In Vitro Cytotoxic Activities of 2-Alkyl-4,6-diheteroalkyl-1,3,5-triazines: New Molecules in Anticancer Research

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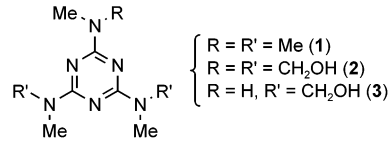
Abstract: The cytotoxic activities of new 2-alkyl-4,6-diheteroalkyl-1,3,5-triazines toward selected tumor cell lines have been evaluated, and for the first time, the potential of 2-alkyl-4,6-dialkoxy-1,3,5-triazines has been shown.

Since the 1970s, several studies have been carried out on the antitumor activity of 2,4,6-tris(*N*,*N*-dialkylamino)-1,3,5-triazines. This follows the discovery that hexamethylmelamine (HMM) **1**[1−3] (Figure 1) is effective against lung, breast, and ovarian cancers but causes severe nausea and vomiting, and some structural analogues of **1** were prepared and tested.[4] These studies pointed out that compounds such as 2,4,6-tris(*N*-methyl-*N*-hydroxymethylamino)-1,3,5-triazine (trimelamol) (**2**) and 2,4-bis(*N*-hydroxymethyl-*N*-methylamino)-6-*N*-methylamino-1,3,5-triazine (**3**), containing hydroxymethyl functionalized amino moieties, showed the highest cytotoxicity[2] (Figure 1).

Although 2,4,6-tris(*N*,*N*-dialkylamino)-1,3,5-triazines are well-known not only in the context of antitumor drugs but also in several other fields of medicinal chemistry,[5−14] no 2-alkyl-4,6-bis(*N*,*N*-dialkylamino)-1,3,5-triazines have ever been considered, perhaps because of their more complex syntheses.[14] Only recently Kukla et al.[15] from Janssen Research Foundation carried out studies on the antiretroviral properties of monoalkyl derivatives of 1,3,5-triazines (**4**) (Figure 1) and in particular against the human immunodeficiency virus (HIV), which is the aetiological agent of acquired immune deficiency syndrome (AIDS) in humans.[15]

These findings and the availability of some 2-alkyl-4,6-diheteroalkyl-1,3,5-triazines prepared in our laboratory in the context of an investigation of new simple and flexible synthetic approaches to 1,3,5-triazine derivatives prompted us to undertake a study of the in vitro cytotoxic activities of **5**−**16** (Figure 2) against some tumor cell lines.

The tested products were selected taking into account the following: (i) **5**−**9** bear dialkylamine substituents and could be somehow related to **1**−**3**; (ii) the available literature data did not allow any hypothesis about the biological properties of **10**−**12**; (iii) the homogeneously



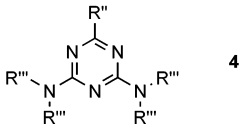


Figure 1.

trisubstituted 1,3,5-triazines **13**−**16** were prepared in order to evaluate the contribution of the alkyl substituents to the biological activity of **5**−**12** in case these last were found cytotoxic.

The preparation of 1,3,5-triazine derivatives **5**,[16] **7**−**9**,[16] **10**−**12**,[17,18] (Figure 2) and **17**−**20**[18] (Figure 3) has been described elsewhere; **6**, **13**, **14**, **16** (Figure 2) were synthesized according to reported procedures,[16−20] while derivatives **21**−**23**[21] (Figure 3) were prepared in the context of this investigation by using, for the first time, a Pd-mediated Stille cross-coupling reaction between 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and suitable organotin compounds;[21] the experimental details of the preparation and characterization of new products are available as Supporting Information. Compound **15** is commercially (Sigma-Aldrich) available.

The cytotoxic activity was evaluated by the MTT assay.[22] (For experimental details, see the Supporting Information.)

In Table 1 the results, reported as IC_{50} values (μM) and obtained in the evaluation of the cytotoxicity of **5**−**12** toward leukemia cell lines L1210 and HL60 and glioma cell line C6, are compared with those provided by the symmetrically substituted 1,3,5-triazine derivatives **13**−**16**.

Although **5**−**9** are structurally related to melamine systems, their activities are negligible (Table 1, entries 1−5) and only small improvements can be obtained when an alkylamino substituent of **13** and **14** is replaced by either an unsaturated or a saturated alkyl chain [Table 1, **13** vs **5**, **8**, **9** (entry 9 vs entries 1, 4, 5) and **14** vs **6** (entry 10 vs entry 2)]. Moreover, the introduction of polar residues to improve the solubility of 1,3,5-triazine derivatives in water caused a complete loss of activity [Table 1, **14** vs **13** (entry 10 vs entry 9) and **6** vs **5** (entry 2 vs entry 1)].

Unexpected and more interesting results were obtained when **10**−**12** were tested (Table 1, entries 6−8) and compared with their symmetrically substituted models **15** and **16** (Table 1, entries 11 and 12). The collected data indicate that (i) for the first time 2,4,6-trialkoxy-1,3,5-triazines (**15** and **16**) were found to be moderately active against the HL60 cell line and that (ii) the replacement of an alkoxy residue by an alkyl moiety (Table 1, **10**−**12** vs **15** and **16**) enhances the cytotoxicity (Table 1, entries 6 and 7 vs entry 11 and entry 8 vs entry 12).

These encouraging results prompted us to further investigate this class of 1,3,5-triazine derivatives; thus,

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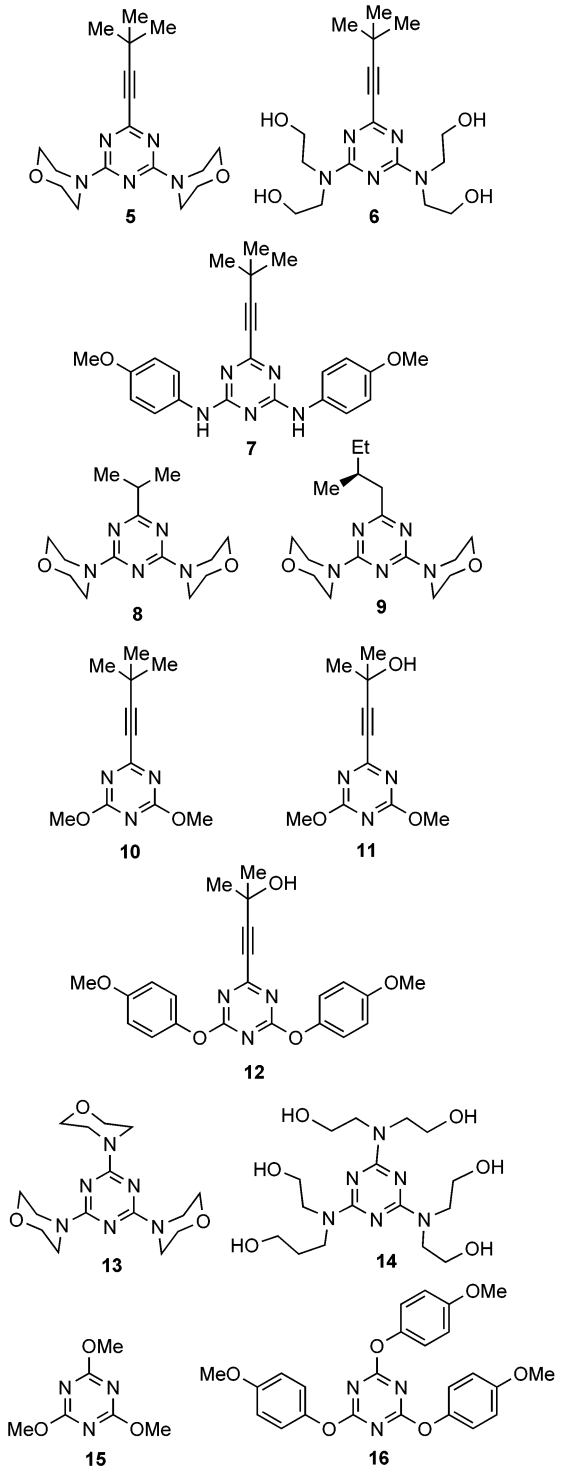

Figure 2.

10 and **12**, together with samples of similar structures (**17**−**23**,[18,21] Figure 3), were submitted to an in vitro antitumor screen program of the National Cancer Institute (NCI) at Bethesda. While **22** and **23** were not found to be positive in the three-cell line (one dose primary anticancer assay) and therefore were not further investigated, the activity of **17**−**21** was evaluated on 60 human tumor cell lines.[23,24] The results of this screening were processed to give an averaged parameter over all cell lines for each test compound, designated as mean graph midpoint (MGM),[25] which can be considered an average antitumor activity parameter

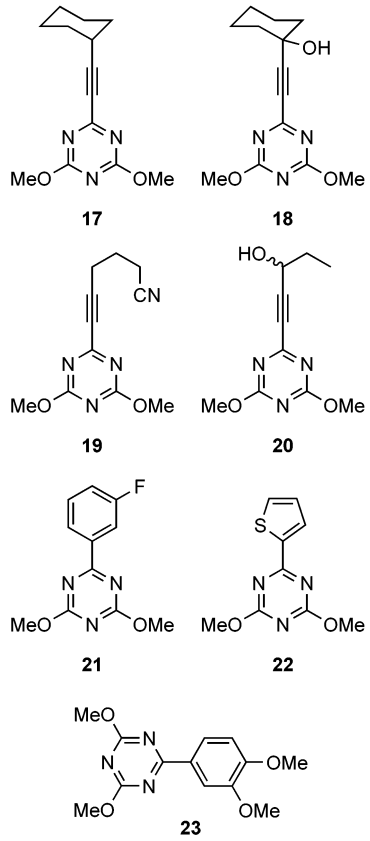

Figure 3.

Table 1. Cytotoxic Activity (IC_{50}, μM) of Compounds **5**−**16** on HL60, L1210, C6 Cell Lines

		HL60		L1210		C6	
entry	compd	48 h	72 h	48 h	72 h	48 h	72 h
1	**5**	182	389	182	120	6607	*a*
2	**6**	*a*	*a*	*a*	*a*	3388	*a*
3	**7**	589	575	*a*	*a*	$>10^4$	$>10^4$
4	**8**	87.1	200	93.3	67.6	141	490
5	**9**	219	141	141	57.5	575	240
6	**10**	7.08	7.08	12.0	9.12	16.6	16.6
7	**11**	15.8	15.1	14.1	12.9	16.2	15.1
8	**12**	17.0	36.3	11.0	14.1	$>10^4$	$>10^4$
9	**13**	129	100	151	110	151	288
10	**14**	*a*	*a*	*a*	*a*	759	*a*
11	**15**	302	339				
12	**16**	70.8	52.5				

[a] No cytotoxic effect.

Table 2. Cytotoxic Activity of Compounds **10**, **12**, **17**−**21** on Selected Human Cancer Cell Lines

compd	−MGM[a]	cell line[b]	IG_{50} (μM)
10	4.58	SR (leukemia)	5.25
12	5.28	SR (leukemia)	0.63
17	5.46	HCT-116 (colon cancer)	0.40
18	5.01	OVCAR-4 (ovarian cancer)	1.86
19	4.82	LOX IMVI (melanome)	2.09
20	4.65	HOP-62 (lung cancer)	4.90
21	4.89	CAKI-1 (renal cancer)	$<10^{-2}$

[a] Mean graph midpoint (calculated from dose−response curves over 60 cell lines for each compound). [b] For each compound, the most sensitive over 60 cell lines was reported.

toward the tested cell lines. For each of the tested compounds the average values (−MGM) are reported in Table 2, together with the most sensitive cell line and the corresponding growing inhibition parameter (IG_{50}, μM). The overall collected data show a moderate and

comparable average cytotoxic activity for structures **10**, **12**, and **17**−**21** toward the investigated cell lines.

More interestingly, the inspection of the IG_{50} concentrations allows us to point out the selectivity of certain products toward selected cell lines. In particular, **12** and **17** were shown to be appreciably active toward cell lines SR (leukemia) and HCT-116 (colon cancer), respectively.

An even more marked effect was observed for **21**, whose active concentration against cell line CAKI-1 (renal cancer) was less than 10^{-2} μM.

To understand the molecular mechanism accountable for the antiproliferative activity of **10**−**12** and **21**, further experiments were carried out taking into account the known properties of some triazines to exert cytotoxic activity by interacting with DNA.[26,27] In particular, it has been shown that some triazine compounds stabilize G-quadruplexes, thus interfering with telomerase activity. On the basis of these findings, the formation of a molecular complex between **10**−**12** and **21** and the DNA macromolecule was investigated by means of circular dichroism (CD) studies.

In detail, increasing amounts of the test compounds ([drug]/[DNA] = 0, 0.02, 0.04, 0.08, and 0.2) were incubated in the presence of salmon testes DNA (1.8×10^{-4} M) in ETN buffer (containing 10 mM Tris, 10 mM NaCl, and 1 mM EDTA, pH 7). Spectra were recorded in the 230−400 nm region at 25 °C.

The CD spectra obtained in the presence of the **10**−**12** and **21** showed no significant differences with respect to that of DNA up to a molar ratio [drug]/[DNA] = 0.2. These results seem to suggest that although DNA is considered the crucial target for many anticancer agents, the antiproliferative effect exerted by **10**−**12** and **21** cannot be ascribed to an interaction with the macromolecule.

In conclusion, our findings outline that (i) the presence of a saturated or unsaturated moiety bonded to the heterocyclic ring via a C−C bond seems to significantly affect the cytotoxic activity of the system, (ii) 2-alkyl-(alk-1′-ynyl,aryl)-4,6-dialkoxy-1,3,5-triazines were for the first time found to be active against selected tumor cell lines and their activity was found to be at least comparable with that of trimelamol and some structural analogues,[1,28] (iii) the structure of the carbon−carbon bonded residue seems to play an important role in the biological activity; this effect is particularly evident in the case of **21**−**23** where the nature of the aromatic C_2 substituent affects dramatically the cytotoxicity, (iv) further investigation is necessary in order to understand the mechanism of action of this new class of 1,3,5-triazine derivative and evaluate their potential as antitumor agents.

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Supporting Information Available: Experimental details of the preparation of **6**, **13**, **14**, **16**, **21**−**23** (Figures 2 and 3) and their characterization data; experimental procedures for the biological tests (cf. Table 1). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Coley, H. M. *N*-(Hydroxymethyl)melamines. *Gen. Pharmacol.* **1997**, *28*, 177−182 and references therein.
(2) Foster, B. J.; Harding, B. J.; Leyland-Jones, B.; Hoth, D. Hexamethylmelamine: critical review of an active drug. *Cancer Treat.* Rev. **1986**, *38*, 197−217.
(3) Ames, M. M. Hexamethylmelamine: pharmacology and mechanism of action. *Cancer Treat. Rev.* **1991**, *18*, 3−14.
(4) Judson, I. R.; Thornton, T. J.; Wilman, D. E. V.; Abel, G.; Rutty, C. J. Synthesis and cytotoxicity of potential tumor-inhibitory analogues of trimelamol (2,4,6-tris[(hydroxymethyl)methylamino]-1,3,5-triazine) having electron-withdrawing groups in place of methyl. *J. Med. Chem.* **1993**, *36*, 4195−4200.
(5) Jarman, M.; Brooks, N.; Thornton, T. J.; Judson, I. R. Stable analogues of the antitumour agent trimelamol retain in vitro cytotoxicity in drug-sensitive and resistant rodent and human cell lines. *Eur J. Cancer* **1994**, *30A*, 1827−1836.
(6) Labrid, C.; Régnier, G. L.; Laubie, M. Almitrine bismesylate: Pharmacological review and structure−activity relationships. *Eur. J. Respir. Dis.* **1983**, *64* (Suppl. 126), 185−189.
(7) D'Atri, G.; Gomarasca, P.; Resnati, G.; Tronconi, G.; Scolastico, C.; Sirtori, C. R. Novel pyrimidine and 1,3,5-triazine hypolipemic agents. *J. Med. Chem.* **1984**, *27*, 1621−1629.
(8) Dhainaut, A.; Regnier, G.; Atassi, G.; Pierre, A.; Leonce, S.; Kraus-Berthier, L.; Prost, J. F. New triazine derivatives as potent modulators of multidrug resistance. *J. Med. Chem.* **1992**, *35*, 2481−2496.
(9) Porter, J. R.; Archibald, S. C.; Brown, J. A.; Childs, K.; Critchley, D.; Head, J. C.; Hutchinson, B.; Parton, T. A. H.; Robinson, M. K.; Shock, A.; Warrellow, J.; Zomaya, A. Discovery and evaluation of *N*-(triazin-1,3,5-yl)phenylalanine derivatives as VLA-4 integrin antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1591−1594.
(10) Hajduk, P. J.; Dinges, J.; Schkeryantz, J. M.; Janowick, D.; Kaminski, M.; Tufano, M.; Augeri, D. J.; Petros, A.; Nienaber, V.; Zhong, P.; Hammond, R.; Coen, M.; Beutel, B.; Katz, L.; Fesik, S. W. Novel inhibitors of Erm methyltransferases from NMR and parallel synthesis. *J. Med. Chem.* **1999**, *42*, 3852−3859.
(11) Wang, Q.; Liu, G.; Shao, R.; Huang, R. Synthesis and antivirus activity of 1,3,5-triazine derivatives. *Heteroat. Chem.* **2003**, *14*, 542−545.
(12) Klenke, B.; Stewart, M.; Barrett, M. P.; Brun, R.; Gilbert, I. H. Synthesis and Biological Evaluation of *s*-Triazine Substituted Polyamines as Potential New Anti-Trypanosomal Drugs. *J. Med. Chem.* **2001**, *44*, 3440−3452.
(13) Henke, B. R.; Consler, T. G.; Go, N.; Hale, R. L.; Hohman, D. R.; Jones, S. A.; Lu, A. T.; Moore, L. B.; Moore, J. T.; Orband-Miller, L. A.; Robinett, R. G.; Shearin J.; Spearing, P. K.; Stewart, E. L.; Turnbull, P. S.; Weaver, S. L.; Williams, S. P.; Wisely, G. B.; Lambert M. H. A New Series of Estrogen Receptor Modulators That Display Selectivity for Estrogen Receptor. *J. Med. Chem.* **2002**, *45*, 5492−5505.
(14) Mylari, B. L.; Withbroe, G. J.; Beebe, D. A.; Brackett, N. S.; Conn, E. L.; Coutcher, J. B.; Oates, P. J.; Zembrowski, W. J. Design and Synthesis of a Novel Family of Triazine-Based Inhibitors of Sorbitol Dehydrogenase with Oral Activity: 1-{4-[3*R*,5*S*-Dimethyl-4-(4-methyl-1,3,5]triazin-2-yl)-piperazin-1-yl]-[1,3,5]-triazin-2-yl}-(*R*) Ethanol. *Bioorg. Med. Chem.* **2003**, *11*, 4179−4188.
(15) Kukla, M. J.; Ludovici, D. W.; Janssen, P. A. J.; Heeres, J.; Moereels, H. E. L. Substituted diamino-1,3,5-triazine derivatives. European Patent Application EP 0 834 507 A1, 1998.
(16) Menicagli, R.; Samaritani, S.; Zucchelli, V. 2-Alkyl-4,6-dialkylamino-1,3,5-triazines via Grignard alkylation of cyanuric chloride: an aged reaction revisited. *Tetrahedron* **2000**, *56*, 9705−9711.
(17) Menicagli, R.; Samaritani, S.; Gori, S. 2-(Alk-1′-ynyl)-4,6-dimethoxy-1,3,5-triazines via Pd-mediated alkynylation of 2-chloro-4,6-dimethoxy-1,3,5-triazine. *Tetrahedron Lett.* **1999**, *40*, 8419−8422.
(18) Samaritani, S.; Menicagli, R. Palladium on Carbon Catalyzed Cross-Coupling between Alk-1-ynes and 2-Chloro-4,6-dialkoxy-1,3,5-triazines. *Tetrahedron* **2002**, *58*, 1381−1386.
(19) Menicagli, R.; Malanga, C.; Peluso, P. Selective mono- or dialkoxylation of 2,4,6-trichloro-1,3,5-triazine in solid−liquid phase transfer conditions. *Synth. Commun.* **1994**, *24*, 2153−2158 and references therein.
(20) Samaritani, S.; Peluso, P.; Malanga, C.; Menicagli, R. Selective Amination of Cyanuric Chloride in the Presence of 18-Crown-6. *Eur. J. Org. Chem.* **2002**, 1551−1555.
(21) Signore, G.; Samaritani, S.; Menicagli, R. Palladium and Nickel-Catalyzed Cross-Coupling Reactions: A Useful Approach to 2-Alkyl-4,6-dialkoxy-1,3,5-triazines. Presented at the 4th Franco-Italian Meeting on Organic Chemistry. Organic Chemistry: New Frontiers and Challenging applications, Annecy, France, May 5−8, 2004; Abstract P25.
(22) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.

(23) Boyd, M. R.; Paull, K. D. Some practical considerations and applications of the National-Cancer-Institute in-vitro anticancer drug discovery screen. *Drug Dev. Res.* **1995**, *34*, 91−109.
(24) Monks, A.; Scudiero, D. A.; Johnson, G. S.; Paull, K. D.; Sausville, E. A. The NCI anti-cancer drug screen: a smart screen to identify effectors of novel targets. *Anti-Cancer Drug Des.* **1997**, *12*, 533−541.
(25) Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. *J. Natl. Cancer Inst.* **1989**, *81*, 1088−1092.
(26) Riou, J. F.; Guittat, L.; Mailliet, P.; Laoui, A.; Renou, E.; Petitgenet, O.; Megnin-Chanet, F.; Helene, C.; Mergny, J. L. Cell senescence and telomere shortening induced by a new series of specific G-quadruplex DNA ligands. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2672−2677.
(27) Gomez, D.; Lemarteleur, T.; Lacroix, L.; Mailliet, P.; Mergny, J. L.; Riou, J. F. Telomerase downregulation induced by the G-quadruplex ligand 12459 in A549 cells is mediated by hTERT RNA alternative splicing. *Nucleic Acids Res.* **2004**, *32*, 371−379.
(28) Jarman, M.; Coley, H. M. Melamine derivatives for use in the treatment of cancer. U.S. Patent 5,854,244, 1998.

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