Antimicrobial Activities of Some Amino Derivatives of 5,7-Dibromo-2-methyl-8-hydroxyquinoline

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The bromine atoms of the title compound, 5,7-dibromo-2-methyl-8-hydroxyquinoline were replaced by the requisite amino compound to afford 6 amino derivatives *viz*: bis(diethylamino)-, bis(dibutylamino)-, bis(dicyclohexylamino)-, dipyrrolidino-, dipiperidino- and dipiperazino derivatives. The antimicrobial activity of these compounds were investigated against selected Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*), Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and yeast (*Candida albicans*). All the compounds showed significant activity against the test microorganisms, from 5–30 times compared to the title compound. It was observed that all derivatives were more effective against Gram positive bacteria. No correlation has been established between the minimum inhibitory (MIC) concentrations of the derivatives and the structural modifications.

Key words 5,7-dibromo-2-methyl-8-hydroxyquinoline; amino derivative; antimicrobial activity

Several derivatives of the quinoline nucleus have found use as chemotherapeutic agents.^{1–3} 8-Hydroxyquinoline, an antiseptic, has been shown to possess marked antimicrobial activity.⁴ The use of quinoline-based chemotherapeutic agents began with isolation of alkaloids of cinchona bark, prominent among which were quinine and cinchonine. Structural modifications resulted in potent antimalarial agents such as chloroquine and primaquine.⁵ Further modifications gave rise to clioquinol (**1**), broxyquinoline (**2**), broxaldine (**3**) and brobenzoxaldine (**4**) which have been used as topical anti-infective agents (Fig. 1). As part of our continued efforts to prepare target compounds with improved antimicrobial activity against the rapidly emerging strains of microorganisms that are resistant to commonly available antibiotics and disinfectants, it was desirable to prepare derivatives of compound **3** by replacing the bromine atoms with various alkylamino substituent. It was found that when **3** was treated with the amine at elevated temperatures or a mixture of the two dissolved in a suitable solvent and heated under reflux, the corresponding derivatives were obtained in very high yields.^{6,7} Thus, under suitable conditions **3** gave the following derivatives, **5–10** (Fig. 2). The synthesis and full characterisation of these derivatives have been reported in a separate communication.⁸

MATERIALS AND METHODS

Test Micro-organism Clinical isolates of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* (bacteria) and *Candida albicans* (yeast) obtained from the medical microbiology unit, University of Nigeria, Nsukka were used for this study. The bacteria isolates represent strains that are resistant to either tetracycline (TCN) or nitrofurantoin (NFT) or to both.

Culture Media Mueller–Hinton agar (oxoid) prepared according to the manufacturer's specifications was used as culture medium for all bacteria and sabouraud dextrose agar (Merck) was used as culture medium for the yeast.

Standard Antibiotics and Other Reagents Standard antibiotics used include TCN (Yatan Pharma, India), nystatin (NYS) and Nitrofurantoin (NFT) (Sigma chemicals), Di-

methylsulphoxide (DMSO) (BDH) was diluted to a concentration of 10% (v/v) before use.

Antimicrobial Screening Test The two-fold dilution cup-plate agar diffusion method⁹ was used to determine the minimum inhibitory concentrations (MIC) of all the derivatives. A known concentration of each derivatives **5–10** or the standard antibiotics (NYS, TCN or NFT) was prepared in 10% (v/v) DMSO. These stock solutions were further serially diluted (2-fold) to obtain various concentrations of each derivative in 10% DMSO. One milliliter of a standardised suspension (10^6 cells/ml) of each test micro-organism was seeded into 20 ml of Mueller–Hinton agar (MHA) or sabouraud dextrose agar (SDA) in sterile petri dishes and allowed to solidify on a flat surface. Using a sterile 6 mm cork-borer, 6 holes were bored on each MHA or SDA plate and

Fig. 1

Fig. 2

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Table 1. MIC Values of 5,7-Dibromo-2-methyl-8-hydroxyquinoline (3), Various Alkylamino Derivatives and Standard Antibiotics against Selected Microorganisms

Compound	Microorganisms/MIC values ($\mu\text{g/ml}$)				
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
3	28.00	2.00	707.95	1779.00	10.00
5	1.74 (16)	3.98 (0.5)	141.25 (5)	N.A	10.00 (1)
6	1.74 (16)	2.00 (1)	398.11 (1.8)	1778.00 (1)	0.35 (30)
7	2.24 (13)	22.39 (0.9)	398.11 (1.8)	501.19 (3.5)	7.10 (1.4)
8	2.82 (10)	39.81 (0.05)	251.19 (3)	501.19 (3.5)	1.58 (6.3)
9	2.82 (10)	8.91 (0.23)	316.23 (2.3)	630.96 (2.8)	7.10 (1.4)
10	7.10 (4)	39.81 (0.05)	141.25 (5)	501.19 (3.5)	2.51 (4)
TCN	N.A	N.A	0.50	N.A	N.T
NFT	2.00	7.94	N.A	N.A	N.T
NYS	N.T	N.T	N.T	N.T	2.82

(), Number of times compounds are as active as the parent compound (3). NA, no activity (resistant); NT, not tested.

the various serially diluted drug solutions (40 μl) introduced into the holes. The plates were allowed to stand for 1 h at room temperature to allow for pre-diffusion after which they were incubated at 37 °C for 24 h (for bacteria) or at 25 °C for 24 h (for the yeast). The resulting inhibition zone diameter (IZD) was measured after the incubation period. A plot of logarithm of drug concentration against IZD² afforded the estimation of the MIC of the drugs according to a reported method.⁹⁾ The experiments were performed in duplicate. Solutions of 10% DMSO without the drugs were used as control.

RESULTS AND DISCUSSION

A summary of the MIC values of 5,7-dibromo-2-methyl-8-hydroxyquinoline (3) and its alkylamino derivatives 5–10 is presented in Table 1.

The strain of *P. aeruginosa* used was resistant to both TCN and NFT; the *E. coli* strain was resistant to NFT alone while the *B. subtilis* and *S. aureus* strains were resistant to TCN alone. All the derivatives showed a significant and remarkable activity against the Gram positive bacteria, *S. aureus* and the yeast, *C. albicans*. The activity of the derivatives was 4–16 times greater than that of the parent compound (3) against *S. aureus*. However, against *B. subtilis*, 3 was surprisingly more active (2–20 times) than most of the derivatives and NFT. The bis(dibutylamino) derivative 6, exhibited the highest antifungal activity as can be seen from the recorded MIC values against *C. albicans*. This compound was 30 times more active than 3 and 8 times more active than the standard NYS. The dipyrrolidino (8) and dipiperazino (10) were 6.3 and 4 times more active than 3, respectively. The Gram negative bacteria, *E. coli* and *P. aeruginosa* were relatively more resistant to the synthesised derivatives than the other micro-organisms. *P. aeruginosa* was the most resistant; even the standard antibiotics (NFT and TCN) were not effective at concentrations of 2000 $\mu\text{g/ml}$. Compounds 7–10 were, however, effective against this organism at fairly mod-

erate concentrations.

A critical evaluation of the results does not indicate a clear pattern or correlation between MIC and the structural modifications. The continuous quest to synthesise new antimicrobial agents or derivatives of existing ones, has almost always been occasioned by resistance of micro-organisms to existing antimicrobial agents. Amongst bacteria, *S. aureus* and *P. aeruginosa* have been consistently identified with antibiotics resistance.^{10–12)} Against this background therefore, the very low MIC of these derivatives against *S. aureus* could be considered interesting. Even though *P. aeruginosa* appears less sensitive to the derivatives, the observation that it is also completely insensitive to the standard antimicrobial agents, (TCN and NFT) highlights the very high resistance of this micro-organism. However, the MIC of certain of the derivatives 7, 8 and 10 against *P. aeruginosa* is such that they be amenable for use as disinfectants and maybe as topical antiseptics, where the possibility of toxicity resulting from systemic absorption of even fairly high concentration is ruled out. In fact, 7, 8 and 10 could be particularly useful as disinfectants since they have a broad spectrum of antimicrobial and antifungal activity.

CONCLUSIONS

It appears from the results of this study that more potent quinoline-based antimicrobial agents can be obtained via structural modifications of 5,7-dibromo-2-methyl-8-hydroxyquinoline. The remarkable efficacy of alkylamino derivatives of this compound against antibiotic-resistant strains of Gram positive and Gram negative bacteria has been established. Based on our current data, some of these derivatives could be used *in vitro*, especially as disinfectants since they have a broad spectrum of activity and are efficacious against highly resistant strains of *P. aeruginosa*. *In vivo* application is not recommended for now, since proper toxicological studies of the derivatives have been concluded.

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