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Thiazole derivatives as inhibitors of cyclooxygenases *in vitro* and *in vivo*

Ghewa A. El-Achkar [a,b], Mariam Jouni [c], May F. Mrad [a], Taghreed Hirz [a], Nehme El Hachem [d], Ali Khalaf [e], Soukaina Hammoud [e], Hussein Fayyad-Kazan [f], Assaad A. Eid [g], Bassam Badran [h], Raghida Abou Merhi [c], Ali Hachem [e], Eva Hamade [c,*], Aïda Habib [a,i,**]

[a] *Department of Biochemistry and Molecular Genetics, Faculty of Medicine, AUB, Beirut, PO Box 11-236, Lebanon*
[b] *INSERM U955, Equipe 12, Faculty of Medicine, University Paris-Est, Creteil, France*
[c] *Genomic and Health Laboratory ER 031/PRASE-EDST, Faculty of Sciences, Lebanese University, Beirut, Lebanon*
[d] *Bioinformatics and Computational Genomics Laboratory, Institut de Recherches Cliniques de Montréal, University of Montreal, Montreal, Quebec, Canada*
[e] *Département de Chimie et de Biochimie, Laboratoire de Chimie Médicinale et des Produits Naturels & PRASE, EDST Lebanese University, Hadath, Lebanon*
[f] *Laboratory of Experimental Hematology, Institut Jules Bordet, Université Libre de Bruxelles, 121, Boulevard de Waterloo, 1000 Bruxelles, Belgium*
[g] *Department of Anatomy, Cell Biology and Physiological Sciences, American University of Beirut, PO Box 11-236, Lebanon*
[h] *Laboratory of Immunology/EDST-PRASE, Lebanese University, Faculty of Sciences, Hadath, Beirut, Lebanon*
[i] *Centre de recherche sur l'inflammation, INSERM UMR 1149-Université Paris Diderot, Faculté de Médecine Xavier Bichat, 16 rue Henri Huchard, F-75018 Paris, France*

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ABSTRACT

Cyclooxygenases (COXs) are important membrane-bound heme containing enzymes important in platelet activation and inflammation. COX-1 is constitutively expressed in most cells whereas COX-2 is an inducible isoform highly expressed in inflammatory conditions. Studies have been carried out to evaluate thiazole derivatives as anti-inflammatory molecules. In this study, we investigated the *in vitro* and *in vivo* effects of two novel thiazole derivatives compound **1** (N-[4-(4-hydroxy-3-methoxyphenyl)-1,3-thiazol-2-yl] acetamide) and compound **2** (4-(2-amino-1,3-thiazol-4-yl)-2-methoxyphenol) on prostaglandin E_2 (PGE_2) production and COX activity in inflammatory settings. Our results reveal a potent inhibition of both compound **1** (IC50 9.01 ± 0.01 μM) and **2** (IC50 11.65 ± 6.20 μM) (Mean ± S.E.M.) on COX-2-dependent PGE_2 production. We also determined whether COX-1 activity was inhibited. Using cells stably over-expressing COX-1 and human blood platelets, we showed that compound **1** is a specific inhibitor of COX-1 with IC50 ($5.56 \times 10^{-8} \pm 2.26 \times 10^{-8}$ μM), whereas compound **2** did not affect COX-1. Both compounds exhibit anti-inflammatory effect in the dorsal air pouch model of inflammation as shows by inhibition of PGE_2 secretion. Modeling analysis of docking in the catalytic site of COX-1 or COX-2 further confirmed the difference in the effect of these two compounds. In conclusion, this study contributes to the design of new anti-inflammatory agents and to the understanding of cyclooxygenase inhibition by thiazole.

1. Introduction

Cyclooxygenases (COXs) are enzymes responsible for the synthesis of prostanoids that have inflammatory and thrombotic effects (Julemont et al., 2004; Patrono et al., 1985; Schror, 1997). COX-1 is the constitutive isoform important for maintaining homeostasis whereas COX-2 is the inducible isoform, highly upregulated by pro-inflammatory mediators. Accumulation of COX-2 protein and increase in prostaglandin (PG) E_2 production are associated with the onset and progression of arthritic diseases (Crofford et al., 1994; Martel-Pelletier et al., 1999). Despite the beneficial effect of COX-2 inhibition, many undesirable side effects persist including gastric irritation result from the inhibition of COX-1 isoform (Abouzid and Bekhit, 2008; Masferrer et al., 1994).

Selective inhibition of COX-2 has potential clinical benefits in cancer and neurological disorders. Novel drug design is carried out with the aim to unveil the therapeutic value of thiazole derivatives which are heterocycles compounds having frequent biological interest.

Thiazoles, or 1,3-thiazoles are five member ring heterocyclic organic compounds with three carbon, one sulfur, and one nitrogen atoms. They are important non-carcinogenic molecules that can be easily metabolized by routine biochemical reactions and exhibit a variety of biological activities manifested by their

* Corresponding author.
** Corresponding author at: Department of Biochemistry and Molecular Genetics, Faculty of Medicine, AUB, Beirut, PO Box 11-236, Lebanon.
E-mail addresses: eva.hamade@ul.edu.lb (E. Hamade), aida.habib@inserm.fr (A. Habib).

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presence in many potent biologically active compounds (Apostolidis et al., 2013; Basile et al., 2012). Woods et al. (2001) demonstrated a potent and selective inhibition of human recombinant COX-2 by a series of thiazole analogs of indomethacin prepared by chemical substitution of carboxyl group with various thiazoles substitutes. A more potent inhibition was observed when the carboxyl group were replaced by an aromatic substitution on the 4-position of the thiazole. These observations, in addition to other reports, strongly suggest that thiazole derivatives when manipulated produce potent anti-inflammatory drugs (Li et al., 2005; Woods et al., 2001).

Recently, we showed that the two synthesized thiazole derivatives (N-[4-(4-hydroxy-3-methoxyphenyl)-1,3-thiazol-2-yl] acetamide) and (4-(2-amino-1,3-thiazol-4-yl)-2-methoxyphenol) significantly inhibited prostaglandin production in lipopolysaccharide (LPS)-treated RAW 264.7 cells (Hamade et al., 2012). In the present study we modified the (N-[4-(4-hydroxy-3-methoxyphenyl)-1,3-thiazol-2-yl] acetamide) molecule by substituting with 2 different side chains R=C_4H_9 for the compound **1** and R=CH_2Ph for the compound **2** and assessed its inhibitory activity on both COX-1 and COX-2 on cells in culture and in mice model of inflammation. Modeling analysis was also performed. We found that compound **1** blocked both COX-2 and COX-1 whereas compound **2** was more selective for COX-2.

2. Material and methods

2.1. Reagents

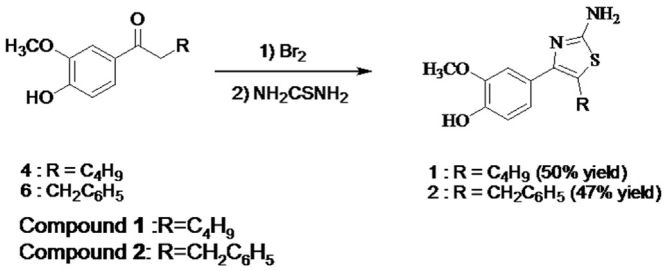
Cell viability assay kit (WST-1) was from Roche Diagnostic (Mannheim, Germany). Ibuprofen was purchased from Calbiochem. NS 398, LPS, anti-β-actin, zymosan A, and arachidonic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). Heat inactivated fetal bovine serum (FBS), penicillin-streptomycin, DMEM, G418 and Dulbecco's phosphate buffered Saline (PBS) were obtained from Invitrogen. Anti-mouse IgG-HRP antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Bradford protein assay reagent and bovine serum albumin (BSA) were from Bio-Rad (Hercules, California, USA). Enhanced chemiluminescence (Amersham, Buckinghamshire, UK). Monoclonal anti COX-1 (COX 111) and anti-COX-2 (COX 214) were raised as previously described (Habib et al., 1993).

2.2. Cell culture

HEK-293 and RAW 264.7 cells (American Type Culture Collection, Rockville, MD, USA) were cultured in DMEM supplemented with 10% FBS, 1% penicillin and 1% streptomycin in a 5% CO_2 humidified incubator at 37 °C. Cells were plated in 6 or 12 well plates for 24 h and pre-treated with different concentrations of inhibitors and/or LPS in serum-free condition. DMSO was used as vehicle and did not exceed 0.1% (v:v).

2.3. Cell viability

Compounds **1** and **2**, which are thiazole derivatives, are shown in Scheme 1. Their preparation is described in Supplementary materials. Cell viability was assessed on cells according to the manufacturer's instructions and expressed as percentage of untreated cells. In brief, 5×10^4 cells were plated in 96-well plates and treated with different concentrations of inhibitors for 24 h.



Scheme 1. Preparation of amino-thiazoles from their corresponding ketones.

2.4. Cyclooxygenase activity

COX activity was tested in RAW cells as described previously (Hamade et al., 2012). Briefly, cells were treated with LPS 100 ng/ml for 24 h washed and incubated with compound **1** or **2** at different concentrations (0.1, 5, 10 and 25 μM) or 10 μM of NS 398 for 30 min in Hanks buffer. 10 μM of arachidonic acid was then added for an additional 30 min. Supernatants were collected and PGE_2 was measured by enzymeimmunoassay (EIA) (Pradelles et al., 1985). Cells were lysed with RIPA buffer (50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P40, 0.5% sodium deoxycholate, 4% protease inhibitors and 1% phosphatase inhibitors) and COX-2 protein levels were detected by western blot as previously described (Habib et al., 1993).

2.5. Blood platelet aggregation

Venous blood was obtained from healthy volunteers who have not ingested any drugs for the last 14 days and after informed consent in accordance with the Institutional Review Board (IRB) of the American University of Beirut (Approval # BioCh.AH.03). The effect of compounds **1** and **2** were tested on the platelet aggregation of washed platelets as described previously (Hamade et al., 2012).

2.6. Over-expression of human recombinant COX-1 in HEK-293 cells and assessment of COX-1 activity

To assess the effect of compounds **1** and **2**, HEK-293 cells stably over expressing human COX-1 were obtained after transfecting HEK-293 with 10 μg of human COX-1 cDNA (kind gift of Colin Funk, Queen's University, Ontario, Canada) (Funk et al., 1991). Cells were maintained for 3 weeks in culture media containing 1 mg/ml G418 and further selected and characterized for enzymatic activity and protein expression of COX-1. Cells were pre-treated in the absence or presence of different concentrations of compounds **1**, **2** or ibuprofen 25 μM in Hanks buffer (pH 7.4), containing 1 mg/ml BSA for 30 min prior to the addition of 25 μM arachidonic acid. After 30 min incubation, PGE_2 was measured in the supernatants. COX-1 protein was determined by western blot in protein extracts (Habib et al., 1993).

2.7. Mice sterile-air pouch model of inflammation

Female C57BL/6 mice (8 weeks) were obtained from the animal care facility at the American University of Beirut. All animal procedures were performed following the recommendations of the IACUC (Institutional Animal Care and Use Committee approval # 12-10-238).

The sterile air pouch model was adapted from Paya et al. (1996) with modifications. The mice were anesthetized with isoflurane and 5 ml volume of sterile air was injected in the subcutaneous tissue of the dorsal midline, below the scapula (Fig. 1). The pouch

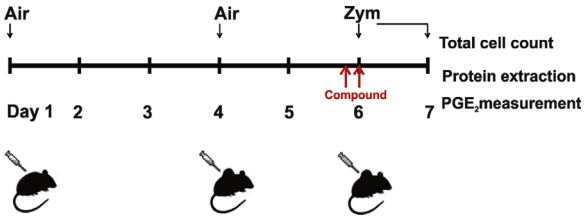


Fig. 1. Outline of the air pouch experiments. On day 1, 5 ml of sterile air was injected in the subcutaneous tissue of the dorsal midline, below the scapula. The pouch was re-inflated with an additional 2.5 ml of sterile air on day 4, to maintain the cavity. On the sixth day after the initial air injection, 0.5 ml of 1% w/v zymosan was injected into the pouch cavity. 24 h after zymosan administration, the mice were sacrificed and the exudates from each mouse were collected by washing the pouch with 1 ml of Hanks buffer containing 0.315% trisodium citrate. The collected exudates (1 ml) contain the recruited inflammatory cells and resident cells. The total number of cells was counted. The exudates were centrifuged at 300*g* for 5 min at +4 °C. The supernatants were collected for PGE_2 measurement. The cell pellets were lysed for protein extraction.

was re-inflated with an additional 2.5 ml of sterile air on day 4, to maintain the cavity. On day 6 after the initial air injection, the mice were injected with 0.5 ml of 1% zymosan A directly into the pouch in order to induce local inflammation. 0.5 ml of compound **1** or **2** (0.7 mg/kg, eq. to 100 μM in an air pouch injected with 0.5 ml total volume) were injected twice in the pouch: two hours prior to zymosan and together with zymosan injection. 24 h after zymosan administration, the mice were sacrificed and the cellular exudate from each mouse was collected by washing the air pouch with 1 ml of Hanks buffer containing 0.315% trisodium citrate. Cells were counted in the collected exudates (1 ml) and further centrifuged at 300*g* for 5 min at 4 °C. PGE_2 was measured in the supernatant as described above and cell pellet were lysed for protein COX analysis.

2.8. Modeling analysis

The 3D structure of COX-2 complexed with celecoxib (PDBID:1EQG) and the ovine COX-1 complexed with ibuprofen (PDBID:1EQG) were selected for docking simulations (Hamade et al., 2012). Water and other heteratoms were removed from the structure. Chain A was retained including ibuprofen and heme group. Hydrogen atoms were added, atom typing and partial charges were assigned using AMBER forcefield (Feng et al., 2009). The coordinates of the binding site were extracted using the co-crystallized ligand, ibuprofen. Docking and scoring: low energy conformations of the chemical compounds were generated using catalyst (Accelrys, Inc.)

The (R)-groups of compounds **1** and **2** were selected; docking simulations were carried out using Autodock 4.2. Each docking simulation was achieved with 10 docking runs with 150 individuals using the Lamarckian genetic algorithm implemented in Autodock and 250,000 energy evaluations. The binding energies were estimated from a new free-energy scoring function based on the AMBER force field, an updated charge-based desolvation term and improved models of the unbound state. The best poses were analyzed and visualized with Discovery Studio visualizer (Accelrys, Inc.).

2.9. Data analysis

Aggregation data was expressed after defining the slope for each aggregation curve, which reflects the rate of platelet reaction, using the Born's method (Li et al., 2005). Curve fitting and calculation of the IC50 values were done using Grafit 7 software (Erithacus software, Staines, UK). PGE_2 concentration in ng/ml was expressed as mean ± S.E.M. for at least 3 different experiments and statistical analysis was performed using *t*-test in Sigma Plot (Systat Software Inc., San Jose, CA), P-values < 0.05 were considered significant. Autoradiograms obtained from western blot analyses were scanned using Epson 1680 pro scanner and the band intensities were quantified by Image J software (NIH, USA).

3. Results

3.1. Compounds **1** and **2** inhibit PGE_2 release in RAW 264.7 cells

Treatment of the Raw 264.7 cells with compound **1** at different concentrations (0.1, 5, 10, 25 μM) inhibited significantly LPS-dependent release of PGE_2 compared to LPS-treated cells. There was a concentration dependent effect starting at 5 μM with a maximal inhibitory effect at 25 μM (Fig. 2A). Similarly, treatment of cells with compound **2** showed an inhibitory effect starting at 10 μM with

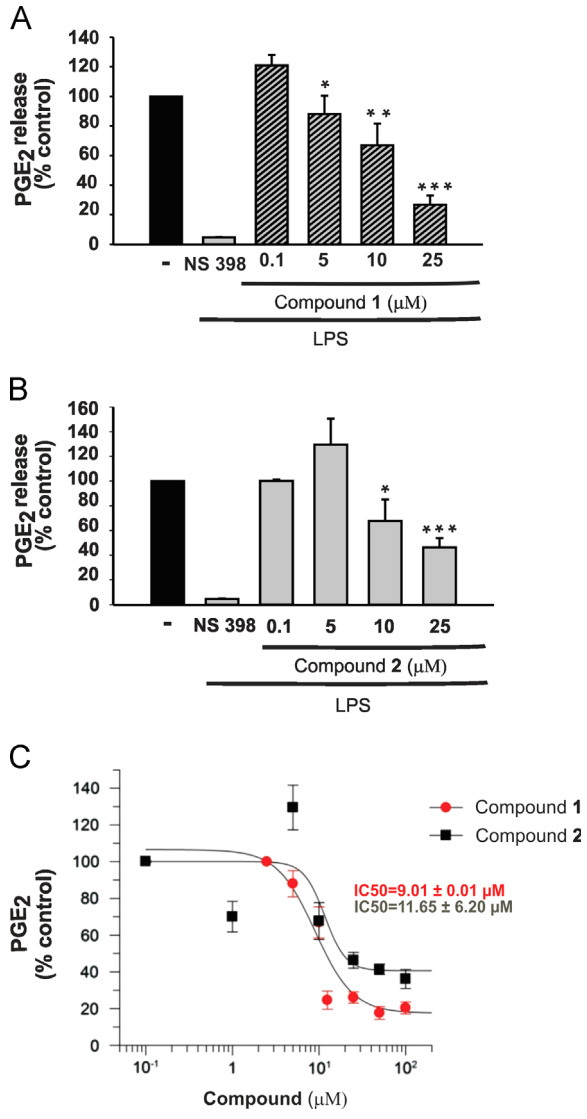


Fig. 2. Dose dependant inhibition of prostaglandin E_2 production by compounds **1** and **2**. (A) and (B) RAW 264.7 cells stimulated with 100 ng/ml of LPS for 24 h were incubated with Hanks buffer and treated for 30 min with compounds **1** and **2** at increasing concentration (0.1, 5, 10 and 25 μM) or with 10 μM of NS 398 and 20 μM of arachidonic acid for additional 30 min. Results are expressed as percentage of secreted PGE_2 in ng/ml and correspond to mean ± S.E.M. of 3 different experiments. (C) Curve fitting for the PGE_2 values obtained from (A) was done using Grafit software. ($n=3$; $^*P<0.05$, $^{***}P<0.001$ *vs* LPS-treated cells).

a maximal inhibitory effect obtained at 25 μM (Fig. 2B). NS 398 a selective COX-2 inhibitor, showed a strong inhibition of PGE_2 release. Both compounds **1** and **2** showed a similar IC50 (9.01 ± 0.01 μM and 11.65 ± 6.20 μM for compounds **1** and **2,** respectively) (Fig. 2C).

3.2. *Compounds **1** and **2** do not modulate LPS-induced COX-2 expression in RAW 264.7 macrophages*

In parallel, we checked whether compound **1** or **2** modified COX-2 expression. Different concentrations of compounds **1** and **2** (5, 10 and 25 μM) were tested on LPS-dependent induction of COX-2. Western blot results revealed no modulation of COX-2 expression by either compound **1** or **2** in RAW 264.7 cells (Fig. 3). Using WST-1 assay, we further show that compound **1** and compound **2** did not affect the viability of the cells (Fig. 4).

3.3. *Compound **1** but not compound **2** inhibits blood platelet aggregation*

We next evaluated the capacity of these compounds to block platelet aggregation. Platelets were pre-treated for 1 min at 37 °C prior to the addition of 25 μM of arachidonic acid and aggregation was followed for 3 min. We first tested high concentrations (6.25, 12.5, and 25 μM). The compound **2** did not show any inhibition of arachidonic acid-dependent platelet aggregation. Compound **1** (3, 10, and 100 nM) with pentyl side chain showed a potent inhibition. Ibuprofen, a well described COX-1/COX-2 non-selective inhibitor, which strongly block platelet aggregation was used as a positive control (Fig. 5) (Hirz et al., 2012).

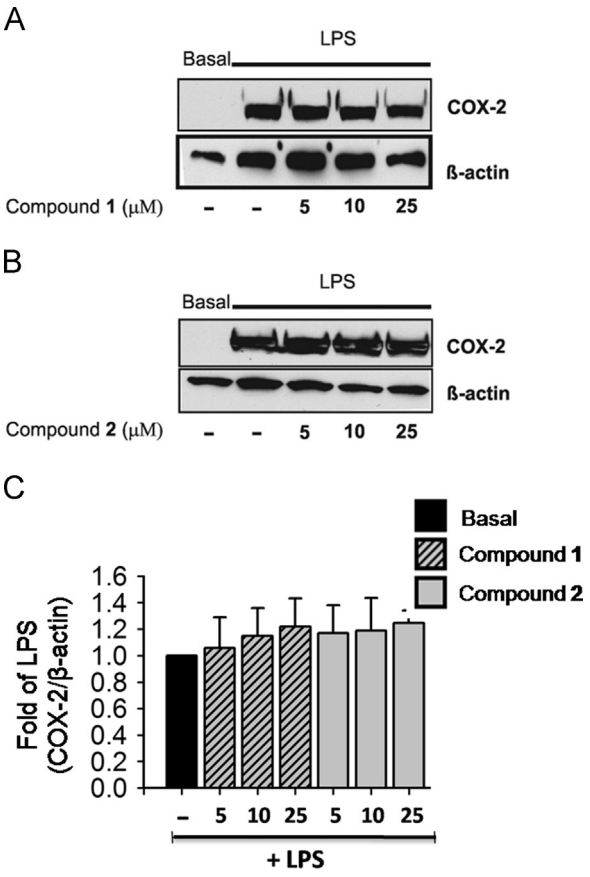


Fig. 3. Compounds **1** and **2** do not modulate COX-2 protein level induced by LPS. RAW 264.7 cells were stimulated with 100 ng/ml LPS and compounds **1(A)** or **2(B)** at 5, 10 and 25 μM, respectively for 24 h. Cells were lysed and analyzed by western blotting using anti-COX-2 and β-actin antibody. Results are representative of 3 different experiments. (C) Protein bands were scanned and its intensity quantified using ImageJ software.

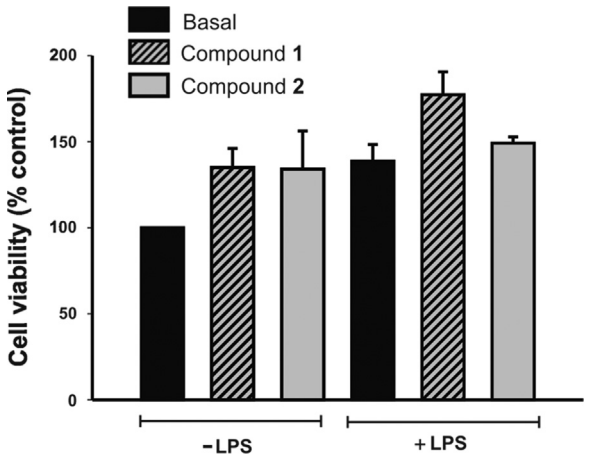


Fig. 4. Cellular viability of RAW 264.7 cells following treatment with compounds **1** and **2**. RAW 264.7 cells were incubated with 25 μM of compound **1** or **2** for 24 h. Cell viability was assessed by WST-1 in comparison to control untreated cells. Results are representative of 3 different experiments performed in triplicates.

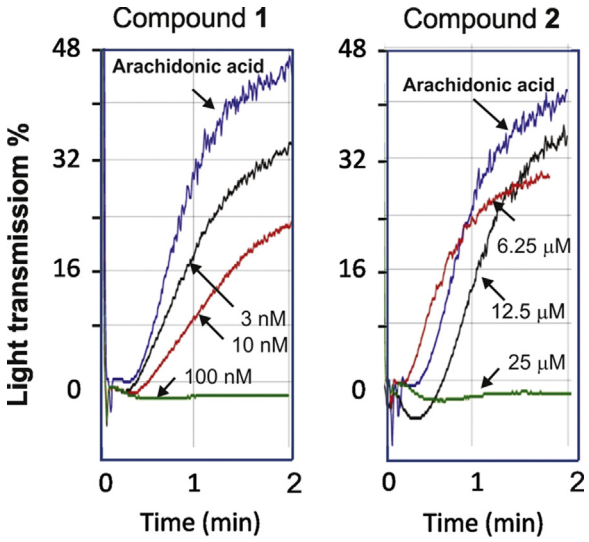


Fig. 5. Aggregation curves of compounds **1** and **2**. Platelet aggregation was performed on human blood washed platelets. Platelets (0.4×10^9plt/ml) were treated with increasing concentrations of compound **1** or **2** for 1 min at 37 °C prior to the addition of 25 μM of arachidonic acid for 3 min under stirring conditions. Results represent aggregation curves of compound **1** and compound **2** and are representative of 4 different experiments.

3.4. *Compound **1** but not compound **2** inhibits PGE_2 levels in HEK-293 cells stably-overexpressing human COX-1*

To further confirm that compound **1** was targeting COX-1 and not other intermediate in targets, we tested the compounds on recombinant COX-1. HEK-293 cells over-expressing human COX-1 were obtained. In these cells, arachidonic acid is metabolized into PGH_2, which is sequentially hydrolyzed into PGE_2. Measurement of PGE_2 under these conditions reflects COX-1 activity. Results showed that compound **1** exerted a strong and significant inhibition of PGE_2 synthesis in HEK-293 over-expressing human COX-1 (IC50 was $5.56 \times 10^{-8} \pm 2.26 \times 10^{-8}$ μM, $n=3$) (Fig. 6A and B), while compound **2** did not show a significant inhibition (data not shown). Ibuprofen strongly blocked COX-1 activity (Fig. 6A). By assessing both platelet aggregation and enzyme activity of

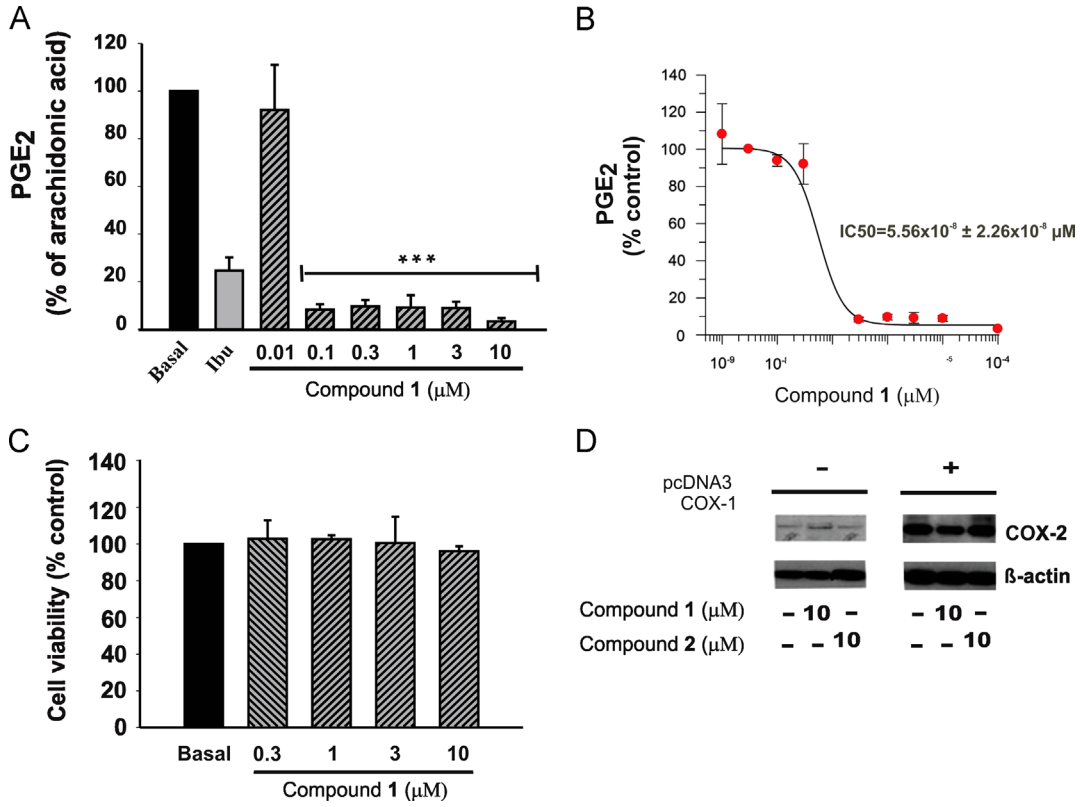


Fig. 6. Inhibition of COX-1 activity. (A) HEK-293 cells stably overexpressing COX-1 were treated for 30 min in the absence or presence of different concentrations of compound **1** and **2** or ibuprofen 25 μM prior to the addition of 25 μM arachidonic acid as described in Section 2. Results are expressed as percentage of the secreted PGE_2 and correspond to mean ± S.E.M. of 3 different experiments (***$P < 0.001$ *vs* LPS-treated cells). (B) HEK-293 cells stably overexpressing COX-1 were treated with different concentrations of compound **1** (0.1, 1, 3, and 10 μM) for 24 h. Cell viability was assessed in comparison to untreated cells as described in the legend for Fig. 4. Results are representative of 3 different experiments. (C) Western blot analysis of COX-1 using selective monoclonal COX-1 in untransfected and pcDNA3-COX-1transfected cells. Results are representative of 3 different experiments. (D) Curve fitting for the PGE_2 values obtained from (A) was done using Grafit software.

recombinant COX-1, we further confirm that compound **1** and not compound **2** blocks COX-1.

Viability of HEK-293 overexpressing cells was not affected during the treatment as shown by WST-1 assay even when compound **1** was incubated for 24 h at high concentrations (Fig. 6C). Moreover, COX-1 protein expression in HEK-293 cells was not modified after treatment with compounds **1** and **2** compared to untreated cells (Fig. 6D).

3.5. *In vivo effect of compounds **1** and **2** on PGE_2 formation and COX-2 expression*

We next investigated the anti-inflammatory effects of compounds **1** and **2** *in vivo* in a mouse model of inflammation. When saline was injected in the air pouch, there was a low accumulation of cells in the air pouch exudates. Injection of zymosan increased the total cell number (Fig. 7A). Compounds **1** and **2** did not change cell infiltration in the pouch exudates (Fig. 7A). The injection of zymosan for 24 h increased PGE_2 levels in exudates from zymosan-injected mice compared to saline alone. The intrapouch injection of 100 μM of compound **1** or **2** decreased significantly PGE_2 levels by 51% and 45% ($P \leq 0.01$, unpaired *t*-test) respectively (Fig. 7B). No effect on COX-2 protein expression was observed (Fig. 7C). Ibuprofen, the COX-1/COX-2 non-selective inhibitor, also decreased PGE_2 formation and was used as a positive control.

3.6. *Modeling analysis*

We finally carried out modeling analysis of compounds **1** and **2** with ovine COX-1 and COX-2 to examine how these compounds dock to the active site of the enzymes, and to determine the amino acids of the active site of the enzymes involved in the interaction with these compounds. Ibuprofen docked into COX-1 and as shown previously (Hirz et al., 2012), the carboxyl group of ibuprofen showed three hydrogen bonds, two with Arginine 120 (guanidine –NH2 group) and one with Tyrosine 355 (p-OH group).

Compounds **1** and **2** were docked nearby Tyrosine 355 and Arginine 120 of the COX-1 catalytic site where compound **1** was found to exhibit three hydrogen bonds one between the methoxy group and OH group of the side chain of Tyrosine 355 (active site of COX-1), two between NH2 group and Serine 530, and Tyrosine 385 amino acids (Fig. 8A). On the other hand, compound **2** was found to exhibit two hydrogen bonds, one between the methoxy group and Arginine 120 (side chain) of active site of COX-1 and the other between amine group and Leucine 352 of COX-1 (amide bond) (Fig. 8B).

The binding scores of compounds **1** and **2** were −6.2 kcal/mol and −6.9 kcal/mol respectively. These scores were comparable to ibuprofen (−8 kcal/mol), which served as a positive control for COX inhibition. Despite the comparable scores of compounds **1** and **2** to ibuprofen, compound **2** did not affect platelet aggregation due to the absence of hydrogen bonding with Tyrosine 355 (Hirz et al., 2012) that indispensible for platelet aggregation/COX-1 activity. A possibility for the absence of this hydrogen bonding can be due to the shielding of the benzyl group.

Compounds **1** and **2** were docked nearby the catalytic site of COX-2 where compound **1** was found to exhibit one hydrogen bond between the amine group and oxygen of peptide bond of Leucine 338 (active site of COX-2) (Fig. 9A), while compound **2** was found to exhibit two hydrogen bonds one between the amine group and oxygen of peptide bond of Methionine 508 (active site of COX-2), and the other between the hydroxyl group and Serine 339 of COX2 (oxygen of amide bond)

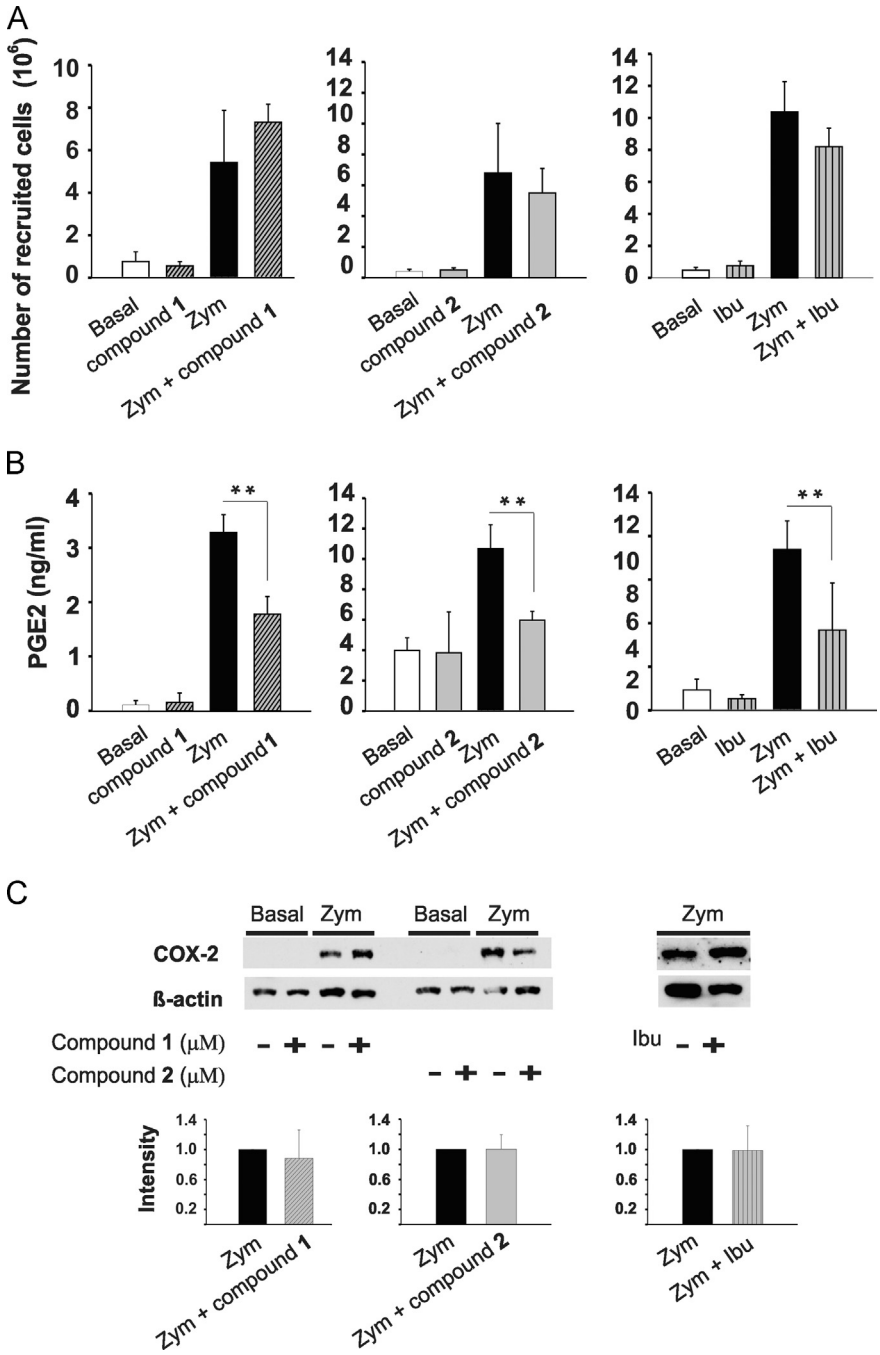


Fig. 7. Effect of compounds **1** and **2** on PGE_2 levels and COX-2 expression in zymosan-injected air pouch model. Drugs were administered two hours before and with zymosan. 24 h after zymosan the total number of cells was counted (A) and PGE_2 levels were measured in exudates using enzymeimmuno assay (B). Cell pellet was lysed using Laemmli buffer solution. Western blotting was performed using COX-2 and β-actin antibodies. Protein band signals were developed and scanned using Epson 1680 pro scanner (C). Data represent means ± S.E.M. (n=4–6 animals); $^{*}P \leq 0.05$ $^{**}P \leq 0.01$ $^{***}P \leq 0.001$. Zym=zymosan/Ibu=ibuprofen.

(Fig. 9B). The docking of celecoxib, known to be a positive inhibitor of COX-2, showed many hydrogen bondings among which are those between the amine group, Leucine 338 (amide bond), Serine 339 side chain, and Glutamine 178 (side chain), in addition to a hydrogen bonding between oxygen of the celecoxib SO_2 and the Arginine 499 (side chain) of the COX-2 active site (Fig. 9C).

Based on those results, we can assume that both compounds exhibit an inhibitory COX-2 activity due to the similarity in the docking features of these compounds compared to celecoxib. The poses for compounds **1** and **2** showed a binding affinity of −7.7 and −8.1 kcal/mol which are relatively similar to celecoxib binding profile as a reference with a binding energy of −12.5 kcal/mol for the best pose.

4. Discussion

COX-2 inhibition is crucial for the anti-inflammatory properties of NSAIDs. Since selective inhibitors of COX-2 spare COX-1 enzymatic activity, COX-2-selective drugs can produce analgesic effects without causing gastrointestinal ulcers despite the increased risk of associated cardiovascular side effects reported (Warner et al., 1999).

We performed this study to analyze the effect of the two novel thiazole derivatives on COX-1 and COX-2 enzymatic activity. We demonstrated that compound **1** was a non-selective COX-1/COX-2 inhibitor, whereas compound **2** was a selective COX-2 inhibitor. The zymosan subcutaneous air pouch model was performed to

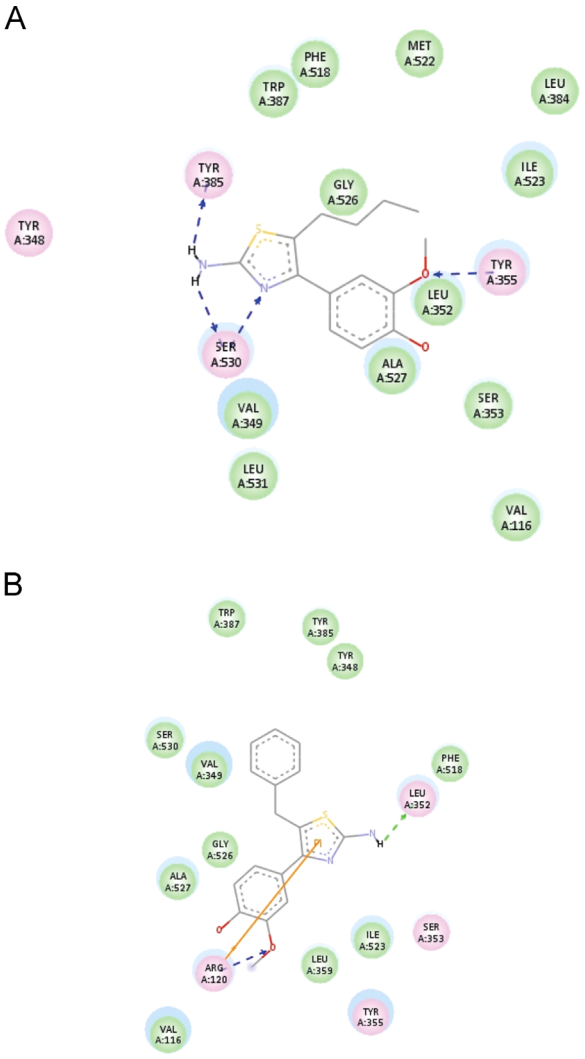


Fig. 8. Docking compounds into ovine COX-1. (A) The amino acids of active site of COX-1 were rendered in violet and green. H-bonds are indicated as blue dashed lines. Results for compound **1** (A), compound **2** (B) are illustrated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

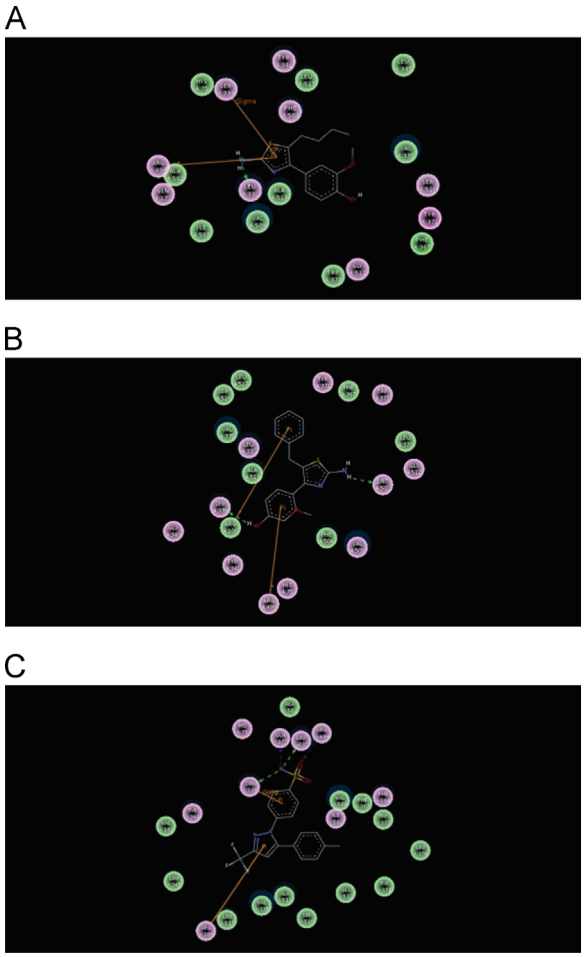


Fig. 9. Docking compounds and celecoxib into ovine COX-2. The amino acids of active site of COX-2 were rendered in violet and green. H-bonds are indicated as blue dashed lines for compounds **1**(A) and **2**(B) while blue and green for celecoxib (C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

investigate the anti-inflammatory effect of the two compounds *in vivo*. The zymosan-dependent migrating cells express COX-2 which is along with mPGES-1, the main enzymes responsible for PGE_2 production in this model (Paya et al., 1997). The groups of mice treated with compound **1** or compound **2** showed a significant decrease in PGE_2 levels without any effect on COX-2 protein expression supporting our *in vitro* findings with a direct effect on PGE_2 synthesis.

Modeling analysis results showed that both compounds are docked nearby Tyrosine 355 and Arginine 120 of the COX-1 catalytic site. Analysis revealed that compound **1** exhibit three hydrogen bonds between methoxy and Tyrosine 355, between NH2 and Serine 530, and between NH2 and Tyrosine 385 while compound **2** exhibited only two hydrogen bonds between phenol and Arginine 120 and between NH2 and the amide bond of Leucine 352. These observations suggest that the absence of the hydrogen bond between the methoxy group in the compound **2** and Tyr 355 of the active site of COX-1 is due to the steric effect of the CH2Ph group, as we described previously for derivatives of 13-hydroxyocatdecadienoic acid and 12-hydroxyeicosatetraenoic acid (Hirz et al., 2012). Consistent with these results, compound **2** did not block the enzymatic activity of COX-1 on platelet aggregation.

When the two compounds were docked in COX-2, we observed that they both interacted with amino acids previously described for selective inhibitors of COX-2 although the 2 compounds interacted with different groups within the active site. Compound **1** exhibit one hydrogen bond between the NH2 group and oxygen of peptide bond of Leucine 338 (active site of COX-2) while compound **2** was found to exhibit two hydrogen bonds one between the NH2 group and oxygen of peptide bond of Methionine 508 (active site of COX-2), and the other between the hydroxyl group and Serine 339 of COX-2 (oxygen of amide bond). Both compounds were able to block COX-2 activity in both *in vitro* assay on Raw 264.7 cells and in air pouch model.

The docking of celecoxib, known to be a positive inhibitor of COX-2, showed many hydrogen bindings among which are those between the amine group, Leucine 338 (amide bond), Serine 339 side chain found for each compound (**1** or **2**), and Glutamine 178 (side chain), in addition to a hydrogen bonding between oxygen of the celecoxib SO_2 and the Arginine 499 (side chain) of the COX-2 active site.

Many thiazole-containing molecules have been described targeting multi-signaling pathways, which may increase the potential side-effect. We have described these molecules as specific anti-cyclooxygenase activities with no cytotoxic effects on cultured cells. Additional investigation is required to characterize their side effects, if any, *in vivo* (Leoni et al., 2014).

In conclusion, our results further confirm a selective role of thiazoles as COX inhibitors. Our study enabled a more advanced characterization of structure-function relationship. Further investigations will be performed in the near future to assess the absence of adverse effect of this thiazole compound **2** on gastro intestinal tract lesions.

Author participation

AK, SH, and A. Hachem designed the molecules and carried out the synthesis. GAE, A. Habib and EH designed the biological studies. GAE, MJ, MFM, TH carried out the biological tests. NH performed docking analysis. GAE, A. Hachem, A. Habib, and EH conceived the study. A. Hachem, A. Habib, HFK, EH, AAE, BB, and RAM participated in problem solving. GAE, AK, A. Hachem, A. Habib and EH analyzed the data and wrote the paper. All authors read and approved the final version of the manuscript.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ejphar.2015.01.008.

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