Egypt. J. Chem. **Vol. 65,** No. 2 pp. 399 - 411 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Convenient Synthesis and Molecular Docking of Novel Pyrido [2,3-*d*] pyrimidines as Potent Antimicrobial Candidates

Alaadin E. Sarhan[1*],Hanaa S. Mohamed[1],Abdulrahman M. Saleh[2], Zeinab M. Nofal[1]

[1] *Therapeutic Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Center,33 El-Behouth St, Dokki, Giza, Egypt.*

[2] *Pharmaceutical Medicinal Chemistry and Drug Design Department, Faculty of Pharmacy(boys), Al-Azhar University,Cairo, Egypt.*

Abstract

New 3-(3,4-dimethoxyphenyl)-1-(thiophen-2-yl) prop-2-en-1-one has been designed as a starting compound to synthesis a novel series of substituted pyrido[2,3-*d*]pyrimidine system incorporated to different Schiff's bases and enamine derivatives as potent antimicrobial compounds**.** Novel synthesized compounds were evaluated for their *in-vitro* antimicrobial potency against different Gram positive and Gram negative bacteria; namely *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella* and *Candida albicans* strain; where they reveal high effectuality at low concentration compared to Trimethoprim. Docking studies declared that new pyrido [2,3-d] pyrimidines completely occupied active pocket of Biotin Carboxylase of both bacterial types and fungal strains acting as selective Fatty Acid Synthesase type II inhibitors. In addition, structure-activity relationship was discussed.

Keywords: Pyrido [2,3-d] pyrimidine,Antimicrobial,Biotin Carboxylase, Molecular Docking

Introduction

Novel compounds with antimicrobial properties is a central research objective today [1]. However, Bacterial infections are the major cause of some high mortality rate diseases. On the other hand, fungi infect many people worldwide every year, most of them cause relatively minor infections but kill at least as many people as tuberculosis or malaria [2]. In addition, microbial infections are becoming more resistant to antibiotics due to years of their overuse and/or misuse, which might lead to a potential global health disaster [3]. This makes the design and development of new antimicrobial candidates with novel chemical structures and/or with different modes of action rather than analogues of the existing ones are necessary for clinical needs [4–5]. In bacteria, the metabolic enzyme is composed of three distinct protein components: biotin carboxylase, biotin carboxyl carrier and carboxyl transferase. So, the antimicrobial drugs that targeting fatty acid synthesis are attractive targets [6]. However, targeting Biotin Carboxylase; that is one portion of the Acetyl-CoA Carboxylase (ACCase); which is responsible for the first step of fatty acid biosynthesis making it a promising broad-spectrum target. Accordingly, the bacterial fatty acid biosynthesis pathway has got much interest for the discovery of novel classes of antibacterial agents targeting fatty acid synthase pathways [7,8], Fig (1).

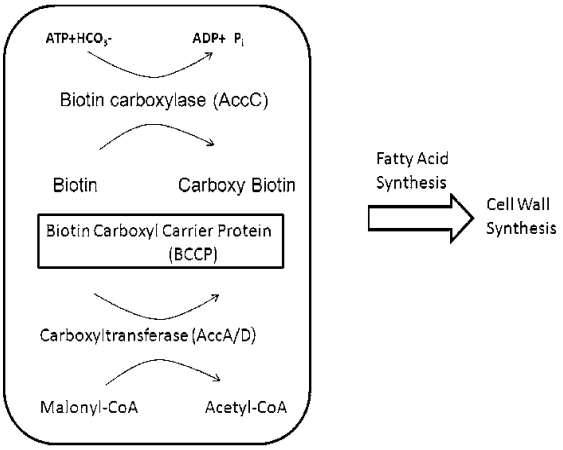


Fig. 1 Biosynthesis Pathway of FAS System

Pyridopyrimidine derivatives (A-D), Fig.(2), [9,10]

*Corresponding author e-mail: dralaa1968@gmail.com.; (Alaadin Sarhan).
Receive Date: 25 July 2021, Revise Date: 08 August 2021, Accept Date: 10 August 2021
DOI: 10.21608/EJCHEM.2021.87454.4223

displayed an excellent selectivity for Biotin Carboxylase especially compounds (A,B), However, compound (A) is lead targeting (ATP) binding sites of Biotin Carboxylase because of its structural similarity to known human targets that lead to excellent selectivity for bacterial target over a range of eukaryotic protein kinases.

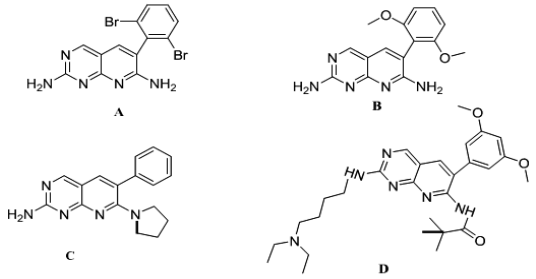


Fig.2 Pyridopyrimidines libraries targeting "Eukaryotic Tyrosine Protein Kinases

Based upon these findings, new pyridopyrimidine analogs were designed, synthesized and screened for their antibacterial, and antifungal activity in-vitro. In addition, molecular docking studies were employed to confirm the interaction behaviors between the prepared compounds and Biotin Carboxylase.

2. Experimental

2.1. Materials

All chemicals were provided by Aldrich companies and were used without additional purifications.

2.2. Chemistry

Elemental microanalyses were carried out at "Micro analytical Unit, Cairo University, using Vario Elementar and were found within ± 0.4% of the theoretical values". All melting points were taken in open capillary tubes using "Electro thermal apparatus 9100" and uncorrected. FT-IR spectra were recorded with a Perkin-Elmer Frontier 400 MHz. 1HNMR and 13C spectra were recorded on a "Bruker Advance TM 500" spectrometer as solutions in" DMSO- d6" at room temperature or "$CDCl_3$". Chemical shifts were expressed in δ (ppm) downfield from TMS as an internal standard and relative to the trace resonance of protonated dimethyl sulfoxide (δ 2.50 ppm),(δ 39.51 ppm) or $CDCl_3$ (δ 7.28 ppm), (δ 77.28 ppm). The mass spectra were measured with "GC Finnegan MAT SSQ-7000 mass spectrometer". Reaction progress was monitored by TLC on "silica gel pre-coated aluminum sheets [Type 60, F 254, Merck, Darmstadt, Germany] and the spots were detected by exposure to UV lamp at λ_{254} nm. The chemical names given for the prepared compounds are according to IUPAC system. The reported yields are based upon pure materials isolated.

3-(3,4-dimethoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (1)

2-acetyl thiophene (0.01 mol) was dissolved in 10 ml of freshly prepared sodium ethoxide, stirring this mixture while adding in portion wise 1.66 g (0.01) mol. of 3,4-dimethoxy benzaldehyde, and keep stirring at room temperature for further 3 h., dry it under reduced pressure, then washed with 10 ml cold water; followed by 10 ml dry ether to obtain chalcone **1**.

Pale yellow crystal (AcOH), m.p.86ºC. Yield (92%); IR (KBr) νmax 1723, 1269, 1205 cm^{-1}; 1HNMR (500 MHz, DMSO-d_6): δ = 3.80 (s, 3H, OCH_3), 3.5 (s, 3H, OCH_3), 6.6-6.9 (dd; 2H, *J* = 8.69 Hz, *J* = 8.2 Hz, thiophene), 7.38 (d, 1H, *J* = 9.64 Hz, H_α), 7.44-7.55 (m, 4H, Ph-H, thiophene H), 7.76 (d, 1H, *J* = 9.63 Hz, H_β); 13C NMR (100 MHz, DMSO-d_6) δ 55.88, 112.96, 115.16, 121.26, 127.10, 127.90, 128.15, 130.72, 134.04, 139.00, 145.15, 164.19, 188.68. EIMS *m/z* 274.33 [M]+ (47.6), 163.08 (88); Anal. Calcd. for $C_{15}H_{14}O_3S$: C, 65.67; H, 5.14; S, 11.69. Found: C, 65.28; H,5.11; S, 11.72.

6-amino-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (2)

1.2 ml (0.01 mol) equivalent to 1.13 g of ethylcyanoacetate was added to continually stirred sodium ethoxide solution; (1.15 g sodium dissolved in 25 ml absolute ethanol); precipitate appears almost immediately; while stirring continue for 15 min, 0.8 g (0.01 M) of thiourea was added portion wise under continuous stirring at room temperature, allow to stir for further 1 h, then mixture allow to reflux for 2h. After cooling down to room temperature, the precipitate was filtered, washed with ethanol and dissolved in warm 5 % KOH. This was treated with glacial acetic acid and formed precipitate was filtered, washed with small amount of AcOH and with a small amount of water and air dried. White solid, m.p.> 300 ºC. Yield (85 %) [**8**].

5-(3,4-dimethoxyphenyl)-7-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidin- 4(1H)-one (3)

2.74 g (0.01 mol) of compound **1** in 15 ml dry DMF, was added 1.43 g (0.01 mol) of compound **2**, reflux for 18 h, precipitated product was filtered of, washed with dry ether.

Pale yellow crystals (MeOH-H_2O), m.p. 261-264 ºC. Yield (72%); IR (KBr) νmax 1680 2115, 3120 cm^{-1}; 1HNMR (500 MHz, DMSO-d_6) δ 3.6 (s, 3H, OCH_3), 3.7 (s, 3H, OCH_3), 6.9-7.9 (m, aromatic, 6H), 8.6 (s, 1H, pyridine-H). 13CNMR (100MHz, DMSO-d_6) δ 56.1, 109.5, 110, 112.5, 120.28, 137, 150, 153, 162, 175; EIMS *m/z* 397.06 $[M]^+$ (27), 260.00 (100); Anal.

Calcd. for $C_{19}H_{15}N_3O_3S_2$: C, 57.42; H, 3.80; N,10.57; S,16.13. Found: C, 57.40; H, 3.77; N, 10.54; S, 16.12

5-(3,4-dimethoxyphenyl)-2-hydrazinyl-7-(thiophen-2-yl)pyrido[2,3-d]pyrimidin-4(3H)-one (4)

3.97g (0.01 mol) pyridopyrimidine **3**, was suspended in 15 ml hydrazine hydrate in closed reflux system fitted with calcium carbonate tube, gently refluxed for 4hrs, allow to cool, precipitate was collected and crystallized.

Brown powder (DMF/EtOH, 1:1), m.p. 268-271ºC. Yield 75% ; IR (KBr)νmax 3344, 3289, 3222, 1677cm^{-1}; 1HNMR (500MHz, DMSO-d_6) δ 3.60 (s, 3H, OCH_3), 3.7 (s, 3H, OCH_3), 5.40 (br s, 2H, NH_2; D_2O exchangeable), 6.14 (br s, 1H, NH; D_2O exchangeable), 6.95–6.99 (m, 3H, thiophene), 7.17–7.34 (m, 3H Ar-H and pyridine-H); 13C NMR (100 MHz, DMSO- d_6) δ 56.2, 112.1, 114.5, 116.5, 120.1, 122.0, 138.1, 152.3, 154.0, 162.20. EIMS m/z 395.11 $[M]^+$ (77). Anal. Calcd. for $C_{19}H_{17}N_5O_3S$: C, 57.71; H, 4.33; N, 17.71; S, 8.11. Found: C, 57.50; H, 4.31; N, 17.70; S, 8.12.

2-(2-(4-substituted-benzylidene)hydrazinyl)-5-(3,4-dimethoxyphenyl)-7-(thiophen-2-yl) pyrido [2,3-d]pyrimidin-4(3H)-one (5a-f)

General procedure: (0.01 mol) of hydrazinyl compound 4, (0.01 mol) of aromatic aldehyde (4-chloro-,4-bromo-,4-fluoro-,4-amino-,4-nitro- and 4-methoxybenzaldehyde) in glacial acetic acid was refluxed for 2hrs. cool, poured over ice water to precipitate the corresponding Schiff's base derivatives ; dried and crystallized.

2-**(2-(4-chloro-benzylidene)hydrazinyl)-5-(3,4-dimethoxyphenyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (5a)**

Gray powder (AcOH:H_2O, 1:1), m.p. 298ºC.Yield; 82; IR (KBr) νmax 3310, 3282, 1692 cm-1; 1HNMR (500 MHz, DMSO-d_6) δ 3.79 (s,3H, -OCH_3), 3.82 (s,3H, -OCH3), 6.96 (s,1H,Ar-H),7.18-7.27(m, 3H, 2Ar-H+Thiophene-H), 7.45–7.50 (m, 3H, 2Ar-H + Pyridine-H),7.76(d, 1H, J=4.9 Hz, thiophene-H), 7.90 (d, 2H, J=8.7 Hz, Ar-H), 8.1 (d, 1H, J=3.2 Hz, Thiophene-H), 8.6 (s, 1H, N=CH), 11.27 (s, 1H, NH, D_2O exchangeable) 11.70 (s, 1H, NH D_2O exchangeable); 13C NMR (100 MHz, DMSO-d_6) δ 55.82, 114.55, 114.89, 116.15, 127.55, 128.46, 129.29, 129.75, 131.17, 131.28, 136.46, 144.45, 151.70, 152.69, 155.92, 161.14, 165.18. EIMS *m/z* 517.10 [M]+ (92), 518.1(13), 517.1(9); Anal. Calcd. for $C_{26}H_{20}ClN_5O_3S$: C, 60.29; H, 3.89; Cl,6.84; N,13.52; S,6.19. Found: C, 60.26; H,3.85; Cl,6.81; N,13.50; S,6.14.

2-(2-(4-bromo-benzylidene)hydrazinyl)-5-(3,4-dimethoxyphenyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (5b)

Dark red crystals (AcOH:H_2O,1:1) ,m.p. 271ºC.Yield 80%; IR (KBr)νmax 3315,3300,1687 cm^{-1}; 1HNMR (500MHz, DMSO-d6) δ 3.6 (s,6H,2-OCH3), 6.8 (s, 1H,Ar-H),7.15-7.22(m, 3H, 2Ar-H+Thiophene-H), 7.55–7.60 (m, 3H, 2Ar-H + Pyridine-H),7.75 (d,1H, J = 4.9 Hz, Thiophene-H),.90 (dd, 2H, J = 8.7, J = 8.2 Hz, Ar-H), 8.2 (d, 1H, J = 3.2Hz , Thiophene-H), 8.2 (s, 1H, N=CH), 11.27 (s,1H, NH, D_2O exchangeable) and 11.70 (s, 1H, NH D_2O exchangeable); 13C NMR (100 MHz, DMSO-d_6) δ 54.45, 113.18, 113.52, 114.78, 126.18, 128.46, 129.29, 129.75, 131.17, 131.28, 136.46,144.45, 151.70, 151.49, 154.62, 160.14, 162.22; EIMS *m/z* 561.04 [M]+ (37), 154.7 (89); Anal. Calcd. for $C_{26}H_{20}BrN_5O_3S$: C,55.52; H, 3.58; N, 12.45; S, 5.70. Found: C, 55.52; H,3.55; N,12.41; S,6.14.

5-(3,4-dimethoxyphenyl)-2-(2-(4-fluoro-benzylidene)hydrazinyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (5c)

Buff powder (AcOH:H_2O, 1:1), m.p.197 ºC. Yield (78%); 1HNMR (500MHz, DMSO-d_6) δ 3.46 (s, 6H, 2-OCH_3), 6.6 (s, 1H,Ar-H),7.22-7.45(m, 3H, 2Ar-H, Thiophene-H), 7.55–7.65 (m, 3H, 2Ar-H, Pyridine-H),7.80 (d,1H, J=4.9 Hz, Thiophene-H), 7.85 (dd,2H, *J* = 8.7; *J* = 8.2Hz, Ar-H), 8.1 (d,1H, *J* = 3.2 Hz, Thiophene-H), 8.6 (s, 1H, N=CH), 11.43 (s, 1H, NH, D_2O exchangeable) and 11.76 (s, 1H, NH D_2O exchangeable); 13C NMR (100 MHz, DMSO-d_6) δ 55.87, 112.46, 114.63, 114.91, 116.28, 121.18, 128.60, 129.33, 130.13, 131.22, 131.33, 136.33, 151.76, 152.74, 160.09, 160.96, 161.48, 164.19; EIMS *m/z* 501.13 $[M]^+$ (22),137.03(97); Anal. Calcd. for $C_{26}H_{20}FN_5O_3S$: C, 62.27; H, 3.79; N, 13.96; S, 6.39.Found: C, 62.25; H, 3.73; N, 13.92; S, 6.32.

2-(2-(4-amino-benzylidene)hydrazinyl)-5-(3,4-dimethoxyphenyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (5d)

Gray crystals (AcOH:H_2O,1:1), m.p. 213-215 ºC. Yield (69%); IR (KBr) ν_{max} 3445, 3215, 1682 cm^{-1}; 1HNMR (500MHz, DMSO-d_6) δ 3.35(s,3H,-OCH_3), 3.43 (s, 3H, OCH_3), 6.85 (s, 1H,Ar-H),7.40-7.62(m, 3H, 2Ar-H, Thiophene-H), 7.65–7.70 (m, 3H, 2Ar-H + Pyridine-H),7.76 (d, 1H, J = 4.9 Hz, Thiophene-H),7.90 (dd, 2H, J=8.7Hz, J = 8.3, Ar-H), 8.3 (d,1H, J=3.2Hz,Thiophene-H), 8.4 (s, 1H, N=CH), 11.70 (s, 1H, NH, D_2O exchangeable),11.85 (s, 1H, NH D_2O exchangeable), 12.1 (br-s, 2H, -NH_2); 13C NMR (100 MHz, DMSO-d_6) δ 55.76, 113.41, 124.73, 125.58, 127.94,129.01, 129.33, 130.75, 131.00, 131.27, 131.91, 134.95, 138.88, 153.68, 159.81. EIMS *m/z* 496.15 $[M]^+$ (13), 364.05 (99); Anal. Calcd. for

$C_{26}H_{22}N_6O_3S$: C, 62.64; H, 4.45; N,16.86; S,6.43. Found: C, 62.61; H, 4.42; N, 16.83; S, 6.40.

5-(3,4-dimethoxyphenyl)-2-(2-(4-nitro-benzylidene)hydrazinyl)-7-(thiophen-2-yl) pyrido[2,3-d]pyrimidin-4(3H)-one (5e)

White crystals (AcOH:H_2O, 1:1),m.p. 228 ^oC. Yield (81%). IR (KBr) ν_{max} 3150, 2875, 1675, 1350,1541 cm^{-1}; ^{1}HNMR (500MHz, DMSO-I) δ 3.35 (s, 6H, 2-OCH_3), 6.5 (s, 1H, Ar-H), 7.20-7.40 (m, 3H, 2Ar-H, Thiophene-H), 7.55–7.65 (m, 3H, 2Ar-H + Pyridine-H), 7.75 (d, 1H, *J* = 4.9Hz, Thiophene-H),7.80 (dd, 2H, *J* =8.7Hz; *J* = 8.3Hz Ar-H), 8.4 (d,1H, J=3.2 Hz, thiophene-H), 8.3 (s, 1H, N=CH), 11.20 (s, 1H, NH, D_2O exchangeable), 11.46 (s, 1H, NH D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO- d_6) δ 114.63, 114.91, 125.57, 126.92, 128.60, 128.98, 129.33, 138.33, 146.45, 154.02, 162.06, 164.27, 165.38; EIMS *m/*z 528.12 [M^+] (34), 137.40 (21), 222.10 (89); Anal. Calcd. for $C_{26}H_{20}N_6O_5S$: C, 59.08; H, 3.81; N, 15.90; S, 6.07. Found: C, 59.04; H, 3.77; N, 15.85; S, 6.04.

2-(2-(4-methoxy-benzylidene)hydrazinyl)-5-(3,4-dimethoxyphenyl)-7-(thiophen-2-yl)pyrido[2,3-d]pyrimidin-4(3H)-one (5f)

White crystals (AcOH:H_2O, 1:1), m.p. >300°C.Yield (55%); IR (KBr) ν_{max}3379, 3368,1677cm^{-1};^{1}HNMR (500MHz, DMSO-d_6) δ 3.45(s,3H,-OCH_3), 3.81 (s, 6H, 2OCH_3),) 6.96 (m, 4H, Ar-H), 7.19–7.22 (m, 2H, Thiophene-H, Pyridine-H), 7.38 (m,4H, Ar-H), 7.57 (s, 1H, N=CH), 7.81 (d, 1H, J = 5.6 Hz, Thiophene-H), 8.06 (d,1H J = 3.8 , Thiophene, 2.31(s, 1H, NH; D_2O exchangeable), 12.99 (s, 1H, NH; D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO- d_6) δ55.22, 107.41, 112.89, 117.67, 128.90, 129.01, 130.24, 130.35, 130.85, 131.68, 142.42, 152.77, 153.32, 154.78, 158.62, 159.54, 175.29; EIMS *m/*z 513.15 [M^+] (100). Anal. Calcd. for $C_{27}H_{23}N_5O_4S$: C, 63.15; H, 4.51; N, 13.64; S, 6.24.Found: C, 63.13; H,4.49; N, 13.62; S, 6.23.

N'-(5-(3,4-dimethoxyphenyl)-4-oxo-7-(thiophen-2-yl)-3,4-dihydropyrido[2,3-d]pyrimidin-2- yl)-N,N-imethylformohydrazonamide. (6).

3.9 gm (0.01) mol. of compound 4,was added 3 ml of dimethylformamide dimethylacetal (DMF- DMA) . Allowed to fuse gently for 10 min.; washed and triturated with dry ether to obtain formohydrazo-namide. Yellow crystals,(ether-washed),m.p. 276 ^oC. Yield (92%); IR (KBr)ν_{max} 3329, 3222, 1677 cm^{-1}; ^{1}HNMR (500MHz, DMSO-d_6) δ 2.9(s,6H,2-CH_3), 3.70(s, 3H, -OCH_3), 3.6 (s, 3H, -OCH_3), 6.25–6.90 (m, 3H, thiophene) 7.15–7.30 (m, 3H Ar-H and pyridine-H),7.9(s,1H,N=CH), 10.2 (br s, 1H, NH; D_2O exchangeable), 11.7(s, 1H, NH; D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ 37.6, 56.1, 112.0, 113.1, 118.0, 121.0, 122.6, 127.6, 128.6, 138.0, 142, 146.0, 153.3, 156.0, 162.3; EIMS *m/*z 450.15 [M^+] (100); Anal. Calcd.for $C_{22}H_{22}N_6O_3S$: C,58.65; H,4.92; N,18.65;S,7.12 Found: C,58.61; H,4.91; N,18.62; S, 7.10.

N'-(5-(3,4-dimethoxyphenyl)-4-oxo-7-(thiophen-2-yl)-3,4-dihydropyrido[2,3-d]pyrimidin-2- yl)-N-substituted-formohydrazonamide (7a-e).

General procedure: In round bottomed flask,(0.01) mol.of compound 6 was mixed well with (0.01 mol) appropriate amine; namely, cyclohexylamine, phenyl amine, 4-aminobenzoic acid, 2-minothiazole and 4-aminobenzenesulfonamid.This mixture allowed to fuse for 20 min. at 100 ^oC, washed and triturated to obtain N-substituted formohyrazonamide.

N-cyclohexyl-N'-(5-(3,4-dimethoxyphenyl)-4-oxo-7-(thiophen-2-yl)-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)formohydrazonamide (7a)

Gray crystals (ethanol),m.p. 198 ^oC. Yield (86%);IR (KBr)ν_{max} 3215, 1682 cm^{-1}; ^{1}HNMR (500MHz, DMSO-d_6) δ1.2(dd,4H J=4.9Hz,J=5.2,cyclohexan), 1.5-1.75(m,4H,cyclohexane) 2.8(m,2H,cyclohexane), 3.60(s, 3H, -OCH3),3.65(s, 3H, -OCH3), 6.42–6.80 (m, 3H, thiophene) 7.15–7.30 (m, 3H, Ar-H and pyridine-H),7.8(s,1H,N=CH), 8.6;9.8 ;10.2 (br s, 3H, NH; D_2O exchangeable);^{13}C NMR (100 MHz, DMSO-d6) δ 24.5, 25.5,33.4, 56.8, 111.2, 120.9, 127.6, 128.6, 138.1,142. 146.0, 153.3, 5,155.8,165.6; EIMS *m/*z 504.19[M^+] (43); Anal. Calcd. for $C_{26}H_{28}N_6O_3S$: C,61.89;H,5.59;N,16.65; S,6.35. Found: C, 61.81; H, 5.55; N,16.62; S, 6.28.

N'-(5-(3,4-dimethoxyphenyl)-4-oxo-7-(thiophen-2-yl)-3,4-dihydropyrido[2,3-d]pyrimidin-2-yl)-N-phenylformohydrazonamide(7b).

Dark yellow crystals (AcOH), m.p.201^oC.Yield (66%); ^{1}HNMR (500MHz, DMSO-d_6) δ 3.81(s, 6H, 2-OCH_3), 6.95–6.98 (m, 2H, Ar-H), 7.18–7.20 (m, 3H, Ar-H), 7.29 (t, 1H, Thiophene-H), 7.37–7.40 (m, 2H, Ar-H), 7.44 (s, 1H, Pyridine-H), 7.71 (d, 1H, J=7.9 Hz, Thiophene-H), 7.76 (dd,1H J=6.6, 1.5 Hz,Ar-H),8.00 (d,H, J= 3.8 Hz, Thiophene-H), 8.11 (s,1H,N=CH), 9.8 (s, 1H, NH; D_2O exchangeable), 11.70 (brs, 2H, 2-NH; D_2O exchangeable), ^{13}C NMR (100 MHz, DMSO-d_6) δ 55.76, 113.41, 124.73, 125.58, 127.94, 129.01, 129.33, 130.75, 131.00, 131.27, 131.91, 134.95, 138.88, 145,153.68, 159.81, EIMS *m/*z 498.15 [M^+] (28),134.07(99); Anal. Calcd. for $C_{26}H_{22}N_6O_3S$: C, 62.64; H, 4.45; N, 16.86; S, 6.43.Found C, 62.61; H, 4.42; N, 16.82; S, 6.39.

4-(N'-(5-(3,4-dimethoxyphenyl)-4-oxo-7-(thiophen-2-yl)-3,4-dihydropyrido[2,3- d]pyrimidin-2-yl)formohydrazonamido)benzoic acid (7c).

Pale yellow crystals (AcOH), m.p.212 ^{o}C ,Yield(78%); IR (KBr) νmax 3447(-OH), 3328 , 3198,1718 (C=O),1682 cm^{-1}; ^{1}HNMR (500MHz, DMSO-d_6) δ 3.75 (s, 6H, 2-OCH3), 6.92–6.95 (m, 2H, Ar-H), 7.12–7.18 (m, 3H, Ar-H), 7.32 (t, 1H, Thiophene-H), 7.32–7.40 (m, 2H, Ar-H), 7.44 (s, 1H, Pyridine-H), 7.70 (d,1H,J=7.9 Hz, Thiophene-H), 7.75 (d,1H, J=6.6 Hz, Ar-H), 8.2 (d,1H, J=3.81Hz,Thiophene-H), 8.12 (s, 1H,N=CH), 9.5 (s,1H, NH; D_2O exchangeable), 11.60 (brs, 2H, 2NH; D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ 54.16, 112.41, 126.13, 129.38, 132.94, 134.01, 134.33, 143.75, 146.00, 150.25,153.68, 158.21, 170.01; EIMS m/z 542.14 [M+] (34),187.06 (100); Anal. Calcd. for $C_{27}H_{22}N_6O_5S$: Calcd. C, 59.77; H, 4.09; N, 15.49; S, 5.91.Found C, 59.71; H, 4.12; N, 15.42; S, 5.89.

N'-(5-(3,4-dimethoxyphenyl)-4-oxo-7-(thiophen-2-yl)-3,4-dihydropyrido[2,3-d]pyrimidin-2- yl)-N-(thiazol-2-yl)formohydrazonamide (7d)

Brown crystals (AcOH),m.p. 286 oC. Yield (81%);1HNMR (500MHz, DMSO-d_6) δ 3.82 (s,3H, OCH_3),3.9(s,3H,-OCH_3),6.2-6.6 (dd, 2H, J = 7.4Hz; J = 8.1Hz, Thiazole),6.9-7.2(m,3H,2-Ar-H; Thiophene-H),7.4(d,1H,J=7.3Hz,,Ar-H),7.6-7.85 (dd, 2H, J = 6.8Hz, 7.2Hz, Thiophene),7.92 (s,1H, N=CH) 8.2 (s, 1H, pyridine), 10.2,10.7,11.2 (3s, 3H, -NH, D_2O exchangeable), ^{13}C NMR (100 MHz, DMSO-d_6) δ 56.1, 111.0, 113.5, 122.0, 128.0, 132.0 , 142.4, 146.0, 150.0, 153.3, 154, 162.0, 163.7; EIMS *m/z* 505.09 [M^{+}] (17), 141.01 (97); Anal. Calcd. For $C_{23}H_{19}N_7O_3S_2$: C, 54.64; H, 3.79; N,19.39; S, 12.68. Found C, 54.61; H, 3.75; N, 19.32; S, 12.69.

N'-(5-(3,4-dimethoxyphenyl)-4-oxo-7-(thiophen-2-yl)-3,4-dihydropyrido[2,3-d]pyrimidin-2- yl)-N-(4-sulfamoylphenyl)formohydrazonamide (7e).

Yellow crystals (AcOH/Water), m.p. 257-259 ^{o}C. Yield (73%); 1HNMR (500MHz, DMSO-d_6) δ3.75 (s, 6H, 2-OCH_3), 6.95–6.98 (m, 2H, Ar-H), 7.18–7.20 (m, 2H, Ar-H), 7.29 (t,1H, Thiophene-H), 7.37–7.40 (m, 2H, Ar-H), 7.44 (s, 1H, Pyridine-H), 7.71 (d, 1H, J=7.9 Hz, Thiophene-H), 7.76 (dd,1H, J = 6.6, 1.5 Hz, Ar-H),7.9 (s, 2H, -NH_2, D_2O exchangeable), 8.1 (d, 1H J=3.8, Hz, Thiophene-H), 8.2 (s, 1H, N=CH), 8.6,9.7, 10.70 (3s, 3H, NH; exchangeable).

2.3. Biological Activity

Targeted pathogenic microorganisms were gained from the American kind culture collection (ATCC; Rockville, MD, USA). The tested organisms were *Staphylococcus aurous* ATCC-47077 (St.), *Bacillus cereus* ATCC-12228 (B.C.), *Escherichia coli* ATCC-25922 (E.C.), *Salmonella typhi* ATCC-15566 (Salm.) and *Candida albicans* ATCC-10231 (C. Alb.).

2.3.1. Antimicrobial assay

Procedure for Agar well diffusion was applied for the purpose of studying antimicrobial activities of our samples under investigation were carried out according to the method described, in which stock cultures of pathogens used were kept on nutrient agar slants at 4 °C. [12,13] .Reference antimicrobial drug Trimethoprim were estimated for its antibacterial and antifungal potency and compared with the tested samples. Seventy micro-liters of bacterial and yeast cells (106 CFU/mL) were spread on plates of nutrient agar media. The wells (6 mm diameter) were excavated on the injected agar plates, then 100 µl of the samples were suspended in DMSO that added up to the wells. The reference antibiotics disks (10 and 30 µg/disk of Trimethoprim) were potted onto surface of agar inoculated plates. The plates were kept at 4 °C for 2h before incubation to permit diffusion to occur. The plates were kept at 37 ℃ for 24 hr. except yeast strain that were incubated at 28 °C for 24hr then followed by measure of the diameter of the inhibition zone (mm), and this was replicate for five times and theaverage was taken [14-17] .

2.3.2. Minimum Inhibitory Concentration (MIC)

"MIC protocols are usually used to evaluate the antimicrobial efficacy of various compounds by measuring the effect of decreasing concentrations of antimicrobial agents over a defined time in terms of microbial population growth inhibition."

Accordingly, our new synthesized compounds with a little modulation for previous reported procedure [16,17] had taken place for evaluating their MIC activity. In summarized, serial dilutions were prepared for the examined materials dissolved in DMSO. 150µL of double strength Mueller Hinton Broth (MH-Broth) medium were loaded in each well of the 98 change number of well micro liter plate followed up by 150µL of the 2-fold appropriate concentration and mixed well to gain the final concentration. After 24h both cultures of the screened bacterialand yeast strain spread as an inoculums of 5 % (V/V) (OD= 0.5 McFarland standard) was inoculated into the respective wells. For the positive growth control, the same inoculums size of each test strain was inoculated in wells that didn't including any of the screened materials. DMSO solution was evaluated as negative control. The plates were statically incubated for 24h at 35^{o}C. We added (30µL) of prepared solution (0.18 %) to each well to work as an electron acceptor aiming at inhibiting bacterial growth for the ease of visibility as a dark blue well, while, presence of growth was noticed by existence of red, pink or purple colour.

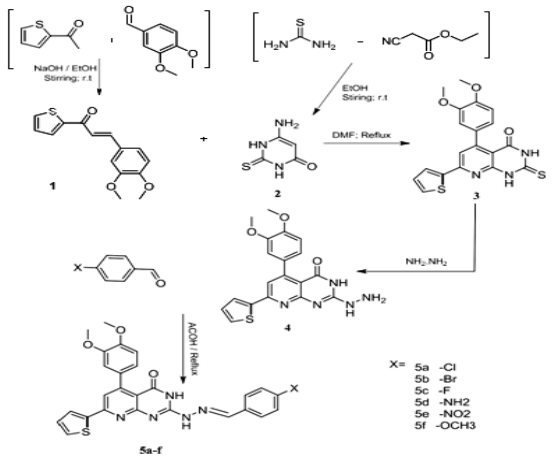
2.4. Molecular Docking Studies

Receptor was prepared for virtual screening, inside the pockets of Fungal and bacterial biotin carboxylase by using MOE 14.0901 Software. Target site selection has been done by protein data bank [18]. The binding sites were generated from the co-crystallized ligand, within crystal protein (PDB codes: 3jzf - 4dq2 -1w96). Protein energy was minimized by applying MMFF94 force fields. 2D structures of tested compounds were drawn using Chem-Bio Draw Ultra16.0 andsaved in MDL-SD file format , From MOE 14.0901 Software, the saved file was opened, 3D structures were protonated and energy Was minimized by applying (0.05 RMSD) k.cal/mol. of MMFF94 force field. Then, minimized structures were prepared for docking using prepared ligand protocol. Molecular Docking process was carried out using CDOCKER protocol, where, the receptor was held rigid while the ligands allowed to be flexible during the refinement. Each molecule was allowed to produce ten different interactions poses with the protein. Accordingly, docking scores (CDOCKER interaction energy) of the best-fitted poses with the active site at Biotin carboxylase was recorded and 3D view was generated by Discovery Studio 2019 Client software.

3. Results and discussion

3.1. Chemistry

Our target starting material 5-(3,4-dimethoxy phenyl)-7-(thiophen-2-yl)-2-thioxo-2,3-dihydro pyrido[2,3-*d*]pyrimidin-4(1H)-one **3** has been synthesized by direct condensation of α,β-unsaturated ketone thiophene derivative **1** with 6-amino-2-thiouracil **2** via electrophilic addition mechanism in dimethylformamide. Compound **1** was easily prepared via stirring at room temperature, while thiouracil **2** obtained in 85% yield from direct addition of ethylcyanoacetate to the alkaline solution of thiourea under continuous stirring at room temperature [11] **(Scheme 1).**



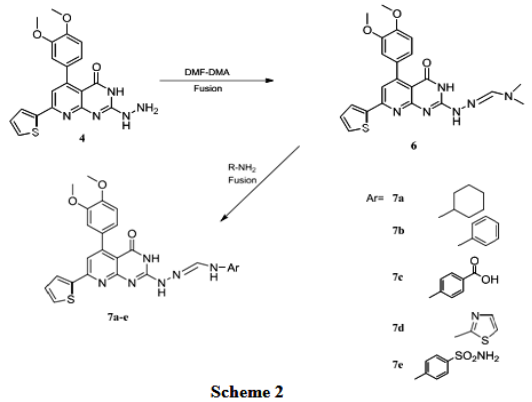
Scheme 1

Compound **3** was clearly assigned via spectroscopic methods and elemental analysis where its NMR data showed two singlet peaks at δ = 3.6, 3.7 ppm corresponding to 6H of two methoxy groups, δ=6.9-7.9 (m, 6H, Ar-H), 8.6 (s, 1H, pyridine-H). EIMS (m/z) 397.06 [M+] (27%). (*cf.* experimental).

Aiming to prepare promising new antimicrobial agents, thioxopyrido[2,3] pyrimidine derivative, compound **3** underwent condensation reaction with hydrazine hydrate to yield hydrazinyl compound **4**, which directly condensed with different aromatic aldehydes, namely, 4-chlorobenzaldehyde,4-bromobenzaldehyde,4-florobenzaldehyde,4-aminobenzaldehyde,4-nitro benzaldehyde and 4-methoxybenzaldehyde to give the corresponding Schiff's bases **5a-f**, **(**Scheme **1).**

1HNMR data for compound **5f** reveals clearly the existence of the compound ,where 3.45(s,3H,-OCH_3), 3.81 (s, 6H, 2OCH_3),) 6.96 (m, 4H, Ar-H),7.19–7.22 (m, 2H, Thiophene-H, Pyridine-H), 7.38 (m,4H, Ar-H), 7.57 (s, 1H, N=CH), 7.81(d, 1H, *J* = 5.6 Hz, Thiophene-H), 8.06 (d, 1H, J = 3.8, Thiophene-H), 12.31 (s, 1H,NH; D_2O) signals were outputted clearly, exchangeable, 12.99 (s, 1H, NH; D_2Oexchangeable).

Continue seeking for new potent antimicrobial structures related to our pyridopyrimidine system via enamine linkage; Hydazinyl **4** underwent fusion for ten minutes with dimethylformamide-dimethylacetal (DMF-DMA) to afford hydrazonamide derivative 6.We got answer for the successful preparation of this important hydrazonamid from nmr confirmation data, that declared the existence of δ (ppm), 2.9(s,6H,2-CH3), 3.6 (s, 3H, -OCH3), 3.7 (s, 3H, - OCH3), 6.25–6.90 (m, 3H, Thiophene) 7.15–7.30 (m,3H Ar-H and pyridine-H),7.9 (s,1H,N=CH),10.2 (br s, 1H, NH; D2O exchangeable), 11.7 (s, 1H, NH; D2O exchangeable). EIMS (m/z) 450.15 [M+] (100%). Compound (**6**) had subjected to react with different amine derivatives namely; cyclohexyl amine, phenyl amine, 4-amino benzoic acid, 2-amino thiazole and 4-aminobenzenesulfonamid; to give a set of compounds **7a-e**, (scheme 2).



Scheme 2

3.2. Biological Evaluation

In this study, series of certain pyrido[2,3-d]pyrimidin-4-one **5a-f** and **7 a-e** have been prepared and evaluated for their biocidal activities against Gram positive bacteria *Staphylococcus aureus* ATCC-47077(St.), *Bacillus-cereus* ATCC-12228 (B.C.), and Gram negative bacteria species , *Escherichia coli*-ATCC-25922(E.C.) , *Salmonella typhi*-ATCC-15566 (Salm.), in addition to *Candida albicans* ATCC-10231 (C. Alb.) as fungi strain.

From resulting and statistical data illustrated in **Table (1)** and **(Fig.3)** ,we can deduce that compound **7c,7d** and **7e** are the most active candidates against *St.aureus* with remarked potency of compound **7d** compared to Trimethoprim,while compound **5f** activity almost the same as referance drug.For *Bacillus cereus* and *Escherichia coli* the same set of compounds **7c,7d** and **7e** showed remarked high antimicrobial activity compared to referance Trimethoprim with relaized supreme activity of **7d** ,while **5f** begin to take part in potency against both two strains.

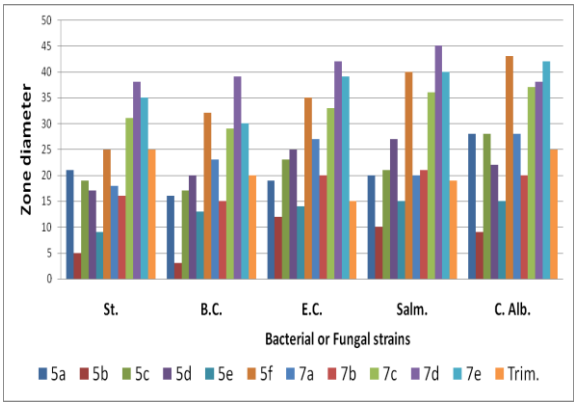


Fig.3 Antimicrobial activity of new synthesized candidates

Table 1. Anti-microbial activity of newly synthesized pyrido[2,3-d]pyrimidine-4-one (mm) , Zone diametr, the well diameter (6 mm) is included

Compound	Gram Positive		Gram Negative		fungi
	St.	*B.C*	*E.C.*	*Salm.*	*C.Alb.*
5a	21	16	19	20	28
5b	5	3	12	10	9
5c	19	17	23	21	28
5d	17	20	25	27	22
5e	9	13	14	15	15
5f	25	32	35	40	43
7a	18	23	27	20	28
7b	16	15	20	21	20
7c	31	29	33	36	37
7d	38	39	42	45	38
7e	35	30	39	40	42
Trimethoprim	25	20	15	19	25

An increasing potent activity had been recorded for the three compounds **7c, 7d** and **7e** together with compound **5f** against Salmonella. compound **5f** was the most potent candidate against Candida Albicans in addition to **7c,7d** and **7e** showed remarked activity compared to reference Trimethoprim. These illations supported by docking studies that aimed to target Biotin Carboxylase of those microbial strains.

From gained result we can confidentially say that the existence of terminal electron donating acidic groups i.e. carboxyl, methoxy and sulfonamide or aromatic character moiety like thiazole ring increases the potency of compounds to be much promising antimicrobials compared to the known Trimethoprim. Minimal Inhibitory Concentration (MIC) of the examined novel pyrido[2,3-d]pyrimidin-4-one derivatives 2-7,were studied. The results in Table (2) were highly encouraging for developing such compound structures according to their high efficiency at comparable low concentration of inhibition effects Fig.(4).

Table 2. Minium Inhibition concentration (MIC) ppm. of new synthesized compounds

Compound	Gram Positive		Gram Negative		fungi
	St.	B.C.	E.C.	Salm.	C.Alb.
5a	80	80	100	100	100
5b	100	100	90	90	90
5c	66	75	70	70	75
5d	30	20	20	15	15
5e	40	45	65	65	65
5f	10	15	10	10	15
7a	20	30	20	18	10
7b	20	20	18	18	15
7c	8	8	10	10	8
7d	10	10	8	8	6
7e	12	13	9	10	12

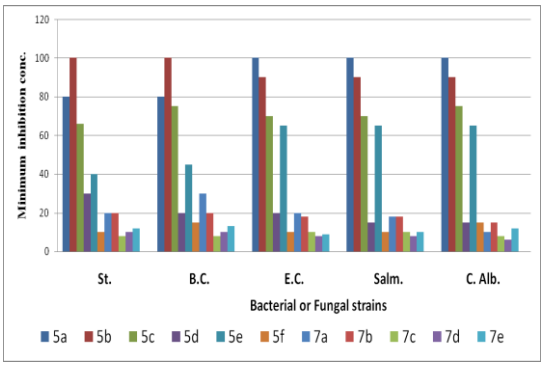


Fig.4 (MIC) ppm. of new synthesized compounds

3.3. Molecular docking studies

We choose our molecular targets by comparing our compounds under investigations with Crystal ligands and determining the essential feature that can bind with critical amino acid of target sites. However, we test our compounds practically against many target sites; then good results determine the suitable protein for doing docking studies.

3.3.1. *Docking process*

For choosing protein target site some processes were done to give insights into molecular binding modes of the tested compounds. Then, crystallographic disorders and unfilled valence atoms were corrected using protein report and utility as well as clean protein options. The rigid of binding site structure of protein was obtained by applying fixed atom constraint. The protein essential amino acids defined and prepared for docking process. These processes to predict the proposed binding mode, affinity, preferred orientation of each docking pose andbinding Free energy (ΔG) of the tested compounds with Biotin carboxylase.

3.3.2. *Interpretation and Discussion*

The binding mode of the reference (Trimethoprim) exhibited an energy binding of (-6.81 kcal/ mol) against *E.coli Biotin carboxylase*, where, pyrimidine-2,4-diamine formed two Pi-alkyl interactions with *Ile157* and *Leu278* respectively, one sulfur-Pi interaction with *Met169*, while the amino groups in positions 2,4 formed three hydrogen bonding with *Glu201, Lys202 and Leu204* with distance of 2.32, 2.12 and 2.32 ºA respectively. A tri-methoxy phenyl moiety interacted with *His236* by Pi-Pi interaction; also the methoxy group in position 5 was binding with *Gly166* by hydrogen bond with distance of 2.46 ºA **Fig. (5).**

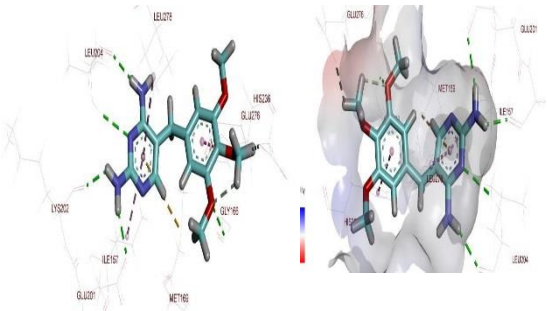


Fig.5 **Trimethoprim docked in *E-coli*, mapping surface showing Trimethoprim occupying the active pocket of *E.coli* Biotin carboxylase**

On the other hand (Trimethoprim) exhibited an energy binding of (-8.24 kcal/mol) with *S. aureus* Biotin carboxylase, where, pyrimidine-2,4-diamine formed two Pi-cation interactions with *Arg125* and *Lys187*. The nitrogen atom in pyrimidine ring and amino group in position 4; formed a hydrogen bonds with *Arg125 and Asp322* with distance of 2.61 ºA for both of them. Also, trimethoxy phenyl moiety interacted with *Trp127* and *Lys147* by Pi-Pi and Pi-cation interactions **Fig.(6)**.

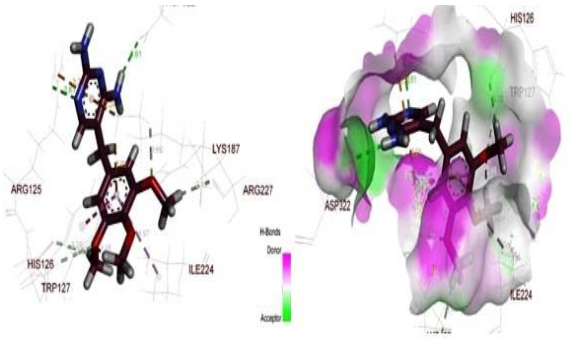


Fig.6 Trimethoprim docked in *S.aureus*, mapping surface showing Trimethoprim occupying theactive pocket of *S.aureus* Biotin carboxylase

Additionally, (Trimethoprim) exhibited inhibition activity against fungi species with an energy binding of (-7.70 kcal/mol) with *Saccharomyces cerevisiae Biotin carboxylase*, where, pyrimidine-2,4-diamine formed Pi- alkyl interaction with *Met393* via amino group in position 2 and attached to *Phe510* by hydrogen bonding with distance of 2.46 ºA, while ,tri-methoxy phenyl moiety interacted with *Trp487* and *Lys73* by Pi-Pi and Pi-alkyl interactions. The 4,5 methoxy groups formed two hydrogen bonds with *Arg76* with distance of 2.74 and 2.15 ºA respectively; **Fig. (7).**

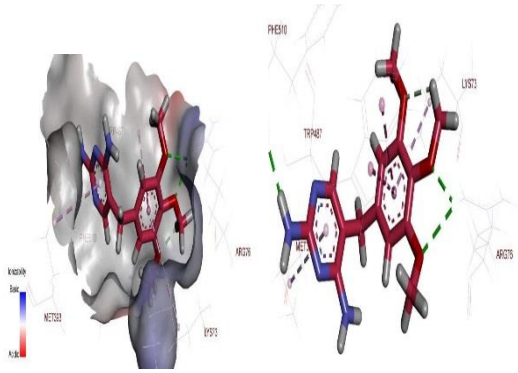


Fig.7 Trimethoprim docked in *S.cerevisiae*, mapping surface showing Trimethoprim occupying theactive pocket of *S.cerevisiae* Biotin carboxylase

The binding mode of the candidate compound **5f** exhibited an energy binding of (-7.25 kcal/ mol) against *E.coli* Biotin carboxylase. The methoxy phenyl ring creating Pi-cation interaction with *Lys238*, while dimethoxy phenyl moiety interacted with *His438* and *His236* by Pi- Pi interactions. The 4-oxo- 3,4-dihydropyrido[2,3-d]pyrimidine nucleus formed two Pi-alkyl interactions with Ile437 and hydrogen bond with Gln233 with a distance of 2.91ºA. Additionally the thiophene ring have two Pi-alkyl and one Pi-Pi interactions with Ile157, Leu278 and Tyr203 Fig. (8).

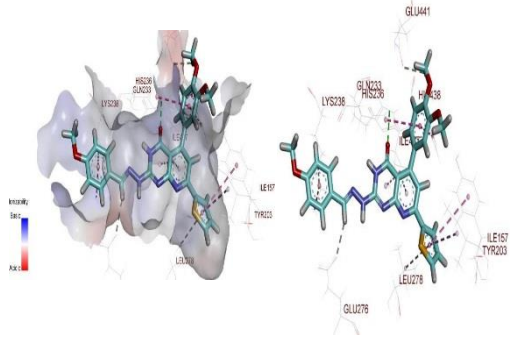


Fig. 8 Compound 5f docked in *E-coli* active pocket of *E.coli* Biotin carboxylase

The binding mode of the candidate compound 5f exhibited an energy binding of (-6.68 kcal/ mol) with S.aureus Biotin carboxylase, inwhich methoxy phenyl ring creating Pi-Pi and Pi- cation interactions with Trp127, Ala228 and Ile224 while the methoxy group creating a hydrogen bond with Asn212 with a distance of 2.11 ºA. The dimethoxy phenyl moiety interacted with Lys187, Arg125 and Asp320 by Pi-cation and Pi-anion interactions, while dimethoxy groups formed two hydrogen bonds with Arg122 and Arg125 with a distance of 1.95 and 2.65 ºA. The 4-oxo- 3,4-dihydropyrido[2,3-d]pyrimidine nucleus formed one hydrogen bond with Lys187 with a distance of 1.94ºA and two Pi-cation interactions with Arg125 and Arg227. Moreover, the thiophene ring have one Pi-anion interaction with Asp322 and weak hydrogen bond between sulfur atom and Arg125 with a distance of 3.02ºA,Fig.(9).

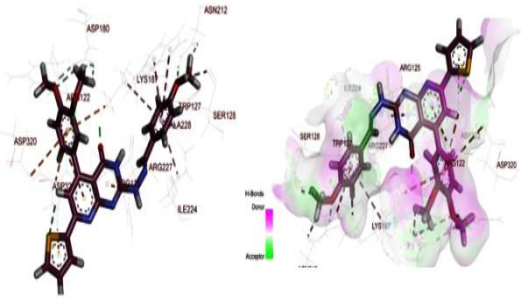


Fig. 9 Compound 5f docked in *S.aureus* with mapping surface showing compound 5f occupying the active pocket of *S.aureus* Biotin carboxylase

Additionally, the binding mode of the candidate compound **5f** exhibited an energy binding of (-5.95 kcal/ mol) with *S.cerevisiae* Biotin carboxylase.; inwhich methoxy phenyl ring creating Pi-Sigma interaction with *Asn398*. The dimethoxy phenyl moiety interacted with *Trp487* and *Lys73* by Pi-Pi and Pi-alkyl interactions, while methoxy group formed one hydrogen bond with*Arg76* in a distance of 2.44 ºA. The 4-oxo- 3,4-dihydropyrido[2,3-d]pyrimidine nucleusformed one Pi-cation interaction with *Glu392* and three Pi-Alkyl interactions with *Pro389* and *Met393*. Additionally, the NH group in hydrazinyl moiety creating hydrogen bonding with *Glu392* with a distance of 2.64 ºA. Moreover, thiophene ring has one Pi-Pi and Pi-alkyl interactions with *Phe510* and *Met393*, while sulfur atom in *Cys454* interacted by sulfur-Pi interaction **Fig. (10).**

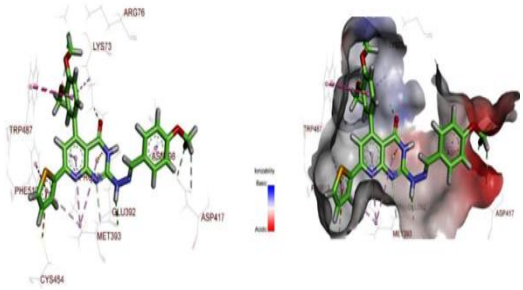


Fig. 10 Compound 5f docked in *Fungal S.cerevisiae*, with mapping surface showing Compound 5f occupying the active pocket of *S.cerevisiae* Biotin carboxylase

The binding mode of the candidate compound **7c** exhibited an energy binding of (-8.46 kcal/ mol) against *E.coli* Biotin carboxylase, where the acidic carboxylic head form two hydrogen bonds with *Gln294* and *Arg338* with a distance of 2.78, 2.70 ºA. The NH group in formohydrazonamido moiety has hydrogen bonding with *His236* with a distance of 2.00 ºA, while 4-oxo- 3,4-dihydropyrido[2,3-d]pyrimidine nucleus formed two Pi-alkyl interactions with *Ile437* and hydrogen bond with *Gln233* in a distance of 2.68 ºA. Also, thiophene ring have two Pi-alkyl and one Pi-Pi interactions with *Ile157*, *Leu278* and *Tyr203*. Moreover, the dimethoxy phenyl moiety creating Pi-Pi interaction with *His236* **Fig. (11).**

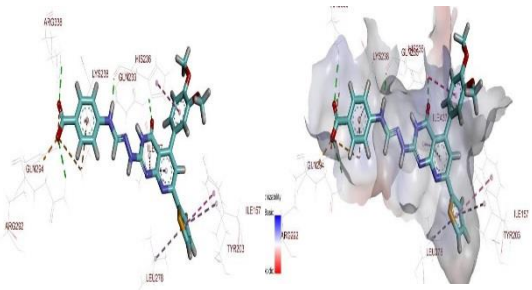


Fig. 11 Compound 7c docked in *E-coli* with mapping surface showing Compound 7c occupying the active pocket of *E.coli Biotin carboxylase*

Binding mode of the candidate compound 7c exhibited an energy binding of (-8.83 kcal/ mol) with S.aureus Biotin carboxylase. The acidic carboxylic head creating a hydrogen bond with Gln116 in a distance of 2.94 ºA. The hydrophobic phenyl moiety formed Pi- alkyl interaction with Leu192 and Pi-Pi interactions with Trp127 and Phe191. The nitrogen atoms in 4-oxo- 3,4-dihydropyrido[2,3-d]pyrimidine nucleus formed three hydrogen bonds with His126

and Lys187 with a distance of 1.97, 2.38, 2.24 ºA; and three Pi-cation interactions with Arg125 and Arg227. Moreover, the thiophene ring have one Pi-anion interaction with *ASP*322,Fig.(12).

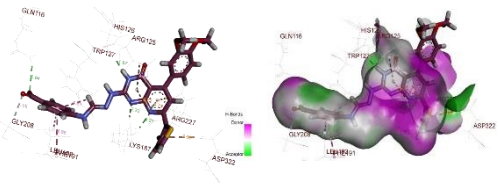


Fig. 12 Compound 7c docked in *S.aureus* Biotin carboxylase, with mapping surface showing compound 7c occupying the active pocket of *S.aureus* Biotin carboxylase

Going ahead discovering our compounds' activities, we record a binding mode for compound 7c with an energy binding of (-8.55 kcal/ mol) with S.cerevisiae Biotin carboxylase, where, acidic carboxylic head form strong ionic bond with Lys451 with a distance of 1.99 ºA. The aromatic phenyl moiety formed Pi-Pi interaction with Phe512, while, -NH group in formohydrazonamido moiety has hydrogen bonding with Ser484 with a distance of 2.57 ºA and two Pi-Pi interactions with Trp487 formed via 4-oxo- 3,4-dihydropyrido[2,3-d]pyrimidine system and thiophene ring Fig. (13).

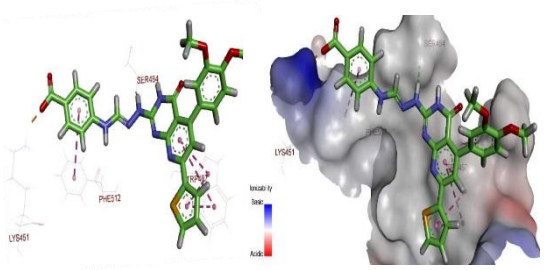


Fig. 13 Compound 7c docked in Fungal Biotin carboxylase with mapping surface showing compound 7coccupying the active pocket of *S.cerevisiae* Biotin carboxylase

The binding mode of the candidate compound **7d** exhibited an energy binding of (-8.96 kcal/ mol) against *E.coli* Biotin carboxylase. where, thiazol-2-yl ring creating hydrogen bond with *Leu204* with a distance of 1.85 ºA and the sulfur atom interacted by Sulfur-Pi interaction with *Tyr203*.Additionally, forming Pi-alkyl interactions with *Leu278*, *Val131* and *Ile157*. The dimethoxy phenyl moiety interacted with *Ile287*, *Lys159* and *Met169* by Pi-Alkyl and Pi-cation interactions. The NH group in formohydrazonamido moiety formedhydrogen bond with *Leu204* with a distance of 2.01 ºA. The 4-oxo-3,4-dihydropyrido[2,3- d] pyrimidine nucleus formed five Pi-Pi and Pi-Alkyl interactions with *His209, His236*, *Leu278* and *Ile287*. Accordingly, the 4-oxo- group creating Hydrogen bonding with *Lys159* with a distance of 2.52 ºA. Moreover, the thiophene ring has one Pi-cation and sulfur-Pi interactions with *Lys238* and *His209* **Fig (14).**

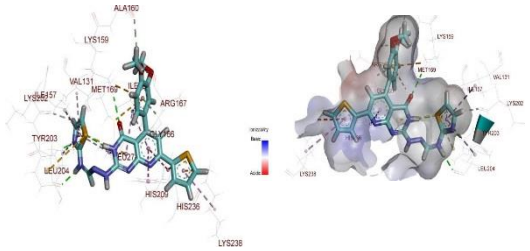


Fig. 14 Compound 7d docked in E-coli with mapping surface showing Compound 7d occupying the active pocket of *E-coli Biotin carboxylase*

The binding mode of the candidate compound **7d** exhibited an energy binding of (-7.40 kcal/ mol) with *S.aureus* Biotin carboxylase. where, thiazol-2-yl ring creating Pi-Pi interaction with *Gly208*. The -NH group in formohydrazonamido moiety formed hydrogenbond with *Arg120* in a distance of 2.25 ºA. The dimethoxy phenyl moiety interacted with *Arg125*, *Lys176* and *Asp320* by Pi-cation and Pi-anion interactions. The 4-oxo- 3,4- dihydropyrido[2,3-d]pyrimidine nucleus formed Pi-cation and Pi-alkyl interactions with *Lys187, Arg122* and *Arg125*. Additionally, thiophene ring creating Pi interactions with *Arg125* and *His126* **Fig. (15).**

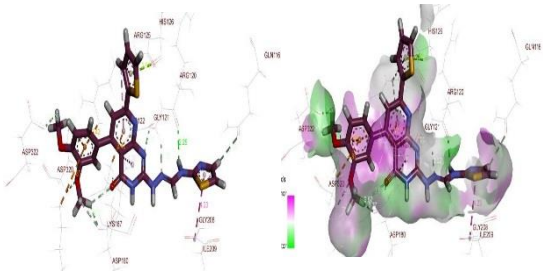


Fig. 15 Compound 7d docked in *S.aureus* with mapping surface showing Compound 7d occupying theactive pocket of *S.aureus* Biotin carboxylase

Going more deeply, compound **7d** exhibited an energy binding of (-7.51 kcal/ mol) with *S.cerevisiae* Biotin carboxylase, inwhich thiazol-2-yl ring creating Pi-cation interaction with *Arg76*. The dimethoxy phenyl moiety interacted with *Lys80* by Pi-cation interactions, while methoxy group forming hydrogen bond with *Asn398* with a distance of 2.25 ºA. The4-oxo- 3,4-dihydropyrido[2,3-d] pyrimidine nucleus formed two Pi-Pi interactions with *Trp487*. Morever, thiophene ring creating Pi-alkyl interaction with *Met393* **Fig (16).**

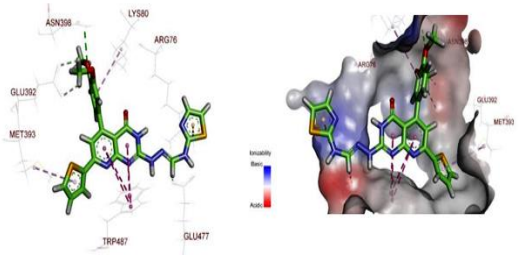


Fig. 16 Compound 7d docked in Fungal Biotin carboxylase, with mapping surface showing compound 7d occupying the active pocket of *S.cerevisiae* Biotin carboxylase

The binding mode of the candidate compound **7e** exhibited an energy binding of (-8.83 kcal/ mol) against *E.coli* Biotin carboxylase. The 4-sulfamoylphenyl moiety creating two hydrogen bonds with *Arg292* and *Arg338* with a distance of 2.55, 2.76 ºA , while the phenyl ring interacted by Pi-cation interaction with *Lys238*. Additionally, the dimethoxy phenyl moiety interacted with *His236* by Pi-Pi interaction. The -NH group informohydrazo-namido moiety formed hydrogen bond with *His236* in a distance of 2.08 ºA. The 4-oxo- 3,4-dihydropyrido[2,3-d]pyrimidine nucleus formed two Pi-Alkyl interactions with *Ile437* and the 4-oxo- group creating hydrogen bonding with *Gln233* with a distance of 2.68 ºA. Moreover, thiophene ring interacted with *Ile157, Leu278* and *Tyr203* by Pi-Pi and Pi-Alkyl interactions **Fig.(17).**

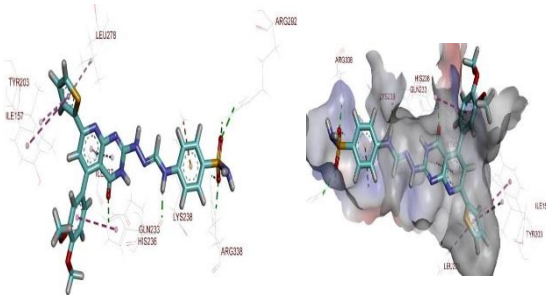


Fig. 17 Compound 7e docked in *E-coli* ,with mapping surface showing Compound 7e occupying the active pocket of *E-coli* Biotin carboxylase

Moreover, the binding mode of the candidate compound 7e exhibited an energy binding of)-7.67 kcal/ mol(with S.aureus Biotin carboxylase. The 4-sulfamoylphenyl moiety creating one hydrogen bond with Gln116 with a distance of 1.86 ºA. The phenyl ring interacted by Pi-alkyl interaction with *Leu192*. Additionally, the dimethoxy phenyl moietyinteracted with *Arg227* and *Asp322* by Pi-cation and Pi-anion interactions. The 4-oxo- 3,4- dihydropyrido[2,3-d]pyrimidine nucleus formed two Pi-cation interactions with *Lys187* and *Arg125*. Moreover, thiophene ring interacted with *Ile224* and *His126* by Pi-alkyl and Pi-sulfur interactions **Fig. (18).**

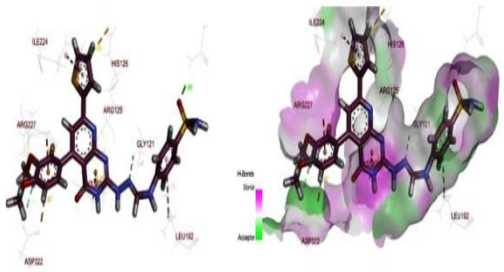


Fig. 18 Compound 7e docked in *S.aureus* with mapping surface showing Compound 7e occupying the active pocket of *S.aureus* Biotin carboxylase

The binding mode of the candidate compound **7e** exhibited an energy binding of (-7.54 kcal/ mol) with *S.cerevisiae Biotin carboxylase*. The 4-sulfamoylphenyl moiety creating one hydrogen bond and Pi-anion interaction with *Asp417* in a distance of 2.13 ºA. The dimethoxy phenyl moiety interacted with *Met393* by Pi- alkyl interaction. Moreover, the thiophene ring interacted with *Trp487, Lys73* and *Arg76* by Pi-Pi, Pi-alkyl and Pi-cation interactions **Fig. (19).**

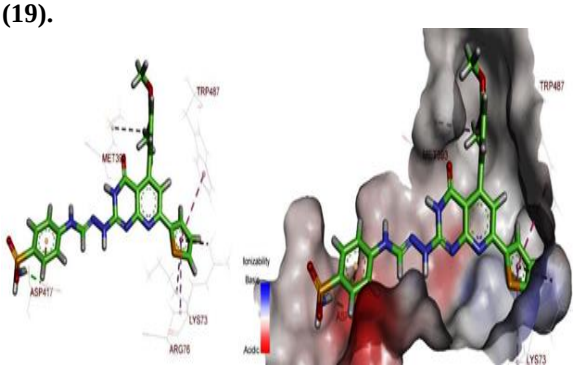


Fig. 19 Compound 7e docked in Fungal Biotin carboxylase, with mapping surface showing Compound 7eoccupying the active pocket of *S.cerevisiae* Biotin carboxylase

Surface mapping and flexible alignment with crystal ligand of compound 7d,fig.(20-22).

The docking scores of the best fitted poses within active site at Biotin Carboxylase, where recorded, Tables (3-5).

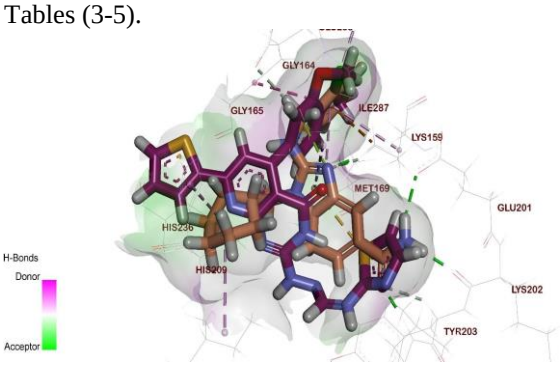


Fig. 20 Surface mapping and flexible alignment with crystal ligand showing compound 7d completelyoccupying the active pocket *E-coli* Biotin carboxylase

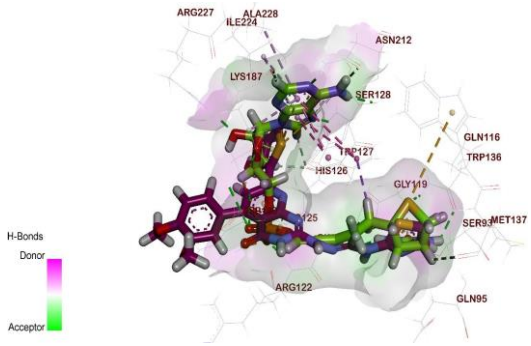


Fig. 21 Surface mapping and flexible alignment with crystal ligand shoeing compound 7d completely occupying the active pocket of *St.aureus* Biotin Carboxylase

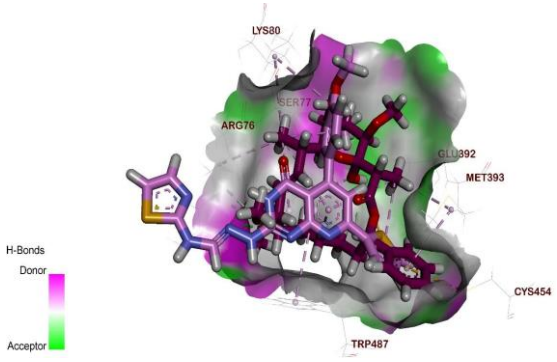


Fig. 22. Surface mapping and flexible alignment with crystal ligand showing compound 7d completely occupying the active pocket of *Fungal* Biotin Carboxylase

Table. 3 Show (ΔG) of tested candidates against (*E.coli Biotin Carboxylase*) target site PDB ID: 3jzf

Compound	interactions		Score (ΔG). K.Cal/ Mole	RMSD (Aº)
	Pi	H.B		
5f	9	2	-7.25	1.69
7c	12	1	-8.46	1.36
7d	1	1	-8.96	1.39
7e	1	1	-8.83	1.25
Tri-methoprim	4	1	-6.81	1.49

Table. 4 Show (ΔG) of tested candidates against against (*S.aureus Biotin Carboxylase*) target site PDBID: 4dq2

Comp.	interactions				Score (ΔG). K.Cal/Mole	RMSD (Aº)
	Pi	ionic	H.B	M-ion		
5f	9	-	1	2	-6.68	1.76
7c	12	-	2	1	-8.83	1.13
7d	1	-	2	1	-7.40	1.37
7e	1	1	1	1	-7.67	1.24
Trimethoprim	4	4	2	1	-8.24	1.59

Table. 5 Show (ΔG) of tested candidates against against (*S.cerevisiae Biotin Carboxylase*) target sitePDB ID: Iw96

Comp.	interactions			M-ion	Score K.Cal/M (ΔG)	RMSD (Aº)
	Pi	ionic	H.B			
5f	9	-	1	2	-5.95	1.24
7c	12	-	2	1	-8.55	1.06
7d	1	-	2	1	-7.57	1.14
7e	1	1	1	1	-7.54	1.78
Tri-methoprim	4	4	2	1	-7.70	1.43

4. Structure–Activity Relationships (SAR)

As outlined in the rationale for the molecular design, we can deduced the SAR of the newly synthesized compounds as potential antimicrobial agents. Firstly, investigating an effect of substitution on the (formohydrazonamido) moiety by different groups revealed that the activity of compounds **5a-e** decreased by incorporating halides and small function groups (eg.NH_2,NO_2) compared to the corresponding member **5f**; that bearing methoxy group which indicating that substitution with a lipophilic bulky group is preferred over small group incorporation. Additionally, by comparison of the activities of compounds **7a- e**, we found that aromatic characters increases in the order of thiazole ring > benzene sulfonamide moiety > benzoic acid or benzene > cyclohexane.

Conclusion

Novel series of substituted pyrido[2,3-d]pyrimidine system incorporated to different schiff's bases and enamine derivatives had been synthesized and evaluated for their in-vitro antimicrobial efficacy

against different gram positive and gram negative bacteria and fugal strain as Fatty acid synthase inhibitors. Antimicrobial assay results and deep docking studies supported by strcture-activity relationship; declares their high effectuality at low concentration compared to Ttrimethoprim as promising antimicrobial agents.

Conflict of interest

This work was carried out under research program of the Pharmaceutical and drug Industries Research Division National Research Center-Egypt. The authors declare that they have no conflict of interest.

Funding

This work funded by the local project of national research center in Egypt under no.12060111

References

1.Marinescu M.,synthesis of antimicrobial benzimidazole-pyrazole compounds and their biological activities. *antibiotics*;10,1002 (2021). doi: 10.3390/antibiotics 10081002

2.Al-Wabli Reem I. et al. Design, Synthesis, and Antimicrobial Activity of Certain New Indole-1,2,4 Triazole Conjugates *molecules.*26, 2292,(2021).

3. Zhang, F.F.; Gan, L.L.; Zhou, C.H. Synthesis, antibacterial and antifungal activities of some carbazole derivatives. Bioorganic Med.Chem. Lett. 20, 1881–1884(2010).

4. Kaku, N.; Morinaga, Y.; Takeda, K.; Kosai, K.; Uno, N.; Hasegawa, H.; Miyazaki, T.; Izumikawa, K.; Mukae, H.; Yanagihara, K.Antimicrobial and immunomodulatory effects of tedizolid against methicillin-resistant Staphylococcus aureus in a murine model of hematogenous pulmonary infection. Int. J. Med. Microbiol. 306, 421–428(2016). doi:10.1016/S0163-7827(02)00007-3.

5. G.Vincent ,Recent Advances in the Inhibition of Bacterial Fatty Acid Biosynthesis. Annual Report in Medicinal Chemistry. 45(10):296-308(2010). doi:10.1016/S0065-7743(10)45018-6.

6. M.H. James, J.C. Cleo, C. Mel et al. Structure −Activity Relationships for a Novel Series of Pyrido [2,3-d]pyrimidine Tyrosine Kinase Inhibitors. J. Med. Chem. 40(15):2296–2303(1997). doi: 10.1021/jm970367n.

7. J.R. Miller et al. A class of selective antibacterial derived from a protein kinase inhibitor pharmacophore.PNAS.2009; 106(6): 1737-1742. doi:10.1073_pnas.0811275106.

8. A. Agnieszka, B. Agata, P. Monika, M. Tomasz, S. Wrobel, M. Dariusz. Synthesis, Experimental and Computational Studies of N- (4-amino-6-oxo- 1,6-dihydropyrimidin-5-yl) benzamide. Letters in Organic chemistry. 15(6):491-502(2018). doi: 10.2174/1570178614666170811123851.

9. J.P. David, V.W. Patrick, J.H. David, J.I. Ynduo, T.L. John. Bacterial fatty-acid biosynthesis: a genomics-driven target for antibacterial drug discovery. Drug Discovery Today.;6(10):537- 544(2001). doi:10.1016/S1359-6446(01)01774-3.

10. D. Payne, M. Gwynn, D. Holmes et al. Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nat Rev Drug Discov. 6: 29- 40(2007). doi:10.1038/nrd2201.

11. G. Yunlei, S. Guanghui, S. Meiling, W. Juan, W. Yi. Prevalence and Therapies of Antibiotic- esistance in Staphylococcus aureus. Front. Cell. Infect. Microbiol. 10(107):1-11(2020). PMID: 5325707.

12. Z.M. Alvand, H.R. Rajabi, A. Mirzaei, A. Masoumiasl. Ultrasonic and microwave assisted extraction as rapid and efficient techniques for plant mediated synthesis of quantum dots: green synthesis, characterization of zinc telluride and comparison study of some biological activities. New J. Chem. 43(38): 15126–15138(2019). doi:10.1039/C9NJ03144H.

13. H.R. Rajabi, R. Naghiha, M. Kheirizadeh, H. Sadatfaraji. Microwave assisted extraction as an efficient approach for biosynthesis of zinc oxide nanoparticles: synthesis, characterization, and biological properties. Materials Science and Engineering. C(78):1109-1118(2017). doi:10.1016/j.msec.2017.03.090.

14. Z.M. Alvand, H.R. Rajab, A. Mirzaei, A. Masoumiasl, H. Sadatfaraji. Rapid and green synthesis of cadmium telluride quantum dots with low toxicity based on a plant-mediated approach after microwave and ultrasonic assisted extraction: Synthesis, characterization, biological potentials and comparison study. Materials Science and Engineering. C(98):535-544(2019).

15. S. Mirsadeghi, H.Zandavar, M. Rahimi, H.F. Tooski, H.R. Rajabi, M. Rahimi-Nasrabadi, E. Sohouli, B. Larijani, S.M. Pourmortazavi. Photocatalytic reduction of imatinib mesylate and imipenem on electrochemical synthesized $Al_2W_3O_{12}$ nanoparticle: Optimization, investigation of electrocatalytic and antimicrobial activity. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 586:124254(2020).

16. R.M. Abdelhameed, M. Abu-Elghait, El-Shahat. (M. ZIF-67@ ZIF-8@ MIL-125-NH2): enhancement the biological and visible-light photocatalytic activity. J. Environ. Chem. Eng. 8(5):104107(2020).

17.P.C. Hannan. Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary mycoplasma species. Vet.Res. 31(4):373-395(2000).

[18] Protein Data Bah.https://www.rcsb.org/nk. Accessed January 12,(2021).