Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 5547–5556
Bioorganic & Medicinal Chemistry

Novel anti-inflammatory agents based on pyridazinone scaffold; design, synthesis and in vivo activity

Khaled Abouzid[a,*] and Salma A. Bekhit[b]

[a]*Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt*
[b]*Department of Pharmacology, Faculty of Veterinary Medicine, University of Alexandria, Edfina, Beheira, Egypt*

Received 11 December 2007; revised 2 April 2008; accepted 4 April 2008
Available online 9 April 2008

Abstract—Herein, we report the design, synthesis, and pharmacological properties of a series of arylethenylpyridazinones **3a**–**h** and arylethylpyridazinone derivatives **3k**–**i** from the corresponding aryloxohexenoic **1a**–**e** and aryloxohexanoic acids **2a**,**d**, respectively. The synthesized compounds were tested for their anti-inflammatory activity in carrageenan-induced rat paw edema model. Compound **3j** demonstrated the greatest in vivo activity with ED_{50} equal to 17 μmol compared with celecoxib with no ulceration on the gastric mucosa. Docking study of the synthesized compounds into the active site of COX-2 revealed a similar binding mode to RS-57067, a COX-2 inhibitor.

1. Introduction

Non-steroid anti-inflammatory drugs (NSAIDs) which inhibit the activity of cyclooxygenases (COXs) are widely used medication for the treatment of pain and inflammation. The common dose limiting toxicity of the classical NSAIDs is the increased risk of gastrointestinal ulceration and hemorrhage.[1a] Cyclooxygenase catalyzes the conversion of arachidonic acid into prostaglandin H2 as the first committed step of prostaglandin biosynthesis. This enzyme has been known as the target of non-steroidal anti-inflammatory drugs (NSAIDs). In 1991 it was recognized that this enzyme existed in two isoforms, designated COX-1, a constitutive enzyme, and COX-2, an enzyme induced in response to a variety of pro-inflammatory stimuli.[1b,2] This recognition led to the hypothesis that the anti-inflammatory and analgesic effects of NSAIDs were due to the inhibition of COX-2, while many of the undesirable side effects of these drugs, particularly gastric irritation, were due to non-selective cyclooxygenase inhibition. The identification of selective inhibitors of COX-2 (coxibs) followed by clinical trials has largely validated this hypothesis. An extensive body of literature has accompanied the rapid development of this field and numerous reviews have been published.[2–4] The three-dimensional crystal structures of both COX-1 and COX-2 have been solved.[5] The two enzymes are highly homologous, with 63% identity and 77% similarity. The COX-1 active site contains an isoleucine residue (Ile523) which is replaced by a valine residue in COX-2 (Val523). This single amino acid difference allows access to a polar side pocket in COX-2, and binding in this pocket, along with a difference in binding kinetics, largely accounts for the selectivity observed with COX-2 inhibitors.[6] Selective cyclooxygenase-2 (COX-2) inhibitors represent a new generation of anti-inflammatory drugs as they demonstrated less gastrointestinal side effects than the classical NSAIDs, which also inhibit the cytoprotective action of COX-1 in the GI tract.[7–9] During the last decade, several selective inhibitors (coxibs) have been reported[10] and many of them have now reached the market such as rofecoxib,[11] celecoxib,[12] valdecoxib,[13] and etoricoxib[14] (Fig. 1). Later on, many publications emerged in this field aiming at finding new lead in this area.[15,16] Clinical experience with marketed COX-2 inhibitors has confirmed the utility of these agents in the treatment of inflammatory pain and symptoms of osteoarthritis and rheumatoid arthritis with an improved gastrointestinal safety profile relative to NSAID comparators. Moreover, withdrawal of rofecoxib from the market recently by its originators due to adverse cardiovascular effects puts a big question mark on the safety profile of other COXIBs in the long-term therapy.[17] Consequently, there

Keywords: Pyridazinone; Anti-inflammatory; Selective COX-2 inhibitors; Docking study.
* Corresponding author. Tel.: +202 2508 0848; fax: +202 2508 0728; e-mail: abouzid@yahoo.com

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.04.007

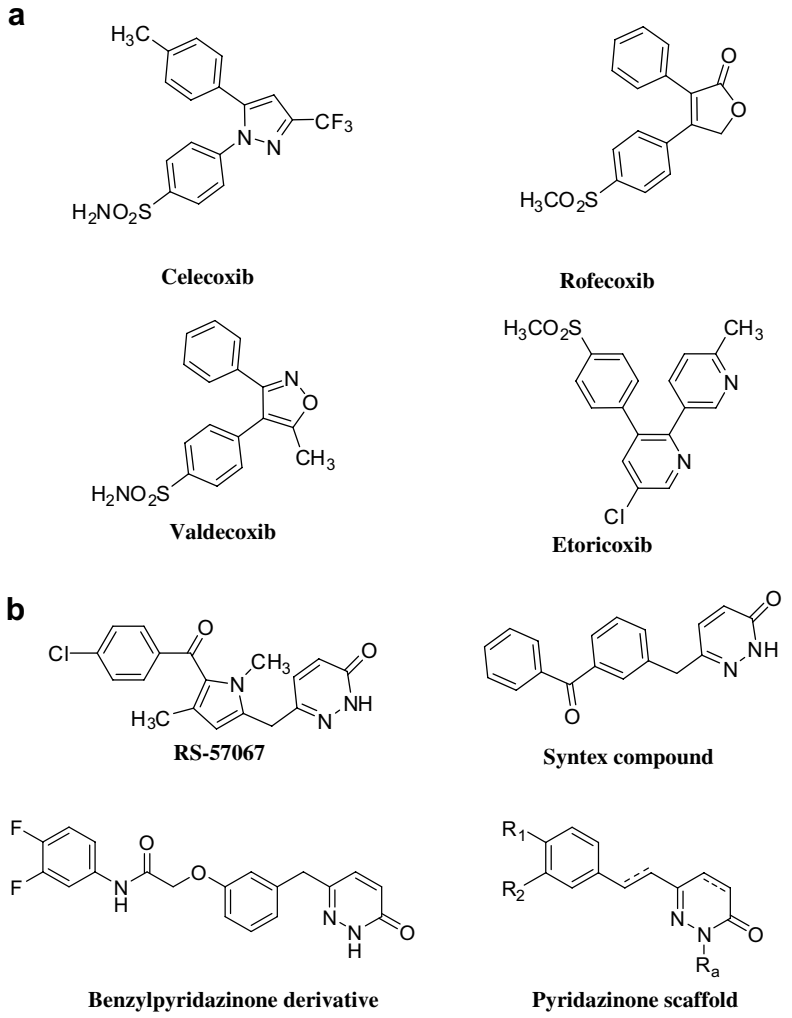


Figure 1. (a) Some famous COXIBs and (b) pyridazinone lead compounds and our pyridazinone scaffold.

is a current need to develop safer anti-inflammatory agents based on alternative templates.

Later on, a number of new indications have been explored clinically with celecoxib and/or rofecoxib. The role of selective COX-2 inhibitors in the prevention and treatment of a variety of cancers has been explored extensively as COX-2 is expressed at high levels in numerous cancer tissues and this expression has correlated with lower survival rates in patients.[18]

Since it has been reported that pyridazinone nucleus was found to be an excellent template for many selective COX-2 inhibitors such as the syntex compound and RS-57067 and benzylpyridazinone derivatives[19–22] have been described. In the same direction, we planned to design new pyridazinone scaffolds as potential anti-inflammatory agents which could act as selective COX-2 inhibitors (Fig. 1).

2. Rational and design

After identification of theX-ray three-dimensional crystal structure of both human COX-1 and COX-2 isozymes and after their exact mechanism became established, molecular design of selective inhibitors became straightforwardly accessible.[23–26]

Taking the reports about the potential cardiovascular dangers posed by COXIBs into cognizance, and the cost and time involved in the discovery of a new drug, our complementary strategy is concerned with the modification of structures of some conventional NSAIDs. This could be verified through conversion of the carboxylic group of NSAIDs into cyclic amide functions aiming to enhance the selectivity of these compounds towards COX-2 enzyme taking advantage of the larger COX-2 active site and thus impart their selectivity. This effort has been aided greatly by the availability of structural information on both COX enzymes.[27]

Based on the literature data in the anti-inflammatory area, pyridazinone skeleton was considered to be an excellent template for novel selective COX-2 inhibitors. In this respect, we describe a new template for COX-2 inhibitors based on pyridazinone system.[22] In these designed compounds we utilized certain feature from non-selective anti-inflammatory drugs such as biphenyl fragment in flurbiprufen and fenbufen, and ethylene di-

oxy or methylene dioxy fragment in the previously reported NSAIDs.[28]

In addition, we investigated further attempts to refine the basic pyridazinone framework designed previously[22] by connecting pyridazinone ring at C(6) with an aryl moiety via ethyl or ethenyl chain instead of a methylene spacer. Also, by placing various substituents on the phenyl ring or on the amidic pyridazinone N(2). Also, partially reduced pyridazinone ring could replace the pyridazinone ring without affecting their binding energy to the active site of COX-2.

Besides, we employed protein docking study to discover structurally novel inhibitors of cyclooxygenase-2 (COX-2) by screening a selected number of pyridazinone derivatives, thus providing an alternative to extensive screening. Also, structural analysis of various anti-inflammatory agents and studying their docking mode in the flexible active site of COX-2 isozyme revealed that certain structural modification of pyridazinone skeleton could be afforded. From the results of virtual screening for many pyridazinone derivatives, a computational model for COX-2 inhibition was constructed and used to decide on candidate inhibitors to be synthesized (see Scheme 1).

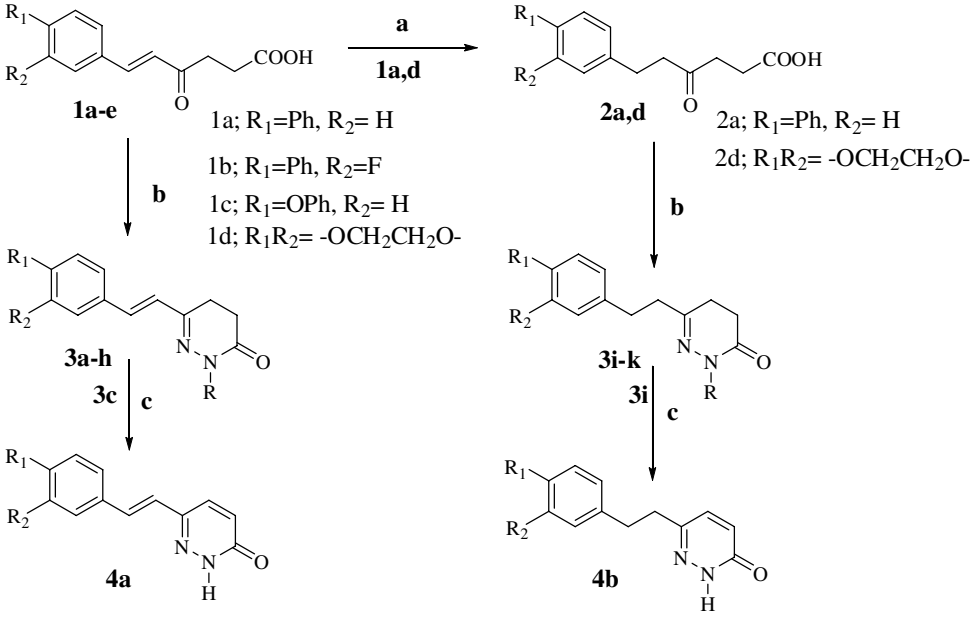
3. Chemistry

We report the synthesis of two series of pyridazinone target compounds from the corresponding keto acids as depicted in the following scheme. Aryloxohexenoic acids represent a key intermediate for the synthesis of the target compounds which was synthesized via condensation of the appropriately substituted araldehyde with levulinic acid according to the method previously reported.[28] Moreover, aryl oxohexenoic acids were reduced with Pd/C to the corresponding oxohexanoic acids. Thereafter, the obtained keto acids **1a**–**e** were cyclized with hydrazine hydrate or its methyl or phenyl derivative in acetic acid to afford the dihydropyridazinone derivatives. On the other hand, oxohexanoic acid derivatives were cyclized more smoothly with hydrazine hydrate or methylhydrazine to provide the corresponding dihydropyridazinones (**3i**–**k**) in refluxing ethanol for 2–3 h. On the other hand, pyridazinones **4a** and **4b** were obtained in moderate yield from the corresponding 4,5-dihydropyridazinones (**3c** and **3i**) under mild conditions with anhydrous $CuCl_2$ in acetonitrile via halogenation and spontaneous HCl elimination under the previously described reaction condition.[29]

Compound	R_1	R_2	R
3a	Ph	H	H
3b	Ph	F	H
3c	OPh	H	H
3d	$-OCH_2CH_2O-$		H
3e	Ph	H	CH_3
3f	$-OCH_2CH_2O-$		CH_3
3g	$-OCH_2O-$		CH_3
3h	Ph	H	Ph
3i	Ph	H	H
3j	$-OCH_2CH_2O-$		H
3k	Ph	H	CH_3
4a	OPh	H	H
4b	Ph	H	H

4. Molecular modeling

A variety of COX-2-ligand crystal structures have been solved over the last years.[23] These X-ray struc-



Scheme 1. Reagents and condition: (a) H_2 Pd/C, ethanol; (b) NH_2NHR/ethanol or acetic acid, reflux; and (c) $CuCl_2$/acetonitrile.

tures provide important information about the relevant interaction possibilities at the COX-2 binding pocket. The first crystal structure of human cyclooxygenase-2, in the presence of a selective inhibitor, is similar to that of cyclooxygenase-1. The structure of the non-steroidal anti-inflammatory drug (NSAID) binding site is also well conserved, although there are differences in its overall size and shape which may be exploited for the further development of selective COX-2 inhibitors. A second COX-2 structure with a different bound inhibitor displays a new, open conformation at the bottom of the NSAID binding site, without significant changes in other regions of the COX-2 structure. These two COX-2 structures provide evidence for the flexible nature of cyclooxygenase, revealing details about how substrate and inhibitor may gain access to the cyclooxygenase active site from within the membrane.[23]

The docking study was performed using Autodock software version 4 according to the reported procedure.[30] The COX-2 structure was obtained from the protein databank (PDB entry: 1CVU). The structures were minimized by Merck Molecular force field (MMFF) to a gradient of 4.5×10^{-5} using Spartan'06. The optimized geometries were exported as Sybyl mol2 file which were exported to Autodock tools. Gasteiger charges were calculated for each molecule and the torsional degrees of freedom were set to the maximum number of rotatable bonds. Water molecules were deleted from the file and hydrogens were added, then Kollman charges were calculated. No further geometry optimization was performed. Non-polar hydrogens were merged with the parent atoms for both the ligands and the receptor.

Structure preparation for the ligands and the receptor was done in Autodock tools. Each docking run was composed of 30 runs of the Lamarckian genetic algorithm (LGA), the number of top individuals to survive to next generation was set to two, and the clustering RMS tolerance was set to 3 Å, all the other docking parameters were kept unmodified from their default values.

Table 1. Predicted docked energy of the best scoring member of the largest cluster obtained for each ligand and experimental ED_{50} values

Compound	Docked energy	ED_{50}
RS-57067	−10.18	
3a	−9.05	24.6
3b	−10.2	N/T
3c	−10.67	28.7
3d	−9.74	27.0
3e	−10.44	32.4
3f	−9.74	N/T
3g	−8.71	34.3
3h	−8.04	38.6
3i	−10.95	**18.7**
3j	−10.72	**17.0**
3k	−10.09	22.3
4a	−10.18	N/T
4b	−10.92	N/T

The predicted docked energy of the best scoring member of the largest cluster obtained for each ligand and the experimental ED_{50} values are given in Table 1.

Figure 2 shows straight line correlation between the ED_{50} and docking energy, this confirms the validity of docking study.

From the binding study we could conclude that most of the ligands exhibit interactions with the amino acids; TRP 328, MET 522, VAL 523, SER 530, ALA 527, and SER 353. It was noticed that the ligands have moved a little away from ARG 513, TYR 355, and ARG 120 (Figs. 3–9). In the active site of COX-2, the amide NH of pyridazinone forms hydrogen bond with SER 530 in addition to extensive van der Waals interactions with many amino acids in contact. On the other hand, both compounds **3i** and **3j** showed the highest binding affinity to the COX2 active site as both compounds possessed the least docking energy compared with the other compounds and at the same time produced the best activity in terms of ED_{50}%. While compound **3k** with a flexible ethyl chain and N-methyl group exhibits little increase in docking energy and lesser ED_{50}% compared with its desmethyl derivative, compound **3h** which has N-phenyl group displayed the highest energy at the binding site. This coincides with the biological data which showed the least activity.

5. Biological screening

5.1. Anti-inflammatory activity

Carrageenan-induced rat paw edema test: Male albino rats weighing 120–150 g (Medical Research Institute, Alexandria University) were used throughout the work. They were kept in the animal house under standard conditions of light and temperature with free access to food and water. The animals were randomly divided into groups of six rats each. The paw edema was induced by subplantar injection of 50 μL of 2% carrageenan solution in saline (0.9%). Indomethacin and the test compounds were dissolved in DMSO and injected subcutaneously in different dose levels of 5, 10, 15, 20 and 25 μmol/kg body weight, 1 h prior to carrageenan injection. DMSO was injected to the control group. The volume of paw edema (mL) was determined by means of water plethysmometer immediately after injection of carrageenan and 4 h later. ED_{50} was calculated for the test compounds and reference drugs through dose response curves by measuring the inhibition of edema volume 4 h after the carrageenan injection.[31] These data were calculated using Microsoft excel software version 2002 and presented in Table 2.

The percentage protection against inflammation was calculated as follows: $V_c - V_d/V_c \times 100$, where V_c is the increase in paw volume in the absence of the test compound (control) and V_d is the increase of paw volume after injection of the test compound. Data were expressed as means ± SEM. Significant differences between the control and the treated groups were obtained

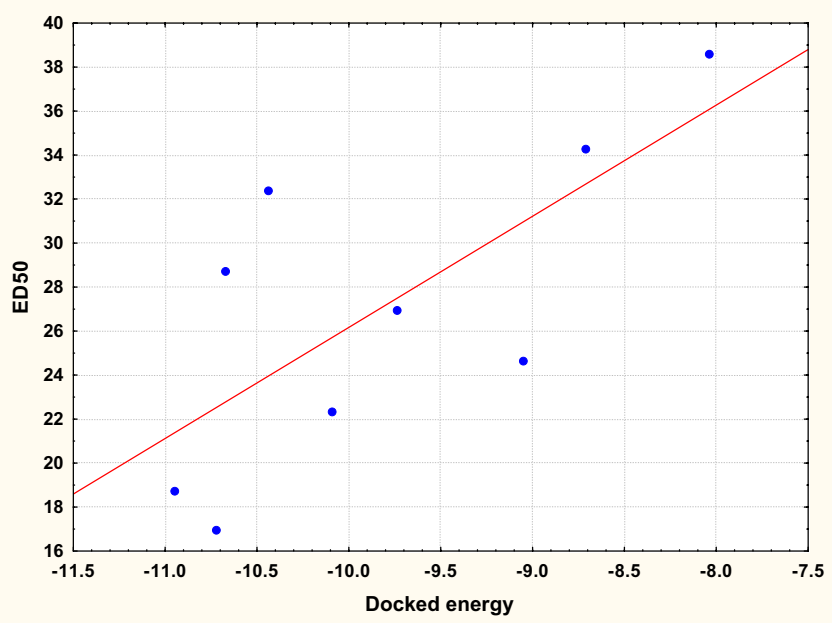


Figure 2. Correlation coefficient between the docked energy and the $ED_{50} = -0.71$ (significant at $P = 0.05$).

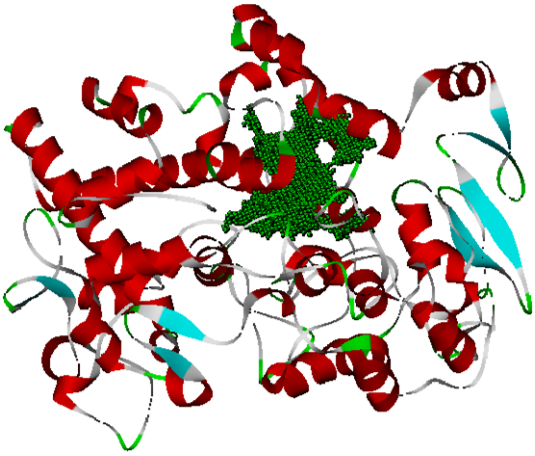


Figure 3. Large binding site of COX-2 appears as a green volume.

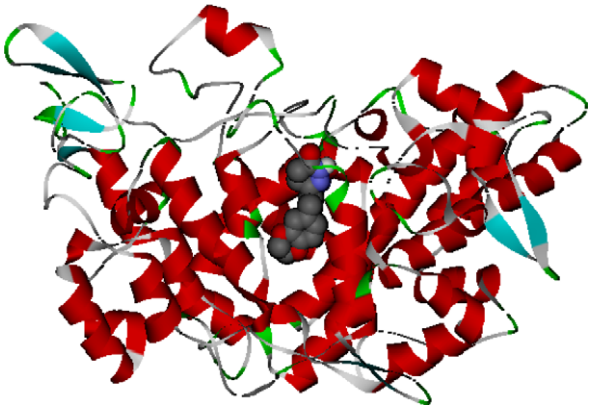


Figure 5. Docking of compound **3j** (spacefill) in the active site of COX-2.

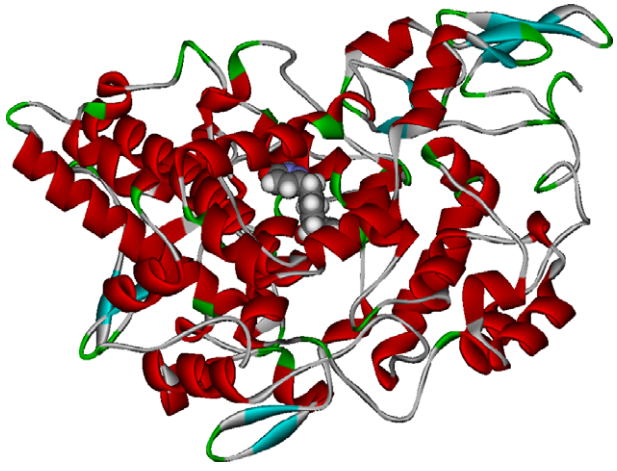


Figure 4. RS-57067 (spacefill) bound in the active site of COX-2.

using Student's *t*-test and *P* values. The differences in results were considered significant when $P < 0.001$. The anti-inflammatory activity of the test compounds relative to that of indomethacin was also determined (Table 3).

5.2. Ulcerogenic effects

Most of the synthesized compounds were evaluated for their ulcerogenic potential in rats.[32] Indomethacin was used as reference standard. Male albino rats (100–120 g) were fasted for 12 h prior to the administration of the compounds. Water was given ad libitum. The animals were divided into groups, each of six animals. The control group received 1% gum acacia orally. Other groups received indomethacin or the test compounds orally in two equal doses at 0 and 12 h for three successive days at a dose of 30 μM/kg per day. Animals were sacrificed by diethyl ether 6 h after the last dose and the stomach was removed. An opening at the greater curva-

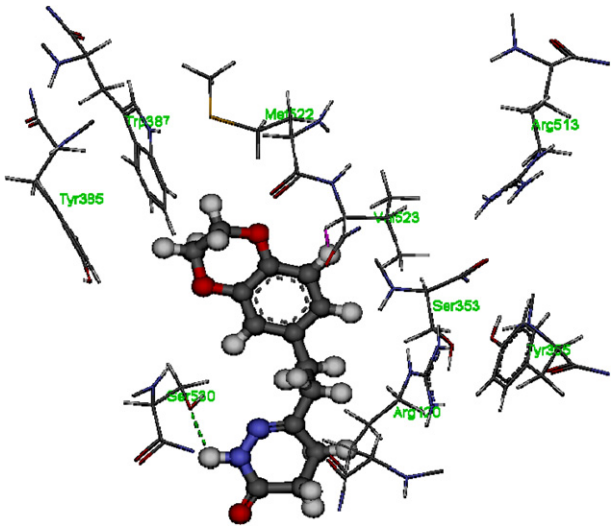


Figure 6. Compound **3j** (ball and stick) docked in the active site of COX-2 showing H bonding between amide H and ser-530 as dotted green line.

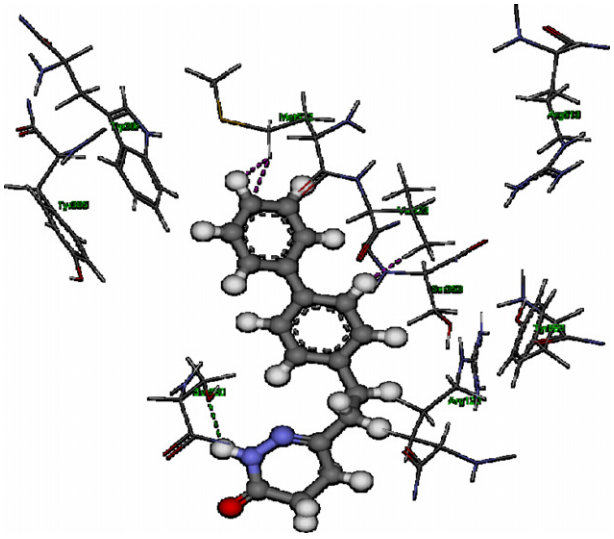


Figure 7. Compound **3i** (ball and stick) docked in the active site of COX-2.

ture was made and the stomach was cleaned by washing with cold saline and inspected with a 3× magnifying lens for any evidence of hyperemia, hemorrhage, definite hemorrhagic erosion, or ulcer. An arbitrary scale was used to calculate the ulcer index which indicates the severity of the stomach lesions.[32] (Table 2). The % ulceration for each group was calculated as follows:

$$\%\ \text{Ulceration} = \frac{\text{Number of animals bearing ulcer in a group}}{\text{Total number of animals in the same group}} \times 100$$

6. Conclusion

The present study describes the synthesis of a series of pyridazinone derivatives linked at C(6) to aryl or biphenyl moieties through two carbon spacers. The synthesized compounds exhibited anti-inflammatory activity and superior gastrointestinal safety profile. The results of biological screening also revealed that compounds **3i** and **3j** in which ethyl spacer between the dihydropyridazinone ring and the aryl moiety exhibits the highest activity compared to the ethenyl analogs.

In addition, two potent and probable selective COX-2 inhibitors **3i** and **3j** have been identified among the test compounds and showed excellent efficacy in animal models of inflammation. Moreover, since it was reported that the safety profile of ulcerogenic test could be considered as a measure for COX-2 selectivity. Accordingly, these compounds could be speculated as selective COX-2 inhibitors.[33] This hypothesis is substantiated by the molecular docking study which demonstrated that docking energy of these compounds are comparable to that of the lead compound RS-57067, which is previously reported as selective COX-2. Also, their binding mode to the active site of COX-2 with amino acids in the vicinity is very close to that of the selective COX-2 inhibitors.

7. Experimental

Melting points were determined with a Stuart Scientific apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR series using KBr cell. ^{1}H NMR and ^{13}C NMR were measured in δ scale on Varian-200 MHz, Gemini-200-software spectrometer and Brücker 300 spectrometers. All the spectra were obtained in solutions in DMSO-d_6 or $CDCl_3$ and referred to TMS. The electron impact (EI) mass spectra were recorded on Finnigan Mat SSQ 7000 (70 eV) mass spectrometer. Analytical thin layer chromatography (TLC) on silica gel plates containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products. All reagents and solvents were purified and dried by standard techniques. Elemental microanalyses were performed at Microanalytical Center, Cairo University, and were within ±4% unless otherwise stated. All chemicals were purchased from Sigma–Aldrich, Germany, and all the solvents were of analytical grades.

7.1. General procedure for preparation of (*E*)-6-(2-arylvinyl)-4,5-dihydropyridazin-3(2H)-ones (3a–d)

To a solution of (*E*)-6-aryl-4-oxohex-5-enoic acids (**1a–d**) (0.071 mmol) in acetic acid (5 mL) hydrazine hydrate 80% (0.71 mmol) was slowly added and the reaction mixture was stirred under reflux for 4 h. The solvent was removed under reduced pressure. The residue was triturated with 10 mL distilled water, filtered and recrystallized from the appropriate solvent.

7.1.1. (*E*)-6-[2-(Biphenyl-4-yl)vinyl]-4,5-dihydropyridazin-3(2H)-one (3a). Compound **3a** was prepared from **1a** and recrystallized from ethanol as white needles. Yield: 71%; mp: 216–217 °C, ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 2.41 (t, 2H, J = 7.85 Hz, CH_2, dihydropyridazinone), 2.81 (t, 2H, J = 7.85 Hz, CH_2, dihydropy-

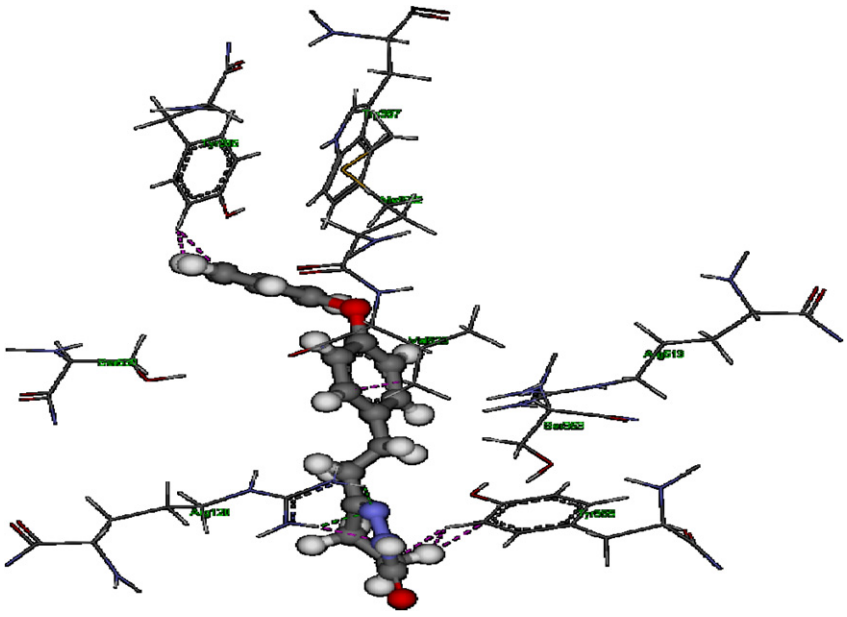


Figure 8. Compound **3c** (ball and stick) docked in the active site of COX-2.

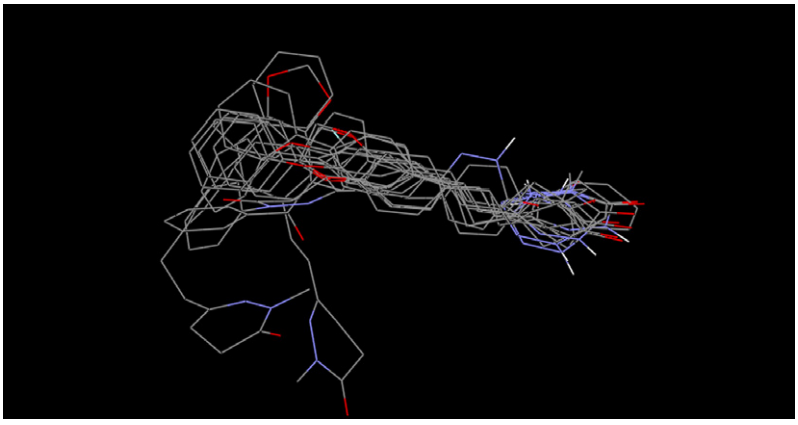


Figure 9. Overlaid docked pyridazinone structures showing that they all bind with the same orientation.

Table 2. Anti-inflammatory (ED_{50}, μmol/kg)[a] and ulcerogenic activity of 6-substituted-4,5-dihydropyridazin-3(2H)-ones (**3a**–**k**) and reference drugs

Compound	ED_{50} (μmol/kg)	% Ulceration
Indomethacin	9.7 ± 0.022	100
Celecoxib	16.7 ± 0.024	0
3a	24.6 ± 0.018	10
3b	N/T	N/T
3c	28.8 ± 0.033	0.0
3d	27.0 ± 0.020	**0.0**
3e	32.4 ± 0.026	20
3f	N/T	N/T
3g	34.3 ± 0.028	10
3h	38.6 ± 0.031	10
3i	**18.7 ± 0.025**	10
3j	**17.0 ± 0.027**	0.0
3k	22.3 ± 0.019	0.0

N/T means not tested.

[a] All data are significantly different from control ($P < 0.001$).

ridazinone), 6.93 (d, 1H, $J = 16.4$ Hz, –CH═), 7.08 (d, 1H, $J = 16.6$ Hz, –CH═), 7.37 (t, 1H, $J = 7.6$ Hz, ArH), 7.47 (t, 2H, $J = 7.6$ Hz, ArH), 7.69–7.71 (m, 6H, ArH), 10.9 (s, 1H, NH), ^{13}C NMR (20, 25, 126, 127, 128, 129, 132, 136, 140, 152, 170). IR (KBr) cm^{-1} 3250, 1686. MS *m/z* 276 (M^+, 43%). Anal. Calcd for $C_{18}H_{14}N_2O$: C, 78.24; H, 5.84; N, 10.14. Found: C, 78.16; H, 5.76; N, 10.21.

7.1.2. (*E*)-6-[2-(2-Fluorobiphenyl-4-yl)vinyl]-4,5-dihydropyridazin-3(2H)-one (3b). Compound **3b** was prepared from **1b** and recrystallized from ethanol as a white solid. Yield: 80%; mp: 188–189 °C, ^{1}H NMR ($CDCl_3$) (200 MHz) δ: 2.54 (t, 2H, $J = 8.0$ Hz, CH_2, dihydropyridazinone), 2.84 (t, 2H, $J = 8.1$ Hz, CH_2, dihydropyridazinone), 6.87 (d, 1H, $J = 16.4$ Hz, –CH═), 7.23 (d, 1H, $J = 16.6$ Hz, –CH═), 7.36 (m, 8H, ArH), 9.0 (s, 1H, NH). IR (KBr) cm^{-1}: 3220, 1680. MS *m/z* 294 (M^+, 87.26%). Anal. Calcd for $C_{18}H_{15}FN_2O$: C, 73.45; H, 5.14; N, 9.52. Found: C, 73.37; H, 4.97; N, 9.35.

7.1.3. (*E*)-6-(4-Phenoxystyryl)-4,5-dihydropyridazin-3(2H)-one (3c). Compound **3c** was prepared from **1c** and

Table 3. Effect of compounds **3i** and **3j** on carrageenan-induced rat paw edema (mL), % protection and activity relative to indomethacin

Test compound	Increase in paw edema (mL) ± SEM[a,b]	% Protection	Activity relative to indomethacin
Control	0.98 ± 0.027	0.0	0.0
Indomethacin	0.25 ± 0.024	74.4	100
Celecoxib	0.22 ± 0.016	77.5	104.23
3i	0.41 ± 0.015	58.16	78.17
3j	0.38 ± 0.012	61.22	82.28

[a] SEM denotes the standard error of the mean.
[b] All data are significantly different from control ($P < 0.001$).

recrystallized from ethanol as a beige solid. Yield: 76%; mp: 173–175 °C, ^{1}H NMR ($CDCl_3$) (300 MHz) δ: 2.55 (t, 2H, $J = 8.0$ Hz, CH_2, dihydropyridazinone), 2.81 (t, 2H, $J = 8.1$ Hz, CH_2, dihydropyridazinone), 6.80 (d, 1H, $J = 16.0$ Hz, –CH═), 7.04 (d, 1H, $J = 16.0$ Hz, –CH═), 7.11–7.45 (m, 9H, ArH), 8.67 (s, 1H, NH). IR (KBr) cm^{-1}: 3210, 1675. MS *m/z* 292 (M^+, 57.2%). Anal. Calcd for $C_{18}H_{16}N_2O_2$: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.41; H, 4.77; N, 9.32.

7.1.4. (*E*)-6-[2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)vinyl]-4,5-dihydropyridazin-3(2H)-one (3d). Compound **3d** was prepared from **1d** and recrystallized from ethanol as off-white solid. Yield: 66%; mp: 179–181 °C, ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 2.36 (t, 2H, $J = 7.85$ Hz, CH_2, dihydropyridazinone), 2.73 (t, 2H, $J = 7.85$ Hz, CH_2, dihydropyridazinone), 4.25 (s, 4H, –OCH $_2$CH$_2$ O–), 6.70 (d, 1H, $J = 16.4$ Hz, –CH═), 6.84 (d, 1H, $J = 8.2$ Hz, ArH), 6.90 (d, 1H, $J = 16.4$ Hz, –CH═), 7.05–7.07 (dd 1H, $J = 1.9$, 8.4 Hz, ArH), 7.09 (d, 1H, $J = 2.2$ Hz, ArH), 10.81 (s, 1H, NH), ^{13}C NMR (20, 25, 64, 65, 115, 117, 120, 126, 130, 144, 145, 151, 167). IR (KBr) cm^{-1}: 3440, 1670. MS *m/z* 258 (M^+, 55.4%). Anal. Calcd for $C_{14}H_{14}N_2O_3$: C, 65.11; H, 5.46; N, 10.85. Found: C, 65.34; H, 5.49; N, 10.67.

7.1.5. (*E*)-6-[2-(Biphenyl-4-yl)vinyl]-2-methyl-4,5-dihydropyridazin-3(2H)-one (3e). Compound **3e** was prepared from **1a**, *N*-methylhydrazine and refluxed for 6 h, recrystallized from ethanol as a faint yellow solid. Yield: 86%; mp: 205–207 °C, ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 2.45 (t, 2H, $J = 8.2$ Hz, CH_2, dihydropyridazinone), 2.82 (t, 2H, $J = 8.2$ Hz, CH_2, dihydropyridazinone), 3.27 (s, 3H, CH_3), 6.91 (d, 1H, $J = 16.4$ Hz, –CH═), 7.06 (d, 1H, $J = 16.6$ Hz, –CH═), 7.32 (t, 1H, $J = 7.6$ Hz, ArH), 7.44 (t, 2H, $J = 7.6$ Hz, ArH), 7.65 (m, 6H, ArH), ^{13}C NMR (20, 27, 38, 126, 127, 128, 129, 132, 130, 138, 152, 167). IR (KBr) cm^{-1}: 1699. MS *m/z* 290 (M^+, 77.1%). Anal. Calcd for $C_{19}H_{18}N_2O$: C, 78.59; H, 6.25; N, 9.65. Found: C, 78.43; H, 6.11; N, 9.34.

7.1.6. (*E*)-6-[2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)vinyl]-2-methyl-4,5-dihydropyridazin-3(2H)-one (3f). Compound **3f** was prepared from **1d**, *N*-methylhydrazine and refluxed for 7 h, recrystallized from aqueous ethanol as a white solid. Yield: 90 %; mp: 176–178 °C, ^{1}H NMR ($CDCl_3$) (300 MHz) 2.49 (t, 2H, $J = 8.1$ Hz, CH_2, dihydropyridazinone), 2.75 (t, 2H, $J = 7.85$ Hz, CH_2, dihydropyridazinone), 3.39 (s, 3H, CH_3), 4.25 (s, 4H, –OCH_2 CH_2O–), 6.74–7.0 (m, 5H, 2-CH═ and 3 ArH). IR (KBr) cm^{-1}: 1661, 1578. MS *m/z* 272 (M^+, 100%). Anal. Calcd for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29, C, 66.05; H, 5.88; N, 10.16.

7.1.7. (*E*)-6-(2-[Benzo[d][1,3]dioxol-5-yl)vinyl]-2-methyl-4,5-dihydropyridazin-3(2H)-one (3g). Compound **3g** was prepared from **1e**, *N*-methylhydrazine and refluxed for 4 h, recrystallized from aqueous ethanol as a white solid. Yield: 87 %; mp: 195–197 °C, ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 2.41 (t, 2H, $J = 7.85$ Hz, CH_2, dihydropyridazinone), 2.77 (t, 2H, $J = 7.85$ Hz, CH_2, dihydropyridazinone), 3.24 (s, 3H, CH_3), 6.04 (s, 2H, –OCH_2 O–), 6.70 (d, 1H, $J = 16.4$ Hz, –CH═), 6.92 (d, 1H, $J = 8.2$ Hz, ArH), 7.01 (d, 1H, $J = 16.4$ Hz, –CH═), 7.05–707 (dd, 1H, $J = 2$, 8.4 Hz, ArH), 7.29 (d, 1H, $J = 1.55$ Hz, ArH), ^{13}C NMR (20, 26, 35, 100, 105, 108, 122, 123, 130, 134, 146, 152, 164). IR (KBr) cm^{-1}: 1640. MS *m/z* 258 (M^+, 48.1%). Anal. Calcd for $C_{14}H_{14}N_2O_3$: C, 65.11; H, 5.46; N, 10.85. Found: C, 65.08; H, 5.79; N, 10.68.

7.1.8. (*E*)-6-[2-(Biphenyl-4-yl)vinyl]-2-phenyl-4,5-dihydropyridazin-3(2H)-one (3h). Compound **3h** was prepared from **1a**, phenylhydrazine and refluxed for 4 h recrystallized from aqueous ethanol as a white solid. Yield: 87 %; mp: 151–153 °C, ^{1}H NMR ($CDCl_3$) (300 MHz) δ: 2.76 (t, 2H, $J = 7.6$ Hz, CH_2, dihydropyridazinone), 2.95 (t, 2H, $J = 7.6$ Hz, CH_2, dihydropyridazinone), 6.91–7.62 (M, 16H, –CH═ and ArH). IR (KBr) cm^{-1}: 1635. MS *m/z* 352 (M^+, 44.4%). Anal. Calcd for $C_{24}H_{20}N_2O$.C, 81.79; H, 5.72; N, 7.95. Found: C, 81.66; H, 5.57; N, 7.69.

7.2. General procedure: 6-(2-arylethyl)-4,5-dihydropyridazin-3(2H)-ones (3i–k)

To a solution of 6-aryl-4-oxohax-5-enoic acid (**2a** or **2d**) (0.071 mmol) in ethanol (10 mL), hydrazine hydrate or *N*-methylhydrazine 80% (0.71 mmol) was added and the reaction mixture was stirred under reflux for 3 h. The reaction mixture was cooled and the precipitate formed was filtered, washed with diethyl ether, and recrystallized from the appropriate solvent.

7.2.1. 6-[2-(Biphenyl-4-yl)ethyl]-4,5-dihydropyridazin-3(2H)-one (3i). Compound **3i** was prepared from **1a** and hydrazine hydrate. Yield: 85 %; mp: 194–196 °C ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 2.26 (t, 2H, $J = 8.1$ Hz, CH_2), 2.46 (t, 2H, $J = 7.9$ Hz, CH_2), 2.58 (t, 2H, $J = 7.9$ Hz, CH_2, dihydropyridazinone), 2.86 (t, 2H, $J = 8.2$ Hz, CH_2, dihydropyridazinone), 7.31–7.35 (m, 3H, ArH), 7.45 (t, 2H, $J = 7.6$ Hz, ArH), 7.57 (d, 2H, $J = 8.2$ Hz, ArH), 7.63 (d, 2H, $J = 7.25$ Hz, ArH), 10.46 (s, 1H, NH), ^{13}C NMR (23, 27, 32, 38, 125, 126, 128, 138, 140, 141, 154, 168). IR (KBr) cm^{-1}: 3247, 1662. MS *m/z* 278 (M^+, 17.1%), 167 (100%). Anal. Calcd for $C_{18}H_{18}N_2O$: 77.67; H, 6.52; N, 10.06. Found: C, 77.38; H, 6.42; N, 9.86.

7.2.2. 6-[2-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)ethyl]-4,5-dihydropyridazin-3(2H)-one (3j). Compound **3j** was obtained from **2d** and recrystallized from dioxan. Yield: 77%; mp: 139–141 °C ^{1}H NMR (DMSO-d_6) (300 MHz)

δ: 2.23 (t, 2H, J = 8.5 Hz, CH_2), 2.41 (t, 2H, J = 7.9 Hz, CH_2), 2.47 (t, 2H, J = 7.25 Hz, CH_2, dihydropyridazinone), 2.68 (t, 2H, J = 7.25 Hz, CH_2, dihydropyridazinone 4.18 (s, 4H, –OCH $_2CH_2O$–), 6.64–6.66 (dd, 1H, J = 2, 8.4, ArH), 6.70 (d, 1H, J = 2.2 Hz, ArH), 6.74 (d, 1H, J = 8.2 Hz, ArH), 10.5 (s, 1H, NH), ^{13}C NMR (20, 25, 64, 65, 115, 117, 120, 126, 130, 144, 145, 151, 167). IR (KBr) cm^{-1}: 3442, 1649,1641,1598. MS *m/z* 260 (M^+, 6.7%), 149 (100%). Anal. Calcd for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.66; H, 6.15; N, 10.88.

7.2.3. 6-(2-(Biphenyl-4-yl)ethyl)-2-methyl-4,5-dihydropyridazin-3(2H)-one (3k). Compound **3k** was prepared from **1a** and recrystallized from ethanol as white crystals. Yield: 91%; mp: 145–146 °C ^{1}H NMR (DMSO-d_6) (300 MHz) δ: 2.31 (t, 2H, J = 8.1 Hz, CH_2), 2.49 (t, 4H, J = 7.9 Hz, CH_2), 2.60 (t, 2H, J = 7.9 Hz, CH_2), 2.86 (t, 2H, J = 8.2 Hz, CH_2), 3.16 (s, 3H, CH_3), 7.31–7.36 (m, 3H, ArH), 7.48 (t, 2H, J = 7.9 Hz, ArH), 7.57 (d, 2H, J = 8.2 Hz, ArH), 7.63 (dd, 2H, J = 8.50, 0.95 Hz, ArH), 10.46 (s, 1H, NH), ^{13}C NMR (24, 27, 32, 36, 39, 125, 126, 128,139,141, 142, 156, 168). IR (KBr) cm^{-1}: 1660. MS 292 (M^+, 33.2%), 167 (100%). Anal. Calcd for $C_{19}H_{20}N_2O$: C, 78.05; H, 6.89; N, 9.58. Found: C, 77.75; H, 6.94; N, 9.79.

7.3. General procedure for dehydrogenation of 4,5-dihydropyridazin-3(2H)-ones (3c and 3i)

To a solution of **3c** or **3i** (0.01 mol) in absolute acetonitrile (25 mL), anhydrous $CuCl_2$ (2.68 g, 0.02 mol) was added and the mixture was refluxed for 30 min. After few minutes, the brown solution turned pale yellow. The product that separated out on cooling was filtered, washed with ether, and crystallized from a suitable solvent.

7.3.1. (*E*)-6-(4-Phenoxystyryl)pyridazin-3(2H)-one (4a). Compound **4a** was prepared from **3c** and recrystallized from acetone as a beige solid. Yield: 64%; mp: 188–190 °C, ^{1} H NMR ($CDCl_3$) (300 MHz) δ: 4.89 (d, 1H, J = 10.5 Hz, –CH═), 5.03 (d, 1H, J = 10.5 Hz, –CH═), 6.82–7.64 (m, 11H, pyridazinone and ArH), 10.4 (s, 1H, NH). IR (KBr) cm^{-1}: 1716. MS *m/z* 290 (M^+, 100%); Anal. Calcd for C $_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.27; H, 4.65; N, 9.88.

7.3.2. 6-(2-(Biphenyl-4-yl)ethyl)pyridazin-3(2H)-one (4b). Compound **4b** was prepared from **3i** and recrystallized from aqueous ethanol as a white solid. Yield: 74%; mp: 180–182 °C ^{1}H NMR ($CDCl_3$) (300 MHz) δ: 2.96–3.03 (t, 4H, J = 6.1 Hz, $2CH_2$), 6.87 (d, 1H, J = 9.6 Hz, CH pyridazinone), 7.08 (d, 1H, J = 9.6 Hz, CH pyridazinone), 7.31–7.58 (m, 9H, ArH), 11.20 (s, 1H, NH). IR (KBr) cm^{-1}: 1710. MS *m/z* 276 (M^+, 3.75%), 167 (100%); Anal. Calcd for $C_{18}H_{16}N_2O$: C, 78.24; H, 5.84; N, 10.14. Found: C, 78.01; H, 5.55; N, 10.40.

Acknowledgments

I thank professor Dr. Ernst Urban, Institute of Pharmacy, Vienna University for his indispensable cooperation and for carrying out NMR spectra. I also thank Dr. Azza Baraka, Department of Pharmacology, Faculty of Medicine, University of Alexandria for her indispensable support in the pharmacological study.

References and notes

1. (a) Allison, M. C.; Howatson, A. G.; Torrance, C. J.; Lee, F. D.; Russel, R. I. *N. Engl. J. Med.* **1992**, *327*, 749; (b) Botting, R. M. *J. Therm. Biol.* **2006**, *31*, 208.
2. Vane, J. R.; Bakhle, Y. S.; Blotting, R. M. *Annu. Rev. Pharmacol. Toxicol.* **1998**, *38*, 97.
3. Dannhardt, G.; Kiefer, W. *Eur. J. Med. Chem.* **2001**, *36*, 109.
4. Isakson, P. C. *Prog. Exp. Tumor Res.* **2003**, *37*, 25.
5. Garavito, R. M.; Mulichak, A. M. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 183.
6. Kurumbail, R. G.; Stevens, A. M.; Gierse, J. K.; McDonald, J. J.; Stegeman, R. A.; Pak, J. Y.; Gildehaus, D.; Miyashiro, J. M.; Penning, T. D.; Seibert, K.; Isakson, P. C.; Stallings, W. C. *Nature* **1996**, *384*, 644.
7. Bombardier, C. *Am. J. Cardiol.* **2002**, *89* (Suppl.), 3-D .
8. Ferreira, S. H.; Moncada, S.; Vane, J. R. *Nature* **1971**, *231*, 237.
9. Murry, M. D.; Brater, D. C. *Annu. Rev. Pharmacol. Toxicol.* **1993**, *33*, 435.
10. Dannhardt, G.; Kiefer, W. *Eur. J. Med. Chem.* **2001**, *36*, 109.
11. Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Doctor, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. M.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347.
12. Talley, J. J.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Koboldt, C. M.; Masferrer, J. L.; Perkins, W. E.; Rogers, R. S.; Shaffer, A. F.; Zhang, Y. Y.; Zweifel, B. S.; Seibert, K. *J. Med. Chem.* **2000**, *43*, 775.
13. Li, J. J.; Norton, M. B.; Reinhard, E. J.; Anderson, G. D.; Gregory, S. A.; Isakson, P. C.; Koboldt, C. M.; Masferrer, J. L.; Perkins, W. E.; Seibert, K.; Zhang, Y.; Zweifel, B. S.; Reitz, D. B. *J. Med. Chem.* **1996**, *39*, 1846.
14. Friesen, R. W.; Brideau, C.; Chan, C.-C.; Charleson, S.; Deschenes, D.; Dube, D.; Ethier, D.; Fortin, T.; Gauthier, J. Y.; Girard, Y.; Gordon, R.; Greig, G.; Riendeau, D.; Savoie, C.; Wang, Z.; Wong, E.; Visco, D.; Xu, L.-J.; Young, R. N. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2777.
15. Chen, Q.-A.; Rao, P. N. P.; Knaus, E. E. *Bioorg. Med. Chem.* **2005**, *13*, 6425.
16. Anana, R.; Rao, P. N. P.; Chenb, Q.-A.; Knausb, E. E. *Bioorg. Med. Chem.* **2006**, *14*, 5259.
17. Dogne, J. M.; Supuran, C. T.; Pratico, D. *J. Med. Chem.* **2005**, *48*, 2251.
18. Grossman, H. B. *Drugs Today* **2003**, *39*, 203.
19. Li, C. S.; Brideau, C.; Chan, C. C.; Savoie, C.; Claveau, D.; Gordon, C. R.; Greig, G.; Gauthier, J. Y.; Lau, C. K.; Riendeau, D.; Therien, M.; Wong, E.; Prasit, P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 597.
20. Biancalani, C. N.; Vergelli, C. C.; Piaz, V. D.; Graziano, A.; Biagini, P.; Ghelardini, C.; Galeotti, N.; Giovannoni, M. P. *J. Med. Chem.* **2006**, *49*, 7826.
21. Beswick, P.; Sharon, B.; Bountra, C.; Brown, T.; Browning, K.; Campbell, I.; Chessell, I.; Clayton, N.; Collins, S.; Corfield, J.; Guntrip, S.; Haslam, C.; Lambeth, P.; Lucas, F.; Mathews, N.; Murkit, G.;

Naylor, A.; Pegg, N.; Pickup, E.; Player, H.; Price, H.; Stevens, A.; Stratton, S.; Wiseman, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5445.
22. Chintakunta, V. K.; Akella, V.; Vedula, M. S.; Mamnoor, P. K.; Mishra, P.; Casturi, S. R.; Vangoori, A.; Rajagopalan, R. *Eur. J. Med. Chem.* **2002**, *37*, 339.
23. Christine, L.; Aaron, M.; Barnett, Jim; Chow, Joan; Chakk, R.; Michelle, B. *Inflamm. Nat. Struct. Biol.* **1996**, *3*, 927.
24. Blobaum, A. L.; Marnett, L. J. *J. Med. Chem.* **2007**, *50*, 1425.
25. Habeeb, A. G.; Rao, P. N.; Knaus, E. E. *J. Med. Chem.* **2001**, *44*, 2921.
26. Soliva, R.; Almansa, C.; Kalko, S. G.; Luque, F. J.; Orozco, M. *J. Med. Chem.* **2003**, *46*, 1372.
27. Trummlitz, G.; van Ryn, J. *Curr. Opin. Drug Discovery Dev.* **2002**, *5*, 550.
28. Abouzid, K.; Frohberg, P.; Lehmann, J.; Decker, M. *Med. Chem.* **2007**, *3*, 433.
29. Csende, F.; Szabó, Z.; Bernáth, G.; Stájer, G. *Synthesis* **1995**, *1995*, 1240.
30. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.
31. DiRosa, M.; Willoughby, D. A. *J. Pharm. Pharmacol.* **1971**, *23*, 297.
32. Abou Zeit-Har, M. S.; Verimer, T.; Long, J. P. *Pharmazie* **1982**, *37*, 593.
33. Naser, M. N. A.; Said, S. A. *Arch. Pharm. Pharm. Med. Chem.* **2003**, *336*, 559.