JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Synthesis, Antimicrobial Evaluation, and Docking Study of Some New Isoxazoline Derivatives Derived from Chalcones

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Abstract: New 2-Isoxazoline derivatives containing furan moieties were synthesized from chalcones as starting materials, followed by antimicrobial activity. Chalcones were synthesized by reacting p-methoxy acetophenone or 3,4-(methylenedioxy)acetophenone with various aldehydes that were synthesized using Claisen-Schmidt condensation. Subsequently, the obtained products underwent cyclization with hydroxylamine hydrochloride to yield the corresponding 2-isoxazoline derivatives. The synthesized isoxazolines have been characterized via ^{1}H-NMR, FTIR, and GC-Mass spectroscopy. The new derivatives were screened for their activity against different bacterial species as well as Candida albicans and exhibited moderate to excellent activity as new antimicrobial agents. A docking study was conducted on most potent derivatives against glucoseamine-6-phosphate synthase (GlcN-6-P), the target enzyme for antimicrobial agents. The study aimed to understand how the discovered derivatives interact with the binding pocket residues of the enzyme.

Key words: Synthesis, Chalcones, Isoxazoline, Claisen-Schmidt, Docking study

Submitted: September 1, 2023. **Accepted**: November 6, 2023.

Cite this: Mahdi IS, Abdula AM, Jassim AMN. Synthesis, Antimicrobial Evaluation, and Docking Study of Some New Isoxazoline Derivatives Derived from Chalcones. JOTCSA. 2024;11(1):217-232.

DOI: https://doi.org/10.18596/jotcsa.1353554.

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1. INTRODUCTION

Heterocyclic compounds are extensively found in nature and are vital to life in different ways. Notably, these substances are significant due to the diverse range of physiological activities linked with this category of compounds, in addition to their great importance in the field of drug discovery (1). Heterocyclic rings exist in numerous important compounds and in the constituents of: vitamins B-complex, antibiotics, chlorophyll, heme, amino acids, enzymes, plant pigments, dye materials, genetic material DNA, etc..(2). Chalcones are naturally occurring substances that belong to the group of bicyclic compounds. They have been the focus of numerous scientists in recent times because of their vast biological capabilities and are considered important intermediates for the synthesis of isoxazolines (3). The word "chalcones" was first used by scientists Kostanecki & Tambor in 1899 (4). Chalcones (1,3 diphenyl-2-propen-1-ones) are derived from two aromatic rings with delocalized π-electron connected by an α,β-unsaturated carbonyl group. These compounds contain a (−C=O−CH=CH−) ketoethylenic group in their composition (5). Isoxazolines are heterocyclic compounds with

five-member rings called azoles with an oxygen atom adjacent to a nitrogen atom and a combined one paired-bond (6). The weak nitrogen-oxygen connections make isoxazolines promising candidates for ring cleavage, thus making it simple to change the substituents in their ring structures, which makes isoxazolines very useful intermediates in many synthetic processes of bioactive compounds (7). Isoxazolines are an important class of heterocyclic compounds that are broadly used in pharmaceuticals and treatments such as: anti-Alzheimer, anticancer (8), anticoagulant (9), anti-inflammatory (10), anti-tuberculosis (11), antidepressant (12), antioxidant (13), antimicrobial (14),(15), antitumor (16), anti-HIV & antiplatelet (17), and ulcer genic activity (18). In this article, we concentrate on the synthesis, characterization, and antimicrobial screening of new isoxazolines against two strains of bacteria; gram positive and gram negative as well as fungi, due to the recent and growing need to find and seek out new antimicrobial agents. The molecular target enzyme for antimicrobial treatment is (GlcN-6-P) synthase (19). A docking analysis was conducted to determine the binding posture of the new antimicrobial drugs and to highlight their mode of action. The docking outcomes improved the potential antimicrobial activity of the new compounds. The interactions between chalcone and isoxazoline derivatives and the active site of glucosamine-6-phosphate synthase were investigated using Autodock (4.2), a useful tool for investigating the binding coalition of tiny ligands to enzyme binding pockets (20).

2. EXPERMENTAL SECTION

2.1. Materials

The chemicals and solvents utilized in this study were: furfural (Sigma-Aldrich, 99%), $NaNO_2$ (Merck, 99%), $CuCl_2.2H_2O$ (Sigma- Aldrich, 99.9%), 4-Chloroaniline (BDH, 99%), 4-Bromoaniline (CDH, 98%), 2,4-Dichloroaniline (Merck, 99%), 2-Chloro-4-nitroaniline (Flouro-chem, 97%), Ethanol (Sigma-Aldrich, ≥99.5%), NaOH (Sigma-Aldrich, 99%), 4-Methoxyacetphenone (Sigma-Aldrich, 99%), 3,4-(Methylenedioxy)acetophenone (Sigma-Aldrich, 99%), $NH_2OH.HCl$ (BDH, 99%).

2.2. Instrumentation

The melting points of the synthesized compounds were accomplished by using an electrothermal capillary apparatus a (digital Stuart scientific SMP30) and were uncorrected, at Al-Mustansiriyah University/ College of Science/ Department of Chemistry/ Iraq. The infrared spectra (FTIR) were recorded on (ALPHA II FTIR Spectrometer-PLATINUM-ATR) (Bruker) in the range (400-4000cm^{-1}) at Al-Mustansiriyah University/ College of Science/ Department of Chemistry/ Iraq. The molecular weight of the prepared compounds was determined by using (Shimadzu model GCMS-QP2010 PLUS) at Samarra University/College of Education for Pure Science/ Iraq.^{1}H-NMR spectra of the prepared compounds were recorded by using TMS as an internal standard on (Bruker Avance Neo 400MHz) NMR spectrometer (Germany) in Al-Basrah University/ College of Education for Pure Science/ Department of Chemistry/ Iraq and (Brukerbiospin GmbH) at (400MHz) & (75MHz) at Gazi Osman Pasa (GOP) University/ Turkey.

2.3. Procedure

Note: All synthesized compounds have been investigated by TLC technique by using hexane:ethylacetate as eluent in different ratios.

2.3.1. Synthesis of 5-Arylfuran-2-carbaldehyde derivatives 1(a-d)

Aniline derivatives (0.136 mol) were dissolved in a mixture consisting of conc. HCl (33.07 mL) and distilled water (22.5 mL). The solution was cooled to 0-5°C, and then added a mixture consisting of sodium nitrite (9.5 gm, 0.138 mol) dissolved in distilled water (25 mL) was added progressively with continuous stirring for 10 min to produce diazonium salt. The solution was filtered and then furan-2-carboxyldehyde (15.4 gm, 0.16 mol) in distilled water (50 mL) was added along with a solution of $CuCl_2.2H_2O$ (5 gm, 0.04 mol) dissolved in distilled water (25 mL) and stirred at a temperature of 10-15°C. The temperature was gradually raised to 40°C and the mixture was stirred for 4h. The reaction progress was monitored by TLC using hexane:ethyl acetate (1:1). The precipitate formed was filtered and washed with sodium hydrogen carbonate solution (5%) and distilled water for several times, then dried and recrystallized with ethanol (21).

Note: In ^{1}H-NMR spectra, the signals at δ 2.5 and 7.26 ppm are for the solvents DMSO-d6 and $CDCl_3$, respectively.

5-(4-chlorophenyl)furan-2-carbaldehyde (1a)

Deep brown powder, yield (64%), m.p 114-116°C (21); FT-IR (cm^{-1}): 3110 (C-H furan), 3059 (C-H aromatic), 2834 (C-H aldehyde), 1673 (C=O), 1661 (C=C furan ring), 1589 (C=C aromatic ring), 1010 (C-Cl). ^{1}H-NMR (400MHz, $CDCl_3$) δ(ppm): 6.83(d,1H,CH furan, *J*=4.0Hz), 7.32(d,1H,CH furan, *J*=4.0Hz), 7.41(d,2H,Ar-H, *J*=8.0Hz), 7.75(d,2H,Ar-H, *J*=8.0Hz), 9.65(s,1H,CO-H aldehyde). Mass (EI) *m/z*: 206 M^+ for $C_{11}H_7ClO_2$.

5-(4-bromophenyl)furan-2-carbaldehyde (1b)
Brown powder, yield (65%), m.p 143-145°C (22); FT-IR (cm^{-1}): 3110 (C-H furan), 3056 (C-H aromatic), 2858 (C-H aldehyde), 1672 (C=O), 1660 (C=C furan ring), 1595 (C=C aromatic ring), 1039 (C-Br). ^{1}H-NMR (400MHz, $CDCl_3$) δ(ppm): 6.84(d,1H,CH furan, *J*=4.0Hz), 7.32(d,1H,CH furan, *J*= 4.0Hz), 7.57(d,2H,Ar-H, *J*=8.0Hz), 7.68(d,2H,Ar-H, *J*=8.0Hz), 9.65(s,1H,CO-H aldehyde). Mass (EI) *m/z*: 250 M^+ for $C_{11}H_7BrO_2$.

5-(2,4-dichlorophenyl)furan-2-carbaldehyde (1c)
Brown powder, yield (79%), m.p 148-150°C (21); FT-IR (cm^{-1}): 3158 (C-H furan), 3079 (C-H aromatic), 2842 (C-H aldehyde), 1677 (C=O), 1663 (C=C furan ring), 1585 (C=C aromatic ring), 1034 (C-Cl). ^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 7.32(d,1H,Ar-H, *J*=12.0 Hz), 7.37(d,1H,CH furan *J*=4.0 Hz), 7.50(d,1H,CH furan *J*=4.0 Hz), 7.96(d,1H,Ar-H, *J*=12.0 Hz), 7.35(s,1H,Ar-H), 9.70(s,1H, CO-H aldehyde). Mass (EI) *m/z*: 240 M^+ for $C_{11}H_6Cl_2O_2$.

5-(2-chloro-4-nitrophenyl)furan-2-carbaldehyde (1d)
Orange powder, yield (75%), m.p 124-126°C; FT-IR (cm^{-1}): 3105 (C-H furan), 3034 (C-H aromatic), 2832 (C-H aldehyde), 1680 (C=O), 1664 (C=C furan ring), 1582 (C=C aromatic ring), $1341_{sym.}$,$1513_{asym.}$(No_2), 1034 (C-Cl).^{1}H-NMR (400 MHz, $CDCl_3$) δ(ppm): 7.41(d,1H,CH furan, *J*=4.0Hz), 7.56(d,1H,CH furan, *J*=4.0Hz), 8.22(d,1H,Ar-H, *J*=8.0Hz), 8.25(d,1H,Ar-H, *J*=8.0Hz), 8.38(s,1H,Ar-H), 9.77 (s,1H,CO-H aldehyde). Mass (EI) *m/z*: 251 M^+ for $C_{11}H_6ClNO_4$.

2.3.2. Synthesis of Chalcone derivatives (General procedure) 2(a-h)
The preparation of chalcone derivatives was accomplished by using the procedure described in the published literature (23). To a solution of methyl ketone (0.001 mol) {4-Methoxy acetophenone or 3,4-(Methylenedioxy)acetophenone} in ethanol (10 mL), sodium hydroxide (40%,1 mL) was added, and the reaction mixture was stirred for 30 min. After that, 0.001 mol of aromatic aldehyde (previously prepared) 1(a-d) was added and stirred for 6-12 h. The completion of the reaction was checked by TLC using hexane:ethyl acetate as eluent (1:1). The precipitate was formed by adding crushed ice, then filtered, dried, and recrystallized with ethanol.

3-(5-(4-chlorophenyl)furan-2-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (2a)
Yellow powder, yield (75%), m.p 169-171 °C; FT-IR (cm^{-1}): 3109 (C-H aromatic), 2839 (C-H aliphatic), 1646 (C=O chalcone), 1603 (CH=CH chalcone), 1587 (C=C aromatic), 1031 (C-Cl). ^{1}H-NMR (400MHz, DMSO-d_6) δ(ppm): 3.89(s,3H,O-CH_3), 7.11(d,2H,Ar-H, *J*=8.0Hz), 7.19(d,1H,CH furan, *J*=4.0Hz), 7.24(d,1H,CH furan, *J*=4.0Hz), 7.54-7.58(m,2H,Ar-H & 1H,CH chalcone), 7.73(d,1H,CH=CH-CO chalcone, *J*=16.0Hz), 7.96(d,2H,Ar-H, *J*=8.0Hz), 8.15(d,2H,Ar-H, *J*=8.0Hz). GC-Mass (EI) *m/z*: 338 M^+ for $C_{20}H_{15}ClO_3$.

3-(5-(4-bromophenyl)furan-2-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (2b)
Yellow powder, yield (76%), m.p 185-187°C; FT-IR (cm^{-1}): 3109 (C-H aromatic), 2939 (C-H aliphatic), 1646 (C=O chalcone), 1599 (CH=CH chalcone), 1587 (C=C aromatic), 1029 (C-Br). ^{1}H-NMR (400MHz, DMSO-d_6) δ(ppm): 3.88(s,3H,-OCH_3), 7.11(d,2H,Ar-H, *J*=8.0Hz), 7.20(d,1H,CH furan, *J*=4.0Hz), 7.27(d,1H,CH furan, *J*=4.0Hz), 7.56(d,1H,CH=CH-CO chalcone, *J*=16.0Hz), 7.69(d,2H,Ar-H, *J*=8.0Hz), 7.75(d,1H,CH=CH-CO chalcone, *J*=16.0Hz), 7.91 (d,2H,Ar-H, *J*=8.0Hz), 8.16(d,2H,Ar-H, *J*=8.0Hz). GC-Mass (EI) *m/z*: 383 M^+ for $C_{20}H_{15}BrO_3$.

3-(5-(2,4-dichlorophenyl)furan-2-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (2c)
Yellow powder, yield (84%), m.p 114-116°C; FT-IR (cm^{-1}): 3112 (C-H aromatic), 2836 (C-H aliphatic), 1648 (C=O chalcone), 1603 (CH=CH chalcone), 1583 (C=C aromatic), 1023 (C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 3.88(s,3H,-OCH_3), 7.11(d,2H,Ar-H, *J*=8.0Hz), 7.25(d,1H,CH furan, *J*=4.0Hz) 7.39(d,1H,CH furan, *J*=4.0Hz), 7.56-7.80(m,1H,CH chalcone & 1H,Ar-H), 8.15(d,2H,Ar-H, *J*=8.0Hz), 8.20(d,1H,CH=CH-CO chalcone, *J*=8.0Hz). GC-Mass (EI) *m/z*: 373 M^+ for $C_{20}H_{14}Cl_2O_3$.

3-(5-(2-chloro-4-nitrophenyl)furan-2-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (2d)
Orange powder, yield (80%), m.p 164-166°C; FT-IR (cm^{-1}): 3110 (C-H aromatic), 2838 (C-H aliphatic), 1648 (C=O chalcone), 1605 (CH=CH chalcone), 1583 (C=C aromatic), $1339_{sym.}$, $1510_{asym.}$(NO_2), 1019 (C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 3.88(s,3H,-OCH_3), 7.11(d,2H,Ar-H, *J*=8.0Hz), 7.31(d,1H,CH furan, *J*=4.0Hz), 7.59(d,1H,CH=CH-CO chalcone, *J*=16.0Hz), 7.65(d,1H,CH furan, *J*=4.0Hz), 7.84(d,1H,CH=CH-CO chalcone, *J*=16.0Hz), 8.15(d,2H,Ar-H, *J*=8.0Hz), 8.25-8.46(m,3H,Ar-H). GC-Mass (EI) *m/z*: 384 M^+ for $C_{20}H_{14}ClO_5$.

1-(benzo[d][1,3]dioxol-5-yl)-3-(5-(4-chlorophenyl)furan-2-yl)prop-2-en-1-one (2e)
Yellow powder, yield (73%), m.p 165-167°C; FT-IR (cm^{-1}): 3108 (C-H aromatic), 2902 (C-H aliphatic), 1644 (C=O chalcone), 1603 (CH=CH chalcone), 1580 (C=C aromatic), 1042 (C-Cl). ^{1}H-NMR (400MHz, DMSO-d_6) δ(ppm): 6.18(s,2H,O-CH_2-O), 7.12(d,1H,Ar-H, *J*=8.0Hz), 7.20 (d,1H,CH furan, *J*=4.0Hz), 7.26(d,1H,CH furan, *J*=4.0Hz), 7.55 (d,2H,Ar-H, *J*=4.0Hz), 7.57(d,2H,Ar-H, *J*=4.0Hz), 7.65(d,1H,Ar-H, *J*=4.0Hz), 7.70(s,1H,Ar-H), 7.86 (d,1H,CH=CH-CO chalcone, *J*=8.0Hz), 7.98 (d,1H,CH=CH-CO chalcone, *J*=8.0Hz). GC-Mass (EI) *m/z*: 352 M^+ for $C_{20}H_{13}ClO_4$.

1-(benzo[d][1,3]dioxol-5-yl)-3-(5-(4-bromophenyl)furan-2-yl)prop-2-en-1-one (2f)
Yellow powder, yield (68%), m.p 180-182°C; FT-IR (cm^{-1}): 3106 (C-H aromatic), 2937 (C-H aliphatic), 1643 (C=O chalcone), 1602 (CH=CH chalcone), 1581 (C=C aromatic), 1022 (C-Br). ^{1}H-NMR (400MHz, DMSO-d_6) δ(ppm): 6.18(s,2H,O-CH_2-O), 7.11(d,1H,Ar-H, *J*=8.0Hz), 7.20(d,1H,CH furan, *J*=4.0Hz), 7.27(d,1H,CH furan, *J*=4.0Hz), 7.55(d,2H,Ar-H, *J*=16.0Hz), 7.67(d,1H,CH=CH-CO chalcone, *J*=12.0Hz), 7.70(s,1H,Ar-H), 7.72(d,2H,Ar-H, *J*=16.0Hz),7.86(d,1H,Ar-H,*J*=8.0Hz), 7.92(d,1H,CH=CH-CO chalcone, *J*=12.0Hz). GC-Mass (EI) *m/z*: 397 M^+ for $C_{20}H_{13}BrO_4$.

1-(benzo[d][1,3]dioxol-5-yl)-3-(5-(2,4-dichlorophenyl)furan-2-yl)prop-2-en-1-one (2g)
Yellow powder, yield (75%), m.p 158-160°C; FT-IR (cm^{-1}): 3111 (C-H aromatic), 2900 (C-H aliphatic), 1645 (C=O chalcone), 1606 (CH=CH chalcone), 1576 (C=C aromatic), 1024 (C-Cl). ^{1}H-NMR (400MHz, DMSO-d_6) δ(ppm): 6.18(s,2H,O-CH_2-O), 7.11(d,1H,Ar-H, *J*=8.0Hz), 7.26 (d,1H,CH furan, *J*=4.0Hz), 7.40(d,1H,CH furan, *J*=4.0Hz), 7.56(s,1H,Ar-H), 7.58-7.64(d,1H,CH=CH-CO chalcone & 1H,Ar-H), 7.76(d,1H,CH=CH-CO chalcone, *J*=16.0Hz), 7.80(d,1H,Ar-H, *J*=4.0Hz), 7.85(d,1H,Ar-H, *J*=8.0Hz), 8.23(d,1H,Ar-H, *J*=8.0Hz). GC-Mass (EI) *m/z*: 387 M^+ for $C_{20}H_{12}Cl_2O_4$.

1-(benzo[d][1,3]dioxol-5-yl)-3-(5-(2-chloro-4-nitrophenyl)furan-2-yl) prop-2-en-1-one (2h)
Yellow powder, yield (78%), m.p 211-213°C; FT-IR (cm^{-1}): 3105 (C-H aromatic), 2911 (C-H aliphatic), 1650 (C=O chalcone), 1607 (CH=CH chalcone), 1586 (C=C aromatic), $1352_{sym.}$, $1477_{asym.}$(NO_2), 1036 (C-Cl). ^{1}H-NMR (400MHz, DMSO-d_6) δ(ppm): 6.19(s,2H,O-CH_2-O), 7.13(d,1H,Ar-H, *J*=8.0Hz), 7.34(d,1H,CH furan, *J*=4.0Hz), 7.61(d,1H,CH=CH-CO chalcone, *J*=16.0Hz), 7.56(d,1H,Ar-H, *J*=4.0Hz), 7.69(d, 1H,CH furan, *J*=4.0Hz), 7.86 (d,1H,CH=CH-CO chalcone, *J*=16.0Hz)), 8.29-8.52(m,3H,Ar-H). GC-Mass (EI) *m/z*: 397 M^+ for $C_{20}H_{12}ClNO_6$.

2.3.3. Synthesis of 3,5-disubstituted aryl-isoxazoline derivatives (General procedure) 3(a-g)
These compounds were obtained according to the modified method reported in (24). Chalcone compounds (0.001 mol) were dissolved in ethanol absolute (10 mL), a solution of hydroxyl amine hydrochloride (0.0015 mol, 0.104 gm) dissolved in aqueous NaOH (40%, 2 mL) was added, and the resulting mixture was refluxed for 15h. The reaction was observed by TLC using hexane:ethyl acetate system (2:1). Crushed ice was added; the precipitate was filtered off, washed with distilled water, dried, and recrystallized with ethanol.

5-(5-(4-chlorophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole (3a)
Light brown powder, yield (60%), m.p 130-133°C; FT-IR (cm^{-1}): 3167 (C-H aromatic), 2837 (C-H aliphatic), 1655 (C=N isoxazoline ring), 1595 (C=C aromatic), 1244 (C-O), 1023 (C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 3.75(dd,1H,Ha-isoxazoline, *J*=4.0, 8.0 Hz), 3.82(s,3H,O-CH_3), 3.80-3.85 (m,1H,Hb-isoxazoline), 5.74-5.79(m appeared as triplet,1H,Hx- isoxazoline), 6.75(d,1H,CH furan, *J*=4.0), 7.02(d,1H,CH furan, *J*=4.0), 7.05(d,2H,Ar-H, *J*=12.0), 7.48(d,2H,Ar-H, *J*=8.0), 7.68-7.73(m,4H,Ar-H). GC-Mass (EI) *m/z*: 353 M^+ for $C_{20}H_{16}ClNO_3$.

5-(5-(4-bromophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole (3b)
Orange powder, yield (49%), m.p 138-140°C; FT-IR (cm^{-1}): 3123 (C-H aromatic), 2836 (C-H aliphatic), 1650 (C=N isoxazoline ring), 1596 (C=C aromatic), 1251 (C-O), 1023 (C-Br). ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 7.75(dd,1H,Ha-isoxazoline, *J*=8.0, 16.0 Hz), 3.83(s,3H,O-CH_3), 3.87(dd,1H,Hb-isoxazoline, *J*=4.0, 8.0 Hz), 5.73-5.78(m appeared as triplet,1H,Hx- isoxazoline), 6.75(d,1H,CH furan, *J*=4.0), 7.02(d,1H,CH furan, *J*=4.0), 7.05(d,2H,Ar-H, *J*=8.0), 7.61(d,2H,Ar-H, *J*=8.0), 7.65(d,2H,Ar-H, *J*=8.0), 7.70(d,2H,Ar-H, *J*=12.0). GC-Mass (EI) *m/z*: 398 M^+ for $C_{20}H_{16}BrNO_3$.

5-(5-(2,4-dichlorophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole (3c)
Brown powder, yield (62%), m.p 86-88°C; FT-IR (cm^{-1}): 3070 (C-H aromatic), 2837 (C-H aliphatic), 1649 (C=N isoxazoline ring), 1597 (C=C aromatic), 1252 (C-O), 1023 (C-Cl). ^{1}H-

NMR (400 MHz, DMSO-d_6) δ(ppm): 3.76(dd,1H,Ha-isoxazoline, *J*=4.0, 8.0 Hz), 3.82(s,3H,O-CH_3), 3.80-3.85(m,1H,Hb-isoxazoline), 5.76-5.81(m appeared as triplet,1H,Hx-isoxazoline), 6.81 (d,2H,Ar-H, *J*=4.0), 7.04(d,2H,Ar-H, *J*=8.0), 7.12(d,1H,CH furan, *J*=4.0), 7.15(d,1H,CH furan, *J*=4.0), 7.29-7.42(m,3H,Ar-H). GC-Mass (EI) *m/z*: 388 M^+ for $C_{20}H_{15}Cl_2NO_3$.

5-(5-(2-chloro-4-nitrophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole (3d)
Brown powder, yield (62%), m.p 86-88°C; FT-IR (cm^{-1}): 3072 (C-H aromatic), 2837 (C-H aliphatic), 1660 (C=N isoxazoline ring), 1598 (C=C aromatic), 1306 sym., 1510 asym. (NO_2), 1246 (C-O),1024 (C-Cl). ^{1}H-NMR (400MHz, DMSO-d_6) δ(ppm): 3.69(d,1H,Ha-isoxazoline, *J*=8.0 Hz), 3.73(s,3H,O-CH_3), 3.82(d,1H,Hb-isoxazoline, *J*=8Hz), 5.70-5.87(m,1H,Hx-isoxazoline), 6.67(d,1H,Ar-H, *J*=8.0), 6.98(d,1H,CH furan, *J*=4.0), 7.12(d,1H,Ar-H, *J*=8.0 Hz), 7.18(d,1H,CH furan, *J*=4.0), 7.34(d,1H,Ar-H, *J*=8.0), 7.64(d,1H,Ar-H, *J*=8.0 Hz), 7.87(d,1H,Ar-H, *J*=8.0 Hz). GC-Mass (EI) *m/z*: 398 M^+ for $C_{20}H_{15}ClN_2O_5$.

3-(benzo[d][1,3]dioxol-5-yl)-5-(5-(4-chlorophenyl)furan-2-yl)-4,5-dihydroisoxazole (3e)
Orange powder, yield (55%), m.p 88-90°C; FT-IR (cm^{-1}): 3121 (C-H aromatic), 2886 (C-H aliphatic), 1608 (C=N isoxazoline ring), 1585 (C=C aromatic), 1254 (C-O), 1040 (C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 3.73(d,2H,CH_2 isoxazoline, *J*=8.0 Hz), 5.74-5.79(t,1H,CH isoxazoline, *J*=12.0 Hz), 6.11(s,2H,O-CH_2-O), 6.76(d,1H,CH furan, *J*=4.0 Hz), 7.02-7.05(m,1H,CH furan & 1H,Ar-H), 7.29(d,1H,CH furan, *J*=4.0 Hz), 7.32(d,1H,Ar-H, *J*=4.0 Hz), 7.49(d,2H,Ar-H, *J*=8.0 Hz), 7.72(d,2H,Ar-H, *J*=8.0 Hz). GC-Mass (EI) *m/z*: 367 M^+ for $C_{20}H_{14}ClNO_4$.

3-(benzo[d][1,3]dioxol-5-yl)-5-(5-(4-bromophenyl)furan-2-yl)-4,5-dihydroisoxazole (3f)
Light brown powder, yield (48%), m.p 96-98°C; FT-IR (cm^{-1}): 3120 (C-H aromatic), 2919 (C-H aliphatic), 1646 (C=N isoxazoline ring), 1607 (C=C aromatic), 1250 (C-O), 1038(C-Br). ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 3.73(d,2H,CH_2 isoxazoline, *J*=12.0 Hz), 5.74-5.79(t,1H,CH isoxazoline, *J*=12.0 Hz), 6.11(s,2H,O-CH_2-O), 7.76(d,1H,CH furan, *J*=4.0 Hz), 7.03-7.05 (m,1H,CH furan & 1H,Ar-H), 7.24(d,1H,Ar-H, *J*=8.0 Hz), 7.31(s,1H,Ar-H), 7.61-7.67(m,4H,Ar-H). GC-Mass (EI) *m/z*: 412 M^+ for $C_{20}H_{14}BrNO_4$.

3-(benzo[d][1,3]dioxol-5-yl)-5-(5-(2,4-dichlorophenyl)furan-2-yl)-4,5-dihydroisoxazole (3g)
Brown powder, yield (65%), m.p 80-83°C; FT-IR (cm^{-1}): 3010 (C-H aromatic), 2891 (C-H aliphatic), 1654 (C=N isoxazoline ring), 1600 (C=C aromatic), 1250 (C-O), 1037 (C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6) δ(ppm): 3.75(d,2H,CH_2 isoxazoline, *J*=12.0 Hz), 5.77-5.82(t,1H,CH isoxazoline, *J*=12.0 Hz), 6.11(s,2H,O-CH_2-O), 6.82(d,1H,CH furan, *J*=4.0 Hz), 7.04(d,1H,Ar-H, *J*=12.0 Hz), 7.16(d,1H,CH furan, *J*=4.0 Hz), 7.83(d,1H,Ar-H, *J*=8.0 Hz), 7.31(s,1H,Ar-H), 7.52(d,1H,Ar-H, *J*=8.0 Hz), 7.74(s,1H,Ar-H), 7.83(d,1H,Ar-H, *J*=8.0 Hz). GC-Mass (EI) *m/z*: 402 M^+ for $C_{20}H_{13}Cl_2NO_4$.

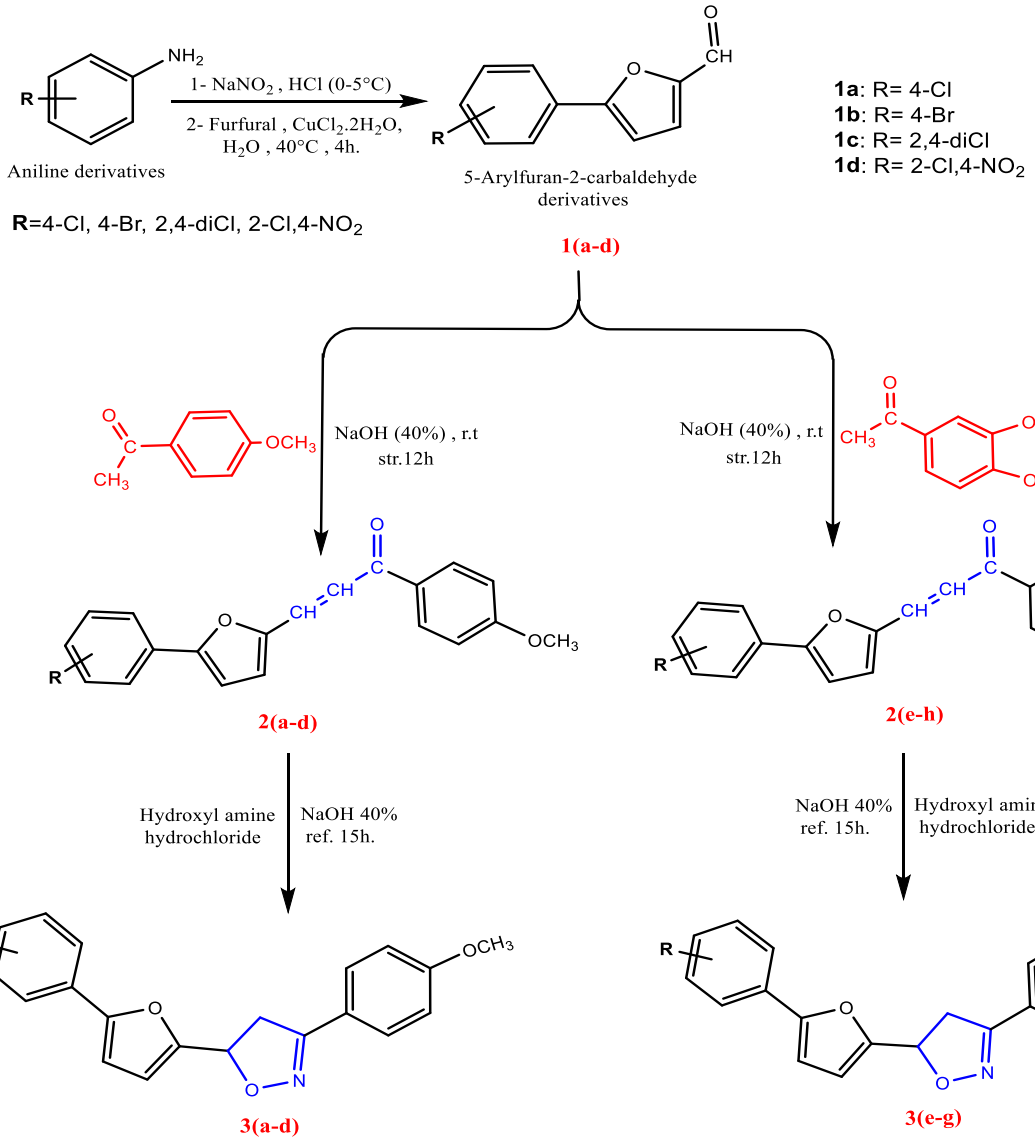
2.4. Biological study

2.4.1. Evaluation of Antibacterial and Antifungal activity
Agar well-diffusion was utilized to study the antimicrobial activity of synthesized compounds against bacterial species: *Staphylococcus aureus* and *Staphylococcus epidermidis* (gram+ve) obtained from the dermatological infection, *Escherichia coli* and *klebsiella* (gram-ve) obtained from the gastrointestinal tract (GIT), as well as *Candida albicans* as fungi. DMSO was used as a solvent and amoxicillin & fluconazole as standard drugs, and the synthesized compounds will be in a solution at a concentration of 10000 µg/ml. This study was performed in the Department of Biology, College of Science, Mustansiriyah University. In brief, the sterilized agar [Muelles-Hitton agar] media were poured into petri dishes and allowed to solidify. The testing bacteria were distributed on the surface of the agar with sterile cotton swab, then holes were made and left it for 15 min. After that, 50µl of each compound solution were placed in the holes and the dishes were incubated at 37°C for 24h. The observed zones of inhibition around holes were measured in millimeters (25).

3. RESULTS AND DISCUSSION

Chalcone derivatives **(2a-2h)** were prepared via analogy method to Claisen-Schmidt condensation reaction as described in literature (26). In brief, acetophenone derivatives reacted with the corresponding 5-Arylfuran-2-carbaldehyde derivatives in ethanol in the presence of aqueous sodium hydroxide; later, the resulting chalcones were cyclized with hydroxylamine hydrochloride to produce isoxazoline derivatives **(3a-3g)** (27). In the following section, we will discuss more

details to characterize the chemical structure of the newly synthesized compounds.



Scheme 1. Synthetic route of isoxazoline derivatives

The results of FTIR spectrum displayed that compound **1a** has weak sharp band at 2834 cm^{-1} attributed to C-H aldehyde, while a strong sharp band at 1673 cm^{-1} belongs to the stretching vibration of carbonyl group in the aldehyde compound. The C=C aromatic stretching vibration of furan ring appears as sharp strong band at 1661 cm^{-1} while the C=C aromatic stretching vibration of aromatic ring appears as medium strong band at 1589 cm^{-1}. The ^{1}H-NMR data of **1a** showed two doublet signals at 6.83 & 7.32 ppm which belong to (CH-CH) of furan ring, and protons of aromatic ring appear as two doublet signals at 7.41 & 7.75 ppm, as well as the presence of singlet signal at 9.65 ppm due to proton CO-H aldehyde. The mass spectrum of aldehyde compounds **1a** giving *m/z*: **206,** which represents the molecular ion **(M^+)** for **($C_{11}H_7ClO_2$)**. FTIR spectrum of chalcone **2b** showed characteristic bands at 1646 cm^{-1} belong to C=O chalcone, 1599 cm^{-1} due to CH=CH chalcone and the CO-H aldehyde band disappeared. The ^{1}H-NMR data of **2b** summarized singlet signal at 3.88 ppm due to -OCH_3, doublet signal at 7.11 ppm belongs to aromatic ring proton. The two doublet signals appeared at 7.20 ppm & 7.27 ppm related to (CH-CH) furan while the doublet signal at 7.56 ppm belongs to CH=CH chalcone. The doublet signal at 7.69 ppm belongs to two aromatic ring protons while the doublet signal at 7.75 ppm related to other proton of CH=CH chalcone and finally the two doublet

signals at 7.91 ppm and 8.16 ppm belong to aromatic ring protons. The molecular ion in GC-mass spectrum showed *m/z*: **383(M^+)** and strongly confirmed the structure **($C_{20}H_{15}BrO_3$)** of the prepared chalcone **2b**. The FTIR spectrum of isoxazoline compound **3b** exhibited characteristic bands at 2836 cm^{-1} due to C-H aliphatic of isoxazoline ring, 1650 cm^{-1} returned to C=N isoxazoline ring and the band that refers to carbonyl of chalcone disappeared. The ^{1}H-NMR spectrum of **3b** showed doublet of doublet signal at 3.75 ppm belonging to Ha-isoxazoline, singlet signal at 3.83 ppm belonging to O-CH_3, doublet of doublet signal at 3.87 ppm due to Hb-isoxazoline. The proton of Hx-isoxazoline appeared as multiplet signal at 5.73-5.78 ppm and disappeared the signals of CH=CH of chalcone. The two doublet signals appeared at 6.75 ppm and 7.02 ppm related to (CH-CH) furan, as well as four doublet signals appearing at: 7.05, 7.61, 7.65, and 7.70 ppm belonging to aromatic rings protons. The GC-Mass spectrum showed *m/z*: **398(M^+)** for **($C_{20}H_{16}BrNO_3$),** which strongly confirmed the structure of isoxazoline **3b**.

Finally, this research included the in vitro assay of antimicrobial activity for the synthesized compounds against two strains of bacteria as well as fungi and exhibited good to moderate activity (according to previous studies, most of the chalcone and isoxazoline compounds have biological activity because they contain active groups in their composition), except chalcone **2g** did not exhibit any activity at a concentration of 10000 µg/ml, as illustrated in Table 1.

Table 1: Antimicrobial activity for the synthesized compounds

	Diameter of Inhibition zone (mm)				
	Gram positive bacteria		**Gram negative bacteria**		**Fungi**
Comp.No.	***Staphylococcus aureus***	***Staphylococcus epidermidis***	***Escherichia coli***	***Klebsiella sp.***	***Candida albicans***
2a	15	10	12	12	14
2b	13	11	12	10	14
2c	16	-	10	10	-
2d	-	12	12	12	15
2e	17	-	11	11	8
2f	16	11	11	11	10
2g	-	-	-	-	-
2h	17	12	12	12	10
3a	15	11	8	11	15
3b	15	13	8	10	15
3c	-	-	8	8	-
3d	12	11	8	9	13
3e	12	10	8	11	13
3f	15	11	8	13	13
3g	14	11	8	10	12
Amoxicilline	23	35	20	22	-
Floconazol	-	-	-	-	13

3.1. Docking study

One of the most significant computational methods for determining the ideal orientation of a small organic molecule to a particular receptor, enzyme, or binding pocket is the docking technique, which is such a flexible process that ligands and receptors must alter their shapes to fit together well (28). A three-dimensional array of structurally recognized receptors, proteins, or enzymes is used in the first phase of docking approaches to determine the best conformations and orientations for ligand binding (22). Scores that are optimally linked with free binding energy are used to rate the exploration of various positions inside the binding pocket (29). The docking study of the potent active derivatives 2h and 3b toward antimicrobial species inside the active site of glucosamine-6-phosphate synthase, antibacterial and antifungal agents' possible targets were investigated. The X-ray shows that the following residues are present in the enzyme's binding pocket, 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 (30), as shown in Fig.1.

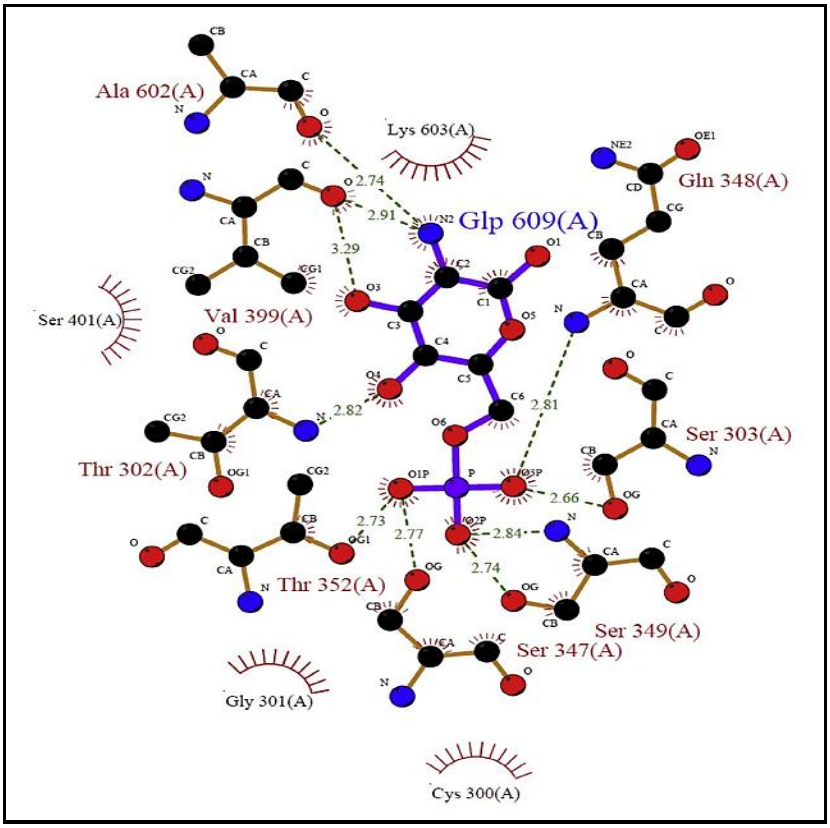
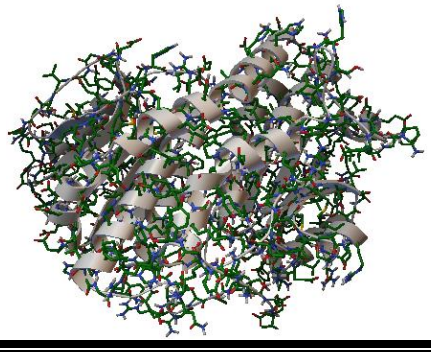
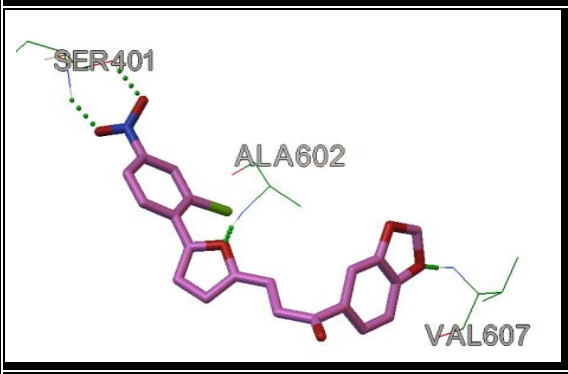


Figure 1. The binding of glucosamine-6-phosphate inside the active site of target enzyme.

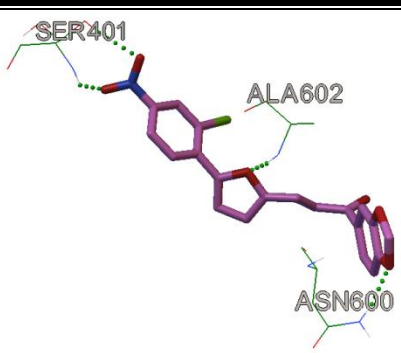
The binding energy of active compounds **2h** and **3b** within the established 3D structure of the target enzyme was assessed using Autodock 4.2. The binding of the best-generated conformers to the target enzyme's binding pocket is illustrated in Figures 2 and 3, respectively.



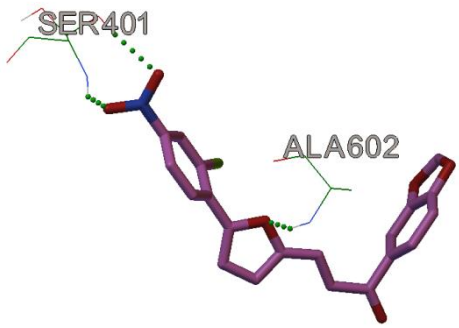
3D structure of glucoseamine-6-phosphate synthase (GlcN-6-P)



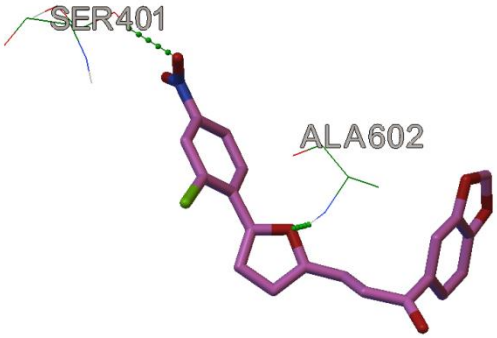
The first generated conformer inside the binding pocket of enzyme



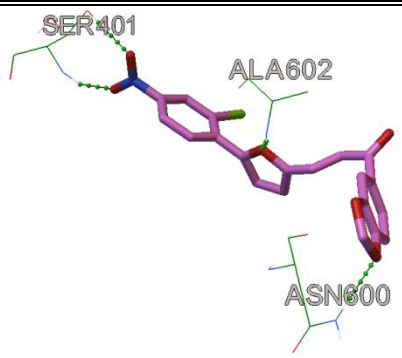
The second generated conformer inside the binding pocket of enzyme

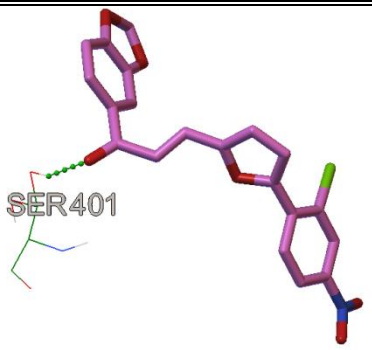


The third generated conformer inside the binding pocket of enzyme



The fourth generated conformer inside the binding pocket of enzyme





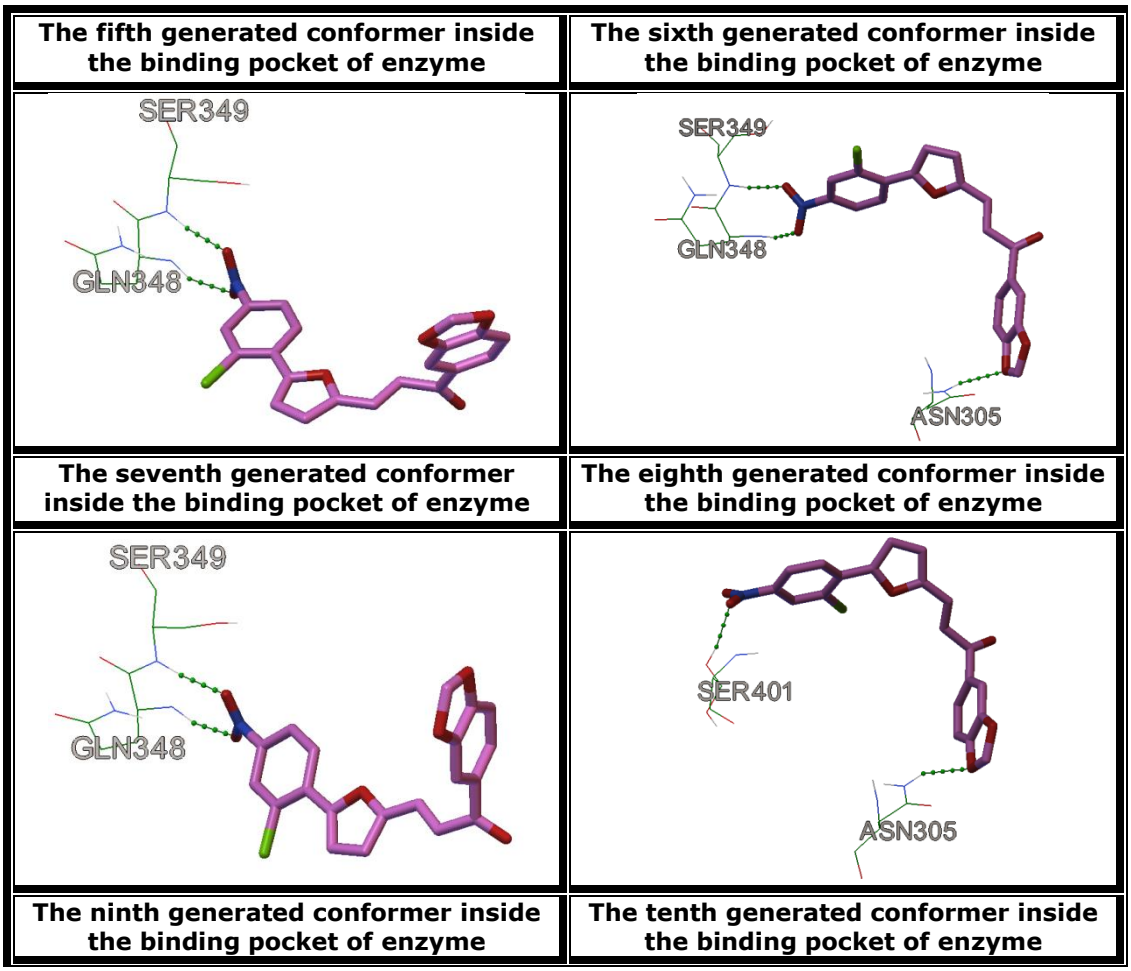
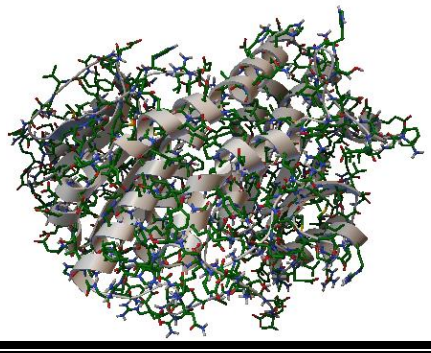
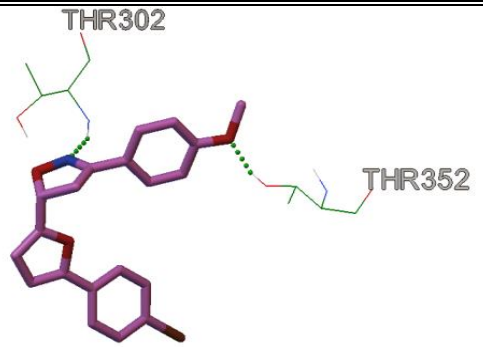


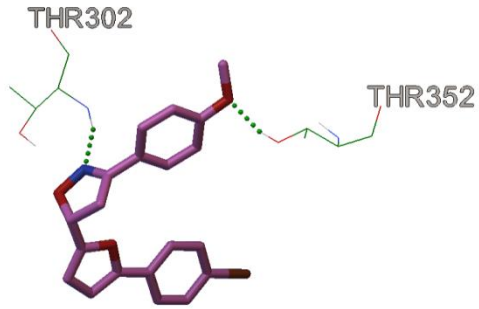
Figure 2. The docking of the best generated conformers of the potent discovered hit 2h inside the binding pocket of glucoseamine-6-phosphate synthase (GlcN-6-P).



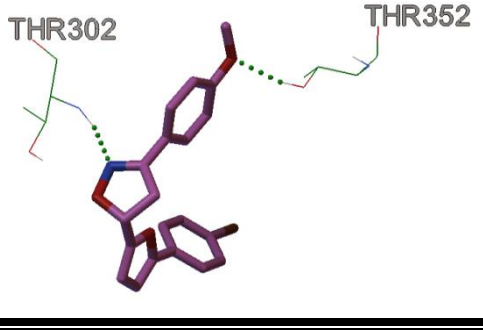
3D structure of glucoseamine-6-phosphate synthase (GlcN-6-P)



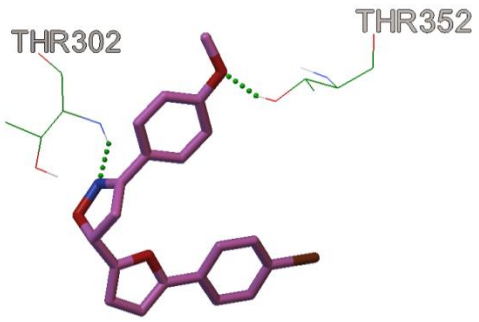
The first generated conformer inside the binding pocket of enzyme



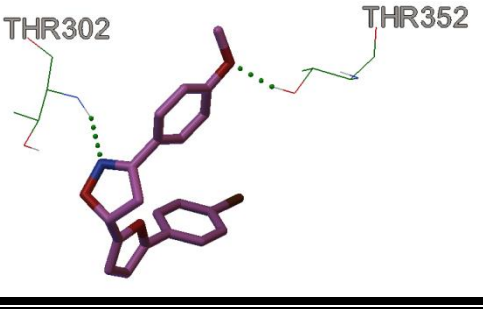
The second generated conformer inside the binding pocket of enzyme



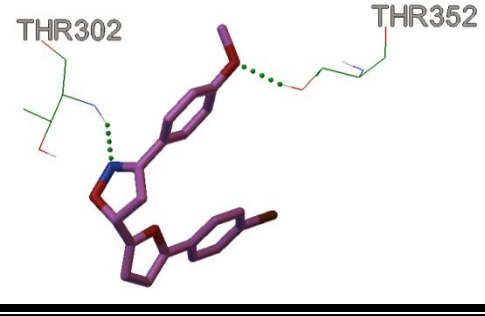
The third generated conformer inside the binding pocket of enzyme



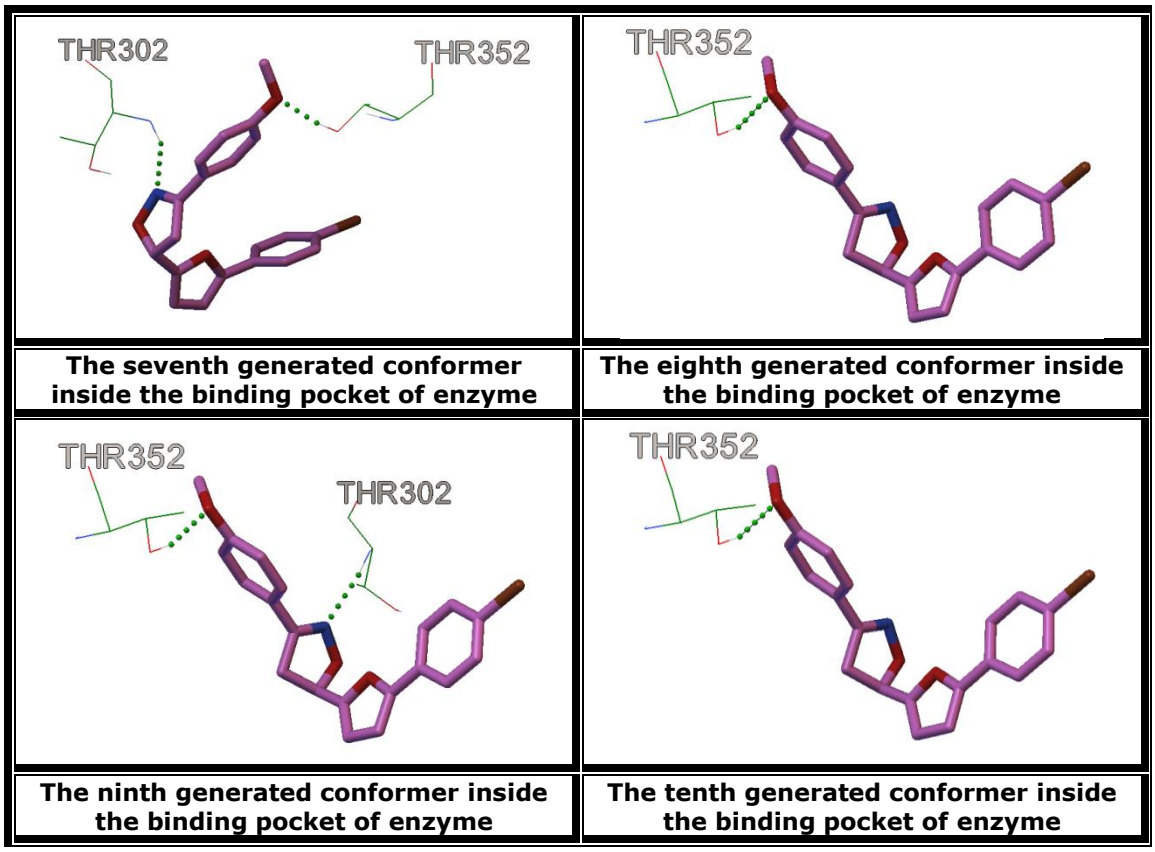
The fourth generated conformer inside the binding pocket of enzyme



The fifth generated conformer inside the binding pocket of enzyme



The sixth generated conformer inside the binding pocket of enzyme



The seventh generated conformer inside the binding pocket of enzyme	The eighth generated conformer inside the binding pocket of enzyme
The ninth generated conformer inside the binding pocket of enzyme	**The tenth generated conformer inside the binding pocket of enzyme**

Figure 3. The docking of the best generated conformers of the potent discovered hit 3b inside the binding pocket of glucoseamine-6-phosphate synthase (GlcN-6-P)

As indicated by molecular docking parameters (Table 2), the high ranking binding energy of compound **2h** was -8.35 kcal mol^{-1} for the best generated conformer. As indicated by Figure 2, the best conformer binds the active site with three H bonds. The intermolecular energy was -9.85 kcal mol^{-1}. Furthermore, the derivative **3b** fits the binding pocket with two hydrogen bonds, the first with HN residue of THR302 and the second with HG1 of THR352. The binding energy of the best generated conformer was -8.13 kcal mol^{-1} with intermolecular energy equal to -9.32 kcal mol^{-1}. The docking results of all generated conformers of compounds **2h** and **3b** within the binding pocket are strongly proportional to the antibacterial activities, as shown in Table 1. The inhibition constant Ki, intermolecular energy, and hydrogen bonds were also determined and depicted in Table 2.

Table 2: Docking parameters of compound 2h and 3b

Compounds	Conformers	Binding Energy (Kcal mol^{-1})	Inhibition constant (μM)	Intermolecular energy ($kcalmol^{-1}$)	H-bonds	Bonding
2h	1	-8.35	$0.752*10^{-3}$	- 9.85	4	SER401:HG:LIG:O ALA602:HN: LIG:O SER401:HN:LIG:O VAL607:HN: LIG:O
	2	-8.03	1.30	- 9.52	4	ASN600:HD22:LIG:O ALA602:HN:LIG:O SER401:HN:LIG:O SER401:HG:LIG:O
	3	-7.84	1.79	- 9.33	3	SER401:HG:LIG:O ALA602:HN:LIG:O SER401:HG:LIG:O
	4	-7.75	2.09	- 9.24	2	ALA602:HN:LIG:O SER401:HG:LIG:O
	5	-7.43	3.55	- 8.93	4	ASN600:HD22:LIG:O ALA602:HN:LIG:O SER401:HN:LIG:O SER401:HG:LIG:O
	6	-6.90	8.80	- 8.39	1	SER401:HG:LIG:O
	7	-6.89	8.90	- 8.38	2	GLN348:HN:LIG:O SER349:HN:LIG:O
	8	-6.87	9.23	- 8.36	3	GLN348:HN:LIG:O ASN305:HD22:LIG:O SER349:HN:LIG:O
	9	-6.81	10.21	- 8.30	2	GLN348:HN:LIG:O SER349:HN:LIG:O
	10	-6.76	11.12	- 8.25	2	ASN305:HD22:LIG:O SER401:HG:LIG:O
3b	1	-8.13	1.10	- 9.32	2	THR302:HN:LIG:N THR352:HG1:LIG:O
	2	-8.06	1.24	- 9.25	2	THR302:HN:LIG:N THR352:HG1:LIG:O
	3	-8.01	1.34	- 9.21	2	THR302:HN:LIG:N THR352:HG1:LIG:O
	4	-7.98	1.43	- 9.17	2	THR302:HN:LIG:N THR352:HG1:LIG:O
	5	-7.95	1.48	- 9.15	2	THR302:HN:LIG:N THR352:HG1:LIG:O
	6	-7.88	1.66	- 9.08	2	THR302:HN:LIG:N THR352:HG1:LIG:O
	7	-7.85	1.76	- 9.04	2	THR302:HN:LIG:N THR352:HG1:LIG:O
	8	-7.84	1.80	- 9.03	1	THR352:HG1:LIG:O
	9	-7.83	1.83	- 9.02	2	THR352:HG1:LIG:O THR302:HN:LIG:N
	10	**-7.79**	**1.69**	**- 8.98**	**1**	**THR352:HG1:LIG:O**

4. CONCLUSION

This project led to the synthesis of new chalcone compounds in order to create new active isoxazoline derivatives. The produced compounds were confirmed via spectroscopic techniques such as FTIR, ^{1}H-NMR, and GC-Mass spectroscopy. The antimicrobial activity was evaluated for new derivatives, and it was found that most of the synthesized compounds exhibited good to moderate antimicrobial activity. The effectiveness was confirmed by studying the docking study of the most potent compounds (**2h** and **3b**) and choosing the best conformation and orientation for these compounds to bind to the binding site of the enzyme glucosamine-6-phosphate synthase (GlcN-6-P synthase), the molecular target enzyme identified in microbial species, in order to clarify the activity of the new derivatives. The docking study promoted the idea that the newly discovered compounds could serve as novel antimicrobial agents.

5. SUPPORTING INFORMATION

Spectral data and antimicrobial figures for the newly synthesized compounds.

6. ACKNOWLEDGMENTS

Thanks to the Department of Chemistry, College of Science, University of Mustansiriyah, Baghdad, Iraq, for supplying them with the chemicals and instruments required for this research.

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