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ORIGINAL RESEARCH

Heterocycles 44. Synthesis, characterization and anticancer activity of new thiazole *ortho*-hydroxychalcones

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Abstract

A novel series of substituted thiazole *ortho*-hydroxychalcones was synthesized to be physico-chemically characterized and evaluated for the anticancer activity. The chalcones were synthesized with 28–68% yields, via Claisen–Schmidt condensation in an ethanolic solution. All the synthesized compounds were purified and characterized by MS, ^{1}H NMR, ^{13}C NMR, IR, and melting points. The cytotoxicity of thiazole *ortho*-hydroxychalcones **3a**–**3o** as well as doxorubicin was determined in a panel of 9 cancer cell lines including sensitive and drug resistant phenotypes. Compounds **3a**, **3b**, **3c**, **3j**, as well as doxorubicin displayed cytotoxic effects in all the 9 tested cancer cell lines with IC_{50} values below 75 μM. The best samples showed IC_{50} values below 10 μM against 5/9 cancer cell lines for **3a**, **3h**, and **3o**, against 7/9 cancer cell lines for **3c** and **3f**, and against 8/9 cancer cell lines for **3j**. Hypersensitivity of all resistant cells towards **3b**, **3g**, **3j**, **3m**, and **3o** was also obtained, suggesting that these compounds are appropriate molecules that could be used to combat drug resistance of cancer cells.

Keywords Thiazole · *ortho*-hydroxychalcones · Cancer · Cytotoxicity · Hypersensitivity · Multidrug resistance.

Introduction

Chalcones, 1,3-diphenyl-2-propene-1-ones, are natural products widely distributed in fruits, vegetables, spices and tea, bearing a benzylideneacetophenone scaffold where the two aromatic rings are joined by a three carbon α,β-unsaturated carbonyl bridge. It is presumed that the double bond in conjugation with the carbonyl group is responsible for the biological activities of the chalcones, as removal of this functionality makes them inactive (Singh et al. 2014). Basically, chalcones are precursors of flavonoids and isoflavonoids (Mahapatra et al. 2015).

The therapeutic potential of chalcones has been highlighted with studies that revealed their anticancer, antimicrobial, antioxidant, anti-inflammatory, anti-hypertensive, hypnotic, MAO-B inhibitors, and anxiolytic properties (Mahapatra et al. 2015; Ashok et al. 2016; Zainuri et al. 2017; Iqbal et al. 2014; Detsi et al. 2009; Narsinghani et al. 2013; Kantevari et al. 2011).

Many naturally occurring chalcones are substituted with hydroxyl groups on the aryl rings. This fact has raised interest in using these compounds as food preservatives because of their antioxidant properties. At present, their

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biological potential is exploited in the design of new bioactive compounds for therapeutic purposes.

The biological properties of the thiazole ring have been intensively studied in the last years and it was observed that this naturally occurring heterocycle possesses multiple pharmacological activities, such as antibacterial, antitumor, anti-inflammatory, and antifungal properties. The thiazole ring is present in the structure of many drugs and also in the structure of new potential candidates for the treatment of various pathologies (Ayati et al. 2015; Leonte et al. 2017; Turan-Zitouni et al. 2016; Mirza et al. 2017; Abhale et al. 2015; Mayhoub et al. 2011; Yahia et al. 2017).

Several compounds containing 2-phenylthiazole moiety in their structures have been found to possess anticancer, anti-inflammatory, analgesic, antitubercular, and antimicrobial properties (Mayhoub et al. 2011; Yahia et al. 2017; Thore et al. 2016; Aliabadi et al. 2010; Abhale et al. 2017).

The aim of this study was to assemble in the same molecule two pharmacophore units recognized to possess anticancer properties: the α,β-unsaturated ketone and the thiazole ring, in order to obtain new compounds with improved therapeutic potential related to the anticancer activity. Previous studies reported by us revealed that the association of the thiazole ring with the chalcone scaffold is beneficial for the anticancer activity (Awoussong et al. 2015). These results encouraged us to synthesize new *ortho*-hydroxychalcones in order to evaluate their antitumor activity.

Materials and methods

Chemistry

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

Nuclear magnetic resonance spectra, ^{1}H NMR and ^{13}C NMR, of the synthesized compounds were recorded on a Bruker Avance DPX-300 NMR spectrometer operating at 600 and 150 MHz, in different deuterated solvents (chloroform-D3, dimethylsulfoxide-D6) and tetramethylsilane as the internal standard; the chemical shifts are expressed in δ ppm.

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

The FT-IR analysis was performed on a 460 Plus spectrometer (Jasco) 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 and 400 cm^{-1} wavelengths at 4 cm^{-1} resolution.

Mass spectra were recorded on Agilent 1100 Ion Trap mass spectrometer operating at 60 eV.

The reactions were monitored by thin layer chromatography (TLC) (silica gel, aluminum sheets 60 F_{254}, Merck) using dichloromethane or dichloromethane:acetone = 25:1 (v/v) as mobile phases.

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.

General procedure for the synthesis of *ortho*-hydroxychalcones 3a–3c

To a solution of 2,4-dihydroxyacetophenone (1 mmol) and a substituted 2-aryl-1,3-thiazole-4-carbaldehyde (1 mmol) dissolved in ethanol (10 mL) and prepared in an ice bath was added drop by drop a cold solution of KOH (0.112 g) in water (0.224 mL). The reaction mixture was stirred in the ice bath for 2 h and then it was stirred at room temperature for another 8 h. The reaction was monitored by TLC using dichloromethane:acetone (25:1) as mobile phase. The reaction mixture was poured on water and acidified with HCl (10% aqueous solution). In all cases, a yellow precipitate was formed, which was filtered and washed with water. The compounds were purified by silica gel column chromatography with dichloromethane:acetone (25:1) as mobile phase.

General procedure for the synthesis of *ortho*-hydroxychalcones 3d–3o

To a solution of the appropriate *ortho*-hydroxyacetophenone (1 mmol) and a substituted 2-aryl-1,3-thiazole-4-carbaldehyde (1 mmol) dissolved in ethanol (10 mL) was added drop by drop a solution of KOH (0.056 g) in water (0.112 mL). The reaction mixture was stirred at room temperature for 10–12 h. The reaction was monitored by TLC using dichloromethane, dichloromethane:acetone (25:1) or dichloromethane:acetone (9:1) as mobile phases. The reaction mixture was poured on water and acidified with HCl (10% aqueous solution). In all cases, a yellow or orange precipitate was formed, which was filtered and washed with water. The compounds were purified by silica gel column chromatography with dichloromethane, dichloromethane: acetone (25:1), dichloromethane:acetone (9:1) or gradient mobile phases, as indicated for each compound.

(E)-1-(2,4-dihydroxyphenyl)-3-(2-phenylthiazol-4-yl)prop-2-en-1-one (3a)

Pale-yellow powder, purified by column chromatography (eluent: dichlorometan:acetone = 25:1), Yield = 30%;

mp:180 °C; $R_f = 0.5$ (eluent: dichloromethane:acetone = 25/1 *v/v*) IR: ν (cm^{-1}): 3092.3 (C-H aromatic), 1598.6 (C=O); ^{1}H NMR (600 MHz, DMSO) δ 13.31 (s, 1H, C2-OH), 12.62 (s, 1H, C4-OH), 8.29 (s, 1H, CH-5 thiazole), 8.11–8.00 (m, 4HH, CH-6, CH-3 vinyl, CH-2′, CH-6′), 6.82 (d, $J = 15.0$ Hz, 1H, CH-2 vinyl), 6.59–6.49 (m, 3H, CH-3′, CH-4′, CH-5′), 6.46 (dd, $J = 8.9$, 2.3 Hz, 1H, CH-5), 6.32 (d, $J = 2.3$ Hz, 1H, CH-3). ^{13}C NMR (151 MHz, DMSO) δ 191.36 (C, C=O), 166.92 (C, C-2 thiazole), 165.65 (C, C-2), 164.93 (C, C-4), 152.54 (C, C-4 thiazole), 135.60 (C, C-1′), 133.65 (CH, C-3 vinyl), 132.90 (CH, C-4′), 130.89 (CH, C-6), 129.34 (CH, C-3′, C-5′), 126.63 (CH, C-2′, C-6′), 125.14 (CH, C-5 thiazole), 122.80 (CH, C-2 vinyl), 112.89 (C, C-1), 108.16 (CH, C-5), 102.34 (CH, C-3); ESI$^+$–MS: *m/z* 324.6 (calculated for $C_{18}H_{14}NO_3S$: 324.06 $[M+H]^+$).

(E)-1-(2,4-dihydroxyphenyl)-3-(2-(4-methoxyphenyl)thiazol-4-yl)prop-2-en-1-one (3b)

Pale-yellow powder, purified by column chromatography (eluent: dichlorometan:acetone = 25:1), Yield = 28%; mp:208 °C; $R_f = 0.5$ (eluent: dichloromethane:acetone = 25/1 *v/v*) IR: ν (cm^{-1}): 3343.96 (OH), 3092.3 (C-H aromatic), 2932 (C-H aliphatic), 1595.81 (C=O), 1585.42 (C=C); ^{1}H NMR (600 MHz, DMSO) δ 13.33 (s, 1H, C2-OH), 10.82 (s, 1H, C4-OH), 8.20 (s, 1H, CH-5 thiazole), 8.08–6.94 (m, 4H, CH-6, CH-3 vinyl, CH-2′, CH-6′), 6.69 (d, $J = 15.0$ Hz, 1H, CH-2 vinyl), 6.06 (d, $J = 8.6$ Hz, 2H, CH-3′, CH-5′), 6.46 (dd, $J = 8.8$, 2.2 Hz, 1H, CH-5), 6.32 (d, $J = 2.2$ Hz, 1H, CH-3), 3.83 (s, 3H, OCH_3). ^{13}C NMR (151 MHz, DMSO) δ 191.38 (C, C=O), 166.68 (C, C-2 thiazole), 165.62 (C, C-2), 165.39 (C, C-4), 161.33 (C, C-4′), 152.33 (C, C-4 thiazole), 135.68 (CH, C-3 vinyl), 132.84 (CH, C-6), 128.26 (CH, C-3′, C-5′), 125.35 (C, C-1′), 124.36 (CH, C-5 thiazole), 122.52 (CH, C-2 vinyl), 114.62 (CH, C-2′, C-6′), 113.14 (C, C-1), 108.56 (CH, C-5), 102.61 (CH, C-3), 55.45 (C, OCH_3); ESI$^+$–MS: *m/z* 354.1 (calculated for $C_{19}H_{16}NO_4S$: 354.08 $[M+H]^+$).

(E)-3-(2-(4-chlorophenyl)thiazol-4-yl)-1-(2,4-dihydroxyphenyl)prop-2-en-1-one (3c)

Intense-yellow powder, purified by column chromatography (eluent: dichlorometan:acetone = 25:1), Yield = 32%; mp:215 °C; $R_f = 0.5$ (eluent: dichloromethane:acetone = 25/1 *v/v*) IR: ν (cm^{-1}): 3366.1 (OH), 3095.19 (C-H aromatic), 1589.06 (C=O), 1518.66 (C=C); ^{1}H NMR (600 MHz, DMSO) δ 13.29 (s, 1H, C2-OH), 10.80 (s, 1H, C4-OH), 8.29 (s, 1H, CH-5 thiazole), 8.11–6.98 (m, 4H, CH-6, CH-3 vinyl, CH-2′, CH-6′), 6.80 (d, $J = 14.9$ Hz, 1H, CH-2 vinyl), 6.58 (d, $J = 6.4$ Hz, 2H, CH-3′, CH-5′), 6.45 (d, $J = 8.0$ Hz, 1H, CH-5), 6.31 (s, 1H, CH-3).^{13}C NMR (151 MHz, DMSO) δ 191.10 (C, C=O), 166.36 (C, C-2 thiazole), 165.58 (C, C-2), 165.26 (C, C-4), 152.43 (C, C-4 thiazole), 135.31 (C, C-4′), 135.26 (C, C-1′), 132.61 (CH, C-3 vinyl), 131.20 (CH, C-6), 129.16 (CH, C-3′, C-5′), 128.09 (CH, C-2′, C-6′), 125.19 (CH, C-5 thiazole), 122.63 (CH, C-2 vinyl), 112.92 (C, C-1), 108.39 (CH, C-5), 102.53 (CH, C-3); ESI$^+$–MS: *m/z* 358.1 ($[M+H]^+$,^{35}Cl), 360.1 ($[M+H]^+$,^{37}Cl) (calculated for $C_{18}H_{13}ClNO_3S$: 358.03 $[M+H]^+$, ^{35}Cl, 360.03$[M+H]^+$, ^{37}Cl).

(E)-1-(2-hydroxy-4-methoxyphenyl)-3-(2-phenylthiazol-4-yl) prop-2-en-1-one (3d)

Intense-yellow powder, purified by column chromatography (eluent: dichlorometane), Yield = 62%; mp:130 °C; $R_f =$ 0.5 (eluent: dichloromethane *v*) IR: ν(cm^{-1}): 3100.01 (C-H aromatic), 2960.8 (C-H aliphatic), 1566 (C=O), 1503 (C=C); ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.49 (s, 1H, C2-OH), 8.09–6.98 (m, 3H, CH-3 vinyl, CH-2′, CH-6′), 6.94 (d, $J = 9.0$ Hz, 1H, CH-6), 6.68 (d, $J = 14.9$ Hz, 1H, CH-2 vinyl), 6.51 (s, 1H, CH-5 thiazole), 6.49–6.46 (m, 3H, CH-3′, CH-4′, CH-5′), 6.50 (dd, $J = 8.9$, 2.5 Hz, 1H, CH-5), 6.46 (d, $J = 2.5$ Hz, 1H, CH-3), 3.85 (s, 3H, OCH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.34 (C, C=O), 169.08 (C, C-2 thiazole), 166.80 (C, C-4), 166.42 (C, C-2), 153.21 (C, C-4 thiazole), 135.68 (C, C-1′), 133.19 (CH, C-3 vinyl), 131.83 (CH, C-4′), 130.69 (CH, C-6), 129.16 (CH, C-3′, C-5′), 126.01 (CH, C-2′, C-6′), 123.22 (CH, C-5 thiazole), 123.02 (CH, C-2 vinyl), 114.36 (C, C-1), 106.88 (CH, C-5), 101.09 (CH, C-3), 55.61 (C, OCH_3); ESI$^+$–MS: *m/z* 338.1 (calculated for $C_{19}H_{16}NO_3S$ 338.2 $[M+H]^+$).

(E)-1-(2-hydroxy-4-methoxyphenyl)-3-(2-(4-methoxyphenyl) thiazol-4-yl)prop-2-en-1-one (3e)

Pale-yellow powder, purified by column chromatography (eluent: dichlorometan), Yield = 60%; mp:154 °C; $R_f = 0.5$ (eluent: dichloromethane *v*) IR: ν (cm^{-1}): 3103.86 (C-H aromatic), 1566. 52 (C = O), 1522.52 (C = C); ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.51 (s, 1H, C2-OH), 8.01 (d, $J =$ 14.8 Hz, 1H, CH-3 vinyl), 6.96 (d, $J = 8.6$ Hz, 2H, CH-2′, CH-6′), 6.94 (d, $J = 9.0$ Hz, 1H, CH-6), 6.66 (d, $J =$ 14.8 Hz, 1H, CH-2 vinyl), 6.45 (s, 1H, CH-5 thiazole), 6.99 (d, $J = 8.6$ Hz, 2H, CH-3′, CH-5′), 6.51 (dd, $J = 8.9$, 2.4 Hz, 1H, CH-5), 6.46 (d, $J = 2.4$ Hz, 1H, CH-3), 3.88 (s, 3H, OCH_3, C4′-OCH_3), 3.86 (s, 3H, C4-OCH3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.42 (C, C = O), 168.94 (C, C-2 thiazole), 166.81 (C, C-4), 166.40 (C, C-2), 161.66 (C, C-4′), 153.03 (C, C-4 thiazole), 135.82 (CH, C-3 vinyl), 131.84 (CH, C-6), 128.56 (CH, C-3′, C-5′), 126.16 (C, C-1′), 122.81 (CH, C-5 thiazole), 122.63 (CH, C-2 vinyl), 114.48 (C, C-1), 114.41 (CH, C-3′, C-6′), 106.86 (CH, C-5), 101.10 (C, C-3), 55.63 (C, C-4-OCH_3), 55.59 (C,

C-4′-OCH_3); ESI^+–MS: *m/z* 368.1 (calculated for $C_{20}H_{18}NO_4S$ 368.3 $[M+H]^+$).

(E)-3-(2-(4-chlorophenyl)thiazol-4-yl)-1-(2-hydroxy-4-methoxyphenyl)prop-2-en-1-one (3f)

Intense-yellow powder, purified by column chromatography (eluent: dichlorometane), Yield = 60%; mp:143 °C; $R_f = 0.5$ (eluent: dichloromethane *v*) IR: $\nu(cm^{-1})$: 3106.62 (C-H aromatic), 1566.52 (C = O), 1503.24 (C = C); ^1H NMR (600 MHz, $CDCl_3$) δ 13.46 (s, 1H, C2-OH), 8.06–6.90 (m, 4H, CH-6, CH-3 vinyl, CH-2′, CH-6′), 6.68 (dd, $J = 14.8$, 1.6 Hz, 1H, CH-2 vinyl), 6.53 (s, 1H, CH-5 thiazole), 6.49–6.41 (m, 2H, CH-3′, CH-5′), 6.51 (dd, $J = 8.9$, 2.1 Hz, 1H, CH-5), 6.49 (d, $J = 16.1$ Hz, 1H, CH-3), 3.86 (s, 3H, OCH3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.26 (C, C=O), 166.64 (C, C-2 thiazole), 166.86 (C, C-4), 166.50 (C, C-2), 153.38 (C, C-4 thiazole), 136.84 (C, C-4′), 135.48 (C, C-1′), 132.43 (CH, C-3 vinyl), 131.81 (CH, C-6), 131.62 (CH, C-5 thiazole), 129.43 (CH, C-3′, C-5′), 128.23 (CH, C-2', C-6'), 123.28 (CH, C-2 vinyl), 114.36 (C, C-1), 106.96 (CH, C-5), 101.12 (CH, C-3), 55.66 (C, OCH_3); ESI^+–MS: *m/z* 372.1 ($[M+H]^+$, ^{35}Cl), 374.1 ($[M+H]^+$, ^{37}Cl) (calculated for $C_{19}H_{15}ClNO_3S$ 372.05 $[M+H]^+$, ^{35}Cl, 374.04 $[M+H]^+$, ^{37}Cl).

(E)-1-(4-bromo-2-hydroxyphenyl)-3-(2-phenylthiazol-4-yl) prop-2-en-1-one (3g)

Intense-yellow powder, purified by column chromatography (eluent: petroleum ether:dichlorometane = 1:2), Yield = 68%; mp:163 °C; $R_f = 0.5$ (eluent: petroleum ether:dichlorometane = 1:2 *v/v*) IR: ν (cm^{-1}): 3103.86 (C-H aromatic), 1564.59 (C=O); ^1H NMR (600 MHz, $CDCl_3$) δ 13.01 (s, 1H, C2-OH), 8.06–8.01 (m, 2H, CH-2', CH-6' overlapped with 8.04 (d, $J = 14.6$ Hz, 1H), CH-3 vinyl), 6.88 (d, $J = 8.6$ Hz, 1H, CH-6), 6.84 (d, $J = 14.8$ Hz, 1H, CH-2 vinyl), 6.58 (s, 1H, CH-5 thiazole), 6.52–6.46 (m, 3H, CH-3′, CH-4′, CH-5′), 6.23 (d, $J = 1.8$ Hz, 1H, CH-3), 6.10 (dd, $J = 8.5$, 1.8 Hz, 1H, CH-5). ^{13}C NMR (151 MHz, $CDCl_3$) δ 193.66 (C, C=O), 169.36 (C, C-2 thiazole), 164.16 (C, C-2), 152.91 (C, C-4 thiazole), 136.18 (C, C-1′), 133.08 (CH, C-3 vinyl), 131.12 (CH, C-4′), 131.03 (C, C-4), 130.96 (CH, C-6), 129.24 (CH, C-3′, C-5′), 126.06 (CH, C-2′, C-6′), 124.28 (CH, C-5 thiazole), 122.55 (C, C-1), 122.38 (CH, C-5), 121.84 (CH, C-3), 119.13 (CH, C-2 vinyl). ESI^+–MS: *m/z* 388.0 ($[M+H]^+$,^{81}Br), 386.1 ($[M+H]^+$, ^{79}Br) (calculated for $C_{18}H_{13}BrNO_2S$ 387.98 $[M+H]^+$,^{81}Br, 385.99 $[M+H]^+$, ^{79}Br).

(E)-1-(4-bromo-2-hydroxyphenyl)-3-(2-(4-methoxyphenyl) thiazol-4-yl)prop-2-en-1-one (3h)

Pale-yellow powder, purified by column chromatography (eluent: petroleum ether:dichlorometane = 1:2), Yield = 68%; mp:140 °C; $R_f = 0.5$ (eluent: petroleum ether: dichlorometane = 1:2 *v/v* IR: ν (cm^{-1}): 3103.86 (C-H aromatic), 1568.81 (C=O); ^1H NMR (600 MHz, $CDCl_3$) δ 13.03 (s, 1H, C2-OH), 8.00 (d, $J = 14.8$ Hz, 1H, CH-3 vinyl), 6.96 (d, $J = 8.6$ Hz, 2H, CH-2′, CH-6′), 6.86 (d, $J = 8.6$ Hz, 1H, CH-6), 6.81 (d, $J = 14.8$ Hz, 1H, CH-2 vinyl), 6.51 (s, 1H, CH-5 thiazole), 6.22 (d, $J = 1.8$ Hz, 1H, CH-3), 6.09 (dd, $J = 8.5$, 1.6 Hz, 1H, CH-5), 6.99 (d, $J = 8.6$ Hz, 2H, CH-3', CH-5'), 3.88 (s, 3H, OCH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 193.68 (C, C=O), 169.16 (C, C-2 thiazole), 164.16 (C, C-2), 161.86 (C, C-4'), 152.60 (C, C-4 thiazole), 136.29 (CH, C-3 vinyl), 131.11 (C, C-4), 130.95 (CH, C-6), 128.61 (CH, C-2′, C-6′), 125.98 (C, C-1′), 123.62 (CH, C-5 thiazole), 122.51 (C, C-1), 122.10 (CH, C-5), 121.81 (CH, C-3), 119.14 (CH, C-2 vinyl), 114.53 (CH, C-3', C-5'), 55.61 (C, OCH_3); ESI^+–MS: *m/z* 418.1 ($[M+H]^+$, ^{81}Br), 416.3 ($[M+H]^+$, ^{79}Br) (calculated for $C_{19}H_{15}BrNO_3S$ 417.99 $[M+H]^+$, ^{81}Br, 416.00 $[M+H]^+$, ^{79}Br).

(E)-1-(4-bromo-2-hydroxyphenyl)-3-(2-(4-chlorophenyl) thiazol-4-yl)prop-2-en-1-one (3i)

Pale-yellow powder, purified by column chromatography (eluent: petroleum ether:dichlorometane = 1:2), Yield = 66%; mp:208 °C; $R_f = 0.5$ (eluent: petroleum ether: dichlorometane = 1:2 *v/v*) IR: ν (cm^{-1}): 3100.96 (C-H aromatic), 1564.59 (C=O); ^1H NMR (600 MHz, Trifluoroacetic acid-D4 and $CDCl_3$ external standard) δ 6.65 (s, 1H, CH-5 thiazole), 6.42 (d, $J = 15.8$ Hz, 1H, CH-3 vinyl), 6.25 (d, $J = 8.5$ Hz, 2H, CH-2', CH-6'), 6.22 (d, $J = 15.8$ Hz, 1H, CH-2 vinyl), 6.12 (d, $J = 8.6$ Hz, 1H, CH-6), 6.99 (d, $J = 8.5$ Hz, 2H, CH-3′, CH-5′), 6.62 (s, 1H, CH-3), 6.55 (d, $J = 8.6$ Hz, 1H, CH-5). ^{13}C NMR (151 MHz, trifluoroacetic acid-D4 and $CDCl_3$ external standard) δ 193.35 (C, C=O), 164.16 (C, C-2 thiazole), 162.45 (C, C-2), 144.05 (C, C-4 thiazole), 143.81 (C, C-4′), 134.15 (C, C-1′), 131.15 (C, C-4), 131.01 (CH, C-3 vinyl), 130.86 (CH, C-6), 129.00 (CH, C-3′, C-5′), 128.86 (CH, C-2′, C-6′), 124.62 (CH, C-5 thiazole), 123.14 (C, C-1), 122.64 (CH, C-5), 121.66 (CH, C-3), 118.10 (CH, C-2 vinyl). ESI^+–MS: *m/z* 424.0 ($[M+H]^+$, ^{37}Cl, ^{81}Br), 422.0 ($[M+H]^+$,^{35}Cl, ^{81}Br and ^{37}Cl, ^{79}Br), 420.0 ($[M+H]^+$, ^{35}Cl, ^{79}Br) (calculated for $C_{18}H_{12}BrClNO_2S$ 423.94 $[M+H]^+$, ^{37}Cl, ^{81}Br, 421.94 $[M+H]^+$,^{35}Cl, ^{81}Br and ^{37}Cl, ^{79}Br, 419.95 $[M+H]^+$, ^{35}Cl, ^{79}Br).

(E)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(2-phenylthiazol-4-yl)prop-2-en-1-one (3j)

Orange-yellow powder, purified by column chromatography (eluent:dichlorometane), Yield = 63%; mp:110 °C; $R_f = 0.5$ (eluent:dichlorometane *v*) IR: ν (cm^{-1}): 3099.05

(C-H aromatic), 1566.88 (C=O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.38 (s, 1H, C2-OH), 8.32 (d, $J = 15.1$ Hz, 1H, CH-3 vinyl), 8.02–6.99 (m, 2H, CH-2', CH-6'), 6.62 (d, $J = 15.1$ Hz, 1H, CH-2 vinyl), 6.48–6.45 (m, 3H, CH-3′, CH-4′, CH-5′), 6.45 (s, 1H, CH-5 thiazole), 6.11 (d, $J = 2.3$ Hz, 1H, CH-5), 5.96 (d, $J = 2.3$ Hz, 1H, CH-3), 3.95 (s, 3H, C6-OCH_3), 3.83 (s, 3H, C4-OCH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.96 (C, C=O), 168.59 (C, C-2 thiazole), 168.56 (C, C-4), 166.46 (C, C-2), 162.81 (C, C-6), 153.90 (C, C-4 thiazole), 134.08 (C, C-1′), 133.42 (CH, C-3 vinyl), 130.60 (CH, C-4′), 130.26 (CH, C-5 thiazole), 129.11 (CH, C-3′, C-5′), 126.85 (CH, C-2′, C-6′), 121.80 (CH, C-2 vinyl), 106.56 (C, C-1), 93.82 (CH, C-3), 91.35 (CH, C-5), 55.96 (C, C-6-OCH_3), 55.61 (C, C-4-OCH_3); ESI$^+$–MS: *m/z* [368.3]$^+$ (calculated for $C_{20}H_{18}NO_4S$ 368.10 [M+H]$^+$).

(E)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(2-(4-methoxyphenyl)thiazol-4-yl)prop-2-en-1-one (3k)

Intense-yellow powder, purified by column chromatography (eluent: gradient dichlorometane followed by dichlorometane:acetone = 25:1), Yield = 65%; mp:139 °C; $R_f = 0.5$ (eluent: dichlorometane:acetone = 25:1 *v/v*) IR: ν (cm^{-1}): 3101.94 (C-H aromatic), 1564.95 (C=O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.40 (s, 1H, C2-OH), 8.30 (d, $J =$ 15.1 Hz, 1H, CH-3 vinyl), 6.94 (d, $J = 8.8$ Hz, 2H, CH-2′, CH-6′), 6.60 (d, $J = 15.1$ Hz, 1H, CH-2 vinyl), 6.96 (d, $J =$ 8.8 Hz, 2H, CH-3′, CH-5′), 6.10 (d, $J = 2.3$ Hz, 1H, CH-5), 5.96 (d, $J = 2.3$ Hz, 1H, CH-3), 3.95 (s, 3H, C6-OCH3), 3.86 (s, 3H, C4′-OCH_3), 3.83 (s, 3H, C4-OCH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.98 (C, C = O), 168.55 (C, C-2 thiazole), 168.43 (C, C-4), 166.40 (C, C-2), 162.69 (C, C-6), 161.58 (C, C-4′), 153.66 (C, C-4 thiazole), 134.25 (CH, C-3 vinyl), 129.96 (C, C-1′), 128.35 (CH, C-2′, C-6′), 126.36 (CH, C-5 thiazole), 121.21 (CH, C-2 vinyl), 114.42 (CH, C-3′, C-5′), 106.55 (C, C-1), 93.81 (CH, C-3), 91.33 (CH, C-5), 55.96 (C, C-6-OCH_3), 55.60 (C, C-4-OCH_3), 55.55 (C, C-4'-OCH_3); ESI$^+$–MS: *m/z* [398.3]$^+$ (calculated for $C_{21}H_{20}NO_5S$ 398.11 [M+H]$^+$).

(E)-3-(2-(4-chlorophenyl)thiazol-4-yl)-1-(2-hydroxy-4,6-dimethoxyphenyl)prop-2-en-1-one (3l)

Intense-yellow powder, purified by column chromatography (eluent: dichlorometane:acetone = 25:1), Yield = 66%; mp:143 °C; $R_f = 0.5$ (eluent: dichlorometane:acetone = 25:1 *v/v*) IR: ν (cm^{-1}): 3095.19 (C-H aromatic), 1562.06 (C=O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.04 (s, 1H, C2-OH), 8.29 (d, $J = 15.1$ Hz, 1H, CH-3 vinyl), 6.93 (d, $J =$ 8.5 Hz, 2H, CH-2', CH-6'), 6.60 (d, $J = 15.1$ Hz, 2H, CH-2 vinyl), 6.45 (s, 1H, CH-5 thiazole), 6.43 (d, $J = 8.5$ Hz, 2H, CH-3′, CH-5′), 6.10 (d, $J = 2.3$ Hz, 1H, CH-5), 5.96 (d, $J = 2.3$ Hz, 1H, CH-3), 3.94 (s, 3H, C6-OCH_3), 3.84 (s, 3H, C4-CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.86 (C, C=O), 168.58 (C, C-2 thiazole), 166.23 (C, C-4), 166.51 (C, C-2), 162.69 (C, C-6), 154.04 (C, C-4 thiazole), 136.56 (C, C-4′), 133.82 (C, C-1′), 131.92 (CH, C-3 vinyl), 130.45 (CH, C-5 thiazole), 129.36 (CH, C-3′, C-5′), 128.02 (CH, C-2′, C-6′), 121.86 (CH, C-2 vinyl), 106.54 (C, C-1), 93.84 (C, C-3), 91.38 (C, C-5), 55.98 (C, C-6-OCH_3), 55.63 (C-C-4-OCH_3); ESI$^+$–MS: *m/z* 402.1 ([M+H]$^+$, ^{35}Cl), 404.1 ([M +H]$^+$, ^{37}Cl) (calculated for $C_{20}H_{17}ClNO_4S$ 402.06 [M+H]$^+$, ^{35}Cl, 404.05[M+H]$^+$, ^{37}Cl).

(E)-1-(2-hydroxy-4-methylphenyl)-3-(2-phenylthiazol-4-yl) prop-2-en-1-one (3m)

Intense-yellow powder, purified by column chromatography (eluent: dichlorometane), Yield = 65%; mp:148 °C; $R_f =$ 0.5 (eluent: dichlorometane *v*) IR: ν (cm^{-1}): 1563.63 (C=O), 1505.16 (C=C); ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.94 (s, 1H, C2-OH), 8.08 (d, $J = 14.9$ Hz, 1H, CH-3 vinyl), 8.05–8.03 (m, 2H, CH-2′, CH-6′), 6.91 (d, $J =$ 8.2 Hz, 1H, CH-6), 6.80 (d, $J = 14.9$ Hz, 1H, CH-2 vinyl), 6.53 (s, 1H, CH-5 thiazole), 6.50–6.46 (m, 3H, CH-3′, CH-4′, CH-5′), 6.84 (s, 1H, CH-3), 6.66 (d, $J = 8.2$ Hz, 1H, CH-5), 2.36 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 193.66 (C, C = O), 169.13 (C, C-2 thiazole), 163.89 (C, C-2), 153.14 (C, C-4 thiazole), 148.33 (C, C-4), 136.18 (C, C-1′), 133.16 (CH, C-3 vinyl), 130.82 (CH, C-4′), 130.09 (CH, C-6), 129.16 (CH, C-3′, C-5′), 126.03 (CH, C-2′, C-6′), 123.48 (CH, C-5 thiazole), 122.95 (C, C-1), 120.34 (CH, C-2 vinyl), 118.66 (CH, C-5), 118.05 (CH, C-3), 22.16 (C, CH_3); ESI$^+$–MS: *m/z* 322.1 calculated for $C_{19}H_{16}NO_2S$ 322.1 [M+H]$^+$).

7-Methyl-2-(2-phenylthiazol-4-yl)-3-((2-phenylthiazol-4-yl) methylene)chroman-4-one (3m')

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (s, 1H, CH-5 thiazole-A), 7.93 (d, $J = 7.7$ Hz, 4H, CH-2, CH-6 phenyl-A), 7.86 (s, 1H, CH vinylene), 7.65 (s, 1H, CH-5 thiazole-B), 7.43–7.32 (m, 7H, CH-3, CH-4, CH-5 phenyl-A and B, CH-5 chroman-4-one), 7.10 (s, 1H, CH-8 chroman-4-one), 6.84 (d, J = 8.0 Hz, 1H, CH-6 chroman-4-one), 6.81 (s, 1H, CH-2 chroman-4-one), 2.31 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 181.91 (C, C=O), 168.60 (C, C-2 thiazole-B), 168.31 (C, C-2 thiazole-A), 159.76 (C, C-8a chroman-4-one), 155.80 (C, C-4 thiazole-B), 152.15 (C, C-7 chroman-4-one), 147.77 (C, C-4 thiazole-A), 133.58 (C, C-1 phenyl-B), 133.02 (C, C-1 phenyl-A), 131.48 (CH, C-4 phenyl-B), 130.66 (CH, C-4 phenyl-A), 130.19 (CH, C-5 thiazole-A), 129.09 (CH, C-3, C-5 phenyl-B), 128.91(CH, C-3, C-5 phenyl-A), 128.63 (C, C-3 chroman-4-one), 127.45 (CH, C-5 chroman-4-one), 126.89 (CH, C-2, C-6 phenyl-B), 126.77 (CH, C-2, C-6 phenyl-A), 125.93 (CH, C-6 chroman-4-one),

123.19 (CH, C vinyl), 119.87 (CH, C-5 thiazole-B), 118.81 (C, C-4a chroman-4-one), 117.40 (CH, C-8 chroman-4-one), 75.02 (CH, C-2 chroman-4-one), 22.10 (C, CH_3). ESI^+–MS: *m/z* 493 (calculated for $C_{29}H_{21}N_2O_2S_2$ 493.1 $[M+H]^+$).

(E)-1-(2-hydroxy-4-methylphenyl)-3-(2-(4-methoxyphenyl) thiazol-4-yl)prop-2-en-1-one (3n)

Pale-yellow powder, purified by column chromatography (eluent: dichlorometane), Yield = 66%; mp:108 °C; R_f = 0.5 (eluent: dichlorometane *v*) IR: ν (cm^{-1}): 3101.94 (C-H aromatic), 1566.49 (C=O), 1523.49 (C=C); ^1H NMR (600 MHz, $CDCl_3$) δ 12.95 (s, 1H, C2-OH), 8.06 (d, J = 14.9 Hz, 1H, CH-3 vinyl), 6.98 (d, J = 8.6 Hz, 2H, CH-2′, CH-6′), 6.91 (d, J = 8.2 Hz, 1H, CH-6), 6.68 (d, J = 14.9 Hz, 1H, CH-2 vinyl), 6.46 (s, 1H, CH-5 thiazole), 6.99 (d, J = 8.6 Hz, 2H, CH-3′, CH-5′), 6.83 (s, 1H, CH-3), 6.66 (d, J = 8.1 Hz, 1H, CH-5), 3.88 (s, 3H, OCH3), 2.36 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 193.61 (C, C=O), 168.99 (C, C-2 thiazole), 163.89 (C, C-2), 161.68 (C, C-4′), 152.93 (C, C-4 thiazole), 148.28 (C, C-4), 136.29 (CH, C-3 vinyl), 130.10 (CH, C-6), 128.59 (CH, C-2′, C-6′), 126.09 (C, C-1′), 122.90 (CH, C-5 thiazole), 122.64 (C, C-1), 120.32 (CH, C-2 vinyl), 118.65 (CH, C-5), 118.06 (CH, C-3), 114.49 (CH, C-3', C-5'), 55.58 (C, OCH_3), 22.16 (C, CH_3); ESI^+–MS: *m/z* 352.1 (calculated for $C_{20}H_{18}NO_3S$ 352.2 $[M+H]^+$).

(E)-3-(2-(4-chlorophenyl)thiazol-4-yl)-1-(2-hydroxy-4-methylphenyl)prop-2-en-1-one (3o)

Intense-yellow powder, purified by column chromatography (eluent: dichlorometane), Yield = 68%; mp:168 °C; R_f = 0.5 (eluent: dichlorometane *v*) IR: ν (cm^{-1}): 3101.94 (C-H aromatic), 1580. 38 (C=O), 1505.16 (C=C); ^1H NMR (600 MHz, $CDCl_3$) δ 12.91 (s, 1H, C2-OH), 8.04 (d, J = 14.9 Hz, 1H, CH-3 vinyl), 6.96 (d, J = 8.5 Hz, 2H, CH-2′, CH-6′), 6.88 (d, J = 8.2 Hz, 1H, CH-6), 6.66 (d, J = 14.9 Hz, 1H, CH-2 vinyl), 6.52 (s, 1H, CH-5 thiazole), 6.45 (d, J = 8.5 Hz, 2H, CH-3′, CH-5′), 6.83 (s, 1H, CH-3), 6.66 (d, J = 8.2 Hz, 1H, CH-5), 2.36 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 193.54 (C, C = O), 166.63 (C, C-2 thiazole), 163.90 (C, C-2), 153.26 (C, C-4 thiazole), 148.41 (C, C-4), 136.82 (C, C-4′), 135.94 (C, C-1'), 131.65 (CH, C-3 vinyl), 130.04 (CH, C-6), 129.40 (CH, C-3′, C-5′), 128.20 (CH, C-2', C-6'), 123.52 (CH, C-5 thiazole), 123.12 (C, C-1), 120.36 (CH, C-2 vinyl), 118.69 (CH, C-5), 118.01 (CH, C-3), 22.18 (C, CH_3); ESI^+–MS: *m/z* 356.1 ($[M+H]^+$, ^{35}Cl), 358.0 ($[M+H]^+$, ^{37}Cl) (calculated for $C_{19}H_{15}ClNO_2S$ 356.1$[M+H]^+$, ^{35}Cl, 358.1 $[M+H]^+$, ^{37}Cl).

Anticancer activity

Cell cultures

In the present study, several cell models including both sensitive and their resistant counterparts were used. Their origins were previously reported. They included drug-sensitive CCRF-CEM leukemia and its multidrug-resistant p-glycoprotein-over-expressing subline CEM/ADR500 (Kimmig et al. 1990; Efferth et al. 2003; Gillet et al. 2004), MDA-MB-231-pcDNA3 breast cancer cells and its resistant subline MDA-MB-231-*BCRP* clone 23 (Doyle et al. 1998), HCT116 ($p53^{+/+}$), colon cancer cells and its knockout clone HCT116 ($p53^{-/-}$), U87MG glioblastoma cells and its resistant subline U87MG.Δ*EGFR* (Kuete et al. 2013a, 2013b, 2013c). To compare tumour with normal cells, HepG2 liver cancer cells and AML12 normal hepatocytes were used (Kuete et al. 2013a, b, c).

Cytotoxicity assays

The resazurin reduction assay (O'Brien et al. 2000) was performed to assess the cytotoxicity of compounds **3a–3o** and doxorubicin as control drug towards various sensitive and drug-resistant cancer cell lines, including the CCRF-CEM and CEM/ADR5000 leukemia, 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.Δ*EGFR* and HepG2 hepatocarcinoma cells and normal AML12 hepatocytes (O'Brien et al. 2000). 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, do not produce fluorescent signals anymore. 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 then were 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 H_2O 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 by linear regression using Microsoft Excel.

Results and discussion

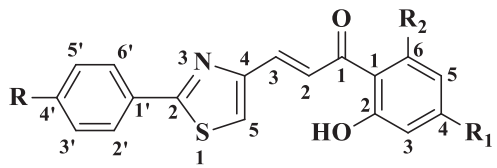
Chemistry

A novel series of *ortho*-hydroxychalcones (Table 1) was synthesized *via* Claisen–Schmidt condensation of equimolar quantity of benzaldehydes (1 equiv) and variously substituted *ortho*-hydroxyacetophenones (1 equiv) in the presence of potassium hydroxide (50% w/v aqueous solution) in an ethanolic solution (Scheme 1) (Awoussong et al. 2015; Mager et al. 1992; Simiti et al. 1991a, 1991b).

If the *ortho*-hydroxyacetophenone was substituted with a single hydroxyl group, 1.66 equiv of KOH were added for 1 equiv of *ortho*-hydroxyacetophenone. If the *ortho*-hydroxyacetophenone was substituted with two hydroxyl groups, the amount of KOH was doubled (3.32 equiv).

The thiazole aldehydes **2a**–**2c** necessary as precursors were synthesized according to a reported method, in two steps. The first step was the Hantzsch condensation of thiobenzamides with 1,3-dichloroacetone, followed by the Sommelet reaction of the obtained compounds (Silberg et al. 1961; Simiti et al. 1991a, b).

Table 1 The structure and yields of the synthesized compounds **3a–o**



Compound	*R*	R_1	R_2	Yield (%)
3a	H	OH	H	30
3b	OCH_3	OH	H	28
3c	Cl	OH	H	32
3d	H	OCH_3	H	62
3e	OCH_3	OCH_3	H	60
3f	Cl	OCH_3	H	60
3g	H	Br	H	68
3h	OCH_3	Br	H	68
3i	Cl	Br	H	66
3j	H	OCH_3	OCH_3	63
3k	OCH_3	OCH_3	OCH_3	65
3l	Cl	OCH_3	OCH_3	66
3m	H	CH_3	H	65
3n	OCH_3	CH_3	H	66
3o	Cl	CH_3	H	68

The synthesized thiazole *ortho*-hydroxychalcones were characterized by mass spectrometry, ^{1}H NMR, ^{13}C NMR, IR, and melting points. The compounds were obtained by this procedure with 28–68% yields.

Based on the reported studies (Ashok et al. 2016; Ragab et al. 2014), the cyclization of chalcones in the presence of different catalysts leads to different products like flavones, flavanones or aurones. Starting from this hypothesis and after observing the formation of different secondary products, in small quantities, in the course of the condensation reaction, we isolated one secondary product **3m′** formed during the Claisen–Schmidt condensation of acetophenone **2e** with thiazole aldehyde **1a** along with the principal product, the thiazole *ortho*-hydroxychalcone **3m**. According to the MS and NMR spectra, the isolated secondary product **3m'** has the structure presented in Fig. 1. We assume that a cyclization of the thiazole *ortho*-hydroxychalcone occurred in the reaction media and the resulted flavanone, possessing an active methylene group, reacted with the thiazole aldehyde **1a** due to the basic reaction conditions.

The structures of the newly synthesized compounds were correlated with the data obtained from mass spectrometry, ^{1}H NMR, ^{13}C NMR, and IR analysis.

In the ^{1}H NMR spectra of the synthesized thiazole *ortho*-hydroxychalcones are present all characteristic signals for the aromatic, aliphatic and vinylic protons. The proton from the 5th position of the thiazole ring appears as a singlet at 8.20–8.29 ppm in case of thiazole chalcones substituted with two hydroxyl groups and at 6.45–6.65 ppm for the other synthesized thiazole chalcones.

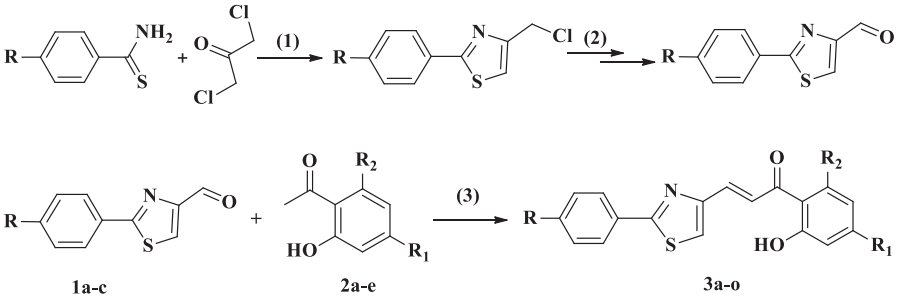
Two characteristic signals corresponding to the vinyl protons are present as doublets (d) in the ^{1}H NMR spectra. The proton located at the second C from the propenone chain appears as a doublet (d) at 6.22–6.94 ppm. The proton located at the third C from the propenone chain appears as a doublet (d) at 6.42–8.32 ppm, in some cases being overlapped with aromatic signals.

All signals corresponding to the protons located on the benzene ring and thiazole ring are present in the aromatic region. In case of the chalcones substituted with methoxyl groups, the corresponding signals of the aliphatic protons are present in the aliphatic area.

The proton from the hydroxyl group is the most deshielded from the spectrum and the signals for this proton appear at chemical shifts between 12.91–14.40 ppm.

The high coupling constant ($J = 14.8$–15.8 Hz) for the two doublets corresponding to the vinylic protons indicates that the chalcones were obtained in the E configuration.

In the ^{1}H NMR spectra of the isolated secondary product, **3m′**, the characteristic signal of the proton from the



Scheme 1 Synthesis of thiazole *ortho*-hydroxychalcones. Reaction conditions: (1) Anhydrous acetone, r.t. 24 h; (2) (**a**) urotropine, chloroform, reflux, 90 min; (**b**) urotropine, acetic acid 50%, reflux, 1 h; (3) KOH 50%, ethanol, r.t. 10–12 h

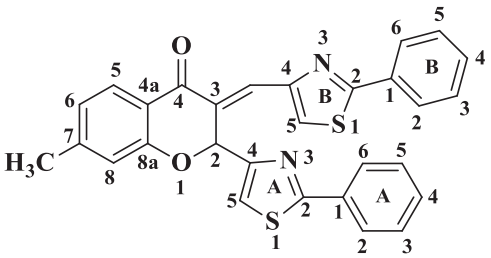


Fig. 1 Isolated secondary product **3m′** formed by the cyclization of the thiazole *ortho*-hydroxychalcone **3m**

hydroxyl group is missing, suggesting that the cyclization of the thiazole *ortho*-hydroxychalcone occurred. The number and the type of the protons, one vinylic proton, one aliphatic proton and different aromatic protons confirm the identity of the secondary product.

In the ^{13}C NMR spectra of the thiazole *ortho*-hydroxychalcones, a characteristic signal at 191–193 ppm indicates the presence of the carbonyl group. In case of the thiazole *ortho*-hydroxychalcones substituted with methoxyl groups, the signals for the aliphatic carbons appear in the aliphatic area of the spectra. All aromatic signals from both benzene rings and the thiazole ring are present, thus confirming the chemical structures of the compounds.

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

In the IR spectra of the synthesized chalcones the stretching vibration of the carbonyl group appears at 1599–1562 cm^{-1}. In some cases, it can also be observed the characteristic band for the C=C bond at 1552–1503 cm^{-1}. The large band between 3100 and 2900 cm^{-1} corresponds to the stretching vibration band of the hydroxyl groups, overlapped with the aromatic C-H bond and aliphatic C-H bond, thus confirming the chemical structures of the compounds.

Anticancer activity

The cytotoxicity of thiazole *ortho*-hydroxychalcones **3a–3o** as well as doxorubicin was determined in a panel of nine cancer cell lines including sensitive and drug resistant phenotypes, as well as in normal AML12 hepatocytes. According to the literature data, the mechanism of action of flavonoids as anticancer agents is based on the action on several targets, including the inhibition of enzymes like tyrosin kinases, aromatases, topoisomerases and glycogen phosphorylases (Ragab et al. 2014). Doxorubicin acts by blocking the topoisomerase II and based on this similar mechanism of action, we chose doxorubicin as the standard compound for the anticancer evaluation.

Results are summarized in Table 2. Compounds **3a**, **3b**, **3c**, **3j**, as well as doxorubicin displayed cytotoxic effects in all the nine tested cancer cell lines with IC_{50} values below 75 µM. Other compounds had selective activities. IC_{50} values ranged from 4.38 (towards CEM/ADR5000 leukemia cells) to 33.68 µM (towards HepG2 hepatocarcinoma cells) for **3a**, from 14.54 (towards CEM/ADR5000 cells) to 73.12µM (towards HepG2 cells) for **3b**, from 3.10 (towards HCT116*(p53$^{-/-}$)* colon carcinoma cells) to 35.29 µM (towards HepG2 cells) for **3c**, from 2.11 (towards HCT116 *(p53$^{-/-}$)* cells) to 58.60 µM (against HepG2 cells) for **3j** and from 0.02 (towards CCRF-CEM cells) to 66.83 µM (towards CEM/ADR5000 cells) for doxorubicin. Significant activity with IC_{50} values 10 µM were obtained for compounds **3a**, **3c–3h**, **3j–3o** in at least two of the nine tested cancer cell lines (Table 2) (Brahemi et al. 2010; Kuete and Efferth 2015). The best samples showed IC_{50} values below 10 µM against 5/9 cancer cell lines for **3a**, **3h** and **3o**, against 7/9 cancer cell lines for **3c** and **3f**, and against 8/9 cancer cell lines for **3j**. This is a clear indication that these compounds are good cytotoxic agents. Apart from **3g** and **3n**, hypersensitivity (degree of resistance or D.R. below 0.90) of CEM/ADR5000 compared to it sensitive parental cell line CCRF-CEM was noted with all other thiazole *ortho*-hydroxychalcones (Table 2) (Mbaveng et al. 2017). This suggests that thiazole *ortho*-hydroxychalcones could be potential inhibitors of P-glycoprotein's expression (Mbaveng et al. 2017). Interestingly, hypersensitivity of all resistant cells towards **3b**, **3g**, **3j**, **3m**, and **3o** was also obtained, suggesting that these compounds are appropriate molecules that could be used to combat drug resistance of cancer cells. It is also important to note that the selectivity indexes of some of the compounds for the normal AML12 hepatocytes versus HepG2 hepatocarcinoma cells are below

Table 2 Cytotoxicity of thiazole *ortho*-hydroxychalcones and doxorubicin towards drug sensitive and resistant cancer cells as determined by resazurin assay

Samples	Cell lines, IC_{50} value in μM and degree of resistance* or selectivity index** (in bracket)									
	CCRF-CEM	CEM/ADR5000	MDA-MB231	MDA-MB231/*BCRP*	HCT116($p53^{+/+}$)	HCT116($p53^{-/-}$)	U87MG	U87MG. Δ*EGFR*	HepG2	AML12
3a	**5.62 ± 0.43**	**4.38 ± 0.20 (0.78)**	**5.34 ± 0.44**	11.58 ± 1.39 (2.17)	**6.19 ± 0.49**	**4.98 ± 0.53 (0.80)**	12.41 ± 1.11	13.94 ± 1.57 (1.12)	33.68 ± 2.85	39.40 ± 4.08
3b	29.71 ± 3.17	14.54 ± 0.96 (0.49)	48.47 ± 5.02	41.83 ± 5.03 (0.86)	21.95 ± 1.73	18.23 ± 2.01 (0.83)	48.23 ± 3.86	27.94 ± 3.16 (0.58)	73.12 ± 5.38	>113.29 (>1.17)
3c	**5.95 ± 0.57**	**4.64 ± 0.27 (0.78)**	**9.34 ± 0.75**	**9.72 ± 1.02 (1.04)**	**7.52 ± 0.55**	**3.10 ± 0.34 (0.41)**	15.62 ± 1.17	**10.08 ± 0.97 (0.65)**	35.29 ± 3.77	44.05 ± 4.98 (1.25)
3d	**89.06 ± 6.88**	**9.26 ± 0.64 (0.10)**	14.12 ± 1.44	14.06 ± 0.98 (1.00)	2.66 ± 0.19	**3.74 ± 0.46 (1.41)**	18.38 ± 1.49	**10.25 ± 1.16 (0.56)**	>118.67	89.79 ± 8.93 (<0.41)
3e	83.03 ± 9.21	**5.09 ± 0.39 (0.06)**	**5.54 ± 0.32**	41.38 ± 3.07 (7.47)	54.06 ± 3.97	**1.76 ± 0.20 (0.03)**	12.76 ± 1.03	40.36 ± 3.29 (3.16)	>108.97	44.87 ± 3.21 (<0.41)
3f	82.32 ± 7.01	**5.34 ± 0.65 (0.06)**	**3.98 ± 0.17**	**10.34 ± 0.88 (2.60)**	**7.82 ± 0.67**	**5.83 ± 0.48 (0.75)**	**9.26 ± 0.75**	**8.22 ± 5.36 (0.89)**	>107.81	55.36 ± 3.75 (<0.52)
3g	**5.10 ± 0.43**	**7.08 ± 0.51 (1.39)**	13.57 ± 0.79	**10.91 ± 1.00 (0.80)**	16.00 ± 0.99	**1.84 ± 0.23 (0.12)**	66.95 ± 4.07	13.16 ± 1.24 (0.20)	>103.90	>103.90
3h	**6.53** ± 0.39	**6.02 ± 0.70 (0.92)**	16.40 ± 1.57	**9.74 ± 0.84**	15.05 ± 1.06	**7.37 ± 0.56 (0.49)**	16.53 ± 1.55	**9.94 ± 1.07 (0.60)**	>96.39	30.48 ± 4.16 (<0.32)
3i	47.49 ± 3.26	41.04 ± 3.09 (0.86)	77.30 ± 5.46	>95.48 (>1.24)	>95.48	19.93 ± 2.07 (<0.21)	>95.48	82.85 ± 6.32 (<0.87)	>98.48	>95.48
3j	**7.14 ± 0.80**	**3.46 ± 0.42 (0.48)**	**4.58 ± 0.33**	**3.57 ± 0.31 (0.78)**	**2.49 ± 0.17**	**2.11 ± 0.36 (0.85)**	**6.43 ± 0.43**	**2.89 ± 0.18 (0.45)**	58.60 ± 4.89	18.01 ± 1.68 (0.31)
3k	**4.26 ± 0.55**	**2.72 ± 0.17 (0.64)**	16.22 ± 1.20	24.89 ± 3.08 (1.53)	48.85 ± 5.02	**1.66 ± 0.19 (0.03)**	76.37 ± 8.17	48.54 ± 5.10 (0.64)	>100.78	83.35 ± 6.73 (<0.39)
3l	**4.06 ± 0.32**	**3.55 ± 0.29 (0.87)**	42.68 ± 2.97	42.50 ± 2.16 (1.00)	42.49 ± 3.72	**1.30 ± 0.24 (0.03)**	41.96 ± 3.20	59.14 ± 4.76 (1.41)	>99.74	38.69 ± 2.88 (0.39)
3m	**10.51 ± 0.87**	**8.74 ± 0.77 (0.83)**	36.27 ± 3.15	16.48 ± 1.43 (0.45)	14.48 ± 1.29	4.36 ± 0.38 (0.30)	85.40 ± 6.15	**8.80 ± 0.58 (0.10)**	>124.58	>124.58
3n	**5.78 ± 0.51**	**6.78 ± 0.69 (1.17)**	17.22 ± 1.86	**199.56 ± 21.79 (11.59)**	11.72 ± 0.77	3.96 ± 0.41 (0.34)	24.71 ± 1.99	**9.27 ± 1.00 (0.38)**	>113.93	48.71 ± 5.12 (0.43)
3o	**8.82 ± 0.63**	**5.52 ±0.38 (0.63)**	16.98 ± 1.32	**8.06 ± 0.65 (0.47)**	13.74 ± 1.25	**1.82 ± 0.30 (0.13)**	21.34 ± 1.64	**8.51 ± 0.74 (0.40)**	>112.66	46.53 ± 3.35 (<0.41)
Doxorubicin	**0.02 ± 0.00**	66.83 ± 2.20 (3341)	**0.07 ±0.00**	**0.43 ± 0.10 (6.14)**	**0.26 ± 0.01**	**0.97 ± 0.02 (3.73)**	**0.14 ± 0.01**	**0.53 ± 0.08 (3.79)**	**2.15 ± 0.03**	**0.48 ± 0.01 (0.22)**

(*): The degree of resistance was determined as the ratio of IC_{50} value in the resistant divided by the IC_{50} in the sensitive cell line; 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 selectivity index was determined as the ratio of IC_{50} value in the normal AML12 hepatocytes divided by the IC_{50} in HepG2 hepatocarcinoma cells. IC_{50} value in bold: Significant cytotoxic effect

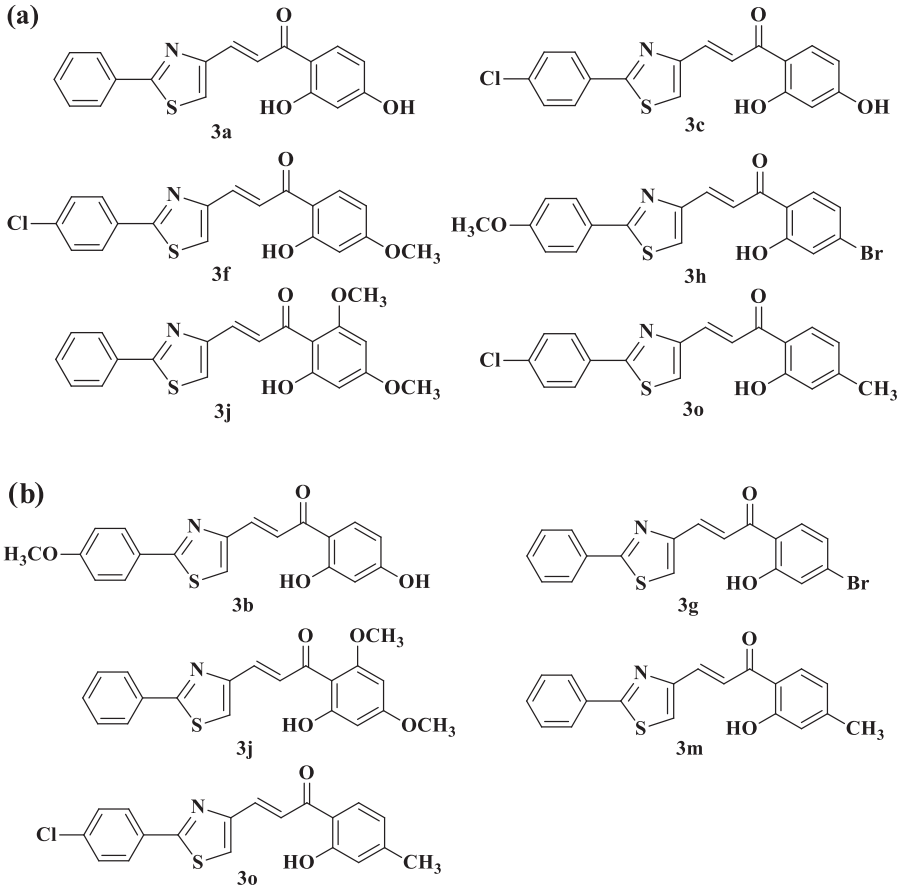


Fig. 2 The structures of the synthesized thiazole *ortho*-hydroxychalcones with the best antiproliferative activity (**a**) compounds with good cytotoxic effects (IC_{50} values below 10 μM) (**b**) compounds that determined hypersensitivity of all resistant cancer cells

1, suggesting their poor selectivity to liver cancer cells; Nonetheless, a keen look of IC_{50} values in AML12 cells versus other cell lines indicate that higher selectivity can be achieved with other cancer types. The overall data highlights the possibility of using the tested synthetics and mostly compounds **3a**, **3c**, **3f, 3h**, and **3j**, and otherwise **3b**, **3g**, **3m**, and **3o** to develop novel drugs to fight drug sensitive and resistant cancers (Fig. 2).

Compounds with two hydroxyl groups on the acetophenone ring (**3a**, **3b**, **3c**) displayed cytotoxic effects in all the nine tested cancer cell lines with IC_{50} values below 75 μm. In the same category of cytotoxic activity, the compound which bears two methoxyl groups on the acetophenone ring (**3j**) is included.

According to the findings, the substituent on the phenylthiazole ring doesn't influence the hypersensitivity of the resistant cells towards the compounds, because compounds containing either methoxyl, chloro, or no substituent on the phenylthiazole ring determined hypersensitivity of the resistant cancer cell lines.

Besides the compounds substituted with a single methoxyl group in the 4th position of the acetophenone ring (**3d**, **3e**, **3f**), the compounds that contained other groups determined hypersensitivity of the resistant cancer cell lines, three of them having no substituent on the phenylthiazole ring (**3g**, **3j**, **3m**).

The most significant cytotoxic activity (IC_{50} values below 10 μm) was obtained for six compounds. In the case of these compounds, the phenylthiazole ring was either not substituted or it was substituted with a chloro or a methoxyl group. The acetophenone ring of these compounds had no substituent in the 6th position, with the exception of compound **3j** which bears a methoxyl group in this position (Fig. 2).

Conclusions

Fifteen new thiazole *ortho*-hydroxychalcones were synthesized by the Claisen-Schmidt condensation between thiazole aldehydes and variously substituted *ortho*-hydroxyacetophenones, in basic media, with 28–68% yields. The reaction conditions for the condensation of thiazole aldehydes and

2,4-dihydroxyacetophenone were optimized, considering the high sensitivity of the compounds due to the presence of two hydroxyl groups on the acetophenone ring. The structures of all synthesized compounds were confirmed by

spectral analysis ^{1}H NMR, ^{13}C NMR, IR, and MS. In general, the synthesized thiazole *ortho*-hydroxychalcones are good antiproliferative agents. Some of the molecules, namely **3a**, **3c**, **3f, 3h** and **3j** as well as **3b**, **3g**, **3m** and **3o** can be used to develop novel cytotoxic drugs to fight drug sensitive and resistant cancers.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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