ELSEVIER

European Journal of Medicinal Chemistry 37 (2002) 267–272

EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY

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Short communication

Pyrrolo[2,1-*c*][1,2,4]triazines from 2-diazopyrroles: synthesis and antiproliferative activity

Patrizia Diana, Paola Barraja, Antonino Lauria, Alessandra Montalbano, Anna Maria Almerico, Gaetano Dattolo, Girolamo Cirrincione *

Dipartimento Farmacochimico Tossicologico e Biologico dell'Università, Via Archirafi 32, 90123 Palermo, Italy

Abstract

Pyrrolo[2,1-*c*][1,2,4]triazines **4a**–**g** were directly obtained from the reaction of 2-diazopyrroles **1a** and **b** with the sodium salts of β-diketones, β-carbonitriles, and β-dinitriles. Only when the 2-diazopyrroles were coupled with ethyl cyanoacetate, it was possible to isolate, together with the pyrrolotriazines, the intermediate hydrazones **3** which, in turn, cyclised to the title ring system. Pyrrolotriazines **4a**–**e** were evaluated for cytotoxic activity against a panel of 60 human cancer cell lines by the National Cancer Institute and some of them demonstrated inhibitory effects in the growth of a wide range of cancer cell lines generally at 10^{-5} M level and in some cases at micromolar concentrations. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: 2-Diazopyrroles; Hydrazones; Pyrrolo-[1,2,4]-triazine; Antiproliferative activity

1. Introduction

Diazoazoles are useful building blocks for the synthesis of polycondensed nitrogen heterocycles of biological interest [1]. In fact, in the course of our studies on new compounds with antineoplastic activity containing the pyrrole and indole moieties, we synthesised 3-triazenopyrroles and 3-triazenoindoles, from the corresponding 3-diazoderivatives, and they have shown remarkable in vitro antileukemic activity [2,3]. The recent synthesis, in preparative scale, of 2-diazopyrroles [4] gave the entry to many other biologically interesting compounds. In fact it was possible to prepare a series of 2-triazenopyrroles, deaza analogues of dacarbazine, 5-(3,3-dimethyl-1-triazenyl)imidazole-4-carboxamide (DTIC), used in therapy as the single most active drug available for the treatment of malignant melanoma and Hodgkin tumours resistant to MOPP therapy (Fig. 1) [5]. One of them showed to be cytotoxic against leukaemia, lymphoma and carcinoma cell lines at micromolar level [6]. We also isolated several derivatives of the new ring system pyrrolo[2,1-*d*][1,2,3,5]tetrazine [7], deaza analogue of the antitumour drug temozolomide, an imidazotetrazinone derivative which is now in the market with the trade name TEMODAL® and is used against malignant melanoma, mycosis fungoides, and brain tumours (Fig. 1) [8]. All the synthesised pyrrolotetrazinones, tested in vitro against a panel of 60 human cancer cell lines, showed potent antiproliferative activity reaching GI_{50} up to nanomolar concentrations [9].

In our attempt to further utilise 2-diazopyrroles as building blocks for the synthesis of pyrrole-fused heterocycles of biological interest, we reacted them with methylene active compounds in order to obtain derivatives of the pyrrolo[2,1-*c*][1,2,4]triazine system of type **4** to evaluate their possible antiproliferative activity. Such a reaction offers wide possibilities to functionalise either the pyrrole and the triazine moieties.

Compounds containing the 1,2,4-triazine portion are found in natural sources and some of them showed biological activity. In particular, 1,2,4-triazines, condensed with one or more heterocycles, found application in many fields as pharmaceutical, herbicides, pesticides, dyes [10]. For example, pyrazolo[5,1-*c*][1,2,4]triazines exhibited antifungal [11] and antitumour activity [12], whilst pyrrolo[2,1-*f*][1,2,4]triazines have shown a pronounced in vitro growth inhibitory

* Corresponding author. Tel.: +39-091-6161606; fax: +39-091-6169999.

E-mail address: gcirrinc@unipa.it (G. Cirrincione).

0223-5234/02/$ - see front matter © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.
PII: S0223-5234(02)01339-9

activity against leukaemic cell lines [13]. Many potential drugs have been modelled on them, particularly in cancer and viral research [14].

2. Results and discussion

A general synthesis of pyrrolotriazine **4** is shown in Fig. 3. The key intermediates for the preparation of the title compounds, the 2-diazopyrroles **1**, were prepared by diazotisation of the corresponding 2-aminopyrroles [4]. 2-Diazopyrroles **1** were reacted, from 0 °C to room temperature (r.t.) in absolute ethanol for 24 h, with the sodium salts of methylene active compounds of type **2**. Unexpectedly pyrrolo[2,1-*c*][1,2,4]triazines **4a**–**g** were directly obtained. In fact the primary coupling products **3**, spontaneously, cyclised to the triazine nucleus. The isolation of the cyclised products from the reaction of diazoazoles with methylene active compounds is not usual at r.t. In fact, under such conditions, most of the diazoazoles led to the hydrazones, sometimes together with the azolotriazine as by products. To obtain good yields of cyclised compounds, it was necessary either to carry out the reaction in refluxing solvent or to heat the intermediate hydrazones in refluxing acetic or sulphuric acid [1]. Instead, the coupling reactions of 2-diazopyrroles with symmetrical species such as acetylacetone (**2a**) and malononitrile (**2b**) gave the corresponding pyrrolotriazines in good to excellent yields (60–90%). Reactions with unsymmetrical compounds such as cyanoacetamide (**2c**) and ethyl cyanoacetate (**2d**) led to the hydrazones that could potentially cyclise with involvement of either the nitrile or the amido/ester group. However, in our case the cyclisation of the nitrile leading to the amino-pyrrolotriazines **4c**–**f** (50–66%) was always observed, and no trace of the oxo-nitrile was detected.

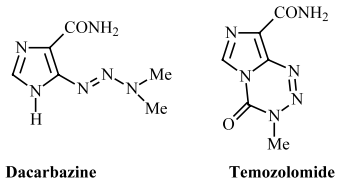

Fig. 1.

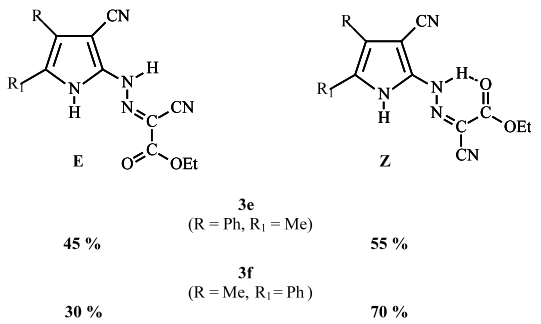

Fig. 2.

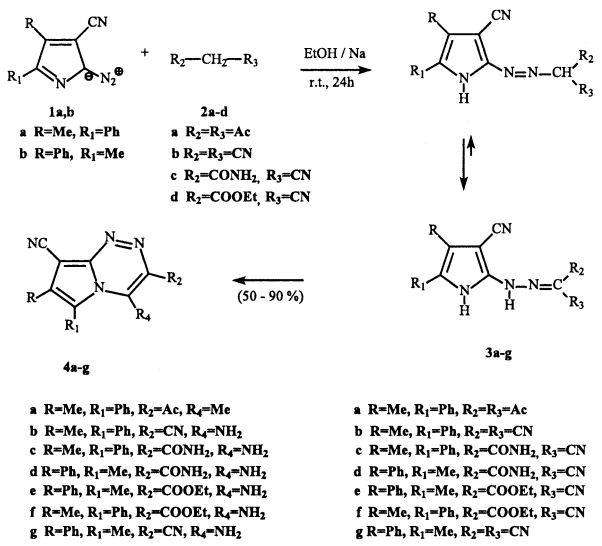

Fig. 3.

Only when the 2-diazopyrroles were coupled with ethyl cyanoacetate (**2d**), it was possible to isolate a mixture of the intermediate hydrazones **3e** and **f** and the pyrrolotriazine derivatives **4e** and **f** derived from their cyclisation. In DMSO solution, the hydrazones **3e** and **f** exist as a mixture of form **E** (**3e** 45% and **3f** 30%) and form **Z** (**3e** 55% and **3f** 70%) because of a geometrical isomerism about the C=N bond (Fig. 2). The **E** and **Z** structures were assigned according to NMR studies carried out on structures of the same type [15]. In fact, form **Z**, which can be stabilised by intramolecular hydrogen bonding, is more abundant than form **E** and its NH signal found at ca. 13 δ confirming the above mentioned strong interaction. The hydrazones **3e** and **f**, in refluxing sulphuric acid, undergo ring closure to the pyrrolotriazines in high yields (84–90%).

Pyrrolotriazines **4a**–**e** were evaluated for cytotoxicity in the NCI's in vitro disease-oriented antitumour screen [16] against a panel of 60 human tumour cell lines derived from leukaemia, lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, breast cancer. The test compounds were evaluated 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 are shown in Table 1, taking into consideration the growth inhibitory power (GI_{50}).

The majority of susceptible tumours was generally inhibited by 10^{-5} M and in some cases by micromolar concentrations of the test compounds. The most sensitive cell line was the ovarian cancer OVCAR-4

Table 1
Inhibition of in vitro tumour cell growth by pyrrolotriazines **4a–e**

Cell line	Cytotoxicity (GI_{50} in μM) [a,b,c]				
	4a	**4b**	**4c**	**4d**	**4e**
Leukaemia					
CCRF-CEM	87.6	>100	17.0	>100	39.8
HL-60 (TB)	ND	>100	7.65	>100	ND
K-562	84.5	>100	26.1	>100	31.8
MOLT-4	68.0	>100	23.4	>100	30.6
RPMI-8226	ND	>100	20.4	>100	22.7
SR	>100	>100	24.8	>100	36.1
Non-small cell lung cancer					
A549/ATCC	>100	>100	17.5	>100	39.9
EKVX	88.7	27.9	3.34	>100	38.0
HOP-62	47.8	32.3	26.1	>100	85.6
HOP-92	>100	18.2	14.5	ND	ND
NCI-H226	ND	16.9	11.0	>100	ND
NCI-H23	37.9	24.4	6.97	>100	38.3
NCI-H322M	>100	>100	20.8	>100	58.4
NCI-H460	>100	>100	14.3	>100	39.8
NCI-H522	35.2	48.9	18.1	>100	27.8
Colon cancer					
COLO 205	>100	>100	16.4	>100	32.9
HCC-2998	57.3	ND	ND	>100	32.5
HCT-116	84.9	78.3	13.5	>100	42.1
HCT-15	>100	88.4	14.8	>100	37.5
HT29	>100	>100	10.5	>100	33.7
KM12	>100	>100	18.6	>100	30.7
SW-620	>100	>100	21.9	>100	40.6
CNS cancer					
SF-268	42.7	28.2	23.1	>100	37.7
SF-295	>100	37.9	15.2	>100	34.1
SF-539	ND	ND	20.9	>100	30.9
SNB-19	ND	ND	ND	>100	21.3
SNB-75	18.8	10.5	17.9	ND	ND
U251	>100	42.2	23.4	>100	38.0
Melanoma					
LOX IMVI	>100	>100	19.7	>100	34.6
MALME-3M	>100	26.9	24.4	>100	20.8
M14	>100	>100	15.3	>100	34.9
SK-MEL-2	>100	51.3	15.7	>100	42.3
SK-MEL-28	>100	>100	15.4	>100	21.6
SK-MEL-5	>100	30.4	15.1	>100	39.9
UACC-257	>100	71.6	17.0	>100	25.4
UACC-62	>100	56.7	5.51	>100	23.9
Ovarian cancer					
IGROV1	>100	52.5	24.7	>100	22.2
OVCAR-3	>100	>100	24.9	>100	29.4
OVCAR-4	16.4	2.77	13.1	>100	10.9
OVCAR-5	>100	>100	24.7	>100	>100
OVCAR-8	>100	24.8	21.5	>100	28.4
SK-OV-3	>100	33.6	21.5	70.4	23.9
Renal cancer					
786-0	>100	84.8	20.9	>100	56.2
A498	>100	>100	13.4	>100	26.8
ACHN	>100	63.6	16.4	>100	29.9
CAKI-1	>100	37.1	16.1	>100	52.6
RXF 393	ND	ND	ND	>100	98.4
SN12C	>100	>100	22.4	>100	37.9
TK-10	80.1	30.5	12.3	>100	37.0
UO-31	>100	28.8	13.3	>100	33.4
Prostate cancer					
PC-3	ND	ND	ND	>100	82.5
DU-145	>100	>100	23.3	>100	83.9
Breast cancer					
MCF7	>100	ND	ND	>100	39.4
NCI/ADR-RES	>100	>100	16.5	>100	38.8
MDA-MB-231/ATCC	54.7	17.1	12.2	>100	51.7
HS 578T	39.8	62.1	22.8	>100	24.6
MDA-MB-435	>100	>100	16.4	>100	32.0
MDA-N	>100	>100	18.5	>100	27.0
BT-549	69.3	28.5	14.8	>100	40.4
T-47D	19.7	19.6	18.4	>100	53.7

[a] Data obtained from NCI's in vitro disease-oriented tumour cells screen.

[b] GI_{50} is the molar concentration causing 50% growth inhibition of tumour cells. Compounds with GI_{50} >100 μM are considered inactive.

[c] ND = Not determined.

against which the active compounds **4** showed GI_{50} in the range 2.77–16.4 μM. The most active compound **4c** was effective against all the tested cell lines with GI_{50} values between 3.34 and 26.1 μM, the best result being against the leukaemic cell line HL-60 (TB), and two non-small lung cell lines EKVX and NCI-H23 with GI_{50} 7.65, 3.34 and 6.97 μM, respectively. Also compound **4e** was active against all the tested cell lines but it was 2–10 times less potent than **4c**. Compound **4b** showed no activity against leukaemic cell lines but was fairly potent against OVCAR-4 with GI_{50} 2.77 μM and the CNS cancer SNB-75 with GI_{50} 10.5 μM. Compound **4a** was inactive against melanoma, renal cancer and ovarian cancer, with the exception of OVCAR-4 cell line, but it showed a moderate to low activity against some of the remaining cell lines. Compound **4d** was inactive against all cell lines with the exception of SK-OV-3, where it exhibited low activity (GI_{50} 70.4 μM). Thus, pyrrolotriazines such as **4c** and **4e** showed an interesting broad spectrum antiproliferative activity.

In order to discern the mechanism of action of pyrrolotriazines **4**, we performed on the NCI screening data, COMPARE computations [17] using **4a–c** and **e** as seed compounds. All compounds had a Pearson correlation coefficients < 0.5 (data not shown) against the 'Standard Agents' database suggesting that these compounds probably act with a different mechanism from those of the Standard Agents and make this class of compounds worthy of great attention. It is our intention to undertake studies directed to elucidate the biochemical action mechanism.

3. Experimental

3.1. Chemistry

All melting points were taken on a Buchi-Tottoli capillary apparatus and are uncorrected; IR spectra were determined with a JASCO FTIR 5300 spectrophotometer; ^{1}H- and ^{13}C-NMR spectra were measured in DMSO-d_6 solutions, (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 $\pm 0.4\%$ of the theoretical values.

3.1.1. General procedure for 2-diazopyrroles *1a* and *b*

2-Diazopyrroles **1a** and **b** were prepared according to the procedure described previously [4], from the corresponding 2-aminopyrroles [18] (3 mmol) dissolved in glacial acetic acid (6 mL) by addition, dropwise at 0 °C under nitrogen atmosphere, of a solution of sodium nitrite (3 mmol) in a small amount of water (1 mL). The mixture was neutralised at 0 °C with a saturated aqueous solution of sodium carbonate and extracted with dichloromethane. The organic layer was dried over sodium sulphate and evaporated under reduced pressure to give the diazo compounds **1a** and **b** which were purified by chromatography on column of silica gel using dichloromethane:ethyl acetate (9:1) as eluant. **1a**: yield 76%, m.p. 72–73 °C; **1b**: yield 80%, m.p. 80–82 °C.

3.1.2. General procedure for pyrrolo[2,1-c][1,2,4]triazines *4a–g*

To a solution of active methylene compound **2a–d** (2 mmol) in anhydrous ethanol (50 mL) was added dropwise a solution of sodium ethoxide (2 mL, 1 N) in ethanol (10 mL) and the mixture was stirred at 0 °C for 15 min. Then, a solution of 2-diazopyrrole **1a–b** (2 mmol) in anhydrous ethanol was added dropwise with stirring at 0 °C. The mixture was stirred at r.t. for 24 h and then was concentrated under reduced pressure. Then, the residue was purified by column chromatography.

The reaction mixture obtained from the reaction of **1a** with acetylacetone (**2a**) was eluted with dichloromethane:ethyl acetate (98:2), the compound eluted was (**4a**) yield 60%, m.p. 176–178 °C; IR cm^{-1} 2226 (CN), 1692 (CO); ^{1}H-NMR δ 2.34 (3H, s, CH_3), 2.39 (3H, s, CH_3), 2.88 (3H, s, CH_3), 7.60–7.62 (5H, m, Ph); ^{13}C-NMR δ 11.5 (q), 17.1 (q), 28.7 (q), 86.7 (s), 114.1 (s), 126.2 (s), 128.3 (d), 128.3 (s), 129.9 (d), 130.2 (s), 131.7 (d), 134.9 (s), 136.6 (s), 140.3 (s), 199.1 (s). Anal. Calc. for $C_{17}H_{14}N_4O$ (290.31): C 70.33, H 4.86, N 19.30. Found: C 70.16, H 4.71, N 19.32%.

The reaction mixture obtained from the reaction of **1a** with malononitrile (**2b**) was eluted with dichloromethane:ethyl acetate (8:2), the compound eluted was (**4b**) yield 90%, m.p. 262–265 °C; IR cm^{-1} 3345 and 3220 (NH_2), 2232 (CN), 2220 (CN); ^{1}H-NMR δ 2.23 (3H, s, CH_3), 7.45 (2H, bs, NH_2), 7.58–7.62 (5H, m, Ph); ^{13}C-NMR δ 10.7 (q), 86.0 (s), 90.2 (s), 106.0 (s), 114.1 (s), 115.8 (s), 121.8 (s), 127.7 (s), 128.5 (d), 130.1 (d), 130.8 (s), 131.6 (d), 143.2 (s). Anal. Calc. for $C_{15}H_{10}N_6$ (274.28): C 65.68, H 3.68, N 30.64. Found: C 65.72, H 3.65, N 30.73%.

The reaction mixture obtained from the reaction of **1a** with cyanoacetamide (**2c**) was eluted with dichloromethane:ethyl acetate (9:1), the compound eluted was (**4c**) yield 64%, m.p. 176–178 °C; IR cm^{-1} 3341 (NH_2), 3294 (NH_2), 2224 (CN), 1666 (CO); ^{1}H-NMR δ 2.25 (3H, s, CH_3), 7.28–7.52 (5H, m, Ph), 7.61 (1H, bs, NH), 7.63 (1H, bs, NH), 11.91 (2H, bs, NH_2); ^{13}C-NMR δ 10.8 (q), 94.2 (s), 116.8 (s), 117.3 (s), 126.0 (d), 126.6 (d), 126.8 (s), 128.7 (d), 128.7 (s), 129.0 (s), 131.7 (s), 145.4 (s), 169.3 (s). Anal. Calc. for $C_{15}H_{12}N_6O$ (292.29): C 61.63, H 4.14, N 28.76. Found: C 61.49, H 4.26, N 28.58%.

The reaction mixture obtained from the reaction of **1b** with cyanoacetamide (**2c**) was eluted with dichloromethane:ethyl acetate (9:1), the compound eluted was (**4d**) yield 66%, m.p. 265–269 °C; IR cm^{-1} 3318 and 3255 (NH_2), 2214 (CN) 1640 (CO); ^{1}H-NMR δ 2.83 (3H, s, CH_3), 7.46–7.67 (5H, m, Ph), 7.70 (1H, s, NH), 8.43 (1H, s, NH), 9.17 (2H, s, NH_2); ^{13}C-NMR δ 12.8 (q), 82.0 (s), 115.0 (s), 118.5 (s), 118.9 (s), 128.5 (d), 128.8 (d), 129.6 (d), 130.9 (s), 131.5 (s), 143.2 (s), 145.51 (s), 168.8 (s). Anal. Calc. for $C_{15}H_{12}N_6O$ (292.29): C 61.63, H 4.14, N 28.76. Found: C 61.78, H 4.06, N 28.62%.

The reaction mixture obtained from the reaction of **1b** with ethyl cyanoacetate (**2d**) was eluted with dichloromethane:ethyl acetate (9:1), the compound eluted was (**4e**) yield 50%, m.p. 252 °C; IR cm^{-1} 3281 (bs NH_2), 2218 (CN), 1685 (CO); ^{1}H-NMR δ 1.33 (3H, t, $J = 7.1$ Hz, CH_3), 2.82 (3H, s, CH_3), 4.35 (2H, q, $J = 7.1$ Hz, CH_2), 7.29–7.50 (5H, m, Ph), 12.15 (1H, s, NH_2), 13.31 (1H, s, NH_2); ^{13}C-NMR δ 11.3 (q), 13.8 (q), 62.3 (t), 77.6 (s), 105.6 (s), 114.3 (s), 115.1 (s), 126.7 (s), 128.2 (d), 128.5 (d), 128.6 (d), 130.4 (s), 132.6 (s), 137.0 (s), 160.9 (s). Anal. Calc. for $C_{17}H_{15}N_5O_2$ (321.33): C 63.54, H 4.70, N 21.80. Found: C 63.37, H 4.58, N 21.78%. Further elution with dichloromethane:methanol (9:1) gave compound (**3e**) yield 42%, m.p. 165 °C; IR cm^{-1} 3259 (NH), 2216 (bs CN); ^{1}H-NMR δ 1.32 (3H, t, $J = 7.0$ Hz, CH_3 **Z**), 1.37 (3H, t, $J = 7.0$ Hz, CH_3 **E**), 2.28 (3H, s, CH_3), 4.32 (2H, q, $J = 7.0$ Hz, CH_2 **Z**), 4.38 (2H, q, $J = 7.0$ Hz, CH_2 **E**), 7.33–7.53 (5H, m, Ph), 11.8 (1H, s, NH **E**), 12.15 (1H, s, NH **E** and **Z**), 13.32 (1H, s, NH **Z**); ^{13}C-NMR δ 11.3 (q), 13.8 (q), 62.3 (t), 77.6 (s), 105.6 (s), 115.8 (s), 120.7 (s), 126.6 (s), 128.2 (d), 128.3 (d), 128.5 (s), 128.6 (d),

132.6 (s), 137.0 (s), 160.9 (s). Anal. Calc. for $C_{17}H_{15}N_5O_2$ (321.33): C 63.54, H 4.70, N 21.80. Found: C 63.52, H 4.53, N 21.63%.

The reaction mixture obtained from the reaction of **1a** with ethylcyanoacetate (**2d**) was eluted with dichloromethane:ethyl acetate (9:1), the compound eluted was (**4f**) yield 60%, m.p. 202–205 °C; IR cm^{-1} 3260 (very broad NH_2), 2233 (CN), 1689 (CO); ^{1}H-NMR δ 1.39 (3H, t, $J = 7.1$ Hz, CH_3), 2.28 (3H, s, CH_3), 4.38 (2H, q, $J = 7.1$ Hz, CH_2), 7.31–7.43 (5H, m, Ph), 12.05 (1H, s, NH), 13.29 (1H, s, NH); ^{13}C-NMR δ 10.8 (q), 13.8 (q), 62.2 (t), 81.3 (s), 105.7 (s), 115.0 (s), 117.7 (s), 124.8 (s), 126.2 (d), 126.9 (d), 128.8 (d), 130.9 (s), 132.5 (s), 137.0 (s), 160.6 (s). Anal. Calc. for $C_{17}H_{15}N_5O_2$ (321.33): C 63.54, H 4.70, N 21.80. Found: C 63.47, H 4.78, N 21.68. Further elution with dichloromethane:methanol (9:1) gave compound (**3f**) yield 35%, m.p. 128–130 °C; IR cm^{-1} 3249 and 3200 (NH_2), 2232 (CN), 2216 (CN), 1685 (CO); ^{1}H-NMR δ 1.28 (3H, t, $J = 7.3$ Hz, CH_3 **Z**), 1.33 (3H, t, $J = 7.3$ Hz, CH_3 **E**), 2.25 (3H, s, CH_3), 4.29 (2H, q, $J = 7.3$ Hz, CH_2 **Z**), 4.34 (2H, q, $J = 7.3$ Hz, CH_2 **E**), 7.30–7.48 (5H, m, Ph), 11.78 (1H, s, NH **A**), 12.16 (1H, s, NH **E** and **Z**), 13.32 (1H, s, NH **Z**); ^{13}C-NMR δ 10.8 (q), 13.8 (q), 62.2 (t), 81.3 (s), 105.7 (s), 115.0 (s), 117.7 (s), 124.8 (s), 126.2 (d), 126.9 (d), 128.7 (s), 128.8 (d), 130.9 (s), 137.0 (s), 160.6 (s). Anal. Calc. for $C_{17}H_{15}N_5O_2$ (321.33): C 63.54, H 4.70, N 21.80. Found: C 63.52, H 4.73, N 21.73%.

The reaction mixture obtained from the reaction of **1b** with malononitrile (**2b**) was eluted with dichloromethane:ethyl acetate (8:2), the compound eluted was (**4g**) yield 80%, m.p. 270 °C; IR cm^{-1} 3418 and 3318 (NH_2), 2230 (CN), 2215 (CN); ^{1}H-NMR δ 2.81 (3H, s, CH_3), 7.47–7.62 (5H, m, Ph), 8.56 (2H, s, NH_2); ^{13}C-NMR δ 12.8 (q), 84.1 (s), 105.8 (s), 114.4 (s), 116.1 (s), 119.9 (s), 128.7 (d), 128.9 (d), 129.5 (d), 130.5 (s), 131.8 (s), 144.3 (s), 144.7 (s). Anal. Calc. for $C_{15}H_{10}N_6$ (274.28): C 65.68, H 3.68, N 30.64. Found: C 65.52, H 3.55, N 30.52%.

*3.1.3. Cyclisation of ethyl 2-cyano-2-(pyrrol-2-yl-hydrazono)acetates **3e** and **f***

A mixture of **3e** and **f** (2 mmol) and concentrated sulfuric acid (20 mL) was kept at r.t. for 1 h. The mixture was then poured into cold water and neutralised by addition of ammonia, and the resulting products were collected by filtration to give the pyrrolotriazine **4e** (84%) and **4f** (90%).

3.2. Pharmacology

3.2.1. Inhibition of tumour cell growth assay

The procedure for the GI_{50} determination was performed according to the NCI protocol [16].

Acknowledgements

This work was financially supported by Ministero dell'Istruzione dell'Università e della Ricerca. A preliminary account of this work was presented at the First Italian-Swiss Meeting on Medicinal Chemistry, Torino, September 1997. We thank the National Cancer Institute (Bethesda, MD) and especially Dr V.L. Narayanan and his team for the antitumour tests reported in this paper.

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