Investigation of the antibacterial and antifungal activity of thiolated naphthoquinones

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Abstract

The WHO has stated that antibiotic resistance is escalating to perilously high levels globally and that traditional therapies of antimicrobial drugs are futile against infections caused by resistant microorganisms. Novel antimicrobial drugs are therefore required. We report in this study on the inhibitory activity of the 1,4-naphthoquinone-2,3-bis-sulfides and 1,4-naphthoquinone sulfides against two bacteria and a fungus to determine their antimicrobial properties. The 1,4-naphthoquinone sulfides have potent activity with a minimum inhibitory concentration (MIC) of 7.8 µg/mL against *Staphylococcus aureus* (Gram +ve), an MIC of 23.4 µg/mL against the fungus, *Candida albicans*, which was better than that of Amphotericin B (MIC = 31.3 µg/mL), and against *Escherichia coli* (Gram −ve) an MIC of 31.3 µg/mL was obtained. The 1,4-naphthoquinone had an MIC of 11.7 µg/mL against *S. aureus* and the 1,4-naphthohydroquinone also had the same activity against *E. coli*.

1. INTRODUCTION

Quinones are a class of natural and synthetic compounds that have several beneficial effects, are aromatic, widespread in nature, and include pigments, antibiotics, vitamins and coenzymes (Castro, Mariani, Panek, Eleutherio, & Pereira, 2008; El-Najjar et al., 2011). Quinones have a wide range of pharmacological activity and have been found in antibacterial, antifungal, anticancer, and antimalarial agents (Janeczko, Demchuk, Strzelecka, Kubiński, & Masłyk, 2016; Polovkovych et al., 2016; Ramirez, Motta-Mena, Cordova, & Garza, 2014; Schuck et al., 2013). Compounds containing thiol have a broad spectrum of biological actions and have generated wide research interest. Some sulfide and sulfoxide quinones have been reported to have antifungal activity (De Paiva, Lima, Figueiredo, & Kaplan, 2004) and synthetic thiol containing derivatives of 1,4-naphthoquinone have been reported as potent antimicrobial and anticancer agents (Braud et al., 2008; Buchkevych, Stasevych, Chervetsova, Musyanovych, & Novikov, 2012; Ibis et al., 2013; Ibis et al., 2016; Tandon, Maurya, Verma, Kumar, & Shukla, 2010).

Antibiotic resistance is escalating to perilously high levels globally according to the WHO. The development and spread of new resistance mechanisms are threatening our capability to

treat ordinary infectious diseases. Antibiotics have become less effective and it has become challenging, and occasionally impossible, to treat infections such as pneumonia, tuberculosis, blood poisoning, gonorrhea, and foodborne diseases (WHO, 2018). Infections from resistant microorganisms spark protracted disease, greater health care costs, and a colossal risk of mortality (WHO, 2018).

Staphylococcus aureus, a Gram-positive bacterium, is known to cause hospital infections globally (Loomba, Taneja, & Mishra, 2010) and methicillin and vancomycin-resistant *S. aureus* are responsible for a high percentage of these infections. The resistance of this organism toward antimicrobial agents is attributed to its ability to form biofilms on biomaterials which makes it challenging to annihilate from the infected host. (Bhattacharya, Bir, & Majumdar, 2015; Loomba et al., 2010).

Escherichia coli, a Gram-negative bacterium, live in the gut of healthy mammals and humans and causes enteric diseases. It is a pathogen that is responsible for significant morbidity and mortality globally. *E. coli* strains can cause enteric/diarrhoeagenic or extraintestinal infections of which the latter are primarily urinary tract (caused by uropathogenic *E. coli*) and sepsis/meningitis (caused by neonatal meningitis *E. coli*) in humans (Clements, Young, Constantinou, & Frankel, 2012).

The opportunistic pathogen, *Candida albicans*, is the primary cause of human fungal infections and it lives commensally within the human body. After host immune response modifications *C. albicans* infection occurs (Monge, Román, Nombela, & Pla, 2006) which can enter the bloodstream and spread to internal organs or can be superficial, affect the skin or mucous membrane, prolong hospital visits and increase the expenses associated with therapy (López-Martínez, 2010). These infections are swiftly escalating especially for immunocompromised people such as neonates, AIDS patients, and transplant patients (Biswas, van Dijck, & Datta, 2007; Zhang, Chen, Niu, Wang, & Xie, 2009).

In this work we report on the screening of thiolated naphthoquinones against two bacterial strains (*E. coli* ATCC 25922 and *S. aureus* ATCC 29213) and a fungal strain (*C. albicans* ATCC 10231).

2. MATERIALS AND METHODS

2.1. Synthesis

The synthesis of the 1,4-naphthoquinone-2,3-bis-sulfides **2–10** shown in Figure 1 (Wellington, Bokako, Raseroka, & Steenkamp, 2012), the 1,4-naphthohydroquinone **11** and the 1,4-naphthoquinoe sulfides **12–20** (Wellington, Gordon, Ndlovu, Kolesnikova, & Steenkamp, 2013) shown in Figure 2, have been reported previously.

2.2. Determination of the in vitro antibacterial and antifungal activity

2.2.1. Bacterial and fungal strains

The fungal and bacterial strains investigated were acquired from the American Type Culture Collection (ATCC) and were sustained on Mueller-Hinton (MH) and Sabouraud Dextrose (SD) agar, respectively. The fungus, *Candida albicans* (ATCC 10231), was cultured overnight in SD broth. The inoculum was adapted by comparison with a McFarland 0.5

standard and diluted with fresh SD broth to around 1 x 10^6 cfu/mL before doing the antifungal assay. The bacteria, Gram-positive *S. aureus* (ATCC 29213) and Gram-negative *E. coli* (ATCC 25922), were cultured in MH broth and the inoculum adjusted to around 1 x 10^6 cfu/mL by comparison with a McFarland 0.5 standard and dilution with MH broth before doing the antibacterial assays.

2.2.2. In vitro antibacterial activity

Assay background

Antibacterial activity was investigated following the method of Eloff (1998) with several adjustments. Sterile distilled water (100 µL) was added to each well of a sterile 96-well microtitre plate. Compounds in triplicate (100 µL) at a concentration of 10 mg/mL were added to the top row of wells, and were serially diluted twofold down the column. This was followed by the subsequent removal of 100 µL from the final well. A volume of 100 µL of the bacterial culture was added to each well of the microtitre plate and the plates were incubated overnight at 37°C. Acetone, broth and the test organism alone were used as negative and growth controls while Gentamicin (Virbac) was used as a positive control. Following incubation, 40 µL of a 0.2 mg/mL solution of *p*-iodonitrotetrazolium violet (INT, Sigma) in water was inserted into each well and incubated for an additional hour or longer at 37°C until color development in the negative control wells. The development of a red formazan color signified bacterial growth while inhibition was signified by a reduction in the color reaction. Minimum inhibitory concentration (MIC) values were documented as the least concentration of the compound to impede growth.

2.2.3. In vitro antifungal activity

Assay background

The procedure of Masoko, Picard, and Eloff (2005) was employed to perform antifungal screening of the compounds. Serial twofold dilutions of the compounds were prepared as for the antibacterial screening. To each well fungal culture in SD broth (100 µL) was added which was followed by the addition of 40 µL of 0.2 mg/mL INT solution and then plates incubation overnight at 25°C. Amphotericin B (Sigma) was used a positive control, with appropriate negative controls. The development of a red formazan color signified antifungal growth and inhibition was signified by a reduction in the color reaction. MIC values were document as the lowest concentration of the compound that impeded growth.

2.3. Lipophilicity

ACD/LogP, a commercially available program, was used to calculate the lipophilicity parameters (Log P) of compounds **1**–**20**. The Log P values for each of the compounds are shown in Tables 1 and 2.

Table 1. In vitro antifungal and antibacterial activity of compounds **1**–**10** expressed as MIC values (μg/mL) and the calculated log *P* values

Compound	*E. coli* (Gram –ve)	*S. aureus* (Gram +ve)	*C. albicans*	Log *P*
1	**31.3**	**11.7**	**62.5**	1.79 ± 0.61
2	>250	**62.5**	145.8	4.11 ± 1.00
3	**93.8**	187.5	>250	4.70 ± 1.00
4	**31.3**	**62.5**	**62.5**	4.19 ± 1.00
5	187.5	>250	>250	4.59 ± 1.00
6	>250	>250	>250	6.58 ± 1.00
7	>250	>250	>250	9.45 ± 1.00
8	>250	**93.8**	>250	5.11 ± 1.00
9	>250	**93.8**	250	2.07 ± 1.00
10	250	>250	145.8	2.42 ± 1.00
Gentamicin	**7.8**	**3.9**	–	−1.89 ± 0.66
Amphotericin B	–	–	**31.3**	0.78 ± 0.83

- Potent activity: MIC ≤10 μg/mL; moderate activity: 11 ≤ MIC ≤ 100; weak activity: MIC >100 μg/mL.

Table 2. In vitro antifungal and antibacterial activity of compounds **11**–**20** expressed as MIC values (μg/mL) and the calculated log *P* values

Compound	*E. coli* (Gram –ve)	*S. aureus* (Gram +ve)	*C. albicans*	Log *P*
11	**11.7**	**46.9**	**62.5**	2.00 ± 0.70
12	**31.3**	**31.3**	**62.5**	6.14 ± 0.73
13	125	**31.3**	**31.3**	6.19 ± 0.73
14	>250	>250	>250	8.65 ± 0.73
15	125	125	125	6.67 ± 0.72
16	**31.3**	**31.3**	**31.3**	7.80 ± 0.72
17	**62.5**	**62.5**	**31.3**	6.58 ± 0.76
18	**31.3**	**7.8**	**23.4**	6.56 ± 0.89
19	>250	250	**62.5**	9.33 ± 0.90
20	125	125	125	8.95 ± 0.93
Gentamicin	**7.8**	**3.9**	–	−1.89 ± 0.66
Amphotericin B	–	–	**31.3**	0.78 ± 0.83

- Potent activity: MIC ≤10 μg/mL; moderate activity: 11 ≤ MIC ≤ 100; weak activity: MIC >100 μg/mL.

3. RESULTS AND DISCUSSION

3.1. Determination of the in vitro antibacterial and antifungal activity

3.1.1. Antibacterial and antifungal activity of the 1,4-naphthoquinone-2,3-bis-sulfides

The synthesis of the 1,4-naphthoquinone-2,3-bis-sulfides **2–10** using Novozym 51003, a commercial laccase, has been reported previously (Wellington et al., 2012). Structures of the synthesized compounds are shown in Figure 1.
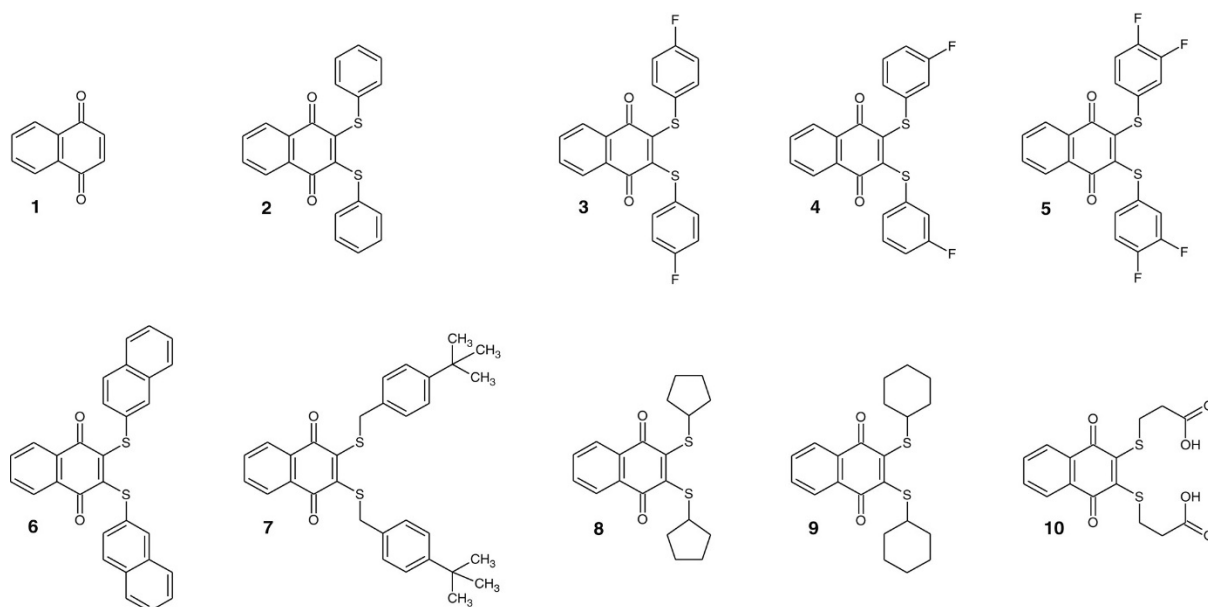

Figure 1. The 1,4-naphthoquinone-2,3-bis-sulfides

The results of the screening are shown in Table 1.

It is apparent from the results in Table 1 that the 1,4-naphthoquinone-bis-sulfides **1–10** were not very active against *E. coli*, *S. aureus,* and *C. albicans* and that only moderate to weak activity was observed. The compounds were more active against *S. aureus* (Gram +ve) than against *E. coli* (Gram −ve) and generally showed much weaker activity against the fungus, *C. albicans*.

Compounds **1** and **4** had the best MIC of 31.3 µg/mL against *E. coli* which was only about fourfold weaker than that of gentamycin (MIC = 7.8 µg/mL). The next best activity was that of **3** with an MIC of 93.8 µg/mL while the rest of the compounds had weak activity.

Compound **1** had the best activity against *S. aureus* with an MIC of 11.7 µg/mL, this was just short of potent activity. This was followed by **2** and **3**, each with an MIC of 62.5 µg/mL, and then **8** and **9** with an MIC of 93.8 µg/mL, the other compounds had weak activity.

Both compound **1** and **4** also had the best activity against *C. albicans* with an MIC of 62.5 µg/mL, this activity was only onefold less than that of Amphotericin B. The other compounds had weak activity.

Overall, **1** had the best activity since it inhibited the two bacteria *E. coli* (MIC = 31.3 µg/mL) and *S. aureus* (MIC = 11.7 µg/mL) as well as the fungus, *C. albicans* (MIC = 62.5 µg/mL). Compound **4** had the second best activity since it inhibited the two bacteria *E. coli* (MIC = 31.3 µg/mL) and *S. aureus* (MIC = 62.5 µg/mL) as well as the fungus, *C. albicans* (MIC = 62.5 µg/mL).

3.1.2. Structure–activity relationship study for antibacterial and antifungal activity

A structure–activity relationship (SAR) was determined by analyzing the activities of the compounds against the bacterial and fungal strains and relating it to their structure. The functional groups essential for activity had to be identified.

The SAR of the compounds for *E. coli* is shown in Figure 3 and compounds **1** and **4** had the best activity (MIC = 31.3 µg/mL). Changing the position of the fluoro group on the phenyl ring from *meta* in **4** to *para* in **3** resulted in a decrease in activity to 93.3 µg/mL. When both fluoro groups are present on the phenyl ring in the *meta* and *para* positions, the activity decreased to weak (MIC = 187.5 µg/mL). The addition of 3-sulfanylpropanoic acid to **1** to afford **10** also resulted in weak activity (MIC = 250 µg/mL).

From these results it is evident that structural modification of **1** was unsuccessful in enhancing the activity against *E. coli* and only two naphthoquinone sulfides, **3** and **4**, had moderate activity against *E. coli*.

From Figure 4 it can be seen that compound **1** again had the best activity (MIC = 11.7 µg/mL). The addition of a phenylthio to **1** to afford **2** and the addition of 3-fluorophenylthio to **1** to afford **4**, resulted in a decrease in activity to 62.5 µg/mL. When a cyclopentylthio was added to **1** to afford **8** and a cyclohexylthio added to **1** to afford **9**, weak activity (MIC = 93.8 µg/mL) was obtained.

Structural modification of **1** was also unsuccessful in enhancing the activity against *S. aureus*. In this case four compounds, **2**, **4**, **8**, and **9**, had moderate activity which was not better than that of **1**.

Compounds **1** and **4** had the best activity (MIC = 62.5 µg/mL) against *C. albicans* (Figure 5). Removal of the fluoro groups from the phenyl ring in **4** and the addition of 3-sulfanylpropanoic acid to **1** to afford **10**, resulted in weak activity (MIC = 145.8 µg/mL) as seen for **2**. A further decrease in activity to 250 µg/mL occurred when the phenyl rings in **2** were replaced with cyclohexyl rings as in **9**.

Structural modification was once again unsuccessful in enhancing the activity since only compound **4** had moderate activity which was the same as for **1**.

3.1.3. Lipophilicity

The calculated lipophilicity value of each of the 1,4-naphthoquinone-2,3-bis-sulfides **1**–**10** (Table 1) was compared with the antibacterial and antifungal activities to determine whether a linear correlation existed between lipophilicity and inhibitory activity.

It is quite apparent from the results (Figures 3-5) that there is definitely not a linear correlation between the calculated log *P* values of the 1,4-naphthoquinone-2,3-bis-sulfides

and the inhibitory activity that is, there is not an increase in inhibitory activity as the lipophilicity increases and vice versa.
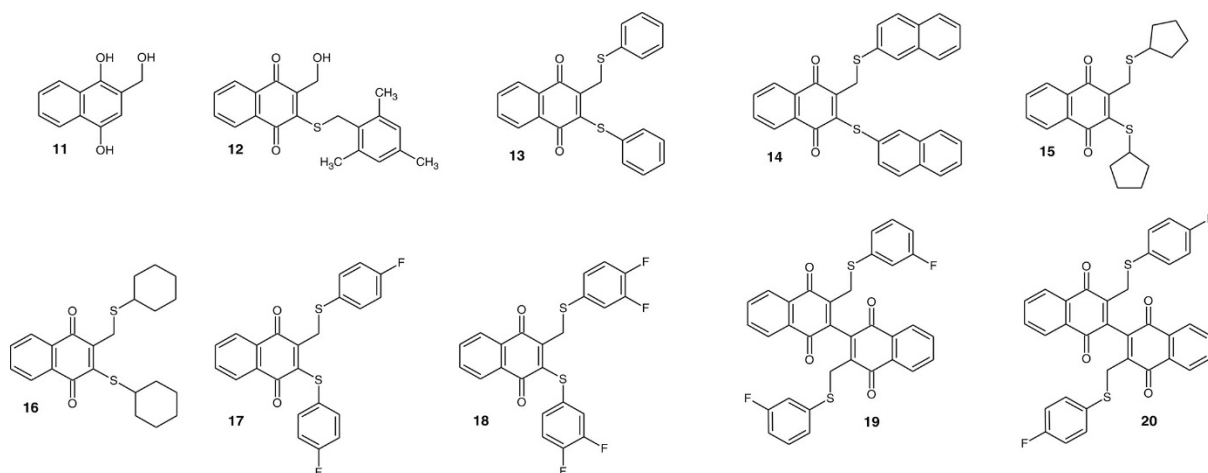

Figure 2. The 1,4-naphthohydroquinone derivative **11**, the 1,4-naphthoquinone monosulfide **12**, the 1,4-naphthoquinone sulfides **13**–**18**, and the 1,4-naphthoquinone sulfide dimers **19** and **20**
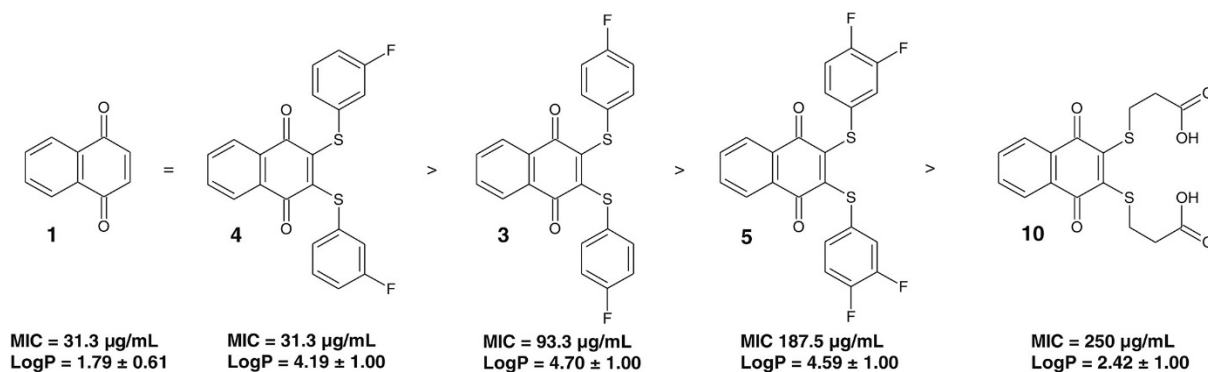

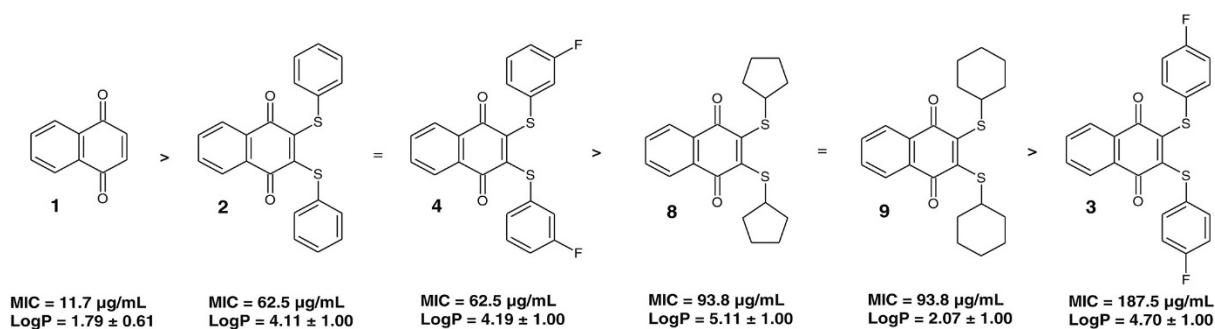**Figure 3.** The SAR of the 1,4-naphthoquinone-2,3-bis-sulfides for *E. coli*

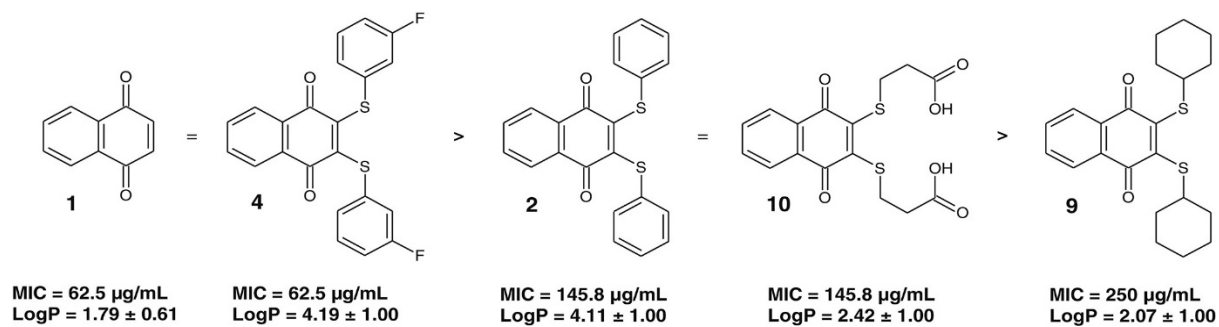**Figure 4.** The SAR of the 1,4-naphthoquinone-2,3-bis-sulfides for *S. aureus*

Figure 5. The SAR of the 1,4-naphthoquinone-2,3-bis-sulfides for *C. albicans*
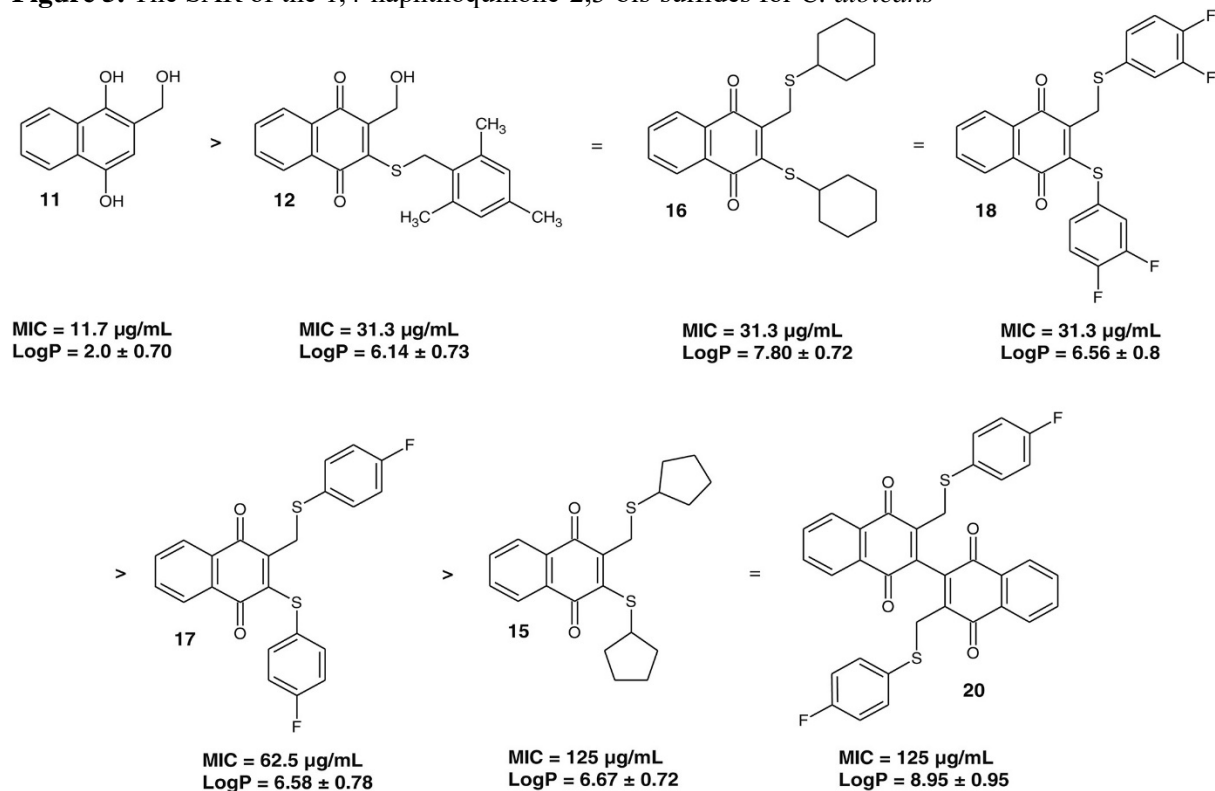

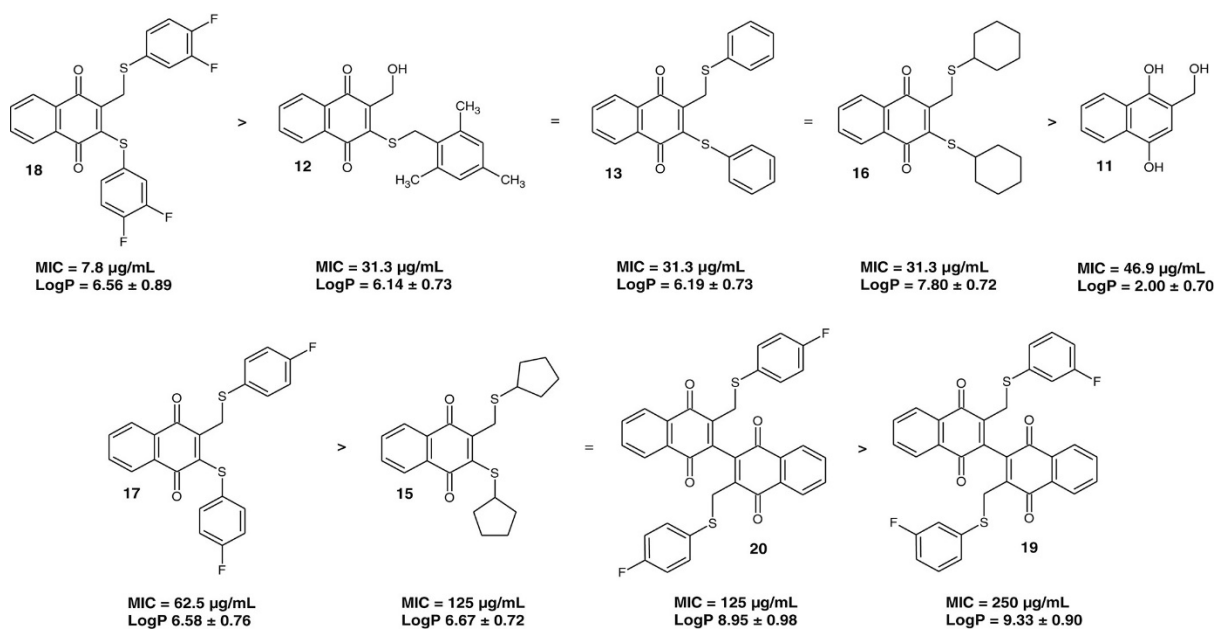**Figure 6.** The SAR of the 1,4-naphthoquinone sulfides for *E. coli*

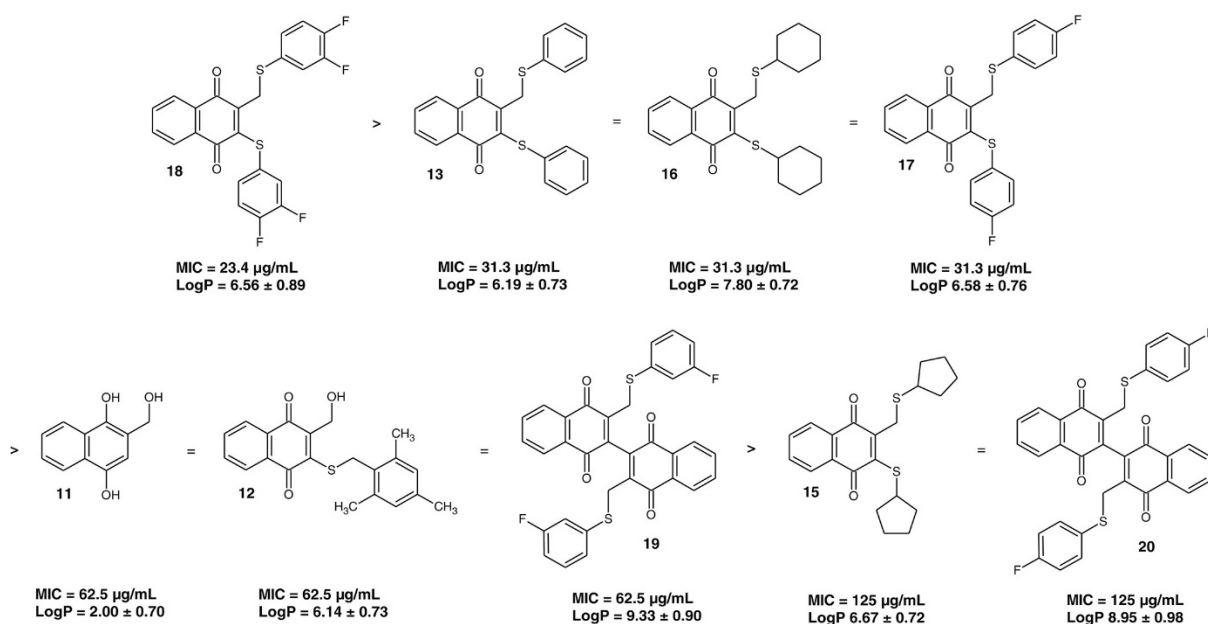**Figure 7.** The SAR of the 1,4-naphthoquinone sulfides for *S. aureus*

Figure 8. The SAR of the 1,4-naphthoquinone sulfides for *C. albicans*

3.2. Determination of the in vitro antibacterial and antifungal activity of the 1,4-naphthoquinone derivative, 1,4-naphthoquinone sulfides and 1,4-naphthoquinone sulfide dimers

The synthesis of the 1,4-naphthohydroquinone **10**, the 1,4-naphthoquinone sulfides **11**–**17**, and the 1,4-naphthoquinone sulfide dimers **18** and **19**, have previously been reported and are shown in Figure 2 (Wellington et al., 2013).

The results of the screening are shown in Table 2.

The 1,4-naphthoquinone sulfides **12–20** were very active against bacterial strains (*E. coli* and *S. aureus*) and the fungus, *C. albicans*, as is evident in Table 2. The compounds appear to be more active against the fungal strain while similar activity was observed against the two bacterial strains.

Compound **11** had the best activity (MIC = 11.7 µg/mL) and almost potent activity which was also close to the activity of gentamycin (MIC = 7.8 µg/mL) against *E. coli*. The next best activity was 31.3 µg/mL for **12**, **16**, and **18** followed by 62.5 µg/mL for **17** while the other compounds had weak activity.

Compound **18** had potent activity against *S. aureus* which was also the best activity and only onefold less than that of gentamycin (MIC = 3.9 µg/mL). The next best activity was 31.3 µg/mL for **12**, **13**, and **16,** 46.9 µg/mL for **11** and 62.5 µg/mL for **17**. The remaining compounds had weak activity.

Compound **18** also had the best activity (MIC = 23.4 µg/mL) against *C. albicans* which was better than that of Amphotericin B (MIC = 31.3 µg/mL). The next best activity was 31.3 µg/mL for **13**, **16**, and **17**, and 62.5 µg/mL for **11**, **12**, and **19**. The remaining three compounds had weak activity.

The compound that had the best activity against both the bacterial strains and the fungal strain is compound **18** which had potent activity (MIC = 7.8 µg/mL) against *S. aureus* and better activity (MIC = 23.4 µg/mL) than Amphotericin B (MIC = 31.3 µg/mL) against *C. albicans*. Compound **16** had the second best activity since it had an activity of 31.3 µg/mL against both bacteria and against the fungus. The third best activity was that of **11** that had almost potent activity (MIC = 11.7 µg/mL) against *E. coli* and moderate activity against *S. aureus* and *C. albicans* with an MIC of 46.3 µg/mL and 62.5 µg/mL, respectively.

3.2.1. SAR study for antibacterial and antifungal activity

The activities of the compounds against the bacterial and fungal strains were analyzed to determine the SAR. This was performed to pinpoint the functional groups that are crucial for activity.

The SAR of the compounds for *E. coli* is shown in Figure 6 from which it is evident that the triol **11** had the best activity (MIC = 11.7 µg/mL). The addition of a substituted arylthio group to **11** to form the monosulfide **12** decreased the activity to 31.3 µg/mL. Structural modification of **11** by the addition of cyclohexylthio groups to form **16,** and the addition of 3,4-difluoro-phenylthio groups to form **18**, also decreased the activity to 31.3 µg/mL. Removal of the *meta* fluoro group on the phenyl ring of **18** resulted in a further decrease in activity to 62.5 µg/mL. The addition of cyclopentylthio groups to form **15** and the formation of the dimer **20** resulted in weak activity (MIC = 125 µg/mL).

Overall, it can be concluded that structural modification of the triol **11** was unsuccessful in enhancing the activity against *E. coli*.

From the SAR of the compounds for *S. aureus* (Figure 7) it is evident that compound **18** having fluoro groups in the *meta* and *para* positions on the phenyl ring had the best activity (MIC = 7.8 µg/mL). The monosulfide **12**, **13**, and **16** had the same activity (MIC = 31.3 µg/mL) while the triol **11** had less activity (MIC = 46.9 µg/mL). It is evident that structural

modification of **11** by the addition of a 3,4-difluoro-phenylthio group as in **18**, the addition of a trimethylbenzylthio group as in **12**, the addition of a phenylthio group as in **13**, and the addition of a cyclohexylthio group as in **16**, had enhanced the activity against *S. aureus*. Removal of the *meta* fluoro group from **18** resulted in a decrease in activity to 62.5 μg/mL for **17**. The addition of a cyclopentylthio group to **11** to form **15**, and the formation of a *para* fluoro dimer **20** from **11** afforded weak activity (MIC = 125 μg/mL). The *meta* fluoro dimer **19** had even weaker activity (MIC = 250 μg/mL).

It may thus be concluded that the *meta* fluoro group in **18** is vital for obtaining potent activity against *S. aureus* and that among the fluoro substituted compounds **18** is the most active having better activity than **17** and both the dimers **19** and **20**.

From the SAR of the compounds for *C. albicans* (Figure 8) it is evident that **18** once again had the best activity (MIC = 23.4 μg/mL). Compounds **13**, **16,** and **17** had the same activity (MIC = 31.3 μg/mL) and the triol **11** had less activity (MIC = 62.5 μg/mL). The removal of the *meta* fluoro group from **18** resulted in a decrease in activity to 31.3 μg/mL. Structural modification of **11** by the addition of a 3,4-difluoro-phenylthio group as in **18**, the addition of a phenylthio group as in **13**, the addition of a cyclohexylthio group as in **16**, and the addition of a 4-fluorop-henylthio group as in 17 had enhanced the activity against *C. albicans*. Removal of the *meta* fluoro group from **18** resulted in a decrease in activity to 62.5 μg/mL for **17**. The addition of a trimethylbenzylthio group to **11** to form **12**, and the formation of the dimer **19**, did not change the activity. Furthermore, the addition of a cyclopentylthio group to **11** to form **15**, and the formation of a *para* fluoro dimer **20** from **11** afforded weak activity (125 μg/mL).

It may thus also be concluded that the *meta* fluoro group in **18** is vital for obtaining moderate activity against *C. albicans* and that among the fluoro substituted compounds **18** is once again the most active having better activity than **17** and both the dimers **19** and **20**.

Overall, it is apparent that the SAR of compounds **11**–**20** against *S. aureus* is similar to that against *C. albicans*.

3.2.2. Lipophilicity

Compound **20** has the highest lipophilicity (log P = 9.33 ± 0.90) and compound **11**, the lowest (log P = 2.00 ± 0.70) as can be seen from the data in Table 2. The log P value of compound **18** having potent activity is 6.56 ± 0.89 but this was not equivalent to that of gentamycin (log P = −1.89 ± 0.66).

The calculated lipophilicity value of each of the 1,4-naphthoquinone sulfides **12**–**20** was compared with the antibacterial and antifungal activities and used for the determining whether a linear correlation between lipophilicity and inhibitory activity exists. From the results it was determined that there is not a linear correlation between the calculated log P values of the compounds and the inhibitory activity that is, there is not an increase in inhibitory activity as the lipophilicity increases and vice versa.

3.3. Comparison of the activity of the 1,4-naphthoquinone-2,3-bis-sulfides 2–10 with the 1,4-naphthoquinone sulfides 12–20

From a comparison of the results of the 1,4-naphthoquinone-2,3-bis-sulfides **2–10** in Table 1 with the results of the 1,4-naphthoquinones sulfides **12–20** in Table 2 it is quite evident that the latter class of compounds were more active against both bacteria and the fungus.

The 1,4-naphthoquinone **1** had better activity (MIC = 11.7 µg/mL) against *S. aureus* than the triol **11** (MIC of 46.9 µg/mL). The triol **11** had better activity (MIC = 11.7 µg/mL) against *E. coli* than the 1,4-naphthoquinone **1** (MIC = 31.3 µg/mL). Both **1** and **11** had the same activity (MIC = 62.5 µg/mL) against *C. albicans*.

Structural modification of the 1,4-naphthoquinone to afford **2–10** did not result in an enhancement of the activity. In contrast, structural modification of the triol **11** did result in enhancement of activity and afforded potent activity (MIC = 7.8 µg/mL) against *S. aureus* and moderate activity (MIC = 23.4 µg/mL) against *C. albicans* which was better than that of triol **11** which had an MIC of 46.9 µg/mL and 62.5 µg/mL, respectively.

From these results it may be concluded that the derivatives of triol **11** are more attractive than those from 1,4-naphthoquinone **1** for further structural modification and study as potential antibacterials and antifungals.

3.4. Possible mechanism of action

Several effects are responsible for the cytotoxic activity of quinones and include adduct formation particularly with enzyme SH groups, DNA damage, inhibition of electron transporters, reactive oxygen species (ROS) generation, and uncoupling of oxidative phosphorylation protein (Freitas et al., 2012; Rahmoun et al., 2013; Silva Jr et al., 2011).

There are two main mechanisms that have been pinpointed. One 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. The other entails the production of the semiquinone radical following one-electron reduction of the quinone ring and its involvement in a redox cycle to provide potent ROS (superoxide anion radical and hydrogen peroxide (Castro et al., 2008).

4. CONCLUSIONS

The 1,4-naphthoquinone-bis-sulfides and 1,4-naphthoquinone sulfides have antibacterial and antifungal properties, but the latter are more effective. The 1,4-naphthoquinone sulfides have potent activity against *S. aureus* and moderate activity against *E. coli* and *C. albicans*. Based on the SAR study it was determined that a fluoro group in the *meta* and *para* position on the phenyl ring affords potent activity against *S. aureus* and moderate activity against both *E. coli* and *C. albicans*. Further studies on the 1,4-naphthoquinone sulfides will be on improving the biological activity of these compounds through structural modification.

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