Iraqi Journal of Science, 2021, Vol. 62, No. 4, pp: 1054-1065
DOI: 10.24996/ijs.2021.62.4.2

ISSN: 0067-2904

Synthesis and Identification of Some New Derivatives of ([Benzyl Thio) Benzimidazole -N- (Methylene-5-Yl)] - 4,5- Di Substituted 1,2,4-Triazole and Evaluation of Their Activity as Antimicrobial and Anti-Inflammatory Agents

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Received: 13/1/2020 Accepted: 29/9/2020

Abstract:

New 2-Mercaptobenzimidazole derivatives were synthesized. 4,5-disubsitituted 1,2,4-Triazole compounds **1b-2c** were synthesized from 2-(benzylthio) benzimidazole compound **a**, which was then reacted with (NaH) in dioxane at a temperature of (0-5 C°) to produce the salt of compound **a**. Then the salt was reacted with ethyl chloro acetate to yield Ethyl 2-(benzylthio) benzimidazole acetate compound **b**. Compound **b** was converted to triazole derivatives by two pathways. The first pathway was reacting compound **b** with semicarbazide, thiosemicarbazide and phenylsemicarbazide in DMSO as a solvent to gain compounds **1b-3b**, which were then cyclized by refluxed with 2N (NaOH) to yield 1,2,4-triazole derivatives compounds **4b-6b**. The second pathway involved the treatment of compound **b** with hydrazine hydrate to produce N-acetohydrazide-2-(benzyl thio) benzimidazole **c.** Compound **c** was refluxed with carbon disulfide (CS_2) in KOH alcoholic solution to obtain the salt compound **1c**. The salt was treated with hydrazine hydrate to yield 1,2,4-triazole derivative compound **2c**. The newly synthesized compounds **b-2c** were identified by FTIR, ^{1}H-NMR and ^{13}C-NMR and their physical properties were measured. Furthermore, their anti-microbial activities were tested against two Gram-positive and two Gram-negative bacteria and against one strain of fungi. Also, some of these synthesized compounds were tested for their anti-inflammatory activities.

Keywords: 2-Mercaptobenzimidazole, semicarbazide, thiosemicarbazide, phenylsemicarbazide 1,2,4-triazole, Anti-Microbial, Anti-inflammatory.

تحضير وتشخيص بعض المشتقات الجديدة من 2-[بنزيل ثايو بنزإيميدازول-N- (مثلين)] -5,4- ثنائي التعويض 4,2,1-ترايازول وتقديرفعاليتها كعوامل مضادة للميكروبات ومضادة للالتهابات

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الخلاصه

حضرت مشتقات جديدة من 2 - مركبتوبنزإيميدازول. 4,5 - ثنائي التعويض 4,2,1 - ترايازول مركبات **1b-2c** قد حظر من 2 - (بنزايل ثايو) بنزإيميدازول مركب **a**, بعد ذلك مفاعلة مركب **a** مع NaH في الدايوكسان عند درجة (0-5 C°) ليعطي ملح المركب **a**. فوعل الملح مع اثيل كلورو

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اسيتيت لينتج اثيل 2 – (بنزيل ثايو) بنزإيميدازول استيت مركب **b** . تم تحويل مركب **b** الى مشتقات الترايازول عن طريق مسارين. المسار الاول يتضمن تفاعل مركب**b** مع (سمي كاربزايد، ثايو سمي كاربزايد وفنيل سمي كاربزايد) في DMSO كمذيب للحصول على مركبات **1b–3b**، بعد ذلك مركبات **1b–3b** تم غلقها حلقيا عن طريق تصعيدها مع 2 N (NaOH) للحصول على مشتقات 4,2,1 – ترايازول مركبات **4b–6b**. المسار الثاني هو معامله مركب **b** مع الهيدرازين للحصول على N– اسيتو هايدرزايد 2 – (بنزايل ثايو) بنزإيميدازول **c**. بعد ذلك تم تصعيد مركب **c** مع (CS_2) في هيدروكسيد البوتاسيوم الكحولي للحصول على ملح **1c**. تم معاملة الملح مع الهيدرازين لإنتاج مشتق 4,2,1 – ترايازول مركب **2c**. هذه المركبات المحظرة الجديدة تم تشخيصها عن طريق [FTIR, ^{1}H–NMR و ^{13}C–NMR] وتم قياس خصائصها الفيزيائية. علاوة على ذلك تم اختبار المركبات المحظرة كمضادات ميكروبية ضد نوعين من بكتريا ايجابية الغرام ونوعين من بكتريا سلبية الغرام وضد نوع واحد من الفطر. كذلك بعض هذه المركبات المحظرة تم اختبارها كمضادات للالتهابات.

Introduction

Inflammation is one of the initial signs of many well-known diseases and described by symptoms including swelling, heat, redness, and pain. In recent times, a small number of benzimidazoles were discovered to have anti-inflammatory and antibacterial activities [1]. Benzimidazole is one of the most exceptional hetero cyclic moieties that produced many effective drugs. A wide range of pharmacological activities were informed by benzimidazole moiety itself and its derivatives [2]. The benzimidazole structure is related with a wide range of biological activities including anti-cancer [3], anti-viral [4], anti-inflammatory [5], anti-oxidant [6] and antimicrobial [7] properties. A countless number of five-membered heterocyclic compounds that contain nitrogen, sulfur and oxygen were synthesized and their numerous pharmacological properties were studied. Of these compounds, 1,2,4-triazoles are known to be used to improve the pharmacokinetic properties (excretion, metabolism, distribution and absorption) of drugs [8]. Furthermore, 1,2,4-triazole derivatives have significant pharmacological properties [9], for example analgesic [10], antifungal [11], antibacterial [12, 13], anticonvulsant [14], anti-inflammatory [15], antiviral [16], antimalarial [17] and antioxidant [18].

Experimental Work

All chemicals used in this work were supplied by Merck, Fluka, BDH and sigma Aldrich chemicals companies. The FTIR spectra were recorded using FTIR 8400s Fourier transitions infrared spectrometer (Shimadzu, Japan), KBr disc in 4000-600 cm^{-1} spectral range, in the Department of Chemistry, College of Science, University of Baghdad. The melting point was recorded using Gallenkamp, electro-thermal apparatus. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on near magnetic resonance Bruker, Ultra-shield (400) MHz. Also, DMSO-d_6 was used as a solvent in the experiment conducted in Isfahan University, Iran. The antimicrobial activity was tested at the Central Service laboratory, College of Education for pure Science (Ibn Al-Haitham), University of Baghdad. The anti-inflammatory activity was detected at the Drug Control Center / Iraqi Ministry of Health.

Synthesis of 2-(benzyl thio) benzimidazole a [19]

The compound 2-marcaptobenzimidazole [2-MBI] (1.5 g, 0.0099 mol.) was dissolved in (5 mL) of absolute ethanol and stirred for about (5 min). Then, KOH alcoholic solution was prepared from (0.560g, 0.0099 mol.) KOH with 3 mL absolute ethanol and added slowly to the solution of [2-MBI], followed by stirring for (10 min). Then, (1.13 mL, 0.0099 mol.) benzyl chloride was added to the solution and left to reflux for (4 h). The reaction mixture was poured on ice water with stirring, filtered to produce a white precipitate and, re-crystallized from ethanol and water (1:1). The physical properties and FTIR spectral data are listed in Table-1.

Synthesis of N-ethyl -2-(benzylthio) benzimidazole acetate b [20].

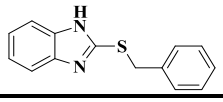
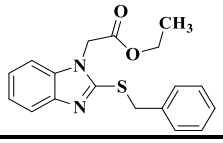
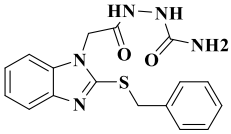
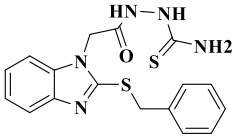
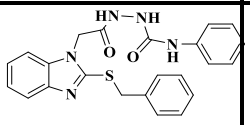
The compound 2-benzyl thiobenzimidazole **a** (0.005 mol., 1.2 g) was mixed with 5 mL dioxane in a round bottom flask and stirred for (5 min). While the precipitate was dissolved and the solution became clear, the round bottom flask was put in ice water bath and NaH 0.2g 0.005mol.) was added slowly, followed by stirring for about 30 min at $0mC^0$. After that, ethyl chloro acetate (0.6 mL, 0.005 mol.) was added drop by drop and stirred in room-temperature overnight. The solution was poured on

iced water and filtered to give an off-white precipitate. The physical properties and FTIR spectral data are listed in Table-1.

Synthesis of 2-[(benzyl thio)benzimidazole -N- (Aceto)] semicarbazide 1b, thiosemicarbazide 2b, and phenylsemicarbazide 3b [21]

Compound **b** (0.5 g, 0.0015mol) was dissolved in (5mL) (DMSO), then (0.0015mol.) of semicarbazide, thiosemicarbazide, and phenylsemicarbazide were added respectively and stirred for some minutes. Then, sodium acetate (0.123g, 0.0015mol) was added to the reaction mixture and refluxed for (18-20 h). Finally, the reaction mixture was poured on ice-cold water. The precipitate was filtered and recrystallized from ethanol to give crystals. Physical properties and FTIR spectral data for compounds **1b-3b** are listed in Table-1.

Table 1-Physical properties and FTIR spectral data for compounds **a-3b**

No.	Compound Structure	Physical Properties			FTIR absorption cm^{-1}				
		M.P C^o	Yield %	Color	ν(N-H_2)	ν(N-H)	ν(C-H) Arom.	ν(C=O)	ν(C=N)
a		186-188	94	White	___	3134	3049	___	1628
b		54-56	82	Off white	___	___	3055	**Ester** 1753	1641
1b		158-160	63	Off white	Asym. 3438 Sym. 3398	3245	3066	**amid** 1695	1650
2b		168-170	75	Off white	Asym. 3468 Sym. 3417	3232	3066	**amid** overlap with ν(C=N)	1620
3b		166-168	64	brown	___	3255	3066	**amid** 1664	1620

Synthesis of 2-[(benzyl thio) benzimidazole -N- (methylene-5-yl)]-4*H*-1,2,4-triazole-3-ol 4b , 4*H*-1,2,4-triazole-3-thiol 5b, and 4-phenyl-1,2,4-triazole-3-ol 6b [8]

Compounds **1b-3b** (0.001mol.) were refluxed with 2N sodium hydroxide solution (25mL) for 10-12 h. The reaction mixture was cooled to room temperature, poured on ice-cooled water, stirred, and neutralized by gradual addition of (1:1) hydrochloric acid. The formed precipitate was filtered and recrystallized from ethanol. Physical properties and FTIR spectral data for compounds **4b-6b** are listed in Table-2.

Synthesis of N- acetohydrazide -2-(benzylthio) benzimidazole c [20]

Compound **b** (0.5g, 0.0015 mol.) was dissolved with (5mL) of ethanol. The mixture was stirred for about (5-10 min), then an excess of 80% hydrazine hydrate was added to the mixture and reflexed for

(6 h). After reflexing, the mixture was poured on ice water and filtered to give a pale white precipitate. The physical properties and FTIR spectral data are listed in Table-2.

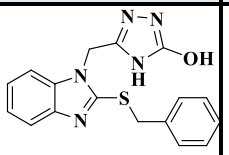
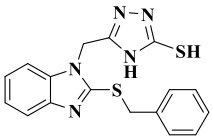
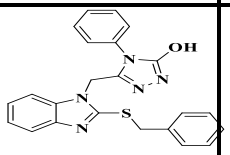
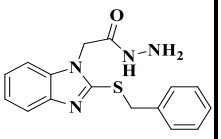
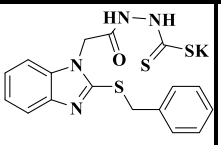
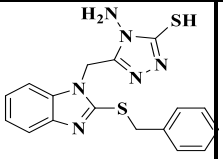
Synthesis of potassium 2-[(benzyl thio) benzimidazole -N- (Aceto)] dithiocarbazate 1c [22]

To a stirred ethanolic solution of KOH (0.071g, 0.0012mol.) in (10mL) absolute ethanol, the hydrazide derivative compound **c** (0.4g, 0.0012mol.) then CS_2 (0.072 mL, 0.0012mol.) were added slowly and stirred overnight. Later, the reaction mixture was filtered and the yellow precipitate was obtained and washed with (20mL) of ether, and dried. The salt compound **1c** was obtained in almost a quantitative yield and employed in the next step without further purification. Physical properties and FTIR spectral data for compound **1c** are listed in Table-2.

Synthesis of 2-[(benzyl thio)benzimidazole-N-(methylene)]-4-amino-1,2,4-triazole-3-thiol 2c [22]

The suspension of potassium salt **1c** (0.5gm, 0.0011mol.) in excess hydrazine hydrate was refluxed until the evolution of H_2S was ceased. After cooling, the reaction mixture was acidified with 10%HCl to yield an off-white precipitate. The precipitate was recrystallized from ethanol to give crystals. Physical properties and FTIR spectral data for compound **2c** are listed in Table-2.

Table 2- physical properties and FTIR spectral data for compounds 4b-2c

No.	Compound Structure	Physical Properties			FTIR absorption cm^{-1}				
		M.P C°	Yield %	Color	ν(N-H)	ν(C-H) Arom.	ν(C-H) Alpha.	ν(C=N)	other
4b		172-174	85	Off white	3290	3068	2964 2885	1662	ν(OH) 3380
5b		178-180	73	Off white	3251	3049	2964 2862	1645	ν(S-H) 2478
6b		178-180	65	Brown	___	3055	2964 2925 2860	1647	ν(OH) 3429
c		134-136	91	Pale white	3288	3060	2958 2925	1637	ν(NH_2) Asym. 3444 Sym. 3390 ν(C=O) Amid 1668
1c		168-170	76	Off white	3222	2979	2935 2894	1629	ν(C=O) amid 1694 ν(C=S) 1494
2c		158-160	86	Off white	___	3064	2964 2925 2866	1645	ν(NH_2) Asym. 3463 Sym. 3434 ν(S-H) 2476

Antimicrobial activities [23]

Some of synthesized compounds were screened *in vitro* for their antimicrobial activities against Gram-positive and Gram-negative bacterial species as well as a fungi strain. The antibacterial activities were tested against two Gram-negative species (***Acinetobacter baumannii*** and ***Pseudomonas aeruginosa***) and two Gram-positive species (***Staphylococcus aureus*** and ***Bacillus subtilis***). The antifungal activities were tested against ***Candida albicans*** species. DMSO with dissolved tested compounds was used as a negative control. Amoxicillin and Fluconazole (800 µg/mL) were used as positive standards to determine the sensitivity of each microbial test. The antimicrobial activities were estimated by measuring the diameter of the zone of inhibition against the tested organisms. A stock solution of (800 µg/mL) was prepared by dissolving 0.004g from the newly synthesized compounds in 5mL DMSO. The diluted microbial culture suspension was spread on NA plates by a swab, while wells with a diameter of 8 mm were punched with a sterile cork borer, where 100µL of the tested compounds' solutions (800µg/ml) was introduced into the holes. The inoculated plates were incubated at 37 C^0 for 24 hours and the resulting zones of inhibition were measured as given in Table-5.

Anti-inflammatory activities [24]

Albino rats of each six weighing (250 ± 50 gm) were provided by the Biotechnology Research Center, AL-Nahrain University, and housed under standardized conditions in the animal house of the Drug Control Center / Iraqi Ministry of Health. Commercial chow was used to feed the animals and they had free entry to water, *ad libitum*. Animals were brought to the laboratory one hour before the experiments; they were divided into five groups (six rats per group), as follows.

- Group (A): refers to the control where six rats were injected with (DMSO) with a dose of 2 ml/kg.
- Group (B): - refers to the reference substance where six rats were injected with Diclofenac Sodium with a dose of (3 mg/kg) dissolved in DMSO.
- Group (C-E): - refers to the test where three groups, with six rats each, were injected independently with the synthesized compounds **3b**, **4b** and **2c**, in doses that are determined below and equivalent to 3 mg/kg body weight of Diclofenac Sodium and dissolved in DMSO.

The anti-inflammatory activities of the synthesized compounds were tested by the paw edema method. An acute inflammation was made by the injection of 0.1ml fresh undiluted egg-white subcutaneously into the plantar side of the left hind paw of the rats, 30 min after the intraperitoneal injection of the synthesized compounds.

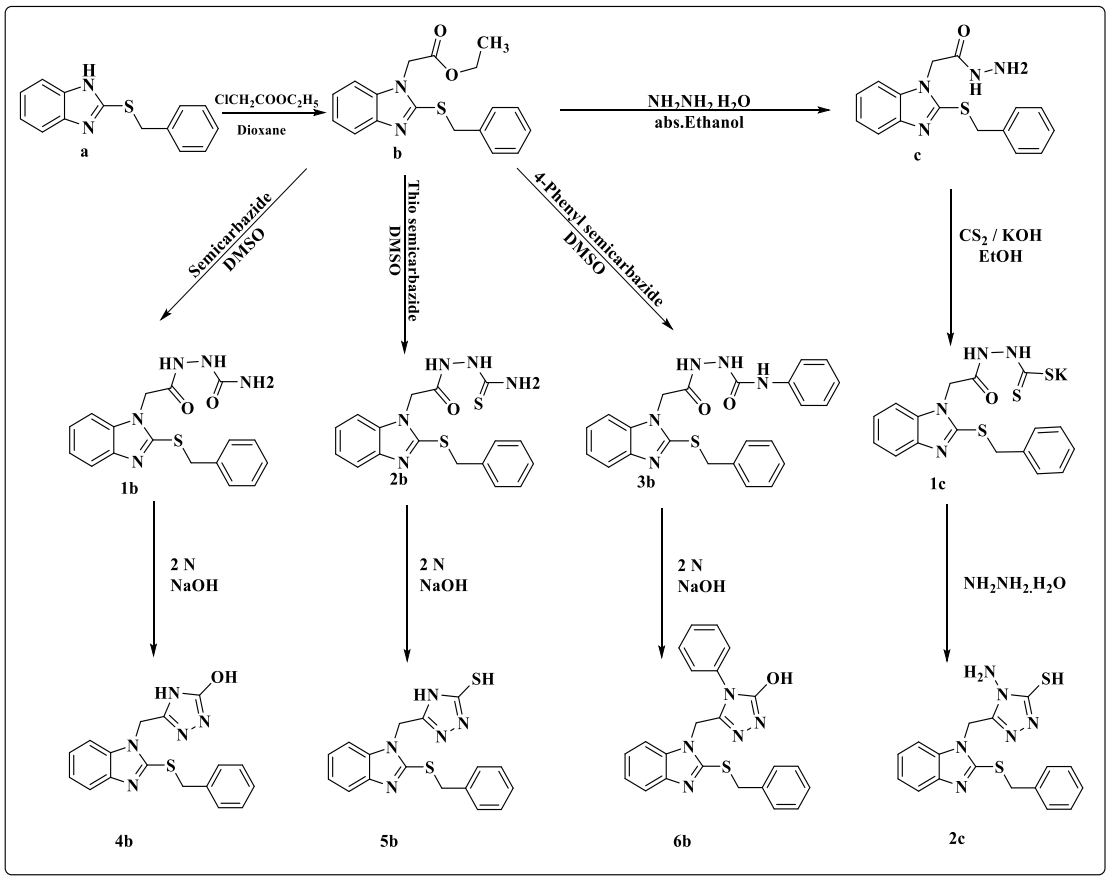
The paw thickness was measured by using a vernier caliper at eight time periods (0, 30, 60, 120, 180, 240, 300 and 360 min) and these measurements were taken after the intraperitoneal injection of the synthesized compounds or the control, which was counted as time zero.

Statistical analysis

The data are expressed as the mean ± SEM and the results were analyzed for statistical significance by using student t-test (two samples that assuming equal variance) for comparison between mean values. Probability (P) values < 0.05 were considered as significant.

Results and Discussion

The synthesis sequence of the preparation of 1,2,4-Triazoles derivatives from 2 mercaptobenzimidazole is shown in Scheme (1).



Scheme (1)

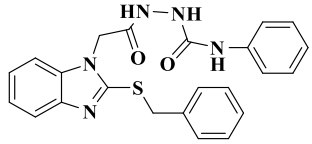
The FTIR spectrum data of compound **a** show absorption bands at 3134 cm^{-1} for stretching vibration of NH[25], 3049 cm^{-1} for ν (CH arom.), 2962, 2860 and 2808 cm^{-1} for ν (CH aliph.), 1628 cm^{-1} for ν (C=N imidazole), and 1512 for ν (C=C arom.). While The FTIR spectrum data of compound **b** show the disappearance of the absorption band 3134 cm^{-1} that belongs to ν (-NH), with new absorption bands that appeared at 1753 $cm^{-1}$for ν (C=O) and 1207 cm^{-1} for ν (C-O), which belong to ester. The FTIR spectrum data of compounds **a** and **b** are listed in Table-1.

Compound **b** was converted to Semicarbazide **1b**, Thiosemicarbazide **2b**, phenylsemicarbazide **3b** and hydrazide **c** derivatives by the reaction of compound **b** with Semicarbazide, Thiosemicarbazide and Phenylsemicarbazide, and in DMSO or hydrazine hydrate in EtOH as solvents, respectively. The FTIR spectral data of compounds **1b-3b** show the disappearance of the absorption band at 1753 cm^{-1}, which belongs to ν (C=O) of the ester. There is a new absorption band at 1695-1664 cm^{-1} which belongs to ν (C=O) of the amid band (1) [26]. There are also new absorption bands at 3468-3438 cm^{-1} and 3417-3398 cm^{-1} which belong to ν (NH_2) Asym. and Sym., respectively. There are also absorption bands at 3255-3232 cm^{-1} which belongs to ν (NH), at 3066 cm^{-1} which belongs to ν (CH) aromatic, and at 1650-1620 cm^{-1} which belongs to ν (C=N) of imidazole ring. The FTIR spectral data of compounds **1b-3b** are listed in Table-1.

The ^{1}H-NMR spectral data of compound **3b** show a singlet signal at δ=4.58 ppm which belongs to 2H due to (-SCH_2), a new singlet signal at δ= 4.72 ppm which belongs to 1H due to (NH-Ar), a singlet signal at δ=5.31 ppm which belongs to 2H due to (-NCH_2), a multiplet signal at δ=7.13-7.46 ppm which belongs to 14H due to (Ar-H), a new singlet signal at δ=9.10 ppm which belongs to 1H due to (CO-NH-N), and a new singlet signal at δ=12.4 ppm which belongs to 2H due to (CO-NH-NH -CO). The ^{1}H-NMR spectral data of compound **3b** are listed in Table-3.

The ^{13}C-NMR spectral data of compound **3b** show a signal at δ=38.67 ppm which belongs to (-SCH_2), a singlet signal at δ=120.87 ppm which belongs to (-NCH_2), a singlet signal at δ=126.77-128.30 ppm which belongs to (Ar), a singlet at δ=149.15ppm due to (C=N) of imidazole ring, a signal at δ=163.15ppm due to (C=O) of amide, and a signal at δ=168.49 ppm due to (N-CO-N). The ^{13}C-NMR spectral data of compound **3b** are listed in Table-3.

Table 3-The ^{1}H-NMR and ^{13}C-NMR spectral data of compound **3b**

Com. NO.	Structure	^{1}H-NMR spectral data (δ ppm)	^{13}C-NMR spectral data (δ ppm)
3b		4.58 (s,2H, S-CH_2);4.72 (s, 1H, NH-Ar); 5.31 (s, 2H,N-CH_2); 7.13-7.46 (m, 9H, Ar-H); 9.10 (s, 1H, CO-NH-N); 12.4 (s, 1H, CO-NH NH - CO).	38.67 (S-CH_2); 120.87 (N-CH_2); 126.77 -128.30 (Ar); 149.15 (C=N imidazole); 163.15 (C=O); 168.49(N-CO-N).

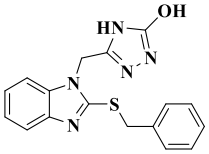
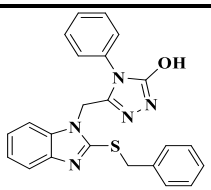
Compounds **1b-3b** were converted to 1,2,4-Triazole derivatives by a cyclization reaction via reflexing with (2N) NaOH to yield 1,2,4-Triazole derivative compounds **4b-6b**.

The FTIR spectral data of compounds **4b-6b** show that there was a disappearance of the absorption bands 3468-3438 cm^{-1} and 3417-3398 cm^{-1} due to ν (NH_2) Asym. and Sym., respectively. Instead of that, there was an appearance of a new band at 3429-3380 cm^{-1} which belongs to ν (OH) for compounds **4b,6b** and 2478 cm^{-1} which belongs to ν (SH) for compound **5b**. The remaining absorption bands are at 3290-3251 cm^{-1} which belongs to ν (NH), at 3068-3049 cm^{-1} which belongs to ν (CH) Aromatic, at 2964-2860 cm^{-1} which belongs to ν (CH) Aliphatic, at 1662-1645 cm^{-1} which belongs to (C=N), and at 1591-1541 cm^{-1} which belongs to (C=C) Aromatic. The FTIR spectral data of compounds **4b-6b** are listed in Table-2.

The ^{1}H-NMR results of compound **4b** show a new singlet signal at δ= 12.4 ppm due to 1H which belongs to (-NH) of triazole ring, while the singlet signal at δ= 3.51ppm is due to 2H and belongs to (-SCH_2), the singlet signal at δ= 4.48 ppm belongs to 2H (-NCH_2), the multiplet at δ= 7.14-7.46 ppm is for 9H (Ar-H), and the singlet signal at δ= 9.84 ppm belongs to 1H for (-OH). The ^{1}H-NMR and The ^{13}C-NMR spectral data of compound **4b** are listed in Table-4.

While the ^{1}H-NMR results of compound **6b** show a singlet signal at δ= 3.50 ppm which belongs to 2H of (-SCH_2), a singlet signal at δ= 4.58 ppm which belongs to 2H (-NCH_2), a multiplet signal at δ= 6.97-7.46 ppm which belongs to 14H for (Ar-H), and a singlet signal at δ= 8.79 ppm due to 1H for (-OH). The ^{1}H-NMR and The ^{13}C-NMR spectral data of compound **6b** are listed in Table-4.

Table 4-The ^{1}H-NMR and ^{13}C-NMR spectral data of compounds **4b-6b**

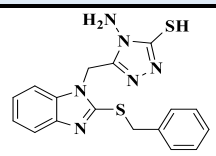
Com. NO.	Structure	^{1}H-NMR spectral data (δ ppm)	^{13}C-NMR spectral data (δ ppm)
4b		3.51 (s,2H, S-CH_2); 4.48 (s, 2H, N-CH_2); 7.14-7.46 (m, 9H, Ar-H); 9.84(s,1H, OH);12.4 (s, 1H, NH triazole).	38.73 (S-CH_2); 113.28 (N-CH_2); 126.76 -128.28 (Ar);137.10 (C=N triazole); 149.15 (C=N imidazole).
6b		3.50 (s,2H, S-CH_2); 4.58 (s, 2H,N-CH_2); 6.97-7.46 (m, 9H, Ar-H); 8.79 (s,1H, OH).	38.66 (S-CH_2); 117.59 (N-CH_2); 126.77 -128.30 (Ar);137.13 (C=N triazole); 149.17 (C=N imidazole).

Compound 10 was synthesized by the reaction of hydrazide compound **c** with CS_2 in ethanolic KOH to give the dithiocarbazate salt **1c** in an excellent yield, which was then cyclized by refluxing with hydrazine hydrate to give a good yield of Triazole derivative **2c**.

The FTIR spectrum data of compound **1c** show absorption bands at 3222 cm^{-1} which belongs to ν (NH), at 2979 cm^{-1} which belongs to ν (CH) Aromatic, at 2935-2984 cm^{-1} which belongs to ν (CH) Aliphatic, and at 1629 cm^{-1} which belongs to ν (C=N) imidazole ring. While compound **2c** shows two absorption bands at 3463;3434 cm^{-1} which belongs to ν (NH_2) Asym. and Sym, respectively, as well as bands at 3064 cm^{-1} which belongs to ν (C-H) Aromatic, at 2476 cm^{-1} which belongs to ν (S-H), and at 1645 cm^{-1} which belongs to ν (C=N)[27]. The FTIR spectrum data of compounds **1c-2c** are shown in Table-2.

The ^{1}H-NMR spectral data of compound **2c** show a singlet signal at δ=3.28 ppm which belongs to 1H due to (-SH), a singlet signal at δ= 4.09 ppm which belongs to 2H for (-SCH_2), a signal at δ= 4.57ppm which belongs to 2H of (-NH_2), a signal at δ= 5.25 ppm which belongs to 2H for (-NCH_2), and a multiplet signal at δ= 7.08-7.44 ppm which belongs to 9H of (Ar-H). The ^{13}C-NMR spectral data of compound **2c** show a signal at δ= 38.69 ppm which belongs to (-SCH_2), a signal at δ= 117.28 ppm which belongs to (-NCH_2), a multiplet signal at δ= 126.76-128.28 ppm which belongs to (Ar), a signal at δ= 149.26 ppm which belongs to (C=N) triazole ring, and a signal at δ= 158.28 ppm which belongs to (C=N) imidazole ring. The ^{1}H-NMR and The ^{13}C-NMR spectral data of compound **2c** are listed in Table-5.

Table 5-The ^{1}H-NMR and ^{13}C-NMR spectral data of compound **2c**

Com. NO.	Structure	^{1}H-NMR spectral data (δ ppm)	^{13}C-NMR spectral data (δ ppm)
2c		3.28(s,1H, SH); 4.09 (s,2H, S-CH_2); 4.57(s,2H, NH_2) 5.25 (s, 2H,N-CH_2); 7.08-7.44 (m, 9H, Ar-H).	38.69 (S-CH_2); 117.28 (N-CH_2); 126.76 -128.28 (Ar);149.26 (C=N triazole); 158.28 (C=N imidazole).

Anti-microbial Activity

Some of 2-mercaptobenzimidazole derivatives (semicarbazide, thiosemicarbazide, phenylsemicarbazide and 1,2,4-triazole derivatives) were screened for anti-microbial activities against two Gram positive (***Staphylococcus aureus*** and ***Bacillus Subtilis*****)** and two Gram negative (***Pseudomonas aeruginosa*** and ***Acinetobacter baumannii*****)** bacteria and one strain of fungi (***Candida albicans***). The results showed that compounds **3b** and **2c** have a moderate to good activity against all species of Gram negative and positive bacteria, but were not effective against the fungal species. While compounds **c**, **1b** and **2b** have a good activity against fungus but not effective against all species of bacteria . Compound **b** was only sensitive against Gram positive bacteria (***Bacillus Subtilis***) and against the fungus, whereas compound **6b** was not effective against Gram negative bacteria (***Acinetobacter baumannii***). Compound **5b** was not effective against all bacterial and fungal species, as shown in Table-6.

Table 6-Results of anti-microbial activity tests of some prepared compounds

No. of comp. and standard (800 µg/ml)	Diameter of inhibition zone (mm)				
	Staphylococcus aureus	Pseudomonas aeruginosa	Bacillus subtilis	Acinetobacter baumannii	Candida Albicans
C	-	-	-	-	-
b	-	-	12	-	20
c	-	-	-	-	18
1b	-	-	-	-	19
2b	-	-	-	-	16
3b	20	21	18	11	-
4b	18	14	15	-	10
5b	-	-	-	-	-
6b	19	11	12	-	11
2c	17	15	14	12	-
Amoxicillin	33	32	33	-	-
Fluconazole	-	-	-	-	25

[Control]: 800µg/ml; Solvent: dimethylsolfoxide

Inhibition Zone: (-) no inhibition; (6-10) weak; (11-18) moderate; (19-30) strong; (30>) very strong.

Anti-inflammatory activities

The Anti-inflammatory activities of the final target compounds **(3b, 4b** and **2c)** were testes using the paw edema method, where an acute inflammation was made by the injection of fresh undiluted egg-white subcutaneously into the plantar side of the left hind paw of Wistar albino rats.

To consider the rationality of this method, the reference compound used to recognize the anti-inflammatory activity profile was Diclofenac Sodium. The tested compounds **(3b, 4b** and **2c)** and the reference drug (Diclofenac Sodium) produced a significant reduction in paw edema thickness as compared to the effect of dimethyl sulfoxide (DMSO), which is used in the control group. The results of the anti-inflammatory activity of the reference and the control are shown in Table-7.

Since p value is < 0.05 by comparing both control (DMSO) and reference (Diclofenac Sodium) groups, this indicates that the paw edema method used in this work is an effective method and can successfully be used for the assessment of the anti-inflammatory effects of the synthesized compounds, as shown in Figure-1.

The results of the anti-inflammatory effects of the tested compounds **(3b, 4b** and **2c)** in comparison to the reference (diclofenac sodium) group and the control (DMSO) group are revealed in Table-8. All the tested compounds **(3b, 4b** and **2c)** effectively limited the increase of the volume of paw edema. The effects of the synthesized compounds started at 120 minutes, a result which is significantly different as compared to the control), and continued till the end of the experiment, with statistically significant reduction (p value less than 0.05) in the thickness of paw edema, as shown in Figure-2.

Table 7-The effects of diclofenac sodium (reference) and dimethyl sulfoxide (control) on the fresh egg white-induced paw edema inflammation in rats

	Time (min)	**DMSO (n=6)**	**Diclofenac Sodium (n=6)**
Paw Thickness (mm)	0	3.73 ± 0.14	3.71 ± 0.23
	30	5.59 ± 0.24	5.42 ± 0.22
	60	6.85 ± 0.10	6.51 ± 0.16*
	120	6.43 ± 0.21	6.25 ± 0.17*
	180	6.55 ± 0.11	6.10 ± 0.13*
	240	5.55 ± 0.10	5.49 ± 0.24
	300	5.40 ± 0.21	4.98 ± 0.24*
	360	5.26 ± 0.16	4.71 ± 0.15*

Data are stated in (mm) paw thickness as (mean ± SEM).
n = number of animals.
Time (0) is the time of i.p. injection of Diclofenac Sodium (reference) and DMSO (control).
Time (30) is the time of injection of fresh egg-white for induction of paw edema.
*Significantly different compared to control: p-value *<0.05.

Table 8-The effects of control, diclofenac sodium and tested compounds 3b, 4b and 2c on eggwhite-induced paw edema inflammation in rats

Time (min)	DMSO (n=6)	Diclofenac Sodium (n=6)	Compound 3b (n=6)	Compound 4b (n=6)	Compound 2c (n=6)
0	3.73 ± 0.14	3.71 ± 0.23	4.36 ± 0.14	3.68 ± 0.12	3.54 ± 0.13
30	5.59 ± 0.24	5.42 ± 0.22	5.32 ± 0.21	5.46 ± 0.10	5.51 ± 0.04
60	6.85 ± 0.10	6.51 ± 0.16 [a]	6.55 ± 0.06 [a]	5.67 ± 0.21 [b]	5.54 ± 0.08 [b]
120	6.43 ± 0.21	6.25 ± 0.17 [a]	6.58 ± 0.10 [a]	5.34 ± 0.09 [b]	5.55 ± 0.23 [b]
180	6.55 ± 0.11	6.10 ± 0.13 [a]	5.92 ± 0.17 [a]	5.12 ± 0.12 [b]	5.50 ± 0.11 [b]
240	5.55 ± 0.10	5.49 ± 0.24	5.49 ± 0.08 [a]	4.82 ± 0.04 [b]	5.13 ± 0.16 [a]
300	5.40 ± 0.21	4.98 ± 0.24 [a]	5.19 ± 0.05 [a]	4.55 ± 0.04 [b]	4.82 ± 0.22 [a]
360	5.26 ± 0.16	4.71 ± 0.15 [a]	5.12 ± 0.12 [a]	4.22 ± 0.11 [b]	4.72 ± 0.07 [a]

Data are stated in (mm) paw thickness as (mean ± SEM).
n = number of animals.
Time (0) is the time of i.p. injection of tested compounds, Diclofenac Sodium and DMSO (control).
Time (30) is the time of injection of fresh egg-white (induction of paw edema).
Non-identical superscripts (a and b) among different groups are considered significantly different p-value <0.05.

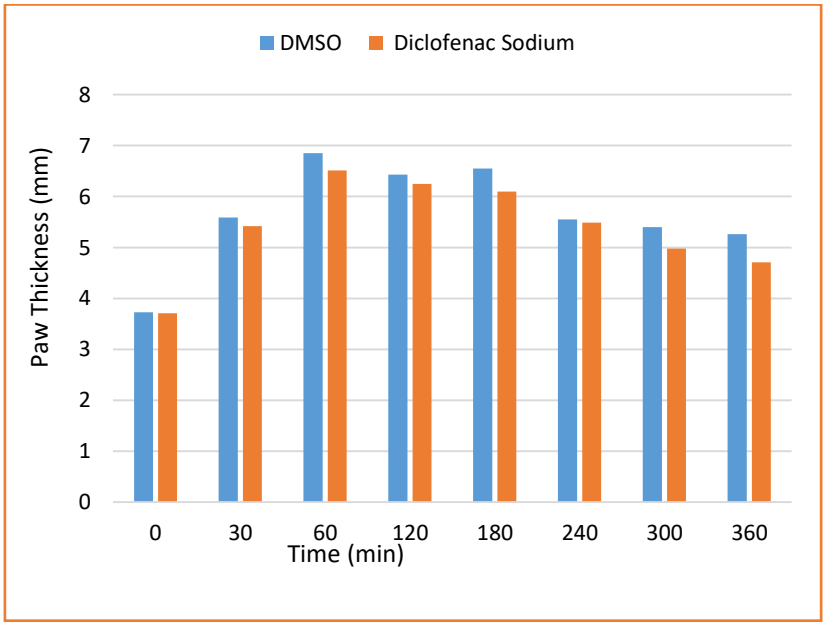


Figure 1-The effect of Diclofenac Sodium and dimethyl sulfoxide on the fresh egg-white induced paw edema in rats Time (30) is the time of egg-white injection

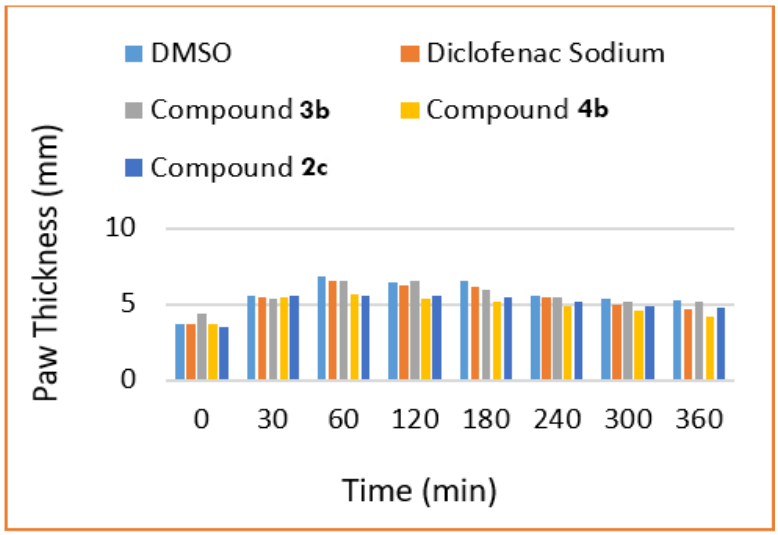


Figure 2-The effect of Diclofenac Sodium, dimethyl sulfoxide, compounds 3b, 4b and 2c on the egg-white induced paw edema in rats

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