Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3174–3176

Synthesis, X-ray crystallographic analysis, and antitumor activity of 1-acyl-3,6-disubstituted phenyl-1,4-dihydro-1,2,4,5-tetrazines

Guo-Wu Rao and Wei-Xiao Hu*

College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou 310014, People's Republic of China

Received 7 January 2005; revised 15 March 2005; accepted 22 March 2005

Abstract—Eleven compounds of 1-acyl-3,6-disubstituted phenyl-1,4-dihydro-1,2,4,5-tetrazines were prepared from 3,6-disubstituted phenyl-1,2-dihydro-1,2,4,5-tetrazines and anhydrides or acyl chlorines, 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 these compounds has an obvious boat conformation and therefore is not homoaromatic. Their antitumor activities were evaluated in vitro by the SRB method for A-549 cell and the MTT method for P-388 cells. The results show that there is one compound which is highly effective against A-549 cells and two compounds which are highly effective against P-388 cells. Thus, this compound possesses potential antitumor activities and is worth researching 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 have been reported, this 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.

Eleven compounds† of 1-acyl-3,6-disubstituted phenyl-1,4-dihydro-1,2,4,5-tetrazines (**2**) were prepared from 3,6-disubstituted phenyl-1,2-dihydro-1,2,4,5-tetrazines (**1**) and anhydrides or acyl chlorines.[3] 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 of the nitrogen is located at the 4 or 2 position (compound **2** or **3**). Their structures were confirmed by single crystal X-ray diffraction and the semi-empirical calculations using the PM3 method.

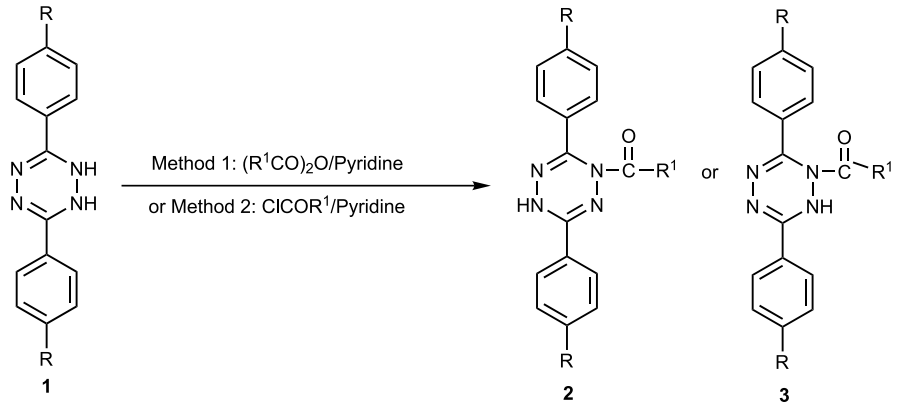
The energy of compounds **2a** and **3a** has been calculated by the semi-empirical calculation of PM3 method in Gaussian 98 procedure.[4] The results show that the Hartree–Fock value of compound **2a** (HF = 0.1475494 a.u.) is lower than that of **3a** (HF = 0.1604914 a.u.). So the structure of compound **2a** is more stable than that of **3a**.

The single-crystal structure of **2f** was determined by X-ray crystallography.[5] The molecular structure of **2f** is illustrated in Figure 1. In **2f**, the N2 = C3 [1.279 (3) Å] and N5 = C6 [1.268 (3) Å] bonds correspond to typical C=N double bonds, and the C6–N1 [1.420 (3) Å], N1–N2 [1.430 (3) Å], C3–N4 [1.367 (3) Å] and N4–N5 [1.391 (3) Å] bond lengths correspond to typical single bonds. Therefore, the tetrazine ring is the 1,4-dihydro structure with the N-substituent group at the 1-position and the N-hydrogen at the 4- and not the 2-position, the compound being 1-isobutyryl-3,6-bis(4-trifluoromethylphenyl)-1,4-dihydro-1,2,4,5-tetrazine (**2f**), rather than the 1,2-dihydro structure (**3f**).

So the products of the reaction of **1** and anhydrides or acyl chlorines have the 1,4-dihydro structure rather

Keywords: 1,2,4,5-Tetrazine; X-ray diffraction; Calculation; Boat conformation; Antitumor.
* Corresponding author. Tel.: +86 571 88320557; fax: +86 571 88320557/85133199; e-mail: huyang@mail.hz.zj.cn
† All new compounds were characterized by IR, ^{1}H NMR, MS, and elemental analysis.

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.03.122



Scheme 1. Route of synthesis.

Table 1. Synthesis of compound (**2**)

Compd.	R	R^1	Yield (%)	Time (h)	Mp (°C)
2a	H	Me	84.9	24	164–165
2b	H	Et	76.6	22	145(d)
2c	H	*i*-Pr	46.3	38	195–196
2d	CF_3	Me	66.7	26	225–227
2e	CF_3	Et	38.6	65	204–205
2f	CF_3	*i*-Pr	30.5	48	207–208
2g	Cl	Me	63.6	14	201–202
2h	Cl	Et	67.2	17	185–186
2i	Cl	*i*-Pr	33.5	22	199–201
2j	H	$ClCH_2$	11.3	25	151–153
2k	CF_3	$ClCH_2$	10.4	63	179–181

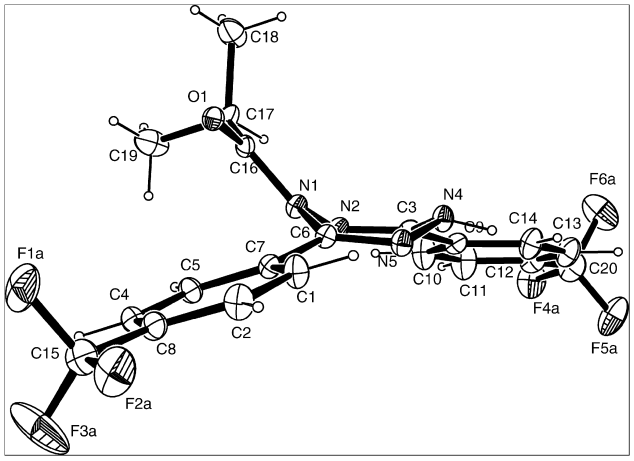
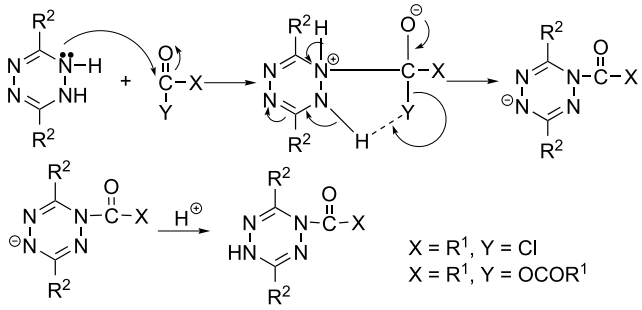


Figure 1. X-Ray structure of **2f**.



Scheme 2. The mechanism of the reaction.

than the 1,2-dihydro structure. The mechanism of the reaction is illustrated in Scheme 2 but warrants further investigation.

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. Homoaromatic structures have been demonstrated by X-ray diffraction for the 1,6-dihydro structures.[6] There still seems to be some doubt whether the 1,4-dihydro structures have homoaromaticity.[7] In **2f**, the atoms N2, C3, N5, and C6 are coplanar, deviations are less than ± 0.0234 (12) Å, and the adjacent N1 and N4 atoms deviate from the plane by 0.4227 (34) Å and 0.3348 (37) Å, respectively. The dihedral angle between N2, C3, N5, C6 plane and N1, N2, C6 plane is 33.87 (27)°, and between N2, C3, N5, C6 plane and C3, N4, N5 plane is 28.15 (15)°. The difference in the dihedral angles is presumably due to a steric effect of the substituent at the 1-position. The central six-membered ring of **2f**, the tetrazine ring, has an obvious boat conformation and therefore is not homoaromatic.

The antitumor activities in vitro for these compounds were evaluated by SRB method for A-549 cell and MTT method for P-388 cell. The results are summarized in Tables 2 and 3.

Table 2. The inhibition ratio for A-549 growth

Compd.	Concentration (mol/L)				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
2a	85.1	0.0	0.0	0.0	0.0
2b	71.5	11.1	6.2	12.3	0.0
2c	83.8	46.6	38.7	29.6	29.5
2d	91.1	63.6	0.0	0.0	0.0
2e	84.7	48.1	21.7	0.0	9.0
2f	79.8	21.0	0.0	0.0	0.0
2j	95.4	95.8	35.3	4.1	13.0
2k	95.5	64.6	0.0	0.0	0.0

Table 3. The inhibition ratio for P-388 growth

Compd.	Concentration (mol/L)				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
2a	62.7	10.9	6.6	5.5	9.2
2b	30.9	9.8	7.6	7.7	8.5
2c	82.5	61.1	52.0	24.9	46.9
2d	50.6	21.5	1.1	5.0	0.0
2e	68.0	21.1	6.7	6.3	0.0
2f	42.2	26.9	15.0	16.2	13.4
2j	99.1	100	3.9	3.6	0.0
2k	98.4	49.4	2.2	11.3	0.0

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 strongly effective. According to this standard, it can be found from Tables 2 and 3 that there is one compound (**2j**) that has a strong affection to A-549 cell and two compounds (**2c** and **2j**) that have strong affection to P-388 cells. So 1-acyl-3,6-disubstituted phenyl-1,4-dihydro-1,2,4,5-tetrazine may have potential antitumor activities. It is a good lead compound that warrants further investigation.

Acknowledgments

The authors thank the National Natural Science Foundation of China (20272053) for financial support and National Center for Drug Screening, Shanghai, China, for evaluation of antitumor activities.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.03.122.

References and notes

1. (a) Neunhoeffer, H. In *Comprehensive Heterocyclic Chemistry, I*; Katritzky, A. R., Ed.; Pergamon: Frankfurt, 1984; Vol. 3, p 531; (b) Sauer, J. In *Comprehensive Heterocyclic Chemistry, II*; Boulton, A. J., Ed.; Elsevier: Oxford, England, 1996; Vol. 6, p 901.
2. (a) Eremeev, A. V.; Tikhomirov, D. A.; Tyusheva, V. A.; Liepins, F. *Khim. Geterotsikl. Soedin.* **1978**, 753(b) Eremeev, A. V.; Tikhomirov, D. A.; Zidermane, A. U.S.S.R. 686,336, 1980.
3. Synthesis of **2f**: 3,6-bis(4-trifluoromethylphenyl)-1,2-dihydro-1,2,4,5-tetrazine (**1**) (5 mmol), prepared according to the procedure of Abdel-Rahman et al.,[8] was dissolved in dichloromethane (20 mL) with stirring. Isobutyric anhydride (10 mmol), pyridine (1 mL), and dichloromethane (20 mL) were added to the mixture in an ice bath. The mixture was stirred at room temperature for 48 h, washed with water, and dried with anhydrous $MgSO_4$. Solvent was removed in vacuo and the residue was recrystallized from ethanol to give the product (**2f**) as a yellow solid. IR (KBr, cm^{-1}): 3444, 3269, 2978, 1655, 1619, 1526, 1472, 1399, 1327, 1318, 1267, 1180, 1128, 1086, 1068, 1011, 991, 850, 701, 604. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.27 (d, $J = 6.9$ Hz, 6H, CH_3), 3.44 (m, $J = 6.9$ Hz, 1H, CH), 7.55–7.87 (m, 8H, Ph), 8.25 (br, 1H, NH). Ms *m/z* (%): 442 (M^+, 10), 444 (23), 443 (100), 373 (19), 372 (78), 145 (9), 43 (30), 41 (14). Anal. Calcd (%) for $C_{20}H_{16}N_4OF_6$: C, 54.30; H, 3.65; N, 12.67. Found: C, 54.19; H, 3.51; N, 12.78. Compounds **2a**–**k** were prepared as described above for **2f**.
4. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B., et al. *Gaussian* 98, Revision A. 7, Pittsburgh, PA: Gaussian Inc., 1998.
5. Crystal data of **2f**. $C_{20}H_{16}F_6N_4O$, $M = 442.37$, Monoclinic, $a = 33.258$ (4), $b = 7.355$ (2), $c = 20.166$ (4) Å, $\beta = 125.690$ (12)°, $U = 4006.4$ (14) $Å^3$, $T = 296$ (2) K, space group $C2/c$, $Z = 8$, $D_c = 1.467$ g/cm^3, μ (Mo-K_α) = 0.132 mm^{-1}, 3798 reflections measured, 3599 unique ($R_{int} = 0.0413$) which were used in all calculations. Fine $R_1 = 0.0410$, $wR(F^2) = 0.1467$ (all data). Full crystallographic details of **2f** have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 255699.
6. Jennison, C. P. R.; Mackay, D.; Watson, K. N.; Taylor, N. J. *J. Org. Chem.* **1986**, *51*, 3034.
7. (a) Neugebauer, F. A.; Krieger, C.; Fischer, H.; Siegel, R. *Chem. Ber.* **1983**, *116*, 2261; (b) Bemis, G. W.; Whittle, R. R.; Mayo, S. L. *Acta Crystallogr., Sect. C* **1984**, *40*, 2076.
8. Abdel-Rahman, M. O.; Kira, M. A.; Tolba, M. N. *Tetrahedron Lett.* **1968**, *35*, 3871.