ANTI-INFLAMMATORY SCREENING OF THIOUREA DERIVATIVES BASED ON MOLECULAR DOCKING STUDIES

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Abstract:

Computer-aided drug design has emerged as an efficient tool of developing candidate drugs for the treatment of many diseases. The thiourea derivatives represent one of the most promising classes of compounds that exhibit various biological properties. The aim of this mini literature review was to analyze the molecular fitting of synthesized thiourea derivatives into the actives sites of COX-1, COX-2 and 5-LOX.

Lipinski's rule of 5 is widely used in rational drug design to predict drug similarity and druglikeness. Among selected compounds with available druglikeness data, all molecules meet criteria for Lipinski's rule except compounds **2**-**4** and **41**-**46**. *In silico* molecular docking analysis were performed in Molecular Operating Environment, OpenEye, AutoDock Tools and AutoDock Vina to explore the binding modes of these compounds into the active sites of target proteins. Within the molecular docking analysis, the interactions of key amino acid residues of enzyme's active sites involved in ligand-protein interactions were investigated.

Based on the results highlighted in this review we can conclude that certain structural features of thiourea derivatives contribute to high binding potential to interact with active sites of COX-1, COX-2 and 5-LOX. It was observed that insertion of carboxyl functional group to the parent compounds increased interaction strength due to formation of additional hydrogen bonds. On the other hand, introduction of pi-reached heterocycles increased the number of hydrophobic interactions that leads to higher binding affinity towards target proteins.

Keywords:

Thiourea Derivatives, Druglikeness, Molecular Docking, COX, 5-LOX.

INTRODUCTION

Computer-aided drug design (CADD) comprises two different approaches, structure based drug design (SBDD) and ligand based drug design (LBDD). SBDD uses knowledge of the target protein structure to calculate interaction energies, while in LBDD, chemical similarity search is performed based on knowledge of known active and inactive molecules [1].

The thiourea derivatives represent one of the most promising classes of compounds that exhibit various biological properties such as anti-inflammatory [2], antiviral [3], anticancer [4], hypoglycemic [5] and antimicrobial [6] activities. Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants, or damaged cells [7].

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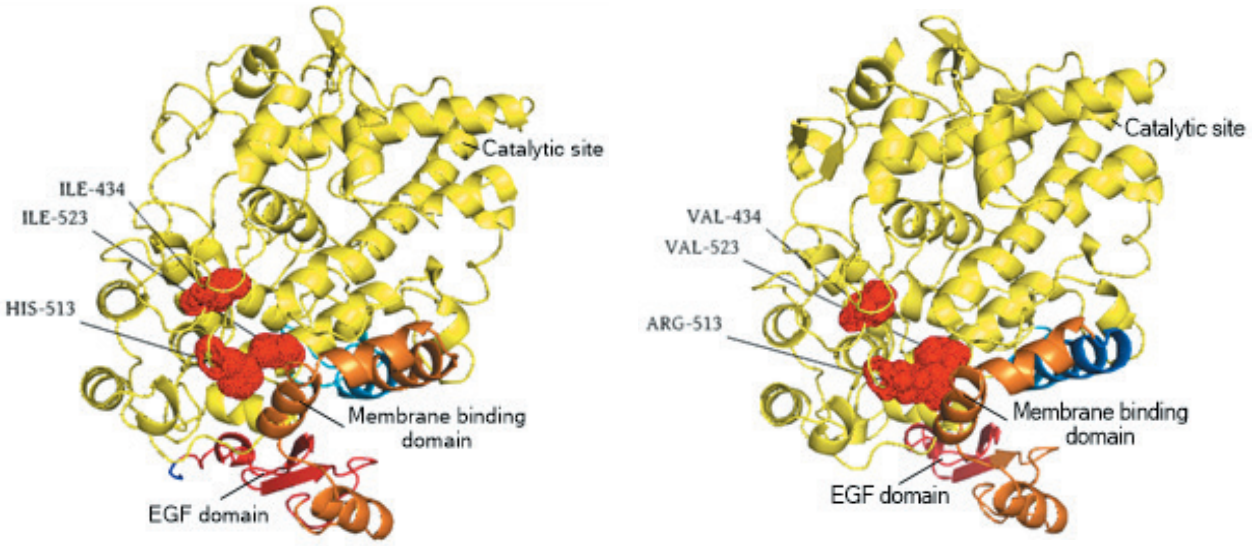

Figure 1 - Molecular structure of human COX-1 (left) and COX-2 (right) enzymes. Membrane binding domain (brown), epidermal growth factor binding domain (red), and catalytic site (yellow) of COX-1 and COX-2

Traditionally, inflammation is treated with non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Long treatment with these drugs is followed by multiple severe side effects, which causes significantly limited efficacy. Due to this fact, there is a constant need to develop new anti-inflammatory drugs [8].

Anti-inflammatory drugs usually act by inhibiting cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) [8] and/or 5-lipoxygenase (5-LOX) [9] COX enzymes mediate the biosynthesis of prostaglandins (PGs) from arachidonic acid (AA) and have 60% of structural homology. COX-1 is a constitutive enzyme responsible for the production of the cytoprotective PG in gastrointestinal tract and kidneys, while COX-2 is an inducible enzyme that is expressed at the site of injury in response to the release of proinflammatory mediators [10]. In addition to the high similarity, the COX-2 active site is approximately 20% larger than binding site of COX-1.

These differences are due to the presence of less voluminous valine at position 523 in COX-2 concerning isoleucine in COX-1 at the same position. COX-2 has an additional hydrophobic side pocket available for drug binding, which is located in the extension of the main pocket. The size of this hydrophobic side pocket is the result of the "replacement" of isoleucine at position 434 and histidine at position 513 in COX-1 with valine and arginine in COX-2, respectively. Mentioned amino acid differences may provide additional interaction in the selective binding of COX-2 inhibitors [11]. Scientists identified an epidermal growth factor binding (EGF) domain, a membrane-binding domain, and two catalytic domains in structures of human COX-1 and COX-2. Both cyclooxygenase isoforms have two active sites within the catalytic domain: peroxidase active site and cyclooxygenase active site (Figure 1) [12].

5-LOX converts the AA to leukotrienes (LTs) that are lipid mediators with strong proinflammatory properties.

This enzyme is associated with acute inflammatory reactions, colon cancer, asthma, atherosclerosis, and pulmonary arterial hypertension [13]. Human 5-LOX comprises two domains, an N-terminal regulatory C2-like domain (residues 1–112) which consists of two antiparallel β-sheets and C-terminal catalytic domain (residues 126–673) which is made up of predominant α-helices and ferrous ion located inside. Non-heme ferrous ion, essential for 5-LOX catalytic ability, is held inside by three conserved histidine residues (His367, His372, His550) and one carboxylate group of the C-terminal Ile673 (Figure 2) [14].

The aim of this mini literature review was to analyze the molecular docking studies to assess anti-inflammatory potential of synthesized thiourea derivatives.

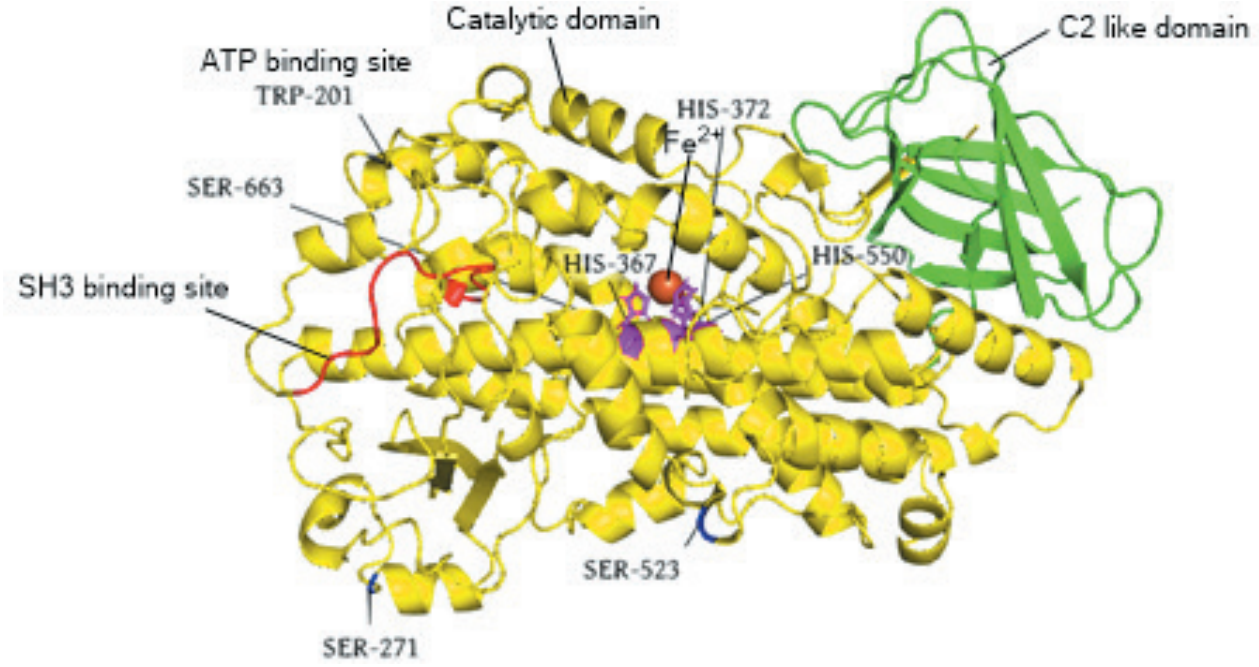

Figure 2 - Molecular structure of human 5-LOX. C2-like domain (green), catalytic domain (yellow), serine phosphorylation sites (blue), Src homology domain 3 (SH3)-binding site (red), a putative ATP binding site near the Trp201 (dark grey), histidine residues (purple), and ferrous ion (brown) of 5-LOX

2. DRUGLIKENESS ANALYSIS

Drug-like compounds are molecules, which contain functional groups and/or have physical properties like majority of known drugs, so they can be biologically active or might show therapeutic potential [15]. Properties such as oral bioavailability or membrane permeability are often correlated to partition coefficient (log P), molecular weight (MW) and number of hydrogen bond acceptors and donors in a molecule.

These parameters are included in Lipinski's rule of 5, and are widely used in predicting drug similarity. The key factors used to describe druglikeness in the Lipinski's rule are molecular weight <500; partition coefficient <5; hydrogen bond donor atoms <5 and hydrogen bond acceptor atoms <10. Molecules that violate these criteria show poor pharmacokinetic properties for oral administration. In this way, the molecules are filtered in the early phase of drug development, which allows focusing on promising compounds [16], [17].

Present research summarizes the molecular modelling studies conducted on selected thiourea derivatives (**1-46**) (Figure 3).

Among the compounds with available druglikeness data (**1-5, 31-46**), all molecules meet criteria for Lipinski's rule except compounds **2-4** and **41-46** (Table 1).

3. MOLECULAR MODELING STUDIES

In silico molecular docking analysis were performed in Molecular Operating Environment [10] [18] [19], OpenEye [19] AutoDock Tools [20] and AudoDock Vina [21] to explore the binding modes of these compounds into the active sites of COX-1, COX-2 and 5-LOX. Within the molecular docking analysis, the interactions of key amino acid residues of enzyme's active sites involved in ligand-protein interactions were investigated.

The key residues involved in binding interactions of flurbiprofen, celecoxib and arachidonic acid during molecular fitting into the active sites of COX-1, COX-2 and 5-LOX were presented in Figures 4 and 5.

3.1. MOLECULAR DOCKING INTO THE COX-1 ACTIVE SITE

The study of El-Kerdawy et al demonstrated that compound **1** bound to the COX-1 with a highest affinity, thereby achieving two hydrogen bonds with Tyr130 and hydrophobic interactions with residues Glu44 and Arg466 [18]. However, flurbiprofen does not achieve any of these binding interactions during molecular fitting into the active site of COX-1.

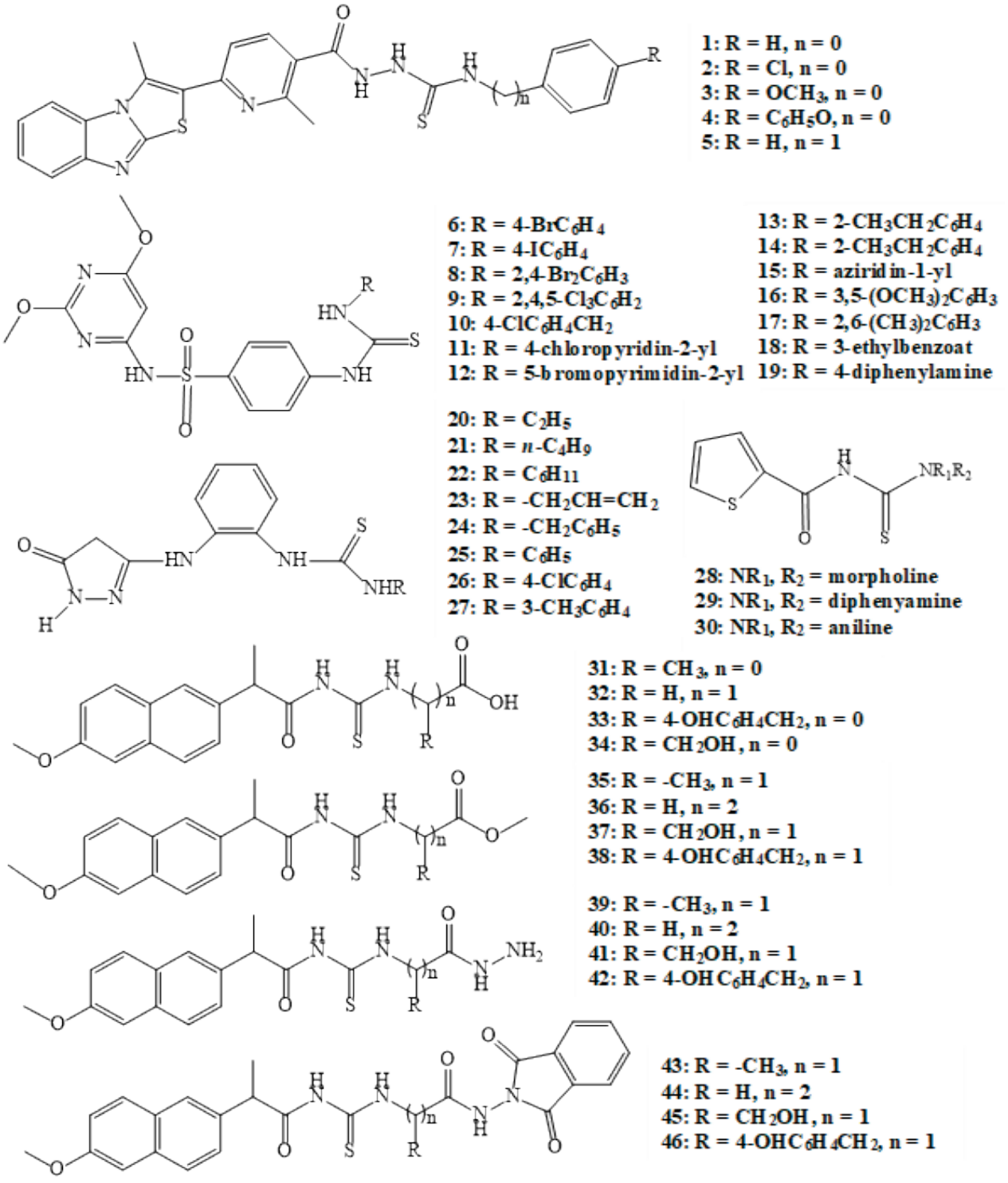

Figure 3 - Analyzed thiourea derivatives

Compound number	Molecular weight (g/mol)	Partition coefficient	Number H-bond acceptors	Number H-bond donors
1	472.6	3.36	7	3
2	507.0	5.47	7	3
3	502.6	4.83	8	3
4	564.7	6.62	8	3
5	486.6	4.77	7	3
31	360.4	2.69	6	3
32	360.4	2.32	6	3
33	376.4	1.66	7	4
34	452.5	3.92	7	4
35	374.5	2.96	6	2
36	374.5	2.58	6	2
37	390.5	1.92	7	3
38	466.5	4.18	7	3
39	374.5	1.54	7	5
40	388.5	1.17	7	5
41	390.5	0.51	8	6
42	466.6	2.77	8	6
43	504.6	3.49	9	3
44	504.6	3.12	9	3
45	520.6	2.46	10	4
46	596.7	4.72	10	4

Table 1 – Druglikeness data of analyzed compounds

3.2. MOLECULAR DOCKING INTO THE COX-2 ACTIVE SITE

Analyzed thiourea derivatives demonstrated different binding patterns during molecular fitting into the active site of COX-2. Table 2 summarizes the binding parameters of selected compound during molecular docking into the active site of COX-2. Celecoxib docked into the COX-2 active site, forming hydrogen bond with residue Gln189, while thiourea derivatives bearing sulphonamide moiety (**1** and **6**) achieved hydrogen bonds with completely different residues. On the other hand, substituted thiourea derivative **23** demonstrated hydrogen bond interactions with residues Tyr385 and Ser350, which were identical as hydrogen bonds achieved by reference diclofenac during molecular fitting into COX-2. It should be also emphasized that derivatives **20** and **22** achieved the same polar interactions with residues Arg120 and Tyr355 as co-crystallized flurbiprofen. Finally, derivative **25** formed even three key hydrogen bonds with residues Arg120, Tyr385, and Ser530. The study of Elhenawy et al illustrated that binding interactions of the naproxen derivatives increased after insertion of an acidic fragment to the initial compound.

Therefore, it was observed that interaction strength decreased in order free acids (**31**-**33**) > methyl esters (**35**-**38**) > hydrazide derivatives (**39**-**42**). Reference naproxen was docked into the COX-2, achieving two hydrogen bonds with residues Tyr385 and Ser530. Among tested compounds free acids derivatives **31** and **38** formed important hydrogen bond interactions with above-mentioned residues. Free acid derivative **32** demonstrated the lowest binding energy of -122.22 kcal/mol, while methyl ester derivative **35** and hydrazide derivative **40** achieved also the lower binding energies of -107.78 and -101.83 kcal/mol, respectively.

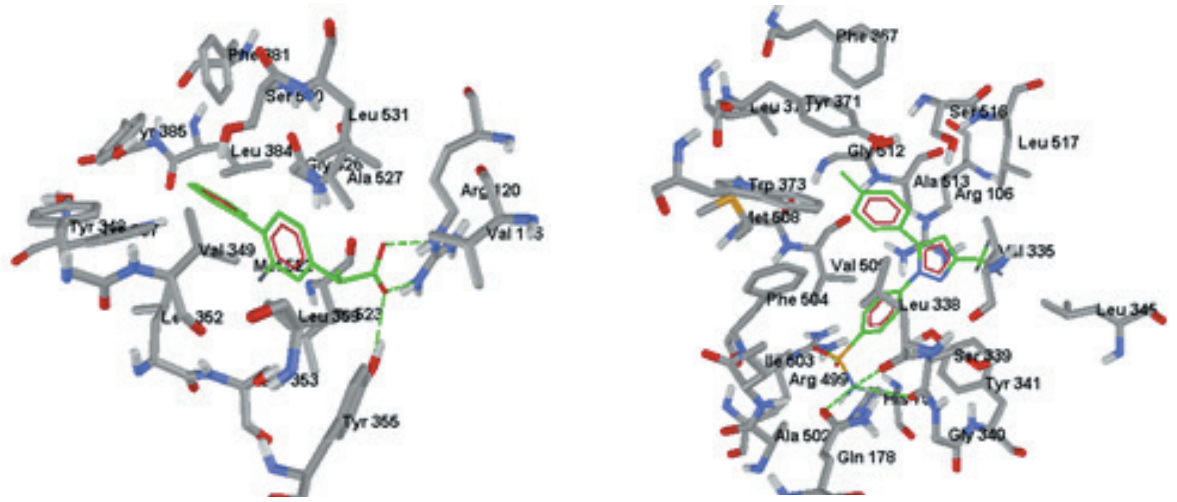

Figure 4 - X-ray crystallographic binding modes of flurbiprofen bound to COX-1 (PDB ID: 3N8Z) (left) and celecoxib bound to COX-2 (PDB ID: 3LN1) (right). Hydrogen bond interactions are shown as dashed green lines

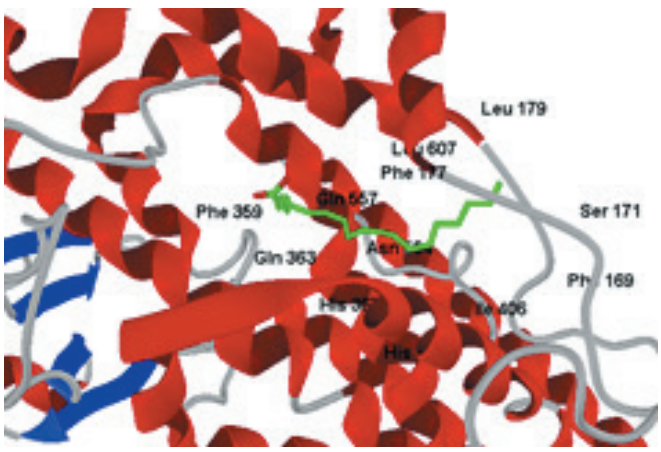

Figure 5 - Close-up view of the key residues involved in binding interactions of arachidonic acid and active site of 5-LOX (PDB ID: 3V99)

3.3. MOLECULAR DOCKING INTO THE 5-LOX ACTIVE SITE

Jacob and Manju showed that thiourea derivatives have a high potential for 5-LOX inhibition. Free binding energies of the analyzed compounds (**28-30**) were approximately -7.00 kcal/mol and were lower in comparison with the reference drug zileuton (-6.43 kcal/mol). Tiophene sulphur atom of tested compounds formed one hydrogen bond with Gln417 residue. Hydrophobic contacts were established with residues Ala157, Thr40, Asn148 and Glu412, while Met145 formed one pi-sulphur interaction with analyzed molecules [20].

4. CONCLUSION

Based on the results highlighted in this review we can conclude that certain structural properties of thiourea derivatives contribute to high binding potential to interact with active sites of COX-1, COX-2 and 5-LOX. It was observed that insertion of carboxyl functional group to the parent compounds increased interaction strength due to formation of additional hydrogen bonds. On the other hand, introduction of pi-reached heterocycles increased the number of hydrophobic interactions that leads to higher binding affinity towards target proteins.

Compound number	PDB code	Hydrogen bond interactions	Hydrophobic interactions	Co-crystal	Reference
1	3LN1	Phe186	Phe184, Gln189, His372, Tyr371, Leu377	Celecoxib	[19]
6		Trp373, Asn386	Ala185, Phe186, Tyr371, His372, Leu377		
20	3PGH	Arg120, Tyr355	Pro86, Val89, Leu93, Val116, Ser119, Val349, Ser353, Glu524, Ala527	Flurbiprofen	[10]
22		Arg120, Tyr355	Val116, Val349, Val523, Ala527, Ser353		
23		Tyr385, Ser530	Val116, Arg120, Tyr348, Val349, Leu352, Tyr355, Phe518, Val523, Gly526, Ala527, Leu531		
25		Arg120, Tyr385, Ser530	Val116, Val349, Leu352, Ser353, Tyr355, Phe381, Trp387, Gly526, Ala527, Leu531		
31	1PXX	Ser530	Gln192, Phe205, Val344, Tyr348, Val349, Leu352, Val523, Ala527	Diclofenac	[21]
33		Ser455	Tyr460, Leu507, Thr521, Leu525		
38		Tyr 385, Met522	Phe209, Val344, Tyr348, Val349, Phe381, Trp387, Leu534		

Table 2 - An overview of key binding interactions achieved between selected compounds and COX-2

5. REFERENCES

[1] V. Tomar, M. Mazumder, R. Chandra, J. Yang and M. Sakharkar, Small Molecule Drug Design, Reference Module in Life Sciences, 2019.

[2] A. Shakeel, "Thiourea Derivatives in Drug Design and Medicinal Chemistry: A Short Review.," J *Drug Des Med Chem*, vol. 2, no. 1, pp. 10-20, 2016.

[3] H. Hu, C. Lin, M. Ao, Ji Y, B. Tang, X. Zhou, M. Fang, J. Zeng and Z. Wu, "Synthesis and biological evaluation of 1-(2-(adamantane-1-yl)-1H-indol-5-yl)-3-substituted urea/thiourea derivatives as anticancer agents," *RSC Advances*, vol. 7, no. 81, pp. 51640-51, 2017.

[4] R. Pingaew, N. Sinthupoom, P. Mandi, V. Prachayasittikul, R. Cherdtrakulkiat, S. Prachayasittikul, S. Ruchirawat and V. Prachayasittikul , "Synthesis, biological evaluation and in silico study of bis-thiourea derivatives as anticancer, antimalarial and antimicrobial agents," *Med Chem Res*, vol. 26, no. 12, pp. 3136-48, 2017.

[5] H. Zhang, Y. Zhang, G. Wu, J. Zhou, W. Huang and X. Hu, "Synthesis and biological evaluation of sulfonylurea and thiourea derivatives substituted with benzenesulfonamide groups as potential hypoglycemic agents," *Bioorg Med Chem Lett*, vol. 19, pp. 1740-4, 2009.

[6] N. Nordin, T. Chai, B. Tan, C. Choi, A. Halim, H. Hussain and Z. Ngaini, "Novel synthetic monothiourea aspirin derivatives bearing alkylated amines as potential antimicrobial agents," *J Chem*, 2017.

[7] R. de Cássia da Silveira e Sá, L. Andrade and D. de Sousa, "A review on anti-inflammatory activity of monoterpenes," *Molecules*, vol. 18, no. 1, pp. 1227-54, 2013.

[8] M. Dvorakova and P. Landa, "Anti-inflammatory activity of natural stilbenoids: A review," *Pharmacol Res*, vol. 124, pp. 126-45, 2017.

[9] S. Fiorucci, R. Meli and G. Cirino, "Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy?," *Biochem Pharmacol*, vol. 62, no. 11, pp. 1433-8, 2001.

[10] A. Moneer, K. Mohammed and H. El-Nassan, "Synthesis of novel substituated thiourea and benzimidazole derivatives containing a pyrazolone ring as antiinflammatory agents," *Chem Biol Drug Des*, vol. 87, no. 5, pp. 784-93, 2016.

[11] W. Smith, D. DeWitt and R. Garavito, "Cyclooxygenases: structural, cellular, and molecular biology," *Annu Rev Biochem*, vol. 69, no. 1, pp. 145-82, 2000.

[12] J. Dhanjal, A. Sreenidhi, K. Bafna, S. Katiyar, S. Goyal, A. Grover and D. Sundar, "Computational Structure-Based De Novo Design of Hypothetical Inhibitors against the Anti- Inflammatory Target COX-2," PloS one, vol. 10, no. 8, p. e0134691, 2015.

[13] M. Halle, T. Christersdottir and M. Back, "Chronic adventitial inflammation, vasa vasorum expansion, and 5-lipoxygenase up-regulation in irradiated arteries from cancer survivors," *Faseb J*, vol. 30, pp. 3845-52, 2016.

[14] Z. He, D. Tao, J. Xiong, F. Lou, J. Zhang, J. Chen, W. Dai, J. Sun and Y. Wang, "Phosphorylation of

5-LOX: The Potential Set-point of Inflammation," *Neurochem Res*, vol. 45, no. 10, pp. 2245-57, 2020.

[15] R. Kadam and N. Roy, "Recent trends in drug-likeness prediction: A comprehensive review of In silico methods," *Indian J Pharm Sci*, vol. 69, no. 5, p. 609, 2007.

[16] C. Lipinski, F. Lombardo, B. Dominy and P. Feeney, "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings," *Adv Drug Deliv Rev*, vol. 46, pp. 3-26, 2001.

[17] D. Clark and S. Pickett, "Computational methods for the prediction of 'drug-likeness'," *Drug Discov Today,* vol. 5, no. 2, pp. 49-58, 200.

[18] M. El-Kerdawy, M. Ghaly, S. Darwish, H. Abdel-Aziz, A. Elsheakh, R. Abdelrahman and G. Hassan, "New benzimidazothiazole derivatives as anti-inflammatory, antitumor active agents: Synthesis, in-vitro and in-vivo screening and molecular modeling studies," *Bioorg Chem*, vol. 83, pp. 250-61, 2019.

[19] M. Ghorab, M. El-Gaby, M. Alsaid, Y. Elshaier, A. Soliman, F. El-Senduny, F. Badria and A. Sherif, "Novel thiourea derivatives bearing sulfonamide moiety as anticancer agents through COX-2 inhibition.," *Anti-Cancer Agents Med Chem,* vol. 17, pp. 1411-25, 2017.

[20] J. Jacob and S. Manju, "Identification and development of thiazole leads as COX-2/5-LOX inhibitors through in-vitro and in-vivo biological evaluation for anti-inflammatory activity," *Bioorg Chem*, vol. 100, p. 103882, 2020.

[21] A. Elhenawy, L. Al-Harbi, G. Moustafa, M. El-Gazzar, R. Abdel-Rahman and A. Salim, "Synthesis, comparative docking, and pharmacological activity of naproxen amino acid derivatives as possible anti-inflammatory and analgesic agents," *Drug Des Devel Ther*, vol. 13, pp. 1773-90, 2019.