Available online at **www. scholarsresearchlibrary.com**

Scholars Research Library

Der Pharmacia Lettre, 2021, 13 (7): 79-92
(http: //scholarsresearchlibrary. com/archive. html)

ISSN 0975-5071
USA CODEN: DPLEB4

Bioactive Scaffolds of Synthesis, *In Silico* Docking Studies, and an *In Vitro* Screenings of Bis-azoles and N-bridged Bis-fused Azoles as Antibacterial Agents

Waleed AMAEl-Enany[1], Sayed M. Riyadh[1,2]

[1]*Departments of Chemistry, University of Cairo, Giza, Egypt*

[2]*Department of Chemistry, University of Taibah, Al-Madinah Al-Munawarah, Saudi Arabia*

[*]***Corresponding author:*** *Waleed AMAEl-Enany, Department of Chemistry, University of* Cairo, Giza, Egypt, *E-mail: waleed_org_chem@yahoo.com.*

ABSTRACT

N',N''-(1,2-Diphenylethane-1,2-diylidene)bis(2-cyanoacetohydrazide), was condensed with benzaldehyde to afford N',N''-(1,2-diphenylethane-1,2-diylidene)bis(2-cyano-3-phenyl acrylohydrazide) as a key intermediate. Bis-pyrazoles containing hydrazone moiety were synthesized by the reaction of N',N''-(1,2-diphenylethane-1,2-diylidene) bis(2-cyano-3-phenylacrylohydrazide with hydrazines. Also, N-bridged bis-fused azoles were synthesized by the addition of N',N''-(1,2-diphenylethane-1,2-diylidene)bis(2-cyano-3-phenylacrylohydrazide with a respective heterocyclic amine. The molecular docking study of the compounds was carried out against the active site of Staphylococcus aureus tyrosyl-tRNA synthetase, this to understand the binding mode of interactions. *In silico* ADMET properties of new azole analogues were also calculated. Moreover, the antibacterial activity of the synthesized hybrids was assessed using the Agar diffusion well method and the results indicated that N',N''-(1,2-diphenylethane-1,2-diylidene)bis(4-amino-8,10-dimethyl-2-phenylpyrido[2`,3`:3,4]pyrazolo[1,5-a]pyrimidine-3-carbohydrazide displayed the best antibacterial activity.

Keywords: Bis-cyanoacetohydrazides, Bis-cyanoacrylohydrazide, Bis-pyrazoles, N-bridged bis-fused azoles, *Staphylococcus aureus* tyrosyl-tRNA synthetase, Docking, Antibacterial activity.

INTRODUCTION

The resistance of antibiotics is still one of the biggest threats worldwide [1]. *Staphylococcus aureus* (*S. aureus*) has been recognized as one of the most harmful bacteria that are responsible for various infections such as bloodstream infections and pneumonia [2]. In the present study, *Staphylococcus aureus* tyrosyl-tRNA synthetase [3] has been chosen as an emerging target for identification of novel inhibitors, consequently, promising antibacterial candidates.

Over the last decades, several bis-azoles have attracted much attention due to their diverse chemo-therapeutic activities such as; antimicrobial [4-8], anticancer [8-11], anti-hypertensive α-blocking agents [12], DPPH radical scavenging and anti-diabetic [13] and anti-anthelmintic agents [14].

Furthermore, the synthetic utility of bis-azoles, as building units for many chain polymers, has been reported [15]. Additionally, by virtue of their high nitrogen content, these compounds have high detonation performance and insensitive to external stimuli. Therefore, bis-azoles could be used as promising candidates for high energy materials [16]. On the other hand, cyanoacetic acid hydrazide represents a versatile intermediate that contains multilateral reactive centers as nitrile group (CN) adjacent to *α*-CH acidic and acid hydrazide moiety (=N-NH-CO). These favorable reactive centers could react with numerous electrophiles and nucleophiles to provide a wide variety of azoles and *N*-bridged fused azoles that exhibiting pharmaceutical properties [17-20].

In our continuous effort for developing new antimicrobial drug candidates [21-30], we have reported here the synthetic routes of novel bis-azoles and bis-fused azoles from bis-cyanoacetohydrazides. In addition, the newly synthesized compounds were screened against *Staphylococcus aureus* tyrosyl-tRNA synthetase using PyRx virtual screening tool.

The obtained results indicated that the studied compounds were docked successfully to the target enzyme with good docking scores ranging from-15.4 to-9.7 kcal/mol. Overall; docking examination results supported that the tested compounds may be considered as potential antibacterial agents against *Staphylococcus aureus* tyrosyl-tRNA synthetase (YRS).

EXPERIMENTAL

Chemistry

An electrothermal Gallenkamp apparatus was used to measure the melting points for the newly synthesized compounds. Pye-Unicam SP300 instrument in potassium bromide discs was used to measure IR spectra. A Varian Mercury VXR-300 spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) was manipulated to measure the ^{1}H NMR and ^{13}C NMR spectra and the chemical shifts were related to that of the solvent. GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers were conducted to record the mass spectra of the samples on the ionizing voltage at 70 eV. Elemental analyses were carried out at the Microanalytical Centre of Cairo University, Giza, Egypt.

Reaction of N', N''-(1,2-diphenylethane-1,2-diylidene) bis (2-cyano-3-phenylacrylohydrazide) (2) with hydrazines: A mixture of *N*', *N*''-(1,2-diphenylethane-1,2-diylidene) bis (2-cyano-3-phenylacrylohydrazide) (2) (0.548 g 1 mmol) and hydrazine hydrate 3a (4 mmol) or phenyl hydrazine 3b (2 mmol) in ethanol was refluxed for 4 hours. The reaction mixture was poured into crushed ice/HCl mixture and the solid products were crystallized from ethanol to give bis-pyrazoles 6a, b.

N', N''-(1, 2-diphenylethane-1, 2-diylidene) bis (5-amino-3-phenyl-1H-pyrazole-4-carbo hydrazide) (6a): Yellow powder (0.49 g, 81%); mp 223°C -225°C; IR (KBr) υ=3310, 3195, 3123 (2NH and NH_2), 1662 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ=6.87 (2H, s, NH_2), 7.21-7.45 (10H, m, Ar–H), 8.15 (1H, s, NH-pyrazole), 10.12 (1H, s, NHCO); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ=108.7, 124.3, 125.2, 126.9, 127.5, 128.8, 129.4, 130.8, 132.4, 133.1, 144.5, 152.8, 168.4; MS m/z (%): 608 [M^{+}] (30), 422 (100), 77 (70). Anal. calcd for $C_{34}H_{28}N_{10}O_2$ (608.24): C, 67.09; H, 4.64; N, 23.01. Found: C, 66.96; H, 4.58; N, 22.97.

N', N''-(1,2-diphenylethane-1,2-diylidene) bis (5-amino-1,3-diphenyl-1H-pyrazole-4-carbo hydrazide) (6b): Yellow powder (0.59 g,78%); mp 212°C -214°C; IR (KBr) υ=3312, 3195, 3122 (2NH and NH_2), 1661 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ=6.88 (2H, s, NH_2), 7.21-7.45 (15H, m, Ar–H), 10.12 (1H, s, NHCO); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ=109.7, 124.3, 125.2, 125.8, 126.9, 127.5, 128.2, 128.8, 129.4, 130.8, 131.7, 132.4, 133.1, 134.9, 144.5, 152.8, 168.4; MS m/z (%): 760 [M^{+}] (40), 77 (100). Anal. calcd for $C_{46}H_{36}N_{10}O_2$ (760.30): C, 72.62; H, 4.77; N, 18.41. Found: C, 72.77; H, 4.53; N, 18.54.

General procedure for the Reaction of N', N''-(1,2-diphenylethane-1,2-diylidene) bis (2-cyano-3-phenylacrylohydrazide) (2) with amines: *N*', *N''*-(1,2-diphenylethane-1,2-diylidene) bis (2-cyano-3-phenylacrylohydrazide) (2) (0.548 g 1 mmol) in ethanol (30 mL) was mixed with appropriate heterocyclic amine (2 mmol) in a molar ratio (1:2). Few drops of acetic acid were added and the

reaction mixture was refluxed for 4–6 hours (monitored through TLC) at 150°C. The reaction mixture was poured into water, and the solid product was collected by filtration followed by washing with ethanol. The crude product was then recrystallized from ethanol/dimethylformamide (DMF) mixture to give pure product.

N',N''-(1,2-diphenylethane-1,2-diylidene)bis(7-amino-5-phenyl-[1,2,4]triazolo[1,5-a] pyrimidine-6-carbohydrazide) (10): Yellow powder (0.54 g, 76%); mp 251°C -253°C; IR (KBr) υ=3436, 3312, 3195 (NH and NH_2), 1662 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ=7.12 (2H, s, NH_2), 7.21-7.43 (10H, m, Ar–H), 8.77 (1H, s, triazole-H), 10.12 (1H, s, NH); MS m/z (%): 712 [M^+] (20), 77 (100). Anal. calcd for $C_{38}H_{28}N_{14}O_2$ (712.25): C, 63.96; H, 3.96; N, 27.51. Found: C, 63.96; H, 4.12; N, 27.67.

N', N''-(1,2-diphenylethane-1,2-diylidene)bis(7-amino-5-phenyltetrazolo[1,5-a]pyrimidine-6-carbohydrazide) (12): Yellow powder (0.69 g,79%); mp266°C -268°C; IR (KBr) υ=3442, 3313, 3195 (NH and NH_2), 1664 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ=7.12 (2H, s, NH_2), 7.21-7.44 (10H, m, Ar–H), 10.13 (1H, s, NH); MS m/z (%): 714 [M^+] (15), 77 (100). Anal. calcd for $C_{36}H_{26}N_{16}O_2$ (714.24): C, 60.50; H, 3.67; N, 31.36. Found: C, 60.66; H, 3.42; N, 31.47.

N', N''-(1,2-diphenylethane-1,2-diylidene)bis(4-amino-2-phenylbenzimidazo[1,2-a]pyrimidine-3-carbohydrazide) (14): Yellow powder (0.62 g, 77%); mp244°C -246°C; IR (KBr) υ=3430, 3197, 3124 (NH and NH_2), 1664 (C=O) cm−1; 1H-NMR (DMSO-d6, 300 MHz): δ=7.13 (2H, s, NH_2), 7.23-7.58 (14H, m, Ar–H), 10.12 (1H, s, NH); MS m/z (%): 810 [M^+] (20), 77 (100). Anal. calcd for $C_{48}H_{34}N_{12}O_2$ (810.29): C, 71.10; H, 4.23; N, 20.73. Found: C, 70.96; H, 4.18; N, 20.87.

N', N''-(1,2-diphenylethane-1,2-diylidene)bis(4-amino-8,10-dimethyl-2-phenylpyrido[2`,3`:3,4] pyrazolo [1,5 a] pyrimidine-3-carbohydrazide) (16): Yellow powder (0.69 g, 80%); mp 273°C -275°C; IR (KBr) υ=3435, 3311, 3195 (NH and NH_2), 1664 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ=2.41 (3H, s, CH_3), 2.58 (3H, s, CH_3), 7.10 (1H, s, pyridine-H), 7.12 (2H, s, NH_2), 7.21-7.45 (10H, m, Ar–H), 10.13 (1H, s, NH); MS m/z (%): 868 [M^+] (15), 77 (100). Anal calcd for $C_{50}H_{40}N_{14}O_2$ (868.35): C, 69.11; H, 4.64; N, 22.57. Found: C, 68.96; H, 4.72; N, 22.43.

N', N''-(1,2-diphenylethane-1,2-diylidene)bis(5-amino-7-phenylthiazolo[3,2-a]pyrimidine-6-carbohydrazide) (18): Yellow powder (0.56 g, 75%); mp260°C -262°C; IR (KBr)=3428, 3313, 3195 (NH and NH_2), 1661 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ=2.99 (1H, s, CH), 5.7 (1H, d, thiazole-H), 6.2 (1H, d, thiazole-H), 7.12 (2H, s, NH_2), 7.21-7.45 (10H, m, Ar–H), 10.13 (1H, s, NH); MS m/z (%): 748 [M^+] (20). Anal. calcd for $C_{40}H_{32}N_{10}O_2S_2$ (748.22): C, 64.15; H, 4.31; N, 18.70; S, 8.56. Found: C, 64.26; H, 4.42; N, 18.57; S, 8.41.

N', N''-(1,2-diphenylethane-1,2-diylidene) bis (4-amino-2-phenylbenzothiazolo[3,2-a]pyrimidine-3-carbohydrazide) (20): Yellow powder (0.63 g, 75%); mp237°C -239°C; IR (KBr) υ=3411, 3309, 3196 (NH and NH_2), 1661 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ=2.95 (1H, s, CH), 7.11 (2H, s, NH_2), 7.21-7.45 (14H, m, Ar–H), 10.12 (1H, s, NH); MS m/z (%): 848 [M^+] (20). Anal. calcd for $C_{48}H_{36}N_{10}O_2S_2$ (848.25): C, 67.91; H, 4.27; N, 16.50; S, 7.55. Found: C, 68.06; H, 4.12; N, 16.62; S, 7.41.

N', N''-(1,2-diphenylethane-1,2-diylidene)bis(4-amino-2-phenylpyrido[1,2-a]pyrimidine-3-carbohydrazide) (22): Yellow powder (0.58 g, 79%); mp270°C -272°C; IR (KBr) υ=3427, 3312, 3195 (NH and NH_2), 1663 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ=2.98 (1H, s, CH), 7.12 (2H, s, NH_2), 7.21-8.01 (14H, m, Ar–H), 10.13 (1H, s, NH); MS m/z (%): 736 [M^+] (15). Anal. calcd for $C_{44}H_{36}N_{10}O_2$ (736.30): C, 71.72; H, 4.92; N, 19.01. Found: C, 71.66; H, 4.72; N, 18.92.

N',N''-(1,2-diphenylethane-1,2-diylidene)bis(4-amino-2-phenyl-2H-pyrimido[1,2-a] pyrimidine-3-carbohydrazide) (24): Yellow powder (0.56 g, 76%); mp 259°C -261°C; IR (KBr) υ=3425, 3197, 3124 (NH and NH_2), 1665 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz): δ=3.02 (1H, s, CH), 7.11 (2H, s, NH_2), 7.21-8.03 (13H, m, Ar–H), 10.10 (1H, s, NH); MS m/z (%): 738 [M^+] (25). Anal. calcd for $C_{42}H_{34}N_{12}O_2$ (738.29): C, 68.28; H, 4.64; N, 22.75. Found: C, 68.36; H, 4.71; N, 22.89.

RESULTS AND DISCUSSION

Chemistry

Our study was inaugurated by Knoevenagel condensation of N',N''-(1,2-diphenylethane -1,2-diylidene)bis(2-cyanoacetohydrazide) (1) [31] with benzaldehyde in boiling ethanolic solution containing catalytic amount of piperidine, to afford the corresponding N',N''-(1,2-diphenylethane-1,2-diylidene)bis(2-cyano-3-phenylac-rylohydrazide) (2). The structure of compound 2 was in agreement with spectroscopic data (Figure 1). Bis(2-cyano-3-phenylacrylohydrazide) 2 was used for the preparation of bis-pyrazoles 6a,b through reaction with hy-drazine hydrate (3a) of phenyl hydrazine (3b) (Figure 1).

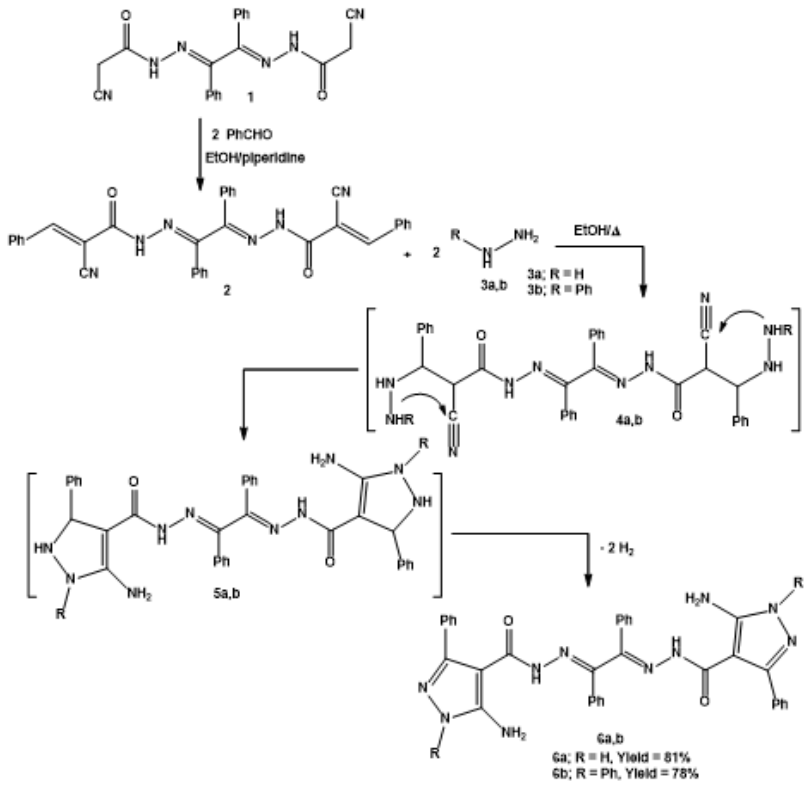

Figure 1: Synthesis of bis-pyrazoles 6a, b.

The mechanistic pathway of this reaction was preceded by sequential addition and molecular hydrogen elimination of non-isolable adduct 4a,b and 5a,b to give novel bis-pyrazoles 6a,b. The spectral date of the isolated products substantiated their structures. For example, the mass spectrum of compound 6a revealed a molecular ion peak [M^+, 608] corresponding to the formula $C_{34}H_{28}N_{10}O_2$. ^{1}H-NMR spectrum showed a pair of singlet signals at 7.10 and 8.14 ppm assignable to (NH2) [32, 33] and (NH-pyrazole) [33], respectively.

Anwar, et al [34] has investigated the reactivity of heterocyclic amines towards activated double bond. The regio-addition of exocyclic amino group (NH2) rather than endocyclic (NH) into acrylonitrile moiety has been confirmed by (^{1}H & ^{15}N) HMBC measurements as well as an X-ray crystal structure determination [34]. To generalize this regio-addition and to implement a more widely applicable approach for

the synthesis of bis-fused azoles, bis(2-cyano-3-phenylacrylohydrazide) 2 was allowed to react with 3-amino[1,2,4]triazole (7) in boiling ethanol, with catalytic amount of acetic acid, to yield the respective N',N''-(1,2-diphenylethane-1,2-diylidene)bis(7-amino-5-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carbohydrazide) (10) rather than its isomeric product 9 (Figure 2).

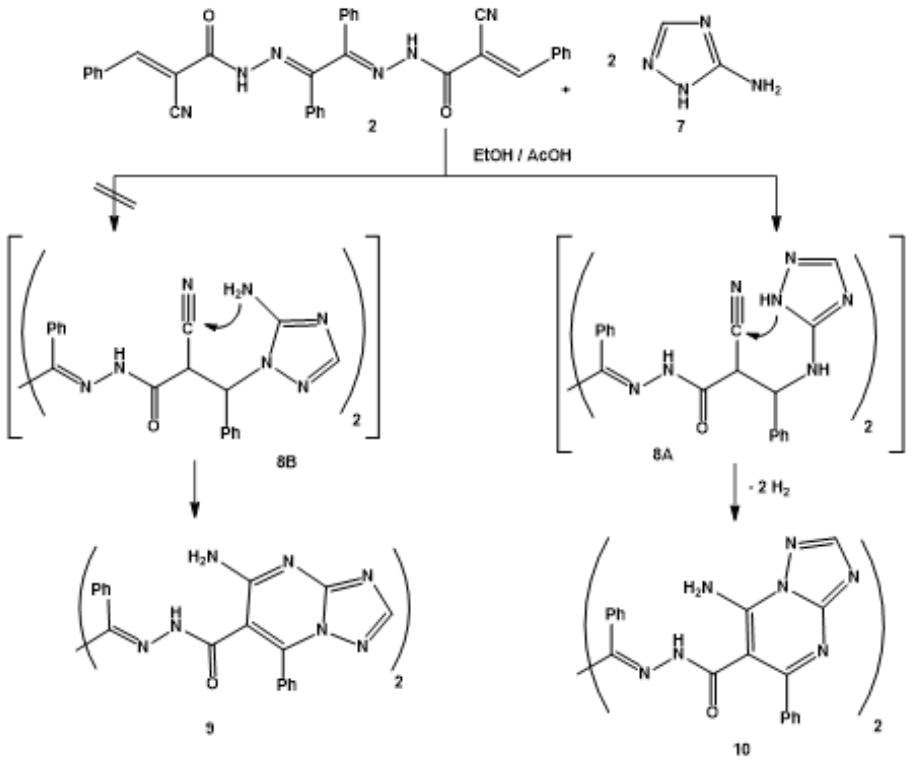

Figure 2: Synthesis of bis-triazolopyrimidine derivative 10.

The strategy of this reaction is depicted by nucleophilic attack of exocyclic amino group with activated acryloni-trile moiety [35] to afford adduct 8a. Intramolecular cyclization and molecular hydrogen elimination of the intermediate 8a furnished product 10 (Figure 2). The structural elucidation for compound 10 was achieved based on its spec-troscopic analysis. IR spectrum of compound 10 revealed the absence of (C≡N) absorption band and the presence of new absorption bands at 3436, 3312 cm-1 assigned to the amino group and another band at 1662 cm-1 due to the carbonyl group. 1H-NMR spectrum displayed down-field singlet signals at 8.77 and 10.12 ppm attributed to CH-pyrazole [36] and NH group, respectively.

Shifting to Figure 3, novel bis(tetrazolo[1,5-a]pyrimidine) 12, bis(benzimidazo-[1,2-a]pyrimidine) 14, and bis(pyrido[2`,3`:3,4]pyrazolo[1,5-a] pyrimidine) 16 were synthesized *via* treatment of bis(2-cyano-3-phenylacrylohydrazide) 2 with 5-amino-1H-[1,2,3,4]tetrazole (11), 2-aminobenzimidazole (13), and 3-amino-4,6-dimethyl-2H-pyrazolo[3,4-b]pyridine (15), respectively, by adopting the same reaction conditions. The assignment of the structures of novel bis-fused azoles 12, 14, 16 was based on analytical and spectral data (In the Expt Section) (figure 3).

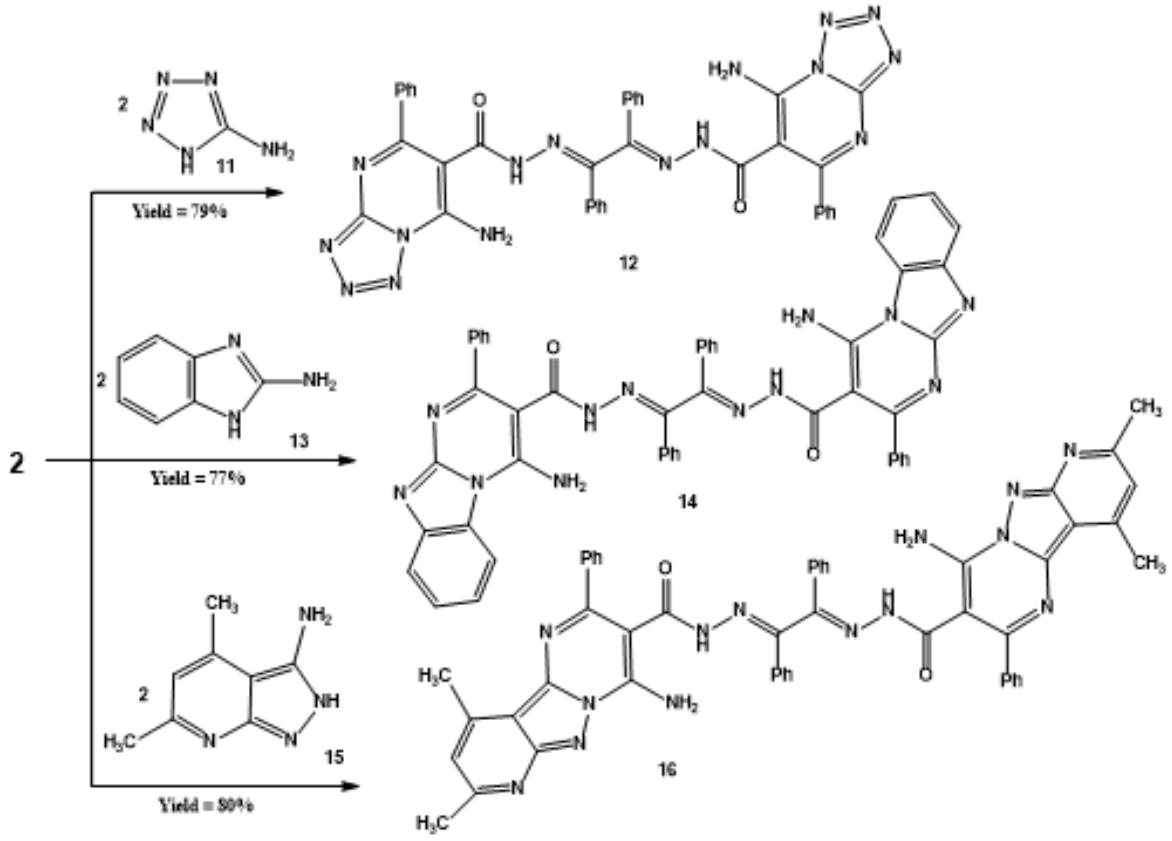

Figure 3: Synthesis of bis-fused pyrimidine derivatives 14, 16, and 18.

To further explore the synthetic utility of bis(2-cyano-3-phenylacrylohydrazide) 2, we investigated it reactivity towards other amino heterocyclic compounds. Thus, treatment of compound 2 with 2-aminothiazole (17), 2-aminobenzothiazole (19), 2-aminopyridine (21), and 2-aminopyrimidine (23) in ethanolic solution with catalytic amount of acetic acid under thermal condition furnished the respective bis(thiazolo[3,2-*a*]pyrimidine) 18, bis(benzothiazolo[3,2-*a*]pyrimidine) 20, bis(pyrido[1,2-*a*]pyrimidine) 22, and bis(pyrimido[1,2-*a*]pyrimidine) 24 (Scheme 4). Elemental analysis and spectral data agreed with the formation of the isolated products.

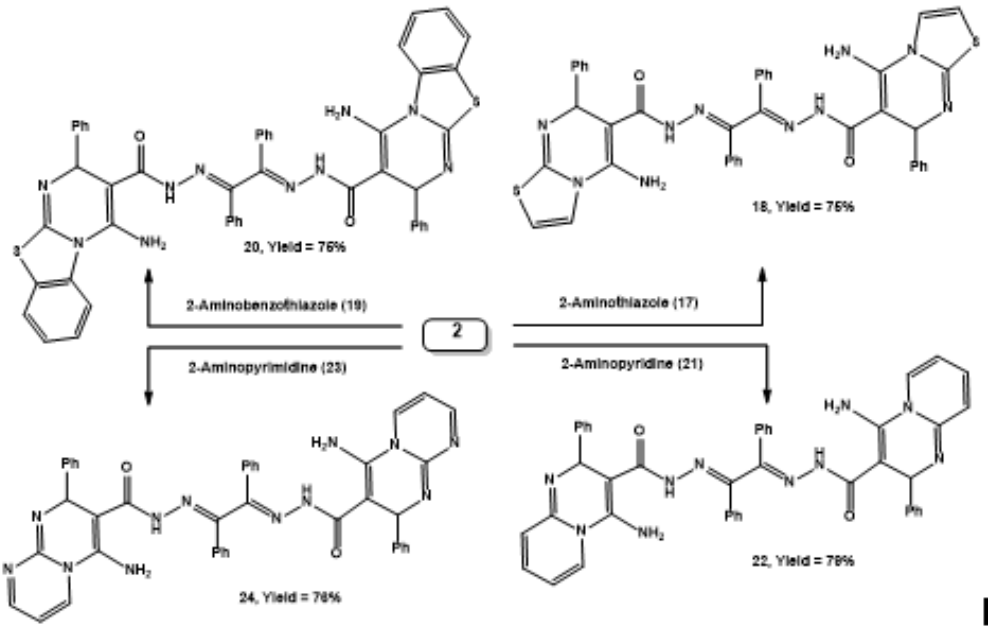

Figure 4: Synthesis of bis-fused pyrimidine derivatives 18, 20, 22 and 24.

In silico docking studies

The newly synthesized compounds were allowed to dock with the active site of *Staphylococcus aureus* tyrosyl-tRNA synthetase, in order to study the binding mode of interactions, using PyRx software. From the data gotten the screened molecules exhibited respectable fitting to the active site of the target enzyme and having binding energy ranging from-15.4 to-9.7 kcal/mol, as represented in Table 1. The 3D interactions between the derivatives and the binding region of the target were visualized using Discovery studio 3.5 as shown in Figure 1.

Compound 6a displayed binding energy (Δ G) of-10.0 kcal/mol and three hydrogen bond interactions with the amino acid residues THR75, THR75 and LEU173 at the distances of 2.95, 1.90 and 2.40 Å respectively. Compound 6b docked to the target though one H-bond with GLN190 with bond length 2.94 Å. In addition, the compound 10 exhibited one H-bond with the residue ASN124 at the distance 2.93 Å. The compound 12 showed three H-bond interactions with the amino acid residues SER82, SER154 and THR171. Compound 14 unveiled binding energy of-13.6 kcal/mol and 3 types of interactions like H-bond, arene-cation and arene-sigma interactions with ILE71, SER82, ARG88 at 3.13, 2.20, 6.93 and 3.69 Å respectively. Moreover, compound 16 with the best binding energy (Δ G)-15.4 kcal/mol and showed the best interactions through H-bonds with the amino acid residues SER82, SER154, TRP197, ASP153 and GLY15. The compounds 18 and 20 formed H-bond interactions with SER82 and THR75 at 2.23 and 3.10 Å respectively. Finally, compounds 22 and 24 exhibited arene-sigma interaction with the residue ILE71 with bond lengths 3.93 and 3.97 Å respectively.

ADMET and drug-likeness prediction

According to Lipinski's rule [37] using Mol inspiration tool as tabulated in Table 2, the tested compounds 6a, 10, 12, 14, 22, and 24 satisfy the Ro5 and meet all criteria of for good permeability and bioavailability displaying rotatable bonds number in the range <10, which means they are flexible [38]. Their n_{OHNH} and n_{ON} values are in the acceptable range except 6a, giving them higher solubility in cellular membranes [39]. The milogp values less than 5, exhibiting them good lipophilicity character. Furthermore, the ADMET parameters [40], as declared in Table 3, show that newly compounds have better Human Intestinal Absorption (%HIA) scores, and good Blood-Brain Barrier (BBB) values, which mean that they could be better absorbed by the human intestine [41]. In addition, all of them exhibited negative AMES toxicity and carcinogenicity test which means that they are safe and non-mutagenic [42] (Figure 4 and Table 1).

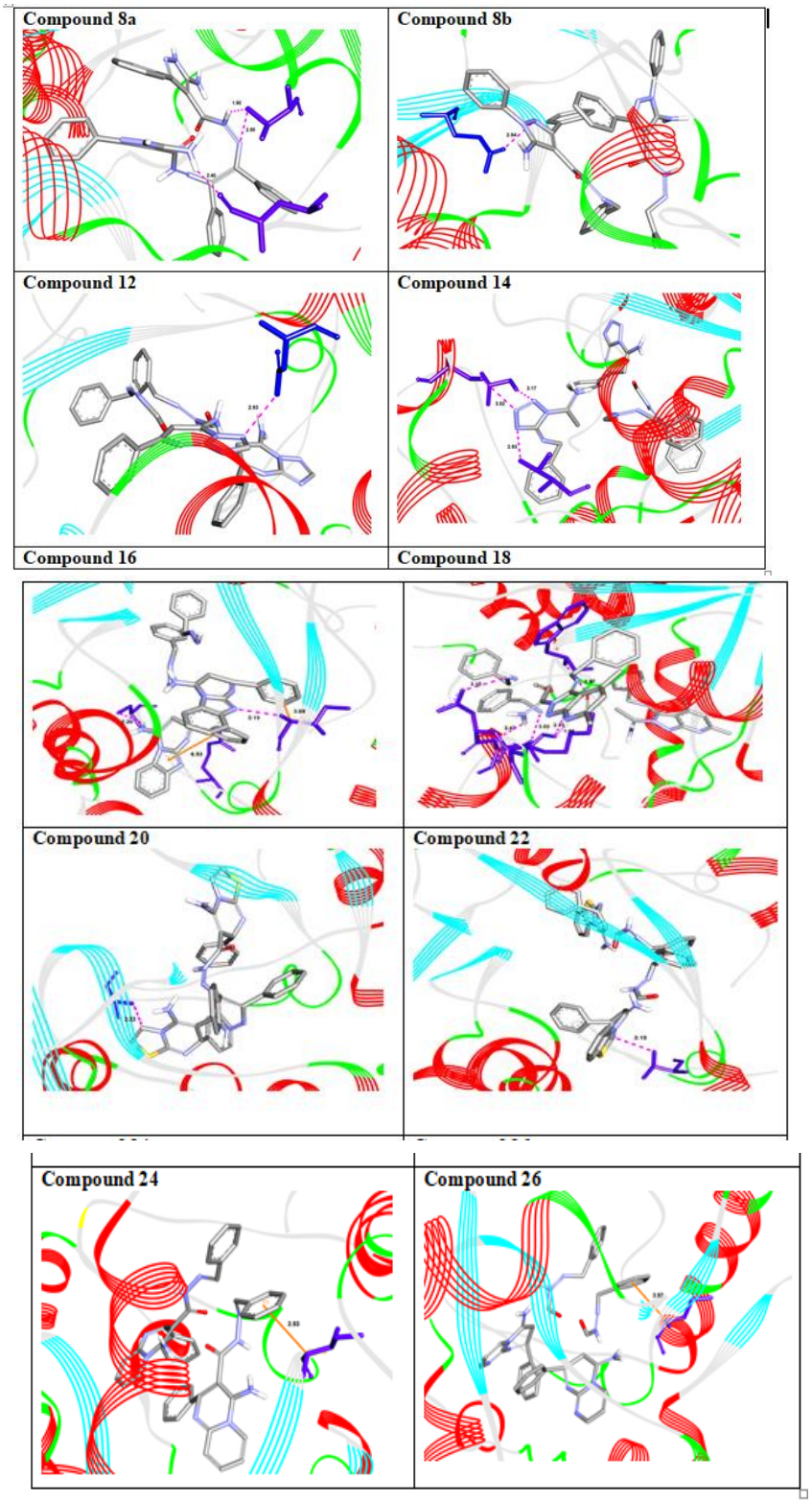

Figure 4: 3D closest interactions between the compounds and the active site residues of *Staphylococcus aureus* tyrosyl-tRNA synthetase.

Compd. No.	Binding energy (ΔG) (kcal/mol)	Intermolecular interactions	Bond length (Å)
Compound 6a	-10	H-bonds	
		THR75:OG1---compound 6a	2.95
		LEU173:O---compound 6a	2.4
		THR75:OG1---compound 6a	1.9
Compound 6b	-11	H-bonds	
		GLN190:OE1---compound 6b	2.94
Compound 10	-13.4	H-bonds	
		ASN124:ND2---compound 10	2.93
Compound 12	-14.7	H-bonds	
		SER82:OG---compound 12	3.17
		SER154:OG---compound 12	3.02
		THR171:OG1---compound 12	2.93
Compound 14	-13.6	H-bonds	
		ILE71:N---compound 14	3.13
		SER82:OG---compound 14	2.2
		arene-cation	
		ARG88:NH1---compound 14	6.93
		arene -sigma	
		ILE71:CA---compound 14	3.69
Compound 16	-15.4	H-bonds	
		SER82:OG---compound 16	2.7
		SER154:OG---compound 16	2.54
		TRP197:N---compound 16	2.97
		ASP153:OD1---compound 16	3.15
		GLY151:O---compound 16	2.47
		SER154:OG---compound 16	3.03
		SER82:OG---compound 16	1.98
Compound 18	-9.7	H-bonds	2.23
		SER82:OG---compound 18	
Compound 20	-11.9	H-bonds	3.1
		THR75:OG1---compound 20	
Compound 22	-10.4	arene -sigma	
		ILE71:CA---compound 22	3.93
Compound 24	-10.6	arene -sigma	
		ILE71:CA---compound 24	3.97

Table 1: The docking binding energies (Δ G), Intermolecular interactions, and bond length between the newly compounds and the active site of the target enzyme.

milogp, logarithm ratio of partition coefficient between n-octanol and water; TPSA, topological polar surface area; MW, molecular weight; n_{ON}, number of hydrogen bond acceptors; n_{OHNH}, number of hydrogen bond donors; $n_{rotb.}$, number of rotatable bonds (Table 2).

Compd. No.	milogp ≤5	TPSA ≤140	MW 130-725	nON 2.0-20.0	nOHNH 0.0-6.0	nrotb. ≤10	Vol A3
6a	4.14	192.3	608.6	12	8	9	534.6
6b	6.82	170.6	760.8	12	6	11	678.2
10	3.62	221.1	712.7	16	6	9	606.7
12	3.34	246.9	714.7	18	6	9	598.4
14	7.7	195.3	810.8	14	6	9	703
16	7.53	221.1	868.9	16	6	9	760.9
18	6.65	169.5	748.9	12	6	9	638.4
20	8.98	169.5	849	12	6	9	726.4
22	5	169.57	736.8	12	6	9	657
24	3.19	195.3	738.8	14	6	9	648.7

Table 2: Physicochemical properties of the title compounds 6a-24.

The pharmacokinetic parameters of compounds are evaluated using admetSAR (Table 3).

	Blood-Brain Barrier (BBB+)	Caco-2 Permeability (Caco2+)	%Human Intestinal Absorption (HIA+)	AMES toxicity	Carcinogenicity
Reference range	-3 to 1.2	<25 poor >500 great	<25 poor >80 high	Nontoxic	Non carcinogenic
6a	0.955	57.14	97.7	Nontoxic	Non carcinogenic
6b	0.975	50	98.42	Nontoxic	Non carcinogenic
10	0.903	54.22	99.69	Nontoxic	Non carcinogenic
12	0.898	54.45	99.69	Nontoxic	Non carcinogenic
14	0.894	53.71	99.66	Nontoxic	Non carcinogenic
16	0.867	53.25	98.53	Nontoxic	Non carcinogenic
18	0.539	58.33	97.77	Nontoxic	Non carcinogenic
20	0.539	58.33	97.77	Nontoxic	Non carcinogenic
22	0.693	53.96	97.56	Nontoxic	Non carcinogenic
24	0.693	53.96	97.56	Nontoxic	Non carcinogenic

Table 3: List of ADMET properties of molecules 6a-24.

The in vitro antibacterial screening

The newly synthesized compounds 10, 12, 14, and 16 were assayed *in vitro* for their antibacterial activity against three Gram-Positive Bacteria: Staphylococcus aureus (SA, RCMB000106), Staphylococcus epidermidis (SE, RCMB010024), Bacillus subtilis (BS, RCMB 000107), and two Gram-negative bacteria: Klebsiella pneumoniae (KP, RCMB000111), Salmonella typhimurium (ST, RCMB010072). Gentamicin, and Ampicillin were used as standard drugs. The microorganisms were tested against the activity of solutions of concentration 30 µg/mL of each compound and using an inhibition zone diameter (IZD) in mm/mg sample as a criterion for the antibacterial activity. The overall measurement results were summarized in Table 4.

Microorganisms					
	Gram-positive Bacteria			Gram-negative Bacteria	
Sample	**SA**	**SE**	**BS**	**KP**	**ST**
10	22.4 ± 0.55	17.2 ± 0.84	21.5 ± 0.62	18.4 ± 0.20	NA
12	22.1 ± 0.57	18.5 ± 0.73	26.4 ± 0.57	NA	NA
14	20.1 ± 0.58	18.3 ± 0.55	23.7 ± 0.90	16.3 ± 0.53	NA
16	26.3 ± 0.31	21.7 ± 0.50	29.3 ± 0.51	18.1 ± 0.75	17.4 ± 0.98
Ampicillin	23.7 ± 0.63	22.4 ± 0.3	32.4 ± 1.2	ND	ND
Gentamicin	ND	ND	ND	22.6 ± 0.63	23.3 ± 0.58

Table 4: Preliminary antibacterial activity for the synthesized compounds 10, 12, 14 and 16 was shown as minimum inhibitory concentration (MIC) in µg/ml of the tested microorganisms. **Abbreviations**: NA: No activity, ND***: not determined, SA*** *(Staphylococcus aureus RCMB000106),* ***SE*** *(Staphylococcus epidermidis RCMB010024),* ***BS*** *(Bacillus subtilis RCMB 000107),* ***KP*** *(Klebsiella pneumoniae RCMB000111),* ***ST*** *(Salmonella typhimurium RCMB010072)*. Values are mean inhibition zone diameter (mm) ± standard deviation of three replicates.

The results in Table 4 revealed that, compound **16** could efficiently inhibit the growth of *Staphylococcus aureus* more than Ampicillin and its inhibition activity against *Gram-negative* bacteria was also comparable to Gentamicin. On the other hand, compounds **10**, **12**, and **14** could effectively, to some extent, inhibit the growth of all tested strains *in vitro*.

The minimum inhibitory concentration (MIC) of compounds 10, 12 and 14 was also determined and the obtained results were shown in Table 5.

Microorganism	10	12	14	16	ST. (30 µg / mL)
Gram positive Bacteria					**Ampicillin**
SA	1.83	3.98	2.9	0.93	0.98
SE	2.48	3.9	3.63	2.04	1.95
BS	1.95	0.49	1.98	1.13	0.49
Gram negative Bacteria					**Gentamicin**
KP	7.63	ND	9.48	1.59	0.98
ST	ND	ND	ND	1.31	0.98

Table 5: The minimum inhibitory concentration of compounds 10, 12, 14 and 16 (µg / mL).***ND:*** *not determined,* ***SA*** *(Staphylococcus aureus RCMB000106),* ***SE*** *(Staphylococcus epidermidis RCMB010024),* ***BS*** *(Bacillus subtilis RCMB 000107),* ***KP*** *(Klebsiella pneumoniae RCMB000111),* ***ST*** *(Salmonella typhimurium RCMB010072.*

The results revealed that, MIC of compound **16** (0.93 µg/mL) was found to be similar to Ampicillin reference drug (0.98 µg/mL) against *Staphylococcus aureus.* Also, a significant MIC values were estimated for compound **16** against *Gram-Positive* and *Gram-Negative* bacteria. Moreover, compounds **10**, **12**, and **14** showed an appreciable broad spectrum of action against both *Gram-Positive* and *Gram-Negative* bacteria. Based on the antimicrobial evaluation, it is expected that compound **16** may be used for the development of new antibacterial agents.

Docking study

To assess the antibacterial activity of the prepared compounds against *Staphylococcus aureus* tyrosyl-tRNA synthetase, the molecular docking approach was performed [43-51]. The intermolecular interactions between the compounds and the active site residues of the enzyme are investigated using PyRx-virtual screening tool of Auto dock 4.2 [52]. The crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase is downloaded from RCSB data bank website (PDB code 1JIJ) [3]. The structure was prepared for further study by removing water molecules then addition and elimination of polar hydrogen atoms. The active site region is identified based on the co-crystallized receptor-ligand complex structure. The 2D structures of compounds are drawn using ChemDraw ultra 7.0, then converted to SDF form using Open Babel tool [53], and saved. The docking algorithm of the compounds was achieved using PyRx tool. Several poses with different binding energies for every single compound were produced after docking process, and the pose with the minimum free binding energy is selected for further study [54]. Discovery studio 3.5 software is used to visualize the 3D interactions between the compounds and the target. Moreover, the physicochemical and ADMET (absorption, distribution, metabolic, excretion and toxicity) properties of compounds are also further evaluated using *in silico* tools such as Mol inspiration and admetSAR web-based servers.

Antibacterial evaluation

The synthesized hybrids were evaluated for potential antibacterial activities against different microbes using the agar diffusion method and were compared to standard reference drugs using the reported methods [55-56].

CONCLUSION

The objective of the present study was to synthesize bis-pyrazoles and *N*-bridged bis-fused azoles comprising basically the hydrazide moiety (=N-NH-CO) and investigate their biological activities. The molecular docking results show that compound 16 has the minimum binding energy (-15.4 kcal/mol) and the best interactions with the target. In addition, the other compounds docked nicely to the active site of the target through various intermolecular interactions. Consequently, the newly synthesized compounds would represent a fruitful matrix for the future development of a new class of bioactive agents against *Staphylococcus aureus* tyrosyl-tRNA synthetase. The antibacterial evaluation results exploring the high potency of the synthesized compounds 10, 12, 14, and 16 against the tested microorganisms with lower MIC values. The docking studies are in agreement with the corresponding experimental antibacterial results.

FUNDING

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through a General Research Project under grant number (R.G.P.1/205/41).

COFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

[1] Pisano M B., Kumar A., Medda R., et al., *NCBI,***2019,**24(15):2815.

[2] Othman I M M.,Elkareem M A M.,Anouar E I H.,et al., *Bioorg Chem,* **2020,** 102:104105.

[3] Qiu X., Janson C A., Smith W W., et al., *Protein Sci,* **2001,** 10(10):2008-2016.

[4] Kheder N. A., Riyadh S.M., Asiry A M., *Chem Pharm Bull (Tokyo),* **2013,** 61(5):504-510.

[5] Fang B., Zhou C H., Rao X C., *Eur J Med Chem,* **2010,** 45(9):4388-4398.
[6] Shaker R. M.., Mahmoud A F., Abdel-Latif F F., *Phos., Sulfur, Silicon,* **2005,** 180, 397-406.
[7] Gomha S M., Kheder N A., Abdelhamid A O., *Molecules,* **2016,** 21(11):1532.
[8] El-Enany W A M A., Gomha S M., Hussein W., *Polycyclic Aromatic Comp,* **2020.**
[9] Gomha S M., Muhammad Z A., El-Reedy A.A. M., *J. Heterocycl Chem,* **2018,** 55(10): 2360-2367.
[10] Gomha S M., Edrees M M, Altalbawy F M A., *Int. J. Mol. Sci,* **2016,** 17(9): 1499.
[11] Holla B S., Poojary K N., Rao B S., *Eur J Med Chem,* **2002,** 37(6):511-7.
[12] El-Enany W A M A., Gomha S M., El-Ziaty A K., *Synth.Commu,* **2019,** 50(1): 85-96.
[13] Gomha S. M., Riyadh S. M., Abdalla M. M., *Curr. Org. Synth,* **2015,** 12(2): 220-228.
[14] Sellanes D., Scarone, L., Mahler G., et al., *Lett. Drug Des. Discov,* **2006,** 3(1):35-43.
[15] Abbas A. A., Rateb N M., *Sulfur Silicon,* **2005,** 180(2):497-511.
[16] Pu K., Wang L., Liu J., RCS adv, **2020,** 10(22): 13185-13195.
[17] Gomha S M., Abdelrazek F M., Abdelrahman A H., *J. Heterocycl. Chem,* **2018,** 55(7): 1729-1737.
[18] Ibrahim H M., Behbehani H., Makhseed S., et al., *Molecules,* **2011,** 16(5):3723-3739.
[19] El-Sayed H A., Abd Elazim M H M., Assy M G., *J. Heterocycl. Chem,* **2020,** 57(4): 1974-1980.
[20] Hosseini H., Bayat M., *Topics Cur. Chem,* **2018**, 376(6):40.
[21] Abu-Melha S., Edrees M M., Riyadh S M., et al., *Molecules,* **2020,** 25(19), 4565-4578.
[22] Gomha S M., Riyadh S M., *Molecules,* **2011,** 16(10): 8244–8256.
[23] Abbas I M., Gomha S M., Elneairy M A A., et al., *J. Serb. Chem. Soc,* **2015,** 80(10):1251-1264.
[24] Gomha S M., Mohamed A M G., Zaki YH., *J. Heterocycl. Chem,* **2018,** 55(5): 1147-1156.
[25] Gomha S M., Hassaneen H M E., *Molecules,* **2011,** 16(8):6549-6560.
[26] Gomha S M., Dawood K M., *J. Chem. Res,* **2014,** 38(9): 515-519.
[27] Abbas I M., Gomha S M., Elaasser M.M., et al., *Turk. J. Chem,* **2015,** 39(2):334-346.
[28] Gomha S M., Abdelrazek F M., Abdelrahman A. H.,et al., *Letters in Drug Design and Discovery*, b 14(7):752-762.
[29] Gomha S M., Muhammad Z A., Al-Hussain S A., *Polycyclic Aromatic Compounds,* **2020.**
[30] Abdelrazek F M., Gomha S M., Shaaban M E B.,et al., *Mini-Reviews in Medicinal Chemistry,* **2019,** 19(6): 527-538.
[31] Sayed, A. R., Gomha, S. M., Abd El-lateef, H. M., et al., *Green Chemistry Letters and Reviews,* **2021,**14(2):180-189.
[32] Kheder N. A., Altalbawy F M A., *Int. J. Pharm. Pharm. Sci,* **2016,** 8(1): 420-427.
[33] Salman A S., *Org. Chem. Int,* **2013.**
[34] Anwar HF, Fleita DH, Kolshorn H, et al., *Arkivoc.* **2006,** 15:133-41.
[35] Al-Mousawi S M., Moustafa M S., Elnagdi M H., *Molecules,* **2011,** 16(5): 3456-3468.
[36] Vevers R J S., *J. Chem. Soc*, **1976**, 421-428.
[37] Lipinski C A, Lombardo F, Dominy B W, et al., *Adv Drug Deliv Rev,* **2001,** 46(1): 3-26.
[38] Abdelmonsef A H., Mohamed M E., El-Naggar M., et al., *Front. Mol. Biosci,* **2020,** 7: 105.
[39] Noser A A., El-Naggar M., Abdelmonsef A H., et al., *Molecules,* **2020,** 25(20): 4780.
[40] Wang Y., Xing J., Xu Y., et al., *Q Rev Biophys,***2015,** 48(4):488-515.
[41] Abdelmonsef A H., *EJMHG,* **2019**.
[42] Gomha S.M., Abdelhady H.A., Abdelmonsef, A.H., et al., *Drug Des Devel Ther,* **2021,** 15: 659-677.
[43] Meng X Y., Zhang H X., Mezei M., Curr Comput Aided Drug Des, **2011,** 7(2):146-157.
[44] El-Maghraby A M., Abdelmonsef A H.,*Egy J Chem,* **2020,** 63(4):1341-58.
[45] Dasari T., Kondagari B., Abdelmonsef A H., et al., *J Bio Struc Dyn,* **2017,** 35(14):3119-39.
[46] Abdelmonsef A H., Dulapalli R., Dasari T., et al., *Comb chem through screen,* **2016,** 19(10):875-92.
[47] Rashdan H R., Shehadi I A., Abdelmonsef A H.,*ACS omega,* **2021,** 6(2):1445-55.

[48] Shehadi I A., Rashdan H R., Abdelmonsef A H., *Comput math meth med*, **2020.**

[49] Abdelmonsef A H., Mosallam A M., *J Heter Chem*, **2020,** 57(4):1637-54.

[50] El-Naggar M., Mohamed M E., Mosallam A M., et al.,*Evol Bioinf*, **2020,**16:1-13

[51] El-Saghier A M., El-Naggar M. Hussein A H., et al., *Frontiers in chemistry*, **2021,** 9:1-4.

[52] Dallakyan S., Olson AJ., *Methods Mol Biol*, **2015,** 1263:243-250.

[53] O'Boyle N. M, Banck M., James C. A, et al., *J Cheminformatics*, **2011,** 3(1): 33.

[54] Rashdan H R M., Abdelmonsef A H., Shehadi I A., et al., *Molecules*, **2020,** 25(21): 4997.

[55] Wayne PA, CLSI, 2012.

[56] Ghorab M M., Alsaid M.S., El-Gaby., et al., Eur J Med Chem, 2016, 124: 299-310.

.