Supporting Information

Synthesis and biological evaluation of novel ^{99m}Tc-labeled palbociclib derivatives targeting cyclin-dependent kinase 4/6 (CDK4/6) as potential cancer imaging agents

Xiaoqing Song†, Qianqian Gan†, Xuran Zhang, Junbo Zhang*

Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University, Beijing, 100875, P. R. China.

* Corresponding Author: No. 19 Xinjiekou Wai Boulevard, Haidian District, Beijing. College of Chemistry, Beijing Normal University, 100875, P. R. (China). E-mail: zhjunbo@bnu.edu.cn

† Author contributions: Xiaoqing Song and Qianqian Gan have contributed equally to this work.

Chemical synthesis

General procedure for the synthesis of **1a-1d**

The synthesis of **1a-1d** was according to the literature reported previously with little modification. In brief, 25 mL formic acid and 13 mL acetic anhydrate were added to a 100 mL flask and stirred at 45 ^{o}C for 1 h. Then aminoalkanoic acid (30 mmol) was added and the mixture was stirred at 95 ^{o}C for 3 h under N_2 atmosphere. After the solvent was removed, the residue was purified via silica gel column chromatography (dichloromethane/methanol = 20/1-10/1) to afford compound **1a-1d** as white powder.

3-Formamidopropanoic acid (1a): Yield, 46%. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.24 (s, 1H), 8.04 (s, 1H), 7.96 (s, 1H), 3.27 (q, *J* = 6.5 Hz, 2H), 2.39 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d6) δ 172.90, 161.26, 33.84, 33.45.

4-Formamidobutanoic acid (1b): Yield, 32%. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.07 (s, 1H), 7.99 (s, 2H), 3.08 (q, *J* = 6.1 Hz, 2H), 2.22 (t, *J* = 7.4 Hz, 2H), 1.58-1.66 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d6) δ 174.15, 161.08, 36.54, 30.99, 24.52.

5-Formamidopentanoic acid (1c): Yield, 44%. ^{1}H NMR (400 MHz, DMSO-d6) δ 11.99 (s, 1H), 7.98 (s, 2H), 3.06 (q, *J* = 6.5 Hz, 2H), 2.20 (t, *J* = 7.2 Hz, 2H), 1.55-1.34 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d6) δ 174.38, 160.95, 36.75, 33.25, 28.53, 21.92.

6-Formamidohexanoic acid (1d): Yield, 70%. ^{1}H NMR (400 MHz, DMSO-d6) δ 11.97 (s, 1H), 7.96 (m, 2H), 3.05 (q, *J* = 6.7 Hz, 2H), 2.18 (t, *J* = 7.4 Hz, 2H), 1.55-1.44 (m, 2H), 1.43-1.36 (m, 2H), 1.29-1.21 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d6) δ 174.42, 160.90, 36.94, 33.59, 28.75, 25.92, 24.17.

General procedure for the synthesis of **2a-2d**

1a (or **1b**-**1d**, 10.2 mmol) and 2,3,5,6-tetrafluoro phenol (11.27 mmol) were dissolved in DMF (5 mL) and stirred for 10 min on ice bath. And then DCC was added and stirred for 10 min on ice bath, followed by stirred at room temperature overnight. Dichloromethane (50 mL) was added to the mixture and the insoluble substance was removed by filtration. Filtrate was washed with water (50 mL × 3) and brine (50 mL) and dried with $MgSO_4$. After the removal of solvent under reduced pressure, the residue was purified via silica gel column chromatography (petroleum ether/ethyl acetate = 1/3) to afford compound **2a**-**2d** as white powder.

2,3,5,6-tetrafluorophenyl 3-formamidopropanoate (2a): Yield, 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.05-6.97 (m, 1H), 6.37 (s, 1H), 3.68 (q, *J* = 6.1 Hz, 2H), 2.96 (t, *J* = 6.1 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.50, 161.58, 147.55-147.29 (m), 145.07-144.96 (m), 141.96-141.74 (m), 139.40-139.25 (m), 103.64 (t, *J* = 22.6 Hz), 33.55, 33.51.

2,3,5,6-tetrafluorophenyl 4-formamidobutanoate (2b): Yield, 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s,1H), 7.05-6.96 (m, 1H), 3,45 (q, *J* = 6.6 Hz, 2H), 2.76 (t, *J* = 7.3 Hz, 2H), 2.07-2.00 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.96, 169.23, 147.35-147.16 (m), 144.92-144.68 (m), 141.86-141.73 (m), 139.38-139.23 (m), 129.56-129.42 (m), 103.23 (t, *J* = 22.9 Hz), 38.59, 30.80, 24.66.

2,3,5,6-tetrafluorophenyl 5-formamidopentanoate (2c): Yield, 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.03-6.94 (m, 1H), 6.03 (s, 1H), 3.34 (q, *J* = 6.6 Hz, 2H), 2.70 (t, *J* = 7.2 Hz, 2H), 1.86-1.75 (m, 2H), 1.70-1.60 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.34, 161.65, 147.42-147.18 (m), 144.99-144.75 (m), 141.92-141.82 (m), 139.43-139.35 (m), 129.71-129.53 (m), 103.20(t, *J* = 22.8 Hz), 37.56, 32.85, 28.70, 21.97.

2,3,5,6-tetrafluorophenyl 6-formamidohexanoate (2d): Yield, 75%. ^{1}H NMR (400 MHz, $CDCl_3$)

δ 8.14 (s, 1H), 7.03-6.94 (m, 1H), 5.90 (s, 1H), 3.31 (q, *J* = 6.7 Hz, 2H), 2.67 (t, *J* = 7.3 Hz, 2H), 1.83-1.75 (m, 2H), 1.62-1.55 (m, 2H), 1.49-1.42 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.47, 161.43, 142.54-142.34 (m), 140.81-140.73 (m), 140.09-139.84 (m), 139.39-139.06 (m), 138.39-138.12 (m), 136.99-136.53 (m), 37.89, 33.17, 29.15, 26.09, 24.35.

General procedure for the synthesis of **3a-3d**

2a (or **2b-2d**, 8.13 mmol) and Burgess reagent (8.13 mmol) were dissolved in dichloromethane (30 mL) and stirred at room temperature overnight. After the solvent was removed under reduced pressure, the residue was purified via silica gel column chromatography (petroleum ether/ethyl acetate = 5/1) to afford compound **3a-3d** as white powder.

2,3,5,6-tetrafluorophenyl 3-isocyanopropanoate (3a): Yield, 57%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08-7.00 (m, 1H), 3.83 (t, *J* = 6.7 Hz, 2H), 3.12 (t, *J* = 6.7 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.77, 158.87, 147.55-147.27 (m), 145.07-144.80 (m), 141.91-141.69 (m), 139.39-139.21 (m), 129.30-129.02 (m), 103.89 (t, *J* = 22.8 Hz), 36.90, 33.57.

2,3,5,6-tetrafluorophenyl 4-isocyanobutanoate (3b): Yield, 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06-6.98 (m, 1H), 3.60-3.57 (m, 2H), 2.91 (t, *J* = 7.2 Hz, 2H), 2.19-2.12 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.34, 157.81 (t, *J* = 5.6 Hz), 147.56-147.29 (m), 145.09-144.81 (m), 142.01-141.81 (m), 139.52-139.30 (m), 129.64-129.50 (m), 103.64 (t, *J* = 24.1 Hz), 40.61 (t, *J* = 6.6 Hz), 29.91, 24.30.

2,3,5,6-tetrafluorophenyl 5-isocyanopentanoate (3c): Yield, 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06-6.96 (m, 1H), 3.49-3.45 (m, 2H), 2.75 (t, *J* = 7.1 Hz, 2H), 2.01-1.91 (m, 2H), 1.89-1.78 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.82, 156.80, 156.85-156.74 (m), 147.45-147.21 (m), 145.02-144.74 (m), 141-97-141.79 (m), 139.44-139.28 (m), 129.61 (t, *J* = 22.9 Hz), 41.20 (t, *J* =

6.8 Hz), 32.44, 28.16, 21.66.

2,3,5,6-tetrafluorophenyl 6-isocyanohexanoate (3d): Yield, 58%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04-6.95 (m, 1H), 3.44-3.40 (m, 2H), 2.71 (t, J = 7.3 Hz, 2H), 1.86-1.71 (m, 4H), 1.65-1.54 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.21, 156.45, 147.40-147.29 (m), 145.05-144.81 (m), 142.02-141.87 (m), 139.51-139.43 (m), 129.89-129.61 (m), 103.33 (t, J = 22.7 Hz), 41.41 (t, J = 6.4 Hz), 33.18, 28.82, 25.74, 23.99.

Ex vivo autoradiography

A mouse bearing MCF-7 tumor was injected 0.2 mL of ^{99m}Tc-**L4** (14.8 MBq), and sacrificed at 4 h post injection. The tumor and muscle were collected immediately, frozen in an optimal cutting temperature compound after washing out the blood with 1 mL of saline, and then cut into 20-μm-thick sections. Tumor and muscle sections were exposed to a phosphorus plate for 12 h and scanned by a storage phosphor system.

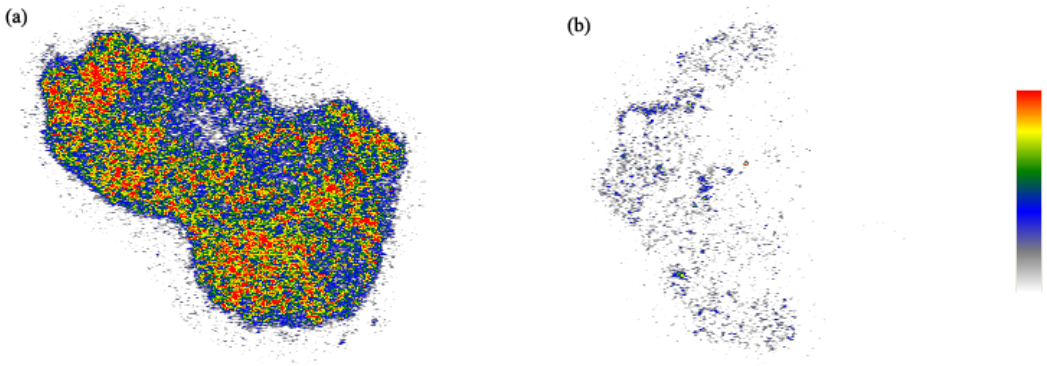

Figure S1 *Ex vivo* autoradiography of ^{99m}Tc-**L4** in tumor (a) and muscle (b).