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Synthesis and antitumor properties of some new *N*-(5-R-benzyl-1,3-thiazol-2-yl)-4,5-dihydro-1*H*-imidazole-2-carboxamides

Volodymyr Ya Horishny[1], Iryna V Drapak[2], Taras I Chaban[2], Yuriy V Ostapiuk[3], Vasyl S Matiychuk[3,*]

1. Department of Pharmaceutical, Organic and Bioorganic Chemistry Danylo Halytsky Lviv National Medical University, Lviv, 79010, Ukraine
2. Department of General, Bioinorganic, Physical and Colloidal Chemistry, Danylo Halytsky Lviv National Medical University, 69 Pekarska, Lviv, 79010, Ukraine
3. Department of Organic Chemistry, Ivan Franko National University of Lviv, 6 Kyryla i Mefodia, Lviv, 79005, Ukraine

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*Corresponding author
Vasyl S Matiychuk

Email:
v_matiychuk@ukr.net

ABSTRACT

New N-(5-R-benzyl-1,3-thiazol-2-yl)-2-morpholin-4-yl-2-oxoacetamides have been prepared in good yields via the reaction of N-(5-R-benzyl-1,3-thiazol-2-yl)-2-chloroacetamides with sulfur and morfoline. These compounds react with ethendiamine to form series of novel N-[5-R-benzyl)-1,3-thiazol-2-yl]-4,5-dihydro-1H-imidazole-2-carboxamides with excellent yields. Anticancer activity screening of synthesized compounds was carried out within the framework of Developmental Therapeutic Program of the National Cancer Institute's (DTP,NCI, Bethesda, Maryland, USA). It was showed that compounds are promising for new anticancer agents search.

Keywords: organic synthesis, thiazole, imidazole, anticancer activity.

INTRODUCTION

2-Aminothiazole and their derivatives are of great importance in the organic and medicinal chemistry field (Nevagi, 2014; Chhabriaet *et al*., 2016; Aejazet *et al*., 2015). 2-Aminothiazole core is privileged structure for the compounds with a broad spectrum of activities, such as antibacterial (Vukovic *et al*., 2008), antifungal (Edwardset *et al*., 2013), antitubercular (Al-Balas *et al*., 2009), anti-HIV (Venkatachalam *et al*., 2001), antioxidant (Chaban *et al*., 2019), pesticidal (Wilkes *et al*., 1991), anti-inflammatory (Holla *et al*., 2003) etc. Among 2-aminothiazole-based compounds 5-benzyl derivatives are of special interest over the last decades. Significant antimicrobal (Khalilet *et al*., 2015) and anticancer activities of these compounds (Krasavin *et al*., 2009; Pokhodylo *et al*., 2014; Choi *et al*., 2011; Schiedel *et al*., 2016; Finiuk *et al*., 2017; Ostapiuk *et al*., 2018) have been reported. Aminothiazole derivatives have been also used as sensitive analytical reagents (Lozynska *et al*., 2015; Tymoshuk *et al*., 2019).

In this work we described the synthesis and anticancer activity of *N*-[5-R-benzyl)-1,3-thiazol-2-yl]-4,5-dihydro-1*H*-imidazole-2-carboxamides.
The latter are the new class of organic compounds and their biological activity is not investigated. However, the synthesis and biological properties of compound with similar structure (**1)** were described. The antimicrobial (Sueleyman *et al*., 2005; Chaudhary *et al*., 2011) and anticancer (Beauchard *et al*., 2009) activity of such compounds were reported. They are also ligands of ad renergic α2 receptor (Saczewski *et al*., 2006), inhibitors of cyclooxygenase (Tanaka *et al*., 1994), and glycogen synthase kinase-3 (Saczewski *et al*., 2006), which can be considered as prominent anticancer targets (Satish and Woodgett, 2008).

MATERIALS AND METHODS

Chemicals and reagents

All chemicals were of analytical grade and commercially available. All reagents and solvents were used without further purification and drying.

Chemistry

All the melting points were determined in an open capillary and are uncorrected. ^{1}H- spectra were recorded on a Varian Mercury 400 (400MHz for ^{1}H). Mass spectra were run using Agilent 1100 series LC/MSD. Agilent Technologies Inc. with an API–ES/APCI ionization mode. The elemental analysis of experimental data on contents of Carbon, Hydrogen and Nitrogen were within ±0.3 % of the theoretical values.

The general procedure for 2-morpholin-4-yl-*N*-aryl-2-thioxoacetamides (2a-e) preparation.

A suspension of 0.01mol of powdered sulfur in 10mL of morpholine was stirred for 5min. To the prepared solutions the chloroacetamides (0.05 mol) was added, and stirred for 60min room temperature. The reaction mixture was poured into 200mL water and left for 24 h. The solid precipitated was filtered off, washed with water (20mL), dried and crystallized from ethanol.

N-(5-benzyl-1,3-thiazol-2-yl)-2-morpholin-4-yl-2-thioxoacetamide (2a)

Yield 86%, mp 188-190°C. ^{1}H NMR (400 MHz, DMSO): δ = 12.61 (s, 1H, NH), 7.50 – 7.16 (m, 6H, C_6H_4, thiazole), 4.10 (s, 2H, $PhCH_2$), 4.08 (d, J = 4.1 Hz, 2H, CH_2), 3.73 (d, J = 4.0 Hz, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.58 (s, 2H, CH_2). Anal.calcd.for $C_{16}H_{17}N_3O_2S_2$: C, 55.31; H, 4.93; N, 12.09. Found: C, 55.12; H, 4.90; N, 12.15.

N-[5-(4-chlorobenzyl)-1,3-thiazol-2-yl]-2-morpholin-4-yl-2-thioxoacetamide (2b)

Yield 93%, mp 238-240°C. ^{1}H NMR (400 MHz, DMSO): δ = 12.65 (s, 1H, NH), 7.36 (d, *J* = 8.2 Hz, 2H, C_6H_4), 7.34 – 7.26 (m, 3H, thiazole, C_6H_4), 4.10 (s, 2H, $ArCH_2$), 4.07 (s, 2H, CH_2), 3.73 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.58 (s, 2H, CH_2). Anal.calcd.for $C_{16}H_{16}ClN_3O_2S_2$: C, 50.32; H, 4.22; N, 11.00. Found: C, 50.49; H, 4.30; N, 10.75.

N-[5-(2-chlorobenzyl)-1,3-thiazol-2-yl]-2-morpholin-4-yl-2-thioxoacetamide (2c)

Yield 99%, mp 191-193°C. ^{1}H NMR (400 MHz, DMSO): δ = 12.67 (s, 1H, NH), 7.59 – 7.39 (m, 2H, C_6H_4), 7.37 – 7.17 (m, 3H, C_6H_4, thiazole), 4.21 (s, 2H, $ArCH_2$), 4.14 – 3.93 (m, 2H, CH_2), 3.72 (s, 2H, CH_2), 3.63 (d, J = 4.1 Hz, 2H, CH_2), 3.57 (s, 2H, CH_2). Anal.calcd.for $C_{16}H_{16}ClN_3O_2S_2$: C, 50.32; H, 4.22; N, 11.00. Found: C, 50.17; H, 4.11; N, 11.15.

2-Morpholin-4-yl-2-thioxo-N-{5-[3-(trifluoromethyl)benzyl]-1,3-thiazol-2-yl}acetamide (2d)

Yield 81%, mp 187-189°C. ^{1}H NMR (400 MHz, DMSO): δ = 12.73 – 12.64 (br.s, 1H, NH), 7.67 (s, 1H, C_6H_4), 7.63-7.54 (br.s, 3H, C_6H_4), 7.38 (s, 1H, thiazole), 4.23 (s, 2H, $ArCH_2$), 4.11 - 4.03 (m, 2H, CH_2), 3.72 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.61 – 3.53 (m, 2H, CH_2). Anal.calcd.for $C_{17}H_{16}F_3N_3O_2S_2$: C, 49.15; H, 3.88; N, 10.11. Found: C, 48.97; H, 3.72; N, 9.99.

N-{5-[2-chloro-5-(trifluoromethyl)benzyl]-1,3-thiazol-2-yl}-2-morpholin-4-yl-2-thioxoacetamide (2e)

Yield 71%, mp 207-209°C. ^{1}H NMR (400 MHz, DMSO): δ = 12.67 (s, 1H, NH), 7.89 (s, 1H, C_6H_3), 7.71 (d, J = 7.3 Hz, 1H, C_6H_3), 7.66 (d, J = 7.0 Hz, 1H, C_6H_3), 7.36 (s, 1H, thiazole), 4.32 (s, 2H, $ArCH_2$), 4.08 (s, 2H, CH_2), 3.73 (d, J = 2.5 Hz, 2H, CH_2), 3.64 (s, 2H, CH_2), 3.59 (s, 2H, CH_2). Anal.calcd.for $C_{17}H_{15}ClF_3N_3O_2S_2$: C, 45.39; H, 3.36; N, 9.34. Found: C, 45.15; H, 3.23; N, 9.25.

The general procedure fot 4,5-dihydro-1*H*-imidazole-2-carboxamides (3a-e) preparation

Method A. 0.0015mol of the corresponding morpholin-4-yl-2-thioxoacetamide 2a-e and 4mL of ethylenediamine was stirred at 50ºC for 30min. The mixture was cooled and poured into the 30mL of water. The precipitate was filtered, washed with water, dried and recrystallized from an alcohol.

Method B. 1g of sulfur was dissolved in ethylenediamine (10 mL), and stirred for 30min. To the formed solution, 0.006mol of the corresponding chloroacetamide was added with constant stirring for 10min. The mixture was continued stirred for 30min, then cooled and poured into the 100mL of water and leave for 1 day. The precipitate was filtered, washed with water, dried and recrystallized from an alcohol.

N-(5-benzyl-1,3-thiazol-2-yl)-4,5-dihydro-1*H*-imidazole-2-carboxamide (3a)

Yield 85%, mp 235°C. ^{1}H NMR (400 MHz, DMSO): δ = 7.31 - 7.17 (m, 5H, C_6H_5), 7.14 (s, 1H, thiazole), 4.01 (s, 2H, $ArCH_2$), 3.83 (s, 4H, $2CH_2$). ESI-MS: m/z 287 $[M+H]^+$; Anal. calcd.for $C_{14}H_{14}N_4OS$: C, 58.72; H, 4.93; N, 19.57. Found: C, 58.45; H, 4.82; N, 19.43.

N-[5-(4-chlorobenzyl)-1,3-thiazol-2-yl]-4,5-dihydro-1*H*-imidazole-2-carboxamide (3b)

Yield 97%, mp 230°C (decomp.). ^{1}H NMR (400 MHz, DMSO): δ = 7.33 (d, *J* = 8.3 Hz, 2H, C_6H_4), 7.24 (d, *J* = 8.3 Hz, 2H, C_6H_4), 7.13 (s, 1H, thiazole), 4.00 (s, 2H, $ArCH_2$), 3.81 (s, 4H, $2CH_2$). Anal.calcd.for $C_{14}H_{13}ClN_4OS$: C, 52.42; H, 4.08; N, 17.46. Found: C, 52.15; H, 4.10; N, 17.67.

N-[5-(2-chlorobenzyl)-1,3-thiazol-2-yl]-4,5-dihydro-1*H*-imidazole-2-carboxamide (3c)

Yield 95%, mp 230°C(decomp.). ^{1}H NMR (400 MHz, DMSO): δ = 7.42 (d, J = 7.4 Hz, 1H, C_6H_4), 7.35 (d, J = 7.3 Hz, 1H, C_6H_4), 7.31 – 7.21 (m, 2H, C_6H_4), 7.12 (s, 1H, thiazole), 4.12 (s, 2H, $ArCH_2$),

3.84 (s, 4H, $2CH_2$). ESI-MS: m/z 321 $[M+H]^+$; Anal. calcd.for $C_{14}H_{13}ClN_4OS$: C, 52.42; H, 4.08; N, 17.46. Found: C, 52.17; H, 3.97; N, 17.50.

N-{5-[3-(trifluoromethyl)benzyl]-1,3-thiazol-2-yl}-4,5-dihydro-1*H*-imidazole-2-carboxamide (3d)

Yield 79%, mp 253-255°C. ^1H NMR (400 MHz, DMSO): δ = 7.57 (s, 1H, C_6H_4), 7.54-7.50 (m, 3H, C_6H_4), 7.17 (s, 1H, thiazole), 4.13 (s, 2H, $ArCH_2$), 3.83 (s, 4H, $2CH_2$). Anal.calcd.for $C_{15}H_{13}F_3N_4OS$: C, 50.84; H, 3.70; N, 15.81. Found: C, 50.60; H, 3.52; N, 15.58.

N-{5-[2-chloro-5-(trifluoromethyl)benzyl]-1,3-thiazol-2-yl}-4,5-dihydro-*1H*-imidazole-2-carboxamide (3e)

Yield 95%, mp>260°C. ^1H NMR (400 MHz, DMSO): δ = 7.78 – 7.56 (m, 2H, C_6H_4), 7.33 (s, 1H), 7.14 (s, 1H, thiazole), 4.09 (s, 2H, $ArCH_2$), 3.81 (s, 4H, $2CH_2$). Anal. calcd.for $C_{15}H_{12}ClF_3N_4OS$: C, 46.34; H, 3.11; N, 14.41. Found: C, 46.50; H, 3.00; N, 14.62.

Pharmacology

Cytotoxic activity against malignant human tumor cells

The tested compounds were added to the culture at a single concentration (10^{-5}M) and the cultures were incubated for 48 h. Endpoint determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each tested compound were reported as the percent growth of the treated cells when compared to the untreated control cells. The percent growth was evaluated spectrophotometrically versus not treated controls. The cytotoxic and/or growth inhibitory effects of the most active compounds were tested *in vitro* against the full panel of about 60 human tumor cell lines at 10-fold dilutions of five concentrations ranging from $10^{-4}$to 10^{-8}M. The 48-h continuous drug exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth.

Using the seven absorbance measurements [time zero, (Tz), control growth in the absence of drug, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percent growth was calculated at each of the drug concentrations levels. Percent growth inhibition was calculated as:

$\frac{(Ti - Tz)}{(C - Tz)} \times 100$ for concentrations for which Ti ≥ Tz

$\frac{(Ti - Tz)}{(Tz)} \times 100$ for concentrations for which Ti <Tz

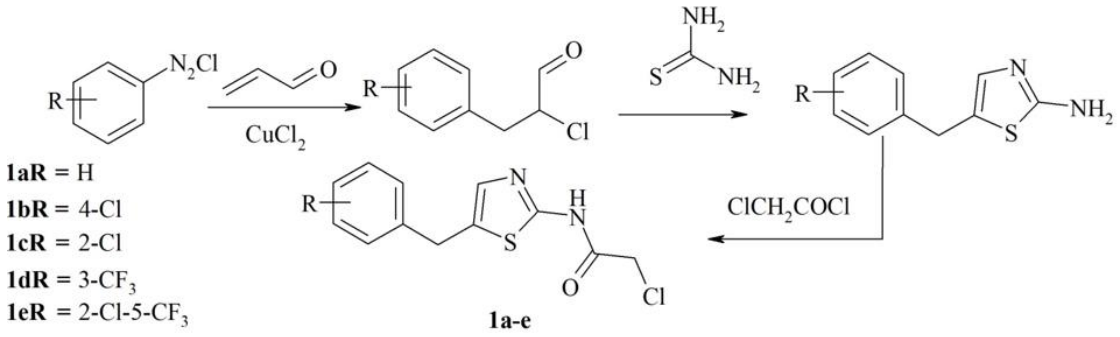
Three dose-response parameters were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from [(Ti − Tz)/(C − Tz)] ×100−50, which is the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from [(Ti − Tz)/Tz] × 100 = -50. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as more or less than the maximum or minimum concentration was tested.

RESULTS AND DISCUSSION

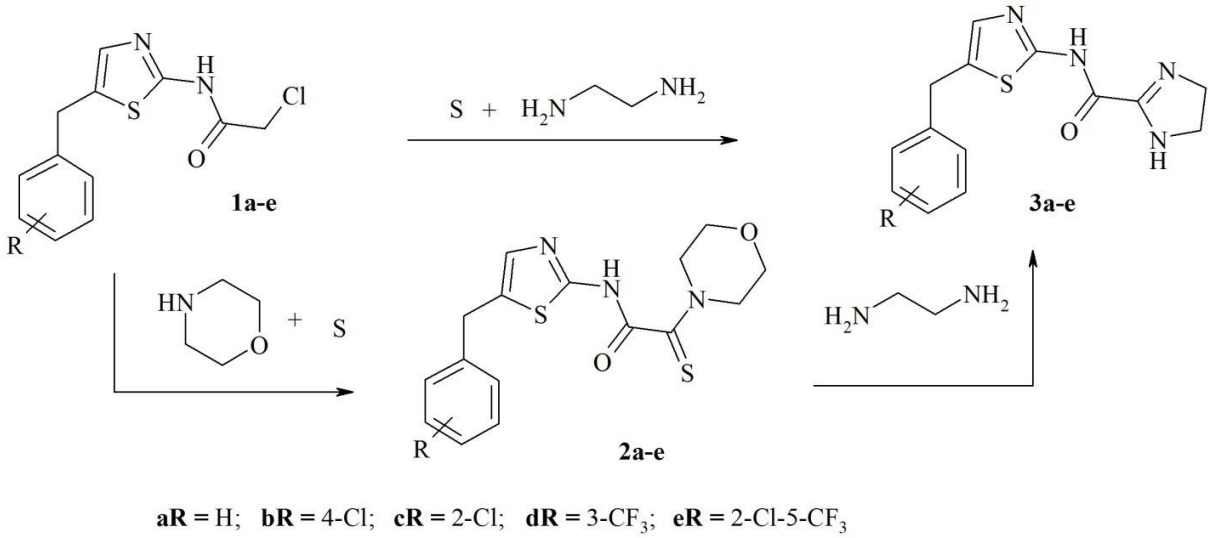
Chemistry

Diazonium salts are important reagents in organic synthesis, which are easily obtained from readily available aromatic amines. The utilization of diazonium salts in the design and synthesis of combinatorial libraries of furane (Obushak *et al*., 2009; Gorak *et al*., 2009; Obushak *et al*., 2008), pyrazole (Matiichuk *et al*., 2008), and 1,2,3-triazole (Obushak *et al*., 2009) derivatives, as well as some fused heterocycles (Chaban *et al*., 2019; Zelisko *et al*., 2015; Chaban *et al*., 2017; Zubkov *et al*., 2010; Klenina *et al*., 2017; Chaban *et al*., 2018) has been shown in our previous works. In this work we developed the new method of the synthesis of N-[5-R-benzyl)-1,3-thiazol-2-yl]-4,5-dihydro-1*H*-imidazole-2-carboxamide **3a–e** based on diazonium salt as a started reagents. At the first stage 2-chloro-N-[5-(R-benzyl)-thiazol-2-yl]-acetamides were prepared via described protocol (**Scheme 1**) (Obushak *et al*., 2004; Ostapiuk *et al*., 2012).

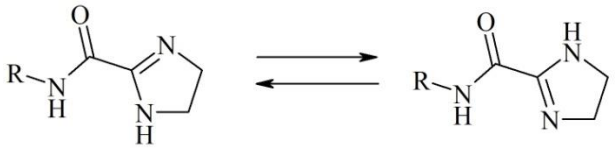
It well known that chloroacetanilides react with sulfur and morfoline to form corresponding monothiooxamides (Yarovenko *et al*., 1999). But chloracetamide derivatives of heterocyclic amines were not investigated in this reaction. So, we study the reaction of chloracetamides **1a-e,** sulfur and morfoline. The optimal conditions for the synthesis of target monothiooxamides were the next: firstly, sulfur was stirred with morfoline for 30min. (this time is needed to obtain a sufficient amount of polysulfides in the reaction mixture);



Scheme 1. Synthesis of 2-chloro-N-[5-(R-benzyl)-thiazol-2-yl]-acetamides.



Scheme 2. Synthesis of N-[5-R-benzyl-1,3-thiazol-2-yl]-4,5-dihydro-1*H*-imidazole-2-carboxamides.



Scheme 3. Tautomeric transformationN-[5-R-benzyl-1,3-thiazol-2-yl]-4,5-dihydro-1*H*-imidazole-2-carboxamides.

after, the corresponding chloroacetyl derivative was added and mixture and stirred for 1 hour. This protocol affords compounds **2a–e** in a very high purity and in excellent yields (**Scheme 2**).

Synthesized N-(5-R-benzyl-1,3-thiazol-2-yl)-2-morpholin-4-yl-2-oxoacetamides **2a–e**were investigated in the reaction with ethendiamine. It was found. that the heating of reagents for 30 min at 50°C led to the closure of the imidazoline ring to form N-[5-R-benzyl)-1,3-thiazol-2-yl]-4,5-dihydro-1*H*-imidazole-2-carboxamides **3a-e**(**Scheme 2**).

We exanimated the possibility of the synthesis of 4,5-dihydroimidazole-2-carboxamides **3a-e** in one-pot reaction of **1,** sulfur, and ethylenediamine (**Scheme 2**). The reaction was carried out by heating in DMF within 5–6 hours, but yields of the final products were lower and required additional crystallizations.

Table I. Overview of the preliminary anticancer assay at single dose concentration of 10μM.

Test compounds	Average growth, %	Range of growth, %	Most sensitive cell line (cancer line/type) GP%	Positive cytostatic effect[a]
2a	100.84	78.99-115.40	UO-31 (RenalCancer) 78.99;	0/60
2b	103.91	80.33-123.38	UO-31 (Renal Cancer) 80.33;	0/60
2c	99.72	69.30-110.27	CCRF-CEM (Leukemia) 69.30; UO-31 (RenalCancer) 72.21	1/60
2d	100.00	74.53-118.71	UACC-62 (Melanoma) 74.77; CAKI-1 (RenalCancer) 79.31; UO-31 (RenalCancer) 74.53	2/60
2e	90.13	67.14-114.39	HOP-92 (Non-Small Cell Lung Cancer)70.37; UACC-62 (Melanoma) 72.57; CAKI-1 (RenalCancer) 67.14; UO-31 (RenalCancer) 69.52	4/60
3a	90.59	57.10-120.12	CCRF-CEM (Leukemia) 57.10;HL-60(TB) (Leukemia) 60.59; K-562 (Leukemia) 70.21; MOLT-4 (Leukemia) 71.88; RPMI-8226 (Leukemia) 59.91; SR (Leukemia) 71.04; UACC-62 (Melanoma) 71.70; A498 (RenalCancer) 61.61;	8/60
3b	80.86	40.80-121.62	CCRF-CEM (Leukemia) 54.37; HL-60(TB) (Leukemiaa) 40.80; K-562 (Leukemia) 48.68; MOLT-4 (Leukemia) 69.14; RPMI-8226 (Leukemia) 58.04;EKVX (Non-Small Cell Lung Cancer) 64.09;NCI-H23 (Non-Small Cell Lung Cancer) 68.17;HCT-15 (Colon Cancer) 67.36; SK-MEL-5 (Melanoma) 62.84;UO-31 (Renal Cancer) 59.68	15/60
3c	41.92	-53.18-95.07	CCRF-CEM (Leukemia) -13.34; HL-60(TB) (Leukemia) 8.60; K-562 (Leukemia) 10.33; MOLT-4 (Leukemia) -17.55; SR (Leukemia) - 19.89; A549/ATCC (Non-Small Cell Lung Cancer) 23.55; NCI-H460 (Non-Small Cell Lung Cancer) 13.67; HCT-116 (Colon Cancer) 36.93; KM12 (Colon Cancer) 35.70; SF-295 (CNS Cancer) 4.89; SF-539 (CNS Cancer) 2.96; LOX IMVI (Melanoma) -53.09; M14 (Melanoma) 30.71; MDA-MB-435 (Melanoma) 30.36; UACC-62 (Melanoma) 38.95; OVCAR-4 (Ovarian Cancer) 31.04; OVCAR-8 (Ovarian Cancer) 34.53; NCI/ADR-RES (Ovarian Cancer) 36.71; 786-0 (Renal Cancer) 45.35; ACHN (Renal Cancer) 33.01; CAKI-1 (Renal Cancer) -53.18; SN12C (Renal Cancer) 38.86; UO-31 (Renal Cancer) -17.10	50/60

[a]Ratio between number of cell lines with percent growth from 0 to75 and total number of cell lines.

The structures of the obtained compounds were confirmed by ^{1}H NMR, mass spectroscopy and elemental analysis. Spectroscopic data of all compounds were in accordance to the proposed structures. In ^{1}H NMR spectra, signals of methylene group protons of N-[5-R-benzyl)-1,3-thiazol-2-yl]-4,5-dihydro-1*H*-imidazole-2-carboxamides appear as a singlets at 3.81-3.84 ppm. Such a character of the spectrum is due to a rapid tautomeric transformation (**Scheme 3**).

For the same reason, the NH protons of the amide group of the 4,5-dihydro-1*H*-imidazole ring do not appear at all. Signals of protons of the methylene group of the benzyl radical are at 4.00 - 4.13 ppm. The 4-H proton signals of the thiazole moiety are at 7.12 - 7.17 ppm.

Table 2. *In vitro* anticancer activity at 60 human tumor cell lines for compound **3c.**

Disease	Celllime	GI_{50}, μM	TGI μM	Disease	Celllime	GI50, μM	TGI μM
Leukemia	CCRF-CEM	**1.56**	>100	**Melanoma**	**LOX IMVI**	**0.15**	**1.12**
	HL-60(TB)	2.80	11.5		MALME-3M	65.9	>100
	K-562	3.93	>100		M14	6.85	>100
	MOLT-4	2.23	>100		MDA-MB-435	7.65	>100
	RPMI-8226	5.38	>100		SK-MEL-2	11.0	55.4
	SR	**1.77**	>100		SK-MEL-28	54.6	>100
NSC	A549/ATCC	2.23	>100		SK-MEL-5	2.81	14.6
lungcancer	EKVX	5.70	>100		UACC-257	5.99	>100
	HOP-62	19.4	58.5		UACC-62	11.6	79.0
	HOP-92	2.85	10.3	**Ovarian**	IGROV1	3.92	>100
	NCI-H226	2.57	80.9	**Cancer**	OVCAR-3	4.99	>100
	NCI-H23	6.98	>100		OVCAR-4	12.3	>100
	NCI-H322M	>100	>100		OVCAR-5	12.2	>100
	NCI-H460	3.20	>100		OVCAR-8	4.60	>100
	NCI-H522	**1.97**	**58.1**		NCI/ADR-RES	6.01	>100
Colon	COLO 205	8.68	>100		SK-OV-3	40.7	>100
Cancer	HCC-2998	16.2	>100	**Renal**	786-0	3.71	35.1
	HCT-116	7.27	>100	**Cancer**	A498	41.3	>100
	HCT-15	6.13	>100		ACHN	3.93	19.6
	HT29	5.81	>100		CAKI-1	3.43	28.3
	KM12	2.89	23.1		RXF 393	4.27	20.3
	SW-620	7.07	>100		SN12C	7.89	21.2
CNS Cancer	SF-268	5.30	>100		TK-10	3.21	9.94
	SF-295	2.71	9.79		UO-31	2.88	15.5
	SF-539	3.95	>100	**Breast**	MCF7	7.73	>100
	SNB-19	2.99	>100	**Cancer**	MDA-MB-231/ATCC	4.81	>100
	SNB-75	**1.57**	4.43		HS 578T	8.47	5.99
	U251	7.83	>100		BT-549	8.33	>100
Prostate	PC-3	6.52	>100		T-47D	5.13	>100
Cancer	DU-145	**1.54**	>100		MDA-MB-468	5.52	>100

Pharmacology

Among newly synthesized compounds substances **2a-e** and **3a-c** were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program for the *in vitro* cell line screening to investigate their anticancer activity. The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers. Primary anticancer assays were performed according to the DTP protocol (NCI USA), which was described elsewhere (Boyd *et al*., 1995; Boyd *et al*., 1997; Shoemaker *et al*., 2006).The results of primary screening are reported as the percent of cancer cell line growth (GP%) (Table I). The range of GP% shows the lowest and the highest values founded for different cancer cell lines.

Tested compounds **2a-e** showed a low antitumor activity in the *in vitro* screening assay. For the compounds **3a** and **3b** the average levels of cell growth (GPmean) were 90.59% and 80.86 % respectively. It should be noted, that selective action of tested compounds was observed towards Leukemia cell lines (range of GP= 57.10–71.88% (compound **3a**) and GP= 35.01–69.14% (**3b**). The most active compound **3c** was found to be effective against 50 cell lines with the average cell growth

Table III. Anticancer selectivity pattern of the most active compound **3c** at the GI_{50} (C, μM) and TGI (C, μM) levels

Cpd	Parameters	Subpanel tumor cell lines								
		L	**NSCLC**	**ColC**	**CNSC**	**M**	**OV**	**RC**	**PC**	**BC**
3c	GI50	2.95	16.1	7.72	4.06	18.5	12.1	9.79	4.03	6.67
	SI*	3.08	0.57	1.18	2.24	0.49	0.75	0.93	2.26	1.36
	TGI	85.25	67.48	89.01	69.3	>100	72.23	31.24	>100	84.33
	SI**	0.91	1.13	0.87	1.12	0.78	1.08	2.49	0.78	0.92

*Selectivity index at the GI50 (C, μM) level; **Selectivity index at the TGI (C, μM) level. L – leukemia, NSCLCC – non-small cell lung cancer, ColC – colon cancer, CNSC – CNS cancer, M – melanoma, OV– ovarian cancer, RC – renal cancer, PC – prostate cancer, BC – breast cancer.

Table IV. Mean growth inhibitory concentration (GI50, μM) of compound **3c** in comparison with 5-FU, Cisplatin and Curcumin.

Cpd	Subpanel tumor cell lines									
	L	**NSCLC**	**ColC**	**CNSC**	**M**	**OV**	**RC**	**PC**	**BC**	**MG-MID**
3c	2.95	16.1	7.72	4.06	18.5	12.1	9.79	4.03	6.67	9.01
5-FU	15.1	>100	8.4	72.1	70.6	61.4	45.6	22.7	76.4	22.60
Cisplatin	6.3	9.4	21.0	4.7	8.5	6.3	10.2	5.6	13.3	9.48
Curcumin	3.7	9.2	4.7	5.8	7.1	8.9	10.2	11.2	5.9	7.41

percent (GPmean) of 41.92%. Moreover, this derivative demonstrated cytotoxic effect on Leukemia cell lines CCRF-CEM (GP=-13.34%), MOLT-4 (GP=-17.55%) and SR (GP=- 19.89%), Melanoma cell lines LOXIMVI (GP =-53.09%), Renal Cancer cell lines CAKI-1 (GP=-53.18%), UO-31 (GP=-17.10%). Significant cytostatic effect was observed toward Leukemia cell lines HL-60(TB) (GP=8.60), CNS Cancer cell lines SF-295(GP=4.89) and SF-539(GP=2.96).

Finally, compound **3c** was selected for an advanced assay against a panel of approximately sixty tumor cell lines at 10-fold dilutions of five concentrations (100μM, 10μM, 1.0μM, 0.1μM and 0.01μM) (Table II). The percentage of growth was evaluated spectrophotometrically versus controls not treated with test agents after 48h exposure and using SRB protein assay to estimate cell viability or growth. Dose–response parameters were calculated for each cell line: GI_{50} – molar concentration of the compound that inhibits 50% net cell growth; TGI – molar concentration of the compound leading to the total inhibition; and LC_{50}– molar concentration of the compound leading to 50% net cell death. Furthermore, a mean graph midpoints (MG_MID) were calculated for GI_{50}, giving an average activity parameter over all cell lines for the tested compound. For the MG_MID calculation, insensitive cell lines were included with the highest concentration tested.

The mentioned derivative demonstrated a high activity toward the SR Leukemia cell lines (GI_{50}=1.77μM), NCI-H522NSC lung cancer cell lines (GI_{50}=1.97μM), SNB-75 CNS Cancer cell lines (GI_{50}= 1.57μM), and DU-145 Prostate Cancer cell lines (GI_{50}=1.97μM**)**. For some cancer cell lines the cytotoxic effect was observed: HOP-92 Non-Small Cell Lung Cancer cell lines LC_5 =62.0 μM; LOX IMVI Melanoma cell lines LC_{50}=4.48μM; and RXF Renal Cancer cell lines LC50=69.5μM.

The selectivity index (SI) obtained by dividing the full panel MG-MID (μM) of the compound **3c** by its individual subpanel MG-MID (μM) was consideredas a measure of compound's selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios greater than 6 indicate high selectivity toward the corresponding cell line, while compounds not meeting ei ther of the criteria are rated non-selective (Rostom, 2006). In this context, the active compound **3c** demonstrates moderate selectivity toward Leukemia cell lines at the GI_{50} levels (SI=3.08) and low selectivity toward Renal Cancer cell lines (SI=2.49) at the TGI levels (Table III).

The screening results revealed that compound **3c** possesses potent *in vitro* antitumor activity, with MG-MID = 9.01 in comparison with standard anticancer agent 5-fluorouracil (5-FU), Cisplatin MG-MID = 22.60 and Curcumin MG-MID = 7.41 (Table IV).

CONCLUSIONS

Here, we have shown the development of new efficient protocol for N-[5-benzyl-1,3-thiazol-2-yl]-4,5-dihydro-1*H*-imidazole-2-carboxamides synthesis. The row of N-[5-R-benzyl-1,3-thiazol-2-yl]-4,5-dihydro-1*H*-imidazole-2-carboxamides was synthesized with high yields. Primary anticancer assay of synthesized compounds was performed at approximately sixty human tumor cell lines panel within DTP protocol (Drug Evaluation Branch, National Cancer Institute, Bethesda, USA). The compounds with significant levels of anticancer activities have been found, that can be used for further optimization. N-[5-(2-chlorobenzyl)-1,3-thiazol-2-yl]-4,5-dihydro-1*H*-imidazole-2-carboxamide (**3c**) could be treated as prospective antitumor agent. The results prove the necessity of further investigations to clarify the features underlying the antitumor effect of tested compounds.

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