Research Article

Synthesis of Novel Acetamide Derivatives and Evaluation of their Antiproliferative Potency against Different Cancer Cell Lines

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ABSTRACT

The objective of this research was to prepare a new acetamide derivatives with anticancer activities. *p*-Toluenesulfonic acid catalyzed the one-pot, a multicomponent reaction involving furochromone carboxaldehyde *I*, cyclohexyl/or phenylisocyanates *1a,b* and amine derivatives (namely: 4-anisidine; 2-anisidine; 4-nitroaniline,4-aminopyridine respectively) *2a-d* for the synthesis of *(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)acetamide derivatives 3a-h* have been described. Also, This methodology was afforded *2-(pyridin-4-ylimino)acetamide derivatives 4a-h* at the reaction of aldehyde derivatives (namely: 1H-indole-3-carbaldehyde; 2-(thiophen-2-yl)-1H-indole-3-carbaldehyde; 3-methoxy-5,6-diphenyl-1H-indole-2-carbaldehyde; 3-hydroxy-5,6-diphenyl-1H-indole-2-carbaldehyde; 8-oxo-2,3-diphenyl-8H-furo[2,3-g]chromene-7-carbaldehyde respectively) *IIa-e* with cyclohexyl/or phenylisocyanates *1a,b* and *p*-aminopyridine. The *in vitro* antiproliferative activity evaluation of the prepared compounds (3a-h) have been assessed against four different human tumor cell lines including human liver HepG2, breast MCF-7, lung A549 and colon HCT116 cancer cell lines. The results revealed that all the prepared compounds did not show any antiproliferative effect towards A549 and HCT116 cell lines. Although compounds 3f, 3d and 3c showed growth inhibitor activity on both HepG2 and MCF-7 cancer cell lines with IC_{50} values near to the standard drug, compound 3e was found to be more potent than the doxorubicin in both liver and breast cancer cell lines. On the other hand, *2-(pyridin-4-ylimino)acetamide* derivatives 4a-h were subjected to a screening system for investigation of their antiproliferative potency against human promyelocytic leukemia (HL-60), lung cancer (A549), breast adenocarcinoma (T-47D) and human colon cancer (LoVo) cell lines in comparison to the traditional anticancer drug cisplatin. Results showed that the highest *in vitro* cytotoxic activity against HL-60 cell line (IC_{50} lower than 12 µg/ml) revealed compounds: 4c, 4d, 4e, 4g. These compounds were chosen for testing their antiproliferative activity against A549 (lung cancer), T-47D (breast adenocarcinoma) and LoVo (human colon cancer cells) cancer cell lines. Compounds (4c and 4e) showed the highest activity against all tested cancer cells. In this research work, a novel acetamide derivatives were prepared.

Keywords: Multicomponent reaction; acetamide derivatives; *p*-toluenesulfonic acid; antiproliferative potency; cancer cell.

INTRODUCTION

In recent decades[1,2], research interests have been focused on a search for more efficient organic synthesis that can rapid generation of complex organic molecules from simple and readily accessible starting materials[3]. The organic medicinal compounds, which are synthesized through stead fast reactions and possesses medicinal activities are generally receive most attention, thus, eventually facilitating the rapid development of new chemical entities (NCEs) available for biological evaluation.

Multicomponent reactions (MCRs)[4] have powerful and bond-forming efficient tool in organic, and medicinal chemistry[5]. Using this strategy, C2-founctionalizations of 1-substituted imidazole have been successfully performed.[6] Considering the continued importance of the imidazole derivatives in both biological and chemical fields, new direct approaches remain highly valuable to the contemporary collection of synthetic methods. Herein we report a multicomponent reaction strategy with various aldehyde and isocyanate.

For many tumor types, established treatments such as cytotoxic chemotherapy and radiotherapy provide only transient therapeutic benefits despite severe side effects. Therefore, the need for better treatments has stimulated research to develop new efficient chemotherapeutic agents for management of cancer (Hassan, 2011)[7].

In the same direction and in continuing effort to find more potent and selective anticancer compounds, herein, we designed and synthesized a series of compounds (**3a-h**).

Their biological activities against three different human liver HepG2, breast MCF-7, lung A549 and colon HCT116 cancer cell lines were evaluated.

Moreover, eight selected 2-(pyridin-4-ylimino) acetamide derivatives 4a-h were subjected to a screening system for investigation of their antitumor potency against human promyelocytic leukemia (HL-60), lung cancer (A549), breast adenocarcinoma (T-47D) and human colon cancer (LoVo) cell lines in comparison to the traditional anticancer drug Cisplatin.

MATERIALS AND METHODS

All starting materials were obtained from commercial suppliers and used without further purification unless otherwise noted. All melting points are uncorrected and were taken on electro-thermal capillary melting point apparatus. Elemental analyses were carried out in the micro analytical 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 Jeol-Ex-300 MHz NMR spectrometer using TMS as an internal standard with (chemical shift. δ= 0 ppm). Mass Spectra were determined on FinniganMatSSQ 7000 mode: EI, 70Ev(Thermo Inst. Sys. Inc., USA). The melting points were measured in degrees centigrade and determined using BucHI 510 apparatus. The purity of the synthesized compounds was tested by thin layer chromatography (TLC), Merck plates. TLC Silica gel 60 F_{254} 25 Aluminum sheets 20 x 20 cm.

General procedure for the preparation of 4,9-dimethoxy-5H-furo[3,2-g] chromen-6-yl derivatives (3a-h)

The furochromone carboxaldehyde **I** (5 mmol) and amine derivatives (namely: 4-anisidine; 2-anisidine; 4-nitroaniline,4-aminopyridine respectively) (5 mmol) were refluxed with a catalytic amount of *p*-toluenesulfonic acid in 5 mL dry toluene for 2 h. and isocyanate derivatives (namely: cyclohexyl/ or phenyl isocyanate) (0.5 mmol) were suspended in 3 mL dry toluene.

The reaction mixture was stirred for 16 h at 100 C. When the reaction was completed, the solvent was evaporated and the resulting residue was dissolved in ethyl acetate and filtered and the crude product was purified.

After completion of the reaction, as indicated by thin-layer chromatography (TLC, ethyl acetate/n-hexane, 2:1), the solid product was separated by filtration followed by washing the residue with ethyl acetate to obtain the pure product ***3a-h***.

2-(4-methoxyphenylimino)-N-cyclohexyl-2-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)acetamide 3a

Following the general procedure, the desired compound was synthesized utilizing *p*-anisidine (1.1 mmol), cyclohexylisocyante (1.10 mmol) and furochromone carbaldehyde**1** (1.50 mg, 1.00 mmol) to give compound **3a**.

White crystals, m.p.164-66 ºC, yield 35%. Mol. formula: $C_{28}H_{28}N_2O_7$, Mol. Wt.: 504.5, analy., calc: C, 66.66; H, 5.59; N, 5.55 found: C, 66.64; H, 5.57; N, 5.57. IR (KBr, cm^{-1}) bands at 1605, 1629 (2C=O) and 3302 (NH). ^{1}H-NMR (DMSO-d6, δ, ppm): 1.09-1.73 (m, 10H, $5CH_2$ cyclohexane); 3.33 (dd, H, CH-N cyclohexane); 3.73, 3.68, 364 (ss, 9H, $3OCH_3$); 5.90(d, 1H, NH, exchangeable D_2O); 6.75- 7.26 (dd, 4H,*p*-anisidine); 6.76, 7.23 (dd, 2H, J=2.01, furan ring) and 8.00 (s, 1H, H_7).

2-(4-methoxyphenylimino)-2-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)-N-phenylacetamide 3b

Following the general procedure, the desired compound was synthesized utilizing *p*- anisidine (1.1 mmol) phenylisocyante (1.10 mmol) and furochromone carbaldehyde**1** (1.00 mmol) to give compound **3b.** Yellow crystals, m.p.147-49 °C, yield 28%. Mol. formula: $C_{28}H_{22}N_2O_7$, Mol. Wt.: 498.5, analy., calcd: C, 67.46; H, 4.45; N, 5.62 found: C, 67.44; H, 4.43; N, 5.64. IR (KBr, cm^{-1}) bands at 1611, 1629 (2C=O) and 3293 (NH). ^{1}H-NMR (DMSO-d6, δ, ppm): 1.12-1.75 (m, 10H); 3.31(d, 1H, CH-NH); 3.71, 3.65, 360 (s, 9H, $3OCH_3$); 5.82 (s, 1H, NH, exchangeable D_2O); 6.34- 7.44 (dd, 9H, arom.); 6.70, 7.35 (dd, 2H, J=2.01, furan ring); and 7.79 (s, 1H, H_7).

2-(2-methoxyphenylimino)-N-cyclohexyl-2-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6 yl) acetamide 3c

Following the general procedure, the desired compound was synthesized utilizing *o*-amino-anisidine (1.1 mmol), cyclohexylisocyante (1.10 mmol) and furochromone carbaldehyde**1** (1.00 mmol) to give compound **3c.** Pale orange solid, m.p.155-57°C, yield 90%. Mol. formula: $C_{28}H_{28}N_2O_7$, Mol. Wt.: 504.5, elemental analy., calcd: C, 66.66; H, 5.59; N, 5.55 found: C, 66.64; H, 5.57; N, 5.53. IR (KBr, cm^{-1}) bands at:1600, 1643 (2C=O) and 3324(NH). ^{1}H-NMR (DMSO-d6, δ, ppm): 1.08-1.76(m, 10H, 5CH2 cyclohexane); 3.33(d, 1H, CH-NH); 3.78, 3.80, 3.82 (sss, 9H, $3OCH_3$); 6.79, 7.81 (dd, 2H, J=2.00, furan ring); 6.77-6.88. (d,4H, *o*-anisidine) ; 8.05 (s, 1H, H_7) and 8.06 (d, 1H, NH exchangeable with D_2O).

2-(2-methoxyphenylimino)-2-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)-N-phenylacetamide 3d

Following the general procedure, the desired compound was synthesized utilizing *o*-amino-anisidine (1.1 mmol), phenylisocyante (1.10 mmol) and furochromone carbaldehyde **1** (1.00 mmol) to give compound **3d**. Orange solid, m.p.-165-67 °C, yield 22%. Mol. formula: $C_{28}H_{22}N_2O_7$, calc. Mol. Wt.: 498.5, elemental analy., :calcd: C, 67.46; H, 4.45; N, 5.62 found: C, 67.44; H, 4.43; N, 5.64. IR (KBr, cm^{-1}) bands at: 1597, 1648 (2C=O) and 3318 (NH). ^{1}H-NMR (DMSO-d6, δ, ppm): 1.08-1.74 (m, 10H, 5CH2 cyclohexane); 3.31(d, 1H, CH- NH); 3.76, 3.77, 3.79 (ss, 9H, $3OCH_3$); 6.43(s, 1H, NH exchangeable with D_2O); 7.28, 7.56 (dd, 2H, J=2.01, furan ring); 7.94 (s, 1H, H_7) and 6.54-8.20 (m, 9H, arom.).

2-(4-nitrophenylimino)-N-cyclohexyl-2-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)acetamide 3e

Following the general procedure, the desired compound was synthesized utilizing *p*-nitroaniline (1.1 mmol), cyclohexylisocyante (1.10 mmol) and furochromone carbaldehyde**1** (1.00 mmol) to give compound **3e**. Reddish brown solid, m.p.154-57°C, yield100 %. Mol. formula: $C_{27}H_{25}N_3O_8$, calc. Mol. Wt.: 519.5, elemental analy., calcd: C, 62.42; H, 4.85; N, 8.09 found: C, 62.40; H, 4.83; N, 8.07. IR (KBr, cm^{-1}) bands at 1612, 1654 (2C=O)

and3320 (NH).^{1}H-NMR (DMSO-d6, δ, ppm): 1.13-1.71 (m, 10H, 5H cyclohexane); 3.37(d, 1H, CH-NH); 3.86, 3.88(s, 6H, 2OCH_3); 5.98 (d, 1H, NH, exchangeable D_2O) 7.36 (s, 1H, H_7); 6.55, 7.67 (dd, 4H, arom.) and 6.76, 7.95 (dd, 2H, J=2.01, furan ring).m/e: 519 (50.0%), 212(10%),118(8 %), 93(100 %)

2-(4-nitrophenylimino)-2-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)-N-phenylacetamide 3f

Following the general procedure, the desired compound was synthesized utilizing *p*-nitroaniline (1.1 mmol), phenylisocyante (1.10 mmol) and furochromonecarboxaldehyde**1** (1.00 mmol) giving compound **3f**. Yellow solid, m.p. 186-88°C, yield 85 %.Mol. formula: $C_{27}H_{19}N_3O_8$, Mol. Wt.: 513.5, elemental analy.:calcd: C, 63.16; H, 3.73; N, 8.18 found: C, 63.14; H, 3.71; N, 8.20. IR (KBr, cm^{-1}) bands at 1596, 1647 (2C=O) and 3321 (NH). ^{1}H-NMR (DMSO-d6, δ, ppm): 3.86, 3.89 (s, 6H, 2OCH_3), 6.92(s, 1H, NH, exchangeable D_2O); 6.64-8.44 (dd, 4H, arom.); 7.22, 8.66 (dd, 2H, J=2.01, furan ring) and 8.66 (s, 1H, H_7). m/e: 513.1 (100.0%), 349(9%), 242(10%), 212(34%), 93(100%).

N-cyclohexyl-2-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)-2-(pyridin-4-ylimino)acetamide 3g: Following the general procedure, the desired compound was synthesized utilizing *p*-amino-pyridine (1.1 mmol); cyclohexylisocyanate (1.10 mmol) and furochromone carboxaldehyde**1** (1.00 mmol) to give compound **3g**. Brown solid, m.p 188-190°C, yield 24%. Mol. formula: $C_{26}H_{25}N_3O_6$, calc. Mol. Wt.: 475.5 calcd: C, 65.67; H, 5.30; N, 8.84 found: C, 65.67; H, 5.30; N, 8.84. IR (KBr, cm^{-1}) band at: 1610, 1645 (2C=O) and 3243 (NH). ^{1}H-NMR (DMSO-d6, δ, ppm): 3.88, 3.91 (ss, 6H, 2OCH_3); 1,11-1.75 (m, 10H, 5H cyclohexane); 3.31(d, 1H, CH- NH); 6.49(d, 1H, NH exchangeable with D_2O), 7.00, 7.56 (dd, 2H, J=2.01, furan ring); 7.95 (s, 1H, H_7) and7.29- 8.09 (d, 4H, pyridine).

2-(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)-N-phenyl-2-(pyridin-4-ylimino) acetamide 3h

Following the general procedure, the desired compound was synthesized utilizing *p*-amino-pyridine (1.1 mmol), phenylisocyante (1.10 mmol) and furochromone carbaldehyde**1** (1.00 mmol) to give compound **3h**. Brownish red solid, m.p.154-56 °C, yield 33%. Mol. formula: $C_{26}H_{19}N_3O_6$, Mol. Wt.: 469.4, elemental analy.: calcd: C, 66.52; H, 4.08; N, 8.95 found: C, 66.50; H, 4.06; N, 8.97. IR (KBr, cm^{-1}) bands at: 1609, 1664 (2C=O) and 3301 (NH).^{1}H-NMR (DMSO-d6, δ, ppm): 3.89; 4.10 (s, 6H, 2OCH_3); 6.49 (s, 1H, NH exchangeable with D_2O); 7.01, 7.56 (dd, 2H, J=2.01, furan ring); 8.00 (s, 1H, H_7) and 7.20-8.23 (d, 4H, pyridine).

General procedures for synthesis of compounds (4a-h)

Various of aldehyde namely: 1H-indole-3-carbaldehyde; 2-(thiophen-2-yl)-1H-indole-3-carbaldehyde; 3-methoxy-5,6-diphenyl-1H-indole-2-carbaldehyde; 3-hydroxy-5,6-diphenyl-1H-indole-2-carbaldehyde; 8-oxo-2,3-diphenyl-8H-furo[2,3-g]chromene-7-carbaldehyde respectively ***IIa-f*** (5 mmol) and 4- aminopyridine (5 mmol) were refluxed with a catalytic amount of *p*-toluenesulfonic acid in 5 mL dry toluene for 2 h. and isocyanate derivatives namely: cyclohexyl/ or phenyl isocyanates (0.5 mmol) were suspended in 3 mL dry toluene. The reaction mixture was stirred for 16 h at 100 °C.

When the reaction was completed, the solvent was evaporated and the resulting residue was dissolved in ethyl acetate and filtered and the crude product was purified. The crude material was purified by ethanol to give the desired products **4a-h.** All products were characterized by IR, ^{1}H-NMR spectroscopy and mass spectrometry.

N-cyclohexyl-2-(1H-indol-3-yl)-2-(pyridin-4-ylimino)acetamide 4a

Following the general procedure, the desired compound was synthesized utilizing *4*-aminopyridine (1.1 mmol), cyclohexylisocyante (1.10 mmol) and 1H-indole-3-carbaldehyde (1.00 mmol) to give compound **4a**. buff solid, m.p.185 °C, yield 70%. Mol. formula: $C_{21}H_{22}N_4O$, Mol. Wt.: 346.4, analy., calc: C, 72.81; H, 6.40; N, 16.17 found: C, 72.79; H, 6.38; N, 16.19. IR (KBr, cm^{-1}) bands at 1624 (C=O) and 3175, 3422 (2NH).^{1}H-NMR (DMSO-d6, δ, ppm): 1.09-1.73 (m, 10H, 5CH_2 cyclohexane); 3.33 (dd, H, CH-N cyclohexane); 5.02(d, 1H, CH-indole); (6.75-7.266.34- 7.44 (m, 4H, arom.); 7.99. 8.02 (dd, 4H, pyridine) and 7.01, 9.87 (dd, 2H, 2NH, exchangeable D_2O).

2-(1H-indol-3-yl)-N-phenyl-2-(pyridin-4-ylimino)acetamide 4b

Following the general procedure, the desired compound was synthesized utilizing *4*-aminopyridine (1.1 mmol), phenylisocyante (1.10 mmol) and 1H-indole-3-carbaldehyde (1.00 mmol) to give compound **4b**. brown solid, m.p.156 °C, yield 65%. Mol. formula: $C_{21}H_{16}N_4O$, Mol. Wt.: 340.4 analy., calcd: C, 74.10; H, 4.74; N, 16.46 found: C, 74.8; H, 4.72; N, 16.48. IR (KBr, cm^{-1}) bands at 1632 (CO) and 3105, 3161 (2NH).^{1}H-NMR (DMSO-d6, δ, ppm): 4.76 (d, 1H, CH-indole); 6.34- 7.44 (dd, 9H, arom.); 7.88. 8.12 (dd, 4H, pyridine) and 7.11, 9.71 (dd, 2H, 2NH, exchangeable D_2O).

N-cyclohexyl-2-(pyridin-4-ylimino)-2-(2-(thiophen-2-yl)-1H-indol-3-yl)acetamide4c

Following the general pocedure, the desired compound was synthesized utilizing *4*-aminopyridine (1.1 mmol), cyclohexylisocyante (1.10 mmol) and 2-(thiophen-2-yl)-1H-indole-3-carbaldehyde (1.00 mmol) to give compound **4c** . green solid, m.p.169 °C, yield 60%. Mol. formula: $C_{28}H_{28}N_2O_7$, Mol. Wt.: 504.5, analy., calcd: C, 66.66; H, 5.59; N, 5.55 found: C, 66.64; H, 5.57; N, 5.53. IR (KBr, cm^{-1}) bands at: 1626 (C=O) and 3179, 3327(2NH). ^{1}H-NMR (DMSO-d6, δ, ppm): 1.03-1.96 (m, 10H, 5CH_2 cyclohexane); 3.49 (dd, H, CH-N cyclohexane); 6.77-6.88(m, 4H, arom.); 7.77, 8,00 (d, 4H, pyridine) and 8.06, 9.54 (dd, 2H, 2NH exchangeable with D_2O).

N-phenyl-2-(pyridin-4-ylimino)-2-(2-(thiophen-2-yl)-1H-indol-3-yl)acetamide 4d

Following the general procedure, the desired compound was synthesized utilizing *4*-aminopyridin (1.1 mmol), phenylisocyante (1.10 mmol) and 2-(thiophen-2-yl)-1H-indole-3-carbaldehyde (1.00 mmol) give compound **4d**. Yellow crystals, m.p.-160 °C, yield 65%. Mol. formula: $C_{25}H_{24}N_4OS$, calc. Mol. Wt.: 428.5 analy., :calcd: C, 70.07; H, 5.64; N, 13.07; S, 7.48 found: C, 70.07; H, 5.64; N, 13.05; S, 7.50. IR (KBr, cm^{-1}) bands at: 1618 (C=O) and 3362 (NH).^{1}H-NMR (DMSO-d6, δ, ppm): 7.24-7.41 (m, 9H, arom., 3H, thiophene); 7.03, 7.98 (dd, 4H, pyridine) and 8.64, 10.53(ss, 2H, 2NH exchangeable with D_2O).

N-cyclohexyl-2-(3-methoxy-5,6-diphenyl-1H-indol-2-yl)-2-(pyridin-4-ylimino)acetamide 4e

Following the general procedure, the desired compound was synthesized utilizing *4*-aminopyridin (1.1 mmol), cyclohexylisocyante (1.10 mmol) and 3-methoxy-5,6-diphenyl-1H-indole-2-carbaldehyde (1.00 mmol) to give compound **4e**.Creamy solid, m.p.210 °C, yield 64%.

Mol. formula: $C_{34}H_{32}N_4O_2$, calc. Mol. Wt.: 528.6 analy.,calcd: C, 77.25; H, 6.10; N, 10.60 found: C, 77.23; H, 6.8; N, 10.62IR (KBr, cm^{-1}) bands at 1648 (2C=O) and 3336, 3430 (2NH).^{1}H-NMR (DMSO-d6, δ, ppm): 1.23-1.98 (m, 10H, 5H cyclohexane); 3.76(d, 1H, CH-NH); 4.26(s, 3H, OCH_3); 6.52, 8.13 (m, 12H, arom.); 6.52, 8.20 (dd, 4H, pyridine) and 8.21, 10.04 (ds, 2H, 2NH, exchangeable D_2O).

2-(3-methoxy-5,6-diphenyl-1H-indol-2-yl)-N-phenyl-2-(pyridin-4-ylimino)acetamide 4f

Following the general procedure, the desired compound was synthesized utilizing *4*-aminopyridin (1.1 mmol), phenylisocyante (1.10 mmol) and 3-methoxy-5,6-diphenyl-1H-indole-2-carbaldehyde (1.00 mmol) giving compound **4f**. Beige needle crystals, m.p.132 °C, yield 165%.

Mol. formula: $C_{34}H_{26}N_4O_2$, Mol. Wt.: 522.6, analy.: calcd: C, 78.14; H, 5.01; N, 10.72 found: C, 78.14; H, 5.01; N, 10.72. IR (KBr, cm^{-1}) bands at 1.660 (C=O) and 3362 (NH). ^{1}H-NMR (DMSO-d6, δ, ppm): 7.02-7.98 (dd,17, arom.); 53, 10.53(ss, 2H, 2NH exchangeable with D_2O). m/e 522 100% as base peak.

2-(3-hydroxy-5,6-diphenyl-1H-indol-2-yl)-N-phenyl-2-(pyridin-4-ylimino)acetamide 4g

The general procedure, the desired compound was synthesized utilizing *p*-amino-pyridine (1.1 mmol); phenylisocyanate (mmol) and 3-hydroxy-5,6-diphenyl-1H-indole-2-carbaldehyde (1.00 mmol) to give compound **4g**. Buff crystals, m.p.208 °C, yield 64%.

Mol. formula: $C_{33}H_{24}N_4O_2$, calc. Mol. Wt.: 508.6 calcd: C, 77.93; H, 4.76; N, 11.02 found: C, 77.91; H, 4.74; N, 11.04. IR (KBr, cm^{-1}) band at:1646 (C=O) ; 3336 (2NH) and 3400 (OH). ^{1}H-NMR (DMSO-d6, δ, ppm) : 4.01(s, 1H, OH exchangeable with D_2O); 7.02- 7.98 (dd,17, arom.); 6.79-7.84 (d,4H, pyridine.) and 10.46, 10.63(ss, 2H, 2NH exchangeable with D_2O).

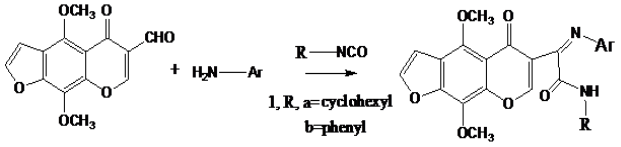
2-(5-oxo-2,3-diphenyl-5H-furo[3,2-g]chromen-6-yl)-N-phenyl-2-(pyridin-4-ylimino)acetamide 4h

Following the General Procedure, the desired compound was synthesized utilizing *p*-amino-pyridine (1.1 mmol), phenylisocyante (1.10 mmol) and; 8-oxo-2,3-diphenyl-8H-furo[2,3-g]chromene-7-carbaldehyde (1.00 mmol) to give compound **4h.** Yellowish brown solid, m.p.165 °C, yield 60%. Mol. formula: $C_{36}H_{23}N_3O_4$, Mol. Wt.: 561.6 analy.: calcd: ***C,*** 76.99; H, 4.13; N, 7.48 found: C, 74.99; H, 4.11; N, 7.50. IR (KBr, cm^{-1}) bands at: 1591, 1653 (2C=O) and 3262 (NH).^{1}H-NMR (DMSO-d6, δ, ppm): 6.82-7.47 (dd, 12, arom.); 6.73-7.81 (d, 4H, pyridine); 7.46(s, 1H, H_7) and 10.49 (s, 1H, 1NH exchangeable with D_2O).

RESULTS AND DISCUSSION

Multi-component reactions[8] were defined as a type of reactions involving at least three different substrates. Consequently, these classes of reactions have found extensive applications in organic, combinatorial, and medicinal chemistry for fast generation of compound libraries[9].

Catalytic of *p*-toluenesulfonic acid was added to a solution of amine derivatives (namely: 4-anisidine; 2-anisidine; 4-nitroaniline,4-aminopyridine respectively) **2a-d**, furochromone carboxaldehyde **I** and isocyante derivatives (namely: cyclohexyl/ or phenyl isocyanates) **1a,b** in methanol. The resulting mixture was stirred at room temperature to give **3a-h** (**Fig. 1**). All products were characterized by melting point, IR, ^{1}H-NMR spectral data, and mass spectroscopy.



I

2 Ar, a= *p*-OCH_3Ph-, *b*=*o*-OCH_3Ph-, c=*p*-NO_2-Ph-, d=C_5H_4N-

3 a, Ar= *p*-OCH_3Ph-, R=cyclohexyl
b, Ar= *p*-OCH_3Ph-, R=phenyl
c, Ar= *o*-OCH_3Ph-, R=cyclohexyl
d, Ar= *o*-OCH_3Ph-, R=phenyl
e, Ar= *p*-NO_2-Ph-, R=cyclohexyl
f, Ar= *p*-NO_2-Ph-, R=phenyl
g, Ar= C_5H_4N-, R=cyclohexyl
h, Ar= C_5H_4N-, R=phenyl

Fig.1

The indole unit exists in numerous privileged pharmaceuticals and natural alkaloids[10]. Our studies initiated with the preparation of ***2-(pyridin-4-ylimino) acetamide derivatives 4a-h*** **(Fig.2)** which could be easily obtained by the reaction of *p*- aminopyridine , aldehyde derivatives (namely: 1H-indole-3-carbaldehyde; 2-(thiophen-2-yl)-1H-indole-3-carbaldehyde; 3-methoxy-5,6-diphenyl-1H-indole-2-carbaldehyde; 3-hydroxy-5,6-diphenyl-1H-indole-2-carbaldehyde; 8-oxo-2,3-diphenyl-8H-furo[2,3-g]chromene-7-carbaldehyde respectively) ***IIa-***

e and isocyanate derivatives(namely: cyclohexyl/ or phenyl isocyanate) ***1a,b*** in methanol. The resulting mixture was stirred at room temperature. All products were characterized by melting point, IR, ^{1}H-NMR spectral data, and mass spectroscopy.

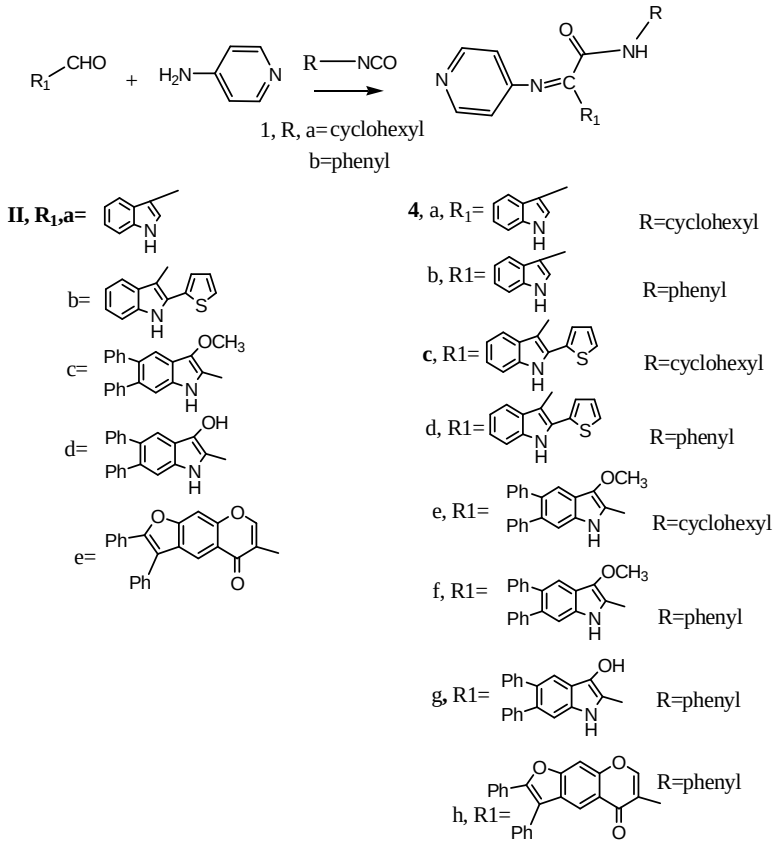


Fig 2

Table 1: *In vitro* antiproliferative activity of Furochromone derivatives against human cancer cell lines

Compound	HepG2	MCF 7	A549	HCT116
3a	N.A.	N.A.	N.A.	N.A.
3g	N.A.	N.A.	N.A.	N.A.
3b	25.90±3.20	N.A.	N.A.	N.A.
3f	6.20±0.60	4.90±0.60	N.A.	N.A.
3d	6.50±0.66	5.80±0.63	N.A.	N.A.
3c	4.30±0.50	4.90±0.49	96.45±10.30	N.A.
3e	3.90±0.40	4.50±0.60	88.00±9.50	N.A.
DMSO	N.A.	N.A.	N.A.	N.A.
Doxorubicin	4.20 ± 0.46	4.70±0.55	5.10±0.50	6.30±0.60

Data were expressed as Mean ±SE of six independent experiments; N.A. : no activity

Anticancer activity

Chemicals

Fetal bovine serum (FBS) and L-glutamine, were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and Sulfo-Rhodamine-B stain (SRB) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich chemical Co. (St. Louis, MO, USA).

1-Anti-proliferative activity for the first group of derivatives (Furochromone)

Anticancer activity screening for the tested compounds utilizing four different human tumor cell lines including liver HepG2, breast MCF-7, lung A549 and colon HCT116 cancer cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). The tumor cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal

calf serum (GIBCO), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37 °C in humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50 x 10^6 were grown in a 25 cm^2 flask in 5 ml of complete culture medium.

In Vitro cytotoxicity assay

The anti-proliferative activity was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previous reported standard procedure (Skehan, 1990). Cells were inoculated in 96-well microtiter plate (10^4 cells/ well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO at 1 mg/ml immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentration of tested compounds (0 - 100 µg/ml) and doxorubicin were added to the cells. Six wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h. at 37°C and in atmosphere of 5% CO_2. After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader at wave length 540 nm. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time.

The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 1. The results were compared to the anti-proliferative effects of the reference control doxorubicin (Li, 1993).[11]

2-In vitro cytotoxic activity for the second group of derivatives (Indoles)

2-(pyridin-4-ylimino)acetamide derivatives **4a-h**. Since all the selected **4a-h** derivatives were soluble in DMSO at concentrations high enough to allow cell experiments, the in vitro cytotoxic activity of these compounds was evaluated by their growth-inhibitory potency against leukemia (HL-60), lung cancer (A549), breast adenocarcinoma (T-47D), human colon cancer cells (LoVo) cancer cell lines in comparison to the traditional anticancer drug Cisplatin.

Tested compounds

Series 1: 4 a, 4b, 4c, 4d, 4e, 4f, 4g, 4h

Series 2: 4c, 4d, 4e, 4g

Compounds from series **1** were tested on the human promyelocytic leukemia (HL-60) and the active compounds which will be included in series **2** were tested on lung cancer (A549), breast adenocarcinoma (T-47D) and human colon cancer cells (LoVo). Reference compound was cisplatin, whereas control of the dissolvent was DMSO, tested in the same concentration as compound probes. Test solutions of the 8 compounds (1 mg/ml) were prepared by dissolving the substances in 100 µl of DMSO completed with 900 µl of tissue culture medium. After wards, the tested compounds were diluted in culture medium to reach the final concentrations of 100, 10, 1, 0.1, 0.01 and 0.001 µg/ml.

Cell lines

Established *in vitro*, human cell line: HL-60 (human promyelocytic leukemia), A549 (lung cancer), T-47D (breast adenocarcinoma) and LoVo (human colon cancer cells) were used. These lines were maintained at the Institute of Immunology and Experimental Therapy, Wroclaw, Poland.

HL-60 cells were cultured in the RPMI 1640 GlutaMAX (Gibco, Scotland, UK) with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose and 10% fetal bovine serum (all from Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and 10% fetal bovine serum (FBS; Thermo-Fisher Scientific Oy, Vataa, Finland). T47D and A549 cells were cultured in RPMI 1640+Opti-MEM (1:1) (both from Gibco, Scotland, UK), supplemented with 2 mM L-glutamine, 5% fetal bovine serum (Thermo-Fisher Scientific Oy, Vataa, Finland). The culture of T47D cells was supplemented with 0.8 mg/L of insulin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

All culture media were supplemented with 100 units/ml penicillin, and 100 µg/ml streptomycin (both from Polfa Tarchomin S.A., Warsaw, Poland). All cell lines were grown at 37°C with 5% CO_2 humidified atmosphere.

Anti-proliferative assay *in vitro*

Twenty four hours prior to the addition of the tested compounds, the cells were plated in 96-well plates (Sarstedt, Germany) at a density of 1 × 10^4 or 0.5 × 10^4 cells per well. The assay was performed after 72 h of exposure to varying concentrations of the tested agents. The *in vitro* cytotoxic effect of all agents was examined using the MTT (HL-60) or SRB (A549, T-47D, LoVo) assay.

The results were calculated as an IC_{50} (inhibitory concentration 50) – the dose of tested agent which inhibits proliferation of 50% of the cancer cell population. Each compound in each concentration was tested in triplicate in a single experiment, which was repeated at least 3 times.

MTT assay[13]

This technique was applied for the cytotoxicity screening against leukemia cells growing in suspension culture. An assay was performed after 72-hours exposure to varying concentrations (from 0.001 to 10 g/ml) of the tested agents. For the last 3-4 hours of incubation 20 µl of MTT solution were added to each well (MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; stock solution: 5 mg/ml, Sigma-Aldrich, Germany). The mitochondria of viable cells reduce the pale yellow MTT to a navy blue formazan. When incubation time was

completed, 80 µl of the lysing mixture were added to each well (lysing mixture: 225 ml dimethyl formamide, POCh, Gliwice, Poland, 67.5 g sodium dodecyl sulphate, Sigma-Aldrich, Germany, and 275 ml of distilled water). After 24 h, when formazan crystals had been dissolved, the optical densities of the samples were read on Synergy H4 photometer (BioTek Instruments, USA) at 570 nm wavelength. Each compound in given concentration was tested in triplicates in each experiment, which was repeated at least 3 times.

SRB assay

This technique was applied for the cytotoxicity screening against lung cancer (A549), breast adenocarcinoma (T-47D), human colon cancer cells (LoVo) cancer cell lines in comparison to the traditional anticancer drug Cisplatin.

Statistical analysis

The results are reported as Mean ± Standard error (S.E.) for at least six times experiments.

RESULTS AND DISCUSSION

1-Anti-proliferative activity for the first group of derivatives (Furochromone)

The anti-proliferative activities were expressed by mean growth inhibitory concentration (IC_{50}). As shown in **table 1**, the anti-proliferative activity of the synthetic compounds was evaluated against human liver HepG2, breast MCF-7, lung A549 and colon HCT116 cancer cell lines using SRB assay, in comparison with doxorubicin as reference drug.

The results revealed that all compounds did not exert any activity against human colon HCT116 and lung A549 cancer cells. The tumor cell line showed normal growth in our culture system and DMSO did not seem to have any noticeable effect on cellular growth. A gradual decrease in viability of cancer cells was observed with increasing concentration of the tested compounds, in a dose-dependent inhibitory effect. For liver HepG2 cancer cells, while compounds **3a** and **3g** had no effect on the cancer cells, Compound **3e** was found to be more potent than the standard drug, doxorubicin anticancer agent with IC_{50} value 3.90±0.40 µg/ml versus 4.20 ± 0.46 µg/ml for doxorubicin. On the other hand, compounds **3f**, **3d** and **3c** were found to be potent anticancer agents had IC_{50} values near to the standard drug (IC_{50}: 6.20±0.60, 6.50±0.66 and 4.30±0.50 µg/ml respectively versus 4.20 ± 0.46 µg/ml for doxorubicin). In the same sense, evaluation the anticancer effect of the tested compounds against human breast MCF-7 cancer cells revealed that although compounds **3a, 3g** and **3b** had no effect on the cancer cells, compounds **3e** was found to be more potent than the doxorubicin with IC_{50} value 4.50±0.60µg/ml versus 4.70±0.55 µg/ml for doxorubicin. Compounds **3f**, **3d** and **3c** showed anticancer activity closed to the standard drug (IC_{50}: 4.90±0.60, 5.80±0.63 and 4.90±0.49 µg/ml respectively, versus 4.70±0.55 µg/ml for doxorubicin). In conclusion, The tested compounds exert anti-carcinogenic activity in liver HepG2 and breast MCF-7 cancer cell lines through reduce the cell proliferation and resulted in significant growth inhibitory. Although compounds **3f**, **3d** and **3c** showed cytotoxicity and growth inhibitor activity on both liver and breast cancer cell lines with IC_{50} values near to the standard drug. Compound **3e** was found to be more potent than the doxorubicin in both liver and breast cancer cell lines. The present study also, reveals that HepG2 cells are more sensitive to the tested compounds than the MCF-7 cells.

2-In vitro cytotoxic activity for the second group of derivatives (Indoles)

The results of the studies on *in vitro* antiproliferative activity are summarized in Tables **2** and **3**.

Data in **Table 2** revealed that the highest in vitro antiproliferative activity against HL-60 cell line (IC_{50} lower than 12 µg/ml) were compounds: **4c**, **4d**, **4e**, **4g**. These compounds were chosen to the next study against the following cell lines: A549 (lung cancer), T-47D (breast adenocarcinoma) and LoVo (human colon cancer cells).

Table 2: Antiproliferative activity of all synthesized indoles against HL-60 (human promyelocytic leukemia).

COMPOUNDS	HL-60 IC_{50} [µg/ml]
4a	44.99 ± 14.87
4b	30.41 ± 7.65
4c	0.92 ± 0.32
4d	2.91 ± 0.34
4e	0.62 ±0.02
4f	57.85 ± 34.92
4g	2.74 ± 0.37
4h	32.36 ± 7.15
CISPLATIN	0.27 ± 0.03

The results in **Table 3** indicated that the highest *in vitro* cytotoxic activity against A549 (lung cancer), T-47D (breast adenocarcinoma) and LoVo (human colon cancer cells) cancer cell lines revealed compounds: **4c**, **and 4e (**IC_{50} between 0.25 – 2.46 µg/ml).

Table 3: Antiproliferative activity of most active indoles derivatives against A549, T-47D and LoVo cancer cell lines.

COMPOUNDS	**A549** IC_{50} [µg/ml]	**T-47D** IC_{50} [µg/ml]	**LoVo** IC_{50} [µg/ml]
4c	2.46 ± 0.37	0.26 ± 0.06	0.40 ± 0.19
4d	4.75 ± 0.29	2.90 ± 0.67	2.26 ± 0.39
4e	2.26 ± 0.37	0.25 ± 0.07	0.41 ± 0.16
4g	7.39 ± 2.17	4.47 ± 2.40	1.98 ± 0.64
CISPLATIN	**2.36 ± 0.53**	**2.27 ± 0.25**	**0.88 ± 0.18**

Structure activity relationship

The antiproliferative potency towards cancer cell lines with respect to the components of the two groups: group I for furochromone derivatives (compounds 3 a-h) and group II for indole derivatives (compounds 3 a-h), were examined. Data on table 1 indicated that compound **3e** showed the highest antiprolefrative potency towards liver and breast cancer cell lines than the standard anticancer drug: doxorubicin. This may be due to the presence of R= cyclohexyl molecule (as hydrophobic) and NO_2 attached with Ar (as polar group) to furochromone. On the other hand, compounds **3f**, **3d** and **3c** showed less antiproliferative activity. This may be attributed to the following: compound 3f contain OCH_3 in Ar (less polar than NO_2), while compounds **3d** and **3c** contain R= phenyl group. On the other hand, results illustrated in table 3 revealed that selected indole derivatives, namely compounds **4c, 4d, 4e, 4g** gave the highest activity against all tested cancer cells. This may be explained as follows: the higher potent compounds **4c** and **4e** were attached with R= cyclohexyl molecule (as hydrophobic) to indoles, while compounds **4d, and 4g** were less active than **4c** and **4e** because the pyridine ring was attached with R= phenyl ring.

CONCLUSION

1- In furochromone group (I):

Compounds **3e** and **3c** showed the highest antiproliferative potency towards liver and breast cancer cell lines with IC_{50} between 3.90 – 4.90 µg/ml.

2-In indoles group (II):

a-Compounds:**4c, 4d, 4e, 4g** showed the highest growth inhibition activity towards all tested cancer cell lines.

b-The most active against all cancer cell lines used were compounds: **4c** and **4e** with IC_{50} between 0.25 – 2.46 µg/ml.

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