Regular Article

Synthesis, *in-Vitro* Cytotoxicity and Antimicrobial Evaluations of Some Novel Thiazole Based Heterocycles

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Condensation of rhodanine (1) with pyrazol-3(2*H*)-one derivatives (2a–f) gave 5-substituted-2-thioxo-1,3-thiazolidin-4-one derivatives (3a–f). Reaction of compound (1) with 2-arylmethylidene-malononitrile (4a–d) yielded the unexpected derivatives (5a–d). The latter compounds were subjected to cyclization reactions with malononitrile under different basic conditions, hydroxylamine hydrochloride and/or thiourea to furnish the fused thiazole derivatives (6a–d) and (8–10a–d). Coupling of (1) with diazotized aromatic amines (11a–c) in pyridine afforded the arylhydrazones (12a–c). Fusion of latter compounds with malononitrile afforded the thiazolopyridazine derivatives (13a–c). The structures of the newly synthesized compounds were elucidated *via* spectral data and elemental analyses. The *in-vitro* cytotoxic activity of compounds (3a–f) against the cell line MCF-7 was evaluated. Also, the synthesized products were investigated for their antibacterial and antifungal activities against six standard organisms including the G^+ bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, G^- bacteria, *Escherichia coli* and *Proteus vulgaris* in addition to fungi, *Candida albicans* and *Aspergillus flavus*.

Key words fused thiazole; cytotoxicity; antibacterial activity; antifungal activity

Introduction

Rhodanines (2-thioxo-1,3-thiazolidin-4-ones) and rhodanine-based molecules are accepted as advantaged heterocycles in drugs discovery processes and in medicinal chemistry.[1,2] They have shown a varied range of pharmacological activities such as antimicrobial,[3–5] anticonvulsant,[6,7] antibacterial, antifungal and insecticidal activities.[8–13] Furthermore, some of them were reported to possess antiviral,[14] antidiabetic,[15,16] antiinflammatory[17,18] and antimalarial[19] activities. Moreover, various substituted rhodanines were found to be anti-tubercular,[20,21] antiproliferative agent against human colon cancer,[22] anti-human immunodeficiency virus (HIV),[23–27] cyclooxygenase (COX-2) inhibitors,[28] potent PTP1B inhibitors,[29] antiglioma and cytotoxicity.[30] Additionally, rhodanine-based molecules have been popular as small molecule inhibitors of numerous targets such as Hepatitis C virus NS3 protease,[31] *β*-lactamase,[32] uridine diphospho-*N*-acetylmuramate/L-alanine ligase,[33] cathepsin D[34] and c-Jun N-terminal kinase (JNK)-stimulating phosphatase-1 (JSP-1),[35] while some of rhodanine derivatives are used for the analysis of certain noble metal ions.[36] Based on these considerations, the present work aimed to synthesize some novel thiazole based heterocycles and to evaluate *in-vitro* for cytotoxic and antimicrobial activities.

Results and Discussion

Chemistry Condensation of rhodanine (**1**) with the appropriate 4-(arylmethylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**2a–f**) in boiling glacial acetic acid containing a catalytic amount of anhydrous sodium acetate afforded the corresponding 5-[4-(arylmethylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ylidene]-2-thioxo-1,3-thiazolidin-4-one (**3a–f**) in a high yield (Chart 1). The structures of the latter compounds were elucidated by their elemental analyses and spectral data (*cf.* Experimental).

Reaction of compound (**1**) with 2-arylmethylidenemalononitrile (**4a–d**) in absolute ethanol in presence of piperidine as a catalyst and stirring at room temperature furnished compounds (**5a–d**) in a good yield. The structures of these compounds were identified to be the unexpected 5-arylmethylidene-2-thioxo-1,3-thiazolidin-4-one not the expected 5-amino-7-aryl-2-thioxo-3,7-dihydro-2*H*-pyrano[2,3-*d*]-1,3-thiazole-6-carbonitrile (**6a–d**) on the basis of their IR, MS, ^{1}H-NMR, ^{13}C-NMR and elemental analyses. The IR spectra of compounds (**5a–d**) revealed the presence of C=O group and

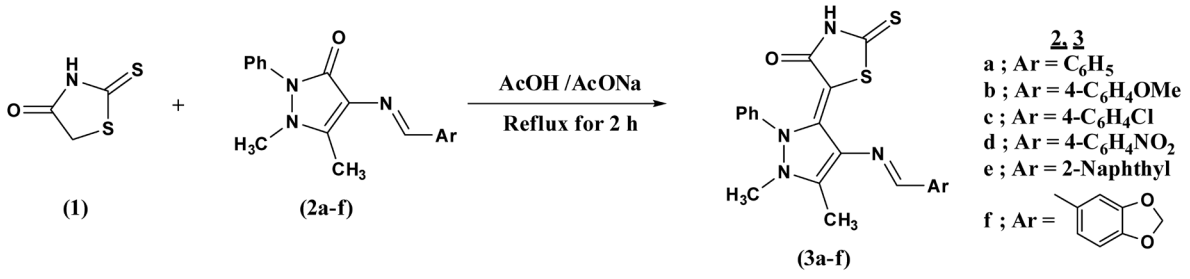

Chart 1. Synthesis of 5-[4-(Arylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ylidene]-2-thioxo-1,3-thiazolidin-4-one Derivatives (**3a–f**)

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there are no bands due to $-NH_2$ or $-C\equiv N$ groups. In addition to the presence of signals due to $=CH$ proton in ^{1}H-NMR. Further verification for the structures of compounds (**5a**–**d**) was their synthesis directly by condensation of rhodanine (**1**) with aromatic aldehydes (**7a**–**d**) in presence of various catalysts as reported[37–42] (identical mp, mixed mp and TLC). This is agreeing with a previous result.[20] However, the pyrano[2,3-*d*]thiazole derivatives (**6a**–**d**) were obtained by the reaction of the 5-arylmethylidene-thiazolidin-4-one derivatives (**5a**–**d**) with malononitrile in boiling ethanol in presence of catalytic amount of triethylamine (Chart 2). The structures of compounds (**6a**–**d**) were excluded on the basis of elemental analyses and spectral data (*cf.* Experimental).

The proposed mechanism for the formation of compounds (**5a**–**d**) can be represented as shown in Chart 3. Initially, rhodanine (**1**) and 2-arylmethylidenemalononitrile (**4a**–**d**) are reacted by Michael addition in presence of base to give the intermediate (**A**), which upon enolization gave the intermediate (**B**). This intermediate was expected to undergo the reverse of a Michael reaction "*β*-elimination of malononitrile molecule—pathway (a)" to yield compounds (**5a**–**d**). The presence of malononitrile in the reaction medium was detected using TLC. The intramolecular *6-exo-dig* cyclization of the intermediate (**B**)—pathway (b) to furnish the pyranothiazole derivatives (**6a**–**d**) not happened under these conditions.

Treatment of compounds (**5a**–**d**) with malononitrile in absolute ethanol in presence of a catalytic amount of ammonium acetate furnished the thiazolo[4,5-*b*]pyridine-6-carbonitrile derivatives (**8a**–**d**). The structures of these compounds were elucidated by their elemental analyses, IR, MS, ^{1}H-NMR and

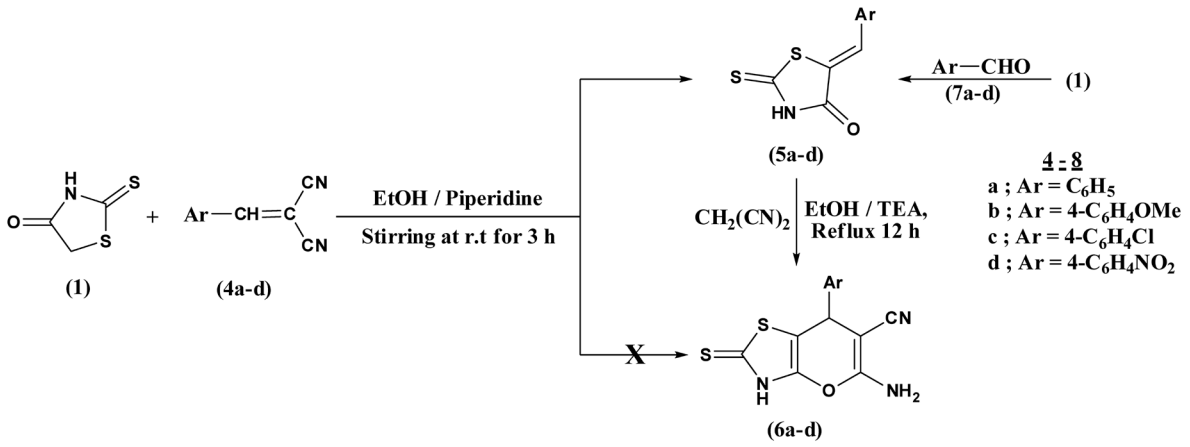

Chart 2. Reaction of Rhodanine (**1**) with 2-Arylidenemalononitrile (**4a**–**d**)

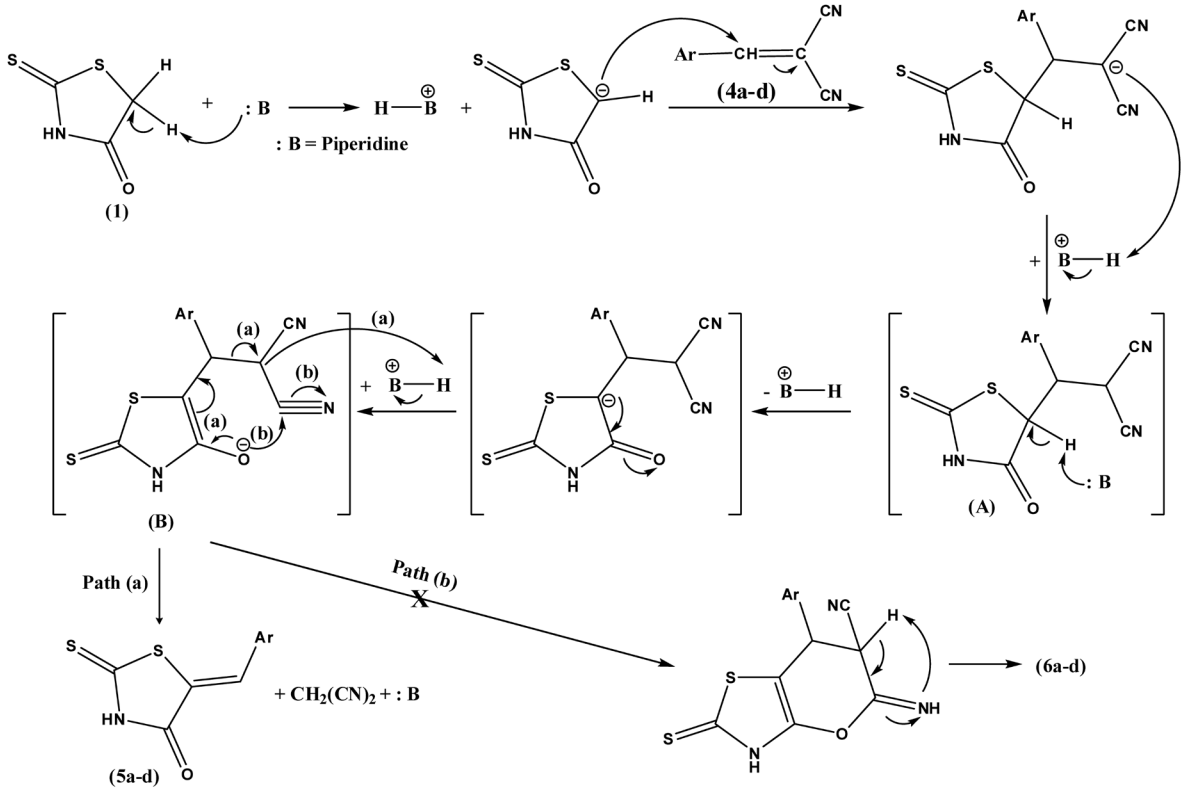

Chart 3. The Proposed Mechanism for the Formation of Compounds (**5a–d**) Not the Expected (**6a–d**)

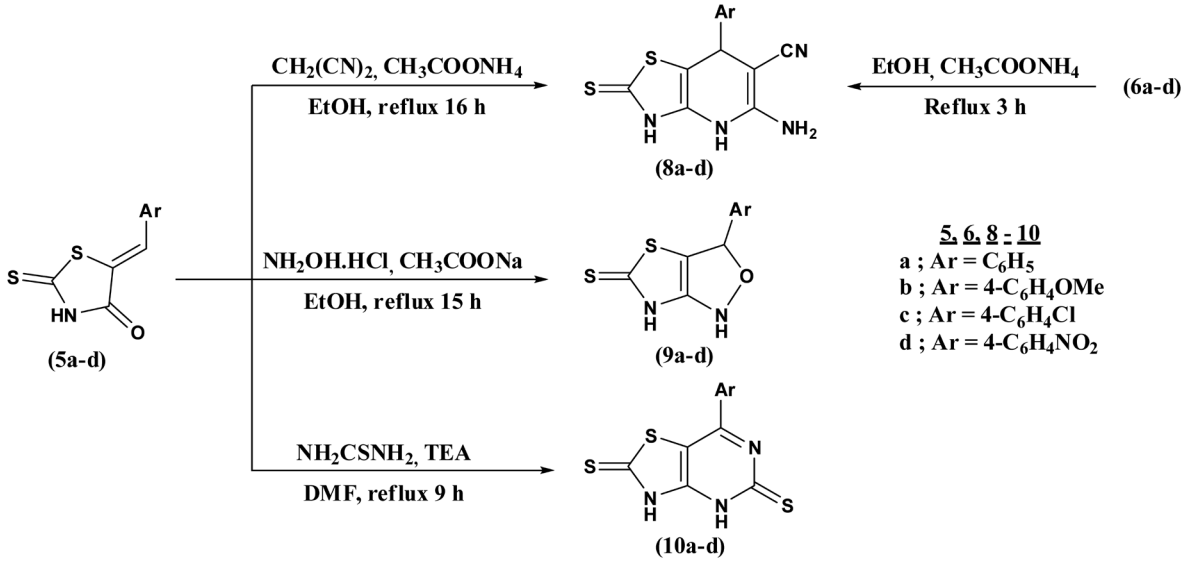

Chart 4. Synthesis of 1,3-Thiazolo[4,5-*b*]pyridine (**8a–d**); 1,3-Thiazolo[4,5-*c*]isoxazole (**9a–d**) and 1,3-Thiazolo[4,5-*d*]pyrimidine (**10a–d**)

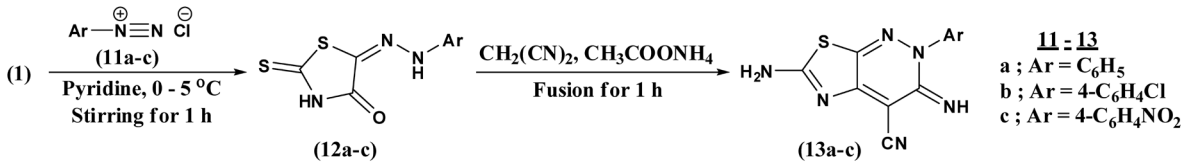

Chart 5. Synthesis of 1,3-Thiazolo[5,4-*c*]pyridazine Derivatives (**13a–c**)

^{13}C-NMR. Compounds (**8a–d**) were also obtained by boiling the pyrano[2,3-*d*]-1,3-thiazole derivatives (**6a–d**) in absolute ethanol containing a catalytic amount of ammonium acetate (identical mp, mixed mp, TLC and spectra). Furthermore, refluxing compounds (**5a–d**) with hydroxylamine hydrochloride in absolute ethanol containing anhydrous sodium acetate gave the 1,3-thiazolo[4,5-*c*]isoxazole derivatives (**9a–d**). In addition, when the arylmethylidene derivatives (**5a–d**) were reacted with thiourea in boiling *N*,*N*-dimethylformamide (DMF) containing few drops of triethylamine, 1,3-thiazolo[4,5-*d*]-pyrimidine derivatives (**10a–d**) were obtained (Chart 4). The spectral data and elemental analyses proved the structures of compounds (**9a–d**) and (**10a–d**) (*cf.* Experimental).

Ultimately, coupling of rhodanine (**1**) with diazotized aromatic amines (**11a–c**) in pyridine at 0°C afforded the arylhydrazone derivatives (**12a–c**) based on their spectral data as well as their synthesis using a reported method[43] (identical mp, mixed mp and TLC). The obtained arylhydrazones have been utilized as starting materials for preparing fused pyridazine ring system. Fusion of the arylhydrazones (**12a–c**) with malononitrile in presence of ammonium acetate over melting point for one hour gave the thiazolo[5,4-*c*]pyridazine derivatives (**13a–c**) (Chart 5). The structures of the latter compounds were confirmed based on elemental analyses and spectral data. Thus, the IR spectra of compounds (**13a–c**) indicated the presence of the absorption bands of the C≡N functional group at 2202–2205 cm^{-1}. Also, the IR spectra revealed the absence of absorption bands due to C=S functional group, as well as a strong smell of H_2S gas was evolved during the reaction (*cf.* Experimental).

Cytotoxicity Evaluation The new synthesized compounds (**3a–f**) were evaluated for human tumour cell growth inhibitory activity against MCF-7 human breast carcinoma cell. The *in-vitro* cytotoxicity evaluation using viability assay was performed at the Regional Centre for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt, using Doxorubicin as reference standard drug. The inhibitory activity results are depicted in Table 1 (*cf.* Experimental). The results of *in-vitro* inhibitory activities of the tested compounds against MCF-7 cancer cell have the following descending order **3a** > **3d** > **3e** > **3c** > **3b** > **3f**.

Table 1. Anticancer IC_{50} Values for Selected Compounds against MCF-7

Compound	MCF-7, IC_{50} values (μg/mL)
3a	7.67 ± 0.6
3b	145 ± 4.9
3c	123 ± 3.1
3d	11.7 ± 0.9
3e	106 ± 2.5
3f	245 ± 8.6
Doxorubicin	0.35 ± 0.03

Antimicrobial Screening The new synthesised compounds were tested for their *in-vitro* antimicrobial activity against six standard organisms including the Gram-positive bacteria, *Staphylococcus aureus* [RCMB 010010] and *Bacillus subtilis* [RCMB 015 (1), NRRL B-543], Gram-negative bacteria, *Escherichia coli* [RCMB 010052, ATCC 25955] and *Proteus vulgaris* [RCMB 004 (1), ATCC 13315] in addition to fungi, *Candida albicans* [RCMB 005003 (1), ATCC 10231] and *Aspergillus flavus* [RCMB 002002]. The antimicrobial screening was performed at the Regional Centre for Mycology and Biotechnology (RCMP) at Al-Azhar University, Cairo,

Table 2. Antimicrobial Screening for the All the Synthesized Compounds

Compound	Inhibition zone diameter (mm)					
	Gram-positive bacteria G^+		Gram-negative bacteria G^-		Fungi	
	Staphylococcus aureus [RCMB 010010]	*Bacillus subtilis* [RCMB 015 (1)] [NRRL B-543]	*Escherichia coli* [RCMB 010052] [ATCC 25955]	*Proteus vulgaris* [RCMB 004 (1)] [ATCC 13315]	*Candida albicans* [RCMB 005003 (1)] [ATCC 10231]	*Aspergillus flavus* [RCMB 002002]
3a	14	14	NA	19	NA	NA
3b	12	18	NA	12	NA	NA
3c	18	22	NA	21	NA	NA
3d	18	14	NA	13	NA	NA
3e	10	18	NA	11	NA	NA
3f	10	17	NA	19	NA	NA
6a	16	16	NA	9	NA	13
6b	16	16	18	14	NA	16
6c	20	18	19	16	18	17
6d	18	16	NA	14	NA	13
8a	10	11	NA	12	NA	9
8b	11	11	NA	15	NA	12
8c	14	9	NA	17	NA	18
8d	12	7	NA	9	NA	21
9a	17	14	NA	20	18	17
9b	18	12	NA	17	13	22
9c	18	16	NA	15	12	16
9d	20	16	NA	15	14	15
10a	18	18	NA	13	13	21
10b	16	16	NA	8	18	8
10c	20	18	NA	15	20	17
10d	20	18	NA	16	17	16
12a	23	14	8	14	15	20
12b	12	16	NA	12	12	NA
12c	18	18	12	12	24	28
13a	14	14	8	NA	15	15
13b	14	12	NA	NA	14	8
13c	13	13	NA	NA	17	8
Gentamycin	24	26	30	25	—	—
Ketoconazole	—	—	—	—	16	20

* NA = No activity; Well diameter of the hole = 6.0 mm (100 μL was tested), RCMB = Regional Center for Mycology and Biotechnology.

Egypt, using Gentamycin (minimum inhibitory concentration (MIC) 4 μg/mL) and Ketoconazole (MIC 100 μg/mL) as reference standard drugs for bacteria and fungi, respectively. The antimicrobial results are depicted in Table 2 (*cf.* Experimental).

The results indicated that five compounds (**6c**, **9d**, **10c**, **10d** and **12a**) showed high antimicrobial activity against *Staphylococcus aureus*, and only one compound (**3c**) showed high antimicrobial activity against *Bacillus subtilis*. On the other hand, all the synthesized compounds except (**6c**, **9d**, **10c**, **10d** and **12a**) showed moderate antimicrobial activity against *Staphylococcus aureus*, as well as all of them except (**3c**, **8c** and **8d**) showed moderate antimicrobial activity against *Bacillus subtilis*, compared with the standard antibiotic Gentamycin.

Also, the results revealed that four compounds (**3a**, **3c**, **3f** and **9a**) showed high antimicrobial activity against *Proteus vulgaris*. On the other hand, twenty compound (**3b**, **3d**, **3e**, **6a**, **6b**, **6c**, **6d**, **8a**, **8b**, **8c**, **8d**, **9b**, **9c**, **9d**, **10a**, **10c**, **10d**, **12a**, **12b** and **12c**) showed moderate antimicrobial activity against *Proteus vulgaris*, with respect to Gentamycin.

In comparison with the well-known antifungal Ketoconazole, seven compounds (**6c**, **9a**, **10b**, **10c**, **10d**, **12c** and **13c**) showed very high antifungal activity against *Candida albicans*, while eight compounds (**9b**, **9c**, **9d**, **10a**, **12a**, **12b**, **13a** and **13b**) showed high antifungal activity against it. Furthermore, four compounds (**8d**, **9b**, **10a** and **12c**) showed very high antifungal activity against *Aspergillus flavus*, as well as ten compounds (**6b**, **6c**, **8c**, **9a**, **9c**, **9d**, **10c**, **10d**, **12a** and **13a**) showed high antifungal activity against *Aspergillus flavus*. Ultimately, seven compounds (**6a**, **6d**, **8a**, **8b**, **10b**, **13b** and **13c**) showed moderate antifungal activity against *Aspergillus flavus*, as shown in Table 2.

In conclusion, some compounds showed congruent results against the most tested microorganisms compared to the standard drugs Gentamycin and Ketoconazole.

Experimental

Chemistry Melting points of the reaction products were determined in open capillary tubes on an electro-thermal melting point apparatus (MEL-TEMP II) and were uncorrected. TLC was used in the monitoring of the progress of all reactions and in the checking of the homogeneity of the synthesized compounds. It was run on TLC aluminium silica gel sheets 60 F_{254} (Merck) using UV light (254 and 365 nm) for

detection. The FTIR were recorded on a Bruker (Model Alpha II) Infrared spectrometer at the Central Lab, Faculty of Science, Ain Shams University, Cairo, Egypt. The ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Bruker Avance (III) 400 MHz spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) spectrometer in dimethyl sulfoxide (DMSO)-d_6 as solvent, using tetramethylsilane (TMS) as internal reference and chemical shifts (δ) are expressed in ppm at the Center for Discovery Research and Development at Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. Mass spectra were recorded at (70 ev) and carried out on direct probe controller inlet part to single quadrupole mass analyzer in Thermo Scientific GCMS Model (ISQ LT) using Thermo X-Caliber Software at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Naser City, Cairo, Egypt. Elemental analyses were performed at the Central Lab, Faculty of Science, Ain Shams University, Cairo, Egypt.

General Procedure for Synthesis of Compounds (3a–f) A mixture of rhodanine (**1**) (0.01 mol, 1.33 g) and 4-(arylmethylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (**2a**–**f**) (0.01 mol) in glacial acetic acid (20 mL) in presence of anhydrous sodium acetate (0.03 mol, 2.5 g) was heated under reflux for 2 h. After cooling, the solid product obtained was filtered off, dried and recrystallized from ethanol to give (**3a**–**f**).

5-[4-(Benzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ylidene]-2-thioxo-1,3-thiazolidin-4-one (**3a**)

Yellow crystals; yield 89.3%; mp 200–202°C. ^{1}H-NMR (DMSO-d_6) δ: 2.51 (3H, –CH_3, s), 3.37 (3H, –**N**CH_3, s), 7.48–7.60 (10H, Ar–H, m), 7.65 (1H, =CH, s), 13.82 (1H, –NH, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 15.40, 39.59, 108.65, 119.75, 125.65, 126.02, 128.65, 128.85, 129.91, 130.93, 131.20, 132.09, 133.44, 163.25, 169.86, 196.18. IR (KBr) υ: 3139 (N–H), 3036 (stretching C–$H_{arom.}$), 2916, 2848 (C–$H_{aliph.}$), 1696 (C=O), 1668 (C=N), 1225 (C=S), 759, 672 (bending C–$H_{arom.}$ monosubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 406 (M^+, 3.80), 347 (4.19), 329 (6.55), 319 (12.49), 316 (3.69), 303 (4.10), 272 (5.82), 134 (100), 103 (5.14), 90 (35.80), 87 (30.67), 77 (26.66), 59 (60.42). *Anal.* Calcd for $C_{21}H_{18}N_4OS_2$ (406.52): C, 62.04; H, 4.46; N, 13.78. Found: C, 62.19; H, 4.42; N, 13.87.

5-[4-(4-Methoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ylidene]-2-thioxo-1,3-thiazolidin-4-one (**3b**)

Yellow crystals; yield 87.5%; mp 238–240°C. ^{1}H-NMR (DMSO-d_6) δ: 2.49 (3H, –CH_3, s), 3.00 (3H, –**N**CH_3, s), 3.81 (3H, –**O**CH_3, s), 7.07–7.55 (9H, Ar–H, m), 9.15 (1H, =CH, s), 13.51 (1H, –NH, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 19.78, 31.35, 56.01, 108.65, 115.49, 123.21, 125.65, 126.04, 127.95, 128.85, 129.35, 130.38, 131.89, 133.05, 161.69, 170.57, 196.26. IR (KBr) υ: 3127 (N–H), 3055 (stretching C–$H_{arom.}$), 2917, 2847 (C–$H_{aliph.}$), 1682 (C=O), 1655 (C=N), 1236 (C=S), 820 (bending C–$H_{arom.}$ *p*-disubstituted ring), 776, 734 (bending C–$H_{arom.}$ monosubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 436 (M^+, 100), 421 (3.76), 405 (8.69), 380 (7.78), 360 (2.69), 345 (13.52), 329 (12.94), 316 (1.30), 302 (1.66), 289 (1.34), 147 (21.31), 134 (3.10), 120 (52.40), 107 (11.30), 91 (18.28), 76 (73.69), 56 (8.86). *Anal.* Calcd for $C_{22}H_{20}N_4O_2S_2$ (436.55): C, 60.53; H, 4.62; N, 12.83. Found: C, 60.71; H, 4.65; N, 12.75.

5-[4-(4-Chlorobenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ylidene]-2-thioxo-1,3-thiazolidin-4-one (**3c**)

Yellow crystals; yield 86.4%; mp 222–224°C. ^{1}H-NMR (DMSO-d_6) δ: 2.45 (3H, –CH_3, s), 3.19 (3H, –**N**CH_3, s), 7.36–7.83 (9H, Ar–H, m), 9.57 (1H, =CH, s), 13.48 (1H, –NH, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 19.51, 34.78, 112.4, 126.55, 127.60, 128.70, 128.85, 129.08, 132.15, 131.50, 135.20, 135.39, 140.85, 144.05, 163.25, 166.65, 198.15. IR (KBr) υ: 3148 (N–H), 3032 (stretching C–$H_{arom.}$), 2919, 2843 (C–$H_{aliph.}$), 1690 (C=O), 1652 (C=N), 1235 (C=S), 822 (bending C–$H_{arom.}$ *p*-disubstituted ring), 726, 674 (bending C–$H_{arom.}$ monosubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 442.5 ($M^+ + 2$, 5.34), 440.5 (M^+, 24.80), 384.5 (31.16), 56 (100). *Anal.* Calcd for $C_{21}H_{17}ClN_4OS_2$ (440.97): C, 57.20; H, 3.89; N, 12.71. Found: C, 57.33; H, 3.92; N, 12.65.

5-[4-(4-Nitrobenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ylidene]-2-thioxo-1,3-thiazolidin-4-one (**3d**)

Orange crystals; yield 88.7%; mp 244–246°C. ^{1}H-NMR (DMSO-d_6) δ: 2.51 (3H, –CH_3, s), 3.26 (3H, –**N**CH_3, s), 7.37–8.30 (9H, Ar–H, m), 9.66 (1H, =CH, s), 13.61 (1H, –NH, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 31.35, 108.65, 125.65, 126.02, 126.25, 127.10, 128.15, 128.85, 132.35, 135.05, 137.20, 140.58, 149.35, 162.09, 168.19, 199.01. IR (KBr) υ: 3169 (N–H), 3040 (stretching C–$H_{arom.}$), 2917, 2847 (C–$H_{aliph.}$), 1699 (C=O), 1668 (C=N), 1523 (NO_2, asym.), 1340 (NO_2, sym.), 1231 (C=S), 844 (bending C–$H_{arom.}$ *p*-disubstituted ring), 763, 680 (bending C–$H_{arom.}$ monosubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 451 (M^+, 12.64), 405 (14.07), 374 (17.29), 360 (9.96), 329 (12.64), 122 (20.63), 91 (25.66), 77 (100). *Anal.* Calcd for $C_{21}H_{17}N_5O_3S_2$ (451.52): C, 55.86; H, 3.79; N, 15.51. Found: C, 55.97; H, 3.84; N, 15.59.

5-[1,5-Dimethyl-4-(naphthalen-2-ylmethyleneamino)-2-phenyl-1*H*-pyrazol-3(2*H*)-ylidene)-2-thioxo-1,3-thiazolidin-4-one (**3e**)

Yellow crystals; yield 89%; mp 258–260°C. ^{1}H-NMR (DMSO-d_6) δ: 2.53 (3H, –CH_3, s), 3.40 (3H, –**N**CH_3, s), 7.58–7.78 (5H, phenyl protons, m), 7.96–8.07 (7H, naphthyl protons, m), 8.19 (1H, =CH, s), 13.91 (1H, –NH, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 19.05, 34.89, 108.65, 119.70, 125.65, 126.17, 126.55, 127.20, 127.69, 128.00, 132.30, 133.54, 134.85, 135.05, 152.25, 166.85, 191.75. IR (KBr) υ: 3138 (N–H), 3048 (stretching C–$H_{arom.}$), 2916, 2835 (C–$H_{aliph.}$), 1699 (C=O), 1670 (C=N), 1224 (C=S), 760, 699 (bending C-$H_{arom.}$ monosubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 456 (M^+, 100), 379 (1.48), 365 (2.91), 338 (1.87), 329 (1.26), 316 (1.44), 302 (4.13), 154 (9.85), 140 (23.92), 127 (21.76), 118 (3.03), 91 (45.61), 77 (31.48). *Anal.* Calcd for $C_{25}H_{20}N_4OS_2$ (456.58): C, 65.76; H, 4.42; N, 12.27. Found: C, 65.90; H, 4.45; N, 12.33.

5-[4-(Benzo[d][1,3]dioxol-5-ylmethyleneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-ylidene]-2-thioxo-1,3-thiazolidin-4-one (**3f**)

Yellow crystals; yield 88.5%; mp 278–280°C. ^{1}H-NMR (DMSO-d_6) δ: 2.49 (3H, –CH_3, s), 3.31 (3H, –**N**CH_3, s), 6.13 (2H, –CH_2, s), 7.07–7.16 (8H, Ar–H, m), 7.54 (1H, =CH, s), 13.73 (1H, –NH, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 18.79, 34.49, 104.95, 108.67, 118.53, 120.01, 125.50, 125.65, 126.02, 126.55, 126.78, 127.75, 129.55, 132.35, 139.80, 140.58, 152.25, 155.48, 166.65, 198.15. IR (KBr) υ: 3141 (N–H), 3042 (stretching C–$H_{arom.}$), 2983, 2892 (C–$H_{aliph.}$),

1687 (C=O), 1627 (C=N), 1217 (C=S), 745, 701 (bending C-$H_{arom.}$ monosubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 450 (M^+, 13.80), 435 (11.04), 391 (16.70), 373 (16.70), 359 (13.83), 330 (10.68), 120 (21.16), 91 (18.64), 77 (8.52), 59 (100). *Anal.* Calcd for $C_{22}H_{18}N_4O_3S_2$ (450.53): C, 58.65; H, 4.03; N, 12.44. Found: C, 58.78; H, 4.08; N, 12.53.

General Procedure for Synthesis of Compounds (5a–d) **Method A:** A solution of compound (**1**) (0.01 mol, 1.33 g) and 2-arylmethylidenemalononitrile (**4a–d**) in absolute ethanol in presence of piperidine as a catalyst was stirred at room temperature for 3 h. The solid product obtained was filtered off, dried and recrystallized from ethanol to afford (**5a–d**).

Method B: According to literature.[37–42]

General Procedure for Synthesis of Compounds (6a–d) A mixture of 5-arylmethylidene-derivatives (**5a–d**) (0.01 mol) and malononitrile (0.01 mol, 0.66 g) was heated for 12 h in (30 mL) of absolute ethanol in presence of few drops of triethylamine under reflux. After cooling at room temperature, the resulted precipitate was filtered off, dried and recrystallized from ethanol to give (**6a–d**).

5-Amino-7-phenyl-2-thioxo-3,7-dihydro-2*H*-pyrano[2,3-*d*]-1,3-thiazole-6-carbonitrile (**6a**)

Reddish brown crystals; yield 84%; mp 248–250°C. ^{1}H-NMR (DMSO-d_6) δ: 4.41 (1H, pyran H-4, s), 6.91 (2H, $-NH_2$, exchangeable with D_2O, s), 7.09–7.44 (5H, Ar–H, m), 7.65 (1H, –NH, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 39.79, 103.89, 116.95, 128.50, 129.28, 137.62, 160.90, 165.37, 189.2. IR (KBr) υ: 3307, 3211, 3150 ($-NH_2$, N–H), 3082 (stretching C–$H_{arom.}$), 2925, 2855 (C–$H_{aliph.}$), 2216 (C≡N), 1643 (C=C), 1236 (C=S), 762, 703 (bending C–$H_{arom.}$ monosubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 287 (M^+, 26.72), 271 (5.35), 245 (6.78), 221 (21.36), 210 (49.78), 205 (8.51), 196 (18.63), 91 (10.87), 82 (12.32), 77 (46.17), 66 (100). *Anal.* Calcd for $C_{13}H_9N_3OS_2$ (287.36): C, 54.34; H, 3.16; N, 14.62. Found: C, 54.49; H, 3.21; N, 14.72.

5-Amino-7-(4-methoxyphenyl)-2-thioxo-3,7-dihydro-2*H*-pyrano[2,3-*d*]-1,3-thiazole-6-carbonitrile (**6b**)

Brown crystals; yield 82.8%; mp 230–232°C. ^{1}H-NMR (DMSO-d_6) δ: 3.83 (3H, $-OCH_3$, s), 4.90 (1H, pyran H-4, s), 7.05 (2H, $-NH_2$, exchangeable with D_2O, s), 7.13–7.62 (4H, Ar–H, m), 7.78 (1H, NH, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 39.68, 52.40, 103.91, 116.95, 128.50, 129.28, 137.62, 150.53, 160.48, 165.37, 189.34. IR (KBr) υ: 3307, 3210, 3150 ($-NH_2$, N–H), 3081 (stretching C–$H_{arom.}$), 2934, 2841 (C–$H_{aliph.}$), 2216 (C≡N), 1644 (C=C), 1230 (C=S), 824 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 317 (M^+, 100), 301 (1.28), 286 (1.42), 275 (5.98), 251 (4.13), 226 (4.95), 210 (1.48), 107 (12.26), 91 (3.96), 66 (12.07). *Anal.* Calcd for $C_{14}H_{11}N_3O_2S_2$ (317.39): C, 52.98; H, 3.49; N, 13.24. Found: C, 53.11; H, 3.51; N, 13.31.

5-Amino-7-(4-chlorophenyl)-2-thioxo-3,7-dihydro-2*H*-pyrano[2,3-*d*]-1,3-thiazole-6-carbonitrile (**6c**)

Buff crystals; yield 79.8%; mp 156–158°C. ^{1}H-NMR (DMSO-d_6) δ: 4.15 (1H, pyran H-4, s), 7.20 (2H, $-NH_2$, exchangeable with D_2O, s), 7.33–7.63 (4H, Ar–H, m), 7.65 (1H, –NH, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 39.37, 103.91, 116.95, 129.28, 129.84, 130.86, 131.90, 137.62, 160.48, 165.37, 188.74. IR (KBr) υ: 3307, 3211, 3150 ($-NH_2$, N–H), 3080 (stretching C–$H_{arom.}$), 2919, 2850 (C–$H_{aliph.}$), 2214 (C≡N), 1645 (C=C), 1238 (C=S), 825 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 323.5 ($M^+ + 2$, 100), 321.5 (M^+, 3.41), 305.5 (6.67), 279.5 (6.72), 210 (1.36), 255.5 (1.29), 230.5 (9.85), 111.5 (9.51), 91 (1.40). *Anal.* Calcd for $C_{13}H_8ClN_3OS_2$ (321.81): C, 48.52; H, 2.51; N, 13.06. Found: C, 48.40; H, 2.55; N, 13.15.

5-Amino-7-(4-nitrophenyl)-2-thioxo-3,7-dihydro-2*H*-pyrano[2,3-*d*]-1,3-thiazole-6-carbonitrile (**6d**)

Dark brown crystals; yield 77.2%; mp 180–182°C. ^{1}H-NMR (DMSO-d_6) δ: 4.49 (1H, pyran H-4, s), 7.22 (2H, $-NH_2$, exchangeable with D_2O, s), 7.32–7.86 (4H, Ar–H, m), 8.01 (1H, –NH, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 56.28, 40.41, 84.05, 117.85, 126.59, 128.37, 142.65, 143.08, 146.65, 151.75, 189.20. IR (KBr) υ: 3351, 3256, 3106 ($-NH_2$, N–H), 3081 (stretching C–$H_{arom.}$), 2918, 2850 (C–$H_{aliph.}$), 2212 (C≡N), 1646 (C=C), 1514 (NO_2 asym.), 1345 (NO_2 sym.), 1230 (C=S), 839 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 332 (M^+, 1.67), 316 (1.23), 290 (2.04), 266 (3.72), 241 (2.70), 210 (2.96), 122 (26.74), 91 (2.07), 66 (2.34), 46 (100). *Anal.* Calcd for $C_{13}H_8N_4O_3S_2$ (332.36): C, 46.98; H, 2.43; N, 16.86. Found: C, 47.01; H, 2.48; N, 16.94.

General Procedure for Synthesis of Compounds (8a–d) **Method A:** A solution of compounds (**5a–d**) (0.01 mol), malononitrile (0.01 mol, 0.66 g) and ammonium acetate (1 g) in absolute ethanol (30 mL) was heated under reflux for 16 h and allowed to cool at room temperature. The solid product obtained was filtered off, washed with water, dried and recrystallized from ethanol to afford the thiazolopyridine derivatives (**8a–d**).

Method B: A solution of equimolar amounts of the pyranothiazole derivatives (**6a–d**) (0.01 mol) and ammonium acetate (0.01 mol) in absolute ethanol (30 mL) was heated for 3 h under reflux. The solid product obtained was filtered off, washed with water, dried and recrystallized from ethanol to give (**8a–d**).

5-Amino-7-phenyl-2-thioxo-2,3,4,7-tetrahydro-1,3-thiazolo-[4,5-*b*]pyridine-6-carbonitrile (**8a**)

Reddish brown crystals; yield 81.3%; mp 183–185°C. ^{1}H-NMR (DMSO-d_6) δ: 4.18 (1H, pyridine H-4, s), 6.95–7.73 (8H, Ar–H, $-NH_2$, –NH pyridine, m), 8.18 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 39.49, 60.92, 103.58, 116.96, 127.01, 128.09, 128.55, 137.60, 140.35, 152.98, 189.87. IR (KBr) υ: 3445, 3317, 3204, 3162 ($-NH_2$, two N-H), 3082 (stretching C–$H_{arom.}$), 2920, 2850 (C–$H_{aliph.}$), 2213 (C≡N), 1627 (C=C), 1233 (C=S), 762, 700 (bending C–$H_{arom.}$ monosubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 286 (M^+, 1.66), 270 (5.33), 244 (18.26), 220 (100), 209 (18.97), 2014 (1.51), 195 (1.68), 91 (4.20), 82 (1.86), 77 (1.77), 66 (1.21). *Anal.* Calcd for $C_{13}H_{10}N_4S_2$ (286.38): C, 54.52; H, 3.52; N, 19.56. Found: C, 54.65; H, 3.57; N, 19.66.

5-Amino-7-(4-methoxyphenyl)-2-thioxo-2,3,4,7-tetrahydro-1,3-thiazolo[4,5-*b*]pyridine-6-carbonitrile (**8b**)

Reddish brown crystals; yield 79.4%; mp 214–216°C. ^{1}H-NMR (DMSO-d_6) δ: 3.74 (3H, $-OCH_3$, s), 4.39 (1H, pyridine H-4, s), 7.21–7.82 (7H, Ar–H, $-NH_2$, –NH pyridine, m), 8.24 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 49.73, 51.08, 57.60, 103.85, 117.58, 121.35, 129.52, 143.05, 145.65, 159.90, 162.00, 192.35. IR (KBr) υ: 3459, 3313, 3207, 3135 ($-NH_2$, two N–H), 3094 (stretching C–$H_{arom.}$), 2917, 2843 (C–$H_{aliph.}$), 2211 (C≡N), 1645 (C=C), 1241 (C=S), 821 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 316 (M^+, 2.09), 300 (67.35), 285 (20.32), 274 (100), 250 (8.95), 225 (8.16), 209 (28.84), 107 (39.84), 91 (47.94), 66 (4.04). *Anal.* Calcd for $C_{14}H_{12}N_4OS_2$ (316.40): C,

53.14; H, 3.82; N, 17.71. Found: C, 53.20; H, 3.87; N, 17.81.

5-Amino-7-(4-chlorophenyl)-2-thioxo-2,3,4,7-tetrahydro-1,3-thiazolo[4,5-*b*]pyridine-6-carbonitrile (**8c**)

Buff crystals; yield 78.1%; mp 128–130°C. ^{1}H-NMR (DMSO-d_6) δ: 4.47 (1H, pyridine H-4, s), 7.46–7.88 (7H, Ar–H, –NH_2, –NH pyridine, m), 8.37 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 40.93, 89.63, 118.37, 123.30, 128.51, 129.80, 136.40, 141.07, 142.49, 195.66. IR (KBr) υ: 3432, 3332, 3204, 3148 (–NH_2, two N-H), 3081 (stretching C–$H_{arom.}$), 2919, 2850 (C–$H_{aliph.}$), 2212 (C≡N), 1648 (C=C), 1236 (C=S), 820 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 320.5 (M^+, 24.18), 322.5 (M^+ + 2, 16.64), 304.5 (46.35), 278.5 (15.72), 209 (13.32), 254.5 (23.22), 229.5 (20.50), 111.5 (40.18), 91 (100). *Anal.* Calcd for $C_{13}H_9ClN_4S_2$ (320.82): C, 48.67; H, 2.83; N, 17.46. Found: C, 48.78; H, 2.86; N, 17.52.

5-Amino-7-(4-nitrophenyl)-2-thioxo-2,3,4,7-tetrahydro-1,3-thiazolo[4,5-*b*]pyridine-6-carbonitrile (**8d**)

Brown crystals; yield 76.4%; mp 135–137°C. ^{1}H-NMR (DMSO-d_6) δ: 4.53 (1H, pyridine H-4, s), 7.58–7.90 (7H, Ar–H, –NH_2, –NH pyridine, m), 8.43 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 41.85, 90.13, 119.08, 123.90, 126.84, 128.79, 130.20, 142.19, 144.90, 195.80. R (KBr) υ: 3432, 3343, 3204, 3108 (–NH_2, two N–H), 3081 (stretching C–$H_{arom.}$), 2922, 2852 (C–$H_{aliph.}$), 2215 (C≡N), 1630 (C=C), 1515 (NO_2 asym.), 1344 (NO_2 sym.), 1231 (C=S), 844 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 331 (M^+, 100), 315 (97.87), 289 (47.05), 265 (10.71), 240 (44.50), 109 (9.51), 122 (42.24), 91 (33.30), 66 (45.07). *Anal.* Calcd for $C_{13}H_9N_5O_2S_2$ (331.37): C, 47.12; H, 2.74; N, 21.13. Found: C, 47.28; H, 2.78; N, 21.21.

General Procedure for Synthesis of Compounds (9a–d) A mixture of 5-arylidene-derivatives (**5a–d**) (0.01 mol) and hydroxylamine hydrochloride (0.01 mol, 0.69 g) in absolute ethanol (30 mL) and anhydrous sodium acetate (0.01 mol, 0.82 g) was heated under reflux for 15 h. After cooling at room temperature, the reaction mixture was poured on ice with continuous stirring. The formed precipitate was filtered off, dried and recrystallized from ethanol to give (**9a–d**).

3-Phenyl-3,6-dihydro-1,3-thiazolo[4,5-*c*]isoxazole-5(1*H*)-thione (**9a**)

Buff crystals; yield 72.1%; mp 256–258°C. ^{1}H-NMR (DMSO-d_6) δ: 5.61 (1H, isoxazole H-3, s), 7.32–7.48 (5H, Ar–H, m), 10.89 (1H, –NH isoxazole, exchangeable with D_2O, s), 11.96 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 39.56, 123.44, 125.71, 128.50, 131.27, 133.82, 145.09, 165.88. IR (KBr) υ: 3149, 3112 (two N–H), 3027 (stretching C–$H_{arom.}$), 2925, 2852 (C–$H_{aliph.}$), 1650 (C=C), 1247 (C=S), 759, 684 (bending C–$H_{arom.}$ monosubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 236 (M^+, 1.58), 205 (19.39), 160 (31.99), 159 (26.40), 145 (27.56), 91 (90.41), 77 (62.80), 76 (100). *Anal.* Calcd for $C_{10}H_8N_2OS_2$ (236.31): C, 50.83; H, 3.41; N, 11.85. Found: C, 50.72; H, 3.38; N, 11.78.

3-(4-Methoxyphenyl)-3,6-dihydro-1,3-thiazolo[4,5-*c*]-isoxazole-5(1*H*)-thione (**9b**)

Yellow crystals; yield 69.8%; mp 210–212°C. ^{1}H-NMR (DMSO-d_6) δ: 3.74 (3H, –OCH_3, s), 5.63 (1H, isoxazole H-3, s), 7.48–7.55 (4H, Ar–H, m), 10.89 (1H, –NH isoxazole, exchangeable with D_2O, s), 12.01 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 39.80, 55.09, 123.98, 126.33, 129.02, 131.76, 133.92, 145.70, 166.45. IR (KBr) υ: 3230, 3139 (two N–H), 3033 (stretching C–$H_{arom.}$), 2930, 2848 (C–$H_{aliph.}$), 1663 (C=C), 1240 (C=S), 824 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 266 (M^+, 100), 235 (57.95), 190 (2.85), 159 (8.53), 107 (65.23), 76 (1.92). *Anal.* Calcd for $C_{11}H_{10}N_2O_2S_2$ (266.34): C, 49.61; H, 3.78; N, 10.52. Found: C, 49.79; H, 3.85; N, 10.61.

3-(4-Chlorophenyl)-3,6-dihydro-1,3-thiazolo[4,5-*c*]-isoxazole-5(1*H*)-thione (**9c**)

Buff crystals; yield 75.3%; mp 296–298°C. ^{1}H-NMR (DMSO-d_6) δ: 5.69 (1H, isoxazole H-3, s), 7.55–7.59 (4H, Ar–H, m), 10.95 (1H, –NH isoxazole, exchangeable with D_2O, s), 12.05 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 40.60, 124.08, 126.67, 129.68, 133.03, 134.42, 146.18, 166.78. IR (KBr) υ: 3189, 3137 (two N–H), 3026 (stretching C–$H_{arom.}$), 2920, 2849 (C–$H_{aliph.}$), 1662 (C=C), 1239 (C=S), 820 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 272.5 (M^+ + 2, 8.17), 270.5 (M^+, 100), 194.5 (3.55), 179.5 (8.17), 159 (40.92), 111.5 (16.31), 91 (9.38), 76 (4.15). *Anal.* Calcd for $C_{10}H_7ClN_2OS_2$ (270.76): C, 44.36; H, 2.61; N, 10.35. Found: C, 44.53; H, 2.59; N, 10.29.

3-(4-Nitrophenyl)-3,6-dihydro-1,3-thiazolo[4,5-*c*]isoxazole-5(1*H*)-thione (**9d**)

Buff crystals; yield 73.9%; mp 270–272°C. ^{1}H-NMR (DMSO-d_6) δ: 5.72 (1H, isoxazole H-3, s), 7.57–7.62 (4H, Ar–H, m), 10.98 (1H, –NH isoxazole, exchangeable with D_2O, s), 12.09 (1H, –NH thiazole, exchangeable with D_2O, s).^{13}C-NMR (DMSO-d_6) δ: 40.88, 124.72, 126.96, 129.87, 133.41, 134.69, 146.37, 167.03. IR (KBr) υ: 3202, 3108 (two N–H), 3015 (stretching C–$H_{arom.}$), 2920, 2849 (C–$H_{aliph.}$), 1657 (C=C), 1512 (NO_2 asym.), 1341 (NO_2 sym.), 1241 (C=S), 843 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 281 (M^+, 28.72), 235 (22.56), 205 (5.21), 159 (14.95), 122 (58.76), 76 (100). *Anal.* Calcd for $C_{10}H_7N_3O_3S_2$ (281.31): C, 42.70; H, 2.51; N, 14.94. Found: C, 42.78; H, 2.54; N, 15.01.

General Procedure for Synthesis of Compounds (10a–d) A mixture of compounds (**5a–d**) (0.01 mol) and thiourea (0.01 mol, 0.76 g) in dimethylformamide (10 mL) in presence of few drops of triethylamine was heated under reflux for 9 h. After cooling at room temperature, the reaction mixture was poured on ice. The formed precipitate was filtered off, dried and recrystallized from the ethanol solvent to give (**10a–d**).

7-Phenyl-1,3-thiazolo[4,5-*d*]pyrimidine-2,5(3*H*,4*H*)-dithione (**10a**)

Brown crystals; yield 70.4%; mp 160–162°C. ^{1}H-NMR (DMSO-d_6) δ: 6.98–7.58 (6H, Ar–H, NH pyrimidine, m), 7.67 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 128.50, 129.32, 130.17, 130.55, 133.00, 134.49, 174.63, 178.04. IR (KBr) υ: 3349, 3212 (two N–H), 3058 (stretching C–$H_{arom.}$), 1599 (C=N), 1237 (C=S), 763, 678 (bending C–$H_{arom.}$ monosubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 277 (M^+, 2.16), 201 (1.75), 200 (1.69), 186 (1.93), 174 (3.24), 147 (12.48), 130 (25.43), 103 (1.21), 91 (100), 77 (1.96), 76 (1.29). *Anal.* Calcd for $C_{11}H_7N_3S_3$ (277.39): C, 47.63; H, 2.54; N, 15.15. Found: C, 47.81; H, 2.49; N, 15.22.

7-(4-Methoxyphenyl)-1,3-thiazolo[4,5-*d*]pyrimidine-2,5(3*H*,4*H*)-dithione (**10b**)

Brown crystals; yield 73.1%; mp 134–136°C. ^{1}H-NMR (DMSO-d_6) δ: 3.86 (3H, –OCH_3, s), 7.12–7.60 (5H, Ar–H, –NH pyrimidine, m), 7.84 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 55.60, 115.05, 128.13, 129.45, 130.34, 130.89, 133.01, 134.69, 174.78, 178.20. IR

(KBr) υ: 3330, 3239 (two N-H), 3074 (stretching C–$H_{arom.}$), 2933, 2834 (C–$H_{aliph.}$), 1600 (C=N), 1241 (C=S), 824 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 307 (M^+, 1.91), 276 (1.31), 231 (100), 200 (4.29), 177 (36.55), 174 (5.47), 133 (1.40), 130 (11.71), 107 (2.01), 76 (2.85). *Anal.* Calcd for $C_{12}H_9N_3OS_3$ (307.41): C, 46.88; H, 2.95; N, 13.67. Found: C, 46.69; H, 2.89; N, 13.58.

7-(4-Chlorophenyl)-1,3-thiazolo[4,5-*d*]pyrimidine-2,5(3*H*,4*H*)-dithione (**10c**)

Buff crystals; yield 69.5%; mp 210–212°C. ^{1}H-NMR (DMSO-d_6) δ: 7.42–7.79 (5H, Ar–H, NH pyrimidine, m), 8.06 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 128.78, 129.71, 130.58, 131.58, 133.28, 134.69, 175.09, 179.27. IR (KBr) υ: 3329, 3247 (two N–H), 3054 (stretching C–$H_{arom.}$), 1618 (C=N), 1220 (C=S), 822 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 313.5 (M^+ + 2, 2.81), 311.5 (M^+, 8.69), 200 (37.38), 196.5 (2.73), 181.5 (2.95), 174 (5.58), 137.5 (1.76), 130 (2.19), 115 (100), 111.5 (10.16). *Anal.* Calcd for $C_{11}H_6ClN_3S_3$ (311.83): C, 42.37; H, 1.94; N, 13.48. Found: C, 42.59; H, 1.98; N, 13.59.

7-(4-Nitrophenyl)-1,3-thiazolo[4,5-*d*]pyrimidine-2,5(3*H*,4*H*)-dithione (**10d**)

Reddish brown crystals; yield 67%; mp 178–180°C. ^{1}H-NMR (DMSO-d_6) δ: 7.51–7.88 (5H, Ar–H, NH pyrimidine, m), 8.32 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 128.91, 129.84, 130.65, 131.90, 133.77, 134.87, 176.23, 180.04. IR (KBr) υ: 3344, 3222 (two N–H), 3076 (stretching C–H_{arom}), 1592 (C=N), 1515 (NO_2 asym.), 1342 (NO_2 sym.), 1234 (C=S), 831 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 322 (M^+, 2.64), 276 (12.48), 246 (100), 231 (1.81), 207 (1.20), 200 (2.18), 192 (1.96), 174 (1.28), 148 (1.45), 130 (9.01), 122 (7.84), 115 (2.88), 91 (2.15), 76 (20.70). *Anal.* Calcd for $C_{11}H_6N_4O_2S_3$ (322.39): C, 40.98; H, 1.88; N, 17.38. Found: C, 40.83; H, 1.92; N, 17.47.

General Procedure for Synthesis of Compounds (12a–c)
Method A: A solution of (**1**) (0.01 mol, 1.33 g) in pyridine (20 mL) was cooled to 0°C, stirred and treated gradually with cooled solution of aryl diazonium chloride (**11a–c**) [prepared from (0.02 mol, 1.4 g) $NaNO_2$ dissolved in (5 mL) water, added with keeping temperature between 0–5°C to (0.01 mol) of amines namely aniline, *p*-chloroaniline and/or *p*-nitroaniline dissolved in (10 mL) of conc. HCl]. After complete addition, the reaction mixture was stirred for 1 h at 0°C. The solid product formed was collected, washed with water, dried and recrystallized from ethanol to give (**12a–c**).

Method B: According to literature.[43)]

5-(2-Phenylhydrazono)-2-thioxo-1,3-thiazolidin-4-one (**12a**)

Orange crystals; yield 93.5%; mp 216–218°C. ^{1}H-NMR (DMSO-d_6) δ: 7.18–7.38 (5H, Ar–H, m), 10.99 (1H, –NH, exchangeable with D_2O, s), 13.67 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 193.05, 165.11, 141.97, 129.48, 126.28, 125.93, 116.08. IR (KBr) υ: 3236, 3160 (two N–H), 3054 (stretching C–$H_{arom.}$), 1695 (C=O), 1597 (C=N), 1212 (C=S), 743, 686 (bending C–$H_{arom.}$ monosubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 237 (M^+, 7.40), 145 (13.17), 160 (16.52), 146 (8.36), 131 (5.83), 106 (16.70), 92 (22.47), 91 (3.77), 77 (100). *Anal.* Calcd for $C_9H_7N_3OS_2$ (237.30): C, 45.55; H, 2.97; N, 17.71. Found: C, 45.76; H, 2.93; N, 17.84.

5-[2-(4-Chlorophenylhydrazono)-2-thioxo-1,3-thiazolidin-4-one (**12b**)

Orange crystals; yield 94%; mp 248–250°C. ^{1}H-NMR (DMSO-d_6) δ: 7.29–7.46 (4H, Ar–H, m), 11.07 (1H, –NH, exchangeable with D_2O, s), 13.82 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 193.13, 165.24, 142.50, 129.75, 126.77, 126.64, 116.30. IR (KBr) υ: 3241, 3189 (two N–H), 3081 (stretching C–$H_{arom.}$), 1698 (C=O), 1602 (C=N), 1216 (C=S), 824 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 273.5 (M^+ + 2, 10.31), 271.5 (M^+, 6.00), 195.5 (8.00), 180.5 (2.81), 160 (9.44), 145 (7.63), 140.5 (19.02), 131 (9.10), 126.5 (7.50), 111.5 (4.85), 76 (100). *Anal.* Calcd for $C_9H_6ClN_3OS_2$ (271.75): C, 39.78; H, 2.23; N, 15.46. Found: C, 39.62; H, 2.28; N, 15.57.

5-[2-(4-Nitrophenylhydrazono)-2-thioxo-1,3-thiazolidin-4-one (**12c**)

Brown crystals; yield 91.8%; mp 186–188°C. ^{1}H-NMR (DMSO-d_6) δ: 7.40–7.88 (4H, Ar–H, m), 11.14 (1H, –NH, exchangeable with D_2O, s), 13.87 (1H, –NH thiazole, exchangeable with D_2O, s). ^{13}C-NMR (DMSO-d_6) δ: 193.42, 167.38, 144.03, 129.50, 126.89, 126.82, 116.90. IR (KBr) υ: 3246, 3191 (two N–H), 3097 (stretching C–H_{arom}), 1691 (C=O), 1595 (C=N), 1551 (NO_2 asym.), 1330 (NO_2 sym.), 1225 (C=S), 840 (bending C–H_{arom} *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 282 (M^+, 10.88), 236 (12.32), 206 (100), 191 (23.02), 160 (10.73), 151 (1.27), 145 (27.97), 137 (6.94), 131 (12.60), 122 (6.31), 91 (11.43), 76 (4.24), 46 (4.96). *Anal.* Calcd for $C_9H_6N_4O_3S_2$ (282.30): C, 38.29; H, 2.14; N, 19.85. Found: C, 38.47; H, 2.21; N, 19.97.

General Procedure for Synthesis of Compounds (13a–c)
An equimolar mixture of compounds (**12a–c**) (0.01 mol) and malononitrile (0.01 mol, 0.66 g) in ammonium acetate (2 g) was fused for 1 h. The reaction mixture was left to stand at room temperature and triturated with ethanol. The solid product was collected by filtration, washed with water, dried and recrystallized from ethanol to give (**13a–c**).

6-Amino-3-imino-2-phenyl-2,3-dihydro-1,3-thiazolo[5,4-*c*]-pyridazine-4-carbonitrile (**13a**)

Dark brown crystals; yield 69%; mp >300°C. ^{1}H-NMR (DMSO-d_6) δ: 6.81–7.41 (8H, Ar–H, =NH, –NH_2, m). ^{13}C-NMR (DMSO-d_6) δ: 102.01, 116.75, 125.45, 126.05, 129.00, 142.95, 151.70, 158.60, 158.75, 162.90. IR (KBr) υ: 3323, 3183 (–NH_2, =NH), 3054 (stretching C–$H_{arom.}$), 2205 (C≡N), 1616, 1596 (C=N), 756, 690 (bending C–$H_{arom.}$ monosubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 268 (M^+, 1.76), 252 (11.24), 191 (39.08), 163 (1.20), 150 (10.24), 118 (6.95), 105 (9.07), 77 (100). *Anal.* Calcd for $C_{12}H_8N_6S$ (268.30): C, 53.72; H, 3.01; N, 31.32. Found: C, 53.55; H, 3.07; N, 31.41.

6-Amino-2-(4-chlorophenyl)-3-imino-2,3-dihydro-1,3-thiazolo[5,4-*c*]pyridazine-4-carbonitrile (**13b**)

Reddish brown crystals; yield 82%; mp >300°C. ^{1}H-NMR (DMSO-d_6) δ: 6.94–7.81 (7H, Ar–H, =NH, –NH_2, m). ^{13}C-NMR (DMSO-d_6) δ: 102.18, 116.97, 125.85, 126.63, 129.17, 143.13, 151.76, 158.56, 158.90, 163.05. IR (KBr) υ: 3321, 3173 (–NH_2, =NH), 3081 (stretching C–$H_{arom.}$), 2202 (C≡N), 1613, 1594 (C=N), 824 (bending C–$H_{arom.}$ *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 304.5 (M^+ + 2, 100), 302.5 (M^+, 59.00), 286.5 (4.40), 267 (3.09), 237.5 (6.41), 228.5 (5.85), 191 (3.53), 111.5 (5.97), 74 (4.85), 65 (1.51). *Anal.* Calcd for $C_{12}H_7ClN_6S$ (302.74): C, 47.61; H, 2.33; N, 27.76. Found: C, 47.75; H, 2.28; N, 27.85.

6-Amino-3-imino-2-(4-nitrophenyl)-2,3-dihydro-1,3-thiazolo[5,4-*c*]pyridazine-4-carbonitrile (**13c**)

Reddish brown crystals; yield 73.7%; mp >300°C. ^{1}H-NMR (DMSO-d_6) δ: 7.14–8.33 (7H, Ar–H, =NH, –NH_2, m). ^{13}C-NMR (DMSO-d_6) δ: 102.76, 117.28, 125.93, 127.00, 129.57, 143.00, 151.95, 158.84, 159.11, 163.23. IR (KBr) υ: 336, 3200, 3191 (–NH_2, =NH), 3097 (stretching C–H_{arom}), 2202 (C≡N), 1616, 1590 (C=N), 1514 (NO_2 asym.), 1334 (NO_2 sym.), 842 (bending C–H_{arom} *p*-disubstituted ring) cm^{-1}. MS (70 ev) *m/z* (%): 313 (M^+, 100), 297 (10.80), 287 (11.05), 267 (2.46), 239 (36.89), 191 (23.09), 122 (9.14), 74 (11.80), 46 (6.40). *Anal.* Calcd for $C_{12}H_7N_7O_2S$ (313.29): C, 46.00; H, 2.25; N, 31.30. Found: C, 46.16; H, 2.31; N, 31.20.

Cytotoxicity Evaluation The new synthesized compounds (**3a**–**f**) were evaluated for human tumour cell growth inhibitory activity against MCF-7 human breast carcinoma cell, which was obtained from VACSERA Tissue Culture Unit. The measurements of cell growth and the viabilities were determined as described in the literature.[44,45] The *in-vitro* cytotoxicity evaluation using viability assay was performed using the standard drug Doxorubicin as reference.

For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100 μL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, U.S.A.) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 24 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, for 24 h, the viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 min. The stain was removed, and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates was measured after gently shaken on Microplate reader (TECAN, Inc., U.S.A.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the microplate reader (Sunrise, TECAN, Inc.) to determine the number of viable cells and the percentage of viability was calculated as $[(ODt/ODc)] \times 100\%$ where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The IC_{50} was estimated from graphic plots of the dose response curve for each conc. using GraphPad Prism Software (San Diego, CA, U.S.A.). The inhibitory activity results are depicted in Table 1.

Antimicrobial Screening The susceptibility tests were performed according to recommendations of National Committee for Clinical Laboratory Standards, 1993. Screening tests regarding the inhibition zone were carried out by the well diffusion method.[46] The inoculum suspension was prepared from colonies grown overnight on an agar plate and inoculated into Mueller–Hinton broth (fungi using malt broth). A sterile swab was immersed in the suspension and used to inoculate Mueller-Hinton agar plates (fungi using malt agar plates). The compounds were dissolved in DMSO at concentration of 10 mg/mL. The inhibition zone was measured around each well after 24 h at 37°C. Controls using DMSO were adequately done. The antimicrobial screening was performed using Gentamycin (MIC 4 μg/mL) and Ketoconazole (MIC 100 μg/mL) as reference standard drugs for bacteria and fungi, respectively. The antimicrobial results are depicted in Table 2.

Conflict of Interest The author declares no conflict of interest.

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