Synthesis of new indole derivatives using one- pot multicomponent reaction with antiproliferative towards normal and cancer cell lines

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Background and objectives
Indoles derivatives are natural products, which are well known for their anticancer activity due to its ability to induce cell death for many cancer cell lines. The aim of this study is to synthesize some new heterocyclic compounds derived from 1H-indole-3-carbaldehyde, malononitrile, and different reagents namely: active methylene derivatives, amine derivatives, and resorcinol. The newly synthesized derivatives were prepared and confirmed by their IR, Mass, and ^{1}NMR spectra; and were also tested for their antiproliferative potency towards human breast cancer (MCF-7) and normal murine fibroblast (BALB/3T3) cell lines.

Materials and methods
The synthesis of new indole derivatives **(3a-e)** was achieved by the three-component reactions of 1H-indole-3-carbaldehyde **(1)**, ethyl 3-oxobutanoate, 5,5-dimethyl cyclohexane-1,3-dione (Dimidone), barbituric, thiobarbituric acid, and cyclohexanone **(2a-e)**, respectively; and malononitrile in the presence of triethylamine. Compound **(4)** was afforded by fusing of compound **(3a)** with thiourea. Also, compounds **(5a, b)** were yielded by the grinding of compounds **(3c, d)** with formic acid. On the other side, one-pot synthesis of **7a-d** has been achieved via three-component of 1H-indole-3-carbaldehyde **(1)**, and malononitrile in the presence of amine derivatives (namely, 2,4-dinitroaniline, 3-nitroaniline, 3-bromoaniline, and 4-methoxyaniline) **(6a-d)**, respectively, by condensation. Also, compounds **(8, 9)** were obtained by refluxing of compounds **(7a, b)** with formic acid. Compound **(10)** was afforded by condensing the mixture of 1H-indole-3-carbaldehyde **(1)** with resorcinol in the presence of malononitrile and triethylamine. The newly synthesized derivatives were tested for their antiproliferative potency towards human breast cancer (MCF-7) and normal murine fibroblast (BALB/3T3) cell lines. Results indicated that they showed significant in-vitro antiproliferative activity.

Results and conclusion
A novel protocol for the preparation of indole derivatives **(3a-e)** using the three-component reactions of 1H-indole-3-carbaldehyde **(1)** with active methylene compounds namely: ethyl 3-oxobutanoate, 5, 5-dimethy cyclohexane-1, 3-dione (Dimidone), barbituric acid, thiobarbituric acid, and cyclohexanone **(2a-e)**; and malononitrile in ethanol and triethylamine as catalyst were proceeded in one step. Also, compounds (**3a, 3c, d)** were reacted with thiourea/and formic acid, respectively, to give compounds (**4 and 5a, b)**. The 3-indole derivatives (**7a-d)** were formed via condensation of the amine derivatives **(6a-d)** with indole aldehydes **(1)** and malononitrile. Compounds (**8 and 9)** were afforded by condensation of compounds (**7b, c)** with formic acid, 2-amino-chromene (**10)** was produced by reaction of resorcinol, 1H-indole-3-carbaldehyde **(1)**, and malononitrile. The in-vitro study showed that most of the prepared compounds gave similar activity towards breast cancer cell line MCF-7 but did not reveal the cytotoxic potency against normal cell line. This means that most of these compounds kill cancer cells but have no or slight effects on normal cells. The newly synthesized derivatives were tested for their antiproliferative potency towards human breast cancer (MCF-7) and normal murine fibroblast (BALB/3T3) cell lines. The results showed that the in-vitro antiproliferative activity of the prepared compounds was significant and could thus be investigated for further *in vivo* and pharmacokinetic studies.

Keywords:
BALB/3T3 cell lines, in-vitro antiproliferative activity, indole, MCF-7, multicomponent reaction

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Introduction

Green chemistry has attracted much interest in organic chemistry [1]. So, organic compounds derived from natural products are useful and can be used as modern drugs. Organic compounds synthesized by using multicomponent reactions (MCRs) are important to proceed with novel preparation methods [2]. MCRs are generally much more environmentally friendly, and offer instantaneous access to large compound libraries [3]. Also, indole is one of the important products in natural and synthetic compounds [4,5]. Indole derivatives have high values in pharmaceutical and medical applications [6]. Also, it is known that heterocyclic systems are very valuable because they are important classes of key structural units in a large number of bioactive molecules used in synthetic drug discovery programs [7].

Materials and methods

Chemistry

All melting points were uncorrected and taken on electrothermal capillary melting point apparatus. The melting points were measured in degree centigrade and determined using BucHI 510 apparatus. Elemental analyses were carried out in the microanalytical unit of the National Research Centre. IR spectra were recorded on a Mattson-5000 FTIR spectrometer using KBr Wafer technique.^{1}H-NMR spectra were determined on a Varian-Gemini-300 MHz and JeolEx-300 MHz NMR spectrometer using TMS as an internal standard with (chemical shift δ, ppm=0). Mass spectra were determined on Finnegan MatSSQ 7000 mode: EI, 70 eV (Thermo Inst. Sys. Inc., NRC, Egypt, Cairo, Dokki). The purity of the synthesized compounds was tested by thin layer chromatography (TLC), Merck plates. TLC silica gel 60 F254 aluminum sheets 20×20 cm.

5-Acetyl-2-amino-4-(1*H*-indol-3-yl)-4*H*-pyran-3-carbonitrile (3a), 2-amino-7,8-dihydro-4-(1*H*-indol-3-yl)-3-isocyano-7,7-dimethyl-4*H*-chromen-5(6H)-one (3b), 7-amino-5-(*1H*-indol-3-yl)-6-isocyano-*1H*-pyrano[2,3-d]pyrimidine-2,4(*3H,5H*)-dione (3c), 7-amino-2,3-dihydro-5-(1H-indol-3-yl)-6-isocyano-2-thioxo-1H-pyrano[2,3-d]pyrimidin-4 (5H)-one (3d), 5,6,7,8-tetrahydro-4-(*1H*-indol-3-yl)-3-isocyano-*4H*-chromen-2-amine(3e).

A mixture of 1H-indole-3-carbaldehyde **(1)** (0.5 mmol), malononitrile (0.5 mmol) and ethyl 3-oxobutanoate, 5,5-dimethyl cyclohexane-1,3-dione (Dimidone), barbituric acid, thiobarbituric acid, and cyclohexanone, respectively, was refluxed in ethanol (3 ml), stirred for 4 h (the progress of the reaction was monitored by TLC). After completion, the reaction mixture was filtered and the precipitate crystallized by ethanol (Fig. 1 and Table 1).

1-(4-amino-2, 5-dihydro-5-(*1H*-indol-3-yl)-2-thioxo-*1H*-pyrano [2, 3-d] pyrimidin-6-yl) ethanone (4).

A mixture of compound **(3a)** (10 mmol) and thiourea (10 mmol) was heated at 180°C in a test tube on sand bath for 4 h. The mixture was allowed to cool at room temperature; the product was solidified by cooling and addition of methanol (50 ml). The precipitate formed was collected by filtration and crystallized by ethanol/chloroform to produce **(4)** (Fig. 2 and Table 1).

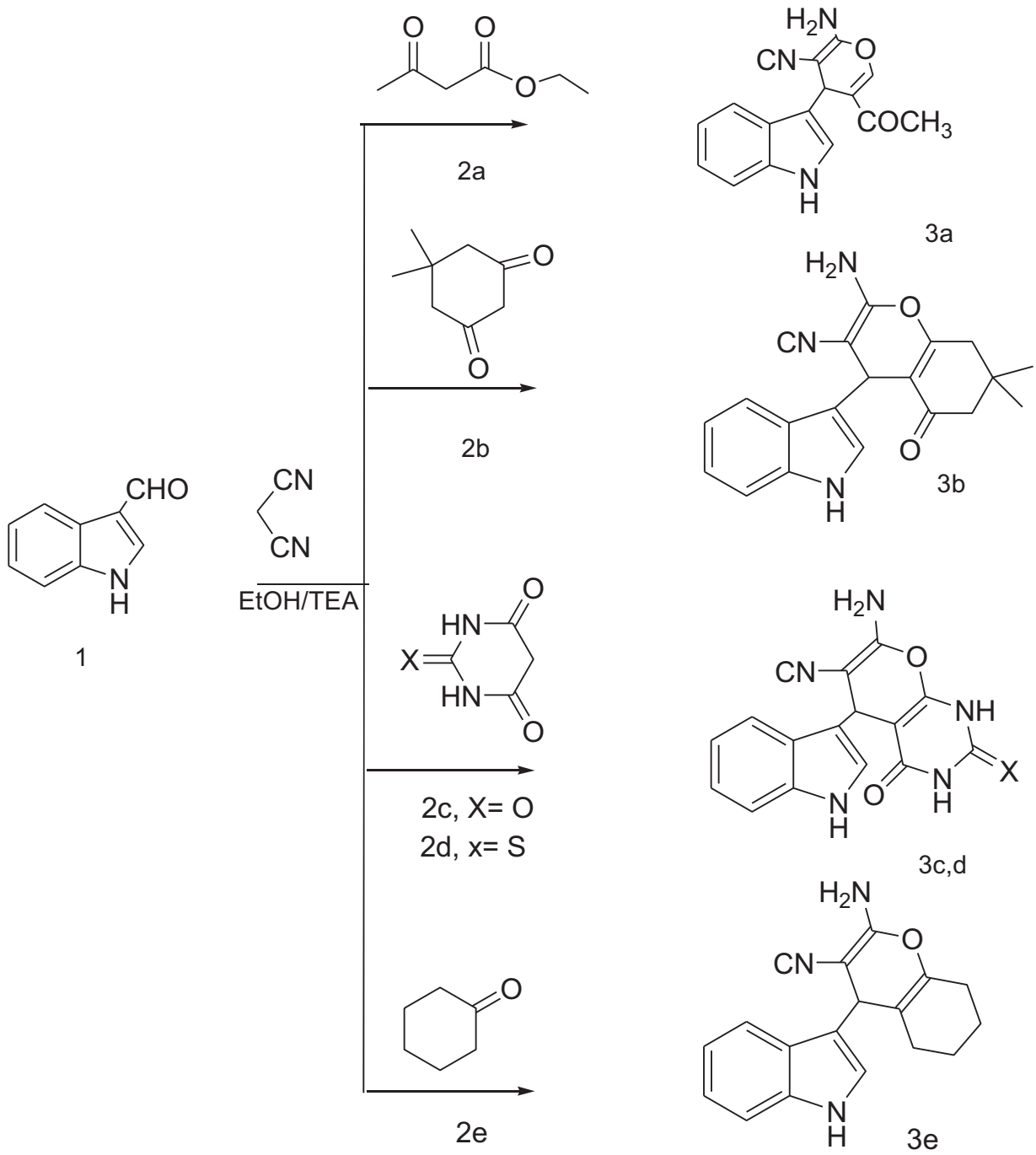
[6, 7-b]-Pyrimidin-9-one-2, 3, 4, 5-tetrahydro-5-(1H-indol-3-yl)-2, 4-dioxo-1H-pyrano [2, 3-d] pyrimidine2, 4(*3H, 5H*)-dione (5a) and [6, 7-b]-Pyrimidin-9-one 2, 3, 4, 5-tetrahydro-5-(*1H*-indol-3-yl)-2-thioxo-1*H*-pyrano [2, 3-d] pyrimidin-4(5*H*)-one (5b).

A mixture of compound **(3c, d)** (1.90 g, 10 mmol), formic acid (10 ml), and catalytic amount of concentrated hydrochloric acid and isopropanol was refluxed for 12 h; the reaction mixture was allowed to cool at room temperature and was poured into cold water (100 ml). The formed solid was collected by filtration, washed by ethanol (20 ml), dried, and crystallized from dimethylformammide/ethanol (2 : 1) (Fig. 3 and Table 1).

[6, 7-b]-Pyrimidin-9-one-2, 3, 4, 5-tetrahydro-5-(1H-indol-3-yl)-2, 4-dioxo-1H-pyrano [2, 3-d] pyrimidine2, 4(*3H, 5H*)-Dione (5a) and [6, 7-b]-Pyrimidin-9-one 2, 3, 4, 5-tetrahydro –5-(*1H*-indol-3-yl)-2-thioxo-1*H*-pyrano [2, 3-d] pyrimidin-4 (5*H*)-one (5b).

A mixture of compound **(3c, d)** (1.90 g, 10 mmol), formic acid (10 ml), and catalytic amount of concentrated hydrochloric acid was grinded, and then isopropanol was added as solvent and the refluxing was carried out for 12 h. The reaction mixture was allowed to cool at room temperature and was poured into cold water (100 ml). The formed solid was collected by filtration, washed by ethanol (20 ml), dried, and crystallized from dimethylformammide/ethanol (2 : 1) (Fig. 3 and Table 1).

Figure 1



Synthesis of compounds **3a-e**.

4-Amino-2-(*1H*-indol-3-yl)-6, 8-dinitroquinoline-3-carbonitrile (7a), 4-amino-2-(*1H*-indol-3-yl)-7-nitroquinoline-3-carbonitrile (7b), 4-amino-6-bromo-2-(*1H*-indol-3-yl) quinoline-3-carbonitrile (7c) and 4-amino-2-(*1H*-indol-3-yl)-6-methoxyquinoline-3-carbonitrile(7d):

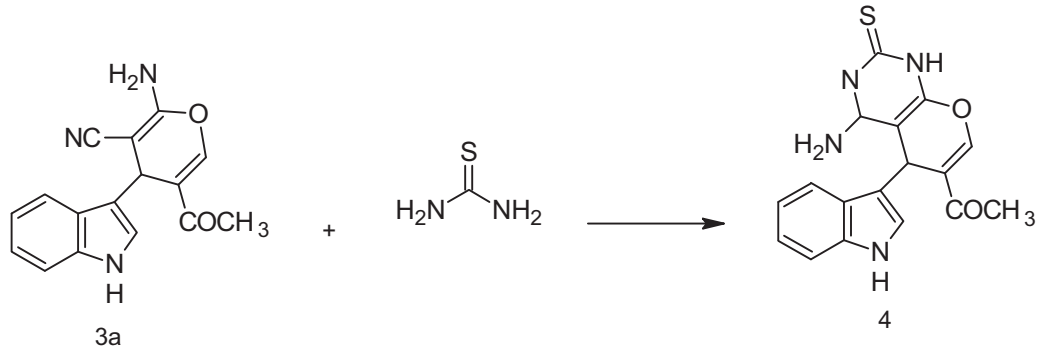
A dried round bottomed flask was filled with a mixture of amine derivatives (namely, 2,4-dinitroaniline, 3-nitroaniline, 3-bromoaniline, and 4-methoxyaniline) **(6a-d)**, respectively (1 mmol), 1H-indole-3-carbaldehyde **(1)** (1 mmol) and malononitrile (3, 66 mg, 1 mmol) in the presence of ethanol (1 mL), followed by triethylamine as catalyst. The mixture was kept under good stirring. The progress of the reaction was monitored by TLC and the formed precipitate was filtered, washed with ethanol, and dried to afford the pure products **(7a-d)** (Fig. 4 and Table 1).

5-(*1H*-indol-3-yl)-7, 9-dinitropyrimido [5, 4-c] quinolin-4(*3H*)-one (8) and 5-(*1H*-indol-3-yl)-9-nitropyrimido [5, 4-c] quinolin-4(*3H*)-one (9).

Table 1 Physical and analytical data of the prepared compounds

Compound number	Formula (molecular weight)	m.p. (°C)	Yield (%)	Analysis (calculated/found, %)		
				C	H	N
3a	$C_{16}H_{13}N_3O_2$ (279.29)	213-5	40	68.81/68.80	4.69/4.45	15.05/14.75
3b	$C_{20}H_{19}N_3O_2$ (333.38,)	170-1	33	72.05/71.89	5.74/5.61	12.60/12.54
3c	$C_{16}H_{11}N_5O_3$ (321.29)	290-2	30	59.81/59.62	3.45/3.35	21.80/21.63
3d	$C_{16}H_{11}N_5O_2S$ (337.36)	320dec	38	56.96/56.42	3.29/3.06	220.76/20.44
3e	$C_{18}H_{17}N_3O$ (291.35)	215-7	37	74.20/73.73	5.88/5.68	14.42/14.32
4	$C_{17}H_{14}N_4O_2S$ (338.38)	180-2	43	60.34/59.77	4.17/4.01	16.56/16.31
5a	$C_{17}H_{11}N_5O_4$ (349.3)	300>dec	63	58.45/58.00	3.17/3.05	20.05/19.99
5b	$C_{17}H_{11}N_5O_3S$ (365.37)	300>dec	31	55.88/55.73	3.03/2.88	19.17/19.12
7a	$C_{18}H_{10}N_6O_4$ (374.31)	259–60	73	57.76/57.16	2.69/2.59	22.45/22.32
7b	$C_{18}H_{11}N_5O_2$ (329.O9)	180-2	48	65.65/65.42	3.37/3.10	21.27/21.12
7c	$C_{18}H_{11}BrN_4$ (363.21)	269–70	60	59.52/59.22	3.05/3.00	15.43/15.23
7d	$C_{19}H_{14}N_4O$ (314.34)	218–20	60	72.60/72.00	4.49/4.29	17.82/16.82
8	$C_{19}H_{10}N_6O_5$ (402.32)	270>dec	91	56.72/56.00	2.51/2.41	20.89/20.45
9	$C_{19}H_{11}N_5O_3$(357.32)	238–40	63	63.86/62.82	3.10/2.90	19.60/19.54
10	$C_{18}H_{13}N_3O_2$(303.31)	200–2	43	71.82/70.28	4.32/4.11	13.85/12.45

Figure 2



Synthesis of compound **4**.

A mixture of compound **(7a or b)** (10 mmol), formic acid (10 ml), and catalytic amount of concentrated hydrochloric acid was heated under reflux for 12 h; the reaction mixture was allowed to cool at room temperature and was poured into cold water (100 ml). The formed solid was collected by filtration, washed by ethanol (20 ml), dried, and crystallized from dimethylformammide/ethanol (2 : 1) (Fig. 5 and Table 1).

2-amino-7-hydroxy-4-(*1H*-indol-3-yl)-*4H*-chromene-3-carbonitrile (10).

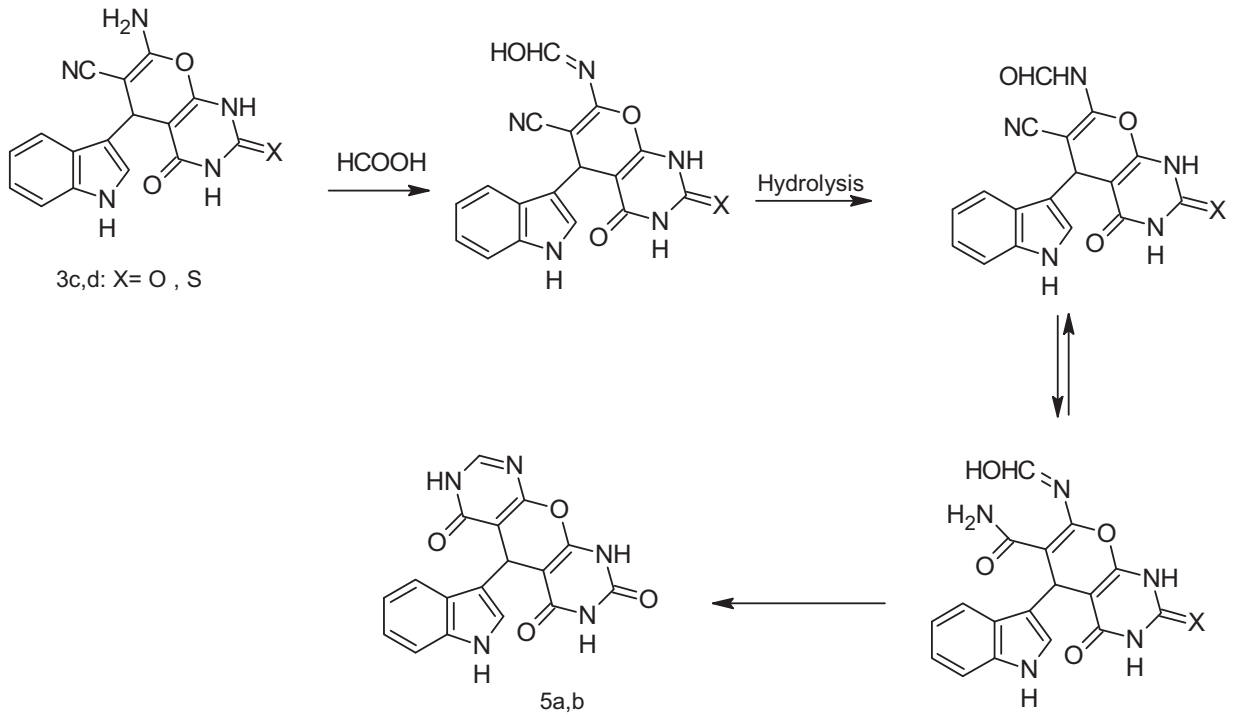
A mixture of 1H-indole-3-carbaldehyde (10 mmol) and resorcinol (10 mmol) and malononitrile (10 mmol) in (10 ml) ethanol was stirred under reflux until the completion of the reaction. The mixture was stirred under reflux until the completion of the reaction, as indicated by TLC (ethyl acetate/n-hexane). Then the reaction mixture was allowed to cool at room temperature .The formed solid was collected by filtration, washed by ethanol (20 ml), dried, and crystallized from ethanol/two drops of chloroform (Fig. 6 and Table 1).

Biological assay

Cells

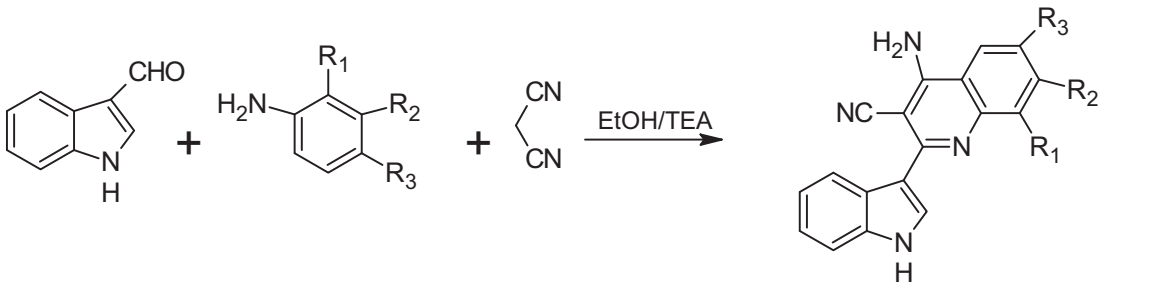
Cell lines, MCF-7 (human breast cancer) and BALB/3T3 (murine fibroblast) are being maintained in the Institute of Immunology and Experimental Therapy, Wroclaw, Poland. All cancer cell lines were obtained from American Type Culture Collection (Rockville, Maryland, USA) and are being maintained in the Institute of Immunology and Experimental Therapy (Wrocław, Poland). MCF-7 cells were cultured in Eagle medium (IIET, Wroclaw, Poland) supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 8 μg/ml of insulin and 1% MEM nonessential amino acid solution 100× (all from Sigma-Aldrich Chemie GmbH, Steinheim,

Figure 3



Synthesis of compounds **5a, b**.

Figure 4



1 6, a, R1=NO2, R2=H, R3= NO2
b, R1=H, R2=NO2 R3= H
c, R1=H, R2=H R3=Br
d, R1=H, R2- H, R3=OCH3

7, a, R1=NO2, R2=H, R3= NO_2
b, R1=H, R2=NO_2 R3= H
c, R1=H, R2=H R3=Br
d, R1=H, R2- H, R3=OCH3

Synthesis of compounds **7a-d**.

Germany). BALB/3T3 cell line was cultured in DMEM (Gibco, UK) supplemented with 2 mM L-glutamine, 10% fetal bovine serum (GE Healthcare, Logan, Utah, USA). All culture media were also supplemented with antibiotics: 100 μg/ml streptomycin (Sigma-Aldrich Chemie GmbH) and 100 U/ml penicillin (PolfaTarchomin SA, Warsaw, Poland). All cell lines were grown at 37°C with 5% CO_2 humidified atmosphere.

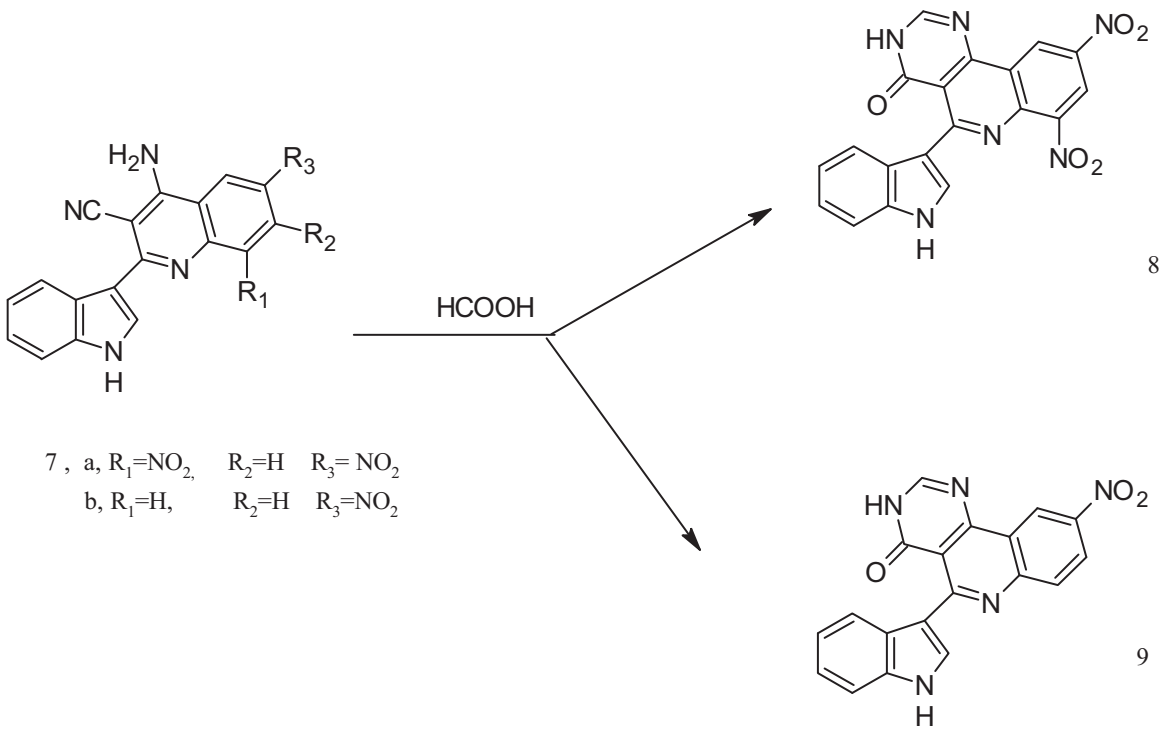
Compounds

Prior to usage, the compounds were dissolved in DMSO (stock solution 10 mg/ml) and culture medium (1 : 9) to the concentration of 1 mg/ml and was subsequently diluted in culture medium to reach the required concentrations (ranging from 100 to 0.1 μg/ml, only one compound **3a** was tested in different range of concentrations from 10 to 0.01 μg/ml, because of the small amount of tested compound).

An antiproliferative assay in vitro

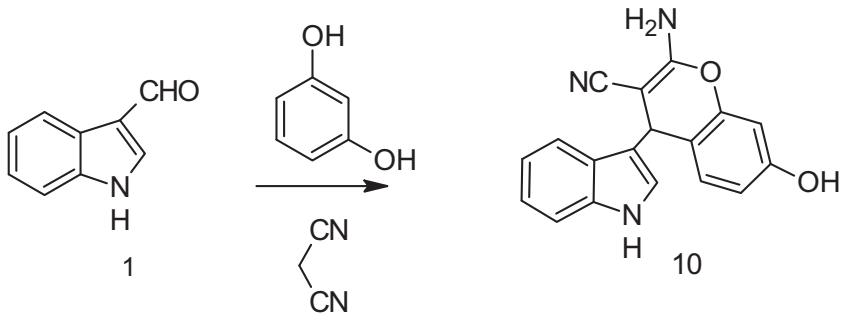
In the time period of 24 h before addition of the tested compounds, the cells were plated in 96-well plates (Sarstedt, Germany) at density of 1×104 cells per well. The assay was performed after 72 h exposure to varying concentrations of the tested compounds. The

Figure 5



Synthesis of compounds **8, 9**.

Figure 6



Synthesis of compound **10**.

in-vitro cytotoxic effect of all compounds was examined using the sulpharhodamine B assay.

Cytotoxic test sulpharhodamine B

The details of this technique were described [8]. The cells were attached to the bottom of plastic wells by fixing them with cold 50% trichloroacetic acid (Sigma-Aldrich Chemie GmbH) on the top of the culture medium in each well. The plates were incubated at 4°C for 1 h then washed five times with tap water. The cellular material fixed with trichloroacetic acid was stained with 0.4% sulpharhodamine B (Sigma-Aldrich Chemie GmbH) dissolved in 1% acetic acid (POCH, Gliwice, Poland) for 30 min. The unbound dye was removed by rinsing (five times) in 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base (POCH) for determination of the optical density (λ=540 nm) in Synergy H4 multimode microplate reader (BioTek Instruments USA). The results were calculated as the IC50 (inhibitory concentration 50%), the concentration of tested compounds that inhibits 50% of the cell populations. The IC50 values were calculated for each experiment separately and the mean±SD values are presented in Table 2. Each compound at each concentration was tested in triplicate in a single experiment, which was repeated three to five times. The results of the studies on antiproliferative activity of tested compounds are summarized in Table 1. In case of compounds **7a-d,**

Table 2 In-vitro antiproliferative potency of the novel derivatives towards breast MCF-7 and normal BALAB/3T3 cell lines

Compounds	MCF-7 IC50±SD (μg/ml)	BALB/3T3 IC50±SD (μg/ml)
Cisplatin	2.97±0.727	2.18±0.600
DMSO	31.63±3.97	6.04±2.07
7a	Nd	Nd
7b	25.23±2.957	52.53±1.05
7c	86.46±11.302	Nd
7d	40.49±1.983	Nd
8	Nd	Nd
9	50.60±8.356	49.72±2.372
3a	Nd	Nd
3b	35.46±2.735	45.90±11.72
3c	Nd	Nd
5a	70.55±14.200	Nd
3d	5.13±0.349	6.65±0.004
5b	6.49±0.496	39.77±7.969
3e	50.10±9.42	Nd

8 and 9, compounds **7b, 9** revealed antiproliferative activity against MCF-7, the IC50 values are 25.23 and 50.60 μg/ml, respectively. However, these compounds show also activity against normal cell line BALB/3T3 (the IC50 values are 52.53 and 49.72 μg/ml, respectively). Moreover, 7c, 7d (the IC50 values are 86.46 and 40.49 μg/ml, respectively) showed weaker or similar antiproliferative activity to **7b, 9,** but they are not active against BALB/3T3 in the used range of concentrations. In contrast, **7a and 8** are not active against both cell lines in the used concentrations. In case of next part of compounds from **3a, 4, and 5a-b,** the **5b** compound revealed selective activity between cancer and normal cell lines. This compound showed high antiproliferative activity against breast cancer cell line MCF-7 as its IC50 value is 6.49 μg/ml and lower activity against normal cell line BALB/3T3 (IC50 value is 39.77 μg/ml) compared to MCF-7. Furthermore, compound 3d (IC50=5.13 μg/ml) revealed similar activity to 5b against cancer MCF-7 cell line, but it is also strongly active against normal cell line BALB/3T3 (IC50=6.65 μg/ml). Compounds 3b, 5a, and 3e showed lower antiproliferative activity than 3d and 5b (the IC50 values are 35.46, 70.55, and 50.10 μg/ml, respectively), 3a is not active in the used concentrations from 10 to 0 and 0.01 μg/ml. The remaining compounds were tested at concentrations ranging from 100 to 0.1 μg/ml. Compounds **3c** and **4** are not active against both cell lines.

Results and discussion

Goal of organic synthesis to the development of economic, sustainable methods is highly desirable. So, one of the most important approaches was one-pot synthesis, which involves the development of MCRs [9,10], where the MDRs are considered as one of the important tools for modern organic synthesis. Also, a series of novel indole that has been synthesized (**3a-e)** had been afforded via three-component reaction, through the reaction of 1H-indole-3-carbaldehyde (**1)**, active methylene compounds namely: ethyl 3-oxobutanoate, 5,5-dimethy cyclohexane-1,3-dione (Dimidone), barbituric acid, thiobarbituric acid, and cyclohexanone (**2a-e)**; and malononitrile in ethanol and triethylamine as catalyst were proceeded in one step. Structural elucidation of compounds (**3a-e)** agreed with [11,12] the spectral data. The IR spectra exhibited absorption band, the appearance of (CN and NH_2 gps.), and disappearance of the (CHO gp.) (Fig. 1 and Table 3).

In contrast, the reaction of fused-pyrimidine in heterocyclic skeleton is found in a broad range of natural products and synthetic molecules with biological uses [13]. For this reason, it is a discovery of new and efficient method for producing complex molecules. Compound (**4)** was obtained by fusing compound (**3a)** with thiourea, compounds **(5a, b)** were obtained upon grinding compounds (**3c** or **3d)** with formic acid, respectively (Figs 2 and 3). Compounds **4** and **5** agreed with comparable results [14] (Figs 2 and 3).

An efficient method for the synthesis of new pyridine derivatives exhibited biological and therapeutic activities [15]. Furthermore, pyridine derivatives have broad applications in coordination chemistry. A one-pot MCR [16] for the synthesis of 3-indole derivatives (**7a-d)** was developed via condensation of amine derivatives (**6a-d)** with indole aldehydes (**1)** and malononitrile. The process was carried out in ethanol and the desired products were obtained in good to excellent yields The structures of pyridine derivatives (**7a-d)** were deduced from their satisfactory spectroscopic data (IR, ^{1}H-NMR, and mass). Compounds **8** and **9** were afforded by condensation of compounds (**7a, b)** with formic acid, and they showed compatible spectral data (^{1}H-NMR, mass spectrum) and elemental analysis (Figs. 4 and 5, Table 3).

The development of new, simple, one-pot synthetic methods for the preparation of both 2-amino-5-hydroxy-chromenes and indole derivatives has become an interesting approach [17]. So, 2-aminochromene derivatives were important organic

Table 3 Spectral characterization of the prepared compounds

Compound number	IR [γ_{max}, cm^{-1}]	^{1}H-NMR (δ, ppm)	Mass (*m/z*, %)
3a	3382, 3327 (NH_2), 3230 (NH) and 2219 (CN) and 1731 (CO),	2.11 (s, 2H, NH_2 exchangeable with D_2O), 2.60 (s, 3H, $COCH_3$),3.97 (s, 1H, 1H-$_4$ pyran), 6.21 (s, 1H, 1H, indole), 6.33 (s, 1H, S, 1H-$_6$ pyran), 7.31 (m, 4H, arom.), 10.11 (s, H, NH)	279 (M^+, 44), 236 (2), 222 (22), 197 (11), 117 (54), 82 (11)
3b	3293, 3164 (NH_2), (NH) and 2183 (CN)	1.13 (s, 6H, $2CH_3$), 2.00, 2.99 (ss, 4H, 2 CH_2, respectively), 2.17 (s, 2H, NH_2, exchangeable with D_2O), 3.94 (s, 1H, 1H-$_4$ pyran), 6.11 (s, 1H, 1H, indole), 7.17 (m, 4H, arom.), 9.40 (s, H, NH)	333.38 (M^+, 33), 318 (21.9), 293 (22), 117 (60)
3c	3443, 3211, 3164 (NH_2, 3NH), and 2183 (CN), 1740 (2 CO)	2.08, 2.11 (s, 2H, NH_2, exchangeable with D_2O), 3.88 (s, 1H, 1H-$_4$ pyran), 7.21 (m, 4H, arom.), 6.23 (s, 1H, 1H, indole), 10.21, 9.00, 8.87 (s, 3H, 3NH, exchangeable with D_2O)	321.09 (M^+, 43), 306, (22%), 281 (33), 205 (43%), 117 (80)
3d	3433 (NH_2), 3155 (3NH), 2272 (CN), 1628 (CO), 1220 (CS)	2, 09 (ss, 2H, NH_2, exchangeable with D_2O), 3.96 (s, 1H, 1H-$_4$ pyran), 6.13 (s, 1H, 1H, indole), 7.22 (m, 4H, arom.), 10.00, 9.03, 8.75 (s, 3H, 3NH, exchangeable with D_2O)	337.36 (M^+, 30), 332 (32), 297 (22), 222 (53), 117 (62)
3e	3293, 3164 (NH_2, NH), 1665 (CO), 2183 (CN)	1.60-2.00 (m, 8H, $4CH_2$), 2.10 (s, 2H, NH_2, exchangeable with D2O), 3.87 (s, 1H, 1H-$_4$ pyran), 6.11 (s, 1H, 1H, indole), 7.17 (m, 4H, arom.), 10.00 (s, H, 1NH, exchangeable with D_2O)	291.14 (M^+, 48.0), 286.14 (44), 176 (77), 251.13 (45), 117 (81)
4	3390, 3164 (NH_2), 3284, 3178 (2NH), and 1617 (CO), 1235 (CS)	2.11 (s, 2H, NH_2 exchangeable with D_2O), 2.34 (s, 3H, CH_3), 3.96, 7.00 (ss, 2H, CH-$_4$, H-$_6$ of pyran ring), 6.08 (s, 1H, indole), 7.22 (m, 4H, arom.), 10.22 8.23 (s, 2H, 2NH, exchangeable with D_2O)	338 (M^+, 33), 296 (43), 281 (11), 166 (60), 117 (77)
5a	3311, 3293, 3164 (4NH), and 1832 (3CO)	3.90 (s, 1H, CH pyran ring), 2.85 (s, 1H, 1NH, exchangeable with D_2O), 7.00 (s, H, pyrimidinone proton), 7.80 (m, 4H, arom.), 10.35, 8.54, 8.00 (s, 3H, 3NH, exchangeable with D_2O)	349.3 (M^+, 65), 234 10), 117 (87)
5b	3333, 3293, 3164 (4NH), and 1783, 1700 (2 CO), 1213(CS)	3.90 (s, 1H, CH pyran ring), 2.11 (s, 1H, 1NH, exchangeable with D_2O), 7.11 (s, H, pyrimidine proton), 7.66 (m, 4H, arom.), 10.35, 9.32, 8.11 (s, 3H, 3NH, exchangeable with D_2O)	365.37 (M^+, 33), 250 (10), 117 (87)
7a	3456, 3321 (NH_2, NH), 2230 (CN)	2.11 (s, 2H, NH_2, exchangeable with D_2O), 6.87(s, H, 1H indole), 7.77, 8.11, 9.00 (m, 6H, arom.), 8.11 (s, 1H, 1NH, exchangeable with D_2O)	374.31 (M^+, 16), 284 (55), 269 (78), 117 (87)
7b	3433, 3218 (NH_2, NH), 2155 (CN)	2.43 (ss, 2H, NH2, exchangeable with D_2O), 6.79 (s, H, 1H indole), 7.80, 8.02, 8.11 (m, 7H, arom.) 8.00 (s, 1H, 1NH, exchangeable with D_2O)	329.09 (M^+, 33), 284 (76), 269 (51), 117 (73)
7c	3430, 3220 (NH_2, NH), 2235 (CN)	2.12 (ss, 2H, NH_2, exchangeable with D_2O), 6.77 (s, H, 1H indole), 7.80, 8.02, 9.11 (m, 7H, arom.). 8.61 (s, 1H, 1NH, exchangeable with D_2O)	362.02 (M^+, 10), 364.01 (97.3), 284 (77), 269 (55), 117 (80)
7d	3430, 3231 (NH_2, NH), 2235 (CN)	2.25 (ss, 2H, NH_2, exchangeable with D_2O), 3.99 (s, 3H, OCH_3), 6.80 (s, H, 1H indole), 7.80, 8.02, 9.11 (m, 7H, arom, 8.22 (s, 1H, 1NH, exchangeable with D_2O)	314.34 (M^+, 10), 284 (60), 269 (45), 117 (68)
8	3332, 3230, 3142 (2NH), 1676-(CO)	6.76 (s, 1H, indole), 7.25 (s, 1H, pyrimidine proton), 7.80, 8.02, 9.11 (m, 6H, arom.), 8.45 (ss, 1H, NH, exchangeable with D_2O)	402.07 (M^+, 20), 312 (47), 117 (71)
9	3320, 3234, 3142 (2NH), 1687-(CO)	6.66 (s, 1H, indole), 7.05 (s, H, pyrimidine proton), 7.80, 8.09, 9.00 (m, 7H, arom.), 8.75, (s, 1H, NH exchangeable with D_2O)	357.32 (M^+, 55), 312 (20), 117 (87)
10	3466, (OH), 3445, 3242 (NH_2, NH), 2200 (CN)	2.85 (ss, 2H, NH_2 exchangeable with D_2O), 4.44 (1H, pyran ring), 6.76 (s, H, 1H indole), 7.80- 8.02 (m, 7H, arom.), 8.87 (s, H, OH, exchangeable with D_2O). 9.11 (ss, 1H, NH, exchangeable with D_2O)	303 (M^+, 54), 272 (20.7), 117 (66)

compounds with biological and pharmacological properties. So, 2-amino-chromene **(10)** was generally preceded by reaction of resorcinol, 1H-indole-3-carbaldehyde **(1),** and malononitrile (Fig. 6). Compound **10** agreed with comparable results [18].

Conclusion

In conclusion, a novel protocol for the preparation of indole derivatives 3a-e using the three-component reactions of aldehyde (**1)** with active methylene compounds namely: ethyl 3-oxobutanoate, 5, 5-dimethy cyclohexane-1, 3-dione (Dimidone), barbituric acid, thiobarbituric acid, and cyclohexanone **(2a-e);** and malononitrile in ethanol and triethyl-amine as catalyst were proceeded in one step. Also, compounds (**3a, 3c, d)** were reacted with thiourea/and formic acid, respectively, to give compounds **(4 and 5a, b)**. The 3-indole derivatives (**7a-d)** were proceeded via condensation of amine

derivatives **(6a-d)** with indole aldehydes (**1**) and malononitrile. Compounds **8** and **9** were afforded by condensation of compounds (**7b, c)** with formic acid. 2-amino-chromene (**10)** was obtained by reaction of resorcinol, 1H-indole-3-carbaldehyde (**1**), and malononitrile. The in-vitro study showed that most of the prepared compounds gave similar activity towards breast cancer cell line MCF-7 but did not reveal the cytotoxic potency against the normal cell line. This means that most of these compounds kill cancer cells but have no or slight effect on normal cells.

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Conflicts of interest
There are no conflicts of interest.

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