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Effect of a Trifluoromethyl Ketone on the Motility of Proton Pump-deleted Mutant of *Escherichia coli* Strain and its Wild-type

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Abstract. *We have recently found that 1-(2-benzoxazolyl)-3,3,3-trifluoro-2-propanone [**TF18**] exhibited the most potent antibacterial activity among 30 trifluoromethyl ketones against various prokaryotes, such as Escherichia coli (E. coli). In the present study, the inhibition of E. coli motility by **TF18** was investigated. **TF18** showed the lowest minimum inhibitory concentration (MIC) and highest inhibitory effect on the motility of E. coli strains. The wild-type E. coli was more sensitive to inhibition of motility than its proton pump-deleted mutant strain at subinhibitory concentrations. These data suggest that one of the targets of the antibacterial effect of the trifluoromethyl ketone is the proton pump system.*

Trifluoromethyl ketones have been shown to be inhibitors of a variety of proteases (1). The strong electron-withdrawing character of the trifluoromethyl group alters the properties of the carbonyl group and increases their electrophilicity (2). Recent efforts have been devoted to discovering new biological activities such as induction (3) and inhibition (4) of apoptosis, cyclooxygenase-2 inhibition (5) and histone deacetylase inhibition (6). We previously reported that a trifluoromethyl ketone derivative [**TF18**] has potent antimicrobial activity against *Escherichia coli* (*E. coli*), *Bacillus megaterium*, *Corynebacterium michiganese* and *Saccharomyces cerevisiae*, but not against Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Serratia marcescens* (7). The combination of the promethazine, an inhibitor of ATP binding cassette (ABC) transporter, with **TF18** was synergistic against the wild-type of *E. coli* strains.

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In this paper, we examined the anti-motility effects of 1-(2-benzoxazolyl)-3,3,3-trifluoro-2-propanone [**TF18**] on two *E. coli* strains with the proton pump-operating and proton pump-deleted mutant-types, respectively.

Materials and Methods

Bacterial strains. Two strains of *E. coli*, AG100 wild-type with the proton pump system and AG100A mutant-type with proton pump deficiency, were kindly provided by Professor Hiroshi Nikaido (University of California, Berkley, USA).

Culture media. Each of the two *E. coli* strains was maintained on minimal-tryptone-yeast extract (MTY) agar plates and cultured in MTY broth media (8). MTY broth media was used for culturing the bacteria with drugs to determine the minimum inhibitory concentration (MIC) values. Phosphate-buffered saline (PBS) was used to dissolve 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye and to dilute **TF18** and bacteria in antimotility experiments.

Chemicals. 1-(2-Benzoxazolyl)-3,3,3-trifluoro-2-propanone [**TF18**] had been previously synthesized (7).

Method for MIC determination. Dilutions of **TF18** were prepared in physiological saline on a 96-well microplate from the left to right side. Overnight preculture of bacteria was diluted 10^4 times in double concentrated MTY broth and 50 μL volume was distributed (about 5 x 10^4 colony forming unit (CFU)/mL) in the wells of the microplate containing the dilutions of **TF18** in 50 μL PBS. The plate was incubated at 37˚C for 24 hours, then the MIC values of **TF18** were determined by examining the wells where the bacteria grew. MTT dye was used to make visible the bacterial growth: after the 24-hour incubation, 10 μL of MTT (5 mg / mL disolved in sterile PBS) was added into the wells and the plate was incubated at 37˚C for 4 hours. Where the bacteria grew, the yellow MTT discoloured to blue formozan derivative through the activity of bacterial NADPH dehydrogenase.

Determination method of antimotility effect by drugs. From the overnight MTY culture of *E. coli*, 100 μL was added to 100 μL of PBS which contained **TF18** in subinhibitory (sub MIC) concentrations such as 10, 50, 90 and 200% of the MIC values. PBS

Table I. *Antimotility effect of TF 18 on E. coli AG100 (wild) and on AG100A (proton pump deficient) strains.*

Concentration of **TF18**	Amount of *E. coli* AG100 (wild) in the evaluated cells (%)*			Amount of *E. coli* AG100A (mutant) in the evaluated cells (%)*		
	Swimming	Tumbling	Non-motile	Swimming	Tumbling	Non-motile
Control	45.5	39.4	15.2	10.5	29.0	60.5
10% MIC	13.3	33.3	53.3	10.3	20.5	69.2
50% MIC	4.5	38.6	56.8	6.9	24.1	69.0
90% MIC	3.0	14.7	82.4	5.6	27.8	66.7
200% MIC	0	16.7	83.3	3.1	15.6	81.3

*approximately 200 cells were counted

without **TF18** was used as a control. The samples were examined right after the addition of **TF18**. One drop of sample was placed on a microscopic slide and covered with an 18-mm square coverslip. The samples were examined under a phase contrast (Zeiss) microscope with 40x objective. Approximately 200 cells of *E. coli* were counted from 6 fields using a hand-tally counter (9, 10). The swimming, tumbling and non-motile cells were separately counted.

Results

It has previously been shown that some trifluoromethyl ketones have an antibacterial effect against *E. coli* (7) and *Helicobacter pylori (H. pylori)* (11). The MIC value of the most effective, **TF18**, on the *E. coli* proton pump-deleted mutant strain was 3.9 mg/L and on the wild-type of *E. coli* 7.8 mg/L, respectively. The compound **TF18** had previously been found to exert the proton pump inhibitory effect (7).

Based on these data, the antimotility experiments were performed on two *E. coli* strains. One of the two strains has a proton pump efflux system, while the other strain has a deleted proton pump due to a mutation (12). The *E. coli* AG100 cells were moderately motile when the swimming and tumbling were measured on the phase contrast microscopy. On the other hand, *E. coli* AG100A (mutant) cells were mainly non-motile.

Inhibition of the motility of *E. coli* AG100 was observed at subinhibitory concentrations of **TF18** (Table I). At 10% MIC (0.78 mg/L), the number of non-motile cells was increased and of the swimming cells was decreased, while the tumbling was not influenced. At 50% MIC (3.9 mg/L), the swimming cells were decreased, while the number of the tumbling and non-motile cells did not change between 50% and 10% MIC. At 90% MIC (7.0 mg/L), 82% of the counted cells were non-motile, while at 200% MIC (15.6 mg/L) swimming cells could not be detected.

In the case of *E. coli* AG100A strains, the swimming cells were decreased with the increase in concentration of **TF18**, while the number of tumbling and non-motile cells were virtually unchanged between 90% (3.5 mg/L) and 10% MIC (0.39 mg/L).

Discussion

Bacterial motility can be related to the virulence of various bacteria (13). Nephro-pathogenic *E. coli* strains can be decreased by the use of proton pump inhibitors, where the active motion of bacteria has a role in the wandering of bacteria from the lower urinary tract *via* the urethra to initiate chronic pyelonephritis. In such a case, the adhesive pili of the bacteria can also have a role in their adhesion to mucous membranes. In clinical studies, it was shown that coadministration of promethazine, an antimotility agent, and an antibiotic resulted in a lower recurrence of chronic infection (14). We suppose that the *in vivo* effect might be more complex than the *in vitro* situation in which changes in the function of pili of bacteria are also involved (15).

In this study, we found that *E. coli* strains operating with the proton pump and its mutant-type were sensitive to the antimotility effect of **TF18**. The wild-type *E. coli* was more sensitive to the antimotility effect of **TF18** than its proton pump-deleted mutant-type. These data indicated that one of the targets of the trifluoromethyl ketone is the proton pump system.

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