

## Supporting Information

Computational screening of antimicrobial peptides for *Acinetobacter baumannii*

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# 1 Supplementary Table A

**Table A.** Details of all the 75 antimicrobial peptides and their experimental protocol used in the present work. Qualitative test set is indicated by blue color, and with a lower bound in MIC.

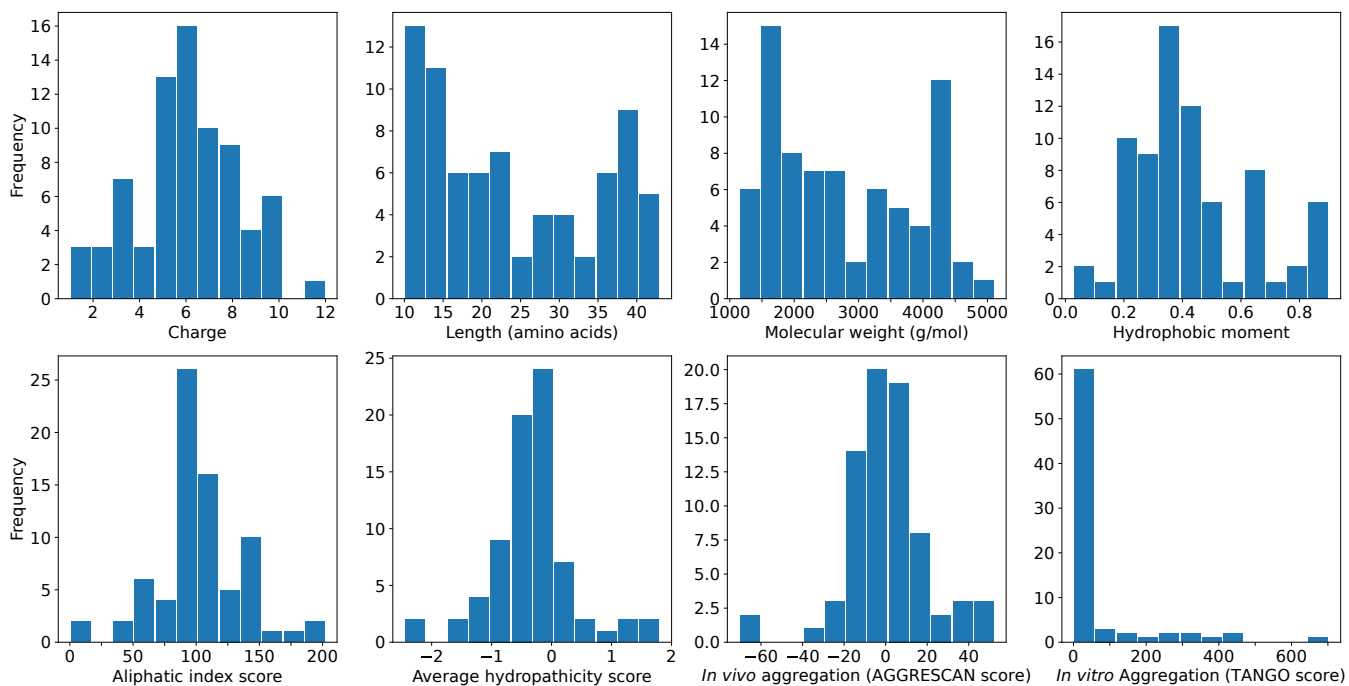
Sl. No	Peptide	Sequence	MIC ( $\mu\text{g/ml}$ )	References	Methods
1	Bactenecin	RLCRIVVIRVCR	64	[1]	CLSI
2	Cecropin A	KWKLFKKIEKVGQNIRDGIIKAGPAVAVVGGQATQIAK	32	[1]	CLSI
3	Cecropin B	KWKIFKKIEKVGVRNIRNGIIKAGPAVAVLGEAKAL	32	[1]	CLSI
4	Cecropin P1	SWLSKTAKKLENSAKKRISSEGIAIAIQGGPR	1.6	[1]	CLSI
5	Histatin 8	KFHEKHHSHRGY	32	[1]	CLSI
6	HNP-1	ACYCRIPACIAGERRYGTCTIYQGRLLWAFCC	50	[1]	CLSI
7	HNP-2	CYCRIPACIAGERRYGTCTIYQGRLLWAFCC	50	[1]	CLSI
8	Indolicidin	ILPWKWPWWPWR	8	[1]	CLSI
9	Magainin I	GIGKFLHSAGKFGKAFVGEIMKS	64	[1]	CLSI
10	Magainin II	GIGKFLHSAKKFGKAFVGEIMNS	256	[1]	CLSI
11	Mastoparan	INLKALALAKKIL	4	[1]	CLSI
12	Melittin	GIGAVLKVLTGLPALISWIKRKRQQ	4	[1]	CLSI
13	$\beta$ -Defensin	DHYNCSVSSGGQCLYSACPIFTKIQTGTCYRGKAKCKC	256	[1]	CLSI
14	LysAB2 P0 <sup>1</sup>	NPEKALEPLIAIQIAIKGMLNGWFTGVGFRKR	237.66	[2]	Conlon et.al. [3]
15	LysAB2 P1 <sup>1</sup>	EKALEKLIAIQKAIKMLNGWFTGVGFRKR	28.48	[2]	Conlon et.al. [3]
16	LysAB2 P2 <sup>1</sup>	EKALEKLIAIQKAIKMLAGWFTGVGARRKR	55.06	[2]	Conlon et.al. [3]
17	LysAB2 P3 <sup>1</sup>	NPEKALEKLIAIQKAIKMLNGWFTGVGFRKR	30.12	[2]	Conlon et.al. [3]
18	LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTE	4	[4]	CLSI
19	Aurein 1.2	GLFDIIKKIAESF -NH <sub>2</sub>	16	[4]	CLSI
20	CAMEL	KWKLFKKIGAVLKVL -NH <sub>2</sub>	2	[4]	CLSI
21	Citropin 1.1	GLFDVIKKVASVIGGL -NH <sub>2</sub>	16	[4]	CLSI
22	Omiganan	ILRWPWPWRK -NH <sub>2</sub>	32	[4]	CLSI
23	r-Omiganan	KRRWPWPWRLI -NH <sub>2</sub>	16	[4]	CLSI
24	Pexiganan	GIGKFLKAKKFGKAFVKILKK -NH <sub>2</sub>	2	[4]	CLSI
25	Temporin A	FLPLIGRVLSGIL -NH <sub>2</sub>	128	[4]	CLSI
26	rr	WLRRIKAWLRR	24.86	[5]	CLSI
27	rr2	WIRRIKWIIRVHK	3.95	[5]	CLSI
28	rr3	WLRRIKAWLRRKRK	31.46	[5]	CLSI
29	rr4	WLRRIKAWLRRIKA	3.73	[5]	CLSI
30	CLP-19	CRKPTFRRLKWKIKFKFKC	80.36	[6]	CLSI
31	C18G <sup>2</sup>	ALWKKLLKLLKSAKKG -NH <sub>2</sub>	3.87	[7]	Wiegand et.al. [8]
32	C18G-Arg <sup>2</sup>	ALWRRLLRLLRSARLG -NH <sub>2</sub>	8.48	[7]	Wiegand et.al. [8]
33	NK-2	KILRGVCKIMRTFLRRISKDILTGKK -NH <sub>2</sub>	2	[9]	CLSI
34	NK27	KILRGVSKIMRTFLRRISKDILTGKK -NH <sub>2</sub>	2	[9]	CLSI
35	N17	KILRGVSKIMRTFLRR -NH <sub>2</sub>	8	[9]	CLSI
36	C20	KKIMRTFLRRISKDILTGKK -NH <sub>2</sub>	8	[9]	CLSI
37	C20-DK	KKIMRTFLRRISKILTGKK -NH <sub>2</sub>	8	[9]	CLSI
38	NK23a	KISKIMRTFLRRISKDILTGKK -NH <sub>2</sub>	4	[9]	CLSI
39	NK23b	KILRGVSKIMRRISKDILTGKK -NH <sub>2</sub>	8	[9]	CLSI
40	NK22b	KILGVSKIMRRISKDILTGKK -NH <sub>2</sub>	16	[9]	CLSI
41	NK23c	KILRGVSKIMRTFLRRILTGKK -NH <sub>2</sub>	4	[9]	CLSI
42	NK19a	KISKIMRTFLRRILTGKK -NH <sub>2</sub>	4	[9]	CLSI
43	NK19b	KILRGVSKIMRRILTGKK -NH <sub>2</sub>	2	[9]	CLSI
44	NK19b-KR	RILRGVSRIMRRILTGR -NH <sub>2</sub>	8	[9]	CLSI
45	NK13	KISKIMRTFLRR -NH <sub>2</sub>	256	[9]	CLSI

Sl. No	Peptide	Sequence	MIC ( $\mu\text{g/ml}$ )	References	Methods
46	Pepcon <sup>2</sup>	FLFSLIPSAIGGLISAFK	37.62	[10]	Wiegand et.al. [8]
47	BP100	KKLFKKILKYL -NH <sub>2</sub>	8.5	[11]	CLSI
48	RW-BP10	RRLFRRLRWL -NH <sub>2</sub>	8.5	[11]	CLSI
49	BP202	KRLFRKILKYL -NH <sub>2</sub>	4.5	[11]	CLSI
50	BP203	KKLFKKILRYL -NH <sub>2</sub>	8.5	[11]	CLSI
51	H4	KFKKLFKKLSPVIGKEFKRIVERIKRFLR	36.34	[12]	CLSI
52	BR001 <sup>3</sup>	KWLFKKIEKVGQNIIRDGIIKAGPAVAVVGGATQIAK -NH <sub>2</sub>	10	[13]	Jorgensen et.al. [14]
53	BR002 <sup>3</sup>	GWLKKIGKKIERVGGHTRDATIQLGIAQQAANVAATAR -NH <sub>2</sub>	10	[13]	Jorgensen et.al. [14]
54	BR003 <sup>3</sup>	GGLKLLGKLEGAGKRVFNAAEKALPVVAGAKALRK	5	[13]	Jorgensen et.al. [14]
55	BR005 <sup>3</sup>	RGFRKHFNKLKVKVHTISETAHVAKDTAVIAGSGAAVVAAT -NH <sub>2</sub>	20	[13]	Jorgensen et.al. [14]
56	BR029 <sup>3</sup>	KWKIFKKIEKAGRNIRDGIIKAGPAVSVVGEAATIIYKYG	20	[13]	Jorgensen et.al. [14]
57	BR031 <sup>3</sup>	GWLRDFGKRIERVGGHTRDATIQAIGVAAQAANVAATVRG	20	[13]	Jorgensen et.al. [14]
58	BR032 <sup>3</sup>	GWLKKIGKKIERVGGHTRDATIQLVLAQAQAANVGPATARG	20	[13]	Jorgensen et.al. [14]
59	BR033 <sup>3</sup>	GWLKKIGKKIERVGGHTRDATIQTIGVAAQAANVAATLKG	20	[13]	Jorgensen et.al. [14]
60	BR034 <sup>3</sup>	GWLKKIFGKKIERVGGHTRDATIQAIGVAAQAANVAATLKG	20	[13]	Jorgensen et.al. [14]
61	BR035 <sup>3</sup>	GWLKKIGKKIERVGGHTRDASIQAIGIAQQAANVAATARG	10	[13]	Jorgensen et.al. [14]
62	BR036 <sup>3</sup>	GWLKKIGKKIERVGGHTRDATIQLVLAQAQAANVAATARG	20	[13]	Jorgensen et.al. [14]
63	BR037 <sup>3</sup>	GLVKKIGKKIERVGGHTRDASIQAIGIAQQAANVAATARG	20	[13]	Jorgensen et.al. [14]
64	Buforin I	AGRGKQGGKVRAKAKTRSSRAGLQFPVGRVHRLLRKGNV	>256	[1]	CLSI
65	Histatin 5	DSHAKRHHGKRVKRFHEKHHSHRGV	>256	[1]	CLSI
66	rr1	WKRRRIKIWKKIR	>438.06	[5]	CLSI
67	C18G-His	ALWHLLHLLHSAHHLG -NH <sub>2</sub>	>31.93	[7]	Wiegand et.al. [8]
68	I10	KKIMRTFLRR -NH <sub>2</sub>	>128	[9]	CLSI
69	NK15	KILRGVSKRILTGKK -NH <sub>2</sub>	>128	[9]	CLSI
70	NK14	KILGVSKRILTGKK -NH <sub>2</sub>	>256	[9]	CLSI
71	NK11	KISKRILTGKK -NH <sub>2</sub>	>256	[9]	CLSI
72	NK10	ISKRILTGKK -NH <sub>2</sub>	>256	[9]	CLSI
73	BR030 <sup>3</sup>	KWKFYKKIERVGGQNIIRDGIIKAGPAVQVGGQPPRYIKENRFYS	>20	[13]	Jorgensen et.al. [14]
74	BR043 <sup>3</sup>	AGFRKRFNKLKVKVHTIKETANVSKDVAIVAGSGVAVGAAMG	>20	[13]	Jorgensen et.al. [14]
75	BR044 <sup>3</sup>	GFRKRFNKLKVKVHTIKETANVSKDVAIVAGSGVAVGAAMG	>20	[13]	Jorgensen et.al. [14]

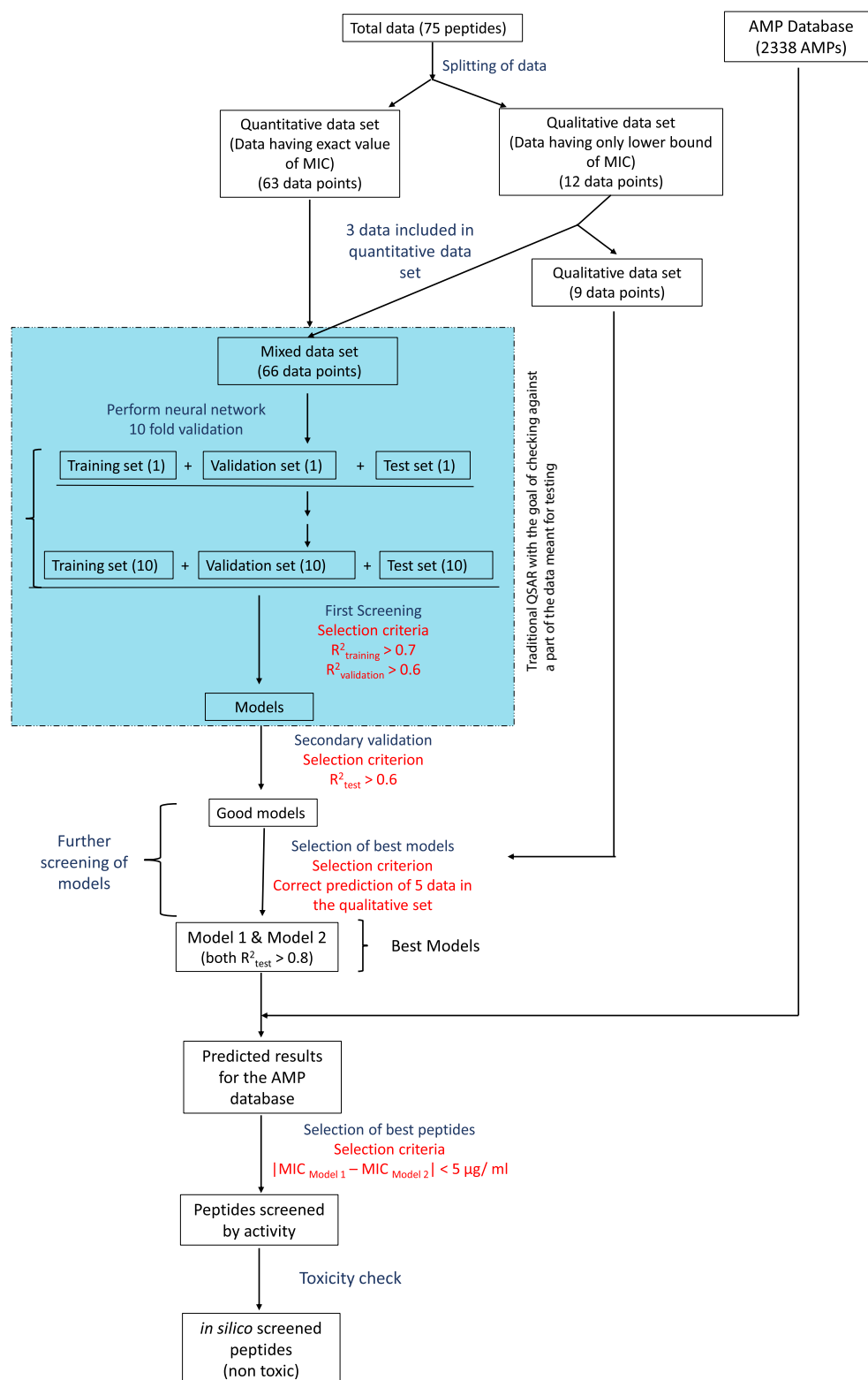
<sup>a</sup>These MIC values are calculated according to CLSI guideline.

<sup>b</sup>These MIC values are calculated according to CLSI and EUCAST guideline.

<sup>c</sup>MIC values obtained from this procedure is comparable with the MIC values obtained according to CLSI guideline.



**Fig A.** Histogram of all the parameters corresponding to the AMPs shown in **Table A in S1 File**. The raw data of these parameters is in **S2 File**.



**Fig B.** Flow chart showing the schematic of how the ANN models were developed. The part embedded in the dotted box is the logic of selecting the best models in traditional QSAR method, if the goal had been to be content with the prediction for the test set with 7 peptides. In such methods, typically a person conducting the test or the person developing the model but chooses to stay blind to this additional test data, eventually compares the performance of the predictions on this small test set. However, in our case, the goal is to make predictions for the 2338 AMPs for which no activity measurements are yet available, a secondary validation criteria was used to screen the models further.

## 2 Supplementary Table B

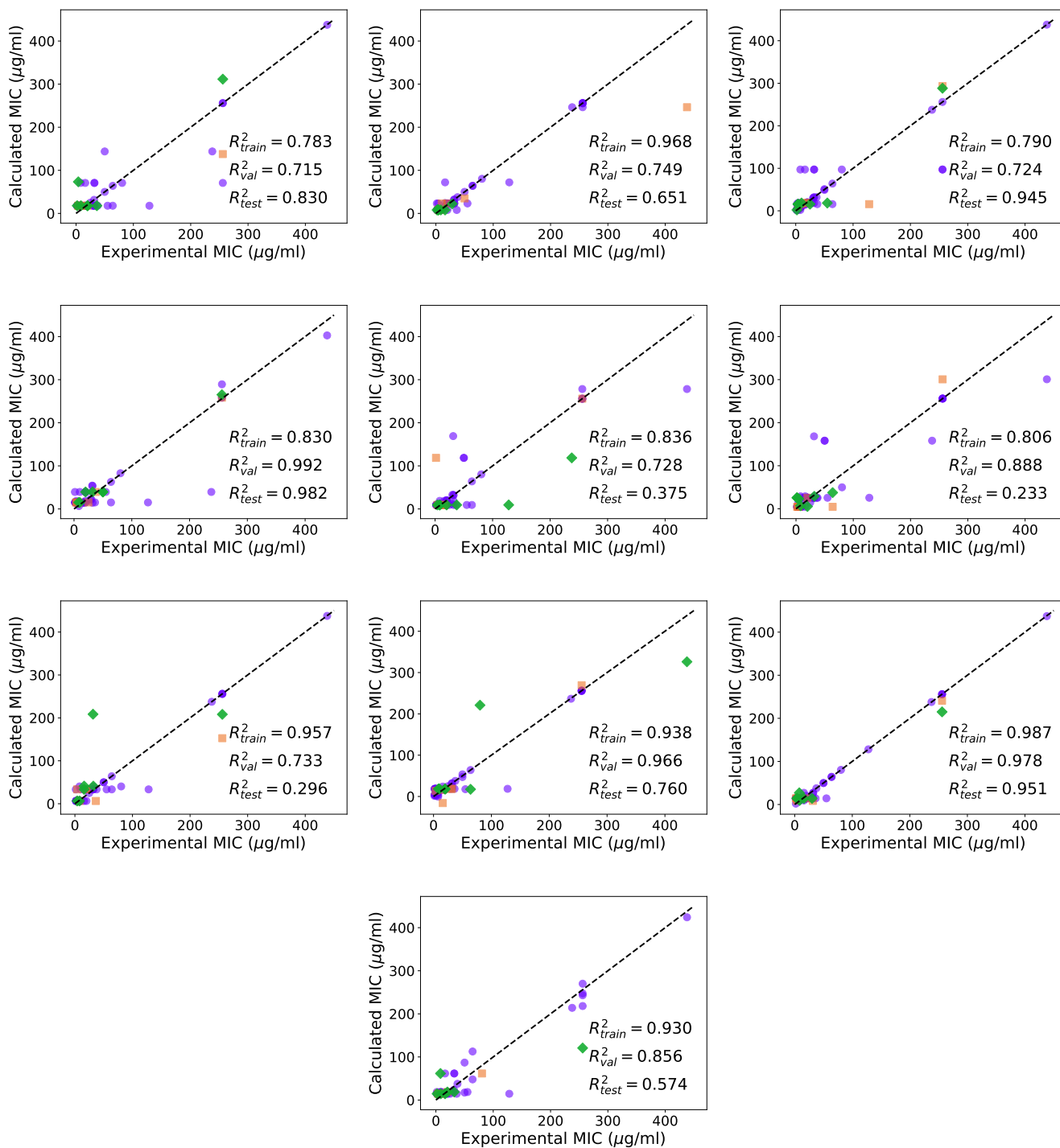
**Table B.** Experimental and predicted MIC values of the 9 qualitative AMP data that were used for an additional test. Before accepting a model, at least 5 of these 9 results were verified to be more than the lower bound suggested by the experiments (to within a factor of 2). An additional condition  $R^2 > 0.6$  was used with the test data set from the quantitative data.

Name	Expt. MIC $\mu\text{g/ml}$	Model-1 MIC $\mu\text{g/ml}$	Model-2 MIC $\mu\text{g/ml}$
C18G-His	>31.9275	75.9	9.4
I10	>128	482.1	437.1
NK15	>128	4.6	14.2
NK14	>256	60.1	14.2
NK11	>256	239.17	215.1
NK10	>256	86.1	14.6
BR030	>20	255.5	214.8
BR043	>20	22.4	14.2
BR044	>20	62.6	14.2

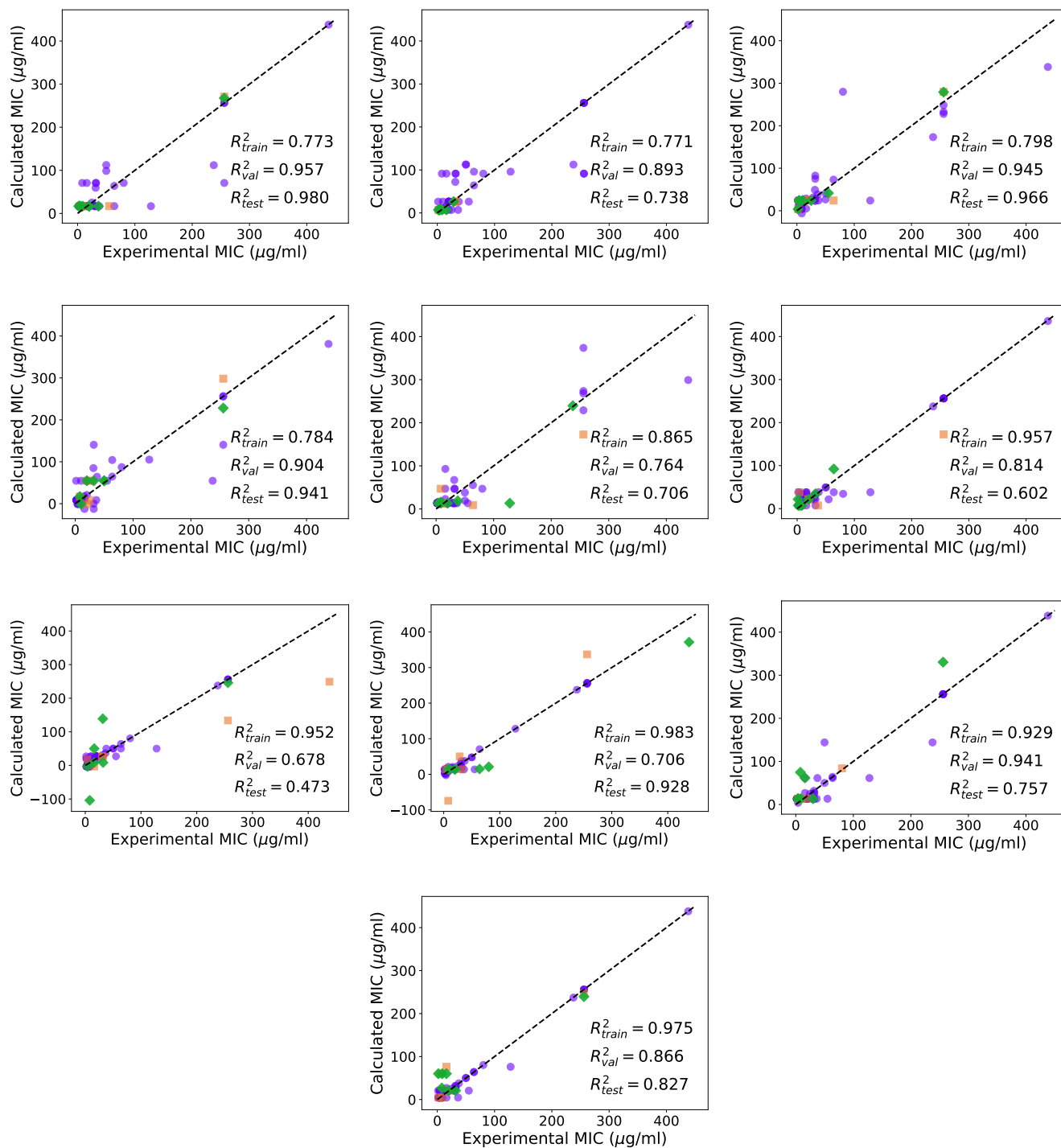
## 3 Supplementary Table C

**Table C.** 10-fold cross validation analysis was performed with a hidden layer between the input and output layers. The hidden layer architecture with 6, 8, and 10 neurons respectively were independently modelled. Here we tabulated the number of times, out of 10,  $R^2_{test} > 0.6$ , as well as the mean squared error (MSE) and standard deviation (SD). The overall error was optimal with the choice of 8 neurons, although all three architectures performed satisfactorily.

	Number of neurons in the hidden layer		
	6 neurons	8 neurons	10 neurons
$\#R^2_{test} > 0.6$	6	9	8
MSE	1963.979	1119.023	1423.350
SD	1984.295	1112.944	1972.955

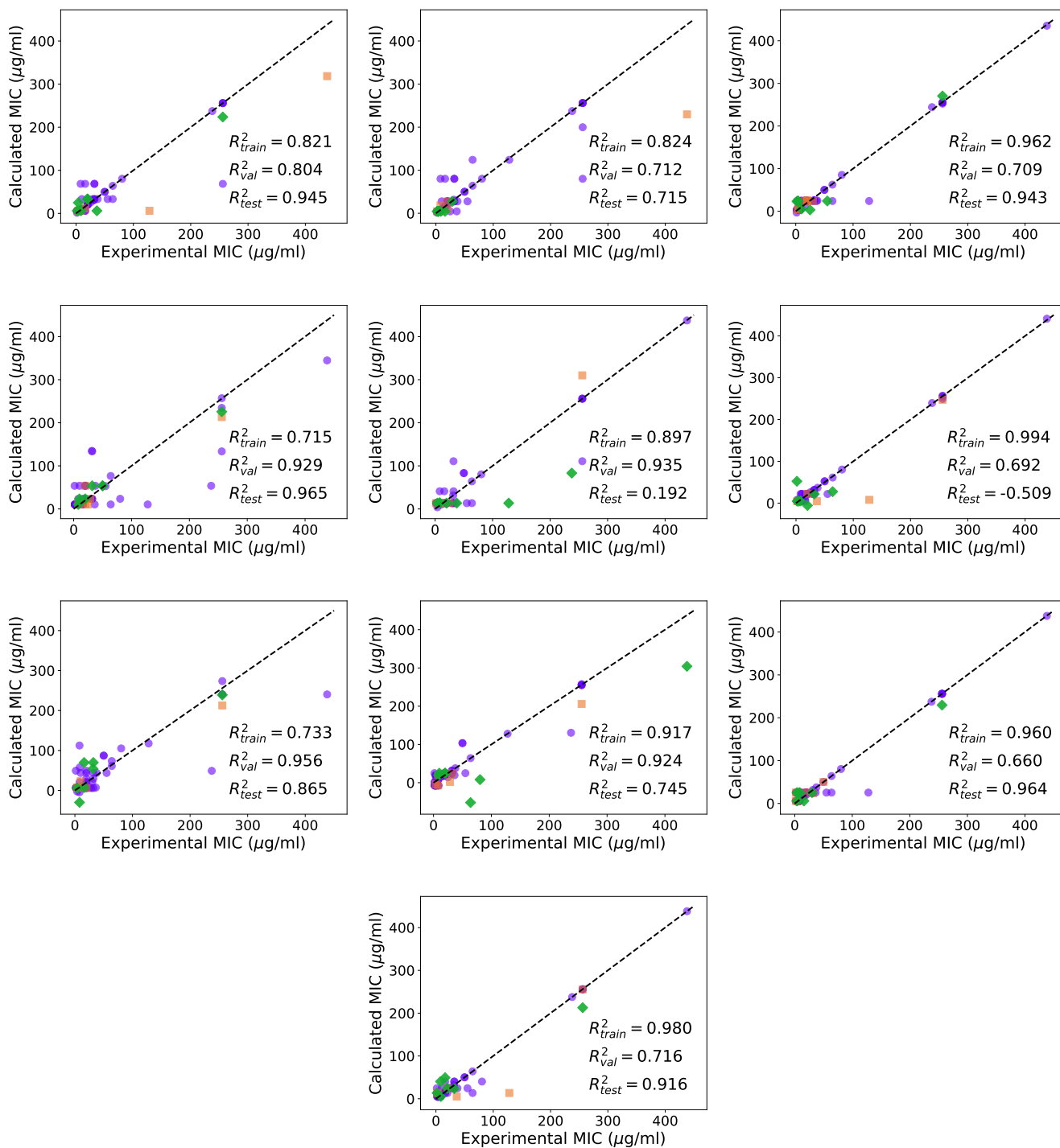


**Fig C.** Comparison of the experimental MIC ( $\mu\text{g/ml}$ ) and predicted MIC ( $\mu\text{g/ml}$ ) values of AMP obtained from the 10 fold cross validation calculation. The neural network is trained with one hidden layer consisting of 6 neurons.

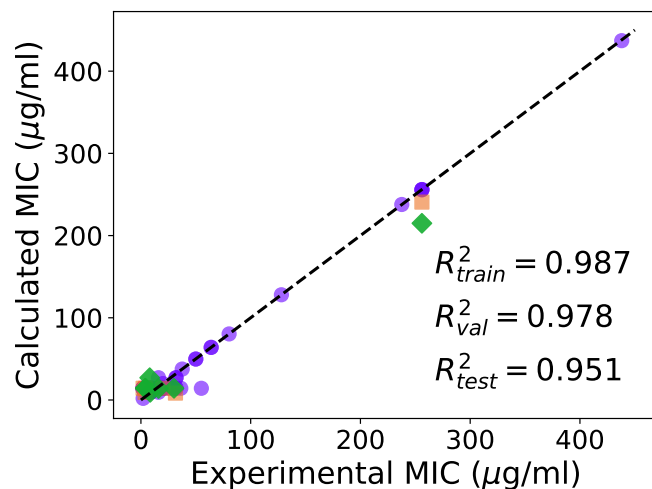


**Fig D.** Comparison of the experimental MIC ( $\mu\text{g/ml}$ ) and predicted MIC ( $\mu\text{g/ml}$ ) values of AMP obtained from the 10 fold cross validation calculation. The neural network is trained with one hidden layer consisting of 8 neurons.

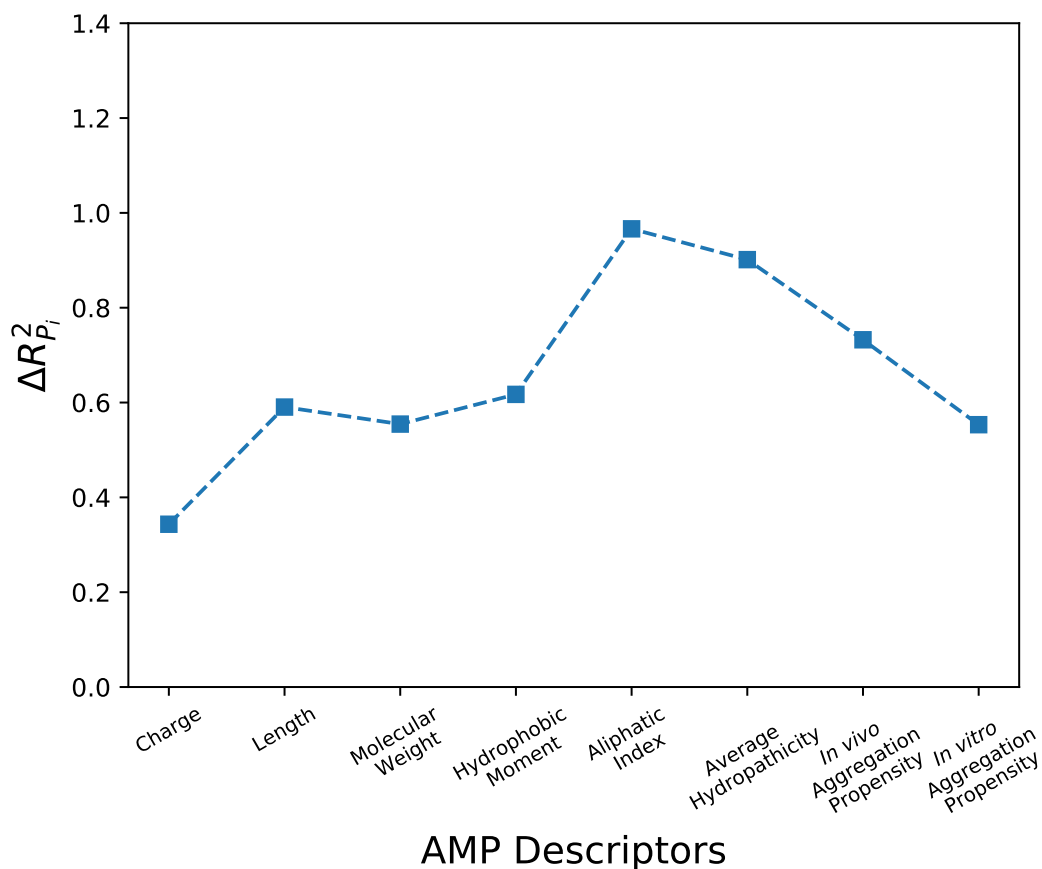




**Fig E.** Comparison of the experimental MIC ( $\mu\text{g/ml}$ ) and predicted MIC ( $\mu\text{g/ml}$ ) values of AMP obtained from the 10 fold cross validation calculation. The neural network is trained with one hidden layer consisting of 10 neurons.



**Fig F.** Comparison of the experimental and calculated MIC ( $\mu\text{g/ml}$ ) of curated AMPs on *A. baumannii* obtained from Model-2, calculated by using 6 hidden neurons. Training (purple circles), validation (orange squares) and test (green diamonds) sets are shown. The data used in the analysis for the peptides given in **Table A in S1 File**. The raw data is in **S2 File**.



**Fig G.** The relative importance of the different parameters is shown for Model-2. Aliphatic index continues to be the most relevant variable, similar to **Fig 3** of the main text.

**Table D.** Comparison of the experimental and predicted MIC values for the MDR *A. baumannii* strains. The results were satisfactory for most of these cases. Polydim-I has extremely low values in one of the parameter used in the model ( $\mu_H$ ), which is not falling in the training range.

Peptide	Expt. MIC $\mu\text{g/ml}$	Model-1 MIC $\mu\text{g/ml}$	Model-2 MIC $\mu\text{g/ml}$	Reference
Agelaia-MPI	39.2	76.1	9.4	[15]
Polybia-MPII	40.4	76.1	9.4	[15]
Polydim-I	> 61.1	-110.9	-172.2	[15]
Con10	70.6	74.8	-174.7	[15]
NDBP-5.8	> 37.8	76.0	9.4	[15]
LS-sarcotoxin	4	20.7	14.2	[16]
LS-stomoxyn	4	76.1	14.2	[16]
BP56	4	4.6	14.2	[17]

## References

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