DESIGN, SYNTHESIS AND MOLECULAR MODELING STUDY OF 1,2,4-TRIAZOLE CARBOHYDRAZIDE DERIVATIVES WITH POTENTIAL ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES

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يتناول البحث تخليق مشتقات , , –تريازول كاربوهيدرازيد **(6a-l)** بهدف التوصل الى مركبات جديدة وفعالة كمضادات للميكروبات ومضادات للالتهابات. وتم التأكد من التركيب البنائى للمركبات المستهدفة بالتحاليل الدقيقة وا ّشعة تحت الحمراء والرنين النووى المغناطيسى للهيدروجين والكربون المشع ومطياف الكتلة. كما تمت دراسة الفاعلية البيولوجية للمركبات المشيدة كمضادات للميكروبات باستخدام العقار المرجعى أمبيسلين و كلوتريمازول واظهرت النتائج ان المركب **6i** مبيسلين ضد *Staphylococcus aureus* لوحظ ان للمركبين **6j, 6l** فاعلية تقارب ا ّمبيسلين ضد *Escherichia coli* ولم تظهر اى من المركبات المحضرة فاعلية ضد *Candida albicans* مقارنة بعقار الكلوتريمازول. تمت دراسة الفاعلية البيولوجية للمركبات المشيدة كمضادات لـ لتهابات باستخدام اختبار(تورم قدم الجرذان بتثير الكراجينين) وقد قورنت النتائج مع عقار Celecoxib كعقار مرجعى وقد أظهرت النتائج أن معظم المركبات تتمتع بفاعلية كمضادات لـ لتهابات ووجد أن المركبين **6l** and **6d** ا تاثيرا كمثبطات للتورم. ولقد تم تقييم التثير التقرحى على المعدة للمركبين **6l** and **6d** ووجد أن لهـا تاثيرا تقرحيا قليلا على المعدة مقارنة ّندوميثاسين. هذا وقد تم دراسة النمذجة الجزيئية للمركبات بهدف وضع تفسير للنتائج البيولوجية التى تم الحصول عليها.

The present investigation is concerned with the synthesis of 1,2,4-triazole carbohydrazide derivatives **(6a-l)** with the objective of discovering novel and potent antimicrobial and anti-inflammatory agents.

The chemical structures of the target compounds were elucidated by elemental analyses, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. The antimicrobial activity of the target compounds were evaluated and compared with ampicillin trihydrate and clotrimazole as references compounds. The results showed that

compound **6i** revealed a similar level of activity as ampicillin against *Staphylococcus aureus*, while compounds **6j** and **6l** exhibited comparable activity against *Escherichia coli*. All compounds were less active against *Candida albicans* when compared with clotrimazole. The results of anti-inflammatory showed that compounds **6d, 6l** possessed higher anti-inflammatory activity than celecoxib in carageenan-induced rat paw edema test with low gastric ulcerogenicity compared with indomethacin. Molecular modeling studies were performed in order to rationalize the obtained biological results.

INTRODUCTION

It has been reported that many compounds having 1,2,4- triazole skeleton possessed interesting biological activities such as antibacterial[1-4], antifungal[5-7], analgesic[8] and anti-inflammatory[8-17].

Non steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for treatment of pain and inflammation in arthritis for decades. NSAIDs reduce pain and swelling associated with arthritis by blocking the metabolism of arachidonic acid by cycloxygenase enzyme COX thereby the production of prostaglandin[18]. Prostaglandins are cytoprotective, administration of NSAIDs in long term may lead to GI ulcers, bleeding and renal disorders.

In the present investigation it seemed of interest to synthesize 5-phenyl-1-(3-pyridyl)1H-1,2,4-triazole-3-carboxylic acid derivatives and study the effect of cyclization on anti-inflammatory activity.

EXPERIMENTAL

Chemistry

Melting points were determined on Electrothermal Melting Point Apparatus and are uncorrected. Elemental microanalyses were performed on Perkin-Elmer, 240 Elemental Analyzer, at the central laboratory, Assiut University. TLC was carried out using silica gel 60 F_{254} precoated (E. Merck, Germany) and were visualized using UV lamp at 254 nm. IR spectra were recorded as KBr disks on a 470 Shimadzu IR Spectrophotometer at the Faculty of Pharmacy, Assiut University. ^{1}H-NMR spectra were recorded on a JEOL JNM-AL 300 FT NMR system. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (TMS) as internal standard. DMSO-d_6 was used as solvent. ^{13}C-NMR spectra were recorded on JEOL-JNM-EX 300, 75.45 MHz. Electrospray ionization-Mass spectra (ESI-MS) were determined on a Finnigan mass spectrometer.

4-(3-Pyridyl)-hydrazono-2-phenyl-2-oxazolin-5-one 3[14]

(Yield 70%).

5-Phenyl-1-(3-pyridyl)-1,2,4-triazole-3-carboxylic acid hydrazide 4[14]

Yield 54%, m.p. 140-142°C.

N^4-Substituted 5-phenyl-1-(3-pyridyl)-1,2,4-triazole-3-carboxylic acid thiosemicarbazide 5a-f[14]

To a suspension of 5-phenyl-1-(3-pyridyl)-1,2,4-triazole-3-carboxylic acid hydrazide **4** (2.8 g, 0.01 mol) in ethanol (40 ml); a solution of the appropriate isothiocyanate (0.01 mol) in ethanol (10 ml) was added. The mixture was refluxed for 2 hrs. The reaction mixture was cooled and the separated solid was filtered, washed with ethanol and crystallized from appropriate solvent.

N`-(3-Substituted-4-phenyl(p-chlorophenyl)thiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl)-1H-1,2,4-triazole-3-carbohydrazide 6a-l

A mixture of **5** (0.002 mol), phenacyl bromide or p-chloro-phenacyl bromide (0.002 mol) and freshly fused sodium acetate (0.25 g, 0.003 mol) in ethanol (25 ml) was heated under reflux for 4-6 hrs. The solvent was evaporated off and the reaction product was allowed to cool. The separated product was filtered off, washed with water and crystallized from a suitable solvent. The physical data of the synthesized compounds **6a-l** are shown in Table 1.

N`-(3-Methyl-4-phenylthiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl)-1H-1,2,4-triazole-3-carbohydrazide 6a

IR (KBr, cm^{-1}): 3410 (NH), 1678 (CO), 1613 (C=N), 1348 (CS).

^{1}H-NMR (DMSO-d$_6$) δ (ppm): 11.15-10.90 (1H, br.s, CONH, exchangeable), 9.00-8.80 (2H, m, pyr.H$_{2,6}$), 8.35-8.00 (2H, m, pyr.H$_{4,5}$), 8.00-7.50 (10H, m,ArH), 6.50-6.30 (1H, s, C5-H of thiazoline), 3.60-3.30 (3H, s, CH$_3$).

Electron impact mass spectrum (EI-MS): m/z 454.3 (M$^+$+1), 476.2 (M$^+$+Na) was consistent with the expected structure.

N`-(3-Methyl-4-p-chlorophenyl-thiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl)-1H-1,2,4-triazole-3-carbohydrazide 6b

IR (KBr, cm^{-1}); 3410 (NH), 1678 (CO), 1610 (C=N), 1394 (CS).

^{1}H-NMR (DMSO-d$_6$) δ (ppm): 11.15-10.95 (1H, br.s, CONH, exchangeable), 9.05-8.80 (2H, m, pyr.H$_{2,6}$), 8.35-8.00 (2H, m, pyr.H$_{4,5}$), 8.00-7.50 (9H, m, ArH), 6.65-6.45 (1H, s, C5-H of thiazoline), 3.60-3.40 (3H, s, CH$_3$). ^{13}C-NMR (DMSO-d$_6$) δ (ppm): 168.16 (CO), 156.47, 155.04, 154.52, 146.41, 139.18, 134.26, 133.95, 125.0 (quaternary cabons), 150.32, 150.0, 133.60, 133.55, 130.42, 129.23, 129.0, 128.83, 128.69, 127.50, 127.60, 126.69, 124.20, 97.69 (CH=), 33.14 (CH$_3$). Electron impact mass spectrum (EI-MS): m/z 488.11 (M$^+$+1), 510.31 (M$^+$+Na).

Table 1: Physical data of the target compounds **(6a-l)**.

Compd. No.	R	R1	m.p. [°C] Solvent of crystal.	Yield [%]	Molecular formula / Mol.Wt	Microanalyses		
						C	H	N
6a	CH_3	H	227-8 aq.DMF	84	$C_{24}H_{19}N_7OS$ 453.52	63.56 63.99	4.22 4.44	21.62 21.31
6b	CH_3	Cl	216-17 aq.DMF	80	$C_{24}H_{18}ClN_7OS$ 487.96	59.07 58.94	3.72 3.73	20.09 20.04
6c	C_2H_5	H	192-3 aq.DMF	83	$C_{25}H_{21}N_7OS$ 467.55	64.22 64.23	4.53 4.63	20.97 21.23
6d	C_2H_5	Cl	244-5 aq.DMF	86	$C_{25}H_{20}ClN_7OS$ 501.99	59.82 59.40	4.02 3.59	19.53 19.45
6e	C_6H_5	H	210-12 Methanol	72	$C_{29}H_{21}N_7OS$ 515.59	67.56 67.57	4.11 4.32	19.02 19.40
6f	C_6H_5	Cl	222-3 Methanol	70	$C_{29}H_{20}ClN_7OS$ 550.03	63.33 63.04	3.67 3.75	17.83 18.50
6g	p-$CH_3C_6H_4$	H	240-1 Methanol	73	$C_{30}H_{23}N_7OS$ 529.62	68.03 68.41	4.38 4.56	18.51 18.90
6h	p-$CH_3C_6H_4$	Cl	246-7 Methanol	76	$C_{30}H_{22}ClN_7OS$ 564.06	63.88 64.21	3.93 4.21	17.38 17.63
6i	p-BrC_6H_4	H	230-1 aq.DMF	79	$C_{29}H_{20}BrN_7OS$ 594.49	58.59 59.00	3.39 3.87	16.49 16.75
6j	p-BrC_6H_4	Cl	228-30 aq.DMF	75	$C_{29}H_{19}BrClN_7OS$ 628.93	55.38 55.68	3.04 3.44	15.59 15.34
6k	c-hexyl	H	192-3 Methanol	67	$C_{29}H_{27}N_7OS$ 521.64	66.77 67.00	5.22 5.61	18.80 19.12
6l	c-hexyl	Cl	241-3 Methanol	65	$C_{29}H_{26}ClN_7OS$ 556.08	62.64 62.79	4.71 4.82	17.63 17.53

N`-(3-Ethyl-4-phenylthiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl)-1H-1,2,4-triazole-3-carbohydrazide 6c

IR (KBr, cm^{-1}): 3410 (NH), 1672 (CO), 1613 (C=N), 1334 (CS).

^{1}H-NMR (DMSO-d$_6$) δ (ppm): 11.15-10.85 (1H, br.s, CONH, exchangeable), 9.05-8.75 (2H, m, pyr.H$_{2,6}$), 8.25-7.95 (2H, m, pyr.H$_{4,5}$), 7.90-7.30 (10H,m,ArH), 6.45-6.25 (1H, s, C5-H of thiazoline), 4.10-3.60 (2H, q,$\underline{CH_2}CH_3$), 1.60-0.90 (3H, t, CH$_2\underline{CH_3}$).

Electron impact mass spectrum (EI-MS): m/z 467.9 (M$^+$+1), 490.01 (M$^+$+Na).

N`-(3-Ethyl-4-p-chlorophenyl-thiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl)-1H-1,2,4-triazole-3-carbohydrazide 6d

IR (KBr, cm^{-1}): 3410 (NH), 1678 (CO), 1613 (C=N), 1365 (CS).

^{1}H-NMR (DMSO-d$_6$) δ (ppm): 11.10-10.90 (1H, br.s, CONH, exchangeable), 9.10-8.75 (2H, m, pyr.H$_{2,6}$), 8.35-7.95 (2H, m, pyr.H$_{4,5}$), 7.90-7.40 (9H, m, ArH), 6.55-6.30 (1H, s, C5-H of thiazoline), 4.10-3.60 (2H, q, C$\underline{H_2}$CH$_3$), 1.40-0.95 (3H, t, CH$_2$C$\underline{H_3}$).

Electron impact mass spectrum (EI-MS): m/z 502.11 (M$^+$+1), 524.21 (M$^+$+Na).

N`-(3-Phenyl-4-phenylthiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl)-1H-1,2,4-triazole-3-carbohydrazide 6e

IR (KBr, cm^{-1}): 3410 (NH), 1673 (CO), 1358 (C=N), 1306 (CS).

^{1}H-NMR (DMSO-d$_6$) δ (ppm): 10.90 (1H, br.s, CONH, exchangeable), 8.90-8.60 (2H, m, pyr.H$_{2,6}$), 8.20-7.90 (2H, m, pyr.H$_{4,5}$), 7.59-7.34 (15H, m, ArH), 6.90 (1H, s, C5-H of thiazoline).

N`-(3-Phenyl-4-p-chlorophenyl-thiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl) 1H-1,2,4-triazole-3-carbohydrazide 6f

IR (KBr, cm^{-1}): 3410 (NH), 1678 (CO), 1358 (C=N), 1313 (CS).

^{1}H-NMR (DMSO-d$_6$) δ (ppm): 10.97 (1H, br.s, CONH, exchangeable), 8.70-8.55 (2H, m, pyr.H$_{2,6}$), 8.30-7.90 (2H, m, pyr.H$_{4,5}$), 7.34-7.59 (14H, m, ArH), 6.90 (1H, s, C5-H of thiazoline).

N`-(3-p-Methylphenyl-4-phenyl-thiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl)-1H-1,2,4-triazole-3-carbohydrazide 6g

IR (KBr, cm^{-1}): 3410 (NH), 1675 (CO), 1358 (C=N), 1313 (CS).

^{1}H-NMR (DMSO-d$_6$) δ (ppm): 10.97 (1H, br.s, CONH, exchangeable), 8.70-8.50 (2H, m, pyr.H$_{2,6}$), 8.30-8.00 (2H, m, pyr.H$_{4,5}$), 7.90-7.10 (14H, m, ArH), 6.90 (1H, s, C5-H of thiazoline), 2.30 (3H, s, CH$_3$).

N`-(3-p-Methylphenyl-4-p-chloro-phenylthiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl)-1H-1,2,4-triazole-3-carbohydrazide 6h

IR (KBr, cm^{-1}): 3410 (NH), 1675 (CO), 1358 (C=N), 1313 (CS).

^{1}H-NMR (DMSO-d$_6$) δ (ppm): 10.97 (1H, br.s, CONH, exchangeable), 8.80-8.50 (2H, m, pyr.H$_{2,6}$), 8.30-8.00 (2H, m, pyr.H$_{4,5}$), 7.90-7.25 (13H, m, ArH), 6.90 (1H, s, C5-H of thiazoline), 2.30 (3H, s, CH$_3$).

N`-(3-p-Bromophenyl-4-phenyl-thiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl)-1H-1,2,4-triazole-3-carbohydrazide 6i

IR (KBr, cm^{-1}): 3410 (NH), 1675 (CO), 1358 (C=N), 1313 (CS).

^{1}H-NMR (DMSO-d$_6$) δ (ppm): 11.30 (1H, br.s., CONH, exchangeable), 8.90-8.60 (2H, m, pyr.H$_{2,6}$), 8.30-8.00 (2H, m, pyr.H$_{4,5}$), 7.90-7.50 (14H, m, ArH), 6.90 ((1H, s, C5-H of thiazoline).

N`-(3-p-Bromophenyl-4-p-chloro-phenylthiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl)-1H-1,2,4-triazole-3-carbohydrazide 6j

IR (KBr, cm^{-1}): 3410 (NH), 1675 (CO), 1358 (C=N), 1313 (CS).

^{1}H-NMR (DMSO-d$_6$) δ (ppm): 11.35 (1H, br.s., CONH, exchangeable), 9.05-8.65 (2H, m, pyr.H$_{2,6}$),

8.40-8.00 (2H, m, pyr.$H_{4,5}$), 7.90-7.60 (13H, m, ArH), 6.90 (1H, s, C5-H of thiazoline).

N`-(3-Cyclohexyl-4-phenylthiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl)-1H-1,2,4-triazole-3-carbo-hydrazide 6k

IR (KBr, cm^{-1}): 3410 (NH), 1675 (CO), 1358 (C=N), 1313 (CS).

^{1}H-NMR (DMSO-d_6) δ (ppm): 10.85 (1H, s, CONH, exchangeable), 8.80-8.60 (2H, m, pyr.$H_{2,6}$), 8.30-8.00 (2H, m, pyr.$H_{4,5}$), 7.90-7.30 (10H, m, ArH), 6.85 (1H, s, C5-H of thiazoline), 3.00-2.45 (1H, hump, c.hexyl), 2.10-0.90 (10H, m, c.hexyl).

N`-(3-Cyclohexyl-4-*p*-chlorophenyl-thiazol-2(3H)-ylidene)-5-phenyl-1-(pyridin-3-yl)-1H-1,2,4-triazole-3-carbohydrazide 6l

IR (KBr, cm^{-1}): 3410 (NH), 1675 (CO), 1358 (C=N), 1313 (CS).

^{1}H-NMR (DMSO-d_6) δ (ppm): 10.85 (1H, s, CONH, exchangeable), 8.80-8.60 (2H, m, pyr.$H_{2,6}$), 8.30-8.00 (2H, m, pyr.$H_{4,5}$), 7.90-7.30 (9H, m, ArH), 6.90 (1H, s, C5-H of thiazoline), 3.20-2.55 (1H, hump, c.hexyl), 2.20-1.10 (10H, m, c.hexyl). Electron impact mass spectrum (EI-MS): m/z 556.01 (M^++1), 578.01 (M^++Na).

Antimicrobial activity

Microbiological screening was carried out at Mycological Center Assiut University. The microdilution susceptibility test in Müller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) were used for the determination of antibacterial and antifungal activities[19]. The utilized test organisms were: *Escherichia coli* (*E. coli*) ATCC 25922 as an example of Gram-negative bacteria, *Staphylococcus aureus* (*S. aureus*) ATCC 19433 as an example of Gram-positive bacteria and *Candida albicans* (*C. albicans*) as yeast-like fungi. Ampicillin trihydrate and clotrimazole were used as standard antibacterial and antifungal agents, respectively. Solutions of the test compounds, ampicillin trihydrate and clotrimazole were prepared in DMSO at concentration of 1600 μg/ml. The twofold dilution of the compounds were prepared (800, 400,.....6.25 μg/ml). The microorganism suspensions at 10^6 CFU/ml (Colony Forming Unit/ml) concentration were inoculated to the corresponding wells. Plates were incubated at 36°C for 24 hrs to 48 hrs. The incubation chamber was kept sufficiently humid. At the end of the incubation period, the minimal inhibitory concentrations (MICs) were determined. MICs were recorded as the minimum concentration of a compound that visually inhibits the growth of tested microorganisms.

Pharmacology
Chemicals and instruments

The following chemicals were used: Celecoxib was provided from EIPICO, Egyptian International Pharmaceutical Industries Co, Egypt. Indomethacin was purchased from Nile Co. (Nile Co., for Drugs and Chem. Industries, Cairo, Egypt).

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Carrageenin, used to induce edema, was purchased from Sigma Chemicals Co. Digital plethysmometer LE7500 (Panlab S.L., Cornella, Barcelona, Spain) was used to measure the volume of paw edema., St. Louis, USA.

Computer program "Prism" was used for carrying statistical analyses. Values of $P< 0.05$ (significant difference) or $P< 0.01$ (highly significant difference) were used as the limit for statistical significance.

Anti-inflammatory activity

Anti-inflammatory activity of the compounds under investigation was studied in rats using carrageenan. A suspension of the tested compounds, and reference drug, celecoxib, in CMC (0.5% w/v in water) was administrated orally to adult male albino rats weighing (100–120 g) in dose level (2.5×10^{-5} mol/kg). Control animals were similarly treated with CMC (0.5% w/v in water).

After 30 minutes 0.1 ml of freshly prepared carrageenan solution (1% in normal saline) was injected into the subplanar region of the right hind paw according to the method of Hernandez-Perez *et al.*[20] The right paw volume was measured by digital plethysmometer at 1,2,3 hrs intervals after administration of the tested compounds.

The difference between the thicknesses of the two paw (right and left) was taken as a measure of edema[21].

Gastric ulcerogenic effect[22]

Adult male albino rats (120-200 g) were divided into 3 groups; each of six animals. Animals were starved for 24 hrs before the experiment but had free access to water. The animals were then treated orally by means of a stomach tube with suspensions of the tested compounds and the reference drug in aqueous solution of carboxymethyl cellulose, CMC, (0.5% w/v) at dose levels of 10, 50, 100 mg/kg. Control animals were treated with an equal volume of 0.5% carboxymethyl cellulose. After 6 hrs, the rats were sacrificed and the stomachs were removed, dissected along the greater curvature and washed with water. The lesions in gastric mucosa were determined by using stereoscopic microscope.

Ulcer was defined as at least one lesion that was 0.5 mm or more in length. Lesion size (mm) was measured along its greatest length and in the case of patches; five such lesions were considered the equivalent of a 1 mm ulcer. The sum of the lesions' lengths in each group of animals was divided by their number and expressed as the gastric ulcer index.

Acute toxicity (LD_{50})[23]

Groups of adult male albino mice, each of six animals (25-30 g) were injected intraperitoneally with graded doses of the tested compounds and the reference drug. The percentage of mortality, in each group of animals, was determined 24 hrs after the injection. Calculation of the LD_{50} was processed by graphical method.

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Molecular docking

All the molecular modeling studies were carried out on Intel Pentium 1.6GHZ processor, 512 MB memory with Windows XP operating system using Molecular Operating Environment (MOE 2007-09 Chemical Computing Group, Canada)[24] as the computational software. All the minimizations were performed with MOE untila RMSD gradient of 0.05 kcal and A^{-1} with MMFF94X force-field and the partial charges were automatically calculated.

The X-ray crystallographic structure of murine COX-2 complexed with celecoxib (PDBID:6COX) was obtained from protein data bank. The enzyme was prepared for docking studies where (i) Ligand molecule was removed from the enzyme active site. (ii) Hydrogen atoms were added to the structure with their standard geometry. (iii) MOE Alpha Site Finder was used for the active sites search in enzyme structure and dummy atoms were created from the obtained alpha spheres.(iv)The obtained model was then used in predicting the ligand-enzyme interactions at the active site.

RESULTS AND DISCUSSION

Chemistry

The synthetic reactions used for the synthesis of compounds **6a-l** are outlined in scheme 1.
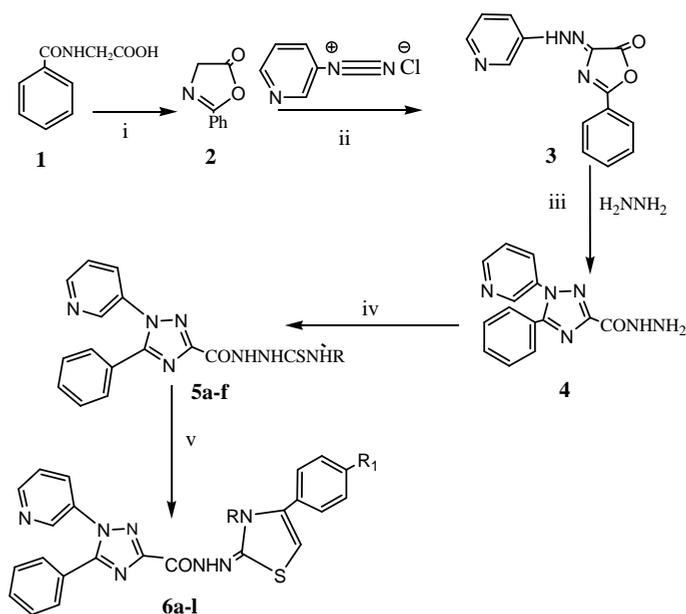
(i) Ac$_2$O / $\triangle$ (ii) NaOAc

(iii) CH$_3$OH (iv) R-NCS / C$_2$H$_5$OH (v) Ph(p-ClPh)COCH$_2$Br/ fused NaOAc/C$_2$H$_5$OH

R: CH$_3$; C$_2$H$_5$; C$_6$H$_5$; C$_6$H$_4$CH$_3$(p); C$_6$H$_4$Br(p); C$_6$H$_{11}$

R$_1$ = H, Cl

Scheme 1: Synthesis of the target compounds **6a-l**.

Reaction of hydrazide **4** with alkyl, arylisothiocyanate in ethanol yielded the corresponding thiosemi-carbazide derivatives **5a-f**. Condensation of compounds **5a-f** with appropriate phenacyl bromide in boiling ethanol containing anhydrous sodium acetate lead to the formation of compounds **6a-l** as one product as confirmed by TLC.

The chemical structures of the reaction products were confirmed by elemental analyses, IR, ^{1}H-NMR, ^{13}C-NMR and MS. IR spectra showed the appearance of characteristic absorption bands at 3410, 1678-1634, 1613-1610 cm^{-1} assigned to NH, CO and CN, respectively.

^{1}H-NMR spectra lacked the signals which appeared at (9.60-10.10) corresponding to reported signals of NHCS[14] and showed a new singlet signal at δ 6.90-6.25 integrated for one proton attributed to the C5-H of the thiazoline ring, in addition to increase number of aromatic protons. Mass spectra (ESI-MS) of compounds **6a-d** and **6l** showed M[+]+1 or M[+]+Na peaks, which were in agreement with their molecular formula.

Antimicrobial activity

Compounds **6a-l** were tested for their *in-vitro* antimicrobial activity against *Escherichia coli (E. coli)* ATCC 25922, *Staphylococcus aureus (S. aureus)* ATCC 19433, and *Candidia albicans (C. albicans)* by the method shown in the experimental part.[19] Ampicillin trihydrate and clotrimazole were used as standard antibacterial and antifungal agents, respectively. Some of the tested compounds showed significant antibacterial and antifungal activities when compared with reference drugs.

The MIC values are generally within the range of 12.5-200 μg/mL against evaluated strains. In comparing their MIC values with ampicillin, it was found that compound **6i** showed a similar level of activity against *Staphylococcus aureus* while the compounds **6b, 6h** possessed one fourth the activity of ampicillin. Compounds **6j** and **6l** exhibited similar activity against *Escherichia coli,* also the rest of compounds revealed activity ranging from one fourth to one eighth activity of ampicillin. Substitution at positions 3 and 4 of thiazoline nucleus with halogenated phenyl rings may increase the antibacterial activity. All compounds were less active against *Candida albicans* when compared with clotrimazole as shown in Table 2.

Anti-inflammatory activity

The preliminary anti-inflammatory activity of the synthesized compounds was evaluated against carrageenan-induced rat paw edema using the method by Hernandez-Perez *et al.*[20] The compounds were tested at 2.5×10^{-5} mol/kg oral dose and compared with celecoxib as reference drug. The results are listed in Table 3. The histograms, Figures (1&2) showed the per cent inhibition of edema induced by reference drug and tested compounds.

Table 2: Minimal inhibitory concentration (MIC µg/ml) of tested compounds.

Compd. No.	*E. coli*	*S. aureus*	*C. albicans*
6a	100	100	>200
6b	200	50	>200
6c	100	100	>200
6d	100	100	>200
6e	100	100	>200
6f	200	100	>200
6g	100	>200	>200
6h	100	50	>200
6i	100	12.5	200
6j	25	>200	>200
6k	100	>200	>200
6l	25	>200	>200
Ampicillin	25	12.5	---
Clotrimazole	--	--	12.5

Table 3: Anti-inflammatory activity of target compounds **6a-l** at 2.5×10^{-5} mol/kg dose level.

Compound	Anti-inflammmatory activity % inhibition ± S.E[(a,b)]		
	1 hr	2 hrs	3 hrs
Control	0.00	0.00	0.00
Celecoxib	54.2 ± 1.56**	68.6 ±1.43**	76.4 ± 1.18**
6a	38.0 ± 2.21**	41.2 ± 1.8**	46.3 ± 2.51**
6b	43.0 ± 2.63**	47.2 ± 2.08**	52.2 ± 1.44**
6c	46.3 ± 2.45**	50.3 ± 3.18*	55.2 ± 3.12**
6d	65.4 ± 2.12**	72.0 ± 1.56**	79.1 ± 1.80**
6e	38.5 ± 3.50**	46.4 ± 1.80**	48.0 ± 2.45*
6f	41.5 ± 2.25*	48.1 ± 6.3**	49.9 ± 2.23**
6g	23.6 ± 5.56*	29.5 ± 6.11**	36.9±5.24**
6h	25.3 ± 4.96*	31.1 ± 1.45**	38.5 ± 2.90*
6i	40.4 ± 3.52**	44.1 ± 3.56**	48.0 ± 3.18**
6j	41.3 ± 1.88**	47.2 ± 2.08**	51.6 ± 3.04*
6k	42.6 ± 1.35**	48.0 ± 2.35**	51.7 ± 1.15**
6l	55.2 ± 1.72**	68.6 ± 1.83**	77.5 ± 1.56**

[a] S.E: standard error
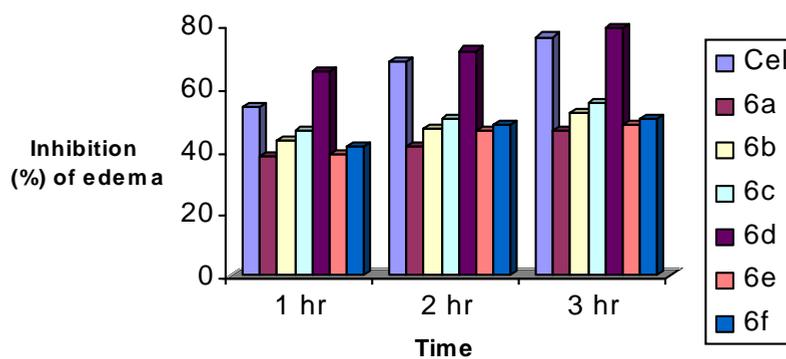[b]* Significant difference at P< 0.05; **Significant difference at P< 0. 01.

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Fig. 1: Inhibition (%) of carrageenan- induced rat paw edema by Celecoxib (Cel), and tested compounds **6a-6f** in treated groups of rats at 2.5×10^{-5} mol/kg
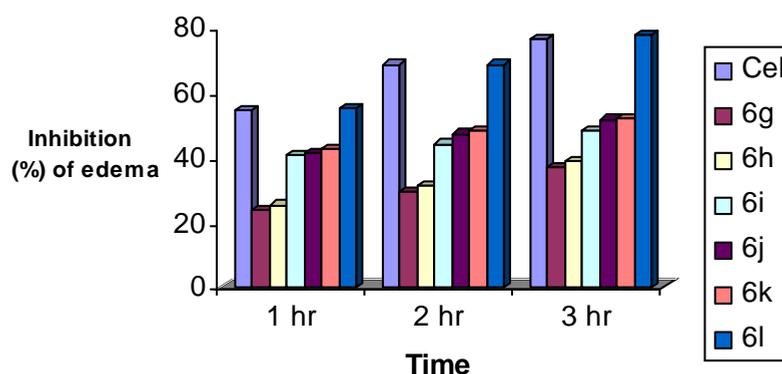

Fig. 2: Inhibition (%) of carrageenan- induced rat paw edema by Celecoxib (Cel), and tested compounds **6g-6l** in treated groups of rats at 2.5×10^{-5} mol/kg.

Results showed that most of the tested compounds exhibited significant ($P < 0.05$) inhibition against carrageenan-induced rat paw edema with inhibition per cent (36.9-97.1%) relative to celecoxib. Among these derivatives, compounds **6d** and **6l** showed maximum inhibition of inflammation (79.1%, 77.5%) and were found to be more potent than celecoxib (76.4%). While derivatives **6g** and **6h** were the least active compounds. From the biological activity studies we concluded that (i) substitution at position 4 of thiazoline ring with *p*-chlorophenyl increases

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activity more than unsubstituted phenyl, (ii) the order of decreasing activity is as follows; ethyl > cyclohexyl > CH_3 > *p*-Br-phenyl > phenyl > *p*- CH_3-phenyl.

In the previous study, the order of decreasing in anti-inflammatory activity of thiosemicarbazides **5a-f** is as follows *p*-Br-phenyl > *p*- CH_3-phenyl > phenyl > cyclohexyl > ethyl > CH_3[14].

Gastric ulceration[22]

The compounds **6d** and **6l** were screened for their ulcerogenic activity at dose level 10, 50, 100 mg/kg. Results indicated that compounds **6d** and **6l** did not induce any ulcerogenic effect at 10 mg/kg dose. At higher doses, the tested compounds exhibited low gastric ulcerogenicity compared with indomethacin, which caused severe ulceration at all dose levels, Table 4.

Acute toxicity (LD_{50})

The acute toxicity of the most active compounds; **6d** and **6l** was determined by calculating their LD_{50} values using graphical method[23]. The LD_{50} of compounds **6d** and **6l** was found to be 100 and 90 mg/kg (i.p.), respectively, while LD_{50} of indomethacin was found to be 50 mg/kg (i.p.). In conclusion compounds **6d** and **6l** showed significant anti-inflammatory activity without ulcerogenic toxicity.

Molecular modeling study

Molecular dockings of compounds **5 a-f**, **6a-l** were performed in order to rationalize the obtained biological results, Besides, molecular docking studies verfied understanding the various interactions between the ligands and enzyme active site in detail. Docking studies of the inhibitors were performed by MOE

Table 4: Ulcerogenic effects of compounds **6d** and **6l** in comparison to indomethacin.

Compound	Dose mg/kg	Ratio of ulcerated animals	Ulcer index (mean ± S.E.)
Indomethacin	10	3/6	(1.35** ± 0.18)
	50	5/6	(1.88** ± 0.15)
	100	6/6	(2.10** ± 0.17)
6d	10	0/6	0.00
	50	0/6	0.00
	100	1/6	(0.50 ± 0.1)
6l	10	0/6	0.00
	50	1/6	(0.50 ± 0.1)
	100	1/6	(0.75* ± 0.12)

*Significant difference at P< 0.05; **Significant difference at P< 0.01.

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(Molecular Operating Environment) using murine COX-2 co-crystallized celecoxib (PDBID:6COX) as a template. We performed 100 docking interactions for each ligand and the top-scoring configuration of each of the ligand-enzyme complexes was selected on energetic grounds.

Docking of compound **6d**, (Figs. 3&4), showed that the ligand was oriented so that the moiety CONHN= was in the vicinity of His 90 residue forming an ionic interaction with both carbonyl group (distance = 2.68 A°) and -N= (distance = 2.71 A°), a hydrogen bond interaction between the NH and Leu 352 (distance = 2.14 A°).

The pyridine, phenyl and triazole rings were located in a hydrophobic pocket surrounded by Phe 518, Ala 527, Val 523, Gly 526, Gly 354 and Met 522. Also thiazoline, *p*-Cl-phenyl rings were surrounded by His 90, Ser 353, Gln192, Arg 513, His 351 and Asp 515. This arrangement is also common to most derivatives **6a-l** with small differences in ionic interaction or hydrophobic pocket. On the other hand, docking of the corresponding thiosemicarbazide compound **5b** showed that the ligand was oriented so that the moiety CONHNH was in the vicinity of His 90 residue forming an ionic interaction with the carbonyl
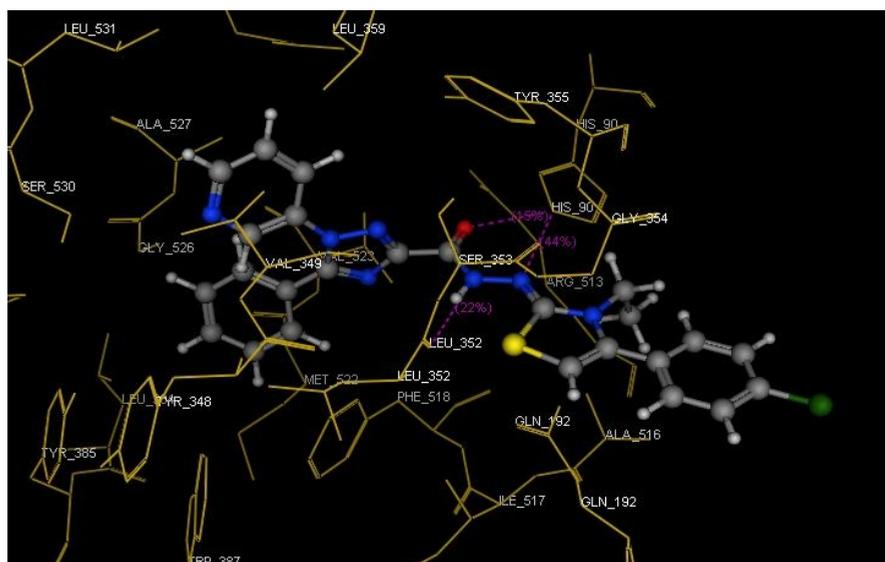

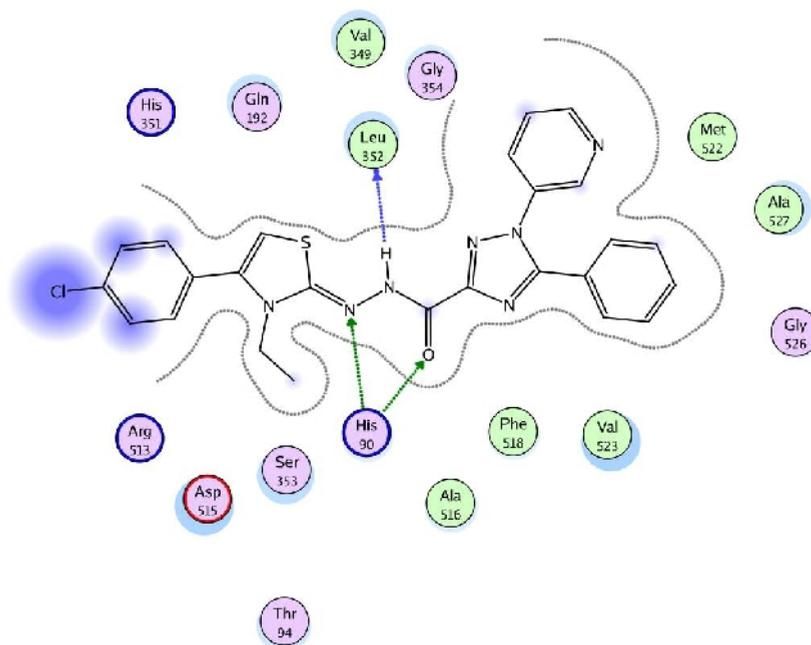**Fig. 3:** 3D docked structure of **6d** (ball and stick) at COX-2 active site.

Fig. 4: Simplified structure of **6d** docked at COX-2 active site.

group (distance = 1.77 A°), a hydrogen bond interaction between the NH and Leu 352 (distance = 1.73 A°). This means similar contacts as **6d** with one missing feature, which in accordance with the anti-inflammatory activity. Docking of compound **6l** showed a hydrogen bond interaction between His 90 and both of the carbonyl (distance = 2..69 A°), and –N= group (distance = 2.70 A°). A hydrogen bond interaction between the NH and Leu 352 (distance = 2.34 A°). Docking of the corresponding thiosemicarbazide **5c** showed ionic interaction between

carbonyl and His 90 (distance = 2.45 A°) and NHCS and Ser 353 (distance = 1.88 A°). Number of hydrogen bond is less than that of the corresponding cyclized form which in accordance with the anti-inflammatory activity.

On the contrary docking study of thiosemicarbazide compound **5f** showed an increased by one feature than that of the corresponding cyclized compound **6j**. The results indicated that compound **5f** showed an ionic interaction between His 90 and both carbonyl and NH (distance = 2.14, 2.24 A°) and NHCS with Ser

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353 (distance = 2.18 A°), while docking of **6j** showed an ionic interaction between His 90 and both carbonyl and NH (distance = 2.78, 2.40 A°), which in accordance with anti-inflammatory activity. This means that there is relation between ionic interaction with the receptor and anti-inflammatory activity.

From the above mentioned data, the molecular modeling studies of the examined compounds showed that they bound to the COX-2 active site with position and orientation very close to that resulting from the crystal structure of celecoxib complex with COX-2 (a hydrogen bond with His 90 (2.91A°), Leu 352 (2.28 A°), Ser 352 (1.58A°).

Conclusion

We reported here the synthesis of 1,2,4-triazole carbohydrazide derivatives. The synthesized compounds were tested for their antimicrobial and anti-inflammatory activity.Compound **6i** was the most active one against *Staphylococcus aureus*, while compounds **6j** and **6l** showed moderate activity against *Escherichia coli.* Most of the tested compounds exhibited significant anti-inflammatory activity in the carrageenan-induced rat paw edema test, with low gastric ulcerogenicity compared with indomethacin. The molecular modeling studies suggested that compounds **6d, 6l** exhibited higher affinity for COX-2 active site and hence increased anti-inflammatory activity than the other derivatives.

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