biomolecules

Rapid Killing and Biofilm Inhibition of Multidrug-Resistant *Acinetobacter baumannii* Strains and Other Microbes by Iodoindoles

Chaitany Jayprakash Raorane †, Jin-Hyung Lee † and Jintae Lee *

School of Chemical Engineering, Yeungnam University, Gyeongsan 38541, Republic of Korea; chaitanyaraorane22@ynu.ac.kr (C.J.R.); jinhlee@ynu.ac.kr (J.-H.L.)

* Correspondence: jtlee@ynu.ac.kr; Tel.: +82-53-810-2533; Fax: +82-53-810-4631

† These authors contributed equally to this work.

Supplementary data

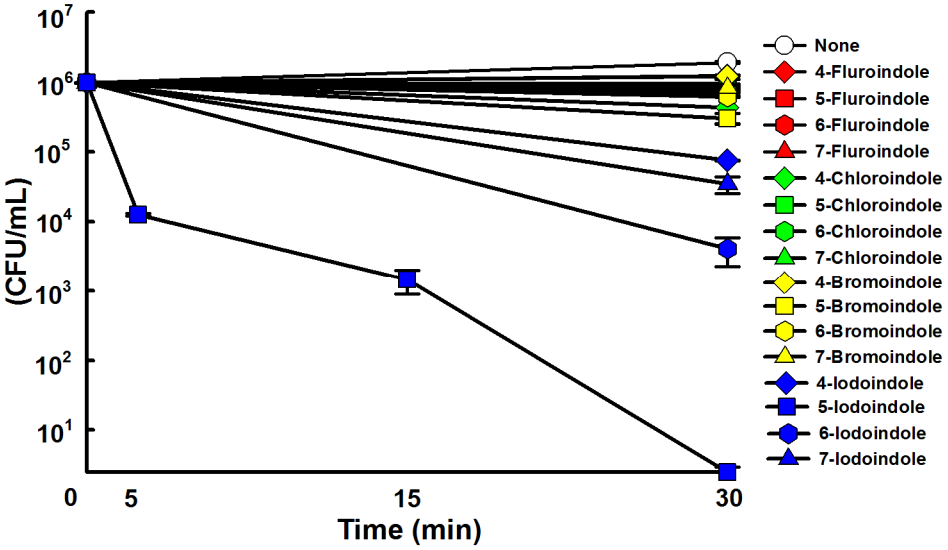

Figure S1. Rapid killing of *Acinetobacter baumannii* ATCC 17978 by halogenated indoles. *A. baumannii* cultures were treated with 0 or 200 μg/ml of the listed halogenated indoles. After incubation, cultures were plated, and CFUs were enumerated. Error bars represent standard deviation.

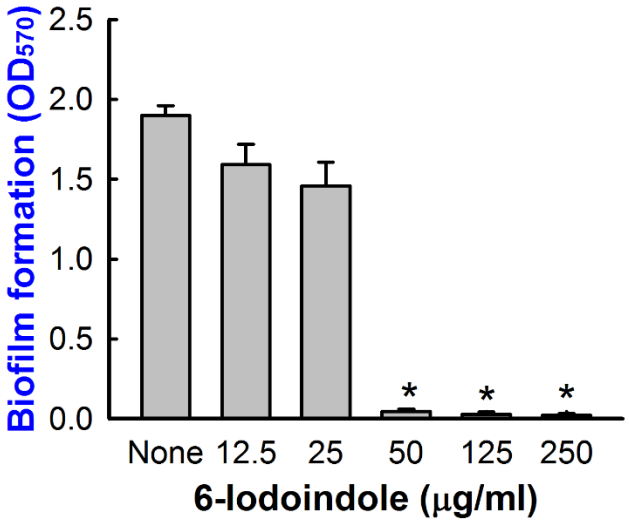

Figure S2. Biofilm inhibition of *A. baumannii* by 6-iodoindole. Biofilm inhibitory effects of 6-iodoindole against *A. baumannii* strains ATCC 17978 after culture for 24 h at 37°C without shaking. Error bars represent standard deviation. *$p < 0.05$ versus untreated controls.

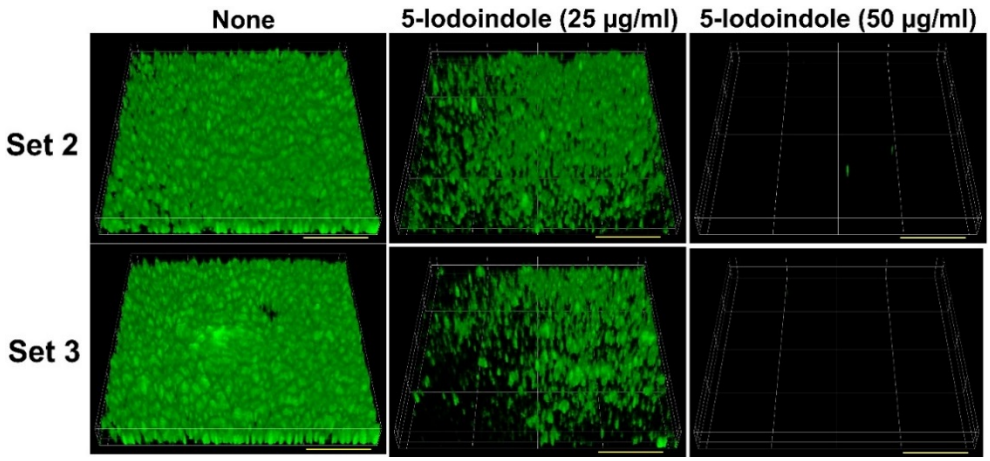

Figure S3. Additional confocal microscopic images showing biofilm inhibition of *A. baumannii*.

Scale bar = 50 µm.

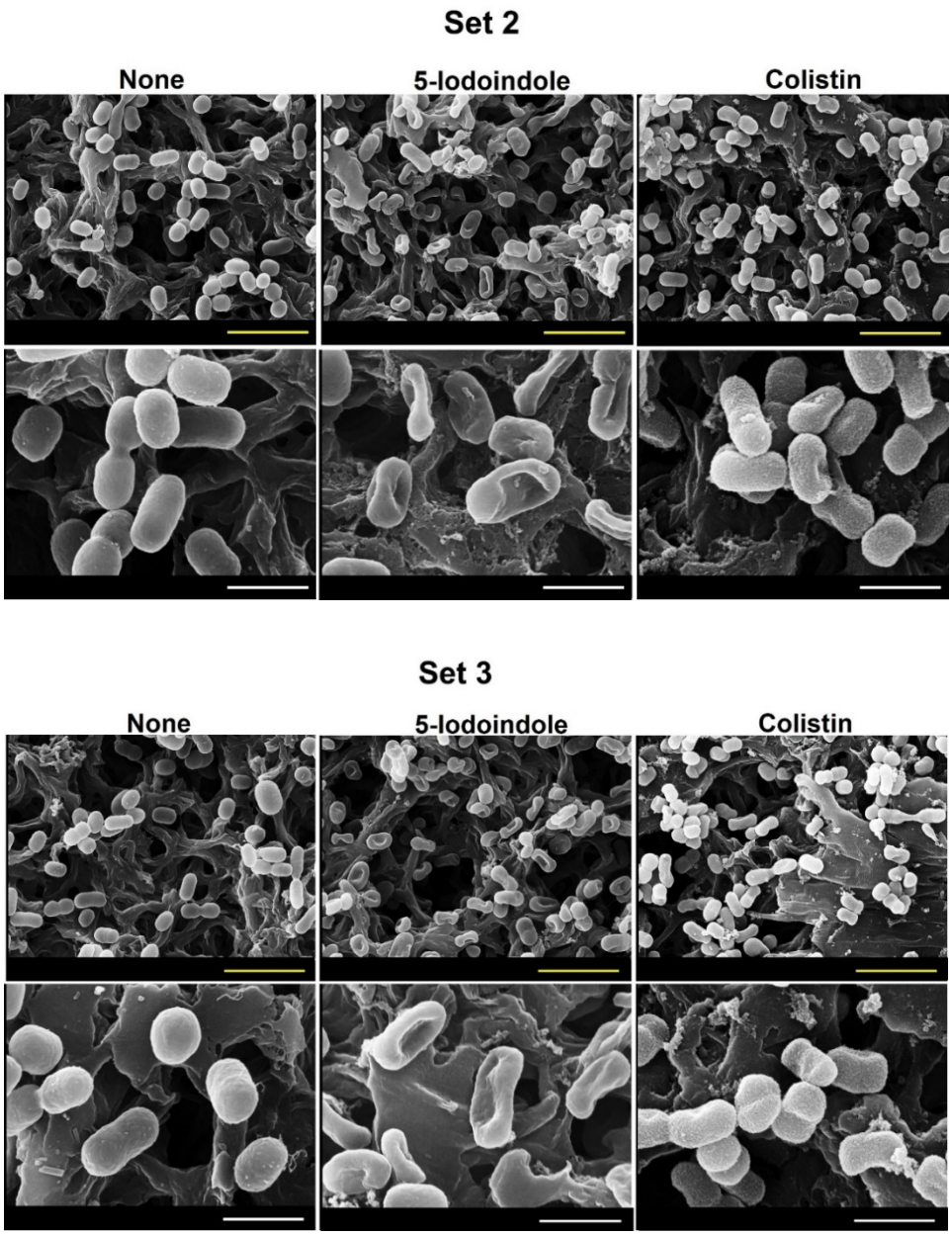

Figure S4. Additional SEM images showing membrane damage by 5-iodoindole in *A. baumannii* cells. Cells were grown with or without 5-iodoindole (125 μg/ml) and or colistin (200 μg/ml; positive control) for 1 h and fixed on nylon membrane filters (0.45 μm). Scale bar: yellow = 3 μm, white = 1 μm.

Table S1. Determination of minimum inhibitory concentrations (MICs) of 5-iodoindole for *A. baumannii* ATCC 17978 and clinical isolates. MIC determinations after culture for 24 h at 37°C in 96-well plates in the presence of 5-iodoindole.

	***A. baumannii* strain**	**5-Iodoindole MIC (µg/ml)**	**Antibiotic**
Reference strain	ATCC 17978	50	Sensitive
	ATCC BAA-1709	50	Sensitive
Clinical isolates obtained from burns patients at the National Rehabilitation Institute of Mexico	A 550	50	MDR
	A 553	50	MDR
	A 556	50	MDR
	A 564	50	MDR
	A 571	50	Sensitive
	A 578	50	MDR
	A 580	50	MDR

Table S2. MICs of halogenated indole derivatives against *A. baumannii* ATCC 17978.

Chemical name	MIC (μg/ml)
4-Bromoindole	125
5-Bromoindole	125
6-Bromoindole	125
7-Bromoindole	125
4-Chloroindole	100
5-Chloroindole	125
6-Chloroindole	125
7-Chloroindole	200
4-Fluroindole	125
5-Fluroindole	125
6-Fluroindole	250
7-Fluroindole	>250
4-Iodoindole	100
5-Iodoindole	50
6-Iodoindole	50
7-Iodoindole	125

Table S3. Antibiofilm and antibacterial activities of indole compounds against *A. baumannii* ATCC 17978. The biofilm screening of *A. baumannii* ATCC 17978 was performed after culture in TSB for 24 h at 37°C in 96-well polystyrene plates in the presence of indole derivatives at 50 µg/ml.

Name	Structure	Biofilm (%)	Cell growth (%)
None		100	100
4-Bromoindole		38 ± 8	32 ± 7
5-Bromoindole		90± 7	66 ± 3
6- Bromoindole		114 ± 8	68 ± 7
7- Bromoindole		106 ± 9	93 ± 1
5-Bromo-3-iodo-7-azaindole		84 ± 9	96 ± 4
4-Chloroindole		25 ± 9	28 ± 5
5-Chloroindole		119 ± 17	50 ± 9
6-Chloroindole		90 ± 5	70 ± 10
7-Chloroindole		98 ± 9	88 ± 2
5,6-Difluro isatin		112 ± 3	92 ± 2
2,3-Dimethyl indole		54 ± 12	80 ± 10
4-Fluroindole		109 ± 3	89 ± 2

Name	Structure	Biofilm (%)	Cell growth (%)
5-Fluroindole		121 ± 14	87 ± 7
6-Fluroindole		118 ± 5	98 ± 2
7-Fluroindole		127 ± 8	91 ± 2
7-Fluoro-5-iodoindole-3-carboxaldehyde		75 ± 4	102 ± 4
Indole		93 ± 7	100 ± 1
4-Iodoindole		40 ± 10	90 ± 11
5-Iodoindole		**4 ± 1**	**2 ± 1**
6-Iodoindole		**6 ± 1**	**5 ± 2**
7-Iodoindole		94 ± 8	79 ± 5
Isatin		75 ± 6	94 ± 2
3-Methyl indole		114 ± 7	81 ± 2
7-Nitroindole		87 ± 10	59 ± 5
5-Nitroisatin		82 ± 3	102 ± 1
5-(Trifluro methoxy) indoline -2,3-dione		92 ± 2	101 ± 3

Table S3. continued