Synthesis of New Derivatives of 1,3,5-Hexahydro-s-triazine as Potential Anticancer Agents

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Abstract□A series of hexahydro-s-triazine derivative were prepared. Cytotoxic activity testing using mouse leukemia cells (p   388) and antimicrobial testing indicated that the compounds which contain isoxazolyl moiety exhibited higher activity than those containing other heterocyclic moieties.

S-Triazine derivatives are reported to show a broad spectrum of biological activity in particular, antimicrobial[1-3] and antitumor[4-7] activities. It is also reported in the literature that some alkyl substituted 1,3,5-hexahydro-s-triazine were prepared as stabilizers for natural ruber latex foam[8], epoxy resin hardness and anticorrosives[9], and few examples of saturated-s-triazines were reported to possess biological properties[10,11]. These observations prompted the synthesis of elsewhere unreported series of substituted 1,3,5-hexahydro-s-triazines derivatives for the purpose of studying their anticancer and antimicrobial activity.

Thus, the target compounds were prepared by one-step reaction of three equivalents of formaldehyde and equivalents of appropriate alkyl or aryl amines in refluxing methanol (Scheme 1). The physical properties and yields are given in Table I.

The structural assignments of the synthesized compounds were determined on the basis of elemental analysis, infrared spectra, ^1H and ^{13}C nmr spectra, and high resolution mass spectrometry.

Attempts to react 2-aminopyrimidine similarly to give 1,3,5-tripyrimidinyl-1,3,5-hexahydro-s-tri-

Scheme 1

Scheme 2   9

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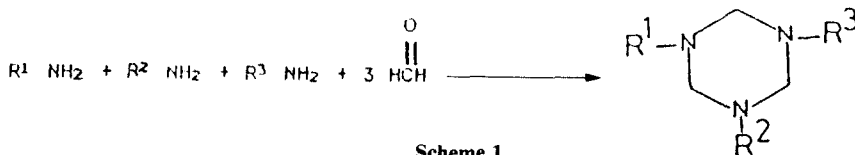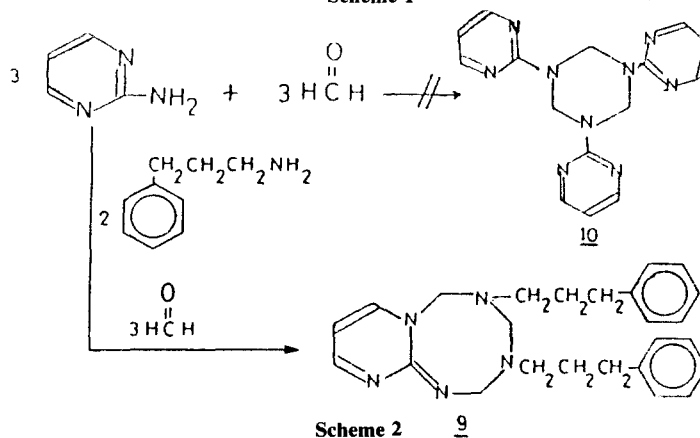
Table I. Analytical data of hexahydro-s-triazine derivatives

Compound	M.P. (°C)	Yield %	Formula	Analysis %	
				Found	Calculated
1	153-154	80	$C_{18}H_{21}N_5O_2$	C 64.27	63.70
				H 6.48	6.24
				N 20.42	20.64
2	103-105	87	$C_{17}H_{19}N_5OS$	C 59.79	59.80
				H 5.32	5.60
				N 20.53	20.51
3	120-122	43	$C_{15}H_{23}N_5O_2$	C 58.49	59.00
				H 7.35	7.59
				N 22.41	22.94
4	68-69	85	$C_{14}H_{21}N_5OS$	C 55.08	54.70
				H 6.81	6.89
				N 23.18	22.78
5*	95-96	56	$C_{24}H_{30}N_3Cl_3$	C 61.33	61.74
				H 6.34	6.48
				N 8.51	9.00
6*	78-80	54	$C_{15}H_{36}N_3Cl_3$	C 48.91	49.38
				H 10.32	9.95
				N 11.07	11.52
7	148-150	65	$C_{16}H_{17}N_5S_2$	C 56.12	55.95
				H 5.09	5.00
				N 20.97	20.40
8	154-155	48	$C_{13}H_{19}N_5S_2$	C 50.42	50.46
				H 6.20	6.19
				N 22.60	22.63
9	62-64	79	$C_{25}H_{31}N_5$	C 74.80	74.76
				H 7.91	7.72
				N 17.50	17.44

*(Reported b.p. 150-160)[12].
**(Reported b.p. 120-126 °C at 15 mmHg)[13].

azine (**10**) was unsuccessful. However, upon treatment of one mole of 2-aminopyrimidine with two mole of phenyl propylamine and three mole of formaldehyde resulted in compound **9** (Scheme 2).

The 100 MHz ^{1}H nmr spectrum of compound **9** in CDCl$_3$ at 25 °C exhibited a sharp singlet at δ 3.65 indicating that an equivalent methylene protons at C-4. Another sharp singlet at δ 4.71 which integrated for four protons indicating the equivalent methylene protons at C-2 and C-6. In addition to the expected protons signals. The structure of compound **9** was also confirmed by high resolution mass spectroscopy and elemental analysis (See EX-

PERIMENTAL).

RESULTS AND DISCUSSION

The ID$_{50}$ values for *in vitro* cytotoxicity of some hexahydro-s-triazine derivatives are listed in Table II. The reliability of the inhibition of growth of tumor cells was demonstrated by the ID$_{50}$ of the most widely used 1-(2-chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea (MeCCNU).

It appears from Table II that the higher activity is obtained when R$_1$, R$_2$ are isoxazolyl and R$_3$ is benzyl moieties. However, when R$_1$, R$_2$ and R$_3$ are

Table II. Cytotoxic Activity of some hexahydro-s-triazine derivatives

Compounds	R_1	R_2	R_3	ID_{50} mmol / l	Relative effectiveness to MeCCNU
MeCCNU	—	—	—	6.67×10^{-3}	1
1			-CH₂Ph	7.48×10^{-4}	8.92 Fold
2			-CH₂Ph	3.16×10^{-3}	2.11 Fold
3			-CH₂CH(CH₃)CH₃	4.21×10^{-3}	1.58 Fold
4			-CH₂CH(CH₃)CH₃	7.93×10^{-3}	0.84 Fold
5	-CH₂Ph	-CH₂Ph	-CH₂Ph	1×10^{-2}	0.66 Fold
6	-CH₂CH(CH₃)CH₃	-CH₂CH(CH₃)CH₃	-CH₂CH(CH₃)CH₃	3.75×10^{-2}	0.18 Fold
7			-CH₂Ph	4.46×10^{-2}	0.15 Fold
8			-CH₂CH(CH₃)CH₃	6.67×10^{-2}	0.10

benzyl moieties the activity was sharply lowered. Replacement of the benzyl group in compound 1 by isobutyl group as in compound 3 results in decrease of the activity to about 6 folds. Displacement of one isoxazolyl group with a thiazoly group as in compound 2 gave low activity when compared with that of compound 1.

Compounds having R_1, R_2, R_3 as isobutyl groups exhibited some potency, if isoxazolyl moiety is present, while the introduction of heterocycle moiety other than isoxazolyl such as two thiazolyl as in compound **8** resulted in suppression of the cyto-

toxic activity. These results reflect the importance of the heterocyclic isoxazolyl moiety in the 1,3,5-hexahydro-s-triazine compounds. The structural similarity of the synthesized triazine derivatives to that of hexamethylmelamine (HMM)[14] and cycloguanil[15] suggested that these compounds may act via dihydrofolate reductase inhibition. These results of anticancer activity are adequate to encourage us to expand testing and to further extending the present work.

The *in vitro* antibacterial activities of the compounds against different bacterial strains are sum-

Table III. MICs of compound 1-8

Compounds	S. aureus	B. subtilis	E. coli	Pr. vulgaris	Ps. aeruginosa	C. albicans
1	75	75	100	50	200	—
2	75	100	100	50	200	—
3	100	100	75	80	200	—
4	75	75	75	75	200	—
5	200	200	200	200	300	—
6	200	200	200	100	300	—
7	100	100	100	50	200	—
8	150	150	150	150	200	—

marized in Table III. It is noteworthy that the replacement of the alkyl or aryl substitution by heterocyclic ring cause an enhancement in activity against all bacteria tested. Compounds 1, 2, 3 and 4 show the highest activity against all tested bacteria and this may be attributed to the presence of the isoxazole ring. All the tested compounds have no activity against Candida albicans.

In conclusion, 1,3,5-hexahydro-s-triazine derivatives were proved to exhibit cytotoxic activity and the result reflect the importance of substituted heterocyclic isoxazolyl moiety rather than other substituents. Also, these compounds showed to have some antimicrobial activity against the tested organisms.

EXPERIMENTAL

Section I: Chemistry

All chemicals were purchased from Fluka AG (Switzerland). Melting points were determined using Mettler FP5 melting point apparatus and are uncorrected. Elemental analyses were done with Perkin-Elmer 240 B elemental analyser. Infrared spectra (KBr) of the compound were obtained from Perkin-Elmer 580 B spectrometer. NMR spectra were obtained from Varian FT 80A NMR spectrometer. The TLC was carried out on precoated silica gel F254 chromatographic plastic sheets. The solvent system consisted of ethyl ether/petroleum ether (3:1).

1,3-bis[3-(5-methylisoxazolyl)]-5-benzyl-1,3,5-hexahydro-s-triazine (1)

The procedure given for the synthesis of compound 1 is the general procedure for the synthesis of 1-9.

To a suspension of 3-amino-5-methylisoxazole

(2.0 mg, 0.02 mol) and benzylamine (1.1 mg, 0.01 mole) in methanol, a solution of formaldehyde 40% (5 ml) was added. The reaction mixture was then heated to reflux for 5 hr. After evaporation the methanol, the solid residue was dissolved in 200 ml of ethyl ether and washed with water three times. The ethereal solution was then dried with anhydrous Na_2SO_4 and then evaporated. The residue was recrystallized from ethylether/pet. ether to give (2.7 gm, 80%) of 1 as colorless solid m.p. 153-154 °C. IR (KBr): 1625 cm^{-1}, ^{1}H NMR (CDCl$_3$) δ: 2.43 (s, 6H, 2CH$_3$), 4.03 (s, 2H, CH$_2$-Ph), 4.52 (s, 4H, methylene protons at C-4 and C-6). 5.03 (s, 2H, methylene protons at C-2), 5.75 (s, 2H, CH), 7.42 (s, 5H, aromatic).

1-[3-(5-methylisoxazolyl)]-3-(2-thiazolyl)-5-benzyl-1,3,5-hexahydro-s-triazine (2)

IR (KBr): 1615 cm^{-1}, ^{1}H NMR (CDCl$_3$) δ: 2.30 (s, 3H, CH$_3$), 3.88 (s, 2H, CH$_2$-Ph), 4.42 (s, 2H, methylene protons at C-6), 4.59 (s, 2H, methylene protons at C-4), 4.63 (s, 2H, methylene protons at C-2), 5.10 (s, 1H, isoxazolyl CH), 6.61 (d, 1H, thiazolyl CH), 7.20 (d, CH, thiazolyl CH), 7.28 (s, 5H, aromatic).

1,3-bis[3-(5-methylisoxazolyl)-S-isobutyl-1,2,3-hexahydro-s-triazine (3)

IR (KBr): 1618 cm^{-1}, ^{1}H NMR (CDCl$_3$) δ: 0.88 (d, 6H, CH$_3$), 1.60 (m, 1H, CH), 2.29 (s, 6H, CH$_3$), 2.49 (d, 2H, CH$_2$), 4.35 (s, 4H, CH$_2$), 5.72 (s, 2H, isoxazolyl CH).

1[3-(5-methylisoxazolyl)]-3-(2-thiazolyl)-5-isobutyl-1,3,5-hexahydro-s-triazine (4)

IR (KBr): 1618 cm^{-1}, ^{1}H NMR (CDCl$_3$) : 0.86 (d, 6H, CH$_3$), 1.65 (m, 1H, CH), 2.30 (s, 3H, CH$_3$), 2.47 (d, 2H, CH$_2$), 4.43 (s, 2H, methylene protons

at C-6), 4.58 (s, 2H, methylene protons C-4), 5.04 (s, 2H, methylene protons C-2), 5.77 (s, 1H, CH, isoxazolyl), 6.62 (d, 1H, CH thiazolyl), 7.17 (s, 1H, CH thiazolyl).

1,3-bis(2-thiazolyl)-S-benzyl-1,3,5-hexahydro-s-triazine (7)

IR (KBr): 1620 cm^{-1}, ^{1}H NMR δ: 3.82 (s, 2H, CH$_2$), 4.60 (s, 4H, methylene protons at C$_4$ and C$_6$), 5.28 (s, 2H, methylene protons at C$_2$), 6.58 (d, 2H, CH), 7.20 (d, 2H, CH), 7.27 (s, 5H, aromatic).

1,3-bis(2-thiazolyl)-5-isobutyl-1,3,5-hexahydro-s-triazine (8)

IR (KBr): 1510 cm^{-1}, ^{1}H NMR (CDCl$_3$) δ: 0.89 (d, 6H, CH$_3$), 1.65 (m, 1H, CH), 2.49 (d, 2H, CH$_2$), 4.67 (s, 4H, methylene protons at C-4 and C-6), 5.28 (s, 2H, methylene protons at C-2), 6.64 (d, 2H, CH thiazolyl), 7.23 (d, 2H, CH thiazolyl).

3,5-Diphenylpropyl-2,4,6-hexahydropyrimido[1,2-a]-1,3,5-tetraazocine (9)

Prepared in a similar manner to that for **1** and obtained as a colorless crystals mp. 62-64 °C (yield 62%). IR (KBr): 1615 cm^{-1}, ^{1}H NMR δ: 1.85 (m, 4H, -CH$_2$), 2.50 (t, 4H, CH$_2$-N), 2.62 (t, 4H, -CH$_2$-Ph), 3.65 (s, 2H, CH$_2$), 4.71 (s, 4H, CH$_2$), 6.5 (t, 1H, CH-pyrimidine), 7.20 (s, 10H, aromatics), 8.29 (d, 2H, CH-pyrimidine). Anal. calcd for C$_{25}$H$_{31}$N$_5$; C, 74.76; H, 7.72; N, 17.44; Found: C, 74.80; H, 7.91; N, 17.50. M$^+$ 401.2571 (C$_{25}$H$_{31}$N$_5$ requires M$^+$, 401.2579).

Section II: In Vitro Cytotoxic Determination

Mouse leukemia cells (P388) were seeded at 3 × 10^5 cell/m*l* in duplicate for each drug concentration in plastic microtiter plate. The synthesized triazine derivatives were added to the cell cultures in 1:10 dilution and incubated for 2 days at 37 °C under a humidified atmosphere. After 48 h of continuous drug exposure, the cells were harvested and counted using a Coulter Counter, Model B (Coulter Electronics Hialeach, FL). A control untreated set of cultures and four duplicate sets of MeCCNU treated cells were included for each separate experiment. A dose-response curve was drawn from the data obtained and ID$_{50}$ was calculated. The ID$_{50}$ defined as the concentration of inhibitor that inhibits the growth of the leukemia cell to 50% of the growth of the control cells.

Section III: Organisms and Media

The test organisms used in this study were stan-dard strains collected from National Collection of Type Culture, Public Health Laboratory, London, Englnd. However, *Candida albicans* standard strain was provided by Mycological Reference Laboratory, School of Tropical Medicine and Hygien London, England.

The antimicrobial activity and minimum inhibitory concentration (MICs) were determined by agar dilution method[15]. Antibiotic Assay Agar No. 1 (oxoid) was used for both growth inhibition and the determination of the minimum inhibitory concentration.

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