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Synthesis and Structure-Activity Relationships of Novel Antiseptics

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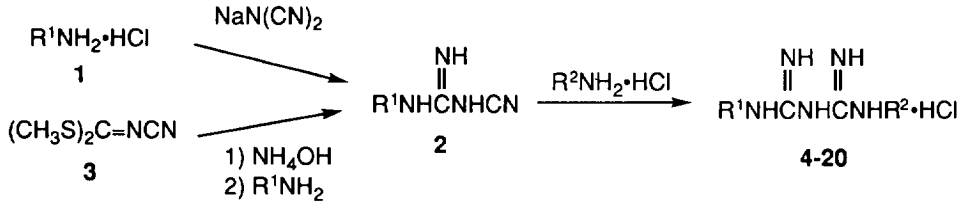
Abstract: For the purpose of developing new antiseptics, we searched for compounds having high biocidal activity covering both gram-positive and gram-negative bacteria. Accordingly, we designed 1,5-disubstituted biguanides and synthesized them using two alternative efficient reaction schemes. The bactericidal activity of these biguanides was assayed by the micro titration plate method. Among the biguanides synthesized, 3,4-dichlorobenzyl derivatives were found to exhibit particularly high bactericidal activity. Ultimately, compound **11** was chosen as a candidate novel antiseptic, which is currently under continued evaluation.

The history of development of antiseptics dates back decades ago. The chronological list includes benzalkonium chloride[1] (1935) and benzethonium chloride[2] (1943), both of which are quaternary ammonium compounds, the amphoteric surfactant alkyldiaminoethylglycine hydrochloride[3] (Tego 51, 1953), the bisbiguanide compound chlorhexidine gluconate[4] (Hibitane, 1954), and more recently polyvinylpyrrolidone iodine complex[5] (1956), all of which are still in broad use today. As to subsequent development, not a single new compound has been launched as an antiseptic during the past forty years. However, each of the above-mentioned commercial antiseptics has its own merit and demerit, with few being suited to all the various clinical applications and methods of use. As typical shortcomings of those commercial antiseptics, it may be pointed out that they are not sufficiently active at their usual concentrations against MRSA[6] which is a principal causative organism of nosocomial infections, nor are they active enough against *Pseudomonas* strains[7] which cause opportunistic infections. It was, therefore, thought that if a more potent, broad-spectrum antiseptic could be developed, it should contribute a great deal to the prevention of infectious diseases.

Accordingly, with low concentration and short-time sterilization as goals of synthesis, we, noticed guanidine, a compound having protein-denaturating activity,[8] subjected it to a variety of side-chain transformations, and scrutinized the structure-activity relationships of the resulting derivatives. It was generally believed that in order that the biguanide group, which can be regarded as the dimer of guanidine, could exhibit antibacterial activity, there must be two biguanide groups within the molecule (bisbiguanide) and that the bactericidal activity of any biguanide derivative[9] itself is low.[10] However, we discovered several compounds having enhanced bactericidal activity and promising to be novel antiseptics among biguanide derivatives. In this paper, we report and discuss the synthesis and structure-activity relationships of biguanide derivatives.

Synthesis

The objective biguanide derivatives (**4-20**) were each synthesized from the corresponding primary amine hydrochloride **1** or *S,S'*-dimethyl *N*-cyanodithioiminocarbonate **3**[11] via cyanoguanidine intermediate **2**[9a), 12] (See Scheme). Thus, cyanoguanidine intermediate **2** was obtained by reacting the corresponding primary amine hydrochloride **1** with sodium dicyanamide[9a), 12] in an equimolar ratio or by treating *S,S'*-dimethyl *N*-cyanodithioiminocarbonate **3** with 1.5 equivalents of aqueous ammonia and reacting it further with 1 equivalent of the corresponding primary amine. The cyanoguanidine intermediate **2** thus obtained was reacted with 1 equivalent each of hydrochlorides of various primary amines to give the objective biguanide derivatives (**4-20**) as hydrochlorides.



Scheme

Evaluating method

The bactericidal concentration (μg/ml) of each compound was determined by microplate assay.[1), 4), 13] The assay procedure comprised exposing a tester strain to each compound for a predetermined time, transferring an aliquot to an antiseptic-inactivating medium to reduce antibacterial activity, post-culturing the sample, and counting surviving organisms. The tester strain inoculum size was 10^6 cfu/ml.

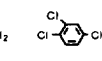
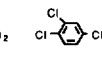
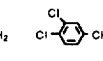
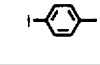
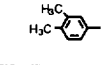
Results of Evaluation

The bactericidal concentration (μg/ml) of each compound after 3 minutes of exposure is shown in Table 1. As tester strains, *Staphylococcus aureus* FDA 209P and methicillin-resistant *Staphylococcus aureus* 57 were chosen from among gram-positive bacteria and *Escherichia coli* NIHJ JC-2, *Pseudomonas aeruginosa* ATCC 10145, *Burkholderia cepacia* 10 from among gram-negative bacteria. As control, data generated with Hibitane are also presented.

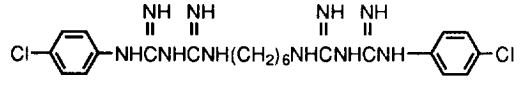
Among the four compounds (**4-7**) synthesized using hexyl, compounds **5** (R^1=3,4-dichlorophenyl) and **7** (R^1=3,4-dichlorobenzyl) showed the broadest bactericidal activity covering all the tester strains. Therefore, with R^1=3,4-dichlorophenyl being fixed, the length of the alkyl side-chain R^2 was varied (**8-10**), but no remarkable increase in activity could be obtained. Then, with R^1=3,4-dichlorobenzyl being fixed, the length of the alkyl side-chain R^2 was similarly varied (**11-13**). As a result, octyl (**11**) was found to give the best result and any longer side-chain rather resulted in attenuation of bactericidal activity (**12, 13**). Aside from them, compounds showing high bactericidal activity (**15, 16, 17, 19,** and **20**) were found among compounds (**14-20**) synthesized by introducing various phenyl groups for R^2 with R^1=3,4-dichlorobenzyl being fixed.

Table 1. Bactericidal Concentrations of Biguanide Derivatives after 3 Minutes Contact (μg/ml)

Compd.	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
R^1									
R^2	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	$(CH_2)_7CH_3$	$(CH_2)_9CH_3$	$(CH_2)_{11}CH_3$	$(CH_2)_7CH_3$	$(CH_2)_9CH_3$
Sa	500	100	>100	50	50	100	100	≦10	50
MRSA	100	100	>100	50	50	20	100	≦10	≦10
Ec	100	20	100	50	50	20	20	≦10	≦10
Pa	20	20	50	50	50	100	20	≦10	100
Bc	100	20	100	100	NT	100	>500	≦10	100

Compd.	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	Hibitane
R^1									
R^2	$(CH_2)_{11}CH_3$								
Sa	100	50	20	20	20	50	20	≦10	100
MRSA	20	100	20	20	20	50	≦10	≦10	100
Ec	20	50	≦10	≦10	≦10	10	≦10	≦10	20
Pa	100	50	20	≦10	≦10	10	≦10	≦10	100
Bc	>500	NT	20	≦10	20	NT	≦10	≦10	>500

Sa = *Staphylococcus aureus* FDA 209P, MRSA = methicillin-resistant *Staphylococcus aureus* 57*, Ec = *Escherichia coli* NIHJ JC-2, Pa = *Pseudomonas aeruginosa* ATCC 10145, Bc = *Burkholderia cepacia* 10*.
* clinical isolates.



Hibitane

Table 2. Bactericidal Concentrations of **11** and Hibitane after 30 Seconds Contact (μg/ml)

Compd.	gram-positive bacteria				gram-negative bacteria		
	Sa	MRSA	Se	Ef	Ec	Sm	Pa
11	20	10	5	10	20	20	10
Hibitane	>160	>160	40	>160	80	40	160

gram-positive bacteria: Sa = *Staphylococcus aureus* FDA 209P, MRSA = methicillin-resistant *Staphylococcus aureus* 57*, Se = *Staphylococcus epidermidis* ATCC 12228, Ef = *Enterococcus faecalis* IFO 12580.
gram-negative bacteria: Ec = *Escherichia coli* NIHJ JC-2, Sm = *Serratia marcescens* IFO 12648, Pa = *Pseudomonas aeruginosa* ATCC 10145.
* clinical isolates.

From among the compounds synthesized above, OPB-2045 (**11**)[14] was finally chosen as the compound for further scrutiny. A variety of efficacy tests revealed that **11** has an extended bactericidal spectrum as well as remarkably increased activity as compared with the conventional antiseptics as far as gram-positive bacteria, inclusive of MRSA, are concerned and that **11** is active against antiseptic-resistant *Pseudomonas* strains at low concentrations, suggesting that this compound may contribute broadly to the prevention of infections in general, inclusive of nosocomial infection. For example, the bactericidal concentration (μg/ml) of **11** and Hibitane after 30 seconds of exposure is shown in Table 2. Still more detailed biological data will be presented in a separate paper. The compound **11** (OPB-2045) is now under intensive development (Phase II).

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14. **11:** ^{1}H NMR (250 MHz, DMSO-d_6): δ 0.84 (3H, t, J = 6.9 Hz), 1.10-1.50 (12H, m), 2.90-3.10 (2H, m), 4.33 (2H, d, J = 6.0 Hz), 6.90-7.20 (3H, m), 7.29 (1H, dd, J = 1.8, 8.3 Hz), 7.50-7.70 (3H, m), 7.92 (1H, bs). Anal. Calcd. for $C_{17}H_{27}Cl_2N_5$•HCl: C, 49.95, H, 6.90, N, 17.13. Found: C, 49.68, H, 7.25, N, 16.98. mp = 178-180 ℃.

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