

THE NEW ANALOGUES OF NITROGEN MUSTARD WITH ONE, TWO OR THREE 2-CHLOROETHYLAMINO FRAGMENTS. REACTIONS WITH NUCLEOPHILES

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Abstract: 1,3,5-Triazines substituted with mono-, di, and tri-[4-(2-chloroethyl)piperazin-1-yl] groups gave products of substitution of chlorine atom when treated with ethanol, phenol, butylamine, toluidine, or thiophenol under mild reaction conditions.

Keywords: 2-[4-(2-chloroethyl)piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine, 2,4-bis[4-(2-chloroethyl)piperazin-1-yl]-6-methoxy-1,3,5-triazine, 2,4,6-tris[4-(2-chloroethyl)piperazin-1-yl]-1,3,5-triazine, alkylation, nucleophilic substitution.

Nitrogen mustards (NMs) e.g., mechlorethamine, chlorambucil, cyclophosphamide and melphalan have been approved as chemotherapeutic agents in the treatment of lymphoma, leukemia, multiple myeloma, ovarian carcinoma. These bifunctional alkylating agents are used extensively for more than 30 years in the treatment of neoplastic and autoimmune diseases (1). The antitumor activity of NMs has been connected with their ability to cross-link the twin strands of DNA, yielded to bifunctional lesions, which if not repaired, can inhibit DNA replication and transcription, eventually leading to cell cycle arrest, apoptosis, and the inhibition of tumor growth. The predominant bifunctional DNA lesions of NM have been reported to involve the distal guanine bases in the opposite strands of 5'-GNC sequences (2). These bifunctional lesions are gifted to blocking DNA replication and transcription, eventually leading to cell death and the inhibition of tumor growth (3). The ability of NM to cross-link DNA results from the presence of two electrophilic centers in each molecule of alkylating reagent. Both of the 2-(chloroethyl) groups presented in NMs are prone to cyclization to an aziridinium ion and consecutively to alkylation of two nucleobases within the DNA duplex (2). Alternatively, following the first alkylation, the second chloroethyl group can be hydrolyzed, giving rise to 2-(hydroxyethyl) monoadducts. Classical NM drugs do not operate selectivity, causing high

levels of inadvertent DNA damage in normal cells, toxic and mutagenic side effects, and in some cases, leading to secondary malignancies (4). Nitrogen mustards, because of their alkylating properties, react also with nucleophilic groups of proteins. Tris-(2-chloroethyl)amine (TCEA), a strong alkylating agent, not only alkylates protein at the hitherto accepted reactive sites but also at the nitrogen of the peptide bond (5).

It has been also reported that therapeutic nitrogen mustard mechlorethamine was covalently bonded to the metal-binding protein metallothionein (MT). The most surprising aspect of this interaction is the selectivity of the alkylating agent for specific residues of MT. Alkylation occurs predominantly in the carboxyl domain of MT, with one molecule of mechlorethamine covalently cross-linking two cysteine residues (6).

DNA-protein cross-linking by nitrogen mustards is not well characterized, probably because of its inherent complexity and the insufficient sensitivity of previous methodologies. If formed, DNA-protein conjugates are likely to contribute to both target and off-target cytotoxicity of nitrogen mustard drugs. DNA repair protein, O-6-alkylguanine DNA alkyltransferase (AGT), can be readily cross-linked to DNA in the presence of nitrogen mustards. Both chlorambucil and mechlorethamine induced the formation of covalent conjugates between ³²P-labeled double-stranded oligodeoxynucleotides and recom-

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binant human AGT protein. The exact chemical structures of AGT-DNA cross-links induced by chlorambucil and mechlorethamine were identified as N-(2-[S-cysteinyl]ethyl)-N-(2-[guan-7-yl]ethyl)-p-aminophenylbutyric acid and N-(2-[S-cysteinyl]ethyl)-N-(2-[guan-7-yl]ethyl)methylamine. Mechlorethamine-induced AGT-DNA conjugates were isolated from protein extracts of AGT-expressing CHO cells but not control cells, demonstrating that nitrogen mustards can cross-link the AGT protein to DNA in the presence of other nuclear proteins (7).

In our previous report (8) we presented the results of synthesis of triazines substituted with one, two or three 2-chloroethylamine moiety **1-3** and evidenced by determination of apoptotic index and cell viability on the standard cell line of mammalian tumor MCF-7 the cytotoxicity for all of the obtained compounds. The studies shown that triazine derivatives **1-3** inhibits 50% of colony formation at the concentration in the range 13,88 μM to 146,79 μM , while the IC₅₀ of chlorambucil is 24,6 μM .

In order to recognize the basic reactivity pathways, herein, we present the studies on alkylation of broad range of nucleophiles with triazine NM **1-3**.

EXPERIMENTAL

Reaction of 2-[4-(2-chloroethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (**1**) with *p*-toluidine (a). General Procedure

To the vigorously stirred solution of 2-[4-(2-chloroethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (**1**) (0.288 g, 1 mmol) in dichloromethane (5 mL) *p*-toluidine (0.214 g, 2 mmol) was added. Stirring was continued for 24 h at room temperature until starting material disappeared and the progress of reaction was monitored by TLC. The solution was washed with water, dried with MgSO₄, and evaporated. 2-[4-[2-(4-Methylphenylamino)ethyl] piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (**4a**) (0.337 g, 94%) was obtained as a pale oil. ¹H-NMR (CDCl₃): δ (ppm): 2.18 (s, 3H, CH₃-C₆H₄); 2.52-2.69 (m, 4H, (CH₂)₂-N-CH₂); 2.82 (t, 2H, N-CH₂-CH₂-NH); 3.11 (t, 2H, N-CH₂-CH₂-NH); 3.16-3.31 (m, 4H, triazine-N-(CH₂)₂); 3.95 (s, 6H, CH₃-O); 6.92 (d, 2H, C₆H₄); 7.12 (d, 2H, C₆H₄). HPLC: (60% CH₃CN, 35% H₂O, 5% CH₃OH, v/v/v), 20 min: R_t = 8.78 min, 90%.

Reaction of 2-[4-(2-chloroethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (**1**) with *n*-butylamine (b)

Starting materials: 2-[4-(2-chloroethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (**1**) (0.288

g, 1 mmol), *n*-butylamine (0.198 ml, 2 mmol). Product: 2-[4-(2-butylaminoethyl)piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (0.311 g, 96%), pale oil.

¹H-NMR (CDCl₃): δ (ppm): 0.88 (t, 3H, CH₃-(CH₂)₃); 1.03-1.44 (m, 4H, CH₃-(CH₂)₂-CH₂); 2.54 (t, 2H, N-CH₂-CH₂-NH); 2.59-2.66 (m, 4H, (CH₂)₂-N-CH₂); 2.73 (t, 2H, N-CH₂-CH₂-NH); 3.11-3.29 (m, 6H, triazine-N-(CH₂)₂ + NH-CH₂-(CH₂)₂-CH₃); 3.94 (s, 6H, CH₃-O).

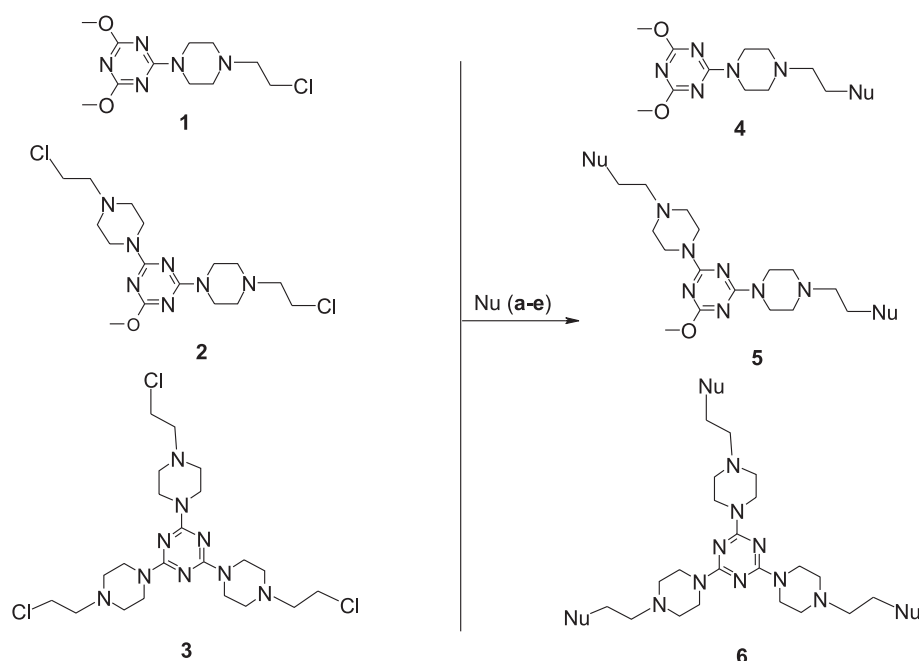
HPLC (60% CH₃CN, 35% H₂O, 5% CH₃OH, v/v/v), 20 min: R_t = 6.13 min, 90%.

Reaction of 2-[4-(2-chloroethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (**1**) with ethanol (c). General Procedure

To the vigorously stirred solution of 2-[4-(2-chloroethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (**1**) (0.288 g, 1 mmol) in ethanol (5 mL), DIPEA (0.175 mL, 1 mmol) and catalytic amount of DMAP (10 mg) was added. Stirring was continued for 24 h at room temperature until starting material dissolved and the progress of reaction was monitored by TLC. The solution was concentrated to dryness, the residue was dissolved in dichloromethane (5 mL) and then washed with water, dried with MgSO₄, filtered and evaporated. 2-[4-(2-Ethoxyethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (**4c**) (0.241 g, 81%) was obtained as an oil. ¹H-NMR (CDCl₃): δ (ppm): 1.12 (t, 3H, CH₃-CH₂-O); 2.38-2.60 (m, 4H, (CH₂)₂-N-CH₂); 2.63 (t, 2H, N-CH₂-CH₂-O); 3.22-3.36 (m, 4H, triazine-N-(CH₂)₂); 3.41 (t, 2H, N-CH₂-CH₂-O); 3.42 (q, 2H, -O-CH₂-CH₃); 3.97 (s, 6H, CH₃-O). HPLC: (60% CH₃CN, 35% H₂O, 5% CH₃OH, v/v/v), 20 min: R_t = 7.93 min, 94%.

Reaction of 2-[4-(2-chloroethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (**1**) with phenol (d). General procedure

To the vigorously stirred solution of phenol (0.094 g, 1 mmol) in dichloromethane (5 mL) DIPEA (0.350 mL, 2 mmol) and 2-[4-(2-chloroethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (**1**) (0.288 g, 1 mmol) were added. Stirring was continued for 24 h at room temperature until all starting material dissolved and the progress of reaction was monitored by TLC. The solution was washed with water, dried with MgSO₄, and evaporated. 2-[4-(2-Phenoxyethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (**4d**) (0.318 g, 92%) was obtained as an oil. ¹H-NMR (CDCl₃): δ (ppm): 2.40-2.62 (m, 4H, (CH₂)₂-N-CH₂); 2.93 (t, 2H, N-CH₂-CH₂-O); 3.23-3.44 (m, 4H, triazine-N-(CH₂)₂); 3.94



Scheme 1. Reaction of triazine derivatives 1-3 with nucleophiles.

(s, 6H, $\text{CH}_3\text{-O}$); 4.01 (t, 2H, $\text{N-CH}_2\text{-CH}_2\text{-O}$); 6.70-6.85 (m, 3H, C_6H_5); 7.05-7.14 (m, 2H, C_6H_5). HPLC: (60% CH_3CN , 35% H_2O , 5% CH_3OH , v/v/v), 20 min: $R_t = 14.22$ min, 93%.

Reaction of 2-[4-(2-chloroethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (1) with thiophenol (e)

Starting materials: 2-[4-(2-chloroethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (1) (0.288 g, 1 mmol), DIPEA (0.350 mL, 2 mmol), thiophenol (0.110 g, 1 mmol). Product: 2-[4-(2-phenylsulfanylethyl)-piperazin-1-yl]-4,6-dimethoxy-1,3,5-triazine (4e), (0.351 g, 97%).

$^1\text{H-NMR}$ (CDCl_3): δ (ppm): 2.47-2.67 (m, 4H, $(\text{CH}_2)_2\text{-N-CH}_2$); 2.59 (t, 2H, $\text{N-CH}_2\text{-CH}_2\text{-S}$); 3.18-3.40 (m, 4H, triazine- $\text{N-}(\text{CH}_2)_2$); 3.18 (t, 2H, $\text{N-CH}_2\text{-CH}_2\text{-S}$); 3.98 (s, 6H, $\text{CH}_3\text{-O}$); 7.12-7.25 (m, 5H, C_6H_5). HPLC: 60% CH_3CN , 35% H_2O , 5% CH_3OH , v/v/v), 20 min: $R_t = 15.02$ min, 89%.

Reaction of 2,4-bis-[4-(2-chloroethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (2) with *p*-toluidine (a)

Starting materials: 2,4-bis-[4-(2-chloroethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (2) (0.404 g, 1 mmol), *p*-toluidine (0.428 g, 4 mmol). Product: 2,4-bis[4-[2-(4-methylphenylamino)ethyl]piperazin-1-yl]-6-methoxy-1,3,5-triazine (5a) (0.491 g, 90%).

$^1\text{H-NMR}$ (CDCl_3): δ (ppm): 2.19 (s, 6H, $2 \times$

$\text{CH}_3\text{-C}_6\text{H}_4$); 2.52-2.74 (m, 8H, $2 \times (\text{CH}_2)_2\text{-N-CH}_2$); 2.82 (t, 4H, $2 \times \text{N-CH}_2\text{-CH}_2\text{-NH}$); 3.15 (t, 4H, $2 \times \text{N-CH}_2\text{-CH}_2\text{-NH}$); 3.11-3.32 (m, 8H, $2 \times$ triazine- $\text{N-}(\text{CH}_2)_2$); 3.90 (s, 3H, $\text{CH}_3\text{-O}$); 6.58-6.61 (m, 4H, $2 \times \text{C}_6\text{H}_4$); 6.89-6.93 (m, 4H, $2 \times \text{C}_6\text{H}_4$). HPLC: (60% CH_3CN , 35% H_2O , 5% CH_3OH , v/v/v), 20 min: $R_t = 7.22$ min, 91%.

Reaction of 2,4-bis-[4-(2-chloroethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (2) with *n*-butylamine (b)

Starting materials: 2,4-bis-[4-(2-chloroethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (2) (0.404 g, 1 mmol), *n*-butylamine (0.396 ml, 4 mmol). Product: 2,4-bis-[4-(2-butylaminoethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (5b) (0.435 g, 91%).

$^1\text{H-NMR}$ (CDCl_3): δ (ppm): 0.88 (t, 6H, $2 \times \text{CH}_3\text{-}(\text{CH}_2)_3$); 1.03-1.45 (m, 8H, $2 \times \text{CH}_3\text{-}(\text{CH}_2)_2\text{-CH}_2$); 2.54 (t, 4H, $2 \times \text{N-CH}_2\text{-CH}_2\text{-NH}$); 2.57-2.70 (m, 8H, $2 \times (\text{CH}_2)_2\text{-N-CH}_2$); 2.70 (t, 4H, $2 \times \text{N-CH}_2\text{-CH}_2\text{-NH}$); 3.10-3.27 (m, 12H, $2 \times$ triazine- $\text{N-}(\text{CH}_2)_2 + \text{NH-CH}_2\text{-}(\text{CH}_2)_2\text{-CH}_3$); 3.94 (s, 3H, $\text{CH}_3\text{-O}$). HPLC (60% CH_3CN , 35% H_2O , 5% CH_3OH , v/v/v), 20 min: $R_t = 6.99$ min, 94%.

Reaction of 2,4-bis-[4-(2-chloroethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (2) with ethanol (c)

Starting materials: 2,4-bis-[4-(2-chloroethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (2) (0.404

Table 1. Reaction of triazine derivatives **1-3** with nucleophiles **a-e**.

Triazine derivatives	Nucleophile	Product	Yield [%]	Purity [%]
1	CH ₃ -C ₆ H ₄ -NH ₂	4a	94	90
	CH ₃ -(CH ₂) ₃ -NH ₂	4b	96	90
	CH ₃ -CH ₂ -OH	4c	81	94
	C ₆ H ₅ -OH	4d	92	93
	C ₆ H ₅ -SH	4e	97	89
2	CH ₃ -C ₆ H ₄ -NH ₂	5a	90	91
	CH ₃ -(CH ₂) ₃ -NH ₂	5b	91	94
	CH ₃ -CH ₂ -OH	5c	80	94
	C ₆ H ₅ -OH	5d	92	96
	C ₆ H ₅ -SH	5e	90	95
3	CH ₃ -C ₆ H ₄ -NH ₂	6a	91	93
	CH ₃ -(CH ₂) ₃ -NH ₂	6b	94	94
	CH ₃ -CH ₂ -OH	6c	77	97
	C ₆ H ₅ -OH	6d	92	96
	C ₆ H ₅ -SH	6e	93	90

g, 1 mmol), DIPEA (0.350 mL, 2 mmol), catalytic amount of DMAP (10 mg), ethanol (5 mL). Product: 2,4-bis-[4-(2-ethoxyethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (**5c**) (0.339 g, 80%). ¹H-NMR (CDCl₃): δ (ppm): 1.11 (t, 6H, 2 × CH₃-CH₂-O); 2.36-2.58 (m, 8H, 2 × (CH₂)₂-N-CH₂); 2.67 (t, 4H, 2 × N-CH₂-CH₂-O); 3.25-3.41 (m, 8H, 2 × triazine-N-(CH₂)₂); 3.44 (t, 4H, 2 × N-CH₂-CH₂-O); 3.58 (q, 4H, 2 × -O-CH₂-CH₃); 3.91 (s, 3H, CH₃-O). HPLC: (60% CH₃CN, 35% H₂O, 5% CH₃OH, v/v/v), 20 min: R_t = 6.97 min, 94%.

Reaction of 2,4-bis-[4-(2-chloroethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (**2**) with phenol (**d**)

Starting materials: 2,4-bis-[4-(2-chloroethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (**2**) (0.404 g, 1 mmol), phenol (0.188 g, 2 mmol) DIPEA (0.700 mL, 4 mmol). Product: 2,4-bis-[4-(2-phenoxyethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (**5d**) (0.478 g, 92%).

¹H-NMR (CDCl₃): δ (ppm): 2.42-2.66 (m, 8H, 2 × (CH₂)₂-N-CH₂); 2.95 (t, 4H, 2 × N-CH₂-CH₂-O); 3.27-3.50 (m, 8H, 2 × triazine-N-(CH₂)₂); 3.90 (s, 3H, CH₃-O); 4.09 (t, 4H, 2 × N-CH₂-CH₂-O); 6.77-6.89 (m, 6H, 2 × C₆H₅); 7.07-7.12 (m, 4H, 2 × C₆H₅). HPLC: (60% CH₃CN, 35% H₂O, 5% CH₃OH, v/v/v), 20 min: R_t = 13.57 min, 96%

Reaction of 2,4-bis-[4-(2-chloroethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (**2**) with thiophenol (**e**)

Starting materials: 2,4-bis-[4-(2-chloroethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (**2**) (0.404 g, 1 mmol), thiophenol (0.220 g, 2 mmol) DIPEA (0.700 mL, 4 mmol). Product: 2,4-bis-[4-(2-phenylsulfanylethyl)-piperazin-1-yl]-6-methoxy-1,3,5-triazine (**5e**) (0.497 g, 90%). ¹H-NMR (CDCl₃): δ (ppm): 2.45-2.69 (m, 8H, 2 × (CH₂)₂-N-CH₂); 2.71 (t, 4H, 2 × N-CH₂-CH₂-S); 3.15-3.42 (m, 8H, 2 × triazine-N-(CH₂)₂); 3.21 (t, 4H, 2 × N-CH₂-CH₂-S); 3.91 (s, 3H, CH₃-O); 7.02-7.35 (m, 10H, 2 × C₆H₅). HPLC: (60% CH₃CN, 35% H₂O, 5% CH₃OH, v/v/v), 20 min: R_t = 14.42 min, 95%.

Reaction of 2,4,6-tris-[4-(2-chloroethyl)-piperazin-1-yl]-1,3,5-triazine (**3**) with *p*-toluidine (**a**)

Starting materials: 2,4,6-tris-[4-(2-chloroethyl)-piperazin-1-yl]-1,3,5-triazine (**3**) (0.521 g, 1 mmol), *p*-toluidine (0.642 g, 6 mmol). Product: 2,4,6-tris-[4-[2-(4-methylphenylamino)ethyl]-piperazin-1-yl]-1,3,5-triazine (**6a**) (0.667 g, 91%).

¹H-NMR (CDCl₃): δ (ppm): 2.15 (s, 9H, 3 × CH₃-C₆H₄); 2.51-2.72 (m, 12H, 3 × (CH₂)₂-N-CH₂); 2.85 (t, 6H, 3 × N-CH₂-CH₂-NH); 3.21 (t, 6H, 3 × N-CH₂-CH₂-NH); 3.19-3.36 (m, 12H, 3 × triazine-N-(CH₂)₂); 6.96-7.02 (m, 6H, 3 × C₆H₄); 7.09-7.17 (m, 6H, 3 × C₆H₄).

HPLC: 60% CH₃CN, 35% H₂O, 5% CH₃OH, v/v/v), 20 min: R_t = 6.40 min, 93%.

Reaction of 2,4,6-tris-[4-(2-chloroethyl)-piperazin-1-yl]-1,3,5-triazine (3) with *n*-butylamine (b)

Starting materials: 2,4,6-tris-[4-(2-chloroethyl)-piperazin-1-yl]-1,3,5-triazine (3) (0.521 g, 1 mmol), *n*-butylamine (0.594 ml, 6 mmol). Product: 2,4,6-tris-[4-[(2-(butylamino) ethyl)-piperazin-1-yl]-1,3,5-triazine (6b) (0.593 g, 94%). ¹H-NMR (CDCl₃): δ (ppm): 0.87 (t, 9H, 3 × CH₃-(CH₂)₃); 1.01-1.54 (m, 12H, 3 × CH₃-(CH₂)₂-CH₂); 2.59 (t, 6H, 3 × N-CH₂-CH₂-NH); 2.61-2.71 (m, 12H, 3 × (CH₂)₂-N-CH₂); 2.77 (t, 6H, 3 × N-CH₂-CH₂-NH); 3.10-3.31 (m, 18H, 3 × triazine-N-(CH₂)₂ + NH-CH₂-(CH₂)₂-CH₃). HPLC (60% CH₃CN, 35% H₂O, 5% CH₃OH, v/v/v), 20 min: R_t = 7.14 min, 94%.

Reaction of 2,4,6-tris-[4-(2-chloroethyl)-piperazin-1-yl]-1,3,5-triazine (3) with ethanol (c)

Starting materials: 2,4,6-tris-[4-(2-chloroethyl)-piperazin-1-yl]-1,3,5-triazine (3) (0.521 g, 1 mmol), DIPEA (0.525 mL, 3 mmol), catalytic amount of DMAP (10 mg), ethanol (10 mL). Product: 2,4,6-tris-[4-[(2-ethoxyethyl)-piperazin-1-yl]-1,3,5-triazine (6c) (0.423 g, 77%). ¹H-NMR (CDCl₃): δ (ppm): 1.11 (t, 9H, 3 × CH₃-CH₂-O); 2.33-2.63 (m, 12H, 3 × (CH₂)₂-N-CH₂); 2.65 (t, 6H, 3 × N-CH₂-CH₂-O); 3.20-3.39 (m, 12H, 3 × triazine-N-(CH₂)₂); 3.44 (t, 6H, 3 × N-CH₂-CH₂-O); 3.48 (q, 6H, 3 × -O-CH₂-CH₃). HPLC: (60% CH₃CN, 35% H₂O, 5% CH₃OH, v/v/v), 20 min: R_t = 7.24 min, 97%.

Reaction of 2,4,6-tris-[4-(2-chloroethyl)-piperazin-1-yl]-1,3,5-triazine (3) with phenol (d)

Starting materials: 2,4,6-tris-[4-(2-chloroethyl)-piperazin-1-yl]-1,3,5-triazine (3) (0.521 g, 1 mmol), phenol (0.282 g, 3 mmol) DIPEA (1.050 mL, 6 mmol). Product: 2,4,6-tris-[4-(2-phenoxyethyl)-piperazin-1-yl]-1,3,5-triazine (6d) (0.638 g, 92%). ¹H-NMR (CDCl₃): δ (ppm): 2.44-2.68 (m, 12H, 3 × (CH₂)₂-N-CH₂); 2.95 (t, 6H, 3 × N-CH₂-CH₂-O); 3.21-3.43 (m, 12H, 3 × triazine-N-(CH₂)₂); 4.03 (t, 6H, 3 × N-CH₂-CH₂-O); 6.71-6.83 (m, 9H, 3 × C₆H₅); 7.09-7.24 (m, 6H, 3 × C₆H₅). HPLC: (60% CH₃CN, 35% H₂O, 5% CH₃OH, v/v/v), 20 min: R_t = 13.76 min, 96%.

Reaction of 2,4,6-tris-[4-(2-chloroethyl)-piperazin-1-yl]-1,3,5-triazine (3) with thiophenol (e)

Starting materials: 2,4,6-tris-[4-(2-chloroethyl)-piperazin-1-yl]-1,3,5-triazine (3) (0.521 g, 1 mmol), thiophenol (0.330 g, 3 mmol), DIPEA (1.050 mL, 6

mmol). Product: 2,4,6-tris-[4-[2-(phenylsulfanyl) ethyl]-piperazin-1-yl]-1,3,5-triazine (6e) (0.690 g, 93%).

¹H-NMR (CDCl₃): δ (ppm): 2.44-2.77 (m, 12H, 3 × (CH₂)₂-N-CH₂); 2.61 (t, 6H, 3 × N-CH₂-CH₂-S); 3.21-3.44 (m, 12H, 3 × triazine-N-(CH₂)₂); 3.22 (t, 6H, 3 × N-CH₂-CH₂-S); 7.02-7.30 (m, 15H, 3 × C₆H₅). HPLC: (60% CH₃CN, 35% H₂O, 5% CH₃OH, v/v/v), 20 min: R_t = 15.21 min, 90%.

RESULTS AND DISCUSSION

Compounds **1-3** with antitumor cytotoxicity towards the standard cell line of the mammalian tumor MCF-7, inducing cell death by apoptosis and necrosis, were treated with nucleophiles. As model nucleophilic substrates in this studies, simple molecule with hydroxylic, amino and sulphhydryl groups were used, mimicking hypothetical more complex intracellular targets. For studies of the interaction with hydroxylic group ethanol and phenol were used. Studies on interaction with amino group were made using *n*-butylamine and *p*-toluidine. Interactions of **1-3** with sulphhydryl group were studied using thiophenol as the model substrate.

It has been found that triazines **1-3** with mono-, bis-, and tris-4-(2-chloroethyl)piperazin-1-yl group gave substitution products in reactions with model nucleophilic reagents under mild reaction conditions.

Substitution proceeded fast and the final products were isolated with good – 77-97% yield and 89-97% purity according to the HPLC determination (Table 1). This confirmed that compounds **1-3** are strong acylating agents, easily reacting with the most of nucleophilic functional groups typical for proteins and nucleic acids.

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