Abstract: Designed as a new group of tricyclic molecules containing the thienocycloheptapyridazinone ring system, a number of 2N-substituted-hexahydrothienocycloheptapyridazinone 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

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 marrow, 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-dihydroinden[1,2-b]pyrroles (1) [5], 1H-benzo[g]indoles (2) [5], thieno[3,2-g]indoles (3) [14], 1,4,5,6-tetrahydrobenzo[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\(^\prime\),3\(^\prime\):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.

|    | a | b | c | d | e | f | g | h | i | j | k | l |
|----|---|---|---|---|---|---|---|---|---|---|---|---|
| R  | H | H | H | H | CH\(_3\) | CH\(_3\) | CH\(_3\) | H | CH\(_3\) | H | CH\(_3\) |
| X  | 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\(^\prime\),3\(^\prime\):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\(_2\)O\(_5\) over Celite\(^\circledR\) gave the ketones 11a,b. A Mannich reaction furnished 12a,b, which were converted with NaCN in CH\(_3\)OH into the nitriles 13a,b. Hydrolysis of these nitriles in refluxing HCl/AcOH led to the \(\gamma\)-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) AlCl₃, CH₂Cl₂, glutaric anhydride, RT, 0.5h; b) DEG, KOH, H₂NNH₂·H₂O, reflux, 3h; c) Toluene, Celite®, P₂O₅, reflux, 2h; d) HN(CH₃)₂·HCl, CH₂O, Ac₂O, 75 °C, 3h; e) MeOH, NaCN, 55 °C, 4h; f) AcOH, HCl 37%, reflux, 3h; g) EtOH an., H₂NNH₂·H₂O, reflux, 3h; h) EtOH an., CH₂O, amine, N₂, 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 (GI₅₀) 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 | 7c | 7d | 7e | 7f | 7g | 7h | 7i | 7j | 7k | 7l |
|-----------------------------------|-----------|----|----|----|----|----|----|----|----|----|----|
| **Non-small cell lung cancer**    |           |    |    |    |    |    |    |    |    |    |    |
| Mean growth %                     |           | 97.85 | **98.09** | 103.17 | 101.55 | 101.33 | 101.88 | 96.66 | 102.97 |
| Range of growth %                 |           | 81.03 to 72.06 to 78.91 | 92.87 to 80.20 | 81.70 to 75.17 | 93.09 to 102.97 |
| Colon cancer                      |           | 107.29 | 105.03 | 107.91 | 105.20 | 104.88 | 104.37 | 101.79 | 123.28 |
| Mean growth %                     |           | 92.60 to 91.34 to 96.71 | 91.23 to 88.76 | 91.22 to 94.33 | 87.28 to 108.32 |
| Range of growth %                 |           | 117.52 | 111.60 | 119.39 | 123.28 | 117.4 | 114.76 | 114.38 | 110.16 |
| **Breast Cancer**                 |           | 103.25 | 100.59 | 107.91 | 105.20 | 104.68 | 104.91 | 101.79 | 108.39 |
| Mean growth %                     |           | 92.60 to 91.34 to 96.71 | 91.23 to 88.76 | 91.22 to 94.33 | 87.28 to 108.32 |
| Range of growth %                 |           | 111.88 | 108.92 | 119.38 | 120.49 | 110.96 | 117.11 | 117.79 | 122.92 |
| **Leukemia**                      |           | 100.46 | **92.31** | 98.55 | 102.95 | 94.13 | 100.01 | 86.67 | 98.54 |
| Mean growth %                     |           | 91.68 to 81.94 to 91.36 | 85.24 to 80.99 | 90.34 to 76.07 | 75.05 to 100.01 |
| Range of growth %                 |           | 107.61 | 113.17 | 111.53 | 120.85 | 112.16 | 94.26 | 94.35 | 112.55 |
| **Renal Cancer**                  |           | 101.77 | 101.13 | 103.67 | **95.02** | 103.58 | 100.19 | 104.36 | 101.80 |
| Mean growth %                     |           | 93.09 to 95.38 to 94.16 | 77.38 to 94.14 | 86.51 to 93.91 | 85.76 to 93.91 |
| Range of growth %                 |           | 110.89 | 108.40 | 114.16 | 114.45 | to113.75 | 121.05 | 94.26 | 112.31 |
| **Melanoma**                      |           | 106.50 | 102.46 | 106.62 | 101.06 | 102.95 | 107.10 | 101.57 | 104.80 |
| Mean growth %                     |           | 100.28 | 88.88 to 101.92 | 92.73 to 87.54 | 92.35 to 91.52 | 93.82 to 93.82 |
| Range of growth %                 |           | to110.39 | **111.60** | **116.2** | 112.29 | to119.80 | 124.26 | 114.53 | 112.20 |
| **Prostate Cancer**               |           | 102.25 | 100.46 | 114.38 | 109.08 | 101.76 | 99.92 | 103.85 | 109.31 |
| Mean growth %                     |           | 98.48 to 93.16 to 108.08 | 104.25 to 99.78 | 96.26 to 102.00 | 102.40 to 116.22 |
| Range of growth %                 |           | 106.02 | 107.76 | 113.91 | 103.76 | 103.58 | 105.70 | 120.68 |
| **CNS Cancer**                    |           | 99.49 | 100.85 | 100.78 | **82.03** | 111.34 | 98.38 | 107.23 | 97.20 |
| Mean growth %                     |           | 91.08 to 77.31 to 89.96 | 72.14 to 80.17 | 85.30 to 81.54 | 82.07 to 97.20 |
| Range of growth %                 |           | 108.91 | 131.16 | 110.76 | 115.77 | 194.64 | 105.97 | 164.17 | 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 | 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). 1H-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/UV254 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$_2$Cl$_2$ (20 mL) cooled with an ice bath, a solution of glutaric anhydride (19 mmol) in dry CH$_2$Cl$_2$ (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$_2$Cl$_2$ (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$_2$Cl$_2$. The combined organic phase was washed with H$_2$O 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$_9$H$_{10}$O$_3$S: 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$_3$S: 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 (CHCl₃-MeOH 9:1); IR (Nujol, \( \nu, \text{ cm}^{-1} \)) 1702 (COOH); \(^1\)H-NMR (CDCl₃, \( \delta \) 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, CH₂), 2.41 (s, 2H, \( J = 6.8 \) Hz, CH₂), 1.74 (m, 4H, \( J = 3.6 \) Hz, 2CH₂); GC-MS \( m/z \): 184 (M⁺); Calcd for C₁₀H₁₂O₃S: 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 (CHCl₃-MeOH 9:1); IR (Nujol, \( \nu, \text{ cm}^{-1} \)) 1693 (COOH); \(^1\)H-NMR (CDCl₃, \( \delta \) ppm): 6.54 (s, 2H, 2CH), 2.77 (t, 2H, CH₂), 2.43 (s, 3H, CH₃), 2.38 (m, 2H, CH₂), 2.38 (m, 2H, CH₂), 1.70 (m, 4H, 2CH₂); GC-MS \( m/z \): 198 (M⁺); Calcd for C₁₀H₁₂O₃S: 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 NaHCO₃ solution (2 × 5 mL), dried (Na₂SO₄), 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 (CHCl₃-MeOH 9:1); IR (film, \( \nu, \text{ cm}^{-1} \)): 1665 (CO). \(^1\)H-NMR (CDCl₃, \( \delta \) 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, CH₂-8), 2.65 (t, 2H, \( J = 6.8 \) Hz, CH₂-5), 2.39 (s, 3H, CH₃), 1.92-1.16 (m, 4H, 2CH₂); GC-MS \( m/z \): 166 (M⁺); Calcd for C₉H₁₀OS: 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 (CHCl₃-MeOH 9:1); IR (film, \( \nu, \text{ cm}^{-1} \)): 1663 (CO); \(^1\)H-NMR (CDCl₃, \( \delta \) ppm): 7.05 (d, 1H, CH), 3.02 (t, 2H, CH₂), 2.69 (t, 2H, CH₂), 2.39 (s, 3H, CH₃), 1.92-1.16 (m, 4H, 2CH₂); GC-MS \( m/z \): 180 (M⁺); Calcd for C₁₀H₁₂OS: 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; Rf: 0.23 (CHCl3-MeOH 9:1); IR (film, ν, cm\(^{-1}\)): 1650 (CO); 1H-NMR (CDCl3), 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, CH2-N⁺CH3), 3.30-3.00 (m, 3H, CH-5, CH2-8), 2.77 (m, 6H, 2CH3). 2.40-1.52 (m, 4H, 2CH2); GC-MS m/z: 259 (M⁺); Calcd for C12H18ClN OS: 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-4-one HCl (12b): Yield 0.14 g (51%) as a crystalline solid: mp 156 °C; Rf: 0.72 (CHCl3-MeOH 9:1); IR (Nujol), ν, cm\(^{-1}\): 1654 (CO); 1H-NMR (CDCl3), δ ppm: 6.96 (s, 1H, CH), 3.05 (t, 2H, CH2), 2.64 (t, 2H, CH2), 2.32 (s, 3H, CH3), 2.02 (m, 4H, 2CH2); GC-MS m/z: ND (M⁺); Calcd for C13H20ClNOS: 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 H2O and afterwards extracted with CH2Cl2 (3 × 5 mL). The resulting organic layer was washed with H2O, brine, dried (Na2SO4), 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; Rf: 0.86 (CHCl3-MeOH 9:1); IR (film, ν, cm\(^{-1}\)): 1660 (CO), 2246 (CN); 1H-NMR (CDCl3), 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, 2CH2), 2.7-2.5 (m, H, CH-5), 2.24-1.68 (m, 4H, 2CH2); GC-MS m/z: 205 (M⁺); Calcd for C11H11NOS: C, 64.36; H, 5.44; N, 6.82; S, 11.62. 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; Rf: 0.90 (CHCl3-MeOH 9:1); IR (Nujol, ν, cm\(^{-1}\)): 1645 (CO), 2237 (CN); 1H-NMR (CDCl3), δ ppm: 7.01 (s, 1H, CH), 3.07 (t, 2H, CH2), 2.7 (m, 2H, CH2), 2.40 (s, 3H, CH3), 2.26 (m, 4H, 2CH2); GC-MS m/z: 219 (M⁺); Calcd for C12H13NOS: 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 H2O and afterwards extracted with CH2Cl2 (4 × 5 mL). The resulting organic layer was washed with H2O, brine, dried (Na2SO4), 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: mp 145-147 °C; \( R_f \): 0.53 (CHCl₃-MeOH 9:1); IR (Nujol, \( \nu \), cm⁻¹): 1660 (CO), 1707 (COOH); \(^1\)H-NMR (CDCl₃), \( \delta \) ppm: 8.27 (bs, 1H, COOH, exchanged with D₂O), 7.33 (d, 1H, \( J = 5.2 \) Hz, CH), 6.92 (d, 1H, \( J = 5.4 \) Hz, CH), 3.36 (m, 5H, 2CH₂, CH-5), 2.63-1.57 (m, 4H, 2CH₂); GC-MS \( m/z \): 224 (M⁺); Calcd for C₁₁H₁₂O₃S: 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 (CHCl₃-MeOH 9:1); IR (Nujol, \( \nu \), cm⁻¹): 1660 (CO), 1708 (COOH); \(^1\)H-NMR (CDCl₃), \( \delta \) ppm: 8.27 (bs, 1H, COOH, exchanged with D₂O), 7.01 (s, 1H, CH), 3.07 (t, 2H, CH₂), 2.70 (m, 2H, CH₂), 2.40 (s, 3H, CH₃), 2.26 (m, 4H, 2CH₂); GC-MS \( m/z \): 239 (M⁺); Calcd for C₁₂H₁₄O₃S: C, 60.48; H, 5.92; S, 13.46. Found: C, 60.56; H, 5.98; S, 13.52.

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

To the solution of acid 14a,b (3 mmol) in anhydrous EtOH (10 mL), H₂NNH₂·H₂O 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 (CHCl₃-MeOH 9:1); IR (KBr, \( \nu \), cm⁻¹): 1674 (CO), 3173 (NH); \(^1\)H-NMR (CDCl₃), \( \delta \) ppm: 8.89 (s, 1H, NH, exchanged with D₂O), 7.26 (d, 1H, \( J = 4.2 \) Hz, CH), 7.04 (d, 1H, \( J = 5.4 \) Hz, CH), 3.10-2.6 (m, 4H, 2CH₂), 2.70-2.20 (m, 3H, CH₂, CH), 2.43-2.13 (m, 2H, CH₂-6), 2.17-1.63 (m, 3H, CH, CH₂), 2.17-1.63 (m, 3H, CH, CH₂); GC-MS \( m/z \): 220 (M⁺); Calcd for C₁₁H₁₂N₂OS: 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 (CHCl₃-MeOH 9:1); IR (KBr, \( \nu \), cm⁻¹): 1680 (CO), 3175 (NH); \(^1\)H-NMR (CDCl₃), \( \delta \) ppm: 8.39 (s, 1H, NH, exchanged with D₂O), 3.10-2.82 (m, 4H, 2CH₂), 2.70-2.20 (m, 3H, CH₂, CH), 2.40 (s, 3H, CH₃), 1.80-2.05 (m, 2H, CH₂); GC-MS \( m/z \): 234 (M⁺); Calcd for C₁₂H₁₄N₂OS: 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 (Na₂SO₄), 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; Rf: 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, 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\(_4\)OS: 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; Rf: 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\(_4\)O\(_2\)S: 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; Rf: 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-9), 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\(_2\)), GC-MS m/z: 413 (M\(^{+}\)); Calcd for C\(_{22}\)H\(_{25}\)F\(_2\)N\(_4\)OS: 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)methyl]-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; Rf: 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\(_3\)), GC-MS m/z: 332 (M\(^{+}\)); Calcd for C\(_{17}\)H\(_{24}\)N\(_4\)OS: C, 61.41; H, 6.03; N, 13.49; 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; Rf: 0.42 (petroleum ether/EtOAc 6.5:3.5); IR (Nujol, ν, cm\(^{-1}\)): 1671 (CO); \(^1\)H-NMR (CDCl\(_3\)), δ ppm: 7.29-6.80 (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\(_3\), 1H, CH\(_3\), 1H, CH); GC-MS m/z: 408 (M\(^{+}\)); Calcd for C\(_{23}\)H\(_{28}\)N\(_4\)OS: 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, \( \nu \), cm\(^{-1}\)): 1671 (CO); \( ^1\)H-NMR (CDCl\(_3\)), \( \delta \) 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\(_4\)O\(_2\)S: 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, \( \nu \), cm\(^{-1}\)): 1665 (CO); \( ^1\)H-NMR (CDCl\(_3\)), \( \delta \) ppm: 7.23-6.86 (m, 6H, 4H, Ar, 1H, CH-10), 4.81 (dd, 2H, CH\(_2\)), 3.74 (s, 3H, OCH\(_3\)), 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\(_4\)OS: 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)methyl]-2,4,4a,5,6,7-hexahydro-3H-thieno[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, \( \nu \), cm\(^{-1}\)): 1667 (CO); \( ^1\)H-NMR (CDCl\(_3\)), \( \delta \) 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\(_3\)); GC-MS \( m/z \): 347 (M\(^+\)); Anal. Calcd for C\(_{18}\)H\(_{26}\)N\(_4\)OS: 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)methyl]-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, \( \nu \), cm\(^{-1}\)): 1659 (CO); \( ^1\)H-NMR (CDCl\(_3\)), \( \delta \) 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, 4CH\(_2\)), 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\(_3\)OS: 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)methyl]-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 (ether); IR (Nujol, \( \nu \), cm\(^{-1}\)): 1667 (CO); \( ^1\)H-NMR (CDCl\(_3\)), \( \delta \) ppm: 6.92 (s, 1H, CH), 4.73 (dd, 2H, \( J = 15.8 \) Hz, CH\(_2\)), 3.12-2.25 (m, 8H, 4CH\(_2\)), 0.91 (d, 3H, CH\(_3\)); GC-MS \( m/z \): 345 (M\(^+\)); Calcd for C\(_{19}\)H\(_{27}\)N\(_3\)OS: C, 66.05; H, 7.88; N, 12.68; S, 9.67. 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, \( \nu \), cm\(^{-1}\)): 1666 (CO); \( ^1\)H-NMR (CDCl\(_3\)), \( \delta \) 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\(_2\)), 3.81-2.74 (m, 8H, 4CH\(_2\), 2CH), 2.00-1.18 (m, 10H, 4CH\(_2\), 2CH), 0.91 (d, 3H, CH\(_3\)); GC-MS \( m/z \): 355 (M\(^+\)); Calcd for C\(_{19}\)H\(_{27}\)N\(_3\)OS: C, 66.05; H, 7.88; N, 12.68; S, 9.67. Found: C, 66.15; H, 7.78; N, 12.08; S, 9.36.
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$^+$); Caled for C$_{16}$H$_{21}$N$_3$O$_2$S: 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 (7I): 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, $\nu$, cm$^{-1}$): 1671 (CO); $^1$H-NMR (CDCl$_3$), $\delta$ 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$^+$); Caled for C$_{17}$H$_{23}$N$_3$O$_2$S: 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.

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