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Original article

Synthesis and antitumor activity of novel 2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine derivatives

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Abstract

The syntheses and antitumor activities of novel 2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine derivatives **4**–**38** are described. All the compounds prepared were screened at the National Cancer Institute (NCI) for their activities against a panel of 60 tumor cell lines and relationships between structure and antitumor activity in vitro are discussed. The triazines **11**, **16**, **20**, **23**, **23** and **34**–**38** exhibited modest or fairly high activity against one or more human tumor cell lines. Prominent compound with remarkable activity (log GI50, $< -8.00 - -5.00$) to all investigated cell lines and highly potent (log GI50 $< -8.00 - -7.64$) against some cell lines of Leukemia (CCRF-CEM, K-562, RPMI-8226, SR), CNS Cancer (SF-539) and Breast Cancer (T-47D) was 2-[2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(5-nitro-2-thienyl)acrylonitrile (**25**). © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: 2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine derivatives; Synthesis; Structures; Antitumor effect

1. Introduction

Despite the existence of considerable number of reports on biological activities of 2,4-diamino-1,3,5-triazine derivatives, only a few reports for preparation of this class of compounds with anticancer activity have been found in the literature [1–6]. In our previous study [6] we have demonstrated that variously substituted 2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine derivatives caused considerable growth inhibition on distinct tumor cell lines, a prominent example being the 6-bromomethyl substituted compound **1a**. The aim of the present investigation was to extend our studies in this class, and explore structure–activity relationships for in vitro antitumor activity.

2. Results and discussion

2.1. Synthesis

The synthetic routes utilised for the preparation of the target compounds **4**–**8**, **9**–**18**, **19**–**34** and **35**–**38** are described in Figs. 1–4, respectively.

Reactions of the previously described [6] 6-bromomethyl- or 6-chloromethyltriazines **1a** and **1b** with azoles (Fig. 1) were carried out according to the known procedure which consists in heating of the substrates in DMSO at 30–40 °C in the presence of NaOH [7] and the desired 2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-6-(heteroarylmethyl)-1,3,5-triazines **4**–**8** were obtained in 25–82% yield. Analogous triazines with cyanomethyl (**9**), methylsulfonylmethyl (**10**), phenylsulfonylmethyl (**11**) and a series of 6-aryl(heteroaryl)-1,3,5-triazines **12**–**15**, **17** and **18** were prepared by reacting biguanides **3a** and **3b** [8] with suitable carboxylic acid esters in methanolic solution (Fig. 2). The above reactions proceeded smoothly at room temperature (**9** and **15**; 76 and 58% yield, respectively) or at reflux (**10**–**14**; 28–72% yield). However, the formation of 2-amino-6-sulfamoylphenyl-1,3,5-triazine (**16**) could only be accomplished upon heating of the substrates in pyridine (42% yield).

The resulting 2-amino-1,3,5-triazines **12** and **15** were further converted to the 2-(1*H*-pyrrol-1-yl)-1,3,5-triazines **17** and **18** (72 and 83% yield, respectively) through the agency of 3,5-dimethoxylthtrahydrofurane in the presence of acetic acid (Fig. 2).

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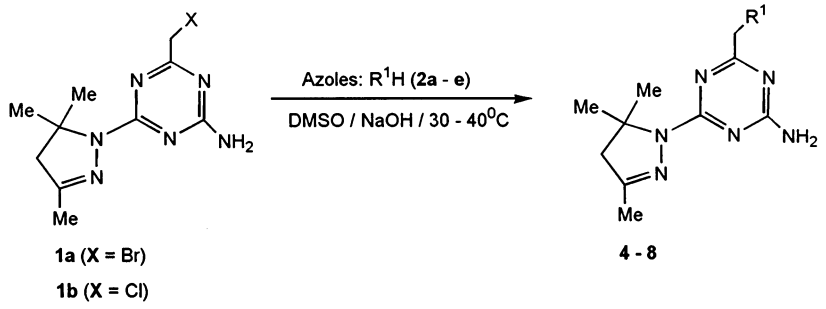
The reaction of [2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]acetonitrile (**9**) with hydroxylamine led to the formation of the expected acetamide oxime **19** (Fig. 3). Finally, a series of 2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazines connected directly to the cyanovinyl pharmacophore were obtained



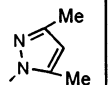
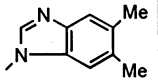
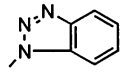
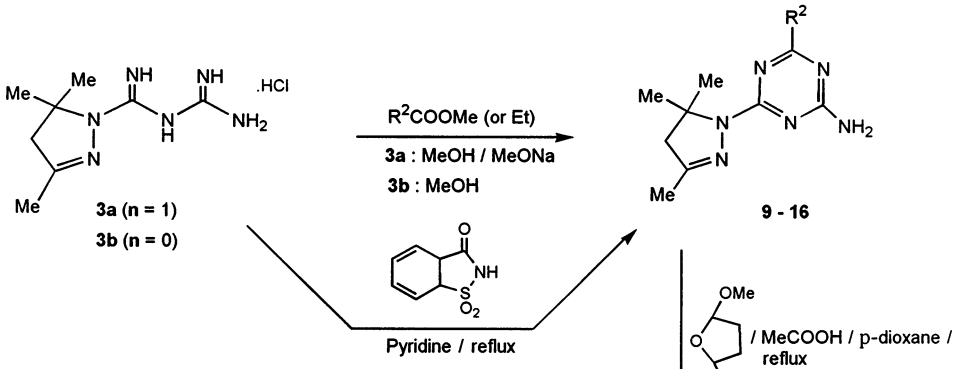
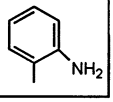
Compd.	4	5	6	7	8
R^1					

Fig. 1.



Compd.	9	10	11	12	13
R^2					

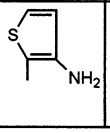
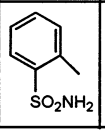
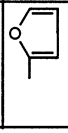
Compd.	14	15	16	17	18
R^2					

Fig. 2.

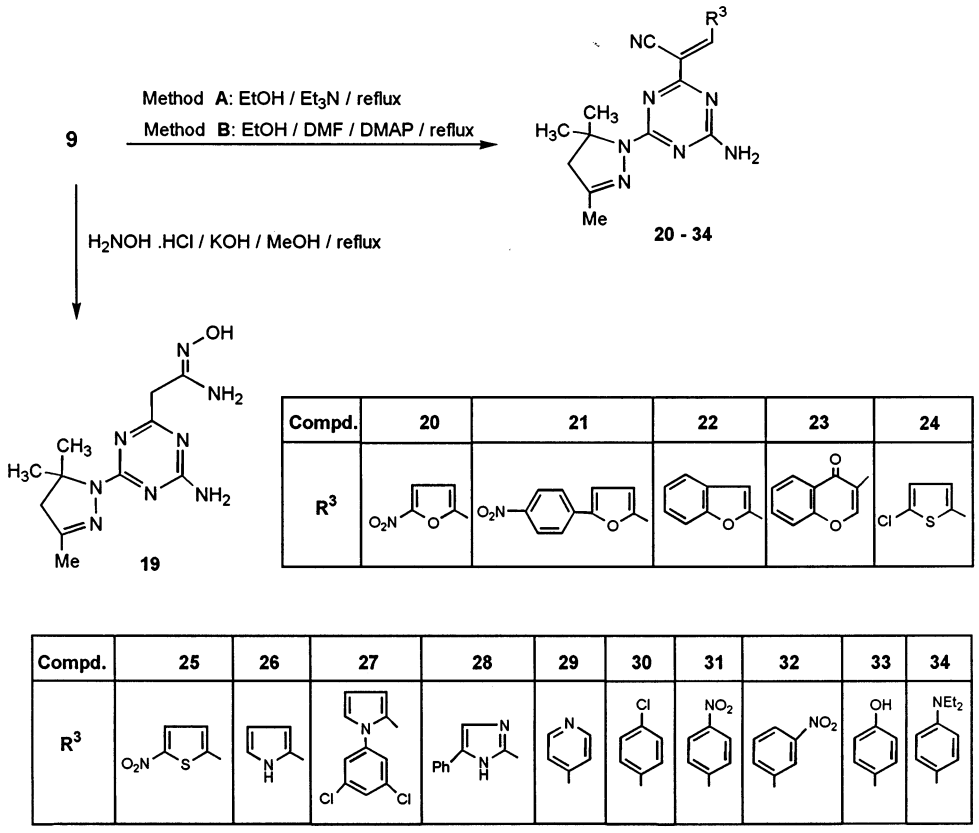


Fig. 3.

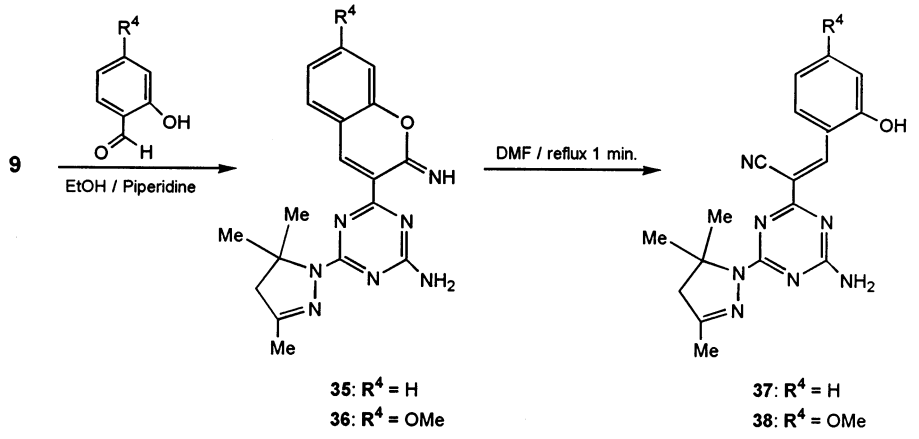


Fig. 4.

from acetonitrile **9** and the variously substituted aromatic or heteroaromatic aldehydes (Fig. 3). These typical Knoevenagel condensations were catalysed by triethylamine or piperidine and upon heating of the substrates in methanol furnished corresponding derivatives **20**–**33** with good (52%) to high (92%) yields. However, the reaction of **9** with 4-dimethylamino-benzaldehyde leading to the formation of **34** required refluxing of the substrates in DMF/EtOH solution in the presence of 4-dimethylaminopyridine (DMAP) as a catalyst. The IR and ^{1}H-NMR spectra of the compounds with cyanovinyl group were determined. Each compound exhibited a single strong absorption of the nitrile group and the single resonance for =CH proton denoting its stereochemical homogenity.

It is well known that the reactions of active acetonitriles with salicylic aldehyde lead to the formation of 2-iminochromene derivatives [9–11]. As depicted in Fig. 4, the analogous reactions of **9** with 2-hydroxybenzaldehyde and 4-methoxy-2-hydroxybenzaldehyde car-

ried out in ethanol at room temperature gave the expected 2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-6-(7R^4-2-imino-2*H*-chromen-3-yl)-1,3,5-triazines (**35**) (R^4 = H; 71% yield) and **36** (R^4 = MeO; 92% yield). Interestingly, we found that the latter compounds could be easily transformed into the desired acrylonitriles **37** and **38** upon short reflux in DMF. We reasoned, that the cleavage of the 2-iminochromenes can proceed via either [1,3] prototropic shift (Fig. 5, pathway A) or [1,5] prototropic shift (pathway B). In order to establish the reaction mechanism, the structures of both reactants and transition states were located and optimised using AM1 Hamiltonian [12]. Calculated reaction barrier for reaction path A (71.1 kcal mol^{-1}) and path B (27.3 kcal mol^{-1}) indicates that [1,5] prototropic shift is largely preferred and a six-membered transition state is involved in the cleavage of 2-iminochromenes **35**–**36**.

All the final products were characterised by IR and NMR spectroscopy as shown in experimental protocols. Elemental analyses were in accordance with the proposed structures.

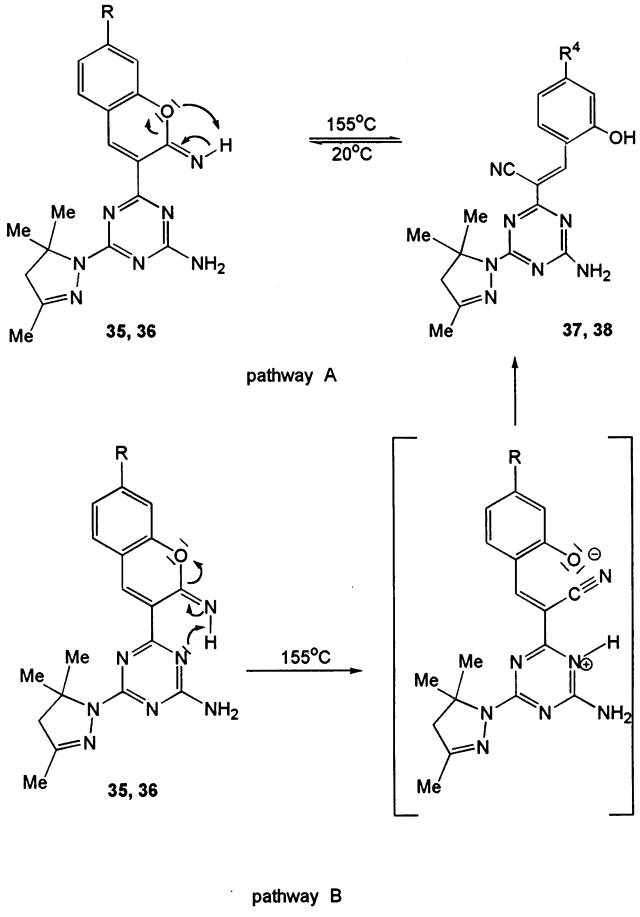


Fig. 5.

2.2. Biology

All compounds **4**–**38** were submitted to the National Cancer Institute (NCI, Bethesda, USA) for testing against a panel of ca. 60 tumor cell lines. Details of this test system and the information, which is encoded by the activity pattern over all cell lines, have been published [13–15]. The antitumor activity of a test compound is reported for each cell line by three parameters: log GI50 value (GI50 = molar concentration of the compound that inhibits 50% net cell growth), log TGI value (TGI = molar concentration of the compound leading to total inhibition of net cell growth), and log LC 50 value (LC 50 = molar concentration of the compound leading to 50% net cell death). Furthermore, a meangraph midpoint (MG_MID) is calculated for each of the mentioned parameters, giving an averaged 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 new selectivity patterns is one of the intentions of the screening program. Selectivity of a compound with respect to one or more cell lines of the screen is characterised by a high deviation of the particular cell line parameter compared to the MG_MID value.

The following is to be noted regarding the tumor cell growth inhibition data with the tested compounds. Replacement of the bromine atom in **1a** by heteroaryl (**4**–**8**) or π-electron groups such as cyano (**9**), methylsulfonyl (**10**), phenylsulfonyl (**11**) or *N*-hydroxyamidine (**19**) resulted in inactive compounds. The introduction of 3-amino-2-thienyl (**14**), 2-sulfamoylphenyl (**16**) or 2-imino-2H-chromen-3-yl (**35**, **36**) substituents into parent structure led to compounds with modest activity (log MG_MID GI50 values in a range between −4.16 and −4.89); Table 1).

Another strategy incorporated into the design of novel analogues was linking the 1,3,5-triazine ring with cyanovinyl group and varying the substituents on, or the nature of, the aromatic (heteroaromatic) ring (compounds **20**–**34**, **37** and **38**). As shown in Table 2, the 5-nitrothienyl (**25**) 3-nitrophenyl (**32**) and 5-nitrofuryl (**20**) derivatives were the most potent compounds in this series (log MG_MID GI50 values −6.59, −6.06 and −5.44, respectively). Elimination of the nitro group from the thiophene derivative **25** yielded vinylene compound **15** with no anticancer activity. Introduction substituents such as chloro (**24**, **30**), hydroxy (**33**) or dimethylamino (**34**) or shifting the nitro substituent in **32** from *meta* to *para* position (compound **31**) led to a dramatic decrease of activity. The loss in potency was also observed when the second aryl was connected to the cyanovinyl pharmacophore (compounds **21** and **27**), which suggests that the lengths of the molecule make major contribution to the variation in cytotoxicity.

Table 1
Overview of the results of the in vitro anticancer screening for selected 2-amino-6-(aryl- or heteroaryl)-1,3,5-triazines **14**, **16**, **35** and **36**

Compound	Number of the cell lines investigated	Number of the cell lines giving positive GI50, TGI and LC_{50}						MG_MID for GI50 and most sensitive cell line
		$-Log_{10}$ GI50		$-Log_{10}$ TGI		$-Log_{10}$ LC_{50}		
		No.	Range	No.	Range	No.	Range	
14	57	32	4.62–4.03	1	4.15	0		4.16, melanoma: M14
16	53	53	5.56–4.13	8	4.39–4.06	0		4.89, colon cancer: HCT-15
35	58	57	4.89–4.49	55	4.54–4.12	44	4.26–4.02	4.72, leukemia: MOLT-4
36	58	57	4.93–4.50	55	4.56–4.02	37	4.25–4.01	4.73, leukemia: MOLT-4

Data obtained from the NCI's in vitro disease—oriented human tumor cells screen [13,14]. The response parameters (mean $-\log_{10}$): GI50, TGI and LC50 are interpolated values representing the molar concentrations at which percentage growth is +50, 0 and −50, respectively. 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 structure of the compounds **20**–**34** containing the cyanovinyl group resembles, to some extend, those of tyrphostins [16,17] and 'stilbenic' tyrosine kinase inhibitors [18–21]. These compounds are conjugated molecules, but they are still flexible enough to permit a wide variety of conformations. Thus, in addition to evaluating the contributions of the aryl (heteroaryl) substituents to bioactivity, the topology of the most active molecules was considered. This work was undertaken to determine these structural features, which contribute to the marked disparity in cytotoxicity between the compounds of this series.

The shapes of active compounds **20**, **25** and **32** and inactive **31** were generated by molecular modelling [12], and the measurements performed are illustrated in Fig. 6. The torsion angles θ_1 and θ_2 were formed between rings **A** and **B** and the adjacent olefinic linkage, respectively, while the distance between 2-amino group at the 1,3,5-triazine ring and nitro group was reflected in the *d* measurement. These angles and the distances *d* determined are collected in Table 3. The data in Table 3 revealed that the θ_1 and θ_2 values for **20** and **25** were substantially lower than for the corresponding phenyl analogues **32** and **31**, respectively. In case of **20** and **25** this means that the position of the polar CN and NO_2 groups in space is completely different from compounds **31** and **32**. This difference can mean that the interaction of ligand compounds with target protein can be completely different. In this case we can assume that compounds **20** and **25** display more favourable interactions on a molecular base.

In conclusion, whatever the mechanism of anticancer action of 2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazines is, the compound **25** with 5-nitrothienyl substituent is superior to 6-bromomethyl derivative **1a** we reported previously, and therefore may prove useful in the development of new chemotherapeutic agents.

3. Experimental

M.p.s are not corrected and were recorded on a Buchi apparatus. IR spectra, KBr pellets, 400–4000 cm^{-1}, were recorded on a Perkin–Elmer 1600 FTIR spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini 200 instrument at 200 and 50 MHz, respectively (chemical shifts are expressed as δ values relative to Me_4Si as standard). Analyses of C, H, N were within $\pm 0.4\%$ of the theoretical values.

*2-Amino-6-(heteroarylmethyl)-4-(3,5,5,-trimethyl-2-pyrazolino)-1,3,5-triazines (**4**–**8**)*

To a solution of the corresponding azole **2a**–**f** (0.02 mol) in DMSO (10 mL) NaOH (1.6 g, 0.04 mol) and bromomethyl- or chlomethyltriazine **1a** or **1b** [6] (0.01 mol) was added and stirred at 30–40 °C for 2 h. After cooling to room temperature water (50 mL) was added dropwise and the suspension was left overnight. The precipitate was collected by filtration, washed with water, dried and purified by crystallisation.

*3.1.1. 2-Amino-6-(1-pyrazolylmethyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine (**4**)*

Yield: 50%, m.p. 183–185 °C (MeOH); IR (KBr): 3440 (NH_2), 1632 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.3 (s, 6H, CH_3), 1.95 (s, 3H, CH_3), 2.75 (s, 2H, CH_2), 5.1 (s, 2H, CH_2), 6.25 (dd, 1H, CH), 6.95 (s, 2H, NH_2), 7.45 (d, 1H, CH), 7.75 (d, 1H, CH) ppm. Anal. ($C_{13}H_{18}N_8$) C, H, N.

*3.1.2. 2-Amino-6-(3,4-dimethyl-1-pyrazolylmethyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine (**5**)*

Yield: 82%, m.p. 183–185 °C (MeOH–water); IR (KBr): 3408 (NH_2), 1632 (C=N) cm^{-1}; ^{1}H-NMR

Table 2
In vitro anticancer data for selected triazinylacrylonitriles **20**, **23**, **25**, **32**, **34**, **37** and **38**

Cell line	$-\text{Log}_{10}$ GI50 (A), $-\log_{10}$ TGI (B) and $-\log_{10}$ LC50 (C) of the in vitro inhibitory activity tests for given compounds																				
	20			**23**			**25**			**32**			**34**			**37**			**38**		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Leukemia																					
CCRF-CEM	5.96			5.72			7.76	7.03	4.43	4.80	4.35		4.56			4.74	4.45	4.15	4.40	4.11	
HL-60 (TB)	5.60	5.20		7.39	5.28	4.55	NT			7.36	5.43		4.45			4.52	4.19		4.78	4.33	
K-562	6.60	6.19	5.57	4.49			>8.00	6.45	4.44	6.29	5.35	4.22				4.29			4.67	4.07	
MOLT-4	5.71	5.27		4.66	4.19		6.86	6.42	4.96	4.70	4.35	4.01	5.37			4.52			4.88	4.47	4.05
RPMI-8226	5.63	5.28		4.75	4.26		7.75	7.08	6.33	6.62	6.22	4.76	4.24			4.77	4.44	4.11	4.38	4.01	
SR	5.59			4.75			7.75	6.82		4.70	4.27		4.91			4.44			4.77	4.45	4.12
Non-small cell lung cancer																					
A549/ATCC	4.88	4.53	4.17	NT			6.04	4.78	4.26	6.28	4.74		4.79	4.49	4.19	4.80	4.50	4.20	4.73	4.35	
EKVX	5.73	5.45	5.16	4.65			6.46	5.60		5.25			4.80	4.24		4.80	4.43	4.06	4.69	4.32	
HOP-62	4.78	4.51	4.25	4.49			6.51	5.76		6.74	4.84		5.04	4.68	4.34	4.69	4.41	4.13	4.62	4.36	4.10
HOP-92	5.74	5.47	5.20	4.44			6.52				5.22		5.54	4.87	4.44	4.69	4.44	4.17	4.50	4.23	
NCI-H23	5.63	5.28	4.23	4.57	4.04		5.96	5.57	5.17	5.69	4.28		4.63	4.18		4.77	4.46	4.15	4.72	4.37	4.02
NCI-H226	5.65	5.16	4.58	4.64			5.82	5.51	5.20	5.63	4.74	4.15	5.54	4.84	4.36	4.76	4.42	4.08	4.78	4.48	4.11
NCI-H322M	4.76	4.51	4.25	4.42			5.00	4.16		5.31			4.89	4.42		4.71	4.32		4.75	4.34	
NCI-H460	5.75	5.37	4.97	4.57			6.69	6.38	5.55	6.47	5.79	4.55	4.81	4.54	4.27	4.71	4.39	4.07	4.76	4.42	4.07
NCI-H522	5.78	5.45		4.58			7.23	6.45	6.14	6.37	5.72	5.24	4.77	4.44	4.10	4.70	4.44	4.18	4.66	4.41	4.16
Colon cancer																					
COLO 205	5.81	5.54	5.27	NT			6.47	5.97	5.26	6.06	4.96	4.29	4.53			4.76	4.50	4.24	4.77	4.51	4.25
HCC-2998	4.80	4.40	4.01	4.52			6.72	6.42	6.12	5.83	5.40	4.95	4.52	4.10		4.71	4.44	4.16	4.71	4.42	4.12
HCT-116	5.87	5.58	5.26	4.69	4.12		6.71	6.37	4.67	6.43	5.13	4.42	4.77	4.42	4.08	4.74	4.49	4.25	4.75	4.50	4.25
HCT-15	5.95	5.51		4.64			7.25	6.49		6.06	4.97					4.71	4.41	4.11	4.75	4.45	4.15
HT29	5.69	5.37	5.04	4.58	4.16		6.62	6.14		6.30			4.92	4.62	4.31	4.70	4.37	4.04	4.72	4.35	
KM12	4.84	4.58	4.29	4.44			6.65	6.09	5.25	6.00	4.95	4.39	5.14			4.80	4.50	4.20	4.81	4.53	4.26
SW-620	5.75	5.43	5.11	4.51			7.49			6.37	4.88	4.14	4.25			NT			NT		
CNS cancer																					
SF-268	5.63	5.03	4.51	4.67			6.39			6.24	4.56		5.17	4.53		4.74	4.43	4.12	4.76	4.42	4.07
SF-295	4.84	4.53	4.23	4.50			5.62	4.61		5.88	4.62		4.81	4.27		4.81	4.51	4.22	4.72	4.37	4.01
SF-539	5.49	4.92	4.45	4.58			7.64	7.24	6.53	6.24	4.92	4.08	5.16	4.70	4.32	4.77	4.51	4.26	4.80	4.54	4.24
SNB-19	4.83	4.55	4.27	NT			5.78	5.29	4.28	6.04	4.11		4.89	4.58	4.27	4.59	4.04		4.45		
SNB-75	5.77	5.25	4.63	NT			6.50	6.06	5.01	6.20	5.05		5.32	4.74	4.37	NT			NT		
U251	5.73	5.42	5.12	4.61			7.24	6.66	6.17	6.43	4.91	4.39	4.96	4.64	4.32	4.74	4.38	4.03	4.57	4.03	
Melanoma																					
LOX IMVI	5.79	5.51	5.24	4.57			6.86	6.52	6.19	6.53	4.94		4.83	4.51	4.20	NT			NT		
MALME-3M	5.70	5.37	5.05	NT			6.42	5.89	5.02	5.32	4.52		4.74	4.39	4.03	4.73	4.47	4.21	4.84	4.50	4.16
M14	5.84	5.56	5.28	4.63			6.63	6.10	5.39	5.85	4.93		5.13	4.68	4.32	4.16	4.44	4.20	4.69	4.45	4.21
SK-MEL-2	5.40	4.90	4.33	4.83	4.37		6.48	5.79	5.30	6.10	5.57	5.09	4.88	4.58	4.27	4.65	4.40	4.15	4.68	4.41	4.14
SK-MEL-5	5.15	4.70	4.32	4.73			6.49	5.86	5.37	5.69	5.11	4.45	4.74	4.43	4.12	4.81	4.54	4.27	4.82	4.55	4.27
SK-MEL-28	5.30	4.72	4.23	4.67			6.77	6.37		5.76	4.56		4.44			4.78	4.51	4.25	4.84	4.53	4.22

Table 2 (*Continued*)

Cell line	$-\text{Log}_{10}$ GI50 (A), $-\log_{10}$ TGI (B) and $-\log_{10}$ LC50 (C) of the in vitro inhibitory activity tests for given compounds																			
	20			**23**			**25**			**32**			**34**			**37**			**38**		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
UACC-257	5.00	4.64	4.32	4.29			6.69	6.27	5.73	5.35	4.64		4.46			4.72	4.41	4.11	4.62	4.23	
UACC-62	5.69	5.32	4.86	NT			6.16	5.67	5.27	5.71	5.19	4.60	4.66			4.77	4.47	4.18	4.76	4.46	4.16
Ovarian cancer																					
IGROV1	5.71	5.42	5.12	4.49			6.83	6.54	6.25	5.84	4.92	4.20	5.46	4.74	4.37	4.64	4.34	4.05	4.62	4.25	
OVCAR-3	5.48	4.95	4.46	4.65			7.30	6.68	6.14	6.21	5.28	4.25	4.76	4.42	4.09	4.83	4.56	4.28	4.82	4.55	4.27
OVCAR-4	5.08	4.68	4.34	4.47			5.74	5.00	4.39	6.12	4.65		5.11	4.61	4.15	4.73	4.47	4.21	4.80	4.52	4.24
OVCAR-5	5.80	5.49	5.18	4.45			5.52	4.79	4.09	5.37			4.58	4.03		4.81	4.52	4.22	4.82	4.47	4.12
OVCAR-8	5.72	5.29	4.75	4.59			7.24	6.68	6.20	6.26	4.52		5.16	4.70	4.35	4.58	4.21		4.48		
SK-OV-3	4.89	4.56	4.24	4.59	4.07		5.10	4.30		5.73	4.65		5.61	4.93	4.46	4.64	4.27		4.57	4.19	
Renal cancer																					
786-O	5.81	5.52	5.24	4.72			6.89	6.54	6.19	6.36	4.98	4.49	4.94	4.63	4.31	4.80	4.54	4.27	4.79	4.52	4.26
A498	4.75	4.50	4.25	4.74	4.20		5.37	4.74		5.92	5.29	4.52	4.84	4.55	4.25	4.61	4.20		4.55	4.16	
ACHN	5.75	5.47	5.20	4.63	4.03		6.77	6.45	6.12	6.06	4.87	4.22	4.87	4.58	4.29	4.73	4.46	4.19	4.77	4.51	4.25
CAKI-1	5.81	5.54	5.27	4.63			6.35	5.35	4.44	6.07	4.08		4.84	4.56	4.28	4.71	4.43	4.16	4.76	4.42	4.07
RXF 393	5.75	5.50	5.24	4.79	4.19		6.72	6.40		6.59	6.08	4.42	5.26	4.74	4.35	4.69	4.40	4.11	4.79	4.48	4.16
SN12C	5.73	5.42	5.22	NT			6.41	5.82	5.34	5.51	4.55		4.56	4.15		4.59	4.14		4.57		
TK-10	5.78	5.51	5.25	4.56			6.68	6.34	5.01	5.76	4.39		4.94	4.53	4.11	4.57	4.23		4.52	4.11	
UO-31	5.73	5.44		4.72	4.01		6.86	6.56	6.25	6.60	5.96	5.08	4.69	4.35	4.02	4.72	4.44	4.15	4.76	4.44	4.13
Prostate cancer																					
PC-3	5.05	4.68	4.34	4.61	4.11		6.84	6.37	4.45	6.35	4.81		4.52			4.80	4.50	4.21	4.42		
DU-145	5.54	4.98	4.47	4.50			6.66	6.27	5.51	6.11	4.81	4.10	5.17	4.57	4.02	4.81	4.49	4.18	4.77	4.42	4.07
Brest cancer																					
MCF7	5.77	5.49	5.22	4.49			6.95	6.43	5.65	6.53	5.91	4.54	4.49			4.61	4.19		4.73	4.34	
NCI/ADR-RES	5.22	4.60	4.10	4.80	4.44	4.09	6.05	4.53		5.68	4.53		5.07	4.61	4.21	4.67	4.33		4.51	4.01	
MDA-MB-435	5.74	5.42	5.09	4.49			6.78	6.39	6.01	6.42	5.76	4.86	4.56			4.76	4.48	4.20	4.77	4.48	4.19
HS 578T	5.38	4.96	4.31	4.62	4.07		6.16			5.43	4.26		5.62	4.94		4.79	4.45	4.11	4.74	4.40	4.06
MDA-MB-231/ATCC	5.74	5.49	5.23	4.52			6.64	6.24	5.95	6.51	5.57	4.42	4.87	4.57	4.27	4.70	4.39	4.08	4.75	4.35	
MDA-N	5.52			4.72	4.07		6.44	5.37		6.27	5.43		4.62			4.82	4.51	4.21	4.77	4.47	4.17
BT-549	5.57	5.11	4.31	NT			6.41	5.61	4.16	6.05	4.14		4.16			4.44	4.17		4.40		
T-47D	5.64	5.29	4.00	4.74	4.16		7.73	7.20	5.92	5.89	4.34		4.80	4.44	4.08	4.80	4.50	4.19	4.80	4.46	4.13
MG_MID	5.45	5.00	4.50	4.67	4.08	4.01	6.59	5.89	4.91	6.06	4.88	4.22	4.84	4.36	4.13	4.70	4.39	4.12	4.69	4.34	4.09

Data obtained from the NCI's in vitro disease—oriented human tumor cells screen [13,14]. The response parameters (mean $-\log_{10}$): GI50, TGI and LC50 are interpolated values representing the molar concentrations at which percentage growth is +50, 0 and −50, respectively. 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. NT: not tested.

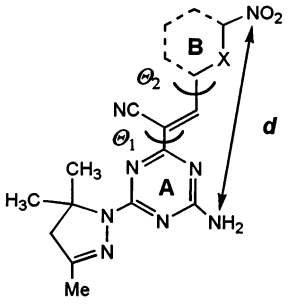


Fig. 6. Indication of the θ_1 and θ_2 torsion angles and the interatomic distance *d*.

Table 3
Certain angles and distances of compounds **20**, **25**, **32** and **31** determined by molecular modelling

Compound	Angle (°)		Interatomic distance *d* (Å)
	θ_1	θ_2	
20	−8.4	−7.0	8.9
25	−4.2	−1.9	9.7
32	−32.7	−38.8	9.37
31	−32.8	−39.1	10.6

(DMSO-d_6): δ 1.27 (s, 6H, CH_3), 1.93 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 2.73 (s, 2H, CH_2), 4.88 (s, 2H, CH_2), 5.80 (s, 1H, CH), 7.0 (d, 2H, NH_2) ppm. Anal. ($C_{15}H_{18}N_8$) C, H, N.

3.1.3. 2-Amino-6-(1-imidazolylmethyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine ***(6)***

Yield: 25%, m.p. 239–241 °C (MeOH–water); IR (KBr): 3408 (NH_2), 1664 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.28 (s, 6H, CH_3), 1.93, (s, 3H, CH_3), 2.74 (s, 2H, CH_2), 4.96 (s, 2H, CH_2), 6.87 (s, 1H, CH), 6.92 (s, 2H, NH_2), 7.14 (s, 1H, CH), 7.64 (s, 1H, CH) ppm. Anal. ($C_{13}H_{18}N_8$) C, H, N.

3.1.4. 2-Amino-6-(5,6-dimethyl-1-benzimidazolylmethyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine ***(7)***

Yield: 70%, m.p. 152–155 °C (MeOH–water); IR (KBr): 3456 (NH_2), 1660 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.1 (s, 6H, CH_3), 1.9 (s, 3H, CH_3), 2.3 (s, 6H, CH_3), 2.7 (s, 2H, CH_2), 5.15 (s, 2H, CH_2), 6.95 (s, 2H, NH_2), 7.2 (s, 1H, CH), 7.4 (s, 1H, CH), 8.1 (s, 1H, CH) ppm. Anal. ($C_{19}H_{24}N_8$) C, H, N.

3.1.5. 2-Amino-6-(1H-benzotriazol-1-ylmethyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine ***(8)***

Yield: 54%, m.p. 242–243 °C (MeOH); IR (KBr): 3375, 3310, 3225 (NH_2), 1650 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 0.76 (s, 6H, CH_3), 188 (s, 3H, CH_3), 2.88 (s, 2H, CH_3), 5.77 (s, 2H, CH_2N), 7.10 (d, 2H, NH_2), 7.38 (t, 1H, CH), 7.52 (t, 1H, CH), 7.81 (d, $J = 8.1$ Hz, 1H, CH), 8.05 (d, $J = 8.1$ Hz, 1H, CH) ppm. Anal. ($C_{16}H_{19}N_9$) C, H, N.

3.2. 2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazyn-6-yl]acetonitrile ***(9)***

To a solution of CH_3ONa in MeOH (7.4 g Na, 200 mL MeOH) biguanide hydrochloride **3a** (69.8 g, 0.3 mol) was added and the reaction mixture was stirred at r.t. for 1 h. Then, a solution of ethyl cyanoethanoate (34.8 g, 0.31 mol) in MeOH (30 mL) was added and stirring was continued at r.t. for 72 h. The precipitate was separated by suction, washed thoroughly with water, dried and purified by crystallisation from 1-butanol: 76% yield, m.p. 246–247 °C, IR (KBr): 3350, 3315, 3200 (NH_2), 2255 (C=N), 1650 (C=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1710 (s, 6H, CH_3), 2.13 (s, 3H, CH_3), 2.85 (s, 2H, CH_2), 3.67 (s, 2H, CH_2), 7.28 (s, 2H, NH_2) ppm. Anal. ($C_{11}H_{15}N_7$) C, H, N.

3.3. 2-Amino-6-(methylsulfonylmethyl, -or phenylsulfonylmethyl, -or 2-furyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazines ***(10–12)***

A solution of 0.02 mol of the corresponding carboxylic acid ester (ethyl methylsulfonylacetate, methyl phenylsulfonylacetate or methyl 2-furancarboxylate) and biguanide **3b** (3.93 g, 0.02 mol) in MeOH (anhydrous, 15 mL) was stirred at ambient temperature for 12 h followed by refluxing for 6 h. After cooling to r.t. and standing overnight, the product that precipitated was separated by suction, washed with MeOH (2 × 2 mL) and purified by crystallisation.

3.3.1. 2-Amino-6-(methylsulfonylmethyl)-4-(3,5,5,-trimethyl-2-pyrazolino)-1,3,5-triazine ***(10)***

Yield: 62%, m.p. 230–232 °C (MeOH); IR (KBr): 3425, 3335 (NH_2), 1650, 1625, (C=N), 1375, 1165 (SO_2) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.65 (s, 6H, CH_3), 2.11 (s, 3H, CH_3), 2.83 (s, 2H, CH_2), 3.23 (s, 3H, CH_3SO_2), 4.27 (s, 2H, CH_2SO_2), 5.77 (s, 2H, NH_2) ppm. Anal. ($C_{11}H_{18}N_6O_2S$) C, H, N.

3.3.2. 2-Amino-6-(phenylsulfonylmethyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine ***(11)***

Yield: 65%, m.p. 245–246 °C (MeOH); IR (KBr): 3425, 3320, 3185 (NH_2), 1655, 1630 (C=N), 1380, 1160 (SO_2) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.28 (s, 6H, CH_3), 1.92 (s, 3H, CH_3), 2.73 (s, 2H, CH_2), 4.39 (s, 2H, CH_2SO_2), 6.95–7.18 (br.s, 2H, NH_2) 7.55–7.85 (m, 5H, Ph) ppm; ^{13}C-NMR (DMSO-d_6): δ 15.80, 25.92, 52.68, 63.51, 64.36, 127.76, 129.24, 133.73, 139.46, 154.59, 162.05, 165.99, 166.72 ppm. Anal. ($C_{16}H_{20}N_6O_2S$) C, H, N.

*3.3.3. 2-Amino-6-(2-furylmethyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine (**12**)*

Yield: 62%, m.p. 275–277 °C (DMF); IR (KBr): 3370, 3310, 3125 (NH_2), 1650, 1630, (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.60 (s, 6H, CH_3), 1.97 (s, 3H, CH_3), 3.35 (s, 2H, CH_2), 6.64 (dd, 1H, H-4), 7.02 (s, 2H, NH_2), 7.14 (d, 1H, H-5), 7.87 (s, 1H, H-3) ppm. Anal. ($C_{13}H_{16}N_6O$) C, H, N.

*3.4. 2-Amino-6-(2-aminophenyl, -or 3-amino-2-thienyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazines (**13** and **14**)*

To a solution of CH_3ONa in MeOH (0.7 g Na, 25 mL MeOH) biguanide hydrochloride **3a** (4.7 g, 0.02 mol) was added and stirred for 1 h. Then, methyl anthranilate (3.1 g, 0.02 mol) or methyl 3-aminothiophene-2-carboxylate (3.2 g, 0.02 mol) was added and the reaction mixture was heated under reflux for 30 h. After cooling to r.t. water (70 mL) was added dropwise and the suspension was left overnight. The precipitate thus obtained was collected by filtration, washed thoroughly with water, dried and purified by crystallisation.

*3.4.1. 2-Amino-6-(2-aminophenyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine (**13**)*

Yield: 72%, m.p. 208–210 °C (EtOH); IR (KBr): 3490, 3260, 3160, (NH_2), 1645, 1625, (C=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.75 (s, 6H, CH_3), 2.12 (s, 3H, CH_3), 2.86 (s, 2H, CH_2), 5.30 (br.s, 2H, NH_2), 6.65–6.75 (m, 3H, NH_2 and 1H arom.), 7.18–7.27 (m, 2H arom.) ppm. Anal. ($C_{15}H_{19}N_7$) C, H, N.

*2-Amino-6-(3-amino-2-thienyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine (**14**)*

Yield: 28%, m.p. 254–256 °C dec.; IR (KBr): 3470, 3280, 3140 (NH_2), 1640, 1625 (C=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.73 (s, 6H, CH_3), 2.11 (s, 3H, CH_3), 2.83 (s, 2H, CH_2), 5.30 (br.s, 4H, NH_2), 6.57 (d, 1H, H-4), 7.27 (d, 1H, H-5) ppm. Anal. ($C_{13}H_{17}S$) C, H, N.

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

A solution of biguamide **3b** (4.9 g, 0.025 mol) and methyl 3-(thien-2-yl-propenoate (4.2 g, 0.025 mol) in MeOH (anhydrous, 25 mL) was stirred at r.t. for 60 h. The product that precipitated was separated by suction and washed MeOH (4 × 3 mL): 58% yield, m.p. 255–257 °C; IR (KBr): 3440, 3290, 3140 (NH_2), 1660, 1630 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.60 (s, 6H, CH_3), 1.97 (s, 3H, CH_3), 2.81 (s, 2H, CH_2), 6.49 (d, $J = 15.7$ Hz, 1H, vinylene), 6.83 (br.s, 2H, NH_2), 7.12 (t, 1H, H-4), 7.42 (d, 1H, H-3), 7.63 (d, 1H, H-5), 7.91 (d, $J = 15.7$ Hz, 1H vinylene) ppm; ^{13}C-NMR (DMSO-d_6): δ 15.83, 26.19, 52.71, 63.46, 126.58, 127.99, 128.41, 129.85, 130.56, 140.32, 153.63, 162.59, 166.27, 166.47 ppm. Anal. ($C_{15}H_{18}N_6S$) C, H, N.

*3.6. 2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-6-(2-sulfamoylphenyl)-1,3,5-triazine (**16**)*

A solution of biguanide **3b** (4.3 g, 0.022 mol) and saccharin (3.7 g, 0.02 mol) in pyridine (24 mL) was heated under reflux for 36 h. The solvent was evaporated under reduced pressure. To the residue water was added (35 mL) and stirred at r.t. for 6 h. The crude product **16** was collected by filtration, washed with water, dried and purified by crystallisation from DMF: 42% yield, m.p. 287–280 °C; IR (KBr): 3425, 3325, 3225, 3165 (NH_2 and SO_2NH_2), 1630 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.57 (s, 6H, CH_3), 1.82 (s, 3H, CH_3), 2.81 (s, 2H, CH_3), 3.34 (s, 2H, NH_2), 6.68 (br.s, 2H, NH_2), 7.58 (br.s, 4H, Ph) ppm; ^{13}C-NMR (DMSO-d_6): δ 15.65, 26.07, 52.61, 63.20, 111.93, 119.06, 122.45, 130.89, 131.47, 155.10, 155.27, 162.46, 166.14 ppm. Anal. ($C_{15}H_{19}N_7O_2S$) C, H, N.

*3.7. 6-Substituted 2-(1-pyrrolyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazines (**17** and **18**)*

A stirred mixture of 2,5-dimethoxytetrahydrofurane (1.58 g, 0.012 mol) the appropriate compound **12** (2.73 g, 0.01 mol) or **15** (3.14 g, 0.01 mol), *p*-dioxane (9 mL) and AcOH (7 mL) was refluxed for 10 h. The solvents were evaporated under reduced pressure. The dry residue was dissolved in boiling EtOH (15 mL) and the solution was left overnight. The solid that deposited of the adequate triazine **17** or **18** was separated by filtration, washed with EtOH (2 × 1 mL) and purified by recrystallisation from DMF.

*3.7.1. 6-(2-Furyl)-2-(1-pyrrolyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine (**17**)*

Yield: 72%, m.p. 206–208 °C; IR (KBr): 1640 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.67 (s, 6H, CH_3), 2.07 (s, 3H, CH_3), 2.95 (s, 2H, CH_2), 6.35 (d, $J = 7.2$ Hz, 2H, H-3.4 pyrrolyl), 6.75 (s, 1H, H-4 furyl), 7.51 (d, $J = 3.5$ Hz, 1H, H-5 furyl), 7.72 (s, 2H, H-2,5 pyrrolyl), 8.03 (d, $J = 3.5$ Hz, 1H, H-3 furyl) ppm. Anal. ($C_{17}H_{18}N_6O$) C, H, N.

*3.7.2. 6-(2-Thienylvinylene)-2-(1-pyrrolyl)-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazine (**18**)*

Yield: 83%, m.p. 181–183 °C; IR (KBr): 1630 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.66 (s, 6H, CH_3), 2.05 (s, 3H, CH_3), 6.35 (br.s, 2H, H-3.4 pyrrolyl), 6.71 (d, $J = 15.5$ Hz, 1H, vinylene), 7.16 (t, 1H, H-4, thienyl), 7.58 (d, $J = 3.3$ Hz, 1H, H-3 thienyl), 7.70–7.76 (m, 3H, CH), 8.25 (d, $J = 15.5$ Hz, 1H, vinylene) ppm. Anal. ($C_{19}H_{20}N_6S$) C, H, N.

3.8. 2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]acetamide oxime (**19**)

To a solution of KOH (2.3 g, 0.04 mol) in MeOH (90 mL) hydroxylamine hydrochloride (2.8 g, 0.04 mol) was added and the resulting suspension was stirred at r.t. for 0.5 h. Then, MeCN **9** (4.9 g, 0.02 mol) was added and the reaction mixture was refluxed for 20 h. After cooling to r.t. and standing overnight, the precipitate was separated by suction, washed with MeOH (4 × 3 mL) and thoroughly with water: 67% yield, m.p. 229–231 °C; IR (KBr): 3485, 3420, 3300, 3155 (NH_2 and OH), 1670, 1640 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.55 (s, 6H, CH_3), 1.96 (s, 3H, CH_3), 2.78 (s, 2H, CH_2), 3.08 (s, 2H, CH_2), 5.36 (s, 2H, NH_2), 6.88 (s, 2H, NH_2), 8.95 (s, 1H, OH) ppm. Anal. ($C_{11}H_{18}N_8O$) C, H, N.

3.9. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(R^3)acrylonitriles (**20**–**33**) (Method A)

To a solution of corresponding aryl -or heteroarylcarbaldehyde (R^3–COH, Scheme 3) (0.01 mol) in EtOH (anhydrous, 30 mL) was added Et_3N (0.3 mL) and [2-amino-4-(3,3,5,-trimethyl-2-pyrazolino-1,3,5-triazin-6-yl]acetonitrile (**9**) (2.45 g, 0.01 mol). The obtained mixture was stirred under reflux for 17–20 h and then left to stand overnight. The solid that precipitated was filtered off, washed with EtOH (4 × 1 mL) and dried. In case of **20**, **21**, **25**, **26**, **29**, **31** and **32** the crude product was further purified by recrystallisation from DMF.

3.9.1. 2-[Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(5-nitro-2-furyl)acrylonitrile (**20**)

Yield: 79%, m.p. 281–283 °C; IR (KBr): 3515, 3400, 3210 (NH_2), 2220 (C≡N), 1615 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.63 (s, 6H, CH_3), 1.99 (s, 3H, CH_3), 2.87 (s, 2H, CH_2), 7.10–7.30 (br.s, 2H, NH_2), 7.65 (d, $J = 4$ Hz, 1H, H-3), 7.87 (d, $J = 4$ Hz, 1H, H-4), 8.28 (s, 1H, vinylene) ppm. Anal. ($C_{16}H_{16}N_8O_3$) C, H, N.

3.9.2. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-[5-(4-nitrophenyl)-2-furyl]-acrylonitrile (**21**)

Yield: 81%, m.p. 331–334 °C; IR (KBr): 3430, 3300, 3140 (NH_2), 2215 (C≡N), 1640 (C=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.64 (s, 6H, CH_3), 1.99 (s, 3H, CH_3), 2.86 (s, 2H, CH_2), 7.05 (s, 2H, NH_2), 7.58 (d, $J = 3.9$ Hz, 1H, H-3), 7.64 (d, $J = 3.9$ Hz, 1H, H-4), 8.16 (m, 2H arom.), 8.28 (s, 1H, vinylene), 8.37 (m, 2H arom.) ppm. Anal. ($C_{22}H_{20}N_8O_3$) C, H, N.

3.9.3. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(2-benzofuranyl)acrylonitrile (**22**)

Yield: 96%, m.p. 286–287 °C; IR (KBr): 3390, 3300, 3215 (NH_2), 2220 (C≡N), 1630, 1610 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.64 (s, 6H, CH_3), 1.99 (s, 3H, CH_3), 2.88 (s, 2H, CH_2), 7.12 (s, 2H, NH_2), 7.33–7.81 (m, 4H arom.), 7.95 (s, 1H, H-3), 8.40 (s, 1H, vinylene) ppm. Anal. ($C_{20}H_{19}N_7O$) C, H, N.

3.9.4. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(4H-4-oxochromen-3-yl)-acrylonitrile (**23**)

Yield: 95%, m.p. 281–283 °C; IR (KBr): 3500, 3265, 3175 (NH_2), 2220 (C≡N), 1660 (C=O), 1635, 1620, (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.63 (s, 6H, CH_3), 1.99 (s, 3H, CH_3), 2.86 (s, 2H, CH_2), 7.20 and 7.37 (2s, 2H, NH_2), 7.58–8.17 (m, 4H arom.), 8.65 (s, 1H, vinylene), 9.22 (s, 1H, H-2 chromonyl) ppm. Anal. ($C_{21}H_{19}N_7O_2$) C, H, N.

3.9.5. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(5-chloro-2-thienyl)acrylonitrile (**24**)

Yield: 96%, m.p. 294–296 °C dec.; IR (KBr): 3500, 3270, 3175 (NH_2), 2215 (C≡N), 1640, 1625 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.61 (s, 6H, CH_3), 1.98 (s, 3H, CH_3), 2.85 (s, 2H, CH_2), 7.00 (s, 2H, NH_2), 7.39 (d, $J = 4$ Hz, 1H, H-3), 7.90 (d, $J = 4$ Hz, H-4), 8.95 (s, 1H, vinylene) ppm. Anal. ($C_{16}H_{16}ClN_7S$) C, H, N.

3.9.6. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(5-nitro-2-thienyl)acrylonitrile (**25**)

Yield: 29%, m.p. > 350 °C; IR (KBr): 3380, 3280, 3230 (NH_2), 2210 (C≡N), 1640, (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.74 (s, 6H, CH_3), 2.13 (s, 3H, CH_3), 2.88 (s, 2H, CH_2), 5.56 (s, 2H, NH_2), 7.21 (d, $J = 4.3$ Hz, 1H, H-3), 7.94 (d, $J = 4.3$ Hz, H-4), 8.51 (s, 1H, vinylene) ppm. Anal. ($C_{16}H_{16}N_8O_2S$) C, H, N.

3.9.7. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(2-pyrrolyl)acrylonitrile (**26**)

Yield: 59%, m.p. 286–287 °C dec.; IR (KBr): 3470, 3330, 3230 (NH_2), 2200 (C≡N), 1635 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.62 (s, 6H, CH_3), 2.14 (s, 3H, CH_3), 2.99 (s, 2H, CH_2), 6.35–6.38 (m, 1H, H-4), 6.91–6.94 (m, 1H, H-5), 7.19 (s, 2H, NH_2), 7.33–7.59 (m, 1H, H-3), 7.59 (s, 1H, vinylene), 14.63 (s, 1H, NH) ppm. Anal. ($C_{16}H_{18}N_8$) C, H, N.

3.9.8. 2-[2-Amino-4-(3,5,5,-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-[1-(3,5-dichlorophenyl)-2-pyrrolyl]-acrylonitrile (**27**)

Yield: 96%, m.p. 320–322 °C; IR (KBr): 3500, 3270, 3180 (NH_2), 2215 (C≡N), 1640, 1625 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.31 (s, 6H, CH_3), 1.94 (s, 3H, CH_3), 2.78 (s, 2H, CH_2), 6.62 (t, 1H, H-4), 7.59–7.72 (m, 4H arom.), 7.88 (t, 1H, H-5), 8.08 (s, 1H, vinylene) ppm. Anal. ($C_{22}H_{20}Cl_2N_8$) C, H, N.

*3.9.9. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(5-phenyl-2-imidazolyl)acrylonitrile (**28**)*

Yield: 92%, m.p. 321–322 °C dec.; IR (KBr): 3500, 3235, 3135 (NH_2) 2220 (C≡N), 1640, 1625 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.64 (s, 6H, CH_3), 1.99 (s, 3H, CH_3), 2.85 (s, 2H, CH_2), 7.10 (s, 2H, NH_2), 7.43–7.55 (m, 3H, Ph), 8.01–8.05 (m, 2H, Ph), 8.18 (s, 1H, vinylene), 8.46 (s, 1H, H-5), 13.37 (s, 1H, NH) ppm. Anal. ($C_{21}H_{21}N_9$) C, H, N.

*3.9.10. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(4-pyridyl)acrylonitrile (**29**)*

Yield: 84%, m.p. 280–282 °C; IR (KBr): 3410, 3210, 3290, 3200 (NH_2), 2220 (C≡N), 1640, 1625 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.76 (s, 6H, CH_3), 2.15 (s, 3H, CH_3), 2.89 (s, 2H, CH_2), 5.61 (s, 2H, NH_2), 7.79 (d, $J = 6$ Hz, 2H, pyridyl), 8.48 (s, 1H, vinylene), 8.80 (d, $J = 6$ Hz, 2H, pyridyl) ppm. Anal. ($C_{17}H_{18}N_8$) C, H, N.

*3.9.11. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(4-chlorophenyl)acrylonitrile (**30**)*

Yield: 92%, m.p. 305–307 °C; IR (KBr): 3500, 3265, 3185 (NH_2), 2220 (C≡N), 1630, 1620 (C=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.77 (s, 6H, CH_3), 2.16 (s, 3H, CH_3), 2.91 (s, 2H, CH_2), 5.90 (s, 2H, NH_2), 7.48 (d, 2H, Ph), 7.99 (d, 2H, Ph), 8.56 (s, 1H, vinylene) ppm. Anal. ($C_{18}H_{18}ClN_7$) C, H, N.

*3.9.12. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(4-nitrophenyl)acrylonitrile (**31**)*

Yield: 56%, m.p. 309–311 °C; IR (KBr): 3385, 3310, 3220 (NH_2), 2220 (C≡N), 1645, 1635 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.63 (s, 6H, CH_3), 1.99 (s, 3H, CH_3), 2.88 (s, 2H, CH_2), 7.15 (s, 2H, NH_2), 8.27 (d, 2H, Ph), 8.51 (d, 2H, Ph), 8.60 (s, 1H, vinylene) ppm. Anal. ($C_{18}H_{18}N_8O_2$) C, H, N.

*3.9.13. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(3-nitrophenyl)acrylonitrile (**32**)*

Yield: 92%, m.p. 292–293 °C dec.; IR (KBr): 3500, 3265, 3175 (NH_2), 2220 (C≡N), 1640, 1620 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.63 (s, 6H, CH_3), 2.0 (s, 3H, CH_3), 2.87 (s, 2H, CH_3), 7.25 (br.s, 2H, NH_2), 7.87 (t, 1H, Ph), 8.33–8.42 (m, 2H, Ph), 8.61 (s, 1H, Ph), 8.87 (s, 1H, vinylene) ppm. Anal. ($C_{18}H_{18}N_8O_2$) C, H, N.

*3.9.14. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(4-hydroxyphenyl)acrylonitrile (**33**)*

Yield: 77%, m.p. 311–313 °C; IR (KBr): 3300, 3215, 3190, (NH_2 and OH), 2215 (C≡N), 1655, 1640 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.63 (s, 6H, CH_3), 1.98 (s, 3H, CH_3), 2.85 (s, 2H, CH_2), 6.95 (d, 2H, Ph), 7.25 (s, 2H, NH_2), 7.92 (d, 2H, Ph), 8.40 (s, 1H, vinylene), 10.55 (s, 1H, OH) ppm. Anal. ($C_{18}H_{19}N_7O$) C, H, N.

*3.10. 2-[2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(4-diethylaminophenyl)arcylonitrile (**34**) (Method B)*

A mixture of **9** (2.45 g, 0.01 mol), 4-diethylaminobenzaldehyde (1.8 g, 0.01 mol), DMAP (0.13, 0.001 mol), DMF (anhydrous, 15 mL) and EtOH (anhydrous, 30 mL) was stirred at reflux for 20 h. After the reaction mixture was concentrated in vacuo, to the resulting dry residue was added EtOH (30 mL) and stirred at r.t. for 1 h. The precipitate was separated by suction, washed with EtOH (5 mL), dried and purified by crystallisation from *p*-dioxane (200 mL): 77% yield, m.p. 291–293 °C dec.; IR (KBr): 3480, 3265, 3180 (NH_2), 2208 (C≡N), 1615 (C=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.22 (t, CH_3), 2.12 (s, 3H, CH_3), 2.85 (s, 2H, CH_2), 3.45 (q, 4H, CH_2), 5.47 (s, 2H, NH_2), 6.69 (d, 2H, Ph), 7.98 (d, 2H, Ph), 8.41 (s, 1H, vinylene) ppm. Anal. ($C_{22}H_{28}N_8$) C, H, N.

*3.11. 2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-6-(2-imino-2H-chromen-3-yl)-1,3,5-triazine (**35**) and its transformation into 2-[2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(2-hydroxyphenyl)-acrylonitrile (**37**)*

To a suspension of the triazinylacetonitrile **9** (2.45 g, 0.01 mol) in EtOH (anhydrous, 30 mL) was added 2-hydroxybenzaldehyde (1.35 g, 0.011 mol) and piperidine (0.4 mL). The obtained solution was stirred at r.t. for 1 h. The product **35** that precipitated was separated by suction, washed with EtOH (3 × 1 mL) and dried in desiccator: 71% yield, m.p. 185–189 °C; IR (KBr): 3465, 3330 (NH_2), 1650, 1615 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 1.72 (s, 6H, CH_3), 2.12 (s, 3H, CH_3) 2.87 (s, 2H, CH_2), 5.65 (s, 2H, NH_2), 7.05–7.45 (m, 4H arom.), 8.37 (s, 1H, H-4 chromenyl), 10.56 (s, 1H, NH) ppm. Anal. ($C_{18}H_{19}N_7O$) C, H, N.

A solution of chromenyltriazine **35** (2.0 g, 0.0057 mol) in DMF (6 mL) was refluxed for 1 min. After cooling to r.t. water (10 mL) was added dropwise with stirring. The acrylonitrile **37** that precipitated was collected by filtration and washed with MeOH (3 × 1 mL): 52% yield, m.p. 217–221 °C; IR (KBr): 3360, 3240 (NH_2), 2215 (C≡N), 1650 (C=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.63 (s, 6H, CH_3), 1.98 (s, 3H, CH_3), 2.85 (s, 2H, CH_2), 6.97 (s, 1H, Ph), 7.12 (s, 3H, NH_2 and OH), 7.20 (d, 1H, Ph), 7.40 (t, 1H, Ph), 8.97 (s, 1H, vinylene) ppm. Anal. ($C_{18}H_{19}N_7O$) C, H, N.

*3.12. 2-Amino-4-(3,5,5-trimethyl-2-pyrazolino)-6-(2-imino-7-methoxy-2H-chromen-3-yl)-1,3,5-triazine (**36**) and its transformation into 2-[2-amino-4-(3,5,5-trimethyl-2-pyrazolino)-1,3,5-triazin-6-yl]-3-(2-hydroxy-4-methoxyphenyl)acrylonitrile (**38**)*

To a suspension of the triazinylacetonitrile **9** (2.45 g,

0.01 mol) in EtOH (anhydrous, 30 mL) was added 2-hydroxy-4-methoxybenzaldehyde (1.5 g, 0.01 mol) and piperidine (0.4 mL). The reaction mixture was heated under reflux for 5 min. After cooling to r.t. and standing overnight, the product **36** that precipitated was separated by suction and washed with EtOH (3 × 1 mL): 92% yield, m.p. 227–229 °C; IR (KBr): 3450, 3200 (NH_2), 1650, 1615 (C=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.61 (s, 6H, CH_3), 1.99 (s, 3H, CH_3), 2.86 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 6.78 (dd, 1H arom.), 6.81 (d, 1H arom.), 7.10 (s, 2H, NH_2), 7.55 (d, 1H arom.), 8.30 (s, 1H, H-4, chromenyl), 8.90 (s, 1H, NH) ppm. Anal. (C_{19} H_{23} N_7O_2) C, H, N.

A solution of obtained chromenyltriazine **36** (2.17 g, 0.0057 mol) in DMF (6 mL) was refluxed for 1 min. After cooling to r.t. water (10 mL) was added dropwise with stirring. The acrylonitrile **38** that precipitated was collected by filtration and washed with MeOH (3 × 1 mL): 56% yield, m.p. 181–185 °C; IR (KBr): 3330, 3205, 3170 (NH_2), 2210 (C≡N), 1640, 1610, (C=N) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.62 (s, 6H, CH_3), 1.98 (s, 3H, CH_3), 2.84 (s, 2H, CH_2), 3.79 (s, 3H, OCH_3), 6.50 (d, $J_{meta} = 2.5$ Hz, 1H, Ph), 6.62 (d,d, $J_{ortho} = 9.5$ Hz, $J_{meta} = 2.5$ Hz, 1H, Ph), 7.10 (s, 3H, NH_2 and OH), 8.24 (d, $J_{ortho} = 9.5$ Hz, 1H, Ph), 8.92 (s, 1H, vinylene) ppm. Anal. ($C_{19}H_{23}N_7O_2$) C, H, N.

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