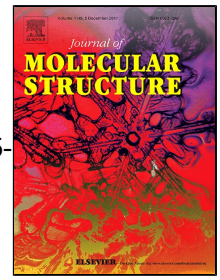
Accepted Manuscript



Synthesis, anti-inflammatory evaluation *in vivo* and docking studies of some new 5-(benzo[b]furan-2-ylmethyl)-6-methyl-pyridazin-*3(2H)*-one derivatives

Youness Boukharsa, Wiame Lakhlili, Jaouad El harti, Bouchra Meddah, Ramata Yvette Tiendrebeogo, Jamal Taoufik, My El Abbes Faouzi, Azeddine Ibrahimi, M'hammed Ansar

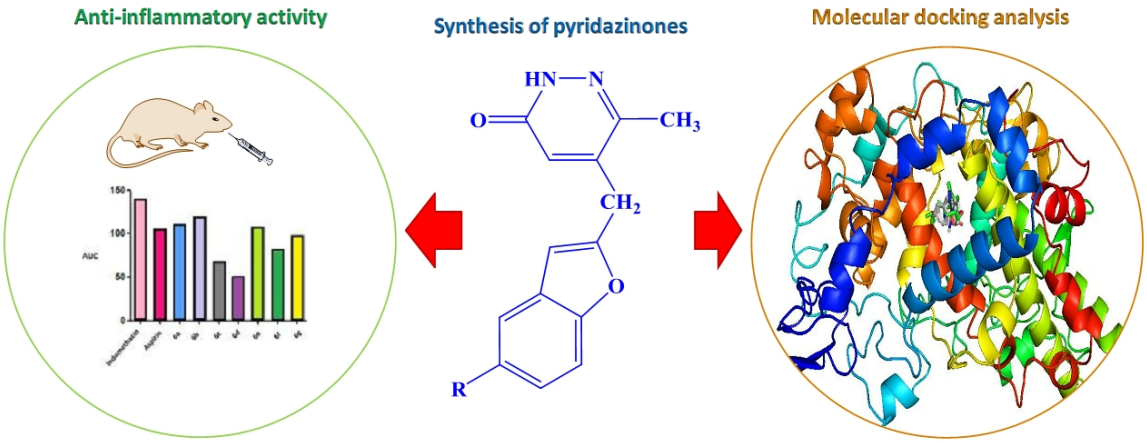
PII:	S0022-2860(17)31291-7
DOI:	10.1016/j.molstruc.2017.09.092
Reference:	MOLSTR 24341
To appear in:	*Journal of Molecular Structure*
Received Date:	22 April 2017
Revised Date:	24 September 2017
Accepted Date:	25 September 2017

Please cite this article as: Youness Boukharsa, Wiame Lakhlili, Jaouad El harti, Bouchra Meddah, Ramata Yvette Tiendrebeogo, Jamal Taoufik, My El Abbes Faouzi, Azeddine Ibrahimi, M'hammed Ansar, Synthesis, anti-inflammatory evaluation *in vivo* and docking studies of some new 5-(benzo[b] furan-2-ylmethyl)-6-methyl-pyridazin-*3(2H)*-one derivatives, *Journal of Molecular Structure* (2017), doi: 10.1016/j.molstruc.2017.09.092

Highlights

- Synthesis of a new series of pyridazin-*3(2H)*-ones derivatives,
- Biological evaluation of anti-inflammatory,
- Compounds **6a**, **6b**, **6e** and **6g** were found to be the most active compound,
- Molecular docking studies showed binding interactions at the active site of COX-2.

Graphical abstract



Synthesis, anti-inflammatory evaluation *in vivo* and docking studies of some new 5-(benzo[b]furan-2-ylmethyl)-6-methyl-pyridazin-*3(2H)*-one derivatives

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Abstract

Seven novel 5-(benzo[b]furan-2-ylmethyl)-6-methyl-pyridazin-*3(2H)*-one derivatives **(6a to 6g)** have been synthesized by the condensation of appropriate 3-(benzofuran-2-ylmethylene)-4-oxopentanoic acid and hydrazine hydrate in ethanol. Structures of all compounds were elucidated by elemental analysis, IR, ^{1}H NMR and ^{13}C NMR. These compounds were tested for their anti-inflammatory activity in carrageenan-induced rat paw edema model. *In silico* molecular docking study has been executed to study the binding interactions of the synthesized compounds with COX-2 protein. Compounds **6a**, **6b**, **6e** and **6g** showed a good anti-inflammatory activity at 50 mg/kg compared with the indometacin at 10 mg/kg and the aspirin at 150 mg/kg and good binding affinity with COX-2.

Keywords: Pyridazin-*3(2H)*-one, benzo[b]furan, anti-inflammatory activity, Molecular docking.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics for the treatment of pain and inflammation. The first NSAID discovered was aspirin which has been used for more than 100 years. However, the molecular mechanism of aspirin and other NSAIDs was elucidated only in the 1970s. Vane proposed that NSAIDs produce their therapeutic and also undesirable effects by inhibition of cyclooxygenase (COX) enzyme in prostaglandin synthetic pathway (1). Until recently, prostaglandin synthesis was thought to result from only one form of COX enzyme however, in the early 1990s, it was discovered that COX exists in two isoforms, COX-l (constitutive form) and COX-2 (inducible form) (2). Thus a new hypothesis has been suggested to explain the effects of NSAIDs; inhibition of COX-l accounts for the undesirable side effects, while inhibition of COX-2 accounts for the therapeutic benefits of NSAIDs (3).

Among these compounds, rofecoxib, celecoxib, valdecoxib and etoricoxib were approved and marketed. However rofecoxib was taking out of the markeft in 2004 because of its cardiac toxicity. Subsequently, some of the other coxibs have been voluntarily withdrawn from the market. On the other hand, some studies have suggested that rofecoxib's adverse cardiac events may not be a class effect but rather an intrinsic chemical property related to its metabolism (4,5).

The use of pyridazinone compounds constitutes an attractive approach for the treatment of several inflammatory disorders. Diverse pyridazinone derivatives have been reported to exhibit a wide range of pharmacological activities (6) such as antihypertensive (7), anticonvulsant (8), cardiotonic (9,10), antibacterial (11), saludiuretic (12), anti-HIV (13), antihistaminic (14), antidepressant (15), phosphodiesterase (PDE) inhibitors (16) and COX-2 inhibitors (17). It has also been reported that pyridazinone derivative have remarkable anticancer activity (18–20). Thus new pyridazinone derivatives are considerable interest in medicinal chemistry and since inflammation is well known to be associated with many diseases (21,22), the protective action of pyridazinone compounds against several diseases could be associated with their anti inflammatory effects.

In this paper, we report synthesis of seven novel pyridazinone derivatives which have been tested for their anti-inflammatory activity *in vivo* in comparison with the indometacine used as reference product. On other hand and to understand the mechanism of the anti-inflammatory activity of the synthesized compounds, the *in silico* approach was used by performing

molecular modeling and docking studies on the X-ray crystal structure of COX-2 in complexe with indometacine (PDB ID: 4COX) using the autodock vina program. These studies provided a potential target for these compounds.

2. Experimental

2.1. Synthesis

Melting points of all synthesized compounds were obtained on a Büchi Melting Point SMP-20 apparatus and were uncorrected. Infrared (IR) spectra were recorded with an IR VERTEX 70 FT-IR (Bruker Optics) spectrometer. ^{1}H Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Avance (400 MHz) spectrometer, using tetramethylsilane (TMS) as internal standard and $CDCl_3$ and DMSO-d_6 as solvent. Mass spectra were recorded on an API 3200 LC/MS/MS mass spectrometer using electrospray ionization (ESI) in positive polarity.

General procedures for the synthesis of compounds 6a-g

To a solution of the substituted phenol **1a-g** (0.5 mol) in 300 ml of 10 N NaOH (3 mol) was heated to 65°C. Then 80 ml of $CHCl_3$ was added in three portions over 15 min. The mixture was heated at reflux in chloroform for 2 h. After cooling, the mixture was acidified to pH 1 with 12 N HCl, the organic layer collected and the aqueous layer extracted with chloroform. The combined chloroform solution was dried and evaporated to give a crude product which was distilled or recrystallized from an appropriate solvent.

To a solution of the substituted 2-hydroxybenzaldehydes **2a-g** (0.15 mol) in N,N-dimethylformamide (100 ml) were added bromoacetaldehyde diethyl acetal (31.5 g, 0.16 mol) and potassium carbonate (42 g, 0.3 mol) at room temperature. The mixture was refluxed for 4 h at 150 °C. After cooling to room temperature, the precipitate was filtered off and the solvent evaporated under reduced pressure. The oily residue was distilled.

A solution of compounds **3a-g** (0.1 mol) in 35 ml of concentrated acetic acid was refluxed for 24 h. After cooling to room temperature, the mixture was evaporated to dryness. The crude product was distilled or recrystallized from an appropriate solvent.

To a solution of appropriate aromatic aldehyde **4a-g** (0.05 mol) and levulinic acid (0.06 mol) were dissolved into 20 ml of concentrated acetic acid and saturated with dry hydrogen chloride. Then, the mixture was stirred for 24 h at room temperature. The precipitate which

was formed was filtered off and washed with ethyl ether. The crude acids were recrystallized from an ethyl acetate.

To a solution of appropriate oxopentanoic acid **5a-g** (0.02 mol) in absolute ethanol (20-30 mL) was added hydrazine hydrate (0.02 mol). The reaction mixture was refluxed for 2h. The solid residue thus obtained was filtered and recrystallized from ethanol. The purity of the compounds was checked by column chromatography on silica gel in the solvent system ethyl acetate.

The analytical data of the products **6a-g** is depicted below.

5-(Benzo[b]furan-2-ylmethyl)-6-methylpyridazin-3(2H)-one (6a).

Obtained as yellow solid, yield 69 %, mp 181–183 °C (ethanol); IR (KBr υmax cm^{-1}), 1604 (C=N), 1662 (C=O), 2900–3000 (C–H); ^{1}H NMR (DMSO-d_6, 400 MHz), d = 2.22 (s, 3H, –N=C–CH_3), 4.08 (s, 2H, –CH_2–), 6.97 (s, 1H, H_4), 6.71 (s, 1H, $H_{3'}$), 7.19-7.58 (m, 4H, $H_{4'}$, $H_{5'}$, $H_{6'}$, $H_{7'}$), 12.71 (ls, 1H, NH); ^{13}C NMR (DMSO-d_6, 300 MHz): δ (ppm) = 18.90 (CH_3), 31.31 (CH_2), 105.19 (C-3'), 111.35 (C-7'), 121.31 (C-4'), 123.39 (C-5'), 124.45 (C-6'), 128.31 (C-9'), 128.62 (C-4), 143.92 (C-2'), 144.69 (C-5), 154.52 (C-8'), 154.79 (C-6), 161.17 (C=O); MS (ESI) *m/z* (relative abundance %): 241.2 $[M-H]^+$ (39,20 %), 263.3 $[M-Na]^+$ (100 %).

5-(5-Methylbenzo[b]furan-2-ylmethyl)-6-methylpyridazin-3(2H)-one (6b).

Obtained as yellow solid, yield 80 %, mp 183–184 °C (ethanol); IR (KBr υmax cm^{-1}), 1603 (C=N), 1666 (C=O), 2900-3000 (C–H); ^{1}H NMR (DMSOd_6, 400 MHz), d = 2.23 (s, 3H, –N=C–CH_3), 2.37 (s, 3H, Ar–CH_3), 4.07 (s, 2H, –CH_2–), 6.58 (s,1H, H_4), 6.64 (s, 1H, $H_{3'}$), 7.07 (dd, 1H, j_1 = 8.41 Hz, j_2 = 1.21 Hz, $H_{6'}$), 7.36 (d, 1H, j = 1.21 Hz, $H_{4'}$), 7.40 (d, 1H, j = 8.41 Hz, $H_{7'}$), 12.74 (ls, 1H, NH); ^{13}C NMR (DMSO-d_6, 300 MHz): δ (ppm) = 18.89 (CH_3-pyr), 21.04 (CH_3-bzfr), 31.21 (CH_2-pyr), 104.85 (C-3'), 111.77 (C-7'), 120.47 (C-4'), 125.30 (C-6'), 127.27 (C-9'), 128.30 (C-5'), 130.50 (C-4), 143.30 (C-2'), 143.67 (C-5), 151.85 (C-8'), 155.90 (C-6), 161.14 (C=O); MS (ESI) *m/z* (relative abundance %): 255.1 $[M-H]^+$ (55,68 %), 276.9 $[M-Na]^+$ (100 %).

5-(5-Ethylbenzo[b]furan-2-ylmethyl)-6-methylpyridazin-3(2H)-one (6c).

Obtained as brown solid, yield 73 %, mp 180–181 °C (ethanol); IR (KBr υmax cm^{-1}), 1605 (C=N), 1661 (C=O), 2900–3000 (C–H); ^{1}H NMR (DMSOd_6, 400 MHz), d = 1.18 (t, 3H, j = 7.50 Hz, –CH_2–CH_3), 2.21 (s, 3H, –N=C–CH_3), 2.65 (q, 2H, j = 7.50 Hz, –CH_2–CH_3), 4.05 (s,

2H, –CH_2–), 6.56 (s, 1H, H_4), 6.64 (s, 1H, $H_{3'}$), 7.08 (dd, 1H, j_1 = 8.40 Hz, j_2 = 1.80 Hz, $H_{6'}$), 7.37 (d, 1H, j = 1.80 Hz, $H_{4'}$), 7.41 (d, 1H, j = 8.40 Hz, $H_{7'}$), 12.76 (ls, 1H, NH); ^{13}C NMR (DMSO-d_6, 300 MHz): δ (ppm) = 16.76 ($\underline{C}H_3$-CH_2-bzfr), 18.88 (CH_3-pyr), 28.56 (CH_3-$\underline{C}H_2$-bzfr), 31.37 (CH_2-pyr), 105.08 (C-3'), 110.97 (C-7'), 119.87 (C-4'), 124.51 (C-6'), 128.28 (C-9'), 128.71 (C-5'), 138.94 (C-4), 143.97 (C-2'), 144.68 (C-5), 153.36 (C-8'), 154.57 (C-6), 161.18 (C=O); MS (ESI) *m/z* (relative abundance %): 269.5 $[M-H]^+$ (32,38 %), 291.3 $[M-Na]^+$ (100 %).

5-(5-Isopropylbenzo[b]furan-2-ylmethyl)-6-methylpyridazin-3(2H)-one (6d).

Obtained as brown solid, yield 82 %, mp 185–186 °C (ethanol); IR (KBr υmax cm^{-1}), 1605 (C=N), 1682 (C=O), 2900–3000 (C–H); ^{1}H NMR (DMSOd_6, 400 MHz), d = 1.24 (d, 6H, j = 6.90 Hz, H_3C–CH–CH_3), 2.10 (s, 3H, –N=C–CH_3), 2.99 (h, 1H, j = 6.90 Hz, H_3C–CH–CH_3), 3.90 (s, 2H, –CH_2–), 7.30–7.76 (m, 5H, H_4, $H_{3'}$, $H_{4'}$, $H_{6'}$, $H_{7'}$), 12.32 (ls, 1H, NH); ^{13}C NMR (DMSO-d_6, 300 MHz): δ (ppm) = 18.89 (CH_3-pyr), 24.87 (2$\underline{C}H_3$-CH-bzfr), 31.37 (CH_2-pyr), 33.84 (2CH_3-$\underline{C}$H-bzfr), 105.20 (C-3'), 110.96 (C-7'), 118.33 (C-4'), 123.15 (C-6'), 128.26 (C-9'), 128.62 (C-4), 143.68 (C-2'), 144.00 (C-5'), 144.69 (C-5), 153.38 (C-8'), 154.56 (C-6), 161.17 (C=O); MS (ESI) *m/z* (relative abundance %): 283.4 $[M-H]^+$ (53,97 %), 337.4 $[M-Na]^+$ (100 %).

5-(5-Methoxybenzo[b]furan-2-ylmethyl)-6-methylpyridazin-3(2H)-one (6e).

Obtained as yellow solid, yield 78 %, mp 190–192 °C (ethanol); IR (KBr υmax cm^{-1}), 1650 (C=O), 2950–3000 (C–H); ^{1}H NMR (DMSOd_6, 400 MHz), d = 2.25 (s, 3H, –N=C–CH_3), 3.74 (s, 3H, –OCH_3), 4.04 (s, 2H, –CH_2–), 6.55 (s, 1H, H_4), 6.64 (s, 1H, $H_{3'}$), 6.82 (dd, 1H, j = 8.70 and 2.71 Hz, $H_{6'}$), 7.08 (d, 1H, j = 2.71 Hz, $H_{4'}$), 7.40 (d, 1H, j = 8.70 Hz, $H_{7'}$), 12.75 (ls, 1H, NH); ^{13}C NMR (DMSO-d_6, 300 MHz): δ (ppm) = 18.86 (CH_3-pyr), 31.38 (CH_2-pyr), 56.12 (OCH_3-bzfr), 103.8 (C-3'), 105.22 (C-4'), 117.64 (C-7'), 117.79 (C-6'), 128.60 (C-9'), 129.90 (C-4), 143.00 (C-2'), 144.90 (C-5), 149.00 (C-8'), 154.20 (C-6), 155.60 (C-5'), 161.15 (C=O).

5-(5-Propoxybenzo[b]furan-2-ylmethyl)-6-methylpyridazin-3(2H)-one (6f).

Obtained as brown solid, yield 87 %, mp 194–196 °C (ethanol); IR (KBr υmax cm^{-1}), 1652 (C=O), 2940–3000 (C–H); ^{1}H NMR (DMSOd_6, 400 MHz), d = 0.96 (t, 3H, j = 6.90 Hz, –OCH_2–CH_2–CH_3), 1.71 (m, 2H, –OCH_2–CH_2–CH_3), 2.21 (s, 3H, –N=C–CH_3), 3.90 (t, 2H, j = 6.90 Hz, –OCH_2–CH_2–CH_3), 4.04 (s, 2H, –CH_2–), 6.55 (s, 1H, H_4), 6.62 (s, 1H, $H_{3'}$), 6.81 (dd, 1H, j = 9.00 and 2.71 Hz, $H_{6'}$), 7.06 (d, 1H, j = 2.71 Hz, $H_{4'}$), 7.38 (d, 1H, j = 9.00 Hz,

$H_{7'}$), 12.74 (ls, 1H, NH); ^{13}C NMR (DMSO-d_6, 300 MHz): δ (ppm) = 10.91 ($\underline{C}H_3$-CH_2-CH_2-bzfr), 18.89 (CH_3-pyr), 22.58 (CH_3-$\underline{C}H_2$-CH_2-bzfr), 31.40 (CH_2-pyr), 70.00 (CH_3-CH_2-$\underline{C}H_2$-bzfr), 104.75 (C-3'), 105.43 (C-4'), 111.78 (C-7'), 113.23 (C-6'), 128.27 (C-9'), 129.29 (C-4), 143.96 (C-2'), 144.68 (C-5), 149.61 (C-8'), 154.46 (C-6), 155.19 (C-5'), 161.17 (C=O); MS (ESI) *m/z* (relative abundance %): 299.4 $[M-H]^+$ (34,09 %), 321.4 $[M-Na]^+$ (100 %).

5-[(5-Chlorobenzo[b]furan-2-yl)methyl]-6-methylpyridazin-3(2H)-one (6g).

Obtained as yellow solid, yield 70 %, mp 198–200 °C (ethanol); IR (KBr υmax cm^{-1}), 1649 (C=O); ^{1}H NMR (DMSOd_6, 400 MHz), d = 2.23 (s, 3H, –N=C–CH_3), 4.11 (s, 2H, –CH_2–), 6.60 (s, 1H, H_4), 6.74 (s, 1H, $H_{3'}$), 7.29 (dd, 1H, *j* = 8.71 and 2.16 Hz, $H_{6'}$), 7.58 (d, 1H, *j* = 8.71 Hz, $H_{4'}$), 7.66 (d, 1H, *j* = 2.16 Hz, $H_{7'}$), 12.76 (ls, 1H, NH); ^{13}C NMR (DMSO-d_6, 300 MHz): δ (ppm) = 18.91 (CH_3-pyr), 31.17 (CH_2-pyr), 106.12 (C-3'), 115.43 (C-7'), 120.51 (C-4'), 124.45 (C-6'), 124.77 (C-5'), 128.32 (C-9'), 130.53 (C-4), 143.63 (C-2'), 144.65 (C-5), 150.27 (C-8'), 155.86 (C-6), 161.13 (C=O); MS (ESI) *m/z* (relative abundance %): 275.3 $[M-H]^+$ (100 %), 297.3 $[M-Na]^+$ (17,61 %).

2.2. Anti-inflammatory activity

The anti-inflammatory activity of the synthesized compounds **6a-g** was evaluated by carrageenan-induced paw edema according to the method described by Winter et al. (23–25).

The carrageenan-induced rat paw edema method is highly reproducible and is the most test widely used primary to evaluate and study anti-inflammatory effect of several compound (26), what justifies our choice of the carrageenan test to evaluate the anti-inflammatory effect of the synthesized compounds.

The adult Wistar rats of both sexes, with weights between 150-280g were used. A total of 10 lots were studied and each lot consists of 6 rats (n = 6). After 18 hours of fasting, the control group received oral administration of orally corn oil (vehicle control, 5 ml/kg), the standard groups received the reference drug (indomethacin at a dose of 10 mg/kg and aspirin at dose of 150 mg/kg), and the test groups received different suspensions of tested pyridazinones (**6a-g)** at a dose of 50 mg/kg. Thirty minutes after feeding, each animal received in the right hind paw, a sub-plantar injection of a suspension of carrageenan in 2.5% NaCl (0.9%), at 0.05 ml.

The volume of the treated paw was measured before and at 30 min, 1, 2, 3 and 4 hours after injection of carrageenan using a plethysmometer LE7500 (Ugo Basile e Italy). The

percentages of inhibition in inflammation were calculated for each animal using the following formula:

$$\% \text{ inhibition} = \frac{(Vn - Vo)Control - (Vn - Vo)Treated}{(Vn - Vo)Control} \times 100$$

Where Vo is volume of the right hind paw measured before injection of carrageenan and Vn is volume of the right hind paw measured at several time-points; at 30 min, 1, 2, 3 and 4 h after carrageenan injection.

2.3. Molecular docking Analysis

The purpose of this section is to provide a potential target for the synthetized compounds by an *in silico* approach using docking studies. The search strategy for potential target was initiated based on several criteria. A biological target involved and validated in the inflammatory process was the first criteria. The second one was availability of the experimental tridimentional strucutre of the target and finally, a target which is complexed with indometacin.

Using these criteria, we were able to propose the cyclooxygenase-2 (COX2) as one of the target protein which was used in this study.

The three dimensional structure of COX-2 has been extensively studied and several complexes of COX-2 are present on the PDB (protein Data Bank) (27,28). For these studies we have chosen to work with the crystal identified by the ID PDB: 4COX (27). Which is the complex of cyclooxygenase-2 with indometacin and that has a resolution of 2.9 Å.

The molecular docking files (ligands + protein active site) were prepared for Autodock vina software, by using AutoDock Tools (v1.5.6) (29,30). The following parameters were adjusted in this preparation step (31): (i) the Gastieger charges and polar hydrogens were added; (ii) the grid-box dimensions were set at 24 Å (X), 21 Å (Y) and 7,75Å (Z); and (ii) the center of the box was positioned at the midpoint of the active site, and the box volume covered the entire active site area plus a significant portion of the protein's solvent-exposed surface.

The following docking parameters were used in Autodock vina:**(a)** all bonds in the inhibitor structures were allowed to rotate freely, except for the multiple bonds and the bonds in aromatic entities; **(b)** the 3D protein structures were considered to be rigid; **(c)** a Lamarckian genetic algorithm was used for searching the conformational space in the active site; **(d)** the default grid spacing was set at 1 Å; **(e)** 100 different conformations were assessed, and the 9

highest scored binding modes were maintained for visual inspection; **(f)** the maximum energy difference between the best and the worst binding modes was set at 3 kcal/mol; **(g)** the scoring function was a stochastic global optimization method inspired chiefly by X-Score (32); and **(h)** visual inspection of the docking results, and image building, were done using PyMOL software (www.pymol.org/funding.html) (33).

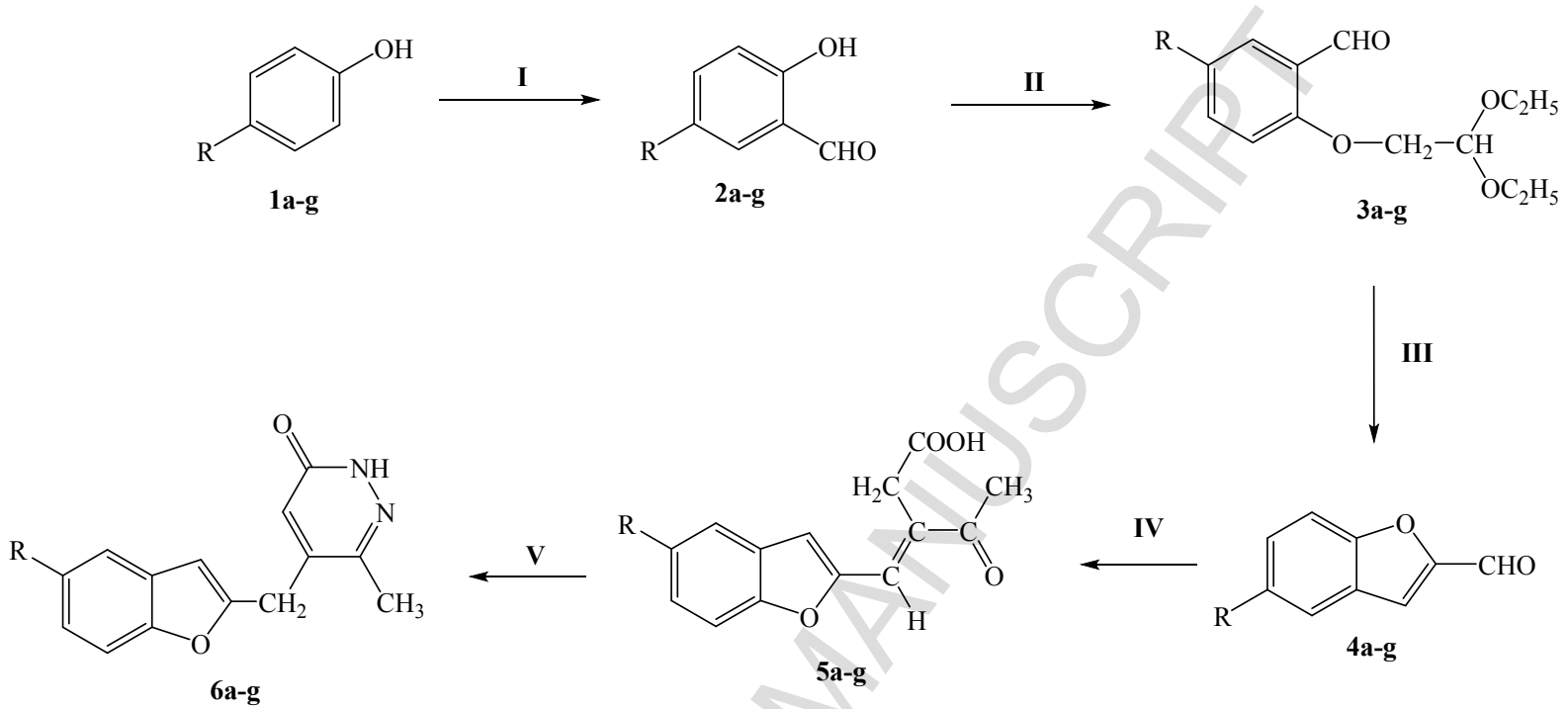
2.4. Statistical Analysis

After checking the homogeneity of variances by ANOVA test, statistical comparisons between control and treated lots were performed using two-way ANOVA test on the individual volume changes before and after injection carrageenan (Vn - Vo). A value of $P<$ 0.001 was considered to be significant. Analysis was performed with GraphPad prism Software (6.0).

3. Results and Discussion

3.1. Synthesis

The pyridazinone derivatives incorporating Benzofuran moiety studied in this work (compounds **6a-g**) were synthesized according to the general procedures outlined in Scheme 1. In the first step, salicylaldehydes **2a-g** were synthesized via Reimer Thieman (34) formylation of the appropriate substituted phenol **1a-g** with $CHCl_3$ and NaOH. Aldehydes **2a-g** has been previously synthesized with very low yield. Briefly, reaction of intermediates **2a-g** with bromacetaldehyde diethyl acetal in the presence of potassium carbonate in DMF (dimethylformamide) yielded compounds **3a-g**. Next, these compounds **3a-g** were cyclized to benzo[b]furan-2-ylcarboxaldehydes **4a-g** by heating in concentrated acetic acid. Benzo[b]furanaldehydes **4a-g** were prepared according to the methods described in the literature (35). Finally, treatment of substituted aldehydes **4a-g** with levulinic acid in the presence of HCl (chlorhydric acid) in acetic acid gave adducts **5a-g**, which were heated by hydrazine hydrate to afford the pyridazin-*3(2H)*-ones **6a-g** (15).



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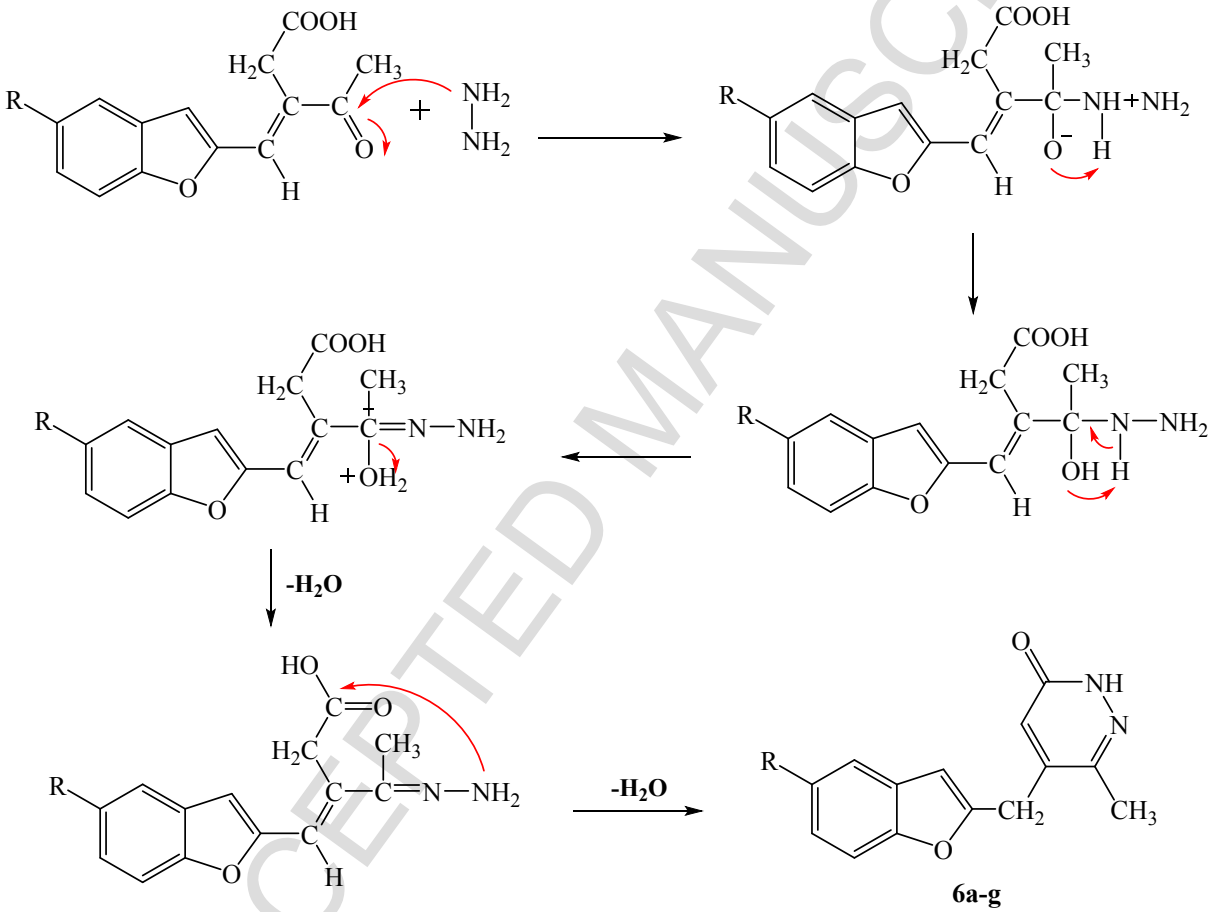
Compound	R
a	H
b	CH_3
c	CH_2CH_3
d	$CH(CH_3)_2$
e	OCH_3
f	$OCH_2CH_2CH_3$
d	Cl

246 **Scheme 1.** Synthesis of target compounds **6a-g**. **Reagents *I***: $CHCl_3$/NaOH 10N, reflux 2h; ***II***: $BrCH_2CH(OC_2H_5)_2/K_2CO_3$/DMF, reflux 4h; ***III***:

247 CH_3COOH, reflux 24h; ***IV***: $H_3CCOCH_2CH_2COOH/CH_3COOH$, reflux 24h; ***V***: H_2NNH_2/EtOH, reflux 2h

The proposed reaction mechanism is reported in Scheme 2. It is carried out according to a condensation reaction of the derivatives of hydrazine with 1,4-dicarbonyl (appropriate oxopentanoic acid), Intermolecular hydrazone formation followed by intramolecular cyclization led to the formation of pyridazinones **6a-g**.

The Pyridazinones are the derivatives of pyridazine which belong to an important group of heterocyclic compounds containing two nitrogen atoms at 1 and 2 positions in a six member ring. In pyridazinone derivatives the amine group (NH) is suitably placed with the carbonyl group and most of the pyridazinone derivatives exhibit tautomerism.



Scheme 2. The proposed mechanism for the formation of products **6a-g**

The chemical structures of all compounds were deduced from their spectral and analytical data. Thus, IR spectrum showed absorption band at υ = 2833-3465 and 1661-1668 cm^{-1} due to NH and carbonyl groups respectively. In addition, the ^1H NMR spectrum revealed the presence of singlet signals at δ = 2.21-2.25 ppm due to (CH_3-pyridazinone), a singlet signals

at δ = 4.04-4.11 ppm attributed to (benzofuran-CH_2 –pyridazinone), a signals at δ = 6.55-7.66 ppm corresponding to aromatic protons of benzofuran-pyridazinone ring and a singlet signals at δ= 12.71-12.76 ppm for NH protons. Moreover, the ^{13}C NMR data exhibited the presence of signals at δ = 18.88-18.90 ppm for (CH_3-pyridazinone) and a signal at δ = 31.28-31.40 ppm for (benzofuran-CH_2-pyridazinone).

3.2. Anti-inflammatory activity

Inflammation is a complex phenomenon with series of interactions between cellular and inflammatory mediators. Regardless of the triggering factor, the progression of edema is expressed by a release of several mediators (36,37). The first phase of inflammation lasts up to 2 h, and involves the participation of histamine, serotonin, and bradykinin; while the second phase, is from 3 to 4 h and mainly sustained by prostaglandins, cytokines and nitric oxide release (23,38).

As illustrated in Table 1, the injection of carrageenan into the sub-plantar tissue in the right hind paw of rats in the control groups caused edema development during 30 minutes, 1, 2, 3 and 4 h after carrageenan injection with a peak volume (1.28 ml) at 4h. This result confirms that experimental carrageenan injection into the rat paw provokes a significant local and acute inflammatory reaction (39).

The single oral treatment with oily tested suspensions of pyridazinones compounds at a dose of 50 mg on carrageenan induced rat paw edema remarkably decreased the level of paw inflammation compared to the negative control group. The average volume of the right paw of the treated group with **6a**, **6b**, **6c**, **6d**, **6e**, **6f** and **6g** compounds at 30min after carrageenan injection were 0.52, 0.57, 0.49, 0.55, 0.59, 0.58 and 0.71 ml respectively and were progressively increased to 0.62, 0.60, 0.57, 0.68, 0.60, 0.68 and 0.64 mL at 2h and to 0.98, 0.90, 0.95, 1.20, 0.97, 1.15 and 0.68 ml (Table 1).

Table 1. Anti-inflammatory activity of the target compounds against the carrageenan-induced paw edema in rats with different time intervals.

Compound	Dose (mg/kg)	Average volume of the right paw (ml)*				
		30min	1h	2h	3h	4h
Control	-	0,79	0,84	0,87	0,91	1,28
Indomethacin	10	0,608	0,58	0,555	0,54	0,56

Aspirin	150	0,71	0,66	0,608	0,64	0,71
6a	50	0,52	0,53	0,62	0,68	0,98
6b	50	0,57	0,51	0,6	0,63	0,9
6c	50	0,49	0,45	0,57	0,61	0,95
6d	50	0,55	0,61	0,68	0,8	1,2
6e	50	0,59	0,54	0,6	0,68	0,97
6f	50	0,58	0,53	0,68	0,78	1,15
6g	50	0,71	0,66	0,64	0,67	0,68

* Each value represents the mean of difference between of volume of paw edema before and after injection carrageenan (Vn - Vo) for six rats (n = 6).

Based on percentages of inhibition values (Figures 1-7), **6a** compound caused the peak effect 4h after carrageenan injection and inhibited edema by 23.43%, however the peak effect of **6b**, **6c**, **6d**, **6e**, **6f** and **6g** was produced at 1h after carrageenan injection with respectively 39,28, 46.42, 27.38, 35.71, 36.90 and 21.42 %. These effects were statistically insignificant (P< 0.05) compared from that produced by 10 mg/kg indomethacin and 150 mg /kg aspirin.

In the presence of indomethacin 10 mg/kg there was a decrease of the volume of edema with a percent inhibition that starting at 23.04% after 30 min to get to 56.25% after 4 hours of injection of carrageenan. Additionally, aspirin at a dose of 150 mg /kg also caused a decrease in edema following kinetic increase and reached a value of 44.53% after 4h. p = 0.26099 (p> 0.05) compared to vehicle treated group.

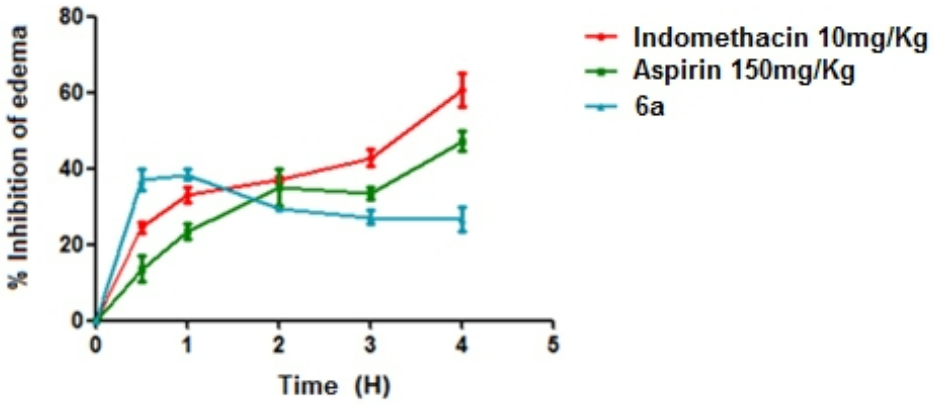
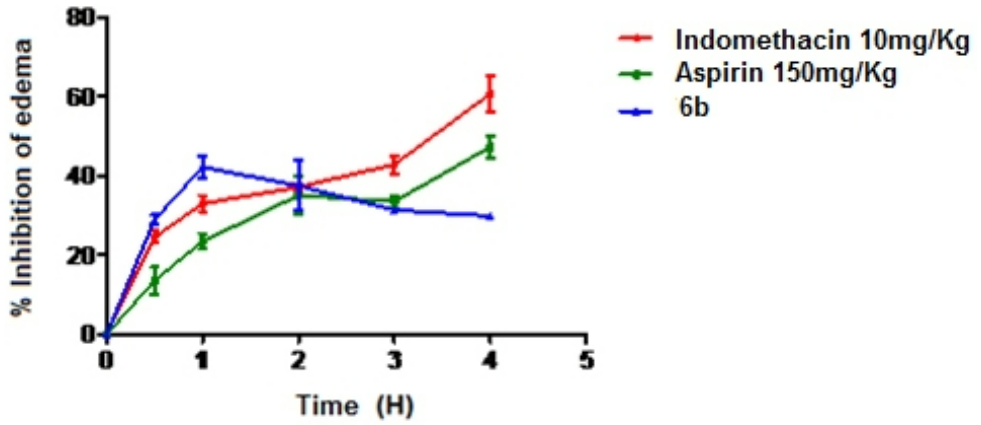
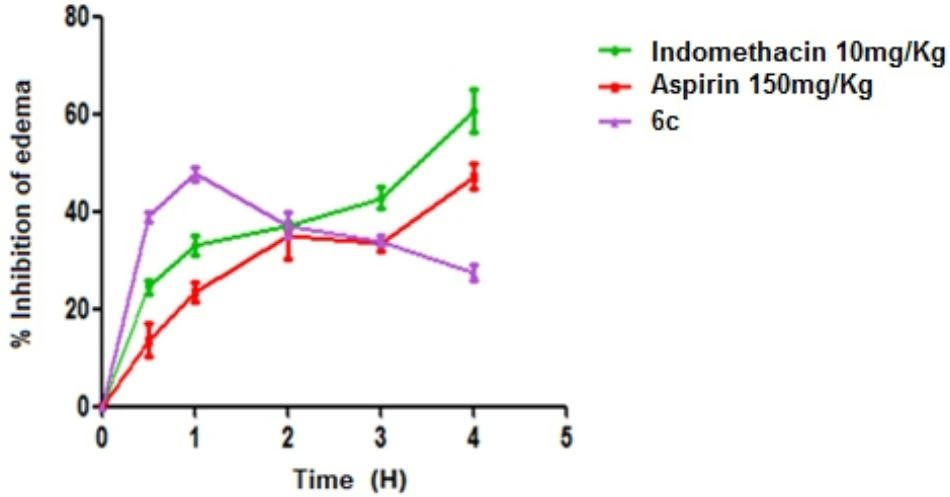


Figure 1. Effect of **6a** on edema induced by carrageenan injection at 2.5% in the right hind paw of the rat.



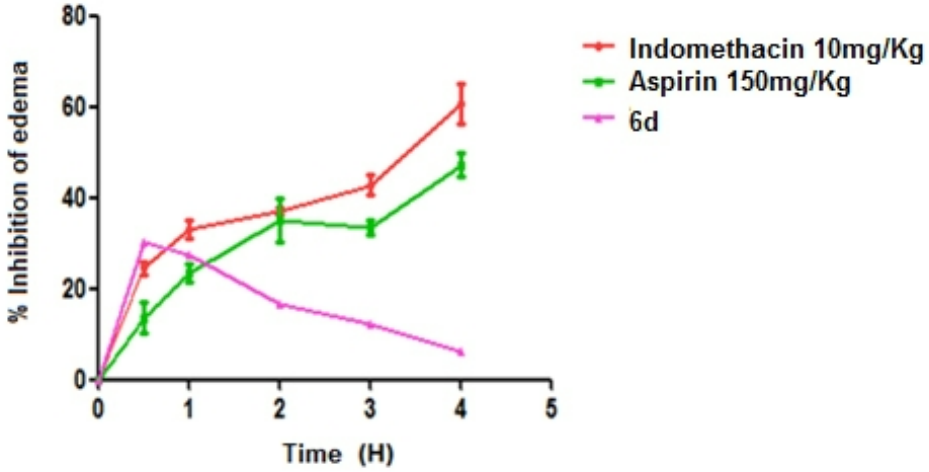
304

305 **Figure 2.** Effect of **6b** on edema induced by carrageenan injection at 2.5% in the right hind
306 paw of the rat.



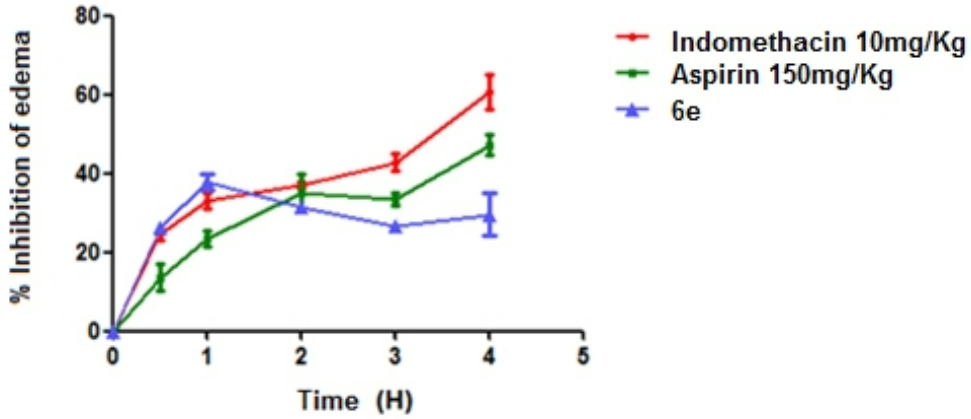
307

308 **Figure 3.** Effect of **6c** on edema induced by carrageenan injection at 2.5% in the right hind
309 paw of the rat.



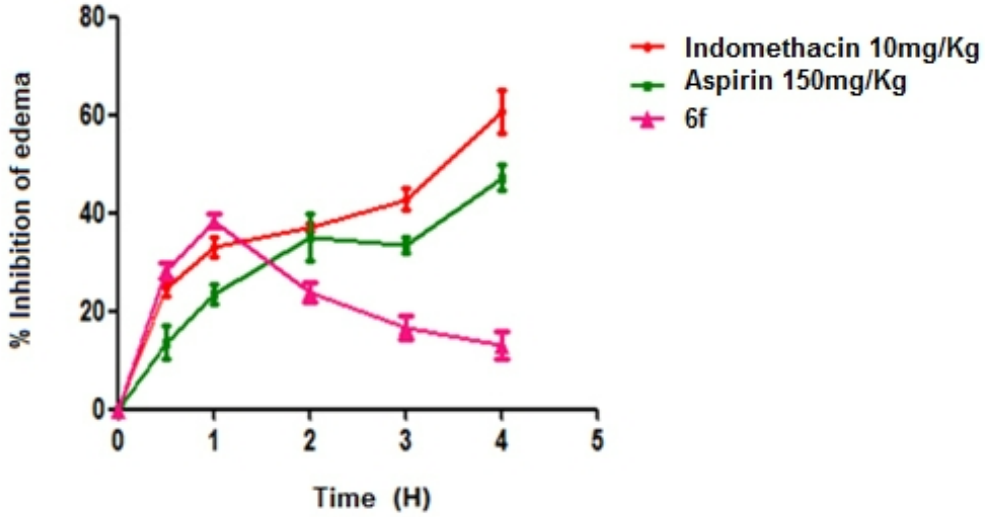
310

311 **Figure 4.**Effect of **6d** on edema induced by carrageenan injection at 2.5% in the right hind
312 paw of the rat.



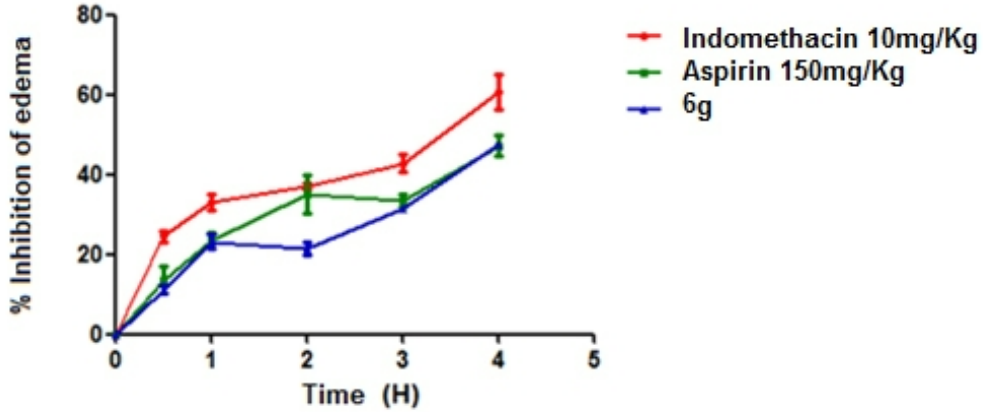
313

314 **Figure 5.** Effect of **6e** on edema induced by carrageenan injection at 2.5% in the right hind
315 paw of the rat.



316

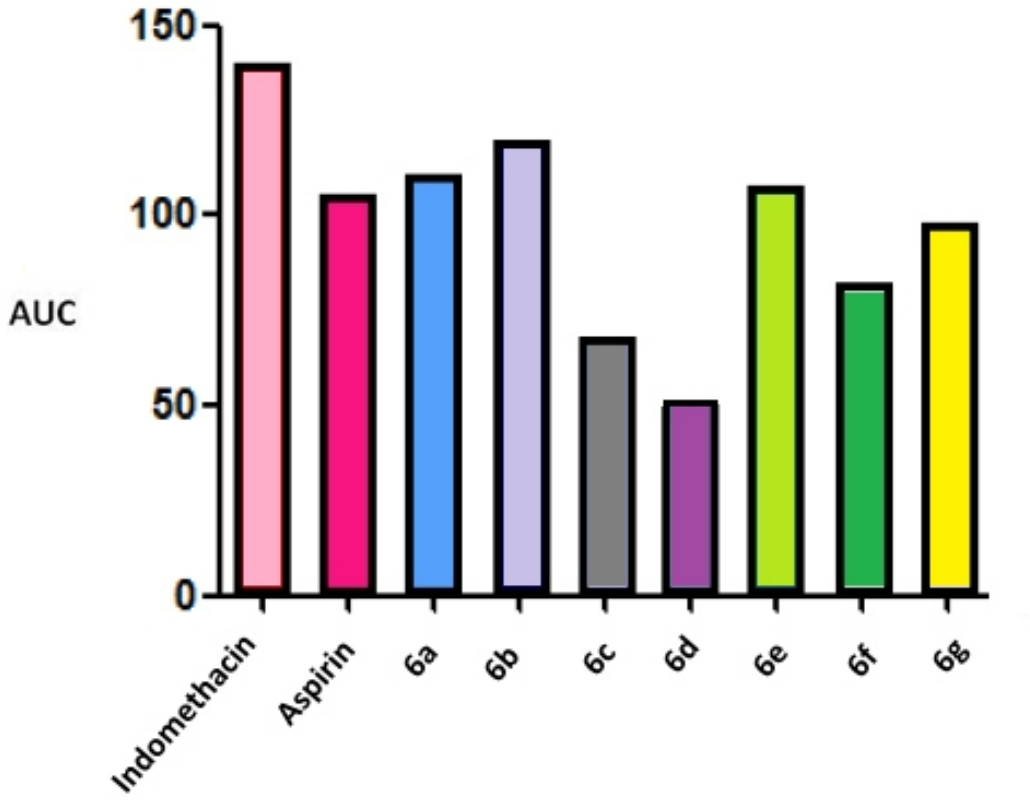
317 **Figure 6.** Effect of **6f** on edema induced by carrageenan injection at 2.5% in the right hind
318 paw of the rat.



319

320 **Figure 7.** Effect of **6g** on edema induced by carrageenan injection at 2.5% in the right hind
321 paw of the rat.

322 Figure 8 shows the difference between the effects of pyridazinones synthesized and reference
323 substances. $p = 0.09$ ($p > 0.05$) for all pyridazinones, concluding that compounds **6a**, **6b**, **6e**
324 and **6g** have a good anti-inflammatory activity, because there is no significant difference
325 between them and the effect of the indomethacin at 10 mg/kg and the aspirin at 150 mg/kg of
326 aspirin. The pyridazinone ring is known for its anti-inflammatory properties and confirmed by
327 this study (40–42).



328

329 **Figure 8.** Area under the curve (AUC) of average percentages of inhibition corresponding to
330 the effect of the pyridazinones (**6a-g**) at 50 mg / kg on carrageenan induced edema in
331 comparison to the two references: Aspirin 150 mg / kg and indomethacin 10 mg / kg.

332 Considering that the production of histamine, serotonin and bradykinin are the main factors
333 responsible for both the first phase of the inflammatory response (lasts up to 2 h), our results
334 suggest that the anti-inflammatory effect of the tested pyridazinones compounds could be
335 related to the inhibition of the release or synthesis of arachidonic acid metabolites. The anti-
336 inflammatory activities of several pyridazinone derivatives have been previously reported.

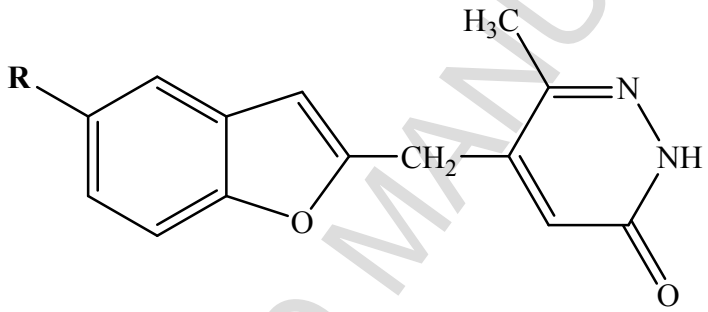
337 Similar results were found by Mogilski et al. (43) and Abouzid et al. (44), which revealed that
338 several pyridazinone derivatives had an excellent inflammatory activity using the carageenan-
339 induced paw edema test. Ochiai et al. (45) also demonstrated that another series of
340 pyridazinone derivatives exhibited significant in-vivo anti-inflammatory. In another series of
341 pyridazinone developed by Gokce et al. (46), compound 7 emerged as a potent anti-
342 inflammatory agent.

Since our findings are preliminary results; further studied need to be carried out to investigate the other specifications such as side effect-activity profiles or toxicological studies of these compounds.

3.3. Molecular docking Analysis

In order to execute the docking of different compounds, the binding and docking sites were validated by the reference product (indomethacin) and Figure 9 shows the overlapping of docked COX2 with the experimental indomethacin (Co-crystaled with COX2). The docking scores (affinity) were between -8,9 and -9,7 kcal/mol for the seven compounds and -10,7 kcal/mol for indometacien (Table 2).

Table 2. Functional groups, Anti-inflammatory and docking score in cyclooxygenase 2 in comparison to indometacin



Compound	R	Anti-inflammatory activity*	Docking score in cyclooxygenase 2 (ID PDB:4COX)
6a	H	23,43	-9,3
6b	CH_3	29,68	-9,7
6c	CH_2CH_3	25,78	-9,6
6d	$CH(CH_3)_2$	6,25	-9,3
6e	OCH_3	24,21	-9,1
6f	OC_3H_7	10,15	-8,9
6g	Cl	46,87	-9,4
Ref	Indometacin	56,25	-10,7

* Anti-inflammatory activitiy exprimed in (% inhibition of edema) at 4h, using 50mg/kg for compounds vs 10mg/kg for indomethacin.

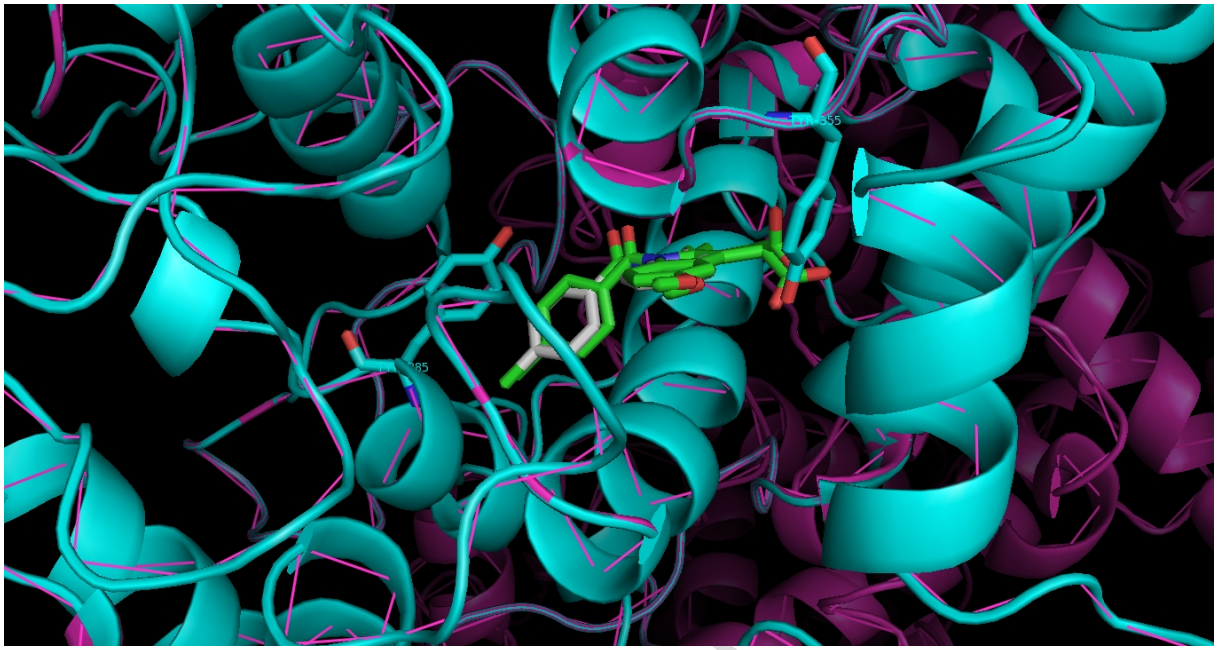


Figure 9. Validation of the docking box. COX2 docked in cyan is superposed at COX2 extracted from PDB (ID PDB 4COX) margenta. Indometacine docked (in white) is superposed at indomethacin co-crystalesed (in green) with COX2 extracted from PDB (ID PDB 4COX) presented in margenta. Tyr355 and Tyr385, two residues of the binding site are shown.

The binding site analysis of the COX2 shows that it is a closed one and do not allow the reception of large molecule. Compound **6f** of our series is the one with the larger radical (**R** = **OC_3H_7**) and in looking at the docking result, we observe that this compound has the lowest affinity (the lowest score) against the compound **6b** (**R=CH_3**) with a methyl radical has the highest score confirms this hypothesis but compound **6a** (**R=H**) despite its small size it has a low score in docking, this is explained by the lack of possibility of making hydrogen bond with hot active site residues (Figure 10).

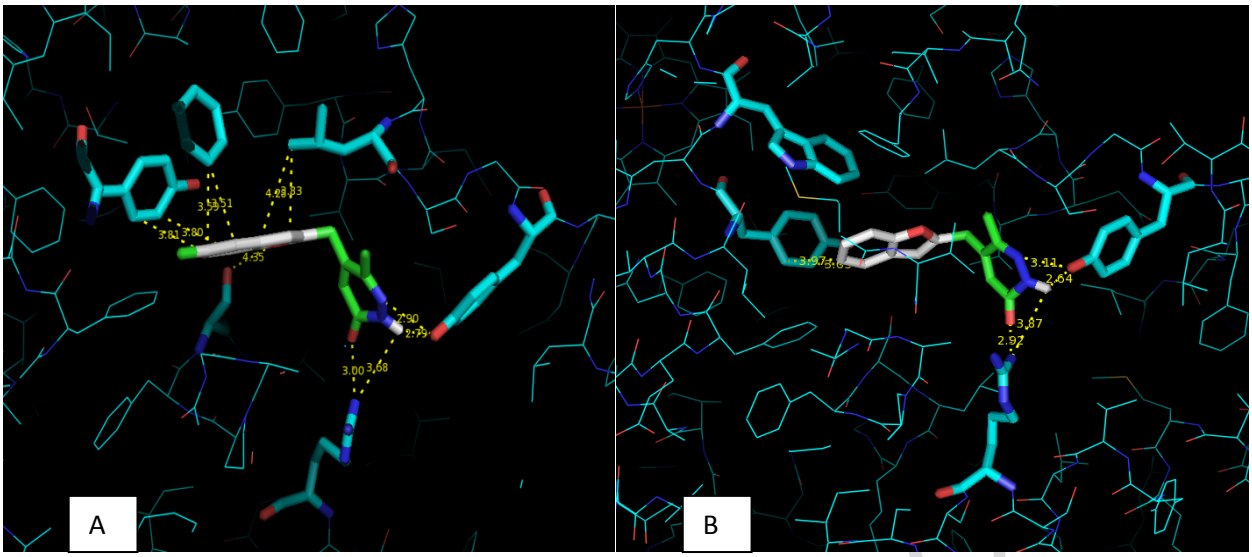


Figure 10. A : Compound (R=CH_3) ; **B** : Compound (R=H)

This observation is in the same way as the biological activity. (29,6875% for CH_3 compound vs 23,4375% for H compound of inhibition. both are has less activity compared to the reference product, as for biological activity as *in silico* analysis.

4. Conclusion

Pyridazinones derivatives showed anti-inflammatory activity at 50 mg /kg compared to the reference products at 10 mg/kg (indomethacin) and at 150 mg/kg (aspirin) with a maximum effect between 1 hour and 2 hours. Synthesized products are faster than reference ones but are had fleeting effects. This fugacious effect may be enhanced by prolonged release dosage forms. Comparison of *in silico* and biological data suggests that COX2 is a potential therapeutic target for our compounds. However, target validation test are required to be undertaken to confirm these results. It will be interesting to study the acute and chronic toxicity effects of these synthesized compounds to evaluate their safety especially the more promising ones (**6a**, **6b**, **6e** and **6g**).

5. Aknowledgments

This work was carried out under National funding from the Moroccan Ministry of Higher education & Scientific research (PPR program) to AI. This work was also supported by a grant from the NIH for H3Africa BioNet to AI.

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