

Aspirin and Other Non-Steroidal Anti-Inflammatory Drugs as Cyclooxygenase Inhibitors: State of the Art, Barriers and Perspectives

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Abstract: Non-steroidal anti-inflammatory drugs (NSAIDs) claimed during last years an increased research interest to establish their cardiovascular safety profile. Generally, NSAIDs inhibit in different degrees both isoforms of cyclooxygenase (COX). Aspirin has a unique property among NSAIDs, namely at low doses it inactivates irreversibly the COX-1 activity in platelets. It is well known that platelets are a significant source of inflammatory mediators and their activation leads to important clinical atherothrombotic vascular events. Atherosclerosis is a chronic inflammatory process. The cardioprotective effect of aspirin resides in its mechanism of action, suppressing the platelet COX-1 dependent thromboxane biosynthesis. There are patients who do not benefit from the cardioprotective effect of aspirin, being labeled as "resistant" to aspirin. The underlying mechanism of aspirin resistance is yet unclear. This review intends to detail recent advances in the field of molecular simulation applied to nonselective non-aspirin NSAIDs and other COX selective inhibitors. Binding studies were performed between the COX-2 enzyme and these molecules. Using 2D-QSAR, it was noticed that the lipophilic bulkier group width-wise is required for a significant biological activity and also, the hydrophobic interactions might be crucial for the potency of some COX inhibitors. In order to understand a meaningful comparison of both classical NSAIDs and newer COX-2 inhibitors, three-dimensional quantitative structure-activity relationships and also molecular docking techniques were applied.

Keywords: Cyclooxygenase inhibitors, molecular simulation, non-steroidal anti-inflammatory drugs (NSAIDs).

1. ASPIRIN AND ANTI-INFLAMMATORY DRUGS - PHARMACODYNAMICS AND PHARMACOKINETICS EVALUATION

Despite its long use in clinical practice, aspirin (acidum acetylsalicylicum) still remains one of the most studied biologically active molecules because of its large spectrum of indications. After its existence for more than 100 years, aspirin is considered the golden standard of antiplatelet therapy [1]. Salicin, the bitter principle of common white willow, was used successfully by Maclagan in 1874 to treat symptoms as pain, fever and inflammation of rheumatic fever [2]. Felix Hoffmann discovered a method to acetylate the hydroxyl group on the benzene ring of salicylic acid to form acetylsalicylic acid [3] and Heinrich Dreser (chief pharmacologist at Bayer) named this final product "Aspirin" [3, 4]. Non-steroidal anti-inflammatory drugs (NSAIDs) claimed during last years an increased research interest to establish their cardiovascular safety profile. Generally, NSAIDs inhibit in different degrees both isoforms of cyclooxygenase (COX). Aspirin has a unique property among NSAIDs, at low doses, it inactivates irreversibly the COX-1 activity in

platelets [5]. So, aspirin inactivates permanently the activity of prostaglandin H-synthase-1 (COX-1) and 2 (COX-2), enzymes (isozymes) catalyzing the first step in prostanoid biosynthesis [6].

From a chemical point of view, aspirin is an O-acetyl derivative of salicylic acid and it is obtained by acylating the hydroxyl group with acetic anhydride, using sulphuric acid as catalyst. The structure and also the synthesis pathway of aspirin are presented in Fig. (1) [7].

1.1. Pathway of Aspirin Actions in Humans

NSAIDs and aspirin have been the topic of many researches. Thus, a number of mechanisms were proposed to explain the effects of aspirin in humans: (i) cyclooxygenase inhibition, (ii) interaction with cyclic nucleotides, (iii) activation of complement components, (iv) altered cellular interactions with prostaglandins, (v) altered leukocyte migration, monocytopenia stimulation and interferon induction [8].

Evidences show that aspirin inhibits lymphocyte activation at a dose of 73 µg/ml and PGE (prostaglandin E) formation at a dose of 1.12 µg/ml [9]. The anti-inflammatory effect of aspirin was described by Vane and Botting [10, 11] inhibiting the synthesis of a pro-inflammatory mediator, namely prostaglandin E₂. Later, it has been demonstrated that aspirin inhibits cyclooxygenase-1 (COX-1) activity by acetylating serine 530, hindering the access of arachidonic acid to the active site. COX type 2 (COX-2) is inhibited by aspirin in a

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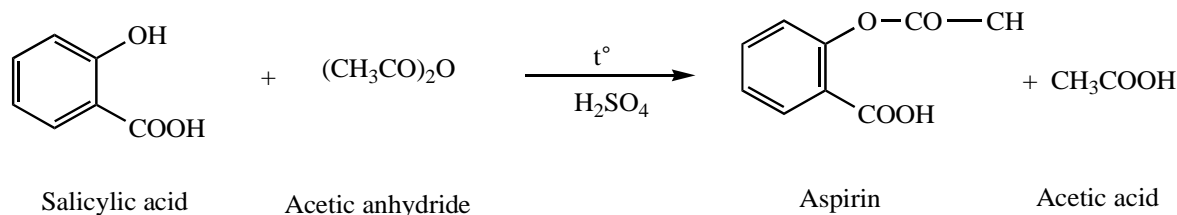


Fig. (1). Aspirin: structure and synthesis.

similar way [12], but is less potent (170-fold less [13]). The sustained and permanent inhibition of platelet COX-1 activity is responsible for the beneficial effects of aspirin in atherothrombotic cardiovascular diseases.

Inhibiting COX isozymes, aspirin interferes with the biosynthesis of cyclic prostanoids (thromboxane A_2 , prostacyclin PGI_2 , prostaglandins E_2 , D_2 and $F_{2\alpha}$), which are obtained by enzymatically catalyzed oxidation of arachidonic acid of cellular membrane phospholipids [14]. Most physiological effects [14] of obtained cyclic prostanoids in the arachidonic acid metabolism are related to one of the following pathways:

- TXA_2 is involved in vasoconstriction, platelet aggregation;
- PGI_2 induces vasodilation, decreases platelet aggregation and, also, increases RBF;
- PGE_2 is responsible for vasodilation, RBF increase, natriuresis, gastric acid production inhibition;
- PGD_2 is involved in RBF increase and also gastric acid production inhibition;
- the simultaneous presence of PGD_2 and $PGF_{2\alpha}$ induces vasoconstriction, bronchoconstriction, uterine contraction increase and also progesterone level decrease.

Syntheses of cyclic prostanoids in the arachidonic acid metabolism pathway (thromboxane A_2 , prostacyclin PGI_2 , prostaglandins E_2 , D_2 and $F_{2\alpha}$) are presented in Fig. (2).

Aspirin acetylates in a selective manner as the hydroxyl group of the serine Ser 530 residue of the enzyme (COX) leading to an irreversible inhibition of COX; thus, a new enzyme has to be elaborated so that new amounts of prostanoids can be produced [3]. In the presence of aspirin, COX-1 is completely inactivated, while COX-2 catalyzes the conversion of arachidonic acid to 15-*R*-hydroxyeicosatetraenoic acid (15-*R*-HETE) and not to PGH_2 [14]. The conversion of arachidonic acid to PGH_2 is essential in obtaining the above mentioned prostanoids. Aspirin blocks prostaglandin synthesis, the beneficial effects of platelet TXA_2 inhibition being superior and the potential prothrombotic state caused by the lack of PGI_2 remaining just in theory [14]. COX inhibition in platelets is irreversible because of the limited mRNA pool and protein synthesis in platelets [14].

Beneficial actions of aspirin on the cardiovascular system are tried to be explained by its antioxidant properties namely: improving endothelial dysfunction [15], inhibiting/protecting LDL from oxidative modification [16], and enhancing nitric oxide production in endothelial cells secondary to prostacyclin synthesis inhibition [14]. COX-2 inhibition reduces inflammation and selective COX-2 antagonists do not affect the gastric mucosa [3].

Fig. (2). Arachidonic acid metabolism via prostaglandin H-synthase (COX) (adapted from ref. [14]).

Legend: PGG_2 : prostaglandin G_2 ; PGH_2 : prostaglandin H_2 ; TXA_2 : thromboxane A_2 ; PGI_2 : prostacyclin; PGE_2 : prostaglandin E_2 ; PGD_2 : prostaglandin D_2 ; $PGF_{2\alpha}$: prostaglandin $F_{2\alpha}$.

Recently, a third distinct COX isozyme, COX-3 was described, and two smaller COX-1 derived proteins, which are presented as potential targets for anti-inflammatory drugs. It was proved that COX-3 mRNA has a significant expression in human at the level of cerebral cortex and of the heart, thus, COX-3 could be a selective target and a central mechanism to control pain and fever [17, 18].

1.2. Absorption and Recommended Doses of Aspirin in Cardiovascular Diseases Management

After oral administration, aspirin (including the enteric-coated aspirin) is quickly absorbed in the upper gastrointestinal tract and it inhibits platelet aggregation immediately; a measurable effect manifesting after 60 minutes following ingestion [14]. Intervening in primary hemostasis, bleeding time increases. The effect of repeated doses of aspirin is cumulative on platelets. If about 20% of cumulative effect from platelets expresses normal COX, hemostasis returns to normal features [14].

The FDA (Food and Drug Administration) recommends dosages of aspirin ranging from 50 mg/d to 1300 mg/d to be comprised in the pharmacological management of atherosclerotic cardiovascular diseases. Actual data are consistent with using aspirin in a dose of 75 – 100 mg daily in the area of cardiovascular disease prevention [19]. Aspirin is efficient at minimum effective dose in following cardiovascular entities [6]: (a) men at high cardiovascular risk, blood hypertension, stable angina, and unstable angina, and severe carotid artery stenosis (75 mg/d), (b) transient ischemic attack and ischemic stroke (50 mg/d); (c) acute myocardial infarction, and acute ischemic stroke (160 mg/d).

1.3. Aspirin Resistance

The debate continues to understand why correctly prescribed and administrated doses of aspirin remain with less/no cardio-protective effect in certain patients. It is yet unclear whether clinical resistance to aspirin is due to dose and type of used antithrombotic agent or to individual/population features [20]. Resistant patients to aspirin may have one or more of the following [21]: (i) receive a low dose of aspirin, (ii) are not compliant to treatment, (iii) have different capacity to absorb aspirin, (iv) underlying genetic condition leading to aspirin ineffectiveness.

At present, there is no accepted golden standard to define aspirin resistance [21]. Potential mechanisms that may determine the appearance of aspirin-resistant TXA₂ biosynthesis are [6]: transient expression of COX-2 in newly formed platelets in case of accelerated platelet turnover; extraplatelet sources of TXA₂ (monocyte/macrophage COX-2); and concomitant administration of another traditional NSAID interferes with aspirin at the level of platelets.

Krasopoulos *et al.* [21] identified 20 studies comprising 2930 patients with cardiovascular disease receiving aspirin as antithrombotic agent. Patients' aspirin status was assessed by several methods: measuring serum TXA₂ in relation to platelet hemostasis, a platelet or collagen adhesion assay, platelet rich plasma aggregometry, whole blood platelet aggregometry, or some combination or modification thereof. 28% of patients were labeled as resistant to aspirin, with higher prevalence in women and in patients with previous renal impairment. 39% of aspirin resistant patients had a

cardiovascular event (*vs* only 16% of sensitive patients, OR=3.85, 95% CI: 3.08-4.80, $p < 0.001$). One of the most important findings of this meta-analysis is that mortality risk in aspirin resistant patients was very high: OR=5.99 (2.28-15.72, $p < 0.003$).

The importance of sensitive platelets to aspirin derives also from the fact that there is a 50% reduction in the risk of myocardial infarction or death from vascular causes in patients with unstable angina receiving aspirin [6]. In acute phases of ischemic stroke, there is an increased TXA₂ biosynthesis, with shorter duration than in acute coronary syndromes, suggesting that other mechanisms intervene in stroke development. However, aspirin started within 48 hours after the onset of symptoms of an acute ischemic stroke brings immediate benefits in terms of clinical non-fatal and fatal events, but not in same rates as in acute coronary syndromes [22].

Snoep *et al.* [23] showed that patients receiving aspirin to prevent recurrent cardiovascular events are still at high risk to develop such events. This might be due to laboratory aspirin resistance, a phenomenon concerning persistent platelet reactivity despite aspirin therapy, which is more likely correlated with clinical resistance to aspirin. The pooled odds ratio of all cardiovascular events in the meta-analysis was 3.8 (95% CI, 2.3-6.1). The estimated prevalence of aspirin resistance in population ranged between 5 and 45% [24]. This large interval is due to the variability of platelet function in population and of biomarker testing, variability referred also as resistance. It has been established that uninhibited platelet COX activity persists in those patients taking aspirin who are younger and heavier and also in those with previous myocardial infarction [24, 25]. There is also gender difference in platelet reactivity: distinct from men, aspirin lowers in women the risk of stroke, with no impact on the risk of myocardial infarction or cardiovascular mortality [24, 26].

Platelets are essential in acute thrombotic events and aspirin reduces by about 25% the risk to develop an acute thrombotic event in high-risk subjects; there is also evidence that about 10 – 20% of the patients receiving aspirin may present one/more recurrences of the arterial thrombotic event [6, 27].

To summarize, The Working Group on Aspirin Resistance of The International Society on Thrombosis and Hemostasis proposed following possible mechanisms of aspirin "resistance" [27] in order: (i) Bioavailability: non-compliance, underdosing, poor absorption, interference: NSAID coadministration, (ii) Platelet function: incomplete suppression of TXA₂ generation, accelerated platelet turnover, stress-induced COX-2 expression in platelets, increased platelet sensitivity to ADP and collagen; (iii) Single nucleotide polymorphisms: receptors: GpIIb-IIIa, collagen receptor, TX receptor, enzymes: COX-1 and 2, TX-synthase; (iv) Platelet interactions with other blood cells: endothelial cells and monocytes provide PGH₂ to platelets and also synthesize their own TXA₂; (v) Other factors: smoking, hypercholesterolemia, lack of exercise, stress, etc.

1.4. Clinical Evidence of Aspirin

In low-risk patients, without known occlusive vascular disease, five primary prevention trials demonstrated that aspirin reduces the risk of myocardial infarction by 30% [28-

33], but with no significant effect on the risk of stroke [28]. Sanmuganathan *et al.* [34] have analyzed data deriving from four primary prevention randomized controlled trials comprising 48540 subjects, of whom 25133 were receiving aspirin, in a dosage of 75 – 500 mg daily. Only in the HOT study [29] among patients were included women. There were significant overall reductions in all cardiovascular events (by 15%) and in myocardial infarction (by 30%), a non-significant reduction in all cause mortality (by 6%), and a non-significant increase in stroke incidence (by 6%) [34]. At coronary event risk 1.5%/year, the five year NNT was 44 to prevent a myocardial infarction, and 77 to prevent a myocardial infarction net of any important bleeding complication. At coronary event risk 1%/year, the NNT was 67 to prevent a myocardial infarction, and 182 to prevent a myocardial infarction net of important bleeding [34]. So, the authors concluded that aspirin has significant benefits (safety and effectiveness) at coronary event risk $\geq 1.5\%$ /year [34].

In another meta-analysis published by Berger *et al.* [35], among 51342 women, in the aspirin arm, there was a 12% reduction in cardiovascular events (OR, 0.88; 95% CI, 0.79 – 0.99; $p = 0.03$) and a 17% reduction in stroke (OR, 0.83; 95% CI, 0.70 – 0.97; $p = 0.02$). There was no significant effect on cardiovascular mortality and myocardial infarction. Men were 44114: in those treated with aspirin, there was a 14% reduction in cardiovascular events (OR, 0.86; 95% CI, 0.78 – 0.94; $p = 0.01$) and a 32% reduction in myocardial infarction (OR, 0.68; 95% CI, 0.54 – 0.86; $p = 0.001$). There was no significant effect on stroke or cardiovascular mortality.

In a recent analysis [36, 37], chemoprevention with aspirin was cost-effective for men over 75 years regardless of the number of cardiovascular risk factors and for 55- and 65-years old men with at least two cardiovascular risk factors. Women 75 years of age with a 2 times increased cardiovascular risk and women 65 years of age with a 5 times increased cardiovascular risk brought forward benefits for being treated with aspirin. By these means, the aspirin treatment for primary prevention of cardiovascular disease is cost-effective when global cardiovascular risk surpasses a certain threshold: $>10\%$ for men and $>15\%$ for women.

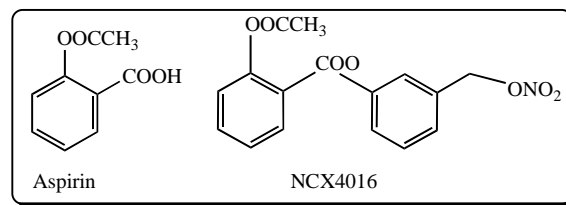
The Antithrombotic Trialists' Collaboration [38] studying the efficiency of antiplatelet therapy established that the risk of major cardiovascular events is reduced by 22% for the whole study population (over 140000 subjects), but diabetic patients (about 5000 patients) seem not to benefit maximally from this kind of therapy: 7% reduction of the risk for major cardiovascular events, but with no statistical significance. The US guideline on diabetes and cardiovascular disease [39] recommends aspirin in primary prevention in all individuals aged > 40 years or with additional risk factors [39]. Europeans do not prescribe aspirin for the primary prevention of myocardial infarction or cardiovascular death, while being indicated for the prevention of stroke [40, 41]. Belch *et al.* [42] concluded that aspirin is not effective in primary prevention of cardiovascular events and mortality in a diabetic population (1276 subjects). Further trials are needed to establish the role of aspirin in diabetic cardiology (aspirin alone or a combination of antiplatelet drugs, dosage) concerning primary prevention issues. Randomized trials have established that aspirin, in secondary prevention, reduces the possibility of non-fatal cardiovascular events by 25 – 30% and fatal events by 15 – 20% [5].

Stroke has been included in the large concept of cardiovascular disease; it is well-known that the great majority of major cerebrovascular events are ischemic and about 15% of them are preceded by a transient ischemic attack [43]. In the Antithrombotic Trialists' Collaboration meta-analysis [38, 43], 18270 patients had a positive history for cerebrovascular disease. Using antiplatelet therapy (a mean of almost 2 and a half years), an absolute risk reduction (ARR) of 36 serious vascular events per 1000 patients was obtained: ARR of 25 non-fatal strokes per 1000 patients ($p < 0.0001$), and smaller, but significant reductions in non-fatal myocardial infarctions ($p = 0.0009$), vascular ($p = 0.04$) and all-cause mortality ($p = 0.002$). Current antiplatelet options in secondary stroke prevention are aspirin alone, aspirin and extended-release dipyridamole, and clopidogrel.

Triple antiplatelet therapy in long term administration leads to an increase of bleeding rates [44]. Aspirin is prescribed in over 90% of patients after surgical myocardial revascularization [45].

2. THE NEWS IN ANTI-INFLAMMATORY DRUGS

NCX 4016, 2-(acetyloxy)benzoic acid 3-[(nitrooxy)methyl]phenyl ester, is a new molecule in which a nitric oxide (NO)-releasing moiety is covalently linked to aspirin. After enzymatic metabolism, NCX 4016 releases both components [46]. This new class of pharmacologic active agents is called NO-NSAIDs (Nitric Oxide Non Steroidal Anti-Inflammatory Drugs) or CINODs (Cyclooxygenase-Inhibiting Nitric Oxide Donors) [46]. Chemical structures [46] of NCX 4016 and Aspirin are shown below:



In their study on healthy subjects, Fiorucci *et al.* [47] showed that NCX 4016 inhibits the cyclooxygenase activity being equally effective with aspirin, with less gastric side effects and preventing monocyte activation. NO aspirin derivatives were meant to achieve a significant degree of gastric protection, but there is the open possibility of vascular protective effects of drug-derived NO [48]. Platelets have a decreased capacity to release NO from organic nitrates. New drugs with a furoxan group as NO-donating moiety with an esteric linkage to the aspirin molecule have been developed in order to overcome the gastric side effects. The NO-release mechanism is unlikely to require the same cellular conditions as that for organic nitrates, suggesting that they might offer a degree of NO-mediated antiplatelet effects to complement those of the aspirin entity [48]. Further on, Turnbull *et al.* [49] showed that NO-aspirins have increased anti-inflammatory properties being candidates in the management of a wide range of inflammatory diseases.

3. APPLICATION OF RATIONAL DRUG DESIGN STUDIES FOR DEVELOPING OF THE MOST SELECTIVE COX-2 INHIBITORS BY 2D-QSAR

Several studies have been focused on a series of compounds with selective COX-2 inhibitory properties because

of their increased anti-inflammatory potency applicable in many systemic inflammatory conditions, with a low-rate of side effects (i.e., gastrointestinal bleeding). It remains of high interest to perform QSAR/QSPR studies upon sets of compounds with increased COX-1 inhibitory activity in order to improve the cardiovascular outcome with as low as possible rate of secondary effects.

Prasanna *et al.* [50] found in their work important requirements (physicochemical and structural) in order to differentiate the COX-1 and COX-2 inhibitory activity in various ligands. Kalgutkar *et al.* [51] showed that derivatization of the carboxylic acid moiety of NSAIDs determines the annulling of the COX-1 inhibitory activity, while COX-2 inhibitory capacity is not significantly affected. The logical consequence was to propose the conversion of classical non-selective NSAIDs to selective COX-2 inhibitors enhancing their anti-inflammatory and analgesic properties with a high safety profile.

In their study, Kant *et al.* [52] showed that ester derivatives of indomethacin have an increased COX-2 inhibitory activity in correlation with thermodynamic and sterimol parameters underlying the fact that the steric effect is essential. It suggests that a lipophilic bulkier group width-wise is required for a significant biological activity.

The phenyl sulfonamide moiety positioned in secondary pocket of enzyme, which consists of amino acid residues Phe518, Gln192, Arg513, Leu352, Ser353 and Val523, is responsible for the selectivity. The unsubstituted phenyl ring positions in a hydrophobic cavity are lined by Tyr385, Trp387, Tyr348, Leu384 and Met522. Interestingly, the indole C-5 CH₃-substituent is located in a hydrophobic region formed by Ile345, Val349, Ala527, Leu531 and Leu534. The hydrophobic interactions of methyl group might be crucial for the potency of 2-sulfonylphenyl-3-phenyl-indole analogs. The study has revealed that atomic Van der Waals volume and atomic masses explain COX-2 inhibitory activity of 2-sulfonyl-phenyl-3-phenyl-indole analogs significantly [53].

The quantification of the structural features of 1,5-diarylpiperazine analogs with various biological activities gave some important structural insights, i.e. Hy (hydrophilic factor) and Mor17v (3D molecular representation of structure based on electron diffraction code). These two physicochemical properties may be helpful in the development of more selective dual COX-2/LOX-5 (lipooxygenase-5) inhibitors [54].

4. GENERAL OVERVIEW OF 3D-QSAR AND MOLECULAR DOCKING METHODS APPLIED TO DEVELOP CYCLOOXYGENASE INHIBITORS

3D-QSAR (three-dimensional quantitative structure activity relationships) is an important component of computer-aided drug design (CADD) methodology [55] to develop new potent COX-2 specific inhibitors, acting as potential anti-inflammatory or anti-cancer drug candidates [56-58]. The aim of 3D-QSAR methods is to identify the spatial properties represented by the similarity or dissimilarity between 3D structure of active site of the enzymes and their inhibitors when electronic and steric fields are considered. 3D-QSAR assumptions are represented by [59]: (i) the modeled compound is directly responsible for the biological effect and not its metabolites or other transformation products,

(ii) the binding of inhibitors to the active site takes place in the same manner for all of them, (iii) the enthalpic processes (steric, electrostatic, hydrogen bonding, etc) are considered.

In 3D-QSAR methods, the biological activity is expressed as the logarithm (with base 10) of the reciprocal of the molar concentration required to produce a standard biological response [59]. In many QSAR studies applied to enzyme inhibitions, the reciprocal logarithm of the constant of inhibition, K_i , is considered and this parameter is correlated with various physicochemical descriptors like: hydrophobicity, molecular volume and surface, Connolly Surface Area [60], Molar Refractivity [61], or ΔG_{solv} (solvation free energies) [62].

4.1. 3D-QSAR-CoMFA/CoMSIA Methods

In CoMFA (Comparative Molecular Field Analyses) [59] method, the alignment (superposition) of bioactive conformers is strongly required [59], with several alignment methods being usually used: atom-to-atom, substructure-to-substructure or field fitting. CoMFA considers that the steric (Lennard-Jones) and electrostatic (Coulombic) components are most important for the interaction between inhibitors and receptor enzymes [59, 63]. The steric and the electrostatic interactions between a probe atom, (sp³-carbon, oxygen and nitrogen) and the ligands are calculated at uniform grid points, and then tabulated (CoMFA matrix) for each molecule. The multivariate statistics such as partial least squares (PLS) method [64], is used to extract the orthogonal principal components of CoMFA matrix [59]. The leave-one-out cross-validation method gives the control criteria: PRESS (Predictable Residual Sum Squares), SD (standard deviation) and q^2 (cross-validate r^2) [59]. The r^2 correlation factor [59], SEE (standard error of estimate) [59] and F (Fisher) [59] are performed by non-cross-validated method [59].

The CoMSIA method [65] includes five different property fields like: steric, electrostatic, hydrophobic, hydrogen bond donor, and acceptor properties. CoMSIA considers the interactions between the ligands (antagonist in our case) and a probe atom having a radius of 1Å, charge of +1 and hydrophobicity of +1, which is placed at the intersections of a surrounding lattice.

Using the CoMSIA method, it is possible to separate the contribution of the steric, electrostatic, hydrophobic, hydrogen bond donor/acceptor descriptors and, at the same time, to evaluate these contributions to the biological activity. Similarly to CoMFA, these five fields can be identified graphically by a contour map. The CoMSIA method is more intuitive and leads itself to a straightforward graphical analysis, compared with the CoMFA method.

4.2. Molecular Docking

Docking can be used to evaluate: (i) correlation between the experimental biological activities with predicted biological activities, (ii) identification of spatial distribution of the inhibitors within the active site, and (iii) predicted binding affinities of the inhibitors to the enzymes active site.

By molecular docking technique [66] a correct fitting of the ligands in the active site of a protein (membrane receptor or enzyme) and also, the prediction of the affinity of the ligand to protein are obtained. When molecular docking is

applied, the knowledge of crystallographic structures of the ligand and also, of the enzyme, is critical. The crucial first step in docking is represented by the identification of binding pockets on enzymes. After identifying the possible binding regions on the enzyme surface, one can then move on to predict which small molecules will bind to a cleft and in what orientation.

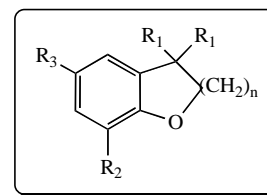
Most docking algorithms consider that the enzyme structure is rigid, according to the high computational cost induced by the flexibility of big molecules, while the ligand is free to move. Usually, in the first step, a library of ligand conformers is generated and in the second step, these conformers are docked into the target, each conformer being treated as a new ligand. Any algorithm able to generate in a correct manner the ligand conformers could be used to generate this library. One of them is represented by genetic algorithms. Genetic algorithms attempt to use the rules of natural selection to subset computationally demanding tasks [67, 68].

4.3. Application of 3D-QSAR and Molecular Docking Studies for Developing New COX-2 Inhibitors

Recently, many research groups have applied 3D-QSAR (CoMFA/ CoMSIA) and molecular docking to develop new inhibitors of COX-2. This review presents the most recent applications of these techniques, when chemical structures substances like thiazolidinedione, benzofuran, furanone and ethers derivatives as COX inhibitors were studied.

In 2006, Mingyue Zheng *et al.* [69] performed a 3D-QSAR and molecular docking analysis of 21 COX-2/5-LOX dual inhibitors, namely, 7-tert-butyl-2,3-dihydro-3,3-dimethylbenzofuran analogues (DHDMBF). In this study of molecular docking, appropriate spatial orientation of

DHDMBF analogs in COX-2 active site was performed. Highly predictive 3D-QSAR models were then successfully set up through CoMFA and CoMSIA techniques based on the binding conformations discovered *via* molecular docking to the crystal structure of COX-2. The modeling results provide a satisfactory explanation for the binding mechanism of DHDMBF analogs with the COX enzyme, and 3D-QSAR models lead to a better understanding of how to design a potent dual inhibitor against the two enzymes. The chemical scaffold of DHDMBF analogs is presented below:



In this study, the authors considered that DHDMBF analogs could be divided into two parts: a polar “anchor” (e.g., carbonyl, amide, amino or hetero ring structure) and a hydrophobic tail. Further, it has been discussed about the most active inhibitor of this series, compound (3) (R1 = Me, R2 = t-Bu, R3 = COphenyl, n = 1, $-\log IC_{50} = 8.15$). By molecular docking it was found that: (i) the phenyl “tail” of compound 3 is involved in interactions with hydrophobic amino acids Ala516, Ile517, Phe518, and Val523 of COX-2; (ii) carbonyl oxygen belonging to compound (3) is able to form two hydrogen bonds with polar residues His90 and Ser353 with bond lengths of 2.59 and 2.62 Å, respectively; (iii) hydrophobic R1 and R2 groups interact with sub-pockets PA2 and PA3, respectively, these interactions being critical for the power of inhibition (Fig. 3).

Fig. (3). The binding models of compound (3) with COX-2. The inhibitor is represented as ball-and-stick and residues within 4 Å around the inhibitor are denoted as stick. Dash lines denote the hydrogen bonds (after M. Zheng [69]).

It was shown that by removing R1 or R2, a drastic decrease of inhibition activity was recorded (e.g. compound **(8)** ($n=1$, R1 = H, R2 = *t*-Bu, R3 = CO(CH₂)₃-*c*-Pr, $-\log IC_{50}$ = 4.82)). All these remarks help us to conclude that the size and shape of hydrophobic "tail" of these compounds play an important role in COX2 inhibition. The final remark of docking study reveals that the phenyl or aliphatic chains with the length of three or four carbon atoms lead to, at least, two strong hydrogen bonds between the "anchor" and residues His90 and Ser353, while too long hydrophobic tails result in weaker hydrogen bonding.

In the same study, for COX-2, a two-component CoMFA model was obtained with cross-validated q^2 of 0.782, conventional r^2 of 0.917, *F* value of 99.484, and standard error of 0.348, while the CoMSIA model (of three descriptors: steric, electrostatic, and hydrophobic) was obtained with a cross-validated q^2 of 0.548 for two components and a conventional r^2 of 0.914. Considering the descriptor contribution to the inhibitory activity, it was concluded that: (i) the sum of steric and hydrophobic fields in the CoMSIA model is approximately equal to the steric contribution in the corresponding CoMFA model, (ii) the distribution of electrostatic fields account for more than half of the total, indicating that electrostatic interaction is essential to the binding of DHDMBF analogs with COX-2.

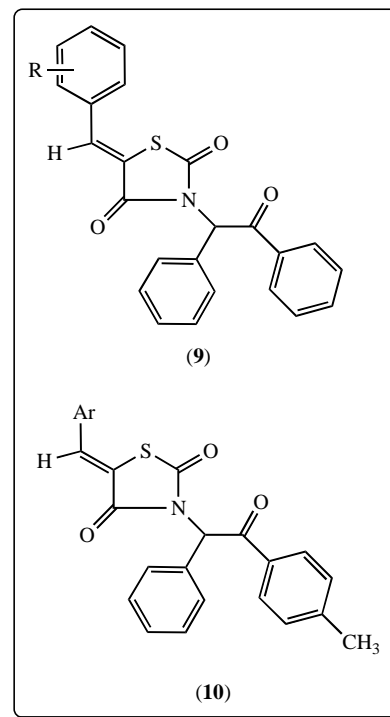
By displaying the CoMFA contour map for COX-2 inhibition it was possible to conclude that large steric favored areas around the R2 group of DHDMBF suggest that more bulky substituents in these positions will significantly improve the biological activity. Increase of biological activity can be obtained when a hydrophobic interaction between the bulky group and the site formed by hydrophobic residues Phe518, Met522, Val523, Gly526, and Ala527 occurs.

At the end of this study it was concluded that compounds with higher COX-2 inhibitory activities, for example, compound **(1)** ($n = 1$, R1 = Me, R2 = *t*-Bu, R3 = CO(CH₂)₃CCH, $-\log IC_{50}$ = 7.82), compound **(3)** ($n = 1$, R1 = Me, R2 = *t*-Bu, R3 = COphenyl, $-\log IC_{50}$ = 8.15), and compound **(18)** ($n = 1$, R1 = Me, R2 = *t*-Bu, R3 = 6-Imidazo[2.1-*b*]thiazolyl, $-\log IC_{50}$ = 7.82), generally bear a bulky group at this position. As revealed, both the steric favorable region around group R2 and the negative charge favorable region near the carbonyl group of DHDMBF analogs should be crucial for designing potent inhibitors against the COX enzymes.

Ahmed Ali *et al.* [70] reported the docking and also three-dimensional qualitative structure selectivity relationship (3D-SSR) studies performed on new 2,4-thiazolidinedione derivatives as selective cyclooxygenase-2 inhibitors, with higher analgesic and anti-inflammatory activity. The chemical scaffold of new 2,4-thiazolidinedione derivatives (compound **(9 (a-1))** and compound **(10(a-p))** is presented below.

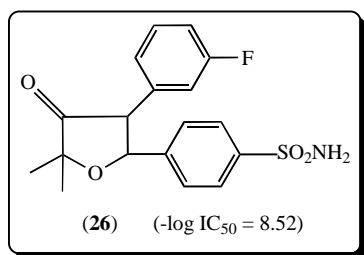
In this study, the docking of the 2,4-thiazolidinedione derivatives into the active site of COX enzymes was carried out using the enzyme parameters obtained from crystallographic structures of the complexes between COX-1 with flurbiprofen (1CX1.pdb) and COX-2 with SC-558 (1CX2.pdb) [71]. The docking results demonstrated that 2,4-thiazolidinediones derivatives show comparable interactions at COX-2 as the co-crystallized SC-558, with certain observations: (i) phen-

ylmethylene group of 2,4-thiazolidinediones derivatives fits into the COX-2 secondary pocket having the same orientation of sulfonamide group of SC-558, (ii) 2,4-thiazolidinedione moiety adopts the position of the central pyrazole ring of SC-558 whereas the second aromatic ring can be superimposed with the bromo-phenyl ring of SC-558, (iii) the third aromatic ring of 2,4-thiazolidinedione adopts the position of the CF3 of SC-558, (iv) it was suggested that the synthesized 2,4-thiazolidinediones derivatives are active COX inhibitors with a clear preference for COX-2.



Devendra Sharad *et al.* [72] identified a series of 5-aryl-2,2-dialkyl-4-phenyl-3(2*H*)-furanone derivatives as selective cyclooxygenase-2 (COX-2) inhibitors by 3D-CoMFA/CoMSIA methods. Considering the importance of alignment in developing 3-D QSAR models in this study, four different alignments of the molecules using compound **(26)** as template structure were proposed: *alignment I* - each analog was aligned to the template by rotation and translation in order to minimize the RMSD between atoms in the template and the corresponding atoms in the analog ($q^2 = 0.502$, $r^2 = 0.922$); *alignment II* - the atoms of the molecules were used for RMS (root mean square) fitting corresponding to the atoms in the template ($q^2 = 0.559$, $r^2 = 0.893$); *alignment III* - centroids rather than the exact superimposition of atoms of the rings were used for RMS fitting to the template ($q^2 = 0.652$, $r^2 = 0.914$); *alignment IV* - both atoms and centroids were selected for superimposition on the template ($q^2 = 0.664$, $r^2 = 0.916$).

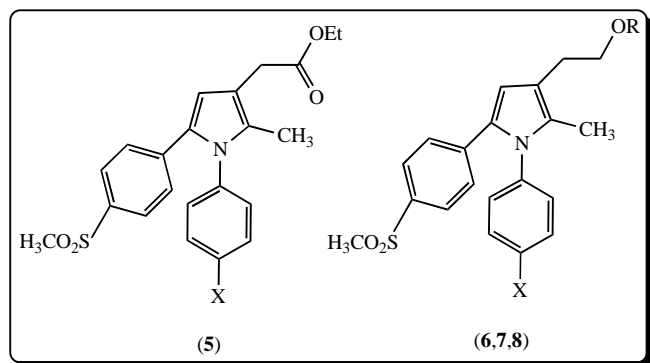
Finally, the statistically significant model was established for 36 molecules in the training set (CoMFA model: $q^2 = 0.664$, r^2 (non-cross-validated square of correlation coefficient) = 0.916, *F* value = 47.341, standard error of prediction = 0.360 and standard error of estimate = 0.180; CoMSIA model: $q^2 = 0.777$, r^2 (non-cross-validated square of correlation coefficient) = 0.905, *F* value = 66.322, standard error of prediction = 0.282 and standard error of estimate = 0.185.



In this study, by displaying the CoMFA steric and electrostatic contour maps, it was noticed that: (i) bulkier substituents at furanone ring are not favorable for steric interactions but the 4-phenyl ring in the vicinity of 5'-F of compound (26) revealed that an increase in activity is anticipated due to the increased steric bulk, (ii) the presence of partial negative charge in the vicinity of 4'-H and 5'-H is associated with increased activity (80% contribution).

The CoMSIA model with the combination of steric, electrostatic, hydrophobic and hydrogen bond donor fields yielded the highest cross-validated $r^2 = 0.777$ with four components, non cross-validated $r^2 = 0.905$, and F value 66.322. The hydrogen bond donor contour maps of CoMSIA near 3''-H of the 5-phenyl ring and also in the vicinity of 3'-F and 4'-H of the 4-phenyl ring indicated the disfavored regions for hydrogen bond donor substituents while in the lower region of the 5-phenyl ring, in the vicinity of 5''-H of compound (26), favored regions for hydrogen bond donor substituents were indicated.

M. Anzini *et al.* [73] performed a complex study including synthesis, biological evaluation and also molecular docking of 1,5-diarylpyrrole-3-alkoxyethyl ethers as selective cyclooxygenase-2 inhibitors with anti-inflammatory and also antinociceptive activities. The chemical scaffolds of compound (5), compound (6), compound (7) and compound (8) are presented below:

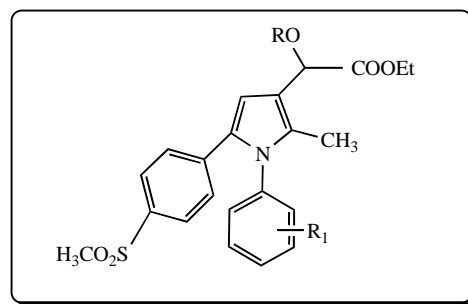


The potential anti-inflammatory and antinociceptive activities of same compound ((7a), X = H, R = Et, $IC_{50} = 0.015$ microM), compound ((8a), X = H, R = n-Pr, $IC_{50} = 0.018$ microM) and compound ((8d) X = F, R = n-Pr, $IC_{50} = 0.030$ microM) were evaluated *in vivo*, where they showed a very good activity against both carrageenan-induced hyperalgesia and edema in the rat paw test. In the abdominal constriction test it was shown that compounds (7a), (8a), and (8d) were able to reduce the number of writhes in a statistically significant manner, leading to the conclusion that compounds (6-8) can be considered interesting candidates for further preclinical studies.

In the present study, the enzyme docking simulations demonstrated that the binding mode of such compounds is characterized by the ether group located into the "carboxylate site" with its oxygen atom involved in a hydrogen bond with the guanidino moiety of Arg120. Moreover, the alkyl portion of the ether side chain was embedded into a lipophilic region defined by Leu93, Val116, and Leu359 (Fig. 4).

The results of this study show that the presence of a 4'-trifluoromethyl or a 4'-fluoro substituent at 1-phenyl ring as in compounds ((6c), X = CF₃, R = Me, $IC_{50} = 0.049$ microM), compound ((6d) X = F, R = Me, $IC_{50} = 0.018$ microM), compound ((7c), X = CF₃, R = Et, $IC_{50} = 0.085$ microM) and compound ((7d), X = F, R = Et, $IC_{50} = 0.047$ microM) and compound ((8c) X = CF₃, R = n-Pr, $IC_{50} = 0.030$ microM) determines a slight decrease in the COX-2 inhibitory activity with the increase in the side chain length, with the exception of compound (8d), while the less bulky ether in the subseries of the 4'-fluoro derivatives (compound (6d)) showed an IC_{50} of 0.018 microM. Also, it was noticed that the 1-aryl substituent showed the usual interaction pathway with the hydrophobic pocket of protein, as well as the aryl moiety at C5 that was in contact with the selectivity site with both hydrophobic interactions and hydrogen bonds.

Also, 1,5-diarylpyrrole derivatives as anti-inflammatory and analgesic agents were kept in mind of M. Biava *et al.* [74]. Authors tested same alcohol and ether derivatives of compounds as possible COX-1/2 inhibitors *in vivo* in the human whole blood (HWB) and *in vivo* for anti-inflammatory activity in mice. The chemical scaffold of 1,5-diarylpyrrole derivatives is presented below:



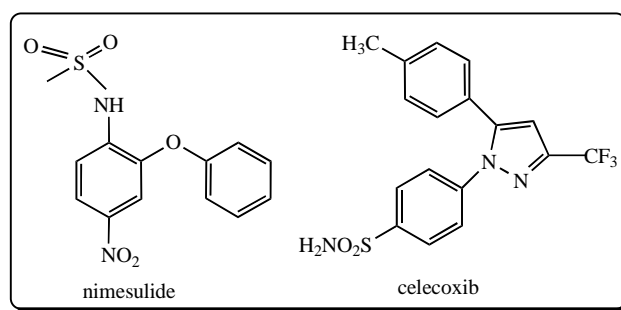
In this study, by docking it was possible to notice certain observations like (i): all the compounds preferred the same orientation within the COX-2 binding site, with the methyl-sulfonyl group located into the selectivity site, (ii) the 5-phenyl ring was accommodated into the hydrophobic pocket, (iii) hydrogen bonds involving the sulfonyl oxygens and the carbonyl group of the ligands, which interacted with Tyr518 and Arg120, respectively, were confirmed as important anchor points for the binding of COX-2 inhibitors; also, it was proved that the additional interactions like hydrogen bond with Ser353 and Van der Waals contacts with the hydrophobic region, are useful to modulate affinity of compounds toward the enzyme, (iv) the side chain at position 3 of the pyrrole ring matching the carboxylate site.

Recently, V. Rajakrishnan *et al.* [75] used the available X-ray crystal structures of the complex of COX-2 with the known inhibitors Diclofenac (PDB ID: 1PXX) and Flurbiprofen (PDB ID: 1HT8) to carry out a molecular simula-

Fig. (4). Graphical representation of the best pose found for compound (7a) (ball and stick notation) within the binding site of COX-2. Hydrogen bond interactions (involving the ether oxygen atom and one of the sulfone oxygens) are coded by black dashed lines. For the sake of clarity, only a few amino acids of the binding site are displayed (after M. Anzini [73]).

tion (molecular modeling, molecular dynamic and molecular docking) study of small peptide inhibitors as potent and selective COX-2 inhibitors.

In this study, the docking methodology was first tested on two known inhibitors (diclofenac and flurbiprofen), which were docked in the crystal structure of active site of COX-2. The docking results showed a free energy of binding (ΔG) of -9.44 kcal/mol, corresponding at K_d value (at 298K) of 0.11 microM for diclofenac and also a free energy of binding of -9.46 kcal/mol, corresponding at K_d value of 0.16 microM for flurbiprofen; thus, the docking methodology gives results that are consistent with the observations. Furthermore, starting from nimesulide and celecoxib (the chemical structures are presented below), the authors designed several tripeptide sequences containing two aromatic rings, and a charged residue at the C-terminal end and each of these tripeptides being docked in COX-2 active site. It was noticed that the tripeptide inhibitor occupies a position similar to that of diclofenac and flurbiprofen, but with a larger set of interacting residues of the COX-2 (Met522, Tyr355, Ser353, Arg120, and His90). The most suitable one (in terms of activity and interactions) was found to have the tryptophan-tyrosine-aspartic acid sequence (WYD, amino acids single-letter codes). The results of tryptophan-tyrosine-aspartic acid tripeptide docking at COX-1/2 active site showed that the tryptophan-tyrosine-aspartic acid tripeptide is 2.6 million times selective for COX-2 over COX-1.



FINAL REMARKS

We considered that the molecular simulation techniques such as: 3D-QSAR (CoMFA, CoMSIA), rational drug design, docking or molecular dynamics will continue to give important information about COX-2 inhibitors design. The descriptors as hydrogen donor or acceptor, the electrostatic or steric fields, the predicted binding energy can improve the knowledge about COX-1/2 inhibition and its application in clinical treatment. A useful approach is to separate the contributions of steric, electrostatic or lipophylic fields to the inhibitors affinity to COX-1/2.

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ABBREVIATIONS

NSAID	= Non-steroidal anti-inflammatory drug
COX	= Cyclooxygenase
PG	= Prostaglandin
TXA ₂	= Thromboxane A ₂
PGI ₂	= Prostacyclin
PGE ₂	= Prostaglandin E ₂
PGD ₂	= Prostaglandin D ₂
PGF _{2α}	= Prostaglandin F _{2α}
RBF	= Renal blood flow
OR	= Odds ratio
CI	= Confidence interval
NNT	= Number needed to treat
QSAR	= Quantitative structure-activity relationships
CoMFA	= Comparative Molecular Field Analysis
CoMSIA	= Comparative Molecular Similarity Indices Analysis

REFERENCES

- Patrono, C.; Rocca, B. Aspirin: Promise and resistance in the new millennium. *Arterioscler. Thromb. Vasc. Biol.*, **2008**, *28*, 25-32.
- Maclagan, T.J. The treatment of acute rheumatism by salicin. *Lancet*, **1876**, *1*, 342-383.
- Vane, J.R.; Botting, R.M. The mechanism of action of aspirin. *Thromb. Res.*, **2003**, *110*, 255-258.
- Dreser, H. Pharmacologisches uber Aspirin (Acetylsalicylsäure). *Pflugers Arch.*, **1899**, *76*, 306-318.
- Patrignani, P.; Capone, M.L.; Tacconelli, S. NSAIDs and cardiovascular disease. *Heart*, **2008**, *94*, 395-397.
- Patrono, C.; Collier, B.; Fitzgerald, G.A.; Hirsh, J.; Roth, G. Platelet-active drugs: the relationship among dose, effectiveness, and side effects: The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest*, **2004**, *126*, 234-264.
- Nutiu, R.; Bolcu, C.; Modra, D.; Albulescu, M.; Iagher, R.; Seiman, C. Practice in organic chemistry, 2nd vol. (in Romanian). Eurostampa Publishing House, Timișoara, **2003**, 37.
- Rumore, M.M.; Aron, S.M.; Hirose, E.J. A review of mechanism of action of aspirin and its potential as an immunomodulating agent. *Med. Hypotheses*, **1987**, *22*, 387-400.
- Morely, J. Mechanism of action of aspirin in inflammation. *Proc. Roy. Soc. Med.*, **1977**, *70*, 32-36.
- Vane, J.R. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat. New Biol.*, **1971**, *231*, 232-235.
- Vane, J.; Botting, R. Inflammation and the mechanism of action of anti-inflammatory drugs. *FASEB J.*, **1987**, *1*, 89-96.
- Wu, K.K. Aspirin and salicylate: an old remedy with a new twist. *Circulation*, **2000**, *102*, 2022-2023.
- Vane, J.R.; Bakhle, Y.S.; Botting, R.M. Cyclooxygenases 1 and 2. *Ann. Rev. Pharmacol. Toxicol.*, **1998**, *38*, 97-120.
- Awtry, E.H.; Loscalzo, J. Aspirin. *Circulation*, **2000**, *101*, 1206-1218.
- Husain, S.; Andrews, N.P.; Mulcahy, D.; Panza, J.A.; Quyyumi, A.A. Aspirin improves endothelial dysfunction in atherosclerosis. *Circulation*, **1998**, *97*, 716-720.
- Steer, K.A.; Wallace, T.M.; Bolton, C.H.; Hartog, M. Aspirin protects low density lipoprotein from oxidative modification. *Heart*, **1997**, *77*, 333-337.
- Chandrasekharan, N.V.; Dai, H.; Roos, K.L.; Evanson, N.K.; Tomcik, J.; Elton, T.S.; Simmons, D.L. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 13926-13931.
- Schwab, J.M.; Beiter, T.; Linder, J.U.; Laufer, S.; Schulz, J.E.; Meyermann, R.; Schluesener, H.J. COX-3 - a virtual pain target in humans? *FASEB J.*, **2003**, *17*, 2174-2175.
- Campbell, C.L.; Smyth, S.; Montalescot, G.; Steinhilber, S.R. Aspirin dose for the prevention of cardiovascular disease. *JAMA*, **2007**, *297*, 2018-2024.
- Biondi-Zoccai, G.; Lotrionte, M. Aspirin resistance in cardiovascular disease carries a worse prognosis, but may indicate pre-existing higher risk. *BMJ*, **2008**, *336*, 166-167.
- Krasopoulos, G.; Brister, S.J.; Beattie, W.S.; Elliot, R.F.; Buchanan, M.R. Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. *BMJ*, **2008**, *336*, 195-198.
- Davi, G.; Patrono, C. Platelet activation and atherothrombosis. *N. Engl. J. Med.*, **2007**, *357*, 2482-2494.
- Snoep, J.D.; Hovens, M.M.C.; Eikenboom, J.C.J.; van der Bom, J.G.; Huisman, M.V. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events. A systematic review and meta-analysis. *Arch. Intern. Med.*, **2007**, *167*, 1593-1599.
- Freedman, J.E. The aspirin resistance controversy: clinical entity or platelet heterogeneity? *Circulation*, **2006**, *113*, 2865-2867.
- Maree, A.O.; Curtin, R.J.; Dooley, M.; Conroy, R.M.; Crean, P.; Cox, D.; Fitzgerald, D.J. Platelet response to low-dose enteric-coated aspirin in patients with stable cardiovascular disease. *J. Am. Coll. Cardiol.*, **2005**, *46*, 1258-1263.
- Ridker, P.M.; Cook, N.R.; Lee, I.M.; Gordon, D.; Gaziano, T.A.; Manson, J.E.; Hennekens, C.H.; Buring, J.E. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. *N. Engl. J. Med.*, **2005**, *352*, 1293-1304.
- Michelson, A.D.; Cattaneo, M.; Eikelboom, J.W.; Gurbel, P.; Kotke-Marchant, K.; Kunicki, T.J.; Pulcinelli, F.M.; Cerletti, C.; Rao, A.K. On behalf of the Platelet Physiology Subcommittee of the Scientific and Standardization Committee of The International Society on Thrombosis and Haemostasis. *J. Thromb. Haemost.*, **2005**, *3*, 1309-1311.
- Patrono, C.; García Rodríguez, L.A.; Landolfi, R.; Baigent, C. Low-dose aspirin for the prevention of atherothrombosis. *N. Engl. J. Med.*, **2005**, *353*, 2373-2383.
- Hansson, L.; Zanchetti, A.; Carruthers, S.G.; Dahlof, B.; Elmfeldt, D.; Julius, S.; Menard, J.; Rahn, K.H.; Wedel, H.; Westerling, S. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomized trial. *Lancet*, **1998**, *351*, 1755-1762.
- Peto, R.; Gray, R.; Collins, R.; Wheatley, K.; Hennekens, C.; Jarrold, K.; Warlow, C.; Hafner, B.; Thompson, E.; Norton, S.; Lilford, J.; Doll, R. Randomised trial of prophylactic daily aspirin in British male doctors. *Br. Med. J. (Clin. Res. Ed.)*, **1988**, *296*, 313-316.
- Steering Committee of the Physicians' Health Study Research Group. Final report on the aspirin component of the ongoing Physicians' Health Study. *N. Engl. J. Med.*, **1989**, *321*, 129-135.
- The Medical Research Council's General Practice Framework. Thrombosis prevention trial: randomised trial of low-intensity oral anticoagulation with warfarin and low-dose aspirin in primary prevention of ischaemic heart disease in men at increased risk. *Lancet*, **1998**, *351*, 233-234.
- Collaborative Group of the Primary Prevention Project. Low-dose aspirin and vitamin E in people at cardiovascular risk: a randomized trial in general practice. *Lancet*, **2001**, *357*, 89-95.
- Sanmuganathan, P.S.; Ghahramani, P.; Jackson, P.R.; Wallis, E.J.; Ramsay, L.E. Aspirin for primary prevention of coronary heart disease: safety and absolute benefit related to coronary risk derived from meta-analysis or randomised trials. *Heart*, **2001**, *85*, 265-271.
- Berger, J.S.; Roncaglioni, M.C.; Avanzini, F.; Pangrazzi, I.; Tognoni, G.; Brown, D.L. Aspirin for the primary prevention of cardiovascular events in women and men. A specific meta-analysis of randomized controlled trials. *JAMA*, **2006**, *295*, 306-313.
- Greving, J.P.; Buskens, E.; Koffijberg, H.; Algra, A. Cost-effectiveness of aspirin treatment in the primary prevention of cardiovascular disease events in subgroups based on age, gender, and varying cardiovascular risk. *Circulation*, **2008**, *117*, 2875-2883.
- Mosca, L. Aspirin chemoprevention: one size does not fit all. *Circulation*, **2008**, *117*, 2844-2846.

- [38] Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ*, **2002**, *324*, 71-86.
- [39] Buse, J.B.; Ginsberg, H.N.; Bakris, G.L.; Clark, N.G.; Costa, F.; Eckel, R.; Fonseca, V.; Gerstein, H.C.; Grundy, S.; Nesto, R.W.; Pignone, M.P.; Plutzky, J.; Porte, D.; Redberg, R.; Stitzel, K.F.; Stone, N.J. American Heart Association, American Diabetes Association. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. *Diabetes Care*, **2007**, *30*, 162-172.
- [40] Ryden, L.; Standl, E.; Bartnik, M.; Van den Berghe, G.; Betteridge, J.; de Boer, M.J.; Cosentino, F.; Jonsson, B.; Laakso, M.; Malmberg, K.; Priori, S.; Ostergren, J.; Tuomilehto, J.; Thrainsdottir, I.; Vanhorebeek, I.; Stramba-Badiale, M.; Lindgren, P.; Qiao, Q.; Priori, S.G.; Blanc, J.J.; Budaj, A.; Camm, J.; Dean, V.; Deckers, J.; Dickstein, K.; Lekakis, J.; McGregor, K.; Metra, M.; Morais, J.; Osterspey, A.; Tamargo, J.; Zamorano, J.L.; Deckers, J.W.; Bertrand, M.; Charbonnel, B.; Erdmann, E.; Ferrannini, E.; Flyvbjerg, A.; Gohlke, H.; Juanatey, J.R.; Graham, I.; Monteiro, P.F.; Parhofer, K.; Pyorala, K.; Raz, I.; Schernthaner, G.; Volpe, M.; Wood, D. Task Force on Diabetes, Cardiovascular Diseases of the European Society of Cardiology (ESC); European Association for the Study of Diabetes (EASD). Guidelines on diabetes, prediabetes, and cardiovascular diseases: executive summary. The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). *Eur. Heart J.*, **2007**, *28*, 88-136.
- [41] Nicolucci, A.; de Berardis, G.; Sacco, M.; Tognoni, G. AHA/ADA vs ESC/EASD recommendations on aspirin as a primary prevention strategy in people with diabetes: how the same data generate divergent conclusions. *Eur. Heart J.*, **2007**, *28*, 1926-1927.
- [42] Belch, J.; MacCuish, A.; Campbell, I.; Cobbe, S.; Taylor, R.; Prescott, R.; Lee, R.; Bancroft, J.; MacEwan, S.; Shepherd, J.; Macfarlane, P.; Morris, A.; Jung, R.; Kelly, C.; Connacher, A.; Peden, N.; Jamieson, A.; Matthews, D.; Leese, G.; McKnight, J.; O'Brien, I.; Semple, C.; Petrie, J.; Gordon, D.; Pringle, S.; MacWalter, R.; and Prevention of Progression of Arterial Disease and Diabetes Study Group, Diabetes Registry Group, and Royal College of Physicians Edinburgh. The prevention of progression of arterial disease and diabetes (POPADAD) trial: factorial randomised placebo controlled trial of aspirin and antioxidants in patients with diabetes and asymptomatic peripheral arterial disease. *BMJ*, **2008**, *337*, 1840.
- [43] Dunn, J.D. Managed care considerations. *Am. J. Manag. Care*, **2008**, *14*, 227-237.
- [44] Sprigg, N.; Gray, L.J.; England, T.; Willmot, M.R.; Zhao, L.; Sare, G.M.; Bath, P.M.W. A randomised controlled trial of triple antiplatelet therapy (aspirin, clopidogrel and dipyridamole) in the secondary prevention of stroke: safety, tolerability and feasibility. *PLoS ONE*, **2008**, *3*, 2852.
- [45] Turley, A.J.; Roberts, A.P.; Morley, R.; Thornley, A.R.; Owens, W.A.; de Belder, M.A. Secondary prevention following coronary artery bypass grafting has improved but remains sub-optimal: the need for targeted follow-up. *Interact. CardioVasc. Thorac. Surg.*, **2008**, *7*, 231-234.
- [46] Gresele, P.; Morni, S. Pharmacologic profile and therapeutic potential of NCX 4016, a nitric oxide-releasing aspirin, for cardiovascular disorders. *Cardiovasc. Drug Rev.*, **2006**, *24*, 148-1468.
- [47] Fiorucci, S.; Mencarelli, A.; Meneguzzi, A.; Lechi, A.; Renga, B.; del Soldato, P.; Morelli, A.; Minuz, P. Co-administration of nitric oxide-aspirin (NCX 4016) and aspirin prevents platelet and monocyte activation and protects against gastric damage induced by aspirin in humans. *J. Am. Coll. Cardiol.*, **2004**, *44*, 635-641.
- [48] Turnbull, C.M.; Cena, C.; Fruttero, R.; Gasco, A.; Rossi, A.G.; Megson, I.L. Mechanism of action of novel NO-releasing furoxan derivatives of aspirin in human platelets. *Br. J. Pharmacol.*, **2006**, *148*, 517-526.
- [49] Turnbull, C.M.; Marcarino, P.; Sheldrake, T.A.; Lazzarato, L.; Cena, C.; Fruttero, R.; Gasco, A.; Fox, S.; Megson, I.L.; Rossi, A.G. A novel hybrid aspirin-NO-releasing compound inhibits TNF α release from LPS-activated human monocytes and macrophages. *J. Inflamm.*, **2008**, *5*, 12.
- [50] Prasanna, S.; Manivannan, E.; Chaturvedi, S.C. Quantitative structure-activity relationship studies of cyclooxygenase inhibitors: a comprehensive analysis. *Drug Dev. Res.*, **2005**, *64*, 220-231.
- [51] Kalgutkar, A.S.; Marnett, A.B.; Crews, B.C.; Rimmel, R.P.; Marnett, L.J. Ester and amide derivatives of the nonsteroidal anti-inflammatory drug, indomethacin, as selective cyclooxygenase-2 inhibitors. *J. Med. Chem.*, **2000**, *43*, 2860-2870.
- [52] Kant, G.; Parate, A.; Chaturvedi, S.C. 2D-QSAR study of indomethacin ester derivatives as cyclooxygenase-2 inhibitors. *Indian J. Pharm. Sci.*, **2006**, *68*, 826-829.
- [53] Gupta, A.K.; Gupta, R.A.; Soni, L.K.; Kaskhedikar, S.G. Exploration of physicochemical properties and molecular modelling studies of 2-sulfonyl-phenyl-3-phenyl-indole analogs as cyclooxygenase-2 inhibitors. *Eur. J. Med. Chem.*, **2008**, *43*, 1297-1303.
- [54] Revathi, S.; Gupta, A.K.; Soni, L.K.; Kavitha, S.; Wagh, R.; Kaskhedikar, S.G. Rationalization of physicochemical characters of 1,5-diarylpiperazine analogs as dual (COX-2/LOX-5) inhibitors: A QSAR approach. *J. Pharm. Biomed. Anal.*, **2006**, *42*, 283-289.
- [55] Reddy, M.R.; Parrill, A.L. Overview of rational drug design, In *Rational drug design: Novel methodology and practical applications*; Parrill, A.L.; Reddy, M.R. Eds.; Oxford University Press: Washington, DC, **1999**; Vol. ACS symposium series 719, pp. 1-11.
- [56] Price, M.L.P.; Jorgensen, W.L. Origin of the selectivity of celecoxib analogs with COX-1 and COX-2 from docking and monte carlo simulations. *J. Am. Chem. Soc.*, **2000**, *122*, 9455-9466.
- [57] Amaravani, M.; Reddy, R.N.; Reddy, G.V.; Reddanna, P.; Reddy, M.R. A comparison of computer aided drug design methods for calculating relative binding affinities of COX-2 inhibitors. *Indian J. Chem.*, **2006**, *45*, 174-181.
- [58] Reddy, R.N.; Mutyala, R.; Aparoy, P.; Reddanna, P.; Rami Reddy, M. Computer aided drug design approaches to develop cyclooxygenase based novel anti-inflammatory and anti-cancer drugs. *Curr. Pharm. Des.*, **2007**, *13*, 3505-3517.
- [59] Oprea, T.I.; Waller, C.L.; Marshall, G.R. Three-dimensional quantitative structure-activity relationship of human immunodeficiency virus (I) protease inhibitors. 2. Predictive power using limited exploration of alternate binding modes. *J. Med. Chem.*, **1994**, *37*, 2206-2215.
- [60] Connolly, M.L. Shape distributions of protein topography. *Biopolymers*, **1992**, *32*, 1215-1236.
- [61] Padron, J.A.; Carrasco, R.; Pellon, R.F. Molecular descriptor based on a molar refractivity partition using Randic-type graph-theoretical invariant. *J. Pharm. Pharm. Sci.*, **2002**, *5*, 258-266.
- [62] Keseru, G.M.; Molnar, L. High-throughput prediction of blood-brain partitioning: a thermodynamic approach. *J. Chem. Inf. Comput. Sci.*, **2001**, *41*, 120-128.
- [63] Cramer, R.D., 3rd; Patterson, D.E.; Bunce, J.D. Recent advances in comparative molecular field analysis (CoMFA). *Prog. Clin. Biol. Res.*, **1989**, *291*, 161-165.
- [64] Ferrara, P.; Gohlke, H.; Price, D.J.; Klebe, G.; Brooks, C.L., 3rd. Assessing scoring functions for protein-ligand interactions. *J. Med. Chem.*, **2004**, *47*, 3032-3047.
- [65] Klebe, G.; Abraham, U.; Mietzner, T. Molecular similarity indices in a comparative analysis (CoMSIA) of drug molecules to correlate and predict their biological activity. *J. Med. Chem.*, **1994**, *37*, 4130-4146.
- [66] Giordanetto, F.; Cotesta, S.; Catana, C.; Trosset, J.Y.; Vulpetti, A.; Stouten, P.F.; Kroemer, R.T. Novel scoring functions comprising QXP, SASA, and protein side-chain entropy terms. *J. Chem. Inf. Comput. Sci.*, **2004**, *44*, 882-893.
- [67] Kurogi, Y.; Guner, O.F. Pharmacophore modeling and three-dimensional database searching for drug design using catalyst. *Curr. Med. Chem.*, **2001**, *8*, 1035-1055.
- [68] Oshiro, C.M.; Kuntz, I.D.; Dixon, J.S. Flexible ligand docking using a genetic algorithm. *J. Comput. Aided Mol. Des.*, **1995**, *9*, 113-130.
- [69] Zheng, M.; Zhang, Z.; Zhu, W.; Liu, H.; Luo, X.; Chen, K.; Jiang, H. Essential structural profile of a dual functional inhibitor against cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX): molecular docking and 3D-QSAR analyses on DHDMBF analogues. *Bioorg. Med. Chem.*, **2006**, *14*, 3428-3437.
- [70] Ali, A.M.; Saber, G.E.; Mahfouz, N.M.; El-Gendy, M.A.; Radwan, A.A.; Hamid, M.A. Synthesis and three-dimensional qualitative structure selectivity relationship of 3,5-disubstituted-2,4-thiazolidinedione derivatives as COX2 inhibitors. *Arch. Pharm. Res.*, **2007**, *30*, 1186-1204.

- [71] Berman, H.M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. The protein data bank. *Nucleic Acids Res.*, **2000**, *28*, 235-242.
- [72] Puntambekar, D.S.; Giridhar, R.; Yadav, M.R. 3D-QSAR CoMFA/CoMSIA studies on 5-aryl-2,2-dialkyl-4-phenyl-3(2H)-furanone derivatives, as selective COX-2 inhibitors. *Acta Pharm.*, **2006**, *56*, 157-174.
- [73] Anzini, M.; Rovini, M.; Cappelli, A.; Vomero, S.; Manetti, F.; Botta, M.; Sautebin, L.; Rossi, A.; Pergola, C.; Ghelardini, C.; Norcini, M.; Giordani, A.; Makovec, F.; Anzellotti, P.; Patrignani, P.; Biava, M. Synthesis, biological evaluation, and enzyme docking simulations of 1,5-diarylpyrrole-3-alkoxyethyl ethers as selective cyclooxygenase-2 inhibitors endowed with anti-inflammatory and antinociceptive activity. *J. Med. Chem.*, **2008**, *51*, 4476-4481.
- [74] Biava, M.; Porretta, G.C.; Poce, G.; Supino, S.; Manetti, F.; Forli, S.; Botta, M.; Sautebin, L.; Rossi, A.; Pergola, C.; Ghelardini, C.; Norcini, M.; Makovec, F.; Giordani, A.; Anzellotti, P.; Cirilli, R.; Ferretti, R.; Gallinella, B.; La Torre, F.; Anzini, M.; Patrignani, P. Synthesis, *in vitro*, and *in vivo* biological evaluation and molecular docking simulations of chiral alcohol and ether derivatives of the 1,5-diarylpyrrole scaffold as novel anti-inflammatory and analgesic agents. *Bioorg. Med. Chem.*, **2008**, *16*, 8072-8081.
- [75] Rajakrishnan, V.; Manoj, V.R.; Subba Rao, G. Computer-aided, rational design of a potent and selective small peptide inhibitor of cyclooxygenase 2 (COX2). *J. Biomol. Struct. Dyn.*, **2008**, *25*, 535-542.

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