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New pyrazolo[1,5-a]pyrimidine, pyrimido[1',2':1,5]pyrazolo[3,4-d]pyridazine and its fused derivatives: synthesis, characterization and antimicrobial evaluation

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Abstract

Use pyrazolo[1,5-a]pyrimidine 4 as a precursor for synthesis of new heterocyclic compounds, where condensation with aromatic aldehydes gives arylidene derivatives 8a-c. Furthermore, when compound 4 is combined with aromatic diazonium chlorides 9a-c, it forms pyrazolo[3,4-d] pyridazines 11a-c. Additionally, reacting The unisolated potassium salt 13 with phenacyl bromide derivatives 14a,b or chloroacetonitrile at room temperature, thieno[3",4":5',6']pyrido[4',3':3,4]pyrazolo[1,5-a]pyrimidines 17a,b or 22 are obtained. Finally, compound 4 reacts with DMFDMA to produce enamine 23. The zone of inhibition measurement was used to gauge the synthetic compounds' in vitro antibacterial activity. The outcomes showed encouraging antibacterial efficacy against the harmful bacterial strains that were examined.

Keywords: pyrazole, antimicrobial, pyrimidine, arylidenes, phencyl bromide, DMFDMA.

Introduction

Heterocyclic derivatives incorporating pyrazolo[1,5-a]pyrimidines moieties show interesting medicinal applications such as antiviral [1], cytotoxic [2], antidepressant [3], antihypertensive[4], analgesic [5], antimicrobial activity [6], antimicrobial activity [7-10]. anticancer activities [11-18]. Notably, several commercially available drugs, as zaleplon, indiplon [19], ocinaplon [20], dinaciclib [21], dorsomorphin [22], anagliptin [23], and pyrazophos [24], incorporate in (**Fig. 1**).Furthermore, there has been extensive research on pyrazolo[1,5-a]pyrimidine analogs uses as inhibitors of kinases [25-29]. Numerous active compounds have been identified, including the B-Raf kinase inhibitor A [30] and the cyclin-dependent kinase inhibitor B [31] (**Fig. 2**).Building upon our previous research [9][32-34], we have decided to investigate the synthesis of novel fused compounds, including pyrazolo[1,5-a]pyrimidine, pyrimido[1',2':1,5]pyrazolo[3,4-d]pyridazine, thieno[3",4":5',6']pyrido[4',3':3,4]pyrazolo[1,5-a]pyrimidines. This will be achieved through the reactions of nucleus pyrazolo[1,5-a]pyrimidine (**4**) with different nucleophile reagents

Experimental

Using a Koffler melting point apparatus, the melting points of all compounds were computed, and the findings were provided without any modifications. Infrared (IR) spectra were gathered using a Nicolet 710 FT-IR spectrometer. Using DMSO-d6 or CDCl3 as solvents, proton nuclear magnetic resonance (1H NMR) and carbon-13 nuclear magnetic resonance (13C NMR) spectra were obtained using a Bruker A Vance III-400 MHz apparatus. The elements microanalysis was performed using a Perkin-Elmer CHN-2400 elemental analyzer. The purity of each component was assessed using TLC plates.

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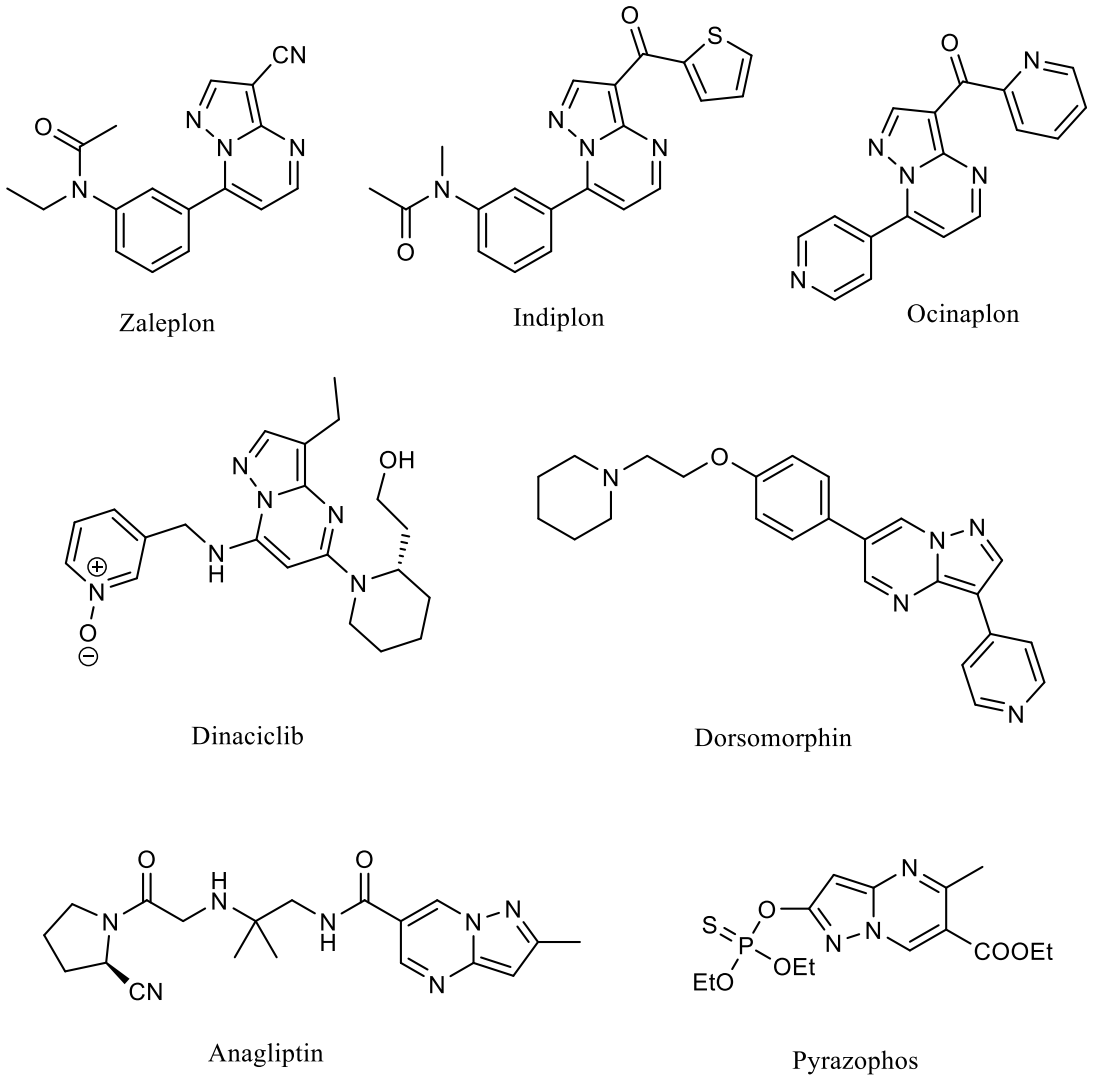


Figure 1. Examples of pharmaceuticals having the pyrazolo[1,5-a]pyrimidine nucleus.

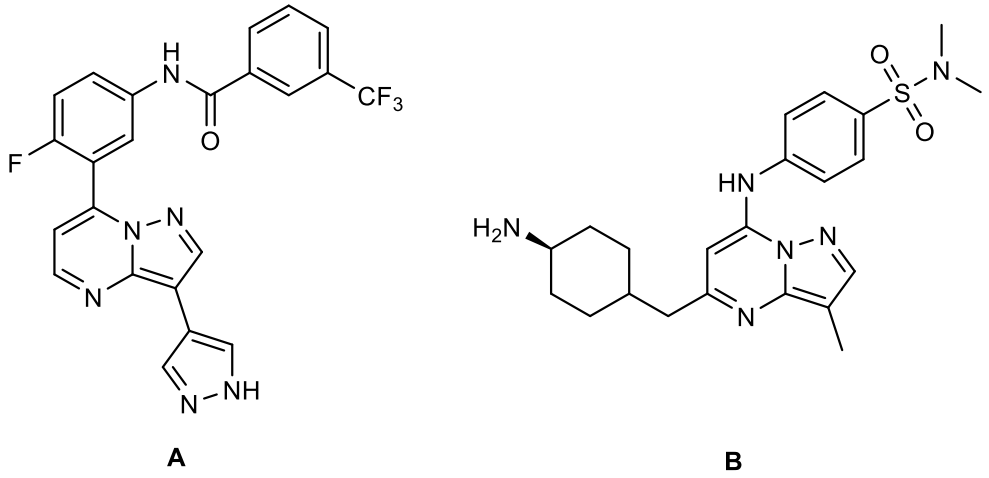


Figure 2. kinase inhibitors with pyrazolo[1,5-a]pyrimidine as their central component.

Chemistry

6-benzoyl-2-(cyanomethyl)-7-methylpyrazolo[1,5-a]pyrimidine-3-carbonitrile (4)

First method: Enamine (5) (2.02 g, 0.01 mol) and benzoyl acetone (**1**) (1.62 g, 0.01 mol) were refluxed for 4 hours in acetic acid (30 ml), cooled off, poured onto ice water (50 ml) and collected by filtration.

Second method: refluxed mixture of 5-aminopyrazole (**3)** (1.47 gm, 0.01 mol) with enamine (**2**) (2.17 gm, 0.01 mol) for 4 hr in acetic acid (30 ml), cooled off, poured onto ice water (50 ml) and collected by filtration.

As pall yellow crystals from ethanol; m.p.138-140 °C; Yield: (2.1g, 69.7%); IR (KBr, cm^{-1}): 2258,2225(2CN) and 1661(C=O);^{1}H NMR (DMSO-d_6, ppm): 2.69 (s, 3H, CH_3), 4.53(s, 2H, CH_2), 7.52 – 8.05(m, 5H, aromatic) and 8.26(s, 1H, CH=N); ^{13}C NMR (DMSO-d6, ppm): 17.5, 24.9, 80.8, 113.1, 116.6, 128.0, 129.0, 130.2, 132.1, 135.7, 146.9, 149.2, 150.5, 159.2 and 165.0; calculated values for $C_{17}H_{11}N_5O$: C, 67.77; H, 3.68; N, 23.24; however, the actual values were: C, 67.19; H, 3.43; N, 22.96.

General procedure for synthesis of arylidenes 8a-c.

Compound (**4**) (3.01g, 0.01mole) and aldehydes **7a-c** (0.01mole) were refluxed together for 4 hours in 30ml of an ethanolic piperidine solution after being cooled off, filtered and then crystallized from the ethanol.

6-benzoyl-2-(1-cyano-2-phenylvinyl)-7-methylpyrazolo[1,5-a]pyrimidine -3-carbonitrile (8a).

Obtained by using benzaldehyde (**7a**) (1.016 ml, 0.01 mole) as yellowish crystals. m.p. 220-222°C; Yield: (2.7g, 9.3%), IR (KBr, cm^{-1}): 2223(CN) and 1654(C=O);^{1}H NMR (DMSO-d6, ppm): 2.64(s, 3H, CH_3), 7.39–7.99(m, 10H, aromatic), 8.16(s, 1H, CH) and 8.29(s, 1H, CH=N); calculated values for $C_{24}H_{15}N_5O$: C, 74.02; H, 3.88; N, 17.98; however, the actual values were: C, 73.39; H, 3.19; N, 17.56.

6-benzoyl-2-(1-cyano-2-(p-tolyl)vinyl)-7-methylpyrazolo[1,5-a]pyrimidine-3-carbonitrile (8b).

Obtained by using 4-methylbenzaldehyde (**7b**) (1.17 ml, 0.01 mole) as brown crystals. m.p.>300 °C; Yield: (2.5 g, 61.9%); IR (KBr, cm^{-1}): 2220(CN) and 1650(C=O);^{1}H NMR (DMSO-d6, ppm): 2.41, 2.64(2s, 6H, $2CH_3$), 7.40 – 7.94(m, 9H, aromatic), 8.15(s, 1H, CH) and 8.24(s, 1H, CH=N); Anal. Calcd. $C_{25}H_{17}N_5O$: C, 74.43; H, 4.25; N, 17.36; Found: C, 74.12; H, 3.98; N, 16.94.

6-benzoyl-2-(1-cyano-2-(4-(dimethylamino)phenyl)vinyl)- 7-methylpyrazolo [1,5-a]pyrimidine-3-carbonitrile (8c).

Obtained by using 4-(dimethylamino)benzaldehyde (**7c**) (1.49g, 0.01 mole) as yellow crystals. m.p.240-242°C; Yield: (3.5g, 80.9%); IR (KBr, cm^{-1}): 2217(CN) and 1658(C=O);^{1}H NMR (DMSO-d6, ppm): 2.7(s, 3H, CH_3), 3.35[s, 6H, $N(CH_3)_2$], 6.85-8.05(m, 9H, aromatic), 8.14(s, 1H, CH) and 8.16(s, 1H, CH=N)); ^{13}C NMR (DMSO-d6, ppm): 24.9, 40.6, 77.6, 93.2, 112.2, 112.4, 114.1, 117.6, 119.8, 129.1, 129.8, 130.1, 132.1, 132.7, 146.5, 147.8, 152.5, 152.8, 153.2 and 164.7; calculated values for. $C_{26}H_{20}N_6O$: C, 72.21; H, 4.66; N, 19.43; however, the actual values were: C, 72.11; H, 4.14; N, 19.17.

General procedure for synthesis of compounds 11a-c.

Stirring the compound (**4**) cooled pyridine solution (30 mL, 3.01g, 0.01 mole), along with the gradually added aryl diazonium chloride **9a-c**, until the solid product developed, filtered, and re-crystallized from the suitable solvent.

3-benzoyl-10-imino-4-methyl-9-phenyl-9,10-dihydropyrimido[1',2':1,5]pyrazolo[3,4-d]pyridazine-7-carbonitrile (11a)

Obtained by using aniline (**9a**) (0.91 ml, 0.01 mole), as brown crystals. m.p. 238-240 °C; Yield: (2.4g, 59.1%); IR (KBr, cm^{-1}): 3236(NH), 2222(CN) and 1652(C=O); ^{1}H NMR (DMSO-d6, ppm): 2.64(s, 3H, CH_3), 7.1-8.08(m, 10H, aromatic), 8.29(s, 1H, CH=N) and 11.99(s, 1H, NH); ^{13}C NMR (DMSO-d6, ppm):17.2, 112.5, 117.4, 119.1, 121.6, 122.4, 127.1, 129.1, 129.6, 132.1, 132.6, 134.5, 139.8, 145.8, 148.1, 155.1, 157.2, 158.1 and 164.1; calculated values for $C_{23}H_{15}N_7O$: C, 68.14; H, 3.73; N, 24.18 however, the actual values were: C, 67.79; H, 3.21; N, 23.95.

3-benzoyl-10-imino-4-methyl-9-(p-tolyl)-9,10-dihydropyrimido[1',2':1,5]pyrazolo[3,4-d]pyridazine-7-carbonitrile (11b)

Obtained by using p-toluidine (**9b**) (1.07 g, 0.01 mole), as pall brown crystals. m.p.258-260 °C; Yield: (2.3 g, 54.8%); IR (KBr, cm^{-1}): 3236(NH), 2223(CN) and 1656(C=O);^{1}H NMR (DMSO-d6, ppm): 2.7, 2.86(2s, 6H, $2CH_3$),7.1-8.04(m, 9H, aromatic), 8.31(s,1H,CH=N), and 11.97 (s, 1H, NH));

^{13}C NMR (DMSO-d6, ppm): 17.5, 20.9, 111.6, 120.6, 121.3, 128.5, 129.1, 129.8, 129.9, 131.4, 132.3, 132.7, 133.1, 139.2, 142.8, 147.1, 154.9, 156.4, 157, and 165.1; calculated values for. $C_{24}H_{17}N_7O$: C, 68.72; H, 4.09; N, 23.38; however, the actual values were: C, 68.42; H, 3.92; N, 23.13.

3-benzoyl-10-imino-9-(4-methoxyphenyl)-4-methyl-9,10-dihydropyrimido[1',2':1,5]pyrazolo[3,4-d]pyridazine-7-carbonitrile (11c)

Obtained by using 4-methoxyaniline (**9c**) (1.23 gm, 0.01 mole), as brown crystals. m.p.>300 °C; Yield: (3.0gm, 68.8%); IR (KBr, cm^{-1}): 3424(NH), 2206(CN) and 1658(C=O);^{1}H NMR (DMSO-d6, ppm): 2.63, 3.75(2s, 6H, 2CH3), 6.95-8.1(m, 9H, aromatic),8.22(s,1H, CH=N) and 11.98 (s, 1H, NH)); ^{13}C NMR (DMSO-d6, ppm): 17.7, 55.7, 111.3, 114.4, 119.8, 121.6, 127.9, 128.9, 129.1, 132.4, 132.7, 133.1, 138.1, 140.1, 145.7, 152.9, 153.4, 154.8 and 163.3; calculated values for $C_{24}H_{17}N_7O_2$: C, 66.20; H, 3.94; N, 22.52; however, the actual values were: C, 66.11; H, 3.62; N, 22.13.

6-benzoyl-2-(1-cyano-2-(dimethylamino)vinyl)-7-methylpyrazolo[1,5-a]pyrimidine-3-carbonitrile (12)

Compound (**4**) was refluxed in dry dioxane (30 ml) with DMF-DMA (1.32 ml, 0.01 mole) for three hours, cooled, recovered by filtering, and recrystallized from ethanol as light brown crystals. m.p.218-220 °C;Yield: (2.2 g, 61.79%); IR (KBr, cm-1): 3072(CH-aromatic), 2926(CH-aliphatic), 2206(C≡N) and 1668(C=O);^{1}H NMR (DMSO-d6, ppm): 2.63(s, 3H, CH_3), 3.38[s, 6H, $N(CH_3)_2$], 7.39(s, 1H, CH-enamine),7.43-7.87(m,5H,phenyl) and 8.12 (s, 1H, CH-pyrimidine); ^{13}C NMR (DMSO-d6, ppm): 27.0, 42.8, 77.2, 78.4, 113.7, 121.2, 128.2, 129.0, 131.3, 132.4, 134.2, 150.1, 152.3, 153.6, 155.8, 164.2 and 169.5; Anal. Calcd. $C_{20}H_{16}N_6O$: C, 67.40; H, 4.53; N, 23.58; Found: C, 67.26; H, 4.19; N, 23.32.

General procedure for synthesis of compounds 18a-b and 23.

At room temperature, compound (**4**) (3.01g, 0.01 mole), potassium hydroxide (0.01 mole), and dimethylformamide (15 ml) were mixed for an hour before adding phenyl isothiocyanate (0.01 mole) and stirring for a further three hours. then mix for 4 hours while adding the chloroacetonitrile (**20**) or **15a–c** phenacyl bromide derivatives (0.01 mole). The mixture was poured over water and ice. The resultant solid was removed through filtration, cleaned with water, and then crystallised again using a 3:1 mixture of ethanol and DMF.

5-amino-8-benzoyl-9-methyl-1-(phenylamino)thieno[3'',4'':5',6']pyrido[4',3':3,4]pyrazolo[1,5-a]pyrimidin-3-yl)(p-tolyl)methanone (18a)

Obtained by using 2-bromo-1-(p-tolyl)ethan-1-one (**15a**) (2.13 gm, 0.01 mole), as brown crystals. m.p.>300 °C; Yield: (3.9g, 68.5%); IR (KBr, cm^{-1}): 3452, 3314(NH_2+NH) and 1685, 1654(2C=O);^{1}H NMR (DMSO-d6, ppm): 2.32, 2.73(s, 6H, $2CH_3$), 7.24(s, 2H, NH_2) 7.45-8.1(m, 9H, aromatic), 8.26(s, 1H, CH=N) and 10.3(s,1H, NH); ^{13}C NMR (DMSO-d6, ppm): 17.4, 21.4, 104.7, 116.7, 122.3, 123.6, 128.6, 129.0, 129.3, 129.5, 129.6, 129.8, 132.4, 133.5, 133.9, 135.8, 139.7, 140.6, 142.1, 144.9, 150.2, 153.4, 154.2, 155.6, 157.1, 166.2 and 192.6 ; calculated values for $C_{33}H_{24}N_6O_2S$: C, 69.70; H, 4.25; N, 14.78; however, the actual values were: C, 69.39; H, 4.12; N, 14.51.

5-amino-3-(4-methoxybenzoyl)-9-methyl-1-(phenylamino)thieno[3'',4'':5',6']pyrido[4',3':3,4]pyrazolo[1,5-a]pyrimidin-8-yl)(phenyl)methanone (18b)

Obtained by using 2-bromo-1-(4-methoxyphenyl)ethan-1-one (**15b**) (2.29 g, 0.01 mole), as brown crystals. m.p.>300 °C; Yield: (3.9g, 68.5%); IR (KBr, cm^{-1}): 3412, 3365 (NH_2+NH) and 1688, 1664(2C=O);^{1}H NMR (DMSO-d6, ppm): 2.45, 3.56(s, 6H, 2CH3), 7.19(s, 2H, NH_2) 7.12-7.69(m, 9H, aromatic), 8.21(s, 1H, CH=N) and 10.12(s,1H, NH); ^{13}C NMR (DMSO-d6, ppm): 17.9, 53.8, 103.9, 114.6, 121.7, 125.4, 128.1, 129.2, 129.4, 129.6, 129.9, 130.7, 132.1, 133.1, 134.1, 136.6, 139.1, 140.5, 141.2, 144.1, 151.1, 153.1, 154.9, 156.2, 157.6 and 164.9calculated values for $C_{33}H_{24}N_6O_3S$: C, 67.79; H, 4.14; N, 14.37; however, the actual values were: C, 67.26; H, 3.96; N, 14.12.

5-amino-8-benzoyl-9-methyl-1-(phenylamino)thieno[3'',4'':5',6']pyrido [4',3':3,4]pyrazolo[1,5-a]pyrimidine-3-carbonitrile (23)

Obtained by using 2-chloroacetonitrile (**20**) (0.629 ml, 0.01 mole), as deep brown crystals. m.p. 248-250 °C; Yield: (2.6g, 54.6%); IR (KBr, cm^{-1}): 3423, 3290(NH_2+NH),2221(CN) and 1657(C=O); ^{1}H NMR (DMSO-d6, ppm): 2.6(s, 3H, CH_3), 3.35(s, 2H, NH_2) 7.1-7.98(m, 10H, aromatic), 8.26(s, 1H, CH=N) and 11.45(s,1H, NH); ^{13}C NMR (DMSO-d6, ppm): 17.9, 105.2, 107.9, 111.2, 117.6, 122.1, 123.5,

128.4, 129.2, 129.6, 129.9, 132.6, 133.9, 135.4, 139.5, 141.8, 146.2, 154.1, 155..8, 157.1 and 166.2; calculated values for $C_{26}H_{17}N_7OS$: C, 65.67; H, 3.60; N, 20.62; however, the actual values were: C, 65.17; H, 3.28; N, 20.13.

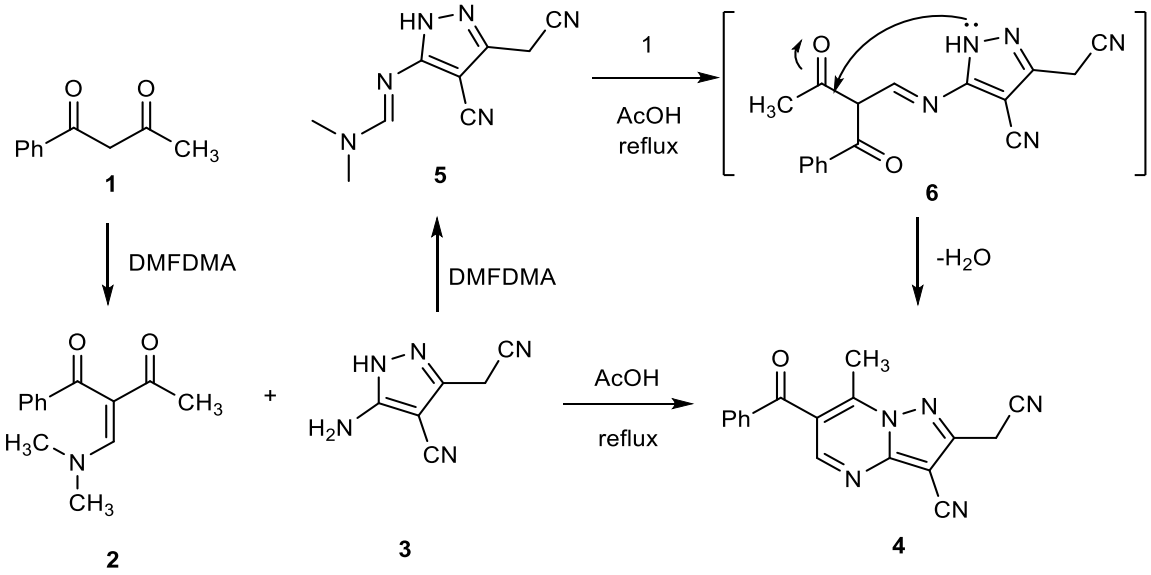
Antimicrobial Susceptibility Test:

Agar well diffusion method was utilized for assessing the antimicrobial potency of the synthesized compounds against test pathogenic microorganisms including Gram-positive bacteria (Bacillus subtilis ATCC 6633 and Staphylococcus aureus ATCC 6538) and Gram-negative bacteria (Escherichia coli ATCC 8739and Pseudomonas aeruginosa ATCC 90274), in addition to fungal strains Aspergillus niger ATCC 16404 and Candida albicans ATCC 10221. The bacterial strains' 24 h old broth cultures were used to inoculate Muller-Hinton plates, which were then tested for antibacterial activity. The antifungal activity was tested using Sabouraud dextrose plates that had previously been treated with a spore solution of several fungi. In pre-inoculated agar plates with an eight mm well diameter, 100 L of the 5% (w/v) test substance were added. Fluconazole (1 mg/mL) and gentamycin (1 mg/mL) were employed as positive controls for microorganisms. [35].

Results and Discussion

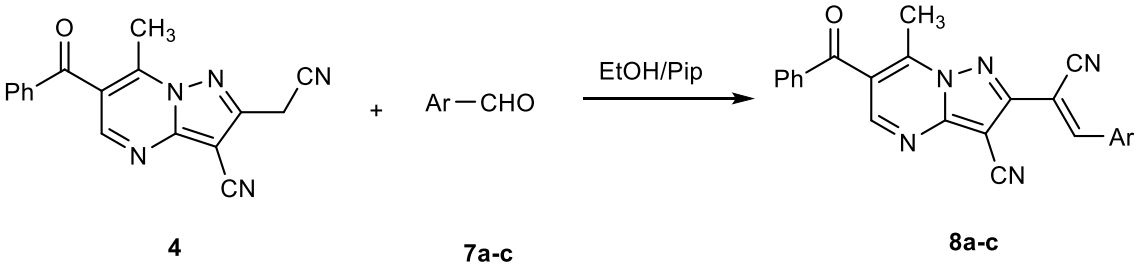
Chemistry

pyrazolo[1,5-a]pyrimidine-3-carbonitrile (**4**) was synthesized by refluxing of enamine (**2**) with 5-aminopyrazole (**3**) in acetic acid. Another rout of synthesis of compound (**4**) by refluxing of enamine (**5**) with benzoyl acetone (**1**) in acetic acid *via* intermediate (**6**) (**scheme 1**). Using spectral data, the structure of pyrazolo[1,5-a]pyrimidine (**4**) was determined. The IR spectrum showed bands for two cyano and carbonyl groups at 2258, 2225, and 1661 cm-1, respectively. ^{1}H NMR showed signals at δ_H 2.69, 3.39, 7.52-7.66 and 8.26 ppm characteristic to CH_3, CH_2, aromatic protons and proton of pyrimidine ring, respectively. ^{13}C NMR spectrum showed signals at δ_C17.5, 24.9, 80.8, 113.1, 116.6, 128.0, 129.0, 130.2, 132.1, 135.7, 146.9, 149.2, 150.5, 159.2 and 165.0 ppm.



Scheme 1; synthesis of pyrazolo[1,5-a]pyrimidine-3-carbonitrile (**4**)

Compound (**4**) is considered a valuable starting material for synthesizing novel heterocyclic compounds. When compound (**4**) was condensed with aromatic aldehydes **7a-c** in ethanolic piperidine solution, it resulted in the formation of arylidenes 8a-c (**Scheme 2**). Arylidene structures were determined through spectral data. In the ^{1}H NMR spectra of compounds 8a-c, the signal corresponding to methylene proton disappeared while new signals appeared around δ_H 7.9 ppm, characteristic to the CH-methine proton adjacent to the pyrimidine proton, along with an increase in aromatic protons. ^{13}C NMR spectra showed vanished the signals characteristic to methylene carbon and emerged new signals around δ_C 146 ppm, characteristic to the C-methine carbon.

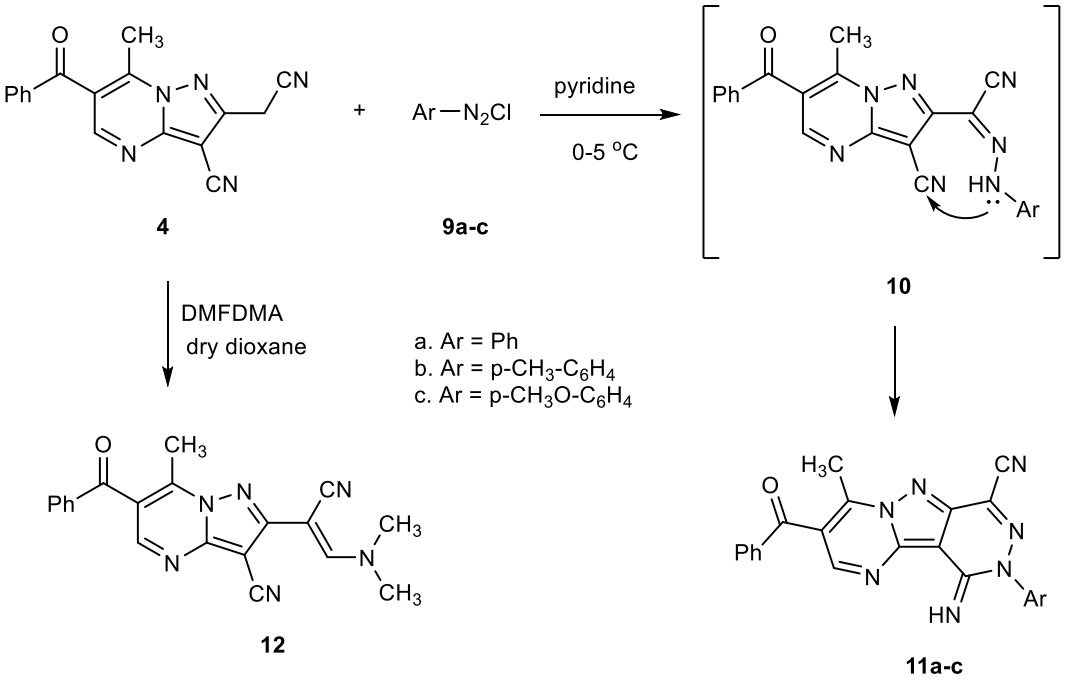


a. Ar = Ph
b. Ar = p-CH_3-C_6H_4
c. Ar = p-$(CH_3)_2$N-C_6H_4

Scheme 2; synthesis of arylidene derivatives **8a-c**

Additionally, the coupling of compounds (**4**) with aromatic diazonium chloride **9a-c** resulted in new derivatives of pyrimido[1',2':1,5]pyrazolo[3,4-d]pyridazine **11a-c** by intramolecular cyclization of **10a-c**. This cyclization happened as a result of the imino group's nucleophilic attack on the cyano group. (**Scheme 3**). The composition of compounds **11a-c** was established through spectral data. IR spectra, a new band appeared above 3200 cm-1, characteristic to NH group, in addition to absorption bands characteristic to the cyano and carbonyl groups. ^{1}H NMR spectra exhibited an exchangeable signal after δ_H 11 ppm, characteristic to imino group. Furthermore, the signals associated with the methylene carbon in ^{13}C NMR spectra vanished, whereas the number of aromatic carbons increased.

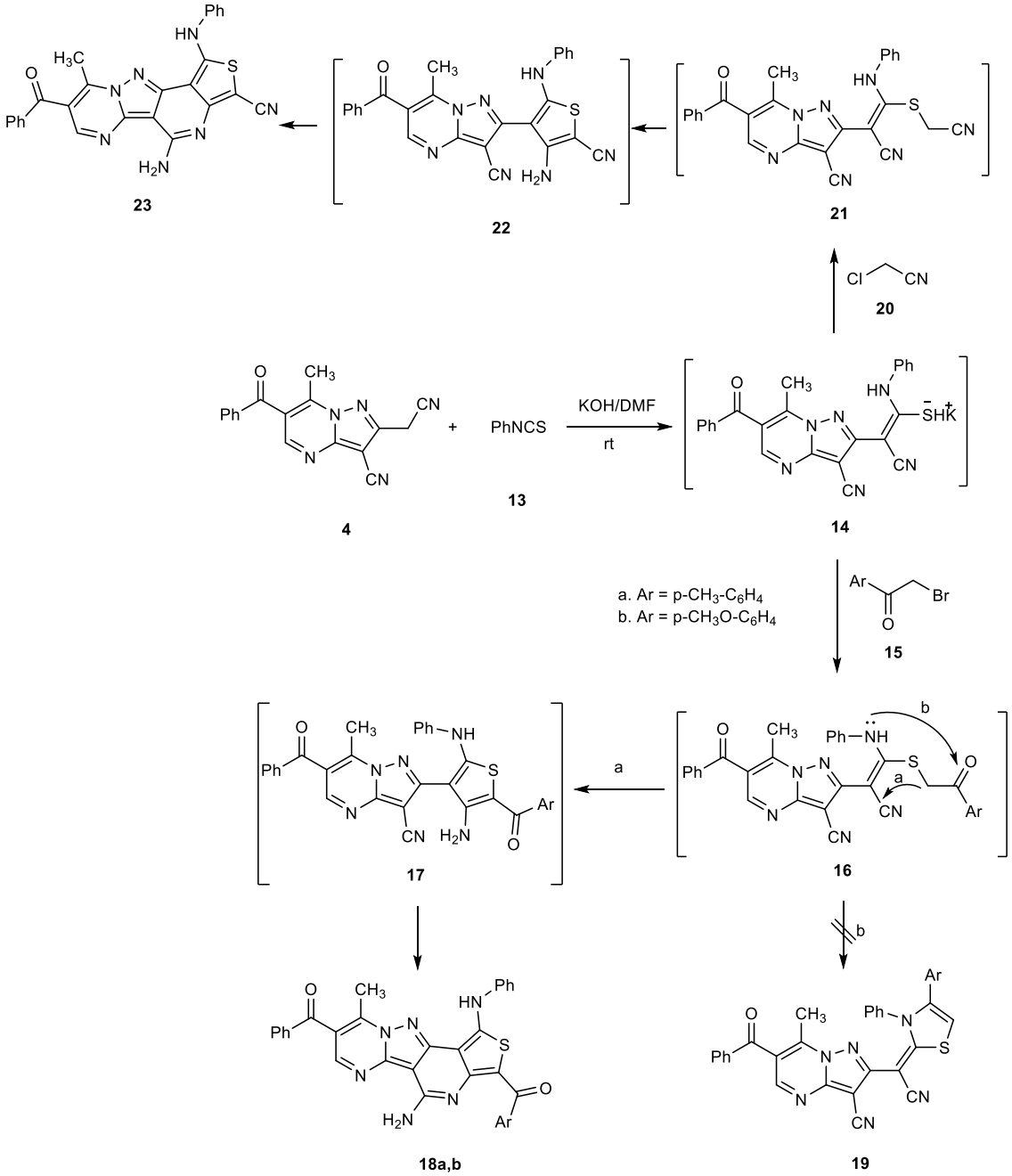
Also, compound (**4**) was refluxed with (DMF DMA) in dry dioxane to produce enamine (**12**) (**Scheme 3**). The enamine's structure was supported by its spectrum data. The singlet signals for $N(CH_3)_2$ and (CH-enamine) occurred at 3.38 and 7.39 ppm, respectively, replacing the methylene group signal that had previously been present in the 1H NMR spectrum. 13C NMR spectra revealed signals at the following C numbers: 27, 42.8, 77.2, 78.4, 113.7, 121.2, 128.2, 129, 131.3, 132.4, 134.2, 150.1, 152.3, 153.6, 155.8, 164.2, and 169.5.



a. Ar = Ph
b. Ar = p-CH_3-C_6H_4
c. Ar = p-CH_3O-C_6H_4

Scheme 3; synthesis ofpyrazolo[3,4-d]pyridazine derivatives **11a-c** and enamine **12**.

Finally, the unisolated potassium salt (**14**) reacted with phenacyl bromide derivatives **15a,b**or chloroacetonitrilein DMF at room temperature to yielded the corresponding pyrido[4',3':3,4]pyrazolo[1,5-a]pyrimidines **18a,b***via* intermediate **17a,b** (pathway a), instead of the expected thiazole derivatives **19a,b***via* intermediate **16a,b** (pathway b) or compound (**23**) *via* intermediates (**21**) and (**22**) (**Scheme 4**). Based on spectral data, the composition of the produced compounds **18a,b** was determined. The IR spectra detected bands at 3452, 3314, 1685, and 1654 cm-1, respectively, that were indicative of amino, imino, and two carbonyl groups. Exchangeable signals at 7.24 and 10.3 ppm, comparable to the amino and imino groups, respectively, were visible in the ^{1}H NMR spectra. The ^{13}C NMR spectra also showed a rise in aromatic carbons, the disappearance of signals unique to the methylene carbon, and the formation of a carbonyl group signal at 166 ppm.



Scheme 4; synthesis ofpyrido [4',3':3,4]pyrazolo[1,5-a]pyrimidine derivatives **18a,b** and **23**.

Antimicrobial activity:

In vitro testing was done on the synthesised compounds (**4**, **8a-c**, **11a-c**, **12**, **18a, b** and **23**) to see if they had any antibacterial or antifungal activity against different strains of bacteria and fungi. As stated in Table 1, the diameter of the inhibition zones (IZ) was measured to evaluate the antibacterial and antifungal activities. Gentamicin served as the study's reference medication. Findings indicated that all of the examined compounds (**4**, **8a-c**, **11a-c**, **12**, **18a, b** and **23**) significantly inhibited the growth of the tested bacterial strains in vitro, with the exception of compound 18a, which did not exhibit antibacterial activity against B. subtilis and P. aeruginosa. Except for compound (**12)**, all of the investigated compounds showed substantial suppression of C. albicans. Comparing the investigated substances to the standard treatment fluconazole, none of the tested substances shown antifungal activity against A. niger.

Table 1: Synthetic chemicals' antimicrobial activity. The inhibitory zones' diameter was expressed in millimeter's (mm).

Sample / Pathogenic microorganism	**4**	**8a**	**8b**	**8c**	**11a**	**11b**	**11c**	**12**	**18a**	**18b**	**23**	**Ref.**
B. subtilis (ATCC 6633)	27	21	23	28	30	29	25	25	NA	25	26	24
S. aureus (ATCC 6538)	17	17	18	18	18	16	18	23	10	22	19	16
E. coli (ATCC 8739)	18	19	20	20	20	18	17	14	19	15	18	15
P. aeruginosa (ATCC 90274)	24	23	24	28	29	27	26	24	NA	24	25	21
C. albicans (ATCC 10221)	21	24	23	26	25	27	24	NA	11	17	24	20
A. niger(ATCC 16404)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	17

Abbreviations: Ref., gentamycin, NA, no activity.

References

1. Sun L, Gao P, Zhan P, Liu X. Pyrazolo [1, 5-a] pyrimidine-based macrocycles as novel HIV-1 inhibitors: a patent evaluation of WO2015123182. *Expert Opin. Ther. Pat.* 26(9), 979–986 (2016).
2. Al-Adiwish WM, Tahir MIM, Siti-Noor-Adnalizawati A, Hashim SF, Ibrahim N, Yaacob WA. Synthesis, antibacterial activity and cytotoxicity of new fused pyrazolo [1, 5-a] pyrimidine and pyrazolo [5, 1-c][1, 2, 4] triazine derivatives from new 5-aminopyrazoles. *Eur. J. Med. Chem.* 64, 464–476 (2013).
3. Gregg BT, Tymoshenko DO, Razzano DA, Johnson MR. Pyrazolo [1, 5-a] pyrimidines. Identification of the privileged structure and combinatorial synthesis of 3-(hetero) arylpyrazolo [1, 5-a] pyrimidine-6-carboxamides. *J. Comb. Chem.* 9(3), 507–512 (2007).
4. El-Feky SA, Abd ZK el-S. Synthesis and antihypertensive activity of novel 1-(4-benzyl-1-phthalazinyl)-pyrazolo [3, 4-d] pyrimidines. *Pharmazie*. 51(8), 540–543 (1996).
5. Raj KKV, Narayana B, Ashalatha B V, Kumari NS. New thiazoles containing pyrazolopyrimidine moiety as possible analgesic agents. *J. Pharmacol. Toxicol*. 1(6), 559–565 (2006).
6. Arias-Gómez A, Godoy A, Portilla J. Functional pyrazolo[1,5-a]pyrimidines: Current approaches in synthetic transformations and uses as an antitumor scaffold. *Molecules*. 26(9), 1–35 (2021).
7. Amin MA, Ismail MM, El-Helby A-G, Bauomy AH, El-Morsy AM. Synthesis and Preliminary Pharmacological Study of Some New Derivatives of 1-Phenyl-4-oxo (1H)-pyrazolo-[3, 4-d] pyrimidine Derivatives. *Alex. J. Pharm. Sci.* 17(1), 1–10 (2003).
8. Fahim AM, Farag AM. Synthesis, antimicrobial evaluation, molecular docking and theoretical calculations of novel pyrazolo [1, 5-a] pyrimidine derivatives. *J. Mol. Struct.* 1199, 127025 (2020).
9. hassan M ibrahem, Hassane AMA. Synthesis, Characterization and in vitro Antibacterial evaluation of New Oxindoles and Spiro-Oxindoles Derivatives. *Egypt. J. Chem.* [Internet]. (2019). Available from: https://ejchem.journals.ekb.eg/article_53680.html.
10. Hassan AS, Morsy NM, Awad HM, Ragab A. Synthesis, molecular docking, and in silico ADME prediction of some fused

pyrazolo[1,5-a]pyrimidine and pyrazole derivatives as potential antimicrobial agents. *J. Iran. Chem. Soc.* [Internet]. (0123456789) (2021). Available from: https://doi.org/10.1007/s13738-021-02319-4.

11. Lane ME, Yu B, Rice A, *et al.* A novel cdk2-selective inhibitor, SU9516, induces apoptosis in colon carcinoma cells. *Cancer Res.* 61(16), 6170–6177 (2001).
12. Fraley ME, Hoffman WF, Rubino RS, *et al.* Synthesis and initial SAR studies of 3, 6-disubstituted pyrazolo [1, 5-a] pyrimidines: a new class of KDR kinase inhibitors. *Bioorg. Med. Chem. Lett.* 12(19), 2767–2770 (2002).
13. Fraley ME, Rubino RS, Hoffman WF, *et al.* Optimization of a pyrazolo [1, 5-a] pyrimidine class of KDR kinase inhibitors: improvements in physical properties enhance cellular activity and pharmacokinetics. *Bioorg. Med. Chem. Lett.* 12(24), 3537–3541 (2002).
14. Wu Z, Fraley ME, Bilodeau MT, *et al.* Design and synthesis of 3, 7-diarylimidazopyridines as inhibitors of the VEGF-receptor KDR. *Bioorg. Med. Chem. Lett.* 14(4), 909–912 (2004).
15. Arora A, Scholar EM. Role of tyrosine kinase inhibitors in cancer therapy. *J. Pharmacol. Exp. Ther.* 315(3), 971–979 (2005).
16. Meijer L, Raymond E. Roscovitine and other purines as kinase inhibitors. From starfish oocytes to clinical trials. *Acc. Chem. Res.* 36(6), 417–425 (2003).
17. Kiss MA, Peřina M, Bazgier V, *et al.* Synthesis of dihydrotestosterone derivatives modified in the A-ring with (hetero)arylidene, pyrazolo[1,5-a]pyrimidine and triazolo[1,5-a]pyrimidine moieties and their targeting of the androgen receptor in prostate cancer. *J. Steroid Biochem. Mol. Biol.* 211(March) (2021).
18. Bondock S, Alqahtani S, Fouda AM. Synthesis and anticancer evaluation of some new pyrazolo [3, 4-d][1, 2, 3] triazin-4-ones, pyrazolo [1, 5-a] pyrimidines, and imidazo [1, 2-b] pyrazoles clubbed with carbazole. *J. Heterocycl. Chem.* 58(1), 56–73 (2021).
19. Hassan AS, Hafez TS, Osman SA. Synthesis, characterization, and cytotoxicity of some new 5-aminopyrazole and pyrazolo [1, 5-a] pyrimidine derivatives. *Sci. Pharm.* 83(1), 27–39 (2015).
20. Drev M, Grošelj U, Mevec Š, *et al.* Regioselective synthesis of 1-and 4-substituted 7-oxopyrazolo [1, 5-a] pyrimidine-3-carboxamides. *Tetrahedron.* 70(44), 8267–8279 (2014).
21. Li Y, Gao W, Li F, *et al.* An in silico exploration of the interaction mechanism of pyrazolo [1, 5-a] pyrimidine type CDK2 inhibitors. *Mol. Biosyst.* 9(9), 2266–2281 (2013).
22. Yu PB, Hong CC, Sachidanandan C, *et al.* Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. *Nat. Chem. Biol.* 4(1), 33–41 (2008).
23. Kato N, Oka M, Murase T, *et al.* Discovery and pharmacological characterization of N-[2-({2-[(2S)-2-cyanopyrrolidin-1-yl]-2-oxoethyl} amino)-2-methylpropyl]-2-methylpyrazolo [1, 5-a] pyrimidine-6-carboxamide hydrochloride (anagliptin hydrochloride salt) as a potent and selective DPP-IV i. *Bioorg. Med. Chem.* 19(23), 7221–7227 (2011).
24. Kaswan P, Pericherla K, Purohit D, Kumar A. Synthesis of 5, 7-diarylpyrazolo [1, 5-a] pyrimidines via KOH mediated tandem reaction of 1H-pyrazol-3-amines and chalcones. *Tetrahedron Lett.* 56(3), 549–553 (2015).
25. Kiessling A, Wiesinger R, Sperl B, Berg T. Selective Inhibition of c-Myc/Max Dimerization by a Pyrazolo [1, 5-a] pyrimidine. *ChemMedChem Chem. Enabling Drug Discov.* 2(5), 627–630 (2007).
26. Selleri S, Bruni F, Costagli C, *et al.* A novel selective GABAA α1 receptor agonist displaying sedative and anxiolytic-like properties in rodents. *J. Med. Chem.* 48(21), 6756–6760 (2005).
27. Mason JS, Morize I, Menard PR, Cheney DL, Hulme C, Labaudiniere RF. New 4-point pharmacophore method for molecular similarity and diversity applications: overview of the method and applications, including a novel approach to the design of combinatorial libraries containing privileged substructures. *J. Med. Chem.* 42(17), 3251–3264 (1999).
28. Ismail NSM, Ali GME, Ibrahim DA, Elmetwali AM. Medicinal attributes of pyrazolo[1,5-a]pyrimidine based scaffold derivatives targeting kinases as anticancer agents. *Futur. J. Pharm. Sci.* [Internet]. 2(2), 60–70 (2016). Available from: http://dx.doi.org/10.1016/j.fjps.2016.08.004.
29. Zhang Y, Liu Y, Zhou Y, *et al.* Pyrazolo [1, 5-a] pyrimidine based Trk inhibitors: Design, synthesis, biological activity evaluation. *Bioorg. Med. Chem. Lett.* 31, 127712 (2021).
30. Berger DM, Torres N, Dutia M, *et al.* Non-hinge-binding pyrazolo [1, 5-a] pyrimidines as potent B-Raf kinase inhibitors. *Bioorg.*

Med. Chem. Lett. 19(23), 6519–6523 (2009).
31. Williamson DS, Parratt MJ, Bower JF, *et al.* Structure-guided design of pyrazolo [1, 5-a] pyrimidines as inhibitors of human cyclin-dependent kinase 2. *Bioorg. Med. Chem. Lett.* 15(4), 863–867 (2005).
32. Othman IMM, Nasr HM, Hassan MI. Synthesis of some novel pyridazine, thienopyridazine, pyrazolopyridine, pyridopyrazolopyrimidine and pyridopyrazolotriazine derivatives with their antimicrobial activity. *Can. Chem. Trans.* 2, 504–517 (2014).
33. El-Qaliei MIH, Mousa SAS, Mahross MH, *et al.* Novel (2-Oxoindolin-3-ylidene) methyl)-1H-pyrazole and their Fused Derivatives: Design, Synthesis, Antimicrobial Evaluation, DFT, Chemical Approach, in Silico ADME and Molecular Docking Studies. *J. Mol. Struct.* , 133299 (2022).
34. El-Qaliei MIH, Mousa SAS, MD Nasr H, A Ìshak E. 5-amino-3-(cyanomethyl)-1H-pyrazole-4-carbonitrile as Precursor for synthesis of some novel pyrazolo [1, 5-a] pyrimidine derivatives. *Egypt. J. Chem.* (2022).
35. Jahangirian, Hossein, et al. Well diffusion method for evaluation of antibacterial activity of copper phenyl fatty hydroxamate synthesized from canola and palm kernel oils. *Digest Journal of Nanomaterials & Biostructures* 8.3: 1263-1270, (2013).