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Designing of promising Tromethamine-Diflunisal-Pyrrole combinations based on COX binding, drug-properties and safety

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Gastric issues that accompany the use of NSAIDs (Non-steroid anti-inflammatory drugs) are always a serious global concern. The inhibition of the Cycloxygenase enzyme (COX) limits the prostaglandin synthesis and thereby facilitates the control of pains, inflammation etc. But this creates gastric issues due to the reduction of mucin formation in the stomach. The present work was performed to create a modification in the structure of NSAID drug Diflunisal, to reduce the gastric effect of acidic moiety in the structure and elevate the overall biological properties. The drug Tromethamine, a base used in acidosis treatment was substituted to reduce the acidic issues. The heterocyclic compound pyrrole was substituted to elevate the properties. Neutral, salt, amide and ester combinations of Tromethamine-Diflunisal were designed, optimized and docked to the crystal structures of COX-1 (PDB ID: 6Y3C) and COX-2 (PDB ID: 5IKR) enzymes, using PyRx software. The combinations with lower COX-1 and COX-2 binding energies relative to Diflunisal were noted. It was analysed if the combinations of Diflunisal, Tromethamine and pyrrole lowers drug-properties or induce toxicities. Pyrrole substitution at position R_4 was not found favourable for COX binding. Among the favourable combinations, DF19 is the Diflunisal-Pyrrole-Tromethamine combination, equally favourable for binding to COX targets.

Keywords: Acidosis, COX-1, COX-2, Diflunisal, Docking, NSAIDs, Pyrrole, Tromethamine

Non-steroid anti-inflammatory drugs (NSAIDs) are recommended worldwide in curing inflammations and pains[1]. Millions of patients administer NSAIDs on regular basis and this indicates the significance of NSAIDs in the medicinal field[2].

The docking target of the NSAIDs is cyclooxygenase enzyme (COX) which speeds up the synthesis of fatty acid prostaglandins. The COX enzyme performs the oxygenation and cyclization of Arachidonic acid, yielding prostaglandins PGE_2, PGF_2 and PGI_2[3]. From so long, prostaglandins are considered as mediators in the treatments related to neurological conditions, pains and inflammations[4]. Prostaglandins prompt the mucin formation and prevent ulceration caused by pepsin or HCl increase in the stomach. When NSAIDs hinders the COX enzymes, the prostaglandin production is reduced. Although it is favourable regarding inflammations and pains, too little concentration of prostaglandins make the stomach lining very vulnerable to acid[5]. The concentration of acidic metabolites during NSAIDs intake could leads to gastric issues and even metabolic acidosis. Renal failure is another after-effect of NSAIDs intake[6]. In a study conducted, chronic kidney patients (belonging to stage 3 to 5) responded in a favourable way to the treatment with $NaHCO_3$, showing improvement in kidneys and metabolic acidosis[7].

In this work, structural modifications were introduced in the structure of Diflunisal (DF) to decrease the gastric issues caused by acidic moiety and increase the biological properties. Tromethamine (Tris or tris(hydroxymethyl)aminomethane), a better $NaHCO_3$ substitute and the hetrocyclic compound pyrrole was chosen for this . Tris is a buffer used in the metabolic acidosis treatment and has favourable pH (7.1-9.1) range[8]. Pyrrole derivatives are well famous for their antimicrobial[9], anti-inflammatory[10], anticancer[11], antiviral[12], antifungal[13] like medicinal characteristics. It is accepted that the anti-inflammatory qualities exhibited by pyrrole groups is due to the inhibition or the hindering of the COX enzyme. NSAIDs like Tolmetin and Keterolac itself are pyrrole derivatives.

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Abbreviations: Asn, Asparagine; COX-1, Cyclooxygenase-1; COX-2, Cyclooxygenase-2; Cys, cysteine; DF, Diflunisal; Gln, Glutamine; Gly, Glycine; LD_{50}, lethal dose fifty percentage; PAINS, Pan-assay interference compounds; Py, Pyrrole; Thr, Threonine; TPSA, Topological polar surface area; Tyr, Tyrosine

Suppl. Data available on respective page of NOPR

All the structures with designed modification were docked to both COX-1 and COX-2 enzymes. The designed combinations include salt, neutral, amide and ester combinations of Diflunisal-Tromethamine. These structures were docked with and without pyrrole groups at the marked positions. All the obtained binding energy values were compared to Diflunisal and the best Diflunisal-Tromethamine-Pyrrole combinations were picked up. The safety and the properties of these combinations were evaluated. From this computational study, the best positions for the pyrrole group substitution were identified. The molecular docking study has helped to choose the favourable combination and the computational analysis saves the huge financial and time loss if the compounds were synthesised directly[14].

Review of Literature

In the past years, DFT has come up as a best substitute to conventional abinitio HF and MP2 method due to it's precise molecular structural predictions. For aromatic molecules, Infrared intensities and Vibrational frequencies can very well be predicted by B3LYP, when compared to Hartree fork and Abinitio[15].

Molecular docking methodology followed by the evaluation of drug associated properties has provided a strong foundation in drug designing and subsequently many works were reported using this methodology. Meraj and co-workers have conducted molecular docking studies of eight analogues of the drug Disopyramidine against the target Human voltage gated sodium channel. Top five lead compounds were identified from docking based on binding affinity. Their property analysis has suggested that these compounds can act better relative to Disopyrmaidine[16]. Through molecular docking and ADMET analysis, Singh and co-workers have identified curcumin as the potential inhibitor of *Plasmodium falciparum* S-adenosyl-L-homocysteine hydrolase relative to noraristeromycin and its derivatives. They have proposed curcumin as an efficient drug candidate against malaria[17]. Lotfy and co-workers have reported sixteen derivatives of 5,5-dimethylthiohydantoin as promising androgen agonists based on molecular docking and Lipinski property analysis. So they proposed these sixteen compounds as promising candidates for prostate cancer treatment[18]. Nisha and co-workers have conducted docking studies followed by ADMET analysis and found a series of lead compounds against Alzheimer's disease. They proposed Acylguanidine 7a as the potential β-Secretase inhibitor that possesses least binding energy and favorable properties.[19] Shah and co-workers have carried out docking studies of flavonoid compounds with cytochrome P450 enzyme aromatase, a target linked to the breast cancer. All the promising compounds in the study showed good ADME properties and among them 6B, 6K, 4K and 2K had least target binding energy and strong binding[20]. Maalik and co-workers has found very strong binding of silibinin and glycyrrhetic acid against targets associated with inflammation namely COX-2, 5β-reductase and phospholipase A2. Out of these two compounds glycyrrhetic acid showed more favorable ADME properties[21].

Docking studies have been carried out on NSAIDs drugs with an aim to reduce its side effects by introducing structural variations. Jones and co-workers have masked the acid moiety of NSAIDs by directly coupling to glucosamide, thereby avoiding cartilage degradation. They have incorporated molecular docking to assess the variation in the binding affinity to COX-1 and COX-2 enzymes. They found Diclofenac-glucosamine and Mefenamic acid-glucosamide bioconjugate exhibiting greater activity toward COX-2 and COX-1, respectively[22]. Gouda and co-workers have designed more than 90 carprofen derivatives. The structure was modified by substitutions and its effect on COX-1 and COX-2 binding was evaluated. They have proposed better target binding structures relative to Carprofen based on binding affinity and properties[23]. Madduluri and Sah have docked the Mefenamic acid bearing N-glycopeptides to COX-2 enzyme to evaluate COX-2 inhibition. Tryptophan derivative had exhibited better activity. Most of the compounds were better than Mefenamic acid in terms of acute toxicity[24].

Synthesis of the potential derivative after the identification of the most promising work through *in silico* evaluation turned out to be successful. Through molecular docking, Gundogdu-Hizliates and co-workers have identified that the synthesised ibuprofen amide and ibuprofen acyl hydrazone derivatives are very promising in COX-2 inhibition. Two of the synthesised compounds were better than Ibuprofen in COX-2 inhibition[25].

Materials and Methods

Molecular Docking

Structures of the designed combinations were drawn and optimized using GaussView[26] and

Gaussion 09 software[27], respectively. These stable structures were docked to COX targets using PyRx software[28]. Crystal structures of two targets namely COX-1 (PDB ID: 6Y3C) and COX-2 (PDB ID: 5IKR) were accessed from the RCSB data bank[29]. The COX-1 model was refined with Yasara energy minimization server[30]. Crystal structures were then validated with SAVES server[31]. Both structures had above 90% amino acids in the most favourable area. Ramachandran plot[32] of refined models are given in (Suppl. Fig. 1). They had above 96 % errat quality (Suppl. Fig. 2 and Suppl. Fig. 3) as per the Errat plots[33]. So the structures were opted for docking after evaluating the validation results. Metapocket server has helped to identify active site in targets and the grid box was set after identifying binding site[34]. Ligplot diagrams plotted with Ligplot+ have helped to identify interacted residues during docking[35].

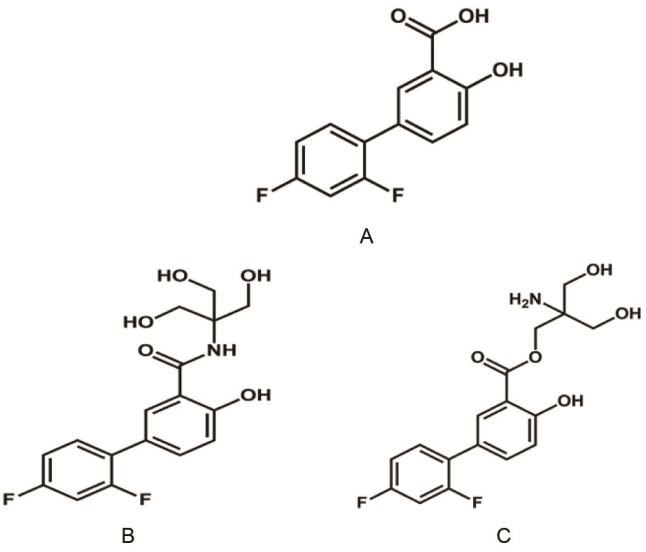

Fig. 1 — 2D structure of (A) Diflunisal; (B) Diflunisal-Tromethamine amide structure; and (C) Diflunisal-Tromethamine ester structure

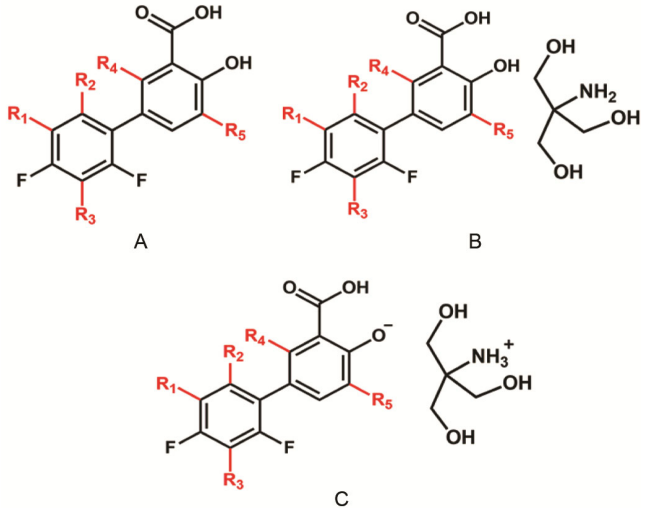

Fig. 2 — The substitutions of pyrrole group on Diflunisal at R_1, R_2, R_3, R_4 and R_5, in the (A) absence; (B) Neutral; and (C) salt combination with Tromethamine

Oral activity and properties

Lipinski rule based oral activity was studied with SwissADME server[36]. Five Lipinski rules are based on size (mass below 500 g/mol), lipohilicity (log P ≤5), hydrogen bond formation (Donors and acceptors ≤ 5 and ≤ 10, respectively), and molar refractivity (40-130).

The studied properties includes Topological polar surface area abbreviated as TPSA[37] with the range in between 20-130 Å^2, 'PAINS alert' pointing out the unspecific fragments bearing structures[38], bioavailability indicated in a score[39], absorption, saturation[40] based on Fraction Csp^3, rotatable bonds and solubility in water[41]. These were evaluated using the SwissADME server to analyze the properties when drug combines with Tromethamine and pyrrole.

Toxicity

Analysis of the median oral lethal dose (LD_{50} in Rats) was done using the server ProTox-II[42]. Based on the LD_{50}, the toxicity class of the combinations were checked.

Results and Discussion

Docking studies of Diflunisal derivatives

Figure 1A gives the 2D structure of Diflunisal. The COX-1 and COX-2 binding energies of Diflunisal were −7.2 kcal/mol and −8.2 kcal/mol, respectively. Through sequential docking, the spontaneous binding of neutral and the salt combination of Diflunisal-Tromethamine was evaluated. For this study, Diflunisal was docked to the COX-1–Tromethamine docked complex and deprotonated Diflunisal was docked to the COX-1-Tromethamine ion docked complex. Similar dcking

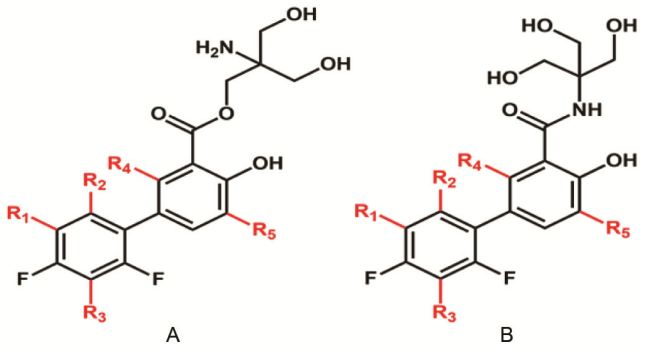

Fig. 3 — The substitutions of pyrrole group at R_1, R_2, R_3, R_4 or R_5 in the (A) Diflunisal-Tromethamine amide structure; and (B) Diflunisal-Tromethamine ester structure

studies were performed on COX-2-Tromethamine and COX-2-Tromethamine ion docked complexes also. The results show that the spontaneous COX binding of Diflunisal has increased when it existed in salt combination with Tromethamine whereas, during its neutral combination with Tromethamine, Diflunisal can bind more spontaneously only to COX-2 enzyme.

Tromethamine was merged into the structure of Diflunisal to form Tromethamine-Diflunisal amide (DF3, Fig. 1B) and Tromethamine-Diflunisal ester (DF4, Fig. 1C) structures. Both combinations have failed to lower the COX-1 binding energy from −7.2 kcal/mol. But in comparison with Diflunisal, Tromethamine-Diflunisal ester structure was more spontaneous to bind with COX-2 enzyme. So the neutral, salt combination or the merging of Tromethamine to Diflunisal (amide) increases the spontaneous COX-2 binding whereas only the salt combination with Tromethamine increased the spontaneous COX-1 binding of drug (Table 1).

Pyrrole substitution in the absence and presence of Tromethamine

Docking results on the COX-1 enzyme is given (Table 2). Five Diflunisal structures namely DF5, DF6, DF7, DF8 and DF9 are those with one pyrrole present at R_1, R_2, R_3, R_4 and R_5, respectively (Fig. 2A). They were docked to the COX-1 enzyme. Except DF8, other four structures had more negative COX-1 binding energy in comparison with Diflunisal. So the substitution of one pyrrole at R_1, R_2, R_3 or R_5 increases the spontaneous COX-1 binding of Diflunisal.

These five structures were docked to COX-1-Tromethamine complex (Fig. 2B). During the neutral combination with Tromethamine, only the substitution of pyrrole at R_1, R_2, R_3 or R_5 increased the spontaneous COX-1 binding of Diflunisal. A neutral combination with Tromethamine alone was not favourable regarding the spontaneous binding (DF1). But the pyrrole substitution at the four mentioned positions has increased the spontaneous binding of drug to COX-1 enzyme.

Table 1 — Binding energy of Diflunisal in the absence, neutral, salt, amide and ester combinations of Tromethamine

No.	Ligand	Target-1	Binding Energy kcal/mol	Target-2	Binding Energy kcal/mol
DF	Diflunisal	COX-1	−7.2	COX-2	−8.2
DF1	Diflunisal	COX-1-Tromethamine complex	−7.2	COX-2-Tromethamine complex	−8.4
DF2	Deprotonated Diflunisal	COX-1-Tromethamine ion complex	−7.3	COX-2-Tromethamine ion complex	−9.0
DF3	Diflunisal-Tromethamine amide	COX-1	−6.9	COX-2	−8.6
DF4	Diflunisal-Tromethamine ester	COX-1	−6.9	COX-2	−8.1

Table 2 — COX-1 binding energy of pyrrole substituted Diflunisal structures in the absence (DF5-DF9), in the neutral combination (DF10-DF14) and in the salt combination with Tromethamine (DF15-DF19)

No.	R_1	R_2	R_3	R_4	R_5	Target-1	BE** (kcal/mol)
DF5	Py*	H	H	H	H	COX-1	−7.7
DF6	H	Py	H	H	H	COX-1	−7.5
DF7	H	H	Py	H	H	COX-1	−7.8
DF8	H	H	H	Py	H	COX-1	−7.1
DF9	H	H	H	H	Py	COX-1	−7.7
DF10	Py	H	H	H	H	Tromethamine - COX-1 Complex	−7.7
DF11	H	Py	H	H	H	Tromethamine - COX-1 Complex	−7.5
DF12	H	H	Py	H	H	Tromethamine - COX-1 complex	−7.7
DF13	H	H	H	Py	H	Tromethamine - COX-1 complex	−7.1
DF14	H	H	H	H	Py	Tromethamine - COX-1 Complex	−7.7
DF15	Py	H	H	H	H	Tromethamine ion - COX-1 Complex	−7.9
DF16	H	Py	H	H	H	Tromethamine ion - COX-1 Complex	−7.7
DF17	H	H	Py	H	H	Tromethamine ion - COX-1 Complex	−7.3
DF18	H	H	H	Py	H	Tromethamine ion - COX-1 Complex	−7.0
DF19	H	H	H	H	Py	Tromethamine ion - COX-1 Complex	−7.9

*Py = Pyrrole group

Table 3 — COX-2 Binding energy of pyrrole substituted Diflunisal structures in the absence of Tromethamine (DF5-DF9), in neutral combination with Tromethamine (DF10-DF14) and in salt combination with Tromethamine (DF15-DF19)

No.	R_1	R_2	R_3	R_4	R_5	Target	Binding Energy (kcal/mol)
DF5	Py*	H	H	H	H	COX-2	−8.4
DF6	H	Py	H	H	H	COX-2	−8.0
DF7	H	H	Py	H	H	COX-2	−8.3
DF8	H	H	H	Py	H	COX-2	−7.3
DF9	H	H	H	H	Py	COX-2	−8.2
DF10	Py	H	H	H	H	Tromethamine - COX-2 Complex	−7.6
DF11	H	Py	H	H	H	Tromethamine - COX-2 Complex	−7.5
DF12	H	H	Py	H	H	Tromethamine - COX-2 complex	−8.4
DF13	H	H	H	Py	H	Tromethamine - COX-2 complex	−7.3
DF14	H	H	H	H	Py	Tromethamine - COX-2 complex	−8.6
DF15	Py	H	H	H	H	Tromethamine ion- COX-2 complex	−7.9
DF16	H	Py	H	H	H	Tromethamine ion- COX-2 complex	−7.7
DF17	H	H	Py	H	H	Tromethamine ion- COX-2 complex	−7.7
DF18	H	H	H	Py	H	Tromethamine ion- COX-2 complex	−7.4
DF19	H	H	H	H	Py	Tromethamine ion- COX-2 complex	−9.0

*Py = Pyrrole group

The binding energy of Tromethamine-Diflunisal salt combination was analysed by docking the five deprotonated structures DF15-DF19 (one pyrrole group at R_1-R_5 respectively) to COX-1-Tromethamine ion complex. Similar to the neutral combination, the pyrrole substitution at R_1, R_2, R_3 or R_5 has increased the spontaneous COX-1 binding of Diflunisal existing in salt combination with Tromethamine.

Thus Pyrrole substitution at R_1, R_2 or R_5 has decreased the COX-1 binding energy to the lowest when Diflunisal existed in salt combination with Tromethamine. Diflunisal with pyrrole at R_3 had least COX-1 binding energy in the absence of Tromethamine. Pyrrole substitution at R_4 was not favourable in any case.

Similar docking study was performed to the COX-2 enzyme also (Table 3). Among the five pyrrole substituted structures (DF5-DF9, Fig. 2A), only DF5 and DF7 had more negative COX-2 binding energy in comparison with Diflunisal. So the substitution of one pyrrole group at R_1 or R_3 increases the spontaneous COX-2 binding of Diflunisal.

In the neutral combination with Tromethamine (Fig. 2B), only the structures DF12 and DF14 had lower COX-2 binding energy in comparison with Diflunisal. So the neutral combination with Tromethamine along with one pyrrole group at R_3 or R_5 increases the spontaneous COX-2 binding of Diflunisal.

Five deprotonated Diflunisal structures (DF15-DF19) were docked to COX-2-Tromethamine ion complex (Fig. 2C) and the salt combination with Tromethamine has decreased the COX-2 binding energy of Diflunisal with pyrrole group at R_5.

Thus Diflunisal with pyrrole at R_1 binds to COX-2 enzyme most spontaneously in the absence of Tromethamine. Diflunisal with one pyrrole at R_3 or R_5 had the least COX-2 binding energy in the presence of Tromethamine. Pyrrole substitution on Diflunisal at R_2 or R_4 is not favourable for COX-2 binding.

Pyrrole substitution on Diflunisal-tromethamine amide and ester structures

Figure 3A & 3B represent the pyrrole substitutions on Diflunisal-Tromethamine amide and ester structures, respectively. Diflunisal-Tromethamine amide (DF3) and ester structures (DF4) were not spontaneous for COX-1 binding in comparison with Diflunisal. So these two structures were docked to COX-1 enzymes after the substitution of one pyrrole at any of the five positions from R_1-R_5 (Table 4). It was found that except DF23, the other four structures had more spontaneous COX-1 binding in comparison with Diflunisal. So the substitution of one pyrrole group at any of the four positions R_1, R_2, R_3 and R_5 increases the spontaneous COX-1 binding of Diflunisal -Tromethamine amide structure.

Pyrrole substitution has made two Diflunisal-Tromethamine ester structures more spontaneous in comparison with Diflunisal namely DF27 and DF29. So the substitution of one pyrrole group at any of the two positions R_1 and R_5 increases the spontaneous COX-1 binding of Diflunisal -Tromethamine ester structure.

Similar docking study was performed on the COX-2 enzyme (Table 4). On docking the five pyrrole substituted Diflunisal-Tromethamine amide structures (Fig. 3A) to COX-2 enzyme, only the structure with pyrrole at R_5 had lower COX-2 energy in comparison with Diflunisal. So only the position R_5 has promoted the spontaneous COX-2 binding of Diflunisal. But the Diflunisal-Tromethamine amide structure got bind to COX-2 enzyme more spontaneously in the absence of pyrrole group.

The Diflunisal–Tromethamine ester structure was not spontaneous for COX-2 binding in comparison with Diflunisal (DF4). But pyrrole substitution at R_1, R_2 or R_3 has made the Diflunisal–Tromethamine ester structure more spontaneous for COX-2 binding.

DF4, DF8, DF13, DF18, DF23 and DF28 were less spontaneous combinations for binding with both COX targets. So these structures cannot be regarded as favourable combinations. The surrounding COX-1 and COX-2 residues involved in the interaction with the structures during docking are given in (Suppl. Table 1) and (Suppl. Table 2), respectively. The best docked complex consisted of hydrogen bonds between structures and targets. But the hydrophobic interactions are predominant.

Analysing the drug-likeness of the discussed structures, only the four structures DF20-DF24 have deviated in one parameter. They showed an excess hydrogen donor from the upper limit 5. But all other four properties set by Lipinski remained in the appropriate range. So the structures studied in this work are orally active (Suppl. Table 3).

Analysing the properties of the studied structures (Table 5), the oral activity exhibited by the combinations was again conformed from the bioavailability score (0.55 or above). Diflunisal-Tromethamine amide and ester forms (DF20-DF29, respectively), are more aqueous soluble than Diflunisal, but they had 8 rotatable bonds. The structures had promising topological polar surface area (TPSA), % absorption and high gastro-intestinal absorption. The combinations DF20-DF29 had slightly higher TPSA (slightly lower % absorption) in comparison with other structures. All the combinations and drug Diflunisal had low fraction Csp^3 values (low saturation). The zero PAINS alert shows that none of the combinations bear fragments that non-specifically bind with other targets.

Drug and the combinations except DF20, DF21, DF22 and DF23, are the structures belonging in toxicity class 4. This means their LD_{50} lie in between 300 – 2000 mg. But the four pyrrole substituted Diflunisal-Tromethamine amides are the structures in lower class 3, (LD_{50} in between 50 – 300 mg) and are less safe in the safer dose of remaining structures (Suppl. Table 4). ProTox-II server and Osiris property explorer had indicated that the structural modifications had not induced cytotoxicity, irritation, carcinogenicity, mutagenicity and they don't affect reproductive organs.

Thus the drug-like and safe combinations of Tromethamine and pyrrole groups that have increased the spontaneous binding of Diflunisal were found out[43-45]. Out of the designed combinations, safe and

Table 4 — COX-1 and COX-2 binding energies of pyrrole substituted Diflunisal-Tromethamine amide (DF20-DF24) and ester structures (DF25-DF29)

No.	R_1	R_2	R_3	R_4	R_5	Target-1	Binding Energy kcal/mol	Target-2	Binding Energy kcal/mol
	Diflunisal-Tromethamine amide					COX-1	−6.9	COX-2	−8.6
DF20	Py*	H	H	H	H	COX-1	−8.0	COX-2	−8.1
DF21	H	Py	H	H	H	COX-1	−7.6	COX-2	−7.6
DF22	H	H	Py	H	H	COX-1	−7.6	COX-2	−7.8
DF23	H	H	H	Py	H	COX-1	−7.0	COX-2	−7.3
DF24	H	H	H	H	Py	COX-1	−7.8	COX-2	−8.4
	Diflunisal-Tromethamine ester					COX-1	−6.9	COX-2	−8.1
DF25	Py	H	H	H	H	COX-1	−7.2	COX-2	−9.2
DF26	H	Py	H	H	H	COX-1	−7.1	COX-2	−8.7
DF27	H	H	Py	H	H	COX-1	−7.5	COX-2	−9.2
DF28	H	H	H	Py	H	COX-1	−7.1	COX-2	−7.6
DF29	H	H	H	H	Py	COX-1	−7.8	COX-2	−7.7

*Py = Pyrrole group

Table 5 — Physico-chemical properties of the studied structural modifications of Diflunisal

No.	Rotational bonds	TPSA (Å^2)	% ABS	Log s (solubility)	Fraction Csp^3	Bioavailability (score)
Diflunisal	2	57.53	89.15	−4.55, Moderately Soluble	0	0.85
Difluinsal ion	2	60.36	88.17	−4.54, Moderately Soluble	0	0.85
DF3	7	110.02	71.04	−3.2, Soluble	0.24	0.55
DF5/DF10	3	73.32	83.70	−5.22, Moderately Soluble	0	0.85
DF6/DF11	3	73.32	83.70	−4.61, Moderately Soluble	0	0.85
DF7/DF12	3	73.32	83.70	−4.61, Moderately Soluble	0	0.85
DF9/DF14	3	73.32	83.70	−4.61, Moderately Soluble	0	0.85
DF15	3	76.15	82.72	−5.22, Moderately Soluble	0	0.56
DF16	3	76.15	82.72	−4.61 Moderately Soluble	0	0.56
DF17	3	76.15	82.72	−4.61, Moderately Soluble	0	0.56
DF19	3	76.15	82.72	−4.61, Moderately Soluble	0	0.56
DF20	8	125.81	65.59	−3.27, Soluble	0.19	0.55
DF21	8	125.81	65.59	−3.27, Soluble	0.19	0.55
DF22	8	125.81	65.59	−3.27, Soluble	0.19	0.55
DF24	8	125.81	65.59	−3.27, Soluble	0.19	0.55
DF25	8	128.8	64.56	−3.47, Soluble	0.19	0.55
DF26	8	128.8	64.56	−3.47, Soluble	0.19	0.55
DF27	8	128.8	64.56	−3.47, Soluble	0.19	0.55
DF29	8	128.8	64.56	−3.47, Soluble	0.19	0.55

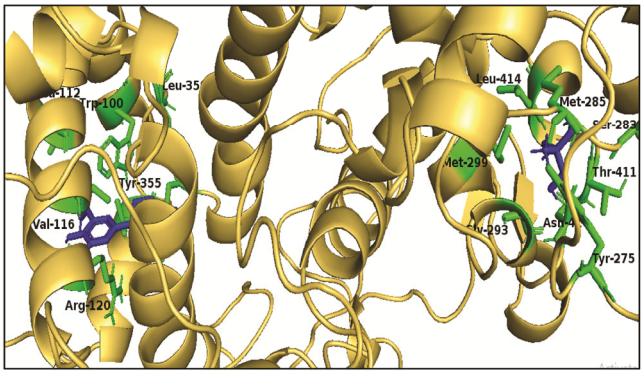

Fig. 4 — Pymol diagram showing the COX-1 residues interacting to the docked DF19 (Blue) and Tromethamine ion (Blue)

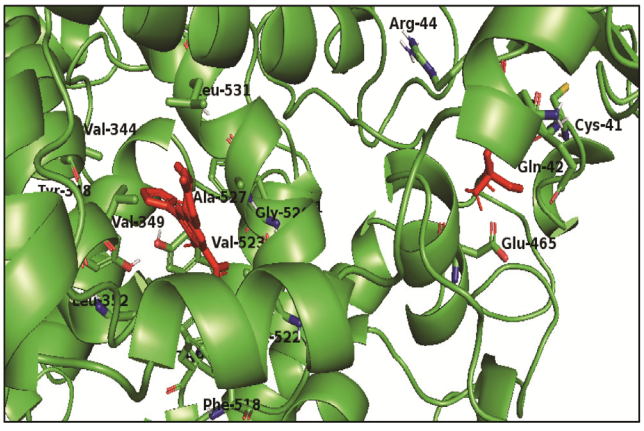

Fig. 5 — Pymol diagram showing the COX-2 residues interacting to the docked DF19 (Blue) and Tromethamine ion (Blue)

orally active combination DF19 was equally favourable for binding to both COX targets. DF19 has pyrole at R_5 and it exists in salt combination with Tromethamine ion. Tromethamine ion formed hydrogen bond with COX-1 residues Gly293, Asn410, Thr411, Tyr275 and COX-2 residues Glu465, Gln42, Arg44, Cys41 in the docking process. The pymol image of the docked complexes of DF19 existing in salt with Tromethamine are given in (Figs 4 & 5).

Conclusion

The present work is a computational molecular docking study undertaken to come up with the structurally modified NSAID drug Diflunisal, with lower gastric problems and increased biological properties. Directly choosing the synthesis and finding out the best structural modification would be very time-consuming procedure that need huge monetary support and often would finish up in crisis. So through the computational drug designing technique, the best Tromethamine-Diflunisal-Pyrrole combinations were found. Neutral, salt, amide and ester combinations of the buffer Tromethamine with drug Diflunisal were evaluated through the obtained COX binding energy values. Pyrrole group (Heterocyclic compound) substitutions were carried out with an aim to enhance biological properties.

DF4, DF8, DF13, DF18, DF23 and DF28 were the failed combinations with less spontaneous binding to

both COX targets. More numbers of the designed derivatives were spontaneous than Diflunisal for COX-1 binding. DF7, DF15, DF19, DF20, DF24 and DF29 are the top combinations with the lowest COX-1 binding energy in comparison with Diflunisal. Similarly DF2, DF25, DF19 and DF27 had the lowest COX-2 binding energy than Diflunisal. Pyrrole substitution at R_4 was not favourable on Diflunisal for COX-1 and COX-2 binding. Analysing the oral activity, properties and toxicity, DF20-DF24 has crossed the maximum limit set for hydrogen donors and they are in the lower toxicity class in comparison with Diflunisal and other structures. The combination of Tromethamine and pyrrole group did not induce cytotoxicity, carcinogenicity, irritation, mutagenicity and reproductive effects in any structures. Thus the derivative DF19 *i.e* Tromethamine-Diflunisal salt with pyrrole group at R_5 is the most favourable, orally active and safe combination that was equally favourable for COX-1 and COX-2 binding.

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Conflict of interest

All authors declare no conflict of interest.

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