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Supplementary Information

Design, synthesis, and biological evaluation of a ^{99m}Tc-labeled small-molecule tracer for PD-L1 imaging

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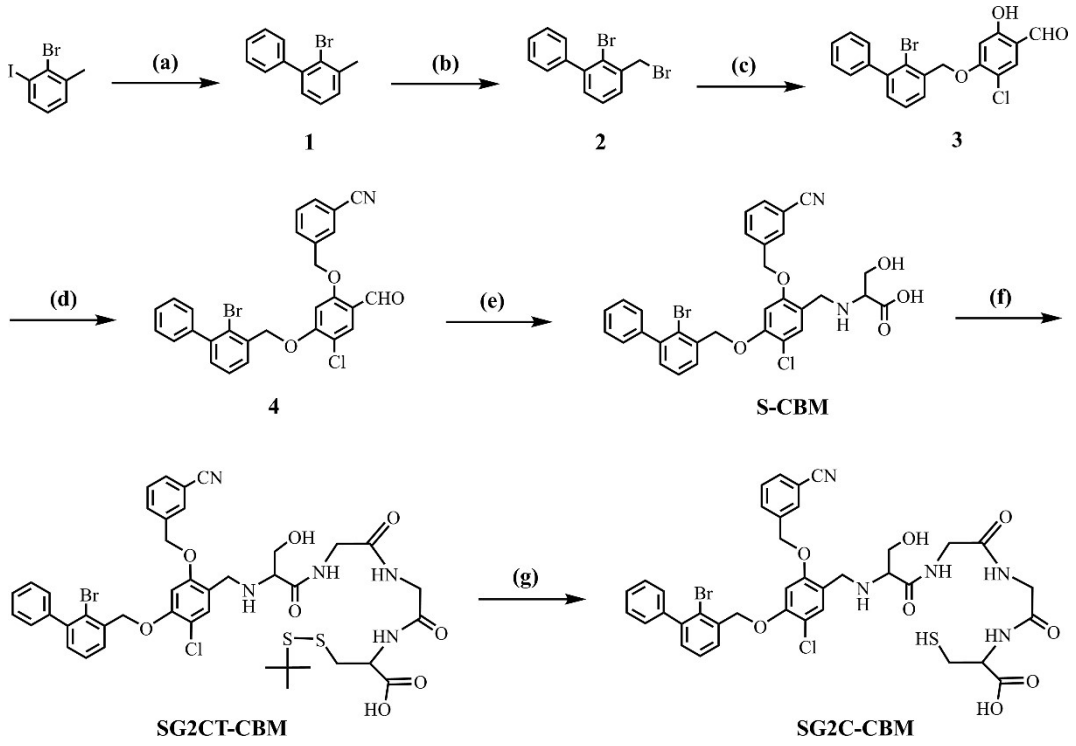
Synthesis of the precursor SG2C-CBM



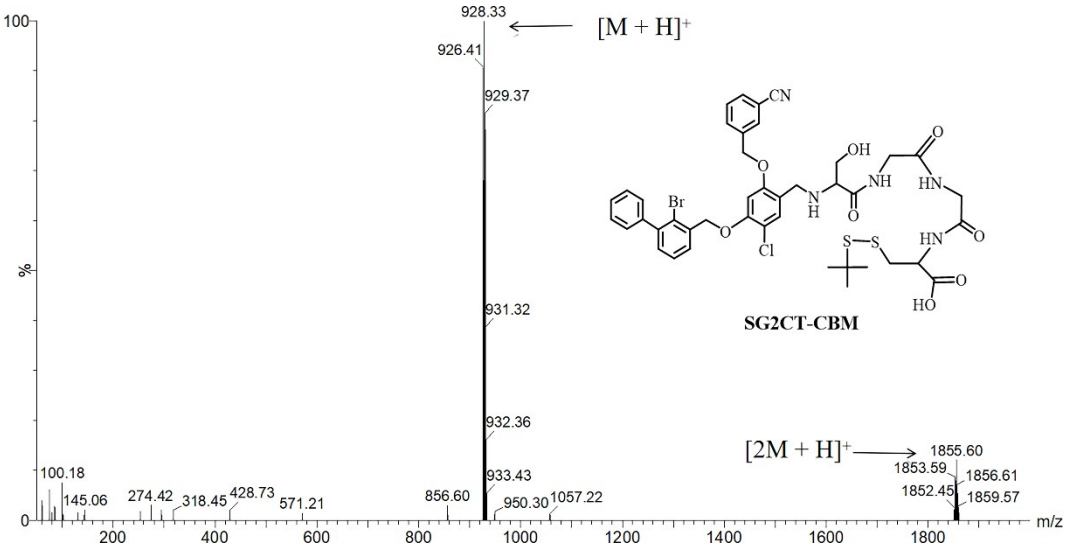
Scheme S1 Synthesis of the precursor SG2C-CBM.

Detailed synthesis procedure of S-CBM has been reported in our previous work (Bioorg Med Chem Lett, 96(2023), 129496. DOI:10.1016/j.bmcl.2023.129496).

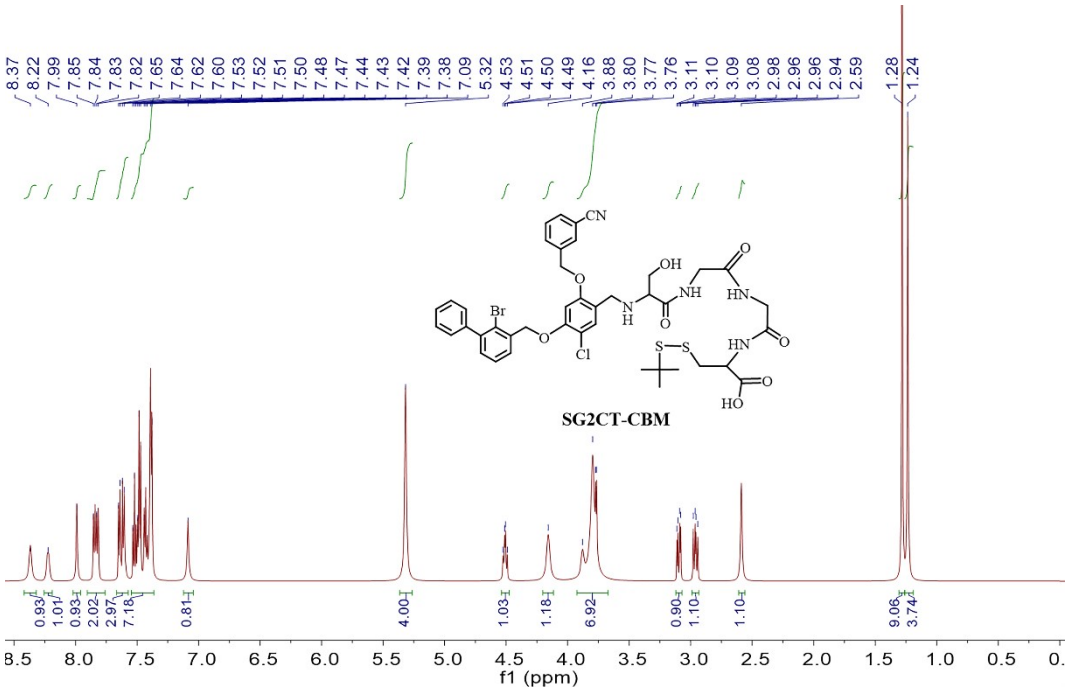
Synthesis of N-[2-(3-cyanobenzene-1-methyleneoxide)-4-(2-bromo-3-phenylbenzyloxy)-5-chlorobenzyl]-N-sericylglycylglycyl (S-tert-butylthio) cysteine (SG2CT-CBM):

The solid phase polypeptide synthesis (SPPS) method was selected to prepare the compound SG2CT-CBM. First of all, resin (450 mg), Fmoc-Cys(StBu)-OH (142 mg, 0.33mmol) and 200 μL *N*, *N*-Diisopropylethylamine (DIPEA) were dissolved in DMF (15 mL), stirred at room temperature for 60 min under a nitrogen atmosphere. After the solvent was removed, 40% methanol solution (6 mL methanol and 9 mL DMF) and 20% piperidine solution (3 mL piperidine and 12 mL DMF) were used to seal the excess resin and remove the Fmoc-protect group of the Fmoc-Cys(StBu)-OH, respectively. Repeating the deprotection step, Fmoc-Gly-Gly-OH (96 mg, 0.27 mmol) and Compound S-CBM (167 mg, 0.27 mmol) were successively connected. A cutting solution (100 mL) containing 1 mL trifluoroacetic acid (TFA) and 99 mL

CH_2Cl_2 was prepared to remove the tripeptide from the resin, and the cutting liquid was collected in a flask after nitrogen bobbling for 1 min × 5. Through rotary evaporation, recrystallization and centrifugation, a white solid (160 mg, 64%) of compound SG2CT-CBM was harvested. ESI-MS (m/z): 928 $[M + H]^+$. ^1H NMR (400 MHz, DMSO-D_6) δ 8.37 (s, 1H), 8.22 (s, 1H), 7.97 (s, 1H), 7.83 (q, J = 4 Hz, 2H), 7.67-7.57 (m, 3H), 7.55-7.36 (m, 7H), 7.09 (s, 1H), 5.32 (s, 4H), 4.51 (q, J = 4 Hz, 1H), 4.16 (s, 1H), 3.93-3.68 (m, 7H), 3.10 (q, J = 4 Hz, 1H), 2.96 (q, J = 4 Hz, 1H), 2.59 (s, 1H), 1.27 (s, 9H), 1.24 (s, 4H).



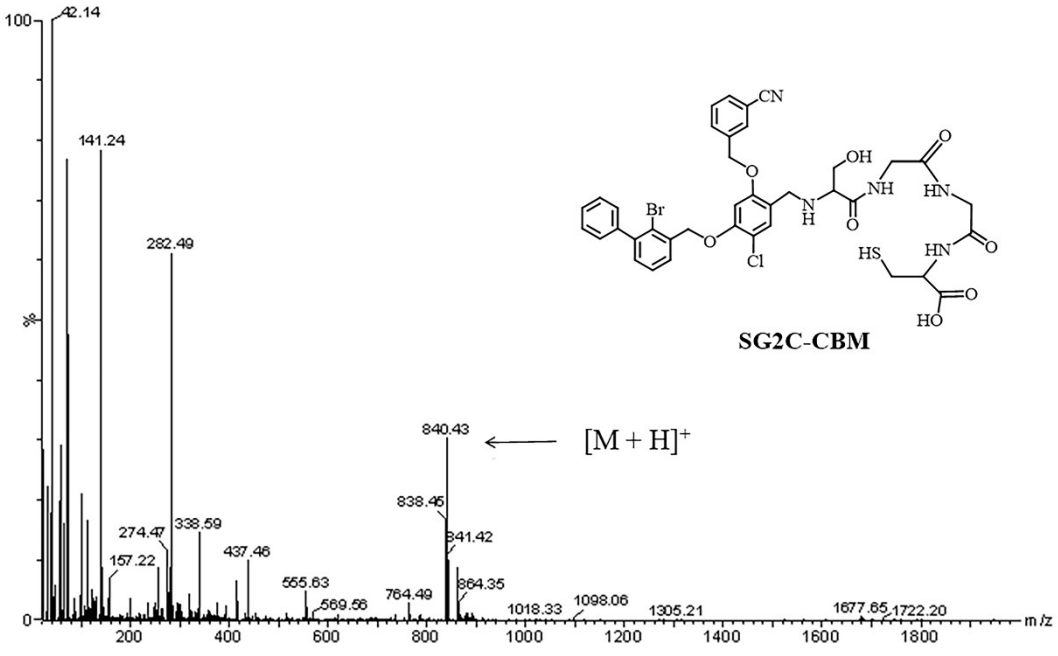
Supplementary Figure. 1 The ESI-MS of compound SG2CT-CBM.



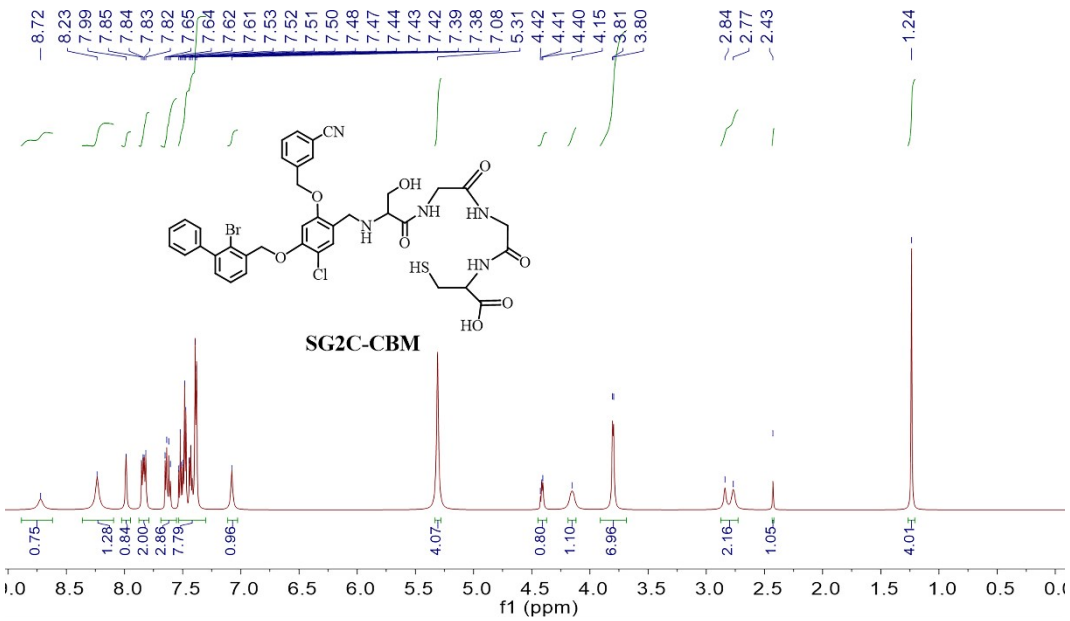
Supplementary Figure. 2 ^{1}H NMR spectrum of compound SG2CT-CBM in DMSO-D_6.

Synthesis of N-[2-(3-cyanobenzene-1-methyleneoxide)-4-(2-bromo-3-phenylbenzyloxy)-5-chlorobenzyl]-N-sericylglycylglycylcysteine (SG2C-CBM):

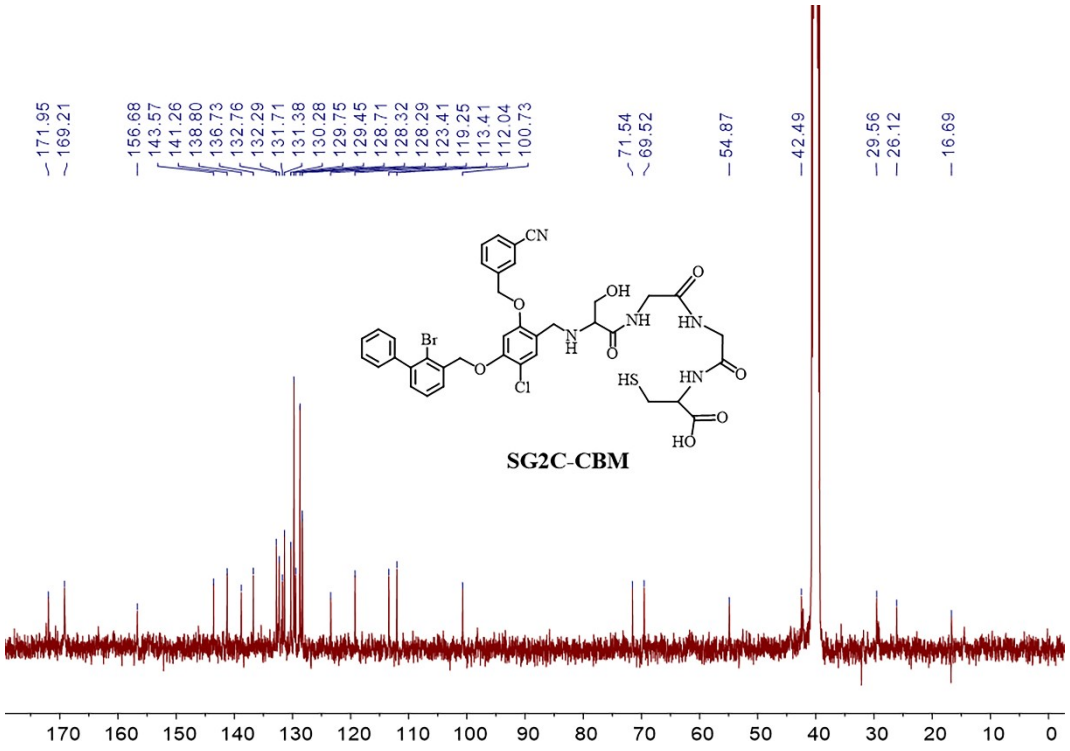
Tri(2-carboxyethyl)phosphine (TCEP) (12.5 mg/mL, 4 mL, 0.175 mmol), a deprotection agent, was added into a solution of compound SG2CT-CBM (30 mg, 0.032 mmol) dissolved in DMF (6 mL) and kept at room temperature for 30 min. Eventually, the pure SG2C-CBM (10 mg, 37.25%) was obtained after purification by semi-preparative HPLC. ESI-MS (m/z): 840 $[M + H]^+$. ^{1}H NMR (400 MHz, DMSO-D_6) δ 8.72 (s, 1H), 8.23 (s, 1H), 7.99 (s, 1H), 7.88-7.79 (m, 2H), 7.69-7.55 (m, 3H), 7.53-7.30 (m, 8H), 7.08 (s, 1H), 5.31 (s, 4H), 4.41 (t, J = 4 Hz, 1H), 4.15 (s, 1H), 3.81 (d, J = 4 Hz, 7H), 2.81 (d, J = 28 Hz, 2H), 2.43 (s, 1H), 1.24 (s, 4H). ^{13}C NMR (101 MHz, DMSO-D_6) δ 171.95, 169.21, 156.68, 143.57, 141.26, 138.80, 136.73, 132.76, 132.29, 131.71, 131.38, 130.28, 129.75, 129.45, 128.71, 128.32, 128.29, 123.41, 119.25, 113.41, 112.04, 100.73, 71.54, 69.52, 54.87, 42.49, 29.56, 26.12, 16.69.



Supplementary Figure. 3 The ESI-MS of compound SG2C-CBM.



Supplementary Figure. 4 ^{1}H NMR spectrum of compound SG2C-CBM in DMSO-D_6.



Supplementary Figure. 5 ^{13}C NMR spectrum of compound SG2C-CBM in DMSO-D_6.

HPLC analysis:

Waters liquid chromatography system consists of a 1525 binary HPLC pump and a Radiomatic 610TR detector. A reversed-phase C18 column (5 mm, 4.6 × 250 mm, DiKMA, Japan) was employed, and the mobile phase of HPLC is composed of deionized water (A) and acetonitrile (B) with 0.1% TFA. The flow rate of the mobile

phase was kept at 1.0 mL/min, and the detection method is shown below.

Table S1 HPLC condition for analysis of compounds.

Time (min)	Flow (mL/min)	H_2O% (0.1% TFA)	CH_3CN% (0.1% TFA)
0	1	60	40
1	1	60	40
25	1	10	90
30	1	10	90
32	1	60	40
35	1	60	40

Table S2 Radio-HPLC condition for analysis of compounds.

Time (min)	Flow (mL/min)	H_2O% (0.1% TFA)	CH_3CN% (0.1% TFA)
0	1	90	10
2	1	90	10
3	1	50	50
15	1	10	90
18	1	90	10
20	1	90	10

Pharmacokinetics parameters:

Table S3 Parameters of [^{99m}Tc]Tc-SG2C-CBM injected in A375-hPD-L1 tumor-bearing female BALB/c nude mice within 180 min.

Parameters	Value
$t_{1/2}\alpha$ (min)	26.452
$t_{1/2}\beta$ (min)	26.457
Tmax (min)	5
CL (%ID/g/min)	0.06
Vz (L/%ID)	2.646
AUC(0-∞) (%ID/g *min)	382.79