Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 2371–2380

BIOORGANIC & MEDICINAL CHEMISTRY

Pyrrolo[2,1-*d*][1,2,3,5]tetrazine-4(3H)-ones, a New Class of Azolotetrazines with Potent Antitumor Activity

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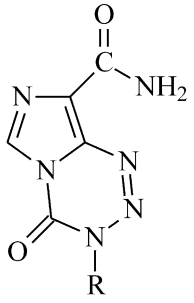
Received 3 January 2003; accepted 28 February 2003

Abstract—Pyrrolo[2,1-d][1,2,3,5]tetrazinones **10a**–**o**, compounds that hold the deaza skeleton of the antitumor drug temozolomide, were prepared by reaction of 2-diazopyrroles **9** and isocyanates. Such a synthetic route represents, among those leading to azolotetrazinones reported so far, the only possible one since attempts to cyclize to the title ring system 2-amino-1-carbamoylpyrroles **11** or the mono substituted 2-triazenopyrrole **12** failed. Compounds **10** were screened at the National Cancer Institute (NCI) for their activity against a panel of about 60 human tumor cell lines. Most of them possess remarkable antineoplastic activity having GI_{50} values in the low micromolar or sub-micromolar range and reaching, in the case of compound **10d**, nanomolar concentrations. The most sensitive cell lines were MDA-N and MDA-MB-435 of the breast sub-panel, and SR, K-562, HL60 (TB) and CCRF-CEM of the leukaemia sub-panel. SAR evaluation and COMPARE computations indicate, for compounds **10**, a mechanism of action different from that of temozolomide.

Introduction

The outstanding antineoplastic activity exhibited by two imidazotetrazinone derivatives, mitozolomide **1** and temozolomide **2**, has attracted remarkable attention on azolotetrazine systems.



1) $R = CH_2CH_2Cl$ **MITOZOLOMIDE**
2) $R = CH_3$ **TEMOZOLOMIDE**

Mitozolomide, synthesized in 1980,[1] is the first azolotetrazine derivative to show excellent antitumor effects in a wide range of murine and xenograft tumors,[2] but in the phase II clinical trials, the recommended dose was too toxic and a deep platelet damage (thrombocytopenia) due to cross-linking of the two strands of the DNA, compromised its clinical use.[3] The 3-methyl congener, temozolomide, showed to be less potent but less toxic than mitozolomide and is currently in the market with the trade name temodal® and is used against malignant melanoma, mycosis fungoides, and brain tumors.[4]

Mitozolomide and temozolomide are prodrugs which undergo, in a nucleophilic micro-environment provided by sequence of guanine residues together with associated water molecules, in the major groove of DNA, ring opening following the nucleophilic attack at C-4 by an activated molecule of water to afford a monoalkyltriazene species, a bioactive alkylating agent. Such reactive entity likely undergoes an S_N2 alkylation of nucleophilic residues in the immediate vicinity such as N-7 and/or O-6 sites of guanine eliminating nitrogen and 5-aminoimidazole-4-carboxamide.[5]

Azolotetrazinones have been prepared through the synthetic routes shown in the Scheme 1. The first route, starting from the diazoazoles **4**, obtained by diazotization followed by neutralization of the corresponding

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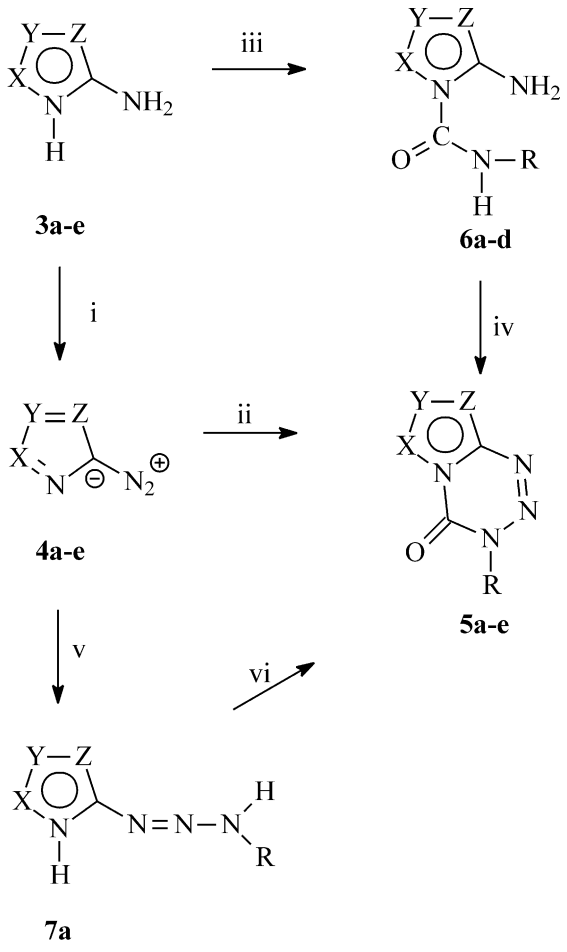
doi:10.1016/S0968-0896(03)00145-7



a X=N, Y=Z=CR (pyrazole)
b X=Y=N, Z=CR (triazole)
c X=N, Y=Z=C-benzofused (indazole)
d X=Z=CR, Y=N (imidazole)
e X=Y=Z=CR (pyrrole)

Scheme 1. Reagents: i $NaNO_2/H^+$, OH^-; ii RNCO; iii RNCO or $ClCO_2Et/RNH_2$ or RNHCOCl; iv $NaNO_2/H^+$; v RNH_2; vi $COCl_2$ or CDI.

azoloamines **3**, upon reaction with alkyl or aryl isocyanate at room temperature in the dark and in a non-hydroxylic solvent, allowed the isolation of the azolotetrazinones **5** in yields from good to excellent. This reaction sequence, that represented the synthetic entry to the azolotetrazinones, was utilized by Ege to obtain pyrazolotetrazinones **5a**, 1,2,3-triazolotetrazinones **5b** and indazolotetrazinones **5c**.[6] Using the same procedure Stevens described the synthesis of many imidazotetrazinones of type **5d** further pyrazolo- and indazolotetrazinone derivatives, and reported the excellent antineoplastic activity of the imidazotetrazinones and pyrazolotetrazinones.[1,7] Cheng, still using the same synthetic pathway prepared two pyrazolotetrazinone derivatives closely related to the active lead compounds imidazotetrazinones and also reported data on their biological evaluation.[8]

The mechanism of this reaction probably involves a stepwise ionic pathway going through an initial nucleophilic attack at the isocyanate carbon, to give a dipolar intermediate which undergoes ring closure, since the reaction is accelerated using polar solvents such as hexamethylphosphoramide as the reaction medium.[1,7]

Later on, alternative syntheses of azolotetrazinones were elaborated. In fact, it was proposed the reaction of azoloamines **3** with isocyanates or carbamoyl chlorides or chloroformates/amines to give the carbamoyl azole derivatives of type **6**. The last two reagents allow, in the preparation of temozolomide, to avoid the use of the volatile and very dangerous methylisocyanate, but only the former has preparative interest. Nitrosation of the intermediates **6** followed by intramolecular coupling led to the azolotetrazinones **5**. This synthetic pathway has the advantage to avoid the use of diazoazoles, compounds notoriously photo and thermo labile and difficult to handle. By the intermediacy of the carbamoyl derivatives **6** were synthesised pyrazolotetrazinones **5a**[9] and imidazotetrazinones **5d**.[10] The yields, in pyrazole series, with some exception, were as good as those observed in the preceding method; in imidazole series, instead, this method leads to the final products with lower yields, sometimes with no preparative interest.

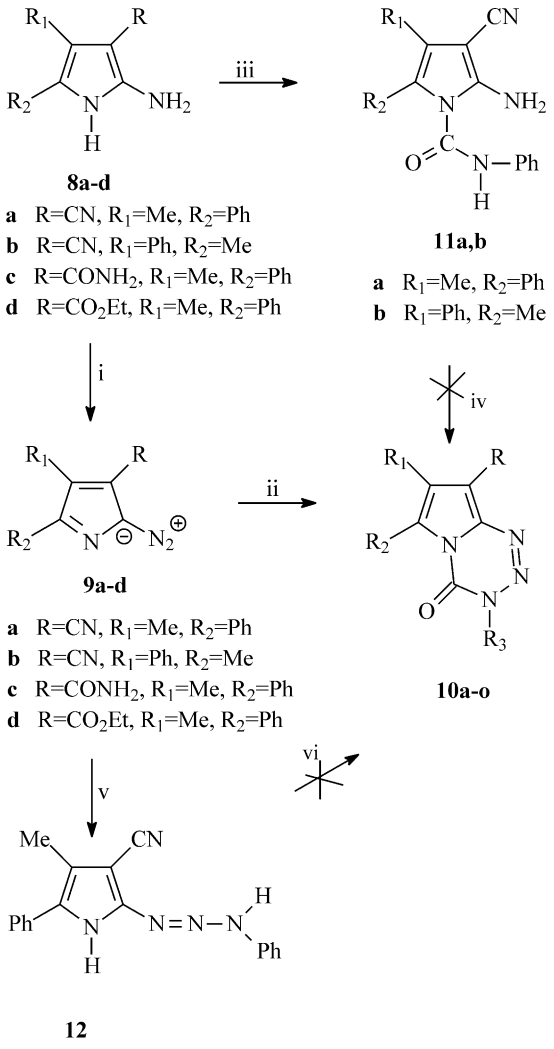
A further synthesis involves the reaction of the diazoazoles **4** with primary amines to give the mono substituted triazenes **7** which undergo carbonylation with phosgene or phosgene equivalents leading to the bicyclic azole systems **5**. By this route were obtained pyrazolotetrazinones **5a** but not imidazotetrazinones **5d**.[9,10]

The pyrrolo[2,1-*d*][1,2,3,5]tetrazine ring system **5e**, having the deaza skeleton of temozolomide, has been prepared by reaction of 2-diazopyrroles **4e** with isocyanates.[11] The key intermediates for this synthesis are the 2-diazopyrroles which were only recently obtained in preparative yields.[12]

In this paper we report the synthesis of a panel of pyrrolo[2,1-*d*][1,2,3,5]tetrazinones of type **10**, by reaction of 2-diazopyrroles with isocyanates which remains the most convenient method to azolotetrazinones, the attempts to obtain them by different routes, and their potent in vitro antitumor activity.

Chemistry

2-Diazopyrroles **9** were isolated, in preparative yields, by diazotization of the corresponding 2-aminopyrroles **8** (Scheme 2). The reaction was carried out in 80% acetic acid with stoichiometric amount of sodium nitrite under nitrogen atmosphere in the dark followed by neutralisation with sodium carbonate. The strict control of the temperature at 0 °C either during diazotization and neutralisation is crucial in obtaining good yields.[12] 2-Diazopyrroles **9a**–**d** were purified by flash chromatography. In the case of 3-carboxamide derivative **8c**, the diazotization also led to a small quantity of 5-methyl-6-phenylpyrrolo[2,3-*d*][1,2,3]triazine-4-one, due to an intramolecular coupling reaction of the diazo group with the carboxamide moiety. It was impossible to obtain pure diazo compound **9c** even after flash chromatography since it slowly but continuously transformed into



10	R	R_1	R_2	R_3
a	CN	Me	Ph	Me
b	CN	Me	Ph	Ph
c	CN	Me	Ph	4-OMe-C_6H_4
d	CN	Me	Ph	4-Cl-C_6H_4
e	CN	Me	Ph	3-Cl-C_6H_4
f	CN	Me	Ph	4-NO_2-C_6H_4
g	CN	Ph	Me	Me
h	CN	Ph	Me	Ph
i	CN	Ph	Me	4-OMe-C_6H_4
j	CN	Ph	Me	4-Cl-C_6H_4
k	CN	Ph	Me	3-Cl-C_6H_4
l	CN	Ph	Me	4-NO_2-C_6H_4
m	CN	Ph	Me	c-Hexyl
n	$CONH_2$	Me	Ph	Ph
o	CO_2Et	Me	Ph	Ph

Scheme 2. Reagents: i $NaNO_2/CH_3COOH$, Na_2CO_3; ii R_3NCO/DMF; iii NaH/THF, PhNCO; iv $NaNO_2/H^+$; v aniline/DCM; vi triphosgene or CDI/THF.

the pyrrolotriazine compound.[11] Thus it was used as crude product obtained from the diazotization.

2-Diazopyrroles were reacted under the same reaction conditions that allowed the isolation of the other azolotetrazinones, that is, with stoichiometric amounts of isocyanates in dichloromethane or ethyl acetate at room temperature. In this case the formation of pyrrolotetrazinones was not observed.

Reaction of 2-diazopyrroles with ten fold excess of isocyanates in dimethylformamide at room temperature in the dark for 24–72 h afforded the pyrrolotetrazinones in acceptable yields (45–60%). The reaction was monitored by IR until the diazo band at ca. 2100 cm^{-1} disappeared. A very easy work up, quenching in icy water and flash chromatography, gave the desired pyrrolotetrazinones **10a–o**. The reduced electrophilicity of the diazo group bounded to the electron rich heterocycle justifies the use of a dipolar aprotic solvent and an excess of reactant.

The structure of pyrrolotetrazinones was confirmed by IR and NMR spectroscopy. In particular, in the IR spectra appeared a band at 1719–1741 cm^{-1} attributable to the carbonyl group of the tetrazinone ring. The ^{13}C NMR spectra showed a pattern compatible with an 1*H*-pyrrole structure, completely different from the pattern of the starting 2*H*-pyrrole structure exhibited by the 2-diazo derivatives.[12] The signals due to the carbonyl carbon of the tetrazinone moiety were found at 140–142 ppm and are in agreement with the chemical shifts observed in the azole series.[13]

In the attempt to avoid the handling of the rather unstable 2-diazopyrroles and to prepare a wider panel of derivatives to explore SAR, we thought to obtain the pyrrolotetrazinone system by the alternative synthesis that involves the carbamoylation of the 2-aminopyrroles and subsequent nitrosation of the corresponding 1-carbamoyl-2-amino derivatives. This alternative route might provide a wider possibility either to functionalize the system and to obtain derivatives unsubstituted at position 6 of the pyrrolotetrazinone system, impossible to obtain by the classical synthesis. In fact, in the preparation of diazo compounds, upon addition of nitrite to the 2-aminopyrroles having the 5 position unsubstituted, the nitrosation might take place at the nucleus and/or the diazonium salt, as soon as formed, couples intramolecularly with the

unsubstituted position of pyrrole. Thus, from the diazotization of 2-amino-3-cyano-4-phenylpyrrole, we could not isolate the corresponding 2-diazopyrrole.[14] On the other hand it was interesting to synthesise a 6-unsubstituted pyrrolotetrazinone derivative since in imidazole series the most active derivatives had no substituent in that position and the introduction of an alkyl group decreased the activity proportionally to the bulkiness of the substituent.[7]

The carbamoyl derivatives in pyrazole and imidazole series were prepared reacting the azolo amines with isocyanate in absence of base or in the presence of triethylamine respectively.[9,10] Such conditions were not suitable for aminopyrroles, in fact in the reaction of these derivatives with isocyanates was observed the involvement of the amino group;[15] whereas under strong basic conditions 2-aminopyrroles were selectively methylated at the ring nitrogen.[16] Thus we reacted the amino derivatives **8a**,**b** with sodium hydride in anhydrous THF. Upon addition of phenylisocyanate the carbamoyl derivatives **11a**,**b** were obtained in excellent yields, (88–97%).

Unfortunately the nitrosation reaction of carbamoyl derivatives **11a**,**b** under several reaction conditions [sodium nitrite in acids (acetic, trifluoroacetic, hydrochloric, sulfuric, nitric, tartaric); isoamyl nitrite in acetic; trifluoroacetic acid, or isoamyl nitrite in DMF and DMAP as base] did not evidence the formation, even in traces, of the pyrrolotetrazinone derivatives **10**.

To complete the picture of the possible synthetic routes to the pyrrolotetrazinones it was undertaken the procedure through the mono-substituted triazene. To this purpose 2-diazopyrrole **9a** was reacted with aniline at room temperature in DCM and in 2 h the monophenyltriazene **12** was quantitatively isolated. Also in this case attempts to cyclise the triazene, at room temperature with triphosgene or carbonyldiimidazole in dry THF in the presence of triethylamine, failed since the formation of pyrrolotetrazinones was not observed. In fact, after 72 h the TLC of the reaction mixture showed many spots likely due to the decomposition of the triazene derivative.

Thus, in conclusion, the only possible synthesis of pyrrolotetrazinones involves the reaction of 2-diazopyrroles with isocyanates.

Biology

The pyrrolotetrazinone derivatives **10a–o** were submitted to the National Cancer Insitute (Bethesda, MD) for testing against a panel of approximately 60 tumor cell lines that have grouped in disease sub-panels including leukaemia, non-small-cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate and breast tumors cell lines. Details of this in vitro test system and the information, encoded by the activity pattern over all cell lines, have been previously reported.[17] The antitumor activity of a test compound is given by three parameters for each cell line; pGI_{50} value (GI_{50} is the molar concentration of the compound that inhibits 50% net cell growth), pTGI value (TGI is the molar concentration of the compound leading to total inhibition of net cell growth), and pLC_{50} value (LC_{50} is the molar concentration of the compound that induces 50% net cell death). Moreover, a mean graph midpoint (MG_MID) is calculated for each of the mentioned parameters, giving an average activity parameter over all cell lines. For the calculation of the MG_MID, insensitive cell lines are included with the highest concentration tested. The discovery of compounds with

Table 1. Overview of the results of the in vitro antitumor screening for compounds **10a–o**[a]

Compd	No. of the cell Lines investigated	No. of cell lines giving positive pGI_{50}[b], pTGI[c], and pLC_{50}[d]								
		pGI_{50}[b]			pTGI[c]			pLC_{50}[d]		
		No.	Range	MG_MID[e]	No.	Range	MG_MID	No.	Range	MG_MID
10a	45	18	4.76–4.01	4.11	1	4.13	4.00	0		
10b	58	58	7.04–4.45	5.72	30	6.51–4.23	4.51	4	6.01–4.36	4.07
10c	58	57	6.71–4.25	5.63	28	6.33–4.20	4.54	5	5.84–4.40	4.09
10d	60	60	>8.00–5.04	7.16	59	7.74–4.08	5.10	31	6.11–4.03	4.22
10e	59	59	6.52–4.49	5.50	27	5.63–4.27	4.45	8	5.22–4.04	4.06
10f	59	59	5.52–4.59	5.04	54	5.00–4.10	4.50	37	4.42–4.02	4.15
10g	59	8	4.37–4.02	4.03	0			0		
10h	59	28	4.54–4.01	4.11	0			0		
10i	59	50	5.43–4.07	4.37	9	4.40–4.03	4.03	1	4.08	4.00
10j	59	54	6.17–4.05	4.72	8	4.80–4.28	4.06	2	4.27–4.18	4.01
10k	55	42	4.90–4.05	4.26	5	4.17–4.01	4.01	0		
10l	55	54	5.72–4.39	4.82	34	5.02–4.02	4.24	12	4.41–4.06	4.04
10m	59	34	6.37–4.01	4.27	7	5.16–4.09	4.04	1	4.11	4.00
10n	53	25	4.89–4.00	4.14	2	4.11–4.05	4.00	0		
10o	58	58	5.88–4.13	5.25	50	5.55–4.10	4.72	43	5.27–4.01	4.33

[a]Data obtained from the NCI's in vitro disease-oriented human tumor cells screen.
[b]pGI_{50} is the−log of the molar concentration that inhibits 50% net cell growth.
[c]pTGI is the−log of the molar concentration giving total growth inhibition.
[d]pLC_{50} is the−log of the molar concentration leading to 50% net cell death.
[e]MG_MID = mean graph midpoint = arithmetical mean value for all tested cancer cell lines. If the indicated effect was not attainable within the used concentration interval, the highest tested concentration was used for the calculation.

new selectivity patterns is one of the targets of the screening program. Selectivity of a compound with respect to a certain cell line of the screen is characterized by a high deviation of the particular cell line parameter compared to the MG_MID value.

An evaluation of the data reported in the Table 1 revealed that compounds belonging to the 8-cyano-7-methyl-6-phenyl series, **10a–f**, are generally more active than the corresponding compounds belonging to the 8-cyano-6-methyl-7-phenyl series, **10g–m**. Surprisingly the 3-methyl derivatives **10a** and **10g** are the least active in terms either of pGI_{50} (mean values 4.11 and 4.03, respectively) and number of sensitive cell lines (18 and 8, respectively). Also surprising is the low activity of the 8-carboxamide derivative **10n** which is active on 25 cell lines having a pGI_{50} mean value 4.14. In the 3-aryl series **10b–f**,**h–l**, the effect of the substituents on the phenyl ring does not play any significant role. In fact in the 8-cyano-6-methyl-7-phenyl series **10b–f**, the most active compound was the 4-chlorophenyl derivative **10d** (MG_MID = 7.16), followed by the unsubstituted phenyl (**10b**), 4-methoxyphenyl (**10c**), and 3-chlorophenyl (**10e**) derivatives which showed similar MG_MID values (5.72, 5.63 and 5.50, respectively). The least active was the 4-nitrophenyl derivative **10f** (MG_MID = 5.04). In the 8-cyano-7-methyl-6-phenyl series **10h–l**, instead, the 4-nitrophenyl derivative **10l** was the most active compound, closely followed by the 4-chlorophenyl substituted derivative **10j** (MG_MID values 4.82 and 4.72, respectively). Derivatives **10i** (4-methoxyphenyl), **10k** (3-chlorophenyl) and **10h** (unsubstituted phenyl) showed decreasing activity (MG_MID values in the range 4.37–4.11).

In the Table 2 are reported the pGI_{50} values of the most active pyrrolotetrazinones and the anticancer agents **1** and **2**. Pyrrolotetrazinones were particularly efficacious against the leukaemia sub-panel. In fact the calculated pGI_{50} MG_MID values of leukaemia sub-panel, related to the active compounds, are always higher than the over all cell lines MG_MID values (ΔMG_MID 0.15–0.62). The most sensitive leukaemia cell lines are SR, K-562, HL60 (TB) and CCRF-CEM cell lines.

Excellent response was obtained in the breast cancer sub-panel in which 3-aryl substituted pyrrolotetrazinones belonging to the 6-phenyl-7-methyl series **10b–f**,**o** had GI_{50} generally in the low micromolar range and in the case of **10d** up to nanomolar concentrations. The most sensitive breast cancer cell lines are MDA-N, MDA-MB-435, and, at lower level, NCI/ADR-RES cell lines.

Against the COLO 205, HCT-116, HT29 and KM12 cell lines of the colon sub-panel as well as the OVCAR-3 of the ovarian sub-panel pGI_{50} was generally above the average.

All the compounds tested, with the exception of **10h**, showed a moderate selectivity with respect to the prostate cancer sub-panel and particularly to the PC-3 cell line.

The 3-cyclohexyl derivative **10m** had a low pGI_{50} mean value (4.27) but showed a remarkable selectivity with respect to CAKI-1 and UO-31 cell lines ($pGI_{50} = 6.37$ and 6.13, respectively), belonging to the renal cancer sub-panel.

It is worthy to note that the pyrrolotetrazinones, with the exception of **10j**, which showed selectivity with respect to LOX IMVI, SK-MEL-5 and UACC-62 cell lines (melanoma sub-panel), did not show any selectivity with respect to the CNS cancer melanoma sub-panels in which temozolomide showed curative properties.

In the Table 3 are reported the pTGI and pLC_{50} of selected compounds which gave positive values with respect to a congruous number of cell lines.

At TGI level the most interesting compound was **10d** either in terms of pTGI (MG_MID = 5.10) and in terms of activity against a wide number of cell lines (59) followed by **10o**, **10c**, **10b** and **10f** (MG_MID in the range 4.72–4.50 and number of cell lines in the range 54–28). Compound **10d** showed good selectivity with respect to the MDA-MB-435 and MDA-N cell lines of the breast cancer sub-panel, to the HCC-2998 and HT29 cell lines of the colon sub-panel, and NCI-H522 of the non-small cell lung cancer sub-panel pTGI of which differed from the MG_MID of two magnitude order or more. Compounds **10b** and **10c** beside the selectivity shown with respect to the same breast cancer cell lines above mentioned, selectively inhibited CCRF-CEM, HL-60(TB) and K-562 of the leukaemia sub-panel.

At LC_{50} level, the best responses were observed in the case of the MDA-MB-435 and MDA-N breast cancer cell lines obtained by **10b** and **10c** and in the case of HCC-2998 colon cancer cell line cell line by **10d**.

Two pyrrolotetrazinone derivatives, **10b** and **10o**, were evaluated as anticancer agents in an in vivo animal model in which polyvinylidene fluoride (PVDF) hollow fibres containing various human cancer cell cultures were implanted intraperitoneally (ip) and subcutaneously (sc) into mice and compounds were administrated by the ip route.[18] The effects of the compounds on reduction of viable cancer cell mass compared to those of controls were determined. Both compounds were tested in the hollow fiber assay against a panel of 12 human cell lines (Non-Small Cell Lung Cancer: NCI-H23, NCI-H522; Colon Cancer: SW-620, COLO-205; CNS Cancer: U251, SF-295; Melanoma: LOX IMVI, UACC-62; Ovarian Cancer: OVCAR-3, OVCAR-5; Breast cancer: MDA-MB-231, MDA-MB-435) as described previously.[19] The compounds were solubilized in 10% DMSO in saline/Tween-80R and administrated intraperitoneally once daily for a total of four doses at each of two dose levels (150 and 100 mg/kg). The day after the last compound dose, the fibers were collected and assessed for viable cell mass. Unfortunately both compounds were inactive under the conditions of test.

The data above described suggest that the mode of action of the pyrrolotetrazinones **10** differs from that of temozolomide. In fact considering that the crucial step

Table 2. Inhibition of in vitro cancer cell lines by pyrrolo[2,1-*d*][1,2,3,5]tetrazine-4(3H)-ones **10**[a] and compounds **1**[a] and **2**[a]

Cell line	pGI_{50}[b]												
	10b	**10c**	**10d**	**10e**	**10f**	**10i**	**10j**	**10k**	**10l**	**10m**	**10o**	**1**	**2**
Leukaemia													
CCRF-CEM	6.05	6.27	7.42	5.44	5.22	5.08	4.74	4.55	5.07	<4.00	5.69	4.2	4.1
HL-60 (TB)	6.61	6.27	7.50	5.48	4.83	5.43	5.35	<4.00	5.72	<4.00	nd[c]	4.5	4.1
K-562	6.63	6.48	7.55	6.27	5.47	4.74	6.17	4.30	4.69	4.05	5.47	4.1	4.1
RPMI-8226	5.88	5.63	7.26	6.20	5.50	4.58	4.63	4.37	4.68	4.32	nd	4.0	4.0
MOLT-4	5.80	5.58	7.20	nd	nd	nd	nd	nd	nd	nd	5.60	4.4	4.1
SR	6.33	6.16	>8.00	6.52	5.33	5.13	5.07	4.90	5.14	4.61	5.88	4.2	4.0
Non-small cell lung cancer													
A549/ATCC	6.21	5.71	7.13	5.35	4.99	<4.00	4.67	nd	nd	4.08	5.00	4.0	4.0
EKVX	5.93	5.75	7.13	5.24	5.05	4.21	4.29	4.48	5.20	4.43	5.24	4.0	4.0
HOP-62	5.39	5.39	6.98	6.05	5.14	4.45	5.15	4.43	4.87	4.83	5.49	4.3	4.0
HOP-92	4.81	<4.00	7.05	4.59	5.09	4.62	<4.00	4.35	4.78	4.62	4.13	4.0	4.0
NCI-H226	5.53	5.80	7.34	5.65	4.90	4.07	4.18	4.31	4.88	4.45	5.10	4.0	4.0
NCI-H23	4.83	5.49	7.09	5.06	5.41	4.09	4.43	<4.00	4.67	<4.00	4.87	4.1	4.0
NCI-H322M	5.47	5.35	7.28	5.41	4.73	4.02	<4.00	4.11	4.64	<4.00	4.80	4.0	4.0
NCI-H460	6.06	5.54	7.39	5.50	5.23	4.29	5.32	<4.00	4.65	<4.00	4.51	4.3	4.0
NCI-H522	nd	nd	7.64	5.71	5.42	4.64	4.96	4.32	4.99	<4.00	5.16	4.0	4.0
Colon cancer													
COLO-205	6.31	5.53	7.72	6.07	4.98	4.47	4.95	4.35	5.15	4.05	5.00	4.0	4.0
HCC-2998	5.60	5.50	7.64	5.46	5.13	4.14	4.97	4.10	4.58	<4.00	4.71	4.0	4.0
HCT-116	6.21	6.30	7.49	5.69	5.18	4.36	4.89	4.15	4.61	4.28	5.83	4.0	4.0
HCT-15	6.14	5.35	7.31	5.42	5.21	4.50	5.00	4.39	4.68	<4.00	5.48	4.0	4.0
HT29	nd	nd	7.55	5.89	5.07	4.36	4.80	4.51	5.05	4.94	5.76	4.0	4.0
KM12	6.40	5.63	7.42	6.05	5.25	4.31	4.62	4.25	4.90	<4.00	5.58	4.0	4.0
SW-620	6.21	6.24	7.32	5.37	4.99	4.57	4.60	4.33	5.11	<4.00	5.70	4.3	4.0
CNS cancer													
SF-268	5.26	5.49	7.12	5.17	4.83	4.08	4.49	4.09	4.90	<4.00	4.88	5.0	4.0
SF-295	6.15	5.69	7.49	5.84	5.45	4.26	5.15	4.40	4.73	4.53	5.20	4.2	4.0
SF-539	6.09	5.88	7.58	5.80	4.84	4.50	4.68	4.48	5.00	4.32	5.46	4.5	4.0
SNB-19	6.01	5.91	7.21	4.99	4.94	4.10	<4.00	nd	nd	4.18	5.40	4.2	4.0
SNB-75	5.59	5.62	nd	5.75	4.70	4.57	4.51	4.51	4.84	4.55	4.72	4.6	4.1
U251	5.78	5.60	7.26	5.63	5.37	4.48	4.86	4.16	5.00	4.27	5.79	4.0	4.0
Melanoma													
LOX IMVI	5.39	5.48	7.26	5.12	4.99	4.47	6.15	4.06	4.76	<4.00	5.49	4.7	4.1
MALME-3M	4.58	5.40	7.33	5.02	4.83	<4.00	4.09	4.06	4.62	<4.00	4.71	4.0	4.0
M14	6.34	6.23	7.61	5.38	4.94	4.09	5.29	nd	nd	4.06	5.40	4.0	4.0
SK-MEL-2	5.38	5.51	7.05	5.90	5.02	4.55	4.57	4.56	5.05	4.50	4.84	4.0	4.0
SK-MEL-28	5.44	6.02	5.25	4.49	4.71	<4.00	<4.00	<4.00	4.49	<4.00	5.45	4.0	4.0
SK-MEL-5	6.33	5.55	7.49	5.42	4.90	4.83	5.94	4.15	4.48	4.14	4.84	4.0	4.0
UACC-257	4.51	4.65	6.05	4.58	4.77	4.07	4.45	<4.00	<4.00	<4.00	4.81	4.0	4.0
UACC-62	5.40	5.42	7.13	5.35	5.21	4.27	6.06	<4.00	4.43	<4.00	5.10	4.3	4.0
Ovarian cancer													
IGROV1	5.83	5.65	7.36	5.60	4.82	4.62	4.49	4.26	4.99	4.22	5.42	4.0	4.0
OVCAR-3	6.40	6.17	7.50	5.78	5.52	4.55	4.79	4.37	4.82	<4.00	5.47	4.0	4.0
OVCAR-4	4.76	4.25	7.04	5.06	5.09	4.54	4.59	4.12	4.98	4.39	4.94	4.2	4.0
OVCAR-5	6.18	5.63	6.73	5.43	4.77	<4.00	<4.00	<4.00	4.56	4.03	5.19	4.0	4.0
OVCAR-8	5.18	5.40	7.16	5.27	4.97	4.56	4.60	4.27	4.96	4.36	5.68	4.0	4.0
SK-OV-3	6.18	5.88	7.05	5.51	4.61	<4.00	4.05	4.42	4.63	4.22	4.95	4.3	4.2
Renal cancer													
786-0	5.78	5.71	7.24	5.53	4.91	4.72	4.74	4.54	5.29	4.61	5.72	4.3	4.0
A498	5.30	5.24	7.56	5.63	5.25	4.42	4.16	4.58	4.70	4.18	4.97	nd	nd
ACHN	4.86	5.13	6.05	5.19	5.31	4.52	5.37	4.27	4.65	4.30	5.37	4.1	4.0
CAKI-1	6.01	5.48	7.07	5.18	4.92	4.34	4.88	<4.00	4.52	6.37	5.62	4.0	4.0
RXF 393	5.70	5.95	7.42	5.73	4.78	4.55	4.55	4.51	4.76	4.70	5.21	4.0	4.0
SN12C	5.72	5.50	7.24	5.42	4.94	4.20	4.45	4.14	4.65	4.01	5.62	4.0	4.0
TK-10	4.45	4.73	6.59	5.00	4.70	4.07	4.11	<4.00	4.55	<4.00	4.89	4.0	4.0
UO-31	5.52	5.54	5.27	5.05	4.95	4.15	4.65	nd	nd	6.13	5.68	4.0	4.1
Prostate cancer													
PC-3	5.76	5.89	7.39	6.06	5.37	4.54	4.65	4.58	5.46	4.69	5.39	4.0	4.0
DU-145	5.76	5.79	7.43	5.46	5.03	4.13	4.41	4.16	5.27	<4.00	5.30	4.0	4.0
Breast cancer													
MCF7	5.85	5.53	6.38	5.40	5.21	4.40	5.12	4.36	4.79	<4.00	5.63	4.2	4.0
NCI/ADR-RES	5.94	5.67	7.56	5.51	5.31	4.18	4.75	<4.00	4.80	<4.00	5.46	4.0	4.0
MDA-MB-231/ATCC	4.67	5.40	7.03	5.80	4.76	4.60	4.44	4.60	5.09	4.67	5.07	4.0	4.0
HS 578T	5.35	5.34	7.40	5.18	4.59	<4.00	4.15	<4.00	4.85	4.01	5.08	4.0	4.0
MDA-MB-435	6.75	6.55	>8.00	6.25	4.89	<4.00	4.51	<4.00	4.61	<4.00	5.00	4.0	4.0
MDA-N	7.04	6.71	>8.00	6.37	4.99	<4.00	4.62	<4.00	4.39	<4.00	5.35	4.0	4.0
BT-549	4.52	5.37	6.56	4.74	4.84	<4.00	4.25	4.05	4.57	<4.00	4.98	4.0	4.0
T-47D	5.38	5.75	5.04	5.37	5.46	4.30	5.21	4.22	4.66	<4.00	5.69	4.0	4.0
MG_MID[d]	5.72	5.63	7.16	5.50	5.04	4.37	4.72	4.26	4.82	4.27	5.25	4.1	4.0

[a]Data obtained from NCI's in vitro disease-oriented tumor cells screen.
[b]pGI_{50} is the −log of the molar concentration causing 50% growth inhibition of tumor cells.
[c]Not determined.
[d]MG_MID = mean graph midpoint = arithmetical mean value for all tested cancer cell lines. If the indicated effect was not attainable within the used concentration interval, the highest tested concentration was used for the calculation.

Table 3. Inhibition of in vitro cancer cell Lines by pyrrolo[2,1-*d*][1,2,3,5]tetrazine-4(3H)-ones **10**[a]

Cell line	pTGI[b]							pLC_{50}[c]						
	10b	**10c**	**10d**	**10e**	**10f**	**10l**	**10o**	**10b**	**10c**	**10d**	**10e**	**10f**	**10l**	**10o**
Leukaemia														
CCRF-CEM	5.37	5.48	<4.00	<4.00	<4.00	4.24	5.15	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
HL-60 (TB)	5.82	5.45	4.48	<4.00	4.22	4.86	nd[d]	<4.00	<4.00	<4.00	<4.00	<4.00	4.08	nd
K-562	6.10	5.89	4.91	5.39	4.51	<4.00	4.88	<4.00	5.16	<4.00	4.52	<4.00	<4.00	4.01
RPMI-8226	4.65	<4.00	5.03	5.58	<4.00	<4.00	nd	<4.00	<4.00	<4.00	5.11	<4.00	<4.00	nd
MOLT-4	4.52	4.47	4.37	nd	nd	nd	5.03	<4.00	<4.00	<4.00	nd	nd	nd	<4.00
SR	4.46	<4.00	4.49	5.57	4.33	<4.00	nd	<4.00	<4.00	<4.00	4.11	<4.00	<4.00	<4.00
Non-small cell lung cancer														
A549/ATCC	4.68	4.82	4.79	<4.00	<4.00	nd	4.66	<4.00	<4.00	4.17	<4.00	<4.00	<4.00	4.33
EKVX	4.92	5.16	4.71	<4.00	4.49	4.62	4.69	<4.00	<4.00	<4.00	<4.00	<4.00	4.12	4.28
HOP-62	<4.00	<4.00	4.87	5.11	4.70	4.35	4.93	<4.00	<4.00	4.33	<4.00	4.34	<4.00	4.43
HOP-92	<4.00	<4.00	4.74	<4.00	4.50	4.34	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
NCI-H226	<4.00	<4.00	6.19	4.90	4.57	4.47	4.59	<4.00	<4.00	4.47	<4.00	4.24	4.06	4.13
NCI-H23	<4.00	<4.00	4.85	<4.00	4.73	4.06	4.40	<4.00	<4.00	4.09	<4.00	4.09	<4.00	<4.00
NCI-H322M	<4.00	<4.00	4.13	<4.00	4.07	4.05	4.53	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	4.27
NCI-H460	4.23	4.81	5.89	<4.00	4.58	<4.00	<4.00	<4.00	<4.00	4.53	<4.00	<4.00	<4.00	<4.00
NCI-H522	nd	nd	7.09	5.07	4.86	4.48	4.10	<4.00	nd	4.42	<4.00	4.39	<4.00	<4.00
Colon Cancer														
COLO-205	5.47	4.97	6.47	5.63	4.64	4.39	4.66	4.47	<4.00	5.11	5.22	4.30	<4.00	4.32
HCC-2998	4.81	4.96	7.18	4.74	4.70	<4.00	4.45	<4.00	<4.00	6.11	<4.00	4.35	<4.00	4.20
HCT-116	4.88	5.58	4.93	4.42	4.72	<4.00	5.55	4.36	<4.00	4.39	<4.00	4.35	<4.00	5.27
HCT-15	<4.00	<4.00	4.92	<4.00	4.61	<4.00	<4.00	<4.00	<4.00	4.28	<4.00	4.12	<4.00	<4.00
HT29	nd	nd	6.78	5.04	4.63	4.12	5.44	<4.00	nd	4.92	4.09	4.22	<4.00	5.12
KM12	4.67	<4.00	5.00	4.83	4.69	<4.00	5.09	<4.00	<4.00	4.16	4.04	4.21	<4.00	4.54
SW-620	<4.00	<4.00	4.61	<4.00	4.29	<4.00	5.37	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	5.05
CNS Cancer														
SF-268	<4.00	<4.00	4.87	<4.00	4.49	4.34	<4.00	<4.00	<4.00	4.19	<4.00	4.14	<4.00	<4.00
SF-295	4.94	5.12	5.66	5.30	4.85	4.13	4.74	<4.00	<4.00	4.38	<4.00	4.37	<4.00	4.31
SF-539	5.44	5.40	6.36	4.98	4.55	4.57	5.27	<4.00	4.40	4.71	<4.00	4.27	4.14	5.09
SNB-19	nd	5.33	4.79	<4.00	4.44	nd	4.82	<4.00	<4.00	4.06	<4.00	<4.00	nd	4.41
SNB-75	5.06	5.07	4.43	5.11	4.40	4.36	4.44	<4.00	<4.00	<4.00	<4.00	4.10	<4.00	4.17
U251	<4.00	4.76	4.91	4.95	4.84	4.67	5.49	<4.00	<4.00	4.31	4.37	4.42	4.33	5.19
Melanoma														
LOX IMVI	<4.00	<4.00	4.89	<4.00	4.66	4.37	<4.00	<4.00	<4.00	4.44	<4.00	4.33	<4.00	<4.00
MALME-3M	<4.00	<4.00	4.46	<4.00	4.51	<4.00	4.32	<4.00	<4.00	<4.00	<4.00	4.19	<4.00	<4.00
M14	4.90	5.15	4.89	<4.00	4.60	nd	4.91	<4.00	<4.00	4.40	<4.00	4.26	nd	4.40
SK-MEL-2	4.27	<4.00	4.88	5.05	4.66	4.54	4.55	<4.00	<4.00	4.20	<4.00	4.31	4.06	4.27
SK-MEL-28	<4.00	<4.00	4.57	<4.00	4.10	<4.00	4.89	<4.00	<4.00	4.12	<4.00	<4.00	<4.00	4.44
SK-MEL-5	4.78	4.77	5.92	4.72	4.60	<4.00	4.56	<4.00	<4.00	5.25	4.16	4.30	<4.00	4.28
UACC-257	<4.00	<4.00	4.68	<4.00	4.32	<4.00	4.48	<4.00	<4.00	4.26	<4.00	<4.00	<4.00	4.15
UACC-62	<4.00	<4.00	4.80	4.44	4.71	<4.00	4.67	<4.00	<4.00	4.32	<4.00	4.35	<4.00	4.31
Ovarian Cancer														
IGROV1	<4.00	<4.00	4.87	<4.00	4.47	4.60	<4.00	<4.00	<4.00	4.06	<4.00	4.13	4.20	<4.00
OVCAR-3	5.29	nd	5.83	5.25	5.00	4.40	4.92	<4.00	<4.00	4.21	<4.00	4.35	<4.00	4.45
OVCAR-4	<4.00	<4.00	4.45	<4.00	4.56	4.42	4.61	<4.00	<4.00	<4.00	<4.00	4.08	<4.00	4.28
OVCAR-5	<4.00	4.48	4.49	<4.00	4.40	4.02	4.73	<4.00	<4.00	<4.00	<4.00	4.03	<4.00	4.36
OVCAR-8	<4.00	4.30	4.86	<4.00	4.59	4.63	5.25	<4.00	<4.00	4.03	<4.00	4.21	4.29	<4.00
SK-OV-3	5.06	5.30	4.52	<4.00	4.10	<4.00	4.62	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	4.29
Renal Cancer														
786-0	4.52	5.08	5.27	<4.00	4.58	4.74	5.48	<4.00	<4.00	<4.00	<4.00	4.24	4.25	5.24
A498	<4.00	<4.00	6.00	4.88	4.53	4.31	4.61	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	4.24
ACHN	<4.00	<4.00	4.08	<4.00	4.79	<4.00	4.82	<4.00	<4.00	<4.00	<4.00	4.35	<4.00	4.41
CAKI-1	<4.00	<4.00	4.56	<4.00	4.28	<4.00	5.18	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	4.63
RXF 393	5.23	5.34	4.61	5.23	4.51	4.32	4.69	<4.00	<4.00	<4.00	<4.00	4.23	<4.00	4.30
SN12C	4.49	4.39	4.88	4.21	4.60	4.16	5.20	<4.00	<4.00	4.22	<4.00	4.27	<4.00	4.65
TK-10	<4.00	<4.00	4.46	<4.00	<4.00	4.05	4.58	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	4.27
UO-31	<4.00	<4.00	4.58	<4.00	4.59	nd	5.33	<4.00	<4.00	4.07	<4.00	4.24	nd	4.94
Prostate Cancer														
PC-3	<4.00	<4.00	4.84	5.00	4.83	5.02	4.82	<4.00	<4.00	4.23	<4.00	4.42	4.41	4.41
DU-145	5.07	5.10	4.74	4.39	4.58	4.63	4.74	<4.00	<4.00	<4.00	<4.00	4.15	4.08	4.36
Breast Cancer														
MCF7	4.35	<4.00	4.63	4.45	4.71	4.16	4.80	<4.00	<4.00	<4.00	<4.00	4.31	<4.00	4.18
NCI/ADR-RES	4.58	5.04	4.82	<4.00	4.18	4.19	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
MDA-MB-231/ATCC	<4.00	4.20	5.80	5.21	4.46	4.57	4.63	<4.00	<4.00	<4.00	<4.00	4.16	4.08	4.24
HS 578T	<4.00	<4.00	4.50	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
MDA-MB-435	6.17	5.95	7.44	nd	4.37	<4.00	4.65	5.20	5.05	4.49	<4.00	<4.00	<4.00	4.31
MDA-N	6.51	6.33	7.74	nd	4.50	<4.00	4.77	6.01	5.84	nd	<4.00	4.02	<4.00	4.35
BT-549	<4.00	<4.00	4.73	<4.00	4.50	4.16	4.61	<4.00	<4.00	<4.00	<4.00	4.16	<4.00	4.23
T-47D	4.06	<4.00	4.48	4.27	4.84	<4.00	5.14	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	4.16
MG_MID[e]	4.51	4.54	5.10	4.45	4.50	4.24	4.72	4.07	4.09	4.22	4.06	4.15	4.04	4.33

[a]Data obtained from NCI's in vitro disease-oriented tumor cells screen.
[b]pTGI is the −log of the molar concentration giving total growth inhibition.
[c]pLC_{50} is the −log of the molar concentration leading to 50% net cell death.
[d]Not determined.
[e]MG_MID = mean graph midpoint = arithmetical mean value for all tested cancer cell lines. If the indicated effect was not attainable within the used concentration interval, the highest tested concentration was used for the calculation.

for the activation of the temozolomide is the ortogonal nucleophilic addition by water at C-4 of the tetrazine nucleus, the bulky phenyl should hinder the attack of the molecule of water at the tetrazinone carbonyl consequently the methyl derivative should be more active than phenyl derivative. Also the phenyl on the position 6 should hinder the approach of the nucleophile to the carbonyl, but compounds **10b–f** are at least one magnitude order more active than compounds **10h–m** bearing the smaller methyl group in the same position. In imidazole series, as already mentioned in this paper, the presence of an hydrogen or a small group bounded to the position 6 is crucial to maintain good activity.

Moreover, at variance with the imidazotetrazinone series in which compounds bearing a cyano group at the position 8 are unactive,[20] pyrrolotetrazinones with a cyano bounded at the same position are more active than the corresponding derivatives bearing, a carbamoyl or an ethoxycarbonyl moieties in spite of the fact that the cyano group is not capable of the intramolecular hydrogen bonding which, in the monomethyl triazene intermediate of the imidazole series, brings about the elimination of the alkylating species.

This evaluation is confirmed by the computerised analysis COMPARE[21] performed for derivative **10b**. In fact the compound showed against temozolomide a Pearson Correlation Coefficient (PCC) very poor, 0.149, at GI_{50} level and even lower, 0.045, at TGI level. In fact there is practically no correlation strongly suggesting a different mechanism of action. COMPARE computations performed for **10b** and **10d** against the NCI 'Standard Agents' Database were negative ($PCC < 0.6$) suggesting that the antiproliferative activity of pyrrolo[2,1-d][1,2,3,5]tetrazines is mechanistically unrelated to that of any known drug and make this class of compounds worthy of great attention. It is our intention to undertake studies directed to elucidate the biochemical mechanism of action.

Experimental

All melting points were taken on a Buchi-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-d_6 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 Biotage FLASH40i chromatography module (prepacked cartridge system). Elemental analysis were within $\pm 0.4\%$ of the theoretical values.

Synthesis of substituted 2-aminopyrroles 8a–d. Derivatives **8a**,**b** were prepared from 1-acetamido-1-phenylpropan-2-one[22] or 2-acetamido-1-phenylpropan-1-one[23] and malonitrile according to the procedure described in the literature.[24] Derivatives **8c**,**d** were prepared from derivative **8a** by acetylation (acetic anhydride) and subsequent reaction with bubbling dry HCl in anhydrous EtOH for 10 h at 0 °C to obtain **8c** or at 60 °C to obtain **8d**, as described previously.[11]

General procedure for the synthesis of 2-diazopyrroles 9a–d. 2-diazopyrroles **9a–d** were prepared by diazotization at 0 °C of the corresponding amines with sodium nitrite in acetic acid followed by neutralization with aqueous Na_2CO_3 as described previously.[11] In the case of the diazotization of **8c** it was impossible to obtain pure **9c** which was reacted without further purification.[11]

General procedure for the synthesis of pyrrolo[2,1-d][1,2,3,5]tetrazine (10a–o). To a solution of **9a–d** (2 mmol) in dry dimethylformamide (10 mL), the suitable isocyanate (20 mmol) in dry dimethylformamide (10 mL) was added dropwise at rt in the dark. The reaction mixture was stirred until the diazo band at $\sim 2110\ cm^{-1}$ disappeared (24–72 h) then poured onto crushed ice. The solid precipitate was filtered off, air dried and purified by flash chromatography with dichloromethane as eluant to give **10a–o**.[11]

10f. Yield 46%; mp 132–134 °C (dec.); IR 2233 (CN), 1741 (CO) 1525 and 1346 (NO_2), 853 and 690 (Ar CH) cm^{-1}; ^1H NMR δ 2.29 (3H, s, CH_3), 7.45–7.53 (5H, m, Ph), 7.92 (2H, d, $J = 9.1$ Hz, C_6H_4), 8.44 (2H, d, $J = 9.1$ Hz, C_6H_4); ^{13}C NMR δ 10.4 (q, CH_3), 93.3 (s, C-3), 112.6 (s, CN), 124.3 (d, Ar) 127.4 (d, Ar), 127.6 (d, Ar), 127.8 (s, C-4), 128.3 (s, Ar), 128.4 (s, C-5), 128.8 (d, Ar), 130.8 (d, Ar), 138.8 (s, C-2), 139.6 (s, CO), 142.6 (s, Ar), 147.1 (s, Ar). Anal. calcd for $C_{19}H_{12}N_6O_3$ (MW 372.34): C, 61.29; H, 3.25; N, 22.57%; found: C, 61.46; H, 3.18; N, 22.69%.

10g. Yield 52%; mp 186–187 °C; IR 2231 (CN), 1734 (CO) 738 and 688 (Ar CH) cm^{-1}; ^1H NMR δ 2.84 (3H, s, CH_3), 3.97 (3H, s, CH_3), 7.43–7.52 (5H, m, Ph); ^{13}C NMR δ 12.9 (q, CH_3), 36.9 (q, CH_3), 92.9 (s, C-3), 112.3 (s, CN), 126.0 (s, C-4), 128.9 (d, Ph), 129.0 (d, Ph), 129.4 (d, Ph), 129.8 (s, Ph), 131.6 (s, C-5), 139.3 (s, C-2), 141.6 (s, CO). Anal. calcd for $C_{14}H_{11}N_5O$ (MW 265.27): C, 63.39; H, 4.18; N, 26.40%; found: C, 63.12; H, 4,07; N, 26.33%.

10i. Yield 60%; mp 170–171 °C; IR 2234 (CN), 1719 (CO), 1253 and 1027 (C–O), 826, 736 and 691 (Ar CH) cm^{-1}; ^1H NMR δ 2.74 (3H, s, CH_3), 3.85 (3H, s, CH_3), 7.47 (2H, d, $J = 8.6$ Hz, Ar), 7.53–7.57 (7H, m, Ar); ^{13}C NMR δ 12.7 (q, CH_3), 55.4 (q, CH_3), 90.2 (s, C-3), 113.0 (s, CN), 114.1 (d, Ar), 126.0 (s, C-4), 128.2 (d, Ar), 128.8 (d, Ar), 129.0 (d, Ar), 129.2 (d, Ar), 129.5 (s, C-5), 129.8 (s, Ar), 130.1 (s, Ar), 139.5 (s, C-2), 141.2 (s, CO), 159.7 (s, Ar). Anal. calcd for $C_{20}H_{15}N_5O_2$ (MW 357.37): C, 67.22; H, 4.23; N, 19.60%; found: C, 66.98; H, 4,37; N, 19.46%.

10l. Yield 51%; mp 153–155 °C; IR 2234 (CN), 1737 (CO), 1521 and 1346 (NO_2), 847, 771 and 698 (ArCH) cm^{-1}; ^1H NMR δ 2.78 (3H, s, CH_3), 7.50–7.66 (5H, m,

Ph), 7.96 (2H, d, $J=8.6$ Hz, C_6H_4), 8.48 (2H, d, $J=8.6$ Hz, C_6H_4); ^{13}C NMR δ 12.9 (q, CH_3), 91.4 (s, C-3), 112.8 (s, CN), 124.5 (d, Ar), 127.0 (s, C-4), 127.7 (d, Ar), 129.0 (d, Ar), 129.2 (d, Ar), 129.3 (d, Ar), 129.7 (s, Ar), 129.8 (s, C-5), 139.2 (s, C-2), 141.0 (s, CO), 142.5 (s, Ar), 147.3 (s, Ar). Anal. calcd for $C_{19}H_{12}N_6O_3$ (MW 372.34): C, 61.29; H, 3.25; N, 22.57%; Found: C, 61.38; H, 3.41; N, 22.33%.

10m. Yield 45%; mp 180–182 °C; IR 2233 (CN), 1723 (CO), 1452 (CH_2), 739 and 698 (Ar CH) cm^{-1}; ^1H NMR δ 1.27–2.03 (11H, m, cyclohexyl), 2.84 (3H, s, CH_3), 7.44–7.57 (5H, m, Ph); ^{13}C NMR δ 13.0 (q, CH_3), 25.0 (t, CH_2), 25.7 (t, CH_2), 31.7 (t, CH_2), 58.1 (d, CH), 92.2 (s, C-3), 112.5 (s, CN), 125.8 (s, C-4), 128.7 (d, Ph), 128.9 (d, Ph), 129.4 (d, Ph), 130.0 (s, Ph), 131.5 (s, C-5), 138.9 (s, C-2), 141.2 (s, CO). Anal. calcd for $C_{19}H_{19}N_5O$ (MW 333.39): C, 68.45; H, 5.74; N, 21.01%; found: C, 68.51; H, 5,91; N, 20.83%.

Preparation of 2-amino-1-carbamoylpyrrole (11a,b). To an ice cooled solution of aminopyrroles **8a**,**b** (5 mmol) in dry THF (50 mL), sodium hydride (50% mineral oil dispersion, 264 mg, 5.5 mmol) is slowly added. After bubbling has ceased, phenylisocyanate (0.54 mL, 5 mmol) was added. The mixture was stirred at rt for 15 min and then heated under reflux for 1 h. The mixture was poured onto crushed ice (500 g). The solid precipitate was filtered air dried and purified by flash chromatography using *n*-hexane/ethyl acetate 6:4 as eluent.

2-Amino-3-cyano-4-methyl-1-(*N*-phenylcarbamoyl)-5-phenylpyrrole (11a). Yield 97%; mp 227–229 °C; IR 3351 (br, NH and NH_2), 2206 (CN), 1706 (CO), 762 and 690 (Ar CH) cm^{-1}; ^1H NMR δ 2.23 (3H, s, CH_3), 7.01 (1H, t, $J=7.9$ Hz, Ph), 7.24–7.52 (9H, m, Ph), 8.67 (1H, s, NH), 8.98 (1H, s, NH), 11.44 (1H, s, NH); ^{13}C NMR δ 10.9 (q, CH_3), 84.4 (s, C-3), 115.4 (s, CN), 115.9 (s, C-4), 118.4 (d, Ph), 122.3 (d, Ph), 123.8 (s, C-5), 126.4(d, Ph), 128.6 (d, Ph), 128.7 (d, Ph), 128.8 (d, Ph), 131.5 (s, C-2), 135.0 (s, Ph), 139.7 (s, Ph), 152.3 (s, CO). Anal. calcd for $C_{19}H_{16}N_4O$ (MW 316.36): C, 72.14; H, 5.10; N, 17.71%; found: C, 71.88; H, 5,31; N, 17.92%.

2-Amino-3-cyano-5-methyl-1-(*N*-phenylcarbamoyl)-4-phenylpyrrole (11b). Yield 88%; mp 234–238 °C; IR 3356 (br, NH and NH_2), 2208 (CN), 1694 (CO), 758 and 695 (Ar CH) cm^{-1}; ^1H NMR δ 2.23 (3H, s, CH_3), 6.96 (1H, t, $J=7.8$ Hz, Ph), 7.28 (2H, t, $J=7.8$ Hz, Ph), 7.45 (2H, d, $J=7.8$ Hz, Ph), 8.64 (5H, bs, Ph), 8.98 (1H, s, NH), 9.16 (1H, s, NH), 11.52 (1H, s, NH); ^{13}C NMR δ 11.3 (q, CH_3), 78.4 (s, C-3), 116.6 (s, CN), 118.1 (d, Ph), 115.9 (s, C-4), 121.7 (d, Ph), 120.9 (s, C-5), 126.2 (d, Ph), 128.1 (d, Ph), 128.4 (d, Ph), 128.7 (d, Ph), 133.5 (s, C-2), 135.0 (s, Ph), 139.6 (s, Ph), 152.4 (s, CO). Anal. calcd for $C_{19}H_{16}N_4O$ (MW 316.36): C, 72.14; H, 5.10; N, 17.71%; found: C, 72.07; H, 4.99; N, 17.77%.

Nitrosation of 2-amino-1-carbamoylpyrroles (11a,b). To an ice cooled solution of 2-amino-1-carbamoyl-pyrroles **11a**,**b** (2 mmol) in acetic acid (80%, 20 mL), sodium nitrite (140 mg, 2 mmol) in water (2 mL) was added. TLC monitoring of the reaction revealed that no traces of pyrrolotetrazinoes **10b**,**h** were formed after 4 days at rt. The same result was obtained when it was used: (a) sodium nitrite in acetic acid and acetic acid/sodium acetate; (b) sodium nitrite in trifluoroacetic acid; (c) sodium nitrite in hydrochloric acid; (d) sodium nitrite in sulphuric acid; (e) sodium nitrite in nitric acid; (f) sodium nitrite in tartaric acid; (g) isoamyl nitrite in acetic acid; (h) isoamylnitrite in trifluoroacetic acid; (i) isoamyl nitrite in DMF and DMAP as base.

3-Cyano-4-methyl-5-phenyl-2-triazenylpyrrole (12). To a solution of 3-cyano-2-diazo-4-methyl-5-phenylpyrrole **9a** (0.63 g, 3 mmol), in dry dichloromethane (30 mL), a solution of aniline (2.73 mL, 30 mmol) in dry dichloromethane (50 mL) was added. The reaction mixture was kept in the dark, at rt and under nitrogen atmosphere, until the diazo stretching band at 2110 cm^{-1} disappeared (2 h). Removal of the solvent, under reduced pressure, gave the 2-triazenylpyrrole **12** in quantitative yields; mp 134–135 °C; IR 3437 e 3246 (NH), 2212 (CN), 766 and 697 (Ar CH) cm^{-1}; ^1H NMR δ 2.25 (3H, s, CH_3), 7.05 (1H, t, $J=7.4$ Hz, Ph) 7.32–7.57 (9H, m, Ph), 12.08 (1H, s, NH), 12.67 (1H, s, NH); ^{13}C NMR δ 10.8 (q, CH_3), 85.3 (s, C-3), 114.5 (d, Ph), 116.6 (s, CN), 118.3 (s, C-4), 122.8 (d, Ph), 127.0 (d, Ph), 127.4 (d, Ph), 127.5 (s, C-5), 128.5 (d, Ph), 129.2 (d, Ph), 131.2 (s, C-2), 141.1 (s, Ph), 146.2 (s, Ph). Anal. calcd for $C_{18}H_{15}N_5$ (MW 301.35): C, 71.74; H, 5.02; N, 23.24%; found: C, 71.54; H, 5,23; N, 23.55%.

Attempted cyclization of 3-cyano-4-methyl-5-phenyl-2-triazenylpyrrole (12). To a solution of the triazenylpyrrole **12** (0.3 g, 1 mmol) in dry THF (30 mL) and TEA (0.1 mL, 1 mmol) trifosgene or 1,1′-carbonyldiimidazole (1 mmol) was added. The mixtures, stirred at rt, after 48 h TLC analysis showed no pyrrolotetrazinone but a very complex sequence of spots due to the decomposition of the starting triazene.

Acknowledgements

This work was financially supported by Ministero dell'Istruzione dell'Università e della Ricerca. A preliminary account of this work was presented the First Italian-Hungarian-Polish Joint Meeting on Medicinal Chemistry, Taormina September 1999. We thank the National Cancer Institute (Bethesda, MD) and especially Dr V.L. Narayanan and his team for the antitumor tests reported in this paper.

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