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Synthesis, structure and anticancer activity of novel 2,4-diamino-1,3,5-triazine derivatives

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Abstract

A series of 2-(4,6-diamino-1,3,5-triazin-2-yl)-2-{[4-(dimethylamino)-phenyl]imino}acetonitriles **19**–**27** have been synthesized by the reaction of 2-(4-amino-6-alkylamino-1,3,5-triazin-2-yl)acetonitriles **10**–**15** with *p*-nitrosodimethylaniline. Unexpectedly, a similar reaction of acetonitriles **10**, **14**, **15**, **17** and **18** with nitrosobenzene led to the formation of 4,6-diamino-*N*-phenyl-1,3,5-triazine-2-carboxamides **28**–**32**. The in vitro antitumor activity of the compounds obtained has been tested and 2-[4-Amino-6-(4-phenylpiperazin-1-yl)-1,3,5-triazin-2-yl]-2{[4-(dimethylamino)phenyl]imino}acetonitrile (**19**) having remarkable activity against melanoma MALME-3 M cell line ($GI_{50} = 3.3 \times 10^{-8}$ M, TGI = 1.1×10^{-6} M) is a leading candidate for further development.

Keywords: 2,4-Diamino-1,3,5-triazine derivatives; Synthesis; Antitumor effect

1. Introduction

1,3,5-triazine derivatives containing various amino groups at position 2, 4 or 6, such as tretamine, furazil and dioxadet, have been known as anticancer drugs [1]. Structural modifications consisting in the replacement of ethyleneimino moiety with either dialkylamino, alkoxy, alkylarylo or hydroxy groups led to discovery of novel chemotherapeutic agents [2–7]. Moreover, an anti-gastric ulcer agent that is commonly used in Japan, irsogladine [2,4-diamino-6-(2,5-dichlorophenyl)-1,3,5-triazine], was shown to possess antiangiogenic properties which result in anticancer effect of the drug [8,9].

As a part of our research program aimed at search for new pharmacophores as antitumor agents, we have previously described syntheses and pronounced anticancer activity of 2,4-diamino-1,3,5-triazine derivatives of type **A** and **B** [10,11] (Fig. 1). The structure of compounds in the former series containing cyanovinyl group bears resemblance to those of tyrphostins [12,13] and "stilbenic" tyrosine kinase inhibitors [14–17].

Encouraged by this result and on the basis of a commonly held view that the CH=N group is bioisosterically equivalent to a CH=CH group, we synthesized novel compounds of general formula **C** (Fig. 1) and investigated their biological activity in four cancer cell lines.

2. Results and discussion

2.1. Chemistry

As outlined in Scheme 1, the reaction of biguanide hydrochlorides **1**–**6** or free bases **7**–**9** with ethyl cyanoacetate carried out in ethanol at room temperature for 1–2 h afforded corresponding acetonitrile derivatives **10**–**18** in 16–76% yields.

The IR spectra of all the acetonitriles **10**–**18** showed C≡N group vibrations in the range of 2255–2259 cm^{-1}, and in ^{1}H-NMR spectra the signals corresponding to CH_2 group appear at 3.63–3.93 ppm.

Condensation of nitrosobenzenes with compounds containing active methylene group was described previously and, de-

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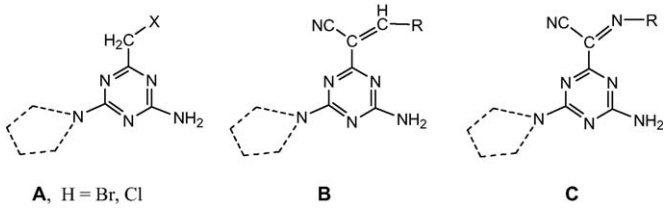
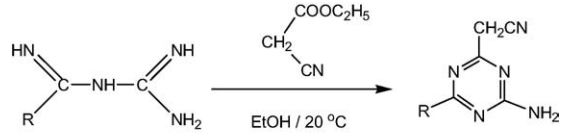
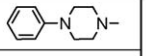
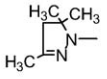
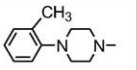
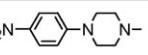
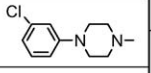
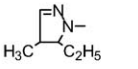
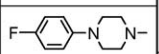
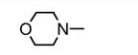
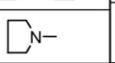


Fig. 1.



Compd.	R	Compd.	R
1, 10		6, 15	
2, 11		7, 16	
3, 12		8, 17	
4, 13		9, 18	
5, 14			

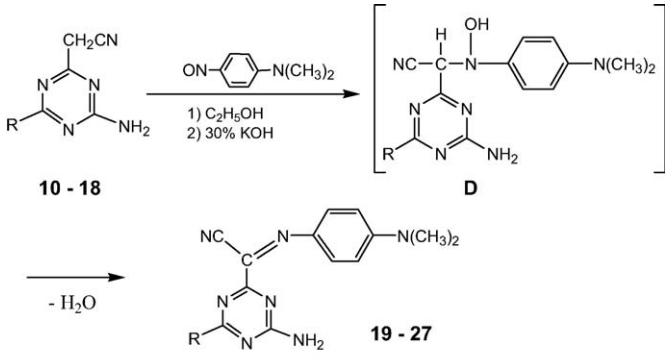
Scheme 1.

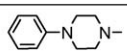
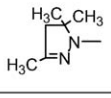
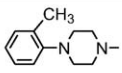
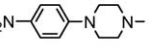
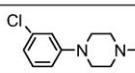
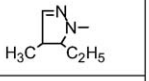
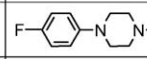
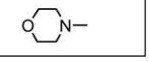
pending on substituents, may lead to the formation of either the imines or nitrones [18–20]. We found that treatment of the acetonitrile derivatives **10**–**18** with *p*-nitrosodimethylaniline in anhydrous ethanol in the presence of KOH yielded the desired iminoacetonitriles **19**–**27** in 36–89% yields. Apparently, the adduct of type **D** formed in the first stage of the reaction sequence underwent a facile imine-forming elimination at room temperature (Scheme 2).

In the IR spectra of these compounds characteristic absorptions in the range of 2201–2204 cm^{-1} attributable to conjugated C=C–**C≡N** groups are present.

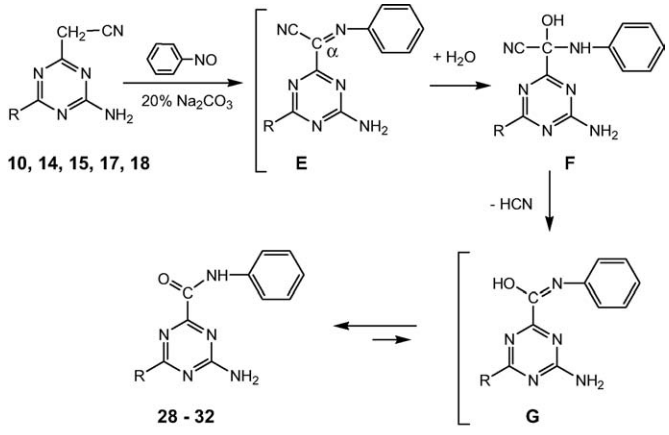
Next, an attempt was made to synthesize the unsubstituted analogues, by the same method as **19**–**27,** using nitrosobenzene. However, no desired iminoacetonitrile was formed and it should be noted that a number of experiments were done to see if condensation with nitrosobenzene would succeed under more vigorous conditions. Higher concentration of KOH and higher reaction temperatures were tried, but the complex mixture of products always contained varying amounts of unreacted starting material and we were unable to separate the products by chromatography.

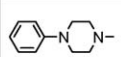
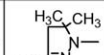
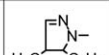
Faced with this unexpected setback, we then performed the reaction of acetonitriles **10**, **14**, **15**, **17** and **18** with nitrosobenzene in DMF or EtOH solution in the presence of 20% aqueous Na_2CO_3. To our surprise, the amide derivatives **28**–**32** were isolated from the reaction mixtures in 20–72% yields. A proposed mechanism for the reaction sequence is presented in Scheme 3. First, the adduct **D** is formed which subsequently loses a water molecule to give iminoacetonitrile **E**. In case of **19**–**27** the presence of strong electron-donating dimethylamino group decreases the electrophilic character of Cα carbon atom which results in a stable iminoacetonitrile **E**. On the other hand, the unsubstituted **E** should be more reactive, and therefore, may undergo addition reaction with water to give the intermediate **F**, in which the leaving group (C≡N) is attached to the carbon atom. Then, the Na_2CO_3-promoted elimination of



Compd.	R	Compd.	R
10, 19		15, 24	
11, 20		16, 25	
12, 21		17, 26	
13, 22		18, 27	
14, 23			

Scheme 2.



Compd.	10, 28	14, 29	15, 30	17, 31	18, 32
R					

Scheme 3.

hydrogen cyanide from **F** gives the final amides **28**–**32** via the iminol **G.**

Structure of the amides **28**–**32** was confirmed by IR, NMR and MS spectroscopic data. For example, in the IR spectrum of compound **30** no absorption at 2000–2200 cm^{-1} attributable to C≡N group is observed. Instead, a strong absorption at 1693 cm^{-1} confirms the presence of C=O group. The ^{1}H-NMR spectrum reveals a signal corresponding to the amide NH proton at 10.12 ppm which shows no correlation to any carbon atom in HSQC (heteronuclear single quantum correlation) spectrum, while in the HMBC (heteronuclear multiple bond correlation) spectrum a correlation of the N**H** proton to the carbon atom of **C**=O group at 158.1 ppm is observed. The MS spectrum of **30** contains a peak of M^+ at $m/z = 325$ (77.2%) which is in agreement with the structure proposed.

2.2. Biology

The in vitro cytotoxic activity of iminoacetonitriles **19**–**27** and amides **28**–**32** was evaluated [21] using human bladder cancer cell line 5637; human pancreatic cancer cell line DAN-G, human breast cancer cell line MCF-7 and human non-small cell lung cancer cell line LCLC-103H. Primary screening of the new compounds was done to indicate whether a substance possesses enough activity at the concentration of 20 μM to inhibit cell growth by 50%.

It should be pointed out that all the amide derivatives **28**–**32** were inactive. On the other hand, the results of primary screening for iminoacetonitriles indicated that compounds **19**, **20**, **21**, **25** and **27** were active. Thus, a secondary screening to determine their potency was performed on a panel of four human cell lines mentioned above. Table 1 lists the IC_{50} values calculated from dose–response data.

As shown in Table 1, the highest cytotoxic activity was found for compound **20** with 4-(2-methylphenyl)piperazin-1-yl substituent at position 6 of triazine ring. Replacement of the 2-methyl group for either 3-chloro (**21**) or 4-nitro (**25**) of the phenyl ring results in reductions in activity. Similarly, unsubstituted analogue **19** was much less active, suggesting that the presence of lipophilic weak electron-donating substituent, such as methyl group, at position 2 of phenyl ring is important for inhibitory activity against the investigated cell lines.

The iminoacetonitriles **19**, **23**, **24**, **26** and **27** and amides **28**–**32** were also selected by the National Cancer Institute (NCI, Bethesda, USA) for testing against a panel of ca. 60 tumor cell lines. Details of this test system have been published [21–23]. Again, amide derivatives proved to be inactive, while iminoacetonitrile **19** bearing (4-phenylpiperazin-1-yl)- substituent at position 6 of triazine ring exhibited remarkable activity and selectivity for melanoma MALME-3 M cell line ($GI_{50} = 3.3 \times 10^{-8}$ M, TGI = 1.1×10^{-6} M and Δ = 2.93) [24].

Table 1
IC_{50} values [μM] in four human cancer cell lines [a]

Compound	Cell line			
	5631	DAN-G	MCF-7	LCLC-103H
19	4.65 ± 1.082	8.93 ± 0.806	> 20	11.3 ± 2.492
20	1.51 ± 0.292	2.17 ± 0.370	5.97 ± 1.497	2.14 ± 0.603
21	6.55 ± 0.483	4.68 ± 0.953	6.86 ± 0.824	7.84 ± 1.235
25	5.43 ± 1.331	12.6 ± 3.041	11.2 ± 2.871	9.59 ± 1.118
27	12.9 ± 0.883	> 20	> 20	> 20

It should be noted that the iminoacetonitrile derivative **24** with 6-(3,5,5-trimethyl-4,5-dihydro-1*H*-pyrazol-1-yl)- substituent at triazine ring was inactive, while the previously described acrylonitriles of type **B** (Fig. 1) required this substituent for their cytotoxic activity [11]. These results are rather surprising from the structure–activity relationships point of view, and may suggest that the mechanisms by which acrylonitriles of type **B** and iminoacetonitriles **19** and **20** exert their antineoplastic effects are different.

3. Experimental protocols

Melting points are not corrected and were recorded on a Buchi apparatus. IR spectra, KBr pellets, 400–4000 cm^{-1}, were recorded on a Satellite FTIR spectrophotometer (Mattson). ^{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 TMS as internal standard). Mass spectra were recorded on Finnigan MAT 95 spectrometer at 70 eV. Analyses of C, H, N were within ± 0.4% of the theoretical values. Biguanides **1** [25], **5** [26,27], **6** [28], **8** [28] and **9** [29], as well as acetonitrile **15** [11] were prepared according to the described procedures.

3.1. Synthesis

3.1.1. General procedure for the preparation of biguanides hydrochlorides *1*–*7*

An equimolar mixture of the appropriate amine hydrochloride and dicyandiamide (68 mmol) in dry 1-butanol (20 ml) was slowly heated with stirring (oil-bath). The mixture began to melt at 90 °C and fused completely at 122–123 °C. Heating was continued over 8 h. After cooling to r.t., stirring was continued for 6 h. The precipitate was separated by suction, washed 1-butanol and isopropyl alcohol, dried and purified by crystallization from MeOH.

*3.1.1.1. N-[imino(4-phenylpiperazin-1-yl)methyl]guanidine hydrochloride **1***. Yield: 70%, m.p. 215–217 °C (Ref. [1], m.p. 243–244 °C). Anal. ($C_{12}H_{19}ClN_6$) C, H, N.

3.1.1.2. N-{imino[4-(2-methylphenyl)piperazin-1-yl]methyl}guanidine hydrochloride 2. Yield: 78%, m.p. 230–232 °C; IR (KBr): 3316, 3204, 2917, 1613, 1548, 1496, 999, 762, 725 cm^{-1}. Anal. ($C_{13}H_{21}ClN_6$) C, H, N.

*3.1.1.3. N-{[4-(3-chlorophenyl)piperazin-1-yl]-(imino)methyl} guanidine hydrochloride **3***. Yield: 61%, m.p. 221–222 °C; IR (KBr): 3308, 3186, 2839, 1649, 1539, 1496, 1239, 1002, 778 cm^{-1}. Anal. ($C_{12}H_{18}Cl_2N_6$) C, H, N.

*3.1.1.4. N-{[4-(4-fluorophenyl)piperazin-1-yl]-(imino)methyl} guanidine hydrochloride **4***. Yield: 55%, m.p. 189–192 °C; IR (KBr): 3322, 3189, 2833, 1644, 1505, 1441, 1237, 1003, 828 cm^{-1}. Anal. ($C_{12}H_{18}ClFN_6$) C, H, N.

*3.1.1.5. N-[imino(pyrrolidin-1-yl)methyl]guanidine hydrochloride **5***. Yield: 47%, m.p. 223–225 °C (Refs. [2,3], m.p. 225–226 °C). Anal. ($C_6H_{14}ClN_5$) C, H, N.

*3.1.1.6. N-[imino(3,5,5-trimethyl-4,5-dihydro-pyrazol-1-yl) methyl]guanidine hydrochloride **6***. Yield: 85%, m.p. 228–232 °C (Ref. [4], m.p. 238–239 °C). Anal. ($C_8H_{17}ClN_6$) C, H, N.

*3.1.2. General procedure for the preparation of biguanides **7–9***

An equimolar mixture of the appropriate amine hydrochloride and dicyandiamide (46.8 mmol) in dry 1-butanol (30 ml) was stirred. The reaction mixture was heated at 90 °C for 40 min and then subsequently heated at 121–123 °C for 8 h. The solid hydrochloride that precipitated was separated by suction, washed with 1-butanol (2 × 5 ml), isopropyl alcohol (4 × 5 ml) and dried. The crude product was dissolved in water (20 ml), treated with active carbon, was heated at 75 °C and filtered. To the filtrate 40% sodium hydroxide was added (4.5 ml). After cooling, the product was separated and was recrystallized from MeOH.

*3.1.2.1. N-{imino{4-(4-nitrophenyl)piperazin-1-yl]methyl}guanidine **7***. Yield: 90%, m.p. 216–220 °C; IR (KBr): 3311, 3157, 2728, 1637, 1555, 1330, 1244, 1115, 999 cm^{-1}. Anal. ($C_{10}H_{14}ClN_3O_2$) C, H, N.

*3.1.2.2. N-[(5-ethyl-4-methyl-4,5-dihydro-pyrazol-1-yl)-(imino) methyl]guanidine **8***. Yield: 75%, m.p. 157–158 °C (Ref. [4], m.p. 189–191 °C). Anal. ($C_8H_{17}ClN_6$) C, H, N.

*3.1.2.3. N-[imino(morpholin-4-yl)methyl]guanidine **9***. Yield: 40%, m.p. 173–176 °C (Ref. [5], m.p. 195–202 °C). Anal. ($C_6H_{13}N_5O$) C, H, N.

*3.1.3. General procedure for the preparation of 2-(4-amino-6-alkylamino-1,3,5-triazin-2-yl)acetonitriles **10–15***

To a solution of sodium ethoxide in ethanol (0.58 g Na, 36 ml ethanol) corresponding biguanide hydrochloride **1–6** (25.4 mmol) was added and the reaction mixture was stirred at r.t. for 3 h. Then ethyl cyanoethanoate (2.71 ml, 25.4 mmol) was added drop-wise and stirring was continued at r.t. for 1 h. The precipitate was separated by suction, washed with ethanol (2 × 5 ml) and purified by crystallization from a suitable solvent.

*3.1.3.1. 2-[4-Amino-6-(4-phenylpiperazin-1-yl)-1,3,5-triazin-2-yl]acetonitrile **10***. Yield: 40%, m.p. 205–208 °C (DMF–water); IR (KBr): 3433, 3323, 3156, 2259, 1653, 1523, 1332 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 3.14–3.19 (m, 4H, CH_2), 3.86 (m, 4H, CH_2), 3.9 (s, 2H, CH_2), 6.83 (d, 1H, CH, J = 7.6 Hz), 6.98 (d, 2H, CH, J = 7.6 Hz), 7.2 (br. s, 2H, NH_2), 7.25 (t, 2H, CH, J = 8.5 Hz) ppm. Anal. ($C_{15}H_{17}N_7$) C, H, N.

*3.1.3.2. 2-{4-Amino-6-[4-(2-methylphenyl)piperazin-1-yl]-1,3,5-triazin-2-yl}acetonitrile **11***. Yield: 34%, m.p. 173–176 °C (MeOH); IR (KBr): 3350, 3325, 2255, 1650, 1632, 1584, 1370 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 2.84–2.89 (m, 4H, CH_2), 3.35–3.89 (m, 4H, CH_2), 3.92 (s, 2H, CH_2), 6.99–7.22 (m, 4H, CH + 2H, NH_2) ppm. Anal. ($C_{16}H_{19}N_7$) C, H, N.

*3.1.3.3. 2-{4-Amino-6-[4-(3-chlorophenyl)piperazin-1-yl]-1,3,5-triazin-2-yl}acetonitrile **12***. Yield: 46%, m.p. 160–162 °C (MeOH); IR (KBr): 3436, 3322, 3155, 2259, 1650, 1553, 1336 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 3.24 (br. s, 4H, CH_2), 3.86 (br. s, 4H, CH_2), 3.92 (s, 2H, CH_2), 6.83 (d, 1H, J = 7.8 Hz), 6.96 (d, 1H, CH, J = 8.3 Hz), 7.01 (s, 1H, CH), 7.13 (br. s, 2H, NH_2), 7.27 (t, 1H, CH, J = 8.1 Hz, J = 8 Hz) ppm. Anal. ($C_{15}H_{16}ClN_7$) C, H, N.

*3.1.3.4. 2-{4-Amino-6-[4-(4-fluorophenyl)piperazin-1-yl]-1,3,5-triazin-2-yl}acetonitrile **13***. Yield: 73%, m.p. 180–182 °C (MeOH); IR (KBr): 3416, 3326, 3153, 2262, 1656, 1548, 1324 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 3.07–3.18 (m, 4H, CH_2), 3.85 (br. s, 4H, CH_2), 3.9 (s, 2H, CH_2), 6.95–7.12 (m, 4H, CH + 2H, NH_2) ppm. Anal. ($C_{15}H_{16}FN_7$) C, H, N.

*3.1.3.5. 2-(4-Amino-6-pyrrolidin-1-yl-1,3,5-triazin-2-yl)acetonitrile **14***. Yield: 76%, m.p. 174–176 °C (MeOH); IR (KBr): 3459, 3339, 3200, 2255, 1660, 1516, 1340 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.95–1.96 (m, 4H, CH_2), 3.5–3.52 (m, 2H, CH_2), 3.58–3.6 (m, 2H, CH_2), 3.63 (s, 2H, CH_2), 5.3 (s, 2H, NH_2) ppm. Anal. ($C_9H_{12}N_6$) C, H, N.

*3.1.3.6. 2-[4-Amino-6-(3,5,5-trimethyl-4,5-dihydro-1H-pyrazol-1-yl)-1,3,5-triazin-2-yl]acetonitrile **15***. Yield: 50%, m.p. 236–237 °C (1-butanol), (Ref. [6] m.p. 246–247 °C); IR (KBr): 3350, 3315, 3200, 2255, 1650, 1539, 1335 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.68 (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.

*3.1.4. General procedure for the preparation of 2-(4-amino-6-alkylamino-1,3,5-triazin-2-yl)acetonitriles **16–18***

To a solution of biguanide **7–9** (30.6 mmol) in dry methanol (48 ml) ethyl cyanoethanoate (3.3 ml, 30.6 mmol) was added drop-wise over 1 h. The reaction mixture was stirred at ambient temperature for 2 h and the product that precipitated was separated by suction, washed with methanol (5 ml) and purified by crystallization.

*3.1.4.1. 2-{4-Amino-6-[4-(4-nitrophenyl)piperazin-1-yl]-1,3,5-triazin-2-yl}acetonitrile **16***. Yield: 59%, m.p. 263–265 °C (DMF-water); IR (KBr): 3455, 3332, 3196, 2259, 1630, 1534,

1322 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 3.56–3.61 (m, 4H, CH_2), 3.88 (br. s, 4H, CH_2), 3.93 (s, 2H, CH_2), 7.06 (d, 2H, CH, $J = 9.5$ Hz), 7.17 (br. s, 2H, NH_2), 8.1 (d, 2H, CH, $J = 9.4$ Hz) ppm. Anal. ($C_{15}H_{16}N_8O_2$) C, H, N.

3.1.4.2. 2-[4-Amino-6-(5-ethyl-4-methyl-4,5-dihydro-1H-pyrazol-1-yl)-1,3,5-triazin-2-yl] acetonitrile ***17***. Yield: 63%, m.p. 278–280 °C (MeOH); IR (KBr): 3503, 3120, 2977, 2257, 1642, 1530, 1378 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.21–1.28 (m, 6H, CH_3), 2.38–2.43 (m, 1H, CH), 2.59–2.67 (m, 1H, CH), 3.25–3.3 (m, 1H, CH), 3.69 (m, 3H, CH + CH_2), 4.22 (s, 1H, CH), 5.41 (br. s, 2H, NH_2) ppm. Anal. ($C_{11}H_{15}N_7$) C, H, N.

3.1.4.3. 2-(4-Amino-6-morpholin-4-yl-1,3,5-triazin-2-yl)acetonitrile ***18***. Yield: 16%, m.p. 178–180 °C (MeOH); IR (KBr): 3369, 3223, 2924, 2259, 1646, 1558, 1384 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 3.64 (s, 2H, CH_2), 3.73 (m, 4H, CH_2), 3.8–3.87 (m, 4H, CH_2), 5.2 (s, 2H, NH_2) ppm. Anal. ($C_9H_{12}N_6O$) C, H, N.

3.1.5. General procedure for the preparation of 2-(4,6-diamino-1,3,5-triazin-2-yl)-2-{[4-(dimethylamino)phenyl] imino}acetonitriles ***19–27***

A mixture of appropriate triazine **10–18** (3.2 mmol) and *p*-nitrosodimethylaniline (0.48 g, 3.2 mmol) in ethanol (80 ml) was refluxed for 0.5 h. Then 30% aqueous potassium hydroxide (0.1 ml) was added. The reaction mixture was stirred at r.t. for 12 h. The solid that precipitated was separated by suction and purified by crystallization from suitable solvent.

3.1.5.1. 2-[4-Amino-6-(4-phenylpiperazin-1-yl)-1,3,5-triazin-2-yl]-2{[4-(dimethylamino)phenyl] imino}acetonitrile ***19***. Yield: 68%, m.p. 243–246 °C (DMF-water); IR (KBr): 3317, 3180, 2914, 2204, 1653, 1614 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 3.11 (s, 6H, CH_3), 3.25 (s, 4H, CH_2), 3.89–3.99 (m, 4H, CH_2), 5.62 (br. s, 2H, NH_2), 6.75 (d, 2H, CH, $J = 9.3$ Hz), 6.91 (t, 1H, CH, $J = 7.3$ Hz, $J = 6.8$ Hz), 6.97 (d, 2H, CH, $J = 7.8$ Hz), 7.28–7.32 (m, 2H, CH), 7.86 (d, 2H, $J = 8.8$ Hz) ppm. EI-MS *m/z* (M^+): 427.2. Anal. ($C_{23}H_{25}N_9$) C, H, N.

3.1.5.2. 2-{4-Amino-6-[4-(2-methylphenyl)piperazin-1-yl]-1,3,5-triazin-2-yl}-2-{[4-(dimethylamino)phenyl]imino}acetonitrile ***20***. Yield: 84%, m.p. 294–296 °C (DMF); IR (KBr): 3473, 3278, 3134, 2202, 1629, 1557 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.33 (s, 3H, CH_3), 2.91 (br. s, 4H, CH_2), 3.08 (s, 6H, CH_3), 3.36 (br. s, 4H, CH_2), 6.87 (d, 2H, CH, $J = 9.3$ Hz), 6.9–7.25 (m, 4H, CH), 7.4 (br. s, 2H, NH_2), 7.61 (d, 2H, CH, $J = 9.1$) ppm. Anal. ($C_{24}H_{27}N_9$) C, H, N.

3.1.5.3. 2-{4-Amino-6-[4-(3-chlorophenyl)piperazin-1-yl]-1,3,5-triazin-2-yl}-2-{[4-(dimethylamino)phenyl]imino}acetonitrile ***21***. Yield: 36%, m.p. 250–254 °C (DMF-water); IR (KBr): 3311, 3213, 2908, 2203, 1644, 1615 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 3.08 (s, 6H, CH_3), 3.29 (br. s, 4H, CH_2), 3.92 (br. s, 2H, NH_2), 3.93 (br. s, 4H, CH_2), 6.58–6.63 (m, 1H, CH), 6.82–7.04 (m, 4H, CH), 7.22–7.3 (m, 2H, CH), 7.62 (d, 1H, CH, $J = 9.1$ Hz) ppm. Anal. ($C_{23}H_{24}ClN_9$) C, H, N.

3.1.5.4. 2-{4-Amino-6-[4-(4-fluorophenyl)piperazin-1-yl]-1,3,5-triazin-2-yl}-2-{[4-(dimethylamino)phenyl]imino}acetonitrile ***22***. Yield: 50%, 236–240 °C (DMF-water); IR (KBr): 3318, 3176, 2906, 2202, 1644, 1557 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 3.08 (s, 6H, CH_3), 3.17 (br. s, 4H, CH_2), 3.94 (br. s, 4H, CH_2), 6.87 (d, 2H, CH, $J = 9.3$ Hz), 6.98–7.14 (m, 4H, CH), 7.16 (br. s, 2H, NH_2), 7.62 (d, 2H, CH, $J = 9.1$ Hz) ppm. Anal. ($C_{23}H_{24}FN_9$) C, H, N.

3.1.5.5. 2-(4-Amino-6-pyrrolidin-1-yl-1,3,5-triazin-2-yl)-2-{[4-(dimethylamino)phenyl]imino} acetonitrile ***23***. Yield: 65%, m. p. 295–299 °C (DMF); IR (KBr): 3407, 3327, 3210, 2201, 1661, 1613 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.98 (s, 4H, CH_2), 3.1 (s, 6H, CH_3), 3.55 (s, 2H, CH_2), 3.75 (s, 2H, CH_2), 5.4 (br. s, 2H, NH_2), 6.75 (d, 2H, CH , $J = 8.3$ Hz), 7.84 (d, 2H, CH, $J = 6.8$ Hz) ppm. Anal. ($C_{17}H_{20}N_8$) C, H, N.

3.1.5.6. 2-[4-Amino-6-(3,5,5-trimethyl-4,5-dihydro-1H-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2-{[4-(dimethylamino)phenyl]imino}acetonitrile ***24***. Yield: 89%, m.p. > 350 °C (DMF-water); IR (KBr): 3398, 3306, 3225, 2202, 1633, 1615 cm^{-1}, ^{1}H-NMR (DMSO-d_6) δ: 1.64 (s, 6H, CH_3), 1.99 (s, 3H, CH_3), 2.86 (s, 2H, CH_2), 3.1 (s, 6H, CH_3), 6.85 (d, 2H, CH, $J = 8.8$ Hz), 7.3 (br. s, 2H, NH_2), 7.6 (d, 2H, CH, $J = 8.3$ Hz) ppm. Anal. ($C_{19}H_{23}N_9$) C, H, N.

3.1.5.7. 2-{4-Amino-6-[4-(4-nitrophenyl)piperazin-1-yl]-1,3,5-triazin-2-yl}-2-{[4-(dimethylamino)phenyl]imino}acetonitrile ***25***. Yield: 60%, m.p. 273–276 °C (DMF-water); IR (KBr): 3348, 3135, 2908, 2201, 1633, 1599 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 3.06 (s, 6H, CH_3), 3.61 (br. s, 4H, CH_2), 3.92 (br. s, 4H, CH_2), 6.84 (d, 2H, CH, $J = 9.1$ Hz), 7.06 (d, 2H, CH, $J = 9.2$ Hz), 7.3 (br. s, 2H, NH_2), 7.6 (d, 2H, CH, $J = 8.9$ Hz), 8.08 (d, 2H, CH, $J = 9$ Hz) ppm. Anal. ($C_{23}H_{24}N_{10}O_2$) C, H, N.

3.1.5.8. 2-[4-Amino-6-(5-ethyl-4-methyl-4,5-dihydro-1H-pyrazol-1-yl)-1,3,5-triazin-2-yl]-2-{[4-(dimethylamino)phenyl]imino}acetonitrile ***26***. Yield: 79%, m.p. 271–274 °C (DMF-water); IR (KBr): 3313, 3179, 2932, 220, 1652, 1613 cm^{-1}, ^{1}H-NMR (DMSO-d_6) δ: 1.1–1.17 (m, 6H, CH_3), 2.29–2.43 (m, 2H, CH_2), 3.04 (s, 6H, CH_3), 3.2–3.23 (m, 1H, CH), 3.49–3.51 (m, 1H, CH), 4.13 (s, 1H, CH), 6.84 (d, 2H, CH, $J = 8.8$ Hz), 7.35 (s, 2H, NH_2), 7.58 (d, 2H, CH , $J = 8.8$ Hz) ppm. Anal. ($C_{19}H_{23}N_9$) C, H, N.

3.1.5.9. 2-(4-Amino-6-morpholin-4-yl-1,3,5-triazin-2-yl)-2-{[4-(dimethylamino)phenyl]imino} acetonitrile ***27***. Yield: 79%, m. p. 239–241 °C (DMF-water); IR (KBr): 3330, 3184, 2858, 2203, 1655, 1614 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 3.11 (s, 6H, CH_3), 3.76 (s, 4H, CH_2), 3.82 (s, 2H, CH_2), 3.99 (s, 2H, CH_2), 5.5 (br. s, 2H, NH_2), 6.75 (d, 2H, CH, $J = 9.3$ Hz), 7.86 (d, 2H, $J = 9.3$ Hz) ppm. Anal. ($C_{17}H_{20}N_8$) C, H, N.

*3.1.5.10. 4-Amino-N-phenyl-6-(4-phenylpiperazin-1-yl)-1,3,5-triazine-2-carboxamide **28***. To a solution of triazine **10** (0.5 g, 1.69 mmol) in ethanol (5 ml) 20% aqueous sodium carbonate (0.4 ml) and nitrosobenzene (0.22 g, 2.03 mmol) were added. The reaction mixture was heated under reflux for 10 min. After cooling to r.t. and standing overnight, the product **28** that precipitated was separated by suction and washed with ethanol, water, dried and purified by crystallization from THF–water: 36% yield, m.p. 237–240 °C; IR (KBr): 3490, 3330, 3139, 1686, 1636, 1515 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 3.2 (t, 4H, CH_2, $J = 5.1$ Hz, $J = 4.8$ Hz), 3.92–3.99 (m, 4H, CH_2), 6.81 (t, 1H, CH, $J = 7.3$ Hz, $J = 7$ Hz), 6.99 (d, 2H, CH, $J = 7.7$ Hz), 7.13 (t, 1H, CH, $J = 7.3$ Hz, $J = 7.7$ Hz), 7.22–7.38 (m, 6H, CH + NH_2), 7.76 (d, 2H, CH, $J = 7.7$ Hz), 10.27 (s, 1H, NH) ppm. Anal. ($C_{20}H_{21}N_7O$) C, H, N.

*3.1.5.11. 4-Amino-N-phenyl-6-pyrrolidin-1-yl-1,3,5-triazine-2-carboxamide **29***. To a solution of triazine **14** (0.3 g, 1.47 mmol) in DMF (4.5 ml) 20% aqueous sodium carbonate (0.36 ml) and nitrosobenzene (0.19 g, 1.76 mmol) were added. The reaction mixture was heated under reflux for 15 min. After cooling to r.t. and standing overnight, the product **29** that precipitated was separated by suction and washed with DMF, water, dried and purified by crystallization from DMF-water: 39% yield, m.p. > 350 °C; IR (KBr): 3322, 3162, 2977, 1655, 1540, 1499 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.9 (s, 4H, CH_2), 3.43–3.59 (m, 4H, CH_2), 7.12 (t, 1H, CH, $J = 7.3$ Hz, $J = 7.3$ Hz), 7.17 (br. s, 2H, NH_2), 7.36 (t, 2H, CH, $J = 7.8$ Hz, $J = 7.3$ Hz), 7.54 (d, 2H, CH, $J = 7.8$ Hz), 10.24 (s, 1H, NH) ppm. Anal. ($C_{14}H_{16}N_6O$) C, H, N.

*3.1.5.12. 4-Amino-N-phenyl-6-[(3,5,5-trimethyl or 5-ethyl-4-methyl)-4,5-dihydro-1H-pyrazol-1-yl]-1,3,5-triazine-2-carboxamides **30–31***. To a solution of triazine **15** or **17** (2.04 mmol) in ethanol (6 ml) 20% aqueous sodium carbonate (0.45 ml) and nitrosobenzene (0.26 g, 2.45 mmol) were added. The reaction mixture was heated under reflux for 24 h. After cooling to r.t. the product that precipitated was separated by suction and washed with ethanol, water, dried and purified by crystallization.

*3.1.5.13. 4-Amino-N-phenyl-6-(3,5,5-trimethyl-4,5-dihydro-1H-pyrazol-1-yl]-1,3,5-triazine-2-carboxamide **30***. Yield: 26%, m.p. 282–283 °C (DMF-water); IR (KBr): 3335, 3226, 2919, 1693, 1637, 1600 cm^{-1}, ^{1}H-NMR (DMSO-d_6) δ: 1.59 (s, 6H, CH_3), 1.98 (s, 3H, CH_3), 2.85 (s, 2H, CH_2), 7.1 (t, 1H, $J = 7.3$ Hz, $J = 7.3$ Hz), 7.2 (br. s, 1H, NH_2), 7.35 (t, 2H, CH, $J = 7.8$ Hz, $J = 7.8$ Hz), 7.45 (br. s, 1H, NH_2), 7.71 (d, 2H, CH, $J = 7.8$ Hz), 10.12 (s, 1H, NH) ppm. EI-MS *m/z* (M^+): 325.1. Anal. ($C_{16}H_{19}N_7O$) C, H, N.

*3.1.5.14. 4-Amino-N-phenyl-6-(5-ethyl-4-methyl-4,5-dihydro-1H-pyrazol-1-yl)-1,3,5-triazine-2-carboxamide **31***. Yield: 72%, m.p. 236–239 °C (DMF-water); IR (KBr): 3504, 3147, 2977, 1691, 1638, 1559 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.1–1.17 (m, 6H, CH_3), 2.3–2.44 (m, 2H, CH_2), 3.23 (m, 1H, CH), 3.56 (s, 1H, CH), 4.15 (s, 1H, CH), 7.12 (t, 1H, CH, $J = 7.3$ Hz), 7.34–7.36 (m, 4H, CH + NH_2), 7.74 (d, 2H, CH, $J = 7.8$ Hz), 10.28 (s, 1H, NH) ppm. Anal. ($C_{16}H_{19}N_7O$) C, H, N.

*3.1.5.15. 4-Amino-6-morpholin-4-yl-N-phenyl-1,3,5-triazine-2-carboxamide **32***. To a solution of triazine **18** (1 g, 4.54 mmol) in DMF (14 ml) 20% aqueous sodium carbonate (1 ml) and nitrosobenzene (0.58 g, 5.45 mmol) were added. The reaction mixture was heated under reflux for 10 min. After cooling to r.t. and standing for 24 h, the product **32** that precipitated was separated by suction and washed with DMF, water, dried and purified by crystallization from DMF-water: 27% yield, m.p. > 350 °C; IR (KBr): 3496, 3334, 2954, 2256, 1702, 1626 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 3.63 (m, 4H, CH_2), 3.7–3.83 (m, 4H, CH_2), 7.11 (t, 1H, CH, $J = 7.32$ Hz, $J = 7.3$ Hz), 7.2 (s, 2H, NH_2), 7.34 (t, 2H, CH, $J = 7.8$ Hz, $J = 7.3$ Hz), 7.73 (d, 2H, CH, $J = 8.3$ Hz), 10.27 (s, 1H, NH) ppm. EI-MS *m/z* (M^+): 300.1. Anal. ($C_{14}H_{16}N_6O_2$) C, H, N.

3.2. Cytotoxicity studies

All reagents were purchased from Sigma (Deisenhofen, Germany) and the cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Brauschweig, Germany). Cell lines used here were: human bladder cancer 5637; human pancreatic cancer DAN-G, human breast cancer MCF-7 and human non-small cell lung cancer LCLC-103H. The culture medium was RPMI-1640 medium containing 2 g/l HCO_3 and 10% FCS. Cells were grown in 75 cm^2 plastic culture flasks (Sarstedt, Nümbrecht, Germany) in a humid atmosphere of 5% CO_2 at 37 °C and passaged shortly before becoming confluent.

For the cytotoxicity studies, 100 μl of a cell suspension were seeded into 96-well microtiter plates (Sarstedt) at a density of 1000 cell per well except for the LCLC-103H cell line, which was plated out at 250 cells per well. One day after plating, the cells were treated with test substance at five concentrations per compound. The 1000-fold concentrated stock solutions in DMF were serially diluted by 50% in DMF to give the feed solutions, which were diluted 500-fold into culture medium. The controls received just DMF. Each concentration was tested in eight wells, with each well receiving 100 μl of the medium containing the substance. The concentration ranges were chosen to bracket the expected IC_{50} values as best as possible. Cells were then incubated for 96 h, after which time the medium was removed and replaced with 1% glutaraldehyde/PBS solution for 20 min. Cells were stored at 4 °C under PBS. Staining with crystal violet was done as previously described [30]. O.D. was measured at $\lambda = 570$ nm with an Anthos 2010 plate reader (Salzburg, Austria).

Corrected T/C values were calculated by the equation:

$$(T/C)_{\mathrm{corr}}(\%) = (\mathrm{O.D._T} - \mathrm{O.D._{c,0}})/(\mathrm{O.D._c} - \mathrm{O.D._{c,0}}) \times 100$$

where $\mathrm{O.D._T}$ is the mean absorbance of the treated cells; $\mathrm{O.D._c}$ the mean absorbance of the controls and $\mathrm{O.D._{c,0}}$ the mean absorbance at the time drug was added. The IC_{50} values were

estimated by a linear least-square regression of the T/C_{corr} values versus the logarithm of the substance concentration; only concentrations that yielded T/C_{corr} values between 10% and 90% were used in the calculation. The reported IC_{50} values are the averages of 3–4 independent experiments and these varied less than 20% from the individual values.

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