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N-Myristoyl Transferase Inhibitors with Antifungal Activity in Quinolinequinone Series: Synthesis, In-silico Evaluation and Biological Assay

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Abstract *A series of anilino and aryl derivatives of quinolinequinone and naphthoquinone were synthesized via Pd catalysed cross-couplings. The results of docking the compound series towards the binding site of fungal N-myristoyl transferase (NMT) indicated that the quinones favourably interacted with the protein at binding free energy ranges of -5.14 to -8.01 kcal/mol. In addition, Candida albican and Candida anthra were susceptible to many of synthesized molecules in vitro, at MIC range of 1.60 -25 µg/ml. However, some of the compounds which had binding interaction with NMT in docking calculations fails to demonstrated measurable antifungal effect; and that highlights the importance of target-ligand complex stability dynamic situations that characterize biological system. Analysis of predicted binding modes revealed interesting structure-activity-relationship that can provide information on activity optimization process.*

Key Words: *Antifungal, quinolinequinones, screening, docking, drug-likeness, binding mode*

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1.0 Introduction

The search for new drugs against fungal infections is receiving enormous research attention due to the rise in fungal infections and microbial resistance to known antifungal drugs (Keyari *et al*. 2013). Quinolinquinones and their carbocyclic analogues have proven to possess wide spectrum of biological activities and this has attracted an increasing interest in the study of these compounds (Porteretal 1972; Fryatt *et al*., 2004; Mulchin *et al*. 2010; Tandon et al. 2005; Egu *et al*., 2017). Synthesis of quinolinequinone and related carbocyclic analogues has been a subject of interest in our research group and herein is their antifungal activities reported.

Recently, computer and computer soft-ware are routinely applied in the design and discovery of new chemotherapeutic agent. The time and cost effectiveness and reliability of results from computational predictions have made the technique a veritable tool in drug design and development (Kubinyi 1998; Egu *et al*., 2014). In this current study, the ability of aniline and aryl derivatives of quinolinequinone and naphthoquinone to interact with N-myristoyl transferase (a validated antifungal protein target) are investigated using docking calculation method. Furthermore, *in-vitro* assay was employed to test susceptibility of *Candida albican* and *Candida anthras* to the compounds.

3.0 Materials and Methods

2.1 Synthesis of quinolinequinone derivatives

The detail synthetic procedures and characterization of the studied compounds has been reported (Egu *et al.* 2014; Egu *et al*., 2017).

2.2 Molecular modeling

The three dimensional chemical structures of the quinolinequinones were generated using the molecular builder interface implemented in molecular operating environment (MOE) software (Chemical Computing Group 2010), energy minimization was carried out using the MMFF94 force field until a gradient of 0.01 kcal/mol was reached and the output file was saved as .mol2. The molecular descriptor calculator included in the QuSAR module of the MOE package was used to compute the molar weight (MW), number of rotatable bonds (NRB), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD) and lipophilicity (log P). The X-ray crystal structure of fungal NMT (PDB code 1IYK) (Berma *et al*. 2000) retrieved from protein databank were also treated and opimised for docking simulation using MOE. AutoDock 4.2.0 was employed to perform the docking calculations (Morris *et al*. 1998). A grid box size of 40 × 40 × 40 A^3 points (spacing between the grid points was ~~of~~ 0.375 A) was used which centered on the mass center (13.82, 47.642, 1.201) of the crystallographic macromolecule encompassing all active site of the atoms. The docking protocol was validated by calculating the root mean square deviation of the docked ligand from the x-ray crystallized ligand. Accerlyl's Discovery Studio Visualizer 3.0 was used to visualize the binding interaction of the protein-ligand complexes.

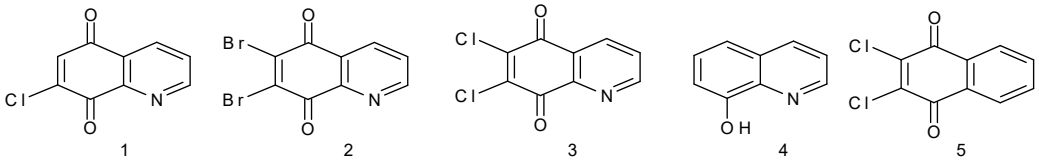
2.3 Minimum inhibitory concentration (MIC) testing

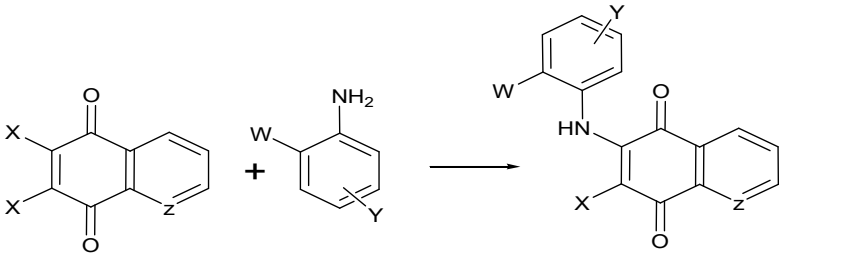
The MIC was evaluated by the broth dilution technique approved by the National Committee for Clinical Laboratory Standards (NCCLS 1993). Serial dilution of 0.1 mg/mL DMSO solution of each sample was carried out to have 0.05, 0.025, 0.0125, 0.00625 mg/mL solutions. Fours drops of each dilution were added to the corresponding cup previously cut in the Mueller Hinton Agar (MHA) plate. The plates were incubated at 37 °C for 24 hours for fungi. The diameter of zone of inhibition was measured and the value was subtracted from the diameter of the borer to give the inhibition zone diameter (IZD). The graph of IZD against the log of concentrations was plotted for each plate containing a specific compound and a microorganism. The anti-log of the intercept on x-axis gives the MIC. The procedure was repeated for ketoconazole which served as a standard/reference drug.

3.0 Results and Discussion

3.1 Synthesis of quinolinequinone derivatives

The key intermediates 7-chloro-5,8-quinolinequinone **1**, 6,7-dibromo-5,8-quinolinequinone **2**, 6,7-dichloro-5,8-quinolinequinone **3** were prepared from 8-hydroxyquinoline **4** (Ryu & Kim 1994; Petrow & Sturgeon 1954). Naphthoquinone **5** was purchased while the other precursors were previously synthesized by our group (Egu *et al.* 2014; Egu *et al*. 2017).

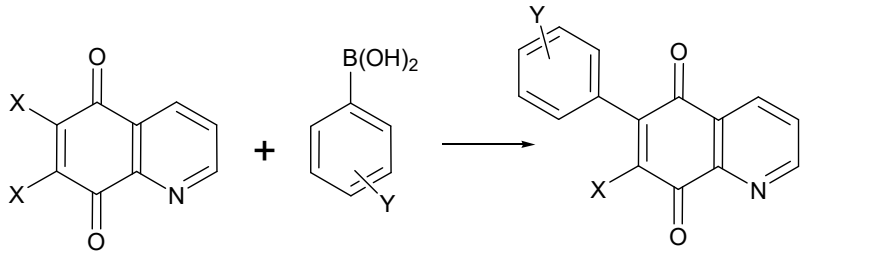




6 = 7-chloro-6-[(4-nitrophenyl)amino]quinoline-5,8-dione,
7 = 7-chloro-6-(phenylamino)quinoline-5,8-dione,
8 = 7-bromo-6-[(4-nitrophenyl)amino]quinoline-5,8-dione,

9 =
7-bromo-6-(phenylamino)quinoline-5,8-dione,
10 = 6,7-bis[(4-chloro-2-nitrophenyl)amino]quinoline-5,8-dione,
11 = 2-chloro-3-((4-nitrophenyl)amino)naphthalene-1,4-dione,
12 = 2-chloro-3-(phenylamino)naphthalene-1,4-dione,

Scheme 1: Synthesis of derivatives of quinolinequinone and naphthoquinone via Buchwald-Hartwig coupling protocol.



X = Br or Cl Y = 3-nitro, H or 4-Br Compounds 13 -21

13 = 6-(4-bromophenyl)-7-chloroquinoline-5,8-quinone**,**
14 = (4-(6-(4-(6-chloro-5,8-dihydroquinolin-7-yl)phenyl)-5,8-dihydroquinolin-7-yl)phenyl)boronic acid**,**
15 = 7-chloro-6-(4-nitrophenyl)quinoline-5,8-dione,
16 = 7-chloro-6-phenylquinoline-5,8-dione,
17 = 7-chloro-6-(3-nitrophenyl)quinoline-5,8-quinone**.**
18 =7-bromo-6-(4-bromophenyl)quinoline-5,8-dione**,**
19 = 6-bromo-7-(4-(7-bromo-5,8-dioxo-5,8-dihydroquinolin-6-yl)quinoline-5,8-dione**,**
20 = **7**-bromo-6-(3-nitrophenyl)quinoline-5,8-dione**,**
21 =7-bromo-6-phenylquinoline-5,8-dione**,**

Scheme 2: Synthesis of derivatives of disubstituted quinolinequinones via Suzuki cross-coupling protocol.

3.2 In-silico study of the quinolinequinones

Many potential drug molecules have failed to achieve commercial value due to bad pharmacokinetic profile. Therefore, the pharmacokinetic properties of potential drugs are examined at early stage of drug discovery to maximize resources (Ntie-Kang *et al*. 2014). Going by the hypothesis made by Lipinski *et al*. 1997), the following criteria highlight orally bioavailable drug molecule: molecular weight (MW) less than 500 Da, lipophilicity (log P) less than 5, hydrogen bond acceptor/donor (HBA/HBD) less than 10/5 and number of rotatable bond (NRB) less than 5. The calculated molecular descriptors for the new quinolinequinones showed that all of them except **10**, **14** and **19**, have physico-chemical parameters in the recommended ranges for drug-likeness (Table 1). The three exceptions failed only in the criteria for MW. However, **14** also overshot in the value required for NRB. The success of bulkier drugs highlights the possibility that none of the new quinolinequinones will pose bioavailability problem (Ryu & Kim 1994).

Table 1: Some physicochemical features of the quinolinequinones

Codes	NRB	MW	HBA	HBD	log P
6	3	329.69	7	1	2.44
7	2	284.7	4	1	2.51
8	3	374.15	7	1	2.65
9	2	329.15	4	1	2.71
10	6	500.25	11	2	4.42
11	3	294.26	6	1	3.04
12	2	249.26	3	1	3.11
13	1	348.58	3	0	3.61
14	4	546.73	8	2	3.85
15	2	314.68	6	0	2.75
16	1	269.68	3	0	2.82
17	2	313.67	6	0	2.46
18	**1**	**392.02**	**3**	**0**	**3.53**
19	**2**	**549.15**	**6**	**0**	**3.85**
20	**2**	**358.12**	**6**	**0**	**2.63**
21	1	313.13	3	0	2.69

Hence, docking of the quinone series was done to continue our search for fungal NMT inhibitors with antifungal effect. The Lamarckian genetic algorithm implemented in AutoDock 4.2 characterized the quinolinequinones to have favourable binding interactions with fungal NMT at binding free energy ranges of -5.14 to 8.01 kcal/mol as shown in Table 2.

Table 2: Docking results for the quinolinequinones

Codes	ΔG (kcal/mol)	K_i(uM)	Ligand efficiency
6	-5.15	167.73	0.22
7	-6.04	37.57	0.3
8	-5.32	125.78	0.23
9	-6.33	23.08	0.32
10	-6.8	10.44	0.32
11	-5.73	62.92	0.17
12	-5.8	55.6	0.26
13	-6.23	27.19	0.33
14	-6.3	24.15	0.32
15	-5.14	171.04	0.23
16	-5.6	78.75	0.22
17	-5.15	167.41	0.23
18	-8.01	1.33	0.4
19	-8.01	1.35	0.25
20	-5.59	80.05	0.25
21	-5.74	61.54	0.3

The docking results suggest each of the quinolinequinones will have a degree of antifungal property since they demonstrated an appreciable level of inhibition constant toward a validated anti-fungal drug target (at μM range). The results obtained from biological testing correlated with the expected hypothesis from docking/theoretical predictions (Table 3). However, discrepancies were observed between the extent of the docking scores of the quinolinequinones and their MIC in *in-vitro* testing. The differences were attributed to interactions with other essential antifungal drug targets other than NMT. In addition, although **7** (K_i = 37.57 μM) and **10** (K_i = 62.92 μM) made binding contact with fungal NMT at rigid-protein-flexible-ligand docking simulation, their failure to exhibit antifungal activity in biological screening underscores the importance of a drug molecule to maintain stable interactions with target receptors at biologically dynamic environment within a time frame sufficient to elicit physiological change. Significant observation was the preference of some of the quinolinequinones for *C. albican* over *C. anthras* and vice-versa which may furnish information on selective medication. It was also observed that for molecules which differ only in position 7 (**6** and **8**, **7** and **9**, **13** and **18**, **17** and **20**), replacing the C7-chlorine with a bromine atom resulted in enhanced binding affinity toward fungal NMT and anti-fungal *in vitro* activities.

Table 3: Antifungal Evaluation of the quinolinequinones determine by diffusion method (Minimum inhibition concentration in μg/mL).

Codes	*C. albican*	*C. anthras*
6	-	12.5
7	-	-
8	1.6	3.2
9	1.6	3.2
10	-	-
11	-	12.5
12	3.2	6.3
13	12.5	12.5
14	25	3.2
15	1.6	3.2
16	-	12.5
17	3.2	6.3
18	6.3	-
19	6.3	3.2
20	25	6.3
21	12.5	3.2

This suggests that the fungal NMT binding site has large surface area; therefore, bulky group can make proper binding contact with NMT and consequently inhibit fungal growth. The later proposal could also account for the significant increase of activity by **19.** It suffice to state that the bulkiness of **19** aided to maintain close binding interaction with the amino acid residues at NMT binding site even in biological dynamic situation (MIC = 6.3 μg/ml and 3.2 μg/ml for *C. albican* and *C. anthras* respectively). The performance of **10**(K_i = 62.92 μM with no significant *in vitro* antifungal activity for both *C. albican* and *C. anthras*), with bulkier group than

bromine at C7 and MW of 500.25 Da, suggest that other factor other than steric may be responsible for binding interactions with NMT and *in vitro* antifungal effect. Analysis of the binding conformation of both compounds (**10** and **19**) shown in Fig 1 as retrieved from the highest populated clusters of docking poses which is generally characterized by the lowest theoretical binding energy showed that both oriented differently and subsequently made binding contact with entirely different amino acid residues. It appeared that the seemingly linear nature of **19**in relation to **10**, enabled it to penetrate deeper into the binding groove to make close interactions within NMT. Although **8** and **9** have theoretical binding inhibition constant at μM range and lowest MICs at *in vitro* screening, we consider **19** as the suitable starting material for the development of antifungal lead compounds whose mechanism of action is by the inhibition of fungal NMT.

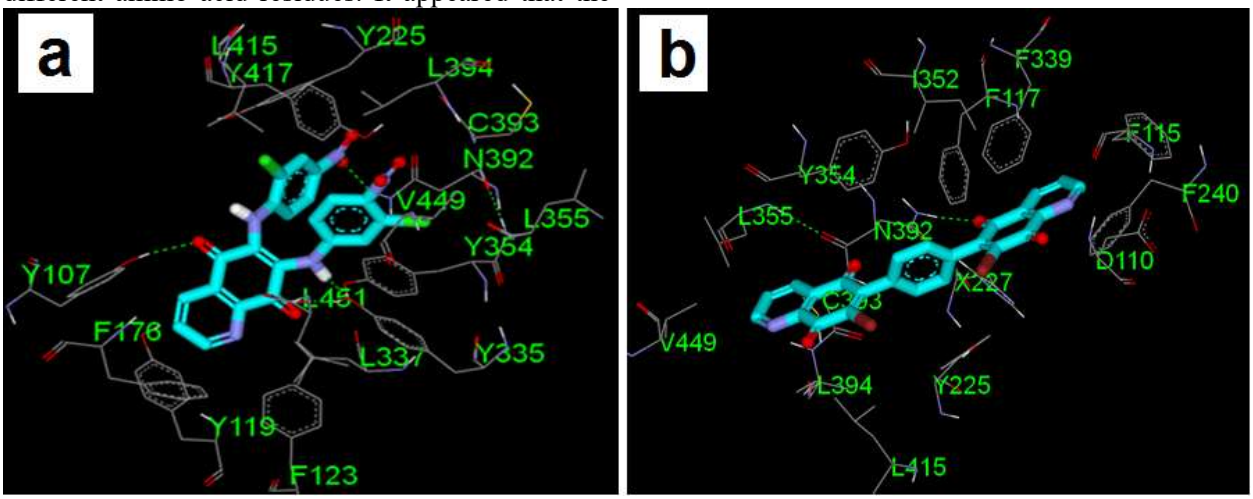


Fig 1. Predicted binding modes for 10 (a) and 19 (b) toward the fungal NMT structure. The atoms are coloured thus; carbon in cyan, hydrogen in white, oxygen in red, nitrogen in blue, chlorine in green, bromine in firebrick. Polar contacts are shown as green dash lines.

4.0 Conclusion

In conclusion, new derivatives of quinolinequinones were found to interact with fungal NMT at micromolar range in docking calculations. In addition, some of the compounds also inhibited the activities of two fungi (*C. albican* and *C. anthras*) at low milligram concentrations. Moreover, one of the quinolinequinones (**19**) appeared to be most suitable to serve as starting material for the development of new antifungal agent that targets NMT.

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