**Supplementary File 1**

**Exploring the repositoryof *de novo* designed bifunctional antimicrobial peptides through deep learning**

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**Supplementary Tables**

**Supplementary file 1a.** Ablation study results of AMPredictor. Best metrics are marked in bold and second-best values are underlined.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Fingerprint** | **Contact Map** | **ESM** | **RMSE** | **MSE** | **Pearson** | **CI** |
| √ | × | × | 0.6431 | 0.4126 | 0.4965 | 0.6399 |
| × | √ | × | 0.7273 | 0.5290 | 0.1958 | 0.5406 |
| × | × | √ | 0.5386 | 0.2901 | 0.7032 | 0.6906 |
| √ | √ | × | 0.6543 | 0.4281 | 0.4768 | 0.6540 |
| √ | × | √ | 0.5356 | 0.2869 | 0.6961 | 0.6942 |
| × | √ | √ | 0.5636 | 0.3176 | 0.6494 | 0.7177 |
| **√** | **√** | **√** | **0.5348** | **0.2860** | **0.7072** | **0.7294** |

**Supplementary file 1b.** Information about five used antiviral classifiers.

|  |  |  |  |
| --- | --- | --- | --- |
| **No.** | **Name** | **Encoding** | **Model** |
| 1 | AVPpred | Motif, alignment, AAC, AAindex | SVM |
| 2 | DeepAVP | One-hot | CNN-LSTM |
| 3 | Deep-AVPpred | Pretrained language model | CNN |
| 4 | ENNAVIA | AAC, modlAMP, AAindex | MLP |
| 5 | AI4AVP | PC6 | CNN |

**Supplementary file 1c.** Sequences and novelty (BLAST E-value) of validated peptides.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Sequence** | **Predicted MIC** | **Length** | **E-value a** | **E-value b** |
| P089 | GLKALMKILALFMAKKIT | 1.644 | 18 | No hits | 4.1 |
| P386 | PIGLKLRAAIAKGLLHALLKGARAD | 1.857 | 25 | 7.5 | 7 |
| P120 | RWKKILGKAGKLLALRSGKLIL | 1.161 | 22 | 2.6 | 2.4 |
| P135 | KWKQFLKKLAKLIATRIAIIIKRRLK | 1.078 | 26 | 0.85 | 5.3 |
| P247 | KKLGKPLFKGLKKILKLFVKM | 1.450 | 21 | 5.3 | No hits |
| P026 | GIHKKLGPIAKKLLKKIAKL | 3.200 | 20 | 2.7 | No hits |
| P039 | GWKPILKKAAKIGKRLTGWLFLNSRVN | 2.270 | 27 | 0.29 | 1.2 |
| P070 | LKWWKKFGRKLARKFRM | 2.410 | 17 | 6.6 | No hits |
| P091 | GRMIRKMFKKLWKILRD | 2.480 | 17 | 5.3 | 4.7 |
| P127 | QKPKKDCGGNLLGMIKKFLK | 3.112 | 20 | No hits | 2.7 |
| P122 | IKARLKLKQRIKLIKIGKTFRRRRTDNM | 2.663 | 28 | No hits | 6.9 |
| P352 | RKFKYKFSKLKRVIFMAK | 3.141 | 18 | No hits | 9.9 |
| P105 | FTLKKLLKKGKKTRKLLTPLIKDHSDAM | 1.855 | 28 | No hits | No hits |
| P316 | MFKKILKKATKVIAGLTGHLFWGTRL | 1.317 | 26 | 0.58 | No hits |
| P244 | GTCKGLLKKLLKGMAKFILK | 1.766 | 20 | 6.8 | No hits |
| P302 | GKWQGLLKMIAKILAK | 1.973 | 16 | 0.37 | 0.58 |
| P185 | FRGRIGKILLKMLAQLAK | 2.103 | 18 | 2.3 | 8.3 |
| P019 | PRLAKLLKGGKYILKKITM | 3.482 | 19 | No hits | 0.048 |
| P252 | GMGSFRKLIKKLAKW | 3.491 | 15 | 2.4 | 2.1 |
| P213 | HKCYKWFIKMLRKFQ | 2.982 | 15 | 7.2 | No hits |
| P036 | KMKTFKKKFLKQFAKRLAGRIYPRFK | 2.325 | 26 | No hits | 4.7 |
| P018 | NWKPKIYRTAKKILKIAGKTL | 2.695 | 21 | 2.5 | 2.1 |
| P168 | KKKKFKKFSKGFQKTLMGMLCMSAAKAW | 2.321 | 28 | No hits | 9.1 |
| P214 | KQKIFQKLAARGILLPLAPFIMERIKW | 2.802 | 27 | No hits | No hits |
| P089 | GLKALMKILALFMAKKIT | 1.644 | 18 | No hits | 4.1 |
| P386 | PIGLKLRAAIAKGLLHALLKGARAD | 1.857 | 25 | 7.5 | 7 |
| P120 | RWKKILGKAGKLLALRSGKLIL | 1.161 | 22 | 2.6 | 2.4 |
| P135 | KWKQFLKKLAKLIATRIAIIIKRRLK | 1.078 | 26 | 0.85 | 5.3 |

a: E-value was from alignment with the training set.

b: E-value was from alignment with DRAMP.

**Supplementary file 1d.** The EC50 (μM) of AMPs inhibiting four enveloped viruses.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **AMP** | **Generator** | **CHIKV** | **HTNV** | **DENV-2** | **HSV-1** |
| P001 | v1 | 1.62 | 2.36 | 5.15 | 2.35 |
| P002 | v1 | 0.37 | 2.08 | 0.99 | 0.93 |
| P076 | v1 | 2.03 | 2.26 | 4.48 | 2.73 |
| P135 | v1 | 6.70 | 0.97 | 0.09 | 0.28 |
| P244 | v2 | 42.08 | 2.64 | 1.96 | 2.30 |

**Supplementary file 1e.** The selectivity index (SI) of three AMPs inhibiting four enveloped viruses.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **AMP** | **CHIKV** | **HTNV** | **DENV-2** | **HSV-1** |
| P001 | 37.27 | **50.36** | 11.71 | 25.72 |
| P002 | **149.21** | 31.75 | **51.05** | **59.01** |
| P076 | 22.64 | 15.68 | 14.90 | 16.90 |

**Supplementary file 1f.** Reported and predicted MICs of some recently mined AMPs.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **No.** | **Peptide** | **Sequence** | **Ref.** | **Reported MIC** | **Predicted MIC** |
| 1 | BigDynorphin | YGGFLRRIRPKLKWDNQKRYGGFLRRQFKVVT | 1 | 14.58 | 7.89 |
| 2 | Apelin-1 | ERPVNLTMRRKLRKHNCLQRRCMPLHSRVPFP | 1 | 41.67 | 13.87 |
| 3 | Apelin-36 | LVQPRGSRNGPGPWQGGRRKFRRQRPRLSHKGPNPF | 1 | 29.17 | 7.75 |
| 4 | vWF-PQR19 | PQRMSRNFVRYVQGLKKKK | 1 | 63.50 | 29.52 |
| 5 | INTb-FTR26 | FTRGKLMSSLHLKRYYGRILHYLKAK | 1 | 25.00 | 6.28 |
| 6 | HSA-GKA38 | GKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKA | 1 | 48.92 | 27.66 |
| 7 | CXC19-VKE44 | VKELIKKWEKQVSQKKKQKNGKKHQKKKVLKVRKSQRSRQKKTT | 1 | 76.00 | 0.76 |
| 8 | FIBg-TWK25 | TWKTRWYSMKKTTMKIIPFNRLTIG | 1 | 76.00 | 3.79 |
| 9 | SCUB1-MPF22 | MFPRSFIKLLRSKVSRFLRPYK | 1 | 60.37 | 4.43 |
| 10 | SCUB1-SKE25 | SKEMFPRSFIKLLRSKVSRFLRPYK | 1 | 22.92 | 4.61 |
| 11 | SCUB3-KHK26 | KHKEMLPKSFIKLLRSKVSSFLRPYK | 1 | 20.83 | 5.87 |
| 12 | SCUB3-MLP22 | MLPKSFIKLLRSKVSSFLRPYK | 1 | 18.23 | 3.36 |
| 13 | SFRP1-KKI32 | KKIVPKKKKPLKLGPIKKKDLKKLVLYLKNGA | 1 | 16.67 | 7.41 |
| 14 | NAPP-LIR38 | LIRIPLHRVQPGRRILNLLRGWREPAELPKLGAPSPGD | 1 | 55.17 | 6.14 |
| 15 | NAPP-LIR23 | LIRIPLHRVQPGRRILNLLRGWR | 1 | 87.42 | 20.82 |
| 16 | Bomidin | MGRFKRFRKKFKKLFKKLS | 2 | 4.00 | 5.43 |
| 17 | CBPZ-GSK24  | GSKPWWWSYFTSLSTHRPRWLLKY  | 3 | 3.00 | 10.41 |
| 18 | XDH-AVA32  | AVAKLPAQKTEVFRGVLEQLRWFAGKQVKSVA  | 3 | 32.00 | 23.51 |
| 19 | c\_AMP660 | IFFRRNKKMAVKVAINGFGRIGRLAFRQMF | 4 | 10.00 | 14.56 |
| 20 | c\_AMP575 | GRYIAKINPDNKKFKTMPSGKKRKGHKMATHKRKKRLRKNRHKKK | 4 | 2.00 | 1.49 |
| 21 | c\_AMP1043 | KQKTLKKVWKLSEKVLIFASAFAKKAGAAEATLVL | 4 | 10.00 | 10.02 |
| 22 | c\_AMP67 | AMTLRKRKFAWYVLSSSLKWLIKKAKKIGVQVCGFE | 4 | 20.00 | 4.80 |
| 23 | c\_AMP69 | AMTSRKRKFVWYVLSSSLKWLIKKAKKIGVQVCGFE | 4 | 10.00 | 4.66 |
| 24 | c\_AMP2041 | SVIWRKLFFIFIKRSGNWIKKVEKRQNLL | 4 | 20.00 | 48.78 |
| 25 | c\_AMP250 | DRDRPECSTMVKYEQKLPSLGKYALKRAIKIKFGRK | 4 | 10.00 | 4.40 |
| 26 | c\_AMP518 | GINLKRKGNIMKKVKNIFHKIANADPMIWGYVMLSESK | 4 | 25.00 | 6.29 |
| 27 | c\_AMP593 | GVPMGSVIKKRRKRMAKKKHRKLLRKTRHQRRNKK | 4 | 25.00 | 2.16 |
| 28 | c\_AMP1655 | RGTCYNRVGLIIRNFSKLKGKKV | 4 | 20.00 | 13.73 |

**Supplementary file 1g.** Minimal inhibitory concentrations (μM) of P076-NH2 and P076.

|  |  |  |
| --- | --- | --- |
| **AMP** | ***S. aureus*** | ***A. baumannii*** |
| ATCC 25923 | MRSA | ATCC 17978 | MDRAB |
| P076-NH2 | 53.12 | 106.32 | 53.12 | 53.12 |
| P076 | 6.64 | 13.29 | 0.21 | 0.21 |

**Supplementary file 1h.** The qRT-PCR primers.

|  |  |  |
| --- | --- | --- |
| **Virus** | **Gene** | **Primers (5’-3’)** |
| HTNV | S | Forward: GAGCCTGGAGACCATCTGReverse: CGGGACGACAAAGGATGT |
| CHIKV | E | Forward: TCTATAACATGGACTACCCGCCCReverse: AGCCAGATGGTGCCTGAGAGT |
| HSV-1 | VP16 | Forward: AATGTGGTTTAGCTCCCGCAReverse: CCAGTTGGCGTGTCTGTTTC |
| DENV-2 | NS5a | Forward: GGTTTTGGGAGCTGGTTGACReverse: ACTCTAAGAAGCGTGCTCCA |

**Supplementary file 1i.** System details of molecular dynamics simulations.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **No.** | **Peptide** | **Membrane** | **Box dimension (nm)** | **Atoms** |
| 1 | P001 | Virus (POPC:POPE:POPS:POPI:DSM:cholesterol = 10:4:1:2:1:2) | 8.66 \* 8.66 \* 11.60 | 81,660 |
| 2 | P002 | 8.66 \* 8.66 \* 11.41 | 80,720 |
| 3 | P076 | 8.66 \* 8.66 \* 11.61 | 81,940 |
| 4 | P001 | Inner membranes of G- bacteria (POPE:POPG:TOCL1 = 7:2:1) | 8.13 \* 8.13 \* 11.60 | 73,644 |
| 5 | P002 | 8.13 \* 8.13 \* 11.41 | 72,702 |
| 6 | P076 | 8.13 \* 8.13 \* 11.61 | 73,721 |
| 7 | P001 | Outer membranes of G- bacteria (outer: 35 ECLIPA; inner: 75 PPPE, 20 PVPG, 5 PVCL2) | 8.05 \* 8.05 \* 12.54 | 84,979 |
| 8 | P002 | 8.15 \* 8.15 \* 11.88 | 82,483 |
| 9 | P076 | 8.07 \* 8.07 \* 12.46 | 84,922 |

**Supplementary file 1j.** Binding free energy between peptides and the lipid bilayers.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Peptide** | **Membrane** | $∆$**Gpolar** | $∆$**Gnonpolar** | $∆$**Gbind** |
| P001 | Virus | -193.00 ± 16.11 | -44.52 ± 2.99 | -229.65 ± 14.35 |
| P002 | Virus | -136.94 ± 22.66 | -53.60 ± 5.12 | -190.54 ± 18.02 |
| P076 | Virus | -195.71 ± 8.81 | -34.98 ± 8.73 | -230.69 ± 8.31 |
| P001 | G- inner membrane | -408.93 ± 35.39 | -47.97 ± 10.82 | -456.89 ± 29.24 |
| P002 | G- inner membrane | -289.58 ± 49.75 | -70.68 ± 31.58 | -360.26 ± 19.00 |
| P076 | G- inner membrane | -404.77 ± 2.89 | -61.02 ± 10.90 | -465.79 ± 11.39 |

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