European Journal of Medicinal Chemistry 54 (2012) 823–833

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Indole based cyclooxygenase inhibitors: Synthesis, biological evaluation, docking and NMR screening

Mónica S. Estevão [a], Luísa C.R. Carvalho [a], Marisa Freitas [b], Ana Gomes [b], Aldino Viegas [a], João Manso [a], Stefan Erhardt [a], Eduarda Fernandes [b], Eurico J. Cabrita [a], M. Manuel B. Marques [a,*]

[a] REQUIMTE-CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Monte de Caparica, 2829-516 Caparica, Portugal
[b] REQUIMTE, Departamento de Química-Física, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal

ARTICLE INFO

Article history:
Received 25 February 2012
Received in revised form
19 June 2012
Accepted 21 June 2012
Available online 29 June 2012

Keywords:
Cyclooxygenase-2 inhibitors
Indole-based library
Anti-inflammatory activity
NMR screening

ABSTRACT

The close structural similarity between the two cyclooxygenase (COXs) isoforms and the absence of selective inhibitors without side effects continues to stimulate the development of novel approaches towards selective anti-inflammatory drugs. In the present study a small library of new indolic compounds involving two different substitutions patterns at the indole scaffold was synthesized. In order to establish a relation between the spatial distribution of known functional groups related with inhibitory activity, two substitution patterns were explored: one with substituents at N-1, C-3, C-5 positions and another at C-2, C-3 and C5 positions. Accordingly, indole positions C-5, C-3 and N-1 were substituted with: sulfonamide or methylsulfone at C-5, *p*-halo-benzyl group at C-3, and an alkyl chain with a trifluoromethyl group at N-1. Alternatively, a *p*-halo-benzyl group was introduced at C-2, leaving the indolic nitrogen free. Inhibitory studies were performed and the activity results obtained against both COXs isoforms were rationalized based on docking and NMR studies. Docking studies show that dialkyation at C-2 and C-3 favors a binding with an orientation similar to that of the known selective inhibitor SC-558. From the tested compounds, this substitution pattern is correlated with the highest inhibitory activity and selectivity: 70% COX-2 inhibition at 50 μM, and low COX-1 inhibition (18 ± 9%). Additionally, Saturation Transfer Difference NMR experiments reveal different interaction patterns with both COXs isoforms that may be related with different orientations of the sulfonamide group in the binding pocket. Despite the moderated inhibitory activities found, this study represents an innovative approach towards COXs inhibitory activity rationalization and to the design of anti-inflammatory drugs.

1. Introduction

The use of aspirin for the treatment of inflammation, fever and pain, dates back to 1897. Since then many non-steroidal anti-inflammatory drugs (NSAIDs) were developed for the treatment of inflammation, such as ibuprofen (**1**), flurbiprofen (**2**), indomethacin (**3**) and diclofenac (**4**) (Fig. 1 A). Cyclooxygenase (COX) was only identified in 1971, as the enzyme involved in the conversion of the arachidonic acid to prostaglandins [1]. NSAIDs were found to inhibit prostaglandin synthesis through COX inhibition and became widely accepted as therapeutic drugs for the treatment of rheumatoid osteoarthritis, arthritis, and pain. Nevertheless, the side effects of these drugs represent a major drawback of its chronic use, involving gastric and intestinal toxicity as well as renal insufficiency. The cause of undesirable side effects of inflammatory drugs was unraveled by the identification of two COX isoforms [2]: COX-1 (performs desirable roles in the protection of gastrointestinal wall) and COX-2 (responsible for various inflammatory diseases [3], promotion of cancer [4] and induction of multi-drug resistance [5]). Both COX isoforms have similar active sites that differ in the presence of a side pocket in COX-2 located above the Arg-120/Tyr-355/Glu-524 constriction [6]. In COX-2 this side pocket is surrounded by Val-523 (Ile-523 in COX-1), and at the bottom of the pocket an Arg-513 (His-513 in most COX-1). The lack of COXs selectivity by the traditional NSAIDs stimulated the development of selective COX-2 inhibitors, taking advantage of these small structural differences between COX-1 and COX-2. A new class of COX-2 inhibitors was developed, the diarylheterocycle derivatives designated as "coxibs", such as celecoxib (**5**), rofecoxib (**6**), valdecoxib (**7**), etoricoxib (**8**) and the coxib-like structure SC-558 (**9**) (Fig. 1 B) [7].

Although coxib compounds benefit from the lack of gastrointestinal toxicity, this risk/benefit balance was recently considered negative following the finding of increased incidence of adverse

* Corresponding author.
E-mail address: mmbmarques@dq.fct.unl.pt (M.M.B. Marques).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.06.040

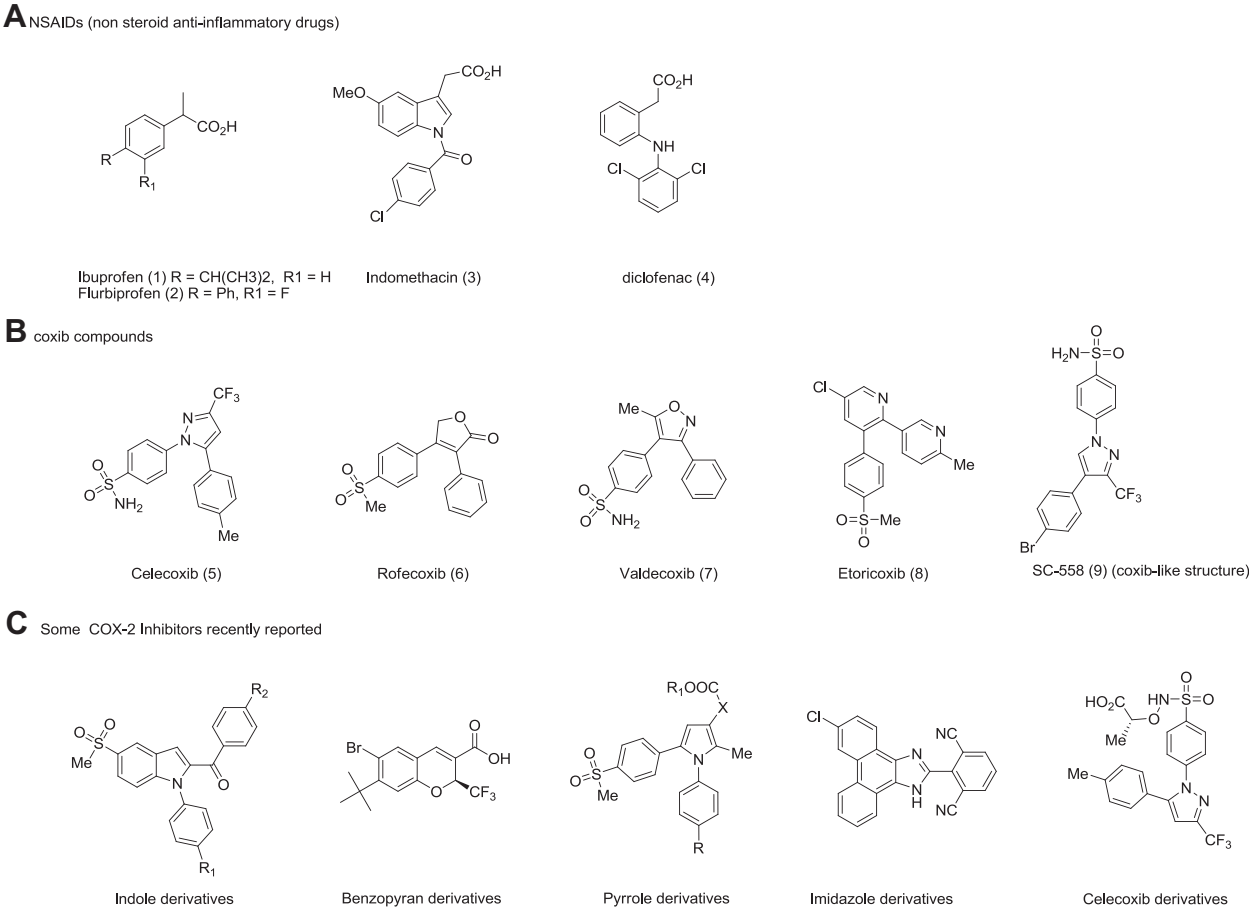


Fig. 1. Chemical structure of some NSAIDs and selective COX-2 inhibitors.

cardiovascular events [8]. Consequently rofecoxib (**6**) was removed from the market due to its cardiac toxicity, limiting the use of these drugs for the treatment of inflammatory diseases. Thus, the discovery of new COX-2 selective inhibitors without cardiac adverse effects, are deemed necessary in the future of anti-inflammatory therapies.

For the development of a new class of inhibitors the choice of a scaffold and of its substitution pattern are of almost importance. When considering the vast amount of work published concerning specific COX-2 inhibitors, several scaffolds have been investigated, such as benzopyran [9], pyrrole [10], imidazole [11], thiazolidine [12], pyrazole (celecoxib) [13] and indole (in particular indomethacin derivatives) [14] (Fig. 1C). From these, the indole ring is considered a privileged structure and an attractive scaffold for drug discovery. Structural diversity can easily be achieved *via* ring substitution and consequently diverse biological activities are associated with indole-derived drug-like molecules, including COX-1 and COX-2 inhibition.

X-ray crystal structures have been crucial to reveal the key interactions between COX-2 residues and important inhibitor functional groups. The crystal structure of mouse COX-2 with the selective inhibitor SC-558 (**9**) (Fig. 1 B) [6a,15], reveals an overall difference between the size and shape of the COX-2 and COX-1 active sites [16]. Several interactions between COX-2 and functional groups present in SC-558 (**9**) and indomethacin (**3**) were identified and are resumed in Fig. 2.

While SC-558 (**9**) penetrates deep in the selective pocket, traditional NSAIDs do not use this pocket [17,18]. Indomethacin (**3**), a non-selective inhibitor of COXs, has an interaction with Arg120 through its carboxylate group at the hydrophobic channel entrance (Fig. 2 b) [15]. This salt bridge is responsible for the traditional NSAIDs anchor for both COX-1 and COX-2, though restricting their selectivity due to limitation of flexibility. Some authors claim that a proper substitution at the C-5 and/or C-6 of the indole ring can increase the steric hindrance with Ile523 (COX-1), while establishing stronger hydrogen bond with Arg513 at the selective pocket of COX-2, and consequently increasing selectivity [14b].

In the present study, the initial strategy envisaged the preparation of an indole library involving substitution of the ring in order to generate a "Y shape" structure similar to indomethacin (**3**) (Fig. 3). Two substitution patterns were considered (A and B, Fig. 3), concerning the inclusion of 3 substituents to afford 3 different interaction regions and relying on modification at Region III. Following detailed analysis of the reported inhibitor structures and theoretical studies, the pattern included substitutions at positions: C-3 (Region I) by a *p*-halobenzyl group in order to fill the hydrophobic channel while exploring other possible hydrophobic interactions with residues at this cavity; C-5 position of the indole ring (Region II). According to previous reports, a methylsulfone [15b] or a sulfonamide seem to be crucial for selectivity, and [6a,16a,15] comparing to indomethacin (**3**) (methoxy group at C-5) would allow an extension of this ring, long enough to fill properly the selective pocket and allowing interaction with key residues (His90, Gln192 and Arg513). Additionally, we planned modification on the nitrogen atom (Region III, pattern A) by an allyl chain carrying a trifluoromethyl group at the end of the chain, on the expectation of getting higher mobility and chain extension. The N-substitution

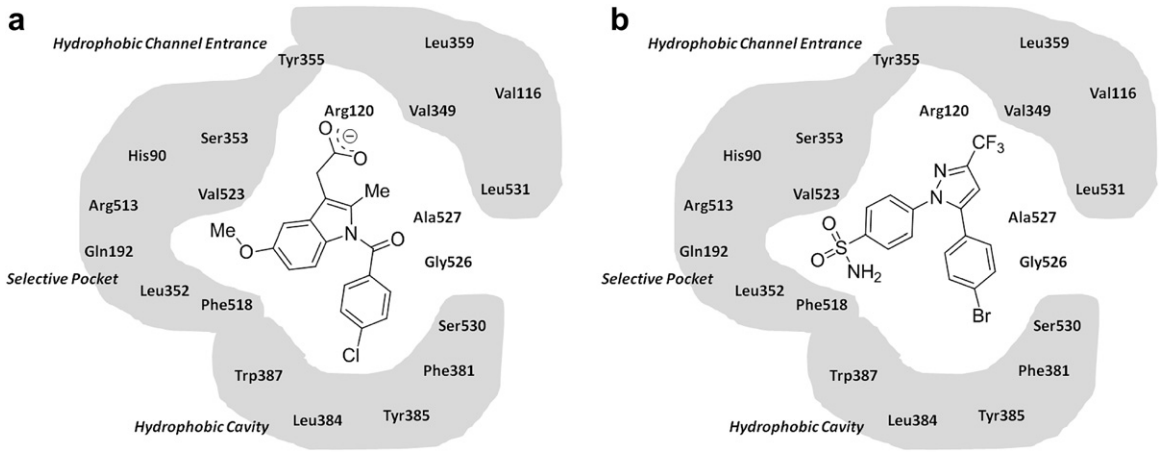


Fig. 2. Schematic representation of SC-558 (COX-2 selective inhibitor) binding to COX-2 (a) and indomethacin (non-selective inhibitor) (b).

intended to increase the selectivity for COX-2 by establishing unfavourable interactions with Arg120 (important for COX-1 inhibition), while enhancing the steric interaction with Ile523 (COX-1). Finally, C-2 substitution was also considered (Region III, pattern B), since we envisaged that a *p*-halobenzyl chain at C-2 would have the ideal shape for filling the hydrophobic pocket.

In order to establish a relation between the spatial distribution of the previously described functional groups, related with COX inhibition, activity studies were performed against both COXs isoforms and the results were rationalized based on docking and NMR studies.

2. Results and discussion

2.1. Chemistry

The first library (Pattern A, Fig. 3) was divided in two groups, those carrying a methylsulfone at C-5 and those with a sulfonamide group at the same position. The library synthesis involved three main steps: the introduction of a sulfonamide/methylsulfone at C-5, for which no direct sulfonylation has yet been reported for the indole nucleus; regioselective alkylation at C-3 and alkylation of N-1. The preparation of sulfones is generally achieved by oxidation of the corresponding sulfides/sulfoxides or *via* displacement of sodium arenesulfinate with a suitable alkyl halide [19].

The 1*H*-indole-5-sulfonamide (**10**) [20] was prepared according to a reported procedure [20], consisting on the chlorosulfonation of 1-acetylindoline (**11**), followed by oxidation and amide hydrolysis in 33% (overall yield). The 5-methylsulfone derivatives **12**, we used 5-iodo-1*H*-indole (**13a**) applying a method reported by Ma and Zhu [21], for the synthesis of aryl sulfones *via* L-proline-promoted CuI-catalyzed coupling reaction of aryl halides with sulfinic acid salts. Thus 5-(methylsulfonyl)-1H-indole (**12**) was prepared in 90% yield (based on recovered 5-iodo-1*H*-indole) (Scheme 1).

The second library (pattern B, Fig. 3) relied on the exploration of substitution at C-2 position of indole which was achieved *via* dialkyaltion of compounds **10** and **12**. Next step consisted on the regioselective alkylation at C-3 of **10** and **12** with different p-halobenzyl bromides.

Applying a one-pot procedure [22], compounds **14a**–**f** were obtained, in moderate yields, by treatment of **10** or **12** [23] with zinc triflate and the corresponding *p*-halobenzyl bromide, in the presence of Hünig's base and tetrabutylammonium iodide. Several attempts were made to improve the yield of compounds **14a**–**f**, such as solvent and reaction temperature.

In order to proceed to N-alkylation, the sulfonamide derivatives would require additional protection-deprotection steps of the amine group. Celecoxib (**5**) and valdecoxib (**7**) possess a sulfonamide group while rofecoxib (**6**) and etericoxib (**8**) have a methylsulfone group (Fig. 1). Thus, at this stage we decided to focus on the methylsulfone derivatives for further functionalization at N-1. To perform the N-alkylation of compounds **14a**–**c**, different approaches were tested such as the classic Mitsunobu reaction conditions [24], using the commercial alcohol (*E*)-4,4,4-trifluorobut-2-en-1-ol, and also the modified Mitsunobu reaction which has been reported for N-alkylation of indoles, involving the use of a phosphorane derivative - (cyanomethylene)

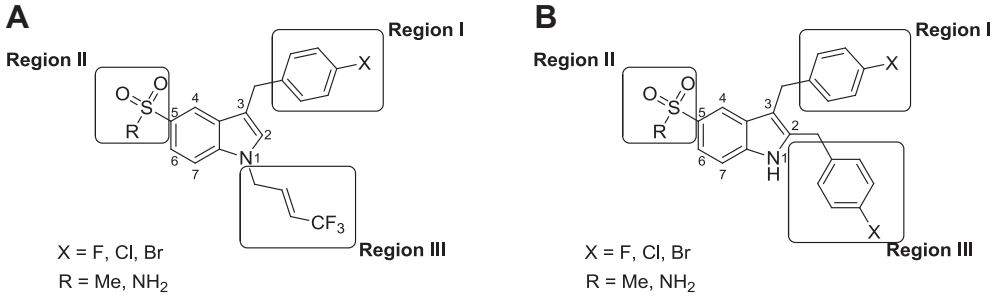
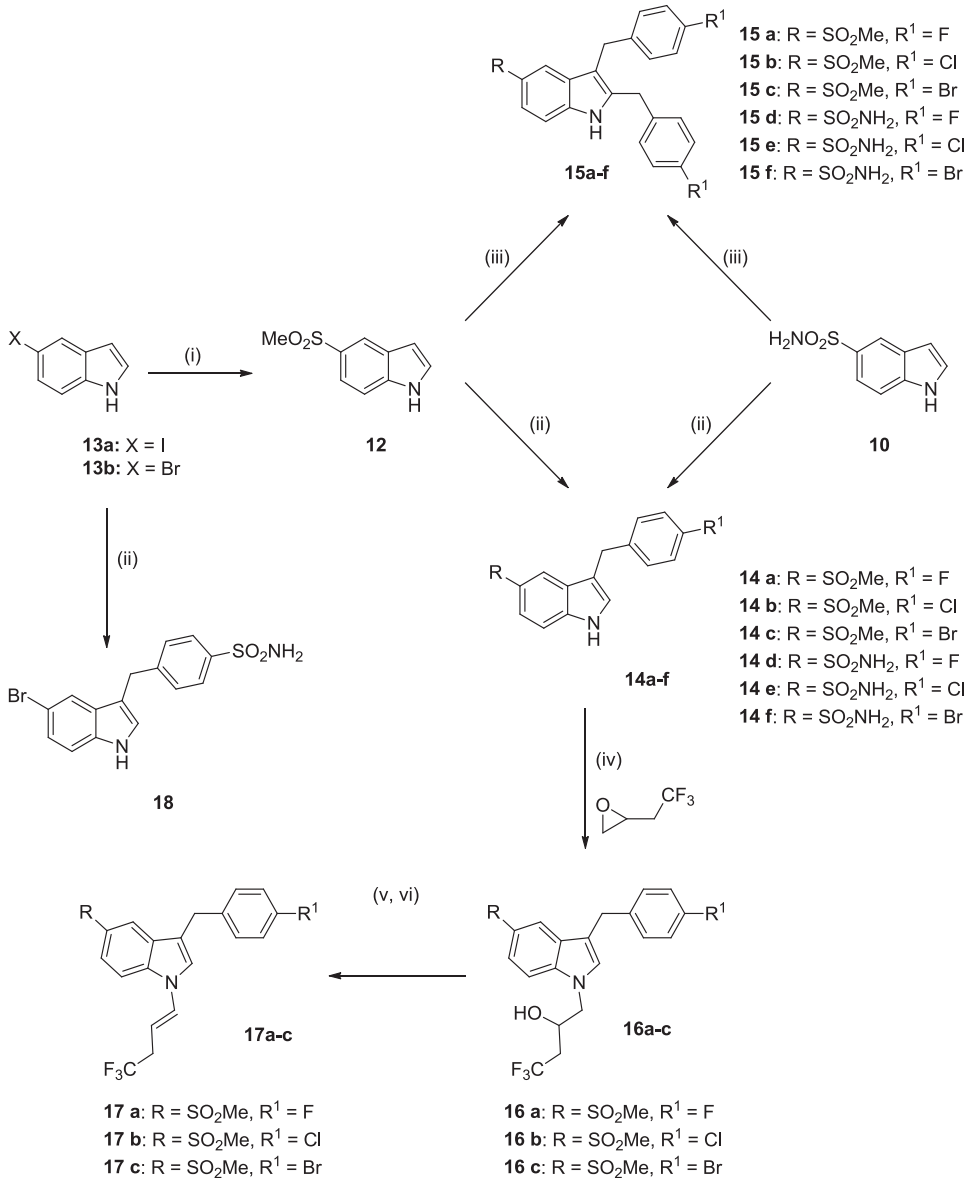


Fig. 3. Proposed substitution pattern for the indole library.



Scheme 1. Synthesis of the indole library: (i) CuI(I), L-proline, $MeSO_2Na$, DMSO, 80 °C, 3 days, 90%; (ii) *p*-halobenzyl bromide (F, Cl, Br) (1 equiv.), $Zn(OTf)_2$, TBAI, DIPEA, toluene, 50 °C, overnight; (iii) *p*-halobenzyl bromide (F, Cl, Br) (3 equiv.), $Zn(OTf)_2$, TBAI, DIPEA, toluene, 70 °C, overnight; (iv) NaH, THF, 0 °C, 45 min, (2,2,2-trifluoro-ethyl)-oxirane, reflux, 2 h; (v) TEA, MsCl, THF, 0 °C, room temperature, 1 h 30 min; (vi) DBU, THF, reflux, 30 min.

trimethylphosphorane [25]. However, these approaches failed to work with indole derivatives **14a**–**c**. Next approach consisted on the introduction of the alkyl chain *via* epoxide opening, using the commercial (2,2,2-trifluoro-ethyl)-oxirane, that afforded compounds **16a**–**c**, followed by mesylation and elimination by treatment with DBU (Scheme 1). Nevertheless, the products isolated, compounds **17a**–**c**, presented a double bond conjugated with the indole ring instead of the desired allylic chain (see Fig. 3).

For the preparation of pattern B (compounds **15a**–**f**), compounds **10** and **12** were dialkylated using the same procedure used to prepare **14a**–**f**, but using an excess of 3 equivalents of the different *p*-halobenzyl bromides. Formation of compounds **15a**–**f** was also observed in small amount during mono-alkylation reaction of **10** and **12**. Inversion of the substituents at indole scaffold was also investigated, and compound **18** was prepared in 79% yield. The commercial 5-bromo-1*H*-indole (**13b**) was alkylated at C-3, by applying the same procedure as described above for compounds **14a**–**f**, using (4-aminosulfonyl)benzyl bromide.

2.2. Inhibition of COX-1 and COX-2— biological assays

Inhibition of COX-1 and COX-2 by the synthesized compounds is expressed as the percent inhibition of control COX-1 or COX-2 activity as displayed in Table 1. The known COX inhibitors indomethacin (**3**) and celecoxib (**5**) were used as positive controls. The inhibitory activity of the studied compounds was first tested at 50 μM, however since some compounds were not active at this concentration the inhibitory activity was also tested at 100 μM. The solubility of the studied compounds, at the tested concentrations, was analyzed before and, as reported in Table 1, some compounds were insoluble at both concentrations and others were only insoluble at 100 μM. Some compounds, like **16b** or **17a** (50 μM, COX-1) displayed inhibition values less than 10%, which we consider to be not significant. Nevertheless, most of the obtained results were well above this percentage. Additionally we observed that some compounds such as **14d**, inhibit both isoforms at the higher concentration to a lesser extent than at the lower. In fact, in

Table 1
Percent inhibition of control COX-1 or COX-2 activity by the 19 synthesized compounds, determined by EIA. Each value represents mean ± SEM of at least 4 experiments performed in duplicate.

Compound	COX-1	COX-2	COX-1	COX-2
	100 μM		50 μM	
14a	INS	INS	INS	INS
14b	28 ± 7	24 ± 16	NA	NA
14c	13 ± 7	26 ± 16	NA	9 ± 5
14d	31 ± 8	20 ± 10	40 ± 9	32 ± 9
14e	19 ± 5	31 ± 8	21 ± 4	32 ± 11
14f	INS	INS	19 ± 7	23 ± 5
15a	NA	NA	–	–
15b	42 ± 1	NA	25 ± 4	24 ± 4
15c	NA	NA	NA	NA
15d	INS	INS	18 ± 9	67 ± 5
15e	INS	INS	41 ± 6	46 ± 1
15f	84 ± 5	NA	35 ± 8	8 ± 4
16a	41 ± 10	9 ± 2	14 ± 11	16 ± 7
16b	NA	34 ± 8	8 ± 4	20 ± 8
16c	20 ± 6	15 ± 9	12 ± 8	23 ± 5
17a	48 ± 11	18 ± 28	8 ± 5	12 ± 7
17b	26 ± 15	23 ± 22	11 ± 10	NA
17c	10 ± 6	19 ± 10	20 ± 9.9	21 ± 6
18	33 ± 8	27 ± 2	11 ± 6	22 ± 1
Positive Controls	10 μM		1 μM	
Celecoxib	–	75 ± 8	NA	–
Indomethacin	–	–	46 ± 5	78 ± 3

NA No activity was found. INS Insoluble compound.

some compounds with low activities, it was difficult to achieve a concentration-dependent effect, due to the high values of SEM obtained.

From the C-5, C-3 disubstituted compounds (**14a**–**f**) only the sulfonamides derivatives **14d**–**f** showed to be active at 50 μM, although not selective. N-1 alkylation, in compounds **16a**–**c** and **17a**–**c**, did not result in an improved activity/selectivity. At 100 μM, compounds **16a** and **17a** (with a methylsulfone at C-5) showed inhibitory activity against COX-1 (41 ± 10% and 48 ± 11%, respectively). Compounds **15a**–**f** allowed investigating the C-5, C-3, C-2 substitution pattern. Compound **15f** showed the strongest inhibition of COX-1 enzyme's activity at the concentration of 100 μM (84 ± 5%). In addition, **15d** resulted in 67 ± 6% (50 μM) of COX-2 inhibition and 18.2 ± 8.5% of COX-1 inhibition, revealing its relative selectivity to inhibit COX-2.

Additionally, compound **18** was evaluated for understanding the relevance of the sulfonamide position within the structure and orientation of the molecule in the binding pocket. However, besides a lower inhibition, no selectivity was found for this compound. Due to solubility issues, some compounds were not tested within the same range of concentrations. Indomethacin (**3**) (1 μM) inhibited COX-1 (46 ± 5%) and COX-2 (78 ± 4%) and the selective COX-2 inhibitor celecoxib (**5**) (10 μM) only inhibited this isoenzyme (75 ± 8%).

2.3. Docking study

All molecules were docked to the active site of both COX-1 and COX-2 using the docking program Autodock4. The docking study suggested that most studied compounds are more active for COX-1 which is in agreement with the observed inhibitory activity. Indeed, compounds **14a**–**f** were all found to be COX-1 selective. They docked inside the binding pocket for both COXs isoforms. Overall, compounds **14a**–**f** bind less strongly to COX-2 than to COX-1. This can be explained by the fact that they use the existing space in COX-1 binding pocket, with the sulfonamide/methylsulfone close to Arg120 and Tyr355, similar to the binding of the acid group in indomethacin (**3**), while the benzyl chain binds in the hydrophobic pocket. The selectivity is reversed for the dialkylated compounds **15a**–**f** when compared to the mono-alkylated ones **14a**–**f**. Compounds **15a**–**f** were the most promising compounds by computational studies, which is almost consistent with the COX-2 inhibition evaluation assays. Due to the increased flexibility introduced by the benzyl group the 2,3-dibenzyl substituted indoles show a potential higher affinity to COX-2. According to the docking results, compounds **15d**–**f**, with a sulfonamide at C-5, are more active than the corresponding methylsulfones **15a**–**c**, what is in accordance with the experimental data. From the **15** series, the computational study indicated that the most promising compounds are the fluorinated compounds **15a** and **15d**, with a methylsulfone and a sulfonamide, respectively. In both cases these compounds strongly bind to COX-2, but not COX-1. Also they do bind in the same orientation as the selective SC-558 inhibitor

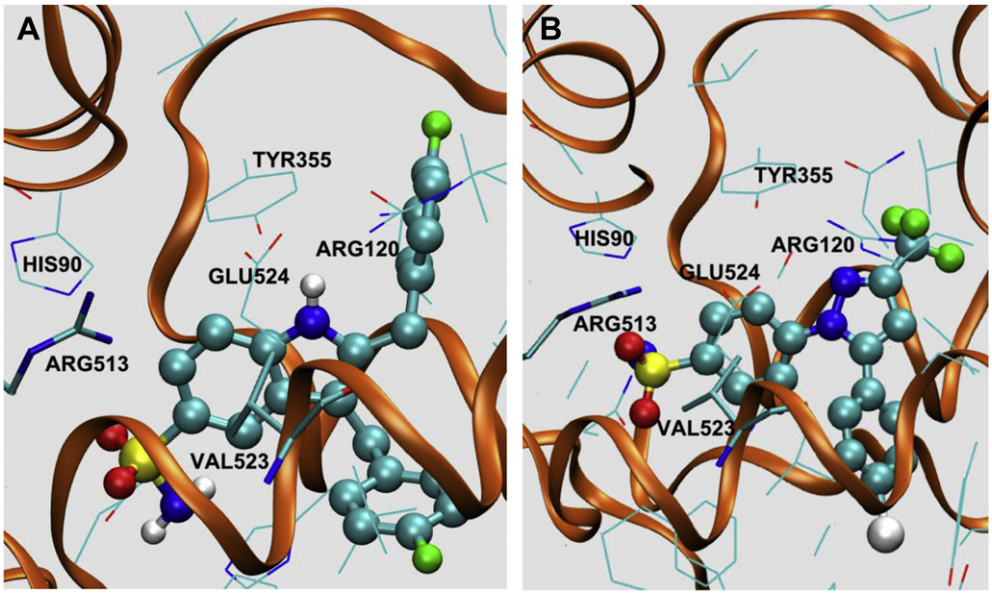


Fig. 4. Docking of compound **15d** (A) and selective inhibitor SC-558 (**9**) (B) in COX-2.

(**9**), such as **15d** in which the sulfonamide group binds to the side pocket next to Val523 and might establish hydrogen bonding *via* the oxygens with Arg513 (Fig. 4 A).

Although compounds **15** only binds inside the COX-2 binding pocket, some very strong interactions in the region of the entrance channel can occur with COX-1. Thus, their strong binding close to the entrance channel suggests that they have the potential to block COX-1 effectively. Moreover, it was found that the binding affinities for COX-2 do not strongly depend on the type of halogen present in the aromatic ring. Nevertheless the same was not observed in the docking for COX-1, where stronger binding is observed for heavier halogens.

For compounds **16a**–**c** and **17a**–**c**, the biological screening results show only low activity. Additionally the docking predicts that they do not fit inside the binding pocket, due to their bulkiness.

2.4. NMR study

In order to have a deeper insight about the mode of interaction of the mono-alkylated (**14a**–**f**) and the di-alkylated compounds (**15a**–**f**) with both enzyme isoforms we have performed saturation-transfer difference NMR (STD-NMR) experiments [26]. We have recently demonstrated that STD-NMR can be effectively used to characterize the binding of the anti-inflammatory drugs ibuprofen, diclofenac and ketorolac to COX-1 and COX-2 [27]. The STD-NMR experiment is based on the nuclear Overhauser effect and in the observation of the ligand resonance signals [26,28]. This technique can be used not only for screening ligands with dissociation constants K_D ranging from ca. 10^{-8} to 10^{-3} M but also to provide insight about the moieties of the ligand that are most important for binding, since it is expected that the regions of the ligand having the strongest contact to the protein will show the most intense STD NMR signals [28].

Due to solubility reasons and considering the percentage of inhibition presented in Table 1 and the results of the docking studies, compounds **14d** and **15d** were chosen for this study. **14d** is a mono-alkylated compound with a higher selectivity for COX-1 and **15d** is, from the di-alkylated series, the one that shows the highest selectivity to COX-2, binding well inside the COX-2 pocket, according to the docking studies. The STD-NMR results are presented in Fig. 5. STD signals were observed for both compounds and for both enzyme isoforms. The fact that STD responses were obtained for all cases is in accordance with **14d** and **15d** binding reversibly to COX-1 and COX-2, as well as with the inhibition and docking results.

When considering the interaction of **14d**, from the comparison of the STD spectra with the reference spectra it can be seen that not all protons gave identical STD responses. For this compound, the most intense STD signals originate from the aryl moiety and the response of protons 6 and 7 of the indole ring is higher in COX-1 than in COX-2. These results may be indicative of a more extended and uniform interaction of **14d** with COX-1 than with COX-2. When considering **15d**, the most intense STD signals originate also from the aryl moieties. In COX-1 there is an appreciable decrease in STD response from proton 4 of the indole ring. This may evidence that the sulfonamide moiety in **15d** may be more important to promote the association with COX-2 than with COX-1 and partly responsible for the selectivity observed in the inhibition and docking studies. Also, contrary to **14d**, it is clear that protons 6 and 7 from the indole ring receive less saturation from the protein, what can be indicative of a different conformation in the binding site. These results are in good agreement with the inhibition and docking studies and they provide an experimental validation of the methodology. This also represents the first NMR study on COXs selectivity with non-commercial compounds.

From the library of 19 new indole based compounds synthesized, the biological tests revealed that the presence of a sulfonamide (**14d**–**f**) was more favorable for interaction with both COXs, being more active than the corresponding methylsulfones (**14a**–**c**). These sulfonamides **14d**–**f** when docked have a similar binding mode as the selective inhibitor SC-558 (**9**), but the docking studies predicted a slight preference for COX-1 binding. Indeed **14d** demonstrated a percent of inhibition higher for COX-1 (40 ± 9) than for COX-2 (32 ± 10) at 50 μM. This observation was further supported by the STD-NMR study that demonstrated that compound **14d**, despite interacting with both COX isoforms, displays a more extended STD response for COX-1, confirming a superior interaction with this enzyme.

These results indicate that the selective inhibitory activity associated with the introduction of a sulfonamide group is

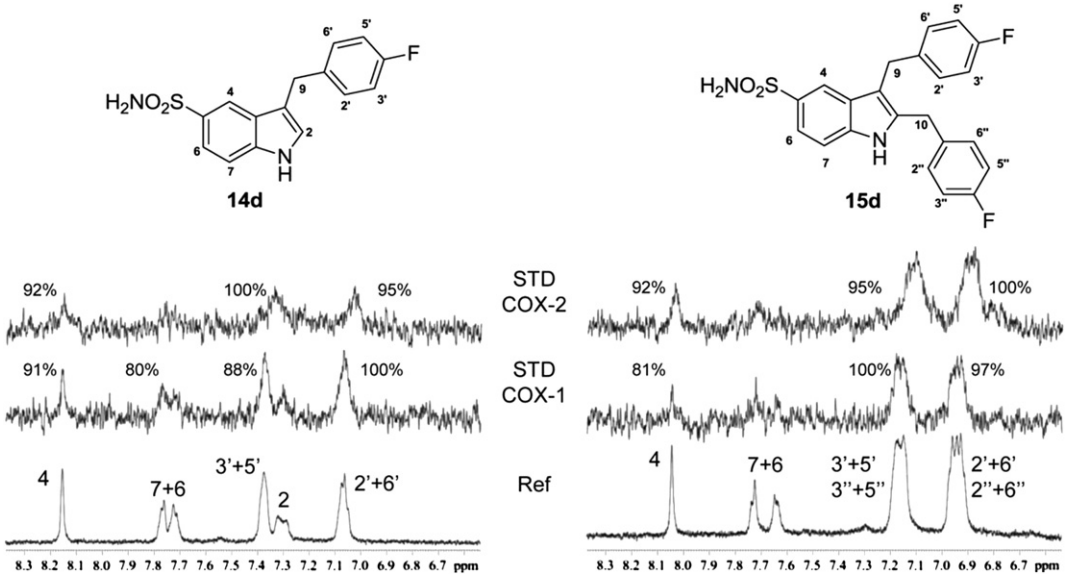


Fig. 5. Expansions of the aromatic region of the STD-NMR spectra of compounds **14d** and **15d** with COX-1 and COX-2 (bottom: reference spectra of **14d** and **15d** with spectral assignments). STD intensities relative to the corresponding reference intensities are shown in each signal as percentage.

dependent on the overall geometry of the molecule. The orientation of the sulfonamide group towards the selective pocket is determined by the shape of the molecule. Thus, the Y shape is necessary for the correct positioning of the groups in the pocket.

With compounds **16a**–**c** and **17a**–**c** (pattern A) we tested if a trifluoromethyl allylic chain would introduce flexibility to the Y shape, when compared to the benzoyl group present in indomethacin (**3**). The presence of the CF_3 group was thought to establish favorable interactions with the key residue Arg120 in COX-2. The biological evaluation of these compounds resulted only in low activity and no selectivity for COX-2. The docking results show that these compounds are too bulky to enter the binding pocket, however interactions can be established and a partial blocking can occur, what would explain the low activity found. This shows that the bulkiness of the substituents is important for binding. If the substituents are too bulky the molecule will not enter the binding site. However once in the binding pocket they need to be bulky enough to orient the sulfonamide to the selective pocket.

The second library prepared (pattern B) envisaged exploration of C-2 substitution with a group that has enough volume to fill the hydrophobic pocket interacting with residues in this region. This substitution pattern (C-2, C-3 and C-5– compounds **15a**–**f**) also leaves the indolic NH free allowing a possible stabilization of the binding with the enzyme *via* hydrogen bonding, which was shown by docking to be important to interact with the hydroxyl group of with Tyr355 (Fig. 4).

As also shown by the calculations, **15a**–**f** bind to COX-2 in a similar manner to that of SC-558 (**9**), and different from indomethacin (**3**). Furthermore, these studies indicate that the volume, nature and shape of the benzyl group at C-2 are the most appropriate for stable binding to hydrophobic pocket. The activity results for compounds **15a**–**f**, confirm that substitution at C-2 with a benzyl group possessing a halogen at the *para* position, is important. The results obtained also indicate that the halogen atom at the aromatic side chain is a key substituent. Compound **15d** was found to be the most promising showing 70% COX-2 inhibition at 50 μM, demonstrating that fluorine seems to be crucial. **15d** demonstrated to have 70% inhibition of COX-2 (67 ± 5.2%), close to celecoxib (**5**) but in a higher concentration, and almost no inhibition for COX-1 (18 ± 8.5%) at 50 μM.

The lack of activity of the methylsulfones **15a** and **15c** is most probably related to the lower electronic density of the sulfone oxygen atoms when compared with the corresponding sulphonamides (**15d**–**f**).

STD-NMR studies performed with **15d** highlighted that the sulfonamide group is indeed important to promote the interaction with COX-2 rather than with COX-1, as evidenced by the close proximity of the indolic proton H4 to COX-2, supporting the observed selectivity in the inhibition evaluation.

3. Conclusion

In conclusion, the synthesized indole library was evaluated against both COX-1 and COX-2 enzymes and compound **15d** was found to be active at 50 μM, showing 70% COX-2 inhibition and low COX-1 inhibition (18 ± 9%). The docking studies performed were in agreement with the experimental data, suggesting that compound **15d** should be the most potent selective COX-2 inhibitor of this series, with a similar orientation inside the binding pocket to the known selective inhibitor SC-558 (**9**). Our results indicate that the introduction of a third substituent on the central indole ring is mandatory to improve selectivity and activity for COX-2. Also, the presence of a sulfonamide at C-5 when combined with dialkyation at C-2 and C-3 favors a binding with an orientation similar to that of the known selective inhibitor SC-558 (**9**). However, the correct orientation of the sulfonamide group to achieve selectivity (towards the selective pocket) results from the correct combination of substituents in a Y shape molecule, since the sulfonamide group interacts preferentially with Arg120. A bulky group at C-2, interacting preferentially with Arg120 (a fluorine atom), prevents the interaction with sulfonamide and directs it to the selective pocket.

These findings are supported by the STD-NMR experiments where different interaction patterns with both COXs isoforms can be related with different orientations of the sulfonamide group in the binding pocket. This study represents an innovative route involving NMR, molecular modeling, synthesis and biological screening towards the understanding of COXs inhibition and rationalization of anti-inflammatory drugs.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points were determined on a Reichert Thermovar apparatus and are uncorrected. ^{1}H- and ^{13}C NMR spectra were recorded in $(CD_3)_2CO$ on a Bruker ARX 400 spectrometer at 400 and 100.62 MHz respectively. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane (TMS). The coupling constants (*J*) are reported in Hertz (Hz). High resolution mass spectra were recorded on an AutoSpecQ spectrometer. IR spectra were run on an FT PerkinElmer 683 instrument, with absorption frequencies expressed in reciprocal centimeters. The progress of all reaction was monitored by Thin-layer chromatography, which was performed on Merck silica gel 60 F254 plates. Flash column chromatography was carried out on Merck silica gel 60 (230–400 mesh). Anhydrous solvents were dried as described [29] and freshly distilled. All the tested compounds possess apurity of at least 95% as determined by HPLC. Analytical HPLC was run on a Merck Hitachi system consisting of an L-7100 pump, Rheodyne type injector, a D-7000 interface and an L-7450 diode array spectrometric detector,equipped with LiChrospher®100 RP-18 column. Eluent system was: 20% A (H_2O/TFA pH 2.5), 80% B (MeOH) to 10% A, 90% B; flow rate = 1 mL/min.

Compound **10** was synthesized according to a previously described methodology [20].

4.1.2. 5-(Methylsulfonyl)-1H-indole (*12*)

To a solution of 5-iodoindole (1 g, 4.11 mmol) in dry DMSO (8.2 mL) were added sodium methanesulfinate (546 mg, 5.35 mmol), copper iodide (157 mg, 0.82 mmol) and L-proline (189 mg, 1.64 mmol) in a sealed tube under argon. The mixture was stirred at 80 °C for 3 days. The reaction was quenched with saturated aqueous NH_4Cl, diluted with distilled water and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate anhydrous, filtered and concentrated. The crude was purified by silica flash chromatography.White solid; 92% yield; mp 166–170 °C; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ ppm 10.79 (s, 1H), 8.22 (s, 1H), 7.68–7.63 (m, 2H), 7.56–7.55 (m, 1H), 6.71–6.70 (m, 1H), 3.06 (s, 3H).

4.1.3. General procedure for the preparation of 3-p-halo-benzylated indoles derivatives (*14a–f* and *18*) and 2,3-p-halo-benzylated indoles derivatives (*15a–f*)*24*

Procedure A: To a solution of 1*H*-indole-5-sulfonamide/5-(methylsulfonyl)-1*H*-indole (2 equiv.), zinc triflate (1.2 equiv.) and tetrabutylammonium iodide (1 equiv.) in dry toluene (ca. 3 mL per mmol of indole) was added *N*,*N*-diisopropylethylamine (2.2 equiv.)

under argon. The reaction mixture was heated at 50 °C for 30 min, followed by addition of the *p*-halo-benzyl bromide (1 equiv.). The mixture was stirred overnight at 50 °C under argon. The reaction was quenched with saturated aqueous NH_4Cl, diluted with distilled water and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate anhydrous, filtered and concentrated. The samples were further purified with silica flash chromatography then PTLC.

Procedure B: To a solution of 1*H*-indole-5-sulfonamide/5-(methylsulfonyl)-1*H*-indole (1 equiv.), zinc triflate (1.2 equiv.) and tetrabutylammonium iodide (1 equiv.) in dry toluene (*ca*. 3 mL *per* mmol of indole) was added *N,N*-diisopropylethylamine (2.2 equiv.) under argon. The reaction mixture was heated at 70 °C for 30 min, followed by addition of the *p*-halo-benzyl bromide (3 equiv.). The mixture was stirred overnight at 70 °C under argon. The reaction was quenched with saturated aqueous NH_4Cl, diluted with distilled water and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate anhydrous, filtered and concentrated. The samples were further purified with silica flash chromatography then PTLC.

4.1.4. 3-(4-Fluorobenzyl)-5-(methylsulfonyl)-1H-indole (**14a**)

Procedure A: White solid; 47% yield; mp 136-136 °C. IR (KBr) ν 3370, 2928, 1503, 1283, 1132, 1090, 762 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ ppm 10.65 (s, 1H), 8.13 (s, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.42–7.28 (m, 3H), 7.03 (t, J = 8.7, 8.7 Hz, 2H), 4.17 (s, 2H), 3.04 (s, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ ppm 162.1 (d, J = 240 Hz, CF), 139.9 (C), 138.0 (C), 132.6 (C), 131.0 (2× CH), 127.7 (C), 126.7 (CH), 120.7 (CH), 120.1 (CH), 117.3 (C), 115.8 (CH), 115.6 (CH), 112.9 (CH), 45.1 (CH_3), 30.8 (CH_2); HR-ESIMS m/z 304.0802 $[M + H]^+$ (calcd for $C_{16}H_{15}FNO_2S$ 304.0808).

4.1.5. 3-(4-Chlorobenzyl)-5-(methylsulfonyl)-1H-indole (**14b**)

Procedure A: White solid; 41% yield; mp 155–157 °C; IR (KBr) ν 3308, 3007, 2926, 1488, 1281, 1141, 1130, 1094, 763 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ ppm 10.67 (s, 1H), 8.12 (s, 1H), 7.66 (d, J = 8.6 Hz, 1H), 7.62 (d, J = 8.6 Hz, 1H), 7.38 (s, 1H), 7.34 (d, J = 8.5 Hz, 2H), 7.30 (d, J = 8.5 Hz, 2H), 4.19 (s, 2H), 3.03 (s, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ ppm 141.0 (C), 139.9 (C), 132.7 (C), 131.0 (2× CH), 129.1 (2× CH), 127.6 (C), 126.8 (CH), 120.8 (CH and C), 120.1 (CH), 116.8 (C), 112.9 (CH), 45.1 (CH_3), 30.9 (CH_2); HR-ESIMS m/z 320.0507 $[M + H]^+$ (calcd for $C_{16}H_{15}ClNO_2S$ 320.0512).

4.1.6. 3-(4-Bromobenzyl)-5-(methylsulfonyl)-1H-indole (**14c**)

Procedure A: White solid; 50% yield; mp 169–171 °C; IR (KBr) ν 3307, 3024, 2926, 1486, 1278, 1140, 1095, 1010, 764 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ ppm 10.69 (s, 1H), 8.11 (s, 1H), 7.66 (d, J = 8.3 Hz, 1H), 7.61 (d, J = 8.3 Hz, 1H), 7.45 (d, J = 7.6 Hz, 2H), 7.38 (s, 1H), 7.29 (d, J = 7.6 Hz, 2H), 4.18 (s, 2H), 3.03 (s, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ ppm 141.4 (C), 139.8 (C), 132.7 (C), 132.1 (2× CH), 131.4 (2× CH), 127.6 (C), 126.8 (CH), 120.8 (CH), 120.0 (CH and C), 116.8 (C), 112.9 (CH), 45.1 (CH_3), 31.0 (CH_2); HR-ESIMS m/z 364.0001 $[M + H]^+$ (calcd for $C_{16}H_{15}BrNO_2S$ 364.0007).

4.1.7. 3-(4-Fluorobenzyl)-1H-indole-5-sulfonamide (**14d**)

Procedure A: White solid; 39% yield; mp 153–155 °C. IR (KBr) ν 3379, 3291, 3251, 1506, 1313, 1147, 1096 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ ppm 10.53 (s, 1H), 8.09 (s, 1H), 7.66 (d, J = 8.3 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.41–7.25 (m, 3H), 7.02 (t, J = 8.0, 8.0 Hz, 2H), 6.33 (s, 2H), 4.14 (s, 2H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ ppm 162.1 (d, J = 240 Hz, CF), 139.1 (C), 138.2 (C), 135.7 (C), 131.0 (2× CH), 127.3 (C), 126.2 (CH), 120.2 (CH), 118.5 (CH), 116.9 (C), 115.7 (2× CH), 112.5 (CH), 30.8 (CH_2); HR-ESIMS m/z 305.0755 $[M + H]^+$ (calcd for $C_{15}H_{14}FN_2O_2S$ 305.0760).

4.1.8. 3-(4-Chlorobenzyl)-1H-indole-5-sulfonamide (**14e**)

Procedure A: White solid; 46% yield; mp 205–206 °C; IR (KBr) ν 3402, 3336, 3267, 1488, 1324, 1154, 1090, 1016, 806 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ ppm 10.55 (s, 1H), 8.08 (s, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.38–7.23 (m, 5H), 6.32 (s, 2H), 4.15 (s, 2H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ ppm 141.1 (C), 139.1 (C), 135.8 (C), 131.9 (C), 131.0 (2× CH), 129.1 (2× CH), 127.3 (C), 126.3 (CH), 120.2 (CH), 118.5 (CH), 116.4 (C), 112.5 (CH), 32.0 (CH_2); HR-ESIMS m/z 321.0459 $[M + H]^+$ (calcd for $C_{15}H_{14}ClN_2O_2S$ 321.0465).

4.1.9. 3-(4-Bromobenzyl)-1H-indole-5-sulfonamide (**14f**)

Procedure A: White solid; 42% yield; mp 220–221 °C. IR (KBr) ν 3392, 3331, 3266, 1485, 1323, 1153, 1099, 1010, 803 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ ppm 10.57 (s, 1H), 8.08 (s, 1H), 7.66 (d, J = 8.5 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.44 (d, J = 8.1 Hz, 2H), 7.34 (s, 1H), 7.26 (d, J = 8.1 Hz, 2H), 6.33 (s, 2H), 4.14 (s, 2H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ ppm 141.7 (C), 139.1 (C), 135.8 (C), 132.1 (2× CH), 131.4 (2× CH), 127.3 (C), 126.4 (CH), 120.2 (CH), 119.4 (C), 118.5 (CH), 116.3 (C), 112.5 (CH), 32.0 (CH_2); HR-ESIMS m/z 364.9954 $[M + H]^+$ (calcd for $C_{15}H_{14}BrN_2O_2S$ 364.9959).

4.1.10. 4-((5-Bromo-1H-indol-3-yl)methyl)benzenesulfonamide (**18**)

Procedure A: The reaction was performed at room temperature. White solid; Yield 79%; mp 157–159 °C; IR (KBr) ν 3401, 2923, 1462, 1325, 1158, 1095, 882 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ ppm 10.36 (s, 1H), 7.80 (d, J = 7.4 Hz, 2H), 7.62 (s, 1H), 7.48 (d, J = 7.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 1H), 7.29 (s, 1H), 7.21 (d, J = 8.4 Hz, 1H), 6.49 (s, 2H), 4.19 (s, 2H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ ppm 146.9 (C), 142.7 (C), 136.5 (C), 130.0 (C), 129.7 (2× CH), 127.0 (2× CH), 125.8 (CH), 124.9 (CH), 121.8 (CH), 114.3 (C), 114.1 (CH), 112.5 (C), 31.6 (CH_2); HR-ESIMS m/z 364.9954 $[M + H]^+$ (calcd for $C_{15}H_{14}BrN_2O_2S$ 364.9959).

4.1.11. 2,3-Bis(4-fluorobenzyl)-5-(methylsulfonyl)-1H-indole (**15a**)

Procedure A: White solid; 9% yield; mp 165–168 °C; IR (KBr) ν 3320, 3227, 2929, 1508, 1291, 1222, 1140 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ ppm 10.61 (s, 1H), 8.02 (s, 1H), 7.60 (d, J = 8.5 Hz, 1H), 7.50 (d, J = 8.5 Hz, 1H), 7.29–7.18 (m, 4H), 7.09–6.89 (m, 4H), 4.24 (s, 4H), 3.01 (s, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ ppm 139.3 (C), 138.8 (C), 138.2 (C), 135.9 (C), 132.9 (C), 131.2 (2× CH), 130.8 (2× CH), 129.0 (C), 120.8 (C), 120.5 (CH), 120.2 (C), 119.7 (CH), 115.9 (4× CH), 113.0 (C), 112.2 (CH), 45.2 (CH_3), 32.0 (CH_2), under the solvent peak (CH_2); HR-ESIMS m/z 412.1177 $[M + H]^+$ (calcd for $C_{23}H_{20}F_2NO_2S$ 412.1183).

4.1.12. 2,3-Bis(4-chlorobenzyl)-5-(methylsulfonyl)-1H-indole (**15b**)

Procedure A: White solid; 7% yield; mp 210–211 °C; IR (KBr) ν 3310, 2918, 1491, 1290, 1142, 1113, 765 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ ppm 10.61 (s, 1H), 8.03 (s, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.51 (d, J = 8.4 Hz, 1H), 7.35–7.12 (m, 8H), 4.25 (s, 4H), 3.01 (s, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ ppm 141.1 (C), 139.2 (C), 138.7 (C), 138.5 (C), 132.9 (C), 132.6 (C), 131.9 (C), 131.2 (2× CH), 130.8 (2× CH), 129.3 (2× CH), 129.1 (2× CH), 128.9 (C), 120.6 (CH), 119.6 (CH), 112.8 (C), 112.2 (CH), 45.2 (CH_3), 32.1 (CH_2), under the solvent peak (CH_2); HR-ESIMS m/z 444.0586 $[M + H]^+$ (calcd for $C_{23}H_{20}Cl_2NO_2S$ 444.0592).

4.1.13. 2,3-Bis(4-bromobenzyl)-5-(methylsulfonyl)-1H-indole (**15c**)

Procedure A: White solid; 15% yield; mp 189–193 °C; IR (KBr) ν 3316, 2922, 1488, 1289, 1142, 1114, 1072, 1011, 765 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ ppm 10.77 (s, 1H), 8.03 (s, 1H), 7.60 (d, J = 8.5 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.42 (d, J = 8.3 Hz, 2H), 7.38 (d, J = 8.3 Hz, 2H), 7.20–7.13 (m, 4H), 4.23 (s, 2H), 4.23 (s, 2H), 3.01

(s, 3H); ^{13}C NMR (100 MHz, *(CD$_3$)$_2$CO*) δ ppm 141.6 (C), 139.2 (2× C), 138.4 (C), 132.9 (C), 132.3 (2× CH), 132.1 (2× CH), 131.5 (2× CH), 131.2 (2× CH), 128.9 (C), 120.5 (CH and C), 119.9 (C), 119.5 (CH), 112.6 (C), 112.2 (CH), 45.1 (CH$_3$), 32.1 (CH$_2$), under the solvent peak (CH$_2$); HR-ESIMS *m/z* 531.9576 [M + H]$^+$ (calcd for $C_{23}H_{20}Br_2NO_2S$ 531.9581).

*4.1.14. 2,3-Bis(4-fluorobenzyl)-1H-indole-5-sulfonamide (**15d**)*

Procedure A: White solid; 11% yield; mp 183–185 °C; IR (KBr) ν 3396, 3267, 3038, 2926, 1507, 1327, 1217, 1154 cm^{-1}; ^{1}H NMR (400 MHz, *(CD$_3$)$_2$CO*) δ ppm 10.46 (s, 1H), 8.01 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.22 (m, 4H), 7.08–6.91 (m, 4H), 6.29 (s, 2H), 4.22 (s, 2H), 4.20 (s, 2H); ^{13}C NMR (100 MHz, *(CD$_3$)$_2$CO*) δ ppm 162.4 (d, *J* = 240 Hz, CF), 162.0 (d, *J* = 239 Hz, CF), 138.3 (CH and C), 138.2 (C), 135.9 (CH and C), 131.2 (2× CH), 130.7 (2× CH), 128.6 (C), 119.9 (CH), 118.1 (CH), 115.8 (4× CH), 112.6 (C), 111.8 (CH), under the solvent peak (2× CH$_2$); HR-ESIMS *m/z* 413.1130 [M + H]$^+$ (calcd for $C_{22}H_{18}F_2N_2O_2S$ 413.1135).

*4.1.15. 2,3-Bis(4-chlorobenzyl)-1H-indole-5-sulfonamide (**15e**)*

Procedure A: White solid; 15% yield; mp 172–173 °C; IR (KBr) ν 3317, 3066, 2909, 1703, 1489, 1317, 1156, 1014, 800 cm^{-1}; ^{1}H NMR (400 MHz, *(CD$_3$)$_2$CO*) δ ppm 10.48 (s, 1H), 8.01 (s, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.33–7.12 (m, 8H), 6.31 (s, 2H), 4.23 (s, 2H), 4.20 (s, 2H); ^{13}C NMR (100 MHz, *(CD$_3$)$_2$CO*) δ ppm 141.2 (C), 138.8 (C), 138.4 (C), 137.9 (C), 135.9 (C), 132.5 (CH), 131.8 (C), 131.1 (2× CH), 130.7 (2× CH), 129.3 (2× CH), 129.0 (2× CH), 128.5 (C), 119.9 (CH and C), 118.0 (CH), 112.3 (CH), 111.8 (C), 32.0 (CH$_2$), under the solvent peak (CH$_2$); HR-ESIMS *m/z* 467.0358 [M + Na]$^+$ (calcd for $C_{22}H_{18}Cl_2N_2NaO_2S$ 467.0364).

*4.1.16. 2,3-Bis(4-bromobenzyl)-1H-indole-5-sulfonamide (**15f**)*

Procedure B: White solid; 89% yield; mp 213–216 °C; IR (KBr) ν 3406, 3336, 3270, 2924, 1487, 1320, 1157, 1011, 802 cm^{-1}; ^{1}H NMR (400 MHz, *(CD$_3$)$_2$CO*) δ ppm 10.49 (s, 1H), 8.01 (s, 1H), 7.62 (d, *J* = 8.3 Hz, 1H), 7.48–7.34 (m, 5H), 7.15 (d, *J* = 7.3 Hz, 4H), 6.30 (s, 2H), 4.21 (s, 2H), 4.19 (s, 2H).; ^{13}C NMR (100 MHz, *(CD$_3$)$_2$CO*) δ ppm 141.6 (C), 139.3 (C), 138.4 (C), 137.8 (C), 135.9 (C), 132.3 (2× CH), 132.0 (2× CH), 131.5 (2× CH), 131.1 (2× CH), 128.5 (C), 120.6 (C), 119.9 (CH and C), 118.0 (CH), 112.3 (C), 111.8 (CH), 32.1 (CH$_2$), under the solvent peak (CH$_2$); HR-ESIMS *m/z* 554.9348 [M + Na]$^+$ (calcd for $C_{22}H_{18}Br_2N_2NaO_2S$ 554.9353).

*4.1.17. General procedure for the preparation of N-alkylated-3-p-halo-benzylated-5-(methylsulfonyl)indoles (**16a–c** and **17a–c**)*

To a solution of 3-*p*-halo-benzylated-5-(methylsulfonyl)indole (1 equiv.) in dry THF (ca. 8 mL per mmol of indole derivative) was added NaH (0.9 equiv.) at 0 °C under argon. The reaction mixture was stirred at that temperature for 45 min (2,2,2-trifluoro-ethyl)-oxirane (1.1 equiv.) was added and mixture was refluxed for 2 h. The reaction was quenched with distilled water and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over sodium sulfate anhydrous, filtered and concentrated. The crude was purified by PTLC (CH_2Cl_2/MeOH 2%) affording compounds **16a–c**.

To a solution of previous compounds in dry THF (ca. 7 mL per mmol of indole derivative), triethylamine (2 equiv.) and methanesulfonyl chloride (1.3 equiv.) were added at 0 °C under argon. The mixture was stirred at that temperature for 30 min, and then allowed to warm at room temperature and stirred for another 1h30. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate, washed with distilled water, dried over sodium sulfate anhydrous, filtered and concentrated. The crude was dissolved in dry THF, and DBU (1.3 equiv.) was added at room temperature under argon. The reaction mixture was refluxed for 30 min. After this time was performed the work-up described above. The crude was purified by silica flash chromatography (CH_2Cl_2) affording **17a–c**.

*4.1.17.1. 1-(3-(4-Fluorobenzyl)-5-(methylsulfonyl)-indol-1-yl)-4,4,4-trifluorobutan-2-ol (**16a**).* White solid; 62% yield; mp 75–79 °C; IR (KBr) ν 3481, 1510, 1292, 1148, 1132 cm^{-1}; ^{1}H NMR (400 MHz, *(CD$_3$)$_2$CO*) δ 8.10 (s, 1H), 7.77–7.63 (m, 2H), 7.36 (m, 3H), 7.04 (t, *J* = 8.1, 8.1 Hz, 2H), 4.53–4.37 (m, 2H), 4.37–4.25 (m, 1H), 4.18 (s, 2H), 3.04 (s, 3H), 2.68–2.36 (m, 2H); ^{13}C NMR (100 MHz, *(CD$_3$)$_2$CO*) δ ppm 162.2 (d, *J* = 240 Hz, CF), 141.2 (C), 137.9 (C), 132.8 (C), 131.1 (4× CH), 126.2 (C), 120.7 (CH), 120.2 (CH), 116.9 (C), 115.7 (CH), 111.5 (CH), 66.1 (CH), 52.7 (CH$_2$), 45.1 (CH$_3$), 39.0 (q, CH$_2$–CF$_3$), 30.7 (CH$_2$); HR-ESIMS *m/z* 430.1095 [M + H]$^+$ (calcd for $C_{20}H_{20}F_4NO_3S$ 430.1100).

*4.1.17.2. 1-(3-(4-Chlorobenzyl)-5-(methylsulfonyl)-indol-1-yl)-4,4,4-trifluorobutan-2-ol (**16b**).* White solid; 46% yield; mp 115–117 °C; IR (KBr) ν 3484, 2926, 1741, 1408, 1290, 1148 cm^{-1}; ^{1}H NMR (400 MHz, *(CD$_3$)$_2$CO*) δ ppm 8.10 (s, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.46–7.22 (m, 5H), 4.53–4.37 (m, 2H), 4.37–4.25 (m, 1H), 4.18 (s, 2H), 3.04 (s, 3H), 2.74–2.30 (m, 2H); ^{13}C NMR (100 MHz, *(CD$_3$)$_2$CO*) δ ppm 140.8 (C), 140.1 (C), 132.8 (C), 132.1 (C), 131.1 (2× CH), 131.0 (CH), 129.1 (2× CH), 128.1 (C), 120.8 (CH), 120.2 (CH), 116.5 (C), 111.5 (CH), 66.1 (CH), 52.7 (CH$_2$), 45.1 (CH$_3$), 39.0 (q, CH$_2$–CF$_3$), 30.8 (CH$_2$); HR-ESIMS *m/z* 446.0799 [M + H]$^+$ (calcd for $C_{20}H_{20}ClF_3NO_3S$ 446.0805).

*4.1.17.3. 1-(3-(4-Bromobenzyl)-5-(methylsulfonyl)-indol-1-yl)-4,4,4-trifluorobutan-2-ol (**16c**).* White solid; 49% yield; mp 139–141 °C; IR (KBr) ν 3486, 2928, 1290, 1146, 1133 cm^{-1}; ^{1}H NMR (400 MHz, *(CD$_3$)$_2$CO*) δ ppm 8.11 (s, 1H), 7.74–7.67 (m, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.37 (s, 1H), 7.29 (d, *J* = 8.0 Hz, 2H), 4.54–4.37 (m, 2H), 4.37–4.24 (m, 1H), 4.16 (s, 2H), 3.05 (s, 3H), 2.76–2.35 (m, 2H); ^{13}C NMR (100 MHz, *(CD$_3$)$_2$CO*) δ ppm 141.3 (C), 140.1 (C), 132.7 (C), 132.1 (2× CH), 131.5 (2× CH), 131.0 (CH), 128.1 (C), 120.7 (CH), 120.2 (CH and C), 116.3 (C), 111.5 (CH), 66.5 (CH), 52.7 (CH$_2$), 45.0 (CH$_3$), 39.0 (q, CH$_2$–CF$_3$), 30.9 (CH$_2$); HR-ESIMS *m/z* 490.0294 [M + H]$^+$ (calcd for $C_{20}H_{20}BrF_3NO_3S$ 490.0299).

*4.1.17.4. 3-(4-Fluorobenzyl)-5-(methylsulfonyl)-1-(4,4,4-trifluorobut-1-en-1-yl)indole (**17a**).* White solid; 66% yield (0.6:0.4, cis/trans); mp 83–86 °C; IR (NaCl) ν 2928, 1674, 1509, 1303, 1137, 759 cm^{-1}; ^{1}H NMR (400 MHz, *(CD$_3$)$_2$CO*) δ ppm 8.15 (s, 2H), 7.92–7.58 (m, 5H), 7.51 (s, 1H), 7.42–7.31 (m, 6H), 7.08–7.00 (m, 4H), 6.01–5.87 (m, 1H), 5.67–5.59 (m, 1H), 4.21 (s, 2H), 4.20 (s, 2H), 3.44–3.29 (m, 2H), 3.30–3.15 (m, 2H), 3.07 (s, 6H); ^{13}C NMR (100 MHz, *(CD$_3$)$_2$CO*) δ ppm 162.2 (d, *J* = 240 Hz, CF), 139.7, 138.9, 137.3, 134.4, 131.1, 129.7, 128.7, 128.3, 125.3, 122.3, 122.1, 120.8, 120.5, 119.5, 115.9, 115.7, 112.1, 111.7, 111.2, 103.6, 45.0 (CH$_3$), 32.7 (q, CH$_2$), 30.6 (CH$_2$); HR-ESIMS *m/z* 412.0989 [M + H]$^+$ (calcd for $C_{20}H_{18}F_4NO_2S$ 412.0994).

*4.1.17.5. 3-(4-Chlorobenzyl)-5-(methylsulfonyl)-1-(4,4,4-trifluorobut-1-en-1-yl)indole (**17b**).* White solid; 83% Yield (0.6:0.4, cis/trans); mp 97–100 °C; IR (NaCl) ν 2928, 1301, 1136 cm^{-1}; ^{1}H NMR (400 MHz, *(CD$_3$)$_2$CO*) δ ppm 8.15 (s, 2H), 7.92–7.59 (m, 5H), 7.54 (s, 1H), 7.45–7.27 (m, 10H), 5.94 (m, 1H), 5.68–5.58 (m, 1H), 4.23 (s, 2H), 4.21 (s, 2H), 3.46–3.28 (m, 2H), 3.28–3.16 (m, 2H), 3.07 (s, 6H); ^{13}C NMR (100 MHz, *(CD$_3$)$_2$CO*) δ ppm 140.3, 140.0, 139.7, 138.9, 134.5, 132.3, 131.1, 129.7, 129.3, 128.7, 128.2, 125.5, 122.3, 122.1, 120.5, 119.0, 112.2, 111.8, 111.2, 103.7, 44.9 (CH$_3$), 35.1 (q, CH$_2$), 32.7 (q, CH$_2$), 30.7 (CH$_3$); HR-ESIMS *m/z* 428.0693 [M + H]$^+$ (calcd for $C_{20}H_{18}ClF_3NO_2S$ 428.0699).

*4.1.17.6. 3-(4-Bromobenzyl)-5-(methylsulfonyl)-1-(4,4,4-trifluorobut-1-en-1-yl)indole (**17c**).* White solid; 69% yield (0.6:0.4, cis/trans); mp 94–97 °C; IR (NaCl) ν 2925, 1301, 1136 cm^{-1}; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ ppm 8.15 (s, 2H), 7.89–7.61 (m, 5H), 7.54 (s, 1H), 7.48–7.42 (m, 4H), 7.38–7.26 (m, 6H), 6.04–5.87 (m, 1H), 5.69–5.58 (m, 1H), 4.21 (s, 2H), 4.20 (s, 2H), 3.45–3.29 (m, 2H), 3.23 (m, 2H), 3.07 (s, 6H); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ ppm 140.8, 139.7, 134.5, 132.2, 131.5, 129.7, 128.8, 128.7, 128.2, 125.5, 122.1, 120.5, 120.3, 118.9, 112.3, 111.8, 111.2, 45.0 (CH_3), 32.7 (q, CH_2), 30.8 (CH_2); HR-ESIMS *m/z* 472.0188 $[M + H]^+$ (calcd for $C_{20}H_{18}BrF_3NO_2S$ 472.0194).

4.2. NMR studies

4.2.1. Samples for NMR

COX-1 from ram seminal vesicles and COX-2 from sheep placenta were purchased from Cayman Chemical (Ann Arbor, MI, USA). The proteins are supplied in 80 mM Tris–HCl, pH 8.0, 0.1% Tween 20 and 300 μM diethyldithiocarbamate (DDC) and were used as such. Stock solutions of compounds **14d** and **15d** (2 mM) were prepared in DMSO-d_6. The samples for STD-NMR experiments were prepared by adding the appropriate amount of ligand stock solution to a 3 mm NMR tube containing the enzyme. Final concentrations were in the range of 3 μM of COX and 300 μM of ligand(s) in a total volume of 300 μL, when necessary volumes were corrected with 80 mM Tris–HCl buffer at pH 8.0.

4.2.2. NMR spectroscopy

All spectra were acquired at 37 °C in a Bruker Avance III spectrometer operating at 600.13 MHz, with a 5 mm triple resonance cryogenic probehead. The STD-NMR spectra were acquired with a standard pulse sequence from the Bruker library with a spin-lock ($T_{1\rho}$) for protein background suppression and water suppression with excitation sculpting with gradients. 1024 transients were acquired in a matrix of 32 k data points in t2 using a spectral window of 12019 Hz centered at 2812.4 Hz. A 2 kHz spin lock filter with a length of 20 ms was used. Selective saturation of protein resonances was performed by irradiating at −300 Hz (on resonance spectrum) using a series of 51 Eburp2.1000 shaped 90° pulses (50 ms, 1 ms delay between pulses), for a total saturation time of 2.5 s. For the off resonance spectrum irradiation was performed at 20,000 Hz. All data was processed with Bruker Topspin 2.1 and the STD spectra were obtained after subtraction of the on-resonance spectra from the off-resonance spectra.

The relative STD effect for a given hydrogen - $(I_{STD}/I_0) \times 100$, where I_0 and I_{STD} are the intensities of the reference (off resonance) and difference (STD-NMR) spectra respectively – was normalized using the highest intensity STD response as a reference for every spectrum.

4.3. COXs inhibition tests

4.3.1. Reagents

The COX-1 and COX-2 assay kit was obtained from Cayman Chemical Co., Ann Arbor, MI, USA.

4.3.2. Equipment

A microplate reader (Synergy HT, BIO-TEK), was used to perform the spectrophotometric readings in COX-1 and COX-2 inhibition assays.

4.3.3. COX-1 and COX-2 inhibition assays

The inhibition of COX-1 (ovine) and COX-2 (human recombinant), by the synthesized compounds was determined in a cell-free system by quantifying the levels of PGF 2α, produced by catalysis of arachidonic acid, using an Enzyme Immunoassay (EIA) kit (COX Inhibitor Screening Assay) supplied by Cayman Chemical Co., Ann Arbor, MI, USA. The known COX inhibitors indomethacin and celecoxib were used as positive controls. The results are expressed as the percent inhibition of control COX-1 or COX-2 activity. For each experimental condition, were performed at least four independent assays, in duplicate.

4.4. Docking studies

For all docking calculations Autodock 4.2 [30] (Release 4.2.2.1 in combination with Autogrid 4.2.2.) was used for the flexible ligand docking into two X-ray crystal structures 2AYL [31] and 3PGH [32] for COX-1 and COX-2, respectively.

Acknowledgments

The authors thank FCT financial support for the project PTDC/QUI/65187/2006 and for the fellowships to M. S. Estevão SFRH/BD/46234/2008, M. Freitas (SFRH/BD/28502/2006), A. Gomes (SFRH/BPD/63179/2009) and Aldino Viegas (SFRH/BD/35992/2007). The NMR spectrometers are part of the National NMR Network (RNRMN) and are funded by Fundação para a Ciência e a Tecnologia (FCT).

References

[1] J.R. Vane, Nat. New Biol. 231 (1971) 232–235.
[2] (a) W.L. Xie, J.G. Chipman, D.L. Robertson, R.L. Erikson, D.L. Simmons, Proc. Natl. Acad. Sci. U.S.A 88 (1991) 2692–2696;
(b) W.L. Smith, R.M. Garavito, D.L. DeWitt, J. Biol. Chem. 271 (1996) 33157–33160;
(c) L.J. Marnett, S.W. Rowlinson, D.C. Goodwin, A.S. Katgutkar, C.A. Lanzo, J. Biol. Chem. 274 (1999) 22903–22906;
(d) R.G. Kurumbail, A.M. Stevens, J.M. Miyashiro, T.D. Penning, K. Seibert, P.C. Isakson, W.C. Stallings, Nature 384 (1996) 644–648.
[3] K. Seibert, Y. Zhang, K. Leahy, S. Hauser, J. Masferrer, W. Perkins, L. Lee, P.C. Isakson, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 12013–12017.
[4] (a) Y.T. Jeon, Y.S. Song, Mini Rev. Med. Chem. 6 (2006) 827–833;
(b) J.-B. Meric, S. Rottey, K. Olaussen, J.-C. Soria, D. Khayat, O. Rixe, J.-P. Spano, Crit. Rev. Oncol./Hematol. 59 (2006) 51–64.
[5] (a) A. Sorokin, Curr. Pharm. Des. 10 (2004) 647–657;
(b) V.A. Patel, M.J. Dunn, A. Sorokin, J. Biol. Chem. 277 (2002) 38915–38920.
[6] (For reviews see). (a) A.L. Blobaum, L.J. Marnett, J. Med. Chem. 50 (2007) 1425–1441;
(b) G. Dannhardt, W. Kiefer, Eur. J. Med. Chem. 36 (2001) 109–126;
(c) A.K. Chakraborti, S.K. Garg, R. Kumar, H.F. Motiwala, P.S. Jadhavar, Curr. Med. Chem. 17 (2010) 1563–1593.
[7] (a) T.D. Penning, J.J. Talley, S.R. Bertenshaw, J.S. Carter, P.W. Collins, S. Docter, M.J. Graneto, L.F. Lee, J.W. Malecha, J.M. Miyashiro, R.S. Rogers, D.J. Rogier, S.S. Yu, G.D. Anderson, E.G. Burton, J.N. Cogburn, S.A. Gregory, C.M. Koboldt, W.E. Perkins, K. Seibert, A.W. Veenhuizen, Y.Y. Zhang, P.C. Isakson, J. Med. Chem. 40 (1997) 1347–1365;
(b) P. Prasit, Z. Wang, C. Brideau, C.C. Chan, S. Charleson, W. Cromilish, D. Ethier, J.F. Evans, A.W. Ford-Hutchinson, J.Y. Gauthier, R. Gordon, J. Gyay, M. Gresser, S. Kargman, B. Kennedy, Y. Leblanc, S. Leger, J. Mancini, G.P. O'Neill, M. Ouellet, M.D. Percival, H. Perrier, D. Riendeau, I. Rodger, P. Tagari, M. Thierien, P. Vickers, E. Wong, L.-J. Xu, R.N. Young, R. Zamboni, Bioorg. Med. Chem. Lett. 9 (1999) 1773–1778.
[8] T. Grosser, S. Fries, G.A. Fitzgerald, J. Clin. Invest. 116 (2006) 4–15.
[9] (a) J. Gierse, M. Nickols, K. Leahy, J. Warner, Y. Zhang, L. C.-Burgos, J. Carter, K. Seibert, J. Masferrer, Eur. J. Pharmacol. 588 (2008) 93–98.
[10] (a) M. Biava, G.C. Porretta, G. Poce, S. Supino, S. Forli, M. Rovini, A. Cappelli, F. Manetti, M. Botta, L. Sautebin, A. Rossi, C. Pergola, C. Ghelardini, E. Vivoli, F. Makovec, P. Anzellotti, P. Patrignani, M. Anzini, J. Med. Chem. 50 (2007) 5403–5411;
(b) M. Anzini, M. Rovini, A. Cappelli, S. Vomero, F. Manetti, M. Botta, L. Sautebin, A. Rossi, C. Pergola, C. Ghelardini, M. Norcini, A. Giordani, F. Makovec, P. Anzellotti, P. Patrignani, M. Biava, J. Med. Chem. 51 (2008) 4476–4481;
(c) M. Biava, G.C. Porretta, G. Poce, C. Battilocchio, F. Manetti, M. Botta, S. Forli, L. Sautebin, A. Rossi, C. Perloga, C. Ghelardini, N. Galeotti, F. Makovec, A. Giordani, P. Anzellotti, P. Patrignani, M. Anzini, J. Med. Chem. 53 (2010) 723–733.
[11] B. Côté, L. Boulet, C. Brideau, D. Claveau, D. Ethier, R. Frenette, M. Gagnon, A. Giroux, J. Guay, S. Guiral, J. Mancini, E. Martins, F. Massé, N. Méthot,

D. Riendeau, J. Rubin, D. Xu, H. Yu, Y. Ducharme, R.W. Friesen, Bioorg. Med. Chem. Lett. 17 (2007) 6816–6820.
[12] A. Zarghi, L. Najafnia, B. Daraee, O.G. Dadrass, M. Hedayati, Bioorg. Med. Chem. Lett. 17 (2007) 5634–5637.
[13] (a) G. Szabó, J. Fischer, A.K. Varga, K. Gyires, J. Med. Chem. 51 (2008) 142–147;
(b) L. Nagarapu, J. Mateti, H.K. Gaikwad, R. Bantu, M.S. Rani, N.J.P. Subhashini, Bioorg. Med. Chem. Lett. 21 (2011) 4138–4140;
(c) M.A.-A. El-Sayed, N.I. Abdel-Aziz, A.A.-M. Abdel-Aziz, A.S. El-Azab, Y.A. Asiri, K.E.H. ElTahir, Bioorg. Med. Chem. 19 (2011) 3416–3424.
[14] (a) O.C. López, J.J.D. Mochón, J.M. Campos, A. Entrena, M.T. Núñez, L. Labeaga, A. Orjales, M.A. Gallo, A. Espinosa, ChemMedChem 2 (2007) 88–100;
(b) S.-J. Wey, M.E. Augustyniak, E.D. Cochran, J.L. Ellis, X. Fang, D.S. Garvey, D.R. Janero, L.G. Letts, A.M. Martino, T.L. Melim, M.G. Murty, S.K. Richardson, J.D. Schroeder, W.M. Selig, A.M. Trocha, R.S. Wexler, D.V. Young, I.S. Zemtseva, B.M. Zifcak, J. Med. Chem. 50 (2007) 6367–6382;
(c) P. Singh, A. Mittal, A. Bhardwaj, S. Kaur, S. Kumar, Bioorg. Med. Chem. Lett. 18 (2008) 85–89;
(d) A.K. Gupta, R.A. Gupta, L.K. Soni, S.G. Kaskhedikar, Eur. J. Med. Chem. 43 (2008) 1297–1303;
(e) A. Koeberle, E.-M. Haberl, A. Rossi, C. Perloga, F. Dehm, H. Northoff, R. Troschuetz, L. Sautebin, O. Werz, Bioorg. Med. Chem. 17 (2009) 7924–7932;
(f) A. Zarghi, A. Tahghighi, Z. Soleimani, B. Daraie, O.G. Dadrass, M. Hedayati, Sci. Pharm. 76 (2008) 361–376;
(g) M. Scholz, A.L. Blobaum, L.J. Marnett, E. Hey-Hawkins, Bioorg. Med. Chem. 19 (2011) 3242–3248.
[15] (a) G. Ermondi, G. Caron, R. Lawrense, D. Longo, J. Comput-Aided Mol. Des. 18 (2004) 683–696;
(b) Y. Harrak, G. Casula, J. Basset, G. Rosell, S. Plescia, D. Raffa, M.G. Cusimano, R. Pouplana, M.D. Pujol, J. Med. Chem. 53 (2010) 6560–6571.
[16] C. Luong, A. Miller, J. Barnett, J. Chow, C. Ramesha, M.F. Browner, Nat. Struct. Biol. 3 (1996) 927–933.
[17] M.L.P. Price, W.L. Jorgensen, J. Am. Chem. Soc. 122 (2000) 9455–9466.
[18] D.K. Bhattacharyya, M. Lecomte, C.J. Riecke, M. Garavito, W.L. Smith, J. Biol. Chem. 271 (1996) 2179–2184.
[19] E. Block, S. Patai, The Chemistry of Functional Groups, Wiley, New York, 1980, Suppl. E, Part. 1, (Chapter 13).
[20] A.L. Borror, E. Chinoporos, M.P. Filosa, S.R. Herchen, C.P. Petersen, C.A. Stern, J. Org. Chem. 53 (1988) 2047–2052.
[21] W. Zhu, D. Ma, J. Org. Chem. 70 (2005) 2696–2700.
[22] X. Zhu, A. Ganesan, J. Org. Chem. 67 (2002) 2705–2708.
[23] Sanofi-Aventis. Pyrimidine hydrazide compounds as PGDS inhibitors. Patent: WO2008/121670 A1, 2008.
[24] S.S. Bhagwat, C. Gude, Tetrahedron Lett. 35 (1994) 1847–1850.
[25] A. Bombrun, G. Casi, Tetrahedron Lett. 43 (2002) 2187–2190.
[26] (a) M. Mayer, B. Meyer, Angew. Chem. Int. Ed. 38 (1999) 1784–1788;
(b) B. Meyer, T. Peters, Angew. Chem. Int. Ed. 42 (2003) 864–890.
[27] A. Viegas, J. Manso, M. Corvo, M.M.B. Marques, E.J. Cabrita, J. Med. Chem. 54 (2011) 8555–8562.
[28] A. Viegas, J. Manso, F. Nobrega, E.J. Cabrita, J. Chem. Educ. 88 (2011) 990–994.
[29] D.D. Perrin, W.L.F. Armarego, D.R. Perrin, Purification of Laboratory Chemicals, second ed., Pergamon, Oxford, 1980.
[30] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson, J. Comput. Chem. 19 (1998) 1639–1662.
[31] K. Gupta, B.S. Selinsky, P.J. Loll, Acta Cryst. D62 (2006) 151–156.
[32] R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Penning, K. Seibert, P.C. Isakson, W.C. Stallings, Nature 384 (1996) 644–648.