Sulfonilamidothiopyrimidone and thiopyrimidone derivatives as selective COX-2 inhibitors: Synthesis, biological evaluation, and docking studies

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · SEPTEMBER 2012

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2012.09.005 · Source: PubMed

European Journal of Medicinal Chemistry 57 (2012) 149–161

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

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

Short communication

Sulfonilamidothiopyrimidone and thiopyrimidone derivatives as selective COX-2 inhibitors: Synthesis, biological evaluation, and docking studies

Livia Basile [a], Susana Álvarez [b], Almudena Blanco [b], Andrea Santagati [c], Giuseppe Granata [c], Patrizia Di Pietro [c], Salvatore Guccione [a,c,*], Mª Ángeles Muñoz-Fernández [b,**]

[a] EtnaLead s.r.l., c/o Etnabuilding, Scuola Superiore di Catania, via S. Nullo 5/i, I-95123 Catania, Italy
[b] Lab. Inmuno-Biología Molecular, Hospital General Universitario Gregorio Marañón, 28007 Madrid, Spain
[c] Department of Drug Sciences, University of Catania, Italy

ARTICLE INFO

Article history:
Received 7 July 2012
Received in revised form
29 August 2012
Accepted 3 September 2012
Available online 10 September 2012

Keywords:
Thiopyrimidone derivatives
Human PBLs
COX-2
Anti-inflammatory
Anti-cancer
PGE_2

ABSTRACT

Newly synthesized sulfonilamidothiopyrimidone derivatives and a subset of 14 sulfonilamidothiopyrimidones and thiopyrimidones selected by an MTT assays cell viability guided selection from an *in house* collection were evaluated to determine the inhibitory effect on the PGE_2 formation in human peripheral blood lymphocytes (PBLs) using commercial ELISA. The newly synthesized sulfonilamidothiopyrimidone derivatives showed interesting pharmacological activities. Preliminary *in vitro* assays showed that compounds **2**–**5** are endowed with very high activity. Compound **2** was the most active as hCOX-2 inhibitor. The observed effects were not due to an inhibition of cell proliferation as proved by the BrdU assay. Western blot of COX-2 confirmed the inhibition on the PGE_2 secretion. Further evidence on the inhibitory potential and selectivity as COX-2 inhibitors of the selected compounds came from the *in vitro* screening. In order to better rationalize the action and the binding mode of these compounds, docking studies were carried out. These studies were in agreement with the biological data. Compounds **2**–**5** were able to fit into the active site of COX-2 with highest scores and interaction energies. Furthermore, compound **2**, which showed an inhibition of around 50% on PGE_2 production, was the best scored in all the docking calculations carried out.

1. Introduction

Inflammation is a process involving multiple factors acting in a complex network. The ingress of leukocytes into the site of inflammation is crucial for the pathogenesis of inflammatory conditions [1]. Neutrophils and macrophages are known to recruit and play pivotal roles in acute and chronic inflammation, respectively [2]. At the inflamed site, the recruited cells are activated to release many inflammatory mediators which elicit the initiation and maintenance of an inflammatory response, causing a change from the acute phase to the chronic phase of inflammation [3]. Chronic inflammation induced by biological, chemical and physical factors has been associated with increased risk of human cancer at various sites. Inflammation facilitates the initiation of normal cells and their growth and progression to malignancy through production of inflammatory oxidants. Appropriate treatment of inflammation with anti-inflammatory agents, inhibitors of oxidant-generating enzymes, and scavengers of oxidants should be explored further for chemoprevention of human cancers especially those known to be associated with chronic inflammation [4].

Non steroidal anti-inflammatory drugs (NSAIDs) are the first choice therapeutic agents for the treatment of inflammation, pain, and fever. Prostaglandins (PGs) are major proinflammatory agents and the suppression of their synthesis at sites of inflammation has been regarded as primarily responsible for the beneficial properties of NSAIDs, although several cyclooxygenase (COX)-independent effects have been described in recent years. Prostaglandin synthase is a bi-functional enzyme that first catalyzes the addition of two molecules of oxygen to arachidonic acid to form the hydroperoxide prostaglandin G2, then reduces

Abbreviations: COX-2, cyclooxygenase-2; PG, prostaglandin; PGG2, hydroperoxy endoperoxide; PGE_2, prostaglandin E_2; PBLs, peripheral blood lymphocytes; BrdU, bromodeoxyuridine; NSAIDs, non steroidal anti-inflammatory drugs; PHA, phytohemagglutinin; PBS, phosphate-buffered saline; BCA, bicinchoninic acid method; NPS, Naproxen; PGH2, prostaglandin H2; PGF2α, prostaglandin; AChE, acetylcholinesterase.

* Corresponding author. Dipartimento di Scienze del Farmaco, V.le a. Doria 6 Ed. 2, Città Universitaria, I-95125 Catania, Italy. Tel.: +39 095 738 4020; fax: +39 095 222239.
** Corresponding author. Tel.: +34 91 5868565; fax: +34 91 586 8018.
E-mail addresses: guccione@unict.it (S. Guccione), mmunoz.hgugm@madrid.salud.org (MªÁ. Muñoz-Fernández).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.09.005

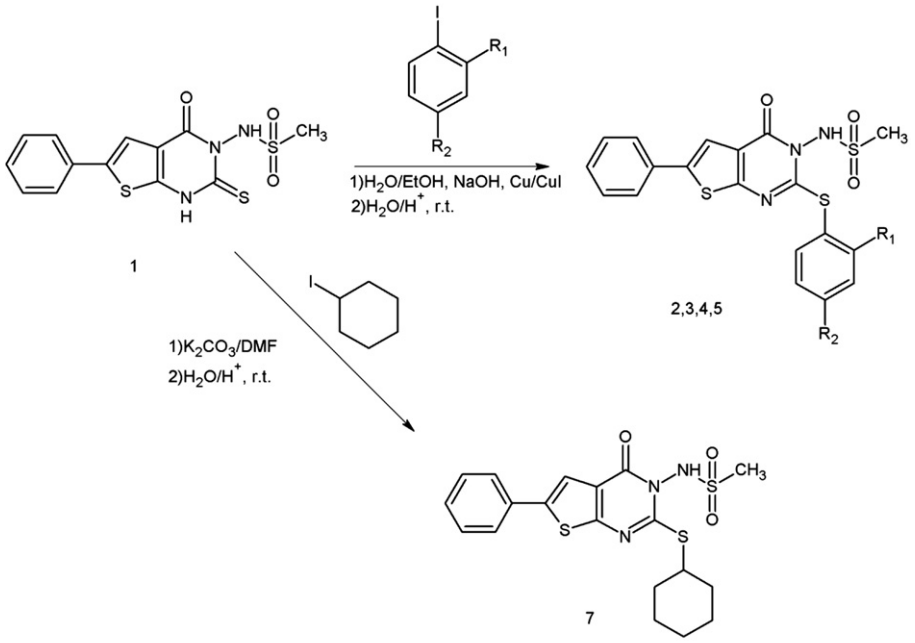
the hydroperoxide to the alcohol, PGH2, by a peroxidase activity [5]. The identification of two different isoforms of the COX enzyme known as COX-1 and COX-2, which are key enzymes in the arachidonic acid metabolism, heralded the start of a new era for research in anti-inflammatory therapy. COX-1, is constitutively expressed in most cells and is responsible for the production of prostaglandins that maintain homeostasis; COX-2 is an inducible isoform that is constitutively present in some organs, such as brain and kidney, but is significantly upregulated in response to a broad range of stimuli in inflammatory conditions [6]. Moreover, the existence of COX-3, a variant of COX-1, has been proposed and is considered to be another target for anti-inflammatory agents [7].

Since COX isozymes discovery, many papers and reviews have been published to describe the structural bases of COX inhibition, and to debate on the therapeutic and adverse effects of worldwide clinically used NSAIDs, included COX-2 selective inhibitors. COX-2 inhibition has been widely investigated, whereas the role of COX-1 in human pathophysiology is mostly not yet well ascertained. As time goes on, the cliché that the constitutively expressed isoform COX-1 is only involved in normal physiological functions, such as platelet aggregation, gastric mucosa protection and renal electrolyte homeostasis is going to be shattered. Recent data show that COX-1, classically viewed as the homeostatic isoform, is actively involved in brain injury induced by pro-inflammatory stimuli including Aβ, lipopolysaccharide, IL-1β, and TNF-α. However, it is clear that in immune system the isoform induced in response to inflammatory stimuli is mainly COX-2.

It was thought that while the antiinflammatory effect of NSAIDs occurs as a result of COX-2 inhibition, many of the undesirable side effects including gastric irritation might be due to the inhibition of the COX-1 isoform [8]. Therefore, several new inhibitors directed toward COX-2 without interfering with COX-1 enzymatic activity at therapeutical doses were developed during the last two decades. These molecules, termed coxibs display improved gastrointestinal safety profile compared to the traditional NSAIDs [9]. However, coxibs were recently withdrawn from the market because of an increased risk of cardiovascular side effects. Selective COX-2 inhibitors so far can be divided into three classes, namely (i) vicinal diaryl heterocycle inhibitors (coxibs), (ii) sulfonanilide inhibitors, and (iii) modified classical nonselective NSAIDs.

Nevertheless, the clinical use of selective COX-2 inhibitors depends on the clinical situation of patients [10]. Till now celecoxib is still available on the market and current evidence suggests that it is an important therapeutic option because of its lower cardiovascular toxicity potential compared to NSAIDs and other coxibs [11]. Recent achievements have also highlighted potential applications of selective COX-2 inhibitors in treatments of cancers [12], and neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease [13].

The aim of this study was to determine the inhibition of sulfonilamidothiopyrimidones **2**–**5**, **7**, **11**, **12**, **31** and thiopyrimidones **19**, **23**, **27**, **B41**, **B43**, **B45** [14] on the generation of COX-2 product in PMA–Ion-stimulated human peripheral blood lymphocytes (PBLs). The newly designed and synthesized compounds **2**–**5**, **7** (Scheme 1) and the MTT-based small focused library **11**, **12**, **31**, **19**,



Compd.	R1	R2
2	F	F
3	COOH	H
4	H	NO_2
5	NO_2	H

Scheme 1. Synthesis of sulfonilamidothiopyrimidone derivatives **2**–**5**, **7**.

23, **27**, **B41**, **B43**, **B45** (Fig. 2) derived from a part of our collection of polycondensed heterocycles (Fig. 2) were conceived as molecular hybrids between the stilbene scaffold in which the C double bond was bioisosterically substituted by sulfur [15] and a condensed 4-quinazolinone or thienopyrimidin-4-one moiety. The former moiety is a motif in the coxibs where these latter two moieties were previously reported as parts of polycondensed anti-inflammatory-analgesic compounds endowed with no or low ulcerogenic effects [16–19] (Fig. 1).

2. Material and methods

2.1. Chemistry. Experimental

^{1}H and ^{13}C NMR spectra were recorded at 200 MHz on a Varian Gemini 200 spectrometer; chemical shifts (δ) are reported in ppm from tetramethylsilane as an internal standard; coupling constants (J) are in Hertz (Hz). IR spectra were recorded on a Perkin Elmer 1600 Series FT-IR in potassium bromide disks. ESI–Mass spectra were acquired on a Thermo Finnigan LCQ-DECA ion trap electrospray mass spectrometer operating in negative ion mode (capillary temperature 230 °C, capillary voltage 46 V, gas flow rate 25 a.u.) scanning from m/z 300 up to 800. Samples were dissolved in H_2O/CH_3OH 20:80 (v:v) containing 1% triethylamine and directly infused in the ion source at 5 μM/ml. Microanalyses for C, H, N and S were obtained from an EA 1108 elemental analyzer Fisons Carlo-Erba instrument. Analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values. Melting points with decomposition (dec.) were determined in open capillary tubes on a SMP1 apparatus (Stuart Scientific Staffordshire) and are uncorrected. The purity of substances was checked by thin layer chromatography on Merck silica gel 60 F-254 plates. All commercial chemicals were purchased from Aldrich, Fluka, Merck, Lancaster and Carlo Erba and were used without further purification.

Compounds **2**–**5** were synthesized with satisfactory yields heating the sulfamido-thioxo **1** with an aryl iodide under basic conditions in the presence of copper powder and iodide (Scheme 1).

The thio-cyclohexyl derivative **7** was obtained in dimethylformide (DMF) at 80 °C in the presence of potassium carbonate. The proposed structures were confirmed by spectral data (IR, ^{1}H NMR, ^{13}C NMR, ESI-Mass) and elemental analysis.

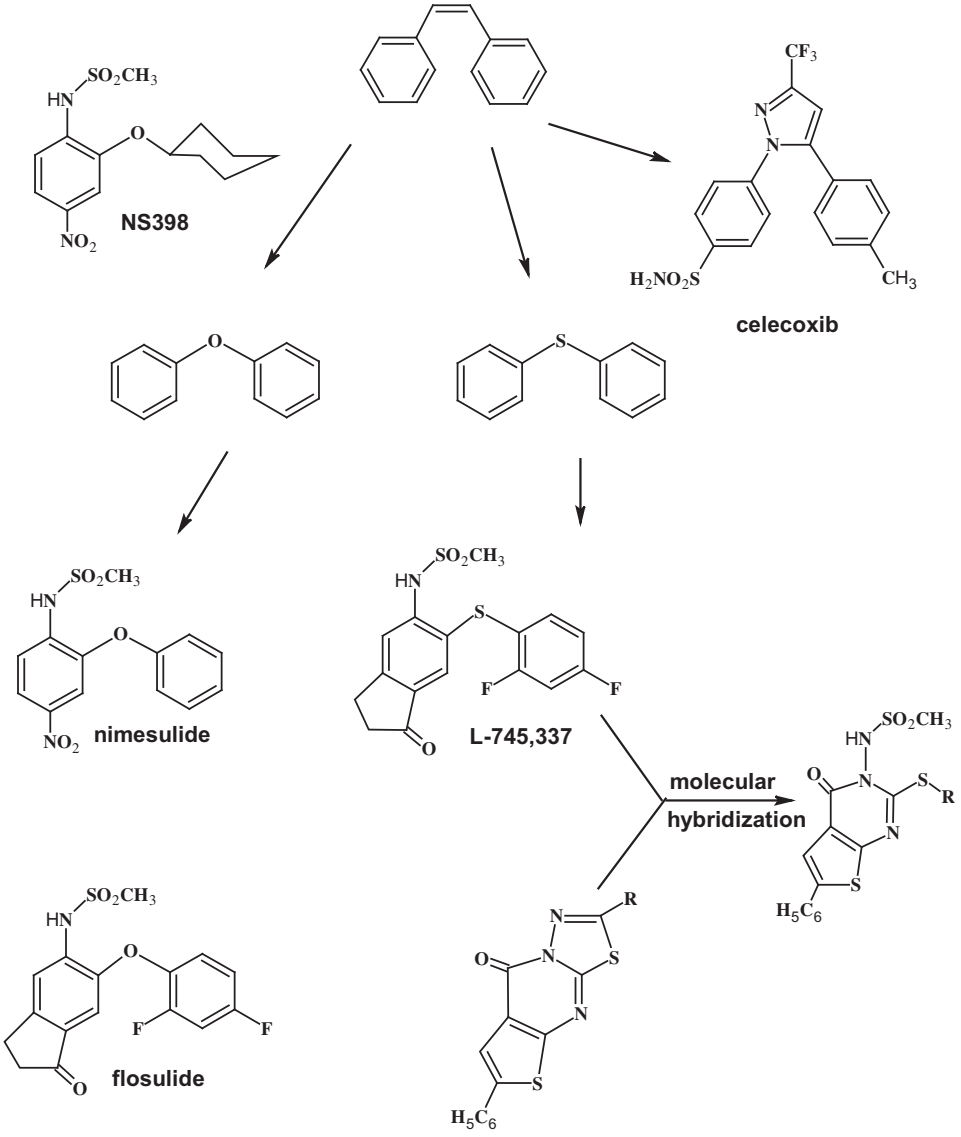


Fig. 1. Rationale for the design.

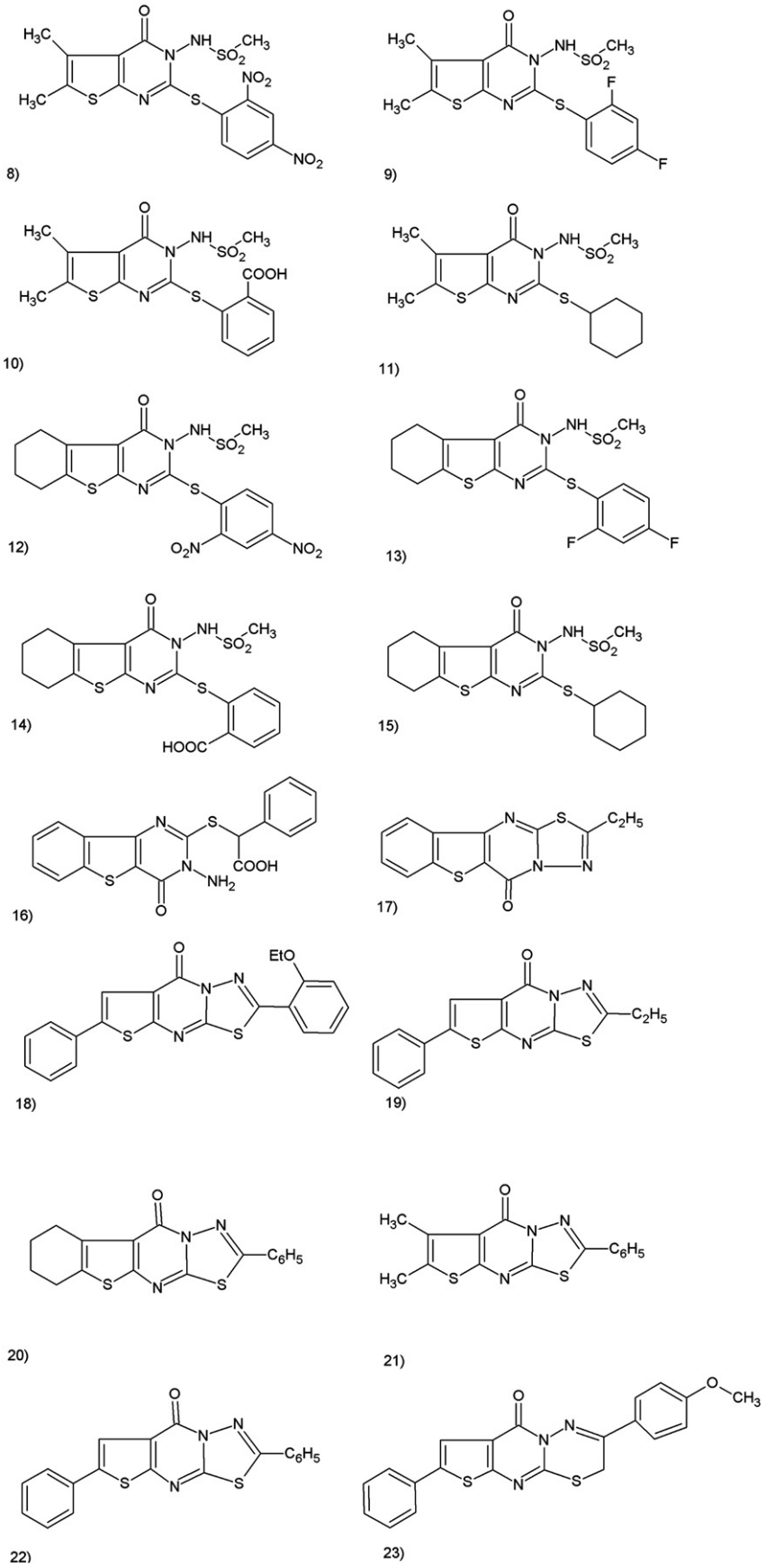


Fig. 2. Dataset of compounds. Selected compounds for COX-2 inhibition assays are in the box.

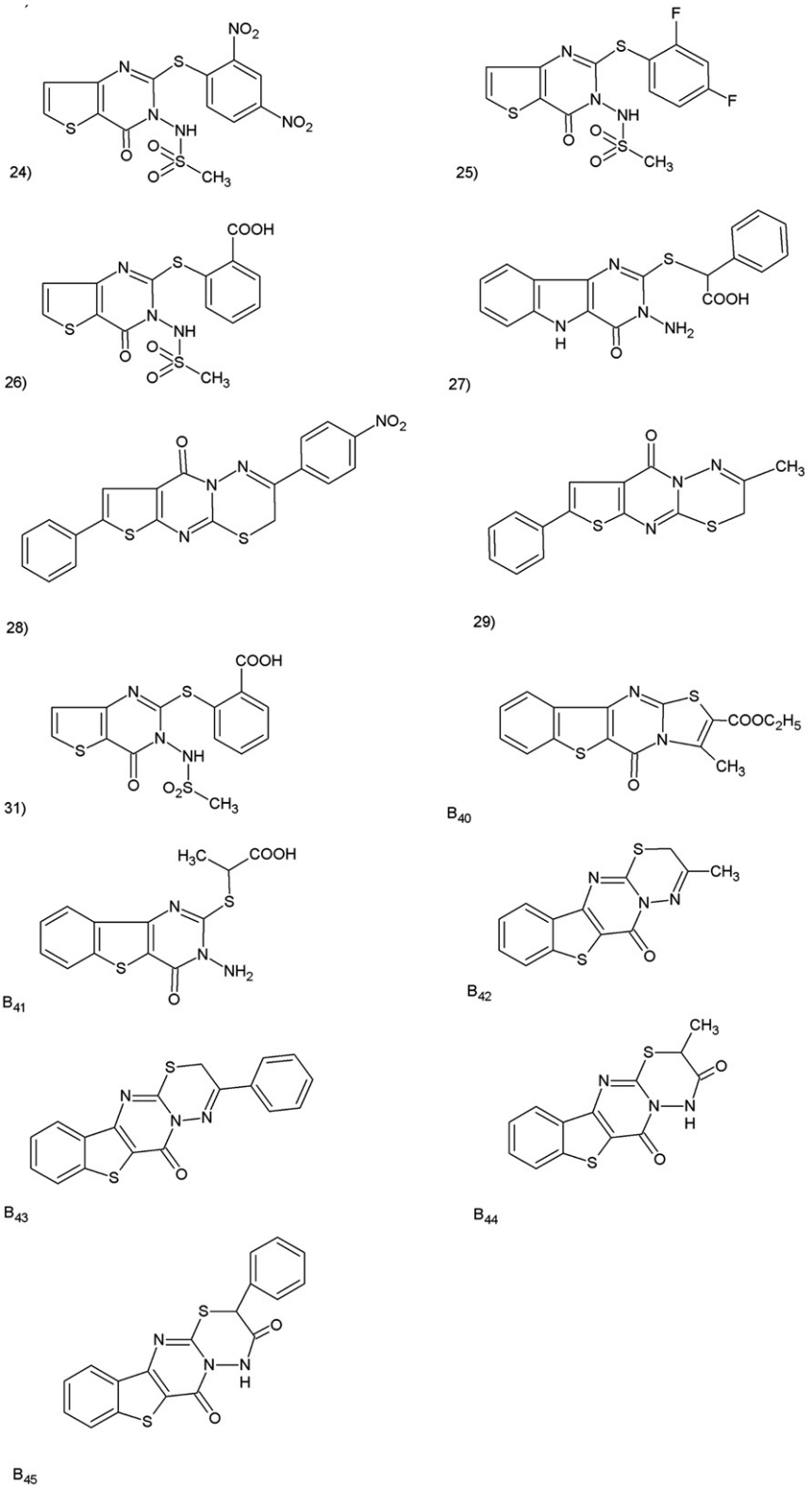


Fig. 2. (*continued*).

Specifically, the ^{1}H NMR spectra showed a typical singlet in the region of 11.0–11.45 ppm attributable to the methanesulfonamide amino group and the multiplet aromatic signals; while that one of the cyclohexyl derivative **7** showed the multiplet of the ten methylenes at 1.20–1.99 ppm and the signal of the proton bonded to the carbon adjacent to sulfur atom appeared at 3.55–3.8 ppm, respectively.

2.1.1. N-[2-[(2,4-difluorophenyl)thio]-4-oxo-6-phenylthieno[2,3-d] pyrimidin-3(4H)-yl]methanesulfonamide (**2**)

To a stirred solution of compound **1** (0.180 g, 0.51 mmol) and sodium hydroxide (40 mg, 1.0 mmol) in ethanol/water 1:1 (40 ml) 2,4-difluoroiodobenzene (0.13 g, 0.51 mmol) and copper powder (30 mg) (I) iodide (20 mg) were added; the mixture was heated at reflux under stirring for 4 h; after cooling to room temperature the mixture was filtered and the filtrate poured in water (150 ml); the resulting solution was filtered and acidified with hydrochloric acid to pH 3–4: the solid separated was collected, washed with water, dried and crystallized from water/dioxane to give pure product **2** as gray powder; yield 35%; m.p. 264–266 °C dec.; I.R. ν [cm^{-1}]: 3182 br (NH), 3090 (C–H benzene), 1690 (C=O), 1345 and 1155 (N–SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.39 (s, 3H, CH_3), 7.26–7.80 (m, 9H, Ar–H and H–thiophene), 11.43 (s, 1H, NH).

^{13}C NMR (TMS): δ 43.94, 105.20 (t, J = 25.32), 112.70 (d, J = 18.30), 116.21, 122.37, 124.00, 126.42, 129.00, 135.78, 137.65 (d, J = 9.68), 139.45, 155.88, 160.00, 160.20, 161.00, 163.10, 164.45. ESI–MS [M − H]$^-$: calcd for $C_{19}H_{13}F_2N_3O_3S_3$: m/z 465; found: m/z 464. Anal. Cald. for $C_{19}H_{13}F_2N_3O_3S_3$ (C, H, N, S): C, 49.02; H, 2.79; N, 9.03; S, 20.66. Found: C, 48.80; H, 2.65; N, 8.85; S, 20.30.

2.1.2. 2-({3-[(Methylsulfonyl)amino]-4-oxo-6-phenyl-3,4-dihydrothieno[2,3-d]pyrimidin-yl}thio)benzoic acid (**3**)

To a stirred solution of the appropriate methanesulphonamide thioxo (compound **1**) [17] (0.180 g, 0.51 mmol) and sodium hydroxide (40 mg, 1.0 mmol) in water (20 ml) 2-iodobenzoic acid (0.130 g 98%, 0.51 mmol), dissolved in a small amount of 10% sodium hydroxide and powder copper (30 mg)/copper (I) iodide (20 mg) were added. The mixture was heated at reflux under stirring for 4 h and then filtered. The resulting solution was cooled to room temperature and acidified with concentrated hydrochloride acid to pH 3–4. The white solid separated was collected, washed with water, dried and purified by chromatography on silica gel column (ethyl acetate/methanol 1:3) to give product **3** as a white powder; yield 50% m.p. 239–241° dec.; I.R. ν [cm^{-1}]: 3300 (NH), 1700 (C=O), 1345 and 1155 (SO_2–N) cm^{-1}; ^{1}H NMR (dimethylformamide-d_6): δ 3.38 (s, 3H, CH_3), 7.32–7.95, (m, 10H, Ar–H and H–thiophene), 11.45 (br s, 1H, NH), 13.15 (br s, 1H, COOH). ^{13}C NMR (TMS): δ 43.44, 118.22, 125.21, 125.34, 125.76, 126.43, 127.21, 128.10, 128.31, 129.18, 129.79, 131.99, 132.26, 132.83, 134.45, 135.69, 168.41, 170.32. ESI–MS [M − H]$^-$: calcd for $C_{20}H_{15}N_3O_5S_3$: m/z 473; found: m/z 472. ESI–MS [M − H]$^-$: calcd for $C_{20}H_{14}N_3O_5S_3Na$ m/z 494. Anal. Cald. for $C_{20}H_{15}N_3O_5S_3$ (C, H, N, S): C, 50.73; H, 3.17; N, 8.88; S, 20.30. Found: C, 50.40; H, 3.22; N, 8.70; S, 19.90.

2.1.3. N-[2-(4-nitrophenyl)thio]-4-oxo-6-phenylthieno[2,3-d] pyrimidin-3(4H)yl]methane sulfonamide (**4**)

To a solution of appropriate methanesulfonamide-thioxo (compound **1**) [17] (0.180 g, 0.51 mmol), and sodium hydroxide (30 mg, 0.75 mmol) water (20 ml) 4-nitroiodobenzene (0.130 g, 98%, 0.51 mmol) in ethanol (20 ml) and powdery copper (30 mg)/copper (I) iodide were added; the mixture was heated at reflux under stirring; after 6 h the mixture was filtered while hot and the filtrate, cooled to room temperature; the resulting solution was filtered and acidified with concentrated hydrochloride acid to pH 3–4: the resulting solid was collected, washed with water, dried and crystallized from ethanol/water to give product **4** as a pure light green powder; yield 60%; m.p. < 100 °C dec.; I.R. ν [cm^{-1}]: 3230 br (NH), 3100 (C–H benzene), 1700 (C=O), 1345 and 1160 (N–SO_2) cm^{-1}; ^{1}H NMR (dimethylsulfoxide-d_6): δ 3.25 (s, 3H, CH_3), 7.35–8.45 (m, 10H, Ar–H and H–thiophene), 11.40 (s, 1H, NH). ^{13}C NMR (TMS): δ 39.53, 116.89, 122.86, 125.45, 125.97, 128.23, 129.36, 129.96, 132.86, 133.67, 134.00, 139.02, 141.03, 141.60, 150.87, 160.23. ESI–MS [M − H]$^-$: calcd for $C_{19}H_{14}N_4O_5S_3$: m/z 474; found: 473. Anal. Cald. for $C_{19}H_{14}N_4O_5S_3$ (C, H, N, S): C, 48.10; H, 2.95; N, 11.81; S, 20.28. Found: C, 48.00; H, 2.75; N, 11.60; S, 20.00.

2.1.4. N-[2-(2-nitrophenyl)thio]-4-oxo-6-phenylthieno[2,3-d] pyrimidin-3(4H)-yl]methanesulfonamide (**5**)

To a solution of appropriate methanesulfonamide-thioxo (compound **1**) [17] (0.180 g, 0.51 mmol), and sodium hydroxide (30 mg, 0.75 mmol) water (20 ml) 2-nitroiodobenzene (0.131 g, 97%, 0.51 mmol) in ethanol (20 ml) and powdery copper (30 mg)/copper (I) iodide were added; the mixture was heated at reflux under stirring; after 6 h the mixture was filtered while hot and the filtrate cooled to room temperature; the resulting solution was filtered and acidified with concentrated hydrochloride acid to pH 3–4: the resulting solid was collected, washed with water, dried and crystallized from ethanol/water to give product **5** as a pure yellow powder; yield 60%; m.p. < 125 °C dec.; I.R. ν [cm^{-1}]: 3220 br (NH), 3090 (C–H benzene), 1700 (C=O), 1350 and 1160 (N–SO_2) cm^{-1}; ^{1}H NMR (dimethylsulfoxide-d_6): δ 3.25 (s, 3H, CH_3), 7.25–8.50 (m, 10H, Ar–H and H–thiophene), 11.37 (s, br, 1H, NH). ^{13}C NMR (TMS):δ 39.49, 117.52, 122.25, 124.84, 125.32, 125.67, 128.71, 129.19, 129.47, 131.79, 132.26, 133.52, 138.02, 140.03, 141.06, 151.88, 160.88. Anal. Cald. for $C_{19}H_{14}N_4O_5S_3$ (C,H,N,S): C, 48.10; H, 2.95; N, 11.81; S, 20.28. Found: C, 48.20; H, 2.80; N, 11.75; S, 20.00.

2.1.5. N-[2-(cyclohexylthio)-4-oxo-6-phenylthieno[2,3-d] pyrimidin-3(4H)-yl]methanesulfonamide (**7**)

A mixture of methanesulphonamide-thioxo **1** [17] (0.180 g, 0.51 mmol), cyclohexyl iodide (0.11 g, 98%, 0.53 mmol, 0.070 ml, d = 1.625) and potassium carbonate (0.145 g) in dimethylformamide (2 ml) was heated at 80 °C under stirring for 4 h; after cooling to room temperature the mixture was treated with hydrochloride acid and poured in water (100 ml): the resulting solid was collected, washed with water, dried and crystallized from petroleum ether to give compound **7** as a pure yellow powder with a 45% yield; m.p.>125 °C dec.; I.R. ν [cm^{-1}]: 3200 (NH), 2925 (H cicloesyl), 1690 (C=O), 1350 and 1155 (N–SO_2); ^{1}H NMR (dimethylsulfoxide-d_6): δ 1.25–2.00 (m, 10H, cyclohexyl), 3.55–3.8 (m, 1H, S–CH), 7.33–7.90 (m, 6H, Ar–H and thiophene–H), 11.22 (s, 1H, NH), Anal. Cald. for $C_{19}H_{21}N_3O_3S_3$ (C, H, N, S): C, 52.40; H, 4.82; N, 9.65; S, 22.09. Found: C, 52.10; H, 4.80; N, 9.35; S, 21.85.

2.2. Cell cultures

Human PBLs were isolated by Ficoll-Hypaque centrifugation. The PBLs were routinely grown in RPMI 1640 (Biochrom KG Seromed, Berlin, Germany) containing 10% heat-inactivated fetal calf serum, 1% penicillin/streptomycin, and 2 mM L-glutamine at 37 °C. Cells were cultured with 2 μg/ml phytohemagglutinin (PHA) and 60 U/ml IL-2 (Murex Diagnostics Corp. Norcross, GA).

2.3. Western blot analyses

Human PBLs were exposed to PMA–Ion in the presence or absence of the inhibitors tested. Cells were washed with phosphate-buffered saline (PBS) and lysed with buffer lysis. Protein contents were measured using the bicinchoninic acid method (BCA protein assay kit from Pierce, Rockford, IL, USA) according to the

manufacturer's instructions. Samples were separated into a 10% SDS polyacrylamide gel and blotted onto a polyvinylidene fluoride membrane (Millipore, Bedford, MA, U.S.A.) by semidry transference blotting. Membranes were blocked overnight at 4 °C using Roti-block (Roth, Karlsruhe) and for another hour at room temperature. Blocked membranes were incubated with primary antibody, a rabbit anti-human COX-2 (Cayman; 1:500), or mouse anti-α-tubulin (Sigma, St. Louis, MO), used as a protein loading control. Visualization of protein bands was performed using enhanced chemiluminescence (ECL) reagents (Amersham Biosciences, UK). Western blot experiments were carried out at least three times.

2.4. Cell viability measurement

To determine the optimum concentration of these novel compounds as well as to assess that their suppressive effect on PGE_2 production was not due to their cytotoxicity, the cell viability was assessed by MTT assay. The method is based on the ability of living cells to reduce MTT tetrazolium salt into MTT formazan by the mitochondrial enzyme succinate dehydrogenase, as reported previously. Briefly, the cells were incubated with MTT (0.15 mg/ml) for 4 h at 37 °C. The amount of MTT formazan product was determined by measuring absorbance with a microplate reader at a test wavelength of 570 nm and reference wavelength of 655 nm.

2.5. Cell proliferation

Human PBLs were grown in the absence or presence of a concentration of 10 μM of the indicated compounds for 24 h in a 37 °C and 5% CO_2 atmosphere. Cell proliferation was measured by quantitating bromodeoxyuridine (BrdU) incorporated into the newly synthesized DNA of replicating cells. In this method BrdU, a thymidine analog, is incorporated into newly synthesized DNA strands of actively proliferating cells. Following partial denaturation of double stranded DNA, BrdU is detected immunochemically allowing the assessment of the population of cells which are synthesizing DNA.

2.6. PGE_2 determination

Supernatants of control or treated human PBLs were tested for PGE_2 production using the PGE_2 EIA kit (Cayman Chemical Company, MI, USA), a competitive enzyme immunoassay.

2.7. Cyclooxygenase-2 (COX-2) assay

Selective Prostaglandin G/H synthase activity was measured *in vitro* using purified COX-1 or COX-2 protein (assay kit No. 560101, Cayman Chemical Company). Compounds **2**–**5** were analyzed with celecoxib as the positive control. The assay is based on the chemical conversion of PGH2 to PGF2α by stannous chloride followed by a PGF2α immunoassay. The assay was carried out in 0.1 M Tris–HCl at pH 8. Overall compound to test was dissolved in dimethyl sulfoxide. Prostaglandin synthesis was initiated by the addition of arachidonic acid to the mixture of reaction. Afterward, stannous chloride was added in order to convert PGH2 to PGF2α and the PGF2α generated was quantified by enzyme immunoassay, EIA. This assay is based on the competition between PGs and a PG-AChE conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells, since the concentration of PG tracer is constant while that of PGs varies. The complex between the antibody and PG binds to a mouse antirabbit monoclonal antibody that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's Reagent is added to the well, containing the substrate to AChE. The product of this enzymatic reaction was determined spectrophotometrically at an absorbance of 405 nm by a Synergy ht, biotek spectromether. It is proportional to the amount of PG tracer in the well. This latter is in turn inversely proportional to the amount of PGs present in the well during the incubation.

2.8. Molecular modeling

Docking studies were carried out on an Intel Core i7, ram memory of 16 GB operating under Linux/Ubuntu 10.04. Side chains flexible ligand–protein docking was carried out by the molecular docking algorithm MolDock Optimizer and the scoring function MolDock [GRID] as in the Molegro Virtual Docker software, version 5 [20,21]. Only torsion angles in the side chains were modified during the minimization; all other properties (including bond lengths and backbone atom positions) were held fixed and a new receptor conformation was generated for each pose after side chains flexible docking calculation. The ligands were built as .pdb files by means of the server PRODRG2 (http://davapc1.bioch.dundee.ac.uk/prodrg/) [22]. Docking of NPS into the hCOX-2 active site as obtained by homology modeling was used to calibrate the system. High scoring poses (Table 1) with the pattern of interactions comparable to the mCOX-2-Naproxen were obtained. Particularly the docking of Naproxen gave a high score pose with high similarity to the entry crystal structure 3NT1 in PDB (Fig. 3); the rmsd between the docked complex and the crystal structure (PDB entry: 3NT1) was around 0.16 Å as calculated by Sybyl-X 1.3 (http://www.tripos.com). Typically, an RMSD value below 2.0 Å represents a good solution for a redocked ligand of course depending on factors such as the ligand size and flexibility.

Protein residues Arg(R) 89, Leu(L) 321, Tyr(Y) 324, Tyr(Y) 354 and Ser(S) 499 in hCOX-2 (human COX-2) were set as flexible; these residues correspond to Arg(R) 120, Leu(L) 352, Tyr(Y) 355, Tyr(Y) 385, Ser(S) 530 which are involved in the inhibitors binding of the homologous mCOX-2 (murine COX-2), as reported by Duggan et al. [23].

Potentials were softened to a value of 1.5 and 0.5 for tolerance and strength, respectively. Preliminary calculations with a tolerance value of 0.9 led to poses with low MolDock scores and unfavorable steric interactions. Increasing the value up to 1.5 led to poses with high scores and no significant unfavorable steric interactions. 70 runs for each molecule were carried out with a population size of 50, maximum iteration of 1500, scaling factor of 0.50, crossover rate of 0.90 and a variation-based termination scheme. The results were in agreement with the experimental data.

Poses within a root-mean square deviation of 1.5 Å were clustered together by means of the "Tabu pose" clustering algorithm.

In order to improve the docking calculations taking into account the interactions with the cofactors, the anion Cl^- and protoporphyrin IX containing Fe as in the mCOX-2 pdb entry (3NT1) were

Table 1
Energy contributions for compounds assayed.

Compound	Total interaction energy (a.u)	Protein–ligand interaction energy (a.u)	Score (a.u)
NPS	−127.26	−126.31	−120.19
2	−126.32	−125.03	−132.07
3	−111.36	−109.30	−122.90
5	−104.87	−103.11	−111.35
4	−86.78	−86.01	−89.26
31	−80.04	−79.95	−91.89
11	−78.29	−77.26	−79.39
23	−33.28	−33.28	−41.74

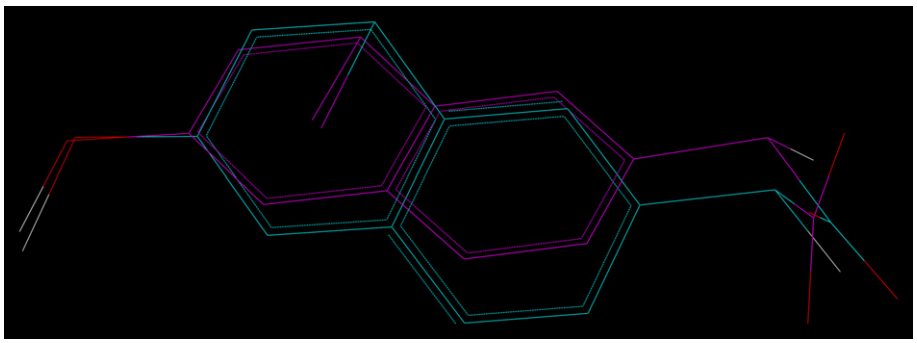


Fig. 3. Light blue: X-ray structure of the complex mCOX-2-Naproxen. Magenta: Highest score pose of Naproxene docked into hCOX-2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

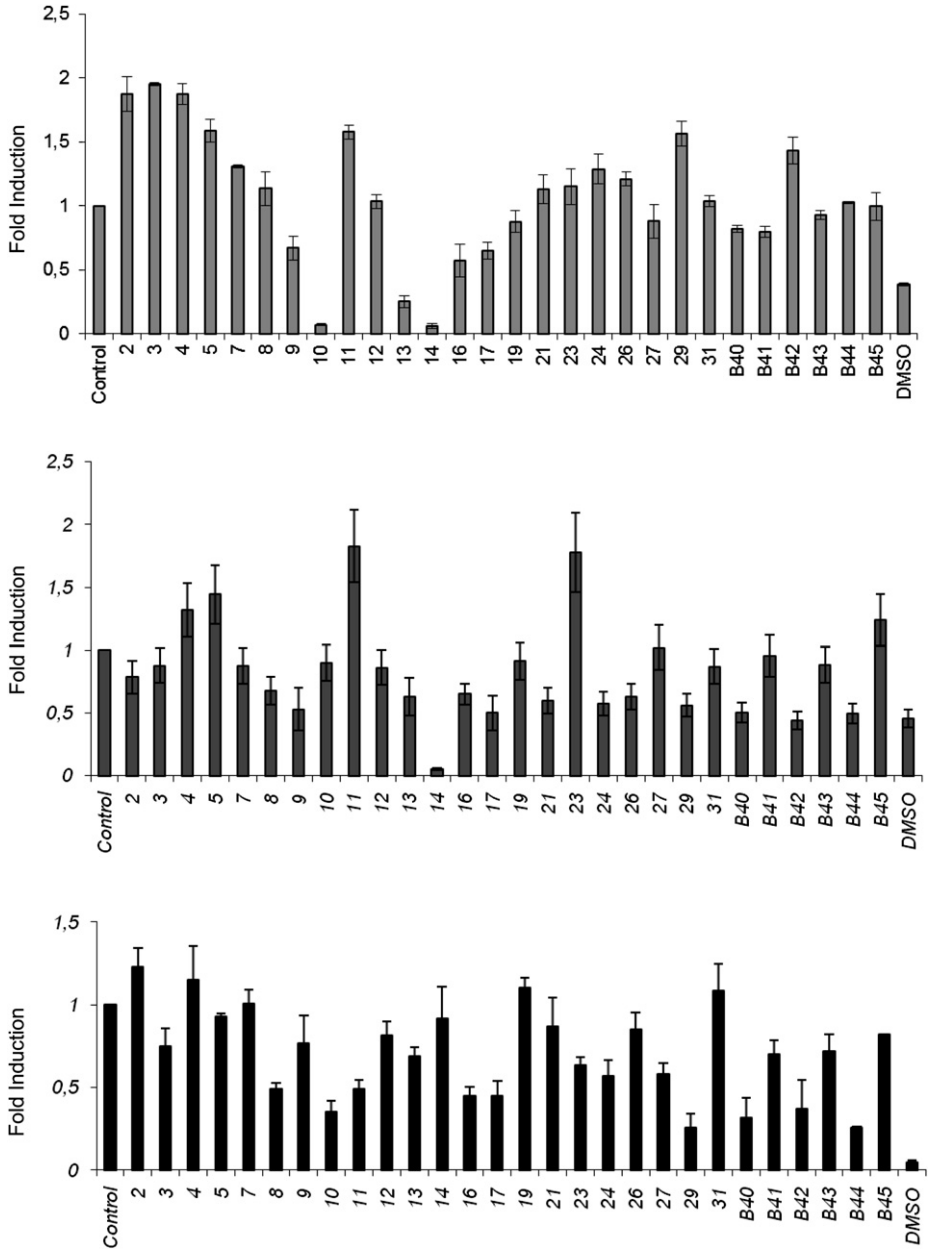


Fig. 4. Cell death of human PBLs. Human cells were treated with IL-2 and different concentrations of the compounds tested (1, 10, and 50 μM). Cell toxicity was measured by MTT assay after 24 h of treatment. DMSO is used as positive control of cell death.

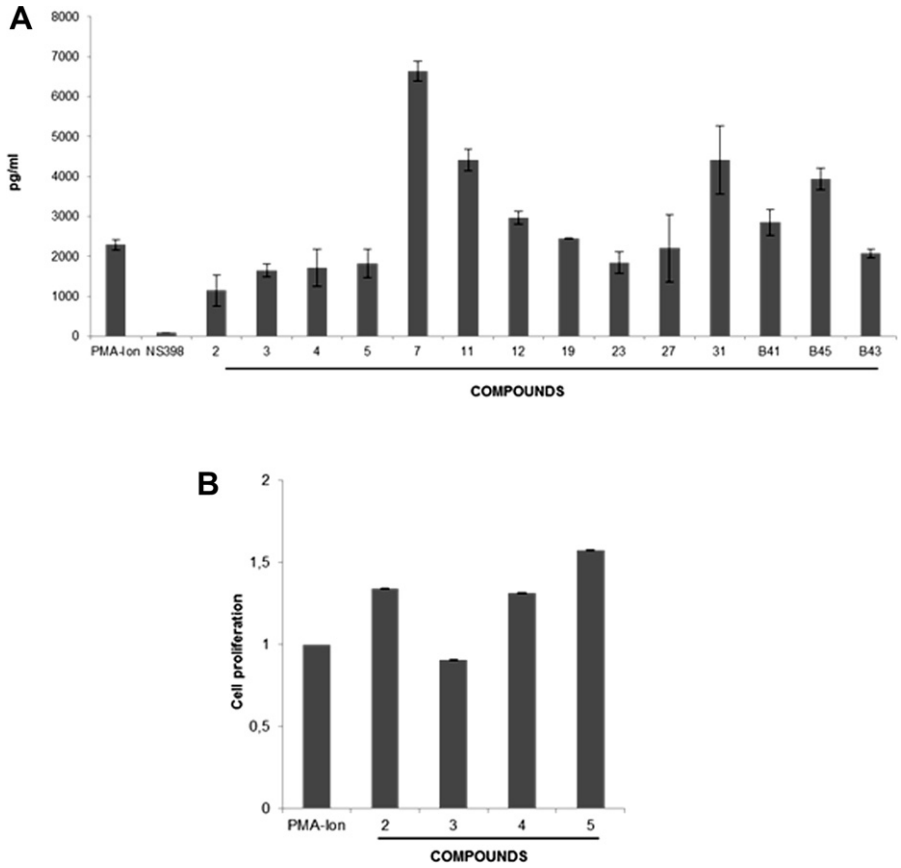


Fig. 5. A. PGE_2 formation as parameter of COX-2 activity. A) PGE_2 production of human PBLs treated with PMA (15 ng/ml)–Ion (10 mM), alone or in combination with different compounds (10 μM) or NS398 (5 μM) measured by ELISA after 24 h of treatment. B. PBLs proliferation in response to sulfonilamidothiopyrimidone derivatives. Human PBLs were exposed to 10 μM of compounds 2, 3, 4, and 5 and 24 h after, cell proliferation was measured by incorporation of BrdU.

Table 2
Favorable and unfavorable interactions of docked compounds.

Compound	Favorable contacts[a]	Energy (a.u)[b]	Unfavorable contacts	Energy (a.u)	Steric interaction energy (a.u)
2	Val 318	−11.11	Arg 89	24.9	−9.71
3	Ala 496	−11.21	Arg 89	63.73	−20.63
	Val 85	−12.01	His 58	7.57	
	Val 318	−13.39	Tyr 324	5.65	
			Tyr 354	18.13	
5	Ala 496	−13.84	Arg 89	90.68	−13.86
	Val 318	−10.64	His 58	2.04	
			Tyr 324	26.99	
			Tyr 354	1.43	
4	Glu 493	−13.15	Arg 89	108.85	44.37
	Val 85	−15.09	Tyr 324	43.22	
			Val 318	7.59	
31	Ala 496	−10.07	Arg 89	124.83	37.53
			His 58	0.41	
			Tyr 324	2.78	
			Val 492	2.27	
11	Ser 322	−10.58	Arg 89	0.79	−20.52
			Leu 328	4.39	
			Tyr 324	67.42	
23	Arg 482	−10.52	Ala 485	27.19	106.66
	Glu 493	−14.93	Arg 89	128.29	
	His 58	−12.98	Val 492	71.75	
	Phe 487	−15.74			
	Ser 322	−16.31			

[a] Only favorable contacts with energies below −10 a.u are listed.
[b] a.u stands for arbitrary units.

added to the model [23]. The cofactors in the hCOX-2 model had the same coordinates as in mCOX-2 (see below). The structure of the hCOX-2 used for the docking calculations was obtained according to Michaux et al. [24,25].

All the docking results were counterchecked by the software FLO (unpublished data) (QXP_FLO program: Thistlesoft, PO Box 227, Colebrook, CT 06021) [26].

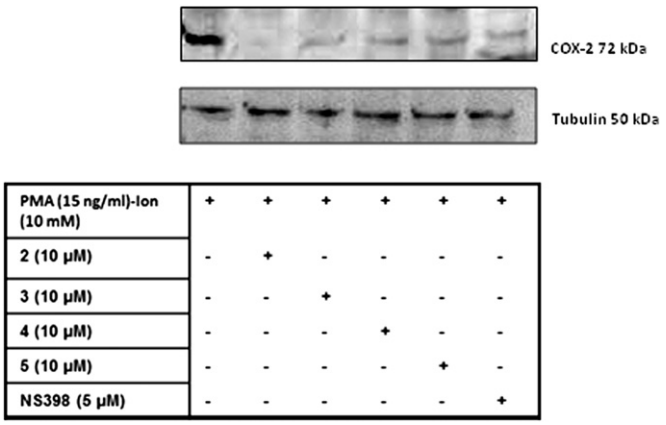


Fig. 6. COX-2 expression in human PBLs. Cell extracts were analyzed by Western blot after treatment with PMA (15 ng/ml)–Ion (10 mM), alone or in combination with the numbered compounds (2, 3, 4, 5) (10 μM) for 20 h at 37 °C. NS398 (5 μM) was used as positive control of COX-2 inhibition. Cells were harvested, lysed, and proteins were separated by SDS–PAGE and analyzed for COX-2. The membrane was reprobed with α-tubulin antibody to confirm equal protein loading.

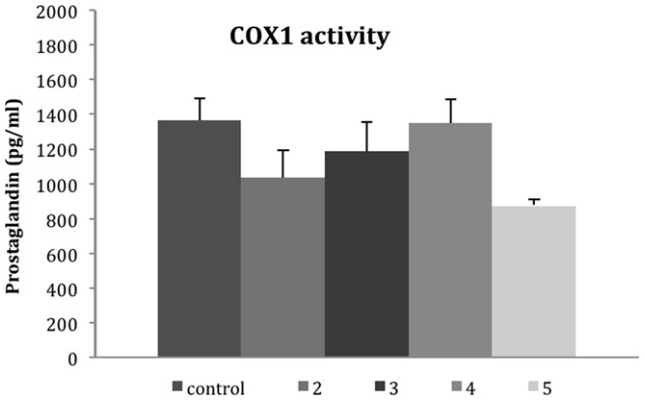


Fig. 7. Effect of sulfanilamidothiopirimidone derivatives on COX-1 enzyme activity *in vitro*. Celecoxib (control) and sulfanilamidothiopirimidone derivatives (final concentration of 5 nM) were added to purified COX-1. Prostaglandin synthesis was initiated by the addition arachidonic acid substrate. The results, expressed as picograms of $PGF_{2\alpha}$ formed are the average of duplicate experiments.

3. Results

3.1. Assessment of cell cytotoxicity and effects on T cell activation

It was previously shown that COX-2 inhibitors as celecoxib were able to act as immunosuppressive drugs modulating T cell activation events such as cell surface expression of activation markers, cytokine production and cell proliferation [27]. Moreover, bioactive molecules by and large are known to exhibit cytotoxic effect to varying levels depending upon their concentration and duration of exposure. To explore the influence of the treatment with these novel compounds on T cell viability, we first addressed whether these compounds were toxic for human PBLs treated with increasing doses of these drugs for 24 h. The cytotoxicity of compounds was estimated by determining the amount of MTT in the culture.

As shown in Fig. 4 at 1 μM most of the tested compounds did not show cell toxicity in PBLs cultures, and at dose of 50 μM, a considerable inhibition in cell viability was observed with the majority of compounds. Having in account these results, we decided to use the dose of 10 μM to study the effects of these compounds on PGE_2 production.

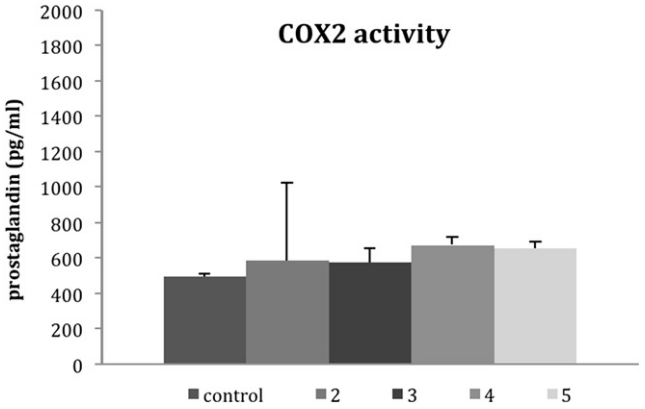


Fig. 8. Effect of sulfanilamidothiopirimidone derivatives on COX-2 enzyme activity *in vitro*. Celecoxib (control) and sulfanilamidothiopirimidone derivatives (final concentration of 5 nM) were added to purified COX-1. Prostaglandin synthesis was initiated by the addition arachidonic acid substrate. The results, expressed as picograms of $PGF_{2\alpha}$ formed are the average of duplicate experiments.

3.2. Effects of sulfonilamidothiopyrimidone derivatives on PGE_2 secretion by human PBLs

The COX-2 activity was evaluated by measuring the PGE_2 levels in PMA–Ion-treated human PBLs. Only, compounds **2**–**5**, **7**, **11**, **12**, **19**, **23**, **27**, **31**, **B41**, **B43**, and **B45** which did not induce cell death at dose of 10 μM were tested (Fig. 5A). The PMA–Ion stimulated cells secreted to the supernatant 2287 pg/ml. We observed that the compounds synthesized by us either inhibited or stimulated PGE_2 formation. Compounds **2**, **3**, **4**, and **5** inhibited PMA–Ion-induced PGE_2 secretion by 20–50%. Compound **2** was the most potent among these inhibitors showing potency higher than 50%, Compounds **23** [28] and **B43** [17] were relatively less potent. In this assay, NS398 (5 μM) was used as positive control of PGE_2 inhibition (Fig. 5A).

Interestingly, compounds **7**, **11** [29], **31** [14], and **B45** [17] increased the PMA–Ion-induced PGE_2 formation. Between them, compound **7** was the most potent among these derivatives and stimulated PGE_2 secretion 2.9-fold. Compounds **7**, **11**, and **31** [14] (Scheme 1, Fig. 2) increased the PGE_2 levels, suggesting an alternate target on respect to hCOX-2. The docking results (Tables 1 and 2) were in agreement with this hypothesis being the interactions with the hCOX-2 weak.

As shown in Fig. 5B, the subject COX-2 inhibitors did not alter PHA-induced T cell proliferation, indicating that the observed effects on PGE_2 secretion are not associated with a decrease of cell proliferation.

3.3. Change in COX-2 expression

It has been previously described that high levels of PGE_2 were not only produced in cell lines as a result of strong COX-2 expression, but also in cells in which COX-2 expression was weak (Effects of COX-2 inhibition on expression of vascular endothelial growth factor and interleukin-8 in lung cancer cells [30]). This could result from reduced catabolism of PGE_2 due to low levels of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), a primary enzyme responsible for PGE_2 metabolism [31]. To check whether low levels of PGE_2 produced by PBLs treated with the novel compounds was due or not to an inhibition of COX-2 expression, we performed Western blot analysis on lysate protein extracted from PMA–Ion-treated cells in presence or not of the four best compounds. NS398 (5 μM) was used again as positive control of COX-2 inhibition. COX-2 expression was strongly detected after PMA–Ion treatment. All the compounds tested, especially compound **2**, were able to diminish COX-2 protein levels, suggesting that the reduced secretion of PGE_2 is due, at least in part, to a lower expression of COX-2 (Fig. 6).

3.4. In vitro inhibitors COX-2 screening

Cyclooxygenase is the rate-limiting enzyme in prostaglandin synthesis. Prostaglandin levels, particularly PGE_2, are often used as a measurement of cyclooxygenase activity. To test whether the inhibition of serum-induced PGE_2 synthesis by sulfonilamidothiopyrimidone derivatives was due to selective inhibition of COX-2 enzyme activity, the effect of these compounds on the activity of purified COX-1 and COX-2 proteins *in vitro* was tested.

COX-1 and COX-2 proteins catalyze the conversion of arachidonic acid into PGG2 (via cyclooxygenase activity) and the subsequent conversion of PGG2 into PGH2 (via peroxidase activity). Cyclooxygenase activity was finally measured by an enzyme immunoassay for PGF2α (see experimental for details).

As shown in Figs. 7 and 8, under identical conditions, compounds **2**–**5** behave as the celecoxib, that reduce the cyclooxygenase-2

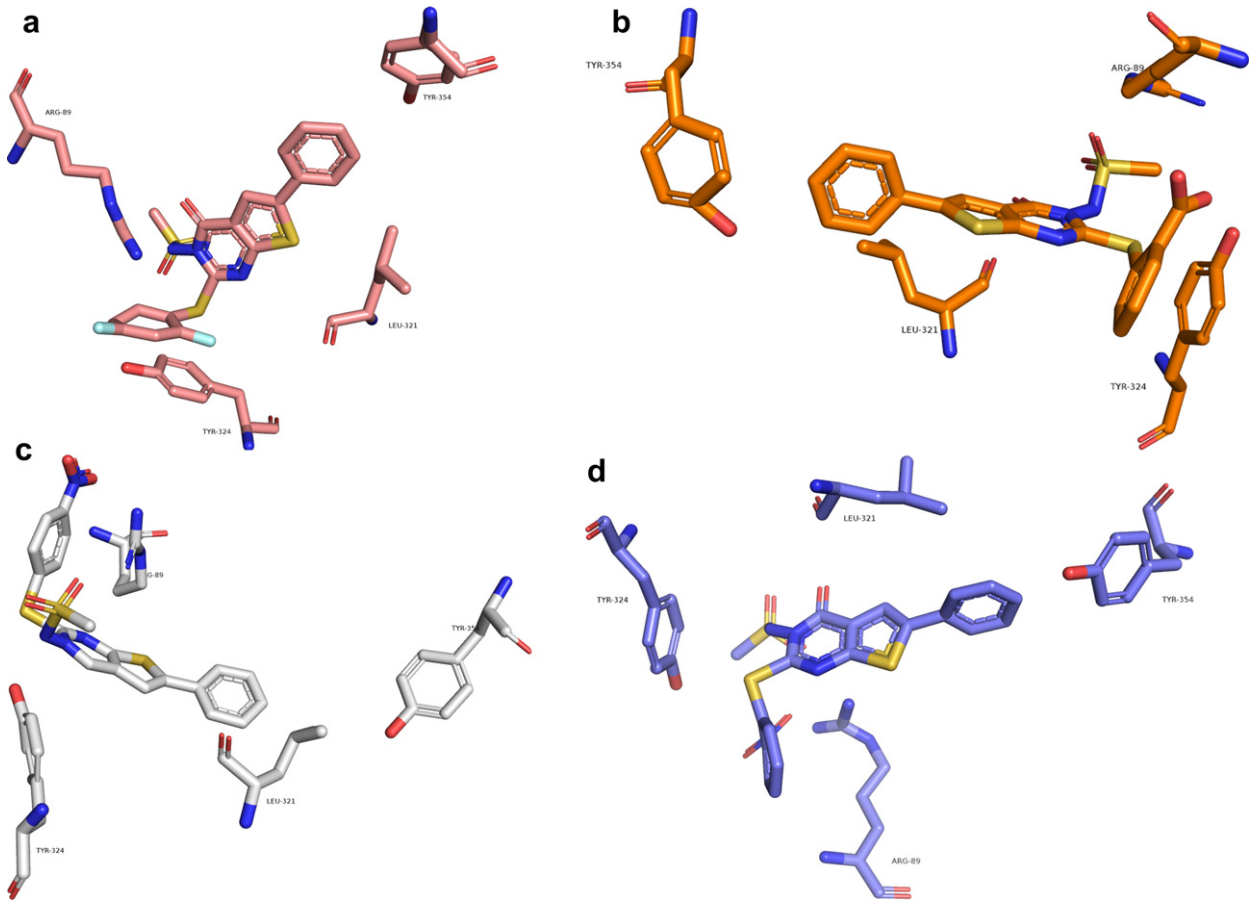


Fig. 9. Flexible docking of compounds **2**–**5** into hCOX-2. Best scoring poses relative to R89, L321, Y324, Y354, Y324. a) Compound **2**. b) Compound **3**. c) Compound **4**. d) Compound **5**.

activity of about 50%, proving to be COX-2 selective inhibitors. In the COX-2 assay the co-presence of these compounds with the ovine COX-2 causes a minor production of PGF2α.

3.5. Docking on hCOX-2

Overall the docking results were essentially in agreement with the biological data in terms of evaluation of interaction energy and docking score (Tables 1 and 2). The most active compounds **2**, **3** and **5** (Fig. 9), show the best interaction energies and highest scores; lowest interaction energies and docking score characterize the poses of compounds **11**, **23** and **31** (Tables 1 and 2). Those three compounds have shown the ability to increase PGE_2 levels probably due to the interaction with another cellular target. Compound **7** which needs further mechanistic studies to finally clarify on its activity (*vide infra*) which is seemingly out of the COX-2 as proved from the low *in vitro* inhibition was not docked.

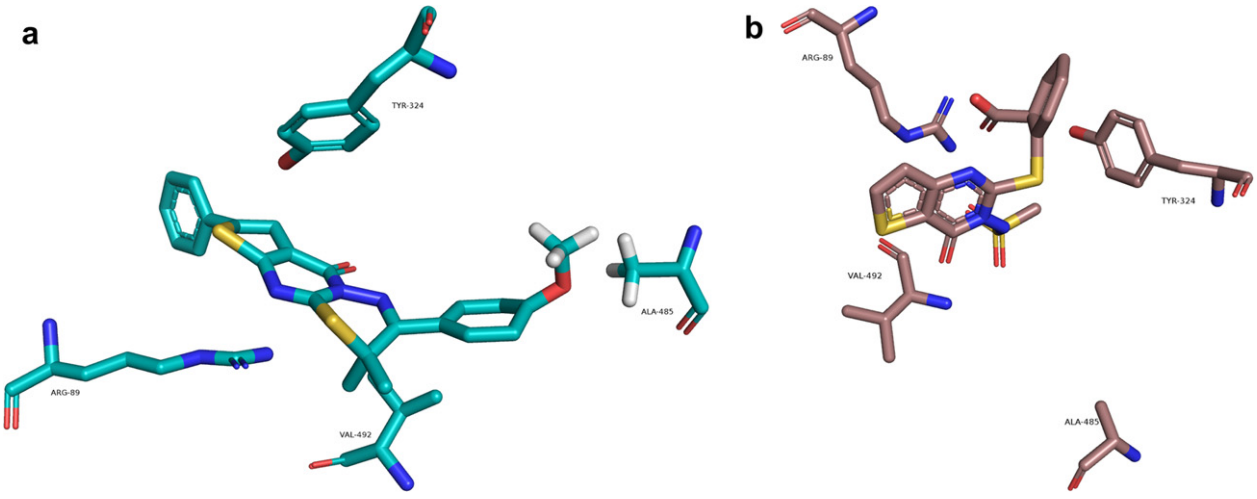


Fig. 10. Compounds **23** (a) and **31** (b) docked into hCOX-2.

Docking of compound **23** on the hCOX-2, was carried out in order to further test our docking protocol; the docking score and the interaction energy were lower than that of the active compounds **2**, **3**, **4** and **5** in agreement with the *in vitro* assays.

Taken all together the data (Table 2) show that the compounds interact by a favorable interaction with Val 318 and Ala 496, which significatively contribute formation of the binding. Hydrophobic interactions involving residues such as Leu, Val, Phe are also important. Binding modes of compounds **23** and **31** are characterized by a significative number of unfavorable interactions due to steric hindrance (Fig. 10). The anomalous PGE_2 promoting action of compounds **11** and **31** might be explained by an interaction with an alternate target.

4. Discussion

Inflammation is in the first instance a self-defense reaction that may, under specific circumstances, develop into a chronic state and become a causative factor in the pathogenesis of a broad range of pathologies. In many chronic and disabling diseases, regardless of their primary etiology, inflammation is the main therapeutic target and, often, the best choice to treat the disease and alleviate the symptoms. Although NSAIDs, which are non-selective COX inhibitors, have been very often used as painkillers [32,33], their side effects such as gastrointestinal hemorrhage remain a serious problem. Although the role of COX-1 seems to be important during neuroinflammation, and its inhibition could be a viable therapeutic approach to treat CNS diseases with a marked inflammatory component, we wanted to determine the effect of new derivatives only on COX-2 activity since the search for new COX-2 inhibitors without the increased risk of gastrointestinal events is a very actual and challenging issue. Newly synthesized sulfonilamidothiopyrimidone **2**–**5** inhibit the COX-2 activity. The antiinflammatory properties were assayed by evaluating the suppression of molecule proinflammatory as PGE_2 in human lymphocytes. PGE_2 synthesized from arachidonic acid by COX and synthases (PGES), acts as both an inflammatory mediator and fibroblast modulator [34]. PGE_2 is the lipid mediator of inflammation in diseases, such as rheumatoid arthritis and osteoarthritis, and is also involved in skin inflammation.

The subject compounds showed potent antiinflammatory activities in the culture of human PBLs. Among them, compounds **2**, **3**, **4**, and **5** have potent anti-inflammatory activity (more than 20% of PGE_2 inhibition), while compounds **23**, and **B43** exhibit marked potency (10–20%). The ability of the sulfonilamidothiopyrimidone derivatives to alter cell proliferation, which is crucial for generation of effective immune responses, was analyzed in the presence of the mitogen PHA. Interestingly, our findings indicate that any of the compounds tested altered significantly cell proliferation in mitogen treated PBLs.

Therefore, due to apparent formation of PGs in diseased tissue, considerable interest is focused on identifying natural and synthetic compounds that can reduce or prevent PGs formation.

Cryer and Feldman reported that cyclooxygenase enzymes are used to measure the anti-inflammatory effects of natural products [35]. Since, COX-2 enzyme is reported to be elevated in cells where inflammation has occurred, the inhibition of this enzyme by any compound could be regarded as one of the indices to judge the anti-inflammatory activity of that compound.

We report here that these inhibitions were partly due to the inhibition at protein expression levels, because COX-2-protein expression was significantly reduced by the treatment with sulfonilamidothiopyrimidone derivatives. Thus, our data suggest that some of these novel compounds tested could act as new synthetic COX-2 inhibitors.

Further evidences on a COX-2 selective inhibition came from *in vitro* screening assay. The cyclooxygenase-2 activity was notably reduced from some of the tested compounds **2**–**5** compared with the standard COX-2 selective inhibitor, celecoxib. These biological results suggest that these new compounds (**2**–**5**) with anti-inflammatory activities are the good candidate as selective anti-COX-2.

Developing drugs that interfere with signaling routes and gene expression, aiming at attenuating the pro-inflammatory response could prove to be a promising avenue.

Overall all the docking results were in agreement with the biological data. Preliminary semi-flexible docking calculations were carried out with MVD 4.2 (data not shown) but no relationship was found with experimental data. Setting flexible residues involved in the docking of Naproxen in mCOX-2 and softening the interaction potential has led to a strong correlation between biological and computational data suggesting a marked ligand-receptor adapting in the binding process. Compound **2** is the most effective inhibitor (inhibition over the 50%), and its predicted affinity is the highest within all the compounds.

Our model was able to predict the ranking of biological data in terms of docking score and binding energies. We found out that hydrophobic interactions with apolar residues of binding site of hCOX-2 are determinant for to have the final inhibitor–enzyme complex. Compounds **7**, **11**, **23** and **31** have bad contacts with polar residues in the pocket due to steric hindrance.

Acknowledgments

We thank Dr. Catherine Michaux (Department of Chemistry, University of Namur, Belgium) for the hCOX-2 structure and Dr. René Thomsen (Molegro ApS Denmark) for the technical support, the helpful suggestions and critically reading and correcting the manuscript. Prof. Giovanni Li Volti (Department of Drug Sciences of the University of Catania) is gratefully acknowledged for the instrumental support and the helpful discussion in the COX assay.

References

[1] I.G. Colditz, Margination and emigration of leucocytes, Surv. Synth. Pathol. Res. 4 (1985) 44–68.
[2] T. Kasama, R.M. Strieter, T.J. Standiford, M.D. Burdick, S.L. Kunkel, Expression and regulation of human neutrophil-derived macrophage inflammatory protein 1 alpha, J. Exp. Med. 178 (1993) 63–72.
[3] M. Gallin, K. Edmonds, J.J. Ellner, K.D. Erttmann, A.T. White, H.S. Newland, H.R. Taylor, B.M. Greene, Cell-mediated immune responses in human infection with *Onchocerca volvulus*, J. Immunol. 140 (1988) 1999–2007.
[4] H. Ohshima, M. Tatemichi, T. Sawa, Chemical basis of inflammation-induced carcinogenesis, Arch. Biochem. Biophys. 417 (2003) 3–11.
[5] W.L. Smith, I. Song, The enzymology of prostaglandin endoperoxide H synthases-1 and -2, Prostaglandins Other Lipid Mediat. 68–69 (2002) 115–128.
[6] J.R. Vane, The fight against rheumatism: from willow bark to COX-1 sparing drugs, J. Physiol. Pharmacol. 51 (2000) 573–586.
[7] L. Parente, M. Perretti, Advances in the pathophysiology of constitutive and inducible cyclooxygenases: two enzymes in the spotlight, Biochem. Pharmacol. 65 (2003) 153–159.
[8] K. Abouzid, S.A. Bekhit, Novel anti-inflammatory agents based on pyridazinone scaffold; design, synthesis and in vivo activity, Bioorg. Med. Chem. 16 (2008) 5547–5556.
[9] Z. Feng, F. Chu, Z. Guo, P. Sun, Synthesis and anti-inflammatory activity of the major metabolites of imrecoxib, Bioorg. Med. Chem. Lett. 19 (2009) 2270–2272.
[10] J.M. Dogne, C.T. Supuran, D. Pratico, Adverse cardiovascular effects of the coxibs, J. Med. Chem. 48 (2005) 2251–2257.
[11] I. Moodley, Review of the cardiovascular safety of COXIBs compared to NSAIDS, Cardiovasc. J. Afr. 19 (2008) 102–107.
[12] F.H. Sarkar, S. Adsule, Y. Li, S. Padhye, Back to the future: COX-2 inhibitors for chemoprevention and cancer therapy, Mini Rev. Med. Chem. 7 (2007) 599–608.
[13] M. Nivsarkar, A. Banerjee, H. Padh, Cyclooxygenase inhibitors: a novel direction for Alzheimer's management, Pharmacol. Rep. 60 (2008) 692–698.
[14] A. Santagati, A. Marrazzo, G. Granata, Synthesis of potential selective COX-2 enzyme inhibitors derived from ethyl ester of 2-isothiocyanato-5-phenyl-3-thiophenecarboxylic acid, J. Heterocyclic Chem. 40 (2003) 869–873.

[15] G. Dannhardt, W. Kiefer, Cyclooxygenase inhibitors – current status and future prospects, Eur. J. Med. Chem. 36 (2001) 109–126.
[16] F. Russo, A. Santagati, M. Santagati, A. Caruso, S. Trombadore, M. Amico-Roxas, Synthesis and pharmacological properties of benzothienothiadiazolopyrimidine derivatives. III, Farmaco Sci. 42 (1987) 437–447.
[17] A. Santagati, G. Granata, A. Marrazzo, M. Santagati, Synthesis and effects on the COX-1 and COX-2 activity in human whole blood ex vivo of derivatives containing the [1]benzothienol-[3, 2-d]pyrimidin-4-one heterocyclic system, Arch. Pharm. (Weinheim) 336 (2003) 429–435.
[18] F. Russo, M. Santagati, A. Santagati, M. Amico-Roxas, R. Bitetti, A. Russo, [Chemical and pharmacological research on derivatives of 2,7-substituted 5H-1,3,4-thia(oxa)diazol[2,3-b]quinazolin-5-one], Farmaco Sci. 36 (1981) 292–301.
[19] F. Russo, M. Santagati, A. Santagati, G. Blandino, [Synthesis and antimycotic activity of derivatives of 1, 3, 4-thiadiazole (3,2a) pyrimidone], Farmaco Sci. 36 (1981) 983–993.
[20] R. Thomsen, M.H. Christensen, MolDock: a new technique for high-accuracy molecular docking, J. Med. Chem. 49 (2006) 3315–3321.
[21] Molegro ApS, Hoegh-Guldbergs Gade 10, Bldg. 1090, DK-8000 Aarhus C, Denmark.
[22] A.W. Schuttelkopf, D.M. van Aalten, PRODRG: a tool for high-throughput crystallography of protein–ligand complexes, Acta Crystallogr. D Biol. Crystallogr. 60 (2004) 1355–1363.
[23] K.C. Duggan, M.J. Walters, J. Musee, J.M. Harp, J.R. Kiefer, J.A. Oates, L.J. Marnett, Molecular basis for cyclooxygenase inhibition by the non-steroidal anti-inflammatory drug naproxen, J. Biol. Chem. 285 (2010) 34950–34959.
[24] C. Michaux, C. Charlier, F. Julemont, X. de Leval, J.M. Dogne, B. Pirotte, F. Durant, A new potential cyclooxygenase-2 inhibitor, pyridinic analogue of nimesulide, Eur. J. Med. Chem. 40 (2005) 1316–1324.
[25] C. Michaux, X. de Leval, F. Julemont, J.M. Dogne, B. Pirotte, F. Durant, Structure-based pharmacophore of COX-2 selective inhibitors and identification of original lead compounds from 3D database searching method, Eur. J. Med. Chem. 41 (2006) 1446–1455.
[26] C. McMartin, R.S. Bohacek, QXP: powerful, rapid computer algorithms for structure-based drug design, J. Comput. Aided Mol. Des. 11 (1997) 333–344.
[27] M.A. Iniguez, C. Punzon, M. Fresno, Induction of cyclooxygenase-2 on activated T lymphocytes: regulation of T cell activation by cyclooxygenase-2 inhibitors, J. Immunol. 163 (1999) 111–119.
[28] A. Santagati, M. Modica, M. Santagati, A. Caruso, V. Cutuli, Synthesis of 2,3-dihydro-3-amino-6-phenyl-2-thioxothieno [2,3-d] pyrimidin-4 (1H)-one and of potential antiinflammatory agents 2-aryl-7-phenyl-3H, 9H-pyrimido [2,1-b]thieno-[2′,3′:4,5]]1,3,4] thiadiazin-9-ones, Pharmazie 49 (1994) 64–65.
[29] G. Granata, S. Barbagallo, A. Perdicaro, A. Marrazzo, A. Santagati, L. Lombardo, V. Cardile, Synthetic approaches to bridgehead nitrogen methanesulfonamide derivatives of 3-amino-2-thioxo-2,3-dihydrothieno[2,3-d]pyrimidin-4(1H)-ones, potential COX-2 selective inhibitors, J. Heterocyclic Chem. 43 (2006) 1099–1104.
[30] Y.M. Zhu, N.S. Azahri, D.C. Yu, P.J. Woll, Effects of COX-2 inhibition on expression of vascular endothelial growth factor and interleukin-8 in lung cancer cells, BMC Cancer 8 (2008) 218.
[31] K.G. Coggins, A. Latour, M.S. Nguyen, L. Audoly, T.M. Coffman, B.H. Koller, Metabolism of PGE2 by prostaglandin dehydrogenase is essential for remodeling the ductus arteriosus, Nat. Med. 8 (2002) 91–92.
[32] L.J. Mengle-Gaw, B.D. Schwartz, Cyclooxygenase-2 inhibitors: promise or peril? Mediators Inflamm. 11 (2002) 275–286.
[33] J.Y. Park, M.H. Pillinger, S.B. Abramson, Prostaglandin E2 synthesis and secretion: the role of PGE2 synthases, Clin. Immunol. 119 (2006) 229–240.
[34] V.C. Sandulache, A. Parekh, H. Li-Korotky, J.E. Dohar, P.A. Hebda, Prostaglandin E2 inhibition of keloid fibroblast migration, contraction, and transforming growth factor (TGF)-beta1-induced collagen synthesis, Wound Repair Regen. 15 (2007) 122–133.
[35] B. Cryer, M. Feldman, Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs, Am. J. Med. 104 (1998) 413–421.