Original Article

IDENTIFICATION OF NEW CYCLOOXYGENASE-2 SELECTIVE INHIBITORS VIA VIRTUAL SCREENING AND COMPLEX-BASED PHARMACOPHORE MAPPING

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ABSTRACT

Background: *New and effective COX-2-targeted medications may cure cancer, inflammation, and other disorders. It's difficult to produce druggable site inhibitors for target protein inhibition that are strong and selective. Such medications need a more informed structure-based or ligand-based drug discovery technique. MOE was used to create a six-point COX-2 selective inhibitor pharmacophore from 43 compounds. The structure includes three aromatic rings, one hydrophobic group (Hyd), one donor (Don), and three H-bond acceptors. A pharmacophore was used to digitally filter the Chembridge database to locate additional structural poses that fulfilled the model's requirements. We found 2090 structurally diverse hits after filtering. Molecular docking was performed on 767 hits after Lipinski's rule of five determined druggability. Twenty compounds with high active site amino acid residue interactions and diverse scaffolds were chosen. The first COX-2 isoform-inhibiting medicines were discovered using structure-based pharmacophores.*

Objectives Using complex-based pharmacophore mapping and virtual screening, this research seeks novel selective COX-2 inhibitors. This involves filtering through a chemical database, defining critical interactions, maximizing hits, and extending drug development for safer and more effective inflammation and pain managem

Study design : *Employing Advanced Computational And Pharmacological Approaches Study*

Duration and place of study :*The study was conducted at Abdul Wali Khan University Mardan from jan 2017 to jan 2018*

Methods: *The investigation used a structure-based drug discovery methodology to find novel cyclooxygenase-2 (COX-2) selective inhibitors. The COX-2 crystal structure was used to create a pharmacophore model, which was then used to virtually search a chemical database. In order to identify potential lead compounds based on interactions with important COX-2 residues, binding energies were computed, and molecular docking was used to refine hits.*

Results: *The goal of the project was to use virtual screening and complex-based pharmacophore mapping to find novel selective COX-2 inhibitors. After the COX-2 crystal structure was used to create a pharmacophore model, 2090 hits were discovered in a database. Following the druggability assessment, 767 hits were chosen for molecular docking. Ultimately, twenty compounds were found to be promising COX-2 inhibitors due to their strong interactions with COX-2 and variety of scaffolds.*

Conclusion: *This study found 20 possible lead drugs that selectively block cyclooxygenase-2 (COX-2) using complex-based pharmacophore mapping and virtual screening. These substances show a variety of scaffolds and robust interactions with COX-2, making them attractive candidates for further research and development as anti-inflammatory and anti-cancer medications with maybe fewer adverse effects.*

Keywords: *Cyclooxygenase-2 (COX-2),Pharmacophore mapping,Virtual screening,Drug discovery*

INTRODUCTION

NSAIDs like aspirin and ibuprofen are the most often prescribed treatments for rheumatic disorders, including osteoarthritis and rheumatoid arthritis, as well as pain and inflammation. Despite the fact that most of these drugs have adverse renal and gastrointestinal consequences [2], they kill 7,000 Americans each year [3]. New methods for making potent anti-inflammatory drugs are needed to reduce NSAID side effects [4].

NASIDs like ibuprofen and aspirin (a non-selective inhibitor) inhibit the COX isoforms COX-1 and COX-2. COX-1 blockage may be deadly. COX-1 is needed for blood flow to the kidneys, platelet aggregation, and protecting the lining of the stomach. COX-2 is made during fever, pain, inflammation, and cancer growth [4].Over the last 20 years, COX-2's selectivity and safety have improved through study. Due to the high risk of cardiovascular adverse effects, many selective COX-2 inhibitors were explored. New types of safe, selective COX-2 inhibitors with fewer cardiovascular side effects were developed after this finding, and valdecoxib and rofecoxib were removed from the market [5]. Inhibitors of COX-2 that are one of a kind are 1, 5-diaryl substituted tetrazoles that have a 4-(methylsulfonyl) phenyl group at position 1 [6]. The most significant COXIB class of selective COX-2 inhibitors is vicinal diaryl heterocycles with p-methylsulfonyl- or p-aminosulfonyl-aryl substituent rings. [5]

Many drug development programs use computational methods for hit identification, lead optimization, etc [7–9]. Virtual screening of ligands or structures is widespread in discovery [10]. Pharmacophores improve targets via their chemical characteristics (functional groups). Rational drug design emphasizes enzyme or receptor activity [8].

Wet labs' poor success rates and exorbitant cost prompted computer methods and extensive in-silico screening. Drug research increasingly requires high-throughput virtual screening to create novel and powerful drug-like compounds [11]. Therefore, virtual screening and complex-based pharmacophore modeling may find new and powerful COX-2 inhibitors. Pharmacophores are three-dimensional representations of tiny chemical molecules used as drugs that may impact target interactions.

Complex-based pharmacophore modeling identifies COX-2-inhibiting characteristics using virtual screening, drug-likeness predictions, molecular docking, protein-ligand binding interactions, binding affinity predictions, and binding energy estimates. The identification of 20 effective lead COX-2 inhibitors shows the importance of this research.

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MATERIALS AND METHODS

A detailed molecular modeling and computational strategy found selective COX-2 inhibitors. Methods summary: MOE constructed a pharmacophore model. The model was based on COX-2's crystal structure with a macrocyclic inhibitor (PDB ID: 1CX2). The model contained hydrophobic groups, hydrogen bond donors, acceptors, and an aromatic ring from crystal structure interaction patterns. A virtual screening: Using the pharmacophore model, a 3D query screened ChemBridge.

This virtual screening looks for COX-2-suppressing pharmacophores. We discovered 2090 structurally diverse hits. Lipinski's Rule of Five was used to evaluate each hit compound's molecular weight, lipophilicity, and hydrogen bond donors and acceptors. Based on these characteristics, 767 hits were examined. COX-2 inhibitor selection is enhanced using molecular docking. The MOE docking method docked hits to COX-2's binding site. Docking scores and binding energies measured the hits' COX-2 active site binding affinity and interactions. Hit Selection: The top conformations of docked compounds were examined, and 20 compounds were chosen for their scaffolds and strong interactions with key COX-2 residues such as His90, Ser353, Gln192, Leu352, Tyr385, Leu352, Ala516, Ser530, Asp515, and Ala527.

RESULTS AND DISCUSSION

Complex-based pharmacophore models are fascinating for uncovering affinity- and selectivity-boost-

ing interaction areas. MOE's pharmacophore-generating tool combined cyclooxygenase's crystal structure with a macrocyclic inhibitor to create a complicated model. MOE and LigPlot determined the chemical properties required to create a protein-ligand-binding pharmacophore model. A pharmacophore model with six primary components—three hydrogen bond acceptors (Acc), one hydrophobic (Hyd), one donor (Don), and one aromatic—was developed using default MOE parameters. Blue, brown, purple, and green are hydrogen bond acceptors, donors, and hydrophobics in Figure 1.

Pharmacophore models were verified with 43 inhibitors—37 active and 6 inactive or least active. We tested 43 test database inhibitors' inhibitory effects [6, 19, 20]. MOE constructor created the test materials' 3D structures, and MOE energy reduction decreased energy [Gradient: 0.05, Force Field: MMFF94X]. Examined all test database chemicals and mapping modes utilizing the six-feature complex-based pharmacophore. Interesting, 36 of 37 active compounds with 90 conformations mapped six pharmacophore model components. Interestingly, no chemical was matched to an inert or least active six-feature complex pharmacophore model. Test database results support our pharmacophore concept. Database pharmacophore screening

ChemBridge compounds with similar features were searched for novel structural poses that fit the pharmacophore model. This method may help medical chemists find drug-like compounds quicker. ChemBridge found 2090 structurally diverse hits with a good six-feature match to the suggested pharmacophore model after screening.

Hit-ligand recovery druggability was assessed using Lipinski's rule of five. According to Lipinski's "rule of five," drug-like molecules exceeding 500 Da, log P values under 5, and hydrogen bond donors and acceptors over 10 have little penetration and absorption. Lipinski's five criteria yielded 767 molecular docking hits due to rigorous implementation.

For all first-retrieved hits, MOE docking was used to increase hit chemicals in cyclooxygenase's binding site. Before docking the first hits, the complex structure ligand was removed and re-docked into the protein binding cavity to validate docking. The co-crystallized and re-docked conformation's RMSD was calculated using MOE's SVL script. According to Figure 2, our protein-ligand docking method matches the experimental binding mode with a 0.5892A^ result. Docking MOE may study drug binding. Similar strategies docked all early hits into cyclooxygenase's binding pocket. By default, MOE stores 10 ligand conformations. All docked molecules' top conformations were in distinct databases. The top 315 poses of the highest-rated conformations for investigation by docking score.

MOE tool LigPlot visualized these 315 hits' protein-binding interactions. Most cyclooxygenase-binding pocket residues, including His 90, Ser353, Gln192, Leu352, Tyr385, Ala516, Ser530, Asp515, and Ala527, interacted with optimistic hits. Approximately 62% of 315 chemicals are bound to protein-critical residues. These 62 compounds' binding energies and affinities were calculated.

MOE used the generalized Born-volume integral (GB-VI) function to compute binding affinities for all 60 compounds, including the complex structural ligand, to find the most probable ligands. Before calculating affinity, each cyclooxygenase-ligand complex's binding pocket was energy-minimized. The binding affinity of each hit was measured in Kcal/mol after energy reduction.

The most intriguing candidates interacted with essential cyclooxygenase residues, had binding energy and affinity equal to the reference ligand in the complex structure, and were visible in the binding cavity. Only 20 of the 62 compounds qualify (Table 1). We think that these lead compounds may be strong, structurally diverse, and new cyclooxygenase inhibitors based on pharmacophore mapping, binding mechanism, affinity, energy, and visual prediction. Table 2 shows these hits' 2D structures.

CONCLUSION

Researchers may target COX-2 to create novel cancer, inflammatory, and other illness therapies. It's difficult to produce druggable site inhibitors for target protein inhibition that are strong and selective. We found potential lead COX-2 inhibitors using these methods. For a test set of 43 COX-2 selective medicines, the MOE pharmacophore modelling tool and the crystal structure of cyclooxygenase (1CX2) were used to create a 3D model. The hypothesis comprises one hydrophobic group (Hyd), one donor (Don), one aromatic ring, and three H bond acceptors. This

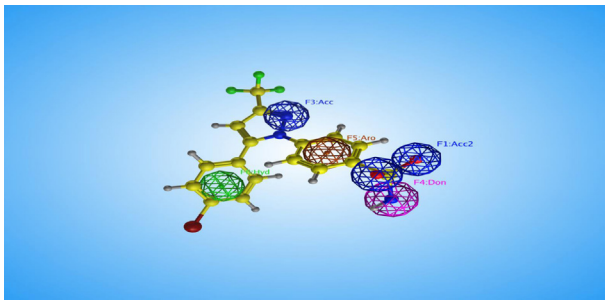


Figure 1: Three-dimensional pharmacophoric features generated from complex structure of Cyclooxygenase

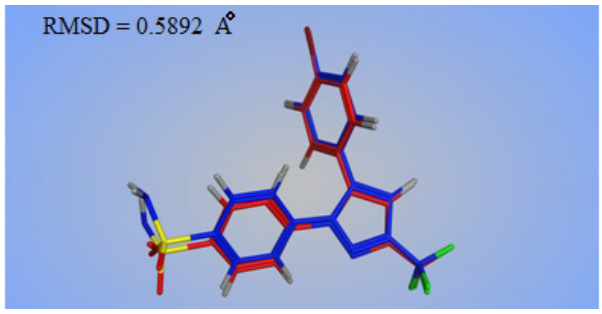


Figure 2: Red Native Co-crystallized ligand and blue dock ligand Docking Analysis

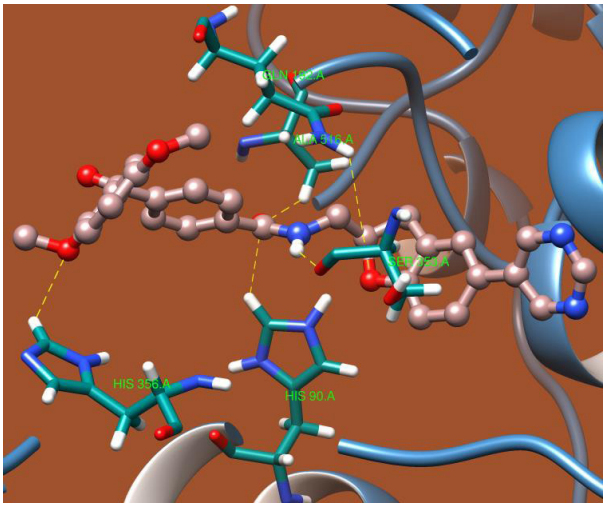


Figure 3: 3D image of compound 1. We can clearly see 5 made by compound 1 with the residue of the binding pocket. The residues His90, His356, Ser353, Ala516 and Gln192 contributed in interactions.

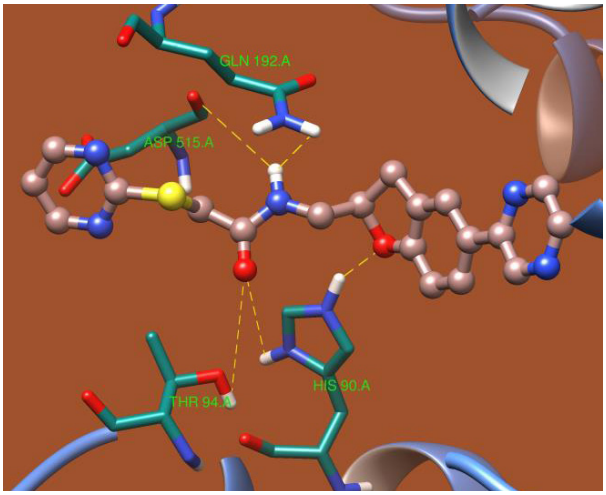


Figure 4: 3D image of compound 2. We can clearly see 5 made by compound 2 with the residue of the binding pocket. The residues His90, Thr94, Asp515 and Gln192 contributed in the interactions.

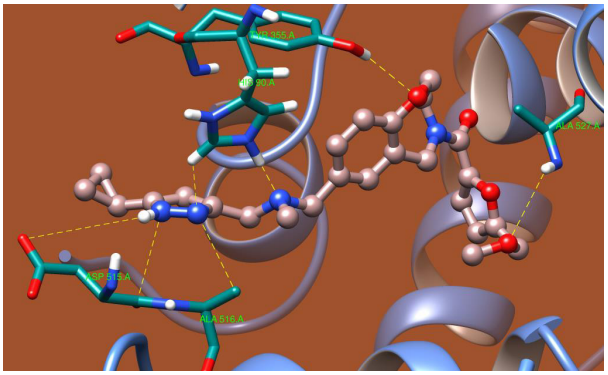


Figure 5: 3D image of compound 3. We can clearly see 7 bonds made by compound 3 with the residue of the binding pocket. The residues His90, Tyr355, Ala527, Ala516 and Asp515 contributed in the interactions.

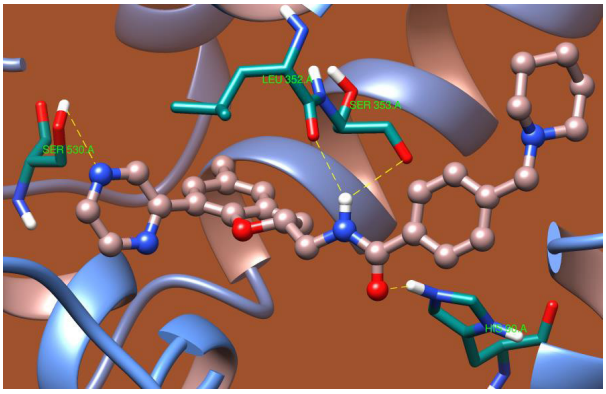


Figure 6: 3D image of compound 4. We can clearly see 4 bonds made by compound 4 with the residues of the binding pocket. The residues His90, Ser353, Leu352 and Ser530 contributed in the interactions

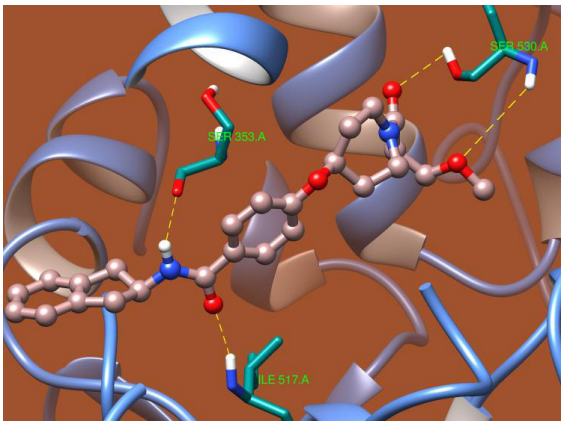


Figure 7: 3D image of compound 18. We can clearly see 4 bonds made by compound 18 with the residues of the binding pocket. The residues Ser353, Ile517and Ser530 contributed in the interactions.

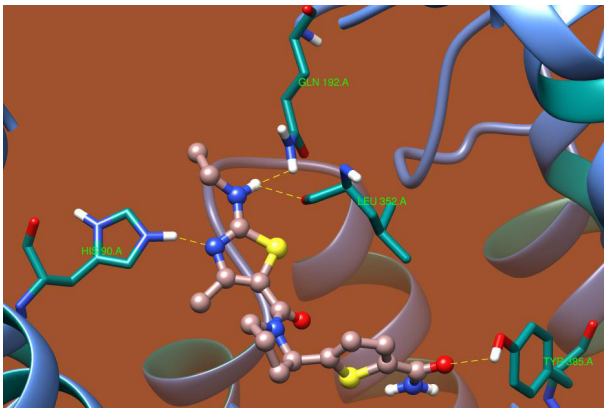


Figure 8: 3D image of compound 19. We can clearly see 4 bonds made by compound 19 with the residues of the binding pocket. The residues His90, Tyr385, Leu352 and Gln192 contributed in the interactions.

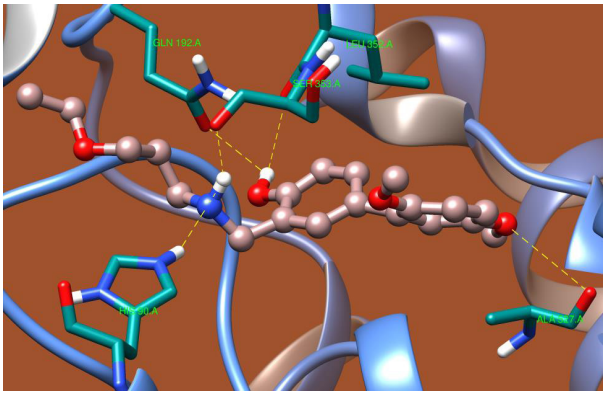


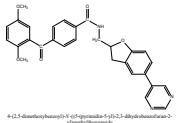
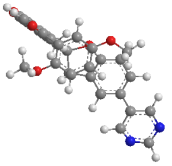
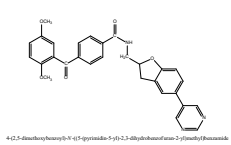
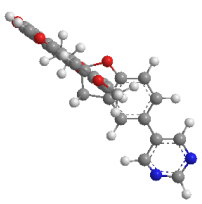
Figure 9: 3D image of compound 20. We can clearly see 5 bonds made by compound 20 with the residues of the binding pocket. The residues His90, Ser353, Ala527, Leu352 and Gln192 contributed in the interactions.

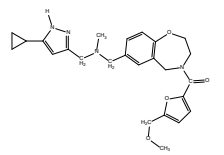
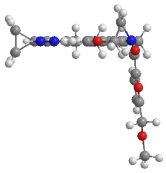
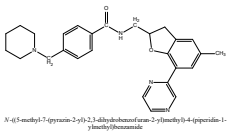
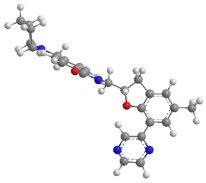
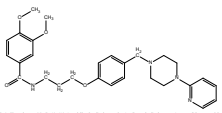
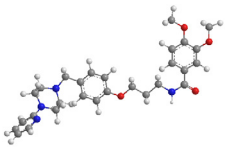
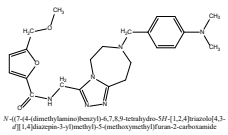
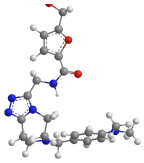
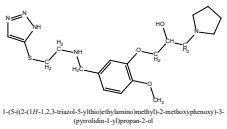
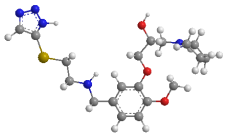
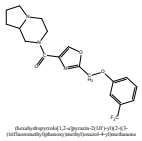
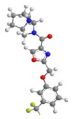
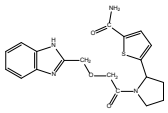
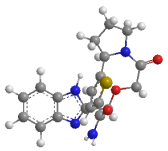
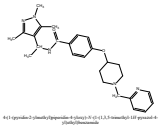
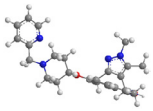
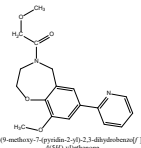
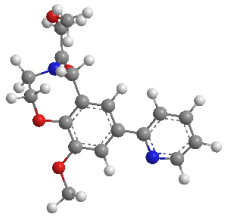
Table 1: ChemBridge database ID, Docking Scores, binding energies, binding affinities and drug like properties of hit compounds.

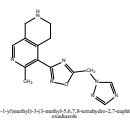
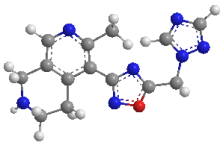
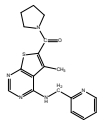
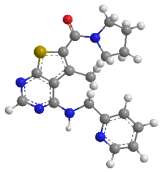
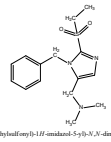
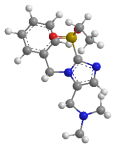
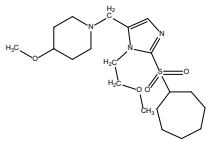
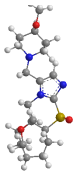
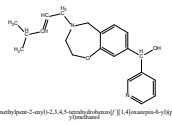
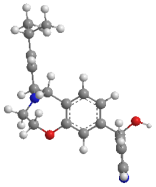
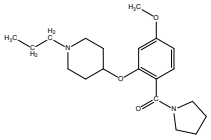
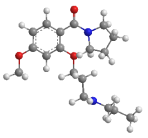
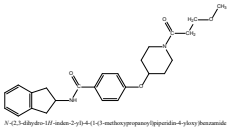
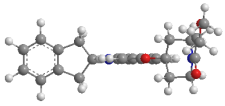
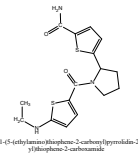
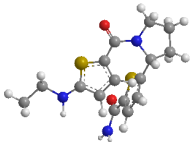
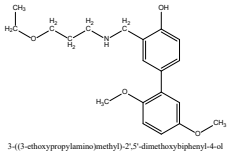
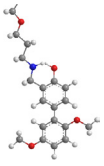
Compound	ChemBridge ID	Docking Score (S)	Binding affinity Kcal/mol	Binding energy Kcal/mol	Drug like properties
1	11030877	-31.1763	-12.711	-23.281	MW. 495.535 g/mol, LogP. 4.125, Don. 1 , Acc. 7
2	19234220	-30.4944	-11.024	-24.064	MW. 379.444 g/mol, LogP. 2.146, Don. 1, Acc. 6
3	21168057	-30.1688	-14.469	-29.595	MW. 450.539 g/mol, LogP. 4.759, Don. 1, Acc. 5
4	21554451	-29.7295	-13.038	-27.826	MW. 142.563 g/mol, LogP. 4.438, Don. 1, Acc. 5
5	17658002	-29.2592	-14.231	-28.550	MW. 490.604 g/mol, LogP. 3.866, Don. 1. Acc. 6

6	18941428	-28.0870	-14.190	-21.193	MW. 438.532 g/mol, LogP. 3.137, Don. 1, Acc. 5
7	15301567	-27.5492	-12.928	-26.585	MW. 407.539 g/mol, LogP. 1.797, Don. 3, Acc. 7
8	19155459	-26.7426	-11.716	-20.431	MW. 395.361 g/mol, LogP. 3.771, Don. 0, Acc. 4
9	12586940	-26.1359	-12.494	-29.887	MW. 384.460 g/mol, LogP. 2.966, Don. 2, Acc. 4
10	13195262	-25.7998	-12.653	-20.413	MW. 447.583 g/mol, LogP. 4.687, Don. 1, Acc. 5
11	15721026	-25.4042	-13.257	-27.096	MW. 362.813 g/mol, LogP. 3.044, Don. 0, Acc. 5
12	15895366	-25.2978	-9.736	-16.902	MW. 297.322 g/mol, LogP. 1.258, Don. 1, Acc. 6
13	11553723	-25.1045	-12.960	-26.541	MW. 353.450 g/mol, LogP. 3.509, Don. 1, Acc. 4
14	21681525	-24.8947	-9.710	-19.400	MW. 307.418 g/mol, LogP. 2.319, Don.0, Acc. 4
15	21537153	-24.6266	-15.214	-27.073	MW. 413.583 g/mol, LogP. 3.170, Don. 0, Acc. 6
16	16448691	-24.5047	-11.519	-22.875	MW. 338.451 g/mol, LogP. 3.932, Don. 1, Acc. 4
17	19233189	-24.4066	-13.133	-23.934	MW. 346.471 g/mol, LogP. 3.184, Don. 0, Acc. 4
18	14708409	-23.9406	-13.776	-25.538	MW. 422.525 g/mol, LogP. 2.990, Don. 1, Acc. 4
19	18861026	-23.4349	-11.747	-22.326	MW. 364.494 g/mol, LogP. 3.117, Don. 2, Acc. 3
20	20805796	-23.1030	-13.187	-25.855	MW. 345.439 g/mol, LogP. 3.859, Don. 2, Acc. 5
21	Reference	-32.2763	-13.398	-25.136	MW. 446.247 g/mol, LogP.4.280 , Don. 1 , Acc.3

The 2D, 3D structures and IUPAC name of 20 novel inhibitors of Cyclooxygense are shown in the Table 2.

S.no	Structure	3D structure
1		
2		

3	 (7-((((5-cyclopropyl-1*H*-pyrazol-3-yl)methyl)(methyl)amino)methyl)-2,3-dihydrobenzo[*f*][1,4]oxazepin-4(5*H*)-yl)(5-(methoxymethyl)furan-2-yl)methanone	
4	 *N*-((5-methyl-7-(pyrazin-2-yl)-2,3-dihydrobenzofuran-2-yl)methyl)-4-(piperidin-1-ylmethyl)benzamide	
5	 3,4-dimethoxy-*N*-(3-(4-(4-(pyridin-2-yl)piperazin-1-yl)methyl)phenoxy)propyl)benzamide	
6	 *N*-((7-(4-(dimethylamino)benzyl)-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*d*][1,4]diazepin-3-yl)methyl)-5-(methoxymethyl)furan-2-carboxamide	
7	 1-(5-((2-(1*H*-1,2,3-triazol-5-ylthio)ethyl)amino)methyl)-2-methoxyphenoxy)-3-(pyrrolidin-1-yl)propan-2-ol	
8	 (hexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl)(2-((3-(trifluoromethyl)phenoxy)methyl)oxazol-4-yl)methanone	
9	 5-(1-(2-((1*H*-benzo[*d*]imidazol-2-yl)methoxy)acetyl)pyrrolidin-2-yl)thiophene-2-carboxamide	
10	 4-(1-(pyridin-2-ylmethyl)piperidin-4-yloxy)-5'-(1,1,3,3-tetramethyl-1*H*-pyrazol-4-yl)methylbenzonitrile	
11	 2-methoxy-1-(9-methoxy-7-(pyridin-2-yl)-2,3-dihydrobenzo[*f*][1,4]oxazepin-4(5*H*)-yl)ethanone	

12	 5-((1*H*-1,2,4-triazol-1-yl)methyl)-3-(3-methyl-5,6,7,8-tetrahydro-2,7-naphthyridin-4-yl)-1,2,4-oxadiazole	
13	 (5-methyl-4-(pyridin-2-ylmethylamino)thieno[2,3-*d*]pyrimidin-6-yl)(pyrrolidin-1-yl)methanone	
14	 1-(1-benzyl-2-(ethylsulfonyl)-1*H*-imidazol-5-yl)-*N*,*N*-dimethylmethanamine	
15	 1-((2-(cycloheptylsulfonyl)-1-(2-methoxyethyl)-1*H*-imidazol-5-yl)methyl)-4-methoxypiperidine	
16	 (*E*)-(4-(4-methylpent-2-enyl)-2,3,4,5-tetrahydrobenzo[*f*][1,4]oxazepin-8-yl)(pyridin-3-yl)methanol	
17	 (4-methoxy-2-(1-propylpiperidin-4-yloxy)phenyl)(pyrrolidin-1-yl)methanone	
18	 *N*-(2,3-dihydro-1*H*-inden-2-yl)-4-(1-(3-methoxypropanoyl)piperidin-4-yloxy)benzamide	
19	 5-(1-(5-(ethylamino)thiophene-2-carbonyl)pyrrolidin-2-yl)thiophene-2-carboxamide	
20	 3-((3-ethoxypropylamino)methyl)-2',5'-dimethoxybiphenyl-4-ol	

six-point structure-based pharmacophore was used to query the Chembridge database in 3D to uncover unique structural poses that satisfied model parameters. We found 2090 structurally diverse hits after filtering. Molecular docking was performed on 767 hits after Lipinski's rule of five determined druggability. Twenty compounds with high active site amino acid residue interactions and diverse scaffolds were chosen. First COX-2 isoform-inhibiting medicines were discovered using structure-based pharmacophores.

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CONFLICT OF INTEREST: Authors declare no conflict of interest

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