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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Abstract:
New 2-Mercaptobenzimidazole derivatives were synthesized. 4,5-disubstituted 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 chloroacetate 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 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 1b-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 (CS2) 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, 1H-NMR and 13C-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.

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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 heterocyclic 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 able 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-marcatobenzimidazole [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 thio benzimidazole 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 0\(^\circ\)C. 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° | Yield % | Color | ν(N-H₂) | ν(N-H) | ν(C-H) Arom. | ν(C=O) | ν(C=N) |
| a   | ![Structure](image.png) | 186-188 | 94 | White | — | 3134 | 3049 | — | 1628 |
| b   | ![Structure](image.png) | 54-56 | 82 | Off white | — | — | 3055 | Ester 1753 | 1641 |
| 1b  | ![Structure](image.png) | 158-160 | 63 | Off white | Asym. 3438 | Sym. 3398 | 3245 | 3066 | amid 1695 | 1650 |
| 2b  | ![Structure](image.png) | 168-170 | 75 | Off white | Asym. 3468 | Sym. 3417 | 3232 | 3066 | amid overlap with ν(C=N) | 1620 |
| 3b  | ![Structure](image.png) | 166-168 | 64 | Brown | — | 3255 | 3066 | amid 1664 | 1620 |

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

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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 e (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$_2$S 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$^\circ$ | Yield % | Color | ν(N-H) | ν(C-H) Arom | ν(C-H) Alpha | ν(C= N) | other |
| 4b  | ![Structure](image) | 172-174 | 85 | Off white | 3290 | 3068 | 2964 2885 | 1662 | ν(OH) 3380 |
| 5b  | ![Structure](image) | 178-180 | 73 | Off white | 3251 | 3049 | 2964 2862 | 1645 | ν(S-H) 2478 |
| 6b  | ![Structure](image) | 178-180 | 65 | Brown | 3055 | 2964 2925 2860 | 1647 | ν(OH) 3429 |
| c   | ![Structure](image) | 134-136 | 91 | Pale white | 3288 | 3060 | 2958 2925 | 1637 | ν(NH$_2$) Asym. 3444 Sym. 3390 ν(C=O) Amid 1668 |
| 1c  | ![Structure](image) | 168-170 | 76 | Off white | 3222 | 2979 | 2935 2894 | 1629 | ν(C=O) amid 1694 ν(C=S) 1494 |
| 2c  | ![Structure](image) | 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 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).
The FTIR spectrum data of compound a show absorption bands at 3134 cm\(^{-1}\) for stretching vibration of NH\([25]\), 3049 cm\(^{-1}\) for \(\nu\) (CH arom.), 2962, 2860 and 2808 cm\(^{-1}\) for \(\nu\) (CH aliph.), 1628 cm\(^{-1}\) for \(\nu\) (C=N imidazole), and 1512 for \(\nu\) (C=C arom.). While The FTIR spectrum data of compound b show the disappearance of the absorption band 3134 cm\(^{-1}\) that belongs to \(\nu\) (-NH), with new absorption bands that appeared at 1753 cm\(^{-1}\) for \(\nu\) (C=O) and 1207 cm\(^{-1}\) for \(\nu\) (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 \(\nu\) (C=O) of the ester. There is a new absorption band at 1695-1664 cm\(^{-1}\) which belong to \(\nu\) (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 \(\nu\) (NH\(_2\)) Asym. and Sym., respectively. There are also absorption bands at 3255-3232 cm\(^{-1}\) which belongs to \(\nu\) (NH), at 3066 cm\(^{-1}\) which belongs to \(\nu\) (CH) aromatic, and at 1650-1620 cm\(^{-1}\) which belongs to \(\nu\) (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 \(\delta=4.58\) ppm which belongs to 2H due to (-SCH\(_2\)), a new singlet signal at \(\delta=4.72\) ppm which belongs to 1H due to (NH-Ar), a singlet signal at \(\delta=5.31\) ppm which belongs to 2H due to (-NCH\(_2\)), a multiplet signal at \(\delta=7.13-7.46\) ppm which belongs to 14H due to (Ar-H), a new singlet signal at \(\delta=9.10\) ppm which belongs to 1H due to (C=O-NH-N), and a new singlet signal at \(\delta=12.4\) 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 \(\delta=38.67\) ppm which belongs to (-SCH\(_2\)), a singlet signal at \(\delta=120.87\) ppm which belongs to (-NCH\(_2\)), a singlet signal at \(\delta=149.15\) ppm due to (C=N) of imidazole ring, a signal at \(\delta=163.15\) ppm due to (C=O) of amide, and a signal at \(\delta=168.49\) 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 ($\delta$ ppm) | $^{13}$C-NMR spectral data ($\delta$ ppm) |
|----------|-----------|---------------------------------------|------------------------------------------|
| 3b       | ![Structure](image) | 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, Aromatic); 9.10 (s, 1H, CO-NH-NH-); 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 refluxing 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 $\delta$= 12.4 ppm due to 1H which belongs to (-NH) of triazole ring, while the singlet signal at $\delta$= 3.51 ppm is due to 2H and belongs to (-SCH$_2$), the singlet signal at $\delta$= 4.48 ppm belongs to 2H (-NCH$_2$), the multiplet at $\delta$= 7.14-7.46 ppm is for 9H (Ar-H), and the singlet signal at $\delta$= 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 $\delta$= 3.50 ppm which belongs to 2H of (-SCH$_2$), a singlet signal at $\delta$= 4.58 ppm which belongs to 2H (-NCH$_2$), a multiplet signal at $\delta$= 6.97-7.46 ppm which belongs to 14H for (Ar-H), and a singlet signal at $\delta$= 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 ($\delta$ ppm) | $^{13}$C-NMR spectral data ($\delta$ ppm) |
|----------|-----------|---------------------------------------|------------------------------------------|
| 4b       | ![Structure](image) | 3.51 (s,2H, S-CH$_2$); 4.48 (s, 2H, N-CH$_2$); 7.14-7.46 (m, 9H, Aromatic); 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       | ![Structure](image) | 3.50 (s,2H, S-CH$_2$); 4.58 (s, 2H,N-CH$_2$); 6.97-7.46 (m, 9H, Aromatic); 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 e 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 $\delta=3.28$ ppm which belongs to 1H due to (-SH), a singlet signal at $\delta= 4.09$ ppm which belongs to 2H for (-SCH$_2$), a signal at $\delta= 4.57$ppm which belongs to 2H of (-NH$_2$), a signal at $\delta= 5.25$ ppm which belongs to 2H for (-NCH$_2$), and a multiplet signal at $\delta= 7.08$-7.44 ppm which belongs to 9H of (Ar-H). The $^{13}$C-NMR spectral data of compound 2c show a signal at $\delta= 38.69$ ppm which belongs to (-SCH$_2$), a signal at $\delta= 117.28$ ppm which belongs to (-NCH$_2$), a multiplet signal at $\delta= 126.76$-128.28 ppm which belongs to (Ar), a signal at $\delta= 149.26$ ppm which belongs to (C=N) triazole ring, and a signal at $\delta= 158.28$ 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       | ![Structure](image) | 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 (semincarbazide, 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) | 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: dimethylsulfoxide

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 tested 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

| Paw Thickness (mm) | Time (min) | DMSO (n=6) | Diclofenac Sodium (n=6) |
|-------------------|------------|------------|------------------------|
|                   | 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<sup>a</sup> | 6.55 ± 0.06<sup>a</sup> | 5.67 ± 0.21<sup>b</sup> | 5.54 ± 0.08<sup>b</sup> |
| 120       | 6.43 ± 0.21 | 6.25 ± 0.17<sup>a</sup> | 6.58 ± 0.10<sup>a</sup> | 5.34 ± 0.09<sup>b</sup> | 5.55 ± 0.23<sup>b</sup> |
| 180       | 6.55 ± 0.11 | 6.10 ± 0.13<sup>a</sup> | 5.92 ± 0.17<sup>a</sup> | 5.12 ± 0.12<sup>b</sup> | 5.50 ± 0.11<sup>b</sup> |
| 240       | 5.55 ± 0.10 | 5.49 ± 0.24              | 5.49 ± 0.08<sup>a</sup> | 4.82 ± 0.04<sup>b</sup> | 5.13 ± 0.16<sup>a</sup> |
| 300       | 5.40 ± 0.21 | 4.98 ± 0.24<sup>a</sup> | 5.19 ± 0.05<sup>a</sup> | 4.55 ± 0.04<sup>b</sup> | 4.82 ± 0.22<sup>a</sup> |
| 360       | 5.26 ± 0.16 | 4.71 ± 0.15<sup>a</sup> | 5.12 ± 0.12<sup>a</sup> | 4.22 ± 0.11<sup>b</sup> | 4.72 ± 0.07<sup>a</sup> |

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](image-url) - 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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