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Synthesis and biological activity evaluation of new functionally substituted 5-arylidene-2,4-dioxotetrahydro-1,3-thiazoles

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Abstract: New functionally substituted 5-arylidene-2,4-dioxotetrahydro-1,3-thiazole-3-carboxylic acid cholesteryl esters were synthesized from 2,4-dioxotetrahydro-1,3-thiazole and evaluated for their *in vitro* cytotoxicity against several human tumor cell lines and one normal lung fibroblast cell line.

Keywords: heterocycles, synthesis, 5-arylidene-2,4-dioxotetrahydro-1,3-thazole-3-carboxylic acid cholesteryl esters, *in vitro* cytotoxicity.

INTRODUCTION

Dioxotetrahydrothiazole derivatives with a carbonyl group at positions 2 and 4 are an important group of heterocyclic compounds with diverse biological activities, *i.e.,* they are also known as anti-neoplastics.[1,2]

Dioxotetrahydrothiazole derivatives have been extensively studied chemically as well as biologically,[3,4] in an effort to generate new translation initiation inhibitors for cancer therapy.[5] 2,4-Dioxotetrahydro-1,3-thiazoles inhibit growth of gastrointestinal,[6,7] biliary and pancreatic adenocarcinoma cells.[8] This study was

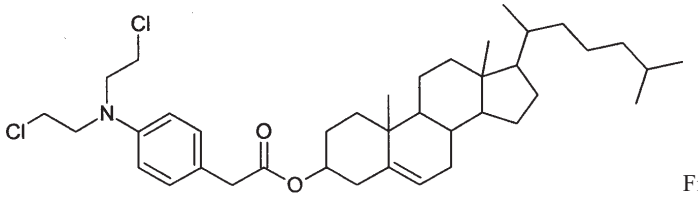

Fig. 1. Phenesterine.

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aimed at the preparation and biological evaluation of new derivatives of 2,4-dioxotetrahydro-1,3-thiazole containing the cholesterole substructure. The cited substructure is present in the known anti-neoplastic phenesterine[9] (Fig. 1).

RESULTS AND DISCUSSION

Several new 5-arylidene-2,4-dioxotetrahydro-1,3-thiazole-3-carboxylic acid cholesteryl esters were prepared according to Scheme 1.The synthesis of 5-arylidene-2,4-dioxotetrahydro-1,3-thiazole-3-carboxylic acid cholesteryl esters was performed in the following steps: condensation of 2,4-dioxotetrahydro-1,3-thiazole (**1**) with appropriate aldehydes, in the presence of morpholine as a catalyst, whereby the 5-arylidene-2,4-dioxotetrahydro-1,3-thiazoles (**2a–e**) were obtained.[10] The 5-arylidene derivatives (**2a**–**e**) were transformed into their potassium salts (**3a**–**e**) using potassium hydroxide in an ethanolic medium. The synthesized derivatives (**3a**–**e**) were isolated in pure form and used in the next step without further purification.[11]

Treatment of **3a**–**e** with cholesteryl chloroformate in refluxing dry acetone yielded the corresponding 5-arylidene-2,4-dioxotetrahydro-1,3-thiazole-3-carboxylic acid cholesteryl esters (**4a**–**e**). All the newly synthesized compounds were characterized by IR, ^{1}H-NMR, MS spectroscopy and microanalyses.

The newly synthesized cholesterol esters **4a**–**e** were preliminarily evaluated for their ability to inhibit the growth of human cervical carcinoma HeLa, breast adenocarcinoma MCF-7, colon adenocarcinoma HT29, melanoma Hs294T, prostate adenocarcinoma PC-3 and lung fibroblast cell line MRC-5.

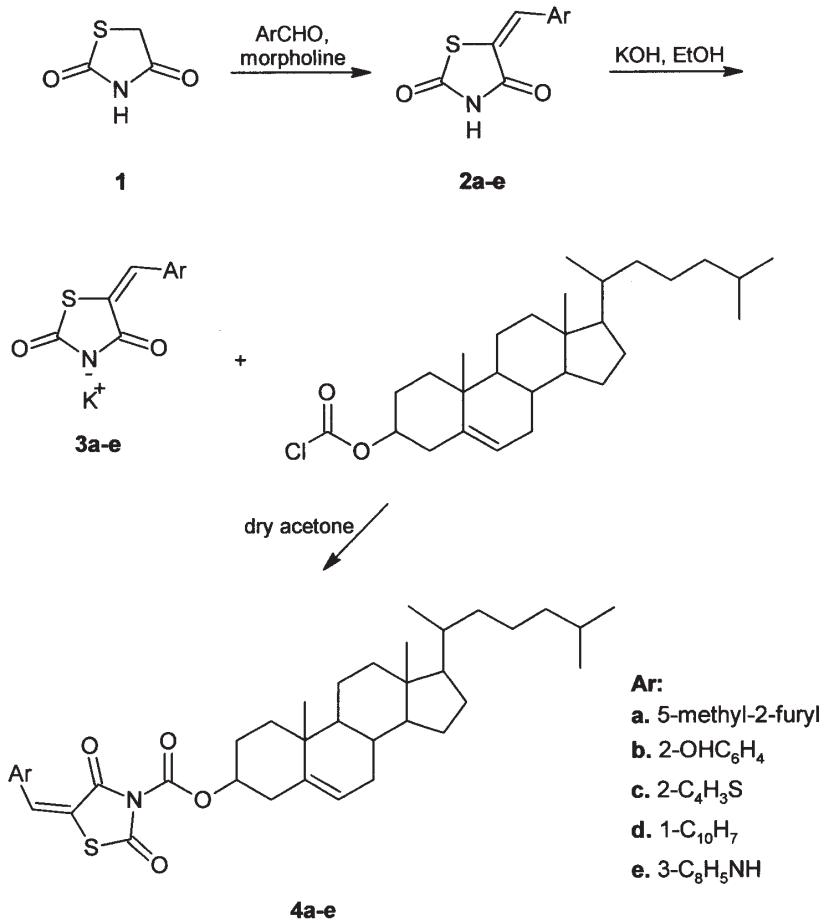
Growth inhibition was evaluated by colorimetric sulphorhodamine B (SRB) assay.[12] Briefly, a single cell suspension was plated into 96-well microtitar plates (Costar, flat bottom) (5×10^3 cells per 180 ml of medium) and preincubated for 24 h at 37 ºC, 5 % CO_2. The tested substances were added to the growth medium, to all wells except for the control and microplates, which were incubated for 48 h. After 48 h, SRB assay was carried out: to all wells 50 % trichloroacetic acid (TCA) was added (50 μl). An hour later, the plates were washed with water and 75 μl of 0.4 % SRB was added. Half an hour later, the plates were washed with citric acid (1 %) and dried.

Finally, 200 μl 10 mmol/dm^3 TRIS (pH 10.5) base was added. The optical density was measured on a microplate reader (Multiscan MCC 340, Labsystems, at 540/690 nm). The growth inhibiton was expressed as a percent of cytotoxicity (CI %) calculated according to the formula: $(1\text{-}A_{TEST}/A_{CONTROL}) \times 100$. A_{TEST} is the absorbance of the tested sample and $A_{CONTROL}$ is the absorbance of the control sample.

The results are presented in Table I. Compound **4a** was inactive against HeLa, MCF-7, HT-29, Hs294T, MRC-5 cell lines but showed cytotoxic activity against PC-3.

Conversely, the analogue **4b** was found to be the most active and inhibited the growth of HeLa, MCF-7, Hs294T and PC-3 cell lines but was inactive against HT-29 and normal lung fibroblast cells.

Compound **4c** showed cytotoxic activity against cervical carcinoma HeLa and PC-3 cell lines and compound **4d** was active against the MRC-5 cell line.



Scheme 1. Reaction path for the synthesis of 5-arylidene-2,4-dioxotetrahydro-1,3-thiazole-3-carboxylic acid cholesteryl esters.

Compound **4e** was completely inactive against MCF-7, HT-29, Hs294T, PC-3 and lung fibroblast MRC-5 cell lines, but showed activity against the HeLa cell line.

TABLE I. IC_{50} values of the compounds tested in SRB assay for 48 h

Compound	$IC_{50}/\mu M$[a]					
	HeLa	MCF-7	HT-29	Hs294T	PC3	MRC-5
4a	>100	>100	>100	>100	1.39	>100
4b	26.72	8.57	>100	14.96	0.80	>100
4c	9.32	>100	58.56	>100	0.58	>100
4d	15.51	>100	>100	>100	4.07	10.09
4e	22.87	>100	>100	>100	>100	>100

[a]IC_{50} is the concentration of compound required to inhibit the cell growth by 50 %.

In conclusion, five novel 5-arylidene-2,4-dioxotetrahydro-1,3-thiazole-3-carboxylic acid cholesteryl esters were synthesized. Compound **4b** was found to be the most active and inhibited the growth of 4 tumor cell lines but was inactive against normal lung fibroblast cells. Of the tumor cells, PC-3 cells were the most sensitive: 4 out of the 5 compounds strongly inhibited their growth.

EXPERIMENTAL

The solvent and all reagents used in this study were purchased from commercial suppliers and were used as received.

The infrared spectra (ν in cm^{-1}) were recorded on a Perkin Elmer FTIR 1725 X spectrophotometer. The ^{1}H-NMR spectra were obtained with a Varian Gemini 200 (200 MHz) instrument, chemical shifts (δ) are given relative to TMS. The mass spectra were obtained on Finnigan MAT-8230 BE spectrometer with EI-CI source at 200 °C. EI : 70 eV, 0.5 mA; CI: 1 mTorr of isobutane, 150 eV, 0.2 mA.

General synthetic procedure

5-Arylidene-2,4-dioxotetrahydro-1,3-thiazole potassium salt (1 mmol) was suspended in dry acetone (20 cm^3) and cholesteryl chloroformate (1 mmol) was added. The reaction mixture was heated under reflux for 2 h, and then filtered and evaporated to dryness. The obtained crystals of 5-arylidene-2,4-dioxotetrahydro-1,3-thiazole-3-carboxylic acid cholesteryl esters were recrystallized from an ethanol–water mixture.

5-(5-Methyl-2-furfurylidene)-2,4-dioxotetrahydro-1,3-thiazole-3-carboxylic acid cholesteryl ester (**4a**)

Yield 46.69 %; m.p. 183 °C (from ethanol–water); IR-spectrum (ν_{max}/cm^{-1}, KBr) 3042, 2950, 1785, 1744, 1702, 1235, 1167 (C–O ester); CIMS: (*m/z* %) 210 100; 369 75; 370 18.0; 578 41.7; 622 0.4 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$ ppm): 2.42 (*s*, 3H), 6.22 (*d*, J = 3.4 Hz, $1H_{furyl}$), 6.76 (*d*, J = 3.4 Hz, $1H_{furyl}$), 7.59 (*s*, 1H). 5.43 (*m*, 1H, =CH), 4.85 (*m*, 1H, O–C–H), 2.52 (*m*, 2H, CH_2 C4), 1.04 (*s*, 3H, CH_3 C19), 0.91 (*d*, 3H, CH_3 C21), 0.88 (*d*, 3H, CH_3 C26 or C27), 0.85 (*d*, 3H, CH_3 C27 or C26), 0.68 (*s*, 3H, CH_3 C18); Anal. Calcd. for $C_{37}H_{51}NO_5S$: C, 71.46; H, 8.27; N, 2.25; S, 5.16; Found: C, 71.10; H, 8.25; N, 2.24; S, 5.19.

5-(2-Hydroxybenzylidene)-2,4-dioxotetrahydro-1,3-thiazole-3-carboxylic acid cholesteryl ester (**4b**)

Yield 36.22 %; m.p. 199 °C (decomp.) (from ethanol–water); IR spectrum, ν, cm^{-1}: 3417 (O–H), 3043 (=C–H), 2950 (C–H), 1761 (C=O), 1708 (C=O), 1330, 1289, 1257, 1223, 1157 (O–CO), 1038; CIMS (*m/z* %) 261 10.2; 369 100; 370 27.0; 371 9.0; 590 1.7; 634 2.2 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$,ppm): 7.29-7.57 (*m*, $4H_{ar}$) 7.99 (*s*, 1H, =CH), 5.42 (*m*, 1H, =CH), 4.52 (*m*, 1H, O–C–H), 2.50 (*m*, 2H, CH_2 C4), 1.05 (*s*, 3H, CH_3 C19), 0.92 (*d*, 3H, CH_3 C21), 0.88 (*d*, 3H, CH_3 C26 or C27), 0.85 (*d*, 3H, CH_3 C27 or C26), 0.68 (*s*, 3H, CH_3 C18); Anal. Calcd. for $C_{38}H_{51}NO_5S$: C, 72.00; H, 8.11; N, 2.21; S, 5.06; Found: C, 71.78; H, 8.09; N, 2.30; S, 5.08.

5-(2-Thenylidene)2,4-dioxotetrahydro-1,3-thiazole-3-carboxylic acid cholesteryl ester (**4c**)

Yield 55.70 %; m.p. 144 °C (from ethanol–water); IR-spectrum (ν_{max}/cm^{-1}, KBr) 3090, 2946, 1777, 1702, 1162 (C–O ester); CIMS (*m/z* %) 212 48; 369 100; 370 28.0; 580 1.7; 624 0.1 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$, ppm): 7.25, 7.43, 7.71 (*m*, $3H_{thiophene}$), 8.11 (*s*, 1H), 5.42 (*m*, 1H, =CH), 4.75 (*m*, 1H, O–C–H), 2.47 (*m*, 2H, CH_2 C4), 1.02 (*s*, 3H, CH_3 C19), 0.91 (*d*, 3H, CH_3 C21), 0.88 (*d*, 3H, CH_3 C26 or C27), 0.85 (*d*, 3H, CH_3, C27 or C26), 0.68 (*s*, H, CH_3 C18); Anal. Calcd. for $C_{36}H_{49}NO_4S_2$: C, 69.30; H, 7.92; N, 2.24; S, 10.28; Found: C, 69.02; H, 7.91; N, 2.23; S, 10.34.

5-(1-Naphthylidene)-2,4-dioxotetrahydro-1,3-thiazole-3-carboxylic acid cholesteryl ester (**4d**)

Yield 86.23 %; m.p. 128 °C (from ethanol–water); IR-spectrum (ν_{max}/cm^{-1}, KBr) 3059, 2946, 1779, 1750, 1702, 1163 (C–O ester); CIMS (*m/z* %) 256 88; 369 100; 370 28.0; 624 8.7; 668 0.1

$(M+H)^+$; ^{1}H-NMR ($CDCl_3$, ppm): 7.48–8.15 (*m*, $7H_{naphthyl}$), 8.68 (*s*, 1H, =C–H), 5.43 (*m*, 1H, =CH), 4.78 (*m*, 1H, O–C–H), 2.50 (*m*, 2H, CH_2 C4), 1.05 (*s*, 3H, CH_3 C19), 0.91 (*d*, 3H, CH_3 C21), 0.88 (*d*, 3H, CH_3 C26 or C27), 0.85 (*d*, 3H, CH_3 C27 or C26), 0.68 (*s*, 3H, CH_3 C18); Anal. Calcd. for $C_{42}H_{53}NO_4S$: C, 75.52; H, 8.00; N, 2.10; S, 4.80; Found: C, 75.30; H, 7.98; N, 2.09; S, 4.82.

5-(3-Indolinylidene)-2,4-dioxotetrahydro-1,3-thiazole-3-carboxylic acid cholesteryl ester (**4e**)

Yield 27.21 %; m.p. 240 °C (decomp.) (from ethanol–water); IR-spectrum (ν_{max}/cm^{-1}, KBr) 3434 (N–H), 3039, 2949, 1776, 1725, 1696, 1164; CIMS (*m/z* %) 369 100; 370 8.0; 657 3.4 $(M+H)^+$; ^{1}H-NMR ($CDCl_3$, ppm): 7.42, 7.71, 8.1, 8.2 (*m*, $4H_{indolyl}$), 7.88 (*s*, 1H), 8.07 (*s*, 1H), 5.43 (*m*, 1H, =CH), 4.83 (*m*, 1H, O–C–H), 2.65 (*m*, 2H, CH_2 C4), 1.04 (*s*, 3H, CH_3 C19), 0.91 (*d*, 3H, CH_3 C21), 0.88 (*d*, 3H, CH_3 C26 or C27), 0.85 (*d*, 3H, CH_3 C27 or C26), 0.68 (*s*, 3H, CH_3 C18); Anal. Calcd. for $C_{40}H_{52}N_2O_4S$: C, 73.13; H, 7.98; N, 4.26; S, 4.88; Found: C, 72.87; H, 7.96; N, 4.24; S, 4.90.

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ИЗВОД

СИНТЕЗА И ПРОЦЕНА БИОЛОШКЕ АКТИВНОСТИ НОВИХ ФУНКЦИОНАЛНО СУПСТИТУИСАНИХ 5-АРИЛИДЕН-2,4-ДИОКСОТЕТРАХИДРО-1,3-ТИАЗОЛА

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Полазећи од 2,4-диоксотетрахидро-1,3-тиазола (**1**) кондензацијом са алдехидима у присуству морфолина као катализатора добијени су одговарајући 5-арилиден-2,4-диоксотетрахидро-1,3-тиазоли (**2a**–**e**), који су затим трансформисани у своје калијумове соли (**3a**–**e**). Калијумове соли су реакцијом са холестерил-хлороформијатом дале одговарајуће холестерил естре 5-арилиден-2,4-диоксотетрахидро-1,3-тиазол-3-карбоксилних киселина (**4a**–**e**). Извршена је процена биолошке активности синтетизованих холестерил естара.

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REFERENCES

1. S. P. Singh, S. S. Parmar, K. Raman, V. I. Stenberg, *Chem. Rev.* **81** (1981) 175
2. K. H. Schmidt-Ruppin, U. Joss, K. Schwieweck, *Oncology* **33** (1976) 229
3. F. G. Brown, *Chem. Rev.* **61** (1961) 463
4. H. W. Lee, B. Y. Kim, J. B. Ahn, H. J. Son, J. W. Lee, S. K. Ahn, C. I. Hong, *Heterocycles* **57** (2002) 2163
5. H. Chen, Y.-H. Fan, A. Natarajan, Y. Guo, J. Iyasere, F. Harbinski, L. Luis, W. Chirst, H. Aktas, J. Halperin, *Bioorg. Med. Chem. Lett*. **14** (2004) 5401
6. H. Sato, S. Ishihara, K. Kawashima, N. Moriyama, H. Suetsugu, H. Kazumori, T. Okuyama, M. A. K. Rumi, R. Fukuda, N. Nagasue, Y. Kinoshita, *Br. J. Cancer* **83** (2000) 1394
7. N. Takahashi, T. Okumura, W. Motomura, Y. Fujimoto, I. Kawabata, Y. Kohgo, *FEBS Lett.* **455** (1999) 135
8. W. Motomura, T. Okumura, N. Takahashi, T. Obara, Y. Kohgo, *Cancer Res.* **60** (2000) 5558

9. E. N. Shkodinskaya, E. M. Kurdyukova, O. S. Vasina, A. Ya. Berlin, *Zh. Obshch. Khim.* **32** (1962) 959
10. K. Popov-Pergal, Ž. Čeković, M. Pergal, *Zh. Obshch. Khim.* **61** (1991) 2112
11. K. Popov-Pergal, Ž. Čeković, M. Pergal, *Sulphur Lett.* **9** (1989) 95
12. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **82** (1990) 1107.