Supporting Information

Antimicrobial Peptide from Wild Bee Venom and Its Synergistic Effect with Antibiotics against *Pseudomonas aeruginosa*

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HYL:

Gly-Ile-Met-Ser-Ser-Leu-Met-Lys-Lys-Leu-Ala-Ala-His-Ile-Ala-Lys-NH_2

Molecular mass (monoisotopic): 1696.98

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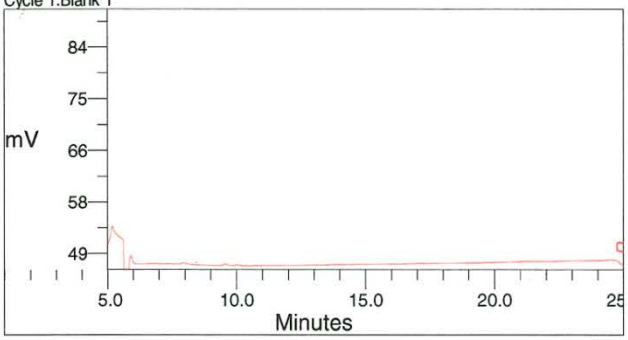
Edman degradation of HYL (residues 1 – 8)

SequencePro™

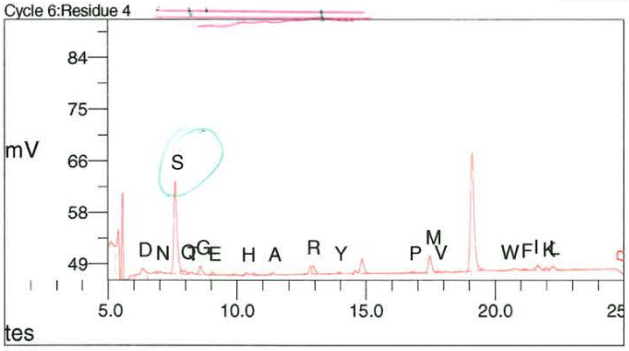
HYL

No lab name
No address

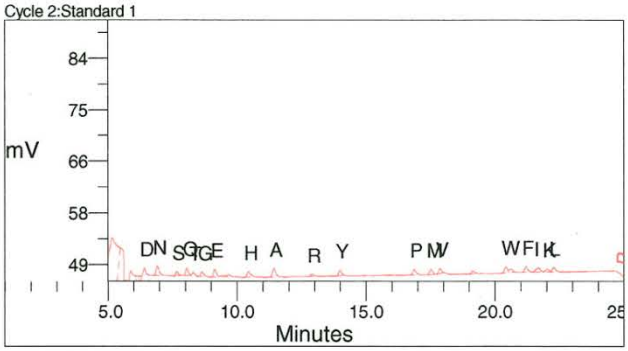
Cycle 1:Blank 1



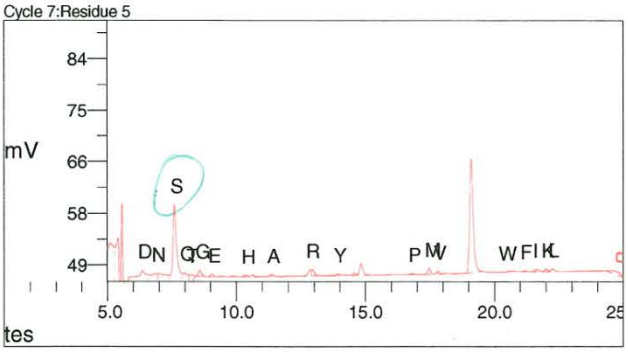
Cycle 6:Residue 4



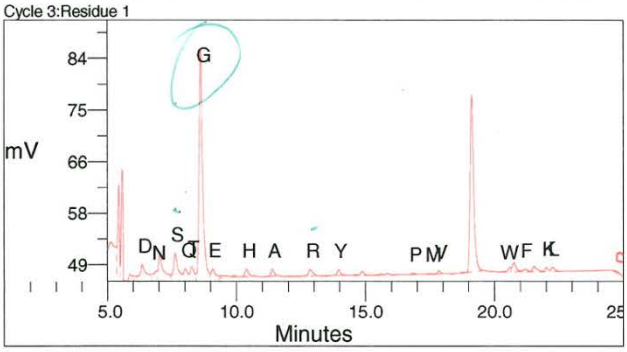
Cycle 2:Standard 1



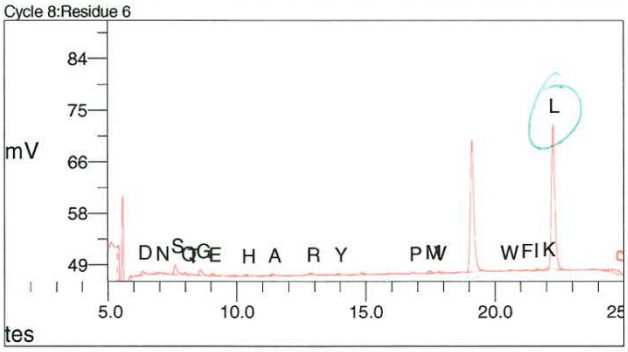
Cycle 7:Residue 5



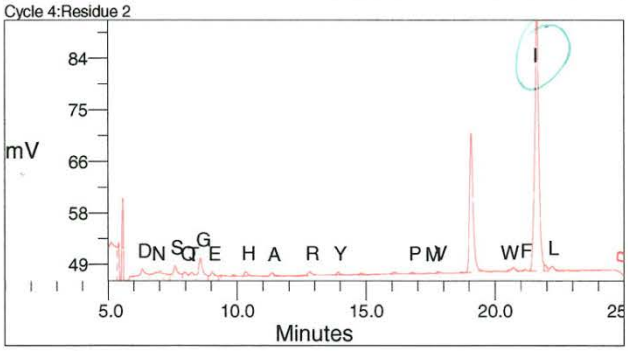
Cycle 3:Residue 1



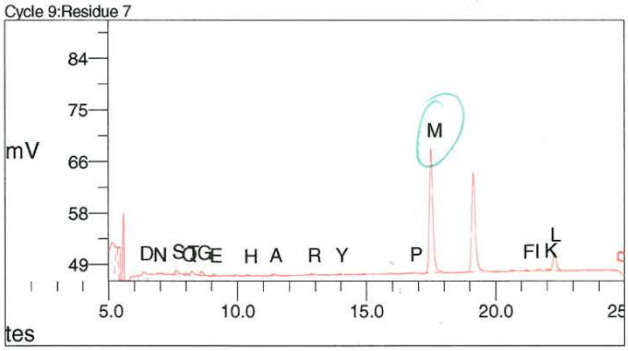
Cycle 8:Residue 6



Cycle 4:Residue 2



Cycle 9:Residue 7



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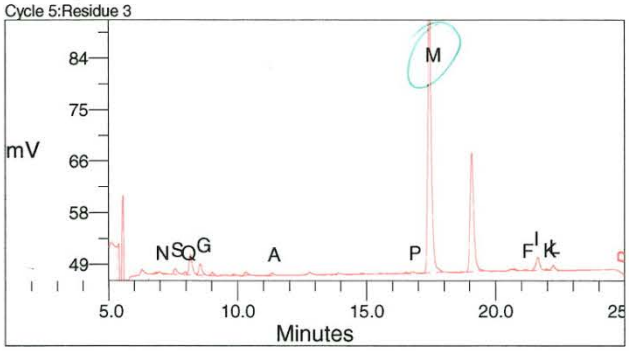

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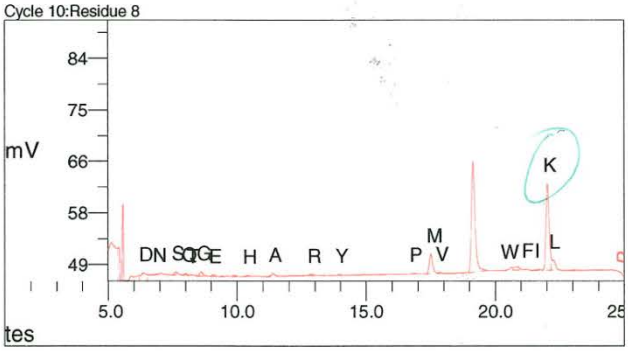
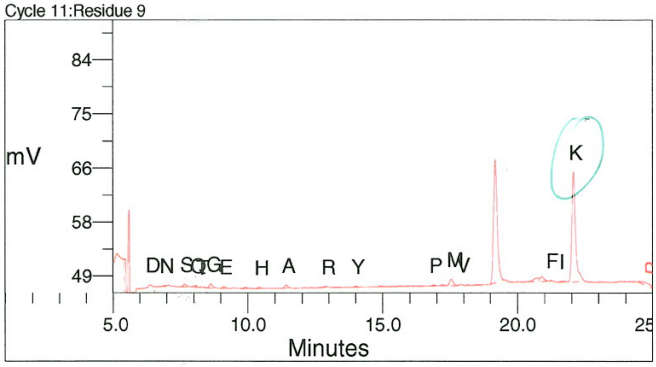


Edman degradation of HYL (residues 9 – 16)

No lab name
No address

Cycle 11:Residue 9



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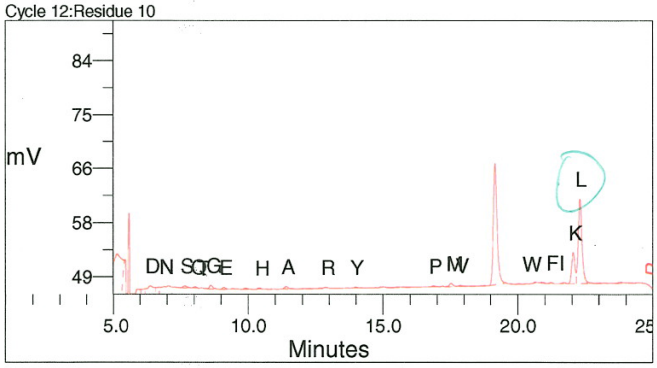

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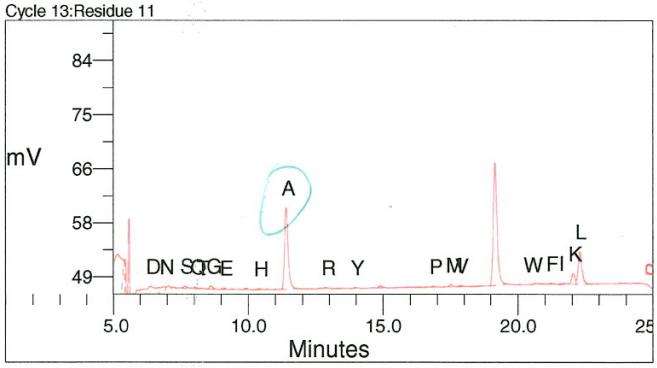

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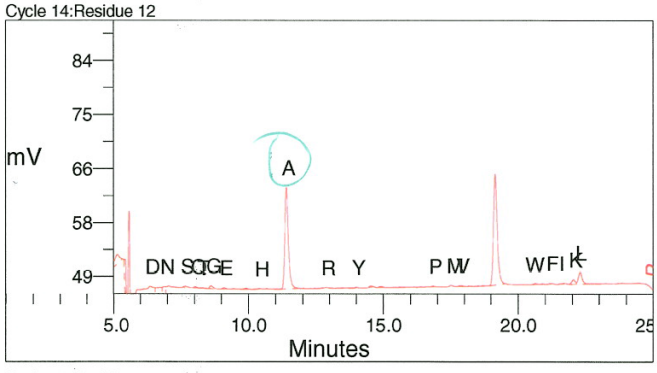

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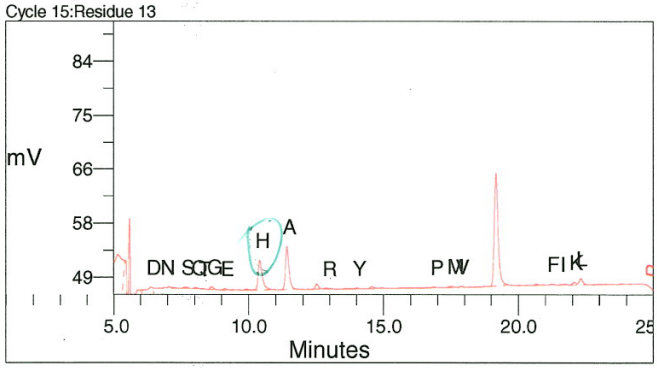

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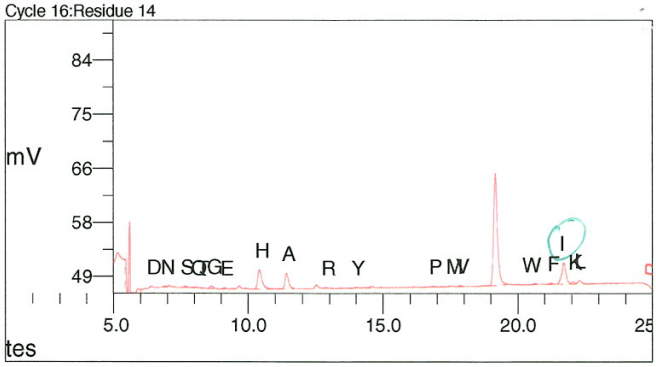

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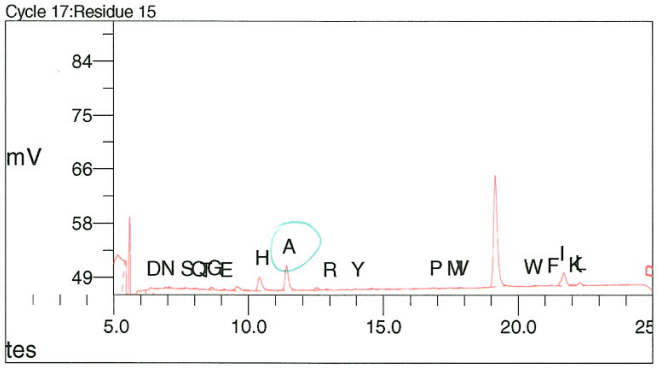

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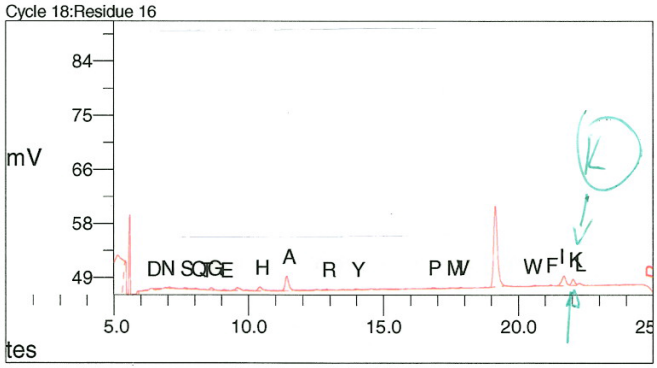

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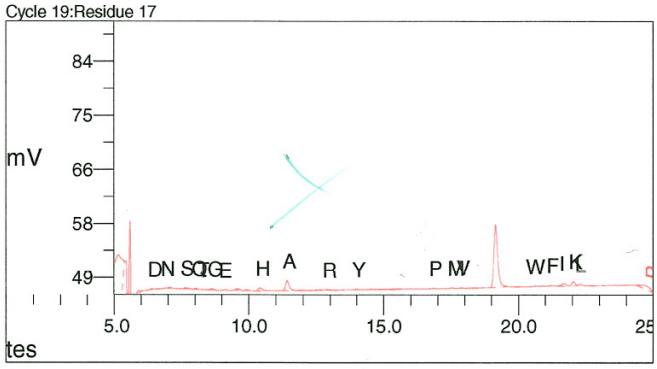
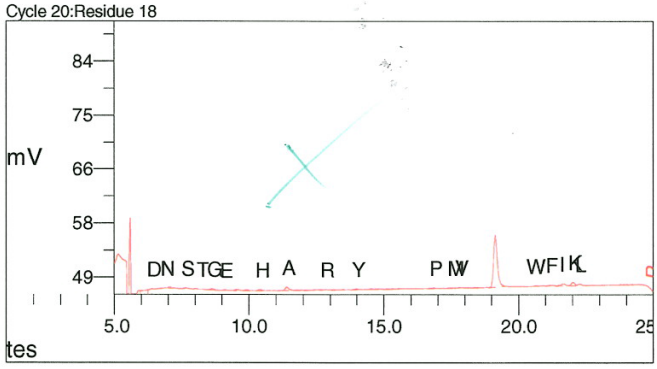
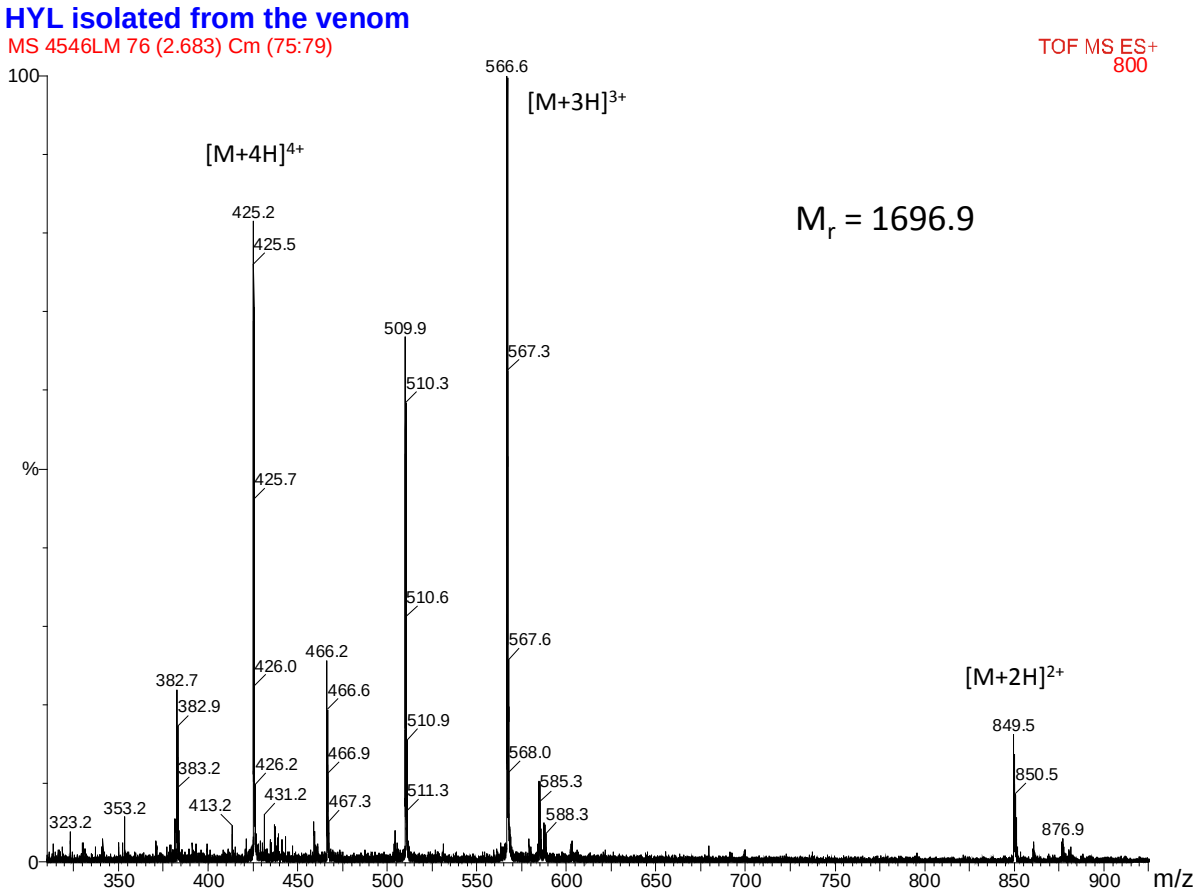


Cycle 20:Residue 18





ESI-QTOF mass spectrum of native HYL

Table S1. NMR constraints and structural statistics for the studied peptides

	HYL	HYL-8	HYL-20
Non-redundant distance and angle constrains			
Total number of NOE constraints	166	259	207
Short-range NOEs			
Intra-residue (i = j)	63	103	79
Sequential (| i - j | = 1)	57	77	47
Medium-range NOEs (1 < | i - j | < 5)	46	78	74
Long-range NOEs (| i - j | ≥ 5)	0	1	7
Torsion angles	-	-	27
Total number of restricting constraints	166	259	234
Total restricting constraints per restrained residue	11.1	17.3	15.6
r.m.s.d. to the mean structure			
All backbone atoms	1.1 Å	0.8 Å	0.3 Å
All heavy atoms	1.5 Å	1.3 Å	0.6 Å

Table S2. Proton NMR parameters of HYL in H_2O+D_2O (9:1) (pH=4.43, T=298 K)

Residue	NH	Hα	Hβ	Other protons
Gly-1	[a]	3.891; 3.857		
Ile-2	8.536 (J=6.8)	4.210	1.854	γ-CH_2: 1.467; 1.204; γ-Me: 0.925; δ-Me: 0.872
Met-3	8.598 (J=9.1)	4.547	2.093; 2.036	γ-CH_2: 2.636; 2.575; SCH_3: 2.112
Ser-4	8.374 (J=4.0)	4.429	3.994; 3.883	
Ser-5	8.482 (J=5.9)	4.375	3.928; 3.895	
Leu-6	8.106 (J=6.4)	4.295	1.639 (2H)	γ-CH: 1.668; δ-Me's: 0.937; 0.877
Met-7	8.067 (J=6.4)	4.360	2.096; 2.045	γ-CH_2: 2.642; 2.555; SCH_3: 2.097
Lys-8	8.147 (J=6.8)	4.225	1.822; 1.782	γ-CH_2 + δ-CH_2:1.477; 1.428; 1.406; ε-CH_2: 2.986
Lys-9	8.140 (J~6.5)	4.247	1.822; 1.782	γ-CH_2 + δ-CH_2:1.477; 1.428; 1.406; ε-CH_2: 2.986
Leu-10	8.189 (J=6.6)	4.293	1.673 (2H)	γ-CH: 1.581; δ-Me's: 0.926; 0.871
Ala-11	8.162 (J=6.5)	4.253	1.382	
Ala-12	8.077 (J~5.5)	4.241	1.349	
His-13	8.395 (J=7.5)	4.680	3.265; 3.178	H2: 8.59; H4: 7.28
Ile-14	8.148 (J=6.8)	4.126	1.826	γ-CH_2: 1.48; 1.166; γ-Me: 0.896; δ-Me: 0.849
Ala-15	8.421 (J=5.9)	4.314	1.392	
Lys-16	8.275 (J=6.9)	4.248	1.832; 1.766	γ-CH_2: 1.451; δ-CH_2: 1.687; ε-CH_2: 2.996; $CONH_2$: 7.589; 7.128

[a] not determined

Table S3. Proton NMR parameters of HYL in 30% TFE (pH=3.97; T=298 K)

Residue	NH	Hα	Hβ	Other protons
Gly-1	[a]	4.028; 3.944		
Ile-2	8.671 (J~3.5)	4.117	1.916	γ-CH_2: 1.590; 1.305; γ-Me: 1.002; δ-Me: 0.964
Met-3	8.390 (J=4.6)	4.462	2.140 (2H)	γ-CH_2: 2.678; 2.618; SCH_3: 2.113
Ser-4	8.264 (J=4.1)	4.262	4.073; 3.996	
Ser-5	8.057 (J=5.0)	4.322	4.101; 3.985	
Leu-6	8.139 (J~3.7)	4.182	1.803 (2H)	γ-CH: 1.745; δ-Me's: 0.963; 0.930
Met-7	8.283 (J~3.0)	4.245	2.223; 2.168	γ-CH_2: 2.765; 2.636; SCH_3: 2.087
Lys-8	7.824 (J=4.2)	4.079	2.022; 1.987	γ-CH_2: 1.494; δ-CH_2:1.742; ε-CH_2: 3.001
Lys-9	7.774 (J=5.3)	4.159	2.107; 2.012	γ-CH_2: 1.505; 1.482; δ-CH_2: 1.726; ε-CH_2: 2.999
Leu-10	8.529 (J~3.5)	4.172	1.872; 1.795	γ-CH: 1.733; δ-Me's: 0.927; 0.891
Ala-11	8.526 (J~3.5)	4.074	1.552	
Ala-12	7.861 (J=4.2)	4.166	1.528	
His-13	8.019 (J=6.6)	4.434	3.520; 3.373	H2: 8.57; H4: 7.298
Ile-14	8.245 (J=6.0)	3.906	1.975	γ-CH_2: 1.726; 1.242; γ-Me: 0.959; δ-Me: 0.892
Ala-15	8.179 (J=4.6)	4.226	1.492	
Lys-16	7.695 (J=7.2)	4.276	1.950; 1.873	γ-CH_2: 1.578; 1.518; δ-CH_2: 1.729; ε-CH_2: 3.030; $CONH_2$: 7.28; 6.96

[a] not determined

Table S4. Proton NMR parameters of HYL-8 in H_2O+D_2O (9:1) (pH=3.36, T=298 K)

Residue	NH	Hα	Hβ	Other protons
Gly-1	[a]	3.89; 3.86		
Ile-2	8.536 (J=6.9)	4.212	1.853	γ-CH_2: 1.473; 1.205; γ-Me: 0.928; δ-Me: 0.874
Met-3	8.598 (J=6.8)	4.543	2.097; 2.038	γ-CH_2: 2.642; 2.576; SCH_3: 2.109
Ser-4	8.380 (J=6.1)	4.424	3.973; 3.874	
Ser-5	8.458 (J=6.1)	4.384	3.928; 3.890	
Leu-6	8.095 (J=6.3)	4.301	1.673; 1.637	γ-CH: 1.616; δ-Me's: 0.938; 0.880
Met-7	8.070 (J=6.4)	4.378	2.093; 2.041	γ-CH_2: 2.640; 2.547; SCH_3: 2.101
Lys-8	8.119 (J=6.2)	4.222	1.831; 1.773	γ-CH_2 + δ-CH_2:1.683; 1.474; 1.402; ε-CH_2: 2.989
Lys-9	8.130 (J=8.0)	4.285	1.831; 1.773	γ-CH_2 + δ-CH_2:1.683; 1.474; 1.402; ε-CH_2: 2.989
Leu-10	8.140 (J=6.8)	4.322	1.652; 1.586	γ-CH: 1.565; δ-Me's: 0.926; 0.871
Lys-11	8.222 (J=6.8)	4.267	1.798; 1.747	γ-CH_2 + δ-CH_2:1.676; 1.435; 1.367; ε-CH_2: 2.985
Lys-12	8.217 (J=6.7)	4.293	1.798; 1.747	γ-CH_2 + δ-CH_2:1.676; 1.435; 1.367; ε-CH_2: 2.985
Ile-13	8.261 (J=7.9)	4.139	1.832	γ-CH_2: 1.490; 1.189; γ-Me: 0.864; δ-Me: 0.850
Ile-14	8.311 (J=7.6)	4.155	1.844	γ-CH_2: 1.470; 1.185; γ-Me: 0.898; δ-Me: 0.844
Ala-15	8.393 (J=6.2)	4.327	1.382	
Lys-16	8.300 (J=6.4)	4.244	1.838; 1.768	γ-CH_2: 1.425; δ-CH_2: 1.687; ε-CH_2: 2.998; $CONH_2$: 7.598; 7.126

[a] not determined

Table S5. Proton NMR parameters of HYL-8 in 30% TFE (pH=4.13, T=298 K)

Residue	NH	Hα	Hβ	Other protons
Gly-1	[a]	4.020; 3.936		
Ile-2	8.687 (J~4.0)	4.122	1.914	γ-CH_2: 1.586; γ-Me: 0.998; δ-Me: 0.947
Met-3	8.408 (J=4.6)	4.448	2.134 (2H)	γ-CH_2: 2.682; 2.611; SCH_3: 2.096
Ser-4	8.275 (J~4.6)	4.235	4.055; 3.983	
Ser-5	8.032 (J=5.0)	4.309	4.091; 3.977	
Leu-6	8.162 (J=4.2)	4.188	1.803 (2H)	γ-CH: 1.728; δ-Me's: 0.966; 0.928
Met-7	8.282 (J~3.8)	4.235	2.233; 2.102	γ-CH_2: 2.751; 2.622; SCH_3: 2.077
Lys-8	7.769 (J=4.2)	4.000	2.015; 1.940	γ-CH_2: 1.476; δ-CH_2:1.739; ε-CH_2: 3.000
Lys-9	7.652 (J=5.6)	4.090	2.034 (2H)	γ-CH_2: 1.457; δ-CH_2: 1.711; 1.645; ε-CH_2: 2.971
Leu-10	8.490 (J=3.3)	4.129	1.866; 1.804	γ-CH: 1.642; δ-Me's: 0.910; 0.884
Lys-11	8.181 (J~3.8)	3.952	1.984; 1.913	γ-CH_2: 1.478; δ-CH_2: 1.692; ε-CH_2: 2.976
Lys-12	7.552 (J=5.7)	4.115	2.032 (2H)	γ-CH_2: 1.500; δ-CH_2: 1.743; 1.657; ε-CH_2: 3.014
Ile-13	7.894 (J=5.9)	3.824	2.078	γ-CH_2: 1.787; 1.180; γ-Me: 0.941; δ-Me: 0.884
Ile-14	8.371 (J=4.8)	3.869	1.939; 1.911	γ-CH_2: 1.679; 1.245; γ-Me: 0.948; δ-Me: 0.850
Ala-15	7.914 (J=4.9)	4.244	1.523	
Lys-16	7.743 (J=6.9)	4.228	1.973 (2H)	γ-CH_2: 1.587; δ-CH_2: 1.746; ε-CH_2: 3.045; $CONH_2$: 7.371; 6.986

[a] not determined

Table S6. Proton NMR parameters of HYL-20 in H_2O+D_2O (9:1) (pH=2.94, T=298 K)

Residue	NH	Hα	Hβ	Other protons
Gly-1	[a]	3.872 (2H)		
Ile-2	8.510 (J=6.2)	4.196	1.844	γ-CH_2: 1.457; 1.192; γ-Me: 0.912; δ-Me: 0.863
Leu-3	8.419 (J=6.5)	4.374	1.578 (2H)	γ-CH: 1.644; δ-Me's: 0.909; 0.846
Ser-4	8.276 (J=6.0)	4.324	3.928; 3.823	
Ser-5	8.323 (J=6.6)	4.368	3.888; 3.830	
Leu-6	8.017 (J=6.1)	4.208	1.539 (2H)	γ-CH: 1.480; δ-Me's: 0.878; 0.808
Trp-7	7.900 br	4.554	3.335; 3.274	NH: 10.16; H2: 7.260; H4: 7.570; H5: 7.129; H6: 7.232; H7: 7.492
Lys-8	7.805 (J=6.2)	4.054	1.731; 1.628	γ-CH_2 + δ-CH_2:1.249; 1.196; ε-CH_2: 2.935
Lys-9	7.895 br	4.175	1.825; 1.762	γ-CH_2 + δ-CH_2:1.666; 1.452; 1.376; ε-CH_2: 2.965
Leu-10	8.044 (J=6.1)	4.271	1.658 (2H)	γ-CH: 1.565; δ-Me's: 0.902; 0.852
Lys-11	8.157 (J=6.6)	4.192	1.75; 1.70	γ-CH_2 + δ-CH_2: 1.63; 1.383; 1.332; ε-CH_2: 2.946
Lys-12	8.109 br	4.260	1.775; 1.73	γ-CH_2 + δ-CH_2: 1.659; 1.418; 1.346; ε-CH_2: 2.964
Ile-13	8.181 br	4.114	1.840	γ-CH_2: 1.493; 1.184; γ-Me + δ-Me: 0.863
Ile-14	8.262 (J~8.0)	4.127	1.837	γ-CH_2: 1.469; 1.177; γ-Me: 0.893; δ-Me: 0.833
Ala-15	8.33 br	4.307	1.387	
Lys-16	8.239 br	4.238	1.843; 1.775	γ-CH_2 + δ-CH_2: 1.688; 1.484; 1.434; ε-CH_2: 3.00; $CONH_2$: 7.564; 7.116

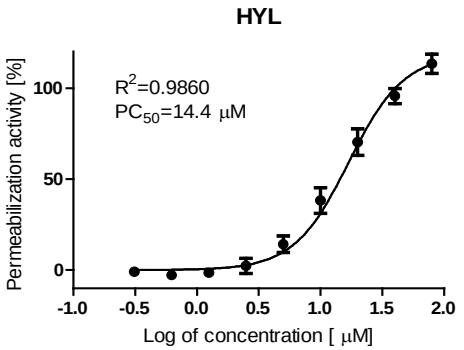
[a] not determined

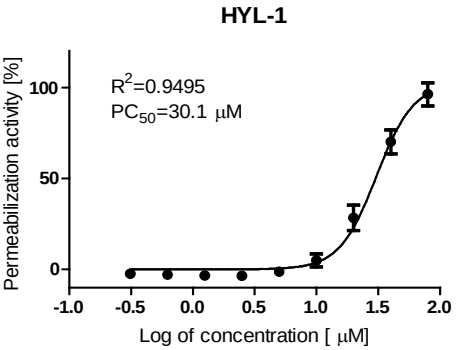
Table S7. Proton NMR parameters of HYL-20 in 30% TFE (pH=2.94; T=298 K)

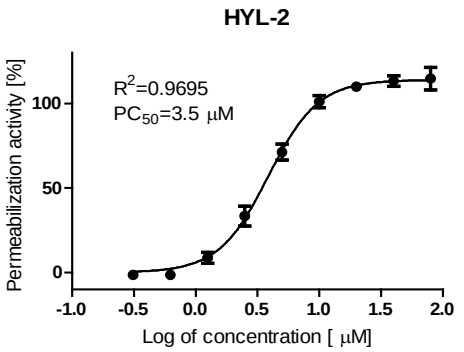
Residue	NH	Hα	Hβ	Other protons
Gly-1	[a]	4.022; 3.945		
Ile-2	8.629 (J=4.6)	4.107	1.894	γ-CH_2: 1.575; 1.289; γ-Me + δ-Me: 0.982
Leu-3	8.086 (J=4.9)	4.223	1.677; 1.642	γ-CH: 1.534; δ-Me's: 0.964; 0.881
Ser-4	8.065 (J=4.2)	4.268	4.074; 3.997	
Ser-5	8.007 (J=5.6)	4.368	4.086; 3.982	
Leu-6	8.031 (J=5.1)	4.209	1.771 (2H)	γ-CH: 1.690; δ-Me's: 0.938; 0.872
Trp-7	8.325 (J=2.8)	4.477	3.397 (2H)	NH: 9.73; H2: 7.19; H4: 7.56; H5: 7.06; H6: 7.19; H7: 7.45
Lys-8	7.794 (J=4.0)	3.848	1.956; 1.919	γ-CH_2: 1.414; δ-CH_2: 1.678; ε-CH_2: 3.027
Lys-9	7.605 (J=6.1)	4.096	2.017 (2H)	γ-CH_2 + δ-CH_2: [a]; ε-CH_2: [a]
Leu-10	8.452 (J=4.0)	4.098	1.850; 1.789	γ-CH: 1.579; δ-Me's: 0.869
Lys-11	8.186 (J=3.0)	3.793	1.672; 1.621	γ-CH_2 + δ-CH_2: 1.547 + 1.298; ε-CH_2: [a]
Lys-12	7.442 (J=5.9)	4.083	1.992; 1.732	γ-CH_2 + δ-CH_2: 1.630; 1.473; ε-CH_2: [a]
Ile-13	7.815 (J=6.3)	3.831	2.059	γ-CH_2: 1.756; 1.177; γ-Me: 0.933; δ-Me: 0.880
Ile-14	8.305 (J=5.1)	3.868	1.903	γ-CH_2: 1.656; 1.236; γ-Me: 0.951; δ-Me: 0.856
Ala-15	7.878 (J=4.9)	4.245	1.510	
Lys-16	7.722 (J=7.0)	4.230	1.967 (2H)	γ-CH_2 + δ-CH_2: 1.748; 1.583; ε-CH_2: 3.046; $CONH_2$: 7.36; 6.96

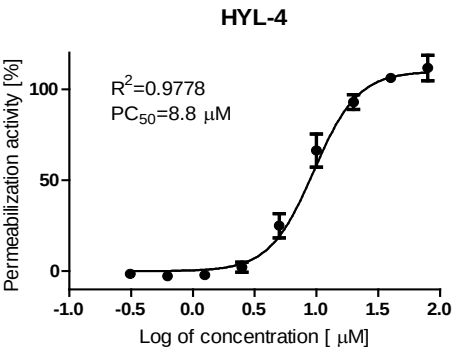
[a] not determined

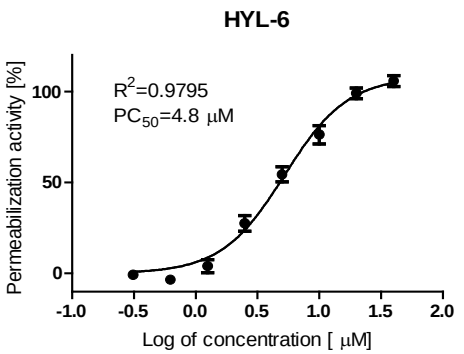
Permeabilization activity of HYL and its analogues plotted against the logarithm of peptide concentrations. PC_{50} values were interpolated from these curves for each peptide.

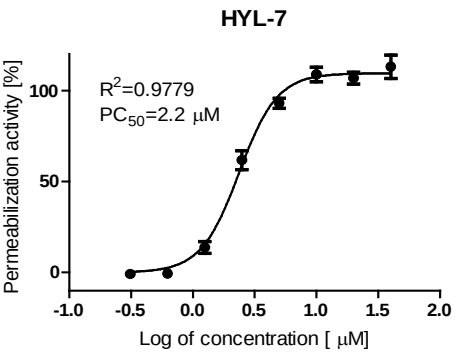


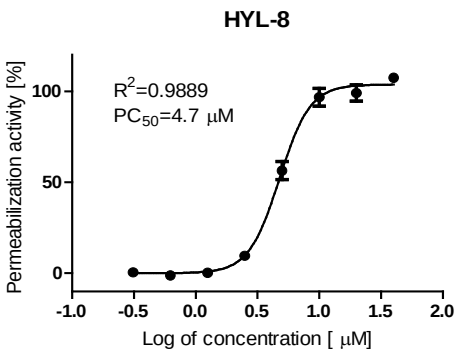


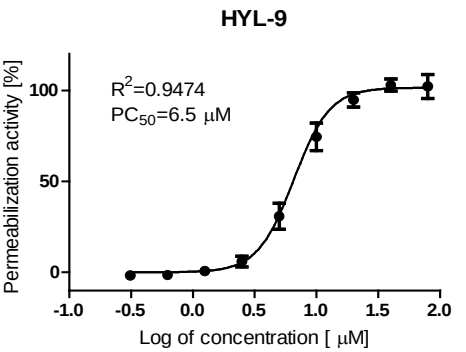


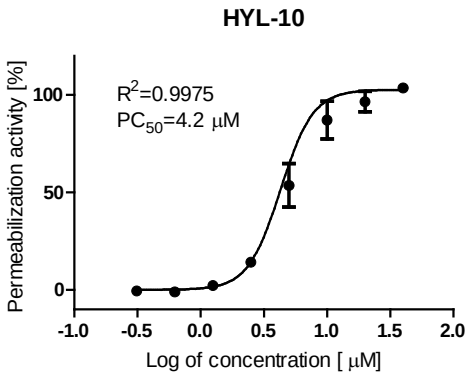


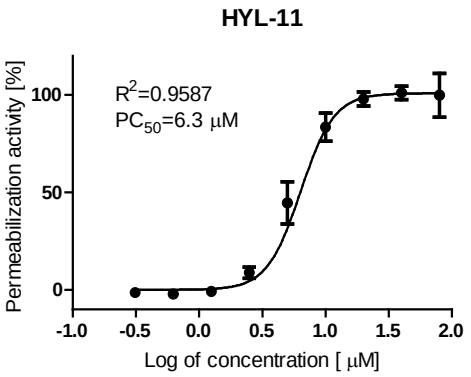


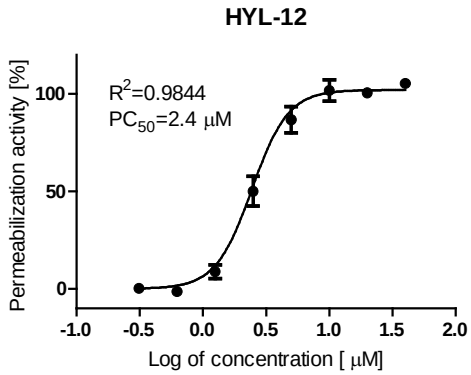


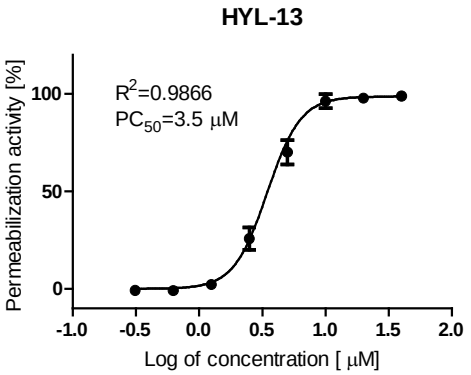


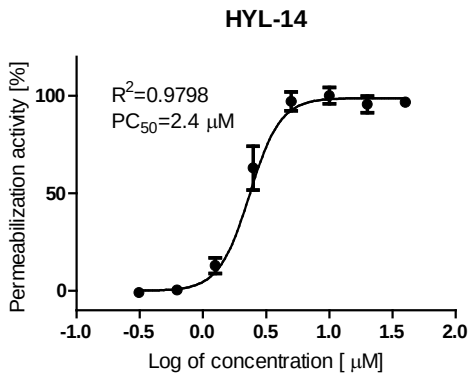



HYL-10
R2=0.9975
PC50=4.2 μM
Permeabilization activity [%]
Log of concentration [μM]


HYL-11
R2=0.9587
PC50=6.3 μM
Permeabilization activity [%]
Log of concentration [μM]


HYL-12
R2=0.9844
PC50=2.4 μM
Permeabilization activity [%]
Log of concentration [μM]


HYL-13
R2=0.9866
PC50=3.5 μM
Permeabilization activity [%]
Log of concentration [μM]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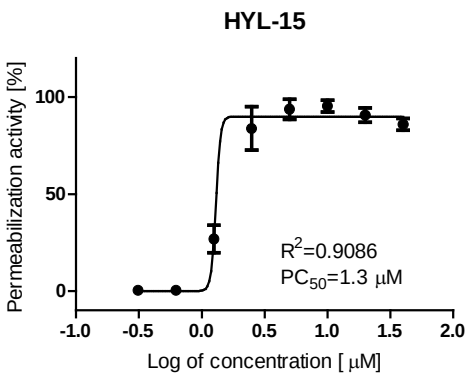
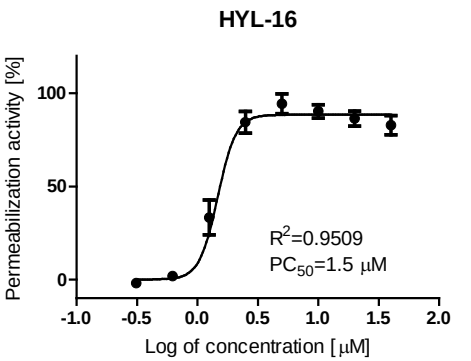
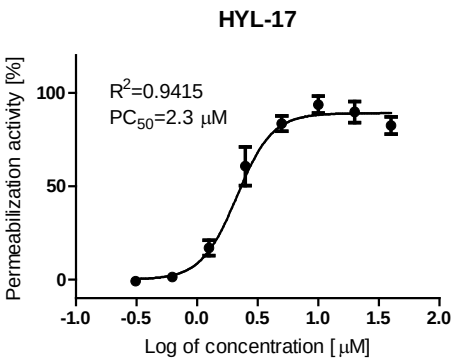
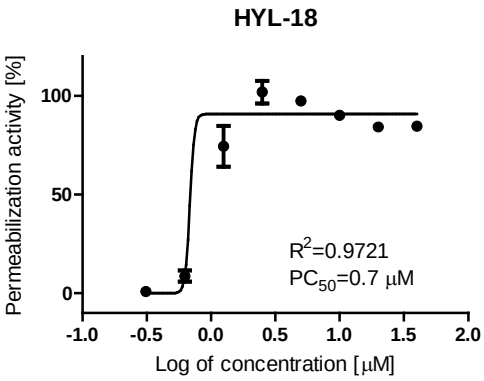
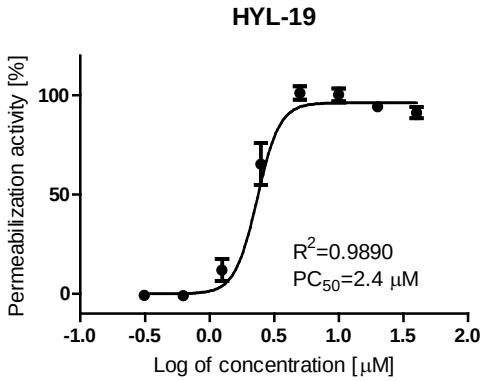
R2=0.9798
PC50=2.4 μM
Permeabilization activity [%]
Log of concentration [μM]

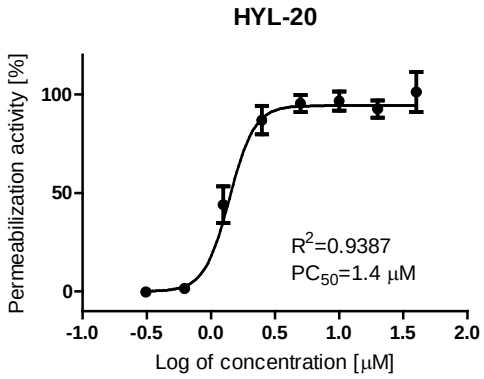

HYL-15
R2=0.9086
PC50=1.3 μM
Permeabilization activity [%]
Log of concentration [μM]

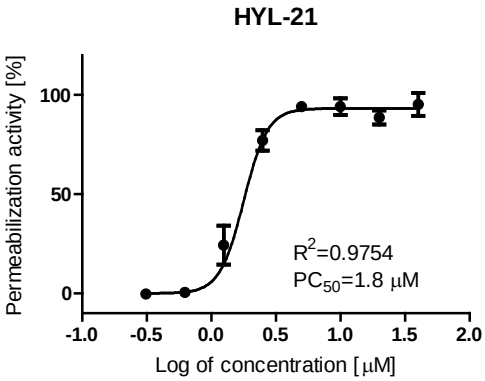

HYL-16
R2=0.9509
PC50=1.5 μM
Permeabilization activity [%]
Log of concentration [μM]

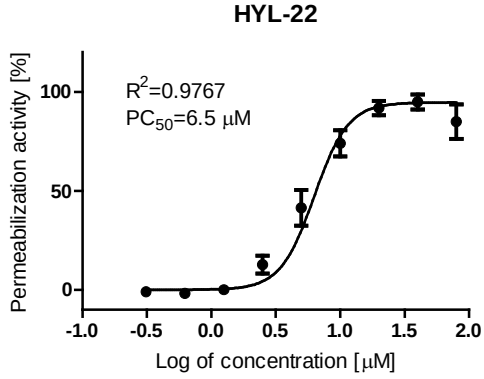

HYL-17
R2=0.9415
PC50=2.3 μM
Permeabilization activity [%]
Log of concentration [μM]

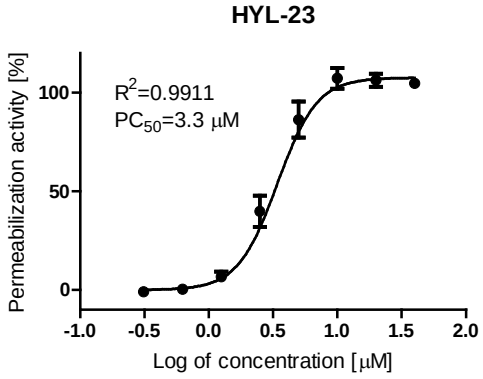

HYL-18
Permeabilization activity [%]
100
50
0
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Log of concentration [μM]
R2=0.9721
PC50=0.7 μM

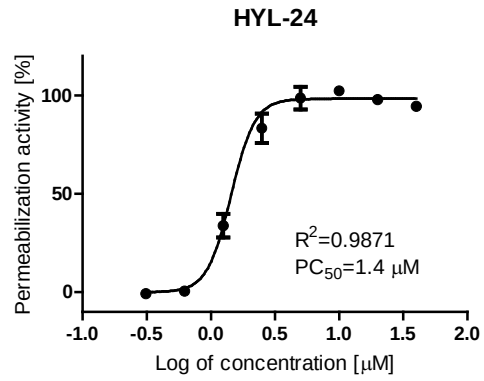

HYL-19
Permeabilization activity [%]
100
50
0
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Log of concentration [μM]
R2=0.9890
PC50=2.4 μM

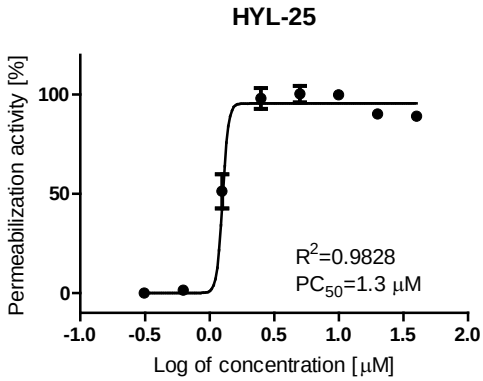

HYL-20
Permeabilization activity [%]
100
50
0
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Log of concentration [μM]
R2=0.9387
PC50=1.4 μM


HYL-21
Permeabilization activity [%]
100
50
0
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Log of concentration [μM]
R2=0.9754
PC50=1.8 μM


HYL-22
Permeabilization activity [%]
100
50
0
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Log of concentration [μM]
R2=0.9767
PC50=6.5 μM


HYL-23
Permeabilization activity [%]
100
50
0
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Log of concentration [μM]
R2=0.9911
PC50=3.3 μM


HYL-24
Permeabilization activity [%]
100
50
0
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Log of concentration [μM]
R2=0.9871
PC50=1.4 μM


HYL-25
Permeabilization activity [%]
100
50
0
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Log of concentration [μM]
R2=0.9828
PC50=1.3 μM