Effects of Trifluoromethyl Ketones on the Motility of *Proteus vulgaris*

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In the present study, we showed the inhibition of motility by trifluoromethyl ketone (TF) derivatives (1—8) in *Proteus vulgaris* (*P. vulgaris*) cultures. Among them, 1-(2-benzoxazoyl)-3,3,3-trifluoro-2-propanone (1) showed a much stronger inhibitory effect on the motility of *P. vulgaris* than other TF compounds at 10% MIC. Our results suggest the possibility of an inhibitory action of TF compounds on the proton motive forces by affecting the action of biological motor and proton efflux in the membranes, resulting in a reduction of the ratio of running and the increased number of tumbling and non-motile cells.

Key words motility; *Proteus vulgaris*; trifluoromethyl ketone; proton pump

We previously reported that 1-(2-benzoxazolyl)-3,3,3-trifluoro-2-propanone (**1**) has potent antimicrobial activity against *Escherichia coli* (*E. coli*), *Bacillus megaterium*, *Corynebacterium michiganese* and yeast, but not against Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Serratia marcescens*.[1)] The combination of the ATP-binding casette (ABC) transporter inhibitor promethazine with **1** was significantly synergistic against *E. coli* strains. **1** also had a synergistic effect with antibiotics in the *E. coli* AG100 strain which has a proton efflux pump that can pump out a wide range of antibiotics, indicating that **1** can exert an inhibitory effect on a proton pump.

In this paper, we examined the effects of trifluoromethyl ketone (TF) derivatives (**1**—**8**) on the motility of *Proteus vulgaris* (*P. vulgaris*).

MATERIALS AND METHODS

Bacterial Strains Clinical isolate of *P. vulgaris* was obtained from the Institute of Clinical Microbiology and Diagnostics, University of Szeged.

Culture Media The *P. vulgaris* strain was maintained on minimal-tryptone-yeast extract (MTY) agar plates and cultured in MTY broth media. Double concentrated MTY broth media was used for culturing bacteria with drugs to determine the MIC values. Phosphate buffered saline (PBS) was used to solve the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye and to dilute the drugs and bacteria in antimotility experiments.

Chemicals 4,4,4-Trifluoro-1-phenyl-1,3-butanedione (**2**) and 3-trifluoroacetylindole (**5**) were obtained from Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan. 3-(2-Benzoxazolyl)-1,1,1-trifluoro-2-propanone (**1**), 1,1,1-trifluoro-3-[2-(4,5-dimethyloxazolyl)]-2-propanone (**3**), 2-trifluoroacetylbenzoxazole (**4**), 1-(2-benzoxazolyl)-2-propanone (**6**), 3-(2-benzimidazolyl)-1,1,1-trifluoro-2-propanone (**7**) and 3-(2-benzothiazolyl)-1,1,1-trifluoro-2-propanone (**8**) were previously synthesized.[2)] Promethazine (Pipolphen®) was used in antimotility experiments as a positive control.[3,4)]

Method for MIC Determination A half dilution of TF compounds was prepared in physiological saline on a 96-well microplate from the left to the right side. The overnight preculture of bacteria was diluted 10^4 times in double concentrated MTY broth, and distributed per 50 μl (about 5×10^4 CFU/ml) in the same amount of dilution of TF compound in a microplate. The plate was incubated at 37 °C for 24 h, then the MIC values of the TF compounds were determined by examining the wells where bacteria grew. The MTT dye was used to make visible the growing of bacteria: after the 24-h incubation, 10 μl of MTT (5 μg/ml dissolved in sterile PBS) was added into the wells, and the plate was incubated at 37 °C for about 3 h. When the bacteria grew, the yellow MTT discolored to blue formozan due to the effect of bacteria's NADPH dehydrogenase.

Method for Determining Antimotility Effect of Drugs After the overnight MTY culture of *P. vulgaris*, 100 μl was added to 900 μl of PBS which contains TF compounds in a subinhibitory (sub MIC) concentration, then 10% of the MIC concentration of TF compounds was administered. PBS with no drug was used as a negative control, and promethazine was used as a positive control.[3,4)] The samples in Eppendorf tubes were incubated for 15 min at 37 °C. One drop of the sample was placed on a microscopic slide and covered with an 18 mm square coverslip. The preparation was examined with a phase contrast Zeiss microscope with 63× water objective in the case of *P. vulgaris*. 200—300 cells of *P. vulgaris* were counted from 4—6 fields using a hand-tally counter. The running, tumbling and non-motile cells were separately counted. The ratio of cells with different types of moving was expressed as a percentage. The average and standard deviation were counted using the Microsoft Excel 2000 Program.

RESULTS

It was previously reported that some TF compounds have an antibacterial effect against *E. coli*[1)] and *Helicobacter pylori* (*H. pylori*).[5)] Here, we studied the antibacterial action of TF compounds (**1**—**8**) on *P. vulgaris*. The structures of the eight TF compounds (**1**—**8**) and their MIC values are presented in Fig. 1 and Table 1, respectively. From these results, **1** and **2** seems to be the most effective antibacterial agents among TF compounds, having the lowest MIC values.

In further experiments, we studied the antimotility action of TF compounds on *P. vulgaris* clinical isolate (Table 2). The average distribution of untreated *Proteus* movement was

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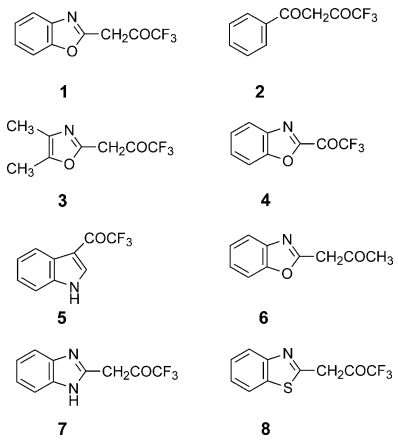

Fig. 1. Chemical Structure of Compounds (**1**—**8**) Tested

Table 1. MIC Values of TF Compounds (**1**—**8**) on *P. vulgaris* Strain

Compound	MIC values in μg/ml
1	6.3
2	12.5
3	156.2
4	625
5	625
6	625
7	1250
8	1250

the following: 64.5±10% running, 28.9±8% tumbling and 6.6±3% non-motile, respectively. In preliminary experiments, the strain of *P. vulgaris* was incubated with 10, 50, and 90% of MIC of **1** for 15 min at 37 °C, respectively, then the suspension was plated on an agar plate. No drug was used as a negative control. Counting the number of colonies grown, there were not significant differences between the treated and untreated samples, indicating that the treatment with the subMIC concentration of **1** in a short period does not influence the growth of bacteria.

Compounds (**5**, **7**, **8**) at 10% of their MIC values partially inhibited the motility. The ratio of moving cells decreased, and one of the tumbling ones significantly increased. The number of non-moving cells increased somewhat only in the sample treated with **5** at 10% of MIC. However, **5**, **7** and **8** had slight solubility problems and formed slight opalescence precipitation which could inhibit the running movement of bacteria. **1** exerted remarkable inhibition of movement: the ratio of non-moving cells increased significantly. In the case of **1**, **2**, **3**, **4** and **6**, the number of tumbling cells also changed, and a concentration-dependent antimotility effect was found in **1**. Among eight TF compounds, **1** was the most promising, with a strong antimotility effect on *P. vulgaris* (Table 2).

Table 2. Antimotility Effect of TF Compounds (**1**—**8**) on *P. vulgaris*

Compound (concentration; μg/ml)	Amount of bacteria (%)		
	Running	Tumbling	Total movement inhibition
Control	64.5±10	28.9±8	6.6±3
1 (10% MIC; 0.63)	49.6±26	26.3±13	24.2±13.5
(50% MIC; 3.15)	0.0±0.0	65.8±35.0	34.3±14.5
(90% MIC; 5.67)	0.0±0.0	9.1±5.2	90.9±27.7
2 (10% MIC; 1.25)	47.6±14.3	38.5±17.7	13.9±7.9
(90% MIC; 11.3)	51.4±14.7	33.8±6.6	14.8±8.7
3 (10% MIC; 15.6)	68.8±14.6	23.6±12.2	7.6±3.3
(90% MIC; 140)	45.1±29.2	37.2±7.2	17.7±12.1
4 (10% MIC; 62.5)	55.0±27.7	34.5±17.9	10.4±4.8
(90% MIC; 563)	3.9±3.7	80.0±21.2	16.1±6.9
5 (10% MIC; 62.5)[a)]	1.2±1.6	72.8±45.2	26.0±15.2
(90% MIC; 563)[a)]	0.3±0.8	91.0±14.2	8.7±2.1
6 (10% MIC; 62.5)	49.5±9.9	32.3±2.9	18.2±8.2
(90% MIC; 563)	6.4±3.4	46.0±21.6	47.6±17.4
7 (10% MIC; 125)[a]	37.8±4.0	53.9±9.8	8.3±2.7
(90% MIC; 1125)[a)]	0.0±0.0	96.1±24.7	3.9±1.4
8 (10% MIC; 125)[a)]	0.0±0.0	92.4±12.8	7.6±2.4
(90% MIC; 1125)[a)]	0.0±0.0	92.8±25.4	7.2±1.5
Promethazine			
(10% MIC; 15.6)	60.3±17.7	30.6±5.6	9.2±2.4
(90% MIC; 140)	4.9±2.8	68.6±41.4	26.5±8.0

a) The compound had solubility problems.

DISCUSSION

P. vulgaris is a peritrichous bacteria which drives the bacterial cell forward in a straight way by counterclockwise rotation. It is known that a flagellated bacterial cell moves by rotation of flagellae, which acts as the biological motor of the bacteria. The energy required for a flagellar motor comes from bacterial proton motive forces. Consequently, flagellae can increase or decrease their rotational speed in relation to the proton motive force due to the generation of pH gradient and electrochemical potential across the membrane.[6)]

It was previously reported that **1** inhibited *H. pylori* growth *in vitro*; however, **1** did not show inhibitory activity against jack bean urease.[5)] *H. pylori* is a spiral-shaped, strongly motile bacterium,[7)] and the motility conferred by the flagella is necessary for colonization in the gastric mucosa and subsequent development of gastritis.[8,9)] Therefore, in the stomach there are some connections between the pathogenecity and the active moving of *H. pylori*. Recent studies have provided evidence that motility in *H. pylori* was also suppressed by proton pump inhibitors such as rabeprazole.[8,9)] It is also suggested that TF compounds might have an inhibitory effect against the *in vitro* motility of *H. pylori*.[10)]

The inhibition of bacterial motility can be related to the virulence of bacteria[11)]: pathogenicity can be decreased in the presence of the TF compounds examined. In the case of nephropathogenic *Proteus* strains, the active moving has a role in the wandering of bacteria from the lower urine tract and bladder to the upper tract *via* the urethrae to cause chronic pyelonephritis. The adhesive pili also have a role in the sticking of bacteria. It was proven in electromicroscopic examination that the function of certain pili can be inhibited by specific compounds: *e.g.* pili-specific phages failed to adsorb.[12)]

In our preliminary experiments on two *E. coli* strains (a wild strain operating with a proton pump and the mutant with a defective proton pump due to a mutation),[13)] we found that the wild strain was more sensitive to the antimotility effect of **1** than the mutant strain.[14)] In both strains, there are many non-moving cells which can be adhered by the pili. After the treatment, the number of non-moving cells de-

creased with the increasing number of tumbling cells. This phenomenon can be explained by a special effect of **1**: it can somehow exert the effect on bacterial pili in both strains.

In this study, we found that some of the TF compounds could inhibit the motility of the bacteria. Based on our results and previous observations, we suggest that (i) TF compounds can inhibit the pathogenic factors (motility) of bacteria by the influence of proton motive forces and (ii) TF compounds also inhibit the efflux of antibiotics administered simultaneously from the cell in order to increase the intrabacterial concentration of the antibiotics, resulting in a synergistic effect between antibiotics and some TF compounds as a resistance modifier.

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