Antibacterial, Antitubercular and Antiviral Activity Evaluations of Some Arylidenehydrazide Derivatives Bearing Imidazo[2,1-b]thiazole Moiety

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ABSTRACT

Objectives: The aim of this study was to determine the probable antibacterial, antitubercular, and antiviral activities of some N\textsuperscript{2}-arylidene-(6-(4-chlorophenyl)imidazo[2,1-b]thiazol-3-yl) acetic acid hydrazides (3a-j). Further structural optimization of the identified lead structures can lead us to new more active potential antibacterial, antitubercular, and antiviral agents.

Materials and Methods: Antibacterial activities of the title compounds against Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 25922. These molecules were also evaluated for their in vitro antitubercular activity against Mycobacterium tuberculosis H37Rv (ATCC 27294) using the BACTEC 460 radiometric system and BACTEC 12B medium. Moreover, all the compounds (3a-j) were also evaluated against some DNA and RNA viruses in Madin-Darby Canine Kidney, Crandell-Rees Feline Kidney (CRFK), Vero, human embryonic lung (HEL) and HeLa cells.

Results: Among the tested compounds, 3i displayed the highest efficacy against S. aureus and E. coli. Compound 3j, 5-nitro-2-furfurylidene derivative showed the highest antituberculosis activity (IC\textsubscript{50}: 6.16 μg/mL and IC\textsubscript{90}: 14.390 μg/mL). Compound 3i showed the most potent antiviral activity against feline corona virus in CRFK cell cultures (antiviral EC\textsubscript{50}: 7.5 μM and SI>13). Furthermore, compounds 3c and 3g displayed activity against herpes simplex virus-1 and vaccinia virus in HEL cell cultures (antiviral EC\textsubscript{50} values of 9; 16 and 20; 14 μM, respectively).

Conclusion: On the basis of aforementioned results, it can be concluded that imidazo[2,1-b]thiazole derivatives bearing hydrazone moieties serve as promising chemical probes to design therapeutic agents with antibacterial, antitubercular, and antiviral properties.

Key words: Imidazo[2,1-b]thiazole, arylidenehydrazide, antibacterial activity, antitubercular activity, antiviral activity

ÖZ

Amaç: Bu çalışmanın amacı, bazı N\textsuperscript{2}-ariliden-(6-(4-klorofenil)imidazo[2,1-b]thiazol-3-yl) asetik asit hidrazitlerinin (3a-j) olası antibakteriyel, antitüberküler ve antiviral aktivitelerinin tayin edilmesidir. Tanımlanmış yapıların ileri yapısal optimizasyonu, bizi daha aktif potansiyel antibakteriyel, antitüberküler ve antiviral ajanlara ulaştırabilir.

Gereç ve Yöntemler: Söz konusu bileşiklerin antibakteriyel aktiviteleri, Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853 ve Escherichia coli ATCC 25922’ye karşı tayin edilmiştir. Bu moleküllerin, Mycobacterium tuberculosis H37Rv (ATCC 27294) karşı in vitro antitüberküler aktiviteleri de BACTEC 460 radiometrik sistem ve BACTEC 12B ortamı kullanılarak tayin edilmiştir. Dahasi, bileşiklerin tümü (3a-j), Madin-Darby Canine Kidney, Crandell-Rees Feline Kidney (CRFK), Vero, insan embriyonik akciğerleri (HEL) ve HeLa hücrelerinde bazı DNA ve RNA virüslerine karşı tayin edilmiştir.
INTRODUCTION
Infectious diseases caused by bacteria have increased dramatically in recent years. Despite many significant advances in antibacterial therapy, the widespread use and misuse of antibiotics have led to the emergence of bacterial resistance to antibiotics, which is a serious threat to public health. On the other hand, tuberculosis (TB), still remains the leading cause of worldwide death among infectious diseases. In 2014, there were an estimated 9.6 million new TB cases: 5.4 million among men, 3.2 million among women and 1.0 million among children. Additionally, viral infections caused by the rapid emergence of antiviral drug resistant strains have become a serious threat globally. Many diseases are actually caused by the different members of DNA- and RNA-containing viruses. Among DNA-containing viruses, the herpes group of viruses, particularly herpes simplex virus-1 (HSV-1) primarily causes encephalitis, stomatitis, ocular infections and HSV-2 primarily causes genital lesions, skin eruptions or cytomegalovirus is related with severe morbidity and mortality in patients at risk for disease because of immune system disabilities and varicella-zoster virus is the ethiological agent of chickenpox and shingles. Influenza (INF) viruses, parainfluenza-3 virus, alphaviruses (e.g. sindbis virus), respiratory syncytial virus (RSV) and vesicular stomatitis virus (VSV) are examples of enveloped single-stranded RNA-containing viruses. VSV causes an economically important disease in horses and cattle. Both RSV and parainfluenza-3 virus are an important cause of respiratory tract infections. Among the heterocyclic rings containing bridgehead nitrogen atom, imidazo[2,1-b]thiazoles derivatives are especially attractive because of their different biological activities such as antibacterial, antituberculosis, antiviral, anticancer, antiinflammatory and diuretic activities. On the other hand, aryldenehydrazide moiety are also associated with various biological properties including antibacterial, antitubercular, antiviral, anticancer, antiinflammatory and analgesic activities.

In continuation of our previous studies on the biological properties of imidazo[2,1-b]thiazole derivatives, in this study, we reported the antibacterial, antitubercular and antiviral activity evaluation of some aryldenehydrazide derivatives bearing imidazo[2,1-b]thiazole moiety.

MATERIALS AND METHODS
Chemistry
All chemicals were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (St. Louis, MO, USA) chemical companies. Using
to determine the actual 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 (IC$_{50}$) in VERO cells at concentrations $6.25$ mg/mL or 10 times the MIC for $M$._tuberculosis $H_{37}$Rv (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 into a formazan product using the Promega CellTiter 96 Non-radioactive Cell Proliferation Assay. Compounds for which the selectivity index (IC$_{50}$/MIC ratio) SI>10 were assumed to possess in vitro activity confirmed in the BACTEC 460 at $6.25$ mg/mL.

**Microplate alamar blue susceptibility assay**

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 $7H9$GC (no Tween 80) in the microplates. BACTEC 12B-passaged inocula were initially diluted $1:2$ in $7H9$GC, and $0.1$ mL was added to wells. Subsequent determination of bacterial titers yielded $1:10^6$ CFU/mL in plate wells for $H_{37}$Rv. Frozen inocula were initially diluted $1:20$ in BACTEC 12B medium followed by a $1:50$ dilution in $7H9$GC. Addition of $1:10$ mL to wells resulted in a final bacterial titers of $2.0 \times 10^5$ CFU/mL for $H_{37}$Rv. 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 $37^\circ C$ until day 4 of incubation, $20$ mL of 10x Alamar Blue solution (Alamar Biosciences/Accumed, Westlake, Ohio, USA) and $12.5$ mL of $20\%$ Tween 80 were added to one B well an done M well, and plates were reincubated $37^\circ C$. Wells were observed at 12 and $24$ h for a color change from blue to pink and for a reading of $\pm 50.000$ 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 $\pm 50.000$ 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 $37^\circ C$, 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) $\times$ $100$. The lowest drug concentration effecting an inhibition of $\pm 90\%$ 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 ($6.25$ mg/mL). The standard vials contained rifampicin ($0.25$ mg/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 $37^\circ C$ and tested daily with a BACTEC instrument. When the GI in the control read at least 30, the increase in GI ($\Delta$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:

\[
\Delta \text{GI} \text{control} > \Delta \text{GI} \text{drug} = \text{susceptible}
\]

\[
\Delta \text{GI} \text{control} < \Delta \text{GI} \text{drug} = \text{resistant}
\]

If a clear susceptibility pattern (the difference of $\Delta$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 $\Delta$GI differences.

**Antiviral activity**

The compounds (3a-j) were evaluated for activity against diverse RNA- and DNA-viruses, using the following cell-based assays:

(a) Madin-Darby Canine Kidney (MDCK) cells infected with INF A/H1N1 subtype (A/Ind/378/05), INF A/H3N2 subtype (A/ Ind/7/87) or INF B (B/Ned/537/05); (b) Crandell-Rees Feline Kidney (CRFK) cells infected with feline coronaviruses (FCoV) or feline herpes virus (FHV); (c) African green monkey kidney (Vero) cells infected with parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B4 virus or Punta toro virus; (d) human embryonic lung (HEL) fibroblast cells infected with HSV-1 or -2, an acyclovir-resistant HSV-1 strain, vaccinia virus, VSV; (e) human cervix carcinoma (HeLa) cells infected with VSV, coxsackie B4 virus or RSV.

To perform the antiviral assays, the virus was added to subconfluent cell cultures in 96-well plates, and at the same time, the test compounds were added at serial dilutions. Appropriate reference compounds were included, i.e. the virus entry inhibitor dextran sulfate 5000, the broad antiviral agent ribavirin, the antiherpetic drug ganciclovir, and the HIV inhibitor azidothymidine. After 3-6 days incubation at $37^\circ C$ or $35^\circ C$ (in the case of INF virus), the cultures were examined by microscopy to score the compounds’ inhibitory effect on virus-induced cytopathic effect or their cytotoxicity. For some viruses, antiviral and cytotoxic activities were confirmed by the clorimetric 3-(4,5-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium cell viability assay.

**RESULTS AND DISCUSSION**

The key intermediate 2 was prepared from ethyl (6-(4-chlorophenyl)imidazo[2,1-b]thiazol-3-yl)acetate hydrobromide (1) and hydrazine hydrate following the literature method. The synthetic route of the compounds is outlined in Scheme 1. Condensation of 2 with appropriate aromatic aldehyde afforded the corresponding $N^2$-arylidene-6-(4-
Compounds 3a-j were evaluated for in vitro antibacterial activity against Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 25922 using the microbroth dilution method. As can be seen in Table 1, 3i (2,4-dichlorobenzylidene derivative) showed the highest activity against S. aureus ATCC 29213 and E. coli ATCC 25922 (MIC: 2 μg/mL, 64 μg/mL, respectively).

Compounds 3a-j were evaluated against M. tuberculosis H$_{37}$Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the MABA. The primary antituberculosis screening was performed in accordance with the protocol of the Tuberculosis Antimicrobial Acquisition and Coordinating Facility Southern Research Institute. Rifampin was used as the control drug in the tests. Compounds demonstrating a percent inhibition of bacterial growth of greater than or equal to 90% in the primary screen were retested against M. tuberculosis H$_{37}$Rv, to determine the actual MIC in the MABA. The MIC 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$_{50}$ using a curve fitting program. Any IC$_{50}$ value of ≤10 μg/mL was considered “Active” for antitubercular activity. Compounds active in the initial screen were tested for cytotoxicity in Vero cells at concentrations 10x the actual MIC in the MABA. The MIC was defined as the lowest concentration at which bacterial growth of greater than or equal to 90% in the primary screen were retested against M. tuberculosis H$_{37}$Rv. Most of the tested compounds showed weakly antitubercular activity and cytotoxicities of the compounds were found to be very high (Table 2).

The compounds (3a-j) were also evaluated against INF A/H1N1 subtype (A/Ned/378/05), INF A/H3N2 subtype (A/HK/7/87), INF B (B/Ned/537/05) in MDCK, FCoV, FHV in CRFK, parainfluenza-3 virus, reovirus-1, sindbis virus, coxsackie B4 virus, punta toro virus in Vero, HSV-1 (KOS), HSV-2 (G), HSV-1 TK KOS ACV, vaccinia virus, VSV, in HEL and VSV, coxsackie B4 virus and RSV in HeLa cell cultures. As can be seen in Table 3, the most active compound was R=2,4-dichlorophenyl substituted 3i. It inhibited FCoV with EC$_{50}$ of 7.5 μM. R=4-hydroxyphenyl substituted derivative 3c, inhibited HSV-1 (KOS), HSV-2 (G), HSV-1 TK KOS ACV, vaccinia virus and VSV with EC$_{50}$ of 9, 27, 32, 16 and 32 μM, respectively. R=3-methoxy-4-hydroxyphenyl substituted 3g showed EC$_{50}$ values of 20 and 14 μM for HSV-1 (KOS) and v virus, respectively (Table 4). However, tested compounds (3a-j) didn’t show any inhibition against INF A/H1N1 subtype (A/Ned/378/05), INF A/H3N2 subtype (A/HK/7/87), INF B (B/Ned/537/05), parainfluenza-3.

| Compound | Assay | IC$_{50}$ (mg/mL) | Activity |
|----------|-------|------------------|----------|
| 3a | MABA | >100 | 100 | Inactive |
| 3b | MABA | >100 | 100 | Inactive |
| 3c | MABA | 22.710 | 33.060 | Weakly active |
| 3d | MABA | 69.170 | >100 | Weakly active |
| 3e | MABA | >100 | >100 | Inactive |
| 3f | MABA | >100 | >100 | Weakly active |
| 3g | MABA | 20.670 | 36.860 | Weakly active |
| 3h | MABA | 44.720 | >100 | Weakly active |
| 3i | MABA | >100 | >100 | Inactive |
| 3j | MABA | 6.16 | 14.390 | Weakly active |

MIC: Minimum inhibitory concentrations, MABA: Microplate Alamar Blue Assay, n.t.: not tested.
virus, reovirus-1, sindbis virus, coxsackie B4 virus, punta toro virus, VSV, coxsackie B4 virus and RSV strains (i.e. minimal antivirally effective concentration ≥5-fold lower than minimal cytotoxic concentration) (Table 5).

CONCLUSION

In this work, a series of arylidenehydrazide derivatives bearing imidazo[2,1-b]thiazole moiety was evaluated for antibacterial, antitubercular and antiviral activities. The results showed that some compounds exhibited antibacterial, antimycobacterial and antiviral activities with different percentage of inhibition. Therefore, we have identified a novel series of imidazo[2,1-b]thiazoles, which may develop into the potential class of antibacterial, anti-tubercular and antiviral agents.

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| Table 3. Anti-feline corona virus and anti-feline herpes virus activity and cytotoxicity of the compounds 3a-j in Crandell-Rees Feline Kidney cell cultures |
| --- |
| **Compound** | **FCoV** | **FHV** |
| 3a | >100 | >100 | >100 |
| 3b | 50.6 | >20 | >20 |
| 3c | 20.7 | >20 | >20 |
| 3d | >100 | >100 | >100 |
| 3e | 4.4 | >4 | >4 |
| 3f | 50.8 | >20 | >20 |
| 3g | 24.5 | >20 | >20 |
| 3h | >100 | >100 | >100 |
| 3i | >100 | 7.5 | 54.8 |
| 3j | 9.7 | >4 | >4 |
| **HHA (µg/mL)** | | | |
| | >100 | 5.3 | 8.8 |
| **UDA (µg/mL)** | | | |
| | >100 | 17.7 | 12.9 |
| **Ganciclovir (µM)** | | | |
| | >100 | >100 | 3.6 |

FCoV: Feline corona virus, FHV: Feline herpes virus, HHA: Hippeastrum hybrid agglutinin, UDA: Urtica dioica agglutinin, MTS: 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-Tetrazolium, *50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay, 50% effective concentration, or concentration producing 50% inhibition of virus-induced, cytopathic effect, as determined by measuring cell viability with the colorimetric formazan-based MTS assay.

| Table 4. Antiviral activity and cytotoxicity of the compounds 3a-j in human embryonic lung cell cultures |
| --- |
| **Compound** | **MCC** | **EC**<sub>50</sub><sup>a</sup> (µM) |
| 3a | >100 | >100 | >100 | >100 |
| 3b | >100 | >100 | >100 | >100 | >100 |
| 3c | ≥100 | 9 | 27 | 32 | 16 | 32 |
| 3d | 100 | >20 | >20 | >20 | >20 | >20 |
| 3e | >100 | >100 | >100 | >100 | >100 | >100 |
| 3f | >100 | >100 | >100 | >100 | >100 | >100 |
| 3g | 500 | 20 | >100 | >100 | 14 | >100 |
| 3h | 100 | >20 | >20 | >20 | >20 | >20 |
| 3i | 100 | >20 | >20 | >20 | >20 | >20 |
| 3j | >100 | >100 | >100 | >100 | >100 | >100 |
| **Brivudin** | >250 | 0.05 | 199 | 10 | 10 | >250 |
| **Ribavirin** | >250 | 2 | 2 | 2 | 10 | >250 |
| **Cidofovir** | >250 | 0.7 | 11 | 3.5 | >250 | >250 |
| **Ganciclovir** | >100 | 0.03 | 0.03 | 0.1 | >100 | >100 |

<sup>a</sup>Required to cause a microscopically detectable alteration of normal cell morphology, <sup>1</sup>Required to reduce virus-induced cytopathogenicity by 50%.
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Conflict of Interest: No conflict of interest was declared by the authors.

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Table 5. Antiviral activity and cytotoxicity of the compounds 3a-j in Vero cell cultures

| Compound | MCC (µM) | EC50 (µM) |
|----------|----------|-----------|
|          | Parainfluenza-3 virus | Reovirus-1 | Sindbis virus | Coxsackie virus B4 | Punta Toro virus |
| 3a       | >100     | >100      | >100         | 100              | 100               |
| 3b       | 100      | >20       | >20          | 20               | 20                |
| 3c       | 20       | >4        | >4           | 4                | 4                 |
| 3d       | >100     | >100      | >100         | >100             | 100               |
| 3e       | 20       | >4        | >4           | 4                | 4                 |
| 3f       | >100     | >100      | >100         | >100             | >100              |
| 3g       | 40       | >8        | >8           | >8               | >8                |
| 3h       | >100     | >20       | >20          | >20              | >20               |
| 3i       | >20      | >20       | >20          | >20              | >20               |
| 3j       | >100     | >100      | >100         | 15               | 100               |
| DS-5000 (µM) | >100     | >100      | >100         | 15               | 20                |
| (S)-DHPA (µM) | >250     | >250      | >250         | >250             | >250              |
| Ribavirin (µM) | >250   | 29        | 146          | >250             | 250               |

*Required to cause a microscopically detectable alteration of normal cell morphology, †Required to reduce virus-induced cytopathogenicity by 50%.
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