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RESEARCH ARTICLE

Two-component, one-pot synthesis of pyrimido[1,2-*b*][1,2,4,5]tetrazines

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Derivatives containing the cyclohepta[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-*b*][1,2,4,5]-tetrazin-6-one system were prepared from the reaction of 3-amino-2-thioxo-1,2,3,5,6,7,8,9-octahydro-4*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one (**5**) or its 2-methylthio derivative **6** with hydrazonoyl chlorides **9**. The mechanism of the studied reactions has been discussed and the biological activity of the isolated products has been evaluated.

Keywords: Hydrazonoyl chlorides; Cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one; Pyrimido[1,2-*b*][1,2,4,5]tetrazine

1. Introduction

Derivatives containing the thienopyrimidine systems were reported to possess several important pharmacological properties [1–5]; in particular the antimicrobial activity of some thieno[2,3:4,5]pyrimido[1,2-*b*][1,2,4,5]tetrazines has been reported [6]. Other differently substituted pyrimido[1,2-*b*][1,2,4,5]tetrazin-6-ones have been recently recognized as human cytomegalovirus (HCMV) protease inhibitors [7,8]. In the light of these considerations and in continuation of our studies regarding the chemistry of hydrazonoyl halides [9], it was thought interesting to investigate their reactions with 3-amino-2-thioxo-1,2,3,5,6,7,8,9-octahydro-4*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one (**5**) or its 2-methylthio derivative **9** in an attempt to develop a series of new derivatives containing the pyrimido[1,2-*b*][1,2,4,5]tetrazine moiety hoping that would enhance the biological activity of this ring system.

2. Results and discussion

The starting material 2-isothiocyanato-5,6,7,8-tetrahydro-4*H*-cyclohept[b]thiophene-3-carboxylic acid ethyl ester (**2**) [9] required for the synthesis of the title compounds was obtained

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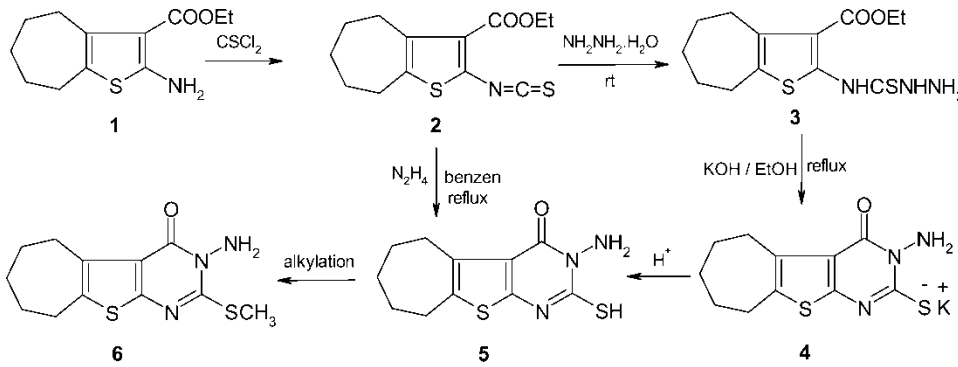
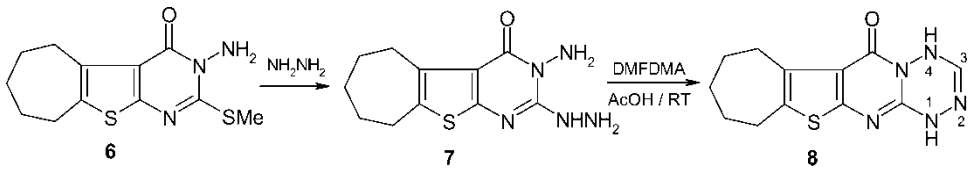


SCHEME 1

from the reaction of amino ester **1** [10] and thiophosgene in acetone at room temperature with subsequent dilution in water (scheme 1). Addition at room temperature of the isothiocyanate **2** to hydrazine hydrate in dichloromethane afforded the thiosemicarbazide derivative **3** which, on refluxing in ethanolic potassium hydroxide solution, gave the potassium salt form of **4**. Acidification of the aqueous solution of the former salt gave 3-amino-2-thioxo-1,2,3,5,6,7,8,9-octahydro-4*H*-cyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one (**5**) in 86 % yield. Compound **5** was also prepared by prolonged heating of the reaction mixture of compound **2** in benzene and hydrazine (98%). The structures of products **2**, **3** and **5** were confirmed by elemental analyses and spectral data (MS, IR, ^{1}H NMR and ^{13}C NMR) (c.f. Experimental). As a representative example, compound **5** revealed a molecular formula $C_{11}H_{13}N_3OS_2$ (M^+ 267). In particular, signals attributable to NH_2, NH and C=O groups were present in IR spectrum. The ^{1}H NMR spectrum exhibited two broad signals at δ 6.34 ppm and 13.68 ppm confirming the presence of the NH_2 and NH groups, respectively. Moreover signals attributable to the methylene protons of **5** were observed in ^{1}H NMR spectrum. The formation of the product was proven using ^{13}C NMR which revealed the presence of the expected sp^3 and the sp^2 carbon atoms at δ (ppm): 27.06, 27.66, 29.10, 32.28, and 115.86, 132.64, 136.44, 146.52, 153.68, 166.50, respectively (c.f. Experimental).

By reaction of the potassium salt **4** or of an alkaline solution of the aminothioxo derivative **5** with iodomethane, the amino methylthio derivative **6** was obtained in 98% yield. Treatment of the latter product **6** with hydrazine hydrate in 2-propanol gave the hydrazine derivative **7** (scheme 2). Structural assignments for the isolated products **6** and **7** were made on the basis of their mass, IR, ^{1}H NMR and ^{13}C NMR data (c.f. Experimental).

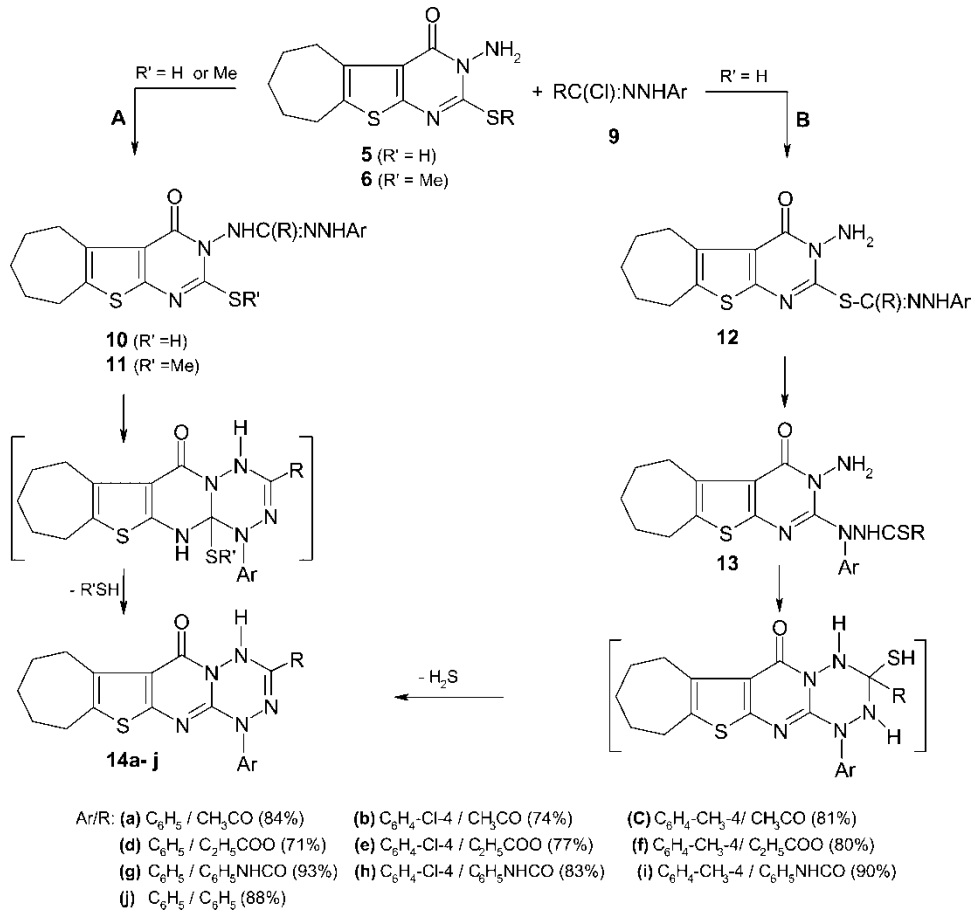
Compound **5** is a versatile intermediate for the preparation of bridgehead nitrogen polyheterocycles due to the presence of two adjacent reactive functional groups. Thus, cyclocondensation of **7** with dimethyl-formamide dimethyl acetal in acetic acid at room temperature for 5 hours led to the formation of 1,7,8,9,10,11-hexa-hydro-4*H*, 6*H*-cyclohepta[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-*b*][1,2,4,5]tetrazin-6-one (**8**) as a parent product of this system and also represents a new heterocyclic ring system (scheme 2).



SCHEME 2

Assignment of the structure to product **8** was obtained by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. Diagnostic signals in the ^{1}H NMR spectrum of compound **8** were a singlet at δ 7.02 ppm attributable to the H-3 proton and two singlets at 9.58 ppm and at 9.76 ppm attributable to the N-1 and N-4 protons, respectively.

Treatment of **5** with hydrazonoyl chlorides **9** in boiling ethanol in the presence of sodium ethoxide gave a single product in each case (scheme 3). The structures of the reaction products were identified as 1,3-disubstituted-1,7,8,9,10,11-hexahydro-4*H*,6*H*-cyclohepta[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-*b*][1,2,4,5]tetrazin-6-one (**14**) (scheme 3). Both spectroscopic data and elemental analyses were consistent with the assigned structures. The infrared spectra of the products **14** exhibited absorption bands in the regions 3249–3301 cm^{-1} and 1662–1684 cm^{-1} due to the NH and C=O groups, respectively. Their mass spectra showed, in addition to the expected molecular ions, peaks at m/z corresponding to [M$^+$−R], [M$^+$−RC(NH)=NNHAr], [M$^+$−RC(=N)NH], [ArN] and [Ar] fragments (c.f. Experimental). The mass spectra of compound **14c** as a typical example of the prepared series showed peaks at 408, 407, 364, 337, 323, 219, 105, 91, and 77. Their ^{1}H NMR spectra revealed the absence of an N-NH$_2$ proton signal present in the spectrum of **5** at δ = 6.34 ppm, and show a common characteristic singlet within δ 9.00 ppm attributable to the N−H proton. In addition, the structure of the products **14** was in agreement with ^{13}C NMR spectra (c.f. Experimental).



SCHEME 3

Further evidence for the assigned structure **14** and in turn the proposed reaction pathway leading to it (Scheme 3) is supported by an alternate synthesis of **14**. Thus, reaction of 3-amino-2-(methylthio)-3,5,6,7,8,9-hexahydro-4*H*-cyclohepta[4,5]-thieno[2,3-*d*]pyrimidin-4-one (**6**) with **9** in pyridine or in ethanol in the presence of triethylamine afforded in each case a product that proved identical in all respect with **14** obtained from **5** and **9**.

The reaction pathway accounting for the formation of products **14a–j** from the reaction of either **5** or **6** with appropriate hydrazonoyl chlorides **9** is outlined in scheme 3. As depicted in scheme 3 there are two possible routes (A and B) for the formation of **14**. In route A (scheme 3) it is suggested that the reactions start with the formation of the hydrazidines **10** or **11**, which in turn cyclizes with concurrent elimination of hydrogen sulfide or methanethiol to give **14**. Alternatively, the reaction of **9** with **5** initially affords the respective thiohydrazonate esters **12** that undergo an *in situ* Smiles rearrangement to yield thiohydrazides **13** [12,13]. Cyclization of **13** with concurrent loss of hydrogen sulfide affords **14** (scheme 3, Route B). In an attempt to distinguish between these two alternative pathways, the reactions of **9** with **6** were further examined. Refluxing of **9** with **6** in pyridine or in ethanol in the presence of triethylamine afforded products identical in all respects with the products **14** obtained from **9** and **5**. This result, together with the fact that all attempts to isolate either the thiohydrazonates **12** or the thiohydrazides **13** under numerous reaction conditions failed, suggests the mechanism proposed in scheme 1 (route A) seems to be consistent with the formation of **14** from reactions of either **5** or **6** with **9**.

3. Biological screening

Compounds **14a, d, f, g, I, j** were tested for their antimicrobial activities against four fungal species namely *Aspergillus fumigatus* (AF), *Penicillium italicum* (PI) and *Syncephalastrum racemosum* (SR) *Candida albicans* (CA) as well as four bacterial species namely *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Bacillus subtilis* (BS) and *Escherichia coli* (EC). The antimicrobial activity was evaluated using the diffusion plate technique. The organisms were tested against the activity of different concentrations of the sample. The fungicide *Terbinafin* and the bactericide *Chloramphenicol* were used as standards under the same conditions. Measurements were taken after 72 h for fungi and 24 h for bacteria. The results showed that the selected compounds **14d** and **14 g** exhibited maximum inhibition against **AF** and **PI**, respectively, whereas compounds **14f** and **14 g** exhibited the highest degree of inhibition against **SA**. All other compounds did not show significant activity against the tested species. The results are summarized in Table 1.

4. Conclusion

In this report, a facile method for the preparation of pyrimido[1,2-*b*][1,2,4,5]tetrazines was described starting from either compound **5** or **6** and hydrazonoyl chlorides **9**. The title compounds were synthesized as new products with anticipated biological values and their structures were confirmed successfully by spectral and elemental analyses.

5. Experimental

All melting points are uncorrected and measured using Electro-thermal IA 9100 apparatus. IR spectra were recorded as potassium bromide pellets on a Nexus 670 spectrophotometer;

Table 1. Antimicrobial activity of some selected compounds.

No.	**14a**			**14d**			**14f**			**14g**			**14i**			**14j**			St.		
C	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1
	Mg/mL			Mg/mL			Mg/mL			Mg/mL			Mg/mL			Mg/mL			Mg/mL		
AF	+	+	+	++	++	++	+	0	0	+	+	+	+	0	0	+	0	0	+++	+++	++
PI	+	+	+	0	0	0	0	0	0	++	++	++	0	0	0	+	+	0	+++	+++	++
SR	+	+	0	0	0	0	0	0	0	+	+	0	+	+	0	0	0	0	+++	+++	+++
CA	+	0	0	+	0	0	0	0	0	+	+	0	+	+	0	+	+	0	+++	++	++
SA	+	+	0	0	0	0	++	++	+	++	++	+	+	0	0	0	0	0	++	++	++
PA	0	0	0	+	+	0	0	0	0	0	0	0	+	+	0	+	+	0	+++	+++	++
BS	+	+	+	+	+	+	+	0	0	+	+	+	+	0	0	+	0	0	+++	+++	++
EC	+	+	+	0	+	+	+	0	0	+	+	+	0	0	0	+	0	0	+++	++	++

Inhibition values = 0.1–0.5 cm beyond control = +; Inhibition values = 0.6–1.0 cm beyond control = ++.
Inhibition values = 1.1–1.5 cm beyond control = + ++; 0 = Not detected.

wave numbers ν (cm^{-1}) are reported. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker 300 MHz (Brock University, Canada). Coupling constants J are reported in Hz and chemical shifts were expressed as part per million; ppm (δ values) against TMS as internal reference. Mass spectra were recorded on EI + Q1 MSLMR UPLR. Microanalytical data were performed by Vario El Elementar apparatus. Organic Microanalysis Section, their results were in agreement with the calculated values.

5.1 *2-Isothiocyanato-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylic acid ethyl ester (2) [10]*

A solution of amino-ester **1** [11] (5.16 g, 21.6 mmol) in dry acetone (80 ml) was added slowly drop-wise over a period of 40 min at room temperature to a stirred solution of thiophosgene (1.6 ml, 97%, d = 1.508, 20.53 mmol), The reaction mixture was stirred at 0 °C for 2 h and at room temperature for additional 1 h. Then, (100 mL) of water was added. The solid separated was collected, washed first with 5% sodium hydroxide and then with water, dried and crystallized from n-hexane to give the isothiocyanate as pale yellow micro-needles. Yield (1.63 g, 58%). Mp: 51–53 °C; IR (KBr) υ (cm^{-1}): 1710 (C=O), 2135 (NCS); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.42 (t, 3H, J = 7.1 Hz, CH_3), 1.56–1.67 (m, 4H, CH_2), 1.81–1.91 (m, 2H, CH_2), 2.69–2.74 (m, 2H, CH_2), 2.93–2.98 (m, 2H, CH_2), 4.36 (q, 2H, J = 7.1 Hz, CH_2). ^{1}H-NMR (d_6-DMSO) δ (ppm): 1.25 (t, 3H, J = 7.1 Hz, CH_3), 1.54–1.57 (m, 4H, CH_2), 1.74–1.78 (m, 2H, CH_2), 2.70–2.73 (m, 2H, CH_2), 2.88–2.91 (m, 2H, CH_2), 4.23 (q, 2H, J = 7.1 Hz, CH_2); ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.40, 26.91, 27.61, 28.08, 29.53, 32.31, 61.08, 128.43, 136.53, 140.08, 162.60; MS: m/z 282.

5.2 *Ethyl 2-(hydrazinocarbonothioyl)-5,6,7,8-tetrahydro-4H-cyclohepta[b]-thiophene-3-carboxylate (3)*

To a stirred solution of hydrazine hydrate (1.4 mL, 28 mmol) in dichloromethane (15 mL) a solution of isothiocyanate **2** (6.97 g, 24.8 mmol) in dichloromethane (15 mL) was added drop-wise at room temperature. After the addition was complete, the mixture was stirred at room temperature for 3 hours, and then the solid was collected, washed with dichloromethane, dried and crystallized from ethanol to afford **3** as yellow crystals. Yield: (2.22 g, 71 %). Mp: 246–248 °C; IR (KBr) υ (cm^{-1}): 3450, 3244, 3147 (NH_2 + NH), 1665 (C=O); ^{1}H-NMR (d_6-DMSO) δ (ppm): 1.30 (t, 3H, J = 7.2 Hz, CH_3), 1.57 (m, 4H, CH_2), 1.81 (m, 2H, CH_2), 2.74 (m, 2H, CH_2), 2.92 (m, 2H, CH_2), 4.31 (q, 2H, J = 7.2 Hz, CH_2), 3.72 (br, 3H, NH and NH_2, exchangeable with D_2O), 12.41 (s, 1H, NH, exchangeable with D_2O); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.40–1.42 (t, 3H, J = 7.1 Hz, CH_3), 1.64–1.68 (m, 4H, CH_2), 1.84–1.86 (m, 2H, CH_2), 2.72–2.76 (m, 2H, CH_2), 3.04–3.08 (m, 2H, CH_2), 4.37–4.44 (q, 2H, J = 7.1 Hz, CH_2), 3.72 (br, 3H, NH and NH_2, exchangeable with D_2O), 12.54 (s, 1H, NH, exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.44, 27.12, 27.98, 28.52, 28.78, 32.37, 61.13, 114.04, 130.42, 136.70, 147.92, 167.27, 171.18; MS: m/z 313 $(M^+)\cdot$ Calcd. (%) for $C_{13}H_{19}N_3O_2S_2$ (313.44): C; 49.82, H; 6.11, N; 13.41, S; 20.46. Found: C; 49.69, H; 6.09, N; 13.43, S; 20.31.

5.3 *3-Amino-2-thioxo-1,2,3,5,6,7,8,9-octahydro-4H-cyclohepta[4,5]thieno[2,3-d] pyrimidin-4-one (5)*

To a warmed ethanolic potassium hydroxide solution [prepared by dissolving (1.12 g, 20 mmol) of KOH in EtOH (50 mL)] was added (6.26 g, 20 mmol) of **3**. The reaction mixture was

refluxed for 1 h. The solid was then collected while hot, dried and poured into water (20 mL), the mixture was acidified with conc. HCl (37%). The reaction mixture was stirred for further 30 minutes at room temperature. The solid that separated was filtered off, washed with 5% sodium bicarbonate, water, cold EtOH (5 mL), dried and crystallized from DMF to afford **5** as colorless crystals; yield: (4.59 g, 86%). Mp: 293–295 °C; IR (KBr) υ (cm^{-1}): 3450, 3249, 3148 (NH_2 + NH), 1671 (C=O); ^{1}H-NMR (d_6-DMSO) δ (ppm): 1.52–1.61 (m, 4H, CH_2), 1.81–1.83 (m, 2H, CH_2), 2.74–2.78 (m, 2H, CH_2), 3.14–3.17 (m, 2H, CH_2), 6.34 (br, 2H, NH_2, exchangeable with D_2O), 13.68 (s, 1H, NH, exchangeable with D_2O). ^{13}C-NMR (d_6-DMSO) δ (ppm): 27.06, 27.66, 29.10, 32.28, 115.86, 132.64, 136.44, 146.52, 153.68, 166.50; MS: m/z 267. Calcd. (%) for $C_{11}H_{13}N_3OS_2$ (267.37): C, 49.41; H, 4.90; N, 15.72; S, 23.98. Found: C, 49.32; H, 4.88; N, 15.74; S, 23.75.

5.4 *3-Amino-2-(methylthio)-3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]thieno-[2,3-d] pyrimidin-4-one (6)*

To a warmed ethanolic potassium hydroxide solution [prepared as described above] was added (6.26 g, 20 mmol) of **5**. The reaction mixture was refluxed for 30 minutes and allowed to cool to room temperature, followed by addition of a solution of methyl iodide (3.12 g, 22 mmol) in EtOH (10 mL). The reaction mixture was heated for 1 h and then was left to stir at room temperature overnight. The solid that separated was filtered off, washed with water (3 × 5 mL), cold EtOH (2 mL) and crystallized from EtOH to afford **6** as colorless crystals; yield: (5.56 g, 99%). Mp: 187–189 °C; IR (KBr) υ (cm^{-1}): 3299, 3178(NH_2), 1679 (C=O); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.43–1.49 (m, 4H, CH_2), 1.68–1.70 (m, 2H, CH_2), 2.50 (s, 3H, CH_3), 2.80 (m, 2H, CH_2), 3.26 (m, 2H, CH_2), 4.71 (br, 2H, NH_2, exchangeable with D_2O). ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.50, 27.24, 27.71, 27.75, 29.83, 32.52, 118.69, 135.93, 136.35, 158.35, 158.56, 160.83. MS: m/z (%) 281. Calcd. (%) for $C_{12}H_{15}N_3OS_2$ (281.40): C; 51.22, H; 5.37, N; 14.93, S; 22.79. Found: C; 51.04, H; 5.38, N; 14.76, S; 22.69.

5.5 *3-Amino-2-hydrazino-3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]thieno-[2,3-d] pyrimidin-4-one (7)*

A mixture of **6** (2.81 g, 10 mmol) and hydrazine hydrate (3.00 g, 60 mmol) was refluxed for 48 hours in 2-propanol (50 mL). After cooling, the solid was collected, washed with ethanol, dried and crystallized from ethanol to afford **6** as colorless powder. Yield: (2.10 g, 79%). Mp: 270–271 °C; IR (KBr) υ (cm^{-1}): 3331, 3180 (NH_2), 1680 (C=O); ^{1}H-NMR (d_6-DMSO) δ (ppm): 1.37–1.62 (m, 4H, CH_2), 1.71–1.91 (m, 2H, CH_2), 2.68–2.77 (m, 2H, CH_2), 3.15–3.18 (m, 2H, CH_2), 5.26 (br, 2H, NH_2, exchangeable with D_2O), 8.12 (br, 1H, NH, exchangeable with D_2O). ^{13}C-NMR (d_6-DMSO) δ (ppm): 27.13, 27.22, 27.71, 28.97, 32.15, 114.06, 129.60, 135.62, 153.35, 158.16, 162.10. MS: m/z (%) 265. Calcd. (%) for $C_{11}H_{15}N_5OS$ (265.34): C; 49.79, H; 5.70, N; 26.39, S; 12.08. Found: C; 49.77, H; 5.73, N; 26.38, S; 12.17.

5.6 *1,7,8,9,10,11-Hexahydro-4H,6H-cyclohepta[4′,5′]thieno[2′,3′:4,5]-pyrimido[1,2-b][1,2,4,5]tetrazin-6-one (8)*

A mixture of **7** (2.65 g, 10 mmol) and *N*, *N*-dimethylformamide dimethyl acetal (1.19 g, 10 mmol) was stirred for 5 hours in acetic acid (5 mL). The solid product, so formed, was collected, dried and crystallized from ethanol to afford **8**. Yield: (0.79 g, 29%). Mp: 218–220 °C; IR (KBr) υ (cm^{-1}): 3231 (NH), 1680 (C=O); ^{1}H-NMR (d_6-DMSO) δ (ppm): 1.56–1.58

(m, 4H, CH_2), 1.71–1.91 (m, 2H, CH_2), 2.69–2.76 (m, 2H, CH_2), 3.12–3.17 (m, 2H, CH_2), 7.02 (s, 1H, CH), 9.58 (br, 1H, NH, exchangeable with D_2O), 9.76 (br, 1H, NH, exchangeable with D_2O). MS: m/z (%) 275. Calcd. (%) for $C_{12}H_{13}N_5OS$ (275.33): C; 52.35, H; 4.76, N; 25.44, S; 11.65. Found: C; 52.42, H; 4.95, N; 25.41, S; 11.53.

6. General synthesis of 1,3-disubstituted-1,7,8,9,10,11-hexahydro-4*H*,6*H*-cyclohepta[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-*b*][1,2,4,5]tetrazin-6-one (14)

Method A: A mixture of equimolar amounts of **5** and the appropriate hydrazonoyl chlorides **9** [14–23] (10 mmol each) in sodium ethoxide solution prepared from sodium metal (0.23 g, 10 mmol) and absolute ethanol (30 mL) was refluxed until methanethiol ceased to evolve (6–12 h). After cooling, the solid that precipitated was filtered and crystallized from the proper solvent. After cooling the reaction mixture, a precipitate was formed, filtered, washed with alcohol and crystallized from the proper solvent to give compounds **14a–j**.

Method B: To a mixture of equimolar amounts of **6** (10 mmol) and the appropriate hydrazonoyl chlorides **9** (10 mmol) in absolute ethanol (30 mL), triethylamine (1.01 g, 10 mmol) was added, and the resulting mixture was refluxed until hydrogen sulfide gas ceased to evolve (3–8 h). The products obtained by this method were found to be identical in all respects (IR, m.p., mixed m.p.) with those obtained by method A.

Method C: A mixture of equimolar amounts of **6** and the appropriate hydrazonoyl chlorides **9** (10 mmol each) in dry pyridine (25 mL) was refluxed for 18 h. After cooling, the reaction mixture was poured into ice-cold hydrochloric acid under stirring. The solid, so formed, was filtered and crystallized from the proper solvent. The products obtained by this method were found to be identical in all respects (IR, m.p., mixed m.p.) with those obtained by method A and B.

6.1 *3-Acetyl-1-phenyl-1,7,8,9,10,11-hexahydro-4H,6H-cyclohepta[4′,5′]-thieno[2′,3′:4,5]pyrimido[1,2-b][1,2,4,5]tetrazin-6-one (14a)*

Yield (3.30 g, 84%). Mp: 217–219 °C (EtOH); IR (KBr) υ (cm^{-1}): 3272 (NH), 1691, 1679 cm^{-1} (C=O). ^{1}H NMR (d_6-DMSO): $\delta = 1.59$ (m, 4H, $2CH_2$), 1.82 (m, 2H, CH_2), 2.53 (s, 3H, CH_3), 2.74 (m, 2H, CH_2), 3.23 (m, 2H, CH_2), 7.24–7.60 (m, 5H, Ar−H), 9.00 (s, 1H, NH). MS, m/z (%): 393. Anal. Calcd. for $C_{20}H_{19}N_5O_2S$ (393.47): C, 61.05; H, 4.87; N, 17.80; S, 8.15; Found: 61.14; H, 4.88; N, 17.82; S, 8.13.

6.2 *3-Acetyl-1-(4-chlorophenyl)-1,7,8,9,10,11-hexahydro-4H,6H-cyclohepta-[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-b][1,2,4,5]tetrazin-6-one (14b)*

Yield: 3.16 g (74%). M.p. 189–191 °C (EtOH); IR (KBr) υ (cm^{-1}): 3263 (NH), 1677 cm^{-1} (C=O). ^{1}H NMR (d_6-DMSO): $\delta = 1.59$ (m, 4H, $2CH_2$), 1.83 (m, 2H, CH_2), 2.52 (s, 3H, CH_3), 2.75 (m, 2H, CH_2), 3.20 (m, 2H, CH_2), 7.53–7.64 (dd, 4H, Ar−H), 9.01 (s, 1H, NH); ^{13}C NMR (d_6-DMSO): $\delta = 27.30, 27.39, 27.83, 29.36, 31.16, 32.32, 118.53, 124.04, 126.16, 127.47, 129.07, 131.99, 134.69, 136.39, 141.13, 143.50, 149.68, 153.08, 160.13, 207.00$. MS, m/z (%): 427. Anal. Calcd. for $C_{20}H_{18}ClN_5O_2S$ (427.92): C, 56.14; H, 4.24; N, 16.37; S, 7.49; Found: 56.22; H, 4.26; N, 16.37; S, 7.52.

6.3 *3-Acetyl-1-(4-methylphenyl)-1,7,8,9,10,11-hexahydro-4H,6H-cyclohepta-[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-b][1,2,4,5]tetrazin-6-one (14c)*

Yield: 3.30 g (81%).M.p. 222–224 °C (EtOH); IR (KBr) υ (cm^{-1}): 3261 (NH), 1678 cm^{-1} (C=O). ^{1}H NMR (d_6-DMSO): $\delta = 1.58$ (m, 4H, $2CH_2$), 1.83 (m, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.73 (m, 2H, CH_2), 3.21 (m, 2H, CH_2), 7.05–7.45 (m, 4H, Ar−H), 9.00 (s, 1H, NH). MS, m/z (%): 407. Anal. Calcd. for $C_{21}H_{21}N_5O_2S$ (407.50): C, 61.90; H, 5.19; N, 17.19; S, 7.87; Found: 61.89; H, 5.21; N, 17.20; S, 7.77.

6.4 *Ethyl (1-phenyl)-6-oxo-1,7,8,9,10,11-hexahydro-4H,6H-cyclohepta[4′,5′]-thieno[2′,3′:4,5]pyrimido[1,2-b][1,2,4,5]tetrazine-3-carboxylate (14d)*

Yield: 3.01 g (71%). M.p. 217–219 °C (Dioxane); IR (KBr) υ (cm^{-1}): 3280 (NH), 1720, 1684 cm^{-1} (C=O). ^{1}H NMR (d_6-DMSO): $\delta = 1.30$ (t, 3H, CH_3), 1.57 (m, 4H, $2CH_2$), 1.82 (m, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.74 (m, 2H, CH_2), 2.81 (m, 2H, CH_2), 3.18 (m, 2H, CH_2), 4.35 (q, 2H, CH_2), 7.26–7.60 (m, 5H, Ar−H), 9.01 (s, 1H, NH). MS, m/z (%): 423. Anal. Calcd. for $C_{21}H_{21}N_5O_3S$ (423.50): C, 59.56; H, 5.00; N, 16.54; S, 7.57; Found: 59.61; H, 5.01; N, 16.55; S, 7.49.

6.5 *Ethyl 1-(4-chlorophenyl)-6-oxo-1,7,8,9,10,11-hexahydro-4H,6H-cyclo-hepta[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-b][1,2,4,5]tetrazine-3-carboxylate (14e)*

Yield: 3.34 g (77%). M.p. 172–174 °C (Dioxane); IR (KBr) υ (cm^{-1}): 3255 (NH), 1724, 1676 cm^{-1} (C=O). ^{1}H NMR (d_6-DMSO): $\delta = 1.31$ (t, 3H, $J = 6.8$ Hz, CH_3), 1.58 (m, 4H, $2CH_2$), 1.83 (m, 2H, CH_2), 2.76 (m, 2H, CH_2), 3.18 (m, 2H, CH_2), 4.37 (q, 2H, $J = 6.8$ Hz, CH_2), 7.26–7.38 (m, 4H, Ar−H), 9.11 (s, 1H, NH). MS, m/z (%): 457. Anal. Calcd. for $C_{21}H_{20}ClN_5O_3S$ (457.94): C, 55.08; H, 4.40; N, 15.29; S, 7.00; Found: C, 55.13; H, 4.42; N, 15.30; S, 7.03.

6.6 *Ethyl 1-(4-methylphenyl)-6-oxo-1,7,8,9,10,11-hexahydro-4H,6H-cyclo-hepta[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-b][1,2,4,5]tetrazine-3-carboxylate (14f)*

Yield: 3.50 g (80%). M.p. 190–192 °C (Dioxane); IR (KBr) υ (cm^{-1}): 3281 (NH), 1721, 1683 cm^{-1} (C=O). ^{1}H NMR (d_6-DMSO): $\delta = 1.30$ (t, 3H, CH_3), 1.57 (m, 4H, $2CH_2$), 1.82 (m, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.74 (m, 2H, CH_2), 2.81 (m, 2H, CH_2), 3.18 (m, 2H, CH_2), 4.35 (q, 2H, CH_2), 7.26–7.38 (dd, 4H, Ar−H), 9.03 (s, 1H, NH). ^{13}C NMR ($CDCl_3/d_6$-DMSO 1:3): $\delta = 13.47, 20.40, 26.47, 26.67, 26.96, 28.90, 31.63, 62.54, 123.87, 128.68, 135.20, 135.56, 135.82, 137.08, 138.60, 139.51, 151.37, 157.21, 159.12$. MS, m/z (%): 437. Anal. Calcd. for $C_{22}H_{23}N_5O_3S$ (437.52): C, 60.40; H, 5.30; N, 16.01; S, 7.33; Found: 60.55; H, 5.31; N, 16.03; S, 7.38.

6.7 *1-(Phenyl)-6-oxo-N-phenyl-1,7,8,9,10,11-hexahydro-4H,6H-cyclohepta-[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-b][1,2,4,5]tetrazine-3-carboxamide (14g)*

Yield: 4.37 g (93%). M.p. 226–228 °C (Dioxane); IR (KBr) υ (cm^{-1}): 3248 (NH), 1682, 1643 cm^{-1} (C=O). ^{1}H NMR (d_6-DMSO): $\delta = 1.59$ (m, 4H, $2CH_2$), 1.83 (m, 2H, CH_2), 2.74 (m, 2H, CH_2), 3.21 (m, 2H, CH_2), 7.16–7.73 (m, 10 H, Ar−H), 9.26 (s, 1H, NH), 10.44 (s, 1H, NH). MS, m/z (%): 470. Anal. Calcd. for $C_{25}H_{22}N_6O_2S$ (470.56): C, 63.81; H, 4.71; N, 17.86; S, 6.81; Found: C, 63.79; H, 4.74; N, 17.89; S, 6.78.

6.8 *1-(4-Chlorophenyl)-6-oxo-N-phenyl-1,7,8,9,10,11-hexahydro-4H,6H-cyclohepta-[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-b][1,2,4,5]tetrazine-3-carboxamide (14h)*

Yield: 4.19 g (83%). M.p. 215–217 °C (Dioxane); IR (KBr) υ (cm^{-1}): 3248 (NH), 1684, 1644 cm^{-1} (C=O). ^{1}H NMR (d_6-DMSO): $\delta = 1.60$ (m, 4H, $2CH_2$), 1.84 (m, 2H, CH_2), 2.74 (m, 2H, CH_2), 3.22 (m, 2H, CH_2), 7.17–7.75 (m, 10 H, Ar−H), 9.27 (s, 1H, NH), 10.45 (s, 1H, NH). MS, m/z (%): 504. Anal. Calcd. for $C_{25}H_{21}ClN_6O_2S$ (505.00): C, 59.46; H, 4.19; N, 16.64; S, 6.35; Found: C, 59.47; H, 4.17; N, 16.65; S, 6.29.

6.9 *1-(4-Methylphenyl)-6-oxo-N-phenyl-1,7,8,9,10,11-hexahydro-4H,6H-cyclohepta-[4′,5′]thieno[2′,3′:4,5]pyrimido[1,2-b][1,2,4,5]tetrazine-3-carboxamide (14i)*

Yield: 4.36 g (90%). M.p. 290–292 °C (Dioxane); IR (KBr) υ (cm^{-1}): 3248 (NH), 1684, 1644 cm^{-1} (C=O). ^{1}H NMR (d_6-DMSO): $\delta = 1.60$ (m, 4H, $2CH_2$), 1.84 (m, 2H, CH_2), 2.74 (m, 2H, CH_2), 3.22 (m, 2H, CH_2), 2.36 (S, 3H, CH_3), 7.17–7.75 (m, 10 H, Ar−H), 9.27 (s, 1H, NH), 10.45 (s, 1H, NH). ^{13}C NMR (d_6-DMSO): $\delta = 20.20, 26.37, 26.89, 28.48, 31.39, 117.79,$ 120.70, 123.90, 124.39, 128.31, 128.62, 134.58, 135.35, 135.45, 136.84, 137.19, 139.77, 141.23, 151.50, 155.19, 159.06, 206.04. MS, m/z (%): 484. Anal. Calcd. for $C_{26}H_{24}N_6O_2S$ (484.58): C, 64.44; H, 4.99; N, 17.34; S, 6.62; Found: C, 64.46; H, 5.00; N, 17.31; S, 6.55.

6.10 *1,3-Diphenyl-1,7,8,9,10,11-hexahydro-4H,6H-cyclohepta[4′,5′]thieno-[2′,3′:4,5]pyrimido[1,2-b][1,2,4,5]tetrazine-6-one (14j)*

Yield: 3.76 g (88%). M.p. 230–232 °C (Dioxane); IR (KBr) υ (cm^{-1}): 3236 (NH), 1681 cm^{-1} (C=O). ^{1}H NMR (d_6-DMSO): $\delta = 1.59$ (m, 4H, $2CH_2$), 1.83 (m, 2H, CH_2), 2.74 (m, 2H, CH_2), 3.22 (m, 2H, CH_2), 7.23–7.88 (m, 10 H, Ar−H), 10.14 (s, 1H, NH). ^{13}C NMR (d_6-DMSO): $\delta = 27.28, 27.39, 27.82, 29.37, 32.32, 118.51, 124.00, 126.13, 127.45, 128.83, 129.05, 129.25,$ 131.97, 134.67, 136.37, 141.11, 143.43, 149.65, 153.04, 160.12. MS, m/z (%): 427. Anal. Calcd. for $C_{24}H_{21}N_5OS$ (427.53): C, 67.43; H, 4.95; N, 16.38; S, 7.50; Found: C, 67.39; H, 4.97; N, 16.38; S, 7.52.

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