

Original article

**ANTIMYCOBACTERIAL PROPERTIES OF SOME
2,5-DISUBSTITUTED-BENZOXAZOLE DERIVATIVES**

**BAZI 2,5-DİSÜBSTİTÜE-BENZOKSAZOL TÜREVLERİNİN
ANTİMİKOBAKTERİYEL AKTİVİTELERİ**

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ABSTRACT

A set of 2-(p-substituted-benzyl)-5-[[4-(p-chloro/fluoro-phenyl)piperazin-1-yl]acetamido]-benzoxazoles was evaluated for in vitro antimycobacterial activity against Mycobacterium tuberculosis and its isolate in comparison with reference drugs. The activity was expressed as minimum inhibitory concentration (MIC) in µg/ml. Compounds showed similar activity against test strains and most of them were more potent against M. tuberculosis than its isolate. Compound 9 exhibited a MIC value of 8 µg/ml against both of the tested microorganisms and had the highest activity among all derivatives. This microbiological assay showed that these benzoxazole derivatives possess a broad spectrum of antimycobacterial activity having MIC values of 8-64 µg/ml against the tested strains.

Key words: Benzoxazoles, Antimycobacterial activity, Mycobacterium tuberculosis

ÖZET

Bazı 2-(p-sübstütüe-benzil)-5-[[4-(p-kloro/floro-fenil)piperazin-1-il]asetamido]-benzoksazol türevi bileşiklerin in vitro antimikobakteriyel aktiviteleri referans ilaçlar ile karşılaştırmalı olarak Mycobacterium

tuberculosis ve izolatına karşı değerlendirilmiştir. Aktiviteler minimum inhibisyon konsantrasyonu (MİK) olarak µg/ml cinsinden ifade edilmiştir. Bileşikler, bakterilere karşı benzer aktivite göstermiş olup izolatına kıyasla M. tuberculosis'e karşı daha etkili oldukları gözlenmiştir. Bileşik 9, 8 µg/ml MİK değeri ile bakterilere karşı tüm türevler içerisinde en etkili türev olarak dikkat çekmiştir. Bu mikrobiyolojik çalışma, aktiviteleri incelenen bileşiklerin test edilen suşlara karşı 8-64 µg/ml MİK değeri aralığında geniş antimikobakteriyel aktiviteye sahip olduğunu göstermiştir.

Anahtar kelimeler: Benzoksazol, Antimikobakteriyel aktivite, *Mycobacterium tuberculosis*

INTRODUCTION

Tuberculosis (TB) is one of the most dangerous and wide spread infectious diseases all over the world. This fact is supported by the new WHO report released in 2011, which stated that there were estimated 8.8 million new cases of TB with 1.45 millions being fatal and more than one third of the global population being infected with *Mycobacterium tuberculosis*, the causative agent of TB . Although most of the cases are located in developing countries, TB has become a serious threat to the modern world due to migration. Especially, rapid development of mycobacterial resistance to conventional drugs is one of the major difficulties in the treatment of tuberculosis. The incidence of tuberculosis has been increasing worldwide, partly due to poverty/inequity and HIV/AIDS pandemic disease, which greatly raises risk of infection. The major health care problem is coincidence of HIV and TB infection together, which is highlighted by the fact that 1.1 million of TB cases and 0.35 million of fatal cases involve HIV positive patients . Currently *M. tuberculosis* strains have been found to be resistant to rifampicin and isoniazid (INH), which are the first line treatments and most powerful antitubercular agents (MDR-TB; multidrug-resistant TB). In addition, strains resistant to fluoroquinolones and to at least one of the injectable drugs including kanamycin, capreomycin and amikacin have been already described (1). Thus it is still necessary to search for new antitubercular agents.

Benzoxazoles are the structural isosteres of natural nucleotides and interact easily with the biopolymers that they constitute an important class of heterocyclic compounds with antimicrobial (2-6), antitubercular (7,8), antiviral (9, 10), antitumor (11-14), and topoisomerase I and II inhibitory (15) activity.

Recently, we have described the synthesis of 2-(*p*-substituted-benzyl)-5-[[4-(*p*-chloro/fluoro-phenyl)piperazin-1-yl]acetamido]benzoxazoles and they were evaluated for antibacterial and antifungal activity against standard strains and drug-resistant isolates in comparison with reference drugs such as ampicillin, gentamycin sulfate, ofloxacin, vancomycin, fluconazole, and amphotericin B trihydrate. In that study, minimum inhibitory concentration (MIC)

of each compound was determined by two-fold serial dilution technique and microbiological results showed that these synthesized compounds possess a broad spectrum of antimicrobial activity having MIC values of 32 - 256 $\mu\text{g/ml}$ against the tested microorganisms (6). In this study, we aimed at assaying antimycobacterial activity of these benzoxazole derivatives (6) and discovering new effective antitubercular agents that have a broad spectrum of antimycobial activity, possessing benzoxazole nucleus in their structure.

Herein, we describe the synthesis of a series of 2-(*p*-substituted-benzyl)-5-[[4-(*p*-chloro/fluoro-phenyl)piperazin-1-yl]acetamido]-benzoxazole derivatives(6) (Figure 1) as a new class of synthetic potent antitubercular agents along with their *in vitro* antitubercular activity tested against *M. tuberculosis* and its isolate.

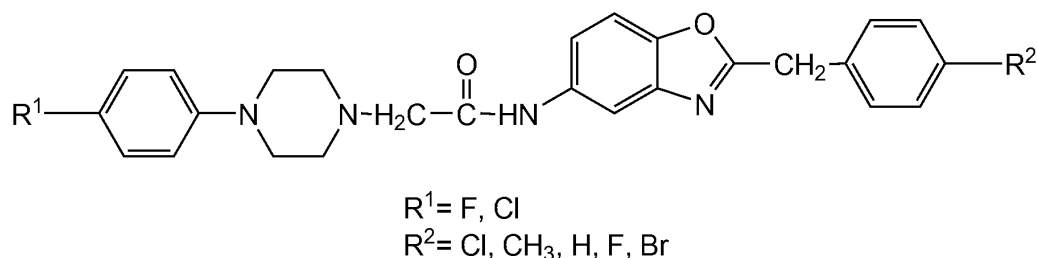


Figure 1. 2-(*p*-substituted-benzyl)-5-[[4-(*p*-chloro/fluoro-phenyl)piperazin-1-yl]acetamido]-benzoxazoles assayed for *in vitro* antimicobacterial activity

MATERIALS AND METHODS

For preparing the target benzoxazole derivatives, firstly, 5-amino-2-(*p*-substituted-benzyl)-benzoxazoles were synthesized by heating 0.02 mol 2,4-diaminophenol·2 HCl with 0.02 mol *p*-substituted phenyl acetic acid in 25 g polyphosphoric acid (PPA) and stirring for 1 - 2 h. At the end of the reaction period, the residue was poured into an ice/water mixture and the solution was neutralized with 10% NaOH. The resulting precipitate was filtered, washed with distilled water, dissolved in boiling ethanol with 0.2 g charcoal, and filtered off. Then distilled water was added to the filtrate slowly in order to stimulate crystallization. The crude compounds were obtained by filtering and drying the crystalline material. Then, chloroacetyl chloride (0.02 mol) was added over a period of 1 h to a stirred, ice-cooled mixture of 5-amino-2-(*p*-substituted-benzyl)-benzoxazole (0.02 mol), sodium bicarbonate (0.02 mol), diethyl ether (40 ml), and water (20 ml). The mixture was continuously stirred overnight at room temperature and filtered. The precipitate was washed with water, 2 N HCl, water, respectively, and the crude product was obtained by drying needles *in vacuo*. Finally, 0.002 mol 5-(2-chloroacetamido)-2-(*p*-substituted-benzyl)-benzoxazole derivatives were added to 0.002 mol *N*-(*p*-chloro/fluoro-phenyl)piperazine and 0.006 mol triethylamine

solution in 3.5 ml *N,N*-dimethylformamide (DMF). The mixture was stirred at room temperature for 24 h. At the end of the reaction time, 5 ml toluene was added, and the reaction medium was evaporated under reduced pressure. The residue was dissolved in chloroform and washed with 5% NaOH three times and later on once with distilled water. The solution was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The residue was dissolved in ethyl acetate and precipitated by adding *n*-hexane. If necessary, recrystallization was performed. Crystalline material (3-12) was dried *in vacuo*. The IR, ¹H-NMR, ¹³C-NMR, Mass spectra and elemental analysis results are in agreement with the proposed structures (6).

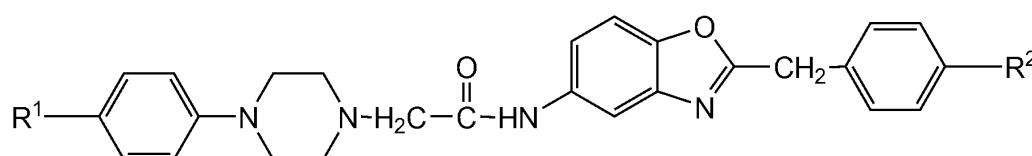
For microbiological assays, standards of INH, ethambutole were dissolved in appropriate solvents recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines (16,17). Stock solutions of the test compounds were prepared in DMSO. Materials used in the microbiology study were; Mueller Hinton agar (MHA) (Merck, Darmstadt, Hesse, Germany), Mueller Hinton broth (MHB) (Merck), RPMI-1640 medium with L-glutamine (Sigma-Aldrich Co.), 3-[*N*-morpholino]-propane-sulfonic acid (MOPS) (Sigma-Aldrich Co.), 96-well microplates (BD, Franklin Lakes, NJ, USA), transfer pipette (Eppendorf, Hamburg, Germany), Isoniazide (Sigma-Germany), Ethambutole (Sigma-Germany), DMSO (Riedel de Haen). Microorganisms used in the assay were *Mycobacterium tuberculosis* H37RV ATCC 27294, *Mycobacterium tuberculosis* isolate. Reference strains and clinical isolates were provided from Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Culture Collection (Ankara, Turkey) and Gazi University Hospital Microbiology Laboratory (Ankara, Turkey), respectively.

Bacterial susceptibility testing was performed according to the guidelines of CLSI M100-S18 (17). MHB was added to each well of the microplates. The bacterial suspensions used for inoculation were prepared at 10⁵ CFU/ml by diluting fresh cultures at McFarland 0.5 density (10⁷ CFU/ml). Suspensions of the bacteria at 10⁵ CFU/ml were inoculated to the two-fold diluted solution of the compounds. A 10-μl bacteria inoculum was added to each well of the microplates. There were 10⁴ CFU/ml bacteria in the wells after inoculations. Microplates were incubated at 37 °C overnight. After incubation, the lowest concentration of the compounds that completely inhibited macroscopic growth was determined and reported as minimum inhibitory concentration (MIC). All solvents and diluents, pure microorganisms and pure media were used in control wells. All experiments were done in triplicate. The data on antimicrobial activity of the compounds and the reference drugs are given in Table 1 as MIC (μg/ml) values.

RESULTS AND DISCUSSION

In this study, it was aimed to identify antimycobacterial activity of 2,5-disubstituted benzoxazoles which were synthesized and assayed for antibacterial and antifungal activity in previous study. We think that a 4-(*p*-chloro/fluoro-phenyl)piperazin-1-yl]acetamido moiety at fifth position of benzoxazole ring will permit these compounds penetrate easier through the lipophilic mycobacterial cell wall for antitubercular activity. In addition, the tested compounds have different substituents at para position of benzyl moiety, the second position of benzoxazole ring, with various electron donating/withdrawing and steric effects to be discussed for antimycobacterial activity.

Table 1. *In vitro* antimycobacterial activity of benzoxazole derivatives in comparison with reference drugs.



Compound	R ¹	R ²	MIC (µg/ml)	
			<i>Mycobacterium tuberculosis</i> H37RVT ATCC 27294	<i>Mycobacterium tuberculosis</i> isolate
3	Cl	Cl	32	8
4	F	Cl	16	8
5	Cl	CH ₃	64	32
6	F	CH ₃	32	16
7	Cl	H	64	16
8	F	H	16	64
9	Cl	F	8	8
10	F	F	8	32
11	Cl	Br	64	8
12	F	Br	16	8
Isoniazide			<0.25	<0.25
Ethambutole			2	2

Compounds 3-12 showed broad antimycobacterial activity against *M. tuberculosis* having MIC values between 8-64 µg/ml which were higher than that of the standards isoniazide and ethambutole. Nevertheless, these compounds had mostly enhanced activity against the isolate of *M. tuberculosis*. Compounds 3, 4, 5, 6, 7, 11 and 12 were more potent against isolate of *M. tuberculosis* than standard strain of *M. tuberculosis*. Compound 9 showed same activity against *M. tuberculosis* and its isolate with a MIC value of 8 µg/ml. Besides, it was the most active derivative in the series against *M. tuberculosis* and its isolate. It possess fluoro atom at benzyl moiety and chloro atom at para position of phenylpiperazine moiety.

Although all of the compounds, 3-12, indicated broad antimycobacterial activity against *M. tuberculosis* and its isolate having MIC values between 8-64 µg/ml, they were less active in comparison with the reference drugs against the tested microorganisms, it can be concluded that different substituents at the C-5 position of the 2-(*p*-substituted-benzyl)benzoxazole nucleus mostly indicated similar antimycobacterial activity. It was observed that the activity of standard drugs against tested microorganisms were higher than benzoxazole derivatives tested in this study.

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