Synthesis and Biological Screening of New Thiadiazolopyrimidine-based Polycyclic Compounds

Alaa M. Alqahtani (✉ amqahtani@uqu.edu.sa)

Umm al-Qura University

Research Article

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Abstract

New tri- and tetra-cyclic compounds based on the thiadiazolopyrimidine ring system were prepared and their antimicrobial activity were estimated. The achieved results evidenced that pyrano-thiadiazolopyrimidine compounds **8a-b** and **9a-b** have substantial efficiencies toward the two strains of Gram-positive bacteria (*S. aureus* and *B. cereus*). While tetracyclic derivatives pyrazolopyrimido-thiadiazolopyrimidine derivatives **16a-b** and **17a-b** displayed prominent efficiencies toward the two strains of Gram-negative bacteria (*E. coli* and *P. aeruginosa*). In addition, compounds **8a-b** and **9a-b** presented good efficacy toward *C. albicans*. The activity of anti-quorum-sensing (anti-QS) inhibition of the new synthesized thiadiazolopyrimidine-based compounds was tested toward *C. violaceum*, where derivatives **16a-b**, **17a-b**, **8b** and **9a** displayed satisfactory activity. Cytotoxic activity of the same derivatives was screened toward various cancer cell lines (MCF-7, PC3, Hep-2 and HepG2) and standard normal fibroblast cell (WI38) by utilizing the MTT assay. The pyrazolopyrimido-thiadiazolopyrimidine derivatives **16a**, **16b**, **17a** and **17b** recorded potent cytotoxic efficacy against MCF-7 cells with IC₅₀ values ranging from 5.69 to 9.36 μ M. Also, the endorsed structural activity relationship (SAR) for the inspected thiadiazolopyrimidine derivatives provided an awareness concerning the chemical structure connected to anticancer efficiency. *In silico* docking studies were implemented toward silence hormone signaling in breast (PDB Code-5NQR). The results are consistent and supportive to the cytotoxic activity.

1. Introduction

Inspired the eminent role of antibiotics use in treating and preventing bacterial infections, the efficiency of antibiotics is inadequate by the increment in the numeral pathogens-resistant to antibiotics. The resistance to antibiotic is viewed as the main risk of public health in the widespread, and led to the increasing in the rate of morbidity and mortality, in addition to more cost treatment [1]. Extensive usage of antibiotics led to the accumulation of microbial resistance [2]. Thus, current antivirulence approaches were established by genetic investigation to diagnose virulence factors for numeral pathogens, where several of these approaches will situate the discerning pressure of pathogens. Moreover, cancer is assumed to be one of the principal sources of death in the world [3]. It is categorized as a tumor cell growth through its ability to disperse through other cell in the body; this progression is known as metastasis that leads most of the time to death [4,5]. Cancer managing includes surgical treatment, radioactive treatment, immunotherapy, chemotherapy and so on. Whereas chemotherapy is considered the utmost important step in the cancer medication protocols. Nevertheless, the lack of selectivity of anticancer agents is the main obstacle to the thriving of cancer medication. One of the further imperative classes of fused heterocyclic moieties has a widespread range of biological effectiveness is the thiadiazolopyrimidine derivatives. The thiadiazolo-pyrimidine nucleus and its substituted products, belonging to the pseudo purine class, had interesting biological profiles, including the antiviral [6], anti-cancer [7,8], antibiofilm [9], antitumor [10], antitubercular [11], antiglycation [12] and antioxidant [13] activities. In last few decades, these analogues were synthesized as PARP1 Inhibitors [14] and STAT3 Inhibitor [15].

A series of 6-cyano-1,3,4-thiadiazolo[3,2-*a*]pyrimidine derivatives was synthesized [16], they showed good binding mode in the active site of STAT3 enzyme inhibitors [17] and to treat breast cancer [18]. The cytotoxic activity [19] was found in 2-alkanesulfinyl/alkanesulfonyl-7-methyl-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one derivatives [20], especially the strong activity was shown by the compounds which have electrophilic substituent group on the 2-position such as alkyl sulfoxide or alkyl sulfone. New series of biologically active sulfonamide derivatives of thiadiazolo[3,2-*a*]pyrimidine were synthesized and investigated for their antitumor activity [21]. Some of them were tested for their *in vitro* and *in vivo* antitumor activities. Abdel Rahman and co-workers [22] synthesized the substituted thiadiazolo[3,2-*a*]pyrimidines and 1,3-disubstituted thiourea. Most of the compounds exhibited potent cytotoxic activity against tumor cell line A549 (non-small cell lung cancer cell line) [23] using sulforhodamine B (SRB) standard method [24]. Recently, Nagaraju and co-workers reported green synthesis [25] and characterization of thiadiazolo[3,2-*a*]pyrimidines *via* the multi-component reaction between chosen substrates of 2-aminothiadiazoles, aldehydes and active methylene compounds in ethanol solvent at room temperature using vanadium oxide loaded on fluorapatite as a robust and sustainable catalyst. Recently, 7-oxo-7*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-5-carboxylate derivatives were conveniently synthesized under mild condition through regioselective cycloaddition reactions [26]. This observation draws our attention to develop the synthesis of some polycyclic heterocyclic compounds containing 1,3,4-thiadiazolo[3,2-*a*]pyrimidine moiety and evaluating their antimicrobial and cytotoxic properties.

2. Results And Discussion

2.1. Chemistry

The reaction of 2-(2-cyanoacetamido)-1,3,4-thiadiazole (**1**) [27] with dimethylformamide-dimethylacetal (DMF-DMA) has been achieved in boiling dioxane to furnish the expected 3-(dimethylamino)acrylonitrile derivative **2** (Scheme 1). Heating of 2-cyano-3-(dimethylamino)-*N*-(1,3,4-thiadiazol-2-yl)acrylamide (**2**) in acetic acid afforded the building block, 6-cyano-7-oxo-7*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine (**3**) which formed by loss of dimethylamine molecule from the intermediate **A**. The structure of **3** was supported by the spectral analyses described in the experimental section.

The thiadiazolo[3,2-*a*]pyrimidine derivative **3** has been employed as a building unit for constructing various functionalized tri- and tetra-cyclic compounds by the reaction with several nitrogen and carbon nucleophiles. Cyclization of thiadiazolopyrimidine **3** with hydrazine hydrate and/or phenylhydrazine was achieved by refluxing in EtOH/DMF mixture to produce the corresponding tricyclic compounds, 3-aminopyrazolo[4,3-*e*]thiadiazolo[3,2-*a*]pyrimidin-4-ones **4** and **5**, respectively (Scheme 2). The chemical structures of **4** and **5** find support from the compatible IR, ¹H NMR, ¹³C NMR and MS analyses (experimental section).

The tricyclic compounds, 6,8-diaminopyridothiadiazolo[3,2-*a*]pyrimidines **6** and **7** were obtained by treatment the thiadiazolo[3,2-*a*]pyrimidine derivative **3** with active nitrile components (namely; malononitrile and ethyl cyanoacetate). The reaction was performed successfully by heating the reactants

in acetic acid and ammonium acetate (Scheme 3). The structures of **6** and **7** were elucidated considering their agreement spectral analyses. The proposed mechanism for the reaction of thiadiazolo[3,2-*a*]pyrimidine compound **3** with activated nitrile involves the nucleophilic addition of nitrile through its methylene group to the cyclic unsaturated nitrile part of compound **3** to constitute the intermediate Michael adduct (**E**). Heterocyclization of intermediate (**E**) was believed to occur by ammonia addition to the nitrile functions to produce the imino-perhydropyridine intermediate (**F**). Tautomerization into the amino-dihydropyridine intermediate (**G**) followed by air oxidation (loss of H₂) led to the formation of pyridothiadiazolo[3,2-*a*]pyrimidine compounds **6** and **7** (Scheme 3).

The pyrano[3,4-*e*]thiadiazolo[3,2-*a*]pyrimidine tricyclic and tetracyclic reagents **8**, **9** and **10** were the products when thiadiazolo[3,2-*a*]pyrimidine derivative **3** reacted with acetylacetone and benzoyl acetone as examples of diketones, acetyl acetonitrile and benzoyl acetonitrile as examples of ketonitriles and two examples of 3-methylpyrazolin-5-ones, respectively (Scheme 4). The reaction was carried out by heating in tetrahydrofuran and initiated by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) protocol. The suggested mechanism is started by Michael addition of enolate carbonyl reagent to β -carbon of the unsaturated nitrile system. The produced intermediate **H** undergoes intramolecular cyclization by addition of enolic-OH function on the nitrile group to form the pyranothiadiazolo[3,2-*a*]pyrimidine ring systems **8a** and **8b**. The structures of compounds **8**, **9** and **10** were secured by IR, ¹H NMR, ¹³C NMR and MS analyses.

The tetracyclic compounds **11**, **12** and **13** were produced from the reaction of thiadiazolo[3,2-*a*]pyrimidine derivative **3** with different α -aminoazole reagents (namely; 5-aminotetrazole, 3-amino-1,2,4-triazole, and/or 5-amino-3-methylpyrazole) as 1,3-binucleophiles (Scheme 5). Finally, the tetracyclic derivatives 6-amino-10-arylazo-pyrazolo[1',5':1,2]pyrimido[5,4-*e*]thiadiazolo[3,2-*a*]pyrimidin-5-ones **16** and **17** were produced from the treatment of thiadiazolo[3,2-*a*]pyrimidine derivative **3** with 3,5-diamino-4-arylazopyrazole **14** and/or 5-amino-4-arylazopyrazol-3-ol **15** compounds, respectively (Scheme 5). In general, the formation of these tetracyclic compounds **11**, **12**, **13**, **16** and **17** requires heating of compound **3** with aminoazole reagent in pyridine for four hours. The chemical structures were secured because of their agreement spectral analyses (IR, ¹H NMR, ¹³C NMR and MS).

2.2. Biological assessment

2.2.1. Antimicrobial and antiquorum-sensing assessment

Antimicrobial activity of the synthesized thiadiazolopyrimidine compounds has been studied toward diverse pathogenic strains such as; Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Bacillus cereus* UW85), Gram-negative bacteria (*Escherichia coli* ATCC 12435 and *Pseudomonas aeruginosa* ATCC 29260) and fungi (*Candida albicans* and *Aspergillus fumigatus* 293). The valuation was applied through the two-fold dilution technique using Ampicillin (antibacterial) and Fluconazole (antifungal) as reference drugs [28,29]. The minimal inhibitory concentration (MICs, μ g/mL) for the synthesized derivatives that prohibit the microbial growth was assigned through visual detection (no turbidity) technique. The presented results (Table 1) for the four established bacterial strains indicated

that compounds **8a-b** and **9a-b** demonstrated significant efficacy toward the two strains of Gram-positive bacteria (*S. aureus* and *B. cereus*). Meanwhile, derivatives **16a-b** and **17a-b** revealed eminent effectiveness toward both *E. coli* and *P. aeruginosa* as Gram-negative bacteria. Furthermore, compounds **8a-b** and **9a-b** showed good effectiveness toward *C. albicans*, but no marked activity was realized toward *A. fumigatus* (Table 1).

Table 1
Antibacterial and antifungal efficacy for the synthesized thiadiazolopyrimidine compounds.

Cpd. NO.	MIC (µg/mL)					
	Gram + ve Bacteria		Gram -ve Bacteria		Fungi	
	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. fumigatus</i>
3	1250	> 1250	> 1250	> 1250	> 1250	> 1250
4	1250	> 1250	> 1250	> 1250	> 1250	> 1250
5	625	625	1250	1250	1250	> 1250
6	> 1250	1250	> 1250	1250	> 1250	> 1250
7	1250	1250	625	1250	> 1250	> 1250
8a	312.5	625	1250	1250	312.5	> 1250
8b	156	312.5	1250	312.5	156	1250
9a	312.5	312.5	625	625	312.5	> 1250
9b	312.5	156	625	625	312.5	> 1250
10a	625	625	1250	312.5	625	> 1250
10b	625	625	312.5	625	> 1250	> 1250
11	312.5	1250	> 1250	1250	1250	> 1250
12	625	> 1250	> 1250	> 1250	> 1250	> 1250
13	1250	> 1250	1250	> 1250	1250	> 1250
16a	1250	> 1250	78	156	> 1250	> 1250
16b	625	> 1250	19.5	78	> 1250	> 1250
17a	625	625	156	156	> 1250	1250
17b	1250	625	19.5	39	1250	1250
Ampicillin	> 1250	1250	19.5	78	-	-
Fluconazole	-	-	-	-	> 1250	> 1250
Ampicillin is the reference drug of bacteria; Fluconazole is the reference of fungi.						

The synthesized thiadiazolopyrimidine-based compounds were screened for their antiquorum-sensing (anti-QS) inhibition activity by utilizing *Chromobacterium violaceum* (ATCC 12472) technique toward catechin as a standard compound [28–30]. Where QS technique of *C. violaceum* was remarked by Violacein (violet pigment) [31,32]. Meanwhile, the reactivity of the synthesized thiadiazolopyrimidine-

containing derivatives as drugs depends on their efficacy to inhibit the liberation of Violacein of QS. The inhibition of QS was determined by the following equation: QS inhibition (mm) = ($r_2 - r_1$). Where, r_1 is the inhibition radius of bacterial growth and r_2 is the inhibition radius for both of growth and pigment. The synthesized compounds **8b**, **9a**, **16a-b** and **17a-b** exhibited adequate anti-QS activities (Table 2).

Table 2
Quorum-sensing inhibitor efficacy of the synthesized thiadiazolopyrimidine derivatives.

Cpd. No.	Diameter of quorum-sensing inhibition (mm) ^a <i>C. violaceum</i>
3	-
4	-
5	-
6	-
7	-
8a	3
8b	12
9a	11
9b	3
10a	13
10b	9
11	5
12	3
13	5
16a	9
16b	12
17a	11
17b	12
Catechin	2
^a No effectiveness (–, < 2 mm inhibition zone); weak effectiveness (2–9 mm); moderate effectiveness (10–15 mm); strong effectiveness (> 15 mm).	

Structural activity relationship. The relation between the structures of synthesized thiadiazolopyrimidine-containing compounds and the achieved antimicrobial results was discussed as follow: (1) Incorporation of pyrazole ring to the thiadiazolopyrimidine skeleton to produce 3-aminopyrazolo-thiadiazolopyrimidinone and 3-amino-phenylpyrazolothiadiazolo-pyrimidinone (compounds **4** and **5**) did not boost the activity toward all screened strains. (2) Introduction of pyran ring to the thiadiazolopyrimidine skeleton as in compounds **8a–b**, **9a–b** and **10a–b** promoted promising antibacterial effectiveness toward *S. aureus* and *B. cereus*. In addition, they demonstrated a remarkable antifungal efficacy toward *C. albicans* and showed no activity against *A. fumigatus*. (3) Both of the tetracyclic pyrazolopyrimido-thiadiazolopyrimidinone derivatives **16a–b** and **17a–b** displayed good results toward *E. coli* and *P. aeruginosa*, which may be attributed to the existence of amino and hydroxyl groups at positions six and nine. (4) Replacement of methyl group in compound **9a** by phenyl ring led to favorable effectiveness toward *B. cereus* (derivative **9b**), while the replacement of the nitrile group on the pyran ring (derivative **9a**) by acetyl group (derivative **8a**) led to eminent antimicrobial effectiveness toward both Gram-positive and Gram-negative bacteria. (5) On the other hand, unfortunately the incorporation of tetrazole and/or triazole moiety into the thiadiazolopyrimidine skeleton (compounds **11** and **12**) diminished the activity toward all examined microbes.

2.2.2. In vitro cytotoxicity evaluation

Cytotoxicity of the prepared tri- and tetracyclic thiadiazolopyrimidine containing compounds were examined toward various cancer cell lines for example liver breast cancer (MCF-7), prostate cancer (PC3), laryngeal carcinoma (Hep-2), carcinoma (HepG2), and standard normal fibroblast cells (WI38) utilizing MTT assay [33] at National Research Centre (Egypt). The cytotoxic results (Table 3) in term of 50% inhibition of cell viability (IC_{50} values) were assessed using the 5-fluorouracil (5-Fu) as standard anticancer drug. The thiadiazolopyrimidine derivatives **16a**, **16b**, **17a** and **17b** recorded the highest cytotoxic efficacy against MCF-7 cell line with IC_{50} values 7.53, 5.69, 9.36 and 8.84 μ M, respectively. In addition, thiadiazolopyrimidine derivatives **8b** and **9b** presented strong efficacy against MCF-7 and Hep-2 cell lines with IC_{50} values ranging from 11.71 to 14.68 μ M. On the other hand, they also displayed moderate activity against HepG2 and PC3 cell lines with based on their related IC_{50} values that ranged from 21.62 to 38.36 μ M. The pyrazolo-thiadiazolopyrimidinone compounds **4** and **5** showed strong activity against HepG2, their IC_{50} values are 15.36 and 19.16 μ M, respectively. Furthermore, compounds **16** and **17** showed moderate effectiveness against the three cell lines following the order: Hep-2 $\gg$ HepG2 $\gg$ PC3.

The suggested structural activity relationship (SAR) for the investigated thiadiazolopyrimidine derivatives gave an awareness concerning the structural countenance associated to anticancer efficacy consequences. (1) The construction of pyrazole ring fused with the thiadiazolopyrimidinone skeleton in compounds **4** and **5** led to strong activity against HepG2 cells, and low effectiveness against the other three tested cells. (2) The incorporation of pyridine ring to the thiadiazolopyrimidine skeleton in compounds **6** and **7** did not provide the desired activity against the tested cell lines. On contrary, fusion of pyran ring to the thiadiazolopyrimidine skeleton in compounds **8b** (substituted with benzoyl group) and

9b (substituted with phenyl group) presented strong anticancer efficacy against MCF-7 and Hep-2 cells and reasonable activity against HepG2 and PC3 cell lines. (3) The construction of pyrazolopyrimidine moiety fused with thiadiazolopyrimidine skeleton to produce the tetracyclic compounds led to enhancement the anticancer activity against MCF-7 cell line. In addition, the compounds **16a** and **16b** having the aminopyrazole nucleus provided cytotoxic efficacy toward MCF-7 cell line higher than their corresponding compounds **17a** and **17b** which contain the hydroxypyrazole nucleus. On the other hand, the derivatives **16b** and **17b** (substituted with 4-anisyl group) displayed higher reactivity than their conjugates **16a** and **17a** that substituted with 4-tolyl group. This is supported by the order of biological anticancer activity toward MCF-7 cell line that monitors the arrangement of substituents [34]. (4) The results of cytotoxicity examination on normal cells (WI38) indicated that compounds **16** and **17** displayed the lowest cytotoxicity with IC_{50} values ranged from 57.86 to 62.26 μ M. (5) The tetracyclic compounds **16a** and **16b** showed promising selectivity as cytotoxic agents for MCF-7 cells with weak cytotoxic effects on normal cells (WI38).

Table 3
Cytotoxic activities of the synthesized derivatives

Cpd. No.	Cytotoxicity IC ₅₀ (μM)				
	HepG2	Hep-2	PC3	MCF-7	WI38
3	82.33 ± 2.19	87.51 ± 2.35	92.81 ± 3.48	77.95 ± 1.37	07.82 ± 1.33
4	15.36 ± 0.45	78.36 ± 2.20	72.31 ± 2.18	61.21 ± 1.11	09.35 ± 0.21
5	19.16 ± 0.52	61.25 ± 1.51	61.83 ± 1.68	54.75 ± 1.48	10.91 ± 0.83
6	62.24 ± 1.07	57.65 ± 1.72	66.26 ± 2.49	49.56 ± 1.25	14.11 ± 0.36
7	36.73 ± 0.17	34.19 ± 0.93	61.83 ± 1.35	39.82 ± 0.68	43.78 ± 0.52
8a	37.12 ± 0.23	22.61 ± 0.21	34.44 ± 0.64	33.42 ± 0.23	27.49 ± 0.60
8b	21.62 ± 0.14	14.65 ± 0.43	30.64 ± 0.57	14.68 ± 0.16	42.82 ± 1.06
9a	33.17 ± 0.36	19.98 ± 0.24	46.43 ± 1.07	22.66 ± 0.29	36.12 ± 0.28
9b	26.78 ± 0.18	11.71 ± 0.36	38.36 ± 0.89	12.84 ± 0.26	48.16 ± 1.47
10a	41.98 ± 1.24	34.04 ± 0.46	40.11 ± 0.36	24.21 ± 0.52	23.26 ± 0.60
10b	37.59 ± 1.26	28.76 ± 1.80	27.26 ± 0.53	27.44 ± 0.49	25.41 ± 0.62
11	42.18 ± 1.17	38.76 ± 0.74	38.10 ± 0.49	31.81 ± 0.67	20.24 ± 0.39
12	44.28 ± 1.35	34.15 ± 0.97	47.38 ± 0.89	36.92 ± 0.29	17.66 ± 0.71
13	28.63 ± 0.41	30.16 ± 0.59	35.56 ± 0.88	57.63 ± 1.40	44.36 ± 1.24
16a	37.59 ± 1.26	32.74 ± 0.36	44.18 ± 0.94	07.53 ± 0.08	60.07 ± 2.41
16b	42.34 ± 0.75	38.25 ± 0.57	47.92 ± 1.48	05.69 ± 0.39	62.26 ± 3.03
17a	38.27 ± 0.17	29.58 ± 0.15	39.40 ± 2.01	09.36 ± 0.23	57.86 ± 1.18
17b	33.54 ± 0.25	18.97 ± 0.40	36.75 ± 0.43	08.84 ± 0.17	58.37 ± 2.29
5-Fu^b	07.20 ± 0.45	05.35 ± 0.23	08.78 ± 0.60	05.58 ± 0.31	10.32 ± 0.62
^b 5-Fluorouracil (5-Fu) is the reference drug for anticancer tests.					

Bleomycin-dependent DNA damage. The prepared polycyclic thiadiazolopyrimidine-based compounds were examined through bleomycin-dependant DNA damage and the results were compared to that of ascorbic acid as a positive control. The outcomes presented the ability of these derivatives to protect the DNA from damage. The capability of compounds **16a-b** and **17a-b** to manifested the best protective effect against DNA damage is indicated by their respectable absorbance values that ranged from 0.031 to 0.053 (Table 4) [35,36].

Table 4
Bleomycin-dependent DNA damage of the synthesized thiadiazolopyrimidine scaffolds.

Cpd. No.	Absorbance ^c
3	0.281 ± 0.22
4	0.165 ± 0.19
5	0.146 ± 0.16
6	0.135 ± 0.14
7	0.132 ± 0.19
8a	0.078 ± 0.13
8b	0.066 ± 0.05
9a	0.064 ± 0.06
9b	0.083 ± 0.08
10a	0.098 ± 0.07
10b	0.119 ± 0.13
11	0.113 ± 0.16
12	0.107 ± 0.08
13	0.128 ± 0.11
16a	0.039 ± 0.07
16b	0.031 ± 0.04
17a	0.053 ± 0.05
17b	0.042 ± 0.03
Ascorbic acid	0.060 ± 0.02
^c Values are mean for three replicates ± SD	

2.2.3. Molecular docking

In silico molecular docking investigation was achieved to evaluate the types of requisite interaction between thiadiazolopyrimidine-based compounds and the crystal structure potent inhibitors of NUDT5 silence hormone signaling in breast (PDB Code-5NQR) [37]. Thiadiazolopyrimidine derivative **3** displayed two types of intermolecular interactions with small binding. The first one result in S atom of thiadiazole ring with Asp 194, and the second result in N-atom of nitrile group with Gly 61 over binding score, S = -4.3922 kcal/mol (Figure S1). The tricyclic compound **4** (pyrazolothiadiazolopyrimidine substituted with

amine function at position-3) revealed two H-bonds. The first H-bond was exhibited between N-atom of pyrimidine nucleus with Arg 84 (2.92 Å) (Figure S2). Wherever, the second H-bond was designed between O-atom of carbonyl group with Arg 84. All of these connections offered binding score, $S = -4.4293$ kcal/mol. The pyrazolothiadiazolopyrimidine compound **5** delivered better binding score ($S = -4.2102$ kcal/mol) through formation of four H-bonds (Figure S3). One H-bond was arisen between the S-atom of thiadiazole ring and Glu 169, the second H-bond was exhibited among N-atom of aminopyrazole moiety and Ser 172, while the third and fourth binding were π - π interactions exhibited between the thiazole and pyrimidine ring with Ile 171.

Nonetheless, the pyrido-thiadiazolopyrimidine derivative **6** presented two H-bonds between Arg 51 with the N-atom of amino pyridine, and Glu 65 with S-atom of thiadiazole ring (Figure S4). Derivative **6** was displayed a weak score of interactions with 5NQR amino- acids ($S = -4.5643$ kcal/mol). Meanwhile, the pyrido-thiadiazolopyrimidine compound **7** revealed two H-bonds between N-atom of the amino pyridine moiety over Asp 194, and O-atom of ester group with Arg 51 of 5NQR over score ($S = -6.6614$ kcal/mol) (Figure S5). The first was H-bond among the N-atom of the amidic moiety with Asp 347, and π - π binding were unveiled among the pyridine ring thru Arg 84 (3.40 Å).

Likewise, aminopyranothiadiazolopyrimidine derivative **8a** displayed two H-bond between S-atom of thiadiazole ring with Asp 194 and N-atom of aminoxazine moiety with Cys 139 over score ($S = -5.7796$ kcal/mol) (Figure S6). While, compound **8b** showed three intermolecular forces came from thiadiazole ring, one H-bond between S-atom of thiadiazole ring with Cys 139 and two π - π interactions with both of Arg 51 and Met 132 over binding score ($S = -5.9214$ kcal/mol) (Figure S7). Also, derivative **9a** demonstrated two H-bonds among S- atom of thiadiazole ring with Asp 194 and N-atom of nitrile group with Gly with binding energy score, $S = -5.3557$ kcal/mol (Figure S8). Compound **9b** established two H-bonds among N-atom of amino group by both Ala 96 and Arg 84 through good score, $S = -6.1407$ kcal/mol (Figure S9). Moreover, aminopyrazolopyrano-thiadiazolopyrimidines **10a** and **10b** exhibited many H-bonds as well as π - π interactions. Derivative **10a** demonstrated three H-bonds between N-atom of amino group with Val 62, N1 of pyrazole ring with Ala 96, and N2 of pyrazole ring with Arg 84 in addition to π - π interaction between thiadiazole ring with Arg 51. All of these bindings were shown energy score, $S = -4.9886$ kcal/mol (Figure S10). Derivative **10b** demonstrated two H-bonds between S-atom of thiadiazole ring with Thr 117, and N1 of pyrazole ring with Ser 172 beside three π - π interactions were displayed between thiadiazole ring with Val 170, pyrazole ring with Ile 171, phenyl ring with Ile 171, respectively. The binding score of **10b** recorded $S = -5.2193$ kcal/mol as shown in (Figure S11). Moreover, the tetracyclic structures 11–13 were revealed reasonable binding scores from (-5.2800 to -5.4772 kcal/mol) through different hydrogen bonds and π - π interactions as exposed in (Table 5, Figure S12, figure S13 and figure S14). Furthermore, amino-pyrazolopyrimidothiadiazolopyrimidines **16** and **17** displayed remarkable binding scores, for example derivative **16a** was displayed binding score, $S = -6.2989$ kcal/mol (Fig. 1) over one H-bond between N-atom of pyrimidine ring with Arg 51, and two π - π interactions between Ter 28 with both pyrimidothiazdiazole ring and pyrimidopyrimidine ring, respectively.

While, compound **16b** exhibited two intermolecular hydrogen bonds between N-atom of aminopyrazole with both Ala 96 and Gln 82 through binding score, $S = -7.7053$ kcal/mol (Fig. 2).

Alternatively, derivative **17a** was presented binding score, $S = -7.4560$ kcal/mol (Figure S15) through one H-bond among O-atom of Hydroxyl group with Asp 194. Finally, derivative **17b** showed one π - π binding among the phenyl ring and Arg 51, and one H-bond between N-atom of aminopyrimidine moiety with Asp 194 over binding score, $S = -7.5846$ kcal/mol (Figure S16). The standard reference drug 5-fluorouracil was subjected to the 5NQR to introduce a comparison in respect to the synthesized derivatives. It presented one intermolecular hydrogen bond only with binding score, $S = -7.4560$ kcal/mol (Figure S17).

Table 5
The binding interaction of the synthesized thiadiazolopyrimidine derivatives.

Code	S(energy score) (Kcal/mol)	Rmsd (refine unit)	Interaction with ligand	Types of Interactions	Distance (Å)
3	-4.3922	1.0737	S of Thiadiazole ring with Asp 194	H-donor	3.13
			N of nitrile group with Gly 61	H-acceptor	3.70
4	-4.4293	1.5133	N of pyrimidine ring with Arg 84	H-acceptor	3.47
			O of Carbonyl group with Arg 84	H-acceptor	3.23
5	-4.7102	0.7663	S of Thiadiazole ring with Glu 169	H-donor	3.34
			N of amino pyrazole with Ser 172	H-donor	2.98
			Thiazole ring with Ile 171	π - π interaction	4.05
			Pyrimidine ring with Ile 171	π - π interaction	4.29
6	-4.5643	1.0767	N of amino pyridine with Arg 51	H-donor	2.98
			S of Thiadiazole ring with Glu 65	H-donor	3.97
7	-5.5334	1.3303	N of amino pyridine with Asp 194	H-donor	3.16
			O of ester group with Arg 51	H-acceptor	2.93
8a	-5.4796	1.2247	S of Thiadiazole ring with Asp 194	H-donor	3.24
			N of amino Oxazine with Cys 139	H-donor	3.96
8b	-5.7214	0.7835	S of Thiadiazole ring with Cys 139	H-donor	3.59
			Thiadiazole ring with Arg 51	π - π interaction	4.00
			Thiadiazole ring with Met 132	π - π interaction	4.22
9a	-5.3557	0.9982	S of Thiadiazole ring with Asp 194	H-donor	3.06
			N of nitrile group with Gly 61	H-acceptor	3.64
9b	-6.4407	1.1913	N of amino Oxazine with Ala 96	H-donor	2.82
			N of amino Oxazine with Arg 84	H-acceptor	3.09

Code	S(energy score) (Kcal/mol)	Rmsd (refine unit)	Interaction with ligand	Types of Interactions	Distance (Å)
10a	-4.9886	0.9778	N of amino Oxazine with Val 62	H-donor	2.97
			N1 of Pyrazole ring with Ala 96	H-donor	3.04
			N2 of Pyrazole ring with Arg 84	H-acceptor	3.18
			Thiadiazole ring with Arg 51	π - π interaction	3.23
10b	-5.2193	1.0414	S of Thiadiazole ring with Thr 117	H-donor	3.71
			NH of pyrazole ring with Ser 172	H-acceptor	3.17
			Thiadiazole ring with Val 170	π - π interaction	3.71
			pyrazole ring with Ile 171		4.38
			Phenyl ring ring with Ile 171	π - π interaction	3.77
11	-5.2800	1.1608		π - π interaction	
			N of Amino group with Met 132	H-donor	3.95
			N of pyrimidine ring with Arg 84	H-acceptor	2.89
			Thiadiazole ring with Gly 97	π - π interaction	3.75
12	-5.4282	1.2159	S of Thiadiazole ring with Glu 93	H-donor	3.18
			O of Carbonyl group with Arg 84	H-acceptor	2.91
13	-5.4772	1.3030	N of amino Pyrimidine with Met 132	H-donor	4.01
			N of Thiadiazole ring with Arg 84	H-acceptor	3.19
			Thiadiazole ring with Gly 97	π - π interaction	3.79
			pyrimidine ring with Gly 97	π - π interaction	3.92
16a	-6.2989	1.3113	N of pyrimidine ring with Arg 51	H-acceptor	3.33
			pyrimidothiazdiazolering with Ter 28	π - π interaction	3.60
			pyrimidopyrimidine ring with Ter 28	π - π interaction	3.67
16b	-7.7053	0.4672	N of aminopyrazole with Ala 96	H-donor	3.09
			N of aminopyrazole with Gln 82	H-acceptor	3.28

Code	S(energy score) (Kcal/mol)	Rmsd (refine unit)	Interaction with ligand	Types of Interactions	Distance (Å)
17a	-7.4560	0.5501	O of Hydroxyl group with Asp 194	H-donor	3.18
17b	-7.5846	0.8285	N of aminopyrimidine with Asp 194	H-donor	3.01
			Phenyl ring with Arg 51	π - π interaction	4.68
5-Fu	-3.8546	0.8432	N1 of pyrimidine ring with Cys139	H-donor	3.93

Finally, the docking technique presented that the derivatives **16a**, **16b**, **17a**, and **17b** were shown a respectable binding score – 6.2989, -7.7053, -7.4560 and – 7.5846 kcal/mol in contrast to 5-Fluorouracil, which exhibited binding score with 5NQR, S = -3.8546 kcal/mol. The two and three dimensional pictures of the most derivatives presented two intramolecular hydrogen bonds which came from their thiadiazole and pyrimidine moieties.

The challenge for sensible docking results is to create what level of conformation for the ligand interactions depending on the binding score with the distinct compounds. All the prepared derivatives contain thiadiazole and pyrimidine moieties that form hydrogen bond linkage with the receptor and chemically disparate amino acids of 5NQR. The immense substituent of larger pocket size of 5NQR can be described as a branched constrained by typically few polar residues, with specific binding site (Asp 194, Ter 28, Met 132, Arg 84, Gly 97 and Cys139) as recorded in the three-dimensional pictures, and offered a proper cavity of the prepared thiadiazolopyrimidine-based compounds.

3. Conclusion

Seventeen tricyclic and tetracyclic compounds incorporating the thiadiazolo[3,2-*a*]pyrimidine ring system are synthesized. The synthetic plan for the preparation of tricyclic compounds, pyrazolothiadiazolo-pyrimidines, pyridothiadiazolo-pyrimidines, and pyranothiadiazolo-pyrimidines, is based on the reactions of 6-cyano-7-oxo-7*H*[1,3,4]thiadiazolo[3,2-*a*]pyrimidine **3** with various nitrogen and carbon nucleophilic reagents. The tetracyclic ring systems in which the pyrimido[5,4-*e*]thiadiazolo[3,2-*a*]pyrimidine skeleton fused with various azoles such as tetrazole, triazole and/or pyrazoles are prepared by cyclization of the building block **3** with different α -aminoazole reagents. The targeted thiadiazolopyrimidine compounds were evaluated for antimicrobial efficacy toward both the two types of bacterial strains in addition to antifungal strains. The results demonstrated that, derivatives **8a-b** and **9a-b** having the pyranothiadiazolopyrimidine nucleus displayed significant activities toward *S. aureus*, *B. cereus*. Meanwhile, derivatives **16a-b** and **17a-b** were displayed prominent efficiencies toward both of *E. coli* and *P. aeruginosa*. The synthesized thiadiazolopyrimidine compounds were tested as antiquorum-sensing, where the derivatives **16a-b**, **17a-b**, **8b** and **9a** demonstrated acceptable activity. The relationship between the chemical structures and the recognized antimicrobial results was suggested. *In vitro* antitumor

efficiency of the synthesized thiadiazolopyrimidine scaffolds was examined toward four cancer cells (MCF-7, PC3, Hep-2, and HepG2) and lung normal cell (WI38) by employing MTT technique. Wherever, the tetracyclic pyrazolopyrimido[5,4-*e*]thiadiazolo[3,2-*a*]pyrimidine derivatives **16a**, **16b**, **17a** and **17b** recorded potent cytotoxic efficacy against MCF-7 cancer cells. Also, the endorsed structural activity relationship for the synthesized thiadiazolopyrimidines provided an awareness concerning their chemical structures and anticancer efficiency. *In silico*-docking studies (PDB Code-5NQR) indicated that compounds **16b** and **17b** recorded the highest binding scores (-7.7053 and -7.5846 kcal/mol).

4. Experimental

4.1. Chemistry

The IR spectra were recorded on Thermo Scientific Nicolet iS10 FTIR spectrometer using KBr discs. The ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained in DMSO- d_6 on JEOL's NMR spectrometer. The mass analyses were performed on Shimadzu Qp-2010 plus mass spectrometer at 70eV. Elemental analyses were estimated on a EuroVector instrument analyzer (EA3000 Series).

Synthesis of 2-cyano-3-(dimethylamino)-N-(1,3,4-thiadiazol-2-yl)acrylamide (**2**)

In 100 mL round bottom flask (RBF), 2-(cyanoacetamido)-1,3,4-thiadiazole (**1**) (1.68 g, 10 mmol) was dissolved in 25 mL dioxane, then *N,N*-dimethylformamide-dimethylacetal (1.20 mL, 10 mmol) was added. The mixture was heated for 4 h and the orange solid that obtained was collected and dried.

Yield 71%, m.p. = 226–227°C, lit. m.p. = 224°C [27]. IR: 3221 (N-H), 2196 ($\text{C}\equiv\text{N}$), 1678 cm^{-1} ($\text{C}=\text{O}$). ^1H NMR: δ 3.13 (s, 3H), 3.23 (s, 3H), 7.91 (s, 1H, $\text{CH}=\text{C}$), 9.04 (s, 1H, thiadiazole-H), 10.26 ppm (s, 1H). Anal. Calcd. for $\text{C}_8\text{H}_9\text{N}_5\text{OS}$ (223.05): C, 43.04; H, 4.06; N, 31.37%. Found: C, 43.13; H, 4.01; N, 31.43%.

Synthesis of 6-cyano-7-oxo-7H-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine (**3**)

A solution of thiadiazolyl-acrylamide compound **2** (2.23 g, 10 mmol) in 25 mL glacial acetic acid was refluxed for 4 h. The solid that obtained after diluted with 50 mL cold water was collected and purified by recrystallization from dioxane.

Yield 56%, m.p. = 264–265°C. IR: 2229 ($\text{C}\equiv\text{N}$), 1690 cm^{-1} ($\text{C}=\text{O}$). ^1H NMR: δ 8.53 (s, 1H, thiadiazole-H), 9.37 ppm (s, 1H, pyrimidine-H). ^{13}C NMR: 94.84, 115.52, 142.30, 159.06, 161.29, 165.56 ppm. MS: m/z (%) = 178 (M^+ , 83.46). Anal. Calcd. for $\text{C}_6\text{H}_2\text{N}_4\text{OS}$ (178.00): C, 40.45; H, 1.13; N, 31.45%. Found: C, 40.31; H, 1.09; N, 31.50%.

Preparation of 3-aminopyrazolo[4,3-*e*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4-ones **4** and **5**

In 50 mL RBF, hydrazine hydrate or phenylhydrazine (3 mmol) was added to a solution of thiadiazolopyrimidine compound **3** (0.53 g, 3 mmol) in EtOH and DMF mixture (3:1). The mixture was

refluxed for 4 h. The solid that formed was collected and recrystallized from EtOH/DMF mixture (1:1) to furnish the conforming pyrazolo[4,3-*e*]thiadiazolo[3,2-*a*]pyrimidine compounds **4** and **5**.

3-Aminopyrazolo[4,3-*e*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4(1H)-one (**4**)

Yield 68%, m.p. = 281–282°C. IR: 3381, 3275, 3211 (NH₂ and NH), 1664 cm⁻¹ (C = O). ¹H NMR: δ 6.58 (s, 2H), 8.36 (s, 1H, thiadiazole-H), 11.80 ppm (s, 1H). ¹³C NMR: 97.34, 144.61, 150.10, 152.08, 161.71, 164.25 ppm. MS: m/z (%) = 208 (M⁺, 56.41). Anal. Calcd. for C₆H₄N₆OS (208.02): C, 34.61; H, 1.94; N, 40.37%. Found: C, 34.44; H, 2.01; N, 40.25%.

3-Amino-1-phenylpyrazolo[4,3-*e*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4(1H)-one (**5**)

Yield 75%, m.p. = 254–255°C. IR: 3370, 3218 (NH₂), 1661 cm⁻¹ (C = O). ¹H NMR: δ 6.44 (s, 2H), 7.42–7.57 (m, 5H), 8.51 ppm (s, 1H, thiadiazole-H). ¹³C NMR: 95.73, 123.50 (2C), 127.29, 129.68 (2C), 138.95, 144.33, 149.70, 152.69, 160.22, 164.38 ppm. MS: m/z (%) = 284 (M⁺, 44.93). Anal. Calcd. for C₁₂H₈N₆OS (284.05): C, 50.70; H, 2.84; N, 29.56%. Found: C, 50.59; H, 2.80; N, 29.48%.

Preparation of 6,8-diamino-5-oxo-pyrido[3,4-*e*][1,3,4]thiadiazolo[3,2-*a*]pyrimidines **6** and **7**

Thiadiazolopyrimidine compound **3** (0.89 g, 5 mmol) and malononitrile (or ethyl cyanoacetate) (0.005 mol) were taken in a 50 mL RBF. Ammonium acetate (1.00 g) and 20 mL acetic acid were then added to the reaction mixture which subjected to reflux for 8 h. The mixture was diluted with 40 mL cold water and the solid that produced was collected and purified by recrystallization from acetic acid.

6,8-Diamino-9-cyano-5-oxo-5H-pyrido[3,4-*e*][1,3,4]thiadiazolo[3,2-*a*]pyrimidine (**6**)

Yield 66%, m.p. = 302–303°C. IR: 3381, 3304, 3248 (NH₂ and N-H), 2211 (C≡N), 1667 cm⁻¹ (C = O). ¹H NMR: δ 7.14 (s, 1H), 8.27 (s, 1H), 8.45 (s, 1H, thiadiazole-H), 8.81 ppm (s, 2H). ¹³C NMR: δ 71.54, 90.33, 114.16, 138.08, 158.69, 162.84, 163.90, 166.21, 167.50 ppm. MS: m/z (%) = 259 (M⁺, 70.11). Anal. Calcd. for C₉H₅N₇OS (259.03): C, 41.70; H, 1.94; N, 37.82%. Found: C, 41.86; H, 1.88; N, 37.72%.

Ethyl 6,8-diamino-5-oxo-5H-pyrido[3,4-*e*][1,3,4]thiadiazolo[3,2-*a*]pyrimidine-9-carboxylate (**7**)

Yield 61%, m.p. = 278–280°C. IR: 3367, 3293, 3241 (NH₂), 1689 (C = O), 1665 cm⁻¹ (C = O). ¹H NMR: δ 1.28 (t, *J* = 7.00 Hz, 3H), 4.28 (q, *J* = 7.00 Hz, 2H), 7.23 (s, 1H), 8.15 (s, 1H), 8.47 (s, 1H, thiadiazole-H), 8.72 ppm (s, 2H). ¹³C NMR: δ 14.30, 61.14, 83.92, 89.68, 139.27, 154.57, 162.30, 163.74, 165.05, 167.58, 168.46 ppm. MS: m/z (%) = 306 (M⁺, 33.27). Anal. Calcd. for C₁₁H₁₀N₆O₃S (306.05): C, 43.13; H, 3.29; N, 27.44%. Found: C, 43.01; H, 3.33; N, 27.46%.

Preparation of 6-aminopyrano[3,4-*e*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-5-ones **8** and **9**

The thiadiazolopyrimidine compound **3** (0.89 g, 5 mmol) was dissolved in 15 mL THF in 50 mL RBF, then the appropriate active methylene compound (namely; acetylacetone, benzoyl acetone, acetyl acetonitrile and benzoyl acetonitrile) (0.005 mol) and DBU (0.45 mL, 3 mol%) were added. The mixture was refluxed for 4 h and then allowed to cool to 25°C. The solid was filtered and recrystallized from THF to produce the conforming pyranothiadiazolopyrimidines **8** and **9**.

9-Acetyl-6-amino-8-methyl-5H,9aH-pyrano[3,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (**8a**)

Yield 72%, m.p. = 226–227°C. IR: 3371, 3288, 3236 (NH₂ and N-H), 1670 (C = O), 1658 cm⁻¹ (C = O). ¹H NMR: δ 2.18 (s, 3H), 2.34 (s, 3H), 5.26 (s, 1H, pyran-H), 6.79 (s, 2H), 8.28 ppm (s, 1H, thiadiazole-H). ¹³C NMR: δ 16.84, 27.50, 42.11, 92.09, 108.02, 138.67, 157.33, 162.46, 164.96, 166.58, 195.91 ppm. MS: m/z (%) = 278 (M⁺, 80.64). Anal. Calcd. for C₁₁H₁₀N₄O₃S (278.05): C, 47.48; H, 3.62; N, 20.13%. Found: C, 47.30; H, 3.70; N, 20.26%.

6-Amino-9-benzoyl-8-methyl-5H,9aH-pyrano[3,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (**8b**)

Yield 76%, m.p. = 239–241°C. IR: 3346, 3294, 3260 (NH₂ and N-H), 1667 cm⁻¹ (C = O). ¹H NMR: δ 2.31 (s, 3H), 5.08 (s, 1H, pyran-H), 6.76 (s, 2H), 7.46–7.73 (m, 5H), 8.23 ppm (s, 1H, thiadiazole-H). ¹³C NMR: δ 16.87, 44.29, 92.28, 105.33, 128.42 (2C), 129.51 (2C), 133.77, 138.15, 139.42, 155.04, 162.71, 165.08, 166.86, 192.60 ppm. MS: m/z (%) = 340 (M⁺, 52.83). Anal. Calcd. for C₁₆H₁₂N₄O₃S (340.06): C, 56.46; H, 3.55; N, 16.46%. Found: C, 56.31; H, 3.50; N, 16.37%.

6-Amino-9-cyano-8-methyl-5-oxo-5H,9aH-pyrano[3,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidine (**9a**)

Yield 68%, m.p. = 264–265°C. IR: 3360, 3292, 3181 (NH₂ and N-H), 2214 (C ≡ N), 1660 cm⁻¹ (C = O). ¹H NMR: δ 2.14 (s, 3H), 5.16 (s, 1H, pyran-H), 6.71 (s, 2H), 8.26 ppm (s, 1H, thiadiazole-H). ¹³C NMR: δ 17.16, 41.51, 79.06, 91.86, 117.63, 138.74, 161.24, 162.89, 165.18, 166.47 ppm. MS: m/z (%) = 261 (M⁺, 45.09). Anal. Calcd. for C₁₀H₇N₅O₂S (261.03): C, 45.97; H, 2.70; N, 26.81%. Found: C, 46.10; H, 2.63; N, 26.70%.

6-Amino-9-cyano-5-oxo-8-phenyl-5H,9aH-pyrano[3,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidine (**9b**)

Yield 62%, m.p. = 258–260°C. IR: 3356, 3270, 3184 (NH₂ and N-H), 2210 (C ≡ N), 1655 cm⁻¹ (C = O). ¹H NMR: δ 5.09 (s, 1H, pyran-H), 6.69 (s, 2H), 7.49–7.56 (m, 5H), 8.18 ppm (s, 1H, thiadiazole-H). ¹³C NMR: δ 40.94, 81.22, 92.19, 118.01, 127.66 (2C), 128.58 (2C), 129.21, 131.40, 138.36, 161.75, 162.50, 165.30, 166.57 ppm. MS: m/z (%) = 323 (M⁺, 58.35). Anal. Calcd. for C₁₅H₉N₅O₂S (323.05): C, 55.72; H, 2.81; N, 21.66%. Found: C, 55.93; H, 2.88; N, 21.90%.

Synthesis of 6-amino-10-methylpyrazolo[4',3':5,6]pyrano[3,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-ones **10a** and **10b**

In 50 mL RBF, each of the 3-methylpyrazolone compound (5 mmol) (was added to a solution of the thiadiazolopyrimidine derivative **3** (0.89 g, 5 mmol in 15 mL THF containing DBU (0.45 mL, 3 mol%). The

mixture was refluxed for 4 h and then left to cool. The solid was collected, dried and purified by recrystallization from EtOH/DMF mixture (1:1) to furnish the cyclic compounds **10a** and **10b**, respectively.

6-Amino-10-methyl-8,10b-dihydro-5H-pyrazolo[4',3':5,6]pyrano[3,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (**10a**)

Yield 64%, m.p. = 310–312°C. IR: 3369, 3281, 2188 (NH₂ and N-H), 1652 cm⁻¹ (C = O). ¹H NMR: δ 2.21 (s, 3H), 4.78 (s, 1H, pyran-H), 6.92 (s, 2H), 8.34 (s, 1H, thiadiazole-H), 12.31 ppm (s, 1H). ¹³C NMR: δ 13.22, 45.69, 95.78, 112.19, 138.93, 140.55, 160.73, 161.48, 163.08, 165.25 ppm. MS: m/z (%) = 276 (M⁺, 30.44). Anal. Calcd. for C₁₀H₈N₆O₂S (276.04): C, 43.47; H, 2.92; N, 30.42%. Found: C, 43.29; H, 2.81; N, 30.55%.

6-Amino-10-methyl-8-phenyl-8,10b-dihydro-5H-pyrazolo[4',3':5,6]pyrano[3,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (**10b**)

Yield 58%, m.p. = 266–267°C. IR: 3344, 3250, 3204 (NH₂ and N-H), 1646 cm⁻¹ (C = O). ¹H NMR: δ 2.23 (s, 3H), 4.73 (s, 1H, pyran-H), 7.08 (s, 2H), 7.47–7.75 (m, 5H), 8.30 ppm (s, 1H, thiadiazole-H). ¹³C NMR: δ 13.31, 45.85, 95.61, 113.96, 122.16 (2C), 126.47, 129.28 (2C), 137.83, 138.78, 144.18, 154.29, 160.44, 162.53, 165.70 ppm. MS: m/z (%) = 352 (M⁺, 39.14). Anal. Calcd. for C₁₆H₁₂N₆O₂S (352.07): C, 54.54; H, 3.43; N, 23.85%. Found: C, 54.68; H, 3.50; N, 23.76%.

Synthesis of tetracyclic compounds **11**, **12**, **13**, **16** and **17**:

Thiadiazolopyrimidine derivative **3** (0.89 g, 5 mmol) was dissolved in 20 mL pyridine followed by addition of the appropriate α-aminoazole reagent (5 mmol) (namely; 5-aminotetrazole, 3-amino-1,2,4-triazole, 5-amino-3-methylpyrazole, 3,5-diamino-4-arylazopyrazole and/or 5-amino-4-arylazopyrazol-3-ol) was added. The mixture was refluxed for 4 h and then diluted with 40 mL cold water. The solid was collected and recrystallized from EtOH/DMF mixture (1:1) to produce the tetracyclic compounds **11**, **12**, **13**, **16** and **17**, respectively.

6-Amino-5H-[1,3,4]thiadiazolo[2',3':2,3]pyrimido[4,5-d]tetrazolo[1,5-a]pyrimidin-5-one (**11**)

Yield 57%, m.p. = 274–275°C. IR: 3341, 3283, 3190 (NH₂ and N-H), 1645 cm⁻¹ (C = O). ¹H NMR: δ 8.38 (s, 1H, thiadiazole-H), 9.08 ppm (s, 2H). ¹³C NMR: δ 89.36, 143.29, 151.18, 154.50, 161.07, 162.74, 164.91 ppm. MS: m/z (%) = 261 (M⁺, 41.20). Anal. Calcd. for C₇H₃N₉OS (261.02): C, 32.19; H, 1.16; N, 48.26%. Found: C, 32.03; H, 1.18; N, 48.35%.

6-Amino-5H-[1,2,4]triazolo[4',3':1,2]pyrimido[5,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (**12**)

Yield 66%, m.p. = 290–291°C. IR: 3343, 3270, 3211 (NH₂ and N-H), 1648 cm⁻¹ (C = O). ¹H NMR: δ 8.37 (s, 1H, thiadiazole-H), 8.61 (s, 1H, triazole-H), 9.16 ppm (s, 2H). ¹³C NMR: δ 91.05, 135.92, 143.63, 148.92, 156.18, 161.84, 162.71, 164.57 ppm. MS: m/z (%) = 260 (M⁺, 73.51). Anal. Calcd. for C₈H₄N₈OS (260.02): C, 36.92; H, 1.55; N, 43.06%. Found: C, 36.80; H, 1.59; N, 43.14%.

6-Amino-9-methyl-5H-pyrazolo[1',5':1,2]pyrimido[5,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (13)

Yield 72%, m.p. = 304–306°C. IR: 3366, 3280, 3178 (NH₂ and N-H), 1655 cm⁻¹ (C = O). ¹H NMR: δ 2.36 (s, 3H), 6.48 (s, 1H, pyrazole-H), 8.38 (s, 1H, thiadiazole-H), 9.25 ppm (s, 2H). ¹³C NMR: δ 13.53, 92.16, 98.42, 142.87, 146.70, 154.06, 157.35, 160.58, 162.50, 163.73 ppm. MS: m/z (%) = 273 (M⁺, 90.32). Anal. Calcd. for C₁₀H₇N₇OS (273.04): C, 43.95; H, 2.58; N, 35.88%. Found: C, 44.14; H, 2.51; N, 35.76%.

6,9-Diamino-10-(4-methylphenylazo)-5H-pyrazolo[1',5':1,2]pyrimido[5,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (16a)

Yield 75%, m.p. > 320°C. IR: 3402, 3370, 3277, 3205 (NH₂ and N-H), 1644 cm⁻¹ (C = O). ¹H NMR: δ 2.32 (s, 3H), 7.36 (d, *J* = 8.50 Hz, 2H), 7.52 (s, 2H), 7.77 (d, *J* = 8.50 Hz, 2H), 8.36 (s, 1H, thiadiazole-H), 8.89 ppm (s, 2H). ¹³C NMR: δ 21.18, 90.49, 100.40, 126.65 (2C), 129.79 (2C), 135.27, 139.38, 141.88, 144.02, 149.76, 157.22, 162.31, 164.80, 166.11 ppm. MS: m/z (%) = 392 (M⁺, 57.30). Anal. Calcd. for C₁₆H₁₂N₁₀OS (392.09): C, 48.97; H, 3.08; N, 35.70%. Found: C, 48.65; H, 3.17; N, 35.84%.

6,9-Diamino-10-(4-methoxyphenylazo)-5H-pyrazolo[1',5':1,2]pyrimido[5,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (16b)

Yield 70%, m.p. > 320°C. IR: 3414, 3361, 3286, 3221 (NH₂ and N-H), 1648 cm⁻¹ (C = O). ¹H NMR: δ 3.78 (s, 3H), 7.05 (d, *J* = 9.00 Hz, 2H), 7.46 (s, 2H), 7.81 (d, *J* = 9.00 Hz, 2H), 8.35 (s, 1H, thiadiazole-H), 8.84 ppm (s, 2H). ¹³C NMR: δ 55.74, 88.65, 97.88, 114.52 (2C), 128.11 (2C), 132.63, 140.37, 143.18, 150.26, 157.76, 159.88, 161.17, 164.92, 167.41 ppm. MS: m/z (%) = 408 (M⁺, 42.88). Anal. Calcd. for C₁₆H₁₂N₁₀O₂S (408.09): C, 47.06; H, 2.96; N, 34.30%. Found: C, 46.90; H, 2.90; N, 34.19%.

6-Amino-9-hydroxy-10-(4-methylphenylazo)-5H-pyrazolo[1',5':1,2]pyrimido[5,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (17a)

Yield 59%, m.p. = 264–265°C. IR: 3370, 3253, 3180 (NH₂ and N-H), 1655 cm⁻¹ (C = O). ¹H NMR: δ 2.32 (s, 3H), 7.35 (d, *J* = 8.50 Hz, 2H), 7.74 (d, *J* = 8.50 Hz, 2H), 8.33 (s, 1H, thiadiazole-H), 8.97 (s, 2H), 11.78 ppm (s, 1H). ¹³C NMR: δ 21.17, 91.08, 103.75, 126.79 (2C), 129.73 (2C), 136.06, 139.23, 143.81, 145.45, 148.94, 153.98, 162.46, 164.57, 166.15 ppm. MS: m/z (%) = 393 (M⁺, 48.29). Anal. Calcd. for C₁₆H₁₁N₉O₂S (393.08): C, 48.85; H, 2.82; N, 32.05%. Found: C, 48.98; H, 2.86; N, 32.12%.

6-Amino-9-hydroxy-10-(4-methoxyphenylazo)-5H-pyrazolo[1',5':1,2]pyrimido[5,4-e][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (17b)

Yield 64%, m.p. = 280–281°C. IR: 3351, 3269, 3172 (NH₂ and N-H), 1658 cm⁻¹ (C = O). ¹H NMR: δ 3.78 (s, 3H), 7.06 (d, *J* = 9.00 Hz, 2H), 7.78 (d, *J* = 9.00 Hz, 2H), 8.32 (s, 1H, thiadiazole-H), 8.94 (s, 2H), 11.90 ppm (s, 1H). ¹³C NMR: δ 55.71, 90.84, 102.60, 114.58 (2C), 128.47 (2C), 131.61, 143.12, 145.04, 152.45,

158.38, 159.73, 163.10, 164.75, 166.69 ppm. MS: m/z (%) = 409 (M⁺, 57.04). Anal. Calcd. for C₁₆H₁₁N₉O₃S (409.07): C, 46.94; H, 2.71; N, 30.79%. Found: C, 46.81; H, 2.78; N, 30.70%.

4.2. Biology

Biological examination procedures are fully deliberated in the supplementary materials file.

Antibacterial and antifungal activity.

The synthesized thiadiazolopyrimidine-based compounds were evaluated and adopted for antibacterial and antifungal effectiveness according to the approved procedure [38,39].

Antiquorum-sensing analysis.

Anti-QS inefficiency was evaluated applied according to the previous technique [40].

MTT cytotoxicity assay

The synthesized compounds were subjected to MTT cytotoxicity assay according to the cited literature [41].

In silico docking

In silico study was achieved to debate the mode of binding for prepared thiadiazolopyrimidine polycyclic compounds with the crystal structure potent inhibitors of NUDT5 silence hormone signaling in breast (PDB Code-5NQR) [37]. The PDB format of protein was designated from homepage of protein data bank and applied through operating MOE v10.2015.10 program.

Declarations

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Figures

Figure 1

The interactions between 16a and (PDB ID: 5NQR).

Figure 2

The interactions between 16b and (PDB ID: 5NQR).

Supplementary Files

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