ANTIBACTERIAL AND FUNGICIDAL ACTIVITIES OF 1,6-BIS[(3-ARYL)-1,2,4-TRIAZOLO[3,4-*B*]-[1,3,4]THIADIAZOLE-6-YL]HEXANES

De-Jiang Li*[a], He-Qing Fu[b]

College of Chemistry and Life Science, *China Three Gorges University, Yichang 443002, P. R. China*[a] and Research Institute of Chemical Engineering, South China University of Technology, Guangzhou 510640, P. R. China [b] **e-mail:** lidejiang999@yahoo.com.cn

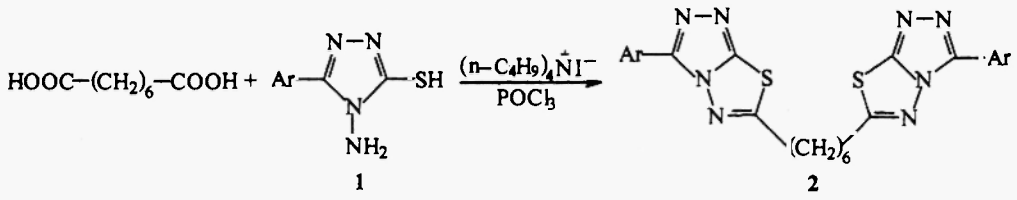
Abstract: Fifteen new 1,6-bis[(3-aryl)-1,2,4-triazolo[3,4-b]-[1,3,4]thiadiazole-6-yl]hexanes were synthesized in high yields by reaction of 3-aryl-4-amino-5-mercapto-1,2,4-triazole with octanedioic acid in the presence of $POCl_3$ and tetrabutylammonium iodide as catalyst. The newly synthesized compounds were characterized by elemental analysis, IR, ^1H NMR and MS. The preliminary antibacterial tests showed that most of them were effective against *S.aureus, E.coli* and *B.subtilis.* **2b, 2c, 2d, 2n** and **2o** exhibited good fungicidal activities against *Cerospora beticola sacc.*

Introduction

Bis[1,2,4-Bis[1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole-4-yl]alkanes were reported to possess antibacterial property (1) and bis[1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazol-3-yl-methoxy]phenylenes possess anticancer activity against a panel of 60 cell lines derived from seven cancer types namely, lung, colon, melanoma, renal, ovarian, and leukemia (2). 2,5-Bis[(3-aryl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridines (3) and 2,6-Bis[(3-aryl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]pyridines (4) endowed with good fungicidal activities against *Cerospora beticola sacc* have been reported from our laboratory. 1,4-Bis[(3-aryl)-1,2,4-triazolo[3,4-*b*]-[1,3,4] thiadiazole-6-yl]butanes (5) and trans-1,2-bis[(3-aryl)-1,2,4-triazolo[3,4-*b*]-[1,3,4] thiadiazole-6-yl]ethenes (6) were found to show significant antibacterial activities. Prompted by these observation and in continuation of our search for bio-active molecules, We designed a facile one-pot method to prepare fifteen new 1,6-bis[(3-aryl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]hexanes by cyclization of 3-aryl-4-amino-5-mercapto-1,2,4-triazoles with octanedioic acid. The synthesis, characterization and the results of antibacterial and fungicidal activities screening studies of the newly synthesized compounds are presented in this paper.

Result and discussion

The synthesis of 1,6-bis[(3-aryl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole-6-yl]hexanes **2** were accomplished in one step with good yields by condensing 3-aryl-4-amino-5-mercapto-1,2,4-triazoles **1** with octanedioic acid in the presence of $POCl_3$ and tetrabutylammonium iodide as catalyst (Scheme 1). Because of the poor solubility of **1** and octanedioic acid in $POCl_3$, the yield of **2** is very low. For example, the yield of **2d** was 36%. However, where the tetrabutylammonium iodide as phase transfer catalyst were utilized and the mixture was first stirred for 3 h at 55-60 □, then refluxed for 8-10 h at 115-120 □, **2a** was obtained in 65% yield (Table **1**).



Scheme 1

The structures of all compounds **2** were established on the basis of elemental analysis and spectral data. The IR spectral data of compounds **2** showed bands at 1610-1635 cm^{-1}, 1230-1265 cm^{-1}, 700-715 cm^{-1} due to C=N, N-N=C and C–S-C, respectively. The ^1H NMR spectra of **2** exhibited multiple signals in the δ 7.24-8.60 range accounting for hydrogen of aryl group, δ 3.33-3.38 range accounting for the 4 hydrogens of $-2SCH_2$, in the δ 1.40-2.08 range with multiple signals accounting for the 8 hydrogens of $-(CH_2)_4-$. With compound **2d** as an example, it exhibited multiple signals in the δ 7.68-7.02, 8.29-8.32 ranges accounting for 8 hydrogens of phenyl group, δ 3.34 accounting for the 4 hydrogens of $-2SCH_2$, δ 1.60-2.07 accounting for the 8 hydrogens of $-(CH_2)_4-$. The EI-MS for compounds **2** exhibited molecular ion peaks. With compound **2d** as an example, it showed a strong molecular ion peak M^+ with m/z 554 and 5% relative abundance.

Table-1 Preparation of 1,6-bis[(3-aryl)-1,2,4-triazolo[3,4-b]-[1,3,4]thiadiazole-6-yl]hexanes **2** from 3-aryl-4-amino-5-mercapto-[1,2,4]triazoles **1**

Entry	Ar	Condition	Yield (%)[a]	m.p. (□)
2a	Ph	115-120□/9 h	70	>250
2b	2-Cl-Ph	115-120□/8 h	62	>250
2c	3-Cl-Ph	115-120□/8 h	64	>250
2d	4-Cl-Ph	115-120□/8 h	65	>250
2e	2-CH_3-Ph	115-120□/10 h	53	>250
2f	3-CH_3-Ph	115-120□/10 h	56	>250
2g	4-CH_3-Ph	115-120□/10 h	52	>250
2h	3-Br-Ph	115-120□/8 h	53	>250
2i	4-Br-Ph	115-120□/9 h	57	>250
2j	2-I-Ph	115-120□/9 h	60	>250
2k	3-I-Ph	115-120□/9 h	65	>250
2l	4-I-Ph	115-120□/9 h	58	>250
2m	4-OCH_3-Ph	115-120□/10 h	64	>250
2n	4-Pyridyl	115-120□/10 h	57	>250
2o	3-Pyridyl	115-120□/10 h	60	>250

[a]Isolated yields based on octanedioic acid.

Compounds **2** were screened for their antibacterial activities against *E. coli*, *S. aureus*, and *B. subtilis* employing the cup-plate method at the concentration of 100 μg/mL in the nutrient agar. The preliminary results indicated that most of compounds express significant antibacterial activity. The results of such studies are given in Table **2**.

The biological activities of compounds **2** were investigated and the results showed that they exhibited fungicidal activities, especially against *Cerospora beticola sacc.*

For example, **2b, 2c, 2d** and **2o** showed 92% of *Cerospora beticola sacc* inhibition of in 50 mg/L (see Table **3**).

Table-2 The antibacterial activities of compounds **2a-2o**

Compd.	*S.aureus*	*E.coli*	*B.subtilis*
2a	+	++	+
2b	+++	+++	++
2c	+++	+++	++
2d	+++	+++	+++
2e	+	-	++
2f	-	+	+
2g	-	-	-
2h	+	-	+
2i	+	+	+
2j	+	-	+
2k	-	-	+
2l	-	-	-
2m	+	-	+
2n	++	+++	+++
2o	+++	++	+++

Zone diamerter of growth inhibition: <10 mm (-), 10 ~ 12 mm (+), 13 ~ 15 mm (+ +), 16 ~ 20 mm (+ + +); Diamater of the cup=8 mm.

Table-3 The Fungicidal Activities **2** (50 mg/L, relative inhibition %)

Compd.	*Gibberella zeae*	*Cerospora beticola sacc*	*Physalospora piricola*
2a	41	72	61
2b	40	93	55
2c	57	95	75
2d	87	97	80
2e	32	75	49
2f	41	72	63
2g	40	70	56
2h	31	75	32
2i	35	80	40
2j	30	81	67
2k	28	74	55
2l	21	80	45
2m	32	86	27
2n	40	96	70
2o	55	94	60

Experimental

Melting points were determined on an X_4 melting point apparatus and were uncorrected. The IR spectra were recorded on a Nicolet Nexus 470 FT-IR spectrophotometer using KBr discs in the range 4000-400 cm^{-1}. ^1H NMR spectra were recorded on a Varian Mercury-Plus 400 NMR spectrometer in CF_3COOD. The chemical shifts are reported as parts per million relative to internal TMS. MS spectra were recorded on a Finnigan Trace GC-MS spetrometer. Elemental Analyses were taken on a Perkin-Elemer-2400-CHN Elemental Analysis Instrument.

Compound 3-Aryl-4-amino-5-mercapto-1,2,4-triazole **1** was prepared from aromatic carboxylic acids by four steps according to the literature (5).

General preparation of 2- A mixture of 3-aryl-4-amino-5-mercapto-1, 2, 4-triazoles (2.2 mmol), octanedioic acid (1.0 mmol), tetrabutylammonium iodide (0.5 mmol) and $POCl_3$ (7 mL) was stirred for 4 h at 55-60 □, then refluxed for 8-10 h at 115-120 □. Excess $POCl_3$ was removed under reduced pressure. The concentrated mass was cooled and poured into crushed ice, neutralised with potassium carbonate. The separated solid was filtered, washed with water, ethanol, then dried. The crude material was recrystallized from ethanol-pyridine, giving the pure products **2a-o**.

2a: white powder, IR (KBr, cm^{-1}): 1626, 1254, 703; ^1H NMR (CF_3COOD, 400 MHz): δ 1.42-2.41 (m, 8H, $(CH_2)_4$), 3.33 (t, 4H, J=7.4, $2SCH_2$), 7.48-7.54 (m, 3H, Ar-H), 7.65-7.72 (m, 3H, Ar-H), 8.28-8.31 (m, 2H, Ar-H); MS (m/z): 486 (M^+, 24%), 311 (12%), 103 (100%). Anal. Calcd For $C_{24}H_{22}N_8S_2$: C, 59.24; H, 4.56; N, 23.03. Found: C, 59.10; H, 4.50; N, 23.17.

2b: white powder, IR(KBr, cm^{-1}): 1631, 1237, 707; ^1H NMR (CF_3COOD, 400 MHz): δ 1.57--2.10 (m, 8H, $(CH_2)_4$), 3.32 (t, 4H, J=7.4, $2SCH_2$), 7.57-7.63 (m, 3H, Ar-H), 7.69-7.73 (m, 3H, Ar-H), 8.21-8.27 (m, 2H, Ar-H); MS (m/z): 554 (M^+, 3%), 345 (20%), 137 (100%). Anal. Calcd For $C_{24}H_{20}N_8S_2Cl_2$: C, 51.89; H, 3.63; N, 20.17. Found: C, 51.74; H, 3.68; N, 20.31.

2c: gray powder, IR (KBr, cm^{-1}): 1615, 1242, 713; ^1H NMR (CF_3COOD, 400 MHz): δ 1.55-2.06 (m, 8H, $(CH_2)_4$), 3.31 (t, 4H, J=7.3, $2SCH_2$), 7.58-7.69 (m, 5H, Ar-H), 8.24-8.27 (m, 3H, Ar-H); MS (m/z): 554 (M^+, 2%), 345 (8%), 137 (100%). Anal. Calcd For $C_{24}H_{20}N_8S_2Cl_2$: C, 51.89; H, 3.63; N, 20.17. Found: C, 51.97; H, 3.51; N, 20.04.

2d: gray powder, IR (KBr, cm^{-1}): 1619, 1230, 710; ^1H NMR (CF_3COOD, 400 MHz): δ 1.60-2.07 (m, 8H, $(CH_2)_4$), 3.34 (t, 4H, J=7.3, $2SCH_2$), 7.68-7.72 (m, 4H, Ar-H), 8.29-8.32 (m, 4H, Ar-H); MS (m/z): 554 (M^+, 5%), 345 (26%), 137 (100%). Anal. Calcd For $C_{24}H_{20}N_8S_2Cl_2$: C, 51.89; H, 3.63; N, 20.17. Found: C, 51.70; H, 3.57; N, 20.29.

2e: white powder, IR (KBr, cm^{-1}): 1632, 1248, 706; ^1H NMR (CF_3COOD, 400 MHz): δ 1.45-2.06 (m, 8H, $(CH_2)_4$), 2.37 (s, 6H, $2CH_3Ph$), 3.30 (t, 4H, J=7.4, $2SCH_2$), 7.61-7.75 (m, 5H, Ar-H), 8.37-8.43 (m, 3H, Ar-H); MS (m/z): 514 (M^+, 3%), 325 (21%), 117 (100%). Anal. Calcd For $C_{26}H_{26}N_8S_2$: C, 60.67; H, 5.09; N, 21.77. Found: C, 60.49; H, 5.14; N, 21.79.

2f: white powder, IR (KBr, cm^{-1}): 1639, 1257, 712; ^1H NMR (CF_3COOD, 400 MHz): δ 1.40-2.08 (m, 8H, $(CH_2)_4$), 2.42 (s, 6H, $2CH_3Ph$), 3.34 (t, 4H, J=7.4, $2SCH_2$), 7.64-7.70 (m, 3H, Ar-H), 7.77-7.81 (m, 2H, Ar-H), 8.42-8.46 (m, 3H, Ar-H); MS (m/z): 514 (M^+, 5%), 325 (20%), 117 (100%). Anal. Calcd For $C_{26}H_{26}N_8S_2$: C, 60.67; H, 5.09; N, 21.77. Found: C, 60.81; H, 5.02; N, 21.59.

2g: white powder, IR (KBr, cm^{-1}): 1641, 1250, 708; ^1H NMR (CF_3COOD, 400 MHz): δ 1.42-2.04 (m, 8H, $(CH_2)_4$), 2.40 (s, 6H, $2CH_3Ph$), 3.31 (t, 4H, J=7.3, $2SCH_2$), 7.75-7.79 (m, 4H, Ar-H), 8.34-8.37 (m, 4H, Ar-H); MS (m/z): 514 (M^+, 7%), 325 (26%),

117 (100%). Anal. Calcd For $C_{26}H_{26}N_8S_2$: C, 60.67; H, 5.09; N, 21.77. Found: C, 60.81; H, 5.02; N, 21.62.

2h: pale yellow powder, IR (KBr, cm^{-1}): 1620, 1341, 705; ^1H NMR (CF_3COOD, 400 MHz): δ 1.41-2.06 (m, 8H, $(CH_2)_4$), 3.30 (t, 4H, J=7.4, $2SCH_2$), 7.64-7.70 (m, 5H, Ar-H), 8.52-8.57 (m, 3H, Ar-H); MS (m/z): 642 (M^+, 1%), 389 (26%), 181 (100%). Anal. Calcd For $C_{24}H_{20}N_8S_2Br_2$: C, 44.73; H, 3.13; N, 17.39. Found: C, 44.89; H, 3.07; N, 17.20.

2i: yellow powder, IR(KBr, cm^{-1}): 1626, 1343, 702; ^1H NMR (CF_3COOD, 400 MHz): δ 1.45-2.04 (m, 8H, $(CH_2)_4$), 3.35 (t, 4H, J=7.4, $2SCH_2$), 7.67-7.71 (m, 4H, Ar-H), 8.60-8.65 (m, 4H, Ar-H); MS (m/z): 642 (M^+, 2%), 389 (31%), 181 (100%). Anal. Calcd For $C_{24}H_{20}N_8S_2Br_2$: C, 44.73; H, 3.13; N, 17.39. Found: C, 44.60; H, 3.15; N, 17.54.

2j: white powder, IR(KBr, cm^{-1}): 1616, 1243, 704; ^1H NMR (CF_3COOD, 400 MHz): δ 1.50-2.06 (m, 8H, $(CH_2)_4$), 3.33 (t, 4H, J=7.4, $2SCH_2$), 7.62-7.67 (m, 3H, Ar-H), 7.71-7.74 (m, 3H, Ar-H), 8.31-8.34 (m, 2H, Ar-H); MS (m/z): 738 (M^+, 1%), 310 (19%), 229 (41%), 102 (100%). Anal. Calcd For $C_{24}H_{20}N_8S_2I_2$: C, 39.04; H, 2.73; N, 15.17. Found: C, 39.19; H, 2.70; N, 15.01.

2k: gray powder, IR(KBr, cm^{-1}): 1621, 1251, 706; ^1H NMR (CF_3COOD, 400 MHz): δ 1.48-2.09 (m, 8H, $(CH_2)_4$), 3.30 (t, 4H, J=7.3, $2SCH_2$), 7.57-7.64 (m, 3H, Ar-H), 7.73-7.78 (m, 2H, Ar-H), 8.42-8.46 (m, 3H, Ar-H); MS (m/z): 738 (M^+, 2%), 310 (11%), 229 (32%), 102 (100%). Anal. Calcd For $C_{24}H_{20}N_8S_2I_2$: C, 39.04; H, 2.73; N, 15.17. Found: C, 39.21; H, 2.67; N, 15.12.

2l: white powder, IR (KBr, cm^{-1}): 1632, 1257, 706; ^1H NMR (CF_3COOD, 400 MHz): δ 1.54-2.04 (m, 8H, $(CH_2)_4$), 3.37 (t, 4H, J=7.4, $2SCH_2$), 7.65-7.71 (m, 4H, Ar-H), 8.51-8.57 (m, 4H, Ar-H); MS (m/z): 738 (M^+, 2%), 310 (24%), 229 (31%), 102 (100%). Anal. Calcd For $C_{24}H_{20}N_8S_2I_2$: C, 39.04; H, 2.73; N, 15.17. Found: C, 38.88; H, 2.80; N, 15.29.

2m: pale yellow powder, IR (KBr, cm^{-1}): 1632, 1223, 715; ^1H NMR (CF_3COOD, 400 MHz): δ 1.59-2.08 (m, 8H, $(CH_2)_4$), 3.44 (t, 4H, J=7.3, $2SCH_2$), 3.89(s, 6H, $2OCH_3$), 7.71-7.76 (m, 4H, Ar-H), 8.28-8.31 (m, 4H, Ar-H); MS (m/z): 546 (M^+, 3%), 341 (6%), 133 (100%). Anal. Calcd For $C_{26}H_{26}N_8S_2O_2$: C, 57.12; H, 4.79; N, 20.50. Found: C, 57.28; H, 4.71; N, 20.37.

2n: brown powder, IR (KBr, cm^{-1}): 1630, 1247, 700; ^1H NMR (CF_3COOD, 400 MHz): δ 1.51-2.05 (m, 8H, $(CH_2)_4$), 3.31 (t, 4H, J=7.3, $2SCH_2$), 7.35-7.39 (m, 4H, Ar-H), 8.31-8.36 (m, 4H, Ar-H); MS (m/z): 488 (M^+, 2%), 312 (14%), 176 (100%), 104 (47%). Anal. Calcd For $C_{22}H_{20}N_{10}S_2$: C, 54.08; H, 4.12; N, 28.67. Found: C, 54.21; H, 4.06; N, 28.50.

2o: brown powder, IR (KBr, cm^{-1}): 1621, 1243, 703; ^1H NMR (CF_3COOD, 400 MHz): δ 1.47-2.03 (m, 8H, $(CH_2)_4$), 3.34 (t, 4H, J=7.4, $2SCH_2$), 7.28-7.35 (m, 5H, Ar-H), 8.35-8.39 (m, 3H, Ar-H); MS (m/z): 488 (M^+, 1%), 312 (9%), 176 (100%), 104 (28%). Anal. Calcd For $C_{22}H_{20}N_{10}S_2$: C, 54.08; H, 4.12; N, 28.67. Found: C, 54.23; H, 4.18; N, 28.79.

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