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Synthesis, Antimicrobial, Antioxidant and Docking Study of Some Novel 3,5- Disubstituted- 4,5- dihydro- 1*H*- pyrazoles Incorporating Imine Moiety

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A NOVEL series of 3,5-Disubstituted-4,5-Dihydro-1*H*-pyrazoles **(3-9)** containing imine moiety were synthesized and characterized using spectral analysis. The synthesized derivatives were *In vitro* screened against several bacterial species, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Acinetobacterbaumannii* as well as *Candida albicans* and revealed moderate to potent activity. The antioxidant study was confirmed for the synthesized derivatives against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical qualitatively using *TLC* technique, and quantitatively by spectrophotometric method. The half maximal inhibitory concentration (IC_{50}) was calculated for the active compounds (**1-5, 8**). Docking study for the potent compounds (**2** and **4)** against glucosamine-6-phosphate synthase, the target enzyme for the antimicrobial agents, was explored to explain the interactions of the discovered hits within the amino acid residues of the enzyme active side. The docking parameters enhanced the activity of new compounds as promising antimicrobial agents.

Keywords: 2-Pyrazoline Schiff base, Antimicrobial, Antioxidant, Docking study.

Introduction

3,5-Disubstituted-4,5-Dihydro-1*H*-pyrazoles are important five membered nitrogen heterocycle, which has been extensively considered by several research groups. The wide pharmacological activities such as antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antitumor and antidepressant[1-3] inspired the chemists to carry out various structural variations in the ring.On the other hand, five membered heterocycles incorporating Schiff base moiety have been synthesized and exhibited a variety of biological activities like antimicrobial, antioxidant, anti-inflammatory, anticancer[4], and so forth. During the last decade, the synthesis and development of a wide spectrum antimicrobial and antioxidant drugs were interested in researchers due to resist untreatable microbes and scavenge free radical, respectively. The extensive literature survey and the continuous attempt of our group for exploring novel antimicrobial and antioxidant agents encourages us to synthesize novel series of 2-pyrazoline derivatives containing imine (Schiff base) moiety. The target compounds (**3-9**) were prepared and screened against several bacterial species (*gram positive and gram negative*) and also against Candida albicans. The antioxidant activity of the synthesized derivatives against 2,2-diphenyl-l-picrylhydrazyl (DPPH) radical by the *TLC* autographic and quantitatively by spectrophotometric assay was achieved. Also, the half maximal inhibitory concentration (IC_{50}) of 2-pyrazoline derivatives was evaluated. Docking Study for the led derivatives (**2** and **4**) against glucosamine-6-phosphate synthase[5], the target enzyme for the antimicrobial agents was achieved to explore and explain the interactions of the discovered hits within the amino acid residues of the enzyme binding pocket. The docking results enhanced the activity of new derivatives

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as promising antimicrobial agents. Autodock 4.2, the effective tool for exploring the binding affinity of small molecule to enzyme target[6] was used to study the interactions between the pyrazoline derivatives and the *GlcN-6-P* synthase binding site.

Experimental

Material

All the chemicals and materials were from Sigma-Aldrich, Fluka and BDH. Melting points were determined on an electrothermal capillary apparatus and are uncorrected. FTIR measurements were recorded on a Shimadzu model FTIR-8400S. Mass spectra were recorded on a Shimadzu GCMS-QP2010 Ultra apparatus. ^{1}HNMR spectra were gained with a Bruker spectrophotometer model ultra-shield at 300 MHz in DMSO-d_6 and $CDCl_3$ solution with the TMS an internal standard. Note: In ^{1}HNMR spectra, the signals at δ 2.5 and 7.26 for DMSO-d_6 and $CDCl_3$ while the signals at δ 3.35 and 1.56 for dissolved water in DMSO-d_6 and $CDCl_3$, respectively.

Synthesis

Synthesis of1-(4-aminophenyl)-3-(1H-pyrrol-2-yl)prop-2-en-1-one (1)

This compound was prepared according to the procedure described in reference[7]. To a solution of 4-aminoacetophenone (1mmol) in ethanol (10 ml), sodium hydroxide (40%, 1ml) was added and the mixture was stirred for 30 minutes. After that pyrrole2-carboxyaldehyde (1 mmol) added and the reaction mixture was stirred overnight. The reaction mixture was allowed to stand at room temperature. The precipitated solid was dried and recrystallized from ethanol. Brown powder, yield 89%, m.p 155-157◦C; IR (cm^{-1}): 3387, 3323 (***NH2***), 3194 (**NH**), 3080 (***C-H aromatic***), 2956, 2845 (***aliphatic C-H***), 1639 (***C=O***), 1624 (*-**HC=CH**-*), 1583 (***C=Caromatic***). 1HNMR (300MHz, DMSO-*d6*) δ (ppm): 4.1 (s, 2H, ***NH2***), 6.3 (d, 1H **,** ***CH=CH***, j =3.01 Hz), 6.7 (d, 2H, ***2 Ar-H pyrrol***, j =***1.41*** Hz***),*** 6.9 (d, 1H***, CH=CH***, j =3.01 Hz) ,7.1-7.2 (d, 2H, ***2 Ar-H***, j =15.48 Hz),7.9 (d, 1H,***Ar-H***, j =***15.47*** Hz***),***7.9 (d, 2H, ***2 Ar-H***, j =***8.51*** Hz***),*** 8.7 (s, 1H, **NH*pyrrole***). Mass (EI) m/e:212 M^+ for $C_{13}H_{12}N_2O$, R.*f*= 0.33 (5:5, Hexane: Ethyl acetate).

Synthesis of 4-(5-(1H-pyrrol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)aniline(2)

This compound was prepared according to modified procedure in reported reference[8]. Reaction mixture of Chalcone compounds (1) (1mmol) in ethanol (10 ml) and excess of hydrazine hydrate 80% (1ml) was refluxed for 6 h. The completion of reaction was checked by *TLC* using ethyl acetate: hexane system (7:3). After that, the reaction mixture was added to crushed ice, the precipitated product was filtered, washed with water and dried. Brown powder, yield 89%, m.p 180-184◦C; IR (cm^{-1}): 3367 (***NH2***),3221 (***NH-pyrazoline***), 3120 **(NH-*pyrrole***) 3080 (***C-H aromatic***), 2949 (**aliphatic C-H**), 1618,1602(***C=N pyrazoline***), 1562 (***C=Caromatic***). ^{1}HNMR (300MHz, DMSO-*d6*) δ (ppm): 2.7(dd, 1H, j= 11.35, 15.85Hz,***H_a-pyrazoline***), 3.0-3.2 (dd, 1H, j=9.96 ,15.93 , ***H_b-pyrazoline***), 4.5-4.6 (t, 1H, ***H_x-pyrazoline***), 5.2 (s, 2H, ***NH2***),6.0(s,1H, ***NH-pyrazoline***), 6.5-7.5 (m, 7H,***7Ar-H)***, 10.6 (s, 1H, ***NH-pyrrole***). Mass (EI) m/e: 226 M^+ for $C_{13}H_{14}N_4$, R.*f*= 0.25(3:7, Hexane: Ethyl acetate).

Synthesis of Schiff bases (3-9)

These compounds were prepared according to the procedure described in published reference[9]. To solution of substituted benzaldehyde (1mmol) in methanol (10ml) with few drops of glacial acetic acid, pyrazoline derivatives (2) (1mmol) was added. The mixture was refluxed for 10-12 h and the reaction was monitored by *TLC* using hexane: ethylacetate system (3:7). The precipitate was filtered and washed with methanol, dried and recrystallized from ethanol.

4-(5-(1H-pyrrol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-N-((1H-pyrrol-2-yl)methylene)aniline (3)

Yellow powder, yield 65 %, m.p178-180◦C; IR (cm^{-1}): 3379 ***(NH-pyrazoline***) , 3090,3032 (***C-Haromatic***) , 2962 (***HC***=N) , 1633(***C=N***), 1604 (***C=N pyrazoline***) ,1572.1541 (***C=Caromatic***) . 1HNMR (300MHz, DMSO-*d6*) δ (ppm): 2.8-2.9 (m, 1H, ***Ha -pyrazoline)*** , 4.1-4.2 (t, 1H, ***H_b -pyrazoline)*** , 4.8 (t, 1H, ***H_x-pyrazoline***), 5.1 (s, 1H, ***NH-pyrazoline***) , 6.0-7.5 (m, 10H, ***Ar-H) ,*** 7.6 (s, ***1H,CH=N***), 7.9 (s, ***1H, NH-pyrrole***), R.f = 0.75(3:7, Hexane: Ethyl acetate).

2-(((4-(5-(1H-pyrrol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)imino)methyl)phenol (4)

Yellow powder, yield 81 %, m.p114-116◦C; IR (KBr , cm^{-1}): 3404 (**OHBroad**) ,3061 (***C-Haromatic***), 2852 (***HC=N***), 1620(***C=N***) , 1597 (***C=Npyrazoline***) , 1570 (***C=Caromatic***). ^{1}H-NMR (400MHz, DMSO) δ (ppm): 2.9-3 (m,***1H, H_a-pyrazoline)***, 4.8 (m, ***1H, H_b-pyrazoline***), 5.9 (m, ***1H, H_x-pyrazoline***),5.1 (s, ***1H, NH- pyrazoline***) , 6.2-8.5 (m, ***11H, Ar-H)***, 8.9 (s, 1H,***OH***) , 9.1 (s, ***1H, HC=N***), 10.3 (s, ***1H, NH-pyrrole***). R.*f*= 0.75(3:7, Hexane: Ethyl acetate).

4-(((4-(5-(1H-pyrrol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)imino)methyl)phenol (5)

Yellow powder, yield 50 %, m.p158-160◦C; IR (KBr ,cm^{-1}):3358 (**OHBroad**),3176 (***aromatic C-H***) , 2964,2850 (***HC=N***), shoulder (***C=N***), 1575 (***C=N pyrazoline***). ^{1}HNMR (300MHz,DMSO-*d6*) δ (ppm): 4.6-4.7 (m, 1H, ***H_a-pyrazoline),*** 5.0 (m, 1H, ***H_b-pyrazoline***), 5.3 (m, 1H, ***H_x-pyrazoline***), 6.1-7.9 (m, 12H, ***11Ar-H, NH-pyrazoline***), 7.9(s, 1H,***OH***),8.5 (s, ***1H, CH***=N), 9.9(s, ***1H, NH-pyrrole***). R.*f*= 0.77(3:7, Hexane: Ethyl acetate).

4-(5-(1H-pyrrol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-N-(4-chlorobenzylidene)aniline (6)

Yellow powder, yield 50 %, m.p140-142◦C; IR (KBr ,cm^{-1}): 3431 ***NH- pyrazoline),*** 3055 (***C-Haromatic***) , 2879 (***HC***=N), 1624 (***C=N***), 1591 (***C=N pyrazoline***). 1HNMR (300MHz, DMSO-*d6*) δ (ppm): 2.4 (m, 1H, ***H_a-pyrazoline***), 2.6 (m,1H, ***H_b-pyrazoline***), 6.62-6.64 (m,1H,***Hx-pyrazoline***), 6.0(s,1H,***NH-pyrazoline***),7.4-7.9(m,11H,11***ArH***),8.2(s, ***1H,CH=N***),8.7(s,***1H,NH-pyrrole***). Mass (EI) m/e: 348.83M^+ for $C_{20}H_{17}N_4Cl$,R.*f*= 0.75(3:7, Hexane: Ethyl acetate).

4-(5-(1H-pyrrol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-N-(4-methoxybenzylidene)aniline (7)

Yellow powder, yield 54 %, m.p163-165◦C; IR (cm^{-1}): 3423***(NH-pyrazoline***), 3066 (***C-Haromatic***) , 2931 (***HC=N***), 1606 (H***C=N***), 1593(***C=N pyrazoline***),1573 (***C=C aromatic***). 1HNMR (300MHz, DMSO-*d6*) δ (ppm): 3.8 (m, 1H, ***H_a-pyrazoline)***,5.1(m, 1H, ***H_a-pyrazoline),*** 5.8 (m, 1H, ***H_x-pyrazoline***), 6.3 (s, 1H, ***NH-pyrazoline***) , 6.7-7.8 (m, 11H, ***11Ar-H) ,*** 8.3(s, ***1H,CH=N***), 9.8(s, ***1H, NH-pyrrole***). Mass (EI) m/e: 344.41M^+ for $C_{21}H_{20}N_4O$, R.*f*= 0.75(3:7, Hexane: Ethyl acetate).

4-(5-(1H-pyrrol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-N-(thiophen-2-ylmethylene)aniline (8)

Brown powder, yield 85 %, m.p 162-164◦C; IR (KBr, cm^{-1}): 3417***(NH-pyrazoline***), 3097 (***C-H aromatic***) , 2920, 2850 (***HC***=N), 1616 (H***C=N),*** 1589(***C=N pyrazoline***), 1500 (***C=Caromatic***). 1HNMR (300MHz, DMSO-*d6*) δ (ppm): 2.8 (m, 1H, ***CHa -pyrazoline)*** , 4.1 (m, 1H, ***CHb -pyrazoline)*** , 4.8 (t, 1H, ***CHx-pyrazoline***), 6.0 (s, 1H, ***NH-pyrazoline***), 6.2-7.9 (m, 10H, ***Ar-H)*** ,7.65 (s, ***1H,CH=N***), 8.8 (s, ***1H, NH-pyrrole***). Mass (EI) m/e: 320.41M^+ for $C_{18}H_{16}N_4S$, R.f = 0.75(3:7, Hexane: Ethyl acetate).

4-(5-(1H-pyrrol-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)-N-(4-nitrobenzylidene)aniline (9)

Yellow powder, yield 95%, m.p163-165◦; IR (cm^{-1}): 3084 (***C-H aromatic***), 3396 (***NH-pyrazoline***), 3228 ***(NH-pyrrole)***, 2872, 2806 (***HC=N***), 1651 (H***C=N***), 1618 (***C=N pyrazoline***),1597(***C=C aromatic***), 1516 -1344 ***(NO_2)***. ^{1}H-NMR (400MHz, DMSO) δ(ppm): 3.0-3.2 (m,***1H, H_a-pyrazoline)***,4.7- 4.8 (m, ***1H, H_b-pyrazoline***) , 5.7- 5.8 (m, ***1H, H_x-pyrazoline***), 6.3 (***s,1H, NH-pyrazoline***), 6.4-7.9 (m, ***11H, Ar-H),*** 8.2 (s, ***1H, CH=N***), 9.2 (s, ***1H, NH-pyrrole***). Mass (EI) m/e: 359.36M^+ for $C_{20}H_{17}N_5O_2$, R.*f*= 0.75 (3:7, Hexane: Ethyl acetate).

Antimicrobial studies

Well diffusion method

The agar well-diffusion method was used to estimate the antimicrobial activity for the synthesized derivatives against various bacterial species *Pseudomonas aeruginosa, Acinetobacterbaumannii* (gram negative), *Staphylococcusaureus (*gram positive)as well as *Candida albicans* (yeast). These isolates were obtained from Department of Biology/ College of Science /Mustansiriyah University. The concentrations for each compound was 1000 µg/ml. Plates were prepared by spreading approximately 10^5 CFU/ml culture broth of each indicator bacterial isolates on Muller Hinton agar surface using sterile cotton swabs. The agar plates were left for about 10 min before aseptically dispensing the 50µl of each compound into the agar wells already bored in the agar plates. The plates were then incubated at 37°C for 24 h. Zones of inhibition were measured and recorded in millimeter diameter. The Dimethyl sulfoxide was used as control [10].

Estimation of Minimal Inhibitory Concentration (MIC)

Serial dilution agar method was used to estimate MIC for compound 8 and 9. Double folded serial dilutions (60, 120, 250, 450) µg/ml to target compounds were freshly prepared from stock solutions. Separately, those dilutions were added to Mueller Hinton agar at 50°C into sterile glass tubes each alone and mixed well then poured into sterile Petri-dishes. Finally cooled to 37ºC and left to solidify at room temperature (25ºC) and kept at 4ºC for use through 24 hours. Few colonies (4-5) from overnight culture of bacteria were transferred to 5ml of normal saline in order to prepare the bacterial suspension and were adjusted

to 0.5 McFarl and turbidity is equal to $1.5x10^8$ CFU/ml. Five microliters of bacterial suspension were drown by micropipette and inoculated plates then left for 10 min. The plates were incubated at 37°C for18-24hours. The result of MIC was recorded for compounds (8) and (9)[11].

Antioxidant study (DPPH. radical scavenging assay)

TLC autographic assay

Few milligrams of synthesized derivative (1-9) and Gallic acid (as astandard) dissolved in methanol were added to the *TLC* plate by extremely small capillary. After drying, *TLC* plates were sprayed with methanolic solution of 0.2 % DPPH. The plates were examined after 30 min of spraying under UV light. Active compounds appear as yellow or blue spots against a purple background[12].

Spectrophotometric (DPPH) assay

This assay was confirmed according to modified procedure in reported reference[13]. At first, from the samples (1-9) and gallic acid (as a standard) at concentration of 5, 10, 15, 25, 50 and 100 µg/mL were taken separately then added to 0.25 mL of DPPH solution (0.13 mg DPPH/ mL methanol) and the volume was complete to 1.5 mL with methanol. The reaction mixture was left to stand for 30 min in dark place. The control contained all reagents without the sample. The DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm against the blank. The capability to scavenge the DPPH radical was calculated using the following equation: DPPH scavenging effect (%) = $(A_0 - A_1/A_0)\times 100$, where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the samples or standards. The results of DPPH scavenging effect (%) were plotted against scavenger concentration to calculate IC_{50}.

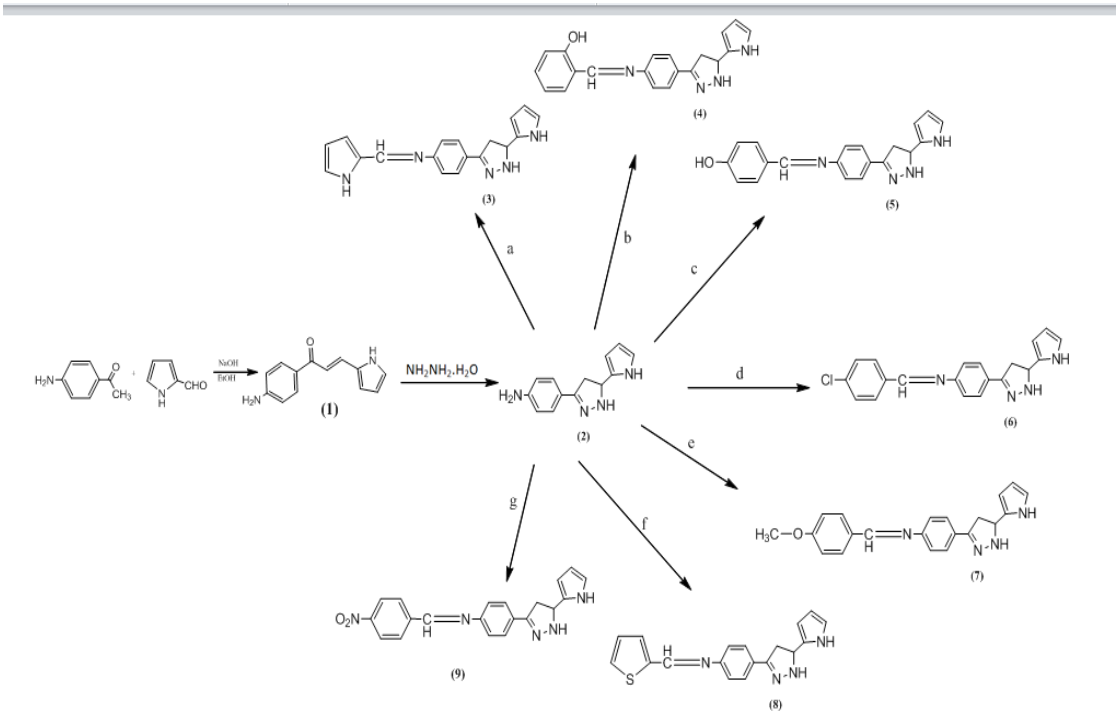
Docking study

Auto Dock 4.2 package tool was used to study the affinity of the potent derivatives (2 and 4) to the binding site of GlcN-6-P synthase as described by the reported reference[14]. The pdb file format of enzyme as receptor was obtained from the RCSB Protein Data Bank (PDB code 1MOQ) and used as a rigid molecule. The water molecules were removed and hydrogens were added to the amino acid residues. The docked compounds were drawn using Chem Draw ultra 7.0 as mol file and the open Babel 2.3.1 software was used to constructing the pdb file. The docking study was achieved using grid dimensions 30.5, 17.5 and -2.2, respectively. Docking algorithm using Lamarckian Genetic was employed with 10 runs, 150 population size, 2,500,000 maximum number of energy evaluations and 27,000 maximum number of generations.

Results and Discussion

Synthesis

Chalcone (1) and the pyrazoline compound (2) were prepared and characterized as described by our previous work [15]. Schiff bases (3-9) were synthesized from the reaction of compound 2 with different aromatic aldehydes in acidic methanolic solution (Scheme1). The structures of obtained compounds were confirmed by spectral analysis (see experimental section). The FT-IR spectra of compound (1) showed the absorption bands at 1639 cm^{-1} and 1624 cm^{-1} regions due to C=O and CH=CH, respectively. The FT-IR spectra of compound (2) showed absorption band of NH-pyrazoline at 3120 cm^{-1}, while the disappearance stretching bands of C=O and CH=CHstrongly confirm the structure. The *FT-IR* spectra of compounds (**3-9**) showed the absorption bands at 1606-1651 cm^{-1}, 1575-1618 cm^{-1} regions due to the stretching vibrations of the ***CH=N*** and ***C=N*** groups, respectively. The disappearance of the NH_2 stretching frequencies strongly enhances the elucidation of prepared compounds. The ^{1}HNMR spectra of compound **1** revealed singlet band at 4.1 ppm due to ***NH_2*** protons, while the signal appears at 6.3,6.9 ppm related to two aromatic protons and ***CH=CH*** of chalcone. The multiplet signal at 6.7-7.9 is due to other aromatic protons. The singlet signal of **NH *pyrrole*** was appeared at 8.7 ppm. The ^{1}HNMR data of compound **2** revealed doublet of doublet signal at 2.7 ppm, doublet of doublet signal at 3.0-3.2 ppm and Tripletsignal at 4.5-4.6 ppm related to H_a, H_b and H_x of pyrazolinering. The protons of ***NH_2*** are appeared at 5.2 ppm as a singlet signal. The multiplet signal showed at 6.5-7.5 ppm is related to aromatic protons as well as the ***NH-***of pyrazoline ring. The singlet signal of *NH-**pyrrole*** is appeared at 10.6 ppm. ^{1}HNMR spectrum of compound **9,** revealed the following signals 3.0-3.2 ppm, 4.7-4.8 ppm and 5.7-5.8 ppm due to H_a, H_b and H_x of pyrazoline ring, while the band of aromatic proton as well as of ***NH-***



Scheme 1. (a) pyrrole-2-carboxaldehyde, EtOH (b) salicylaldhyde, EtOH (c)p-hydroxybenzaldehyde, EtOH(d) p-chlorobenzaldehyde, EtOH (e)p-methoxybenzaldehyde, EtOH (f) 2-thiophenecarboxaldehyde, EtOH (g) p-nitrobenzyaldehyde, EtOH.

pyrazoline is appeared as a multiplet signal at 6.3-7.9 ppm. Another two singlet signal is appeared at 8.2 ppm and 9.2ppm are related to ***CH=N*** and ***NH-pyrrole,*** respectively. The mass spectra are consistent with the molecular ion peak values of the prepared compounds

Antimicrobial activity

The *in vitro* assay of the synthesized compounds (1-9) against different pathogenic bacteria and yeast were achieved using 1000µg/ml concentration as illustrated by Table 1. The activity of compounds (1-9) was evaluated against *Staphylococcus aureus* (gram positive bacteria), *Pseudomonas aeruginosa* and *Acinetobacterbaumannii*(gram negative bacteria), and *Candida albicans* (yeast). All the prepared compounds revealed promising activity against the

TABLE 1. *In Vitro* antimicrobial inhibition zone (mm) of the synthesized compounds.

Compound	*S.aureus*	*P.aeruginosa*	*Acinetobacter*	*C. albicans*
1	20	16	16	18
2	22	13	18	14
3	20	14	14	20
4	16	15	16	20
5	18	10	5	20
6	18	23	25	22
7	30	11	18	25
8	22	17	20	30
9	24	14	20	14
Amoxicillin	17	16	16	-

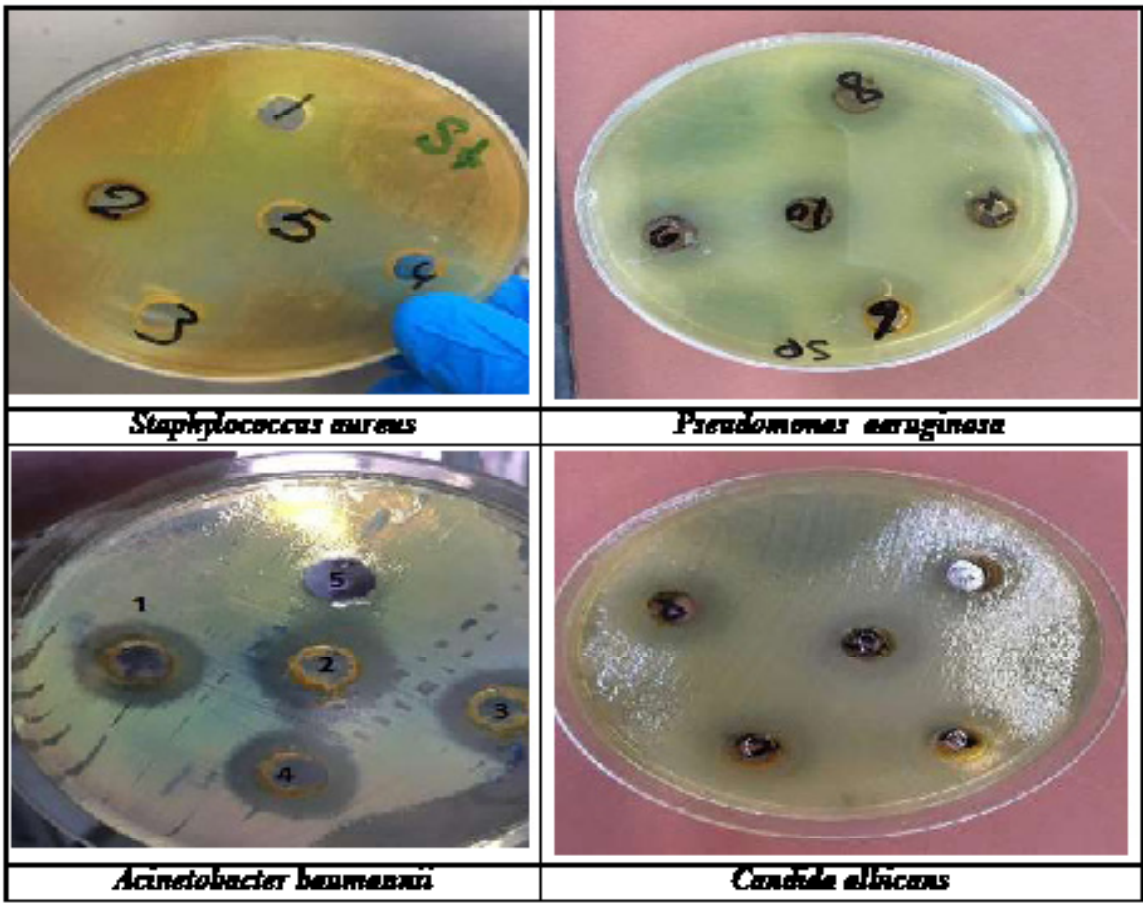


Fig. 1. Antimicrobial activity of pyrazoline derivatives 1a(1), 2a (2),3a(7) ,4a(6) ,5a(8),6a(9) ,7a(5) ,8a(4) , 9a (3), against *S.aureus, P.aeruginosa, Acinetobacter baumannii* **and** *C. albicans.*

different species. Figure 1 shows the antimicrobial activities of selected synthesized derivatives. The minimum inhibitory concentration of compound 8 and 9 was 250 µg/ml.

Antioxidant activity

The scavenging properties of all the synthesized derivatives (1-9) were evaluated against DPPH radical using TLC autographic assay. The synthesized compounds dissolved in methanol solvent were distributed on TLC plate using spotting capillary. After drying and spraying the DPPH solution, the active compounds (1, 2, 3, 4, 5 and 8) appeared as yellow or blue spots with purple background. The scavenging activity of the lead derivatives (1, 2, 3, 4, 5 and 8) was determined using spectroscopic method as described by the

TABLE 2. The results of IC_{50}.

Gallic Acid	16.3
1	84.77
2	10.79
3	37.25
4	26.86
5	41.62
6	-
7	-
8	73.1
9	-

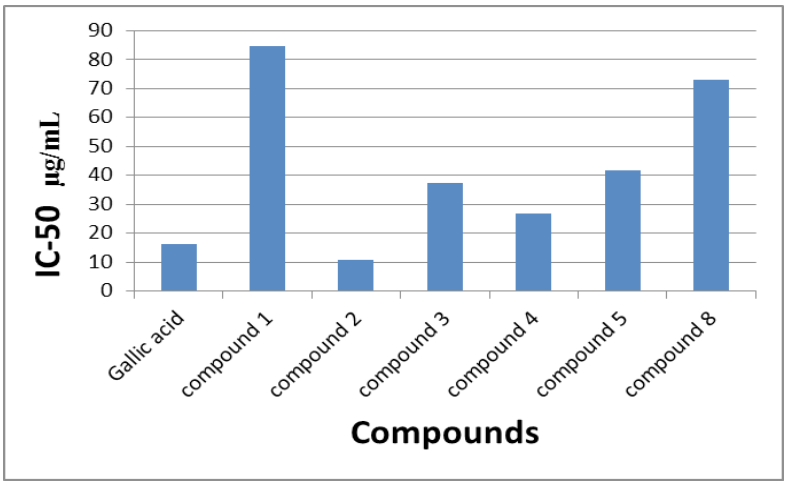


Fig. 2. The result of IC_{50}.

indicated reference[13]. The IC_{50} was calculated from the plotting of DPPH scavenging activity against different concentrations of each tested compound (5, 10, 15, 25, 50 and 100) µg/ml (Table 2, Fig. 2).

The scavenging activity of compounds 2, 3, 4, 5 and 8 revealed promising results compared with compound 1. Our suggestion for this observation may be related to presence of pyrazoline core in the discovered hits. Compound **2** shows the most potent scavenging activity due to cooperation of free amino group, which has high electronegativity, and pyrazoline core. On the other hand, the scavenging of compound 4 and 5 associated with the free hydroxyl group (OH) as well as the pyrazoline system. Compound **8** has no free group so its reveal low scavenging activity.

Docking study

The docking approach of the potent active pyrazoline 2 and pyrazoline derivative 4 against the active site of glucosamine-6-phosphate synthase, the potential target for antibacterial

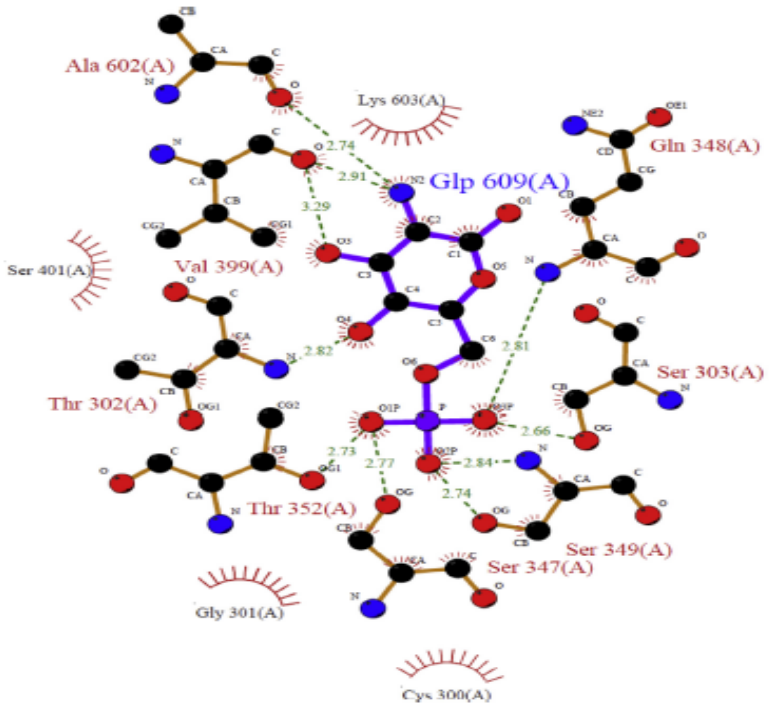
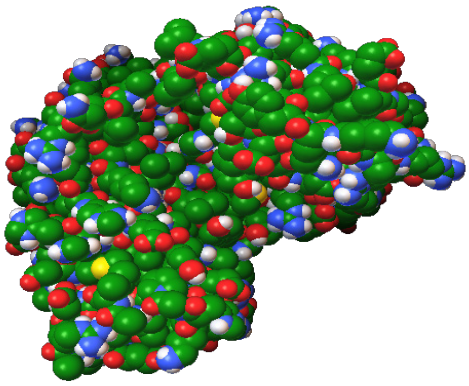


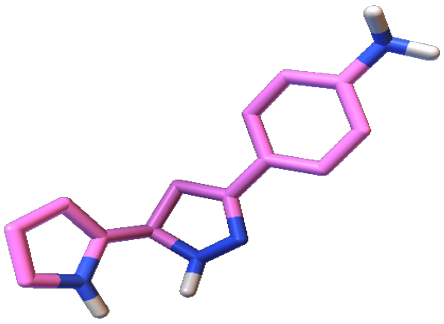
Fig. 3. Ligplot of GlcN-6-P showing the binding of glucosamine-6-phosphate in an active site of enzyme.

and antifungal agents was explored. As indicated by the X-ray, the binding pocket of the enzyme include the following residues, Cys 300, Gly 301, Thr 302, Ser 303, Ser 347, Gln 348, Ser 349, Thr 352, Val 399, Ser 401, Ala 602 and Lys 603 as shown in Fig. 3[16].

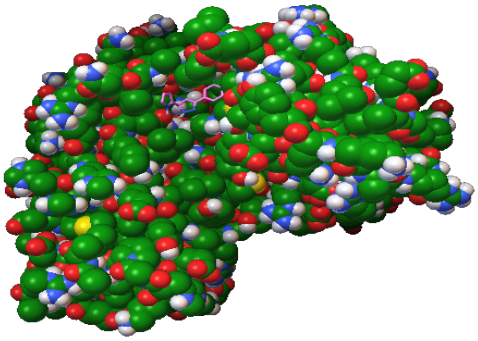
The binding energy of active compounds inside the known three dimensional structure of the specific enzyme was explored by using Autodock 4.2. The binding of the best building conformers for compound 2 and 4 inside the binding pocket of L-Glutamine: D-fructose-6-phosphate amidotransferase is illustrated in Fig. 4.



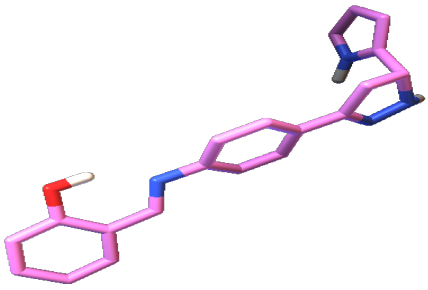
3D structure of glucoseamine-6-phosphate synthase (GlcN-6-P)



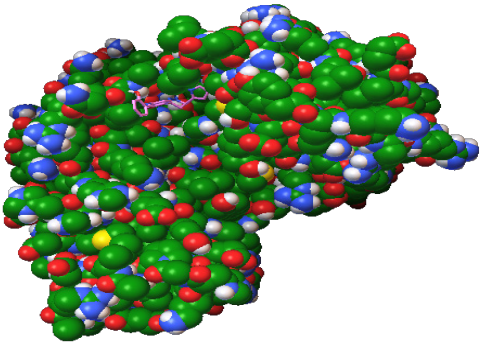
Compound 2



The best conformer of compound 2 inside binding pocket of GlcN-6-P.



Compound 4



The best conformer of compound 4 inside binding pocket of GlcN-6-P

Fig. 4. The docking of the best generated conformers of the potent discovered hits (2 and 4) inside the binding pocket of L-Glutamine: D-fructose-6-phosphate amidotransferase (GlcN-6-P).

As indicated by molecular docking parameters (Table 3), the high ranking binding energies of the generated conformer was- 7.67 and 7.57 kcal mol-

TABLE 2. Docking parameters of compounds (2 and 4).

Bonding	H-bonds	Intermolecular energy (kcalmol^{-1})	Inhibition constant (μM)	Binding Energy (Kcal mol-^{1})	Compounds	
THR302:HN: LIG:N	1	-8.56	2.39	-7.67	1	2
THR302:HN: LIG:N	1	-8.56	2.42	-7.66	2	
THR302:HN: LIG:N	1	-8.52	2.57	-7.63	3	
THR302:HN: LIG:N	1	-8.46	2.85	-7.56	4	
THR302:HN: LIG:N	1	-8.41	3.08	-7.52	5	
THR302:HN: LIG:N	1	-8.40	3.15	-7.51	6	
LIG:H : GLU488:OE2 THR302:HN: LIG:N	2	-8.29	3.83	-7.39	7	
THR302:HN: LIG:N	1	-8.26	3.96	-7.37	8	
THR302:HN: LIG:N	1	-8.10	5.23	-7.21	9	
THR302:HN: LIG:N	1	-7.98	6.43	-7.08	10	
LIG:H: ASN600:O LIG:H: LYS603:O	2	-9.06	2.82	-7.57	1	4
LIG:H: LYS603:O ALA602:HN: LIG:N	2	-8.85	4.07	-7.35	2	
LIG:H: VAL605:O ASP354:HN: LIG:O	2	-8.73	4.93	-7.24	3	
LIG:H: LYS603:O ALA602:HN: LIG:N	2	-8.69	5.28	-7.02	4	
LIG:H: LYS603:O ALA602:HN: LIG:N	2	-8.65	5.07	-7.15	5	
LIG:H: LYS603:O ALA602:HN: LIG:N	2	-8.56	6.54	-7.07	6	
LIG:H: LYS602:O ALA602:HN: LIG:N	2	-8.56	6.54	-7.07	7	
LIG:H: LYS603:O ALA602:HN: LIG:N	2	-8.49	7.389	-7.00	8	
LIG:H: LYS603:O ALA602:HN: LIG:N	2	-8.49	7.83	-7.00	9	
LIG:H: LYS603:O	1	-8.49	7.47	-6.99	10	

[1]for compound 2 and 4, respectively. The docking results of all generated conformers of compounds within the binding pocket are strongly enhancing antibacterial and antifungal activities as depicted in Table 1. Furthermore, the inhibition constant Ki, intermolecular energy and hydrogen bonds were also determined and recorded in Table 3.

Conclusion

The present research summarized the synthesis of novel 3,5-disubstituted-4,5-dihydro-1*H*-pyrazole derivatives containing imine moiety as promising antimicrobial agents. The scavenging activity of the potent antioxidant derivatives was determined by using DPPH radical. On the other hand, docking approach using Autodock 4.2 was achieved to explore the binding state of ligand inside the glucosamine-6-phosphate synthase pocket for the potent discovered hits.

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تحضير ودراسة الفعالية المضادة للميكروبات والمضادة للاكسدة ودراسة الارتباط لعدد جديد من مشتقات البايرازولين المعوضة الحاوية على مجموعة الايمين

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تضمن البحث تحضير عدد جديد من مشتقات البايرازولين الثنائية التعويض والمحتوية على مجموعة الايمين وتشخيصها بأستخدام الطرق الطيفية. وقد تم دراسة الفعالية المضادة للميكروبات حيث اظهرت المركبات المحضرة فعالية متوسطة الى فعالية عالية تجاه الاصناف الميكروبية المستخدمة. كذلك فقد تم دراسة الفعالية المضادة للأكسدة تجاه جذور DPPH نوعياً بأستخدام طريقة كروماتوغرافيا الورقة وكمياً بأستخدام الطرق الطيفية حيث وتم حساب قيمة IC_{50} للمركبات الفعالة. بالاضافة الى ذلك فقدتضمن البحث دراسة الارتباط Docking study (للمركبات التي اظهرت فعالية عالية تجاه الميكروبات) داخل الموقع الفعال لانزيم Glucoseamine-6-phosphate synthase المستهدف من قبل المضادات الميكروبية.