COX-1 Inhibitors: Beyond Structure Toward Therapy

Paola Vitale, Andrea Panella, Antonio Scilimati, and Maria Grazia Perrone

Department of Pharmacy - Pharmaceutical Sciences, University of Bari "A. Moro", 70125 Bari Italy

Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/med.21389

Abstract: Biosynthesis of prostaglandins from arachidonic acid (AA) is catalyzed by cyclooxygenase (COX), which exists as COX-1 and COX-2. AA is in turn released from the cell membrane upon neopathological stimuli. COX inhibitors interfere in this catalytic and disease onset process. The recent prominent discovery involvements of COX-1 are mainly in cancer and inflammation. Five classes of COX-1 inhibitors are known up to now and this classification is based on chemical features of both synthetic compounds and substances from natural sources. Physicochemical interactions identification between such molecules and COX-1 active site was achieved through X-ray, mutagenesis experiments, specific assays and docking investigations, as well as through a pharmacometric predictive model building. All these insights allowed the design of new highly selective COX-1 inhibitors to be tested into those disease models in which COX-1 is involved. Particularly, COX-1 is expressed at high levels in the early to advanced stages of human epithelial ovarian cancer, and it also seems to play a pivotal role in cancer progression. The refinement of COX-1 selective inhibitor structure has progressed to the stage that some of the inhibitors described in this review could be considered as promising active principle ingredients of drugs and hence part of specific therapeutic protocols. This review aims to outline achievements, in the last 5 years, dealing with the identification of highly selective synthetic and from plant extracts COX-1 inhibitors and their theranostic use in neuroinflammation and ovarian cancer. Their gastrotoxic effect is also discussed.

 Med. Res. Rev., 00, No. 0, 1–32, 2016

Key words: COX-1 inhibitors; natural compounds and dietary phytochemicals; docking studies; neuroinflammation; ovarian cancer

1. INTRODUCTION

COXs, even if their discovery dates back to many years ago, still constitute the target of many research efforts. It is mainly justified by the continuous unravel of COXs involvements (particularly COX-1) in novel physiological and pathological events with an early-marked inflammatory component (i.e., cancer and neurological and neurodegenerative diseases).[1] These

Correspondence to: Antonio Scilimati and Maria Grazia Perrone, Department of Pharmacy - Pharmaceutical Sciences, University of Bari "A. Moro", Via Orabona 4, 70125 Bari, Italy. E-mail: antonio.scilimati@uniba.it; mariagrazia.perrone@uniba.it

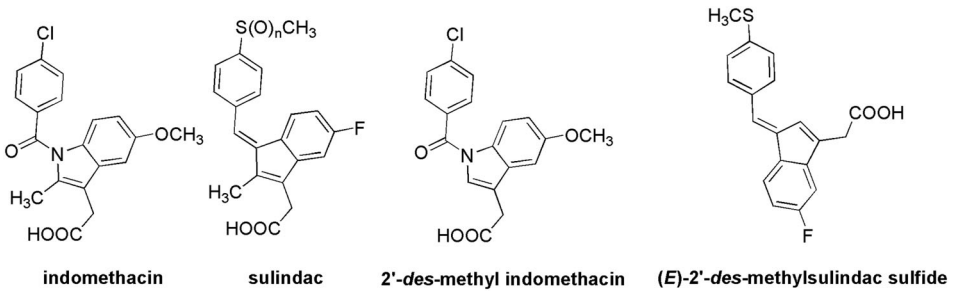


Figure 1. Indomethacin, sulindac sulfide (n = 0), and their corresponding *des*-methyl derivatives.

efforts are also due to the increasing number of compounds, reported in the last years (2010–2015), endowed with a remarkable COX-1 inhibitory potency, selectivity, and affinity.

Cyclooxygenase (COX) catalytic site has an innate plasticity. In consequence, known COX-1 inhibitors have quite different chemical features. Common structural requirements among those selective COX-1 inhibitors were also identified by extensive structure–activity relationship (SAR) studies, docking investigations, and building a pharmacometric predictive model.[2] Based on such characteristics, COXs inhibitors can be grouped as (i) carboxylic acids, (ii) diarylheterocycles, (iii) phenazones (pyrazolones, oxicams), (iv) carbo (or sulfo)-amides, and some natural products endowed with COXs inhibitory activity not belonging to those classes, but present in plants and dietary phytochemicals.

Most of the latest published reviews and papers dealt with COX-2 role and the effects of its inhibition in human health and diseases.[3–5] Herewith, we would summarize the 2010–2015 achievements, obtained both on the novel and relevant chemical entities development as selective COX-1 inhibitors and considering the COX-1 inhibition as a theranostic target.

2. NEW NOTEWORTHY COX-1 SELECTIVE INHIBITORS (2010–2015)

A. Carboxylic Acids

Most of traditional nonsteroidal anti-inflammatory drugs (*t*NSAIDs) of "carboxylic acids" class interacts with COX-1 by forming a salt bridge with R120 located at catalytic site gate. Such an interaction orientates the aromatic portion of these molecules toward Y385, at the upper part of the COX active site. Y385 radical is involved in the first step of the bis-oxygenation reaction of the arachidonic acid (AA).

Sulindac sulfoxide ($n = 1$, Fig. 1), belonging to this class, is a NSAID that shows cancer chemopreventive activity in animal models.[6,7] It is a prodrug, being reduced to the active sulfide metabolite (n = 0, Fig. 1) by colonic microflora. Several structural modifications were made at the carboxyl, benzylidene, and 5′-position of the indene ring. As a result, several sulindac derivatives were identified as potent and selective inhibitors modifying the benzylidene group. The replacement of the 5′-fluoro or carboxy group was found to be less tolerated by the plasticity of the COX-1 catalytic site. (*E*)-2′-*des*-methylsulindac sulfides (*E*-DMSSs) were also found to selectively inhibit COX-1.[8,9]

The *des*-methylsulindac sulfide analogues were found to be potent inhibitors of COX-1 in human ovarian carcinoma cells (*h*OVCAR-3). *E*-DMSSs were weakly toxic toward *h*OVCAR. Their antiproliferative action is much more high (100-fold) than their inhibition of COX-1. *E*-DMSSs could be helpful to study *in vivo* COX-1 biology and could be optimized as therapeutic agents targeting COX-1.

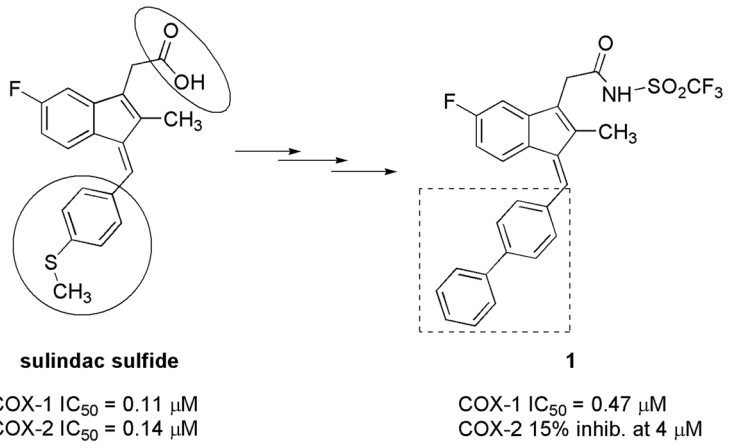


Figure 2. Structural modifications to find novel DMSSs as COX-1 inhibitors.

Increasing the size and hydrophobicity of the *E*-DMSS aryl group, by replacing the methylsulfonylbenzylidene with biphenylmethylidene (Fig. 2), the potency and selectivity of COX-1 inhibition are significantly improved.[8] The introduction of a fluorine or trifluoromethyl on the biphenyl ring did not improve the inhibitory potency, which instead was achieved increasing the molecule hydrophilicity, by introducing one or more nitrogen atoms into the biphenyl moiety. The inhibitory activity against COX-1 was retained fusing the biphenyl to a fluorene, but an increased COX-2 inhibition was observed. The introduction of one alkyl at Cα of the carboxyl group reduced the inhibitory activity.

In the benzylidene series, the conversion of the carboxylic acid functionality into a non-ionizable ester or amide reduced the inhibitory potency, even if their COX-1 selectivity was maintained. Methyl and isopropyl ester and substitution at Cα- of the carboxy group provided a novel series of esters and acids. In particular, ethyl esters of the α,α-disubstituted compounds retained the COX-1 selectivity, but with a reduced potency.

The SAR study was completed by synthesizing a series of substituted alkyl and aryl sulfonimides as carboxylic acid bioisosteres. Biphenylmethylidene-trifluoromethylsulfonimide (**1**), among all the prepared *E*-DMSSs, was the most potent and selective inhibitor (COX-1 IC_{50} = 0.47 μM; 15% inhibition of COX-2 at 4 μM).[8]

B. Diarylheterocycles

The diarylheterocycles as COX inhibitors were deeply studied. In particular, the choice of the heterocycle core ring received a remarkable attention. The isoxazole, thiazole, pyrazole, triazole, thiophene, and furanone rings were used as a central ring in the preparation of several COX-1 inhibitors.[10] The heterocycle core rings mostly used for the preparation of COX-1 inhibitors will be further on described.

1. Isoxazoles

The isoxazole nucleus was subjected to an extensive SAR investigation to definitely identify COX-1 catalytic site requirements for COX-1 potent and selective inhibition. For this reason, either one group or more groups linked to such a ring were replaced taking into consideration their size, steric, and electronic features. The isoxazole is the central heterocycle of potent and selective COX-1 inhibitors such as mofezolac,[11] **P6**, **P9**, **P10**, and **2**–**5** (Fig. 3).[12,13]

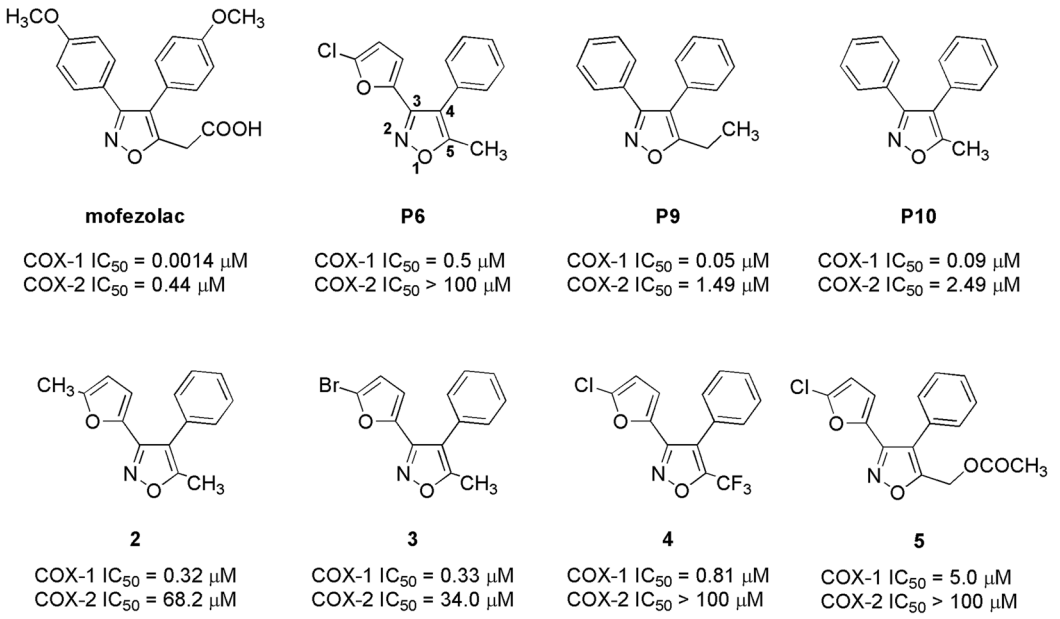
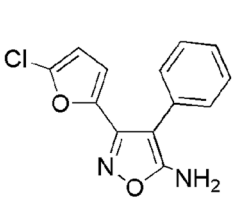


Figure 3. Mofezolac and some **P6** analogues.

An extensive SAR study[13] using **P6** as a lead showed that (i) the furan group is an important moiety for COX-1 inhibition selectivity, (ii) the substituent size of the furan (chloro/bromo atom or methyl group in place of a hydrogen) determined a marked COX-1 selectivity, and (iii) the introduction of a CF_3 in place of a methyl group gives a more lipophilic selective COX-1 inhibitor, with a high COX-2/COX-1 selectivity index (SI = COX-2 IC_{50}/COX-1 IC_{50}).

In summary, COX-1 selectivity driven essential elements are the concomitant presence of 5-methyl (or 5-CF_3), 4-phenyl, and 5-chloro(-bromo or -methyl)furan-2-yl groups on the isoxazole ring.[13] Moreover, **2**, **3**, and **4** inhibitors by inhibiting COX-1-dependent thromboxane (TXA_2) biosynthesis were demonstrated to affect *in vitro* platelet aggregation. To have a stronger and slowly reversible binding to the COX-1 active site, it has been necessary to replace the chlorine of **P6** with CH_3 (**2**) or a Br (**3**) and to introduce a 5-trifluoromethyl group in place of the 5-methyl of **P6**. *In vivo* experiments of **4** in mice show that the inhibition of platelet-derived TXA_2 is preferred to the PGI_2-protective vascular effects.[13]

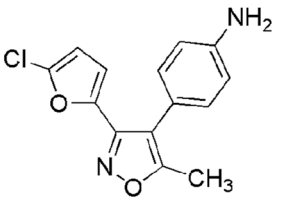
The importance of the presence of the oxygen atom in the substituent linked to isoxazole-C_3 on the inhibition of COX-1 activity and COX SI has also been supported by docking studies.[14] Both O_1-furan oxygen atom and N_2-isoxazole nitrogen atom of compounds **6** and **7** are within H-bonding distance with the OH group of S353 (Fig. 4). The $O_1\cdots O$ and $N_2\cdots O$ atoms are separated by 2.6 Å and 2.8 Å, respectively, and show a favorable geometry for H-bonding. The furan oxygen of **6** also accepts a weak H-bond from the OH group of Y355. The isoxazole oxygen of **6** is involved in a further H-bond with the NH_2 group of R120, which is a key amino acid in the binding of substrates, such as AA, and inhibitors with a carboxylic acid function. The substitution of methyl with an amino group on isoxazole-C_5 increases COX-1 inhibitory activity and selectivity of **6** compared to **P6**. In fact, 5-amino-3-(5-chlorofuran-2-yl)-4-phenylisoxazole (**6**) was the most potent inhibitor of the series (IC_{50} = 1.1 μM, Fig. 4), determined in *h*OVCAR-3 expressing only *h*COX-1. Docking experiments have rationalized its



6

COX-1 IC_{50} = 1.1 μM
COX-2 IC_{50} > 50 μM

A

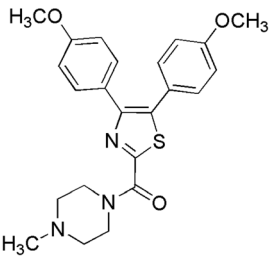


7

COX-1 IC_{50} = 4.3 μM
COX-2 IC_{50} > 50 μM

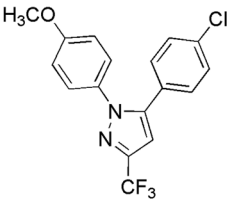
B

Figure 4. Chemical structures of **6** and **7**, their COXs IC_{50} values from a colorimetric inhibitory assay, and docking plots: (**A**) for **6** and (**B**) **7** in COX-1 active site, respectively.[7]



FR122047

COX-1 IC_{50} = 0.028 μM
COX-2 IC_{50} = 65 μM



SC-560

COX-1 IC_{50} = 0.007 μM
COX-2 IC_{50} = 74.9 μM

Figure 5. Chemical structure of **FR122047** and **SC-560** and their COXs IC_{50} values.

potency displaying that **6** interacts with constriction residues R120 and Y355 at the base of the active site, as well as an interaction with S530 at the top of the pocket.[14]

SC-560, **FR122047** (Fig. 5), **mofezolac**, **P6**, **P9**, and **P10** (Fig. 3) as examples of highly selective COX-1 inhibitors have in common a five-member heteroaromatic central ring (thiazole in **FR122047,** pyrazole in **SC-560**, and isoxazole in **mofezolac**, **P6, P9**, **and P10**). In general, two aromatic rings, often 4-methoxyphenyls, linked at adjacent atoms of a five-member heteroaromatic ring are important in the target-compound definition, although not essential: **FR122047** and **mofezolac** have two 4-methoxyphenyls linked to central heterocyclic ring, whereas **SC-560** has only one 4-methoxyphenyl, **P6** brings a phenyl on the isoxazole-C_4 and a 5-chlorofuran on isoxazole-C_3, capable of H-bonding by its oxygen atom with the hydroxyl group of COX-1 S530 that is the COX amino acid acetylated by aspirin; **P9** and **P10**, analogues of **P6**, bring two phenyls at C_3 and C_4, respectively, and are still preferential COX-1 inhibitors.

2. Pyrazoles

Isoxazole core ring role in COX-1 inhibition was elucidated by preparing a set of new diarylheterocycles.[15] Replacing the isoxazole with isothiazole or pyrazole determines a drastic decrease in COX-1 inhibitory activity. The replacement of the isoxazole oxygen atom with NH (O-NH) provides a series of "*N*H-pyrazoles" synthesized as analogues of the correspondent isoxazoles.[15] The O-NH exchange was found to be unfavorable for COX-1 inhibitory

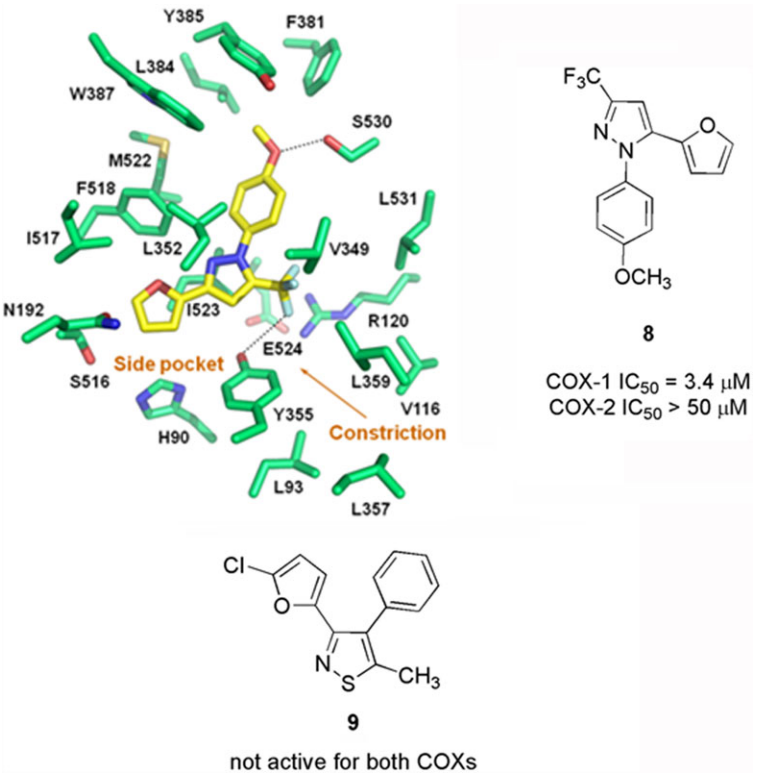


Figure 6. 5-(Furan-2-yl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazole (**8**), its COXs IC_{50} values, and docking plots; 3-(5-chlorofuran-2-yl)-5-methyl-4-phenyl isothiazole (**9**).

activity and selectivity. On the contrary, among the *N*-aryl-substituted pyrazoles, the 5-(furan-2-yl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1*H*-pyrazole (**8**), in which the phenyl bears an electron-donating group (EDG = OCH_3) was found to selectively inhibit COX-1 activity (IC_{50} = 3.4 μM; 28% inhibition of COX-2 at 50 μM), in contrast to its inactive analogue, 5-(furan-2-yl)-1-phenyl-3-(trifluoromethyl)-1*H*-pyrazole, which does not have the methoxy EDG (Fig. 6).[15]

Molecular docking of **8** into the active site of COX-1 allowed to identify some further critical interactions between the inhibitor and the constriction ring residues R120 and Y355 located at the base of the active site, as well as van der Waals contacts with Y385 and W387 at the top of the pocket. In addition, the 3-(5-chlorofuran-2-yl)-5-methyl-4-phenyl isothiazole (**9**),[15] in which the isoxazole-oxygen atom was exchanged with a sulfur atom, was not able to inhibit at all both COX isoforms. Once again, proving the importance of such an oxygen atom presence, at least in the first phase of the inhibitor recognition process by the isoenzymes.

3. Triazoles

Triazole nucleus has also been used, as a central ring, to prepare COXs inhibitors. Most efforts were made to develop a number of 1,2,4-triazoles endowed with biological activity such as **FK881**[16] (3-methoxy-1,5-bis(4-methoxyphenyl)-1*H*-1,2,4-triazole, Fig. 7), which is found to be a specific COX-1 inhibitor exerting a potent analgesic effect without inducing gastrointestinal (GI) toxicity. The pharmacological profile of **FK881** was investigated and compared to a NSAID and COXIBs (selective COX-2 inhibitors). The effects of **FK881** on the activity of human whole

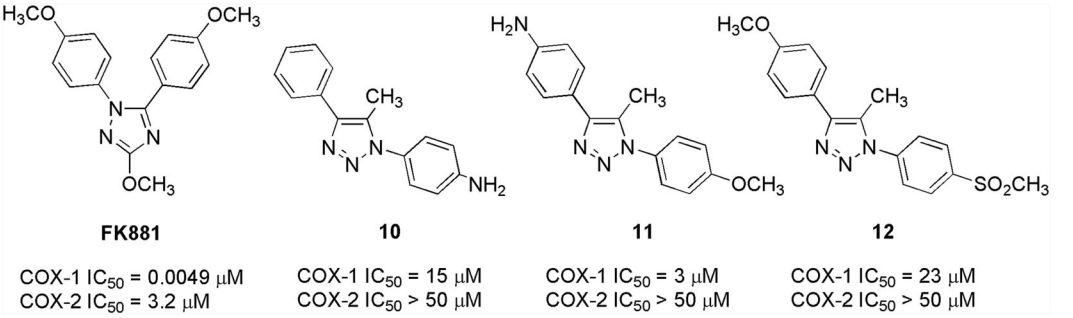


Figure 7. 1,2,4- and 1,2,3-triazoles and their COXs inhibitory activities [**FK881** IC_{50} values are by human whole blood assay (HWBA).[16] **10**–**12** IC_{50} values are by a colorimetric assay (Cayman kit)][2].

blood COX isozymes were detected, and its platelet COX-1 IC_{50} and monocyte COX-2 IC_{50} values were 0.0049 and 3.2 μM, respectively. **FK881** COXs SI was equal to 653.

FK881 had r*h*COX-1 $IC_{50} = 0.064$ μM and r*h*COX-2 IC_{50} value exceeded 100 μM, SI > 1562. **FK881** anti-inflammatory activity in rat carrageenan-induced paw edema (acute inflammatory model) is dose dependent ($ED_{30} = 22$ mg/kg). It also inhibited paw swelling associated with adjuvant arthritis ($ED_{50} = 17$ mg/kg). Furthermore, **FK881** dose dependently inhibited acetic acid induced writhing in mice ($ED_{50} = 19$ mg/kg) (acute pain model) and adjuvant arthritis hyperalgesia in rats ($ED_{50} = 1.8$ mg/kg) (chronic pain model). However, unlike traditional NSAIDs, **FK881** was better tolerated at GI tract, although its antipyretic effect was weak. Analgesic activity of **FK881** resulted to be correspondent to that of classical NSAIDs in preclinical animal model, and may be useful in treating symptoms of rheumatoid arthritis and osteoarthritis, having a substantially improved GI side-effect profile.[16]

A series of 1,4-diarylheterocycles bearing the 1,2,3-triazole (regioisomer of 1,2,4-triazole, a chemical portion of **FK881**) as a core ring was prepared. Based on the COXs inhibition data, the 1,2,3-triazole moiety is not able to establish productive interactions, as in the case of **FK881**. By considering the percentage inhibition of COXs activity by the novel triazoles, it seems that the 1,2,3-triazole as a core ring is not able to form H-bonds similar to the isoxazole, even when in the molecule is present a furan substituent. This outcome seems to depend also upon the molecule volume, electronic and steric features of the substituents present on one or both the aryls linked to the triazole. The aryl to which a specific substituent is bonded is also particularly important. A selective and potent COX-1 inhibition was instead obtained when on the *para*-position of N_1-aryl there is SO_2CH_3 and the C_4-aryl is an anisole (**12**, COX-1 $IC_{50} = 23$ μM), or even better when OCH_3 is on the *para*-position of phenyl of N_1 and the aniline is on triazole-C_4 (**11**, COX-1 $IC_{50} = 3$ μM), or a NH_2 is of the N_1-phenyl and the C_4-aryl is a phenyl (**10**, COX-1 $IC_{50} = 15$ μM). As in the isoxazole, 1,2,3-triazoles bearing a NH_2 group were particularly potent and selective COX-1 inhibitors. In fact, **10** and **11** are the most potent and selective COX-1 inhibitors of this series with IC_{50} values of 15 and 3 μM, respectively.[2]

C. Benzamides

TFAP [*N*-(5-amino-2-pyridinyl)-4-trifluoromethylbenzamide, Fig. 8] is the aryl-benzanilidic structure prototype, bearing two aryls in *E*-like geometry.[17] Although **TFAP** has some promising properties as a potent analgesic agent without gastric damage, it caused a red coloration of urine after oral administration in mice.[18] Ultraviolet-Visible (UV–VIS) spectra and liquid chromatography tandem-mass spectrometry (LC–MS/MS) analyses performed on mice urine samples and metabolite candidates, revealed that the diaminopyridine metabolite,

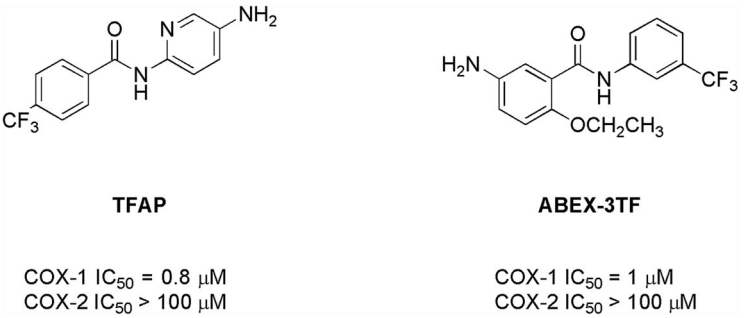


Figure 8. **TFAP** and **ABEX-3TF** chemical structures and their COXs IC_{50} values.

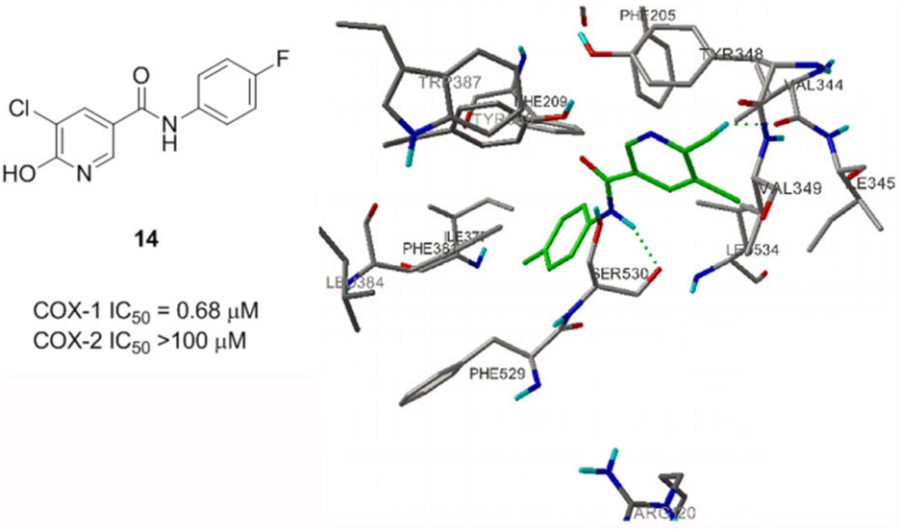


Figure 9. Docking binding mode of **14** in the COX-1 active site.

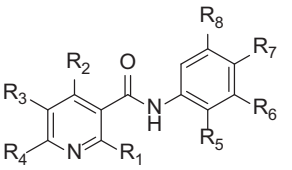
derived from **TFAP** amide bond hydrolysis, was responsible for the purple color of urine samples. The modification of the diaminopyridine skeleton of **TFAP** led to the preparation of the 5-amino-2-ethoxy-*N*-(substituted)benzamide (**ABEX**) series that do not present the problem of colored metabolite. As a result of such a structural modification and after *in vitro* and *in vivo* testing of **ABEX** compounds, a novel COX-1 selective inhibitor, 5-amino-2-ethoxy-*N*-(3-trifluoromethylphenyl)benzamide (**ABEX-3TF**) was identified. **ABEX-3TF** shown a better analgesic activity than indomethacin and did not cause coloration of urines.[19]

Successively, a set of novel *N*-phenyl-nicotinamide (**13**) selective inhibitors of COX-1 was prepared (Table I).[20] Their IC_{50} values ranged between 0.68 and 95 μM.

In particular, the compound bearing R_3 = Cl and R_4 = OH substituent on the pyridine ring (**Entry 8** Table I and **14** in Fig. 9) was the most potent COX-1 inhibitor (0.68 ± 0.07 μM) with a high selectivity (COX-2 IC_{50} > 100 μM). Furthermore, the inhibitory capability seems to be modulated by the R_7-substituent on the phenyl ring (IC_{50} values are in the order CH_3 > Br > OCH_3 > Cl > F).

Overall, these SAR results allowed to ascertain that in the *N*-phenylnicotinamide series (**13**) (i) EWG (i.e., halogen) substituents on the *para* position of the phenyl of *N*-arylnicotinamides increased in most cases the inhibitory activity and (ii) compounds bearing a hydroxy-substituted

Table I. Selected *N*-phenylnicotinamides Particularly Active as COX-1 Inhibitors



13

									IC_{50} (μM)	
Entry	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	COX-1	COX-2
Indomethacin									0.15 ± 0.04	10 ± 2
1	OH	H	H	H	H	H	F	H	32 ± 3	>100
2	OH	H	H	H	H	H	Cl	H	28 ± 5	>100
3	H	H	H	OH	H	H	F	H	3.5 ± 0.6	>100
4	H	H	H	OH	H	H	Cl	H	9.2 ± 1.0	>100
5	H	H	H	OH	H	H	Br	H	30 ± 5	>100
6	H	H	H	OH	H	H	OCH_3	H	86 ± 15	>100
7	H	H	H	CH_3	H	H	F	H	70 ± 11	>100
8	H	H	Cl	OH	H	H	F	H	0.68 ± 0.07	>100
9	H	H	Cl	OH	H	H	Cl	H	2.7 ± 0.3	>100
10	H	H	Cl	OH	H	H	Br	H	74 ± 13	>100
11	H	H	Cl	OH	H	H	CH_3	H	95 ± 6	>100
12	H	H	Cl	OH	H	H	OCH_3	H	44 ± 10	>100
13	H	H	Cl	OH	Cl	H	Cl	H	16 ± 4	>100
14	H	H	Cl	OH	H	Cl	H	Cl	29 ± 6	>100

pyridine and EWG (e.g., *para*-halogen) substituted phenyl rings were found to be selective COX-1- inhibitors.

Docking experiments supported the inhibitory potency based on the bonds established between **14** and COX-1 catalytic site. The *N*-phenylnicotinamide skeleton amide made a hydrogen bond with S530. In addition, the hydroxy group exhibited a hydrogen bond with V344, supporting the importance of its presence in this set of *N*-phenylnicotinamides.[20]

3. DOCKING SIMULATION OUTCOMES

Pharmacophore modeling is an interesting tool in drug discovery as an attempt to identify new ligands. This method looks at chemical and physical interactions between an active molecule and a target protein.

The focus on the synthesis of selective COX-1 inhibitors found its rationale in the recently more deeply exploited COX-1 important role in inflammatory syndromes and oncology (particularly, neuroinflammation[15] and ovarian cancer[21]).

Throughout docking studies, it has been possible to clarify the disposition of a number of COX-1 inhibitors into the enzyme active site. Concerning diarylheterocycle inhibitors with isoxazole as a core ring, compound **3** (Fig. 3) shows the preference for two possible alternative binding mode (**A** and **B**, Fig. 10) in the COX-1 catalytic site.

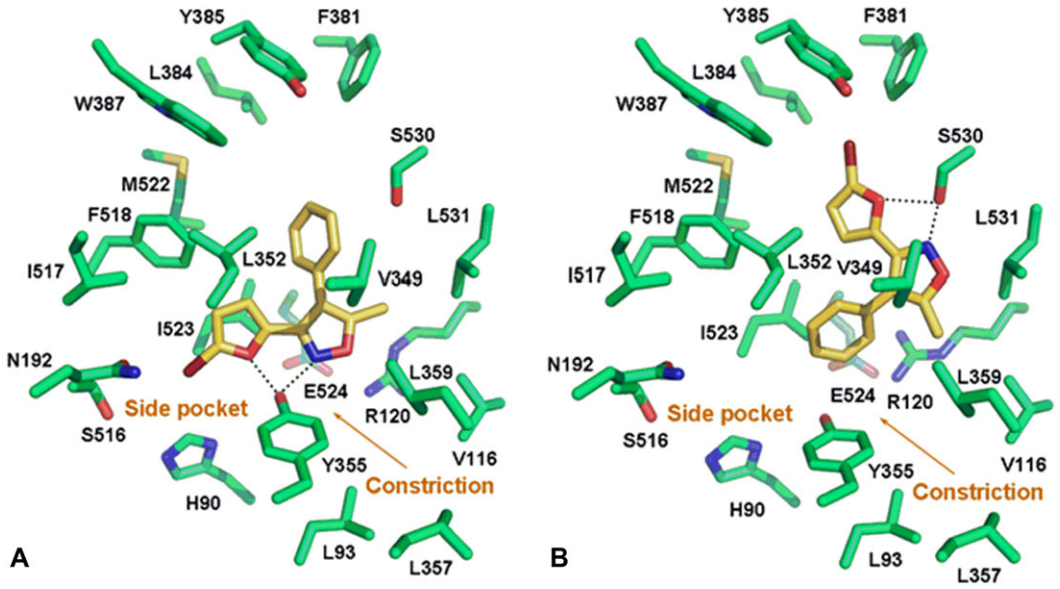


Figure 10. Binding mode **A** and binding mode **B** of compound **3** (yellow) into the COX-1 binding site.

In pose **A**, the isoxazole is located near the opening of the COX-1 active site and, the furan oxygen and isoxazole nitrogen atoms of the inhibitor make H-bonding with the OH group of Y355. The phenyl ring at the isoxazole-C_4 forms hydrophobic interaction with I523, G526, and V349. Moreover, the 5-bromofuran of **3** is oriented toward the side pocket of the protein active site and makes interaction with H90, L352, I517, F518, and I523. In pose **B**, the isoxazole is located at the apex of the active site, and the furan oxygen and isoxazole nitrogen atoms of the inhibitor make a bifurcated H-bond with the OH group of S530.[22] The phenyl ring forms hydrophobic interaction with L352, I523, and Y355 and the 5-bromofuran is oriented as in pose **A** and makes interaction with M522, F518, L352, W387, Y385, L384, and F381. The 5-methyl is located in a small hydrophobic cleft where it makes interaction with V349, L359, V116, and L531 residues.

Concerning **5** (Fig. 3), the carbonyl oxygen of the 5-acetoxy establishes a H-bond with the guanidinium group of R120 (Fig. 11), typically of substrates and carboxylic acid containing inhibitors; the 4-phenyl ring and the 5-chlorofuran moiety insert into the hydrophobic pocket framed by I523, G526, and V349 and the side pocket, respectively.

The isoxazole **3** has a rapidly reversible inhibition mechanism, in contrast with **5** that has a slowly reversible inhibition mechanism. The latter inhibitory mechanism found its rationale, by docking studies, considering that being **5** located near the opening of the COX-1 active site, typically seen with substrates and inhibitors belonging to the "carboxylic acid" class. The simultaneous presence of 5-methyl, 4-phenyl, and 3-(halofuran)-linked to the isoxazole seems essential, but not exclusive to design COX-1 selective inhibitors. The substitution of the chloro with bromo atom or with the methyl on the furan, and 5-CF_3 instead of 5-methyl group on the isoxazole, generates a molecule that binds more tightly COX-1.[13] Main docking study outcomes for **3** and **5** are depicted in Figure 11, which represent the key points interactions of these selective COX-1 inhibitors with the COX-1 active site.

3D-Quantitative structure–activity relationships of mofezolac, one of the most active COX-1 inhibitor, pharmacophoric model confirmed the "four points interactions hypothesis": one hydrogen bond acceptor, one hydrophobic, and two aromatic functions. In particular,

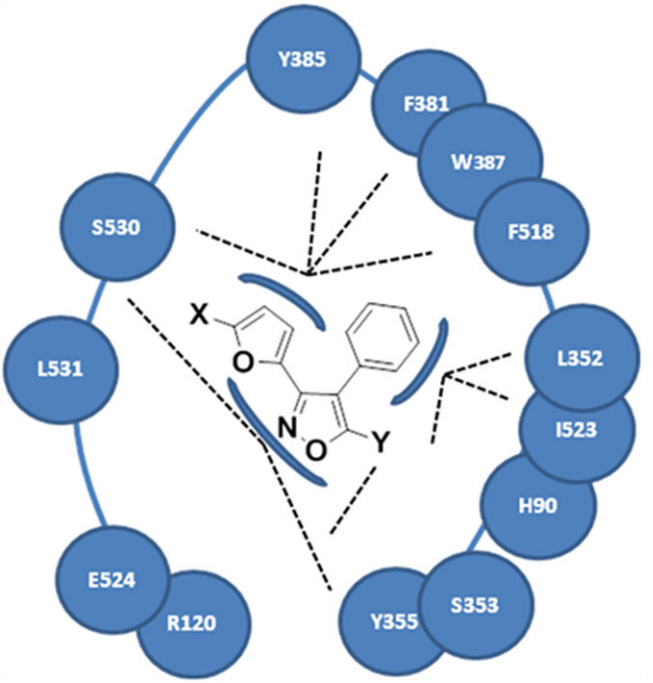


Figure 11. Diarylheterocycles **3** (X = Br and Y = CH_3) and **5** (X = Cl and Y = CH_2OCOCH_3) schematic surrounding in COX-1 binding site.

the enhanced inhibitory activity seems related to the negative charge due to the presence of the -CH_2COOH moiety bond to the isoxazole core ring. In addition, the two aromatic 4-methoxyphenyl rings contribute to COX-1 inhibitory activity and any substitution on these groups would interfere with the best mapping of the molecule on the pharmacophore.[23]

In the interaction of inhibitors with COX-1, the nature of the groups linked to the heteroaromatic core ring affects their disposition into the active site. In fact, the substitution of the acetoxy group of **5** with a NH_2 group of compound **6** determines the "horizontal flipping" of the molecule where the furan oxygen atom and isoxazole nitrogen atom of the inhibitor make H-bonding with the OH group of Y355 (Figs. 4 and 12). The isoxazole oxygen of **6** is involved in a further H-bond with the NH_2 group of R120, which plays a crucial role in binding substrates and inhibitors bearing a carboxylic acid function moiety. Finally, the NH_2 group at isoxazole-C_5 of **6** establishes an H-bond with the S530 OH group, which adopts a "down" position during the flexible docking simulation. Unexpectedly, the NH_2 group at *para*-position of phenyl ring of **7** also interacts with S530 OH group, since this residue switches in an "up" conformation. It is worth noting that the extensive H-bonding network between **6** and the COX-1 active site provides a tight anchor for the compound, explaining its higher inhibitory potency than **7** (Fig. 4). The phenyl ring at isoxazole-C_4 of both compounds is oriented toward the apex of the COX-1 active site and forms hydrophobic interactions with residues L352, F381, L384, Y385, W387, F518, M522, and G526. Importantly, the 5-chlorofuran moiety of **6** and **7** is oriented toward the side pocket (residues 513–520) of COX-1 and makes hydrophobic contacts with residues H90, L352, I517, F518, and I523.[14]

It has been shown that the nature of groups linked to the core ring can force inhibitor disposition into the COX-1 active site.[14] Docking studies in which the isoxazole is replaced

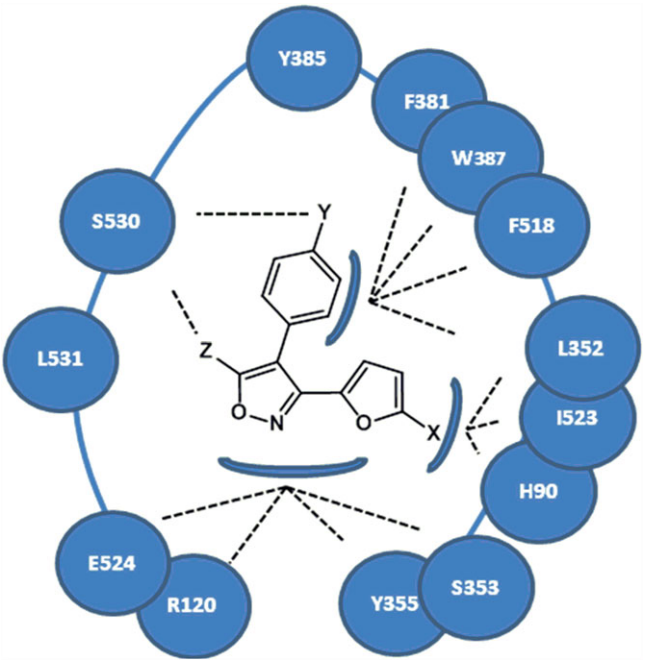


Figure 12. Key points interactions, by docking experiments, of diarylheterocycles **6** (X = Cl and Y = H, Z = NH_2) and **7** (X = Cl and Y = NH_2, Z = CH_3).

with a pyrazole ring bearing almost the same linked groups show the same disposition of compounds **6** and **7** (Figs. 4 and 12).

In fact, the furan ring and trifluoromethyl group in **8** are located at the opening of the COX-1 active site in proximity to the constriction residues Y355 and R120 (Fig. 13). The furan ring makes hydrophobic contacts with L352, F518, and I523 amino acids, and specifically the furan oxygen atom makes a hydrogen bond with Y355. In addition, the trifluoromethyl forms a bidentate hydrogen bond with R120. The 4-methoxyphenyl is oriented toward the top of the COX active site and forms hydrophobic interactions with F518, M522, and I523 amino acids, whereas its methoxy substituent makes Van der Waals contacts with F381, L384, Y385, and W387. The absence of an oxygen atom in the core ring of pyrazole may be a possible explanation for most of known pyrazoles inactivity toward both COX isoforms.[15]

Docking investigations with *N*-phenylnicotinamides **13** (Table I) allowed to ascertain that the two aromatic rings should be in the *s-trans* configuration around the amide bond to ensure the COX-1 activity and selectivity. From the analysis of further data related to benzamide-based compounds, *s-cis* configuration presence decreases selectivity toward COX-1 over COX-2, even if the *s-cis* conformation appears more similar to compounds such as Mofezolac and **FR122047** and other diarylheterocycles (Figs. 3 and 5).[23]

It is noteworthy the predictive pharmacometric model built by Volsurf program, developed as an attempt to gain more deeply insights on the chemical moieties, determinant the right interactions between COX-1 and its selective inhibitors.[2]

Interestingly, such a model allowed to predict the preferential COX-1 inhibitory activity of compound **12** (Fig. 7), even if it bears the sulfamoyl group. Generally, its presence in similar molecules confers COX-2 selectivity. Thus, this means that the presence of a sulfonamide, another group conferring COX-2 selectivity, or methyl sulfamoyl group in these molecules does not assure COX-2 prevalent inhibition over COX-1.

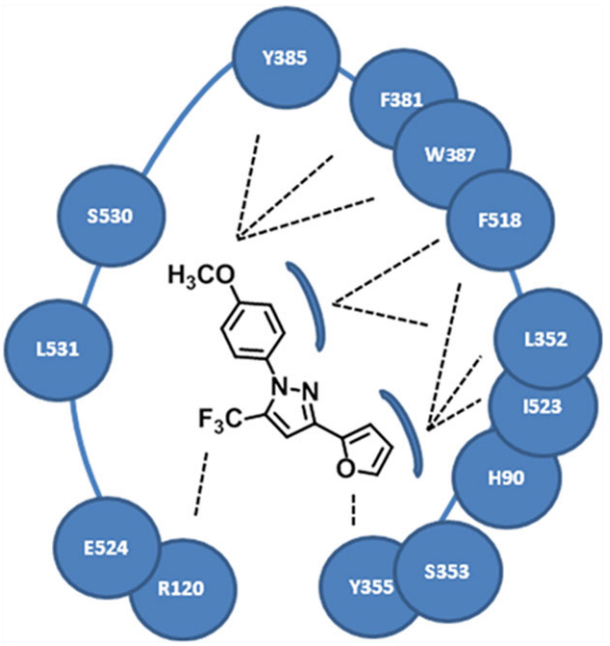


Figure 13. COX-1 amino acids involved in the interactions with pyrazole **8** by docking experiments.

4. NATURAL COMPOUNDS

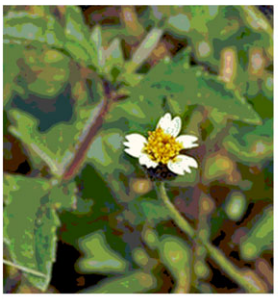
Several secondary metabolites and catabolites arising from natural products possess a pharmacological activity. The acquisition of bioactive compounds from natural products requires the development of high-throughput approaches.[24]

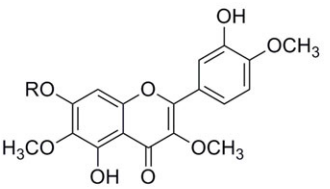
Several studies have proven the anti-inflammatory properties of some plant extracts, in turn sources of substances already used to treat inflammatory syndromes, pain, fever, etc. Such extracts are normally complex mixtures of compounds and, more importantly, also contain many nonactive components together with bioactive molecules, whose identification is still a challenge.

The procedure usually used to discover COX-1 inhibitors, and other biologically active compounds, is bioassay-guided fractionation through column chromatography separations, or by preparative ultrafiltration-high performance liquid chromatography (HPLC).[25]

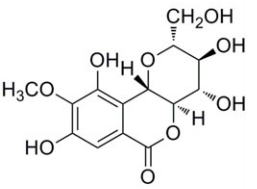
A. Flavonoids

Standardized ethanol or ethyl acetate extracts of *Tridax procumbens* aerial parts (Fig. 14) were found to be endowed with analgesic, antipyretic, and antiarthritic activities in *in vivo* models. They also show *in vitro* COX inhibitory activity. The highest COX-1 and COX-2 inhibition at 50 μg/μL was found with *T. procumbens* ethyl acetate extracts, mainly due to the presence of centaureidin (**15**), centaurein (**16**), and bergenin (**17**) (Fig. 14). Bergenin exhibited the highest COXs inhibitory property, followed by centaureidin. Centaurein inhibited very weakly COX-1 and COX-2 activity. In addition, centaureidin and bergenin were found to be preferential COX-1 inhibitors, like curcumin.[26] Bergenin, centaureidin, and centaurein were tested at 100 μM and exhibited a COX-1 inhibition percentage (%) of 70, 61, and 36, respectively, and of 41, 30, and 21 for COX-2. Thirty micromolar curcumin inhibited 59% of COX-1 activity and



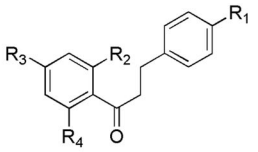


15 (R = H)
16 (R = β-D-glucopyranosyl)

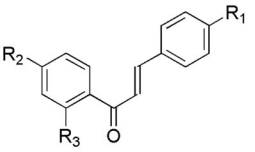


17

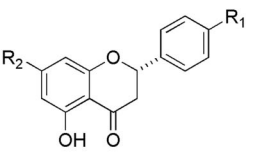
Figure 14. *Tridax procumbens* plant image and some compounds isolated from its leaves.



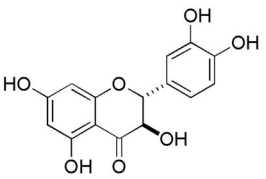
18 (R_1, R_4 = OH; R_2, R_3 = OCH_3)
19 (R_1, R_2 = OCH_3; R_4, R_3 = OH)
20 (R_1 = H; R_2, R_4= OCH_3; R_3 = OH)
21 (R_1 = H; R_2, R_3, R_4 = OCH_3)



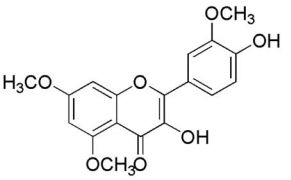
22 (R_1, R_3 = OH; R_2 = OCH_3)
23 (R_1, R_2, R_3 = OCH_3)



24 (R_1 = H; R_2 = OCH_3)
25 (R_1 = H; R_2 = OH)
26 (R_1, R_2 = OCH_3)



27



28

Figure 15. *Nectandra amazonum* image and flavonoids isolated from its leaves.

20% of COX-2. Eleven flavonoids isolated from *Nectandra amazonum* (Lauraceae) exhibited a dose-dependent selective COX-1 inhibitory activity (Fig. 15, Table II).

Dihydrochalcones **18–21**, chalcones **22–23**, and flavonol **28** showed better COX-1 inhibitory activities (IC_{50} = 1.6–36.5 μM). Flavones **24–26** displayed a lower inhibitory activity. Flavonol **27** had no effect on COXs activity. Dihydrochalcone **18**, with two OH at C_4 and $C_{2'}$, was the most potent COX-1 inhibitor (IC_{50} = 1.6 μM). The inhibitory activity decreased in the absence of OH at C_4, as in compounds **19–21**. As observed, also for compounds **18–21**, chalcone **23** structurally different from **22** by the presence of two additional OCH_3 at C_4 and $C_{2'}$ exhibited a lower activity than **22**. Autodock Vina program was used to dock the compound structures within the active site of the COX-1 (PDB: 3N8V). COX-1-R120 (or Y355) and S530 were found to be the key residues to dock the most active flavonoids, indicating that such interactions might interfere with the formation of prostaglandin PGH_2 in the COX-1 active site.[27]

B. Pamir Mountain Plants Extracts

Some anti-inflammatory, pain killer, or febrifuge plants of the Pamir Mountain in northeastern Afghanistan have been studied. COX-1 selective inhibitory activity (Table III) was detected in

Table II. COX-1/COX-2 IC_{50} Values of Flavonoids Isolated From *Nectandra amazonum* (Lauraceae)[21]

Compound	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)
18	1.6	>500
19	8.1	>500
20	22.7	145
21	31.3	214
22	10.1	75.1
23	36.5	84.5
24	145	246
25	84.6	312
26	453	>500
27	>500	>500
28	17.8	124
Ibuprofen	2.7	1.8
Celecoxib	8.3	0.05
Aspirin	0.4	2.5

Table III. COXs IC_{50} Values of Some Pamir Mountain Plants Extracts[22]

Extract source	COX-1 IC_{50} (μM)
Artemisia persica	0.5
Dragocephalum paulsenii	0.5
Ephedra intermedia	3.8
Hyoscyamus pusillus	0.7
Nepeta parmiriensis	0.7
Rumex patientia subsp. pamiricus	3.5

Table IV. COX Inhibition Percentage (%) at 30 μM Final Concentration of the Chemical Constituents (**29–36**) Isolated From *Rumex nepalensis* Roots and Their COXs IC_{50} Values (in Square Brackets)[28]

Compound	COX-1 inhibition (%) [IC_{50} (μM)]	COX-2 inhibition (%) [IC_{50} (μM)]
29	29	42
30	32	49
31	55[39]	76 [23]
32	13	22
33	41	25
34	57 [40]	73 [26]
35	68 [27]	59 [32]
36	32	36
Curcumin	59 [35]	20 [79]
Indomethacin	98 [0.2]	51
Celecoxib	13	96 [0.15]

ethanol extracts of several types of plants. The observed *in vitro* activities support the therapeutic uses of some plant species in the traditional medicine system of the Pamir Mountain.

Similarly, chloroform and ethyl acetate extracts of *Rumex nepalensis* roots were tested in acute inflammation mouse models and on purified COX-1 and COX-2 isoenzymes (Fig. 16, Table IV).[28]

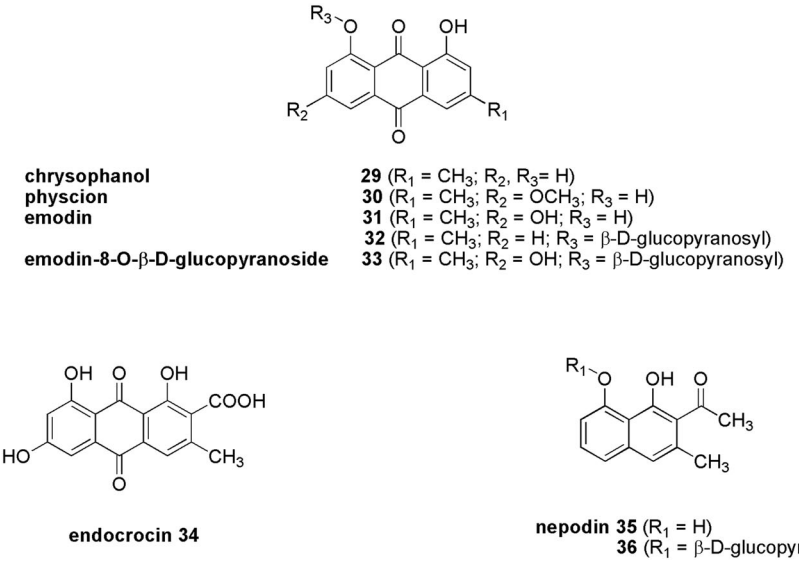


Figure 16. Structures of some substances (**29–36**) isolated from *Rumex nepalensis* roots.

C. Rumex nepalensis Extracts

Six anthraquinones and two naphthalene derivatives were isolated from ethyl acetate extract of *R. nepalensis* roots. Chrysophanol (**29**), physcion (**30**), emodin (**31**), emodin-8-O-β-D-glucopyranoside (**33**), endocrocin (**34**), and nepodin (**35**) were identified. Emodin (**31**) exhibited a higher potent inhibitory effect on COX-2, followed by endocrocin (**34**). Nepodin (**35**) showed a preferential COX-1 inhibitory effect. Emodin, endocrocin, and nepodin also revealed a noteworthy topical anti-inflammatory activity in mice. Interestingly, nepodin showed better radical scavenging activity (antioxidant properties) than trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and ascorbic acid against 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical traps. The anti-inflammatory effect of *R. nepalensis* roots was assumed to be COX inhibition mediated by anthraquinones and naphthalene derivatives and through the radical scavenging activities of the naphthalene derivatives.[28]

D. Dietary Phytochemicals

The effect on COXs activity in *in vitro* assays and cardiovascular toxicity of a variety of active dietary components has been estimated,[29] and most of them moderately inhibits COX-1 (Table V). Six of eight dietary phytochemicals selectively inhibited COX-1 activity. Naringenin and quercetin, two flavonols, were not COX-1/COX-2 inhibitors (COXs $IC_{50} > 400$ μM).

Epigallocatechin-3-gallate polyphenol, from green tea, exhibited the most potent inhibitory effect against the ratio TXB_2/6-keto-PGF1α under physiological and pathological conditions, an unexpected cardioprotective property that merits further investigations. This finding is consistent with a number of recent epidemiologic studies, suggesting that regular consumption of green tea might provide cardioprotective effects.

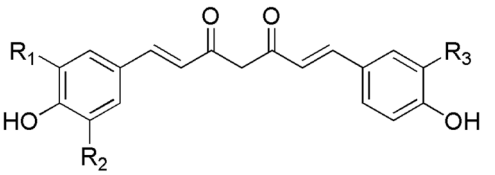
1. Curcuminoids

Four curcuminoids [curcumin, demethoxycurcumin (**37**), bis-demethoxycurcumin (**38**) and 1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-(1*E*,6*E*)-1,6-heptadiene-3,

Table V. COXs IC_{50} Values[a] and the Corresponding Selectivity Index (SI) of Some Dietary Phytochemicals Compared to Celecoxib (Preferential COX-2 Inhibitor) and Aspirin (Preferential COX-1 Inhibitor)

Compound	Source	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)	SI
Celecoxib	–	95	0.02	0.0002
Aspirin	White willow	5	18	3.6
Apigenin	Celery	94	146	1.5
Curcumin	Curry	330	NA	–
Genistein	Soybean	10	256	25.6
Epigallocatechin-3-gallate (EGCG)	Green tea	18	29	1.6
Kaempferol	Broccoli	111	236	2.1
Naringenin	Orange	NA	NA	–
Quercetin	Black tea	NA	NA	–
Resveratrol	Grape	3	8	2.7

[a]The enzyme inhibition by the compounds was determined using a colorimetric COX (ovine) inhibitor screening assay kit (Cayman Chemicals).
NA, not active.



Curcumin (R_1, R_3= OCH_3; R_2= H)
37 (R_1, R_2 = H; R_3 = OCH_3)
38 (R_1, R_2, R_3= H)
39 (R_1, R_2, R_3= OCH_3)

Figure 17. Chemical structures of four curcuminoids from turmeric.

5-dione (**39**), Fig. 17] with COX-1 inhibitory activity were identified in turmeric by a new methodology based on the use of COX-1-functionalized magnetic nanoparticles.[24]

2. Cannabinoids

Anti-inflammatory properties of various cannabinoids have been verified by various *in vivo* and *in vitro* studies. Some endocannabinoids (ECs) protect the colon from inflammation, a very early stage of bowel disease and colorectal cancer. However, the overall mode of action for the anti-inflammatory effects of cannabinoids is not yet completely clarified.[30]

ECs, such as anandamide, have structural similarities to AA. Then, ECs might be COXs substrates, resulting in the production of PG ethanolamides and PG glycerol esters.[31] Recently, the inhibitory effects of different naturally occurring cannabinoids were evaluated in an *in vitro* COX enzyme inhibition assay (Fig. 18).[32] In particular, cannabidiol, tetrahydrocannabinol (Δ^9-THC, **40**), tetrahydrocannabinolic acid (Δ^9-THCA-A, **41**), cannabidiolic acid (CBDA, **42**), cannabigerol (CBG, **43**), and cannabigerolic acid (CBGA, **44**) were found to affect COX enzyme activity, interfere with the action of NSAIDs, but none of those cannabinoids showed high COXs selectivity (Fig. 18).[33]

Looking at the known selective COX-1 inhibitors structures, it is evident that the identification of the chemical moieties determinants for selective COX-1 inhibition is not possible and consequently more systematic and well designed structure–activity studies are necessary.

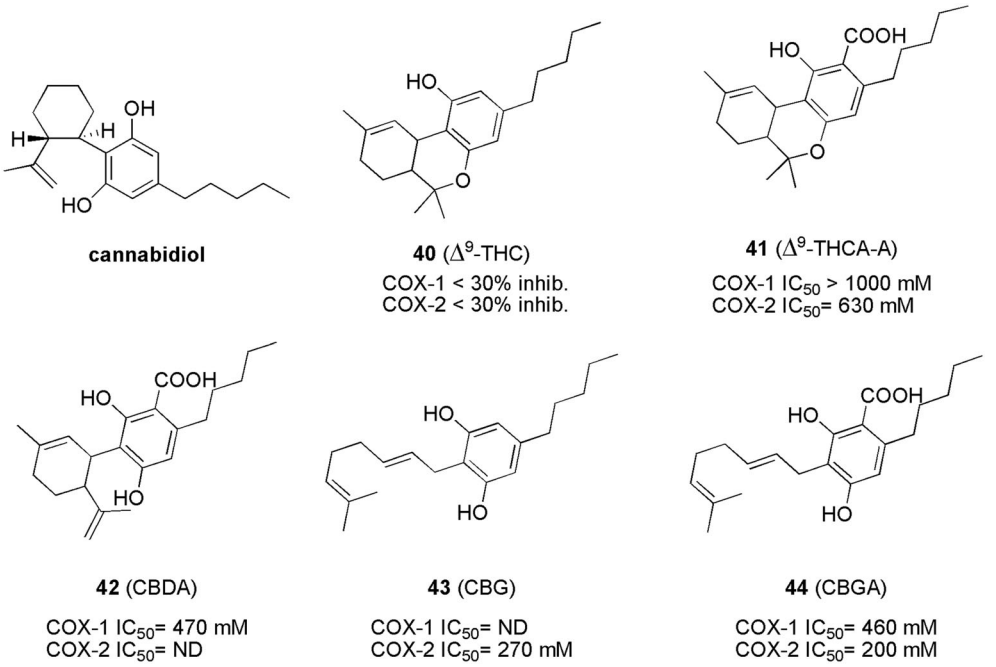


Figure 18. Chemical structures of the cannabinoids Δ^9-THC (**40**), Δ^9-THCA-A (**41**), CBDA (**42**), CBG (**43**), and CBGA (**44**) and their COXs IC_{50} values.

Recently, it was attempted to draw, for each of the five chemical classes in which all the COXs inhibitors can be grouped, a structure with the indications of the necessary physicochemical characters able to switch the selectivity toward one of the other COX isoform.[10] Some substituent features have been, however, identified among the class of diarylheterocycles.

In particular, it seems important for COX-1 selectivity:

1. the presence in the molecules of two (hetero)aromatic moieties linked to a heterocycle central ring;
2. the presence of nitrogen and/or an oxygen atoms capable to act as H-bond acceptors (NH_2, $NHCH_3$, OCH_3, furan oxygen, etc.);
3. in the sulindac and benzilidene derivatives structures a rigid conformation is preferred;
4. fluorinated groups introduction seems to have a role in COX-1 inhibitory activity but its contribution should be further investigated;
5. CH_3SO_2- and NH_2SO_2- removal reverts the activity in favor of COX-1 isoform. Almost all the COX-2 inhibitors bear one of the two groups.

5. COX-1 EXPLOITATION IN SELECTED DISEASES

A. COX-1 Inhibition and Cancer

1. Tumor Microenvironment and Epithelial-to-Mesenchymal Transition

It is well known that in mature platelets the only isoform present is COX–1 and that low dose of aspirin irreversibly inactivates platelet COX-1 through selective acetylation of the enzyme S530.

Activated platelets seem to have a central role in the regulation of angiogenic-regulating factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epithelial growth factor (EGF), transforming growth factor (TGF-β), angiostatin, and insulin-like growth factor (IGF),[34] which mediate the cross-talk between tumor cells and tumor microenvironment (TME).[35] Tumor cells and TME maintain complex bidirectional interactions which have a deep influence on cancer progression and contribute to almost all of cancer hallmarks.[36]

Inhibition of tumor cell–microenvironment interactions is emerging as a promising strategy for cancer treatment. Abnormal microcirculation in tumors leads to a hostile microenvironment characterized by hypoxia, which makes tumor cells highly aggressive, metastatic, and resistant to radiotherapy and most conventional chemotherapeutic agents.[37] Hypoxia, one common characteristic of the microenvironment of tumors, through activation of the hypoxia inducible factor (HIF), is at the center of the growth dynamics of tumor cells.[38] In addition, all mediators released from activated platelets may have a role in the upregulation of HIF expression.[39] Targeting platelet-hypoxia cross-talk could be a novel approach to modify TME and in this direction aspirin may have a role in shaping TME.

Aspirin alters TME in T-cell lymphoma mouse model, and its oral administration to mice, as a prophylactic measure, is accompanied by biophysical, biochemical, and immunological alterations of pH, level of dissolved O_2, and glucose of the TME.[40]

Thus, aspirin suppressing platelets activation, via COX-1 inhibition, may offer a feasible way to block the communication between tumor cells and TME, and rebalance the ratio of platelet release of pro- and anti-angiogenic factors, thus ''normalizing'' tumor vasculature and shaping TME. Consequently, this will produce a reduced aggressiveness and progression of the tumor and an enhancement of the therapeutic treatment efficacy due to the improvement of the sensitivity.[41]

It has been attributed to aspirin, the chemotherapeutic effects on the metastatic process by platelet-related COX-1 signaling pathway inhibition. Epithelial-to-mesenchymal transition (EMT) is the starting event of metastasis formation, consisting of the acquisition of mesenchymal cell characteristics of tumor cells that lose their epithelial connotations.[42] This event is characterized from an augmented motility and matrix invasion of the tumor cells. Malignant cells break away from the primary tumor site and enter the bloodstream or lymphatic vessels, becoming circulating tumor cells (CTCs). In some cases, before the onset of clinical symptoms, CTCs reach a secondary organ in the very first stage of cancer, thus compromising the prognosis. Platelets were, recently, found to induce EMT in CTCs.

The direct interactions of platelets with CTCs determine their activation and the secretion of α-granules, which contain TGF-β and PDGF at concentrations several fold higher than in most cell types.[43] Moreover, EMT is promoted also by platelet-secreted PDGF as in the case of prostate cancer cells.[44] In addition to platelet-derived PDGF, TGF-β signaling may increase the expression of PDGF in cancer cells, which acts in a sequential autocrine or paracrine manner to promote sustained EMT.[45] Considering all these studies, it has been hypothesized that aspirin in consequence of COX-1 inhibition may represent a new therapeutic approach to treat metastatic cancer through the modulation of the platelet-related EMT of CTCs (Fig. 19).[46]

Due to the phenomenon known as "aspirin resistance," a considerable number of patients do not respond to aspirin. As a consequence, COX-1 inhibitors alternative to aspirin are needed.[22]

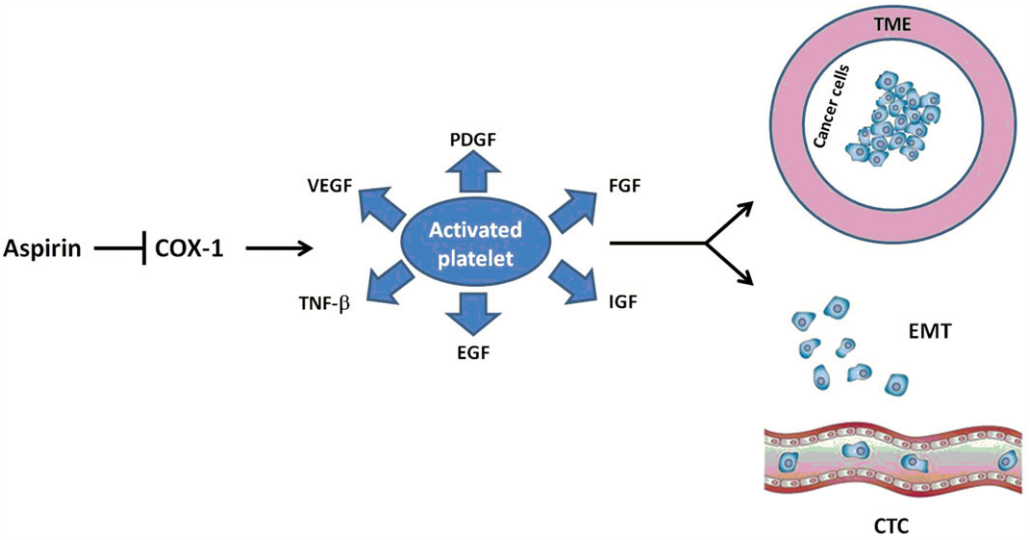


Figure 19. Aspirin suppressing platelets activation, via COX-1 inhibition, modifies the tumor microenvironment and modulates the platelet-related EMT of CTCs.

2. Selective COX-1 Inhibition: Theranostic Agents for Early Ovarian Cancer Diagnosis and Treatment

Early diagnosis of ovarian cancer is nowadays a challenge to increase the patient 5-year survival rate for this malignancy. Despite the introduction of intensive surgical treatments and advances in the use of novel therapeutic agents, new diagnostic biomarkers are welcome as an attempt to reduce the morbidity and mortality caused from advanced stage of ovarian cancer. Most epithelial ovarian cancer cells express high levels of COX-1 rather than COX-2, therefore COX-1 has been recently proposed as an ideal biomarker for the ovarian cancer detection.

An impressive work on the role of COX-1 in high-grade serous ovarian cancer has been published providing additional insights into COX-1 part in this pathology. COX-1 protein was moderately to highly expressed in 99% of high-grade tumors and COX-1 expression was significantly higher than COX-2 in high-grade tumors, and across all serous tumors compared to endometrioid, mucinous, and clear cell tumors. Moreover, it was demonstrated that the downregulation of COX-1 gene expression inhibits multiple protumorigenic pathways and that knockdown of COX-1 inhibits protumorigenic functions such as cell viability, clonogenicity, and migration/invasion in COX-1 expressing ovarian cancer cells.[47] All these data support the idea of COX-1 as an ovarian cancer biomarker.

[^{18}F]-Fluorine-containing selective COX-1 inhibitors have been developed as a positron emission tomography (PET) radiotracer imaging agents targeting COX-1. 3-(5-Chlorofuran-2-yl)-5-(fluoromethyl)-4-phenylisoxazole ([^{18}F]-**P6**) has been proposed as a COX-1 inhibitor radiotracer to detect ovarian cancer in *in vivo* PET computerized tomography.

[^{18}F]-**P6** is a selective and potent COX-1 inhibitor [IC_{50} = 2.0 μM (purified *o*COX-1) and 1.37 μM (*h*OVCAR-3 cell COX-1)] that shows a selective uptake in *in vivo* PET/CT imaging experiments in COX-1-expressing ovarian carcinoma (*h*OVCAR-3) tumor xenografts as compared with the normal leg muscle tissue (Fig. 20).[21]

In addition, VU0487836 (Fig. 21) identification provided the basis to further develop radiotracers to facilitate radiologic imaging of ovarian cancer expressing elevated levels of COX-1.[48]

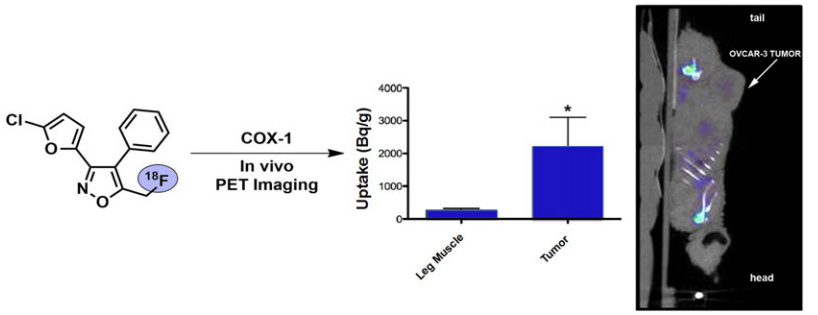
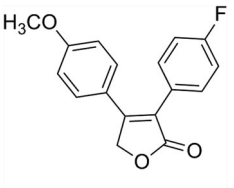


Figure 20. *In vivo* PET imaging of COX-1-expressing tumor by **[^{18}F]-P6**. Tumor-bearing female nude mice were dosed by i.p. injection with compound **[^{18}F]-P6** (100 μL, 7.4 MBq, intraperitoneal injection) under anesthesia. At 4 h post injection, the animals were imaged in the microPET/CT instrument (30-minute acquisition). OVCAR-3 tumor and normal leg muscles were removed and amount of compound **[^{19}F]-P6** was determined by LC-MS. The plot shows the increased unlabeled compound **[^{19}F]-P6** in COX-1-expressing OVCAR-3 tumors versus normal leg muscle (n = 4, p = 0.01) (*statistical significance).



VU0487836

Figure 21. Chemical structure of the selective COX-1 inhibitor VU0487836.

3. COX Inhibitors as Chemosensitizers

Latest advancement in the therapeutic treatment of ovarian cancer can not be neglected. Selective COX inhibitors have been defined as good chemosensitizers because of their capacity to suppress the P-glycoprotein (P-gp) expression, mainly responsible for multidrug resistance facilitating the drug cell efflux, thus increasing the cytotoxic effects of chemotherapeutics.

This effect was observed when paclitaxel is administered in combination with the highly selective COX-1 inhibitor **SC-560**. The co-treatment proved to be a powerful therapeutic tool to promote awareness of paclitaxel resistant ovarian tumors by suppressing the expression/MDR1 P-gp.[49] Further studies are needed to better clarify the mechanism of this COX inhibition mediated chemosensitization.[50]

B. Neuroinflammation

The term neuroinflammation describes the role of inflammatory processes in the pathophysiology of most neurodegenerative diseases. A prevailing response to all types of central nervous system injuries (e.g., those of disease, trauma, chemicals, and drugs) is the activation of microglia in which COX-1 is predominately localized, thereafter COX-1 can have an important role in the neuroinflammatory process. On the contrary, COX-2, being localized in neurons, might be a major player in conditions in which the neurons are directly challenged.

As neuroinflammation is considered the first step of many chronic neurodegenerative conditions [51–53] (i.e., Alzheimer's and Parkinson's diseases, amyotrophic lateral sclerosis, multiple sclerosis, traumatic brain injury, HIV dementia, and prion diseases), a number of clinical trials were accomplished and in most of these studies COX-1 was found to exert a prominent role.

As an exemplificative case,[54] in the brain of Alzheimer's disease (AD) patients, COX-1 expressing microglia were found surrounding amyloid plaques; in a small trial, the use of indomethacin had beneficial effect in protecting AD patients from cognitive decline; the use of low-dose aspirin is associated with reduced risk of AD. On the other hand, a COX-2 upregulation was observed in the early AD stages and a downregulation in the advanced ones.[54] Furthermore, randomized trials with the preferential COX-2 inhibitors celecoxib or rofecoxib had no effect.[54]

Microglia activation is an important hallmark in neuroinflammation. Different stimuli may activate microglia, including LPS, beta-amyloid, prion, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), neuronal injury, and microglia-derived factors.

In particular, the interaction of LPS with its receptors (Toll-like receptor4-CD14) activates intracellular signaling pathways (i.e., NF-kB, MAPKs, and JAK-STAT), which leads to the activation of microglia and the induction of transcription genes coding for proinflammatory mediators (including iNOS, NADPH oxidase, and COX-2) and the consequent release of cytokines (IL-1beta, IL-6 and TNF-alpha), chemokines (monocyte chemotactic protein 1), nitric oxide, and PGs, which are associated with the neuroinflammation. PGs derive from the COX catalytic activity through the bis-oxygenation of AA, in turn released upon neurotransmitters, neuromodulators, or inflammatory stimulation. In addition, LPS and other inflammatory stimuli activate matrix metalloproteinases, which regulate blood brain barrier, and, consequently, cause infiltration of peripheral leukocytes into the brain. The inflammatory response is exacerbated by the peripheral leukocytes recruitment determining the neuronal damage.

Then, the hypothesis would be COX-1 being constitutively expressed in microglia produces PGE_2, a proinflammatory PG, as a primary rapidly response to inciting stimuli. This effect was confirmed in COX-1-deficient mice treated with LPS and in wild-type mice treated with the selective COX-1 inhibitor **SC-560** and LPS. Under the same circumstances, COX-2-deficient mice treated with LPS and the selective COX-2 inhibitor, celecoxib, alter the inflammatory response, whereas treated wild-type mice did not alter the inflammatory response. COX-2 inhibition seems to afford neuroprotection catalyzing the lipoxins and resolvins biosynthesis. COX-2, mainly present in pyramidal neurons, mostly participates in increasing PGs synthesis in response to neuronal insults, such as ischemia and excitotoxicity. Hence, COX-1 is responsible for primary response to inflammatory stimuli, whereas COX-2 is responsible for the secondary response. Vice versa, upon neuronal damage, COX-2 is responsible for primary response, whereas COX-1 is responsible for the secondary response, upon microglia activation. Then, selective COX-1 inhibition is essential in neuroinflammation. NF-kB is another crucial player in inflammation, immunity, cell proliferation, and apoptosis. NF-kB is sequestered in the cytoplasm in complexes with the inhibitory molecule known as $I\kappa B\text{-}\alpha$, in which phosphorylation and degradation is a necessary step in the activation of NF-kB. NF-kB activation and translocation to the nucleus determine the transcription of genes of cytokines and chemokines involved in the inflammatory response (Fig. 22).

Interestingly, in LPS-stimulated murine N13 cells, the selective COX-1 inhibition markedly reduces PGE_2 biosynthesis due to three concomitant synergic events, the reduction of both cPGES mRNA and COX-1 expression extent, and COX-1 catalytic activity inhibition.[55,56]

The direct link between NF-kB activation and COX-1 activity is demonstrated by the absence of an increase in NF-kB activation in LPS-injected COX-$1^{-}/^{-}$ mice with respect to the corresponding COX-$1^{+}/^{+}$ mice treated with LPS. In LPS-stimulated murine N13 microglial cells the activation of NF-κB is remarkable reduced in the presence of COXs inhibitors (**P6** and **P10)** due to the inhibition of the phosphorylation of $I\kappa B\text{-}\alpha$.[55,56] It is noteworthy that **P6** and **P10** in this model did not affect at all the COX-2 expression (Fig. 23).

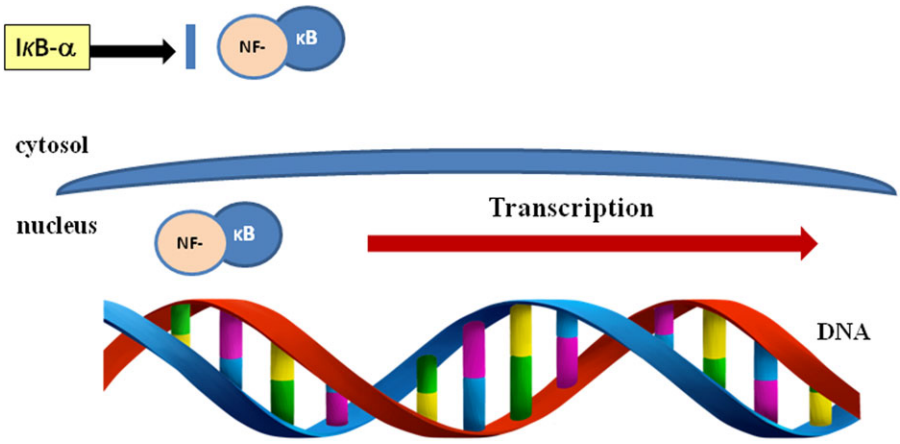


Figure 22. Simplified mechanism of NF-kB activation.

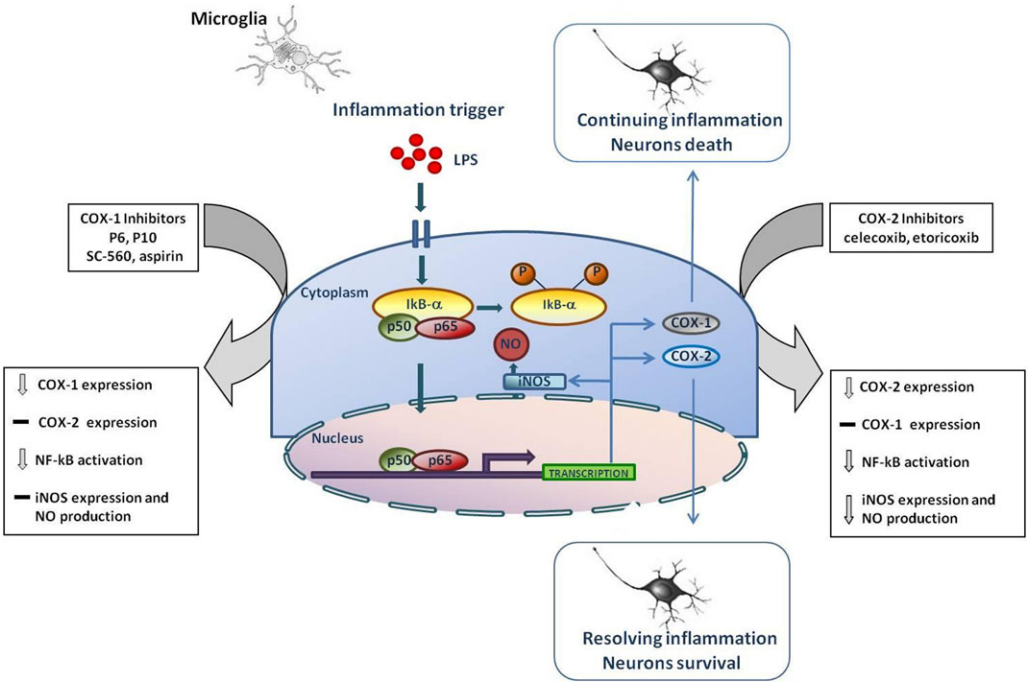


Figure 23. Effects of some selective COX-1 inhibitors and COXIBs (preferential COX-2 inhibitors) in LPS-stimulated N13 murine microglial cells (one of the worldwide used models of neuroinflammation).

Furthermore, inducible Nitric Oxide Synthase (iNOS), NADPH oxidase, and myeloperoxidase (MPO), expressed in glial cell, are the major sources of reactive oxygen species in the neuroinflammation. In LPS-injected COX-1-ablated mice, iNOS, NADPH oxidase, and MPO levels are less markedly increased compared to COX-$1^{+/+}$ LPS-administrated mice (Fig. 24). Similar results were obtained in LPS-treated microglial cell lines in which the presence of COX-1 inhibitors reduced iNOS expression as well as NO production.

Protein carbonyls and nitrotyrosine levels are oxidative damage hallmarks. In LPS-stimulated COX-$1^{-/-}$ mice, nitrotyrosine immunoreactive cells were very few with respect

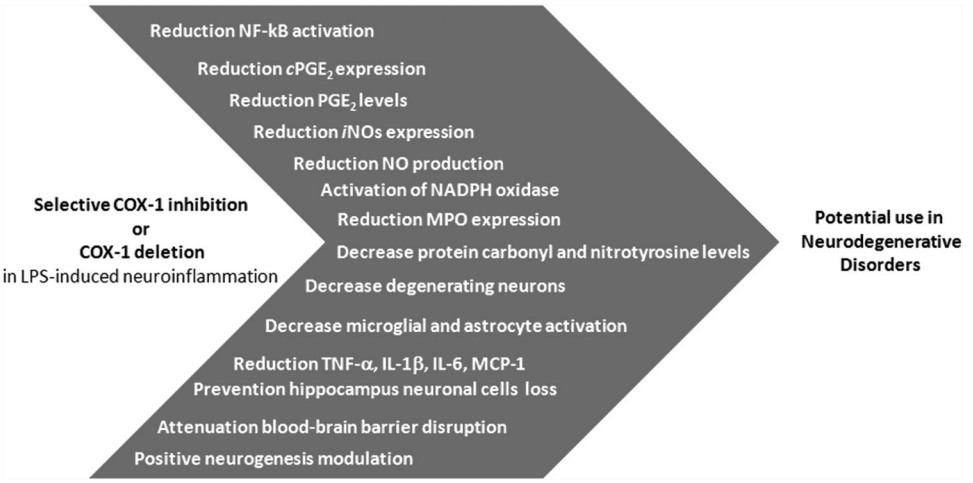


Figure 24. COX-1 involvement panel in *in vitro* and *in vivo* models of neuroinflammation.

to COX-$1^{+}/^{+}$ mice; also protein carbonyls were low in the COX-1-ablated mice compared to the wild-type but unfortunately their level in LPS-stimulated COX-$1^{-}/^{-}$ mice was similar to the vehicle-injected COX-$1^{-}/^{-}$.

A strong modification of gene expression involved in inflammatory response, learning, and memory is caused by LPS injection in brain. Comparing the corresponding wild-type mice, LPS-induced leukocyte infiltration was less severe in COX-$1^{-}/^{-}$ mice, while a significant increase was observed in COX-$2^{-}/^{-}$ mice and a significant increase in COX-$2^{-}/^{-}$ mice compared to their respective wild-type mice. These changes were accompanied by a differential expression of specific chemokines and blood–brain barrier disruption.[57]

LPS administration also causes neuronal damage: cell loss and gliosis occur in hippocampus of COX-$1^{+}/^{+}$ mice, whereas COX-$1^{-}/^{-}$ mice show decreased degenerating neurons after LPS administration. This reduced neuronal damage is due to a decreased glial response, evaluated through the analysis of CD11b, CD45, and the microglia marker glial fibrillary acidic protein.[55]

Moreover, an increase in proliferation and differentiation of hippocampal progenitor cells was observed when a COX-1 gene deletion was achieved in neuroinflammed adult mouse brain.[58]

Nowadays, efforts are directed to investigate a possible repositioning of some NSAIDs with preferential COX-1 selectivity to support and enrich the knowledge on the effects of the selective COX-1 inhibition and to better clarify their efficacy in neuroinflammation.

C. Gastrotoxicity

PGs have a protection role in gastric mucosa due to their effects on mucus and bicarbonate production, surface hydrophobicity, mucosal blood flow, and possible endothelial and epithelial cellular protection.[59] The COXs tissue distribution suggests that the constitutive isoenzyme (COX-1) is critical for physiological functions of GI mucosa, whereas the inducible COX-2 acts under pathological conditions. COX-1 is the principal isoform in the GI apparatus of a variety of species where it is principally localized into parietal cells.[60] It is found in the mucosal epithelium, vascular endothelium, smooth muscle cells of the tunica muscularis, mucosal epithelium of the gastric fundus, corpus, antrum and/or pylorus, duodenum, jejunum, ileum, caecum, and colon (Fig. 25).[61]

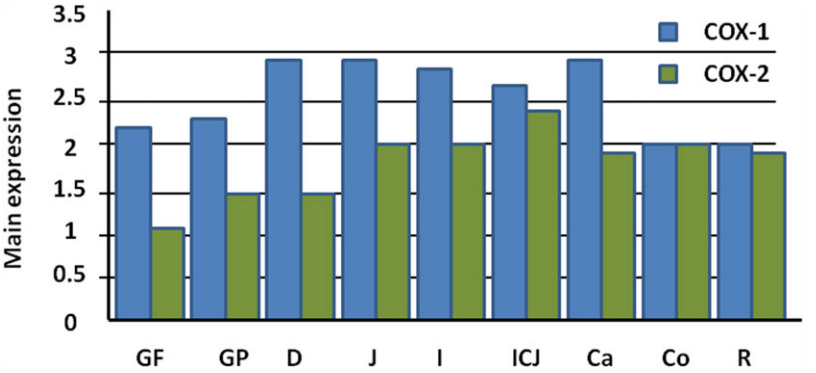


Figure 25. COX-1 and COX-2 expression score in the lamina propria of some anatomic compartments. GF, gastric fundus; GP, gastric pylorus; D, duodenum; J, jejunum; I, ileum; ICJ, ileocecal junction; Ca, caecum; Co, colon; R, rectum.

However, in humans, COX-1 is highly expressed in small intestine while in gastric fundus/antrum its expression is lower. Ulcerogenic effects have been noted only by using nonselective *t*NSAIDs, while by using separately COX-1 or COX-2 inhibitors no GI damage was observed, thus demonstrating that GI toxic effects are due to the contemporary inhibition of both isoforms. Nonselective *t*NSAIDs are responsible for gastric damage due to the contribution of the inhibition of the two isoforms: COX-1 inhibition determines a reduced blood flow while COX-2 inhibition increases leukocyte adherence to the vascular endothelium.[62]

A chronic administration (50 mg/kg for 5 days) of **P6**, a selective COX-1 inhibitor (Fig. 3), in male CD1 mice determined a transient mucosal hyperemia that disappeared upon suspension of the treatment and did not seem to display any significant gastric damage if compared with aspirin-treated mice, which showed breaks in the epithelial barrier and a marked alteration of foveolae and gastric glands (Fig. 26).[63]

High-dose administration (320 mg/kg) of **FK881** (Fig. 7), another selective COX-1 inhibitor, does not cause any ulceration in gastric mucosa, confirming that each isoform helps to maintain mucosal integrity, and subsequently to the COX-1 inhibition, COX-2 upregulation may determine the PGs production to an extent enough to prevent GI injury.[64]

6. CONCLUSION AND REMARKS

It is well known that *t*NSAIDs exert their therapeutic action by COX-2 inhibition, and that their side effects such as the stomach irritation and ulceration are due to COX-1 inhibition.

However, there are no evidences that a highly selective inhibition of COX-1 is the cause of the gastric injury because COX-1 knockout mice do not spontaneously develop gastric lesions and the separate administration of the selective COX-1 inhibitor **SC-560** and selective COX-2 inhibitor celecoxib did not cause gastric damage in rats. Instead, they produce ulcers if administered together.

These findings were explained by considering that the inhibition of only COX-1 induces an upregulation of COX-2, which in turn produces a sufficient PGE_2 quantity to preserve the integrity of the gastric mucosa. Thus, confirming that the simultaneous inhibition of the two COXs is responsible for the formation of the gastric damage.

Then, COX-1 inhibition cannot be confined to the gastrotoxicity, as a side effect of *t*NSAIDs. In fact, low dose of aspirin proven to be beneficial, by inhibiting platelet COX-1, in primary and secondary prevention of cardiovascular diseases. Nowadays, increasing evidences

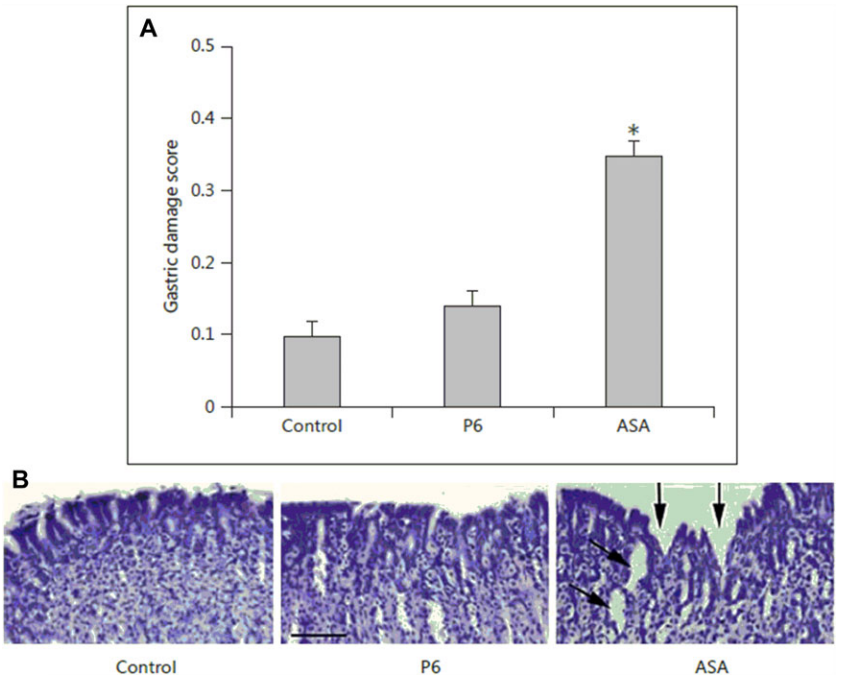


Figure 26. (**a**) Gastric damage scores of controls and **P6**- and **ASA**-treated mice. *$p < 0.01$ ASA versus control. (**b**) Histological observations of gastric mucosa of controls and **P6**- and **ASA**-treated mice (scale bar = 50 μm; arrows show altered foveolae and gastric glands). The gastric damage score was calculated by measuring the length of the ulcers in millimeters.

show that the long-term use of aspirin is associated with reduced risk of some types of cancer and diseases such as colon cancer and Alzheimer's disease.

From a structural point of view, COX-1 and COX-2 isoforms share 60–65% sequence identity within species and about 85–90% sequence identity among different species. They are bifunctional enzymes catalyzing the bis-oxygenation of AA. COX-1 and COX-2 are homodimers of 70 kDa subunits. Each monomer plays a different role. One monomer lacks the heme and behaves as an allosteric moiety and its partner monomer is the catalytic monomer.

Then, the design of highly selective COX-1 or COX-2 inhibitors should also take into account that the two isoforms active site is a long hydrophobic channel, in which more than 50 residues are involved in the substrate or inhibitor recognition. This justifies the different chemical classes, at least five, to which the known inhibitors belong.

Among the COX-1 inhibitors, listed in this review, it is evident that some structural key elements should be present in the molecules. In the case of isoxazole and triazole scaffolds, the presence of one or two methoxy groups is determinant for the COX-1 selectivity, as well as an amino group. For substances from natural source, a number of hydroxyl groups bond to aromatic or not aromatic parts of the molecules are necessary.

Up to 15 years ago, only **FR122047**, mofezolac, and **SC-560** were known as preferential COX-1 inhibitors. Then, during the last 10 and 5 years, a number of other selective COX-1 inhibitors were identified and several studies tried to differentiate the different roles of the two COX isoforms in the human physiology and some pathologies. COX-1 is constitutively expressed in most of body districts for homeostatic functions. Since COX-2 activity is stimulated by inflammatory and mitogen stimuli, it is commonly viewed as the target of anti-inflammatory drugs. Successively, COX-2 was detected as constitutively expressed in some body cells/tissues

overcoming the paradigm of COX-1 constitutive and COX-2 inducible. Actually, several selective COX-1 inhibitors are known. They were tested in several diseases models, proving to have a pharmacodynamic behavior good enough to become a drug and in some cases also an acceptable pharmacokinetic profile. For none of the novel COX-1 inhibitors a complete preclinic investigation has been reported with the exception of mofezolac that reached the market and is used to treat human algesia. It is available in Japan as Disopain®. The scope of this review is not only to stimulate the development of novel highly selective COX-1 inhibitors but also to trigger extensive pharmaceutical and pharmacological investigations in the human diseases in which COX-1 has a central part in the onset and/or progression of the disease. For now, neuroinflammation and ovarian cancer represent reasonable targets.

ACKNOWLEDGMENTS

This work was financially supported by My First AIRC Grant-MFAG 2015 (Project Id. 17566).

CONFLICT OF INTEREST

The authors declare no financial conflicts of interest.

REFERENCES

1. Perrone MG, Scilimati A, Simone L, Vitale P. Selective COX-1 inhibition: A therapeutic target to be reconsidered. Curr Med Chem 2010;17(32):3769–3805.
2. Perrone MG, Vitale P, Panella A, Fortuna CG, Scilimati A. General role of the amino and methylsulfamoyl groups in selective cyclooxygenase(COX)-1 inhibition by 1,4-diaryl-1,2,3-triazoles and validation of a predictive pharmacometric PLS model. Eur J Med Chem 2015;94:252–264.
3. Regulski M, Regulska K, Prukała W, Piotrowska H, Stanisz B, Murias M. COX-2 inhibitors: A novel strategy in the management of breast cancer. Drug Discov Today 2015;pii:S1359-6446(15)00455-459.
4. Asghar W, Jamali F. The effect of COX-2-selective meloxicam on the myocardial, vascular and renal risks: A systematic review. Inflammopharmacology 2015;23(1):1–16.
5. Patrignani P, Patrono C. Cyclooxygenase inhibitors: From pharmacology to clinical read-outs. Biochim Biophys Acta 2015;1851(4):422–432.
6. Thompson HJ, Jiang C, Lu J, Mehta RG, Piazza GA, Paranka NS, Pamukcu R, Ahnen DJ. Sulfone metabolite of sulindac inhibits mammary carcinogenesis. Cancer Res 1997;57(2):267–271
7. Reddy BS, Kawamori T, Lubet RA, Steele VE, Kelloff GJ, Rao CV. Chemopreventive efficacy of sulindac sulfone against colon cancer depends on time of administration during carcinogenic process. Cancer Res 1999;59(14):3387–3391.
8. Liedtke AJ, Crews BC, Daniel CM, Blobaum AL, Kingsley PJ, Ghebreselasie K, Marnett LJ. Cyclooxygenase-1-selective inhibitors based on the (*E*)-2′-Des-methyl-sulindac sulfide scaffold. J Med Chem 2012;55(5):2287–2300.
9. Felts AS, Ji C, Stafford JB, Crews BC, Kingsley PJ, Rouzer CA, Washington MK, Subbaramaiah K, Siegel BS, Young SM, Dannenberg AJ, Marnett LJ. Desmethyl derivatives of indomethacin and sulindac as probes for cyclooxygenase-dependent biology. ACS Chem Biol 2007;2(7): 479–483.
10. Vitale P, Scilimati A, Perrone MG. Update on SAR studies toward new COX-1 selective inhibitors. Curr Med Chem 2015;22(37):4271–4292.
11. Goto K, Ochi H, Yasunaga Y, Matsuyuki H, Imayoshi T, Kusuhara H, Okumoto T. Analgesic effect of mofezolac, a non-steroidal anti-inflammatory drug, against phenylquinone-induced acute pain in mice A new antiinflammatory agent, selectively inhibits prostaglandin G/H

synthase/cyclooxygenase (COX-2) activity *in vitro*. Prostaglandins Other Lipid Mediat 1998;56(4):245–254.

12. Di Nunno L, Vitale P, Scilimati A, Tacconelli S, Patrignani P. Novel synthesis of 3,4-diarylisoxazole analogues of valdecoxib: Reversal cyclooxygenase-2 selectivity by sulfonamide group removal. J Med Chem 2004;47(20):4881–4890.
13. Vitale P, Tacconelli S, Perrone MG, Malerba P, Simone L, Scilimati A, Lavecchia A, Dovizio M, Marcantoni E, Bruno A, Patrignani P. Synthesis, pharmacological characterization, and docking analysis of a novel family of diarylisoxazoles as highly selective cyclooxygenase-1 (COX-1) inhibitors. J Med Chem 2013;56(11):4277–4299.
14. Vitale P, Perrone MG, Malerba P, Lavecchia A, Scilimati A. Selective COX-1 inhibition as a target of theranostic novel diarylisoxazoles. Eur J Med Chem 2014;74:606–618.
15. Perrone MG, Vitale P, Malerba P, Altomare A, Rizzi R, Lavecchia A, Di Giovanni C, Novellino E, Scilimati A. Diarylheterocycle core ring features effect in selective COX-1 inhibition. ChemMedChem 2012;7(4):629–641.
16. Imanishi J, Morita Y, Yoshimi E, Kuroda K, Masunaga T, Yamagami K, Kuno M, Hamachi E, Aoki S, Takahashi F, Nakamura K, Miyata S, Ohkubo Y, Mutoh S. Pharmacological profile of FK881(ASP6537), a novel potent and selective cyclooxygenase-1 inhibitor. Biochem Pharmacol 2011;82(7):746–754.
17. Kakuta H, Zheng X, Oda H, Harada S, Sugimoto Y, Sasaki K, Tai A. Cyclooxygenase-1-selective inhibitors are attractive candidates for analgesics that do not cause gastric damage. Design and in vitro/in vivo evaluation of a benzamide-type cyclooxygenase-1 selective inhibitor. J Med Chem 2008;51(8):2400–2411.
18. Kakuta H, Fukai R, Xiaoxia Z, Ohsawa F, Bamba T, Hirata K, Tai A. Identification of urine metabolites of TFAP, a cyclooxygenase-1 inhibitor. Bioorg Med Chem Lett 2010;20(6):1840–1843.
19. Fukai R, Zheng X, Motoshima K, Kakuta H. Significance and creation of novel cyclooxygenase-1 (COX-1) selective inhibitors. J Pharm Soc Jpn 2011;131(3):347–351.
20. Shi L, Li ZL, Yang Y, Zhu ZW, Zhu HL. Design of novel *N*-phenylnicotinamides as selective cyclooxygenase-1 inhibitors. Bioorg Med Chem Lett 2011;21(1):121–124.
21. Perrone MG, Malerba P, Uddin MJ, Vitale P, Panella A, Crews BC, Daniel CK, Ghebreselasie K, Nickels M, Tantawy MN, Manning HC, Marnett LJ, Scilimati A. PET radiotracer [^{18}F]-P6 selectively targeting COX-1 as a novel biomarker in ovarian cancer: Preliminary investigation. Eur J Med Chem 2014;80:562–568.
22. Evangelista V, Manarini S, Di Santo A, Capone ML, Ricciotti E, Di Francesco L, Tacconelli S, Sacchetti A, D'Angelo S, Scilimati A, Sciulli MG, Patrignani P. De novo synthesis of cyclooxygenase-1 counteracts the suppression of platelet thromboxane biosynthesis by aspirin. Circ Res 2006;98(5):593–595.
23. Balaji B, Hariharan S, Shah DB, Ramanathan M. Discovery of potential and selective COX-1 inhibitory leads using pharmacophore modelling, in silico screening and in vitro evaluation. Eur J Med Chem 2014;86:469–480.
24. Zhang Y, Shi S, Chen X, Peng M. Functionalized magnetic nanoparticles coupled with mass spectrometry for screening and identification of cyclooxygenase-1 inhibitors from natural products. J Chrom B Anal Technol Biomed Life Sci 2014;960:126–132.
25. Cao H, Yu R, Choi Y, Ma ZZ, Zhang H, Xiang W, Lee DY, Berman BM, Moudgil KD, Fong HH, van Breemen RB. Discovery of cyclooxygenase inhibitors from medicinal plants used to treat inflammation. Pharmacol Res 2010;61(6):519–524.
26. Jachak SM, Gautam R, Selvam C, Madhan H, Srivastava A, Khan T. Anti-inflammatory, cyclooxygenase inhibitory and antioxidant activities of standardized extracts of *Tridax procumbens* L. Fitoterapia 2011;82(2):173–177.
27. Valdés-Barrera ID, Cuca-Suarez LE, Coy-Barrera ED. *Nectandra amazonum*-derived flavonoids as COX-1 inhibitors: In vitro and docking studies. Nat Prod Commun 2014;9(5):649–652.

28. Jeppesen AS, Soelberg J, Jäger A. Antibacterial and COX-1 inhibitory effect of medicinal plants from the Pamir Mountains, Afghanistan. Plants 2012;1:74–81.
29. Li H, Zhu F, Sun Y, Li B, Oi N, Chen H, Lubet RA, Bode AM, Dong Z. Select dietary phytochemicals function as inhibitors of COX-1 but not COX-2. PLoS One 2013;8(10):e76452.
30. Ruhaak LR, Felth J, Karlsson PC, Rafter JJ, Verpoorte R, Bohlin L. Evaluation of the cyclooxygenase inhibiting effects of six major cannabinoids isolated from *Cannabis sativa*. Biol Pharm Bull 2011;34(5):774–778.
31. Kozak KR, Rowlinson SW, Marnett LJ. Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. J Biol Chem 2000;275(43):33744–33749.
32. Yu M, Ives D, Ramesha CS. Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2. J Biol Chem 1997;272(34):21181–21186.
33. Takeda S, Misawa K, Yamamoto I, Watanabe K. Cannabidiolic acid as a selective cyclooxygenase-2 inhibitory component in cannabis. Drug Metab Dispos 2008;36(9):1917–1921.
34. Radziwon-Balicka A, Moncada de la Rosa C, Jurasz P. Platelet-associated angiogenesis evaulating factors: A pharmacological perspective. Can J Physiol Pharmacol 2012;90(6):679–688.
35. Goubran HA, Burnouf T, Radosevic M, El-Ekiaby M. The platelet-cancer loop. Eur J Intern Med 2013;24(5):393–400.
36. Fang H, Declerck YA. Targeting the tumor microenvironment: From understanding pathways to effective clinical trials. Cancer Res 2013;73(16):4965–4977.
37. Bar EE, Lin A, Mahairaki V, Matsui W, Eberhart CG. Hypoxia increases the expression of stem-cell markers and promotes clonogenicity in glioblastoma neurospheres. Am J Pathol 2010;177(3):1491–1502.
38. Tsai YP, Wu KJ. Hypoxia-regulated target genes implicated in tumor metastasis. J Biomed Sci 2012;14(19):102
39. Rossi Sartori-Cintra A, Sampaia de Mara C, Argolo DL, Bellini Coimbra I. Regulation of hypoxia-inducible factor-1a (HIF-1a) expression by interleukin-1b (IL-1b), insulin-like growth factors I (IGF-I) and II (IGF-II) in human osteoarthritic chondrocytes. Clinics (Sao Paulo) 2012;67(1):35–40.
40. Kumar A, Vishvakarma NK, Tyagi A, Bharti AC, Singh SM. Anti-neoplastic action of aspirin against a T-cell lymphoma involves an alteration in the tumour microenvironment and regulation of tumour cell survival. Biosci Rep 2012;32(1):91–104.
41. Su BB, Chen JH, Shi H, Chen QQ, Wan J. Aspirin may modify tumor microenvironment via antiplatelet effect. Med Hypotheses 2014;83:148–150.
42. Yu M, Ting DT, Stott SL, Wittner BS, Ozsolak F, Paul S, Ciciliano JC, Smas ME, Winokur D, Gilman AJ, Ulman MJ, Xega K, Contino G, Alagesan B, Brannigan BW, Milos PM, Ryan DP, Sequist LV, Bardeesy N, Ramaswamy S, Toner M, Maheswaran S, Haber DA. RNA sequencing of pancreatic circulating tumour cells implicates WNT signalling in metastasis. Nature 2012;487(7408):510–513.
43. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell 2011;20(5):576–590.
44. Bao B, Wang Z, Ali S, Kong D, Li Y, Ahmad A, Banerjee S, Azmi AS, Miele L, Sarkar FH. Notch-1 induces epithelial-mesenchymal transition consistent with cancer stem cell phenotype in pancreatic cancer cells. Cancer Lett 2011;307(1):26–36.
45. Gotzmann J, Fischer AN, Zojer M, Mikula M, Proell V, Huber H, Jechlinger M, Waerner T, Weith A, Beug H, Mikulits W. A crucial function of PDGF in TGF-beta-mediated cancer progression of hepatocytes. Oncogene 2006;25(22):3170–3185.
46. Lou XL, Deng J, Deng H, Ting Y, Zhou L, Liu YH, Hu JP, Huang XF, Qi XQ. Aspirin inhibit platelet-induced epithelial-to-mesenchymal transition of circulating tumor cells. Biomed Rep 2014;2(3):331–334.
47. Wilson AJ, Fadare O, Beeghly-Fadiel A, Son DS, Liu Q, Zhao S, Saskowski J, Uddin MJ, Daniel C, Crews B, Lehmann BD, Pietenpol JA, Crispens MA, Marnett LJ, Khabele D. Aberrant

over-expression of COX-1 intersects multiple pro-tumorigenic pathways in high-grade serous ovarian cancer. Oncotarget 2015;6(25):21353–21368.

48. Uddin MJ, Elleman AV, Ghebreselasie K, Daniel CK, Crews BC, Nance KD, Huda T, Marnett LJ. Design of fluorine-containing 3,4-diarylfuran-2(5H)-ones as selective COX-1 inhibitors. ACS Med Chem Lett 2014;5(11):1254–1258.
49. Perrone MG, Vitale P., Panella A, Ferorelli S, Contino M, Lavecchia A, Scilimati A. Isoxazole-based scaffold inhibitors targeting cyclooxygenase(COX)s. Chem Med Chem, 2016 in press.
50. Lee JP, Hahn HS, Hwang SJ, Choi JY, Park JS, Lee IH, Kim TJ. Selective cyclooxygenase inhibitors increase paclitaxel sensitivity in taxane-resistant ovarian cancer by suppressing P-glycoprotein expression. J Gynecol Oncol 2013;24(3):273–279.
51. Yermakova AV, Rollins J, Callahan LM, Rogers J, O'Banion MK. Cyclooxygenase-1 in human Alzheimer and control brain: Quantitative analysis of expression by microglia and CA3 hippocampal neurons. J Neuropathol Exp Neurol 1999;58(11):1135–1146.
52. Aïd S, Bosetti F. Gene expression of cyclooxygenase-1 and Ca(2+)-independent phospholipase A(2) is altered in rat hippocampus during normal aging. Brain Res Bull 2007;73(1–3):108–113.
53. Barrio JR1, Satyamurthy N, Huang SC, Petric A, Small GW, Kepe V. Dissecting molecular mechanisms in the living brain of dementia patients. Acc Chem Res 2009;42(7):842–850.
54. Choi SH, Aid S, Bosetti F. The distinct roles of cyclooxygenase-1 and -2 in neuroinflammation: Implications for translational research. Trends Pharmacol Sci 2009;30:174–181.
55. Choi SH, Langenbach R, Bosetti F. Genetic deletion or pharmacological inhibition of cyclooxygenase-1 attenuate lipopolysaccharide-induced inflammatory response and brain injury. FASEB J 2008;22:1491–501.
56. Calvello R, Panaro MA, Carbone ML, Cianciulli A, Perrone MG, Vitale P, Malerba P, Scilimati A. Novel selective COX-1 inhibitors suppress neuroinflammatory mediators in LPS-stimulated N13 microglial cells. Pharmacol Res 2012;65:137–148.
57. Choi SH, Aid S, Choi U, Bosetti F. Cyclooxygenases-1 and -2 differentially modulate leukocyte recruitment into the inflamed brain. Pharmacogenomics J 2010;10:448–457.
58. Russo I, Amornphimoltham P, Weigert R, Barlati S, Bosetti F. Cyclooxygenase-1 is involved in the inhibition of hippocampal neurogenesis after lipopolysaccharide-induced neuroinflammation. Cell Cycle 2011;10:2568–2573.
59. Wallace JL, Bell CJ. Gastroduodenal mucosal defence. Curr Opin Gastroenterol 1996;12:503–511.
60. Jackson LM1, Wu KC, Mahida YR, Jenkins D, Hawkey CJ. Cyclooxygenase (COX) 1 and 2 in normal, inflamed, and ulcerated human gastric mucosa. Gut 2000;47(6):762–770.
61. Haworth R, Oakley K, McCormack N, Pilling A. Differential expression of COX-1 and COX-2 in the gastrointestinal tract of the rat. Toxicol Pathol 2005;33(2):239–245.
62. Hiratsuka T, Futagami S, Tatsuguchi A, Suzuki K, Shinji Y, Kusunoki M, Shinoki K, Nishigaki H, Fujimori S, Wada K, Miyake K, Gudis K, Tsukui T, Sakamoto C. COX-1 and COX-2 conversely promote and suppress ischemia-reperfusion gastric injury in mice. Scand J Gastroenterol 2005;40(8):903–913.
63. Perrone MG, Lofrumento DD, Vitale P, De Nuccio F, La Pesa V, Panella A, Calvello R, Cianciulli A, Panaro MA, Scilimati A. Selective cyclooxygenase-1 inhibition by P6 and gastrotoxicity: Preliminary investigation. Pharmacology 2015;95(1–2):22–28.
64. Tanaka A, Araki H, Hase S, Komoike Y, Takeuchi K. Up-regulation of COX-2 by inhibition of COX-1 in the rat: A key to NSAID-induced gastric injury. Aliment Pharmacol Ther 2002;16(2): 90–101.

Paola Vitale *received her degree with full marks in Chemistry and Pharmaceutical Technology at the University of Bari (Italy) in 2000. She was awarded as the best graduate of the Faculty of*

Pharmacy of Bari in 2000. During her Ph.D. studies, she spent 6 months to study new heterocycles as NSAIDs by the human whole blood assay under the supervision of Prof. Patrignani at the Centre of Studies on Aging (Chieti, Italy). In 2004, she obtained her Ph.D. in Medicinal Chemistry at the University of Bari, in collaboration with Prof. L. Di Nunno and Prof. A. Scilimati. From 2004, she was research fellow (2 years) at the University of Bari for "mechanism investigations and new synthetic methodologies for pharmacologically active isoxazoles". In 2006, she became researcher at the University of Bari, teaching Organic Chemistry. During the last years, her work has been object of international patents, peer-reviewed articles and reviews on international journals, book chapters, and oral communications at national and international Organic Chemistry congress and meetings. Currently, her scientific interests are focused mainly on reaction mechanisms and structural investigation of heterocyclic systems and on applied biocatalysis to the stereoselective synthesis of heterocycles and new APIs.

Andrea Panella *is graduated cum laude in "Pharmacy" at the University of Bari (Italy) in 2012 discussing the thesis in Medicinal Chemistry entitled: "2-alcoxyquinolinic derivatives as potential agents able to revert MultiDrugResistance." He started his Ph.D. in Medicinal Chemistry in 2013 under the guidance of Proff. Scilimati and M.G. Perrone. He spent one year of his Ph.D. in the Research Unit of Prof. W. Smith at the University of Michigan, Ann Arbor to learn COX-1 expression, purification, and cocrystallization with its inhibitors.*

Antonio Scilimati *graduated cum laude in Chemistry at the University of Bari (Italy). He received his Ph.D. in the field of enzymatic catalysis under the supervision of Prof. Charles J. Sih at the University of Wisconsin (Madison, USA). Then, he spent 4 years as a Qualified Person at MerckSerono plant producing recombinant drugs. Back to the University of Bari as an Associate Professor to teach "Chemical, Pharmaceutical and Toxicological Analysis of Drugs". Currently, he is still at the University of Bari, focusing his research interest on the identification of theranostic agents targeting cyclooxygenase-1 as a novel biomarker in oncology and neuroinflammation.*

Maria Grazia Perrone *is graduated cum laude in "Medicinal Chemistry" at the University of Bari (Italy) in 2000. She received her Ph.D. in Medicinal Chemistry in 2004. During the doctorate studies, she was at the Department of Food Science, University of Bologna to learn "microorganisms handling for drug active principle ingredient (API) production". Then, she joined the Research Unit of Prof. C. Syldatk at the Institut für Bioverfahrenstechnik, Lehrgebiet Physiologische Microbiologie at the Stuttgart University (Germany). In this period, she worked with different strains of yeast as API drug production biocatalysts. Since 2006 she is a full-time researcher in Medicinal Chemistry at the Department of Pharmacy - Pharmaceutical Sciences of the University of Bari. She is the principle investigator of a funded research projects 2013–2018. Her scientific interests target the central nervous system disorders and oncology in which the cyclooxygenase is a biomarker.*