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Antibacterial and Antiproliferative Activity of Cationic Fullerene Derivatives

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Abstract—We examined the antibacterial and antiproliferative activities of alkylated C_{60}-bis(*N,N*-dimethylpyrrolidinium iodide) derivatives. The fullerene derivatives inhibited bacteria and cancer cell growth effectively. However, the fullerene derivatives with a long alkyl chain did not show antibacterial activity.

Introduction

An alarming increase in antimicrobial and antitumor resistances is one of the most serious problems in medicinal chemistry. These threats provide motivations to search for new types of lead compounds to be used as medicine. Generally, the acquisition of the drug resistance for completely new type of compounds seems to be difficult.

Fullerene, a condensed aromatic ring compound with an extended π-conjugated system, is a new type of organic compound that was discovered in 1985.[1] This compound has a cage-like shape, and a great deal of attention has been focused on its unique properties.

The biological effects of fullerene and its derivatives are also of interest.[2] Some biological activities based on their unique physical properties and chemical reactivities have been reported. For example, DNA scissions[3,4] and oxidation of biological materials[5,6] depend on photo-induced active oxygen production by the fullerene.[7,8] Other interesting biological effects of fullerene derivatives have also been reported.[9–12] We intend to develop fullerene derivatives as a new type of lead compound to be used as medicine.

We have reported that cationic fullerene derivatives, alkylated C_{60}-bis(*N,N*-dimethylpyrrolidinium iodide), inhibit *Escherichia coli* growth, but anionic fullerene derivatives, regio isomer of $C_{60}(C(COOH)_2)_2$, did not. We also have shown that the mechanism of growth suppression is respiratory chain inhibition.[13,14] The respiratory chain is located in the membrane. A compound with a long alkyl chain seems to be easily incorporated into the biological membrane. These encouraged us to investigate the antibacterial and antiproliferative activities of alkylated C_{60}-bis(*N,N*-dimethylpyrrolidinium iodide) derivatives.

In this report, we studied the antibacterial and antiproliferative activities of C_{60}-bis(*N,N*-dimethylpyrrolidinium iodide) regio isomers, **1–3**, and C_{60}-bis(2-alkyl-*N,N*-dimethylpyrrolidinium iodide) derivatives, **4–6**.

Results and Discussion

Fullerene derivatives

Reparation of **1–3**, main regio isomers of C_{60}-bis(*N,N*-dimethylpyrrolidinium iodide), have been reported (Fig. 1).[14]

Preparation of alkylated C_{60}-bis(*N,N*-dimethylpyrrolidinium iodide) derivatives[15]

Fullerene derivatives, C_{60}-bis(2-alkyl-*N*-methylpyrrolidine) were synthesized from C_{60}, *N*-methylglycine, and

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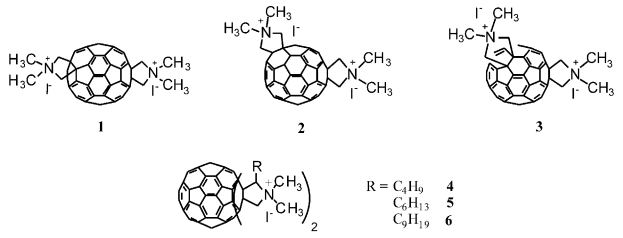

Figure 1. Structure of fullerene derivatives.

the corresponding aldehyde. Bis-adduct, a mixture of the regio-isomer, was purified by silica gel column chromatography. It was then treated with methyl iodine to give C_{60}-bis(2-alkyl-*N,N*-dimethylpyrrolidinium iodide), **4**–**6**.

4–**6** were identified by ^{1}H NMR and FAB-mass. The results of high-resolution FAB-mass; **4**; $C_{76}H_{37}N_2$ (observed) 977.2994 (error, +3.8) **5**; $C_{80}H_{45}N_2$ (observed) 1033.3638 (error, +5.5) **6**; $C_{86}H_{57}N_2$ (observed) 1117.4430 (error, −9.2).

Antibacterial activity

Antibacterial activity of the fullerene derivatives in vitro was tested against Gram-positive bacteria, according to NCCLS guidelines as minimal inhibitory concentration (MIC) (Table 1). The fullerene derivative was added as a DMSO solution.

With an identical effect on respiratory chain activity,[15] the regio isomers of C_{60}-bis(*N,N*-dimethylpyrrolidinium iodide), **1**, **2**, and **3**, had excellent antibacterial activity, which was comparable with that of vancomycin (VCM). The antibacterial effect of the three regio isomers was not significantly different. These findings indicate that it is not necessary to separate the regio isomers to study their biological activities. C_{60}-bis(2-alkyl-*N,N*-dimethylpyrrolidinium iodide) (alkyl: *n*-C_4H_9, **4**, *n*-C_6H_{13}, **5**) also showed antibacterial activity, but both were less effective. These derivatives, **1** to **5**, inhibited the growth of vancomycin-resistant *Enterococcus faecalis*. In *E. coli* growth inhibition, **4** was more effective than C_{60}-bis(*N,N*-dimethylpyrrolidinium iodide).[15] This difference may be explained as follows. First, C_{60}-bis(*N,N*-dimethylpyrrolidinium iodide), which was used for the *E. coli* experiment, was a mixture of regio isomers. Second, *E. coli* is Gram-negative bacteria; therefore, its permeability into the cell is different from that of the Gram-positive strain.

In contrast to **1**–**5**, derivatives with a long alkyl chain, **6**, had no antibacterial activity. In the respiratory chain inhibition, **6** was inactive. MIC of the anionic fullerene derivatives, which did not have respiratory chain inhibition activity,[13] was over 100 μg/mL. These results indicated that the mechanism of antimicrobial activity is a respiratory chain inhibition and that appropriate lipophilicity of the cationic derivatives was suitable for the inhibition of the respiratory chain and for antibacterial activity.

Taso et al.[16] are currently studying the antibacterial activity of carboxy fullerenes. They have shown that its action is achieved by insertion into the cell walls and disruption of the cell structure. This mechanism is different from the respiratory chain inhibition. The bacteriostatic effect of fullerene derivatives has been reported by Bosi et al.,[17] but the mechanism was not investigated.

Antiproliferative activity

The antiproliferative activities of fullerene derivatives were evaluated and compared with well-known anticancer agents by using a panel of 36 human cancer cell lines. The antiproliferative activity was measured by the MTT assay, giving the fullerene derivative concentration required for 50% growth inhibition (GI_{50}).[18] The average of the logarithm of GI_{50} (MID-GI_{50}) is presented in Table 2. Compounds **1**–**6** were effective in the antiproliferative activity, which was comparable to that of cisplatin (MID-GI_{50} = −5.30). In case of antibacterial activity, **6** was ineffective. It seems that the mechanism of antiproliferative activity is not the same as that of antibacterial activity and/or that the distribution of these derivatives in a mammalian cell is different from that in bacteria.

The antiproliferative activity spectra of fullerene derivatives for tumor cell lines were compared with well-known anticancer agents using the NCI-developed COMPARE program.[18] It has been shown that agents operating by a similar mechanism give comparable spectra and show a high correlation coefficient between the two agents. Table 2 shows the highest correlation coefficient values (r_{max}) and corresponding anticancer agents with fullerene derivatives. All r_{max} values were less than 0.75, and some were less than 0.5. When r_{max} is less than 0.5, a compound is a completely new type of antiproliferative agent whose working mechanism is

Table 1. Antibacterial activity of fullerene derivatives

	MIC (μg/mL)						
	1	2	3	4	5	6	VCM
S. aureus 209P JC-1	1.56	0.78	3.12	6.25	6.25	>100	1.56
S. aureus M133 (MRSA)	0.78	1.56	3.12	6.25	12.5	>100	1.56
S. aureus M126 (MRSA)	3.12	1.56	3.12	6.25	12.5	>100	1.56
S. eidermidis ATCC 14990	6.25	3.12	3.12	6.25	12.5	>100	3.12
E. hirae ATCC 8043	12.5	6.25	6.25	6.25	25	>100	3.12
E. faecalis W-73	12.5	6.25	6.25	6.25	50	>100	3.12
E. faecium vanA (VRE)	12.5	6.25	6.25	12.5	12.5	>100	>100
E. faecalis NCTC 12201 (VRE)	12.5	3.12	6.25	6.25	25	>100	>100

Table 2. Antiproliferative activity of fullerene derivatives

Fullerene	MID-GI_{50}	Highest correlation coefficient (r_{max})	Anticancer agent
1	−5.44	0.490	Bleomycin
2	−5.18	0.523	Taxol
3	−5.18	0.534	Actinomycin-D
4	−5.42	0.701	Actinomycin-D
5	−5.48	0.709	Vincristine
6	−5.61	0.637	Taxol

different from that of well-defined anticancer agents. In the case of $0.5 < r_{max} < 0.75$, the mechanism of antiproliferative activity may be different from that of well-defined anticancer agents.[18]

Tabata et al. have reported the anticancer activity of a fullerene derivative with laser photo-irradiation.[19] A laser photo-irradiated fullerene derivative produces active oxygen to kill cancer cells. The action was not replicated in the present experiment because photo-irradiation was not applied.

In conclusion, the data obtained from this study indicate that the cationic fullerene derivatives are new and effective antibacterial and antitumor agents.

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