Design, synthesis and biological evaluation of 1,3,5-triazine derivatives as potential inhibitors of dihydrofolate reductase.

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Abstract: Being aware of the crucial importance of diaminotriazine pharmacophore for binding to dihydrofolate reductase; two different series of diaminotriazine derivatives were designed, synthesized and evaluated as potential inhibitors of human dihydrofolate reductase (DHFR). The first series were 4,6-diamino-2,2-dimethyl-1,2-dihydro-1-phenyl-1,3,5-triazine derivatives and the second series were 2,4-diamino-6-phenyl-1,3,5-triazine derivatives. Triazine derivatives with lipophilic N-cyclohexyl substitution in position 4 displayed the highest potency in antitumor testing. These findings were in accordance with the results of the molecular modeling and docking simulation studies. These compounds would be potential anticancer lead agents. The synthesized compounds were screened for qualitative (zone of inhibition) and quantitative (MIC) antibacterial activity by agar cup plate method. The tested compounds were very weak or not active aginst *Escherichia Coli* and *Sarcina Lutea*.
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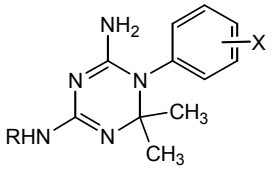
1. Introduction

Dihydrofolate reductase (DHFR) is an important enzyme in the folate cycle which supplies one-carbone unites for the biosynthesis of deoxythymidine monophosphate (dTMP).[1] Inhibition of DHFR leads to decrease cell growth and cell death.[2] Enzyme inhibition is effective because binding affinities of substrate analogues are so great that they are not readly displaced by natural substrate.[3] Hence this effect forms one of the important bases in cancer chemtherapy.[4]

DHFR is the major target of drug development against several diseases such as cancer [5,6], bacterial [7,8] and parasitic [9,10] infection.

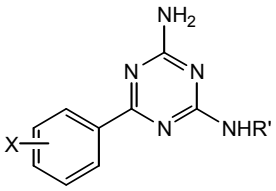
2,4-diamino-5,6-dihydrotriazine derivatives were found to interfere with folic acid metabolism.[11] Although these substances are not very active against bacterial DHFR, they have shown definit promise in cancer chemotherapy.[11,12] 1,3,5-Triazine derivatives having various amino groups at positions 2, 4 or 6 have been known as anticancer drugs. [13,14]

Aiming at discovering new antitumor agents, two different series of diaminotriazine derivatives have been synthesized and evaluated as anticancer agents that inhibiting dihydrofolate reductase (DHFR) enzyme. The first series was the 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-1,3,5-triazine derivatives (I) and the second series was the 2,4-diamino-6-phenyl-1,3,5-triazine derivatives (II).



(I)

4,6-diamino-1,2-dihydro-2,2-dimethyl -1-phenyl-1,3,5-triazine derivatives



(II)

2,4-diamino-6-phenyl-1,3,5-triazine derivatives

Different synthetic strategie were reported for synthesis of 4,6-diamino-2,2-dialkyl-1,2-dihydro-1,3,5-triazine derivatives (I). The first strategy was the three-component synthesis which involves condensation of an arylamine hydrochloride, dicyandiamide and a ketone or an aldehyde.[15,16] Another synthetic method involved the condensation of an arylbiguanide and a ketone or an aldehyde.[17-20]

2,4-diamino-1,3-5-triazine derivatives (II) were usually synthesized by using both solution- and solid-phase approaches.[21]

Recently nitrile compounds were synthesized in situ from reaction of aldehyde derivatives with iodine in aqueous ammonia. One-pot synthesis of 2,4-diamino-6-phenyl-1,3-5-triazine derivatives was achieved by reaction of resulted nitrile derivatives with dicyandiamide in aqueous KOH.[22]

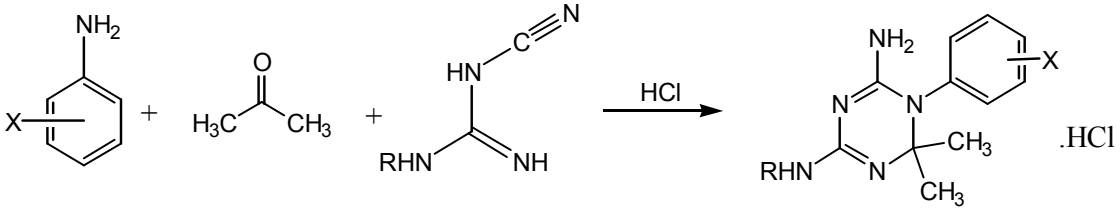
The N-cyclohexyltriazine derivatives were designed and synthesized to increase the lipophilicity of triazines and to create a new series of triazines that may have a different binding affinity to the active site of DHFR and consequently a different antitumor activity.

The present study reports the synthesis, molecular modeling and a comparison of cytotoxic activity of synthesized triazine analogues (I) and (II) on Ehrlich Ascites Carcinoma (EAC) cells. Furthermore, it reports the docking of synthesized compounds into DHFR enzyme and the correlation between the docking scores of synthesized triazines and their perlimenary in vivo antitumor activity.

2. Results and Discussion

Chemistry

As outlined in scheme (1), the one-pot, three component cyclocondensation reaction between different aniline derivatives, acetone, and dicyandiamide (or N-cyclohexyldicyandiamide) provide a direct access to synthesize the 4,6-diamino-1,2-dihydro-2,2-dimethyl-1,3,5-triazine derivatives 3a-3f or 4b-4d respectively.[19]



1a-1f

1a X=-H
1b X=4-Cl
1c X=3-NO_2
1d X=4-Cl, 3-NO_2
1e X=4-CH_3
1f X=4-F

2a-2b

2a R=-H
2b R=-C_6H_{11}

3a-3f and 4b-4d

3a-3f (R=-H)	**4b-4d (R=-C_6H_{11})**
3a X=-H	4b X=4-Cl
3b X=4-Cl	4c X=3-NO_2
3c X=3-NO_2	4d X=4-Cl, 3-NO_2
3d X=4-Cl, 3-NO_2	
3e X=4-CH_3	
3f X=4-F	

Scheme 1. Synthesis of 4,6-diamino-2,2-dimethyl-1,2-dihydro-1,3,5-triazine derivatives

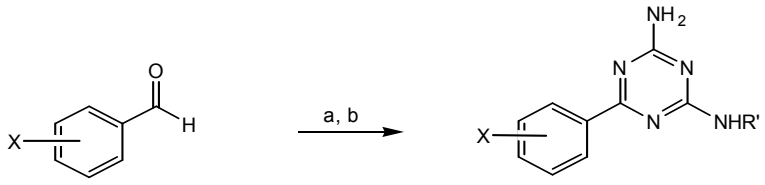
For the second series of the 2,4-diamino-1,3,5-triazine derivatives 5a-5g and 7b-7d, different benzaldehyde derivatives were transformed to nitriles in situ by using iodine in aqueous ammonia followed by reaction of resulted nitrile derivatives with dicyandiamide (or N-cyclohexyldicyandiamide) in a strong alkaline media using KOH to afford 2,4-diamino-1,3,5-triazine derivatives 5a-5g and 7b-7d as shown in scheme (2). [22]

The N-cyclohexyl derivatives 4b-4d and 7b-7d were designed and synthesized to increase the lipophilicity of triazines and to create a new series of triazines that would have a different binding affinity to the active site of DHFR and consequently a different antitumor activity.

The N-cyclohexyl derivatives of triazines were synthesized by using cyclohexyldicyandiamide which was prepared by the reaction between cyclohexylamine hydrochloride and sodium dicyandiamide.[23]

Biological activity

Synthesized compounds were examined for their antitumor activity. An *in vivo* study was carried out using EAC on mice.[24] The results of the present study showed a remarkable antitumor effect of the tested compounds 4b-4d and 7b-7d against EAC in albino mice.



5a-5g **6a-6g and 7b-7d**

5a X=-H
5b X=4-Cl
5c X=3-NO_2
5d X=4-Cl, 3-NO_2
5e X=3-Cl
5f X=4-F
5g X=2-Cl

6a-6g (R=-H)
6a X=-H
6b X=4-Cl
6c X=3-NO_2
6d X=4-Cl, 3-NO_2
6e X=3-Cl
6f X=4-F
6g X=2-Cl

7b-7d (R=-C_6H_{11})
7b X=4-Cl
7c X=3-NO_2
7d X=4-Cl, 3-NO_2

Scheme 2. Synthesis of 2,4-diamino-1,3,5-triazine derivatives. Reagents and conditions; (a) I_2, aq. NH_3, rt. (b) dicyandiamide or N-cyclohexyldicyandiamide, KOH.

The study was made among three groups of mice (n = 5). The groups comprised of: a) tumor-bearing mice. b) tumor-bearing mice injected for 21 days with a daily dose of 10 mg/Kg body weight of synthesized compounds and 5-fluorouracil (5FU) as a reference standard cytotoxic agent. c) control mice (normal). It was noted that: In the 6th day after inoculation of Ehrlich cells in mice, increase in body weight and ascites was observed clearly, also mice became slow and inactive. Mice received compounds 4b-4d and 7b-7d were more active and more protected against ascites and show no increase in weight than control mice. The % inhibition of tumor growth was calculated compared to control group by weighing the solid tumors isolated from inoculated mice on day-22 (Tables 1 and 2). Mice received compounds 4b, 4c, 4d, 7c and (5FU) showed 100% inhibition of tumor growth.

A screening of antibacterial activities using a Gram negative (*Eschericia Coli*) and a Gram positive bacteria (*Sarcina Lutea*) was performed for graded concentrations (16-1024 µg/ml) of synthesized compounds. The diameters of the inhibition zone corresponding to the MICs were measured.

Positive control using only inoculation and negative control using only DMSO in the cavity were carried out. From analysis of the rsults, it was noticed that compounds 4b, 4c, 4d and 6b exhibited very weak antibacterial activities while other compounds were inactive. The values of MIC in µg/ml of compounds 4b, 4c and 5b against *Eschericia Coli* were 128, 512 and 512 respectively. The values of MIC in µg/ml of compounds 4b, 4c and 5b against *Sarcina lutea* were 128, 256 and 512 respectively.

Table 1. Tumour reducing activity of 4,6-diamino-1-phenyl-1,2-dihydro-2,2-dimethyl-1,3,5-triazine derivatives (I) and 5-fluorouracil (5FU) as a reference cytotoxic agent

Compound number	3a	3b	3c	3d	3e	3f	4b	4c	4d	5FU
% inhibition of tumour growth	23	38	16	15	12	10	100	100	100	100

Table 2. Tumour reducing activity of 2,4-diamino-6-phenyl-1,3,5-triazine derivatives (II) and 5-fluorouracil (5FU) as a reference cytotoxic agent

Compound number	6a	6b	6c	6d	6e	6f	6g	7b	7c	7d	5FU
% inhibition of tumour growth	18	2	7	4	16	18	16	85	100	90	100

Molecular Modeling studies

To understand the obtained pharmacological data on a structural basis, the affinity of synthesized triazine derivatives to DHFR was analyzed by molecular modeling and docking techniques.

X-ray crystal structure of human DHFR (code: 3GHC) is available through the RCSB Protein Data Bank.[25] The active site of DHFR was located (figure 1) and the affinities of synthesized compounds (ligands) to DHFR (protein) were determined by studying the ligands docking scores into their active site, and the docking is measured in kcal/mol. The lower the docking score, the better the affinity of the ligand to the active site. The docking of prepared

triazine derivatives into the active site of DHFR was determined using a docking algorithm with potentials of mean force (PMF). The molecular docking package (Molgro Virtual Docker) [26] to predict possible binding of various ligands within DHFR active site was used.

It has been observed that in The first series; the 4,6-diamino-1,2-dihydro-2,2-dimethyl-1,3,5-triazine derivatives (I) showed lower docking scores and better activity data than the second series; the 2,4-diamino-1,3,5-triazine derivatives (II).

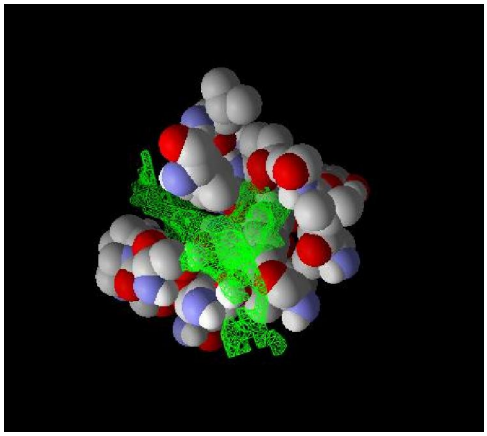


Figure 1. The active site of protein DHFR (deepest grooves shown in green).

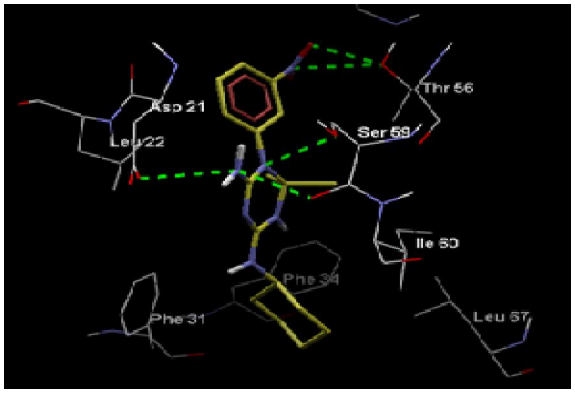


Figure 2. Docking of compound (4c) into the active site of protein DHFR

Moreover it was observed that the series of prepared triazine derivatives which have N-cyclohexyl side-chain showed the lowest docking scores and the best antitumor activity against EAC. The determined binding affinities for compounds 4b-4d and 7b-7d (4b: -120; 4c: -118; 4d: -120; 7b: -106; 7c: -122; 7d: -112) were in good agreement with the experimentally determined pharmacological activity data. This activity was explained by studying the attraction forces between synthesized triazine derivatives and the amino acids located in the active site of DHFR. A representative example of such attraction forces between compound 4c and DHFR is shown in figure (2).

Compound (4c) makes H-bonds with Thr56, Ser59 and Leu22. The cyclohexyl side chain was perfectly impeded in a lipophilic groove in the active site and surrounded by three lipophilic amino acid; Phe34, Phe31 and Ile60 which made a hydrophobic attraction force with the cyclohexyl group.

3. Experimental

Chemistry

All chemicals used in synthesis were purchased from Aldrich-Sigma Chemical Co. or Fluka Co. They were used without further purification. Melting points were determined on a Stuart, SMP 30 melting point apparatus and were uncorrected. NMR spectra were run on a 300 MHz Varian Mercury spectrometer. The chemical shift (δ) values are expressed in parts per million (ppm) relative to tetramethylsilane as an internal standard and DMSO-d_6 as a solvent; signals were characterized as s (singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). Mas spectra were generated on Shimadzu GC-MS-QP 2010 plus (electron impact, ionization energy, 70eV) elemental analyses were obtained on the Micro Analytical Centre Cairo Univ. Giza, Egypt. The results were found to be in good agreement (± 0.4%) with the calculated values. Reactions were monitored by thin-layer chromatography (TLC) using precoated aluminium-backed plates (Merck silica gel 60 F_{254}) visualised under UV light λ = 254 nm).

N-cyclohexyldicyandiamide (2b)

Cyclohexylamine hydrochloride (9.04 g, 0.066 mol), sodium dicyanimide (5.93 g, 0.066) and butanol (25 ml) were heated at reflux for 6h. The cooled suspension was filtered and the filtrate was evaporated. The residual syrup solidifies on treatment with dioxan. The white precipitate was purified by crystallization from water to afford (14) (9.5 g, 86.71%), as a white shiny white crystalline solid, mp 166-167°C (lit.[23] 166 °C).

General procedure for the synthesis of compounds 1a-1f and 4b-4d

A mixture of aromatic amine (0.02 mol), dicyandiamide or N-cyclohexyldicyandiamide (0.022 mol), acetone (60 ml) and conc. HCl (1.86 ml) was stirred overnight at room temperature. The formed white solid was collected, washed with acetone and crystalized from aqueous ethanol suitable solvent to afford the pure product. In this manner the following compounds were prepared.

4,6-Diamino-1-phenyl-1,2-dihydro-2,2-dimethyl-1,3,5-triazine hydrochloride (1a)

From aniline (1.86 g, 0.02 mol), dicyandiamide (1.85 g, 0.022 mol), 1a (4.40 g, 90%), was obtained as a white crystalline solid, m. r. 202-204°C (lit.[15] 200-

203°C). ^{1}HNMR (DMSO-d_6, ppm) δ: 9.12 (s, 1H, NH), 7.53-7.52 (m, 3H, Ph 3,4,5-H_3), 7.37 (d, *J* = 7.8 Hz, 2H, Ph 2,6-H_2), 3.34 (s, 4H, 2 NH_2), 1.33 (s, 6H, 2 CH_3).

4,6-Diamino-1-(4-chlorophenyl)-1,2-dihydro-2,2-dimethyl-1,3,5-triazine hydrochloride (1b)

From 4-chloroaniline (2.55 g, 0.02 mol), dicyandiamide (1.85 g, 0.022 mol), 1b (4.60 g, 84%), was obtained as a white crystalline solid, m. r. 214-215°C (lit.[15] 210-215°C). ^{1}HNMR (DMSO-d_6, ppm) δ: 9.32 (s, 1H, NH), 7.58 (d, *J* = 8.6 Hz, 2H, Ph 3,5-H2), 7.41 (d, *J* = 8.6 Hz, 2H, Ph 2,4-H_2), 3.36 (s, 4H, 2 NH_2), 1.33 (s, 6H, 2 CH_3).

4,6-Diamino-1-(3-nitrophenyl)-1,2-dihydro-2,2-dimethyl-1,3,5-triazine hydrochloride (1c)

From 3-nitroaniline (2.76 g, 0.02 mol), dicyandiamide (1.85 g, 0.022 mol), 1c (3.65 g, 61%), was obtained as a yellow crystalline solid, m. r. 206-207°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 8.44 (d, *J* = 8.2 Hz, 1H, Ph 4-H), 8.27 (s, 1H, Ph 2-H), 7.84-7.83 (m, 2H, Ph 5,6-H_2), 3.31 (s, 4H, 2 NH_2), 1.51 (s, 3H, CH_3), 1.49 (s, 3H, CH_3). ^{13}C NMR (300 MHz, DMSO-d_6, ppm) δ: 158.2, 149.3, 137.7, 132.2, 126.1, 125.24, 117.6, 114.3, 70.5, 27.9, 27.7; EI-MS: *m/z* 263.20 ($C_{11}H_{15}N_6O_2$, [M^+]).

4,6-Diamino-1-(4-chloro-3-nitrophenyl)-1,2-dihydro-2,2-dimethyl-1,3,5-triazine hydrochloride (1d) From 4-chloro-3-nitroaniline (3.54 g, 0.02 mol), dicyandiamide (1.85 g, 0.022 mol), 1d (4.95 g, 74%), was obtained as a yellow needled crystalline solid, m. r. 218-220°C (lit.[19] 218-222°C). ^{1}HNMR (DMSO-d_6, ppm) δ: 8.07 (s, 1H, Ph 2-H), 7.86 (d, *J* = 8.6 Hz, 1H, Ph 5-H), 7.69 (d, *J* = 8.6 Hz, 1H, Ph 6-H), 3.31 (s, 4H, 2 NH_2), 1.50 (s, 6H, 2CH_3).

4,6-Diamino-1-(4-methlphenyl)-1,2-dihydro-2,2-dimethyl-1,3,5-triazine hydrochloride (1e)

From p-toluidine (2.14 g, 0.02 mol), dicyandiamide (1.85 g, 0.022 mol), 1e (4.0 g, 77%), was obtained as a rectangular colourless crystalline solid, m. r. 206-208°C (lit.[15] 206-208 °C). ^{1}HNMR (DMSO-d_6, ppm) δ: 7.35 (d, *J* = 8.6 Hz, 2H, Ph 2,6-H_2), 7.16 (d, *J* = 8.6 Hz, 2H, 3,5-Ph H_2), 2.17 (s,3H, CH_3), 1.50 (s, 6H, 2CH_3).

4,6-Diamino-1-(4-fluorophenyl)-1,2-dihydro-2,2-dimethyl-1,3,5-triazine hydrochloride (1f)

From 4-fluroaniline (2.22 g, 0.02 mol), dicyandiamide (1.85 g, 0.022 mol), 1f (2.85 g, 52%), was obtained as a white solid, m. r. 222-224°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 9.18 (s, 1H, NH), 7.45 (d, *J* = 7.5 Hz, 2H, Ph 3,5-H_2), 7.36 (d, *J* = 7.5 Hz, 2H, Ph 2,6-H_2), 1.33 (s, 6H, 2CH_3). ^{13}C NMR (300 MHz, DMSO-d_6, ppm) δ: 158.2, 155.8, 134.3, 132.9, 131.5, 124.1, 117.6, 115.8, 70.1, 29.5, 27.7; EI-MS: *m/z* 236.00 ($C_{11}H_{14}FN_5$, [M^+]).

6-Amino-4-cyclohexylamino-1-(4-chlorophenyl)-1,2-dihydro-2,2-dimethyl-1,3,5-triazine hydrochloride (4b)

From 4-chloroaniline (2.55 g, 0.02 mol), N-cyclohexyldicyandiamide (3.34 g, 0.022 mol), 4b (3.72 g, 50%), was obtained as a white crystalline solid, m. r. 266-267°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 9.78 (br s, 1H, NH), 7.41 (d, *J* = 8.40 Hz, 2H, Ph 3,5-H_2), 7.33 (d, *J* = 8.40 Hz, 2H, Ph 2,4-H_2), 3.42 (br s, 1H, NH), 1.81-1.66 (m, 5H, cyclohexyl-H_5), 1.53-1.22 (m, 12H, 6H 2 x CH_3 and 6H-cyclohexyl-H_6); EI-MS: *m/z* 334.41 ($C_{17}H_{25}{}^{35}ClN_5$, [M^+]), 336.41 ($C_{17}H_{25}{}^{37}ClN_5$, [M^+]).

6-Amino-4-cyclohexylamino-1-(3-nitrophenyl)-1,2-dihydro-2,2-dimethyl-1,3,5-triazine hydrochloride (4c)

From 3-nitroaniline (2.76 g, 0.02 mol), N-cyclohexyldicyandiamide (3.34 g, 0.022 mol), 4c (3.80 g, 50%), was obtained as a white crystalline solid, m. r. 230-232°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 9.03 (br s, 1H, NH), 8.37 (d, *J* = 8.20 Hz, 1H, Ph 4-H), 8.22 (s, 1H, Ph 2-H), 7.89 (d, *J* = 8.20 Hz, 1H, Ph 6-H), 7.81 (t, *J* = 8.20 Hz, 1H, Ph 5-H), 3.82 (1H, br s, NH), 1.82-1.68 (m, 5H, cyclohexyl-H_5), 1.39-1.05 (m, 12H, 6H 2 x CH_3 and 6H-cyclohexyl-H_6). ^{13}C NMR (300 MHz, DMSO-d_6, ppm) δ: 156.3, 148.4, 137.6, 132.1, 126.1, 125.2, 117.7, 114.1, 70.5, 49.1, 32.9, 27.8, 27.6, 24.9, 24.6; EI-MS: *m/z* 345.00 ($C_{17}H_{25}N_6O_2$, [M^+]).

6-Amino-4-cyclohexylamino-1-(4-chloro-3-nitrophenyl)-1,2-dihydro-2,2-dimethyl-1,3,5-triazine hydrochloride (4d)

From 4-chloro-3-nitroaniline (3.54 g, 0.01 mol), N-cyclohexyldicyandiamide (3.34 g, 0.022 mol), 4d (1.70 g, 41%), was obtained as a white crystalline solid, m. r. 224-225°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 9.07 (s, 1H, NH), 8.21 (d, *J* = 2.6 Hz, 1H, Ph 2-H), 7.91 (d, *J* = 8.60 Hz, 1H, Ph 5-H), 7.77 (dd, *J* = 2.60, 8.60 Hz, 4H, Ph 6-H), 3.81 (br s, 1H, NH), 1.82-1.70 (5H, m, cyclohexyl-H_5), 1.45-1.22 (12H, m, 6H 2 x CH_3 and 6H-cyclohexyl-H_6). ^{13}C NMR (300 MHz, DMSO-d_6, ppm) δ: 156.0, 155.1, 147.6, 138.3, 132.4, 126.0, 119.5, 116.6, 65.4, 50.2, 32.8, 29.4, 29.3, 25.0, 24.5; EI-MS: *m/z* 379.65 ($C_{17}H_{24}{}^{35}Cl\ N_6O_2$, [$M^+$]), 381.65 ($C_{17}H_{24}{}^{37}Cl\ N_6O_2$, [$M^+$]).

General procedure for synthesis of compounds (6a-6g) and (7b-7d)

A solution of aromatic aldehyde (0.02 mol), iodine (5.60 g, 0.022 mol) in ammonia water (180 ml of 28 % solution) and THF (20 ml) was stirred at room temperature for 1-2h. The dark solution became colorless at the end of the reaction. A mixture of dicyandiamide or N-cyclohexyldicyandiamide (0.022 mol) and KOH (2.46 g, 0.044 mol) was then added. The reaction mixture was heated at reflux for 48-72 h. The product was filtered off and crystalized from

aqueous ethanol to afford pure product. In this manner the following compounds were prepared.

2,4-Diamino-6-phenyl-1,3,5-triazine (6a)

From benzaldehyde (2.12 g, 0.02 mol) and dicyandiamide (1.85 g, 0.022 mol), 6a (1.80 g, 40%), was obtained as a white solid, m. r. 218-220°C (lit.[22] 220-222°C). ^{1}HNMR (DMSO-d_6, ppm) δ: 8.34 (m, 2H, Ph 2,6-H_2), 7.53 (m, 3H, Ph 3,4,5-H_3), 3.45 (br s, 4H, 2NH_2).

2,4-Diamino-6-(4-chlorophenyl)-1,3,5-triazine (6b)

From 4-chlorobenzaldehyde (2.82 g, 0.02 mol), and dicyandiamide (1.85 g, 0.022 mol), 6b (3.40 g, 77%), was obtained as a light-sensitive white crystalline solid, m. r. 101-102°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 7.89 (d, *J* = 8.8 Hz, 2H, Ph 2,6-H_2), 7.67 (d, *J* = 8.8 Hz, 2H, Ph 3,5-H_2), 3.32 (4H, br s, 2NH_2); EI-MS: *m/z* 221.10 ($C_9H_8{}^{35}ClN^5$, [M^+], EI-MS *m/z*: 223.10 ($C_9H_8{}^{37}ClN_5$, [M^+]).

2,4-Diamino-6-(3-nitrophenyl)-1,3,5-triazine (6c)

From 3-nitrobenzaldehyde (3.00 g, 0.02 mol) and dicyandiamide (1.85 g, 0.022 mol), 6c (3.52 g, 76%) was obtained as a White crystalline solid, m. r. 106-108°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 8.74 (s, 1H, Ph 2-H), 8.53 (d, *J* = 8.4 Hz, 1H, Ph 4-H), 8.31 (d, *J* = 8.4 Hz, 1H, Ph 6-H), 7.88 (t, *J* = 8.4 Hz, 1H, Ph 5-H). ^{13}C NMR (300 MHz, DMSO-d_6, ppm) δ: 148.4, 139.0, 131.7, 128.5, 127.9, 117.5, 113.4; EI-MS: *m/z* 231.15 ($C_9H_8N_6O_2$, [M^+]).

2,4-Diamino-6-(4-chloro-3-nitrophenyl)-1,3,5-triazine (6d)

From 4-chloro-3-nitorobenzaldehyde (3.72 g, 0.02 mol) and dicyandiamide (1.85 g, 0.022 mol), 6d (3.96 g, 74%), was obtained as a light-sensitive yellow crystalline solid, m. r. 142-144°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 8.48 (s, 1H, Ph 2-H), 8.13 (d, *J* = 9.1 Hz, 1H, Ph 5-H), 7.88 (d, *J* = 9.1 Hz, 1H, Ph 6-H). ^{13}C NMR (300 MHz, DMSO-d_6, ppm) δ: 166.7, 148.8, 148.2, 137.1, 130.3, 126.3, 120.8, 119.2, 97.1; EI-MS *m/z*: 266.35 ($C_9H_7{}^{35}ClN_6O_2$, [M^+]), EI-MS: *m/z* 268.25 ($C_9H_7{}^{37}ClN_6O_2$, [M^+]).

2,4-Diamino-6-(3-chlorophenyl)-1,3,5-triazine (6e)

From 3-chlorobenzaldehyde (2.82 g, 0.02 mol) and dicyandiamide (1.85 g, 0.022 mol), 6e (3.0 g, 68%), was obtained as a light-sensitive white crystalline solid, m. r. 128-129°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 7.91 (s, 1H, Ph 2-H), 7.85 (d, *J* = 7.8 Hz, 1H, Ph 4-H), 7.58 (d, *J* 7.8 Hz, 1H, Ph 6-H); (t, *J* = 7.8 Hz, 1H, Ph 5-H); EI-MS: *m/z* 221.00 ($C_9H_8N_5{}^{35}Cl$, [M^+]), EI-MS *m/z*: 223.00 ($C_9H_8N_5{}^{37}Cl$, [M^+]).

2,4-Diamino-6-(4-fluorophenyl)-1,3,5-triazine (6f)

From 4-fluorobenzaldehyde (2.48 g, 0.02 mol) and dicyandiamide (1.85 g, 0.022 mol), 6f (2.60 g, 63%), was obtained as a light-sensitive white crystalline solid, m. r. 48-49°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 7.96-7.92 (m, 2H, Ph 3,5-H_2), 7.23-7.29 (m, 2H, Ph 2,6-H2). ^{13}C NMR (300 MHz, DMSO-d_6, ppm) δ: 148.3, 139.0, 131.7, 128.5, 127.9, 117.5, 113.3; EI-MS: *m/z* 205.00 ($C_9H_8N_5F$, [M^+]).

2,4-Diamino-6-(2-chlorophenyl)-1,3,5-triazine (6g)

From 2-chlorobenzaldehyde (2.82 g, 0.02 mol) and dicyandiamide (1.85 g, 0.022 mol), 6g (3.0 g, 68%) was obtained as a light-sensitive white crystalline solid, m. r. 50-52°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 7.87 (d, *J* = 8.89 Hz, 1H, Ph 3-H), 7.75-7.76 (m, 2H, Ph 5,6-H_2), 7.57 (d, *J* = 8.25 Hz, 1H, Ph 4-H). ^{13}C NMR (300 MHz, DMSO-d_6, ppm) δ: 135.8, 135.5, 135.1, 130.5, 128.5, 116.4, 112.5; EI-MS: *m/z* 221.10 ($C_9H_8{}^{35}Cl\ N_5$, [M^+]), 223.10 ($C_9H_8{}^{37}ClN_5$, [M^+]).

2-Amino-4-cyclohexylamino-6-(4-chlorophenyl)-1,3,5-triazine (7b)

From 4-chlorobenzaldehyde (2.82 g, 0.02 mol) and N-cyclohexyldicyandiamide (3.34 g, 0.022 mol) 7b (2.60 g, 43%), was obtained as a light-sensitive yellow crystalline solid, m. r. 104-105°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 7.59 (d, *J* = 8.60 Hz, 2H, Ph 3,5-H_2), 7.41 (d, *J* = 8.60 Hz, 1H, Ph 2,4-H_2), 6.50 (br s, 2H, NH_2), 1.75-1.50 (m, 5H, cyclohexyl-H_5), 1.28-1.05 (m, 6H, cyclohexyl-H_6). ^{13}C NMR (300 MHz, DMSO-d_6, ppm) δ: 160.7, 139.0, 134.6, 130.2, 118.9, 110.6, 49.8, 32.6, 25.5, 24.8; EI-MS: *m/z* 303.00 ($C_{15}H_{18}{}^{35}ClN_5$, [M^+]), 305.00 ($C_{15}H_{18}{}^{37}ClN_5$, [M+]).

2-Amino-4-cyclohexylamino-6-(3-nitrophenyl)-1,3,5-triazine (7c)

From 3-nitorobenzaldehyde (3.00 g, 0.02 mol) and N-cyclohexyldicyandiamide (3.34 g, 0.022 mol) 7c (4.0 g, 64%), was obtained as a light-sensitive white solid, m. r. 111-112°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 8.75 (s, 1H, Ph 2-H), 8.54 (d, *J* 8.10 Hz, 1H, Ph 4-H), 8.31 (d, *J* 8.10 Hz, 1H, Ph 6-H), 7.87 (t, *J* 8.10 Hz, 1H, Ph 5-H), 6.71 (br s, 2H, NH_2), 6.51 (br s, 1H, NH), 1.75-1.49 (m, 5H, cyclohexyl-H_5), 1.30-1.18 (m, 6H, 6H-cyclohexyl-H_6). ^{13}C NMR (300 MHz, DMSO-d_6, ppm) δ: 160.8, 148.3, 138.9, 131.7, 128.4, 127.8, 118.9, 117.5, 113.4, 50.0, 32.9, 25.5, 24.8; EI-MS: *m/z* 314.24 ($C_{15}H_{18}N_6O_2$, [M^+]).

2-Amino-4-cyclohexylamino-6-(4-chloro-3-nitrophenyl)-1,3,5-triazine (7d)

From 4-chloro-3-nitorobenzaldehyde (3.72 g, 0.02 mol), and N-cyclohexyldicyandiamide (3.34 g, 0.022 mol) 7d (3.20 g, 46%), was obtained as a light-sensitive yellow crystalline solid, m. r. 104-105°C. ^{1}HNMR (DMSO-d_6, ppm) δ: 8.89 (d, *J* = 8.40 Hz, 1H, Ph 5-H), 8.50 (d, *J* = 2.00 Hz, 1H, Ph 2-H), 8.15 (dd, *J* = 2.00, 8.40 Hz, 1H, Ph 4-H), 6.50 (br s, 2H, NH_2), 1.75-1.50 (m, 5H, cyclohexyl-H_5), 1.28-1.05 (m, 6H, cyclohexyl-H_6). ^{13}C NMR (300 MHz, DMSO-d_6, ppm) δ: 165.3, 160.8, 148.8, 137.1, 130.3, 125.2, 120.8, 118.8, 97.1, 49.8, 32.9, 25.5, 24.8; EI-MS: *m/z*

348.20 ($C_{15}H_{17}{}^{35}ClN_6O_2$, [$M^+$]), 350.25 ($C_{15}H_{17}{}^{37}ClN_6O_2$, [$M^+$]).

Preliminary evaluation of antitumor activity

Cells of Ehrlich ascites tumour were obtained from National Cancer Institute, Cairo, Egypt. The experimental animals, adult female albino mice (9-12 g) were used throughout the study. All animals were inoculated with (2 x 106) cells/mouse on day "0" except normal group, and treatment started 24 h after inoculation, at a single dose of 1.0 mg/mouse/day, i.p. All treatments were given for 21 days. The desired concentration of tumour cells was obtained by dilution with saline solution (0.9% sodium chloride solution).[24]

Tumour reducing activity

Ehrlich ascites tumour cells (0.2 ml prepared in 0.9% sodium chloride solution) were injected into the mice peritoneal cavity and (10 mg/Kg body weight/day) of the synthesized compounds (5 mice/group) and 5-fluorouracil were injected i.p. from day-1 to day-21. Animals were observed for the development of ascites tumour and death due to tumour burden. On day-21 solid tumour was isolated, weighed and % reduction of tumour growth was calculated compared to the control group, as shown in tables (1 and 2).

Antibacterial activity

The antibacterial activity of tested samples (1-20) was determined by agar cup plate method [27] using two organisms such as *Escherichia Coli* 10408 (E.C.) and *Sarcina Lutea* 10449 (S. L.).

This method based on diffusion of antibacterial compounds from reservoir bore containing graded concentrations of the tested compounde ranging from 16 to 1024 µg/ml (prepared by serial dilution from stock solution contains 10 mg/ml in DMSO with water) to the surrounding inoculated nutrient agar medium so that the growth of microorganisms was inhibited as circular zone around the bore.

The graded concentrations (16-1024 µg/ml) of the synthesized compounds were placed in a bore made in petri dishes which containing different organisms and incubated at 37^o C for 24 h.

The MIC was defined as the lowest concentration of the tested sample allowing no visible growth. The inhibition zone around the bore was measured after 24 h. The inhibition zones were calculated from the mean of three different experiments.

Conclusions

A comparison study of the synthesis, biological activity and docking simulation between two series of diaminotriazine derivatives has been reported. The first series (I); 4,6-diamino-2,2-dimethyl-1,2-dihydro-1-phenyl-1,3,5-triazines and the second one (II); 2,4-diamino-6-phenyl-1,3,5-triazines were synthesized by one pot synthetic strategy. The antitumor activity results showed that compounds of series (I) have non-significant higher inhibitory activity than compounds in series (II). Triazine derivatives having cyclohexyl side chain (4b-4d and 7b-7d) in both series exhibited significant inhibitory activity in tumor growth due to hydrophobic attraction force between the cyclohexyl ring and three lipophilic amino acids; Phe34, Phe31 and Ile60 in the active site of DHFR. The pharmacological findings are in good agreement with predicted docking scores obtained by docking simulation studied which allow a mean of qualitative ranking of new, not yet synthesized derivatives.

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