Pyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5]tetrazine-4(3*H*)-ones, a new class of temozolomide heteroanalogues

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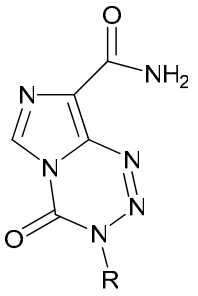
Dedicated to Prof Nicolò Vivona on the occasion of his 70th anniversary

Abstract
Twelve derivatives of new ring system pyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5]tetrazine were prepared in good yields by reaction of 2-diazo-3-ethoxycarbonyl-pyrrolo[3,2-*b*]pyridine with alkyl- or aryl-isocyanates. Nine derivatives, screened by the National Cancer Institute (Bethesda, MD) for the *in vitro* one dose primary anticancer assay against a panel of about 60 human tumor cell lines, showed no significant activity.

Keywords: Temozolomide Mitozolomide, pyrrolo[2,1-*d*][1,2,3,5]tetrazinones; Antitumor activity, pyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5]tetrazines

Introduction

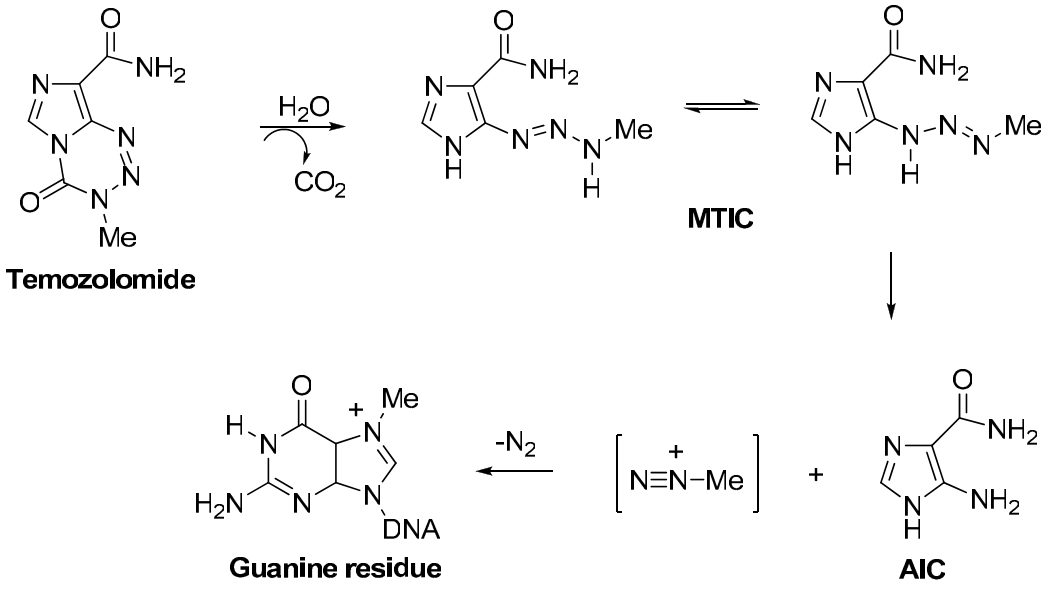
In the past two decades azolotetrazinones have been the focus of medicinal chemists because of the outstanding antineoplastic activity shown by two imidazotetrazine derivatives temozolomide, and mitozolomide. The lead compound, mitozolomide, has demonstrated antitumor activity in patients with small cell carcinoma of the lung and malignant melanoma; however, severe and unpredictable myelosuppression effects precluded its further development.[1-3]



Mitozolomide R=CH_2CH_2Cl
Temozolomide R=CH_3

Figure 1. Azolotetrazinones

Temozolomide (TMZ), a 3-methyl analog of mitozolomide, is currently on the market with the trade name Temodal® and is used against malignant melanoma, mycosis fungoides, and brain tumors.[4-6] TMZ is a prodrug that undergoes spontaneous degradation at physiologic pH to form the 5-(3-methyl-1-triazeno)-imidazole-4-carboxamide (MTIC), which subsequently fragments into 5-aminoimidazole-4-carboxamide (AIC) and the DNA-methylating agent, methyldiazonium. This latter likely undergoes an S_N2 alkylation at the N-7 and/or O-6 sites of a guanine residue (Scheme 1).[7,8]



Temozolomide **MTIC** **Guanine residue** **AIC**

Scheme 1. Temozolomide mechanism of activation.

More recently temozolomide and its esters are primarily indicated for the treatment of malignant glioma.[9-11]

We have previously reported on the synthesis and the antitumor activity of pyrrolo[2,1-*d*][1,2,3,5]tetrazine-4(3*H*)-ones (**1**) which exhibited a significant growth inhibition efficacy in many cancer cell lines, with GI_{50} values in the low micromolar or sub-micromolar range and reaching, in some cases nanomolar concentrations.[12,13]

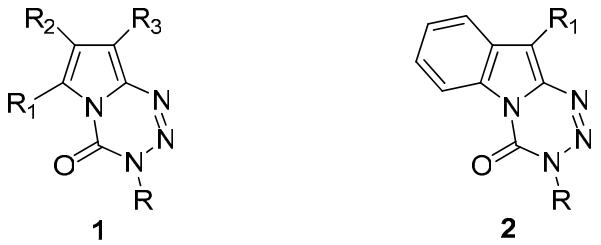


Figure 2. Pyrrolo-tetrazinones (**1**) and indolo-tetrazinones **(2**).

Pyrrolotetrazinones, did not show any selectivity with respect to the CNS cancer and melanoma sub-panels against which temozolomide showed curative properties, however they exhibited excellent responses in the breast cancer and leukaemia sub-panels.[13] Nanostructured lipid carrier (NLC) loaded with pyrrolotetrazinones tested *in vitro* against hepatocellular carcinoma (HuH-6 and HuH-7) and human prostate cancer (PC-3) cell lines have shown an enhancement of cytotoxicity.[14] SAR studies as well as the computerised analysis COMPARE[15] indicated that pyrrolotetrazinones have a mode of action different from that of temozolomide. Studies directed to elucidate the biochemical mechanism of action of this class of compounds indicated that they interfere with the microtubule network, block mitosis and that mitochondria and caspases play a central role in the activation of the executioner phase of pyrrolotetrazinones-induced apoptosis.[16]

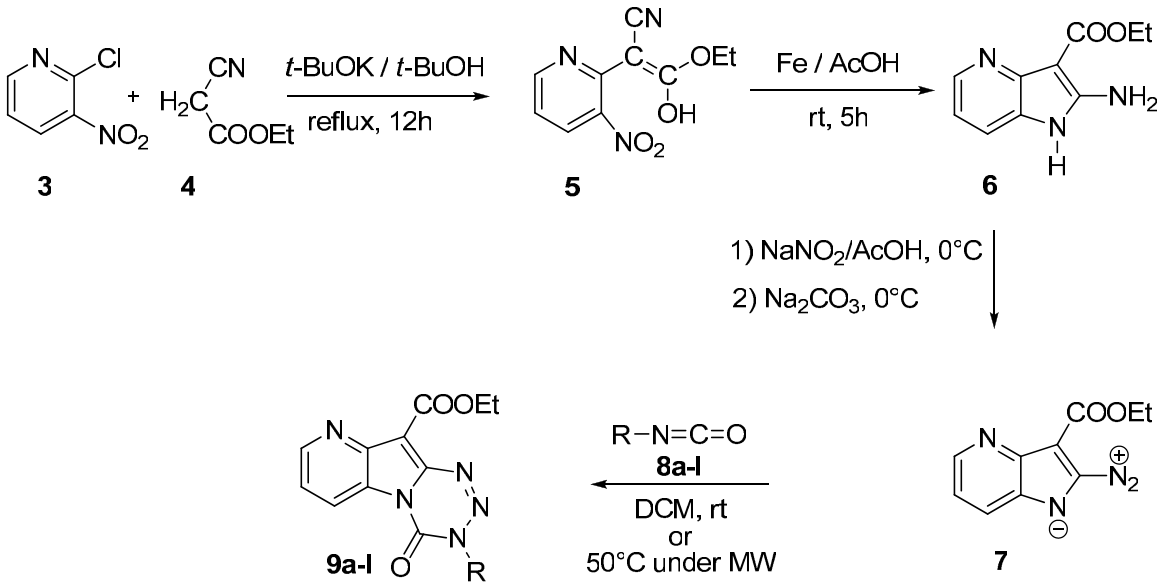
Benzocondensation of the pyrrolotetrazinone ring led to [1,2,3,5]tetrazino[5,4-*a*]indole-4-one (**2**) derivatives which showed antiproliferative activity in the micromolar range. These latter, at variance with pyrrolotetrazinones, exhibited good selectivity with respect to the CNS sub-panel in which the lead compound, as already stated, showed curative properties.[17]

Encouraged by these results we thought it was interesting to verify whether the condensation of the pyrrolotetrazine system with a pyrido moiety increases the antineoplastic activity.

In this paper we focus our attention on the synthesis of the new ring system pyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5]tetrazine **9** by reaction of the key intermediate 2-diazo-3-ethoxycarbonyl-pyrrolo[3,2-*b*]pyridine **7** with alkyl- or aryl-isocyanates.

Results and Discussion

The synthesis of pyridopyrrolo[1,2,3,5]tetrazines **9a-l** was accomplished by the route depicted in Scheme 2.



[a] For designation of R in **8a-l** and **9a-l**, see Table 1.

Scheme 2[a]

Reaction between the 2-chloro-3-nitropyridine **3** and the potassium enolate of ethyl cyanoacetate **4** gave derivative **5** in high yield (92%). Reduction of this latter with iron and acetic acid at room temperature yielded the 2-amino-3-ethoxycarbonyl-pyrrolo[3,2-*b*]pyridine **6** (yield 80%). The 2-diazo-3-ethoxycarbonyl-pyrrolo[3,2-*b*]pyridine **7** was obtained, in excellent yield (96%), by diazotization of the corresponding amine **6.** The reaction was carried out in 80% acetic acid with stoichiometric amount of sodium nitrite under nitrogen atmosphere in the dark followed by neutralization with sodium carbonate. The strict control of the temperature at 0°C both during diazotization and neutralization is crucial in obtaining a good yield. The structure of 2-diazo-pyrrolo[3,2-*b*]pyridine **7** was confirmed by analytical and spectral data (IR, ^{1}H and ^{13}C NMR). In particular the IR spectra showed a sharp and strong band at 2198 cm^{-1}.

The pyridopyrrolo-tetrazines **9a-l** have been prepared in moderate to good yields (35-80%) by reaction of the diazo **7** with stoichiometric amounts of proper isocyanates **8a-l** in DCM at room temperature for 12-48 h. The same reactions carried out under microwave irradiation, with a CEM discover apparatus, gave the pyridopyrrolo-tetrazines **9a-l** with higher yield (62-95%) in a shorter time (3 min) (Table 1). The structure of all derivatives **9** was confirmed by spectroscopic data.

Table 1. Pyridopyrrolo-tetrazines **9a-l**

Entry	Isocyanate	R	Product	Yield (%) CM[a]	Yield (%) MM[b]
1	**8a**	C_6H_5	**9a**	55	75
2	**8b**	C_6H_{11}	**9b**	80	95
3	**8c**	*o*-Cl-C_6H_4	**9c**	38	62
4	**8d**	*m*-Cl-C_6H_4	**9d**	40	69
5	**8e**	*p*-Cl-C_6H_4	**9e**	40	70
6	**8f**	*o*-OMe-C_6H_4	**9f**	45	75
7	**8g**	*m*-OMe-C_6H_4	**9g**	47	76
8	**8h**	CH_2CH_2Cl	**9h**	42	70
9	**8i**	CH_2-C_6H_5	**9i**	57	86
10	**8j**	*o*-Me-C_6H_4	**9j**	40	68
11	**8k**	*m*-Me-C_6H_4	**9k**	35	62
12	**8l**	*p*-Me-C_6H_4	**9l**	38	65

[a]**CM**= Conventional method; [b]**MM**= Microwave method

Biological screenings were performed, on nine selected pyridopyrrolotetrazines (**9a**, **9b 9c**, **9d**, **9e**, **9f**, **9g**, **9k**, **9i**), by the National Cancer Institute (Bethesda, MD), at one dose concentration (10^{-5} M), for the *in vitro* disease-oriented antitumor screenings against a panel of about 60 human tumor cell lines that have grouped in disease sub-panel including leukaemia, non-small lung, colon, central nervous system, melanoma, ovarian, renal, prostate, and breast tumors cell lines.[18] The results obtained take into consideration the growth inhibitory power (GI_{50}). None of the tested compounds showed significant activity.

Conclusions

In conclusion the annellation of the pyrido moiety to the pyrrolotetrazine system, also regarded as the aza-substitution in position 4 of the [1,2,3,5]tetrazino[5,4-*a*]indole-4-ones led to the new ring system pyrido[2',3':4,5]pyrrolo[2,1*d*][1,2,3,5]tetrazine which was devoid of significant activity.

Experimental Section

General Procedures. All melting points were taken on a Büchi-Tottoli capillary apparatus and are uncorrected; IR spectra were determined in bromoform with a Jasco FT/IR 5300 spectrophotometer; ^1H and ^{13}C NMR spectra were measured at 200 and 50.3 MHz, respectively

in DMSO-d6 or $CDCl_3$ solution, using a Bruker AC series 200 MHz spectrometer (TMS as internal reference). Column chromatography was performed with Merck silica gel 230-400 Mesh ASTM or with Büchi Sepacore chromatography module (prepacked cartridge system). Elemental analyses (C, H, N) were within ±0.4% of the theoretical values. Microwave experiments were carried out using a CEM Discover LabmateTM microwave apparatus.

Synthesis of 2-(3-nitropyridin-2-yl)-3-ethoxy-3-hydroxyacrylonitrile (5). To a stirred solution of *t*-BuOK (0.85 g, 7.6 mmol) in *t*-BuOH (10 ml) ethyl cyanoacetate **4** (0.94 g, 8.3 mmol) was added. After 5 min, a solution of 2-chloro-3-nitropyridine **3** (0.60 g, 3.8 mmol) in *t*-BuOH was added and the mixture heated to reflux for 12 h. The red solution was cooled to room temperature and the solvent was evaporated under reduced pressure. The residue was washed with 1M hydrochloric acid, water and recrystallized from methanol to give compound **5** (0.82 g) as orange crystals, m.p. 136-137°C, yield 92%. IR: ν 3556 (OH), 2190 (CN) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.26 (t, *J = 7.0 Hz*, 3H), 4.23 (q, *J = 7.0 Hz*, 2H), 7.05 (d, *J = 6.1 Hz*, 1H), 8.40-8.49 (m, 2H), 14.49 (bs, OH); ^{13}C NMR (DMSO-d_6): δ 14.3 (q), 60.2 (t), 112.2 (d), 116.2 (s), 125.9 (s), 134.5 (s), 138.8 (d), 142.0 (d), 146.1 (s), 168.3 (s). *Anal*. Calcd for $C_{10}H_9N_3O_4$: C, 51.07; H 3.86; N, 17.87. Found: C, 51.27; H 3.69; N, 17.78.

Synthesis of 2-amino-3-(ethoxycarbonyl)pyrrolo[3,2-*b*]pyridine (6). Iron powder (1.50 g, 26.9 mmol) was added to a solution of **5** (1.01 g, 4.3 mmol) in acetic acid (7.5 ml). The mixture was stirred at room temperature for 5 h and the solid formed was collected and recrystallized from ethanol to give amine **6** (0.71 g): brown powder, m.p.111-112°C, yield 80%. IR: ν 3557 and 3280 (NH_2 and NH), 1691 (CO) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.28 (t, *J = 7.1 Hz*, 3H), 4.25 (q, *J = 7.1 Hz*, 2H), 6.86 (dd, *J = 7.8 5.0 Hz*, 1H), 7.39 (dd, *J = 7.8 1.4 Hz*, 1H), 8.08 (dd, *J = 5.0 1.4 Hz*, 1H). ^{13}C NMR (DMSO-d_6): δ 15.3 (q), 58.6 (t), 84.5 (s), 114.8 (d), 115.9 (d), 127.1 (s), 141.3 (d), 145.5 (s), 156.1 (s), 165.7 (s). *Anal*. Calcd for $C_{10}H_{11}N_3O_2$: C, 58.53; H 5.40; N, 20.48. Found: C, 58.64; H 5.20; N, 20.22.

Synthesis of 2-diazo-3-ethoxycarbonyl-pyrrolo[3,2-*b*]pyridine (7). To a solution of 2-amino-3-ethoxycarbonyl-pyrrolo[3,2-*b*]pyridine **6** (0.62 g, 3 mmol) in glacial acetic acid (6 ml) a solution of sodium nitrite (3 mmol) in a small amount of water (1 ml) was added dropwise at 0°C under a nitrogen atmosphere. The mixture was neutralized at 0°C with saturated Na_2CO_3 and the yellow solid precipitated was filtered off and dried under vacuum and in the dark. The crude product, quickly shaken in cyclohexane and filtered off, gave 2-diazo-pyrrolo-pyridine **7** (0.62 g): brown powder, m.p. 162°C dec., yield 96%. IR: ν 2198 (N_2^+), 1714 (CO) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.34 (t, *J = 7.0 Hz*, 3H), 4.35 (q, *J = 7.0 Hz*, 2H), 6.99-8.72(m, 3H). ^{13}C NMR (DMSO-d_6): δ 14.6 (q), 59.1 (t), 114.6 (s), 114.8 (d), 129.9 (s), 131.1 (s), 140.5 (d), 143.1 (s), 150.9 (d), 162.0 (s). *Anal*. Calcd for $C_{10}H_8N_4O_2$: C, 55.56; H 3.73; N, 25.91. Found: C, 55.24; H 3.96; N, 25.72

General procedure for the synthesis of pyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5] tetrazine-4-(3*H*)-ones (9a-l)

A. Conventional method (CM)

To a solution of **7** (0.86 g, 2 mmol) in anhydrous DCM (10 ml), the suitable isocyanate **8a-l** (2 mmol) in anhydrous DCM (10 ml) was added dropwise at room temperature in the dark under a nitrogen atmosphere. The reaction mixture was stirred for 12-48 h, then the solvent was evaporated under reduced pressure and the residue purified by chromatography with DCM as eluent to give **9a-l** (yields 35-80%).

B. Microwave method (MM)

A mixture of **7** (0.43 g, 1 mmol), the suitable isocyanate **8a-l** (1 mmol), and anhydrous DCM (5 ml) was irradiated at power of 150 W, temperature of 50°C for 3 min. The solvent was evaporated under reduced pressure and the residue purified by chromatography with DCM as eluent to give **9a-l** (yields 62-95%).

Ethyl 3-phenyl-4-oxo-3,4-dihydropyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5]tetrazine-10-carboxylate (9a). Yellow powder, m.p. 162-163°C, yield 55% (CM), 75% (MM). IR: ν 1736 (CO), 1711 (CO) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.40 (t, *J = 7.1 Hz*, 3H), 4.48 (q, *J = 7.1 Hz*, 2H), 7.62-7.69 (m, 6H), 8.76 (d, *J = 8.3 Hz*, 1H), 8.95 (d, *J = 3.6 Hz*, 1H). ^{13}C NMR (DMSO-d_6): δ 14.4 (q), 60.7 (t), 121.7 (d), 122.9 (s), 123.8 (d), 124.3 (s), 126.7 (2xd), 129.1 (2xd), 129.5 (d), 137.3 (s), 140.8 (s), 141.1 (s), 143.3 (s), 149.8 (d), 161.6 (s). *Anal*. Calcd for $C_{17}H_{13}N_5O_3$: C, 60.89; H 3.91; N, 20.89. Found: C, 60.69; H 4.13; N, 20.79.

Ethyl 3-cyclohexyl-4-oxo-3,4-dihydropyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5] tetrazine-10-carboxylate (9b). Yellow powder, m.p. 213-214°C, yield 80% (CM), 95% (MM). IR: ν 1705 (CO), 1650 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.05-2.11 (m, 14H), 4.63 (q, *J = 7.1 Hz*, 2H), 7.54 (dd, *J = 8.5 4.6 Hz*, 1H), 8.82 (dd, *J = 5.0 1.5 Hz*, 1H), 9.02 (dd, *J = 4.6 1.5 Hz*, 1H). ^{13}C NMR ($CDCl_3$): δ 14.5 (q), 25.0 (t), 25.7 (2xt), 31.9 (2xt), 58.3 (d), 61.6 (t), 105.2 (s), 121.4 (d), 123.1 (s), 124.4 (d), 140.4 (s), 143.9 (s), 150.7 (d), 156.7 (s), 161.7 (s). *Anal*. Calcd for $C_{17}H_{19}N_5O_3$: C, 59.81; H 5.61; N, 20.52. Found: C, 59.63; H 5.82; N, 20.32.

Ethyl 3-(2-chlorophenyl)-4-oxo-3,4-dihydropyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5] tetrazine-10-carboxylate (9c). Yellow powder, m.p. 139°C, yield 38% (CM), 62% (MM). IR: ν 1745 (CO), 1711 (CO) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.41 (t, *J = 7.1 Hz*, 3H), 4.50 (q, *J = 7.1 Hz*, 2H), 7.67-7.86 (m, 5H), 8.75 (d, *J = 8.2 Hz*, 1H), 8.98 (d, *J = 3.8 Hz*, 1H). ^{13}C NMR (DMSO-d_6): δ 14.3 (q), 60.9 (t), 105.7 (s), 122.2 (d), 123.1 (s), 123.9 (d), 128.6 (d), 130.3 (d), 130.6 (d), 131.5 (s), 132.3 (d), 134.3 (s), 139.8 (s), 140.0 (s), 142.7 (s), 150.1 (d), 160.9 (s). *Anal*. Calcd for $C_{17}H_{12}ClN_5O_3$: C, 55.22; H 3.27; N, 18.94. Found: C, 55.39; H 3.16; N, 18.83.

Ethyl 3-(3-chlorophenyl)-4-oxo-3,4-dihydropyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5] tetrazine-10-carboxylate (9d). Yellow powder, m.p. 145°C, yield 40% (CM), 69% (MM). IR: ν 1739 (CO), 1712 (CO) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.39 (t, *J = 6.9 Hz*, 3H), 4.48 (q, *J = 6.9 Hz*, 2H), 7.70-7.81 (m, 5H), 8.75 (d, *J = 8.2 Hz*, 1H), 8.95 (d, *J = 3.5 Hz*, 1H). ^{13}C NMR (DMSO-d_6): δ 14.3 (q), 60.8 (t), 104.8 (s), 121.9 (d), 123.0 (s), 123.8 (d), 125.5 (d), 126.6 (d),

129.5 (d), 130.9 (d), 133.1 (s), 138.4 (s), 140.0 (s), 140.5 (s), 142.7 (s), 150.0 (d), 161.0 (s). *Anal*. Calcd for $C_{17}H_{12}ClN_5O_3$: C, 55.22; H 3.27; N, 18.94. Found: C, 55.12; H 3.43; N, 18.81.

Ethyl 3-(4-chlorophenyl)-4-oxo-3,4-dihydropyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5] tetrazine-10-carboxylate (9e). Yellow powder, m.p. 136-137°C, yield 40% (CM), 70% (MM). IR: ν 1732 (CO), 1712 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.52 (t, *J = 7.1 Hz*, 3H), 4.65 (q, *J = 7.1 Hz*, 2H), 7.55-7.68 (m, 5H), 8.82 (dd, *J = 8.3 1.4 Hz*, 1H), 9.05 (dd, *J = 3.9 1.4 Hz*, 1H). ^{13}C NMR ($CDCl_3$): δ 14.5 (q), 61.9 (t) 100.0 (s), 107.5 (s), 122.1 (d), 123.8 (s), 124.5 (d), 127.0 (2xd), 129.6 (2xd), 135.3 (s), 135.6 (s), 139.0 (s), 140.3 (s), 151.0 (d), 161.4 (s). *Anal*. Calcd for $C_{17}H_{12}ClN_5O_3$: C, 55.22; H 3.27; N, 18.94. Found: C, 55.17; H 3.33; N, 18.87.

Ethyl 3-(2-methoxyphenyl)-4-oxo-3,4-dihydropyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5] tetrazine-10-carboxylate (9f). Yellow powder, m.p. 157-158°C, yield 45% (CM), 75% (MM). IR: ν 1743 (CO), 1709 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.52 (t, *J = 7.1 Hz*, 3H), 3.85 (s, 3H), 4.65 (q, *J = 7.1 Hz*, 2H), 7.12-7.21 (m, 2H), 7.47-7.61 (m, 3H), 8.81 (d, *J = 8.7 Hz*, 1H), 9.04 (d, *J = 3.9 Hz*, 1H). ^{13}C NMR ($CDCl_3$): δ 14.5 (q), 56.0 (q), 61.8 (t), 112.2 (s), 112.3 (d), 121.1 (d), 121.8 (d), 123.6 (s), 124.5 (d), 125.4 (s), 129.0 (d), 132.1 (d), 139.6 (s), 140.2 (s), 143.7 (s), 150.8 (d), 155.1 (s), 161.6 (s). *Anal*. Calcd for $C_{18}H_{15}N_5O_4$: C, 59.18; H 4.14; N, 19.17. Found: C, 59.04; H 4.22; N, 19.20.

Ethyl 3-(3-methoxyphenyl)-4-oxo-3,4-dihydropyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5] tetrazine-10-carboxylate (9g). Yellow powder, m.p. 156-157°C, yield 47% (CM), 76% (MM). IR: ν 1745 (CO), 1711 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.53 (t, *J = 7.0 Hz*, 3H), 3.89 (s, 3H), 4.62 (q, *J = 7.0 Hz*, 2H), 7.07-7.24 (m, 3H), 7.46-7.60 (m, 2H), 8.83 (d, *J = 8.4 Hz*, 1H), 9.05 (d, *J = 4.5 Hz*, 1H). ^{13}C NMR ($CDCl_3$): δ 14.5 (q), 55.6 (q), 61.8 (t), 107.1 (s), 11.7 (d), 115.5 (d), 118.1 (d), 122.0 (d), 123.8 (s), 124.5 (d), 130.1 (d), 137.8 (s), 139.2 (s), 140.3 (s), 143.7 (s), 150.9 (d), 160.2 (s), 161.5 (s). *Anal*. Calcd for $C_{18}H_{15}N_5O_4$: C, 59.18; H 4.14; N, 19.17. Found: C, 59.26; H 4.05; N, 19.09.

Ethyl 3-(2-chloroethyl)-4-oxo-3,4-dihydropyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5] tetrazine-10-carboxylate (9h). Yellow powder, m.p. 116-117°C, yield 42% (CM), 70% (MM). IR: ν 1743 (CO), 1714 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.51 (t, *J = 7.1 Hz*, 3H), 4.04 (t, *J = 6.1 Hz*, 2H), 4.63 (q, *J = 7.1 Hz*, 2H), 4.84 (t, *J = 6.1 Hz*, 2H), 7.57 (dd, *J = 8.5 4.6 Hz*, 1H), 8.81 (dd, *J = 8.5 1.5 Hz*, 1H), 9.05 (dd, *J = 4.6 1.5 Hz*, 1H). ^{13}C NMR ($CDCl_3$): δ 14.5 (q), 40.9 (t), 50.6 (t), 61.8 (t), 121.9 (d), 123.4 (s), 124.4 (d), 135.2 (s), 139.5 (s), 140.8 (s), 143.6 (s), 150.9 (d), 161.5 (s). *Anal*. Calcd for $C_{13}H_{12}ClN_5O_3$: C, 48.53; H 3.76; N, 21.77. Found: C, 48.37; H 3.82; N, 21.67.

Ethyl 3-benzyl-4-oxo-3,4-dihydropyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5]tetrazine-10-carboxylate (9i). Yellow powder, m.p. 150-152°C, yield 57% (CM), 86% (MM). IR: ν 1745 (CO), 1712 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.47 (t, *J = 7.1 Hz*, 3H), 4.57 (q, *J = 7.1 Hz*, 2H), 5.61 (s, 2H), 7.18-7.53 (m, 6H), 8.76 (dd, *J = 8.5 1.5 Hz*, 1H), 8.95 (dd, *J = 4.6 1.5 Hz*, 1H). ^{13}C NMR ($CDCl_3$): δ 14.5 (q), 53.6 (t), 61.8 (t), 121.6 (d), 122.9 (s), 124.4 (d), 123.2 (s), 127.2 (2xd), 128.9 (d), 129.0 (2xd), 134.5 (s), 139.2 (s), 140.5 (s), 150.8 (d), 158.2 (s), 161.8 (s). *Anal*. Calcd for $C_{18}H_{15}N_5O_3$: C, 61.89; H 4.33; N, 20.05. Found: C, 61.67; H 4.56; N, 20.16

Ethyl 3-(2-methylphenyl)-4-oxo-3,4-dihydropyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5] tetrazine-10-carboxylate (9j). yellow powder, m.p. 181-182°C, yield 40% (CM), 68% (MM). IR: ν 1743 (CO), 1714 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): 1.51 (t, *J = 7.0 Hz*, 3H), 2.31 (s, 3H), 4.63 (q, *J = 7.0 Hz,* 2H), 7.23-7.75 (m, 5H), 8.78-9.04 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 14.5 (q), 17.8 (q), 61.8 (t), 107.1 (s), 121.9 (d), 123.7 (s), 124.5 (d), 127.3 (d), 128.0 (d), 130.7 (d), 131.5 (d), 135.6 (s), 135.9 (s), 139.4 (s), 140.2 (s), 143.7 (s), 150.9 (d), 161.5 (s). *Anal*. Calcd for $C_{18}H_{15}N_5O_3$: C, 61.89; H 4.33; N, 20.05. Found: C, 61.74; H 4.41; N, 20.15.

Ethyl 3-(3-methylphenyl)-4-oxo-3,4-dihydropyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5] tetrazine-10-carboxylate (9k). Yellow powder, m.p. 168-169°C, yield 35% (CM), 62% (MM). IR: ν 1744 (CO), 1711 (CO) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.40 (t, *J = 7.1 Hz*, 3H), 2.44 (s, 3H), 4.48 (q, *J = 7.1 Hz,* 2H), 7.39-7.72 (m, 5H), 8.75 (dd, *J = 8.5 1.4 Hz*, 1H), 8.95 (dd, *J = 4.5 1.4 Hz*, 1H). ^{13}C NMR (DMSO-d_6): δ 14.4 (q), 20.9 (q), 60.7 (t), 104.2 (s), 121.6 (d), 122.9 (s), 123.8 (d), 127.0 (d), 128.9 (d), 130.1 (d), 131.5 (d), 131.7 (s), 137.2 (s), 138.7 (s), 140.6 (s), 142.9 (s), 149.8 (d), 161.1 (s). *Anal*. Calcd for $C_{18}H_{15}N_5O_3$: C, 61.89; H 4.33; N, 20.05. Found: C, 61.93; H 4.28; N, 20.11.

Ethyl 3-(4-methylphenyl)-4-oxo-3,4-dihydropyrido[2',3':4,5]pyrrolo[2,1-*d*][1,2,3,5] tetrazine-10-carboxylate (9l). Yellow powder, m.p. 154°C, yield 38% (CM), 65% (MM). IR: ν 1743 (CO), 1712 (CO) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.52 (t, *J = 7.1 Hz*, 3H), 2.48 (s, 3H), 4.65 (q, *J = 7.1 Hz,* 2H), 7.27-7.57 (m, 5H), 8.83 (d, *J = 8.4 Hz*, 1H), 9.03 (d, *J = 4.5 Hz*, 1H). ^{13}C NMR ($CDCl_3$): δ 14.5 (q), 21.3 (q), 61.8 (t), 100.0 (s), 106.8 (s), 121.9 (d), 123.7 (s), 124.5 (d), 125.7 (2xd), 130.0 (2xd), 134.3 (s), 139.3 (s), 140.0 (s), 143.8 (s), 151.0 (d), 161.5 (s). *Anal*. Calcd for $C_{18}H_{15}N_5O_3$: C, 61.89; H 4.33; N, 20.05. Found: C, 61.82; H 4.42; N, 19.98.

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