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RESEARCH ARTICLE

Design, Antimicrobial Testing, and Molecular Docking Studies of New Chalcone and Pyrimidine Derivatives Based on 2-phenyl-1H-pyrazol-3(2H)-one

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Abstract: ***Background & Objectives*:** Heterocyclic pyrimidine and pyrazole rings have attracted the interest of medicinal chemists because of their pharmacological potential including antimicrobial activity. Based on molecular hybridization, new chalcones **6a-g** and pyrimidines **7a-g** based on a pyrazole scaffold were designed.

***Methods*:** The synthesis of these compounds involved mild condensation reactions between compound **4** and various aromatic aldehydes in a mixture of ethanol/NaOH (95:5 v/v) to give the corresponding chalcones **6a-g**. These chalcones were further reacted with urea in the presence of a base in ethanol to produce the pyrimidine derivatives **7a-g**. These new candidates were screened for their *in vitro* antimicrobial activities and molecular docking studies were evaluated.

***Results*:** The antibacterial and antifungal studies of all synthesized compounds against the strains tested showed that compounds **6c, d**, and **7c, d** exhibited the highest antibacterial and antifungal activities. In addition, the structure-activity relationship and docking studies are discussed.

***Conclusion*:** The synthesized compounds **6c, 6d**, **7c, and 7d** showed the highest antibacterial and antifungal activities against the tested strains.

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1. INTRODUCTION

According to the World Health Organization (WHO), bacterial infections are among the top ten greatest threats to humanity [1]. As we enter more diverse ecosystems, new serious variants of known species are discovered each year along with increasing bacterial resistance to current antimicrobial agents, leading to potentially devastating consequences [2]. For this reason, extensive research has been directed towards the discovery of new structures with potent biological activity to minimize the emergence of these drug-resistant bacteria [3-5]. In this regard, the use of different products has been evaluated over time. Natural products and their derivatives have been described as a relevant source of bioactive compounds for drug research [6, 7]. Chalcones (1,3-diphenyl-2-propen-1-ones) belong to a group of natural compounds that have attracted significant interest from medicinal chemists for their specific therapeutic uses. Chalcones are the precursors of flavonoids [8, 9] and their derivatives have been implicated in various biological activities, including antibacterial [10, 11] antimalarial [12], anti-inflammatory [13], antioxidant [14], anticancer [15-17], *etc*.

Meanwhile, literature searches have also revealed that pyrimidine has been used extensively as the central unit for the synthesis of a variety of novel bioactive molecules [18]. Its derivatives have occupied an important position in medicinal chemistry due to their wide application as drugs and drug intermediates. It has been reported that compounds comprising the pyrimidine ring exhibit a wide range of pharmacological activities. In addition, various analogues of pyrimidines have been found to possess antimicrobial [19, 20], anti-inflammatory [21], analgesic, anticonvulsant, and anti-Parkinsonian activities [22].

In connection with our aim to synthesize and evaluate the biological activity of new potent bioactive compounds [23-33], we wanted to develop a new series of chalcone and pyrimidine derivatives containing 2-phenyl-1H-pyrazol-3(2H)-one moiety. Our scope in this study was to construct novel

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pyrazole-pyrimidine derivatives *via* the merge between pyrimidine core and pyrazole nucleus which are important scaffolds as antimicrobial agents with glucosamine-6-phosphate synthase (GlcN-6-P) inhibitory effect. The antibacterial and antifungal activities of each chalcone **6a-g** and pyrimidine derivative **7a-g** were evaluated using an agar well diffusion assay by measuring the mean diameter of the zone of inhibition. Furthermore, molecular docking studies were performed in our study to accurately predict the optimized conformations for both the newly synthesized compounds (as ligands) and protein targets to form a stable complex.

2. MATERIALS AND METHODS

2.1. Chemistry

All chemical reagents used for the synthesis were purchased from Sigma Aldrich (Somatco Trading Co. Ltd., Sakaka, Aljouf, Saudi Arabia) and used without further purification. The progress of the reaction was controlled using thin layer chromatography (TLC) on F254Merck plates (Darmstadt, Germany) precoated with silica gel. Spot visualization was performed with UV irradiation at 350-380 nm. Melting points (m.p.) of the synthesized compounds were determined on a Gallenkamp electrothermal melting point apparatus and are uncorrected. The elemental analyses (C, H, and N) of the synthesized compounds were performed on the CE 440 Elemental Analyzer-Automatic Injector (Exeter Analytical, Inc., USA) at the Microanalytical Center of Cairo University. IR spectra were recorded on a Shimadzo infrared spectrophotometer (Research Laboratory, Chemistry Department, Jouf University) using potassium bromide disks. ^{1}H, ^{13}C-NMR spectra were recorded on a Varian Mercury VXR-300 NMR spectrometer (Palo Alto, CA) at 400 and 125 MHz in dimethyl sulfoxide-d 6 (DMSO-d_6). Chemical shifts are given in ppm (δ) relative to the internal standard TMS. Mass spectra were obtained at 70 eV on a Shimadzu GCMS-QP 1000EX spectrometer.

2.1.1. Synthesis of 4-((4-(4-chlorophenyl)-2-oxobut-3-en-1-yl)diazenyl)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (6c)

80% yield as pale yellow crystals; mp 225-227^{o}C; U.V (λ_{max}) 365 nm; IR (KBr): ν (cm^{-1}), 2870-3154 (CH_2, CH), 1653-1699 (2C=O), 1652-1674 (C-N) pyrazolo ring, 1623 (CH═CH),1537 (N=N); ^{1}H NMR (δ, DMSO-d6): 2.22 (s,3H,CH_3), 2.40 (s, 3H, CH_3), 2.59 (s, 2H, CH_2), 3.02 (s, 1H, CH), 3.27 (s, 1H, CH), 6.94 (d, *J* =9.11 *Hz*, 2H, ArH), 6.99 (d, *J* =9.14*Hz*, 2H, ArH), 7.45 (d, *J* = 8.46 *Hz*, 2H, ArH), 7.69 (d, *J* = 15.62 *Hz*, 1H, H),) 7.79 (d, *J* = 15.62 *Hz*, 1H, H), 7.67 (d, *J* = 15.63 *Hz*, 1H, ArH) , 8.02 (d, *J* =9.13 *Hz*, 2H, ArH); ^{13}C NMR (DMSO-d_6): 27.04, 36.23 ($2CH_3$),61.25(CH_2), 114.78,159.13(C=C),123.03,124.01,125.55, 126 .45, 127.09, 129.29, 134.29 ,142.37(CH=CH), 164.86,195.93(2C=O); MS (ESI) *m/z*: calcd for $C_{21}H_{19}ClN_4O_2$ [M]: 394.85, found: 394.55; Analysis calculated for: $C_{21}H_{19}ClN_4O_2$ (394.85): C, 63.88; H, 4.85; Cl, 8.98; N, 14.19.Found : C, 63.90; H, 4.88; Cl, 8.99; N, 14.21.

2.1.2. Synthesis of 1,5-dimethyl-4-((4-(4-nitrophenyl)-2-oxobut-3-en-1-yl)diazenyl)-2-phenyl-1H-pyrazol-3(2H)-one (6d)

78% yield as orange crystals; mp 200-202^{o}C; U.V (λ_{max}),373 nm; IR (KBr): ν (cm^{-1}), 2870-3154 (CH_2, CH), 1653-1699 (2C=O), 1652-1674 (C-N) pyrazolo ring, 1630 (CH═CH),1537 (N=N); ^{1}H NMR (δ, DMSO-d6): 2.23 (s,3H,CH_3), 2.42 (s, 3H, CH_3), 2.60 (s, 2H, CH_2), 3.04 (s, 1H, CH), 3.27 (s, 1H, CH), 6.92 (d, *J* =9.99 *Hz*, 2H, ArH), 6.98 (d, *J* =9.12*Hz*, 2H, ArH), 7.42 (d, *J* = 8.44 *Hz*, 2H, ArH), 7.68 (d, *J* = 15.61 *Hz*, 1H, H), 7.73 (d, *J* = 8.42 *Hz*, 2H, ArH), 7.65 (d, *J* = 15.61 *Hz*, 1H, H) , 8.02 (d, *J* =9.11 *Hz*, 2H, ArH); ^{13}C NMR (DMSO-d_6): 27.04, 36.23 ($2CH_3$),61.25(CH_2), 114.78, 159.13 (C=C),123.03,124.01,125.55, 126 .45, 127.09, 129.29, 134.29 ,142.37(CH=CH), 164.87, 195.96 (2C=O); MS (ESI) *m/z*: calcd for $C_{21}H_{19}N_5O_4$ [M]: 405.41, found: 405; Analysis calculated for: $C_{21}H_{19}N_5O_4$ (405.41): C, 62.22; H, 4.72; N, 17.27.Found : C, 62.26; H, 4.72; N, 17.30.

2.1.3. Synthesis of 4-((((6-(4-chlorophenyl)-2-hydroxy-1,6-dihydropyrimidin-4-yl)methyl)diazenyl) -1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (7c)

66% yield as Orange crystals; mp 280-282^{o}C; U.V (λ_{max}) 357 nm; IR (KBr): ν (cm^{-1}), 1667 (C=O), 1610-1575 (C-N) pyrazolo ring, 1510 (N=N), 3313-3172 (NH & OH); ^{1}H NMR (δ, DMSO-d_6): 2.15 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.58 (s, 2H, CH_2), 4.57 (s, 1H, OH), 4.88 (d,1H,CH), 5.09 (s, 1H, NH), 6.83 (d, *J* =9.90 *Hz*, 1H, ArH), 7.19 (d, *J* =9.90 *Hz*, 2H, ArH), 7.23 (d, *J* =9.3*Hz*, 2H, ArH), 7.76(d, *J* = 8.43 *Hz*, 2H, ArH), 7.74(d, *J* = 15. 59 *Hz*, 2H, ArH), 8.02 (d, *J* =9. 11 *Hz*, 2H, ArH);^{13}C NMR (DMSO-d_6): 27.04, 36.23 ($2CH_3$),61.25 (CH_2), 51.65 , 118.64 (CH-CH), 114.78, 159.13 (C=C) ,123.03, 124.01, 125.55, 126 .45, 127.09, 129.29 (C=C), 141.12(C-N),155.04(C=N),165.01 (C=O);MS (ESI) *m/z*: calcd for $C_{22}H_{21}ClN_6O_2$ [M]: 436.89,Found: 436; Analysis calculated for $C_{22}H_{21}ClN_6O_2$ (436.89); Calcd: C, 60.48; H, 4.84; Cl, 8.11; N, 19.24;Found: C, 60.50; H, 4.92; Cl, 8.14; N, 19.26 .

2.1.4. Synthesis of 4-(((2-hydroxy-6-(4-nitrophenyl)-1,6-dihydropyrimidin-4-yl)methyl)diazenyl)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (7d)

69% yield as red crystals; mp 233-237 ^{o}C; U.V (λ_{max})377 nm; IR (KBr): ν (cm^{-1}), 1653 (C=O), 1652-1674 (C-N) pyrazolo ring, 1517 (N=N), 3210 (NH), 3403(OH); ^{1}H NMR (δ, DMSO-d_6): 2.15 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.58 (s, 2H, CH_2), 4.57 (s, 1H, OH), 4.94 (d,1H,CH), 5.09 (s, 1H, NH), 6.85 (d, *J* =9.89 *Hz*, 1H, ArH), 7.21 (d, *J* =9.92 *Hz*, 2H, ArH), 7.25 (d, *J* =9.2*Hz*, 2H, ArH), 7.77(d, *J* = 8.44 *Hz*, 2H, ArH), 7.75(d, *J* = 15. 62 *Hz*, 2H, ArH), 8.02 (d, *J* =9. 12 *Hz*, 2H, ArH);^{13}C NMR (DMSO-d_6): 27.04, 36.23 ($2CH_3$),61.25 (CH_2), 51.65 , 118.64 (CH-CH), 114.78, 159.13(C=C) ,123.03, 124.01, 125.55, 126 .45, 127.09, 129.29 (C=C), 141.12(C-N),155.04(C=N),165.01 (C=O);MS (ESI) *m/z*: calcd for $C_{22}H_{21}N_7O_4$ [M]: 447.45,Found: 447; Analysis calculated for $C_{22}H_{21}N_7O_4$ (447.45); Calcd: C, 59.05; H, 4.73; N, 21.91;Found: C, 59.08; H, 4.75; N, 21.93.

2.2. Antimicrobial Activity

In this work, the *in vitro* antimicrobial activity (antibacterial and antifungal) of all synthesized compounds **4**, **6a-g**, and **7a-g** was evaluated using a Kirby–Bauer disk diffusion method [34]. Plates impregnated with gram-positive (*Bacillus subtilis and Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) were incubated at 35-37°C for 24-48 h. The standard discs of benzylpenicillin (Pencillin-G) and fluconazole served as positive controls for antibacterial activity and antifungal, respectively. Filter discs impregnated with 10 µL of solvent (distilled water, chloroform, and DMSO) were used as negative controls. All experiments were repeated and performed in triplicate in case of a significant difference in the results and the mean inhibition diameters were measured in mm/mg sample.

2.3. Molecular Docking Simulation Study

Crystal structure of glucosamine-6-phosphate synthase enzyme (GlcN-6-P). Preparation of the downloaded enzyme and the target compounds were performed according to reported method [35]. It was prepared as a receptor by removing water and co-crystallized ligands and ions, then protonated using the Pymol software ver. 2.5.1. Validation of docking protocol had been done by redocking the cocrystallized ligand iside GlcN-6-P with RMSD = 1.1342. Meanwhile, the newly synthesized candidates were converted to 3D by chemdraw, and were optimized by using the MMFF94 force field by Avogadro Software. Blind docking was done by the use of CB-DOCK2. CB-Dock predicts protein cavities and measures the centers and sizes of top N (n = 5 by default) cavities. The profiles of interaction and visualization were performed for the best-docked complexes using Discovery Studio software.

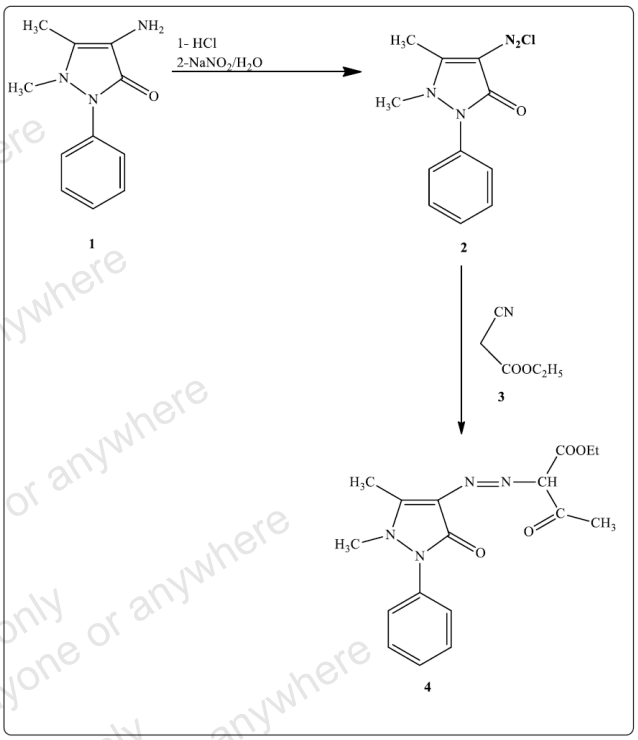
3. RESULTS AND DISCUSSION

3.1. Chemistry

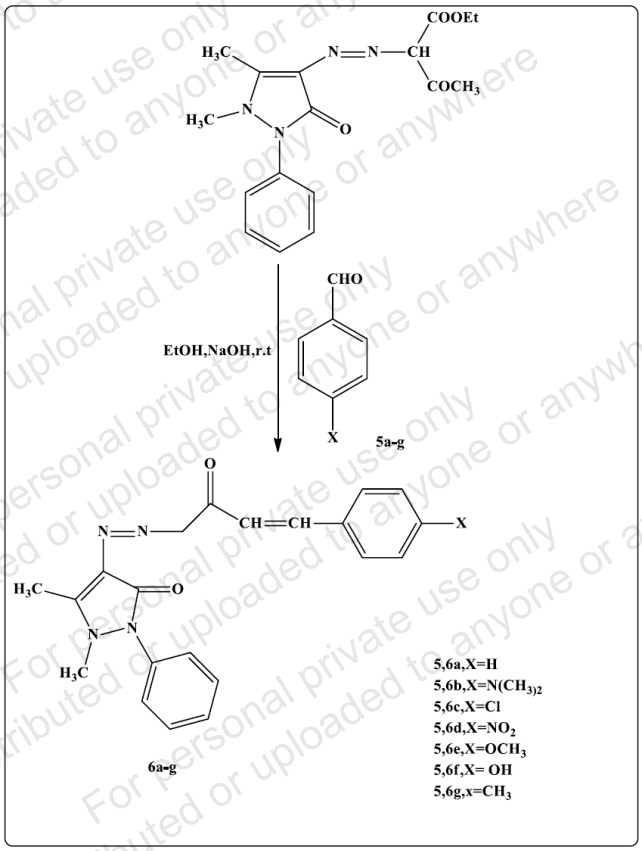
In this work, a new series of chalcones **6a-g** and pyrimidine derivatives **7a-g** based on -phenyl-1*H*-pyrazol-3(2H)-one units were designed and their biological activity was evaluated. Thus, after treatment with HNO_2 generated in situ from $NaNO_2$/HCl at 0-5^oC, 4-amino antipyrine **1** was converted into its corresponding diazonium salt 2 by diazotization [36]. Reaction of the diazonium salt **2** with ethyl acetoacetate in a mixture of sodium acetate and ethanol solutions afforded ethyl 2-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)diazenyl)-3-oxobutanoate **4** as an orange colored crystal powder. The formation of the monoazo dye **4** is shown in Scheme **1**.

The synthesis of chalcones and pyrimidine derivatives was performed following the steps shown in Scheme **2**.

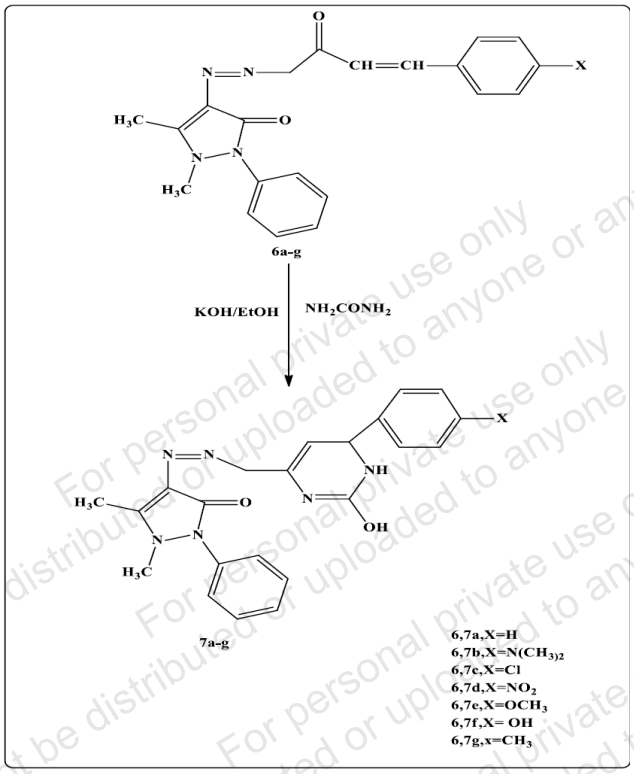
The reaction proceeded *via* the Claisen-Schmidt condensation reaction [27] between the synthesized azo dye **4** and the aromatic benzaldehyde derivatives **5a-g** in dilute ethanolic sodium hydroxide solution at room temperature [28]. Then, compounds **7a-g** were synthesized by reacting the prepared chalcones **6a-g** with urea and potassium hydroxide in ethanol (Scheme **3**).



Scheme 1. The chemical structures of the new monoazodye **4**.



Scheme 2. Synthesis of chalcone derivatives **6a-g**.



Scheme 3. Synthesis of pyrimidine derivatives **7a-g**.

The purity of all synthesized compounds was determined by thin layer chromatography (TLC) and elemental analysis. All synthesized compounds **4, 6a-g**, and **7a-g** were obtained in moderate to good yields (64–77%) and their spectral data (IR, ^{1}H NMR, ^{13}C NMR) were in full agreement with the proposed structures. The IR spectrum of chalcones **6a-g** indicated the existence of absorption bands in the respective regions of 2870-3154 cm^{-1}, 1653–1699 cm^{-1}, 1652-1674 cm^{-1}, 1537 cm^{-1}, and 1620-1630 cm^{-1} corresponding to (CH_2, CH), C=O, C-N (pyrazole ring), N=N, and CH=CH groups.

On the other hand, the IR spectrum of compounds **7a-g** showed bands at 1516, 1653, 1652-1674 and 3403 cm^{-1}, 3210 cm^{-1} and corresponding to N=N, C=O group, C-N pyrazole ring and, OH, and NH groups respectively.

The ^{1}H-NMR spectrum of compounds **6a-g** showed signals varying from 2.21 and 2.59 ppm corresponding to CH_2 and CH_3 groups. All multiplet depicted at δ = 7.2-8.02 ppm were assigned the aromatic protons present in compounds **6a-g**. In addition, the ^{1}H NMR spectrum of compounds **7a-g** showed signals at 2.15, 4.05, and 13.7 ppm for CH_3, CH_2, and NH, respectively. The ^{1}H NMR spectra of these pyrimidine derivatives showed signals at 7.2-8.02 ppm, each corresponding to aromatic protons. The results showed that all analytical and spectral data of the synthesized compounds were consistent with the proposed structures.

3.2. Biological Evaluation

3.2.1. Antimicrobial Activity and Structure-activity Relationship (SAR)

The newly synthesized compounds **4**, **6a-g**, and **7a-g** tested *in vitro* for their antibacterial activity against two-gram positive bacteria: namely: *Bacilli subtilis,* Staphylococcus aureus, and two gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Benzylpenicillin (Penicillin-G) was used as a reference to evaluate the potency of the tested compounds under the same conditions (Table **1**). The mean inhibition zone diameter (MIZ, mm/mg sample) (n=3) was determined as a parameter of the antibacterial activity. As shown in Table **1**, tested compounds **6b-6f**, **7c**, **7d**, **7f** showed moderate to high antibacterial potential against all screened bacteria with an inhibition zone range (MIZ = 5-16 mm/mg). The chalcone derivatives **6c**, **6d**, and pyrimidines **7c**, **7d** (MIZ = 10-17 mm/mg) displayed the highest antibacterial activity and showed a comparable potential to penicillin-G (MIZ = 12-16 mm/mg). The structure-activity relationship (SAR) of the newly constructed compounds suggested that the withdrawing effect of the substituent group attached to the para position of the phenyl ring of the synthesized compounds induced an increase in their antimicrobial activity compared to the others. For example, replacing the hydrogen atom on the phenyl group in compounds **6a** and **7a** with electron-withdrawing groups such as nitro **6d**, **7d**, and chloro **6c**, **7c** showed a significant improvement against gram-positive and gram-negative bacteria. The results showed that the presence of electron donating groups such as dimethylamino group at the para position of the phenyl ring in compounds **6b** and **7b** slightly reduced their biological activities against the bacteria tested. Furthermore, the compounds with the hydroxyl group **6f** and the methyl group **6g**, **7g** showed the lowest antibacterial activity compared to those bearing electron-withdrawing groups substituents at the para-position of the phenyl ring. The exception was presented in compound **7f**, which showed good antibacterial activity against *Pseudomonas aeruginosa.* Besides, the target compounds **4**, **6a-g** and **7a-g** were evaluated for their antifungal potential using *Aspergillus flavus*, *Candida albicans*, *Aspergillus Fumigates* and *Penicillium Marneffei* as fungal strains using fluconazole as a standard. The antifungal results are presented in Table **1**, which shows that all of the new compounds exhibited antifungal potential against all fungal species tested. Compound **7b** showed good antifungal activity against *Aspergillus Fumigates* and *Candida albicans*. It is worth noting that compounds **6c**, **6d**, **7c**, and **7d** were the most active antifungal candidates with MIZ = 9-19 mm/mg.

3.2.2. Molecular Docking Simulation Study

The prepared compounds **4**, **6a-g**, and **7a-g** were docked within the active site of glucosamine-6-phosphate synthase (GlcN-6-P) to predict their mechanism of action. The crystal structure of GlcN-6-P with the cocrystallized ligand was downloaded from the Protein Data Bank (PDB: ID 1XFF). Validation of docking protocol was done by redocking the cocrystallized ligand iside GlcN-6-P with RMSD = 1.1342 (Fig. **S1**). Data obtained from the docking study, including binding energy scores (kcal/mol), hydrogen bonding interactions between functional groups and amino acid residues, are listed in Table **2**. The chalcone derivative **6b** recorded a binding energy score (-16.90 kcal/mol), showing four hydrogen bonds with amino acids GLN9 and ARG216. In addition, compound **2b** within GlcN-6-P involved the interaction with ALA58, LEU63, LEU17, PRO62, ALA13, ALA38 and PRO166 amino acid residues through pi-alkyl and pi-sigma interactions (Fig. **1**).

Table 1. Antimicrobial assessment of the new synthesized compounds4, 6a-g, and 7a-g.

Compound no.	Gram-positive Bacteria		Gram-negative Bacteria		Fungi			
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus flavus*	*Candida albicans*	*Aspergillus Fumigates*	*Penicillium Marneffei*
Mean inhibition zone diameter (mm/mg sample) (n=3) ± SD								
Control (DMSO)	0	0	0	0	-	-	-	-
Benzylpenicillin (Pencillin-G)	13.00 ± 0.24	14.00 ± 0.42	12.00 ± 0.06	16.00 ± 0.10	-	-	-	-
Fluconazole	-	-	-	-	20.00 ± 0.26	18.00 ± 0.04	20.00 ± 0.62	21.00 ± 0.09
4	-	-	-	1.11 ± 0.08	9.13 ± 0.34	11.14 ± 0.15	12.12 ± 0.53	13.11 ± 0.82
6a	-	-	-	7.12 ± 0.05	10.11 ± 0.47	10.14 ± 0.24	10.11 ± 1.3	9.13 ± 1.31
6b	12.12 ± 0.06	11.13 ± 0.39	9.11 ± 0.27	9.14 ± 0.27	14.13 ± 0.61	12.12 ± 0.32	11.11 ± 0.84	8.10 ± 0.76
6c	11.14 ± 0.13	16.12 ± 1.10	13.13 ± 0.04	15.12 ± 0.23	19.14 ± 0.09	17.11 ± 1.22	15.13 ± 0.63	10.11 ± 0.59
6d	12.12 ± 0.22	15.13 ± 0.53	14.11 ± 0.17	17.14 ± 1.59	17.12 ± 1.94	12.14 ± 0.48	16.13 ± 1.58	5.14 ± 0.98
6e	11.14 ± 0.38	13.11 ± 1.28	5.12 ± 1.28	13.12 ± 0.06	13.14 ± 2.04	11.11 ± 0.07	10.10 ± 1.18	9.13 ± 2.26
6f	7.12 ± 0.90	10.14 ± 1.25	11.11 ± 0.92	9.12 ± 0.27	12.13 ± 1.26	14.12 ± 0.05	13.14 ± 0.07	12.11 ± 0.58
6g	-	-	10.12 ± 1.21	11.14 ± 2.61	10.11 ± 0.99	13.14 ± 0.14	13.13 ±	8.12 ± 0.18
7a	-	-	-	8.12 ± 0.09	11.11 ± 0.07	14.14 ± 0.16	14.11 ± 0.32	15.13 ± 1.33
7b	11.13 ± 0.94	-	8.11 ± 0.34	12.12 ± 0.56	13.13 ± 1.05	17.14 ± 1.63	19.13 ± 0.87	14.11 ± 0.22
7c	12.11 ± 1.08	13.13 ± 0.23	13.12 ± 0.45	10.11 ± 1.27	18.14 ± 0.67	18.11 ± 0.88	16.12 ± 0.48	13.13 ± 0.83
7d	12.14 ± 1.69	12.11 ± 2.95	12.12 ± 1.78	14.11 ± 0.07	9.14 ± 1.18	12.13 ± 0.99	12.11 ± 0.56	10.12 ± 1.27
7e	-	7.10 ± 0.55	12.11 ± 1.94	6.12 ± 2.48	13.11 ± 1.77	12.12 ± 1.11	15.11 ± 1.63	9.10 ± 0.22
7f	8.11 ± 1.88	10.14 ± 2.13	11.13 ± 0.89	14.11 ± 2.47	11.13 ± 0.68	13.11 ± 2.27	6.10 ± 1.53	11.13 ± 2.07
7g	10.13 ± 1.67	-	7.12 ± 0.45	12.14 ± 0.94	10.11 ± 0.54	12.13 ± 0.56	9.11 ± 1.51	6.14 ± 0.65

Table 2. Docking simulation data for compounds 4, 6a-g, and 7a-g within GlcN-6-P active site.

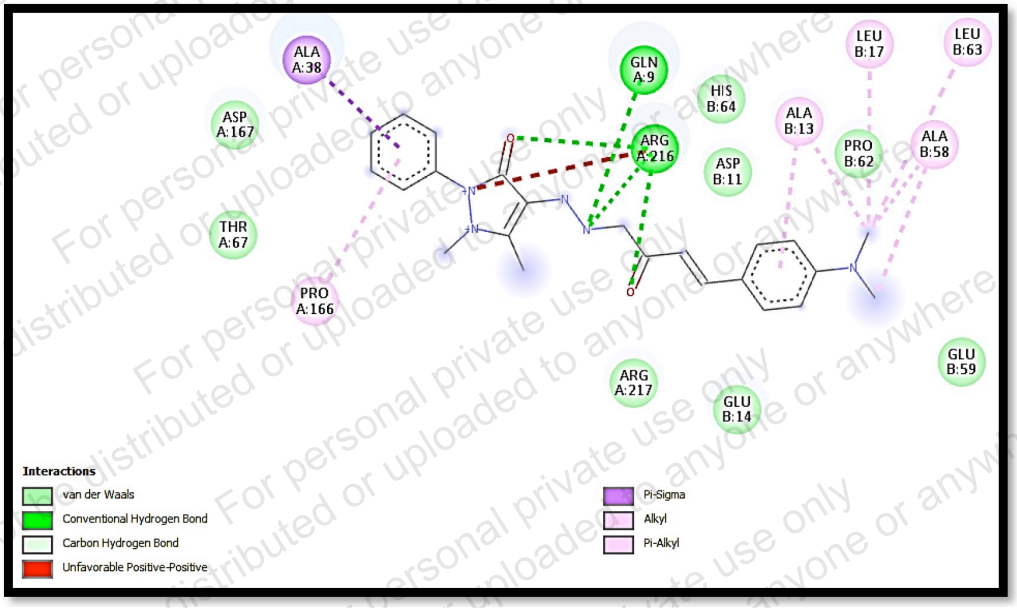
Compound No.	Docking Score Kcal/mol	Number of Bonds	Type of Interactions	Amino Acids	Function Group
4	-11.69	3	H-bond	THR200	C=O
			Pi-Alkyl	PRO198	Phenyl
			Pi-Alkyl	MET184	Phenyl
6a	-10.26	3	Pi-Alkyl	PRO198	Phenyl
			Pi-Alkyl	ARG22	Phenyl
			Pi-Alkyl	LEU194	Phenyl
6b	-16.90	12	Pi-Alkyl	ALA58	N-CH3
			Pi-Alkyl	ALA58	N-CH3
			Pi-Alkyl	LEU17	N-CH3
			Pi-Alkyl	ALA13	N-CH3
			Pi-Alkyl	ALA13	Phenyl ring
			Pi-Alkyl	PRO166	Phenyl ring
			Pi-Sigma	ALA38	Phenyl ring
			H-bond	ARG216	C=O
			H-bond	ARG216	NH
			H-bond	ARG216	Pyrazole C=O
			H-bond	GLN9	NH

(Table 2) Contd….

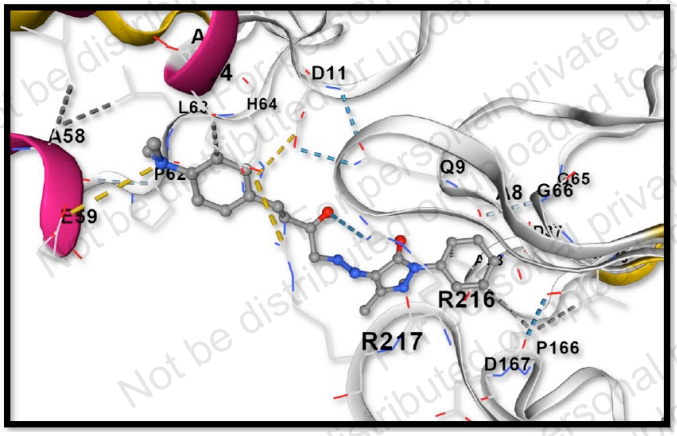
Compound No.	Docking Score Kcal/mol	Number of Bonds	Type of Interactions	Amino Acids	Function Group
6c	-19.52	9	H-bond H-bond H-bond H-bond Pi-Alkyl Pi-Alkyl Pi-Alkyl Pi-Alkyl Van der Waals	ALA38 GLY65 ALA8 THR67 ARG217 ARG216 ARG216 ALA38 PRO166	Pyrazole C=O Pyrazole C=O NH NH Cl Cl Phenyl Phenyl CH_2
6d	-19.99	7	H-bond H-bond H-bond H-bond H-bond Pi-alkyl Pi-alkyl	GLN9 ALA38 GLY66 PRO166 THR67 ARG217 ARG217	NO2 C=O C=O NH NH Phenyl Phenyl
6e	-16.82	1	Pi-alkyl	PRO198	Phenyl
6f	-14.77	4	H-bond H-bond Pi-Alkyl Pi-Alkyl	GLN9 GLY66 PRO166 ASP167	N=N C=O Phenyl Phenyl
6g	-12.58	2	H-bond H-bond	ARG217 ARG21	C=O OCH_3
7a	-13.59	5	H-bond H-bond Pi-Alkyl Pi-Alkyl Van der Waals	ALA38 GLY66 ARG217 ARG216 GLN9	Pyrazole C=O Pyrazole C=O Phenyl Phenyl CH_3
7b	-13.21	3	Amide-Pi stacked Pi-Alkyl Pi-Alkyl	ALA13 PRO52 HIS64	Phenyl CH_3 CH_3
7C	-18.27	10	H-bond H-bond H-bond H-bond H-bond H-bond Pi-Anion Pi-Anion Pi-Anion Pi-Anion	GLY66 GLN9 GLY66 VAL36 PRO166 THR67 ASP11 ASP37 GLU39 ASP37	NH NH Pyrimidine N OH Pyrimidine NH Pyrimidine NH Pyrazole N Pyrazole N Pyrazole 37 Phenyl
7d	-18.55	7	H-bond H-bond H-bond H-bond Van der Waals Pi-alkyl Attractive charge	GLN9 VAL36 THR67 ARG216 THR215 ARG216 ASP11	NH OH OH NO_2 NO_2 Phenyl Pyrazole N

(Table 2) Contd….

Compound No.	Docking Score Kcal/mol	Number of Bonds	Type of Interactions	Amino Acids	Function Group
7e	-16.39	2	H-bond Van der Waals	ARG202 ARG202	NH Phenyl
7f	-16.14	4	H-bond Van der Waals Pi-alkyl Pi-Pi	ARG202 ARG202 PRO21 TYR25	NH Phenyl Phenyl Phenyl
7g	-15.24	2	Pi-alkyl Pi-Pi	PRO21 TYR25	Phenyl Phenyl

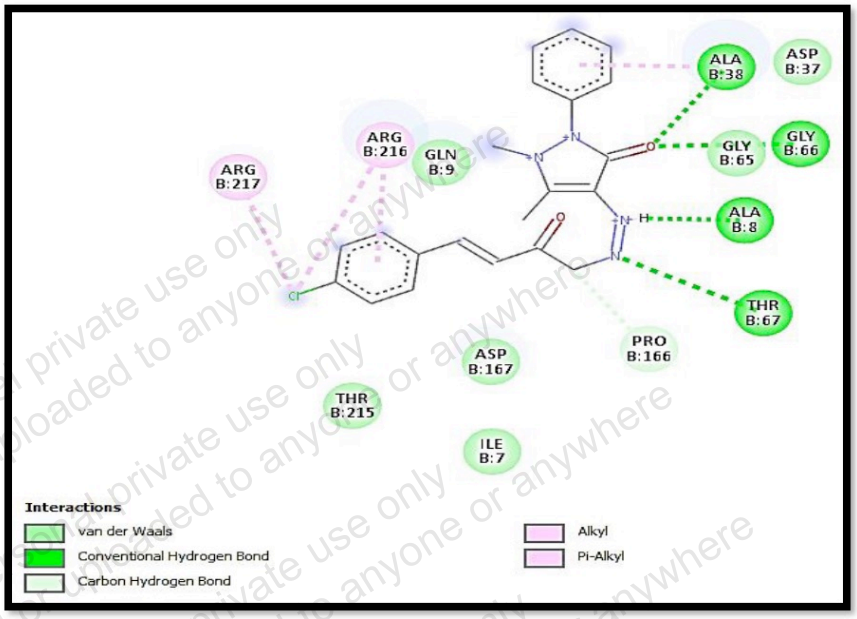


(A)

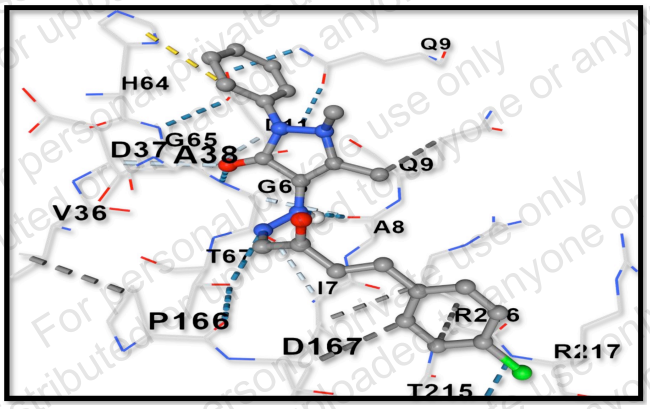


(B)

Fig. (1). Binding mode of compound **6b** within GlcN-6-P active site. (**A**) 2D binding mode (**B**) 3D binding mode. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).



(A)



(B)

Fig. (2). Binding mode of compound **6c** within Glc-N-6-P active site. (**A**) 2D binding mode (**B**) 3D binding mode. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

In addition, the chalcone derivative **6c** showed four H-bonding interactions with Ala38, GLY66, ALA8 and THR67 and showed other pi-alkyl interactions with ARG217, ARG216 and ALA38 and a Van der Waals bond with PRO166 (Fig. **2**).

Furthermore, compound **6d** formed five hydrogen bonds with GLN9, ALA38, GLY66, PRO166 and THR67 and displayed Pi-alkyl interactions with ARG217 and ARG216 as shown in Fig. (**S2**).

The pyrimidine derivative **7c** displayed an excellent fit within the active site with a binding energy score = -18.27 Kcal/mol. This compound formed 6 H-bonding interactions with GLN9, GLY66, VAL36, PRO166 and THR67. In addition, compound **7c** showed pi-anion interactions with ASP11, GLU39 and ASP37 and Amide-Pi stacked with PRO166 (Fig. **S3**).

On the other hand, compound **7d** displayed Formed conventional hydrogen bonding with ARG216, THR67, VAL36 and GLN9 amino acid residues, pi-alkyl bonding with ARG216 and van der Waals interaction with THR215 (Fig. **S4**).

CONCLUSION

In this study, novel chalcones **6a-g** and pyrimidine **7a-g** derivatives containing 2-phenyl-1H-pyrazol-3(2H)-one moiety were successfully synthesized and their chemical structures were identified and confirmed by different spectral techniques. All synthesized compounds were tested *in vitro* for their antimicrobial activities against gram-positive, gram-negative bacteria and fungi. The synthesized compounds **6c**, **6d**, **7c**, and **7d** showed the highest antibacterial and antifungal activities against the tested strains. The structure-activity relationship suggested that the presence of electron-

withdrawing groups such as nitro (**6d**, **7d**) and chloro (**6c**, **7c**) at the para position of the phenyl moiety of the synthesized compounds significantly enhanced their antibacterial and antifungal activity compared to those, which carry electron-donating groups as substituents. Results of molecular docking studies have also supported *in vitro* antimicrobial testing.

LIST OF ABBREVIATIONS

WHO = World Health Organization

TLC = Thin Layer Chromatography

m.p. = Melting Points

MIZ = Mean Inhibition Zone

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All the data and supportive information is provided within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

REFERENCES

[1] Prestinaci, F.; Pezzotti, P.; Pantosti, A. Antimicrobial resistance: A global multifaceted phenomenon. *Pathog. Glob. Health,* **2015**, *109*(7), 309-318. http://dx.doi.org/10.1179/2047773215Y.0000000030 PMID: 26343252

[2] Doron, S.; Gorbach, S. *Bacterial infections: overview*; International Encyclopedia of Public Health, **2008**, p. 273.

[3] Pendleton, J.N.; Gorman, S.P.; Gilmore, B.F. Clinical relevance of the ESKAPE pathogens. *Expert Rev. Anti Infect. Ther.,* **2013**, *11*(3), 297-308. http://dx.doi.org/10.1586/eri.13.12 PMID: 23458769

[4] Levy, S.B.; Marshall, B. Antibacterial resistance worldwide: Causes, challenges and responses. *Nat. Med.,* **2004**, *10*(Suppl.12), S122-S129. http://dx.doi.org/10.1038/nm1145 PMID: 15577930

[5] Okeke, I.N.; Klugman, K.P.; Bhutta, Z.A.; Duse, A.G.; Jenkins, P.; O'Brien, T.F.; Pablos-Mendez, A.; Laxminarayan, R. Antimicrobial resistance in developing countries. Part II: strategies for containment. *Lancet Infect. Dis.,* **2005**, *5*(9), 568-580. http://dx.doi.org/10.1016/S1473-3099(05)70217-6 PMID: 16122680

[6] Naman, C.B.; Leber, C.A.; Gerwick, W.H. Modern natural products drug discovery and its relevance to biodiversity conservation. *Microbial Resources*; Elsevier: Amsterdam, **2017**, pp. 103-120.

[7] Koehn, F.E.; Carter, G.T. The evolving role of natural products in drug discovery. *Nat. Rev. Drug Discov.,* **2005**, *4*(3), 206-220. http://dx.doi.org/10.1038/nrd1657 PMID: 15729362

[8] Yang, H.M.; Shin, H.R.; Cho, S.H.; Bang, S.C.; Song, G.Y.; Ju, J.H.; Kim, M.K.; Lee, S.H.; Ryu, J.C.; Kim, Y.; Jung, S.H. Structural requirement of chalcones for the inhibitory activity of interleukin-5. *Bioorg. Med. Chem.,* **2007**, *15*(1), 104-111. http://dx.doi.org/10.1016/j.bmc.2006.10.007 PMID: 17064909

[9] Gupta, D.; Jain, D.; Trivedi, P. Recent advances in chalcones as antiinfective agents. *Int. J. Chem. Sci.*, **2010**, *8*, 649-654.

[10] Alcaráz, L.E.; Blanco, S.E.; Puig, O.N.; Tomás, F.; Ferretti, F.H. Antibacterial activity of flavonoids against methicillin-resistant Staphylococcus aureus strains. *J. Theor. Biol.,* **2000**, *205*(2), 231-240. http://dx.doi.org/10.1006/jtbi.2000.2062 PMID: 10873434

[11] Xu, M.; Wu, P.; Shen, F.; Ji, J.; Rakesh, K.P. Chalcone derivatives and their antibacterial activities: Current development. *Bioorg. Chem.*, **2019**, *91*, 103133. http://dx.doi.org/10.1016/j.bioorg.2019.103133 PMID: 31374524

[12] Liu, M.; Wilairat, P.; Go, M.L. Antimalarial alkoxylated and hydroxylated chalcones: Structure-activity relationship analysis. *J. Med. Chem.,* **2001**, *44*(25), 4443-4452. http://dx.doi.org/10.1021/jm0101747 PMID: 11728189

[13] Lee, Y.H.; Jeon, S.H.; Kim, S.H.; Kim, C.; Lee, S.J.; Koh, D.; Lim, Y.; Ha, K.; Shin, S.Y. A new synthetic chalcone derivative, 2-hydroxy-3′,5,5′-trimethoxychalcone (DK-139), suppresses the Toll-like receptor 4-mediated inflammatory response through inhibition of the Akt/NF-κB pathway in BV2 microglial cells. *Exp. Mol. Med.,* **2012**, *44*(6), 369-377. http://dx.doi.org/10.3858/emm.2012.44.6.042 PMID: 22382990

[14] Haraguchi, H.; Inoue, J.; Tamura, Y.; Mizutani, K. Antioxidative components of *Psoralea corylifolia* (Leguminosae). *Phytother. Res.,* **2002**, *16*(6), 539-544. http://dx.doi.org/10.1002/ptr.972 PMID: 12237811

[15] Bonakdar, A.P.S.; Vafaei, F.; Farokhpour, M.; Esfahani, M.H.N.; Massah, A.R. Synthesis and anticancer activity assay of novel chalcone-sulfonamide derivatives. *IJPR,* **2017**, *16*, 565.

[16] Caamal-Fuentes, E.; Peraza-Sánchez, S.; Torres-Tapia, L.; Moo-Puc, R. Isolation and identification of cytotoxic compounds from Aeschynomene fascicularis, a Mayan medicinal plant. *Molecules,* **2015**, *20*(8), 13563-13574. http://dx.doi.org/10.3390/molecules200813563 PMID: 26213910

[17] Go, M.; Wu, X.; Liu, X. Chalcones: An update on cytotoxic and chemoprotective properties. *Curr. Med. Chem.,* **2005**, *12*(4), 483-499. http://dx.doi.org/10.2174/0929867053363153 PMID: 15720256

[18] Avupati, D.; Yejella, P. A review on pyrimidine scaffold. *World J Pharm Res Technol,* **2014**, *3*, 1563-1587.

[19] Mallikarjunaswamy, C.; Mallesha, L.; Bhadregowda, D.G.; Pinto, O. Studies on synthesis of pyrimidine derivatives and their antimicrobial activity. *Arab. J. Chem.,* **2017**, *10*, S484-S490. http://dx.doi.org/10.1016/j.arabjc.2012.10.008

[20] Abd El-Aleam, R.H.; George, R.F.; Hassan, G.S.; Abdel-Rahman, H.M. Synthesis of 1,2,4-triazolo[1,5-a]pyrimidine derivatives: Antimicrobial activity, DNA Gyrase inhibition and molecular docking. *Bioorg. Chem.,* **2020**, *94*, 103411. http://dx.doi.org/10.1016/j.bioorg.2019.103411 PMID: 31711767

[21] Tageldin, G.N.; Fahmy, S.M.; Ashour, H.M.; Khalil, M.A.; Nassra, R.A.; Labouta, I.M. Design, synthesis and evaluation of some pyrazolo[3,4-d]pyrimidine derivatives bearing thiazolidinone moiety as anti-inflammatory agents. *Bioorg. Chem.,* **2018**, *80*, 164-173. http://dx.doi.org/10.1016/j.bioorg.2018.06.013 PMID: 29929077

[22] Amr, A.E.G.E.; Omar, M.A.A.; Abdalla, M.M. Analgesic, anticonvulsant and antiparkinsonian activities of some synthesized 2, 6-bis (tetracarboxamide)-pyridine and macrocyclic tripeptide derivatives. *Int. J. Pharmacol.,* **2016**, *12*(2), 74-80. http://dx.doi.org/10.3923/ijp.2016.74.80

[23] Elkanzi, N.A.A.; Kadry, A.M.; Ryad, R.M.; Bakr, R.B.; Ali El-Remaily, M.A.E.A.A.; Ali, A.M. Efficient and recoverable bio-organic catalyst cysteine for synthesis, docking study, and antifungal activity of new bio-active 3,4-Dihydropyrimidin-2(1 *H*)-ones/thiones under microwave irradiation. *ACS Omega,* **2022**, *7*(26), 22839-22849. http://dx.doi.org/10.1021/acsomega.2c02449 PMID: 35811927

[24] Elkanzi, N.A.A.; El Azab, I.H.; Bakr, R.B. Design, synthesis, and *in silico* molecular docking study of some novel thiochromene derivatives with antimicrobial potential. *Polycycl. Aromat. Compd.,* **2022**, *42*(9), 6760-6779. http://dx.doi.org/10.1080/10406638.2022.2041052

[25] Bakr, R.B.; Elkanzi, N.A.; Ghoneim, A.A.; Moustafa, S. Synthesis, molecular docking studies and *in vitro* antimicrobial evaluation of novel pyrimido [1, 2-a] quinoxaline and triazino [4, 3-a] quinoxaline derivatives. *Heterocycles,* **2018**, *96*, 1941-1957.

[26] Elkanzi, N.A.A.; Bakr, R.B. Microwave assisted, antimicrobial activity and molecular modeling of some synthesized newly pyrimidine derivatives using 1, 4-diazabicyclo[2.2.2]octane as a catalyst. *Lett. Drug Des. Discov.,* **2020**, *17*(12), 1538-1551. http://dx.doi.org/10.2174/1570180817999200802033351

[27] Hrichi, H.; Elkanzi, N.A.A.; Bakr, R.B. Novel β-lactams and thiazolidinone derivatives from 1, 4-dihydroquinoxaline Schiff's base: Synthesis, antimicrobial activity and molecular docking studies. *Chem. J. Moldova,* **2020**, *15*(1), 86-94. http://dx.doi.org/10.19261/cjm.2019.647

[28] Abdelgawad, M.A.; Al-Sanea, M.; Musa, A.; Elmowafy, M.; El-Damasy, A.K.; Azouz, A.A.; Ghoneim, M.M.; Bakr, R.R. Docking study, synthesis, and anti-inflammatory potential of some new pyridopyrimidine-derived compounds. *J. Inflamm. Res.,* **2022**, *15*, 451-463. http://dx.doi.org/10.2147/JIR.S343263 PMID: 35125880

[29] Abdelgawad, M.A.; Elkanzi, N.A.A.; Musa, A.; Ghoneim, M.M.; Ahmad, W.; Elmowafy, M.; Abdelhaleem Ali, A.M.; Abdelazeem, A.H.; Bukhari, S.N.A.; El-Sherbiny, M.; Abourehab, M.A.S.; Bakr, R.B. Optimization of pyrazolo[1,5-a]pyrimidine based compounds with pyridine scaffold: Synthesis, biological evaluation and molecular modeling study. *Arab. J. Chem.,* **2022**, *15*(8), 104015. http://dx.doi.org/10.1016/j.arabjc.2022.104015

[30] Alanazi, M.A.; Arafa, W.A.A.; Althobaiti, I.O.; Altaleb, H.A.; Bakr, R.B.; Elkanzi, N.A.A. Green design, synthesis, and molecular docking study of novel quinoxaline derivatives with insecticidal potential against *Aphis craccivora*. *ACS Omega,* **2022**, *7*(31), 27674-27689. http://dx.doi.org/10.1021/acsomega.2c03332 PMID: 35967065

[31] Abdelgawad, M.A.; Musa, A.; Almalki, A.H.; Alzarea, S.I.; Mostafa, E.M.; Hegazy, M.M.; Mostafa-Hedeab, G.; Ghoneim, M.M.; Parambi, D.G.T.; Bakr, R.B.; Al-Muaikel, N.S.; Alanazi, A.S.; Alharbi, M.; Ahmad, W.; Bukhari, S.N.A.; Al-Sanea, M.M. Novel phenolic compounds as potential dual EGFR and COX-2 inhibitors: Design, semisynthesis, *in vitro* biological evaluation and *in silico* insights. *Drug Des. Devel. Ther.,* **2021**, *15*, 2325-2337. http://dx.doi.org/10.2147/DDDT.S310820 PMID: 34103896

[32] Elkanzi, N.A.; Bakr, R.B.; Ghoneim, A.A. Design, synthesis, molecular modeling study, and antimicrobial activity of some novel pyrano [2, 3-b] pyridine and pyrrolo [2, 3-b] pyrano [2.3-d] pyridine derivatives. *J. Heterocycl. Chem.,* **2019**, *56*, 406-416.

[33] El Azab, I.H.; Bakr, R.B.; Elkanzi, N.A.A. Facile one-pot multicomponent synthesis of pyrazolo-thiazole substituted pyridines with potential anti-proliferative activity: Synthesis, *in vitro* and *in silico* studies. *Molecules,* **2021**, *26*(11), 3103. http://dx.doi.org/10.3390/molecules26113103 PMID: 34067399

[34] Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C.; Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.,* **1966**, *45*(Suppl. 4), 493-496. http://dx.doi.org/10.1093/ajcp/45.4_ts.493 PMID: 5325707

[35] Aouf, A.; Bouaouina, S.; Abdelgawad, M.A.; Abourehab, M.A.S.; Farouk, A. In silico study for algerian essential oils as antimicrobial agents against multidrug-resistant bacteria isolated from pus samples. *Antibiotics,* **2022**, *11*(10), 1317. http://dx.doi.org/10.3390/antibiotics11101317 PMID: 36289975

[36] Kolb, V.M. *Green Organic Chemistry and its Interdisciplinary Applications*; CRC Press, **2017**. http://dx.doi.org/10.1201/9781315371856