Rapid Lead Discovery Through Iterative Screening of One

Bead One Compound Libraries

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Supplementary Material and Methods

Fmoc-protected amino acids were purchased from AnaSpec (Fremont, CA). TentaGel resin was purchased from Rapp Polymere GmbH (Germany). All other reagents were purchased from Sigma-Aldrich or Alfa Aesar, unless otherwise specified. All of the chemical reagents and solvents from commercial sources were used without further purification. 5 mL and 10 mL disposable reaction columns (Intavis AG) were used as reaction vessels for solid phase synthesis. Syntheses of peptoids under microwave conditions were performed in a 1500 W microwave oven (GE model JE 1860BH04) with 10% power. HPLC was carried out on a Waters systems equipped with a Waters 1525 binary HPLC pumps and a 2487 dual λ absorbance detector, or a 2998 photodiode array detector. The mobile phase comprised of buffer A (H₂O containing 20% CH₃CN and 0.1% trifluoroacetic acid (TFA)) and buffer B (CH₃CN containing 0.1% TFA). Analytical HPLC was conducted using a Vydac C-18 column (5 μm, 250 x 4.6 mm, Alltech, Deerfield, IL) at a flow rate of 1.0 mL/min with UV detection at 220 nm. MS and MS/MS (MALDI-TOF) were performed on a 4800 Proteomics Analyzer (Applied Biosystems) with α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix. All steps involving water utilized distilled water filtered through a Barnstead Nanopure filtration system (Thermo Scientific).

Library synthesis

Peptoid linker synthesis with DIC: The linker was synthesized using standard peptoid synthesis methods with microwave acceleration¹ and 2M solutions of DIC, bromoacetic acid, and the corresponding amines in DMF.

First library (structure shown in Fig. 1): Tentagel Rink-amide beads (1 g, 90 μm, 0.20 mmol/g, Rapp-Polymere GmbH, Germany) were swelled in DMF for 2 hours before use. Fmoc was deprotected by 20% piperidine for 30 min. Beads were washed thoroughly with DMF. Peptoid linker (see Fig. 1) was synthesized using standard peptoid submonomer synthesis¹. Solutions of bromoacetic acid (2M in DMF) and DIC (2M in DMF) were used for the acylation step and amine solutions (1M in DMF) was used for amination step. For the variable region, the beads were first split into three portions, coupled with 2-Bromoacetic acid, (S)-2-bromopropanoic acid-d₄² and (R)-2-bromopropanoic acid respectively. For bromoacetic acid, 2M solution of DIC and 2M solution of bromoacetic acid were used as described above. For bromopropanoic acids, BTC³ was used as coupling reagent. BTC (92.1 mg, 0.31 mmol) was dissolved in 5 ml anhydrous THF in a glass vial. Bromopropionic acid (89 μl, 0.95 mmol) was then added to the vial and the whole vial was kept in a -20 °C freezer for 15 min. Beads were washed using DCM, DMF and then THF respectively 5 times each, then 2:1 THF/DIPEA (750 μl THF, 375 μl DIPEA, 2.2 mmol) was added to the beads. The solution was shaken gently. 2,4,6-Trimethylpyridine (356 μl, 2.7 mmol), was added to the cold solution of bromopropionic acid with BTC. A white precipitate was formed following the addition. The white suspension was then applied to the beads and the reaction vessel was put on a shaker for 2 hours at room temperature. The solution in the vessel should be a pale yellowish suspension during the whole course of the reaction. A darker color is an
indication of excessive heat released during the initial addition of the acid chloride solution. The beads were washed with DCM five times and then DMF 5 times. The chloranil test was used to monitor the completion of the reaction. All three portions of beads were then pooled together and the beads were incubated with a 2M solution of the corresponding amine in DMF at 60°C overnight. The completion of the reaction was monitored by the chloranil and silver acetate tests.

**Second library** (structure shown in Fig. 3): Tentagel Rink-amide beads (0.5 g, 90 μm, 0.23 mmol/g, Rapp-Polymere GmbH, Germany) were swelled in DMF for 2 hours before use. Fmoc was deprotected using 20% piperidine for 30 min. Beads were washed thoroughly with DMF. Peptoid linker (see Fig. 3) was synthesized using standard peptoid sub-monomer synthesis conditions. Solutions of bromoacetic acid (2M in DMF) and DIC (2M in DMF) were used for the acylation step and amine solutions (1M in DMF) was used for amination step. For the variable region, the beads were first split in three portions, coupled with 2-Bromoacetic acid, (R)-2-bromo-4-methylpentanoic acid, (S)-2-bromopropanoic acid-d₂ and (R)-2-bromopropanoic acid respectively. For bromoacetic acid, a 2M solution of DIC and 2M solution of bromoacetic acid were used as described above. For chiral acids, BTC was used as coupling reagent. BTC (92.1 mg, 0.31 mmol) was dissolved in 5 ml anhydrous THF in a glass vial. The corresponding bromo acid (0.95 mmol) was then added to the vial and the whole vial was kept in a -20 °C freezer for 15 min. Beads were washed using DCM, DMF and then THF respectively for 5 times each, then 2:1 THF/DIPEA (750 μl THF, 375 μl DIPEA, 2.2 mmol) was added to the beads, which were then shaken gently. 2,4,6-Trimethylpyridine (356 μl, 2.7 mmol), was added to the cold solution of bromopropionic acid with BTC and white precipitate was formed following the addition. The white suspension was then applied to the beads and the reaction vessel was put on a shaker for 2 hours at room temperature. The beads were washed with DCM five times, then DMF for 5 times. The chloranil test was used to monitor the completion of the reaction. All three portions of beads were then pooled together and the beads were incubated with 2M solution of the corresponding amine in DMF at 60°C overnight. The completion of the reaction was monitored by the chloranil and silver acetate tests. Butane-1,4-diamine was used in mono-MTT protected form, the MTT protection group was deprotected by treatment with 1% TFA in DCM for 5 minutes at 4 °C.

**Synthesis of the derivative library with a proline-core structure**

**Derivative library with proline-core structure** (structure shown in Fig. 6): Tentagel Rink-amide beads (1 g, 160 μm, 0.50 mmol/g, Rapp-Polymere GmbH, Germany) were swelled in DMF for 2 hours before use. Fmoc was deprotected by 20% piperidine for 30 min. Beads were washed thoroughly with DMF. Peptoid linker containing sarcosine (see Fig. 6) was synthesized using standard peptoid sub-monomer synthesis. Solutions of bromoacetic acid (2M in DMF) and DIC (2M in DMF) were used for the acylation step and amine solutions (1M in DMF) was used for amination step. For the first position of the variable region, the peptoid unit was constructed by 1M DIC and 2M bromoacetic acid solution in DMF was use for acylation. Amination was completed by incubating corresponding amine or hydrazide (as shown in Fig. 6 Box X) as 1M solution in DMF with
beads at 50 °C for 2 hours. After completion of the first peptoid unit, the beads were all washed thoroughly with DMF and split in two portions. F-moc-cis-4-azidoproline was added to one portion and fmoc-trans-4-azidoproline to the other. DIC plus Oxyma was used as coupling reagent. A solution of proline:DIC:Oxyma 1:1:1 was used. The final concentration of each reagent was 0.5M. After incubating with the beads for two hours, this step was repeated one more time to ensure the completeness of the coupling. Each portion was then further split in two, resulting in four equal portions of beads, namely libraries A, B, C and D. All four libraries were kept separate from this point. All four libraries were first deprotected by 20% piperidine in DMF for 30 min. For library A and C, the secondary amine of the proline ring was acylated by DIC and bromo acetic acid as described above. In the amination step, beads were split in 18 portions. Primary amines and hydrazides (as listed in Fig. 6 Box Z) were used as a 1M DMF solution. Protected and unprotected amino acids (NH$_2$-Tyr(2-Cl-Trt)-OH, NH$_2$.Trp(For)-OH, NH$_2$.Gln-OH, NH$_2$.Ser(Trt)-OH, NH$_2$.Leu-OH, NH$_2$.Phe-OH) were used as 0.5 M 1:1 amino acid: DIEA, DMF solutions. Amination was done by incubating beads with the corresponding solution at 50 °C for 2 hours and checked using the chloranil test. After amination, the beads were treated with 4 eq. of Fmoc-CI and 10 eq. of DIEA in DMF to protect the secondary amine formed at the N-terminus. The 4-azido group on the proline ring was then reduced using 30 mM TCEP in PBS buffer (pH = 7.5) overnight. Bromoacetic acid and DIC were used to acylate the amino group reduced from azide. After acylation, beads were washed with aqueous buffer to prevent the formation of activated acid or anhydride at position Z. Amines and hydrazides (as listed in Fig. 6 Box Y) were used as 1 M solution to perform the amination. After amination, Fmoc at position Z was deprotected by 20% piperidine. Beads were then washed with DMF and then incubated with 0.1M dimethylethylendiamine (DMEDA) water solution for 2 hours to remove the formyl protection group on tryptophan. Finally, 1% of TFA in DCM was used to remove all other protecting groups on serine and tyrosine. For library B and D, position Z was constructed as for library A and C. Afterwards, beads were first separated into 21 portions. Four portions were treated with the four corresponding alkynes (N-benzyl-N-methylprop-2-yn-1-amine, ethynylcyclopropane, dodec-1-yne, but-3-yn-1-ylbenzene) (10 eq.) using Cu$_2$SO$_4$ (10 eq.) and ascorbic acid (30 eq.) as catalyst in 1:1 tBuOH: water for 24 hours respectively. The other 17 portions were reduced by TCEP using the protocol described above. Out of 17 portions, 12 portions were reacted with the corresponding acid or acid chloride to form amide bonds. Specifically, eight portions were coupled with the acids ((E)-2-methylbut-2-enoic acid, 2-(2-methoxyphenyl)propanoic acid, thiophene-2-carboxylic acid, butyric acid, 2-(1,3-dioxoisindolin-2-yl)acetic acid, 2-(3,4-dichlorophenyl)acetic acid, 2-(6-methoxynaphthalen-2-yl)propanoic acid, 2-phenylacetic acid) using DIC as coupling reagent and 4 eq. of the acid, respectively. Four portions were treated with 4 eq. acid chlorides (4-methoxybenzenesulfonyl chloride, 4-phenoxysterbenzenesulfonl chloride, 4'-chloro-[1,1'-biphenyl]-4-sulfonyl chloride, methanesulfonl chloride) in DCM solution with equivalent of DIEA added as base. The other five portions were reacted with the five amino acid as described above. All protecting groups were deprotected as for library A and C.
**Chloranil test**

All reagent should be prepared fresh.

Solution A: 2% Chloranil (118-75-2) in DMF

Solution B: 2% acetaldehyde (75-07-0) in DMF

Solution A 100 microliter with solution B 100 microliter were mixed before the test in a 1.5ml tube, then the beads were dropped in and gently shake. If the beads turn blue within 5 min, it indicates the presence of secondary amine on the surface of the beads.

**Silver acetate test**

AgOAc 50 mg was dissolved in 10 ml 0.5M acetic acid solution. Amine solution during the amination of PTA or peptoid were dropped in 0.3 ml of above solution and tested for AgBr precipitation. If AgBr precipitation formed, reaction was then allowed with fresh amine solution for a extended period of time till shown negative in silver acetate test which indicate none observable bromide were further replaced by amine during reaction.
Table S1: Letter codes for initial library decode.

| Structure | Letter code | Structure | Letter code |
|-----------|-------------|-----------|-------------|
| ![Structure](image1) | J | ![Structure](image2) | G |
| ![Structure](image3) | F | ![Structure](image4) | A |
| ![Structure](image5) | L | ![Structure](image6) | B |
| ![Structure](image7) | D | ![Structure](image8) | H |
| ![Structure](image9) | I | ![Structure](image10) | S |
| ![Structure](image11) | K | ![Structure](image12) | R |
| ![Structure](image13) | E | ![Structure](image14) | N |
| ![Structure](image15) | C | | |

### Table S2.
Sequences and K<sub>0s</sub> (μM) of 96 hits identified from initial screening. Each side chain is assigned with a letter code. See Table S2 for letter codes. NB: no binding detected. ? : Sequence cannot be decoded.

|    | K<sub>d</sub> | R/S/N 1 | R/S/N 2 | R/S/N 3 | R/S/N 4 | K<sub>d</sub> | R/S/N 1 | R/S/N 2 | R/S/N 3 | R/S/N 4 |
|----|--------------|---------|---------|---------|---------|--------------|---------|---------|---------|---------|
| KYG-1 | 110 | R | F | N | B | S | J | S | D | KYG-49 | >150 | R | E | N | A | N | D | S | I |
| KYG-2 | 22 | N | F | N | B | R | D | S | J | KYG-50 | >150 | S | N | I | H | N | F | R | C |
| KYG-3 | 60 | N | L | N | A | N | K | R | E | KYG-51 | >150 | N | E | S | B | N | K | N | G |
| KYG-4 | 35 | R | G | N | C | N | B | S | I | KYG-52 | >150 | S | H | N | K | S | C | R | K |
| KYG-5 | 30 | N | E | N | L | N | H | R | D | KYG-53 | >150 | N | E | S | D | N | K | N | H |
| KYG-6 | 60 | N | K | N | B | R | G | S | F | KYG-54 | >150 | N | B | N | H | R | L | S | D |
| KYG-7 | 61 | N | E | N | C | R | B | R | E | KYG-55 | >150 | N | K | N | H | R | I | N | L |
| KYG-8 | 61 | S | I | N | R | A | S | F | KYG-56 | >150 | S | I | N | L | N | F | R | C |
| KYG-9 | 67 | N | K | N | B | N | D | R | J | KYG-57 | >150 | S | A | N | I | R | E | N | A |
| KYG-10 | 72 | N | F | N | B | N | A | R | K | KYG-58 | >150 | S | C | R | E | N | K | N | E |
| KYG-11 | 72 | N | G | N | I | R | L | S | E | KYG-59 | >150 | R | D | R | B | ? | ? | ? | ? |
| KYG-12 | 73 | N | J | N | F | S | J | R | L | KYG-60 | >150 | N | K | N | B | N | D | S | I |
| KYG-13 | 79 | N | L | N | H | R | D | S | H | KYG-61 | >150 | N | L | N | G | N | K | R | A |
| KYG-14 | 88 | N | I | N | D | S | E | R | J | KYG-62 | >150 | N | L | N | H | N | A | N | J |
| KYG-15 | 90 | N | B | N | A | N | F | S | E | KYG-63 | >150 | S | E | N | F | S | D | R | J |
| KYG-16 | 91 | N | B | N | G | S | A | S | A | KYG-64 | >150 | N | E | N | C | R | A | N | A |
| KYG-17 | 95 | N | F | R | E | N | H | S | L | KYG-65 | >150 | N | C | N | A | R | J | N | J |
| KYG-18 | 102 | N | F | N | D | S | I | N | G | KYG-66 | >150 | N | H | N | D | N | K | N | A |
| KYG-19 | 103 | N | D | N | C | R | C | N | L | KYG-67 | >150 | R | L | N | K | R | I | ? | ? |
| KYG-20 | 112 | N | A | N | B | R | K | N | I | KYG-68 | >150 | N | F | F | N | F | N | K | R | A |
| KYG-21 | 125 | N | A | R | K | N | H | N | C | KYG-69 | >150 | N | I | I | N | I | I | S | B |
| KYG-22 | 125 | N | B | N | E | N | K | S | G | KYG-70 | >150 | N | C | N | F | N | F | S | J |
| KYG-23 | 135 | N | J | N | A | R | B | N | K | KYG-71 | >150 | N | I | N | C | S | L | N | E |
| KYG-24 | 135 | N | F | R | G | N | D | R | G | KYG-72 | >150 | N | B | N | H | N | G | ? | ? |
| KYG-25 | 150 | N | K | S | A | N | I | R | A | KYG-73 | >150 | N | J | N | C | N | I | S | H |
| KYG-26 | >150 | N | F | N | L | N | F | N | D | KYG-74 | >150 | N | K | N | F | N | A | S | H |
| KYG-27 | >150 | N | F | S | A | S | B | N | B | KYG-75 | >150 | N | F | N | C | R | L | R | L |
| KYG-28 | >150 | N | K | N | G | R | C | N | L | KYG-76 | >150 | N | B | S | L | N | J | R | E |
| KYG-29 | >150 | N | L | N | H | R | C | N | H | KYG-77 | >150 | N | H | N | F | R | L | N | G |
| KYG-30 | >150 | N | F | N | B | N | E | ? | ? | KYG-78 | >150 | N | I | R | E | S | C | N | C |
| KYG-31 | >150 | N | R | K | N | B | R | L | N | D | KYG-79 | >150 | N | L | N | D | S | K | R | J |
| KYG-32 | >150 | N | C | N | L | N | D | N | F | KYG-80 | N | B | A | N | B | R | E | S | C |
| KYG-33 | >150 | N | D | N | K | R | G | N | E | KYG-81 | N | B | R | F | N | H | N | B | S | B |
| KYG-34 | >150 | N | D | N | H | N | I | N | A | KYG-82 | N | B | N | L | R | L | S | I | N | G |
| KYG-35 | >150 | N | K | N | A | S | I | N | G | KYG-83 | N | C | N | F | R | D | ? | ? | ? |
| KYG-36 | >150 | N | A | N | D | N | F | N | C | KYG-84 | N | B | S | J | N | B | N | E | R | D |
| KYG-37 | >150 | N | L | N | I | S | G | N | I | KYG-85 | N | B | A | N | E | R | C | R | J |
| KYG-38 | >150 | N | D | N | B | N | A | R | D | KYG-86 | N | B | G | N | A | N | F | R | I |
| KYG-39 | >150 | N | A | N | F | ? | ? | ? | KYG-87 | N | B | K | S | H | N | D | N | I |
| KYG-40 | >150 | N | G | R | J | N | F | R | J | KYG-88 | N | B | C | N | H | S | H | N | H |
| KYG-41 | >150 | N | K | N | G | S | A | N | A | KYG-89 | N | B | N | B | N | H | S | A |
| KYG-42 | >150 | R | J | N | C | N | D | S | H | KYG-90 | N | K | N | B | ? | ? | ? | ? |
| KYG-43 | >150 | N | E | N | G | S | D | N | F | KYG-91 | N | B | R | D | N | B | S | E | N | I |
| KYG-44 | >150 | N | F | N | L | N | E | R | D | KYG-92 | N | B | S | L | S | E | N | C | N |
| KYG-45 | >150 | N | H | N | K | S | A | N | E | KYG-93 | N | B | N | H | N | D | N | L | N | C |
| KYG-46 | >150 | S | L | R | A | N | C | N | C | KYG-94 | N | B | N | E | N | L | R | E | S | H |
| KYG-47 | >150 | N | H | N | H | N | J | N | H | KYG-95 | N | B | N | D | S | J | S | H | N | F |
| KYG-48 | >150 | S | G | N | G | S | F | R | G | KYG-96 | N | A | N | B | ? | ? | ? | ? |
Table S3: Letter codes for the second library.

| Structure | Letter code | Structure | Letter code |
|-----------|-------------|-----------|-------------|
| ![Structure](image1) | J | ![Structure](image2) | G |
| ![Structure](image3) | F | ![Structure](image4) | A |
| ![Structure](image5) | T | ![Structure](image6) | B |
| ![Structure](image7) | D | ![Structure](image8) | H |
| ![Structure](image9) | M | ![Structure](image10) | O |
| ![Structure](image11) | P | ![Structure](image12) | Q |
| ![Structure](image13) | I | ![Structure](image14) | N |
| ![Structure](image15) | E | ![Structure](image16) | R |
| ![Structure](image17) | U | ![Structure](image18) | S |
| ![Structure](image19) | C | ![Structure](image20) | L |
| ![Structure](image21) | V |             |             |
Table S4: Sequences and $K_d$ (μM) of 71 hits identified from the second screen. Each side chain is assigned with a letter code. See Table S3 for letter codes.

|     | $K_d$ (μM) | KYG-97 | KYG-98 | KYG-99 | KYG-100 | KYG-101 | KYG-102 | KYG-103 | KYG-104 | KYG-105 | KYG-106 | KYG-107 | KYG-108 | KYG-109 | KYG-110 | KYG-111 | KYG-112 | KYG-113 | KYG-114 | KYG-115 | KYG-116 | KYG-117 | KYG-118 | KYG-119 | KYG-120 | KYG-121 | KYG-122 | KYG-123 | KYG-124 | KYG-125 | KYG-126 | KYG-127 | KYG-128 | KYG-129 | KYG-130 | KYG-131 | KYG-132 |
|-----|-----------|--------|--------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|     |           | 1      | 2      | 3      | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       |
| #   |           | 1      | 2      | 3      | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       | 3       | 4       | 1       | 2       |
| KYG-97 | 0.5      | F      | J      | N      | I      | S      | C      | KYG-133 | 14.3 | E      | V      | S      | F      | R      | J      | KYG-130 | 14.5 | Q      | C      | N      | F      | S      | U      | KYG-128 | 8.7 | P      | V      | R      | D      | N      | U      | KYG-135 | 14.6 | O      | T      | N      | G      | S      | C      | KYG-127 | 9.1 | D      | B      | S      | Q      | O      | S      | H      | KYG-136 | 14.9 | G      | H      | R      | O      | N      | T      | KYG-125 | 9.5 | E      | H      | N      | Q      | L      | A      | KYG-137 | 15.9 | F      | T      | L      | E      | N      | A      | KYG-123 | 1.1 | F      | B      | S      | Q      | N      | H      | KYG-138 | 15.9 | Q      | V      | N      | I      | R      | B      | KYG-121 | 2.3 | O      | C      | S      | O      | S      | J      | KYG-139 | 16.2 | D      | J      | S      | Q      | S      | J      | KYG-121 | 2.4 | O      | U      | S      | G      | R      | C      | KYG-140 | 16.4 | F      | A      | R      | P      | N      | B      | KYG-115 | 3.4 | O      | T      | R      | O      | S      | T      | KYG-141 | 16.4 | F      | C      | N      | O      | S      | A      | KYG-113 | 3.7 | O      | U      | R      | F      | N      | A      | KYG-142 | 17   | I      | B      | R      | M      | N      | T      | KYG-107 | 4.6 | P      | U      | N      | G      | N      | T      | KYG-143 | 18.3 | I      | A      | N      | G      | R      | C      | KYG-108 | 4.9 | Q      | C      | N      | Q      | N      | H      | KYG-144 | 19.2 | F      | U      | N      | D      | N      | J      | KYG-109 | 5.8 | M      | J      | L      | P      | R      | A      | KYG-145 | 19.3 | P      | J      | N      | O      | R      | C      | KYG-110 | 6.1 | E      | C      | N      | E      | R      | A      | KYG-146 | 19.5 | M      | B      | N      | D      | S      | T      | KYG-111 | 6.3 | Q      | U      | S      | Q      | S      | C      | KYG-147 | 19.7 | E      | U      | R      | E      | N      | A      | KYG-112 | 7.4 | O      | A      | L      | P      | N      | B      | KYG-148 | 21.6 | D      | C      | S      | D      | N      | V      | KYG-113 | 8.2 | P      | U      | R      | E      | S      | J      | KYG-149 | 22.6 | E      | U      | N      | D      | N      | J      | KYG-114 | 8.3 | M      | H      | N      | M      | N      | U      | KYG-150 | 22.7 | Q      | J      | N      | F      | R      | A      | KYG-115 | 8.7 | Q      | T      | R      | O      | N      | J      | KYG-151 | 23.5 | E      | T      | N      | M      | R      | T      | KYG-116 | 8.8 | I      | T      | S      | D      | N      | A      | KYG-152 | 23.6 | I      | A      | R      | M      | N      | B      | KYG-117 | 8.9 | G      | U      | S      | G      | N      | T      | KYG-153 | 23.6 | D      | B      | L      | F      | N      | H      | KYG-118 | 9.3 | M      | T      | R      | Q      | N      | H      | KYG-154 | 24.5 | D      | C      | S      | P      | S      | B      | KYG-119 | 9.3 | M      | H      | S      | E      | N      | U      | KYG-155 | 25.6 | Q      | H      | N      | D      | S      | V      | KYG-120 | 9.6 | P      | B      | S      | O      | N      | T      | KYG-156 | 25.7 | M      | T      | N      | F      | S      | V      | KYG-121 | 9.8 | G      | J      | N      | E      | N      | C      | KYG-157 | 26.1 | O      | B      | S      | P      | S      | T      | KYG-122 | 10.6 | G      | J      | N      | D      | S      | C      | KYG-158 | 27.3 | D      | C      | S      | G      | S      | B      | KYG-123 | 10.7 | D      | A      | N      | P      | N      | B      | KYG-159 | 28.9 | D      | H      | N      | D      | S      | B      | KYG-124 | 11.6 | D      | C      | N      | Q      | S      | V      | KYG-160 | 29.6 | F      | H      | L      | O      | S      | T      | KYG-125 | 11.8 | Q      | B      | S      | E      | R      | V      | KYG-161 | 30.1 | O      | U      | N      | D      | N      | C      | KYG-126 | 12.1 | Q      | T      | S      | F      | S      | U      | KYG-162 | 30.2 | O      | U      | S      | P      | S      | V      | KYG-127 | 12.1 | M      | H      | N      | P      | N      | H      | KYG-163 | 30.5 | I      | B      | R      | E      | R      | T      | KYG-128 | 13.3 | Q      | V      | S      | F      | N      | B      | KYG-164 | 30.7 | D      | B      | N      | E      | N      | J      | KYG-129 | 13.4 | M      | H      | L      | M      | N      | T      | KYG-165 | 30.8 | P      | C      | N      | F      | R      | C      | KYG-130 | 13.6 | G      | A      | S      | Q      | L      | U      | KYG-166 | 30.9 | I      | T      | N      | P      | N      | H      | KYG-131 | 13.7 | I      | C      | N      | O      | N      | C      | KYG-167 | 31.7 | I      | H      | N      | M      | S      | T      | KYG-132 | 13.8 | I      | H      | L      | E      | R      | T      |
Figure S1: KYG-1 MS, MS/MS and HPLC
Figure S2 KYG-2 MS, MS/MS and HPLC
Figure S3 KYG-3 MS, MS/MS and HPLC
Figure S4 KYG-4 MS, MS/MS and HPLC
Figure S5 KYG-5 MS, MS/MS and HPLC
Figure S6 KYG-97 MS, MS/MS and HPLC
Figure S7 KYG-98 MS, MS/MS and HPLC
Figure S8 KYG-99 MS, MS/MS and HPLC
Figure S9 KYG-100 MS, MS/MS and HPLC
Figure S10 KYG-101 MS, MS/MS and HPLC
Figure S12 KYG-169 MS, MS/MS and HPLC
Figure S13 KYG-170 MS, MS/MS and HPLC
Figure S14 KYG-171 MS, MS/MS and HPLC
Figure S15 KYG-173 MS, MS/MS and HPLC
Figure S16 KYG-NC MS, MS/MS and HPLC
Figure S17 KYG-175 MS and HPLC
Figure S18 KYG-176 MS and HPLC

[Diagram showing mass spectrometry and high-performance liquid chromatography (HPLC) results for KYG-176.]
Figure S19 KYG-177 MS and HPLC
Figure S20 KYG-178 MS and HPLC
Figure S21 KYG-179 MS and HPLC
Figure S22 KYG-180 MS and HPLC
Figure S23 KYG-181 MS and HPLC
Figure S24 KYG-182 MS and HPLC
Figure S25 KYG-183 MS and HPLC
Figure S26 KYG-184 MS and HPLC
Figure S27 KYG-185 MS and HPLC
Figure S28 KYG-186 MS and HPLC
Figure S29 KYG-187 MS and HPLC
Figure S30 KYG-188 MS and HPLC
Figure S31 KYG-189 MS and HPLC
Figure S32 KYG-190 MS and HPLC

| % Composition | Minutes |
|---------------|---------|
| 0.00          | 2.00    |
| 0.02          | 4.00    |
| 0.04          | 6.00    |
| 0.06          | 8.00    |
| 0.08          | 10.00   |
| 0.10          | 12.00   |
| 0.12          | 14.00   |
| 0.14          | 16.00   |
| 0.16          | 18.00   |
| 0.18          | 20.00   |
| 0.20          |         |
| 0.22          |         |
| 0.24          |         |
| 0.26          |         |
| 0.28          |         |
| 0.30          |         |
| 0.32          |         |

m/z

- Cl
- OMe
- NHO
- F
- NO

Chemical structure of KYG-190.
Figure S33 KYG-191 MS and HPLC
Figure S35 KYG-193 MS and HPLC
Figure S36 KYG-194 MS and HPLC
Figure S37 KYG-195 MS and HPLC
Figure S38 KYG-196 MS and HPLC
Figure S39 KYG-197 MS and HPLC
Figure S40 KYG-198 MS and HPLC
Figure S41 KYG-199 MS and HPLC
Figure S42 KYG-200 MS and HPLC
Figure S43 KYG-201 MS and HPLC
Figure S44 KYG-202 MS and HPLC
Supplemental References

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