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Original article

Synthesis of heteroaromatic analogues of (2-aryl-1-cyclopentenyl-1-alkylidene)-(arylmethyloxy)amine COX-2 inhibitors: effects on the inhibitory activity of the replacement of the cyclopentene central core with pyrazole, thiophene or isoxazole ring

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Abstract

Several heteroaromatic analogues of (2-aryl-1-cyclopentenyl-1-alkylidene)-(arylmethyloxy)amine COX-2 inhibitors, in which the cyclopentene moiety was replaced by pyrazole, thiophene or isoxazole ring, were synthesized, in order to verify the influence of the different nature of the central core on the COX inhibitory properties of these kinds of molecules. Among the compounds tested, only the 3-(*p*-methylsulfonylphenyl) substituted thiophene derivatives **17** and **22**, showed a certain COX-2 inhibitory activity, accompanied by an appreciable COX-2 versus COX-1 selectivity. Only one of the 1-(*p*-methylsulfonylphenyl)pyrazole compounds (**16**) displayed a modest inhibitory activity towards both type of isoenzymes, while the pyrazole 1-(*p*-aminosulfonylphenyl) substituted **12** proved to be significantly active only towards COX-1. All the isoxazole derivatives were inactive on both COX isoforms.

Keywords: Antiinflammatory drug; COX-2 inhibitor; Aryl-substituted methyleneaminoxymethyl moiety; Heteroaromatic COX-2 inhibitor

1. Introduction

Non-steroidal antiinflammatory drugs (NSAIDs) are mainly used in the treatment of pain and inflammation related to a large variety of pathologies [1]. Their mechanism of action involves the inhibition of the production of prostaglandins by the enzyme cyclooxygenase (COX) [2]. Prostaglandins are among the most important mediators of inflammation. They promote blood vessel dilation, and vascular permeability, causing the typical redness, heat, and swelling phenomena involved in inflammation. Moreover, they promote pain transmission from nociceptors to the brain by increasing the sensitivity of the nerve endings. However, prostaglandins also play a cytoprotective role in the gastrointestinal tract, and they are necessary for normal platelet aggregation and renal function. Therefore, a reduction in the production of circulating prostaglandins leads to numerous side effects, the most important being gastrointestinal ulcers [3].

The double nature (i.e. inflammation reduction vs. gastrolesivity) of the effects caused by early NSAIDs acting as COX inhibitors was explained by the discovery of two isoforms of the COX enzyme [4,5]. One isoform (COX-1) is constitutive and regularly expressed, produ-

Abbreviations: NSAID, non-steroidal antiinflammatory drug; COX, cyclooxygenase; MAOMM, methyleneaminoxymethylmoiety; PGE2, prostaglandin E2; TMEDA, tetramethylethylenediamine; DMF, dimethylformamide; THF, tetrahydrofuran.

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cing prostaglandins involved in the cytoprotection of the gastrointestinal tract. The other isoform (COX-2) is associated with inflammatory states and is generally absent from all tissues, unless induced by inflammation mediators. Therefore, the inducible isoform COX-2 constitutes the real target for antiinflammatory drugs. In fact, molecules which are capable of being selective COX-2 inhibitors, without affecting the constitutive isoform (COX-1), have proved to be excellent drugs in the treatment of inflammatory pathologies and, most importantly, are devoid of side effects which are typical of earlier non-selective NSAIDs.

A common structural backbone of most COX-2 selective inhibitors consists of two aryl groups linked to adjacent atoms of a central ring which can be homocyclic or heteroaromatic, one of which is substituted in the *para* position with either an aminosulfonyl ($-SO_2NH_2$) or a methylsulfonyl ($-SO_2Me$) group. There are also examples of potent COX-2 inhibitors that possess cycloalkyl [6], alkoxy [7] or phenoxy [7,8] moieties in the non-sulfonyl containing 'aryl' region. The central rings most commonly found within this class of molecules are cyclopentene (**1**, Fig. 1) [6], thiophene (**2**) [9], pyrazole (**3**) [10,11], furanone (**4**) [12] and isoxazole (**5**) [13]. The first examples of selective COX-2 inhibitors which entered the market were celecoxib (**3**) and rofecoxib (**4**) [10–12].

We have previously described the synthesis and antiinflammatory activity of a series of analogues of the 1,2-diarylcyclopentene COX-2 inhibitor **1**, in which the *para*-fluoro substituted aryl has been replaced by variously substituted oxime-ether groups (**6**, Fig. 1) [14,15], on the basis of the fact that the oxime motif can provide a similar enzyme or receptor-binding affinity as noted for an aryl moiety in several drug pharmacophores [16–19].

Some of these new arylcyclopentene oxime-ethers showed COX-2 inhibitory properties, with satisfactory levels of activity and COX-2 vs COX-1 selectivity. Among the new compounds, the benzyl-substituted one containing the methylsulfonyl moiety (**7**, Fig. 2) and its methylenedioxybenzyl analogue (**8**) appeared to be the most active of the series (Table 1) [14,15]; on the contrary, their aminosulfonyl analogues (**9** and **10**) proved to be devoid of any activity. These data, together with the observation that most of the already known tricyclic COX-2 inhibitors (**2**–**5**, Fig. 1) possess a central ring of a heteroaromatic nature, prompted us to study in depth this type of oxime-ether COX-2 inhibitors, synthesizing some heteroaromatic analogues of methylsulfonyl compounds of type **6** (Fig. 1), in which the central cyclopentene ring is replaced by a pyrazole (**14**–**16**), a thiophene (**17**–**22**), or an isoxazole (**23**–**25**) ring (Fig. 2). In addition, we prepared the *p*-aminosulfonylphenyl-substituted pyrazole derivatives **11**–**13** (Fig. 2), since this sulfonamidic moiety was typical of the commercially available pyrazole-based COX-2 inhibitor celecoxib (**3**, Fig. 1).

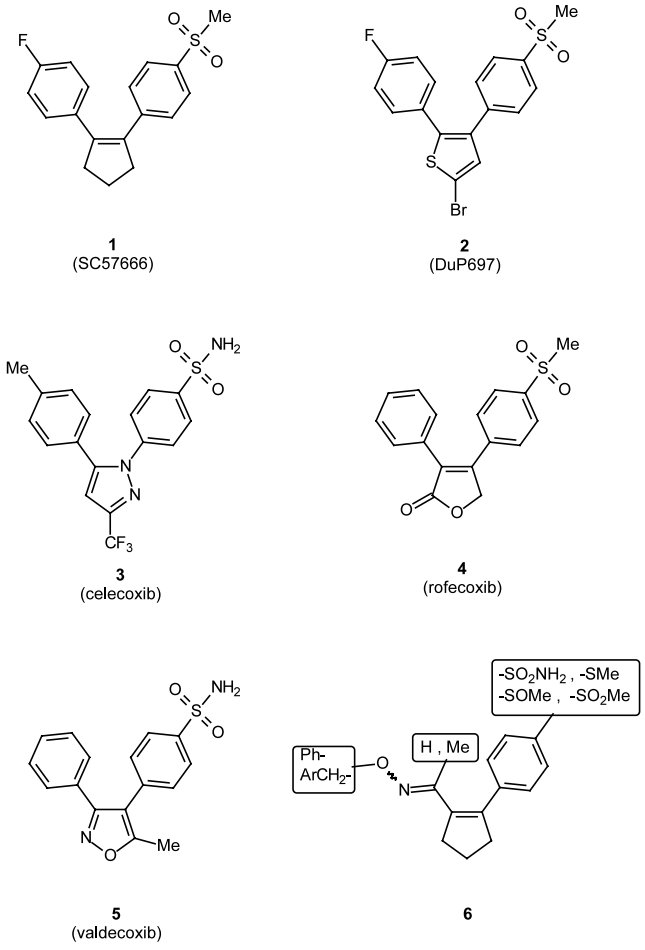
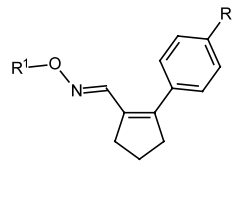


Fig. 1. Structures of some COX-2 selective antiinflammatory drugs characterized by diaryl-substituted carbocyclic or heterocyclic 5-membered ring (**1**–**5**), together with the general structure (**6**) of the previously studied MAOM analogues of type **4** drugs.

2. Chemistry

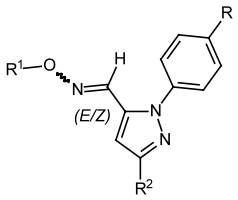
The synthesis of the sulfonamidic 3-trifluoromethyl-substituted pyrazoles **11** and **12** (Fig. 3) started from the α,β-unsaturated ketone **26** [20] which, by condensation with 4-(aminosulfonyl)phenylhydrazine hydrochloride [21] in refluxing ethanol, afforded the regioisomeric mixture of the two pyrazoles **27** and **28**. The isomer **27** was separated by column chromatography, subjected to α-lithiation with *n*-BuLi/TMEDA [22], and quenched, firstly with DMF, and then with a saturated aqueous solution of ammonium chloride, affording the α-formylated pyrazole **29**. Condensation of **29** with the appropriate *O*-benzyl hydroxylamine hydrochloride afforded compounds **11** and **12**.

For the preparation of the 3-methyl-substituted pyrazole **13** (Fig. 3), the β-diketone **30** was condensed



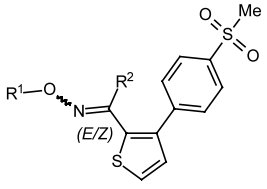
7-10

cmpd	R	R^1
7	SO_2Me	$PhCH_2$
8	SO_2Me	$(3,4\text{-}OCH_2O)C_6H_3CH_2$
9	SO_2NH_2	$PhCH_2$
10	SO_2NH_2	$(3,4\text{-}OCH_2O)C_6H_3CH_2$



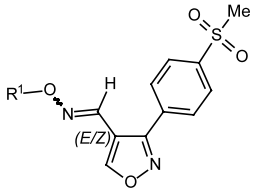
11-16

cmpd	R	R^1	R^2	config
11	SO_2NH_2	$PhCH_2$	CF_3	*E*
12	SO_2NH_2	$(3,4\text{-}OCH_2O)C_6H_3CH_2$	CF_3	*E*
13	SO_2NH_2	$(3,4\text{-}OCH_2O)C_6H_3CH_2$	CH_3	*E*
14	SO_2Me	$PhCH_2$	H	*E*/*Z* (1:1)
15	SO_2Me	$(3,4\text{-}OCH_2O)C_6H_3CH_2$	H	*E*
16	SO_2Me	Ph	H	*E*



17-22

cmpd	R^1	R^2	config
17	$PhCH_2$	H	*E*
18	$(3,4\text{-}OCH_2O)C_6H_3CH_2$	H	*E*
19	$(3,4\text{-}OCH_2O)C_6H_3CH_2$	H	*Z*
20	$PhCH_2$	Me	*Z*
21	$(3,4\text{-}OCH_2O)C_6H_3CH_2$	Me	*Z*
22	Ph	Me	*Z*



23-25

cmpd	R^1	config
23	$PhCH_2$	*Z*
24	$(3,4\text{-}OCH_2O)C_6H_3CH_2$	*E*/*Z* (1:4)
25	Ph	*E*/*Z* (1:3)

Fig. 2. Structures of the more active methylsulfonyl-MAOM compounds of type **6** (**7**, **8**) and of their aminosulfonyl analogues (**9**, **10**) previously described, together with the structures of the heteroaromatic analogues of type **6** compounds, described in this work (**11**–**25**).

with 4-(aminosulfonyl)phenylhydrazine hydrochloride [21] in refluxing ethanol [23], to give the pyrazole derivative **31** as the only regioisomer. Further condensation of **31** with 3,4-(methylenedioxy)benzyloxyamine hydrochloride afforded the oxime-ether derivative **13** with the *E* configuration.

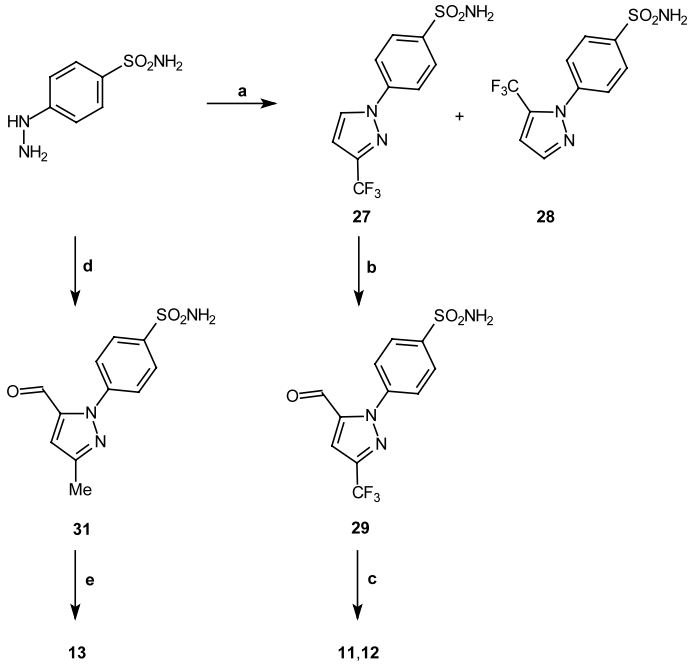
Pyrazole derivatives lacking substituents in the 3 position but possessing a methylsulfonyl group in the *para* position of the aromatic substituent (**14**–**16**), were synthesized as shown in Fig. 4. Condensation of 4-(methylthio)phenylhydrazine **32** [24] with the bis-dimethylacetale of malonaldehyde afforded the *N*-aryl-substituted pyrazole **33**. Regioselective α-lithiation of **33** with *n*-BuLi/DMF [25], followed by sequential quenching with a solution of HCl, afforded the α-formylated pyrazole **34**. Selective oxidation of the methylthio moiety to a methylsulfonyl group was obtained using oxone as the oxidant, to give pyrazole derivative **35**, which was then condensed with the appropriate arylmethyloxy- or phenoxy-amine hydrochloride to afford the corresponding oximes **14**–**16**. After purification, compound **14** was still obtained as a 1:1 E/Z mixture and was tested as such, whereas compounds **15** and **16** were obtained and tested as pure *E* diastereoisomers.

Thiophene derivatives **17**–**22** were prepared as reported in Fig. 5. The cross-coupling reaction of 3-bromothiophene with 4-(methylthio)phenylboronic acid under Suzuki conditions [26] gave 3-[4-(methylthio)phenyl]thiophene **36** [27] which constitutes the branch point of the synthetic scheme, since it was the common intermediate of different pathways for the synthesis of both aldoximes **17**–**19** and ketoximes **20**–**22**. Vilsmeier reaction of **36** with $DMF/POCl_3$, afforded the 2-formylated thiophene compound **37**. Subsequent oxidation with oxone of the methylthio group of **37** to a methylsulfonyl one gave compound **38**, which was

Table 1
COX-1 and COX-2 inhibitory activity of compounds **7**–**25**

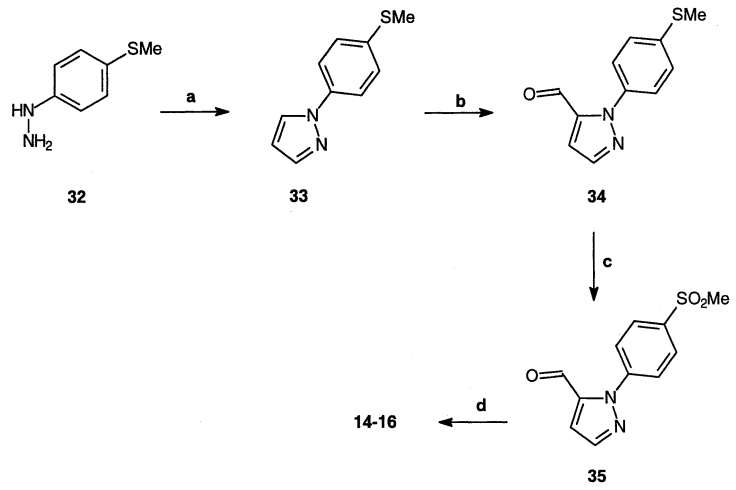
Compound	M.p. (°C)	Crystn solvent [a]	Formula [b]	In vitro inhibitory activity [c]	
				COX-1	COX-2
7 [d]				29%	1.9
8 [d]				27%	0.41
9 [e]				0	19%
10 [e]				0	12%
11	– [f]	–	$C_{18}H_{15}N_4O_3SF_3$	21%	17%
12	96–98	A	$C_{19}H_{15}N_4O_5SF_3$	2.4	7%
13	166–168	B	$C_{19}H_{18}N_4O_5S$	20%	20%
14	103–105	C	$C_{18}H_{17}N_3O_3S$	0	5%
15	137–139	D	$C_{19}H_{17}N_3O_5S$	22%	20%
16	124–125	E	$C_{17}H_{15}N_3O_3S$	112	43
17	139–141	D	$C_{19}H_{17}NO_3S_2$	18%	1.7
18	149–151	D	$C_{20}H_{17}NO_5S_2$	11%	11%
19	131–133	D	$C_{19}H_{17}NO_3S_2$	0	12%
20	93–95	D	$C_{20}H_{19}NO_3S_2$	10%	18%
21	98–100	D	$C_{21}H_{19}NO_5S_2$	0	28%
22	140–142	A	$C_{19}H_{17}NO_3S_2$	0	11
23	128–130	D	$C_{18}H_{16}N_2O_4S$	4%	0
24	153–155	C	$C_{19}H_{16}N_2O_6S$	4%	21%
25	138–140	B	$C_{17}H_{14}N_2O_4S$	0	0
3 (celecoxib)				1.04	0.02

[a] A = hexane–Et_2O, B = ^{i}PrOH, C = MeOH, D = EtOH, E = Et_2O.
[b] All compounds were analysed for C, H, and N.
[c] Data are indicated as IC_{50} (μM) for the more active compounds or as inhibition percentage (%) at a concentration of 10 μM for compounds showing IC_{50} values >1 mM.
[d] See Ref. [12].
[e] See Ref. [13].
[f] Vitreous solid.



a: $EtOCH{=}CHCOF_3$ (**26**), EtOH, reflux; **b**: 1) n-BuLi, DMF; 2) NH_4Cl; **c**: $PhCH_2ONH_2{\cdot}HCl$ or (3,4-$OCH_2O)C_6H_3CH_2ONH_2{\cdot}HCl$, $H_2O/CHCl_3$; **d**: $CH_3COCH_2COCH(OEt)_2$ (**30**), MeOH, reflux; **e**: (3,4-$OCH_2O)C_6H_3CH_2ONH_2{\cdot}HCl$, $H_2O/CHCl_3$.

Fig. 3. Synthesis of the aminosulfonyl type 3-trifluoromethyl (**11**, **12**) and 3-methyl (**13**) pyrazoles.

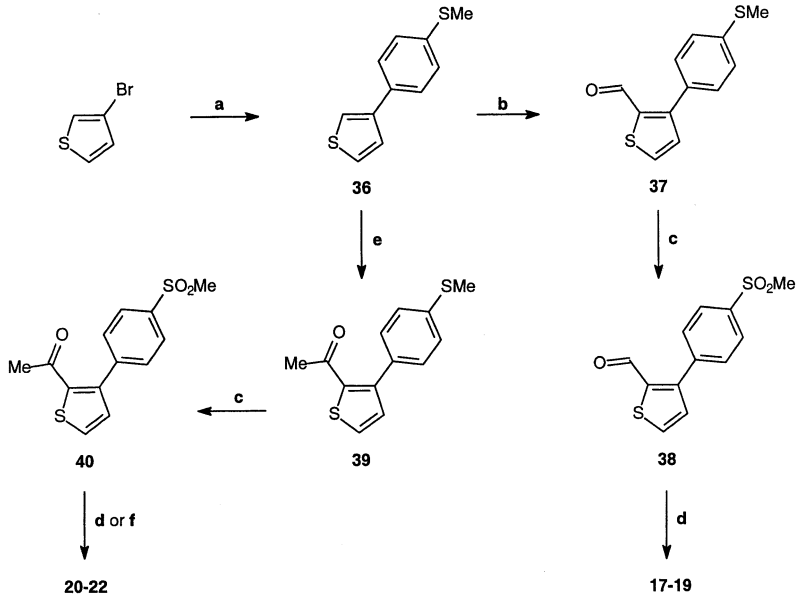


a: $(MeO)_2CHCH_2CH(OMe)_2$, EtOH, reflux; **b**: 1) n-BuLi, DMF, 2) HCl; **c**: oxone, THF/MeOH; **d**: $PhCH_2ONH_2$·HCl or $(3,4\text{-}OCH_2O)C_6H_3CH_2ONH_2$·HCl or $PhONH_2$·HCl, EtOH, reflux.

Fig. 4. Synthesis of methylsulfonyl type pirazoles (**14**–**16**).

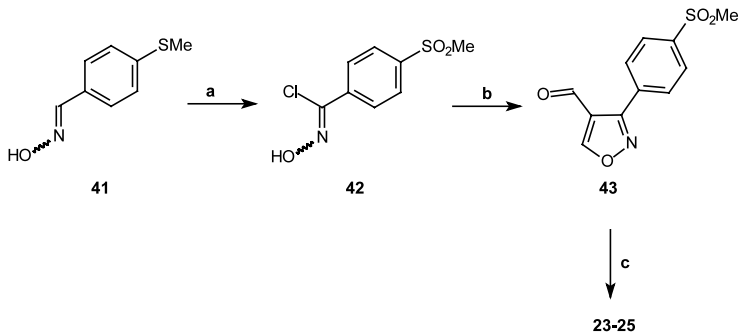
condensed with *O*-benzylhydroxylamine hydrochloride to afford aldoxime **17** with the *E* configuration, or with *O*-(3,4-methylenedioxybenzyl)hydroxylamine hydrochloride to give a mixture of the aldoximes **18** and **19** with the *E* and *Z* configuration, respectively, which were separated by crystallisation. Acetylation of intermediate **36** with acetyl chloride in the presence of tin(IV) chloride [28], afforded the methyl ketone derivative **39**, which was then submitted to oxone oxidation to yield the methylsulfonyl compound **40**. Final condensation of **40** with the appropriate *O*-substituted arylhydroxylamine hydrochloride afforded, after purification, ketoximes **20-22** with the *Z* configuration.

The synthesis of isoxazole derivatives **23**–**25** started with the oxime **41**, obtained by oximation of 4-(methylthio)benzaldehyde [29] (Fig. 6), which was submitted to oxidation/chlorination [30] in the presence of oxone and aqueous HCl. The chlorooximate **42** thus obtained was then treated with triethylamine and 3-(dimethylamino)acrolein [31], affording the 4-formyl



a: 4-(methylthio)phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3 2M, toluene, reflux; **b**: DMF, $POCl_3$; **c**: oxone, THF/MeOH; **d**: $PhCH_2ONH_2$·HCl or $(3,4\text{-}OCH_2O)C_6H_3CH_2ONH_2$·HCl, EtOH, reflux; **e**: AcCl, $SnCl_4$, benzene; **f**: $PhONH_2$·HCl, EtOH, reflux.

Fig. 5. Synthesis of thiophene derivatives **17**–**22**.



a: oxone, HCl, DMF; b: $Me_2NCH=CHCHO$, NEt_3, toluene; c: $PhCH_2ONH_2 \cdot HCl$ or $(3,4\text{-}OCH_2O)\text{-}C_6H_3CH_2ONH_2 \cdot HCl$ or $PhONH_2 \cdot HCl$, EtOH, reflux.

Fig. 6. Synthesis of isoxazole derivatives **23**–**25**.

isoxazole derivative **43**. Final condensation of **43** with the appropriate *O*-substituted hydroxylamines gave the corresponding oximic derivatives **23**–**25** as a single *Z*-isomer (for **23**) or diastereomeric *E*/*Z* mixtures (for **24** and **25**) which were purified as such.

The configuration around the oximic double bond of **11**–**25** was assigned on the basis of the chemical shift values of the aldoximic (for **11**–**19** and **23**–**25**) or methylketoximic (**20**–**22**) proton signal in their ^{1}H-NMR spectra. In fact, in compounds with the *E* configuration this signal, due to the paramagnetic effect of the more proximal oxygen, lies at lower fields than in the corresponding *Z*-isomers [14,15].

3. Biopharmacology

The in vitro inhibitory activity of heteroaromatic compounds **11**–**25** towards COX-1 and COX-2 was evaluated by measuring prostaglandin E2 (PGE2) production in U937 cell lines for COX-1 and activated J774.2 macrophages for COX-2. The results are reported in Table 1, together with those obtained in the same types of tests with celecoxib (**3**), chosen as the reference compound, and with the previously studied cyclopentenyl analogues **7**–**10** [14,15].

4. Results and discussion

As far as COX-1 inhibitory properties are concerned, only the 4-(aminosulfonylphenyl) pyrazole derivative **12** and the 4-(methylsulfonylphenyl) pyrazole **16** showed IC_{50} values in the micromolar range (2.42 and 112 μM, respectively, vs. 1.04 μM of celecoxib **3**). All the other compounds proved to be practically devoid of any COX-1 inhibitory activity. A modest activity or a complete inactivity on the same enzyme had already been found also for compounds **7**–**10** [14,15].

As far as COX-2 inhibitory properties are concerned, only thiophene derivative **17** proved to possess an appreciable activity, with an IC_{50} value of 1.7 μM, which is very close to the one previously found for the cyclopentenyl analogue **7** [14]. Compounds **16** and **22** proved to be slightly active with IC_{50} values lower than 50 μM, whereas the other derivatives proved to be either only scarcely active (**13**, **15**, **21**, **24**) or practically inactive (**11**, **12**, **14**, **18**, **19**, **20**, **23**, **25**).

From the results obtained at the COX-2 level with the new methylsulfonyl derivatives **14**–**25**, it appeared that, among the heteroaromatic rings utilized in constructing the new compounds, only the pyrazolic and the thiophenic ones, as in the cases of **16** and **17**, **22**, respectively, are able to replace, more or less effectively, the cyclopentenic system of compounds of type **6** (Fig. 1). Furthermore, for the thiophenic compounds (**17**, **22**), the activity appears to be linked to the type of oxime-ether side chain, which allows a good interaction with the COX-2 active site only when it is of the unsubstituted benzyl aldoximic type with the *E* configuration, as in compound **17**. The introduction of a methylenedioxy substituent on the aromatic ring of the oximic side chain of **17** causes a dramatic reduction in the inhibitory activity, independently of the type of configuration around its double bond (see **18** and **19**). Incidentally, also thiophene compound **22**, which may be considered as structurally related to compound **19** as far as the *Z*-configuration is concerned, but possesses an oximic side chain which is less bulky, even though substituted with a methyl group on the oximic carbon atom, is still capable of exerting a certain inhibitory action. An influence of the steric factors on the activity results also for pyrazole derivatives **14**–**16** for which only the one less hindered on the oximic side-chain (**16**) possesses a certain activity on both types of COX-enzymes.

As for compounds **11**–**13**, which may be considered as related to the inactive cyclopentenyl derivatives **9** and **10**, since they possess the aminosulfonyl substituent on the non-oximic aromatic ring, it appears that the replacement of the cyclopentene ring of **9**,**10** with the heteroaromatic system (i.e. the pyrazole) present in one of the better known aminosulfonyl COX-2 inhibitors,

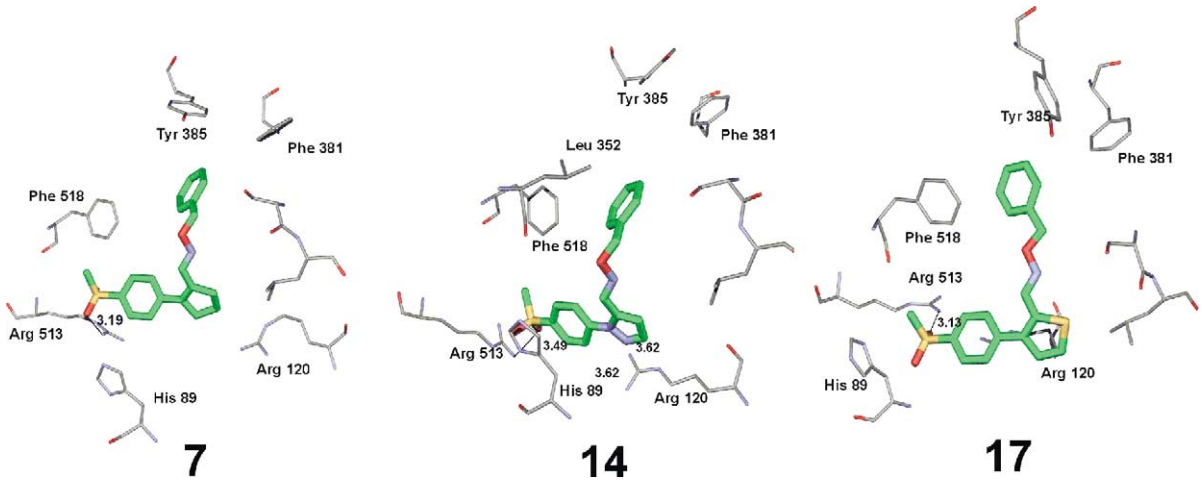


Fig. 7. Compounds **7**, **14** and **17** docked into the catalytic site of COX2. The most important residues are labeled.

celecoxib **3**, arouses a modest inhibitory activity only towards the COX-1 enzyme, and only in the case in which the phenyl ring linked to the oximic side chain presents the methylenedioxy substituent (see **12** vs. **11**).

A possible explanation for the different effects due to the various central cores of the oximic compounds studied, can be found in a study of the docking with the active site of the COX-2 enzyme of the series of benzyloximic derivatives to which most of the active compounds (**7**, IC_{50} 1.9 μM and **17**, IC_{50} 1.7μM) belong.

Fig. 7 indicates that *O*-benzyl oximes with the *E* configuration (**7**, **14**, **17**) interact with the active site of the enzyme in an analogous spatial orientation. It appears that in the case of the more active compounds (**7** and **17**), the enzyme-inhibitor complex is stabilised by an interaction between the sulfonic group and the Arg513 (distance 3.19 and 3.13 Å, respectively) and by a hydrophobic interaction between the benzylic group and the Tyr385; this interaction is strengthened by the Phe381 in the case of **7**, and by the Phe518 in the case of **17**. As regards the inactive compound **14**, the N2 of the pyrazole ring interacts with the Arg120 (N–N 3.62 Å) and this modifies the orientation of the inhibitor in the enzyme active site, thus disturbing the interaction with the Arg513 (3.49 Å) and preventing the hydrophobic stabilisation of the aromatic ring with the Tyr385.

As regards the isoxazole derivative **23** and the *Z*-isomer of pyrazole, which takes part of the mixture of **14** (1:1), the study of docking indicates their inability to fit in the active site of the enzyme without producing important deformations of the molecules.

5. Conclusions

These results indicate that in the class of COX-2 inhibitors of the oxime-ether type, the pentatomic central system plays an important role on its own in determining the biological activity. The results reported here indicate that, among the heteroaromatic systems tested, the thiophene ring is the one which gives results of a certain interest. Furthermore, among the thiophene and pyrazole derivatives, the steric factors related to the size of the oximic side-chain also seems to have a significant effect on the COX inhibition process. As regards the benzyloximic compounds with the *E*-configuration which are active towards COX-2 (**7**, **17**), the docking studies seem to explain the inhibitory properties in terms of particular interactions with specific aminoacidic residues in the catalytic site of the enzyme.

6. Experimental protocols

6.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparison of compounds were taken as paraffin oil mulls or as liquid films on a Unicam Mattson 1000 FT-IR spectrometer. ^{1}H-NMR spectra of all compounds were obtained with a Gemini 200 spectrometer operating at 200 MHz, in a ca. 2% solution of $CDCl_3$. Analytical TLCs were carried out on 0.25 mm layer silica gel plates containing a fluorescent indicator; spots were detected under UV light (254 nm). Column chromatography was performed using 70–230 mesh silica gel. Mass spectra were detected with a Hewlett Packard 5988A spectrometer. Evaporation was made in vacuo (rotating evaporator); the *O*-arylmethyloxyamines to be used for the preparation of the oxime-ethers **11**–**25**, not commercially available, were prepared following the synthetic method described in Ref. [32]. Na_2SO_4 was always used as the drying agent. Elemental analyses were performed in our analytical laboratory and agreed with the theoretical values to within ±0.4%.

*6.1.1. Synthesis of 4-[3-(trifluoromethyl)-1-pyrazolyl]benzenesulfonamide (**27**)*

A suspension of 4-sulfonylamidophenylhydrazine·HCl (2.63 g, 11.8 mmol) in anhydrous EtOH (60 mL) was treated with a solution of 4-ethoxy-1,1,1-trifluoro-3-buten-2-one **26** (2.0 g, 12 mmol) in anhydrous EtOH (10 mL). The resulting solution was stirred under reflux for 4h and cooled at room temperature (r.t.), and the solvent was then evaporated to dryness to yield a crude residue consisting of a mixture of regioisomers **27** and 4-[5-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (**28**) in a ratio of 60:40, which were separated by column chromatography eluting with a 4:6 hexane–AcOEt mixture. **27** (40%): m.p. 177–179 °C ($CHCl_3$); ^{1}H-NMR δ 6.43 (br, 2H, SO_2NH_2), 6.67 and 8.0 (2d, 2H, $J = 2.6$ Hz, H4 and H5 pyrazole), 7.76 and 7.96 ppm (2d, 4H, $J = 8.6$ Hz, $ArSO_2NH_2$); MS $m/z = 291$ (M^+). $C_{10}H_8F_3N_3O_2S$ (C, H, N). **28** (20%): ^{1}H-NMR δ 6.12 (br, 2H, SO_2NH_2), 6.83 and 7.71 (2d, 2H, $J = 1.5$ Hz, H4 and H5 pyrazole), 7.60 and 8.03 ppm (2d, 4H, $J = 8.2$ Hz, $ArSO_2NH_2$).

*6.1.2. Synthesis of 4-[5-formyl-3-(trifluoromethyl)-1-pyrazolyl]benzenesulfonamide (**29**)*

A solution of *n*-BuLi 1.6M in hexane (6.4 mL, 10 mmol) was added dropwise under argon to a stirred and cooled solution (−78 °C) of **27** (1.0 g, 3.4 mmol) and TMEDA (10 mmol) in anhydrous THF (50 mL). The resulting mixture was stirred at 0 °C for 30 min and then cooled to −78 °C and treated with DMF (1.1 mL). After 6 h at r.t., the resulting suspension was quenched with 300 mL of saturated NH_4Cl solution. The aqueous phase was extracted three times with AcOEt, and the combined organic phases were washed with water and brine, dried and evaporated to dryness. The crude residue was subjected to column chromatography eluting with a 95:5 CH_2Cl_2–MeOH mixture to yield pure **29** (38%): m.p. 122–124 °C (Et_2O–hexane); ^{1}H-NMR δ 5.32 (br, 2H, SO_2NH_2), 7.38 (s, 1H, H4 pyrazole), 7.68 and 8.09 (2d, 4H, $J = 8.7$ Hz, $ArSO_2NH_2$), 9.90 ppm (s, 1H, CHO); MS $m/z = 319$ (M^+).

*6.1.3. Synthesis of 4-(5-formyl-3-methyl-1-pyrazolyl)benzenesulfonamide (**31**)*

A cooled (10 °C) solution of 4-sulfonylamidophenylhydrazine hydrochloride (1.0 g, 4.5 mmol) in MeOH (110 mL) was treated with a solution of 1,1-diethoxyacethylacetone **30** (1.20g, 6.4 mmol) in MeOH (30 mL). After the addition was complete, the mixture was refluxed for 1 h and cooled at r.t., and the solid precipitate was filtered off. Evaporation of the solvent yielded **31** as a crude product, which was used in the subsequent reaction without any further purification. **31**(37%); ^{1}H-NMR δ 2.40 (s, 3H, Me), 6.90 (s, 1H, H4 pyrazole), 7.68 and 7.98 (2d, 4H, $J = 9.6$ Hz, Ar), 9.80 ppm (s, 1H, CHO); MS $m/z = 265$ (M^+).

*6.1.4. General procedure for the preparation of 4-{5-[(E)-((arylmethyloxy)imino)methyl]-3-(trifluoromethyl)-1-pyrazolyl}benzenesulfonamides (**11**, **12**) and 4-{5-[(E)-((3,4-methylenedioxyphenylmethyloxy)imino)methyl]-3-(methyl)-1-pyrazolyl}benzenesulfonamide (**13**)*

A two-phase mixture of aldehyde **29** or **31** (1.5 mmol) in $CHCl_3$ (15 mL) and the appropriate *O*-arylmethyloxyamine hydrochloride (1.51 mmol) in H_2O (5 mL), was stirred at r.t. for 16 h. The organic phase was then separated and the aqueous solution was extracted twice with $CHCl_3$. Evaporation of the dried and filtered combined organic extracts afforded a residue consisting almost exclusively of the corresponding *E* oxime-ethers **11**, **12** and **13** which were purified by column chromatography eluting with a 95:5 $CHCl_3$–MeOH mixture, for **11** and **12** and by crystallisation for **13**. (*E*)-**11** (36%): ^{1}H-NMR δ 4.95 (br, 2H, SO_2NH_2), 5.17 (s, 2H, $PhCH_2O$), 7.06 (s, 1H, H4 pyrazole), 7.35–7.68 (m, 5H, Ph), 7.60 and 8.05 (2d, 4H, $J = 8.9$ Hz, $ArSO_2NH_2$), 8.04 ppm (s, 1H, CH=N). (*E*)-**12** (35%): ^{1}H-NMR δ 4.93 (br, 2H, SO_2NH_2), 5.03 (s, 2H, $ArCH_2O$), 5.98 (s, 2H, OCH_2O), 6.81 (m, 3H, Ar), 7.04 (s, 1H, H4 pyrazole), 7.60 and 8.04 (2d, 4H, $J = 8.7$ Hz, Ar-SO_2NH_2), 8.02 ppm (s, 1H, CH=N); MS (FAB) $m/z = 469$ ($M+H^+$). (*E*)-**13** (30%): ^{1}H-NMR δ 2.36 (s, 3H, Me), 5.02 (s, 2H, $ArCH_2O$), 5.88 (s, 2H, OCH_2O), 6.60 (s, 1H, H4 pyrazole), 6.81 (s, 3H, Ar), 7.57 and 7.96 (d, 2H, $J = 7.9$ Hz, $ArSO_2NH_2$), 8.04 ppm (s, 1H, CH=N); MS $m/z = 414$ (M^+).

*6.1.5. Synthesis of 1-[4-(methylthio)phenyl]-pyrazole (**33**)*

A solution of tetramethoxypropane (7.40g, 45 mmol) and 4-methylthiophenylhydrazine·HCl (8.60g, 45.2 mmol) in anhydrous EtOH (70 mL) was refluxed for 2 h. The solvent was evaporated and the residue was suspended in a 5% aqueous solution of NaOH and extracted three times with AcOEt. Combined organic extracts were dried, filtered and evaporated to yield a crude product which was crystallised from hexane to give pure **33** (54%): m.p. 72–73 °C (hexane); ^{1}H-NMR δ 2.51 (s, 3H, SMe), 6.46 (br, 1H, H4 pyrazole), 7.33 and 7.61 (2d, 4H, $J = 8.2$ Hz, Ar), 7.72 (br, 1H, H3 pyrazole), 7.88 ppm (d, 1H, $J = 2.4$ Hz, H5 pyrazole); MS $m/z = 190$ (M^+). $C_{10}H_{10}N_2S$ (C, H, N).

*6.1.6. Synthesis of 1-[4-(methylthio)phenyl]-pyrazole-5-carbaldehyde (**34**)*

A 1.6 M solution of *n*-BuLi in hexane (6.6 mL) was slowly added under argon to a solution of **33** (2.0 g, 10.5 mmol) in dry THF (20 mL). The suspension was stirred mechanically for 2 h at r.t. and then treated with DMF (0.80 mL, 11 mmol). The mixture was stirred for an additional hour, poured into 1 N aqueous HCl (10 mL) and the organic solvent was evaporated. The resulting

aqueous mixture was basified to pH 8 with 1N aqueous NaOH and extracted three times with AcOEt. Combined organic extracts were filtered and evaporated to yield a viscous oil which was subjected to column chromatography eluting with 8:2 hexane–AcOEt mixture to give pure **34** (38%): ^{1}H-NMR δ 2.54 (s, 3H, SCH_3), 7.10 and 7.75 (2d, 2H, $J = 2.0$ Hz, H4 and H3 pyrazole), 7.33–7.44 (m, 4H, Ar), 9.87 ppm (s, 1H, CHO); MS $m/z = 218$ (M^+). $C_{11}H_{10}N_2OS$ (C, H, N).

6.1.7. Synthesis of 1-[4-(methylsulfonyl)phenyl]-pyrazole-5-carbaldehyde (35)

A cooled (0 °C) solution of **34** (1.0g, 4.6 mmol) in a 1:1 THF–MeOH mixture (28 mL), was treated with a solution of oxone (3.4 g, 5.5 mmol) in H_2O (15 mL). After 6h of vigorous stirring at r.t., the mixture was poured into AcOEt and the organic phase was washed with H_2O and brine, filtered and evaporated to afford the crude methylsulfonyl derivative **35** which was purified by crystallisation from *i*-PrOH (41%): m.p. 142–144 °C; ^{1}H-NMR δ 3.12 (s, 3H, SO_2CH_3), 7.20 (d, 1H, $J = 1.3$ Hz, H4 pyrazole), 7.75 and 8.10 (2d, 4H, $J = 8.4$ Hz, Ar), 7.84 (d, 1H, $J = 1.3$ Hz, H3 pyrazole), 9.94 ppm (s, 1H, CHO); MS $m/z = 250$ (M^+). $C_{11}H_{10}N_2O_3S$ (C, H, N).

6.1.8. General procedure for the preparation of 1-[4-(methylsulfonyl)phenyl]-pyrazole-5-carbaldehyde O-arylmethyl- (14, 15) and O-phenyl-oximes (16)

A solution of **35** (0.4 g, 1.6 mmol) and the appropriate arylmethyloxyamine or phenylmethyloxyamine (1.6 mmol), as hydrochlorides, in anhydrous EtOH (20 mL) was heated for 2 h at reflux temperature, the solvent was then evaporated and the residue was crystallised from the appropriate solvent to yield pure oxime-ethers as single *E*-isomers **15**,**16** or a 1:1 *E*/*Z* mixture **14**. (*E*/*Z*)-**14** (58%): ^{1}H-NMR (*E* isomer) δ 3.09 (s, 3H, SO_2CH_3), 5.16 (s, 2H, OCH_2Ph), 6.83 and 7.73 (2d, 2H, $J = 1.6$ Hz, H4 and H3 pyrazole), 7.31–7.43 (m, 5H, Ph), 7.64 and 8.03 (2d, 4H, $J = 8.6$ Hz, *Ar*SO_2CH_3), 8.11 ppm (s, 1H, CH=N). ^{1}H-NMR (*Z* isomer) δ 3.11 (s, 3H, SO_2CH_3), 5.31 (s, 2H, OCH_2Ph), 7.31 and 7.76 (2d, 2H, $J = 1.6$ Hz, H4 and H3 pyrazole), 7.32–7.43 (m, 6H, Ph and CH=N), 7.69 (d, 2H, $J = 8.6$ Hz, *Ar*-SO_2CH_3), 8.09 (d, 2H, $J = 8.6$ Hz, *Ar*SO_2CH_3). For the *E*/*Z* mixture of **14**: MS $m/z = 355$ (M^+). (*E*)-**15** (49%): ^{1}H-NMR δ 3.09 (s, 3H, SO_2CH_3), 5.04 (s, 2H, OCH_2Ph), 5.97 (s, 2H, OCH_2O), 6.80–7.25 (m, 4H, *Ar*CH_2O and H4 pyrazole), 7.64 and 8.05 (2d, 4H, $J =$ 8.6 Hz, $ArSO_2$), 7.74 (d, 1H, $J = 1.8$ Hz, H3 pyrazole), 8.09 ppm (s, 1H, CH=N). (*E*)-**16** (21%): ^{1}H-NMR δ 3.12 (s, 3H, SO_2CH_3), 6.98 and 7.81 (2d, 2H, $J = 1.8$ Hz, H4 and H3 pyrazole), 7.07–7.43 (m, 5H, Ph), 7.74 and 8.13 (2d, 4H, $J = 8.4$ Hz, $ArSO_2$), 8.41 ppm (s, 1H, CH=N).

6.1.9. Synthesis of 3-[4-(methylthio)phenyl]thiophene (36)

Compound **36** was prepared as reported previously [27]. A mixture of 3-bromothiophene (5.15 g, 25 mmol) and 4-methylthiophenylboronic acid (4.12 g, 24.5 mmol), in a 1:1 toluene–EtOH mixture (80 mL) and aqueous 2 M Na_2CO_3 (18 mL, 36 mmol) was treated at r.t. with $Pd(PPh_3)_4$ (0.4 g) and then refluxed under argon with stirring for 12 h. Evaporation of the organic phase gave an aqueous residue which was taken up in AcOEt. The organic phase was washed with H_2O, filtered and evaporated to afford a crude residue which was crystallised from EtOH to yield pure **36** (49%): ^{1}H-NMR δ 2.48 (s, 3H, SMe), 7.18–7.53 (m, 7H, Ar).

6.1.10. Synthesis of 3-[4-(methylthio)phenyl]thiophene-2-carbaldehyde (37)

A cooled (0 °C) and stirred mixture of DMF (1.65 mL, 21.2 mmol) and anhydrous CH_2Cl_2 (30 mL) was treated dropwise with $POCl_3$ (10 mL, 11 mmol). After 1 h at 0 °C, a solution of **36** (1.45 g, 7.0 mmol) in anhydrous CH_2Cl_2 (20 mL) was added. After 2h at r.t., the mixture was treated with ice and the pH was adjusted to 5 with solid AcONa. The aqueous phase was extracted with Et_2O and the organic extracts were dried and evaporated to yield an oily residue which was purified by column chromatography eluting with a 1:9 petroleum ether–AcOEt mixture to give pure **37** (60%): ^{1}H-NMR δ 2.52 (s, 3H, SMe), 7.16 and 7.71 (2d, 2H, $J = 4.9$ Hz, H4 and H5 thiophene), 7.33 (s, 4H, Ar), 9.83 ppm (s, 1H, CHO). $C_{12}H_{10}OS_2$ (C, H, N).

6.1.11. 3-[4-(Methylsulfonyl)phenyl]thiophene-2-carbaldehyde (38)

Compound **38** was synthesized from **37** following the same procedure described above for the preparation of **35**. Compound **38** (71%): m.p. 152–154 °C (EtOH); ^{1}H-NMR δ 3.12 (s, 3H, SO_2CH_3), 7.25 and 7.81 (2d, 2H, $J = 4.9$ Hz, H4 and H5 thiophene), 7.68 and 8.07 (2d, 4H, $J = 8.0$ Hz, Ar), 9.86 ppm (s, 1H, CHO). $C_{12}H_{10}O_3S_2$ (C, H, N).

6.1.12. General procedure for the synthesis of 3-[4-(methylsulfonyl)phenyl]thiophene-2-carbaldehyde O-arylmethyl oximes (17–19)

Oxime-ethers **17**–**19** were synthesized from **38** and the appropriate arylmethyloxyamine hydrochloride following the same procedure described above for the preparation of **14**–**16**. While **17** was purified by crystallisation of the crude reaction product, **18** and **19** were separated by column chromatography. (*E*)-**17** (43%): m.p. 137–139 °C (EtOH); ^{1}H-NMR δ 3.08 (s, 3H, SO_2CH_3), 5.18 (s, 2H, PhCH_2), 7.13 (d, 1H, $J = 5.1$ Hz, H4 thiophene), 7.07–7.43 (m, 5H, Ph), 7.37–7.62 (m, 6H, *Ph*CH_2 and H5 thiophene), 7.78 and 8.01 (2d, 4H, $J = 8.4$ Hz, *Ar*SO_2CH_3), 8.21 ppm (s, 1H, CH=N).

(*E*)–**18** (34%): ^{1}H-NMR δ 3.08 (s, 3H, SO_2CH_3), 5.04 (s, 2H, ArCH_2), 5.93 (s, 2H, OCH_2O), 6.80–7.54 (m, 7H, H4 and H5 thiophene and $ArCH_2$ and $ArSO_2$), 7.95 (d, 2H, $J = 8.0$ Hz, $ArSO_2CH_3$), 8.15 ppm (s, 1H, CH=N). (*Z*)-**19** (36%): ^{1}H-NMR δ 3.08 (s, 3H, SO_2CH_3), 5.20 (s, 2H, ArCH_2), 5.91 (s, 2H, OCH_2O), 6.80–7.11 (m, 4H, H4 thiophene and $ArCH_2$), 7.49–7.60 (m, 3H, H5 thiophene and $ArSO_2$), 7.56 (s, 1H, CH=N), 7.98 ppm (d, 2H, $J = 8.0$ Hz, $ArSO_2CH_3$).

*6.1.13. Synthesis of 2-acetyl-3[4-(methylthio)phenyl]thiophene (**39**)*

A cooled (0 °C) and stirred mixture of **36** (1.0 g, 4.8 mmol) and AcCl (0.38 g, 4.8 mmol) in anhydrous C_6H_6 (20 mL) was treated dropwise with $SnCl_4$ (1.26 g, 4.84 mmol). After 2h at r.t., the mixture was quenched with 5% aqueous HCl (10 mL). The organic phase was separated, washed with water, dried and evaporated to give a residue which was crystallised from hexane-AcOEt to yield pure **39** (35%): m.p.: 79–82 °C;^{1}H-NMR δ 2.20 (s, 3H, CH_3CO), 2.53 (s, 3H, SCH_3), 7.05 and 7.54 (2d, 2H, $J = 5.1$ Hz, H4 and H5 thiophene), 7.31 (s, 4H, Ar); MS $m/z = 248$ (M^+). $C_{13}H_{12}OS_2$ (C, H).

*6.1.14. Synthesis of 2-acetyl-3[4-(methylsulfonyl)phenyl]thiophene (**40**)*

Compound **40** was synthesized from **39** following the same procedure described above for the preparation of **35**. Compound **40** (40%): m.p.: 141–143 °C (EtOH); ^{1}H-NMR δ 2.34 (s, 3H, CH_3CO), 3.12 (s, 3H, SO_2CH_3), 7.08 (d, 1H, $J = 4.9$ Hz, H4 thiophene), 7.58–7.63 (m, 3H, Ar and H5 thiophene), 8.0 ppm (d, 2H, $J = 7.8$ Hz, Ar). $C_{13}H_{12}O_3S_2$ (C, H).

*6.1.15. General procedure for the preparation of (Z)-2-acetyl-3[4-(methylsulfonyl)phenyl] thiophene O-arylmethyl- (**20, 21**) and O-phenyl-oximes (**22**)*

Compounds **20**–**22** were synthesized from **40** and the appropriate arylmethyloxyamine hydrochlorides (for the preparation of **20** and **21**) or phenoxyamine hydrochloride (for the preparation of **22**) following the same procedure described above for the preparation of **14**–**16**.

(*Z*)-**20** (45%): ^{1}H-NMR δ 1.92 (s, 3H, CH_3), 3.08 (s, 3H, SO_2CH_3), 5.18 (s, 2H, ArCH_2), 7.08 (d, 1H, $J = 5.1$ Hz, H4 thiophene), 7.30–7.36 (m, 6H, H5 thiophene and $PhCH_2$), 7.55 and 7.90 ppm (2d, 4H, $J = 8.2$ Hz, $ArSO_2$); (*Z*)-**21** (47%):^{1}H-NMR δ 1.90 (s, 3H, CH_3), 3.09 (s, 3H, SO_2CH_3), 5.06 (s, 2H, ArCH_2), 5.98 (s, 2H, OCH_2O), 6.81–6.87 (m, 3H, $ArCH_2$), 7.09 and 7.32 (2d, 2H, $J = 5.3$ Hz, H4 and H5 thiophene), 7.58 and 7.95 ppm (2d, 4H, $J = 8.2$ Hz, $ArSO_2$). (*Z*)-**22** (50%): ^{1}H-NMR δ 2.15 (s, 3H, CH_3), 3.09 (s, 3H, SO_2CH_3), 7.01–7.32 (m, 6H, H4 thiophene and Ph), 7.42 (d, 1H, $J = 5.1$ Hz, H5 thiophene), 7.62 and 8.0 ppm (2d, 4H, $J = 8.1$ Hz, $ArSO_2CH_3$).

*6.1.16. Synthesis of 4-(methylthio)benzaldehyde oxime (**41**)*

A solution of hydroxylamine hydrochloride (5.48 g, 78.8 mmol) in H_2O (30 mL) was added dropwise to a cooled (0 °C) and stirred solution of NaOH (4.89 g, 122 mmol) in H_2O (30 mL). The resulting mixture was then treated with 4-thiomethylbenzaldehyde (10 g, 150 mmol) in EtOH (60 mL). The reaction was followed by TLC until the disappearance of the aldehyde. The mixture was concentrated and the pH was adjusted to 4 with 10% aqueous HCl. The aqueous phase was extracted with Et_2O. Combined organic extracts were dried and evaporated to afford a crude residue which was purified by crystallisation from hexane. Compound **41** (46%): m.p. 115–117 °C; IR ν_{max} (nujol) $\nu_{C=N}$ 1591; ^{1}H-NMR δ 2.51 (s, 3H, SCH_3), 7.24 and 7.50 (2d, 4H, $J = 8.4$ Hz, Ar), 8.10 ppm (s, 1H, CH=N). C_8H_9NOS (C, H, N).

*6.1.17. Synthesis of 4-(methylsulfonyl)benzhydroximic acid chloride (**42**)*

Oxone (6.5 g, 11 mmol) was added at 0 °C to a stirred solution of **41** (1 g, 6 mmol) in a 0.5 N HCl (0.25 mL) aqueous solution and DMF (10 mL), and the resulting mixture was stirred at r.t. for 30 min. The reaction mixture was poured into cold water (25 mL) and extracted with $CHCl_3$. The organic layers were washed with brine, dried and evaporated. Removal of $CHCl_3$ gave a crude residue which was purified by crystallisation from AcOEt. Compound **42** (39%): m.p. 183–185 °C; ^{1}H-NMR δ 3.08 (s, 3H, SO_2CH_3), 7.98 and 8.06 ppm (2d, 4H, $J = 8.6$ Hz, Ar). $C_8H_8NO_3SCl$ (C, H, N).

*6.1.18. Synthesis of 3-[4-(methylsulfonyl)phenyl]isoxazole-4-carbaldehyde (**43**)*

A solution of **42** (500 mg, 2.14 mmol) was added dropwise to a stirred solution of 3-dimethylaminoacrolein (0.11 mL, 1.1 mmol) and triethylamine (0.29 mL, 2.1 mmol) in THF (13 mL). The reaction mixture was stirred for 2 h and then evaporated. The crude residue was taken up with $CHCl_3$. The organic layer was washed with brine, filtered and evaporated, and the residue was purified by column chromatography using $CHCl_3$ as the eluant. Compound **43** (45%): m.p. 135–138 °C (EtOH); ^{1}H-NMR δ 3.11 (s, 3H, SO_2CH_3), 8.09 (s, 4H, Ar), 9.17 (s, 1H, H5 isoxazole), 10.08 ppm (s, 1H, CHO); MS $m/z = 251$ (M^+). $C_{11}H_9NO_4S$ (C, H, N).

*6.1.19. General procedure for the preparation of 3-[4-(methylsulfonyl)phenyl]isoxazole-4-carbaldehyde O-arylmethyl- (**23, 24**) and O-phenyl-oximes (**25**)*

Oximes **23**–**25** were synthesized from **43** and the appropriate aryloxyamine hydrochloride following the same procedure described above for **14**–**16**. (*Z*)-**23** (45%): ^{1}H-NMR δ 3.11 (s, 3H, SO_2CH_3), 5.34 (s, 2H, PhCH_2), 7.28 (s, 1H, CH=N), 7.40 (s, 5H, $PhCH_2$), 7.82 and 8.11 (2d, 4H, $J = 8.1$ Hz, $ArSO_2$), 9.37 ppm (s, 1H,

H5 isoxazole). (*E*/*Z*)-**24** (50%): ^{1}H-NMR (*Z* isomer) δ 3.12 (s, 3H, SO_2CH_3), 5.22 (s, 2H, ArCH_2), 5.98 (s, 2H, OCH_2O), 6.80–6.90 (m, 3H, *Ar*CH_2), 7.27 (s, 1H, CH=N), 7.82 and 8.11 (2d, 4H, $J = 8.2$ Hz, *Ar*SO_2), 9.35 ppm (s, 1H, H5 isoxazole). ^{1}H-NMR (*E* isomer) δ 3.10 (s, 3H, SO_2CH_3), 4.99 (s, 2H, ArCH_2), 5.98 (s, 2H, OCH_2O), 6.80–6.90 (m, 3H, *Ar*CH_2), 7.82 and 8.11 (2d, 4H, $J = 8.2$ Hz, *Ar*SO_2), 8.05 (s, 1H, CH=N), 8.75 ppm (s, 1H, H5 isoxazole). (*E*/*Z*)-**25** (56%): ^{1}H-NMR (*Z* isomer) δ 3.13 (s, 3H, SO_2CH_3), 7.13–7.43 (m, 5H, PhO), 7.53 (s, 1H, CH=N), 7.87 and 8.15 (2d, 4H, $J =$ 8.6 Hz, $ArSO_2$), 9.59 ppm (s, 1H, H5 isoxazole). ^{1}H-NMR (*E* isomer) δ 3.13 (s, 3H, SO_2CH_3), 7.13–7.40 (m, 5H, PhO), 7.84 and 8.12 (2d, 4H, $J = 8.6$ Hz, $ArSO_2$), 8.37 (s, 1H, CH=N), 8.93 ppm (s, 1H, H5 isoxazole).

6.2. Biopharmacological methods

6.2.1. Enzyme assays

All compounds **11**–**25** were tested following the procedure described previously [14,15] in intact cell assays to verify their capacity to inhibit PGE2 production, considered as an index of activity on COX-1 and COX-2 enzymes. For the COX-1 assay, 1.5×10^6 resting U937 human cells were incubated with the test compounds for 30 minutes in the presence of 10 μM arachidonic acid. Tubes were then centrifuged and the PGE2 content in the supernatant was measured by a commercial immunoenzymatic assay (Amersham). The COX-2 assay was performed in accordance with the method described by Mitchell et al. [33] with minor modifications as suggested by Grossman et al. [34]. Murine J774.2 cells were pretreated for 1 h with 300 μM aspirin to inactivate endogenous constitutive COX-1, and were then stimulated with LPS to induce COX-2 expression. After overnight incubation, cells were treated for 45 min with the different test compounds. Supernatants were then collected and PGE2 was measured as described above. All compounds were tested in duplicate. For each product a stock solution was prepared in DMSO at a concentration of 100 mM. Linear regression was used to calculate IC_{50} values. SEM were lower than 10% in all experiments.

6.3. Docking studies

The study of COX2-inhibitor complexes was performed using the DOCK program of Kuntz and coworkers [35]. This program was designed in order to calculate the different ways in which a ligand can dock into the active site of a protein and to select the best ones. The starting-point of the calculation are the crystallographic structures of murine COX2 complexed with SC-558, a selective COX2 inhibitor. The DOCK program stoichastically generates a large number of possible interaction conformations and assigns them a score which indicates how good the interaction is. During this search the protein molecule is maintained rigid, while some torsion angles of the ligand are allowed to rotate [36,37].

The COX2-inhibitor complexes were subsequently minimised, using MacroModel program [38] to solve any conflicts due to the rigidity of aminoacids of the site during docking. The procedure used to refine the complex involved 100 ps of molecular dynamics with a constraint of 0,02 kcal on the protein backbone while the ligand and the residues of the binding site were free. The simulation temperature was 300 K, the timestep 1.5 fs. the molecular dynamics simulation was followed by a minimization in which no kind of restraint was applied. The calculation was performed using AMBER forcefield with distance dependent electrostatic treatment and dielectric constant 4.0.

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