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RESEARCH PAPER

Synthesis, characterization and biological evaluation of thioureas, acylthioureas and 4-thiazolidinones as anticancer and antiviral agents

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ABSTRACT

In this study, thiourea derivatives [**1-4**] were synthesized by using 2-amino-4-substituted pyridine compounds and these compounds have been used as the starting materials for synthesis of 2-imino-1,3-thiazolidin-4-one ring [**5, 6**]. Two different procedures for 4-thiazolidinone ring closure and synthesis method were optimized. The synthesized compounds were identified by the help of elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data while the purities of them were proved with TLC. Synthesized compounds were evaluated for their antiviral and anticancer activity. Antiviral activity against *Murine norovirus, Yellow fever, Enterovirus* and *Chikungunya* strains of the test compounds were investigated and EC_{50} values of these compounds were determined higher than 0,3 μM. Cytotoxicity of test compounds was examined on NIH3T3 cell line. When the anticancer activity of test compounds was examined against PC-3, A549, HeLa, HT-29, MCF-7, SJSA1 and K562 cell lines, the percent proliferation values of these compounds were observed over 61% for all cell lines.

Keywords: Acylthioureas; anticancer; antiviral; tautomerism; thiourea; 2-amino-1,3-thiazol-4-one; 2-imino-1,3-thiazolidin-4-one.

1. INTRODUCTION

Cancer is expressed as an uncontrolled cell division and spread of abnormal cells whose normal cellular functions are damaged. Furthermore, cancer is a major health problem worldwide and seen as the second cause of death after cardiovascular diseases (1). Recently, some research studies have shown that infections with some parasites, viruses and bacteria are risk factors for several types of cancer in humans. Infectious agents such as hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) have been found to be associated with many cancers (2). The relationship among inflammation, infection which is caused by a virus, and cancer makes it important to discover new antiviral and anti-inflammatory agents.

Many studies have shown that thioureas have a broad spectrum of biological effects profile including antiviral (3), antitubercular (4, 5), antibacterial (6), antifungal (7), antimycobacterial (8), anti-inflammatory (9), anticancer (10), antitumor (11) and antimalarial (12) activity.

Thiourea is a versatile reagent for the synthesis chemistry and is an important building block in the synthesis of heterocyclic compounds. Acyl thiourea derivatives have increasing

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importance due to the electronic structure of these molecules, resulting in their wide biological effect spectrum and biologically active metal complex. Acyl thiourea compounds, cambinol and tenovin-1 are small molecule inhibitors of the NAD^+-dependent family of protein deacetylases that is one of the targets in the treatment of cancer and neurodegenerative diseases (13, 14). In the other research, CID 1067700 carrying acyl thiourea structure has been reported as an inhibitor of nucleotide binding by Ras-related GTPases (15). In addition, *N*-substituted phenylthioureas have been found that have antitumoral effects against skin cancer (16, 17).

4-Thiazolidinone derivatives among heterocyclic ring systems have been reported to possess various biological properties such as anticancer (18), anti-inflammatory (19, 20), antibacterial (21, 22), antioxidant (23), anticonvulsant (24), antiviral (25), antimicrobial (26) and antifungal activities (27). The following 1,3-thiazolidin-4-on derivatives are used in medicine: ralitoline (anticonvulsant), piprozolin (choleretic), etozolin, ozolinone (diuretic), dexetozolin (antihypertensive), mezolidon (antiulcer) (28-30).

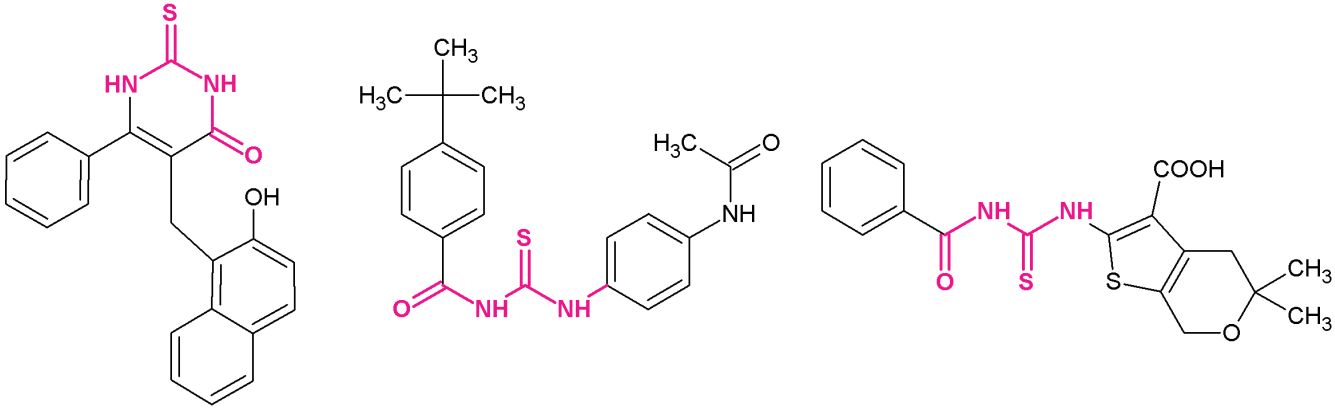
Different tautomers of the same compounds may have various biological effects from each other and identification of tautomerism is quite important for the heteroaromatic system. Aromaticity of heteroaromatic system for a drug with an amino-imino tautomerism is affected by this tautomerism and biological effects may change with new groups that have capable of H-bonding interaction, formed by tautomerism (31). In this study, our aim is to synthesize 2-imino-1,3-thiazolidin-4-on derivatives using two different procedures and to examine the tautomerism of synthesized compounds. Besides, we decided to evaluate the antiviral and anticancer activity of the synthesized thiourea and 4-thiazolidinone derivatives.

2. RESULTS AND DISCUSSION

2.1. Chemistry

There are several methods used for the synthesis of thiazolidinone derivatives. One of these methods is cyclization of chloroacetamides in the presence of ammonium thiocyanate (32, 33) while the other one is cyclization of thioureas with ethyl bromoacetate in the presence of sodium acetate (34, 35). Synthesized 4-thiazolidinone derivatives by using different methods have five possible tautomeric forms and these forms are given in Figure 3 (36).

2-Imino-1,3-thiazolidin-4-one derivatives are our target compounds in this study and one of the tautomers of 4-thiazolidinone ring. We have synthesized these compounds by using two different methods as given in Scheme 1. We have at first preferred the cyclization of chloroacetamides to synthesize these compounds in the presence of NH_4SCN. Synthesis of 2-chloro-*N*-[4-methylpyridin-2-yl] acetamide was carried out by the reaction of 2-amino-4-methylpyridine (5 mmol) with chloroacetyl chloride (6 mmol) in the presence of TEA (6 mmol) and DCM (15 ml) at 50-60°C. Red powder substance was obtained with a yield of 35% and melting point of this compound was observed as 114°C (37). A solution of 2-chloro-*N*-[4-methylpyridin-2-yl]acetamide (5 mmol) and ammonium thiocyanate (10 mmol) in 20 ml absolute ethanol were refluxed for 6 h and the crude product was obtained by evaporating ethanol. Compound **5'** was obtained as a brown powder with a yield



Cambinol Tenovin-1 CID 1067700

Figure 1. Biologically active acyl thiourea derivatives.

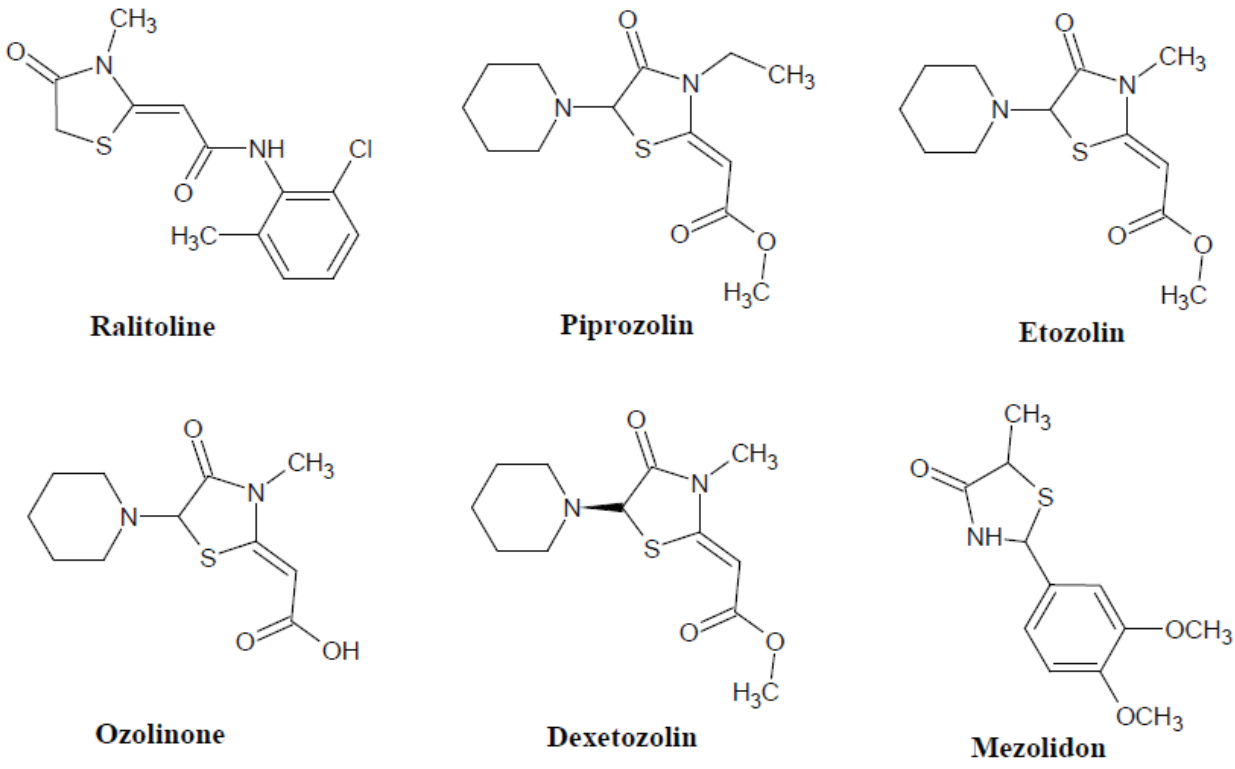


Figure 2. Drugs bearing 1,3-thiazolidin-4-one ring used in medicine.

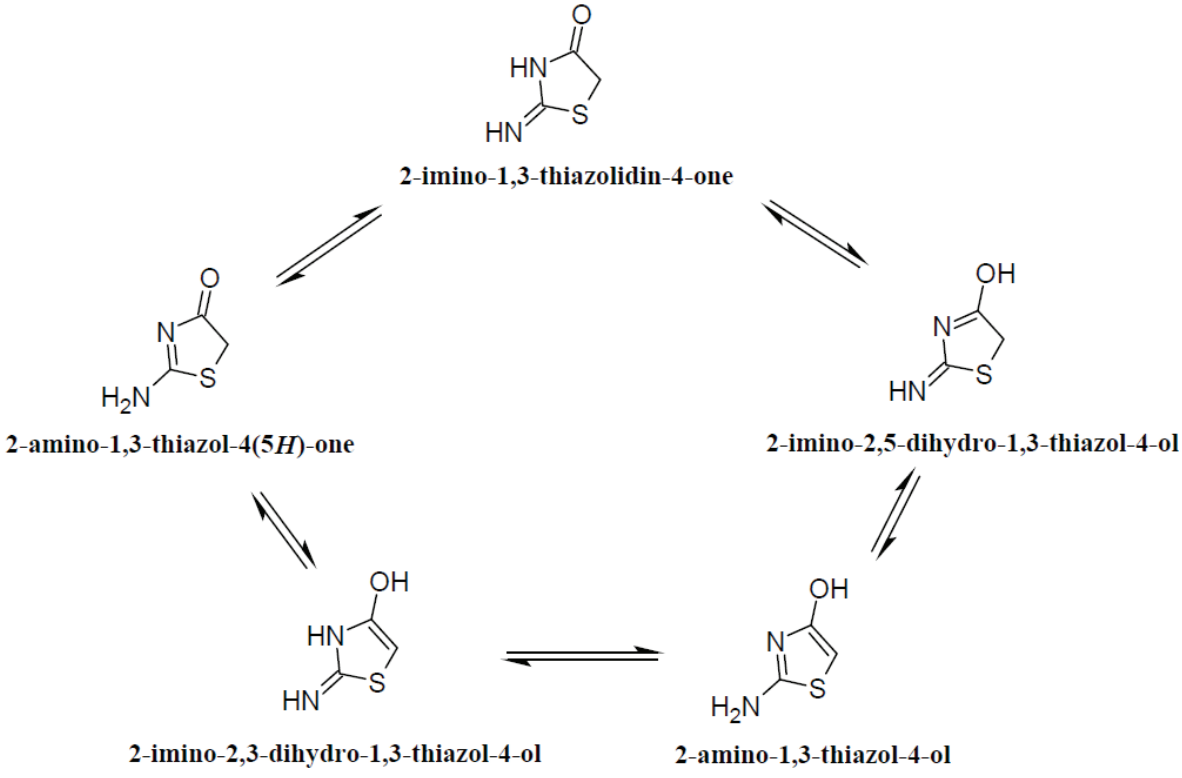


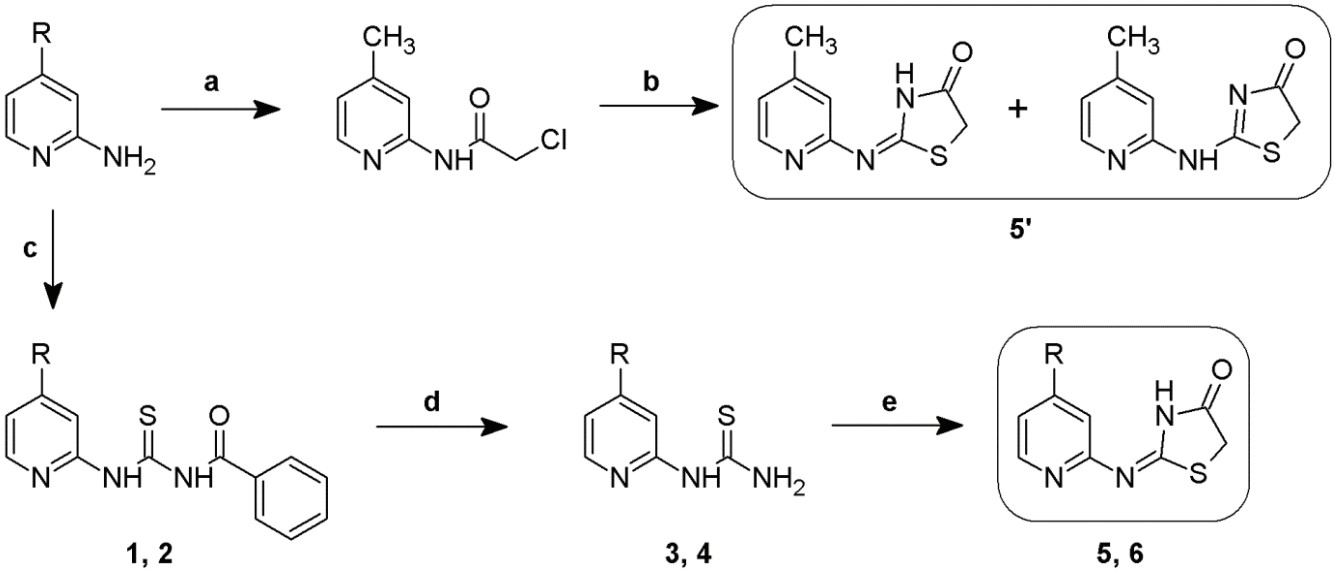
Figure 3. Tautomeric forms of 1,3-thiazolidin-4-one ring system.

of 52% and melting point of this compound was observed between 241-243°C. Positive and negative ion mass spectra of compound **5'** were obtained by using electrospray ionization technique. Molecular ion peak of compound **5'** was observed at 205.929 Da (calculated: 206.039 Da) for negative ion spectra and 207.965 Da (calculated: 208.054 Da) for positive ion spectra.

At the same time, the target compound **5** was synthesized performing a stepwise reaction protocol from *N*-(4-methylpyridine-2-yl)thiourea as outlined in Scheme 1 and we compared physical and spectral findings of compounds **5** and **5'**.

When ^{1}H-NMR spectrum of compound **5'** is examined, two peaks with 2H and 1H integrations were detected for -CH_2- protons located in the fifth position of 4-thiazolidinone ring at 3.81 ppm and 3.71 ppm, respectively (38, 39) while signals at about 2.47 ppm with 3/2H integration and 2.32 ppm with 3H integration that were attributed to the –CH_3 protons located in the fourth position of pyridine. In addition, two N-H peaks with 1H and 1/2 H integrations were detected at 11.95 ppm and 9.62 ppm, respectively (Figure 4). Since it is known that N-H peak was observed about 12 ppm and 9-10 ppm for 2-imino-1,3-thiazolidin-4-one (40, 41) and 2-amino-1,3-thiazol-4(5*H*)-one (42), respectively, these findings showed that these tautomeric forms were together with 1:2 ratio in our synthesized product. After these results, we decided to synthesize 1,3-thiazolidin-4-one ring by using the other method that cyclization of thioureas with ethyl bromoacetate in the presence of sodium acetate in ethanol. White powder substance was obtained with a yield of 74% and melting point of this compound was observed as 244°C. When we compared IR spectra of compound **5** and **5'**, we saw that there was 93% similarity between these spectra (Figure 5).

When ^{1}H-NMR spectrum of compound **5** is examined, signals at 3.81 ppm and 2.31 ppm were attributed to the -CH_2- and –CH_3 protons, respectively. Moreover, we observed a broad singlet peak for N-H proton of 4-thiazolidinone ring at 11.92 ppm that was noticed to exchange with D_2O in the ^{1}H NMR spectrum (Figure 4). As a result, we determined that 2-imino tautomeric form is obtained by cyclization of thioureas whilst mixture of two tautomeric forms is obtained by using chloroacetamides as a starting compound (Scheme 1).



Scheme 1. Two different synthesis procedures for 1,3-thiazolidin-4-one ring.

Reagents and conditions: **a)** $ClCOCH_2Cl$, TEA, DCM; **b)** NH_4SCN, EtOH, reflux; **c)** NH_4SCN, C6H5COCl, dry acetone, 80°C reflux; **d)** 1N NaOH, MeOH, 100°C, reflux; **e)** CH_3COONa, $BrCH_2COOC_2H_5$, EtOH, 100°C, reflux. (R=CH_3 for compounds 1,3,5 ; R=Cl for compounds 2,4,6)

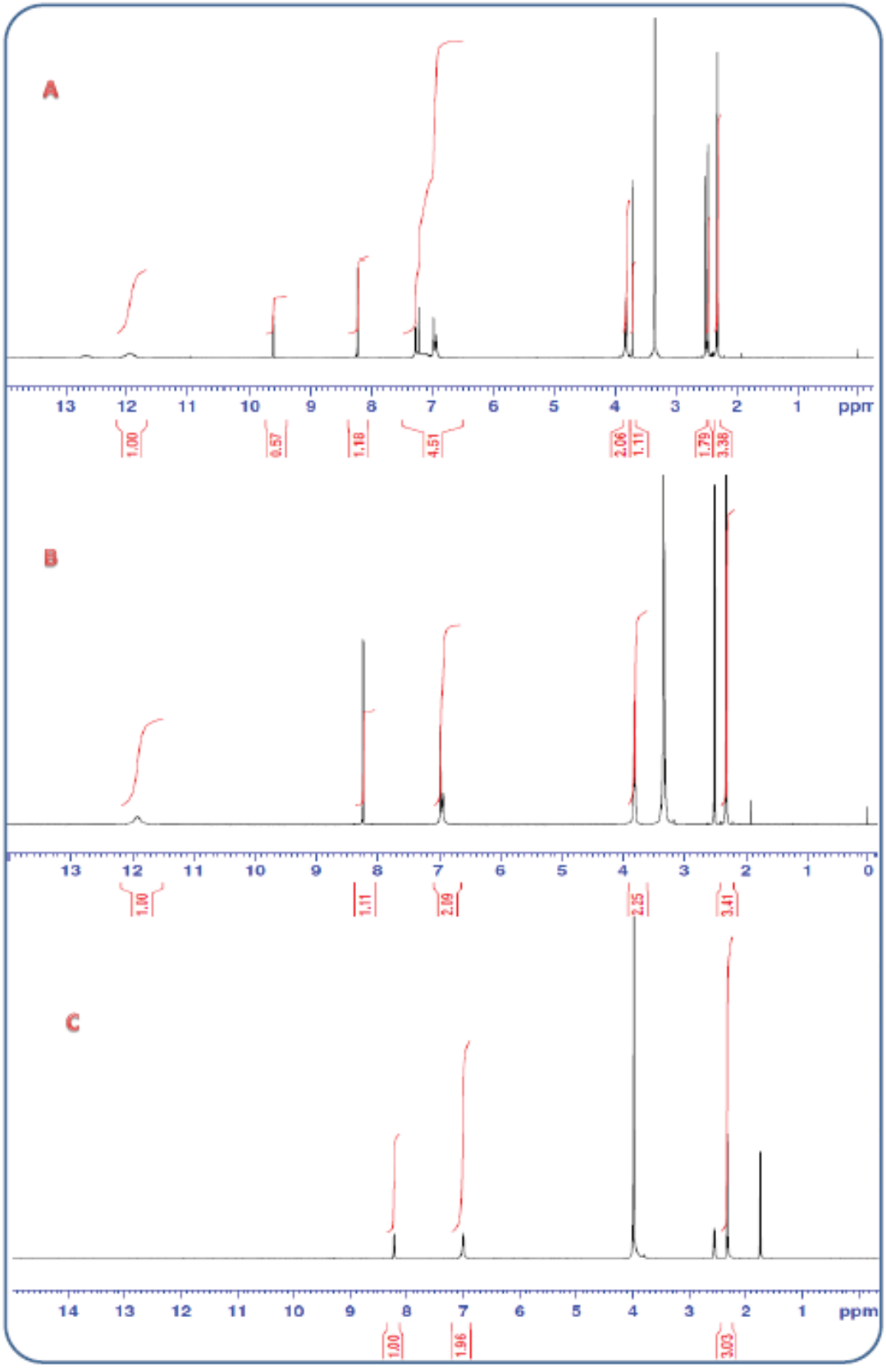


Figure 4. A) ^{1}H-NMR Spectrum of compound **5'**, B) ^{1}H-NMR Spectrum of compound **5**, C) D_2O Exchange spectrum of compound **5**.

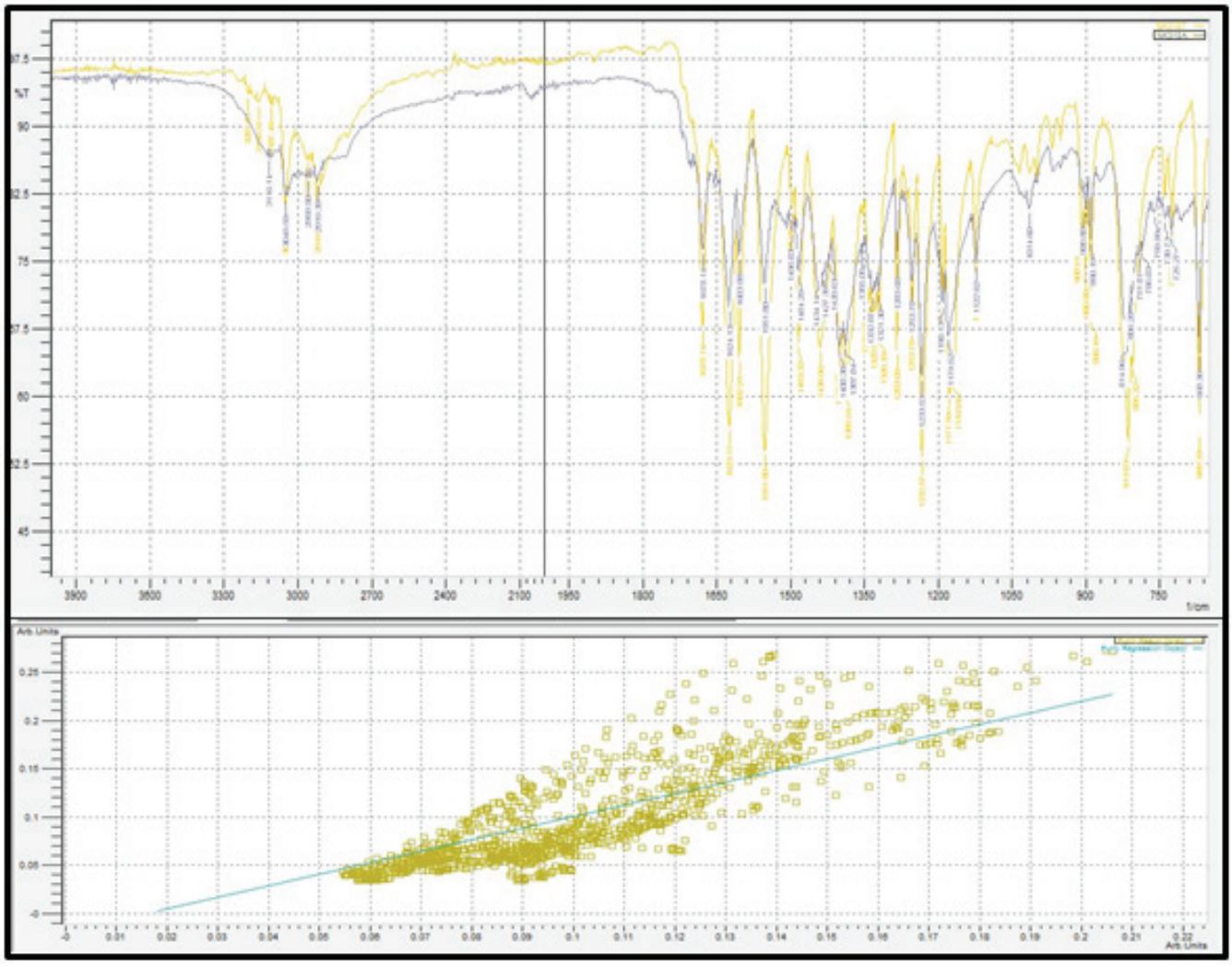


Figure 5. Comparison of IR spectra of compounds **5** and **5'**.

Table 1. Physical and analytical data of compounds **5** and **5'**.

		Compound 5'	**Compound 5**
Yield (%)		52	74
Appearance		Brown powder substance	White powder substance
M.p. (°C)		241-243	244
Rf [petroleum ether - acetone (30:70, v/v)]		0.49	0.49
Elemental analysis (Calculated / Found)	**C**	48.68* / 48.91	52.16 ** / 51.72
	H	4.99 / 4.58	4.38 / 4.65
	N	18.92 / 18.32	20.27 / 20.04
	S	14.44 / 13.21	15.47 / 15.20

* Calculated according to $C_9H_9N_3OS$. 4/5 H_2O

** Calculated according to $C_9H_9N_3OS$

In the light of these findings, we decided to synthesize the target compounds by using thioureas as starting materials. *N*-(4-Substituted pyridine-2-yl)thioureas [**3**, **4**] were obtained by hydrolysis of *N*-(4-substituted pyridine-2-yl)-*N*'-benzoyl thioureas [**1**, **2**] which were synthesized from 2-amino-4-methylpyridine and 2-amino-4-chloropyridine as starting compounds. 2-Imino-1,3-thiazolidin-4-one [**5**, **6**] derivatives were obtained by heating the compound **3** and **4** with ethyl bromoacetate in the presence of sodium acetate in ethanol.

All synthesized compounds were checked for purity and identity using TLC and elemental analysis. All newly synthesized compounds were characterized by their melting points, FTIR, ^{1}H NMR and mass spectral data, whereas ^{13}C NMR spectra were also recorded for compounds **5** and **6**.

The IR spectra of compounds **1** and **2** have C=O, C=S and N-H stretching bands of acylthiourea group at 1674-1675 cm^{-1}, 1237-1249 and 1165 cm^{-1}, and 3256-3274 cm^{-1}, respectively (7, 43). In the ^{1}H-NMR spectra of compound **2**, resonances assigned to the N^{1}-H and N^{2}-H protons of thiourea were detected between 8.83-13.28 ppm as three broad singlets, which are supported by the literature (7, 44, 45). When we examined the IR spectra of compounds **3** and **4**, we detected two C=S stretching bands at 1106-1170 cm^{-1} and 1216-1235 cm^{-1}, and N-H stretching bands at 3084-3188 cm^{-1}, whereas C=O stretching bands of the acylthioureas disappeared. In the ^{1}H-NMR spectra of compound **4**, NH proton of thiourea was observed at 10.65 ppm while NH_2 protons have two singlet peaks at 9.05 ppm and 10.38 ppm (46). In the IR spectra of compounds **5** and **6**, the disappearance of C=S stretching bands of the thioureas, detection of C=O stretching band and C-S-C stretching band at 1678-1694 cm^{-1} and 666-669 cm^{-1}, respectively (32, 47) and observation of thiazolidinone NH peak at 11.92 ppm in the ^{1}H-NMR spectra of compound **5** is an evidence for ring closure form of 2-imino-1,3-thiazolidin-4-one. Another support for 2-imino-1,3-thiazolidin-4-one ring closure was the detection of a signal at 3.81-3.87 ppm, due to the presence of -CH_2- protons located in the fifth position of the 4-thiazolidinone ring, furthermore, NH proton of compound **5** was observed to exchange with D_2O in the spectrum. ^{13}C-NMR spectra of compounds **5** and **6** showed the presence of new carbonyl peak (**C**=O) due to 4-thiazolidinone ring at 172.72 ppm. Moreover, the chemical shift of -CH_2- carbon for these compounds were detected in the range of 35.60-36.37 ppm (48). The mass spectral analysis of the synthesized compounds was performed under electro-spray ionization. Mass spectra confirmed the molecular weights and empirical formula of compounds **1**-**6** for both negative and positive ion mass spectra. Our target compounds that are 2-imino-1,3-thiazolidinones gave elemental analysis data consistent to assigned structures.

2.2. Biological Activity Studies

2.2.1. Antiviral Activity

Results of antiviral assays against *Murine norovirus*, *Yellow fever virus*, *Enterovirus* and *Chikungunya* virus strains for synthesized compounds **1**-**6** were given in **Table 2**. EC_{50} values for all strains of these compounds were determined higher than 0.3 μM. EC_{50} value of compounds **2** and **6** could not be calculated for *Yellow fever virus* and *Enterovirus*, respectively because their dose-response curve did not show a linear increase in studied concentration range. Tested compounds did not show 50 % or higher antiviral activity than the control group for all strains in studied concentration range, so their cytotoxicity could not be calculated.

Table 2. Results of antiviral activity for all synthesized compounds.

Compound	*Murine norovirus*		*Yellow fever*		*Enterovirus*		*Chikungunya*	
	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
1	>0.4	0.2±0	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4
2	>0.3	0.2±0	-	>0.3	>0.3	>0.3	>0.3	>0.3
3	>0.6	0.1±0.03	>0.6	>0.6	>0.6	>0.6	>0.6	>0.6
4	>0.5	0.2±0.08	>0.5	0.2±0.02	>0.5	>0.5	>0.5	>0.5
5	>0.5	0.2±0.07	>0.5	0.2±0.0001	>0.5	>0.5	>0.5	>0.5
6	>0.4	0.1±0.07	>0.4	>0.4	-	>0.4	>0.4	0.2±0.03

2.2.2. Anticancer Activity

In this study, human chronic myeloid leukemia cell line K562 (ATCC, CCL243), human breast cancer cell line MCF-7 (ATCC, HTB22), human colon cancer cell line HT-29 (ATCC, HTB-38), human osteosarcoma cell line SJSA1 (ATCC, CRL-2098) human lung cancer cell line A549 (ATCC, CCL-185), human prostate cancer cell line PC-3 (ATCC, CRL-1435), human cervical carcinoma cell line HeLa (ATCC, CCL-2) were used and proliferation values of test compounds were evaluated at 10 μM dose (**Tablo 3**). Additionally, cytotoxicty results against mouse fibroblast cell line NIH3T3 (ATCC, CRL-1658) at 10 μM dose of compounds **1-6** were given in **Table 3** and their proliferation values were observed in the range of 29.79-98.19 %. The most active compound was identified as compound **4** by 38.92% and 35.96% inhibition in HT-29 and SJSA1 cell lines, respectively. This compound was observed non-toxic to NIH3T3 mouse fibroblast cells with high survival rate (92.01%). Other remarkable inhibitions were observed with compounds **2** (32.29% inhibition vs. HT-29) and **6** (35.82% inhibition vs. HeLa) at 10 μM dose. These compounds appeared as safe towards NIH3T3 fibroblasts.

2.3. Prediction of Drug-Likeness and Absorption, Distribution, Metabolism, and Excretion (ADME) Properties of Compounds 1-6.

Oral bioavailability and appropriate drug delivery are quite important for new drug candidates intended for oral use (49). Many of the drug candidates with poor pharmacokinetic profiles fail at development stages (50). A low and variable bioavailability is not a desired property in the development of a drug candidate (51). Accordingly, a computational study for prediction of ADME properties of the molecules was performed by using Molinspiration online property calculation toolkit for determination of topological polar surface area (TPSA), absorption (%ABS), lipophilicity and simple molecular descriptors used by Lipinski in formulating his "rule of five" (52, 53). Table 4 represents a calculated percentage of absorption (%ABS), topological polar surface area (TPSA) and Lipinski parameters of the compounds **1**-**6**. Percentage of absorption (%ABS) was estimated using the equation: %ABS=109-(0.345xTPSA), according to Zhao *et al* (54). TPSA was also calculated using Molinspiration online property calculation toolkit according to the fragment-based method of Ertl *et al* (55). Polar surface area, together with lipophilicity, is an important property of a molecule in transport across biological membranes. Too high TPSA values give rise to a poor bioavailability and absorption of a drug. According to the above criterions, calculated percentages of absorption for compounds **1**-**6** ranged between 90.3 and 91.4%. Number of hydrogen bond acceptors varied 3 to 4 while the number of hydrogen bond donors varied 1 to 3 for all synthesized compounds. All these findings related to Lipinski parameters showed that compounds **1**-**6** have zero violations to Lipinski rule of five.

Table 3. Cytotoxicity and anticancer activity of compounds **1**-**6** in selected cancer cell lines.

	% Proliferation (at 10 μM dose)							
Compound	**NIH3T3**	**PC-3**	**A549**	**HeLa**	**HT-29**	**MCF-7**	**SJSA1**	**K562**
1	29.79	78.11	80.12	100.17	89.44	113.08	81.51	99.19
2	78.12	93.41	89.29	78.77	67.71	91.61	79.87	102.52
3	48.17	70.03	73.26	99.63	89.72	107.44	89.85	98.34
4	92.01	77.26	76.26	80.89	61.08	79.60	64.04	113.51
5	75.73	78.85	82.09	121.72	89.11	108.47	92.63	105.75
6	98.19	82.47	74.46	64.18	81.81	97.97	81.26	109.22

Table 4. Predicted ADME, Lipinski parameters and molecular properties of compounds **1**-**6**.

Compound	**MW**	**Volume**	**TPSA**	**%ABS**	***n*-ROTB**	***n*-ON**	***n*-OHNH**	***mi* LogP**	***n* Violations**
1	271.35	239.51	54.02	90.4	4	4	2	2.52	0
2	291.76	236.48	54.02	90.4	4	4	2	2.75	0
3	167.24	148.00	50.94	91.4	2	3	3	1.11	0
4	187.66	144.97	50.94	91.4	2	3	3	1.34	0
5	207.26	175.18	54.35	90.3	1	4	1	0.92	0
6	227.68	172.16	54.35	90.3	1	4	1	1.14	0

3. CONCLUSION

In conclusion, we used two different synthesis procedures for 4-thiazolidinone ring closure and compared the structure of the obtained compounds by using physical, analytical and spectral data. So we determined that 2-imino tautomeric form is obtained by cyclization of thioureas even though a mixture of two tautomeric forms is obtained by using chloroacetamides as a starting compound. As a result, we observed that different tautomers of the 4-thiazolidinone ring could be obtained when the synthesis method was changed.

Biological properties of compounds **1**-**6** were evaluated as anticancer and antiviral agents. Antiviral activity against *Murine norovirus*, *Yellow fever virus*, *Enterovirus* and *Chikungunya* virus strains of the all synthesized compounds were evaluated but a significant antiviral activity at subtoxic concentrations was not observed. Cytotoxic property against NIH3T3 cell line and anticancer activity against K562, MCF-7, HT-29, SJSA1, A549, PC-3, HeLa cell lines of compounds **1**-**6** were evaluated at 10 μM dose. Non-toxic compounds **2** (against HT-29 cell line), **4** (against HT-29 and SJSA1 cell lines) and **6** (against HeLa cell line) showed that cell growth inhibition between 32.29 and 38.92%.

4. MATERIAL AND METHODS

4.1. Synthetic Chemistry

All solvents and reagents were obtained from commercial sources and used without purification. The purity of the compounds was confirmed by the thin-layer chromatography (TLC) performed on Merck silica gel 60 F254 aluminum sheets (Merck, Darmstadt, Germany), using developing systems: S1: petroleum ether/acetone (60:40 v/v); S2: dichloromethane/ethyl acetate/ethanol (60:30:10 v/v/v) and S3: petroleum ether /acetone (30:70 v/v) . Spots were detected under UV light at λ= 254 and 366 nm. All melting points (°C, uncorrected) were determined using Schmelzpunktbestimmer SMP II basic model melting point apparatus. Elemental analyses were obtained using Leco CHNS-932 and are consistent with the assigned structures. Infrared spectra were recorded on a Shimadzu FTIR 8400S and data are expressed in wavenumbers ν (cm^{-1}). NMR spectra were recorded on Brüker 300 MHz Ultrashield TM at 300 MHz for ^{1}H-NMR and 600 MHz for ^{13}CNMR (decoupled), the chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane (TMS) using DMSO-d_6 as solvent. ESI positive and negative ionization (low resolution) mass spectra of the synthesized compounds were obtained using AB SCIEX API 2000 LC–MS/MS instrument.

General procedure for the synthesis of N-(4-substituted pyridin-2-yl)-N'-benzoyl thioureas [**1**-**2**]

A solution of benzoyl chloride (0.012 mol) in dry acetone (30 ml) was added, dropwise, to a suspension of NH_4SCN (0.0132 mol) in acetone (20 ml). The mixture was stirred until the formation of benzoyl isothiocyanate for 1 h. Then 2-amino-4-substituted pyridine derivative (0.01 mol), dissolved in acetone (20 ml) was slowly added to the reaction flask, with constant stirring. The solution was heated under reflux for 3 h. The reaction mixture was then poured onto ice. Solids of *N*-(4-substituted pyridin-2-yl)-*N'*-benzoyl thioureas were collected by filtration and finally purified by recrystallization from methanol.

N-[(4-Methylpyridin-2-yl)carbamothioyl]benzamide [**1**]

Yield 74%. M.p. 165°C (lit.154-155°C (56)). TLC Rf: 0.58 (S1). IR, ν (cm^{-1}): 3274 (NH str), 1674 (C=O str), 1249, 1165 (C=S str). LC–MS-(ESI): Calculated: M_{mi}: 271.078, $(M+H)^+$: 272.085, $(M-H)^-$: 270.071. Found: $(M+H)^+$: 272.004, $(M-H)^-$: 270.123.

N-[(4-Chloropyridin-2-yl)carbamothioyl]benzamide [**2**]

Yield 70%. M.p. 145-146°C. TLC Rf: 0.85 (S2). IR, ν (cm^{-1}): 3256 (NH str), 1675 (C=O str), 1237, 1165 (C=S str). ^{1}H NMR, δ (ppm): 7.43-7.45 (dd, *J=5.4 Hz and 1.8 Hz*, 1H, Pyr-H), 7.54-7.72 (m, 4H, Pyr-H and Ar-H), 7.97-8.00 (m, 2H, Ar-H), 8.44 (d, *J=5.4 Hz*, 1H, Pyr-H), 8.83 (br s, NH), 12.05 (br s, NH), 13.28 (br s, NH). LC–MS-(ESI): Calculated: M_{mi}: 291.023, $(M+H)^+$: 292.031. Found: $(M+H)^+$: 291.976.

General procedure for the synthesis of N-(4-substituted pyridin-2-yl)thioureas [**3**-**4**]

Compound **1** or **2** (0.005 mol) was dissolved in methanol (20 ml) and solution of aqueous sodium hydroxide (1N) (5 ml) was added. The solution was heated under reflux for 6 h, then the mixture was poured onto crushed ice and neutralized with 1N hydrochloric acid (pH~7). The precipitated crude product was washed with water and purified by crystallization with methanol.

N-(4-Methylpyridin-2-yl)thiourea [**3**]

Yield 84%. M.p. 220-222°C (lit.210-212°C (57)). TLC Rf: 0.48 (S1), 0.64 (S3). IR, ν (cm^{-1}): 3219, 3189 (NH str), 1235, 1170 (C=S str). LC–MS-(ESI): Calculated: M_{mi}: 167.052, $(M+H)^+$: 168.059, $(M-H)^-$: 166.044. Found: $(M+H)^+$: 168.114, $(M-H)^-$: 165.965.

N-(4-Chloropyridin-2-yl)thiourea [**4**]

Yield 75%. M.p. 197°C. TLC Rf: 0.75 (S2). IR, ν (cm^{-1}): 3265, 3112 (NH str), 1216, 1106 (C=S str). ^{1}H NMR, δ (ppm): 7.16-7.19 (dd, *J=5.6 Hz, 2.1 Hz and 1.8 Hz*, 1H, Pyr-H), 7.27 (d, *J=1.5 Hz*, 1H, Pyr-H), 8.23 (d, *J=5.4 Hz*, 1H, Pyr-H), 9.05 (br s, NH), 10.38 (br s, NH), 10.65 (br s, NH). LC–MS-(ESI): Calculated: M_{mi}: 186.997, $(M+H)^+$: 188.004, $(M-H)^-$: 185.990. Found: $(M+H)^+$: 188.031, $(M-H)^-$: 186.055.

General procedure for the synthesis of 2-[(4-substituted pyridin-2-yl)imino]-1,3-thiazolidin-4-one [**5-6**]

Anhydrous sodium acetate (0.02 mol) and ethyl bromoacetate (0.005 mol) were added into the ethanolic solution of compound **3** or **4** (0.005 mol). The reaction mixture was boiled for 7 h under reflux conditions. At the end of the reaction, the substance precipitated at room temperature was filtered, dried and purified by crystallization with ethanol.

(2Z)-2-[(4-Methylpyridin-2-yl)imino]-1,3-thiazolidin-4-one [**5**]

Yield 74%. M.p. 244°C. TLC Rf: 0.49 (S3). IR, ν (cm^{-1}): 3157 (NH str), 1678 (C=O str), 666 (C-S-C str). ^{1}H NMR, δ (ppm): 2.31 (s, 3H, -CH_3), 3.81 (s, 2H, S-CH_2-), 6.93-6.98 (m, 2H, Pyr-H), 8.23 (d, *J=5.4 Hz*, 1H, Pyr-H), 11.92 (br s, 1H, thiazolidinone NH). ^{13}C NMR, δ (ppm): 21.01 (CH_3), 36.37 (SCH_2), 118.15, 120.69, 146.89, 149.46 (Pyr-C), 172.82 (C=O). Anal. calc. for $C_9H_9N_3OS$ (207.25 g/mol): C, 52.16; H, 4.38; N, 20.27; S, 15.47%. Found C, 51.72; H, 4.65; N, 20.04; S, 15.20%. LC–MS-(ESI): Calculated: M_{mi}: 207.047, $(M+H)^+$: 208.054, $(M-H)^-$: 206.039. Found: $(M+H)^+$: 208.068, $(M-H)^-$: 205.933.

(2Z)-2-[(4-Chloropyridin-2-yl)imino]-1,3-thiazolidin-4-one [**6**]

Yield 68%. M.p. 267°C. TLC Rf: 0.53 (S2). IR, ν (cm^{-1}): 3148 (NH str), 1694 (C=O str), 669 (C-S-C str). ^{1}H NMR, δ (ppm): 3.87 (s, 2H, S-CH_2-), 7.24-7.26 (m, 2H, Pyr-H), 8.37 (d, *J=6 Hz*, 1H, Pyr-H). ^{13}C NMR, δ (ppm): 35.60 (SCH_2), 118.46, 120.05, 144.59, 148.70 (Pyr-C). Anal. calc. for $C_8H_6ClN_3OS$ (227.67 g/mol): C, 41.55; H, 2.79; N, 18.17; S, 13.86%. Found C, 41.95; H, 2.75; N, 17.89; S, 13.37%. LC–MS-(ESI): Calculated: M_{mi}: 226.992, $(M+H)^+$: 227.999, $(M-H)^-$: 225.985. Found: $(M+H)^+$: 228.035, $(M-H)^-$: 225.996.

4.2. Biological Methods

4.2.1. Antiviral Assays

The antiviral activity of the selected compounds against the different viruses was determined using an MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium]-based cytopathic effect (CPE) reduction assay. Antiviral assays were performed in DMEM medium supplemented with 2% FCA and other components depending on the cell line. Cells were seeded in a 96-well plate (Falcon, BD Biosciences) and allowed to adhere overnight. The next day they were infected with virus in the presence (or absence) of a dilution series of compounds. Cells were incubated (37°C, 5% CO_2, 95-99% relative humidity) until complete CPE was observed in infected untreated cells. Subsequently, the assay medium was aspirated, replaced with 75 µl of a 5% MTS (Promega) solution (MTS-phenazinemethosulfate (MTS/PMS) stock solution [(2 g/L MTS (Promega, Leiden, The Netherlands) and 46 mg/L PMS (Sigma–Aldrich, Bornem, Belgium) in PBS at pH 6–6.5)] was diluted 1/20 in MEM (Life Technologies, Gent, Belgium)) in phenol red-free medium, and incubated for 1.5 h. Absorbance was measured at a wavelength of 498 nm (Safire2, Tecan), with the optical densities (OD values) reaching 0.6-0.8 for the untreated, uninfected controls. The optical density values were converted to control percentages and logarithmic interpolation was used to calculate the EC_{50} and CC_{50}.

The EC50 was defined as the compound concentration that (i) protected 50% of cells from virus-induced CPE or (ii) reduced RNA copies by 50%. Adverse effects of the drug on the host cell were also assessed by means of the MTS method, by exposing uninfected cells to the same concentrations of the compounds. The CC50 was defined as the compound concentration that reduced the number of viable cells by 50%.

4.2.1.1. Chikungunya Virus-Cell-Based Antiviral Assay

Chikungunya virus (Indian Ocean strain 899), kindly provided by C. Drosten (Institute of Virology, University of Bonn, Germany), was grown on Vero A cells. Serial dilutions of the test compounds were made in 100 µl assay medium [MEM Rega3 (cat. no. 19993013; Invitrogen), 2% FCS (Integro), 5 ml of 200 mM L-glutamine, and 5 ml of 7.5% NaHCO3] in a 96-well microtiter. Subsequently, 50 µl of a 4 x virus dilution in assay medium was added, followed by 50 µl of a cell suspension. This suspension, with a cell density of 25 000 cells/50 µl, was prepared from a Vero A cell line subcultured in cell growth medium (MEM Rega3, supplemented with 10% FCS, 5 ml of L-glutamine, and 5 ml $NaHCO_3$) at a ratio of 1:4 and grown for 7 days in 150 cm^2 tissue culture flasks (Techno Plastic Products). The assay plates were returned to the incubator for 6-7 days a time at which maximal virus-induced cell death or cytopathic effect (CPE) is observed in untreated, infected controls.

4.2.1.2. Norovirus-Cell-Based Antiviral Assay

RAW 264.7 cells (1 x 10^4 cells/well) were seeded in a 96-well plate in 100 µl medium (DMEM medium supplemented with 10% FCS, 1% $NaHCO_3$, 1% L-glutamine, 1% sodium pyruvate, 1% P/S and 2% Hepes) and infected with MNV (virus strain MNV-1.CW1 kindly provided by Dr. Herbert Virgin, Washington University, St. Louis, USA) at a multiplicity of infection (MOI) of 0.001 in the presence (or absence) of a dilution series of compounds. Cells were incubated for 3 days, i.e. until complete CPE was observed in infected untreated cells.

4.2.1.3. Enterovirus-Cell-Based Antiviral Assay

Enterovirus 71 strain BrCr (EV71 BrCr), a gift from F. van Kuppeveld (Universiteit Utrecht, The Netherlands), was grown on RD cells. Cells were seeded at a density of 2.5 x 10^4 cells per well (100 µL) in 96-well cell culture plates. These assays were performed using MEM Rega3 medium supplemented with 2% FBS, 2 mM-glutamine and 0.075% $NaHCO_3$. Cells were allowed to adhere overnight, after which serial dilutions of the compound were made in the supernatant and 100 µl of a 2 x virus dilution in assay medium was added. The cultures were subsequently incubated for 4 days at 37°C, until complete virus-induced CPEs were observed in the untreated and infected virus control conditions (VC).

4.2.1.4. Yellow Fever Virus-Cell-Based Antiviral Assay

Yellow Fever Virus (Stamaril; Aventis Pasteur MSD, Brussels, Belgium) was grown on Huh-7 cells. Cells were seeded at a density 5.5 x 10^3 cells/well in 100 µL assay medium (DMEM medium supplemented with 10% FCS, 1% NEAA and 2% Hepes) in 96-well cell culture plates and allowed to adhere overnight. Antiviral assays were performed in DMEM medium supplemented with 2% FCS, 1% NEAA and 2% Hepes.

Serial dilutions of the compound were made in the supernatant and 100 µl of a 2 x virus dilution in assay medium was added. The cultures were subsequently incubated for 5 days at 37°C, until complete virus-induced CPEs were observed in the untreated and infected virus control conditions (VC).

4.2.2. Anticancer Assay

4.2.2.1. Cell culture

Human breast cancer cell line MCF-7 (ATCC, HTB22), human colon cancer cell line HT-29 (ATCC, HTB-38), human osteosarcoma cell line SJSA1 (ATCC, CRL-2098) human lung cancer cell line A549 (ATCC, CCL-185), human prostate cancer cell line PC-3 (ATCC, CRL-1435), human cervical carcinoma cell line HeLa (ATCC, CCL-2), human leukemic cell line, K562 (ATCC, CCL-243) and mouse fibroblast cell line NIH3T3 (ATCC, CRL-1658) were maintained in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (Fetal bovine serum), 1% L-Glutamine and penicillin/streptomycin (Gibco) at 37°C in a humidified incubator with 5% CO_2.

4.2.2.2. Cell viability assay

Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cells (1 × 10^4 cells/well) were seeded onto 96-well plates and incubated overnight. Then, the cells were treated with compounds (at 10 µM concentration) for 48 h. After the incubation period, MTT was added into each well to a final concentration of 0.5 mg/mL and incubated for 4 h. The culture medium was then removed and 100 µL of the SDS buffer was added to solubilize the purple formazan product. Absorbances at wavelengths of 570 and 630 nm were measured by a microplate reader (Biotek, Winooski, VT).

For human leukemic cell line, K562 (ATCC, CCL-243) cells, 200 µl (1x10^4 cells) of cell suspension was plated in each well of a 96-well plate. After the incubation for 12 h, the cells were treated with 10 µM concentrations of compounds. An equal volume of DMSO was added to the control wells, and the cells were cultured an additional 32 h. After the incubation period, 20 µL MTT was added to each well to a final concentration of 5 mg/mL and incubated at 37 ^{0}C for 4 h. Plates were then centrifuged at 400 × g for 10 min. Supernatants were removed from the wells and 200 µL of the SDS buffer was added into wells to solubilize the purple formazan product. Absorbances at wavelengths of 570 and 630 nm were measured by a microplate reader (Biotek, Winooski, VT, USA).

Viability was calculated as follows: Cell Viability (%)= (OD test /OD control) x 100%.

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Tiyoüreler, açiltiyoüreler ve 4-tiyazolidinonların sentezi, karakterizasyonu ve antikanser ve antiviral etkilerinin değerlendirilmesi

ÖZET

Bu çalışma kapsamında 2-amino-4-sübstitüe piridin bileşiklerinden hareketle sentezlenen tiyoüre türevleri [**1**-**4**], 2-imino-1,3-tiyazolidin-4-on halkalarının [**5**, **6**] sentez başlangıç maddelerini oluşturmaktadır. 4-Tiyazolidinon halka kapaması ve sentezi için iki farklı yöntem optimize edilmiştir. Sentezlenen bileşiklerin yapıları elementel analiz, IR, ^{1}H-NMR, ^{13}C-NMR ve kütle spektrumu ile kanıtlanmış, saflıkları ise İTK ile kontrol edilmiştir. Elde edilen bileşikler antiviral ve antikanser etkinlikleri açısından değerlendirilmiştir. *Murine norovirus*, *Yellow fever*, *Enterovirus* ve *Chikungunya* suşlarına karşı antiviral etkileri incelenen bileşiklerin 0,3 µM'dan yüksek EC_{50} değerine sahip oldukları tespit edilmiştir. Sitotoksisiteleri NIH3T3 hücre hattı üzerinde incelenen bileşiklerin antikanser etkileri PC-3, A549, HeLa, HT-29, MCF-7, SJSA1 ve K562 hücre hatlarına karşı incelenmiş %61'in üzerinde proliferasyon değerine sahip oldukları gözlemlenmiştir.

Anahtar kelimeler: Açiltiyoüre; antikanser; antiviral; tautomerizm; tiyoüre; 2-amino-1,3-tiyazol-4-on; 2-imino-1,3-tiyazolidin-4-on.

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