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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME BENZIMIDAZO-1,2,4-TRIAZOLE DERIVATIVES AS ANTIMICROBIAL AND ANTI-INFLAMMATORY AGENTS

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تم فى هذا البحث تشييد ثلاث مجموعات جديدة وهى: ن' (اريل ميثيليدين) ٢ (١يد ٤،٢،١ تريازولو[٣،٢ أ]بنزإميدازول ٢ يل سلفانيل)أسيتوهيدرازيد (أحد عشر مركبا" **4a-k**) ، ن' (ألفا أريل إيثيليدين) ٢ (١يد ٤،٢،١ تريازولو[٣،٢ أ]بنزإميدازول ٢ يل سلفانيل)أسيتوهيدرازيد (أربعة مركبات **5a-d**) و٢ ({٥ (ألكيل سلفانيل) ٤،٣،١ أوكساديازول ٢ يل]ميثيل}سلفانيل) ١يد ٤،٢،١ تريازولو[٣،٢ أ]بنزإميدازول (خمسة مركبات **7a-e**) وذلك من خلال تفاعل المركب (**1**) ١يد ٤،٢،١ تريازولو[٣،٢ أ]بنزإميدازول ٢ ثيون مع خلات بروموميثيل لتكوين المركب (**2**) خلات ميثيل (١يد ٤،٢،١ تريازولو[٣،٢ أ]بنزإميدازول ٢ يل سلفانيل) الذى تمت إزابته وغليانه مع هيدرات الهيدرازين لتكوين المركب (**3**) ٢ (١ يد ٤،٢،١ تريازولو[٣،٢ أ]بنزإميدازول ٢ يل سلفانيل)أسيتوهيدرازيد. وعند تكثيف المركب (**3**) مع الالدهيدات الأروماتية ومشتقات الأسيتوفينونات اعطى المركبات (**4a-k**) و(**5a-d**) على الترتيب. ولكن عند معالجة المركب (**3**) مع ثنائى كبريت الكربون فى وجود هيدروكسيد البوتاسيوم نتج عن ذلك المركب (**6**) ٥ [(١ يد ٤،٢،١ تريازولو[٣،٢ أ]بنزإميدازول ٢ يل سلفانيل)ميثيل] ٤،٣،١ أوكساديازول ٢(٣ يد) ثيون وهذا المركب عند معاملته مع هاليدات الألكيل المختلفة أعطى السلسلة (**7a-e**). ولقد إختبرت درجة نقاوة جميع المركبات الجديدة بواسطة كروماتوجرافيا الطبقة الرقيقة كما تم التحقق من التراكيب البنائية لها اعتمادا على نتائج طرق التحاليل الطيفية المختلفة (الأشعة تحت الحمراء، الرنين النووى المغناطيسى ومطياف الكتلة) بالإضافة الى التحليل الكمى الدقيق لعناصر تلك المركبات. وقد تم اختبار الفاعلية البيولوجية لجميع المركبات المستهدفة كمضادات للبكتريا والفطريات والإلتهابات بالمقارنة بعقار الأمبيسللين والفلوكونازول والإندوميثاسين على التوالى. هذا الى جانب دراسة الإرساء الجزيئى لأحد المركبات (**5c**).

*Three new series of N`-(aryl or heteroarylmethylidene)-2-(1H-1,2,4-triazolo[2,3-a]benzimidazol-2-ylsulfanyl) acetohydrazides **(4a-k)**, N`-(α-arylethylidene)-2-(1H-1,2,4-triazolo[2,3-a]benzimidazol-2-ylsulfanyl) acetohydrazides **(5a-d)**, and 2-({[5-(alkyl or aralkylsulfanyl)-1,3,4-oxadiazol-2-yl]methyl}sulfanyl)-1H-1,2,4-triazolo[2,3-a]benzimidazoles **(7a-e)** were synthesized. Reaction of compound **(1)** with methyl bromoacetate afforded **(2)**, which when refluxed with hydrazine hydrate yielded **(3)**. The latter was condensed with aromatic aldehydes and substituted acetophenones to afford compounds **(4a-k)** and **(5a-d)** respectively. Treatment of compound **(3)** with carbon disulfide in the presence of potassium hydroxide resulted in the formation of **(6)**. The latter was alkylated with the appropriate alkyl or aralkyl halides to afford compounds **(7a-e)**. The purity of all new compounds was checked by TLC and elucidation of their structures was confirmed by IR, ^{1}HNMR, and mass spectrometry along with elemental microanalyses. All the target compounds were evaluated for their in-vitro antimicrobial and in-vivo anti-inflammatory activities in comparison with ampicillin, fluconazole, and indomethacin as reference drugs respectively. In addition to molecular docking of compound **5c** was performed.*

INTRODUCTION

Rheumatic diseases are the most prevalent causes of disability, and commercially available non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for reducing pain and swelling associated with inflammation[1]. However, their therapeutic uses are often limited by common side effects, such as gastrointestinal haemorrhage[2], gastric ulceration and renal injury[3]. In addition, it is known that bacterial infections often produce

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pain and inflammation[4]. On the other hand, benzimidazole derivatives were reported to possess a wide spectrum of biological effects such as antibacterial[5], antifungal[6], analgesic and anti-inflammatory[7&8]. Moreover, 1,2,4-triazoles and their condensed derivatives constitute an important class of organic compounds with analgesic-anti-inflammatory[9&10] and antimicrobial activities[11&12]. Literature survey reveals that 1,2,4-triazolobenzimidazole derivatives showed pronounced antifungal[13], antibacterial[14&15] and analgesic-anti-inflammatory activities[16]. In addition, hydrazone derivatives also exhibit potent antimicrobial and analgesic-anti-inflammatory activities[17-20]. On the other hand, recent studies reported substituted 1,3,4-oxadiazoles as antimicrobial[21&22], analgesic and anti-inflammatory compounds[23&24]. In view of the aforementioned data and in continue to a previous work about the chemical and biological properties of 1,2,4-triazolo[2,3-a]benzimidazole[14&16], the present work aims at the synthesis of 1,2,4-triazolo[2,3-a]benz-imidazoles incorporating acetohydrazones and 1,3,4-oxadiazole structures along with evaluation of their *in-vitro* antimicrobial and *in-vivo* anti-inflammatory activities. In addition, substantiation of the molecular docking of certain compounds was carried out.

MATERIALS AND METHODS

Melting points were determined on an electrothermal melting point apparatus [Stuart Scientific, model SMP3, England, UK], and were uncorrected. Pre-coated silica gel plates (kieselgel 0.25 mm, 60G F254, Merck, Germany) were used for TLC monitoring of reactions. The developing solvent systems of $CHCl_3/CH_3OH$ (9.5:0.5 and 8:2 *V/V*) were used and the spots were detected at 254 nm wavelength using ultraviolet lamp (Spectroline, model CM-10, USA). The target compounds were crystallized from ethanol unless otherwise specified.

IR spectra (KBr discs) were recorded on a shimadzu IR-470 spectrometer (Shimadzu, Kyoto, Japan) at Faculty of Pharmacy, Assiut University, Assiut. ^{1}H-NMR Spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz, Varian, CA, USA) at Faculty of Pharmacy, Assiut University, Assiut. Chemical shifts are expressed in δ-value (ppm) relative to TMS as an internal standard, using DMSO-d_6, unless otherwise specified, as a solvent, and deuterium oxide was used for the detection of exchangeable protons.

Mass spectra were recorded with JEOL JMS600 mass spectrometer (JEOL, Tokyo, Japan). Elemental microanalyses were performed on a Perkin-Elmer 240 elemental analyzer (Perkin-Elmer, USA) at the unit of Microanalysis, Faculty of Science, Cairo University.

Most of the required chemicals were of commercial grade: *o*-phenylenediamine (Aldrich, Germany), bromine (Rankem, India), sodium cyanide, potassium carbonate and potassium hydroxide (El Nasr Pharm. Company, Egypt), hydroxylamine sulphate (Aldrich, Germany), alkyl halides (Fluka, Switzerland) carrageenan (Sigma, USA), carbon disulphide (Riedel-de Haën, Germany), indomethacin (Liometacin® vial, Nile Company, Egypt), ampicillin (Epicocillin® vial, EIPICO, Cairo, Egypt), fluconazole (Treflucan®, EIPICO, Cairo, Egypt), sodium carboxymethylcellulose (NaCMC) (El Nasr Pharm. Company, Egypt) and normal saline (Almottahedoon Pharma Company, Egypt) were obtained from the local market.

The starting materials 1,2-diaminobenzimidazole and 1,2,4-triazolo[2,3-a]benzimidazole-2-thione **(1)**; were prepared according to reported procedures[25,14&16].

Chemistry

Synthesis of methyl (1*H*-1,2,4-triazolo[2,3-a] benzimidazol-2-ylsulfanyl) acetate (2)

To a suspension of 1*H*-1,2,4-triazolo[2,3-a]benzimidazole-2-thione **(1)** (9.5 g, 0.05 mole) and potassium carbonate (6.9 g, 0.05 mole) in dry acetone (100 mL), methyl bromo acetate (4.61 mL, 0.05 mole) was added. The reaction mixture was stirred for 8 hrs at ambient temperature. Acetone was evaporated and the residue was treated with water, filtered, dried and crystallized from ethanol to afford the product as colorless crystals.

Yield: 13.0 g (94%), m.p. 206-208°C, R_f: 0.9 (Chloroform : Methanol 9.5:0.5).

IR [ν cm^{-1}]: 3440 (NH); 1729 (C=O); 1589, 1472, 1453 (C=N/C=C).

^{1}HNMR [60 MHz, δ ppm DMSO-d_6]: 3.69 (3H; s; C<u>H</u>$_3$), 4.00 (2H; s; C<u>H</u>$_2$), 7.09-7.77 (4H; m; C_6<u>H</u>$_4$), and 11.87 (1H; br s; N<u>H</u>, exchangeable with D_2O).
Microanalysis for $C_{11}H_{10}N_4O_2S$ (262.05): Calculated/Found: 50.37/50.12 (%C), 3.84/3.61 (%H), 21.36/21.00 (%N).

Synthesis of 2-(1*H*-1,2,4-triazolo[2,3-a]benzimidazol-2-ylsulfanyl) acetohydrazide (3)

To a stirred solution of methyl (1*H*-1,2,4-triazolo[2,3-a]benzimidazol-2-ylsulfanyl) acetate **(2)** (5.25 g, 0.02 mole) in absolute ethanol (50 mL), hydrazine hydrate 99% (0.97 mL, 0.02 mole) was added. The reaction mixture was refluxed for 4 hrs, and then cooled. The precipitated product was filtered, washed with cold ethanol, dried, and crystallized from ethanol as white crystals.
Yield: 4.5 g (85.7%), m.p. 235-237°C, R_f: 0.7 (Chloroform : Methanol 8:2).
IR [ν cm^{-1}]: 3430, 3250, 3120 (NH); 1642 (C=O), 1613, 1593, 1529, 1474, 1453 (C=N/C=C).

^{1}HNMR [60 MHz, δ ppm DMSO-d_6]: 3.87 (2H; s; C<u>H</u>$_2$), 4.29 (2H; s; N<u>H</u>$_2$), 7.20-8.03 (4H; m; C_6<u>H</u>$_4$), 9.26 (1H; brs; exchangeable CON<u>H</u>,), and 12.20 (1H; brs; exchangeable N<u>H</u> of triazole ring).
Microanalysis for $C_{10}H_{10}N_6OS$ (262.29): Calculated/Found: 45.79/45.49 (%C), 3.84/3.71 (%H), 32.04/32.31 (%N), 12.22/12.54 (%S).

Synthesis of *N*`-(aryl or heteroarylmethylidene)-2-(1*H*-1,2,4-triazolo[2,3-a]benzimidazol-2-ylsulfanyl) acetohydrazides (4a-k)

A suspension of 2-(1*H*-1,2,4-triazolo[2,3-a]benzimidazol-2-ylsulfanyl) acetohydrazide **(3)** (0.52 g, 0.002 mole) in ethanol (10 mL) and the appropriate aryl aldehyde (0.002 mole) was heated under reflux for 4-8 hrs. The reaction mixture was cooled and the precipitated product was filtered, washed with cold ether and crystallized from ethanol. Yields, m.p., elemental analyses, IR, ^{1}HNMR and mass spectral data are listed in tables I and II.

Table I: Physicochemical properties of compounds **(4a-k)**.

Comp No.	Ar	Yield %	M.p.°C	R_f	M.F. (M.Wt.)	Microanalyses Calculated/Found			
						C%	H%	N%	S%
4a*	C_6H_5	94	252-254	0.85	$C_{17}H_{14}N_6OS$ (350.40)	58.27 58.19	4.03 3.98	23.98 24.02	9.15 9.04
4b	*p*.BrC_6H_4	96	258-261	0.78	$C_{17}H_{13}BrN_6OS$ (429.29)	47.56 47.31	3.05 2.88	19.58 19.51	7.47 7.59
4c	*p*.ClC_6H_4	88	238-240	0.77	$C_{17}H_{13}ClN_6OS$ (384.84)	53.06 52.98	3.40 3.14	21.84 21.49	8.33 8.39
4d	*m*.ClC_6H_4	91	232-234	0.83	$C_{17}H_{13}ClN_6OS$ (384.84)	53.06 52.89	3.40 3.26	21.84 21.54	…..
4e	*p*.$CH_3C_6H_4$	93	260-262	0.72	$C_{18}H_{16}N_6OS$ (364.42)	59.32 59.00	4.43 4.16	23.06 22.89	8.80 9.04
4f	*p*.CH_3O C_6H_4	86	236-238	0.69	$C_{18}H_{16}N_6O_2S$ (380.42)	56.83 56.61	4.24 4.10	22.09 21.96	8.43 8.40
4g	*p*.isopropylC_6H_4	81	246-247	0.77	$C_{20}H_{20}N_6OS$ (392.48)	61.20 60.87	5.14 5.33	…….	8.17 8.04
4h	*p*.$NO_2C_6H_4$	95	269-271	0.74	$C_{17}H_{13}N_7O_3S$ (395.40)	51.64 51.40	3.31 3.11	24.80 24.63	8.11 8.20
4i	*p*.HOC_6H_4	85	277-279	0.90	$C_{17}H_{14}N_6O_2S$ (366.40)	55.73 55.67	3.85 3.57	22.94 22.85	…..
4j	2-thienyl	83	234-235	0.85	$C_{15}H_{12}N_6OS_2$ (356.43)	50.55 50.30	3.39 3.49	23.58 23.39	17.99 18.03
4k	*m*-OCH_2O-*p*-C_6H_3	87	267-269	0.84	$C_{18}H_{14}N_6O_3S$ (394.41)	54.81 54.50	3.58 3.60	21.31 21.51	…..

* Compound **4a** was purified by column chromatography (Silica gel 60, Fluka, Switzerland), using Chloroform : methanol (9.5:0.5) as a mobile phase.

Table II: ^{1}HNMR, IR and Mass* Spectral data of compounds **(4a-k)**.

Comp No.	Ar	^{1}HNMR [δ ppm DMSO-d_6]**	IR [ν cm^{-1}]		
			NH	C=O	C=N & C=C
4a	C_6H_5	4.00, 4.50 (2H; two s; C<u>H</u>$_2$), 7.03-7.80 (9H; m; C_6<u>H</u>$_4$ & C_6<u>H</u>$_5$), 7.93, 8.16 (1H; two s; C<u>H</u>), 11.10, 11.30 (1H; two br s; N<u>H</u>N), and 11.46 (1H; br s; N<u>H</u> of triazole ring).	3490, 3180	1666	1614, 1585, 1474, 1453
4b	*p*.BrC_6H_4	3.90, 4.36 (2H; two s; C<u>H</u>$_2$), 6.86-7.70 (8H; m; C_6<u>H</u>$_4$ & C_6<u>H</u>$_4$), 7.80, 8.06 (1H; two s; C<u>H</u>), 11.53, 11.76 (1H; two br s; N<u>H</u>N), and 12.01 (1H; br s; N<u>H</u> of triazole ring).	3445, 3160	1660	1624, 1585, 1503, 1472, 1453
4c	*p*.ClC_6H_4	4.03, 4.40 (2H; two s; C<u>H</u>$_2$), 7.06-7.83 (8H; m; C_6<u>H</u>$_4$ & C_6<u>H</u>$_4$), 7.90, 8.13 (1H; two s; C<u>H</u>), 11.60, 11.76 (1H; two br s; N<u>H</u>N), and 12.33 (1H; br s; N<u>H</u> of triazole ring).	3450, 3180	1658	1618, 1587, 1472, 1456
4d	*m*.ClC_6H_4	4.00, 4.46 (2H; two s; C<u>H</u>$_2$), 6.93-7.66 (8H; m; C_6<u>H</u>$_4$ & C_6<u>H</u>$_4$), 7.86, 8.10 (1H; two s; C<u>H</u>), 11.33, 11.53 (1H; two br s; N<u>H</u>N), and 12.00 (1H; br s; N<u>H</u> of triazole ring).	3390, 3160	1661	1615, 1589, 1557, 1474
4e	*p*.$CH_3C_6H_4$	2.33 (3H; s; C<u>H</u>$_3$), 3.96, 4.46 (2H; two s; C<u>H</u>$_2$), 7.10-7.73 (8H; m; C_6<u>H</u>$_4$ & C_6<u>H</u>$_4$), 7.86, 8.16 (1H; two s; C<u>H</u>), 11.50, 11.73 (1H; two br s; N<u>H</u>N), and 12.10 (1H; br s; N<u>H</u> of triazole ring).	3470, 3180	1667	1613, 1586, 1482, 1454
4f	*p*.CH_3O C_6H_4	3.76 (3H; s; OC<u>H</u>$_3$), 4.00, 4.43 (2H; two s; C<u>H</u>$_2$), 6.63-7.76 (8H; m; C_6<u>H</u>$_4$ & C_6<u>H</u>$_4$), 7.83, 8.06 (1H; two s; C<u>H</u>), 11.36, 11.50 (1H; two br s; N<u>H</u>N), and 12.20 (1H; br s; N<u>H</u> of triazole ring).	3425, 3200	1664	1622, 1593, 1500, 1486, 1452
4g	*p*.isopropylC_6H_4	1.23 (6H; d; CH(C<u>H</u>$_3$)$_2$), 1.36-1.56 (1H; m; C<u>H</u>(CH$_3$)$_2$), 4.00, 4.43 (2H; two s; C<u>H</u>$_2$), 6.93-7.83 (8H; m; C_6<u>H</u>$_4$ & C_6<u>H</u>$_4$), 7.90, 8.10 (1H; two s; C<u>H</u>), 11.50, 11.63 (1H; two br s; N<u>H</u>N), and 12.13 (1H; br s; N<u>H</u> of triazole ring).	3465, 3200	1670	1625, 1549, 1489, 1465
4h***	*p*.$NO_2C_6H_4$	4.10, 4.43 (2H; two s; C<u>H</u>$_2$), 7.10-7.40 (9H; m; C_6<u>H</u>$_4$ & C_6<u>H</u>$_4$ and C<u>H</u>), 12.07, 12.23 (1H; two br s; N<u>H</u>N), and 12.56 (1H; br s; N<u>H</u> of triazole ring).	3325, 3185	1668	1622, 1584 1503, 1486, 1451
4i	*p*.HOC_6H_4	3.93, 4.36 (2H; two s; C<u>H</u>$_2$), 6.53-7.70 (8H; m; C_6<u>H</u>$_4$ & C_6<u>H</u>$_4$), 7.76, 8.00 (1H; two s; C<u>H</u>), 9.60 (1H; br s; O<u>H</u>), 11.37, 11.60 (1H; two br s; N<u>H</u>N), and 12.10 (1H; br s; N<u>H</u> of triazole ring).	3610-3160 (& OH)	1652	1622, 1589 1502, 1458
4j	2-thienyl	3.93, 4.40 (2H; two s; C<u>H</u>$_2$), 6.90-7.93 (7H; m; C_6<u>H</u>$_4$ & C_4<u>H</u>$_3$S), 8.09, 8.43 (1H; two s; C<u>H</u>), 11.36, 11.56 (1H; two br s; N<u>H</u>N), and 11.76 (1H; br s; N<u>H</u> of triazole ring).	3250, 3160	1659	1621, 1576 1532, 1454
4k	*m*-OCH_2O-*p*-C_6H_3	3.93, 4.33 (2H; two s; C<u>H</u>$_2$), 5.93 (2H; s; OC<u>H</u>$_2$O), 6.56-7.66 (7H; m; C_6<u>H</u>$_4$ & C_6<u>H</u>$_3$), 7.70, 8.03 (1H; two s; C<u>H</u>), 11.40, 11.56 (1H; two br s; N<u>H</u>N), and 12.16 (1H; br s; N<u>H</u> of triazole ring).	3465, 3290	1650	1623, 1585, 1482, 1455

* EIMS [*mlz* (%)]: **4a**: [350 (M^+, 1)], **4b**: [430 (M+2, 4.04), 428 (M^+, 3.9)], **4c**: [387 (*M+2*, 1.3)], **4e**: [364 (M^+, 6.7)], **4f**: [380 (M^+, 5.1)], **4g**: [392 (M^+, 5.3)], **4h**: [395 (M^+, 3.3)], 4j: [356 (M^+, 2.2)].

** protons of NH and OH groups are exchangeable by D_2O.

*** IR spectrum of compound **4h** (ν cm^{-1} KBr) showed bands at 1326 and 1537 cm^{-1} due to NO_2 group.

Synthesis of *N*`-(α-arylethylidene)-2-(1*H*-1,2,4-triazolo[2,3-a]benzimidazol-2-ylsulfanyl) acetohydrazides (5a-d)

To a suspension of 2-(1*H*-1,2,4-triazolo[2,3-a]benzimidazol-2-ylsulfanyl) acetohydrazide **(3)** (0.52 g, 0.002 mole) in ethanol (10 mL) and the appropriate acetophenone derivative (0.002 mole), 2 drops of glacial acetic acid were added, then the reaction mixture was heated under reflux for 6-8 hrs, then cooled. The precipitated product was filtered, dried and recrystallized from ethanol. Yields, m.p., elemental analyses, IR, ^{1}HNMR and mass spectral data are listed in tables III and IV.

Synthesis of 5-[(1*H*-1,2,4-triazolo[2,3-a]benzimidazol-2-ylsulfanyl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (6)

2-(1*H*-1,2,4-Triazolo[2,3-a]benzimidazol-2-ylsulfanyl) acetohydrazide **(3)** (2.62 g, 0.01 mole) was dissolved in a solution of potassium hydroxide (0.56 g, 0.01 mole) in 20 mL aqueous ethanol (70%), carbon disulfide (0.60 mL, 0.01 mole) was added. The reaction mixture was refluxed for 7 hrs then the reaction mixture was cooled and acidified with hydrochloric acid (35%). The precipitate was filtered, washed with water, dried and recrystallized from ethanol.

Yield: 2.60 g (84.5%), m.p. 231-232°C, R_f: 0.76 (Chloroform : Methanol 8:2).

IR [ν cm^{-1}]: 3445, 3300 (NH); 1608, 1574, 1535, 1475 (C=N/C=C), 1250 (C=S).

^{1}HNMR [60 MHz, δ ppm DMSO-d_6]: 4.30 (2H; s; C$\underline{H}_2$), 6.86-7.73 (4H; m; $C_6\underline{H}_4$), and 12.31 (1H; br s; N$\underline{H}$ of triazole), 14.41 (1H, br s, N$\underline{H}$ of oxadiazole). NH protons of triazole and NH of oxadiazole rings were exchanged with D_2O.

EIMS [*mlz* (%)]: 304 (M^+, 14.6), 272 (1.6), 271 (4.5), 204 (4.9), 190 (100), 158 (25.9).

Microanalysis for $C_{11}H_8N_6OS_2$ (304.02): Calculated/Found: 43.41/43.18 (%C), 2.65/2.53 (%H), 27.61/27.30 (%N).

Synthesis of 2-({[5-(alkyl or aralkylsulfanyl)-1,3,4-oxadiazol-2-yl]methyl}sulfanyl)-1*H*-1,2,4-triazolo[2,3-a]benzimidazoles (7a-e)

To a suspension of 5-[(1*H*-1,2,4-triazolo [2,3-a]benzimidazol-2-ylsulfanyl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione **(6)** (0.61 g, 0.002 mole) and potassium carbonate (0.28 g, 0.002 mole) in dry acetone (10 mL), the appropriate alkyl or aralkyl halide (0.002 mole) was dropped portionwise. The reaction mixture was stirred for 6-8 hrs at ambient temperature. Acetone was evaporated; the residue was treated with water and then extracted with chloroform (3×15 mL). The chloroform extract was washed with water and dried over anhydrous magnesium sulfate. Chloroform layer was filtered, evaporated and the residue was crystallized from ethanol. Yields, m.p., elemental analyses, IR, ^{1}HNMR and mass spectral data are listed in tables V and VI.

Biological screening

1- Antimicrobial screening

a) Antibacterial activity

Organisms and culture conditions

The used bacterial cultures were obtained from Assiut University Mycological Center (AUMC), Assiut University. The synthesized compounds (**1**, **2**, **3**, **4a-k**, **5a-d**, **6** and **7a-e**) were tested for their *in-vitro* antibacterial activity, in comparison to ampicillin as a reference drug using the standard agar cup diffusion method[26] against six bacterial species representing both Gram positive and Gram negative strains: *Serratia marcescens* (AUMC B55), *Pseudomonas aeruginosa* (AUMC B73), *Escherichia coli* (AUMC B53), *Staphylococcus aureus* (AUMC B54), *Bacillus cereus* (AUMC B52) and *Micrococcus luteus* (AUMC B112).

Materials and method

Bacterial strains were individually cultured for 48 hrs in 100 mL conical flasks containing 30 mL Nutrient Agar (NA). Assay was done in 10 cm sterile Petri dishes in which one mL bacterial suspension and 15 mL of NA were poured. Plates were shaken gently to homogenize the inocula. After solidification of the media, 5 mm cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer. The test compounds (**1**, **2**, **3**, **4a-k**, **5a-d**, **6** and **7a-e**) and ampicillin were dissolved in dimethyl sulfoxide (100 μmol/mL) and were pipeted in the cavities. In addition, other cavities were pipeted with the solvent (DMSO) and served as a negative control. The seeded plates were incubated at 28 ± 2°C for 48 hrs. The radii of inhibition zones (in mm) of triplicate sets were measured and the results are cited in table VII.

Table III: Physicochemical properties of compounds **(5a-d)**.

Comp No.	R	Yield %	M.p.°C	R_f	M.F. (M.Wt.)	Microanalyses Calculated/Found			
						C%	H%	N%	S%
5a	H	80	274-275	0.86	$C_{18}H_{16}N_6OS$ (364.11)	59.32 58.95	4.43 4.65	23.06 22.80	…..
5b	Br	75	270-272	0.89	$C_{18}H_{15}BrN_6OS$ (442.02)	48.77 48.70	3.41 3.15	18.96 19.06	7.23 7.23
5c	Cl	82	271-272	0.67	$C_{18}H_{15}ClN_6OS$ (398.07)	54.20 54.09	3.79 3.62	21.07 20.77	8.04 7.87
5d	OCH_3	85	268-270	0.80	$C_{19}H_{18}N_6O_2S$ (394.12)	57.85 57.69	4.60 4.68	21.31 21.49	8.13 8.43

Table IV: ^{1}HNMR, IR and Mass* Spectral data of compounds **(5a-d)**.

Comp. No.	R	^{1}HNMR[δ ppm DMSO-d_6]**	IR [ν cm^{-1}]		
			N-H	C=O	C=N & C=C
5a	H	2.30 (3H; s; C<u>H</u>$_3$), 4.00, 4.43 (2H; two s; SC<u>H</u>$_2$), 7.00-7.86 (9H; m; C_6<u>H</u>$_4$ and C_6<u>H</u>$_5$), 10.73,11.06 (1H; two br s; N<u>H</u>N) and 12.00 (1H; br s; N<u>H</u> of triazole ring).	3445, 3225	1656	1628,1605, 1584, 1457
5b	Br	2.26 (3H; s; C<u>H</u>$_3$), 4.03, 4.43 (2H; two s; SC<u>H</u>$_2$), 7.00-7.83 (8H; m; C_6<u>H</u>$_4$ and C_6<u>H</u>$_4$), 10.80 and 11.00 (1H; two br s; N<u>H</u>N) and 12.20 (1H; br s; N<u>H</u> of triazole ring).	3425, 3270	1661	1611,1585, 1533,1474, 1452
5c	Cl	2.16 (3H; s; C<u>H</u>$_3$), 3.86, 4.33 (2H; two s; SC<u>H</u>$_2$), 6.96-7.73 (8H; m; C_6<u>H</u>$_4$ and C_6<u>H</u>$_4$), 10.53,11.00] (1H; two br s; N<u>H</u>N) and 12.10 (1H; br s; N<u>H</u> of triazole ring).	3445, 3200	1667	1621,1609, 1574, 1453
5d	OCH_3	2.23 (3H; s; C<u>H</u>$_3$), 3.76 (3H; s; OCH_3), 3.93, 4.50 (2H; two s; SC<u>H</u>$_2$), 6.66-7.83 (8H; m; C_6<u>H</u>$_4$ and C_6<u>H</u>$_4$), 10.23,10.96 (1H; two br s; N<u>H</u>N) and 11.93 (1H; br s; N<u>H</u> of triazole ring).	3445, 3240	1666	1624,1609, 1587, 1453

* EIMS [*mlz* (%)]: **5c**: [400 (*M+2*, 0.8), 398 (M^+, 2.4)], **5d**: [394 (M^+, 1.5)].
** protons of NH groups are exchangeable by D_2O.

Table V: Physicochemical properties of compounds **(7a-e)**.

Comp No.	R^1	Yield %	M.p.°C	R_f	M.F. (M.Wt.)	Microanalysis Calculated/Found		
						C%	H%	N%
7a	$-CH_2CH_2CH_3$	70	144-146	0.93	$C_{14}H_{14}N_6OS_2$ (346.07)	48.54 48.21	4.07 4.13	24.26 23.99
7b	$-CH(CH_3)_2$	65	160-161	0.88	$C_{14}H_{14}N_6OS_2$ (346.07)	48.54 48.25	4.07 4.04	24.26 23.97
7c	$-CH_2CH=CH_2$	67	145-147	0.87	$C_{14}H_{12}N_6OS_2$ (344.05)	48.82 48.83	3.51 3.80	24.40 24.50
7d	$-CH_2C_6H_5$	72	208-210	0.74	$C_{18}H_{14}N_6OS_2$ (394.07)	54.81 54.58	3.58 3.91	21.30 21.36
7e*	$-CH_2CH_2C_6H_5$	75	180-181	0.72	$C_{19}H_{16}N_6OS_2$ (408.08)	55.86 55.92	3.95 4.20	20.57 20.56

* recrystallized from acetone.

Table VI: ^{1}HNMR, IR and Mass* Spectral data of compounds **(7a-e)**.

Comp No.	R^1	^{1}HNMR[δ ppm DMSO-d_6]**	IR [ν cm^{-1}]	
			N-H	C=N & C=C
7a**	$-CH_2CH_2CH_3$	1.00 (3H; t; C<u>H</u>$_3$), 1.53-1.76 (2H; m; SCH$_2$C<u>H</u>$_2$), 3.13 (3H; t; SC<u>H</u>$_2$CH$_2$), 4.53 (2H; s; SC<u>H</u>$_2$), 7.03-7.66 (4H; m; C_6<u>H</u>$_4$), and 11.8 (1H; br s; <u>NH</u> of triazole).	3455	1613,1585, 1557,1469
7b**	$-CH(CH_3)_2$	1.43 (6H; d; two C<u>H</u>$_3$), 3.5-4.03 (1H; m; SC<u>H</u>), 4.56 (2H; s; SC<u>H</u>$_2$), 7.00-7.66 (4H; m; C_6<u>H</u>$_4$), and 11.83 (1H; br s; <u>NH</u> of triazole).	3440	1618,1597, 1575,1456
7c	$-CH_2CH=CH_2$	3.70 (2H; d; SC<u>H</u>$_2$CH), 4.52 (2H; s; SCH$_2$), [4.96, 5.22] (2H; d.d; CHC<u>H</u>$_2$), 5.43-6.20 (1H; m; SCH$_2$C<u>H</u>), 7.00-7.56 (4H; m; C_6<u>H</u>$_4$), and 11.92 (1H; br s; <u>NH</u> of triazole).	3390	1621,1586, 1469,1453
7d	$-CH_2C_6H_5$	4.33 (2H; s; SC<u>H</u>$_2$C$_6$H$_5$), 4.50 (2H; s; SC<u>H</u>$_2$), 7.00-7.60 (9H; m; C_6<u>H</u>$_4$ and C_6<u>H</u>$_5$), and 12.06 (1H; br s; <u>NH</u> of triazole).	3450	1618,1591, 1461,1454
7e	$-CH_2CH_2C_6H_5$	3.00, 3.36 (4H; two t; SC<u>H</u>$_2$C<u>H</u>$_2$C$_6$H$_5$), 4.50 (2H; s; SC<u>H</u>$_2$), 7.00-7.65 (9H; m; C_6<u>H</u>$_4$ and C_6<u>H</u>$_5$), and 12.16 (1H; br s; <u>NH</u> of triazole).	3440	1615,1587, 1459,1454

* EIMS [*mlz* (%)]: **7a**: [346 (M^+, 2.7)], **7d**: [394 (M^+, 1.61)].
** Protons of NH groups are exchangeable by D_2O.
*** recorded in $CDCl_3$.

Table VII: Antimicrobial activity of the compounds **(1, 2, 3, 4a-k, 5a-d, 6** and **7a-e)**.

Organism / Compd.[a]	Zones of inhibition (mm.) against: Bacteria: Gram-positive: *B. Cereus*	*S. aureus*	*M. luteus*	Gram-negative: *E. coli*	*P. aerogin.*	*S. marces.*	Fungi: *T. rubrum*	*C. albicans*	*M. canis*	*F. oxysp.*	*A. flavus*	*P. chrys.*
DMSO	--	--	--	--	--	--	--	--	--	--	--	--
Ampicillin	30	26	17	26	16	29	--	--	--	--	--	--
Fluconazole	--	--	--	--	--	--	10	--	28	33	22	16
1	9	8	--	8	8	--	--	12	--	--	--	--
2	--	10	--	--	--	--	--	--	--	--	--	--
3	--	9	--	--	--	--	--	--	--	--	--	--
4f	--	9	--	13	--	--	--	--	--	--	--	--
4g	--	9	--	12	--	--	--	--	--	--	--	--
4h	--	9	--	12	--	--	--	--	--	--	--	--
4i	--	8	--	10	10	--	--	--	--	--	--	--
4j	--	8	--	10	10	--	--	--	--	--	--	--
4k	8	--	--	8	--	--	--	--	--	--	--	--
6	14	10	10	11	10	8	--	10	--	8	--	--
7b	--	--	--	--	--	--	14	--	12	--	--	--
7c	--	--	--	--	--	--	16	--	--	--	--	--

(--) no inhibition
[a]100 µmole mL^{-1} in DMSO
Compounds **4a-e**, **5a-d**, **7a**, **7d** and **7e** were completely inactive.

b) Antifungal activity

Organisms and culture conditions

The used Sabouraud Agar (SA) media were prepared in Assiut University Mycological Center (AUMC), Assiut University. The test compounds (**1**, **2**, **3**, **4a-k**, **5a-d**, **6** and **7a-e**) were evaluated for their antifungal activity *in-vitro*, in comparison to fluconazole as a reference drug using the standard agar cup diffusion method[27] against six pathogenic, phytopathogenic, or food poisoning fungal species: *Trichophyton rubrum* (Castellani) Sabouraud (AUMC 1804), and *Candida albicans* (Robin) Berkhout (AUMC 418), *Microsporum canis* (AUMC 5451), *Fusarium oxysporum* Schlechtendal (AUMC 209), *Aspergillus flavus* (AUMC 1276) and *Penicillium chrysogenum* (AUMC 283).

Materials and method

Spore suspension in sterile distilled water was prepared from 7 days old culture of the test fungi growing on Sabouraud's dextrose broth (30 mL) media in 100 mL conical flasks. The final spore concentration was nearly 5×10^4 spores/mL. About 15 mL of the growth medium was introduced on sterilized Petri dishes of 10 cm diameter and inoculated with 1 mL of spore suspension. Plates were shaken gently to homogenize the inocula. After solidification of the media, 5 mm cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer and was filled with the solutions of the test compounds (**1**, **2**, **3**, **4a-k**, **5a-d**, **6** and **7a-e**) and fluconazole (100 µmol/mL in DMSO). In addition, other cavities were impregnated with the solvent (DMSO) and served as a negative control. The seeded plates were incubated at 28±2°C for 7 days. The radii of inhibition zones (in mm) of triplicate sets were measured at successive intervals during the incubation period and the results are cited in table VII.

The minimum inhibitory concentrations (MICs)

The test compounds giving positive results, (**4f**, **4i**, **4j**, **6**, **7b** and **7c**) were diluted with DMSO to prepare a series of descending concentration down to 0.02 mg/mL. Diluted solutions were similarly assayed as mentioned before and the least concentration (below which no activity) was recorded. The squares of inhibition zone diameters were plotted against log concentrations of the test compounds, extrapolation of the resulting straight line to intersect with log concentration scale in the curve corresponded to log MIC, and MIC was obtained as antilog[26&27], and the results are cited in table VIII.

Table VIII: Antimicrobial activity of some of the compounds (MIC, mg mL^{-1}).

Zones of inhibition mm. & MICs (mg/mL) against:					
Organism / Compd.	Bacteria			Fungi	
	Gram-positive	Gram-negative			
	M. luteus	*E. coli*	*P. aerogin.*	*T. rubrum*	*C. albicans*
DMSO	--	--	--	--	--
Ampicillin	10(5.0)	22(2.5)	12(2.5)	--	--
Fluconazole	--	--	--	10(30)	0
1	--	8	8	--	12(19.2)
4f	--	12(19.0)	--	--	--
4i	--	10	10(9)	--	--
4j	--	10	10(9)	--	--
6	9(3.8)	11	8(3.8)	--	10(30)
7b	--	--	--	11(2.1)	--
7c	--	--	--	8(2.1)	--

2- Anti-inflammatory activity

Male adult albino rats (120–150 g) were obtained from the animal house (Faculty of Medicine, Assiut University, Egypt). Animals were housed in separate cages 3 animals each, in temperature-controlled rooms at 25°C. Animals were allowed free access to rodent chow and water and maintained at a 12 hrs light/dark cycle. Work was conducted in accordance with the internationally accepted principles for laboratory animals' use and care as found in the European Community Guidelines[28] and Institutional Ethical Committee Approval was obtained.

The test compounds (**1**, **2**, **3**, **4a-k**, **5a-d**, **6** and **7a-e**) and the reference drug were suspended in 1% NaCMC in normal saline. Suspensions of the test compounds, reference drug and 1% NaCMC-saline solution (negative control) were injected *i.p.* (1 mL each).

The anti-inflammatory activity of the synthesized compounds (**1**, **2**, **3**, **4a-k**, **5a-d**, **6** and **7a-e**) was evaluated according to the carrageenan induced paw edema method in comparison to indomethacin as a reference drug[29]. The test is based on pedal inflammation in rat paws induced by subplantar injection of carrageenan suspension (0.2 mL of 1% solution in normal saline) into the right hind paw of the rats. Male adult albino rats were divided into groups of four animals each. The rat paw thickness was measured with a Vernier caliper (SMIEC, Shangahai, China) before and 1 hr after carrageenan injection to detect the carrageenan induced inflammation. The test compounds (**1**, **2**, **3**, **4a-k**, **5a-d**, **6**, **7a-e** and indomethacin), at a dose of 28 µmole/Kg, were injected *i.p.* to twenty five different groups of rats 1h after carrageenan injection. In addition, a control group received the vehicle 1% NaCMC solution in normal saline. The difference between the thicknesses of the two paws was taken as a measure of edema inhibition. The measurement was carried out at 0.5, 1, 2, 3, 4 and 5 hrs after injection of the test compounds, reference drug and control. The results are listed in table IX.

Table IX: Inhibitory effect of test compounds (**1**, **2**, **3**, **4a-k**, **5a-d**, **6** and **7a-e**) and indomethacin upon carrageenan induced paw edema in rats.

Compd.	Edema inhibition (%) ± S.E.					
	0.5 hr	1 hr	2 hrs	3 hrs	4 hrs	5 hrs
Negative control	--	--	--	--	--	--
Indomethacin	28.64±0.17***	40.26±0.17***	40.26±0.17***	42.40±0.17***	74.52±0.17***	78.80±0.29***
1	4.25±0.17	10.49±0.17**	19.06±0.17***	46.90±0.29***	46.90±0.29***	51.18±0.33***
2	10.96±0.29	31.91±0.17***	40.47±0.17***	46.90±0.29***	46.90±0.29***	68.31±0.29***
3	15.44±0.44*	31.91±0.17***	31.91±0.17***	31.91±0.17***	46.90±0.29***	46.90±0.29***
4a	16.78±0.14*	20.34±0.14**	41.76±0.14***	41.76±0.14***	47.11±0.29***	47.11±0.14***
4b	16.55±0.14*	20.13±0.29**	36.19±0.29***	41.54±0.14***	46.90±0.29***	57.60±0.14***
4c	27.52±0.14***	35.97±0.29***	41.33±0.14***	46.68±0.14***	52.03±0.14***	52.03±0.14***
4d	22.15±0.29***	30.84±0.14***	41.54±0.14***	52.25±0.14***	52.25±0.14***	68.31±0.29***
4e	16.78±0.14*	36.40±0.29***	41.76±0.14***	47.11±0.29***	52.46±0.14***	63.17±0.14***
4f	21.70±0.14***	30.41±0.14***	30.41±0.14***	35.76±0.29***	51.82±0.14***	51.82±0.14***
4g	27.96±0.14***	36.40±0.29***	41.76±0.14***	57.82±0.29***	63.17±0.14***	68.52±0.29***
4h	27.29±0.14***	41.11±0.14***	46.47±0.29***	57.17±0.14***	67.88±0.29***	78.59±0.14***
4i	16.55±0.14*	41.54±0.14***	46.90±0.29***	52.25±0.14***	57.60±0.29***	62.96±0.14***
4j	22.37±0.29***	36.40±0.29***	41.76±0.14***	47.11±0.29***	63.17±0.14***	63.17±0.14***
4k	22.37±0.14***	36.40±0.29***	41.76±0.14***	52.46±0.14***	52.46±0.14***	63.17±0.14***
5a	27.52±0.14***	46.68±0.29***	52.03±0.14***	62.74±0.14***	62.47±0.14***	84.15±0.14***
5b	16.78±0.14*	36.40±0.29***	52.46±0.14***	52.46±0.14***	63.17±0.14***	68.52±0.29***
5c	27.29±0.14***	30.41±0.14***	46.47±0.29***	46.47±0.14***	62.53±0.14***	89.29±0.29***
5d	21.70±0.29***	25.05±0.14***	46.47±0.29***	46.47±0.14***	67.88±0.29***	83.94±0.14***
6	4.03±0.17	18.84±0.17**	35.97±0.29***	35.97±0.29***	42.40±0.33***	42.90±0.33***
7a	6.71±0.17	36.40±0.29***	40.69±0.17***	40.69±0.17***	47.11±0.29***	72.81±0.17***
7b	0.00±0.29	25.70±0.29***	29.98±0.17***	29.98±0.17***	38.54±0.13***	40.69±0.17***
7c	0.00±0.29	25.70±0.29***	29.98±0.17***	29.98±0.33***	32.12±0.17***	40.69±0.17***
7d	17.67±0.17**	21.20±0.29***	29.76±0.17***	29.76±0.17***	40.47±0.17***	40.47±0.17***
7e	17.45±0.33**	20.99±0.33***	35.97±0.29***	53.10±0.17***	61.67±0.17***	68.09±0.29***

* significant difference at $P < 0.05$ vs. control value (student's-t-test).
** significant difference at $P < 0.01$ vs. control value (student's-t-test).
*** significant difference at $P < 0.001$ vs. control value (student's-t-test).

Molecular docking

Docking and molecular studies were carried out at the Department of medicinal chemistry, Faculty of pharmacy, Assiut university, Assiut, Egypt. All molecular modeling studies were carried out on an Intel Pentium 1.6 GHz processor, 512 MB memory with Windows XP operating system using Molecular Operating Environment[30] as the computational software. All minimizations were performed with MOE until a RMSD gradient of 0.01 Kcal mol^{-1} $Å^{-1}$ with MMFF94X force-field and the partial charges were automatically calculated.

The X-ray crystallographic structure of murine COX-2 complexed with indomethacin (PDB ID: 4COX) and the X-ray crystallographic structure of ovine COX-1 complexed with flurbibrufen (PDB ID: 1CQE), were 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 the 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 key intermediate 1*H*-1,2,4-triazolo[2,3-a]benzimidazole-2-thione; compound **1** was prepared according to a reported procedure and its structure was confirmed by matching its physical and spectral data with the reported one[14&16].

Treatment of compound **1** with methyl bromoacetate in acetone and potassium carbonate afforded a new key intermediate; methyl (1*H*-1,2,4-triazolo[2,3-a]benzimidazol-2-ylsulfanyl) acetate; compound **2**, which when refluxed with hydrazine hydrate yielded 2-(1*H*-1,2,4-triazolo[2,3-a]benzimidazol-2-ylsulfanyl) acetohydrazide; compound **3 (**Scheme 1**)**. Reaction of compound **1** with methyl bromoacetate afforded the S-substituted ester as *S*-alkylation supersedes the *N*-alkylation due to the difference in nucleophilicity as reported by[31].

IR spectrum of compound **2** showed NH band at 3440 cm^{-1} while IR spectrum of compound **3** showed bands at 3430, 3250 and 3120 cm^{-1} due to NH and $NHNH_2$ functions. Also IR spectra of compounds **2** and **3** showed bands at 1729 and 1642 cm^{-1} due to carbonyl function derived from ester and hydrazide structures, respectively. ^{1}HNMR spectrum of compound **2** showed singlet signals derived from ester group at 3.69 ($-OCH_3$) and 4.00 ($-SCH_2$) ppm. ^{1}HNMR spectrum of compound **3** showed new signals derived from hydrazide structure appeared at 4.29 ($-NHN\underline{H}_2$) and 9.26 ($-N\underline{H}NH_2$) ppm integrating for two protons and one proton, respectively (exchangeable with D_2O).

The target compounds; **4a-k** and **5a-d**, were synthesized by condensation of compound **3** with aryl (heteroaryl) aldehydes or substituted acetophenones, respectively (Scheme 1).

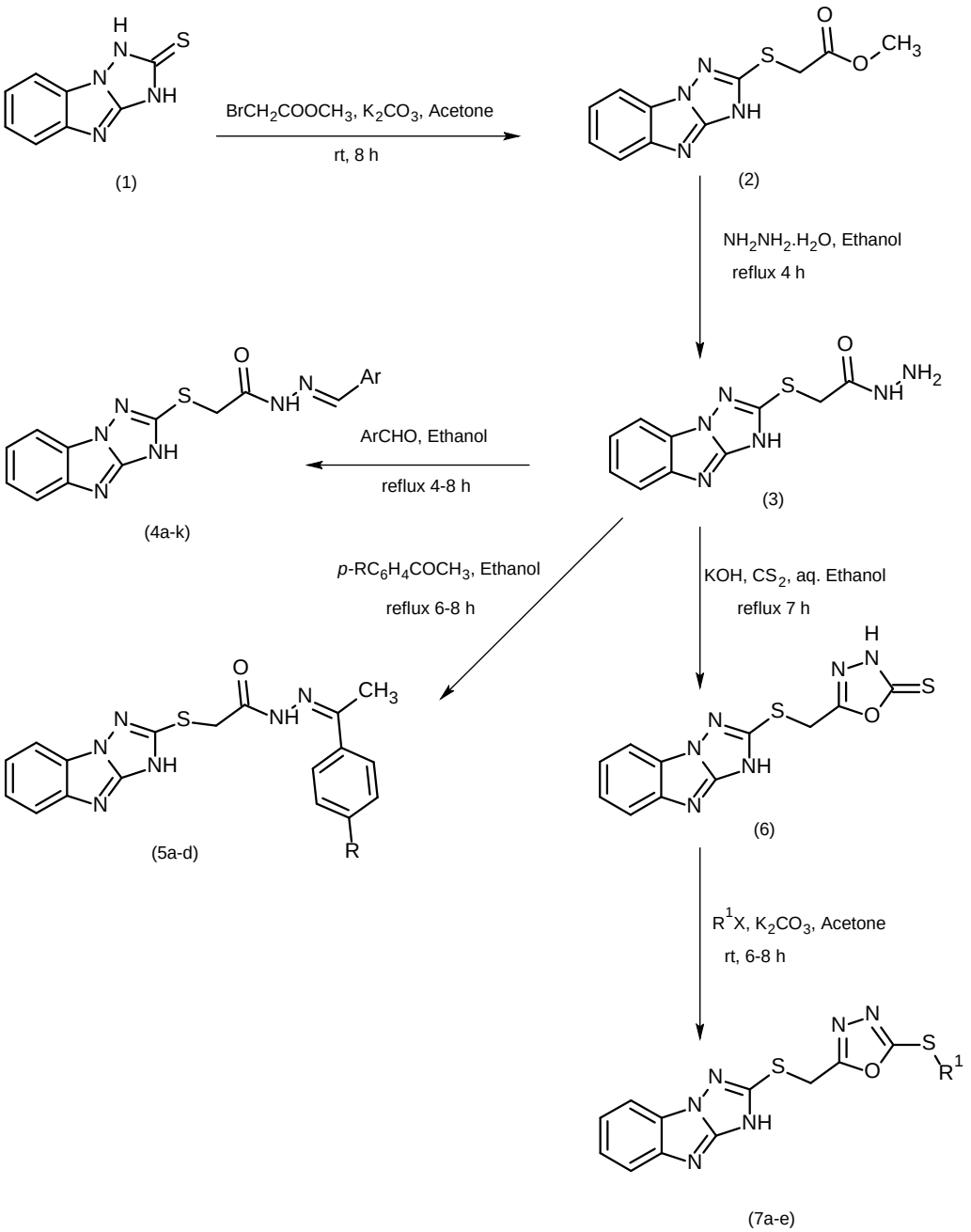
The structures of compounds **4a-k** and **5a-d** were confirmed by IR and ^{1}HNMR as well as elemental analyses. The IR spectra of compounds **4a-k** showed bands in the 3490-3160 cm^{-1} regions for the NH groups. The C=O groups of compounds **4a-k** absorbed in the 1670-1650 cm^{-1} regions while, IR spectra of compounds **5a-d** showed bands in the 3445-3200 cm^{-1} regions for the NH groups. The C=O groups of compounds **5a-d** absorbed in the 1667-1656 cm^{-1} regions.

^{1}HNMR spectra of compounds **4a-k** and **5a-d** displayed additional signals due to the aromatic ring derived from aldehyde or acetophenone moieties in the aromatic region, and the signal belonging to $–NH_2$ group of the hydrazide structure disappeared.

^{1}HNMR spectra of compounds **4a-k** showed two sets of signals each belonging to the $–SCH_2$ group, -N=CH group and –NH-N group of *cis* and *trans*-conformers at (3.90 and 4.46), (7.76 and 8.43) and (11.10 and 12.23) ppm, respectively. The upfield lines of $–SCH_2$, -N=CH and –NH- protons were assigned to *cis*-conformer of the amide structure and downfield lines of the protons of the same groups were assigned to *trans*-conformer of the amide structure[32].

It was reported that the compounds having arylidene-hydrazide structure may exist as *E/Z* geometrical isomers about –C=N double bond and as *cis/trans* amide conformers [32&3]. Besides

the compounds containing imine bond are present in higher percentage in dimethyl-d_6 sulfoxide solution in the form of geometrical *E* isomer about –C=N double bond. The *E* geometrical isomers of these compounds undergo a rapid *cis/trans* amide equilibrium, in which the *cis* conformer predominates, while the *Z* isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond[3].



Ar = C_6H_5, *p*-BrC_6H_4, *p*-ClC_6H_4, *m*-ClC_6H_4, *p*-$CH_3C_6H_4$, *p*-$CH_3OC_6H_4$, *p*-isopropylC_6H_4, *p*-$NO_2C_6H_4$, *p*-HOC_6H_4, 2-thienyl, *m*-OCH_2O-*p*-C_6H_3.
R = H, Br, Cl, CH_3O
R^1 = $CH_3CH_2CH_2$, $(CH_3)_2CH$, $CH_2CH{=}CH_2$, $C_6H_5CH_2$, $C_6H_5CH_2CH_2$

Scheme 1: Synthetic route of compounds **2**, **3**, **4a-k**, **5a-d**, **6** and **7a-e**.

The investigation of ^{1}HNMR spectra of compounds **4a-k** demonstrated that these hydrazones behaved similarly in dimethyl-d_6 sulfoxide solution and no signal belonging to *Z* isomer was observed. On the other hand, the *cis/trans* conformers of *E* isomer were present in the dimethyl-d_6 sulfoxide solutions. Similarly, ^{1}HNMR spectra of compounds **5a-d** showed two singlets at about (3.86 and 4.50 ppm) of C$\underline{H}_2$ group of the thioacetyl moiety and two singlets at about (10.23 and 11.06 ppm) of –NH-N group[33].

The EIMS of compounds **3** and **4b** showed the molecular ion peaks at *m/z* 262 (69.06%) and *m/z* 428 (3.91%), respectively.

On the other hand, compounds **7a-e** were prepared through treatment of compound **3** with carbon disulfide in the presence of potassium hydroxide resulted in the formation of compound **6** which was alkylated with different alkyl or aralkyl halides in acetone and potassium carbonate.

The structure of compounds **6** and **7a-e** were confirmed by IR, ^{1}HNMR, MS as well as elemental analyses. IR spectrum of compound **6** showed two bands at 3445 and 3300cm^{-1} (2NH) stretching, a strong band at 1250 cm^{-1} (C=S) stretching and it was devoided of absorption bands around 1642 cm^{-1} of the carbonyl group. ^{1}HNMR spectrum of compound **6** displayed no signals belonging to –N$\underline{H}_2$ and -CON$\underline{H}$ groups derived from hydrazide structure; instead, new singlet signal appeared at 14.41 exchangeable with D_2O due to –NH of oxadiazole ring.

IR spectra of compounds **7a-e** showed a broad band at 3390-3455 cm^{-1} due to NH stretching, while their ^{1}HNMR spectra approved the absence of –NH signal of oxadiazole ring at 14.41 ppm. Moreover the *S*-alkyl groups give patterns in accordance with the expected structures as shown in table VI.

Biological screening

1- Antimicrobial activities

a) Antibacterial activity

Results of the antibacterial activity for compounds (**1**, **2**, **3**, **4a-k**, **5a-d**, **6** and **7a-e**), table VII indicated that *Micrococcus luteus* and *Serratia marcescens* were resistant to the tested compounds except for compound **6**, while *Staphylococcus aureus* and *Escherichia coli* were the most sensitive organisms to the tested compounds since they showed 31-50% antibacterial activity of that exerted by ampicillin. Compounds **4a-e**, **5a-d** and **7a-e** were completely inactive against the used organisms. Also, the test compounds were inactive against *Bacillus cereus* and *Pseudomonas aeruginosa* except for compounds **1**, **4i**, **4j**, **4k** and **6** which showed 27-63% antibacterial activity compared to ampicillin. The results indicated that compound **6** was the most active one giving 28-63% activity in comparison with ampicillin.

b) Antifungal activity

Results of the antifungal activity of the test compounds (**1**, **2**, **3**, **4a-k**, **5a-d**, **6** and **7a-e**), table VII indicated that most of the test compounds were completely inactive against the used fungal species except for compounds **1**, **6**, **7b** and **7c**. Compounds **7b** and **7c** were more active than fluconazole against *Trichophyton rubrum* showing 140 and 160% activity, respectively. Also, compound **7b** exihibited 43% of the antifungal activity of fluconazole against *Microsporum canis* but compound **6** exhibited 24% of fluconazole activity against *Fusarium oxysporum*. Moreover, it was observed that fluconazole was inactive against *candida albicans* strain while, compounds **1** and **6** were active against this strain.

The minimum inhibitory concentrations (MICs)

Results of the MICs of the test compounds (**4f**, **4i**, **4j**, **6**, **7b** and **7c**), table VIII indicated that compound **6** was active against *Micrococcus luteus* at MIC value 3.8 mg/mL lower than that of ampicillin with comparable inhibition zone while ampicillin MIC values were lower than those of **4f**, **4i**, **4j** and **6** against *Escherichia coli* and *Pseudomonas aeruginosa*. Again compounds **7b** and **7c** were more active than fluconazole with MIC values 2.1 mg/mL against *Trichophyton rubrum*. Moreover, compound **1** was more active than compound **6** with MIC value 19.2 mg/mL against *candida albicans*.

It is noteworthy to mention that, compound **1** displayed moderate antibacterial activity against most strains except *Micrococcus luteus* and *Serratia marcescens* with good antifungal activity against *candida*

albicans strain. Conversion of compound **1** to the acetate ester **2** or acetohydrazide **3** abolished both antibacterial and antifungal activities. Introduction of arylmethylidene moieties in compounds **4a-k** enhanced the antibacterial activity only in compounds **4f-k,** while they showed no activity against fungal strains. On the other hand, hydrazones of 2-(1*H*-1,2,4-triazolo[2,3-a]benzimidazol-2-ylsulfanyl) acetohydrazide **5a-d** showed no activity against all bacterial and yeast strains. The cyclization of hydrazide (**3)** to 1,3,4-oxadiazole derivative (**6)** resulted in moderate to good activities against all bacterial strains and only against *candida albicans* and *Fusarium oxysporum* of fungal strains while the alkylation abolished its antibacterial or antifungal activity against the tested organisms except for compound **7b** and **7c** which showed good antifungal activity against *Trichophyton rubrum*.

2-Anti-inflammatory activity

Results of anti-inflammatory activity for the test compounds (**1**, **2**, **3**, **4a-k**, **5a-d**, **6** and **7a-e**), table IX revealed that all the test compounds showed a gradual increase of the anti-inflammatory activity up to its maximum after 5 hrs. Compounds **2**, **4d**, **4e**, **4g-k**, **5a-d**, **7a** and **7d** were the most active ones showing 80-113% anti-inflammatory activity of indomethacin, while the other compounds showed a moderate activity (51-73%) at 5 hrs. Moreover, it was noted that compounds **4h** and **7a** exhibited, almost, comparable results to those of indomethacin, while compounds **5a**, **5c** and **5d** were more active than indomethacin giving 107-113% anti-inflammatory activity at 5 hrs.

According to the results in table IX, it can be concluded that compound **1** displayed a moderate anti-inflammatory activity in comparison to indomethacin. Conversion of compound **1** into acetate ester **2** increased the activity while the latter decreased when the acetate ester moiety was converted to acetohydrazide one **3**. Introduction of aryl-methylidene moieties, compounds **4a-k,** enhanced the activity again except for compound **4a** with unsubstituted phenyl ring. Compound **4h** with *p*-NO_2 substituent was the most active one in the series **4b-I** giving comparable effect to indomethacin. Substitution with *m*-Cl, *p*-isopropyl, *p*-CH_3 and *p*-OH groups (**4d**, **4g**, **4e** and **4i**) gave excellent activity while substitution with *p*-Br, *p*-Cl and *p*-CH_3O groups (**4b**, **4c** and **4f**) gave moderate to good one. Compounds comprising thiophene and 1,3-benzodioxole moieties (**4j** and **4k**) gave comparable activities to those having phenyl ring **4a-k**. Condensation of acetohydrazide moiety with unsubstituted or substituted acetophenone (*p*-Cl and *p*-CH_3O), **5a-d** resulted in more active compounds than those containing an azomethine –N=CH–group (**4a-c** and **4f**) and indomethacin as a reference drug. Cyclization of acetohydrazide moiety to 1,3,4-oxadiazole-2-thione didn't enhance the activity, however, S-alkylation of the 1,3,4-oxadiazole ring with *n*-propyl moiety produced comparable activity to that of indomethacin. Moreover, replacement by isopropyl or allyl moiety (**7b** and **7c**) decreased the activity. Also, replacement of *n*-propyl group with phenethyl moiety (**7e**) resulted in excellent activity which is not the case with the benzyl substituent (**7d**).

Molecular Modeling Study

Molecular dockings to COX-2 as well as conformational alignment studies of compound **5c** were performed in order to rationalize the obtained anti-inflammatory results and to help in understanding the various interactions between the ligand and enzyme active site in details. Furthermore, molecular docking of this compound was performed to COX-1 to predict its selectivity.

Docking studies of the inhibitor were performed by MOE (Molecular Operating Environment) using murine COX-2 co-crystallized with indomethacin (PDB ID: 4COX) as a template, and COX-1: (PDB ID: 1CQE)[34]. We performed 100 docking iterations and the top-scoring configuration of the ligand-enzyme complexes was selected on energetic grounds.

Docking of compound **5c** to COX-2**,** figure 1 showed that the ligand was oriented so that the carbonyl moiety was in the vicinity of Arg120 residue forming hydrogen bonding with the guanidinium side chain (distance = 1.78 Å). A hydrogen bond interaction between the ligand imidazole nitrogen and the Ser530 side chain (distance = 2.30 Å). The *p*-chlorophenyl moiety was located in the hydrophobic pocket of Leu93, Tyr355, Val116

and Leu359 residues. The 1,2,4-triazolobenzimidazole ring surrounded by Val349, Ala527, Ser530, Tyr385 and Trp387 residues. The score of docking was -11.77 kcal/mole.

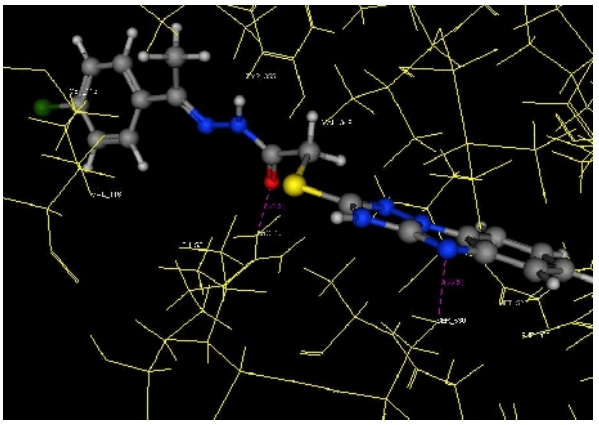


Fig. 1: 3D representation of docking pose of compound **5c** in the active site of murine COX-2 viewed using molecular operating environment (MOE) module.

Conformational superposition of indomethacin (from the X-ray crystal structure of indomethacin-COX-2 complex) and compound **5c** (from the docking simulation) is shown in figure 2. The superposition showed that their hydrophilic (carbonyl group of acetate) and hydrophobic groups (*p*-chlorophenyl and benzimidazole moieties) overlapped with each other. Compound **5c** aligned with indomethacin in a manner that explained the orientation of the compound in the active site of COX-2.

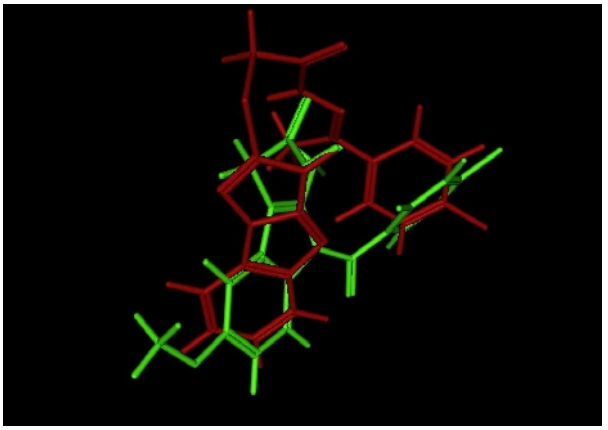


Fig. 2: Conformational alignment of indomethacin from the crystal structure of indomethacin-murine COX-2 complex (green) and that of compound **5c** from the docking simulation (red).

Docking of compound **5c** to COX-1 active site, figure 3 showed that the ligand was oriented so that the nitrogen of thioacetamide moiety was in the vicinity of Tyr355 residue forming hydrogen bonding with the hydroxyl side chain (distance = 1.78 Å). The *p*-chlorophenyl moiety of the ligand was located in the vicinity of the hydrophobic pocket of Arg120, Leu115, Ile89 and Val116. 1,2,4-triazolobenzimidazole moiety surrounded by Val349, Leu352, Ser353, Gly526, Trp387, Ala527, Ser530 and Met522 residues. The score of docking was -10.46 kcal/mole.

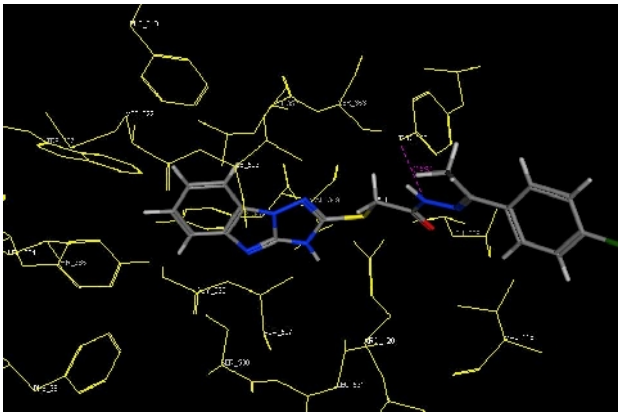


Fig. 3: 3D representation of docking pose of compound **5c** in the active site of ovine COX-1 viewed using molecular operating environment (MOE) module.

From the above mentioned data, the molecular modeling studies of the examined compound **5c** showed that, it binds to the COX-2 active site with position and orientation very close to that resulting from the crystal structure of indomethacin complex with COX-2[35]. Consequently, these observations provided a good explanation for the observed potent inhibitory activity of compound **5c**. Moreover**,** the molecular modeling studies of the examined compound **5c** in the COX-1 active site indicated that the compound was probably non selective anti-inflammatory agent.

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