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Synthesis, structure analysis, and antitumor activity of 3,6-disubstituted-1,4-dihydro-1,2,4,5-tetrazine derivatives

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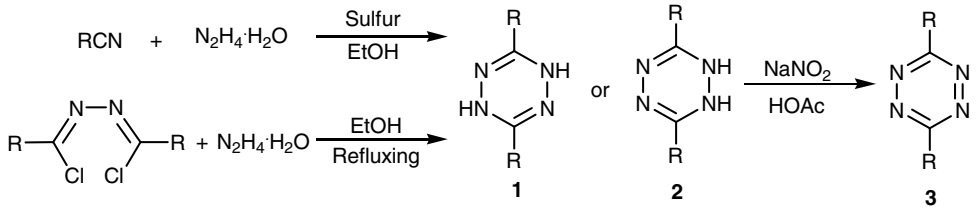
Abstract—Fourteen compounds of 3,6-disubstituted-1,4-dihydro-1,2,4,5-tetrazine derivatives were prepared and their structures were confirmed by single-crystal X-ray diffraction and the semi-empirical calculation of PM3 method. This reaction yields the 1,4-dihydro derivatives rather than the 1,2-dihydro derivatives. The central six-membered ring of 1,4-dihydro-1,2,4,5-tetrazine has a chair conformation and therefore is not homoaromatic. Their antitumor activities were evaluated in vitro by SRB method for A-549 and BEL-7402 cells, and MTT method for P-388 and HL-60 cells. The results show that there is one compound which is highly effective against P-388 cells and one compound which is highly effective against HL-60 cells. So it is a kind of compound which possesses potential antitumor activities and is worth to research further.

1,2,4,5-Tetrazine derivatives have a high potential for biological activity, possessing a wide range of antiviral and antitumor properties, and these derivatives have been widely used in pesticides and herbicides.[1] 1,2,4,5-Tetramethyl-3,6-bis(phenylethynyl)-1,2,4,5-tetrazine has been suggested as an antitumor agent.[2] Although no data about antitumor activities were reported, it was the first indication that this kind of compound may possess potential antitumor activity. We are interested in whether changing the structure is possible to improve the antitumor activity or not.

Dihydro-1,2,4,5-tetrazine has four isomers, namely 1,2-, 1,4-, 1,6-, and 3,6-dihydro-1,2,4,5-tetrazine. There still seems to be much confusion over the structures of 1,2- and 1,4-dihydro-1,2,4,5-tetrazines, and the same compound is often formulated as both structures. For example, the CAS number of both 3,6-diphenyl-1,2-dihydro-1,2,4,5-tetrazine and 3,6-diphenyl-1,4-dihydro-1,2,4,5-tetrazine is 14478-73-0.[6a,b] In most cases, the dihydro structure which would be the first reaction product is presented, or authors have formulated their compounds in the dihydro structure which seemed to be the most accepted at that time.[1a] Most scientists[3] believe that the dihydro structure is 1,2-dihydro-1,2,4,5-tetrazine.

Fourteen compounds[4] of 3,6-disubstitutedihydro-1,2,4,5-tetrazines (**1** or **2**) and 3,6-disubstituted-1,2,4,5-tetrazines (**3**) were prepared. The route of synthesis is shown in Scheme 1. The results are summarized in Table 1. However, IR, ^{1}H NMR, and MS studies failed to prove whether the hydrogen or the nitrogen is located



Scheme 1. Route of synthesis.

Keywords: 1,4-Dihydro-1,2,4,5-tetrazine; Structure analysis; Antitumor activity; Chair conformation.
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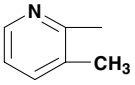
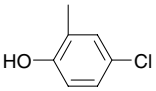
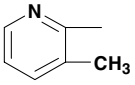
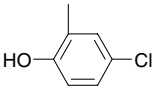
at the 4 or 2 positions (compounds **1** or **2**). Their structures were confirmed by single-crystal X-ray diffraction and the semi-empirical calculations using the PM3 method.

Table 1. Synthesis of compounds (**1**) and (**3**)

Compound	R	Yield (%)	Mp (°C)
1a	Ph	83.2	190–192[3b]
1b	*p*-CF_3Ph	85.5	213–216
1c	*p*-ClPh	71.1	250–252[5]
1d	*p*-$ClC_6H_4CH_2$	32.0	193–194
1e		50.3	211–213
1f	*o*-ClPh	54.4	208–210[6]
1g		35.1	250 (d)
3a	Ph	98.8	195–196[7]
3b	*p*-CF_3Ph	99.5	186–188[8]
3c	*p*-ClPh	87.4	228–230[9]
3d	*p*-$ClC_6H_4CH_2$	91.2	131–132
3e		47.2	179–180
3f	*o*-ClPh	51.8	178–180[6]
3g		33.2	239–241

The single-crystal structure of **1d** was determined by X-ray crystallography.[10] The molecular structure of **1d** is illustrated in Figure 1. In **1d**, the N(2a)═C(1a)[1.272 (3) Å] bonds correspond to typical C═N double bonds, and the N(1a)–N(2a_3)[1.502 (4) Å], N(2a)–N(1a_3) [1.502 (4) Å], and N(1a)–C(1a)[1.422 (4) Å] bond lengths correspond to typical single bonds. Therefore, the tetrazine ring is the 1,4-dihydro structure, the compound being 3,6-bis(4-chlorobenzyl)-1,4-dihydro-1,2,4,5-tetraz ine (**1d**), rather than the 3,6-bis(4-chlorobenzyl)-1,2-dihy dro-1,2,4,5-tetrazine (**2d**). So the products (**1** or **2**) have the 1,4-dihydro structure rather than the 1,2-dihydro structure.

The energy of compounds **1a**, **2a**, **1d**, and **2d** has been calculated by the semi-empirical calculation of PM3 method in Gaussian 98 procedure.[11] The results show that the value of Hartree–Fock of compound **1a** (HF = 0.2003401 a.u.) and **1d** (HF = 0.1676137 a.u.) is lower than that of **2a** (HF = 0.2125709 a.u.) and **2d** (HF = 0.1814932 a.u.), respectively. So the structures of compounds **1a** and **1d** are more stabilized than those of **2a** and **2d**.

Homoaromatic structures have been demonstrated by X-ray diffraction for the 1,6-dihydro structures.[12] There still seems to be some doubt as to whether the 1,4-dihydro structures have homoaromaticity.[13] In **1d**, the atoms

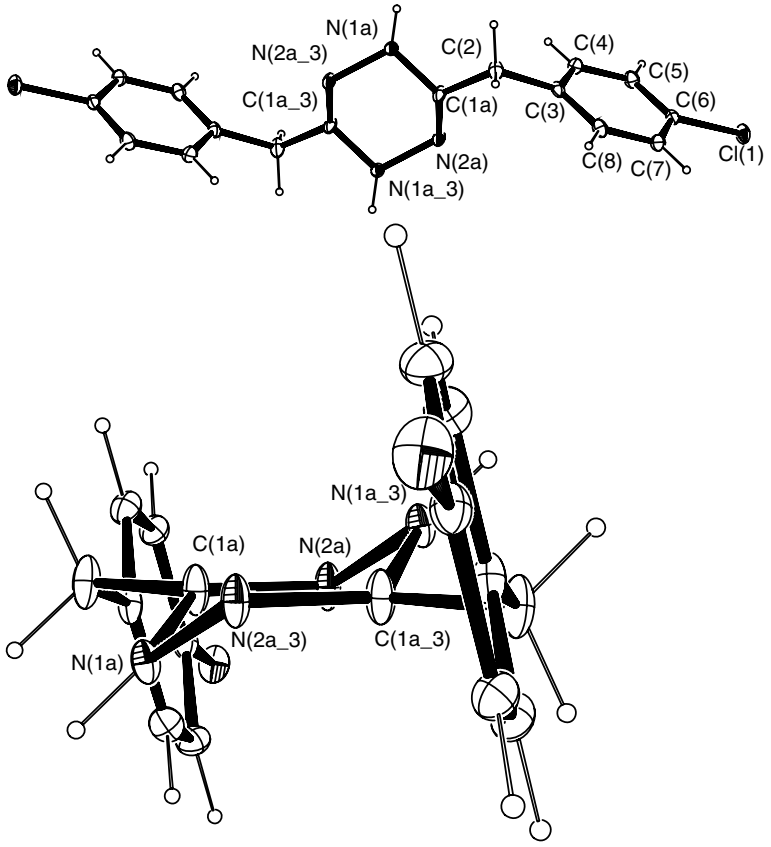


Figure 1. X-ray structure of **1d**.

C(1a), N(2a), C(1a_3) and N(2a_3) are coplanar, and the adjacent N(1a_3) and N(1a) atoms deviate from the plane by 0.5798(63) Å and −0.5798(63) Å, respectively. The dihedral angle between C(1a), N(2a), C(1a_3), N(2a_3) plane and N(1a), C(1a), N(2a_3) plane or N(1a_3), C(1a_3), N(2a) plane is 43.03 (34)°. The

Table 2. The inhibition ratio for A-549 growth

Compound	Concentration (mol/L)				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1a	55.8	67.2	5.4	10.2	0.0
1b	70.3	22.0	0.0	0.0	0.0
1c	73.4	2.0	4.7	0.0	7.1
1d	64.5	17.8	3.0	10.1	7.8
1e	2.6	3.6	0.0	0.0	0.0
1g	65.3	80.3	0.0	0.0	0.0
3a	60.4	9.5	20.3	27.1	29.7
3b	51.5	44.4	33.0	19.5	9.7
3c	52.3	35.1	29.0	19.4	16.1
3d	58.4	29.1	9.3	8.9	0.0
3e	93.5	9.8	0.0	0.0	0.0
3f	8.2	2.8	5.6	14.6	12.6
3g	7.6	62.3	16.4	0.0	0.0

Table 3. The inhibition ratio for P-388 growth

Compound	Concentration (mol/L)				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1a	87.6	69.0	0.0	0.0	0.0
1b	47.7	36.9	15.2	13.8	9.1
1c	65.9	51.0	36.0	19.4	4.9
1d	48.8	15.5	4.4	2.9	0.0
1e	0.0	0.0	0.0	0.0	0.0
1g	89.0	0.0	0.0	0.0	0.0
3a	15.5	0.0	0.0	0.0	0.0
3b	40.2	2.5	0.0	0.0	0.0
3c	0.0	0.0	0.0	0.0	0.0
3d	65.8	57.1	13.1	1.1	8.7
3e	34.9	0.0	0.0	0.0	0.0
3f	0.0	0.0	0.0	0.0	0.0
3g	**92.5**	**90.7**	**91.2**	**88.0**	**82.8**

Table 4. The inhibition ratio for HL-60 growth

Compound	Concentration (mol/L)				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1a	82.1	16.1	2.9	2.6	17.2
1e	96.3	13.6	2.9	2.6	17.2
3a	35.1	16.4	13.9	13.9	0.0
3b	43.7	7.0	5.8	1.4	0.0
3c	66.7	12.2	0.0	4.0	0.0
3d	85.4	22.3	13.5	21.1	7.6
3e	**94.3**	**96.6**	**7.0**	**9.1**	**4.2**

Table 5. The inhibition ratio for BEL-7402 growth

Compound	Concentration (mol/L)				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1a	54.0	14.3	0.0	0.0	0.0
1e	94.9	8.3	0.0	0.0	0.0
3a	41.7	7.4	0.0	0.0	0.0
3b	21.3	12.6	0.0	0.0	0.0
3c	27.4	0.1	0.0	0.0	3.4
3d	61.2	0.0	0.0	0.0	6.0
3e	93.0	23.8	0.0	0.0	2.8

central six-membered ring of **1d**, the tetrazine ring, has an obvious chair conformation and therefore is not homoaromatic.

The antitumor activities in vitro for these compounds were evaluated by SRB method for A-549 and BEL-7402 cells, and MTT method for P-388 and HL-60 cells. The results are summarized in Tables 2–5.

Usually, when the concentration of the compound solution is 10^{-6} mol/L, the inhibition ratio of the solution to cancer cell growth is more than 50%, or when the concentration of the compound solution is 10^{-5} mol/L, the inhibition ratio of the solution to cancer cell growth is more than 85%, the compound is considered as strongly effective. According to this standard, it can be found from Tables 2–5 that there is one compound (**3g**) that has a very strong effect against to P-388 cells. The inhibition ratio of the solution to cancer cell growth is more than 80% in 10^{-8} mol/L. And there is one compound (**3e**) that has a strong affect against HL-60 cells.

The different group of 3,6-positions has prodigious diversity of antitumor activity. Changing the group of 3,6-positions is possible to improve the antitumor activity. So 1,4-dihydro-1,2,4,5-tetrazine is a kind of compound which may have potential antitumor activities. It is a good lead compound that warrants further investigation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.04.066.

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4. Synthesis of **1f** or **1 g**: azine (10 mmol) was dissolved in ethanol (20 ml) with stirring. Hydrated hydrazine (12 mmol, 80%) was added to the mixture in reflux conditions. The mixture was refluented for 45 min, cooled, filtered, and washed with ethanol (3× 10 ml). Solvent was removed in vacuo and the residue was recrystallized from ethanol or chloroform to give the product as a yellow solid. Synthesis of **1d**: (4-Chloro-phenyl)-acetonitrile (50 mmol) and sulfur (1.0 g) were dissolved in anhydrous ethanol (15 ml) with stirring in nitrogen. Hydrated hydrazine (10 ml, 80%) was added to the mixture in an ice bath. The mixture was stirred at room temperature for 2 h and then reflux conditions for 1–2 h, cooled, filtered, and washed with anhydrous ethanol (3 × 10 ml). Solvent was removed in vacuo and the residue was recrystallized from ethanol or chloroform to give the product (**1d**) as a colorless solid. IR (KBr, cm^{-1}): 3446, 3267, 3182, 1678, 1491, 1442, 1417, 1235, 1209, 1083, 1014, 978, 932, 834, 741, 659, 586, 499, 429. ^{1}H NMR (400 MHz, $CDCl_3$)δ: 3.48 (s, 4H, CH_2), 6.13 (br, 2H, NH), 7.17–7.32 (m, 8H, Ph). MS *m/z* (%): 333 (M^+, 11), 334 (27), 332 (40), 127 (31), 125 (100), 116 (16), 90 (13), 89 (40), 63 (15). Anal. Calcd for $C_{16}H_{14}N_4Cl_2$: C, 57.67; H, 4.23; N, 16.81. Found: C, 57.82; H, 4.17; N, 16.79.Compounds of **1a**–**e** were prepared as described above for **1d**. Synthesis of **3d**: compound **1d** (10 mmol) was suspended in a 10% solution of $NaNO_2$ (25 ml) with stirring. Ether (15 ml) was added to the mixture, a 10% solution of CH_3COOH (15 ml) was dropped to the mixture. The mixture was stirred at room temperature for 5 h, filtered and the residue was recrystallized from ethanol to give the product (**3d**) as a red solid. IR (KBr, cm^{-1}): 3050, 1607, 1553, 1491, 1378, 1088, 1014, 852, 785. ^{1}H NMR (400 MHz, $CDCl_3$)δ: 4.57 (s, 4 H, CH_2), 7.30–7.35 (m, 8H, Ph). MS *m/z* (%): 331 (M^+, 0.73), 151 (34), 116 (100), 89 (27), 50 (8). Anal. Calcd for $C_{16}H_{12}N_4Cl_2$: C, 58.02; H, 3.65; N, 16.92. Found: C, 58.34; H, 3.61; N, 16.73. Compounds of **3a**–**e** were prepared as described above for **3d**.
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10. Crystal data of **1d**. $C_8H_7ClN_2$, M = 166.61, Monoclinic, a = 5.833 (1), b = 5.131 (3), c = 26.250 (6) Å, β = 93.05 (2)°, U = 784.5 (5) Å^3, T = 293 (2) K, space group P 21/n, Z = 4, D_c = 1.411 g/cm^3, μ(Mo-K$_\alpha$) = 0.415 mm^{-1}, 3798 reflections measured, 3599 unique (R_{int} = 0.0413) which were used in all calculations. Fine R_1 = 0.0437, wR (F^2) = 0.1421 (all data). Full crystallographic details of **1d** have been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC **255695**.
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