

## SUPPORTING INFORMATION - BIOLOGY

### Microwave-Assisted One-Pot Synthesis and Anti-Biofilm Activity of 2-Amino-1*H*-imidazole/Triazole Conjugates

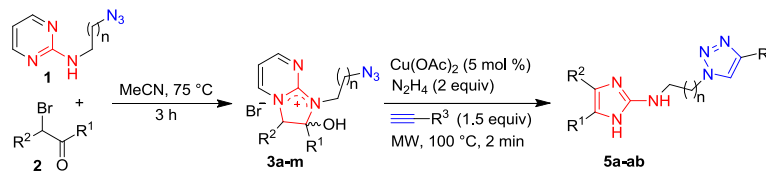
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**Table S1.** Scope of the microwave-assisted one-pot synthesis of the 2-AIT framework and anti-biofilm activity against *S. Typhimurium* and *P. aeruginosa*.<sup>a</sup>



Compounds	R1	R2	n	R3	Yield 3	Yield 5	<i>Salmonella Typhimurium</i> ATCC14028										<i>Pseudomonas aeruginosa</i> PA14																
							Compounds 3					Compounds 5					Compounds 3					Compounds 5											
							BIC50 <sup>f</sup> (μM)	95% confidence interval for BIC50	IC50 <sup>f</sup> (μM)	95% confidence interval for IC50	bioscreen <sup>d</sup> 20μM	biocreen 40μM	BIC50 (μM)	95% confidence interval for BIC50	IC50 (μM)	95% confidence interval for IC50	bioscreen 20μM	biocreen 40μM	BIC50 (μM)	95% confidence interval for BIC50	IC50 (μM)	95% confidence interval for IC50	bioscreen 20μM	biocreen 40μM	BIC50 (μM)	95% confidence interval for BIC50	IC50 (μM)	95% confidence interval for IC50	bioscreen 20μM	biocreen 40μM			
3a, 5a	Ph	H	2	Ph			71,71	51,8 to 99,4	>400				39,97	32,3 to 49,5	>400				23,52	18,9 to 29,3	365,5	255,1 to 523,8	-	-	19,02	8,8 to 41,0	>400						
3b, 5b	Ph	H	1	Ph	89	84	142,6	100,0 to 203,2	>400				36,48	22,9 to 58,1	>400				29,29	21,1 to 40,6	>400				>400		>400					+	
3c, 5c	4-BrC <sub>6</sub> H <sub>4</sub>	H	1	Pr	90	94	45,06	37,8 to 53,7	162,6	123,6 to 213,9			25,32	20,8 to 30,8	~94,50				43,56	37,6 to 50,5	>400				48,69	35,4 to 66,9	>400						
3d, 5d	4-BrC <sub>6</sub> H <sub>4</sub>	H	2	Pr	83	75	19,48	13,7 to 27,8	>400				35,33	29,8 to 41,9	~96,34				63,27	48,9 to 81,8	133,1	87,14 to 203,2			27,23	15,1 to 49,1	>400				-		
3c, 5e	4-BrC <sub>6</sub> H <sub>4</sub>	H	1	Hept	90	73							188,8	134,6 to 264,9	>400										>400		>400						
3d, 5f	4-BrC <sub>6</sub> H <sub>4</sub>	H	2	C(CH <sub>3</sub> ) <sub>2</sub> (NH <sub>2</sub> )	83	75							30,87	27,5 to 34,6	~91,37										21,54	18,3 to 25,3	20,08	14,1 to 28,6	+		+		
3c, 5g	4-BrC <sub>6</sub> H <sub>4</sub>	H	1	c-Pr	90	80							2,022	1,4 to 2,9	2,372	0,9 to 6,3									71,62	21,8 to 234,8	>400						
3c, 5h	4-BrC <sub>6</sub> H <sub>4</sub>	H	1	c-Hex	90	71							8,367	6,7 to 10,4	18,45	14,6 to 23,3									12,5*		26,2	16,0 to 42,8					
3c, 5i	4-BrC <sub>6</sub> H <sub>4</sub>	H	1	4-MeC <sub>6</sub> H <sub>4</sub>	90	73							>400		>400										>400		>400						
3d, 5j	4-BrC <sub>6</sub> H <sub>4</sub>	H	2	4-pentylC <sub>6</sub> H <sub>4</sub>	83	80							>400		>400										>400		>400						
3c, 5k	4-BrC <sub>6</sub> H <sub>4</sub>	H	1	4-MeOC <sub>6</sub> H <sub>4</sub>	90	66							91,2	40,4 to 205,9	>400										42,7	22,4 to 81,7	>400						
3e, 5l	3,4-diClC <sub>6</sub> H <sub>3</sub>	H	1	Pr	75	85	15,26	11,7 to 19,8	>400				35,64	26,3 to 48,3	>400				46,6	37,5 to 58,0	196,0	156,0 to 246,2			27,38	18,2 to 41,3	>400					-	
3f, 5m	3,4-diClC <sub>6</sub> H <sub>3</sub>	H	2	Pr	77	91	12,52	9,6 to 16,3	>400		+		31,78	22,2 to 45,4	60,85	37,6 to 98,4			50,45	41,8 to 60,9	120,3	94,52 to 153,2			24,41	16,8 to 35,5	>400						
3e, 5n	3,4-diClC <sub>6</sub> H <sub>3</sub>	H	1	c-Hex	75	89							55,21	22,5 to 135,8	>400										~25*		>400						
3f, 5o	3,4-diClC <sub>6</sub> H <sub>3</sub>	H	2	c-Hex									26,75	17,8 to 40,1											>400		>400						
3f, 5p	3,4-diClC <sub>6</sub> H <sub>3</sub>	H	2	4-tert BuC <sub>6</sub> H <sub>4</sub>	77	85							17,78	9,8 to 32,4	>400											>400		>400				~12,5*	
3e, 5q	3,4-diClC <sub>6</sub> H <sub>3</sub>	H	1	4-heptylC <sub>6</sub> H <sub>4</sub>	75	84							>800		>400											>400		>400					
3f, 5r	3,4-diClC <sub>6</sub> H <sub>3</sub>	H	2	CH <sub>2</sub> NMe	77	81							10,84	9,1 to 13,0	67,51	47,7 to 95,7									8,135	4,4 to 15,0	6,661	5,3 to 8,4	+				
3f, 5s	3,4-diClC <sub>6</sub> H <sub>3</sub>	H	2	thiophen-3-yl	77	91							2,009	1,5 to 2,8	5,432	0,9 to 33,5									3,8**	2,6 to 5,6	>400						
3g, 5t	4-FC <sub>6</sub> H <sub>4</sub>	H	1	c-Pr	69	80	172,8	130,7 to 228,4	>400				93,3	73,3 to 118,8	>400				18,95	14,2 to 25,3	>400				~50*		>400						
3g, 5u	4-FC <sub>6</sub> H <sub>4</sub>	H	1	c-Pr-CH <sub>2</sub>	69	68							128,3	49,2 to 334,2	>400										32,9	16,1 to 67,1	>400						-
3h, 5v	morpholino-methanone	H	1	Pr	76	39	>400		>400				>400		>400				332,8	247,3 to 447,9	>400				>400		>400						
3i, 5w	morpholino-methanone	H	2	Pr	65	45	>400		>400				>400		>400				349,2	271,0 to 449,9	>400				>400		>400						
3j, 5x	naphth-2-yl	H	1	4-BuC <sub>6</sub> H <sub>4</sub>	69	64	22,43	18,1 to 27,7	66,34	49,8 to 88,4	+	0	>400	324,2 to 854,2	>400				42,61	37,5 to 48,5	189,1	144,3 to 247,7		+	>400		>400						
3k, 5y	CHPh <sub>2</sub>	H	1	tert Bu			34,14	27,9 to 41,7	>400				30,87	27,5 to 34,6	~91,04				23,1	18,2 to 29,3	210,6	147,1 to 301,4			>400	6,0 to 19,9	137,7	65,9 to 287,9				-	
3k, 5z	CHPh <sub>2</sub>	H	1	c-Pen									8,367	6,7 to 10,4	>400											>400		>400					
3l, 5aa	Ph	Ph	2	Hept	75	84	169,4	117,2 to 244,7	>400				10,84	9,1 to 13,0	>400				~200*		>400				>400		>400						
3m, 5ab	4-ClC <sub>6</sub> H <sub>4</sub>	4-Me C <sub>6</sub> H <sub>4</sub>	1	c-Pen	72	56	49,07	37,8 to 63,7	>400				6,464	4,9 to 8,6	>400				115,2	87,3 to 152,1	~384,6				>400		>400						

<sup>a</sup>All reactions were conducted on a 0.25 mmol scale of **3b-m**, applying hydrazine hydrate (2 equiv), acetylene (1.5 equiv), Cu(OAc)<sub>2</sub> (5 mol%) in EtOH/H<sub>2</sub>O (4:1) (1 mL); the mixture was irradiated in a sealed tube at a ceiling temperature of 100 °C and 35 W maximum power for 2 min; isolated yields are given.

<sup>b</sup>BIC<sub>50</sub>: compound concentration at which the biofilm formation is inhibited with 50%; 95% confidence intervals are provided in Table S2.

<sup>c</sup>IC<sub>50</sub>: compound concentration at which the planktonic growth is inhibited with 50%; 95% confidence intervals are provided in Table S2. Effect of the compounds on the planktonic growth curves are also provided in Table S2.

<sup>d</sup>o: the planktonic growth is completely or almost completely inhibited when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor;

+: the planktonic growth is retarded when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor;

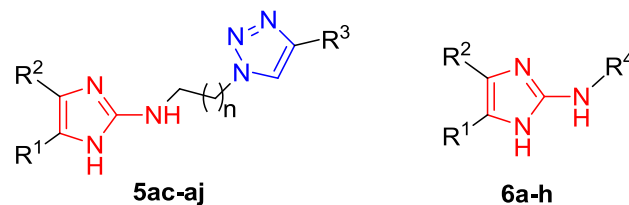
-: the planktonic growth is not or only slightly affected when the bacteria are grown in the presence of the indicated concentration of biofilm inhibitor;

No symbol indicated: effect not determined.

\*The compound is not able to completely prevent biofilm formation, as the dose response curve reaches a steady state level at about 50% biofilm inhibition.

\*\*With increasing concentrations, the dose response curve reaches a maximum of 90 % biofilm inhibition at a concentration of ~25 μM. At higher concentrations the % inhibition decreases again.

**Table S2.** Effect of incorporation of a triazole moiety in the long 2*N*-alkyl chain of 5-phenyl-2-aminoimidazoles on the anti-biofilm activity against *S. Typhimurium*, *E. coli*, *P. aeruginosa* and *S. aureus*.



compound	R1	R2	n	R3	R4	<i>Salmonella Typhimurium</i> ATCC14028				<i>Escherichia coli</i> TGI				<i>Pseudomonas aeruginosa</i> PA14				<i>Staphylococcus aureus</i> SH100			
						IC50 <sup>a</sup> (μM)	95% confidence interval for IC50	BIC50 <sup>b</sup> (μM)	95% confidence interval for BIC50	IC50 (μM)	95% confidence interval for IC50	BIC50 (μM)	95% confidence interval for BIC50	IC50 (μM)	95% confidence interval for IC50	BIC50 (μM)	95% confidence interval for BIC50	IC50 (μM)	95% confidence interval for IC50	BIC50 (μM)	95% confidence interval for BIC50
<b>5ac</b>	4-ClC <sub>6</sub> H <sub>4</sub>	H	3	Bu		23,9	11,9 to 48,0	56,9	16,9 to 81,6	12,2	3,5 to 42,5	19	9,5 to 38,1	5,0*	3,1 to 8,2	>400		~94,7		134,2	114,0 to 158,1
<b>6a</b>	4-ClC <sub>6</sub> H <sub>4</sub>	H			Dec	>400		>400		>400		>400		>400		>400		~ 200,8		236	198,3 to 280,9
<b>5ad</b>	4-FC <sub>6</sub> H <sub>4</sub>	H	3	Bu		186,9	57,12 to 611,7	>400		~100		340	170,0 to 681,2	3,7*	2,6 to 5,2	>400		304,3	116,9 to 792,6	390,8	334,4 to 456,7
<b>6b</b>	4-FC <sub>6</sub> H <sub>4</sub>	H			Dec	>400		>400		~ 199,5		~400		19,0**	7,0 to 51,5	>400		~75,0		~ 88,8	
<b>5ae</b>	4-OMeC <sub>6</sub> H <sub>4</sub>	H	3	Bu		114,5	61,5 to 213,2	>400		71	59,5 to 84,7	~75		6,9*	4,2 to 11,3	159,7	107,9 to 236,1	~150,0		130,9	95,7 to 179,2
<b>6c</b>	4-OMeC <sub>6</sub> H <sub>4</sub>	H			Dec	49,7	24,5 to 100,7	>400		47,3	20,0 to 111,9	>400		>400		>400		~ 55,7		32,9	21,8 to 49,5
<b>5af</b>	3,4-diClC <sub>6</sub> H <sub>3</sub>	H	2	Bu		28,1	14,0 to 56,4	>400		6,7	2,2 to 20,3	~25		3,1	2,0 to 4,8	>400		~ 50,1		349,3	54,5 to 2238
<b>6d</b>	3,4-diClC <sub>6</sub> H <sub>3</sub>	H			Non	38,2	17,5 to 83,5	>400		9,6	6,1 to 15,3	>400		14,86**	6,3 to 35,1	>400		~ 70,9		43,4	23,5 to 80,0
<b>5ag</b>	3,4-diClC <sub>6</sub> H <sub>3</sub>	H	3	Bu		41,5	20,1 to 85,8	>400		10,3	3,6 to 29,3	~25		6,6*	4,9 to 9,0	>400		45,7	22,3 to 93,8	125,4	110,5 to 142,4
<b>6e</b>	3,4-diClC <sub>6</sub> H <sub>3</sub>	H			Dec	46,5	21,5 to 100,4	>400		44,8	15,9 to 126,0	~50		>400		>400		~ 91,4		43,9	30,4 to 63,2
<b>5ah</b>	naphth-2-yl	H	2	Bu		137,2	52,16 to 360,9	>400		52,7	15,7 to 176,3	>400		3,6*	2,1 to 5,9	>400		>400		>400	
<b>6f</b>	naphth-2-yl	H			Non	>400		>400		>400		>400		>400		>400		~201,9		440,8	119,5 to 1627
<b>5ai</b>	naphth-2-yl	H	2	Pen		9,7	3,6 to 25,8	>400		13,2	6,8 to 25,6	~25		1,3*	0,9 to 2,1	>400		36,7	17,9 to 75,1	109,5	77,1 to 155,4
<b>6g</b>	naphth-2-yl	H			Dec	>400		>400		187,6	106,7 to 330,4	>400		>400		>400		~ 141,1		141,4	108,2 to 184,8
<b>5aj</b>	CHPh <sub>2</sub>	H	2	Hept		31,9	8,7 to 117,2	>400		40,2	22,6 to 71,4	~100		37,4	13,80 to 101,2	>400		51,9	28,8 to 93,8	~ 51,0	
<b>6h</b>	CHPh <sub>2</sub>	H			Dodec	>400		>400		~200		>400		24,61*	13,1 to 46,3	>400		~ 55,0		42,1	32,3 to 54,7

<sup>a</sup>BIC<sub>50</sub>: compound concentration at which the biofilm formation is inhibited with 50%; 95% confidence intervals are provided in Table S2.

<sup>b</sup>IC<sub>50</sub>: compound concentration at which the planktonic growth is inhibited with 50%; 95% confidence intervals are provided in Table S2.

\*With increasing concentrations, the dose response curve reaches a maximum of 70 to 90 % biofilm inhibition at a concentration between 6,25 and 50 μM. At higher concentrations the % inhibition decreases again.

\*\*With increasing concentrations, the dose response curve reaches a maximum of 50 to 60 % biofilm inhibition at a concentration between 12,5 and 25  $\mu\text{M}$ . At higher concentrations the % inhibition decreases again.