Synthesis, X-ray crystallographic analysis and antitumor activity of 3,6-bis(substituted phenyl)-1,4-bis(substitutedphenylsulfonyl)-1,4-dihydro-1,2,4,5-tetrazine

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Seven compounds (**3a–g**) of 3,6-bis(substitutedphenyl)-1,4-bis(substitutedphenylsulfonyl)-1,4-dihydro-1,2,4,5-tetrazine were synthesised via reaction of *N*-arylsulfonyl-α-chloro-substituted phenyl hydrazone and triethyl amine. Their structures were confirmed by IR, ^{1}H NMR, MS, elemental analysis, and **3b** was confirmed by single-crystal X-ray diffraction. It was proved they are 1,4-dihydro-*s*-tetrazine derivatives. The central six-membered ring of **3b** has an obvious boat conformation. Their antitumor activities were evaluated *in vitro* by the SRB method for A-549 cell and the MTT method for P-388 cell. The results show that none of the compounds has good activities, but the substituents have a clear effect on their antitumor activity.

Keywords: crystal structure, antitumor activity, 1,4-dihydro-*s*-tetrazine

There are several reviews on the use of compounds containing the 1,2,4,5-tetrazine skeleton as pharmaceuticals.[1,2] For example, 3-amino-6-aryl-1,2,4,5-tetrazines showed modest antimalarial activity, some hexahydro-*s*-tetrazines have useful analgesic and antiflammatory activity. For a series of tetrahydro-*s*-tetrazines the antibacterial and antifungal activities have been evaluated.

Recently our research team found that some s-tetrazine derivatives have good antitumor activites, especially 1,4-dihydro-*s*-tetrazine-1,4-dicarboxamides.[3-7] To further investigate how the substituents located at 3,6 positions and 1,4 positions of 1,4-dihydro-*s*-tetrazine ring influence their antitumor activities, seven compounds were designed and synthesised.The chemical structures and synthetic route of the target compounds were shown as Fig 1.

When preparing **1a–g**, the literature method was modified with using aryl sulfonyl hydrazide reacting with aryl-carbonyl chloride instead of using aryl carbonyl hydrazide reacting with arylsulfonyl chloride.[8] When preparing **2a–g** from **1a–g**, the literature method was used with using $SOCl_2$ as chloridising agent.[8] Finally, the **3a–g** including five new compounds were prepared by using Et_3N as base to dehydrochlorination and cyclisation.[9,10] The mechanism of the reaction could be deduced as Fig.2. The preparation is summarised in Table 1.

Table 1 the preparation of **3a–g**

Entry	X	Y	m.p/°C Lit.	Yield/ %
3a	H	CH_3	157–159 (156–157)[9]	47.2
3b*	CH_3	CH_3	183–185	40.6
3c*	Cl	CH_3	180–181.5	22.4
3d	H	H	152–154 (151–156)[10]	40.0
3e*	CH_3	H	165–166	41.6
3f*	Cl	H	154–155	18.8
3g*	CH_3O	H	141–143	32.3

*New coupounds.

In addition to IR, NMR, MS, and EA, their structures were confirmed by X-ray crystallography of compound **3b**. (Fig.3). The crystal data of **3b** is summarised in Table 2. The molecular structure shows that two phenyl groups located at the 3,6-position of the *s*-tetrazine ring, and two phenyl sulfonyl groups were at the 1,4-positions. The bond lengths of C7 = N1and C8 = N3 are 1.249(10) Å and 1.265(10) Å respectively, which corresponds to a C=N double bonds; the bonds lengths of N1–N2 and N3–N4 are 1.462(8) Å and 1.399(9) Å, which corresponds to N–N single bond; and bond lengths of C7–N4

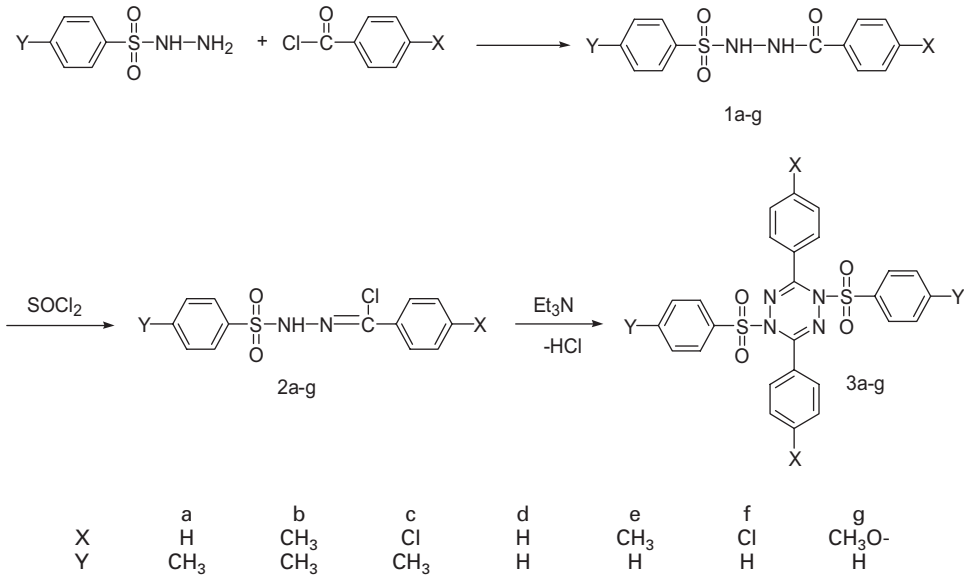

Fig. 1 The synthetic route of the target compounds and their chemical structures.

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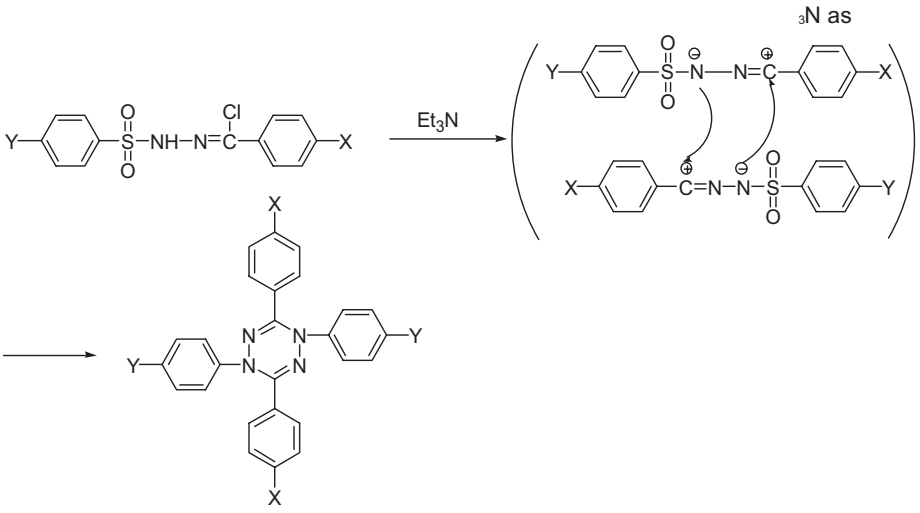

Fig. 2 The mechanism of the reaction.

Table 2 The crystal data of **3b**

Empirical formula	$C_{30}H_{28}N_4O_4S_2$
Formula weight	572.68
Temperature	295(2) K
Wavelength	0.71073Å
Crystal system, space group	Orthorhombic, P c a 21
Unit cell dimensions	*a* = 22.474(4)Å α = 90.000(17)°. *b* = 10.366(3)Å β = 90.000(12)°. *c* = 24.994(4)Å γ = 90.000(18)°.
Volume	5823(2) Å^3
Z, Calculated density	8, 1.307 Mg/m^3
Absorption coefficient	0.225 mm^{-1}
F(000)	2400
Crystal size	0.40 × 0.35 × 0.10 mm
Theta range for data collection	1.63 to 25.17°.
Index ranges	0< = *h*< = 26, 0< = *k*< = 12, –29< = *l*< = 1
Reflections collected/unique	5626/5626 [R_{int} = 0.0000]
Completeness to2 θ = 25.17	100.0 %
Max. and min. transmission	0.9779 and 0.9155
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parametres	5626/1/729
Goodness-of-fit on F^2	0.975
Final R indices [I>2 σ(I)]	R_1 = 0.0391, wR_2 = 0.0947
R indices (all data)	R_1 = 0.1909, wR_2 = 0.1417
Absolute structure parametre	0.16(15)
Extinction coefficient	0.00020(12)
Largest diff. peak and hole	0.248 and –0.216 e Å^{-3}

and C8–N2 are 1.389(10) Å and 1.406(10) Å respectively, which correspond C–N single bond. It therefore confirms that **3b** is a 1,4-dihydro-*s*-tetrazine derivative.

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.[11] There still seems to be some doubt whether the 1,4-dihydro structures have homoaromaticity.[12] In **3b** the atoms N1, C7, N3 and C8 are coplanar,

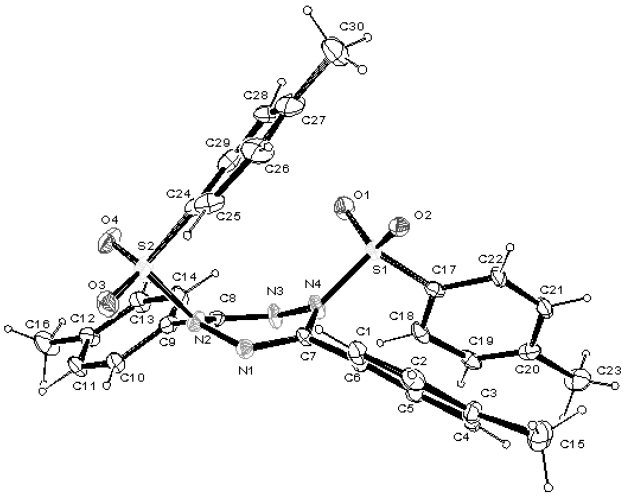

Fig. 3 Molecular structure of **3b**, shown with 30 % probability displacement ellipsoids.

deviations are less than ±0.0034Å, and the adjacent N2 and N4 atoms deviate from the plane by 0.2527 Å and 0.1244 Å, respectively. The central six-membered ring of **3b**, the tetrazine ring, has obvious boat confirmation and therefore is not homoaromatic.

Compounds **3a–g** were evaluated for their antitumour activity in vitro by method MTT for P-388 cell and SRB for A-549 cell. The results were summarised in Table 3. Usually, when the concentration of the compound solution is 10^{-6}mol/l, the inhibition rate of the solution to cancer cell growth is more than 50 %, the compound is considered as to be effective. According to this standard, it can be found from Table 3 that there is no one compound of them to effective to both

Table 3 inhibition of *in vitro* tumor cell growth by the compounds **3a–g**

Compd.	Rate of inhibition of p-388 C(tetrazine)/(mol/l)					Rate of inhibition of A-549 C(tetrazine)/(mol/l)				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
3a	0	0	2.4	0.1	0.1	70.0	4.5	15.7	7.1	3.9
3b	0	0	0	2.3	0	0	8.0	3.1	34.9	36.6
3c	2.2	0	0	14.9	0	75.1	21.6	0	13.4	24.8
3d	0	16.6	0	2.0	0.5	63.1	0	0	0	0
3e	22.3	6.9	0	11.9	0	47.1	0	0	0	0
3f	57.9	56.2	25.7	0	4.2	64.6	47.2	13.6	11.2	39.6
3g	37.8	14.9	0	2.8	3.5	63.3	15.6	3.0	0	0.4

P-388 and A-549 cell. But comparing the **3d–g**, when Y is same, their substituents X are H, CH_3, Cl and CH_3O–. It seems that electrowithdrawing group (Cl) could be favourable to their antitumour activity.

Crystallographic data for the structure reported in this paper has been deposited at the Cambridge Crystallographic Data Centre (CCDC No. 289461). Copies can be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 44 1223 336 033; e-mail deposit@ccdc.cam.ac.uk).

Experimental

Compounds **1a–g** were synthesised by modified literature method.[8] The synthesis of compounds **2a–g** were carried out as described in the literature.[8] Solvents and reagents were commercially available without further purification. X-ray single diffraction was carried out with an Enraf-Nonius CAD-4 diffractometer by the Analysis centre of Fu-Dan University. The data was collected and refined by CAD-4 EXPRESS and refined using SHELXS97 and SHELXL97. Melting points used an XRC-1 apparatus and are uncorrected. IR spectra were recorded from KBr discs on a Nicolex FI-IR-170 instrument. ^{1}H NMR spectra were run on a Bruker AC400 (400 MHz) spectrometer using TMS as internal standard and $CDCl_3$ as solvent. Mass spectra were obtained on a HP5989A spectrometer at an ionising voltage of 70ev by electron impact. Elemental analyses were performed on a Carlo ERBA-1106 instrument.

3,6-diphenyl-1,4-di(p-tolylsulfonyl)-1,4-dihydro-1,2,4,5-tetrazine (**3a**): Triethylamine (0.8 g, 7.8 mmol) and dried THF (48 ml) were mixed and cooled to –10 °C. The **2a** (*N*′-[chloro(phenyl)methylene] -4-methylbenzenesulfonohydrazide) (1.2 g, 3.9 mmol) in THF (12 ml) was added dropwise with stirring. Then the mixture was heated to 20–25 °C and kept stirring at this temperature for 12 h, using TLC (benzene/ethyl acetate = 8: 2) to follow the reaction. After reaction, the precipitate (triethylamine chloride) was filtrated off. The filtrate was concentrated to remove the solvent. To the residual was added absolute ethanol (6 ml) to give about 1 g of a yellow precipitate. The solid was filtered, and recrystallised from absolute ethanol twice to give 0.5 g product, yield 47.2 %. M.p.157–159 °C (Lit[9] 156–157 °C). IR (KBr, cm^{-1}) 1596 m (C=N), 1370 s (SO_2), 1335 s (ring), 1174 s(SO_2). ^{1}H NMR ($CDCl_3$ δ ppm): 7.75 (d, 4H, *J* = 8.0 Hz), 7.50 (d, 4H, *J* = 8.0 Hz), 7.35–7.42 (m, 10H, ArH), 2.48 (s, 6H). MS (*m/z*, %). 38 (M-155, 1.85), 155 (57.98) 105 (39.34), 91 (100), 77 (24.5), 65 (28.9).

3,6-ditolyl-1,4-di(p-tolylsulfonyl)-1,4-dihydro-1,2,4,5-tetrazine (**3b**): Following the method used for **3a** with triethylamine (1.4 g, 13.6 mmol) in 30 ml of THF and 2.5 g (7.8 mmol) of **2b** in 30 ml of THF and recrystallised from absolute ethanol/ethyl acetate twice to give 0.6 g (40.6 %) of **3b** as yellow crystal. M.p. 183–185 °C. IR (KBr, cm^{-1}): 1594 m (C=N), 1364 s (SO_2), 1326 s (ring), 1173 s (SO_2). ^{1}H NMR ($CDCl_3$ δ ppm): 7.76 (d, 4H, *J* = 8.4 Hz), 7.40 (d, 4H, *J* = 8.4 Hz), 7.35 (d, 4H, *J* = 8.0 Hz), 7.20 (d, 4H, J = 8.0 Hz), 2.47 (s, CH_3, 6H), 2.40 (s, CH_3, 6H). MS (*m/z*, %): 417 (M-155, 0.78), 155 (52.7), 119 (32.9), 91 (100), 65 (30.2). Anal. Calcd. for $C_{30}H_{28}N_4O_4S_2$ (572.71): C, 62.92; H, 4.93; N, 9.78.Found: C, 62.72; H, 4.90; N, 9.80.

3,6-Di (4-chlorophenyl)1,4-di(p-tolyl sulfonyl)1,4-dihydro-s-tetrazine (**3c**): Following the method used for **3a**, with triethylamine (1.6 g, 15.58 mmol) in 30 ml of THF, and 3.0 g (8.7 mmol) of **3c** in 30 ml THF and recrystallised with acetone/ethyl acetate twice to give 0.6 g (22.4 %) of **3c** as yellow crystal. M.p. 180–181.5 °C. IR (KBr, cm^{-1}): 1593 m (C=N), 1385 s (SO_2), 1322 m (ring), 1193 s (SO_2), 1092 s (Ar–Cl). ^{1}H NMR ($CDCl_3$ δ ppm): 7.74 (d, 4H, *J* = 8.4 Hz), 7.43 (d, 4H, *J* = 6.4 Hz), 7.40 (d, 4H, *J* = 8.4 Hz), 7.38 (d, 4H, *J* = 6.8 Hz), 2.48 (s, 6H). MS: *m/z* (%) 457 (M-155, 0.05), 155 (64.23), 137 (47.09), 91 (100), 77 (13.79), 65 (28.17). Anal. Calcd. for $C_{28}H_{22}Cl_2N_4O_4S_2$ (613.55): C, 54.81; H, 3.61; N, 9.13. found: C, 54.81; H, 3.21; N, 8.76.

3,6-diphenyl-1,4-di(phenylsulfonyl)-1,4-dihydro-1,2,4,5-tetrazine (**3d**): Following the method used for **3a**, with triethyl amine (2.4 g, 23.3 mmol) in 35 ml of THF and 4.5 g (15.3 mmol) of **2d** in 30 ml of THF and recrystallised with acetone/ethyl acetate to give 1.5 g (40 %) of **3d** as a yellow crystal. M.p. 152–154 °C (Lit[10] 151–156 °C). IR (KBr, cm^{-1}): 1567 m (C=N), 1386 s (SO_2), 1323 s (ring), 1187 s (SO_2). ^{1}H NMR ($CDCl_3$ δ ppm): 7.87 (d, 4H, *J* = 7.2 Hz), 7.70 (t, 2H, *J* = 7.2 Hz), 7.57 (t, 4H, *J* = 8.0 Hz), 7.52 (d, 2H, *J* = 7.6 Hz), 7.49 (d, 4H, *J* = 8.0 Hz), 7.39 (t, 4H, *J* = 7.6 Hz). MS: *m/z* (%) 517 (M, 4.92), 375 (30.84).234 (18.85), 125 (24.2), 103 (100), 77 (28.9)

3,6-di(p-tolyl)-1,4-di(phenylsulfonyl)-1,4-dihydro-1,2,4,5-tetrazine (**3e**): Following the method used for **3a**, with triethylamine (1.8 g, 17.5 mmol) in 35 ml of THF, and 3.0 g (9.7 mmol) of **2e** in 30 ml THF and recrystallised with absolute ethanol/ethyl acetate twice to give 1.1 g (41.6 %) of **3e** as yellow crystal. M.p. 165–166 °C. IR (KBr, cm^{-1}): 1610 m (C=N), 1372 s (SO_2), 1336 s (ring), 1183 s (SO_2). ^{1}H NMR ($CDCl_3$) δ ppm: 7.88 (d, 4H, *J* = 7.6 Hz), 7.69 (t, 2H, *J* = 7.4 Hz), 7.56 (t, 4H, *J* = 8.0 Hz), 7.38 (d, 4H, *J* = 8.0 Hz), 7.19 (d, 4H, *J* = 8.0 Hz), 2.39 (s, 6H). MS (*m/z* %) 545 (M^+, 0.37), 403 (1.47), 141 (46.7) 119 (65.2), 91 (29.9), 77 (100). Anal. Calcd. for $C_{28}H_{24}N_4O_4S_2$ (544.66): C, 61.75; H, 4.44; N, 10.29 Found: C, 61.42; H, 4.51; N, 10.01

3,6-di(4-chlorophenyl)-1,4-di(phenylsulfonyl)-1,4-dihydro-1,2,4,5-tetrazine (**3f**): Following the method used for **3a**, with triethylamine (1.5 g, 14.5 mmol) in 30 ml of THF, and 3.0 g (9.1 mmol) of **2f** in 25 ml THF and recrystallised from absolute ethanol/ethyl acetate twice to give 0.5 g (18.8 %) of **3f** as yellow crystal. M.p. 154–155 °C. IR (KBr, cm^{-1}): 1597 m (C=N), 1369 s (SO_2), 1328 (ring), 1186 s (SO_2) ^{1}H NMR ($CDCl_3$ δ ppm): 7.86 (d, 4H, *J* = 7.6 Hz), 7.73 (t, 2H, *J* = 7.6 Hz), 7.59 (t, 4H, *J* = 8.0 Hz), 7.41 (d, 4H, *J* = 8.4 Hz), 7.37 (d, 4H, *J* = 8.4 Hz). MS *m/z* (%): 443 (M-141, 2.15), 141 (80.1), 102 (20.6), 77 (100), 51 (25.9) Anal. Calcd. for $C_{26}H_{18}C_{l2}N_4O_4S_2$ (585.50): C, 53.34; H, 3.10; N, 9.57; Found: C, 52.98; H, 3.06; N, 9.67.

3,6-di(4-methoxyphenyl)-1,4-di(phenylsulfonyl)-1,4-dihydro-1,2,4,5-tetrazine (**3g**): Following the method used for **3a**, with 2.0 g (19.4 mmol) of triethylamine in 30 ml of THF, and 4.2 g (12.4 mmol) of **2g** in 25 ml THF and recrystallised with absolute ethanol/ethyl acetate twice to give 1.2 g (32.3 %) of **3g** as yellow crystal. M.p. 141–143 °C. IR (KBr, cm^{-1}): 1607 m (C=N), 1368 s (SO_2), 1332 s (ring), 1183 s (SO_2), 1028 (Ar-OCH_3) ^{1}H NMR ($CDCl_3$ δ ppm): 7.90 (d, 4H, *J* = 7.6 Hz), 7.68 (t, 2H, *J* = 7.6 Hz), 7.57 (t, 4H, *J* = 7.6 Hz), 7.46 (d, 4H, *J* = 8.8 Hz), 6.89 (d, 4H, *J* = 8.8 Hz), 3.84 (s, 6H). MS *m/z* (%): 435 (M-141, 0.11), 88 (4.95), 70 (18.5), 61 (18.9), 43 (100). Anal. Calcd. for $C_{28}H_{24}N_4O_6S_2$ (576.66): C, 58.32; H, 4.19; N, 9.72.Found: C, 58.31; H, 4.16; N, 9.82.

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