Scientific paper

Synthesis and Evaluation of Novel Pyrrolo[2,3-*d*] and Thieno[2,3-*d*]Pyridazinones as *in Vitro* Antiproliferative Agents

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Dedicated to Professor Branko Stanovnik on the occasion of his 70th birthday

Abstract

Two different types of novel heterocyclic-fused pyridazinones like pyrrolo[2,3-*d*] and thieno[2,3-*d*] derivatives were synthesized starting from their isoxazolo[3,4-*d*] precursors by oxidative cleavage with CAN followed by cyclocondensation of the five-membered system using bi-functional nucleophiles. The final compounds were preliminarily screened *in vitro* as antiproliferative agents under the protocols of the NCI using three human cell lines of CNS, lung and breast cancers. None of the compounds was able to reduce the growth at value < 32% which was the cut-off for a more in depth in vitro screening.

Keywords: Thieno[2,3-*d*]pyridazinones, pyrrolo[2,3-*d*]pyridazinones, synthesis, in vitro antiproliferative agents.

1. Introduction

The pyrrolopyridazinone sub-unit is well represented among heterocyclic compounds displaying antitumor and antiviral activities.

Townsend et al.[1–5] reported several series of purine nucleoside analogues containing the pyrrolo[2,3-*d*]pyridazin-7-one system as heterocyclic core. Some of these compounds, like the prototype **1** (Figure 1), showed significant antiproliferative and antiviral activities *in vitro* and low cytotoxicity. Very recently the same group reported a series of isosteric pyrrolotriazines active against human cytomegalovirus and herpes simplex virus.[6] These compounds were synthesized as analogues of Sangivamycin **2**. This natural compound, which is active against L 1210 leukemia, P 338 leukemia and Lewis lung carcinoma, has been in clinical trials against several types of human cancers.[7, 8] Further examples of pyrrolopyridazinones (or fused analogues) endowed with antitumor and/or antiviral activities (compounds **3**, **4** and **5**) were described by authors from different countries active both in industry[9] and in academia.[10–12] Our continuing interest in the chemistry and pharmacology of pyridazin-3(2*H*)-one derivatives and heterocyclic-fused analogues[13–15] led us to undertake a research program aimed to synthesize novel examples of pyrrolo[2,3-*d*]pyridazin-7-ones, as well as of the corresponding thieno[2,3-*d*] derivatives to evaluate their *in vitro* antiproliferative effect. Thus in this work we report the preliminary results obtained in this area.

2. Results and Discussion

The target compounds of general structure (**6**) (Figure 1) were synthesized following a well established procedure described by us in some previous papers.[13–15] The key intermediates are the 5-acetyl-4-nitropyridazinones **8a-h** (Scheme 1) which are easily obtained from the isoxazolopyridazinones **7a-h** by oxidative cleavage of the fi-

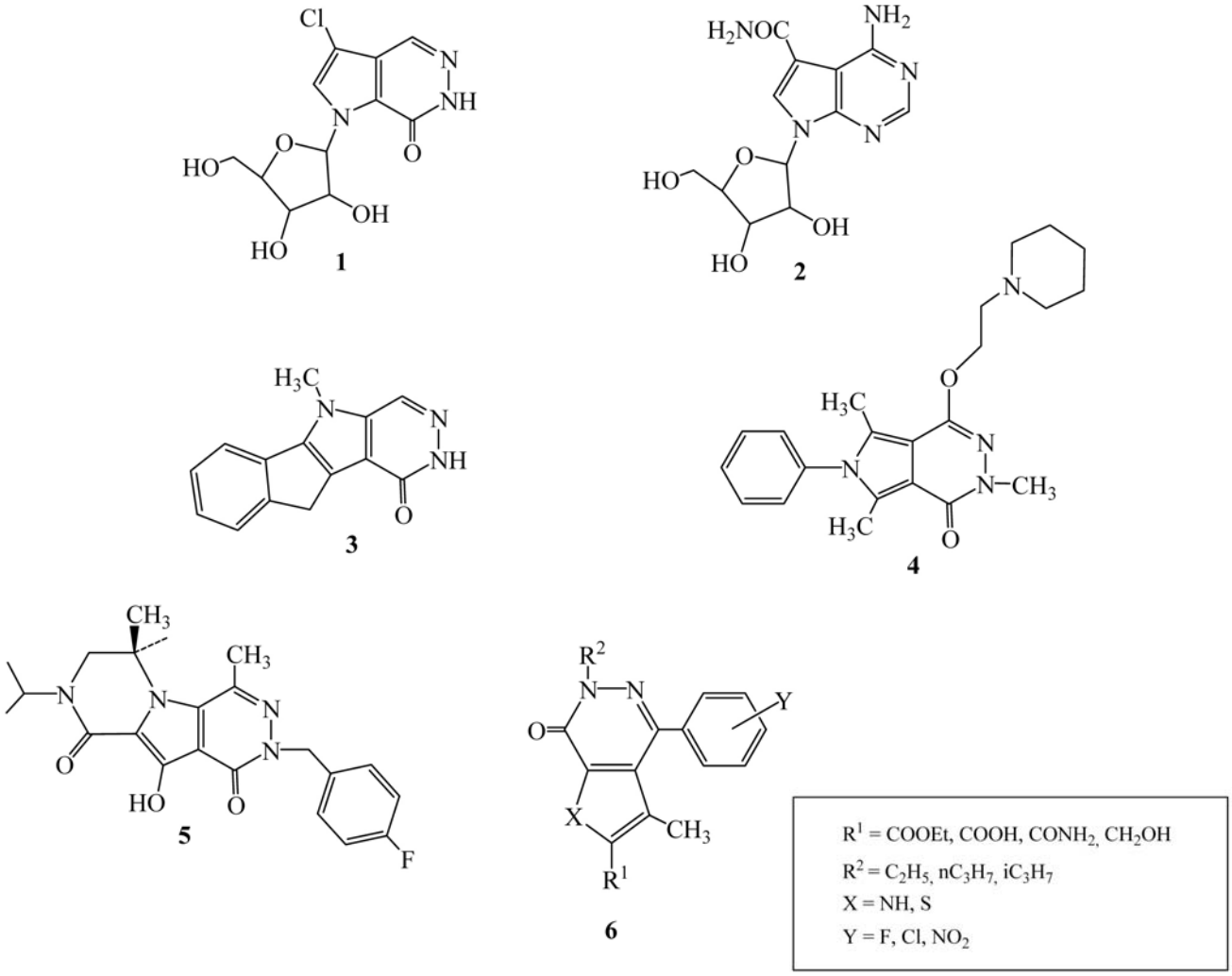
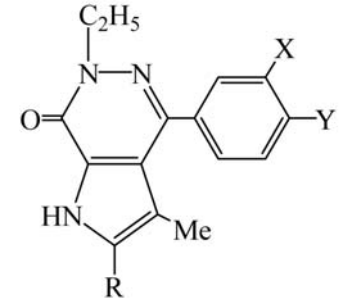


Figure 1. Structure of compounds displaying antitumoral and antiviral activities.

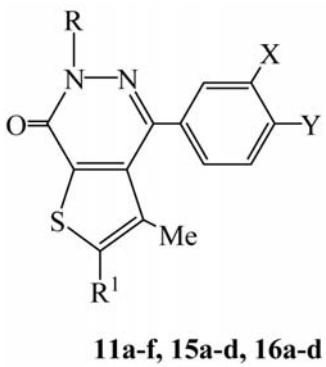
Table 1. Evaluation of antiproliferative activity of compounds **10a-d**, **12a-d**, **13a-e** and **14a,b** on three different cell lines (MCF7, NCI-H460 and SF268)

Comp	R	X	Y	% of growth[a] BREAST (MCF7)	LUNG (NCI-H460)	CNS (SF-268)
10a	COOEt	H	H	54	89	80
10b	COOEt	H	F	93	98	121
10c	COOEt	F	H	99	97	98
10d	COOEt	H	Cl	67	90	106
12a	COOH	H	H	NT[c]	NT[c]	NT[c]
12b	COOH	H	F	101	98	112
12c	COOH	F	H	116	101	133
12d	COOH	H	Cl	104	99	108
13a	$CONH_2$	H	H	101	98	83
13b	$CONH_2$	H	F	105	101	96
13c	$CONH_2$	F	H	NT[c]	NT[c]	NT[c]
13d	$CONH_2$	H	Cl	64	100	78
13e	COZ[b]	H	H	NT[c]	NT[c]	NT[c]
14a	CH_2OH	H	H	103	97	124
14b	CH_2OH	H	F	88	92	102



[a] Compounds were tested at 100 μM; [b] Z = 2-methylaziridin-1-yl; [c] Not tested

Table 2. Evaluation of antiproliferative activity of compounds **11a-f**, **15a-d** and **16a-d** on three different cell lines (MCF7, NCI-H460 and SF268)

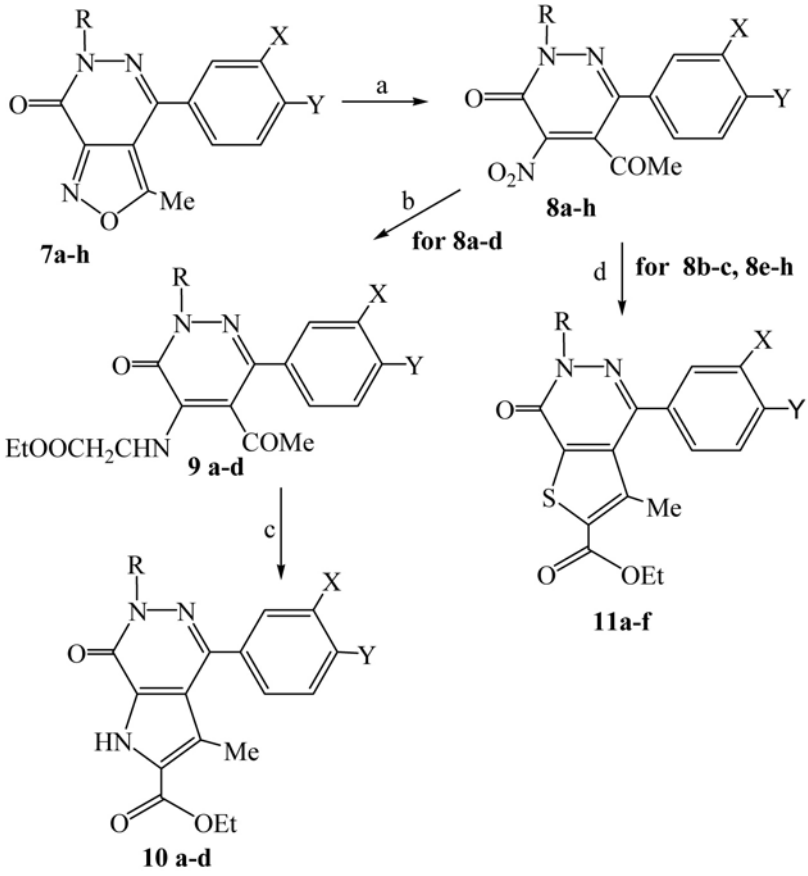


11a-f, 15a-d, 16a-d

Comp	R	R^1	X	Y	% of growth[a] BREAST (MCF7)	LUNG (NCI-H460)	CNS (SF-268)
11a	Et	COOEt	H	F	67	101	97
11b	Et	COOEt	F	H	75	99	75
11c	Et	COOEt	NO_2	H	58	93	43
11d	n-Pr	COOEt	H	H	NT[b]	NT[b]	NT[b]
11e	i-Pr	COOEt	H	H	75	75	70
11f	i-Pr	COOEt	NO_2	H	NT[b]	NT[b]	NT[b]
15a	Et	COOH	H	F	114	100	139
15b	Et	COOH	F	H	87	90	103
15c	Et	COOH	NO_2	H	117	101	132
15d	i-Pr	COOH	NO_2	H	NT[b]	NT[b]	NT[b]
16a	Et	$CONH_2$	H	F	113	98	115
16b	Et	$CONH_2$	F	H	110	102	130
16c	Et	$CONH_2$	NO_2	H	109	92	126
16d	i-Pr	$CONH_2$	NO_2	H	NT[b]	NT[b]	NT[b]

[a] Compounds were tested at 100 μM; [b] Not tested

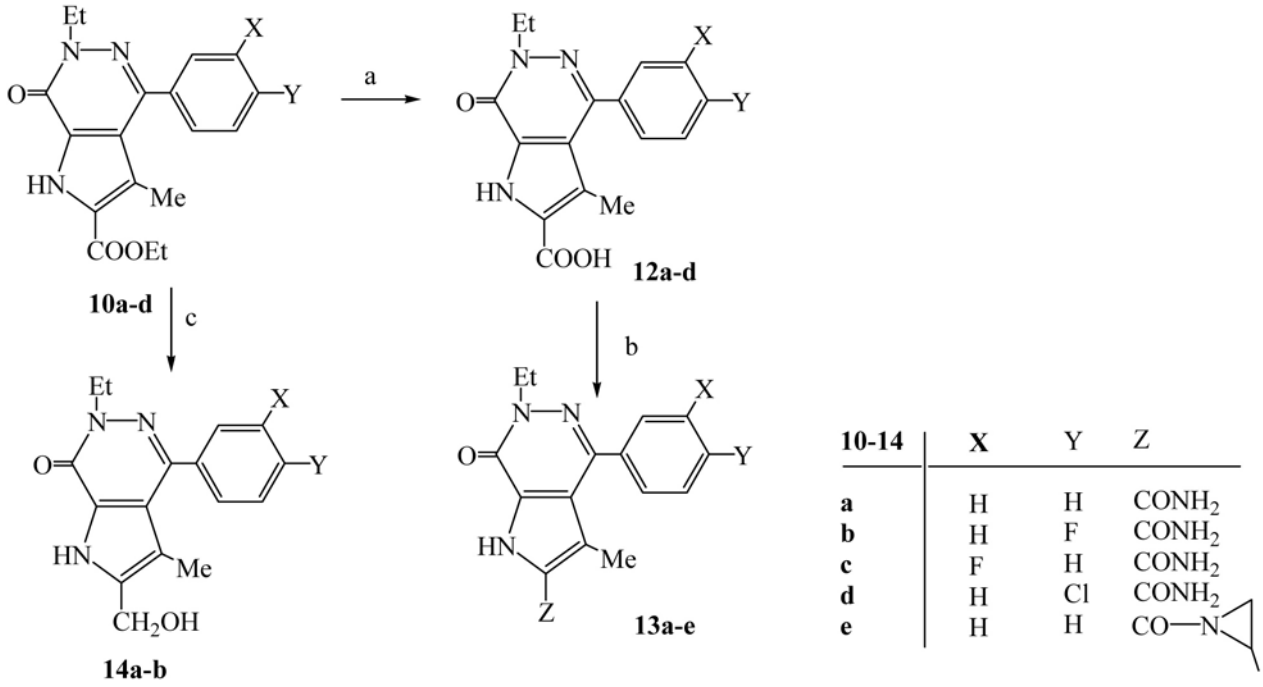
ve-membered system with ceric ammonium nitrate (CAN). With the exception of **7h**, all the precursors of type **7** were previously described by us.[14,15] Likewise compounds **8a-g** were reported in our foregoing papers.[14,16–18] The unknown **7h** was easily prepared by alkylation of the 2-unsubstituted analogue[14] with 2-iodopropane (see experimental). In compounds **8** the nitro group is a very good leaving group and can be easily replaced under



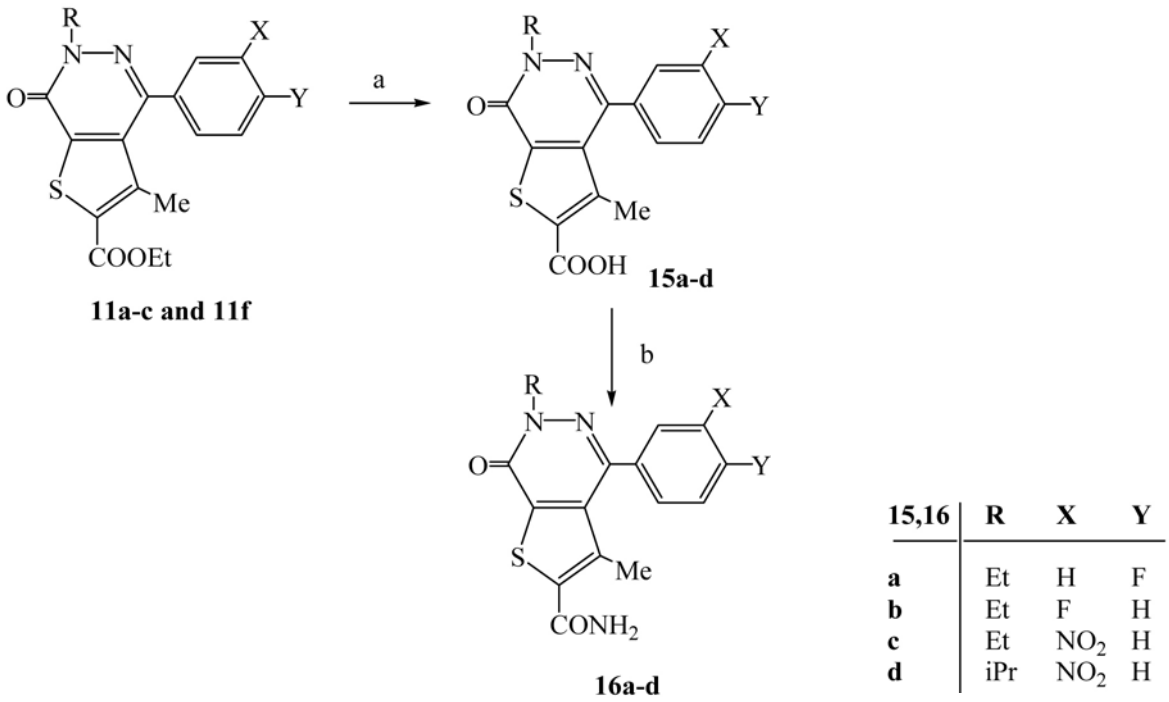
11	R	X	Y
a	Et	H	F
b	Et	F	H
c	Et	NO_2	H
d	nPr	H	H
e	iPr	H	H
f	iPr	NO_2	H

7-10	R	X	Y
a	Et	H	H
b	Et	H	F
c	Et	F	H
d	Et	H	Cl
e	Et	NO_2	H
f	nPr	H	H
g	iPr	H	H
h	iPr	NO_2	H

Scheme 1. a: CAN, 65% HNO_3, 50% CH_3COOH, 60 °C; b: glycine ethylester hydrochloride, EtOH, 45 °C; c: NaOEt/EtOH, 40 °C; d: ethyl-2-mercaptoacetate, EtOH, rt.



Scheme 2. a: 6N NaOH, EtOH, 50 °C; b: 1) $SOCl_2$, 60 °C, 2) NH_3 or methylaziridine; c: $NaBH_4$



Scheme 3. a: 6N NaOH, EtOH, 50 °C; b: 1) $SOCl_2$, reflux; 2) NH_3

mild conditions and in high yields by a variety of *O*-, *N*- and *S*-nucleophiles. In our case, treatment with glycine ethyl ester in ethanol afforded the intermediates **9a-d** which, in turn, were smoothly converted into the pyrrolo[2,3-*d*]pyridazinones **10a-d** by briefly heating with sodium ethoxide in ethanol at 40 °C. Among these compounds **9d**, **10a** and **10d** were previously described.[14,17] When the precursors **8b-c** and **8e-h** were treated with ethyl thioglycolate in alcoholic medium the thienopyridazinone esters **11a-f** were directly isolated in good yields.

The ester group of compounds **10** was hydrolyzed using 6N NaOH in ethanol affording the corresponding carboxylic acids **12a-d** (**12a** was already reported[19]), which, in turn, were converted into the corresponding amides **13a-e** (**13a**[19]) through the intermediate chlorides, by treatment with ammonia or the appropriate amine (Scheme 2). Reduction of the ester group with sodium borohydride afforded the primary alcohols **14a-b** in good yields.

Several examples of amides in the thienopyridazinone series (compounds **16b-d**) were prepared from the esters **11** (Scheme 3), using the same experimental conditions described for the conversion of the pyrrolopyridazinones esters **10**.

Compounds **10-16** were tested in vitro as antiproliferative agents following the protocols optimised by the Development Therapeutic Program (DPT) of the National Cancer Institute (Bethesda, USA). On the basis on this program the novel compounds are evaluated on 60 human tumor cell lines. Since researchers from NCI found that 95% of active compounds on one of the 60 cell lines can be identified using three cell lines only, at the present the novel compounds are screened on the following cell lines: MCF7 (breast cancer), NCI-H460 (lung cancer) and SF-268 (CNS cancer).

Thus our compounds were tested at 100 micromoles concentration against the three selected cell lines and the results are depicted in Tables 1 and 2.

Unfortunately, all synthesized products showed a very low activity and they were not able to reduce the growth of anyone of the cell lines at values < 32% that is the limit given by NCI for further evaluation in the full panel of 60 cell lines.

The ester **11c** was the only compound which approached the limit of 32% growth inhibition against the SF-286 cell line (CNS). Taking into account that the corresponding carboxylic acid **15c** and the amide **16c** were completely ineffective against all the three cell lines, it seems that lipophilicity could play a role in inducing antiproliferative properties in the present series.

3. Conclusions

In conclusion, we synthesized a new series of pyrrolo[2,3-*d*]pyridazin-7-ones and thieno[2,3-*d*]pyridazin-7-ones derivatives and we evaluated their *in vitro* antiproliferative effect. Unluckily, the preliminary results showed that all new products are not able to reduce the growth of the cell lines till the values given by NCI to continue evaluation. Taking into account that the compound that more closely approached the limit of 32% is the ester **11c** and that its analogues bearing a polar function (the carboxylic acid **15c** and the amide **16c**) are inactive, we hypothize that the lipophilicity was important for activity. With this in mind the synthesis of analogues bringing the ester function and the 3-nitrophenyl fragment is in progress.

4. Experimental

All melting points were determined on a Buchi apparatus and are uncorrected. ^{1}H-NMR spectra were recorded with Avance 400 instruments (Bruker Biospin, version 002 with SGU). Chemical shifts are reported in ppm, using the solvent as internal standard. Extracts were dried over Na_2SO_4 and the solvents were removed under reduced pressure. E. Merck F-254 commercial plates were used for analytical TLC to follow the course of the reaction. Silica gel 60 (Merck 70–230 mesh) was used for column chromatography.

6-Isopropyl-3-methyl-4-(3-nitrophenyl)isoxazolo[3,4-*d*]pyridazin-7(6*H*)-one 7h. A suspension of 3-methyl-4-(3-nitrophenyl)isoxazolo[3,4-*d*]pyridazin-7-(6*H*)-one (200 mg, 0.74 mmoles), K_2CO_3 (250 mg, 1.8 mmoles), 2-iodopropane (305 mg, 1.8 mmoles) and anhydrous DMF (3mL) was stirred at 100 °C for 1 h. Treatment with ice-cold water (20 mL) afforded **7h** as crude precipitate (87% yield). The analytical sample was obtained by crystallization from ethanol: mp 208–210 °C.

^{1}H NMR ($CDCl_3$): δ 8.45–8.30 (m, 2H, aromatic), 8.00 (d, J = 7.4 Hz, 1H, aromatic), 7.80–7.65 (m, 1H, aromatic), 5.40 (m, 1H, $CH(CH_3)_2$), 2.60 (s, 3H, CH_3), 1.40 (d, J = 7.6 Hz, 6H, $CH(CH_3)_2$).

Anal. Calcd. for $C_{15}H_{14}N_4O_4$: C, 57.32; H, 4.49; N, 17.83. Found: C, 57.59; H, 4.81; N,17.58.

5-Acetyl-2-isopropyl-4-nitro-6-(3-nitrophenyl)pyridazin-3(2*H*)-one 8h. To a stirred suspension of **7h** (150 mg, 0.48 mmoles) in 50% acetic acid (5 mL) and 65% HNO_3 (0.45 mL), ceric ammonium nitrate (1.9 g, 3.47 mmoles) was added portionwise in 45 min, maintaining the temperature at 55–60 °C. After dilution with ice-cold H_2O (50 mL) and standing for 1 h the precipitate **7h** (48% yield) was collected by suction and purified by column chromatography (eluent: toluene/ethyl acetate 8:2); mp = 109–111 °C, crystallization solvent ethanol.

^{1}H NMR ($CDCl_3$): δ 8.45–8.20 (m, 2H, aromatic), 8.00 (d, J = 7.5 Hz, 1H, aromatic), 7.80–7.62 (m, 1H, aromatic), 5.58–5.30 (m, 1H, $CH(CH_3)_2$), 2.28 (s, 3H, CH_3), 1.60–1.32 (m, 6H, $CH(CH_3)_2$). Anal. Calcd. for $C_{15}H_{14}N_4O_6$: C, 52.03; H, 4.07; N, 16.18. Found: C, 51.93; H, 4.19; N, 16.05.

General procedure for compounds 9a-c. To a cooled solution of glycine ethylester hydrochloride (2.52 mmoles) in water (2.5 mL), 6N NaOH (0.5 mL) was added dropwise until pH 9.0. The solution was saturated with NH_4Cl and extracted with ethyl ether (4 × 20 mL). The organic layer was dried on anhydrous sodium sulfate and evaporated in vacuo. The residual oil was dissolved in ethanol (2mL) and the appropriate 4-nitro derivative **8** (0.33 mmoles) was added. The suspension was stirred at 45 °C for 20 min. After cooling, the crude precipitate was filtered off.

Ethyl 2-(5-acetyl-2-ethyl-3-oxo-6-phenyl-2,3-dihydropyridazin-4-ylamino)acetate 9a. Yield 45%, mp 116–118 °C, crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 7.40 (s, 5H, aromatic), 4.40 (d, J = 7.7 Hz, 2H, CH_2–NH), 4.37–4.15 (m, 4H, N–CH_2–CH_3 and O–CH_2–CH_3), 1.80 (s, 3H, CH_3), 1.45–1.24 (m, 6H, N–CH_2–CH_3 and O–CH_2–CH_3). Anal. Calcd. For $C_{18}H_{21}N_3O_4$: C, 62.96; H, 6.16; N, 12.24. Found: C, 62.52; H, 5.97; N, 11.97.

Ethyl 2-[5-acetyl-2-ethyl-6-(4-fluorophenyl)-3-oxo-2,3-dihydropyridazin-4-ylamino]acetate 9b. Yield 48%, mp 127–128 °C, crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 8.42 (exch br s, 1H, NH), 7.52–7.35 (m, 2H, aromatic), 7.25–7.04 (m, 2H, aromatic), 4.53–4.38 (m, 2H, CH_2–NH), 4.25–4.12 (m, 4H, N–CH_2–CH_3 and O–CH_2–CH_3), 1.83 (s, 3H, CH_3), 1.50–1.23 (m, 6H, N–CH_2–CH_3 and O–CH_2–CH_3). Anal. Calcd. For $C_{18}H_{20}FN_3O_4$: C, 59.83; H, 5.58; N, 11.63. Found: C, 60.02; H, 5.41; N, 11.87.

Ethyl 2-[5-acetyl-2-ethyl-6-(3-fluorophenyl)-3-oxo-2,3-dihydropyridazin-4-ylamino]acetate 9c. Yield 53%, mp 95–96 °C, crystallization solvent: ethanol .

^{1}H NMR ($CDCl_3$): δ 7.45–7.15 (m, 4H, aromatic), 4.50–4.41 (m, 2H, NH–CH_2), 4.36–4.15 (m, 4H, N–CH_2–CH_3 and O–CH_2–CH_3), 1.85 (s, 3H, CH_3), 1.42–1.23 (m, 6H, N–CH_2–CH_3 and O–CH_2–CH_3). Anal. Calcd. For $C_{18}H_{20}FN_3O_4$: C, 59.83; H, 5.58; N, 11.63. Found: C, 60.05; H, 5.61; N, 11.77.

General procedure for compounds 10b,c. To a solution of the appropriate compound **9** (0.25 mmoles) in anhydrous ethanol (2 mL) a solution of sodium ethoxide prepared dissolving sodium (1.5 mmoles) in anhydrous ethanol (1.5 mL) was added. The mixture was stirred for 10 min at room temperature. Dilution with ice-cold water (8 mL) and acidification with 6N HCl afforded the desired **10** as a precipitate which was filtered off.

Ethyl 6-ethyl-4-(4-fluorophenyl)-3-methyl-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyridazine-2-carboxylate 10b. Yield 83%, mp 219–220 °C, crystallization solvent: ethanol .

^{1}H NMR ($CDCl_3$): δ 7.56–7.43 (m, 2H, aromatic), 7.35–7.10 (m, 2H, aromatic), 4.48–4.32 (m, 4H, N–CH_2–CH_3 and O–CH_2–CH_3), 2.18 (s, 3H, CH_3), 1.45–1.35 (m, 6H, N–CH_2–CH_3 and O–CH_2–CH_3). Anal. Calcd. For $C_{18}H_{18}FN_3O_3$: C, 62.97; H, 5.28; N, 12.24. Found: C, 63.21; H, 5.19; N, 12.47.

Ethyl 6-ethyl-4-(3-fluorophenyl)-3-methyl-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyridazine-2-carboxylate 10c. Yield 72%, mp 164–166 °C, crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 7.58–7.42 (m, 1H, aromatic), 7.35–7.15 (m, 3H, aromatic), 4.55–4.23 (m, 4H, N–CH_2–CH_3 and O–CH_2–CH_3), 2.20 (s, 3H, CH_3), 1.52–1.40 (m, 6H, N–CH_2–CH_3 and O–CH_2–CH_3). Anal. Calcd. For $C_{18}H_{18}FN_3O_3$: C, 62.97; H, 5.28; N, 12.24. Found: C, 63.01; H, 5.35; N, 12.07.

General procedure for compounds 11a-f (11c[14]). A solution of ethyl-2-mercaptoacetate (1.0 mmoles) in absolute ethanol (1 mL) was added to a solution of sodium ethoxide prepared dissolving sodium (1.0 mmoles) in absolute ethanol (1.5 mL). The mixture was stirred at room temperature for 20 min. After evaporation in vacuo the residue was treated with a suspension of compound **8** (0.33 mmoles) in absolute ethanol (2 mL). After 20 min stirring the reaction mixture was diluted with ice- cold water (25 mL) and the precipitate **11** collected by suction.

Ethyl 6-ethyl-4-(4-fluorophenyl)-3-methyl-7-oxo-6,7-dihydrothieno[2,3-*d*]pyridazine-2-carboxylate 11a. Yield = 47% , mp = 185–188 °C dec., crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 7.50–7.38 (m, 2H, aromatic), 7.30–7.15 (m, 2H, aromatic), 4.51–4.23 (m, 4H, N–CH_2CH_3 and O–CH_2–CH_3), 2.20 (s, 3H,CH_3), 1.48–1.32 (m, 6H, N–CH_2–CH_3 and O–CH_2–CH_3). Anal. Calcd. For $C_{18}H_{17}FN_2O_3S$: C, 59.99; H, 4.75; N, 7.77. Found: C, 59.73; H, 4.98; N, 8.09.

Ethyl 6-ethyl-4-(3-fluorophenyl)-3-methyl-7-oxo-6,7-dihydrothieno[2,3-*d*]pyridazine-2-carboxylate 11b. Yield = 35% , mp = 176 °C, crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 7.65–7.55 (m, 1H, aromatic), 7.35–7.05 (m, 3H, aromatic), 4.39–4.18 (m, 4H, N–CH_2CH_3 and O–CH_2–CH_3), 2.50 (s, 3H, CH_3), 1.56–1.25 (m, 6H, N–CH_2–CH_3 and O–CH_2–CH_3). Anal. Calcd. For $C_{18}H_{17}FN_2O_3S$: C, 59.99; H, 4.75; N, 7.77. Found: C, 59.85; H, 4.48; N, 7.94.

Ethyl -3-methyl-7-oxo-4-phenyl-6-propyl-6,7-dihydrothieno[2,3-*d*]pyridazine-2-carboxylate 11d. Yield = 35%, mp > 300 °C, crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 7.60–7.40 (H, aromatic), 4.40–4.20 (m, 4H, N–CH_2–CH_2–CH_3 and O–CH_2–CH_3), 2.18 (s, 3H, CH_3), 2.00–1.80 (m, 2H, N–CH_2–CH_2–CH_3), 1.40 (t, J = 7.6 Hz, 3H, O–CH_2–CH_3), 1.00 (t, J = 7.5 Hz 1H, CH_2–CH_2–CH_3). Anal. Calcd. For $C_{19}H_{20}N_2O_3S$: C, 64.02; H, 5.66; N, 7.86. Found: C, 64.24; H, 5.95; N, 8.03.

Ethyl 6-isopropyl-3-methyl-7-oxo-4-phenyl-6,7-dihydrothieno[2,3-*d*]pyridazine-2-carboxylate 11e. Yield = 38%, mp = 153–155 °C, crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 7.60–7.40 (m, 5H, aromatic), 5.50–5.35 (m, 1H, CH–$(CH_3)_2$), 4.40 (q, J = 7.7 Hz, 2H, O–CH_2–CH_3), 2.20 (s, 3H, CH_3), 1.50–1.30 (m, 9H,

$(CH_3)_2$–CH and O–CH_2–CH_3). Anal. Calcd. For $C_{19}H_{20}N_2O_3S$: C, 64.02; H, 5.66; N, 7.86. Found: C, 63.74; H, 5.80; N, 7.73.

Ethyl-6-isopropyl-3-methyl-4-(3-nitrophenyl)-7-oxo-6,7-dihydrothieno[2,3-*d*]pyridazine-2-carboxylate 11f. Yield = 35%, mp = 164–165 °C, crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 8.45–8.35 (m, 2H, aromatic), 7.85–7.65 (m, 2H, aromatic), 5.54–5.46 (m, 1H, CH–$(CH_3)_2$), 4.40 (q, J = 7.6 Hz, 2H, CH_2), 2.21 (s, 3H, CH_3), 1.48–1.35 (m, 9H, CH_2–CH_3 and $(CH_3)_2$–CH). Anal. Calcd. For $C_{19}H_{19}N_3O_5S$: C, 56.85; H, 4.77; N, 10.47. Found: C, 56.68; H, 4.72; N, 10.22.

General procedure for 12b-d. To a solution of the appropriate derivative **10** (0.2 mmoles) in ethanol (2 mL), 2N NaOH (4 mL) was added and the mixture was stirred at 50 °C for 5 h. After concentration in vacuo, the residue was diluted with ice-cold water. Acidification with 6N HCl afforded compound of type **12** as a precipitate which was collected by filtration.

6-Ethyl -4-(4-fluorophenyl)-3-methyl-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyridazine-2-carboxylic acid 12b. Yield 77%, mp > 300 °C, crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 7.68–7.50 (m, 2H, aromatic), 7.40–7.18 (m, 2H, aromatic), 4.20 (q, J = 7.5 Hz, 2H, CH_2–CH_3), 2.00 (s, 3H, CH_3), 1.30 (t, J = 7.5 Hz, 3H, CH_2–CH_3). Anal. Calcd. For $C_{16}H_{14}FN_3O_3$: C, 60.95; H, 4.48; N, 13.33. Found: C, 61.12; H, 4.37; N, 13.09.

6-Ethyl -4-(3-fluorophenyl)-3-methyl-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyridazine-2-carboxylic acid 12c. Yield 92% , mp > 300 °C crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 7.64–7.45 (m, 1H, aromatic), 7.40–7.20 (m, 3H, aromatic), 4.50 (q, J = 7.5 Hz, 2H, CH_2–CH_3), 2.30 (s, 3H, CH_3), 1.50 (t, J = 7.5 Hz, 3H, CH_2–CH_3). Anal. Calcd. For $C_{16}H_{14}FN_3O_3$: C, 60.95; H, 4.48; N, 13.33. Found: C, 61.18 ; H, 4.60; N, 13.6.

4-Chlorophenyl-6-ethyl-3-methyl-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyridazine-2-carboxylic acid 12d. Yield 79%, mp > 300 °C, crystallization solvent: ethanol.

^{1}H NMR (DMSO-d_6): δ 7.58 (s, 4H, aromatic), 4.20 (q, J = 7.5 Hz, 2H, CH_2–CH_3), 2.00 (s, 3H, CH_3), 1.26 (t, J = 7.5 Hz, 3H, CH_2–CH_3). Anal. Calcd. For $C_{16}H_{14}ClN_3O_3$: C, 57.93; H, 4.25; N, 12.67. Found: C, 58.05; H, 4.30; N, 13.09.

General procedure for compounds 13b-e. The carboxylic acid **12** (0.16 mmoles) was suspended in $SOCl_2$ (0.5 mmoles) and the mixture was stirred at 60 °C for 4 h. After evaporation in vacuo, the residue was cooled and treated with the opportune amine (1.5 mmoles). The obtained precipitate was washed with water and collected by filtration.

6-Ethyl-4-(4-fluorophenyl)-3-methyl-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyridazine-2-carboxamide 13b. Yield 68% , mp > 300 °C crystallization solvent: ethanol.

^{1}H NMR (DMSO-d_6): δ 7.70 (exch br s, 2H, NH_2), 7.65–7.55 (m, 2H, aromatic), 7.45–7.25 (m, 2H, aromatic), 4.20 (q, J = 7.5 Hz, 2H, CH_2–CH_3), 2.00 (s, 3H, CH_3), 1.30 (t, J = 7.5 Hz, 3H, CH_2–CH_3). Anal. Calcd. For $C_{16}H_{15}FN_4O_2$: C, 61.14; H, 4.81; N,17.83. Found: C, 61.38; H, 4.59; N, 17.60.

6-Ethyl-4-(3-fluorophenyl)-3-methyl-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyridazine-2-carboxamide 13c. Yield = 62%, mp > 300, crystallization solvent: ethanol.

^{1}H NMR (DMSO-d_6): δ 7.50–7.20 (m, 4H, aromatic), 4.20 (q, J = 7.5 Hz, 2H, CH_2–CH_3), 2.00 (s, 3H, CH_3), 1.30 (t, J = 7.5 Hz, 3H, CH_2–CH_3). Anal. Calcd. For $C_{16}H_{15}FN_4O_2$: C, 61.14; H, 4.81; N,17.83. Found: C, 61.42; H, 4.99; N, 17.97.

4-(4-chlorophenyl)-6-ethyl-3-methyl-7-oxo-6,7-dihydro-1*H*-pyrrolo[2,3-*d*]pyridazine-2-carboxamide 13d. Yield = 84%, mp > 300, crystallization solvent: ethanol.

^{1}H NMR (DMSO-d_6): δ 7.53 (s, 4H, aromatic), 4.20 (q, J = 7.5 Hz, 2H, CH_2–CH_3), 3.90 (exch br s, 2H, NH_2), 2.05 (s, 3H, CH_3), 1.20 (t, J = 7.5 Hz, 3H, CH_2–CH_3). Anal. Calcd. For $C_{16}H_{15}ClN_4O_2$: C, 58.10; H, 4.57; N, 16.94. Found: C, 58.34; H, 4.84; N, 16.73.

6-ethyl-3-methyl-2-[(2-methylaziridin-1-yl)carbonyl]-4-phenyl-1,6-dihydro-7*H*-pyrrolo[2,3-*d*]pyridazin-(6*H*)-one 13e. Yield = 53%, mp = 70–73, crystallization solvent: cyclohexane.

^{1}H NMR ($CDCl_3$): δ 7.50 (s, 5H, aromatic), 4.40 (q, J = 7.5 Hz, 2H, N–CH_2–CH_3), 2.85–2.75 (m, 1H, $CHCH_3$), 2.55–2.44 (m, N–CH_2CH), 2.22–2.18 (m, N–CH_2CH), 2.18 (s, 3H, CH_3), 1.40 (t, J = 7.5 Hz, 3H, N–CH_2–CH_3), 1.25 (d, J = 7.1 Hz, 3H, CH–CH_3). Anal. Calcd. For $C_{19}H_{20}N_4O_2$: C, 67.84, H, 5.99; N, 16.66. Found: C, 67.79; H, 6.22; N, 16.38.

General procedure for compounds 14a,b. To a stirred and cooled solution of **11** (0.23 mmoles) in DMSO (4 mL) and H_2O (0.2 mL), sodium borohydride (9.2 mmoles) was added portionwise. The mixture was stirred for additional 12 h at 110 °C. After cooling the precipitate **14** was filtered off.

6-ethyl-2-(hydroxymethyl)-3-methyl-4-phenyl-1,6-dihydro-7*H*-pyrrolo[2,3-*d*]pyridazin-7-one 14a.

Yield = 90%, mp > 300 °C, crystallization solvent: cyclohexane.

^{1}H NMR ($CDCl_3$): δ 7.57 (s, 5H, aromatic), 4.82 (s, 2H, CH_2–OH), 4.40 (q, J = 7.5 Hz, 2H, N–CH_2CH_3, 1.83 (s, 3H, CH_3), 1.48 (t, J = 7.5 Hz, 3H, CH_2–CH_3). Anal. Calcd. For $C_{16}H_{17}N_3O_2$: C, 67.83; H, 6.05; N,14.83. Found : C, 67.95; H, 6.27; N, 15.08.

6-ethyl-4-(4-fluorophenyl)-2-(hydroxymethyl)-3-methyl-1,6-dihydro-7*H*-pyrrolo[2,3-*d*]pyridazin-7-one 14 b. Yield = 38%, mp > 300, crystallization solvent: cyclohexane.

^{1}H NMR ($CDCl_3$): δ 7.65–7.55 (m, 2H, aromatic), 7.38–7.24 (m, 2H, aromatic), 4.85 (s, 2H, CH_2–OH), 4.80 (exch br s, 1H, OH), 4.40 (q, J = 7.5 Hz, 2H, N–CH_2CH_3, 1.90 (s, 3H,CH_3), 1.50 (t, J = 7.5 Hz, 3H, N–CH_2–CH_3). Anal. Calcd. For $C_{16}H_{16}FN_3O_2$: C, 63.78; H, 5.35; N, 13.95. Found: C, 64.02; H, 5.16; N, 14.19.

General procedure for compounds 15a-d. To a solution of compound **10** (0.17 mmoles) in ethanol (3 mL), 6N NaOH (2 mL) was added and the mixture was stirred at 50 °C for 4 h. After concentration in vacuo the residue was diluted with ice-cold H_2O (10 mL) and acidified with 6N HCl. The precipitated **15** was collected by suction.

6-ethyl-4-(4-fluorophenyl)-3-methyl-7-oxo-6,7-dihydrothieno[2,3-*d*]pyridazine-2-carboxylic acid 15a. Yield = 58%, mp = 270 °C dec., crystallization solvent: ethanol.

^{1}H NMR (DMSO-d_6): δ 7.54–7.46 (m, 1H, aromatic), 7.38–7.25 (m, 2H, aromatic), 4.45–4.35 (m, 2H, N–CH_2–CH_3), 3.20 (exch br s, 1H, OH), 2.20 (s, 3H,CH_3), 1.50 (t, J = 7.5 Hz, 3H, N–CH_2–CH_3). Anal. Calcd. For $C_{16}H_{13}FN_2O_3S$: C, 57.82; H, 3.94; N, 8.43. Found: C, 57.63; H, 4.19; N,8.70.

6-ethyl-4-(3-fluorophenyl)-3-methyl-7-oxo-6,7-dihydrothieno[2.3-*d*]pyridazine-2-carboxylic acid 15b. Yield = 84%, mp = 285 °C dec., crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 7.60–7.40 (m, 1H, aromatic), 7.28–7.15 (m, 3H, aromatic), 4.36 (q, J = 7.6 Hz, 2H, N–CH_2CH_3), 2.20 (s, 3H, CH_3), 1.42 (t, J = 7.6 Hz, 3H, N–CH_2CH_3). Anal. Calcd. For $C_{16}H_{13}FN_2O_3S$: C, 57.82; H, 3.94; N, 8.43. Found: C, 57.69; H, 4.11; N,8.47.

6-ethyl-3-methyl-7-oxo-4-(3-nitrophenyl)-6,7-dihydrothieno[2,3-*d*]pyridazine-2-carboxylic acid 15c. Yield = 86%, mp = 255–257 °C, crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 8.43–8.37 (m, 2H, aromatic), 7.90–7.73 (m, 2H, aromatic), 4.90 (exch br s, 1H, OH), 4.40 (q, J = 7.5 Hz, 2H, NCH_2CH_3), 2.20 (s, 3H, CH_3), 1.40 (t, J = 7.5 Hz, 3H, N–CH_2CH_3). Anal. Calcd. for $C_{16}H_{13}N_3O_5S$: C, 53.48; H, 3.65; N, 11.69. Found: C, 53.72; H, 3.99; N,11.47.

6-isopropyl-3-methyl-7-oxo-4-(3-nitrophenyl)-6,7-dihydrothieno[2,3-*d*]pyridazine-2-carboxylic acid 15d. Yield = 42%, mp = 144–146 °C, crystallization solvent: ethanol.

^{1}H NMR ($CDCl_3$): δ 8.46–8.35 (m, 2H, aromatic), 7.84–7.62 (m, 2H, aromatic), 5.58–5.42 (m, 1H, $CH(CH_3)_2$), 3.00 (exch br s, 1H,OH), 2.23 (s, 3H, CH_3), 1.45 (d, J = 7.5 Hz, 6H, $(CH_3)_2CH$). Anal. Calcd. for $C_{17}H_{15}N_3O_5S$: C, 54.68; H, 4.05; N, 11.25. Found: 54.51; H, 4.12; N, 11.46.

General procedure for 16a-d. Compound of type **15** (0.25 mmoles) was suspended in $SOCl_2$ (16 mmoles). The mixture was refluxed for 4 h. After cooling the excess of reagent was evaporated in vacuo and the crude residue after cooling was treated with a cold solution of 30% aqueous ammonia. The precipitate **16** was filtered off.

6-Ethyl-4-(4-fluorophenyl)-3-methyl-7-oxo-6,7-dihydrothieno[2,3-*d*]pyridazine-2-carboxamide 16a. Yield = 68%, mp = 264–265 °C, crystallization solvent: ethanol.

^{1}H NMR (DMSO-d_6): δ 8.00 (exch br s, 2H, NH), 7.60–7.56 (m, 2H, aromatic), 7.41–7.22 (m, 2H, aromatic), 4.28 (q, J = 7.5 Hz, 2H, N–CH_2CH_3), 1.90 (s, 3H, CH_3), 1.30 (t, J = 7.5 Hz, 3H, N–CH_2CH_3). Anal. Calcd. for $C_{16}H_{14}FN_3O_2S$: C, 57.99; H, 4.26; N, 12.68: Found: C, 58.18; H, 4.41; N, 12.74.

6-Ethyl-4-(3-fluorophenyl)-3-methyl-7-oxo-6,7-dihydrothieno[2,3-*d*]pyridazine-2-carboxamide 16b. Yield 75% , mp = 242–244 °C, crystallization solvent: ethanol.

^{1}H NMR (DMSO-d_6): δ 7.90 (exch br s, 2H, NH), 7.60–7.30 (m, 4H, aromatic), 4.20 (q, J = 7.5 Hz 2H, N–CH_2CH_3), 1.90 (s, 3H, CH_3), 1.30 (t, J = 7.5 Hz, 3H, N–CH_2CH_3). Anal. Calcd. for $C_{16}H_{14}FN_3O_2S$: C, 57.99; H, 4.26; N, 12.68: Found: C, 57.83; H, 4.52; N, 12.86.

6-Ethyl-3-methyl-4-(3-nitrophenyl)-7-oxo-6,7-dihydrothieno[2,3-*d*]pyridazine-2-carboxamide 16c. Yield 65% , mp = 231–233, crystallization solvent ethyl ether

^{1}H NMR (DMSO-d_6): δ 8.40–7.80 (m, 4H, aromatic), 4.20 (q, J = 7.5 Hz, 2H, N–CH_2CH_3), 1.90 (s, 3H, CH_3), 1.30 (t, J = 7.5 Hz, 3H, N–CH_2CH_3). Anal. Calcd. for $C_{16}H_{14}N_4O_4S$: C, 53.62; H, 3.94; N, 15.63. Found: C,53.46; H, 4.21; N, 15.43.

6-Isopropyl-3-methyl-4-(3-nitrophenyl)-7-oxo-6,7-dihydrothieno[2,3-*d*]pyridazine-2-carboxamide 16d. Yield 65% , mp = 263–265, crystallization solvent ethanol

^{1}H NMR (DMSO-d_6): δ 8.40–8.36 (m, 1H, aromatic), 8.05–7.50 (m, 3H, aromatic), 5.32–5.20 (m, 1H, $(CH_3)_2$–CH), 1.90 (s, 3H, CH_3), 1.32–1.23 (m, 6H, $(CH_3)_2$–CH). Anal. Calcd. for $C_{17}H_{16}N_4O_4S$: C, 54.83; H, 4.33; N, 15.04. Found: C, 55.06; H, 4.02; N, 15.38.

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6. References

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Povzetek

Sintetizirali smo nove pirolo[2,3-*d*]- in tieno[2,3-*d*]-kondenzirane piridazinske derivate. Sinteza temelji na oksidativnem razcepu izoksazolo[3,4-*d*]piridazinskih prekurzorjev s CAN, ki mu sledi ciklokondenzacija z dinukleofili. Končni produkti so bili testirani *in vitro* na antiproliferacijsko aktivnost pri treh vrstah človeških celic, vendar nobena od spojin ni bistveno zmanjšala rasti.