SYNTHESIS UNDER MICROWAVE IRRADIATION AND MOLECULAR DOCKING OF SOME NOVEL BIOACTIVE THIADIAZOLES

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Abstract: A novel series of fused imidazole were prepared from reaction of 2-bromoacetyl-3-phenyl-1,3,4-thiadiazole with various heterocyclic amines under microwave irradiation. The structures of all the novel products were elucidated based on elemental analysis and spectral data. In addition, the biological activity of the newly synthesized compounds was evaluated and the results obtained indicate their potency as anti-inflammatory, analgesic and anti- ulcer agents. The binding mechanism of the most active compounds was studied using MOE to analyze the molecular interactions.

Keywords: 1,3,4-thiadiazoles, fused imidazoles, heterocyclic amines, microwave irradiation, anti-inflammatory, analgesic and anti- ulcer agents.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have a wide clinical use for the treatment of respiratory tract infections and fever. The two isoforms of cyclooxygenase (COX) are poorly distinguishable by most of the classical NSAIDs and these agents actually inhibit COX-1 extensively, besides COX-2, leading to gastrointestinal injury, suppression of TXA2 formation and platelet aggregation. The combination of these interactions is probably the reason for gastrointestinal bleeding as the most serious complication of these drugs. Human COX-2 enzyme isoform is a 604 amino acid heme protein [1, 2] that has specific key amino acids that are conserved from human to zebrafish [2]. Among these conserved Arg106, Tyr341, Tyr371, and Ser516. Through this catalytic active site the enzyme catalyzes the bioconversion of arachidonic acid to the signaling prostaglandins which are involved in the process of inflammation, pain and fever [2-4]. Inhibiting the catalytic function of the enzyme relies upon blocking these conserved essential amino

acids. Recently, researchers reported 1, 3, 4-thiadiazole derivatives that exhibited analgesic and anti-inflammatory activities (Figure 1). 5-Arylamino-1,3,4-thiadiazol-2(3*H*)-one,3-(5-bromo-2-thienyl)-1-phenyl-4-[3-acetyl-5-(N-substitutedacetamido)-2,3-dihydro-1,3,4-thiadiazol-2-yl]-1*H*-pyrazol, 5-arylamino substituted 3-nicotinoyl/isonicotinoyl-1,3,4-thiadiazol-2(3*H*)-one and 2-(2-naphthyloxymethyl)-5-substituted amino-1,3,4-thiadiazole derivatives showed anti-inflammatory and analgesic activities [5]. A series of novel spiro-thioxanthene and spiro-xanthene-9'2-[1,3,4]thiadiazole derivatives were synthesized and tested for anti-inflam matory and analgesic activities and showed significant activity compared to comparable to ibuprofen as standard drug [6]. Some 6-substituted-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole derivatives were synthesized and screend for anti-inflammatory activity [7]. A series of 2-(6- methyl-benzofuran-3-ylmethyl)-imidazo[2,1-b][1,3,4]thiadiazoles have been tested for their *in vivo* analgesic, anti-inflammatory activities and showed good anti-inflammatory activity[8]. On the other hand, microwave-assisted organic synthesis is a tool by which we can achieve goals in a few minutes with high yield as compared to conventional heating [9-13].

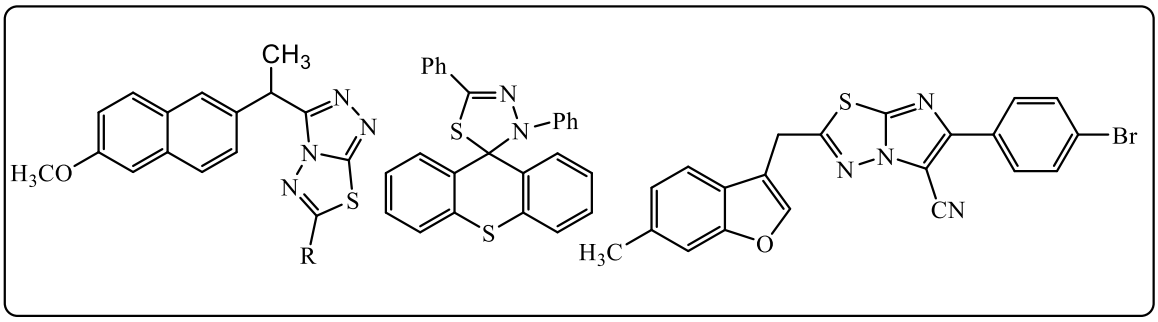


Figure1. Structures of literature lead compounds

As a part of our research interest towards developing new routes for the synthesis of a variety of heterocyclic systems with promising biological and pharmacological activities[14-26], we report in the present work the synthesis of a new series of 1,3,4-thidiazoles as anti-inflammatory, analgesic and anti- ulcer agents (Figure 2).

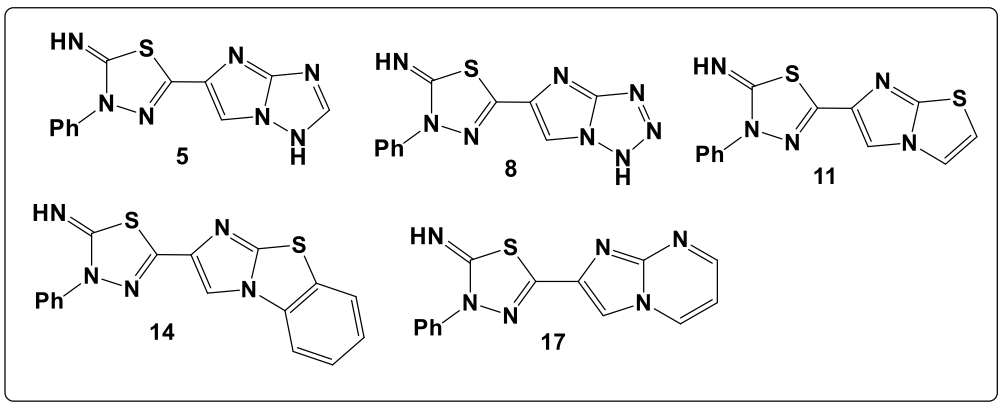


Figure 2. Structures of the proposed target compounds

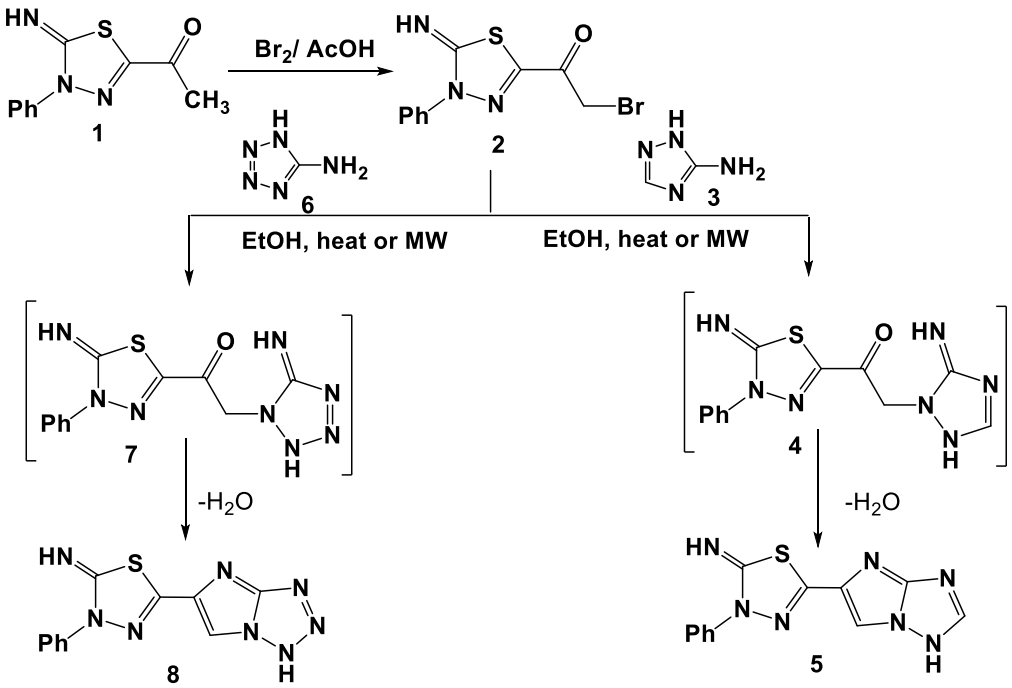
2. Results and discussion

2.1. Chemistry

The starting compound, 2-bromo-1-(5-imino-4-phenyl-4,5-dihydro-1,3,4-thiadiazol-2-yl) ethanone (**2**) and was prepared *via* reacting the 1-(5-imino-4-phenyl-4,5-dihydro-1,3,4-thiadiazol-2-yl)ethanone (**1**) [27] with bromine in glacial acetic acid.

Reaction of 2-bromoacetylthiadiazole **2** with some heterocyclic amines was examined in ethanol using both thermal heating and microwave irradiation for comparison. Thus reaction of compound **2** with 5-amino-1,2,4-triazole (**3**) yielded the imidazo[1,2-b]triazole derivative **5** (Scheme 1). The structure of compound **5** was evidenced by microanalysis and spectral (IR, ^{1}H NMR, Mass) data. For example, the IR spectra of compound **5**, revealed two absorption bands at ύ = 3406 and 3251 cm^{-1} attributed to the 2NH groups, respectively. Also, ^{1}H NMR displayed four singlet signals at δ = 7.14, 7.91, 10.75 and 11.12 ppm assignable for the imidazole-H5, triazole-H3, and 2NH (D_2O exchangeable) protons, respectively, in addition to the expected signals characteristic for the phenyl protons. The mass spectra of compound **5** revealed a peak corresponding to its molecular ion at m/z 283.

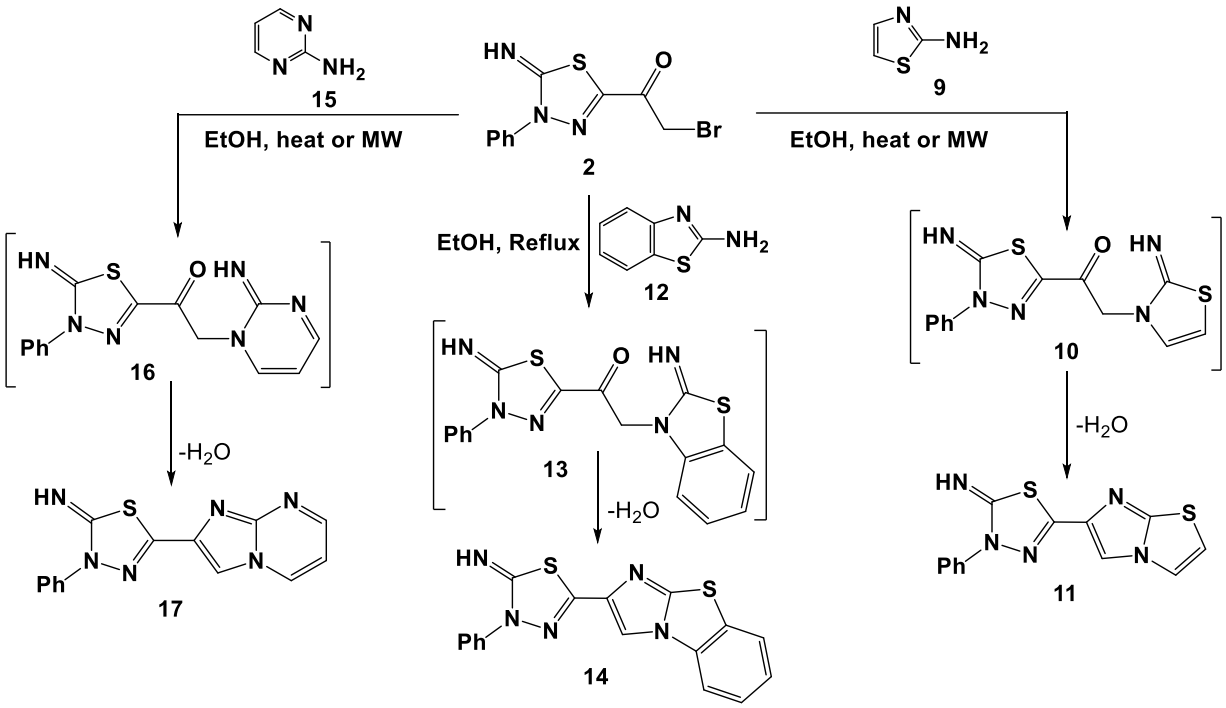
Similarly, treatment of **2** with 5-amino-1,2,3,4-tetrazole (**6**) under the same reaction conditions afforded the imidazo[1,2-b][1,2,4]tetrazole derivative **8**. The structres of **8** was established on the basis of elemental analyses as well as spectral data (see Experimental data).



Scheme 1: Synthesis of compounds **5** and **8**

Also, treatment of **2** with 2-aminothiazole (**9**) under the same reaction conditions afforded the imidazo[2,1-b]thiazole derivative **11** as depicted in Scheme 2.

Similarly, treatment of **2** with 2-aminobenzo[d]thiazole (**12**) and 2-aminopyrimidine (**15**) under the same reaction conditions afforded the fused imidazole derivatives **14** and **17** respectively (Scheme 2). The structure of the products **11, 14** and **17** were elucidated based on elemental and spectral data (IR, ^{1}H NMR, Mass). ^{1}H NMR of **11** as example, displayed two doubles and two singlet signals at δ = 7.14 (d, J = 5.1 Hz, 1H, thiazole-H2), 7.14 (d, J = 5.1 Hz, 1H, thiazole-H2), 7.94 (d, J = 5.1 Hz, 1H, thiazole-H3), 8.66 (s, 1H, Imidazole-H) and 10.75 (br s, D_2O exchangeable, 1H, NH), in addition to the expected signals characteristic for the phenyl protons. The mass spectra of products **11**, **14** and **17** revealed in each case a molecular ion peak which is consistent with the assigned structure (see experimental). Generally, the use of microwave irradiation was more efficient than thermal heating as it decreases the reaction time and increases the yield of the products **5, 8, 11, 14** and **17** as illustrated in Table 1.



Scheme 2: Synthesis of compounds **11, 14** and **17**

Table 1. Comparison between conventional heating and microwave irradiation for synthesis of compounds **5, 8, 11, 15** and **17**.

Compound No.	Reaction Times		Reaction Yields (%)	
	Conventional Methods	Microwave	Conventional Methods	Microwave
5	5 h	3 min	68	78
8	4 h	3 min	70	80
11	8 h	6 min	67	77
14	7 h	5 min	72	80
17	3 h	2 min	70	83

2.2. Pharmacological activity

Anti-nociceptive (analgesic) effect:

Different chemical compounds that were tested in this study depicted a significant analgesic effect than that of standard drug (Aspirin) (Table 2). Besides that, their effect were lasted for 2 hours after administration and increasing with time in either the period or the onset of actions. Compound **11** expressed a more powerful analgesic effect than other deferent tested compounds. In addition, Compound **11** presented a more powerful analgesic effect than the Aspirin by 27.6%, after 2 hours from administration.

Anti-inflammatory effect:

The anti-inflammatory effect of the chemical compounds (Table 3 & 4) exhibited a more pronounced effect than that of standard drug (Indomethacin). Furthermore, their intensity persisted along the 4 hours after administration. As a confirmatory calculation, Table 4 showed their edema, inhibition and potency percentage against the standard drug that confirms their noticeable effect. Compounds **11** and **17** showed the highest anti-inflammatory effect in relation with different tested chemical compounds. Moreover, compounds **11** and **17** showed the highest anti-inflammatory effect in comparison with Indomethacin by 117% and 106.3% respectively after 4 hours.

Anti-ulcerogenic effect:

Table 5 & 6 presented the significant anti-ulcerogenic effect of different chemical compounds against control positive group but not potent as standard drug (Esomeprazole) in either U.I., ulcer score or inhibition percent. Compound **17** showed more anti-ulcerogenic effect than the different tested compounds in relation with Esomeprazole by 70.37% after 3 weeks of protection and 4 hours from Indomethacin administration.

Table 2. Anti-nociceptive (analgesic) effect of different compounds in albino mice.

	Time (seconds)		
Groups	**Zero time**	**60 minutes**	**120 minutes**
Control	10.56±0.12	10.61±0.13	10.49±0.09
Aspirin	10.38±0.14	16.24±0.11*	19.18±0.18*
2	10.3±0.1	21.41±0.21*#	25.33±0.22*#
5	10.4±0.15	21.52±0.14*#	25.2±0.08*#
8	10.37±0.13	21.32±0.24*#	25.16±0.18*#
11	10.26±0.09	23.46±0.2*#	26.51±0.21*#
14	10.31±0.11	21.06±0.12*#	25.28±0.17*#
17	10.23±0.17	21.22±0.1*#	24.91±0.13*#

Values were expressed as means ± S.E of 6 animals. As compared with normal control (*) group and standard (#) group (one-way ANOVA followed by Tukey post hoc test) at $P < 0.05$.

Table 3. Anti-inflammatory effect of different compounds in albino rats**.**

	Edema paw thickness (mm)			
Groups	**1 hr**	**2 hr**	**3 hr**	**4 hr**
Control positive	7.12±0.06	7.51±0.01	7.93±0.04	6.81±0.02
Indomethacine	5.61±0.03*	6.52±0.08*	5.69±0.07*	4.58±0.06*
2	3.44±0.04*#	4.38±0.01*#	3.61±0.08*#	2.6±0.05*#
5	3.51±0.07*#	4.6±0.02*#	3.55±0.04*#	2.5±0.08*#
8	3.49±0.01*#	4.71±0.03*#	3.75±0.05*#	2.67±0.09*#
11	3.02±0.03*#	4.1±0.04*#	3.12±0.09*#	2.11±0.03*#
14	3.66±0.06*#	4.69±0.07*#	3.65±0.02*#	2.7±0.08*#
17	3.21±0.05*#	4.08±0.06*#	3.2±0.02*#	2.22±0.07*#

Values were expressed as means ± S.E of 6 animals. As compared with control positive (*) group and standard (#) group (one-way ANOVA followed by Tukey post hoc test) at $P < 0.05$.

Table 4. Anti-inflammatory effect (%) of different compounds in albino rats after 4 hours.

Groups	Edema%	Inhibition%	Potency%
Control positive	100	-	-
Indomethacine	67.25	32.75	100
2	38.17	61.83	188.79
5	36.71	63.29	193.25
8	39.2	60.8	185.64
11	30.98	69.02	210.74
14	39.64	60.36	184.3
17	32.59	67.41	205.83

Table 5. Ulcer Index (U.I) and ulcer scores of different compounds in albino rats**.**

Groups	U.I	Ulcer score
Control positive	22.45±0.19	4
Esomeprazole	4.91±0.1*	1
2	10.32±0.13*$^{\#}$	3
5	10.22±0.16*$^{\#}$	3
8	9.82±0.09*$^{\#}$	2
11	6.65±0.07*$^{\#}$	1
14	8.75±0.1*$^{\#}$	2
17	10.03±0.18*$^{\#}$	3

Values were expressed as means ± S.E of 8 animals. As compared with control positive (*) group and standard ($^{\#}$) group (one-way ANOVA followed by Tukey post hoc test) at $P < 0.05$

Table 6. Ulcer inhibition (%) of different compounds in albino rats**.**

Groups	Ulcer inhibition (%)
Control positive	-
Esomeprazole	78.12
2	54.03
5	54.47
8	56.25
14	61.02
11	55.32
17	70.37

2.3. Molecular Modeling:

The target molecule COX-2 enzyme crystal (PDB ID: 3ln1) was selected among different crystal structures available through the RCSB protein data bank website (PDB), subsequently it was downloaded and validated. The validation process was performed through flexible docking of the co-crystallized celecoxib molecule within its binding pocket and measuring the RMSD. The validation resulted in an RMSD value of 0.036 Å and 0.2226 Å for the amino acid residues of the active site and the co-crystallized ligand respectively (Figure 3).

Upon docking the selected most active compounds **11** and **17** the best poses for each compound displayed interactions with a range of the key amino acids in the active site (Figure 4 and 5) with varying binding energy scores (table 7).

To begin with compound **11** the hydrogen atom of the ligand's imine group was found to interact with the lone pair of electrons of the phenolic oxygen atom in Tyr341 (Figure 4a), or the alcoholic oxygen atom in Ser516 (Figure 4b). Moreover, in one pose of the ligand **17** an interaction was noted between the lone pair of the alcoholic oxygen atom of the Ser516 residue and the hydrogen atom of the imine group of the ligand (Figure 5a). On the other hand, in another pose the ligand **17** displayed; a hydrogen bond interaction between the hydrogen atom of the ligand's imine group and the oxygen of the amide backbone of the Pro71 residue, and another interaction through the phenyl ring and the nitrogen atom of the thiadiazole ring with the cationic side chain of the Arg106 amino acid (Figure 5b).

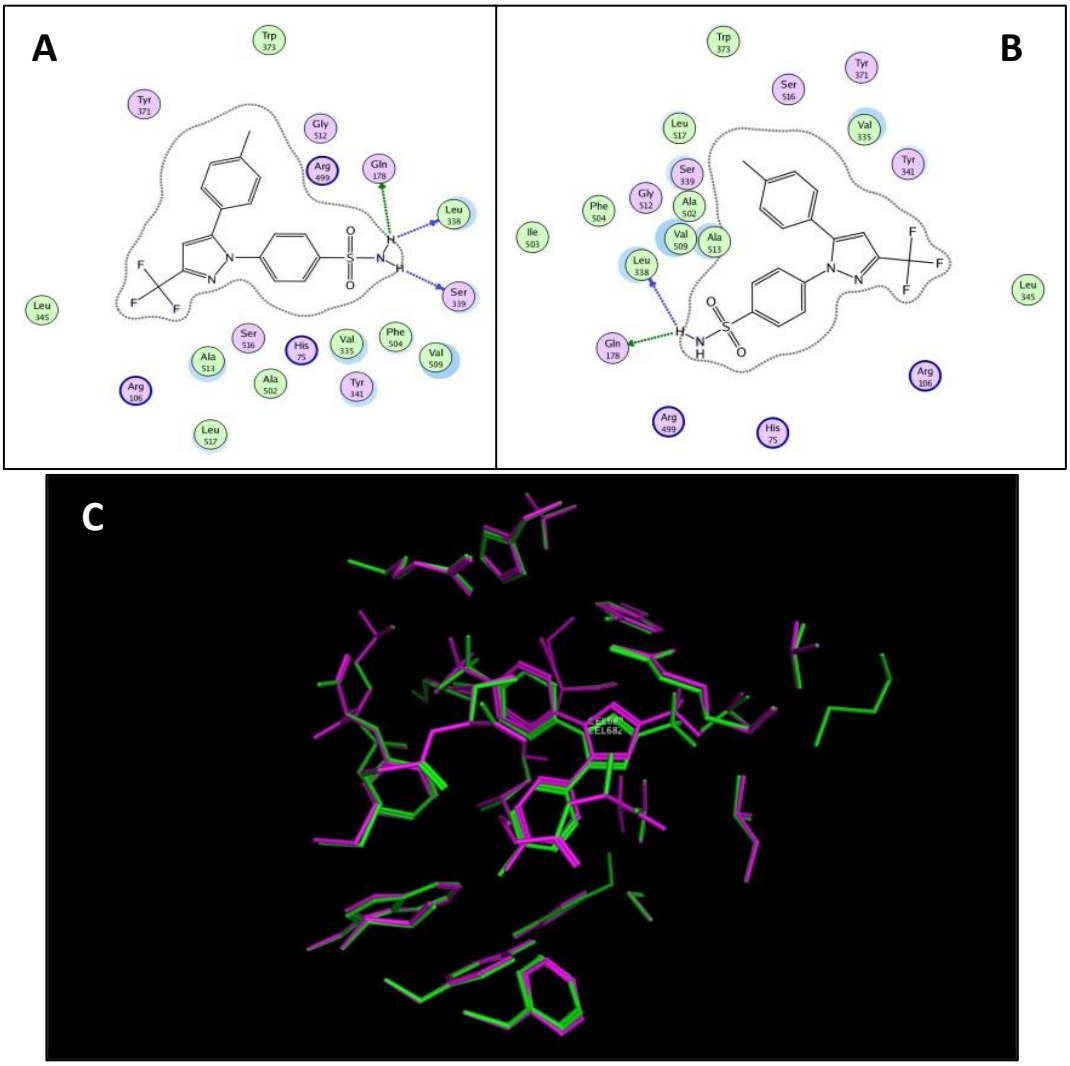


Figure 3. The virtual binding interactions between celecoxib and the active site of COX-2 enzyme (PDB ID: 3ln1). Figures 1a & 1b illustrate the original celecoxib interactions according to the downloaded coordinates and after the validation process respectively. Figure 1c depicts the superposed active site with the bound celecoxib before the validation process (colored in green) and after it (colored in magenta).

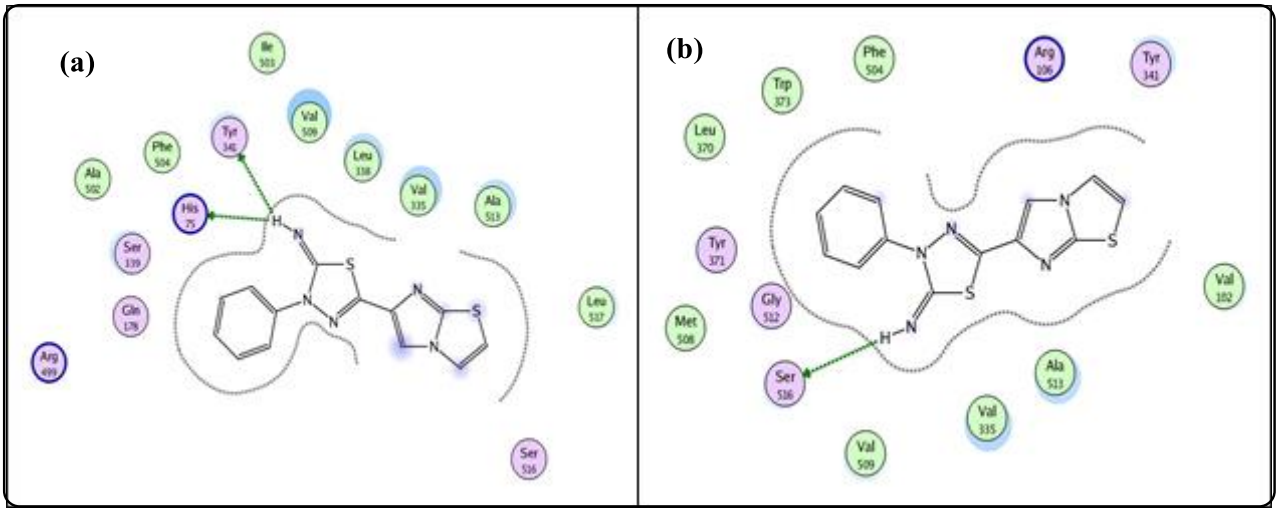


Figure 4. Binding interactions of the selected best poses of the compound **11** with the COX-2 enzyme active site.

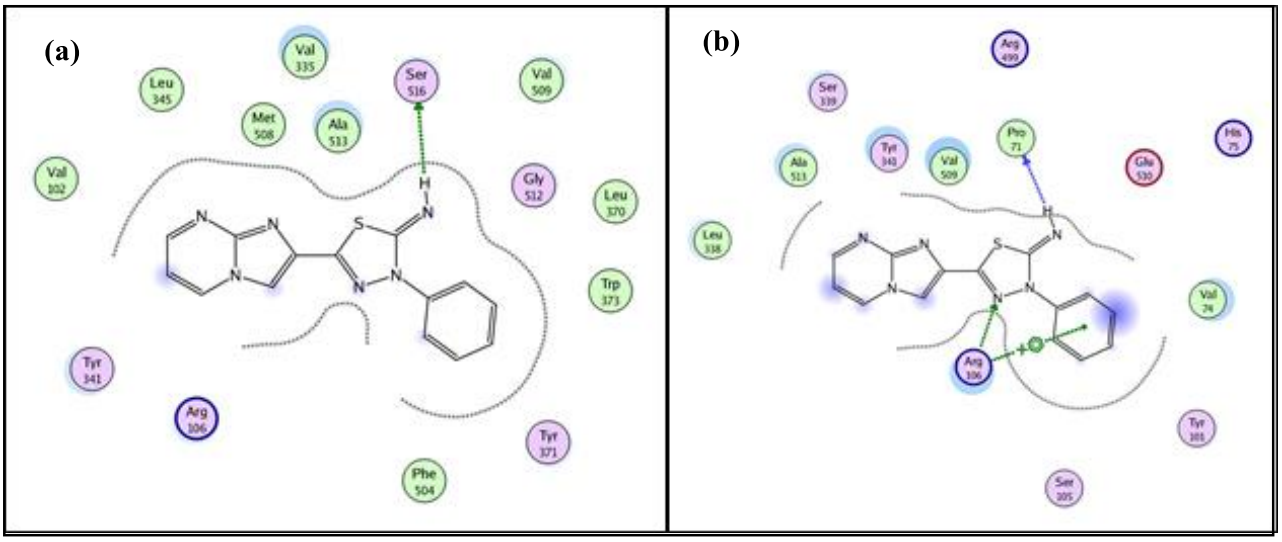


Figure 5. Binding interactions of the selected best poses of the compound **17** with the COX-2.

Table 7. Interactions of the selected most active compounds' best poses with key amino acids in the active site and their binding energy scores.

Ligand Name	Score (Kcal/mol)	Key amino acids interacting with the ligand
11	-10.96	Tyr 341
11	-10.57	Ser 516
17	-10.80	Ser 516
17	-10.86	Arg 106

3. Experimental Section

3.1. Chemistry

Melting points were measured with an IA 9000-series digital melting-point apparatus (Bibby Sci. Lim. Stone, Staffordshire, UK). Solvents were generally distilled and dried by standard literature procedures prior to use. IR spectra were recorded in potassium bromide discs on FTIR 8101 PC infrared spectrophotometers (Shimadzu, Tokyo, Japan). NMR spectra were recorded on a Mercury VX-300 NMR spectrometer (Varian, Inc., Karlsruhe, Germany) operating at 300 MHz (^{1}H NMR) and run in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCeMS-QP1000 EX mass spectrometer (Tokyo, Japan) at 70 eV. Microwave reactions were performed with a Millstone Organic Synthesis Unit with a touch control terminal (MicroSYNTH, Giza, Egypt) and a continuous focused microwave power delivery system in a pressure glass vessel (10 mL) sealed with a septum under magnetic stirring. The temperature of the reaction mixture was monitored using a calibrated infrared temperature control under the reaction vessel, and control of the pressure was performed with a

pressure sensor connected to the septum of the vessel. Elemental analyses were carried out at the Microanalytical Centre of Cairo University, Giza, Egypt. All reactions were followed by TLC (Silica gel, Merck).

Synthesis of 2-Bromo-1-(5-imino-4-phenyl-4,5-dihydro-1,3,4-thiadiazol-2-yl)ethanone (2)

Bromine solution in 1mL acetic acid (1.57g, 10 mmol) was added portionwise into a stirred solution of 2-acetylthiadiazole derivatives **1** (10 mmol) in 20 mL acetic acid. After complete addition, the reaction mixture was stirred for 2-4h (monitored by TLC). The reaction mixture was left to give solid product which collected by filtration, dried and recrystallized from EtOH to give product **2** as yellow solid, (83% yield), mp 110-112 °C; IR (KBr) ν_{max} 3429 (NH), 1664 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.43 (s, 3H, CH_3), 7.37-7.92 (m, 5 H, Ar-H), 10.64 (s, br, 1H, NH); MS m/z (%) 298 (M^++2, 7), 296 (M^+, 9), 178 (37), 149 (40), 117 (73), 76 (37), 43 (100). Anal. Calcd. for $C_{10}H_8BrN_3OS$ (296.96): C, 40.28; H, 2.70; N, 14.09. Found: C, 40.19; H, 2.62; N, 14.01%.

General procedure for the reaction of 2-bromoacetylthiadiazole 2 with heterocyclic amines 3, 6, 9, 12 and **15:**

Method A: A solution of 2-bromoacetylthiadiazole **2** (0.298g, 1 mmol) and the appropriate heterocyclic amines **3, 6, 9, 12** and **15** (1 mmol) in ethanol (20 mL) was refluxed for 3-8 h (monitored by TLC). The reaction mixture was left to cool and the formed solid product was collected by filtration, washed with water, dried and recrystallized from proper solvent to give the corresponding pure products **5, 8, 11, 14** and **17**.

Method B: Repetition of the same reactions of method A with heating in a microwave oven at 500 W and 120 °C for a period of time. The reaction mixture was treated similar to method A to obtain products identical in all respects with those separated from method A. Compounds **5, 8, 11, 14** and **17** with their physical constants and spectral data are depicted as shown below:

5-(1*H*-Imidazo[1,2-b][1,2,4]triazol-5-yl)-3-phenyl-1,3,4-thiadiazol-2(3*H*)-imine (5). Yellow solid, (75% yield), mp 203-205 °C (DMF); IR (KBr) ν_{max} 3406, 3251 (2NH), 3085, 2979 (2CH), cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.14 (s, 1H, imidazole-H5), 7.28-7.82 (m, 5H, Ar-H), 7.91 (s, 1H, triazole-H3), 10.75, 11.12 (2s, br, 2H, 2NH); MS m/z (%) 283 (M^+, 13), 257 (59), 234 (67), 211 (14), 171 (47), 137 (72), 111 (29), 95 (23), 84 (44), 71 (32), 57 (91), 43 (100). Anal. Calcd. for $C_{12}H_9N_7S$ (283.31): C, 50.87; H, 3.20; N, 34.61. Found: C, 51.15; H, 3.50; N, 34.50%.

5-(1*H*-Imidazo[1,2-e]tetrazol-5-yl)-3-phenyl-1,3,4-thiadiazol-2(3*H*)-imine (8). Yellow solid, (75% yield), mp 163-165 °C (EtOH); IR (KBr) ν_{max} 3416, 3247 (2NH), 3094 (CH), 1611 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.11 (s, 1H, imidazole-H5), 7.28-8.60 (m, 5H, Ar-H), 10.80, 11.61 (2s, br, 2H, 2NH); MS m/z (%) 284 (M^+, 17), 236 (45), 171 (45), 143 (78), 109 (45), 97 (66), 71 (34), 69 (75), 57 (71), 43 (100). Anal. Calcd. for $C_{11}H_8N_8S$ (284.3): C, 46.47; H, 2.84; N, 39.41. Found: C, 46.32; H, 3.35; N, 39.30%.

5-(Imidazo[2,1-b]thiazol-6-yl)-3-phenyl-1,3,4-thiadiazol-2(3*H*)-imine (11). Green solid, (75% yield), mp 184-185 °C (EtOH/DMF); IR (KBr) ν_{max} 3417 (NH), 1630 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.29-7.89 (m, 5H, Ar-H), 7.14 (d, J = 5.1 Hz, 1H, thiazole-H2), 7.94 (d, J = 5.1 Hz, 1H, thiazole-H3), 8.66 (s, 1H, imidazole-H), 10.75 (s, br, 1H, NH); MS m/z (%) 299 (M^+, 18), 287 (57), 259 (74), 205 (38), 157 (24), 106 (82), 89 (43), 76 (50), 43 (100). Anal. Calcd. for $C_{13}H_9N_5S_2$ (299.37): C, 52.16; H, 3.03; N, 23.39. Found: C, 52.03; H, 3.00; N, 23.30%.

5-(Benzo[*d*]imidazo[2,1-*b*]thiazol-2-yl)-3-phenyl-1,3,4-thiadiazol-2(3*H*)-imine (14).
Brown solid, (73% yield), mp 178-180 °C (EtOH); IR (KBr) ν_{max} 3413 (NH), 3063, 2965 (CH), 1612 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.96-8.51 (m, 8H, Ar-H), 8.44 (s, 1H, benzothiazole-H8), 9.04 (s, 1H, imidazole-H), 11.43 (s, br, 1H, NH); MS m/z (%) 349 (M^+, 18), 348 (44), 346 (34), 344 (100), 233 (78), 219 (64), 207 (53), 172 (9), 103 (63), 77 (86). Anal. Calcd. for $C_{17}H_{11}N_5S_2$ (349.43): C, 58.43; H, 3.17; N, 20.04. Found: C, 58.73; H, 3.45; N, 20.35%.

5-(Imidazo[1,2-a]pyrimidin-2-yl)-3-phenyl-1,3,4-thiadiazol-2(3*H*)-imine (17). Yellow solid, (78% yield), mp 153-155 °C (EtOH); IR (KBr) ν_{max} 3443 (NH), 3086, 2968 (CH), 1590 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.07-7.97 (m, 6H, Ar-H), 8.65 (s, 1H, imidazole-H), 8.80 (d, 1H, pyrimidine-H5), 8.99 (d, 1H, pyrimidine-H7), 11.00 (s, 1H, NH),; MS m/z (%) 294 (M^+, 13), 248 (57), 173 (30), 144 (34), 105 (32), 90 (42), 81 (39), 79 (54), 57 (43), 44 (100). Anal. Calcd. for $C_{14}H_{10}N_6S$ (294.33): C, 57.33; H, 3.42; N, 28.56. Found: C, 57.42; H, 3.30; N, 28.45%.

3.2. Pharmacological screening

Material and Methods:

Material:

Animals:

Adult male albino Sprague Dawely rats and mice weighing 120-130 g and 20-22 g respectively were obtained from the National Research Centre Animal House (Giza, Egypt) and were housed in a standard polypropylene cages and kept under constant environmental factors and equal light-dark

cycles. The animals were served commercially normal rat standard dietand water *ad libitum*.All surgeries were done under deep sodium pentobarbital anesthesia to minimize suffering

***Ethics Statement*:**

The experiments were carried out in agreement with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH No. 85: 23, revised 1996) and Institutional Animal Ethical Committee (IAEC) and complies with the guidelines from the Canadian Council on Animal Care.

Drugs:

Standard drugs used as a control:

Indomethacin (Indocid) was obtained from El Kahera pharmaceutical industrial company, Giza, Egypt; Aspirin (Aspirin) from Bayer Limited Egypt LLC, Cairo, Egypt and Esomeprazole (Esmopump) from Organo Pharma, El Obour, Egypt.

Methods:

Anti-nociceptive (Analgesic) test:

This test was done by hot plate (Ugo Basile, Italy) method at 52 °C (± 0.1 °C) for estimation of different compounds effect [28]. 21 groups of mice (n = 6) were adapted for 3 consecutive days before the test by putting them in the hot plate device adopted at room temperature for 15 minutes [29,30]. Inactivity to show analgesic reaction, like licking the paws or skipping out the hot plate was documented at 60 and 120 minutes after 1 hour from i.p administration of 19different compounds (10 mg/kg) after dissolving in DMSO, Aspirin (200mg/kg) (positive control) and DMSO (negative control) [31]. Reaction time for thermal pain was documented before administration of test compounds and aspirin (0 time).

Anti-inflammatory test:

This experiment was carried out by carrageenan induced rat paw edema [32] for evaluating the anti-inflammatory properties of the different compounds. Rats were divided into 21 groups (n=6), i.p administration of 19 different compounds (10mg/kg), and DMSO (negative control), while indomethacine (20mg/kg) (positive control) were administered once orally by gastric tube [32].All rats were injected with 0.1ml of 1% carrageenan solution in saline at the sub planter area of the right hind paw after 1 hour from different compounds and indomethacine administration. Each rat, its paw thickness was measured by planimeter before carrageenan administration. After

administration of carrageenan, the paw thickness of each rat was measured every 1 hour for 4 hours. Edema rate, inhibition, potency were calculated as follows [34].

$$\textbf{Edema (\%)} = \frac{\text{Control thickness mean - treated thickness mean}}{\text{Control thickness mean}} \times 100$$

$$\textbf{Inhibition (\%)} = \frac{\text{Control edema (\%) mean - treated edema (\%) mean}}{\text{Control edema (\%) mean}} \times 100$$

$$\textbf{Potency (\%)} = 100 - \frac{\text{Inhibition(\%) indomethacine - Inhibition(\%) treated group}}{\text{Inhibition(\%) indomethacine}} \times 100$$

Anti-ulcerogenic test:

A- Ulcer induction:

Treatments (21 groups; n=8) with thedifferent19 compounds (10mg/kgb.w. dissolved in DMSO), standard (Esomeprazole) (20mg/kg b.w dissolved in saline) and control positive (DMSO) were kept on for 21 days before ulcer induction (indomethacin administration). These were orally administered once daily with *ad libitum*facility of food and water during the experiment period. Gastric ulceration was induced in the ratsaccording to the technique illustrated by Sayanti et al. [35]. In brief, ratswere administered a single oral dose of indomethacin (30mg/kg b.w.), andthey were banned from food buthad a free access to water 24 hour before indomethacin administration. Different degrees of ulcers have demonstrated after 4 hours of indomethacin administration.

B- Stomach isolation:

After 4 hours of ulcer induction, rats were sacrificed. Rats' abdomen was surgically opened and the stomach excised and opened along the greater curvature. The washed stomachs were conserved in 0.1 M phosphate saline buffer (pH 7.4) before the macroscopic examination.

C- Ulcer quantification:

Scores of ulcers in the indomethacin treated rats were calculated using the method of Szabo and Hollander [36]. Washed stomachs were pinned on a corkboard and the ulcers were scored by dissecting microscope with square-grid eyepiece according to grading on a 0-5 scale as presented in Table 8.

Table 8. Ulcer scores and scores description.

Score	Description
0	normal mucosa
1	Vascular congestions
2	One or two lesions
3	Severe lesions
4	Very severe lesions
5	Mucosa full of lesions

Areas (mm^2) of mucosal damage were signified as a percentage of the total surface area of the stomach. Mean ulcer score for each animal was represented as ulcer index (U.I) and the inhibition percentage against ulceration was obtained using the following equation:

U.I. = [Ulcerated area/total stomach area] × 100.

%Ulcer inhibition = [U.I. in control − U.I. in test] × 100/U.I. in control.

Statistical analysis:

The differences between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey post hoc test determined by SPSS software program, version 21.Values are expressed as mean ± S.E. The level of statistical significance was taken at $P < 0.05$.

Molecular Modeling [37-40**]**

The molecular modeling study was performed using the MOE software version 2008.10. The procedures for the target preparation, ligand preparation and docking process that were performed are discussed in this section.

Target preparation:

The atomic coordinates for the target protein were downloaded from the protein data bank website (PDB ID: 3ln1). Hydrogen atoms were added and charges were adjusted using the protonate 3D tool of the MOE. The parameters were set to the default values for the protonate 3D tool. Thereafter, water molecules and all ligands other than the celecoxib were removed. Next, three subunits of the tetramer were removed, and only one subunit -with its co-crystallized celecoxib- was kept. Afterwards, the site finder tool was used to locate the active site of the remaining subunit, with the guidance of the co-crystallized celecoxib and after optimizing the settings for the site finder. Then, dummy atoms were created based upon the alpha centers -that were generated by the site finder

tool- in the vicinity of the active pocket. Finally, the target was validated through docking of the co-crystallized celecoxib, using the dummy atoms to guide the dock tool in locating the binding site. The default settings of the dock tool were selected for the placement and initial scoring methods. The post-placement refinement was adjusted to the forcefield, using an RMS gradient of 0.0001 kcal/mol in addition to deactivating the fix receptor option. Lastly, the final scoring methodology was set to London dG scoring function.

Ligand preparation:

The selected most active ligands were built using the MOE builder tool and a library of their structures was constructed. Afterwards, the library was submitted to the conformational search tool to find the ligands' least energy conformers. The parameters were set to the default settings of the conformational search tool; except for the RMS (root mean square) gradient was set to a value of 0.001 kcal/mol. Subsequently, the conformation with the least potential energy for each ligand was selected and used for docking.

Docking:

The library of the energy minimized most active ligands of interest was supplied to the docking tool, using the same parameters utilized in the validation process. The results of the flexible docking were analyzed based upon interactions with essential amino acids in the active site and scores generated by the dock tool of the MOE.

4. Conclusion

In the investigation described above, a new series of 5-heteroaryl-1,3,4-thiadiazole derivatives was synthesized *via* reaction of 2-bromoacetylthiadiazole with various heterocyclic amines under microwave irradiation. Different spectroscopic methods and elemental analyses were used to confirm the structures of the newly synthesized compounds. The anti-inflammatory properties of some of the prepared compounds were evaluated. The results demonstrate that selected members of this series, including **11** and **17**, show excellent activities against all tested anti-inflammatory compared with the standard indomethacine, Esomeprazole, aspirin for anti-inflammatory, Anti-nociceptive (analgesic), Anti-ulcerogenice. The binding mechanism of the most active compounds was studied using MOE to analyze the molecular interactions. The molecular docking study confirmed high binding affinities -21.53 and -21.66, kcal/mol for **11** and **17** respectively.

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