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ORIGINAL RESEARCH

Synthesis and evaluation of new imidazo[2,1-*b*]thiazoles as antituberculosis agents

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ABSTRACT

New *N*'-(arylidene)-2-[6-(4-bromophenyl)imidazo[2,1-*b*]thiazol-3-yl]acetohydrazides (**3a**-**i**) were synthesized by reacting 2-[6-(4-bromophenyl)imidazo[2,1-*b*]thiazol-3-yl]acetohydrazide with different aromatic aldehydes. The structures of the title compounds were established by spectral data (IR, ^{1}H NMR, ^{13}C NMR) and elemental analyses. The synthesized compounds were evaluated for *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv employing the BACTEC 460 radiometric system. The compounds exhibited varying degrees of inhibition in the *in vitro* primary screening that was conducted at a concentration of 6.25 μg/ml. Among the synthesized compounds [6-(4-bromophenyl)imidazo[2,1-*b*]thiazol-3-yl]acetic acid 2,4-dinitrobenzylidenehydrazide (**3e**) was found to be the most active compound *in vitro* with MIC of 6.25 μg/ml.

Keywords: Imidazo[2,1-*b*]thiazole, hydrazone, antimycobacterial activity

INTRODUCTION

Tuberculosis (TB), which is caused by *Mycobacterium tuberculosis*, remains one of the deadliest communicable diseases for humans, and it has been identified by the World Health Organization (WHO) as one of the three priority diseases for drug research and development. In 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease, 360 000 of whom were HIV-positive (1). However, this problem has become serious as *Mycobacterium tuberculosis* developed resistance against both the first line as also the second line drugs. Due to this, there is emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *M. tuberculosis* all over the world (2).

TB may become an incurable disease. Therefore, there is an urgent need of finding newer anti-mycobacterial agents to combat this problem. A number of studies on the synthesis and biological activities of the condensed imidazo[2,1-*b*]thiazoles has been reported since the discovery of novel broad spectrum antihelmintic and immunomodulator drug, Levamisole (I) (3). In addition, the imidazo[2,1-*b*]thiazole derivatives of Levamisole have been reported as potential antitubercular (II) (4, 5), anticancer (III) (6), acetylcholinesterase (AChE)

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inhibitor (IV) (7), cardiodepressant (V) (8), anthelmintic and anti-inflammatory (VI) (9), anti-infectious (VII) (10), antiviral (VIII) (11) agents (**Figure 1**).

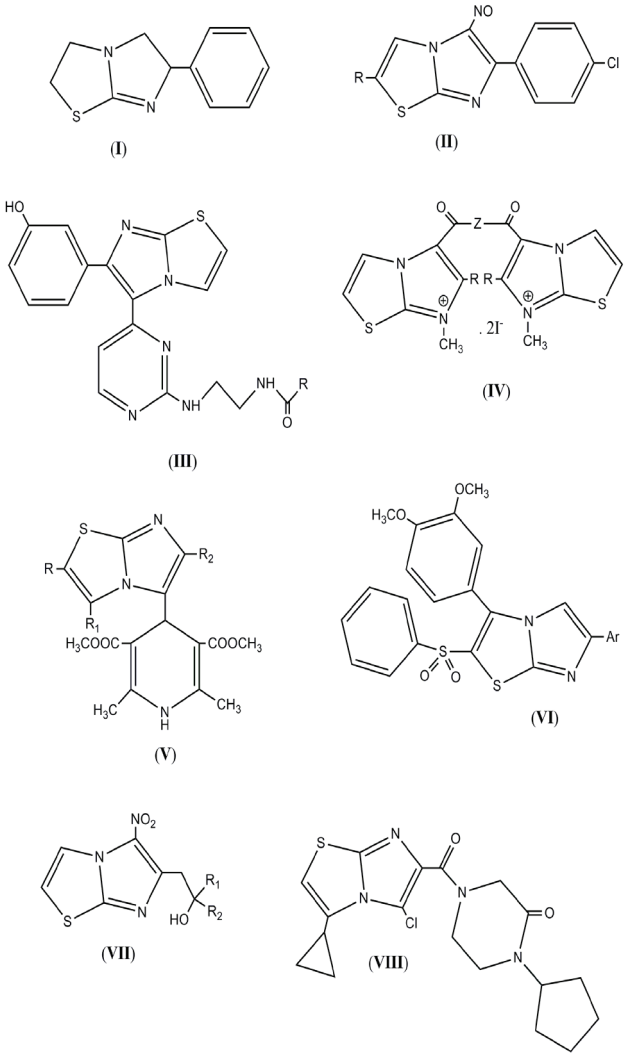


Figure 1. Chemical structures of some bioactive molecules bearing an imidazo[2,1-*b*]thiazole core.

On the other hand, hydrazide-hydrazones (12-17) have been a subject of interest in medicinal chemistry as a group with a wide range of biological properties including antimycobacterial activity. In view of these facts and as a continuation of our research on the antitubercular (18-21) and the other biological properties of imidazo[2,1-*b*]thiazoles, we here report the synthesis, structural determination and antitubercular activity evaluation of some new compounds bearing imidazo[2,1-*b*]thiazole moiety (**Figure 2**).

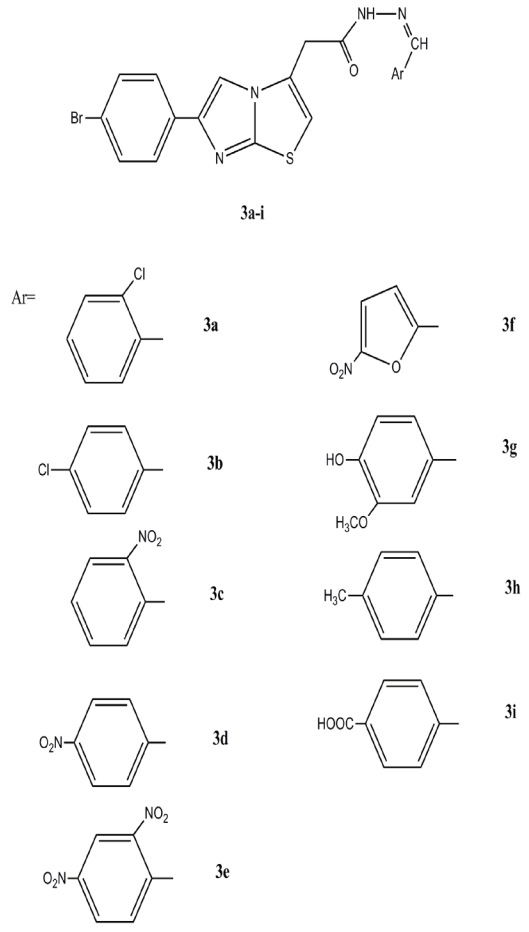


Figure 2. *N*'-(arylidene)-2-[6-(4-bromophenylimidazo[2,1-*b*]thiazol-3-yl]acetohydrazide derivatives (**3a-i**).

MATERIALS AND METHODS

Chemistry

Melting points were determinated with a Büchi B-540 melting point apparatus (Flawil, Switzerland) in open capillaries and are uncorrected. Elemental analyses were performed on a LECO CHNS 932 elemental analyser (St. Joseph, Michigan). IR spectra were recorded on KBr discs, using a Perkin Elmer Model 1600 FT–IR spectrophotometer (Norwalk, Connecticut, USA). ^{1}H-NMR spectra were obtained on Bruker DPX 400 (400 MHz) spectrophotometer (Rheinstetten, Germany) using DMSO–d_6.

Preparation of ethyl 6-(4-bromophenyl)imidazo[2,1-*b*] thiazole-3-acetate hydrobromide (1)

Compound **1** was obtained according to the procedure described by Robert *et al.* (22).

Preparation of 2-[6-(4-bromophenyl)imidazo[2,1-*b*] thiazol-3-yl]acetohydrazide (2)

Compound **2** was prepared according to the procedure described by Kühmstedt *et al.* (23).

General procedure for preparation of *N*'-(arylidene)-2-[6-(4-bromophenyl)imidazo[2,1-*b*]thiazol-3-yl] acetohydrazide derivatives (3a-i)

A solution of 0.005 mol of **2** and equimolar amount of appropriate aldehyde in 30 ml of ethanol was heated for 5h. The precipitate obtained was filtered off, washed with boiling ethanol.

N'-(2-chlorobenzylidene)-2-[6-(4-bromophenyl) imidazo[2,1-b]thiazol-3-yl]acetohydrazide ***(3a)***

Yield: 97%, M.p. 255-258°C, IR (KBr) ν_{max} (cm^{-1}): 3204, 3135, 3065 (N-H, ar C-H); 1689 (C=O); 1595 (hydrazone C=N). ^{1}H NMR (400 MHz, DMSO-d_6): 3.95, 4.37 (2s, 2H, CH_2CO), 7.11 (s, 1H, C_2-H), 7.39- 7.47 (m, 2H, ar $C_{3,5}$-H), 7.53-7.59 (m, 3H, Br-Ph $C_{3,5}$-H, ar C_4-H), 7.76-7.81 (m, 2H, Br-Ph $C_{2,6}$-H), 7.95, 8.04 (2dd, 1H, *J*=7.81, 7.32 Hz, *J*=1.95, 1.96 Hz, ar C_6-H), 8.30, 8.31 (2s, 1H, C_5-H), 8.46, 8.64 (2s, 1H, N=CH), 11.84, 12.00 (2s, 1H, CONH). For $C_{20}H_{14}BrClN_4OS$ calculated: C 50.70, H 2.98, N 11.83; found: C 50.37, H 3.24, N 12.16.

N'-(4-chlorobenzylidene)-2-[6-(4-bromophenyl) imidazo[2,1-b]thiazol-3-yl]acetohydrazide ***(3b)***

Yield: 97%, M.p. 257-259°C, IR (KBr) ν_{max} (cm^{-1}): 3197, 3131, 3068 (N-H, ar C-H); 1681 (C=O); 1596 (hydrazone C=N). ^{1}H NMR (400 MHz, DMSO-d_6): 3.92, 4.34 (2s, 2H, CH_2CO), 7.09 (s, 1H, C_2-H), 7.49, 7.51 (2d, 2H, *J*=8.52, 8.87 Hz, ar $C_{3,5}$-H), 7.55, 7.57 (2d, 2H, *J*=8.51, 7.13 Hz, Br-Ph $C_{3,5}$-H), 7.71-7.80 (m, 4H, Br-Ph $C_{2,6}$-H, ar $C_{2,6}$-H), 8.05, 8.24 (2s, 1H, N=CH), 8.26, 8.28 (2s, 1H, C_5-H), 11.68, 11.79 (2s, 1H, CONH). For $C_{20}H_{14}BrClN_4OS$ calculated: C 50.70, H 2.98, N 11.83; found: C 50.73, H 2.56, N 11.97.

N'-(2-nitrobenzylidene)-2-[6-(4-bromophenyl)imidazo[2,1-b] thiazol-3-yl]acetohydrazide ***(3c)***

Yield: 94%, M.p. 242-244°C, IR (KBr) ν_{max} (cm^{-1}): 3566, 3446 (cry. O-H); 3196, 3131, 3109, 3062 (N-H, ar C-H); 1672 (C=O); 1566 (hydrazone C=N). ^{1}H NMR (400 MHz, DMSO-d_6): 3.71, 4.10 (2s, 2H, CH_2CO), 6.86, 6.87 (2s, 1H, C_2-H), 7.32, 7.33 (2d, 2H, *J*=8.40, 7.82 Hz, Br-Ph $C_{3,5}$-H), 7.40, 7.44 (2d, 1H, *J*=7.49, 7.10, Hz, ar C_4-H), 7.52-7.56 (m, 3H, Br-Ph $C_{2,6}$-H, ar C_5-H), 7.80, 7.84 (2d, 1H, *J*=8.61, 8.49 Hz, ar C_6-H), 7.89 (d, 1H, *J*=7.69 Hz, ar C_3-H), 8.06 (s, 1H, C_5-H), 8.20, 8.41 (2s, 1H, N=CH), 11.70, 11.87 (2s, 1H, CONH). ^{13}C-NMR [100 MHz, δ ppm, DMSO-d_6]: 32.69, 34.16 (CH_2), 109.91, 110.11 (C_5), 111.34, 111.59 (C_2), 120.58, 120.67 (Br-Ph C_4), 125.46, 125.60 (ar C_3), 126.97, 127.36 (C_3), 127.44, 127.50 (Br-Ph $C_{2,6}$), 128.92, 129.13 (ar C_6), 129.48 (ar C_1), 131.44, 131.61 (ar C_5), 132.39, 132.42 (Br-Ph $C_{3,5}$), 134.39, 134.48 (Br-Ph C_1), 134.43, 134.69 (ar C_4), 140.00, 143.46 (N=CH), 145.58, 145.69 (C_6), 148.94, 149.03 (ar C_2), 149.64, 149.71 (C_{7a}), 164.60, 170.26 (CONH). For $C_{20}H_{14}BrN_5O_3S.½ H_2O$ calculated: C 48.69, H 3.06, N 14.19; found: C 48.70, H 3.00, N 14.30.

N'-(4-nitrobenzylidene)-2-[6-(4-bromophenyl)imidazo[2,1-b] thiazol-3-yl]acetohydrazide ***(3d)***

Yield: 93%, M.p. 266-268°C, IR (KBr) ν_{max} (cm^{-1}): 3563 (cry. O-H); 3193, 3143, 3076 (N-H, ar C-H); 1681 (C=O); 1595 (hydrazone C=N). ^{1}H NMR (400 MHz, DMSO-d_6): 3.96, 4.39 (2s, 2H, CH_2CO), 7.11 (s, 1H, C_2-H), 7.56 (d, 2H, *J*=8.46 Hz, Br-Ph $C_{3,5}$-H), 7.77, 7.79 (2d, 2H, *J*=8.44, 7.05, Hz, Br-Ph $C_{2,6}$-H), 7.97, 8.00 (2d, 2H, *J*=8.80, 8.81 Hz, ar $C_{2,6}$-H), 8.12, 8.16 (2s, 1H, C_5-H), 8.27, 8.28 (2d, 2H, *J*=8.68, 9.11 Hz, ar $C_{3,5}$-H), 8.35, 8.85 (2s, 1H, N=CH), 11.92, 12.02 (2s, 1H, CONH). For $C_{20}H_{14}BrN_5O_3S.H_2O$ calculated: C 47.81, H 3.20, N 13.94; found: C 47.14, H 2.55, N 14.29.

N'-(2,4-dinitrobenzylidene)-2-[6-(4-bromophenyl) imidazo[2,1-b]thiazol-3-yl]acetohydrazide ***(3e)***

Yield: 94%, M.p. 254-257°C, IR (KBr) ν_{max} (cm^{-1}): 3110 (N-H, ar C-H); 1674 (C=O); 1600 (hydrazone C=N). ^{1}H NMR (400 MHz, DMSO-d_6): 3.90, 4.28 (2s, 2H, CH_2CO), 7.02 (2s, 1H, C_2-H), 7.47 (d, 2H, *J*=8.41 Hz, Br-Ph $C_{3,5}$-H), 7.68 (d, 2H, *J*=8.34 Hz, Br-Ph $C_{2,6}$-H), 8.19, 8.22 (2s, 1H, C_5-H), 8.30 (d, 1H, *J*=8.75 Hz, ar C_6-H), 8.40-8.61 (m, 2H, ar C_5-H, N=CH), 8.70 (d, 1H, *J*=2.23 Hz, ar C_3-H), 13.06 (s, 1H, CONH). For $C_{20}H_{13}BrN_6O_5S$ calculated: C 45.38, H 2.48, N 15.88; found: C 45.35, H 1.98, N 16.27.

N'-(5-nitro-2-furfurylidene)-2-[6-(4-bromophenyl) imidazo[2,1-b]thiazol-3-yl]acetohydrazid ***(3f)***

Yield: 51%, M.p. 250-252°C, IR (KBr) ν_{max} (cm^{-1}): 3201, 3019 (N-H, ar C-H); 1674 (C=O); 1566 (hydrazone C=N). ^{1}H NMR (400 MHz, DMSO-d_6): 3.97, 4.33 (2s, 2H, CH_2CO), 7.01, 7.11 (2s, 1H, C_2-H), 7.24, 7.29 (2d, 1H, *J*=3.93, 3.95 Hz, furan C_3-H), 7.56, 7.58 (2d, 2H, *J*=8.60, 8.98 Hz, Br-Ph $C_{3,5}$-H), 7.75-7.81 (m, 3H, Br-Ph $C_{2,6}$-H ve furan C_4-H), 8.02, 8.19 (2s, 1H, N=CH), 8.25, 8.27 (2s, 1H, C_5-H), 12.02, 12.10 (2s, 1H, CONH). For $C_{18}H_{12}BrN_5O_4S$ calculated: C 45.58, H 2.55, N 14.77; found: C 45.40, H 2.70, N 13.93.

N'-(3-methoxy-4-hydroxybenzylidene)-2-[6-(4-bromophenyl) imidazo[2,1-b]thiazol-3-yl]acetohydrazide ***(3g)***

Yield: 86%, M.p. 256-258°C, IR (KBr) ν_{max} (cm^{-1}): 3489 (O-H); 3138, 3108, 3058 (N-H, ar C-H); 1682 (C=O); 1611 (hyrazone C=N). ^{1}H NMR (400 MHz, DMSO-d_6): 3.78 (s, 3H, ar C_3-OCH_3), 3.87, 4.31 (2s, 2H, CH_2CO), 6.80, 6.82 (2s, 1H, C_2-H), 7.07-7.12 (m, 2H, ar $C_{2,5}$-H), 7.27 (d, 1H, *J*=9.52 Hz, ar C_6-H), 7.54, 7.57 (2d, 2H, *J*=8.50, 8.63 Hz, Br-Ph $C_{3,5}$-H), 7.74, 7.79 (2d, 2H, *J*=8.50, 8.45 Hz, Br-Ph $C_{2,6}$-H), 7.94, 8.11 (2s, 1H, N=CH), 8.26, 8.28 (2s, 1H, C_5-H), 9.38 (broad s, 1H, ar C_4-OH), 11.12, 11.83 (2s, 1H, CONH). For $C_{21}H_{17}BrN_4O_3S$ calculated: C 51.97, H 3.53, N 11.54; found: C 52.39, H 3.48, N 11.74.

*N'-(4-methylbenzylidene)-2-[6-(4-bromophenyl) imidazo[2,1-b]thiazol-3-yl]acetohydrazide (**3h**)*

Yield: 93%, M.p. 245-246°C, IR (KBr) ν_{max} (cm^{-1}): 3139, 3078 (N-H, ar C-H); 1680 (C=O); 1604 (hydrazone C=N). ^{1}H NMR (400 MHz, DMSO-d_6): 2.34 (s, 3H, ar C_4-CH_3), 3.90, 4.32 (2s, 2H, CH_2CO), 7.09 (s, 1H, C_2-H), 7.25 (d, 2H, *J*=7.91 Hz, ar $C_{3,5}$-H), 7.54-7.63 (m, 4H, Br-Ph $C_{3,5}$-H, ar $C_{2,6}$-H), 7.76, 7.79 (2d, 2H, *J*=8.51, 8.60 Hz, Br-Ph $C_{2,6}$-H), 8.03, 8.20 (2s, 1H, N=CH), 8.27, 8.28 (2s, 1H, C_5-H), 11.55, 11.65 (2s, 1H, CONH). ^{13}C-NMR [100 MHz, δ ppm, DMSO-d_6]: 21.89 (CH_3), 32.71, 34.22 (CH_2), 109.85, 110.15 (C_5), 111.15, 111.39 (C_2), 120.56, 120.67 (Br-Ph C_4), 127.23 (ar C_1), 127.45, 127.51 (Br-Ph $C_{2,6}$), 127.23, 127.62 (C_3), 127.77, 127.97 (ar $C_{3,5}$), 130.30 (ar $C_{2,6}$), 132.22, 132.39 (Br-Ph $C_{3,5}$), 134.39, 134.50 (Br-Ph C_1), 140.62, 140.85 (ar C_4), 145.54, 145.69 (C_6), 144.62, 147.95 (N=CH), 149.63, 149.72 (C_{7a}), 164.04, 169.79 (CONH). For $C_{21}H_{17}BrN_4OS$ calculated: C 55.64, H 3.78, N 12.36; found: C 55.79, H 3.84, N 12.21.

*N'-(4-carboxybenzylidene)-2-[6-(4-bromophenyl) imidazo[2,1-b]thiazol-3-yl]acetohydrazide (**3i**)*

Yield: 94%, M.p. 293-295°C, IR (KBr) ν_{max} (cm^{-1}): 3470 (O-H); 3080 (N-H, ar C-H); 1670 (C=O); 1602 (hyrazone C=N). ^{1}H NMR (400 MHz, DMSO-d_6): 3.95, 4.37 (2s, 2H, CH_2CO), 7.11 (s, 1H, C_2-H), 7.55 (d, 2H, *J*=8.43 Hz, Br-Ph $C_{3,5}$-H), 7.77 (d, 2H, *J*=8.46 Hz, Br-Ph $C_{2,6}$-H), 7.83-7.97 (m, 4H, ar $C_{2,6}$-H, ar $C_{3,5}$-H), 8.02, 8.12 (2s, 1H, N=CH), 8.30, 8.32 (2s, 1H, C_5-H), 11.83, 11.95 (2s, 1H, CONH), 13.12 (broad s, 1H, COOH). For $C_{21}H_{15}BrN_4O_3S$ calculated: C 52.18, H 3.13, N 11.59; found: C 52.35, H 3.18, N 11.39.

Biological activity

Antimycobacterial assay

In vitro evaluation of antituberculosis activity

Primary screening was conducted at 6.25 μg/ml against *Mycobacterium tuberculosis* H37Rv in BACTEC 12B medium using a broth microdilution assay the Microplate Alamar Blue Assay (MABA) (24). Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system (25). Compounds effecting <90 % inhibition in the primary screen were not generally evaluated further. Compounds demonstrating at least 90 % inhibition in the primary screen were re-tested at lower concentrations against *M. tuberculosis* H37Rv in order to determine the actual minimum inhibitory concentration (MIC) using MABA. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90 % relative to the controls. Concurrently with the determination of MICs, compounds were tested for cytotoxicity (IC50) in VERO cells at concentrations ≤ 6.25 μg/ml or 10 times the MIC for *M. tuberculosis* H37Rv (solubility in media permitting). After 72 h exposure, viability was assessed on the basis of cellular conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a formazan product using the Promega CellTiter 96 Non-radioactive Cell Proliferation Assay. Compounds for which the selectivity index (IC50: MIC ratio) SI >10 were assumed to possess *in vitro* activity confirmed in the BACTEC 460 at 6.25 μg/ml.

Microplate alamar blue susceptibility assay (MABA)

Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument, Meriden, Connecticut, USA) 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. Subsequent determination of bacterial titers yield 1×10^6 CFU/ml in plate wells for H37Rv. 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 a final bacterial titers of 2.0×10^5 CFU/ ml for H37Rv. Wells containing drug only were used to detect autofluorescence of compounds. Addition 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× Alamar Blue solution (Alamar Biosciences/ Accumed, Westlake, Ohio, USA) and 12.5 μl of 20% Tween 80 were added to one B well an done M well, and plates were reincubated 37°C. Wells were observed at 12 and 24 h 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, Massachusetts, USA) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. If the B wells became pink by 24 h, reagent was added to the entire plate. If the well remained blue or ≤ 50,000 FU was measured, additional M and B wells were tested daily until a color change occurred, at which time reagents were 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 had 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 triplicate B wells) × 100. The lowest drug concentration effecting an inhibition of ≥90% was considered the MIC.

BACTEC radiometric method of susceptibility testing

Inocula for susceptibility testing were either from a positive BACTEC isolation vial with a growth index (GI) of 500 or more, or a suspension of organisms isolated earlier on a conventional medium. The culture was well mixed with a syringe and 0.1 ml of a positive BACTEC culture was added to each of the vials containing the test compounds (6.25 μg/ml). The Standard vials contained rifampicin (RMP) (0.25 μg/ml). A control vial was inoculated with a 1:100 dilution of the culture. Each vial was tested immediately on a BACTEC instrument to provide CO_2 in the headspace. The vials were incubated at 37°C and tested daily with a BACTEC instrument. When the GI in the control read at least 30, the increase in GI (Δ GI) from the previous day in the control was compared with that in the drug vial. The following formula was used to interpret the results:

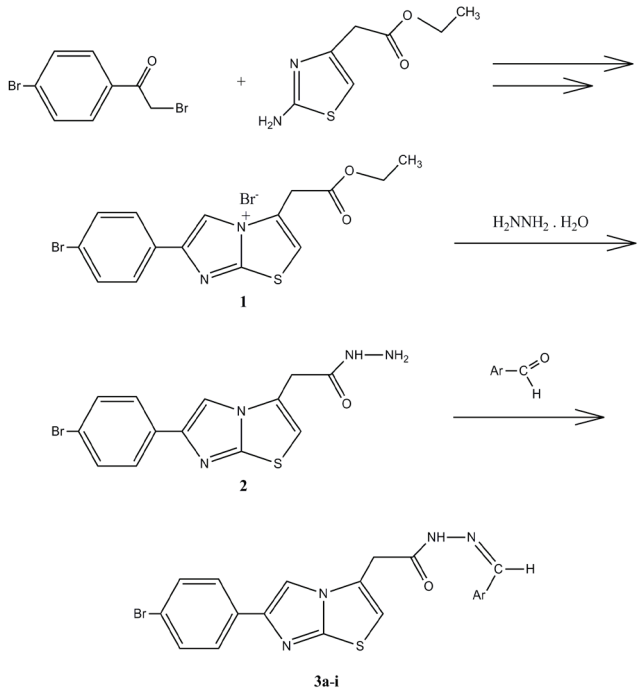
Δ GI control > Δ GI drug = susceptible

Δ GI control < Δ GI drug = resistant

If a clear susceptibility pattern (the difference of Δ GI of control and the drug bottle) was not seen at the time the control GI was 30 the vials were read for 1 or 2 additional days to establish a definite pattern of Δ GI differences.

RESULTS AND DISCUSSION

In this work, we synthesized a series of *N*'-(arylidene)-2-[6-(4-bromophenyl)imidazo[2,1-*b*]thiazol-3-yl] acetohydrazides. The general procedure for the preparation of target compounds **3a-i** were described in Scheme 1.



Scheme 1. The synthetic route for preparation of **3a**-**i**.

The starting compounds **1**, **2** were prepared according to the literature methods. Thus ethyl 6-(4-bromophenyl) imidazo[2,1-*b*]thiazole-3-acetate hydrobromide **1** (22, 26). was reacted with hydrazine hydrate to give the 2-[6-(4-bromophenyl)imidazo[2,1-*b*]thiazol-3-yl] acetohydrazide **2** (23, 27). The reaction of appropriate aromatic aldehydes with **2** yielded [6-(4-bromophenyl) imidazo[2,1-*b*]thiazol-3-yl]acetic acid arylidenehydrazides (**3a-i**) (28). Structures of the synthesized compounds were established on the basis of IR, ^{1}H-NMR, ^{13}C-NMR spectral data. IR spectra of the compounds showed characteristic bands at 3204-3080 cm^{-1} and 1689-1670 cm^{-1} corresponding to N-H and C=O groups, respectively. Compounds **3g** and **3i** showed broad absorption band at 3489 cm^{-1} and 3470 cm^{-1} for O-H, in their respective IR spectra. The absorption bands associated with other functional groups appeared in the expected regions. The ^{1}H-NMR spectral data were also consistent with the assigned structures. ^{1}H-NMR spectra of compounds revealed the presence of two geometric isomers as concluded from the NH, N=CH and CH_2 protons resonating as double singlets at about δ 12.02-11.12 / 12.10-11.40, 8.36-7.80 / 8.85-7.96 and δ 3.97-3.71 / 4.39-4.10 ppm (29), respectively. The signals of these protons appeared as a pair of signals owing to different steric arrangement of hydrazide-hydrazone functionality in the stereoisomers. This steric arrangement might be attributed to the hindered

rotation of azomethine linkage (30). It is assumed that the N=CH double bond restricts rotation and gives rise to the formation of *E* and *Z* isomers. ^{13}C-NMR spectra of **3c** and **3h** chosen as prototypes verified the proposed hyrazide-hydrazone structure.

Compounds **3a-3i** were tested for *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}R_V$ using the BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA). Rifampicin was used as the standard in the antimycobacterial assays. As shown in Table 1, all of the compounds exhibited varying degrees of inhibition in the *in vitro* primary screening that was conducted at 6.25 μg/ml. The compounds which exhibited < 90% inhibition in the primary screen (MIC >6.25 μg/ml) were not evaluated further. Compounds **3a**, **3c**, **3e** and **3f** effecting >90% inhibition in the primary screen at 6.25 μg/ml were re-tested at lower concentrations against *Mycobacterium tuberculosis* $H_{37}R_V$ to determine the actual MIC using MABA. MIC values of these compounds were found to be 6.25 or >6.25 μg/ml (Table 2). The level I and level II antimycobacterial activity results showed that the most active compound **3e** against *Mycobacterium tuberculosis* $H_{37}R_V$ at 6.25 μg/ml. The same compound was also tested for cytotoxicity (IC_{50}) in VERO cells at concentrations equal to and greater than the MIC for *Mycobacterium tuberculosis* $H_{37}R_V$. The IC_{50} value was found at a concentration level of >10 μg/ml for compound **3e** and the resulting selectivity index (SI= IC_{50}/MIC) was calculated as >1.6 (Table 3), showing that this compound not only displayed a considerable antimycobacterial activity, but also had a remarkable cytotoxicity.

Table 1. Primary antimycobacterial activity screening results of **3a**-**3i** (Level I)

Compound	Ar	MIC (μg/ml)	Assay	Inhibition (%)
3a	C_6H_4Cl(2-)	<6.25	Alamar	95
3b	C_6H_4Cl(4-)	>6.25	Alamar	89
3c	$C_6H_4NO_2$(2-)	<6.25	Alamar	91
3d	$C_6H_4NO_2$(4-)	>6.25	Alamar	52
3e	$C_6H_3NO_2$(2,4-)	<6.25	Alamar	99
3f	5-nitro-2-furyl	<6.25	Alamar	98
3g	$C_6H_3(OCH_3)(OH)$(3,4-)	>6.25	Alamar	84
3h	$C_6H_4CH_3$(4-)	>6.25	Alamar	1
3i	C_6H_4COOH(4-)	>6.25	Alamar	86
Rifampicin	-	0.25	Alamar	98

Table 2. MIC assay results of **3a**, **3c**, **3e** and **3f** (Level II)

Compound	MIC (μg/ml)	Inhibition (%)
3a	>6.25	95
3c	>6.25	91
3e	6.25	99
3f	>6.25	98

Table 3. Cytotoxicity assay results of **3a**, **3c**, **3e** and **3f** (Level II)

Compound	MIC (μg/ml)	Inhibition (%)	IC_{50} (μg/ml)	SI
3a	>6.25	95	9.8	-
3c	>6.25	91	1.05	-
3e	6.25	99	>10	>1.6
3f	>6.25	98	9.88	-

A remarkable activity was found in compound **3f** carrying 5-nitro-2-furyl moiety. Compound **3a** having orto chlorophenyl was more active than the corresponding orto nitrophenyl derivative. The most active disubstituted derivative was **3e** having 2,4-dinitrophenyl moiety. The results obtained show that some of the prepared and tested compounds especially the chlorophenyl/nitrophenyl/furyl derivatives may be considered promising for the development of new antitubercular agents.

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Yeni imidaz o[2,1-*b*]tiyazol türevlerinin sentezi ve antitüberküler etkilerinin değerlendirilmesi

ÖZ

2-[6-(4-Bromofenil)imidazo[2,1-*b*]tiyazol-3-il]asetohidrazit ile farklı aromatik aldehitlerin reaksiyonundan yeni *N*'-(ariliden)-2-[6-(4-bromofenil)imidazo[2,1-*b*]tiyazol-3-il] asetohidrazitler (**3a**-**i**) elde edilmiştir. Elde edilen bileşiklerin yapıları spektral veriler (IR, ^{1}H NMR, ^{13}C NMR) ve elementel analiz ile saptanmıştır. Sentezlenen bileşiklerin *Mycobacterium tuberculosis* H37Rv'ye karşı antimikobakteriyel aktiviteleri BACTEC 460 radyometrik sistem kullanılarak tayin edilmiştir. Bileşikler, 6.25 µg/ml konsantrasyonda gerçekleştirilen *in vitro* primer tarama testlerinde değişik derecelerde inhibisyon göstermişlerdir. Sentezlenen bileşikler arasında [6-(4-bromofenil)imidazo[2,1-*b*]tiyazol-3-il]asetik asit 2,4-dinitrobenzilidenhidrazit (**3e**), *in vitro* 6.25 µg/ml MIC değeri ile aktivitesi en yüksek bileşik olarak bulunmuştur.

Anahtar kelimeler: İmidazo[2,1-*b*]tiyazol, hidrazon, antimikobakteriyel aktivite

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