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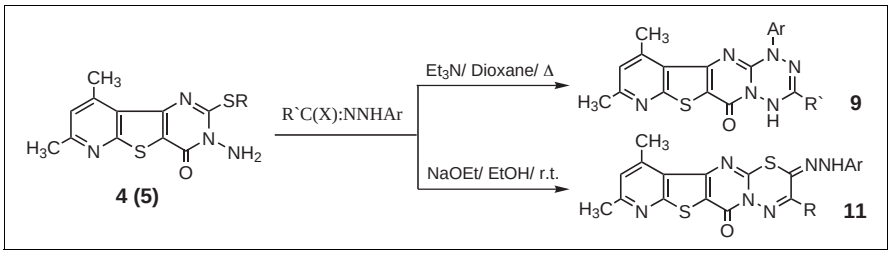

The reaction between 3-amino-2,3-dihydro-7,9-dimethyl-2-thioxo-pyrido[3',2':4,5]thieno[3,2-*d*]-pyrimidin-4(1*H*)-one **4** or its 2-methylthio derivative **5** with hydrazonoyl halides **6** in dioxane in the presence of triethylamine under reflux has followed heterocyclization reaction to yield pyrido[3",2":4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-ones **9**. On the other hand, reaction of compound **4** with hydrazonoyl halides **6** in sodium ethoxide at room temperature led to formation of hydrazonothioate compounds **10**. The latter on treatment with glacial acetic acid produced tetracyclic compounds, namely 2-arylhydrazonopyrido[3",2":4',5']thieno [3',2':4,5]pyrimido[2,1-*b*][1,3,4]thiadiazinones **11**. An alternative method was carried out to prove the structure of product **11**. The mechanism of the reaction under study was proposed and the products were screened for their biological activity.

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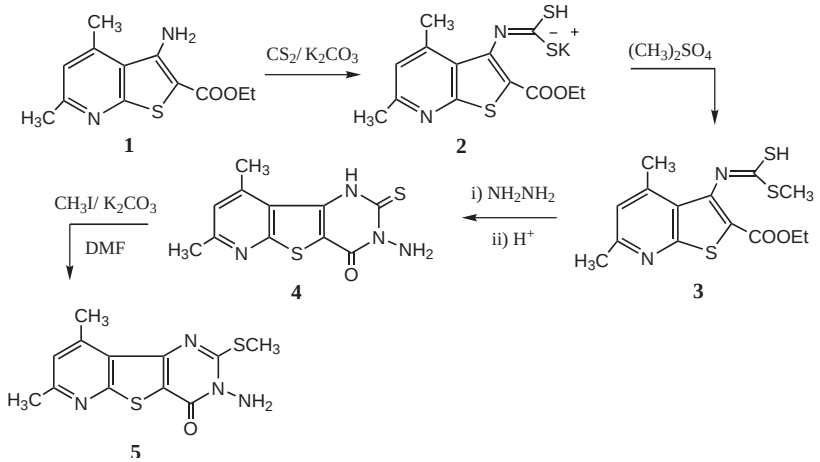
Introduction.

Fused pyrimidines have interesting biological activities such as inhibitors of phosphodiesterase7 (PDE7) [1] and potent selective adenosine receptor antagonists (A_{2B}) [2]. In particular, pyridothienopyrimidines an example of triheterocyclic system have attracted much attention because of interesting biological activities [3-7]. Moreover, annelated [1,2,4,5]tetrazines were identified to be one of the most potent inhibitors of the human Cytomegalovirus protease [8]. On the other hand, thiadiazine nucleus has been reported for different biological activities [9] including antituberculostatic, antiviral and anthelmenthic. Encouraged by the above and in continuation to our work on the synthesis of bridgehead nitrogen heterocycles [10-13] we report herein direct, efficient and regioselective route for the synthesis of the new tetraheterocyclic systems namely, pyrido[3",2":4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-ones (**9**) and pyrido[3",2":4',5']thieno[3',2':4,5]pyrimido-[2,1-*b*][1,3,4]thiadiazinones (**11**). The biological activity of compounds **9** was also evaluated.

Results and Discussion.

The required starting materials namely 3-amino-2,3-dihydro-7,9-dimethyl-2-thioxo-pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(1*H*)-one **4** and its 2-methylthio derivative **5** have not been reported hitherto. They were prepared in good overall yields according to the reaction sequence depicted in Scheme 1.

Scheme 1



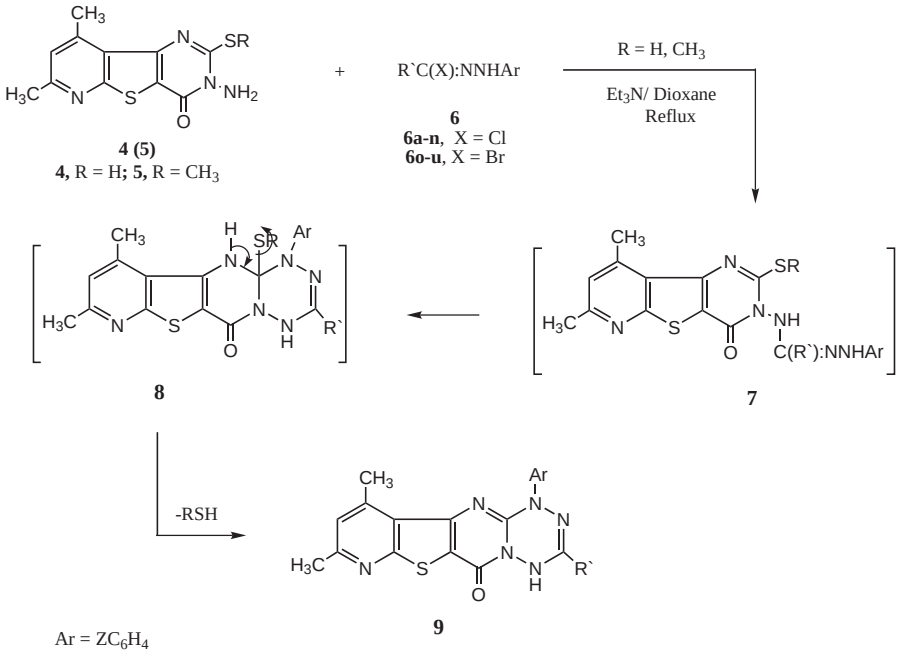
Treatment of ethyl 3-amino-4,6-dimethyl-thieno[2,3-*b*]-pyridine-2-carboxylate **1** [14] with carbon disulfide in the presence of anhydrous potassium carbonate, followed by methylation of the product **2** with dimethyl sulfate gave the corresponding methyl dithiocarbonate **3**. The latter reacts with hydrazine hydrate at room temperature followed by acidification furnished the starting material **4**. Methylation of the latter with methyl iodide in dimethyl-formamide in the presence of anhydrous potassium carbonate afforded the corresponding 2-methylthio derivative **5**. The structures of both **4** and **5** were evidenced by their spectral data (mass, IR, ^{1}H NMR) and microanalysis (see Experimental).

Reaction of 3-amino-2,3-dihydro-7,9-dimethyl-2-thioxo-pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(1*H*)-one **4** with hydrazonoyl halides **6** in dioxane in the presence of triethylamine at reflux till hydrogen sulfide gas ceased to evolve afforded the corresponding tetracyclic compounds **9**. The isolated products were assigned the structure of pyrido[3",2":4',5']thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5] tetrazin-6(4*H*)-ones **9**. The structures of the compounds **9** were established on the basis of spectral and microanalyses data (see Experimental). A plausible mechanism to account for the formation of **9** is shown in Scheme 2.

It is suggested that, reactions of **4** with hydrazonoyl halides **6** presumably proceed through initial nucleophilic attack by the amino group of pyrimidine nucleus to give the transient hydrazine derivatives **7** which subsequently undergo *in situ* cyclization with concurrent elimination of hydrogen sulfide to give the respective annelated tetrazines **9** as the end products. The formation of **7** is analogous to the reactions of hydrazonoyl halides with hydrazines, which were reported to give the corresponding hydrazidines [15]. The assignment of the proposed mechanism and the formation of **9** were further manifested by alternate synthesis. Thus, treatment of 2-methylthio derivative **5** with each of hydrazonoyl halides **6a,b,g** in dioxane in the presence of triethylamine at reflux led to evolution of methanethiol and the formation of products that proved to be identical in all respects (mp, mixed mp and IR) with compounds **9a,b,g** (Scheme 2).

Our interest was extended to study the effect of the basicity of the medium on the reaction of compound **4** with hydrazonoyl halides **6**. Thus, treatment of 3-amino-2,3-dihydro-7,9-dimethyl-2-thioxo-pyrido[3',2':4,5]thieno-

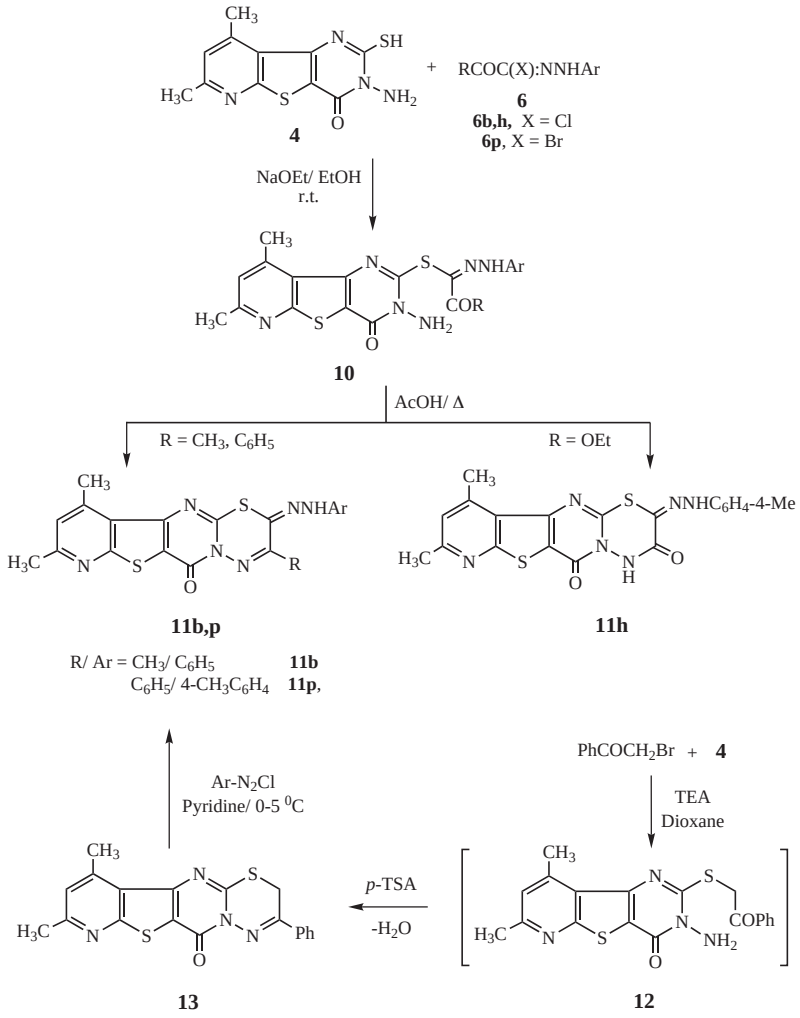
Scheme 2



R`/ Z	Compd. No.	R`/ Z	Compd. No.	R`/ Z	Compd. No.
Ph/ H	**6a, 9a**	COOEt/ 4-CH_3	**6h, 9h**	COPh/ H	**6o, 9o**
$COCH_3$/ H	**6b, 9b**	COOEt/ 4-Cl	**6i, 9i**	COPh/ 4-CH_3	**6p, 9p**
$COCH_3$/ 4-CH_3	**6c, 9c**	COOEt/ 4-NO_2	**6j, 9j**	COPh/ 4-NO_2	**6q, 9q**
$COCH_3$/ 4-Cl	**6d, 9d**	CONHPh/ H	**6k, 9k**	COPh/ 3-Cl	**6r, 9r**
$COCH_3$/ 4-OCH_3	**6e, 9e**	CONHPh/ 4-CH_3	**6l, 9l**	COPh/ 4-Cl	**6s, 9s**
$COCH_3$/ 4-NO_2	**6f, 9f**	CONHPh/ 4-Cl	**6m, 9m**	2-Thienoyl/ 4-Cl	**6t, 9t**
COOEt/ H	**6g, 9g**	CONHPh/ 4-NO_2	**6n, 9n**	2-Furoyl/ 4-Cl	**6u, 9u**

[3,2-*d*]pyrimidin-4(1*H*)-one **4** with hydrazonoyl halides **6** in ethanol in the presence of sodium ethoxide at room temperature was found to give products which are completely different from compounds **9**. On the basis of spectral (IR, ^{1}H NMR, Mass) and elemental analyses data (see Experimental), the compounds isolated were assigned structure **10** (Scheme 3).

Scheme 3



To account for the formation of products **10** isolated from the studied reaction, it is assumed that the thiol tautomer of compound **4** undergoes 1,3-addition to the nitrilimine (generated *in situ* by elimination of HX from the corresponding hydrazonoyl halides **6** in the presence of a base) to give directly the hydrazonothioate esters **10** (Scheme 3). Next, we directed our interest to investigate cyclization of hydrazonothioate esters **10** isolated from the foregoing reactions. Thus, refluxing the latter in glacial acetic acid yielded the respective cyclized products namely, 2-arylhydrazonopyrido[3",2":4',5']thieno[3',2': 4,5]-pyrimido[2,1-*b*][1,3,4]thiadiazin-6-ones **11** (Scheme 3). The structure of the latter products was elucidated on the basis of their spectral and elemental analyses data. For example, the IR spectra of **11** revealed the absence of one of the carbonyl and NH_2 absorption bands that were present in the spectra of their precursors **10**. Also, the ^{1}H NMR spectra of **11** revealed no signal assignable to the NH_2 protons. However, they revealed the presence of signal assigned to the hydrazono NH proton signal at δ 11.2 ppm (see Experimental). The structure of **11** was further proved *via* an alternative method (Scheme 3). Thus, reaction of compound **4** with phenacyl bromide in dioxane in the presence of triethyl amine at reflux led to formation of compound **12**. The latter undergoes dehydrative cyclization on treatment with *p*-toluene-sulphonic acid to give compound **13**. The structure of

compound **13** was substantiated by spectral (Mass, IR, and ^{1}H NMR) and elemental analyses data (See Experimental). Coupling of compound **13** with *p*-toluenediazonium chloride at 0-5 °C in pyridine led to formation of product which is identical in all respects (mp, mixed mp and IR) with compound **11p**.

Antimicrobial Activity.

The compounds **9b-d, 9g, 9i-l** and **9o-p** were tested for their antimicrobial activities using four fungi species namely *Aspergillus fumigatus* **AF**, *Penicillium italicum* **PI**, *Syncephalastrum racemosum* **SR** and *Candida albicans* **CA**. Also, four bacteria species namely, *Staphylococcus aureus* **SA**, *Pesudomonas aeruginosa* **PA**, *Bacillus subtilis* **BS** and *Escherichia coli* **EC** were tested. The organisms were tested against the activity of solutions of concentration of 5 mg/ml of each compound and using inhibition zone diameter (IZD) in cm as criterion for the antimicrobial activity. The fungicide Terbinafin and the bactericide Chloramphenicol were used as references to evaluate the potency of the tested compounds under the same conditions. The results are depicted in Table 1.

Table 1

Antimicrobial Activity of the Products[a] **9b-d, 9g, 9i-l** and **9o-p**

Micro-organism/ IZD (cm)*

Compound No.	AF	PI	SR	CA	SA	PA	BS	EC
9b	+	0	+	0	0	+	0	0
9c	0	0	+	+	0	+	0	0
9d	0	0	+	0	0	0	+	0
9g	0	+	0	+	0	+	0	0
9i	0	0	+	+	0	0	+	0
9j	+	0	+	+	0	0	+	+
9k	+	+	+	+	0	0	0	0
9l	+	+	+	0	0	+	+	0
9o	+	+	+	0	0	0	0	0
9p	+	+	+	0	0	+	+	0
CA[b]					1.0	2.8	2.6	1.0
TE[c]	3.0	3.6	3.6	3.0				

[a]; The concentration of the solution 5.0 mg/ml was tested. [b] Chloramphenicol; [c], Terbinafin. *IZD beyond control/ (sign): 1.1-1.5 cm/ (+++); 0.6-1.0 cm/ (++); 0.1-0.5 cm/ (+); 0 cm/ (-).

The results revealed that compound **9k-l** and **9o-p** exhibited a degree of inhibition against **AF**, **PI** and **SR** while compounds **9c, 9i** and **9k** have inhibition effect against **SR** and **CA**. The biological activities of the other compounds against the tested organisms are weak, however, the activities of the tested compounds are much less than that of standard antifungal and antibacterial agents used.

Conclusion.

We have encountered a novel series of pyrido-[3",2":4',5']thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-ones and 2-arylhydrazonopyrido[3",2":4',5']-thieno[3',2':4,5]pyrimido[2,1-*b*][1,3,4]thiadiazinones that preserve a degree of functionality and potential interest of biological evaluation.

EXPERIMENTAL

Melting points were recorded on Gallenkamp apparatus and are uncorrected. Infrared spectra (KBr) were determined on a Pye Unicam SP-3000 infrared spectrophotometer. ^{1}H NMR was determined on a Varian Gemini 300 spectrometer (300 MHz) in DMSO-d_6 with TMS as internal standard. Mass spectra were recorded on a GCMS-QP 1000 EX Schimadzu spectrometer. Elemental analyses were carried out at the Microanalytical center, University of Cairo, Giza, Egypt. Hydrazonoyl halides **6** [16-24] were prepared by literature methods. They are toxic and cause skin irritation, so proper safety precautions should be taken and it is recommended to protect hands with rubber gloves. The biological evaluation of the products was carried out at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

Synthesis of 3-Amino-2,3-dihydro-7,9-dimethyl-2-thioxo-pyrido-[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(1*H*)-one (**4**).

A mixture of ethyl 3-amino-4,6-dimethyl-thieno[2,3-*b*]-pyridine-2-carboxylate (**1**) [14] (50 g, 20 mmol), carbon disulfide (20 ml) and anhydrous potassium carbonate (60 g) was dissolved in 150 ml DMF and stirred for 12 h at room temperature. The reaction mixture was heated further on a water bath for 12 h, cooled and poured into ice-water. The reaction mixture was treated dropwise with dimethyl sulfate (30 ml) under stirring then allowed to stand at room temperature for 12 h and extracted with chloroform. The solvent was evaporated under reduced pressure and the crude product (**3**) was obtained. Compound (**3**) was treated with hydrazine hydrate (80%, 20 ml), and the reaction mixture was stirred for 3 h at room temperature then poured into ice-water. The reaction mixture was acidified with ice-cold hydrochloric acid. The solid formed was collected by filtration, washed with water, dried and recrystallized from dioxane to give pure (**4**); 3.9 g (70%), mp 306 °C; ir: 3359, 3218 (NH_2), 3316 (NH), 1652 (CO) cm^{-1} ; ^{1}H nmr δ (DMSO-d_6) 2.39 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 5.7 (s, 2H, NH_2) 7.2 (s, 1H, pyr-H), 11.2 (s, 1H, NH); ms, *m/z* (%) 279 (M^++1, 16), 278 (M^+, 100), 247 (90), 175 (21), 131 (14), 77 (10).

Anal. Calcd. For $C_{11}H_{10}N_4OS_2$: C, 47.46; H, 3.62; N, 20.13; S, 23.04. Found: C, 47.21; H, 3.51; N, 19.97; S, 22.87.

Synthesis of 3-Amino-7,9-dimethyl-2-methylthio-pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin -4(3*H*)-one (**5**).

To a stirred solution of 3-amino-2,3-dihydro-7,9-dimethyl-2-thioxo-pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(1*H*)-one (**4**) (2.78 g, 10 mmol) in 50 ml DMF in the presence of anhydrous K_2CO_3 (2.07 g, 15 mmol), was added methyl iodide (1.42 g, 10 mmol). The reaction mixture was stirred overnight at room temperature then poured into ice-water. The solid formed was filtered, washed with water, dried and recrystallized from

dioxane to give pure (**5**); 2.1 g (72%), mp 234 °C; ir: 3351, 3211 (NH_2), 1642 (CO) cm^{-1}; ^1H nmr δ (DMSO-d_6) 2.39 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.87 (s, 3H, SCH_3), 5.71 (s, 2H, NH_2) 7.2 (s, 1H, pyr-H); ms, *m/z* (%) 292 (M^+, 7), 263 (100), 247 (70), 205 (59), 175 (27), 131 (27), 64 (25).

Anal. Calcd. for $C_{12}H_{12}N_4OS_2$: C, 49.29; H, 4.14; N, 19.16; S, 21.93. Found: C, 49.20; H, 4.15; N, 18.98; S, 21.73.

Synthesis of 9,11-Dimethyl-1,3-disubstituted-pyrido[3'',2'':4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-ones (**9a-u**).

Method A: To a mixture of equimolar amounts of **4** and the appropriate hydrazonoyl halides **6** (10 mmol) in 50 ml dioxane were added triethylamine (1.4 mL, 10 mmol). The reaction mixture was refluxed till all of the starting materials have disappeared and hydrogen sulfide gas ceased to evolve (6-10 h, monitored by TLC). The solvent was evaporated and the residue was treated with methanol. The solid that formed was filtered and recrystallized from dioxane to give compounds **9a-u**.

Method B: Treatment of **5** with hydrazonoyl halides **6a,b,g** following the same procedure described in method A, led to formation of products which were found to be identical in all respects (mp mixed mp and IR) with **9a,b,g**.

9,11-Dimethyl-1,3-diphenyl-pyrido[3'',2'':4',5']thieno[3',2':4,5]-pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9a**).

This compound was obtained in 3.37 g (77%), mp 256 °C; ir: 3351 (NH), 1670 (CO) cm^{-1}; ^1H nmr (DMSO-d_6) δ 2.59 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 7.2-7.8 (m, 11H, Ar-H), 9.21 (s, 1H, NH); ms, *m/z* (%) 439 (M^++1, 12), 438 (M^+, 100), 230 (14), 77 (65).

Anal. Calcd. for $C_{24}H_{18}N_6OS$: C, 65.74; H, 4.14; N, 19.17; S, 7.31. Found: C, 65.72; H, 4.17; N, 19.05; S, 7.29.

3-Acetyl-9,11-dimethyl-1-phenyl-pyrido[3'',2'':4',5']thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9b**).

This compound was obtained in 2.9 g (72%), mp 284 °C; ir 3274 (NH), 1697, 1644 (2CO) cm^{-1}; ^1H nmr (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.56 (s, 3H, $COCH_3$), 7.11 (s, 1H, Pyr-H), 7.3-7.8 (m, 5H, Ar-H), 9.00 (s, 1H, NH); ms, *m/z* (%) 405 (M^++1, 21), 404 (M^+, 79) 230 (26), 97 (48), 55 (100).

Anal. Calcd for $C_{20}H_{16}N_6O_2S$: C, 59.39; H, 3.99; N, 20.78; S, 7.93. Found: C, 59.38; H, 4.05; N, 20.58; S, 7.91.

3-Acetyl-9,11-dimethyl-1-(4-methylphenyl)pyrido[3'',2'':4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9c**).

This compound was obtained in 3.13 g (75%), mp 282 °C; ir: 3278 (NH), 1705, 1653 (2CO) cm^{-1}; ^1H nmr (DMSO-d_6) δ 2.37 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.54 (s, 3H, Ar-CH_3), 3.56 (s, 3H, $COCH_3$), 7.16 (s, 1H, Pyr-H), 7.3-7.56 (m, 4H, Ar-H), 9.00 (s, 1H, NH); ms, *m/z* (%) 419 (M^++1, 29), 418 (M^+, 100), 376 (76), 230 (18), 91 (27).

Anal. Calcd. for $C_{21}H_{18}N_6O_2S$: C, 60.27; H, 4.34; N, 20.08; S, 7.66. Found: C, 60.21; H, 4.35; N, 20.01; S, 7.63.

3-Acetyl-1-(4-chlorophenyl)-9,11-dimethyl-pyrido[3'',2'':4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9d**).

This compound was obtained in 3.2 g (73%), mp 270 °C; ir: 3255 (NH), 1697, 1655 (2CO) cm^{-1}; ^1H nmr (DMSO-d_6) δ 2.43 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 3.56 (s, 3H, $COCH_3$), 7.17 (s, 1H, Pyr-H), 7.55-7.73 (m, 4H, Ar-H), 9.01 (s, 1H, NH); ms, *m/z* (%) 440 (M^++2, 42), 439 (M^++1, 23), 438 (M^+, 100), 396 (96), 230 (20), 111 (34).

Anal. Calcd. for $C_{20}H_{15}ClN_6O_2S$: C, 54.73; H, 3.44; N, 19.15; S, 7.31. Found: C, 54.70; H, 3.45; N, 18.96; S, 7.29.

3-Acetyl-9,11-dimethyl-1-(4-methoxyphenyl)pyrido[3'',2'':4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9e**).

This compound was obtained in 3.25 g (75%), mp 246 °C; ir: 3278 (NH), 1693, 1647 (2CO) cm^{-1}; ms, *m/z* (%) 434 (M^+, 2), 230 (12), 101 (23), 86 (100).

Anal. Calcd for $C_{21}H_{18}N_6O_3S$: C, 58.05; H, 4.18; N, 19.34; S, 7.38. Found: C, 58.11; H, 4.16; N, 19.08; S, 7.37.

3-Acetyl-9,11-dimethyl-1-(4-nitrophenyl)pyrido[3'',2'':4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9f**).

This compound was obtained in 3.24 g (72%), mp 238 °C; ir: 3452 (NH), 1683, 1647 (2CO) cm^{-1}; ms, *m/z* (%) 450 (M^+, 50), 369 (40), 230 (39), 115 (56), 58 (100).

Anal. Calcd. for $C_{20}H_{15}N_7O_4S$: C, 53.45; H, 3.36; N, 21.82; S, 7.13. Found: C, 53.44; H, 3.34; N, 21.73; S, 7.11.

Ethyl 9,11-dimethyl-6-oxo-1-phenyl-pyrido[3'',2'':4',5']thieno-[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazine-3-carboxylate (**9g**).

This compound was obtained in 3.39 g (78%), mp 272 °C; ir: 3278 (NH), 1720, 1697 (2CO) cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.21 (t, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 4.36 (q, 2H, CH_2), 7.12 (s, 1H, Pyr-H), 7.15-7.68 (m, 5H, Ar-H), 9.21 (s, 1H, NH); ms, *m/z* (%) 435 (M^++1, 27), 434 (M^+, 100), 306 (28), 230 (24), 105 (32), 77 (79).

Anal. Calcd. for $C_{21}H_{18}N_6O_3S$: C, 58.05; H, 4.18; N, 19.34; S, 7.38. Found: C, 58.01; H, 4.17; N, 19.20; S, 7.23.

Ethyl 9,11-dimethyl-1-(4-methylphenyl)-6-oxo-pyrido[3'',2'':4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazine-3-carboxylate (**9h**).

This compound was obtained in 3.6 g (80%), mp 268 °C; ir: 3271 (NH), 1722, 1687 (2CO) cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.23 (t, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 2.55 (s, 3H, Ar-CH_3), 4.38 (q, 2H, CH_2), 7.12 (s, 1H, Pyr-H), 7.15-7.68 (m, 4H, Ar-H), 9.22 (s, 1H, NH); ms, *m/z* (%) 449 (M^++1, 40), 448 (M^+, 97), 320 (29), 230 (28), 131 (12), 91 (100).

Anal. Calcd. for $C_{22}H_{20}N_6O_3S$: C, 58.92; H, 4.49; N, 18.74; S, 7.15. Found: C, 58.91; H, 4.50; N, 18.43; S, 7.11.

Ethyl 1-(4-chlorophenyl)-9,11-dimethyl-6-oxo-pyrido[3'',2'':4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazine-3-carboxylate (**9i**).

This compound was obtained in 3.6 g (77%), mp 264 °C; ir: 3290 (NH), 1728, 1674 (2CO) cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.32 (t, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 4.39 (q, 2H, CH_2), 7.18 (s, 1H, Pyr-H), 7.55-7.73 (m, 4H, Ar-H), 9.42 (s, 1H, NH); ms, *m/z* (%) 470 (M^++2, 42), 469 (M^++1, 33), 468 (M^+, 85), 340 (44), 230 (43), 111 (100), 77 (22).

Anal. Calcd. for $C_{21}H_{17}ClN_6O_3S$: C, 53.79; H, 3.65; N, 17.92; S, 6.84. Found: C, 53.67; H, 3.54; N, 17.71; S, 6.81.

Ethyl 9,11-dimethyl-1-(4-nitrophenyl)-6-oxo-pyrido[3'',2'':4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazine-3-carboxylate (**9j**).

This compound was obtained in 3.35 g (70%), mp 255 °C; ir: 3247 (NH), 1701, 1654 (2CO) cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.35

(t, 3H, CH_3), 2.53 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 4.42 (q, 2H, CH_2), 7.24 (s, 1H, Pyr-H), 7.93 (d, 2H, Ar-H), 8.38 (d, 2H, Ar-H), 9.58 (s, 1H, NH); ms, *m/z* (%) 480 (M^++1, 35), 479 (M^+, 100), 351 (30), 230 (28), 122 (22).

Anal. Calcd. for $C_{21}H_{17}N_7O_5S$: C, 52.60; H, 3.57; N, 20.45; S, 6.69. Found: C, 52.53; H, 3.46; N, 19.26; S, 6.60.

9,11-Dimethyl-1-phenyl-3-(*N*-phenylcarbamoyl)-pyrido[3",2":4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9k**).

This compound was obtained in 3.94 g (82%), mp 264 °C; ir: 3367, 3282 (2NH), 1689, 1651 (2CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 2.41 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 7.16 (s, 1H, Pyr-H), 7.27-7.82 (m, 10H, Ar-H), 9.35 (s, 1H, NH), 10.54 (s, 1H, NH); ms, *m/z* (%) 482 (M^++1, 36), 481 (M^+, 100), 362 (32), 230 (14), 77 (56).

Anal. Calcd. for $C_{25}H_{19}N_7O_2S$: C, 62.36; H, 3.98; N, 20.36; S, 6.66. Found: C, 62.34; H, 3.95; N, 20.03; S, 6.64.

9,11-Dimethyl-1-(4-methylphenyl)-3-(*N*-phenylcarbamoyl)-pyrido[3",2":4',5']thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9l**).

This compound was obtained in 3.96 g (80%), mp 224 °C; ir: 3394, 3282 (2NH), 1689, 1678 (2CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 2.42 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 2.62 (s, 3H, Ar-CH_3), 7.16 (s, 1H, Pyr-H), 7.21-7.76 (m, 9H, Ar-H), 9.32 (s, 1H, NH), 10.45 (s, 1H, NH); ms, *m/z* (%) 496 (M^++1, 34), 495 (M^+, 100), 376 (38), 230 (17), 91 (80).

Anal. Calcd. for $C_{26}H_{21}N_7O_2S$: C, 63.02; H, 4.27; N, 19.79; S, 6.47. Found: C, 62.34; H, 4.11; N, 19.54; S, 6.38.

1-(4-Chlorophenyl)-9,11-dimethyl-3-(*N*-phenylcarbamoyl)-pyrido[3",2":4',5']thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9m**).

This compound was obtained in 4.38 g (85%), mp 286 °C; ir: 3392, 3257 (2NH), 1689, 1652 (2CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 2.42 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 7.16 (s, 1H, Pyr-H), 7.24-7.78 (m, 9H, Ar-H), 9.42 (s, 1H, NH), 10.21 (s, 1H, NH); ms, *m/z* (%) 517 (M^++2, 15), 382 (23), 286 (28), 230 (17), 188 (39), 119 (51), 77 (96).

Anal. Calcd. for $C_{25}H_{18}ClN_7O_2S$: C, 58.19; H, 3.52; N, 19.00; S, 6.21. Found: C, 58.12; H, 3.30; N, 18.57; S, 6.15.

9,11-Dimethyl-1-(4-nitrophenyl)-3-(*N*-phenylcarbamoyl)-pyrido[3",2":4',5']thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9n**).

This compound was obtained in 4.21 g (80%), mp 224 °C; ir: 3306, 3275 (2NH), 1686, 1655 (2CO) cm^{-1}; ms, *m/z* (%) 527 (M^++1, 2), 526 (M^+, 6), 351 (2), 230 (17), 101 (25), 86 (100).

Anal. Calcd. for $C_{25}H_{18}N_8O_4S$: C, 57.03; H, 3.45; N, 21.28; S, 6.09. Found: C, 57.18; H, 3.12; N, 21.03; S, 6.16.

3-Benzoyl-9,11-dimethyl-1-phenyl-pyrido[3",2":4',5']thieno-[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9o**).

This compound was obtained in 3.31g (71%), mp 284 °C; ir: 3251 (NH), 1701, 1660 (2CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 2.39 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.38 (s, 1H, Pyr-H), 7.2-8.1 (m, 10H, Ar-H), 9.51 (s, 1H, NH); ms, *m/z* (%) 467 (M^++1, 25), 466 (M^+, 64), 230 (14), 105 (100), 77 (60).

Anal. Calcd. for $C_{25}H_{18}N_6O_2S$: C, 64.36; H, 3.89; N, 18.01; S, 6.87. Found: C, 64.30; H, 3.88; N, 17.76; S, 6.84.

3-Benzoyl-9,11-dimethyl-1-(4-methylphenyl)-pyrido[3",2":4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9p**).

This compound was obtained in 3.36 g (70%), mp 266 °C; ir: 3240 (NH), 1697, 1651 (2CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.55 (s, 3H, Ar-CH_3), 6.38 (s, 1H, Pyr-H), 7.2-8.16 (m, 9H, Ar-H), 9.53 (s, 1H, NH); ms, *m/z* (%) 481 (M^++1, 31), 480 (M^+, 98), 230 (18), 105 (100), 77 (36).

Anal. Calcd. for $C_{26}H_{20}N_6O_2S$: C, 64.98; H, 4.20; N, 17.49; S, 6.67. Found: C, 64.90; H, 4.22; N, 17.11; S, 6.53.

3-Benzoyl-9,11-dimethyl-1-(4-nitrophenyl)-pyrido[3",2":4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9q**).

This compound was obtained in 3.58 g (70%), mp 226 °C; ir: 3255 (NH), 1697, 1655 (2CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 2.39 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.38 (s, 1H, Pyr-H), 7.21-7.96 (m, 9H, Ar-H), 9.51 (s, 1H, NH); ms, *m/z* (%) 511 (M^+, 8), 230 (22), 105 (100), 77 (76).

Anal. Calcd. for $C_{25}H_{17}N_7O_4S$: C, 58.70; H, 3.35; N, 19.17; S, 6.27. Found: C, 58.31; H, 3.30; N, 19.06; S, 6.23.

3-Benzoyl-1-(3-chlorophenyl)-9,11-dimethyl-pyrido[3",2":4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9r**).

This compound was obtained in 3.5 g (70%), mp 278 °C; ir: 3229 (NH), 1697, 1655 (2CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 2.39 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.48 (s, 1H, Pyr-H), 7.21-7.96 (m, 9H, Ar-H), 9.61 (s, 1H, NH); ms, *m/z* (%) 503 (M^++2, 11), 501 (M^+, 12), 230 (18), 105 (100).

Anal. Calcd. for $C_{25}H_{17}ClN_6O_2S$: C, 59.94; H, 3.42; N, 16.78; S, 6.40. Found: C, 59.84; H, 3.27; N, 16.64; S, 6.21.

3-Benzoyl-1-(4-chlorophenyl)-9,11-dimethyl-pyrido[3",2":4',5']-thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9s**).

This compound was obtained in 3.5 g (70%), mp 260 °C; ir: 3433 (NH), 1690, 1647 (2CO) cm^{-1}; ms, *m/z* (%) 501 (M^+, 2), 230 (18), 105 (19), 86 (100).

Anal. Calcd. for $C_{25}H_{17}ClN_6O_2S$: C, 59.94; H, 3.42; N, 16.78; S, 6.40. Found: C, 59.90; H, 3.36; N, 16.55; S, 6.31.

1-(4-Chlorophenyl)-9,11-dimethyl-3-(2-thienoyl)-pyrido[3",2":4',5']thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9t**).

This compound was obtained in 3.6 g (71%), mp 276 °C; ir: 3248 (NH), 1693, 1624 (2CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 2.39 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.38 (s, 1H, Pyr-H), 7.2-8.1 (m, 7H, Ar-H), 9.51 (s, 1H, NH); ms, *m/z* (%) 506 (M^+, 5), 278 (12), 230 (26), 111 (100), 63 (10).

Anal. Calcd. for $C_{23}H_{15}ClN_6O_2S_2$: C, 54.49; H, 2.98; N, 16.58; S, 12.65. Found: C, 54.38; H, 2.91; N, 16.23; S, 12.54.

1-(4-Chlorophenyl)-9,11-dimethyl-3-(2-furoyl)-pyrido[3",2":4',5']thieno[3',2':4,5]pyrimido[2,1-*c*][1,2,4,5]tetrazin-6(4*H*)-one (**9u**).

This compound was obtained in 3.43 g (70%), mp 260 °C; ir: 3252 (NH), 1693, 1643 (2CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 2.39 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.38 (s, 1H, Pyr-H), 7.2-8.1 (m, 7H, Ar-H), 9.51 (s, 1H, NH); ms, *m/z* (%) 492 (M^++2, 17), 490 (M^+, 40), 230 (22), 95 (100).

Anal. Calcd. for $C_{23}H_{15}ClN_6O_3S$: C, 56.27; H, 3.08; N, 17.12; S, 6.53. Found: C, 56.10; H, 3.01; N, 16.95; S, 6.40.

Synthesis of Hydrazonothioate esters **10**.

To a stirred solution of (5 mmol) sodium ethoxide (prepared by adding 0.115 g sodium to 20 ml absolute ethanol) was added compound **4** (1.39 g, 5 mmol). The solution was left stirring for about 10 min. then the appropriate hydrazonoyl halide **6b,h,p** was added. The reaction mixture was left stirring at room temperature for about 10 hrs. The precipitate formed was collected by filtration and recrystallized from DMF to give products **10b,h,p**.

1-[3-Amino-7,9-dimethyl-4-oxo-pyrido[3',2':4,5]thieno[3,2-d]-pyrimidin-2-yl]thio-1-(*N*-phenylhydrazono)-2-oxo-propane (**10b**).

This compound was obtained in 1.51 g (70%), mp 260 °C; ir: 3386, 3236 (NH_2), 3143 (NH), 1743, 1631 (2CO) cm^{-1}; ms, *m/z* (%) 438 (M^+, 52), 403 (74), 320 (74), 114 (74), 56 (100).

Anal. Calcd. for $C_{20}H_{18}N_6O_2S_2$: C, 54.78; H, 4.14; N, 19.16; S, 14.62. Found: C, 54.58; H, 4.01; N, 18.92; S, 14.46.

Ethyl 2-[3-amino-7,9-dimethyl-4-oxo-pyrido[3',2':4,5]thieno-[3,2-*d*]pyrimidin-2-yl]thio-2-[(*N*-(4-methylphenyl)hydrazono)]-ethanoate (**10h**).

This compound was obtained in 1.88 g (78%), mp 297 °C; ir: 3381, 3226 (NH_2), 3183 (NH), 1743, 1631 (2CO) cm^{-1}; ms, *m/z* (%) 482 (M^+, 52), 407 (35), 320 (24), 165 (100), 152 (57).

Anal. Calcd. for $C_{22}H_{22}N_6O_3S_2$: C, 54.75; H, 4.60; N, 17.41; S, 13.29. Found: C, 54.34; H, 4.52; N, 17.13; S, 13.10.

2-[3-Amino-7,9-dimethyl-4-oxo-pyrido[3',2':4,5]thieno[3,2-*d*]-pyrimidin-2-yl]thio-2-[(*N*-(4-methylphenyl)hydrazono)]-1-phenylethanone (**10p**).

This compound was obtained in 1.88 g (73%), mp 290 °C; ir: 3394, 3232 (NH_2), 3140 (NH), 1741, 1631 (2CO) cm^{-1}; ms, *m/z* (%) 514 (M^+, 26), 462 (47), 320 (24), 122 (55), 105 (84), 77 (100).

Anal. Calcd. for $C_{26}H_{22}N_6O_2S_2$: C, 60.68; H, 4.31; N, 16.33; S, 12.46. Found: C, 60.61; H, 4.13; N, 16.20; S, 12.41.

Synthesis of 2-Arylhydrazono-3-substituted-pyrido[3",2":4',5']-thieno[3',2':4,5]pyrimido[2,1-*b*][1,3,4]thiadiazin-6-ones (**11**).

A solution of each of compound **10b,h,p** (5 mmol) in 20 ml glacial acetic acid was heated under reflux for about 1 hr. The precipitate formed was collected by filtration and recrystallized from DMF to give the respective **11b,h,p**.

2-Phenylhydrazono-3,9,11-trimethyl-pyrido[3",2":4',5']thieno-[3',2':4,5]pyrimido[2,1-*b*][1,3,4]thiadiazin-6-one (**11b**).

This compound was obtained in 1.47 g (70%), mp > 300 °C; ir: 3233 (NH), 1632 (CO) cm^{-1}; ms, *m/z* (%) 420 (M^+, 68), 354 (63), 236 (73), 180 (100), 122 (74), 63 (94).

Anal. Calcd. for $C_{20}H_{16}N_6OS_2$: C, 57.12; H, 3.84; N, 19.99; S, 15.25. Found: C, 57.03; H, 3.75; N, 19.88; S, 15.04.

9,11-Dimethyl-3-hydroxy-2-(4-methylphenylhydrazono)-pyrido-[3",2":4',5']thieno[3',2':4,5]pyrimido[2,1-*b*][1,3,4]thiadiazin-6-one (**11h**).

This compound was obtained in 1.7 g (78%), mp > 300 °C; ir: 3387, 3228 (2NH), 1643, 1660 (2CO) cm^{-1}; ms, *m/z* (%) 436 (M^+, 22), 389 (22), 295 (40), 178 (53), 111 (100), 55 (91).

Anal. Calcd. for $C_{20}H_{16}N_6O_2S_2$: C, 55.03; H, 3.69; N, 19.25; S, 14.69. Found: C, 56.94; H, 3.61; N, 19.20; S, 14.64.

9,11-Dimethyl-2-(4-methylphenylhydrazono)-3-phenyl-pyrido-[3",2":4',5']thieno[3',2':4,5]pyrimido[2,1-*b*][1,3,4]thiadiazin-6-one (**11p**).

This compound was obtained in 1.99 g (80%), mp > 300 °C; ir: 3263 (NH), 1632 (CO) cm^{-1}; ms, *m/z* (%) 496 (M^+, 60), 366 (52), 267 (64), 175 (56), 111 (92), 84 (100).

Anal. Calcd. for $C_{26}H_{20}N_6OS_2$: C, 62.88; H, 4.06; N, 16.92; S, 12.91. Found: C, 62.58; H, 4.00; N, 16.67; S, 12.62.

Synthesis of 9,11-Dimethyl-3-phenyl-pyrido[3",2":4',5']thieno-[3',2':4,5]pyrimido[2,1-*b*][1,3,4]thiadiazin-6-one (**13**).

To an equimolecular amounts (0.005 mol) of compound **4** and phenacyl bromide in dioxane, triethyl amine (0.7 ml, 0.005 mol) was added. The reaction mixture was refluxed for 15 min. followed by addition of *p*-toluenesulphonic acid (0.86 g, 0.005 mol) and the reaction mixture was further refluxed for 2 hrs. The precipitate that formed was collected by filtration, dried and recrystallized from acetic acid

This compound was obtained in 1.32 g (70%), mp 252 °C; ir: 1704 (CO) cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 2.59 (s, 3H, CH_3), 2.84 (s, 3H, CH_3), 4.42 (s, 2H, CH_2), 7.28 (s, 1H, Pyr-H), 7.59-8.08 (m, 5H, Ar-H); ms, *m/z* (%) 379 (M^++1, 29), 378 (M^+, 100), 318 (6), 275 (33), 247 (20), 231 (10), 204 (7), 175 (12), 105 (10), 77 (24), 51 (15).

Anal. Calcd. for $C_{19}H_{14}N_4OS_2$: C, 60.30; H, 3.73; N, 14.80; S, 16.94. Found: C, 60.10; H, 3.42; N, 14.66; S, 16.36.

Coupling of 9,11-Dimethyl-3-phenyl-pyrido[3",2":4',5']thieno-[3',2':4,5]pyrimido[2,1-*b*][1,3,4]thiadiazin-6-one (**13**).

To a solution of compound **13** (1.89 g, 0.005 mol) in 20 ml pyridine was added the diazonium chloride solution, prepared as usual by diazotizing aniline (5 mmoles) in hydrochloric acid (3 ml, 6 *M*) with sodium nitrite (0.35 g, 5 mmoles) in 10 ml water portionwise with stirring and cooling. After complete addition, the reaction mixture was left for 12 hrs. The precipitate that formed was collected by filtration, washed with water, dried and then recrystallized from DMF to give pure **11p**.

Antimicrobial Assay.

The Antimicrobial activity of compounds **9a-u** was studied using four fungal species together with four bacterial species. It was assayed biologically using a spore suspension of the fungal species (one ml of sterile water containing approximately 10^8 conidia) or spreading bacterial suspension over a solidified malt agar medium. The layer was allowed to set for 30 min. A solution of each of the tested compounds (5 mg/ml) was placed onto sterile 5 mm filter paper discs and allowed to dry, then the discs were placed onto the center of the malt agar plate and incubated at the optimum incubation temperature 28 °C. A clear zone around the disc was taken as an indication of the inhibition of the best organism growth. The size of the clear zone is proportional to the inhibitory action of the compound under investigation. The fungicide terbinafin and the bactericide chloroamphenicol were used as standard under the same conditions. Measurements were considered after 72 h for fungi and 24 h for bacteria.

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