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Original article

SYNTHESIS OF SOME (5-CHLORO-2(3H)-BENZOTHIAZOLINONE-3-YL) ACETO/PROPANOHYDRAZIDES TOWARDS ANTIMICROBIAL AND ANTIVIRAL ACTIVITY

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Abstract

In this study, new eleven hydrazone derivatives of (5-chloro-2(3H)-benzothiazolinone-3-yl) aceto/propanohydrazide have been synthesized. Structures of the synthesized compounds have been elucidated by IR and ^{1}H-NMR spectral data and their elemental analyses. All compounds were screened for their antiviral and antimicrobial activities aganist various virus, bacteria and fungi strains.

Key words: *5-Chloro-2(3H)-benzothiazolinone, Hydrazone, Synthesis, Antimicrobial activity, Antiviral activity.*

Bazı (5-Kloro-2(3H)-Benzotiyazolinon-3-il)aseto/propanohidrazitlerin Antimikrobiyal ve Antiviral Aktiviteleri

Bu çalışmada (5-kloro-2(3H)-benzotiyazolinon-3-il)aseto/propanohidrazitin onbir yeni hidrazon türevi sentezlenmiştir. Sentezlenen bileşiklerin yapıları IR ve ^{1}H-NMR spektral verileri ve elementel analiz sonuçları ile açıklanmıştır. Sentezlenen tüm bileşiklerin antiviral ve antimikrobiyal aktiviteleri çeşitli virus, bakteri ve funguslara karşı değerlendirilmiştir.

Anahtar kelimeler: *5-Kloro-2(3H)-benzotiyazolinon, Hidrazon, Sentez, Antimikrobiyal aktivite, Antiviral aktivite.*

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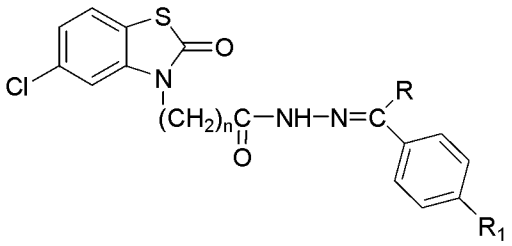
INTRODUCTION

Many of screening efforts have been made to find new antimicrobial and antiviral agents from synthetic or natural compounds in that a variety of newly synthesis active compounds with different molecular targets have been recognize to control infectious caused by bacteria, fungi and viruses (1-6). Also, during the past several years, the emergence of organisms resistant to nearly all classes of antimicrobial agents has become a serious public health concern (7,8). In general, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents (9).

There has been considerable interest in the chemistry of 2(3H)-benzothiazolinone ring system, a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activity such as antimicrobial (10,11), anticonvulsant (12,13), anti-inflammatory and analgesic (14-17) activities. Meantime, various hydrazide and hydrazone derivatives have shown antiplatelet (18), antinociceptive (19), antimicrobial (20-23), cytotoxic and antiviral (24) activities.



Scheme 1. (5-Chloro-2(3H)-benzothiazolinone-3-yl)aceto/propanohydrazides

Therefore, in this study, our aim was to prepare some new hydrazone derivatives using the biologically active 5-chloro-2(3H)-benzothiazolinone ring as a starting compound. The new compounds (Scheme 1) were tested against a series of standard gram-positive, gram-negative bacteria, a yeast-like fungi and viral strains to determine their minimum inhibiting concentrations.

MATERIALS AND METHODS

Apparatus

Melting points were determined with an Electrothermal-9200 digital melting point apparatus and are uncorrected.

The IR spectra of the compounds were recorded on a Perkin–Elmer 1330 IR spectrophotometer. The ^{1}H-NMR spectra were recorded on a Bruker 500 FT NMR spectrometer using tetramethylsilane as the internal standard and DMSO-d_6 solvent. All chemical shifts were recorded as δ (ppm).

Microanalyses for C, H, and N were performed by TUBITAK Analytical Laboratory, Ankara, Turkey and were within the range of 0.4 % of theoretical values.

Chemistry

All the chemicals used for the synthesis of the compounds were purchased from either Aldrich Chemicals or E. Merck AG. 5-Chloro-2(3H)-benzothiazolinone was synthesized according to the procedures previously published procedures (25,26).

Synthesis of ethyl (5-chloro-2(3H)-benzothiazolinone-3-yl)acetate (2) (26)

5-Chloro-2(3H)-benzothiazolinone (20 mmol) was dissolved in 30 ml of acetone, and 22 mmol of potassium carbonate was added. Ethyl bromoacetate (22 mmol) was added to the final mixture and refluxed for 4 h, cooled to 0°C, poured into 100 ml of ice–water mixture and stirred for 1 h at 0–10°C. The precipitate formed in the meantime was filtered and washed with water, dried and crystallized from ethanol.

Synthesis of methyl (5-chloro-2(3H)-benzothiazolinone-3-yl) propionate (3) (27)

5-Chloro-2(3H)-benzothiazolinone (20 mmol) was dissolved in 30 ml of methanol and 22 mmol of TEA was added. And then methyl acrylate (22 mmol) was added to the final mixture and refluxed for 6h, cooled to 0°C, poured into 100 ml of ice–water mixture and stirred for 1 h at 0–10°C. The precipitate was filtered, washed with water, dried, and crystallized from ethanol.

Synthesis of (5-chloro-2(3H)-benzothiazolinone-3-yl)aceto/propanohydrazide (4)

Ethyl (5-chloro-2(3H)-benzothiazolinone-3-yl) acetate/propionate (5 mmol) and 10 mmol of 98% hydrazine hydrate were dissolved in 20 ml of ethanol. The final mixture was stirred for 6h at room temperature. At the end of this period, the reaction mixture was poured into 50 ml of water with stirring. The precipitate formed was filtered, and washed with water. The solid material was dried at room temperature.

General procedure for the preparation of hydrazones of (5-chloro-2(3H)-benzothiazolinone-3-yl) aceto/propanohydrazide (5)

(5-chloro-2(3H)-benzothiazolinone-3-yl)acetohydrazide/(5-chloro-2(3H)-benzothiazolinone-3-yl)propanohydrazide (18 mmol) was dissolved in 50 ml of ethanol and 18 mmol of an appropriate benzaldehyde/acetophenone derivative and a few drop acetic acid added, then the final mixture was refluxed for 4-8 h. The precipitate formed was filtered and washed with hot alcohol and dried at room temperature.

Microbiological studies

Test materials

Compounds were dissolved in dimethylsulphoxide (DMSO; Merck) at a final concentration of 512 μg ml^{-1} and sterilized by filtration using 0.22μm Millipore (MA 01730, USA) and used as the stock solutions. The stock solutions of the agents were prepared in medium according to the Clinical and Laboratory Standards Institute (28).

Microorganisms

Standard strains of the following bacteria, namely *Escherichia coli* (ATCC 35218), *Pseudomonas aeroginosa* (ATCC 10145), *Klepsiella pneumoniae* (RSKK 574), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), and *Bacillus subtilis* (ATCC 6633) for the determination of antibacterial activity, and standard strains of *Candida albicans* (ATCC 10231) for the determination of antifungal activity were used.

Inoculum preparation

Mueller-Hinton Broth (MHB; Difco) and Mueller-Hinton Agar (MHA; Oxoid) were applied for growing and diluting of the bacteria. Sabouraud liquid medium (SLM; Oxoid) and Sabouraud

dextrose agar (SDA; Oxoid) were applied for growing and diluting of the fungi. The medium RPMI-1640 with L-glutamine was buffered pH: 7 with 3-[N-morpholino]-propansulfonic acid (MOPS). Before the test, strains of fungi and bacteria were cultured on media and passaged at least twice to ensure purity and viability at 35°C for 24 to 48 h. Culture suspensions were prepared according to the CLSI; formerly NCCLS M27-A (29). The bacterial suspensions used for inoculation were prepared at 10^5cfu ml^{-1} by diluting fresh cultures at McFarland 0.5 density (10^8cfu ml^{-1}). The fungi suspension was prepared by the spectrophotometric method of inoculum preparation at a final culture suspension of $2.5x10^3$cfu ml^{-1} (28).

Table 1. Physicochemical data of **5a-k**.

Comp.	R	R_1	n	Crys. Sol.	Yield %	Mp [°C]	Calcd/Found
5a	H	H	1	Isopropanol	32	280-282	C 55.57/55.62 H 3.50/3.65 N 12.15/11.77
5b	H	4-Cl	1	Methanol	38	292-294	C 50.14/49.84 H 2.92/2.94 N 11.05/10.80.
5c	CH_3	4-Cl	1	Acetone	42	202-204	C 51.75/51.37 H3.32/3.23 N10.66/10.28.
5d	CH_3	4-OCH_3	1	Toluene	61	222-224	C 55.45/55.24 H 4.14/4.52 N 10.78/10.72.
5e	H	H	2	Toluene	61	181-182	C 56.74/56.82 H 3.92/3.55 N 11.68/11.57
5f	H	4-CH_3	2	Toluene	80	177-178	C 57.83/58.02 H 4.31/4.53 N 11.24/11.13
5g	H	4-OCH_3	2	Ethanol	62	200-202	C 55.45/55.46 H 4.14/4.36 N 10.78/10.72
5h	CH_3	H	2	Toluene	86	177-178	C 57.83/57.40 H 4.31/4.47 N 11.24/11.14
5i	CH_3	4-Cl	2	Toluene	82	176-178	C 52.95/52.61 H 3.70/3.41 N 10.29/9.82
5j	CH_3	4-CH_3	2	Toluene	47	178-179	C 58.83/58.50 H 4.68/4.69 N 10.83/10.85
5k	CH_3	4-OCH_3	2	Acetone	80	180-181	C 56.50/56.83 H 4.49/4.66 N 10.40/9.97

Antibacterial and antifungal tests

The microdilution method was employed for antibacterial and antifungal activity tests. Media were placed into each 96 wells of the microplates. Compounds solutions at 512µg ml^{-1} were

added into first raws of microplates and two fold dilutions of the compounds (512-0.25 μg ml^{-1}) were made by dispensing the solutions to the remaining wells. 10μl culture suspensions were inoculated into all the wells. The sealed microplates were incubated at 35ºC for 24 h and 48 h in humid chamber. The lowest concentration of the compounds that completely inhibit macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported (1).

Table 2. IR and ^{1}H-NMR spectral data of the compounds **5a-k**.

Comp.	IR (KBr) cm^{-1}	^{1}H-NMR (ppm, δ)
5a	3179, 1683	DMSO-d_6, 11.79 (1H, s, NH), 8.23, 8.04 (1H, s, =CH), 7.75-7.70 (3H, m, phenyl-$H^{2,6}$, H^7), 7.57, 7.53 (1H, d, H^4), 7.47-7.41 (3H, m, phenyl-$H^{3,4,5}$), 7.28, 7.25 (1H, d, H^6), 5.19, 4.77 (2H, s, CH_2CO)
5b	3180, 1697, 1681	DMSO-d_6, 11.93, 11.85 (1H, s, NH), 8.22, 8.03 (1H, s, =CH), 7.77 (2H, d, phenyl-$H^{2,6}$), 7.72 (1H, d, H^7), 7.56, 7.52 (1H, d, H^4), 7.51-7.47 (2H, m, phenyl-$H^{3,5}$), 7.28, 7.25 (1H, d, H^6), 5.19, 4.77 (2H, s, CH_2CO)
5c	3189, 1691	DMSO-d_6, 11.10, 11.04 (1H, s, NH), 8.04, 7.71 (1H, d, H^7), 7.90, 7.55 (1H, d, H^4), 7.87 (2H, d, phenyl-$H^{2,6}$), 7.82 (2H, d, phenyl-$H^{3,5}$), 7.40, 7.25 (1H, dd, H^6), 5.22, 4.72 (2H, s, CH_2CO), 2.28, 2.26 (3H, s, CH_3).
5d	3228, 1689	DMSO-d_6, 10.93, 10.85 (1H, s, NH),8.05, 7.71 (1H, d, H^7), 7.91, 7.54 (1H, d, H^4), 7.81 (2H, d, phenyl-$H^{2,6}$), 7.75 (2H, d, phenyl-$H^{3,5}$), 7.40, 7.25 (1H, dd, H^6), 5.20, 4.70 (2H, s, CH_2CO), 3.78, 3.77 (3H, s, OCH_3), 2.25, 2.24 (3H, s, CH_3).
5e	3196, 1680, 1661	DMSO-d_6, 11.46, 11.43 (1H, s, NH), 8.12, 7.92 (1H, s, =CH), 7.79, 7.62 (1H, d, H^7), 7.67, 7.51 (2H, m, phenyl-$H^{2,6}$), 7.60 (1H, d, H^4), 7.42 (3H, m, phenyl-$H^{3,4,5}$), 7.25 (1H, dd, H^6), 4.25 (2H, s, CH_2CO), 3.02, 2.65 (2H, t, N-CH_2).
5f	3183, 1681, 1664	DMSO-d_6, 11.39, 11.33 (1H, s, NH), 8.07, 7.89 (1H, s, =CH), 7.69, 7.63 (1H, d, H^7), 7.59 (1H, d, H^4), 7.40 (2H, d, phenyl-$H^{2,6}$), 7.26- 7.23 (1H, d, H^6), 7.17 (2H, d, phenyl-$H^{3,5}$), 4.24 (2H, s, CH_2CO), 3.01, 2.63(2H, t, N-CH_2), 2.32, 2.31(3H, s, CH_3).
5g	3182, 1677	DMSO-d_6, 11.32, 11.27 (1H, s, NH), 8.05, 7.86 (1H, s, =CH), 7.69, 7.63 (1H, d, H^7), 7.61, (2H, d, phenyl-$H^{2,6}$), 7.59 (1H, d, H^4), 7.26,7.24(1H, dd, H^6), 6.98 (2H, d, phenyl-$H^{3,5}$), 4.24 (2H, m, CH_2CO), 3.80, 3.77 (3H, s, OCH_3), 3.00, 2.63 (2H, t, N-CH_2).
5h	3261, 1680, 1660	DMSO-d_6, 10.61, 10.42 (1H, s, NH), 7.76 (2H, m, phenyl-$H^{2,6}$), 7.69, 7.62 (1H, d, H^7), 7.58 (1H, d, H^4), 7.67 (3H, m, phenyl-$H^{3,4,5}$), 7.26, 7.22 (1H, dd, H^6), 4.25 (2H, s, CH_2CO), 3.06, 2.78 (2H, t, N-CH_2), 2.21, 2.18 (3H, s, CH_3).
5i	3192, 1672	DMSO-d_6, 10.66, 10.47 (1H, s, NH), 7.78, 7.65 (2H, d, phenyl-$H^{2,6}$), 7.69, 7.61 (1H, d, H^7), 7.56 (1H, d, H^4), 7.46, 7.39 (2H, d, phenyl-$H^{3,5}$), 7.26, 7.21 (1H, dd, H^6), 4.26, 3.43(2H, m, CH_2CO), 3.05, 2.78 (2H, t, N-CH_2), 2.20, 2.17 (3H, s, CH_3).
5j	3188, 1665	DMSO-d_6, 10.55, 10.37 (1H, s, NH), 7.69, 7.62 (1H, d, H^7), 7.66 (2H, d, phenyl-$H^{3,5}$), 7.56 (2H, d, phenyl-$H^{2,6}$), 7.54 (1H, s, H^4), 7.26, 7.24 (1H, dd, H^6), 4.25 (2H, m, CH_2CO), 3.05, 2.76 (2H, t, N-CH_2), 2.33, 2.30 (3H, s, CH_3), 2.17, 2.15 (3H, s, phenyl-CH_3).
5k	3175, 1685, 1667	DMSO-d_6, 10.52, 10.34 (1H, s, NH), 7.92 (2H, d, phenyl-$H^{2,6}$), 7.74, 6.88 (1H, d, H^7), 7.81, 7.56 (1H, d, H^4), 7.69 (2H, d, phenyl-$H^{3,5}$), 7.25, 7.21 (1H, dd, H^6), 4.69, 4.23 (2H, m, CH_2CO), 3.82 (3H, s, OCH_3), 3.03, 2.74 (2H, t, N-CH_2), 2.16, 2.13 (3H, s, CH_3).

Cytotoxicity and antiviral tests

Cell line and growth condition

Vero cell line (African green monkey kidney) and MDBK (Madin-Darby Bovine Kidney) used in this study was obtained from Department of Virology, Faculty of Veterinary, Ankara University (Ankara-Turkey). The culture of the cells were grown in EMEM (Eagle's Minimal Essential Medium) enriched with 10% fetal calf serum (FCS, Biochrom, Germany), 100 mg ml^{-1} of streptomycin and 100 IU ml^{-1} of penicillin in a humidified atmosphere of 5% CO_2 at 37°C. The cells were harvested using trypsin solution (Bipco Life Technologies, UK).

Test Viruses

In order to determine the antiviral activity of the compounds, as DNA viruses; *Herpes simplex* Type-1 (*HSV*-1) virus, as RNA viruses; *Para-influenza*-3 *virus* (*PI*-3) were used. The test viruses were obtained from Department of Virology, Faculty of Veterinary, Ankara University.

Antiviral Activity

The antiviral activities of the compounds were determined as previously described (2). Media (EMEM) were placed into each 96 wells of the microplates (Greiner^R, Germany). Stock solutions of the compounds were added into first raws of microplates and two-fold dilutions of the compounds (512-0.25μg ml^{-1}) were made by dispensing the solutions to the remaining wells. Two-fold dilution of each material was obtained according to Log_2 on the microplates. Acyclovir (Biofarma) and oseltamivir (Roche) were used as the control agents. Strains of *HSV*-1 and *PI*-3 titers were calculated as $TCID_{50}$ and they were inoculated into all the wells. The cells were evaluated using cell culture microscope (x400), comparing with treated-untreated control cultures and with acyclovir and oseltamivir as the control agents. Consequently, maximum CPE (Cytopathogen Effect) concentrations as the indicator of antiviral activities of the compounds were determined.

Cytotoxicity

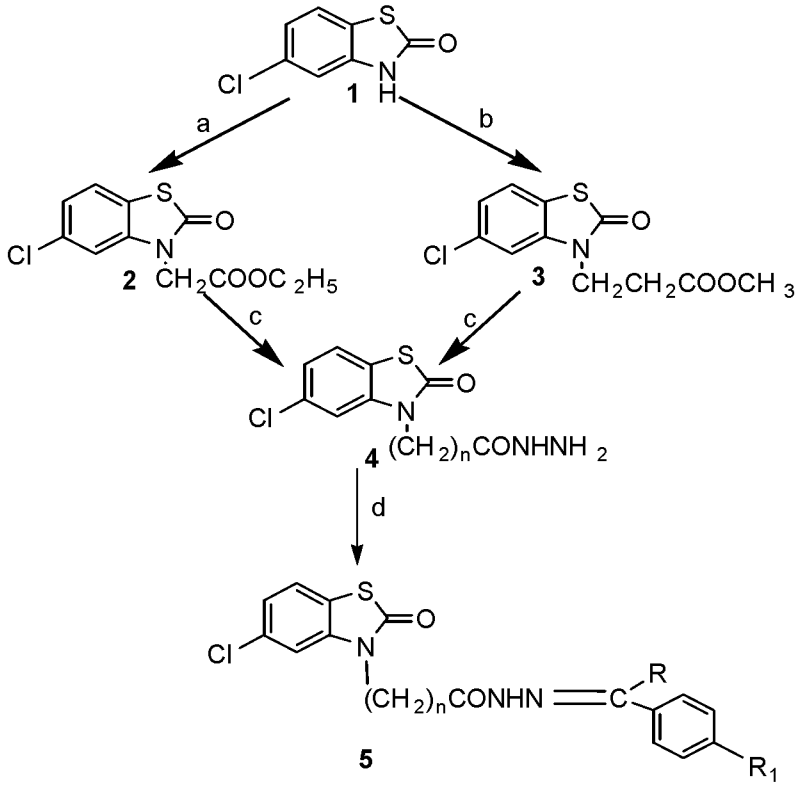
The maximum non-toxic concentrations (MNTCs) of each sample were determined by the method described previously Özçelik *et.al.*(3), based on cellular morphologic alteration. Several concentrations of each sample were placed in contact with confluent cell monolayers and incubated in 5% CO_2 at 37°C for 48 h. MNTC were determined by comparing treated and controlling untreated cultures (3).

RESULTS AND DISCUSSION

Chemistry

The synthetic route of the title compounds is illustrated in Scheme 2. Synthesis of the compounds was started by obtaining 5-chloro-2(3H)-benzothiazolinone from 2,5-dichloronitrobenzene (25, 26). Compound 5-chloro-2(3H)-benzothiazolinone **1** was then reacted with ethyl bromoacetate to obtain ethyl (5-chloro-2(3H)-benzothiazolinone-3-yl) acetate **2 (26)**. Ethyl (5-chloro-2(3H)-benzothiazolinone-3-yl) propionate **3** was prepared by the reaction of **1** with methyl acrylate (27). This compound was synthesized by a Micheal addition type reaction. (5-Chloro-2(3H)-benzothiazolinone-3-yl)aceto/propanohydrazide **4** was obtained by the reaction of **2** or **3** with hydrazine hydrate (15). The hydrazide thus obtained was reacted with benzaldehyde /acetophenone derivatives to obtain the title compounds **5**. Structures of the compounds synthesized have been elucidated by IR and ^{1}H-NMR spectral data and microanalysis. Physical and spectral data of the synthesized compounds are given in

experimental part. The compounds displayed characteristic N-H and C=O bands at 3260-3182 cm^{-1} and 1697-1679 or 1667-1660 cm^{-1}, respectively.

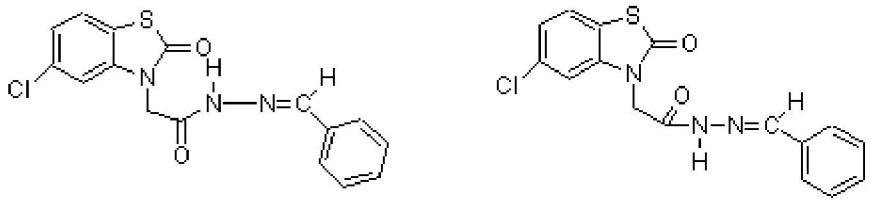


n: 1, 2 R: H, CH_3; R_1:H, Cl, CH_3, OCH_3

a: $BrCH_2COOC_2H_5$, K_2CO_3, acetone, reflux, 4 h; **b**: CH_2=$CHCOOCH_3$, TEA, CH_3OH, reflux, 6 h, **c**: NH_2NH_2, C_2H_5OH, 6 h, rt; **d**: Ar-CHO / $ArCOCH_3$, C_2H_5OH, CH_3COOH (cat.), reflux, 3 h.

Scheme 2. Synthetic route of the title compounds

The ^{1}H-NMR spectra of these compounds recorded in DMSO-d_6 showed two signals. Corresponding to NH-, H^4, H^6, H^7 and -CH_2- peaks indicating that these compounds are present in two conformer, if one incline to consider free rotation about C2'-C1'bond of N^3-$C^{2'}$-$C^{1'}$-O part of the compounds (Scheme 3). There could be two conformers because N-H signals at 11.85-10.34 and 11.93-10.52 ppm could be assigned as synperiplanar and antiperiplanar conformer , respectively (Scheme 3). The two forms were found to be in the ratio (1/3 or 1/4) as calculated from the integration values, chemical shifts and *J*-constants of the NH- and -CH_2- signals. This can be thought of about *E/Z* isomers due to the presence of CO-NH group. Evaluation of tune signal of indicate presence of the *E* isomers in the mixture. The signal of *E* isomer was displaced downfield in relation to that of the *Z* isomer (30). The signals are considered that two stereoisomeric forms of the compounds are due to minor synperiplanar CO-NH conformation, and a major antiperiplanar CO-NH conformation as depicted in Scheme 3 (24, 31, 32).



Scheme 3. Stereoisomeric forms of compounds

Biological Activity

The compounds are tested against three gram-positive (*S. aureus*, *B. subtilis*, and *E. faecalis*) and three gram-negative bacteria (*P. aeruginosa*, *E. coli*, and *K. pneumoniae*). The antifungal activities of compounds were evaluated *in vitro* against a yeast-like fungi *C. albicans*. The microdilution method was employed for antibacterial and antifungal activity tests. Ampicillin (AMP) and ofloxacin (OFX) were used as posivite control against bacteria and ketoconazole (KET) against fungi. Both antibacterial and antifungal activities and minimum inhibition concentrations (MICs; μg ml^{-1}) value of the compounds were illustrated in Table 3. The compounds inhibited growth of the bacteria and fungi with MICs between 16 and 512 μg ml^{-1}. (5-Chloro-2(3H)-benzothiazolinone-3-yl)propanohydrazide derivatives displayed better antimicrobial activity than (5-chloro-2(3H)-benzothiazolinone-3-yl)acetohydrazide derivatives. Among the tested compounds, **5e-5j** are the most effective compounds at 16 μg ml^{-1} against *B. Subtilis* and they are effective against *E. faecalis* with a MIC value of 32 μg ml^{-1}.

Table 3. Antibacterial and antifungal activities of the compounds as MIC values.

Compounds	**Microorganisms**						
	E. coli	***P. aeruginosa***	***K. pneumoniae***	***S. aureus***	***E. faecalis***	***B. subtilis***	***C. albicans***
5a	>512	>512	>512	>512	>512	>512	>512
5b	>512	>512	>512	>512	>512	>512	>512
5c	>512	>512	>512	>512	>512	>512	>512
5d	>512	>512	>512	>512	>512	>512	>512
5e	64	128	64	64	32	16	64
5f	64	128	64	64	32	16	64
5g	64	128	64	64	32	16	64
5h	64	128	64	64	32	16	64
5i	64	128	64	64	32	16	64
5j	64	128	64	64	32	16	64
5k	64	128	64	64	>512	>512	>512
AMP	2	-	2	<0.5	0.25	0.5	-
OFX	0.12	1	<0.5	0.5	1	1	-
KET	-	-	-	-	-	-	2

AMP: Ampicilline, OFX: Ofloxasine, KET: Ketoconazole, -: No activity observed.

The same compounds are effective against gram-negative *E.coli*, *K. pneumoniae* and gram-positive *S. aureus*, and against yeast-like fungi *C. albicans* with a MIC value of 64 μg ml^{-1}. In comparison, **5a-5d** had no activity to the references and **5e-5j**.

The antiviral activities as well as cytotoxicity were tested against DNA virus *Herpes simplex* Type-1 (*HSV*-1) and RNA virus *Parainfluenza*-3 *virus* (*PI*-3) using Vero (African green monkey kidney) and MDBK (Madin-Darby Bovine Kidney) cell line cultures, in which acyclovir (16-≤0.25 μg ml^{-1}) and oseltamivir (32- ≤0.25 μg ml^{-1}) were employed as references. Results of antiviral activity of the compounds are shown in Table 4. All the compounds showed activity at 4-64 μg ml^{-1} concentration range against DNA virus *HSV*-1 and only compound **5d** effected at 8-32 μg ml^{-1} concentration range against RNA virus *PI*-3.

Compounds **5a, 5e, 5h, 5j** were found active against tested DNA viruses at range of 2-16 μg ml^{-1}. **5h** (4-16μg ml^{-1}) and **5a** (8-16μg ml^{-1}) showed better antiviral activity than that of other tested compounds, which were not as active as acyclovir (16<0.25μg ml^{-1}). Additionally, these finding showed MNTC values of these compounds, 32 μg ml^{-1} was observed for **5h** and **5a.** Compound **5h** influenced higher than the other compounds, which means less toxic in comparison to the reference. **5b** and **5c** (8 μg ml^{-1}), showed higher MNTCs than other compounds. However, antiviral activities of these compounds were seen at concentrations of 1-4μg ml^{-1} and 2-4μg ml^{-1} respectively. Our results showed us that these compounds was effective to DNA virus *HSV-1*.

Table 4. Cytotoxicity on MDBK and Vero cells as well as antiviral activity against HSV-1 and PI-3 results of the compounds (**5a-k**).

	MDBK Cells (μg ml^{-1})			*Vero Cells (μg ml^{-1})*		
	MNTC (μg ml^{-1})	*CPE Inhibitory Concentration*		*MNTC (μg ml^{-1})*	*CPE Inhibitory Concentration*	
		HSV-1			*PI*-3	
Compounds		**Max.**	**Min.**		**Max.**	**Min.**
5a	32	16	8	1	-	-
5b	8	4	1	16	-	-
5c	8	4	2	1	-	-
5d	128	64	16	64	32	8
5e	16	8	4	2	-	-
5f	8	-	-	2	-	-
5g	64	-	-	8	-	-
5h	32	16	4	4	-	-
5i	8	-	-	1	-	-
5j	16	8	2	2	-	-
5k	64	-	-	32	-	-
Acyclovir	16	16	<0.25	-	-	-
Oseltamivir	-	-	-	32	32	<0.25

-: No activity observed, MNTC: Maximum non-toxic concentration, CPE: Cytopathogenic effect

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