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New Synthesised Aminoanthraquinone Derivatives and Its Antimicrobial and Anticancer Activities (Route II)

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Abstract

A series of aminoanthraquinone derivatives were synthesized *via* two reaction steps. The starting material of 1,4-dihydroxyanthraquinone (**1**) was subjected toamination and the major product obtained was then further reacted under reduction, methylation and acylation separately to produce 2-(butylamino)anthracene-1,4-dione (**2**), 2-(butylamino)-1-hydroxy-4-methoxyanthracene-9,10-dione (**9**), 2-(butylamino)-1,4-dimethoxyanthracene-9,10-dione (**10**), 3-(butylamino)-4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1-yl-acetate (**11**), 2-(butylamino)-4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1-yl-acetate (**12**) and 2-(butylamino)-9,10-dioxo-9,10-dihydroanthracene-1-yl-acetate (**13**). Aminoanthraquinone**13** exhibitedstrong antimicrobial activities toward Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Candida albicans* and *Escherichia coli* with MIC values of 0.1, 0.1, 0.1 and 0.5 mg/mL respectively. Aminoanthraquinones **9**, **10** and **13** showed strong cytotoxicity against both MCF-7 (IC_{50} 2.0-11.0μg/mL)and Hep-G2 (IC_{50} 1.1-14.0μg/mL)cell lines.

Keywords: 1,4-Dihyroxyanthraquinone; amination; aminoanthraquinone; substitution; antimicrobial; cyctotoxic, MCF-7, Hep-G2

1. Introduction

1,4-Dihydroxyanthraquinone or quinizarin(**1**) has been largely used as an intermediate for the synthesis of potential drugs. Naturally derived and synthetic anthraquinones, especially their amino derivatives, showed significant biological activities as cited in our previous report [1]. Structural modification of anthraquinone**1** has also been shown increase their bioactivity [1-5].

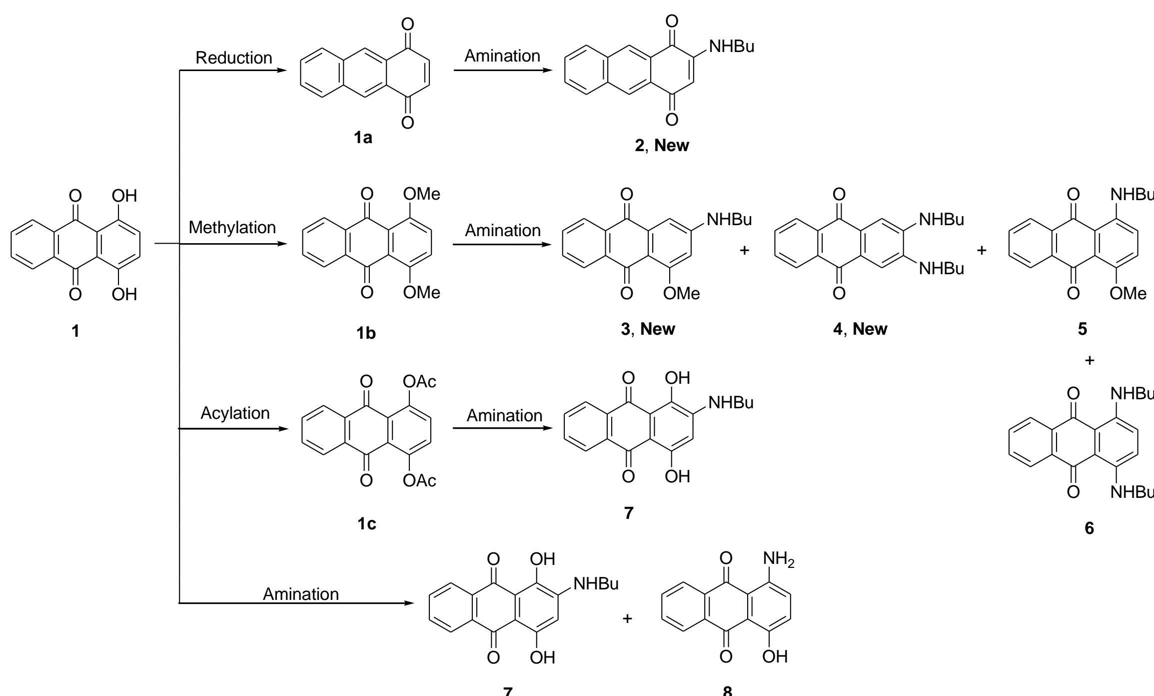
Recently, Wang *et al.* been synthesised naphthalene polyamines, anthracene polyamines and anthraquinone polyamines and evaluated the potential of these compounds as anti-Alzheimer agents, against acetylcholinesterase and butyrylcholinesterase. The anthraquinione polyamines showed good inhibition towards acetylcholinesterase with IC_{50} values of 1.50-11.13 μM whereas naphthalene and anthracene polyamines showed selectivity for butyrylcholinesterase with IC_{50} values of 4.39-47.55 and 0.016-0.657 μM respectively. The inhibition ability was affected by the chain length of the polyamine whereby chain length of four to six carbons in between the two nitrogen atoms were the optimum length that showed the most potent activity against butyrylcholinesterase [6].

Hence by modifying the active site such as the hydroxyl functionality using simple reactions to produce new derivatives of aminoanthraquinone is interesting and worthwhile to be studied. Based on the promising bioactivities, the aim of this work was to synthesise new aminoanthraquinone derivatives with potential as anticancer and antimicrobial agents.It was reported that the shorter amines would reduce the cytotoxic effects [7] whereas the use of diamines or longer amine chains allow the possibility of forming side products [5,6]. Therefore, a simple straight chained butylamine was chosen for this study.

In our previous report, we managed to synthesise four known aminoanthraquinones (**5**, **6**, **7** and **8**) together with three new aminoanthraquinones (**2**, **3** and **4**) *via* two reaction steps. 1,4-Dihydroxyanthraquinone (**1**) was first treated under methylation, acylation or reduction separately and then followed by the attachment of the amino group to the anthraquinone ring *via* amination (named as Route I). The final formation of the aminoanthraquinone derivatives were dependent on the effects of the 1,4-disubstituted anthraquinones whether the substituent was a dihydroxy (**1**), dione (**1a**), dimethyl (**1b**) or diacyl (**1c**) group (Scheme 1). We also proposed that all the aminoanthraquinones were produced through a nucleophilic substitution mechanism [1].

In this report, we used the same type of reaction but in a *vice-versa* sequence (Route II) where the final formation of aminoanthraquinone derivatives were based on the amino groups itself which was installed earlier in the first step (Scheme 2). It is also expected that the reactions proceeded through the same mechanism as established before.The synthesised aminoanthraquinones were then further tested for cytotoxicity and antimicrobial activity.

Scheme 1. Synthesised aminoanthraquinones from Route I.



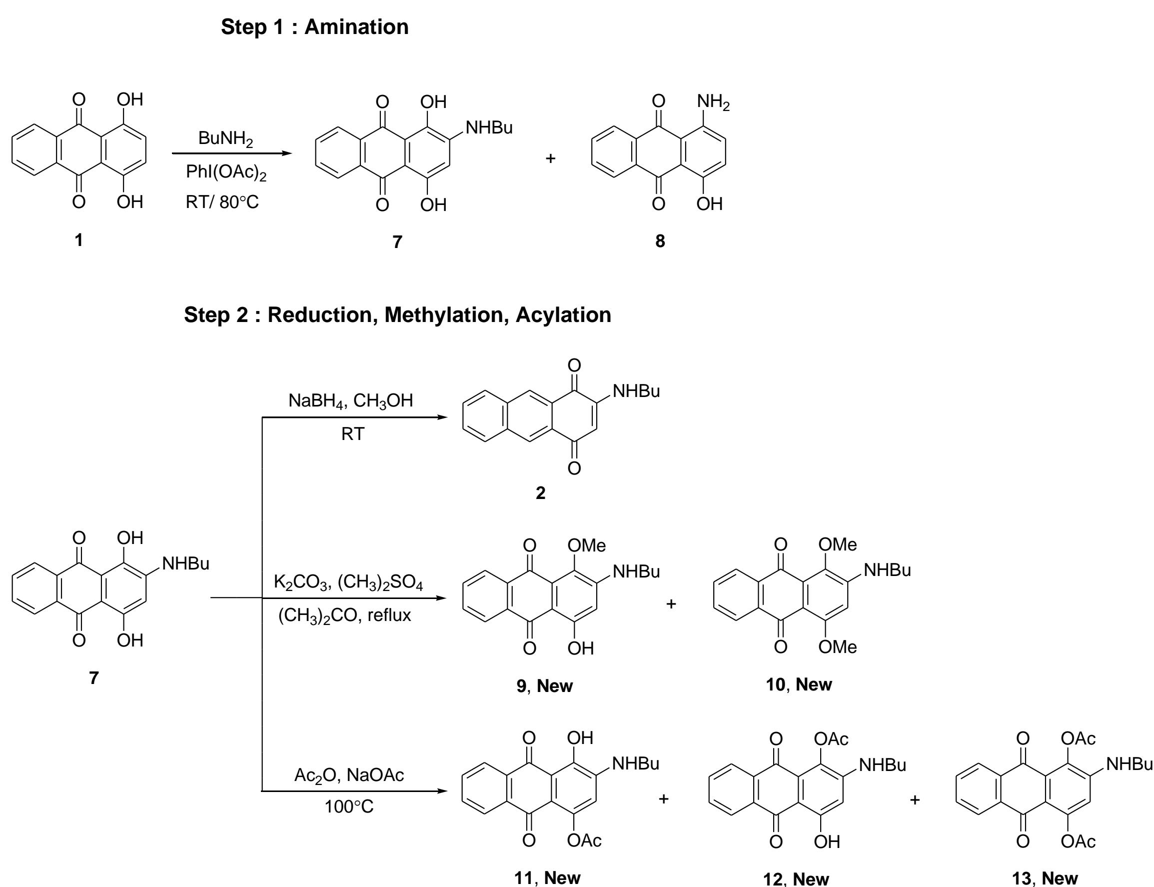
2. Results and Discussion

Aminoanthraquinone derivatives were successfully synthesized *via* two simple reaction steps, an amination and followed with either methylation, reduction or acylation separately to give six anthraquinone derivatives **2**, **9**, **10**, **11**, **12** and **13** (Scheme 2). All new compounds synthesised were elucidated using mp, IR, MS, 1D NMR, 2D NMR and compared with data available in the literature.

*2.1. Amination of 1,4-dihydroxyanthraquinone (**1**)*

Amination reactions were attempted using the procedure reported by Teich*et al.* [7]. Amination of anthraquinone**1** gave a mixture of known products, 1-butylamino-4-hydroxyanthracene-9,10-dione (**7**) and 2-(butylamino)-1,4-dihydroxyanthracene-9,10-dione (**8**) in overall 70-90% yield (Scheme 2). The effects of catalyst, reaction time, the proposed mechanism and the spectroscopic data have been published by our group recently [1]. The position of substituted butylamino and OH groups in **8** were confirmed by HMBC analysis where it showed the ^{3}J correlation between H-1' with C-2, OH-1 with C-9a and C-2, OH-4 with C-4a, C-3 and also H-3, with C-4 and C-4a (Figure 1). The major product of aminoanthraquinone **7** was chosen for the second reaction step.

Scheme 2. Two-step synthesis of aminoanthraquinones (Route II).



*2.2. Reduction, Methylation and Acylation of Aminoanthraquinone (**7**)*

Applying the method reported by Hua *et al*., reduction of 2-(butylamino)-1,4-dihydroxyanthracene-9,10-dione (**7**) in the presence of 5 equiv. of $NaBH_4$ was unsuccessful [8]. Even though a higher amount of reducing agent (15 equiv.) was used, only a small amount of **2** was obtained (3% yield) with 89% of starting material **7** being recovered in the reaction. In comparison, amino derivative **2** was also obtained in moderate yield (46-60%) from Route I where the opposite sequence was applied towards anthraquinone**1**. This is probably due to the early substitution of NHBu group which was stabilised by the resonance of the ring through the internal H-bonding between OH, NH and C=O groups which allowed compound **7** to be more stable compared to **1** (Figure 2). Spectroscopic data for compound **2** has been discussed previously.The position of the butylaminoand the ketone groupsof **2** was confirmed by the HMBC experiment and the additional selected correlations areindicated in Figure 1.

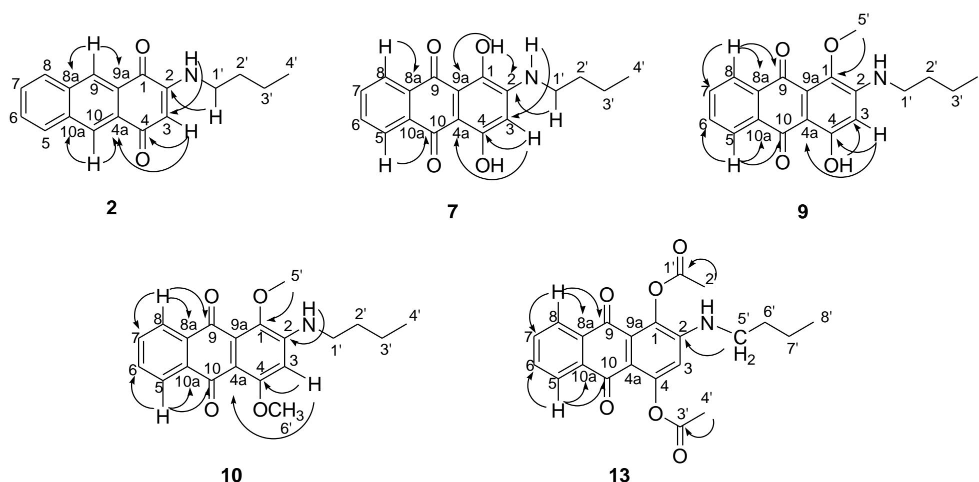


Figure 1. Selected HMBC correlations of aminoanthraquinones**2**,**7**,**9**,**10**and**13**.

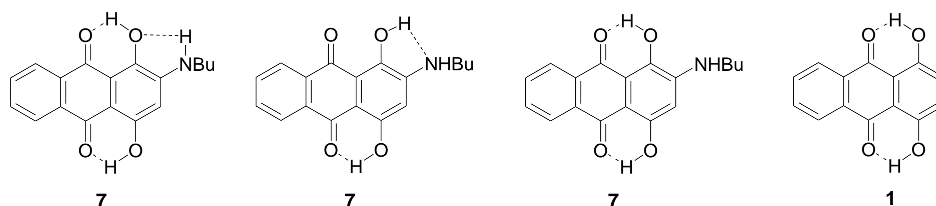


Figure 2. Internal H-bonding in aminoanthraquinones **1** and **7**.

The methylation of **7** with $(CH_3)_2SO_4$in the presence of K_2CO_3 was accomplished in 2 hours of reaction in acetone at 60°C using a procedure adapted from Sugimoto *et al.* [9]. Compounds 2-(butylamino)-1-hydroxy-4-methoxyanthracene-9,10-dione (**9**) and 2-(butylamino)-1,4-dimethoxyanthracene-9,10-dione (**10**), both as orange powders, were obtained in 32% and 25% yields respectively.It is believed that the compound **9**is produced first as an intermediate before further reaction to produce compound **10**. Increasing the reaction time to 24 hours gave only the dimethylated product of aminoanthraquinone **10** in 63% yields.

The IR spectrum of aminoanthraquinone **9** indicated a broad absorbance around 3373 cm^{-1} probably due to an overlap of OH and NH functional groups whereas for the aminoanthraquinone **10**, a sharp band was observed corresponding to the NH group only. The EIMS analysis gave a molecular ion peak of *m/z* 352 for compound **9** and *m/z* 339 for compound **10** which matched with the calculated molecular weight of $C_{19}H_{19}NO_4$ and $C_{20}H_{21}NO_4$ respectively.

The ^{1}H NMR spectrum of aminoanthraquinone **9** showed the presence of one chelated OH proton at δ14.10 ppm, proving that only one hydroxy group was methylated. The existence of methoxy protons were observed as a singlet at δ3.87 ppm. Signals for H-3 and NH were assigned at δ6.26 and5.52 ppm respectively. According to the ^{13}C NMR of **9**, 19 carbons were identified where five signals at higher field represented the methoxy and butyl carbons at 42.8, 30.9, 20.3, 13.9 and 61.0 ppm for C-1', C-2', C-3', C-4' and C-5' respectively.

The DEPT analysis supported the existence of two methyls, three methylenes, five methines and nine quaternary carbons in the structure of compound **9**. The COSY analysis showed the correlation between methyl and methylenes of butylamino and also the correlation of H-6 and H-7 to H-5 and H-8. Further HMBC experiments confirmed that the H-5' (δ 3.87 ppm) of methoxy had a ^3J correlation with C-1 (δ 142.9 ppm) whereas hydroxy proton had a ^2J correlation with C-4 (δ 164.1 ppm) and a ^3J correlation with C-3 (δ 100.4 ppm) and C-4a (δ 105.3 ppm). The ^4J correlation was also observed indicating the interaction between OH (δ 14.09 ppm) and C-2 (δ 150.9 ppm)(Figure 1).

The ^{1}H spectrum for **10** showed the disappearance of both chelated hydroxy protons and the presence of two methoxy groups which were proven by the existence of six protons of two methyl groups at δ 3.97 and δ3.83 ppm. The ^{13}C NMR spectrum gave twenty carbons where two signals at δ 184.4 and δ180.0 ppm corresponded to carbonyl groups and two

signals at δ 61.0 and δ56.5 ppm were assigned to the methoxy carbons. The COSY showed similar correlations as compound **9**. The position of substituted dimethoxy groups was established by HMBC analysis and the selected correlations were shown in Figure 1. It was observed that H-3 had a ^{2}J correlation with C-2 and C-4 and also a ^{3}J correlation with C-4a and C-1. The ^{3}J interactionbetween H-5 with C-10 and H-8 with C-9 were also seen in the spectrum.

Treatment of compound **7** with excess amount of Ac_2O in the presence of NaOAc at high temperatures produced a mixture of monoacylated aminoanthraquinones, 3-(butylamino)-4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1-yl-acetate (**11**) and 2-(butylamino)-4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1-yl-acetate (**12**)together with the diacylated aminoanthraquinone, 2-(butylamino)-9,10-dioxo-9,10-dihydroanthracene-1-yl-acetate (**13**).**C**ompounds **11**, **12** and **13** were obtained in 25, 34 and 15% yields respectivelywhen the acylation was performed for 7 hours. Increasing the reaction time to 24 hours also increased the yield of **13**(81%) with a small amount of **12** (5%) and no trace of **11**.

The presence of ester and ketone functionalities of **7**, **8** and **9** were proven by IR data which showed strong bands around 1772 and 1667 cm^{-1}. This was supported by the MS spectrum that gave an*m/z* of353 that matched with the calculated M^{+}, $C_{20}H_{19}NO_5$ for compounds **11** and **12**.On the other hand, the MS spectrum of compound **13** showed an *m/z* of 395 that matched the molecular formula of $C_{22}H_{21}NO_6$.

The ^{1}H NMR analysis of both **11** and **12** showed the presence of one methyl of the acetyl group at δ 2.43 and δ 2.47 ppm and also a chelated proton of the OH group at δ 13.09 and δ 13.47 ppm respectively. The OHpeak of compound **11** that was next to the substituted NHBu appeared at lower chemical shifts due to more internal H-bonding compared to the structure of **12**. However in the ^{1}H NMR spectrum of **13**, two methyls of the acetyl group were observed at δ 2.50 and δ 2.46 ppm with no trace of the chelated proton at the very low field region. Based on the ^{13}C NMR spectra from all compounds, the value at δ 170.0 ppm was assigned to the C=O of ester whereas C=O of ketones that are close to the acetyl group appeared at δ 180.9 ppm for compound **11**, δ 180.6 ppm for compound **12**, δ 181.3 and δ 181.1 ppm for compound **13**.

The HMQC experiment of **13** showed the correlation between H-3', H-2' and H-4' to their respective carbons. The HMBC analysis of **13** gave a ^{3}J correlation between H-5 and H-8

with C-9 and C-10 together with a correlation of H-5’ with C-2. The ^{2}J correlation was also observed between H-2’ and H-4’ with C-1’and C-3’ respectively (Figure 1).

2.3. Biological activity

Antimicrobialactivityof the compounds were carried out using the disc diffusion test against four types of microbes,Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa, Candida albicans and Escherichia coli*and then followed by obtaining the minimum inhibition concentration (MIC). Cytotoxic assays were conducted on Human estrogen receptor (ER+) positive breast cancer (MCF-7) and human hepatocarcinoma (Hep-G2) cell lines. Due to insufficient amounts of compounds **11** and **12**, only aminoanthraquinones **9**, **10** and **13** were subjected to the antimicrobial and cytotoxic assays. The biological activities of compound **2** have been reported earlier by our group and the results showed that compound **2** was not active towards all four microbes but it exhibited very strong cytotoxicity towards MCF-7 and Hep-G2 cell lines with IC_{50} values of 1.1 and 1.2 μg/mL respectively.

In this work, only aminoanthraquinone**13** with concentrationsof 0.5-1.0 mg/mL inhibited the growth of all four microbes. Lower concentrations only affected the growth of *P. aeruginosa*. Further MIC testson **13** showed good inhibition with minimum concentration values in range of 0.1-0.5 mg/mL (Table 1). Based on the previous and current data, it can be concluded that the absence of methoxy group (compounds **2** and **6**) or the presence of monomethoxy (compounds **5** and **9**) or dimethoxy groups (compound **10**) did not inhibit the growth of microbes. Only an acyl substituted aminoanthraquinone **13** showed potential as an antimicrobialagent.

Table 1. Antimicrobial analysis of aminoanthraquinone **13**.

	Diffusion diameter (cm)			
Bacteria/Yeast Concentration (mg/mL)	**MRSA**	***P. aeruginosa***	***C. albicans***	***E. coli***
0	-	-	-	-
0.1	-	0.6	-	-
0.5	0.7	0.7	0.5	0.6
1.0	0.9	0.8	0.6	0.7
MIC (mg/mL)	0.1	0.1	0.1	0.5

Table 2. Cytotoxic activity of aminoanthraquinones**9**,**10**and**13**.

Aminoanthraquinone	IC_{50} (*μ*g/mL)	
	MCF-7	**Hep-G2**
9	7.0	14.0
10	11.0	9.0
13	2.0	1.1

The aminoanthraquiniones**9**, **10** and **13** were active against both cell lines tested. Compound **13** showed very strong activity with IC_{50} values of 2.0 and 1.1 *μ*g/mL for MCF-7 and Hep-G2 cell lines respectively. Increasing the number of methoxy groups (compound **10**) also increased the activity against Hep-G2 cell line but decreased the cytotoxicity against the MCF-7 cell line. In contrast, aminoanthraquinone **9** showed an opposite trend. Both dimethylated and diacetylated aminoanthraquinones **10** and **13** were more active towards Hep-G2 compared to MCF-7. Therefore it can be concluded that the presence of amino substituent together with the methoxy or carboxyl groups enhanced the cytotoxic activity of the compounds and have potential use as anticancer agents.

3. Experimental Section

3.1 General

Allchemicals and solvents are commercially available and were of analytical grade and used without purification unless otherwise stated. All reactions were monitored by TLC, using Silica gel 60 F245 (Merck KGaA) precoatedaluminium backed plates and visualised by UV and H_2SO_4 solution. All organic extracts were dried over Na_2SO_4 and evaporated using a rotary evaporator. Column chromatography was performed on silica gel 60 (100-150 mesh). Melting points wererecorded by digital melting point equipment (Electrothermal IA9000 Series). The IR spectra were obtained by Perkin-Elmer FT-IR Model Spectrum 100 series spectrophotometer using UATR techniques and the adsorption bands were measured in the range of 280-4000 cm^{-1}. MS spectra were recorded using Shimadzu model QP5050A series. The1D NMR and 2D NMR spectra were run ona JEOL machine at 400 MHz or 500 MHz.Chemical shifts, δ were recorded in ppm relative to TMS signal. The coupling constants J are given in Hz.

*3.2 Amination of **1***

Butylamine (0.94 mol) was added dropwise to a mixture of**1** (1.0 g, 4.2mmol) and $PhI(OAc)_2$ (4.6mmol) at RT and stirred for 6 hours. The reaction mixture, 10 M HCl(25 mL) and $NaHCO_3$ (84 mL) were added to ice cold water (84 mL) successively. The resulting solution was extracted with EtOAc (3 x 25 mL). The organic layer was washed with H_2O (3 x 25 mL), dried and evaporated. The crude product was then chromatographed on silica gel (DCM-petroleum ether, 4:1) to give compounds **7** and **8**.

2-(Butylamino)-1,4-dihydroxyanthracene-9,10-dione (**7**). Dark pink powder (176.0 mg, 90%); mp 158.0-158.4ºC; ν_{max} (UATR) 3378, 3308, 2949, 1635, 1565, 1516, 1459, 1417, 1260, 1156 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 14.18 (1H, s, O*H*), 13.83 (1H, s, O*H*), 8.23 (1H, d, *J* 6.9 Hz, *H*-aromatic), 8.19 (1H, d, *J* 6.9 Hz, *H*-aromatic), 7.71 (1H, t, *J* 5.8 Hz, *H*-aromatic), 7.64 (1H, t, *J* 5.8 Hz, *H*-aromatic), 5.98 (1H, s, *H*-aromatic), 5.53 (1H, br. s, N*H*), 3.13-3.19 (2H, m, $NHCH_2CH_2$), 1.62-1.70 (2H, m, $CH_2CH_2CH_2$), 1.41-1.47 (2H, m, $CH_2CH_2CH_3$), 0.98 (3H, t, *J* 6.9 Hz, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 183.2, 177.8, 166.5, 153.7, 147.7, 134.6, 134.0, 132.3, 132.2, 126.4, 126.2, 110.3, 102.9, 100.2, 42.7, 30.7, 20.3, 13.8; *m/z* (EIMS) 311 (M^+, $C_{18}H_{17}NO_4$ requires 311).

1-(Butylamino)-4-hydroxyanthracene-9,10-dione (**8**). Dark purple powder (31.5 mg, 38%); mp 127-128ºC (Lit. 122-123ºC [10]); IR (UATR) 3170, 2954, 2926, 2853, 1616, 1580, 1463, 1233, 1162 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 13.71 (1H, s, O*H*) 10.29 (1H, br. s, N*H*), 8.30 (2H, t, *J* 8.0 Hz, *H*-aromatic), 7.77 (1H, t, *J* 5.7 Hz, *H*-aromatic), 7.70 (1H, t, *J* 5.7 Hz, *H*-aromatic), 7.20 (2H, d,*J* 4.6 Hz,*H*-aromatic), 3.36 (2H, t, *J* 6.9 Hz, $NHCH_2CH_2$), 1.70-1.77 (2H, m, $CH_2CH_2CH_2$), 1.47-1.55 (2H, m, $CH_2CH_2CH_3$), 0.99 (3H, t, *J* 6.9 Hz, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 187.5, 182.0, 156.8, 147.7, 135.5, 134.2, 132.7, 132.5, 129.0, 126.7, 126.4, 124.1, 113.8, 108.4, 42.7, 31.6, 20.4, 13.9; *m/z* (EIMS) 295 (M^+, $C_{18}H_{17}NO_3$ requires 295).

*3.3 Reduction of**7***

Compound **7** (0.1 g, 0.32 mmol) was dissolved in MeOH (3 mL) and stired homogenously under nitrogen atmosphere. $NaBH_4$ (3.2 mmol) was added slowly to the reaction mixture and stirred for 22 hours. Another 1.6 mmol of $NaBH_4$ was added to the reaction mixture and stirred for another 2 hours. Water (0.5 mL) was added to the reaction mixture and the pH was

adjusted to 5-6 with 1M HCl. Methanol was evaporated and the residue of the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layer was dried, filtered and evaporated under vacuum. The crude product was purified by column chromatography (DCM-petroleum ether, 4:1) to produce compound **2**.

2-(Butylamino)anthracene-1,4-dione (**2**). Yellow powder (3 mg, 3%); mp 173.4-173.9°C; ν_{max} (UATR) 3325, 2926, 1677, 1573, 1504, 1456, 1406, 1319, 1250 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 8.55 (1H, s, *H*-aromatic), 8.54 (1H, s, *H*-aromatic), 7.98 (2H, t, *J* 7.8 Hz, *H*-aromatic), 7.61 (2H, dt, *J*6.9, 14.9 Hz, *H*-aromatic), 6.00 (1H, br. s, N*H*), 5.84 (1H, s, C*H*=C), 3.19 (2H, q,*J* 6.9 Hz, $NHCH_2CH_2$), 1.65-1.70 (2H, m, $CH_2CH_2CH_2$), 1.39-1.47 (2H, m, $CH_2CH_2CH_3$), 0.97 (3H, t, *J* 6.9 Hz, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 182.7, 181.5, 149.1, 135.8, 134.0, 130.2, 130.0, 129.8, 129.7, 129.1, 128.7, 127.7, 127.6, 102.7, 42.4, 30.4, 20.3, 13.8; *m/z* (EIMS) 279 (M^+, $C_{18}H_{17}NO_2$ requires 279).

3.4 Methylation of ***7***

A mixture of **7** and, K_2CO_3 (3.53 mmol) and $(CH_3)_2SO_4$ (4.5 mmol) in acetone (4 mL) was refluxed for 2 hours. The cooled reaction mixture was added to 25 mL 2 M HCl and extracted with EtOAc (3 x 25 mL).The organic layer was dried and evaporated. The crude product was purified by column chromatography (DCM-petroleum ether, 3:1) to produce compounds **9** and **10**.

2-(Butylamino)-1-hydroxy-4-methoxyanthracene-9,10-dione(**9**). Orange powder (33.1 mg, 32%); mp 118.2-118.6°C; ν_{max} (UATR) 3373, 2928, 1665, 1581, 1412, 1352, 1235, 1152, 1002 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 14.09 (1H, s, O*H*), 8.23 (1H, d, *J* 6.9 Hz, *H*-aromatic), 8.21 (1H, d, *J* 6.9 Hz, *H*-aromatic), 7.64-7.67 (2H, m,*H*-aromatic), 6.26 (1H, s, *H*-aromatic), 5.52 (1H, br. s, N*H*), 3.87 (3H, s, OCH_3) 3.22 (2H, q,*J* 6.9 Hz, $NHCH_2CH_2$), 1.64-1.71 (2H, m, $CH_2CH_2CH_2$), 1.40-1.49 (2H, m, $CH_2CH_2CH_3$), 0.97 (3H, t, *J* 6.9 Hz, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 184.7, 182.8, 164.1, 150.9, 142.9, 134.4, 134.4, 133.7, 133.3, 127.1, 126.1, 122.7, 105.3, 100.4, 61.0, 42.8, 30.9, 20.3, 13.9; *m/z* (EIMS) 325 (M^+, $C_{19}H_{19}NO_4$ requires 325).

2-(Butylamino)-1,4-dimethoxyanthracene-9,10-dione(**10**). Orange powder (68.3 mg, 63%); mp 152.2-152.4ºC; ν_{max} (UATR) 3330, 2924, 1662, 1581, 1464, 1358, 1233 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 8.18 (1H, d, *J* 8.0 Hz, *H*-aromatic), 8.10 (1H, d, *J* 6.9 Hz, *H*-aromatic), 7.66 (1H, t, *J* 6.9 Hz, *H*-aromatic), 7.60 (1H, t, *J* 6.9 Hz, *H*-aromatic), 6.33 (1H, s, *H*-aromatic), 5.30 (1H, br. s, N*H*), 3.97 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.19 (2H, q, *J* 6.9 Hz, $NHCH_2CH_2$), 1.62-1.69 (2H, m, $CH_2CH_2CH_2$), 1.39-1.49 (2H, m, $CH_2CH_2CH_3$), 0.95 (3H, t, *J* 6.9 Hz, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 184.7, 182.8, 164.1, 150.9, 142.9, 134.4, 134.4, 133.7, 133.3, 127.1, 126.1, 122.7, 105.3, 100.4, 61.0, 42.8, 30.9, 20.3, 13.9; *m/z* (EIMS) 339 (M^+, $C_{20}H_{21}NO_4$ requires 339).

3.5 Acylation of **7**

A mixture of compound **7**(0.42 mmol), Ac_2O (14 mmol), and NaOAc (0.42 mmol) was stirred at 100°C for 24 h. The resulting solution was poured to ice cold water (25 mL) and then extracted with CH_2Cl_2 (3 x 25 mL). The combined organic layer was dried and evaporated under vacuum. The crude product was purified by column chromatography(DCM-petroleum ether, 3:1) to produce compound **11**, **12** and **13**.

3-(Butylamino)-4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1-yl-acetate (**11**). Orange oil (28.3 mg, 25%); ν_{max} (UATR) 3017, 1773, 1667, 1591, 1429, 1174 cm^{-1}; δ_H (500 MHz, $CDCl_3$)13.09 (1H, s, O*H*), 8.29 (1H, dd, *J*9.2,4.6 Hz, *H*-aromatic), 8.22 (1H, dd, *J*9.2,4.6 Hz, *H*-aromatic), 7.81 (2H, t, *J* 4.6 Hz, *H*-aromatic), 7.24 (1H, br. s, N*H*), 7.20 (1H, s, *H*-aromatic), 2.43 (3H, s, $COCH_3$), 1.87-92 (2H, m, $NHCH_2CH_2$), 1.47-1.54 (2H, m, $CH_2CH_2CH_2$), 1.26-1.34 (2H, m, $CH_2CH_2CH_3$), 0.89 (3H, t, *J*6.9 Hz, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 118.1, 180.9, 170.0, 169.1, 161.4, 157.3, 135.3, 134.9, 134.5, 134.0, 132.3, 127.7, 127.3, 126.9, 115.8, 30.3, 22.6, 21.1, 20.2, 13.8; *m/z* (EIMS) 353 (M^+, $C_{20}H_{19}NO_5$ requires 353).

2-(Butylamino)-4-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1-yl-acetate (**12**). Yellow oil (39.1 mg, 25%); ν_{max} (UATR) 3015, 1772, 1667, 1590, 1366, 1170 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 13.47 (1H, s, O*H*), 8.29 (1H, dd, *J* 5.7, 2.2 Hz, *H*-aromatic), 8.22 (1H, dd, *J* 5.7-2.2 Hz, *H*-aromatic), 7.81 (2H, t, *J* 4.6 Hz, *H*-aromatic), 7.30 (1H, br. s, N*H*), 7.24 (1H, s, *H*-aromatic), 2.47 (3H, s, $COCH_3$), 1.89-1.95 (2H, m, $NHCH_2CH_2$), 1.46-1.55 (2H, m,

$CH_2CH_2CH_2$), 1.26-1.35 (2H, m, $CH_2CH_2CH_3$), 0.88 (3H, t, J6.9 Hz, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 188.8, 180.6, 170.0, 169.6, 157.7, 142.9, 138.4, 135.4, 134.4, 134.1, 133.0, 132.3, 127.6, 123.2, 117.1, 30.2, 22.4, 21.2, 20.1, 13.9; *m/z* (EIMS) 353 (M^+, $C_{20}H_{19}NO_5$ requires 353).

2-(Butylamino)-9,10-dioxo-9,10-dihydroanthracene-1,4-diyl diacetate(**13**). Yellow powder (81.3 mg, 64%); mp 103.4-104.1ºC; ν_{max} (UATR) 3535, 2936, 1772, 1667, 1587, 1361, 1168, 1011 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 8.17 (2H, dd, J 5.8, 3.5Hz, *H*-aromatic), 7.77 (2H, dd, J 5.7, 3.4Hz, *H*-aromatic), 7.30 (1H, br. s, N*H*), 5.29 (1H, s, *H*-aromatic), 2.50 (3H, s, $COCH_3$), 2.46 (3H, s, $COCH_3$), 1.87-91 (2H, m, $NHCH_2CH_2$), 1.46-1.54 (2H, m, $CH_2CH_2CH_2$), 1.26-1.33 (2H, m, $CH_2CH_2CH_3$), 0.89 (3H, t, J 6.9 Hz, CH_2CH_3); δ_C (125 MHz, $CDCl_3$) 181.3, 181.1, 170.0, 169.3, 168.7, 168.4, 145.4, 134.5, 134.4, 133.4, 133.3, 127.6, 127.2, 127.0, 125.9, 53.5, 30.2, 22.7, 21.2, 21.1, 20.1, 13.8; *m/z* (EIMS) 395 (M^+, $C_{22}H_{21}NO_6$ requires 395).

3.6 Antimicrobial test

Nutrient agar (20 g) was suspended in 1 L of distilled water and stirred. It was boiled to dissolve homogenously and autoclaved at 121°C for 20 min. The agar was allowed to cool to 50°C and poured into sterile disposable petri dishes. Four microbes, methicillin-resistant *Staphylococcus aureaus* (MRSA), *Pseudomonas aeruginosa*, *Candida albicans* and *Escherichia coli* were inoculated into prepared nutrient broth and incubated at 37°C for overnight. The suspension of the microbes in the broth was inoculated on the nutrient agar using sterile cotton bud. The sterile 6 mm paper discs were impregnated with the synthesised compounds in concentrations of 20, 10, 5, 2, 1, 0.5 and 0.1 mg/mL and allowed to soak for 1 min. The paper discs were removed, dried and placed on the surface of agar plates inoculated with the microbial cultures. Each synthesised compound was tested in triplicate. Paper discs impregnated with acetone were used as a negative control. The petri dishes were incubated in an inverted position at 37°C for 24 h. The zones of inhibitions (clear area without bacterial growth) were measured in cm [11].

The minimum inhibitory concentration (MIC),is considered the lowest concentrationof the sample which inhibits the visible growth of microbe. The compound was dissolved in a minimum quantity of acetone to get a stock solution (20 mg/mL). MRSA, *P. aeruginosa*,

C.albicans and *E. coli* seeded broth were prepared in nutrient broth. Compound dilutions were made serially from the stock solution to produce 0.1, 0.5, 0.1 and 0 mg/mL used as control. The tubes were incubated in bath shaker at 37ºC for 24 hours and the MIC values were recorded by obtaining the optical densities of each broth using the UV spectrophotometer at 600 nm [12].

3.7 Cytotoxic Assays

The MCF-7 (estrogen receptor positive human breast) and Hep-G2 (human hepatocellular liver carcinoma) cancer cells were purchased from ATCC. The cells were grown and maintained in RPMI 1640 media, supplemented with 10% fetal calf serum (FCS) and 1% antibiotic penicillin-streptomycin in an atmosphere of 5% CO_2 at 37°C. Themedium was used to dilute the cells toaconcentration of 5×10^5cells/mL. From this cells suspension, 100 μL of various concentrations of the synthesised compounds were pipetted into a 96-well micro titer plate and incubated in 37°C, 5% CO_2 incubator for 72 h. The various concentration used were 100, 50, 25, 12.5, 6.25, 3.125, 1.56μg/mL. The assay of each concentration of synthesised compounds was performed in triplicate and the control wells of untreated population were also included. After three days, the fraction of surviving cells was determined relative to the untreated cells population by the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method where the viability of cells was measured by 20 μL of blue formazan crystals of MTT solution (5 mg/mL in phosphate-buffered saline, PBS) added to each well followed by incubation in 37°C, 5% CO_2 incubator for 3-4 h. 100 μL of cells suspension or cells monolayer in each microtiter was removed from each well. The plate was left at room temperature for 30 min before reading the absorbance. The absorbance was read with the multiwell scanning spectrophotometer (ELISA reader) test wavelength of 570 nm and reference wavelength of 630 nm. The cytotoxic index used was IC_{50} which is the concentration that yields 50% inhibition of the cells compared with untreated control [13].

4. Conclusions

Five new aminoanthraquinone derivatives (**9**, **10**, **11**, **12** and **13**) were successfully synthesised from the amination and either reduction, alkylation or acylation of 1,4-dihydroxyanthraquinone. Aminoanthraquinone **13** showed strong antimicrobial activity against four microbes tested (MRSA, *P. aeruginosa*, *C. albicans* and *E. coli*).

Aminoanthraquinones **9**, **10** and **13** showed strong cytotoxic activity towards both MCF-7 and Hep-G2 cell lines.

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