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Synthesis and preliminary anticancer activity of new 1,4-dihydro-3-(3-hydroxy-2-naphthyl)-4-substituted-5*H*-1,2,4-triazoline-5-thiones

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Abstract

1,4-Dihydro-3-(3-hydroxy-2-naphthyl)-4-substituted-5*H*-L2,4-triazolinc-5-thiones were synthesized. The structures of original eight compounds were confirmed by elemental analysis,^{1}H NMR and mass spectral methods. One of the compounds (**3a**) was tested in vitro for its anticancer activity against 52 human tumor cell lines.

Keywords: 1,2,4-Triazoline-5-thione derivatives; Synthesis; Anticancer activity

1. Introduction

A series of 3,4-disubstituted-l,2,4-triazoline-5-thiones were synthesized by us [1]. In this study, these compounds were screened for in vitro anti-tumor activity by National Institutes of Health National Cancer Institute and one of them [1,4-dihydro-3-(3-acetyloxy-2-naphthyl)-4-ethyl-5*H*-l,2,4-triazoline-5-thione (**3a**)] was found valuable to research. In this point of view, we synthesized new l,2,4-triazoline-5-thiones and their acetylated and benzoylated derivatives. Thus, eight new compounds were obtained for anticancer drug development.

2. Chemistry

The compounds were synthesized in several steps (Fig. 1). First l,2,4-triazoline-5-thiones **2a**–**f** were formed by the cyclization in alkaline medium of the thiosemicarbazides **1a**–**f**, which were obtained by the condensation of 3-hydroxy-2-naphthoic acid hydrazide with alkyl–aryl isothiocyanates [2,3]. Some of the physical properties of these compounds were shown in Table 1.

For obtaining benzoylated derivatives $\mathbf{3a_1}$ and $\mathbf{3a_2}$, **2a** was heated in benzoyl chloride and 2,6-dichlorobenzoyichloride [4], respectively, and **2b** was heated in acetic anhydride to obtain acetylated derivative **3b** [5].

The structures of the new compounds were confirmed using UV, ^{1}H NMR and mass (for only **2c** which was selected as a prototype) spectral data besides their elemental analysis.

According to the UV spectroscopic data (Table 1), strong bands, which observed at 229.8–233.4 nm, characterized to K band of triazole ring.

The ^{1}H NMR spectra of **2a**–**f** displayed the OH and NH resonance at 10.15–10.55 and 13.87–14.20 ppm, respectively [6,7]. The signal of these protons were not observed because of removel of these protons by acetylation–benzoylation for **3b**, $\mathbf{3a_1}$ and $\mathbf{3a_2}$. The other protons were shown in Table 2.

The mass spectrum of **2c** revealed the molecular ion peak (100%). The important common fragments [5] were shown in Fig. 2.

3. Experimental

3.1. Chemistry

Chemicals used in the experiments were commercially available and were used without further purification.

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Melting points were determined in glass capillary tubes on a Büchi 530 apparatus and unconnected. Elemental analyses were performed with Leco-932 elemental analyzer. UV spectra were obtained on a Shimadzu UV 2100S spectrophotometer (1 mg/100 ml in ethanol). The proton nuclear magnetic resonance (^{1}H NMR) spectra were run on a Bruker AVANC DPX 400 spectrometer in DMSO-d_6 with tetramethylsilane as the internal reference: chemical shift values (δ) are expressed in ppm. Mass spectra were taken on a Fisons Instruments VG Platform II LS–MS spectrometer.

*3.1.1. General procedure for the preparation of 1,4-dihydro-3-(3-hydroxy-2-naphthyl)-4-substituted-5H-1,2,4-triazoline-5-thiones(**2a–f**)*

A solution of 3-hydroxy-2-naphthoic acid hydrazide

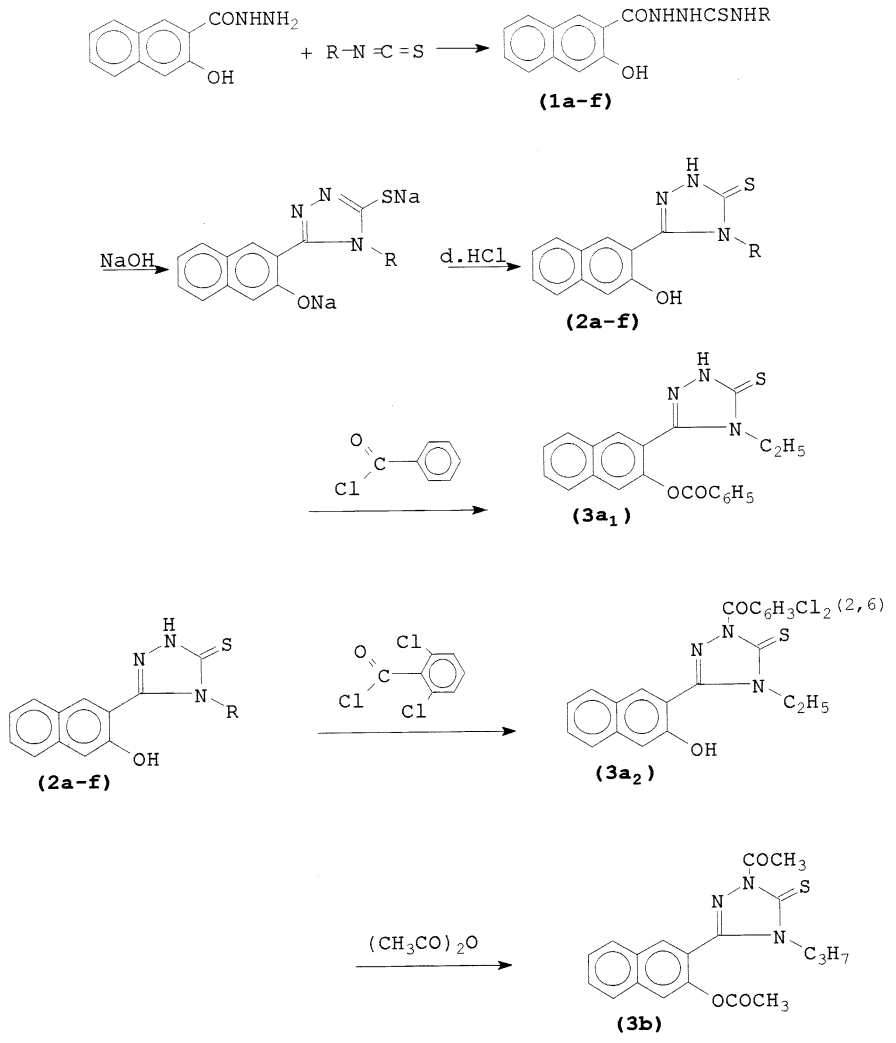
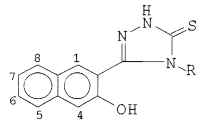


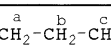
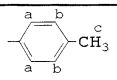
Fig. 1. Synthesis of the compounds.

Table 1
Physical constants of **2b–f**

Composition	Yield (%)	M.p. (°C)	Molecular formula (mwt.)	UV(λ_{max}) ε_{max}	Analyses (calculated/found)		
					C	H	N
2b	87.5	233	$C_{15}H_{15}N_3OS$ 285.37		63.14 63.77	5.30 5.35	14.72 14.57
2c	94.9	298	$C_{18}H_{13}N_3OS$ 319.38	233.0 (13925)	67.69 66.96	4.10 4.17	13.15 12.78
2d	79.2	239–243	$C_{18}H_{12}FN_3OS$. 1/2($C_2H_5OH{\cdot}H_2O$) 369.42	232.7 (77430)	61.77 61.18	4.37 4.30	11.37 11.79
2e	89.2	301	$C_{18}H_{12}FN_3OS$ 337.38	232.7 (32591)	64.08 64.41	3.59 3.95	12.46 11.95
2f	96.3	290	$C_{19}H_{15}N_3OS$ 333.41	232.8 (13370)	68.45 68.42	4.53 4.79	12.60 12.51

Table 2
Characteristic ^{1}H NMR chemical shifts of **2b–f**



Comp.	R	^{1}H-NMR (DMSO-d$_6$), δ ppm
2b		13.87 (1H, s, NH), 10.55 (1H, s, OH), 8.01 (1H, s, C_1H), 7.89 (1H, d, C_8H), 7.79 (1H, d, C_5H), 7.51 (1H, t, C_6H), 7.36 (2H, t, C_7H and C_4H), 3.87 (2H, t, 2H$_a$), 1.51 (2H, m, 2H$_b$), 0.63 (3H, t, 3H$_c$)
2c		14.06 (1H, s, NH), 10.15 (1H, s, OH), 8.02 (1H, s, C_1H), 7.79 (1H, d, C_8H), 7.65 (1H, d, C_5H), 7.44 (1H, t, C_6H), 7.36-7.28 (6H, m, C_7H, 2H$_a$, 2H$_b$ and H$_c$), 7.05 (1H, s, C_4H)
2d		14.16 (1H, s, NH), 10.22 (1H, s, OH), 8.02 (1H, s, C_1H), 7.83 (1H, d, C_8H), 7.65 (1H, d, C_5H), 7.44 (1H, t, C_6H), 7.40-7.25 (4H, m, C_7H, H$_b$, H$_c$ and H$_d$), 7.16 (1H, t, H$_a$), 7.05 (1H, s, C_4H)
2e		14.14 (1H, s, NH), 10.28 (1H, s, OH), 8.03 (1H, s, C_1H), 7.80 (1H, d, C_8H), 7.67 (1H, d, C_5H), 7.45 (1H, t, C_6H), 7.41-7.32 (2H, m, C_7H and H$_c$), 7.28 (1H, d, H$_d$), 7.21 (1H, t, H$_b$), 7.10 (1H, d, H$_a$), 7.07 (1H, s, C_4H)
2f		14.00 (1H, s, NH), 10.18 (1H, s, OH), 8.00 (1H, s, C_1H), 7.79 (1H, d, C_8H), 7.66 (1H, d, C_5H), 7.45 (1H, t, H$_6$), 7.31 (1H, t, C_7H), 7.17 (2H, d, 2H$_b$), 7.13 (2H, d, 2H$_a$), 7.06 (1H, s, C_4H), 2.24 (3H, s, 3H$_c$)

in ethanol was heated until dissolved, treated with equimolar appropriate isothiocyanate, refluxed for 3 h and precipitated thiosemicarbazide was recrystallized from ethanol [2].

A solution of appropriate thiosemicarbazide **1a–f** in 2 N sodium hydroxide was refluxed for 2 h. After cooling, cone, hydrochloric acid was added and precipitated product **2a–f** was filtered, washed several times with distilled water, finally recrystallized from ethanol [1].

*3.1.2. General procedure for the preparation of 1,4-dihydro-1-acetyl-3-(3-acetyloxy-2-naphthyl)-4-propyl-5H-l,2,4-triazoline-5-thione (**3b**)*

A solution of **2b** in acetic anhydride was refluxed at 150–160 °C for 30 min. After cooling, distilled water was added and allowed to stand overnight. The resulting precipitate was filtered, washed with distilled water and recrystallized from methanol [5]. $C_{19}H_{19}N_3O_3S$, 86%, m.p. 159 °C. Elemental analysis (Calc./Found%): 61.77/62.33 (C), 5.18/5.59 (H), 11.38/11.46 (N), 8.68/8.42 (S).^{1}H NMR (δ, ppm): 0.67 (3H, s, CH_3), 1.57 (2H, m, CH_2-CH_3), 2.24 (3H, s, $OCOCH_3$), 2.65 (3H, s, $NCOCH_3$), 3.94 (2H, t, N-CH_2), 7.66 (1H, t, C_6H of napht.), 7.73 (1H, t, C_6H of napht.), 7.99 (1H, s, C_4H of napht.), 8.06 (1H, d, C_8H of napht.), 8.11 (1H, d, C_8H of napht.), 8.42 (1H, s, C_1H of napht.).

*3.1.3. General procedure for the preparation of 1,4-dihydro-3-(3-benzoyloxy-2-naphthyl)-4-ethyl-5H-1,2,4-triazoline-5-thione (**3a$_1$**)*

Benzoyl chloride was added to a solution of **2a** in acelone and refluxed at 120–140 °C for 6 h. After cooling, the precipitated product was recrystallized from ethanol [4]. $C_{21}H_{17}N_3O_2S$, 55%, m.p. 197–199 °C. Elemental analysis (Calc./Found%): 67.18/67.38 (C), 4.57/4.22 (H), 11.19/10,77 (N), 8.54/8.00 (S).^{1}H NMR (δ, ppm): 1.13 (3H, t, CH_3), 4.00 (2H, q, CH_2), 7.60 (2H, t, m-protons to carbonyl on benzoyl), 7.66–7.77 (3H, m, p-proton to carbonyl on benzoyl and C_7H C_6H of napht.), 7.99 (2H,d, o-protons to carbonyl on

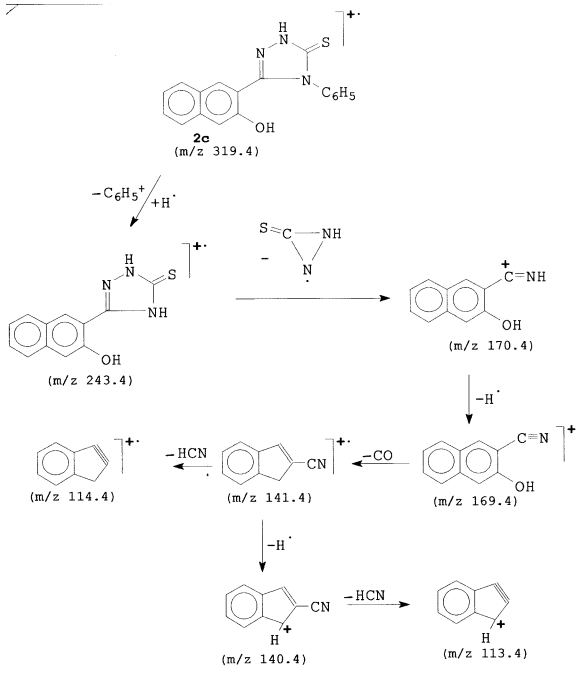


Fig. 2. Common mass fragmentation patterns of **2c**.

benzoyl), 8.07 (1H, d, C_5H of napht.), 8.13 (2H, d, C_4H and C_8H of napht.), 8.42 (1H, s, C_1H of napht.), 13.86 (1H, s, NH).

*3.1.4. General procedure for the preparation of 1,4-dihydro-1-(2,6-dichlorobenzoyl)-3-(3-hydroxy-2-naphthyl)-4-ethyl-5H-1,2,4-triazoline-5-thione (**3a$_2$**)*

2,6-Dichlorobenzoyl chloride was added to a solution of **2a** in acelone and refluxed at 120–140 °C for 2 h. The precipitated product was filtered and recrystallized from ethanol [4]. $C_{21}H_{15}Cl_2N_5O_2S$, 96%, m.p. 210 °C. Elemental analysis, (Calc./Found%): 56.77/56.77 (C), 3.40/3.31 (H), 9.46/9.33 (N), 7.21/7.22 (S).^{1}H NMR (δ, ppm): 1.15 (3H, t, CH_3), 4.10 (2H q, CH_2), 7.37 (2H, t, m-protons to chlorines on benzoyl and C_4H of napht.), 7.53 (1H, t, C_7H of napht.), 7.64–7.73 (3H, m, o-protons to chlorines on benzoyl and CgH of napht.), 7.82 (1H, d, C_5H of naphl.), 7.94 (1H; d, C_8H of napht.), 8.06 (1H, s, C_1H of naphl.), 10.93 (1H, s, OH).

3.2. Anti-tumor activity screen

The NCl's in vitro anti-tumor screen consisled of 52 human tumor cell lines (Table 3) against which **3a** was tested at a minimum of five concentrations at 10-fold dilutions. A 48 h continuous drug exposure protocol was used, and a sulforhodamine B (SRB) protein assay was used lo estimate cell viability or growth. The measured effect of the **3a** on a cell line was currently calculated according lo one or the other of the following two expressions:

$$\text{If } (\text{Mean } \text{Od}_{\text{test}} - \text{Mean } \text{Od}_{\text{tzero}}) \geq 0$$

$$\text{PG} = 100 \times (\text{Mean } \text{Od}_{\text{test}} - \text{Mean } \text{Od}_{\text{tzero}}) / (\text{Mean } \text{Od}_{\text{ctrl}} - \text{Mean } \text{Od}_{\text{tzero}})$$

$$\text{If } (\text{Mean } \text{Od}_{\text{test}} - \text{Mean } \text{Od}_{\text{tzero}}) < 0$$

$$\text{PG} = 100 \times (\text{Mean } \text{Od}_{\text{test}} - \text{Mean } \text{Od}_{\text{tzero}}) / \text{Mean } \text{Od}_{\text{tzero}}$$

Mean Od_{tzero} is the average of optical density measurements SRB-derived color just before exposure of cells to the test compound. Mean Od_{test} is the average of optical density measurements SRB-derived color after 48 h exposure of cells to the test compound. MeanOd_{ctrl} is the average of optical density measurements SRB-derived color after 48 h with no exposure of cells to the test compound. PG is the percentage growth, the calculated measurement of effect.

4. Results and discussion

Table 3 shows all tested human tumor cell lines. Table 4 represents the experimental data collected against six cell lines for anti-tumor activity screen. Out of 52 cell lines studied, only six were presented here. The first column describes the subpanel and cell line involved. The second column lists the Mean Od_{tzero} and Mean $Od_{ctrl.}$ The third column lists the mean optical densities (Mean Od_{test}) for each of five different concentrations and their calculaled percent growth in paranthesis. Each concentration was expressed as the $\log_{10}$ (molar or μg/ml). The next column lists the response parameters GI50 (%50 growth inhibition), TGI (total growth inhibition) and LC50 (lethal concentration), these parameters were interpolated values representing the concentrations at which the PG was + 50, 0 and − 50, respectively. In the case which these response parameters can not be obtained by interpolation, the value given for each response parameter is the highest concentration tested and is preceded by a '>' sign. The last column lists the $\log_{10}$ of the values in fourth column and these values were calculated from the curves in Fig. 3. The values extending to the smaller

Table 3
Tested panels and cell lines

Panel	Cell line
Leukemia	CCRF-CEM, HL-60(TB), K-562. MOLT-4, RPMI-8226, SR
Non-small cell lung cancer	EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522
Colon cancer	HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620
CNS cancer	SF-268, SF-295. SF-539, U251
Melanoma	LOX fMVI, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257
Ovarian cancer	IGROVI, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3
Renal cancer	786-0, A498, ACHN, CAKI-1, RXF 393, TK-10, UO-31
Prostate cancer	PC-3, DU-145
Breast cancer	MCF7, NCI/ADR-RES; MDA-MB-231/ATCC, HS 578T, MDA-MB-435, MDA-N

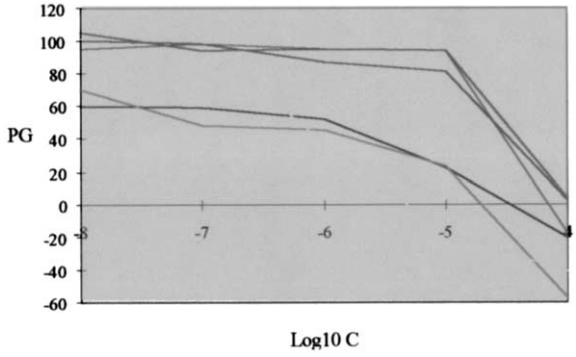


Fig. 3. Dose–response curves of selected six cell lines.

Table 4
In vitro testing results of **3a** for selected six cell lines out of 52 lines

Cell line	Mean Od_{tzero} (Od_{ctrl})	Mean Od_{test} (percent growth)					Response parameters			$\log_{10}$ GI50	$\log_{10}$ TGI	$\log_{10}$ LC50
		−8.0	−7.0	−6.0	−5.0	−4.0	GI50	TGI	LC50			
CCRF-CEM	0.207 (0.836)	0.587 (60)	0.581 (59)	0.533 (52)	0.350 (23)	0.166(−20)	1.16E−0.6	3.42E−0.5	>1.00E−0.4	−5.94	−4.47	> −4.00
HL-60(TB)	0.737 (2.117)	1.704 (70)	1.398 (48)	1.356 (45)	1.062 (24)	0.314(−57)	8.05E−08	1.95E−05	8.10E−05	−7.09	−4.71	−4.09
SR (Leukemia)	0.695 (1.995)	2.005(101)	1.984 (99)	1.180 (37)	0.573 (−18)	0.414 (−41)	6.23E−0.7	4.79E−0.6	>1.00E−0.4	−6.21	−5.32	> −4.00
NCI-H460 (lung)	0.280 (1.357)	1.305 (95)	1.337 (98)	1.302 (95)	1.289 (94)	0.232 (−17)	2.47E−0.5	6.98E−0.5	>1.00E−0.4	−4.61	−4.16	>4.00
SF-268 (CNS)	0.469 (1.294)	1.293 (100)	1.280 (98)	1.185 (87)	1.136 (81)	0.495 (3)	2.49E−0.5	>1.00E−0.4	>1.00E−0.4	4.60	>4.00	> −4.00
MCF7 (breast)	0.273 (1.219)	1.268 (105)	1.164 (94)	1.174 (95)	1.165 (94)	0.307 (4)	3.08E−0.5	>1.00E−0.4	>1.00E−0.4	−4.51	>4.00	>4.00
									Mean of 52 lines:	−4.68	−4.17	−4.01

values from the mean value of 52 cell lines represent sensitivity of cell line to the test agent in excess of the average sensitivity of all tested cell lines. The values to the higher values represent sensitivity less than the mean.

The values at either limit were also calculated in the mean used for the $\log_{10}$ Gl50, $\log_{10}$ TGI and $\log_{10}$ LCSO. Therefore, the mean was given in the Table 4 may not be the actual mean of the G150 for instance. For this reason, we referred to this value as the mean (-4.68). According to mean values, the principal response parameters lay about (-7.09) to (-4.00). The values within > -4 are uneffective [8].

The dose–response curves (Fig. 3 which was drawn for only six cell lines) were created by plotting the percent growths against the $\log_{10}$ of the corresponding concentrations for 52 cell lines. Horizontal lines were provided at the PG values of $+50$, 0 and -50. The concentrations corresponding to these points where the curves cross these lines were the GI50, TGI and LC50.

Acknowledgements

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