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Research Article:

In Silico Design and SAR Studies Evaluation of New Amide Compounds as COX-1 and 2 Inhibitors

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Abstract

Background: The Nonsteroidal anti-inflammatory drugs still are indispensable in the treatment of pain and inflammation, but the available agents have limitations due to their gastrointestinal toxicity or cardiovascular risk. In this study, molecular docking was used to screen for new amide-based COX inhibitors that demonstrated an improved selectivity profile. **Methods:** A series of 132 amide derivatives of four molecular scaffolds (ortho-, meta-, para-aminobenzoic acid, and para-phenylenediamine) was designed and docked to COX-1 and COX-2 enzymes. Binding energies, selectivity indices and pharmacokinetics were calculated with the help of in silico methods. **Results:** Several such compounds showed better COX-2 binding than some commercial NSAIDs. PPDA+Z14 showed the best COX-2 binding affinity: (9:7 kcal=mol) with mild selectivity (2:4kcal=mol). The PPDA core displayed a general trend for enhanced COX-2 selectivity, and the introduction of halogen atoms or bulky aromatic substituents improved the affinity. All 25 prioritized compounds were both Lipinski Rule of 5 compliant and had good ADME profiles. **Conclusions:** In the present work, we have shown via computational approaches that promising amide-based lead compounds with a balanced COX-2 selectivity profile can be identified, which might provide anti-inflammatory effects with a low risk of cardiovascular events compared to very selective coxibs. The combination of the PPDA scaffold with selected substituents is therefore suggested as a privileged structure for the design of potentially less toxic anti-inflammatory agents, deserving of experimental investigation.

1. Introduction

During prostaglandin synthesis, cyclooxygenase enzymes mediates the rate-determining step involved in converting arachidonic acid to PGH2. PGH2 is a substrate for the synthesis of prostanoids such as prostaglandins, which participate in inflammation, pain, fever and several homeostatic mechanisms (1). Two isoforms of the cyclooxygenase, COX-1 and COX-2, are expressed in diverse tissues and related to different physiological functions. Widely expressed COX-1 has been shown to play a role in maintaining gastric mucosal integrity, in regulation of renal blood flow, and in hemostasis (2). In contrast, COX-2 expression is mainly induced by inflammatory stimuli such as cytokines, growth factors, and tumor-promoting agents and as such, COX-2 has been implicated as a key factor in pathologic inflammatory response and pain (3).

Classic over the counter NSAIDs including aspirin, ibuprofen, and diclofenac are non-specific inhibitors of both COX enzymes. Despite being effective anti-inflammatory and analgesic agents, the inhibition of COX-1 by these drugs leads to undesirable effects, including gastrointestinal disturbances in the form of ulcers and hemorrhage, and renal toxicity (3). These adverse effects result from the inhibition of the COX-1-mediated protective prostaglandin synthesis in stomach and kidney cells (4). It has been reported that 15-30% of chronic users of NSAIDs experience adverse events to the gastrointestinum, out of leads to serious complications in 1-2% per year even including perforation, ulcer and bleeding. The realization that COX-2 has a dominant role in inflammation, together with crystallographic studies showing structural differences in the isoforms, led to the design of COX-2 selective inhibitors (coxibs). The most important structural difference is the replacement of isoleucine-523 in COX-1 with the much smaller valine-523 in COX-2, which creates a second hydrophobic binding site in COX-2 that can bind even larger chemical moieties (5). This understanding led to the development of selective agents (celecoxib, rofecoxib

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and etoricoxib) with proven lesser gastrointestinal toxicity than non-selective NSAIDs in clinical trials (6).

Yet the clinical use of COX-2-selective inhibitors has been marred by concerns over cardiovascular safety (5). Several coxibs have been withdrawn from the market or had their indications restricted because of an increased risk of thrombotic events, e.g. myocardial infarction and stroke, with long-term treatment (7). These events are the result of a preferential inhibition of COX-2-derived prostacyclin (PGI2) in the endothelial cells lining blood vessels, without inhibition of COX-1 derived thromboxane A2 (TXA2) in platelets; thereby creating a prothrombotic environment. Thus, there is still a requirement in the art for anti-inflammatory agents with optimal COX-2 selectivity to minimize or avoid the gastrointestinal toxicity associated with non-selective NSAIDs and which have acceptable cardiovascular safety (8).

Amides are an attractive class of compounds for the development of COX inhibitors due to their good metabolic stability, ease of synthesis, and wide range of structural diversity (8). The amide group is a stable linking group that is not subjected to rapid hydrolysis unlike ester-containing prodrugs which require metabolic transformation. In addition, amides are able to hydrogen bond both as donors and acceptors, and may interact with amino acids in the active site of an enzyme (9). Systematic variation of groups that are attached to an amide core enable structure-activity relationship studies for improved potency and selectivity (10).

Computer-aided drug design remains the key for compound screening in pharmaceutical investigation by means of molecular docking while providing valuable structural information of drug molecules in an incredibly short time (10). Molecular docking predicts computationally the orientation of small molecules in the active site of protein and their binding possibilities which can be predicted by shape complementarity, electrostatic complementarity and hydrogen bonds. These computational methodologies direct the choice of compounds for synthesis and biological evaluation, and if sufficiently validated, bring significant acceleration and cost reduction to drug discovery (11).

In the current study, the molecular docking technique was used to predict the activity of novel amide compounds as COX-1 and COX-2 inhibitors. The scientists synthesized 132 compounds based on 4 different molecular scaffolds, 33 diversified carboxylic acid-derived amide substituents. This all-round strategy made it possible to select the best framework-substituent sets with a relatively strong COX-2 binding and COX-2 selectivity. The molecular scaffolds were the ortho-aminobenzoic acid (OABA), the meta-aminobenzoic acid (MABA), the para-aminobenzoic acid (PABA) and the para-phenylenediamine (PPDA). The research goals were to: (1) identify lead candidates with improved COX-2 binding relative to known inhibitors, (2) study the structural determinants for selectivity, (3) understand the molecular basis of isoform-selective binding, and (4) identify potential candidates for synthesis and biological evaluation.

2. Materials and Methods

A full library consisting of 132 amides was were constructed and energy minimized by using ChemDraw (version 16.0.0.82(68)) software. The optimized structures of the molecules were then uploaded to the Mcule.1-click-docking (MCD) web service (https://mcule.com) to perform molecular docking simulations (12). The dockings were carried out by the Mcule.1-click-docking web service on two enzyme models, cyclooxygenase-1 (prostaglandin G/H synthase 1, PDB ID: 3N8Y) and cyclooxygenase-2 (prostaglandin G/H synthase 2, PDB ID: 3NTG). The docking scores were in kcal/mol and the active site dimensions were X: 33.2262, Y: -45.3426, Z: -3.5976 for the 3N8Y enzyme and X: 26.4813, Y: 22.0785, Z: 16.1282 for the 3NTG enzyme. Prioritization of compounds was performed based on binding energy scores obtained from docking analyses and analyzing the spatial fit of ligands in target enzymes by molecular visualizations by using Discovery Studio Visualizer (BIOVIA) v. 20.1.0.19295 (13).

Table 1 summarizes the 31 carboxylic acid-based substituents applied in this work. A detailed analysis was conducted for docking results for both target enzymes with all designed ligands.

The compound library was systematically designed based on 4 diverse core scaffolds the ortho-aminobenzoic acid (OABA), the meta-aminobenzoic acid (MABA), the para-aminobenzoic acid (PABA) and the para-phenylenediamine (PPDA) which are represented in **Table 2**. Each scaffold was diversified by amide linkages with 33 different carboxylic acid fragments (Z1-Z33). Promising lead compounds were selected by a rational visual inspection together with a conformational analysis of the docked compounds. For every compound, all four highest-affinity binding poses produced by docking simulations were thoroughly analyzed. This evaluation allowed the determination of whether the best binding pose and the best energetically conformation in the enzyme active site was reached by comparing the ligand orientation and interaction profile with one or more reference NSAID molecules that were used as a control. The best docking scores for the poses with the lower energies were noted. This structural analysis was carried out using the BIOVIA Discovery Studio Visualizer (14).

Table 1. The carboxylic acid-derived substituents

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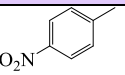
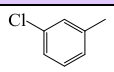
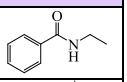
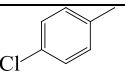
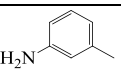
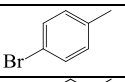
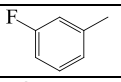
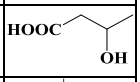
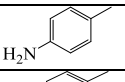
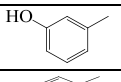
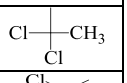
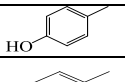
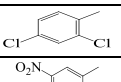
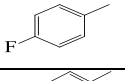
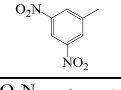
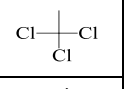
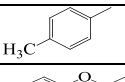
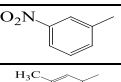
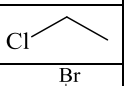
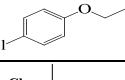
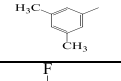
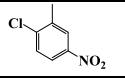
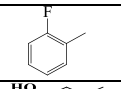
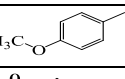
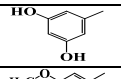
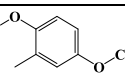
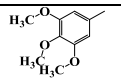
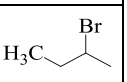
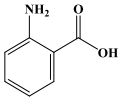
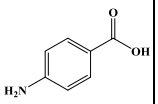
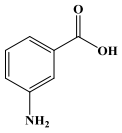
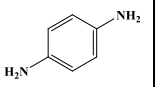
Z1		Z12		Z23	
Z2		Z13		Z24	
Z3		Z14		Z25	
Z4		Z15		Z26	
Z5		Z16		Z27	
Z6		Z17		Z28	
Z7		Z18		Z29	
Z8		Z19		Z30	
Z9		Z20		Z31	
Z10		Z21		Z32	
Z11		Z22		Z33	

Table 2. The scaffolds used in the designing the new compounds

Ortho-amino benzoic acid (OABA)		Para-amino benzoic acid (PABA)	
Meta-amino benzoic acid (MABA)		Para-Phenylenediamine (PPDA)	

Six known inhibitors (four non-selective agents, diclofenac, meloxicam, ibuprofen, piroxicam, and two COX-2-selective inhibitors, celecoxib, and etoricoxib) were docked for comparison along with the designed compounds against both enzyme targets. Subsequently, the compounds with better docking score were analyzed for their computational pharmacokinetic properties using the web based SwissADME (15) (http://www.swissadme.ch/). The molecular structures of the selected candidates were submitted to the web server and the predicted ADME (absorption, distribution, metabolism, and excretion) properties.

3. Results and Discussion

3.1. Docking results for NSAIDs drugs

In the current molecular docking, a full series of 132 designed amide derivatives as potential COX-1 and COX-2 inhibitors was assessed. The compounds were designed based on 33 different carboxylic acid derived substituents (Z1-Z33) and four core scaffolds, the ortho-aminobenzoic acid (OABA), the meta-aminobenzoic acid (MABA), the para-aminobenzoic acid (PABA) and the para-phenylenediamine (PPDA). This rational design principle allowed for the full SAR investigation to serve as leadcandidates with significantly improved COX-2 selectivity profiles.

3.2. Validation of docking protocol

The docking procedure was initially validated by using known clinical NSAIDs. The reference compounds have binding energies, which are consistent with their physiological bioactivities and clinical selectivity (**Table 3**).

Table 3. Docking results for reference NSAID compounds

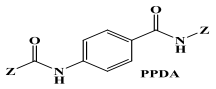
Drug	COX-1 (3N8Y) (kcal/mol)	COX-2 (3NTG) (kcal/mol)	Selectivity Index	Clinical Profile
Diclofenac	-7.8	-8.4	0.6	Modest COX-2 preference
Ibuprofen	-7.2	-7.2	0.0	Non-selective
Meloxicam	-5.3	-7.5	2.2	Preferential COX-2
Piroxicam	-7.0	-8.0	1.0	Weak COX-2 preference
Celecoxib	-2.9	-9.1	6.2	Highly selective COX-2
Etoricoxib	-3.1	-9.2	6.1	Highly selective COX-2

Excellent linear correlations were displayed between the respective binding energies scores of the reference compounds and their known pharmacological activities (**Table 3**). Non-selective NSAIDs, such as ibuprofen demonstrated similar binding affinities towards both COX isoforms (−7.2 kcal/mol for both), while diclofenac had a mild preference towards COX-2 (selectivity index: 0.6 kcal/mol). By contrast, very potent/selective COX-2 inhibitors (celecoxib and etoricoxib) were two totally different binding modes characterized by very weak COX-1 binding (−2.9 and −3.1 kcal/mol, respectively) and very strong COX-2 binding (−9.1 and −9.2 kcal/mol, correspondingly) giving rise to selectivity indices higher than 6.0 kcal/mol. These findings are in close agreement with clinical results, supporting the predictive value of our docking protocol to reproduce COX selectivity profile (16).

3.3. Analysis of molecular docking for amide derivatives

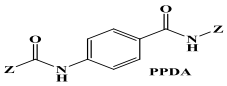
The docking simulations for a comprehensive library of 132 amide derivatives have been completed and analyzed (**Table 4** and **5**). These derivatives were derived from the 4 core molecular scaffolds, each functionalized with 33 distinct carboxylic acid substituents (Z1-Z33).

Table 4. The docking results of the designed amide derivatives with Cox1(3N8Y) enzyme



Z	OABA	MABA	PABA	PPDA
1	-8.2	-6.9	-5.0	-4.3
2	-6.9	-6.6	-5.9	-6.8
3	-6.5	-6.2	-4.9	-5.6
4	-6.9	-6.6	-5.9	-6.8
5	-7.5	-7.4	-6.8	-6.5
6	-7.4	-7.2	-7.6	-6.7
7	-8.7	-7.5	-7.5	-6.4
8	-6.5	-6.2	-6.8	-6.7
9	-7.7	-7.9	-7.9	-6.3
10	-6.1	-6.8	-5.9	-5.1
11	-5.9	-6.9	-6.9	-5.9
12	-8.4	-8.1	-8.3	-7.5
13	-5.9	-6.9	-6.9	-5.9
14	-8.7	-8.0	-8.1	-7.3
15	-8.5	-8.1	-7.6	-7.1
16	-5.9	-6.9	-6.9	-5.9
17	-7.2	-6.6	-5.5	-2.2
18	-5.9	-6.9	-6.9	-5.9
19	-8.6	-8.1	-7.3	-6.6
20	-8.6	-8.2	-8.1	-6.9
21	-7.3	-7.4	-6.6	-6.2
22	-5.9	-6.9	-6.9	-5.9
23	-4.9	-6.5	-6.5	-4.9
24	-7.6	-7.5	-7.4	-7.6
25	-5.1	-5.4	-6.9	-4.7
26	-6.5	-5.8	-6.5	-5.3
27	-5.9	-5.4	-5.7	-5.8
28	-5.6	-4.8	-6.8	-5.8
29	-4.4	-5.8	-6.1	-5.6
30	-6.1	-5.8	-6.5	-6.8
31	-5.1	-5.4	-6.9	-4.7
32	-6.5	-5.8	-6.5	-5.3
33	-5.9	-5.4	-5.7	-5.8

Table 5. The docking results of the designed amide derivatives with Cox2 (3NTG) enzyme.



Z	OABA	MABA	PABA	PPDA
1	-8.4	-8.4	-7.3	-9.3
2	-5.7	-6.9	-7.9	-6.8
3	-6.3	-6.5	-7.8	-6.9
4	-7.6	-7.5	-7.9	-7.6
5	-8.1	-7.9	-7.3	-9.0
6	-8.2	-8.2	-7.8	-9.1
7	-8.5	-8.4	-7.9	-9.7
8	-5.7	-6.9	-7.9	-6.8
9	-8.9	-8.9	-8.1	-9.3
10	-8.2	-8.4	-7.1	-8.9
11	-6.3	-6.5	-7.8	-6.9
12	-8.4	-8.3	-7.8	-9.8
13	-5.7	-6.9	-7.9	-7.4
14	-8.4	-8.3	-8.1	-9.7
15	-8.1	-8.7	-7.7	-9.5
16	-5.5	-5.8	-7.6	-7.9
17	-8.3	-8.0	-7.2	-7.8
18	-6.8	-5.4	-5.4	-6.8
19	-8.9	-8.8	-8.3	-9.4
20	-8.3	-8.4	-7.7	-9.4
21	-8.3	-8.3	-7.1	-8.9
22	-6.8	-5.4	-5.4	-6.8
23	-5.6	-6.7	-6.5	-5.1
24	-9.0	-9.1	-8.8	-9.1
25	-6.8	-5.4	-5.4	-6.8
26	-5.6	-6.7	-6.5	-5.1
27	-5.7	-7.2	-5.8	-5.3
28	-9.0	-8.9	-8.6	-9.0
29	-5.5	-5.8	-7.6	-7.9
30	-6.2	-5.6	-7.9	-7.7
31	-5.5	-5.8	-7.2	-6.5
32	-4.6	-4.7	-7.2	-5.7
33	-6.8	-5.4	-5.4	-6.8

All standard NSAIDs (SN) ligands in the COX-1 (3N8Y) active site interacted with ALA 496 AA residue. In the same vein, three of the SNs were attached to only three of the following binding residues (TYR 324, VAL 85, SER 499, VAL 318, and LEU 321) whereas one or two SNs were bound four of these residues (SER 322, MET 491, TYR 354, and ILE 492). The ten residues define however the active/binding site to which the ligand binds with this enzyme (17) (Figure 1). Meanwhile the SNs were active over the COX-2 (3NTG) active site forming interactions with five conserved AA shared including: ALA 496, VAL 318, TRP 356, VAL 492, SER 322. Moreover, three of the SNs bind six (LEU 328, LEU 500, LEU 353, LEU 321, HIS 58, and TYR 324) and exclusively with COX-2 selective inhibitors, seven other residues (SER 322, GLY 495, TYR 354, ARG 482, MET 491, PHE 487, and GLN 161) selectively bound. These residues are the 12 functional binding site through which the ligand binds with this enzyme (18) (**Figure 1**).

As can be seen, docking against COX showed that 17 of these compounds had better docking scores than the reference NSAIDs against COX-1, while 8 compounds had better binding scores against COX-2 than the reference compounds (highlighted in Tables 1 and 2), the analysis of docking results presented in **Tables 1** and **2** out of 132 amide compounds evaluated. The 17 COX-1 lead compounds were predicted to make binding interactions with 7-10 of the ten AA residues that anchor the standard NSAIDs and with an additional 4-6 residues in the active pocket of the enzyme. These additional interactions seem to increase the binding affinity of the test compounds towards the COX-1 enzyme as shown in **Figure 2**. Likewise, the 8 lead COX-2 compounds also formed binding with 7-10 of the 12 AA residues that holds the standard NSADs and in addition interacted with 3-5 other AA residues in the active pocket of the enzyme. These multiple contacts might contribute to the increased binding affinity of the test compounds to the COX-2 enzyme (**Figure 2**).

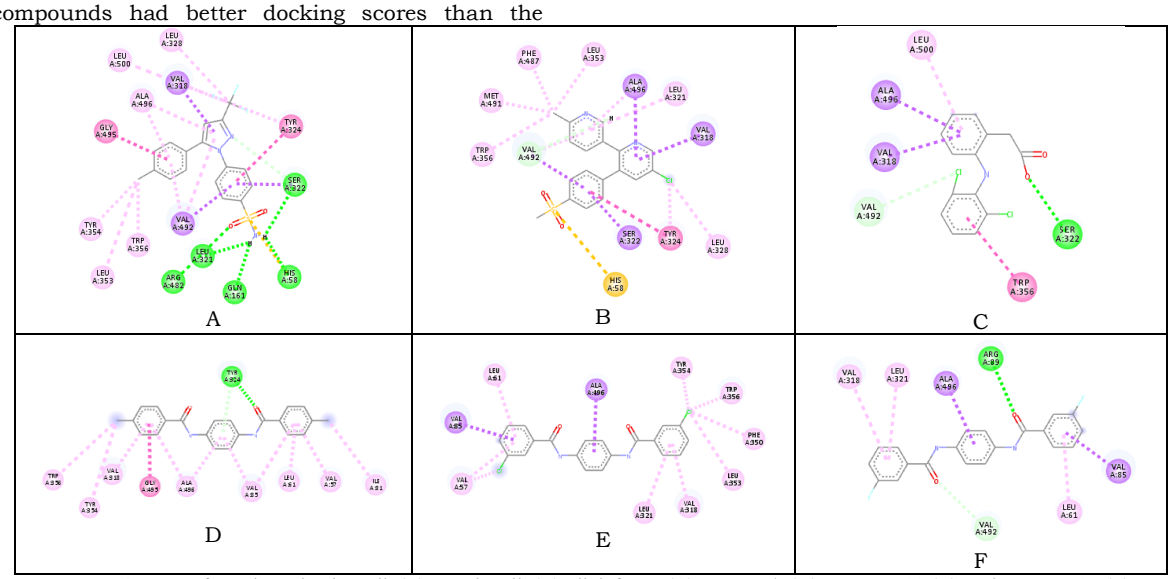


Figure 1. Poses for selected celecoxib (A), Etoricoxib (B), diclofenac (C) PPDA+Z7 (D), PPDA+Z12 (E) and PPDA+Z14 (F) with the COX 2 (3NTG) enzyme.

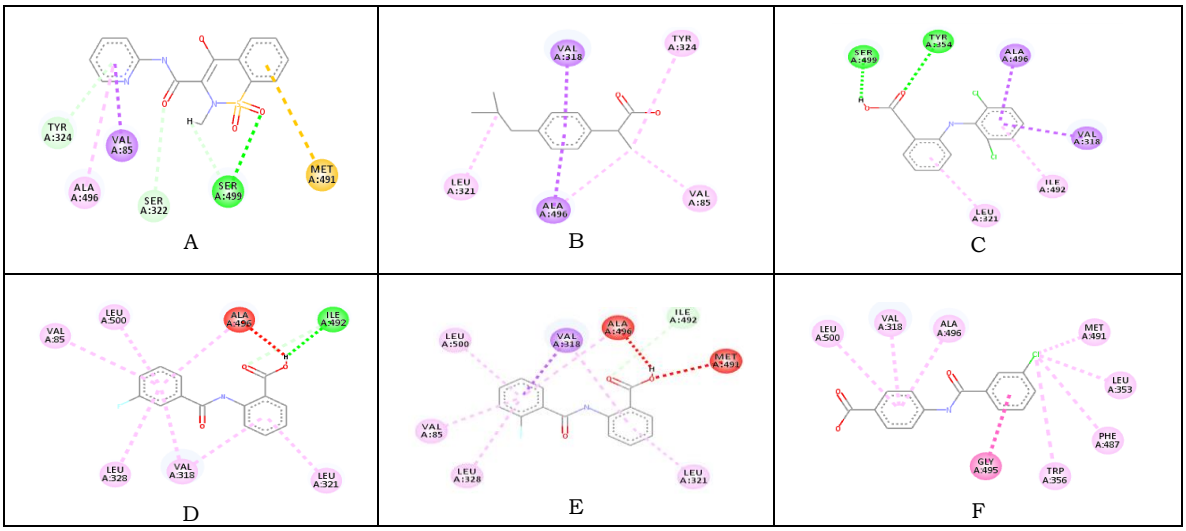


Figure 2. Poses for selected piroxicam (A), ibuprofen (B), diclofenac (C) Ortho+Z14 (D), Meta+Z20 (E) and Para+Z12 (F) with the COX 1 (3N8Y) enzyme

3.4. Structure-activity relationships

3.4.1. Scaffold-dependent selectivity patterns

The selectivity indices (SI) were calculated as ΔG(COX-1) - ΔG(COX-2). None of the synthesized compounds attained the coxib-like extreme selectivity (SI> 6 kcal/mol). These selectivity indices (although slightly lower than celecoxib and etoricoxib) should be considered relative to absolute binding energies. The leading PPDA compounds possess COX-2 binding affinities (-9.3 to -9.8 kcal/mol) that are comparable to or higher than those of marketed coxibs, implying they might have a higher COX-2 potency. This concurrence of large absolute COX-2 affinity with moderate selectivity indices may be suitable for an optimal therapeutic performance, conferring potent anti-inflammatory activity with acceptable gastrointestinal safety margin (19).

The core scaffold was found to be essential for governing the COX selectivity patterns. The PPDA scaffold also showed the best preference for COX-2 over COX-1 for all kind of substituents, which means that the large fused aromatic moiety of the PPDA skeleton with the two amide linking points might have an ideal geometry for entering the COX-2 active site as the absence of the carboxylic acid moiety that is needed for their taking up in the enzyme pocket. The dual amide functionality could be involved in simultaneous binding to several amino acids residues in the binding pocket which would contribute to higher affinity and selectivity (20,21).

Unlike the aminobenzoic acid grands (OABA, MABA, PABA), the obtained compounds presented a better dual inhibition profile, although more balanced (with regard to their orth, meta and para positioned). These scaffolds are believed to bind to highly conserved residues within both isoforms of COX, rendering them less selective. The ortho substitution pattern of OABA induces conformational restriction of the molecule during binding, while meta and para isomers can rotate more freely when docked inside the active site (22,23).

Molecular docking analysis showed compounds binding deeper into the COX-1 (3N8Y) active site and where most NSAIDs interact with ALA 496. In addition, the interactions with TYR 324, VAL 85, SER 499, VAL 318, and LEU 321 were common (for some) of the high affinity ligands. These interactions correspond to the major determinants of binding to COX-1 for inhibition (17). In the COX-2 (3NTG) pocket, every selective inhibitor was stably bounded with ALA 496, VAL 318, TRP 356, VAL 492, and SER 322. Notably, additional interaction with LEU 328, LEU 500, LEU 353, and TYR 324 were the 2 in 1 anti-inflammatory agents 5 that exhibited high COX-2 selectivity, which is also in line with the reported structural depictions (18,23).

3.4.2. Substituent effects on binding affinity

Both binding affinity and selectivity were largely dependent on the type and location of substituent on the carboxylic acid group. Halogen substituents especially with a fluorine atom (Z6, Z14, Z20) show a general trend of enhancement in COX-2 binding in different scaffolds. The high electronegativity of the fluorine atom combined with a small van der Waals radius allows the formation of attractive electrostatic interactions and dipole-dipole interactions within the enzyme active site without causing large steric effects (24-28). Fluorinated and halogenated substituents were also linked with selectivity, maybe by halogen-bonding, or by electron-withdrawing effects, which stabilize ligand-enzyme complex. In addition, electron withdrawing substituents (for example, fluorine improved binding, perhaps by polarizing the amide bond or through involvement in electrostatic interactions.

Disubstituent bulky aromatic such as Z9 (Cl and NO2) showed scaffold-dependant results. Together with PPDA, Z9 gave rise to compounds that exhibited a remarkable COX-2 selective profile, presumably by effectively filling the COX-2 side pocket formed by Val523. This side pocket (which is narrowed by the bulkier Ile523 residue in COX-1) is the main structural determinant for achieving COX-2 selectivity (6,20,25). The presence of bulky aromatic substituents (Z7, Z12, Z19, Z24) led to a uniform increase in the COX-2 affinity irrespective of the scaffold, probably by increasing the van der Waals interactions and occupying the COX-2 side pocket more effectively.

Hydroxyl-bearing substituents (Z5, Z15, and Z21) were hydrogen bond donors and acceptors, enabling hydrogen bonding interactions with polar residues of the active site, which enhanced the affinity. The meta-hydroxyphenyl moiety in Z15 was found to be particularly potent, affording balanced COX inhibitors that can be applied where dual isoform activity is desired. In contrast, the aliphatic moieties Z25-Z33 which are theoretically smallest of groups showed the weakest binding affinities in most of the tested aminothiazoles, inferring that the contact surface is minimal to provide the sufficient interaction with the active site (30). The large, flat aromatic substituents were essential for potent COX-2 binding in all the scaffolds. On the other hand, aliphatic or smaller (Z25–Z33) groups led to poor docking scores. This points to the need for a large hydrophobic interaction in the COX-2 cavity.

3.4.3. Molecular basis of selectivity

The selectivity trends observed can be explained by known structural distinctions between COX-2 and COX-1. The replacement of isoleucine-523 in COX-1 by the smaller valine-523 in COX-2 generates a second binding pocket, about 25% larger in COX-2. This enlarged pocket allows for larger substituents, which would otherwise sterically hinder in the COX-1 binding site (6,20). The extended aromatic nature and dual amide nature of the PPDA scaffold seems to be ideally suited to take advantage of this structural distinction. Moreover, small differences in the positioning of active site residues of the two isoforms determine the shape of hydrogen bonding networks. The selective compounds found in this study most likely also form hydrogen bonds with ARG513, PHE518, and other COX-2 specific resides, which are not present or are differently placed in COX-1. Together these interactions, and complementary hydrophobic contacts in the Val523 pocket, explain the observed selectivity (29,30,32).

3.5. Evaluation of the pharmacokinetic profile

The in silico ADME prediction by SwissADME indicated that all the 25 superior lead compounds obeyed Lipinski's Rule of Five well which suggested good drug-likeness and good oral bioavailability (**Table 6**). This is a definite advantage compared to certain existing NSAIDs having poor pharmacokinetics (31,33).

Table 6. Pharmacokinetic properties of the tested compounds predicted by SwissADME.

No.	Comp.	Physicochemical Properties			Lipophilic activity	Agues Solubility	Drugs kinetics			Drug-likeness
		Molecular weight g/mol	No. of HBA	No. of HBD	Log Po/w	Log S	GI availability	BBB penetration	CYP 450	Lipinski
1	Ortho+Z1	286.24	5	2	1.19	-3.83	high	no	no	yes
2	Ortho+Z7	255.27	3	2	1.97	-4.08	high	yes	no	yes
3	Ortho+Z12	275.69	3	2	1.94	-4.38	high	yes	no	yes
4	Ortho+Z14	259.23	4	2	1.88	-3.94	high	yes	no	yes
5	Ortho+Z15	257.24	4	3	1.12	-3.64	high	no	no	yes
6	Ortho+Z19	269.30	3	2	2.25	-4.38	high	yes	no	yes
7	Ortho+Z20	259.23	4	2	1.77	-3.94	high	yes	no	yes
8	Meta+Z9	320.68	5	2	1.29	-4.18	high	no	no	yes
9	Meta+Z12	275.69	3	2	1.97	-4.03	high	yes	no	yes
10	Meta+Z14	259.23	4	2	1.82	-3.59	high	yes	no	yes
11	Meta+Z15	257.24	4	3	1.45	-3.29	high	no	no	yes
12	Meta+Z19	269.30	3	2	2.25	-4.03	high	yes	no	yes
13	Meta+Z20	259.23	4	2	1.91	-3.35	high	yes	no	yes
14	Para+Z9	320.68	5	2	1.41	-3.08	high	no	no	yes
15	Para+Z12	275.69	3	2	1.97	-3.50	high	yes	no	yes
16	Para+Z14	259.23	4	2	1.86	-3.07	high	yes	no	yes
17	Para+Z20	259.23	4	2	1.91	-3.70	high	yes	no	yes
18	PPDA+Z1	406.35	6	2	1.81	-4.40	low	no	yes	yes
19	PPDA+Z7	344.41	2	2	3.05	-4.90	high	yes	yes	Yes
20	PPDA+Z9	475.24	6	2	1.58	-4.97	low	no	yes	yes
21	PPDA+Z12	385.24	2	2	3.18	-5.48	high	yes	yes	yes
22	PPDA+Z14	352.33	4	2	2.80	-4.61	high	yes	yes	yes
23	PPDA+Z15	348.35	4	4	1.99	-4.01	high	no	yes	yes
24	PPDA+Z19	372.46	2	2	3.55	-5.41	high	yes	yes	yes
25	PPDA+Z20	352.33	4	2	2.65	-4.53	high	yes	yes	yes

The molecular weight of the compounds ranged between 255.27 and 475.24 g/mol and most of them were in the range considered as suitable for oral absorption (300-400 g/mole). The numbers of hydrogen bond acceptors and donors were 2-6 and 2-4, respectively, all acceptable numbers. The lipophilicity, i.e., Log P value was between 1.12 and 3.55, and most of the compounds had intermediate lipophilicity (1.5–3.0) which is considered as an optimum value for permeation through membrane and systemic circulation (15,33,34).

Aqueous solubility (Log S) ranging from -3.08 to -5.78 indicated that most of the compounds are soluble/insoluble in water. Although this may pose formulation problems, it is typical of the lipophilic character necessary for COX inhibition and similar to that of current NSAIDs. Predicted gastrointestinal absorption was high in 24 of 25 compounds, and PPDA+Z1 was the only one with low GI absorption, possibly due to its high molecular weight (406. 35 g/mol) and H-bond acceptor number (6). Blood-brain barrier (BBB) penetration predictions were dramatic: while compounds from the PPDA series mostly had no BBB penetration, they potentially do offer peripheral inflammation without CNS side effects. Meanwhile, some of the OABA~, MABA~, and PABA derivatives were found to be BB permeable and this may be an advantage in neuroinflammatory diseases, but may necessitate a close scrutiny for CNS related side effects (15,34).

Predictions of interaction with CYP450 enzymes showed that the majority of compounds (20/25) are not predicted to inhibit CYP450, representing low potential for metabolic drug-drug interactions. The five compounds predicted to be CYP450 inhibitors (mainly PCGA derivatives) should be further evaluated in preclinical development to determine clinical relevance. However, the fact that most leads do not inhibit CYP450 is a positive safety feature that may facilitate saying safe supervision when co-administered with other drugs commonly administered to patients in need of anti-inflammatory treatment (34).

3.6. Comparison with existing therapeutics

Considering marketed NSAIDs and coxibs as the benchmark, the first two compounds of this study reveal also a maximum of three possible ones. The absolute COX-2 binding energies of the best PPDA derivatives (-9.3 to -9.8 kcal/mol) are comparable or higher than those of celecoxib and etoricoxib (-9.1 to -9.2 kcal/mol), which indicates at least equal or better COX-2 inhibitory potency. Although the selectivity indices are somewhat lower (2.3-3.3 vs. 6.1-6.2 kcal/mol), this may in fact translate into a clinical benefit by guaranteeing enough COX-1 sparing to mitigate the gastrointestinal damage and not achieving the extreme COX-2 selectivity that has been related to cardiovascular risk (7,8).

The amide bond used in our design of the compound gives more metabolic stability as compared to the ester containing prodrugs which need enzymatic activation. This direct-acting mechanism may allow for more predictable pharmacokinetics, potentially resulting in less variability between patients in terms of therapeutic effect (10,30). Furthermore, the structural diversity of our compound collection makes an excellent starting point for future programs by providing multiple back-up candidates and options for optimization, unlike mono-molecule development programmes which are highly vulnerable to attrition.

Despite the valuable predictive information that can be obtained from molecular docking, there are several caveats. Docking scoring functions are approximations of the binding free energy based on overly simplified models that might not consider all entropic contributions, protein flexibility and solvent effects (19,31,32). Therefore, the scores of docking need to be considered as an indicator of ranking for each compound, but not as an absolute prediction of the biological activity. Experimental validation in vitro and in vivo with COX-1 and COX-2 enzymatic tests yet to prove predicted selectivity profiles and to obtain quantitative IC_{50} figures.

4. Conclusions

Herein, the molecular docking study performed on 132 amides derivatives belonging to four different scaffolds, allowed us to discover several leads with better binding affinity towards COX-2 and good selectivity in comparison to known NSAIDs. The work defines well pronounced SAR showing that the PPDA scaffold in combination with halogenated or bulky aromatic substituents yields compounds that are remarkably COX-2 selective with high absolute binding affinity. The dual inhibitor PPDA+Z14 stands as the best candidate with the highest COX-2 binding energy in the whole series (-9.7 kcal/mol) along with a moderate affinity for COX-1 (-7.3 kcal/mol) leading to a selectivity index of 2.4 kcal/mol. Other leader candidates such as PPDA+Z12, PPDA+Z9 and PPDA+Z7 achieved similar profiles in terms COX-2 binding energy. These are as good as or better than the binding affinities of marketed coxibs, while selecting only moderately rather than extremely that may translate into better cardiovascular safety.
Structure-activity relationships analysis indicated fluorine substituted (Z6, Z14, Z20) and bulky aromatic groups such as (Z9) consistently improve COX-2-selectivity by establishing favourable electrostatic interactions. The PPDA template was better for achieving COX-2 selectivity owing to its longer aromatic system and two amide groups which can bind to more than one residue within the binding pocket of COX-2 specific isoform. In silico ADME calculation also proved that the all 25 chosen compounds satisfied Lipinski's Rule of Five and the physiochemical properties of the compounds are like that of drug molecule which can be helpful in oral administration. The prediction of high intestinal absorption accompanied by low CYP450 inhibitory potential for the majority of the examined derivatives indicates their potential as compounds with favorable pharmacokinetics and low propensity to cause metabolic drug-drug interactions.

5. Acknowledgements

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6. Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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تصميم حاسوبي وتقييم العلاقة بين البنية والفعالية البيولوجية لمركبات أميدية جديدة كمثبطات لإنزيمي– السيكلوأوكسيجيناز1و2

الخلاصة

الخلفية: لا تزال الأدوية المضادة للالتهاب غير الستيرويدية لا غنى عنها في علاج الألم والالتهاب، لكن العوامل المتاحة لها قيود بسبب سميتها المعدية المعوية أو مخاطرها القلبية الوعائية. في هذه الدراسة، تم استخدام الإرساء الجزيئي للكشف عن مثبطات جديدة لإنزيمات COX قائمة على الأميد والتي أظهرت ملفاً تحسيناً في الانتقائية. **الطرق:** تم تصميم سلسلة من 132 مشتقاً أميدياً من أربعة سقالات جزيئية (حمض أورثو-أمينوبنزويك، وميتا-أمينوبنزويك، وبارا-أمينوبنزويك، وبارا-فينيلين ثنائي الأمين) وإرساؤها على إنزيمات COX-1و .COX-2تم حساب طاقات الارتباط ومؤشرات الانتقائية والحرائك الدوائية بمساعدة الطرق الحاسوبية. **النتائج:** أظهرت عدة مركبات من هذا القبيل ارتباطاً أفضل بإنزيم COX-2مقارنة ببعض الأدوية المضادة للالتهاب غير الستيرويدية التجارية. أظهر المركب PPDA+Z14 أفضل ألفة ارتباط بإنزيم :COX-2 (9.7- كيلو كالوري/مول) مع انتقائية معتدلة (2.4 كيلو كالوري/مول). أظهر هيكل PPDA اتجاهاً عاماً نحو تعزيز انتقائيةCOX-2 ، وأدى إدخال ذرات الهالوجين أو المستبدلات الأروماتية الضخمة إلى تحسين الألفة. جميع المركبات الـ25 ذات الأولوية كانت متوافقة مع قاعدة ليبينسكي للخمسة ولها ملامح ADME جيدة. **الاستنتاجات:** في العمل الحالي، أظهرنا عبر المناهج الحاسوبية إمكانية تحديد مركبات رائدة واعدة قائمة على الأميد مع ملف انتقائية متوازن لإنزيمCOX-2 ، والتي قد توفر تأثيرات مضادة للالتهاب مع خطر منخفض لأحداث القلب والأوعية الدموية مقارنة بمثبطات الكوكسيب شديدة الانتقائية. لذلك يُقترح الجمع بين سقالة PPDA مع مستبدلات مختارة كبنية مميزة لتصميم عوامل مضادة للالتهاب يحتمل أن تكون أقل سمية، وتستحق التحقيق التجريبي.

الكلمات المفتاحية: الإرساء الجزيئي; إنزيمCOX-1; إنزيمCOX-2 ; المثبطات الانتقائية ; المشتقات الأميدية