Synthesis of Novel 2-metylamino-4-substituted-1,3-thiazoles with Antiproliferative Activity

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In the context of the continuous and increased interest in new biologically active thiazole analogues in medicinal chemistry, we proposed a research project that involves the synthesis of several agents bearing a 2-amino-thiazole core fused with thiophene or substituted 3-formylchromone and the investigation of their antiproliferative potential. Thus, a few new N'-2-(2-methylamino)thiazol-4-yl)acetohydrazide-hydrazones have been synthesized through the condensation of a hydrazide fragment with thiophene or different substituted 3-formylchromones. The structures of the newly obtained compounds have been confirmed by the combined use of elemental analysis and spectroscopic data (MS, ^{1}H NMR and ^{13}C NMR). The new molecules have undergone antiproliferative evaluation using MTT assay in three in vitro models, namely Hs578T, HeLa and Hep2G. The condensation of the thiazolic hydrazide-hydrazones with a chromone moiety has proven to be effective in respect to the antiproliferative activity, especially in the case of compound ***3b****, which showed excellent results on all three cancer cell lines used.*

Keywords: 2-Methyl-aminothiazole; Hydrazid-hydrazone; Antitumoral; 3-Formylchromones

There is a continuous and increased interest in new biologically active thiazole analogues in the field of medicinal chemistry. Synthetic thiazole derivatives have proven to exhibit a wide variety of biologic activities such as: antitumor [1-10], antibacterial [11], anti-prion [12], anti-tubercular [13] and anti-inflammatory [14]. Moreover, 2-aminothiazole scaffold represents the building block for potent anticancer agents [1-10], being also the core interest of our study. Various reports revealed that thiophene moiety carrying chemotherapeutic agents exhibit promising antineoplasic activity [15-16].

Benzopyrones (coumarines and flavonoids) derivatives have emerged from the background of anticancer drug development [17-20] since flavopiridol (HMR 1275, L86-8275), a flavonoid derived from an Indian plant, has proven to be highly effective against various cancer cell lines (with a concentration in the range of nM) and entered the clinical trials as the first inhibitor of cyclin-dependent kinases [17]. Subsequently, 3-formylchromones have been reported to be endowed with malignant-cell specific cytotoxicity [10].

With respect to the biological activity, thiophene and formylchromones can be fused with other heterocyclic compounds giving rise to more potent agents [15-22]. Also, the recognition gained by the N-N linkage recently led to the development of some hydrazide-hydrazone derived compounds that have undergone various heterocyclic transformations, having efficient growth inhibitory effects [22-25].

In view of these observations, the various pharmacological properties of thiophene, 3-formylchromone and hydrazide-hydrazone derivatives, have instigated us to explore the synthesis and screening of several potential antiproliferative agents, bearing a 2-aminothiazole core fused with thiophene or substituted 3-formylchromone moieties through a hydrazide-hydrazone bridge.

Therefore, we report herein, the scope of the current study, the synthesis, chemical characterization and the evaluation of the antiproliferative effect using MTT assay, of the newly obtained compounds.

Experimental part

Chemicals, equipment and methods

Solvents and reagents for MTT assay were obtained from commercial sources. The melting points were taken with an Electrothermal melting point meter and are uncorrected. For the synthesis of the hydrazide-hydrazone derivatives a CEM Discover microwave synthesizer model number 908010 has been used. Analytical thin layer chromatography (TLC) was used to monitor the reaction progress and was carried out on precoated Silica Gel $60F_{254}$ sheets using ethyl-acetate-heptane 7:3 system and UV absorbtion for visualization. Yields were not optimized. The ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on a Bruker Avance NMR spectrometer operating at 400 MHz or 500 MHz and 100 MHz, respectively, and were in accordance with the assigned structures. Chemical shift values were reported relative to tetramethylsilane (TMS) as internal standard. The samples were prepared by dissolving the compounds in DMSO-d_6 (δ_H = 2.51ppm, δ_C = 40.02 ppm) as solvent and the spectra were recorded using a single excitation pulse of 12μs (^{1}H NMR) and 8μs (^{13}C NMR), respectively. GC-MS analyses were performed

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with an Agilent gas chromatograph 6890 equipped with an apolar Macherey Nagel Permabond SE 52 capillary column. Elemental analysis was registered with a Vario El CHNS instrument.

Ethyl 2-(2-(methylamino)thiazol-4-yl)acetate (**1**). Accurately weighed, 5.00 g (0.033 mol) N-methylthiourea were dissolved in 20 mL ethanol in a 50 mL one neck round bottom flask. 2.8 mL (0.033 mol) of 4-choloro-acetoacetate were added to this solution. The reaction mixture has been heated under reflux for 3h. After cooling, the alcohol was evaporated using a rotary evaporator (Buchi RotavaporR-210). The solid formed was dissolved in distilled water and the *p*H was brought to 7 with a saturated solution of sodium bicarbonate. The precipitated solid was collected by filtration under low pressure, recrystallized from ethanol and dried to give orange crystals.

Yield: 92%; light orange solid; m.p. 104 °C; Anal. Calcd. for $C_8H_{12}N_2O_2S$: C, 47.98; H, 6.04; N, 13.99; S, 16.01. Found: C, 47.83; H, 6.09; N, 13.76; S, 16.20; ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.13-7.11 (1H, m, C_5-thiazole-H), 6.31 (1H, s, -NH-), 4.26 (2H, s, -CH_2-), 3.41 (2H, s, -CH_2-), 3.02 (3H, s, CH_3-), 2.11 (3H, s, -CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 167.3 (C=O), 165.7 (C_2-thiazole), 156.9 (C_4-thiazole), 123.3 (C_5-thiazole), 43.6 (CH_2), 39.7 (CH_2), 31.5 (CH_3), 14.2 (CH_3); HRMS (*m* / *z*): 201.082 ($M+H^+$).

Procedure for the synthesis of the intermediate compound:2-(2-(methylamino)thiazol-4-yl)acetohydrazide (**2**). 8 mL hydrazine hydrate (100% solution) were added to 4.00 g of compound **1** (20 mmol) and the mixture was stirred at room temperature for 5h. The reaction was monitored by TLC. After the reaction was completed, the formed solid product was collected by filtration. The resulted product was recrystallized from ethanol.

Yield: 55%; white crystals; m.p. 146 °C; Anal. Calcd. for $C_6H_{10}N_4OS$: C, 38.7; H, 5.41; N, 30.08; S, 17.22. Found: C, 38.54; H, 5.53; N, 30.11; S, 17.18; ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 7.14-7.10 (1H, m, C_5-thiazole-H), 5.21 (4H, s, -NH-, -NH-, -NH_2), 3.45 (2H, s, -CH_2-), 3.01 (3H, s, CH_3-); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 172.3 (C=O), 167.7 (C_2-thiazole), 154.8 (C_4-thiazole), 123.1 (C_5-thiazole), 43.6 (CH_2), 31.5 (CH_3); HRMS (*m* / *z*): 186.151 ($M+H^+$).

*General procedure for the synthesis of compounds **3a-d**:* 2 mmol (0.372 g) of **2** were dissolved in 3 mL ethanol and 2 mmol of corresponding aldehyde were added. The reaction mixture has been refluxed under microwave irradiation (100°C, 150W) for 20 min. The formed precipitate was collected by filtration and purrified through recristallization from absolute methanol.

2-(2-methylamino)thiazol-4-yl)-N'-(thiophen-2-methylen)-acetohydrazide (**3a**): Yield: 80%; m.p. 180 °C; Anal. Calcd. for $C_{11}H_{12}N_4OS_2$: C, 47.12; H, 4.31; N, 19.98; O, 5.71; S, 22.87. Found: C, 47.34; H, 4.17; N, 19.80; O, 5.67; S, 22.68; ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 11.48 (1H, s, CO-NH-), 8.16 (1H, s, =CH-), 7.64 (1H, d, C_3-thiophene-H), 7.44 (1H, d, C_5-thiophene-H), 7,39 (1H, t, C_4-thiophene-H), 7.14-7.10 (1H, m, C_5-thiazole-H), 6.33 (1H, s, -NH-), 3.39 (2H, s, -CH_2-), 2.79 (3H, s, CH_3-); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 171.3 (C=O), 169.8 (C_2-thiazole), 146.4 (C_4-thiazole), 141.9 (=CH-), 138.8 (C_2-thiophene), 130.8 (C_5-thiophene), 128.9 (C_3-thiophene), 128.3 (C_4-thiophene), 102.5 (C_5-thiazole), 39.9 (CH_2), 31.5 (CH_3); HRMS (*m* / *z*): 281.7 ($M+H^+$).

2-(2-methylamino)thiazol-4-yl)-N'-((6-methyl-4-oxo-4H-chromenyl)methylen)-acetohydrazide (**3b**):Yield: 82%; m.p. 234 °C; Anal. Calcd. for $C_{17}H_{16}N_4O_3S$: C, 61; H, 5.12; N, 15.81; S, 9.05. Found: C, 61.12; H, 5.07; N, 15.71; S, 9.01; ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 11.62 (1H, s, CO-NH-), 8.35 (1H, s, =CH-), 7.91 (1H, s, C_5-chromenyl-H), 7.67 (1H, d, C_7-chromenyl-H), 7.64-7.62 (1H, m, C_2-chromenyl-H), 7.47-7.39 (1H, m, C_8-chromenyl-H), 7.23-7.11 (1H, m, C_5-thiazole -H), 6.37 (1H, s, -NH-), 3.84 (2H, s, -CH_2-), 2.79 (3H, s, CH_3-), 2.451 (3H, s, C_6-chromenyl-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 172.1 (C=O), 170.0 (C=O chromenyl), 169.9 (C_2-thiazole), 167.1 (C_2-chromenyl), 162.3 (C_4-thiazole), 154.2 (C_{8a}-chromenyl), 140.8 (=CH-), 138.1 (C_7-chromenyl), 136.5(C_6-chromenyl), 126.4 (C_5-chromenyl), 122.2 (C_5-thiazole), 119.0 (C_{4a}-chromenyl), 117.9 (C_8-chromenyl), 115.6 (C_3-chromenyl), 40.1 (CH_2), 31.5 (CH_3), 20.0 (CH_3-chromenyl); HRMS (*m* / *z*): 357.310 ($M+H^+$).

2-(2-methylamino)thiazol-4-yl)-N'-((6-chloro-4-oxo-4H-chromenyl)methylene)-acetohydrazide (**3c**): Yield: 83%; m.p. 220 °C; Anal. Calcd. for $C_{16}H_{13}ClN_4O_3S$: C, 54.47; H, 4.03; N, 14.95; S, 8.55. Found: C, 54.31; H, 4.11; N, 14.81; S, 8.50; ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 11.56 (1H, s, -CO-NH-), 8.85 (1H, s, =CH-), 8.07-8.05 (1H, m, C_7-chromenyl-H), 7.92-7.89 (1H, m, C_5-chromenyl-H), 7.79-7.82 (1H, m, C_8-chromenyl-H), 7.46-7.39 (1H, m, C_2-chromenyl-H), 6.37 (1H, s, C_5-thiazole-H), 4.38 (1H, s,-NH-), 3.46 (2H, s, -CH_2-), 2.78 (3H, s, CH_3-); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 172.6 (C=O), 171.8 (C=O chromenyl), 168.7 (C_2-thiazole), 166.3 (C_2-chromenyl), 162.1 (C_4-thiazole), 154.2 (C_{8a}-chromenyl), 140.5 (=CH-), 137.9 (C_7-chromenyl), 135.8(C_6-chromenyl), 126.2 (C_5-chromenyl), 121.9 (C_5-thiazole), 119.4 (C_{4a}-chromenyl), 117.6 (C_8-chromenyl), 115.8 (C_3-chromenyl), 41.2 (CH_2), 31.5 (CH_3); HRMS (*m* / *z*): 377.213 ($M+H^+$).

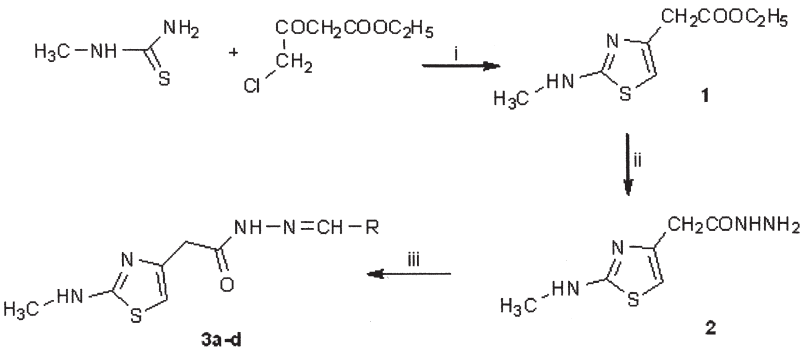
2-(2-methylamino)thiazol-4-yl)-N'-((4-oxo-4H-chromenyl)methylene)-acetohydrazide (**3d**): Yield: 79%; m.p. 200 °C; Anal. Calcd. for $C_{16}H_{14}N_4O_3S$: C, 59.98; H, 4.74; N, 16.46; S, 9.42. Found: C, 59.82; H, 4.68; N, 16.38; S, 9.35; ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 11.51 (1H, s, -CO-NH-), 8.79 (1H, s, =CH-), 8.36-8.12 (1H, m, C_5-chromenyl-H), 7.86 (1H, m, C_7-chromenyl-H), 7.73- 7.72 (1H, m, C_8-chromenyl-H), 7.57-7.54 (1H, m, C_6-chromenyl-H), 7.47-7.39 (1H, m, C_2-chromenyl-H), 6.37 (1H, s, C_5-thiazole-H), 3.41 (1H, s, -NH-), 3.37 (2H, s, -CH_2-), 2.79 (3H, s, CH_3-); ^{13}C-NMR (100 MHz, DMSO-d_6, ppm): δ 173.1 (C=O), 172.8 (C=O chromenyl), 167.9 (C_2-thiazole), 166.2 (C_2-chromenyl), 163.7 (C_4-thiazole), 154.2 (C_{8a}-chromenyl), 140.3 (=CH-), 134.9 (C_7-chromenyl), 126.1 (C_6-chromenyl), 125.7 (C_5-chromenyl), 121.8 (C_5-thiazole), 119.3 (C_{4a}-chromenyl), 118.0 (C_8-chromenyl), 117.1 (C_3-chromenyl), 42.7 (CH_2), 31.4 (CH_3); HRMS (*m* / *z*): 343($M+H^+$).

Measurement of Antiproliferative Activity (MTT Assay)

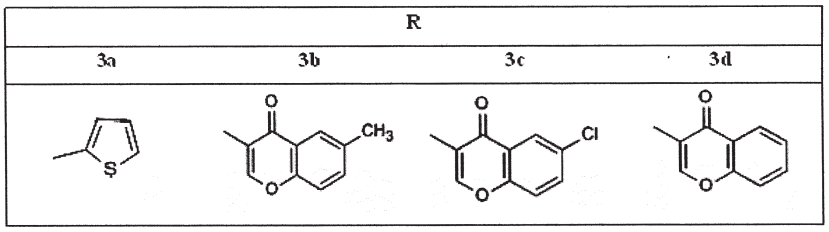
Our target compounds have undergone an *in vitro* antiproliferative screening at Ion Chiricută Oncology Institute, Cluj-Napoca. They have been tested in six different dose concentrations: 500 μg / mL, 250 μg / mL, 25 μg / mL, 2.5 μg / mL, 1.25 μg / mL and 0.25 μg / mL respectively.

The antiproliferative activity of the newly synthesized compounds was determined through the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, by monitoring the activity of mithocondrial dehydrogenase in living cells. The experiment was effectuated using 2 x 10^3 cells per well in 96-well plates overnight, to achieve 60% confluence and measured with a Biotek Synergy HT Microplate Plate Reader (Biotek, Bucharest, Romania) in a volume of 200 μL per well.

The MTT assay was performed at 48 h after the treatment with the newly obtained agents. The culture medium was removed prior the performing of the MTT assay. After this, the cells were washed with phosphate buffer saline and



Scheme 1. Synthesis of new 2-methyl-aminothiazolyl-4 acetohydrazide-hydrazones **3a-d**
i: absolute C_2H_5OH, reflux, 3h; ii:H_2N-NH_2, stirring at room temperature, 6h; iii: R-CHO/C_2H_5OH, MW (100°C, 150W), 20 min



150 mL Haks salt containing MTT (Sigma), at the final concentration 455 mg of MTT per mL of Haks salt, were added into each well. The cells have undergone 2h incubation under standard conditions, followed by the removal of the MTT solution. After this, 200 mL of dimethylsulfoxide were added in each well. In the end, the absorption has been measured at 490 nm, using the microplate reader.

Statistical Analysis

The obtained data have been analyzed using GraphPad Prism 6.0 Free trial (Graph Pad Software Inc., http://www.graphpad.com).

Results and discussions

Chemicals

Thiazoles can be without difficulty synthesized and give countless adjustment possibilities by several reaction modes in different positions, due to their intense reactivity. This has been comprehensively documented.

We synthesized a series of four 2-methyl-aminothiazolyl-4-acetohydrazide-hydrazones through the condensation of a hydrazide fragment with thiophene or different substituted 3-formylchromones.

The synthesis of the intermediate compounds **1**, **2** and the aimed compounds **3a-d** are depicted in scheme 1. Compound **1** has been synthesized by undergoing the general method known as Hantzsch thiazole synthesis, from N-methylthiourea and 4-chloroacetoacetate. In order to obtain the aimed products, it was first necessary to synthesize hydrazide **2**, by the reaction of the corresponding ethyl ester **1a** with hydrazine hydrate. The condensation reaction of compound **2** with thiophen-2-carbaldehyde, 6-methyl-4-oxo-4H-chromene-3-carbaldehyde, 6-chloro-4-oxo-4H-chromene-3-carbaldehyde or 4-oxo-4H-chromene-3-carbaldehyde, gave the target compounds **3a-d**.

The structures of the newly synthesized compounds have been confirmed by elemental analysis and spectroscopic data (MS, ^{1}H NMR and ^{13}C NMR).

Cytotoxic Activity

The MTT values after 48h incubation with the novel synthesized compounds on human cervix adenocarcinoma HeLa, liver hepatocellular carcinoma Hep2G and breast adenocarcinoma Hs578T cell lines are presented as percentage of control in relation with log(compound concentration, μg/mL). GraphPad Prism free trial was used for IC_{50} determination.

All the tested compounds revealed dose-effect relationship at 48h after treatment, each compound having specific cell type sensitivity, the data being presented in figure 1-3 and table 1. Compounds **3b** and **3c** were equipotent in regard to the activity towards Hep2G cell line, **3b** having an IC_{50} value of 5.077 μM and **3c** an IC_{50} of 5.095 μM. A difference in the efficiency for the synthesized compounds was observed on HeLa cell line, **3b** showing remarkable antiproliferative effect (IC_{50} of 0.823 6 μM), whereas **3c** although exhibiting a good antiproliferative effect, ranked second in potency with an IC_{50} of 2.212 μM. **3a** had a moderate antiproliferative effect against all three cell lines, its effect being ranked as follows: Hela>Hs578T>Hep2G, its IC_{50} values ranging from 7.451 μM to 16.439 μM, being less potent than **3b** and **3c** on all three cell lines. **3d** has been the least effective compound of the four tested, showing loss of activity on HeLa and Hep2G cell line, however, having better antiproliferative results (IC_{50} of 34.832 mM) on Hs578T than **3c** (loss of activity with an IC_{50} of 130.22 μM).

A previous study that reveals the antitumoral effect in breast cancer cell models (MCF-7 cells) of thiophene and thiazole derived compounds bearing a hydrazide-hydrazone scaffold, has established that the addition of a

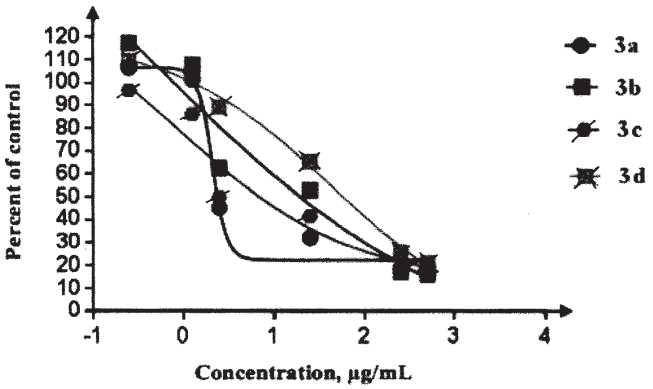


Fig. 1. Antiproliferative effect of compounds 3a-d as measured by MTT assay on HeLa cells after 48h incubation, at concentrations between 0.25μg/mL and 500 μg/mL

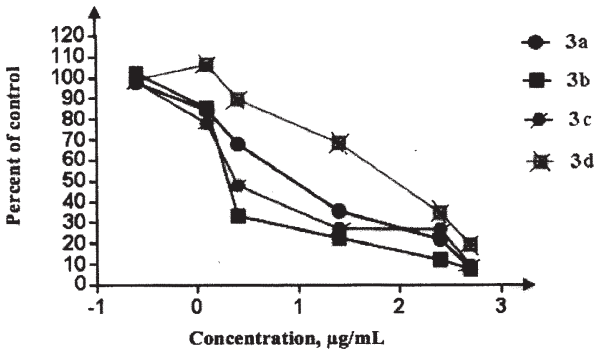


Fig. 2. Antiproliferative effect of compounds 3a-d, measured by MTT assay on Hep2G cells after 48h incubation, at concentrations between 0.25µg/mL and 500 µg/mL

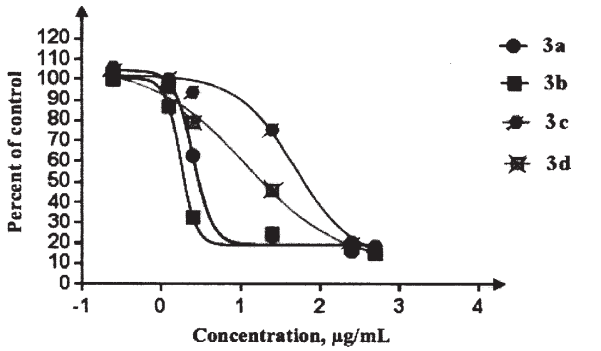


Fig. 3. Antiproliferative effect of compounds 3a-d as measured by MTT assay on Hs578T cells after 48h incubation, at concentrations between 0.25µg/mL and 500 µg/mL

Cell	Compound	IC_{50}(µM)	Hill	R^2
Hela	**3a**	7.451	0.521	0.9656
	3b	0.823	0.191	0.9734
	3c	2.212	0.358	0.8369
	3d	136.29	12.87	0.7927
Hep2G	**3a**	16.439	0.452	0.6778
	3b	5.077	0.503	0.8650
	3c	5.095	0.782	0.9111
	3d	405.306	0.528	0.9720
Hs578T	**3a**	9.051	0.177	0.9525
	3b	4.950	0.277	0.9170
	3c	130.222	0.625	0.9618
	3d	34.832	0.160	0.9720

Table 1
ANTIPROLIFERATIVE EFFECT MEASURED BY MTT ASSAY ON HeLa, Hep2G AND Hs578T CELLS, AFTER 48h INCUBATION WITH THE SELECTED COMPOUNDS

thiophene, benzothiophene or thiazole ring to the biological active sulphone moiety, leads to a decrease in the activity of the compounds (IC_{50} ranged from 49.3 µM to 87.8 µM) [25]. Concerning the IC_{50} determined for **3a**, our compound bearing a thiophene moiety, the values ranged from 7.451 µM to 16.439 µM. Thereupon, the better antiproliferative results on a more invasive breast-cancer cell line (Hs578T) could account for the presence of the 2-aminothiazole core in its structure, the two biologically active moieties acting sinergistically. Furthermore, a series of alpha-(N)-heterocyclic derivatives bearing a hydrazone moiety has been tested for the antiproliferative activity [15]. The more potent representatives assessed against all cell lines were 3 and 5-methylthiophene-2-carboxaldehyde derivatives with an IC_{50} ranging from 1.63 µM to 26.5 µM [15]. Likewise, the concluded results of the antiproliferative activity of latter mentioned compounds, could be a consequence of the presence of the heterocyclic rings that prevail in the structure.

A difference in cellular sensitivity regarding the second group of newly synthesized compounds, represented by **3b**, **3c** and **3d**, was observed. The three agents bearing 3-formylchromone scaffold in their structure, exhibited different potency towards all three cell lines. The substitution of 3-formylchromone in C-6 position has proven to be effective, **3b** and **3c** having a more prominent antiproliferative effect on Hela and Hep2G cell lines than the parent compound, **3d**, bearing in its structure an unsubstituted 3-formylchromone nucleus, our results being in agreement with a similar study made by Masami Kawase et al. (2007) [10]. They have studied the antitumor effect of 3-formylchromones on different cell lines (HSC-2, HSC-3, HSG, HL-60) through an MTT assay after 24h, and obtained a cytotoxic concentration (CC_{50}/ µM) ranging from 8 µM to 546 µM, stating that the lowest cytotoxic effect belongs to the parent unsubstituted 3-formylchromone derived molecules. The results could be explained by the fact that the presence of a methyl group or chlorine on 3-formylchromone nucleus increases lipophilicity and therefore enhances the internalization of the drug in the cancer cell through the lipophilic membrane. Conversely, the results from the triple negative breast cancer cell line Hs578T are conflicting with the aformentioned hypothesis. They highlight that the presence of an electron withdrawing substitute on 3-formylchromone leads to a decrease in the biological activity (**3c** losing its activity with an IC_{50} of 130.222 µM). In addition to this, the presence of an electron inducing group such as methyl (**3b** with an IC_{50} of 4.950 µM) increased the compounds' activity tremendously in comparison to the unsubstituted 3-formylchromone derivative (**3d** with an IC_{50} of 34.832 µM).

However, compound **3b** had a better antiproliferative effect than the natural compound epigallocatechin gallate widely involved in studies relevant to inhibition of cell proliferation. In brief, according to a previous study made by the research group of Ion Chiricută Cancer Institute, Cluj-Napoca, epigallocatechin gallate exhibits an IC_{50} of 15.8 µM on Hs578T cell line after a 48h treatment (MTT assay) [26]. The presence of a chromone derivative in the structure of the compounds **3b-d**, does not account for an anti/pro-oxidant mechanism through radical scavanging as established for epigallocatechin gallate [26, 27], on the grounds that the molecular-core 3-formylchromone has no notable antioxidant activity, not even in high doses [28]. The differences in the antiproliferative activity observed among the compounds of the same series (3-formylchromone derivatives) show that even if they had exhibited an anti/pro-oxidative mechanism, it could not have been solely responsible for the effect against the cell proliferation.

Conclusions

One by one, beginning with the chemical synthesis, then the recognized and anticipated biological, especially

antiproliferative effects, several derivatives are worthy of specific mentions: 2-(2-methylamino)thiazol-4-yl)-N'-(thiophen-2-methylen)-acetohydrazide and 2-(2-methylamino)thiazol-4-yl)-N'-((4-oxo-4H-chromenyl)methylene)-acetohydrazide. The MTT test that has been conducted represents just a preliminary screening of the antiproliferative activity of the synthesized compounds. Consequently, the mechanism of action of these derivatives remains unelucidated. However, some of the tested substances exhibited great antiproliferative effect. The excellent results of compounds **3a** and **3b** on the triple negative breast cancer cell line Hs578T deserve special mention. Thereupon, the mechanism of action and the specific role of each substituent require further investigation.

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References

1.ZHANG, C., LUNDGREN, K., YAN, Z., ARANGO, M., PRICE, S., HUBER, A., HIGGINS, J., TROCHE, G., SKAPTASON, J., KOUDRIAKOVA, T., NONOMIYA, J., YANG, M., O'CONNOR, P., BENDER, S., LOS, G., LEWIS, C., Mol. Cancer. Ther., 7, 2008, p. 818.
2. JINYEONG, K., YEOJU, M., SEUNG, W.H., Bull. Korean Chem. Soc., 32, 2011, p. 2893.
3. JUNG, K. Y., KIM, S. K., GAO, Z. G., GROSS, A. S., MELMAN, N., JACOBSON, K. A., KIM, Y. C., Bioorg. Med. Chem., 12, 2004, p. 613.
4. ROMAGNOLI, R., BARALDI, P. G., BRANCALE, A., RICCI, A., HAMEL, E., BORTOLOZZI, R., BASSO, G., VIOLA, G., J. Med. Chem., 54, 2011, p. 5144.
5. KIM, K. S., KIMBALL, S. D., MISRA, R. N., RAWLINS, D. B., HUNT, J. T., XIAO, H. Y., LU, S., QIAN, L., HAN, W. C., SHAN, W., MITT, T., CAI, Z. W., POSS, M. A., ZHU, H., SACK, J. S., TOKARSKI, J. S., CHANG, C. Y., PAVLETICH, N., KAMATH, A., HUMPHREYS, W. G., MARATHE, P., BURSUKER, I., KELLAR, K. A., ROONGTA, U., BATORSKY, R., MULHERON, J. G., BOL, D., FAIRCHILD, C. R., LEE, F. Y., WEBSTER, K. R., J. Med. Chem., 45, 2002, p. 3905.
6.SCHENONE, S., ZANOLI, S., BRULLO, C., CRESPAN, E., MAGA, G., Curr. Drug Ther., 3, 2008, p. 158.
7. LU, Y., LI, C. M., WANG, Z., CHEN, J., MOHLER, M. L., LI, W., DALTON, J. T., MILLER, D. D., J. Med. Chem., 54, 2011, p. 4678.
8. GORCZYNSKI, M. J., GREMBECKA, J., ZHOU, Y., KONG, Y., ROUDAIA, L., DOUVAS, M. G., NEWMAN, M., BABER, G., BIELNICKA, I. Z., CORPORA, T., SHI, J., SRIDHARAN, M., LILIEN, R., DONALD, B. R., SPECK, N. A., BROWN, M. L., BUSHWELLE, J. H., Chem. Biol., 14, 2007, p. 1186.
9. GOMHA, S. M., KHALIL, K. D., Molecules, 17, 2012, p. 9335.
10. KAWASE, M., TANAKA, T., KAN, H., TANI, S., NAKASHIMA, H., SAKAGAMI, H., In Vivo, 21, 2007, p. 829.
11. SINGH, N., SHARMA, U. S., SUTAR, N., KUMAR, S., SHARMA, U. K., J. Chem. Pharm. Res., 2, 2010, p. 691.
12. GHAEMMAGHAMI, S., MAY, B. C., RENSLO, A. R., PRUSINER, S. B., J. Virol., 84, 2010, p. 3408.
13.LU, X., LIU, X., WAN, B., FRANZBLAU, S. G., CHEN, L., ZHOU, C., YOU, Q., Eur. J. Med. Chem., 49, 2012, p. 164.
14. SHARMA, R. N., XAVIER, F. P., VASU, K. K., CHATURVEDI, S. C., PANCHOLI, S. S., J. Enzyme Inhib. Med. Chem., 24, 2009, p. 890.
15. JHA, K. K., KUMAR, S., TOMER, I., MISHRA, R., J. Pharm. Res., 5, 2012, p. 560.
16. DAI, X. Y., ZENG, X. X., PENG, F., HAN, Y. Y., LIN, H. J., XU, Y. Z., ZHOU, T., XIE, G., DENG, Y., MAO, Y. Q., YU, L. T., YANG, L., ZHAO, Y. L., Cell. Physiol. Biochem., 29, 2012, p. 281.
17. SENDEROWICZ, A. M., Invest. New Drugs., 17, nr. 3, 1999, p. 313.
18. VALDAMERI, G., GENOUX-BASTIDE, E., PERES, B., GAUTHIER, C., GUITTON, J., TERREUX, R., WINNISCHOFER, S. M. B., ROCHA, M. E. M., BOUMENDJEL, A., DI PIETRO, A., J. Med. Chem., 55, 2012, p. 966.
19.KHADEM, S., MARLES, R. J., Molecules, 17, 2012, p. 191.
20.KIM, Y., KIM, W. J., CHA, E. J., Korean J. Physiol. Pharmacol., 15, 2011, p. 279.
21. MOHAREB, R. M., FLEITA, D. H., SAKKA, O. K., Molecules, 16, 2011, p. 16.
22. ROLLAS, S., KÜÇÜKGÜZEL, S. G., Molecules, 12, 2007, p. 1910.
23. NARASIMHAN, B., KUMAR, P., SHARMA, D., Acta Pharm. Sciencia, 52, 2010, p. 169.
24. GÜRSOY, E., GÜZELDEMIRCI-ULUSOY, N., Eur. J. Med. Chem., 42, 2007, p. 320.
25. AL-SAID, M. S., BASHANDY, M. S, AL-QASOUMI, S. I., GHORAB, M. M., Eur. J. Med. Chem. 46, 2011, p. 137.
26. BRAICU, C., PILECKI, V., BALACESCU, O., IRIMIE, A., BERINDAN NEAGOE, I., Int. J. Mol. Sci., 12, 2011, p. 9342.
27. HSU, Y. C., LIOU, Y. M., J. Cell Physiol., 226, nr. 10, 2011, p. 2721.
28. DIAS, M. M., MACHADO, N. F., MARQUES, M.P., Food Funct., 2, nr. 10, 2011, p. 595.

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