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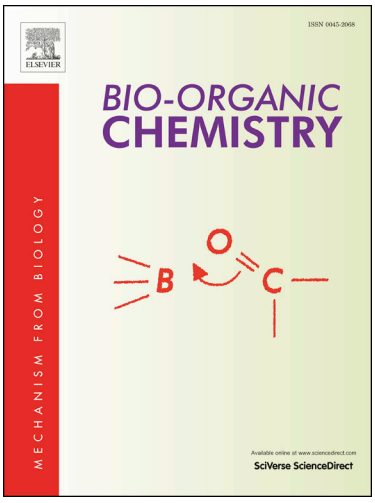

New imidazolone derivatives comprising a benzoate or sulfonamide moiety as anti-inflammatory and antibacterial inhibitors: Design, synthesis, selective COX-2, DHFR and molecular-modeling study

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New imidazolone derivatives comprising a benzoate or sulfonamide moiety as anti-inflammatory and antibacterial inhibitors: Design, synthesis, selective COX-2, DHFR and molecular-modeling study

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Abstract

New imidazol-5-one derivatives **12a,b** and **12e, f**, **14a,b** and **16a,b** were synthesized and screened for their in *vivo* anti-inflammatory activity using a standard acute carrageenan-induced rat paw oedema method. All the tested compounds exhibited good anti-inflammatory activity; especially compound **12f** which produced the maximum effect of 49.0 % compared to the standard drug, celecoxib, (43.1%). The most active anti-inflammatory agents **12a**, **12e**, and **12f** were studied for their interactions with enzyme COX-2 compared to celecoxib. The study showed that, compound **12e** exhibited a high selectivity towards COX-2 inhibition with IC_{50} = 0.087 μM. Moreover, the antibacterial screening indicated that some synthesized compounds showed good antibacterial activity toward the Gram-negative bacteria *Escherichia coli*. Additionally, compounds **7**, **12a**, **12f**, and **12** showed a good binding affinity with enzyme dihydrofolate reductase (DHFR) whereas compound **12f** has a higher inhibitory effect on DHFR than the tested compounds **7**, **12a** and **12h**. On the other hand, the combination between these tested compounds and sulfadiazine as a reference drug (10 μM compound + 1 μM reference), showed that compound **12h** has higher potency (0.078±0.002) than sulfadiazine (0.135±0.004). In addition, docking analysis was performed and it confirmed the presented results.

Keywords: Imidazol-5-ones; Synthesis; COX-2 inhibitors; Anti-inflammatory; DHFR inhibitors, Antibacterial, DHFR.

1. Introduction

Inflammation is a process which occurs when the tissues are injured by foreign organisms such as bacteria, viruses, trauma, toxins, heat, or any other cause[1,2]. It is an essential immune response by the host that allows for the removal of harmful stimuli as well as the healing of damaged tissue. Inflammation can be acute and chronic. However, acute inflammation is short-duration and its response in the body is not dangerous. It represents the early physical reaction followed by healing. Chronic inflammation, on the other hand, is longer in duration than acute inflammation and is caused by a variety of diseases especially cancer, neurodegenerative disorders and cardiovascular diseases [3,4]. The function of inflammation is that the damaged cells release chemicals including histamine, bradykinin, leukotrienes, prostaglandins (PGs), serotonin and others. These chemicals cause the blood vessels to leak fluid into the tissues causing swelling. This helps to isolate the foreign substance and prevent further contact with body tissues [5,6].

Steroid drugs prevent the synthesis of leukotrienes and PGs through the phospholipase A2 pathway. Also they are highly effective for chronic inflammatory conditions [7-10]. On the other hand, non-steroidal anti-inflammatory drugs (NSAIDs) can block specific prostaglandins (PGs) synthesis through the cyclooxygenase enzymes (COX-1 and COX-2) and lipoxygenase (5-LOX) inhibition [11,12]. NSAIDs are frequently used for arthritis, rheumatism and to alleviate pain in daily life but they have side effects such as gastric ulcer and renal impairment [13,14]. Selective COX-2 inhibitors were marketed as safer agents with no gastric ulcer risks compared to NSAIDs [15-17].

Celecoxib was the first selective COX-2 inhibitor (coxisb) to appear as a safer replacement for NSAIDs (non-selective COX-1/COX-2 inhibitors) but it has some side effects. So some celecoxib analogs were synthesized with higher therapeutic value and lower side effects through increasing specificity toward COX-2 rather than COX-1 [18-20].

Thus, it is important to find new anti-inflammatory agents to inhibit a selective COX-2 with a higher potential for clinical use and lesser adverse effects like Celecoxib. It's worth mentioning that antibacterial as well as anti-infammatory agents have a

significant function in pharmacological and medicinal chemistry. Both chemotherapeutic and anti-inflammatory agents are required to treat bacterial infections with inflammatory disorders.

Additionally, the antibacterial activity is one of the most important activities for inspecting novel antibacterial agents that control the most resistant pathogenic types of bacteria due to the over-use of antibiotics [21,22]. A remarkable example of Gram-negative bacteria is *pseudomonas aeruginosa* (*P. aeruginasa*) which is found abundantly in nature, for instance: plants, water, and soil. Pseudomonades are fairly common pathogens involved in infections acquired in a hospital setting by patients who are already hospitalized with another illness or condition, or people who have a weak immune system. Infections can occur in any part of the body and the symptoms depend on which part of the body is infected [23-27]. On the other hand, *Staphylococcus aureus* (*S. aureus*) is an example of Gram-positive bacteria which is a normal inhabitant of the respiratory tract and on the skin. *S. aureus* at skin infection causes sinusitis and food poisoning besides producing virulence factors such as potent toxins [28]. Moreover, *Streptococcus mutans* (*S. mutans*) is another type of Gram-positive in cocci bacteria. These arbitrary anaerobes are usually found in the human oral cavity and are a major contributor to tooth decay. *S. mutans* are mesophiles that grow at temperatures between 18-40 ^{o}C. In addition, they are cariogenic microorganisms that break down sugar to produce an acid that demineralizes teeth. The result of this conversion is that the coating of the tooth breaks down then the calcium molecule dissolves generating a hole [29].

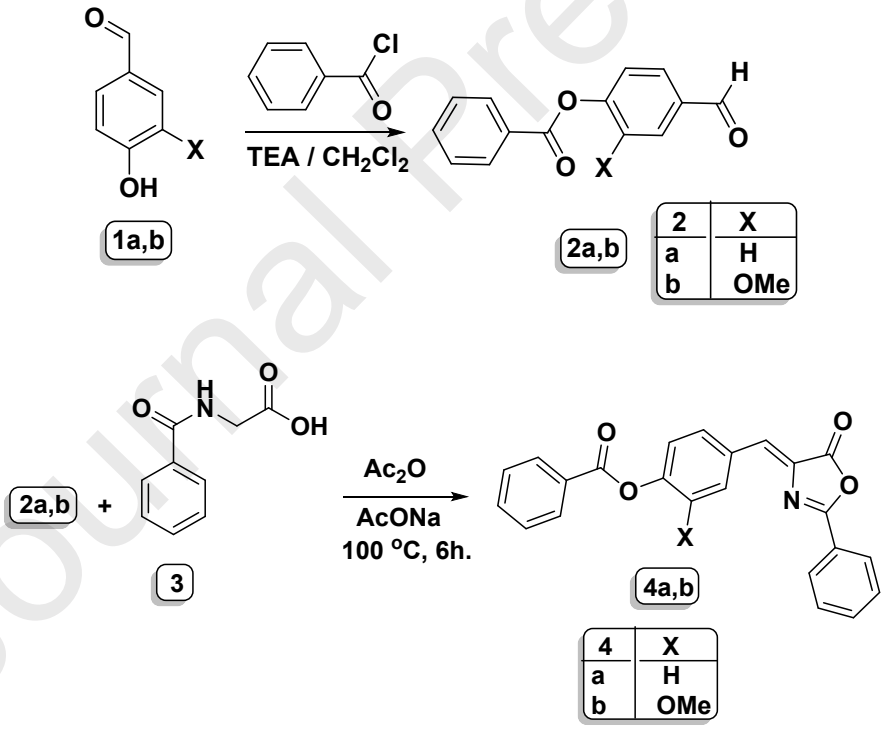
It was reported that several imidazolone derivatives have a wide range of biological activities like analgesic, anti-inflammatory [30-34] and antibacterial activities [35-38] among others [39-43]. Based on the above-mentioned information, and extension to synthesize bioactive molecules of interest biological activity [44-50] motivated us to find compounds having dual antimicrobial and anti-inflammatory activities. Thus, in the present study, we synthesize some new substituted imidazol-5-one analogs with comparable antibacterial and anti-inflammatory potencies. Also, we investigated them regarding their in *vivo* anti-inflammatory activity against acute carrageenan-induced paw oedema in wistar albino rats and determined their ability to inhibit selective cyclooxygenase (COX-2) to develop new anti-inflammatory drugs with greater activity and lesser side effects. Additionally, we evaluated them against

some types of pathogen bacteria and determine dihydrofolate reductase (DHFR) at different concentrations to provide a biochemical mechanism of action.

2. RESULTS AND DISCUSSION

2.1. Chemistry

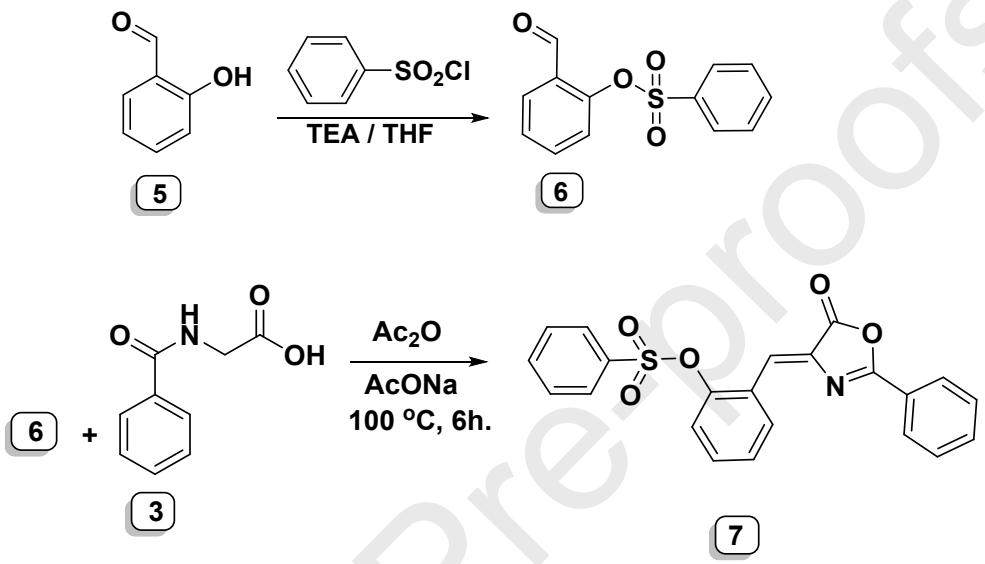
Cyclocondensation of benzoylglycine (**3**) with 4-formyl arylbenzoates **2a, b** [which is prepared by alkylating 4-hydroxybenzaldehyde derivatives **1a, b** with benzoyl chloride and triethylamine in dichloromethane] in acetic anhydride containing anhydrous sodium acetate in water bath at 100 ^{o}C afforded products **4a, b** (Scheme 1). The IR spectrum of **4a** showed absorption bands at 1738 and 1647 cm^{-1} corresponding to ester and carbonyl groups, respectively. Compound **4a** is characterized by the presence of a singlet signal at δ = 8.18 ppm assigned to vinylic proton in the ^{1}H NMR spectrum. Also, the mass spectrum of **4a** exhibited a correct molecular ion peak at m/z = 369 (M^{+}) corresponding to the molecular formula $C_{23}H_{15}NO_4$.



Scheme 1. Synthetic routes of 4-[(5-oxo-2-aryloxazol-4(5*H*) ylidene) methyl]-phenyl benzoate **4a,b.**

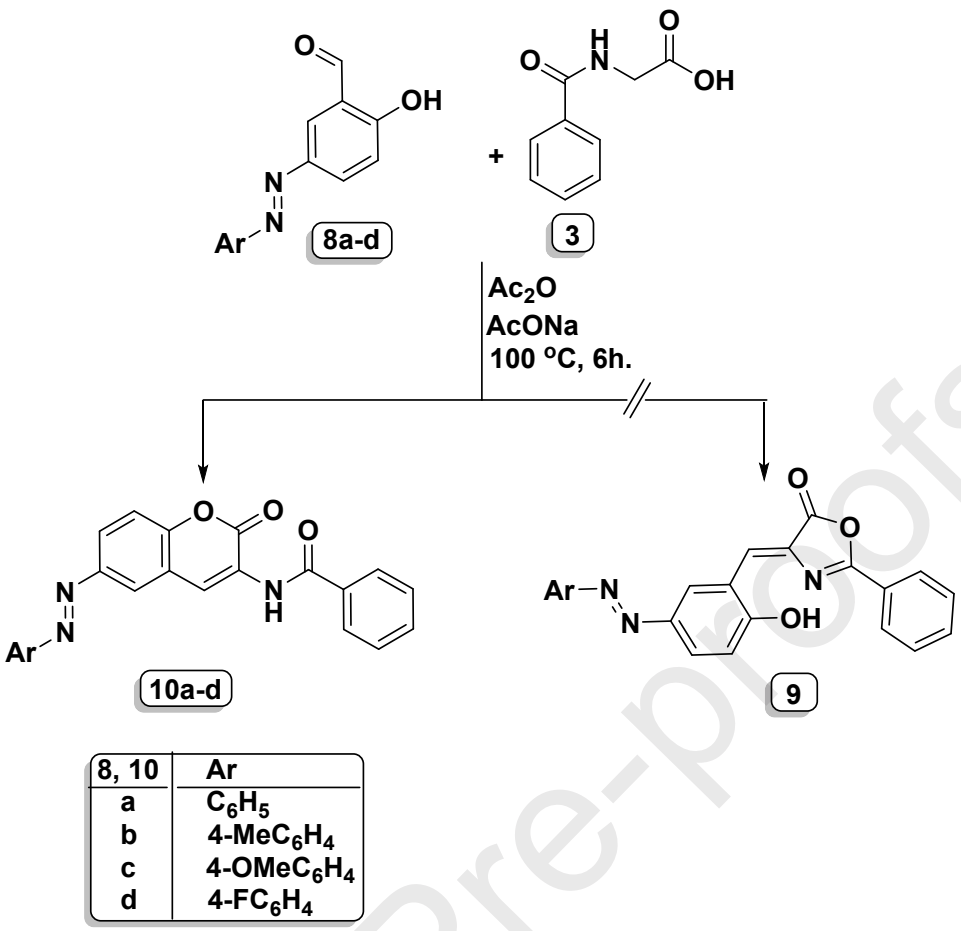
Moreover, cyclocondensation of compound **3** with 2-formylphenyl benzenesulfonate (**6**) [which is prepared by alkylating 2-hydroxybezaldehyde with

benzenesulfonyl chloride and triethylamine in tetrahydrofuran (THF)] in acetic anhydride concerning anhydrous sodium acetate afforded the new product **7** (Scheme 2). The ^{1}H NMR spectrum of **7** showed a singlet signal at δ = 8.76 ppm due to vinylic proton, besides the signals corresponding to aryl protons in the expected region. Also the IR spectrum implied the presence of carbonyl group at 1653 cm^{-1}.



Scheme 2. Synthesis of 2-[(5-oxo-2-phenyloxazol-4(5*H*)-ylidene) methyl] phenyl benzenesulfonate **7.**

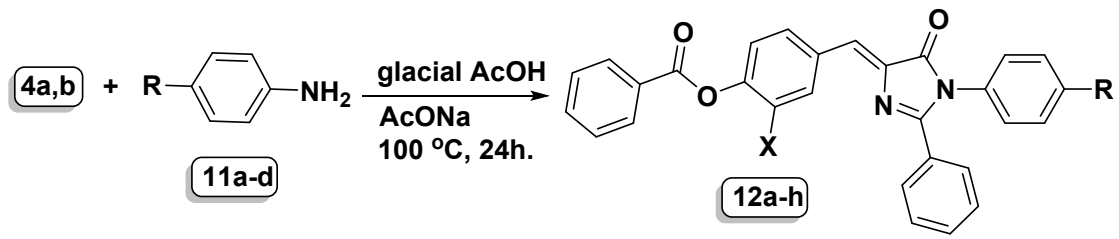
Additionally, compound **3** was condensed with arylazosalicyladehydes **8a-d** under the reaction conditions to produce compounds **10a-d** (Scheme 3). The structures of the prepared compounds were screened by spectral data and elemental microanalyses. The IR spectrum of **10d** taken as a representative example of the prepared series showed characteristic peaks at 3330, 1721 and 1670 cm^{-1} corresponding to NH and two carbonyl groups, respectively. The ^{1}H NMR spectrum of **10d** revealed two singlet signals at δ = 8.78 and 9.76 ppm attributed to chromene and NH protons, along with signals corresponding to aryl protons. Elemental analysis and spectral data are in agreement with the suggested structure **10d**.



8, 10	Ar
a	C_6H_5
b	4-MeC_6H_4
c	4-$OMeC_6H_4$
d	4-FC_6H_4

Scheme 3. Synthetic route of *N*-(2-oxo-6-(aryldiazenyl)-2*H*-chromen-3-yl)-benzamides **10a-d.**

Heating of compounds **4a, b** with different aromatic amines **11a-d** in glacial acetic acid and anhydrous sodium acetate in a water bath at 100 °C produced compounds **12a-h** (Scheme 4). The chemical structure of the products was confirmed on the bases of their analytical and spectroscopic data. The IR spectrum of **12e** taken as a typical example of the prepared series showed absorption bands at 3332, 1725



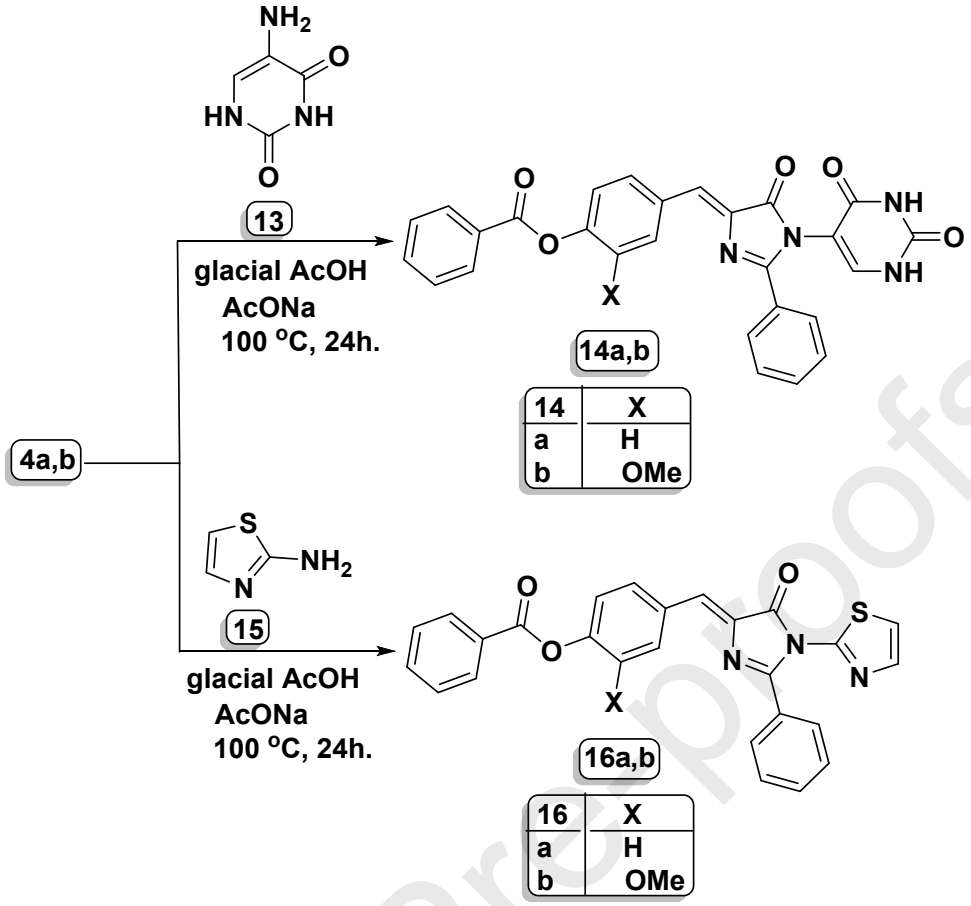
11	R
a	SO_2NH_2
b	COOH
c	COMe
d	OH

12	X	R	12	X	R
a	H	SO_2NH_2	e	OMe	SO_2NH_2
b	H	COOH	f	OMe	COOH
c	H	COMe	g	OMe	COMe
d	H	OH	h	OMe	OH

Scheme 4. Synthesis of 5-oxo-2-phenylimidazol-4-ylidenes **12a-h.**

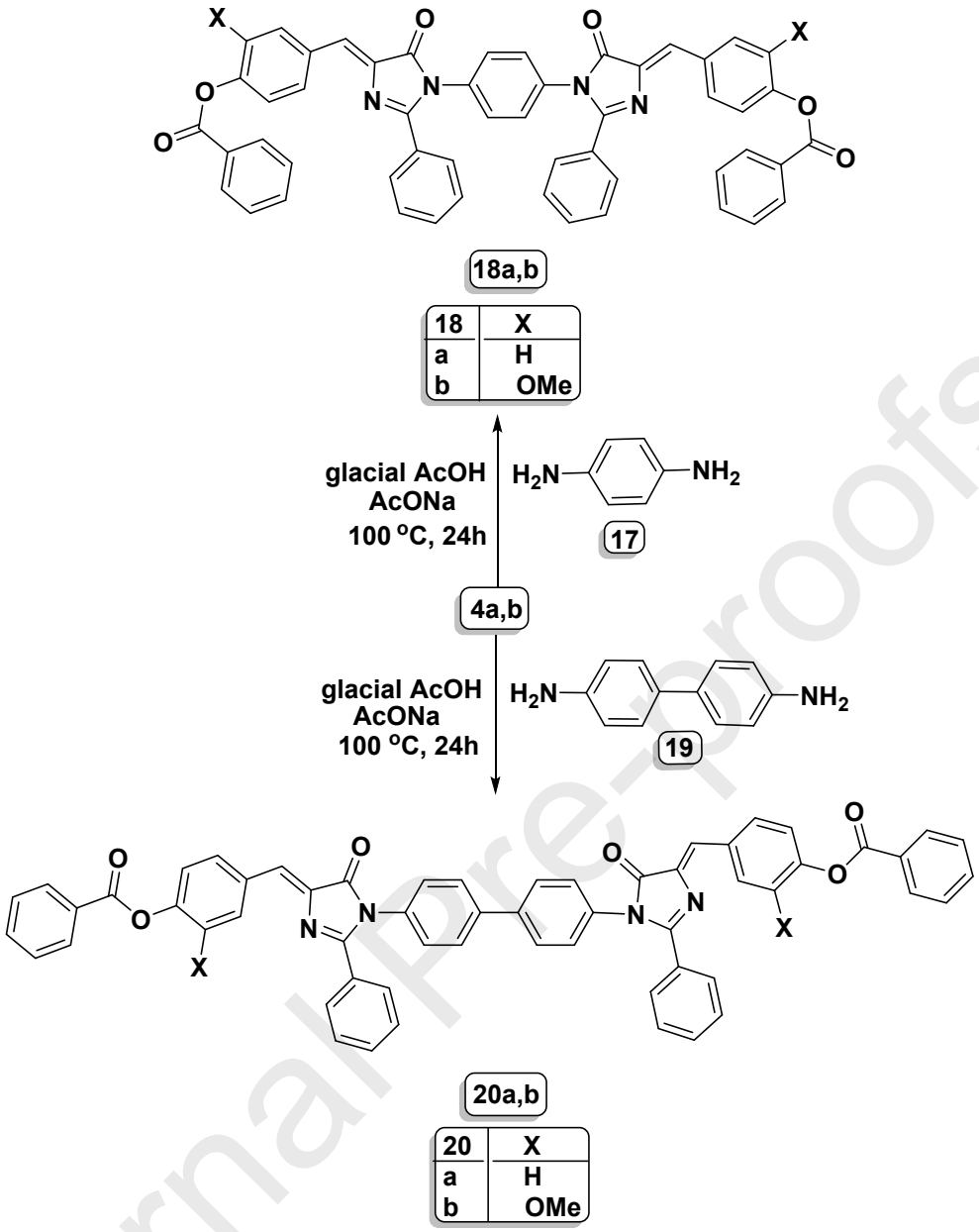
and 1639 cm^{-1} revealed to amino and two carbonyl groups, respectively. The ^1H NMR spectrum displayed a signal at δ = 3.86 ppm assigned to methoxy protons and a D_2O exchangeable signal at δ = 7.37 ppm attributed to NH_2 protons. Moreover, a singlet signal at δ = 8.32 ppm due to vinylic proton. Its ^{13}C NMR spectrum showed characteristic signals at δ = 55.8, 116.1, 163.7 and 169.1 ppm owing to methoxy, vinylic and carbonyl carbons, respectively. Also, the mass spectrum of **12e** presented a correct molecular ion peak at m/z = 553 (M^+) which is consistent with the molecular formula $C_{30}H_{23}N_3O_6S$.

Similarly, compounds **4a,b** were heated with each of 5-aminopyrimidine-2,4(1*H*,3*H*)-dione (**13**) and thiazol-2-amine (**15**) under the same reaction conditions and this produced compounds **14a, b** and **16a, b**, respectively (Scheme 5). The structure of the products was confirmed *via* their elemental analysis and spectral data (see exp.).



Scheme 5. Synthesis of compounds **14** and **16.**

On the other hand, the reaction of compounds **4a, b** with each of benzene-1,4-diamine (**17**) and benzidine (**19**) in glacial acetic acid containing anhydrous sodium acetate in a water bath at 100 °C produced bis-compounds **18a, b**, and **20a, b** respectively, (Scheme 6). The structure of the new products was deduced from their elemental analyses and spectral data. For example, the IR spectrum of **20b** showed characteristic absorption bands at 1737 and 1640 cm^{-1} corresponding to four carbonyl groups. Furthermore, two singlet signals at δ = 3.87 and 8.12 ppm due to the two methoxy and vinylic protons appeared in its ^{1}H NMR spectrum besides the expected signals of aryl protons. The elemental analyses together with the spectral data are provided in the proposed structure **20**.



Scheme 6. Synthesis of bis-imidazol-5-ones **18** and **20.**

2.2. Screening of in *vivo* anti-inflammatory activity: Carrageenan-induced paw edema in rats

The new imidazol-5-one derivatives **12a,b**, **12e**, **12f**, **14a**, **14b**, **16a** and **16b** were evaluated in *vivo* anti-inflammatory activity using acute carrageenan-induced rat paw edema and celecoxib (as a reference drug) according to the reported procedure [51] using a dose of compounds (50 mg/Kg b.w.). The anti-inflammatory activity was measured based on a change in paw thickness (mm) at 0, 1, 2, 3, 4 and 5 hours after injection of carrageen as shown in Table 1.

At the beginning of the experiment at 0 and 0.5h, all the tested compounds were rather weak and ineffective, but after the first hour the effect of the tested compounds increased throughout all the time intervals. Thus, compounds **12e** and **12f** showed more potent anti-inflammation efficiency among the rest of the compounds where compound **12e** proved to be very close in inhibition (41.8%) to the reference drug celecoxib after the five hours. On the other hand, compound **12f** showed remarkable anti-inflammatory activity (49.0%) compared to the standard celecoxib (43.1%). Compounds **12b** and **14a** exhibited weak anti-inflammatory activity (18.9 and 10.3%) whereas the rest of the tested compounds namely **12a**, **14b**, **16a** and **16b** exhibited moderate anti-inflammatory activity (34.0, 27.3, 34.6 and 22.4%) respectively, compared to celecoxib (43.1%) at the end of experiment time (Table 2 and Fig. 1).

Table 1: The anti-inflammatory activity of tested compounds **12a**, **12b**, **12e**, **12f**, **14a**, **14b**, **16a**, **16b** and celecoxib (50 mg/Kg b.w)

Compound no.	Mean value of paw edema thickness (mm) ± SEM						
	Zero time	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Control –Ve	3.89 ± 0.10**	4.79 ± 0.11	6.19 ± 0.09***	7.80 ± 0.10***	9.48 ± 0.10***	8.95 ± 0.10***	8.79 ± 0.07***
Celecoxib	4.35 ± 0.17	4.74 ± 0.17	5.63 ± 0.18	6.51 ± 0.21	5.82 ± 0.15	5.31 ± 0.18	5.00 ± 0.16
12a	4.05 ± 0.12	4.76 ± 0.12	5.63 ± 0.11	6.86 ± 0.12	6.35 ± 0.12**	6.04 ± 0.12***	5.80 ± 0.10***
12b	4.33 ± 0.12	4.95 ± 0.13	5.73 ± 0.13	6.74 ± 0.14	7.96 ± 0.16***	7.43 ± 0.12***	7.13 ± 0.11***
12e	4.18 ± 0.09	4.38 ± 0.09	4.89 ± 0.09***	5.71 ± 0.10***	6.67 ± 0.08***	5.87 ± 0.09***	5.12 ± 0.06
12f	4.09 ± 0.11	4.57 ± 0.11	5.29 ± 0.10	6.28 ± 0.12	5.44 ± 0.10	4.79 ± 0.14**	4.48 ± 0.1**
14a	4.20 ± 0.13	4.98 ± 0.13	6.01 ± 0.14	7.27 ± 0.14***	8.67 ± 0.13***	8.20 ± 0.12***	7.88 ± 0.13***
14b	4.46 ± 0.14	4.86 ± 0.15	5.47 ± 0.15	6.34 ± 0.014	7.46 ± 0.16***	6.69 ± 0.13***	6.39 ± 0.13***
16a	4.45 ± 0.25	4.72 ± 0.24	5.25 ± 0.23	5.94 ± 0.24**	6.83 ± 0.23***	6.23 ± 0.25***	5.75 ± 0.26***
16b	3.95 ± 0.08	4.84 ± 0.09	6.06 ± 0.08**	7.54 ± 0.10***	7.14 ± 0.08***	6.94 ± 0.08***	6.82 ± 0.10***

Data analyzed using ANOVA and expressed as Mean ± SEM of four animals for each group

**Significant difference with celecoxib at $P< 0.05$

*** Highly significant difference with celecoxib at $P< 0.01$

Table 2: Percentage of inhibition of oedema % in rat using all tested compounds at time interval 1,3 and 5h

Compound no.	Percentage of inhibition of oedema %		
	1h	**3h**	**5h**
Celecoxib	9.1	38.6	43.1
12a	9.1	33.0	34.0
12b	7.4	16.0	18.9
12e	21.0	29.6	41.8
12f	14.5	42.6	49.0
14a	2.9	8.5	10.3
14b	11.6	21.3	27.3
16a	15.2	28.0	34.6
16b	2.1	24.7	22.4

The anti-inflammatory activity (inhibition %) is expressed according to the equation % inhibition = $(M_c\text{-}M_t/M_c)$ x 100, where M_t represents the mean increase in paw thickness in rats treated with the tested compound and M_c represents the mean increase in paw thickness in control group.

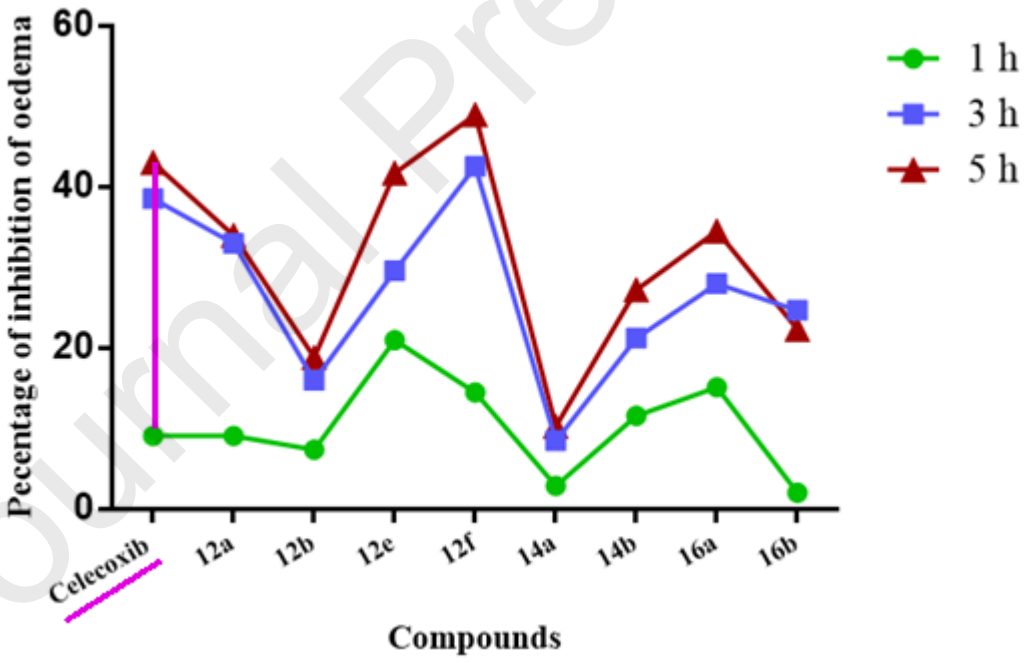


Fig. 1. The percentage of oedema inhibition of the tested compounds and standard drug celecoxib

2.3. Structure activity relationship (SAR)

The results presented in Table 1 show that the anti-inflammatory activity of compound **12e** is very close to the reference drug (celecoxib) due to the similarity in their structures which contain sulfonamide (SO_2NH_2) moiety, but compound **12f** showed a higher activity than celecoxib and this may be due to the presence of carboxylic moiety (COOH), which is a more ionizable group, instead of sulfonamaide (SO_2NH_2) moiety (Fig. 2).

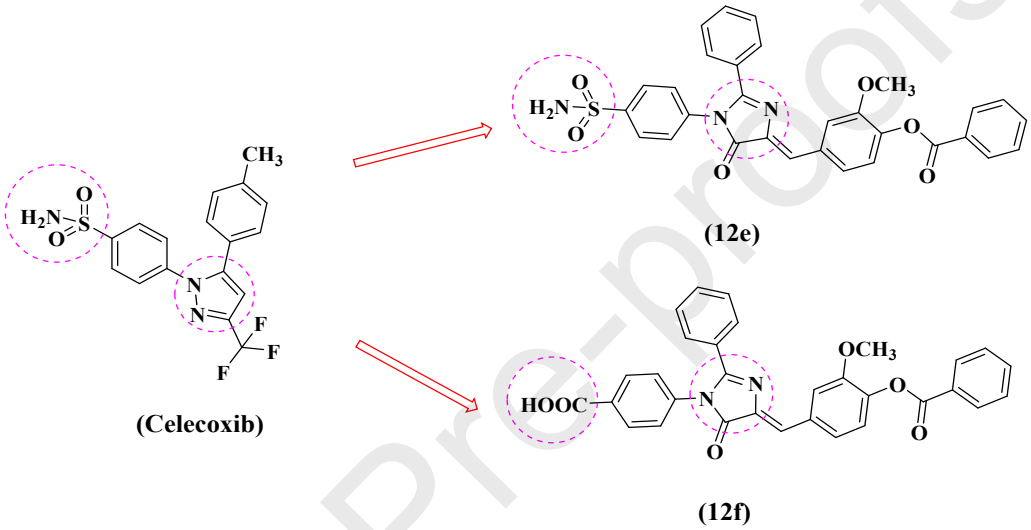


Fig. 2. Structure activity relationship (SAR) of compounds **12e** and **12f** with standard drug celecoxib

2.4. Enzyme inhibition of cyclo-oxygenase-2 (COX-2)

Additionally, compounds **12a**, **12e** and **12f** were tested for inhibiting COX-2 enzyme and inhibiting activity was determined by measuring IC_{50}. It was observed that compounds **12a, 12e** and **12f** inhibit COX-2 enzyme more than the reference drug celecoxib. Compound **12e** has more inhibiting activity compared to the rest of the tested compounds and this may be due to the similarity in structure with celecoxib (Table 3).

Table 3: Inhibition of some tested compounds **12a**, **12e**, **12f** and celecoxib on cyclo-oxygenase enzyme (COX-2)

Compound no.	COX-2 IC_{50} (µM)
12a	0.09
12e	0.087
12f	0.092
Celecoxib	0.11

2.5. Antibacterial evaluation

Some selected newly synthesized compounds **4a, b**, **7**, **12a, b**, **12e, f**, **12h**, **14a**, **14b**, and **16a** were screened in *vitro* for their antibacterial activity against *Staphylococcus aureus* and *Streptococcus mutans* (Gram-positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Gram-negative bacteria) using nutrient agar medium. Ampicillin and gentamicin were used as standard drugs for Gram-positive and Gram-negative, respectively. The antimicrobial activity of the synthesized compounds was determined using agar well diffusion method [52], and the diameters (mm) of inhibition zones are shown in Table 4.

The results depicted in Table 4 show that the toxicity of most of the tested compounds against *Staphylococcus aureus* and *Streptococcus mutans* is higher than *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Compound **12a** is the most toxic compound against *Staphylococcus aureus* and *Streptococcus mutans* with activity index (184 and 94%) respectively, compared to standard drug ampicillin. It's also the only tested compound to exhibit toxicity against *Pseudomonas aeruginosa* which showed moderate activity with activity index (48%) compared to standard drug gentamicin. Compounds **7**, **12f** and **12h** showed good toxicity against *Staphylococcus aureus* with activity index (80, 84 and 80%) respectively, whereas compounds **12b** and **16a** showed moderate toxicity against *Staphylococcus aureus*. Also, compounds **12e**, **12f**, **12h**, **14a**, **14b**, and **16a** showed moderate activity against *Streptococcus mutans*.

Table 4: Antibacterial activity of some tested compounds **4a**, **4b**, **7**, **12a**, **12b**, **12e**, **12f**, **12h**, **14a, 14b** and **16a** against Gram-positive and negative bacteria.

Compound no.	Diameter inhibition zone in mm (% activity index)				
	Gram-negative bacteria			Gram-positive bacteria	
	Escherichia coli (ATCC:9637)	***Klebsiella pneumonia (ATCC:10031)***	***Pseudomonas aeruginosa (ATCC:27853)***	***Staphylococcus aureus* (ATCC:6538)**	***Streptococcus mutans (ATCC:25175)***
4a	NA	NA	NA	NA	NA
4b	NA	NA	NA	NA	NA
7	NA	NA	NA	17.6 ± 0.6 (80%)	NA
12a	NA	NA	14.6 ± 0.5 (48%)	40.6 ± 0.6 (184%)	28.3 ± 0.5 (94%)
12b	NA	NA	NA	11.3 ± 0.5 (51%)	NA
12e	NA	NA	NA	NA	15.3 ± 0.5 (51%)
12f	NA	NA	NA	18.6 ± 0.6 (84%)	16.6 ± 0.6 (55%)
12h	NA	NA	NA	17.6 ± 0.6 (80%)	14.3 ± 0.5 (47%)
14a	NA	NA	NA	NA	21.6 ± 0.6 (72%)
14b	NA	NA	NA	NA	17.6 ± 0.6 (58%)
16a	NA	NA	NA	10.6 ± 0.5 (48%)	13.6 ± 0.5 (45%)
Gentamicin	27±0.5 (100%)	25±0.5 (100%)	30±0.5 (100%)	-	-
Ampicilin	-	-	-	22±0.1 (100%)	30±0.5 (100%)

- Zone of inhibition is expressed in the form of mean± standard deviation (mm).
- NA: No activity
- Well diameter (6mm).
- 100 µ l was tested.

2.6. Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of compounds **7**, **12a**, **12e**, **12f**, **12h**, **14a** and **14b** with high antibacterial activity showed that compound **12a** has both a higher toxicity (40.6±0.6, 184%) and a minimum inhibitory concentration (MIC = 62.5 µg/ml) among all the tested compounds against *Staphylococcus aureus* compared to the standard drug ampicillin.

Table 5: Minimum inhibitory concentration of some tested compounds **7**, **12a**, **12e**, **12f**, **12h**, **14a** and **14b**

Compound no.	Minimum inhibitory concentration (MIC) in µg/ml	
	Gram positive bacteria	
	Staphylococcus aureus (ATCC:6538)	*Streptococcus mutans (ATCC:25175)*
7	Stock	-
12a	62.5	250
12e	-	stock
12f	250	250
12h	250	-
14a	-	250
14b	-	250
Ampicilin	62.5	62.5

Stock concentration 1 mg/ml

Concentrations unit of MIC are represented as µg/ml

2.7. Enzyme inhibition of dihydrofolate reductase (DHFR)

Compounds **7**, **12a**, **12f** and **12h** which have good MIC values were introduced to enzyme assay against dihydrofolate reductase (DHFR) enzyme to determine inhibitory activity expressed as IC_{50} using sulfadiazine as a reference drug. The results showed that compound **12f** has an inhibitory effect on DHFR higher than the other tested compounds **7, 12a** and **12h**. Combining some synthesized compounds with sulfadiazine (10 µM compound + 1 µM reference) indicated that all tested compounds have higher inhibitory effect toward DHFR. Compound **12f** showed the highest inhibiting activity compared to the standard drug and the other tested compounds. This may be due to presence of carboxylic group. Also, compounds **12a** and **12h** have an inhibiting effect higher than the standard drug sulfadiazine.

Table 6: Inhibition of some tested compounds **7**, **12a**, **12f**, **12h** and sulfadiazine on dihydrofolate reductase (DHFR)

Compound no.	DHFR IC_{50} (µM)	Combination (10 µM compound+ 1 µM reference)
7	0.593 ± 0.013	0.306 ± 0.003
12a	0.792 ± 0.019	0.133 ± 0.003
12f	0.285 ± 0.006	0.078 ± 0.002
12h	0.347 ± 0.009	0.124 ± 0.003
Sulfadiazine	0.135 ± 0.004	-

2.8. In silico studies

2.8.1. Molecular docking study

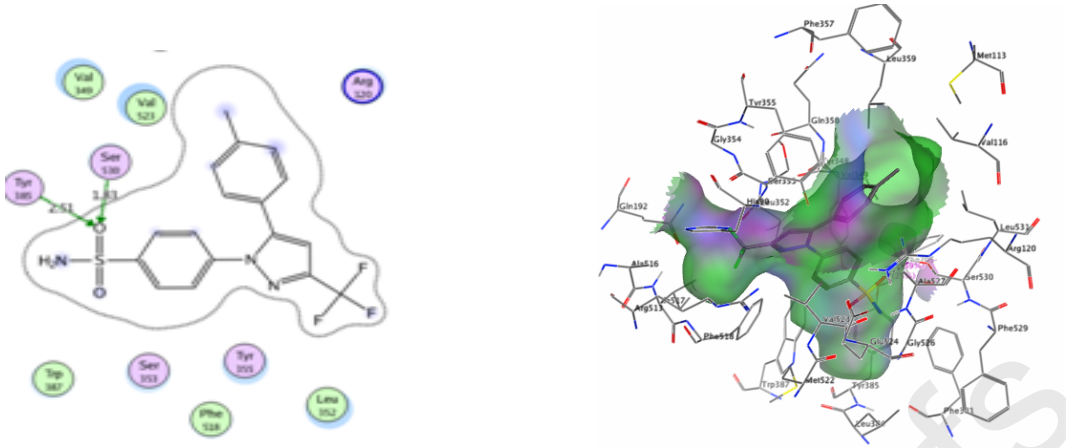
Molecular docking study was performed to identify the most preferred binding mode of compound into the enzyme binding active site. Celecoxib is known as an anti-inflammatory standard drug by inhibiting COX-2 enzyme, so the synthesized new target compounds **12e** and **12f** were docked with COX-2 enzyme (pdb code: 4cox) compared to celecoxib utilizing molecular operating environment (MOE. 2009.10) which showed the possible interactions of the compounds with active site of enzyme (Table 7).

From the results presented in table 7, it was found that the standard drug celecoxib showed two hydrogen bond interactions one of them between oxygen of SO_2 group and Ser530 with bond length 1.83 Å and the other between SO_2 and Tyr385 with bond length 2.51 Å with binding energy (S = -13.6661) Kcal/mol (Fig. 3). Moreover, compound **12e** also showed two bond interactions, the first is hydrogen bond between NH_2 which is attached to SO_2 moiety in **12e** and OH of Tyr385 (3.67 Å), the other bond is arene-cation between phenyl ring of **12e** and Arg120 with bonding energy equal to -16.1076 Kcal/mol (Fig. 4). In spite of this, compound **12f** has the highest potency against COX-2 than each of compound **12e** and the standard drug celecoxib as clarified by five interactions one of them for arene-arene interaction between phenyl ring of **12f** and Arg120 and the remaining four are hydrogen bonds. The first hydrogen bond is between oxygen of methoxy group in **12f** and

Table 7: The protein- ligand interactions of compounds **12e**, **12f** and celecoxib (standard drug) with 4cox active site pocket.

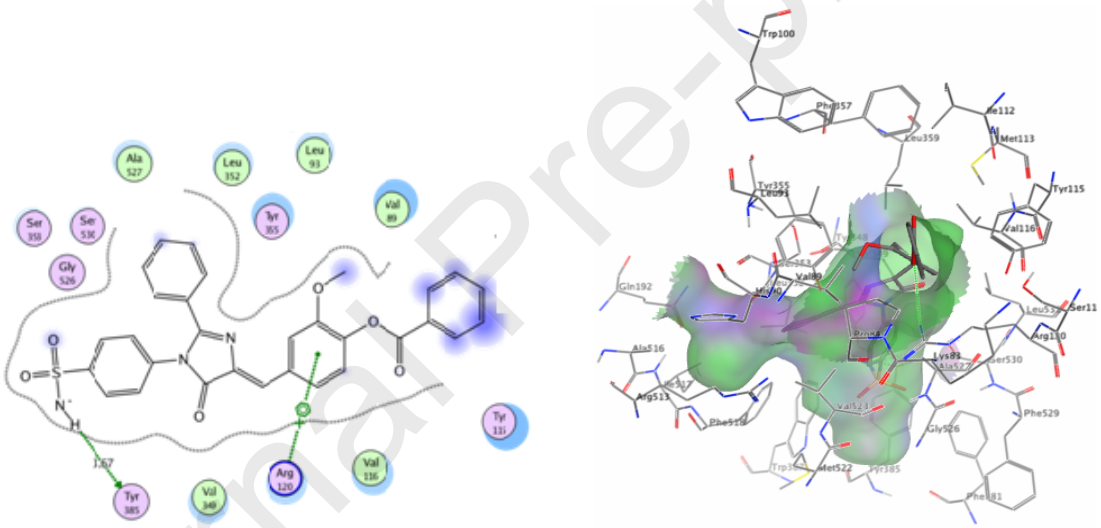
Compound no.	Binding energy (S) Kcal/mol	Groups of the ligand involved in the interaction	Amino acids involved in the interaction	Type of the bond and length of hydrogen bonds Å
Celecoxib	-13.6661	SO_2	Ser530	**Polar 1.83 Å**
		SO_2	Tyr385	**Polar 2.51 Å**
12e	-16.1076	NH	Tyr385	**Polar 3.67 Å**
		Phenyl ring	Arg120	**Basic (Arene-cation interaction)**
12f	-15.2095	OCH_3	Arg120	**Basic 2.50 Å**
		OCH_3	Tyr 355	**Polar 2.13 Å**
		CO	Arg513	**Basic 2.19 Å**
		CO	His90	**Basic 2.69 Å**
		Phenyl ring	Arg120	**Basic (arene-arene interaction)**

Arg120 (2.50 Å), and the second is also between the oxygen atom of methoxy group and Tyr355 (2.13 Å). The remaining two hydrogen bonds are between carbonyl function of benzoate moiety in **12f** with each of Arg513 (2.19 Å) and His90 (2.69 Å) with binding energy S = -15.2095 Kcal/mol (Fig. 5). The results obtained in these docking studies suggested that the synthesized compounds **12e** and **12f** were fit on the active site of the enzyme to form the binding interactions as indicated by their docking pattern compared to that of celecoxib. Overall, these interactions and binding patterns into 4cox active site explain the remarkable COX-2 inhibitory activity of these compounds.



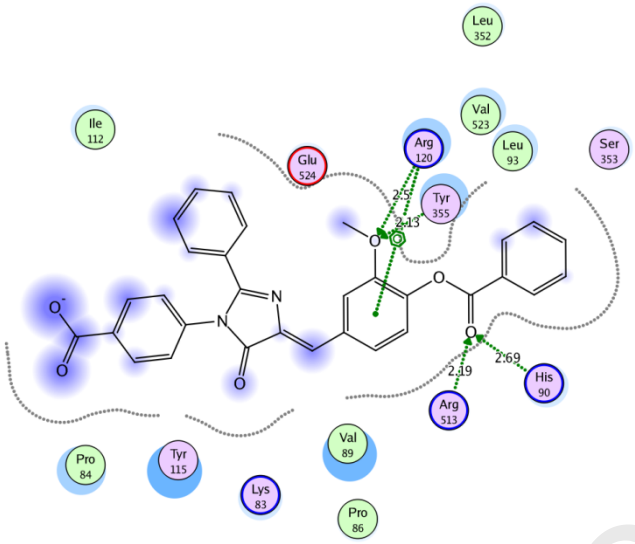
2D structure 3D structure

Fig. 3. The binding pattern of celecoxib (2D and 3D) structure with the active site of cyclo-oxygenase enzyme (PDB code = 4cox).

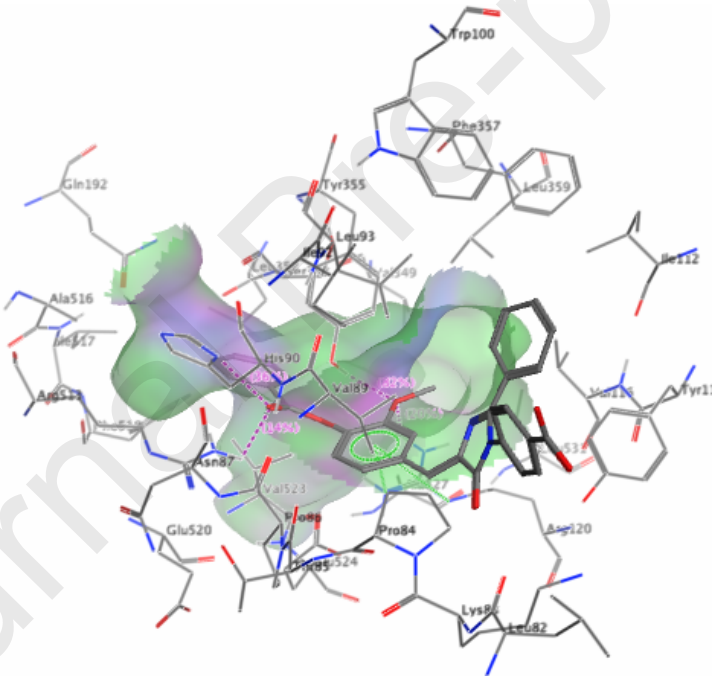


2D structure 3D structure

Fig. 4. The binding pattern of compound **12e** (2D and 3D) structure with the active site of cyclo-oxygenase enzyme (PDBcode = 4cox).



2D structure



3D structure

Fig. 5. The binding pattern of compound **12f** (2D and 3D) structure with the active site of cyclo-oxygenase enzyme (PDBcode = 4cox).

2.8.2. Lipiniski's rule of five (the effect of lipophilic and steric parameters)

Compounds **12a**, **12e**, **12f** and **12h** have topological polar surface area (TPSA) of 127.51, 136.74, 105.50 and 88.43 Å^2, respectively. These values denoted that the studied compounds can permeate cell membranes [53] (Table 8). Compound **12h** is

more capable of permeating cell membrane than the other studied compounds and this is due to its containing the hydroxyl moiety (more polar group) which is highly absorbable through gastrointestinal (GI) tract.

Table 8: Lipiniski's parameters and TPSA of the more potent cytotoxic compounds

Compound no.	Lipinisk's Parameters						
	TPSA (Å²)	**Log P**	**MW**	**nHBD**	**nHBA**	**GI absorption**	**No. of viol**
12a	127.51	4.09	523.56	1	7	Low	1 violation: MW > 500
12e	136.74	4.03	553.59	1	8	Low	1 violation: MW > 500
12f	105.50	4.46	494.49	1	7	High	0
12h	88.43	4.76	490.51	1	6	High	0

nHBD = number of hydrogen bond donor
nHBA = number of hydrogen bond acceptor
GI absorption = Gastrointestinal absorption

3. Conclusion:

The present study investigates the synthesis of novel 5-imidazol-5-one analogs with comparable antibacterial and anti-inflammatory potencies which were analyzed by the suitable methods such as IR, ^{1}H NMR, ^{13}C NMR and MS. All spectral data were following postulated structures. All the tested compounds exhibited good anti-inflammatory activity, especially compound **12f** which produced the maximum effect of 49.0 % compared to the standard drug, celecoxib (43.1%). Also, compounds **12a**, **12e** and **12f** were studied for their interactions with enzyme COX-2. Additionally, the most active antibacterial agents **7, 12a, 12f** and **12h** exhibited good binding affinity with enzyme dihydrofolate reductase (DHFR). The inhibitory effect of compound **12f** on DHFR is higher than the other tested compounds **7**, **12a** and **12h**. On the other hand, combining these tested compounds with sulfadiazine as a reference drug indicated that compounds **12a, 12f** and **12h** have more potency than sulfadiazine.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points were determined on an Electrothermal (9100) apparatus and are uncorrected. The IR spectra were recorded as KBr pellets on a Perkin Elmer 1430 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in deuterated dimethylsulfoxide at 300 and 75 MHz, respectively, on a Varian Gemini NMR spectrometer using tetramethylsilane as an internal reference and the results are expected as δ value. Mass spectra were taken on a Shimadzu GCMS-QP 1000 Ex mass spectrometer at 70 eV. Elemental analyses were carried out at the Microanalyses Center of Cairo University, Giza, Egypt. Anti-inflammatory carried out in Mansoura pharmacy, Egypt. Antibacterial activity was performed at the central laboratory of biochemistry at Cairo University, Giza, Egypt. Compounds **2a,b** and **6** were prepared according to the procedure in the literature [54, 55].

4.1.2. Synthesis of compounds 4a,b.

A mixture of benzoylglycine (**3**) (0.01 mol), aromatic aldehydes **2a,b** and fused sodium acetate (0.015 mol) in acetic anhydride (5 ml) was heated in a water bath (100 ^{o}C), for 2 hours. The reaction mixture was allowed to cool at room temperature and poured on cold water. The solid was collected by filtration and recrystallized from an ethanol-dioxane mixture.

4.1.2.1. 4-((5-Oxo-2-phenyloxazol-4(5*H*)-ylidene)methyl)phenyl benzoate (4a).

Yellow crystals; yield 71%; m.p = 218 °C; IR (KBr) 1738 (CO), 1647 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ = 7.47 (d, 2H, J = 8.7 Hz, Ar), 7.60-8.16 (m, 10H, Ar), 8.18 (s, 1H, CH), 8.40 (d,2H, J = 8.7 Hz, Ar); m/z = 369 (M^{+}, 2.5%), 106 (7.7%), 105 (100%), 77 (34.6%); Anal. Calcd for $C_{23}H_{15}NO_4$: C, 74.79; H, 4.09; N, 3.79. Found: C, 74.62; H, 4.25; N, 3.56%.

4.1.2.2. 2-Methoxy-4-((5-oxo-2-phenyloxazol-4(5*H*)-ylidene)methyl)phenyl benzoate (4b).

Yellow crystals; yield 67%; m.p = 230 °C; IR (KBr) 1784 (CO), 1653 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ = 3.85 (s, 3H, OCH_3), 7.25-7.80 (m, 10H, Ar), 7.82 (d, 1H,

J = 7.5 Hz, Ar), 8.03 (s, 1H, CH), 8.09 (d,1H, *J* = 7.2 Hz, Ar), 8.18 (s, 1H, CH, Ar); m/z = 399 (M^+, 4.6%), 398 (17.4%), 106 (10.7%), 105 (100%), 77 (34.9%); Anal. Calcd for $C_{24}H_{17}NO_5$: C, 72.17; H, 4.29; N, 3.51. Found: C, 72.34; H, 4.13; N, 3.72%.

4.1.3. Synthesis of 2-((5-oxo-2-phenyloxazol-4(5*H*)-ylidene)methyl)phenyl benzenesulfonate (7).

A mixture of compound **3** (0.01 mol), 2-formylphenyl benzenesulfonate **6** (0.01 mol) and fused sodium acetate (0.015 mol) was heated at 100 °C in acetic anhydride (5 ml) for 2 hours. The mixture was allowed to cool at room temperature and poured on cold water. The solid so formed was filtrated off and recrystallized from a mixture of ethanol-dioxane.

2-((5-Oxo-2-phenyloxazol-4(5*H*)-ylidene)methyl)phenyl benzenesulfonate (7).

Yellow crystals; yield 54%; m.p = 150 °C; IR (KBr) 1653 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ = 6.96-8.13 (m, 14H, Ar), 8.76 (s, 1H, CH); Anal. Calcd for $C_{22}H_{15}NO_5S$: C, 65.18; H, 3.73; N, 3.45; S, 7.91. Found: C, 65.35; H, 3.57; N, 3.68; S, 7.73%.

4.1.4. Synthesis of compounds 10a-d.

A mixture of compound **3** (0.01 mol), arylazosalicyaldehdes **8a-d** (0.01 mol) and fused sodium acetate (0.015 mol) was heated in acetic anhydride at 100 °C for 3 hours. The reaction mixture was allowed to cool at room temperature and poured onto cold water. The solid which collected by filtration was recrystallized from an ethanol-dioxane mixture to give compounds **10a-d.**

4.1.4. 1. *N*-(2-Oxo-6-(phenyldiazenyl)-2*H*-chromen-3-yl)benzamide (10a).

Yellow crystals; yield 66%; m.p > 300 °C; IR (KBr) 3327 (NH), 1717 (CO), 1664 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ = 7.42-8.07 (m, 12H, Ar), 8.32 (s, 1H, CH, Ar), 8.90 (s, 1H, CH, Ar), 9.81 (s, 1H, NH); Anal. Calcd for $C_{22}H_{15}N_3O_3$: C, 71.54; H, 4.09; N, 11.38. Found: C, 71.71; H, 4.25; N, 11.16%.

4.1.4. 2. *N*-(2-Oxo-6-(*p*-tolyldiazenyl)-2*H*-chromen-3-yl)benzamide(10b).

Yellow crystals; yield 56%; m.p > 300 °C; IR (KBr) 3320 (NH), 1725 (CO), 1658 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ = 2.32 (s, 3H, CH_3), 7.21-8.06 (m, 11H, Ar),

8.41 (s, 1H, CH, Ar), 8.72 (s, 1H, CH, Ar), 9.64 (s, 1H, NH); Anal. Calcd for $C_{23}H_{17}N_3O_3$: C, 72.05; H, 4.47; N, 10.96. Found: C, 72.21; H, 4.30; N, 10.73%.

4.1.4. 3. *N*-(6-((4-Methoxyphenyl)diazenyl)-2-oxo-2*H*-chromen-3-yl) benzamide (10c).

Yellow crystals; yield 64%; m.p > 300 °C; IR (KBr) 3335 (NH), 1716 (CO), 1668 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 3.81 (s, 3H, OCH_3), 7.32-8.11 (m, 11H, Ar), 8.21 (s, 1H, CH, Ar), 8.68 (s, 1H, CH, Ar), 9.62 (s, 1H, NH); Anal. Calcd for $C_{23}H_{17}N_3O_4$: C, 69.17; H, 4.29; N, 10.52. Found: C, 69.01; H, 4.46; N, 10.30%.

4.1.4. 4. *N*-(6-((4-Fluorophenyl)diazenyl)-2-oxo-2*H*-chromen-3-yl)benzamide (10d).

Yellow crystals; yield 62%; m.p > 300 °C; IR (KBr) 3330 (NH), 1721 (CO), 1670 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 7.44-8.05 (m, 11H, Ar), 8.34 (s, 1H, CH, Ar), 8.78 (s, 1H, CH, Ar), 9.76 (s, 1H, NH); Anal. Calcd for $C_{22}H_{14}FN_3O_3$: C, 68.21; H, 3.64; N, 10.85. Found: C, 68.36; H, 3.46; N, 10.64%.

4.1.5. Synthesis of compounds 12a-h

A solution of the appropriate compound **4a,b** (0.01 mol) was refluxed in glacial acetic acid (10 ml) with aromatic amine derivatives **11a-d** (0.01 mol) containing a catalytic amount of fused sodium acetate for 3 hours. The solid product that precipitated by cooling was filtered off and recrystallized from ethanol-dioxane mixture.

4.1.5.1. 4-((5-Oxo-2-phenyl-1-(4-sulfamoylphenyl)-1,5-dihydro-4*H*-imidazol-4-ylidene)-methyl)phenyl benzoate (12a).

Yellow crystals; yield 66%; m.p > 300 °C; IR (KBr) 3316 (NH_2), 1701 (CO), 1637 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 7.01 (s, 2H, NH_2), 7.42 (d, 2H, J = 8.4 Hz, Ar), 7.52-7.81 (m, 10H, Ar), 7.82 (d, 2H, J = 8.1 Hz, Ar), 8.01 (d,2H, J = 8.7 Hz, Ar), 8.22 (d, 2H, J = 8.1 Hz, Ar), 8.31 (s, 1H, CH); Anal. Calcd for $C_{29}H_{21}N_3O_5S$: C, 66.53; H, 4.04; N, 8.03; S, 6.12.Found: C, 66.36; H, 4.20; N, 8.26; S, 6.29%.

4.1.5.2. 4-(4-(4-(Benzoyloxy)benzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)-benzoic acid (12b).

White crystals; yield 68%; m.p = 292 °C; IR (KBr) 3431 (OH), 1732 (CO), 1684 (CO), 1637 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 7.31 (d, 2H, *J* = 7.8 Hz, Ar), 7.44-7.61 (m, 10H, Ar), 7.74 (d, 2H, *J* = 8.1 Hz, Ar), 8.11 (d, 2H, *J* = 8.4 Hz, Ar), 8.20 (d, 2H, *J* = 7.8 Hz, Ar), 8.26 (s, 1H, CH), 10.31 (s, 1H, OH); m/z = 488 (M^+, 1.0%), 487 (2.7%), 368 (9.1%), 264 (2.3%), 223 (2.6%), 120 (8.5%), 106 (7.8%), 105 (100%), 77 (30.0%); Anal. Calcd for $C_{30}H_{20}N_2O_5$: C, 73.76; H, 4.13; N, 5.73. Found: C, 73.59; H, 4.30; N, 5.51%.

4.1.5.3. 4-((1-(4-Acetylphenyl)-5-oxo-2-phenyl-1,5-dihydro-4*H*-imidazol-4-ylidene)-methyl)phenyl benzoate (12c).

Yellow crystals; yield 62%; m.p = 264 °C; IR (KBr) 1737 (CO), 1715 (CO), 1680 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 2.60 (s, 3H, CH_3), 7.37-7.65 (m, 10H, Ar), 7.75 (d, 2H, *J* = 6.6 Hz, Ar), 8.01 (d, 2H, *J* = 8.4 Hz, Ar), 8.02 (s, 1H, CH), 8.15 (d,2H, *J* = 7.8 Hz, Ar), 8.46 (d, 2H, *J* = 8.7 Hz, Ar); Anal. Calcd for $C_{31}H_{22}N_2O_4$: C, 76.53; H, 4.56; N, 5.76. Found: C, 76.69; H, 4.39; N, 5.53%.

4.1.5.4. 4-((1-(4-Hydroxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4*H*-imidazol-4-ylidene)-methyl)phenyl benzoate (12d).

Yellow crystals; yield 67%; m.p = 269 °C; IR (KBr) 3435 (OH), 1747 (CO), 1677 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 7.10 (d, 2H, *J* = 8.7 Hz, Ar), 7.21 (d, 2H, *J* = 8.7 Hz, Ar), 7.34-7.71 (m, 10H, Ar), 7.82 (d, 2H, *J* = 8.4 Hz, Ar), 8.12 (s, 1H, CH), 8.22 (d, 2H, *J* = 8.1 Hz, Ar), 9.87 (s, 1H, OH); Anal. Calcd for $C_{29}H_{20}N_2O_4$: C, 75.64; H, 4.38; N, 6.08. Found: C, 75.46; H, 4.55; N, 6.31 %.

4.1.5.5. 2-Methoxy-4-((5-oxo-2-phenyl-1-(4-sulfamoylphenyl)-1,5-dihydro-4*H*-imidazol-4-ylidene)methyl)phenyl benzoate (12e).

Yellow crystals; yield 70%; m.p = 284 °C; IR (KBr) 3332 (NH_2), 1725 (CO), 1639 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 3.86 (s, 3H, OCH_3), 7.37 (s, 2H, NH_2), 7.39-7.57 (m, 10H, Ar), 7.59 (d, 2H, *J* = 7.8 Hz, Ar), 7.65 (s, 1H, Ar), 7.89 (d, 1H, *J* = 7.8 Hz, Ar), 8.05 (d, 2H, *J* = 8.1 Hz, Ar), 8.13 (d, 1H, *J* = 7.8 Hz, Ar), 8.32 (s, 1H, CH); ^{13}C NMR (DMSO-d_6) δ = 55.8, 66.2, 116.1, 120.2, 123.3, 125.4, 126.5, 127.2, 128.0, 128.3, 128.8, 128.9, 129.7, 131.5, 133.0, 134.0, 137.1, 138.1, 141.1, 143.6, 150.8, 160.2, 163.7, 169.1; m/z = 553 (M^+, 3.9%), 449 (2.1%), 258 (18.2%), 179 (5.5%), 106 (7.5%), 105 (100%), 77 (16.5%); Anal. Calcd for $C_{30}H_{23}N_3O_6S$: C, 65.09; H, 4.19; N, 7.59; S, 5.79. Found: C, 65.26; H, 4.01; N, 7.36; S, 5.63%.

4.1.5.6. 4-(4-(4-(Benzoyloxy)-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)benzoic acid (12f).

Yellow crystals; yield 58%; m.p > 300 °C; IR (KBr) 3433 (OH), 1730 (CO), 1691 (CO), 1638 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 3.86 (s, 3H, OCH_3), 7.29 (d, 2H, *J* = 8.1 Hz, Ar), 7.35-7.43 (m, 10H, Ar), 7.53 (s, 1H, Ar), 7.62 (d, 2H, *J* = 7.8 Hz, Ar), 7.96 (d, 1H, *J* = 8.1 Hz, Ar), 8.13 (d, 1H, *J* = 8.1 Hz, Ar), 8.33 (s, 1H, CH), 10.22 (s, 1H, OH); Anal. Calcd for $C_{31}H_{22}N_2O_6$: C, 71.81; H, 4.28; N, 5.40. Found: C, 71.63; H, 4.45; N, 5.18%.

4.1.5.7. 4-((1-(4-Acetylphenyl)-5-oxo-2-phenyl-1,5-dihydro-4*H*-imidazol-4-ylidene)-methyl)-2-methoxyphenyl benzoate (12g).

Yellow crystals; yield 66%; m.p = 273 °C; IR (KBr) 1740 (CO), 1720 (CO), 1687 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 2.61 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 7.37 (d, 2H, *J* = 8.1 Hz, Ar), 7.42-7.74 (m, 10H, Ar), 7.77 (d, 2H, *J* = 7.5 Hz, Ar), 7.89 (s, 1H, Ar), 8.02 (d, 1H, *J* = 8.1 Hz, Ar), 8.13 (d,1H, *J* = 7.5 Hz, Ar), 8.32 (s, 1H, CH); Anal. Calcd for $C_{32}H_{24}N_2O_5$: C, 74.41; H, 4.68; N, 5.42. Found: C, 74.59; H, 4.51; N, 5.63%.

4.1.5.8. 4-((1-(4-Hydroxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4*H*-imidazol-4-ylidene)-methyl)-2-methoxyphenyl benzoate (12h).

Yellow crystals; yield 69%; m.p = 230 °C; IR (KBr) 3434 (OH), 1744 (CO), 1684 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 3.85 (s, 3H, OCH_3), 6.82 (d, 2H, *J* = 8.7 Hz, Ar), 7.09 (d, 2H, *J* = 8.7 Hz, Ar), 7.29 (s, 1H, Ar), 7.38-7.60 (m, 10H, Ar), 7.62 (d, 1H, *J* = 7.8 Hz, Ar), 8.13 (d, 1H, *J* = 8.1 Hz, Ar), 8.32 (s, 1H, CH), 9.81 (s, 1H, OH); Anal. Calcd for $C_{30}H_{22}N_2O_5$: C, 73.46; H, 4.52; N, 5.71. Found: C, 73.29; H, 4.70; N, 5.49%.

4.1.6. Synthesis of compounds 14a,b and 16a,b.

A solution of the appropriate compound **4a,b** (0.01 mol) was refluxed in glacial acetic acid (10 ml) with each of aromatic amine **13** and **15** (0.01 mol) containing a catalytic amount of fused sodium acetate for 4 hours. The solid so formed by cooling at room temperature was filtered off and recrystallized from ethanol-dioxane mixture.

4.1.6.1. 4-((1-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-5-oxo-2-phenyl-1,5-dihydro-4*H*-imidazol-4-ylidene)methyl)phenyl benzoate (14a).

Yellow crystals; yield 62%; m.p > 300 °C; IR (KBr) 3233 (NH), 3120 (NH), 1735 (CO), 1685 (CO), 1650 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 7.44 (d, 2H, *J* = 8.7 Hz, Ar), 7.53-7.84 (m, 10H, Ar), 8.01 (s, 1H, CH), 8.14 (s, 1H, CH), 8.43 (d, 2H, *J* = 8.7 Hz, Ar), 11.41 (s, 1H, NH), 11.52 (s, 1H, NH); m/z = 478 (M^+, 14.7%), 374 (3.0%), 214 (5.7%), 171 (4.9%), 116 (7.4%), 106 (7.6%), 105 (100%), 89 (4.3%), 77 (31.3%); Anal. Calcd for $C_{27}H_{18}N_4O_5$: C, 67.78; H, 3.79; N, 11.71.Found: C, 67.60; H, 3.62; N, 11.93%.

4.1.6.2. 4-((1-(2,4-Dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-5-oxo-2-phenyl-1,5-dihydro-4*H*-imidazol-4-ylidene)methyl)-2-methoxyphenyl benzoate (14b).

Yellow crystals; yield 66%; m.p > 300 °C; IR (KBr) 3280 (NH), 3115 (NH), 1737 (CO), 1717 (CO), 1663 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 3.86 (s, 3H, OCH_3), 7.31-7.76 (m, 10H, Ar), 7.82 (d, 1H, *J* = 7.5 Hz, Ar), 7.90 (s, 1H, Ar), 8.01 (s, 1H, CH), 8.11 (s, 1H, CH), 8.21 (d,1H, *J* = 7.5 Hz, Ar), 11.21 (s, 2H, 2NH); Anal. Calcd for $C_{28}H_{20}N_4O_6$: C, 66.14; H, 3.96; N, 11.02. Found: C, 66.31; H, 3.80; N, 11.25%.

4.1.6.3. 4-((5-Oxo-2-phenyl-1-(thiazol-2-yl)-1,5-dihydro-4*H*-imidazol-4-ylidene)-methyl)-phenyl benzoate (16a).

Yellow crystals; yield 68%; m.p = 216 °C; IR (KBr) 1737 (CO), 1660 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 7.48 (d, 2H, *J* = 8.7 Hz, Ar), 7.61-7.78 (m, 10H, Ar), 8.14 (d, 1H, *J* = 8.1 Hz, Ar), 8.18 (s, 1H, CH), 8.26 (d, 1H, *J* = 7.8 Hz, Ar), 8.41 (d, 2H, *J* = 9 Hz, Ar); Anal. Calcd for $C_{26}H_{17}N_3O_3S$: C, 69.17; H, 3.80; N, 9.31; S, 7.10.Found: C, 69.35; H, 3.63; N, 9.54; S, 7.27%.

4.1.6.4. 2-Methoxy-4-((5-oxo-2-phenyl-1-(thiazol-2-yl)-1,5-dihydro-4*H*-imidazol-4-ylidene)methyl)-phenyl benzoate (16b).

Yellow crystals; yield 64%; m.p = 210 °C; IR (KBr) 1735 (CO), 1649 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 3.87 (s, 3H, OCH_3), 7.36 (s, 1H, Ar), 7.39 (d, 1H, *J* = 8.4 Hz, Ar), 7.59-7.69 (m, 10H, Ar), 7.71 (d, 1H, *J* = 8.4 Hz, Ar), 7.91 (d, 1H, *J* = 8.4 Hz, Ar), 8.09 (d, 1H, *J* = 8.4 Hz, Ar), 8.21 (s, 1H, CH); Anal. Calcd for

$C_{27}H_{19}N_3O_4S$: C, 67.35; H, 3.98; N, 8.73; S, 6.66. Found: C, 67.19; H, 3.82; N, 8.52; S, 6.49%.

4.1.7. Synthesis of compounds 18a,b and 20a,b.

A solution of the appropriate compound **4a,b** (0.02 mol) was refluxed in glacial acetic acid (10 ml) with each of benzene-1,4-diamine (**17**) (0.01 mol) and benzidine (**19**) (0.01 mol) containing fused sodium acetate (0.015 mol). The solid product that precipitated was filtered off and recrystallized from DMF.

4.1.7.1. 1,4-Phenylenebis(5-oxo-2-phenyl-1,5-dihydro-4*H*-imidazole-1-yl-4-ylidene))-bis(methanylylidene))bis(4,1-phenylene) dibenzoate (18a).

Yellow crystals; yield 56%; m.p > 300 °C; IR (KBr) 1731 (2CO), 1644 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 7.24-8.06 (m, 32H, Ar), 8.14 (s, 2H, 2CH); Anal. Calcd for $C_{52}H_{34}N_4O_6$: C, 77.03; H, 4.23; N, 6.91. Found: C, 77.19; H, 4.05; N, 6.69%.

4.1.7.2. 1,4-Phenylenebis(5-oxo-2-phenyl-1,5-dihydro-4*H*-imidazole-1-yl-4-ylidene))-bis(methanylylidene))bis(2-methoxy-4,1-phenylene) dibenzoate (18b).

Yellow crystals; yield 61%; m.p > 300 °C; IR (KBr) 1725 (2CO), 1640 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 3.86 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 7.37-8.16 (m, 30H, Ar), 8.21 (s, 2H, 2CH); Anal. Calcd for $C_{54}H_{38}N_4O_8$: C, 74.47; H, 4.40; N, 6.43. Found: C, 74.29; H, 4.23; N, 6.66%.

4.1.8.1. 4-(-(1-(4'-(4-(-4-(Benzoyloxy)benzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)-[1,1'-biphenyl]-4-yl)-5-oxo-2-phenyl-1,5-dihydro-4*H*-imid-azol-4-ylidene)methyl)phenyl benzoate (20a).

Yellow crystals; yield 60%; m.p > 300 °C, IR (KBr) 1731 (2CO), 1645 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 7.31-8.11 (m, 36H, Ar), 8.22 (s, 2H, 2CH); Anal. Calcd for $C_{58}H_{38}N_4O_6$: C, 78.54; H, 4.32; N, 6.32. Found: C, 78.38; H, 4.14; N, 6.55%.

4.1.8.2. 4-(-(1-(4'-(4-(4-(Benzoyloxy)-3-methoxybenzylidene)-5-oxo-2-phenyl-4,5-dihydro-1*H*-imidazol-1-yl)-[1,1'-biphenyl]-4-yl)-5-oxo-2-phenyl-1,5-dihydro-4*H*-imidazol-4-ylidene)methyl)-2-methoxyphenyl benzoate (20b).

Yellow crystals; yield 64%; m.p > 300 °C; IR (KBr) 1737 (2CO), 1640 (2CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ = 3.87 (s, 6H, $2OCH_3$), 7.31-8.11 (m, 34H, Ar), 8.12 (s,

2H, 2CH); Anal. Calcd for $C_{60}H_{42}N_4O_8$: C, 76.10; H, 4.47; N, 5.92. Found: C, 76.27; H, 4.65; N, 5.69%.

4. 2. Biological study methods

4.2.1. In *vivo* anti-inflammatory assay

The in *vivo* anti-inflammatory activity of some synthesized compounds using celecoxib as a reference drug was elevated using in *vivo* carrageenan-induced rat paw edema (50mg/kg) and the measurement of paw thickness was done after 0, 0.5, 1, 2, 3, 4 and 5h of carrageenan injection [51].

4.2.2. Ethical approval: *Obtained from Institutional Animal Care and Use Committee (CU-IACUC), Cairo University.* ***Approval number (CUIF7618).***

4.2.3. Methodology of COX-2

The screen for COX-2 inhibitory activity was performed according to Ayaoub *et al*. [56]. Briefly, all positive compounds, and controls were solubilized in DMSO and assayed in triplicate at 25 μM concentration. COX-2 (Sigma-Aldrich) was added in 180 μL of the assay buffer containing 5 mM hematin (Sigma-Aldrich), 100 mM Tris-HCl buffer, pH 8.0. After addition of the test compound or positive control (10 μL), the reaction mixture was incubated for 5 min at room temperature, the reaction was started by the addition of 5 μL of arachidonic acid solution (Sigma-Aldrich) dissolved in methanol and *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD). After incubation for 1 h, the reaction mixture had its absorbance measured at 610 nm. Compounds that inhibited 50% of COX-2 activity had its IC_{50} values calculated by using sigma plot software.

4.2.4. Antimicrobial assay

The antimicrobial activity of synthesized compounds was determined using agar well diffusion method [52]. All the selected compounds were tested *in vitro* for their antibacterial activity against *staphylococcus aureus* and *Streptococcus mutans* (Gram-positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* and *klebsiella* (Gram-negative bacteria) using nutrient agar medium. Ampicillin and Gentamicin were used as standard drugs for Gram-positive and Gram-negative respectively. DMSO was used as a solvent control. The compounds were tested at a concentration of 15 mg/ml against both bacterial and fungal strains.

4.2.5. Method of testing

The sterilized media was poured onto the sterilized Petri dishes (20-25 ml, each Petri dish) and allowed to solidify at room temperature. Microbial suspension was prepared in sterilized saline equivalent to McFarland 0.5 standard solution (1.5x 10^5 CFU mL^{-1}) and its turbidity was adjusted to OD = 0.13 using spectrophotometer at 625 nm. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension and was flooded on the dried agar surface then allowed to dry for 15 minutes with lid in place. Wells of 6 mm diameter was made in the solidified media with the help of sterile borer. 100 µL of the solution of the tested compound was added to each well with the help of micropipette. The plates were incubated at 37 °C for 24 hrs in case of antibacterial activity. This experiment was carried out in triplicate and zones of inhibition were measured in mm. scale.

4.2.6. Methodology of MIC

For each strain, three to five isolated colonies were selected from the fresh agar plate and were transferred into a tube containing 3-4 ml of sterile broth medium. The bacterial suspension was mixed well and incubated at 35-37 °C for 2-6 h. The turbidity of the bacterial suspension should be equal to or greater than the turbidity of a McFarland Standard 0.5. After that, 1 mg of the tested compound (antimicrobial agent) was dissolved in 1 ml DMSO and two-fold serial dilution was done using broth medium. A fixed volume of the prepared bacterial inoculum was added to each tube and incubated for at 37 °C 16-20 h. The MIC is defined as the lowest concentration of the antimicrobial agent that inhibits visible growth of the tested isolate as observed with the unaided eye[57].

4.2.7. Methodology of DHFR

1- Methotrexate was diluted to 100-fold dilution with DHFR assay buffer; also test sample was diluted to 100X in an appropriate solvent.

2- (Diluted methotrexate and 2 µL of the test sample) added into wells assigned as sample screening (S), enzyme control (EC) or inhibitor control (IC), respectively.

3- Dihydrofolate reductase enzyme solution was prepared by adding 2 µL dihydrofolate reductase with 798 µL DHFR Assay Buffer, then 98 µL of diluted

dihydrofolate reductase was added into desired well(s) containing the test samples, enzyme control or inhibitor control, also 100 µL of DHFR assay buffer was added to desired well(s) as background control.

4- (40-Fold dilution of NADPH stock solution) was prepared with DHFR assay buffer, mixed by vortex then kept on ice, the diluted NADPH was added as 40 µL to each well that containing the test samples, enzyme control, inhibitor control or background control.

5- Wells mixed and incubated at room temperature for 10-15 min, avoid light.

6- DHFR substrate was prepared as 15-fold dilution with DHFR assay buffer, vortex briefly and kept on ice, then 60 µL of diluted DHFR substrate added to each well containing the test samples, enzyme control, inhibitor control or background control and wells mixed.

7- Absorbance was measured immediately at 340 nm in kinetic mode at room temperature and obtained two values for the absorbance (A_1 and A_2) at two-time points (t_1 and t_2) in the linear range of the plot.

8- The slope was calculated for all test inhibitor samples [S] and enzyme control [EC] by dividing the net ΔA (A_1-A_2) values with the time Δt (t_2-t_1). Subtract the solvent control or inhibitor background control readings from its paired sample readings.

$$\% \text{ Relative inhibition} = \frac{\text{Slope of [EC] - Slope of [S]}}{\text{Slope of EC}} \times 100$$

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Highlights:

A series of imidazol-5-ones, bearing a sulfonamide or carboxyl moiety was designed and synthesized.

- COX-2 inhibition of the imidazol-5-ones was tested *in vitro.*
- Compounds **12e** and **12f** showed higher potency than celecoxib.
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New imidazolone derivatives comprising benzoate or sulfonamide moiety as anti-inflammatory and antibacterial inhibitors: Design, synthesis, selective COX-2, DHFR and molecular modeling study

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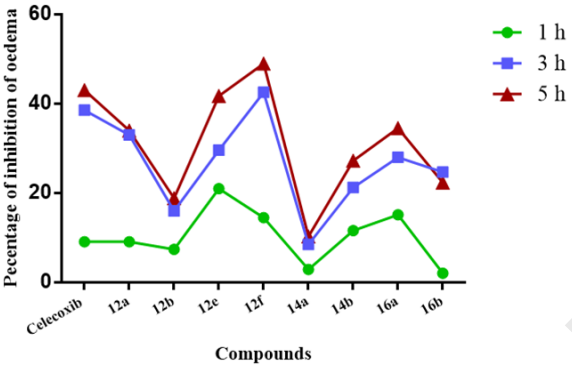
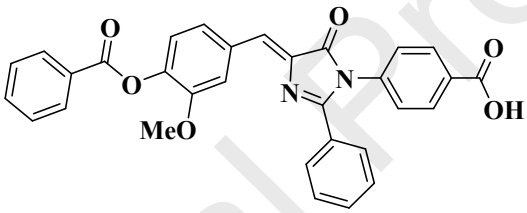


The percentage of oedema inhibition of the tested compounds and standard drug celecoxib

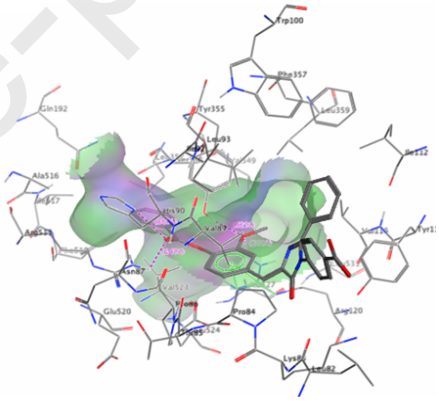
inhibition of oedema %
12f = 49
Celecoxib = 43.1



12f

DHFR IC_{50} = 0.285± 0.006
Combination (10 µM12f+1µM reference) = 0.078± 0.002

Enzyme Inhibition on COX-2
12f = 0.092
Celecoxib = 0.11



Molecular docking of 12f

Conflicts of Interest: The authors declare no conflict of interest.