Turkish J.Pharm. Sci
1(1), 55-64, 2004

ACTIVITY OF 1-[(2-AMINOPHENYL)THIO]-1-PHENYL-2-NITROALKANE DERIVATIVES AGAINST *HELICOBACTER PYLORI*

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Abstract

In this study, anti-Helicobacter pylori activity of previously synthesized 1-[(2-aminophenyl)thio]-1-phenyl-2-nitroethane ***1*** *and 1-[(2-aminophenyl)thio]-1-phenyl-2-nitropropane* ***2*** *derivatives has been examined. Anti-Helicobacter pylori activity of these compounds was investigated in-vitro agar dilution method. Ampicillin and ofloxazin were used as references. According to the results anti-Helicobacter pylori activities of the compounds are not close to that of ampicillin and ofloxazin. However, compound* ***1b*** *was found as having a comparable activity to reference compounds.*

Key Words: *1-[(2-aminophenyl)thio]-1-phenyl-2-nitroalkane derivatives, β-nitrostyrenes, anti-Helicobacter pylori activity.*

1-[(2-Aminofenil)tiyo]-1-fenil-2-nitroalkan Türevlerinin Helicobacter pylori'ye Karşı Aktiviteleri

Bu çalışmada sentezleri daha önceden yapılan 1-[(2-aminofenil)tiyo]-1-fenil-2-nitroetan ***1*** *ve 1-[(2-aminofenil)tiyo]-1-fenil-2-nitropropan* ***2*** *türevlerinin anti-Helicobacter pylori aktiviteleri test edilmiştir. Bileşiklerin anti-Helicobacter pylori aktiviteleri in-vitro agar dilution metodu ile araştırılmıştır. Ampisilin ve ofloksazin referans bileşikler olarak kullanılmıştır. Sonuçlara göre bileşiklerin anti-Helicobacter pylori aktivitesi ampisilin ve ofloksazine yakın değildir. Buna rağmen* ***1b*** *bileşiği referans bileşiklerle kıyaslanabilir aktiviteye sahip bulunmuştur.*

Anahtar kelimeler : *1-[(2-aminofenil)tiyo]-1-fenil-2-nitroalkan türevleri, β-nitrostirenler, anti-Helicobacter pylori aktivite.*

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Introduction

The etiology of peptic ulceration is thought to be multifactorial, and various kinds of drugs are used in clinical therapy. For example representative drugs include antiacid and anticholinergic agents, which are applied to the treatment of patients with gastric ulcer in order to neutralize gastric acid or to inhibit its secretion, but their clinical usage is restricted by disadvantages such as short duration and side effect (1).

More than 60% of the world's population is infected with the pathogenic Gram-negative bacterium *Helicobacter pylori* (1,2). Since the initial isolation from gastric disease patients (3) the clinical importance of *Helicobacter pylori* has been continued for about 20 years. It is widely accepted that *Helicobacter pylori* is a major causative factor in chronic gastritis, peptic ulcer and certain malignant peptic complications. Eradication of *Helicobacter pylori* results in a significant decrease in the recurrence rate in peptic ulcer patients (4-11).

There is a number of certain alkylthio and arylthio derivatives showing anti-*Helicobacter pylori* activity in literature (12-14). Recently selective anti-*Helicobacter pylori* acting 2-[[(2-piridyl)metyl]thio]-1H-benzimidazoles **3** (Figure 1) were synthesized (12) .

The synthesis and anti-*Helicobacter pylori* activity of a novel, potent cephem **4** (Figure 1) derivatives are described by Yoshida and Co-workers (13,14). Compound **4** have been included a (5-methyl-1,3,4-thiadiazol-2-yl)thio moiety at the 3-position of cephem.

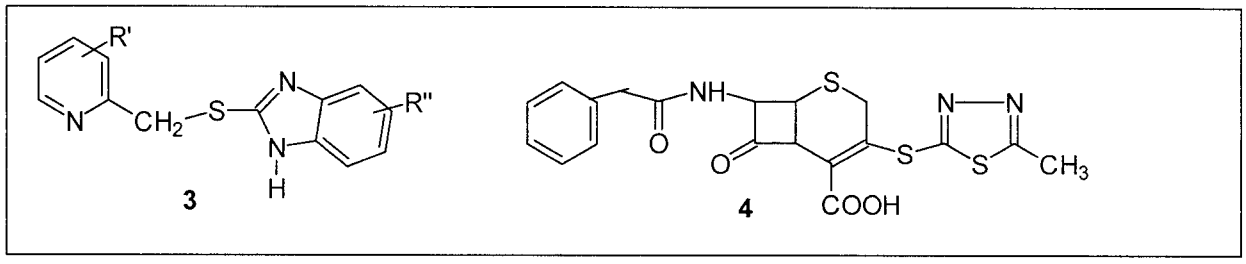


Figure 1. Certain arylthio compounds in the literature

Helicobacter pylori infection has been treated with antimicrobial agents in addition gastric antisecretory drugs (15). These therapies are not entirely successful. Furthermore, there remain problems such as drug resistance, side effects, and non-compliance (16-20). Therefore, great interest is developed to novel agents suitable for a single therapy treatment. A possible strategy for obtaining these products is to combine an antisecretory pharmacore with an anti-*Helicobacter pylori* agent in a single molecule.

In view of these observations, it was thought worthwhile to evaluate the anti-*Helicobacter pylori* of some previously synthesized 1-[(2-aminophenyl)thio]-1-phenyl-2-nitroethane **1** (Figure 2) and 1-[(2-aminophenyl)thio]-1-phenyl-2-nitropropane **2** (Figure 2) derivatives.

1-[(2-aminophenyl)thio]-1-phenyl-2-nitroethane **1** (Figure 2) and 1-[(2-aminophenyl)thio]-1-phenyl-2-nitropropane **2** (Figure 2) derivatives are the addition product of β-Nitrostyrenes, β-methyl-β-nitrostyrenes and 2-aminothiophenole. β-Nitrostyrenes and β-methyl-β-nitrostyrenes exhibit potent antibacterial and antifungal activities (21-23). Similarly, the addition products of β-nitrostyrenes possessed antibacterial and antifungal activities (24-26). Michael type addition reaction of β-nitrostyrenes and β-metyl-β-nitrostyrenes with various aromatic and aliphatic thiol groups have been investigated by our research groups (27-29).

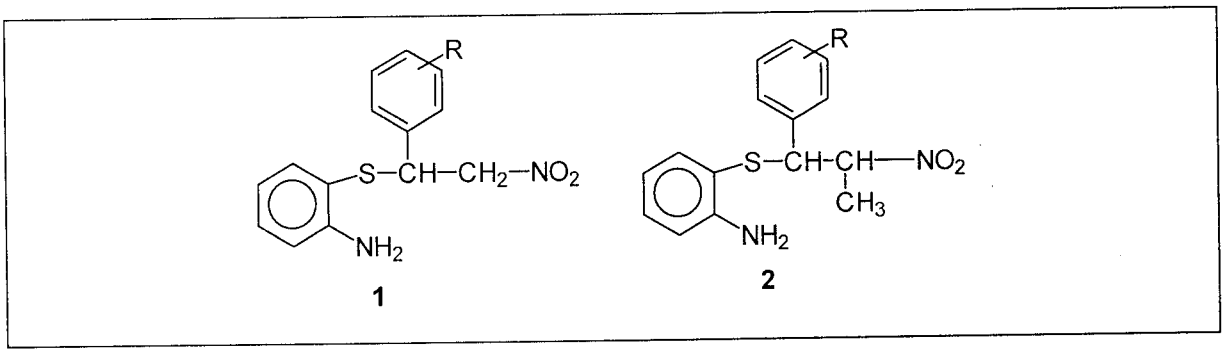


Figure 2. Structure of the tested compounds **1** and **2**.

Experimental

Chemistry

β-Methyl-β-nitrostyrene derivatives were synthesized in our laboratory according to literature (30, 31). Synthesis of 1-[(2-aminophenyl)thio]-1-phenyl-2-nitroethane **1** and 1-[(2-aminophenyl)thio]-1-phenyl-2-nitropropane **2** derivatives have been reported in our previous study (27, 28).

Microbiology

Material and Methods

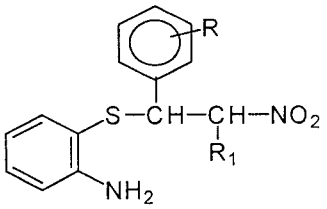
For the activity assessment *in vitro* agar dilution method was employed (32, 33). The 18 compounds were diluted with Mueller Hinton Agar (MHA) supplemented with 5% sheep blood at 45°C to prepare the concentration of 125, 62.5, 31.25, 15.62, 7.8, 3,9 μg/ml by using a serial 2-fold dilution method. *Helicobacter pylori* NCTC 11637 strain was grown for 3 days in MHA supplemented with 5% sheep blood at 37°C in a microaerophilic jar system (Anaerocult C, Merck-1.16275). The cells, which were suspended in saline and adjusted to McFarland 2.0 (10^7 cfu/ml) were applied to each plate with the use of a replication device. The MIC values were evaluated after 3 days incubation under microaerophilic atmosphere with high humidity.

Microorganism mixtures in 10 mL dimethylsulphoxide (DMSO), microorganism and media were used as controls. Microorganisms were obtained from Hacettepe University, Faculty of Medicine Department of Microbiology and Clinical Microbiology. Minimum inhibitory concentrations (MICs) of *Helicobacter pylori* of tested compounds are shown as μM/ml in Table 2.

Results and Discussion

The synthesis and structural elucidation of the 1-[(2-aminophenyl)thio]-1-phenyl-2-nitroethane **1a-j** and 1-[(2-aminophenyl)thio]-1-phenyl-2-nitroethane **2a-h** derivatives (Figure 2) were published in our previous papers (27, 28).

TABLE 1. Synthesized 1-[(2-aminophenyl)thio]-1-phenyl-2-nitroethane **1a-j** and 1-[(2-aminophenyl)thio]-1-phenyl-2-nitropropane **2a-h** derivatives



Com.	R	R_1
1a	H	H
1b	4-Br	H
1c	4-Cl	H
1d	4-NO_2	H
1e	4-CH_3	H
1f	3-OCH_3	H
1g	4-OCH_3	H
1h	4-OH, 3-OCH_3	H
1i	4-$NHCOCH_3$	H
1j	4-OC_2H_5	H
2a	H	CH_3
2b	4-Br	CH_3
2c	4-Cl	CH_3
2d	4-CH_3	CH_3
2e	4-OCH_3	CH_3
2f	4-OH, 3-OCH_3	CH_3
2g	4-OC_2H_5	CH_3
2h	4-$N(CH_3)_2$	CH_3

Anti- Helicobacter pylori Activity

Antibacterial activity of 1-[(2-aminophenyl)thio]-1-phenyl-2-nitroethane (**1**) and 1-[(2-aminophenyl)thio]-1-phenyl-2-nitropropane (**2**) derivatives were evaluated against

Helicobacter pylori. *In vitro* agar dilution method was used for anti-*Helicobacter pylori* activity test. Ampicillin and ofloxazin were used as standart compounds. The results of screening studies are given in Table 2.

TABLE 2. Minimal Inhibitory Concentration (MICs) of **1a-j** and **2a-h** against *Helicobacter pylori* NCTC 11637.

Compound	MIC's of synthesized compounds against *Helicobacter pylori* NCTC 11637
1a	$2{,}27x10^{-1}$
1b	$8{,}46x10^{-2}$
1c	$4{,}05x10^{-1}$
1d	$1{,}95x10^{-1}$
1e	$4{,}43x10^{-1}$
1f	$4{,}11x10^{-1}$
1g	$4{,}11x10^{-1}$
1h	$3{,}90x10^{-1}$
1i	$3{,}58x10^{-1}$
1j	$3{,}93x10^{-1}$
2a	$4{,}32x10^{-1}$
2b	$3.39x10^{-1}$
2c	$3{,}86x10^{-1}$
2d	$3{,}74x10^{-1}$
2e	$4{,}12x10^{-1}$
2f	$3{,}91x10^{-1}$
2g	$3{,}91x10^{-1}$
2h	$3{,}73x10^{-1}$
Ampicillin	$3.43x10^{-4}$
Ofloxazin	$6.64x10^{-4}$

It has been recognized that eradication of *Helicobacter pylori* is most rational approach to prevent recurrence of idiopathic digestive ulcers. Aiming to discover potent anti-*Helicobacter pylori* agents, we have tested activity of 1-[(2-aminophenyl)thio]-1-phenyl-2-nitroethane **1** and 1-[(2-aminophenyl)thio]-1-phenyl-2-nitropropane **2** derivatives against *Helicobacter pylori.*

The anti- *Helicobacter pylori* activity of these compounds are shown in Table 2. As seen in Table 2 effects of derivatives **1** and **2** on *Helicobacter pylori* NCTC 11637 have been found too weak to compare with that of ampicillin and ofloxazin. Among the derivatives, compound **1b** was found to be the most active compound. But also its activity was found less than the standards. These results demonstrated that aromatic thiol group could be changed for optimization of the anti-*Helicobacter pylori* activity. Introduction of thio-heterocyclic group such as 2-mercaptomethylbenzimidazole and its analog to the 1-position may be improved the activity. Further studies are in progress on this subject.

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received : 25.03.2004

accepted : 29.04.2004