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Synthesis and antiproliferative activity of [1,2,3,5]tetrazino-[5,4-*a*]indoles, a new class of azolo-tetrazinones

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Abstract—Eight derivatives of the new ring system [1,2,3,5]tetrazino[5,4-*a*]indole-4-one **7**, were synthesised in good yields by reaction of 2-diazoindoles with alkyl or aryl isocyanates. Compounds **7** were screened at National Cancer Institute (NCI) for their activity against a panel of approximately 60 human tumour cell lines. Some of them showed antiproliferative activity having generally GI_{50} in the micromolar range. The most sensitive cell lines were SF-295, SNB-75 and SF-539 of the CNS cancer sub-panel, SR of the leukaemia sub-panel, UACC-62 of the melanoma sub-panel and OVCAR-4 of the ovarian cancer sub-panel.

1. Introduction

Through the last two decades extensive studies have been carried out on azolo-tetrazinones. In fact a large number of papers on pyrazolo-, triazolo-, indazolo- and imidazo-tetrazinones have appeared,[1,2] and in particular the antitumour properties of the latter heterocyclic ring system were exhaustively described. For example, mitozolomide (**1**), 8-carbamoyl-3-(2-chloroethyl)-imidazo-[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one, was the first azolo-tetrazinone to show remarkable antitumour activity,[3] but its severe delayed toxicity, revealed in the phase II clinical trials, compromised its clinical use.[4] Substitution of the 3-(2-chloroethyl) group on the 3 position of the 1,2,3,5-tetrazine moiety, with a methyl substituent led to temozolomide (**2**), which proved to be less potent but also less toxic than its 2-chloroethyl congener, being now in the market with the trade name of Temodal® and used against malignant melanoma, mycosis fungoides and brain tumours.[5]

Most of the synthesis of azolo-tetrazinones of type **4** involve the treatment of the corresponding diazoazoles **3** with alkyl or aryl isocyanates at room temperature in a nonhydroxylic organic solvent, often for prolonged reaction times (Chart 1).[6] The mechanism of this type

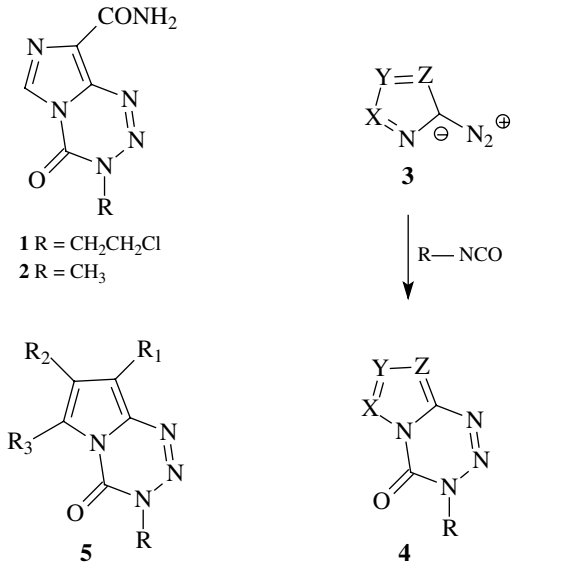

Chart 1.

Keywords: Diazoindoles; Azolotetrazinones; Antiproliferative activity; Tetrazino-indoles.
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of cycloaddition is not completely clarified. It probably involves a stepwise ionic pathway going through an initial nucleophilic attack at the isocyanate carbon, originating a dipolar intermediate, which undergoes ring closure. A support to this mechanism is provided by the fact that the reaction is accelerated using a polar solvent such as hexamethylphosphoramide as the reaction medium.[7,8]

As part of our ongoing program devoted to the synthesis of new molecules containing the pyrrole or the indole moieties possessing antiproliferative activity, we recently reported the synthesis of the pyrrolo[2,1-*d*][1,2,3,5]tetrazine ring system using this synthetic route.[9]

However, for the cycloaddition of the isocyanates to the 2-diazopyrroles, more severe reaction conditions were requested, because of the reduced electrophilicity of the diazo group bound to the pyrrole nucleus, the most electron rich azole ring.

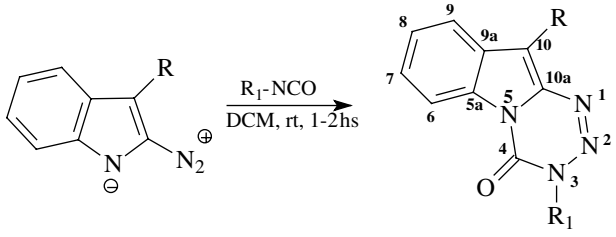
The pyrrolo-tetrazinones, screened against a panel of approximately 60 human tumour cell lines by the NCI of Bethesda, showed potent antiproliferative effects, generally with GI_{50} values at low micromolar or submicromolar concentrations and in some cases reaching GI_{50} in the nanomolar range. Pyrrolo-tetrazinones showed excellent responses in the breast cancer and leukaemia sub-panels and did not exhibit any selectivity with respect to CNS cancer and melanoma sub-panels, in which temozolomide gave the best therapeutic performances. SAR studies as well as the computerised analysis COMPARE[10]indicated that pyrrolo-tetrazinones have a mode of action different from that of temozolomide and is unrelated to that of any known antitumour drug.[11]

2. Results and discussion

2.1. Chemistry

Considering our interest also in the indole chemistry, and having in mind the promising antiproliferative activity shown by pyrrolo-tetrazinones we focused our attention on the synthesis of the new ring system [1,2,3,5]tetrazino[5,4-*a*]indole to verify whether the benzocondensation could improve the already remarkable biological activity of the pyrrolo-tetrazinones.

Tetrazino-indoles are unknown probably because of the unavailability of their precursors, the 2-diazoindoles. In fact only recently the diazotisation and subsequent neutralisation of the unstable and difficult to handle 2-aminoindoles, under suitable reaction conditions, led, in preparative yields, to the latest class of diazoazoles, the 2-diazoindoles.[12] Interestingly, NMR studies and semi-empirical calculations indicated that the negative charge of the zwitterionic species 2-diazoindoles is mainly located on the nitrogen of the indole ring, thus differing from 2-diazopyrroles, 3-diazopyrroles and 3-diazoindoles in which it was mainly found on the *ipso*-carbon.[12]



6a,b **7a,h**

6: **a** R=COOEt, **b** R=Ph
7: **a** R = COOEt R' = Me; **b** R = COOEt R' = Ph;
c R = COOEt R'= CH_2CH_2Cl; **d** R = COOEt R' = 4-MeO-Ph;
e R = Ph R' = Me; **f** R = R'= Ph; **g** R = Ph R' = CH_2CH_2Cl;
h R = Ph R' = 4-MeO-Ph.

Scheme 1.

Thus, 2-diazoindoles **6** were reacted with equimolecular amounts of alkyl or aryl isocyanates. The reactions were carried out in DCM at room temperature and went to completion in a shorter time (1–2 h) compared to that of the pyrrole series. The indolo[2,1-*d*][1,2,3,5]-tetrazinone derivatives **7**, after an easy work up and purification by flash chromatography were obtained in 60–84% yields (Scheme 1). The reaction conditions were milder than those employed in the case of 2-diazopyrroles and were similar to those used in azole series.

The ready reactivity of 2-diazoindoles in the cycloaddition to isocyanates further confirms the location of the negative charge on the N-1 nitrogen which promotes the initial nucleophilic attack to the isocyanate carbon.

The structure of tetrazino-indoles **7a**–**h** was confirmed by IR and NMR spectroscopic data. In particular, the IR spectra showed an intense stretching absorption at 1655–1738 cm^{-1} due to the carbonyl group of the tetrazine moiety; the ^{13}C NMR spectra showed a pattern of signals compatible with an 1*H*-indole structure while the singlets of the carbonyl carbon were found in the range 140.8–142.2 ppm in agreement with those observed in pyrrole and azole series.[11,13]

2.2. Biology

Biological screenings were performed on selected indoloterazinones (**7a**,**b**,**d**,**e**,**f**,**h**) by the NCI of Bethesda and their cytotoxicity was evaluated in the in vitro disease-oriented antitumour screenings against a panel of approximately 60 human tumour cell lines derived from leukaemia, nonsmall lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, breast cancer. The test compounds were evaluate using five concentrations at 10-fold dilutions, the highest being 10^{-4} M and the others 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} M. The results obtained take into consideration the growth inhibitory power (GI_{50}).[14]

An evaluation of the data reported in Table 1 revealed that the most interesting compounds bear the ethoxycarbonyl functionality at the position 10. In fact compound **7d** resulted the most active both in terms of number of

Table 1. Overview of the results of the in vitro antitumour screening for indolotetrazinones **7**[a]

Compound	N[b]	pGI_{50}[d]		
		n[c]	Range	MG_MID[e]
7a	58	12	4.65–4.00	4.06
7b	58	34	5.45–4.00	4.27
7d	58	52	4.90–4.00	4.50
7e	57	29	5.58–4.00	4.26
7f	57	13	4.89–4.00	4.10
7h	53	14	4.76–4.00	4.10

[a] Data obtained from NCI's in vitro disease-oriented human tumour cells screen.
[b] N is the number of cell lines investigated.
[c] n is the number of cell lines giving positive pGI_{50}.
[d] pGI_{50} is the $-\log$ of the molar concentration that inhibits 50% net cell growth.
[e] MG_MID = mean graph midpoint = arithmetical mean value for all tested cancer cell lines. If the identical effect was not attainable within the used concentrated interval, the highest tested concentration was used for the calculation.

sensitive cell lines and of MG_MID value followed by compounds **7b** and **7e** with similar MG_MID values and number of active cell lines. Compounds **7a**,**f** and **7h** resulted to be less interesting being active only against 12–14 cell lines with low MG_MID value 4.06–4.10.

In the Table 2 the pGI_{50} values of the screened tetrazino-indoles **7** are reported.

Compound **7b** showed to be selective with respect to the leukaemia sub-panel being active against all the cell lines at micromolar concentrations. The most sensitive resulted SR and K-562 cell lines with pGI_{50} 5.10 and 4.78, respectively. Other cell lines that showed GI_{50} in the low micromolar range are SF-295 (pGI_{50} = 5.45) cell line belonging to the CNS cancer sub-panel, melanoma Lox IMVI cell line (pGI_{50} = 5.10) and SK-OV-3 (pGI_{50} = 5.45) and T-47D (pGI_{50} = 5.04) belonging to

Table 2. Inhibition of in vitro cancer cell lines for indolotetrazinones **7**[a]

Cell line	pGI_{50}[b]					
	7a	**7b**	**7d**	**7e**	**7f**	**7h**
Leukaemia						
CCRF-CEM	<4.00	4.71	4.85	<4.00	<4.00	<4.00
HL-60 (TB)	<4.00	4.62	4.68	<4.00	<4.00	<4.00
K-562	<4.00	4.78	4.59	<4.00	4.62	<4.00
MOLT-4	<4.00	4.64	4.35	<4.00	4.44	<4.00
RPMI-8226	<4.00	4.60	4.51	<4.00	4.13	ND[c]
SR	<4.00	5.10	4.77	<4.00	4.55	<4.00
Non small cell lung cancer						
A549/ATCC	<4.00	4.31	4.36	4.10	4.19	<4.00
EKVX	4.00	<4.00	4.46	4.42	<4.00	<4.00
HOP-62	4.45	4.06	4.74	4.63	<4.00	<4.00
HOP-92	<4.00	<4.00	4.85	4.58	<4.00	4.20
NCI-H226	<4.00	<4.00	4.57	4.93	<4.00	4.76
NCI-H23	<4.00	4.12	4.72	4.47	<4.00	4.66
NCI-H322M	<4.00	<4.00	4.25	<4.00	<4.00	<4.00
NCI-H460	<4.00	4.19	4.04	<4.00	<4.00	<4.00
NCI-522	ND	ND	ND	4.17	<4.00	4.03
Colon cancer						
COLO-205	<4.00	4.39	<4.00	<4.00	<4.00	<4.00
HCC-2998	<4.00	4.23	<4.00	<4.00	<4.00	ND
HCT-116	<4.00	4.13	4.26	<4.00	<4.00	<4.00
HT29	ND	ND	ND	<4.00	<4.00	<4.00
HCT-15	<4.00	4.23	<4.00	<4.00	<4.00	4.44
KM12	<4.00	4.22	4.06	<4.00	<4.00	<4.00
SW-620	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
CNS cancer						
SF-268	4.17	<4.00	4.79	4.45	<4.00	<4.00
SF-295	4.40	5.45	4.88	4.42	4.05	<4.00
SF-539	4.50	4.03	4.70	5.52	4.27	ND
SNB-19	<4.00	<4.00	4.66	ND	ND	ND
SNB-75	4.43	4.57	4.84	4.86	4.79	4.74
U251	4.10	4.01	4.55	4.52	<4.00	<4.00
Melanoma						
LOX IMVI	<4.00	5.10	4.76	<4.00	<4.00	<4.00
MALME-3M	<4.00	<4.00	4.51	4.39	<4.00	4.50
M14	<4.00	4.07	4.02	<4.00	<4.00	4.47
SK-MEL-2	<4.00	<4.00	4.47	<4.00	4.41	<4.08
SK-MEL-28	<4.00	<4.00	4.10	4.61	<4.00	<4.00

(*continued on next page*)

Table 1 (*continued*)

Cell line	pGI_{50}[b]					
	7a	**7b**	**7d**	**7e**	**7f**	**7h**
SK-MEL-5	<4.00	4.80	4.23	<4.00	<4.00	<4.00
UACC-257	<4.00	<4.00	4.18	<4.00	<4.00	<4.00
UACC-62	<4.00	4.84	4.72	4.51	<4.00	4.05
Ovarian cancer						
IGROV1	<4.00	4.10	4.76	4.06	<4.00	<4.00
OVCAR-3	<4.00	4.37	4.38	4.04	<4.00	<4.00
OVCAR-4	<4.00	4.29	4.92	5.58	4.71	ND
OVCAR-5	<4.00	<4.00	4.57	<4.00	<4.00	<4.00
OVCAR-8	<4.00	<4.00	4.88	4.25	<4.00	<4.00
SK-OV-3	4.19	5.45	4.90	4.59	<4.00	<4.00
Renal cancer						
786-0	4.05	4.20	4.77	4.54	4.27	<4.00
A498	<4.00	<4.00	4.51	4.46	<4.00	<4.00
ACHN	4.15	<4.00	4.60	4.20	<4.00	<4.00
CAKI-1	<4.00	4.08	4.68	4.58	<4.00	<4.00
RXF 393	4.65	4.51	4.69	ND	ND	ND
SN12C	<4.00	<4.00	4.15	<4.00	<4.00	<4.00
TK-10	4.24	<4.00	4.59	4.51	<4.00	<4.00
UO-31	<4.00	4.14	4.56	<4.00	4.18	4.05
Prostate cancer						
PC-3	<4.00	<4.00	4.66	ND	ND	ND
DU-145	<4.00	<4.00	4.12	<4.00	<4.00	<4.00
Breast cancer						
MCF7	<4.00	4.34	4.70	<4.00	<4.00	<4.00
NCI/ADR-RES	<4.00	<4.00	4.60	<4.00	<4.00	<4.00
MDA-MB-231/ATCC	<4.00	<4.00	4.68	4.03	<4.00	4.52
HS 578T	<4.00	<4.00	4.70	4.54	<4.00	<4.00
MDA-MB-435	<4.00	4.05	<4.00	<4.00	<4.00	4.65
MDA-N	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
BT-549	<4.00	<4.00	4.70	4.28	<4.00	<4.00
T-47D	<4.00	5.04	4.61	4.42	4.89	4.12
MG_MID[d]	4.06	4.27	4.50	4.26	4.10	4.10

[a] Data obtained from NCI's in vitro disease-oriented tumour cells screen.
[b] pGI_{50} is the −log of the molar concentration causing 50% growth inhibition of tumour cells.
[c] ND = 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.

the ovarian and breast cancer cell line respectively. The introduction of a methoxy substituent in the position 4 of the 3-phenyl group led to compound **7d**, that showed a MG_MID 4.50, higher than all the other derivatives; it was active against a wider range of cell lines 52 out of 58 although none of them reached the low micromolar values. In fact the most sensitive cell lines resulted SK-OV-3 and OVCAR-4 (pGI_{50} = 4.90 and 4.88, respectively) of the ovarian cancer; the SNB-75 (pGI_{50} = 4.84) of the CNS cancer sub-panel, HOP-92 (pGI_{50} = 4.85) and CCRF-CEM (pGI_{50} = 4.85) belonging to the nonsmall cell lung cancer and leukaemia sub-panel, respectively. Derivative **7e** is completely inactive against leukaemia, colon and most of melanoma cancer cell lines, but it was selective with respect to the CNS cancer sub-panel. In fact the calculated pGI_{50} MG_MID value of the CNS sub-panel, is higher than the overall cell lines MG_MID value (ΔMG_MID −0.49). The most sensitive cell lines are SF-539 and SNB-75 having pGI_{50} 5.52 and 4.86, respectively. It also showed GI_{50} in the low micromolar range (pGI_{50} = 5.58) against OVCAR-4 cell line of the ovarian cancer. Compounds **7a**,**f** and **7h** showed against the active cell line pGI_{50} values in the range 4.89–4.00.

It is worthy to note that the indolo-tetrazinones, at variance with pyrrolo-tetrazinones, showed good selectivity with respect to the CNS sub-panel in which temozolomide showed curative properties.

Recently has been reported that indolo-tetrazinone **7d** possesses the 'so called' biophore 13, associated with differential cytostatic activity in between MCF-7 and MDA-MB-231 cells.[15]

3. Experimental

All melting points were taken on a Büchi–Tottoli capillary apparatus and are uncorrected; IR spectra were determined with a Jasco FT/IR 5300 spectrophotometer; ^{1}H and ^{13}C NMR spectra were measured in DMSO-d_6 solutions unless otherwise specified (TMS as internal

reference), at 200 and 50.3 MHz respectively, using a Bruker AC series 200 MHz spectrometer. Column chromatography was performed with Merck silica gel 230–400 mesh ASTM. Elemental analyses (C, H, N) were within ±0.4% of the theoretical values.

3.1. General procedure for the preparation of 2-diazoindoles 6a,b

2-Diazoindoles **6** were synthesised in preparative yields (50–60%) as previously reported by us, by diazotisation of the corresponding 2-aminoindoles with sodium nitrite in acetic acid at 0 °C, and subsequent neutralisation with aqueous sodium carbonate.[12]

3.2. General procedure for the synthesis of [1,2,3,5]-tetrazino[5,4-*a*]indoles 7a–h

To a solution of the diazoderivatives **6a**,**b** (5 mmol) in dry dichloromethane (15 mL), the suitable isocyanate (5 mmol) dissolved in the same solvent (5 mL) was added dropwise keeping the temperature at 0 °C. The reactions were allowed to reach rt and stirred for additional 1–2 h. The solvent was removed in vacuo and the crude material purified by column chromatography (DCM as eluant). Recrystallisation from ethanol gave the title compounds as yellow solids.

3.2.1. Ethyl 3-methyl-4-oxo-3,4-dihydro[1,2,3,5]tetrazino-[5,4-*a*]indole-10-carboxylate 7a. Yield: 60%; mp 154–155 °C; IR 1738 (CO), 1690 (CO) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.50 (3H, t, $J = 7.3$ Hz, CH_2CH_3), 4.05 (3H, s, CH_3), 4.49 (2H, q, $J = 7.3$ Hz, CH_2CH_3), 7.51 (1H, t, $J = 6.2$ Hz, H-8), 7.56 (1H, t, $J = 6.2$ Hz, H-7), 8.30–8.42 (2H, m, H-6 and H-9); ^{13}C NMR ($CDCl_3$): δ 14.4 (q), 36.6 (q), 61.0 (t), 105.2 (s), 116.1 (d), 122.6 (d), 126.5 (d), 127.1 (s), 127.2 (d), 128.6 (s), 138.7 8 (s), 141.2 (s, CO), 162.6 (s, CO). Anal. Calcd for $C_{13}H_{12}N_4O_3$: C, 57.35; H, 4.44; N, 20.58. Found: C, 57.30; H, 4.43; N, 20.56.

3.2.2. Ethyl 4-oxo-3-phenyl-3,4-dihydro[1,2,3,5]tetrazino-[5,4-*a*]indole-10-carboxylate 7b. Yield: 65%; mp 140–141 °C; IR 1738 (CO), 1697 (CO) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.53 (3H, t, $J = 6.9$ Hz, CH_2CH_3), 4.49 (2H, q, $J = 6.9$ Hz, CH_2CH_3), 7.46–7.57 (4H, m, H-8, H-4′, H-3′ and H-5′), 7.60 (1H, dt, $J = 8.2$, 2.1 Hz, H-7), 7.69 (2H, dd, $J = 8.2$, 1.8 Hz, H-2′ and H-6′), 8.36 (1H, dd, $J = 8.2$, 2.1 Hz, H-6), 8.46 (1H, dd, $J = 8.2$, 2.1 Hz, H-9); ^{13}C NMR ($CDCl_3$): δ 14.4 (q), 61.2 (t), 106.3 (s), 106.4 (d), 122.8 (d), 125.9 (2 × d), 125.9 (s), 126.6 (d), 127.2 (s), 127.6 (d), 129.1 (2 × d), 129.2 (d), 129.4 (s), 137.2 (s), 140.8 (s, CO), 162.4 (s, CO). Anal. Calcd for $C_{18}H_{14}N_4O_3$: C, 64.67; H, 4.22; N, 16.76. Found: C, 64.63; H, 4.20; N, 16.74.

3.2.3. Ethyl 3-(2-chloroethyl)-4-oxo-3,4-dihydro[1,2,3,5]-tetrazino[5,4-*a*]indole-10-carboxylate 7c. Yield: 84%; mp 127–128 °C; IR 1726 (CO), 1655 (CO) cm^{-1}; ^1H NMR: 1.42 (3H, t, $J = 7.1$ Hz, CH_2CH_3, 4.10 (2H, t, $J = 6.1$ Hz, CH_2), 4.45 (2H, q, $J = 7.1$ Hz, CH_2CH_3), 4.72 (2H, t, $J = 6.1$ Hz, CH_2), 7.62 (1H, dt, $J = 6.9$, 2.1 Hz, H-8), 7.66 (1H, dt, $J = 6.9$, 2.1 Hz, H-7), 8.33 (1H, dd, $J = 6.9$, 2.1 Hz, H-6), 8.41 (1H, dd, $J = 6.9$, 2.1 Hz, H-9); ^{13}C NMR: 14.3 (q), 41.6 (t), 50.3 (t), 60.6 (t), 103.4 (s), 115.9 (d), 122.1 (d), 126.4 (d), 126.6 (s), 127.1 (d), 128.4 (s), 138.9 (s), 141.1 (s, CO), 162.1 (s, CO). Anal. Calcd for $C_{14}H_{13}ClN_4O_3$: C, 52.43; H, 4.09; N, 17.47. Found: C, 52.39; H, 4.06; N, 17.42.

3.2.4. Ethyl 3-(4-methoxyphenyl)-4-oxo-3,4-dihydro-[1,2,3,5]tetrazino[5,4-*a*]indole-10-carboxylate 7d. Yield: 70%; mp 160–162 °C; IR 1724 (CO), 1694 (CO) cm^{-1}; ^1H NMR: 1.42 (3H, t, $J = 7.2$ Hz, CH_2CH_3, 3.80 (3H, s, OCH_3), 4.47 (2H, q, $J = 7.2$ Hz, CH_2CH_3), 7.16 (2H, d, $J = 9.2$, Hz, H-3′ and H-5′), 7.62 (2H, d, $J = 9.2$ Hz, H-2′ and H-6′), 7.64–7.71 (2H, m, H-7 and H-8), 8.33–8.48 (2H, m, H-6 and H-9); ^{13}C NMR: 14.4 (q), 55.6 (q), 60.6 (t), 105.4 (s), 114.2 (2 × d), 116.2 (d), 122.2 (d), 126.4 (d), 126.7 (s), 127.0 (d), 126.2 (2 × d), 128.8 (s), 130.2 (s), 130.1 (s), 141.2 (s, CO), 159.7 (s), 162.3 (s, CO). Anal. Calcd for $C_{19}H_{16}N_4O_4$: C, 62.63; H, 4.43; N, 15.38. Found: C, 62.60; H, 4.41; N, 15.37.

3.2.5. 3-Methyl-10-phenyl[1,2,3,5]tetrazino[5,4-*a*]indol-4-(3*H*)-one 7e. Yield: 60%; mp 180–182 °C; IR: 1712 (CO) cm^{-1}; ^1H NMR ($CDCl_3$): 4.00 (3H, s, CH_3), 7.40 (1H, dt, $J = 7.3$, 1.2 Hz, H-8), 7.48 (1H, dt, $J = 7.9$, 2.3 Hz, H-4′), 7.55 (2H, dt, $J = 7.9$, 2.3 Hz, H-3′ and H-5′), 7.62 (1H, dt, $J = 7.3$, 1.2 Hz, H-7), 7.88 (2H, dd, $J = 7.9$, 2.3 Hz, H-2′ and H-6′), 8.06 (1H, dd, $J = 7.3$, 1.2 Hz, H-6), 8.53 (H, dd, $J = 7.3$, 1.2 Hz, H-9); ^{13}C NMR ($CDCl_3$): 35.9 (q), 116.5 (d), 117.1 (s), 121.1 (d), 125.3 (d), 126.7 (d), 127.5 (s), 128.1 (d), 128.8 (2 × d), 129.2 (s), 129.9 (2 × d), 130.9 (s), 134.6 (s), 142.2 (s, CO). Anal. Calcd for $C_{16}H_{12}N_4O$: C, 69.55; H, 4.38; N, 20.28. Found: C, 69.50; H, 4.42; N, 20.26.

3.2.6. 3,10-Diphenyl-3,4-dihydro[1,2,3,5]tetrazino[5,4-*a*]-indol-4-(3*H*)-one 7f. Yield: 60%; mp 185–187 °C; IR: 1730 (CO) cm^{-1}; ^1H NMR: 7.50–7.64 (10H, m, H-7 and H-8, H-2′ and H-6′, H-3′ and H-5′, H-4′, H-2″ and H-6″, H-4″), 7.69 (2H, dt, 2H, dt, $J = 7.7$, 2.3 Hz, H-3″ and H-5″), 8.13 (1H, dd, $J = 7.7$, 1.2 Hz, H-6), 8.56 (1H, dd, $J = 7.7$, 1.2 Hz, H-9); ^{13}C NMR: 116.8 (d), 118.6 (s), 121.5 (d), 125.5 (d), 126.0 (2 × d), 127.5 (d), 127.7 (s), 128.4 (d), 128.7 (d), 128.9 (2 × d), 129.1 (2 × d), 130.1 (2 × d), 130.1 (s), 131.0 (s), 134.0 (s), 137.6 (s), 141.8 (s, CO). Anal. Calcd for $C_{21}H_{14}N_4O$: C, 74.54; H, 4.17; N, 16.56. Found: C, 74.50; H, 4.12; N, 16.54.

3.2.7. 3-(2-Chloroethyl)-10-phenyl-3,4-dihydro[1,2,3,5]-tetrazino[5,4-*a*]indol-4-(3*H*)-one 7g. Yield: 82%; mp 158–160 °C; IR: 1710 (CO) cm^{-1}; ^1H NMR: 3.98 (2H, t, $J = 6.2$ Hz, CH_2), 4.71 (2H, t, $J = 6.2$ Hz, CH_2), 7.45 (1H, dt, $J = 8.0$, 1.2 Hz, H-8), 7.50 (1H, dt, $J = 7.4$, 1.2 Hz, H-4′), 7.60 (2H, dt, $J = 7.4$, 1.2 Hz, H-3′ and H-5′), 7.61 (1H, dt, $J = 8.0$, 1.2 Hz, H-7), 7.88 (2H, dd, $J = 7.4$, 1.2 Hz, H-2′ and H-6′), 8.06 (1H, dd, $J = 8.0$, 1.2 Hz, H-6), 8.52 (1H, dd, $J = 8.0$, 1.2 Hz, H-9); ^{13}C NMR: 41.4 (t), 49.6 (t), 116.5 (d), 118.1 (s), 121.3 (d), 125.5 (d), 127.4 (d), 127.6 (s), 128.3 (d), 128.8 (2 × d), 129.5 (s), 129.9 (2 × d), 130.7 (s), 134.1 (s), 142.1 (s, CO). Anal. Calcd for $C_{17}H_{13}ClN_4O$: C, 62.87; H, 4.03; N, 17.25. Found: C, 62.90; H, 4.08; N 17.27.

3.2.8. 3-(4-Methoxyphenyl)-10-phenyl-3,4-dihydro-[1,2,3,5]-tetrazino[5,4-*a*]indol-4-(3*H*)-one 7h. Yield: 60%; mp 192–194 °C, IR: 1726 (CO) cm^{-1}; ^1H NMR ($CDCl_3$): 3.81 (3H, s, CH_3), 7.01 (2H, dd, $J = 6.7$, 2.0 Hz, H-3″ and H-5″), 7.40–7.60 (7H, m, H-7 and H-8, H-3′ and H-5′, H-4′, H-2″ and H-6″) 7.85 (2H, dt, $J = 7.7$, 1.7 Hz, H-2′ and H-6′), 8.03 (1H, dt, $J = 7.6$, 1.1 Hz, H-6), 8.52 (1H, dt, $J = 7.6$, 1.1 Hz, H-9); ^{13}C NMR ($CDCl_3$): 55.6 (q), 114.3 (2 × d), 116.8 (d), 118.3 (s), 121.4, (d), 125.5 (d), 127.4 (d), 127.4 (s), 127.4 (2 × d), 127.8 (s), 128.4 (d), 128.9 (2 × d), 130.0 (2 × d), 130.4 (s), 130.9 (s), 133.0 (s), 142.0 (s, CO), 159.8 (s). Anal. Calcd for $C_{22}H_{16}N_4O_2$: C, 71.73; H, 4.38; N, 15.21. Found: C, 71.73; H, 4.42; N, 15.24.

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References and notes

1. (a) Ege, G.; Gilbert, K. *Tetrahedron Lett.* **1979**, 4253; (b) Ege, G.; Gilbert, K.; Maurer, K. *Chem. Ber.* **1987**, *120*, 1375.
2. See, for example: (a) Lunt, E.; Stevens, M. F. G.; Stone, R.; Wooldridge, K. R. H. British Patent 2104522A; (b) Stevens, M. F. G.; Hickman, J. A.; Stone, R.; Gibson, N. W.; Baig, E.; Lunt, E.; Newton, C. G. *J. Med. Chem.* **1984**, *27*, 196; (c) See: Ref. 7; (d) Cheng, C. C.; Elslager, E. F.; Werbel, L. M.; Priebe, S. R.; Leopold, W. R. *J. Med. Chem.* **1986**, *29*, 1554.
3. Hickman, J. A.; Stevens, M. F. G.; Gibson, N. W.; Langdon, S. P.; Fizames, C.; Lavelle, F.; Atassi, G.; Lunt, E.; Tilson, R. M. *Cancer Res.* **1985**, *45*, 3008.
4. Harding, M.; Northcott, D.; Smith, J.; Stuart, N. S. A.; Green, J. A.; Newlands, E. S. *Eur. J. Cancer.* **1988**, *57*, 113.
5. (a) Newlands, E. S.; Stevens, M. F. G.; Wedge, S. R.; Wheelhouse, R. T.; Brock, C. *Cancer Treat. Rep.* **1997**, *23*, 35; (b) Bleehem, N. M.; Newlands, E. S.; Lee, S. M.; Tatcher, N.; Selby, P.; Calvert, A. H.; Rustin, G. J. S.; Brampton, M.; Stevens, M. F. G. *J. Clin. Oncol.* **1995**, *13*, 910; (c) O'Reilly, S. M.; Newlands, E. S.; Glaser, M. G.; Brampton, M.; Rice-Edwards, J. M.; Illinworth, R. D.; Richards, P. G.; Kennards, C.; Colquhoun, I. R.; Lewis, P.; Stevens, M. F. G. *Eur. J. Cancer* **1993**, *29A*, 940; (d) Newlands, E. S.; Blackledge, G. R. P.; Slack, J. A.; Rustin, G. J. S.; Smith, D. B.; Stuart, N. S. A.; Quarterman, C. P.; Hoffman, R.; Stevens, M. F. G. *Br. J. Cancer* **1992**, *65*, 287.
6. Stevens, M. F. G.; Hickman, J. A.; Stone, R.; Gibson, N. W.; Baig, G. U.; Lunt, E.; Newton, C. G. *J. Med. Chem.* **1984**, *27*, 196.
7. Stevens, M. F. G. In *New Avenues in Developmental Cancer Chemotherapy*. In *Bristol–Myers Cancer Symposium*; Harrap, K. R., Connors, T. A., Eds.; Academic: Orlando, FL, USA, 1987; Vol. 8, p 335.
8. Lunt, E.; Newton, C. G.; Smith, C.; Stevens, G. P.; Stevens, M. F. G.; Straw, C. G.; Walsh, R. J. A.; Warren, P. J.; Fizames, C.; Lavelle, F.; Langdom, S. P.; Vickers, L. M. *J. Med. Chem.* **1987**, *30*, 357.
9. Diana, P.; Barraja, P.; Lauria, A.; Almerico, A. M.; Dattolo, G.; Cirrincione, G. *Synthesis* **1999**, 2082.
10. Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 1088.
11. Diana, P.; Barraja, P.; Lauria, A.; Montalbano, A.; Almerico, A. M.; Dattolo, G.; Cirrincione, G. *Bioorg. Med. Chem.* **2003**, *11*, 2371.
12. Barraja, P.; Diana, P.; Lauria, A.; Almerico, A. M.; Dattolo, G.; Cirrincione, G. *Helv. Chim. Acta* **2001**, *84*, 2212.
13. Wheelhouse, R. T.; Wilman, D. E. V.; Thomson, W.; Stevens, M. F. G. *J. Chem. Soc., Perkin Trans. 1* **1995**, 249.
14. (a) Grever, M. R.; Sherpatz, S. A.; Chabner, B. A. *Semin. Oncol.* **1992**, *19*, 622; (b) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langely, J.; Cronise, P.; Vaigro-Woiff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1991**, *83*, 757; (c) Boyd, M. R.; Paull, K.; Rubinstein, L. R. In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery*; Valeriote, F. A., Corbett, T., Baker, L., Eds.; Kluwer Accademic: Amsterdam, 1992; Vol. 11.
15. Cunningham, A. R.; Cunningham, S. L.; Day, B. W. *Bioorg. Med. Chem.* **2003**, *11*, 5249.