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Synthesis and Cytotoxic Activities of Azanaphthoquinone Annelated Pyrrolo Hydrazone Derivatives

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Abstract

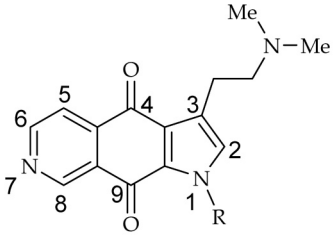
We are interested in the synthesis of azanaphthoquinone annelated pyrrolo hydrazone derivatives as cytostatic compounds. The synthetic pathway was started from the commercially available 5-hydroxyisoquinoline by 3-steps reaction to obtain 1*H*-pyrrolo[3,2-*g*]isoquinoline-4,9-dione. *N*-Alkylation of nitrogen atom in pyrrole ring was carried out under basic conditions with different side chains to obtain mono-substituted azanaphthoquinone annelated pyrroles with 2 to 4-carbon side chains in moderate to good yields. The hydrazone derivatives were synthesized by condensation reaction of mono-substituted products **5** with hydrazine under basic conditions. The reactions occurred regioselectively at C-4 to give products **6**. The synthesized compounds were purified by column chromatography and characterized by spectroscopic techniques including ^{1}H NMR, ^{13}C NMR, IR, UV and LC-MS. The antiproliferative activity of the synthesized compounds was evaluated on cervical carcinoma: KB/HeLa by using xCELLigence from Roche. The results show that the mono-substituted products with 2-carbon side chain (**5b**) exhibited a very good activity with IC_{50} value of 0.008 μM. The hydrazone **6a** showed higher inhibitory activity with IC_{50} value of 0.282 μM compared to the mono-substituted derivative **5a**. The side chains with cyclic amine are of interest for the further studies.

Keywords: Anticancer agents, Azanaphthoquinone, Intercalating agents

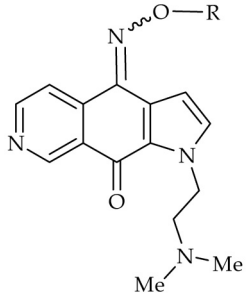
1. Introduction

The anthracyclines antibiotics and their analogues have been known as a key class of compounds for cancer chemotherapy (1). Four mechanisms have been suggested for the mode of action of the anthracyclines including free radical-induced DNA damage (2) stabilization of the DNA-topoisomerase II complex (3) disruption of lipid layers (4) and inhibition of DNA and RNA synthesis (5). However, the major drawback of using these agents is organ toxicity (mostly blood, bone marrow and heart). For these reasons many efforts are focusing on the development of anthracyclines analogues and new core structures which show lower organ toxicity for example Mitoxantrone (6-7).

Azanaphthoquinone annelated pyrrole core structures were developed by H. Spreitzer (8). The mono- and di-substituted derivatives **1** were synthesized (9). The biological evaluation of the synthesized compounds with human cancer cell lines showed promising cytotoxicity as shown in Table 1. As a part of their studies, the synthesis of oxime derivatives **2** have been reported pre viously (10). The oximes **2** showed remarkable biological activity with four different cancer cell lines. However, the easy metabolic cleavage of oxime moiety is the major drawback of these target molecules. The carbinol derivatives of azanaphthoquinone annelated pyrroles were synthesized. However, this series of compounds exhibited only moderate activity (11). As a part of our studies on the design and synthesis of pharmaceutical active compounds as potential anticancer compounds, we herein report on the synthesis of azanaphthoquinone annelated pyrrolo hydrazone derivatives **3** (Figure 1) to overcome the disadvantages of oxime derivatives. Replacing of nitrogen-oxygen bond in oxime with nitrogen-nitrogen bond could enhance the stability of drug from metabolic cleavage. The length of side chain and the functional group in the end of side chain could affect the interaction between drug and DNA strand. Therefore, different side chains will be attacthed to the core structure in an attempt to improve the potency of activity.



1a : R = CH_2CH_2aziridine
1b : R = $CH_2(CH_2)_3$aziridine
1c : R = $CH_2(CH_2)_5$aziridine



2a : R = $CH_2CH_2NMe_2$
2b : R = CH_2CH_2OH
2c : R = Me
2d : R = H
2e : R = CH_2COOEt

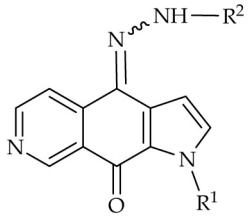


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Figure 1. The structure of azanaphthoquinone annelated pyrrole derivatives.

Table 1. The antiproliferative activities of compounds **1** and **2** on four different human cancer cell lines expressed in IC_{50} (μM).

Cell	IC_{50} (mM)							
	1a	**1b**	**1c**	**2a**	**2b**	**2c**	**2d**	**2e**
KB/HeLa (Cervix)	2.87	1.29	> 12	0.67	0.66	1.21	> 12	> 12
NCl-H460 (Lungs)	> 12	1.28	> 12	0.23	0.13	0.79	> 12	> 12
SF-268 (CNS)	7.12	4.68	> 12	1.23	1.39	2.43	> 12	> 12
SKOV-3 (Ovaries)	8.09	4.34	> 12	0.94	0.48	2.24	> 12	> 12

2. Materials and Methods

^{1}H NMR spectra were recorded on a Bruker AVANCE (300 MHz) spectrometer. The residue of the non-deuterated solvent was used as internal standard which was related to tetramethylsilane with δ = 7.26 ppm for $CDCl_3$. ^{13}C NMR spectra were recorded on a Bruker AVANCE (75 MHz) with the residue of the non-deuterated solvent peak as the internal standard, δ = 77.0 ppm for $CDCl_3$. The IR spectra were recorded on Perkin-Elmer FT-IR spectroscopy Spectrum RXI. Mass spectrometric analyses were recorded on a LC-MS Bruker Daltonics DataAnalysis 3.3. All reactions and atmospheric pressure distillations were performed under a positive pressure of dry nitrogen. Reaction flasks were dried at 120 °C for 2 h and connected with N_2-line when they were still warm. Extracts were dried over anhydrous magnesium sulfate ($MgSO_4$). Solvents were removed by rotary evaporator at water aspirator pressure. A trace amount of solvent was further removed under vacuum (Ca. 0.01 mmHg).

Thin layer chromatography (TLC) was performed with Merck silica gel 60 PF_{254} plate (Merck-Nr 1.05554: 0.2 mm, 20 x 20 cm) or Merck aluminium oxide plate (Merck-Nr 1.05550: 0.2 mm, 20 x 20 cm). Chromatography was performed using Merck silica gel 60, 70-230 mesh ASTM, Nr 1.07734 or aluminium oxide activated basic, 50-200 Micron (Acros Organics Nr 189990010).

2.1 Synthesis of 1-[2-(dimethylamino)ethyl]-1*H*–pyrrolo[3,2-*g*]isoquinoline-4,9-dione (5a)

NaH (60% suspension in mineral oil, 300 mg, 6.7 mmol) was suspended with DMF (3 ml). The mixture was cooled to 0 °C then 1*H*-pyrrolo[3,2-*g*] isoquinoline-4,9-dione (**4**) (493 mg, 2.5 mmol) in DMF (4 ml) was added dropwise. The reaction mixture was stirred at 0 °C for additional 30 min. 2-Chloro-*N,N*-dimethylethylamine hydrochloride (589 mg, 3.8 mmol) in DMF (4 ml) was added dropwise. The reaction mixture was allowed to stir at 67 °C for 4 h. Water (5 ml) was added to quench the reaction and the solvent was removed in *vaccuo*. The crude product was purified by column chromatography (aluminium oxide) eluting with gradient EtOAc to EtOAc/MeOH; 8/2 to give product **5a** (602 mg, 89%) as an orange solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.30 (s, 1H), 9.00 (d, *J* = 5.0 Hz, 1H), 7.90 (d, *J* = 5.0 Hz, 1H), 7.14 (d, *J* = 2.8 Hz, 1H), 6.78 (d, *J* = 2.8 Hz, 1H), 4.67 (t, *J* = 6.5 Hz, 2H), 2.84 (t, *J* = 6.5 Hz, 2H), 2.38 (s, 6H) ppm; ^{13}C NMR (75 Hz, $CDCl_3$): δ = 179.7, 175.4, 154.9, 148.0, 139.2, 133.1, 129.8, 128.7, 126.8, 119.0, 107.2, 59.3, 47.2, 45.5 ppm; IR (KBr Disc): ν_{max} = 2924, 1639, 1616, 1018, 618, 467 cm^{-1} (10).

2.2 Synthesis of 1-(2-(pyrrolidin-1-yl)ethyl)-1*H*-pyrrolo[3,2-g]isoquinoline-4,9-dione (5b)

NaH (60% suspension in mineral oil, 177 mg, 4.4 mmol) was suspended with DMF (3 ml). The mixture was cooled to 0 °C then 1*H*-pyrrolo[3,2-*g*]isoquinoline-4,9-dione (**4**) (323 mg, 1.6 mmol) in DMF (4 ml) was added dropwise. The reaction mixture was stirred at 0 °C for additional 30 min. 2-Pyrrolidinylethylchloride hydrochloride (419 mg, 2.5 mmol) in DMF (3 ml) was added dropwise. The reaction mixture was allowed to stir at 67 °C for 4 h. Water (5 ml) was added to quench the reaction and the solvent was removed in *vaccuo*. The crude product was purified by column chromatography (silica gel) eluting with gradient EtOAc to EtOAc/MeOH; 8/2 to give product **5b** (190 mg, 39%) as an orange solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.36 (s, 1H), 9.01 (d, *J* = 4.8 Hz, 1H), 7.97 (d, *J* = 4.8 Hz, 1H), 7.31 (d, *J* = 2.4 Hz, 1H), 6.80 (d, *J* = 2.4 Hz, 1H), 4.81 (t, *J* = 6.9 Hz, 2H), 3.30 (t, *J* = 6.9 Hz, 2H), 3.02 (br s, 4H), 1.98 (br s, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.7, 175.4, 154.9, 148.0, 139.2, 133.1, 129.8, 128.7, 126.8, 119.0, 107.2, 55.3, 53.9, 47.2, 23.5 ppm; IR (KBr Disc): ν_{max} = 3312, 2924, 1667, 1616, 1498, 1385, 1246, 1054, 707, 614 cm^{-1}. MS: Calcd. for $C_{17}H_{18}N_3O_2$ (M^+ + H): 296.1399; found 296.1090.

2.3 Synthesis of 1-[3-(dimethylamino) propyl]-1*H*–pyrrolo[3,2-*g*]isoquinoline-4,9-dione (5c)

NaH (60% suspension in mineral oil, 611 mg, 15.3 mmol) was suspended with DMF (10 ml). The mixture was cooled to 0 °C then 1*H*-pyrrolo[3,2-*g*] isoquinoline-4,9-dione (**4**) (1.1237 g, 5.7 mmol) in DMF (10 ml) was added dropwise. The reaction mixture was stirred at 0 °C for additional 30 min. 3-Dimethylamino-1-propyl chloride hydrochloride (1.3293 g, 8.3 mmol) in DMF (10 ml) was added dropwise. The reaction mixture was allowed to stir at 67 °C for 18 h. Water (10 ml) was added and the mixture was exhaustively extracted with EtOAc (5x100 ml). The combined organic phase was dried ($MgSO_4$) and removed in *vaccuo*. The crude product was purified by column chromatography (aluminium oxide) eluting with EtOAc to give product **5c** (863 mg, 55%) as a brown solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.32 (s, 1H), 8.95 (d, *J* = 4.8 Hz ,1H), 7.91 (d, *J* = 4.8 Hz,1H), 7.06 (d, *J* = 2.7 Hz, 1H), 6.74 (d, *J* = 2.7 Hz, 1H), 4.48 (t, *J* = 6.9 Hz, 2H), 2.62 (t, *J* = 6.9 Hz, 2H), 2.20 (s, 6H), 2.00 (p, *J* = 6.9 Hz, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.0, 175.0, 154.4, 148.1, 139.2, 132.2, 129.8, 128.9, 126.7, 119.3, 108.2, 55.0, 47.0, 45.0, 29.0 ppm; IR (KBr Disc): ν_{max} = 2923, 1636, 1616, 1248, 1015, 623, 476 cm^{-1}; MS: Calcd. for $C_{16}H_{18}N_3O_2$ (M^+ + H): 284.1399; found 284.1139.

2.4 Synthesis of 1-(3-hydroxypropyl)-1*H*-pyrrolo[3,2-*g*]isoquinoline-4,9-dione (5d)

The solution of 2-(3-bromopropoxy)-tetrahydro-2*H*-pyran (644 mg, 2.9 mmol) in DMF (2 ml) was added dropwise to the mixture of 1*H*-pyrrolo[3,2-*g*] isoquinoline-4,9-dione (**4**) (243 mg,1.2 mmol) and Na_2CO_3 (340 mg, 3.2 mmol) in DMF (6 ml) at 0 °C (12). Then reaction mixture was stirred at 40 °C 18 h. Afterward, water (10 ml) was added and the mixture was exhaustively extracted with EtOAc (5x100 ml). The combined organic phase was dried ($MgSO_4$) and removed in *vaccuo*. The crude product was purified by column chromatography (silica gel) eluting with EtOAc to give alkylated product (182 mg, 55%) which was hydrolyzed by using *p*-TsOH monohydrate (250 mg, 1.32 mmol) in MeOH (5 ml) to furnish compound **5d** in quantitative yield as an orange liquid (13).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.37 (s, 1H), 9.91 (d, *J* = 4.8 Hz, 1H), 8.04 (d, *J* = 4.8 Hz, 1H), 7.16 (d, *J* = 2.7 Hz, 1H), 6.77 (d, *J* = 2.7 Hz, 1H), 4.70-4.50 (m, 2H), 3.95-3.75 (m, 2H), 2.20-2.05 (m, 2H) ppm;

^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.5, 175.0, 154.4, 148.1, 139.6, 132.3, 129.9, 128.9, 127.0, 119.0, 108.2, 63.8, 47.0, 32.0 ppm; IR (KBr Disc): ν_{max} = 3412, 1738, 1657, 1617, 1497, 1377, 1248, 1034, 936, 706, 613 cm^{-1}.

2.5 Synthesis of 1-(4-(hydrobutyl)-1*H*-pyrrolo[3,2-g]iso-quinoline-4,9-dione (5e)

The solution of 2-(4-bromobutoxy)-tetrahydro-2*H*-pyran (284 mg, 1.2 mmol) in DMF (1 ml) was added dropwise to the mixture of 1*H*-pyrrolo[3,2-*g*]isoquinoline-4,9-dione (**4**) (79 mg, 0.4 mmol) and Na_2CO_3 (150 mg, 1.0 mmol) in DMF (2 ml) at 0 °C. Then reaction mixture was stirred at 40 °C 18 h. Afterward, water (10 ml) was added and the mixture was exhaustively extracted with EtOAc (5x100 ml). The combined organic phase was dried ($MgSO_4$) and removed in *vaccuo*. The crude product was purified by column chromatography (silica gel) eluting with gradient EtOAc/Hexane; 6/4 to give alkylated product (56 mg, 40 %) which was hydrolyzed by using *p*-TsOH monohydrate (180 mg, 0.96 mmol) in MeOH (4 ml) to compound **5e** in quantitative yield as an orange liquid.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.34 (s, 1H), 9.00 (d, *J* = 4.8 Hz, 1H), 7.94 (d, *J* = 4.8 Hz, 1H), 7.05 (d, *J* = 2.7 Hz, 1H), 6.77 (d, *J* = 2.7 Hz, 1H), 4.52-4.48 (m, 2H), 3.81-3.71 (m, 2H), 2.05-1.91 (m, 2H) 1.79-1.62 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.5, 175.0, 154.4, 148.0, 139.5, 131.5, 129.9, 128.8, 127.0, 118.9, 108.2, 66.8, 49.5, 28.0, 25.4 ppm; IR (KBr Disc): ν_{max} = 3411, 1637, 1618, 1376, 1245 900-620 cm^{-1}; MS: Calcd. for $C_{15}H_{14}N_2NaO_3$ (M^+ + Na): 293.0902; found 293.0647.

2.6 Synthesis of 4-(2-(2-(dimethylamino) ethyl)hydrazono)-1-(2-(dimethylamino)ethyl)-1*H*-pyrrolo[3,2-*g*] isoquinoline-4,9-dione (6a)

The solution of 2-(*N*,*N*-dimethylamino)ethylhydrazine (156 mg, 1.2 mmol) in EtOH (0.7 ml) was slowly added to the mixture of compound **5a** (276 mg, 1.0 mmol) and triethylamine (0.5 ml, 3.6 mmol) in THF (2 ml) at room temperature (14). The reaction mixture was stirred at room temperature for 2 h. Then the second portion of the hydrazine (156 mg, 1.2 mmol) in EtOH (0.7 ml) was added. The reaction mixture was stirred for an additional 18 h. After that solvent was removed in *vaccuo*. The crude product was purified by column chromatography (Aluminium oxide) eluting with EtOAc to give product **6a** (104 mg, 29%) as an orange liquid.

^{1}H NMR (300 MHz,$CDCl_3$): δ = 9.33 (s, 1H), 8.73 (d, *J* = 5.4 Hz, 1H), 7.74 (d, *J* = 5.1 Hz, 1H), 7.05 (d, *J* = 2.7 Hz, 1H), 6.90 (d, *J* = 2.4 Hz, 1H), 5.65 (br s, 1H), 4.40-4.60 (m, 2H), 2.91 (t, *J* = 6.5 Hz, 2H), 2.66 (t, *J* = 6.5 Hz, 2H), 2.38-2.48 (m, 2H), 2.35 (s, 6H), 2.27 (s, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.0, 157.5, 152.5, 149.5, 142.5, 131.8, 128.5, 126.5, 120.9, 118.5, 107.5, 60.0, 55.8, 47.1, 45.6, 45.4, 42.0 ppm; IR (in MeOH) ν_{max} = 3434, 2911, 2733, 2367, 1651, 1591, 1460, 1376, 1258, 1209, 1040, 919, 723 cm^{-1}; MS: Calcd. for $C_{19}H_{31}N_6O$ (M^+ + 5H): 359.2559; found 359.1944.

2.7 Synthesis of 4-(2-(2-(dimethylamino) ethyl)hydrazono)-1-(2-(pyrrolidin-1-yl)ethyl)-1*H*-pyrrolo[3,2-*g*] isoquinoline-4,9-dione (6b)

The solution of 2-(*N*,*N*-dimethylamino) ethylhydrazine (404 mg, 3.9 mmol) in EtOH (0.7 ml) was slowly added to the mixture of compound **5b** (190 mg, 0.6 mmol) and triethylamine (0.2 ml, 1.5 mmol) in THF (3 ml) at room temperature. The reaction mixture was stirred at room temperature for 2 h. Then the second portion of the hydrazine (410 mg, 3.9 mmol) in EtOH (0.7 ml) was added. The reaction mixture was stirred for an additional 2 h. After that solvent was removed in *vaccuo*. The crude product was purified by column chromatography (aluminium oxide) eluting with EtOAc to give product **6b** (71 mg, 30%) as an orange liquid.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.34 (s, 1H), 8.76 (d, *J* = 5.1 Hz, 1H), 7.77 (d, *J* = 5.1 Hz, 1H), 7.11

(d, J = 2.4 Hz, 1H), 6.42 (d, J = 2.4 Hz, 1H), 4.75-4.45 (m, 2H), 3.17 (q, J = 5.3 Hz, 2H), 2.96 (t, J = 6.9 Hz, 2H), 2.70 (br s, 4H), 2.49 (t, J = 5.7 Hz, 2H), 2.40 (s, 6H), 1.90 (br s, 4H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.0, 157.5, 152.5, 149.5, 142.5, 131.8, 128.5, 126.5, 120.6, 118.5, 107.5, 56.0, 55.9, 54.1, 47.5, 44.7, 42.0, 23.5 ppm; IR (in CH_2Cl_2) ν_{max} = 3376, 2926, 2853, 1651, 1593, 1427, 1399, 913, 762 cm^{-1}; MS: Calcd. for $C_{21}H_{33}N_6O$ (M^+ + 5H): 385.5263; found 385.2137.

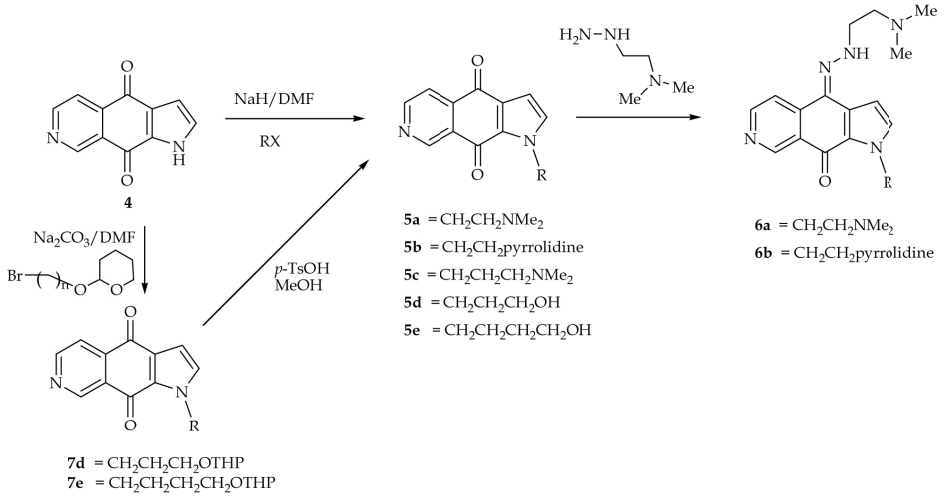
2.8 Biological evaluation

Antiproliferative acitivity of the synthesized compounds was evaluated with human cancer cell line: KB/HeLa (cervical carcinoma). The concentration of compounds that inhibit 50% (IC_{50}) of cell proliferation after 48 h was detected by automatic xCELLigence System (Roche) using the data from at least two independents MTT cytotoxicity assays and reported in μM.

3. Results and Discussion

The core structure azanaphthoquinone annelated pyrrole skeletion; 1*H*-pyrro[3,2-*g*]isoquinoline-4,9-dione (**4**), was synthesized by 3-steps reaction starting form the commercially available 5-hydroxyisoquinonline (10). The side chains were introduced by alkylation reaction of compound **4** with 2 to 4-carbon side chains under a basic condition at 67 ºC (4 h) to give mono-substituted azanaphthoquinone annelated pyrroles **5** in moderate to good yields. The condensation reaction of **5** with hydrazine derivative in THF/EtOH using triethylamine as a base at room temperature (24 h) occurred regioselectively at C-4 position to furnish the target molecules **6** as shown in scheme 1. The shifting of the ^{13}C NMR signal of C-4 in compound **5a** from 179.7 ppm to 157.5 ppm in product **6a** confirms that the hydrazine moiety was condensed into the mono-substituted compound **5a** at C-4. The structure elucidation of **6a** was accomplished by 2D NMR and NOE experiment. Irradiation of methylene group in hydrazone moiety showed a significant nuclear Overhauser enhancement of hydrogen atom at C-5 thus indicating the condensation at C-4. We suggested that the steric effect of the first side chain probably prevent the condensation at C-9.

The condensation of compound **5c**-**5e** was also studied, however it was not successful. We believed that the steric hindrance of the mono-substituted product with 3 and 4-carbon side chains cause the failure of the attachment of the hydrazine side chain.



Scheme 1. The synthetic pathway of azanaphthoquinone annelated pyrrolo hydrazone derivatives.

The biological evaluation of compounds **5a** (10), **5b**, **5c**, **5e**, **6a** and **6b** were screened for antiproliferative activity against human cancer cell line: KB/HeLa (cervical carcinoma) compared to standard compound, Mitoxantrone. The concentration of compounds that inhibit 50% (IC_{50}) of cell proliferation after 48 h were detected by automatic xCELLigence System (Roche) using the data from at least two independent MTT cytotoxic assays. The results of the cytotoxic assays are shown in Table 2. Interestingly, the mono-substituted product with 2-(pyrrolidin-1-yl)ethyl group (**5b**) showed the highest inhibition with IC_{50} value of 0.008 μM. Compound **5c** was found to be the second most active compound with IC_{50} value of 0.141 μM. The amino group is significantly more potent than the one with hydroxyl group. In order to determine whether the hydrazone formation leads to improved cytotoxic activity, compound **6a** and **6b** were included in the screening. The results showed that compound **6a** showed higher inhibitory activity, while compound **6b** showed lower inhibitory activity compared to the mono-substituted derivatives **5**. In comparison to Mitoxantrone, compound **5b**, **5c** and **6a** exhibited remarkable activity. To extend compound library, introducing of 2- and 3-carbon side chains with cyclic amine group such as pyrrolidine and piperidine should be investigated. The study of stability of hydrazone **6a** is in progress.

Table 2. The antiproliferative activities of compounds **5** and **6** on cervical carcinoma: KB/HeLa expressed in IC_{50} (μM).

Compounds	5a	5b	5c	5e	6a	6b	Mitoxantrone
IC_{50} (μM)	11.040	0.008	0.141	7.400	0.282	26.282	0.799

4. Conclusion

In conclusion, a series of mono-substituted azanaphthoquinone annelated pyrroles and the hydrazone derivatives were successfully synthesized. Six synthesized compounds were screened for cytotoxic activity against KB/HeLa cell line. The results showed that this series of compounds exhibited promising cytotoxicity. The mono-substituted product with 2-(pyrrolidin-1-yl)ethyl group (**5b**) showed very good activity with IC_{50} of 0.008 μM. While, the longer side chains exhibited lower activity. Moreover, the hydrazone **6a** show higher activity, while hydrazone **6b** showed lower activity than mono-substituted derivatives **5**. Therefore, the modification of the target molecules needs to be investigated by introducing of 2 and 3-carbon side chains with cyclic amine group. The study of stability of hydrazone **6a** is on going.

5. Acknowledgement

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