

HETEROCYCLES 45. SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 3-INDOLYL-1-PYRIDYL-2-PROPENONES AS ANTICANCER AGENTS

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Abstract

A series of seven 3-indolyl-1-pyridyl-2-propenones were synthesized *via* Claisen-Schmidt condensation with 68.8-91.8% yields. All the synthesized compounds were purified and characterized by melting points, IR, ¹H NMR, ¹³C NMR and HRMS. The cytotoxicity of the synthesized 3-indolyl-1-pyridyl-2-propenones **1-7** and doxorubicin, used as positive control, was determined in a panel of nine human cancer cell lines including both sensitive and drug-resistant phenotypes, as well as in normal AML12 hepatocytes. Compounds **3**, **4** and **7** displayed half maximal inhibitory concentration (IC₅₀) values below 100 μM in all tested cancer cell lines meanwhile, other compounds displayed selective activities.

Rezumat

O serie de 3-indolil-1-piridil-2-propenone **1-7** au fost sintetizate prin reacția de condensare Claisen-Schmidt cu randamente cuprinse între 68,8 și 91,8%. Toți compușii sintetizați au fost purificați și caracterizați prin punctele de topire și prin metode spectrale: IR, ¹H RMN, ¹³C RMN și HRMS. Citotoxicitatea compușilor sintetizați a fost evaluată pe nouă linii celulare canceroase, atât pe fenotipuri sensibile, cât și pe fenotipuri rezistente la medicația actuală, utilizând doxorubicina ca substanță antiproliferativă de referință. Citotoxicitatea compușilor a fost testată și pe hepatocite normale. Compușii **3**, **4** și **7** au prezentat valori ale concentrațiilor minime inhibitorii (CI₅₀) mai mici de 100 μM pe toate liniile celulare testate, în timp ce alți compuși au prezentat activități selective.

Keywords: chalcones, anticancer agents, cytotoxicity, selectivity

Introduction

The development of new anticancer agents is currently a worldwide health issue, since cancer has become one of the leading causes of mortality in the entire world [25, 28]. One of the major problems that occur in anti-cancer drug therapy is the lack of selectivity of drugs that lead to cancer chemotherapy failure. In addition, many cancer cell lines have become resistant to the available drugs [28]. It is therefore important to design new molecules which have different structures from the ones currently used in therapy, which could act selectively on cancer cells.

Several researches showed that natural products are promising antineoplastic agents, many of them presenting low toxicity and good selectivity [6, 8, 9]. An increasing number of natural products are currently promising anticancer drug candidates or key structures for the

design of new synthetic/semisynthetic analogues with enhanced activities.

Chalcones are organic compounds with natural origins possessing the general structure 1,3-diaryl-2-propen-1-one. They are intensively studied in the medicinal field, because of their remarkable biological potential. In plants, chalcones have multiple roles, being involved in natural defence mechanisms and in different biosynthetic pathways, since they are the biosynthetic precursors of flavonoids and isoflavonoids [15, 25-27, 31].

Starting from the general structure of natural chalcones, new synthetic analogues were obtained and evaluated for their biological potential. Polyhydroxylated and indole derived chalcones were shown to have anti-inflammatory activity [20, 30]. Some chalcone analogues containing heterocyclic moieties such as pyridine or furane possess inhibitory activity against *Mycobacterium*

tuberculosis [14]. Several multi-substituted chalcones were found to have antifungal activities [12]. Chalcones with chlorine and fluorine atoms on ring A and electron-donating groups on ring B exhibited strong antimalarial activities against both chloroquine-resistant strain and chloroquine-sensitive strain. Chalcone derivatives containing fluorine, bromine or nitro groups have been reported to possess good anti-leishmanial activity [2, 13, 17]. Besides these activities, the anticancer potential of various heterocyclic chalcone analogues is largely reported [1, 5, 11, 24]. Structure-activity relationships were established based on the biological evaluation of synthetic chalcones containing various substituents grafted on the two aromatic rings. The antioxidant activity of chalcones and their flavonoidic derivatives is due to different mechanisms such as free radical scavenging, hydrogen donation, singlet oxygen quenching and metal ion chelation [19].

Synthetic analogues of chalcones possessing different heterocyclic rings such as thiazole, pyrazole and indole proved to be effective anticancer agents, even on cancer resistant cell lines [5, 25]. Indole based chalcones showed interesting cytotoxic activity and selectivity, but several studies are necessary to be performed in order to establish their mechanism of action [4, 11]. Being aware of the biological potential of indole, pyridine and chalcone moieties, we oriented our research to the synthesis of new 3-indolyl-1-pyridyl-2-propenones with various structural modifications, in order to evaluate their anticancer potential.

Materials and Methods

All chemicals and reagents used in this study were purchased from Sigma-Aldrich, Fluka and Merck Chemical Co. The progress of the reactions was monitored by thin layer chromatography (TLC), which was performed on aluminium sheet pre-coated with silica gel, kieselgel 60 F₂₅₄ (Merck) and the spots were visualized under a UV lamp at 254 nm. Preparative chromatographic purifications were performed using Merck Kieselgel 60 Å column chromatography.

Melting points of synthesized compounds were determined in open capillary tubes, with an Electro-thermal IA 9000 digital apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu Fourier transform infrared (FTIR) spectrophotometer. ¹H and ¹³C NMR spectra were recorded with Bruker WM-400 in dimethylsulphoxide (DMSO-d₆) at 400 and 100 MHz, respectively, using tetramethylsilane as the internal standard. Chemical shifts were recorded as δ (ppm). Coupling constant (J) are referred in Hertz (Hz). The following abbreviations for peak patterns were used: s - singlet, d - doublet, dd - double doublet, t - triplet, q - quartet, brs - broad signal.

Mass spectra were obtained using a Bruker FTMS 4.7T BioAPEX II. Spectra facilities were carried out by analytical instruments facility division of the

Faculty of Science, University of Lorraine, France. Compounds **3b-e** was obtained by alkylation of indol-3-carbaldehyde **3a** with different alkylating agents and compounds **1-7** were synthesized by Claisen-Schmidt condensation, according to the previous literature [29].

General procedure for the synthesis of compounds **3b-e** and **1-7**

Synthesis of indol-3-carbaldehyde derivatives (**3b-e**)

A mixture of indole-3-carbaldehyde **3a** (10 mmol), the appropriate alkylating reagent **2b-e** (10.85 mmol of methyl iodide/ethyl iodide/allyl bromide/benzyl chloride respectively), anhydrous K₂CO₃ (1.4 g) and N,N-dimethylformamide (10 mL) was stirred vigorously at room temperature for 1 hour and then refluxed for 16 - 24 h. After completion of the reaction as monitored by TLC (eluent petroleum ether:ethyl acetate 4:1 v/v), the cooled reaction mixture was poured into water (40 mL) and the precipitated solid was collected by filtration and air dried. After the purification with the appropriate eluent mixture petroleum ether:ethyl acetate 4:1 v/v, the pure compounds **3b-e** were obtained (Figure 1).

Synthesis of 3-indolyl-1-pyridyl-2-propenones (**1-7**)

A mixture of indol-3-carbaldehyde derivative **3a-e** (1.06 mmol) and the appropriate acetylpyridine (1.58 mmol) in anhydrous methanol (15 mL) was refluxed in the presence of piperidine (1.58 mmol) for 20 - 26 h. The reaction course was monitored by TLC using as eluent a mixture of dichloromethane:acetone 9:1 v/v. The formed precipitate was filtered, washed with ice-cold methanol and air dried for 24 h to obtain a pure bright orange or yellow powder **1, 4, 5, 6, 7**, except for compounds **2** and **3** which have been purified by column chromatography on silica gel (Merck, 230 - 400 mesh) using as eluent a mixture of dichloromethane:acetone 9:1 v/v to afford the pure products as orange powder.

E-3-(1*H*-Indol-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one (**1**)

Yield 72.03%, bright orange powder, mp 273 - 274°C; IR (KBr, cm⁻¹): ν_{max} 3090 (NH), 1648 (C=O), 1604 (C=N), 1563 (C=N), 1544, 1542; ¹H NMR (400 MHz, dimethyl sulfoxide DMSO-d₆): δ 7.24 (m, 1H, Ar-H); 7.27 (m, 1H, m, Ar-H); 7.52 (d, 1H, *J* = 1.2 and 6.8 Hz, Ar-H); 7.58 (d, 1H, *J* = 15.6 Hz, H_α); 7.97 (d, 2H, *J* = 6.0 Hz, Ar-H); 8.11 (d, 1H, *J* = 1.2 and 6.8 Hz, Ar-H); 8.18 (d, 1H, *J* = 15.2 Hz, H_β); 8.83 (d, 2H *J* = 6.4 Hz, Ar-H); 12.03 (1H, s, NH); ¹³C NMR (100 MHz, DMSO-d₆): δ 112.5; 112.8; 114.7; 120.5; 121.3; 121.4; 122.9; 125.0; 134.4; 137.6; 140.8; 144.7; 150.6; 188.4; ESIMS (Bruker FTMS 4.7T BioAPEX II, CHCl₃/ACN) *m/z* = 249.1019 (M+H)⁺, calculated for C₁₆H₁₃N₂O⁺: 249.09.

E-3-(1-Methylindol-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one (**2**)

Yield 90.57%, orange powder, mp 152 - 154°C; IR (KBr, cm⁻¹): ν_{max} 1644 (C=O), 1592 (C=N), 1561 (C=C),

1543; ¹H NMR (400 MHz, DMSO-d₆): δ 3.875 (s, 3H, NCH₃), 7.321 (m, 1H, Ar-H), 7.344 (m, 1H, Ar-H), 7.574 (d, 1H, *J* = 15.2 Hz, H_α), 7.577 (dd, 1H, *J* = 8.8 and 6.4 Hz, Ar-H), 7.989 (brs, 2H, Ar-H), 8.071 (d, 1H; *J* = 15.6 Hz, H_β), 8.145 (dd, 1H, *J* = 7.2 and 0.8 Hz, Ar-H), 8.168 (s, 1H, Ar-H), 8.950 (brs, 2H, Ar-H); ¹³C NMR (100 MHz, DMSO-d₆): δ 33.1; 110.9; 111.8; 114.6; 120.6; 121.7(3C); 122.9; 125.5; 137.7; 138.1; 140.1; 144.6; 150.6(2C); 188.3; ESIMS (Bruker FTMS 4.7T BioAPEX II, CHCl₃/ACN) *m/z* = 263.1176 (M+H)⁺, calculated for C₁₇H₁₅N₂O⁺: 263.11.

E-3-(1-Ethylindol-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one (3)

Yield 79.73%, bright orange powder, mp 128 - 130°C; IR (KBr, cm⁻¹): ν_{max} 1650 (C=O), 1594 (C=N), 1565 (C=C), 1406; ¹H NMR (400 MHz, DMSO-d₆): δ 1.43 (t, 3H, N-CH₂-CH₃); 4.28 (m, 2H, N-CH₂-CH₃); 7.28 (m, 1H, Ar-H); 7.31 (m, 1H, Ar-H); 7.58 (d, 1H, *J* = 15.2 Hz, H_α); 7.63 (d, 1H, *J* = 1.2 and 6.8 Hz, Ar-H); 7.97 (d, 2H, *J* = 6.0 Hz, Ar-H); 8.08 (d, 1H, *J* = 15.2 Hz, H_β); 8.13 (d, 1H, *J* = 1.2 and 6.8 Hz, Ar-H); 8.26 (s, 1H, s, Ar-H); 8.83 (d, 2H, *J* = 6.4 Hz, Ar-H); ¹³C NMR (100 MHz, DMSO-d₆): δ 15.0; 40.9; 110.9; 112.0; 114.7; 120.7; 121.4; 121.6; 122.9; 125.7; 135.9; 137.1; 140.1; 144.7; 150.6; 188.3; ESIMS (Bruker FTMS 4.7T BioAPEX II, CHCl₃/ACN) *m/z* = 277.1332 (M+H)⁺, calculated for C₁₈H₁₇N₂O⁺: 277.13.

E-3-(1-Allylindol-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one (4)

Yield 91.80%, bright orange powder, mp 103 - 104°C; IR (KBr, cm⁻¹): ν_{max} 1650 (C=O), 1595 (C=N), 1566 (C=C), 1548; ¹H NMR (400 MHz, DMSO-d₆): δ 4.91 (d, 2H, *J* = 5.6 Hz, N-CH₂-CH=CH₂); 5.11 (dd, 1H, *J* = 16.8 and 1.2 Hz, N-CH₂-CH=CH₂); 5.22 (dd, 1H, *J* = 10.4 and 1.2 Hz, N-CH₂-CH=CH₂); 6.06 (m, 1H, N-CH₂-CH=CH₂); 7.28 (m, 1H, Ar-H); 7.32 (m, 1H, Ar-H); 7.57 (dd, 1H, *J* = 1.2 and 6.8 Hz, Ar-H); 7.60 (d, 1H, *J* = 15.6 Hz, H_α); 7.97 (d, 2H, *J* = 6.0 Hz, Ar-H); 8.08 (d, 1H, *J* = 15.6 Hz, H_β); 8.15 (d, 1H, *J* = 1.2 and 6.8 Hz, Ar-H); 8.20 (s, 1H, Ar-H); 8.83 (d, 2H, *J* = 6.4 Hz, Ar-H); ¹³C NMR (100 MHz, DMSO-d₆): δ 48.5; 111.2; 112.2; 115.1; 117.6; 120.7; 121.4; 121.7; 123.0; 125.7; 133.4; 136.5; 137.4; 140.0; 144.6; 150.6; 188.4; ESIMS (Bruker FTMS 4.7T BioAPEX II, CHCl₃/ACN) *m/z* = 289.1329 (M+H)⁺, calculated for C₁₉H₁₇N₂O⁺: 289.13.

E-3-(1-Benzylindol-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one (5)

Yield 78.00%, yellow powder, mp 178 - 180°C; IR (KBr, cm⁻¹): ν_{max} 1714 (C=O), 1595 (C=N), 1565 (C=C), 1406; ¹H NMR (400 MHz, DMSO-d₆): δ 5.518 (s, 2H, Ar-CH₂-), 7.269 - 7.302 (m, 5H, Ar-H), 7.326 (m, 1H, Ar-H), 7.344 (m, 1H, Ar-H), 7.600 (m, 1H, Ar-H), 7.609 (d, 1H, *J* = 15.2 Hz, H_α), 7.981 (d, 2H, *J* = 5.6 Hz, Ar-H), 8.091 (d, 1H; *J* = 15.6 Hz, H_β), 8.115 (m, 1H, Ar-H), 8.373 (s, 1H, Ar-H), 8.830 (brs, 2H, Ar-H); ¹³C NMR (100 MHz, DMSO-d₆, Me₄Si): δ 49.6; 111.4; 112.4; 115.3; 120.7; 121.5; 121.7; 123.1;

125.8; 127.2; 127.7; 128.7; 136.8; 137.0; 137.4; 139.9; 144.6; 150.6; 188.4; ESIMS (Bruker FTMS 4.7T BioAPEX II, CHCl₃/ACN) *m/z* = 339.1486 (M+H)⁺, calculated for C₂₃H₁₉N₂O⁺: 339.4.

E-3-(1*H*-Indol-3-yl)-1-(pyridin-3-yl)prop-2-en-1-one (6)

Yield 72.80%, bright orange powder, mp 197 - 198°C; IR (KBr, cm⁻¹): ν_{max} 3088 (NH), 1650 (C=O), 1585 (C=N), 1560 (C=C); ¹H NMR (400 MHz, DMSO-d₆): δ 7.239 (m, 1H, Ar-H), 7.269 (m, 1H, Ar-H), 7.507 (dd, 1H, *J* = 6 and 2.4 Hz, Ar-H), 7.599 (dd, 1H, *J* = 8.0 and 7.6 Hz, Ar-H), 7.657 (d, 1H, *J* = 15.6 Hz, H_α), 8.113 (d, 1H, *J* = 15.6 Hz, H_β), 8.151 (dd, 1H, *J* = 6.8 and 2.4 Hz, Ar-H), 8.175 (s, 1H, Ar-H), 8.459 (d, 1H, *J* = 7.6 Hz, Ar-H), 8.805 (d, 1H, *J* = 4.8 Hz, Ar-H), 9.295 (d, 1H, *J* = 2 Hz, Ar-H), 11.995 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-d₆): δ 112.4; 112.8; 115.1; 120.5; 121.2; 122.8; 123.8; 125.1; 133.6; 133.8; 135.6; 137.5; 139.8; 149.2; 152.6; 187.8; ESIMS (Bruker FTMS 4.7T BioAPEX II, CHCl₃/ACN) *m/z* = 249.1020 (M+H)⁺, calculated for C₁₆H₁₃N₂O⁺: 249.09.

E-3-(1-Methylindol-3-yl)-1-(pyridin-3-yl)prop-2-en-1-one (7)

Yield 68.84%, bright orange powder, mp 156 - 157°C; IR (KBr, cm⁻¹): ν_{max} 1648 (C=O), 1585 (C=N), 1556 (C=C); ¹H NMR (400 MHz, DMSO-d₆): δ 3.87 (s, 3H, NCH₃); 7.32 (m, 1H, Ar-H); 7.34 (m, 1H, Ar-H); 7.50 (dd, 1H, *J* = 6.0 and 6.4 Hz, Ar-H); 7.59 (dd, 1H, *J* = 1.2 and 6.8 Hz, Ar-H); 7.64 (d, 1H, *J* = 15.2 Hz, H_α); 8.07 (d, 1H, *J* = 15.2 Hz, H_β); 8.16 (s, 1H, Ar-H); 8.18 (dd, 1H, *J* = 1.2 and 6.8 Hz, Ar-H); 8.46 (dd, 1H, *J* = 1.2 and 6.4 Hz, Ar-H); 8.81 (dd, 1H, *J* = 1.6 and 6.0 Hz, Ar-H); 9.29 (d, 1H, *J* = 2.0 Hz, Ar-H); ¹³C NMR (100 MHz, DMSO-d₆): δ 33.1; 110.8; 111.8; 115.0; 120.7; 121.5; 122.8; 123.8; 125.5; 133.6; 135.6; 137.2; 138.0; 138.1; 149.2; 152.6; 187.7; ESIMS (Bruker FTMS 4.7T BioAPEX II, CHCl₃/ACN) *m/z* = 263.1176 (M+H)⁺, calculated for C₁₇H₁₅N₂O⁺: 263.12.

Cytotoxicity study

The resazurin reduction assay was performed to assess the cytotoxicity of compounds **1-7** and doxorubicin was used 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, HCT116p53^{+/+} colon cancer cells and its resistant subline HCT116p53^{-/-}, 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, 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 × 10⁴ cells was placed in each well of a 96-well cell culture plate (Thermo Scientific,

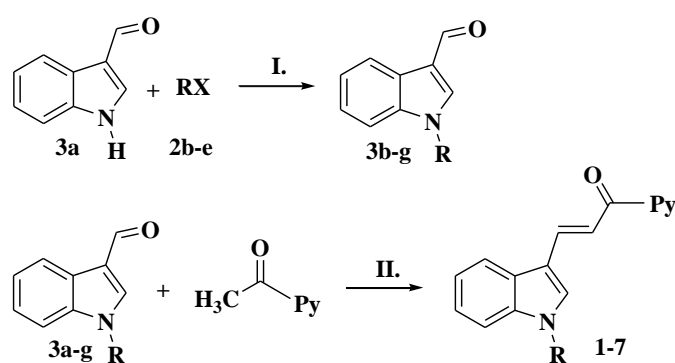
Langensfeld, Germany) in a total volume of 200 μL . Cells were allowed to attach overnight and then were treated with different concentrations of compounds. For suspension cells, aliquots of 2×10^4 cells *per well* were seeded in 96-well-plates in a total volume of 100 μL . The studied compound was immediately added in varying concentrations in an additional 100 μL of culture medium to obtain a total volume of 200 μL *per 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 M 2000 Pro™ 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

The synthetic route using Claisen-Schmidt condensation of these target compounds **1-7** is outlined in Figure 1. Indol-3-carboxaldehydes **3a-g** (1 equiv.) and appropriate acetylpyridine (1.5 equiv.) in anhydrous methanol were refluxed in the presence of piperidine (1.5 equiv.) affording the corresponding 3-indolyl-1-pyridyl-2-propenones **1-7**. Compounds **2** and **3** were purified by column chromatography while compounds **1**, **4-7** were precipitated directly, filtered and washed with ice-cold methanol and air dried.



Compound	R	Py
1	H	
2b/3b/2	CH_3	
2c/3c/3	CH_2CH_3	
2d/3d/4	$\text{CH}_2\text{CH}=\text{CH}_2$	
2e/3e/5	$\text{CH}_2-\text{C}_6\text{H}_5$	
3f/6	H	
3g/7	CH_3	

Figure 1.

Synthesis of 3-indolyl-1-pyridyl-2-propenones **1-7**

Reaction conditions: **I.** anhydrous K_2CO_3 , N,N -dimethylformamide, r.t. 1 h and then reflux 16 - 24 h;

II. piperidine, dry methanol, reflux 20 - 26 h

3-Indolyl-1-pyridyl-2-propenones **1-7** were obtained by the above mentioned procedure with 68.8 - 91.8% yields. Their structures were confirmed by melting points, IR, ^1H NMR, ^{13}C NMR and HRMS analysis. In the IR spectra of compounds **1-7**, the stretching vibrations of the carbonyl groups appeared at $1714 - 1644 \text{ cm}^{-1}$. The characteristic bands for the $\text{C}=\text{C}$ bond of the propenone chain appeared at $1566 - 1556 \text{ cm}^{-1}$, while the stretching vibrations of the $\text{C}=\text{N}$ bond were present at $1604 - 1563 \text{ cm}^{-1}$. The ^1H NMR spectra of the N -unsubstituted 3-indolyl-1-pyridyl-2-propenones showed characteristic signals of the NH proton at approximately 12 ppm. All synthesized compounds present characteristic signals of the two vinylic protons H_α and H_β of the propenone chain as two doublets at $\delta_{\text{H}} 7.57 - 7.65$ (H_α) and $\delta_{\text{H}} 8.07 - 8.11$ (H_β). The high coupling constant ($J = 15.2 - 15.6 \text{ Hz}$) of the two doublets corresponding to protons H_α and H_β of the propenone chain indicates that the 3-indolyl-1-pyridyl-propenones were obtained with E configuration [29].

Aromatic protons of the pyridine and indole rings were found between 7.24 to 9.29 ppm, where the H-2 of the indole ring is also found as a singlet at 8.15 - 8.37 ppm. [22]. In case of the N -substituted indolyl propenones with aliphatic residues (N -methyl, N -ethyl, N -allyl), the corresponding signals of the aliphatic protons were present in the aliphatic area. In the ^{13}C NMR spectra of the 3-indolyl-1-pyridyl-2-propenones, characteristic signals at 187.7 - 188.4 ppm were indicative of the conjugated carbonyl group [29] while C_α and C_β were found around 122.8 - 123.0 ppm and 138.1 - 144.7 ppm, respectively. Other signals corresponding to the unsaturated/aromatic sp^2 carbons located in the vinyl group and benzene ring respectively were located around 110.8 - 144.7 ppm. In the high resolution ESI MS spectra, the molecular ions $[\text{M}+\text{H}]^+$ are present for all the synthesized compounds and the obtained experimental values are in accordance with the corresponding theoretically calculated molecular weights. Based on all these data,

the structures of **1-7** were univocally determined as shown in Figure 1. The synthesized compounds **1** [29], and **6** [18, 21] were previously described.

Cytotoxicity study

The cytotoxicity of compounds **1-7** and doxorubicin was first determined in a panel of nine human cancer cell lines including both sensitive and drug-resistant phenotypes, as well as in normal AML12 hepatocytes (Table I). Compounds **3**, **4** and **7** displayed IC_{50} values below 100 μM in all tested cancer cell lines, meanwhile other compounds displayed selective activities.

In CEM/ADR5000 leukaemia cells, significant activity with IC_{50} values below 10 μM [3, 10] were obtained with compounds **3** (9.62 μM) and **7** (9.90 μM). It is worth noting that CEM/ADR5000 cells were highly resistant to doxorubicin contrary to the test compounds **3**, **4** and **7**. Hypersensitivity (degree of resistance or D.R. below 0.90) [16] CEM/ADR5000 compared to its sensitive parental cell line CCRF-CEM was noted with compounds **3**, **4** and **7**, suggesting that they might have inhibitory effect on P-glycoprotein's expression [16]. Besides, hypersensitivity was obtained with **3** towards resistant MDA-MB231/BCRP cells [7], HCT-116($p53^{-/-}$) and U87MG. $\Delta EGRF$ cells as well as with **4** and **7** in MDA-MB231/BCRP cells compared to

their respective sensitive counterparts MDA-MB231 cells, HCT116($p53^{+/+}$) cells and U87MG cells. It is also important to note that the selectivity indexes of compounds **3**, **4** and **7** for the normal AML12 hepatocytes versus hepatocarcinoma HepG2 cells are above 1, suggesting their good selectivity to liver cancer cells. The overall data highlights the good activity of compounds **3**, **4** and **7**. These compounds are potential cytotoxic agents that could be used or explored more to develop novel drugs to fight drug sensitive and resistant cancers. A lipophilic molecule with positive charge promotes mitochondrial membrane adhesion that eventually leads to apoptosis when incubated at high micromolar concentration [23]. In case of the 3-indolyl-1-pyridyl-2-propenones, the nitrogen atoms can be protonated under the influence of the mitochondrial membrane potential which is generated by proton pumps, and the compounds become molecules with positive charge that can adhere to the mitochondrial membrane. Moreover, in case of compounds **3**, **4** and **7** an enhancement of the cytotoxicity and selectivity was observed, which could be explained by the increase in the lipophilic character of these molecules due to the substitution of the hydrogen atom from the indole ring by the ethyl, allyl and methyl groups, respectively.

Table I

Cytotoxicity of the studied compounds (**1-7**) and doxorubicin towards drug sensitive and resistant cancer cells as determined by resazurin assay

Cell lines	IC_{50} value in μM and degree of resistance							Doxorubicin
	1	2	3	4	5	6	7	
CCRF-CEM	> 100	> 100	35.34 \pm 3.61	44.47 \pm 3.23	84.10 \pm 6.39	> 100	28.66 \pm 1.05	0.02 \pm 0.00
CEM/ADR5000	> 100	> 100	9.62 \pm 0.77	13.03 \pm 2.01	> 100	> 100	9.90 \pm 1.00	122.96 \pm 10.94
(D.R.)*			(0.27)	(0.29)			(0.35)	(6,683.00)
MDA-MB231	> 100	> 100	48.21 \pm 3.56	22.26 \pm 1.81	> 100	> 100	63.36 \pm 4.91	0.13 \pm 0.01
MDA-MB231/BCRP	> 100	> 100	46.26 \pm 3.29	16.44 \pm 1.11	> 100	> 100	49.66 \pm 2.97	0.79 \pm 0.08
(D.R.)*			(0.96)	(0.74)			(0.78)	(6.14)
HCT116($p53^{+/+}$)	> 100	> 100	24.54 \pm 2.78	34.92 \pm 3.58	> 100	> 100	33.14 \pm 1.76	0.48 \pm 0.06
HCT116($p53^{-/-}$)	> 100	> 100	16.08 \pm 2.01	35.98 \pm 2.10	> 100	> 100	24.67 \pm 1.09	1.78 \pm 0.08
(D.R.)*			(0.66)	(1.03)			(0.74)	(3.73)
U87MG	> 100	> 100	19.58 \pm 0.88	24.98 \pm 2.18	> 100	> 100	23.30 \pm 1.86	0.26 \pm 0.03
U87MG. $\Delta EGRF$	> 100	> 100	18.01 \pm 0.84	30.72 \pm 2.06	> 100	> 100	42.24 \pm 3.19	0.98 \pm 0.07
(D.R.)*			(0.92)	(1.23)			(1.81)	(3.79)
HepG2	> 100	> 100	42.87 \pm 4.42	29.03 \pm 2.87	> 100	> 100	40.57 \pm 3.12	4.56 \pm 0.48
AML12 (S.I)**	> 100	> 100	66.23 \pm 5.67	48.43 \pm 2.80	> 100	> 100	> 100	52.90 \pm 4.09
			(1.55)	(1.67)			(> 2.46)	(11.59)

(*): The degree of resistance (D.R.) 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. $\Delta EGRF$ were used as the corresponding resistant counterpart for CCRF-CEM, MDA-MB-231-*pcDNA*, HCT116 ($p53^{+/+}$), U87MG respectively; (**): The selectivity index (S.I.) 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 activity [3]

Conclusions

In summary, seven indole-based chalcones analogues with various substituents (**1-7**) were prepared using Claisen-Schmidt condensation. The synthesized compounds were tested against several cancer cell lines including both sensitive and their resistant counterparts. The overall data highlights the good activity of compounds

3, **4** and **7**. These compounds are potential cytotoxic agents that could be used or explored more to develop novel drugs to fight drug sensitive and resistant cancers.

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Conflict of interest

The authors declare no conflict of interest.

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