Heterocycl. Commun., Vol. 17(1-2), pp. 69–71, 2011 • Copyright © by Walter de Gruyter • Berlin • Boston. DOI 10.1515/HC.2011.015

Synthesis and anticancer activities of 1,4-bis(6-aryl-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole-3-yl)benzene derivatives

Hao-Yu Ding and De-Jiang Li*

Hubei Key Laboratory of Natural Products Research and Development, China Three Gorges University, Yichang 443002, P.R. China

*Corresponding author
e-mail: lidejiang999@yahoo.com.cn

Abstract

1,4-Bis(6-aryl-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl)-benzenes (**3**) were synthesized in high yields by reaction of bis(4-amino-5-mercapto-1,2,4-triazol-3-yl)benzene (**1**) with carboxylic acids **2** in the presence of $POCl_3$ and tetrabutylammonium iodide as catalyst. The MTT assay indicated that compounds **3a–i** possess inhibitory activity against the proliferation of HepG-2 (liver cancer), A549-1 (lung cancer) and 231-2 (breast cancer).

Keywords: anticancer activity; synthesis; triazolothiadiazoles.

Introduction

Bis-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazoles have been reported to possess antibacterial properties (Holla et al., 1998) and anticancer activities against a panel of 60 cell lines derived from seven cancer types, namely lung, colon, melanoma, renal, ovarian, and leukemia (Holla et al., 2002). Pyridines containing a bis-1,2,4-triazolo[3,4-*b*]-[1,3,4]-thiadiazole moiety (Li and Fu, 2006; Li et al., 2006a,b) endowed with good fungicidal activities against *Cercospora beticola* Sacc. have been reported from our laboratory. Butanes and *trans*-ethenes containing the bis-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole moiety (Li et al., 2006a,b, 2007) show significant antibacterial activities. In a previous paper, we reported the synthesis of a series of 1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazoles by the reaction of 3-aryl-4-amino-5-mercapto-1,2,4-triazoles with dicarboxylic acids. Prompted by our previous results and in continuation of our search for bioactive molecules, we designed a facile method to prepare new 1,4-bis(6-aryl-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl)benzene derivatives **3** by cyclization of bis-(4-amino-5-mercapto-1,2,4-triazol-3-yl)benzene (**1**) with carboxylic acids **2**. The synthesis, characterization, and the results of fungicidal activity screening studies of the newly synthesized compounds are presented in this paper.

Results and discussion

As shown in Scheme 1, terephthaloylhydrazide was prepared by hydrazinolysis of dimethyl terephthalate. The reaction of terephthaloylhydrazide with CS_2 in the presence of KOH in absolute ethanol gave potassium terephthaloyldithiocarbazate. The reaction of this intermediate product with hydrazine hydrate yielded bis(4-amino-5-mercapto-1,2,4-triazol-3-yl)benzene (**1**). This four-step preparation of **1** has been described previously (Li and Fu, 2007a,b, 2008).

The desired 1,4-bis(6-aryl-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl)benzenes **3** were synthesized in good yields by condensation of compound **1** with dicarboxylic acids in the presence of $POCl_3$ and tetrabutylammonium iodide as catalyst. Because of poor solubility of **1** and carboxylic acids in $POCl_3$, the yields of products **3** were rather low for the reactions conducted in the absence of the catalyst. For example, the yield of **3a** was 35%. However, in the presence of tetrabutylammonium iodide as a phase transfer catalyst the desired product **3a** was obtained in 82% yield. The structures of all compounds **3** were established on the basis of elemental analysis and spectral data. The IR spectra of compounds **3** show bands at 1621–1630 cm^{-1}, 1236–1253 cm^{-1}, and 695–706 cm^{-1} due to the presence of C=N, N-N=C, and C-S-C moieties, respectively. The ^1H NMR spectra of **3** exhibit multiple signals in the range of δ 7.6–8.8 for protons of the aryl groups. The EI mass spectra of compounds **3** exhibit molecular ion peaks. For example, the spectrum of **3a** shows a molecular ion peak at the expected value *m/z* 478 with a 21% relative abundance.

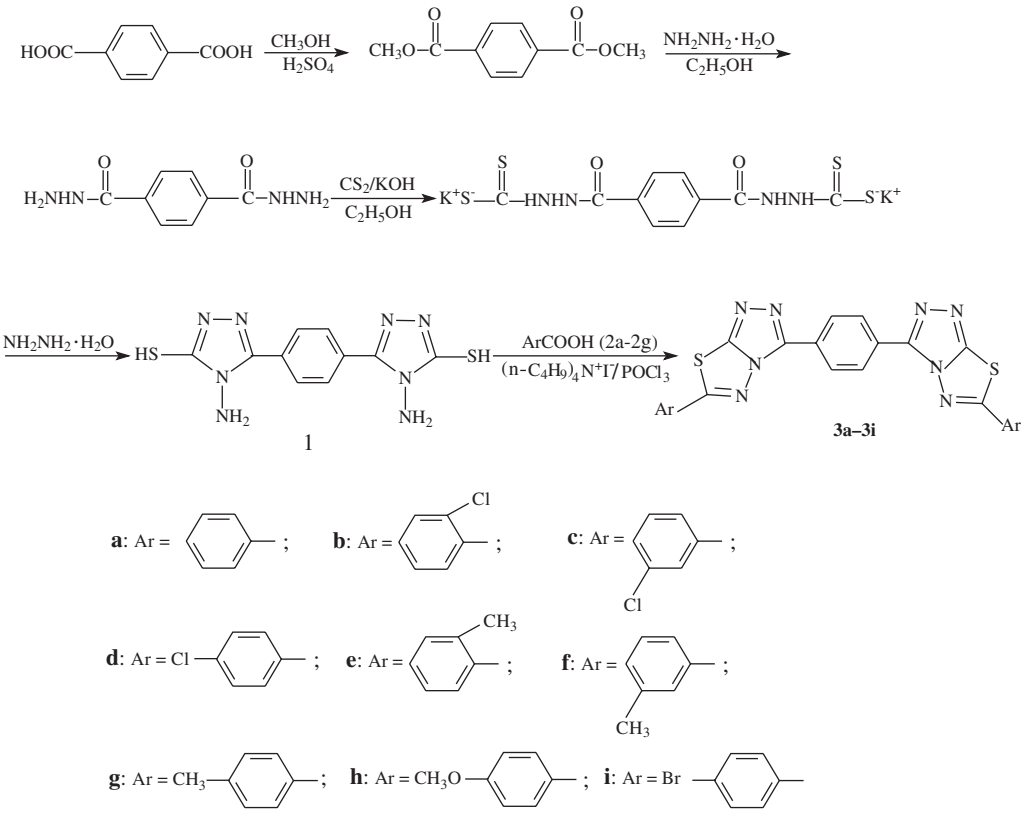
The MTT assay indicated that compounds **3a–i** possess inhibitory activity against the proliferation of three cancer cells, including HepG-2 (liver cancer), A549-1 (lung cancer) and 231-2 (breast cancer). Compound **3d** is highly active against the HepG2 and 231-2 cells (Table 1).

Experimental

The IR spectra were recorded in the range of 4000–400 cm^{-1} on a Nicolet Nexus 470 FT-IR spectrophotometer using KBr discs. ^1H NMR spectra were recorded at 400 MHz in CF_3COOD on a Varian Mercury-Plus 400 NMR spectrometer. The chemical shifts are reported as parts per million relative to internal TMS. Electron impact mass spectra were recorded on a Finnigan Trace GC-MS spectrometer. Elemental analyses were obtained on a Perkin-Elmer-2400-CHN instrument.

General preparation of compounds 3a–i

A mixture of bis(4-amino-5-mercapto-1,2,4-triazol-3-yl)benzene (**1**, 2.2 mmol), carboxylic acid (**2a–g**, 1.0 mmol), tetrabutylammonium



Scheme 1 Synthesis of compounds **3a–i**.

iodide (0.5 mmol), and $POCl_3$ (8 ml) was stirred for 3 h at 55–60°C and then heated under reflux for 10–14 h at 115–120°C. After cooling, excess $POCl_3$ was removed under reduced pressure. The concentrated mass was poured onto crushed ice, and the mixture was neutralized with potassium carbonate. The separated solid was filtered, washed with water, ethanol, and dried. The crude material was crystallized from a mixture of ethanol and pyridine to yield pure title compound **3a–i**.

1,4-Bis(6-phenyl-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl)-benzene (3a) Light yellow powder; yield 82%; ^{1}H NMR: δ 8.82–8.80 (m, 4H, Ar-H), 8.12–8.10 (m, 4H, Ar-H), 7.84–7.78 (m, 6H, Ar-H); IR: 1621, 1253, 698 cm^{-1}; MS: *m/z* 478 (M^+, 21%), 303 (18%), 121 (100%). Analysis calculated for $C_{24}H_{14}N_8S_2$: C, 60.24; H, 2.95; N, 23.42. Found: C, 60.31; H, 2.87; N, 23.56.

1,4-Bis[6-(2-chlorophenyl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl]benzene (3b) Light yellow powder; yield 83%; ^{1}H NMR: δ 8.76–8.45 (m, 4H, Ar-H), 8.11–8.03 (m, 5H, Ar-H), 7.64–7.29 (m, 3H, Ar-H); IR: 1623, 1257, 702 cm^{-1}; MS: *m/z* 546 (M^+, 12%), 548 (M^++2, 6%), 137 (86%), 111 (100%). Analysis calculated for $C_{24}H_{12}N_8S_2Cl_2$: C, 52.66; H, 2.21; N, 20.47. Found: C, 52.72; H, 2.31; N, 20.40.

Table 1 Inhibitory activities of compounds **3a–i** against three types of cancer cells.

Compound	IC_{50}/(μg l^{-1})		
	HepG-2	A549-1	231-2
3a	35.61	40.2	39.2
3b	30.28	39.5	33.5
3c	31.2	38.2	33.6
3d	25.8	36.1	23.1
3e	36.2	42.1	42.3
3f	37.3	43.5	43.5
3g	35.1	46.1	40.6
3h	30.4	4.08	42.3
3i	29.1	45.3	40.1

1,4-Bis[6-(3-chlorophenyl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl]benzene (3c) Yellow powder, yield 80%, ^{1}H NMR: δ 8.49–8.38 (m, 5H, Ar-H), 8.07–8.01 (m, 3H, Ar-H), 7.56–7.31 (m, 4H, Ar-H); IR: 1627, 1246, 700 cm^{-1}; MS-EI: *m/z* 546 (M^+, 10%), 548 (M^++2, 5%), 137 (90%), 111 (100%). Analysis calculated for $C_{24}H_{12}N_8S_2Cl_2$: C, 52.66; H, 2.21; N, 20.47. Found: C, 52.72; H, 2.25; N, 20.40.

1,4-Bis[6-(4-chlorophenyl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl]benzene (3d) Yellow powder, yield 85%, ^{1}H NMR: δ 8.52–8.46 (m, 4H, Ar-H), 8.11–8.08 (m, 4H, Ar-H), 7.82–7.69 (m, 4H, Ar-H); IR: 1631, 1239, 705 cm^{-1}; MS-EI: *m/z* 546 (M^+, 20%), 548 (M^++2, 8%), 137 (83%), 111 (100%). Analysis calculated for $C_{24}H_{12}N_8S_2Cl_2$: C, 52.66; H, 2.21; N, 20.47. Found: C, 52.501; H, 2.27; N, 20.57.

1,4-Bis[6-(2-methylphenyl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl]benzene (3e) Light yellow powder; yield 79%; ^{1}H NMR: δ 8.35–8.32 (m, 5H, Ar-H), 8.27–8.21 (m, 3H, Ar-H), 7.71–7.62 (m, 4H, Ar-H), 2.46 (s, 6H, $2CH_3$); IR: 1621, 1236, 700 cm^{-1}; MS: *m/z* 506 (M^+, 8%), 135 (92%), 117 (100%). Analysis calculated for $C_{26}H_{18}N_8S_2$: C, 61.64; H, 3.58; N, 22.12. Found: C, 61.52; H, 3.63; N, 22.01.

1,4-Bis[6-(3-methylphenyl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl]benzene (3f) Light yellow powder; yield 80%; ^{1}H NMR: δ 8.35–8.32 (m, 5H, Ar-H), 8.31–8.25 (m, 3H, Ar-H), 7.81–7.76 (m, 4H, Ar-H), 7.71–7.63 (m, 4H, Ar-H), 2.51 (s, 6H, $2CH_3$); IR: 1628, 1241, 700 cm^{-1}; MS: *m/z* 506 (M^+, 8%), 135 (92%), 117 (100%). Analysis calculated for $C_{26}H_{18}N_8S_2$: C, 61.64; H, 3.58; N, 22.12. Found: C, 61.56; H, 3.60; N, 22.03.

1,4-Bis[6-(4-methylphenyl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl]benzene (3g) Light yellow powder; yield 83%; ^{1}H NMR: δ 8.35–8.32 (m, 8H, Ar-H), 7.58–7.52 (m, 4H, Ar-H), 2.56 (s, 6H, $2CH_3$); IR: 1630, 1236, 707 cm^{-1}; MS: *m/z* 506 (M^+, 15%), 135 (83%), 117 (100%). Analysis calculated for $C_{26}H_{18}N_8S_2$: C, 61.64; H, 3.58; N, 22.12. Found: C, 61.56; H, 3.60; N, 22.03.

1,4-Bis[6-(4-methoxyphenyl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl]benzene (3h) Light yellow powder; yield 84%; ^{1}H NMR: δ 8.45–8.31 (m, 8H, Ar-H), 7.38–7.32 (m, 4H, Ar-H), 4.03 (s, 6H, $2OCH_3$); IR: 1627, 1236, 700 cm^{-1}; MS: *m/z* 538 (M^+, 8%), 151 (78%), 107 (100%). Analysis calculated for $C_{26}H_{18}N_8O_2S_2$: C, 57.98; H, 3.37; N, 20.80. Found: C, 57.81; H, 3.46; N, 20.71.

1,4-Bis[6-(4-bromophenyl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl]benzene (3i) Yellow powder; yield 77%; ^{1}H NMR: δ 8.37–8.29 (m, 8H, Ar-H), 7.91–7.78 (m, 4H, Ar-H), 3.91 (s, 6H, $2OCH_3$); IR: 1626, 1231, 706 cm^{-1}; MS: *m/z* 634 (M^+, 5%), 636 (M^++2, 4%), 181 (70%), 155 (100%). Analysis calculated for $C_{24}H_{12}N_8S_2Br_2$: C, 45.31; H, 1.90; N, 17.61. Found: C, 45.41; H, 2.10; N, 17.41.

Acknowledgements

We gratefully acknowledge financial support of this work by the Science Foundation of Hubei Province Education Department, China (Project no. C2010027).

References

Holla, B. S.; Gonsalves, R.; Shenoy, B. Studies on some N-bridged heterocycles derived from bis-[4-amino-5-mercapto-1,2,4-triazol-3-yl]alkanes. *Il Farmaco* **1998**, *53*, 574–578.

Holla, B. S.; Poojary, K. N.; Rao, B. S.; Shivananda, M. K. New bis-aminomercaptotriazoles and bis-triazolothiadiazoles as possible anticancer agents. *Eur. J. Med. Chem*. **2002**, *37*, 511–517.

Li, D. J.; Fu, H. Q. Synthesis and fungicidal activities 2,5-bis[(3-aryl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridines. *Heterocycl. Commun.* **2006**, *12*, 383–388.

Li, D. J.; Long, D. Q.; Fu, H. Q. The synthesis and fungicidal activities of 2,6-bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl] pyridines. *Phosphorus Sulfur Silicon* **2006a**, *181*, 2079–2087.

Li, D. J.; Zhu, S. P.; Fu, H. Q. Synthesis and antibacterial activities of 1,2-bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl] ethenes. *Heterocycl. Commun.* **2006b**, *12*, 449–452.

Li, D. J.; Fu, H. Q. Synthesis and antibacterial activities of 1,5-bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pentanes. *Heterocycl. Commun.* **2007a**, *13*, 347–352.

Li, D. J.; Fu, H. Q. Synthesis and antibacterial activities of 1,7-bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]heptanes. *Heterocycl. Commun.* **2007b**, *13*, 407–412.

Li, D. J.; Long, D. Q.; Fu, H. Q. The synthesis and antibacterial activities of 1,4-bis[(3-aryl)-1,2,4-triazolo [3,4-*b*]-[1,3,4]thiadiazole-6-yl]butanes. *Phosphorus Sulfur Silicon* **2007**, *182*, 1307–1314.

Li, D. J.; Fu, H. Q. Antibacterial and fungicidal activities of 1,8-bis[(3-aryl)-s-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]octanes. *Heterocycl. Commun.* **2008**, *14*, 69–76.

Received April 19, 2011; accepted May 26, 2011