Synthesis of Novel 4,6-Di(substituted)amino-1,2-dihydro-1,3,5-triazine Derivatives as Topical Antiseptic Agents

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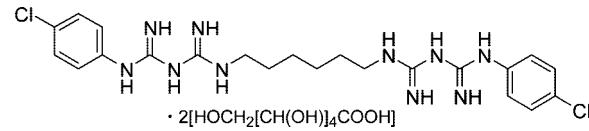
Abstract: A series of novel 4,6-di(substituted)amino-1,2-dihydro-1,3,5-triazine derivatives designed to have ClogP of 5.1−7.5 was synthesized and evaluated for their antiseptic properties by MIC and MBC tests against Gram-positive and Gram-negative bacteria, including MRSA, VRE, and *P. aeruginosa*. Among these compounds, 4-alkyl-6-aralkyl derivatives having ClogP of 6.6−7.1 and 4-alkyl-6-aryl or 4,6-dialkyl derivatives with ClogP of 6.0−6.4 showed pronounced antibacterial activities in both tests.

A large number of antibacterial drugs, such as antibiotics and synthetic or semisynthetic antibacterial agents, have been developed and used clinically to combat various infectious diseases, and these drugs have contributed dramatically to human health. On the other hand, bacteria that have gained resistance against these drugs, including so-called MRSA[a] and VRE, are recently becoming more prevalent and causing serious social problems, such as nosocomial infections, opportunistic infections, and so on.

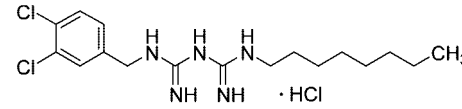
Although one of the useful ways to reduce these risks is to prevent infections beforehand by applying appropriate, fast-acting antiseptics or disinfectants topically on the human body, such as on the hands or skin, or in the medical environment, no new compound has been developed as an effective topical antiseptic or disinfectant for more than 50 years. Thus, since the discovery of benzalkonium chloride (1935), benzethonium chloride (1943), alkyldiaminoethylglycine hydrochloride (1953), CHG (Figure 1, 1954), and povidone-iodine (1956), these agents have continued to be widely used despite their unsatisfactory antiseptic potential and spectrum. Furthermore, many bacteria are now said to be becoming resistant[1] even to these agents. Therefore, there is an urgent need to develop new compounds having highly potent antiseptic property against wide variety of bacteria, including various drug-resistant organisms.

In one such attempt, Tsubouchi et al.[2] reported in 1997 that they had succeeded in modifying the antiseptic potential of CHG by chemical modification focused on its biguanide structure and identified the N^1,N^5-disubstituted biguanide derivative **1** (OPB-2045,[2] Figure 1) as a candidate for clinical testing, but as far as we are aware, it has not yet been put to practical use.

We now report on our approach to develop a new antiseptic agent for topical use, and the synthesis and antiseptic properties of novel 4,6-di(substituted)amino-dihydrotriazine derivatives are presented.



Chlorhexidine gluconate (Clog P=6.18 (free base))



1 (Clog P=6.19 (free base))

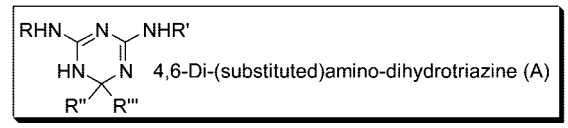


Figure 1. Biguanide-type antiseptics and synthetic target (**A**).

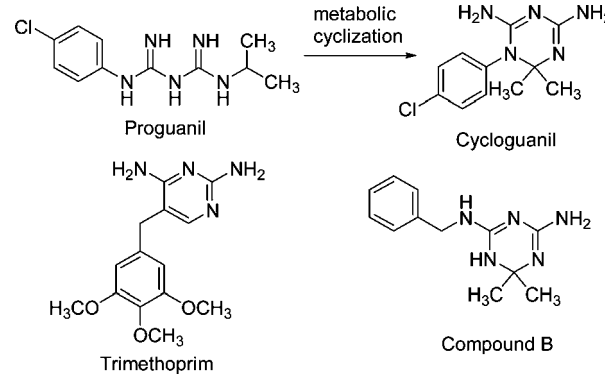


Figure 2. Structures of proguanil, cycloguanil, trimethoprim, and compound **B**.

4,6-Di(substituted)amino-dihydrotriazine structure (**A**, Figure 1) is thought to be one of the cyclized forms of biguanide antiseptic agents such as CHG or **1**, which stimulated our interest to study the antiseptic potential of this skeleton. Syntheses of a large number of compounds possessing this skeleton have been reported so far, especially since the discovery of cycloguanil in 1946 as an active metabolite of a biguanide-type antimalarial agent proguanil[3] (Figure 2), and various biological activities such as antimalarial, antibacterial, antitumor, antitoxoplasmic, anthelmintic, anticoccidium activities, and so on have been described.[4] However, it is well-known that these activities mainly depend on their inhibition of dihydrofolate reductase. Therefore, it is anticipated that even if many such compounds have antibacterial activity, this mode of action should not be bactericidal but rather bacteriostatic because of their inhibition of folic acid metabolism. To our knowledge, no folate inhibitor has been reported to have acute bactericidal properties and therefore has not been applied as antiseptic drug for topical use. According to our own experiments, for example, it was shown that trimethoprim, a representative antifolate antibacterial agent with a diaminopyrimidine structure (Figure 2), had antibacterial activity only in the MIC test but not in the MBC test, which is often used[2] to evaluate acute bactericidal properties (data not shown). A simple 4-amino-6-benzylamino-dihydrotriazine compound (**B**)[5] (Figure 2) was prepared as the acetate to examine its antibacterial property, but this was inactive even in our MIC test.

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[a] Abbreviations: MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococcus; ClogP, calculated log P; CHG, chlorhexidine gluconate; *S. aur*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; *P. aerug*, *Pseudomonas aeruginosa*.

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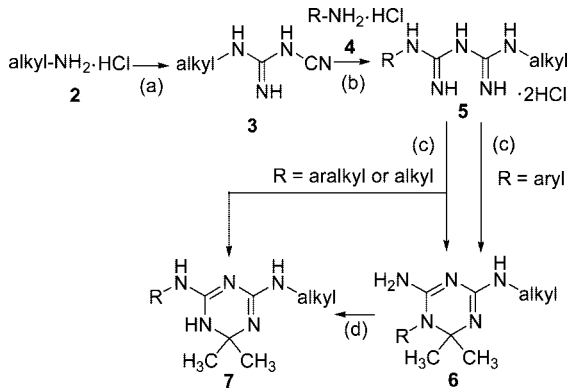
Scheme 1[a]



[a] Reagents and conditions: (a) $NaN(CN)_2$, CH_3CN, reflux; (b) xylene, reflux, concentrated HCl; (c) acetone, MeOH, concentrated HCl, reflux; (d) 5 M NaOH, EtOH (aq), reflux.

To generate compounds possessing acute bactericidal properties, we have introduced a concept that a suitable balance between molecular hydrophilicity and hydrophobicity is necessary for a compound to interact or participate with the bacterial cell wall to kill bacteria instantly. Thus, we designed hitherto unknown, novel 4,6-di(substituted)amino-dihydrotriazine derivatives (**A**) with ClogP [6] of 5−7 as an index because the free bases of CHG and **1** have ClogP of 6.18 and 6.19, respectively. Compounds with a suitable combination of aryl, aralkyl, and alkyl side chains at the two amino groups were prepared, and their antiseptic activity was evaluated by MIC and MBC tests for Gram-positive bacteria, *S. aur* 209PJC and MRSA 97-115, and for Gram-negative bacteria, *E. coli* NIHI JC-2 and *P. aerug* PAO-1. MIC and MBC values were determined, after 24 h of incubation and after 1, 3, and 5 min of contact, with the test organisms, respectively.

4,6-Di(substituted)amino-1,2-dihydrotriazine derivatives (**7**) were conveniently synthesized as shown in Scheme 1. The primary alkylamine hydrochloride (**2**) was reacted with sodium dicyanamide to give the cyanoguanidine (**3**). Reaction of **3** with another primary amine hydrochloride (**4**) gave a key intermediate biguanide (**5**) as the dihydrochloride. When cyclized with acetone in the presence of acid (e.g., hydrochloric acid) under reflux, two types of compounds **6** and **7** were obtained depending on the substituent R. In the case where R was an aryl group, the 4-alkylamino-6-amino-1-aryl-1,2-dihydro-1,3,5-triazin-6-amine (**6**) was obtained, in which the aryl group was incorporated in the triazine ring as an N^1-substituent. However, heating **6** with sodium hydroxide in ethanol resulted in isomerization to the desired 4,6-di(substituted)amino-1,2-dihydrotriazine (**7**) with two exocyclic substituted-amino groups. On the other hand, in the case where R in **5** is an aralkyl or an alkyl group, cyclization with acetone gave a mixture of **6** (minor) and **7** (major). Although **7** can be isolated by column chromatography, it was possible to obtain **7** as a single compound by treating the mixture with sodium hydroxide. Compounds **6** and **7** were able to be isolated as acid salts by treating with suitable acids. In the case of **7**, treatment of the free base with wet carboxylic esters easily produced the carboxylic acid salts, e.g., acetic acid salt from ethyl acetate, by rapid hydrolysis of the esters due to the strong basicity of **7**.

According to the above-mentioned general procedure, **8**−**36** having ClogP between 5.1 and 7.5 were designed and synthesized using a dimethyl structure for R″ and R‴ of the target (**A**) because it was easy and economical to prepare. It was also thought to be desirable to avoid the added complexity arising from the introduction of an asymmetric center at the C2 position. Most of the dihydrotriazine derivatives reported here were obtained as the acetates with some exceptions (Table 1). MIC

Table 1. Antibacterial Activity (MIC) of Compounds **7** (**8**−**36**) and CHG

				MIC (μg/mL)			
compd[a]	R[b]	alkyl	ClogP[c]	*S. aur*[d]	MRSA[e]	*E. coli*[f]	*P. aerug*[g]
8	Bzl	undecyl	7.43	0.4	0.8	100	100
9	Bzl	decyl	7.02	0.4	0.4	12.5	25
10	Bzl	nonyl	6.60	0.8	1.6	12.5	12.5
11	Bzl	octyl	6.18	0.8	1.6	12.5	25
12	4-Me-Bzl	decyl	7.50	0.8	0.8	100	100
13	4-Me-Bzl	nonyl	7.08	0.8	0.8	25	50
14	4-Me-Bzl	octyl	6.67	0.4	0.4	12.5	12.5
15	4-Me-Bzl	heptyl	6.25	0.8	1.6	25	50
16	4-MeO-Bzl	undecyl	7.31	0.4	0.8	100	100
17	4-MeO-Bzl	decyl	6.89	0.4	0.8	25	50
18[h]	4-MeO-Bzl	nonyl	6.47	0.8	1.6	25	50
19	4-CF_3−Bzl	octyl	7.10	0.4	0.8	50	25
20	2-Cl-Bzl	octyl	6.74	0.8	0.8	25	25
21	2-Cl-Bzl	octyl	6.74	0.8	0.8	25	25
22	Ph-CH_2CH_2	decyl	7.15	0.8	0.8	25	25
23	Ph-CH_2CH_2	nonyl	6.74	0.4	0.8	12.5	12.5
24	Ph-CH_2CH_2	octyl	6.32	0.8	1.6	25	25
25[i]	4-MeO-Ph-CH_2CH_2	decyl	7.03	0.4	0.8	50	50
26	Ph	decyl	6.81	0.4	0.4	12.5	100
27	Ph	nonyl	6.39	0.8	0.8	12.5	12.5
28	Ph	octyl	5.97	1.6	1.6	25	50
29	Ph	heptyl	5.55	6.3	6.3	50	100
30	octyl	octyl	7.22	0.8	1.6	100	>100
31	heptyl	heptyl	6.38	0.8	0.8	12.5	50
32	octyl	hexyl	6.38	0.8	0.8	12.5	50
33	octyl	pentyl	5.97	1.6	1.6	25	25
34	octyl	butyl	5.55	1.6	3.1	25	50
35	octyl	propyl	5.13	1.6	6.3	100	100
36	dodecyl	ethyl	6.31	0.8	1.6	12.5	50
	CHG		6.18	0.2	3.1	1.6	50

[a] Evaluated as acetates. [b] Bzl = benzyl; Ph = phenyl. [c] Free base. [d] *S. aur* 209PJC. [e] MRSA 97-115. [f] *E. coli* NIHJ JC-2. [g] *P. aerug* PAO-1. [h] Mesylate. [i] Hydrochloride.

Table 2. Bactericidal Activity (MBC) of Compounds **7** (**9**−**36**) and CHG

		MBC (μg/mL)						MBC (μg/mL)			
compd	contact (min)	*S. aur*[a]	MRSA[b]	*E. coli*[c]	*P. aerug*[d]	compd	contact (min)	*S. aur*[a]	MRSA[b]	*E. coli*[c]	*P. aerug*[d]
9	1	6.3	12.5	25	3.1	**23**	1mn	25	25	12.5	12.5
	3	6.3	6.3	12.5	3.1		3	12.5	12.5	3.1	3.1
	5	6.3	6.3	6.3	1.6		5	6.3	6.3	3.1	1.6
10	1	12.5	12.5	6.3	6.3	**24**	1	50	>100	6.3	6.3
	3	6.3	6.3	6.3	3.1		3	12.5	>100	3.1	6.3
	5	6.3	6.3	3.1	3.1		5	12.5	>100	3.1	3.1
11	1	25	>50	12.5	25	**25**	1	50	25	3.1	3.1
	3	12.5	50	6.3	12.5		3	12.5	6.3	1.6	3.1
	5	6.3	50	6.3	12.5		5	6.3	6.3	1.6	1.6
13	1	6.3	25	6.3	12.5	**27**	1	12.5	25	12.5	6.3
	3	3.1	12.5	1.6	3.1		3	6.3	25	6.3	3.1
	5	3.1	12.5	1.6	3.1		5	6.3	25	3.1	3.1
14	1	6.3	25	12.5	12.5	**28**	1	25	25	12.5	25
	3	3.1	25	6.3	6.3		3	6.3	12.5	6.3	12.5
	5	3.1	12.5	6.3	3.1		5	6.3	12.5	6.3	6.3
15	1	25	50	25	25	**31**	1	6.3	25	6.3	3.1
	3	12.5	50	12.5	12.5		3	6.3	25	3.1	1.6
	5	12.5	50	12.5	12.5		5	3.1	12.5	3.1	1.6
17	1	12.5	25	12.5	12.5	**32**	1	12.5	12.5	12.5	6.3
	3	6.3	6.3	6.3	3.1		3	6.3	6.3	6.3	3.1
	5	3.1	6.3	6.3	3.1		5	6.3	6.3	6.3	1.6
18	1	6.3	>100	12.5	25	**33**	1	3.1	6.3	12.5	6.3
	3	6.3	>100	6.3	12.5		3	3.1	3.1	6.3	3.1
	5	3.1	>100	3.1	12.5		5	3.1	3.1	6.3	3.1
19	1	12.5	6.3	3.1	12.5	**34**	1	25	25	50	50
	3	6.3	6.3	3.1	6.3		3	12.5	12.5	25	25
	5	6.3	6.3	3.1	6.3		5	6.3	12.5	12.5	12.5
20	1	3.1	6.3	3.1	6.3	**36**	1	3.1	25	12.5	3.1
	3	3.1	6.3	1.6	1.6		3	3.1	12.5	3.1	3.1
	5	3.1	6.3	1.6	1.6		5	1.6	6.3	3.1	3.1
21	1	12.5	12.5	3.1	6.3	CHG	1	62.5	1000	62.5	>500
	3	6.3	6.3	3.1	3.1		3	62.5	250	31.3	>500
	5	6.3	6.3	1.6	1.6		5	62.5	125	15.6	>500
22	1	50	>50	12.5	25						
	3	12.5	>50	12.5	12.5						
	5	12.5	>50	12.5	12.5						

[a] *S. aur* 209PJC. [b] MRSA 97-115. [c] *E. coli* NIHJ JC-2. [d] *P. aerug* PAO-I.

and MBC data evaluated for these compounds are shown in Tables 1 and 2, respectively.

Generally speaking, most of the compounds synthesized had potent antibacterial properties comparable to CHG in the MIC test (Table 1) and were superior to the CHG in the MBC test (Table 2), irrespective of the kind of hydrophobic *N*-substituents or *N*-alkyl chain-length. However, it seems that compounds with a relatively high ClogP (e.g., **8**, **12**, and **16** in the aralkyl-alkyl compounds; **26** in phenyl-alkyl compounds; **30** in dialkyl compounds) and those with a low ClogP value (e.g., **29**, **35**) tend to show reduced activity against Gram negative bacteria despite the fact that they still have potent activity against Gram positive bacteria in the MIC test. Compounds that showed MIC values less than 100 μg/mL (Table 1) were further evaluated by the MBC test (Table 2). Although some aralkyl-alkyl compounds having slightly high or low ClogP values, such as **11**, **15**, **18**, **22**, and **24**, were less effective against MRSA compared to other bacteria including ordinary *S.aur*, it was very interesting to note that these compounds generally had faster-acting and more-potent bactericidal activity than CHG, as indicated by the results of MBC test (Table 2). It is noted that the difference of substituents on the phenyl ring and the alkyl chain length does not seem to affect the antimicrobial activities, as can be seen from **9**−**36**. Therefore, ClogP of the whole molecule seemed to be a very important determinant factor of the antiseptic properties of this series. For example, 4-alkyl-6-aralkyl-dihydrotriazine derivatives having ClogP of 6.6−7.1 and 4-alkyl-6-aryl or 4,6-dialkyl derivatives with ClogP of 6.0−6.4 are expected to have a reasonably good potential as antiseptic agents.

Table 3. Bactericidal Activity (MBC) of Compounds **14**, **32**, and CHG

		MBC (μg/mL)			
compd	contact (min)	MRSA[a]	MRSA[b]	VRE[c]	*P. aerug*[d]
14	1	50	50	25	6.3
	3	50	50	25	6.3
	5	25	25	12.5	3.1
32	1	50	50	25	3.1
	3	25	25	25	3.1
	5	25	25	12.5	1.6
CHG	1	500	500	>1000	125
	3	250	500	>1000	31.3
	5	250	500	>1000	31.3

[a] MRSA 97-53. [b] MRSA KM 97-108. [c] VRE 49. [d] *P. aerug* no. 12.

MBC effects of **14** and **32** against different strains of MRSA and *P. aerug* and a VRE strain were further evaluated in comparison to CHG. As shown in Table 3, these compounds showed potent bactericidal properties against every strain including VRE. In contrast, CHG was far less potent than **14** and **32** and appeared to be inactive against the VRE used.

In conclusion, our work has shown that the novel 4,6-di(substituted)amino-1,2-dihydro-1,3,5-triazine derivatives synthesized had remarkable and acute bactericidal properties and thus may be promising new antiseptics, which are to be developed after a 50-year absence of this type of drug. Further studies on antiseptic properties including mode of action of these analogues [e.g., 1,2-dihydro-2,2-dimethyl-6-(4-methylben-

zyl)amino-4-octylamino-1,3,5-triazine D-gluconate (HM-242)] are currently in progress.

Supporting Information Available: Details on the synthetic procedures, compound characterization, and MIC and MBC test methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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