

Synthesis and Antibacterial Activities of Bis[(3-benzyloxyphenyl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole derivatives

Lang-xi Hu, De-jiang Li

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

ABSTRACT

Bis[(3-benzyloxyphenyl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole derivatives **3** were synthesized in high yields by reaction of 3-benzyloxyphenyl-4-amino-5-mercapto-1,2,4-triazole **1** with dicarboxylic acid **2** in the presence of POCl₃ and tetrabutylammonium iodide as catalyst. The preliminary antibacterial tests showed that most of them were effective against *S.aureus*, *E.coli* and *B.subtilis*.

Keywords: 1,2,4-triazole, bis[1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole, synthesis, antibacterial activities.

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-meth-oxy]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 new bis[(3-benzyloxyphenyl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole derivatives by cyclization of 3-benzyloxyphenyl-4-amino-5-mercapto-1,2,4-triazole with dicarboxylic acid. The synthesis, characterization and the results of fungicidal activities screening studies of the newly synthesized compounds are presented in this paper.

RESULTS AND DISCUSSION

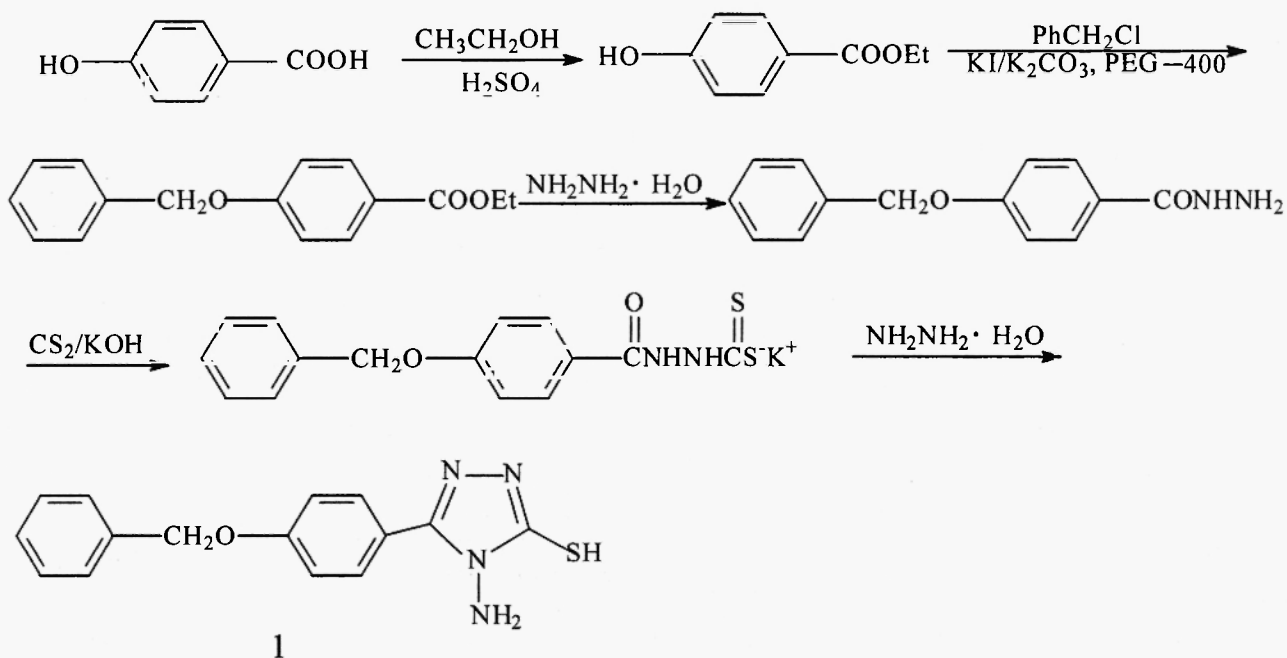
The benzyloxybenzoylhydrazide were prepared by hydrazinolysis of the ethyl benzyloxybenzoate. The reaction of benzyloxybenzoylhydrazide with CS₂/KOH in absolute ethanol gave potassium benzyloxybenzoyldithiocarbamate and

then the reaction of potassium benzyloxybenzoyldithiocarbazate with hydrazine hydrate afforded 3-benzyloxyphenyl-4-amino-5-mercapto-1,2,4-triazole **1** (Scheme 1).

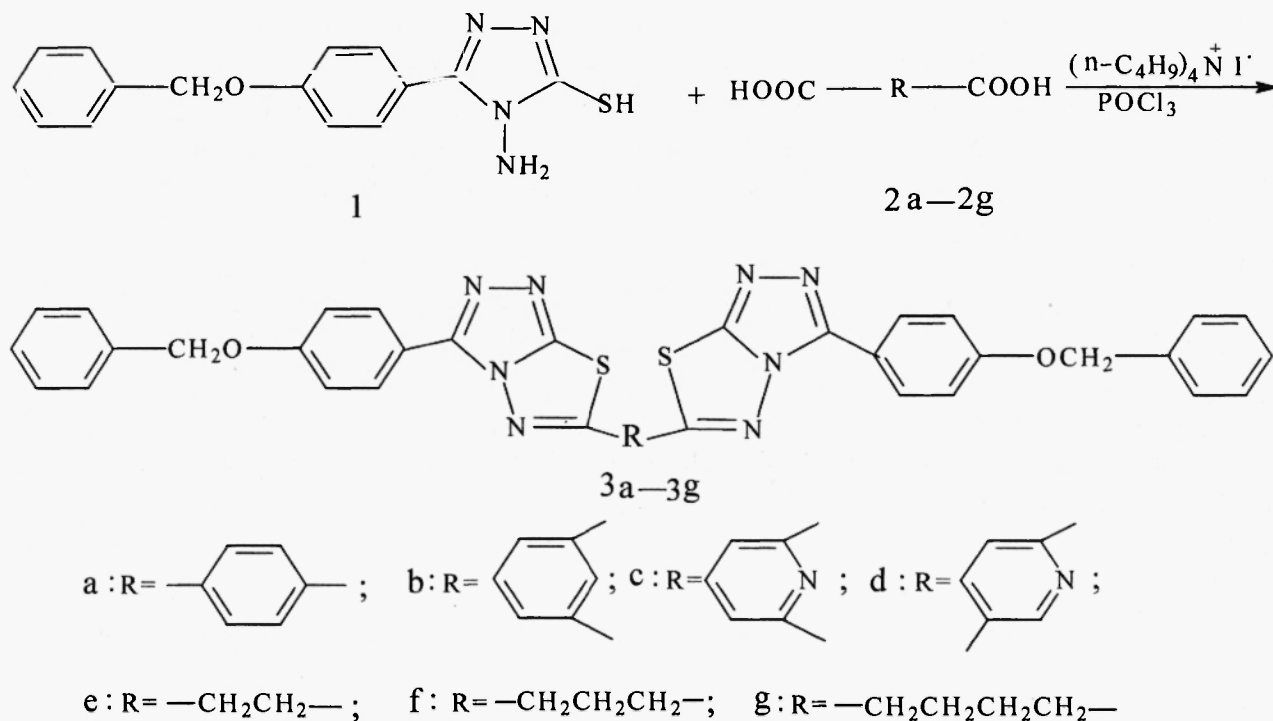
The synthesis of bis[(3-benzyloxyphenyl)-1,2,4-triazolo[3,4-*b*]-[1,3,4]thiadiazole derivatives **3** was accomplished in one step with good yields by condensing 3-benzyloxyphenyl-4-amino-5-mercapto-1,2,4-triazoles **1** with dicarboxylic acids in the presence of POCl_3 and tetrabutylammonium iodide as catalyst (Scheme-2). Because of the poor solubility of **1** and dicarboxylic acids in POCl_3 , the yield of **3** is very low. For example, the yield of **3a** was 30%. However, where the tetrabutylammonium iodide as phase transfer catalyst were utilized and the mixture was first stirred for 4 h at 55-60°C, then refluxed for 12 h at 115-120°C, **3a** was obtained in 80% yield.

The structures of all compounds **3** were established on the basis of elemental analysis and spectral data. The IR spectral data of compounds **3** showed bands at 1620-1635 cm^{-1} , 1230-1260 cm^{-1} , and 695-710 cm^{-1} due to C=N, N=N=C and C-S-C, respectively. The ^1H NMR spectra of **3** exhibited multiple signals in the δ 8.40-7.21 range accounting for hydrogen of aryl group. With compound **3a** as an example, it exhibited multiple signals in the δ 8.36-8.32, 7.46-7.27 ranges accounting for the 22 hydrogens of phenyl groups, δ 5.26 range accounting for the 4 hydrogens of $-\text{OCH}_2$. The EI-MS for compounds **3** exhibited molecular ion peaks. For example, **3a** showed strong molecular ion peak M^+ with m/z 690 and 15% relative abundance.

Scheme 1



Scheme 2



Compounds 3 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 1.

Table 1
The antibacterial activities of compounds 3a-3g

Compound.	<i>S.aureus</i>	<i>E.coli</i>	<i>B.subtilis</i>
3a	++	++	++
3b	++	++	++
3c	+++	+++	++
3d	+++	+++	+++
3e	+	-	+
3f	+	+	+
3g	+	-	-

Zone diameter of growth inhibition: <10 mm (-), 10 ~ 12 mm (+), 13 ~ 15 mm (+ +), 16 ~ 20 mm (+ + +);

Diameter of the cup=8 mm.

EXPERIMENTAL

Melting points were determined on an X₄ 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 CF₃COOD. The chemical shifts are reported as parts per million relative to internal TMS. MS spectra were recorded on a Finnigan Trace GC-MS spectrometer. Elemental Analyses were taken on a Perkin-Elmer-2400-CHN Elemental Analysis Instrument.

Preparation of compound 1

Compound 3-benzyloxyphenyl -4-amino-5-mercapto-1,2,4-triazole **1** was prepared from aromatic carboxylic acids by four steps according to the literature (7-9).

Preparation of compound 3

A mixture of compound 3-benzyloxyphenyl -4-amino-5-mercapto-1,2,4-triazole (2.2 mmol), bicarboxylic acid (1.0 mmol), tetrabutylammonium iodide (0.5 mmol), and POCl₃ (7 mL) was stirred for 4 h at 55-60°C, and then refluxed for 10-14 h at 115-120°C. Excess POCl₃ was removed under reduced pressure. The concentrated mass was cooled and poured into crushed ice, and neutralized with potassium carbonate. The separated solid was filtered, washed with water, ethanol, and then dried. The crude material was recrystallized from a mixture of ethanol and pyridine to afford pure title compounds 3a-3g.

Compound 3a

Light yellow powder, ¹H NMR (CF₃COOD, 400 MHz): δ 8.36-8.32 (m, 6H, Ar-H), 7.46-7.27 (m, 16H, Ar-H), 5.26 (s, 4H, 2OCH₂); IR (KBr, cm⁻¹): 1631, 1250, 695. MS-EI (*m/z*): 690 (M⁺, 15%), 409 (25%), 319 (52%), 91 (100%). Elemental Anal. Calcd. For C₃₈H₂₆O₂N₈S₂: C, 66.07; H, 3.77; N, 16.22. Found: C, 66.14; H, 3.84; N, 16.15.

Compound 3b

Yellow powder, ¹H NMR (CF₃COOD, 400 MHz): δ 8.40-8.35 (m, 5H, Ar-H), δ 8.27-8.11 (m, 4H, Ar-H), 7.40-7.24 (m, 17H, Ar-H), 5.22 (s, 4H, 2OCH₂); IR (KBr, cm⁻¹): 1622, 1239, 703. MS-EI (*m/z*): 690 (M⁺, 12%), 409 (21%), 319 (42%), 91 (100%). Elemental Anal. Calcd. For C₃₈H₂₆O₂N₈S₂: C, 66.07; H, 3.77; N, 16.22. Found: C, 66.12; H, 3.61; N, 16.33.

Compound 3c

Yellow powder, ¹H NMR (CF₃COOD, 400 MHz): δ 8.39-8.32 (m, 6H, Ar-H), δ 8.22-8.15 (m, 5H, Ar-H), 7.35-7.21 (m, 14H, Ar-H), 5.18 (s, 4H, 2OCH₂); IR (KBr, cm⁻¹): 1631, 1242, 710. MS-EI (*m/z*): 691 (M⁺, 10%), 410 (17%), 319 (50%), 91 (100%). Elemental Anal. Calcd. For C₃₇H₂₅O₂N₉S₂: C, 64.24; H, 3.64; N, 18.22. Found: C, 64.35; H, 3.52; N, 18.09.

Compound 3d

Yellow powder, ¹H NMR (CF₃COOD, 400 MHz): δ 8.36-8.25 (m, 6H, Ar-H), δ 8.18-8.04 (m, 8H, Ar-H), 7.31-7.17 (m, 11H, Ar-H), 5.20 (s, 4H, 2OCH₂); IR (KBr, cm⁻¹): 1627, 1251, 710. MS-EI (*m/z*): 691 (M⁺, 17%), 410 (29%), 319 (57%), 91 (100%). Elemental Anal. Calcd. For C₃₇H₂₅O₂N₉S₂: C, 64.24; H, 3.64; N, 18.22. Found: C, 64.12; H, 3.70; N, 18.33.

Compound 3e

Yellow powder, $^1\text{H NMR}$ (CF_3COOD , 400 MHz): δ 8.40-8.28 (m, 6H, Ar-H), 7.42-7.21 (m, 12H, Ar-H), 5.21 (s, 4H, 2OCH_2), 4.01 (s, 4H, $-\text{SCH}_2\text{CH}_2\text{S}-$); IR (KBr, cm^{-1}): 1625, 1242, 702. MS-EI (m/z): 642 (M^+ , 8%), 361 (41%), 271 (62%), 91 (100%). Elemental Anal. Calcd. For $\text{C}_{34}\text{H}_{26}\text{O}_2\text{N}_8\text{S}_2$: C, 63.53; H, 4.08; N, 17.43. Found: C, 63.69; H, 4.18; N, 17.32.

Compound 3f

Yellow powder, $^1\text{H NMR}$ (CF_3COOD , 400 MHz): δ 8.30-8.22 (m, 8H, Ar-H), 7.51-7.37 (m, 10H, Ar-H), 5.19 (s, 4H, 2OCH_2), 2.63 ~ 2.70 (m, 2H, CH_2), 3.57 (t, $J=7.5$, 4H, 2SCH_2); IR (KBr, cm^{-1}): 1627, 1239, 706. MS-EI (m/z): 656 (M^+ , 5%), 376 (25%), 286 (49%), 91 (100%). Elemental Anal. Calcd. For $\text{C}_{35}\text{H}_{28}\text{O}_2\text{N}_8\text{S}_2$: C, 64.02; H, 4.27; N, 17.07. Found: C, 64.16; H, 4.17; N, 17.12.

Compound 3g

Yellow powder, $^1\text{H NMR}$ (CF_3COOD , 400 MHz): δ 8.41-8.29 (m, 7H, Ar-H), 7.31-7.20 (m, 11H, Ar-H), 5.17 (s, 4H, 2OCH_2), 3.38 (t, 4H, $J=7.5$, 2SCH_2), 2.21 (t, $J=7.3$, 4H, CH_2CH_2); IR (KBr, cm^{-1}): 1627, 1239, 706. MS-EI (m/z): 670 (M^+ , 4%), 390 (21%), 300 (42%), 91 (100%). Elemental Anal. Calcd. For $\text{C}_{36}\text{H}_{30}\text{O}_2\text{N}_8\text{S}_2$: C, 64.48; H, 4.47; N, 16.72. Found: C, 64.39; H, 4.40; N, 16.82.

ACKNOWLEDGMENTS

We gratefully acknowledge financial support of this work by the Science Foundation of Hubei Province Education Department, China (Project No. D200660001).

REFERENCES

1. B. S. Holla, R. Gonsalves, S. Shenoy, *Il Farmco* **53**, 574 (1998).
2. B. S. Holla, K. N. Poojary, B. S. Rao, M. K. Shivananda, *Eur. J. Med. Chem.*, **37**, 511 (2002).
3. D. J. Li, H. Q. Fu, *Heterocycl. Commun.*, **12**, 383 (2006).
4. D. J. Li, D. Q. Long, H. Q. Fu, *Phosphorus, Sulfur, Silicon*, **181**, 2079 (2006).
5. D. J. Li, D. Q. Long, H. Q. Fu, *Phosphorus, Sulfur, Silicon*, **182**, 1307 (2007).
6. D. J. Li, S. P. Zhu, H. Q. Fu, *Heterocycl. Commun.*, **12**, 449 (2006).
7. D. J. Li, H. Q. Fu, *Heterocycl. Commun.*, **13**, 347 (2007).
8. D. J. Li, H. Q. Fu, *Heterocycl. Commun.*, **13**, 407 (2007).
9. D. J. Li, H. Q. Fu, *Heterocycl. Commun.*, **14**, 69 (2008).

