Investigation of the Antimicrobial and Anticancer Activity of Aminonaphthoquinones

Kevin W. Wellington,[a*] Natasha I. Kolesnikova,[a] Nomgqibelo B.P. Nyoka[b] and Lyndy J. McGaw[b]

[a]CSIR Biosciences, P O Box 395, Pretoria, South Africa

[b]Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Sciences, University of Pretoria, Onderstepoort 0110, South Africa.

kwwellington@gmail.com

Tel.: +2712-841-3933; fax: +2712-841-2195

Abstract

In this study we report on the inhibitory activity of synthesized aminonaphthoquinones against two bacterial and one fungal species to determine their antimicrobial properties. A minimum inhibitory concentration (MIC) of 7.8 µg/mL was obtained against the fungus, *Candida albicans*, which was better than that of Amphotericin B (MIC = 31.25 µg/mL). *Escherichia coli* (Gram -), was inhibited at a MIC of 23.4 µg/mL and *Staphylococcus aureus* (Gram +) at a MIC of 31.3 µg/mL. The aminonaphthoquinones were also screened against HCT116 colon, PC3 prostate and HepG2 liver cancer cell lines to evaluate their cytostatic effects. They had potent activity (GI_{50} = 5.87-9.90 µM) which was about 3-6-fold better than that of parthenolide (GI_{50} = 25.97 µM) against the prostate cancer cell line. These compounds were generally more selective for cancer cells than for normal human lung fetal fibroblasts (WI-38).

Keywords

Aminonaphthoquinones; antibacterial activity; antifungal activity; minimum inhibitory concentration; anticancer activity.

1. INTRODUCTION

According to the WHO, antimicrobial resistance threatens the successful prevention and treatment of a continually growing range of infections caused by bacteria, fungi, parasites and viruses (WHO). Standard treatments of antimicrobial drugs are ineffective against infections caused by resistant microorganisms that are adept at enduring assault by antibacterial drugs (e.g. antibiotics), antifungals, antivirals, and antimalarials, thus increasing the risk of spread to others. Infections from resistant microorganisms trigger prolonged illness, higher health care costs, and an immense threat of mortality (WHO).

Prostate cancer is the second most prevalent cancer in men globally. Around 910 000 cases of cancer were as a result of prostate cancer in 2008 and the disease accounts for around 14% of all new cancer cases in men. It is predicted that the number of cases will almost double (to 1.7 million) by 2030 (WCRF; Globocan). Surgical or radiation therapy is used to cure prostate cancer in its early stage. There is currently no curative treatment option for patients that are diagnosed with a locally advanced or metastatic disease (Albertsen et al. 2008; So et al. 2005). There is thus an urgent need for drugs that can prevent and/or treat prostate cancer metastasis.

Colorectal cancer is the third most common cancer, the fourth leading cause of cancer-related death and is responsible for about 1 400 000 new cases and about 700 000 deaths globally (Arnold 2016). Chemotherapy and radiotherapy are often additional treatments due to surgical

resection being insufficient for many patients. Unfortunately the commonly used chemotherapeutic agents are toxic and have adverse side effects and potentially curative chemotherapy may be declined by the patient (Schnell 2003; Venook 2005). Improved treatments with fewer adverse effects and reduced or no toxicity are required for patients suffering from colorectal cancer.

In 2012, 782 000 new cancer cases were attributed to liver cancer (hepatocellular carcinoma) which is the sixth most common cancer in the world (WCRF liver cancer). Patients with liver cancer only have two medical treatments available to them. One is resection for non-cirrhotic patients, and the other is liver transplantation for cirrhotic patients (Breitenstein et al. 2009). High rates of recurrence after resection coupled with poor prognosis have led to most patients with an advanced form of the disease not being eligible for surgery (Zender et al. 2006). Development of resistance to chemotherapy has further increased mortality rates. Novel drugs that can by-pass resistance mechanisms are thus required.

The 1,4-naphthoquinones are the most significant and broadly dispersed chemical class in the quinone family. Their derivatives have been reported to have a range of biological activities such as antiallergic (Lien et al. 2002), antibacterial (Yildirim et al. 2017; Janeczko et al. 2018; Novais et al. 2018), anticancer (Benites et al. 2018; Manickam et al. 2018), dual anticancer and antibacterial (Bayrak et al. 2017), antifungal (Huang et al. 2002), anti-inflammatory (Tandon et al. 2004; Sasaki et al. 2002), antithrombotic (Jin et al. 2004; Yuk et al. 2000), antiplatelet (Lien et al. 2002; da Silva et al. 2002), antiviral (Inbaraj and Chignell 2004; Yuk et al. 2000; Tandon et al. 2002; Ilina et al. 2002), apoptotic (Kim et al. 2003a; Kim et al. 2003b; Gao et al. 2004), lipoxygenase inhibitory (Richwien and G. Wurm 2004; Wurm and S. Schwandt 2003, radical scavenging (Song et al. 2000) and anti-ringworm (Inbaraj and Chignell 2004) activities.

In previous work we reported on the laccase-catalyzed synthesis of aminonaphthoquinones and their anticancer activity against the MCF7 breast, HeLa cervical, UACC62 melanoma, and TK10 renal cancer cell line (Wellington et al. 2012). The results of this previous study prompted us to investigate the anticancer activity of the aminonaphthoquinones against HCT116 colon, HepG2 liver and PC3 prostate cancer cell lines. The broad biological activities of the 1,4-naphthoquinones led us to investigate the antibacterial and antifungal properties of the aminonaphthoquinones against a fungal strain (*C. albicans* ATCC 10231) and two bacterial strains (*E. coli* ATCC 25922, *S. aureus* ATCC 29213).

2. MATERIALS AND METHODS

2.1. Synthesis

The synthesis of the aminonaphthoquinones **2-12** has been previously reported (Wellington et al. 2012).

2.2. Determination of the in vitro antibacterial and antifungal activity

The growth inhibitory effects of the compounds were tested in triplicate (with the entire assays repeated to confirm results) against *Staphylococcus aureus* (Gram +, [ATCC 29213]), *Escherichia coli* (Gram -, [ATCC 25922]) and *Candida albicans* (ATCC 10231). A broth

microdilution assay was used to determine the minimum inhibitory concentration (MIC) for each of the compounds.

2.2.1 In vitro antibacterial activity

Antibacterial activity was carried out following the procedure of Eloff (1998) with some modifications.

2.2.2 In vitro antifungal activity

The method of Masoko et al. (2005) was used to conduct antifungal screening of the samples.

2.3 Determination of the anticancer activity and cytotoxicity

2.3.1 Anticancer evaluation

Assay background

The growth inhibitory effects of the compounds were tested in triplicate in a 3-cell line panel consisting of colon (HCT116), prostate (PC3) and liver (HepG2) cancer cells by the SRB assay. The SRB assay was developed by Skehan and colleagues to measure drug-induced cytotoxicity and cell proliferation (Skehan et al. 1990).

2.3.2 Cytotoxicity evaluation

The cytotoxic effects of the compounds were tested in triplicate using the Sulforhodamine B (SRB) assay on the human fetal lung fibroblast WI-38 "normal cell line". Three parameters such as 50% cell growth inhibition (GI_{50}), total cell growth inhibition (TGI) and the lethal concentration that kills 50% of cells (LC_{50}) were also determined during the screening process which was done in triplicate. Emetine, a natural product alkaloid known for its toxicity, was used as a standard (Akinboye and Bakare 2011).

2.4 Lipophilicity

The commercially available program, ACD/LogP, was used to calculate the lipophilicity parameters (Log *P*) of compounds **1-12**. The Log *P* values for each of the aminonaphthoquinones are shown in Table 1.

3. RESULTS

3.1 Determination of the in vitro antibacterial and antifungal activity

3.1.1 Antibacterial and antifungal activity of the aminonaphthoquinones

Compound **1** is the substrate that was modified in the laccase-catalysed reaction to afford a series of aminonaphthoquinones **2-12**. The structures of the synthesized compounds are shown in Figure 1.

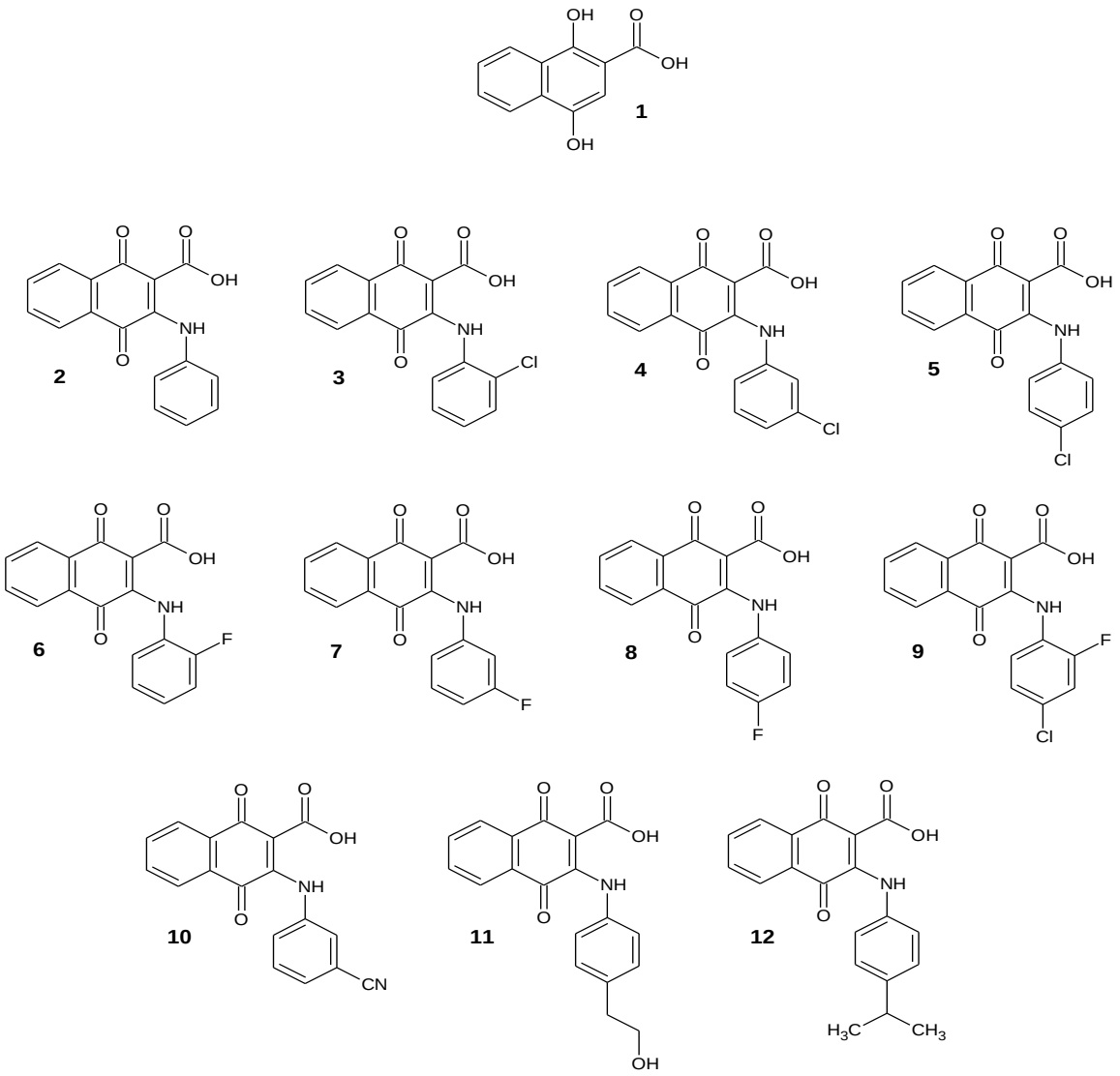

Figure 1. The 1,4-naphthohydroquinone **1** and the synthesized aminonaphthoquinones **2-12** (Wellington et al. 2012).

The results of the screening are shown in Table 1.

Table 1. *In vitro* antifungal and antibacterial activity of the aminonaphthoquinones expressed as MIC values (μg/mL) and the calculated log *P* values.

Compound	*E. coli* (Gram –)	*S. aureus* (Gram +)	*C. albicans*	Log *P*
1	187.5	**62.5**	**62.5**	2.79 ± 0.26
2	**62.5**	125	**62.5**	2.41 ± 1.00
3	125	125	**31.3**	2.43 ± 1.00
4	**31.3**	**62.5**	125	2.95 ± 1.00
5	125	> 250	125	2.91 ± 1.00
6	**62.5**	**46.9**	**7.8**	1.88 ± 1.00
7	**62.5**	**31.3**	**62.5**	2.41 ± 1.00
8	125	**31.3**	**62.5**	2.37 ± 1.00
9	**62.5**	250	**15.6**	2.43 ± 1.00
10	**93.8**	125	**31.3**	2.03 ± 1.00
11	**93.8**	125	125	1.06 ± 1.00
12	**23.4**	**31.3**	**62.5**	3. 26 ± 1.00
Gentamicin	**7.81**	**3.91**	**-**	-1.89 ± 0.66
Amphotericin B	-	-	**31.25**	0.78 ± 0.83

Potent activity: MIC ≤ 10 μg/mL; Moderate activity: 11 ≤ MIC ≤ 100; Weak activity: MIC > 100 μg/mL.

3.1.2 Lipophilicity

For the determination of a direct correlation between lipophilicity and inhibitory activity, the calculated lipophilicity value of each of the aminonaphthoquinones (Table 1) was compared with the antibacterial and antifungal activities.

From the data in Table 2 compound **12** has the highest lipophilicity (log *P* = 3.26 ± 1.00) and compound **11**, the lowest (1.06 ± 1.00). The log *P* values of compounds **10**, **3**, **5** and **7** having potent activity, are in the range 2.01 ± 1.00 to 2.01 ± 1.00. Two of the compounds, **10** (log *P* = 2.03 ± 1.00**)** and **7** (log *P* = 2.41 ± 1.00), have log *P* values almost equivalent to that of parthenolide (log *P* = 2.42 ± 0.42).

3.2 Anticancer activity

The compounds were screened in triplicate against HCT116 colon, PC3 prostate and HepG2 liver cancer cell lines using the Sulforhodamine B (SRB) assay (Skehan et al. 1990). The anticancer agent, parthenolide, was used as a positive control because it induces cytotoxicity in colorectal, prostate and liver cancer cell lines (Hayashi et al. 2011; Zhang et al. 2004; Carlisi et al. 2010). Three parameters such as 50% cell growth inhibition (GI_{50}), total cell growth inhibition (TGI) and the lethal concentration that kills 50% of cells (LC_{50}) were determined during the screening process (Table 2).

Table 2. *In vitro* anticancer screening against HCT116 colon , PC3 prostate and HepG2 liver cancer cell lines as well as normal human fetal lung fibroblasts (WI-38) expressed as GI_{50}, TGI and LC_{50} values (μM).

	HCT116 colon				PC3 prostate				HepG2 liver				WI-38 Lung Fibroblast			
Cpd	**GI_{50}**	**TGI**	**LC_{50}**	**SI**	**GI_{50}**	**TGI**	**LC_{50}**	**SI**	**GI_{50}**	**TGI**	**LC_{50}**	**SI**	**GI_{50}**	**TGI**	**LC_{50}**	**Log *P****
1	N.D.	N.D.	N.D.	N.D	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.79 ± 0.26
2	57.76	>100	>100	0.8	34.75	>100	>100	1.4	42.11	75.62	>100	1.1	48.10	99.27	>100	1.92 ± 1.00
3	55.47	>100	>100	1.3	**9.22**	65.85	96.29	3.1	33.74	65.01	96.29	0.9	28.74	64.36	99.98	2.43 ± 1.00
4	54.88	>100	>100	0.9	**27.81**	84.98	>100	1.9	40.17	69.16	98.16	1.3	51.67	91.22	>100	2.95 ± 1.00
5	54.12	>100	>100	0.6	**9.90**	65.52	>100	3.2	**29.80**	61.49	93.18	1.1	32.13	77.06	>100	2.91 ± 1.00
6	54.84	>100	>100	1.1	**16.06**	99.45	>100	3.8	**19.72**	58.51	97.30	3.1	60.36	>100	>100	1.88 ± 1.00
7	56.21	>100	>100	0.5	**5.95**	43.69	99.54	5.0	**13.62**	50.17	86.72	2.2	29.50	66.97	>100	2.41 ± 1.00
8	58.74	>100	>100	0.6	**16.50**	91.76	>100	2.2	34.73	66.12	97.50	1.1	37.00	85.67	>100	2.37 ± 1.00
9	70.14	>100	>100	1.0	42.32	>100	>100	1.7	42.66	73.80	>100	1.7	71.56	>100	>100	2.43 ± 1.00
10	51.43	>100	>100	0.2	**5.87**	33.85	87.80	1.5	**13.74**	52.83	91.92	0.6	**8.80**	45.31	86.90	2.03 ± 1.00
11	57.53	>100	>100	0.9	34.26	>100	>100	1.6	**29.64**	66.28	>100	1.8	54.62	>100	>100	1.06 ± 1.00
12	55.45	>100	>100	0.9	47.59	>100	>100	1.1	37.91	75.33	>100	0.7	51.46	96.04	>100	3.26 ± 1.00
Parth	**35.67**	61.82	87.98	-	**25.97**	54.77	83.57	-	**6.96**	38.11	95.03	-	-	-	-	2.42 ± 0.42
Emetine	0.07	0.98	>100	-	0.05	0.45	>100	-	<0.01	0.10	>100	-	<0.01	0.06	0.64	4.85 ± 0.60

Compound = cpd; N.D. = not determined; Path = parthenolide; Potent Activity, p: GI_{50} or TGI < 10 μM; Moderate Activity, m: < 30 μM GI_{50} or TGI > 10 μM; Weak Activity, w: > 30 μM GI_{50} or TGI < 100 μM; Inactive, i: GI_{50} or TGI > 100 μM; Value > 100 indicates absence of activity;* Calculated; SI = selectivity Index.

3.2.3 Cytotoxicity evaluation

The cytotoxic effects of the compounds were determined by screening against a normal human fetal lung fibroblast cell line (WI-38) using the SRB assay. Three parameters such as 50% cell growth inhibition (GI_{50}), total cell growth inhibition (TGI) and the lethal concentration that kills 50% of cells (LC_{50}) were determined in triplicate. Emetine, an alkaloid known for its toxicity, was used as a standard (Akinboye and Bakare 2011). The selectivity index (SI) values were determined in order to evaluate the selectivity of the compounds for cancer cells over normal human lung fibroblasts and the results are shown in Tables 1 and 2.

4. DISCUSSION

4.1 Determination of the in vitro antibacterial and antifungal activity

From the results in Table 1 it is evident that the 1,4-naphthohydroquinone acid **1** and the aminonaphthoquinones **2-12** had both antibacterial and antifungal activity. *E. coli* was more susceptible to these compounds than *S. aureus* additionally *C. albicans* was the most susceptible of all the test organisms. The weakest activity (187.5 μg/mL) was observed for **1** against *C. albicans* while the best activity (7.8 μg/mL) was observed for **6** against *E.coli*.

Compound **12** had the best activity against *E. coli* (MIC = 23.4 μg/mL) followed by **4** (MIC = 31.3 μg/mL). The activity of compound **12** was only about 3-fold less than that of gentamicin (MIC = 7.81 μg/mL). Compounds **2**, **6 7** and **9** had the same activity (MIC = 62.5 μg/mL) and compounds **10** and **11** also had similar activity (MIC = 93.8 μg/mL). The inhibitory activities of most of the compounds were less than 100 μg/mL.

The best inhibitory activity against *S. aureus* was 31.3 µg/mL for compounds **7**, **8** and **12** which is about 10-fold less than that of gentamicin (MIC = 3.91 µg/mL). The next best activity was that of compounds **6** (MIC = 46.9 µg/mL), and **1** and **4** that had the same activity (MIC = 62.5 µg/mL).

Compound **6** had the best and most potent activity (7.8 µg/mL) against *C. albicans* which was about 4-fold better than that of amphotericin B (MIC = 31.25 µg/mL). This was followed by **9** (MIC = 15.6 µg/mL) which had activity about 2-fold better than that of amphotericin B. Compounds **3** and **10** had the same inhibitory activity (MIC = 31.3 µg/mL) as that of amphotericin B. Compounds **1**, **2**, **7**, **8** and **12** all had the same activity (MIC = 62.5 µg/mL) which was 2-fold less than that of amphotericin B. The inhibitory activities of most of the compounds were also less than 100 µg/mL against *C. albicans*.

4.1.2 Structure-activity relationship study for antibacterial and antifungal activity

In order to determine whether there was a structure-activity relationship (SAR), the activities of the compounds against the bacterial and fungal strains were analysed to identify the functional groups that are essential for activity. In Figure 2 below the SAR for *C. albicans* is shown.

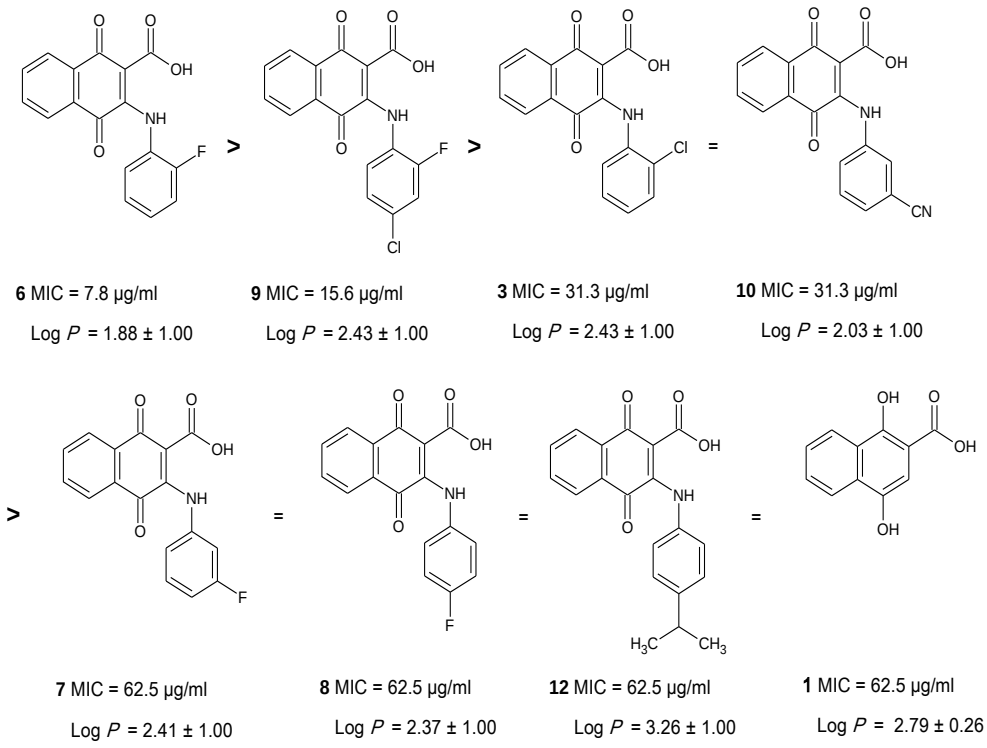

Figure 2. The SAR of the aminonaphthoquinones for *C. albicans*.

Compound **6**, with a fluoro group on the phenyl ring, had potent antifungal activity (MIC = 7.8 µg/mL). When a chloro group is added in the *para* position of the phenyl ring in **6** to afford **9**, the activity decreased to moderate (MIC = 15.6 µg/mL). The replacement of the fluoro group in

6 with a chloro group to afford **3** did not result in equivalent activity to that of **6** but rather a decrease (MIC = 31.3 µg/mL) . The addition of a nitrile group in the *meta* position of the phenyl ring as in **10** afforded the same activity. When a fluoro group was added in the *meta* position of the benezene ring as in **7**, the activity decreased (MIC = 62.5 µg/mL). The addition of a fluoro group as in **8** and the addition of an isopropyl group as in **12**, both in the *para* position on the phenyl ring, also afforded the same activity as that of **7** (62.5 µg/mL). The results showed that mono-substitution with a F atom at the *ortho*-position of the aminobenzene ring afford compound **6** with the most potent activity whereas those with a F substitution at the *para*- and *meta*-position (compounds **7** and **8**) exhibited decreased activities. Furthermore, a substitution with other functional groups (CN, Cl, and isopropyl) at the same or at a different position, also lead to decreased activities of the compounds.

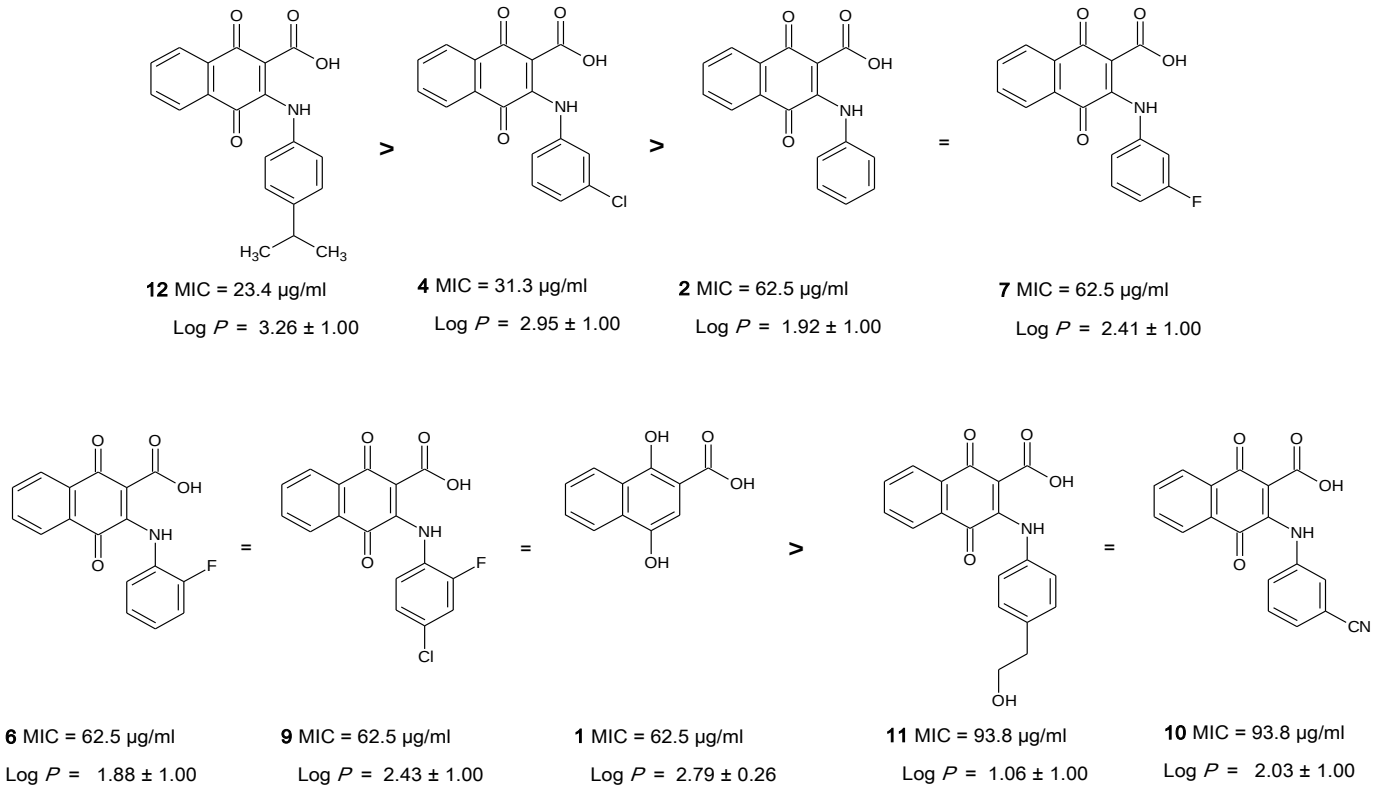

Figure 3. The SAR of the aminonaphthoquinones for *E. coli*.

The SAR of the aminonaphthoquinones for *E. coli* is shown in Figure 3. Compound **12**, having an isopropyl group in the *para* position on the phenyl ring, had the best activity (MIC = 23.4 µg/mL). The next best activity was that of **4** having a chloro group in the *meta* position on the phenyl ring which resulted in a decrease in activity to 31.3 µg/mL. A further decrease in activity to 62.5 µg/mL occurred when there was no substituent on the phenyl ring as in **2**. The addition of a fluoro group in the *meta* position on the phenyl ring of **2** afforded **7** which gave the same activity (MIC = 62.5 µg/mL) and not that of **4** (MIC = 31.3 µg/mL). A change in the position of the fluoro group from *meta* in **7** to *ortho* in **6** and the addition of a chloro group to the *para*

position of **6** to afford **9** also afforded the same activity (MIC = 62.5 µg/mL). The presence of an ethyl hydroxyl group in the *para* position of the phenyl ring as in **11** resulted in a further decrease in activity to 93.8 µg/mL. A nitrile group in the *meta* position of the benzene ring as in **10** afforded the same activity and not that of **4** (MIC = 31.3 µg/mL).

From the results it is evident that 12 having an isopropyl group at the *para* position on the aminobenzene ring gave the best activity and that substitution with other functional groups (CN, Cl, F and ethyl hydroxyl) at the same or at a different position, also lead to decreased activities of the compounds.

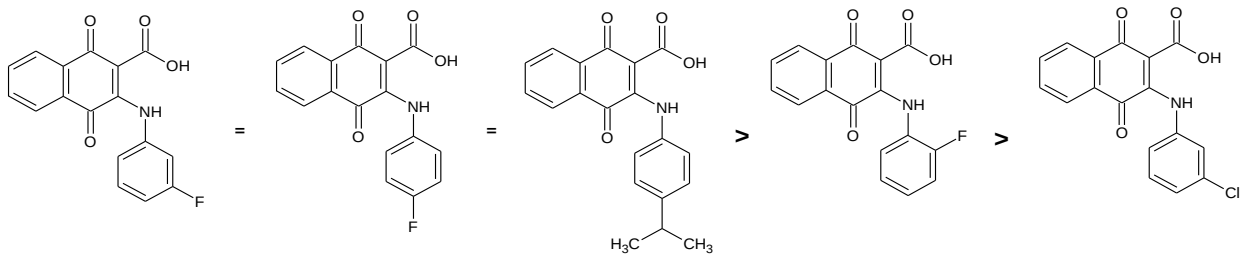

Figure 4. The SAR of the aminonaphthoquinones for *S. aureus*.

From the SAR of the aminonaphthoquinones for *S. aureus* (Figure 4) it can be seen that compounds **7** and **8** having fluoro groups in the *meta* and *para* positions on the phenyl ring respectively, and **12** having an isopropyl group in the *para* position, had the best activity (MIC = 31.3 µg/mL). A fluoro group in the *ortho* position on the phenyl ring resulted in a decrease in activity to 46.9 µg/mL. The replacement of the fluoro group in **7** with a chloro group as in **8** resulted in a further decrease in activity to 62.5 µg/mL.

It thus evident that fluoro and isopropyl groups on the phenyl ring favour inhibitory activity against *S. aureus*.

4.2 Lipophilicity

LogP is the most commonly used measure of lipophilicity and is defined as the partition coefficient of a molecule between an aqueous and lipophilic phase, usually octanol and water (Silverman 1992). *LogP* is used to predict drug-likeness (Lipinski 2004). Small molecule drugs in particular are dependent on lipophilicity to cross biological membranes through passive transport. Pharmacological activity is affected by lipophilicity in addition to pharmacokinetic properties such as absorption, distribution, metabolism, excretion, and toxicity (ADME/Tox) (Silverman 1992).

It is evident from the results that there is not a direct correlation between the calculated log *P* values of the aminonaphthoquinones and the inhibitory activity i.e. there is not an increase in inhibitory activity as the lipophilicity increases and vice versa.

4.3 Anticancer activity

From the results in Table 2 it is evident that the aminonaphthoquinones exhibit anticancer activity and were most effective against the prostate cancer cell line based on the GI_{50} values.

The compounds had weak activity against the colon cancer cell line which was also weaker than that of parthenolide (GI_{50} = 35.67 μM). Several compounds (**10**, **7**, **3** and **5**) had potent activity (GI_{50} = 5.87-9.90 μM) against the PC3 prostate cancer cell line which was about 3-6-fold better than that of parthenolide (GI_{50} = 25.97 μM). Compounds **10** and **7** are at least 4-fold more active than parthenolide. Compounds **4**, **6** and **8** had moderate activity and the rest only had weak activity. Only compounds **10** (TGI = 33.85 μM) and **7** (TGI = 43.69 μM) had a better TGI than that of parthenolide (TGI = 54.77 μM).

Several compounds, **5-7**, **10**, and **11,** only had moderate activity (GI_{50} = 13.62-29.80 μM) against the liver cancer cell line which was weaker than that of parthenolide (GI_{50} = 6.96 μM).

4.3.1 Structure-activity relationships for anticancer activity

From the SAR of the aminonaphthoquinones for the prostate cancer cell line (Figure 5) the best activity (GI_{50} = 5.87 μM) was observed for compound **10** having a nitrile group in the *meta* position on the phenyl ring. Substituting the nitrile group in **10** with a fluoro group to afford **7** resulted in a slight decrease in activity (GI_{50} = 5.95 μM). A chloro functional group in the *ortho* position on the phenyl ring as in **3** (GI_{50} = 9.22 μM) and another in the *para* position as in **5** (GI_{50} = 9.90 μM), resulted in a further decrease in activity.

The replacement of the chloro group in **3** with a fluoro group did not give equivalent activity, instead the activity decreased from potent (GI_{50} = 9.22 μM) to moderate (GI_{50} = 16.06 μM**)** by more than 50%. The replacement of the chloro group in **5** with a fluoro group as in **6** also did not give equivalent activity, instead the activity decreased from potent (GI_{50} = 9.90 μM) to moderate (GI_{50} = 16.50 μM**)** by more than 50%. The replacement of the fluoro group in **7** with a chloro group as in **4** also did not give equivalent activity. Here too, the activity decreased from potent (GI_{50} = 5.95 μM) to moderate (GI_{50} = 27.81 μM**)** but this time by about 4.5-fold.

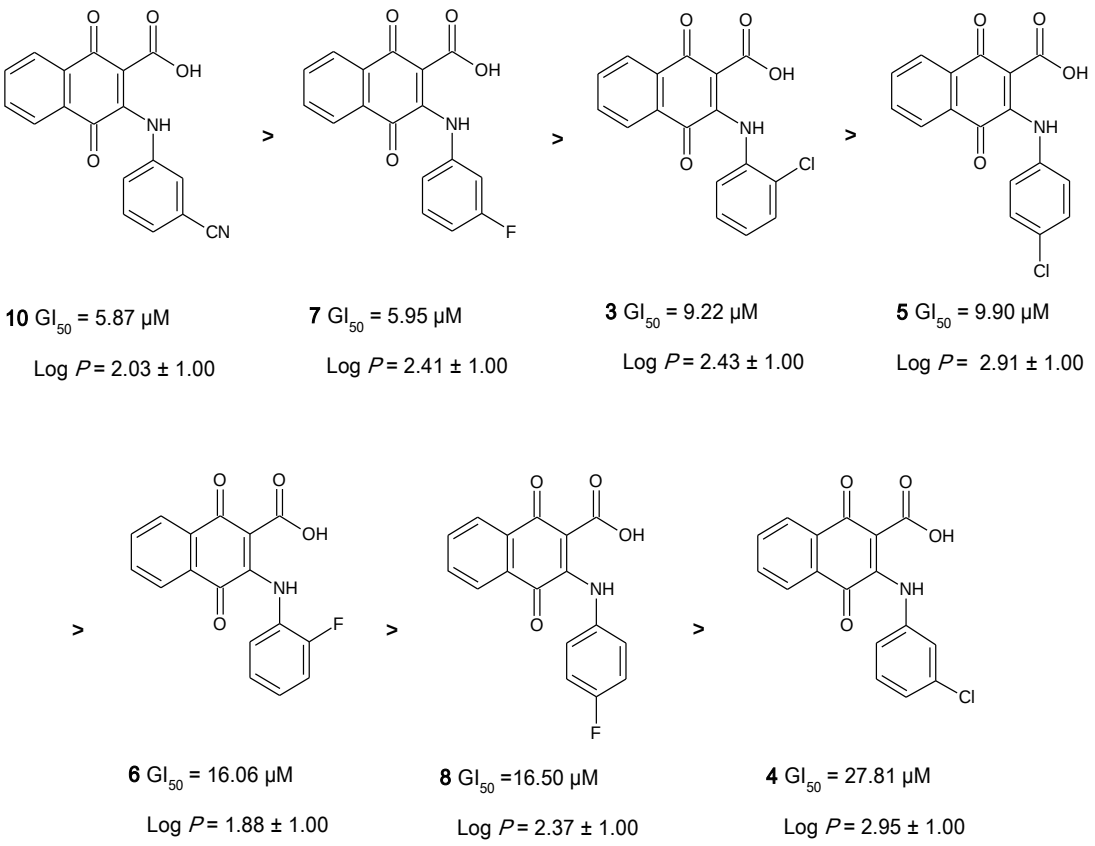

Figure 5. The SAR of the aminonaphthoquinones for the PC3 prostate cancer cell line.

Amongst the compounds having fluoro groups on the phenyl ring, **7** (GI_{50} = 5.95 μM, *meta* fluoro group) afforded potent activity while **6** (GI_{50} = 16.06 μM *ortho* fluoro group**)** and **8** (GI_{50} =16.50 μM, *para* fluoro group**)** only afforded moderate activity. A different trend was observed for the compounds having chloro groups on the phenyl ring, **3** (GI_{50} = 9.22 μM, *ortho* chloro group) had slightly better potent activity than **5** (GI_{50} = 9.90 μM, *para* chloro group) and both these compounds had much better activity than **4** (GI_{50} = 27.81 μM, *meta* chloro group).

From these results it is evident that compounds having chloro (*ortho* and *para* position), fluoro (*meta* position) and nitrile (*meta* position) groups on the phenyl ring favour potent activity against the prostate cancer cell line.

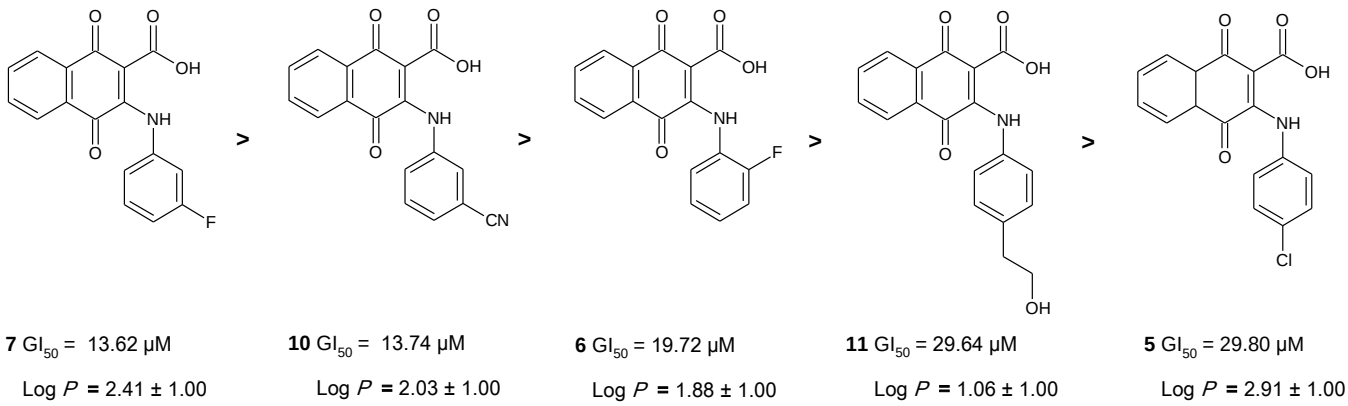

Figure 6. The SAR of the aminonaphthoquinones for the HepG2 liver cancer cell line.

Only moderate activity was obtained against the HepG2 liver cancer cell line for the aminonaphthoquinones. It is evident from the SAR for HepG2 liver cancer in Figure 6 that a fluoro group in the *meta* position on the phenyl ring as in **7** afforded the best activity (GI_{50} = 13.62 μM). The replacement of the fluoro group with a nitrile group as in **10** led to a slight decrease in activity (GI_{50} = 13.74 μM). A change in the position of the fluoro group from *meta* as in **7** to *ortho* as in **6** resulted in about a 50% decrease in activity. A chloro and an ethyl hydroxyl group both in the *para* position of the phenyl ring as in **11** (GI_{50} = 29.64 μM) and **5** (GI_{50} = 29.80 μM) respectively, led to more than a 100% decrease in activity when compared with **7** having the *meta* fluoro group.

From these results it can be seen that for anticancer activity for both the PC3 prostate cancer and HepG2 liver cancer cell lines, the fluoro, chloro and nitrile groups, in either the *ortho* or *meta* position on the phenyl ring, are essential for activity and that the SAR is similar. The PC3 prostate cancer cell line was more susceptible to **7** (*meta* fluoro group) and **10** (*meta* nitrile group) than the HepG2 liver cancer cell line. Compound **10** (*meta* nitrile group) was the most active against the PC3 prostate cancer cell line and **7** (*meta* fluoro group) most active against HepG2 liver cancer cell line.

4.3.2 Lipophilicity

The results in Table 2 indicate that that there is not a direct correlation between the anticancer activity of these compounds and their lipophilicity (calculated log *P* values).

4.4 Cytotoxicity evaluation

The selectivity index (SI) values were determined in order to evaluate the selectivity of the compounds for cancer cells over normal human lung fibroblasts and the results are shown in Table 2.

Compound **10** is slightly more selective for prostate cancer than for the human lung fetal fibroblasts. Compound **3** is at least 3-fold more selective for prostate cancer cells while compound **7** is at least 5-fold more selective for prostate cancer than for fibroblasts.

From these results it is evident that the aminonapthoquinones are not as cytotoxic as emetine against the fibroblasts. Overall, these compounds are generally more active against prostate cancer cells than against normal human fetal lung fibroblasts.

4.5 Possible mechanisms of action

The cytotoxic activity of quinones is due to a series of effects such as reactive oxygen species (ROS) generation, inhibition of electron transporters, uncoupling of oxidative phosphorylation protein, DNA damage, and adduct formation particularly with enzyme SH groups (Rahmoun et al. 2013; Freitas et al. 2012; Silva-Jr et al. 2011). Two major mechanisms have been identified. One is the generation of the semiquinone radical after one-electron reduction of the quinone ring and its participation in a redox cycle to give potent ROS (superoxide anion radical and hydrogen peroxide). The other is that quinones, as potent electrophiles, are able to react with the thiol group of glutathione with depletion of its reduced form and enhancement of oxidative stress (Castro et al. 2008).

5. CONCLUSIONS

The aminonaphthoquinones have antibacterial, antifungal and anticancer properties. They have potent activity (MIC = 7.8 μg/mL) against the fungus, *C. albicans*. Based on the SAR study it was determined that a fluoro group in the *ortho* position on the aminobenzene ring affords potent activity against *C. albicans*. The aminonaphthoquinones also have potent cytostatic effects (GI_{50} = 5.87-9.90 μM) against the PC3 prostate cancer cell line and moderate cytostatic effects against the HepG2 liver cancer cell line. The compounds are also more selective for the prostate cancer cell line than for normal human lung fibroblasts (WI-38). These results inspire further studies which will focus on improving the biological activity and also determining the mechanism of action of these compounds against the bacterial and fungal strains and also the cancer cell lines.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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