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RESEARCH ARTICLE

Studies on Thiazolyliminothiazoline Derivatives as Potential Antitubercular Agents

Zafer Asım KAPLANCIKLI*, Gülhan TURAN-ZITOUNI*, Ahmet ÖZDEMİR*°

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Summary

In this study, eight new ethyl {2-[3,4-diaryl-3H-thiazol-2-ylidenamino]thiazol-4-yl}acetate were synthesized by reacting ethyl [2-(3-aryl(thiouredio)thiazol-4-yl]acetate and phenacyl bromides in ethanol. The solid was filtered and recrystallized from ethanol. The chemical structures of the synthesized compounds were proven by elemental analysis, IR, 1H-NMR and MS spectral data. The compounds were evaluated for in vitro antituberculosis activity against Mycobacterium tuberculosis $H_{37}Rv$ using the BACTEC 460 radiometric system and BACTEC 12B medium. The preliminary results showed that all of the tested compounds were inactive against the test organism.

***Key Words**: Thiazole, acetic acid ethyl esters, antitubercular activity*

Potansiyel Antitüberküler Ajanlar Olarak Tiyazoliliminotiyazolin Türevleri Üzerine Yapılan Çalışmalar

Özet

Bu çalışmada, etil [2-(3-aril(tiyoürediyo)tiyazol-4-il]asetat ve fenaçil bromürler etanol içinde reaksiyona sokularak sekiz adet yeni etil {2-[3,4-diaril-3H-tiyazol-2-ilidenamino] tiyazol-4-il}asetat sentezlenmiştir. Katı ürün süzülerek ayrılmış ve etanolden kristallendirilmiştir. Sentezlenen bileşiklerin kimyasal yapıları elemental analiz, IR, ^{1}H-NMR ve MS sonuçları ile aydınlatılmıştır. BACTEC 460 radyometrik sistem ve BACTEC12B ortamından yararlanılarak in vitro Mycobacterium tuberculosis $H_{37}Rv$'e karşı bileşiklerin antitüberküler aktiviteleri ölçülmüştür. Test edilen tüm bileşiklerin, ön deneme sonuçları, Mycobacterium tuberculosis'e karşı aktif olmadıkları gözlenmiştir.

***Anahtar Kelimeler:** Tiyazol, Asetik asid etil esterleri, Antitüberküler aktivite*

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INTRODUCTION

According to alarming data from the World Health Organisation, tuberculosis has spread to every corner of the globe. As much as one-third of the world's population is currently infected and more than 5000 people die from tuberculosis every day. A large number of the infected people are carriers of the latent form, which creates a potentially dangerous source of the illness for the future. The HIV pandemic has led to the rapid growth of the tuberculosis epidemic and increased the likelihood of people dying from tuberculosis (1,2). The emergence of

* Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 26470, Eskişehir, Turkey
° Corresponding author e-mail: ahmeto@anadolu.edu.tr

multidrug-resistant (MDR) strains of *M. tuberculosis* that are resistant to the two most effective drugs, isoniazid (INH) (3) and rifampicin (4) have reaffirmed tuberculosis as a primary public health threat. In addition, strains that are even more resistant than MDR, the so-called widely drug resistant, have recently been described (5). So there is urgently need for new chemotherapeutic agents to combat the emergence of resistance and shorten the duration of treatment to improve patient compliance (6).

Thiazole derivatives, because of their unique chemical properties, are suitable starting materials for designing combinatorial series of heterocyclic compounds and modeling structures of potential biologically active compounds. Thus, research in this area has high practical value (7–16).

The development of new antitubercular agents is the principal goal of our group. Lately, we have studied a number of structurally different compounds, such as the derivatives of thiazolylhydrazone (16), triazole (17), pyrazoline (18), hydrazide (19,20). The aim of this paper is to synthesis, antimycobacterial evaluation study of ethyl {2-[3,4-diaryl-3H-thiazol-2-ylideneamino]thiazol-4-yl}acetate.

MATERIAL AND METHODS

Chemistry

All reagents were used as purchased from commercial suppliers (Aldrich Chemical Co.) without further purification. Melting points were determined by using an Electrothermal 9100 digital melting point apparatus and were uncorrected. The compounds were checked for purity by TLC on silica gel 60 F_{254}. Spectroscopic data were recorded on the following instruments: Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser; IR, Shimadzu 435 IR spectrophotometer; ^{1}H-NMR, Bruker 400 MHz NMR spectrometer in DMSO-d_6 using TMS as internal standard; GC-MS was performed with an Agilent Technologie 6890N GC apparatus (equipped with a 12m x 0.20 mm dimethylpolysiloxane capillary column) linked to an Agilent 5973 EIMS mass spectrometer.

General procedure for synthesis of the compounds

Preparation of ethyl [2-(3-aryl(thiouredio)thiazol-4-yl]acetate (1)

A mixture of ethyl (2-aminothiazol-4-yl) acetate (0.1 mol) and 4-substituted phenylisothiocyanate (0.1 mol) in ethanol was refluxed for 2 h. The solid was filtered and recrystallized from ethanol.

Preparation of ethyl {2-[3,4-diaryl-3H-thiazol-2-ylidenamino]thiazol-4-yl}acetate (2a-h)

Ethyl [2 - (3 - aryl(thiouredio)thiazol - 4 - yl]acetate (**1**) (0.001 mol) and appropriate α-bromoacetophenone (0.001 mol) in absolute ethanol was refluxed for 4-5 h. The solid was filtered and recrystallized from ethanol. Some characteristics of the synthesized compounds are given in Table 1.

Table 1. Some characterizations of the compounds

Comp.	R_1	R_2	Mol. For.	Yield (%)	M.p. (°C)	M.W.
2a	H	OCH_3	$C_{23}H_{21}N_3O_3S_2$	69	188	451
2b	H	NO_2	$C_{22}H_{18}N_4O_4S_2$	74	210	466
2c	CH_3	OCH_3	$C_{24}H_{23}N_3O_3S_2$	68	126	465
2d	CH_3	NO_2	$C_{23}H_{20}N_4O_4S_2$	63	117	480
2e	OCH_3	CH_3	$C_{24}H_{23}N_3O_3S_2$	72	113	465
2f	OCH_3	NO_2	$C_{23}H_{20}N_4O_5S_2$	75	206	496
2g	OCH_3	OCH_3	$C_{24}H_{23}N_3O_4S_2$	63	137	481
2h	NO_2	OCH_3	$C_{23}H_{20}N_4O_5S_2$	76	196	496

***Ethyl {2 - [3 - phenyl - 4 - (4 - methoxyphenyl) - 3H - thiazol - 2 - ylideneamino]thiazol - 4 - yl} acetate* (2a)**

IR [n cm^{-1}, KBr]: 1728 (C=O), 1637-1592 (C=N, C=C). - ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.23 (3H, t, *J*=7.1 Hz), 3.68 (2H, s), 3.70 (3H, s), 4.13 (2H, q, *J*=7.1 Hz), 6.80 (2H, d, *J*=6.8 Hz), 6.84 (2H, s), 7.09 (2H, d, *J*=6.8 Hz), 7.25-7.28 (2H, m), 7.34-7.45 (3H, m). - MS (*m/z*): 451 (M$^+$, 100%), 422, 379, 378, 309, 263, 210. For $C_{23}H_{21}N_3O_3S_2$ calculated: 61.18% C, 4.69% H, 9.31% N; found: 61.22% C, 4.77% H, 9.36% N.

***Ethyl {2-[3-phenyl-4-(4-nitrophenyl)-3H-thiazol-2-ylideneamino]thiazol-4-yl} acetate* (2b)**

IR [n cm^{-1}, KBr]: 1734 (C=O), 1625-1585 (C=N, C=C). - ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.21 (3H, t, *J*=7.1 Hz), 3.71 (2H, s), 4.15 (2H, q, *J*=7.1 Hz), 6.85-8.60 (11H, m). - MS (*m/z*) 466 (M$^+$, 100%), 437, 420, 393, 394, 347, 324, 277. For $C_{22}H_{18}N_4O_4S_2$ calculated: 56.64% C, 3.89% H, 12.01% N; found: 56.61% C, 3.84% H, 12.05% N.

***Ethyl {2 - [3 - (4 - methylphenyl) - 4 - (4 - methoxyphenyl)-3H-thiazol-2-ylideneamino] thiazol-4-yl} acetate* (2c)**

IR [n cm^{-1}, KBr]: 1727 (C=O), 1630-1575 (C=N, C=C). - ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.23 (3H, t, *J*=7.1 Hz), 2.32 (3H, s), 3.68 (2H, s), 3.71 (3H, s), 4.13 (2H, q, *J*=7.1 Hz), 6.78-6.83 (4H, m), 7.08-7.16 (4H, m), 7.19-7.23 (2H, m). - MS (*m/z*) 465 (M$^+$, 100%), 450, 436, 393, 392, 323, 277, 224. For $C_{24}H_{23}N_3O_3S_2$ calculated: 61.91% C, 4.98% H, 9.02% N; found: 61.93% C, 5.03% H, 8.99% N.

***Ethyl {2-[3-(4-methylphenyl)-4-(4-nitrophenyl)-3H-thiazol-2-ylideneamino]thiazol-4-yl} acetate* (2d)**

IR [n cm^{-1}, KBr]: 1732 (C=O), 1624-1570 (C=N, C=C). - ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.21 (3H, t, *J*=7.1 Hz), 2.32 (3H,s), 4.12 (2H, q, *J*=7.1 Hz), 3.71 (2H, s), 7.16-7.35 (5H, m), 7.48-7.65 (3H, m), 8.12-8.18 (2H, m). - MS (*m/z*): 480 (M$^+$, 100%), 451, 434, 408, 407, 361, 338, 291. For $C_{23}H_{20}N_4O_4S_2$ calculated: 57.49% C, 4.19% H, 11.66% N; found: 57.56% C, 4.24% H, 11.75% N.

***Ethyl {2-[3-(4-methoxyphenyl)-4-(4-methylphenyl)-3H-thiazol-2-ylideneamino] thiazol-4-yl} acetate* (2e)**

IR [n cm^{-1}, KBr]: 1729 (C=O), 1631-1564 (C=N, C=C). - ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.23 (3H, t,

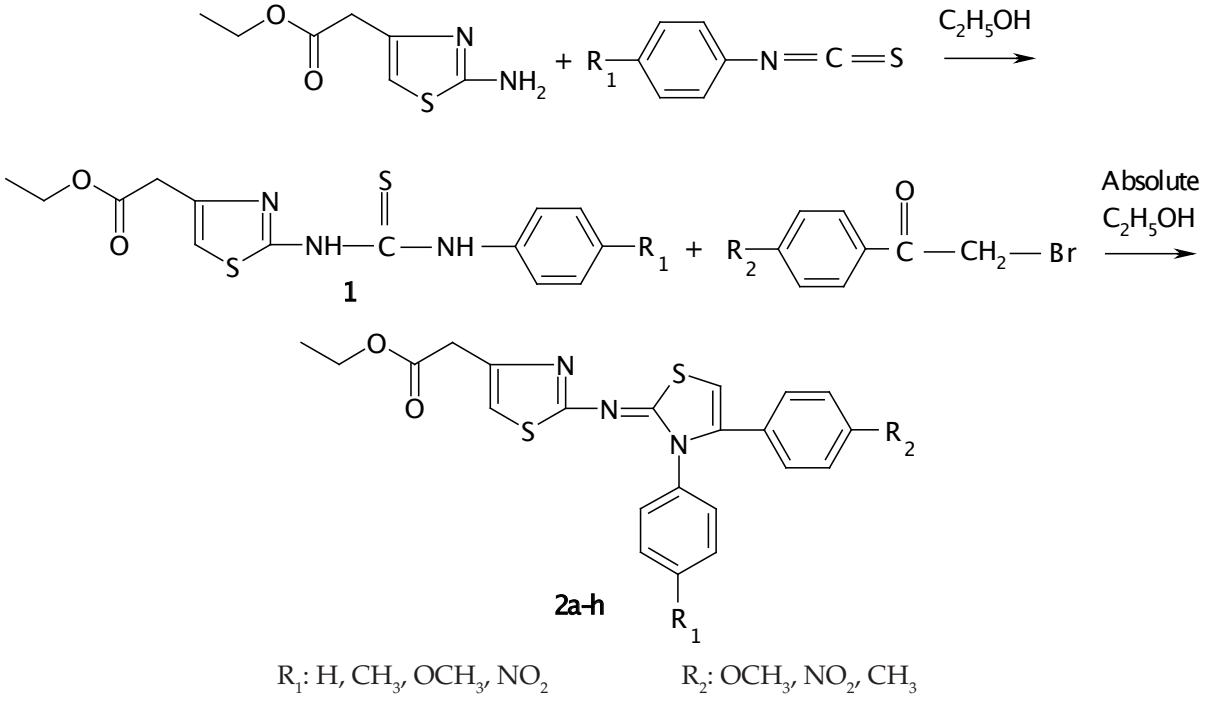

Figure 1. The general synthesis

J=7.1 Hz), 2.24 (3H, s), 3.69 (2H, s), 3.77 (3H, s), 4.13 (2H, q, *J*=7.1 Hz), 6.83-7.21 (10H, m). - MS (*m/z*): 465 (M^+, 100%), 450, 436, 420, 392, 224. For $C_{24}H_{23}N_3O_3S_2$ calculated: 61.91% C, 4.98% H, 9.02% N; found: 61.89% C, 5.04% H, 9.11% N.

***Ethyl {2-[3-(4-methoxyphenyl) - 4 - (4-nitrophenyl) - 3H-thiazol-2-ylideneamino] thiazol-4-yl} acetate* (2f)**

IR [n, cm^{-1}, KBr]: 1733 (C=O), 1633-1582 (C=N, C=C). - ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.23 (3H, t, *J*=7.1 Hz), 3.70 (2H, s), 3.74 (3H, s), 4.13 (2H, q, *J*=7.1 Hz), 6.87 (1H, s), 6.92-6.99 (4H, m), 7.17 (1H, m), 7.25 (2H, d, *J*=8.9 Hz), 7.54 (2H, d, *J*=8.9 Hz). - MS (*m/z*) 496 (M^+, 100%), 481, 467, 423, 407, 376, 352, 337, 291. For $C_{23}H_{20}N_4O_5S_2$ calculated: 55.63% C, 4.06% H, 11.28% N; found: 55.48% C, 3.93% H, 11.19% N.

***Ethyl {2-[3-(4-methoxyphenyl)-4-(4-methoxyphenyl) - 3H - thiazol - 2 - ylideneamino]thiazol - 4 - yl} acetate* (2g)**

IR [n, cm^{-1}, KBr]: 1723 (C=O), 1637-1585 (C=N, C=C). - ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.24 (3H, t, *J*=7.1 Hz), 3.69 (2H, s), 3.71 (6H, s), 4.15 (2H, q, *J*=7.1 Hz), 6.79-6.85 (4H, m), 7.09-7.18 (4H, m), 7.20-7.24 (2H, m). - MS (*m/z*) 481 (M^+, 100%), 466, 451, 412, 392, 326, 276, 221. For $C_{24}H_{23}N_3O_4S_2$ calculated: 59.86% C, 4.81% H, 8.73% N; found: 59.92% C, 4.78% H, 8.69% N.

***Ethyl {2-[3-(4-nitrophenyl)-4-(4-methoxyphenyl)-3H-thiazol-2-ylideneamino] thiazol-4-yl} acetate* (2h)**

IR [n, cm^{-1}, KBr]: 1722 (C=O), 1618-1573 (C=N, C=C). - ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.21 (3H, t, *J*=7.1 Hz), 3.79 (2H, s), 3.85 (3H, s), 4.12 (2H, q, *J*=7.1 Hz), 7.08 (2H, d, *J*=8.8 Hz), 7.28 (1H, s), 7.56 (2H, d, *J*=8.8 Hz), 7.86 (2H, d, *J*=9.3 Hz), 8.23 (3H, t, *J*=9.3 Hz). - MS (*m/z*): 496 (M^+, 100%), 467, 423, 407, 359, 331, 306, 285. For $C_{23}H_{20}N_4O_5S_2$ calculated: 55.63% C, 4.06% H, 11.28% N; found: 55.70% C, 4.11% H, 11.25% N.

MICROBIOLOGY

1. In vitro evaluation of antituberculosis activity

The primary screen was conducted against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) (21). Compounds were tested in 10 twofold dilutions, from 100 µg/mL to 0.19 µg/mL. Compounds effecting <90% inhibition in the primary screening were not generally evaluated further. The MIC value was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. This value was determined from the dose-response curve as the IC_{90} using a curve fitting program. Any IC_{90} value of ≤ 10 µg/mL was considered "Active" for antitubercular activity. Compounds active in the initial screen were tested for cytotoxicity in VERO cells. After 72 h exposure, viability was assessed using the CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTT) reagent from Promega. Cytotoxicity was determined from the dose-response curve as the IC_{50} using a curve fitting program. Concurrent with the determination of MICs, compounds were tested for IC_{50} in VERO cells at concentrations 10x the MIC for *M. tuberculosis* $H_{37}Rv$.

1.a. Microplate Alamar Blue Assay (MABA)

Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. Initial drug dilutions were prepared in either dimethyl sulfoxide or distilled deionized water, and subsequent twofold dilutions were performed in 0.1 mL of 7H9GC (no Tween 80) in the microplates. BACTEC 12B-passaged inocula were initially diluted 1:2 in 7H9GC, and 0.1 mL was added to wells. The determination of bacterial titer yielded 1×10^6 CFU/mL in plate well for $H_{37}Rv$. Frozen inocula were initially diluted 1:20 in BACTEC 12B medium followed by a 1:50 dilution in 7H9GC. Addition of 1/10 mL to wells resulted in final bacterial titer of 20×10^5 CFU/mL for $H_{37}Rv$. Wells containing drug only were used to detect autofluorescence of compounds. Additional control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37°C. Starting at day 4 of incubation, 20 µL of 10 x alamarBlue solution (Alamar Biosciences/Accumed, Westlake, Ohio) and 12.5 µL of 20% Tween

Table 2. Primary in vitro antituberculosis activity screening results of the compounds

Compounds	IC_{90} (µg/mL)	IC_{50} (µg/mL)	Activity
2a	> 100	> 100	Inactive
2b	> 100	> 100	Inactive
2c	> 100	> 100	Inactive
2d	> 100	> 100	Inactive
2e	> 100	70.337	Weakly active
2f	> 100	> 100	Inactive
2g	> 100	> 100	Inactive
2h	> 100	> 100	Inactive
Rifampin	0.125	> 100	Control Agent

80 were added to one B well and one M well, and plates were reincubated at 37°C. Wells were observed 12 and 24 h later for a color change from blue to pink and for a reading of ≥50,000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, Mass.) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. If the B wells become pink after 24 h, the reagent is being added to the entire plate. If the well-remain blue or ≥50,000 FU is measured, additional M and B wells are tested daily until a color change occurred, when reagents are added to all remaining wells. Plates were then incubated at 37°C, and results were recorded at 24 h post-reagent addition. Visual MICs were defined as the lowest concentration of drug that prevented a color change. For fluorometric MICs, a background subtraction was performed on all wells with a mean of triplicate M wells. Percent inhibition was defined as (1 - (test well FU/mean FU of triplicate B wells) x 100). The lowest drug concentration effecting an inhibition of ≥90% was considered the MIC.

1.b. BACTEC *radiometric assay*

A total of 1/10 ml of BACTEC 12B-passaged inoculum was delivered without prior dilution into 4 mL of test medium. The determination of bacterial titer yielded average titer of 1×10^5 CFU/ml of BACTEC 12B medium for $H_{37}Rv$. Frozen inocula were initially diluted 1:20 in BACTEC 12B medium, and then 0.1 mL was delivered to the test medium. This yielded 5.0×10^5 CFU per BACTEC vial for $H_{37}Rv$. Twofold drug dilutions were prepared in either dimethylsulfoxide or deionized water and delivered via a 0.5-mL insulin syringe in a 50-µL volume. Drug-free control vials consisted of solvent with bacterial inoculum and solvent with a 1:100 dilution of bacterial inoculum (1:100 controls). Vials were incubated at 37°C, and the growth index (GI) was determined in a BACTEC 460 instrument (Becton-Dickinson) until the growth index (GI) of the 1:100 controls reached at least 30. All vials were read the following day, and the growth index (GI) and daily change in growth index (GI) (ΔGI) were recorded for each drug dilution. The MIC was defined as the lowest concentration for which the ΔGI was less than the ΔGI of the 1:100 control. If the growth index (GI) of the test sample was greater than 100, the sample was scored as resistant even if the ΔGI was less than the ΔGI of the 1:100 control.

RESULTS AND DISCUSSION

In this study, eight new compounds were synthesized. Ethyl [2-(3-aryl(thiouredio)thiazol-4-yl]acetate (**1**) were prepared by reacting ethyl (2-aminothiazol-4-yl)acetate with 4-substituted phenylisothiocyanate in according to the method described in the literature[10]. The reaction of ethyl [2-(3-aryl(thiouredio)thiazol-4-yl]acetate (**1**) and phenacyl bromides gave the ethyl {2-[3,4-diaryl-3H-thiazol-2-ylideneamino]thiazol-4-yl}acetate **(2a-h)** as shown in Figure 1.

The structures of compounds **(2a-h)** were confirmed by IR, ^{1}H-NMR and MS spectral data. The IR data were very informative and provided evidence for the formation of the expected structures. C=O, C=N and C=C functions absorbed strongly in the expected regions: C=O at 1734-1722 cm^{-1}, C=N and C=C at 1639-1556 cm^{-1}, respectively. In the ^{1}H-NMR spectra of the compounds, the signal due to the *CH_3-$\underline{CH_2}$-O-CO-* methylene protons present in all compounds appeared at 4.12–4.15 ppm, as quartet. The CH_3 protons of ester were observed at 1.21-1.24 ppm as triplet. The *CO-$\underline{CH_2}$-* methylene protons present in all compounds appeared at 3.68-3.79 ppm, as singlet. All the other aromatic and aliphatic protons were observed at expected regions. The mass spectra (MS)

of compounds (**2a-h**) are also in agreement with their molecular formula.

The compounds **2a-h** were also evaluated for antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) using the BACTEC 460 radiometric system and BACTEC 12B medium. The preliminary results indicated that all of the tested compounds were inactive against the test organism.

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