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Synthesis and Structure Investigations of Potential Sedative and Anticonvulsant Hydroxy- and Acetoxy-*N*-(3-oxobutyl)-pyrido[2,3-*d*]pyridazinones[a]

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Summary. Novel *N*-(3-oxobutyl)-hydroxy- and acetoxypyrido[2,3-*d*]pyridazinones were synthesized and tested *in vivo* for their sedative and anticonvulsant activity. The *Michael*-type reaction of quinolinic acid hydrazide and methyl vinyl ketone afforded a mixture of two isomers, 5-hydroxy-N^7-(3-oxobutyl)-pyrido[2,3-*d*]pyridazin-8(7*H*)-one and 8-hydroxy-N^6-(3-oxobutyl)-pyrido[2,3,-*d*]pyridazin-5-(6*H*)-one, in a ratio of 2:1 which were separated by crystallization. Subsequent acetylation of both isomers yielded the corresponding 5- and 8-acetoxy compounds. The structures of the compounds were proven and completely assigned on the basis of ^{1}H, ^{13}C, ^{15}N NMR, and 1D NOE difference spectra as well as 2D C,H-correlation experiments. Preliminary pharmacological tests showed low acute toxicity with a $LD_{50} > 1000\,mg/kg$ in the mouse and sedative activity for the title compounds. 5-Acetoxy-N^7-(3-oxobutyl)-pyrido[2,3-*d*]pyridazin-8(7*H*)-one displayed a borderline anticonvulsant activity in the metrazole test model.

Keywords. Pyrido[2,3-*d*]pyridazinones, acetoxy- and hydroxy-*N*-(3-oxobutyl)-; Structure elucidation; NMR spectroscopy; Toxicity; Anticonvulsant activity.

Introduction

A number of metabolites collectively named kynurenines affecting neuronal function originate from the tryptophan metabolism [1, 2]. 2,3-Pyridinedicarboxylic acid (quinolinic acid, *QA*) and 4-hydroxy-2-quinolinecarboxylic acid (kynurenic acid, *KA*) are two important products formed in the course of the kynurenine pathway (Scheme 1).

[a] Dedicated to Prof. Dr. *M. Tišler*, Ljubljana, on the occasion of his 75th birthday

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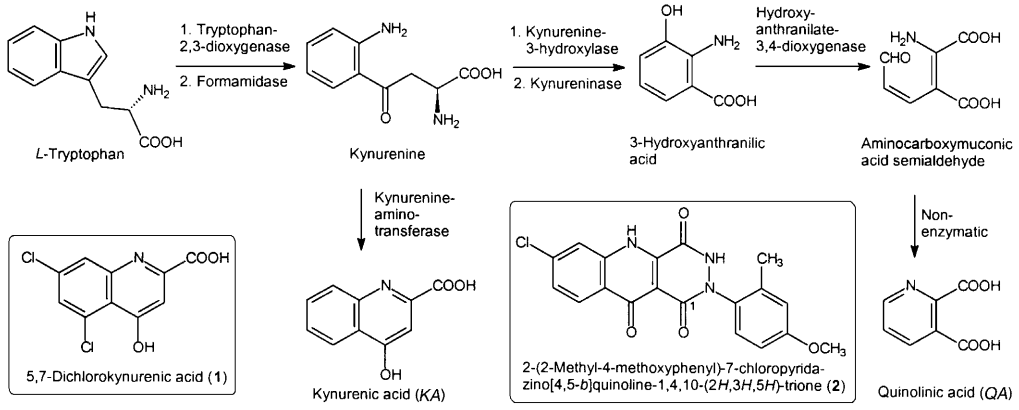


Scheme 1. Formation of quinolinic acid and kynurenic acid from *L*-tryptophan; structures of kynurenic acid analogues

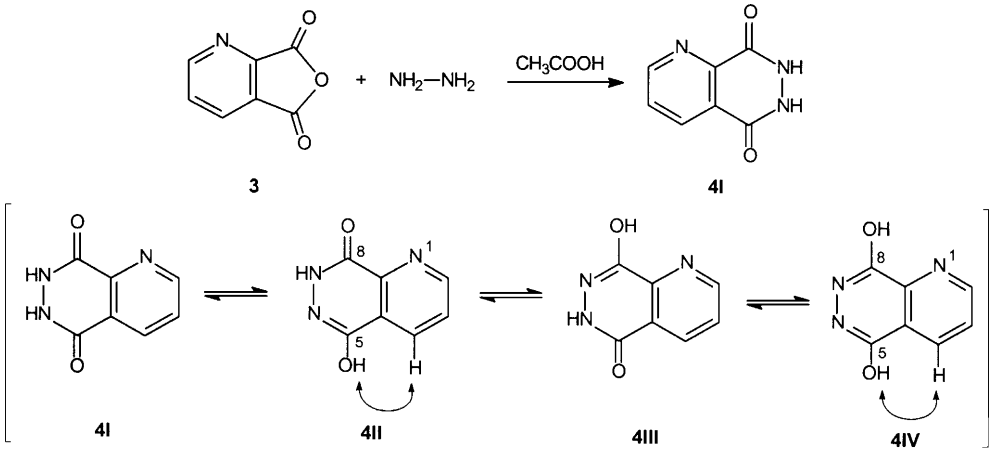
QA, produced among others in small amounts in the central nervous system by activated brain macrophages and microglia, is known as a selective *N*-methyl-*D*-aspartate (*NMDA*) receptor agonist and exerts manyfold excitatory effects [3, 4]. In higher concentrations *QA* is a very strong endogenous excitotoxin that causes convulsions and is associated with neurodegenerative disorders and neuronal cell death, *e.g.* in stroke, AIDS dementia, *Huntington*'s disease, and epilepsy [5–8]. In contrast, *KA* is an endogenous antagonist at *NMDA* receptors and a neuroprotective agent which counteracts with the excitatory and neurotoxic effects of *QA* [9].

In search of potent agents for the treatment of neurodegenerative diseases, a series of compounds structurally related to *KA* have been synthesized and tested [10]. Among others, dichlorokynurenic acid (**1**) and the benzofused quinolinic hydrazide **2** (Scheme 1) are highly active *NMDA* receptor antagonists [11, 12]. Compound **2** combines elements of both neuroactive tryptophan metabolites *QA* and *KA*.

In the present contribution we report on the synthesis and preliminary pharmacological evaluation of the novel *N*-(3-oxobutyl) substituted hydroxy- and acetoxypyrido[2,3-*d*]pyridazinones **7**, **8**, **13**, and **16**. The hydroxypyridopyridazinone skeleton of these compounds is on the one hand structurally related to the pyridopyridazinedione moiety of the tricyclic antagonist **2**; on the other hand, it can be regarded as a cyclic amide-imidic acid analogue of the excitotoxin *QA*. The title compounds were tested *in vivo* for sedative and anticonvulsant activity. Moreover, the regioselectivity of the *Michael*-type addition of quinolinic hydrazide to methyl vinyl ketone was studied.

Results and Discussion

The starting material for the synthesis of the target compounds **7**, **8**, **13**, and **16** was quinolinic hydrazide (**4**) which was prepared from quinolinic anhydride (**3**) and hydrazine in good yield according to Ref. [14]. As shown in Scheme 2, **4** could exist in the four tautomeric forms **4I** (hydrazide, 2 NH), **4IV** (azino-diol, 2 OH), and **4II**, **III** (amide-imidic acid, NH/OH).



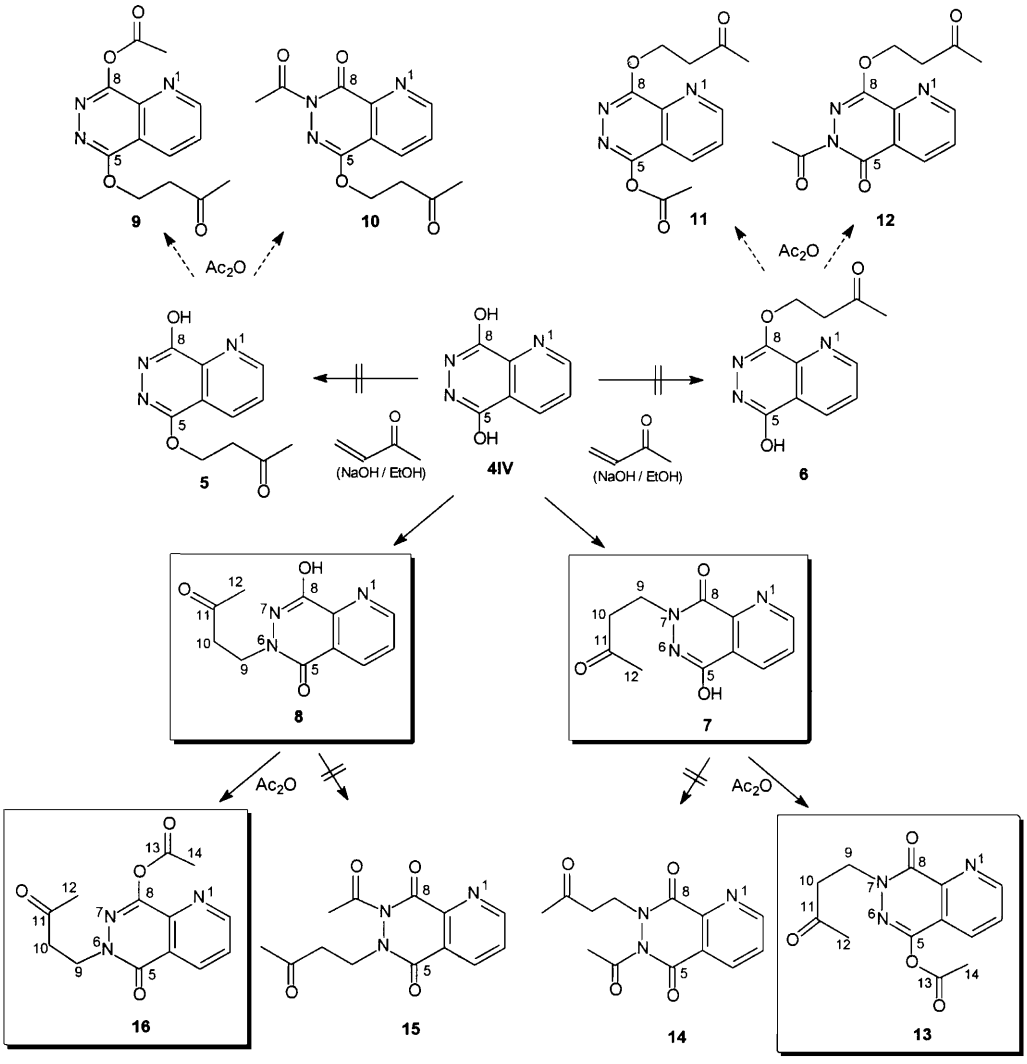
Scheme 2. Synthesis and tautomers of quinolinic acid hydrazide

In the ^{1}H NMR spectrum of **4** in *DMSO*-d_6 at 298 K the signals of the OH and/or NH functions of the possible tautomers coincide in a broad singlet at 11.62 ppm with intensity 2. Attempts to separate the possible NH/OH signals by change of the solvent were not successful. However, the observed mutual NOEs between the doublet of a doublet signal of the aromatic H-4 at 8.49 ppm and the broad singlet at 11.62 ppm demonstrated the existence of tautomers **4II** and/or **4IV** with a hydroxy group in position 5 each (Scheme 2). The ^{13}C spectrum of **4** showed one set of seven signals and pointed to a rapid equilibrium between the NH/OH tautomers of **4** and/or the predominance of one tautomer. The chemical shifts of C-5 at 152.7 ppm and C-8 at 142.1 ppm, whose assignment were proved by long-range C,H-correlation experiments, suggest the prevailing existence of the azino-diol form **4IV** [15].

4 was reacted with methyl vinyl ketone as described for maleic hydrazide in Ref. [16]. In order to increase the reactivity of the ambident N,O-nucleophile **4**, the *Michael*-type addition was carried out in ethanolic sodium hydroxide. The reaction afforded a mixture of two 1:1-adducts (**7** and **8**) in a ratio of 2:1. The main product **7** was separated from the minor product **8** by crystallization. Subsequent action of acetic anhydride on **7** and **8** in the presence of a catalytic amount of pyridine yielded the corresponding acetyl-(3-oxobutyl) substituted isomers **13** and **16**.

A priori, the isolated products could have been generated by addition of the oxygen atoms at C-5 or C-8 and/or the ring nitrogen atoms N-6 or N-7 of **4** to the enone. Consequently, the products could be at first 5- or 8-(oxobutyloxy)-pyrido-pyridazinoles **5**, **6**, or tautomers thereof. In case of the more probable *N*-substitution, 7- or 6-(oxobutyl)-pyridopyridazinones **7**, **8**, or tautomers had to be expected (Scheme 3). The subsequent acetylation of **5**–**8** could also have yielded *O*- and/or *N*-acetyl compounds in each case. Hence, eight possible formulas **9**–**16** had to be considered for the prepared acetylated adducts, which finally proved as **13** and **16** (Scheme 3).

Earlier, *Feuer* and *Harmetz* [16] had found by chemical proof that the *Michael* addition of maleic hydrazide to various enones produces *N*-monosubstituted products. They established in the same manner that the acetylation of maleic



Scheme 3. Possible products of the reaction of **4** with methyl vinyl ketone and subsequent acetylation with acetic anhydride

hydrazide yields exclusively acetoxypyridazinones [17]. In case of the so far unknown substituted quinolinic hydrazide derivatives **7**, **8**, **13**, and **16**, one- and two-dimensional NMR analyses allowed to establish unambiguously the correct structures. The complete assignment of all proton, carbon, and nitrogen signals (Tables 1, 2) was achieved on basis of ^{1}H, ^{13}C, ^{15}N NMR, and 1D NOE spectra as well as 2D C,H-correlation experiments over one and multiple bonds.

Structure elucidation of the acetoxy-(3-oxobutyl)-pyridopyridazines **13** *and* **16**

The acetylated adducts had to be derivatives of definite tautomers of **4**. Hence, the NMR analyses of **13** and **16** were carried out first. The procedure is demonstrated in detail on the example of adduct **13**.

Table 1. NMR data of compounds **13** and **16** (360 MHz ^{1}H/90 MHz ^{13}C/36 MHz ^{15}N, *DMSO*-d_6); numbering: see Scheme 3

	5-Acetoxy-7-(oxobutyl)-pyrido[2,3-*d*]pyridazin-8(7*H*)-one (**13**)				8-Acetoxy-6-(oxobutyl)-pyrido[2,3-*d*]pyridazin-5(6*H*)-one (**16**)			
	^{13}C δ/ppm	^{1}H δ/ppm; J_{HH}/Hz	Relevant long-range correlations	^{15}N δ/ppm; $^2J_{NH}$/Hz	^{13}C δ/ppm	^{1}H δ/ppm; J_{HH}/Hz	Relevant long-range correlations	^{15}N δ/ppm; $^2J_{NH}$/Hz
1	–	–		− 66.0 d (J = 12.3)	–	–		− 76.3 d (J = 11.3)
2	157.2	9.14 dd (J = 4.5, 1.6)	H-4		155.7	9.13 dd (J = 4.6, 1.7)	H-3, H-4	
3	128.2	7.94 dd (J = 8.2, 4.5)	H-2, H-4		127.6	7.91 dd (J = 8.1, 4.6)	H-2	
4	132.6	8.35 dd (J = 8.2, 1.6)	H-2, H-3		135.4	8.62 dd (J = 8.1, 1.7)	H-2	
4a	121.9	–	H-2, H-3		124.9	–	H-3	
5	143.0	–	CH_3-14, H-4		158.5	–	CH_2-9, H-4	
6	–	–		− 80.4 s	–	–		− 195.1 s
7	–	–		− 191.6 s	–	–		− 77.0 s
8	157.2	–	H-2, H-4		144.8	–	CH_3-14, CH_2-9, H-4	
8a	143.7	–	CH_2-9, H-4		140.4	–	H-2, H-4	
9	45.7	4.29 t (J = 7.1)			45.3	4.25 t (J = 7.1)		
10	40.8	2.95 t (J = 7.1)			40.8	2.95 t (J = 7.1)		
11	206.3	–	CH_3-12, CH_2-9, CH_2-10		206.3	–	CH_3-12, CH_2-10, CH_2-9	
12	29.8	2.13 s			29.9	2.15 s		
13	168.9	–	CH_3-14		168.7	–	CH_3-14	
14	20.3	2.45 s			20.1	2.43 s		

The chemical shifts of protons as well as of the hydrogen bearing carbon atoms of the acetoxy-(3-oxobutyl)-pyridopyridazine **13** could easily be obtained from ^{1}H, ^{13}C NMR, and C,H-correlation spectra (Table 1). However, these data did not suffice to determine the position of oxobutyl and acetyl group at the oxygen and nitrogen atoms in positions 5–8 beyond any doubt. These questions could be answered on basis of the concerted evaluation of heteronuclear multiple bond correlation, NOE, and ^{15}N NMR experiments.

The HMBC spectra enabled the assignment of the quaternary carbon atoms, including C-5 and C-8 of the pyridazine ring (Table 1). Moreover, the methyl protons of the acetyl group (CH_3-14) correlated over four bonds with the pyridazine C-5. Thus, the acetyl group had to be attached either to O-5 or to N-6 which applied to isomers **11**–**14** (Scheme 3). The observed correlation between the CH_2-9 protons of the oxobutyl substituent and C-8a indicated the linkage of the oxobutyl residue to N-7 or O-8, which applied again to the formulas **11**–**14**. The correct location of the acetoxy residue at C-5 was determined by 1D NOE measurements. Among others, these experiments revealed the spatial vicinity of the aromatic H-4 and the methyl protons of the acetoxy group (CH_3-14), implying that N-6 must be an imidic nitrogen atom as in case of adducts **11** and **13**, respectively.

Natural abundance ^{15}N NMR spectroscopy decided the still remaining open question of the correct position of the oxobutyl substituent (N-7 or O-8). The proton-coupled ^{15}N NMR spectrum of **13** showed a doublet at − 66.0 ppm and

Table 2. NMR data of compounds **7** and **8** (360 MHz ^{1}H/90 MHz ^{13}C/36 MHz ^{15}N, *DMSO*-d_6); numbering: see Scheme 3

	5-Hydroxy-7-(oxobutyl)-pyrido[2,3-*d*]pyridazin-8(7*H*)-one (**7**)				8-Hydroxy-6-(oxobutyl)-pyrido[2,3-*d*]pyridazin-5(6*H*)-one (**8**)			
	^{13}C δ/ppm	^{1}H δ/ppm; J_{HH}/Hz	Relevant long-range correlations	^{15}N δ/ppm; $^2J_{NH}$/Hz	^{13}C δ/ppm	^{1}H δ/ppm; J_{HH}/Hz	Relevant long-range correlations	^{15}N δ/ppm; $^2J_{NH}$/Hz
1	–	–		− 65.8 d (J = 13.4)	–	–		− 70.9 d (J = 12.3)
2	154.2	9.07 dd (J = 4.5, 1.7)	H-4		154.7	9.10 dd (J = 4.5, 1.7)	H-3, H-4	
3	127.5	7.87 dd (J = 8.1, 4.5)	H-2		126.9	7.86 dd (J = 8.1, 4.5)	H-2	
4	132.9	8.32 dd (J = 8.1, 1.7)	H-2		140.4	8.55 dd (J = 8.1, 1.7)	H-2	
4a	121.4	–	H-3		124.9	–	H-3	
5	149.3	–	H-4		157.3	–	CH_2-9, H-4	
6	–	–		− 74.6 s	–	–		− 190.8 s
7	–	–		− 187.3 s	–	–		− 80.4 s
8	156.3	–	CH_2-9, H-4		150.6	–	CH_2-9	
8a	144.3	–	H-2, H-4		140.4	–	H-4	
9	45.1	4.18 t (J = 7.2)			44.8	4.27 t (J = 7.2)		
10	41.2	2.91 t (J = 7.2)			41.2	2.90 t (J = 7.2)		
11	206.6	–	CH_3-12, CH_2-10		206.6	–	CH_3-12, CH_2-10	
12	29.8	2.15 s			29.9	2.14 s		
OH	–	11.89 s, b				11.40 s, b		

two singlets at − 80.4 and − 191.6 ppm. Hence, the fused ring system contains three different types of nitrogen atoms which ruled out the structure of **11** with two approximately equivalent nitrogens in the pyridazine moiety. The signal of the pyridine nitrogen at − 66.0 ppm is splitted up into a doublet by the $^2J_{NH}$ (12.3 Hz) coupling with H-2. The chemical shift value of − 80.4 ppm is in good accordance with the typical shift range of imidic nitrogens of type N-6. The chemical shift of − 191.6 ppm is characteristic for a N-disubstituted amide nitrogen such as N-7 bearing the oxobutyl residue [18, 19]. Together these facts prove that the structure of the acetylated adduct represents 5-acetoxy-7-(3-oxobutyl)-pyrido[2,3-*d*]pyridazin-8(7*H*)-one (**13**).

The analogous NMR analysis of the second acetylated adduct resulted in the structure of 8-acetoxy-6-(3-oxobutyl)-pyrido[2,3-*d*]pyridazin-5(6*H*)-one (**16**, Scheme 3); Table 1 lists the complete NMR data.

Structure elucidation of the hydroxy-(3-oxobutyl)-pyridopyridazines **7** *and* **8**

With the knowledge of the structures of **13** and **16** in mind, the structure of their precursors could be easily predicted: they had to be the corresponding 5- and 8-hydroxy compounds **7** and **8** (Scheme 3). Nevertheless, we were able to prove the expected structures of the isomers **7** and **8** by similar NMR analyses as described above independently. The decision about the O- or N-substitution in the pyridazine ring was made by ^{15}N NMR and HMBC experiments. The proton-coupled ^{15}N NMR

spectra of both compounds showed signals for three types of nitrogens with typical chemical shift values for pyridine, imidic, and disubstituted amide type nitrogens [18, 19]. Moreover, only the signals of the pyridine nitrogens at − 65.8 ppm for **7** and − 70.9 ppm for **8** are splitted into narrow doublets with $^2J_{NH}$ of about 13 Hz. Hence, no NH functions are present in these molecules, indicating the 5- or 8-hydroxy form of **7** and **8** as well as oxobutyl substitution at the nitrogens N-7 or N-6, respectively. Long-range C,H-correlations *via* ^3J(C,H) of CH_2-9 with C-8 in case of **7** and C-5 in case of **8** answered the question of the correct location of the oxobutyl residue at N-7 and N-6. In case of adduct **7**, an observed mutual NOE between the broad singlet at 11.89 ppm and the aromatic H-4 confirmed the existence of the 5-hydroxy tautomer.

The complete assigned proton, carbon, and nitrogen chemical shifts of adducts **7** and **8** are listed in Table 2. They are consistent with the structures of 5-hydroxy-7-(3-oxobutyl)-pyrido[2,3-*d*]pyridazin-8(7*H*)-one (**7**) and 8-hydroxy-6-(3-oxobutyl)-pyrido[2,3-*d*]pyridazin-5(6*H*)-one (**8**).

As expected, the alkali-catalyzed *Michael*-type addition of the ambident anions of **4** to the β-carbon of methyl vinyl ketone took place regioselectively under attack of the more nucleophilic N-6 and N-7, yielding exclusively the isomeric *N*-(oxobutyl) hydroxy compounds **7** and **8**. Subsequent acetylation gave exclusively *O*-acetoxy derivatives with amide-imidic acid substructure.

Pharmacological testing

The preliminary pharmacological testing of pyrido[2,3-*d*]pyridazinones **7**, **8**, **13**, and **16** showed promising results [20]. All compounds were assessed for acute toxicity; their LD_{50} in mice was found to be >1000 mg/kg, thus they are relatively nontoxic. The test compounds displayed sedative activity. In the *Irwin* test with mice the spontaneous activity, motoric, and sensomotoric response as well as the rightening reflex were suppressed by all four compounds **7**, **8**, **13**, and **16** after oral administration of 100 mg per kg weight. The 5-acetoxy compound **13** applied in a dose of 300 mg/kg *p.o.* in mice exhibited borderline anticonvulsant activity in the metrazole convulsion model. Further pharmacological tests are in progress.

Experimental

Melting points were determined on a Kofler melting point apparatus and are uncorrected. Thin-layer chromatograms (TLC) were run on TLC plastic sheets with silica gel 60 F254 (E. Merck, Darmstadt); elution systems: CH_2Cl_2:MeOH = 8:2 (ES 1), toluene:MeOH = 8:2 (ES 2). The spots were detected by visual examination under UV light (254 and 366 nm). Infrared spectra were recorded with a Perkin Elmer 2000 FTIR spectrophotometer in KBr discs; frequencies are reported in cm^{-1} (s = strong, m = medium, w = weak). NMR spectra were aquired on a Bruker 360 MHz NMR spectrometer equipped with an Aspect 3200 computer system and operating at an observation frequency of 360.98 MHz for ^{1}H, 90.59 MHz for ^{13}C, and 36.45 MHz for ^{15}N. 1D and 2D NMR experiments were performed using a reverse geometry 5 mm broad-band probehead. The HH-COSY [21], HSQC [22], HMBC [23], and 1D NOE difference [24] experiments were performed using the standard pulse programs. The HMBC experiments were optimized for J(C,H) of 2, 4, 8, and 10 Hz (delays of 250, 125, 62.5, and 50 ms, respectively). All NOEs were measured in samples degassed by the freeze-pump-thaw technique. 15–25 mg of the substances were dissolved in 0.5 ml of deuterated *DMSO* and measured at 298 K. The chemical shifts are reported as δ units (ppm) with *TMS* as internal standard for ^{1}H and

^{13}C NMR spectra. For the natural abundance ^{15}N NMR spectra, nitromethane in *DMSO*-d_6 (50% v/v solution) was used as external standard ($\delta = 0.0$ ppm). The ^{15}N NMR spectra were obtained from solutions containing 180 mg of the respective compound and 0.03 *M* Cr(*acac*)$_3$. Elemental analyses (C, H, N) were performed by the Chair of Organic Chemistry, Faculty of Chemistry and Chemical Engineering, University of Ljubljana; the results agreed favourably with the calculated values.

Pyrido[2,3-d]pyridazine-5,8(6H,7H)-dione (**4**; $C_7H_5N_3O_2$)

4 was prepared according to Ref. [14]. Yield: 5.1 g (85%); m.p.: 311°C (Ref. [14]: 311–312°C). TLC (ES 1): $R_f = 0.37$; IR (KBr): $\nu = 3426$ m, 1700 s, 1648 m, 1590 m, 1419 m, 1318 m, 1302 s, 1040 m, 820 m cm^{-1}; ^1H NMR (*DMSO*-d_6): $\delta = 7.85$ (dd, $J = 8.0$, 4.5 Hz, 1H, H-3), 8.49 (dd, $J = 8.0$, 1.6 Hz, 1H, H-4), 9.09 (dd, $J = 4.5$, 1.6 Hz, 1H, H-2), 11.62 (s, broad, 2H, OH/NH) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 124.7$ (C-4a), 127.1 (C-3), 134.5 (C-4), 142.1 (C-8), 152.7 (C-5), 154.7 (C-2), 157.2 (C-8a) ppm.

5-Hydroxy-7-(3-oxobutyl)-pyrido[2,3-d]pyridazin-8(7H)-one (**7**; $C_{11}H_{11}N_3O_3$)
and 8-hydroxy-6-(3-oxobutyl)-pyrido[2,3-d]pyridazin-5(6H)-one (**8**; $C_{11}H_{11}N_3O_3$)

To a solution of 3.3 g (20 mmol) of **4** in 25 cm^3 EtOH, 1.7 g (22 mmol) of methyl vinyl ketone and one drop of 20% aqueous NaOH were added. The reaction mixture was refluxed for 4 h. After cooling to room temperature, the precipitated crystals were filtered of; recrystallization from EtOH/Et_2O afforded 2.0 g (42.9%) of **7**.

M.p.: 220–225°C; TLC (ES 1): $R_f = 0.48$; IR (KBr): $\nu = 2960$ m, 1710 s, 1662 s, 1590 s, 1483 m, 1375 m, 1255 m, 1162 m, 824 m cm^{-1}; ^1H, ^{13}C, and ^{15}N NMR: see Table 2.

Cooling of the filtrate to 0°C afforded 1.0 g (21.5%) of **8**; m.p.: 127°C (EtOH/Et_2O); TLC (ES 1): $R_f = 0.42$; IR (KBr): $\nu = 3117$ m, 1714 s, 1643 s, 1561 m, 1485 m, 1376 m, 1169 m, 798 m cm^{-1}; ^1H, ^{13}C, and ^{15}N NMR: see Table 2.

5-Acetoxy-7-(3-oxobutyl)-pyrido[2,3-d]pyridazin-8(7H)-one (**13**; $C_{13}H_{13}N_3O_4$)
and 8-acetoxy-6-(3-oxobutyl)-pyrido[2,3-d]pyridazin-5(6H)-one (**16**; $C_{13}H_{13}N_3O_4$)

Acetic acid anhydride (3 cm^3) and one drop of pyridine were added to 0.23 g (1 mmol) of **7**. The reaction mixture was heated to reflux for a few minutes. After cooling to room temperature, **13** crystallized and was obtained in quantitative yield.

M.p.: 151°C (EtOH/Et_2O); TLC (ES 2): $R_f = 0.63$; IR (KBr): $\nu = 2975$ m, 1769 s, 1703 s, 1677 s, 1599 s, 1430 m, 1330 s, 1196 m, 1088 m, 890 m, 814 s, 687 m cm^{-1}; ^1H, ^{13}C, and ^{15}N NMR: see Table 1.

The same procedure applied to **8** (1 mmol) afforded **16** in quantitative yield; m.p.: 78°C (EtOH/Et_2O); TLC (ES 2): $R_f = 0.65$; IR (KBr): $\nu = 2980$ m, 1767 s, 1711 s, 1668 s, 1578 m, 1194 s, 1143 m, 1097 m, 833 m, 791 m cm^{-1}; ^1H, ^{13}C, and ^{15}N NMR: see Table 1.

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