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ORIGINAL ARTICLE

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

İmidazo[2,1-*b*]tiyazol Çekirdeği Taşıyan Bazı Arilidenhidrazit Türevlerinin Antibakteriyel, Antitüberküler ve Antiviral Aktivite Tayinleri

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ABSTRACT

Objectives: The aim of this study was to determine the probable antibacterial, antitubercular, and antiviral activities of some N^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_{50}: 6.16 µg/mL and IC_{90}: 14.390 µg/mL). Compound **3i** showed the most potent antiviral activity against feline corona virus in CRFK cell cultures (antiviral EC_{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_{50} values of 9; 16 and 20; 14 µM, respectively).

Conclusion: On the basis of aforementioned results, it can be conluded 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^2-ariliden-(6-(4-klorofenil)imidazo[2,1-*b*]tiyazol-3-il) 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. Dahası, bileşiklerin tümü **(3a-j)**, Madin-Darby Canine Kidney, Crandell-Rees Feline Kidney (CRFK), Vero, insan embriyonik akciğeri (HEL) ve HeLa hücrelerinde bazı DNA ve RNA virüslerine karşı tayin edilmiştir.

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Bulgular: Test bileşikleri arasında, **3i**, *S. aureus* ve *E. coli*'ye karşı en yüksek etkinliği göstermiştir. 5-Nitro-2-furfuriliden türevi **3j** bileşiği, en yüksek antitüberküler aktivite göstermiştir (IC_{50}: 6.16 µg/mL ve IC_{90}: 14.390 µg/mL). Bileşik **3i**, en güçlü antiviral aktiviteyi CRFK hücre kültürlerinde feline corona virüse karşı göstermiştir (antiviral EC_{50}: 7.5 µM ve SI>13). Ayrıca, **3c** ve **3g** bileşikleri HEL hücre kültürlerinde, herpes simpleks virüs-1 ve aşı virüsüne karşı aktivite göstermişlerdir (antiviral EC_{50} değerleri sırasıyla 9; 16 ve 20; 14 µM'dir).

Sonuç: Yukarıda sözü edilen sonuçlara dayanarak, hidrazon çekirdeği taşıyan imidazo[2,1-*b*]tiyazol türevleri, antibakteriyel, antitüberküler ve antiviral özelliklere sahip terapötik ajanlar tasarlamak için umut verici kimyasal problar olarak yarar sağlayabilir.

Anahtar kelimeler: İmidazo[2,1-*b*]tiyazol, arilidenhidrazid, antibakteriyel aktivite, antitüberküler aktivite, antiviral aktivite

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.[1,2] 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.[3] Additionally, viral infections caused by the rapid emergence of antiviral drug resistant strains have become a serious threat globally.[4] 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.[5,6] 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.[7] Both RSV and parainfluenza-3 virus are an important cause of respiratory tract infections.[8,9]

Among the heterocyclic rings containing bridgehead nitrogen atom, imidazo[2,1-*b*]thiazoles derivatives are especially attractive because of their different biological activities such us antibacterial,[10] antituberculosis,[11] antiviral,[12] anticancer,[13] antiinflammatory[14] and diuretic[15] activities. On the other hand, arylidenehydrazide moiety are also associated with various biological properties including antibacterial,[16] antitubercular,[17] antiviral,[18] anticancer,[19] antiinflammatory and analgesic[20] activities.

In continuation of our previous studies on the biological properties of imidazo[2,1-*b*]thiazole derivatives,[21-27] in this study, we reported the antibacterial, antitubercular and antiviral activity evaluation of some arylidenehydrazide 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 a Büchi B-540 melting point apparatus (Flawil, Switzerland) with open capillaries, melting points were determined and are uncorrected. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyser. Infrared spectra were recorded (in KBr) using a Perkin Elmer Spectrum 100 fourier transform infrared (FTIR) spectrometer and Shimadzu IRAffinity-1 FTIR spectrophotometer. ^{1}H and ^{13}C-nuclear magnetic resonance spectra were obtained on Varian UNITY INOVA 500 MHz spectrometer using dimetil sulfoxide-d_6 as an internal standard. All chemical shifts were reported as δ (ppm) values and spin-spin couplings (J) were exposed in Hz. MS (ESI-) were determined on a Finnigan LCQ Advantage Max mass spectrometer.

General synthesis of N^2-arylidene-(6-(4-chlorophenyl) imidazo[2,1-b]thiazol-3-yl)acetic acid hydrazides (**3a-3j**)[28]

A solution of 0.005 mol compound **2** and 0.005 moL of an appropriate aromatic aldehyde in 100 mL ethanol was heated under reflux for 5 h. The precipitate obtained was purified either by recrystallization from ethanol or by washing with hot ethanol.

Biological activity

Antibacterial activity

Minimum inhibitory concentrations (MICs) were determined by the microbroth dilution method using the National Committee for Clinical Laboratory Standards recommendations.[29] Mueller-Hinton broth (Oxoid, Hemakim, Turkey) was used as the test medium. An inoculum of approximately $5x10^5$ CFU cm^{-3} was delivered per well. Serial twofold dilutions of the test compounds (128-0.25 µg/mL) and extra dilutions (256-0.25 µg/mL) for antibiotic standards were prepared. Plates were incubated for 16-20 h at 35°C in an ambient air incubator. The lowest concentration of the test compounds inhibiting visible growth was taken as the MIC value.

Antitubercular activity

In vitro evaluation of antitubercular activity

Primary screening was conducted at 6.25 mg/mL against *Mycobacterium tuberculosis* $H_{37}Rv$ in BACTEC 12B medium using a broth microdilution assay the Microplate Alamar Blue Assay (MABA).[30] Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system.[31] 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* $H_{37}Rv$ in order

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 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 $1x10^6$ 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 7H9GC. Addition of 1/10 mL to wells resulted in a final bacterial titers of $2.0x10^5$ 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˚C. Starting at 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˚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) x 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 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˚C and tested daily with a BACTEC instrument. When the GI in the control read at least 30, the increase in GI (ΔGI) from the previous day in the control was compared with that in the drug vial. The following formula was used to interpret the results:

ΔGI control > ΔGI drug = susceptible

ΔGI control < ΔGI drug = resistant

If a clear susceptibility pattern (the difference of ΔGI 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 ΔGI differences.

Antiviral activity

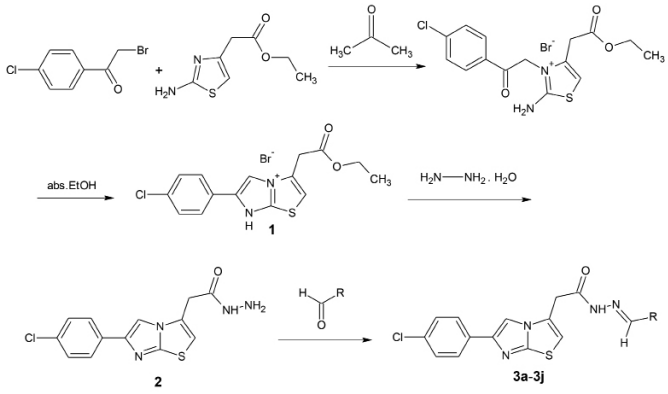
The compounds (**3a**-**j**) were evaluated for activity against diverse RNA- and DNA-viruses, using the following cell-based assays[32]: (a) Madin-Darby Canine Kidney (MDCK) cells infected with INF A/H1N1 subtype (A/Ned/378/05), INF A/H3N2 subtype (A/HK/7/87) or INF B (B/Ned/537/05); (b) Crandell-Rees Feline Kidney (CRFK) cells infected with feline corona virus (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 cervixcarcinoma Henrietta Lacks (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˚C (or 35˚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 colorimetric 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.[33] 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-

chlorophenyl)imidazo[2,1-*b*]thiazol-3-yl)acetic acid hydrazides (**3a-j**).[28]



Scheme 1. Synthesis of the title compounds **(3a-j)**

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[29]. 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[30]. 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_{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 IC_{50} in Vero cells. 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 cytotoxicity in Vero cells at concentrations 10x the MIC for *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 virüs 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

Table 1. Antibacterial activity of compounds 3a-j (MIC mg/mL)

Comp./ *microorg.	R	A	B	C
3a	C6H5	128	>128	128
3b	$C_6H_4(OH)(2-)$	>128	>128	>128
3c	$C_6H_4(OH)(4-)$	128	128	>128
3d	$C_6H_4(OCH3)(4-)$	64	128	128
3e	$C_6H_4(NO_2)(4-)$	>128	>128	>128
3f	$C_6H_4(N(CH_3)_2)(4-)$	128	>128	128
3g	$C_6H_3(OCH_3)(OH)(3,4-)$	128	>128	>128
3h	$C_6H(OCH_3)_2(2,5-)$	>128	>128	128
3i	$C_6H(Cl_2)(2,4-)$	32	>128	64
3j	5-nitro-2-furyl	128	128	128
Amikacin	-	1	1	2

MIC: Minimum inhibitory concentrations, *A: *Staphylococcus aureus* ATCC 29213, B: *Pseudomonas aeruginosa* ATCC 27853, C: *Escherichia coli* ATCC 25922

Table 2. Antimycobacterial activity screening results of 3a-j (MIC mg/mL)

Compound	Assay	IC_{50} (mg/mL)	IC_{90} (mg/mL)	Activity
3a	n.t.	n.t.	n.t.	n.t.
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
Rifampicin			0.125	

MIC: Minimum inhibitory concentrations, MABA: Microplate Alamar Blue Assay, n.t.: not tested

virus, reovirus-1, sindbis virus, coxsackie B4 virus, punta toro virüs, 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	CC_{50}[a] (μM)	EC_{50}[b] (μM)	
		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 virüs, FHV: Feline herpes virüs, HHA: Hippeastrum hybrid agglutinin, UDA: Urtica dioica agglutinin, MTS: 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethonyphenol)-2-(4-sulfophenyl)-2H-Tetrazolium, [a]50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric, formazan-based MTS assay, [b]50% effective concentration, or concentration producing 50% inhibition of virus-induced, cytopathic effect, as determined by measuring e cell viability with the colorimetricformazan-based MTS assay

Table 4. Antiviral activity and cytotoxicity of the compounds 3a-j in human embryonic lung cell cultures

Compound	MCC[a] (μM)	EC_{50}[b] (μM)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Herpes simplex virus-1 TK KOS ACV^r	Vaccinia virus	Vesicular stomatitis virus
3a	>100	>100	>100	>100	>100	>100
3b	>100	>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	1.1	3.5	>250	>250
Ganciclovir	>100	0.03	0.03	0.1	>100	>100

[a]Required to cause a microscopically detectable alteration of normal cell morphology, [b]Required to reduce virus-induced cytopathogenicity by 50%

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

Compound	MCC[a] (μM)	EC_{50}[b] (μM)				
		Parainfluenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
3a	>100	>100	>100	>100	>100	>100
3b	100	>20	>20	>20	>20	>20
3c	20	>4	>4	>4	>4	>4
3d	>100	>100	>100	>100	>100	>100
3e	20	>4	>4	>4	>4	>4
3f	>100	>100	>100	>100	>100	>100
3g	40	>8	>8	>8	>8	>8
3h	100	>20	>20	>20	>20	>20
3i	≥20	>20	>20	>20	>20	>20
3j	100	>20	>20	>20	>20	>20
DS-5000 (μM)	>100	>100	>100	15	>100	20
(S)-DHPA (μM)	>250	>250	>250	>250	>250	>250
Ribavirin (μM)	>250	29	146	>250	>250	112

[a]Required to cause a microscopically detectable alteration of normal cell morphology, [b]Required to reduce virus-induced cytopathogenicity by 50%

Conflict of Interest: No conflict of interest was declared by the authors.

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