Anticancer Screening and Structure Activity Relationship Study of Some Semicarbazides and 1,2,4-Triazolin-5-ones

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Abstract: In this paper some semicarbazide and 1,2,4-triazolin-5-one derivatives are evaluated *in vitro* for their anticancer activity towards human tumour cell line: ovary (TOV 112D), lung (A 549), breast (T47D) and uterus (Hela). Compounds **2-4** have been found to show the most effective *in vitro* activity against ovary cancer cell line. For compound **4** the growth inhibition (80% and 70%) is observed in two concentrations (100 μg/ml and 50 μg/ml). The cytotoxic effect of examined compounds seems to be dose-dependent and time-dependent. Compound **7** has GI=80% values towards the breast cancer cell line in concentration of 100 μg/ml. Structure-activity relationship (SAR) studies are carried out for all the compounds of the series. Compounds **2**-**4** show an activity profile that can be improved through medicinal chemistry strategies.

Keywords: Anticancer activity, Cytotoxicity, Isocyanate, Semicarbazide, Synthesis, 1,2,4-Triazol-5-one.

1. INTRODUCTION

Cancer is an important issue of clinical medicine and pharmacology. At present, a wide range of cytotoxic drugs with different mechanisms of action are used to treat human cancer, either alone or in combination. Many compounds are in different phases of clinical trials. The main drawback of the cytotoxic drugs is that they do not discriminate between cancerous and normal cell types and are accompanied by toxic side effects that are often cumulative and are also accompanied by doses limiting.

Recently, 1,2,4-triazole and its derivatives have attracted attention as anticancer agents [1-5]. It was reported that compounds having triazole moieties, such as Verozole, Letrozole and Anastrozole, appear to be very effective aromatase inhibitors that are also very useful for preventing breast cancer [6-10]. Some triazoles can inhibit HER2 tyrosine kinase phosphorylation in the breast cancer cell [11,12]. Semicarbazides, linear analogues, commonly applied in the 1,2,4-triazole synthesis, are also an important class of compounds of diverse biological properties [13,14]. Some of them exhibit a promising cytotoxic activity profile [15,16].

On the other hand, pyrrole and its derivatives have shown to possess antitumor activity [17]. 2,4-Disubstituted brominated pyrroles, which are brominated at position 3 and 5 along with 2,3,4-trisubstituted pyrroles have demonstrated potent cytotoxic activity *in vitro* against a variety of murine and human suspended and solid tumor models [18]. Distamycin is a well known antibiotic, which contains three pyrrole rings and can selectively recognize AT region in DNA minor groove [19]. Netropsin is a basic oligopeptide isolated from *Streptomyces netropsis* which is composed of 1-methylpyrrole-2-carboxamide moieties linked to guanidinoacetamidino and amidinopropyl [20].

In the previous paper 1,6-bis(1,2,4-triazol-5-one)hexane derivatives were screened *in vitro* for their antiproliferative and anticancer activity in human tumor cell lines derived from breast and lung carcinoma cells. Some of them were found to be moderate effective against tested cell line in concentration 50 μg/ml [21]. In continuation of our research in the domain of heterocyclic compounds herein, semicarbazide and 1,2,4-triazolin-5-one derivatives are tested *in vitro* against human ovary, lung, breast and uterus cancer cell lines. Additionally, the geometry of all compounds is optimized by the semi-empirical (AM1) method. Selected descriptors are applied for SAR studies which provides indications for the synthesis of new potentially active compounds.

2. MATERIAL AND METHOD

2.1. Chemistry

Preparation, physicochemical and spectroscopic characterization of the compounds **1**-**7** have been described elsewhere. All compounds were characterized by elemental analysis and spectroscopic methods and the data was described in the previous papers [22,23].

2.1.1. 4-Ethyl-1[(1-methylpyrrol-2-yl)acetyl]semicarbazide (1)

Yield: 78%; M.p: 165-166 °C [22].

2.1.2. 4-(4-Bromophenyl)-1-[(1-methylpyrrol-2-yl)acetyl] semicarbazide (2)

Yield: 86%; M.p: 200-201 °C [22].

2.1.3. 4-Ethyl-3-[(1-methylpyrrol-2-yl)methyl]-1,2,4-triazolin-5-one (3)

Yield: 90%; M.p: 150-151 °C [22].

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2.1.4. 4-(4-Bromophenyl)-3-[(1-methylpyrrol-2-yl)methyl]-1,2,4-triazolin-5-one (4)

Yield: 89%; M.p: 180-181 °C [22].

2.1.5. 4,4'-Bis(3-methyl-1,2,4-triazolin-5-on-4-yl)diphenylmethane (5)

Yield: 83%; M.p: 308-310 °C [23].

2.1.6. 4,4'-bis[3-(1-methylpyrrol-2-yl)methyl-1,2,4-triazolin-5-on-4-yl]diphenylmethane

Yield: 81%; M.p: 225-227 °C [23].

2.1.7. 4,4'-Bis(3-benzyl-1,2,4-triazolin-5-on-4-yl)diphenylmethane (7)

Yield: 69%; M.p: 120-121 °C [23].

2.2. Inhibition of Tumour Cell Growth Assay

The semicarbazides **1**, **2** and 1,2,4-triazol-5-ones **3-7** were evaluated *in vitro* for their anticancer activity in human tumour cell line: human ovary carcinoma (TOV 112D - ATCC CRL-11731), human lung carcinoma (A 549 - ECACC 86012804), human breast carcinoma (T47D - ECACC 85102201) and human uterus carcinoma (Hela - ECACC 93021013). Additionally, the primary cell line to normal human skin fibroblasts (HSF) was used in this experiment.

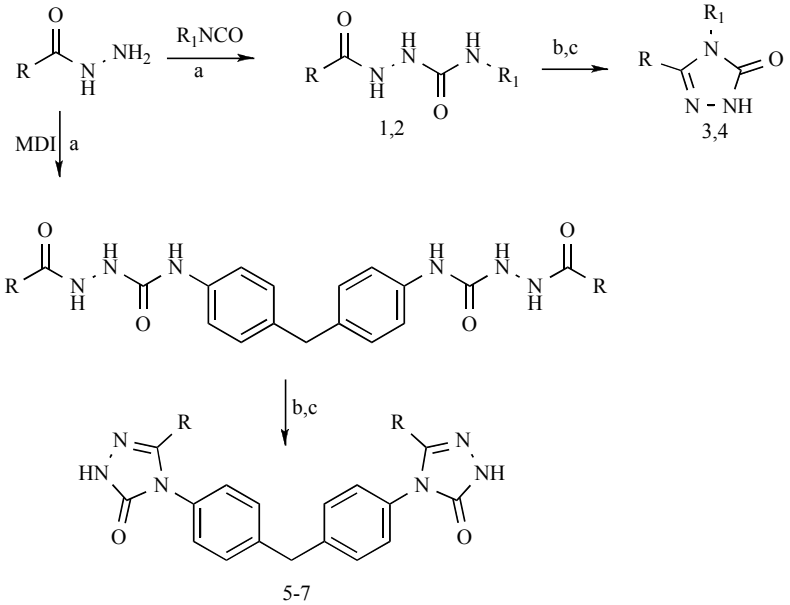
In the current protocol each cell line was inoculated at 10^4 cells per ml density on a microlitre plate at RPMI medium. Test compounds were then added at three examined concentrations (10, 50 and 100 μg/ml) and cultures were incubated for 24, 48 and 72 h. End-point determinations were made with 5-bromo-2-deoxy-uridine (BrdU) labeling and detection kit III (Roche) on Elisa reader (BIO-TEC Instruments USA). The growth percentage was evaluated spectrophotometrical versus untreated controls using cell viability of growth assay. Results for each spectrophotometrical measure were reported as percent of growth inhibition in comparison to untreated ones. All experiments were done in triplicate.

2.3. Computational Methods

The molecular modeling was performed using HYPERCHEM software packet and VEGA ZZ [24,25]. The energy and geometry of the derivatives were optimized by the AM1 semi-empirical method [26]. In order to perform structure-activity relationship (SAR) studies, some descriptors were calculated: the highest occupied molecular orbital energy (E_{HUMO}), the lowest occupied molecular orbital energy (E_{LUMO}), dipole moment (μ), Polar Surface Area (PSA), polarizability (P), refractivity (R) and logarithm of n-octanol/water partition coefficient (logP).

3. RESULTS AND DISCUSSION

The investigated semicarbazides **1** and **2** were obtained in the reaction of 1-methylpyrrol-2-ylacetic acid hydrazide with ethyl and 4-bromophenyl isocyanate respectively. The cyclization reaction of these compounds led to 1,2,4-triazolin-5-ones **3**, **4** [22]. Compounds **5-7** were obtained in the intermolecular dehydrative cyclization of appropriate bis-



For **1, 3**: R= 1-methylpyrrol-2-ylmethyl, $R^1 = C_2H_5$

For **2, 4**: R= 1-methylpyrrol-2ylmethyl, $R^1 = 4\text{-}BrC_6H_4$

For **5**: R= CH_3

For **6**: R= 1-methylpyrrol-2-ylmethyl

For **7**: R= $CH_2C_6H_5$

Scheme 1. Synthesis of semicarbazide and 1,2,4-triazolin-5-one derivatives.

Reagents and conditions: a) diethyl ether, room temperature; b) 2% NaOH, reflux: c) 3M HCl.

Table 1. Inhibition of Cancer Cell Line *In Vitro* by Selected Compounds at Concentrations 100 μg/ml and 50 μg/ml

Time of incubation (h)		Growth inhibition factor GI (%)									
Compound		**2**		**3**		**4**		**6**		**7**	
Doses		I	II	I	II	I	II	I	II	I	II
Cell line											
Normal cell line HSF	24	0	0	0	0	10	0	0	0	10	0
	48	0	0	0	1	0	0	0	0	10	0
	72	0	0	0	1	0	0	0	0	10	0
Cancer cell line											
TOV 112D	24	20	50	10	45	50	25	0	0	0	0
	48	30	75	15	50	75	50	25	15	50	5
	72	30	85	50	70	80	70	50	15	50	5
A549	24	0	5	0	0	10	5	10	5	5	20
	48	0	5	0	5	25	10	25	5	5	2
	72	5	5	0	5	25	15	50	15	10	10
T 47D	24	5	0	5	0	3	1	10	0	10	0
	48	5	0	10	5	5	1	15	0	20	0
	72	0	0	10	5	10	2	25	0	80	0

I-Concentration of 100 μg/ml, which corresponds to concentration of 0.28 mM for compound **2**, 0.48 mM for compound **3**, 0.30 mM for compound **4**, 0.19 mM for compound **6**, and 0.24 mM for compound **7**.
II-Concentration of 50 μg/ml, which corresponds to concentration of 0.14 mM for compound **2**, 0.24 mM for compound **3**, 0.15 mM for compound **4**, 0.09 mM for compound **6**, and 0.12 mM for compound **7**.

semicarbazides [23]. The synthetic pathway followed for the preparation of the title compounds was presented in Scheme **1**.

All compounds were evaluated *in vitro* for their cytotoxic activity against four cell lines: human ovary carcinoma (TOV 112D), human lung carcinoma (A 549), human breast carcinoma (T47D) and human uterus carcinoma (Hela). Beside human skin fibroblast (HSF) was included in the cytotoxicity study. Compounds were tested at three concentrations (100, 50, 10 μg/ml). Results for each test compound were reported as the percentage of growth (GI %) of the treated cells compared to the untreated control cells. According to the data listed in Table **1** compounds **2-4, 6** and **7** have potential to reduce the growth of the ovary cancer cell line (TOV 112D). For compounds **2-4** growth inhibitory values towards ovary cancer cell line at two concentrations (100 and 50 μg/ml) were found to be the most effective against this cancer line and their factors were in the range 50-85% (Fig. **1**). Cytotoxic effect of the compound **4** towards cancer ovary cell line seemed to be time and dose-dependent (Fig. **2**). The highest inhibition was observed for compound **2** (GI=85%) after 72h on incubation. Only for compounds **4** inhibition in concentration 10 μg/ml (GI=10%) was observed. Compounds **6** and **7** show 50% inhibition at concentration 100 μg/ml. Compound **6** show tumor cell growth inhibition against lung cancer line (A 549) in concentration 100 μg/ml (GI=50%). Only compound **7** was found the most active against breast cancer line (T 47D); its GI was 80% in concentration 100 μg/ml. Whereas compound **1**and **5** proved to be inactive against tested cell line *in vitro* (GI < 50%). Additionally, all compounds exhibited no cytotoxic activity against human uterus carcinoma (Hela) cell lines (GI≤25%) and no or low toxicity against normal fibroblast cultures (HSF) with GI≤15%.

For studies of structure-activity analysis some descriptor were calculated (Table **2**). An important descriptor is the lipophilicity log P which determines the capability of biological active substances to pass through biological membranes. Lipohilicity is directly connected with the Polar Surface Area (PSA). Molecules with a polar surface area greater than 140 $Å^2$ permeate cell membranes to the lesser extent. The tested compounds had PSA values ranged from 52.68 $Å^2$ to 115.26 $Å^2$. For compounds **3** and **4** showing the highest proliferative activity against ovarian cancer cells, these values were the lowest (52.68 $Å^2$ and 52.98 $Å^2$). However, the biologically active compound **2** was characterized by a high PSA values (115.26 $Å^2$). The log P were in the range from -1.95 (for compound **2**) to 2.30 (for compound **6**). It seems that the log P and PSA are not decisive factors for the antitumor activity of tested compounds, similarly as volume, molar mass and dipole moment (Table **2**). Analysis of LUMO and HUMO energies indicates that the studied compounds are stable. The SAR study revealed that anticancer activity against ovary carcinoma cell line of tested compounds depends on the nature of a substituent. Among the semicarbazides the 4-bromophenyl moiety was more favorable than the ethyl

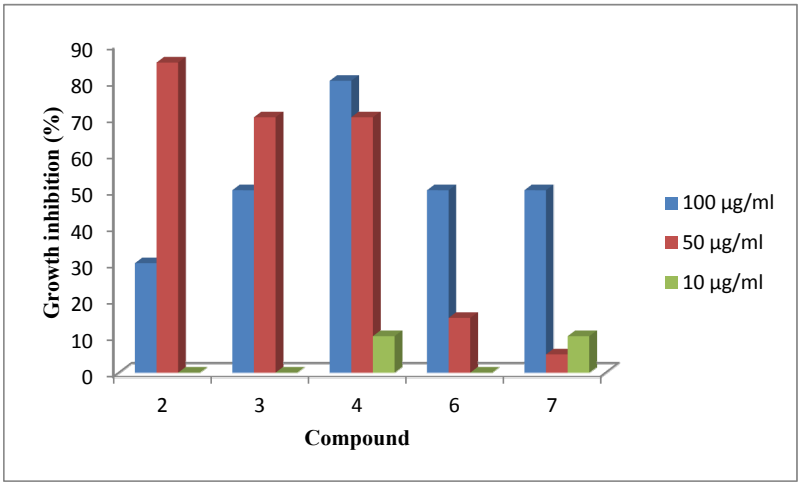


Fig. (1). Inhibition of *in vitro* human ovary cell line (TOV 112D) growth (expressed as GI percentage) by the most active compounds in three examined concentrations (100 µg/ml, 50 µg/ml and 10 µg/ml) after 72h incubation.

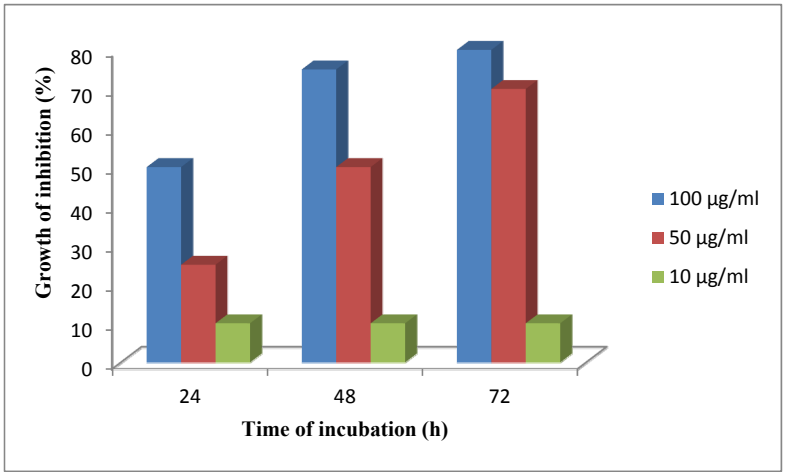


Fig. (2). Inhibition of *in vitro* human ovary cell line (TOV 112D) growth (expressed as GI percentage) by tested compound **4** in three examined concentrations (100 µg/ml, 50 µg/ml and 10 µg/ml) and time of incubation.

Table 2. Physicochemical and Topological Parameters of Compounds 1-7

No	PSA [Å^2]	*V* [Å^3]	M [amu]	µ [D]	logP	P [A^3]	R [A^3]	E_{LUMO} [eV]	E_{HOMO} [eV]	DELH [eV]
1	77.49	267.96	351.20	1.92	-1.75	32.28	88.88	-0.278	-8.688	8.410
2	115.26	210.64	224.26	2.03	-1.95	23.67	61.51	-0.605	-8.565	7.960
3	52.98	247.84	333.19	4.34	1.66	30.88	82.32	-0.453	-8.735	8.282
4	52.68	193.68	206.25	3.65	-0.47	22.26	59.67	0.485	-8.892	9.377
5	98.33	318.58	362.39	3.65	0.19	38.84	107.50	-0.298	-8.991	8.693
6	96.69	463.37	514.59	4.12	2.30	58.16	165.06	-0.271	-8.968	8.697
7	103.78	469.21	520.59	5.99	-0.20	57.70	159.83	-0.274	-8.603	8.329

group. In case of cyclic derivatives mono-triazoles turned out to be more active than bis-triazoles.

CONCLUSION

Some semicarbazides and 1,2,4-triazolin-5-ones were tested *in vitro* against ovary carcinoma (TOV 112D), lung carcinoma (A 549), breast carcinoma (T47D) and uterus carcinoma (Hela) cell lines. The preliminary results showed that some of these excellent growth inhibition activity against ovary, breast and lung cancer cell line. Compounds **2**-**4** were found be the most effective *in vitro* against ovary cancer cell line in two examined concentrations. Compound **7** had comparable GI values towards the breast cancer cell

line in concentration of 100 μg/ml. All compounds showed no or low cytotoxic activity against human utereus cell line and human ski fibroblast cells-primary cancer cells. The obtained results prove the necessity for further investigations to clarify the features underlying the antitumor potential of tested compounds. The most effective compounds have been selected for further studies. These results provide useful information about mechanisms of action of above-mentioned compound. The preliminary study on the molecular mechanism of activity of tested compounds shows that the anticancer activity of derivatives results from the competitive inhibition of receptor tyrosine kinases.

CONFLICT OF INTEREST

Declared none.

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Declared none.

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