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Supporting Information

The Activity of Small Urea-γ-AApeptides Toward Gram-Positive Bacteria

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Supporting Information

1. General information

Rink amide MBHA resins (0.7 mmol/g, 200–400 mesh) were purchased from Chem-Impex Int'l Inc. The solid phase syntheses of all compounds were carried out in a peptide reaction vessel on a Burrell Wrist-Action shaker. Solvents and other chemicals were ordered from either Fisher Scientific or Sigma-Aldrich, and were used without further purification. All compounds were analyzed and purified using the Waters Breeze 2 HPLC system under 215 nm of UV detector equipped with both analytical and preparative modules. The desired fractions were lyophilized on a Labcono lyophilizer.

2. Synthesis of *γ*-AApeptide building blocks

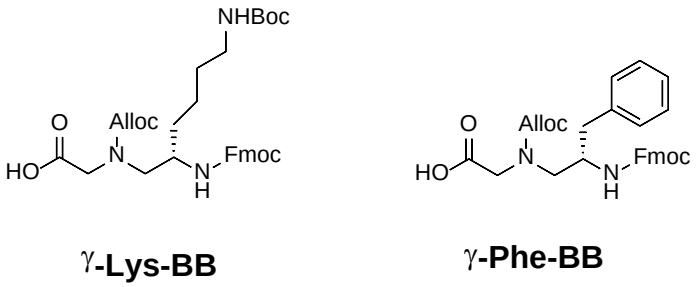


Figure S1. γ-AApeptide building blocks.

Both γ-AApeptide building blocks were used in synthesis of sequences on the solid phase, and their procedure of synthesis was reported in previously paper.[1]

3. Synthesis of small linear compounds on solid phase

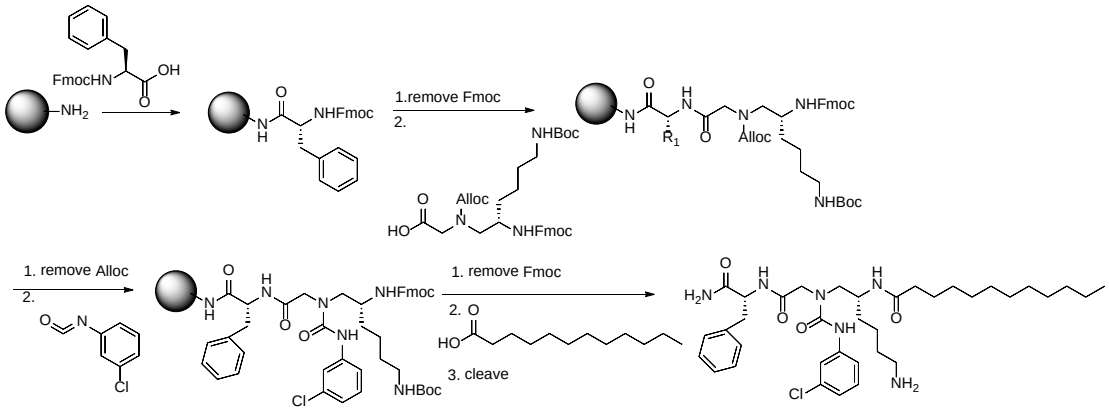
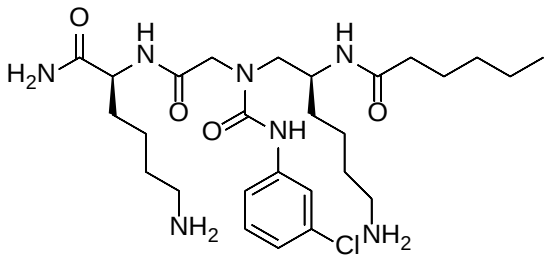


Figure S1. Synthesis of compound **10**.

Synthetic procedure of the compound **10**: 200 mg Rink-amide (MBHA) resin (0.14 mmol) was treated with 3 mL 20% piperidine/DMF (v/v) solution for 15 min (× 2) to remove the Fmoc protection group, followed by DMF (2 mL × 3) and DCM (2 mL × 3) wash. The attachment of Fmoc-L-Phenylalanine to the resin was achieved by adding Fmoc-L-Phenylalanine (155 mg, 0.4 mmol), DIC (101 mg, 114 μL, 0.8 mmol), and HOBt (122 mg, 0.8 mmol) in 3 mL DMF to the reaction vessel, and the reaction was allowed to shake at room temperature for 3 h. The solution was drained, and the beads were washed with DCM (3 mL × 3) and DMF (3 mL × 3). After that, beads were treated with 3 mL 20% piperidine/DMF (v/v) solution for 15 min (× 2) to remove the Fmoc protection group, followed by DMF (2 mL × 3) and DCM (2 mL × 3) wash. Then **γ**-Lys-BB (238 mg, 0.4 mmol), DIC (101 mg, 114 μL, 0.8 mmol), and HOBt (122 mg, 0.8 mmol) in 3 mL DMF was added to the reaction vessel, and the reaction was allowed to shake at room temperature for 3 h. The solution was drained, and the beads were washed with DCM (3 mL × 3) and DMF (3 mL × 3). After that, the resin was treated with $Pd(PPh_3)_4$ (24 mg, 0.02 mmol) and $Me_2NH.BH_3$ (70 mg, 1.2 mmol) in 3 mL DCM for 10 min (×2) to remove the alloc protein group, then washed with DCM (3 mL x3) and DMF (3 mL ×3). Next, 3-chlorophenyl isocyanate (77 mg, 61 μL, 0.5 mmol) and DIPEA (65 mg, 87 μL, 0.5 mmol) in 3 mL DCM were added to the resin and allowed to react for 30 min at room temperature, and then the solution was drained. After DMF (2 mL ×3) and DCM (2 mL ×3) wash, beads were treated with 3 mL 20% piperidine/DMF (v/v) solution for 15 min (× 2) to remove the Fmoc protection group, followed by wash with DMF (2 mL ×3) and DCM (2 mL ×3). Subsequently, lauric acid (80 mg, 0.4 mmol), DIC (101 mg, 114 μL, 0.8 mmol), and HOBt (122 mg, 0.8 mmol) in 3 mL DMF were added to the reaction vessel and reacted for 3 h. After the solution was drained, the beads were washed with DMF (2 mL ×3) and DCM (2 mL ×3), followed by the incubation with 4 mL cocktail of 1:1 TFA: DCM 1:1 (v/v) for 2 h to achieve cleavage and global deprotection of the compound. After the solvent was removed in vacuo, the residue was analyzed and purified on the Waters HPLC system, and the desired fraction was lyophilized to give the pure product **10**.

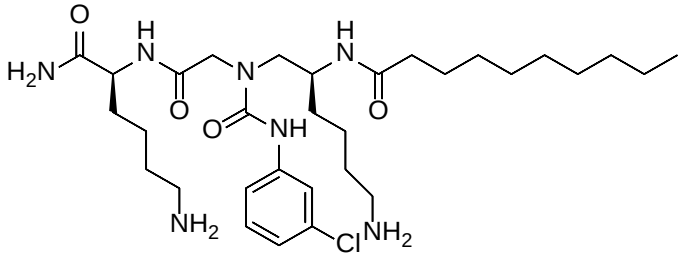
Synthesis of other compounds: The other compounds were synthesized following the similar procedure of compound **10**.

Compound 1



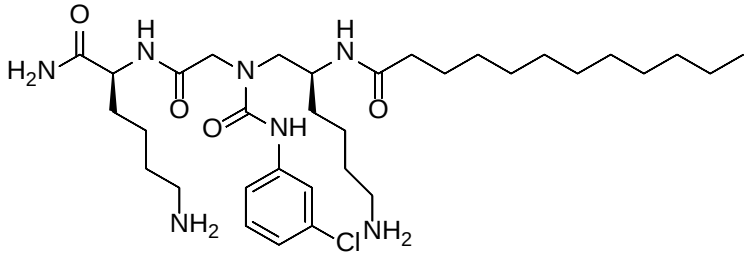
HRMS (ESI) $C_{27}H_{46}ClN_7O_4$ [M+H]+ calc'd = 568.3372; found = 568.2142.

Compound 2



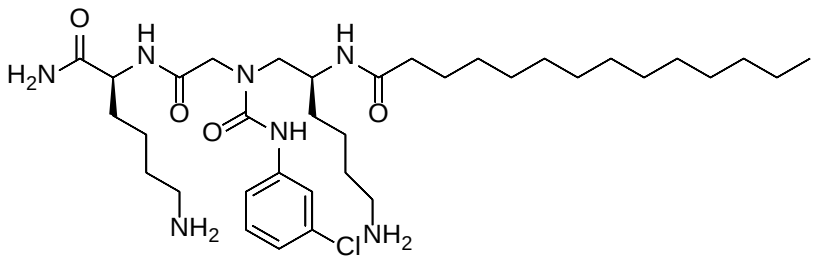
HRMS (ESI) $C_{31}H_{54}ClN_7O_4$ [M+H]+ calc'd = 624.3998; found = 624.4067.

Compound 3



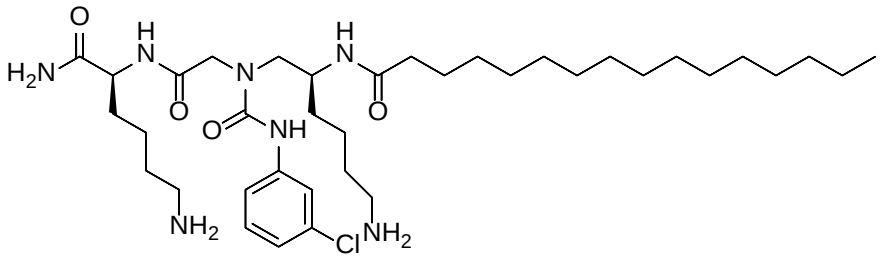
HRMS (ESI) $C_{33}H_{58}ClN_7O_4$ [M+H]+ calc'd = 652.4312; found = 652.4197.

Compound 4



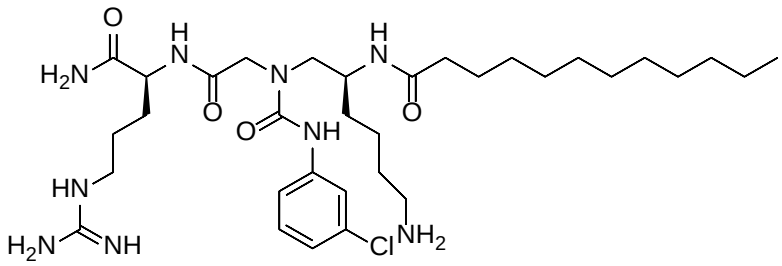
HRMS (ESI) $C_{35}H_{62}ClN_7O_4$ [M+H]+ calc'd = 680.4625; found = 680.4877.

Compound 5



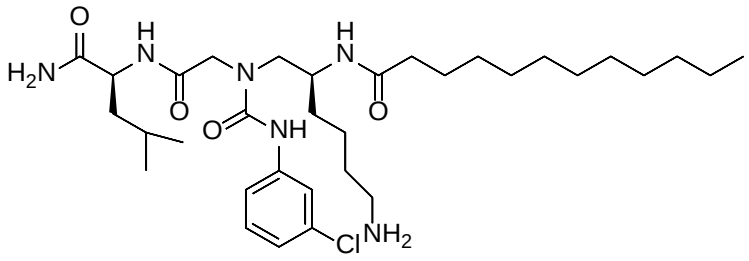
HRMS (ESI) $C_{37}H_{66}ClN_7O_4$ [M+H]+ calc'd = 708.4938; found = 780.5051.

Compound 6



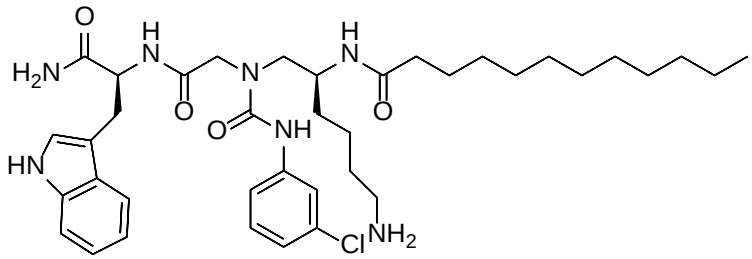
HRMS (ESI) $C_{33}H_{58}ClN_9O_4$ [M+H]+ calc'd = 680.4373; found = 680.4452.

Compound 7



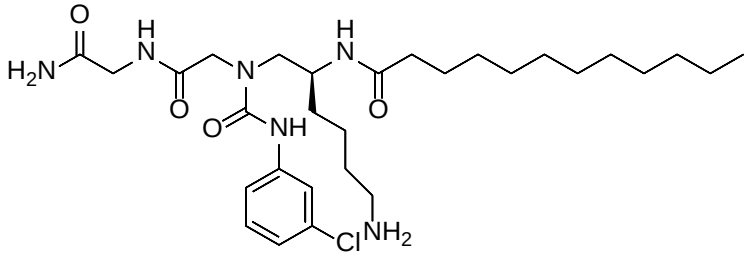
HRMS (ESI) $C_{33}H_{57}ClN_6O_4$ [M+H]+ calc'd = 637.4203; found = 637.4524.

Compound 8



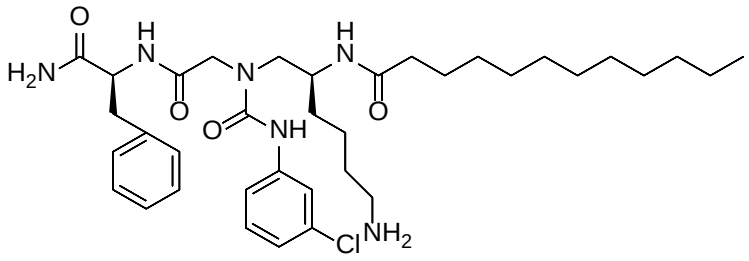
HRMS (ESI) $C_{38}H_{56}ClN_7O_4$ [M+H]+ calc'd = 710.4155; found = 710.4207.

Compound 9



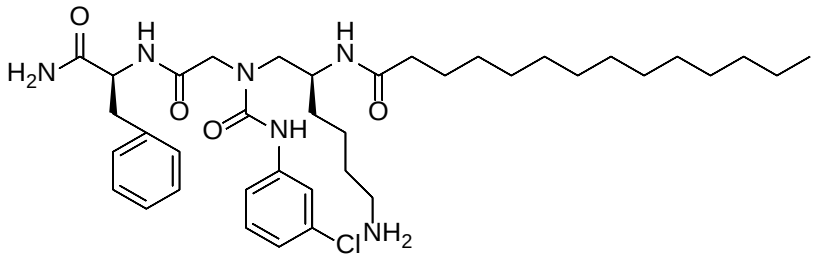
HRMS (ESI) $C_{29}H_{49}ClN_6O_4$ [M+H]+ calc'd = 581.3577; found = 581.3633.

Compound 10



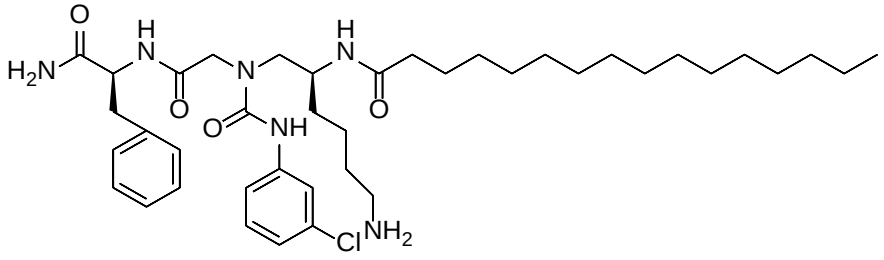
HRMS (ESI) $C_{36}H_{55}ClN_6O_4$ [M+H]+ calc'd = 671.4046; found = 671.4178.

Compound 11



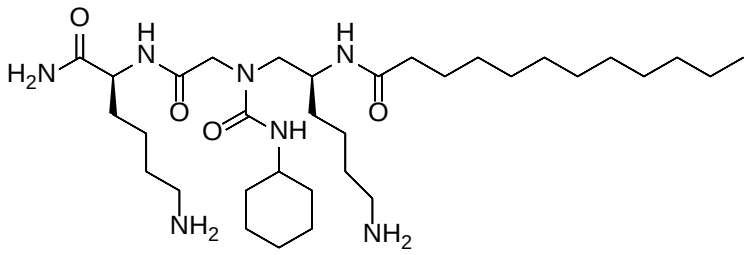
HRMS (ESI) $C_{38}H_{59}ClN_6O_4$ [M+H]+ calc'd = 699.4359; found = 699.5469.

Compound 12



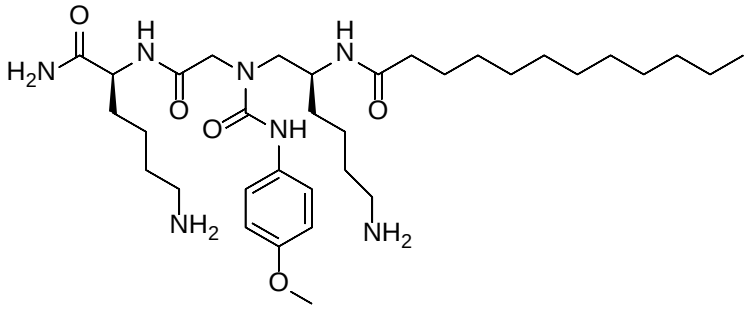
HRMS (ESI) $C_{40}H_{63}ClN_6O_4$ [M+H]+ calc'd = 727.4672; found = 727.5967.

Compound 13



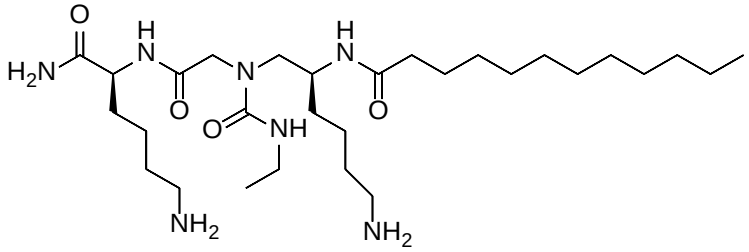
HRMS (ESI) $C_{33}H_{65}N_7O_4$ [M+H]+ calc'd = 624.5171; found = 624.6178.

Compound 14



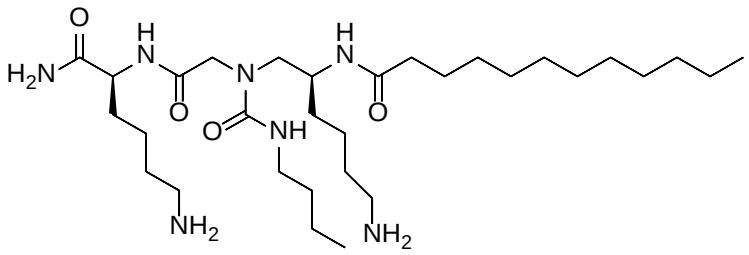
HRMS (ESI) $C_{34}H_{61}N_7O_5$ [M+H]+ calc'd = 648.4807; found = 648.6791.

Compound 15



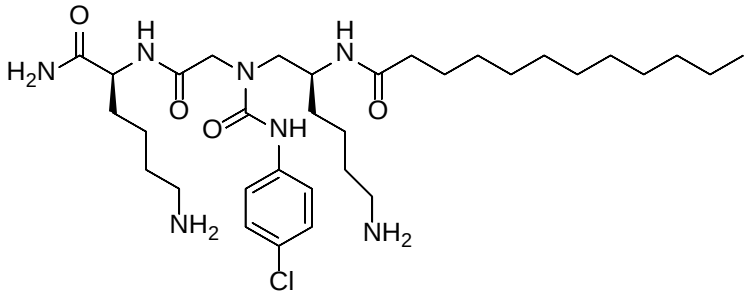
HRMS (ESI) $C_{29}H_{59}N_7O_4$ [M+H]+ calc'd = 570.4701; found = 570.6543.

Compound 16



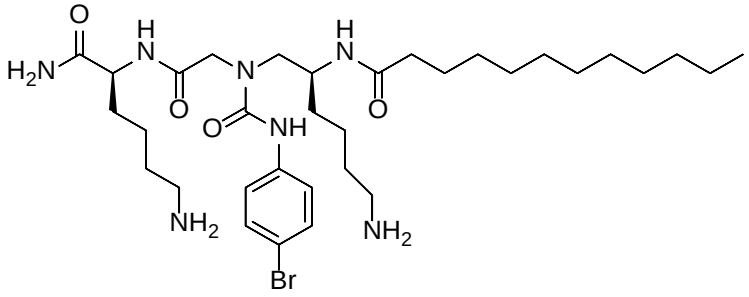
HRMS (ESI) $C_{31}H_{63}N_7O_4$ [M+H]+ calc'd = 598.5014; found = 598.7013.

Compound 17



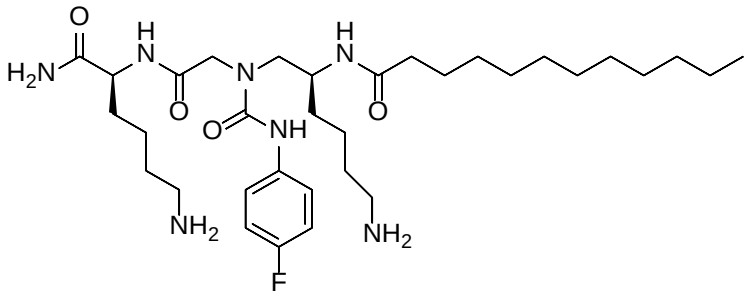
HRMS (ESI) $C_{33}H_{58}ClN_7O_4$ [M+H]+ calc'd = 652.4311; found = 652.4368.

Compound 18



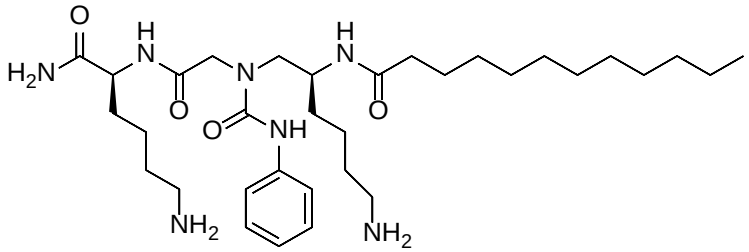
HRMS (ESI) $C_{33}H_{58}BrN_7O_4$ [M+H]+ calc'd = 696.3806; found = 696.4045.

Compound 19



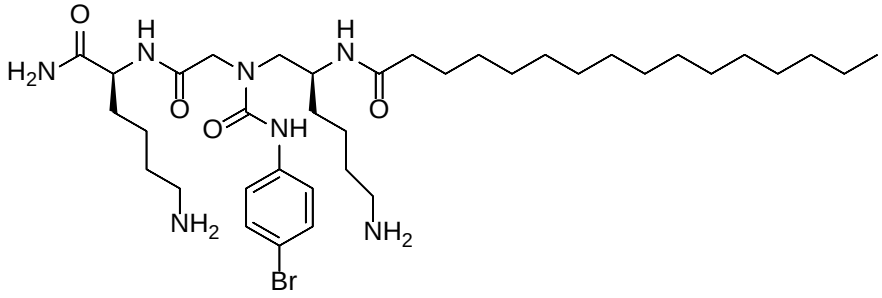
HRMS (ESI) $C_{33}H_{58}FN_7O_4$ [M+H]+ calc'd = 636.4607; found = 636.4589.

Compound 20



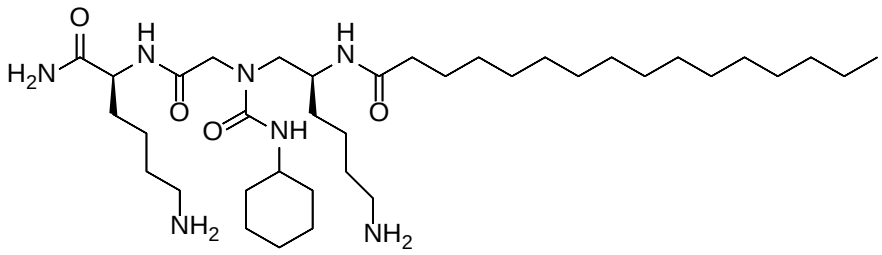
HRMS (ESI) $C_{33}H_{59}N_7O_4$ [M+H]+ calc'd = 618.4701; found = 618.5063.

Compound 21



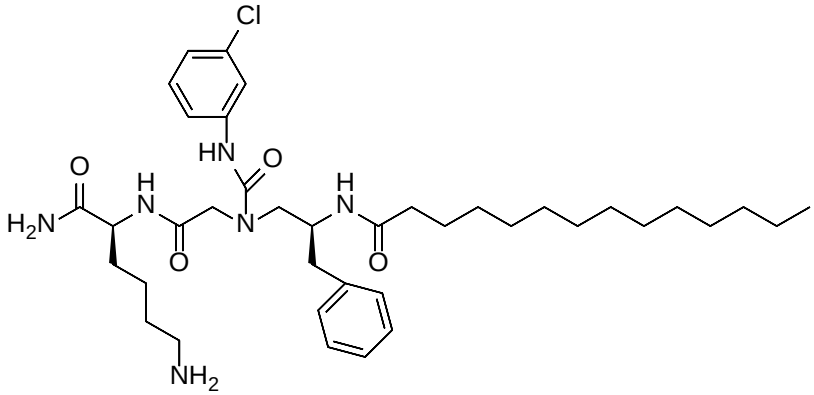
HRMS (ESI) $C_{37}H_{66}BrN_7O_4$ [M+H]+ calc'd = 752.4432; found = 752.4867.

Compound 22



HRMS (ESI) $C_{37}H_{73}N_7O_4$ [M+H]+ calc'd = 680.5797; found = 680.6102.

Compound 23



HRMS (ESI) $C_{38}H_{59}ClN_6O_4$ [M+H]+ calc'd = 699.4359; found = 699.5927.

4. Minimum inhibitory concentrations (MICs) assays.[2]

All compounds were tested against -three different bacteria strains: Methicillin-resistant *S. aureus* (MRSA, ATCC 33591), Methicillin-resistant *S. epidermidis* (MRSE, RP62A), vancomycin-resistant *Enterococcus faecalis* (ATCC 700802). One colony of each bacteria was incubated in 4 mL TSB buffer overnight at 37 °C, then diluted 100 times and incubated for another 6 hours to mid-logarithmic phase. All compounds were diluted in 96-wells plate with 50 µL 2-fold serial dilution, then 50 µL of diluted bacterial medium (1×10^6 CFU/mL) was added to each well. After 20 hours incubation at 37 °C, absorption at 600 nm wavelength was read on a Biotek Synergy HT microtiter plate reader. Minimum inhibitory concentrations were determined as the lowest concentrations that inhibit bacteria growth completely.

5. Time kill assays.[2]

Bacteria MRSA suspensions were incubated at 37 °C to mid-logarithmic phase and diluted to 1×10^6 CFU/mL, then mixed with compound **10** (12.5, 6.25, 3.125 μg/mL). The mixtures were incubated at 37 °C for 10 min, 30 min, 1 h and 2 h respectively, then diluted by 10^2 to 10^4 folds and 100 μL was spread on TSB agar plates. Numbers of bacteria colonies were counted after 20 hours incubation at 37 °C.

6. Drug resistance assays. [3]

After MICs assay against MRSA, bacteria from the well which contained 1/2 MIC were diluted to 1×10^6 CFU/mL for next MIC measurement. The measurement was repeat for 14 passages.

7. Hemolytic assays.[2]

Fresh red blood cells (RBCs) was washed with 1× PBS buffer and centrifuged 10 min at 3500 rpm less than 3 times until the supernatant was clear, then RBCs in the bottom layer was diluted into 5% v/v suspension in 1× PBS. 50 μL of compounds were diluted in 96-wells plate with 2-fold serial dilution and mixed with 50 μL RBCs suspension, then incubated for 1 hour at 37 °C. The mixture was then centrifuged for 10 min at 3500 rpm. 30 μL of the supernatant was added to 100 μL PBS, then absorbance of mixture was read on a Biotek Synergy HT plate reader at 410 nm and 540 nm. The hemolysis activity was calculated by the formula % hemolysis = $(Abs_{sample}-Abs_{PBS})/(Abs_{Triton}-Abs_{PBS})x100\%$. 1% and 5% Triton X-100 were used as positive control and 1× PBS buffer was used as negative control.

8. Fluorescence microscopy.[2]

Both propidium iodide (PI) and 4', 6'-diamidino-2-phenylindole dihydrochloride (DAPI) fluorescent dyes were used in the study. Bacteria MRSA suspensions were incubated at 37 °C to mid-logarithmic phase and diluted 100 folds, then incubated with compound **10** for 2 hours at 37 °C. After centrifugation for 15 min at 5000 rpm, cell pellets were washed with 1× PBS buffer, and incubated with PI (5 μg/mL) for 15 min on ice in the dark, then washed 2 times with PBS. Then DAPI (10 μg/mL) was also applied the same way. The pellets were then diluted in 100 μL PBS and 10~20 μL was applied on chamber slides and observed under Zeiss Axio Image Zloptical microscope using 100× oil-immersion objective.

9. HPLC analysis of compounds 1− 23.

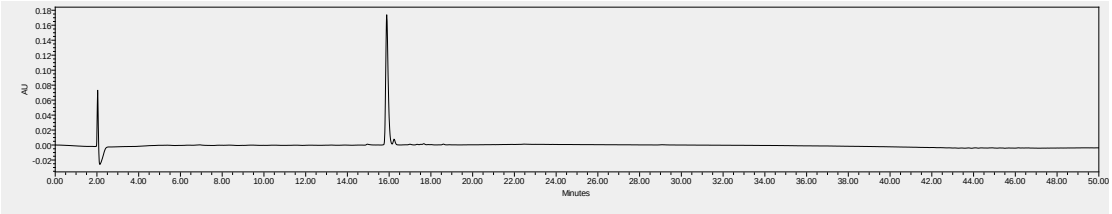
Compound Name	Retention time(min)	Purity (%)
1	15.77	96.80
2	23.11	99.59
3	23.64	99.32
4	23.67	97.8
5	28.71	99.58
6	21.74	99.52
7	28.05	99.60
8	27.95	97.35
9	24.98	99.86
10	30.51	96.51
11	34.46	96.40
12	36.01	97.11
13	36.47	99.33
14	24.23	97.51
15	21.82	95.32
16	24.14	95.99
17	24.92	99.17
18	22.28	99.26
19	35.98	99.57
20	21.53	99.60
21	28.03	99.78

22	28.12	99.01
23	37.35	95.20

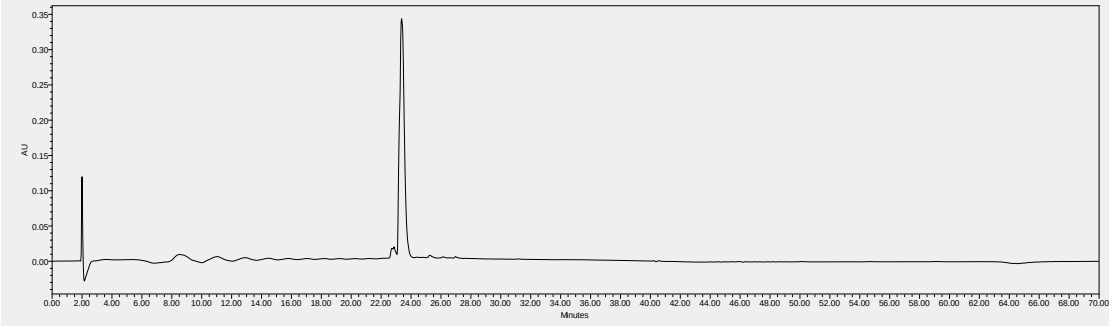
Table S1. HPLC purities and retention time of compounds **1− 23**

HPLC spectra of compounds **1− 23**

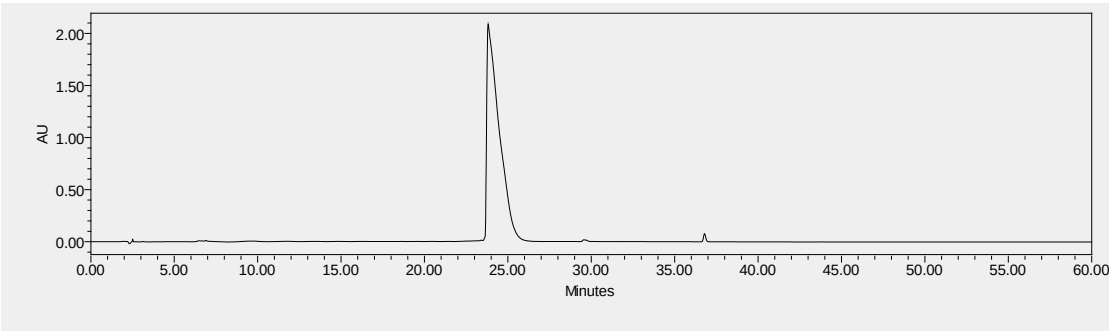
Compound 1



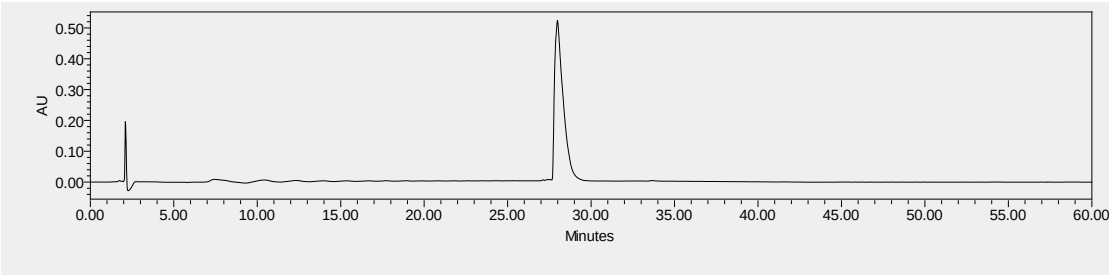
Compound 2



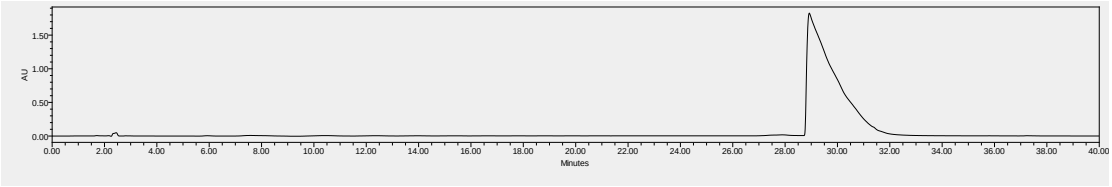
Compound 3



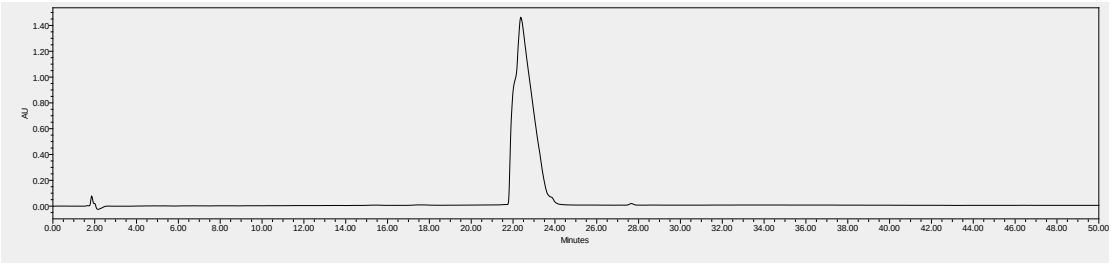
Compound 4



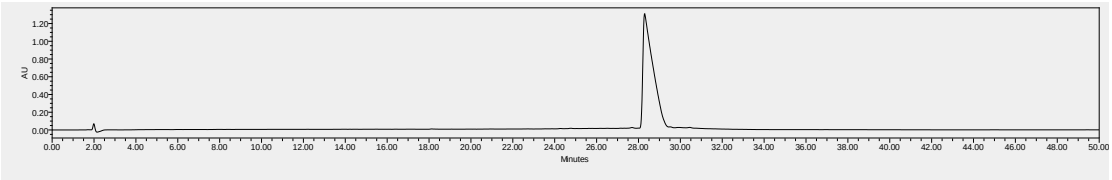
Compound 5



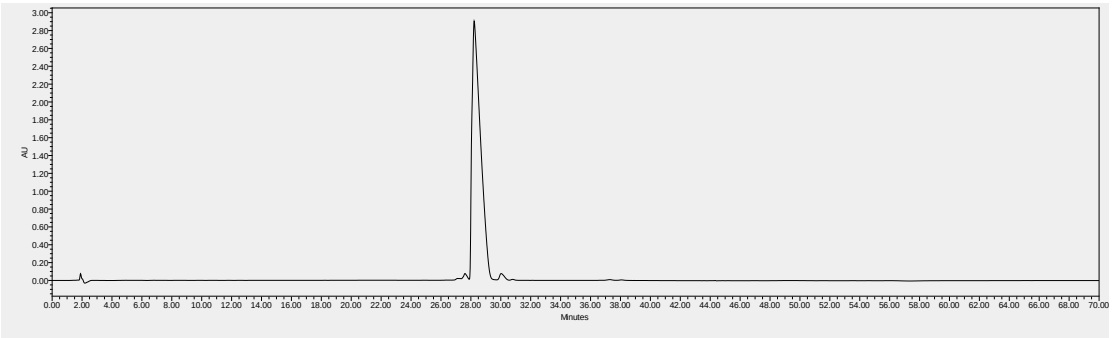
Compound 6



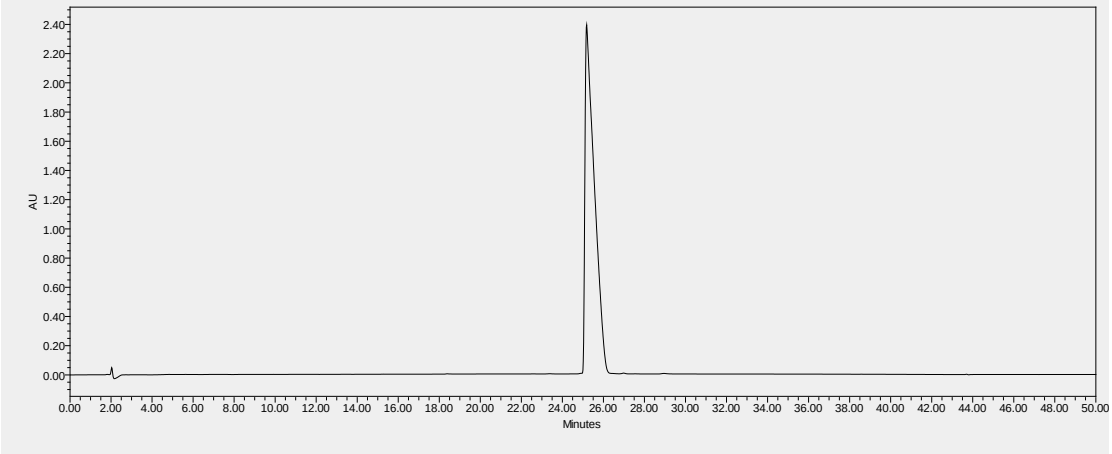
Compound 7



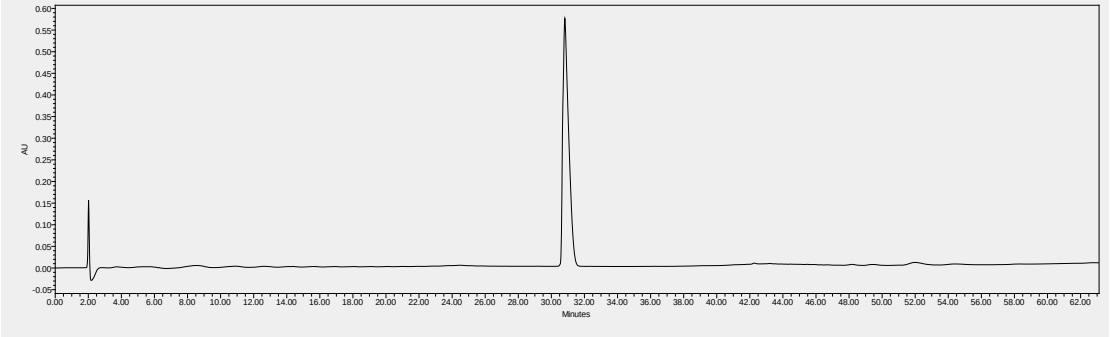
Compound 8



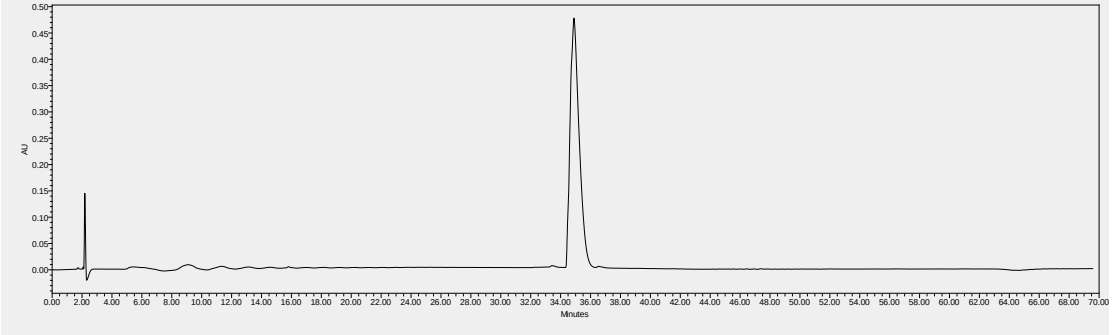
Compound 9



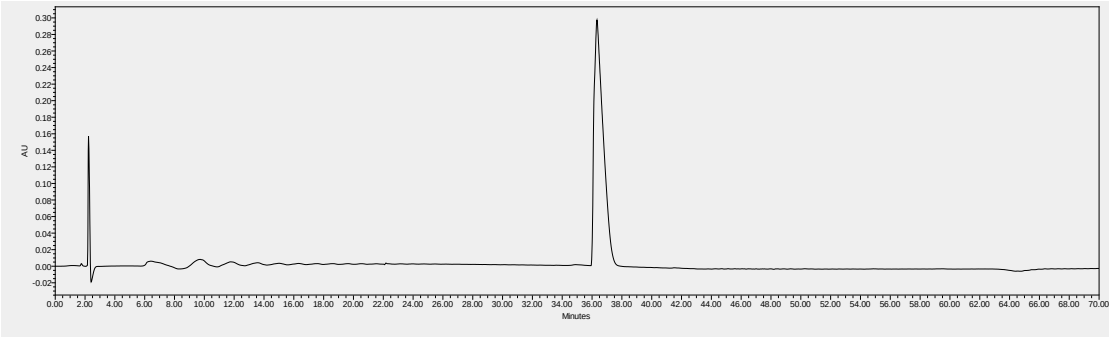
Compound 10



Compound 11



Compound 12



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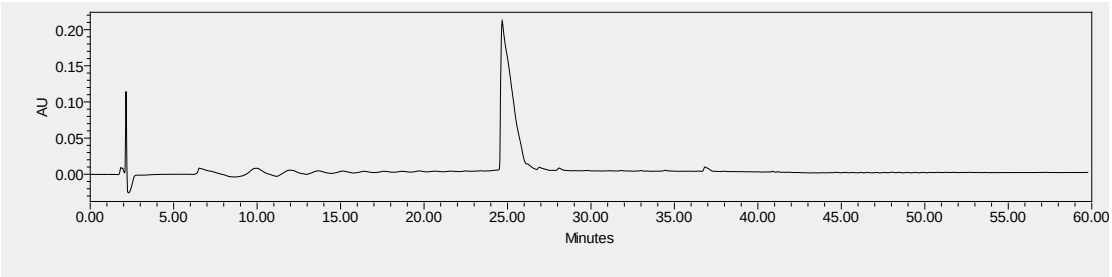

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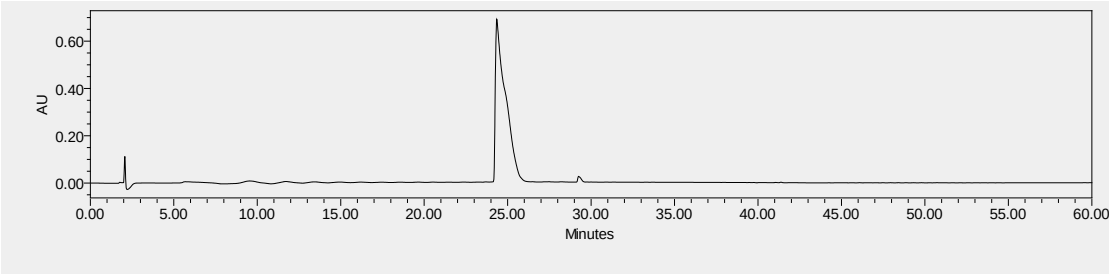

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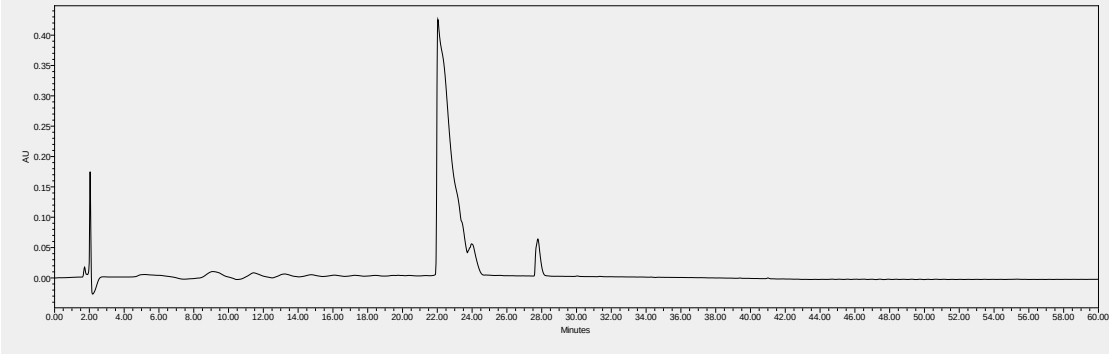

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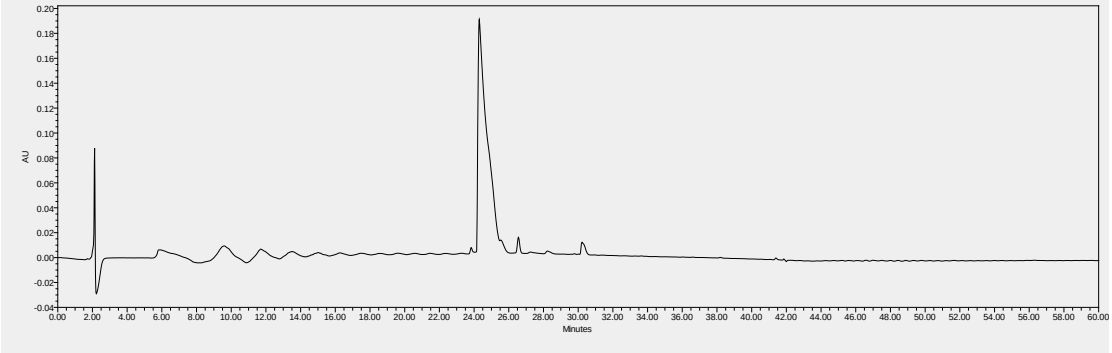

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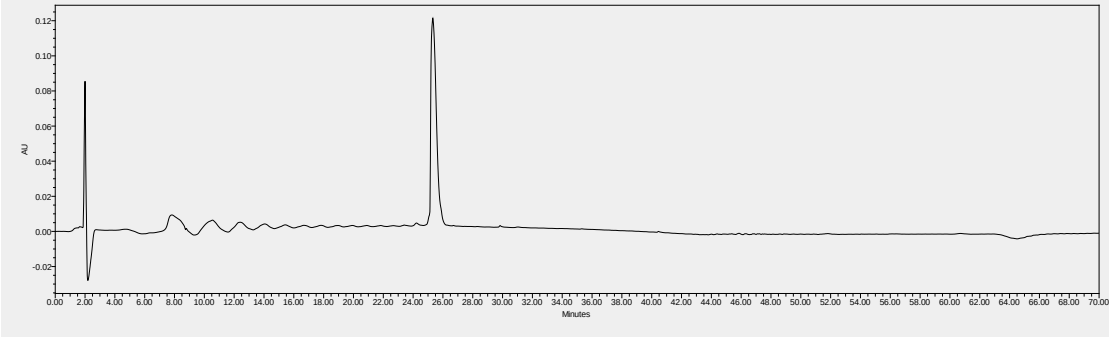

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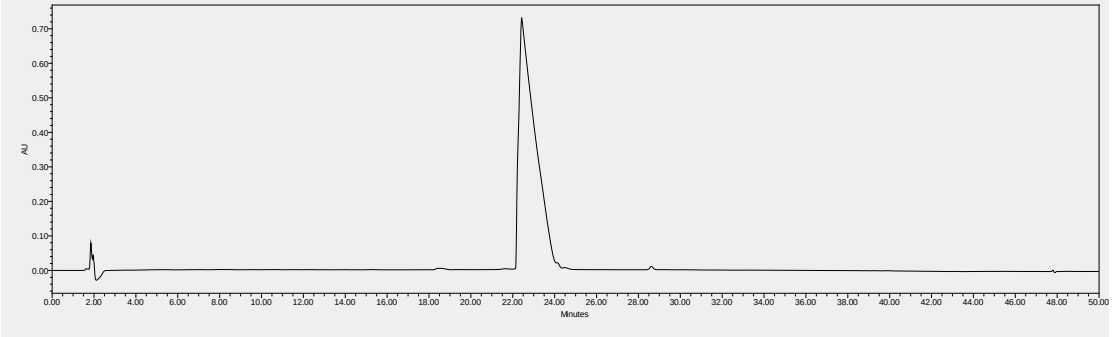

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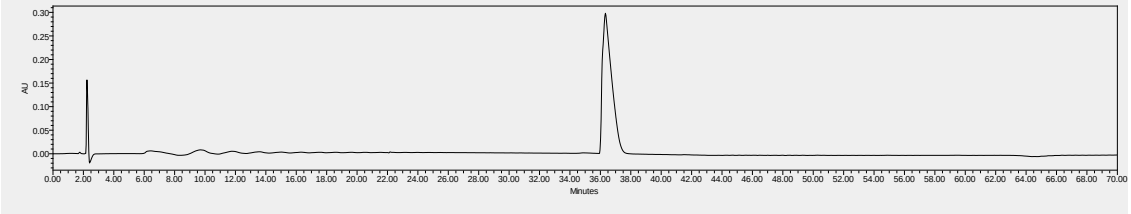

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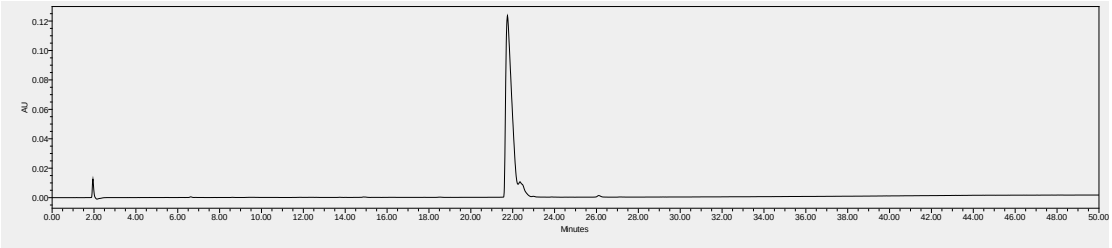

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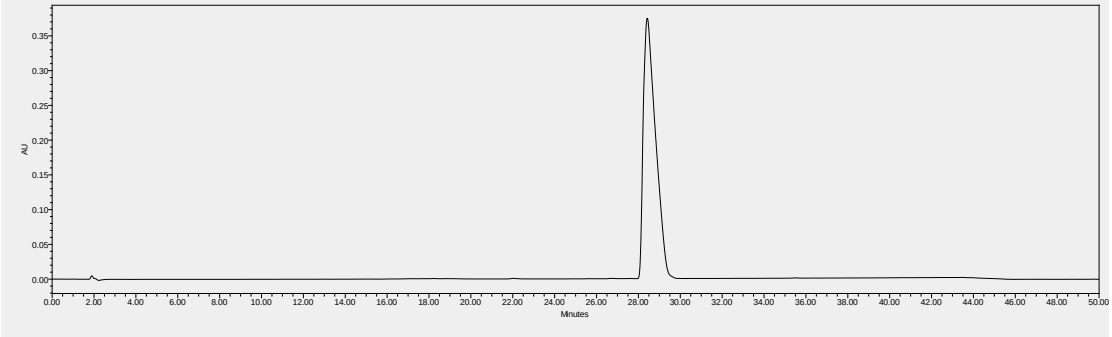

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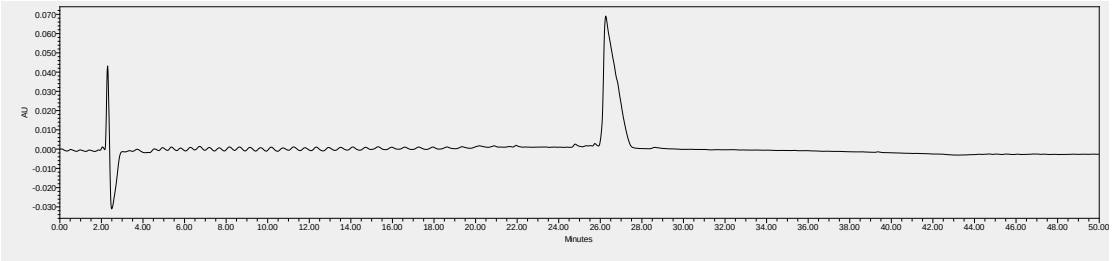

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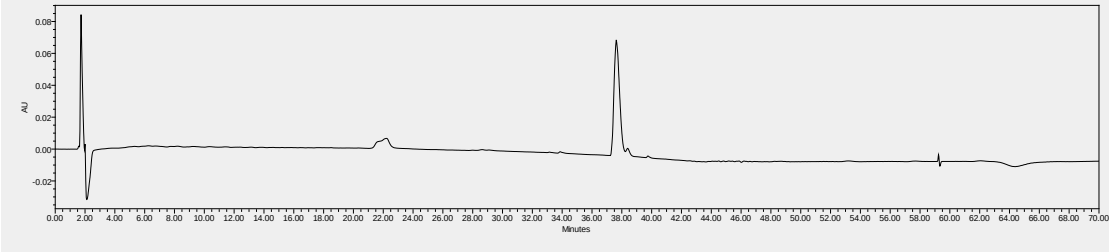

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