https://doi.org/10.31925/farmacia.2020.3.15 **ORIGINAL ARTICLE**

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, ^{1}H NMR, ^{13}C 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 topoisomerases 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.

Rezumat

O serie de noi aurone tiazolice **2a-o** au fost sintetizate, caracterizate și evaluate pentru potențialul antiproliferativ. Au fost aplicate diverse metode de ciclizare oxidativă a *orto*-hidroxicalconelor tiazolice, cu diverși agenți oxidanți, și s-a observat faptul că agentul oxidant cel mai eficient pentru obținerea auronelor tiazolice a fost acetatul de mercur(II). Noile aurone tiazolice s-au obținut cu randamente cuprinse între 70% și 86%. Toți compușii sintetizați au fost purificați și caracterizați prin metode spectrale (IR, ^{1}H RMN, ^{13}C RMN și SM). Citotoxicitatea auronelor tiazolice a fost evaluată pe nouă linii celulare canceroase utilizând doxorubicina ca substanță antiproliferativă de referință. Compușii **2a**, **2i** și **2e** au prezentat cele mai bune efecte citotoxice. Studiul interacțiunilor dintre compușii sintetizați și topoizomerazele I și II sugerează faptul că molecula țintă a auronelor este topoizomeraza I, precum și importanța tiazolului în stabilirea unor legături de hidrogen cu His367 și cu Arg364 din această enzimă.

Keywords: thiazole aurone, antiproliferative activity, topoisomerase

Introduction

Cancer is one of the main causes of death world-wide 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, consequentially, 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).

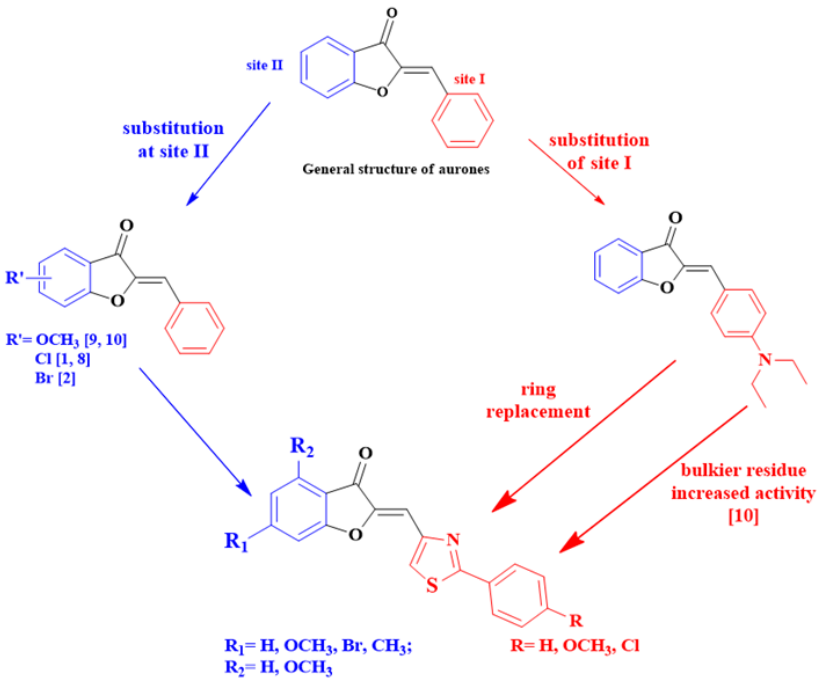


Figure 1.
The design strategy for the target thiazole aurones

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 substituted 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 F_{254}, 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 = 9:1 (v/v) or dichloromethane:acetone = 25:1 (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 ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMQC 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-d_6); 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 Mureș, 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; ^{1}H NMR (600 MHz, $CDCl_3$)s 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); ^{13}C NMR (151 MHz, $CDCl_3$) 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, C-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 $C_{18}H_{14}NO_2S^+$: 308.07).

*The cyclization of thiazole ortho-hydroxychalcone **1a** with hydrogen peroxide in alkaline environment*

A solution of 30% hydrogen peroxide (0.42 mL) was added to a mixture of 2-phenylthiazole *ortho*-hydroxychalcone **1a** (0.6 mmol, 0.19 g), ethanol (1.8 mL) and a 20% aqueous solution of sodium hydroxide (1.8 mL) with continuous stirring at room temperature for 48 h. The reaction mixture was acidified with acetic acid and the solid that was formed was filtered, washed with water and purified by column chromatography. The reaction was monitored by TLC, using dichloromethane:acetone = 25:1 (*v/v*) as the mobile phase. The structural analysis revealed the formation of an

unexpected product, the corresponding hydroxyflavone **4a** as the main product.

*3-Hydroxy-2-(2-phenylthiazol-4-yl)-4H-chromen-4-one (**4a**):* mp: 203 - 204°C; ^{1}H NMR (600 MHz, DMSO) δ 10.17 (s, 1H, hydroxyl), 8.55 (s, 1H, CH-5 thiazole), 8.13 (d, 1H, CH-5 chromen-4-one), 8.07 (d, 2H, CH-2', CH-6'), 7.82 (t, 1H, CH-6 chromen-4-one), 7.76 (d, 1H, CH-8 chromen-4-one), 7.57 (m, 3H, CH-3', CH-4', CH-5'), 7.49 (t, 1H, CH-7 chromen-4-one). ^{13}C NMR (151 MHz, DMSO) δ 172.99 (C, C=O), 167.77 (C, C-2 thiazole), 154.81 (C, C-8a chromen-4-one), 146.91 (C, C-4 thiazole), 141.89 (CH, C-3 chromen-4-one), 139.74 (C, C-1'), 134.36 (CH, C-7 chromen-4-one), 132.77 (CH, C-2 chromen-4-one), 131.37 (CH, C-4'), 129.90 (CH, C-3', C-5'), 126.97 (CH, C-2', C-6'), 125.37 (CH, C-5 thiazole), 125.12 (CH, C-5 chromen-4-one), 124.01 (CH, C-6 chromen-4-one), 122.39 (C, C-4a chromen-4-one), 118.95 (CH, C-8 chromen-4-one). ESI$^+$-MS: *m/z* 322.00 (calculated for $C_{18}H_{12}NO_3S$ 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 aurones, 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-o** with mercury(II) acetate in pyridine*

The thiazole *ortho*-hydroxychalcone **1a-o** (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-yl)methylene)benzofuran-3(2H)-one (**2a**)*: bright-yellow powder, purified by column chromatography (eluent: dichlorometane:acetone = 25:1), Yield = 80%; mp: 169.1 - 170.8°C; R_f = 0.5 (eluent: dichloromethane: acetone = 25:1 *v/v*). IR: ν(cm^{-1}): 3093.26 (C-H aromatic), 1593.88 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) 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-C**H**=C<, CH-5). ^{13}C NMR (151 MHz, $CDCl_3$) 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-**C**H=C<). ESI$^+$-MS: *m/z* 306.30 (calculated for $C_{18}H_{12}NO_2S$ 306.05 $[M+H]^+$).

*(Z)-2-((2-(4-methoxyphenyl)thiazol-4-yl)methylene)-benzofuran-3(2H)-one (**2b**):* yellow powder, purified by column chromatography (eluent: dichlorometane: acetone = 25:1), Yield = 76%; mp:212.4 - 213.7°C; R_f = 0.5 (eluent: dichloromethane:acetone = 25:1 *v/v*). IR: ν(cm^{-1}): 3093.26 (C-H aromatic), 1597.73 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) 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-C**H**=C<, CH-5), 7.01 (d, 2H, CH-3', CH-5', *J* = 8.9 Hz), 3.89 (s, 3H, OC**H**$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) 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-**C**H=C<), 55.4 (C-O**C**H$_3$). ESI$^+$-MS: *m/z* 336.50 (calculated for $C_{19}H_{14}NO_3S$ 336.06 $[M+H]^+$).

*(Z)-2-((2-(4-chlorophenyl)thiazol-4-yl)methylene)-benzofuran-3(2H)-one (**2c**):* yellow powder, purified by column chromatography (eluent: dichlorometane: acetone = 25:1), Yield = 78%; mp: 197.1 - 198.0°C; R_f = 0.5 (eluent: dichloromethane:acetone = 25:1 *v/v*). IR: ν(cm^{-1}): 3046.98 (C-H aromatic), 1599.66 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) 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-C**H**=C<). ^{13}C NMR (151 MHz, $CDCl_3$) 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, C-3', C-5'), 127.9 (CH, C-2', C-6'), 124.7 (CH, C-4), 123.9 (CH, C-5 thiazole), 123.7 (CH, C-5), 121.8 (C, C-9), 112.9 (CH, C-7), 106.0 (CH, thiazole-**C**H=C<). ESI$^+$-MS: *m/z* 340.7 ($[M+H]^+$,^{35}Cl), 342.1 ($[M+H]^+$,^{37}Cl) (calculated for $C_{18}H_{11}ClNO_2S$ 340.01 $[M+H]^+$,^{35}Cl, 342.00 $[M+H]^+$,^{37}Cl).

*(Z)-6-methoxy-2-((2-phenylthiazol-4-yl)methylene)-benzofuran-3(2H)-one (**2d**):* pale-yellow powder, purified by column chromatography (eluent: dichlorometane:acetone = 50:1), Yield = 85%; mp: 190.4 - 191.3°C; R_f = 0.5 (eluent: dichloromethane:acetone = 50:1 *v/v*). IR: ν(cm^{-1}): 3107.72 (C-H aromatic), 1604.48 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09 (s, 1H, CH-5 thiazole), 8.02 - 8.00 (m, 2H, CH-2', CH-6'), 7.72 (d, 1H, CH-4, *J* = 8.2 Hz), 7.49 - 7.47 (m, 3H, CH-3', CH-4', CH-5'), 7.18 (s, 1H, CH-7), 6.79 - 6.77

(m, 2H, CH-5, thiazole-C**H**=C<), 3.95 (s, 3H, OC**H**$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 182.5 (C, C=O), 168.4 (C, C-2 thiazole), 167.7 (C, C-8), 167.5 (C, C-6), 149.5 (C, C-2), 148.5 (C, C-4 thiazole), 133.0 (C, C-1'), 130.5 (CH, C-4'), 129.0 (CH, C-3', C-5'), 126.7 (CH, C-2', C-6'), 125.9 (CH, C-4), 123.1 (CH, C-5 thiazole), 115.0 (C, C-9), 112.2 (CH, C-5), 105.3 (CH, C-7), 96.8 (CH, thiazole-CH=C<), 56.0 (C-O**C**H$_3$). ESI$^+$-MS: *m/z* 336.2 (calculated for $C_{19}H_{13}NO_3S$ 336.07 $[M+H]^+$).

*(Z)-6-methoxy-2-((2-(4-methoxyphenyl)thiazol-4-yl)-methylene)benzofuran-3(2H)-one (**2e**):* pale-orange powder, purified by column chromatography (eluent: dichlorometane:acetone = 25:1), Yield = 70%; mp: 192.0 - 193.4°C; R_f = 0.5 (eluent: dichloromethane: acetone = 25:1 *v/v*). IR: ν(cm^{-1}): 3122.19 (C-H aromatic), 1604.48 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 (s, 1H, CH-5 thiazole), 7.93 (d, 2H, CH-2', CH-6', *J* = 8.6 Hz), 7.70 (d, 1H, CH-4, *J* = 8.4 Hz), 7.13 (s, 1H, CH-7), 6.98 (d, 2H, CH-3', CH-5', *J* = 8.6 Hz), 6.76 - 6.75 (m, 2H, CH-5, thiazole-C**H**=C<), 3.94 (s, 3H, OC**H**$_3$-C-6), 3.88 (s, 3H, OC**H**$_3$-C-4'). ^{13}C NMR (151 MHz, $CDCl_3$) δ 182.5 (C, C=O), 168.4 (C, C-2 thiazole), 167.5 (C, C-8), 167.4 (C, C-6) 161.4 (C, C-2), 149.2 (C, C-4'), 148.4 (C, C-4 thiazole), 128.2 (CH, C-2', C-6'), 126.0 (C, C-1'), 125.8 (CH, C-4), 122.4 (CH, C-5 thiazole), 115.1 (C, C-9), 114.3 (CH, C-3', C-5'), 112.1 (CH, C-5), 105.4 (CH, C-7), 96.8 (CH, thiazole-**C**H=C<), 56.06 (O**C**H$_3$-C-6), 55.43 (O**C**H$_3$–C-4'). ESI$^+$-MS: *m/z* 366.1 (calculated for $C_{20}H_{16}NO_4S$ 366.08 $[M+H]^+$).

*(Z)-2-((2-(4-chlorophenyl)thiazol-4-yl)methylene)-6-methoxybenzofuran-3(2H)-one (**2f**):* pale-yellow powder, purified by column chromatography (eluent: dichlorometane:acetone = 50:1), Yield = 70%; mp: 212.6 - 213.5°C; R_f = 0.5 (eluent: dichloromethane: acetone = 50:1 *v/v*). IR: ν(cm^{-1}): 3113.51 (C-H aromatic), 1608.34 (C=O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 (s, 1H, CH-5 thiazole), 7.93 (d, 2H, CH-2', CH-6', *J* = 8.5 Hz), 7.71 (d, 1H, CH-4, *J* = 9 Hz), 7.44 (d, 2H, CH-2', CH-5', *J* = 8.5 Hz), 7.12 (s, 1H, CH-7), 6.77 - 6.76 (m, 2H, CH-5, thiazole-C**H**=C<), 3.95 (s, 3H, OC**H**$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 182.5 (C, C=O), 168.4 (C, C-2 thiazole), 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, C-5 thiazole), 115.0 (C, C-9), 112.2 (CH, C-5), 105.0 (CH, C-7), 96.8 (CH, thiazole-**C**H=C<), 56.1 (C-O**C**H$_3$). ESI$^+$-MS: *m/z* 370.1 ($[M+H]^+$,^{35}Cl), 372.1 ($[M+H]^+$,^{37}Cl) (calculated for $C_{19}H_{13}ClNO_3S$ 370.03 $[M+H]^+$,^{35}Cl, 372.03 $[M+H]^+$,^{37}Cl).

*(Z)-6-bromo-2-((2-phenylthiazol-4-yl)methylene)-benzofuran-3(2H)-one (**2g**):* yellow powder, purified by column chromatography (eluent: dichlorometane: acetone = 25:1), Yield = 78%; mp: 220.5 - 221.0°C; R_f = 0.5 (eluent: dichloromethane:acetone = 25:1 *v/v*). IR: ν(cm^{-1}): 2944.77 (C-H aromatic), 1602.56 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (s, 1H, CH-5 thiazole), 8.04 - 8.03 (m, 2H, CH-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, CH-3', CH-4', CH-5'), 7.38 (d, 1H, CH-5, *J* = 8.1 Hz), 7.31 (s, 1H, thiazole-C**H**=C<). ^{13}C NMR (151 MHz, $CDCl_3$) δ 183.0 (C, C=O), 167.9 (C, C-2 thiazole), 166.0 (C, C-8), 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, C-5 thiazole), 120.9 (C, C-9), 116.6 (CH, C-7), 107.2 (CH, thiazole-**C**H=C<). ESI$^+$-MS: *m/z* 384.0 ($[M+H]^+$,^{81}Br), 382.1 ($[M+H]^+$, ^{79}Br) (calculated for $C_{18}H_{11}BrNO_2S$ 383.96 $[M+H]^+$,^{81}Br, 381.99 $[M+H]^+$, ^{79}Br).

*(Z)-6-bromo-2-((2-(4-methoxyphenyl)thiazol-4-yl)-methylene)benzofuran-3(2H)-one (**2h**):* bright-yellow powder, purified by column chromatography (eluent: dichlorometane:acetone = 25:1), Yield = 86%; mp: 222.1 - 222.8°C; R_f = 0.5 (eluent: dichloromethane: acetone = 25:1 *v/v*). IR: ν(cm^{-1}): 3115.44 (C-H aromatic), 1596.77 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) 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-C**H**=C<), 6.98 (d, 2H, CH-3', CH-5', *J* = 8.4 Hz), 3.87 (s, 3H, OC**H**$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) 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, C-4 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, C-5 thiazole), 121 (C, C-9), 116.6 (CH, C-7), 114.4 (CH, C-3', C-5'), 107.4 (CH, thiazole-**C**H=C<), 55.5 (C-O**C**H$_3$). ESI$^+$-MS: *m/z* 414.1 ($[M+H]^+$,^{81}Br), 412.1 ($[M+H]^+$, ^{79}Br) (calculated for $C_{19}H_{13}BrNO_3S$ 413.97 $[M+H]^+$,^{81}Br, 411.99 $[M+H]^+$,^{79}Br).

*(Z)-6-bromo-2-((2-(4-chlorophenyl)thiazol-4-yl)-methylene)benzofuran-3(2H)-one (**2i**):* orange powder, purified by column chromatography (eluent: dichlorometane:acetone = 25:1), Yield = 71%; mp: 238.8 - 239.8°C; R_f = 0.5 (eluent: dichloromethane:acetone = 25:1 *v/v*). IR: ν(cm^{-1}): 3094.23 (C-H aromatic), 1599.66 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (s, 1H, CH-5 thiazole), 7.93 (d, 2H, CH-2', CH-6', *J* = 8.1 Hz), 7.67 (d, 1H, CH-4, *J* = 8 Hz), 7.55 (s, 1H, CH-7), 7.44 (d, 2H, CH-3', CH-5', *J* = 8.1 Hz), 7.39 (d, 1H, CH-5, *J* = 8 Hz), 7.22 (s, 1H, thiazole-C**H**=C<). ^{13}C NMR (151 MHz, $CDCl_3$) δ 182.9 (C, C=O), 166.6 (C, C-2 thiazole), 166 (C, C-8), 149.2 (C, C-4 thiazole), 147.5 (C, C-2), 136.6 (C, C-4'), 131.5 (C, C-1'), 134.4 (C, C-6), 129.3 (CH, C-2', C-6'), 127.9 (CH, C-3', C-5'), 127.5 (CH, C-5), 125.6 (CH, C-4), 124.3 (CH, C-5 thiazole), 120.9 (C, C-9), 116.6 (CH, C-7), 106.9 (CH, thiazole-**C**H=C<). ESI$^+$-MS: *m/z* 418.0 ($[M+H]^+$,^{35}Cl, ^{79}Br), 420.0 ($[M+H]^+$,^{35}Cl, ^{81}Br and ^{37}Cl, ^{79}Br), 422.0 ($[M+H]^+$,^{37}Cl, ^{81}Br), (calculated for $C_{18}H_{10}BrClNO_2S$ 417.93 $[M+H]^+$,^{35}Cl, ^{79}Br, 419.93 $[M+H]^+$,^{35}Cl, ^{81}Br and ^{37}Cl, ^{79}Br, 421.93 $[M+H]^+$,^{37}Cl, ^{81}Br,).

*(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 dichlorometane and dichlorometane:acetone = 25:1), Yield = 72%; mp: 210.3 - 211°C; R_f = 0.5 (eluent: dichloromethane:acetone = 25:1 *v/v*). IR: ν(cm^{-1}): 3003.59 (C-H aromatic), 1593.88 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.01 - 7.99 (m, 3H, CH-5 thiazole, CH-2', CH-6'), 7.49 - 7.47 (m, 3H, CH-3', CH-4', CH-5'), 7.10 (s, 1H, thiazole-CH=C<), 6.38 (s, 1H, CH-7), 6.13 (s, 1H, CH-5), 3.94 (s, 3H, OC**H**$_3$-C-4), 3.91 (s, 3H, OC**H**$_3$-C-6). ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.1 (C, C=O), 169.1 (C, C-6), 168.9 (C, C-4), 167.6 (C, C-2 thiazole), 159.5 (C, C-8), 149.6 (C, C-4 thiazole), 148.6 (C, C-2), 133.1 (C, C-1'), 130.4 (CH, C-4'), 129.0 (CH, C-3', C-5'), 126.7 (CH, C-2', C-6'), 122.5 (CH, C-5 thiazole), 105.4 (C, C-9), 104.3 (CH, thiazole-**C**H=C<), 94.1 (CH, C-5), 89.4 (CH, C-7), 56.2 (O**C**H$_3$-C-4), 56.1 (O**C**H$_3$-C-6). ESI$^+$-MS: *m/z* 366.1 (calculated for $C_{20}H_{16}NO_4S$ 366.07 [M+H]$^+$).

*(Z)-4,6-dimethoxy-2-((2-(4-methoxyphenyl)thiazol-4-yl)methylene)benzofuran-3(2H)-one (**2k**):* yellow powder, purified by column chromatography (eluent: gradient of dichlorometane:acetone = 25:1 and dichlorometane:acetone = 9:1), Yield = 74%; mp: 214.6 - 215.3°C; R_f = 0.5 (eluent: dichloromethane: acetone = 25:1 *v/v*). IR: ν(cm^{-1}): 2989.12 (C-H aromatic), 1593.88 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 - 7.92 (m, 3H, CH-2 thiazole, CH-2', CH-6'), 7.07 (s, 1H, thiazole-C**H**=C<), 6.96 (d, 2H, CH-3', CH-5', *J* = 8.8 Hz), 6.37 (s, 1H, CH-7), 6.12 (s, 1H, CH-5), 3.94 (s, 3H, OC**H**$_3$-C-4), 3.91 (s, 3H, OC**H**$_3$-C-6), 3.86 (s, 3H, OC**H**$_3$-C-4'). ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.2 (C, C=O), 169.0 (C, C-6), 168.9 (C, C-4), 167.4 (C, C-2 thiazole), 161.4 (C, C-4'), 159.4 (C, C-8), 149.3 (C, C-4 thiazole), 148.5 (C, C-2), 128.2 (CH, C-2', C-6'), 126.1 (C, C-1'), 121.8 (CH, C-5 thiazole), 114.3 (CH, C-3', C-5'), 105.4 (C, C-9), 104.4 (CH, thiazole-**C**H=C<), 94.1 (CH, C-5), 89.4 (CH, C-7), 56.2 (O**C**H$_3$-C-4), 56.1 (O**C**H$_3$-C-6), 55,4 (O**C**H$_3$-C-4'). ESI$^+$-MS: *m/z* 396.1 (calculated for $C_{21}H_{18}NO_5S$ 396.08 [M+H]$^+$).

*(Z)-2-((2-(4-chlorophenyl)thiazol-4-yl)methylene)-4,6-dimethoxybenzofuran-3(2H)-one (**2l**):* pale-yellow powder, purified by column chromatography (eluent: gradient of dichlorometane and dichlorometane:acetone = 25:1), Yield = 80%; mp: 234.3 - 235.8°C; R_f = 0.5 (eluent: dichloromethane:acetone = 25:1 *v/v*). IR: ν(cm^{-1}): 3015.16 (C-H aromatic), 1590.99 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.00 (s, 1H, CH-5 thiazole), 7.92 (d, 2H, CH-2', CH-6', *J* = 8.3 Hz), 7.43 (d, 2H, CH-3', CH-5', *J* = 8.3 Hz), 7.08 (s, 1H, thiazole-C**H**=C<), 6.39 (s, 1H, CH-7), 6.15 (s, 1H, CH-5), 3.96 (s, 3H, OC**H**$_3$-C-4), 3.92 (s, 3H, OC**H**$_3$-C-6). ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.1 (C, C=O), 169.1 (C, C-6), 168.9 (C, C-4), 166.2 (C, C-2 thiazole), 159.6 (C, C-8), 149.8 (C, C-4 thiazole), 148.7 (C, C-2), 136.4 (C, C-4'), 131.6 (C, C-1'), 129.3 (CH, C-3', C-5'), 127.9 (CH, C-2', C-6'), 122.6 (CH, C-5 thiazole), 105.4 (C, C-9), 104.0 (CH, thiazole-**C**H=C<), 94.2 (CH, C-5), 89.5 (CH, C-7), 56.3 (O**C**H$_3$-C-4), 56.2 (O**C**H$_3$-C-6). ESI$^+$-MS: *m/z* 400.00 ([M+H]$^+$,^{35}Cl), 402.00 ([M+H]$^+$,^{37}Cl) (calculated for $C_{20}H_{15}ClNO_4S$ 400.03 [M+H]$^+$,^{35}Cl, 402.03 [M+H]$^+$, ^{37}Cl).

*(Z)-6-methyl-2-((2-phenylthiazol-4-yl)methylene)-benzofuran-3(2H)-one (**2m**):* bright-yellow powder, purified by column chromatography (eluent: dichloro-metane:acetone = 25:1), Yield = 82%; mp: 200.8 - 201.5°C; R_f = 0.5 (eluent: dichloromethane:acetone = 25:1 *v/v*). IR: ν(cm^{-1}): 3128.94 (C-H aromatic), 1593.88 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.10 (s, 1H, CH-5 thiazole), 7.99 - 8.01 (m, 2H, CH-2', CH-6'), 7.68 (d, 1H, CH-4, *J* = 7.8 Hz), 7.49 - 7.44 (m, 3H, CH-3', CH-4', CH-5'), 7.20 (s, 1H, thiazole-C**H**=C<), 7.13 (s, 1H, CH-7), 7.03 (d, 1H, CH-5, *J* = 7.8 Hz), 2.50 (s, 3H, C**H**$_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 183.8 (C, C=O), 167.7 (C, C-2 thiazole), 166.5 (C, C-8), 149.5 (C, C-4 thiazole), 149.2 (C, C-2), 148.0 (C, C-6), 133.0 (C, C-1'), 130.5 (CH, C-4'), 129.1 (CH, C-3', C-5'), 126.7 (CH, C-2', C-6'), 125.0 (CH, C-5), 124.4 (CH, C-4), 123.4 (CH, C-5 thiazole), 119.5 (C, C-9), 113.1 (CH, C-7), 105.9 (CH, thiazole-**C**H=C<), 22.7 (**C**H$_3$). ESI$^+$-MS: *m/z* 320.1 (calculated for $C_{19}H_{14}NO_2S$ 320.07 [M+H]$^+$).

*(Z)-2-((2-(4-methoxyphenyl)thiazol-4-yl)methylene)-6-methylbenzofuran-3(2H)-one (**2n**):* yellow powder, purified by column chromatography (eluent: dichloro-metane:acetone = 25:1), Yield = 80%; mp: 204.0 - 204.9°C; R_f = 0.5 (eluent: dichloromethane:acetone = 25:1 *v/v*). IR: ν(cm^{-1}): 2958.27 (C-H aromatic), 1608.34 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (s, 1H, CH-5 thiazole), 7.94 (d, 2H, CH-2', CH-6', *J* = 8.8 Hz), 7.69 (d, 1H, CH-4, *J* = 7.8 Hz), 7.18 (s, 1H, thiazole-C**H**=C<), 7.13 (s, 1H, CH-7), 7.04 (d, 1H, CH-5, *J* = 7.8 Hz), 6.98 (d, 2H, CH-3', CH-5', *J* = 8.8 Hz), 3.88 (s, 3H, OCH_3), 2.51 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 183.8 (C, C=O), 167.5 (C, C-2 thiazole), 166.5 (C, C-8), 161.4 (C, C-4'), 149.2 (C, C-4 thiazole), 149.0 (C, C-2), 147.9 (C, C-6), 128.2 (CH, C-2', C-6'), 125.9 (C, C-1'), 124.9 (CH, C-5), 124.3 (CH, C-4), 122.7 (CH, C-5 thiazole), 119.5 (C, C-9), 114.3 (CH, C-3', C-5'), 112.9 (CH, C-7), 105.9 (CH, thiazole-**C**H=C<), 55.4 (O**C**H$_3$-C-4'), 22.58 (CH$_3$-C-6). ESI$^+$-MS: *m/z* 350.1 (calculated for $C_{19}H_{14}NO_2S$ 350.08 [M+H]$^+$).

*(Z)-2-((2-(4-chlorophenyl)thiazol-4-yl)methylene)-6-methylbenzofuran-3(2H)-one (**2o**):* pale-orange powder, purified by column chromatography (eluent: dichloro-metane:acetone = 50:1), Yield = 84%; mp: 238.1 - 239.7°C; R_f = 0.5 (eluent: dichloromethane:acetone = 50:1 *v/v*). IR: ν(cm^{-1}): 2921.63 (C-H aromatic), 1593.88 (C=O). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (s, 1H, CH-5 thiazole), 7.95 (d, 2H, CH-2', CH-6', *J* = 8.4 Hz), 7.71 (d, 1H, CH-4, *J* = 7.8 Hz), 7.46 (d, 2H, CH-3', CH-5', *J* = 8.4 Hz), 7.19 (s, 1H, thiazole-C**H**=C<), 7.15 (s, 1H, CH-7), 7.07 (d, 1H, CH-5, *J* = 7.8 Hz),

2.53 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 183.8 (C, C=O), 166.6 (C, C-2 thiazole), 166.3 (C, C-8), 149.7 (C, C-4 thiazole), 149.3 (C, C-2), 148.2 (C, C-6), 136.5 (C, C-1'), 131.6 (C, C-4'), 129.3 (CH, C-3', C-5'), 127.9 (CH, C-2', C-6'), 125.1 (CH, C-5), 124.4 (CH, C-4), 123.5 (CH, C-5 thiazole), 119.5 (C, C-9), 113.1 (CH, C-7), 105.6 (CH, thiazole-**C**H=C<), 22.7 (**C**H_3-C-6). ESI^+-MS: *m/z* 354.01 ($[M+H]^+$,^{35}Cl), 356.01 ($[M+H]^+$,^{37}Cl) (calculated for $C_{19}H_{13}ClNO_2S$ 354.03 $[M+H]^+$,^{35}Cl, 356.03 $[M+H]^+$,^{37}Cl).

Biology

Cell cultures. Cancer cell models tested included both sensitive cells and their resistant counterparts. Their origins were previously reported. They included drug-sensitive CCRF-CEM leukaemia and its multidrug-resistant P-glycoprotein-over-expressing subline CEM/ADR5000 [12, 17, 22], MDA-MB-231-pcDNA3 breast cancer cells and its resistant subline MDA-MB-231-*BCRP* clone 23 [11], HCT116 ($p53^{+/+}$), colon cancer cells and its knockout clone HCT116 ($p53^{-/-}$), U87MG glioblastoma cells and its resistant subline U87MG.-Δ*EGFR* [23-25]. To compare tumour with normal cells, HepG2 liver cancer cells and AML12 normal hepatocytes were used [23-25].

Cytotoxicity Assays. Resazurin reduction assay [35] was performed to assess the cytotoxicity of the thiazole aurones and doxorubicin as the control drug towards various sensitive and drug-resistant cancer cell lines, including the CCRF-CEM and CEM/ADR5000 leukaemia, MDA-MB231 breast cancer cells and its resistant subline MDA-MB231/*BCRP*, HCT116$p53^{+/+}$ colon cancer cells and its resistant subline HCT116$p53^{-/-}$, U87MG glioblastoma cells and its resistant subline U87MG.*ΔEGFR* and HepG2 hepatocarcinoma cells and normal AML12 hepatocytes. The assay is based on the reduction of the indicator dye, resazurin, to the highly fluorescent resorufin by viable cells. Non-viable cells rapidly lose their metabolic capacity to reduce resazurin and, thus, no longer produce fluorescent signals. Briefly, adherent cells were detached by treatment with 0.25% trypsin/EDTA (Invitrogen, Darmstadt Germany) and an aliquot of 1×10^4 cells was placed in each well of a 96-well cell culture plate (Thermo Scientific, Langenselbold, Germany) in a total volume of 200 µL. Cells were allowed to attach overnight and were then treated with different concentrations of compounds. For suspension cells, aliquots of 2×10^4 cells *per* well were seeded in 96-well-plates in a total volume of 100 µL. The studied compound was immediately added in varying concentrations in an additional 100 µL of culture medium to obtain a total volume of 200 µL/well. After 72 h, resazurin (Sigma-Aldrich, Schnelldorf, Germany) (20 µL, 0.01% w/v) in distilled water was added to each well and the plates were incubated at 37°C for 4 h. Fluorescence was measured on an Infinite M2000 ProTM plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength 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. IC_{50} values represent the compound concentrations required to inhibit 50% of cell proliferation and were calculated from a calibration curve. The presented IC_{50} 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).

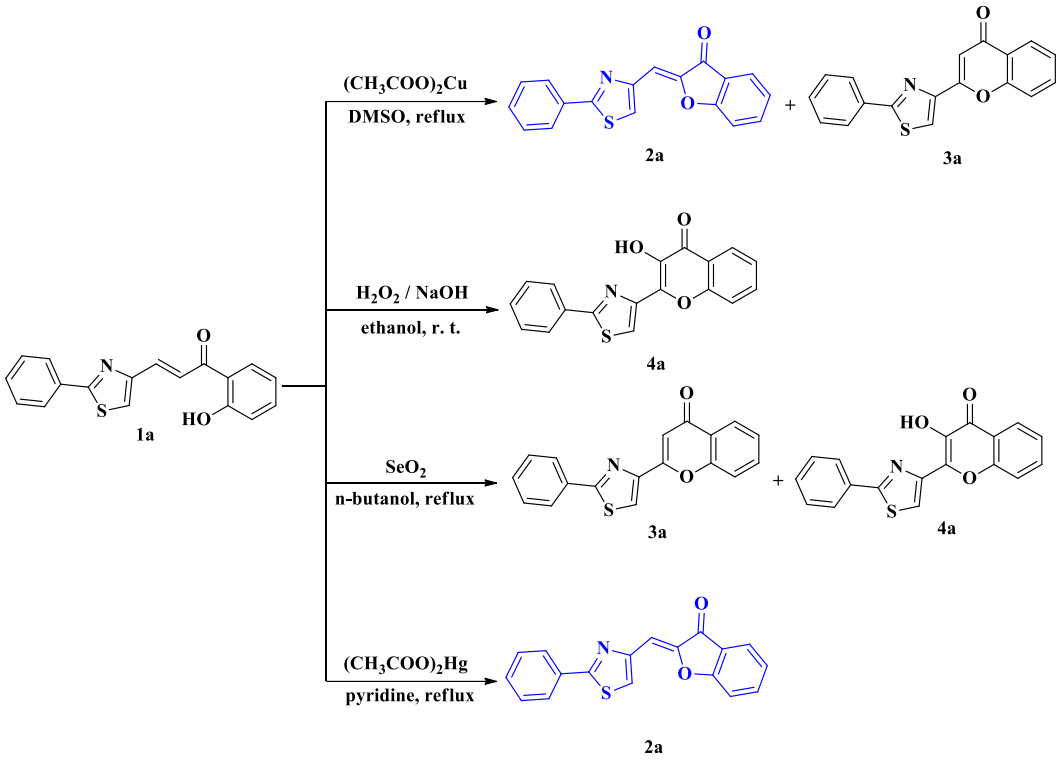
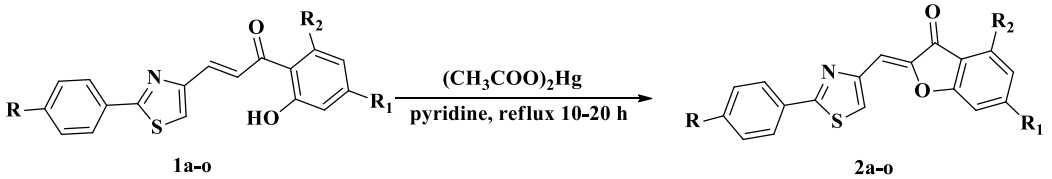


Figure 2.

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**



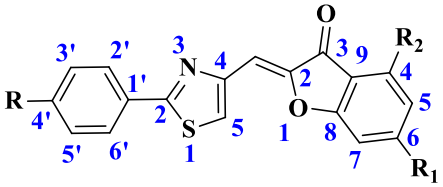
R = H, OCH_3, Cl
R_1= H, OCH_3, Br, CH_3
R_2= H, OCH_3

Figure 3.

The oxidative cyclization of thiazole *ortho*-hydroxychalcones with mercury(II) acetate in pyridine

Table I

The structures and yields of the synthesized compounds **2a-o**



Compound	R	R_1	R_2	Yield (%)
2a	H	H	H	80
2b	OCH_3	H	H	76
2c	Cl	H	H	78
2d	H	OCH_3	H	85
2e	OCH_3	OCH_3	H	70
2f	Cl	OCH_3	H	70
2g	H	Br	H	78
2h	OCH_3	Br	H	86
2i	Cl	Br	H	71
2j	H	OCH_3	OCH_3	72
2k	OCH_3	OCH_3	OCH_3	74
2l	Cl	OCH_3	OCH_3	80
2m	H	CH_3	H	82
2n	OCH_3	CH_3	H	80
2o	Cl	CH_3	H	84

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 are found at 104.0 - 112.8 ppm in the case of *Z*-aurones and respectively 119.8 - 122.2 ppm for *E*-aurones [13]. In our case, in the ^{13}C NMR spectra of the synthesized aurones, all exocyclic vinylic carbons have chemical shifts below 107.4 ppm, which indicates the *Z* configuration. Moreover, in the ^{1}H NMR spectra of aurones, the vinylic protons appear at chemical shifts below 8 ppm, which is characteristic also for the *Z* isomeric forms of aurones [13].

In the ^{1}H NMR spectra of the synthesized thiazole aurones there are characteristic signals for the aromatic, aliphatic and vinylic protons. The proton from the 5^{th} position of the thiazole ring appears as a singlet at 7.99 - 8.77 ppm. One characteristic signal corresponding to the vinylic proton appears as a singlet in the ^{1}H NMR spectra between 6.95 and 7.26 ppm.

The signals corresponding to the protons located on the benzene ring and thiazole ring are present in the aromatic region. In the case of the aurones substituted with methoxy or methyl groups, the corresponding signals of the aliphatic protons are present in the aliphatic area.

In the ^{13}C NMR spectra of the thiazole aurones, a characteristic signal at 180 - 207 ppm indicates the presence of the carbonyl group from the benzofuranone moiety. In the case of thiazole aurones substituted with methoxy or methyl groups, the signals for the aliphatic carbons are present in the aliphatic area of the spectra. All aromatic signals, from both the benzene ring and the thiazole ring, are present in the spectra.

The ESI-MS spectra reveal the presence of molecular ions in the positive ionization mode, $[M+H]^+$, for all the synthesized compounds.

In the FT-IR spectra of the synthesized aurones, the stretching vibration of the carbonyl group appears at 1609 - 1590 cm^{-1}. The bands present in the region 3130 - 2912 cm^{-1} correspond to the stretching vibration of the aromatic and aliphatic C-H bonds.

In the ^{1}H NMR spectra of the unexpected hydroxyl-flavone **4a** the characteristic signals for the vinylic protons that were present in the spectra of the *ortho*-hydroxychalcone are no longer present, because 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 ^{13}C 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 ^{1}H NMR spectra of the unexpected compound **3a** there is no characteristic signal for a phenolic hydroxylic proton, thus indicating that this compound is another cyclization product of the *ortho*-hydroxy-chalcone **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 ^{1}H NMR and ^{13}C 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.Δ*EGFR* were used as the corresponding resistant counterpart for CCRF-CEM, MDA-MB-231-*pcDNA*, HCT116 (*p53*$^{+/+}$), U87MG respectively. The determined IC_{50} 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 IC_{50} value in the resistant type divided by the IC_{50} in the sensitive cell line. The selectivity index (SI) was determined as the ratio of IC_{50} value in the normal AML12 hepatocytes divided by the IC_{50} in HepG2 hepatocarcinoma cells [33]. Irrelevant data, such as IC_{50} higher than 50 μM were replaced with a hyphen. The related indexes which derived from these values were displayed as hyphen as well. The significant cytotoxic effect expressed as low IC_{50} 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 (IC_{50} = 66.83 ± 2.20 μM), compound **2a** showed the most promising result (IC_{50} = 5.85 ± 0.46 μM), whereas against the sensitive phenotype, compound **2e** showed the best result (IC_{50} = 13.18 ± 1.03 μM), but still moderate, referred to the positive control (IC_{50} = 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 (IC_{50} = 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

Cytotoxicity of thiazole aurones **2a-o** and doxorubicin towards the leukaemia cells and the breast adenocarcinoma cells, expressed as IC_{50} values (μM), with the degree of resistance (DR)

Samples	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 (IC_{50} = 15.67 ± 1.11 μM and 14.55 ± 1.31 μM, respectively). However, the IC_{50} values are higher than the one displayed by doxorubicin (IC_{50} = 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 benzofuranone 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 IC_{50} values are not comparable to the ones of the reference drug.

Compound **2e** is also one of the only two compounds from our series that displayed cytotoxic activity against the hepatocarcinoma cells with a IC_{50} value of 18.75 ± 1.17 μM.

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

Table III

Cytotoxicity of thiazole aurones **2a-o** and doxorubicin towards the colon carcinoma cells and the glioblastoma cells, expressed as IC_{50} values (μM), with the degree of resistance (DR)

Samples	Colon carcinoma cells			Glioblastoma cells		
	HCT116*(p53$^{+/+}$)*	HCT116*(p53$^{-/-}$)*	DR	U87MG	U87MG. *ΔEGFR*	DR
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	1.57
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**	**0.14 ± 0.01**	**0.53 ± 0.08**	**3.79**

Table IV

Cytotoxicity of thiazole aurones **2a-o** and doxorubicin towards the hepatocarcinoma cells and the normal hepatocytes, expressed as IC_{50} values (μM), with the selectivity index (SI)

Samples	Hepatic cells		
	HepG2	AML12	SI
2a	-	> 131.13	-
2b	-	> 119.38	> 1.17
2c	-	> 117.99	> 1.35
2d	-	> 119.38	> 1.54
2e	18.75 ± 1.17	> 109.56	> 5.84
2f	38.51 ± 3.22	> 108.39	> 2.81
2g	-	> 104.45	-
2h	-	> 96.86	-
2i	-	> 95.94	> 1.15
2j	-	> 109.57	-
2k	-	> 101.25	> 1.29
2l	-	> 100.24	-
2m	-	> 125.36	> 1.61
2n	-	> 114.59	> 1.08
2o	-	> 113.30	> 1.09
Doxorubicin	**2.15 ± 0.03**	**0.48**	**0.22**

Molecular Docking

The results of the molecular docking study evaluating the interactions between compounds **2a-o** and human topoisomerases 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 (Ki) 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 Mean ΔG (kcal/mol)	NoC	ΔG (kcal/mol)	Ki (μM)	2 Å cluster Mean ΔG (kcal/mol)	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

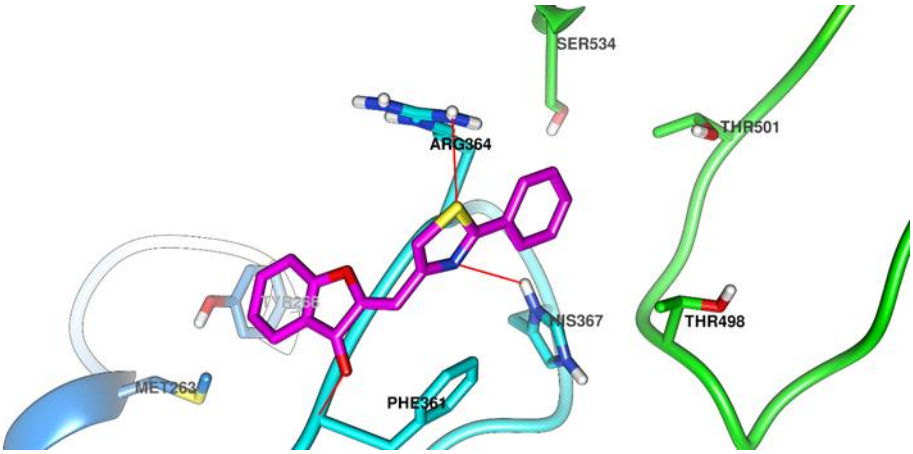


Figure 4.
Compound **2a** (carbon atoms depicted in magenta) bound to the human topoisomerase I

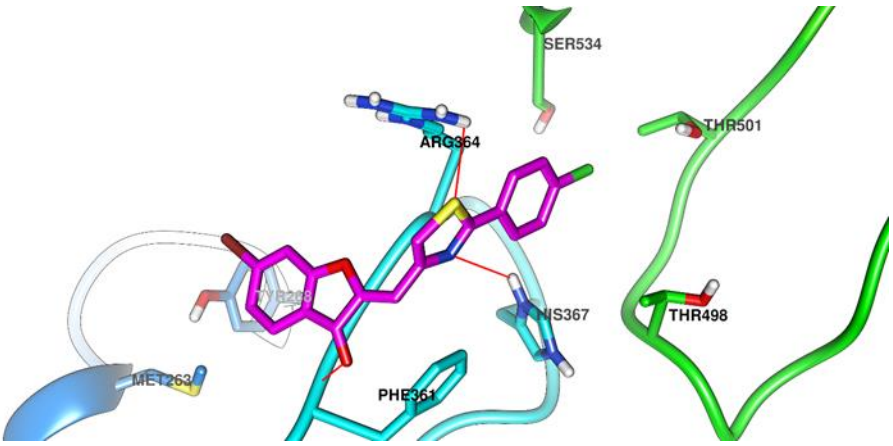


Figure 5.
Compound **2i** (carbon atoms depicted in magenta) bound to the human topoisomerase I

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-l**).

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 ΔG = -6.01 kcal/mol for compound **2i** and ΔG = -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 adeno-carcinoma cells, the resistant phenotype (MDA-MDB 231/BRCP). Compound **2e**, which contains two metoxy groups, one on the phenyltiazole 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 revelled 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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