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Original article

Synthesis, structural characterization and antitumor activity of novel 2,4-diamino-1,3,5-triazine derivatives

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Abstract – The syntheses, structural elucidation based on NMR spectroscopy and X-ray analysis of **8** as well as antitumor activities of novel 2,4-diamino-1,3,5-triazine derivatives **5** and **7**–**22** are described. Screenings performed at NCI showed that most derivatives possessed a moderate to strong growth inhibition activity on various tumor panel cell lines between 0.148 and 56.2 μM concentrations. 2-Amino-6-bromomethyl-4-(3,5,5-trimethyl-2-pyrazoline)-1,3,5-triazine **11** showed the most potent antitumor activity with the mean midpoint values of $\log_{10}$ GI50, $\log_{10}$ TGI50 and $\log_{10}$ LC50 of all tests equal to -5.26, -4.81 and -4.37, respectively and therefore, it can be considered as a lead structure for further development of anticancer agents. © 2000 Éditions scientifiques et médicales Elsevier SAS

2,4-diamino-1,3,5-triazine derivatives / X-ray structure analysis / antitumor effect

1. Introduction

Numerous 2,4-diamino-1,3,5-triazines possess various biological activity, but many of their derivatives are still with unexplored pharmacological properties. However, several reports describe their potential as cardiotonic [1], neuroleptic [2] nootropic [3], antihistaminergic [4], tuberculostatic [5], anti-HIV [6, 7], antiviral [8] and anticancer [9–11] agents. In this context and in connection with a research program on 2,4-diamino-1,3,5-triazine chemistry and biological activities undertaken in our laboratories years ago [7], we have considered that novel, suitably substituted, 2,4-diamino-1,3,5-triazines may act as potential anticancer agents.

2. Results

2.1. Synthesis

The synthetic routes utilized for the preparation of the target compounds **5–18** and **19–22** are described in *figures 1* and *2*, respectively.

Synthesis of triazine derivatives **5–12** were performed according to a well known procedure [12] consisting of the reactions of either biguanide hydrochlorides **1–4** (Method A) or their free bases **1a–4a** (Method B) with suitably substituted carboxylic acid esters.

The 6-chloromethyl- or 6-bromomethyl-triazines **8** and **11** were subsequently subjected to the reactions with selected N, O or S nucleophiles to afford products **13** and **14–16**. Among them 7,8-dihydroimidazo[1,2-*a*]-1,3,5-triazine-2,4-dithione [13], considered to be 5-aza-7-deaza-purine [14], was used.

Hydrolysis of the chloromethyl derivative **8** carried out in aqueous alkaline medium at ambient temperature led to the formation of a mixture of the alcohol **17** and ether **18** in 35 and 46% yields, respectively.

The mercaptylation of **8** with thiourea gave the desired product **20**. The method consists in initial formation of the isothiouronium salt **19**, followed by alkaline hydrolysis in the presence of hydrazine. Bearing in mind that the previously described analogous mercaptylation of 4-amino-6-piperidino-2-chloromethyl-1,3,5-triazine

* Correspondence and reprints.

led to the formation of corresponding disulfide rather than the anticipated thiol [11], hydrazine was used to prevent possible oxidation of the mercaptomethyl derivative **20**.

Further reaction of the thiol **20** with ethyl chloroacetate in the presence of EtONa gave ester **21**, which upon treatment with biguanide **1a** afforded the sulfide **22**.

All of the final compounds were characterized by IR, NMR and elemental analysis and all were in accordance with the proposed structures.

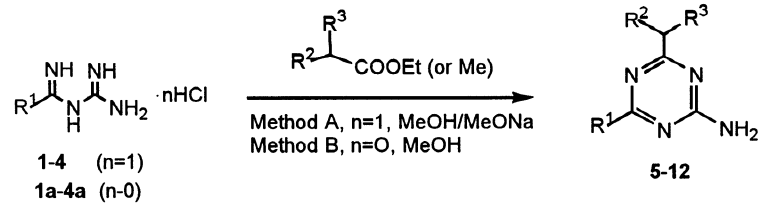
An intriguing feature of the ^{1}H-NMR spectrum of the compound **8** run in $CDCl_3$ at 22°C was pairing of some signals from protons in the vicinity of the triazine moiety. Thus, methylene protons were found as two broadened signals at 4.26 and 4.36 ppm, respectively, while the NH_2 occurred as a broad singlet at 6.0 ppm. When the temperature of the ^{1}H- and ^{13}C-NMR experiments was decreased to -30°C, both spectra exhibited doubled signals arising from all protons and carbons. For example, protons of the NH_2 appeared as four resonances at 5.25, 6.33, 6.83 and 7.51 ppm. Moreover, two distinct signals were found in ^{15}N-NMR spectrum for exocyclic nitrogen atom at -294 and -296 ppm. From the N,H heterocorrelated HSQC spectrum it followed that signals at 5.25 and 7.51 ppm correlated to the nitrogen atom at -296 ppm ($^1J(^{15}\text{N}.^1\text{H}) = 90$ Hz) and these at 6.33 and 6.83 correlated to the nitrogen atom at -294 ppm ($^1J(^{15}\text{N}.^1\text{H}) = 94$ Hz). Such type of coupling constants is known to be strongly dependent on the hybridization of the nitrogen atom. Approximate percentage of *s* character in a hybrid orbital was calculated using the following equation [15]:

$$\%s = 0.43 \cdot \left|{}^1J(\text{N.H})\right| - 6$$

and amounted to 33%, thereby suggesting sp^2 hybridization of the exocyclic nitrogen atom.

Therefore, we have also performed quantum chemical calculations of energy as functions of C6–N7 enterring bond rotation using AM1 semiempirical method [16]. The rotational energy profile showed a global minimum at dihedral (κ = N1–C6–N7–N8) angle = -8.9° and a second minimum ca. -166.6°. The energy difference between the two conformers was ca. 1.6 kcal mol^{-1}, with $\kappa = -8.9$° the preferred dihedral angle. The energy barrier between these conformations was ca. 5 kcal mol^{-1}. These findings confirm that in solution, compound **8** may adopt two major rotamer populations **A** and **B** (*figure 3*) while in the solid state, rotamer **A** is present exclusively due to intermolecular packing forces (vide infra).

All the above evidence points to a conclusion that doubling of the signals in NMR spectra resulted from hindered rotation of pyrazoline ring with respect to 1,3,5-triazine ring system. It is worth noting that a



1-4 (n=1)
1a-4a (n-0)

5-12

Compd.	R^1	R^2	R^3
1, 5	Me_2N	H	Cl
2a, 6		H	Cl
3, 7		H	Cl
4, 8		H	Cl

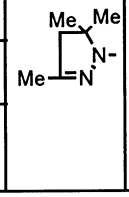
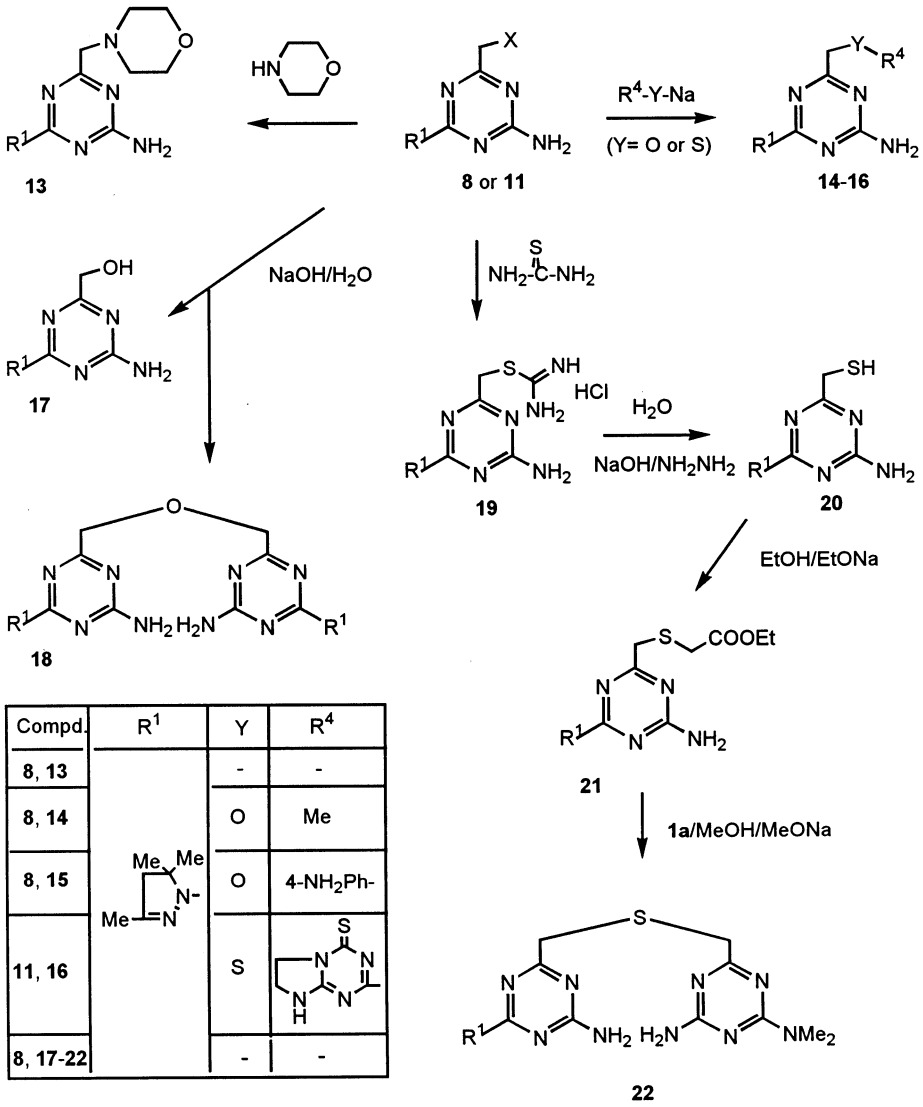
Compd.	R^1	R^2	R^3
4, 9		Me	Cl
4, 10		H	F
4, 11		H	Br
4a, 12		H	$MeSO_2$

Figure 1.



Compd.	R^1	Y	R^4
8, 13		-	-
8, 14		O	Me
8, 15		O	4-NH_2Ph-
11, 16		S	
8, 17-22		-	-

Figure 2.

similar effect was not observed in NMR spectra run at 22° for other 1,3,5-triazine derivatives obtained, which might be explained by either lower energy barrier to the rotation or, which is more probable, overlapping of the signals attributed to CH_2 bridges for corresponding rotamers.

2.2. Crystal structure of **8**

The 1,3,5-triazine ring is slightly non-planar with C2, N5 located in the mean plane, N1, C4 0.03 Å below and N3, C6 0.03 Å above this plane. The C–N bond lengths within this ring show large discrepancies, ranging from 1.321 to 1.364 Å, however the pattern of bond lengths within the ring compares well with that observed in the other diamino-*s*-triazine derivative, where similar influence of the substituents on the ring geometry could be expected [17]. The N12 amino group lies in the plane of the *s*-triazine ring and C2–N12 bond length of 1.314 Å indicates significant conjugation of the N12 lone electron pair with the π-system of the aromatic ring. All endocyclic torsion angles of the 3,5,5-trimethyl-2-pyrazoline fragment are smaller than 3°. Average deviation of atoms from the least-squares plane calculated

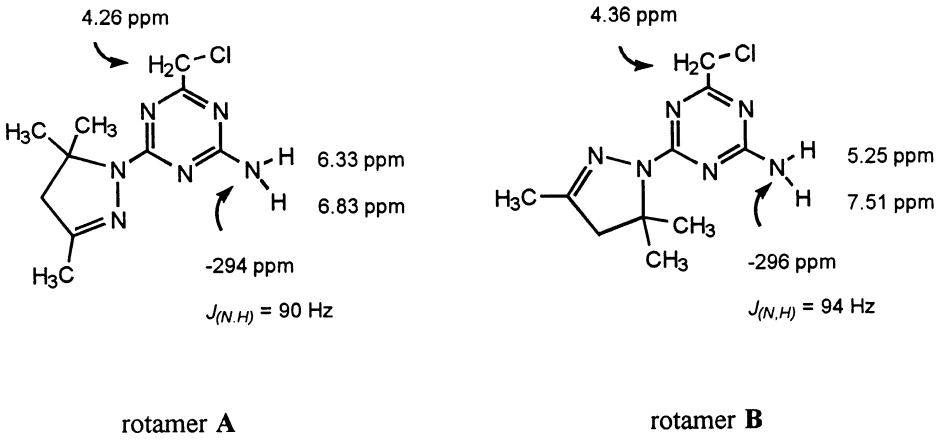


Figure 3.

through the five atoms constituting the pyrazoline ring is 0.013 Å, with C14 lying in the plane of the ring and C6 deviating 0.179(2) Å from the plane of the ring. Atoms N5, N1, N7, C6 are coplanar and atoms N8 and C11 are displaced from their plane by −0.035(4) and 0.309(5) Å which results in significant piramidalization of N7. This deformation is also reflected in deviation of N7 (0.091(3) Å) from the plane through its three substituents and in the values of the N5C6N7C11 and N1C6N7N8 torsion angles which are −15.5(4)° and −1.3(3)°, respectively.

The molecules in the crystal join via N–H···N hydrogen bonds forming tapes as shown in *figures 4* and *5*. Both hydrogen atoms of the N12 amino group are involved in this interaction and N1 and N3 atoms of the 1,3,5-triazine ring act as acceptors.

2.3. Biology

Evaluation of anticancer activity was performed on all compounds described herein at the National Cancer Institute (NCI) of Bethesda, following the known in vivo disease oriented antitumor screening program against a panel of 62 tumor cell lines derived from nine cancer types (leukemia, lung, colon, brain, melanoma, ovarian, prostate, renal and breast) according to standard protocol [18]. In each test, dose–response curves for each cell line were measured with five different drug concentrations and the concentration causing 50% cell growth inhibition (GI50), total cell growth inhibition (TGI), 0% growth) and 50% cell death (LC50, −50% growth) compared with the control, was calculated.

Data of the most active compounds **5**–**8**, **11**, **13**, and **16** are recorded in *table I*.

Among the 2-chloromethyl series **5**–**8**, the 3,5,5-trimethylpyrazoline derivative **8** showed significant activity against most of the cell lines with the exception of prostate cancer, while its analogues bearing dimethylamine (**5**), morpholine (**6**) or 3-methylpyrazoline (**7**) were less active. It was concluded from these results that a significant antineoplastic activity was associated with 3,5,5-trimethylpyrazoline moiety. Therefore, further structural modifications conserved this ring system and a highly active **8** served as a point of departure. Thus, a set of compounds **9**–**22** varied on 6-methylene group was produced. The introduction of any of the substituents F, OH, $MeSO_2$, OMe and SH, however, re-

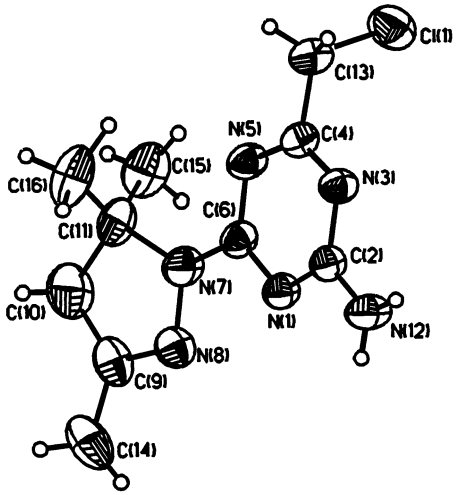


Figure 4. Atom labeling of **8**.

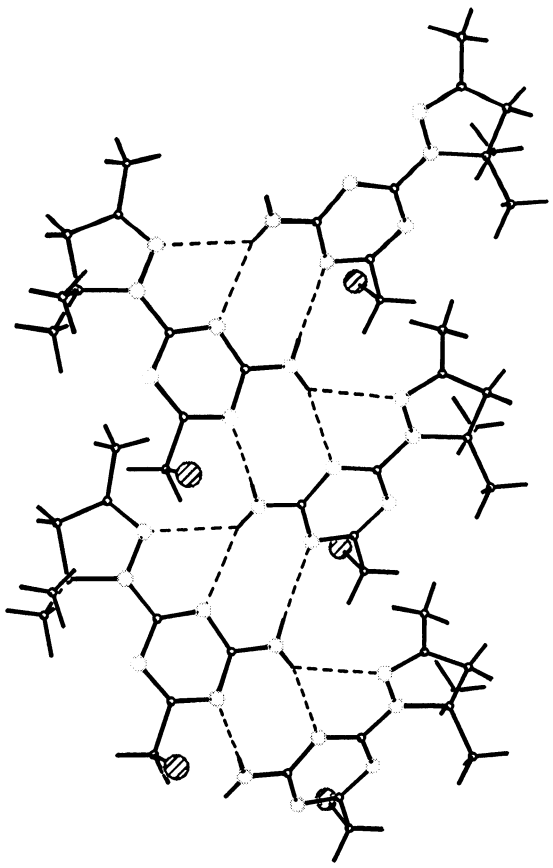


Figure 5. H-bonded tape of molecules **8**.

sulted in considerable decreasing of the antitumor activity. Also, ether (**18**) or thioether (**22**) groups were associated with very poor activity.

The only substituents which gave indisputable activity were Br (**11**), morpholine (**13**) and 7,8-dihydroimidazo[1,2-*a*]triazine-2,4-dithione (**16**), with the former being most active ($\log_{10}$ GI50 ranges from -4.25 to -6.83).

For the compound **11**, the mean logarithmic value of GI50 in all cell lines is -5.42; this value is the midpoint of the bar graph. Compound **11** was found to exhibit moderate selectivity towards renal cell lines CAKI-1 at the TGI level with a selectivity ratio of 39. This selectivity was retained at the LC50 level with a ratio of 49. From the pattern of the mean graph it is clear that **11** also demonstrates a greater than average activity towards some cell lines of lung cancer (NCI-H522 and HOP-92) as well as melanoma cell line LOX IMVI.

In conclusion, the high degree of activity of compounds **8** and **11** raises the potentiality of their further derivatization in the hope of finding even more active and selective cytotoxic agents.

3. Experimental protocols

3.1. Synthesis

Melting points are uncorrected and were recorded on a Buchi apparatus. IR spectra were recorded on a Perkin–Elmer FT spectrometer. 1D ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini XL-200 instrument at 200 and 50 MHz, respectively. Two-dimensional NMR spectra were recorded on a Varian Unity Plus 500 instrument. The results of elemental analyses for C, H and N were within $\pm 0.4\%$ of the theoretical values.

*3.2. 2-Amino-4-R^2-6-haloalkyl-1,3,5-triazines **5**–**11***

3.2.1. Method A

To a solution of sodium methoxide in methanol (2.3 g Na, 90 mL methanol) corresponding biguanide hydrochloride **1**, **2** or **3** (0.101 mol) [21] was added and the reaction mixture was stirred at r.t. for 3 h. Then, a suitably substituted acetic acid methyl ester (*figure 1*) was added dropwise. The reaction mixture was stirred for 24 h and subsequently heated under reflux for 6 h. After cooling to r.t., the product that precipitated was collected by filtration, washed with methanol (2×5 mL) and water and purified by crystallization from suitable solvent (*table II*).

3.2.2. Method B

To a solution of biguanide **4a** (10.0 g, 0.059 mol) in ethanol (100 mL) suitably substituted acetic acid ethyl ester (*figure 1*) was added dropwise over 1 h. The reaction mixture was stirred at ambient temperature for 12 h and the product that precipitated was separated by suction, washed with ethanol (10 mL) and purified by crystallization.

2-Amino-6-(methylsulfonyl)-4-(3,5,5-trimethyl-2-pyrazolino-1,3,5-triazine **12** was obtained according to method B, with the exception that the reaction mixture was heated under reflux for 8 h.

Table I. In vitro anticancer data for compounds **5–8**, **11**, **13** and **16**[a]

Cell line	$-\log_{10}$ GI50 (A), $-\log_{10}$ TGI (B) and $-\log_{10}$ LC50 (C) of the in vivo inhibitory activity tests for given compounds																				
	5			**6**			**7**			**8**			**11**			**13**			**16**		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Leukemia																					
CCRF-CEM	4.76	4.23		4.73	4.34		4.83	4.36		5.11	4.56	4.06	6.63	6.22	5.46	4.52	4.04				
HL-60	4.72	4.11		4.86	4.45	4.04	4.92	4.40		5.62	5.21		6.33	5.65		4.82	4.52	4.23			
K-562	4.54	4.07		4.63	4.26		4.74	4.49	4.23	4.67	4.32		5.46								
MOLT-4	4.73			4.57			4.71	4.16		4.76	4.37		5.89	5.50							
RPMI-8226	4.34			4.49			4.41			4.59			5.71								
SR	4.60	4.18		4.67	4.21		4.68	4.21		4.88	4.31		5.54	4.77		4.52					
Non-small cell lung cancer																					
A 549/ATCC	4.10						4.24			4.40			4.90	4.33							
EKVX	4.74	4.38	4.02	4.75	4.42	4.90	4.79	4.45	4.10	5.19	4.51	4.06	5.43	4.89	4.24	4.50			4.56		
HOP-62	4.76	4.33		4.74	4.35		4.94	4.53	4.12	5.44	4.72	4.10	4.79	4.58	4.27	4.34			4.85	4.55	4.26
HOP-92	4.68	4.34		4.77	4.49	4.21	4.71	4.41	4.11	4.84	4.52	4.20	6.07	5.64	5.26	4.48			4.73	4.35	
NCI-H 226	4.43			4.43			4.52			4.51			5.54	4.98	4.47				4.82	4.27	
NCI-H 23	4.61	4.16		4.75	4.45	4.16	4.71	4.39	4.06	4.80	4.47	4.13	5.30	4.74	4.34				4.32		
NCI-H 322M	4.54	4.11		4.07			4.30			4.35			4.76	4.47	4.18				4.58		
NCI-H 460	4.48			4.75	4.39	4.02	4.68	4.31					5.32	4.79	4.39						
NCI-H 522	4.96	4.60	4.24	5.47	4.95	4.34	5.71	5.31	4.72	5.86	5.23	5.21	6.63	6.00	5.38	4.85	4.54	4.22			
Colon cancer																					
COLO 205	4.53	4.21		4.75	4.49	4.23	4.73	4.44	4.15	4.78	4.47	4.17	5.38	4.82	4.39	4.22			4.41		
HCC-2998	4.34			4.75	4.44	4.13	4.51			4.52	4.17										
HCT-116	4.26			4.67	4.18		4.61	4.06		4.73	4.31		5.51	4.92	4.29						
HCT-15	4.53	4.03		4.80	4.46	4.12	4.76	4.36		4.75	4.39	4.02	5.46	4.87	4.37				4.49		
HT-29	4.39			4.49			4.52			4.55	4.07		5.19	4.66	4.22						
KM 12	4.54			4.49			4.79	4.26		4.90	4.47	4.04	5.05	4.66	4.30						
SW-620	4.55	4.06		4.61	4.22		4.63	4.12		4.77	4.44	4.11	5.51	5.08	4.55						
CNS cancer																					
SF-268	4.57			4.47			4.49			4.70	4.14		5.18	4.60	4.11				4.81	4.45	4.09
SF-295	4.51			4.53			4.52			4.71	4.26		4.90	4.60	4.29				4.64	4.13	
SF-539	4.59	4.22		4.64	4.28		6.61	4.24		4.72	4.47	4.21	5.51	5.08	4.53				4.39		
SNB-19													4.74	4.36					4.42		
SNB-75	6.04	4.95		4.47	4.01		4.73	4.13		4.76	4.24		5.04	4.67	4.33				4.81	4.40	
U 251	4.45			4.65	4.27		4.53			4.79	4.40	4.05	5.54	5.03	4.51				4.41		
Melanoma																					
LOX IMVI	5.10	4.66	4.20							5.75	5.47	5.20	6.53	5.97	5.42	4.83	4.55	4.26	4.36		
M 14	4.15			4.57			4.60	4.09		4.71	4.24		5.14	4.60	4.12				4.17		
SK-MEL-2							4.58			4.54			4.80	4.52	4.24				4.77	4.20	
SK-MEL-28	4.30			4.69	4.34		4.64	4.25		4.72	4.24		4.95	4.63	4.32						
SK-MEL 5	4.53	4.04		4.70	4.34		4.83	4.49	4.16	4.82	4.50	4.19	4.98	4.65	4.31	4.13					
UACC-257	4.06			4.20			4.16			4.32			4.95	4.63	4.32						
UACC-62	4.72	4.38	4.04	4.80	4.50	4.21	4.82	4.51	4.20	4.94	4.60	4.25	5.17	4.71	4.35	4.58			4.37		
MALME-3M													5.61	5.12	4.51						

(*Continued*)

Table I. (Continued)

Cell line	$-\log_{10}$ GI50 (A), $-\log_{10}$ TGI (B) and $-\log_{10}$ LC50 (C) of the in vivo inhibitory activity tests for given compounds																				
	5			**6**			**7**			**8**			**11**			**13**			**16**		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Ovarian cancer																					
IGROV 1	4.87			4.84	4.49	4.13	5.15	4.59	4.12	5.35	4.72	4.20				4.77	4.43	4.09	4.82	4.29	
OVCAR-3	4.75	4.46	4.04				4.87	4.45	4.03	5.05	4.80	4.14	5.47	4.87	4.42						
OVCAR-4	4.71	4.31	4.00	4.76	4.48	4.19	4.77	4.49	4.21	5.36	4.80	4.36	4.86	4.57	4.27	4.69	4.34				
OVCAR-5	4.18	4.42	4.13	4.70	4.33		4.70	4.24		4.59	4.22		5.43	4.85	4.32						
OVCAR-8	4.45			4.46			4.68	4.31		4.83	4.51	4.19	5.34	4.78	4.36				4.74	4.33	
SK-OV-3	4.25			4.34			4.44			4.50			4.73	4.47	4.22				4.72	4.15	
Prostate cancer																					
PC-3	4.13			4.14			4.44			4.31			5.36	4.77	4.37						
DU-145				4.42			4.25			4.49			5.60	5.07	4.51				4.52		
Renal cancer																					
786-0	4.65	4.23		4.85	4.53	4.53	5.00	4.64	4.28	5.52	5.07	4.59	6.49	5.97	5.43	4.82	4.51	4.19	4.03		
A 498	4.55	4.15		4.48			4.37			4.88			4.87	4.58	4.29				4.40		
ACHN	4.80	4.40		4.78	4.49	4.19	4.76	4.41	4.07	5.27	4.74	4.33	5.45	4.88	4.43	4.50			4.35		
CAKI-1	5.26	4.74	4.33	4.78	4.52	4.25	5.04	4.67	4.32	5.57	5.19	4.66	6.83	6.47	6.11	4.69	4.40	4.10	4.60	4.21	
RXF-393	4.58			4.44			4.30			4.49									4.81	4.41	4.02
SN 12C	4.36			4.44			4.49			4.66	4.33		5.28	4.69	4.15						
TK-10	4.14			4.20			4.26			4.29			5.42	4.93	4.46				4.13		
DU-31	4.91	4.58	4.26	4.76	4.50	4.24	5.11	4.69	4.33	5.51	5.05	4.48	6.50	5.84	5.33	4.74	4.48	4.21			
Breast cancer																					
MCF 7	4.40						4.42			4.44			5.61	4.95	4.43				4.29		
MCF 7/ADR-RES	4.46	4.06		4.54	4.05		4.62	4.13													
MDA-MB-231/ATCC	4.55	4.14		4.35	4.10		4.40			4.51	4.10		5.23	4.73	4.36				4.53		
HS 578 T				4.54									4.86	4.42						4.38	
MDA-MB-435	4.60						4.58			4.68			5.02	4.67	4.33				4.81		
BT-549	4.33			4.42			4.49			4.53			4.98	4.65	4.32						
T-47/D	4.40			4.53			4.51			4.51			5.41	4.89	4.27						
NCI/ADR-RES	4.22			4.50			4.12			4.33			4.75	4.29					4.66	4.27	
MDA-N				4.26						4.84	4.46	4.07	4.94	4.59	4.25				4.52		
$\neq\neq\neq$	1.50	0.80	0.30	0.90	0.75	0.29	1.08	1.09	0.67	1.05	1.16	1.08	1.41	1.56	1.69	0.68	0.48	0.24	0.50	0.47	0.25

[a] The response parameters (mean −log10): GI50, TGI and LC50 are interpolated volumes representing the concentrations at which percentage growth is +50, 0 and −50, respectively. $\neq\neq\neq$, the reported data represent the logarithmic difference between the parameter value referred to the most sensible cell line and the same mean parameter; $\neq\neq$ is considered low if <1, moderate if >1 and <3, high if >3.

3.3. 2-Amino-6-(morpholinomethyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine **13**

To a solution of morpholine (3.5 g, 0.34 mol) in benzene (60 mL) chloromethyl-triazine **8** was added. The reaction mixture was refluxed for 5 h with stirring. After cooling to r.t., morpholine hydrochloride that precipitated was separated by filtration and the filtrate was evaporated to dryness under reduced pressure. The residue was washed with water (20 mL), collected by filtration and purified by crystallization from methanol–water.

3.4. 2-Amino-6-methoxymethyl-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine **14**

3.4.1. Method A

A solution of sodium methoxide (0.46 g Na, 40 mL methanol) was treated with chloromethyl-triazine **8** (3.82 g, 0.015 mol) and the resulting mixture was heated under reflux for 8 h. After cooling to r.t. water (60 mL) was added dropwise and the solution was left overnight. The solid that deposited was separated by filtration and purified by recrystallization from isopropanol.

3.4.2. Method B

To a suspension of the compound **8** (3.82 g, 0.015 mol) in methanol (30 mL), 7% aqueous sodium hydroxide (10 mL) was added. The reaction mixture was heated under reflux for 6 h. Then, the solution was concentrated under reduced pressure to a volume of 25 mL, treated with water (25 mL) and the resulting solution was allowed to stand at r.t. for 12 h. The solid that precipitated was collected by filtration and purified by crystallization from isopropanol.

3.5. 2-Amino-6-(4-aminophenoxymethyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine **15**

To a solution of 4-aminophenol (3.3 g, 0.03 mol) in anhydrous dioxane (50 mL) sodium (0.69 g, 0.03 mol) was added and the reaction mixture was heated under reflux for 6 h. Then, triazine **8** (6.37 g, 0.025 mol) was added and the resulting solution was refluxed for 14 h. Then, charcoal (0.2 g) was added and the solution was allowed to stand at r.t. overnight. The charcoal and side products that precipitated were separated by suction and the filtrate was concentrated under reduced pressure to a volume of 20 mL. The solution thus obtained was treated with water (80 mL) and the crude product **15**

Table II. Physico–chemical properties of the triazine derivatives **5–22**

Compd.	Yield (%)	M.P. (°C)	Analysis for
5	60	175–176 (MeOH)	$C_6H_{10}ClN_5$
6	51	179–180 (*i*-propanol)[a]	$C_8H_{12}ClN_5O$
7	74	214–215 (MeOH)	$C_8H_{11}CLN_6$
8	69	225–226 (H_2O)	$C_{10}H_{15}ClN_6$
9	66	196–198 (ethanol)	$C_{11}H_{17}ClN_6$
10	49	250–253 (dec.) (EtOH)	$C_{10}H_{15}FN_6$
11	58	275–278 (dec.) (EtOH)	$C_{10}H_{15}BrN_6$
12	60	230–232 (MeOH)	$C_{11}H_{18}N_6O_2S$
13	70	169–171 (dec.) (MeOH/H_2O)	$C_{14}H_{23}N_7O$
14	80 (Method A) 82 (Method B)	226–227 (*i*-propanol))	$C_{11}H_{18}N_6O$
15	53	225–227 (toluene)	$C_{16}H_{21}N_7O$
16	48	217–221 (dec.) (FMF/H_2O)	$C_{15}H_{20}N_{10}S_2$
17	35	230–232 (EtOH)	$C_{10}H_{16}N_6O$
18	46	326–327 (dec.) (DMF)	$C_{20}H_{30}N_{12}O$
19	85	226–227 (dec.) (MeOH)	$C_{11}H_{19}ClN_8S$
20	88	224–226 (H_2O)	$C_{10}H_{16}N_6S$
21	78	155–157 (EtOH)	$C_{14}H_{22}N_6O_2S$
22	72	223–225 (MeOH/H_2O)	$C_{16}H_{25}N_{11}S$

[a] Ref. [11] m.p. 178°C.

Table III. Spectroscopic data for the triazine derivatives **5–22**

Comp.	IR (KBr)	^{1}H-NMR ($CDCl_3$)	^{13}C-NMR ($CDCl_3$)
5[a]	3385, 3320, 3175, 1645, 1590,1560	2.49 (s, 6H, CH_3), 4.27 (s, 2H, CH_2), 6.95 (s, 2H, NH_2)	35.6, 46.7, 165.3, 168.8, 171.9
6[a]	3330. 3152, 2855, 1648, 1520, 1440	3.55–3.65 (m, HH), 3.65–3.75 (m, 4H), 4.3 (s, 2H, CH_2) 7.05 (s, 2H, NH_2)	43.1, 46.5, 65.9, 164.6, 166.9, 172.3
7	3490, 3275,3140, 1640, 1620, 1570, 1550, 1520	2.51 (s, 3H,CH_3), 3.26, (t, 2H), 4.37 (t, 2H), 4.69 (s, 2H), 6.29 (s, 2H, NH_2)	16.3, 35.8, 45.2, 46.0, 111.8, 158.6, 161.9, 166.9
8	3325, 3210, 1635, 1665, 1555, 1535	1.61 (s, 6H, CH_3), 2.04 (s, 3H, CH_3)	16.3, 26.5, 46.1, 53.6, 64.3, 155.5, 162.4, 172.1
9[a]	3372, 3319, 3230, 2965, 1657, 1534, 1469, 1378, 1336,	1.55 (s, 6H, CH_3), 1.7 (d, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.85 (s, 2H, CH_2), 4.45 (s, 2H, CH_2), 4.68 (q, 1H, CH), 7.0 (d, 2H, NH_2)	
10	3354, 3321, 3218, 2974, 1656, 1569, 1534, 1469, 1451	1.65 (s, 6H, CH_3), 2.1 (s, 3H, CH_2), 2.76 (s, 2H, CH_2), 5.1 (d, 2H, CH_2, $J_{HF} = 48$ Hz), 6.2 (s, 2H, NH_2)	
11	3323, 3216, 2963, 1657, 1535, 1467, 1448, 1379, 1332	1.58 (s, 6H, CH_3), 2.0 (s, 3H, CH_2), 2.81 (s, 2H, CH_2), 4.15 (s, 2H, CH_2), 5.3 (s, 2H, NH_2)	
12	3425, 3335, 3115, 1665, 1625, 1570, 1515, 1375, 1302, 1165, 1122	1.65 (s, 6H, CH_3), 2.11 (s, 3H, CH_3), 2.83 (s, 2H, CH_2), 3.23 (s, 3H, CH_3), 4.27 (s, 2H, CH_2), 5.77 (s, 2H, NH_2),	
13	3325, 3210, 1660, 1640, 1625, 1555, 1530, 1114	1.66 (s, 6H, CH_3), 2.11 (s, 3H, CH_3), 2.59 (t, 4H, CH_2), 3.41 (s, 2H, CH_2), 3.77 (t, 4H, CH_2), 5.39 (s, 2H, NH_2)	
14	3360, 3315, 3205, 1650, 1615, 1560, 1530, 1190, 1070	1.65 (s, 6H, CH_3), 2.1 (s, 3H, CH_3), 2.81 (s, 2H, CH), 3.48 (s, 3H, CH_3), 5.59 (s, 2H, NH_2)	16.4, 2.3, 53.7, 58.9, 64.1, 74.3, 154.9, 162.2, 166.2, 173.8
15	3460, 3400, 3300, 3200, 1640, 1625, 1570, 1540, 1505, 1225	1.55 (s, 6H, CH_3), 2.08 (s, 3H, CH_3) 2.78 (s, 2H, CH_2), 3.12 (s, 2H, NH_2) 4.8 (s, 2H, CH_2), 5.74 (s, 2H, NH_2), 6.51–6.81 (m, 4H)	
16	3424, 3338, 3159, 2964, 1638, 1526, 1457, 1410, 1379, 1263	1.55 (s, 6H, CH_3), 2.1 (s, 3H, CH_3), 2.8 (s, 2H, CH_2), 3.6 (m, 2H, CH_2), 4.0 (s, 2H, CH_2), 4.15 (m, 4H, CH_2), 6.9 (s, 2H, NH_2), 9.2 (s, 1H, NH)	
17	3450, 3325, 3215, 166o, 1640, 1570, 1560, 1535, 1085	1.66 (s, 6H, CH_3), 2.12 (s, 3H, CH_3), 2.84 (s, 2H, CH_2), 3.01 (t, 1H, OH), 4.49 (d, 2H, CH_2), 5.43 (s, 2H, NH_2)	16.3, 26.6, 53.7, 63.5, 64.1, 155.4, 161.7, 163.3, 175.7
18	3375, 3300, 3215, 1640, 1625, 1580, 1549, 1138		
19[a]	3320, 3205, 3155, 3050, 2970, 2770, 2730, 1655, 1590, 1575, 1540	1.57 (s, 6H, CH_3), 2.01 (s, 3H, CH), 2.84 (s, 2H, CH_2), 3.01 (t, 1H, OH), 4.49 (d, 2H, CH_2), 5.43 (s, 2H, NH_2)	
20	3325, 3210, 2540, 1655, 1635, 1555, 1535	1.66 (s, 6H, CH_3), 1.98 (t, 1H, SH), 2.09 (s, 3H, CH_3), 2.81 (s, 3H, CH_2) 3.51 (d, 2H, CH_2), 5.53 (s, 2H, NH_2)	
21	3385, 3305, 3205, 1720, 1640, 1630, 1570, 1530,	1.27 (t, 3H, CH_3), 165 (s, 6H, CH_3), 2.1 (s, 3H, CH_3), 2.81 (s, 2H, CH_2), 3.36 (s, 2H, CH_2), 3.64, (s, 2H, CH_2), 4.18 (q, 2H, CH_2), 5.44 (s, 2H, NH_2)	
22	3320, 3160, 1655, 1630, 1560, 1525	1.58 (s, 6H, CH_3), 2.07 (s, 3H, CH_3), 2.99 (s, 2H, CH_2), 3.11 (s, 3H, N–CH_3) 3.17 (s, 3H, N–CH_3), 3.83 (s, 4H, CH_2–S–CH_2), 8.58 (s, 2H, NH_2), 8.63 (s, 2H, NH_2)	

[a] NMR spectrum run in DMSO-d_6.

that precipitated was collected by filtration and purified by crystallization from toluene.

3.6. [2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl-methylthio]-7,8-dihydroimidazo[1,2-a]-1,3,5-triazine-4H(6H)-thione **16**

To a solution of 7,8-dihydroimidazo[1,2-*a*]1,3,5-triazine-2,4-dithione [13] (1.86 g, 0.01 mol) in methanolic potassium hydroxide (2.1 g KOH, 12 mL methanol) bromomethyl-triazine **11** (3.06 g, 0.01 mol) was added and the reaction mixture was stirred at r.t. for 0.5 h. The potassium salt of **16** that precipitated was separated by suction, washed with methanol (5 mL) and dried at r.t. The resulting solid was suspended in water (10 mL) and neutralized with 10% hydrochloric acid to give crude product **16** that was purified by crystallization from dimethylformamide–water.

3.7. [2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]methanol **17** and bis [2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-ylmethyl] ether **18**

To a suspension of the triazine **8** (12.7 g, 0.05 mol) in water (80 mL) was added 16% aqueous sodium hydroxide and the reaction mixture was stirred at ambient temperature for 12 h followed by refluxing for 6 h. After cooling to r.t., product **18** that precipitated was collected by filtration, washed with water (6 × 5 mL) (the aqueous filtrate which contained product **17** was used for further workup), dimethylformamide (4 mL) and ethanol (2 × 3 mL) and purified by crystallization from ethanol.

Aqueous filtrate from the experiment described above was neutralized with 15% hydrochloric acid, filtered with charcoal and evaporated under reduced pressure to dryness. The dry residue thus obtained was extracted with chloroform (100 mL). The organic layer was concentrated to a small volume and the crude product **17** that precipitated was collected by filtration and purified by crystallization from dimethylformamide.

Table IV. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($A^2 \times 10^3$) for **8**[a]

	x	*y*	*z*	U_{eq}
Cl(1)	2145(1)	4860(1)	323(1)	66(1)
N(1)	4061(2)	8959(2)	1928(1)	39(1)
C(2)	3051(3)	7534(3)	2052(1)	38(1)
N(3)	3019(2)	6028(2)	1723(1)	41(1)
C(4)	4039(3)	6108(3)	1250(1)	39(1)
N(5)	5145(2)	7393(2)	1089(1)	45(1)
C(6)	5129(3)	8786(3)	1465(1)	40(1)
N(7)	6304(3)	10114(3)	1352(1)	53(1)
N(8)	6278(3)	11586(2)	1725(1)	47(1)
C(9)	7635(3)	12556(3)	1606(1)	52(1)
C(10)	8780(5)	11852(6)	1130(2)	84(1)
C(11)	7916(3)	10088(3)	953(1)	53(1)
N(12)	2042(3)	7591(3)	2524(1)	53(1)
C(13)	3948(3)	4543(3)	843(1)	48(1)
C(14)	7957(5)	14245(4)	1922(2)	72(1)
C(15)	7305(5)	10100(5)	317(1)	71(1)
C(16)	9120(4)	8514(5)	1080(2)	78(1)

[a] U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor.

Table V. Selected bond lengths (Å) and angles (°) for **8**

Bond lengths			
Cl(1)–C(13)	1.786(3)	C(6)–N(7)	1.356(3)
N(1)–C(6)	1.327(3)	N(7)–N(8)	1.401(3)
N(1)–C(2)	1.345(3)	N(7)–C(11)	1.506(3)
C(2)–N(12)	1.314(3)	N(8)–C(9)	1.276(3)
C(2)–N(3)	1.364(3)	C(9)–C(10)	1.480(4)
N(3)–C(4)	1.321(3)	C(9)–C(14)	1.486(4)
C(4)–N(5)	1.327(3)	C(10)–C(11)	1.534(4)
C(4)–C(13)	1.503(3)	C(11)–C(15)	1.507(4)
N(5)–C(6)	1.358(3)	C(11)–C(16)	1.514(4)
Bond angles			
C(6)–N(1)–C(2)	115.1(2)	N(8)–N(7)–C(11)	113.0(2)
N(12)–C(2)–N(1)	117.9(2)	C(9)–N(8)–N(7)	108.3(2)
N(12)–C(2)–N(3)	117.9(2)	N(8)–C(9)–C(10)	114.0(2)
N(1)–C(2)–N(3)	124.3(2)	N(8)–C(9)–C(14)	121.0(3)
C(4)–N(3)–C(2)	113.6(2)	C(10)–C(9)–C(14)	125.0(3)
N(3)–C(4)–N(5)	128.1(2)	C(9)–C(10)–C(11)	105.3(2)
N(3)–C(4)–C(13)	116.2(2)	N(7)–C(11)–C(15)	110.2(2)
N(5)–C(4)–C(13)	115.7(2)	N(7)–C(11)–C(16)	111.4(2)
C(4)–N(5)–C(6)	112.9(2)	C(15)–C(11)–C(16)	110.8(3)
N(1)–C(6)–N(7)	117.8(2)	N(7)–C(11)–C(10)	99.3(2)
N(1)–C(6)–N(5)	125.6(2)	C(15)–C(11)–C(10)	111.3(3)
N(7)–C(6)–N(5)	116.6(2)	C(16)–C(11)–C(10)	113.3(3)
C(6)–N(7)–N(8)	117.6(2)	C(4)–C(13)–Cl(1)	109.0(2)
C(6)–N(7)–C(11)	128.2(2)		

3.8. S-[2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-ylmethyl] isothiourea hydrochloride **19**

A suspension of chloromethyl-triazine **8** (1.,7 g, 0.05 mol) and thiourea (3.9 g, 0.051 mol) in methanol (70 mL) was heated under reflux for 5 h. After cooling to r.t., the solid that precipitated was collected by filtration, washed with methanol (3 × 4 mL) and dried in a desiccator to give pure isothiouronium salt **19**, which was used for further transformations.

3.9. [2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]methanethiol ***20***

To a suspension of isothiouronium salt **19** (9.92 g, 0.03 mol) in water (40 mL), 5% aqueous sodium hydroxide and 98% hydrazine hydrate (5 mL) were subsequently added and the reaction mixture was heated under reflux for 1 h. After cooling to r.t., pH of the solution was adjusted to 7.5 with 1% hydrochloric acid. The crude product **20** that precipitated was washed with water and purified by recrystallization from water.

3.10. Ethyl [2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-ylmethylthio] acetate ***21***

Compound **20** (3.78 g, 0.015 mol) was dissolved in a solution of sodium ethoxide (0.016 mol) in ethanol (40 mL) and to the resulting solution ethyl chloroacetate (2.0 g, 0.016 mol) was added dropwise. The reaction mixture was heated under reflux for 35 h. After cooling to r.t. water was added (50 mL) and heating was continued for an additional 2 h. The solid that precipitated was separated by suction and purified by crystallization from ethanol.

3.11. 2-Amino-6-[(2-amino-4-dimethylamino-1,3,5-triazin-6-yl)-methyl thiomethyl]-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine ***22***

To a solution of sodium methoxide (0.4 g Na, 60 mL methanol), biguanide **1a** (2.0 g, 0.012 mol) and triazine **21** (3.4 g, 0.01 mol) were subsequently added. The resulting suspension was heated under reflux for 9 h, cooled to r.t. and treated with water (30 mL). After standing overnight, product **22** that precipitated was collected by filtration and purified by crystallization from methanol–water.

Physico–chemical properties of the 1,3,5-triazine derivatives **5**–**22** obtained are presented in *table I* and their spectroscopic data are shown in *table III*.

4. X-ray structure analysis

Crystal data for $C_{10}H_{15}N_6Cl$ (**8**): monoclinic, space group $P2_1/n$, $a = 7.3982(6)$, $b = 7.5814(5)$, $c = 22.680(1)$ Å, $\beta = 90.737(5)°$, $V = 1272.0(1)$ Å^3, $Z = 4$, $d_x = 1.330$ g cm^{-3}, $T = 293$ K. Data were collected on Kuma KM-4 diffractometer for crystal with dimensions $0.5 \times 0.4 \times 0.3$ mm up to $2\theta_{max} = 130°$. Out of 2165 measured reflections, 2123 were independent and used in further calculations. The structure was solved by direct methods with the program SHELXS-86 [19]. Full-matrix least-squares refinement was carried out on F^2 with SHELXL-93 [20]. Hydrogen atoms have been located on ΔF map and their parameters included in the refinement process. Final R indices for reflections with $I > 2\sigma(I)$ and 215 refined parameters are: $R_1 = 0.0558$, $wR_2 = 0.1462$ ($R_1 = 0.0635$, $wR_2 = 0.1680$ for all data). Final atomic coordinates, bond lengths and bond angles are listed in *table IV* and *table V*, respectively. Atom labeling is shown in *figure 4*.

Supplementary data

Further details of the crystal structure investigation may be obtained from the Director of the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, on quoting the full journal citation.

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References

[1] Kosary I., Kosztreiner E., Rabloczky G., Kurhy M., Eur. J. Med. Chem. 24 (1989) 97–105.

[2] Kreutzberger A., Schlafer J., Arch. Pharm. (Weinheim) 321 (1998) 827–830.

[3] Kreutzberger A., Kochanowski R., Arch. Pharm. (Weinheim) 321 (1989) 837–840.

[4] Mohr R., Buschauer A., Schunack W., Arch. Pharm. (Weinheim) 319 (1986) 878–885.

[5] Dobhi T.P., Shah V.H., Parkh A.R., Indian J. Pharm. Sci. 54 (1992) 109–111.

[6] Kukla M.J., Ludovici D., Jonssen P.A.J., Heeres J., Moereels H., Emiel L., Eur. Pat. Appl. EP 834 507, Chem. Abstr. 128 (1998) 257449.

[7] Brzozowski Z., Acta Pol. Pharm.-Drug Res. 55 (1998) 49–56.

[8] Angelucci R., Anteni D., Giraldi P.N., Longemann W., Naunini G., Experientia 19 (1963) 234–236.

[9] Stivens M.F.G., Bliss E.A., Brown T.B., Mckenzie S.M., Eur. J. Med. Chem.-Chim. Ther. 19 (1984) 372–379.

[10] Mayumi O., Kawahara N., Goto D., Wakabayashi Y., Ushiro S., Yoshida S., Izumi H., Kuwano M., Sato Y., Cancer Res. 56 (1996) 1512–1516.

[11] Hayashi S., Furukawa M., Fujino Y., Yoshimatsu S., Chem. Pharm. Bull. 17 (1969) 329–334.

[12] Overberger C.G., Michelotti F.W., Corabateas P.M., J. Am. Chem. Soc. 79 (1957) 942–948.

[13] F. Saczewski, M. Gdaniec, Liebigs Ann. Chem. (1987) 721–724.

[14] Prisbe E.J., Verheyden J.P.H., Moffatt J.G.M., J. Org. Chem. 43 (1978) 4774–4784.

[15] Binsch G., Lambert J.B., Roberts B.W., Roberts J.D., J. Am. Chem. Soc. 86 (1964) 5564–5570.

[16] Quantum chemical calculations were performed using semiempirical AM1 method as implemented into SPARTAN program, version 5.0 (1997), Wavefunction Inc., Suite 370, 18401 Von Karman Ave, Irvine CA 92612, USA and installed on a Silicon Graphics O2 workstation.

[17] Perrakis A., Hempel A., Hamodrakas S.J., Camerman N., Tsitsa P., Antoniadou-Vyzas E., Camerman A., J. Cryst. Spectr. Res. 23 (1993) 821–824.

[18] Monks A., Scudeiro D., Skehan P., J. Nat. Cancer Inst. 83 (1991) 757–761.

[19] G.M. Sheldrick, SHELXS-86: Program for the Solution of Crystal Structures. University of Göttingen, Germany, 1986.

[20] G.M. Sheldrick, SHELXL-93: Program for the Refinement of Crystal Structures. University of Göttingen, Germany, 1993.

[21] Brzozowski Z., Kamiński Z., Kozakiewicz I., Angielski S., Rogulski J., Acta Pol. Pharm. 36 (1979) 401–410.