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Original article

Synthesis, antiviral and anticancer activity of some novel thioureas derived from *N*-(4-nitro-2-phenoxyphenyl)-methanesulfonamide

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ABSTRACT

Due to a continuing effort to develop new antiviral agents, a series of 1-[4-(methanesulfonamido)-3-phenoxyphenyl]-3-alkyl/aryl thioureas **3a–i** have been synthesized by the reaction of alkyl/aryl isothiocyanates with 4-amino-2-phenoxymethanesulfonanilide. These derivatives were structurally characterized by the use of spectral techniques and evaluated for their anticancer and antiviral activities. None of the tested compounds showed significant anticancer properties on A549 and L929 cell lines. All synthesized compounds **3a–i** were evaluated in vitro against HIV-1 (IIIB) and HIV-2 (ROD) strains in MT-4 cells, as well as other selected viruses such as HSV-1, HSV-2, Coxsackie virus B4, Sindbis virus and varicella–zoster virus using HEL, HeLa and Vero cell cultures. Compound **3b** was able to block HIV replication with almost 100% maximum protection at 125 μg/ml, and IC_{50} values of 54.9 μg/ml and 65.9 μg/ml against HIV-1 and HIV-2, respectively.

1. Introduction

HIV infection and AIDS have been implicated as the first diseases for which the discovery of drugs has been performed entirely via a rational drug design approach. Current treatment regimens are based on the use of two or more drugs from different classes of inhibitors termed highly active antiretroviral therapy (HAART). Some thiourea compounds were reported to be non-nucleoside inhibitors (NNIs) of the reverse transcriptase (RT) enzyme of the human immunodeficiency virus (HIV) [1]. High throughput screening studies revealed that certain bis(aryl)thioureas carrying an amide functionality, as exemplified by the structure shown below, could be considered as inhibitors of herpes viruses such as HSV, CMV and VZV [2]. In addition, some thiourea derivatives were reported to be potent inhibitors of influenza virus neuraminidase, Coxsackie virus B4 and thymidine kinase positive varicella–zoster virus (TK^+ VZV, OKA strain) [3,4].

Nimesulide is a representative of the methanesulfonanilide class of inhibitors, which display an interesting degree of COX-2 selectivity. COX-2 plays a pathogenic role in carcinogenesis and supports tumor cell growth. Literature results demonstrate that selective COX-2 inhibitors such as nimesulide suppress the telomerase activity of gastric cancer cells [5] and exhibit antiproliferative effects on colorectal cancer cell lines [6]. Su and et al. reported some novel nimesulide analogs which were evaluated as selective aromatase expression regulator in breast cells [7,8]. Some thiourea derivatives have been reported to possess anticancer activity against Erlich carcinoma and K562 human leukemia cells [9]. Moreover, some clinical studies indicated a significant correlation between viral infections and some proliferative disorders.

These findings encouraged us to go further with our ongoing studies on thiourea derivatives. Therefore, a novel series of 1-[4-(methanesulfonamido)-3-phenoxyphenyl]-3-alkyl/aryl thioureas were designed by the modification of the reported [2] bis(aryl)thiourea earlier. The intentional modifications were: *i.* introduction of alkyl and aryl groups at **R** position; trying several linkers (**L**); *ii.* introduction of a phenoxy ring; *iii.* replacement of acylamido group with methanesulfonamido functionality (Fig. 1). All synthesized compounds were screened in vitro against HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cells, as well as other selected viral strains (such as HSV, CMV, VZV) using HeLa, Vero, HEL cells cytomegalovirus (CMV), varicella–zoster virus (VZV) in human embryonic lung (HEL) cells and anticancer activity together with cytotoxicity on A 549 and L 929 cell lines.

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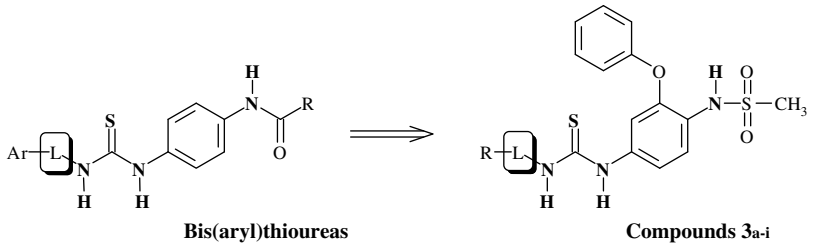
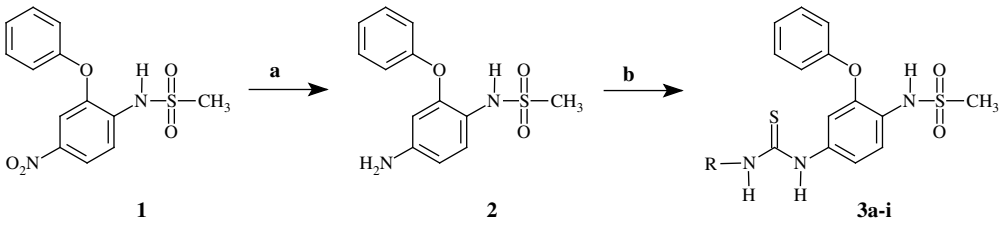


Fig. 1. The newly designed thiourea derivatives **3a–i**.



Scheme 1. Synthetic route to compounds **3a–i**. Reagents and conditions: (**a**) $SnCl_2$ + HCl/MeOH, reflux; (**b**) R-NCS/dry acetone, reflux.

2. Result and discussion

2.1. Chemistry

4-Amino-2-phenoxymethanesulfonanilide **2** was prepared by the reaction of 4-nitro-2-phenoxymethanesulfonanilide (Nimesulide) with $SnCl_2$ solution in 5.4 ml of concentrated HCl in boiling methanol [10]. This solution was refluxed and the resulting product was obtained after evaporation of methanol and subsequent neutralization with $NaHCO_3$ (5%). 1-[4-(Methanesulfonamido)-3-phenoxyphenyl]-3-alkyl/aryl thioureas **3a–i** were synthesized by the reaction of substituted alkyl/aryl isothiocyanates with compound **2** in dry acetone (Scheme 1). This type of reactions were reported to be performed in certain dry solvents or mixtures [11,12]. In the present study, dry acetone was tried and found to be useful. The physical and spectral data of thioureas **3a–i** are given in Tables 1 and 2. FTIR spectra exhibited N–H and C=S streching vibrations at 3228–3371 and 1325–1392 cm^{-1}, respectively. ^1H NMR spectral findings provide evidence for the formation of thiourea function by displaying resonances at 7.67–9.4 and 9.19–10.23 ppm due to thiourea R–NH–CS and CS–NH–Ar function, respectively [13] as well as the lack of resonances attributable to NH_2 function [10]. High resolution mass spectra (HRMS) confirmed the molecular weights and empirical formulae of the compounds **3a–i**, with less than 8 mmu bias between calculated and experimental *m/z* values of either molecular or fragment ions (Table 1). Ionization mode was electron impact (EI) in most cases, whereas some compounds (**3e**, **3g** and **3i**) did not give molecular ion peaks by using this technique. These compounds were analyzed using fast atomic bombardment (FAB) procedure giving exact MH^+ peaks instead of M^+ in 3-nitrobenzyl alcohol matrix.

2.2. Biological activity

2.2.1. Antiviral activity

Compounds **3a–i** were tested for antiviral activity and cytotoxicity in various viral test systems, according to previously published procedures [14–18]. Thiourea derivatives **3a–i** were evaluated for their anti-HIV activity. With one exception, none of the synthesized compounds showed any specific activity against HIV-1 (III_B) or HIV-2 (strain ROD) in MT-4 cells. Compound **3b** had IC_{50} values of 54.9 μg/ml and 65.9 μg/ml against HIV-1 and HIV-2, respectively. Although only weakly active, this compound was able to block the HIV replication effectively with almost 100% maximum protection at 125 μg/ml (from virus-induced cytopathogeneity). It would be worthwhile to design several analogues of this compound to optimize its anti-HIV potency and this will be the subject of our future research. Based on the experience with this type of

Table 1
Some physicochemical characteristics of thiourea derivatives **3a–i**.

Compounds	R	Formula	M.p. (°C)	N–H, C=S	Log *P*[a]	HR MS (m/z)	
						Calculated	Found
3a	CH_3	$C_{15}H_{17}N_3O_3S_2$	205-9	3350, 3267, 1338	2.73	352.0784 (FAB)	352.0791 (MH^+)
3b	C_2H_5	$C_{16}H_{19}N_3O_3S_2$	145-51	3356, 1336	3.19	365.0868 (EI)	365.0881 (M^+)
3c	C_3H_7	$C_{17}H_{21}N_3O_3S_2$	120-4	3333, 1392	3.51	379.1024 (EI)	379.1054 (M^+)
3d	$CH_2CH{=}CH_2$	$C_{17}H_{19}N_3O_3S_2$	193-4	3335, 1386	3.20	377.0868 (EI)	377.0855 (M^+)
3e	$CH_2C_6H_5$	$C_{21}H_{21}N_3O_3S_2$	129-30	3306, 1323	3.94	428.1097 (FAB)	428.1122 (MH^+)
3f	$CH_2CH_2C_6H_5$	$C_{22}H_{23}N_3O_3S_2$	138-9	3323,3228,1325	4.33	441.1181 (EI)	441.1143 (M^+)
3g	$CH_2CH_2C_6H_4$ Cl (4)	$C_{22}H_{22}ClN_3O_3S_2$	183-7	3356, 1336	4.88	476.0864 (FAB)	476.0870 (MH^+) 478.0829 ($MH+2$)
3h	C_6H_{11}	$C_{20}H_{25}N_3O_3S_2$	204-5	3371, 3234, 1338	4.23	420.1410 (FAB)	420.1417 (MH^+)
3i	C_6H_4 NO_2 (4)	$C_{20}H_{18}N_4O_5S_2$	109-14 (dec.)	3321, 1371	4.10	459.0791 (FAB)	459.0792 (MH^+)

[a] Calculation of log *P* values were performed using ALOGPS 2.102 log *P*/log *S* calculation software http://www.vcclab.org.

Table 2
^{1}H NMR spectral data of **3a–i**.

Compounds	^{1}H NMR (DMSO-d_6, ppm)
3a	2.86 (d, 3H, NH–CH_3); 2.94 (s, 3H, SO_2–CH_3); 7.04–7.42 (m, 8H, Ar–H); 7.66 (bs, 1H, NH–CH_3); 9.22 (s, 1H, NH); 9.51 (bs, 1H, NH).
3b	1.04 (t, 3H, N–CH_2–CH_3); 2.94 (s, 3H, SO_2–CH_3);3.30 (q, 2H, N–CH_2–CH_3); 7.03–7.42 (m, 8H, Ar–H); 7.69 (bs, 1H, NH–CH_2–CH_3); 9.20 (bs, 1H, NH); 9.41 (bs, 1H, NH).
3c	0.81 (t, 3H, N–CH_2–CH_3); 1.46 (m, 2H, N–CH_2–CH_3); 2.93 (s, 3H, SO_2–CH_3); 3.35 (q, 2H, N–CH_2–CH_2–CH_3); 7.05–7.42 (m, 8H, Ar–H); 7.70 (bs, 1H, NH–CH_2); 9.20 (bs, 1H, NH); 9.42 (bs, 1H, NH).
3d	2.93 (s, 3H, SO_2–CH_3); 4.05 (t, 2H, N–CH_2–CH=); 5.03 (d, 1H, –CH=CH_2, $J = 17.2$ Hz, *trans*); 5.08 (d, 1H, –CH=CH_2, $J = 10.3$ Hz, *cis*); 5.76–5.85 (m, 1H, –CH=CH_2), 7.05–7.41 (m, 8H, Ar–H); 7.80 (bs, 1H, NH–CH_2); 9.22 (bs, 1H, NH); 9.52 (bs, 1H, NH).
3e	2.93 (s, 3H, SO_2–CH_3); 4.66 (d, 2H, HN–CH_2–C_6H_5); 7.06–7.40 (m, 13H, Ar–H); 8.13 (bs, 1H, NH–CH_2); 9.22 (bs, 1H, NH); 9.59 (s, 1H, NH).
3f	2.78 (t, 2H, NH–CH_2 –CH_2–); 2.93 (s, 3H, SO_2–CH_3); 3.63 (q, 2H, NH–CH_2 –CH_2–); 7.04–7.41 (m, 13H, Ar–H); 7.70 (bs, 1H, NH–CH_2); 9.21 (s, 1H, NH); 9.53 (bs, 1H, NH).
3g	2.81 (m, 2H, NH–CH_2 –CH_2–); 2.96 (s, 3H, SO_2–CH_3); 3.34 (m, 2H, NH–CH_2–CH_2–); 7.05–7.44 (m, 12H, Ar–H); 7.75 (bs, 1H, NH–C_6H_4–); 9.29 (s, 1H, NH); 9.58 (bs, 1H, NH).
3h	1.10–1.25 (m, 10H, cyclohexyl CH_2); 1.80 (s, 1H, N–CH-cyclohexyl); 2.93 (s, 3H, SO_2–CH_3); 7.06–7.41 (m, 8H, Ar–H); 7.57, 7.59 (2b, 1H, NH-cyclohexyl); 9.19 (bs, 1H, NH); 9.33 (s, 1H, NH).
3i	2.96 (s, 3H, SO_2–CH_3); 7.06–8.12 (m, 12H, Ar–H); 9.2–9.4 (bs, 1H, NH–C_6H_4–); 10.23 and 10.29 (bs, 2H, NH).

molecules, thioureas are considered to act on the allosteric site of HIV-1 RT, and a certain degree of flexibility might be required for binding to HIV-1 RT. The absence of anti-HIV potency in most of the compounds was probably due to their inability to exist in butterfly-like conformation.

The compounds were also evaluated for in vitro antiviral activity against herpes simplex virus [HSV-1 (strain KOS), thymidine kinase-deficient (TK^-) strain of HSV-1 resistant to **acyclovir** (ACV^R), HSV-2 (G)], varicella–zoster virus (VZV) [OKA strain and a thymidine kinase deficient (TK^-) VZV (07/1 strain)], cytomegalovirus (CMV) [AD-169 strain and Davis strain], vaccinia virus (VV), vesicular stomatitis virus (VSV) in HEL cell cultures; VSV, Coxsackie virus B4, respiratory syncytial virus (RSV) in HeLa cell cultures and parainfluenza-3 virus, Reo virus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus Vero cell cultures. Results are presented in Tables 3–5. As a result of broad spectrum antiviral screening of **3a–i**, the derivatives, which had minimal antivirally effective concentration (MIC) less than one-fifth of minimal cytotoxic concentration (if $5 \times \text{MIC} < \text{MCC}$), were considered active. There was only one derivative (compound **3i**) which showed marginal activity towards several viruses in HEL cell cultures. This derivative with 4-nitrophenyl moiety showed inhibitory potency against vaccinia virus and vesicular stomatitis virus by an MIC value of 8 μg/ml whilst affecting the HEL cells with an MCC of 40 μg/ml, which led up to a selectivity index (SI = MCC/MIC) of 5. It is currently unclear whether the activity is due to its toxicity in the cell culture or to a real antiviral effect. Compound **3i** was found to be inactive against HSV-1 (KOS) and HSV-2 (G) strains.

No activity was observed against the strains which were tested with compounds **3a–i** in HeLa cell cultures.

The test compounds were also screened for their antiviral potency against varicella–zoster virus (VZV) [OKA strain and a thymidine kinase deficient (TK^-) VZV (07/1 strain)], cytomegalovirus (CMV) [AD-169 and Davis strains]. However, no activity was observed with any of the screened compounds, except for compounds **3c** and **3d** which had an inhibitory effect on CMV with an EC_{50} value of approximately 55 ug/ml, and on VZV **(3d)** with an EC_{50} value of 49 to 59 μg/ml, and a MCC >100 μg/ml. These derivatives are less potent than the standard drugs cidofovir and ganciclovir.

Table 3
Cytotoxicity and antiviral activity of compounds in HEL, HeLa and Vero cell cultures.

Compounds	HEL cell cultures						HeLa cell cultures				Vero cell cultures						
	Min cytotoxic conc.[a] (μg/ml)	Min inhibitory conc.[b] (μg/ml)					Min cytotoxic conc.[a] (μg/ml)	Min inhibitory conc.[b] (μg/ml)			Min cytotoxic conc.[a] (μg/ml)	Min inhibitory conc.[b] (μg/ml)					
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK KOS ACV^R		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus		Para i influenza-3 virus	Reo virus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
3a	>100	>100	>100	100	>100	>100	>100	100	>100	>100	>100	>100	>100	>100	>100	>100
3b	>200	120	120	40	>200	120	200	>40	>40	>40	200	≥24	>40	>40	>40	>40
3c	200	>40	>40	>40	>40	>40	200	>40	>40	40	40	>8	>8	>8	>8	>8
3d	200	>40	>40	>40	>40	>40	200	>40	>40	40	200	≥24	>40	>40	>40	>40
3e	40	>8	>8	>8	>8	>8	40	>8	>8	>8	40	>8	>8	>8	>8	>8
3f	40	>8	>8	>8	>8	>8	40	>8	>8	>8	8	>1.6	>1.6	>1.6	>1.6	>1.6
3g	>100	>100	>100	>100	>100	>100	100	>20	>20	>20	100	>20	>20	>20	>20	>20
3h	40	>8	>8	>8	>8	>8	40	>8	>8	>8	8	>1.6	>1.6	>1.6	>1.6	>1.6
3i	40	>8	>8	8	8	8	40	>8	>8	>8	40	>8	>8	>8	>8	>8
Brivudin (μM)	>250	0.08	10	2	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250	>250
Ribavirin (μM)	>250	250	250	150	150	>250	>250	30	150	50	>250	150	150	>250	>250	250
Acyclovir (μM)	>250	0.4	0.4	>250	>250	50										
Ganciclovir (μM)	>100	0.032	0.0064	100	>100	2.4										
(S)-DHPA (μM)							>250	150	150	>250	>250	50	250	>250	>250	>250

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

Table 4
Cytotoxicity and antiviral activity of compounds **3a–i** against cytomegalovirus (CMV), varicella–zoster virus (VZV) in human embryonic lung (HEL) cells.

Compounds	Antiviral activity vs. CMV EC_{50} (μg/ml)[a]		Antiviral activity vs. VZV EC_{50} (μg/ml)[a]		Cytotoxicity (CMV) CC_{50} (μg/ml)		Cytotoxicity (VZV) CC_{50} (μg/ml)	
	AD-169 strain	Davis strain	TK^+ VZV OKA strain	TK^- VZV 07/1 strain	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]
3b	>100	>100	>100	>100	>100	>100	>100	>100
3c	54.7	54.7	>20	>20	>100	56	100	56
3d	54.7	54.7	58.5	49	>100	82.4	>100	82.4
3e	>4	>4	>4	>4	20	45.2	20	45.2
3f	>20	>20	>20	>20	100	48	≥100	48
3h	>4	>4	>20	>0.8	20	33.6	≥4	82.4
3i	54.7	54.7	>20	>20	>100	>100	100	>100
Ganciclovir	1.4	1.7	1.0	15	400	80	>50	190
Cidofovir	0.24	0.37	0.0095	12.6	400	57	>50	244

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU).
[b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[c] Cytotoxic concentration required to reduce cell growth by 50%.

2.2.2. Anticancer activity

Cytotoxic and anticancer effects of synthesized compounds were tested at four different concentrations. Compound **3a**, as shown in Fig. 2, caused 10–20% cytotoxic effect at the highest concentration after a 4 day incubation period. The anticancer activity results of 1-[4-(methanesulfonamido)-3-phenoxyphenyl]-3-alkyl/aryl thioureas **3a–i** on A549 lung cell growth, showed that three compounds (**3b**, **3c** and **3h**) derived from ethyl, propyl and cyclohexyl isothiocyanates inhibited proliferation of A549 lung cell by inhibiting 10–20% of cells at 10 μM. (Fig. 2) [19]. As known, the lipophilicity of the compounds plays an important role during the penetration of these compounds into the cells and increases the activity. Log *P* values of the synthesized compounds were calculated using ALOGPS 2.102 log *P*/log *S* calculation software (Table 1). These results demonstrated that there were no direct correlations between the inhibitory effect of three compounds (**3b**, **3c** and **3h**) against A549 lung cell and the lipophilicity of these compounds.

3. Experimental

3.1. Chemistry

3.1.1. Instrumentation and chemicals

All chemical compounds were purchased from Fluka. Nimesulide was generously donated by Sanovel Pharmaceuticals (Turkey). Melting points were determined with Büchi-530 apparatus. The IR spectra were obtained with a Shimadzu FTIR–8400. ^{1}H NMR spectra in DMSO-d_6 were recorded on a Brüker Avance-DPX-400 spectrometer (400 MHz). HREI–mass spectra were performed using a Jeol JMS-700 instrument. Merck silica gel 60 F254 plates were used for analytical TLC.

SMILES were generated from the structures using the ACD/ChemSketch version 8.0 molecular editor (http://www.acdlabs.com) and then log *P* values were calculated using ALOGPS 2.102 log *P*/log *S* calculation software [20,21]. The calculated log *P* values for all the compounds are given in Table 1.

Table 5
Cytotoxicity and antiviral activity of compounds **3a–i** against HIV-I(III_B) and HIV-II(ROD).

Compounds	HIV-1 (III_B)		HIV-2 (ROD)	
	IC_{50} (μg/ml)	CC_{50} (μg/ml)	IC_{50} (μg/ml)	CC_{50} (μg/ml)
3a	>125	>125	>125	>125
3b	54.85[a]	>125	65.90[a]	>125
3c	>89.33	89.33	>89.33	89.33
3d	>87.20	101.38	>12.80	101.38
3e	20.15	61.63	>18.30	61.63
3f	>20.78	20.78	>20.78	20.78
3g	>14.83	14.83	>14.83	14.83
3h	>35.30	35.30	>35.30	35.30
3i	>15.17	15.17	>15.17	15.17

[a] For compound **3b** maximum protection (%) values at 125 μg/ml were 97% (HIV-1) and 89.5% (HIV-2).

*3.1.2. Preparation of 4-amino-2-phenoxymethanesulfonanilide **2***

Nimesulide (0.0032 mole) was dissolved in boiling methanol and $SnCl_2$ (0.016 mole) solution in 5.4 ml of concentrated HCl was carefully added. This solution was refluxed for 30 min. and the resulting product was obtained after evaporation of methanol followed by neutralization with $NaHCO_3$ (5%). Crystals from ethanol; m.p. 159–160 °C (159–160 °C [10])

*3.1.3. Synthesis of 1-[4-(methanesulfonamido)-3-phenoxyphenyl]-3-alkyl/aryl thioureas **3a–i***

A dry acetone solution of 4-amino-2-phenoxymethanesulfonanilide **2** and equimolar substituted alkyl/aryl isothiocyanates in dry acetone was heated under reflux for 5 h. The completion of reaction was checked by TLC (benzene:acetone, 70:30, v/v) . The precipitate obtained was filtered off and recrystallized twice with dry methanol/ethanol.

3.1.4. Antiviral activity

Compounds **3a–i** were tested for antiviral activity and cytotoxicity in various viral test systems, according to previously published procedures [14–18]. The synthesized compounds were tested against human immunodeficiency virus [HIV-1 (IIIB) and HIV-2 (ROD)], vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytical virus, parainfluenza-3 virus, reovirus, Sindbis virus, Punto Toro virus, herpes simplex virus type 1 and 2 and vaccinia virus-induced cytopathicity at subtoxic concentrations in MT-4 cells, HeLa, Vero or Hel cell culture. **Brivudin**, **(S)-DHPA**, **ribavirin**, **acyclovir**, cidofovir and **ganciclovir** were used as the reference compounds.

3.1.5. Anticancer activity

The synthesized compounds were tested for their anticancer activities and cytotoxicity properties. The CellTiter 96 Aqueous ONE Solution (Promega, Madison, WI) was used to evaluate cellular viability utilizing reduction of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS). A 549 and L 929 cell line were used to test both anticancer effects and cytotoxicity. Cells were routinely grown in a 75 mm flask in an environment containing 5% CO_2 and passed every 3 days. Cell viability was analyzed using the MTS assay.

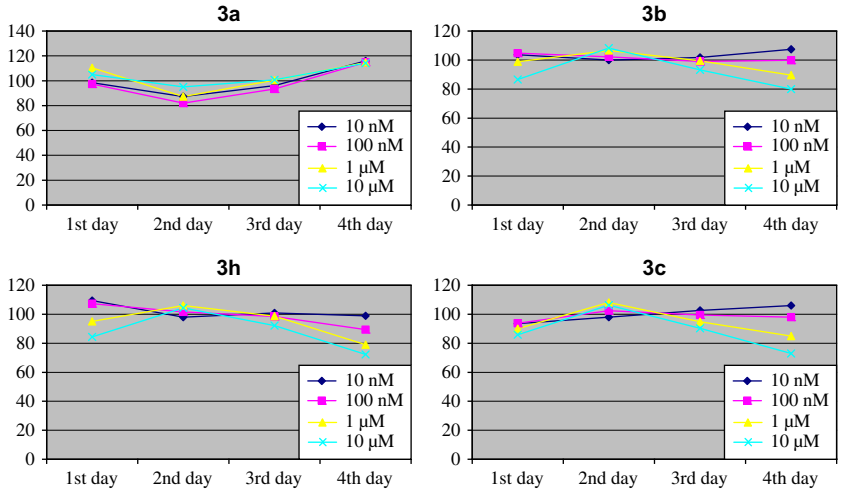


Fig. 2. The growth inhibitory effect of compounds **3a–c**, **3h** against A549 lung cell.

5000 Cells were plated in each well of a 96-well tissue culture plate. After 24 h of growth, the medium was replaced with fresh medium containing compounds **3a–i** at different concentration levels (10 nM, 100 nM, 1 μM and 10 μM) and the cells were grown for 4 days [19].

The MTS assay was performed according to the protocol provided by the Manufacturer. In short, 20 μL of MTS solution was added to each well, and cells were incubated at 37 °C for 1–3 h. The absorbance (at 490 nm) of each well was determined. Data are presented as a percentage of the values obtained from cells cultured under the same conditions in the absence of test compounds. For the time course study of cytotoxicity, L 929 cells were treated with compounds **3a–i** with the same dose used to detect anticancer activity. Cell viability was analyzed 1–4 days after the initiation of treatment, using the MTS assay.

Although all test compounds were dissolved in DMSO and the final concentration of DMSO was 0.1%, the solvent showed no activity in these assays at the level that was used for screening. For comparison of the anticancer activity and cytotoxicity observed with the test compounds, doxorubicin and taxol were selected as standard drugs.

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