Egypt. J. Chem. Vol. 67, SI: M. R. Mahran, pp.529– 536 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Novel Acrylamide-Pyrazole Conjugates: Design, Synthesis and Antimicrobial Evaluation

Mamdouh A. Abu-Zaied [a], Galal H. Elgemeie [b], Reham A. Mohamed-Ezzat [c]

[a] Green Chemistry Department, Chemical Industries Research Institute, National Research Centre, Dokki, Giza,12622, Egypt

[b] Chemistry Department, Faculty of Science, Helwan University, Helwan, Cairo,11795, Egypt

[c] Chemistry of Natural & Microbial Products Department, Pharmaceutical and Drug Industries Research Institute, National Research Center, Dokki, Giza, Cairo, 12622, Egypt

In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract

A novel series of acrylamide-pyrazole conjugates were synthesized using a new strategy starting from 3-isobutyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **1**. The reaction pathway was performed via unexpected routes to generate new pyrazole derivatives through the reaction of pyrazole methylene malononitrile **3a** or pyrazole acrylic acid ethyl ester derivative **3b** respectively with 2-Cyano-*N*-aryl acetamide derivatives **4a-e** to yield the unexpected phenyl-1*H*-pyrazol-4-yl)-*N*-aryl-acrylamide derivatives **7a-e**. The structures of the synthesized compounds were confirmed via elemental and spectroscopic analysis (IR, ^{1}H NMR, ^{13}C NMR). The antibacterial and antifungal activities of the compounds were estimated against some species of Gram (−ve) bacteria such as Klebsiella pneumoniae and Gram (+ve) bacteria that are experiencing a high rate of antibiotic resistance nowadays and against Candida albicans fungus. Compound **7c** showed activities against *Escherichia coli* (inhibition zone 23±1 mm, and *Klebsiella* pneumoniae (inhibition 21±1mm)as compared to Gentamicin (inhibition zone 27±0.1 mm) & (inhibition zone 29±0.5 mm), and exhibiting potent inhibitory activity toward candida albicans fungus (inhibition zone 25±1 mm) compared to Nystatin (inhibition zone 28±0.2). Most of the novel synthesized pyrazoles showed significant potencies as compared to standard antibiotics.

Keywords: Pyrazole, Aryl-acrylamide, Antibacterial activity, Antifungal activity.

1. Introduction

The need for novel antibiotic medicines has arisen due to resistance to currently existing antibiotics. The resistance of infections to currently approved antibiotics has increased; those infections were caused by various species as Staphylococcus aureus, and Pseudomonas aeruginosa. Acquisition of drug resistance genes is the main factor behind the spread and emergence of resistant bacterial species leading to a broad range of antibiotic resistance (e.g., Cephalosporin-resistant mutant beta-lactamases discovered in numerous bacterial organisms) [1]. While the rate and degree of resistance exhibited by bacteria to various antimicrobial agents differ, resistance has ultimately developed to all anti-microbial agents [2]. Different chemicals that are effective against a certain disease-causing bacterium must be accessible in order to stop or slow the development of a resistant pathogen population. Therefore, it is necessary to synthesize novel compounds that can enter the pathogenic bacterial cell and either kill it or stop its growth without also negatively impacting their host [1].

The pyrazole core is a basis of biologically significant molecules with a wide range of pharmacological and biological properties [3,4]. Condensed heterocyclic derivative transformations are also of theoretical interest for the development of novel synthetic techniques and the investigation of the connections between the reactivity and chemical structure of organic compounds. Heterocyclic ligands based on pyrazoles can have a variety of biological uses. For instance, a number of the compounds have been produced with high efficiency as potential antibacterial or antifungal agents [5].

The pyrazole nucleus existence in various structures results in diversified applications in several fields. Additionally, pyrazole derivatives possess various potencies as antiviral [6], anti-inflammatory [7], anti-diabetic [8], anti-glaucoma [9], and antimicrobial activities [10]. Furthermore, pyrazole pro-drugs have been verified to sustain remarkable anticancer potency [11]. Moreover, pyrazole nucleus is a unique structural moiety necessary for medicinal chemistry and a main scaffold for many drugs (Fig.1) [12-15].

*Corresponding author e-mail: mamdouh_abozaid@yahoo.com

Receive Date: 25 December 2023, Revise Date: 20 January 2024, Accept Date: 04 March 2024

DOI: 10.21608/ejchem.2024.258180.9074

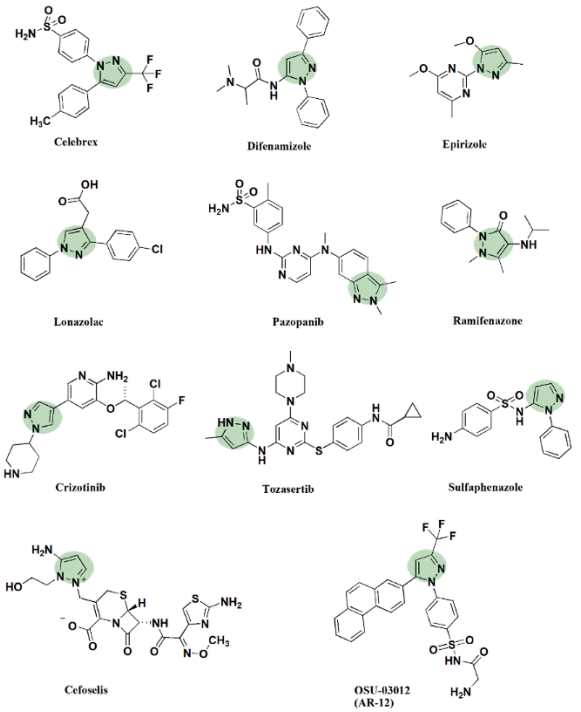


Fig. 1. Pyrazole-based drugs

It's worth to note that various pyrazole derivatives which possess several activities have been synthesized [16-22]. On the other hand, acrylamide moiety possesses therapeutic potential for targeting various diseases [23]. Recently starting from acrylamide, we have synthesized several heterocycles which have various potencies as anti-SARS-CoV-2 [24], antitumor [24, 25] and other applications [26-29].

Thus, here in, we have synthesized novel pyrazoles using acrylamide derivatives to generate significant bioactive molecules containing pyrazole moiety.

2. Experimental

2.1. Chemical Methods

Using a GallenKamp melting point device, all melting points were determined. At the National Research Centre, Cairo, Egypt, the ^{1}H and ^{13}C NMR spectra were measured using a Jeol-500 MHz/ spectrometer in DMSO-d_6 or $CDCl_3$, using $Si(CH_3)_4$ as an internal standard. The Microanalytical Unit at Cairo University, Faculty of Science, performed the elemental analysis. TLC was used to track the reactions progress using aluminium sheets covered in silica gel F_{254} (Merck). Detection was impacted while viewing under a short-wavelength UV lamp. Compound **1** was synthesised in accordance with our earlier publication [30].

2.1.1. General procedure for synthesizing 3a,b

To a solution of pyrazole-4-carbaldehyde derivative **1** (10 mmol) in absolute ethanol (10 mL) containing 3drops of triethylamine, active methylene compounds (malononitrile**2a**, ethyl cyanoacetate**2b** (10 mmol) was added, and stirred for 5 min. The solid precipitate was formed filtered off and recrystallized from the ethanol to give compounds **3a**,**b**

*2.1.1.1. 2-((3-isobutyl-1-phenyl-1H-pyrazol-4-yl) methylene) malononitrile(**3a**)*

White solid; (EtOH); yield (90%); m.p. 169 °C; IR (KBr, cm^{-1}) υ 3132 (C-H aromatic), 2950 (CH), 2216, 2210 (2CN), 1612 (C=N), 1594 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.90 (d, J = 6.4 Hz, 6H, 2xCH_3), 1.94-1.96 (m, 1H, CH), 2.65 (d, J = 7.2 Hz, CH_2), 7.4٥-7.84 (m, ٥H, C_6H_5),8.08 (s, 1H, vinylic-H), 9.01 (s, 1H, pyrazole H-5). Anal. Calcd. for. $C_{17}H_{16}N_4$ (276.34): C, 73.89; H, 5.84; N, 20.27. Found: C, 73.80; H, 5.75; N, 20.18%.

*2.1.1.2. 2-Cyano-3-(3-isobutyl-1-phenyl-1H-pyrazol-4-yl)-acrylic acid ethyl ester (**3b**)*

white solid; (EtOH); Yield 92%; m.p. 117 °C; IR (KBr, cm^{-1}) ν3029(CH aromatic), 2954 (CH), 2215(CN), 1715(C=O), 1605 (C=N). ^{1}HNMR (500 MHz, DMSO-d_6): δ 0.98 (d, 6H, J = 6.9 Hz, 2xCH_3), 1.40 (t, 3H, J = 6.9 Hz, CH_3 ester), 2.03-2.04 (m, 1H, CH), 2.69 (d, 2H, J = 6.8 Hz, CH_2), 4.37 (q, 2H, CH_2 ester), 7.37 (t, 1H, J = 7.7 Hz, aromatic H-4), 7.48 (t, 2H, J = 7.65 Hz, aromatic H-3, H-5), 7.74 (d, 2H, J = 8.4 Hz, aromatic H-2, H-6), 8.17 (s, 1H vinylic-H), 8.98 (s, 1H, pyrazole H-5); ^{13}C NMR (125 MHz, DMSO-d_6): δ 14.33 (CH_3 ester), 22.59 (2C, 2xCH_3), 29.38 (CH), 35.05 (CH_2), 62.44 (CH_2 ester), 98.63 (pyrazole C-4), 115.88 (vinylic C-2), 116.95 (CN), 119.92 (2C, Ar-c), 127.95 (Ar-C), 128.94 (2C, Ar-C), 129.71 (pyrazole C-5), 139.03 (Ar-C), 145.47 (pyrazole C-3), 156.99 (vinylic C-1), 163.03 (C=O). Anal. Calcd. For. $C_{19}H_{21}N_3O_2$ (323.39): C, 70.57; H, 6.55; N, 12.99. Found: C, 70.45; H, 6.56; N, 12.90%.

2.1.2. General Procedure for the Synthesis of 2-Cyano-3-(3-isobutyl-1-phenyl-1H-pyrazol-4-yl)-N-aryl-acrylamide (٧a-e)

A solution of 2-Cyano-*N*-aryl-acetamide**4a** or **4b** (10 mmol each) in ethanol-water mixture (1:1 ratio) contained sodium hydroxide (10 mmol) was treated with pyrazole methylene malononitrile derivative **3a** or pyrazole acrylic acid ethyl ester derivative **3b** (10 mmol each) and heated under reflux. A period of twelve hours was required for complete reaction. The reaction mixture then, was left to cool at room temperature, the solid precipitate was collected by filtration, dried and recrystallized from an appropriate solvent to give compounds **7a-e**

2.1.2.1. 2-Cyano-3-(3-isobutyl-1-phenyl-1H-pyrazol-4-yl)-N-phenyl-acrylamide (7a).

white solid; (EtOH); Yield 92%; m.p.١٧٢ °C; IR (KBr, cm^{-1}) υ 3459 (NH), 3058 (C-H aromatic), 2956

(CH), 2215 (CN), 1667 (C=O), 1616 (C=N), 1599 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.97 (d, 6H, J = 6.8 Hz,2xCH_3), 1.99-2.03 (m, 1H, CH), 2.75 (d, 2H, J = 7.2 Hz, CH_2), 7.14-7.86 (m, 10 H, 2C_6H_5), 8.14 (s, 1H, vinylic-H), 9.04 (s,1H, pyrazole H-5), 10.28 (br, s, D_2O exch., 1H, NH). Anal . Calcd. For. $C_{23}H_{22}N_4O$ (370.45): C, 74.57; H, 5.99; N, 15.12. Found: C, 74.48; H, 5.88; N, 15.10 %.

2.1.2.2. 2-Cyano-3-(3-isobutyl-1-phenyl-1H-pyrazol-4-yl)-N-(4-methoxy-phenyl)-acrylamide (7b)

white solid; (EtOH); Yield 95%; m.p.١٨٠ °C; IR (KBr, cm^{-1}) υ 3436 (NH), 3054 (C-H aromatic), 2966 (CH), 2217 (CN), 1669 (C=O), 1604 (C=N), 1594 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.99 (d, 6H, J = 6.65 Hz, 2xCH_3), 1.90-1.93 (m, 1H, CH), 2.67 (d, 2H, J = 8.24 Hz, CH_2), 3.68 (s, 3H, CH_3), 6.71-7.80 (m, 9H, C_6H_5, C_6H_4), 8.26 (s, 1H, vinylic-H), 9.98 (s, 1H, pyrazole H-5), 10.42 (br, s, D_2O exch., 1H, NH), Anal. Calcd. For. $C_{24}H_{24}N_4O_2$ (400.47): C, 71.98; H, 6.04; N, 13.99: Found: C, 71.86; H, 6.14; N, 13.89 %.

2.1.2.3. 2-Cyano-3-(3-isobutyl-1-phenyl-1H-pyrazol-4-yl)-N-p-tolyl-acrylamide (7c)

white solid; (EtOH); Yield 95%; m.p. 195 °C; IR (KBr, cm^{-1}) υ 3451 (NH), 3029 (C-H aromatic), 2966 (CH), 2217 (CN), 1654 (C=O), 1607 (C=N), 1592 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.99 (d, 6H, J = 5.7 Hz, 2xCH_3), 2.05-2.08 (m, 1H, CH), 2.34 (s, 3H, CH_3), 2.71 (d, 2H, J = 6.65 Hz, CH_2), 7.1٨-7.٧٥ (m, 9H, C_6H_5, C_6H_4), 8.35 (s, 1H, vinylic-H), 8.9٠ (s, 1H, pyrazole H-5), 10.81 (br, s, D_2O exch., 1H, NH). Anal. Calcd. For. $C_{24}H_{24}N_4O$ (384.47): C, 74.97; H, 6.29; N, 14.57; Found: C, 74.87; H, 6.18; N, 14.48 %.

2.1.2.4. N-(4-Chloro-phenyl)-2-cyano-3-(3-isobutyl-1-phenyl-1H-pyrazol-4-yl)-acrylamide (7d)

white solid; (EtOH); Yield 95%; m.p. 218 °C; IR (KBr, cm^{-1}) υ 3462 (NH), 3036 (C-H aromatic), 2948 (CH), 2218 (CN), 1653 (C=O), 1610 (C=N), 1597 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.9٣ (d, 6H, J = 6.65 Hz, 2xCH_3), 1.98-2.٠١ (m, 1H, CH), 2.7٠ (d, 2H, J = 7.15 Hz, CH_2), 7.38 (d, 3H, J = 9.1Hz, C_6H_5), 7.53 (t, 2H, J = 8.6, 7.6 Hz, C_6H_5), 7.68 (d, 2H, J = 8.6 Hz, C_6H_4), 7.80 (d, 2H, J = 7.6 Hz, C_6H_4), 8.10 (s, 1H, vinylic-H), 8.99 (s, 1H, pyrazole H-5), 10.36 (br, s, D_2O exch., 1H, NH). Anal. Calcd. For. $C_{23}H_{21}ClN_4O$ (404.89): C, 68.23; H, 5.23; Cl, 8.76; N, 13.84; Found: C, 68.12; H, 5.13; Cl, 8.68; N, 13.75 %.

2.1.2.4. N-(4-Bromo-phenyl)-2-cyano-3-(3-isobutyl-1-phenyl-1H-pyrazol-4-yl)-acrylamide (7e)

white solid; (EtOH); Yield 95%; m.p. ٢٢4-٢٢5 °C; IR (KBr, cm^{-1}) υ 3455 (NH), 3045 (C-H aromatic), 2960 (CH), 2210 (CN), 1663 (C=O), 1602 (C=N), 1591 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.93 (d, 6H, J = 6.7 Hz, 2xCH_3), 1.99-2.02 (m, 1H, CH), 2.71 (d, 2H, J = 7.15 Hz, CH_2), 7.52-7.63 (m, 9H, C_6H_5, C_6H_4), 8.10 (s, 1H, vinylic-H), 9.00 (s, 1H, pyrazole H-5), 10.36 (br, s, D_2O exch., 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 22.84 (2C, 2xCH_3), 28.91 (CH), 34.3 (CH_2), 103 (pyrazole C4), 115.9 (C=CH), 117.45 (CN), 119.82 (2C, Ar-C), 123.41 (2C, Ar-C), 128.15 (Ar-C), 128.74 (Pyrazole C-5), 130.36 (2C, Ar-C), 132.05 (2C,Ar-C), 138.22 (2C, Ar-C), 142.15 (Ar-C), 156.28 (Pyrazole C-3), 159.11 (-CH=C), 161.01 (C=O). Anal. Calcd. For. $C_{23}H_{21}BrN_4O$ (449.34): C, 61.48; H, 4.71; Br, 17.78; N, 12.47. Found: C, 61.38; H, 4.60; Br, 17.68; N, 12.38%.

4.2. Antimicrobial assay:

The agar well diffusion method was utilized to ascertain the antimicrobial activity of the produced compounds [31]. Using nutritional agar medium, all of the compounds were evaluated for their antibacterial activity against Gram (+ve) bacteria (Streptococcus mutans and Staphylococcus aureus), Gram (−ve) bacteria (Pseudomonas aeruginosa and Klebsiella), and Escherichia coli. Standard medications for Gram (−ve) bacteria and Gram (+ve) bacteria were Gentamicin and Ampicillin, respectively. The solvent control was DMSO. The compounds were evaluated against bacterial and fungal strains at a dosage of 15 mg/ml.

2.2.1. Method of testing:

After filling each 20–25 ml sterilized Petri dish with the sterilized media, the dishes were left to harden at room temperature. The turbidity of the microbial suspension was adjusted to OD= 0.13 using a spectrophotometer set to 625 nm. The suspension was made in sterile saline, which was equivalent to the McFarland 0.5 standard solution (1.5 x 105 CFU mL^{-1}). The ideal time to regulate the turbidity of the inoculum solution was fifteen minutes. After that, a sterile cotton swab was dipped into the suspension, inundated on the dried agar surface, and let to dry for fifteen minutes with a lid on. Using a sterile borer, wells of 6 mm in diameter were created in the solidified material. Using a micropipette, 100 µL of the tested compound's solution was applied to each well. For a full day, the plates were incubated at 37°C to check for antibacterial activity. The zones of inhibition were measured in triplicate and reported on a millimetre scale throughout this experiment.

The study was approved under no. 6447082023 by the Medical Research Ethics Committee (MERC) federal (accurance no. : FWA 00014747)

3. Results and discussion.

3.1. Chemistry

The target and intermediate compounds were synthesized using the procedures shown in Scheme 1. Therefore,3-isobutyl-1-phenyl-1*H*-pyrazole-4-

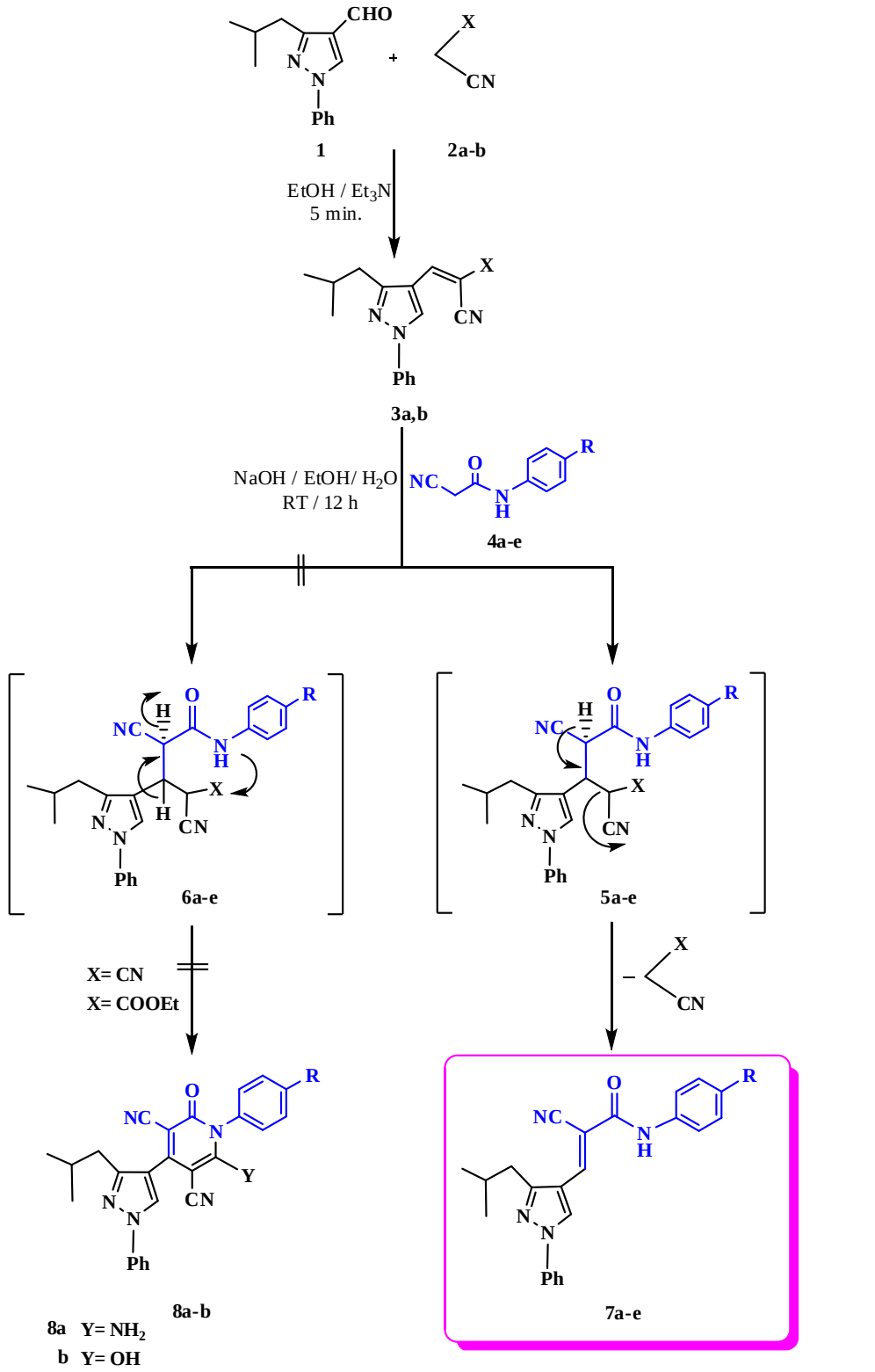
carbaldehyde **1** is condensationally reacted with active methylene compounds (malononitrile **2a**, ethyl Cyanoacetate **2b**), using triethylamine as a catalyst afforded pyrazole malononitrile **3a** /and pyrazole acrylic acid ethyl ester derivatives **3b** respectively**.** The spectroscopic and microanalysis data revealed the structure of compound **3a-b**. The IR spectra of **3a** indicated the disappearance of aldehydic carbonyl group, which detected at the parent compound **1,** with the appearance of two new absorption bands at 2216, 2210 cm^{-1} (2CN). Moreover, the ^{1}H NMR spectra of compound **3a** showed the singlet signal at δ 8.08 corresponding to (vinylic-H).

Reaction of pyrazolylmethylenemalononitrile/ ethyl cyanoacetate **3a**,**3b** with 2-Cyano-*N*-aryl-acetamide derivatives **4a-e** did not yield the expected pyridine derivatives **8a,b**. The products obtained from this reaction proved to be phenyl-1*H*-pyrazol-4-yl)-*N*-aryl-acrylamide derivative**7a-e** via studying its spectra (^{1}H NMR, ^{13}C NMR, IR). Mechanism for this reaction is accomplished in scheme 1. The reaction initiates via the nucleophilic attack of active methylene group of the 2-Cyano-*N*-aryl-acetamide **4a-e** to double bond of compounds **3a**,**b** to give Michael addition adduct followed by the elimination of a molecule of malononitrile or ethyl cyanoacetate. Thus, the IR spectrum of compound **7a** as a representative example showed the presence of a characteristic -NH band at 3459 $cm^{-1}$and 1667 cm^{-1} (CONH). Its ^{1}H NMR spectrum indicated the existence of a signal at 10.28 ppm (1H, NH D_2O exchangeable) and singlet signal at 7.86 ppm (vinylic-H). Because there is less steric hindrance in product 7, it is possible that it is the thermodynamically controlled product and that it forms instead of the N-aryl-2-pyridone 8.

3.2. Antimicrobial evaluation.

The novel compounds (**3a,b** & **7a-e**) were estimated for their *in vitro* anti-bacterial efficacy against some species of Gram (−ve) bacteria, such as *Escherichia coli* (ATCC:10536), Acinetobacter *baumannii* (ATCC:10536), *Klebsiella pneumonia* (ATCC:10536), and *Pseudomonas aeruginosa*, along with two Gram (+ve) bacteria, as *Streptococcus mutans* (ATCC:25175) and Staphylococcus aureus (ATCC:13565).

Meanwhile, their efficacy against the fungus Candida albicans (ATCC:10231) was evaluated. To estimate the preliminary antibacterial and antifungal potencies, the agar diffusion assay was utilized. Nystatin was utilized as a conventional antifungal medication. Standard medications such as Gentamicin, Tigecycline, and Ampicillin were also employed to combat Gram (-ve) and Gram (+ve) bacterial strains. The average diameter of the inhibition zones surrounding the disks, measured in millimetres, was used to represent the results. As accomplished in table 1, compound **3a** displayed activity against *Acinetobacter baumannii* (inhibition zone 19±1mm)as compared to Tigecycline (inhibition zone 23±0.4 mm). On the other hand, compounds **3a** &**7a** are also revealed fungal zone of inhibition with the value 22±1mm against the *Candida albicans* as compared to Nystatin (28±0.2). Among these compounds, compound **7b** showed activities against *Escherichia coli* (inhibition zone 22±1 mm, and *Klebsiella* pneumonia (inhibition zone25±1mm), compared to Gentamicin (inhibitionzone27±0.1 mm) & (inhibition zone 29±0.5 mm), respectively. In addition, **7b** showed activities *Acinetobacter baumannii* of (inhibition zone 18±1mm &20±1mm) as compared to Tigecycline (inhibition zone 23±0.4 mm). Both compounds also revealed fungal zone of inhibition with the value23±1mm against the *Candida albicans* compared to Nystatin (28±0.2) Meanwhile, compound **7c** showed activities against *Escherichia coli* (inhibition zone 23±1 mm, and *Klebsiella* pneumoniae (inhibition 21±1mm)as compared to Gentamicin (inhibition zone 27±0.1 mm) & (inhibition zone 29±0.5 mm), respectively. In addition, it indicated activity against *Acinetobacter baumannii* (inhibition zone 21±1 mm as compared to Tigecycline (inhibition zone 23±0.4 mm), however it reveals no inhibition zones on the tested gram positive species. This compound also revealed fungal zone of inhibition with the value 25±1mm against the *Candida albicans* compared to Nystatin (28±0.2). Furthermore, compound **7d** exhibited activities against the *Escherichia coli* (inhibition zone 15±1 mm) compared to Gentamicin (inhibition zone 27±0.1 mm). Compound **7e** showed remarkable activities against *Escherichia coli* (inhibition zone 21±1 mm, and *Klebsiella pneumonia* (inhibition zone 23±1mm)compared to Gentamicin (inhibition zone 27±0.1 mm) & (inhibition zone 29±0.5 mm), respectively. Also, it showed remarkable activities against gram positives species such as *Staphylococcus aureus* and *Streptococcus mutans* with inhibition zones 20±1 & 22±1 as compared with Ampicillin with inhibition zone 29±0.2 & 22±0.1 mm respectively. This compound also indicated fungal zone of inhibition with the value 22±1mm against the *Candida albicans* compared to Nystatin (28±0.2).



Scheme 1. Synthesis of (pyrazol-4-yl)-*N*-aryl-acrylamide derivatives 7a-e

Table 1. Determination of the antimicrobial activity of compounds (3a,b & 7a-e) against different antibacterial and fungal strains.

Compound / Microorganism	3a	3b	7a	7b	7c	7d	7e	Standard antibiotic
Gram negative bacteria								
Escherichia coli	NA	NA	NA	22±1	23±1	15±1	21±1	Gentamicin 27±0.1
Klebsiella pneumoniae	NA	NA	NA	25±1	21±1	NA	23±1	Gentamicin 29±0.5
Pseudomonas aeruginosa	NA	NA	NA	NA	NA	NA	NA	Gentamicin 32±0.4
Acinetobacter baumannii	19±1	NA	NA	18±1	21±1	NA	NA	Tigecycline 23±0.4
Gram positive bacteria								
Staphylococcus aureus	NA	NA	NA	NA	NA	NA	20±1	Ampicillin 29±0.2
Streptococcus mutans.	NA	NA	NA	NA	NA	NA	22±1	Ampicillin 22±0.1
Fungi								
Candida albicans	22±1	NA	21±1	23±1	25±1	NA	22±1	Nystatin 28±0.2

NA: No activity;

- The expression for zone of inhibition is represented by mean± standard deviation (mm).

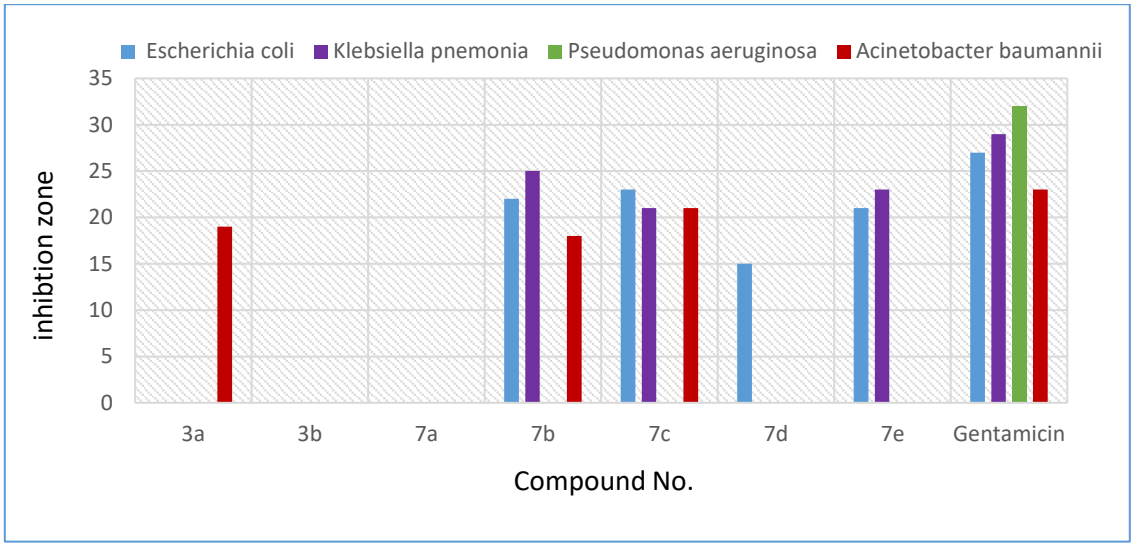


Fig. 2. The anti-bacterial activities of the novel synthesized compounds and Gentamicin (standard drug) were compared against Gram –ve bacteria.

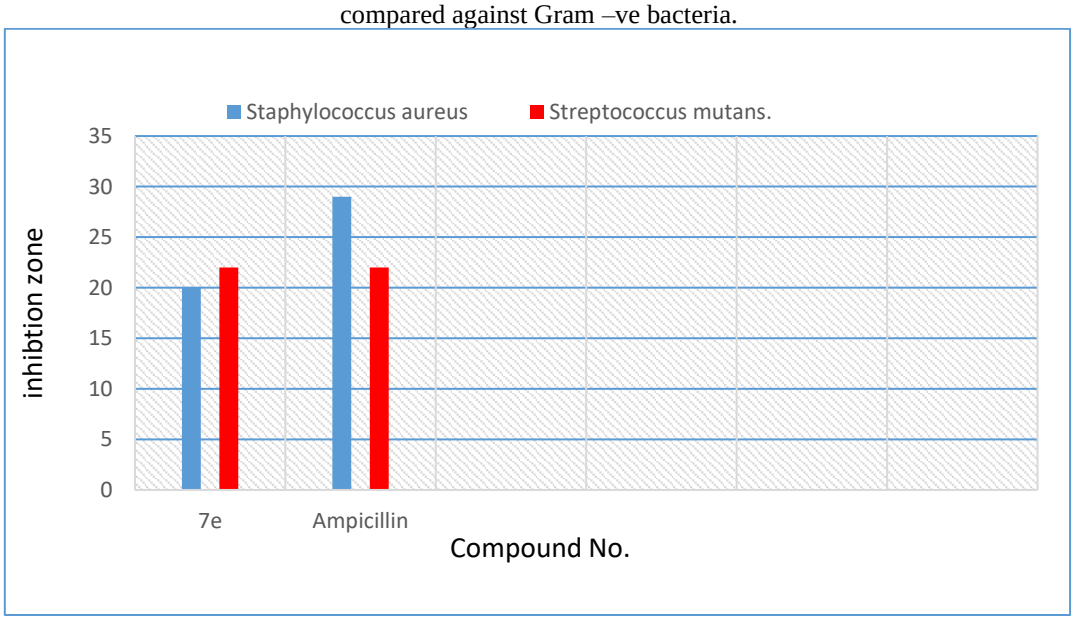


Fig. 3. The anti-bacterial activities of the novel synthesized compounds and Ampicillin (standard drug) were compared against Gram +ve bacteria.

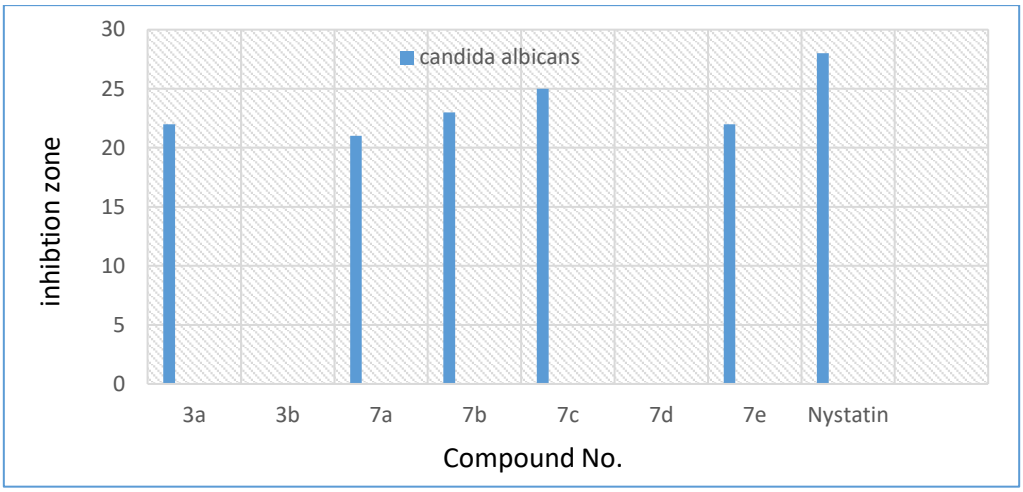


Fig. 4. The anti-fungal activities of the novel synthesized compounds (3a, 3b & 7a-e) and Nystatin (standard drug) were compared against the *candida albicans* fungus.

4. Conclusions

New acrylamide-pyrazole conjugates were designed and synthesized via unexpected reaction pathway utilizing the synthesized 2-(3-isobutyl-1-phenyl-1*H*-pyrazol-4-ylmethylene)-malononitrile and 2-cyano-3-(3-isobutyl-1-phenyl-1*H*-pyrazol-4-yl)-acrylic acid ethyl ester. The structures of compounds were confirmed using spectroscopic techniques. The antimicrobial activities of the novel structures were evaluated which revealed promising results for developing these new pyrazoles as potent antimicrobial agents as compared to standard antibiotics.

5. Conflict of interest

There is no conflict of interest

6. References

[1] L. Li, X. Chen, S. T. Cutler.Pyrazole antimicrobial agents. US6673923B2.

[2] H. S. Gold & R. C. Moellering, Jr. Antimicrobial-drug resistance. *New Eng. J Med.* 1996, 335(19):1445-1453. DOI: 10.1056/NEJM199611073351907.

[3] M. H. Abdellattif, E. O. Hamed, N. K. R. Elhoseni, *et al.* Synthesis of novel pyrazolone candidates with studying some biological activities and in-silico studies. *Sci Rep* 2023, **13**, 19170. DOI:10.1038/s41598-023-43575-z.

[4] E.O. Hamed, M. G. Assy, N. H. Ouf, D. A. Elsayed& M. H. Abdellattif. Cyclization of *N*-acetyl derivative: Novel synthesis—Azoles and azines, antimicrobial activities, and computational studies. *Heterocycl.Commun.*2022, **28**, 35–43. DOI:10.1515/hc-2022-0004.

[5] Y. Kaddouri. New *N*-alkylated heterocyclic compounds as prospective NDM1 inhibitors: Investigation of in vitro and in silico properties. *Pharmaceuticals* 2022, **15**, 803. DOI:10.3390/ph15070803.

[6] G. Ouyang, Z. Chen, X. J. Cai, B. A. Song, P. S. Bhadury, S. Yang, L. H. Jin, W. Xue, D.Y. Hu, & S. Zeng. Synthesis and antiviral activity of novel pyrazole derivatives containing oxime esters group, *Bioorg. Med. Chem.* 2008, **16,** 9699-9707.DOI:10.1016/j.bmc.2008.09.070.

[7] M. Mantzanidou, E. Pontiki, & D. Hadjipavlou-Litina.Pyrazoles and Pyrazolines as Anti-Inflammatory Agents.*Molecules (Basel, Switzerland)*.2021, **26**, 3439. DOI:10.3390/molecules26113439.

[8] M. J. Naim, O. Alam, M. J. Alam, M. Shaquiquzzaman, M. M. Alam & V. G. M. Naidu. Synthesis, docking, in vitro and in vivo antidiabetic activity of pyrazole-based 2,4-thiazolidinedione derivatives as PPAR-γ modulators. *Archiv der Pharmazie*. 2018, **351** (3-4), e1700223. DOI:10.1002/ardp.201700223.

[9] R. Kasımoğulları, M. Bülbül, B. S. Arslan& B. Gökçe. Synthesis, characterization and antiglaucoma activity of some novel pyrazole derivatives of 5-amino-1,3,4-thiadiazole-2-sulfonamide. *Eur. J. Med. Chem.* 2010, **45**, 4769–4773. DOI:10.1016/j.ejmech.2010.07.041.

[10] M. Marinescu. Synthesis of Antimicrobial Benzimidazole-Pyrazole Compounds and Their Biological Activities.*Antibiotics (Basel, Switzerland)*.2021, **10**, 1002. DOI:10.3390/antibiotics10081002.

[11] G. H. Elgemeie& R.A. Mohamed-Ezzat (2022b).New Strategies Targeting Cancer Metabolism, pp. 1–619. Publisher: Elsevier (Amsterdam). ISBN: 978-0-12-821783-2.
DOI: 10.1016/C2019-0-00369-X.

[12]R.Sivaramakarthikeyan, S.Iniyaval, V.Saravanan, W. Lim, C. Mai & C. Ramalingan. Molecular Hybrids Integrated with Benzimidazole and Pyrazole

Structural Motifs: Design, Synthesis, Biological Evaluation, and Molecular Docking Studies. *ACS Omega* 2020, **5**, 10089–10098. DOI:10.1021/acsomega.0c00630.

[13] G. H. Elgemeie, M. A. Abu-Zaied, Hebishy, N. Abbas and M. Hamed. A First Microwave-Assisted Synthesis of a New Class of Purine and Guanine Thioglycoside Analogs. *Nucleosides Nucleotides,* 2016, **35**, 459-478. DOI: 10.1080/15257770.2016.1202964.

[14] N. Devi, R. Shankar, V. Singh. 4-Formyl-Pyrazole-3-Carboxylate: A Useful Aldo-X Bifunctional Precursor for the Syntheses of Pyrazole-fused/Substituted Frameworks, *J. Heterocycl. Chem.*,2018, **55**, 373-390. DOI:10.1002/jhet.3045.

[15] H. Kumar, K.K. Bansal& A. Goyal. Synthetic Methods and Antimicrobial Perspective of Pyrazole Derivatives: An Insight, Anti-Infective Agents 2020, **18**,207-223. DOI:10.2174/2211352517666191022103831.

[16] M. A. Abu-Zaied &G. H. Elgemeie. Novel synthesis of new pyrazolethioglycosides as pyrazomycin analogues, *Nucleosides Nucleotides*. 2019, **38**, 374-389, DOI: 10.1080/15257770.2018.1554220.

[17] M. A. Abu-Zaied & G. H. Elgemeie. Synthesis of the first novel pyrazolethioglycosides as deaza ribavirin analogues, *Nucleosides Nucleotides.* 2017, **36**, 713-725, DOI: 10.1080/15257770.2017.1378817.

[18] G. H. Elgemeie, R. A. Mohamed, Microwave chemistry: Synthesis of purine and pyrimidine nucleosides using microwave radiation, *J. Carbohydr. Chem.* 2019, **38**, 1-47. DOI:10.1080/07328303.2018.154343.

[19] G. H. Elgemeie, S. A. Alkhursani, R. A. Mohamed, New synthetic strategies for acyclic and cyclic pyrimidinethione nucleosides and their analogues, *Nucleosides Nucleotides.* 2019, **38**, 12−87.DOI:10.1080/15257770.2018.1498511.

[20] G. H. Elgemeie, A. M. Salah, N. S. Abbas, H. A. Hussein, R. A. Mohamed, Pyrimidine non-nucleoside analogs: A direct synthesis of a novel class of *N*-substituted amino and *N*-sulfonamide derivatives of pyrimidines, *Nucleosides Nucleotides.* 2017, **36**, 213-223. DOI:10.1080/15257770.2016.1257808.

[21] G. H. Elgemeie, M. A. Abu-Zaied, S. A. Loutfy, 4-Aminoantipyrine in carbohydrate research: Design, synthesis and anticancer activity of thioglycosides of a novel class of 4-aminoantipyrines and their corresponding pyrazolopyrimidine and pyrazolopyridinethioglycosides, *Tetrahedron*, 2017, **73**, 5853-5861. DOI:10.1016/j.tet.2017.08.024.

[22] G. H. Elgemeie, M. Abu-Zaied, & P.G. Jones. Crystal structure of 4-{[(cyano-imino)(methyl-sulfanyl)methyl]amino}-1,5-dimethyl-2-phenyl-2,3-di-hydro-1*H*-pyrazol-3-one. *Actacrystallographica. Section E, Crystallographic communications*, 2015, 71(Pt1),104–106. DOI:10.1107/S2056989014027601.

[23] G. Autore, A. Caruso, S. Marzocco, B. Nicolaus, C. Palladino, A. Pinto, A. Popolo, M. S.Sinicropi,G. Tommonaro, & C. Saturnino. Acetamide derivatives with antioxidant activity and potential anti-inflammatory activity. *Molecules (Basel,Switzerland).*2010, **15**, 2028–2038. DOI:10.3390/molecules15032028.

[24] R. A. Mohamed-Ezzat, G. H. Elgemeie, Discovery and Synthesis of Novel Bio-Isostere of Purine Analogues Inhibiting SARS-CoV-2. *Egypt.J.Chem.*,2023, ***66***, 167-185. DOI:10.21608/ejchem.2023.196504.7681.

[25] R. A. Mohamed-Ezzat, B. M. Kariuki, G. H. Elgemeie, Unexpected products of the reaction of cyanoacetylhydrazones of aryl/heteryl ketones with hydrazine: A new route to aryl / heterylhydrazones, X-ray structure, and in vitro anti-proliferative activity against NCI 60-cell line panel. *Egypt. J. Chem.*, 2023, **66**, 225-239.DOI:10.21608/ejchem.2023.197330.7682.

[26] G. H. Elgemeie, A. M. Salah, N. S. Abbas, H. A. Hussein, R. A. Mohamed. Nucleic acid components and their analogs: Design and synthesis of novel cytosine thioglycosideanalogs, *Nucleosides &Nucleotides*. 2017, **36**, 139-150. DOI: 10.1080/15257770.2016.1231318.

[27] G. H. Elgemeie, R. A. Mohamed, H. A. Hussein& P.G. Jones.Crystal structure of*N*-(2-amino-5-cyano-4-methylsulfanyl-6-oxo-1,6-dihydropyrimidin-1-yl)-4-bromobenzene-sulfonamide dimethylformamidemonosolvate, *Acta Crystallogr E Crystallogr Commun.* 2015, E71, 1322–1324. DOI:10.1107/S2056989015018903.

[28] R. A. Mohamed-Ezzat, G. H. Elgemeie, &P. G. Jones. Crystal structures of (*E*)-2-amino-4-methylsulfanyl-6-oxo-1-(1-phenylethylideneamino)-1,6-dihydro-pyrimidine-5-carbonitrile and (*E*)-2-amino-4-methyl-sulfanyl-6-oxo-1-[1-(pyridin-2-yl)ethylideneamino]-1,6-dihydropyrimidine-5-carbonitrile. *ActaCrystallogr E CrystallogrCommun.* 2021, **77**, 547-550. DOI:10.1107/S2056989021004126**.**

[29] R. A. Mohamed-Ezzat, A.H. Hashem& S. Dacrory. Synthetic strategy towards novel composite based on substituted pyrido[2,1-*b*][1,3,4]oxadiazine-dialdehyde chitosan conjugate with antimicrobial and anticancer activities. *BMC Chemistry*.2023, **17**, 88. DOI:10.1186/s13065-023-01005-1.

[30] M.A. Abu-Zaied, E.M. El-Telbani, G.H. Elgemeie, G.A.M. Nawwar, Synthesis and in vitro anti-tumor activity of new oxadiazolethioglycosides, *Eur. J. Med. Chem.* 2011, **46**, 229-235. DOI: 10.1016/j.ejmech.2010.11.008.

[31] A. C. Scott (1989). Laboratory control of antimicrobial therapy. In: Collee JG *et al*. eds. *Practical Medical Microbiology*, 13th Edition. Edinburgh: Churchill Livingstone, 161.