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RESEARCH ARTICLE

Synthesis, Antibacterial Evaluation and Docking Study of Some New Fused Pyrido-Pyrimidine and Naphthyridine Cycles

Mohammed Hashim Abed Al Nabi, Ahmed Mutanabbi Abdula, Hamid H. Mohammed*

Department of Chemistry, College of Science, Mustansiriyah University, Baghdad, Iraq.

Corresponding Author: Hamid H. Mohammed

Abstract

New fused pyrido-pyrimidine and naphthyridine cycles were synthesized and characterized using spectral analysis. Initially, 2-cyano-N-(1-phenylethylidene) acetohydrazide (2) was prepared from the reaction of cyanoacetic acid hydrazide (1) with acetophenone then cyclized to 4,6-diamino-2-oxo-1-((1-phenylethylidene)amino)-1,2-dihy-dropyridine-3-carbonitrile (3) by the action of malononitrile and triethyl amine in dioxin. Fused pyridopyrimidine cycles (4-6) were obtained from the reaction of compound 3 with formic acid, glacial acetic acid and propionic acid, respectively in the presence of $POCl_3$ as a catalyst. The novel cycles (7-9) were synthesized from the cyclization reaction of compound 3 with benzoyl chloride, phenylisocyanate and phenylisothiocyanate in presence of pyridine. Naphthyridine cycles (10-13) were obtained by cyclization of compound 3 with some of aliphatic ketones namely; acetone, cyclohexanone, 2-butanone and 4-methyl-2-pentanone, respectively in presence of $FeCl_3$ as catalyst. The antimicrobial activity of the synthesized derivatives was evaluated against several bacterial species as well as candida albicans. Docking study was also achieved to explore the binding affinity of the potent discovered hit (9) inside the binding pocket of glucosamine-6-phosphate synthase, a target enzyme for the antimicrobial agents.

Keywords: *Naphthyridine Cycles; Fused Pyrido-Pyrimidine; Antibacterial.*

Introduction

Fused pyrimidine rings are among a wide variety of nitrogen heterocycles that have been explored for developing biologically important molecules due to their pharmaceutical activities [1, 3]. This is evident in particular from publications of regular reviews on the chemistry of systems where the pyrimidine ring is fused to various heterocycles [4, 5]. Many articles dealing with fused pyrimidines have illustrated that these derivatives exhibited several activities such as antimicrobial, antitumor, antimalarial, and antihypertensive, vasodilator and anti-allergic [6, 8].

On the other hand, naphthyridine also represent one of the important fused nitrogen compounds with diver's biological activities [9]. Several articles reported naphthyridine with antibacterial, anticancer and anti-inflammatory activity [10, 11]. The large interest in fused nitrogen heterocycles during the last decade encourage as to synthesize novel pyridopyrimidine and naphthyridine derivatives. The novel compounds were characterized using *IR* and *1HNMR* techniques. The antimicrobial activities against several bacterial species as well as candida albicans were evaluated. Docking study using Autodock 4.2 tool was explored to study the interactions between the potent hit [9] and the binding residue inside the active site of glucosamine-6-phosphate synthase, the specific enzyme for the antimicrobial reagents [12]. The docking parameters illustrated that the active compound fits the binding pocket and strongly enhanced the *in vitro* assay.

Experimental

Chemical Materials

All reactants and solvents used in this study were reagents grade and they are available from Sigma-Aldrich and Fluka companies.

Melting points were determined in open capillary tubes using Stuarts, SMP30 melting point apparatus and are uncorrected. Infrared spectra (FT-IR) were recorded using a Shimadzu FT-IR8400S spectrophotometer. ^{1}HNMR spectra were recorded on a Bruker, Ultra Shield 400Mhz, spectrometer (Switzerland) using DMSO-d_6 as a solvent with a tetramethylsilane (TMS) as an internal standard. All progress of the reactions and checking the purity were performed with thin layer chromatography (TLC) technique and revealed by mixture of n-hexane and ethyl acetate (3: 2) as eluent.

Synthetic Part

Preparation of Cyanoacetic Acid Hydrazide (1) [13]

To ethyl cyanoacetate (1.13g, 0.01mol) in ethanol (5ml), hydrazine hydrate (0.50g, 0.01mol) was added and stirred until complete solution in ice bath. The white product obtained was purified by recrystallization from ethanol. White powder, yield 96%, m.p 106-108 ◦C; *IR* (cm^{-1}): 3408, 3348 (*NH$_2$*), 3308 (*NH*), 2929 (*C-H*) aliphatic, 2260 (*C≡N*), 1683 (*C=O*), 1618 (*N-H*) bending. *R.f* = 0.95.

Synthesis of 2-cyano-N-(1-phenylethylidene) acetohydrazide (2) [14]

To a solution of cyanoacetic acid hydrazide (**1**) (0.99g, 0.01mol) in 1, 4-dioxane (30ml), acetophenone (1.20g, 0.01mol) was added. The reaction mixture was heated under reflux for 10 h then left to cool. The solid product formed upon pouring onto ice water was collected by filtration, and purified by recrystallization from ethanol. White powder, yield 91%, m.p 148-150 ◦C; *IR* (cm^{-1}): 3354 (*N-H*), 3169 (*C-H*) aromatic, 2937 cm^{-1} (*C-H*) aliphatic, 2260 (*C≡N*), 1678 (*C=O*), 1651 (*N-H*) bending, 1573 (*C=N*), 1552 (*C=C*). *^{1}HNMR* (300MHz, DMSO-d6) δ (ppm): 2.09 (s, 3H, *CH$_3$*), 3.53 (s, 2H, *CH$_2$*), 6.44-7.26 (m, 5H, *ArH*), 8.08 (s, 1H, *N-H*). *R.f* = 0.56.

Synthesis of 4, 6-diamino-2-oxo-1-((1-phenylethylidene) amino)-1, 2-dihydropyridine-3-carbonitrile (3) [15]

Equimolecular amounts of compound **2** (2.01g, 0.01 mol) and malononitrile (0.66g, 0.01mol) in 1,4-dioxane (30 mL) containing triethylamine (1.5 mL) was heated under reflux for 5 h. The reaction mixture was left to cool and evaporated under vacuum. The remaining product was triturated with ethanol and the formed solid product was collected by filtration, and purified by recrystallization from 1, 4-dioxane. Greenish brown powder, yield 76%, m.p 220-222 ◦C; IR (cm^{-1}): 3335, 3404 (*NH$_2$*), 3180 (C-*H*) aromatic, 3138 (*C-H*) aliphatic, 2212 (*C≡N*), 1681 (*C=O*), 1651 (*C=N*), 1573 (*C=C*). *^{1}HNMR* (300MHz, DMSO-d6) δ (ppm): 2.13 (s, 3H, *CH$_3$*), 3.98 (s, 2H, *NH$_2$*), (s, 2H, *NH$_2$*), 5.96 (s, 1H, *pyrido ring*), 6.50-7.46 (m, 5H, *ArH*). *R.f* = 0.14.

Synthesis of pyrido[4,3-*d*] pyrimidine-4,5(3*H*,6*H*)-dione Derivatives (4-6)[16]

A mixture of compound **3** (2.67g, 0.01mol) was dissolved in aliphatic carboxylic acid (formic acid, glacial acetic acid, or propionic acid) (5mL), then $POCl_3$ (0.2mL) was added quickly. The mixture was refluxed for 12 h then cooling with ice water (50 mL). The mass of white precipitate was collected after adding fused K_2CO_3 to neutralize the acid, dried, then recrystallized from ethanol to give compounds **4**, **5** and **6**, respectively.

7-amino-6-((1-phenylethylidene) amino) pyrido[4,3-*d*]pyrimidine-4,5(3*H*,6*H*)-dione (4)

Dark brown powder, yield 79%, m.p 192-194◦C; *IR* (cm-1): 3466, 3335 (*NH$_2$*), 3236 (*NH*), 3198 (*C-H*) aromatic, 2972 (*C-H*) aliphatic, 1677 (*C=O*) *pyrimidine ring*, 1649 (*C=O*) *pyridine ring*, 1614 (*C=N*), 1587 (*C=C*). *^{1}HNMR* (300MHz, DMSO-d6) δ (ppm): 2.18 (s, 3H, *CH$_3$*), 4.92 (s, 2H, *NH$_2$*), 6.43 (s, 1H, *pyridine ring*), 6.95-7.54 (m, 10H, *ArH*), 9.34 (s, 1H, *N=CH*) 12.46 (s, 1H, *=NH*). *R.f* = 0.71.

7-amino-2-methyl-6-((1-phenylethylidene) amino) pyrido[4,3-*d*] pyrimidine-4,5(3*H*,6*H*)-dione (5)

Dark brown powder, yield 67%, m.p 100-102◦C; *IR* (cm^{-1}): 3432, 3335 (*NH$_2$*), 3279 (*NH*), 3102 (*C-H*) aromatic, 2910 (*C-H*) aliphatic, 1678 (*C=O*) *pyrimidine ring*, 1640 (*C=O*) *pyridine ring*, 1604 (*C=N*), 1552 (*C=C*). *R.f* = 0.32.

7-amino-2-ethyl-6-((1-phenylethylidene) amino) pyrido [4, 3-*d*] pyrimidine-4, 5(3*H*, 6H)-Dione (6)

Yellow powder, yield 82%, m.p 110-112 ◦C; *IR* (cm^{-1}): 3437, 3384 (*NH$_2$*), 3230 (*NH*), 3105 (*C-H*) aromatic, 2929 (*C-H*) aliphatic, 1687 (*C=O*) *pyrimidine ring*, 1649 (*C=O*) *pyridine ring*, 1610 (*C=N*), 1566 (*C=C*). *R.f* = 0.49.

Synthesis of 7-amino-2-phenyl-6-((1-phenylethylidene) amino) pyrido [4, 3-*d*] pyrimidine-4, 5(3*H*, 6H)-dione (7) [17]

A mixture of compound **3** (2.67g, 0.01mol) and benzoyl chloride (1.40g, 0.01mol) in pyridine (20 mL) was refluxed for 18 h. The solid product formed upon pouring onto ice-water is collected by filtration and recrystallized from ethanol. Brown powder, yield 80%, m.p 241-243°C; *IR* (cm^{-1}): 3454, 3381, (*NH2*), 3294 (*NH*), 3105 (*C-H*) aromatic, 2937 (*C-H*) aliphatic, 1683 (*C=O*) *pyrimidine ring*, 1649 (*C=O*) pyridine ring, 1600 (C=N), 1581 (C=C).^{1}HNMR (300MHz, DMSO-d6) δ (ppm): 2.17 (s, 3H, CH_3), 4.61 (s, 2H, NH_2), 6.44 (s, 1H, pyridine ring), 6.74-7.90 (m, 10H, ArH), 12.78 (s, 1H, =NH). *R.f* = 0.84.

Synthesis of 1*H*-pyrido [4, 3-*d*] pyrimidin-5-one Derivatives (8-9) [17]

A mixture of compound **3** (2.67g, 0.01mol) and phenyl isocyanate (1.19g, 0.01mol) or phenyl isothiocyanate (1.35g, 0.01mol) in ethanol (10 mL) was refluxed for 5 h. The solid product formed upon pouring onto ice water was collected by filtration and washed with distilled water and recrystallized from ethanol.

7-amino-6-((1-phenylethylidene) amino)-2-(phenylimino)-2, 3-dihydropyrido [4, 3-*d*] pyrimidine-4, 5(1*H*, 6H)-Dione (8)

Pale brown powder, yield 81%, m.p 235-237°C; IR (cm-1): 3429, 3332, (NH_2), 3244 (NH), 3117 (C-H) aromatic, 2910 (C-H) aliphatic, 1682 (C=O) pyrimidine ring, 1670 (C=O) pyridine ring, 1604 (C=N), 1550 (C=C). *R.f* = 0.39.

7-amino-6-((1-phenylethylidene) amino)-2-(phenylimino)-4-thioxo-2, 3, 4, 6-tetrahydropyrido[4,3-*d*]pyrimidin-5(1*H*)-one (9)

Brown powder, yield 79%, m.p 246-248 °C; IR (cm-1): 3423, 3331 (NH_2), 3213 (NH), 3160 (C-H) aromatic, 2926 (C-H) aliphatic, 1708 (C=O) pyrimidine ring, 1680 (C=O) pyridine ring, 1597 (C=N), 1577 (C=C), 1512 (C=S). *R.f* = 0.29.

Synthesis of 4, 7-diamino-6H-[1, 6] naphthyridin-5-one Derivatives (10-13) [18]

To a mixture of compound **3** (2.67 g, 0.01 mol) and corresponding ketone (10 mL); (acetone, 2-butanone, 4-methyl-2-pentanone or cyclohexanone) placed in a round bottom flask connected to a reflux condenser, $FeCl_3$ (1.62g, 0.01mol) was added. The mixture was heated at 120 °C for 24 h with continues stirring. After cooling to room temperature, the remaining solids were treated with NaOH solution (2 $mol.L^{-1}$, 8 mL) and the mixture was heated at reflux for 24 h. On cooling to room temperature, the reaction mixture was extracted with $CHCl_3$ (3 x 8 mL) and the organic layers were combined then dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the solid obtained was recrystallized from ethanol.

4, 7-diamino-2-methyl-6-((1-phenylethylidene) amino)-1, 6-naphthyridin-5(6*H*)-one (10)

Dark brown powder, yield 62%, m.p 65-67°C; IR (cm-1): 3440, 3347 (NH_2), 3195 (C-H) aromatic, 2985 (C-H) aliphatic, 1683 (C=O), 1610 (C=N), 1572 (C=C).^{1}HNMR (300MHz, DMSO-d6) δ (ppm): 2.14 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 4.30 (s, 2H, NH_2), 4.63 (s, 2H, NH_2), 6.30 (s, 1H, pyridino ring), 6.51 (s, 1H, pyridine ring) , 6.88-7.47 ppm (m, 5H, ArH). *R.f* = 0.21.

4, 7-diamino-2, 3-dimethyl-6-((1-phenylethylidene) amino)-1, 6-naphthyridin-5(6*H*)-one (11)

Yellowish brown powder, yield 57%, m.p 50-52°C; IR (cm-1): 3435, 3345 (NH_2), 3117 (C-H) aromatic, 2920 (C-H) aliphatic, 1679 (C=O), 1620 (C=N), 1590 (C=C).^{1}HNMR (300MHz, DMSO-d6) δ (ppm): 2.14 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 4.23 (s, 2H, NH_2), 4.57 (s, 2H, NH_2), 6.27 (s, 1H, pyridino ring), 6.84-7.64 (m, 5H, ArH). *R.f* = 0.35.

4, 7-diamino-3-isopropyl-2-methyl-6-((1-phenylethylidene) amino)-1, 6-naphthyridin-5(6*H*)-one (12)

Light brown powder, yield 55%, m.p 61-63°C; IR (cm-1): 3433, 3335 (NH_2), 3076 (C-H) aromatic, 2918 (C-H) aliphatic, 1675 (C=O), 1608 (C=N), 1556 (C=C).

^{1}HNMR (300MHz, DMSO-d6) δ (ppm): 1.03-1.09 (s, 6H, $2CH_3$), 2.13 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.60 (m, 1H, ***CH***$(CH_3)_2$), 4.15 (s, 2H, NH_2), 4.43 (s, 2H, NH_2), 6.46 (s, 1H, pyridino ring), 6.64-7.38 ppm (m, 5H, ArH). *R.f* = 0.33.

3, 10-diamino-2-((1-phenylethylidene) amino)-6, 7, 8, 9-tetrahydrobenzo[*b*] [1, 6] naphthyridin-1(2*H*) - one (13)

Dark brown powder, yield 77%, m.p 69-71°C; IR (cm-1): 3432, 3388 (NH_2), 3100 (C-H) aromatic, 2958 (C-H) aliphatic, 1677 (C=O), 1602 (C=N), 1573 (C=C). *R.f* = 0.42.

Antimicrobial Activity

Applying the agar plate diffusion technique some of the synthesized compounds were screened *in vitro* for antibacterial activity against gram +ve species (*Staphylococcus aureus* and *Staphylococcus epidermidis*), and gram –ve species (*Escherichia coli* and *Klebsiella pneumniae*) as well as *Candida albicans*. Prepared agar and petri-dishes were sterilized by autoclaving for 15 minute at 121°C. The ager plates were surface inoculated uniformly from the broth culture of the tested microorganisms.

In the solidified medium suitably spaced apart holes were made (6 mm in diameter) then filled with 100 µl of the prepared compounds. The synthesized compounds (**3**-**7**, **9**-**10**, **11**, **13**) were dissolved in DMSO in concentration of 10^{-3} mol.L^{-1}.These plates were incubated at 37°C for 24 h. The inhibition zones caused by the various compounds on the bacteria were examined [19].

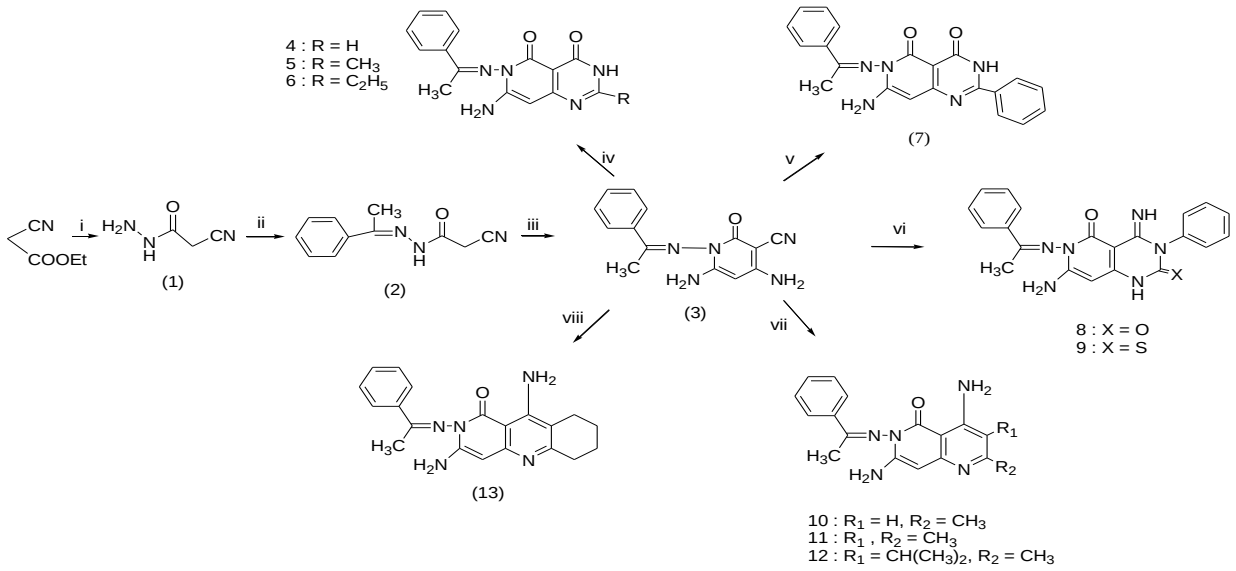
Docking Study

Auto Dock 4.2 package tools was used to study the affinity of the potent derivative (9) 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 ChemDraw 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 [20].

Results and Discussion

Synthesis and Characterization

Compound **1** was prepared by reaction of ethyl cynoacetate and hydrazine hydride in ethanol as described by Scheme 1.



Scheme (1): (i) : N_2H_4, Ethanol ; **(ii)** : acetophenone , dioxane, 10 hs ; **(iii)** : $CH_2(CN)_2$, NEt_3, dioxane, 5hs ; **(iv)** : RCOOH, $POCl_3$, 18hs: **(v)** : benzoyl chloride , pyridine, 18 hs. ; **(vi)** : phenylisocyanate or phenylisothiocyanate , pyridine , 16 hs; **(vii)** : acetone or 2-butanone or 4-methyl-2-butanone , $FeCl_3$, heat, 48 hs. ; **(viii)** : cyclohexanone , $FeCl_3$, heat, 48 hs

The *IR* spectra of compound **1** shows stretching bands symmetrical and unsymmetrical at 3408 and 3348 cm^{-1} related to NH_2 group, 3308 cm^{-1} for ***NH***, 2929 cm^{-1} for ***C-H*** aliphatic, 2260 cm^{-1} for ***C≡N***, stretching band at 1683 cm^{-1} for ***C=O*** and 1618 cm^{-1} for ***N-H*** bending. Compound **2** was synthesized by condensation of acid hydrazide derivative **1** with acetophenone, the *IR* spectra of compound **2** shows stretching bands at 3354

cm^{-1} for ***NH*** group, 3169 cm^{-1} for aromatic ***C-H***, 2937 cm^{-1} for aliphatic ***C-H***, 2260 cm^{-1} related to ***C≡N*** group and band at 1678 cm^{-1} for carbonyl group, while the bands appeared at 1573 and 1552 cm^{-1} related to stretching frequencies of ***C=N*** and ***C=C*** groups, respectively. The disappearances of NH2 stretching bands strongly confirm the product formation. The ***^{1}HNMR*** spectrum of compound **2** shows a singlet signal at δ = 2.09 ppm related to three protons of ***CH_3*** group, singlet signal at δ = 3.53 ppm of ***CH_2*** protons, multiplet signal at δ = 6.44-7.26 ppm for the aromatic protons, while the singlet of ***NH*** proton appeared at δ = 8.08 ppm. The reaction of derivative **2** with malononitrile afforded compound **3**.

The *IR* spectrum of compound **3** shows stretching bands symmetrical and unsymmetrical at 3404, 3335 cm^{-1} for ***NH_2***, 3180 cm^{-1} for aromatic ***C-H***, 3138 cm^{-1} for aliphatic ***C-H***, 2212 cm^{-1} for ***C≡N***, 1681 cm^{-1} for ***C=O***, 1651 cm^{-1} for ***C=N*** group and band at 1573 cm^{-1} for ***C=C***. The ***^{1}HNMR*** spectrum of compound **3** shows singlet signal at δ = 2.13 ppm related to CH_3 group, singlet signal at δ = 3.98 ppm for ***NH_2***, while the two singlet signals at δ = 4.37 and 5.96 ppm related to ***NH_2*** and ***1H*** of pyrido ring. The other aromatic protons appeared as multiplet at δ = 6.50-7.46 ppm. Novel pyrido[4,3-*d*]pyrimidine-4,5(3*H*,6*H*)-dione derivatives **4-6** were synthesized from the reaction of compound **3** with acetic acid, formic acid and propionic acid, respectively in presence of $POCl_3$ as depicted in reported reference. The target derivatives **(4-6)** were characterized by FT-IR and ^{1}HNMR technique.

IR spectrum of compounds **4-6** shows the disappearance of ***C≡N*** stretching band, while the other bands depicted in experimental part strongly confirm the structures of the products. The ***^{1}HNMR*** of compound **4** shows the singlet signal at δ = 2.18 ppm related to methyl group, while the signal of ***NH_2*** appeared as broad singlet at δ = 4.92 ppm. The singlet signal at δ = 6.43 ppm of pyridine ring ***(1H)***, multiplet at δ = 6.95-7.54 of aromatic protons (***4H)***, singlet at δ = 9.34 ppm of ***N=CH*** (1H) and δ = 12.46 ppm related to ***NH*** group strongly confirm the structures of the synthesized compounds. Compound 3 was also considered as key for the synthesis of pyrimidine derivatives **7-9** as described in ***Scheme 1***. Reaction of compound **3** with benzoyl chloride in presence of pyridine yielded pyrido [4, 3-d] pyrimidine-4, 5(3H, 6H)-Dione derivative **7**. Treatment of compound **3** with phenyl isocyanate in pyridine for 16 h led to the fused pyrimidino derivative **8**, while the reaction with phenyl isothiocyanate in the same solvent for 16 h afforded derivative **9** in 81 and 79% yield, respectively. Compounds 7-9 were characterized by *IR* and *^{1}HNMR* technique. *IR* spectra show the disappearance of ***C≡N*** stretching band, while the other characteristic bands depicted in experimental section (***see section 2.2.5 and 2.2.6***).

The *^{1}HNMR* spectrum of compound **7** shows a singlet signal at δ = 2.17 ppm related to CH_3 group, another singlet at δ = 4.61 ppm of ***NH_2*** group, and the third singlet at δ = 6.44 ppm related to one proton of pyridine ring. The multiplet signal at 6.74-7.90 ppm related to aromatic protons (***10H***) and the singlet signal at δ = 12.78 ppm for =NH group strongly confirm the structure. Cyclization of compound **3** with acetone, 2- butanone, 4-methyl-2-pentanone and cyclohexanone in presence of Lewis acids as catalyst and NaOH afforded the pyridines derivatives (**10-13)**, respectively. 6H-[1,6]Naphthyridin-5-one derivatives **(10-13)** were characterized using FT-IR and ^{1}HNMR analysis. ***IR*** spectra of synthesized compounds **(10-13)**, shows the disappearance of ***C≡N*** stretching band and the other characteristic bands shows experimental part strongly confirm the structures of desired compounds.

The *^{1}HNMR* spectrum of compound **10** shows the singlet signal at δ = 2.14 and 2.62 ppm related to two methyl groups, while the two singlet signals appeared at 4.30 and 4.63 ppm related to two amino groups. The proton of pyridine ring was indicated at 6.30 ppm, while the singlet signal at δ = 6.51 ppm related to pyridine ring (1H). the aromatic rings appeared as multiplet signal at 6.88-7.47 ppm (5H). The *^{1}HNMR* spectrum of compound **11** and **12** as illustrated in experimental part (**see section 2.2.7**) enhanced the elucidation structure of compounds.

Antimicrobial activity

With the development of new strains of bacteria resistant to many currently available antibiotic treatments, there is increasing interest in the discovery of new antibacterial agents.

Antimicrobial resistance refers to microorganism that develop the ability to inactivate, exclude or block the inhibition or lethal mechanism of the antimicrobial agents.

The results of the preliminary screening test are listed in Table 1. The *in vitro* assay reveal that compound **9** have the highest activity against all microbial species and can be considered the potent discovered hit.

Table 1: *In Vitro* antimicrobial inhibition zone (mm) of the synthesized compounds

Comp.	Gram positive		Gram negative		Fungi
	S. aureus	S. epidermidis	E. coli	Klebsiella pneumniae	Candida albicans
3	-	-	14	-	13
4	9	6	12	14	-
5	9	-	8	10	9
6	-	14	14	-	14
7	-		14	-	22
9	20	23	20	16	25
10	-	-	14		16
11	12	18	-	16	10
13	8	18	-	-	-

Docking Study

The docking study of the potent active derivative compound 9 against the binding pocket of glucosamine-6-phosphate synthase, the target enzyme for the antimicrobial agents was achieved. As illustrated by the crystallographic image, the active site of the enzyme consist of the following residues, Lys 603, Ala 602, Ser 401, Val 399, Thr 352, Ser 349, Gln 348, Ser 347, Ser 303, Thr 302, Gly 301 and Cys 300 as shown in Figure 1 [12].

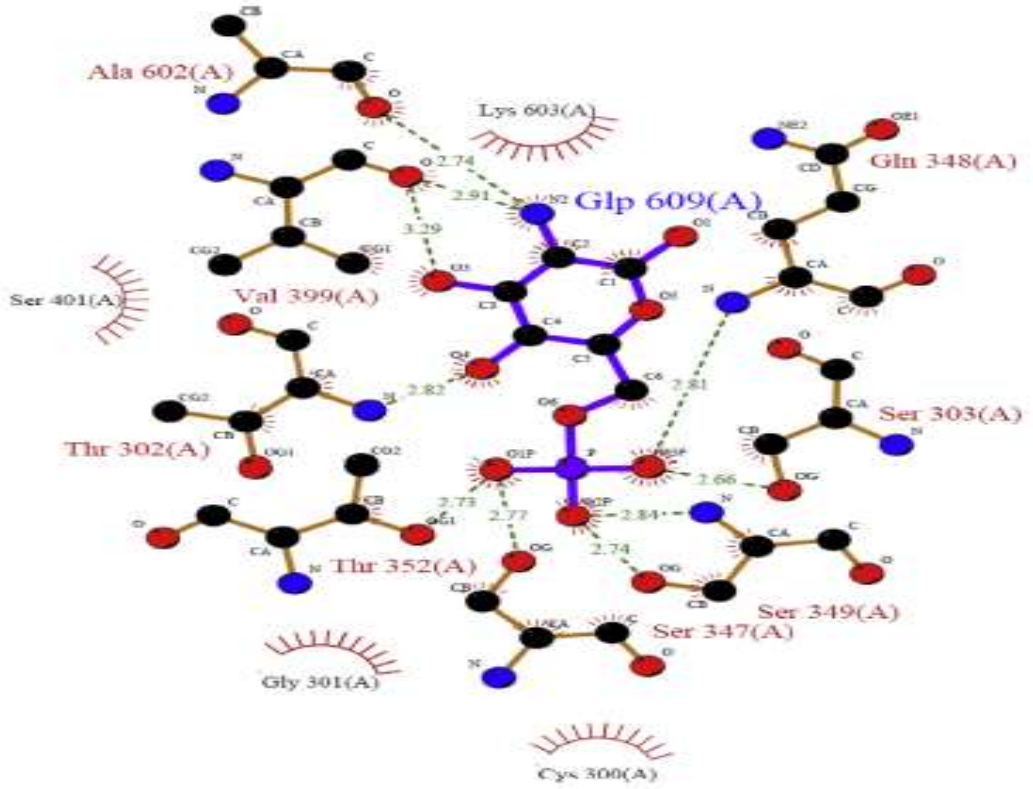
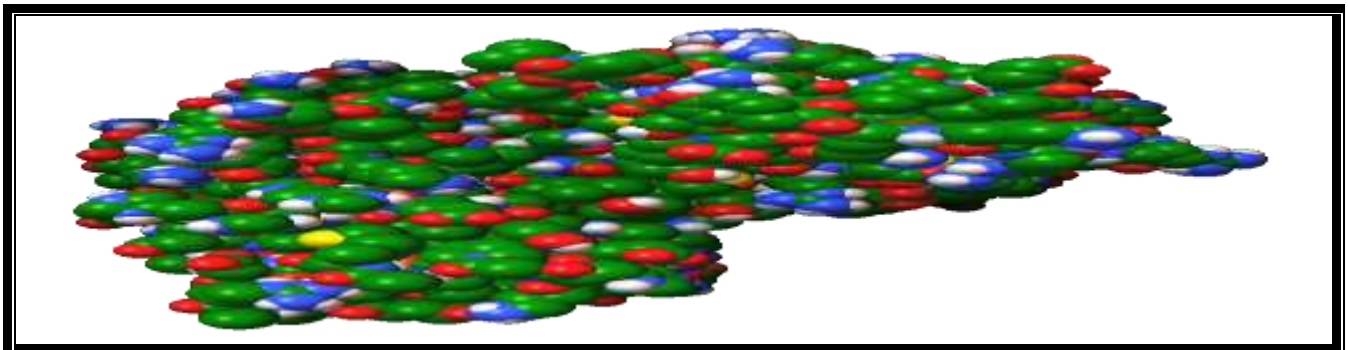
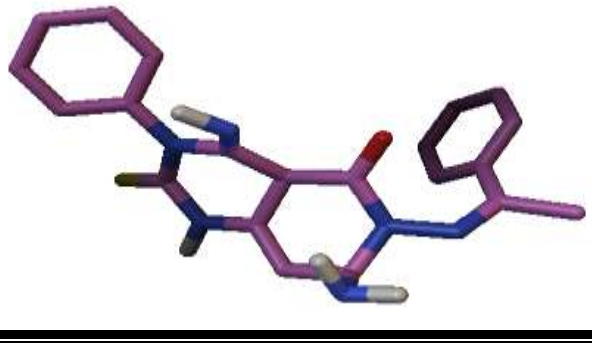


Figure 1: Ligplot of GlcN-6-P showing the binding of glucosamine-6-phosphate in an active site of enzyme

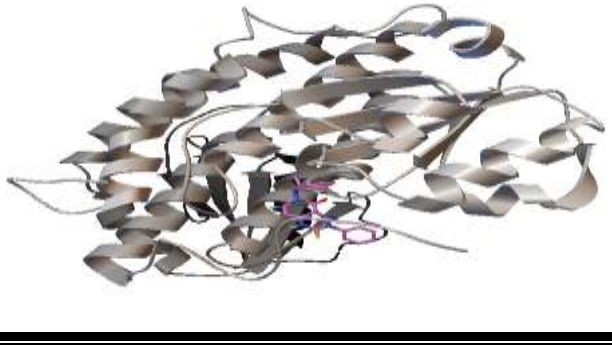
Auto dock 4.2 was used to explore the binding energy of active compounds inside the known three dimensional structure of the specific enzyme. The binding of the best building conformer for compound 9 inside the binding pocket of L-Glutamine: D-fructose-6-phosphate amidotransferase are illustrated in Figure 2.



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



Compound 9



The best conformer of compound 9 inside binding pocket of GlcN-6-P

Figure 2: The docking of the best generated conformers of the potent discovered hit (9) inside the binding pocket of L-Glutamine: D-fructose-6-phosphate amidotransferase (GlcN-6-P)

As indicated by molecular docking parameters, the high ranking binding energies of the generated conformer was -8.90 kcal mol^{-1}, while the Intermolecular energy was -10.09 kcal mol^{-1}. The best conformer fits the active site with two hydrogen bonds, the first one with ALA602 and the second with THR302 residues as depicted in Table 2. The docking results of compound 9 within the binding pocket are strongly enhancing the *In Vitro* assay.

Table 2: docking parameters of pyrido [4, 3-*d*] pyrimidin-5(1*H*)-one (9) inside the target enzyme

compounds		Binding Energy (Kcal mol^{-1})	Inhibition constant 10^2x (nM), *(μM)	Intermolecular energy (kcalmol^{-1})	H-bonds	Bonding
9	1	-8.90	3.00	-10.09	2	LIG:H :ALA602:O THR302:HN: LIG:O
	2	-8.88	3.11	-10.07	2	LIG:H :ALA602:O THR302:HN: LIG:O
	3	-8.56	5.29	-9.76	0	-
	4	-8.40	6.97	-9.59	2	LIG:H :ALA602:O THR302:HN: LIG:O
	5	-8.37	7.36	-9.56	2	LIG:H :ALA602:O THR302:HN: LIG:O
	6	-8.35	7.51	-9.55	3	LIG:H :ALA602:O LIG:H :GLU488:OE2 THR302:HN: LIG:O
	7	-7.90	1.63*	-9.09	2	LIG:H :GLU488:OE2 THR302:HN: LIG:O
	8	-7.88	1.66*	-9.08	2	LIG:H :GLU488:OE2 THR302:HN: LIG:O
	9	-7.86	1.72*	-9.06	2	LIG:H :GLU488:OE2 THR302:HN: LIG:O
	10	-7.77	2.01*	-8.96	1	THR352:HG1: LIG:N

Conclusion

The present research summarized the synthesis new fused pyridopyrimidine and naphthyridine cycles. As expected, the novel derivatives exhibited moderate to potent activity against several bacterial species as well as Candida Albicans. Docking approach was achieved to exploring the binding of the discovered hit inside the binding site of glucosamine-6-phosphate synthase, the target enzyme for the antimicrobial agents.

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