Synthesis and Cytotoxic Activity of Hexahydro-1,3,5-triazine Derivatives through Ring Condensation

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As a part of a research program for pharmacologically interacting sym-triazine derivatives with hexahydrotriazine ring, novel hexahydrotriazine derivatives (**1**–**13**) were successfully synthesized. The synthesis of hexahydrotriazines was carried out from the assembly of three molecules of various amines and three molecules of formaldehyde by "1 + 1 + 1 + 1 + 1 + 1" cycloaddition. The synthesis mechanism is probably passes through imines, which trimerizes to give the hexahydrotriazine ring. All synthesized hexahydrotriazines (**1**–**13**) were screened for their cytotoxic activity by cancer cells. As a result, compound **1**–**9** reduced the viability of T98G cells in a concentration-dependent manner. At a concentration of 100 μM, compound **9** exhibited the greatest cytotoxicity at 94.6%. The reason is that compound **9** is C_5H_5N that has a resonance hybrid structure and exhibits aromaticity. It is considered to be active because it has a structure that is a raw material for pyridine derivatives as mainly medicines and pesticides.

Keywords: Hexahydrotriazine, Sym-triazine, Cytotoxic activity, Cyclocondensation, Pharmaceutical activity

Introduction

Hexahydrotriazines as symtriazines are important building blocks in high-explosive compounds[1–4] and are reported to show a broad spectrum of biological activity in particular, antitumor, antimicrobial, and anticancer activities.[5] They are also used as herbicides, carcenolytics, and growth stimulating and antidote activity.[6] An increasing interest with hexahydrotriazine core is attracted by hexahydrotriazine derivatives, as witnessed in a recent review on their pharmaceutical activity properties. Until now, despite the less literature on hexahydrotriazines, they represent a useful class of organic compounds. The close similarity with hexahydrotriazine core suggested them as potential capability of modifying substitutes of this ubiquitous moiety. As a part of a research program related to the synthetic study of pharmacologically interesting compounds and good chelating agents for transition metal ions, we were in the middle of an experiment about 1,5,3,7-diazadiphosphocine-1,5-dicarboxylic acids obtained from the synthesis of an unusual medium-sized ring heterocyclic ligand with carboxylic-amino-phosphonic donating groups.[7–11] This heterocyclic ligand results from the assembly of two molecules of amine, two molecules of H_3PO_2, and four molecules of formaldehyde; its striking feature is that each atom of this eight-membered ring is originated from eight single different molecules, representing a formal "1 + 1 + 1 + 1 + 1 + 1 + 1 + 1" cyclocondensation.[12]

But according to a graduate student's not to laughable reaction except H_3PO_2, we fortunately were able to synthesize hexahydrotriazine, not 1,5,3,7-diazadiphosphocine-1,5-dicarboxylic acid. Here we report the synthesis of hexahydrotriazine derivatives from the assembly of three molecules of various amines[aniline, 4-ethylaniline, 6-methylpyridin-2-amine, 3-aminoquinoline, 2-furfurylamine, 2-(aminomethyl)-thiophene, thiazol-2-amine, benzylamine, 3-picolylamine, 1-(3-aminopropyl)pyrrolidin-2-one,[13] 3-hydrazino-1,2-benzisothiazole 1,1-dioxide,[14] L-cysteine,[15] glucosamine[16]] and three molecules of formaldehyde by "1 + 1 + 1 + 1 + 1 + 1" cyclocondensation originated from six single different molecules. The synthesis mechanism is probably passes through imines, which trimerizes to give the hexahydrotriazine ring. In recent decades, ultrasound has been used more and more frequently in organic synthesis. The use of ultrasound in organic transformations is well known because it can enhance the reaction rate and can alter selectivity performance of the reaction.[17–19] Comparing with traditional methods under conventional condition, this method is more convenient and easily controlled.[20,21] It can also felicitates reactions at ambient conditions that otherwise require higher temperature, pressure, or concentrations; to the best of our knowledge, the use of ultrasound has been extended to the synthesis of hexahydrotriazine derivatives, but they did not get enhanced yield for the reaction.[22]

Experimental

Chemical Experimental. All chemicals were purchased from commercial suppliers and used without further purification unless otherwise stated. All solvents used in the reaction were freshly distilled from a suitable dehydrating agent under nitrogen. All solvents used in the chromatography

were purchased and used without further purification. ^{1}H NMR spectra were recorded on Varian Mercury 400 MHz Fourier Transform Nuclear Magnetic Resonance (Palo alto, CA, USA) and 100 MHz for ^{13}C, with the chemical shift (δ) reported in parts per million (ppm) downfield relative to tetra-methyl silane and the coupling constants (*J*) quoted in Hz. Chloroform-d and dimethyl sulfoxide-d_6 were used as a solvent and an internal standard. Mass spectra were recorded with an HP 5890 (Hewlett Packard, Palo Alto, CA, USA) GC/Mass (70 eV, EI). Melting points were measured on a MEL-TEMP II apparatus (Triad Scientific, Manasquan, NJ, USA) and were uncorrected. Elemental analyses were measured on a Thermo Scientific Flash 2000 (Thermo Scientific, Waltham, MA, USA), Thin-layer chromatography (TLC) was performed on DC-Plastikfolien 60, F254 (layer thickness 0.2 mm; Merck, Darmstadt, Germany) plastic-backed silica gel plates and visualized by UV light (254 nm). Chromatographic purification was carried out using Kieselgel 60 (60–120 mesh, Merck).

General procedure for the synthesis of 1,3,5-hexahydrotriazine derivatives. Amine (0.03 mol) was dissolved in 25 mL of ethanol in a 100 mL one necked round-bottomed flask. Formaldehyde (0.11 mL, 0.03 mol), trimethylamine (0.041 mL, 0.03 mol) as a catalyst were added in mixture. The temperature of condensation reaction is the reflux temperature of ethanol, the solvent of the reaction. After mixing for 7 h, the reaction mixture was cooling in room temperature, observed by TLC to the point of completion. After recrystallization of the mixture with ethanol, the residual solution is removed by a vacuum rotary evaporator. The products were purified by column chromatography to give the compound **1**–**13**.

1,3,5-Triphenyl-1,3,5-triazine 1**.** Yield: 56.5%; mp: 150∼151 °C; Rf: 0.64 (TLC eluent; n-Hexane: E.A = 5:3); Mass (70 eV), *m/z* (rel. int.%): 315.17 (100), 316.18 (22.7), 317.18 (2.5); ^{1}H NMR (Chloroform-d, 400 MHz): δ 7.21 (dd, *J* = 7.4 Hz and 3.4 Hz, 6H), 7.02 (d, *J* = 7.4 Hz, 6H), 6.86 (t, *J* = 7.4 Hz, 3H), 4.89 (s,6H); ^{13}C NMR (Chloroform-d, 100 MHz): δ 148.45, 129.08, 120.032, 116.980, 67.13; Anal. Calcd. for $C_{21}H_{21}N_3$: C, 79.97; H, 6.71; N, 13.32; Found: C, 80.01; H, 6.72; N,13.27.

1,3,5-Tribenzyl-1,3,5-triazine 2**.** Yield: 60.5%; mp: 159∼160 °C; Rf: 0.58 (TLC eluent; n-Hexane: E.A = 5:1); Mass (70 eV), *m/z* (rel. int.%): 402.55(100), 403.26(26.0), 404.26(3.2); ^{1}H NMR (Chloroform-d, 400 MHz): δ 7.05 (d, *J* = 7.3 Hz, 6H), 6.95 (d, *J* = 8.8 Hz, 6H), 4.78 (s, 6H), 4.89 (s, 6H), 2.51–2.57 (m, 6H), 1.15–1.19 (m, 9H); ^{13}C NMR (Chloroform-d, 100 MHz): δ 150.43, 138.25, 130.42, 115.56, 82.12, 24.42, 15.52; Anal. Calcd. for $C_{27}H_{33}N_3$: C, 81.16; H, 8.32; N, 10.52; Found: C, 81.13; H, 8.34; N, 10.53.

1,3,5-Tris(6-methylpyridin-2-yl)-1,3,5-triazine 3**.** Yield: 60.2%; mp: 121∼122 °C; Rf: 0.58 (TLC eluent; n-Hexane: E.A = 5:4); Mass (70 eV), *m/z* (rel. int.%): 360.21 (100), 361.22 (22.5), 362.21 (3.4); ^{1}H NMR (dimethyl sulfoxide-d_6, 400 MHz): δ 7.86 (dd, *J* = 7.2 Hz and 3.2 Hz, 3H), 6.86 (d, *J* = 7.4 Hz, 3H), 6.48 (d, *J* = 7.4 Hz, 3H), 5.56 (s, 6H), 2.32 (s, 9H); ^{13}C NMR (dimethyl sulfoxide-d_6, 100 MHz): δ 158.45, 155.23, 138.32, 118.32, 101.21, 82.11, 24.22; Anal. Calcd. for $C_{21}H_{24}N_6$: C, 69.97; H, 6.71; N, 23.31; Found: C, 69.96; H, 6.73; N, 23.30.

1,3,5-Tri(quinolin-3-yl)-1,3,5-triazine 4**.** Yield: 66.4%; mp: 196∼197 °C; Rf: 0.52 (TLC eluent; n-Hexane: E.A = 3:5); Mass (70 eV), *m/z* (rel. int.%): 468.21 (100), 469.21 (32.4), 470.21 (2.7); ^{1}H NMR (dimethyl sulfoxide-d_6, 400 MHz): δ 8.50 (s, 3H), 8.32 (d, *J* = 8.0 Hz, 3H), 7.65 (d, *J* = 7.4 Hz, 3H), 7.32–7.43 (m, 6H), 7.11 (s, 3H), 4.76 (s, 6H); ^{13}C NMR (dimethyl sulfoxide-d_6, 100 MHz): δ 149.12, 148.32, 145.42, 133.22, 128.98, 125.32, 88.42; Anal. Calcd. for $C_{30}H_{24}N_6$: C, 76.90; H, 5.16; N, 17.94; Found: C, 76.88; H, 5.19; N, 17.93.

1,3,5-Tris(furan-2-ylmethyl)-1,3,5-triazine 5**.** Yield: 66.8%; mp: sticky oil; Rf: 0.52 (TLC eluent; n-Hexane: E.A = 5:3); Mass (70 eV), *m/z* (rel. int.%): 327.16 (100), 328.16 (19.5), 329.17 (1.8); ^{1}H NMR (dimethyl sulfoxide-d_6, 400 MHz): δ 7.56 (d, *J* = 7.3 Hz, 3H), 6.35 (dd, *J* = 7.2 Hz and 3.2 Hz, 3H), 6.17 (d, *J* = 7.4 Hz, 3H), 3.80 (s, 6H), 3.62 (s, 6H); ^{13}C NMR (dimethyl sulfoxide-d_6, 100 MHz): δ 148.22, 140.02, 110.04, 772.22, 50.42; Anal. Calcd. for $C_{18}H_{21}N_3O_3$: C, 66.04; H, 6.47; N, 12.84; O, 14.66; Found: C, 66.01; H, 6.50; N, 12.82; O, 14.68.

1,3,5-Tris(thiophen-2-ylmethyl)-1,3,5-triazine 6**.** Yield: 65.6%; mp: sticky oil; Rf: 0.61 (TLC eluent; n-Hexane: E.A = 5:3); Mass (70 eV), *m/z* (rel. int.%): 375.09 (100), 376.09 (20.5), 377.07 (13.6); ^{1}H NMR (dimethyl sulfoxide-d_6, 400 MHz): δ 7.36 (d, *J* = 7.3 Hz, 3H), 6.94–6.89 (m, 6H), 4.03 (s, 6H), 3.85 (s, 6H); ^{13}C NMR (dimethyl sulfoxide-d_6, 100 MHz): δ 140.22, 127.22, 125.55, 77.22, 50.12; Anal. Calcd. for $C_{18}H_{21}N_3S_3$: C, 57.57; H, 5.64; N, 11.19; S, 25.61; Found: C, 57.58; H, 5.62; N, 11.20; S, 25.60.

1,3,5-Tri(thiazol-2-yl)-1,3,5-triazine 7**.** Yield: 72.3%; mp: 161∼162 °C; Rf: 0.54 (TLC eluent; n-Hexane: E.A = 5:3); Mass (70 eV), *m/z* (rel. int.%): 336.03 (100), 338.02 (13.6), 337.03 (13.0); ^{1}H NMR (dimethyl sulfoxide-d_6, 400 MHz): δ 7.05 (d, *J* = 7.4 Hz, 3H), 6.63 (d, *J* = 7.2 Hz, 3H), 3.3 2 (s, 6H); ^{13}C NMR (dimethyl sulfoxide-d_6, 100 MHz): δ 155.02, 137.01, 112.38, 83.13; Anal. Calcd. for $C_{12}H_{12}N_6S_3$: C, 42.84; H, 3.60; N, 24.98; S, 28.58; Found: C, 42.82; H, 3.62; N, 24.97; S, 28.59.

1,3,5-Tribenzyl-1,3,5-triazine 8**.** Yield: 66.5%; mp: sticky oil; Rf: 0.52 (TLC eluent; n-Hexane: E.A = 5:1); Mass (70 eV), *m/z* (rel. int.%): 357.22 (100), 358.22 (26.0), 359.23 (2.7); ^{1}H NMR (Chloroform-d, 400 MHz): δ 7.30–7.20 (m, 15H), 3.65 (s, 6H), 2.16 (s, 6H); ^{13}C NMR (Chloroform-d, 100 MHz): δ 140.32, 130.22, 125.21, 80.22, 58.21; Anal. Calcd. for $C_{24}H_{27}N_3$: C, 80.63; H, 7.61; N, 11.75; Found: C, 80.61; H, 7.63; N, 11.75.

1,3,5-Tris(pyridin-3-ylmethyl)-1,3,5-triazine 9**.** Yield: 72.6%; mp: sticky oil; Rf: 0.54 (TLC eluent; MeOH = 1);

Mass (70 eV), *m/z* (rel. int.%): 360.21 (100), 361.21 (22.7), 362.21 (2.5); ^{1}H NMR (Chloroform-d, 400 MHz): δ 8.50 (s, 3H), 8.47 (d, *J* = 7.2 Hz, 3H), 7.62 (d, *J* = 7.4 Hz, 3H), 7.21 (dd, *J* = 7.2 Hz and 3.4 Hz, 3H), 3.64 (s, 6H), 2.16(s, 6H); ^{13}C NMR (Chloroform-d, 100 MHz): δ 152.12, 150.32, 148.45, 140.12, 124.03, 80.12, 58.21; Anal. Calcd. for $C_{21}H_{24}N_6$: C, 69.97; H, 6.71; N, 23.32; Found C, 69.96; H, 6.73; N, 23.31.

1,1′,1″-((1,3,5-Triazine-1,3,5-triyl)tris(propane-3,1-diyl)) tris(pyrrolidin-2-one) 10**.** Yield: 30.5%; mp: sticky oil; Rf: 0.53 (TLC eluent; MeOH: E.A = 3:5); Mass (70 eV), *m/z* (rel. int.%): 462.33 (100), 463.34 (26.0), 464.34 (2.7); ^{1}H NMR (dimethyl sulfoxide-d_6, 400 MHz): δ 5.75 (s, 6H), 3.18–3.38 (m, 18H), 1.89–2.21 (m, 18H); ^{13}C NMR (dimethyl sulfoxide-d_6, 100 MHz): δ 158.45, 80.32, 55.45, 52.81, 50.22, 28.52, 25.52, 18.43; Anal. Calcd. for $C_{24}H_{42}N_6O_3$: C, 62.31; H, 9.15; N, 18.17; O, 10.37; Found: C, 62.28; H, 9.18; N, 18.15; O, 10.39.

3,3′,3″-((1,3,5-Triazine-1,3,5-triyl)tris(azanediyl))tris (benzo[d]isothiazole 1,1-dioxide) 11**.** Yield: 10.5%; mp: sticky oil; Rf: 0.53 (TLC eluent; n-Hexane: E.A = 5:3); Mass (70 eV), *m/z* (rel. int.%): 633.12 (100), 634.13 (26.3), 635.12 (12.7); ^{1}H NMR (dimethyl sulfoxide-d_6, 400 MHz): δ 7.32–7.35 (m, 7H), 7.22–7.26 (m, 11H), 5.05 (s, 6H); ^{13}C NMR (dimethyl sulfoxide-d_6, 100 MHz): δ 182.77, 137.34, 128.45, 128.41, 127.14, 127.11, 127.05, 75.10; Anal. Calcd. for $C_{24}H_{21}N_9O_6S_3$: C, 45.93; H, 3.37; N, 20.07; O, 15.29; S, 15.31; Found: C, 45.93; H, 3.38; N, 20.06; O, 15.30; S, 15.30.

(2S,2′S,2″S)-2,2′,2″-(1,3,5-triazine-1,3,5-triyl)tris (3-mercaptopropanoic acid) 12**.** Yield: 20.2%; mp: sticky oil; Rf: 0.43 (TLC eluent; MeOH: E.A = 5:2); Mass (70 eV), *m/z* (rel. int.%): 399.06 (100), 401.06 (13.3), 400.06 (12.9); ^{1}H NMR (dimethyl sulfoxide-d_6, 400 MHz): δ 4.64–4.79 (m, 7H), 4.28 (d, *J* = 7.4 Hz, 6H), 3.29–3.40 m, 18H); ^{13}C NMR (dimethyl sulfoxide-d_6, 100 MHz): δ 169.78, 62.30, 48.45, 32.07; Anal. Calcd. for $C_{12}H_{21}N_3O_6S_3$: C, 36.08; H, 5.30; N, 10.52; O, 24.02; S, 24.08; Found: C, 36.06; H, 5.32; N, 10.52; O, 24.03; S, 24.07.

1,3,5-Triglucosyl-1,3,5-triazine 13**.** Yield: 30.5%; mp: sticky oil; Rf: 0.53 (TLC eluent; MeOH: E.A = 3:5); Mass (70 eV), *m/z* (rel. int.%): 573.24 (100), 574.24 (22.7), 575.24 (3.1); H NMR (dimethyl sulfoxide-d_6, 400 MHz): δ 4.95 (d, *J* = 7.4 Hz, 5H), 4.82 (s, 6H), 3.32–3.62 (m, 15H), 3.06–3.17 (m, 6H); ^{13}C NMR (dimethyl sulfoxide-d_6, 100 MHz): δ 92.67, 89.08, 76.12, 71.96, 71.53, 69.63, 69.53, 60.25, 56.67, 54.23, 46.63; Anal. Calcd. for $C_{21}H_{39}N_3O_{15}$: C, 43.98; H, 6.85; N, 7.33; O, 41.84; Found: C, 43.95; H, 6.88; N, 7.35; O, 41.82.

General procedure for the synthesis of 1,3,5-hexahydrotriazine derivatives by ultrasound assisted method. A mixture of amine (0.03 mol) and formaldehyde (0.11 mL, 0.03 mol) was dissolved in 25 mL of ethanol in a 100 mL one necked round-bottomed flask introduced into sonicator. The reaction mixture was stirred with gentle refluxing for 30 min. The temperature of condensation reaction is the reflux temperature of ethanol, the solvent of the reaction. After completion of reaction (as monitored by TLC), reaction solvent was removed by vacuum rotary evaporator. The reaction mixture was purified by column chromatography to give the compound **1**, **5**–**7**. We tried different reactions other than the four suggested reactions, but they did not proceed and we no longer performed different reactions under different reaction conditions. The result of these reactions was not satisfied and shown in Table 1.

MTT assay. Cytotoxicity is the effect of being toxic to cells caused by toxic agent. Cytotoxicity activity was monitored using 3-(4,5-dimethyl-2-thiazolil)-2, 5-diphenyl-2H-tetrazolium bromide (MTT). Cancer cells (T98G) and Nuff (human newborn foreskin fibroblast) were purchased from the Korean Cell Line Bank on March 14, 2015, and cytotoxicity tests were conducted in the Department of Genetics, Dong-A University School of Medicine. Cell cytotoxicity of the compounds were adjusted by MTT. First, compounds were dissolved in dimethyl sulfoxide. Then, compounds were dissolved in the free-medium immediately before treatment with the cells, followed by 70% sonication, and the material was filtered through a 0.45 μM filter (Millipore, Billerica, MA, USA). All cells were seeded into 96-well plates at a density of 1×10^4 cells/well prior to treatment. Next day, medium was replaced with complement medium containing various concentration compounds at 37 °C in a humidified atmosphere of CO_2 incubator. After 24 h, media replaced the 100 μL of thiazolyl blue tetrazolium solution (Sigma Chemicals, St. Louis, MO, USA) at 5 mg/mL in 1X phosphate buffered saline and cells incubated for 4 h at 37 °C. Thiazolyl blue tetrazolium–formazan was dissolved in 100 μL dimethyl sulfoxide, and the absorbance at 550 nm was adjusted using a microplate reader. The cell viability (%) was calculated as (O.D.$_{550}$ of treated cells/O.D.$_{550}$ of nontreated cells) × 100.

First, when the synthesized compound **1**–**9** was administered to normal cells (Nuff) at 25 and 50 μM concentrations and analyzed by MTT analysis, there was almost no toxicity to normal cells. (Figure 1) So then, the synthesized compound **1**–**9** were administered to Cancer cell (human

Table 1. Yields of 1,3,5-triazacyclohexanes by sonochemistry.

Compounds	Yield (%)[a]	Compounds	Yield (%)[a]	Compounds	Yield (%)[a]
1	46.5	**6**	55.8	**11**	-
2	-[b]	**7**	52.6	**12**	-
3	-	**8**	-	**13**	-
4	-	**9**	-		
5	46.2	**10**	-		

[a] Isolated yield.
[b] No reaction; after reaction, TLC was unable to determine the product's spot.

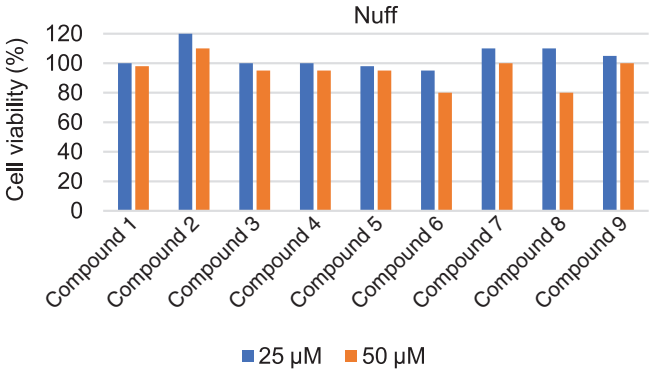


Figure 1. Cytotoxic activity of the 1,3,5-triazacyclohexane derivative compounds against Normal cell line at various concentration.

glioblastoma, T98G) and analyzed by MTT assay Experimental results shown in Figure 2 and Table 2. Human glioblastoma (T98G) showed the following activities. At a low concentration of 12.5 μM, compound **5**,**6** exhibited greater than 80% cytotoxicity, compound **2** showed a low cytotoxicity of 30.8%, Also the same concentrates, the values for T98G were 60.3% for compound **1**, 58.1% for compound **3**, 74.5% for compound **4**, 53.3% for compound **7**, 64.9% for compound **8**, 79.7% for compound **9**. At a concentration of 25 μM, compound **4**,**5**,**6**,**8**,**9** exhibited greater than 80% cytotoxicity, 83.0% for compound **4**, 94.4% for compound **5**, 89.0% for compound **6**, 80.2% for compound **8**, 87.7% for compound **9**. Compound **2** showed a low cytotoxicity as well. Compound **2** showed of 36.0%. Also the same concentrates, the values were 61.4% for compound **1**, 67.7% for compound **3**, 57.0% for compound **7**. At a concentration of 50 μM cytotoxicity graphs look like 25 μM, the values were 64.5% for compound **1**, 45.1% for compound **2**, 68.5% for compound **3**, 94.6% for compound **4**, 94.3% for compound **5**, 89.2% for compound **6**, 60.0% for compound **7**, 84.1% for compound **8**, and 90.4% for compound **9**. In particular, at a concentration of 100 μM, shows that greatest cytotoxicity is the compound **9** (94.6%), followed by compound **4**,**5** (94.1%). Also the same concentrates, the values were 75.7% for compound **1**, 55.9% for compound **2**, 79.8% for compound **3**, 90.1% for compound

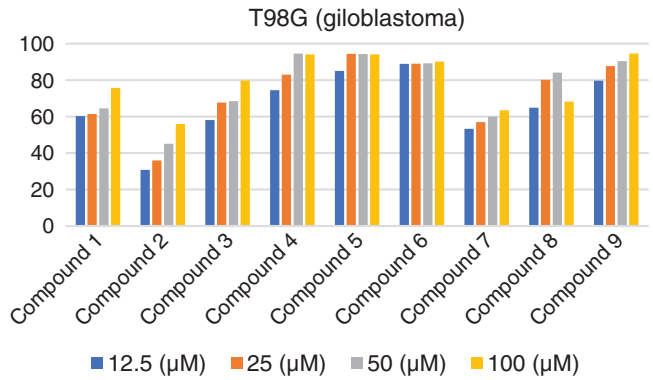


Figure 2. Cytotoxic activity of the 1,3,5-triazacyclohexane derivative compounds against T98G cell line at various concentration.

Table 2. Summary of cytotoxic activity of the compounds against T98G cell line at various concentrations.

	Cytotoxic activity (%)			
	Concentration (μM)			
Compounds	12.5 μM	25 μM	50 μM	100 μM
1	60.3	61.4	64.5	75.7
2	30.8	36.0	45.1	55.9
3	58.1	67.7	68.5	79.8
4	74.5	83.0	94.6	94.1
5	85.0	94.4	94.3	94.1
6	88.8	89.0	89.2	90.1
7	53.3	57.0	60.0	63.5
8	64.9	80.2	84.1	68.2
9	79.7	87.7	90.4	94.6

6, 63.5% for compound **7**, 68.2% for compound **8**. For the IC_{50} values, compound **1** was 6.30 μM, compound **2** was 67.61 μM, compound **3** was 7.08 μM, compound **4** was 5.12 μM, compound **7** was 7.59 μM, compound **9** was 2.82 μM, and compound **5**, **6**, **8**'s IC_{50} values could not be unpredictable (Table 3; Supporting Information).

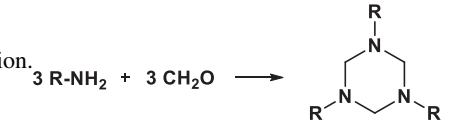
Results and Discussion

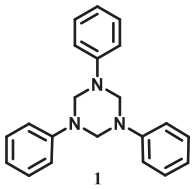
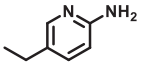
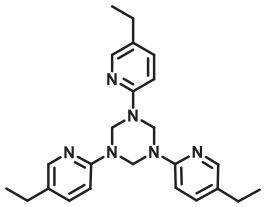
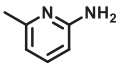
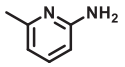
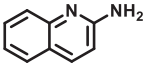
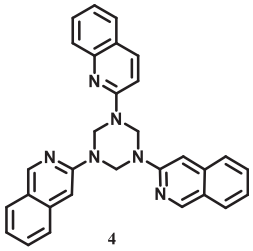
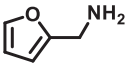
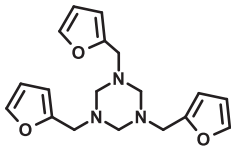
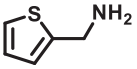
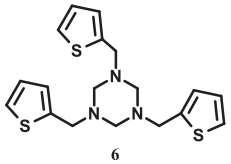
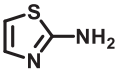
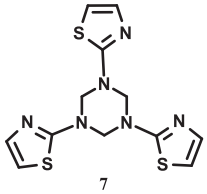
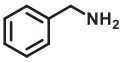
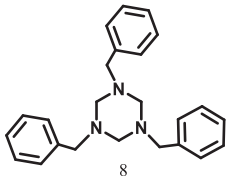
The 1,3,5-hexahydrotriazines are the subject of several structure studies considering their use in the industrial and pharmacological chemistry. The symmetrically substituted triazines product number **1**–**13**, were synthesized from the condensation reaction of various amines and formaldehyde. These compounds are stable at room temperature and adequate yield with a trans parent color. This heterocyclic compound results from the assembly of three molecules of amine and three molecules of formaldehyde; its striking feature is that each atom of this six-membered ring is originated from six single different molecules, representing a formal 1 + 1 + 1 + 1 + 1 + 1; cyclocondensation. The obtained yield is satisfactory in spite of the number of essential steps involved in the significant transformation and of the ring size, usually unfavorable for entropic

Table 3. IC_{50} of the 1,3,5-triazacyclohexane derivative compounds against T98G cell line at various concentration.

Compounds	Cell lines	IC_{50} values (μM)[a]
1	T98G	6.30
2		67.61
3		7.08
4		5.12
5		>100
6		>100
7		7.59
8		>100
9		2.82

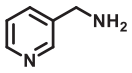
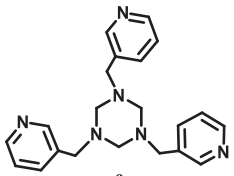
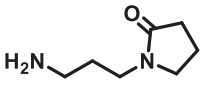
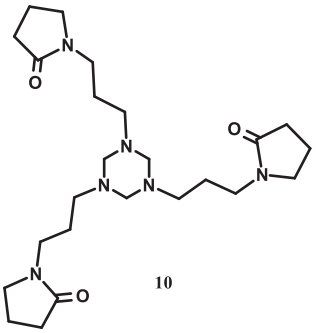
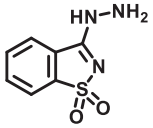
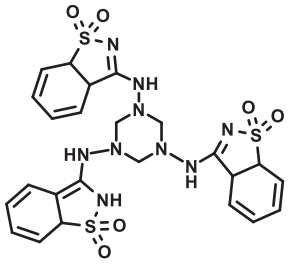
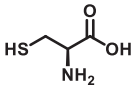
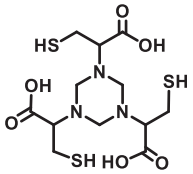
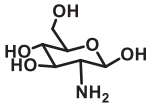
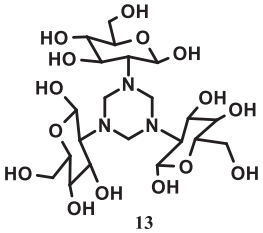
[a] $p < 0.0001$.

Table 4. Structures and yields of 1,3,5-triazacyclohexanes by thermal reaction. 

Compounds	Amines	Structures	Yield (%)[a]
1		 1	56.5
2		 2	60.5
3			60.2
4		 4	66.4
5		 5	66.8
6		 6	65.6
7		 7	72.3
8		 8	66.5 [24]

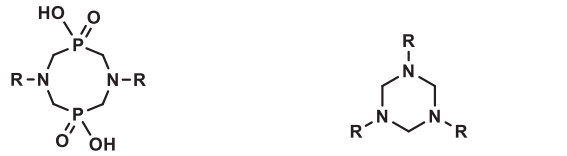
(*continued overleaf*)

Table 4 (*continued*)

Compounds	Amines	Structures	Yield (%)[a]
9		 **9**	72.6 [25]
10		 **10**	30.5
11		 **11**	10.5
12		 **12**	20.2
13		 **13**	30.5

[a] Isolated yield.

reasons. The reaction mechanism about formation of hexahydrotriazines is that the first reaction step begins with the lone-pair electron of amine attacking the carbon of formaldehyde. Subsequently, through a series of reversible reaction processes, intermediate imine is formed. It can then be inferred from the reaction steps of amine, imine, and formaldehyde. Finally, hexahydrotriazine as a final product is produced. In fact, hexahydrotriazines synthesized by amines with various functional groups are expected to have high biological activity. Evidence that hexahydrotriazines were synthesized is as follows (Table 4). In this study, various amines were used to react with formaldehyde and have physiological activity. In bioactivity, to investigate the effect of compounds in T98G, the cells were treated with designated concentrations of compounds for 24 h. The cytotoxicity of compound **1**–**9** was measured by MTT assays (Figure 2, Table 2). As a result, compound **1**–**9** reduced the viability of T98G cells in a concentration-dependent manner. At a concentration of 100 μM, compound **9** exhibited the greatest cytotoxicity at 94.6%. The reason is that compound **9** is C_5H_5N that has a resonance hybrid structure and exhibits aromaticity. It is considered to be active because it



Scheme 1. Structures of 1,5,3,7-diazadiphosphosines[23] and hexahydrotriazines (**1**–**13**)

has a structure that is a raw material for pyridine derivatives as mainly medicines and pesticides. Synthetic compound **3** has pyridine ring directly attached to the N atoms of the triazine ring, but product compound **9** is a form where the pyridine rings can be freely rotated by methylene group as you can see. With this structural difference, there is a clear difference in cytotoxicity activity. To solve this problem, we will demonstrate this effect through a structure-activity relationship in the near future. We expressed the aforementioned sentence based on this thought. Although it cannot be accurately expressed, the high cytotoxicity activity (94.6%) of product 9 can be described as the role of the N atom of pyridine attached to a freely rotating methylene group. Synthesis of hexahydrotriazine derivatives was performed by means of the effect of ultrasound in forming acoustic cavitation in reaction solution and resulting in the initiation or enhancement of the chemical activity in the reaction solution. The result of these reactions was not satisfied and shown in Table 1. Ethanol is not a suitable solvent for this condensation reaction. However, the results of the first reaction of sonochemistry were not good, so we stopped it, and we performed cyclocondensation with ethanol, which melts the starting material well, as a solvent (Scheme 1).

Conclusion

We think that there is an immense need for new compounds with new mode of drug action, for treatment of human disease. In conclusion, hexahydrotriazine derivatives (**1**–**13**) were obtained in 10–72% yields by using a one-pot reaction of various amines and formaldehyde. The preparation is very easy, the molecule being assembled in one single reaction from readily available and cheap starting materials. The most outstanding result is that compound **9** exhibited the greatest cytotoxic activity (94.6%) at a concentration of 100 μM. Generally highly active pesticides and pharmaceuticals have pyridine ring, compound **9** with pyridine ring has a resonance hybrid structure and exhibits aromaticity. The need for new pharmaceutical continues to be a still standing challenge.

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Supporting Information. Additional supporting information may be found online in the Supporting Information section at the end of the article.

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