Supporting Information

Preparation, in vitro and in vivo evaluation, and molecular dynamics (MD) simulation studies of novel F-18 labeled tumor imaging agents targeting focal adhesion kinase (FAK)

Yu Fang\textsuperscript{a,b}, Dawei Wang\textsuperscript{a}, Xingyu Xu\textsuperscript{a}, Gila Dava\textsuperscript{a}, Jianping Liu\textsuperscript{a}, Xiang Li\textsuperscript{a}, Qianqian Xue\textsuperscript{a}, Huan Wang\textsuperscript{a}, Jiangshan Zhang\textsuperscript{b}, Huabei Zhang\textsuperscript{a,*}

\textsuperscript{a} Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, P. R. China.

\textsuperscript{b} College of Chemistry and Chemical Engineering, Anyang Normal University, No. 436 Xian’ge Avenue, Anyang 455000, P. R. China

Corresponding Author:

For H. Zhang: phone +86-10-58802012, E-mail hbzhang@bnu.edu.cn.
H-NMR of 5:
$^1$H-NMR of 7a:
$^{13}$C-NMR of 7a:
ESI-MS Spectrum, XXY-1-2

#:1 Ret.Time:Averaged 1.227-1.680(Scan#:47-64)
Base Peak:630.90(273052) Polarity:Pos Segment1 - Event1
Intensity

Peak of isotope
$^1$H-NMR of 7b:
$^{13}$C-NMR of 7b:
ESI-MS of 7b:
$^1$H-NMR of $[^{19}\text{F}]_2$
$^{13}$C-NMR of $[^{19}\text{F}]_{2}$
$^{19}$F-NMR of $[^{19}$F]2
ESI-MS of $[^{19}\text{F}]_2$

#1
Ret. Time: Averaged 1.33-1.547674-3.541-5.59
Base Peak: 477.1 (227966), Palmitoyl, Pos, Segment 1 - Enteral

ESI-MS Spectrum, XXY-1-2-F

Peak of isotope
$^1$H-NMR of $[^{19}\text{F}]_3$
$^{13}$C-NMR of $[^{19}F]_3$
$^{19}$F-NMR of $[^{19}F]_3$
ESI-MS of $[^{19}\text{F}]_3$
$^1$H-NMR of $[^{19}\text{F}]0$ (including the chemical structure of $[^{19}\text{F}]1$)

\begin{center}
\includegraphics[width=\textwidth]{hnmr.png}
\end{center}

the chemical structure of $[^{18}\text{F}]1$

\begin{center}
\includegraphics[width=0.5\textwidth]{structure.png}
\end{center}
$^{13}$C-NMR of [F$^1$]1 a
ESI-MS of $[^{19}\text{F}]$1

The chemical structure and the related spectrum of $[^{19}\text{F}]$1, and the chemical structure of its corresponding F-18 labeled radiotracer $[^{18}\text{F}]$1, were cited from our published work:

D. Wang, Y. Fang, H. Wang, X. Xu, J. Liu and H. Zhang, RSC Advance, 2017, 7, 22388-22399.

(Note: The $[^{19}\text{F}]$1 in this work was named 8a in the above published work, and its corresponding F-18 labeled radiotracer $[^{18}\text{F}]$1 was named $[^{18}\text{F}]$-8a in the above published work.)
Table S1

Biodistribution in S180-tumor-bearing mice after intravenous injection of $^{18}$F[I] $^{a,b}$ (logP = 3.32±0.04)

| Organs       | 5       | 15      | 30      | 60      | 120     |
|--------------|---------|---------|---------|---------|---------|
| Blood        | 6.62±0.25 | 4.73±0.82 | 2.19±0.01 | 1.93±0.10 | 0.81±0.01 |
| Brain        | 1.20±0.12 | 0.89±0.18 | 1.19±0.18 | 1.51±0.12 | 0.83±0.14 |
| Heart        | 1.23±0.15 | 2.44±0.22 | 2.45±0.46 | 1.97±0.29 | 1.61±0.20 |
| Liver        | 3.29±0.02 | 1.99±0.23 | 1.75±0.32 | 1.57±0.30 | 0.50±0.06 |
| Spleen       | 1.98±0.03 | 1.65±0.11 | 2.28±0.12 | 1.74±0.19 | 0.92±0.34 |
| Lung         | 8.73±1.26 | 8.96±1.35 | 7.39±1.29 | 4.23±0.57 | 3.81±0.44 |
| Kidney       | 0.70±0.09 | 1.62±0.23 | 4.58±0.44 | 6.39±0.79 | 7.37±0.64 |
| Muscle       | 1.38±0.21 | 1.47±0.19 | 1.73±0.20 | 1.97±0.25 | 1.59±0.27 |
| Bone         | 1.88±0.13 | 1.95±0.25 | 3.79±0.42 | 4.16±0.46 | 4.71±0.53 |
| Intestine $^b$ | 0.06±0.02 | 0.43±0.07 | 0.28±0.06 | 0.11±0.02 | 0.34±0.08 |
| Stomach $^b$ | 0.05±0.01 | 0.23±0.03 | 0.42±0.03 | 0.37±0.09 | 1.35±0.05 |
| Tumor        | 3.39±0.25 | 3.69±0.51 | 3.71±0.43 | 3.23±0.14 | 3.11±0.22 |
| Tumor/Muscle | 2.46     | 2.51     | 2.14     | 1.64     | 1.96     |
| Tumor/Bone   | 1.80     | 1.89     | 0.98     | 0.78     | 0.66     |
| Tumor/Blood  | 0.55     | 0.78     | 1.69     | 1.67     | 3.84     |

$^a$ The preparation and the related biological evaluation results of $^{19}$F[I] and its corresponding F-18 labeled radiotracer $^{18}$F[I], were cited from our published work: D. Wang, Y. Fang, H. Wang, X. Xu, J. Liu and H. Zhang, RSC Advance, 2017, 7, 22388-22399. (Note: The $^{19}$F[I] in this work was named 8a in the above published work, and its corresponding F-18 labeled radiotracer $^{18}$F[I] was named $^{18}$F-8a in the above published work. The chemical structures of $^{19}$F[I] and $^{18}$F[I] were shown in the Supplementary Materials.)

The in vivo biodistribution studies of $^{18}$F[I] in S180-tumor-bearing mice, were performed in the same batch with the F-18 radiotracers in this work, and were cited from the above published work. What’s more, we have checked some errors in the calculation of the logP and a few of biodistribution data of the $^{18}$F[I] from our original experimental data. Therefore, we have made corrections in these tables and adjusted some discussions in the corresponding positions in the manuscript.

$^b$ Expressed as % injected dose per gram (%ID/g) unless otherwise indicated. Data are the average for five mice ± standard deviation.

Table S2

Biodistribution in S180-tumor-bearing mice after intravenous injection of $^{18}$F[2] $^{a,b,c}$ (logP = 2.97±0.10)

| Organs     | 5       | 15      | 30      | 60      | 120     | 30 (blockade) $^c$ |
|------------|---------|---------|---------|---------|---------|-------------------|
| Blood      | 6.17±0.36 | 4.36±0.62 | 3.08±0.26 | 3.24±0.25 | 2.31±0.18 | 2.86±0.31 |
| Brain      | 2.52±0.44 | 1.70±0.13 | 2.32±0.31 | 2.05±0.32 | 1.83±0.32 | 1.96±0.20 |
| Heart      | 2.37±0.15 | 2.81±0.09 | 2.93±0.14 | 2.88±0.23 | 2.61±0.25 | 2.48±0.08 |
| Liver      | 3.52±0.49 | 3.36±0.56 | 2.59±0.20 | 2.36±0.46 | 1.73±0.25 | 2.89±0.52 |
| Spleen     | 2.94±0.01 | 2.34±0.14 | 2.68±0.28 | 1.93±0.04 | 1.61±0.21 | 2.53±0.14 |
| Lung       | 10.45±0.52 | 6.13±0.48 | 2.84±0.34 | 2.61±0.20 | 1.94±0.32 | 2.35±0.23 |
| Kidney     | 5.67±0.12 | 5.62±0.27 | 5.95±0.11 | 6.12±0.12 | 6.73±0.11 | 5.83±0.68 |
| Muscle     | 1.87±0.28 | 2.25±0.20 | 2.39±0.41 | 2.37±0.14 | 2.47±0.18 | 2.64±0.13 |
| Organs         | 5   | 15  | 30  | 60  | 120 |
|---------------|-----|-----|-----|-----|-----|
| Blood         | 6.48±0.92 | 4.19±0.33 | 2.41±0.15 | 2.35±0.22 | 2.13±0.14 |
| Brain         | 2.54±0.11 | 2.43±0.07 | 1.91±0.10 | 2.50±0.12 | 2.57±0.07 |
| Heart         | 2.48±0.18 | 1.61±0.24 | 1.26±0.09 | 1.63±0.09 | 1.86±0.13 |
| Liver         | 4.14±0.12 | 4.65±0.28 | 3.74±0.11 | 2.24±0.16 | 1.85±0.04 |
| Spleen        | 2.87±0.24 | 2.11±0.19 | 1.47±0.13 | 1.25±0.11 | 0.98±0.07 |
| Lung          | 10.63±0.61 | 6.44±0.73 | 4.86±0.30 | 4.09±0.63 | 3.53±0.32 |
| Kidney        | 2.07±0.16 | 3.49±0.12 | 4.39±0.07 | 5.89±0.48 | 6.50±0.91 |
| Muscle        | 3.10±0.05 | 2.55±0.23 | 2.07±0.10 | 2.58±0.29 | 2.65±0.20 |
| Bone          | 3.16±0.16 | 2.57±0.03 | 3.07±0.10 | 3.90±0.17 | 3.50±0.34 |
| Intestine b   | 0.90±0.06 | 2.24±0.12 | 4.62±0.04 | 4.78±0.02 | 5.72±0.13 |
| Stomach b     | 1.54±0.08 | 1.44±0.06 | 1.54±0.10 | 1.56±0.16 | 1.52±0.19 |
| Tumor         | 3.08±0.20 | 2.80±0.33 | 3.24±0.07 | 2.97±0.47 | 2.88±0.44 |
| Tumor/Muscle  | 0.99  | 1.10 | 1.57 | 1.54 | 1.46  |
| Tumor/Bone    | 0.98  | 1.09 | 1.06 | 1.02 | 1.11  |
| Tumor/Blood   | 0.47  | 0.67 | 1.34 | 1.26 | 1.35  |

\*Expressed as % injected dose per gram (%ID/g) unless otherwise indicated. Data are the average for five mice ± standard deviation. \*Expressed as % injected dose per organ (%ID).
$[^{18}F]_2$–normal saline, $37^\circ$C, 1 h

$[^{18}F]_2$–normal saline, $37^\circ$C, 2 h

$[^{18}F]_2$–murine plasma, $37^\circ$C, 1 h

$[^{18}F]_2$–murine plasma, $37^\circ$C, 2 h

$[^{18}F]_3$–normal saline, $37^\circ$C, 1 h

$[^{18}F]_3$–normal saline, $37^\circ$C, 2 h
Fig. 51. The HPLC chromatogram of F-18 labeled radiotracers in normal saline and murine plasma at 37 °C after 1 h and 2 h, respectively. The HPLC profile data were exported from the *.lcd files by Shimadzu LCSolution Software, and were graphically interpreted and coordinate translated by Origin 8.0.

The in vitro stability of [18F]1 was shown in our published work: D. Wang, Y. Fang, H. Wang, X. Xu, J. Liu and H. Zhang, RSC Advance, 2017, 7, 22388-22399.
Fig. S2. The docking studies on the interaction of $^{19}$F1, $^{19}$F2, and $^{19}$F3 with the FAK. (Most of the residues in the binding pocket of the FAK were shown as “Lines”, while the key residue which have hydrogen-bonding interactions with the F-19 standards were shown as “Ball and Stick”, the F-19 standards were shown as “Capped Sticks”, and the hydrogen bonds were shown as “Yellow Dotted Line”).