Egypt.J.Chem. **Vol. 63**, No. 1. pp. 225 - 238 (2020)

Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Synthesis, Antimicrobial, Antioxidant and Docking Study of Novel 2H-1,4-Benzoxazin-3(4H)-One Derivatives

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A NOVEL series of 1,4-benzoxazinone derivatives were synthesized and characterized using FT-IR , 1H-NMR, 13C-NMR and Mass spectroscopy. These compounds were in vitro screened against several bacterial species gram positive and gram negative as well as Candida albicans and found exhibiting moderate to potent activity. The antioxidant study was confirmed for the synthesized derivatives against 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical. Docking study for the potent compound 8 against glucosamine-6-phosphate synthase , the target enzyme for the antimicrobial agents was explored to explain the interactions of the discovered hits with in the amino acid residues of the enzyme active side. The docking parameters enhanced the activity of new compound as promising antimicrobial agents.

Keywords: Antimicrobial, Antioxidant, Docking study, 2-Aminophenol, Benzoxazinone, Hydrazide

Introduction

In recent years, heterocyclic compounds had been received considerable attention due to their biological and pharmacological importance [1]. Heterocyclic compounds are of great importance to the life, because many natural products such as hormones and antibiotics have structural subunits [2]. Nitrogen-containing heterogeneous compounds play an important role in medical chemistry by assisting in various biological processes [3]. Benzoxazines are heterocyclic organic compounds that contain oxazine ring (a heterocyclic six-membered ring with oxygen and nitrogen atom) is attached to a benzene ring. The chemistry and pharmacology of benzoxazinone derivatives have been of great interest because of its various biological activity [4]. The benzoxazinone derivatives have received the attention of medicinal chemists due to their wide range of biological activities which include anti-cancer, anti-inflammatory, anti-leukemic, antimalarial, antipyretic, anticonvulsant, antiphlogistic, analgesic and antimicrobial activities [5-7]. N-alkylation of 1,4-benzoxazinone was performed to synthesize N-ester with ethyl chloroacetate in presence of anhydrous potassium carbonate, the above compound was also used to synthesize hydrazide compound by the treatment of this compound with hydrazine hydrate. Synthesize of some heterocyclic compounds by the treatment of hydrazide derivative with different chemical reagents. As mentioned heterocyclic compounds were synthesized from hydrazide derivatives may be beneficial as bioactivities.

Materials and Methods

Materials and physical measurements

All starting materials and solvents were obtained from Sigma-Aldrich and used without any additional purification. Melting points were measured on an electro thermal capillary apparatus and are uncorrected. FTIR spectrum were recorded on a Shimadzu model FTIR-8400S. Mass spectra were obtained on a Shimadzu GCMS-QP2010 Ultra apparatus. ^{1}HNMR and ^{13}CNMR spectra were achieved with a Bruker spectrophotometer model

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Received 13/9/2019; Accepted 10/11/2019
DOI: 10.21608/ejchem.2019.16920.2030

ultra-shield at 300 MHz in DMSO-d6 solution with the TMS as internal standard.

Synthesis of 2-chloro-N-(2-hydroxyphenyl) acetamide (FH)

This compound was prepared according to the procedure described in reference [8]. A mixture of *o*-amino phenol (2.18 g,0.02 mol) and anhydrous K_2CO_3(0.4g,0.003 mol) in dry acetone (25ml), chloroacetyl chloride (2.26ml,0.02 mol) was added drop-wise at (0-5 °C). The reaction mixture was allowed to stirr at room temp. for 1h and heated under reflux for 6h after cooling, the solution was poured into ice-cold water. The precipitate formed was filtered off and recrystallized from ethanol.
Dark brown powder ; yield 92%, m.p. 130-132 °C, IR (ν/cm^{-1}) : 3367(NH), 3010 (aromatic C-H), 2875,2991 (aliphatic C-H), 3190 (OH Broad), 1654(C=O) amide. ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.39 (s, 2H, CH_2), 6.77-7.90 (m, 4H, Ar-H), 9.45 (s,1H,NH), 9.97(s,1H,OH). ^{13}CNMR (DMSO-d_6) δ: 42.83, 114.70, 118.59, 123.20, 124.33, 125.08, 147.12, 164.08. MS, *m/z* [*M*]$^+$: 185(185.5) found (calcu.) for $C_{8H8O2}NCl$.

Synthesis of 2H-1,4-Benzoxazin-3(4H)-one (1)

A mixture of 2-chloro-N-(2-hydroxyphenyl) acetamide (1.85g,0.01 mol) and anhydrous K_2CO_3 (1.38g,0.01 mol) was dissolved in DMF (25ml) then refluxed for 10 h. The solvent was evaporated under reduced pressure and poured into ice-cold water. The precipitate was separated by filtration, washed with water, dried and recrystallized from ethanol.

Pale red powder ; yield 88 %, m.p. 170-171 °C., IR (ν/cm^{-1}) : 3182(NH), 3059 (aromatic C-H), 2856,2982 (aliphatic C-H), 1699(C=O) amide. ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.55(s,2H,CH_2), 6.87-6.96 (m, 4H, Ar-H), 10.71(s,1H,NH). MS, *m/z* [*M*]$^+$: 149 (149.14) found (calcu.) for C_{8H7NO2}.

Synthesis of ethyl 2-(3-oxo-2,3-dihydro-4H-benzo[b][1,4]oxazin-4-yl) acetate (2)

This compound was prepared according to the procedure described in reference [9]. A mixture of compound **1** (1.49g,0.01 mol), ethyl chloroacetate (1.2ml,0.01 mol) and anhydrous K_2CO_3 (2g,0.014 mol) in dry acetone (50ml) was refluxed for 15 h. The mixture was cooled, then poured into ice-cold water. The formed precipitate was separated by filtration, washed with water, dried and recrystallized from ethanol.

Pale orange powder ; yield 85%, m.p. 68-70 °C, IR (ν/cm^{-1}) : 3072 (aromatic C-H), 2854,2983 (aliphatic C-H), 1735 (C=O)ester,1676 (C=O) amide,1213 (C-O). ^{1}H-NMR (DMSO-d_6) δ (ppm):1.19 (t,3H,CH_3), 4.15(q,2H,CH_2), 4.47(s,2H,CH_2), 4.71(s,2H,CH_2) (oxazine ring), 6.92-7.03 (m, 4H, Ar-H).

Synthesis of 2H-1,4-benzoxazine-3-one-4-yl-aceticacid hydrazide (3)

This compound was prepared according to the procedure described in reference [10]. A mixture of compound **2** (0.001mol) in absolute ethanol (25ml), hydrazine hydrate 80% (0.002mol) was added and then refluxed for10h. The mixture was concentrated, cooled and poured into ice-cold water. The solid was filtered, dried and recrystallized from absolute ethanol.

White powder ; yield 60%, m.p. 163-165 °C, IR(ν/cm^{-1}) : 3331(NH), 3255,3213(NH_2), 3055 (aromatic C-H), 2850,2970 (aliphatic C-H), 1683, 1668 (C=O) amide. ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.27 (s,2H,CH_2), 4.47 (s,2H,NH_2), 4.69(s,2H,CH_2)(oxazine ring), 6.85-7.03(m,4H, Ar-H), 9.33 (s,1H,NH).MS, *m/z* [*M*]$^+$: 221 (221.21) found (calcu.) for $C_{10H11N3}O_3$.

Synthesis of Schiff bases compounds 4-6

These compounds were prepared according to the procedure described in reference [11]. To a solution of aromatic aldehydes (4-chlorobenzaldehyde, thiophene-2-carbaldehyde and pyrrol-2-carbaldehyde) (0.01 mol) in absolute ethanol (30ml) with few drops of glacial acetic acid, acid hydrazide compound **3** (0.01 mol) was added, refluxed for 8-10 h, then cooled and filtered. The obtained products were recrystallized from absolute ethanol.

N'-(4-chlorobenzalidene)-2-(3-oxo-2,3-dihydro-4H-benzo[b][1,4]oxazin-4-yl) acetohydrazide (4). White powder ; yield 88%, m.p. 272-274 °C, IR (ν/cm^{-1}) : 3180 (NH), 3057 (aromatic C-H), 2852,2949 (aliphatic C-H), 1697, 1668 (C=O) amide, 1614 (C=N). ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.48 (s, 2H,CH_2), 4.91(s, 2H,CH_2) (oxazine ring), 6.76-7.92 (m, 8H,Ar-H), 8.31 (s,1H, CH=N),11.83 (s,1H, NH). MS, *m/z* [*M*]$^+$: 343 (343.76) found (calcu.) for $C_{17H14}ClN_3O_3$.

2-(3-oxo-2,3-dihydro-4H-benzo[b][1,4] oxazine-4-yl)-N'- (thiophen-2-ylmethylene) acetohydrazide (5). White powder ; yield 80%,

m.p. 276-278 °C, IR (ν/cm^{-1}) : 3169 (NH), 3051 (aromatic C-H), 2850,2949 (aliphatic C-H), 1695, 1662 (C=O) amide, 1606 (C=N). ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.64 (s, 2H,CH_2), 4.97(s, 2H,CH_2) (oxazine ring), 7.01-7.81 (m, 7H,Ar-H), 8.22(s, 1H, CH=N), 11.81(s,1H,NH). MS, *m/z* [*M*]$^+$: 315 (315.34) found (calcu.) for $C_{15H13N3}O_3S$.

N'-((1H-pyrrol-2-yl)methylene)-2-(3-oxo-2,3-dihydro-4H-benzo[b][1,4]oxazin-4-yl)acetohydrazide (6). Gray powder ; yield 73%, m.p. 258-260 °C, IR (ν/cm^{-1}) : 3207 (NH), 3049 (aromatic C-H), 2890,2989 (aliphatic C-H), 1683, 1645 (C=O) amide, 1622 (C=N). ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.62 (s, 2H,CH_2), 5.04 (s, 2H,CH_2) (oxazine ring), 6.13-7.86 (m,7H,Ar-H), 8.05 (s, 1H, CH=N), 11.40 (s,1H, NH), 11.49 (s,1H, NH Pyrrole ring).

Synthesis of Lactam compounds 7, 8

These compounds were prepared according to the procedure described in reference [12]. A mixture of compounds **4,5** (0.01 mol) and triethylamine (0.02 mol) in dioxane (30ml). Chloroacetyl chloride (0.02 mol) was added drop wise with stirring for 30 min at room temperature. The reaction mixture was heated under reflux for 8 h, the solvent was evaporated by reduced pressure and poured into ice-cold water, then the precipitate was separated by filtration, washed with water, dried and recrystallized from ethanol.

N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-2-(3-oxo-2,3-dihydro-4H-benzo[b][1,4] oxazine-4-yl) acetamide (7). Brown powder ; yield 65%, m.p. 155-157 °C, IR (ν/cm^{-1}) : 3238 (NH), 3030 (aromatic C-H), 2890,2955 (aliphatic C-H), 1730 (C=O) β lactam, 1683, 1651 (C=O) amide. ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.12 (s, 2H,CH_2), 4.89 (s, 2H,CH_2), (oxazine ring), 5.07-5.48 (dd,2H,CH),7.02-7.96 (m,8H,Ar-H),10.41 (s,1H,NH).^{13}CNMR(DMSO-d_6) δ: 46.36,66.97,91.33,115.78,116.68,122.66,123.72,128.47,128.63,128.84,129.14,130.28,131.44,137.05,144.54,164.37, 165.65,167.28. MS, *m/z* [*M*]$^+$: 419 (420.24) found (calcu.) for $C_{19H15}Cl_{2N3}O_4$.

N-(3-chloro-2-oxo-4-(thiophen-2-yl)azetidin-1-yl)-2-(3-oxo-2,3-dihydro-4H-benzo[b][1,4] oxazin-4-yl)acetamide (8). Brown powder ; yield 55%, m.p. 134-136 °C,IR (ν/cm^{-1}) : 3105 (NH) , 3003 (aromatic C-H), 2880,2964 (aliphatic C-H), 1732 (C=O) β lactam, 1708, 1683 (C=O) amide. ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.23 (s, 2H,CH_2), 5.18 (s, 2H,CH_2) (oxazine ring), 5.66-5.26 (dd,2H,CH), 7.02-8.86 (m,7H, Ar-H),10.07 (s,1H, NH). MS, *m/z* [*M*]$^+$: 390 (391.82) found (calcu.) for $C_{17}H_{14}ClN_3O_4S$.

Synthesis of N-(1,5-dioxo-3-(1H-pyrrol-2-yl)-1,5-dihydrobenzeno[e][1,3]oxazepin-4(3H)-yl-2-(3-oxo-2,3-dihydro-4H-benzo[b][1,4]oxazine-4-yl) acetamide (9)

This compound was prepared according to the procedure described in reference [13]. A mixture of compound **6** (0.001 mol) and Phthalic anhydride (0.001 mol) in dry benzene (20ml). The mixture was refluxed for 15 h, excess solvent was distilled, the precipitate was filtered and recrystallized from ethanol.

Pale brown powder ; yield 64%, m.p. 182-184 °C, IR (ν/cm^{-1}) : 3207,3192 (NH), 3020 (aromatic C-H), 2854,2953 (aliphatic C-H), 1741 (C=O) lactone, 1683 (C=O) lactam. ^{1}H-NMR(DMSO-d_6) δ(ppm) :4.63 (s, 2H,CH_2) 5.05 (s, 2H,CH_2) (oxazine ring), 6.13-8.06 (m, 12H,Ar-H + CH-N), 11.39 (s,1H, NH), 11.47 (s,1H, NH Pyrrole ring).

Synthesis of 2-(2- (3-oxo-2,3-dihydro -4H – benzo [b] [1,4] oxazin -4- yl) acetyl)-N-phenyl hydrazine-1-carboxamide (10)

This compound was prepared according to the procedure described in reference [14]. To a solution of compound **3** (0.01mol) in dioxane (20ml), phenyl isocyanate (0.01mol) was added. The reaction mixture was heated under reflux for 10 h. The reaction mixture was left to stand at room temperature overnight. The solid product which separated was filtered off, dried and recrystallized from dioxane to give compound **10**.

White powder ; yield 84%, m.p. 224-226 °C, IR (ν/cm^{-1}) : 3275, 3140 (NH), 3059 (aromatic C-H), 2850,2951 (aliphatic C-H), 1693,1683,1664 (C=O)amide. ^{1}H-NMR, (DMSO-d_6) δ (ppm):4.63 (s, 2H,CH_2), 4.71 (s, 2H,CH_2)(oxazine ring), 6.94 -7.53 (m, 9H,Ar-H), 8.19 (s, 1H,CONHPh), 8.68 (s,1H, CONH), 10.08 (s, 1H,NHCO). ^{13}C NMR (DMSO-d_6) δ: 66.10, 66.96, 115.48, 116.39, 118.54, 121.99, 122.58, 123.67, 128.61, 128.85, 139.42, 144.60, 155.16, 164.49,166.91, 170.42. MS, *m/z* [*M*]$^+$: 340 (340.33) found (calcu.) for $C_{17}H_{16}N_4O_4$.

Synthesis of compounds 11, 12

These compounds were prepared according to the procedure described in reference [15]. A mixture of compound **3** (0.002 mol) and appropriate

alkyl halide, namely, p-toluene sulphonyl chloride or benzoyl chloride (0.002 mol) in dioxane (20ml) was refluxed for 10 h. The formed precipitate was filtered, dried and recrystallized from suitable solvent to give compounds **11, 12** respectively.

4-methyl-N'-(2-(3-oxo-2,3-dihydro-4H-benzo[b][1,4]oxazin-4-yl)acetyl) benzene sulfono hydrazide (11). White powder ; yield 58%, m.p. 206-208 °C,IR (ν/cm^{-1}): 3302,3203 (NH),3055 (aromatic C-H), 2941 (aliphatic C-H), 1693, 1670 (C=O) amide, 1342-1170 (SO_2). ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.42 (s,3H,CH_3), 4.45 (s, 2H,CH_2), 4.76 (s, 2H,CH_2)(oxazine ring), 6.62-7.78 (m, 8H,Ar-H), 9.77 (s, 1H, NH-CO),10.41 (s, 1H, NH-SO_2). MS, *m/z* [*M*]$^+$: 375 (375.39) found (calcu.) for $C_{17}H_{17}N_3O_5S$.

N'-(2-(3-oxo-2,3-dihydro-4H-benzo[b][1,4] oxazin-4-yl)acetyl) benzohydrazide (12)

White powder ; yield 91%, m.p. 275-277 °C, IR (ν/cm^{-1}): 3180 (NH), 3037 (aromatic C-H), 2852,2958 (aliphatic C-H), 1707,1680,1651 (C=O) amide. ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.66 (s, 2H,CH_2) 4.72 (s, 2H,CH_2)(oxazine ring) 7.04-7.95 (m, 9H,Ar-H), 10.32 (s,1H, NH), 10.46 (s,1H, NHCOPh) . MS, *m/z* [*M*]$^+$: 325 (325.31) found (calcu.) for $C_{17}H_{15}N_3O_4$.

Synthesis of N-(2,5-dioxopyrrolidin-1-yl)-2-(3-oxo-2,3-dihydro-4H-benzo[b][1,4]oxazin-4-yl) acetamide (13)

This compound was prepared according to the procedure described in reference [16]. A mixture of compound **3** (0.002 mol), succinic anhydride (0.002 mol) in glacial acetic acid (30ml) was refluxed for 15h and then poured into ice-cold water. The precipitate formed was separated by filtration, washed with water, dried and recrystallized from ethanol.

White powder ; yield 73%, m.p. 228-230 °C, IR (ν/cm^{-1}): 3186 (NH), 3055 (aromatic C-H), 2852,2958 (aliphatic C-H), 1722,1685 (C=O) amide. ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.42 (m,4H,CH_2), 4.58 (s, 2H,CH_2), 4.69 (s,2H, CH_2) (oxazine ring), 6.79-7.01 (m, 4H,Ar-H), 10.18 (s,1H,NH). ^{13}CNMR (DMSO-d_6) δ: 28.68,42.18, 66.81, 114.96, 116.37, 122.55, 128.79, 128.84, 144.59, 164.31, 170.03, 173.46. MS, *m/z* [*M*]$^+$: 303 (303.27) found (calcu.) for $C_{14}H_{13}N_3O_5$.

Synthesis of 4-(2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethyl)-2H-benzo[b][1,4] oxazin-3(4H)-one (14)

This compound was prepared according to the procedure described in reference [16]. A mixture of compound **3** (0.002 mol) and acetyl acetone (0.002 mol) in absolute ethanol (30ml) was refluxed for 16h. The reaction mixture was cooled and then poured into ice-cold water. The precipitate formed was separated by filtration, dried and recrystallized from absolute ethanol .

White powder ; yield 53%, m.p. 93-95 °C, IR (ν/cm^{-1}): 3005 (aromatic C-H), 2887,2960 (aliphatic C-H), 1681(C=O) amide, 1383 (CH_3). ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.25 (s,3H, CH_3), 2.73 (s,3H,CH_3), 4.73 (s,2H, CH_2), 5.42 (s,2H,CH_2) (oxazine ring), 6.29 (s,1H,CH), 6.97-7.17 (m, 4H,Ar-H). MS, *m/z* [*M*]$^+$: 285 (285.29) found (calcu.) for $C_{15}H_{15}N_3O_3$.

Synthesis of2 -(3-oxo-2,3-dihydro-4H-benzo[b][1,4]oxazin-4-yl) acetic acid (15)

This compound was prepared according to the procedure described in reference [17]. To a solution of compound **2** (0.01mol) was dissolved in absolute ethanol (25ml), then sodium hydroxide (5%, 20 ml) was added. The reaction mixture was refluxed on a water bath for 4 h. After that cooled to room temperature. Then solution of dilHCl was added drop-wise with stirring until formation of precipitate. That precipitate was filtered off and washed with cold water, dried and then recrystallized from absolute ethanol.

White powder ; yield 55%, m.p. 226-228 °C, IR (ν/cm^{-1}): 3091 (aromatic C-H), 3146 (OH Broad) carboxylic acid, 2835,2987 (aliphatic C-H), 1728 (C=O) acid,1699 (C=O) (oxazine ring). ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.63 (s,2H,CH_2),4.69 (s,2H,CH_2) (oxazine ring), 7.03 (m,4H,Ar-H), 10.7 (s,1H,OH). MS, *m/z* [*M*]$^+$: 206 (207.18) found (calcu.) for $C_{10}H_9NO_4$.

Antimicrobial study

In vitro antimicrobial testing effects of 1,4-benzoxazinone derivatives were estimated against *gram-positive* bacteria, namely, *Staphylococcus aureus, Staphylococcus epidermidis*, and against *gram-negative* bacteria, namely, *Klebsiella spp., Escherichia coli* as well as *Candida albicans*. The antimicrobial activity was determined using the agar well diffusion method by measuring the inhibition zone in mm [18]. The old Muller-Hinton broth cultures of test bacteria were swabbed on sterile Muller-Hinton agar plates using sterile cotton swab followed by punching wells of 6 mm with the help of sterile cork borer. Compounds **FH and 1-15** (10 mg/

ml of DMSO), and control (DMSO) were added to, respectively. The plates were allowed to stand for 1 h at room temperature and incubated at 37°C for 24 h in upright position, and the zone of inhibition was recorded.

Antioxidant study

Radical scavenging activity of some of the synthesized compounds **3-15** were evaluated for in vitro quantitatively using spectroscopic method against (1,1-diphenyl-2-picryl hydrazyl) DPPH and compared with Ascorbic acid (as standard). DPPH is a stable free radical, that can easily accept a hydrogen radical or an electron to become a stable molecule. The basic reaction between an antioxidant (H-A) and (DPPH) can be described in [DPPH + (H - A) → DPPH - H + A]. The DPPH assay was done according to modified procedure in reference [19]. Methanolic DPPH solution (0.3 mmol, 1 ml) was added to sample solution in DMSO (3 ml) at concentration of (12.5,25,50,100) µg/ml. The reaction mixture was shaken and the absorbance at 517 nm was taken after 30 min of incubation in the dark at room temperature. The percentage antiradical activity was calculated as follows:

% antiradical activity = [Ac-As / Ac]×100 : where Ac is the control absorbance and As is samples absorbance or standard. Methanol was used as the solvent and ascorbic acid as the standard.

The IC_{50} is the molar concentration of a given inhibitor, which reduces the respective uninhibited reaction rate to one half and is indicative of the inhibitory potency of compounds and was calculated from the plotting of DPPH scavenging activity against four different concentrations of each tested compound.

Docking Study

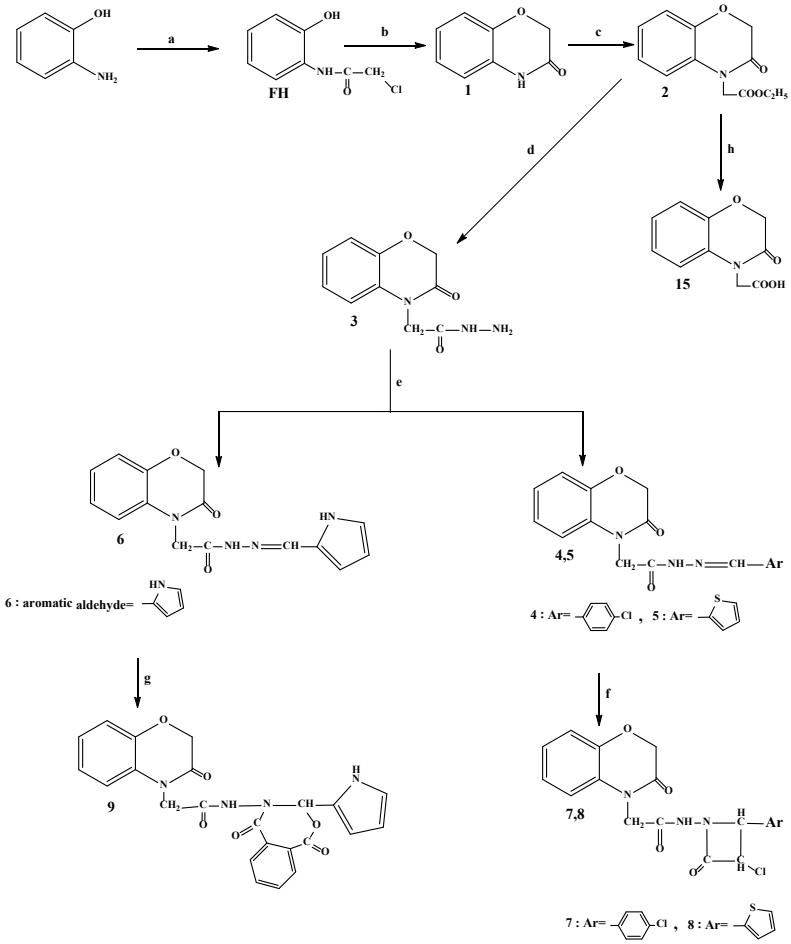
Auto Dock 4.2 package software was used to specify the affinity of the potent benzoxazinone derivative **8** to the binding site of GlcN-6-P synthase. The pdb file of enzyme as receptor was downloaded from the RCSB Protein Data Bank (PDB code 1MOQ) and used as a fixed molecule [20]. Water molecules were eliminated and hydrogens were added to the protein amino acid. The docked compound was drawn using Chem Draw ultra 7.0 as mol file, while the open Babel 2.3.1 software was used to build the pdb file. The X, Y and Z coordinates were specified as 30.5, 17.5 and -2.2, respectively. Docking algorithm using Lamarckian Genetic was employed with 10 runs, 150 population size and 2.500.000 maximum number of energy evaluations, while the maximum number of generations was 27.00.

Results and Discussion

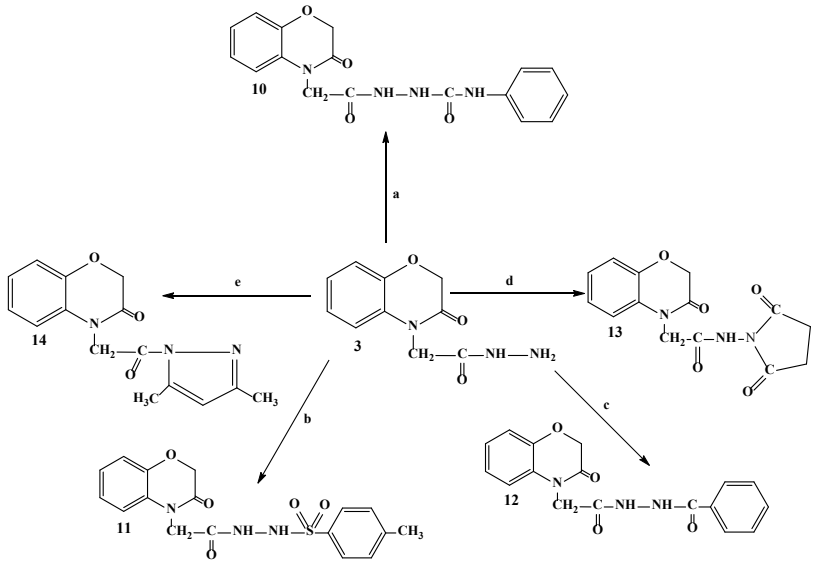
Chemistry

The synthetic route for the synthesis of derivatives 1,4-benzoxazinone is shown in Schemes **1 and 2**. Firstly, the starting material *o*-amino phenol was converted to the intermediate *o*-chloro acetamidophenol through acetylation reaction with chloro acetylchloride at (0–5) °C in acetone and anhydrous K_2CO_3. The spectrum IR of *o*-chloro acetamidophenol indicated a band at 1654 cm^{-1} for carbonyl of amide and band at 3367 cm^{-1} for (NH). The spectrum ^{1}HNMR of compound **FH** showed a singlet at 9.97 ppm due to (OH), singlet at 9.45 ppm due to (NH) and multiplet signal at (6.77-7.90) ppm due to aromatic protons. Then the cyclization of *o*-chloro acetamidophenol with anhydrous K_2CO_3 in refluxing DMF to give compound **1**.The spectrum IR of the compound **1** indicated a band at 1699 cm^{-1} for carbonyl of amide and band at 3182 cm^{-1} for (NH). The spectrum ^{1}HNMR of the compound **1** showed a singlet at 10.71 ppm due to (NH) while the multiplet signal at (6.87- 6.96) related to aromatic protons. The ester compound **2** was identified by the appearance of the following bands at (1735 cm^{-1}) for the ester carbonyl band and disappearance of (N-H) stretching for amide. ^{1}HNMR for compound **2** showed a triplet signal at 1.19 ppm due to (CH_3) and quartet signal at 4.15 ppm due to (CH_2). The ester compound **2** was reacted with excess amount of hydrazine hydrate to produce hydrazide compound **3**, the hydrazide compound was identified by disappearance of ester carbonyl band and the appearance of the following bands (3331) cm^{-1} and (3257,3213) cm^{-1} for (NH),(NH_2) respectively. ^{1}HNMR for compound **3** showed a singlet at 9.33 ppm due to (NH) and a singlet at 4.69 ppm due to (NH_2). Compound **3** was used to prepare a number of heterocyclic compounds as indicated in schemes **1and 2**. The Schiff bases compounds **4-6** gave new IR absorption like (CH=N) at (1606-1622) cm^{-1} and showed absence of (NH_2) stretching vibrations. ^{1}HNMR spectra of compounds **4-6** showed multiplet signal at (6.13-7.92) ppm and (8.02-8.31) ppm due to aromatic protons and (CH=N) respectively. Compounds **7,8** were synthesized by the reaction of compounds **4,5** with chloro acetylchloride in the presence of triethylamine in dioxane. The IR spectra showed stretching bands at (3238, 3105) cm^{-1} due to (NH) and the carbonyl of the new ring appeared at (1730, 1732) cm^{-1}, respectively. ^{1}HNMR for compounds **7,8** showed a singlet at (10.43,10.07) ppm due to (NH) and doublet doublet signal at ((4.94-5.58),

(5.26-5.66)) ppm for CH respectively.Compound **9** was synthesized by the reaction of compound **6** with phthalic anhydride. The IR spectrum of the compound **9** exhibited the absence of absorption band at 1622 cm^{-1} as azomethine (C=N) and presence of a new band at 1741 cm^{-1} due to (C=O) group as lactone. ^{1}HNMR for the compound **9** showed a singlet at (11.47,11.39) ppm due to (NH pyrrole +C-NH-N) and mlutiplet signal at (6.13-8.06) ppm due to (aromatic protons + CH-N). While compounds **10-12** were synthesized by the reaction of compound **3** with (phenyl isocyanate, p-toluenesulphonyl chloride, benzoyl chloride). These compounds were identified by disappearance of the (NH_2) stretching frequencies which strongly enhances the formation of compounds **10-12**. The ^{1}HNMR spectrum of the compound **10** revealed singlet signal at (8.19, 8.68, 10.08) ppm due to (NH) protons, while compounds **11,12** revealed singlet signal at (9.77,10.41), (10.32,10.46) ppm due to (NH) protons respectively. Also compounds **13,14** were identified by disappearance of (NH_2) band and the appearance of the following bands 1722 cm^{-1} for carbonyl of succinimide & (1685,1681) cm^{-1} for carbonyl of amide respectively, ^{1}HNMR for compound **13** showed a singlet at 10.18 ppm due to (NH) and multiplet signal at 2.42 ppm due to ($2CH_2$). While ^{1}HNMR for the compound **14** showed a singlet at 6.29 ppm due to (CH) and singlet singlet signal at (2.25, 2.73) ppm due to (CH_3). Hydrolysis of ester to the compound **15** was carried out by the reaction of compound **2** with NaOH, FT-IR spectrum of the compound **15** showed the disappearance of the (C=O) ester band of compound **2** and appeared the broad peak of the (OH) of carboxylic acid at 3146 cm^{-1} and (C=O) band of acid at 1728 cm^{-1}. The ^{1}HNMR spectrum of compound **15** showed the following, singlet at 4.69 ppm due to (CH_2) of oxazine ring, singlet at 4.63 ppm due to (N-CH_2) and singlet at 10.7 ppm due to (OH).



Scheme 1. (a) chloroacetyl chloride,K_2CO_3,Aceton (b) K_2CO_3,DMF(c) ethyl chloroacetate, K_2CO_3,Aceton (d) N_2H_4, EtOH (e) aromatic aldehydes, EtOH,G.A.A (f) chloroacetyl chloride,Et_3N,Dioxane (g) phthalic anhydride,Benzene (h) 5%NaOH, EtOH.



Scheme 2. (a) phenyl isocyanate, Dioxane (b) p-toluene sulphonyl chloride, Dioxane (c) benzoyl chloride, Dioxane (d) succinic anhydride, G.A.A (e) acetyl acetone, EtOH.

Antimicrobial activity

In this work it has been used the Amoxicillin drug as standard antibacterial, Ketoconazole as standard antifungal for comparison with the benzoxazinone derivatives. DMSO was used as solvent for all compounds and as control. The results of these studies are summarized in **Table 1**, **Figure 1** it was observed that the benzoxazinone derivatives showed higher activity against *gram positive* bacteria compared with *gram negative* bacteria, In addition compounds **FH,1,2,7,8,10,12 and 15** showed good inhibition toward *Candida albicans*.

TABLE 1: Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of chemical compounds against the pathological strains based on well diffusion assay

Comp.	inhibition zone (mm) at 10 mg/ml against				
	Gram positive		Gram negative		Fungi
	S.aureus	*S.epidermidis*	*E.coli*	*Klebsiellaspp*	*C. albicans*
FH	13	16	14	12	11
1	12	14	12	12	13
2	11	-	11	-	10
3	12	11	-	-	-
4	14	15	-	-	-
5	-	10	-	-	-
6	10	12	-	-	-
7	13	12	13	13	11
8	13	14	14	15	14
9	-	-	-	-	-
10	11	-	11	12	12
11	13	12	-	-	-
12	13	13	-	-	12
13	-	-	-	-	-
14	11	11	12	15	-
15	10	10	-	13	12
DMSO	-	-	-	-	-
Amoxicillin	21	10	16	18	-
ketoconazole	0	0	0	0	17

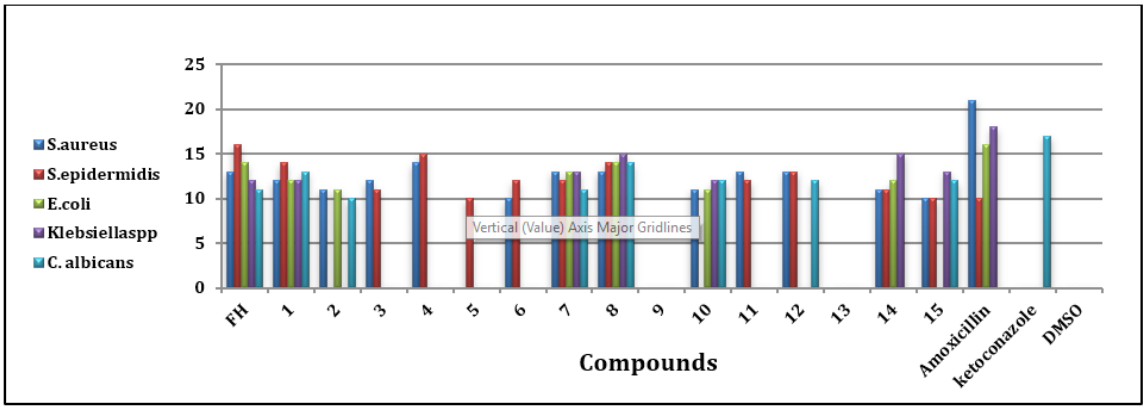


Figure 1: comparison between the effect of each compounds and reference antibiotic (Amoxicillin) for each type of bacteria and reference antifungal (Ketoconazole) for candida albicans.

Antioxidant activity

DPPH radical scavenging is considered a good *in vitro* model and is widely used to conveniently assess antioxidant efficacy. DPPH has absorption at 517 nm which disappears when DPPH is reduced by an antioxidant compound or a radical species to become a stable diamagnetic molecule. As a result, the color changes from purple to yellow [21]. This change in color is taken as an indicator of the ability of hydrogen to donate to tested compounds. The reducing abilities of the examined compounds were determined by their interaction with the free stable radical 1,1-diphenyl-2-picryl-hydrazine (DPPH) at four different concentrations for 30 min. the scavenging activity and IC_{50} results of some of synthetized compounds showed in Table 2 :

The results in **Figure 5**, **Table 2** above showed, compounds **8, 9 and 12** the most active compounds of the series, showed promising antioxidant activity having IC_{50} value of (33.81,36.60) **µg/ml** and (36.41) **µg/ml** respectively, in DPPH radical scavenging assay in comparison with ascorbic acid (IC_{50} =36.48 **µg/ml**). From the results **Table 2 in Figures (2-4)**, there is significance different in scavenging activity in concentrations (12.5, 25 and 50) µg/ml between ascorbic acid and compound **11** but there is no significance different in concentration (100) µg/ml between them, may be this related that the increase in scavenging activity or antioxidant ability of component with increase the concentration of this component. On the other hand, the compound **3** showed exhibit excellent antioxidant properties in concentrations (12.5, 25) µg/ml the potential is comparable with antioxidant activity of ascorbic acid activity, because the compound **3** has free amino group (the high electronegativity of nitrogen atom leads to capturing the free radical). Also, the compounds **4, 5, 6 and 7** showed scavenging activity in concentrations (100,50) µg/ml is very low compared with ascorbic acid because they have significance differences in comparison with ascorbic acid, but in concentrations (12.5,25) µg/ml there is no significance difference between compounds **4, 5, 6 and 7** and ascorbic acid so the compounds **4, 5, 6 and 7** are active in this concentration compared with ascorbic acid. While, compounds **10, 13, 14 and 15** didn't show any scavenging activity in comparing with ascorbic acid where the range of scavenging was (41-64) in all sample, are summarized in **Figures (2-4)**.

TABLE 2 : The results of scavenging activity and IC_{50} of some synthesized compounds 3-15

Comp.	scavenging activity % in (12.5 - 100) µg/ml Ascorbic acid = 24 – 83 % Comp.	IC_{50} Ascorbic acid = 36.48 Comp.
3	29 – 73 %	48.96
4	15 – 72 %	50.61
5	12 – 65 %	40.68
6	13 – 64%	41.62
7	15 – 71 %	51.68
8	10 – 75 %	33.81
9	10 – 81 %	36.60
10	7 – 64 %	47.29
11	15 – 74 %	49.15
12	9 – 75 %	36.41
13	9 – 41 %	43.29
14	12 – 61 %	43.64
15	14 – 57 %	43.18

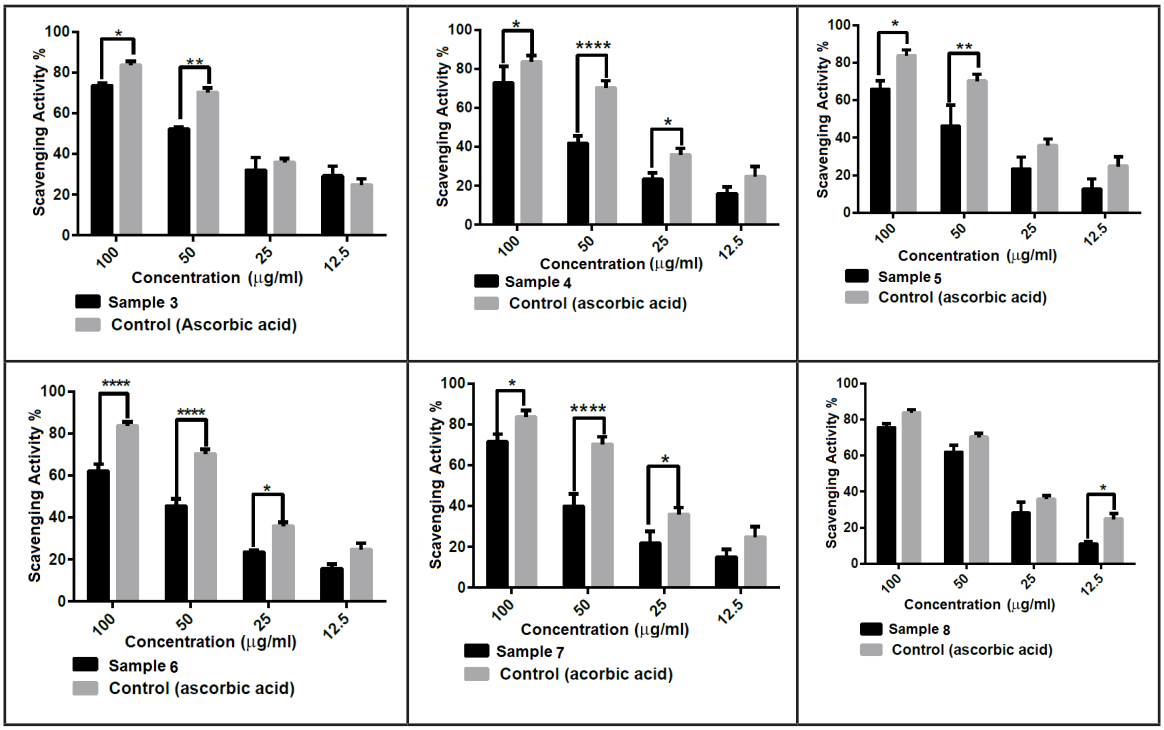


Figure 2 : % scavenging activity of the compounds 3,4,5,6,7 and 8 using DPPH.

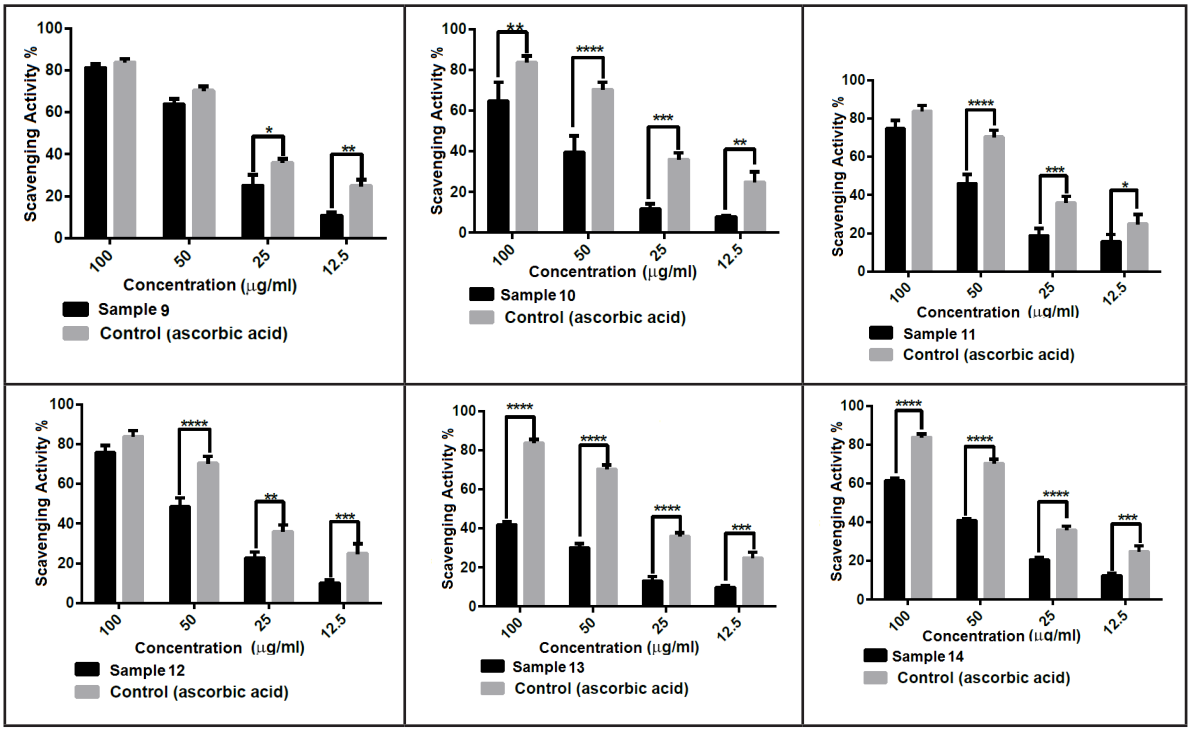


Figure 3 : % scavenging activity of the compounds 9,10,11,12,13 and 14 using DPPH.

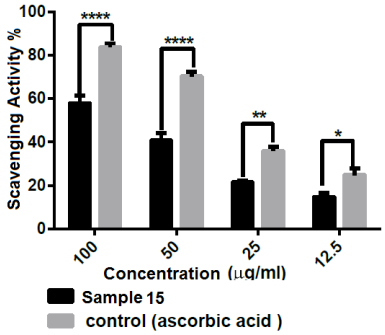


Figure 4 : % scavenging activity of the compound 15 using DPPH.

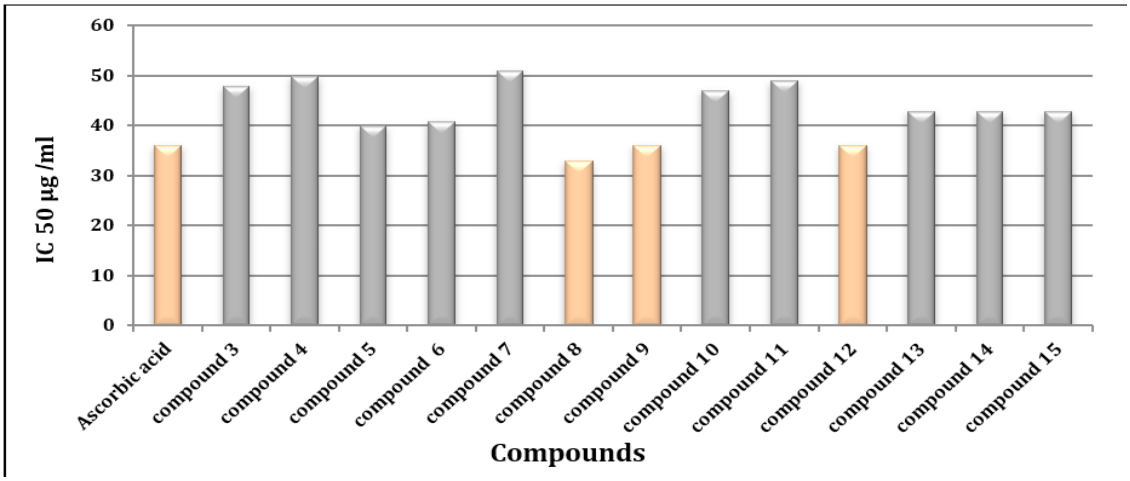


Figure 5: The result of IC_{50}

Docking Study

The docking study of the potent active benzoxazinone derivative **8** toward antimicrobial species inside the active pocket of L-Glutamine: D-fructose-6-phosphate amido transferase, the active target for antimicrobial agents was explored. As described by the X-ray study, the binding pocket of target enzyme including the following subsequent residues, cysteine 300, glycine 301, threonine 302, serine 303, serine 347, glutamine 348, serine 349, threonine 352, valine 399, serine 401, alanine 602 and lysine 603 as shown in **Figure 6**.

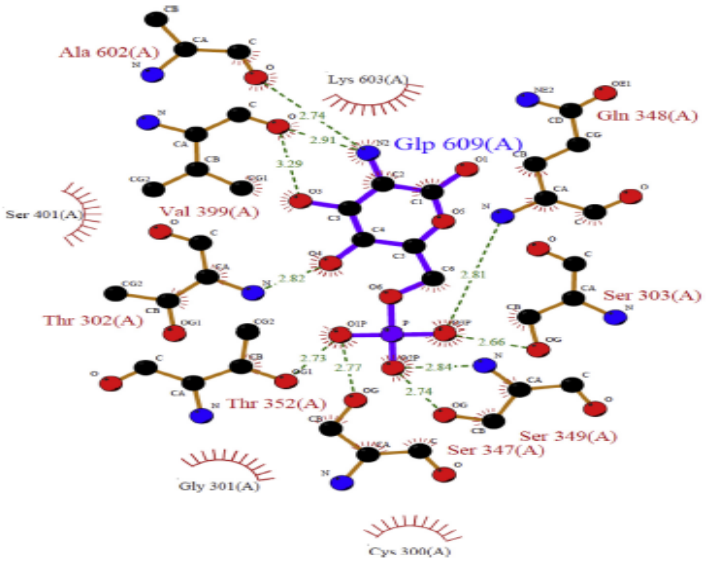
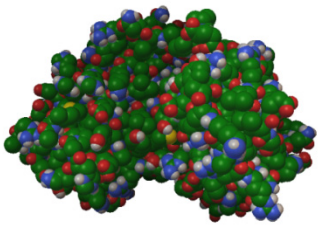
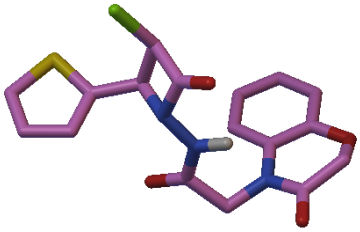


Figure 6 : Ligplot of GlcN-6-P showing the binding of glucosamine-6-phosphate in an active site of enzyme.

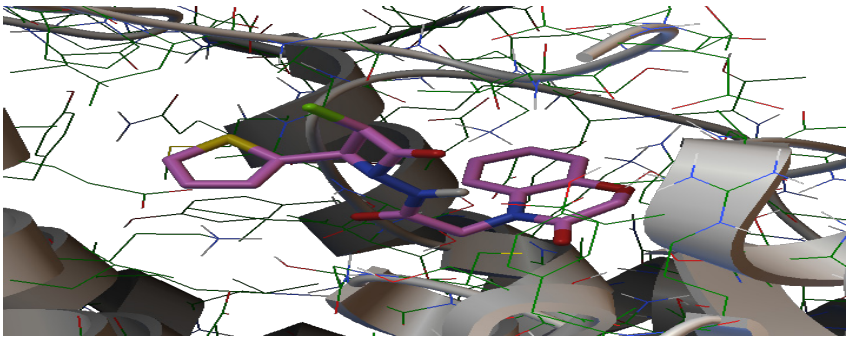
The binding energy of active compound inside the known three-dimensional structure of the specific enzyme was explored by using auto dock 4.2 [22]. The binding of the best building conformers for compound **8** inside the binding pocket of L-Glutamine: D-fructose-6-phosphate amido transferase is illustrated in **Figure 7**.



3D structure of GlcN-6-P



Structure of compound 8



Best generated conformer inside the binding site of GlcN-6-P

Figure 7 : The docking of the best generated conformer of compound 8 inside the binding pocket of L-Glutamine: D-fructose-6-phosphate amidotransferase (GlcN-6P).

The docking parameters for the ten generated conformers by the Auto dock 4.2 are illustrated in **Table 3**. The binding energy of the best generated conformer is (-7.14) Kcal mol^{-1} with (-8.34) Kal mol^{-1} intermolecular energy. The calculated inhibition constant (Ki) was (5.82**)** µM as determined by the docking approach.The best conformer bonds the enzyme pocket with two hydrogen bonding the first one with THR 302 while the second with the SER 349 residue. The docking results enhanced the activity of new derivative as promising antimicrobial agents.

TABLE 3: Docking parameters of compound 8

Compound 8	Binding Energy (Kcal mol^{-1})	Inhibition constant (µM)	Intermolecular energy ($kcalmol^{-1}$)	H-bonds	Bonding
1	-7.14	5.82	-8.34	2	THR302:HN:LIG:O SER349:HN:LIG:O
2	-6.53	16.47	-7.72	2	GLY301:HN:LIG:O THR302:HN:LIG:O
3	-6.40	20.52	-7.59	2	LIG:H: ALA602:O SER401:HN:LIG:O
4	-6.27	25.21	-7.47	2	ALA602:HN:LIG:O VAL605:HN:LIG:O
5	-6.24	26.60	-7.43	1	ALA602:HN:LIG:O
6	-6.22	27.39	-7.42	1	ALA602:HN:LIG:O
7	-6.15	30.79	-7.35	1	ALA602:HN:LIG:O
8	-6.08	34.98	-7.27	1	ALA602:HN:LIG:O
9	-5.72	64.51	-6.91	1	VAL605:HN:LIG:O
10	-5.54	86.35	-6.74	1	VAL605:HN:LIG:O

Conclusion

The present research summarized the synthesis of novel 1,4-benzo**x**azinone derivatives**.** The antimicrobial study of these derivatives against some gram positive and gram negative species as well as against C. albicans was studied using the well diffusion method. The novel derivatives **7 and 8** revealed excellent and highest activity against all

kinds of bacteria and C. albicans . On the other hand, the scavenging activity of the potent antioxidant derivatives was determined by using DPPH radical. The benzoxazinone derivative compound **8** has high anti-oxidant activity even higher than ascorbic acid. Also**,** 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. Finally it can be concluded that this class of molecules certainly holds great promise towards the pursuit to discover novel class of antibacterial, antifungal and antioxidant agents.

Acknowledgment

The authors would like to thank, Mustansiriyah University (www. uomustansiriyah. edu. iq) Baghdad, Iraq for its support in the present work.

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