PYRIDAZINE AND ITS RELATED COMPOUNDS, PART 39. SYNTHESIS AND ANTIMICROBIAL EVALUATION OF SOME THIENOPYRIDAZINE BASED HETERCYCLE

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The starting material, 5-amino-3,4-diphenylthieno[2,3-c]pyridazine-6-carbonitrile was subjected to subsequent reactions with aromatic and heteroaromatic aldehydes, benzylchloride, cyclohexanone, phenyl isothiocyanate, hydroxylamine hydrochloride and hydrazine hydrate to afford new polycyclic compounds. The new synthesized compounds were confirmed by their infrared, mass spectrum, ^{1}H-NMR, and elemental analyses, and further screened for antimicrobial activity.

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INTRODUCTION

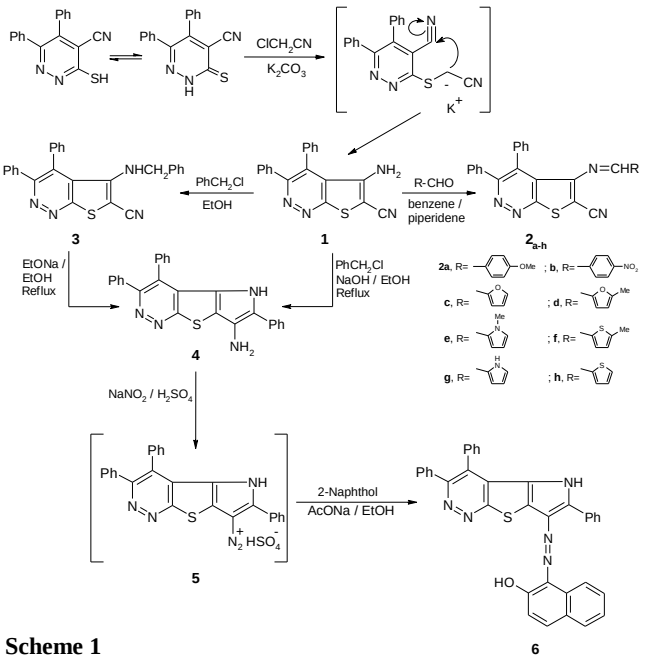
Pyridazine derivatives and heterocyclic-annulated pyridazines continue to attract interest due to a wide spectrum of biological and therapeutic effects. They are used as analgesic,[1] antibacterial,[2] anti-inflammatory,[3] antihypertensive,[4] antihistaminic,[5] anti-nociceptive agents,[6] as well as platelet aggregation inhibitors.[7] Thieno-pyridazines[8] have been reported to possess considerable antiasthmatic[9] and fibrinolytic activities.[10] Also, the enami-nonitrile serves as a good synthon for construction of a many condensed heterocyclic systems.[11] Based on these considerations, our interest was focused on synthesizing new heterocyclic compounds including thienopyridazine moiety with suitable substituents, these structures all on different derivatives related to compounds of biological and pharmacological interest to be obtained. The biological activity of some of the synthesized compounds has been screened.

RESULTS AND DISCUSSION

The starting material, 5-amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carbonitrile **1** was synthesized following the literature procedure by refluxing a solution of 4-cyano-5,6-diphenylpyridazine-3(2*H*)-thione with chloroacetonitrile in dry acetone in presence of potassium carbonate.[12,13]

The reaction of 5-amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carbonitrile **1** with an equimolecular quantity of aromatic and / or heteroaromatic aldehydes in benzene gave 5-arylidene- and 5-heteroarylideneaminothieno-pyridazine derivatives **2$_{a-h}$** (**Scheme 1**).

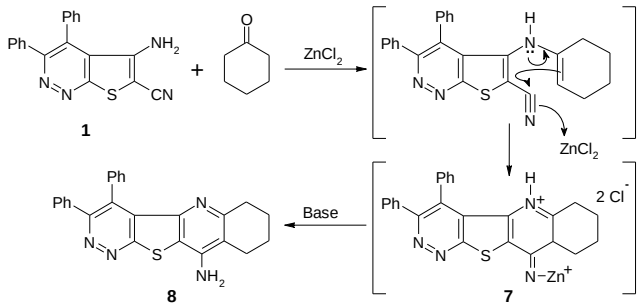
A mixture of compound **1** and benzyl chloride was heated in ethanol under reflux afforded 5-benzylamino derivative **3**, which undergoes cyclization upon heating under reflux in presence of sodium ethoxide in ethanol gave 7-amino-3,4,6-triphenyl-5H-pyrrolo[2`,3`:4,5]thieno-[2,3-*c*]pyridazine **4**. Alternatively, compound **4** was obtained directly upon refluxing a solution of compound **1** in ethanol with benzyl chloride in presence of sodium hydroxide. Reaction of compound **4** with nitrous acid followed the normal course of diazotization yielding clear diazonium salt solution **5**, which underwent coupling with 2-naphthol in ethanolic solution in presence of sodium acetate trihydrate as buffered solution to give the corresponding azo derivative **6** (**Scheme 1**).



Scheme 1

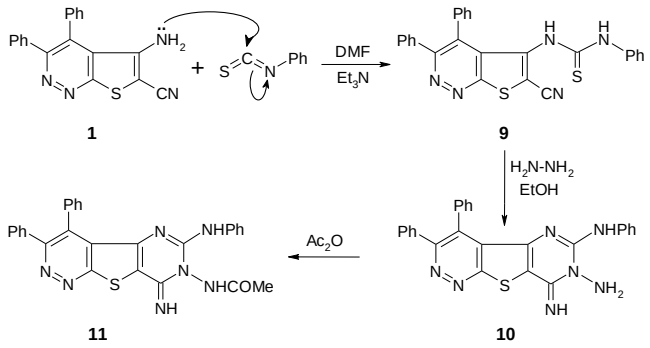
Treatment of compound **1** with cyclohexanone in presence of one molar equivalent of anhydrous zinc chloride led to the separation of 1:1 complex of the expected amino derivative with zinc chloride **7**. 10-Amino-3,4-diphenyl-

6,7,8,9-tetrahydropyridazino[4`,3`:4,5]thieno[3,2-*b*]quinoline **8** was liberated by treatment of the resulting complex with alkali and extracted with benzene[14,15] (**Scheme 2**).



Scheme 2

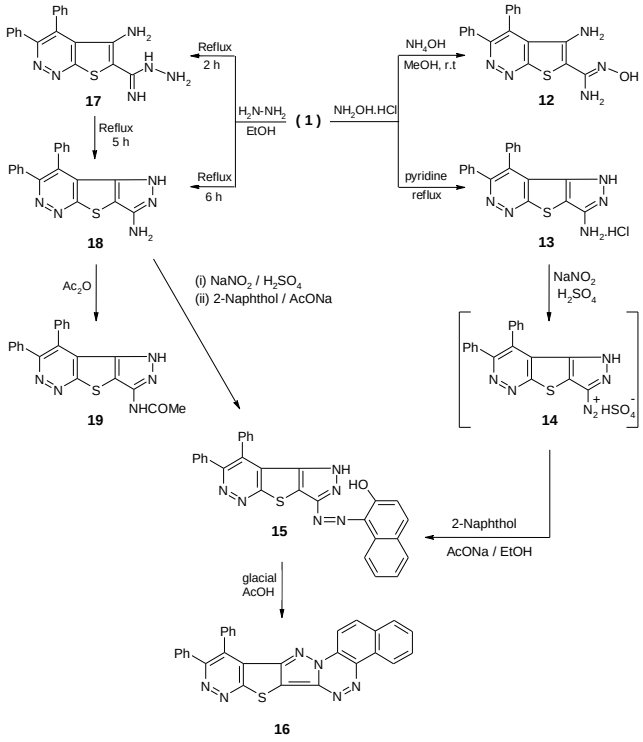
Treatment of compound **1** with phenyl isothiocyanate in dimethylformamide as solvent in the presence of triethylamine at room temperature for 18 h led to the corresponding N,N`-di-substituted thiourea **9**, which was transformed into 7-amino-6-anilino-8-imino-3,4-diphenylpyrimido[4`,5`:4,5]thieno[2,3-*c*]pyridazine **10** by the reaction with hydrazine hydrate (98 %) in boiling ethanol. Treatment of compound **10** with acetic anhydride yielded the 7-acetyl-amino derivative **11** (**Scheme 3**).



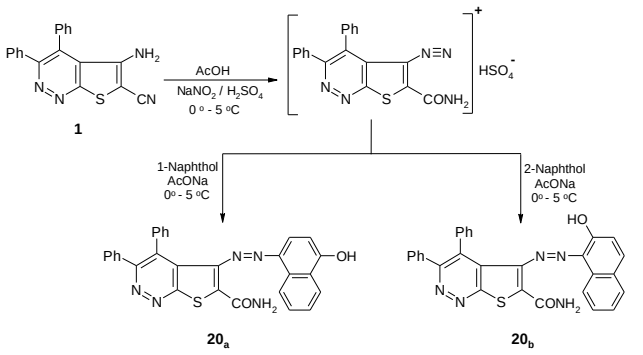
Scheme 3

The reaction of 5-amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carbonitrile **1** with hydroxylamine hydrochloride was carried out in methanol containing ammonium hydroxide at room temperature to give the corresponding 6-carboxamidoxime derivative **12**. Further attempted reaction in boiling pyridine for 5 h afforded 3-amino-7,8-diphenyl-1*H*-pyrazolo[3`,4`:4,5]thieno[2,3-*c*]-pyridazine hydrochloride **13**, *via* the initial formation of **12** followed by intermediate intramolecular nucleophilic attack of amino group at position 5 to the oxime nitrogen atom with elimination of one molecule of water. Diazotization reaction of compound **13** with nitrous acid followed the normal course yielding clear diazonium salt solution **14**, which underwent coupling with 2-naphthol in ethanol in presence of sodium acetate trihydrate as buffered solution to give the corresponding azo derivative **15**. The latter compound underwent readily cyclization upon heating in glacial acetic acid at reflux temperature to give **16**, which was formed by elimination of one molecule of water (**Scheme 4**).

Compound **1** was reacted with hydrazine hydrate (98 %) in refluxing ethanol for 2 h to give 5-amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carboximidohydrazide **17**, while reflux-ing for 6 h gave 3-amino-7,8-diphenyl-1H-pyrazolo[3`,4`:4,5]thieno[2,3-*c*]pyridazine **18**. Also, compound **18** was afforded by heating the imido derivative **17** in ethanol under reflux for 5 h. Additional evidences for the structure of pyrazolothienopyridazine derivative **18** was proved by its reaction with acetic anhydride to give the corresponding 3-acetylamino derivative **19**, and with $NaNO_2$ / H_2SO_4 to give the diazotized aminopyrazolothieno-pyridazine, which upon coupling with 2-naphthol in presence of sodium acetate trihydrate as buffered solution in ethanol (50 %) gives **15** (**Scheme 4**).



Scheme 4



Scheme 5

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Table 1. Antimicrobial activity of the synthesized compounds

Compd. No.	Zone of inhibition					
	Staphylococcus Aurous	***Bacillus subtitles***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Candida albicans***	***Aspergillums Niger***
2_a	++	++	++	-	++	+++
2_b	++	++	-	-	+++	++
2_c	-	-	++	-	+++	++++
2_d	+	++	++	-	++++	++
2_e	-	++	++	-	++++	++++
2_f	-	+	++	+	+++	+++
2_g	++	++	++	-	+++	-
2_h	+	++	++	-	+++	-
9	+	++	+	++	++	++++
12	++	+	+	+	+++	++
17	++	++	-	-	++	++
19	++	++	-	-	+	++
20_b	++	++	-	+	+++	-
Ciprofloxacin	++++	++++	++++	++++	-	-
Nystin	-	-	-	-	++++	++++

The concentration of the all synthesised compounds and the two references was 0.30 mg 0.10 mL^{-1} of dimethylformamide. Zone of inhibition: + = < 15 mm; ++ = 15-24 mm; +++ = 25-34 mm; ++++ = 35-44 mm; - = no inhibition.

Diazotization of the 5-amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carbonitrile **1** was carried out, followed by coupling with 1- and 2-naphthol to give the azo derivatives **20_a** and **20_b**, respectively (**Scheme 5**). The structure of the prepared compounds was confirmed on the basis of spectroscopic data (IR, mass, ^{1}H-NMR spectra) and elemental analysis as described in the experimental part.

Screening for antimicrobial activities

Applying the agar plate diffusion technique,[16] the newly synthesised compounds were screened in vitro for antimicrobial activity against Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), yeast (*Candida albicans*), and fungi (*Aspergillus niger*).

In this method,a standard 5 mm sterilised filter paper disk impregnated with the compound (0.3 mg / 0.1 ml of dimethylformamide) was placed on agar plate seeded with the tested organism. The plates were incubated for 24 h at 37 °C for bacteria and 28 °C for fungi. The inhibition zone of bacteria and fungi growth around the disk were determined. The screening results are given in (**Table I**).

The results indicated that seven synthesized compounds **2_a**, **2_b**, **2_g**, **12, 17, 19** and **20_b** showed moderate antimicrobial activity against the examined Gram positive bacteria *Staphylococcus aureus*. On the other hand, ten synthesized compounds **2_a**, **2_b**, **2_d**, **2_e**, **2_g**, **2_h**, **9**, **17**, **19** and **20_b** showed moderate antimicrobial activity against the examined Gram positive bacteria *Bacillus subtilis*. Only the six synthesised compounds **2_a**, **2_b**, **2_g**, **17**, **19** and **20_b** showed moderate antimicrobial activity against the both examined Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*.

On the other hand, seven compounds **2_a**, **2_c**, **2_d**, **2_e**, **2_f**, **2_g** and **2_h** and one compound **9** showed moderate antimicrobial activity against the examined Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, respectively.

In addition, two compounds **2_d** and **2_e** and three compounds **2_c**, **2_e** and **9** showed very high antifungal activity against the examined fungi *Candida albicans* (yeast) and *Aspergillus niger*, respectively. Only one compound **2_e** showed very high antifungal activity against the both examined fungi *Candida albicans* and *Aspergillus niger*. The results revealed also that seven synthesised compounds **2_b**, **2_c**, **2_f**, **2_g**, **2_h**, **12** and **20_b** and two synthesised compounds **2_a** and **2_f** showed high antifungal activity against the examined fungi *Candida albicans* and *Aspergillus niger*, respectively. Only one synthesised compound **2_f** showed high antifungal activity against the both examined fungi *Candida albicans* and *Aspergillus niger*.

In conclusion, results of antimicrobial activity revealed that the synthesised compounds showed moderate and / or very high antimicrobial activity against bacteria and fungi, respectively. It could be concluded from these results that the biologically active synthesised compounds are nearly as active as the standard antibacteria Ciprofloxacin against the both tested Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). On the other hand, the biologically active synthesised compounds are active as the standard Fungicid Nystin against the both tested fungi *Candida albicans* and *Aspergillus niger*.

EXPERIMENTAL

Melting points were determined in open glass capillaries and are uncorrected. Elemental analysis (CHN) were carried out using a Perkin-Elmer 240 C Microanalyzer the Microanalytical Laboratory-Cairo University.

The IR spectra of compounds were recorded on a Perkin-Elmer spectrophotometer model 1430 as potassium bromide pellets and frequencies are reported in cm^{-1}. The ^{1}H-NMR spectra were recorded on a Perkin-Elmer R12B spectrometer 200 MHz and chemical shifts δ are in ppm relative to internal TMS, and mass spectra were recorded on a mass spectrometer HP model MS 5988 El 70 ev. Reactions were routinely followed by thin layer chromatography (TLC) on silica gel; F_{254} aluminum sheets (Merck). The spots were detected by UV irradiation at 254–365 nm.

General procedure for the synthesis of 5-arylidene- and 5-heteroarylideneamino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carbonitriles, 2_{a-h}.

A mixture of compound **1** (0.5 g, 1.52 mmol) and the appropriate aromatic and heteroaromatic aldehydes (1.52 mmol) in benzene (10 mL) was heated under reflux for 3 h in presence of piperidine (0.5 mL). The solvent was then evaporated under reduced pressure, and the solid product was recrystallised from ethanol to give **2_{a-h}**.

5-[[(4-Methoxyphenyl)methylidene]amino]-3,4-diphenylthieno-[2,3-*c*]pyridazine-6-carbonitrile, 2_a.

Yield: (0.51 g, 75 %); m.p. 154°-156 °C; IR: 2246 (C≡N), 1678 (C=N), 2926, 1448 (–OCH_3); MS (*m/z* %): 446 (M^+, 2.72 %), 329 (M^+ - C_8H_8O, 100 %); Anal Calcd. For $C_{27}H_{18}N_4OS$: C, 72.62; H, 4.06; N, 12.55; Found: C, 72.30; H, 4.13; N, 12.27 %.

5-[[(4-Nitrophenyl)methylidene]amino]-3,4-diphenylthieno[2,3-*c*]-pyridazine-6- carbonitrile, 2_b.

Yield: (0.52 g, 74 %); m.p. 128° - 129 °C; IR: 2248 (C≡N), 1675 (C=N), 1516 (NO_2, asym.), 1341 (NO_2, sym.); MS (*m/z* %): 461 (M^+, 1.81 %), 329 (M^+ - $C_7H_5NO_2$, 100 %); Anal Calcd. For $C_{26}H_{15}N_5O_2S$: C, 67.66; H, 3.28; N, 15.18; Found: C, 67.96; H, 3.22; N, 15.42 %.

5-[[(Furan-2-yl)methylidene]amino]-3,4-diphenylthieno[2,3-*c*]-pyridazine-6-carbonitrile, 2_c.

Yield: (0.49 g, 79 %); m.p. 212° - 214 °C; IR: 2245 (C≡N), 1665 (C=N); MS (*m/z* %): 407 (M^+ +1, 6.89 %), 103 (M^+ - $C_{17}H_9N_3OS$, 100 %), 77 (64.59 %); Anal. Calcd. For $C_{24}H_{14}N_4OS$: C, 70.92; H, 3.47; N, 13.79; Found: C, 70.58; H, 3.55; N, 13.51 %.

5-[[(5-Methylfuran-2-yl)methylidene]amino]-3,4-diphenylthieno-[2,3-*c*]pyridazine-6-carbonitrile, 2_d.

Yield: (0.54 g, 84 %); m.p. 196° - 198 °C; IR: 2973 (–CH_3), 2245 (C≡N), 1666 (C=N); MS (*m/z* %): 420 (M^+, 0.64 %), 329 (M^+ - C_6H_6O, 100 %); Anal Calcd. For $C_{25}H_{16}N_4OS$: C, 71.41; H, 3.84; N, 13.33; Found: C, 71.76; H, 3.90; N, 13.07 %.

5-[[(1-Methyl-1*H*-pyrrol-2-yl)methylidene]amino]-3,4-diphenylthieno[2,3- *c*]-pyridazine-6-carbonitrile, 2_e.

Yield: (0.52 g, 82 %); m.p. 209° - 210 °C; IR: 2976 (–CH_3), 2241 (C≡N), 1665 (C=N); MS (*m/z* %): 419 (M^+, 0.23 %), 329 (M^+ - C_6H_7N, 100); Anal Calcd. For $C_{25}H_{17}N_5S$: C, 71.57; H, 4.09; N, 16.70; Found: C, 71.23; H, 4.18; N, 16.41 %.

5-[[(5-Methylthiophene-2-yl)methylidene]amino]-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carbonitrile, 2_f.

Yield: (0.53 g, 80 %); m.p. > 300 °C; IR: 2965 (–CH_3), 2246 (C≡N), 1664 (C=N); MS (*m/z* %): 436 (M^+, 0.13 %), 329 (M^+ - C_6H_6S, 100 %); Anal Calcd. For $C_{25}H_{16}N_4S_2$: C, 68.78; H, 3.70; N, 12.84; Found: C, 69.10; H, 3.62; N, 12.58 %.

3,4-Diphenyl-5-[[(1*H*-pyrrol-2-yl)methylidene]amino]thieno-[2,3-*c*]pyridazine-6-carbonitrile, 2_g.

Yield: (0.48 g, 78.5 %); m.p. 208° - 210 °C; IR: 3281 (–NH), 2243 (C≡N), 1664 (C=N); MS (*m/z* %): 405 (M^+, 4.80 %), 57 (M^+ - $C_{14}H_{10}N_2$, - $C_8H_5N_3$, 100 %); Anal Calcd. For $C_{24}H_{15}N_5S$: C, 71.09; H, 3.73; N, 17.27; Found: C, 70.76; H, 3.65; N, 17.51 %.

3,4-Diphenyl-5-[[(thiophene-2-yl)methylidene]amino]thieno-[2,3-*c*]pyridazine-6-carbonitrile, 2_h.

Yield: (0.49 g, 76 %); m.p. 140° - 142 °C; IR: 2247 (C≡N), 1673 (C=N); MS (*m/z* %): 422 (M^+, 0.44 %), 329 (M^+ - C_5H_4S, 100 %); Anal Calcd. For $C_{24}H_{14}N_4S_2$: C, 68.22; H, 3.34; N, 13.26; Found: C, 68.59; H, 3.29; N, 13.55 %.

5-Benzylamino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carbonitrile, 3.

A solution of equimolar mixture of compound **1** (0.5 g, 1.52 mmol) and benzyl-chloride (0.2 g, 1.52 mmol) in absolute ethanol (10 mL) was heated under reflux for 3 h. The solvent was evaporated, and the residue was treated with petroleum ether 40° - 60°C. The solid precipitate was filtered off, dried, and recrystallised from ethanol to give **3**. Yield: (0.44 g, 69.5 %); m.p. 192° - 194 °C; IR: 3284 (–NH), 2922 (–CH_2–), 2197 (C≡N); MS (*m/z* %): 419 (M^+ +1, 46.2 %), 149 (M^+ - C_6H_5, - $C_{14}H_{10}N$, 100 %); Anal Calcd. For $C_{26}H_{18}N_4S$: C, 74.62; H, 4.33; N, 13.39; Found: C, 74.95; H, 4.25; N, 13.13 %.

7-Amino-3,4,6-triphenyl-5*H*-pyrrolo[2`,3`:4,5]thieno[2,3-*c*]pyridazine 4.

Method A

To a solution of equimolar mixture of compound **1** (0.5 g, 1.52 mmol) and benzyl-chloride (0.2 g, 1.52 mmol) in absolute ethanol (10 mL), NaOH (0.5 g) was added.

The reaction mixture was heated under reflux for 6 h, cool to room temperature, poured into ice water, and neutralised with HCl. The precipitate was filtered off, washed with water, dried, and recrystallised from ethanol to give **4**. Yield: (0.29 g, 45.5 %); m.p. 155°-156 °C; IR: 3442, 3319, 3177 (–NH_2, –NH); MS (*m/z* %): 418 (M^+, 30.8 %), 149 (M^+ - C_6H_5, - $C_{14}H_{10}N$, 100 %); Anal Calcd. For $C_{26}H_{18}N_4S$: C, 74.62; H, 4.33; N, 13.39; Found: C, 74.99; H, 4.24; N, 13.11 %.

Method B

To a solution of sodium ethoxide [(0.1 g) of Na in (10 mL) absolute ethanol], compound **3** (0.5 g, 1.19 mmol) was added. The reaction mixture was refluxed for 5 h, cool to room temperature, poured into ice water, and neutralized with HCl. The precipitate was filtered off, washed with water, dried, and recrystallised from ethanol to give **4**. It was identical with the compound prepared by method A (m.p and mixed m.p). Yield: (0.34 g, 68 %).

7-[(2-Hydroxynaphthalen-1-yl)diazenyl]-3,4,6-triphenyl-5*H*-pyrrolo[2`,3`:4,5]thieno[2,3-*c*]pyridazine, 6.

Compound **4** (0.5 g, 1.19 mmol) was dissolved in concentrated H_2SO_4 (5 mL), cooled to 0° - 5°C in an ice bath, then a solution of $NaNO_2$ (0.16 g, 2.39 mmol) in H_2O (5 mL) was added with stirring, keeping the temperature at 0° - 5°C. The above mixture was added drop by drop to a solution of 2-naphthol (0.17 g, 1.19 mmol) and sodium acetate (1.0 g) in ethanol (50 %, 10 mL) with stirring for 1.5 h. The precipitate was filtered off, washed with water, dried, and recrystallised from ethanol to give **6**. Yield: (0.36 g, 52.7 %); m.p. 104° - 105°C; IR: broad band around 3198 (–OH, –NH), 1599 (N=N), 1242 (C–O, phenolic); MS (*m/z* %): 573 (M^+, 0.17 %), 63 (M^+ - $C_{32}H_{22}N_4OS$, 100 %); Anal Calcd. For $C_{36}H_{23}N_5OS$: C, 75.37; H, 4.04; N, 12.21; Found: C, 75.75; H, 4.12; N, 12.50 %.

10-Amino-3,4-diphenyl-6,7,8,9-tetrahydropyridazino[4`,3`:4,5]-thieno[3,2- *b*]quinoline, 8.

To a solution of compound **1** (0.5 g, 1.52 mmol) in cyclohexanone (10 mL), anhydrous zinc chloride (0.04 g, 1.52 mmol) was added, and the reaction mixture was heated under reflux for 30 minute. Evaporate the solvent under reduced pressure, and dissolve the residue in NaOH (10 mL, 40 %), then extract with benzene. The benzene layer was dried over anhydrous $MgSO_4$, and evaporated to give **8**. Yield: (0.35 g, 57 %); m.p. 189° - 190 °C; IR: 3500, 3358 (–NH_2), 2929 (CH, aliphatic), 1657 (C=N); MS (*m/z* %): 408 (M^+, 3.78 %), 77 (M^+ - $C_{19}H_{15}N_4S$, 100 %); Anal Calcd. For $C_{25}H_{20}N_4S$: C, 73.50; H, 4.94; N, 13.72; Found: C, 73.82; H, 4.85; N, 13.45 %.

***N*-(6-Cyano-3,4-diphenylthieno[2,3-*c*]pyridazin-5-yl)-*N*`-phenylthiourea, 9.**

Method A

To a solution of compound **1** (0.5 g, 1.52 mmol) in dimethylformamide (10 mL), phenyl isothiocyanate (0.21 g, 1.52 mmol) and triethylamine (0.5 mL) were added, the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was poured into water, then the solid product was filtered off, washed with water, dried, and recrystallised from ethanol to give **9**. Yield: (0.51 g, 73 %); m.p. 218° - 219 °C; IR: 3451, 3333 (two –NH), 2243 (C≡N), 1240 (C=S); MS (*m/z* %): 463 (M^+, 0.02 %), 329 (M^+ - C_7H_6NS, 100 %); ^{1}H-NMR (DMSO-d_6): 8.09 (s, 1H, NH), 7.90 (s, 1H,–NHPh), 7.17-7.39 (m, 15H, 3Ph); Anal Calcd. For $C_{26}H_{17}N_5S_2$: C, 67.36; H, 3.70; N, 15.11; Found: C, 67.01; H, 3.62; N, 15.40 %.

Method B

To a solution of compound **1** (0.5 g, 1.52 mmol) in pyridine (10 mL), phenyl isothio-cyanate (0.21 g, 1.52 mmol) was added. The reaction mixture was heated under reflux for 6 h. After cooling, the reaction mixture was poured into ice water, then the solid product was filtered off, dried, and recrystallised from ethanol to give **9**, which identical with that prepared by method A (m.p and mixed m.p). Yield: (0.5 g, 71 %).

7-Amino-6-anilino-8-imino-3,4-diphenylpyrimido[4`,5`:4,5]thieno[2,3-*c*]pyridazine, 10.

To a solution of compound **9** (0.5 g, 1.08 mmol) in ethanol (10 mL), hydrazine hydrate (98 %, 1.08 mmol) was added. The reaction mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure, and the residue was treated with water. The solid product was filtered off, dried, and recrystallised from ethanol to give **10**. Yield: (0.36 g, 72.5 %); m.p. 190° - 191 °C; IR: broad bands around 3318, 3185 (–NH_2, =NH, –NH), 1664 (C=N); MS (*m/z* %): 463 (M^+ +2, 0.27 %), 371 (M^+ - C_6H_6N, 100 %); Anal Calcd. For $C_{26}H_{19}N_7S$: C, 67.66; H, 4.15; N, 21.24; Found: C, 67.31; H, 4.22; N, 21.51 %.

7-Acetylamino-6-anilino-8-imino-3,4- diphenylpyrimido-[4`,5`:4,5]thieno[2,3-*c*]-pyridazine, 11.

A solution of compound **10** (0.5 g, 1.08 mmol) in acetic anhydride (10 mL) was refluxed for 5 h. The solvent was evaporated under reduced pressure, and the solid product was recrystallised from acetic acid to give **11**. Yield: (0.45 g, 83 %); m.p. 204° - 205 °C; IR : broad band around 3444 (=NH, –NH, –NH amide), 2919 (–CH_3), 1734 (C=O amide), 1668 (C=N); MS (*m/z* %): 503 (M^+, 0.29 %), 63 (M^+ - $C_{24}H_{20}N_6OS$, 100%); Anal Calcd. For $C_{28}H_{21}N_7OS$: C, 66.78; H, 4.20; N, 19.47; Found: C, 67.15; H, 4.11; N, 19.76 %.

5-Amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carboxamid-oxime, 12.

To a solution of compound **1** (0.5 g, 1.52 mmol) in methanol (10 mL), hydroxylamine hydrochloride (0.11 g, 1.52 mmol) and ammonium hydroxide (0.5 mL) were added. The reaction mixture was stirred at room temperature for 24 h. The solid product was filtered off, dried, and recrystallised from ethanol to give **12**. Yield: (0.29 g, 54 %); m.p. 212° - 213°C; IR: 3459 (–OH), 3380, 3292, 3229, 3158 (two –NH_2), 1661 (C=N); MS (*m/z* %): 362 (M^+ +1, 12.2 %), 51 (M^+ - $C_{15}H_{12}N_5OS$, 100 %); ^{1}H-NMR (DMSO-

d_6) : 9.27 (s, 1H, OH), 7.14-7.39 (m, 10H, 2Ph), 5.74 (s, 2H, 6-NH_2), 4.09 (s, 2H, 5-NH_2); Anal Calcd. For $C_{19}H_{15}N_5OS$: C, 63.14; H, 4.18; N, 19.38; Found: C, 63.52; H, 4.24; N, 19.09 %.

3-Amino-7,8-diphenyl-1*H*-pyrazolo[3`,4`:4,5]thieno[2,3-*c*]pyridazine hydrochloride, **13.**

To a solution of compound **1** (0.5 g, 1.52 mmol) in pyridine (10 mL), hydroxylamine hydrochloride (0.11 g, 1.52 mmol) was added. The reaction mixture was heated under reflux for 5 h. The solvent was evaporated under reduced pressure, and the residue was treated with water. The solid product was filtered off, dried, and recrystallised from ethanol to give **13**. Yield: (0.41 g, 71 %); m.p. > 300 °C; IR: 3296, 3155 (–NH_2, –NH), 1673 (C=N); MS (*m/z* %): 380 (M^+, 0.03 %), 382 (M^+ +2, 0.10 %), 304 (M^+ - CH_3ClN_2, 100 %); Anal Calcd. For $C_{19}H_{14}ClN_5S$: C, 60.07; H, 3.71; N, 18.44; Found: C, 60.40; H, 3.79; N, 18.19 %.

3-[(2-Hydroxynaphthalen-1-yl)diazenyl]-7,8-diphenyl-1*H*-pyrazolo[3`,4`:4,5]thieno-[2,3-*c*]pyridazine, **15.**

Method A

Compound **13** (0.5 g, 1.32 mmol) was dissolved in concentrated H_2SO_4 (5 mL), cooled to 0° - 5°C in an ice bath, then a solution of $NaNO_2$ (0.18 g, 2.63 mmol) in H_2O (5 mL) was added with stirring, keeping the temperature at 0° - 5°C. The above mixture was added drop by drop to a solution of 2-naphthol (0.19 g, 1.32 mmol) and sodium acetate (1.0 g) in ethanol (50 %, 10 mL) with stirring for 1.5 h. The precipitate was filtered off, washed with water, dried, and recrystallised from ethanol to give **15**. Yield: (0.49 g, 76 %); m.p. 201° - 202 °C; IR: broad band around 3181(–OH, –NH), 1670 (C=N), 1623 (N=N), 1243 (C–O, phenolic); MS (*m/z* %): 499 (M^+ +1, 1.03 %), 77 (M^+ - $C_{23}H_{13}N_6OS$, 100 %); Anal Calcd. For $C_{29}H_{18}N_6OS$: C, 69.86; H, 3.64; N, 16.86; Found: C, 69.55; H, 3.69; N, 16.58 %.

Method B

Compound **18** (0.5 g, 1.45 mmol) was dissolved in concentrated H_2SO_4 (5 mL), cooled to 0° - 5°C in an ice bath, then a solution of $NaNO_2$ (0.2 g, 2.91 mmol) in H_2O (5 mL) was added with stirring, keeping the temperature at 0° - 5°C. The above mixture was added drop by drop to a solution of 2-naphthol (0.21 g, 1.45 mmol) and sodium acetate (1.0 g) in ethanol (50 %, 10 mL) with stirring for 1.5 h. The precipitate was filtered off, washed with water, dried, and recrystallised from ethanol to give **15**, which identical to the compound prepared by method A (m.p and mixed m.p). Yield (0.51 g, 70.5 %).

3,4-Diphenylnaphtho[2,1- *e*]pyridazino[4``,3``:4`,5`]thieno[2`,3`:4,5]pyrazolo[3,2-*c*][1,2,4]triazine, 16.

A solution of compound **15** (0.5 g, 1.0 mmol) in glacial acetic acid (10 mL) was heated under reflux for 3 h. The solvent was then evaporated under reduced pressure, and the solid product was recrystallised from ethanol to give **16**. Yield: (0.2 g, 41.5 %); m.p. 211° - 212°C; IR: 1644 (C=N), 1558 (N=N), and there is no bands in the –OH and –NH regions; MS (*m/z* %): 480 (M^+, 0.35 %), 371 (M^+ - C_6H_5, - N_2, -2 H_2, 100 %); Anal Calcd. For $C_{29}H_{16}N_6S$: C, 72.48; H, 3.36; N, 17.49; Found: C, 72.80; H, 3.45; N, 17.76 %.

5-Amino-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carboximidohydrazide, 17.

To a solution of compound **1** (0.5 g, 1.52 mmol) in ethanol (10 mL), hydrazine hydrate (98 %, 1.52 mmol) was added, and the reaction mixture was heated under reflux for 2 h. The solvent was then evaporated under reduced pressure, and the residue was treated with water. The solid product was filtered off, dried, and recrystallised from ethanol to give **17**. Yield: (0.38 g, 69 %); m.p. 179° - 180 °C; IR: broad bands around 3350, 3158 (two –NH_2, =NH, –NH), 1670 (C=N); MS (*m/z* %): 360 (M^+, 10.36 %), 287 (M^+ - NH_2, - CH_4N_3, 100 %); ^{1}H-NMR (DMSO-*d_6*): 8.16 (s, 1H, –NH), 8.05 (s, 2H, –NH_2), 7.05-7.83 (m, 10H, 2Ph), 5.38 (s, 1H, =NH), 4.16 (s, 2H, 5-NH_2); Anal Calcd. For $C_{19}H_{16}N_6S$: C, 63.31; H, 4.48; N, 23.32; Found: C, 63.01; H, 4.55; N, 23.05 %.

3-Amino-7,8-diphenyl-1*H*-pyrazolo[3`,4`:4,5]thieno[2,3-*c*]pyridazine, **18.**

Method A

To a solution of compound **1** (0.5 g, 1.52 mmol) in absolute ethanol (10 mL), hydrazine hydrate (98 %, 1.52 mmol) was added, and the reaction mixture was heated under reflux for 6 h. The solvent was then evaporated under reduced pressure, and the residue was treated with water. The solid product was filtered off, dried, and recrystallised from ethanol to give **18**. Yield: (0.37 g, 70 %); m.p. 250° - 251°C; IR: 3356, 3295, 3206 (–NH_2, –NH), 1658 (C=N); MS (*m/z* %): 343 (M^+, 22.6 %), 51 (M^+ - $C_{15}H_{10}N_5S$, 100 %); Anal Calcd. For $C_{19}H_{13}N_5S$: C, 66.45; H, 3.81; N, 20.40; Found: C, 66.80; H, 3.75; N, 20.65 %.

Method B

A solution of compound **17** (0.5 g, 1.39 mmol) in absolute ethanol (10 mL) was heated under reflux for 5 h. The solvent was concentrated, and the solid product was filtered off, and dried to give **18**. It was identical with that prepared by method A (m.p and mixed m.p). Yield: (0.36 g, 75.2 %).

3-Acetylamino-7,8-diphenyl-1*H*-pyrazolo[3`,4`:4,5]thieno-[2,3-*c*]pyridazine, 19.

A solution of compound **18** (0.5 g, 1.45 mmol) in acetic anhydride (10 mL) was refluxed for 5 h. The solvent was then evaporated under reduced pressure, and the solid product was recrystallised from acetic acid to give **19**. Yield: (0.46 g, 82 %); m.p. 170° - 171 °C; IR: 3413, 3168 (–NH pyrazolo, –NH amide), 2923 (–CH_3), 1749 (C=O amide), 1675 (C=N); MS (*m/z* %): 385 (M^+, 100 %); ^{1}H-NMR (DMSO-*d_6*): 11.94 (s, 1H, NH-pyrazolo), 9.71 (s, 1H, –NHCO), 7.24-7.47 (m, 10H, 2Ph), 2.02 (s, 3H, CH_3); Anal Calcd. For $C_{21}H_{15}N_5OS$: C, 65.44; H, 3.92; N, 18.17; Found: C, 65.10; H, 3.85; N, 17.90 %.

General Procedure for the synthesis of 5-[(hydroxynaphthalen-1-yl)diazenyl]-3,4-diphenylthieno[2,3-*c*]pyridazine-6-carboxamide, $20_{a,b}$.

Compound **1** (0.5 g, 1.52 mmol) was dissolved in acetic acid (90 %, 5 mL), cooled to 0° - 5°C in an ice bath, then a solution of $NaNO_2$ (0.21 g, 3.04 mmol) in concentrated sulphuric acid (5 mL) was added with stirring, keeping the temperature at 0° - 5°C. The above mixture was added drop by drop to a solution of 1- and/or 2-naphthol (0.22 g, 1.52 mmol) and sodium acetate (1.0 g) in ethanol (50 %, 20 mL) with stirring for 1.5 h. The precipitate was filtered off, washed with water, dried, and recrystallised from ethanol to give $\mathbf{20_{a,b}}$.

5-[(4-Hydroxynaphthalen-1-yl)diazenyl]-3,4-diphenylthieno-[2,3-*c*]pyridazine-6-carboxamide, 20_a.

Yield: (0.46 g, 61 %); m.p. 249°- 250 °C; IR: 3390 (–OH), 3300, 3168 (–NH_2, amide), 1674 (C=O, amide), 1594 (N=N), 1201 (C–O, phenolic), there is no absorption bands referred to C≡N group; MS (*m/z* %): 504 (M^+ +3, 0.09 %), 78 (M^+ - $C_{23}H_{13}N_5O_2S$, 100 %); Anal Calcd. For $C_{29}H_{19}N_5O_2S$: C, 69.44; H, 3.82; N, 13.96; Found: C, 69.75; H, 3.89; N, 13.68 %.

5-[(2-Hydroxynaphthalen-1-yl)diazenyl]-3,4-diphenylthieno-[2,3-*c*]pyridazine-6-carboxamide, 20_b.

Yield: (0.49 g, 64 %); m.p. > 300 °C; IR: 3381 (–OH), 3307, 3168 (–NH_2, amide), 1682 (C=O, amide), 1604 (N=N), 1245 (C–O, phenolic), there is no absorption bands referred to C≡N group; MS (*m/z* %): 499 (M^+ -2, 0.26 %), 394 (M^+ - C_7H_5N, - H_2, 100 %); Anal Calcd. For $C_{29}H_{19}N_5O_2S$: C, 69.44; H, 3.82; N, 13.96; Found: C, 69.78; H, 3.91; N, 14.22 %.

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