Cell Chemical Biology, Volume *24*

Supplemental Information

Rational Design of Selective Allosteric Inhibitors of PHGDH and Serine Synthesis with Anti-tumor Activity

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Supporting Information

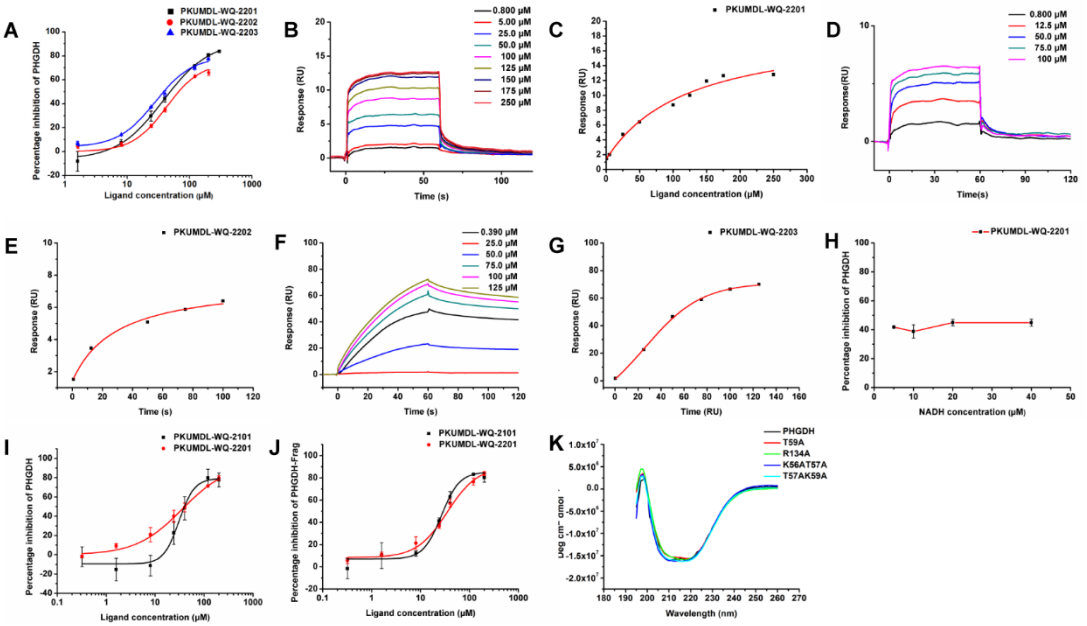


Figure S1, Related to Figure 1. Dose-response curves of the discovered inhibitors and CD spectra. (A) Dose-response curves for PKUMDL-WQ-2201, PKUMDL-WQ-2202, and PKUMDL-WQ-2203. Enzyme inhibition assay showed that the IC_{50} values for PKUMDL-WQ-2201, PKUMDL-WQ-2202, and PKUMDL-WQ-2203 were 35.7 ± 8.6, 42.0 ± 6.9, and 28.1 ± 1.3 μM, respectively. The curves were fitted by Hill equation and the results are expressed as the mean ± SD (n=3). (B-G) Compounds directly bound to PHGDH. B, D, F, SPR direct binding curves at the indicated concentrations of PKUMDL-WQ-2201, PKUMDL-WQ-2202 and PKUMDL-WQ-2203. C, E, G, The K_D values for PKUMDL-WQ-2201, PKUMDL-WQ-2202 and PKUMDL-WQ-2203 were 66.9 ± 1.9, 29 ± 11, and 7.22 ± 0.03 μM, respectively. The K_D values were simulated by using affinity fitting in Biacore T200 Evaluation Software. (H) Competitive assay of PKUMDL-WQ-2201 with the cofactor NADH. PKUMDL-WQ-2201 and the cofactor do not competitively bind to the same site. Increasing the NADH concentration did not led to a significant decrease of PKUMDL-WQ-2201 inhibition ability. (I-J) Dose-response curves of PKUMDL-WQ-2101 and PKUMDL-WQ-2201 for full length of PHGDH and PHGDH containg the substrate binding domain and the nucleotide binding domain. The IC_{50} values of PKUMDL-WQ-2101 and PKUMDL-WQ-2201 for full length of PHGDH were 34.8 ± 3.6 and 35.7 ± 8.6, respectively (I), while the IC_{50} values of PKUMDL-WQ-2101 and PKUMDL-WQ-2201 for PHGDH fragment were 30.1 ± 2.4 and 35.0 ± 6.9 μM, respectively (J). The curves were fitted by Hill equation and the results are expressed as the mean ± SD (n=3). (K) CD spectra of WT PHGDH and its mutants. Despite some mutations, all the mutants retained their secondary structures.

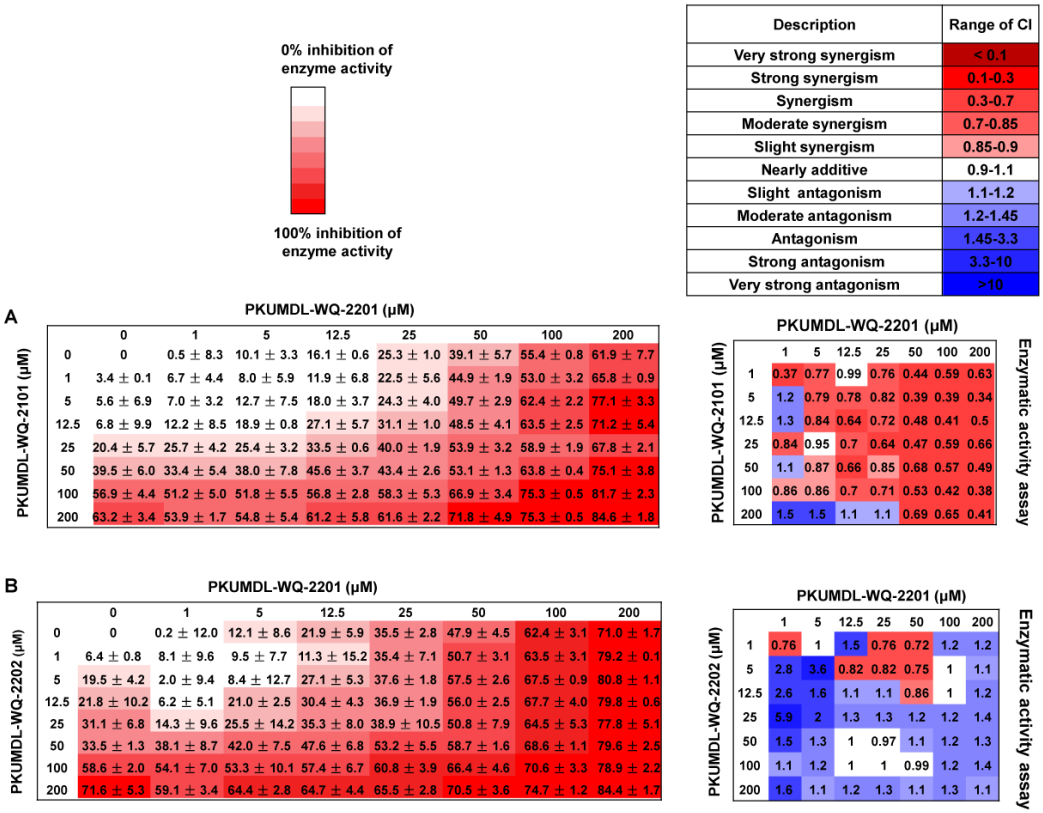


Figure S2, Related to Figure 1. Synergism between inhibitors binding in the different or the same site. (A) Percent PHGDH inhibition observed after treatment of PKUMDL-WQ-2101 + PKUMDL-WQ-2201 combinations in matrix format (Left). CI Values Calculated for PKUMDL-WQ-2101 + PKUMDL-WQ-2202 combinations with Combosyn Software (Right). (B) Percent PHGDH inhibition observed after treatment of PKUMDL-WQ-2201 + PKUMDL-WQ-2202 combinations in matrix format (Left). CI Values Calculated for PKUMDL-WQ-2201 + PKUMDL-WQ-2202 combinations with Combosyn Software (Right). Values are heat mapped with white equal to 0% inhibition of enzyme activity and red equal to 100% inhibition of enzyme activity. CI values are heat mapped with lowest values in red and highest values in blue. Error represents the SD of three replicates.

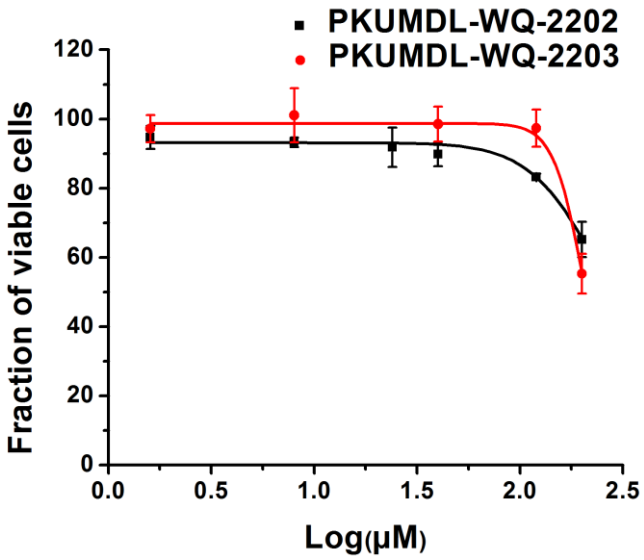


Figure S3, Related to Figure 2. MTT assays of PKUMDL-WQ-2202 and PKUMDL-WQ-2203 for MDA-MB-468 cells. EC_{50} values of PKUMDL-WQ-2202 and PKUMDL-WQ-2203 were all larger than 200 μM. The curves were fitted by Hill equation and the results are expressed as the mean ± SD (n=3).

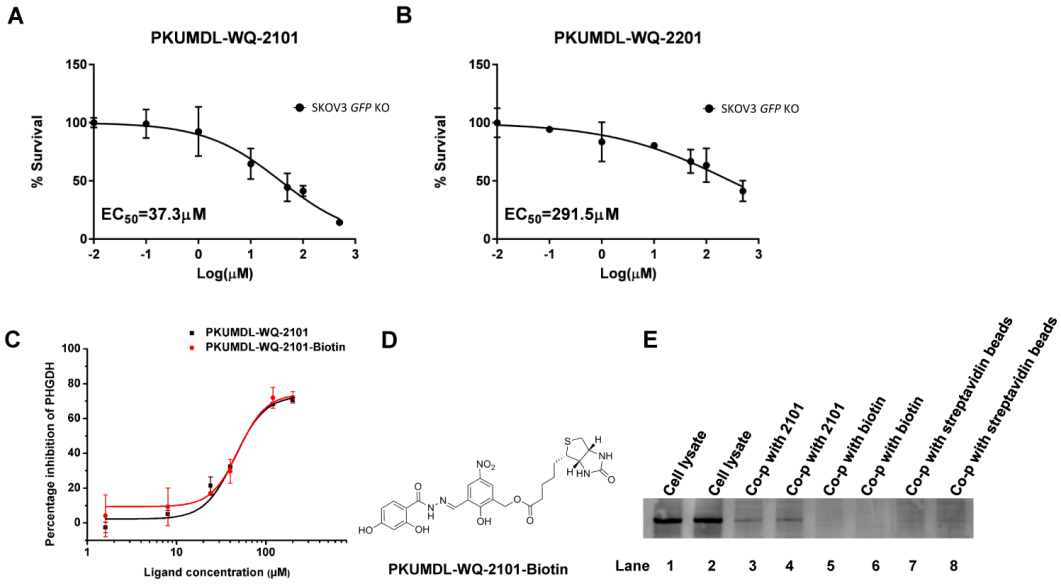


Figure S4, Related to Figure 3. PKUMDL-WQ-2101 and PKUMDL-WQ-2201 selectively bound in PHGDH in cells. (A) Dose response curve of SKOV3 *GFP* KO control cells treated with PKUMDL-WQ-2101 or (B) PKUMDL-WQ-2201 with 0-200μM concentrations for 24 hours. (C) Enzyme inhibition assay for biotinylated PKUMDL-WQ-2101. The IC_{50} values for PKUMDL-WQ-2101 and biotinylated PKUMDL-WQ-2101 were 44.9 ± 3.1 and 49.5 ± 6.1 μM, respectively. (D-E) Pull down assay of biotinylated PKUMDL-WQ-2101 targeting endogenous PHGDH in MDA-MB-468. (D) Structure of biotinylated PKUMDL-WQ-2101. (E) PHGDH was pulled down in MDA-MB-468 cell lysates by biotinylated PKUMDL-WQ-2101 and analyzed by western blot with anti-PHGDH (Lanes 3, 4). PHGDH endogenous expression quantity in 15 μl 2 mg/ml cell lysate supernatant (lanes 1, 2), and PHGDH pulled down by biotin (Lanes 5, 6) and free streptavidin beads (Lanes 7, 8) were also analyzed by western blot with anti-PHGDH and used as controls. Co-p, co-precipitate. Data represent the mean ± SEM independent experiments.

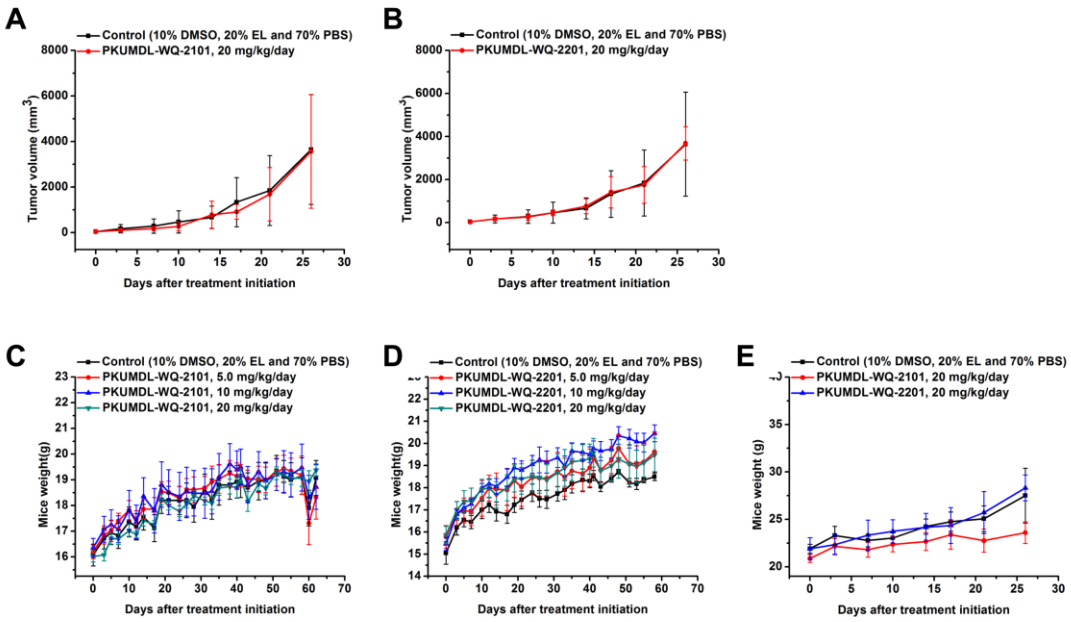


Figure S5, Related to Figure 5. PKUMDL-WQ-2101 and PKUMDL-WQ-2201 did not affect the tumor growth of MDA-MB-231 xenografts, and influence the weight of mice for both MDA-MB-468 and MDA-MB-231 xenografts. Compared with the control group, the growth of tumors were not influenced by the 26-days treatment of PKUMDL-WQ-2101 (A) and PKUMDL-WQ-2201 (B). The weight of mice in MDA-MB-468 xenografts were not influcenced by the 60-days treatment of PKUMDL-WQ-2101 (C) and PKUMDL-WQ-2201 (D). (E) The weight of mice in MDA-MB-231 xenografts were not influcenced by the 26-days treatment of PKUMDL-WQ-2101 and PKUMDL-WQ-2201. Data represent the mean ± SEM independent experiments.

Table S1, Related to Figure 1.

SPECS ID of Compounds.

Compound names used in the current study	SPECS ID
PKUMDL-WQ-2101	AG-205/06965007
PKUMDL-WQ-2201	AM-879/41890886
PKUMDL-WQ-2202	AN-465/14952021
PKUMDL-WQ-2203	AN-967/15490027

Supplemental Experimental Procedures

Chemicals

Compounds were purchased from SPECS. The purity of these compounds from SPECS database was more than 90% and for most compounds greater than 95% (confirmed by the supplier, using NMR, LC-MS, or both; data available through the website.; for our hits, high resolution mass spectra (HRMS) and 400M ^{1}H-NMR data for PKUMDL-WQ-2101, PKUMDL-WQ-2201, PKUMDL-WQ-2202 and PKUMDL-WQ-2203 were reconfirmed and provided in the SI). PKUMDL-WQ-2101 and PKUMDL-WQ-2201were re-synthesized in-house and confirmed by HRMS and 400M ^{1}H-NMR (synthetic methods were shown in Methods). Biotinylated PKUMDL-WQ-2101 was also synthesized in-house and confirmed by HRMS and 400M ^{1}H-NMR (synthetic methods were shown in Methods and validation spectra were shown in supplement figures).

Molecular cloning, protein expression and purification

The full-length *PHGDH* or *PSAT1* open reading frame (Seajet Scientific, Beijing, China) was amplified by polymerase chain reaction (PCR). The PCR fragments were ligated into the pET21a (+) vector, confirmed by DNA sequencing (Genewiz, Beijing, China), and transformed to the BL21 (DE3) strain of *Escherchia coli* (*E. coli*). Recombinant cell was cultivated at 37℃ until the OD_{600} reached 0.6-0.8. Then, PHGDH or PSAT1 expression was induced and the cells were grown for another 8 h at 25℃. Cells were harvested by centrifugation (6000 rpm, 15 min) and broken by sonication. Insoluble material was separated by centrifugation (17000 rpm, 30 min) and the supernatant was purified using a nickel-nitrilotriacetic column (HisTrap HP; GE Healthcare) and then a gel-filtration column (Sephacryl S-200 HR, GE Healthcare). The final purity of the protein was >95% as judged by SDS−PAGE. Protein concentrations were measured via Nanodrop 2000 (Thermo Scientific, USA).

CRISPR-Cas9 sgRNA design

To design specific sgRNAs to target *PHGDH*, an online tool called E-CRISP (Heigwer et al., 2014) software database e-crisp.org was used. Target sequences complementary to the gRNA, followed by a PAM sequence of NGG, were generated which is required for double stranded DNA cuts by Cas9 nuclease. sgRNAs were ranked by off-target effects and target-site homology using the BowTie2 alignment program. Outputs of sgRNAs specific to the *PHGDH* genomic sequence were generated with an average specificity score, annotation score, and efficacy score. This resulted in 5 sgRNAs with a score of 100% across all scores. One sgRNA (GCTCTGAGCCTCCTTGGTGC) efficiently knocked out *PHGDH* and used for this study.

Cell transduction and puromycin selection

The day before transduction, SKOV3 cells were plated in 3 x 10cm^2 tissue culture dishes. The day of transduction, 4 mL of DMEM supplemented with 10% FBS, 100U/ml penicillin and 100mg/ml streptomycin was added to one dish, while 4mL of LentiCRISPR-PHGDH or LentiCRISPR-EGFP were added to the other dishes of SKOV3 cells. Next, 2.5 μl of 1 mg/ml polybrene transfection reagent (Millipore) was added to each plate. Viraly infected SKOV3 cells and control plate were incubated at 37˚C. After 24 hours, media from each plate was aspirated and fresh RPMI-1640 (sigma), 10% FBS, 100U/ml penicillin, 100mg/ml streptomycin with 2 μg/ml puromycin was added for 3 days. Virally infected LentiCRISPR-PHGDH and LentiCRISPR-EGFP plates were compared to the non-transduced plate to ensure complete cell death of those cells. To ensure complete gene knockout of transduced cell lines, cell cloning by serial dilution was performed using a 96-well plate on puromycin-resistant cells. After 4 days, wells were checked for single cells. Those wells containing single cells were allowed to reach confluency for 3 weeks and subsequently seeded in 6-well then 10cm^2 plates.

Enzyme assay

Due to the unavailability of PHGDH direct-substrate phosphohydroxypyruvate (PHP), the enzyme activity of PHGDH was measured accompanied with the upstream of PSAT1 catalytic reaction. The activity of recombinant PHGDH was measured by monitoring the reduced nicotinamideadenine dinucleotide (NADH) to nicotinamideadenine dinucleotide (NAD^+) change in fluorescence emission at 456 nm

(excitation at 338 nm).

To evaluate the effects of compounds on PHGDH activity, compounds were first pre-incubated with enzyme samples in the assay buffer (25 mM HEPES, pH 7.1, 400 mM KCl, 5 μM phosphopyridoxa (PLP), 0.5 mM α-ketoglutarate, 150 μM NADH, 1 mM DTT) for 10 min at 25℃, then the reaction was started by adding L-phospho-O-serine (Pser). Each compound was dissolved in DMSO at a final concentration of 5%, which did not affect the assay signal. Fluorescence signals were recorded for 3 min with a kinetics mode program using on a plate reader (Synergy, Biotek). IC_{50} values were obtained by fitting the data to a three-parameter Hill model of the graph of log dose against percentage inhibition from at least three sets of experiments. Percentages of inhibition were calculated according to the following equation:

$$\frac{Vo - Vi}{Vo - Vn}$$

in which Vo and Vi represent the maximum reaction rate of the enzyme incubated without or with compounds, and Vn represents the maximum degradation rate of NADH.

Surface Plasmon Resonance (SPR) experiments

The binding affinities of compounds towards PHGDH were assayed using the SPR-based Biacore T200 instrument (GE Healthcare). PHGDH was immobilized on a CM5 sensor chip by using standard amine-coupling at 25℃ with running buffer PBS-P (20 mM phosphate buffer, 2.7 mM NaCl, 137 mM KCl, 0.05% surfactant P-20, pH 7.4), respectively, as described previously. A reference flow cell was activated and blocked in the absence of PHGDH. In the direct binding experiments between PHGDH and compounds, PHGDH immobilization level was fixed at 800 response units (RU), and then different concentrations of compounds containing 5% DMSO were serially injected into the channel to evaluate binding affinity. Regeneration was achieved by extended washing with the running buffer after each sample injection. The equilibrium dissociation constants (K_D) of the compounds were obtained by fitting the data sets to 1:1 Langmuir binding model using Biacore T200 Evaluation Software.

Circular dichroism

WT PHGDH and its mutants were dissolved to a final concentration of 0.2 mg/ml in PBS buffer (20 mM phosphate buffer, 2.7 mM NaCl, 137 mM KCl, pH 7.4). CD spectra was recorded using 1-mm quartz cuvettes for the far ultraviolet region (190-260 nm) on a MOS 450 AF/CD (Biologic, France) at 25℃. The spectra were corrected by subtracting a buffer blank and were averaged over three accumulations and smoothed by standard noise reduction provided with the instrument.

Pull down assays

Biotinylated PKUMDL-WQ-2101 targeting endogenous PHGDH in MDA-MB-468 cells was performed using M-280 streptavidin Dynabeads (Invitrogen). As described in the protocol of manufacturer, 100 μl suspension of beads was first washed three times with 100 μl PBS buffer (20 mM phosphate buffer, 2.7 mM NaCl, 137 mM KCl, pH 7.4); 100 μl 60 μM biotinylated PKUMDL-WQ-2101 (5% DMSO) or biotin (5% DMSO) was then added to the beads, gently rotated at 25℃ for 1h, washed three times with 200 μl PBST buffer (PBS buffer, 0.5% Tween 20), blotted with 100 μl 5% BSA buffer (final concentration 1 mg/ml) and washed three times with 200 μl PBST buffer; MDA-MB-468 cells were lysed according to the instruction of NP40 (Invitrogen), and 200 μl 20 mg/ml cell lysate supernatant was then added to free streptavidin beads, biotin immobolized beads and PKUMDL-WQ-2101-biotin immobolized beads, respectively, gently rotated at 25℃ for 1h. Another 80 μl 2 mg/ml cell lysate supernatant was directly mixed with 20 μl 5x SDS loading buffer, boiled at 95℃for 10 min and collected for further western blot analysis; after three times wash with PBST buffer, the beads were finally resuspended in 80 μl washing buffer and 20 μl 5x SDS loading buffer, followed by heating at 80℃for 10 min.

Proteins were resolved by 12% SDS/PAGE and transferred on to polyvinylidenedifluoride(PVDF) membranes (Millipore) for western blot analysis. The membranes were then blocked with 30 ml TBST buffer (136.9 mM NaCl, 2.68 mM KCl, 24.8 mM Tris (pH 7.4) with HCl, and 0.1% Tween 20) contained 5% dry milk while being gently shaken at 25℃for 1h. After blocking, the membranes were incubated with a 1:500 dilution of mouse monoclonal PHGDH antibody (Santa Cruz) and then 1:500 dilution of horseradish

peroxidase-conjugated goat anti-mouse IgG antibody (Santa Cruz). PHGDH was detected by chemiluminescence (Vigorous Biotechnology).

Two biological replicate samples were generated and analyzed for PKUMDL-WQ-2101 and cell lysate supernatant.

Metabolite extraction

For culture from adherent SKOV3 cells, media was quickly aspirated. Next, 1mL of extraction solvent (80% methanol/water) cooled to -80˚C was added immediately to each well and the dishes were then transferred to -80˚C for 15 min. After, the plates were removed and cells were scraped into the extraction solvent on dry ice. All metabolite extractions were centrifuged at 20,000*g* at 4˚C for 10 min. Finally, the solvent in each sample was evaporated using a speed vacuum for metabolite analysis. For polar metabolite analysis, the cell metabolite extract was dissolved in 15 µl methanol/acetonitrile (1:1 *v/v*) (LC-MS optima grade, Thermo Scientific). Samples were centrifuged at 20,000*g* for 10 min at 4˚C and the supernatants were transferred to Liquid Chromatography (LC) vials. The injection volume for polar metabolite analysis was 2 µL.

Liquid chromatography

An Xbridge amide column (100 x 2.1mm i.d., 3.5 µm; Waters) is employed on a Dionex (Ultimate 3000 UHPLC) for compound separation at room temperature. Mobile phase A is 5mM ammonium acetate and, pH 6.0, and mobile phase B is 100% acetonitrile. The gradient is linear as follows: 0 min, 85% B; 1.5 min, 85% B; 5.5 min, 35% B; 10 min, 35% B; 10.5 min, 35% B; 14.5 min, 35% B; 15 min, 85% B; and 20 min, 85% B. The flow rate was 0.15 mL/min from 0 to 10 min and 15 to 20 min, and 0.3 mL/min from 10.5 to 14.5 min. All solvents are LC-MS grade and purchased from Fisher Scientific.

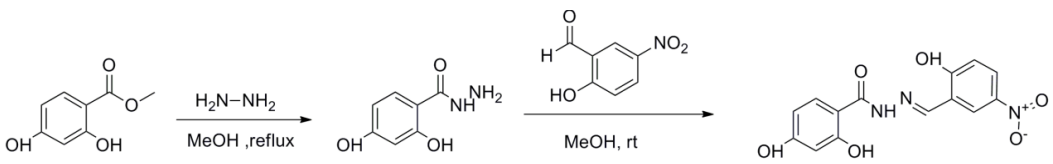
Mass spectrometry

The Q ExactivePlus MS (Thermo Scientific) is equipped with a heated electrospray ionization probe (HESI) and the relevant parameters are as listed: evaporation temperature, 120˚C; sheath gas, 30; auxillary gas, 10; sweep gas, 3; spray voltage, 3.6kV for positive mode and 2.5kV for negative mode. Capillary temperature was set at 320˚C, and S lens was 55. A full scan range from 60 to 900 (m/z) was used. The resolution was set at 70,000. The maximum injection time was 200 ms. Automated gain control (AGC) was targeted at 3,000,000 ions.

Compound characterization data

1H and 13C NMR spectra were recorded on a Bruker Ascend 400 MHz spectrometer. The chemical shift values (δ) are reported in ppm relative to tetramethylsilane as internal standard. 1H NMR spectra are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constant (J values) in Hz and integration. High resolution mass spectra were recorded on a Bruker Apex IV FTMS mass spectrometer using ESI (electrospray ionization). Toluene was distilled from Na0 and benzophenone. Dichloromethane were dried over 4Å molecular sieves. All other reagents were purchased from commercial sources and used as received.

Synthesis of PKUMDL-WQ-2101

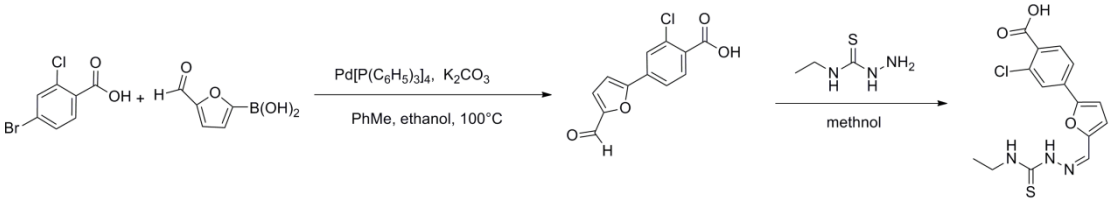


2,4-dihydroxybenzohydrazide. Hydrazine hydrate85% (2.031g, 34.5 mmol) was added to a solution of methyl 2,4-dihydroxybenzoate (1.781g, 11.5 mmol) in methanol (50 ml) and the mixture was stirred and refluxed overnight. The solvent evaporated under reduce pressure and the resulting residual was recrystallised from methanol to give the title compounds as a white solid （1.546g, 80%）.1H-NMR (400

MHz, DMSO): 6.35 (2H, m), 7.77 (1H, d, J=9.20 Hz), 10.06 (1H, s), 10.69 (1H, s), 11.86 (1H, s).

(E)-2,4-dihydroxy-N'-(2-hydroxy-5-nitrobenzylidene)benzohydrazide. A mixture containing 2,4-dihydroxybenzohydrazide (1.681g, 10.0 mmol) and 2-hydroxy-5-nitrobenzaldehyde (1.670g, 10.0 mmol) was stirred at rt in methanol (50ml) for 7 h. The solvent was eliminated in vacuo and the resulting residual was recrystallised from methanol to give the title compound as an orange solid (2.378g, 75%).1H-NMR(DMSO): 6.33 (1H, d, J=1.80 Hz), 6.39 (1H, dd, J=1.98, 8.92 Hz), 7.12 (1H, d, J=8.99 Hz), 7.81 (1H, d, J=8.75 Hz), 8.18 (1H, dd, J=2.64, 9.20 Hz), 8.57 (1H, d, J=2.57 Hz), 8.73 (1H, s), 10.26 (1H, s), 12.02 (1H, s), 12.15 (1H, s), 12.32 (1H, s). HRMS (ESI): calcd for C14H12N3O6, [$(M+H)^+$], 318.07261, found 318.07239.

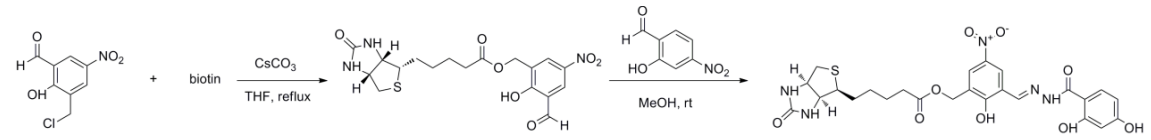
Synthesis of PKUMDL-WQ-2201



2-chloro-4-(5-formylfuran-2-yl)benzoic acid. To a solution of 5-formyl-2-furanboronic acid (0.524g, 2.23 mmol) in a mixture (7/3) of dry toluene and ethanol (10 mL), 4-bromo-2-chlorobenzoic acid(0.312g, 2.23 mmol), Pd(PPh3)4 (0.057g, 0.223 mmol) and K2CO3 (0.771g, 5.58 mmol) was added(Kong et al., 2012). The mixture was stirred and heated at 100 ℃ under argon protection. After the reaction was completed monitored by TLC, the mixture was cooled to room temperature and 100 ml 2N HCl was added. The organic solvents were removed under vacuum, and the residue was extracted with EtOAc (3 × 100 mL) and washed with brine. The organic phase was finally dried over Na2SO4 and evaporated to dryness to give a crude compound that was purified through flash chromatography to afford the product as an orange solid(0.251 g, 45%). 1H-NMR (400 MHz, DMSO): 7.55 (1H, d, J=3.76 Hz), 7.70 (1H, d, J=3.76 Hz), 7.92 (2H, m), 8.05 (1H, d, J=1.28 Hz), 9.67 (1H, s).

(Z)-2-chloro-4-(5-((2-(ethylcarbamothioyl)hydrazono)methyl)furan-2-yl)benzoic acid. A mixture containing2-chloro-4-(5-formylfuran-2-yl)benzoic acid (0.100g, 0.40 mmol) and N-ethylhydrazinecarbothioamide (0.048g, 0.40 mmol) was stirred at rt in methanol (20ml) for 7 h. The solvent was eliminated in vacuum and the resulting residual was recrystallised from methanol to give the title compound as an orange solid (0.126g, 90%).1H-NMR(DMSO): 1.18 (3H, t, J=7.08 Hz), 3.62 (2H, m, J=6.81 Hz), 7.13 (1H, d, J=3.65 Hz), 7.39 (1H, d, J=3.60 Hz), 7.87 (2H, q, J=9.09 Hz), 7.98 (1H, s), 8.01 (1H, s), 8.39 (1H, t, J=5.85 Hz), 11.54 (1H, s), 13.40 (1H, s). HRMS (ESI): calcd for C15H15ClN3O3S, [$(M+H)^+$], 352.05226, found 352.05098

Synthesis of biotinylated PKUMDL-WQ-2101



3-formyl-2-hydroxy-5-nitrobenzyl 5-((3aS, 4S, 6aR)-2-oxohexahydro-1H-thieno [3, 4-d] imidazol-4-yl) pentanoate. A mixture of biotin (0.098 g, 0.40 mmol), THF (10 ml) and CsCO3 (0.086 g, 0.40 mmol) was heated to reflux for 30 min, followed by the addition of3-(chloromethyl)-2-hydroxy-5-nitrobenzaldehyde (0.055 g, 0.40 mmol). The mixture was keep heated to reflux for additional 4 h. After reaction has been completed, the solvent was eliminated in vacuum and the resulting residual was recrystallised from methanol/water to give the title compound as a white solid （0.063 g, 45%.1H-NMR (400 MHz, DMSO):1.56 (6H, m), 2.27 (1.9H, t, J=7.32 Hz), 2.90 (2H, m), 3.24 (2H, m), 4.26 (1.0H, t, J=5.46 Hz), 4.48 (3H, m), 7.34 (1H, s),8.54 (1.9H, dd, J=2.76, 16.61 Hz), 10.34 (1H, s), 12.08 (1H, s). 3-((E)-(2-(2, 4-dihydroxybenzoyl)hydrazono)methyl)-2-hydroxy-5-nitrobenzyl 5-((3aS, 4S, 6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate. A mixture containing 4-chloro-2-formyl-3-hydroxy-6-nitrobenzyl 5 - ((3aS, 4S, 6aR)-2-oxohexahydro-1H-thieno- [3,

4-d]imidazol-4-yl)pentanoate (0.037 g, 0.09 mmol) and 2-hydroxy-4-nitrobenzaldehyde (0.014 g, 0.09 mmol) was stirred at rt in methanol (10ml) for 7 h. The resulting orange precipitate was filtered washed with additional methanol and dried to afford the final product as an orange solid(0.021 g, 41%).1H-NMR (400 MHz, DMSO): 1.52 (6H, m), 2.22 (2H, t, J=7.34 Hz), 2.77 (1H, q, J=6.07 Hz), 2.86 (1H, d, J=12.44 Hz), 3.18 (2H, m), 4.17 (1H, t, J=5.29 Hz), 4.42 (3H, m), 6.36 (1H, d, J=1.92 Hz), 6.41 (1H, dd, J=1.82, 9.22 Hz), 6.98 (1H, s), 7.81 (1H, d, J=8.64 Hz), 8.18 (1H, d, J=2.44 Hz), 8.48 (1H, d, J=2.44 Hz), 10.34 (1H, s), 12.00 (1H, s), 12.24 (1H, s), 13.36 (1H, s). HRMS (ESI): calcd for C25H28N5O9S, [$(M+H)^+$], 574.16077, found 574.16079.

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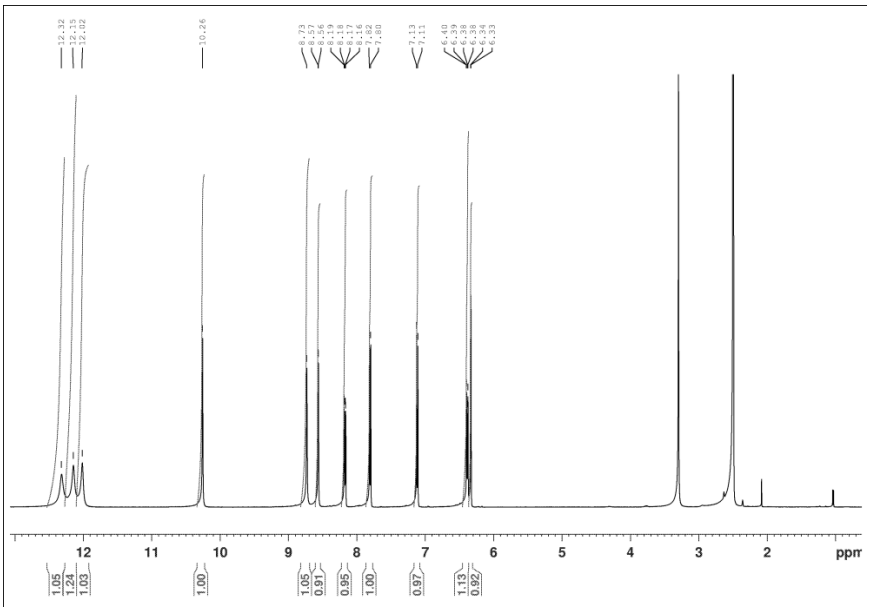
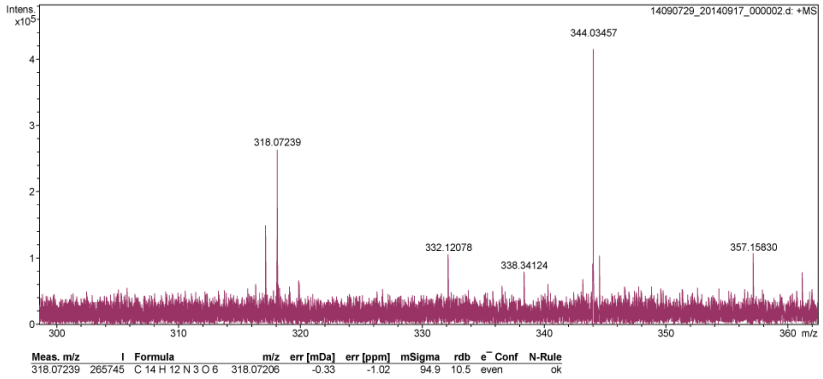
Kong, X., Qin, J., Li, Z., Vultur, A., Tong, L., Feng, E., Rajan, G., Liu, S., Lu, J., Liang, Z., et al. (2012). Development of a novel class of B-RafV600E-selective inhibitors through virtual screening and hierarchical hit optimization. Org. Biomol. Chem. *10*, 7402-7417.

Supplemental ^{1}H NMR and HRMS Spectra

^{1}H NMR spectra of PKUMDL-WQ-2101 (Solvent:DMSO)

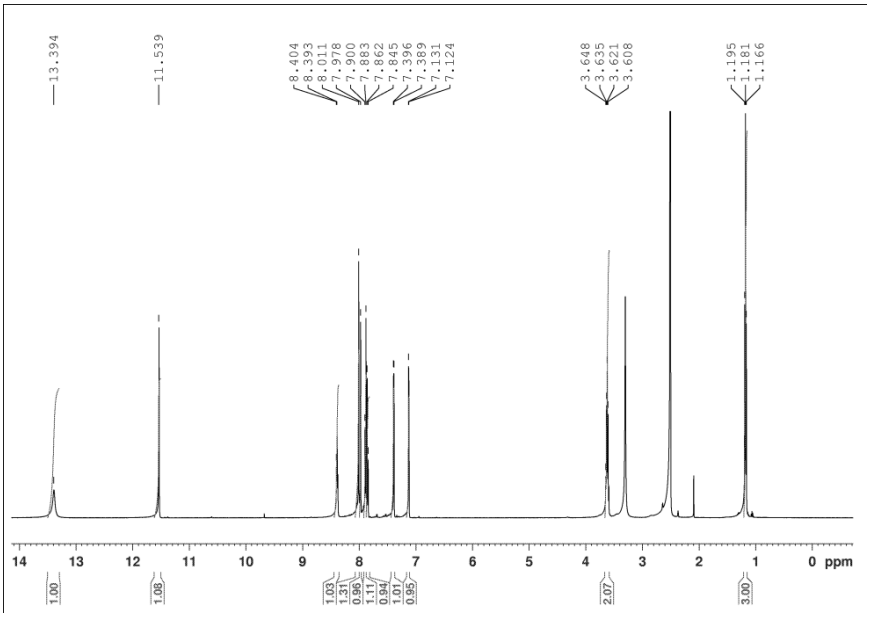


HRMS of PKUMDL-WQ-2101

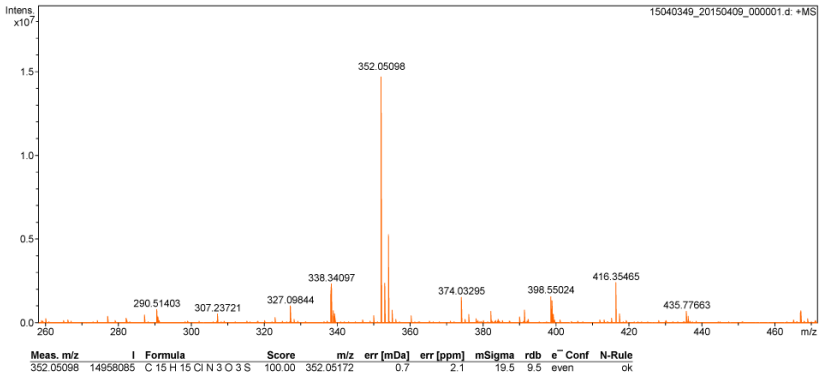


Meas. m/z	I	Formula	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
318.07239	265745	C 14 H 12 N 3 O 6	318.07206	-0.33	-1.02	94.9	10.5	even	ok

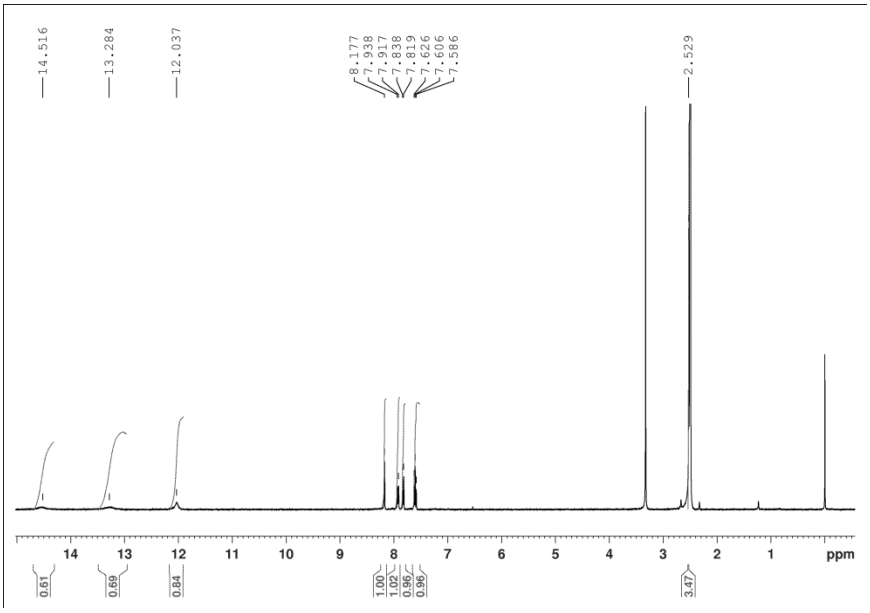
^{1}H NMR spectra of PKUMDL-WQ-2201 (Solvent:DMSO)



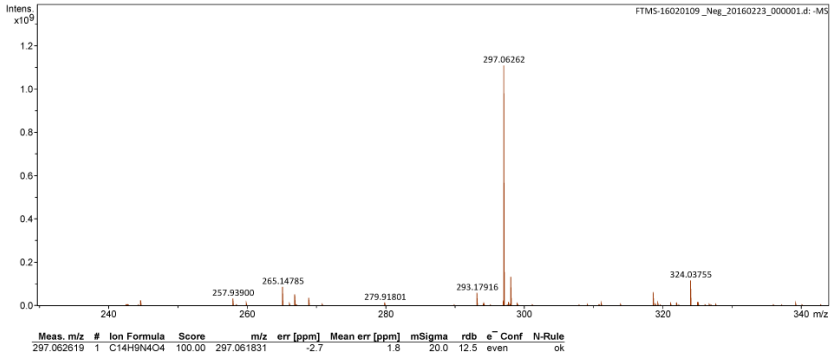
HRMS of PKUMDL-WQ-2201



^{1}H NMR spectra of PKUMDL-WQ-2202 (Solvent:DMSO)

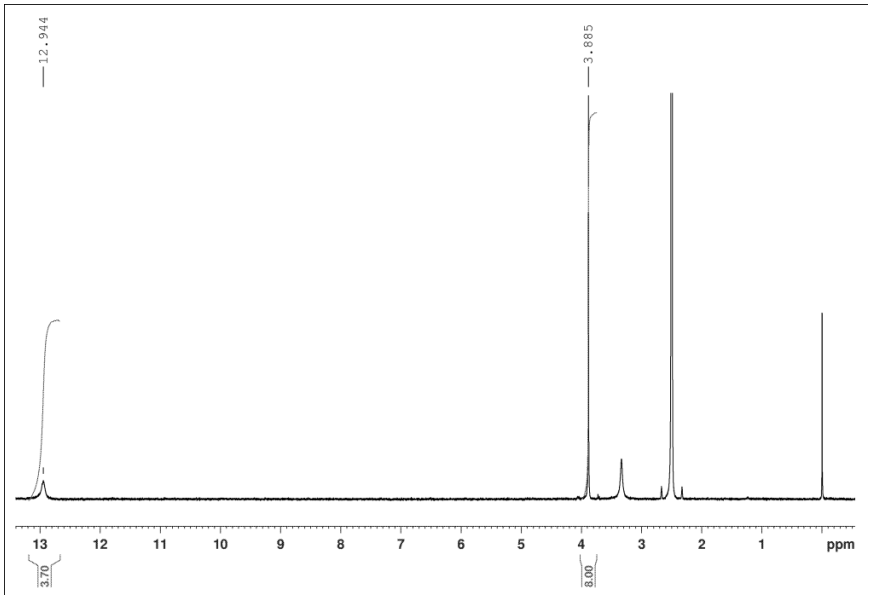


HRMS of PKUMDL-WQ-2202

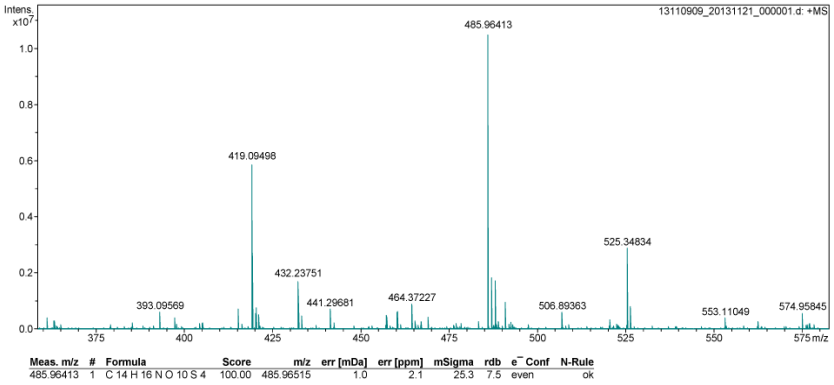


Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
297.062619	1	C14H9N4O4	100.00	297.061831	-2.7	1.8	20.0	12.5	even	ok

^{1}H NMR spectra of PKUMDL-WQ-2203 (Solvent:DMSO)

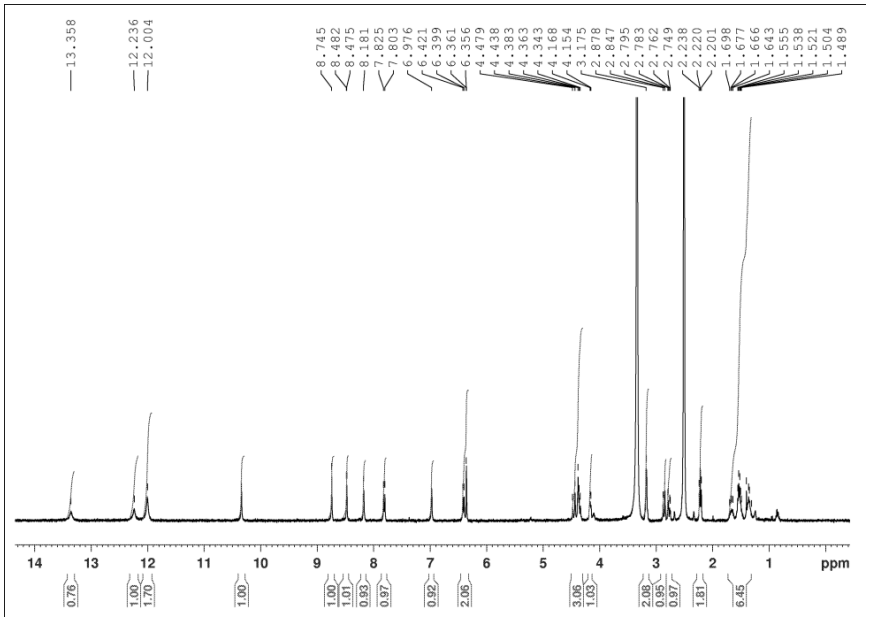


HRMS of PKUMDL-WQ-2203

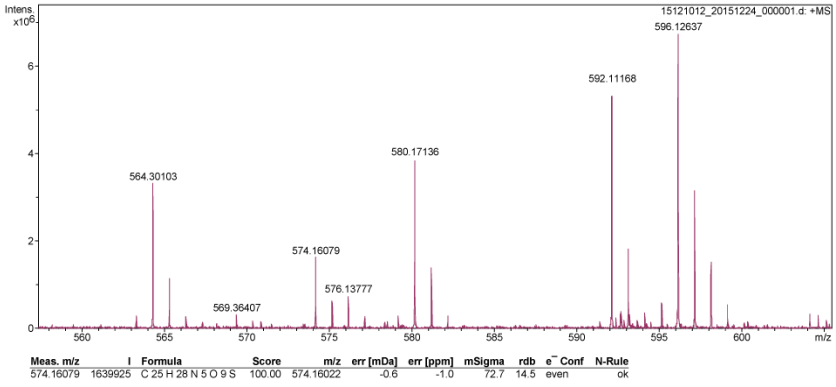


Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
485.96413	1	C 14 H 16 N O 10 S 4	100.00	485.96515	1.0	2.1	25.3	7.5	even	ok

^{1}H NMR spectra of biotinylated PKUMDL-WQ-2201 (Solvent:DMSO)



HRMS of biotinylated PKUMDL-WQ-2201



Meas. m/z	I	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
574.16079	1639925	C 25 H 28 N 5 O 9 S	100.00	574.16022	-0.6	-1.0	72.7	14.5	even	ok