tetrahydropyrimidine ring. The replacement of acidic group from our previously reported compounds by aromatic ring at position C_5 did not show better anti-inflammatory activity but probably increased the COX-2 inhibitory activity. These preliminary results indicate that the presence of $–SCH_3$ group showed medium COX-2 inhibitory activity which by replacement with $–SO_2CH_3$ or $–SO_2NH_2$ group in our further work may increase the activity. The presence of different moieties O, S and NH at position C_2 had minimal effect on COX-2 activity, however compound containing sulfur moiety showed higher inhibition.

Table 4 In vitro percent inhibition of COX-2 and COX-1 enzymes by compounds 4A1-4C9

Sr. no.	Comp. code	COX-2 inhibition (%)[a]	COX-1 inhibition (%)[a]
1	4A1	NI[b]	ND[d]
2	4A2	NI	ND
3	4A3	36.5[c]	NI
4	4A4	NI	ND
5	4A5	NI	ND
6	4A6	35.88[c]	ND
7	4A7	NI	ND
8	4A8	NI	ND
9	4A9	NI	ND
10	4A10	NI	ND
11	4B1	75[c]	NI
12	4B2	NI	ND
13	4B3	30.78 ± 13.53	NI
14	4B4	NI	ND
15	4B5	NI	ND
16	4B6	NI	ND
17	4B7	NI	ND
18	4B8	NI	ND
19	4B9	25.3[c]	NI
20	4B10	NI	ND
21	4B11	53.29 ± 14.88	2.17[c]
22	4C1	NI	ND
23	4C2	NI	ND
24	4C3	NI	ND
25	4C4	NI	ND
26	4C5	NI	ND
27	4C6	19.96 ± 8.58	5.97[c]
28	4C7	19.43 ± 10.7	NI
29	4C8	NI	ND
30	4C9	NI	ND
31	Celecoxib	50.79 ± 6.93	NI

[a] Data are indicated as percentage of inhibition at 50 μM ± SEM

[b] No inhibition obtained

[c] Reading obtained by one experiment only

[d] Not determined

COX-1 and COX-2 are two identified COX isoenzymes that share 60% homology and have the same affinity to convert arachidonic acid to prostaglandin. While COX-1 is constitutive and important for maintenance of physiological homeostasis, COX-2 is inducible upon inflammation. In this context, it is therefore desirable that any inhibitors targeted to COX-2 show minimum COX-1 inhibitory activity. Hence, the compounds showing COX-2 inhibition were further evaluated for their inhibitory activity on COX-1 isoform. None of the compounds at 50 μM, were found to inhibit COX-1 activity irrespective of their inhibitory activity on COX-2.

To confirm and validate the overall study, we again performed the docking studies of new 4,5,6-tripheny-1,2,3,4-tetrahydropyrimidine derivatives on COX-2 enzyme. The presence of additional ring helped the compounds to fit into binding cavity of the enzyme, thus increasing the hydrophobic rewards (Fig. 5). However, the replacement of acid group by aromatic ring did not show the hydrogen bonding in the hydrophobic pocket of the enzyme. Thus, this validation indicates that replacement of acid group with phenyl ring containing hydrogen donating group may increase the COX-2 inhibitory activity.

Conclusion

The docking study of 1,2,3,4-tetrahydroyrimidine analogues has been described with the aim to develop combined structure-ligand based pharmacophore using Glide XP energetic terms. The generated features were used to

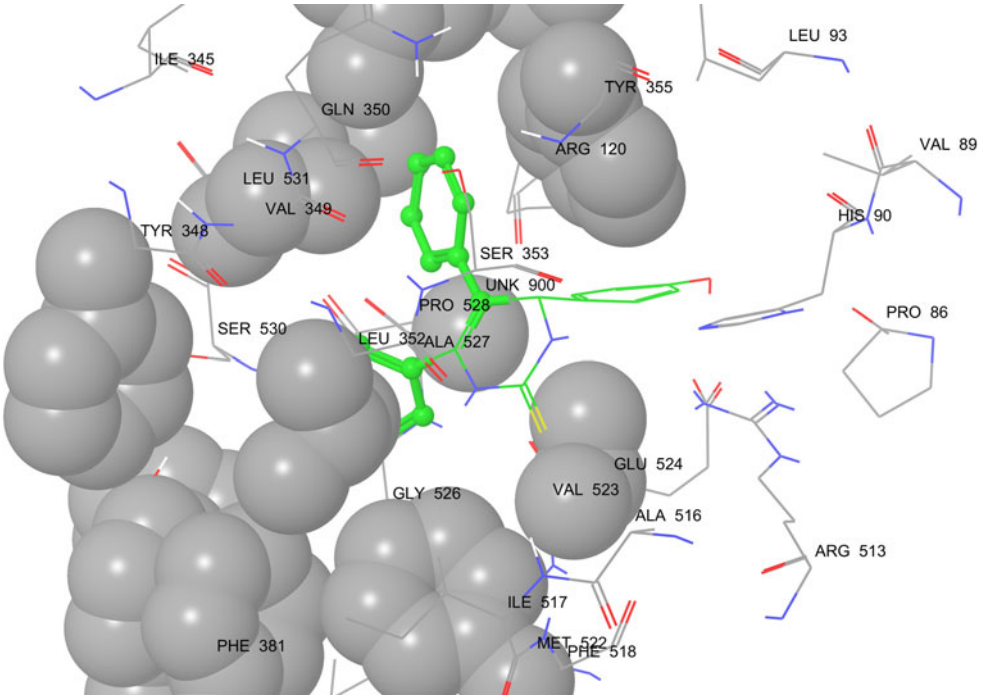


Fig. 5 The docked pose of the compound **4B3** in active site of COX-2 enzyme showing the binding of phenyl rings in hydrophobic space

design the new compounds. The thirty new 4,5,6-triphenyl-1,2,3,4-tetrahydropyrimidine derivatives were synthesized and evaluated for COX-2 and COX-1 inhibition. Of the 30 compounds screened, two compounds **4B1** and **4B11** displayed potent and selective COX-2 inhibition. The compounds did not inhibit COX-1 activity irrespective of their inhibitory activity to COX-2. The data from the study suggests that 4,5,6-triphenyl-1,2,3,4-tetrahydropyrimidine derivatives possess potential for the design of future molecules with the modification on $–SCH_3$ group as well as phenyl ring at C_5 position of 1,2,3,4-tetrahydroyrimidine core to increase COX-2 inhibition. Further, the development of E-pharmacophore is a useful method for design of new compounds and can be applied for screening of compounds.

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