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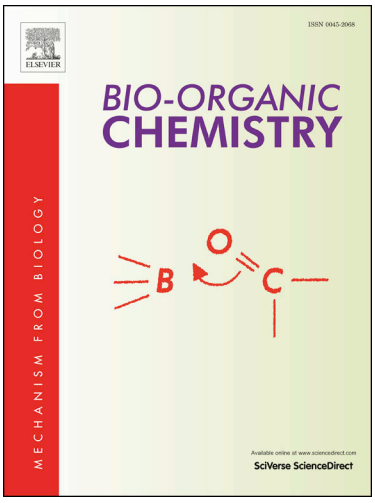


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# Synthesis, molecular docking and antimicrobial activity of new fused Pyrimidine and Pyridine derivatives

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**Abstract**: Synthesis of some new heterocyclic ring systems incorporated pyrimidine and pyridine moieties starting from 1-(furan-2-*yl*)-3-(thiophen-2-*yl*) chalcone was achieved. The structure of the new compounds was interpreted by spectral studies and ESI-MS analysis. Antimicrobial investigations of the designated compounds were performed towards some harmful pathogenic microbes. Antimicrobial tests proved that compound **11** unveiled a greater antimicrobial activity than other designed compounds. Docking of compound **11** into active site of DNA gyrase B chain displayed binding-energy of -13.05 kJ $mol^{-1}$ and distance at 3.18 $A^o$. Furthermore, docking investigation was approved for the goal compounds into DNA gyrase B chain and exhibiting binding energy extended from-13.05 to -20.48 kJ $mol^{-1}$.

**Keywords**: Pyrimidine, pyridine, chalcones, docking, DNA gyrase B chain, antimicrobial.

## 1. Introduction

Pyrimidine and pyridine ring systems are very important classes of compounds owing to their wide-spectrum of biological activities. Nitrogen aromatic pyrimidine, pyridine, and their analogs occur in nature and they show energetic role in the pitch of synthetic heterocyclic chemistry [1]. Such heterocyclic compounds are extensively used for many applications in medicinal science. Many of their derivatives are used as antimicrobial [2-10], antiviral [11-13], antitumor [14-21] and anti-oxidant [22, 23] agents in veterinary medicinal (Fig 1).

**Fig.1**

Chalcone derivatives have invited the attention of medicinal chemists due to their high potential as chemical sources for designing and developing promising drugs [24, 25]. Some of the attracted chalcones were approved for clinical use, Metochalcone was marketed as a choleretic drug [26, 27]. Hesperetin is a metabolite of hesperidin which has better bioavailability, and as presented clinical trials of hesperidin methyl chalcone for chronic venous lymphatic insufficiency and hesperidin trimethyl chalcone for trunk or branch varicosis [28,29]. As well as sofalcone proved as an anti-ulcer agent that improves the amount of mucosal prostaglandin [30, 31]. Also, Ro-09-0410 and its prodrug, Ro-09-0415 were tested for rhinovirus infections [32] (Fig 2).

**Fig.2**

Furthermore, chalcone constituents are central initial ingredients for the synthesis of unlike units of heterocyclic compounds, for example, pyrimidines, thiophenes, and pyrazolines, etc. Several of these structures are extremely bioactive and are extensively used as pharmaceuticals [33-36]. As part of our principal research program focused on the synthesis of a variety of heterocycles molecules for biological evaluation [37-41]. herein, we report the synthesis of some heterocyclic ring systems incorporated with pyrimidine and pyridine moieties starting from 1-(furan-2-*yl*)-3-(thiophen-2-*yl*) chalcone. Antimicrobial investigations of the designated compounds were performed towards some harmful pathogenic microbes. Furthermore, docking examination was accomplished for the goal molecules to DNA gyrase B chain using MOE 2008.10 program.

## 1. Results and Discussion

### 1.1. Chemistry

Chalcone represent a significant synthetic intermediate candidate, mainly as a building unit for the construction of several heterocyclic structures. Starting compound 1-(furan-2-*yl*)-3-(thiophen-2-*yl*) chalcone **4**, was prepared as a previously reported method [42, 43] by the Claisen-Schmidt reaction of 2-actylfurane with 2-thiophenecaboxaldhyde in the presence of catalytic KOH, Scheme 1.

**Scheme 1**

The actions of chalcone **4** towards some primary heteroaryl amines having $NH_2$ group connected at the α-site respect to the ring nitrogen (1,3-N, N-nucleophiles) was considered. Consequently, chalcone **4** used for the synthesis of azolopyrimidines (**10**, **11** and **15**), benzimidazothiazine **12** and pyrazolopyridine **13** compounds, Scheme 2.

**Scheme 2**.

Refluxing of chalcone **4** with 3-amino-1,2,4-triazole **5** in basic medium of potassium hydroxide, afforded 5-(furan-2-*yl*)-7-(thiopen-2-*yl*)-[1,2,4]triazolo[1,5-*a*]pyrimidine. The construction of **10** was established based on spectroscopic methods IR, NMR, and Mass. IR spectra not shown significant information. The $^{1}$H NMR spectrum is characterized by the singlet-signals at δ 7.85 and 7.31 ppm of H-2 of the triazole ring and H-6 for the pyrimidine respectively. Additionally, the $^{13}$C-NMR of compound **10** display thirteen carbons.

Also, chalcone **4** react with 5-amino-1,2,3,4-tetrazole monohydride **6** in basic condition to afford 4,7-dihydro-5-(furan-2-*yl*)-7-(thiopen-2-*yl*)[1,2,4,5]tetrazolo[1,5-a] pyrimidine **11**. The construction of compound **11** was established based on spectroscopic tools; IR, NMR, and Mass. IR spectra presentation absorption band at 3160.30 $cm^{-1}$ distinguishing for NH proton. Also, the $^{1}$H-NMR data showed six aromatic protons and two doublets, with coupling constant *J*=3.82Hz, at δ 5.1 and 6.6 ppm for 2H of pyrimidine and a single proton at 10.38 ppm assigned for the NH of pyrimidine. Further, its $^{13}$C NMR spectrum showed four singles at δ 62.43, 102.57, 152.65 and 158.60 ppm which indicated to aliphatic carbon, C=C, and C=N in

pyrimidine ring, respectively. In addition to eight carbons of the furanyl and thienyl rings (6CH & $2C_q$) appeared at 117.76, 120.88, 135.77, 136.18, 136.24, 136.52, 153.29, and 155.28 ppm.

Reaction of cyclic thiourea, namely 2-mercaptobinzemidzole **7** with chalcone **4** afford *2H*-benzo[4,5]-imidazo[2,1-b][1,3]thiazine derivatives **12**. The structure of this compound was interpreted based on IR, NMR, and Mass. IR spectra have shown 3155.40, 3111.66, 1623.44 and 1564.63 $cm^{-1}$ which indicated to aromatic-CH, aliphatic-CH, C=N, and C=C respectively. Accordioning to $^1H$ NMR spectrum two-doublets, with *J*=8.16 Hz, at δ 6.44 and 6.65 ppm for thiazine protons, in addition to the ten aromatic protons. Furthermore, the $^{13}C$ NMR spectrum of compound **12** displaying eighteen carbons.

The reaction of 5-amino-1, 2-dihydro-3*H*-pyrazolo-3-one **8** with chalcone **4** in basic condition only fused pyridine **13** was isolated. The product, 6-(furan-2-*yl*)-4-(thiopen-2-*yl*)-*1H*-pyrazolo[3,4-*b*]pyridine-3-ol **13** was interpreted based IR, NMR, and Mass tools. IR spectra have shown a broad peak at 3300-3000 $cm^{-1}$ which indicate the occurred of OH group. Also, its $^1H$ NMR displayed singlet at δ 6.7 ppm for pyridine proton besides to six-aromatic protons and two signals at 10.53 and 11.43 ppm for -OH and -NH. Additionally, the $^{13}C$ NMR of **13** display distinguishing fourteen carbons. The structure of possible pyrazolopyrimidine **14** was excluded as a result of the absence of *H-2* of pyrazole ring.

Furthermore, chalcone **4** react with 4-((4-nitrophenyl)diazenyl)-*1H*-pyrazole-3,5-diamine afford one separated product. The product was recognized as 5-(furan-2-*yl*)-3-((4-nitrophenyl)diazinyl)-7-(thiopen-2-*yl*)pyrazolo[1,5-a]pyrimidin-2-amine **15**. IR of **15** viewing two bands owing to amino and nitro groups. Additionally, the $^1H$ NMR of compound **15** exposed a new singlet at δ 7.0 owing to pyrimidine-proton and $^{13}C$ NMR spectrum revealed eighteen carbons.

The behavior of chalcone **4** towards 3-cyanoacetyl indole **16** was considered. Thus, the reaction of compound **4** with **16** in excess ammonium acetate and refluxing in acetic acid afforded 6-(furan-2-*yl*)-2-(*1H*-indol-3-*yl*)-4-(thiophen-2-*yl*)nicotinonitrile **19**. IR of the product exposed two characteristic bands owing to NH and CN groups at 3389 and 2204 $cm^{-1}$ respectively. Moreover, the $^1H$ NMR of compound **19** display singlet at

δ 6.8 due to a proton of the construction of a new pyridine ring and $^{13}C$ NMR spectrum revealed twenty-two characteristic carbons.

Also, chalcone **4** react with ethylcyanoacetate or cyanoacetamide afford a single product. Which was recognized as 6-(furan-2-*yl*)-4-(thiophen-2-*yl*-1,2-dihydro-2-oxo-pyridine-3-carbonitrile **20**. The spectral data of compound **20** was in a good agreement with the literature values [44], Scheme 3.

Chlorination of compound **20** using $POCl_3/PCl_5$ furnished 2-chloro-6-(furan-2-*yl*)-4-(thiophen-2-*yl*) pyridine-3-carbonitrile **23**. Chloropyridine **23** was established based on IR, NMR, and Mass tools. IR of compound **23** exhibited characteristic absorption-band for (CN) at 2226 $cm^{-1}$. As well as, the loss of C=O group of starting 2-oxo-pyridine-3-carbonitrile **20**. The $^1H$ NMR **23** presented singlet at δ 6.72 (H-5 of pyridine), and its mass displayed molecular ion peak at m/z (%): 286 ($M^+$, 5%).

Reaction of compound **20** with ethyl chloroacetate in basic medium at room temperature afford Ethyl 2-((3-cyano-6-(furan-2-*yl*)-4-(thiophen-2-*yl*) pyridin-2-*yl*) oxy) acetate **24**. The structure of **24** was established based on IR, NMR, and Mass. IR spectra of compound **24** showed two absorption bands distinguishing for (C≡N) at 2217 $cm^{-1}$ and C=O group at 1749 $cm^{-1}$. The $^1H$ NMR of compound **24** showed new characteristic triplet and quartet signals at δ 0.85 and 3.84 ppm for ethyl group and singlet signal at δ 4.59 ppm for methylene protons. Its $^{13}C$ NMR at δ 24.24 (Me), 71.29 ($CH_2$), 73.83 ppm ($CH_2$) respectively. And its mass displayed molecular ion peak at m/z: 335 ($M^+1$).

Treatment of the chloropyridine derivative **23** with hydrazine in dioxane give 6-(furan-2-*yl*)-2-hydrazinyl-4-(thiophen-2-*yl*) pyridine-3-carbonitrile **25**. The hydrazinyl derivative **25** was established based on IR, NMR, and Mass. The IR spectra of hydrazinyl **25** showed characteristic bands for (CN) at 2201 $cm^{-1}$ and at 3203, 3160 for ($NH-NH_2$). The $^1H$ NMR of compound **25** disclosed new characteristic signals at δ 6.72 and 6.82 ppm ($NH-NH_2$) group and singlet at δ 6.70 ppm for pyridine-proton. And its mass disclosed molecular ion peak at m/z: 283 ($M^+1$).

Furthermore, treatment of chloropyridine **23** with sodium azide afford 2-chloro-6-(furan-2-*yl*)-3-(*2H*-tetrazol-5-*yl*)-4-(thiophen-2-*yl*) pyridine **27** instead of compound

**26**. The structure of **27** was established based on IR, NMR, and Mass. IR spectra of compound **27** showed absorption band distinguishing for (NH) at 3293 $cm^{-1}$ and loss of CN group. $^{1}H$ NMR of **27** exhibited a new characteristic broad signal at δ 6.58 ppm (NH) group. $^{13}C$ NMR display a characteristic signal at δ 171.79 ppm (C-5 of new construction tetrazole ring). And its mass showed molecular ion peak at m/z: 331 ($M^{+}2$).

Ester **24** was treated with hydrazine to give 2-((3-cyano-6-( furan-2-*yl*)- 4-( thiophen-2-*yl*) pyridin-2-*yl*) oxy) acetohydrazid **28**. The acetohydrazid derivative **28** was interpreted based on IR, NMR, and Mass. IR spectra of **28** showed characteristic bands for (CN) at 2206 $cm^{-1}$ and at 1660 $cm^{-1}$ for (C=O). $^{1}H$ NMR **28** disclosed characteristic broad signals at δ 4.60 and 5.26 ppm (NH-$NH_2$) group and singlet at δ 4.19 ppm for methylene-protons (its $^{13}C$ NMR at δ 73.93 ppm). And its mass disclosed molecular ion peak at m/z: 322 ($M^{+}$-18).

**Scheme 3**.

Behavior of acetohydrazid **28** towards isatin and 4-nitrobenzaldehyde was considered as outlined in scheme 4. The interpretations of the isolated compounds **31** and **32** were an agreement with spectroscopic analysis; IR, NMR, and Mass (see experimental section).

**Scheme 4**.

**2.2. In Silico Molecular Docking Screening**

Modeling studies are essential to recognize the mechanisms of actions of drugs, methods of interactions with designed compounds, and to integrate all trial indication reported. Which are essential to find a reliable and exact image of biologically energetic compounds. Thus, offer novel visions to plan novel medicinal compounds. Docking investigation was approved for the goal synthetic molecules into DNA gyrase B chain using MOE 2008.10 program. From the data gotten the new targets under investigation displayed respectable fitting to the binding position of the protein surface and having binding energy extended from-13.05 to -20.48 kJ $mol^{-1}$ in contrast to the ligand (Novobiocin complexes). Which displayed binding energy of-25.34 kJ $mol^{-1}$, showed arene- cation interaction between benzene ring of chromen moiety and Arg 75 and formed five Hydrogen-bonds with the amino-acid residues; a) hydrogen of $NH_2$ group

with Asp 72 in distance 2.75 A$^{o}$ (36%); b) hydrogen of OH group with Asn 45 in distance 2.78 A$^{o}$ (68%); c) oxygen atom of C=O group formed two hydrogen bonds with Arg 135 in distance 2.90 A$^{o}$ (23%) and3..02 A$^{o}$ (23%); d) hydrogen of OH group with Asp 80 in distance 2.52 A$^{o}$ (81%) (Fig 3).

**Fig 3**

Compound **11** displayed binding-energy of -13.05 kJ mol$^{-1}$ and arene-cation contact between thiophene and Arg 75 beside forming one H-bond with the amino acid residues; a) hydrogen of NH group with Asp 72 in distance 3.18 A$^{o}$ (11%) (Fig 4).

**Fig 4**

Compound **25** displayed binding-energy of -17.18 kJ mol$^{-1}$ and arene-cation interface between furan and Arg 75 beside forming four Hydrogen-bonds with the amino acid; a) hydrogen of NH group with Asp 72 in distance 3.01A$^{o}$(14%) b) hydrogen of $NH_2$ group with Asp 72 in distance 2.69(70%) A$^{o}$ c) nitrogen of $NH_2$ group with Thr 166 and Gly 76 in distance 2.93(33%) and 3.01(33%) A$^{o}$ respectively (Fig 5).

**Fig 5**

Compound **27** revealed binding-energy of -20.48 kJ mol$^{-1}$ and arene-cation interaction between furan and Arg 75 beside forming one H-bond with the amino acid; a) hydrogen of NH group with Asp 72 in distance 2.77 A$^{o}$ (33%) (Fig 6).

**Fig 6**

Compound **28** exhibited binding-energy of -18.11 kJ mol$^{-1}$ and arene-cation interface between thiophene and Arg 75 beside forming two H-bonds with the amino acid residues; a) hydrogen of $NH_2$ group with Asp 45 in distance 2.88 A$^{o}$ (24%); b) nitrogen of $NH_2$ group with Tyr 94 in distance 2.97 A$^{o}$ (18%) (Fig 7).

**Fig 7**

Compound **31** unveiled binding-energy of -17.24 kJ mol$^{-1}$ and formed three H-bonds with the amino acid residues; a) hydrogen of NH group with Asp 48 in distance 3.03 A$^{o}$ (11%); b) nitrogen of hydrazine moiety and O-atom of C=O group with Lys 109 in distance 2.60 A$^{o}$ (43%) and 3.11 A$^{o}$ (17%) (Fig 8).

**Fig 8**

**Table 1**

### 2.3. Evaluation of antimicrobial activity

Data presented in Table **2** displayed that the diameters of inhibition zone of all tested synthesized compounds against all tested strains, results found that all compounds were indeed capable of halting microbial growth and have inhibition effects toward evaluated microbial strains. Antimicrobial susceptibility tests proved that compound **11** unveiled a greater antimicrobial activity than other compounds (Table 2).

The acquired results detected the highest diameters of the inhibition zone that was caused by compound **11** were 18, 16, 15 and 13 mm for *E. coli, L. monocytogenes, C. albicans,* and *A. niger*, respectively using the disc diffusion method, while by using well diffusion assay the dimeters were 21, 18, 17, and 16 mm, respectively. Conversely, the lowest antimicrobial effect was for compound **28**, results exhibited that the zone of inhibition diameters using disc diffusion assay toward *E. coli, L. monocytogenes, C. albicans,* and *A. niger* were 5, 4, 4, and 3 mm, and 8, 7, 8 and 5 mm using well diffusion assay. Furthermore, the experimental marks pointed out the diameter of the clear zone using the well-diffusion method are wider than the disc diffusion method. Furthermore, the investigational marks revealed that the tested Gram-negative bacterial species were more sensitive than Gram-positive species to all tested synthesized compounds. Furthermore, IC50% has been calculated for each compound as illustrated in Table 3 and some pictures of disc of the inhibition zone were demonstrated in Fig. 9.

**Table 2**

**Table 3**

**Fig.9**

### Minimum inhibitory concentration

To assess the antimicrobial effectiveness and MIC values for each tested compound against evaluated microbial lineages. Fig.10 illustrated the biocidal activity and MIC values of three distinct concentrations of synthesized compound **11** against evaluated species. The MIC value for inactivation of *E.coli* was 50 µg/mL within 30 min of time intervals, while *L. monocytogenes* was taken 100 µg/mL at 5 min. MIC values for *C.*

*albicans* and *A. niger* were taken the same dose and contact time (100 µg/mL within 15 min).

**Fig 10**

Regarding compound **25**, results demonstrated that 100 µg/mL within 15 and 30 min is considered the effective dose toward *E.coli* and *L. monocytogenes*. Whereas MIC values for *C. albicans* and *A. niger* were 150 µg/mL within 5 min (Fig. 11).

**Fig 11**

Data illustrated graphically in Figs. 12 and 13 pointed out that both compounds **27** and **28** have the same MIC values and inactivation rate against evaluated microbial lineages and also were indeed able to prevent microbial growth. Thus, all four *studied species* were susceptible to effective concentrations of compound **27** and **28**, MIC values toward all tested strains were 150 µg/mL within 5 min for *E.coli* and 15 min for the other evaluated strains.

**Fig 12**

**Fig 13**

Results presented in Fig. 14 revealed that 150 µg/mL within 15 min of compound **31** were required to wholly inhibit bacterial growth of *E.coli* and L. monocytogenes, while *C. albicans* and *A. niger* needed longer time (30 min)

**Fig 14**

**2.4. Structure activity relationship**

In connection with the found activity values with the structural-moiety of the most active designed molecules, it was approved that five applicants of the synthesized compounds were the greatest activity. Antimicrobial activity of the synthesized compounds was further investigated by the molecular docking approach, a method of simulation of fitting ligands into binding site(s) of macromolecular targets. The marks

revealed that compound **11** with tetrazolopyrimidine substructure and with a binding energy of -13.05 kJ $mol^{-1}$ and distance at 3.18 $A^o$ revealed higher antimicrobial activity than other compounds. On the other hand, compound **25** with pyridine substructure bearing 2-hydrazide and 3-carbonitrile groups which have binding-energy -17.18 kJ $mol^{-1}$ revealed good antimicrobial activity. Also, compound **27** incorporating pyridine with tetrazole substituted ring at 3-position, in the present work, display more activity than compound **31** and **28** respectively [2-6]. Compound **28** with acetohydrazid moiety exposed less activity than the other compounds.

## 3. Experimental

### 3.1. General Considerations

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Microwave reactions were performed with a domestic microwave oven Panasonic NN-SC688S, 120 V / 60 Hz, 1200W. Monitoring the reaction and checking the purity of the final products were carried out by thin layer chromatography (TLC) using silica gel precoated aluminum sheets (60 F254, Merck) and visualization with Ultraviolet light (UV) at 365 and 254 nm. Melting point (°C) were measured in open glass capillaries using stuart SMP30 advanced digital apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Buker NMR spectrometer at 400.18 MHz for $^1H$ and 100.62 MHz for $^{13}C$ or on a Buker Ascend 850 NMR spectrometer at 850.15 MHz for $^1H$ and 213.77 MHz for $^{13}C$ (Nuclear magnetic resonance center, KAU, Jeddah, KSA) or on a Buker Avance 500 NMR spectrometer at 500.13 MHz for $^1H$ and 125.75 MHz for $^{13}C$ at 25°C (Research Unit, College of Pharmacy, Prince Sattam Bin Abdulaziz University, AlKharj, KAS). The chemical shifts are expressed in (ppm) downfield from tetramethylsilane (TMS) as internal standard; coupling constants *(J)* are expressed in Hz. Deuteriochloroform ($CDCl_3$) and duteriodimethylsulphoxide (DMSO-$d_6$) were used as solvents. The splitting patterns were designated as: s (singlet), d (doublet), t (triplet), q (quarter) and (multiplet). Electrospray ionization mass spectra (ESI-MS) were recorded on Mass Spectrometry Impact II™, Bruker (college of pharmacy, KAU, Jeddah, KSA).

### 3.2. Chemistry

*3.2.1.* Synthesis of 1-(furan-2-yl)-3-(thiophen-2-yl) prop-2-en-1-one chalcone**.**

*Conventional method:* KOH (0.16 gm, 3 mmol), was dissolved in $H_2O$ (2.5 ml) and EtOH (10 ml) at room temperature. (0.224 gm, 2 mmol) 2-thiophenecaboxaldhyde **1** was added to the solution. Then, 2-acetyl furan **2** (0.220 gm, 2 mmol) was added over 10 min. The mixture was stirred for 5 h at room temperature. A solid product was poured into water (ice-cold) and neutralized with dil. HCl. After neutralization, the solid was filtered, washed with cold EtOH, dried and recrystallized from ethanol to give chalcone **4**.

*Microwave irradiation method:* Repetition of the same scale reactions of the conventional method. The reaction mixture was microwave irradiated for 4 min at 200 watts at 150°C. Completion of the reaction was identified by observing on TLC plates (ethylacetate:petroleum ehther; 1:6, Rf: 0.3). After completion of the reaction was treated similar to a conventional method to obtain chalcone **4**. Mol. formula: $C_{11}H_8O_2S$, conventional method yields 90%, microwave 86%, yellow crystals, m.p.74°C. Characterization was the same as reported method [42, 43].

3.2.2. General Method for the synthesis of compounds **10**, **11**, **12**, **13**, and **15**.

A mixture of chalcone **4** (0.20 gm, 1 mmol) and the appropriate heterocyclic amine **5, 6, 7, 8** and **9** (1.2 mmol) in DMF (10 ml) in the presence (26 mg, 0.1 mmol) of KOH was refluxed for 0.45–2h at 110°C. The reaction monitored through TLC (petroleum ether: ethyl acetate 8:2, Rf: 0.2, 0.3, 0.25, 0.3, 0.25, respectively), reaction mixture was allowed to cool to ambient temperature, then poured into ice-water and the solid product was collected by filtration followed by washing with ethanol. The crude product was then recrystallized from appropriate solvent to give pure products **10, 11, 12, 13** and **15**, respectively.

3.2.2.1. *5-( Furan-2-yl)-7-(thiophen-2-yl) [1,2,4] triazolo[1,5-a] pyrimidine* **10**

Pink solid, (DMF); yield 85%, m.p. 150-153°C. IR (KBr, ν, cm-1): 3190- 3101(Ar-H), 1706 (C=N), 1590 (C=C). $^1$H NMR (DMSO-$d_6$, δ ppm) 5.91 (dd, 1H, H-4, furan, *J*=5.1Hz), 6.56 (dd, 1H, H-4, thiophen, *J*=9.35 Hz), 6.81 (dd, 1H, H-3, furan, *J*=3.4 Hz), 7.22 (dd, 1H, H-3, thiophen, *J*=2.2 Hz), 7.30 (dd, 1H, H-5, thiophen, J=6.0 Hz), 7.31 (s, 1H, H-5, pyrimidine), 7.81 (dd, 1H, H-5, furan, *J*=5.01 Hz), 7.85 (s, 1H, H-3, triazole); $^{13}$C NMR (DMSO $d_6$, δ ppm) 105.47 (CH, C-5, pyrimidine), 117.22 (CH, C-C-3, furan), 118.30 (CH, C-4, furan), 132.32 (CH, C-4, thiophen), 133.90 (CH, C-3,

thiophen) , 137.35 (CH, C-5, thiophen), 139.55 (C, C-2, thiophen), 145.37 (CH, C-5, furan) , 150.80 (C, C-2, pyrimidine), 154.97 (CH,C-3, triazole), 155.51 (C, C-2, furan) , 159.50 (C, C-6, pyrimidine), 159.90 ( C,C-4, pyrimidine); ESI-MS, *m/z:* 269.0 [M+1]; Analysis Calcd. for $C_{13}H_8N_4OS$ (268): C, 58.20; H, 3.01; N, 20.88; Found C, 58.32; H, 3.08; N, 20.81.

3.2.2.2. *5-( Furan-2-yl)-7-(thiophen-2-yl)-4,7-dihydrotetrazolo[1,5-a] pyrimidine* **11**

white solid (acetone); yield 80%, m.p. 244-246°C. IR (KBr, ν, cm-1): 3160 (NH), 3115-3086 (Ar-H), 1667 (C=N) 1610 (C=C); $^1$H NMR (DMSO-$d_6$, δ ppm) 5.15 (dd, 1H, H-5, pyrimidine, *J*=5.3 Hz), 6.31(dd, 1H, H-4, furan, *J*=6.73 Hz), 6.61 (d, 1H, H-4, pyrimidine *J*=3.82 Hz), 6.71 (dd, 1H, H-4, thiophen, *J*=8.58 Hz), 6.75 (d, 1H, H-3, furan, J=3.4 Hz), 6.94 (dd, 1H, H-3, thiophen, *J*=4.33 Hz), 7.24 (dd, 1H, H-5, thiophen, *J*=4.59 Hz), 7.45(d, 1H, H-5, furan, *J*=1.36 Hz), 10.38 (1H, exchangeable with $D_2O$, NH); $^{13}$C NMR (213 MHz, DMSO-$d_6$) $\delta_C$ = 62.43 (CH, C-4, pyrimidine), 102.57 (CH, C-5, pyrimidine), 117.76 (CH, C-4, furan), 120.88 (CH, C-3, furan), 135.77 (CH, C-4, thiophen), 136.18 (CH, C-3, thiophen), 136.24 (CH, C-5, thiophen), 136.52 (C, C-2, thiophen), 152.65 (C, C-6, pyrimidine), 153.29 (CH, C-5, furan), 155.28 (C, C-2, furan), 158.60 (C, C-2, pyrimidine). ESI-MS, *m/z:* 274.2 [M+3], 271.5 [$M^+$]; Analysis Calcd. For $C_{12}H_9N_5OS$ (271): C, 53.13; H, 3.34; N, 25.81; Found C, 53.22; H, 3.30; N, 25.74.

3.2.2.3. *2-( Furan-2-yl)-4-(thiophen-2-yl) 2H-benzo [4,5] imidazolo[2,1-b] thiazine* **12** yellow crystals (DMF) in yield 88%, m.p 230°C; IR (KBr, ν, cm-1): 3155-3111 (Ar-H), 1623(C=N), 1564 (C=C); $^1$H NMR (DMSO $d_6$, δ ppm) 6.15 (dd, 1H, H-4, furan, *J*=5.27 Hz), 6.44 (d, 1H, H-4, thiazine, *J*=8.16 Hz), 6.65 (dd, 1H, H-5, thiazine, *J*=8.16 Hz), 6.79 (dd, 1H, H-4, thiophen, J=8.67 Hz,), 6.88 (m, 3H, H-3, furan& 2H, Ar-H), 7.16 (dd, 1H, H-3, thiophen J=4.76 Hz), 7.24 (d, dd, 1H, H-5, thiophen J=8.84 Hz), 7.44 (dd, m, 3H, H-5, furan& 2H, Ar-H); $^{13}$C NMR (DMSO $d_6$, δ ppm) 112.27 (CH, C-4, thiazine), 113.44 (CH, C-6, benzimidazole) , 119.51 (CH, C-4, furan), 121.72 (CH, C-3, furan), 121.83 (CH, C-9, benzimidazole), 123.27 (CH, C-5, thiazine), 124.98 (CH, C-8, benzimidazole), 127.09 (CH, C-7, benzimidazole), 128.39 (CH, C-4, thiophen), 130.23(CH, C-3, thiophen), 131.46 (CH, C-5, thiophen), 131.74 (C, C-5, benzimidazole), 132.98 (C, C-6, thiazine), 148.77 (C, C-2, thiophen), 149.41 (CH, C-5, furan), 150.85 (C, C-4, benzimidazole), 154.24 (C, C-2, furan), 173.34 (C, C-2,

thiazine). ESI-MS, *m/z:* 338.29 [M+2], 336.43 [$M^+$]; Analysis Calcd. For $C_{18}H_{12}N_2OS_2$: C, 64.26; H, 3.60; N, 8.33; Found C, 64.37; H, 3.39; N, 8.22.

3.2.2.4. *6-(Furan-2-yl)-4-(thiopen-2-yl)-1H-pyrazolo[3,4-b]pyridine-3-ol* **13**

Black crystals (DMF); yield 67%, m.p. 288°C; IR (KBr, ν, cm-1): br. 3000- 3100 (OH and NH), 1592 (C=N), 1544 ( C=C); $^1$H NMR (DMSO-$d_6$, δ ppm) 5.91 (dd, 1H, H-4, furan, *J*= 4.93 Hz),6.43 (d, 1H, H-4, thiophen, *J*= 3.23 Hz), 6.47 (dd, 1H, H-3, furan, *J*=8.67 Hz), 6.71 (s,1H, H-5, pyridine), 7.00 (dd, 1H, H-3, thiophen, *J*=5.61Hz), 7.11 (d, 1H, H-5, thiophen, *J*=.68 Hz, 1H), 7.50 (dd, 1H, H-5, furan), 10.53 (s, 1H, exchangeable with $D_2O$, OH), 11.43 (s, 1H, exchangeable with $D_2O$, NH); $^{13}$C NMR (DMSO $d_6$, δ ppm) 105.38 (C, C-3, pyridine), 115.31 (CH, C-3, furan), 116.67 (CH, C-4, furan), 119.05 (CH, C-5, pyridine), 135.13 (CH, C-4, thiophen), 135.49 (CH, C-3, thiophen), 138.14 (CH, C-5, thiophen), 114.65 (C, C-4, pyridine), 114.93 (C, C-2, thiophen), 151.31 (CH, C-5, furan), 154.99 (C, C-2, furan), 159.18(C, C-3, pyrazole), 160.61 (C, C-6, pyridine), 161.12 (C, C-2, pyridine). ESI-MS, *m/z:* 384.04 [M+1], 383.30 [$M^+$]; Analysis Calcd. For $C_{14}H_9N_3O_2S$: C, 59.35; H, 3.20; N, 14.83; Found C, 59.77; H, 3.12; N, 14.49.

3.2.2.5. *6-(Furan-2-yl)-3-((4-nitrophenyl)diazinyl)-7-(thiopen-2-yl)pyrazolo[1,5-a]pyrimidin-2-amine* **15**

Red crystals; yield 60%, m.p. 270°C; IR (KBr, ν, cm-1): br. 3200 ( NH), 1690 ( C=N), 1640 and 1440 (NO2), 1548 ( C=C); 1H NMR (DMSO-$d_6$, δ ppm) (dd, 1H, H-4, furan, *J*=3.20 Hz), 6.79 (dd, 1H, H-4, thiophen, *J*=8.76 Hz), 6.85 (dd, 1H, H-3, furan, *J*=2.76 Hz), 7.00 (s,1H, H-5, pyrimidine), 7.03 (br. exchangeable with $D_2O$, $NH_2$), 7.37(m, 4H, Ar-H), 7.53 (dd, 1H, H-3, thiophen, *J*= 4.92 Hz), 7.68 (d, 1H, H-5, thiophen, *J*=8.48 Hz), 8.00 (dd, 1H, H-5, furan, *J*=3.12 Hz), $^{13}$C NMR (DMSO $d_6$, δ ppm) 109.32 (CH, C-5, pyrimidine), 121.04 (CH, C-3, furan), 121.32 (CH, C-4, furan), 124.83 (CH, C-4, thiophen), 129.61 (2CH, C-2&C-6, benzene), 132.92 (2CH, C-3&C-5, benzene), 135.80 (CH, C-3, thiophen),136.63 (CH, C-5, thiophen), 137.54 (C, C-2, thiophen), 141.54 (C, C-4, pyrimidine), 141.72 (C, C-4, pyrazole), 143.54 (C, C-4, benzene), 147.25 (CH, C-5, furan), 153.85 (C, C-1, benzene), 154.21(C, C-2, pyrimidine), 156.01 (CH, C-2, furan), 159.76 (C, C-3, pyrazole), 165.25 (C, C-6, pyrimidine). ESI-MS, *m/z:* 432.06 [M+1], 431.08 [$M^+$]. Analysis Calcd. For $C_{20}H_{13}N_7O_3S$: C, 55.68; H, 3.04; N, 22.73; Found C, 55.76; H, 3.01; N, 22.55.

3.2.3. *6-(Furan-2-yl)-2-(1H-indol-3-yl)-4-(thiophen-2-yl)nicotinonitrile* **19**

A mixture of 3-cyanoacetylindole **16** (2 mmol, 0.368 gm) and ammonium acetate (8 mmol, 0.62 gm) in acetic acid AcOH (10 ml) was heated until complete dissolve. Then, add chalcone **4** (2 mmol, 0.202 gm) and refluxed for 12 h. The reaction motoring by TLC (ethylacetate:petroleum ehther; 1:6, Rf: 0.2). After the completion of reaction allowed to cool at room temperature. The solid precepted was crystallized from AcOH to afford the title compound as black crystals, yield 65 %, m.p. 236-238°C; IR (KBr, ν, cm-1): 3389 (NH-Indole), 3150, 3098, 3081(CH-Ar), 2204 (CN), 1634, 1603 (C=N), 1558(C=C); $^{1}$H NMR (DMSO $d_6$, δ ppm) 5.90 (dd, 1H, H-4, furan, *J*=5.1 Hz), 6.35 (m, 2H,Ar-H), 6.45 (dd, 1H, H-4, thiophen, *J*=8.67 Hz), 6.55 (dd, 1H, H-3, furan, *J*=3.4 Hz), 6.64 (dd, 1H, H-3, thiophen, *J*=7.48 Hz), 6.80 (s,1H, H-5, pyridine), 7.05 (m, 2H,Ar-H), 7.13 (dd, 1H, H-5, thiophen, *J*=1.50 Hz), 7.51 (d, 1H, H-2, Indole, J=2.80 Hz), 7.54 (d, 1H, H-5, furan, J=7.65 Hz), 10.97 (s, exchangeable with $D_2O$, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, δ ppm) 101.95 (C, C-3, pyridine), 115 (CH, C-3, benzene), 115.95 (CH, C-3, Indole), 116.13 (C, CN), 116.19 (CH, C-3, furan), 116.53 (CH, C-4, furan), 122.73 (CH, C-5, pyridine), 124.38 (CH, C-5, benzene), 125.21 (CH, C-6, benzene),125.95 (CH, C-4, benzene), 129.95 (CH, C-4, thiophen), 132.10 (CH, C-3, thiophen), 132.41 (CH, C-2, Indole), 133.55 (CH, C-5, thiophen), 133.84 (C, C-1, benzene), 139.75 (C, C-2, thiophen), 140.64 (C, C-2, benzene), 149.60 (C, C-4, pyridine), 150.21 (CH, C-5, furan), 153.53 (C, C-2, furan), 155.44 (C, C-2, pyridine), 161.73 (C, C-6, pyridine). ESI-MS, *m/z:* 368.07 [M+1], 367.42 [$M^+$]. Analysis Calcd. For $C_{22}H_{13}N_3OS$: C, 71.92; H, 3.57; N, 11.44; O; Found C, 71.92; H, 3.57; N, 11.44.

3.2.2.1. *6-(Furan-2-yl)-4-(thiophen-2-yl-1,2-dihydro-2-oxo-pyridine-3-carbonitrile* **20**

A mixture of chalcone (10 mmol, 2.04 gm) and ethyl cyanoacetate **17** (1.13gm, 10 mmol) or cyanoacetamide **18** (2.10gm, 10 mmol) in n-butanol (50 ml) containing ammonium acetate (6.16gm, 80 mmol) was heated under reflux for 3h or 5h respectively. The reaction mixture was concentrated into its half volume and left to cool. Then, the solid separated upon cooling was filtered, washed with water and recrystallized from DMF to afford a compound **20** as yellow crystals, yield 60 % or 40% respectively, m.p. 300-303°C. The spectral data of compound was in good agreement with the literature values[36].

3.2.2.2. *2-Chloro-6-(furan-2-yl)-4-(thiophen-2-yl) pyridine-3-carbonitrile* **23**

A solution mixture of compound **20** (2.68 gm, 10 mmol), phosphorus oxychloride (10 ml, 0.10 mole) and phosphorous pentachloride (0.5 gm, 2 mmol) were fused on boiling water bath for 10 h. After the reaction was completed, TLC (ethylacetate:petroleum ehther; 1:6, Rf: 0.2)., the solution mixture was cooled and poured gradually into crushed ice. The obtained precipitate was filtered off and dried and purified by silica gel column chromatography with a gradient elution of ethyl acetate/n-hexane to afford **23**. as yellow crystals, yield 64% m.p. 163-165°C; IR (KBr, ν, cm-1): 3132, 2916 (CH-Ar), 2226 (CN), 1603 (C=N), 1565 (C=C); $^{1}$H NMR ($CDCl_3$, δ ppm) 6.08 (dd, 1H, H-4, furan, *J*=5.1 Hz), 6.70 (dd, 1H, H-4, thiophen, *J*=7.8 Hz), 6.72 (s,1H,H-5, pyridine), 6.78 (dd, 1H, H-3, furan, *J*=4.08 Hz), 7.07 (dd, 1H, H-3, thiophen, *J*=6.12 Hz), 7.09 (dd, 1H, H-5, thiophen, *J*=2.21 Hz), 7.45 (dd, 1H, H-5, furan, *J*=1.10 Hz); $^{13}$C NMR ($CDCl_3$, δ ppm) 110.37 (C, C-3, pyridine), 120.10 (CH, C-3, furan), 120.90 (C, CN), 122.09 (CH, C-4, furan), 122.81 (CH, C-5, pyridine), 136.00 (CH, C-4, thiophen), 137.24 (CH, C-3, thiophen), 137.30 (CH, C-5, thiophen), 143.54 (C, C-2, thiophen), 152.74 (C, C-4, pyridine), 154.93 (CH, C-4, furan), 157.97 (C, C-2, pyridine), 158.14 (C, C-2, furan), 161.88 (C, C-6, pyridine). ESI-MS, *m/z:* 286 [$M^+$]. Analysis Calcd. For $C_{14}H_7ClN_2OS$: C, 58.64; H, 2.46; Cl, 12.36; N, 9.77; Found C, 58.78; H, 2.41; N, 9.54.

3.2.2.3. *6-(Furan-2-yl)-2-hydrazinyl-4-(thiophen-2-yl) pyridine-3-carbonitrile* **25**

A mixture of the chloropyridine derivative **23** (2.86 g mmol) and hydrazine hydrate 80% (1.57 ml, 50 mmol) in dioxane (10 ml) was heated under reflux for 12 h, TLC, (ethylacetate:petroleum ehther; 1:6, Rf: 0.3). (ethylacetate:petroleum ehther; 1:6, Rf: 0.3). The formed precipitate was filtered off, dried and recrystallized from methanol to give the hydrazine derivative **25** as yellow crystals, yield 64% m.p. 200°C; IR (KBr, ν, cm-1): 3203, 3160 (NH-$NH_2$), 2974 (CH-Ar), 2201 (CN), 1600 (C=N), 1572 (C=C); $^{1}$H NMR ($CDCl_3$, δ ppm) 6.00 (dd, 1H, H-4, furan, *J*=5.2 Hz), 6.54 (dd, 1H, H-4, thiophen, *J*=8.68 Hz, 1H), 6.61 (dd, 1H, H-3, furan, *J*=3.32 Hz, 1H), 6,70 (s,1H,H-5, pyridine), 6.72 (s, exchangeable with $D_2O$, 2H, $NH_2$), 6.82 (s, exchangeable with $D_2O$, H, NH), 6.98 (m, 1H, H-3, thiophen), 7.14(m, 1H, H-5, thiophen, 7.90 (m, 1H, H-5, furan, J=1.12 Hz); $^{13}$C NMR ($CDCl_3$, δ ppm) 111.77 (C, C-3, pyridine), 120.66 (CH, C-3, furan), .120.93 (C, CN), 121.10 (CH, C-4, furan), 123.04 (CH, C-5, pyridine), 139.16 (CH, C-4, thiophen), 139.132 (CH, C-3, thiophen), 139.99 (CH, C-5, thiophen),

148.40 (C, C-2, thiophen), 155.27 (C, C-4, pyridine), 156.74 (CH, C-5, furan), 158.03 (C, C-2, pyridine), 163.21 (C, C-2, furan), 163.50 (C, C-6, pyridine). ESI-MS, *m/z:* 283.05 [M+1], 282.32 [$M^+$]. Analysis Calcd. For $C_{14}H_{10}N_4OS$: C, 59.56; H, 3.57; N, 19.85; Found C, 59.73; H, 3.51; N, 19.76.

3.2.2.4. *2-Chloro-6-(furan-2-yl)-3-(2H-tetrazol-5-yl)-4-(thiophen-2-yl)pyridine* **27**

A mixture of the chloropyridine **23** (0.286 gm, 1 mmol) in 10 ml of DMF and then add (0.195 gm, 3 mmol) of sodium azide in 5 ml of water was refluxed for 8 h, After the reaction was completed, TLC, (ethylacetate:petroleum ehther; 1:6, Rf: 0.3), the precipitate was removed by filtration and filtrate poured into water (ice-cold) and neutralized with dil. AcOH. After neutralization, the solid was filtered, washed with water, crystallized from ethanol to give **27** as a red powder in a yield (53%) m.p. 160 °C. IR (KBr, ν, cm-1): 3293 (NH), 3114, 3112 (CH-Ar), 1647 (N=N), 1597 (C=N), 1547,1539 (C=C), 703(C-Cl); 1H NMR (DMSO-$d_6$, δ ppm) 6.25 (dd, 1H, H-4, furan, *J*=5.2 Hz), 6.58 (br, exchangeable with $D_2O$, 1H, NH), 6.70 (s,1H,H-5, pyridine), 6.77 (dd, 1H, H-4, thiophen, *J*=3.4 Hz), 6.84 (dd, 1H, H-3, furan, *J*= 8.8 Hz), 7.38 (dd, 1H, H-3, thiophen, *J*=4.8 Hz), 7.38 (dd, 1H, H-5, thiophen, *J*=6.15 Hz), 7.45 (m, 1H, H-5, furan, *J*=1.4 Hz); $^{13}$C NMR (DMSO-$d_6$, δ ppm) 116.28 (CH, C-3, furan), 122.43 (CH, C-4, furan), 123.18 (CH, C-5, pyridine), 127.76 (CH, C-4, thiophen), 139.01 (CH, C-3, thiophen), 139.29 (CH, C-5, thiophen), 139.59 (C, C-3, pyridine), 140.21 (C, C-2, thiophen), 148.02 (C, C-2, pyridine), 155.44 (C, C-4, pyridine), 156.66 (CH, C-5, furan), 160.96 (C, C-2, furan), 162.36 (C, C-6, pyridine), 171.79 (C, C-5, tetrazole). ESI-MS, *m/z:* 329 [$M^+$]. Analysis Calcd. For $C_{14}H_8ClN_5OS$: C, 50.99; H, 2.45; Cl, 10.75; N, 21.24; Found: C, 51.08; H, 2.41; N, 21.19.

3.2.2.5. *Ethyl 2-((3-cyano-6-(furan-2-yl)-4-( thiophen-2-yl) pyridin-2-yl) oxy) acetate* **24**

A mixture of **20** (2.68gm, 10 mmol) and (1.52 gm, 11 mmol) potassium carbonate was stirred in dry DMF (20 ml) for 1h, followed by the addition of ethyl chloroacetate (1.44 gm, 10 mmol), the reaction mixture was stirred at room temperature for 24 h., then poured into ice-water and acidified with HCl. to give the crude product which purified by silica gel column chromatography with a gradient eluent of ethyl acetate/ petroleum ether to afford **24** as yellow crystals, yield: 95%; m.p.124 °C. IR (KBr, ν, cm-1): 2976 (CH-Ar), 2217 (CN), 1749 (C=O), 1599 (C=N), 1547, 1539 (C=C); $^1$H NMR ($CDCl_3$,

δ ppm) 0.85 (t, 3H, Me, *J*=19.04 Hz), 3.84 (q, 2H, $CH_2$, *J*= 21.33 Hz), 4.59 (s, 2H, $CH_2$), 6.15 (dd, 1H, H-4, furan, *J*=5.1 Hz), 6.69 (dd, 1H, H-3, furan, *J*=3.99 Hz), 6.81 (dd, 1H, H-4, thiophen, *J*=8.84 Hz), 6.85 (s,1H, H-5, pyridine), 7.16 (dd 1H, H-3, thiophen, *J*=6.12 Hz), 7.18 (dd, 1H, H-5, thiophen, *J*=2.29 Hz), 7.57 (dd, 1H, H-5, furan, *J*=6.8 Hz, 1H); $^{13}$C NMR ($CDCl_3$, δ ppm) 24.24 (Me), 71.29 ($CH_2$), 73.83 ($CH_2$), 100.16 (C, C-3, pyridine), 120.82 (CH, C-4, furan), 122.54 (C, C-CN), 122.69 (CH, C-4, furan), 125.62 (CH, C-5, pyridine), 138.73 (CH, C-4, thiophen), 139.43 (CH, C-3, thiophen), 139.76 (CH, C-5, thiophen), 147.38 (C, C-2, thiophen), 155.00 (C, C-4, pyridine), 158.37 (CH, C-5, furan), 158.93 (C, C-2, furan), 162.09 (C, C-6, pyridine), 174.05 (C, C-2, pyridine), 178.23 (C=O). ESI-MS, *m/z:* 355.06 [M+1], 353.25 [M-1]. Analysis Calcd. For $C_{18}H_{14}N_2O_4S$: C, 61.01; H, 3.98; N, 7.91; Found: C, 61.22; H, 3.92; N, 7.86.

3.2.2.6. *2-((3-Cyano-6-( furan-2-yl)- 4-( thiophen-2-yl) pyridin-2-yl) oxy) acetohydrazid* **28**

The compound **24** (3.54 gm, 10 mmol) was added to hydrazine hydrate 80% (20 mmol, 0.627 ml) in absolute ethanol (20 ml) and refluxed for 7 h, the reaction monitoring by TLC (ethylacetate:petroleum ehther; 1:6, Rf: 0.24). After finishing concentrated the reaction mixture was and cooling, the precipitate obtained was filtered off, crystallized from ethanol to give **28** as white crystals, yield: 95%; m.p. 245°C; IR (KBr, ν, cm-1): 3132, 2916 (CH-Ar), 2206 (CN), 1660 (C=O), 1603 (C=N), 1565 (C=C); $^1$H NMR (DMSO-$d_6$, δ ppm) 4.19 (s, 2H, $CH_2$), 4.60 (s, exchangeable with $D_2O$, 2H, $NH_2$), 5,26 (s, exchangeable with $D_2O$, H, NH), 5.87 (dd, 1H, H-4, furan, *J*= 4.85 Hz), 6.44 (m, 2H, H-4, thiophen, H-3, furan), 6.71 (s,1H, H-5, pyridine), 7.08 (m, 3H, H-3& H-5 thiophen, H-5, furan,); $^{13}$C NMR (DMSO-$d_6$, δ ppm) 73.93 ($CH_2$), 99.42 (CH, C-3, pyridine), 120.30 (CH, C-3, furan), 123.20 (C, CN), 123.85 (CH, C-4, furan), 125.42 (CH, C-5, pyridine), 139.05 (CH, C-4, thiophen), 140.53 (CH, C-3, thiophen), 141.42 (CH, C-5, thiophen), 146.35 (C, C-2, thiophen), 156.92 (C, C-4, pyridine), 158.07 (CH, C-5, furan), 158.64 (C, C-2, furan), 161.11 (C, C-6, pyridine), 173.39 (C, C-2, pyridine), 176.98 (C=O). ESI-MS, *m/z:* 341.36 [M+1]. Analysis Calcd. For $C_{16}H_{12}N_4O_3S$: C, 56.46; H, 3.55; N, 16.46; Found : C, 56.55; H, 3.32; N.

3.2.3. *2-((3-Cyano-6-(furan-2-yl)-4-(thiophen-2-yl)pyridin-2-yl)oxy)-N'-(2-oxoindolin-3-ylidene)acetohydrazide* **31**

A mixture of the compound **28** (0.34 gm, 1 mmol) and isatin **29** (0.147 gm, 1 mmol) in 20 ml ethanol containing drops of acetic acid was refluxed at the water bath. The reactions motoring by TLC (ethylacetate:n-hexane; 2:5, Rf: 0.24). After the completion of reactions, allowed to cool at room temperature. then left overnight at room temperature. The formed precipitate was filtered, dried and recrystallized from methanol to give the title compound **32** as yellow crystals, yield: 96%; m.p.246 °C; IR (KBr, ν, cm-1): 3100- 3300 (br. 2NH), 2226 (CN), 1705, 1715 (2C=O), 1603 (C=N), 1565 (C=C); $^{1}$H NMR (DMSO-$d_6$, δ ppm) 5.22 (s, 2H, $OCH_2CO$), 6.26 (dd, 1H, H-4, furan, *J*=5.2 Hz), 6.54 (d, H-4, thiophene, *J*=7.84 Hz), 6.68 (t, 1H, H-4, benzene, *J*=15.28 Hz, 1H), 6.83 (m, 1H, H-3, furan), 6,96 (dd, 1H, H-3, thiophene, J=8.84 Hz), 7.02 (t, 1H, H-5, benzene, *J*=15.32 Hz), 7.22 (s,1H, H-5, pyridine), 7.51 (m, 1H, H-5, thiophene *J*=0.92 Hz), 7.69 (m, 2H, H-3& H-5, benzene), 7.77 (d, 1H, H-5, furan, *J*=7.68 Hz), 10.74 (s, exchangeable with $D_2O$, NH), 11.18 (s, exchangeable with $D_2O$, NH); $^{13}$C NMR ($CDCl_3$) 89.99 ($CH_2$, $OCH_2CO$), 110,98 (C, C-3 pyridine), 111.47 (CH, C-3, furan), 113.74 (CH, C-5, pyridine), 114.12 (CH, C-4, furan), 115.99 (C, CN), 116.33 (C,C-2, benzene), 122.54 (CH, C-6, benzene), 127.21 (CH, C-4, benzene), 129.65 (CH, C-4, thiophene), 131.17 (2CH, C-3, thiophene & C-3, benzene), 131.99 (2CH, C-5, thiophene& C-5, benzene), 133.68 (C,C-2, thiophene), 137.12 (C, C-1, benzene), 144.85 (CH,CH=N), 147.24 (CH, C-5, furan), 148.77 (C, C-4, pyridine) , 149.30 (C, C-2, furan), 151.81 (C, C-6, pyridine), 164.55 (C, C-2, pyridine), 164.55 (C, C=O), 168.81 (C, C=O). ESI-MS, *m/z:* 470.06 [M+1], 469.48 [$M^+$]. Analysis Calcd. For $C_{24}H_{15}N_5O_4S$: C, 61.40; H, 3.22; N, 14.92; Found: C, 61.47; H, 3.13; N, 14.87.

3.2.4. *2-((3-Cyano-6-(furan-2-yl)-4-(thiophen-2-yl)pyridin-2-yl)oxy)-N'-(4-nitrobenzylidene)acetohydrazide* **32**

A mixture of the compound **27** (0.34 g, 1 mmol) and 4-nitrobenzaldehyde **30** (1 mmol) in absolute ethanol (20 ml) was refluxed at the water bath. The reactions motoring by TLC (ethylacetate:n-hexane; 1:3, Rf: 0.36).. After the completion of reactions allowed to cool at room temperature. then left overnight at room temperature. The formed precipitate was filtered, dried and recrystallized from DMF to give the title compound.

(DMF) Whit crystals, yield: 96%; m.p. 273°C.; IR (KBr, ν, cm-1): 3300 (NH), 2221 (CN), 1695 (C=O), 1603 (C=N), 1550 and 1400 ($NO_2$), 1565 (C=C); $^1$H NMR (DMSO $d_6$, δ ppm) 5.21 (s, 2H, $OCH_2CO$), 6.26 (dd, 1H, H-4, furan, *J*=5.2 Hz), 6.75 (d, 1H, H-4, thiophene, *J*=3.28 Hz), 6.93 (m, 1H, H-3, furan),, 7.18 (s,1H, H-5, pyridine), 7.57 (m, 5H, H-2, H-6,H-3& H-5 benzene& H-3, thiophene), 7.57 (s,1H, N=CH), 7.75 (m, 1H, H-5, furan), 11.56 (s, exchangeable with $D_2O$, NH); $^{13}$C NMR ($CDCl_3$, δ ppm) 63.71($OCH_2CO$), 89.20 (C, C-3, pyridine), 110.06 (CH, C-3, furan), 112.97 (CH, C-5, pyridine), 113.17 (CH, C-4, furan), 115.45 (C, CN), 124.04 (2CH, C-2& C-6, benzene), 127.89 (2CH, C-3& C-5, benzene), 128.85 (CH, C-4, thiophene), 130.32 (CH, C-3, thiophene), 131.14 (CH, C-5, thiophene), 136.34 (C, C-2, thiophene), 140.18 (C, C-1, benzene), 141.72 (C, C-4, pyridine), 146.46 130.31 (C, C-5, furan), 147.81 (CH, CH=N), 147.92 (C, C-4, benzene), 148.51(C, C-2, furan), 151.08 (C, C-6, pyridine), 164.26 (C, C-2, pyridine), 168.76 (C=O) ESI-MS, *m/z:* 474.06 [M+1], 473.34 [$M^+$], 472.33[M-1]. Analysis Calcd. For $C_{23}H_{15}N_5O_5S$: C, 58.35; H, 3.19; N, 14.79; Found: C, 58.49; H, 3.11; N, 14.72.

**3.3. In Silico Molecular Docking Screenings.**

Docking estimation of the most active antibacterial compounds **11, 25, 27, 28** and **31** was achieved by Molecular Operating Environment (MOE) 2008.10 releases of Chemical Computing Group, Montreal, Canada (http://www. chemcomp. com.). The program operated on an Intel(R) Core (TM) i3-32100 CPU@3.10G*Hz* 3.09 G*Hz* processor, 3.41 GB of RAM, Microsoft Windows XP.

Docking was done against the active site of the protein molecular surface of DNA gyrase B chain (PDB ID: 1KIJ) in complex with Novobiocin (was downloaded from a protein data bank (http://www. rcsb. org/-pdb) (PDB ID: 1KIJ) [45].

The DNA gyrase enzyme has a crucial role in bacterial cell viability [46]. DNA gyrase B) possesses the ATPase activity, providing a sufficient amount of energy for the DNA supercoiling [47] DNA gyrase remains an attractive target due to its presence across all microbes [48]. Targeting DNA gyrase with an inhibitor is a new strategy for dealing with antimicrobial resistance by disrupting DNA synthesis, leading to cell death and reducing the development of resistance [49].

The protein crystal construction was prepared for docking *via* removing of water molecules, addition and elimination of polar hydrogen atoms then separation of the active pocket. The active site was measured to be the site where co-crystalline ligand namely, Novobiocin complexes (PDB ID: 1KIJ). The crystal structure of Gyrase B 43K

ATPase domain in complex with novobiocin, is one of the most potent inhibitors of gyrase showing large conformational changes of the subdomains within the dimer [50]. The co-crystalline ligand was re-docked in the active pocket to ensure the docking process was effective and the active pocket was saved to be used for docking simulation of the designated new compounds.

The building of the selected compounds for docking was drawn in Chem Draw Ultra 10.0 (ChemOffice package) and saved. Before the molecular docking, preparation steps must be done as follow; a) converting the 2D structure of ligands to their 3D form; b) addition and removing of polar hydrogen atoms; c) energy minimized using the MMFF94x force field until a RMSD (Root-mean-square deviation) of atomic position gradient of 0.01 Kcal $mol^{-1}$ $Å^{-1}$ was gotten and saved as MOE. MMFF94x was reported as an efficient force field for minimizing ligand-protein complexes [51].

The docking Algorithm was done by MOE-DOCK default. It uses a flexible, rigid technique for posing the molecule inside the cavity. All rotatable bonds of ligands are allowed to undergo free rotation to be placed into the rigid receptor binding site. The docking scores were expressed in negative energy terms; the lower the binding free energy, the better the binding affinity [52], and the ligand interactions (hydrogen bonding and hydrophobic interaction) with DNA gyrase B chain was determined [53].

### 3.3.1. Evaluation of antimicrobial activity.

### 3.3.2. Preparation of working stock solution

By dissolving 500 mg of each synthesized compound in 2 ml DMSO, stock solutions of five synthetic compounds (**11**, **25**, **27**, **28** and **31**) were prepared and ready to be used. Four prospective pathogenic microbes were examined against three distinct concentrations (50, 100 and 150 µg/mL) of each synthesized compound.

### 3.3.2. Microorganisms used and preparation

Four microbial species used in the present study were as follows: *Escherichia coli* ATCC 25922 as an example for Gram-negative bacteria, *Listeria monocytogenes* ATCC 25152 as an example for Gram-positive bacteria, *Candida albicans* ATCC 10231 as an example for yeast; and *Aspergillus niger* ATCC 6275 as an example for fungi. All bacterial and fungal species were cultured into Tryptic Soya broth and Malt extract broth for incubation overnight at 37 $C^{o}$, and harvested in the mid-log phase. For 20 minutes, each culture tube (falcon 50 ml) was centrifuged at 4000 rpm. The supernatant was discarded and the pellet cells were taken and washed three times to

remove any undesired particulates using phosphate-buffered saline. Final cells concentration of culture suspension was $2.5x10^6$ CFU/mL [54].

**3.3.3. Antimicrobial susceptibility testing**

**a. Kirby–Bauer test (well and disc diffusion)**

Well and disc diffusion assays were performed by disseminating 100µL on the surface of Müller Hinton agar (MHA) (BBLTM, Germany) plates from each prospective microbial pathogen culture. For well diffusion assay, the wells (6 mm in diameter) were punctured with such a sterile driller upon the surface of MHA media and 100 µL of each antiseptic solution studied was inserted into each well [55]. Whilst the sterile filter paper discs (6 mm in diameter) were saturated with 100 µL of the tested antiseptic and placing the discs onto the surface of MHA plates. After appropriate incubation, the diameters of clear zones were measured in mm using a scale.

**b. Minimum inhibitory concentrations (MIC)**

The MIC values of each synthesized compound were studied against the target microbial pathogens according to Gharib et al [56]. In brief, 100 µL of each fresh microbial culture was injected into 10 mL sterile distilled water tubes that containing different concentrations (50, 100 and 150 µg/mL) of tested antiseptics. While control tubes for each tested strain were free from disinfectants. The specific conditions of all experiments were at room temperature and shaking at 250 rpm. Samples were taken from each tube with four different time intervals (5, 15, and 30 min) for counting of the viable bacterial cells. The densities of living cells before (initial count) and after exposure to synthesized compounds were counted using the pour plate method. All experiments were performed in triplicate and repeated at least twice on different days [57].

**Author Contributions**: Radwan .M. A. A. conceived the research project, participated in all steps of the research, interpreted the results, discussed the experimental data and prepared the manuscript; Maha A. Alshubramy do the experimental part and, participated in all steps of the manuscript. Bahaa A. Hemdan prepared antimicrobial work. Dina S. El-Kady makes docking studies. All authors read, discussed and approved the manuscript.

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**Conflict of interest**

The authors declare no conflict of interest.

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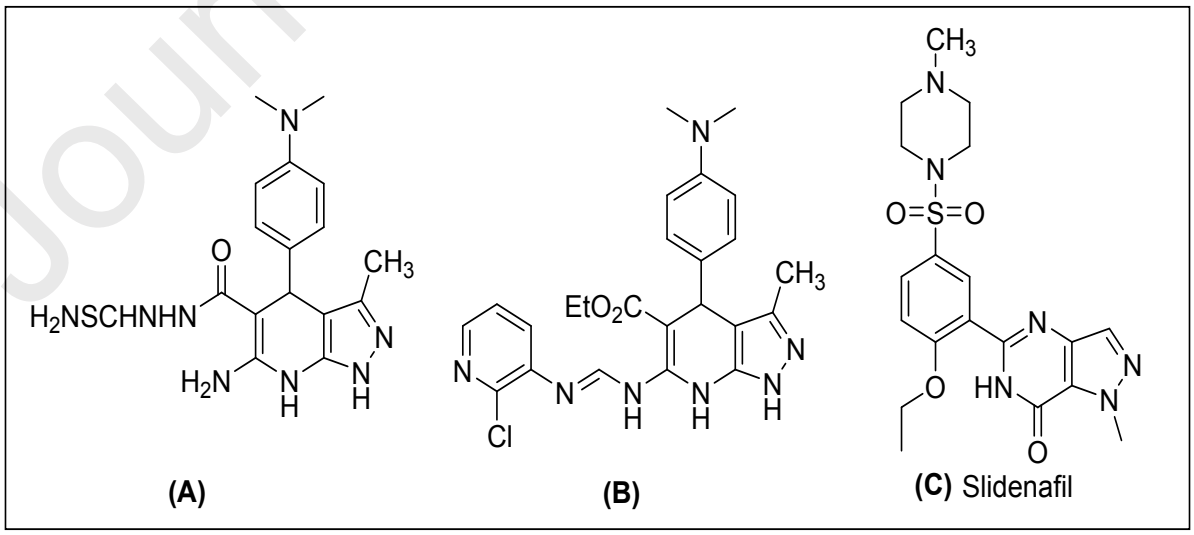
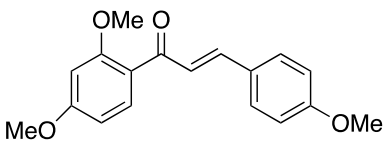
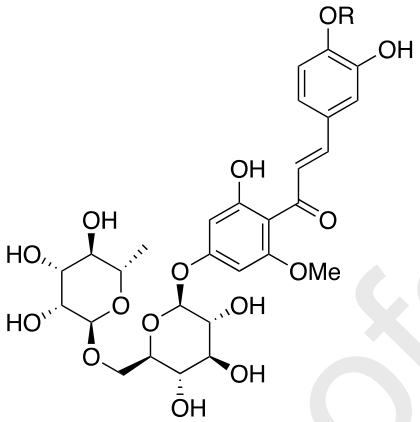


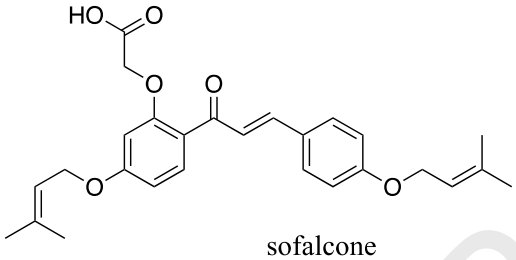

**Fig.1 Examples of bioactive fused Pyrimidine and Pyridine compounds**



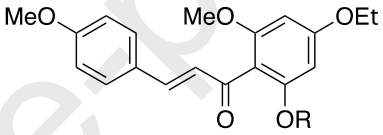

Metochalcone




Hesperidin methylchalcone, R=H
Hesperidin trimethylchalcone , R=Me

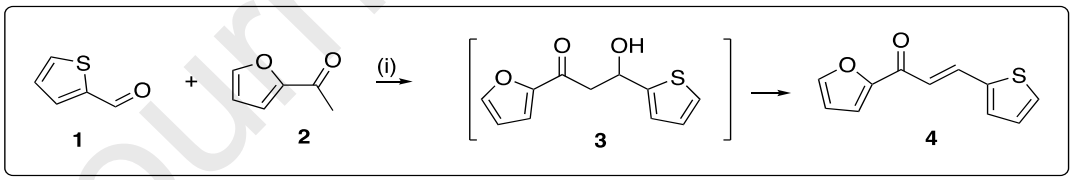



sofalcone

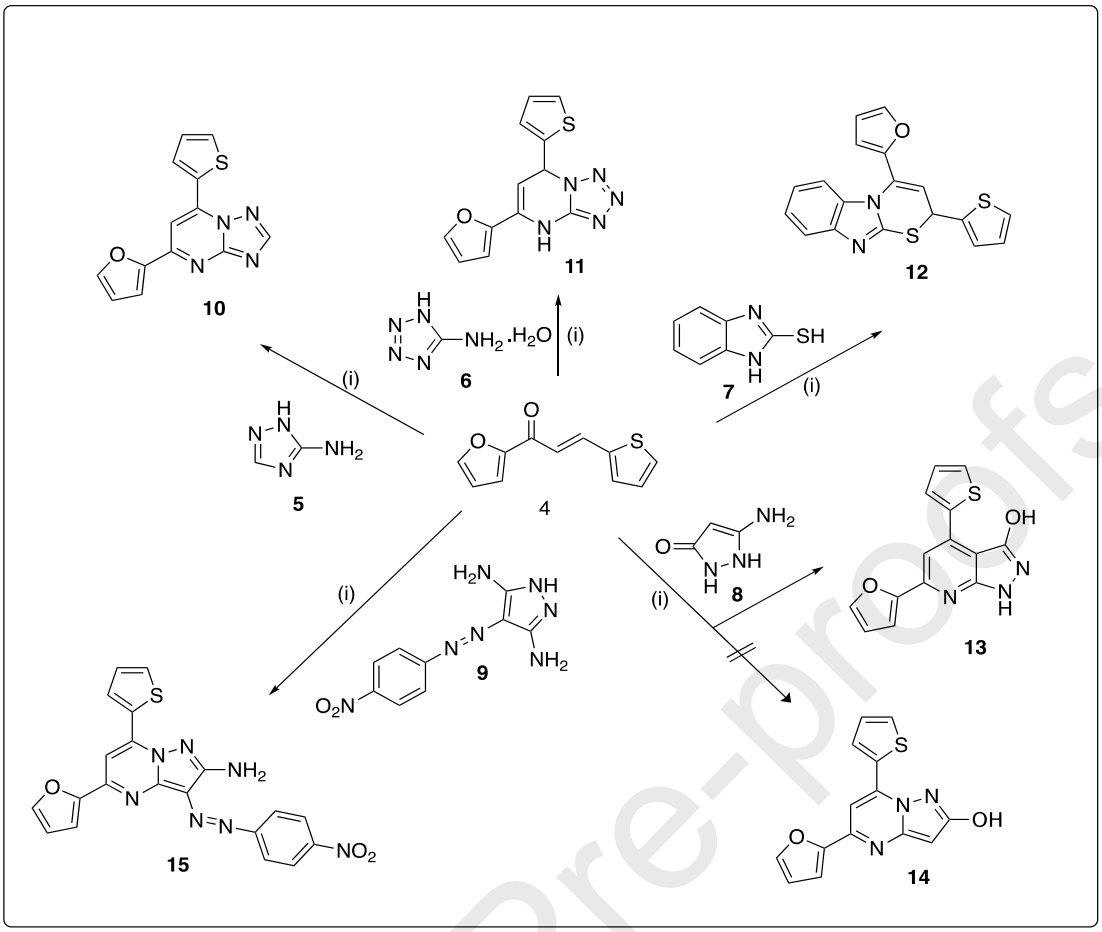



Ro 09-0410, R=H
Ro 09-0415, R=$PO_3Na_2$

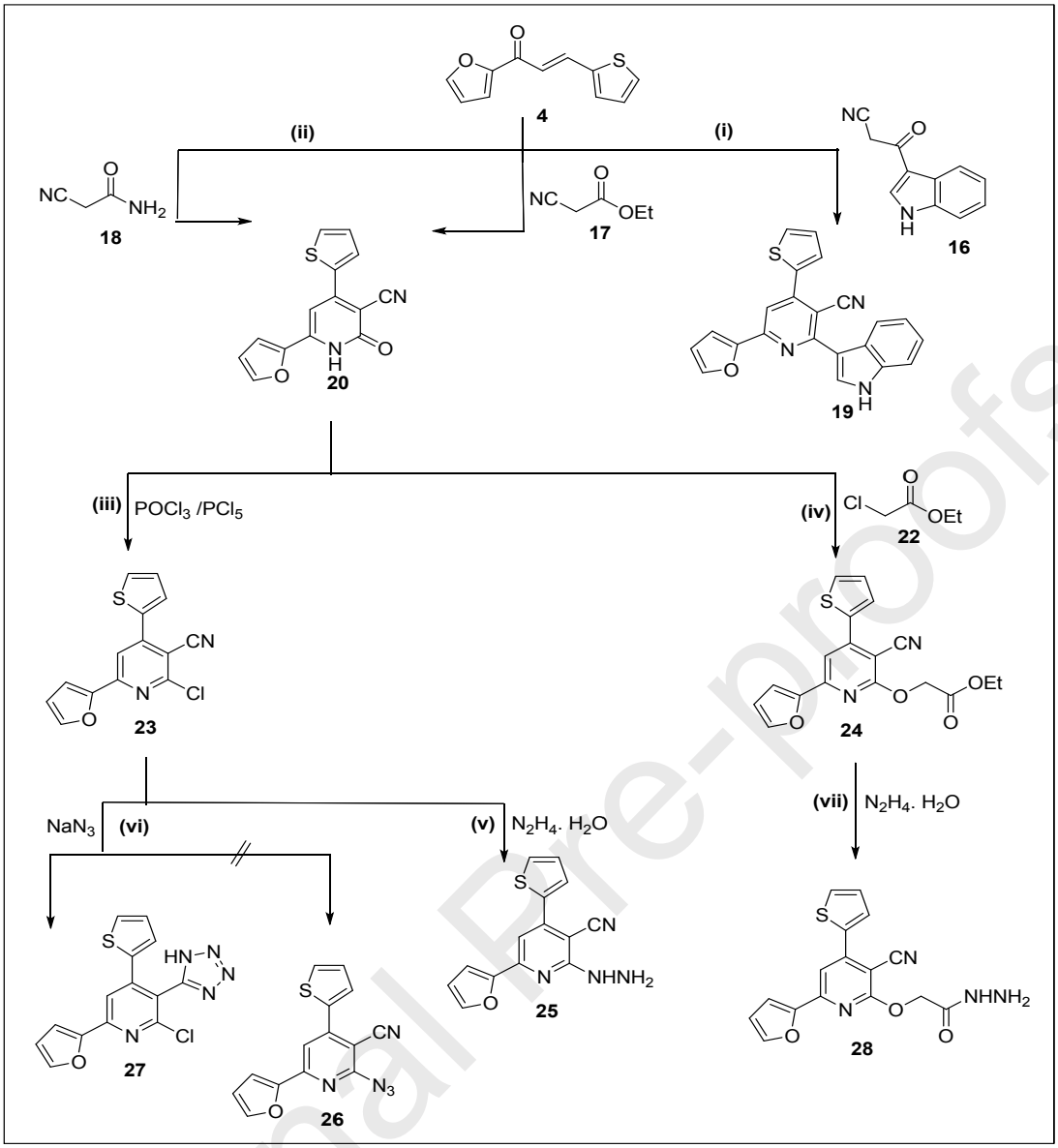
**Fig.2 Some chalcones that have been marketed or clinically tested**



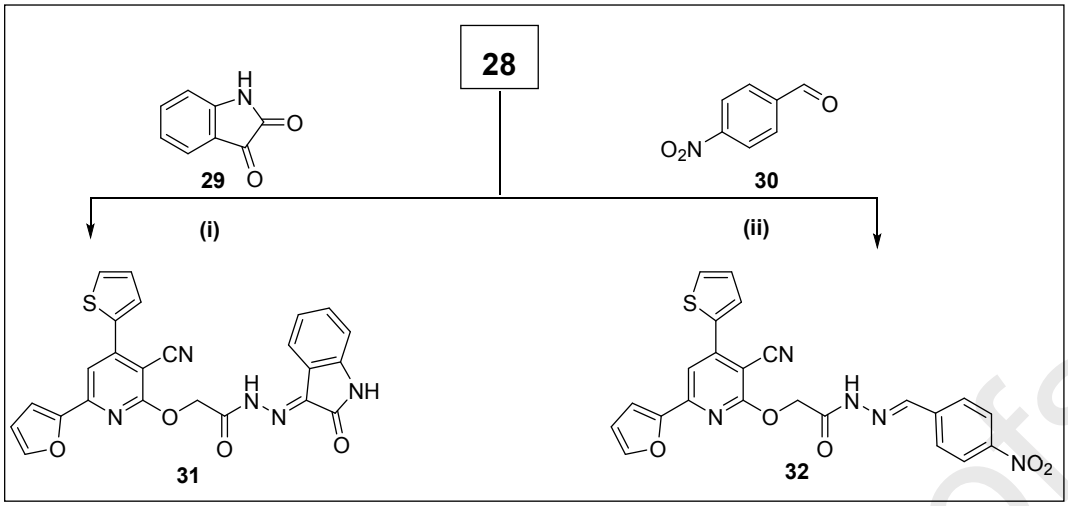

**Scheme 2** *Reagents and conditions:* (i) KOH, EtOH, stirring 5 h**.**




**Scheme 3** *Reagents and conditions:* (i) KOH**,** DMF, reflux.




**Scheme 3** *Reagents and conditions:* (i) $NH_4OAc$, AcOH, reflux 12 h. (ii) $NH_4OAc$, n-butanol reflux 3h/ 5h. (iii) fusion at 80, 10 h. (iv) $K_2CO_3$, DMF, stirring, 24 h. (v) dioxane, reflux 12 h. (vi) $H_2O$, DMF, reflux 8 h. (vii) EtOH, reflux 7 h.




**Scheme 4** Reagents and conditions: (i) EtOH, AcOH, reflux 2 h (ii) EtOH, reflux.

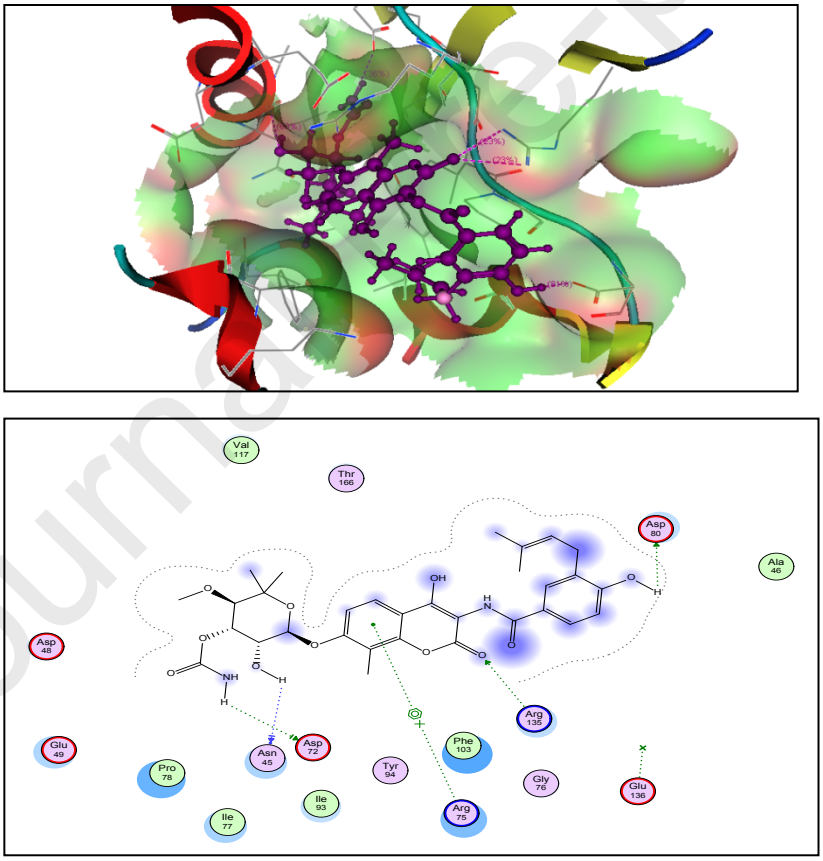



**Fig 3**: Redocking of **Novobiocin** into active site of DNA grase B chain active site.

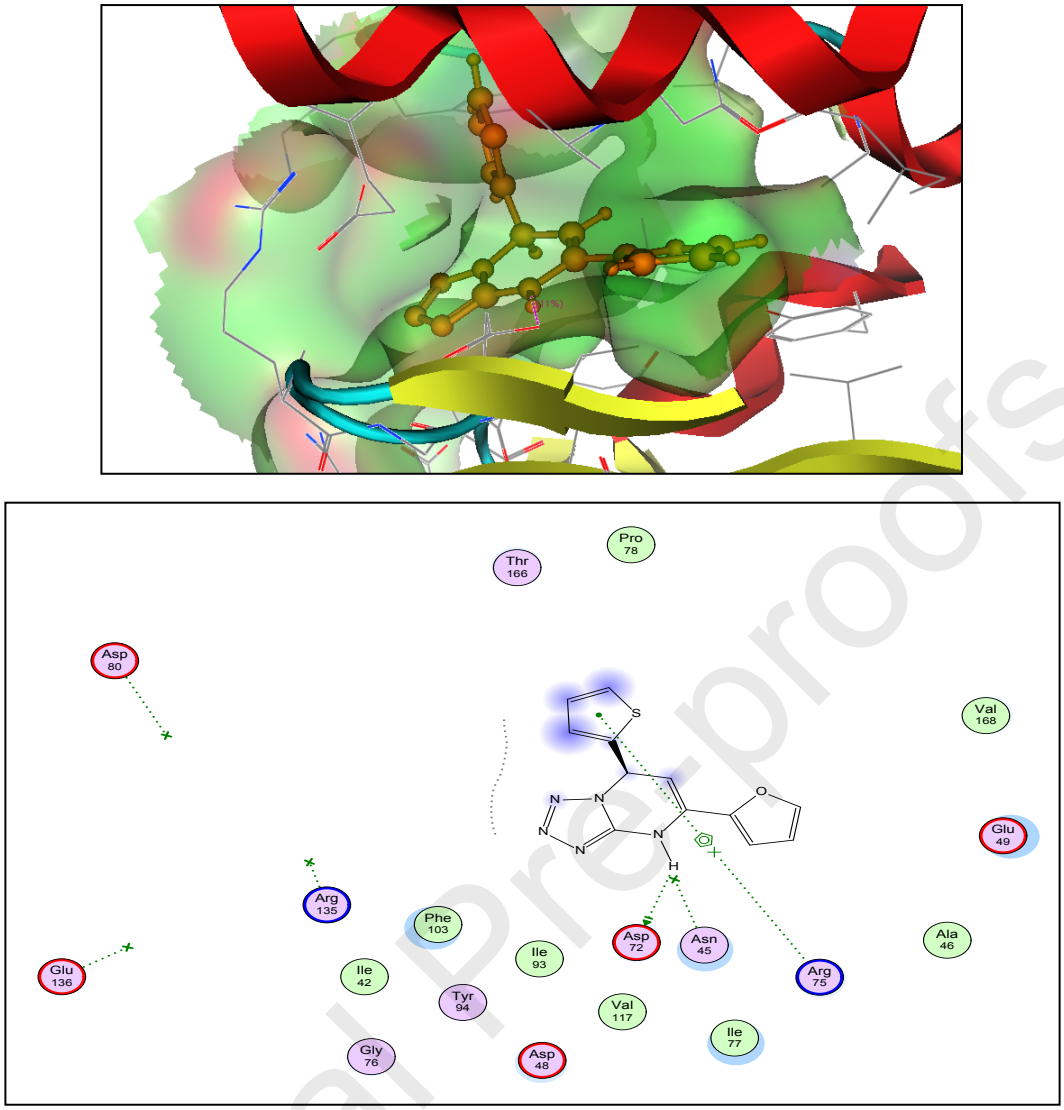
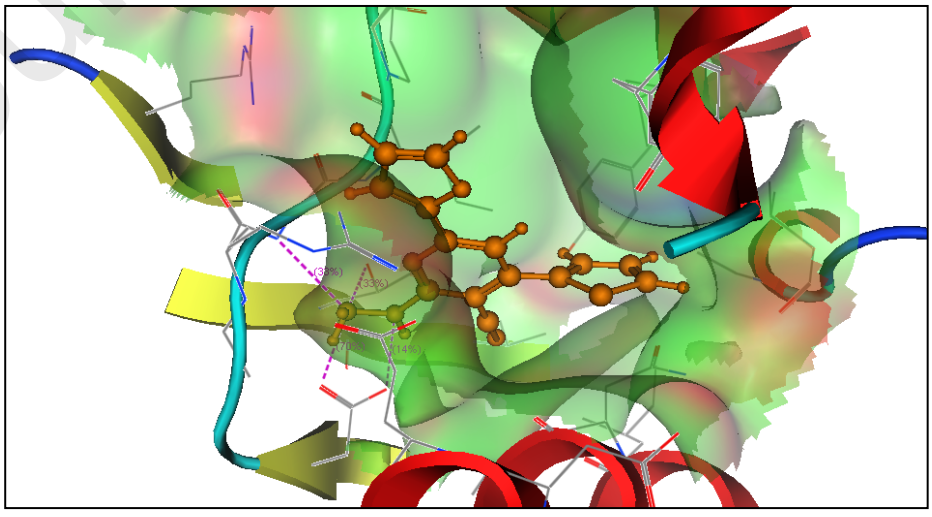



**Fig 4:** Docking of compound **11** into active site of DNA grase B chain active site.



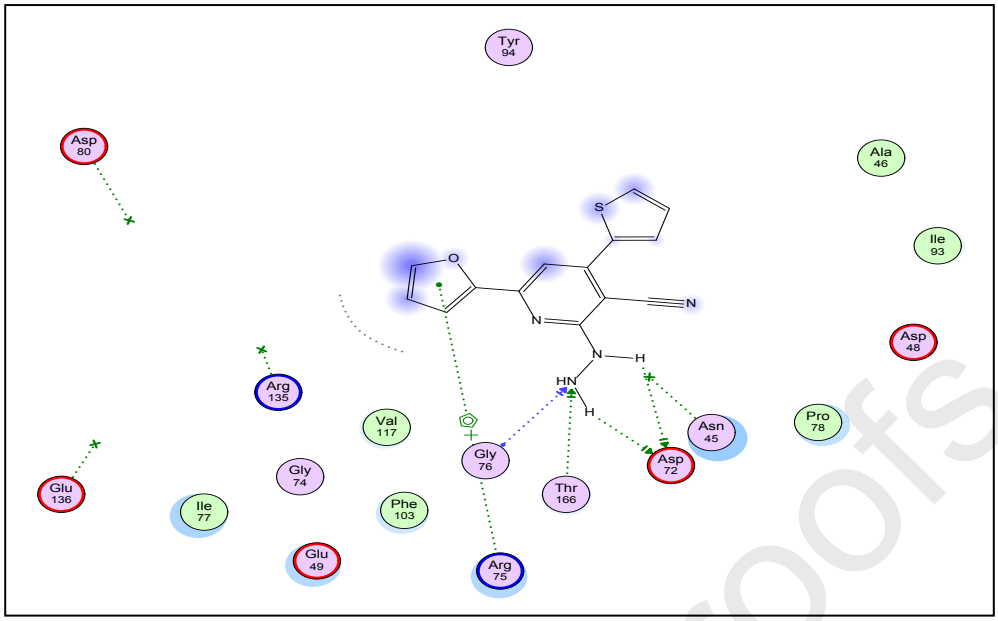



**Fig 5:** Docking of compound **25** into active site of DNA grase B chain active site.

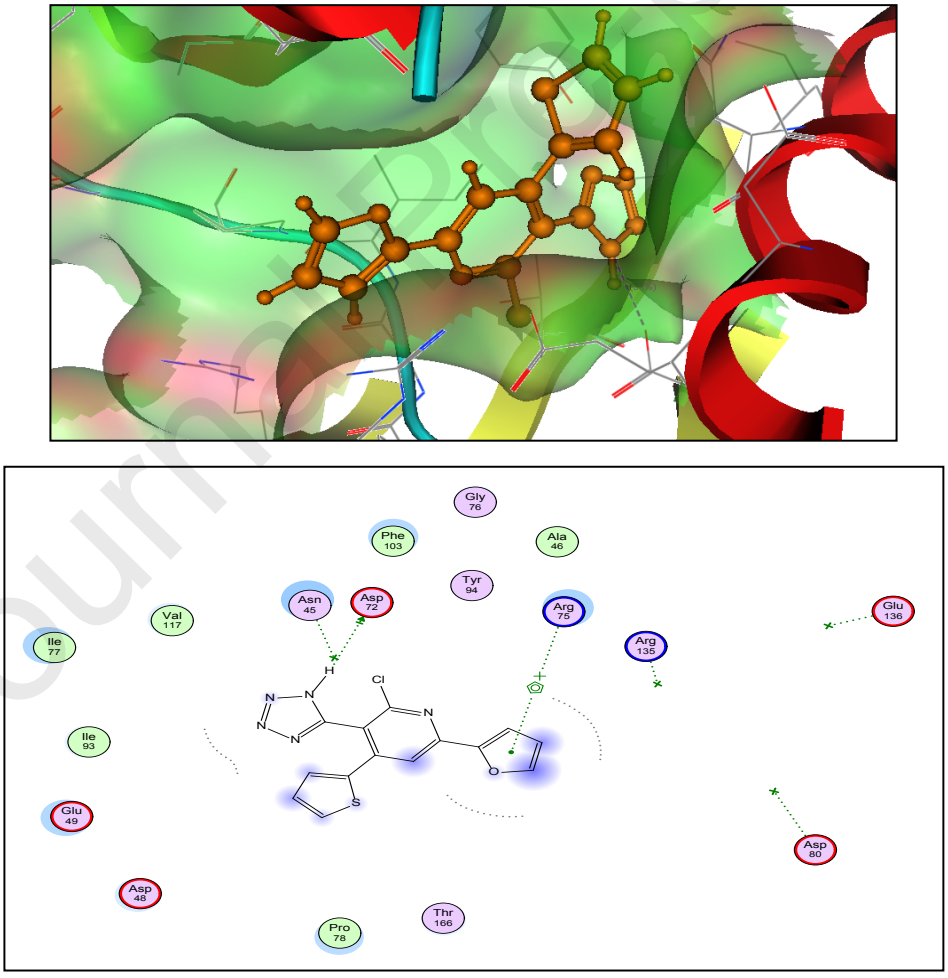



**Fig 6:** Docking of compound **27** into active site of DNA grase B chain active site.

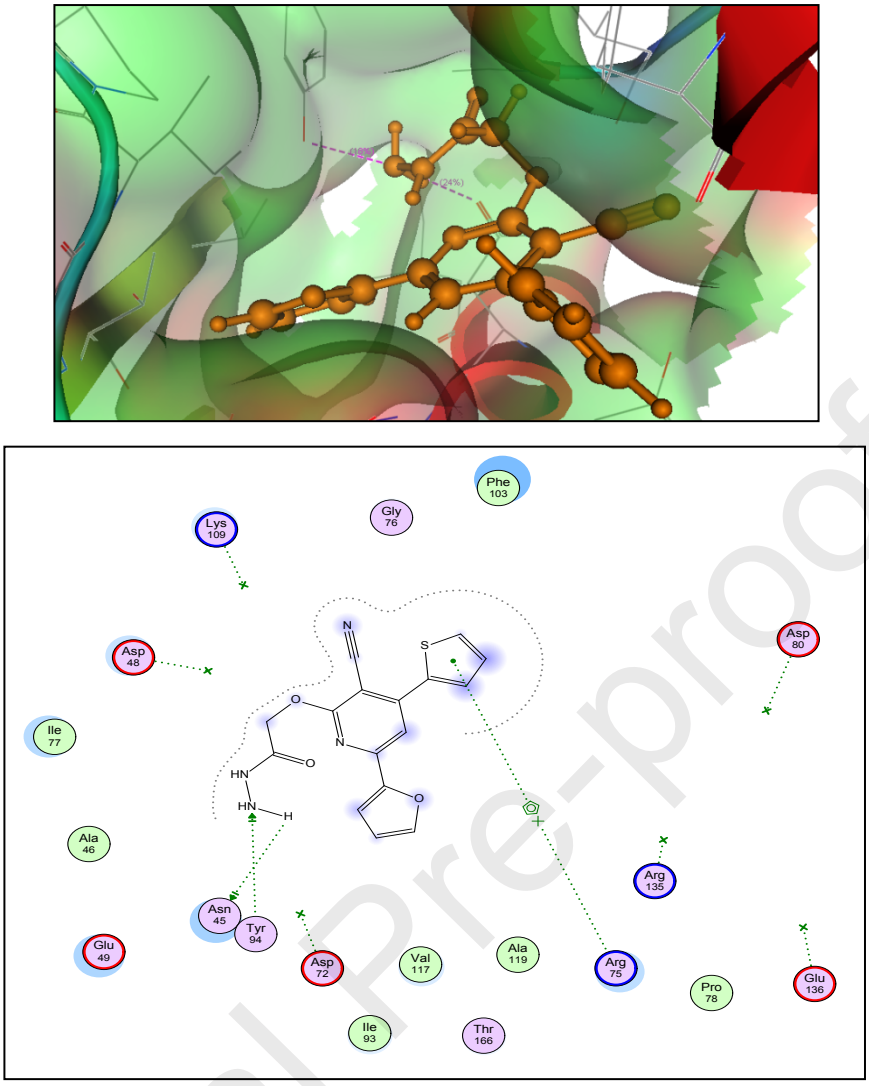



**Fig 7:** Docking of compound **28** into active site of DNA grase B chain active site.

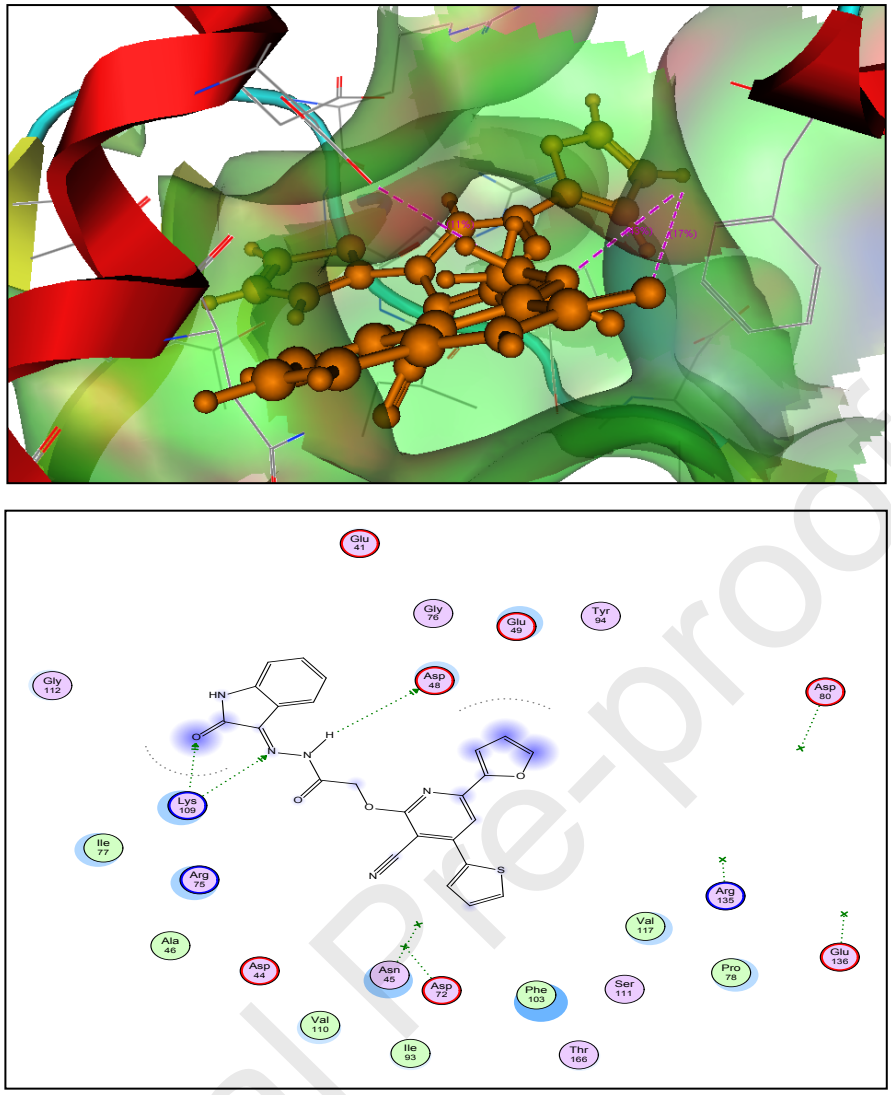



**Fig 8:** Docking of compound **31** into active site of DNA grase B chain active site.

**Table 1**: Binding energies and distance of docked compounds into DNA grase B chain active site.

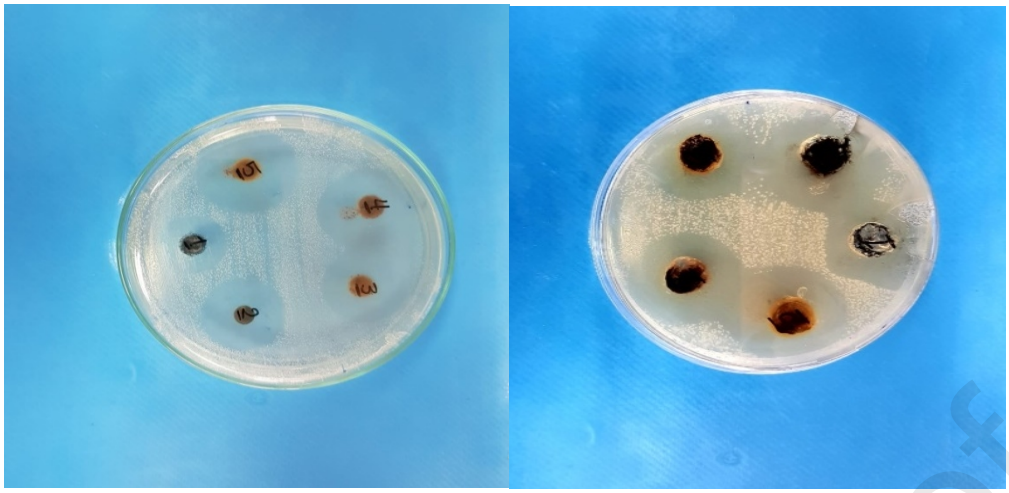
| **Compounds NO.** | **Binding energy KJ mol$^{-1}$** | **Main atoms from the compounds** | **Residue involved** | **Distance A°** |
|---|---|---|---|---|
| **11** | -13.05 | hydrogen atom of NH group | Asp 72 | 3.18 |
| **25** | -17.18 | hydrogen atom of NH group<br>hydrogen atom of $NH_2$<br>nitrogen atom of $NH_2$ group | Asp 72<br>Asp 72<br>Thr 166, Gly 76 | 3.01<br>2.69<br>2.93, 3.01 |
| **27** | -20.48 | hydrogen atom of NH group | Asp 72 | 2.77 |
| **28** | -18.11 | hydrogen atom of $NH_2$ group<br>nitrogen atom of $NH_2$ group | Asp45<br>Tyr 94 | 2.88<br>2.97 |
| **31** | -17.24 | hydrogen atom of NH group<br>nitrogen atom of hydrazine moiety<br>oxygen atom of C=O group | Asp 48<br>Lys 109<br>Lys 109 | 3.03<br>2.60<br>3.11 |

**Table 2.** Antimicrobial activities and inhibition zone diameters of synthesized compounds toward tested pathogenic microbiome.

| Tested synthetized compounds | Diffusion assays | Tested pathogenic microbiome | | | |
|---|---|---|---|---|---|
| | | ***E. coli*** | ***L. monocytogenes*** | ***C. albicans*** | ***A. niger*** |
| **11** | **Disc** | 18 ±0.16 | 16 ±0.28 | 15 ±0.22 | 13 ±0.16 |
| | **Well** | 21 ±0.20 | 18 ±0.19 | 17 ±0.15 | 16 ±0.22 |
| **25** | **Disc** | 14 ±0.28 | 12 ±0.23 | 11 ±0.22 | 10 ±0.19 |
| | **Well** | 16 ±0.21 | 16 ±0.19 | 13 ±0.15 | 12 ±0.22 |
| **27** | **Disc** | 13 ±0.16 | 12 ±0.24 | 11 ±0.24 | 10 ±0.15 |
| | **Well** | 16 ±0.20 | 15 ±0.11 | 13 ±0.12 | 12 ±0.28 |
| **28** | **Disc** | 5 ±0.23 | 4 ±0.28 | 4 ±0.28 | 3 ±0.24 |
| | **Well** | 8 ±0.18 | 7 ±0.17 | 8 ±0.16 | 5 ±0.21 |
| **31** | **Disc** | 13 ±0.26 | 12 ±0.21 | 11 ±0.21 | 10 ±0.17 |
| | **Well** | 15 ±0.22 | 14 ±0.18 | 13 ±0.27 | 11 ±0.23 |
| **Amphotericin** | **Disc** | -- | -- | 18 ±0.22 | 23 ±0.16 |
| | **well** | -- | -- | 21 ±0.15 | 25 ±0.22 |
| **Ampicillin** | **Disc** | -- | 17 ±0.23 | -- | -- |
| | **Well** | -- | 19 ±0.19 | -- | -- |
| **Gentamicin** | **Disc** | 23 ±0.22 | -- | -- | -- |
| | **Well** | 26 ±0.15 | -- | -- | -- |

**Table 3.** IC50% for each designed compound

| **Tested microbes** | **$IC_{50\%}$ values of tested compounds within 5 min** | | | | |
|---|---|---|---|---|---|
| | **11** | **25** | **27** | **28** | **32** |
| ***E.coli*** | 21.61 | 46.20 | 26.40 | 55.61 | 46.12 |
| ***L.monocyutogenes*** | 10.00 | 33.33 | 49.00 | 35.09 | 14.73 |
| ***C. albicans*** | 60.35 | 33.02 | 46.65 | 33.63 | 37.08 |
| ***A.niger*** | 57.86 | 42.74 | 51.37 | 27.75 | 41.06 |



1. Disc of inhibition 2. Well of inhibition

**Fig.9** Some pictures of disc of inhibition zone

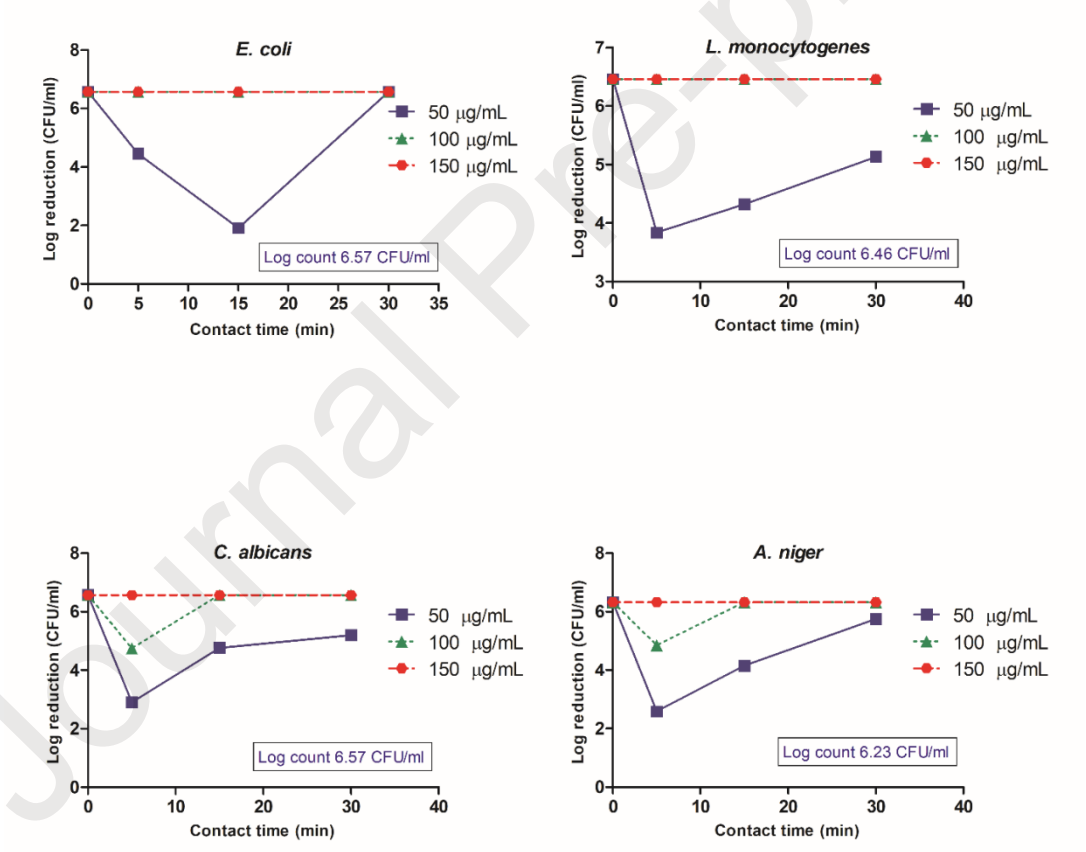



**Fig 10 MIC** values of compound **11** toward evaluated pathogenic organisms at distinct time intervals.

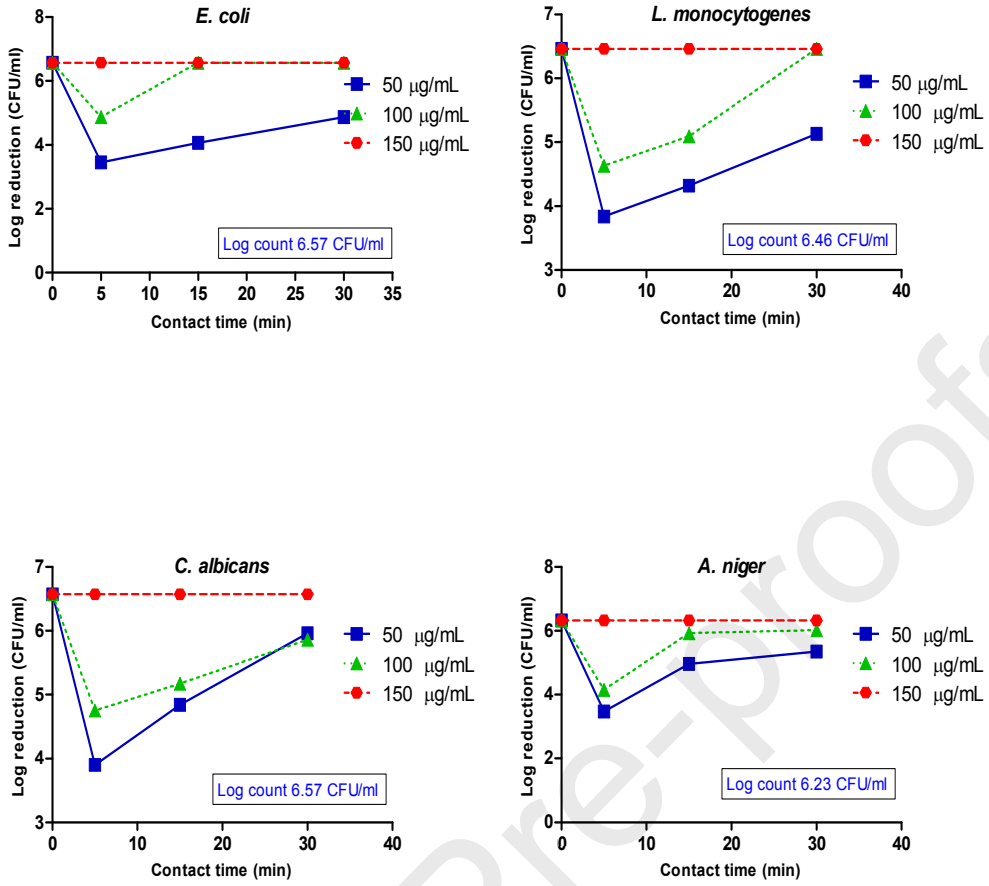



**Fig 11** MIC values of compound **25** toward evaluated pathogenic organisms at distinct time intervals.

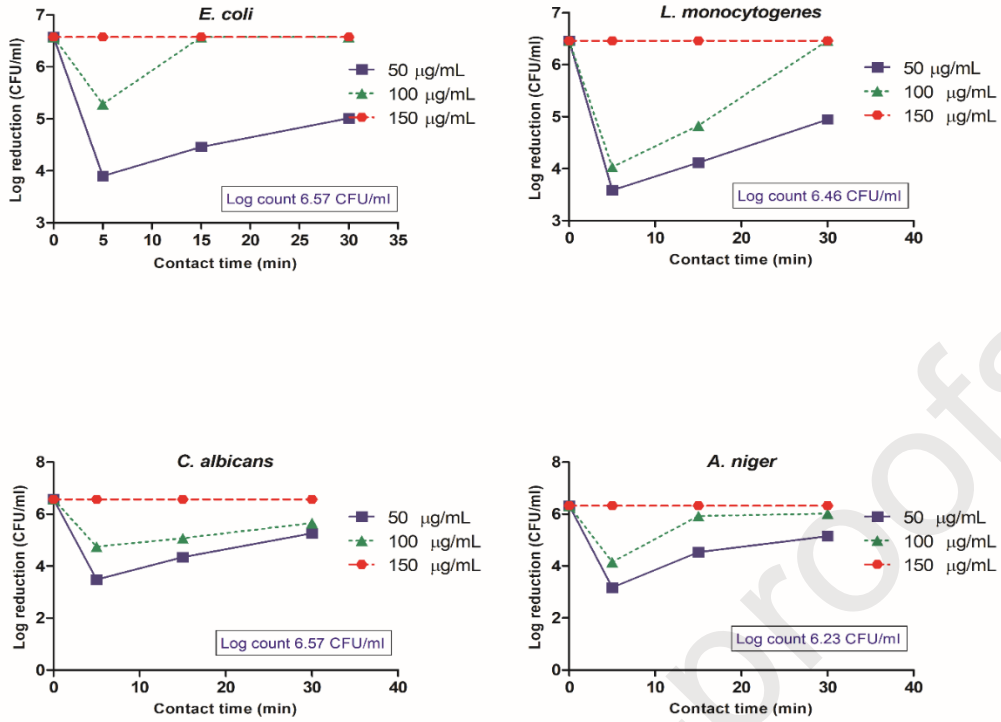



**Fig 12** MIC values of compound **27** toward evaluated pathogenic organisms at distinct time intervals.

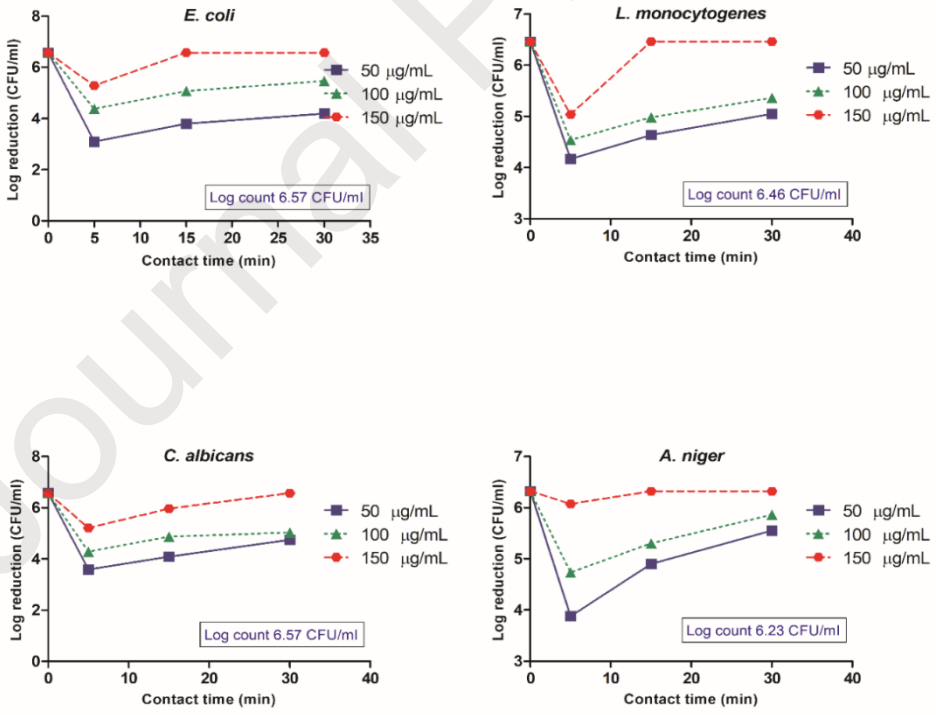



**Fig 13** MIC values of compound **28** toward evaluated pathogenic organisms at distinct time intervals.

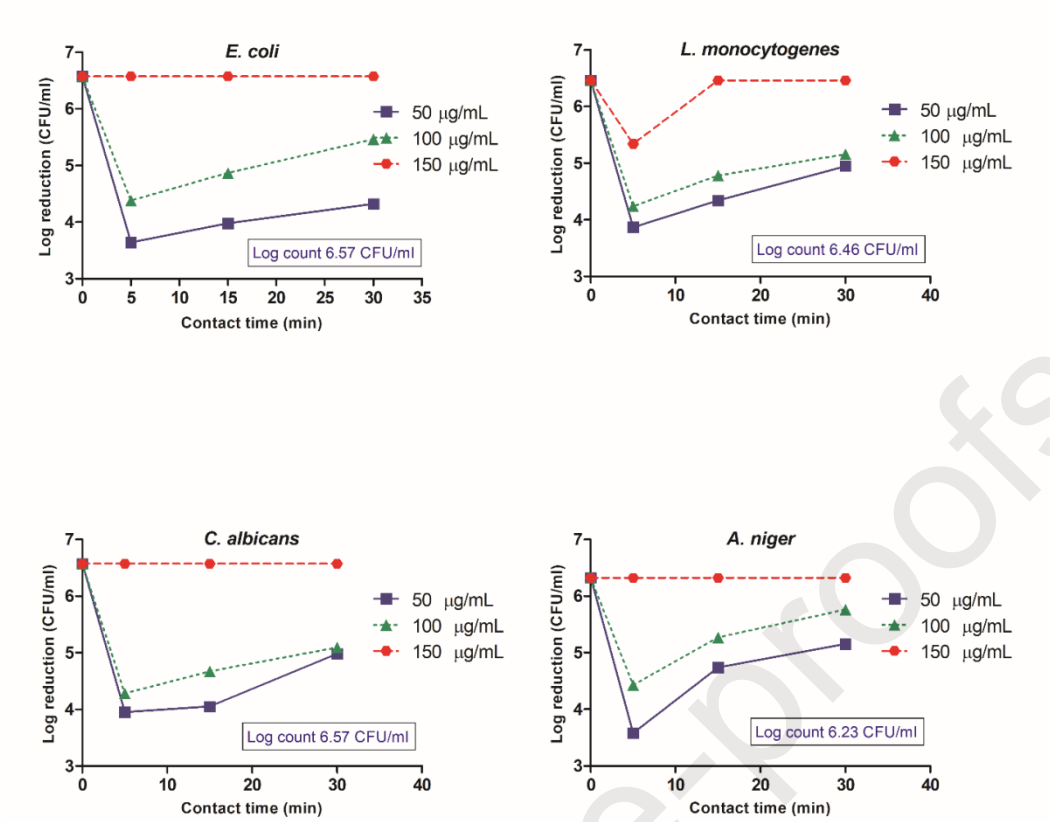
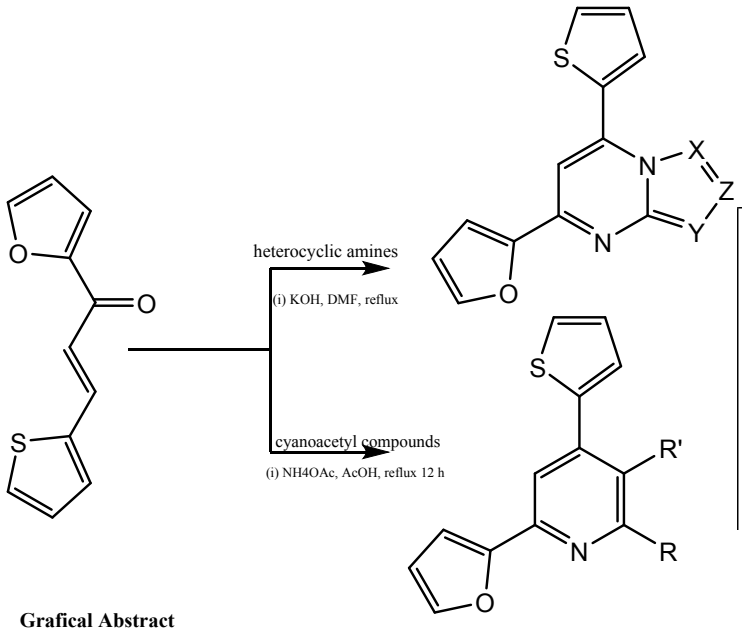
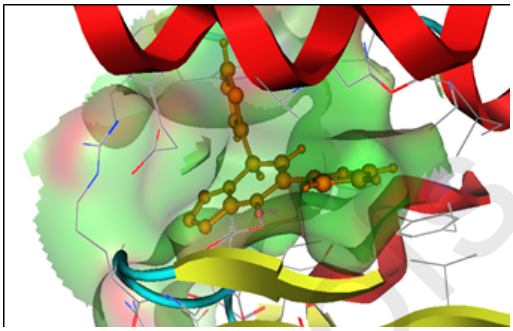



**Fig 14** MIC values of compound **31** toward evaluated pathogenic organisms at distinct time intervals.




**Grafical Abstract**



Docking of compound **11**

**Highlight**

1- Synthesis of new heterocyclic ring systems incorporation pyrimidine and pyridine moieties starting from heterochalcone.
2- Antimicrobial investigations of the designated compounds.
3- Antimicrobial tests proved that compound **11** unveiled a greater antimicrobial activity than other designed compounds.
4- Docking examination was approved for the goal compounds into DNA grase B chain and exhibiting binding energy extended from-13.05 to -20.48 kJ $mol^{-1}$.

**Conflict of interest**

The authors declare no conflict of interest.