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Article

Synthesis and Cytotoxicity of Novel Hexahydrothieno-cycloheptapyridazinone Derivatives

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Abstract: Designed as a new group of tricyclic molecules containing the thienocycloheptapyridazinone ring system, a number of 2*N*-substituted-hexahydrothieno-cycloheptapyridazinone derivatives were synthesized and their biological activity evaluated. Among the synthesized compounds, derivatives **7d** and **7h** were found to possess cytotoxic activity against non-small cell lung cancer and central nervous system cancer cell lines, respectively.

Keywords: pyridazinones; synthesis; cytotoxicity

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1. Introduction

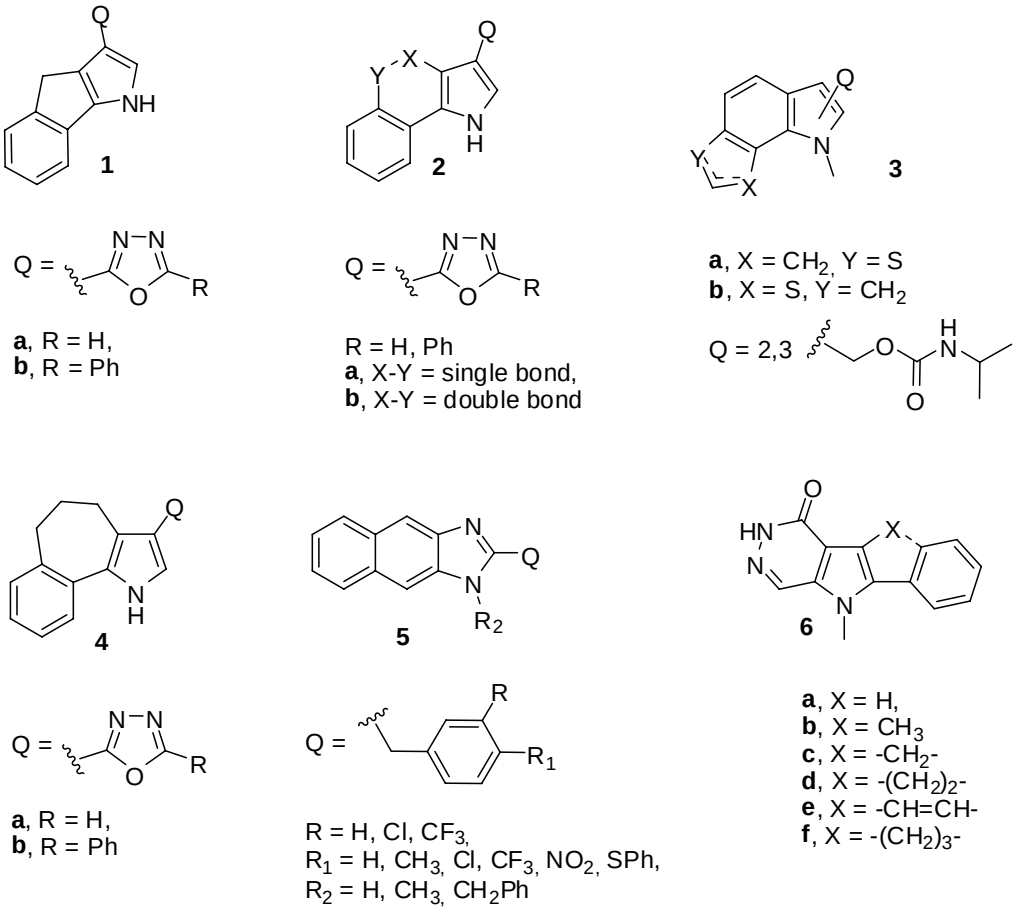
Among diseases, cancer is not a single pathological state but a broad group of diseases characterized by a high proliferative index and the spread of aberrant cells from their site of origin [1]. Clinically, the therapeutic treatment of cancer is a combination of surgery and/or radiotherapy with chemotherapy [2,3].

Current chemotherapy consists of cytotoxic (cell-killing) agents and anti-hormonal drugs, which reduce the proliferation of the tumors [2,3]. The therapeutic use of anticancer drugs is complicated by systemic toxicity, usually observed in the bone narrow, the gastrointestinal (GI) tract and hair, and by development of resistance. Therefore, the search for novel chemical structures with broader therapeutic windows and acceptable resistance profiles is being actively pursued.

In discovering anticancer compounds, a notable role is played by polycondensed heterocycles containing the pyridazinone moiety [4]. A wide spectrum of pharmacological activities has been reported for these compounds. These include anticancer [5], antihypertensive, anti-thrombotic and antiulcerative properties [6-9]. Pyridazinone derivatives also possess affinity for benzodiazepine receptors [10] and the ability to inhibit the human matrix metalloproteinase [11] and aldose reductase [12,13] enzymes.

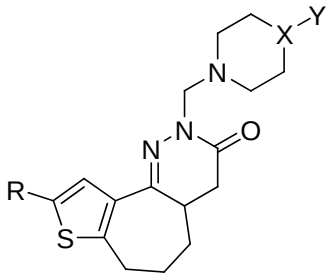
A major interest in our group is the design, synthesis and evaluation of new antiproliferative compounds as candidate cytotoxic and anticancer agents. In recent years, we have reported the synthesis of novel derivatives of 1,4-dihydroindeno[1,2-*b*]pyrroles (**1**) [5], 1*H*-benzo[*g*]indoles (**2**) [5], thieno[3,2-*g*]indoles (**3**) [14], 1,4,5,6-teterahydrobenzo[6,7]cyclohepta[1,2-*b*]pyrroles (**4**) [5], naphto[2,3-*d*]imidazoles (**5**) [15], pyrrole[2,3-*d*]pyridazinones (**6**) [16] and their cytotoxic activities in the NCI preclinical antitumor screen (Figure 1).

Figure 1. Chemical structures of some known anticancer agents synthesized by our group.



In continuation of our research in this field, we describe herein the synthesis of novel 2,4,4a,5,6,7-hexahydro-3*H*-thieno[2',3':6,7]cyclohepta[1,2-*c*]pyridazinones bearing substituted piperazine, piperidine and morpholine moieties, using a random screening approach [17], and the antitumor activities of the resulting compounds **7a-l** reported in Table 1.

Table 1. Novel thienocyclohepta[1,2-*c*]pyridazinones **7a-l**.

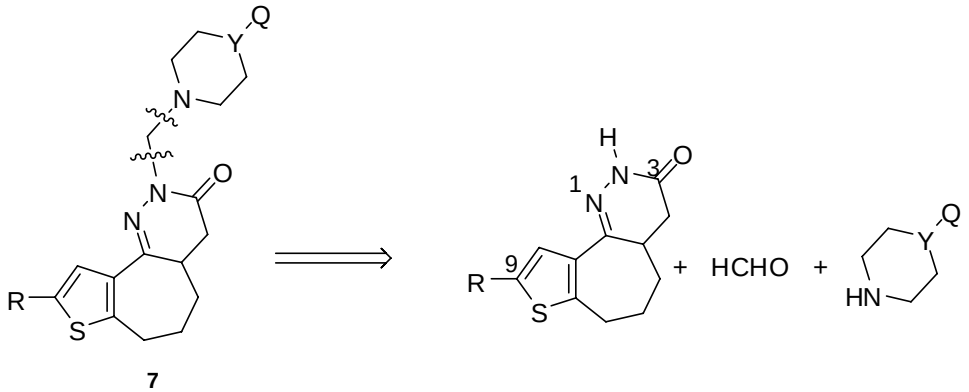


7	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**
R	H	H	H	H	CH_3	CH_3	CH_3	CH_3	H	CH_3	H	CH_3
X	N	N	N	N	N	N	N	N	CH_2	CH_2	O	O
Y	Ph	*o*-OCH_3-Ph	*o*-F-Ph	CH_3	Ph	*o*-OCH_3-Ph	*o*-F-Ph	CH_3	CH_3	CH_3	-	-

2. Chemistry

The retrosynthetic analysis shown in Figure 2 shows how novel pyridazinone derivatives could be prepared by condensation of a tricyclic ring system with formaldehyde and the appropriate substituted piperazine synthon or its isosteres such as methylpiperidine and morpholine.

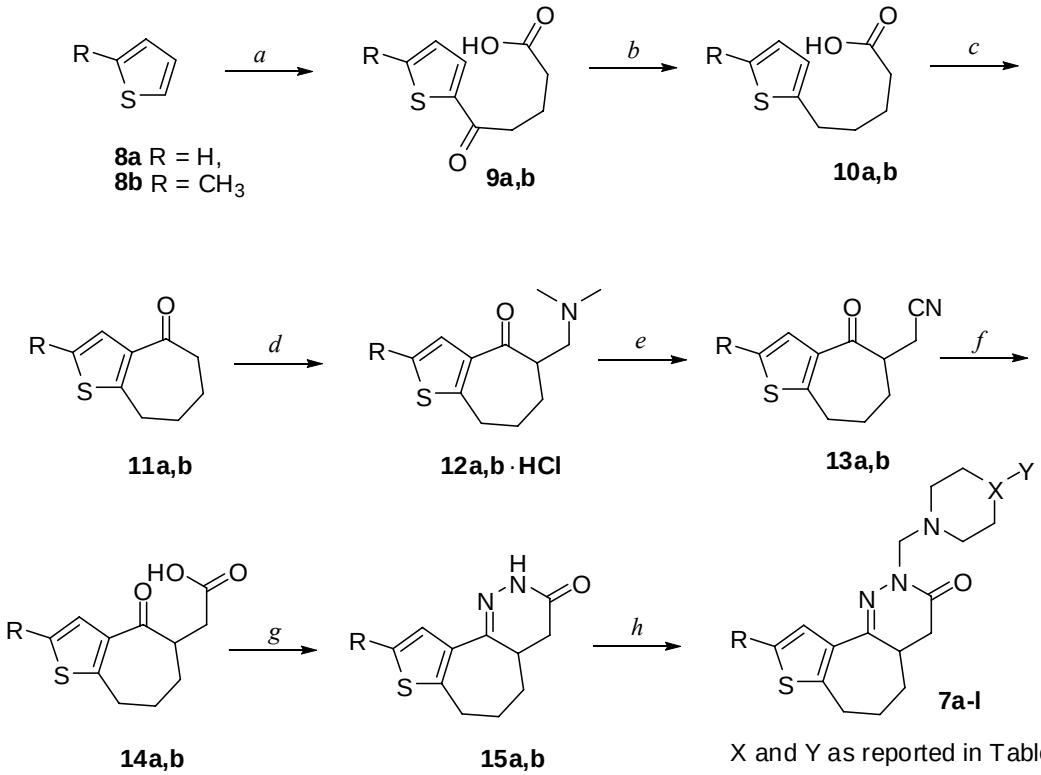
Figure 2. The retrosynthetic analysis of the target compounds **7**.



Accordingly, the new 2,4,4a,5,6,7-exahydro-3*H*-thieno[2',3':6,7]cyclohepta[1,2-*c*]pyridazin-3-one derivatives **7a-l** were synthesized in according to Scheme 1. The reaction of thiophenes **8a,b** with glutaric anhydride to give ketoacids **9a,b** was followed by Wolff- Kishner reduction to **10a,b**, whose cyclization with P_2O_5 over Celite® gave the ketones **11a,b**. A Mannich reaction furnished **12a,b**, which were converted with NaCN in CH_3OH into the nitriles **13a,b**. Hydrolysis of these nitriles in refluxing HCl/AcOH led to the γ-ketoacids **14a,b**; condensation of the latter with hydrazine hydrate afforded the pyridazinones **15a,b**.

Preparation of the target compounds **7a-l** was accomplished by treatment of the pyridazinones **15a,b** with formaldehyde and appropriate amines.

Scheme 1. Synthesis of novel pyridazinone derivatives **7a-l**.



Reagents and conditions: *a)* $AlCl_3$, CH_2Cl_2, glutaric anhydride, RT, 0.5h; b) DEG, KOH, $H_2NNH_2 \cdot H_2O$, reflux, 3h; *c)* Toluene, Celite®, P_2O_5, reflux, 2h; *d)* $HN(CH_3)_2 \cdot HCl$, CH_2O, Ac_2O, 75 °C, 3h; *e)* MeOH, NaCN, 55 °C, 4h; *f)* AcOH, HCl 37%, reflux, 3h; *g)* EtOH an., $H_2NNH_2 \cdot H_2O$, reflux, 3h; *h)* EtOH an., CH_2O, amine, N_2, reflux, 8h.

3. Results and Discussion

A new series of twelve substituted pyridazinones **7a-l** were synthesized and eight of them (**7c-e,h-l**) were evaluated at a single concentration of 10^{-5} M (10 μM) for their antitumor activities. The evaluation established a primary screening where compounds were tested to determine their growth inhibitory properties against sixty different human tumor cell lines *in vitro* [18-20]. The compounds were added at a single concentration and the cell culture was incubated for 48 h. End point determinations were made using a protein binding dye, sulforhodamine B (SRB), which was used to estimate cell viability or growth [21]. The results for each compound are reported as percent growth of treated cells when compared to untreated control cells (Tables 2-3). Range of growth % shows the lowest and the highest growth % found among different cancer cell lines, where all tested compounds have demonstrated being scarcely active or completely inactive in the antitumor screening *in vitro* (Table 2). 5-Fluorouracil (5-FU) was used as reference compound with the mean growth inhibitory effect (GI_{50}) of 2.45×10^{-5} M which corresponds in logarithmic scale to 4.61 [22].

Table 2. Anticancer screening data of selected pyridazinone derivatives **7c,d,e,h,i,j,k,l**.[a]

Panel/Cell Lines	**Compounds**							
Non-small cell lung cancer	**7c 7d**		**7e**	**7h**	**7i 7j**		**7k**	**7l**
Mean growth %	97.85	**98.09**	103.17	101.55	101.33	101.88	96.66	102.97
Range of growth %	81.03 to 113.17	72.06 to 120.26	78.91 to 108.81	92.87 to 109.80	80.20 to110.82	81.70 to 119.13	75.17 to 113.32	93.09 to 112.16
Colon cancer								
Mean growth %	107.29	105.03	109.01	104.88	103.17	104.91	101.79	123.28
Range of growth %	100.56 to 117.52	96.19 to 111.60	97.01 to 119.39	93.70 to 123.28	93.76 to 117.4	95.50 to 112.76	93.53 to 114.38	99.22 to 110.16
Breast Cancer								
Mean growth %	103.25	100.59	107.91	105.20	104.68	107.49	101.71	108.39
Range of growth %	92.60 to 113.98	91.34 to 109.30	96.71 to 121.53	91.23 to 120.16	88.76 to122.29	91.22 to 124.23	94.33 to 110.47	87.28 to 129.10
Ovarian Cancer								
Mean growth %	104.72	101.64	108.09	102.84	109.28	102.64	103.69	104.24
Range of growth %	99.67 to 111.88	98.15 to 108.92	95.14 to 119.38	96.73 to 120.49	92.31to 170.96	94.48 to 117.11	92.28 to 117.79	96.32 to 122.92
Leukemia								
Mean growth %	100.46	**92.31**	98.55	102.95	94.13	100.01	86.67	98.54
Range of growth %	91.68 to 107.61	81.94 to 113.17	91.36 to 111.53	85.24 to 120.85	80.99 to	90.34 to 112.16	76.07 to 94.26	75.05 to 112.55
Renal Cancer								
Mean growth %	101.77	101.13	103.67	**95.02**	103.58	100.19	104.36	101.80
Range of growth %	93.09 to 110.89	95.38 to 108.40	94.16 to 114.16	77.38 to 114.45	94.14 to113.75	86.51 to 121.05	93.91 to 114.99	85.76 to 112.31
Melanoma								
Mean growth %	106.50	102.46	106.62	101.06	102.95	107.10	101.57	104.80
Range of growth %	100.28 to110.39	88.88 to 111.60	101.92 to116.24	92.73 to 112.29	87.54 to119.80	92.35 to 124.26	91.52 to 114.53	93.82 to 112.20
Prostate Cancer								
Mean growth %	102.25	100.46	114.38	109.08	101.76	99.92	103.85	109.31
Range of growth %	98.48 to 106.02	93.16 to 107.76	108.08 to 120.68	104.25 to 113.91	99.78 to 103.76	96.26 to 103.58	102.00 to105.70	102.40 to 116.22
CNS Cancer								
Mean growth	99.49	100.85	100.78	**82.03**	111.34	98.38	107.23	97.20
Range of growth %	91.08 to 108.91	77.31 to 131.16	89.96 to 110.76	72.14 to 115.77	80.17 to 194.64	85.30 to 105.97	81.54 to 164.17	82.07 to 106.60

[a] Assay at 1-dose 10^{-5} M (10 μM) concentration.

Nevertheless, compounds **7d** and **7h** displayed a higher anti-proliferative activity in the non-small cell lung cancer cell line EKVX and in the CNS cancer cell line SNB-75, which showed growth inhibitions of 27.94% and 27.86%, respectively (Table 3). Compounds **7k** and **7l** also showed cell

growth inhibitory activity, even if weaker than the one expressed by **7d** and **7h**. In particular, compound **7l** was found to be active as growth % inhibitor of the leukemia cell line RPMI-8226 with a value of 24.95%; the derivative **7k** was active on leukemia cell line SR with a value of 24.83% and non-small cell lung cancer cell line EKVX with a value of 23.93%. Moreover, **7e** was found to be active as growth % inhibitor of the non-small cell lung cancer cell line EKVX with a value of 21.09%. Finally, **7c** and **7i** showed a growth % inhibitor of non-small cell lung cancer cell line HOP-92 with values of 18.97, and 19.80%, respectively.

Table 3. *In vitro* cancer lines growth % inhibition of pyridazinones **7d,e,h,k,l**.

Compd	Panel/Cell Lines						
	Non-small cell lung cancer		Leukemia Renal		cancer		CNS cancer
	EKVX*	HOP-92	SR	RPMI-8226	CAKI-1	UO-31	SNB-75*
7d	27.94	20.09					22.69
7e	21.09						
7h					22.62	22.23	27.86
7k	23.93		24.83				
7l				24.95			

***** The most sensitive cell lines.

4. Conclusions

As part of our continuous search for potential biologically active compounds, a series of pyridazinone derivatives were synthesized and assessed for their anticancer activity. It was found that all new eight compounds tested showed weak or incomplete activity without significant differences between 9-substituted and unsubstituted derivatives. Specifically, two of them showed scant activity, while others showed no activity in the cell growth inhibition assay against sixty different human cancer cell lines panel *in vitro*. From these data, we may conclude compounds **7d** and **7h** were the most effective molecules for anti-proliferative activity, specifically in non-small cell lung cancer and CNS cancer respectively, so they might be useful as leads for designing new compounds with potential antitumoral activity. This structure was derived from pyridazinone with hydrogen or methyl group in the 9-position linked to the 4-methylpiperazine moiety by a methylene spacer. The obtained results prove the necessity for further investigations to clarify the molecular mechanisms involved in antitumor activities to acquire more information about the structural requirements for enhancing anticancer activities and minimizing neurotoxicities, the synthesis of more new derivatives with different substituents at other positions is needed.

5. Experimental

5.1. General

Melting points were determined using a Reichert-Köfler hot-stage apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer Paragon 500 FT IR spectrophotometer (KBr pellets, in Nujol mulls, as well as in film). ^{1}H-NMR spectra were recorded on a Varian XL 200 FT

NMR spectrometer using $CDCl_3$ as solvent, unless otherwise specified. Chemical shifts are reported in δ or ppm and coupling constants (*J*) in Hertz (Hz), downfield from tetramethylsilane (TMS). Multiplicities are recorded as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Reactions were monitored by analytical thin-layer chromatography (TLC) using SiO_2 Polygram SIL and ALOX N/UV_{254} precoated plastic sheets and with visualization by irradiation with a UV lamp and/or iodine vapor for detection. Flash chromatography was performed using Merck silica gel type 60 (230-400 mesh ASTM). Electron ionization mass spectra (70 eV) were recorded on a Hewlett-Packard 5790-5970 MSD gas chromatograph/mass spectrometer. Atmospheric Pressure Ionization Electrospray (APIES) mass spectra, when reported, were obtained on a Agilent 1100 series LC/MSD spectrometer. All moisture sensitive reactions were performed under nitrogen atmosphere, using oven-dried glassware. Anhydrous DCM, THF and DMF was obtained from Aldrich, Lancaster or Merck. All starting materials and reagents were commercially available from Aldrich, Lancaster and Avocado. Evaporation was performed *in vacuo* (rotary evaporator). Anhydrous sodium or magnesium sulfate was always used as the drying agent. Elemental analyses were performed in a Perkin-Elmer 240C elemental analyzer, and the results were within ± 0.4% of the theoretical values, unless otherwise noted.

5.2. General procedure for the synthesis of 5-oxopentanoic acids **9a,b**

To a suspension of anhydrous $AlCl_3$ (17.54 mmol) in dry CH_2Cl_2 (20 mL) cooled with an ice bath, a solution of glutaric anhydride (19 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise under a N_2 atmosphere, and the whole mixture was stirred at RT for 0.5 h. Then a solution of thiophene **8a,b** (17 mmol) in dry CH_2Cl_2 (15 mL) was added dropwise and the reaction mixture stirred at the same temperature for an additional 0.5 h. The mixture was poured into crushed ice and conc. HCl was slowly added followed by warming until the suspended materials dissolved. The aqueous phase was separated and extracted with CH_2Cl_2. The combined organic phase was washed with H_2O and then extracted with 2N NaOH aqueous solution (5 × 7 mL): the solid separated upon acidification of the alkali layer, was filtered off and air dried to yield the desired product.

5-(Thiophen-2-yl)-5-oxopentanoic acid (**9a**): Yield 2.12 g (60%) as a cream solid: mp 93-94 °C; R_f: 0.48 ($CHCl_3$-MeOH 9:1); IR (Nujol, ν, cm^{-1}): 1696 (COOH), 1654 (CO); ^{1}H-NMR ($CDCl_3$), *δ* ppm: 7.74 (d, 1H, *J* = 4 Hz, CH), 7.64 (d, 1H, *J* = 5.2 Hz, CH), 7.15 (t, 1H, *J* = 3.6 Hz, CH), 3.04 (t, 2H, *J* = 7 Hz, CH_2), 2.53 (t, 2H, *J* = 7.4 Hz, CH_2), 2.09 (quint., 2H, *J* = 7 Hz, CH_2); GC-MS *m/z*: 198 (M^+); Calcd for $C_9H_{10}O_3S$: C, 54.53; H, 5.08; S, 16.17. Found: C, 54.42; H, 4.89; S, 16.08.

5-(5-Methyl-2-thienyl)-5-oxopentanoic acid (**9b**): Yield 2.07 g (60%) as a cream solid: mp 105-107 °C; R_f: 0.65 ($CHCl_3$-MeOH 9:1); IR (Nujol, ν, cm^{-1}): 1693 (COOH), 1650 (CO); ^{1}H-NMR ($CDCl_3$), *δ* ppm: 7.54 (d, 1H, *J* = 3.6 Hz, CH), 6.80 (d, 1H, *J* = 3 Hz, CH), 2.95 (t, 2H, *J* = 7.4 Hz, CH_2), 2.54 (s, 3H, CH_3), 2.5 (t, 2H, CH_2), 2.09 (qu, 2H, CH_2); GC-MS *m/z*: 212 (M^+); Calcd for $C_{10}H_{12}O_3S$: C, 56.58; H, 5.70; S, 15.11. Found: C, 56.49; H, 5.63; S, 15.21.

5.3. General procedure for the synthesis of pentanoic acids **10a,b**

A mixture of 5-oxopentanoic acid **9a,b** (2.00 g, 9.3 mmol), diethylene glycol (DEG, 24 mL), potassium hydroxide (0.035 mol) and hydrazine hydrate (0.045 mol) was refluxed with a Dean-Stark apparatus for 3 h. The solution, after cooling to RT, was poured into cold water (50 mL), washed with ether, acidified with 6 N HCl and then extracted with ether (4 × 5 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo* to give the title compounds.

5-(2-thienyl)pentanoic acid (**10a**): Yield 1.50 g (82%) as a yellow amber solid: mp 41 °C; R_f: 0.72 ($CHCl_3$-MeOH 9:1); IR (Nujol, ν, cm^{-1}): 1702 (COOH); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.11 (d, 1H, J = 4 Hz, CH), 6.93 (d, 1H, J = 4 Hz, CH), 6.79 (t, 1H, J = 3.8 Hz, CH), 2.77 (t, 2H, J = 6.6 Hz, CH_2), 2.41 (s, 2H, J = 6.8 Hz, CH_2), 1.74 (m, 4H, J = 3.6 Hz, $2CH_2$); GC-MS *m/z*: 184 (M^+); Calcd for $C_{10}H_{12}O_3S$: C, 58.67; H, 6.56; S, 17.14. Found: C, 58.59; H, 6.49; S, 17.23.

5-(5-Methyl-2-thienyl)pentanoic acid (**10b**): Yield 1.50 g (82%) as yellow amber solid: mp 47-49 °C; R_f: 0.72 ($CHCl_3$-MeOH 9:1); IR (Nujol, ν, cm^{-1}): 1693 (COOH); ^{1}H-NMR ($CDCl_3$), δ ppm: 6.54 (s, 2H, 2CH), 2.77 (t, 2H, CH_2), 2.43 (s, 3H, CH_3), 2.38 (m, 2H, CH_2), 1.70 (m, 4H, $2CH_2$); GC-MS *m/z*: 198 (M^+); Calcd for $C_{10}H_{12}O_3S$: C, 60.57; H, 7.12; S, 16.17. Found: C, 60.49; H, 7.06; S, 16.12.

5.4. General procedure for the synthesis of ketones **11a,b**

To a solution of pentanoic acid **10a,b** (13 mmol) in toluene (35 mL), were added Celite® (4.52 g) and phosphorus pentoxide (23 mmol). The mixture was refluxed for 2 h, then cooled and filtered. The filtrate was washed with 5% aqueous $NaHCO_3$ solution (2 × 5 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*, to give the ketones as oils.

5,6,7,8-Tetrahydro-4H-cyclohepta[b]thiophen-4-one (**11a**): Yield 0.78 g (59%) as a yellow brown oil; R_f: 0.87 ($CHCl_3$-MeOH 9:1); IR (film, ν, cm^{-1}): 1665 (CO). ^{1}H-NMR ($CDCl_3$), δ ppm: 7.33 (d, 1H, J = 5.4 Hz, CH-2), 6.91 (d, 1H, J = 5.4 Hz, CH-3), 3.03 (t, 2H, J = 5.2 Hz, CH_2-8), 2.65 (t, 2H, J = 6.8 Hz, CH_2-5), 2.39 (s, 3H, CH_3), 1.92-1.16 (m, 4H, $2CH_2$); GC-MS *m/z*: 166 (M^+); Calcd for $C_9H_{10}OS$: C, 65.02; H, 6.06; S, 19.29. Found: C, 65.08; H, 6.12; S, 19.21.

5,6,7,8-Tetrahydro-2-methyl-4H-cyclohepta[b]thiophen-4-one (**11b**): Yield 0.79 g (59%) of the compound **7** as yellow brown oil which was used for the next step without further purification. R_f: 0.88 ($CHCl_3$-MeOH 9:1); IR (film, ν, cm^{-1}): 1663 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.05 (d, 1H, CH), 3.02 (t, 2H, CH_2), 2.69 (t, 2H, CH_2), 2.39 (s, 3H, CH_3), 1.92 (m, 4H, $2CH_2$); GC-MS *m/z*: 180 (M^+); Calcd for $C_{10}H_{12}OS$: C, 66.63; H, 6.71; S, 17.79. Found: C, 66.57; H, 6.63; S, 17.71.

5.5. General procedure for the synthesis of Mannich bases **12a,b**

Acetic anhydride (39 mmol) was added dropwise to a solution of dimethylamine hydrochloride (10 mmol) and 37% formaldehyde (29 mmol) at 85-90 °C and the mixture was stirred for 0.5 h. Then tetrahydrocyclohepta[*b*]thiophen-4-one **11a,b** (7 mmol) was added to the mixture and the whole stirred

at 75 °C for 3h. After cooling, the mixture was evaporated under reduced pressure and the resulting crude residue was crystallized from acetone (**12a**) or triturated with diisopropyl ether (**12b**) to afford the desired product.

5,6,7,8-Tetrahydro-5-dimethylaminomethyl-4H-cyclohepta[b]thiophen-4-one·HCl (**12a**): Yield 0.76 g (42%) as a crystalline solid; mp 155 °C; R_f: 0.23 ($CHCl_3$-MeOH 9:1); IR (film, ν, cm^{-1}): 1650 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.37 (d, 1H, J = 5.4 Hz, CH), 7.04 (d, 1H, J = 5.4 Hz, CH), 4.02-3.59 (m, 2H, CH_2-N^+H $(CH_3)_2$), 3.30-3.00 (m, 3H, CH-5, CH_2-8), 2.77 (m, 6H, $2CH_3$). 2.40-1.52 (m, 4H, $2CH_2$); GC-MS *m/z*: 259 (M^+); Calcd for $C_{12}H_{18}ClNOS$; C, 55.48; H, 6.98; Cl, 13.65; S, 12.34. Found: C, 55.57; H, 6.92; Cl, 13.69; S, 12.39.

2-Methyl-5,6,7,8-tetrahydro-5-dimethylaminomethyl-4H-cyclohepta[b]thiophen-4one·HCl (**12b**): Yield 0.14 g (51%) as a crystalline solid: mp 156 °C; R_f: 0.72 ($CHCl_3$-MeOH 9:1); IR (Nujol), ν, cm^{-1}): 1654 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 6.96 (s, 1H, CH), 3.05 (t, 2H, CH_2), 2.64 (t, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.02 (m, 4H, $2CH_2$); GC-MS *m/z*: ND (M^+); Calcd for $C_{13}H_{20}ClNOS$: C, 57.02; H, 7.36; Cl, 12.95; N, 5.12; S, 11.71. Found: C, 57.10; H, 7.39; S, 11.65.

5.6. General procedure for the synthesis of nitriles **13a,b**

To a solution of the Mannich base **12a,b** (4 mmol) in methanol (8 ml), an aqueous solution of NaCN (22 mmol, 10 ml) was dropwise added, at RT, and the mixture was stirred at 55 °C for 4 h, then poured onto cold H_2O and afterwards extracted with CH_2Cl_2 (3 × 5 mL). The resulting organic layer was washed with H_2O, brine, dried (Na_2SO_4), filtered and evaporated *in vacuo*.

5,6,7,8-Tetrahydro-5-cianomethyl-4H-cyclohepta[b]thiophen-4-one (**13a**): Yield 0.62 g (75%) as a dark oil; R_f: 0.86 ($CHCl_3$-MeOH 9:1); IR (film, ν, cm^{-1}): 1660 (CO), 2246 (CN); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.43 (d, 1H, J = 5.4 Hz, CH), 7.03 (d, 1H, J = 4.8 Hz, CH), 3.38-2.80 (m, 4H, $2CH_2$), 2.7-2.5 (m, H, CH-5), 2.24-1.68 (m, 4H, $2CH_3$); GC-MS *m/z*: 205 (M^+); Calcd for $C_{11}H_{11}NOS$: C, 64.36; H, 5.44; N, 6.82; S, 15.62. Found: C, 64.32; H, 5.49; N, 6.91; S, 11.69.

2-Methyl-5,6,7,8-Tetrahydro-5-cianomethyl-4H-cyclohepta[b]thiophen-4-one (**13b**): Yield 0.49 g (47%) as an amorphous dark solid: mp 77-78 °C; R_f: 0.90 ($CHCl_3$-MeOH 9:1); IR (Nujol, ν, cm^{-1}): 1645 (CO), 2237 (CN); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.01 (s, 1H, CH), 3.07 (t, 2H, CH_2), 2.7 (m, 2H, CH_2), 2.40 (s, 3H, CH_3), 2.26 (m, 4H, $2CH_2$); GC-MS *m/z*: 219 (M^+); Calcd for $C_{12}H_{13}NOS$: C, 65.72; H, 5.97; N, 6.39; S, 14.62. Found: C, 65.72; H, 5.93; S, 14.67.

5.7. General procedure for the synthesis of acids **14a,b**

To a solution of nitrile **13a,b** (3.5 mmol) in AcOH (3.6 ml), HCl conc. (2.5 ml) was dropwise added at RT, then the reaction mixture was refluxed for 3 h (TLC). After cooling to RT, the mixture was diluted with cold H_2O and afterwards extracted with CH_2Cl_2 (4 × 5 mL). The resulting organic layer was washed with H_2O, brine, dried (Na_2SO_4), filtered and evaporated *in vacuo*.

4-Oxo-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophen-5-acetic acid (**14a**): Yield 0.67 g (85%) as a brown dark solid: mp145-147 °C; R_f: 0.53 ($CHCl_3$-MeOH 9:1); IR (Nujol, ν, cm^{-1}): 1660 (CO), 1707 (COOH); ^{1}H-NMR ($CDCl_3$), δ ppm: 8.27 (bs, 1H, COOH, exchanged with D_2O), 7.33 (d, 1H, J = 5.2 Hz, CH), 6.92 (d, 1H, J = 5.4 Hz, CH), 3.36 (m, 5H, $2CH_2$, CH-5), 2.63-1.57 (m, 4H, $2CH_2$); GC-MS *m/z*: 224 (M^+); Calcd for $C_{11}H_{12}O_3S$: C, 58.91; H, 5.38; S, 14.30. Found: C, 58.97; H, 5.43; S, 14.38.

2-Methyl-4-oxo-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophen-5-acetic acid (**14b**): Yield 0.88 g (82%) as a coffee-black solid: mp 145-147 °C. R_f: 0.68 ($CHCl_3$-MeOH 9:1); IR (Nujol, ν, cm^{-1}): 1660 (CO), 1708 (COOH); ^{1}H-NMR ($CDCl_3$), δ ppm: 8.27(bs, 1H, COOH, exchanged with D_2O), 7.01 (s, 1H, CH), 3.07 (t, 2H, CH_2), 2.7 (m, 2H, CH_2), 2.40 (s, 3H, CH_3), 2.26 (m, 4H, $2CH_2$); GC-MS *m/z*: 239 (M^+); Calcd for $C_{12}H_{14}O_3S$: C, 60.48; H, 5.92; S, 13.46. Found: C, 60.56; H, 5.98; S, 13.52.

5.8. General procedure for the synthesis of pyridazinones **15a,b**

To the solution of acid **14a,b** (3 mmol) in anhydrous EtOH (10 mL), $H_2NNH_2 \cdot H_2O$ 80% (3 mmol) was added dropwise and the resulting mixture was refluxed for 3 h. After cooling at room temperature, the solvent was evaporated *in vacuo.*

2,4,4a,5,6,7-Hexahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]pyridazin-3-one (**15a**): Yield 0.59 g (87%) as a brown dark solid: mp 147-148 °C; R_f: 0.66 ($CHCl_3$-MeOH 9:1); IR (KBr, ν, cm^{-1}): 1674 (CO), 3173 (NH); ^{1}H-NMR ($CDCl_3$), δ ppm: 8.89 (s, 1H, NH, exchanged with D_2O), 7.26 (d, 1H, J = 4.2 Hz, CH), 7.04 (d, 1H, J = 5.4 Hz, CH), 3.10-2.6 (m, 4H, $2CH_2$), 2.70-2.20 (m, 3H, CH_2, CH), 2.43-2.213(m, 2H, CH_2-6), 2.17-1.63 (m, 3H, CH, CH_2); GC-MS *m/z*: 220 (M^+); Calcd for $C_{11}H_{12}N_2OS$: C, 59.97; H, 5.49; N, 12.72; S, 14.56. Found: C, 59.92; H, 5.43; N, 12.67; S, 14.48.

9-Methyl-2,4,4a,5,6,7-hexahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]pyridazin-3-one (**15b**): Yield 0.78 g (85%) as a colorless solid scales: mp 179-182 °C; R_f: 0.68 ($CHCl_3$-MeOH 9:1); IR (KBr, ν, cm^{-1}): 1680 (CO), 3175 (NH); ^{1}H-NMR ($CDCl_3$), δ ppm: 8.39 (s, 1H, NH, exchanged with D_2O), 3.10-2.82 (m, 4H, $2CH_2$), 2.70-2.20 (m, 3H, CH_2, CH), 2.40 (s, 3H, CH_3), 1.80-2.05 (m, 2H, CH_2); GC-MS *m/z*: 234 (M^+); Calcd for $C_{12}H_{14}N_2OS$: C, 61.51; H, 6.02; N, 11.96; S, 13.69. Found: C, 61.45; H, 6.07; N, 11.91; S, 13.63.

5.9. General procedure for the synthesis of 2-N-substituted-2,4,4a,5,6,7-hexahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]pyridazin-3-one derivatives **7a-l**

To a solution of pyridazinone (0.91 mmol) in anhydrous ethanol (5 mL), 37% formaldehyde (11 mmol) and appropriate amines (2 mmol) were added and the mixture was refluxed under nitrogen atmosphere for 8 h. The reaction mixture was cooled to RT, then the solvent was evaporated under reduced pressure and the residue was taken-up in water and extracted with chloroform (4 × 5 mL). The combined organic phase was dried (Na_2SO_4), filtered and concentrated *in vacuo*, to give a crude oil which was purified by flash chromatography (FC).

2-N-[(4-N-Phenylpiperazin-1-yl)methyl]-2,4,4a,5,6,7-hexahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]pyridazin-3-one (**7a**): FC: petroleum ether/EtOAc 6.5:3.5; Yield 0.19 g (52%) as a beige solid with mp 121-123 °C; R_f: 0.35 (petroleum ether/EtOAc 6.5:3.5); IR: (KBr, ν, cm^{-1}): 1661 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.28 (d, 1H, J = 5.8 Hz, CH), 7.05 (d, 1H, J = 5.6 Hz, CH), 7.00-6.80 (m, 5H, Ar-H),4.84 (dd, 2H, J = 13.2 Hz, 2H, CH_2), 3.38-3.12 (m, 4H, $ArN(CH_2)_2$,), 3.07-2.63 (m, 6H, , 2H, CH_2-7, 4H, $N(CH_2)_2$), 2.78-2.60 (m, 2H, CH_2-4), 2.50-2.30 (m, 2H, CH_2-6), 2.06-1.70 (m, 3H, CH-4a, CH_2-5); GC-MS *m/z*: 395 (M^+); Calcd for $C_{22}H_{26}N_4OS$: C, 66.97; H, 6.64; N, 14.20; S, 8.13. Found: C, 66.85; H, 6.53; N, 13.99; S, 8.02.

2-N-[(4-(o-Methoxy-phenylpiperazin-1-yl)methyl]-2,4,4a,5,6,7-hexahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]pyridazin-3-one (**7b**): FC: petroleum ether/EtOAc 6.5:3.5; Yield 0.186 g (48%) as a glassy solid with mp 50-51 °C; R_f: 0.23 (petroleum ether/EtOAc 6.5:3.5); IR (Nujol, ν, cm^{-1}): 1669 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.20 (d, 1H, J = 5.4 Hz, CH-9), 6.94 (d, 1H, J = 5.4 Hz, CH-10), 6.90-6.70 (m, 4H, Ar-H), 4.75 (dd, 2H, CH_2), 3.74 (s, 3H, OCH_3), 3.15-2.75 (m, 10H, $5CH_2$, Ar-$N(CH_2)_2$, CH_2, $N(CH_2)_2$; 2,70-2.20 (m, 4H, $2CH_2$), 2.00-1.60 (m, 3H, CH-4a, CH_2-5); GC-MS *m/z*: 425 (M^+); Anal. Calcd for $C_{23}H_{28}N_4O_2S$: C, 65.07; H, 6.65; N, 13.20; S, 7.55. Found: C, 64.86; H, 6.53; N, 13.09; S, 7.43.

2-N-[(4-(o-Fluoro-phenylpiperazin-1-yl)methyl]-2,4,4a,5,6,7-hexahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]pyridazin-3-one (**7c**): FC: petroleum ether/EtOAc 6.5:3.5; Yield 0.217 g (58%) as a white solid with mp 170 °C; R_f: 0.32 (petroleum ether/EtOAc 6.5:3.5); IR (Nujol, ν, cm^{-1}): 1671 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.28 (d, 1H, J = 5.6 Hz, CH), 7.04 (d, 1H, J=5.6 Hz, CH), 7.13-6.86 (m, 4H, Ar-H), 4.82 (dd, 2H, J = 13 Hz, CH_2), 3.20-2.85 (m, 10H, $ArN(CH_2)_2$, CH_2, $N(CH_2)_2$), 2.76-2.30 (m, 4H, $2CH_2$), 2.05-1.70 (m, 7H, 3H, CH_2, CH); GC-MS *m/z*: 413 (M^+); Calcd for $C_{22}H_{25}F\ N_4OS$: C, 64.05; H, 6.11; F, 4.61; N, 13.58; S, 7.77. Found: C, 64.17; H, 6.03; N, 13.49; S, 7.65.

2-N-[(4-(o-Methylpiperazin-1-yl)methy]-2,4,4a,5,6,7-hexahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]pyridazin-3-one (**7d**): FC: petroleum ether/EtOAc 6.5:3.5; Yield 0.020 g (5.3%) as a brown solid with mp 118 °C; R_f: 0.32 (petroleum ether/EtOAc 6.5:3.5); IR (Nujol, ν, cm^{-1}): 1673 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.29 (d, 1H, J = 5.4 Hz, CH-9), 7.05 (d, 1H, J = 5.2 Hz, CH-10), 5.26 (m, 2H, CH_2-a), 3.10-2.86 (m, 6H, CH_2, $N(CH_2)_4$), 2.68-1.72 (m. 14H, $N(CH_2)_2$, $3CH_2$, CH_3,CH); GC-MS *m/z*: 332 (M^+); Calcd for $C_{17}H_{24}N_4OS$: C, 61.41; H, 7.28; N, 16.85; S, 9.64. Found: C, 61.39; H, 7.13; N, 16.73; S, 9.48.

9-Methyl-2-N-[(4-N-phenylpiperazin)methyl]-2,4,4a,5,6,7-hexahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]pyridazin-3-one (**7e**): FC: petroleum ether/acetone 6.5:3.5; Yield 0.070 g (20%) as a beige solid with mp 51-52 °C; R_f: 0.42 (petroleum ether/EtOAc 6.5:3.5); IR (Nujol, ν, cm^{-1}): 1671 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.35-6.80 (m, 6H, 5H, Ar, 1H, CH-10), 4.81 (dd, 2H, J = 13,2 Hz, 2H, CH_2-a), 3.35-3.08 (t, 4H, $ArN(CH_2)_2$,), 3.00-2.80 (m, 6H, 4H, $N(CH_2)_2$, 2H, CH_2), 2.75-1.67 (m, 10H, 6H, CH_2, 1H, CH_3, 1H, CH); GC-MS *m/z*: 408 (M^+); Calcd for $C_{23}H_{28}N_4OS$: C, 67.61; H, 6.91; N, 13.71; S, 7.85. Found: C, 67.52; H, 6.56; N, 13.58; S, 7.79.

9-Methyl-2-N-[(4-(o-methoxy-phenylpiperazin-1-yl)methyl]-2,4,4a,5,6,7-hexahydro-3H-thieno [2',3':6,7]cyclohepta[1,2-c]pyridazin-3-one (**7f**): FC: petroleum ether/EtOAc 6.5:3.5; Yield 0.090 g (24%) as a glassy solid with mp 51-53 °C; R_f: 0.23 (petroleum ether/EtOAc 6.5:3.5); IR (KBr, ν, cm^{-1}): 1671 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 6.97-6.65 (m, 5H, 4H, Ar, 1H, CH-10), 4.73 (dd, 2H, CH_2), 3.74 (s, 3H, OCH_3), 3.12-2.72 (m, 10H, 4H, $ArN(CH_2)_2$, 2H, CH_2, 4H, $N(CH_2)_2$; 2,38 (s, 3H, CH_3), 2.62-1.62 (m, 7H, 6H, CH_2, 1H, CH); GC-MS *m/z*: 439 (M^+); Calcd for $C_{24}H_{30}N_4O_2S$: C, 65.72; H, 6.89; N, 12.77; S, 7.31. Found: C, 65.79; H, 6.78; N, 12.65; S, 7.43.

9-Methyl-2-N-[(4-(o-fluoro-phenylpiperazin-1-yl)methyl]-2,4,4a,5,6,7-hexahydro-3H-thieno [2',3':6,7]cyclohepta[1,2-c]pyridazin-3-one (**7g**): FC: petroleum ether/EtOAc 6.5:3.5; Yield 0.217 g (47%) as a white solid with mp 138-140 °C; R_f: 0.29 (petroleum ether/EtOAc 6.5:3.5); IR (KBr, ν, cm^{-1}): 1665 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.23-6.86 (m, 6H, 4H, Ar, 1H,CH-10), 4.81 (dd, 2H, CH_2), 3.18-3.02 (t, 4H, $ArN(CH_2)_2$, 2.97-2.02 m, 6H, 4H, $N(CH_2)_2$, 2H, CH_2), 2.41 (s, 3H, CH_3),2.75-2.72 (m, 7H, 6H, CH_2, 1H, CH); GC-MS *m/z*: 426 (M^+); Calcd for $C_{23}H_{27}F\ N_4OS$: C, 64.76; H, 6.38; F, 4.45; N, 13.13; S, 7.52. Found: C, 64.67; H, 6.25; F, 4.38; N, 13.19; S, 7.63.

9-Methyl-2-N-[(4-methylpiperazin-1-yl)methy]-2,4,4a,5,6,7-hexahydro-3H-tieno[2',3':6,7] cyclohepta [1,2-c]pyridazin-3-one (**7h**): FC: petroleum ether/EtOAc 6.5:3.5; Yield 0.012 g (5%) as a brown solid with mp 136-137 °C; R_f: 0.46 (ether); IR (Nujol, ν, cm^{-1}): 1667 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 6.97 (s, 1H, CH), 5.24 (dd, 2H, CH_2), 3.15-2.84 (m, 6H, $3CH_2$), 2.41 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.73-2.22 (m, 6H, $3CH_2$), 2.10-1.72 (m, 5H, $2CH_2$, CH); GC-MS *m/z*: 347 (M^+); Anal. Calcd for $C_{18}H_{26}N_4OS$: C, 62.40; H, 7.56; N, 16.17; S, 9.25. Found: C, 62.35; H, 7.48; N, 16.24; S, 9.36.

2-N-[(4-Methylpiperidin-1-yl)methy]-2,4,4a,5,6,7-hexahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c]-pyridazin-3-one (**7i**): FC: petroleum ether/EtOAc 6.5:3.5; Yield 0.17 g (45%) as a white solid with mp 87 °C; R_f: 0.33 (petroleum ether/EtOAc 6.5:3.5); IR (Nujol, ν, cm^{-1}): 1659 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.2 (d, 1H, J = 5.0 Hz, CH-9), 7.3 (d, 1H, J = 5.2 Hz, CH-10), 4.74 (dd, 2H, J = 12.8 Hz, CH_2), 3.13-2.25 (m, 8H, CH_2-7, $N(CH_2)_2$, CH_2-4), 2.05-1.18 (m, 10H, $4CH_2$, 2CH), 0.91 (d, 3H, CH_3); GC-MS *m/z*: 331 (M^+); Calcd for $C_{18}H_{25}N_3OS$: C, 65.22; H, 7.60; N, 12.68; S, 9.67. Found: C, 65.15; H, 7.48; N, 12.57; S, 9.49.

9-Methyl-2-N-[(4-methylpiperidin-1-yl)methy]-2,4,4a,5,6,7-hexahydro-3H-thieno[2',3':6,7]cyclohepta [1,2-c]pyridazin-3-one (**7j**): FC: petroleum ether/ethyl acetate 6.5:3.5; Yield 0.190 g (51%) as a white solid with mp 133-135 °C; R_f: 0.42 (petroleum ether/EtOAc 6:4); IR (Nujol, ν, cm^{-1}): 1667 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 6.92 (s, 1H, CH),4.73 (dd, 2H, J = *15.8* Hz, CH_2), 3.12-2.25 (m, 8H, $4CH_2$), 2.41 (s, 3H, CH_3), 2.05-1.18 (m, 10H, $4CH_2$, 2CH), 0.91 (d, 3H, CH_3); GC-MS *m/z*: 345 (M^+); Calcd for $C_{19}H_{27}N_3OS$: C, 66.05; H, 7.88; N, 12.16; S, 9.28. Found: C, 66.15; H, 7.78; N, 12.08; S, 9.36.

2-N-[(4-Morpholine-1-yl)methyl]-2,4,4a,5,6,7-hexahydro-3H-thieno[2',3':6,7]cyclohepta[1,2-c] pyridazin-3-one (**7k**): FC: petroleum ether/ acetone 6.5:3.5; Yield 0.23 g (78%) as a brown solid with mp 80 °C; R_f: 0.44 (petroleum ether/acetone 7:3); IR (Nujol, ν, cm^{-1}): 1666 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 7.25 (d, 1H, J = 4.8 Hz, CH-9), 7.04 (d, 1H, J = 5Hz, CH-10), 4.73 (dd, 2H, J = 13.2 Hz, CH_2),

3.7 (t, 4H, J = 4.4 Hz, $O(CH_2)_2$), 3.10 (m, 11H, CH_2-7, $N(CH_2)_2$, CH_2-4, CH_2-6, CH-4a), 2.00-1.75 (m, 2H, CH_2-5); GC-MS *m/z*: 319 (M^+); Calcd for $C_{16}H_{21}N_3O_2S$: C, 60.16; H, 6.63; N, 13.16; S, 10.04. Found: C, 60.02; H, 6.56; N, 13.02; S, 9.89.

9-Methyl-2-[(4-morpholine-1-yl)methyl]-2,4,4a,5,6,7-hexahydro-3H-thieno[2',3':6,7] cyclohepta[1,2-c]pyridazin-3-one (**7l**): FC: petroleum ether/acetone 6.5:3.5; Yield 0.180 g (46%) as a white solid with mp 108-110 °C; R_f: 0.46 (petroleum ether/acetone 7:3); IR (KBr, ν, cm^{-1}): 1671 (CO); ^{1}H-NMR ($CDCl_3$), δ ppm: 6.90 (s, 1H, CH), 4.72 (dd, 2H, J = 14.6 Hz, CH_2), 3.69 (t, 4H, J = 4.4 Hz, $2CH_2$), 2.91 (t, 2H, J = 6.6 Hz, CH_2), 2.71 (t, 4H, J = 4.2 Hz, $2CH_2$), 2.41 (s, 3H, CH_3), 2.64- 1.78 (m, 7H, $3CH_2$ CH); GC-MS *m/z*: 333 (M^+); Calcd for $C_{17}H_{23}N_3O_2S$: C, 61.23; H, 6.95; N, 12.60; S, 9.62. Found: C, 61.15; H, 6.84; N, 12.54; S, 9.51.

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Sample Availability: Samples of the compounds **7g** and **7l** are available from the authors.