



## Antibacterial Activity of Some New Azole Compounds

Hossein Sadeghpour<sup>a,\*</sup>, Yones Ghasemi<sup>b,c</sup>, Zahra Rezaie<sup>a,b</sup>, Soghra Khabnadideh<sup>a,b</sup>,  
Zahra Falahzadeh<sup>b</sup>

<sup>a</sup>Department of Medicinal Chemistry, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>b</sup>Pharmaceutical Sciences Research Center, and <sup>c</sup>Department of Biotechnology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

---

### Abstract

We have previously synthesized new N-alkylated azoles (1b-7b) as antifungal agents. Here, we evaluated the antibacterial activities of these compounds against two gram positive and two gram negative bacteria by broth microdilution method. The aminothiazole derivative (7b) showed an excellent antibacterial activity. Other compounds exhibited moderate to good antibacterial activity. All compounds are more active against gram positive than against gram negative bacteria.

**Keywords:** Aminothiazole; Antibacterial; Benzimidazole; Benzotriazole.

**Received:** May 28, 2012; **Accepted:** July 10, 2012.

---

### 1. Introduction

Due to the changes in the culture and life style, new diseases are emerging among the human population such as cancers and AIDS which increase the risk of bacterial infections [1]. Increasing resistance of microorganisms to currently available antimicrobial drugs is the major cause of morbidity and mortality throughout the world. Thus, development of novel antimicrobial drugs is still in demand [2]. Among several classes of antimicrobial agents, azoles have very good antibacterial properties and nitroimidazoles are the most known among them. Other azoles ring such as triazole [3, 4], benzimidazole [5-8], and pyrazole [9] are investigated as potent antibacterial agents.

The best known antimicrobial property for N-alkylated azoles is their antifungal activity, and their mechanism of action is the inhibition of 14- $\alpha$ -demethylase (CYP51) [10]. To develop new azole derivatives as potential antifungal agents, previously, we have synthesized novel N-alkylated azoles including benzimidazole, benzotriazole and aminothiazole derivatives that had moderate antifungal activity [11]. In the present study, antibacterial activity of these new N-alkylated azoles was evaluated against two gram positive and two gram negative bacteria by borath microdilution method.

### 2. Material and methods

#### 2.1. Chemistry

A group of N-alkylated azole derivatives (1b-6b) bearing different length of alkyl chain

---

\*Corresponding Author: Hossein Sadeghpour, Department of Medicinal Chemistry, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran  
Tel: (+98) 711-2425395; Fax: (+98) 711-2424126  
Email: sadeghpurh@sums.ac.ir

were synthesized using previously published methods [11]. Briefly, benzimidazole or benzotriazole ring, alkyl bromides, tetraethyl ammonium bromide (TEAB), sodium hydroxide and potassium carbonate in acetonitrile were refluxed for 48 h. The reaction mixture was then filtered and concentrated. The organic compound was extract between water and chloroform, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude compound was purified by plate chromatography on silica gel using petroleum ether and ethyl acetate as solvent to obtain the final products [11] (Scheme 1).

For synthesis of aminothiazol derivative (7b), we use the previously published method in two steps. In brief, the amide intermediate was prepared by adding 3-chloropropionyl chloride to a solution of 2-aminothiazole in the presence of triethylamine (TEA) and 1,4-dioxane as solution and refluxed for 5 h. Then, it was purified by extraction between dichloromethane and water. In the second step, piperazine 2-ethanol was reacted with the amide intermediate in a similar condition as benzimidazole and benzotriazole compounds reacted with bromoalkanes [11] (Scheme 2).

## 2.2. Tested organisms

The antibacterial activities of the desired compounds were screened against the

following bacterial stains: gram-negative bacteria *proteus vulgaris* (PTCC1312), *escherichia coli* (PTCC1338), and gram-positive bacteria *bacillus subtilis* (PTCC1023), and *staphylococcus epidermis* (PTCC1114). The strains under study were obtained from the Persian Type Culture Collection. Bacteria were maintained on nutrient agar.

## 2.3. Antibacterial activity

*In vitro* antimicrobial activity was evaluated by the minimum inhibitory concentration (MIC) using broth microdilution method. Ampicillin and metronidazole were used as positive control and the solvent was applied for negative control. In order to detect MIC, first The Mueller-Hinton broth powder (Merck, 21 g) was suspended in distilled water (100 ml) and heated until it dissolved completely. The medium were autoclaved at a pressure of 4 lb/inc<sup>2</sup> and 121 °C for 20 min. Serial dilutions of the selected compound (0.5-128.0 µg/ml) were prepared in a sterile 96-well microtitre trays using Mueller-Hinton broth media. Stock inoculums were prepared by suspending some colonies of the examined bacteria in 3 ml of sterile water, and adjusting the turbidity of the inoculums to 0.5 McFarland standards at 600 nm wavelength (this yields stock suspension of 1-1.5×10<sup>8</sup> cells/ml). Then, this suspension



**Scheme 1.** Synthesis of N-alkylated benzimidazole and benzotriazole derivatives (1b-6b).

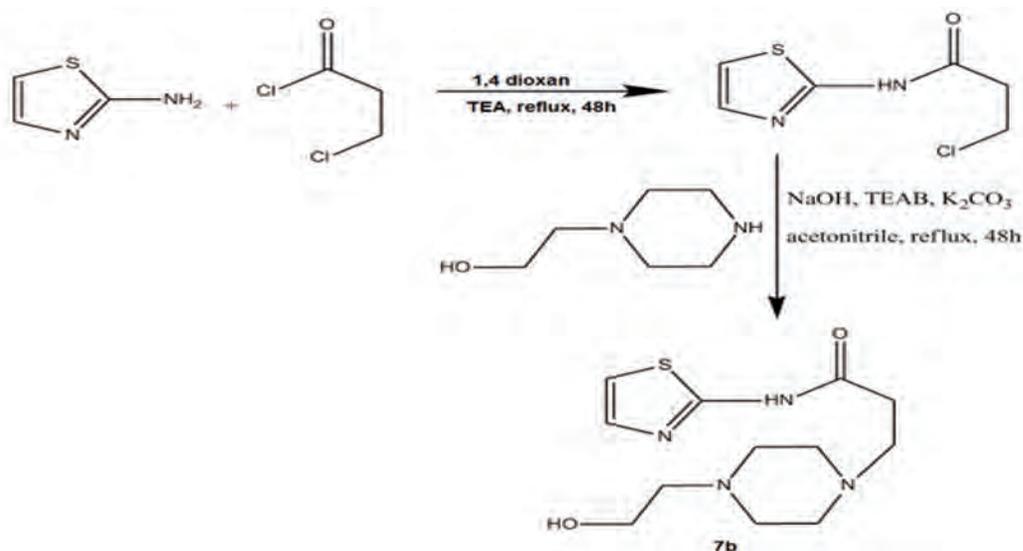
**Table 1.** *In vitro* antibacterial screening of the compounds 1b–7b (MIC values are given in µg/ml).

Compounds	Bacterial strains tested			
	<i>Proteus vulgris</i> (PTCC1312)	<i>Escherichia Coli</i> (PTCC1338)	<i>Bacillus subtilis</i> (PTec1023)	<i>Staphylococcus epidermis</i> (PTCC1114)
1b	16	32	8	8
2b	32	32	64	64
3b	32	64	32	32
4b	32	64	64	32
5b	8	16	8	4
6b	8	16	8	16
7b	16	8	4	4
Ampicillin	2	0.05	2	0.05
Metronidazole	16	64	16	8

was diluted up to 100 ml with borath media and the final concentration of bacteria confirmed at  $1-1.5 \times 10^6$  cells/ml. The test compound was dissolved in DMSO and a concentration of 512 µg/ml and 0.1 ml of this solution was added in the second well, which was serially diluted with 0.1 ml media. The first well (containing 0.1 ml borath media) was defined as the blank well. A fixed volume of 0.1 ml bacteria suspension was added in all wells which were incubated at 37 °C for 24 h. After 24 h, the turbidity of 96-well microtitre tray wells were measured by Elisa reader. The MIC<sub>50</sub> was determinate as the concentration of the first well witch there was less than 50% turbidity of bacterial suspension relative to blank well.

### 3. Results and discussion

The antibacterial activity was determined by microdilution assay and the MIC<sub>50</sub> values are shown in Table 1. There are 3 categories of central nucleus that are aminothiazole, benzotriazole and benzimidazole. The most potent compound was 7b which has the aminothiazole moiety. On the other hand, from the MIC values can concluded that if the benzotriazole nucleus exist in the structure, the antibacterial activity will be increased. More lipophilic bezotriazol derivatives had more antibacterial activity. Among the benzimidazole analogues compound 1b is the most active. In this group antibacterial activity decreased when lipophilicity is increased. Another point of interest is that

**Scheme 2.** Synthesis of N-alkylated aminothiazol derivative (7b).

on the basis of the observed MIC values of these compounds, gram positive bacteria are generally more sensitive than gram negative bacteria which can be correlated with their cell wall structure. The antibacterial effect of aminothiazol derivative was even more than metronidazole.

#### 4. Conclusion

The preliminary study on the selected compounds demonstrated that these compounds have good antibacterial effects on the gram positive bacteria. This could initiate further study for synthesis of new compounds with these three nucleuses especially aminothiazol derivatives.

#### Acknowledgments

The authors wish to thank Dr. N. Montazeri Najafabadi and Dr. A. Gholami for collaborating in antibacterial tests. This manuscript has been obtained from a Pharm. D. student thesis (Zahra fallahzadeh).

#### References

- [1] Nath AR, Reddy MS. Design, synthesis, antibacterial and antifungal activity of novel 2-[(E)-2-aryl-1-ethenyl]-3-(2-sulfanyl-1H-benzimidazole-5-yl)-3,4-dihydro-4-quinolinones. *E J Chem* 2012; 9: 1481-9.
- [2] Sharma PK, Chandak N, Kumar P, Sharma C, Aneja KR. Synthesis and biological evaluation of some 4-functionalized-pyrazoles as antimicrobial agents. *Eur J Med Chem* 2011; 46: 1425-32.
- [3] Sarmah KN, Sarmah NK, Patel TV, Kurmi KB. Synthesis, characterization, antimicrobial studies of certain triazole containing s-triazine derived compound. *Internat J Computat Engineering Res* 2012; 2: 289-93.
- [4] Kutkowska J, Modzelewska-banachiewicz B, Zoilkowska G, Rzeski W, Urbanik-sypniewska T, Zwolska Z, Prus M. Antimicrobial activity of 3,4-disubstituted-1,2,4-triazole. *Acta Poloniae Pharm Drug Res* 2005; 62: 303-6.
- [5] Khalafi-Nezhad A, Soltani Rad MN, Mohabatkar H, Asrari Z, Hemmateenejad B. Design, synthesis, antibacterial and QSAR studies of benzimidazole and imidazole chloroaryloxyalkyl derivatives. *Bioorganic & Amp; Med Chem* 2005; 13: 1931-8.
- [6] Yadav S, Kumar P, De Clercq E, Balzarini J, Pannecouque C, Dewan SK, Narasimhan B. 4-[1-(Substituted aryl/alkyl carbonyl)-benzimidazol-2-yl]-benzenesulfonic acids: synthesis, antimicrobial activity, QSAR studies, and antiviral evaluation. *Eur J Med Chem* 2010; 45: 5985-97.
- [7] Kucukbay H, Yilmaz U, Sireci N, Onganer A. Synthesis and antimicrobial activities of some bridged bis-benzimidazole derivatives. *Turk J Chem* 2011; 35: 561-71.
- [8] Singh N, Pandurangan A, Kavita Rana PA, Ahmad A, Tiwari AK. Benzimidazole a short review of their antimicrobial activities. *Internat Current Pharm J* 2012; 1: 119-27.
- [9] Xu LL, Zheng CJ, Sun LP, Miao J, Piao HR. Synthesis of novel 1,3-diaryl pyrazole derivatives bearing rhodanine-3-fatty acid moieties as potential antibacterial agents. *Eur J Med Chem* 2012; 48: 174-8.
- [10] Sun QY, Xu JM, Cao YB, Zhang WN, Wu QY, Zhang DZ, Zhang J, Zhao HQ, Jiang YY. Synthesis of novel triazole derivatives as inhibitors of cytochrome P450 14- $\alpha$ -demethylase (CYP51). *Eur J Med Chem* 2007; 42: 1226-33.
- [11] Khabnadideh S, Rezaei Z, Pakshir K, Zomorodian K, Ghafari N. Synthesis and antifungal activity of benzimidazole, benzotriazole and aminothiazole derivatives. *Res Pharm Sci* 2012; 7: 65-72.