Synthesis and biological screening of new thiadiazolopyrimidine-based polycyclic compounds

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Novel tri- and tetra-cyclic compounds based on the thiadiazolopyrimidine ring system were synthesized, and their antimicrobial activity was estimated. The obtained results evidenced the substantial efficiencies of pyrano-thiadiazolopyrimidine compounds 8a–b and 9a–b toward the two strains of gram-positive bacteria (*S. aureus* and *B. cereus*). Besides, tetracyclic pyrazolopyrimidothiadiazolopyrimidine 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 displayed good efficacy toward *C. albicans*. The activity of antiquorum sensing (anti-QS) inhibition of the newly synthesized thiadiazolopyrimidine-based compounds toward *C. violaceum* was tested, suggesting satisfactory activity for derivatives 16a–b, 17a–b, 8b, and 9a. The cytotoxic activity of these derivatives was screened toward various cancer cell lines (MCF-7, PC3, Hep-2, and HepG2) and standard normal fibroblast cells (WI38) by utilizing the MTT assay. The pyrazolopyrimidothiadiazolopyrimidine derivatives 16a, 16b, 17a, and 17b showed potent cytotoxic efficacy against the MCF-7 cells with the IC_{50} values ranging from 5.69 to 9.36 µM. Also, the endorsed structural activity relationship (SAR) of the inspected thiadiazolopyrimidine derivatives provided a correlation between the chemical structure and anticancer efficiency. The in silico docking studies were implemented for silencing the hormonal signaling in the breast (PDB Code-5NQR). The results were found to be consistent with the cytotoxic activity.

Inspired by the important role of antibiotics in the treatment and prevention of bacterial infections, the efficiency of the drugs is inadequate with the increase in the number of pathogens resistant to the antibiotics. The resistance to antibiotics is the main risk to public health and leads to an increase in the rate of morbidity and mortality in addition to the high cost of treatment. The extensive use of antibiotics causes the accumulation of microbial resistance. Thus, the current antivirulence approaches were established by genetic investigation to diagnose the virulence factors of numerous pathogens, where several methods were used to situate the pressure of the pathogens. Moreover, cancer is considered one of the primary causes of death in the world. It is defined as the growth of the tumor cell through its ability to disperse through other cells in the body by a progression termed as metastasis that leads to death in most cases. Cancer therapeutics include surgical treatment, radioactive treatment, immunotherapy, chemotherapy, etc. Chemotherapy is considered the most important step in the cancer treatment protocol. Nevertheless, the lack of selectivity of anticancer agents is the main limitation to the development of cancer medication. Thiadiazolopyrimidine derivatives are an important class of fused heterocyclic moieties with widespread biological effectiveness. The thiadiazolo-pyrimidine nucleus and its derivatives, belonging to the pseudo purine class, show interesting biological profiles, including antiviral, anticancer, antibiofilm, antitumor, antitubercular, antiglycation and antioxidant activities. In the past few decades, these analogues were synthesized as PARP1 inhibitors and STAT3 inhibitors.

A series of 6-cyano-1,3,4-thiadiazolo[3,2-a]pyrimidine derivatives showing a good binding mode in the active site of STAT3 enzyme inhibitors was synthesized to treat breast cancer. The 2-alkanesulfinyl/alkanesulfonyl-7-methyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one derivatives showed good cytotoxic activity, with exceptionally strong activity for the compounds containing electrophilic substituents, such as alkyl sulfoxide or alkyl sulfone, on the 2-position. A new series of biologically active sulfonamide derivatives of thiadiazolo[3,2-a]pyrimidine was synthesized and investigated for their antitumor activity. Some of them were...
tested for the in vitro and in vivo antitumor activities. Abdel Rahman and coworkers22 synthesized substituted thiadiazolo[3,2-a]pyrimidines and 1,3-disubstituted thiourea. Most of the compounds exhibited potent cytotoxic activity against the tumor cell line A549 (non-small cell lung cancer cell line)23 using the sulforhodamine B (SRB) standard method24. Recently, Nagaraju and coworkers reported the green synthesis25 and characterization of thiadiazolo[3,2-a]pyrimidines via the multi-component reaction between the chosen 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. Also, 7-oxo-7H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-5-carboxylic acid derivatives were conveniently synthesized under mild conditions through regioselective cycloadition reactions26. This observation drew attention to the synthesis of polyheterocyclic compounds containing 1,3,4-thiadiazolo [3,2-a]pyrimidine moiety and evaluation of their antimicrobial and cytotoxic properties.

Results and discussion

Chemistry. The reaction of 2-(2-cyanoacetamido)-1,3,4-thiadiazole (1)27 with dimethylformamide-dimethylacetel (DMF-DMA) was performed in boiling dioxane to synthesize 3-(dimethylamino)acrylonitrile derivative 2 (Fig. 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-7H-[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 absorptions of nitrile and cyclic carbonyl functions were recorded in the IR spectrum at 2229 and 1690 cm\(^{-1}\), respectively. In \(^1\)H NMR spectrum, the proton of pyrimidine ring resonates singlet at δ 8.51 ppm. The 13C NMR spectrum displayed ten carbon signals corresponding to twelve carbon atoms. The characteristic carbon signal of conjugated cyclic carbonyl group was recorded at δ 164.38 ppm.

The thiaadiazolo[3,2-a]pyrimidine derivative 3 was employed as a building unit for the construction of various functionalized tri- and tetra-cyclic compounds via reaction with nitrogen and carbon nucleophiles. The cyclization of thiaadiazoloprymidines 3 with hydrazine hydrate and/or phenylhydrazine was achieved by refluxing in EtOH/DMF mixture to produce the corresponding tricyclic compounds 3-aminoypyrazolo[4,3-e][thiaadiazolo][3,2-a]pyridymidine 4 and 5, respectively (Fig. 2). The chemical structures of 4 and 5 were characterized by IR, \(^1\)H NMR, \(^13\)C NMR, and MS analyses (experimental section). The IR spectra of compounds 4 and 5 did not show any absorption related to the nitrile function. The \(^1\)H NMR spectrum of 5 showed singlet at δ 6.44 ppm for two protons corresponding to amino group (–NH\(_2\)). The aromatic protons were observed as multiplet at δ 7.42–7.57 ppm. The proton of thiadiazole ring resonates singlet at δ 8.51 ppm. The \(^13\)C NMR spectrum displayed ten carbon signals corresponding to twelve carbon atoms. The characteristic carbon signal of conjugated cyclic carbonyl group was recorded at δ 164.38 ppm.

The tricyclic 6,8-diaminopyrothiadiazolo[3,2-a]pyrimidines 6 and 7 were obtained by the treatment of thiaadiazolo[3,2-a]pyrimidine derivative 3 with active nitrile components (namely, malononitrile and ethyl cyanoacetate). The reaction was conducted by heating the reactants in acetic acid and ammonium acetate (Fig. 3). The structures of 6 and 7 were elucidated from the results of the spectral analyses. The proposed mechanism for the reaction of thiaadiazolo[3,2-a]pyrimidine compound 3 with activated nitrile involves the nucleophilic addition of nitrile through its methylene group to the cyclic unsaturated nitrile of compound 3 to yield the intermediate Michael adduct (E). The heterocyclization of the intermediate (E) was assumed to occur by the addition of ammonia to the nitrile groups to produce the imino-perhydropryidine intermediate (F). The tautomerization leading to the aminodihydropyridine intermediate (G), followed by air oxidation (loss of H\(_2\)) results in the formation of the pyrothiadiazolo[3,2-a]pyrimidine compounds 6 and 7 (Fig. 3).

![Figure 1. Preparation of 6-cyano-7-oxo-7H-[1,3,4]thiadiazolo[3,2-a]pyrimidine (3).](image-url)
The pyrano[3,4-e]thiadiazolo[3,2-a]pyrimidine tricyclic and tetracyclic compounds 8, 9, and 10 were obtained by the reaction of thiadiazolo[3,2-a]pyrimidine derivative 3 with acetylacetone and benzoyl acetone as examples from diketones, acetyl acetonitrile and benzoyl acetonitrile as examples from ketonitriles and 3-methylpyrazolin-5-ones, respectively (Fig. 4). The reaction was carried out by heating the reactants in tetrahydrofuran, which was initiated by using the 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) protocol. The suggested mechanism involves the Michael addition of the enolate carbonyl reagent to the β-carbon of the unsaturated nitrile system. The produced intermediate undergoes intramolecular cyclization by the addition of enolic-OH functionality to the nitrile group in order to yield the pyranothiadiazolo[3,2-a]pyrimidine ring systems 8a and 8b. The structures of compounds 8, 9 and 10 were confirmed by the IR, 1H NMR, 13C NMR, and MS analyses. Accordingly, the 1H NMR spectrum of 8a (as a typical example) exhibited two singlet signals at δ 2.18 and 2.34 ppm to identify the protons of methyl and acetyl groups, respectively. The proton of pyran ring is recorded at δ 5.26 ppm as singlet signal. The proton of amino function (-NH₂) resonate as singlet at δ 6.79 ppm. The singlet at δ 8.28 was attributed to the proton of thiadiazole ring. The 13C NMR spectrum displayed eleven carbon signals. The characteristic carbon signal of acetyl-carbonyl carbon is observed at δ 195.91 ppm. The mass analysis recorded a molecular ion peak at m/z = 278.

The construction of pyrimidine nucleus fused with the building unit 3 has been explored through the reactions with various cyclic nitrogen 1,3-binucleophiles. Thus, the tetracyclic compounds 11, 12, and 13 were produced by 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 (Fig. 5). Finally, the tetracyclic derivatives, 6-amino-10-arylazo-pyrazolo[1′,5′:1,2]pyrimido[5,4-e]thiadiazolo[3,2-a]...
Figure 4. Synthesis of pyrothiadiazolo[3,2-a]pyrimidine derivatives 8, 9 and 10.

Figure 5. Reaction of thiadiazolopyrimidine derivative 3 with 3-aminotriazole, 5-aminotetrazole, and/or 5-aminopyrazoles.
determined by the following equation: 

\[ QS \text{ inhibition (mm)} = (r_2 - r_1) \]

where \( r_1 \) is the inhibition radius of the bacterial growth and \( r_2 \) is the inhibition radius for growth as well as the release of the pigment. The compounds 8a–b, 9a–b, and 3-amino-phenylpyrazolothiadiazolo-pyrimidinone (compounds 4 and 10) achieved antimicrobial results was discussed as follows: (1) The incorporation of a pyrazole ring to the thiadiazolopyrimidine skeleton to produce 3-aminopyrazolo-thiadiazolopyrimidinone and 3,5-diamino-4-arylazopyrazole 14 and/or 5-amino-4-arylazopyrazol-3-ol 15, respectively (Fig. 5). In general, the formation of the tetracyclic compounds 11, 12, 13, 16, and 17 involves the heating of compound 3 with the aminoazole reagent in pyridine for four hours. The chemical structures were characterized from the consistent data obtained from the spectral analyses (IR, 1H NMR, 13C NMR, and MS).

**Biological assessment.**

**Antimicrobial and antiquorum-sensing assessment.** The antimicrobial activity of the synthesized thiadiazolopyrimidine compounds was 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 assessment was performed through the two-fold dilution technique using Ampicillin (antibacterial) and Fluconazole (antifungal) as the reference drugs28,29. The minimal inhibitory concentration (MICs, µg/mL) of the synthesized derivatives for prohibiting microbial growth was determined through the visual detection (no turbidity) technique. The obtained results (Table 1) for the four bacterial strains indicated that the 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 gram-negative bacteria, such as E. coli and P. aeruginosa. Furthermore, compounds 8a–b and 9a–b showed good effectiveness toward C. albicans but no marked activity against A. fumigatus (Table 1).

The synthesized thiadiazolopyrimidine-based compounds were screened for their antiquorum-sensing (anti-QS) inhibition activity by the Chromobacterium violaceum (ATCC 12472) technique using catechin as the standard compound20–30. The QS technique of C. violaceum was remarked by the detection of violacein (violet pigment)10,12. Meanwhile, the reactivity of the synthesized thiadiazolopyrimidine-containing derivatives as drugs depends on their efficiency to inhibit the liberation of violacein during the QS technique. The QS inhibition was determined by the following equation: QS inhibition (mm) = (\( r_1 - r_2 \)), where \( r_1 \) is the inhibition radius of the bacterial growth and \( r_2 \) is the inhibition radius for growth as well as the release of the pigment. The compounds 8b, 9a, 16a–b, and 17a–b exhibited remarkable anti-QS activities (Table 2).

**Structural activity relationship.** The relationship between the structures of the synthesized thiadiazolopyrimidine-containing compounds and achieved antimicrobial results was discussed as follows: (1) The incorporation of a pyrazole ring to the thiadiazolopyrimidine skeleton to produce 3-aminoazopyrazolo-thiadiazolopyrimidinone and 3-amino-phenylpyrazolothiadiazolo-pyrimidinone (compounds 4 and 5) did not boost the activity toward all the screened bacterial strains. (2) The introduction of a pyran ring to the thiadiazolopyrimidine skeleton in compounds 8a–b, 9a–b and 10a–b promoted promising antibacterial effectiveness against S. aureus and B. cereus. In addition, they demonstrated remarkable antibacterial efficacy against C. albicans and no significant activity against A. fumigatus. (3) The tetracyclic pyrazolopyrimido-thiadiazolopyrimidinone derivatives 16a–b and 17a–b displayed good results against E. coli and P. aeruginosa, which may be attributed to the existence of

| Cpd. no. | MIC (µg/mL) |
| --- | --- |
| **Gram +ve bacteria** | **Gram –ve bacteria** | **Fungi** |
| | S. aureus | B. cereus | E. coli | P. aeruginosa | C. albicans | A. fumigatus |
| 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 | 1250 | 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 |

Table 1. Antibacterial and antifungal efficacy for the synthesized thiadiazolopyrimidine compounds. Ampicillin is the reference drug of bacteria; Fluconazole is the reference of fungi.
amino and hydroxyl groups at the six and nine positions. (4) The replacement of the methyl group in compound 9a by the phenyl ring caused favorable effectiveness against \( B.\) cereus (derivative 9b), while the replacement of the nitrile group on the pyran ring (derivative 9a) by the acetyl group (derivative 8a) led to eminent antimicrobial effectiveness toward gram-positive as well as gram-negative bacteria. (5) On the other hand, the incorporation of tetrazole and/or triazole moieties to the thiadiazolopyrimidine skeleton (compounds 11 and 12) diminished the activity toward all the examined microbes.

In vitro cytotoxicity evaluation. The cytotoxicity of the prepared tri-and tetra-cyclic thiadiazolopyrimidine compounds were examined toward various cancer cell lines, such as liver and breast cancer (MCF-7), prostate cancer (PC3), laryngeal carcinoma (Hep-2), carcinoma (HepG2), and standard normal fibroblast cells (WI38) using the MTT assay at the National Research Centre (Egypt). The cytotoxicity (Table 3) toward 50% inhibition of the cell viability (IC\(_{50}\) values) was assessed using 5-fluorouracil (5-Fu) as the standard anticancer drug. The thiadiazolopyrimidine derivatives 16a, 16b, 17a, and 17b exhibited the highest cytotoxic efficacy against the MCF-7 cell lines with the IC\(_{50}\) values of 7.53, 5.69, 9.36, and 8.84 µM, respectively. The thiadiazolopyrimidine derivatives 8b and 9b presented strong efficacy against the MCF-7 and Hep-2 cell lines, with the IC\(_{50}\) values ranging from 11.71 to 14.68 µM. Also, these compounds displayed moderate activity against the HepG2 and PC3 cell lines, as observed from their IC\(_{50}\) values ranging from 21.62 to 38.36 µM. The pyrazolo-thiadiazolopyrimidinone compounds 4 and 5 showed strong activity against the HepG2 cells with the IC\(_{50}\) values of 15.36 and 19.16 µM, respectively. Furthermore, compounds 16 and 17 showed moderate effectiveness against the three cell lines by following the order: Hep-2 > HepG2 > PC3.

The suggested structural activity relationship (SAR) of the thiadiazolopyrimidine derivatives suggested the structural countenance associated with the anticancer efficacy. (1) The pyrazole ring fused with the thiadiazolopyrimidinone skeleton in compounds 4 and 5 caused strong activity against the HepG2 cells and low effectiveness against the other three tested cells. (2) The incorporation of pyridine to the thiadiazolopyrimidine skeleton in compounds 6 and 7 did not offer the desired activity against the tested cell lines. In contrast, the fusion of the pyran ring to the thiadiazolopyrimidine skeleton in compounds 8b (substituted with benzoyl group) and 9b (substituted with phenyl group) presented strong anticancer efficacy against the MCF-7 and Hep-2 cells and reasonable activity against the HepG2 and PC3 cell lines. (3) The construction of the pyrazolopyrimidine moiety fused with the thiadiazolopyrimidine skeleton to produce tetracyclic compounds led to the enhancement of the anticancer activity against the MCF-7 cell lines. In addition, compounds 16a and 16b possessing an aminopyrazole nucleus exhibited higher cytotoxic efficacy against the MCF-7 cell lines than their corresponding compounds 17a and 17b containing a hydroxypyrazole nucleus. Also, the derivatives 16b and 17b (substituted with the 4-anisyl group) displayed higher reactivity than their conjugates 16a and 17a containing the 4-tolyl group. This is supported by order of biological anticancer activity toward MCF-7 cell lines on tuning the substituents. (4) The results of the cytotoxicity examination on normal cells (WI38) indicated that compounds 16 and 17 displayed the lowest cytotoxicity with the IC\(_{50}\) values ranging from 57.86 to 62.26 µM. (5) The tetracyclic compounds 16a

| Cpd. no. | Diameter of quorum-sensing inhibition (mm)a |
|----------|---------------------------------------------|
| 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                                           |

Table 2. Quorum-sensing inhibitor efficacy of the synthesized thiadiazolopyrimidine derivatives. a No effectiveness (–, <2 mm inhibition zone); weak effectiveness (2–9 mm); moderate effectiveness (10–15 mm); strong effectiveness (> 15 mm).
and 16b showed promising selectivity as cytotoxic agents against the MCF-7 cells with weak cytotoxic effects on normal cells (WI38).

Bleomycin-dependent DNA damage. The prepared polycyclic thiadiazolopyrimidine-based compounds were examined through the bleomycin-dependant DNA damage, and the results were compared to that of ascorbic acid as a positive control. The obtained data reflected the ability of these derivatives to protect the DNA from damage. The capability of compounds 16a–b and 17a–b to manifest the best protective effect against DNA damage was indicated by the corresponding absorbance values ranging from 0.031 to 0.053 (Table 4).

### Table 3. Cytotoxic activities of the synthesized derivatives. *5-Fluorouracil (5-Fu) is the reference drug for anticancer tests.

| Cpd. no. | Cytotoxicity IC<sub>50</sub> (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*   | 07.20 ± 0.45                  | 05.35 ± 0.23 | 08.78 ± 0.60 | 05.58 ± 0.31 | 10.32 ± 0.62 |

### Table 4. Bleomycin-dependent DNA damage of the synthesized thiadiazolopyrimidine scaffolds. *Values are mean for three replicates ± SD.

| Cpd. no. | Absorbance* |
|---------|-------------|
| 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 |
Molecular docking. The in silico molecular docking studies were conducted to evaluate the types of requisite interaction between the thiadiazolopyrimidine-based compounds and the crystal structure of the potent inhibitors of NUDT5 sHormone signaling in breast cancer (PDB Code-5NQR)\(^2\). The thiadiazolopyrimidine derivative 3 displayed two types of intermolecular interactions with low binding effects. The first type of interaction bonds the S atom of the thiadiazole ring with Asp 194, and the second interaction bonds the N-atom in the nitrile group with Gly 61 over a binding score S of −4.3922 kcal/mol (Fig. S1). The tricyclic compound 4 (pyrazolothiadiazolopyrimidine substituted with the amine functional group at position-3) showed two H-bonds resulting from the bonding of the N atom of pyrimidine with Arg 84 (2.92 Å) (Fig. S2) and that of the O atom of the carbonyl group with Arg 84. The resultant binding score S was found to be −4.4293 kcal/mol. The pyrazolo-thiadiazolopyrimidine compound 5 exhibited a better binding score (S = −4.2102 kcal/mol) through the formation of four H-bonds (Fig. S3). One of the H-bond resulted from the bonding of the S atom of the thiadiazole ring with Glu 169, the second bond formed between the N atom of the aminopyrazole moiety and Ser 172, while the third and fourth bonds were π–π interactions of the thiazole and pyrimidine rings with Ile 171.

Nonetheless, the pyridothiadiazolopyrimidine derivative 6 presented two H-bonds corresponding to the bonding of the N atom of aminopyridine with Arg 51 and that of the S atom of thiadiazole with Glu 65 (Fig. S4). The derivative 6 displayed weak interactions with the 5NQR amino acids (S = −4.5643 kcal/mol). Meanwhile, the pyridothiadiazolopyrimidine compound 7 revealed two H-bonds between the N atom of the aminopyridine moiety with Asp 194, and the O atom of the ester group with Arg 51 of 5NQR (S = −6.6614 kcal/mol) (Fig. S5). An H-bonding was formed by the N atom of the amide moiety with Asp 347, and a π–π bond was observed between the pyridine ring and Arg 84 (3.40 Å).

Similarly, the aminopyranothiadiazolopyrimidine derivative 8a displayed two H-bonds resulting from the bonding of the S atom of thiadiazole with Arg 194 and N atom of the aminoxazine moiety with Cys 139 (S = −5.7966 kcal/mol) (Fig. S6). While, compound 8b showed three intermolecular forces resulting from the thiadiazole ring, one H-bond formed by the S atom of thiadiazole with Cys 139, and two π–π interactions with Arg 51 and Met 132 over a binding score S of −5.9214 kcal/mol (Fig. S7). Also, derivative 9a demonstrated two H-bonds resulting from the bonding of the S atom of thiadiazole with Arg 194 and N atom of the nitrile group with Gly along with a binding energy score, S of −5.3557 kcal/mol (Fig. S8). Compound 9b exhibited two H-bonds between the N atom of the amine group bonded to Ala 96 and Arg 84 through a good score S of −6.1407 kcal/mol (Fig. S9). Moreover, the aminopyrazolopyrano-thiadiazolopyrimidines 10a and 10b exhibited H-bonds and π–π interactions. The derivative 10a demonstrated three H-bonds between the N atom of the amino group and Val 62, N1 of pyrazole and Ala 96, and N2 of pyrazole and Arg 84 in addition to the π–π interaction between the thiadiazole ring and Arg 51. The bonds resulted in an overall energy score (S) of −4.9886 kcal/mol (Fig. S10). Besides the three π–π interactions displayed by thiadiazole with Val 170, pyrazole with Ile 171, and phenyl with Ile 171, the derivative 10b demonstrated two H-bonds formed by the S atom in thiadiazole with Thr 117, and N1 of the pyrazole ring with Ser 172. The binding score of 10b was found to be −5.2193 kcal/mol, as shown in Fig. S11. Moreover, the tetracyclic structures 11–13 revealed reasonable binding scores from −5.2800 to −5.4772 kcal/mol resulting from different hydrogen bonds and π–π interactions (Table 5, Figs. S12, S13, and S14). Furthermore, the aminopyrazolothiadiazolopyrimidines 16 and 17 displayed remarkable binding scores. For instance, derivative 16a exhibited a binding score of −6.2989 kcal/mol (Fig. 6) attributed to the H-bonding of the N atom of pyrimidine with Arg 51, and the two π–π interactions of Ter 28 with pyridothiadiazolo-diazole and pyrimidopyrimidine, respectively. Compound 16b exhibited two intermolecular hydrogen bonds resulting from the N atom of aminopyrazole with Ala 96 and Glu 82 through a binding score S of −7.7053 kcal/mol (Fig. 7).

Alternatively, derivative 17a showed a binding score of −7.4560 kcal/mol (Fig. S15) resulting from the H-bond of the O atom in the hydroxyl group with Asp 194. Finally, derivative 17b showed π–π binding between the phenyl ring and Arg 51, and an H-bond between the N atom of the aminopyrimidine moiety and Asp 194 over a binding score of −7.5846 kcal/mol (Fig. S16). The standard reference drug 5-fluourouracil was subjected to 5NQR for a comparative study of the synthesized derivatives. The drug presented an intermolecular hydrogen bond with a binding score of −7.4560 kcal/mol (Fig. S17).

Finally, the docking technique showed that the derivatives 16a, 16b, 17a, and 17b gave respectable binding scores of −6.2989, −7.7053, −7.4560, and −7.5846 kcal/mol, respectively, in contrast to 5-Flourouracil exhibiting a binding score of −3.8546 kcal/mol with 5NQR. The two-and three-dimensional images of most of the derivatives presented two intramolecular hydrogen bonds resulting from the thiazole and pyrimidine moieties. The challenge in the docking method is the development of the level of conformation for the ligand interactions in distinct compounds depending on the binding scores. All the synthesized derivatives possess thiazole and pyrimidine moieties that form hydrogen bonds with the receptors and chemically disparate amino acids of 5NQR. The large pocket size of 5NQR was constrained by typically few polar residues with specific binding sites (Asp 194, Ter 28, Met 132, Arg 84, Gly 97 and Cys139), as observed from the three-dimensional images, and offered a proper cavity for the synthesized thiazolopyrimidine-based compounds.

Conclusion

Seventeen tricyclic and tetracyclic compounds containing the thiazolopyrimidine ring system were synthesized. The synthetic strategy for the preparation of the tricyclic compounds, pyrazolothiadiazolo-pyrimidines, pyridothiadiazolo-pyrimidines, and pyranothiadiazolo-pyrimidines, was based on the reactions of 6-cyano-7-oxo-7H- [1,3,4] thiazolopyrimidines 3 with various nitrogen containing nucleophilic reagents. The tetracyclic ring systems formed from pyrimido[5,4-e]thiazolopyrimidine skeleton fused with various azoles, such as tetrazole, triazole and/or pyrazoles, were prepared by the cyclization of the building block 3 with different α-aminooazole reagents. The targeted thiazolopyrimidine compounds were
| 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 Carboxyl 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        |
|      |                           |                    | Thiadiazole 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 pyrdine 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 Arg 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 Arg 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 Arg 194 | H-donor              | 3.06        |
|      |                           |                    | N of nitrile group with Glu 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        |
| 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 | π–π interaction    | 4.38        |
|      |                           |                    | Phenyl ring ring with Ile 171 | π–π interaction    | 3.77        |
| 11   | - 5.2800                  | 1.1608             | 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 Carboxyl 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        |
|      |                           |                    | pyrimidothiazolopyrimidering 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        |
| 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 Cys 139 | H-donor              | 3.93        |

Table 5. The binding interaction of the synthesized thiadiazolopyrimidine derivatives.
evaluated for their antimicrobial efficacy against the two types of bacterial strains along with the antifungal strains. The results demonstrated that the derivatives 8a–b and 9a–b with the pyranothiadiazolopyrimidine nucleus displayed significant activities against S. aureus and B. cereus. Meanwhile, the derivatives 16a–b and 17a–b showed prominent efficiencies against E. coli and P. aeruginosa. The synthesized thiadiazolopyrimidine compounds were tested by antiquorum-sensing, where the derivatives 16a–b, 17a–b, 8b and 9a demonstrated acceptable activities. The relationship between the chemical structures and recognized antimicrobial results was determined. The in vitro antitumor efficiency of the synthesized thiadiazolopyrimidine scaffolds toward the four cancer cells (MCF-7, PC3, Hep-2, and HepG2) and lung normal cell (WI38) was examined by employing the MTT technique. The tetracyclic pyrazolopyrimido[5,4-e]thiadiazolo[3,2-a]pyrimidine derivatives 16a, 16b, 17a and 17b recorded potent cytotoxic efficacy against the MCF-7 cancer cells. Also, the endorsed structural activity relationship of the synthesized thiadiazolopyrimidines provided a correlation between their chemical structures.

Figure 6. The interactions between 16a and (PDB ID: 5NQR).

Figure 7. The interactions between 16b and (PDB ID: 5NQR).
and anticancer efficiency. The in silico-docking studies (PDB Code-5NQR) indicated that the compounds 16b and 17b exhibited the highest binding scores (-7.7053 and -7.5846 kcal/mol).

**Experimental Chemistry.** The IR spectra were recorded using the Thermo Scientific Nicolet iS10 FTIR spectrometer using KBr discs. The 1H NMR (500 MHz) and 13C NMR (125 MHz) spectra were obtained in DMSO-d6 using the JEOL’s NMR spectrometer. The mass analyses were performed at 70 eV on the Shimadzu Qp-2010 plus mass spectrometer. The elemental analyses were conducted using the EuroVector instrument analyzer (EA3000 Series).

**Synthesis of 2-cyano-3-(dimethylamino)-N-(1,3,4-thiadiazol-2-yl)acrylamide (2).** In a 100 ml round bottom flask (RBF), 2-(cyanoacetamido)-1,3,4-thiadiazole (1) (1.68 g, 10 mmol) dissolved in 25 ml of THF was treated with N,N-dimethylformamidemethylacetal (1.20 ml, 10 mmol). The mixture was heated for 4 h after, which the product was formed as an orange solid that was collected and purified by recrystallization in acetic acid.

**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 obtained solid was diluted with 50 ml of cold water, which was collected and purified by recrystallization in dioxane.

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

**Synthesis of 6,8-diamino-5-oxo-pyrido[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) and malononitrile (or ethyl cyanoacetate) (0.005 mol) were taken in a 50 ml RBF. To this solution, the thiadiazolopyrimidine compound (0.89 g, 5 mmol) was dissolved in 15 ml THF taken in a 50 ml RBF. To this solution, the appropriate active methylene compound (namely, acetylacetone, benzoyl acetone, acetylacetonitrile, 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 up to 25 °C. The solid was filtered and recrystallized using THF to form the conforming pyranothiadiazolopyrimidines 8 and 9.
6-Amino-9-cyano-8-methyl-5H,9aH-pyrano[3,4-c][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one (9a).

Yield 72%, m.p. = 226–227 °C. IR: 3371, 3281, 3184 (NH2 and N–H), 3072, 2835, 2041, 1648, 1460, 1450, 1380, 1370, 1125, 1043, 1030, 968, 945, 768, 760, 725 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 (1H, thiadiazole-H). 13C NMR: δ 16.87, 44.29, 92.09, 118.01, 127.66 (2C), 128.58 (2C), 129.21, 131.40, 138.36, 161.75, 162.50, 165.30, 165.57 ppm. MS: m/z (%) = 278 (M⁺, 27%).

Analysis: Calcd. for C15H9N5O2S (323.05): C, 55.72; H, 2.81; N, 21.66%. Found: C, 55.93; H, 2.88; N, 21.90%.

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

Yield 76%, m.p. = 239–241 °C. IR: 3458, 3392, 3340, 2929, 2849, 1754, 1668, 1584, 1505, 1438, 1362, 1340, 1147, 1060, 1035, 1023, 972, 928, 876, 745 cm⁻¹ (C=O). 1H NMR: δ 2.18 (s, 3H), 2.34 (s, 3H), 4.78 (s, 1H, pyran-H), 7.08 (s, 2H), 7.47–7.56 (m, 5H), 8.34 ppm (1H, thiadiazole-H). 13C NMR: δ 13.22, 45.85, 92.09, 118.01, 127.66 (2C), 128.58 (2C), 129.21, 131.40, 138.36, 161.75, 162.50, 165.30, 165.57 ppm. MS: m/z (%) = 261 (M⁺, 45.09). Anal. Calcd. for C16H12N6O2S (352.07): C, 54.54; H, 3.43; N, 27.14%. Found: C, 54.29; H, 3.48; N, 27.10%.

Synthesis of 6-amino-10-methylpyrazolo[4,3-c][1,3,4]thiadiazolo[3,2-a]pyrimidin-5-ones, 10a and 10b.

In a 50 ml RBF, each of the 3-methylpyrazolone compounds (5 mmol) was mixed with 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 cooled down to room temperature. The resultant solid was collected, dried, and purified by recrystallization in an EtOH/DMF mixture (1:1) to furnish the cyclic compounds 10a and 10b, respectively.

6-Amino-5H-[1,2,4]triazolo[4,3-c][1,3,4]thiadiazolo[3,2-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, 1H). 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 C₁₅H₁₁N₅O₂S (326.02): C, 52.19; H, 3.43; N, 23.65%. Found: C, 52.48; H, 3.45; N, 23.71%.

Synthesis of tetracyclic compounds 11, 12, 13, 16 and 17.

The thiadiazolopyrimidine derivative 3 (0.89 g, 5 mmol) was dissolved in 20 ml pyridine, followed by the 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). The mixture was refluxed for 4 h and then diluted with 40 ml of cold water. The solid was collected and recrystallized in an EtOH/DMF mixture (1:1) to produce the tetracyclic compounds 11, 12, 13, 16 and 17, respectively.
6.48 (s, 1H, pyrazole-H), 8.38 (s, 1H, thiadiazole-H), 9.25 ppm (s, 2H). 13C NMR: δ 13.55, 92.16, 98.42, 142.87, 146.70, 150.06, 153.73, 160.58, 162.50, 163.73 ppm. MS: m/z (%) = 273 (M⁺, 90.32). Anal. Calcd. for C₁₁H₁₁N₉O₂S (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, 3269, 3186 (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 (%) = 393 (M⁺, 57.00). Anal. Calcd. for C₁₆H₁₁N₁₀O₂S (393.09): 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: 3404, 3361, 3264, 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 C₁₆H₁₂N₁₀O₃S (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: 3351, 3269, 3172 (NH2 and N–H), 1658 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, 150.45, 150.54, 157.48, 162.46, 164.57, 166.15 ppm. MS: m/z (%) = 392 (M⁺, 57.30). Anal. Calcd. for C₁₆H₁₂N₁₀O₃ (392.09): 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 (%) = 403 (M⁺, 57.04). Anal. Calcd. for C₁₆H₁₂N₁₀O₃ (403.08): C, 46.94; H, 2.71; N, 30.79%. Found: C, 46.81; H, 2.78; N, 30.70%.

**Biology.** The biological examination procedures are fully deliberated in the supplementary material.

**Antibacterial and antifungal activity.** The synthesized thiaadiazolopyrimidine-based compounds were evaluated and adopted for antibacterial and antifungal effectiveness using the approved procedure⁶⁶,⁶⁹,⁷⁰,⁸⁹. The anti-QS inefficiency was evaluated using the previous technique mentioned in the literature⁹⁰. The synthesized compounds were subjected to the MTT cytotoxicity assay on the basis of the cited literature⁹¹.

**In silico docking.** The in silico study was conducted to debate the mode of binding in the prepared thiaadiazolopyrimidine polycyclic compounds with the crystal structure potent inhibitors of NUDT5 silence hormone signaling in breast cancer (PDB Code-5NQR)⁹². The PDB format of the protein was designated from the homepage of the protein data bank and applied using the MOE v10.2015.10 program.

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Author contributions

A.M.A. who prepared the new compounds in the manuscript in Lab. The paid biological tests were carried out at National Research Centre (Egypt). The author wrote and reviewed the manuscript text.

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The author declares no competing interests.

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