HETEROCYCLES 47. SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NEW THIAZOLE AURONES AS ANTIPROLIFERATIVE AGENTS

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Abstract

New substituted thiazole aurones 2a-o were synthesized and evaluated for their anticancer activity. A screening of methods based on the oxidative cyclization of ortho-hydroxychalcones, with different agents, was applied in order to find the optimal way for the synthesis of these compounds. The best oxidizing agent proved to be mercury(II) acetate and it allowed the synthesis of the thiazole aurones with yields of 70-86%. All synthesized compounds were purified and characterized by ESI-MS, ¹H NMR, ¹³C NMR and IR. The cytotoxicity of the thiazole aurones was determined in a panel of nine cancer cell lines using doxorubicin as control. Compounds 2a, 2i and 2e displayed the best cytotoxic activities. The interactions between aurones 2a-o and topoisomerasases I and II were assessed by means of the molecular docking study and their target molecule is predicted to be topoisomerase I. The evaluation of the results revealed the importance of the thiazole ring for establishing a hydrogen bond with His367 and Arg364.

Keywords: thiazole aurone, antiproliferative activity, topoisomerase

Introduction

Cancer is one of the main causes of death worldwide and its frequency increases year by year. Cancer affects many types of cells, leading to various and multiple forms of tumours which can develop in both children and adults. Most drugs used in the cancer therapy nowadays are not selective and they determine the death of healthy and cancerous cells alike [13, 21, 37]. Therefore, new drugs with higher selectivity for cancerous cells and, consequently, fewer side effects are still needed. Many naturally occurring compounds with different structures have showed anticancer activity. In the past years, flavonoids have been found to be a group of potential anticancer agents [37, 46]. Evidence suggests
that one of the anticancer effects displayed by some flavonoids is based on the inhibition of topoisomerases.

Some of the discovered and tested flavonoids were proved to be topoisomerase I inhibitors, whereas others were proved to inhibit both types of topoisomerases, acting by stabilizing in vitro the topoisomerase-DNA complexes. Topoisomerases are essential enzymes that play a pivotal role in the over-winding or under-winding of the DNA, caused by the intertwined nature of its double-helical structure [27, 29, 30].

Aurones, compounds from the family of flavonoids, contribute to the bright yellow colour of some flowering plants such as cosmos, snapdragon and dahlia. Among the various subclasses of flavonoids, aurones have not been extensively studied for their biological activities [3, 6, 10, 13, 21, 37]. However, the existing data from the literature that refers to the anticancer activity of aurones and their synthetic analogues is very promising [7, 10, 13, 20, 21, 26, 37]. Alsayari et al. showed that aurones exhibit a broad spectrum of anticancer mechanisms by interacting with various targets, such as cyclin dependent kinase, histone deacetylase, topoisomerase, adenosine receptor or telomerase [2].

Aurones are compounds that contain a benzofuranone ring linked to a benzene ring by a vinylic carbon bridge [13, 21, 37]. This suggests that there are two sites on the aurone core, where modifications can be made, in order to obtain substituted analogues with enhanced anticancer activity (Figure 1).

The biological results obtained by Cheng et al. suggest that the presence of a moiety that contains a nitrogen atom enhanced the anticancer activity of the tested compounds. Moreover, the presence of a bulkier substituent close to this nitrogen atom increased the activity. A deeper analysis revealed that the nitrogen atom should not be part of a strong electron withdrawing group, such as an amide or a nitrile [7]. Based on these findings, we proposed the synthesis of a new series of aurones, substituted on site I, with a scaffold where the nitrogen atom is part of a π-excessive heterocyclic ring, without bonding the nitrogen atom with a bulkier group, so the nitrogen will not be deprived of electrons and it will not lose the ability to form a hydrogen bond with the Arg364 or His367 from the active site of topoisomerase I, as suggested by the molecular docking studies.

We guided our research towards the thiazole ring, because previous studies reported in literature stated that the benzene ring bound to the benzofuranone through the vinylic carbon is not mandatory and it can be replaced with other cyclic compounds containing nitrogen, some of these compounds exhibiting remarkable activity [10, 44]. Thiazole is already a common moiety found in the structure of certain molecules with anticancer activity [1, 14, 16, 19, 42], thus the research concerning the biological properties of the thiazole ring is ongoing and warrants further investigation. The hydrocarbonic substitution of the thiazole in position 2 was realized with a phenyl ring which, in some compounds from our series, was substituted in para position.

Among the reported aurones with anticancer activity, two types of substitution can be found on site II, in
terms of volume of the substituent linked directly to the benzofuranone. Here, substitutions can be made on one of the four available positions from the benzene fragment of benzofuranone. Most of the compounds reported in literature are substitute with small residues, such as chlorine, methoxy, hydroxyl or amino groups [7, 10, 13, 26] or, less often, with bulky substituents containing various aromatic rings [5]. We have chosen small substituents, inspired by the idea that insertion of the phenylthiazole system on substitution site I, already extended the molecules in our series and we wanted to avoid obtaining molecules with a higher molecular weight which can decrease the permeability through cell membranes [28].

Since the anticancer activity of thiazole aurones has not been extensively studied, this investigation deals with the synthesis, evaluation of the antiproliferative activity and assessment of the theoretical interactions of some new synthetic aurones analogues with the human topoisomerases I and II.

Materials and Methods

Chemistry

All used chemicals, solvents and reagents, were of 95 - 99% purity grade and purchased from Alfa Aesar and Sigma Aldrich, Germany. Normal and deuterated solvents were used as received without further purifications.

The reactions were monitored by performing TLC (silica gel, aluminium sheets 60 F254, Merck) using dichloromethane, dichloromethane:acetone = 25:1 (v/v) or dichloromethane:acetone = 9:1 (v/v) as mobile phases. The TLC plates were analysed using a UV lamp, at 254 nm or 365 nm wavelengths.

The compounds were purified by column chromatography, using different solvents mixtures (dichloromethane, dichloromethane:acetone = 25:1 (v/v) or dichloromethane:acetone = 9:1 (v/v)) as mobile phases, as indicated for each individual compound, in the experimental part.

Purity of compounds was checked preliminary by TLC and then using RP-HPLC coupled with MS spectrometry (Agilent 1100 series) in order to confirm that the purification of the target compounds was successfully achieved.

Mass spectra were recorded on an Agilent 1100 Ion Trapp SL mass spectrometer (Agilent, Santa Clara, CA, USA), operated in positive ionization mode detection, using an electrospray ionization source at 70 eV.

Melting points were determined in an open glass capillary tube on an Electrothermal 9000 IA digital apparatus.

The 1H NMR, 13C NMR, 1H-1H COSY and 1H-13C HMBC spectra were recorded on a Bruker (Ascend, BioSpin, Germany) Avance spectrometer operating at 600 MHz and 151 MHz, respectively, in different deuterated solvents (chloroform-d, dimethylsulfoxide-d6); the chemical shifts are expressed in δ ppm.

The FT-IR analysis was performed on a 460 Plus spectrometer (Jasco) at the University of Medicine, Pharmacy, Science and Technology of Târgu Mureş, Romania, Department of Analytical Chemistry and Drug Analysis by using the Spectra Manager software. The solid sample was introduced in the ATR device’s slot and the IR spectra were recorded between 4000 cm⁻¹ and 400 cm⁻¹ wavelengths at 4 cm⁻¹ resolution.

General synthetic procedures

The cyclization of thiazole ortho-hydroxychalcone 1a with copper(II) acetate in DMSO

2-Phenylthiazole ortho-hydroxychalcone 1a (0.91 mmol, 0.28 g) was added to a stirred solution of copper(II) acetate (0.655 mmol, 0.13 g) in DMSO (2.8 mL) at room temperature and refluxed for 12 h on an electric mantle. The reaction was monitored by TLC, using dichloromethane:acetone = 25:1 (v/v) as the mobile phase. This showed that an unexpected product was formed in an approximate molar ratio of 1:1, alongside the aurone 2a. We identified the product as the corresponding flavone 3a, based on spectral data.

2-(2-Phenylthiazol-4-yl)chroman-4-one (3a): mp: 189 - 190°C; 1H NMR (600 MHz, CDCl3) δ 8.24 (dd, J = 7.9, 1.2 Hz, 1H, CH-5 chromen-4-one), 8.04 (s, 1H, CH-5 thiazole), 8.01 (dd, J = 6.5, 2.9 Hz, 2H, CH-2’, CH-6’), 7.72 - 7.69 (m, 1H, CH-7), 7.55 (d, J = 8.3 Hz, 1H, CH-8 chromen-4-one), 7.50 - 7.47 (m, 3H, CH-3’, CH-4’, CH-5’), 7.42 (t, J = 7.5 Hz, 1H, CH-6 chromen-4-one), 7.39 (s, 1H, CH-3 chromen-4-one); 13C NMR (151 MHz, CDCl3) δ 178.82 (C, C=O), 169.42 (C, C-2 thiazole), 158.71 (C, C-9 chromen-4-one), 156.21 (C, C-2 chromen-4-one), 148.74 (C, C-4 thiazole), 134.09 (C, C-1’), 132.83 (CH, CH-5 thiazole), 131.03 (CH, C-7 chromen-4-one), 129.26 (CH, C-3’, C-5’), 126.91 (CH, C-2’, C-6’), 126.01 (CH, C-4’), 125.48 (CH, C-5 chromen-4-one), 124.25 (CH, C-6 chromen-4-one), 120.41 (C, C-10 chromen-4-one), 118.11 (CH, C-8 chromen-4-one), 108.64 (CH, C-3 chromen-4-one). MS: m/z 308.30 [M+H]+ (calculated for C18H13NO2S2: 308.07).
unexpected product, the corresponding hydroxyflavone 4a as the main product.

3-Hydroxy-2-(2-phenylthiazol-4-y1)-4H-chromen-4 one (4a): mp: 203 - 204°C; 1H NMR (600 MHz, DMF) δ 10.17 (s, 1H, hydroxyl), 8.55 (s, 1H, CH-5 thiazole), 8.13 (d, 1H, CH-5 thiazole-4-one), 8.07 (d, 2H, CH-2', CH-6'), 7.82 (t, 1H, CH-6 thiazole-4-one), 7.76 (d, 1H, CH-8 thiazole-4-one), 7.57 (m, 3H, CH-3', CH-4', CH-5'), 7.49 (t, 1H, CH-7 thiazole-4-one), 1.34 (CH, CH-3 thiazole-4-one), 1.32, 1.37 (CH, C-4'), 129.90 (CH, C-3', C-5'), 126.97 (CH, C-2', C-6'), 125.37 (CH, C-5 thiazole-4-one), 120.31 (CH, C-6 thiazole-4-one), 122.39 (C, C-4a thiazole-4-one), 118.95 (CH, C-8 thiazole-4-one). EI* MS: m/z 322.00 (calculated for C9H8N2O5S 322.05 [M+H]+).

The cyclization of thiazole ortho-hydroxychalcone 1a with selenium dioxide

A mixture of freshly sublimed selenium dioxide (1.98 mmol) and 2-phenylthiazole ortho-hydroxychalcone 1a (0.75 mmol, 0.23 g) dissolved in n-butanol (3.30 mL) was heated under reflux for 24 h. The obtained turbid solution was filtered while hot to remove the selenium metal. The reaction was monitored by TLC, using dichloromethane:acetone = 25:1 (v/v) as the mobile phase and this showed that the obtained products were not auroes, but other products from the flavonoid family, which we later identified as the corresponding flavone 3a and the corresponding hydroxyflavone 4a, based on spectral data.

The cyclization of thiazole ortho-hydroxychalcone 1a-0 with mercury(II) acetate in pyridine

The thiazole ortho-hydroxychalcone 1a-0 (1 mmol) was mixed with mercury(II) acetate (1.1 mmol) in 10 - 20 mL pyridine at room temperature. The mixture was refluxed and stirred at 110°C for 10 - 20 h in an oil bath. The cooled reaction mixture was poured into ice cold water and acidified with HCl 10%. The formed yellow precipitate was filtered and purified by column chromatography with different solvents mixtures as mobile phases, as indicated for each individual compound.

(Z)-2-(2-phenylthiazol-4-y1)methylene benzofuran-3(2H)-one (2a): bright-yellow powder, purified by column chromatography (eluent: dichloromethane:acetone = 25:1). Yield = 80%; mp: 169.1 - 170.8°C; Rf = 0.5 (eluent: dichloromethane:acetone = 25:1 v/v). IR: ν(cm⁻¹): 3093.26 (C-H aromatic), 1593.88 (C=O). 1H NMR (600 MHz, CDCl3) δ 8.16 (s, 1H, CH-5 thiazole), 8.02 - 8.00 (m, 2H, CH-2', CH-6'), 7.82 (d, 1H, CH-4, J = 7.6 Hz), 7.68 (t, 1H, CH-6), 7.48 (m, 3H, CH-3', CH-4', CH-5'), 7.34 (d, 1H, CH-7, J = 8.2 Hz), 7.25 (m, 2H, thiazole-CH=C=C, CH-5). 13C NMR (151 MHz, CDCl3) δ 184.3 (C, C=O), 167.8 (C, C-2 thiazole), 166.0 (C, C-8), 149.4 (C, C-2), 147.6 (C, C-4 thiazole), 136.9 (CH, C-6), 133.0 (C, C-1'), 130.5 (CH, C-4'), 129.0 (CH, C-3', C-5'), 126.8 (CH, C-2', C-6'), 124.8 (CH, C-4'), 123.8 (CH, C-5 thiazole), 123.7 (CH, C-5), 121.9 (C, C-9), 112.9 (CH, C-7), 106.4 (CH, thiazole-CH=C=C). ESI-MS: m/z 306.30 (calculated for C9H8N2O5S 306.05 [M+H]+).

(Z)-2-(2-(4-methoxyphenyl)thiazol-4-y1)methylene benzofuran-3(2H)-one (2b): yellow powder, purified by column chromatography (eluent: dichloromethane:acetone = 25:1). Yield = 76%; mp: 212.4 - 213.7°C; Rf = 0.5 (eluent: dichloromethane:acetone = 25:1 v/v). IR: ν(cm⁻¹): 3093.26 (C-H aromatic), 1597.73 (C=O). 1H NMR (600 MHz, CDCl3) δ 8.11 (s, 1H, CH-5 thiazole), 7.96 (d, 2H, CH-2', CH-6', J = 8.9 Hz), 7.84 (d, 1H, CH-4, J = 7.7 Hz), 7.69 (t, 1H, CH-6), 7.35 (d, 1H, CH-7, J = 8.4 Hz). 7.27-7.24 (m, 2H, thiazole-CH=C=C, CH-5), 7.01 (d, 2H, CH-3', CH-5', J = 8.9 Hz), 3.89 (s, 3H, OCH3). 13C NMR (151 MHz, CDCl3) δ 184.3 (C, C=O), 167.6 (C, C-2 thiazole), 165.9 (C, C-8), 161.5 (C, C-2), 149.1 (C, C-4'), 147.5 (C, C-4 thiazole), 136.9 (CH, C-6), 128.3 (CH, C-2', C-6'), 125.9 (C, C-1'), 124.7 (CH, C-4), 123.6 (CH, C-5), 123.1 (CH, C-5 thiazole), 121.9 (C, C-9), 114.4 (CH, CH-3', CH-5'), 112.9 (CH, C-7), 106.5 (CH, thiazole-CH=C=C), 55.4 (C-OCH3). ESI-MS: m/z 336.50 (calculated for C9H8O2N2S 336.06 [M+H]+).

(Z)-2-(2-(4-chlorophenyl)thiazol-4-y1)methylene benzofuran-3(2H)-one (2c): yellow powder, purified by column chromatography (eluent: dichloromethane:acetone = 25:1). Yield = 78%; mp: 197.1 - 198.0°C; Rf = 0.5 (eluent: dichloromethane:acetone = 25:1 v/v). IR: ν(cm⁻¹): 3046.98 (C-H aromatic), 1599.66 (C=O). 1H NMR (600 MHz, CDCl3) δ 8.14 (s, 1H, CH-5 thiazole), 7.92 (d, 2H, CH-2', CH-6', J = 8.2 Hz), 7.81 (d, 1H, CH-4, J = 7.6 Hz), 7.67 (t, 1H, CH-6), 7.44 (d, 2H, CH-3', CH-5', J = 8.2 Hz), 7.32 (d, 1H, CH-7, J = 8.3 Hz), 7.24 (t, 1H, CH-5), 7.20 (s, 1H, thiazole-CH=C=C). 13C NMR (151 MHz, CDCl3) δ 184.2 (C, C=O), 166.3 (C, C-2 thiazole), 165.9 (C, C-8), 149.5 (C, C-2), 147.6 (C, C-4 thiazole), 137.0 (CH, C-6), 136.5 (C, C-1'), 131.5 (C, C-4'), 129.3 (CH, CH-3', C-5'), 127.9 (CH, CH-2', C-6'), 124.7 (CH, CH-4), 123.9 (CH, CH-5 thiazole), 123.7 (CH, C-5), 121.8 (C, C-9), 112.9 (CH, C-7), 106.0 (CH, thiazole-CH=C=C). ESI-MS: m/z 340.7 ([M+H]+), 342.1 ([M+H]++), 342.0 ([M+H]++).
IR: ν(cm⁻¹)

1H NMR (600 MHz, CDCl₃) δ 8.11 (s, 1H, CH₅-thiazole), 8.04 - 8.03 (m, 2H, CH₂-2', CH-6'), 7.67 (d, 1H, CH-4, J = 8.1 Hz), 7.55 (s, 1H, CH-7), 7.51 - 7.50 (m, 3H, CH₃-3', CH₄', CH₅'), 7.38 (d, 1H, CH-5, J = 8.1 Hz), 7.31 (s, 1H, thiazole-CH=C=). 13C NMR (151 MHz, CDCl₃) δ 183.0 (C, C=O), 167.9 (C, C=O), 160.0 (CH, CH-2'), 149.0 (C, C-4 thiazole), 147.5 (C, C-2), 132.9 (C, C-1'), 131.4 (C, C-6), 130.6 (CH, C-4'), 129.1 (CH, C-2', C-6'), 127.4 (CH, C-5), 126.8 (CH, C-3', C-5'), 125.6 (CH, C-4), 124.2 (CH, CH-5 thiazole), 120.9 (C, C-9), 116.6 (CH, C-7), 107.2 (CH, thiazole-CH=C=). ESI⁺-MS: m/z 384.0 [M+H]+, 382.1 ([M+H]+), 379.1 [M+H]+, 376.1 [M+H]+.

(Z)-6-bromo-2-[(2-(4-chlorophenyl)thiazol-4-yl)methylen]-benzofuran-3(2H)-one (2h): orange powder, purified by column chromatography (eluent: dichloromethane:acetone = 25:1). Yield = 78%; mp: 232.1 - 222.8°C; Rf = 0.5 (eluent: dichloromethane:acetone = 25:1 v/v). IR: ν(cm⁻¹): 3115.44 (C-H aromatic), 1596.77 (C=O). 1H NMR (600 MHz, CDCl₃) δ 8.04 (s, 1H, CH-5 thiazole), 7.93 (d, 2H, CH-2', CH-6', J = 8.4 Hz), 7.67 (d, 1H, CH-4, J = 8 Hz), 7.55 (s, 1H, CH-7), 7.38 (d, 1H, CH-5, J = 8 Hz), 7.23 (s, 1H, thiazole-CH=C=), 6.98 (d, 2H, CH₃-3', CH₅', J = 8.4 Hz), 3.87 (s, 3H, OCH₃). 13C NMR (151 MHz, CDCl₃) δ 183.0 (C, C=O), 167.8 (C, C-2 thiazole), 165.9 (C, C-8), 161.6 (C, C-4'), 148.9 (C, CH₂ thiazole), 147.3 (C, C-2), 131.3 (C, C-1'), 128.3 (CH, C-2', C-6'), 127.4 (CH, C-5), 125.9 (C, C-6), 125.6 (CH, C-4), 123.6 (CH, C₅ thiazole), 121 (C, C-9), 116.6 (CH, C-7), 114.4 (CH, C-3', C-5'). 107.4 (CH, thiazole-CH=C=), 55.5 (C-OCH₃). ESI⁺-MS: m/z 414.1 ([M+H]+), 412.1 ([M+H]+), 409.1 ([M+H]+), 405.1 ([M+H]+).

(Z)-6-bromo-2-[(2-(4-chlorophenyl)thiazol-4-yl)methylen]-benzofuran-3(2H)-one (2h): orange powder, purified by column chromatography (eluent: dichloromethane:acetone = 25:1). Yield = 78%; mp: 221.6 - 213.5°C; Rf = 0.5 (eluent: dichloromethane:acetone = 50:1 v/v). IR: ν(cm⁻¹): 3113.51 (C-H aromatic), 1608.34 (C=C=O). 1H NMR (600 MHz, CDCl₃) δ 8.07 (s, 1H, CH-5 thiazole), 7.93 (d, 2H, CH₂-2', CH-6', J = 8.5 Hz), 7.71 (d, 1H, CH-4, J = 9 Hz), 7.44 (d, 2H, CH₂-2', CH₅', J = 8.5 Hz), 7.12 (s, 1H, CH-7), 6.77 - 6.76 (m, 2H, CH₂, thiazole-CH=C=), 3.95 (s, 3H, OCH₃). 13C NMR (151 MHz, CDCl₃) δ 182.5 (C, CH₂-3'), 168.4 (C, CH₂-2'), 167.5 (C, C-8), 166.3 (C, C-6), 149.7 (C, C-4 thiazole), 148.6 (C, C-2'), 136.4 (C, C-1'), 131.6 (C, C-4'), 129.3 (CH, C-3', C-5'), 127.9 (CH, C-2', C-6'), 125.9 (CH, C-4), 123.2 (CH, C₅ thiazole), 115.0 (C, C-9), 112.2 (CH, CH₂-5), 105.0 (CH, C-7), 96.8 (CH, thiazole-CH=C=), 56.1 (C-OCH₃). ESI⁺-MS: m/z 370.1 [M+H]+, 372.1 ([M+H]+, 371.1 ([M+H]+). 35Cl) (calculated for C₁₉H₁₁BrN₂O₃ 370.03 [M+H]+, 372.03 [M+H]+, 371.03 [M+H]+). 35Cl).

References:

(Z)-6-bromo-2-[(2-phenylthiazol-4-yl)methylen]-benzofuran-3(2H)-one (2g): yellow powder, purified by column chromatography (eluent: dichloromethane:acetone = 25:1). Yield = 78%; mp: 220.5 - 221.0°C; Rf = 0.5 (eluent: dichloromethane:acetone = 25:1 v/v). IR: ν(cm⁻¹): 2944.77 (C-H aromatic), 1602.56 (C=O).

(Z)-6-bromo-2-[(2-phenylthiazol-4-yl)methylen]-benzofuran-3(2H)-one (2g): yellow powder, purified by column chromatography (eluent: dichloromethane:acetone = 25:1). Yield = 78%; mp: 220.5 - 221.0°C; Rf = 0.5 (eluent: dichloromethane:acetone = 25:1 v/v).
(Z)-4,6-dimethoxy-2-((2-phenylthiazol-4-yl)methylene)benzofuran-3(2H)-one (2j): pale-yellow powder, purified by column chromatography (eluent: gradient of dichloromethane and dichloromethane:acetone = 25:1). Yield = 72%; mp: 210.3 - 211°C; Rf = 0.5 (eluent: dichloromethane:acetone = 25:1 v/v). IR (v cm⁻¹): 3003.59 (C-H aromatic), 1593.88 (C=C=O). 1H NMR (600 MHz, CDCl₃) δ 8.01 - 7.99 (m, 3H, CH=CH-), 7.49 - 7.47 (m, 3H, CH=CH-), 7.04 - 7.12 (s, 1H, thiazole-CH=C=), 6.94 (d, 2H, CH=CH₂), 6.92 (d, 1H, CH-), 6.59 (s, 3H, OCH₃), 3.97 (s, 3H, OCH₃). 13C NMR (151 MHz, CDCl₃) δ 180.1 (C, C=O), 169.1 (C, C=O), 168.9 (C, C=O), 167.6 (C, C=O), 159.5 (C, C=O), 149.6 (C, C=O), 148.6 (C, C=O), 133.1 (C, C=O), 130.4 (C, C=O), 129.0 (CH, C=O), 126.7 (CH, C=O), 122.5 (CH, C=O), 105.4 (C, C=O), 104.3 (C, C=O), 94.1 (CH, C=O), 89.4 (CH, C=O), 56.2 (OCH₃), 56.1 (OCH₃), 37.0 (S, OCH₃). EI-MS: m/z 366.1 (calculated for C₂₉H₂₂O₆S 366.07 [M+H⁺]).

(Z)-6-methyl-2-((2-phenylthiazol-4-yl)methylene)-6-methylbenzofuran-3(2H)-one (2m): bright-yellow powder, purified by column chromatography (eluent: dichloromethane:acetone = 25:1). Yield = 82%; mp: 200.8 - 201.5°C; Rf = 0.5 (eluent: dichloromethane:acetone = 25:1 v/v). IR (v cm⁻¹): 3128.94 (C-H aromatic), 1593.88 (C=C=O). 1H NMR (600 MHz, CDCl₃) δ 8.10 (s, 1H, CH=CH-), 7.99 - 8.01 (m, 2H, CH=CH₂), 7.68 (d, 1H, CH=CH₂), J = 7.8 Hz), 7.49 - 7.44 (m, 3H, CH=CH-), 7.20 (s, 1H, thiazole-CH=C=), 7.13 (s, 1H, CH=CH), 7.03 (s, 1H, CH=CH), 7.82 (s, 1H, CH=CH), 7.50 (s, 3H, C=O). 13C NMR (151 MHz, CDCl₃) δ 183.8 (C, C=O), 167.7 (C, C=O), 166.5 (C, C=O), 149.5 (C, C=O), 149.2 (C, C=O), 148.0 (C, C=O), 133.0 (C, C=O), 130.5 (C, C=O), 129.1 (C, C=O), 126.7 (CH, C=O), 125.0 (CH, C=O), 124.4 (CH, C=O), 123.8 (CH, C=O), 119.5 (C, C=O), 113.1 (CH, C=O), 105.9 (CH, thiazole-CH=C=), 22.7 (CH₃). EI-MS: m/z 320.1 (calculated for C₂₉H₂₂O₆S 320.07 [M+H⁺]).
length of 544 nm and an emission wavelength of 590 nm. Each assay was done at least twice with six replicates each. The viability was evaluated based on a comparison with untreated cells. IC₅₀ values represent the compound concentrations required to inhibit 50% of cell proliferation and were calculated from a calibration curve. The presented IC₅₀ are expressed as an average value of determinations, where the extreme values are found in a ±10% interval of the resulted average value.

Molecular Docking. The topoisomerases used as targets in the molecular docking study were taken from Protein Data Bank (PDB - www.rcsb.org). Both proteins were isolated from Homo sapiens and their three dimensional structures were obtained after X-ray diffraction. For topoisomerase I (PDB entry code 1SC7) the Cartesian coordinates of the centre of the search space were set to x = 98.714, y = 1.491, z = 10.193, while for topoisomerase II (PDB entry code 4G0U) coordinates of the centre of the search space were set to x = 31.335, y = 90.761, z = 48.009 [40, 43]. The binding sites were identified, thanks to the co-crystallized ligands in the original macromolecule structures from PDB, using AutoDock Tools 1.5.6 [34]. The search space was considered as cube, with the length of the sides x = y = z = 60, for both targets, to ensure similar experimental conditions.

The processing of the files containing the ligands and the targets was performed according to the previous reported protocol [32, 41]. The molecular docking study was carried using AutoDock 4.2 [34] in batch mode using in-house written scripts. 200 docked poses were generated for each ligand. Visualization and analysis of the docking results were performed using UCSF Chimera [36]. The sequence homology analysis of the two proteins was performed using Clustal Omega [38].

Results and Discussion

Chemistry

Data from literature describes various methods for the synthesis of aurones, using ortho-hydroxychalcones as starting material, by an oxidative cyclization reaction with different oxidizing agents: copper(II) acetate [3], hydrogen peroxide [37], selenium dioxide [37] and mercury(II) acetate [13].

The thiazole ortho-hydroxychalcones 1a-o were obtained as previously reported by our group, via a Claisen-Schmidt condensation of thiazole aldehydes with ortho-hydroxyacetophenones in an alkaline environment [4, 8, 31, 39, 45].

In order to choose the optimal method for the synthesis of the desired products 2a-o, an initial screening of methods was performed to obtain compound 2a using as starting material compound 1a. An overview of the results that we obtained applying the methods stated above for the synthesis of compound 2a is presented in Figure 2, which shows that different reaction products were formed. Their structures were elucidated based
on spectral analysis, as presented in the Materials and Methods section.

When copper(II) acetate was used as the oxidizing agent and dimethylsulfoxide (DMSO) as solvent, the formation of both the aurone 2a and the corresponding flavone 3a in an approximate 1:1 molar ratio took place (Figure 2).

Next, when applying the oxidative cyclization of ortho-hydroxychalcones with hydrogen peroxide with alkaline catalysis, we noticed the formation of the corresponding hydroxyflavone 4a (Figure 2). In the literature, this method is reported for the synthesis of aurones or hydroxyflavones [37, 39].

Another oxidizing agent used to convert ortho-hydroxychalcones to flavonoid derivatives mentioned in literature is selenium dioxide [37]. In our case, the cyclization of the thiazole ortho-hydroxychalcones with selenium dioxide leads to hydroxyflavone 4a and flavone 3a in an approximate 1:1 molar ratio (Figure 2). The results of this screening showed that the best method for the synthesis of thiazole aurones is the oxidative cyclization of thiazole ortho-hydroxychalcones with mercury(II) acetate in pyridine (Figure 2). We applied this method for the synthesis of the whole new series of thiazole aurones 2a-o (Figure 3, Table I).

The screening of the oxidative cyclization of 2-phenylthiazole ortho-hydroxychalcone 1a with different oxidizing agents in order to obtain the desired thiazole aurone 2a

The oxidative cyclization of thiazole ortho-hydroxychalcones with mercury(II) acetate in pyridine
Previous studies reported that the oxidative cyclization of ortho-hydroxychalcones with mercury(II) acetate in pyridine leads exclusively to the formation of the thermodynamically more stable Z-aurones [13]. In our case, the NMR spectral data also indicates that the aurones, synthesized by the same procedure, are in the Z-isomeric form, as detailed below. According to the literature data, the chemical shifts of the exocyclic vinylic carbons have chemical shifts below 8 ppm, which is characteristic also for the aromatic region. In the case of the aurones, the vinylic protons appear as a singlet in the aromatic region. The signals corresponding to the hydroxylic proton, thus indicating that this compound was elucidated based on our previous researches regarding the cyclization reaction took place. There is one characteristic signal for the hydroxyl group proton, signal that confirms the formation of the hydroxyflavone 4a, as opposed to that of the corresponding aurone 2a. In addition, in the 13C NMR spectra of the hydroxyflavone 4a, with the exception of one signal characteristic for the carbonyl group, all signals are present in the aromatic area, including the ones corresponding to C-2 and C-3 which are not aromatic.

In the 1H NMR spectra of the unexpected compound 3a there is no characteristic signal for a phenolic hydroxyl proton, thus indicating that this compound is another cyclization product of the ortho-hydroxychalcone 1a, formed in the reaction medium along with the corresponding aurone 2a (Figure 2). The structure of compound 3a was elucidated based on our previous researches regarding the cyclization reaction of thiazole ortho-hydroxychalcones into their...
corresponding flavonoid derivatives, in different reaction conditions [9]. Both 1H NMR and 13C NMR spectral data of compound 3a are entirely found in the spectral characterization of a thiazole flavone previously synthesized by our group in different reaction conditions [9] and this fact determined us to conclude that the reaction product is the flavone 3a.

### Biological activity

The cytotoxicity of novel thiazole aurones 2a-o was evaluated in a panel of nine cancer cell lines including sensitive and drug resistant phenotypes, as well as in normal AML12 hepatocytes. Doxorubicin was used as reference drug, based on the similarity of mechanisms of action with the literature reported flavonoids [6, 20]. Cell lines CEM/ADR5000, MDA-MB-231-BCRP, HCT116 (p53+/−) and U87MG.AEGRF were used as the corresponding resistant counterpart for CCRF-CEM, MDA-MB-231-pcDNA, HCT116 (p53+/−), U87MG respectively. The determined IC50 values in the cytotoxic assay and the degree of resistance or the selectivity index are presented in Tables II-IV. The degree of resistance (DR) was determined as the ratio of IC50 value in the resistant type divided by the IC50 in the sensitive cell line. The selectivity index (SI) was determined as the ratio of IC50 value in the normal AML12 hepatocytes divided by the IC50 in HepG2 hepatocarcinoma cells [33]. Irrelevant data, such as IC50 higher than 50 μM were replaced with hyphen. The related indices which derived from these values were displayed as hyphen as well. The significant cytotoxic effect expressed as low IC50 values were highlighted in bold.

Overall, the highest cytotoxic effect was found against the leukaemia cells. In terms of activity, the results are moderate compared to doxorubicin, on the sensitive phenotype. Against the leukaemia cells doxorubicin-resistant phenotype (IC50 = 66.83 ± 2.20 μM), compound 2a showed the most promising result (IC50 = 5.85 ± 0.46 μM), whereas against the sensitive phenotype, compound 2e showed the best result (IC50 = 13.18 ± 1.03 μM), but still moderate, referred to the positive control (IC50 = 0.02 ± 0.00 μM). For compound 2a, the degree of resistance is low on leukaemia cells (DR = 0.36), this value being the best for the compounds from the current series.

In terms of substitutions on this aurone’s scaffold, compound 2a, the compound with the best cytotoxic activity on the leukaemia resistant cell lines, does not have any substituent on the phenylthiazole system, nor on the benzofuranone ring. The next best cytotoxic activities were also displayed by compounds that don’t have any substituents on the benzofuranone ring, namely, compounds 2b and 2c, compound 2c exhibiting better activity than compound 2b. Compound 2c is substituted on the phenylthiazole system with the chlorine group, an electron withdrawing substituent, whereas compound 2b is substituted on the phenylthiazole system with the methoxy group, an electron donating substituent. These results could indicate that substitution with an electron donating group decreases the cytotoxic activity of the compounds and the substitution with an electron withdrawing group is favourable.

On the breast adenocarcinoma cells, the activity of compounds 2a-o is negligible, with the exception of compound 2i, which exhibited the best activity against the MDA-MB231/BCRP strain (IC50 = 5.43 ± 3.17 μM). Moreover, the activity of this compound against the MDA-MB231/BCRP resistant strain, in comparison to the MDA-MB231 sensitive strain is improved, having the degree of resistance of 0.11.

In the structure of this compound, we can also find chlorine on the phenylthiazole system, as well as bromine, another electron withdrawing group, on the benzofuranone ring.

### Table II

| Samples | Cytotoxicity of thiazole aurones 2a-o and doxorubicin towards the leukaemia cells and the breast adenocarcinoma cells, expressed as IC50 values (μM), with the degree of resistance (DR) |
|---------|---------------------------------------------------------------------------------------------------|
| Leukaemia cells | Breast adenocarcinoma cells |
| CCRF-CEM | CEM/ADR5000 | DR | MDA-MB231 | MDA-MB231/BCRP | DR |
| 2a | 16.36 ± 0.98 | 5.85 ± 0.46 | 0.36 | - | - |
| 2b | 28.79 ± 1.86 | 20.27 ± 1.87 | 0.70 | - | - |
| 2c | 17.74 ± 2.01 | 13.48 ± 0.88 | 0.76 | - | - |
| 2d | 25.89 ± 2.19 | - | - | - | - |
| 2e | 13.18 ± 1.03 | 53.48 ± 3.42 | 1.42 | - | - |
| 2f | 29.62 ± 3.11 | 18.65 ± 1.37 | 0.88 | 36.04 ± 2.15 | - |
| 2g | 28.15 ± 1.56 | 26.03 ± 2.11 | 1.23 | - | - |
| 2h | 16.21 ± 1.24 | 34.60 ± 3.29 | 2.61 | - | - |
| 2i | 21.05 ± 1.95 | 42.33 ± 3.42 | 1.65 | 51.11 ± 3.09 | 5.43 ± 3.17 | 0.11 |
| 2j | - | - | - | - | - |
| 2k | 28.61 ± 1.94 | 44.29 ± 2.84 | 1.55 | - | - |
| 2l | - | - | - | 22.27 ± 1.07 | - |
| 2m | 22.14 ± 1.09 | 25.95 ± 1.18 | 1.17 | - | - |
| 2n | 39.68 ± 2.67 | 41.91 ± 3.65 | 1.06 | - | - |
| 2o | 18.13 ± 1.89 | 33.59 ± 2.36 | 1.85 | - | - |
| Doxorubicin | 0.02 ± 0.00 | 66.83 ± 2.20 | 3341 | 0.07 ± 0.00 | 0.43 ± 0.10 | 6.14 |
The anticancer activity of the novel synthesized compounds is overall more intense on the resistant colon carcinoma cells HCT116(p53+/−) than on the sensitive phenotype HCT116(p53+/+). Compound 2e and 2n showed the best results on the HCT116(p53+/−) resistant phenotype (IC50 = 15.67 ± 1.11 μM and 14.55 ± 1.31 μM, respectively). However, the IC50 values are higher than the one displayed by doxorubicin (IC50 = 0.97 ± 0.02 μM).

These two compounds have a methoxy group on the phenylthiazole system, but they also have electron donating groups on the benzofuranone ring. Compound 2e is substituted with a methoxy group on the benzo-furanone ring, whereas compound 2n is substituted with a methyl group on this moiety. On the glioblastoma cells, compound 2e was the only one from our series which displayed a noticeable activity, yet the IC50 values are not comparable to the ones of the reference drug.

Promisingly, all compounds manifest cytotoxic activity against the normal hepatocytes at high concentrations, reaching a selectivity index value of 5.84 for compound 2e.

| Samples | Colon carcinoma cells | Glioblastoma cells | DR |
|---------|-----------------------|--------------------|----|
|         | HCT116(p53+/−)        | HCT116(p53+/+)     |     |
| 2a      |                      |                    |    |
| 2b      |                      |                    |    |
| 2c      | 36.52 ± 2.48         | -                  |    |
| 2d      | 33.55 ± 2.64         | -                  |    |
| 2e      | 15.67 ± 1.11         | 27.38 ± 1.67       | 42.88 ± 3.77 |
| 2f      |                      |                    |    |
| 2g      | 26.35 ± 1.97         | -                  |    |
| 2h      | 31.84 ± 2.07         | -                  |    |
| 2i      | 38.81 ± 1.30         | -                  |    |
| 2j      |                      |                    |    |
| 2k      |                      |                    |    |
| 2l      | 33.17 ± 1.19         | -                  |    |
| 2m      | 29.08 ± 1.45         | -                  |    |
| 2n      | 14.55 ± 1.31         | -                  |    |
| 2o      | 39.76 ± 2.84         | -                  |    |
| Doxorubicin | 0.26 ± 0.01 | 0.97 ± 0.02 | 3.73 |

Table IV

Cytotoxicity of thiazole aurones 2a-o and doxorubicin towards the hepatocarcinoma cells and the glioblastoma cells, expressed as IC50 values (μM), with the degree of resistance (DR).

| Samples | Hepatic cells | 2a | 2b | 2c | 2d | 2e | 2f | 2g | 2h | 2i | 2j | 2k | 2l | 2m | 2n | 2o | Doxorubicin |
|---------|---------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|            |
|         | HepG2         | -  | -  | > 119.38 | > 117.99 | > 119.38 | 18.75 ± 1.17 | > 109.56 | > 109.56 | > 108.39 | > 104.45 | > 96.86 | > 95.94 | > 104.25 | > 100.24 | > 125.36 | > 114.59 | > 113.30 |
|         | AML12         | 131.13 | 1.17 | > 117.99 | > 119.38 | > 117.99 | > 119.38 | > 109.56 | > 114.59 | > 104.45 | > 96.86 | > 95.94 | > 104.45 | > 119.38 | > 125.36 | > 114.59 | > 113.30 |
|         | SI            | -  | -  | > 119.38 | > 117.99 | > 119.38 | 18.75 ± 1.17 | > 109.56 | > 109.56 | > 108.39 | > 104.45 | > 96.86 | > 95.94 | > 104.25 | > 100.24 | > 125.36 | > 114.59 | > 113.30 |

Molecular Docking

The results of the molecular docking study evaluating the interactions between compounds 2a-o and human topoisoermerases I and II are presented in Table V as binding affinity expressed as the variation of the Gibbs free energy (ΔG), the consequent inhibition constant (K_i) and the energetically analysis and sterically dispersion of the predicted poses from the 2 Å cluster containing the top binding conformation. It is important to notice the amino acids which are in the vicinity of the studied binding site, because they could also influence the binding of compounds 2a-o. In the proximity of the R_1 substituent, there are no amino acids with which it could interact; therefore its presence on the aurone’s moiety does not influence the affinity for the enzyme. It may influence other parameters, such as lipophilicity, penetration or susceptibility to metabolism, but not the affinity for the enzyme.
Table V

Binding affinity of the compounds 2a-o to the catalytic site of topoisomerases I and II expressed as variation of free Gibbs energy (kcal/mol) and inhibition constant (µM). For the best pose of each compound, the analysis of the 2 Å cluster of which it belongs is presented.

| Compound | Topoisomerase I | Topoisomerase II |
|----------|----------------|-----------------|
|          | ΔG (kcal/mol) | Ki (µM) | 2 Å cluster | ΔG (kcal/mol) | Ki (µM) | 2 Å cluster |
|          | Mean | NoC | Mean | NoC |
| 2a       | -7.38 | 3.89 | -7.33 | 31 | -5.87 | 49.80 | -5.76 | 6 |
| 2b       | -6.73 | 11.66 | -6.58 | 16 | -5.81 | 55.11 | -5.72 | 57 |
| 2c       | -7.16 | 5.64 | -6.74 | 28 | -5.75 | 60.98 | -5.59 | 70 |
| 2d       | -7.29 | 4.53 | -6.85 | 3 | -5.41 | 108.25 | -5.30 | 44 |
| 2e       | -6.51 | 16.91 | -6.22 | 5 | -5.65 | 72.19 | -5.65 | 2 |
| 2f       | -7.03 | 7.03 | -6.73 | 38 | -5.66 | 70.98 | -5.53 | 114 |
| 2g       | -6.84 | 9.69 | -6.71 | 26 | -6.00 | 39.99 | -5.97 | 2 |
| 2h       | -6.90 | 8.75 | -6.31 | 12 | -5.97 | 42.07 | -5.83 | 43 |
| 2i       | -7.36 | 4.03 | -6.90 | 20 | -6.01 | 39.32 | -5.88 | 120 |
| 2j       | -7.15 | 5.74 | -7.04 | 4 | -5.47 | 97.82 | -5.30 | 24 |
| 2k       | -6.42 | 19.68 | -6.02 | 12 | -5.57 | 82.63 | -5.47 | 14 |
| 2l       | -6.69 | 12.48 | -6.40 | 12 | -5.47 | 97.82 | -5.32 | 13 |
| 2m       | -7.22 | 5.10 | -7.18 | 10 | -5.81 | 55.11 | -5.64 | 21 |
| 2n       | -6.76 | 11.09 | -6.55 | 5 | -5.99 | 40.67 | -5.86 | 62 |
| 2o       | -7.21 | 5.19 | -7.06 | 23 | -5.91 | 46.55 | -5.79 | 105 |

ΔG = variation of the free Gibbs energy, Ki = inhibition constant, NoC = number of conformations

Figure 4.
Compound 2a (carbon atoms depicted in magenta) bound to the human topoisomerase I

Figure 5.
Compound 2i (carbon atoms depicted in magenta) bound to the human topoisomerase I
The presence of a substituent R$_2$ on the aurone scaffold will determine the repulsion of the respective fragment from the Phe361-Arg362 peptide bridge, leading to a decrease in the binding affinity of a ligand substituted in this manner. This argument can explain why some compounds with a R$_2$ substituent have reduced affinity for the enzyme (compounds 2j-I).

The phenyl fragment is oriented towards a pocket containing multiple polar amino acids (Thr498, Thr501, Ser534), but the distance between them is too big, so interaction with them is impossible, even if the nucleus carries a polar, but small substituent. The development of novel compounds targeting topoisomerase I could be directed to substitution of the phenyl nucleus with polar, but bulkier fragments in order to determine interaction with one of these amino acids with hydroxyl in their structure.

Regarding the interaction of our compounds with topoisomerase II, we can say that the binding affinity of compounds 2a-o is modest, indicating that this enzyme cannot be considered a target for the present series of compounds. Overall, binding affinity of the compounds is ranging between $\Delta G = -6.01$ kcal/mol for compound 2i and $\Delta G = -5.41$ kcal/mol for compound 2d.

The sequence homology analysis of the two topoisomerases revealed just a few conserved residues between the two structures. The 20.09% relative identity is concordant with the literature reports that suggest that topoisomerases have developed from different ancestral enzymes, followed different evolutionary paths and had some lateral gene transfer. This hypothesis could justify the large difference of binding affinity of our compounds for the two topoisomerases [15, 18].

Conclusions

A screening of methods regarding the optimal way to synthesize the new thiazole aurones 2a-o via the oxidative cyclization of ortho-hydroxychalcones was applied. The most convenient protocol is based on using mercury(II) acetate, because by applying this method, we got the highest amounts of desired products and the secondary by-products were found only in traces. The yields for obtaining the wanted thiazole aurones 2a-o varied between 70% and 86%. The structures of all synthesized compounds were confirmed by spectral analysis 1D NMR ($^1$H, $^13$C), 2D NMR (COSY, HMQC), IR and MS. Some of the newly synthesized thiazole aurones showed cytotoxic activity. Compound 2a, the unsubstituted aurone, displayed good anticancer activity on the leukaemia resistant phenotype (CEM/ADR5000) and compound 2i, substituted with chlorine on the phenyl-thiazole system and with bromine on the benzofuranone, displayed good anticancer activity on the breast adenocarcinoma cells, the resistant phenotype (MDA-MB 231/BRC). Compound 2c, which contains two metoxy groups, one on the phenylthiazole system and the other one on the benzofuranone, displayed the broadest cytotoxic spectra, with moderate activities, but presenting a good selectivity index.

Using the molecular docking study, the interactions between our compounds and topoisomerase I and II were evaluated. The evaluation of the results revealed the importance of the thiazole ring for establishing a hydrogen bond with His367 and Arg364. Moreover, the analysis of the docked poses revealed that a substitution on position 6 of the benzofuranone ring is unfavourable in terms of interaction with topoisomerase I. Regarding topoisomerase II, the molecular docking studies revealed that this is not the target of our compounds.

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Conflict of interest

The authors declare no conflict of interest.

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