# *Synthesis and evaluation of radiobrominated benzimidazole-quinoline derivatives for PDGFRβ imaging*

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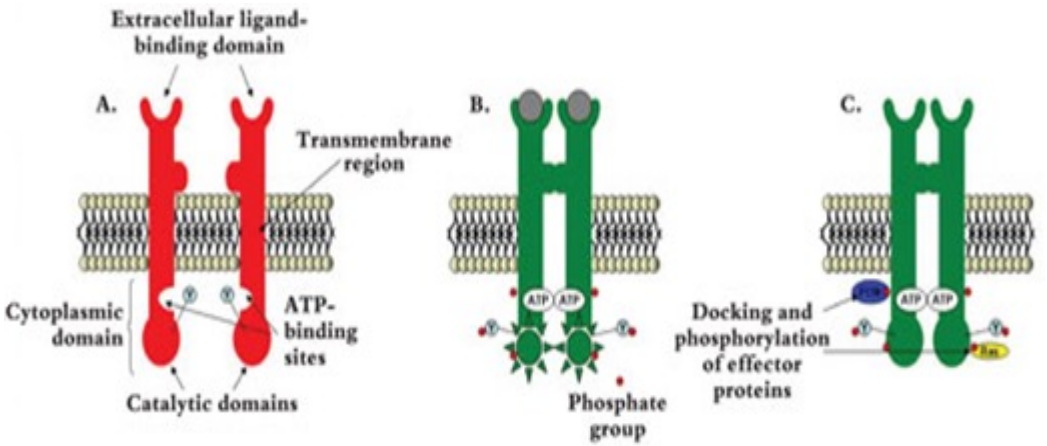
# General introduction

PDGFRβ, EGFR, and VEGFR are family of receptor tyrosine kinases.



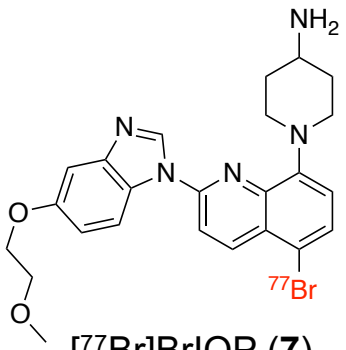

* *US Pharm.* ***2008****; 33(10)(Oncology suppl):3-14*

PDGFRβ could be a target for cancer therapy and tumor-imaging agents.

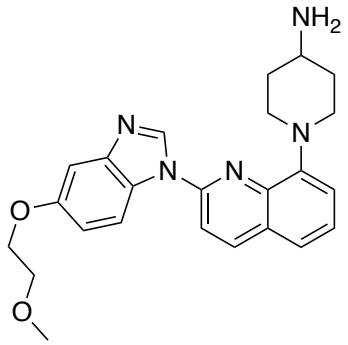
Radiolabeled TKI might be meaningful to:

1. evaluate the drug sensitivity,
2. assess the mutation status of receptor target,
3. be a useful tool in drug development.

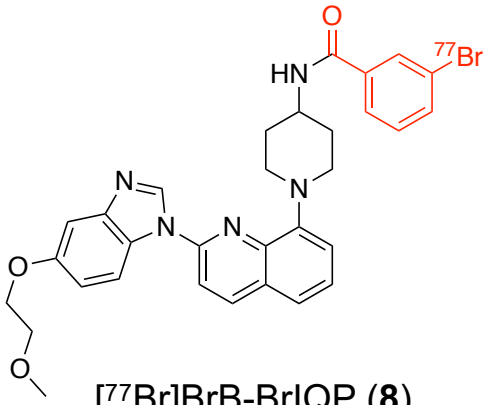
# Strategy




[$^{77}$Br]BrIQP (**7**)




IQP (**1**)




[$^{77}$Br]BrB-BrIQP (**8**)

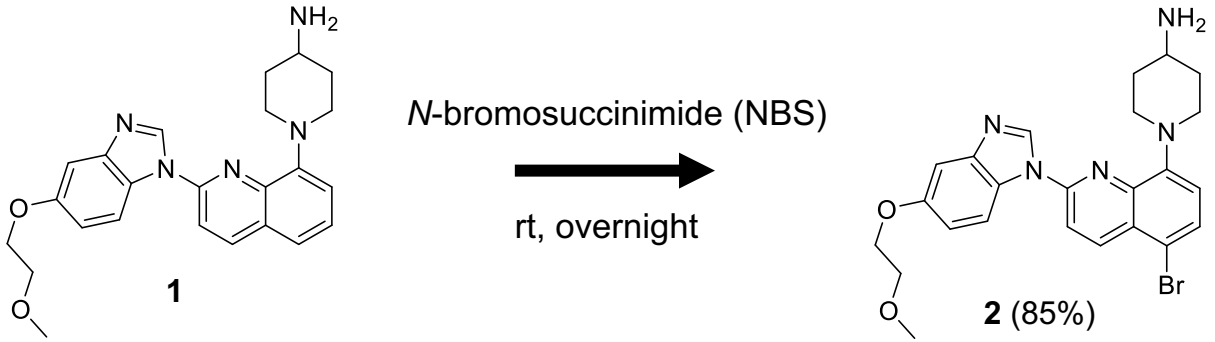
$IC_{50}$ IQP:
PDGFRβ = 1 nM
PDGFRα = 10 nM
Other kinases = > 1,000 nM

$^{76}$Br ($t_{1/2}$ : 16.1 h)
$^{77}$Br ($t_{1/2}$ : 57.0 h)

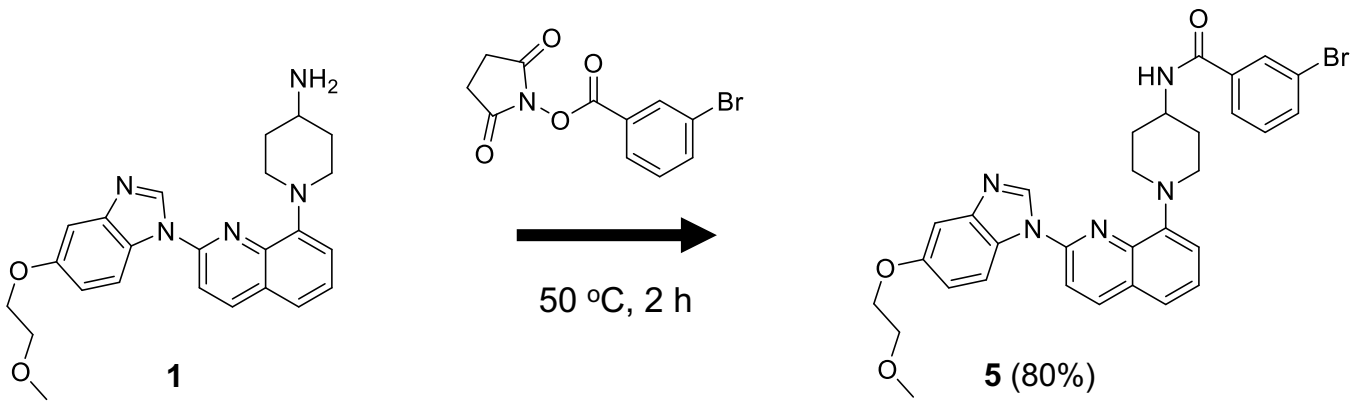

[1]*Cancer Res.* ***2005***, *65, 957-966*
[2]*Onco Targets Ther.* ***2014***, *7, 1215-1221*

# Synthesis of reference compounds

1. Synthesis of BrIQP (**2**)

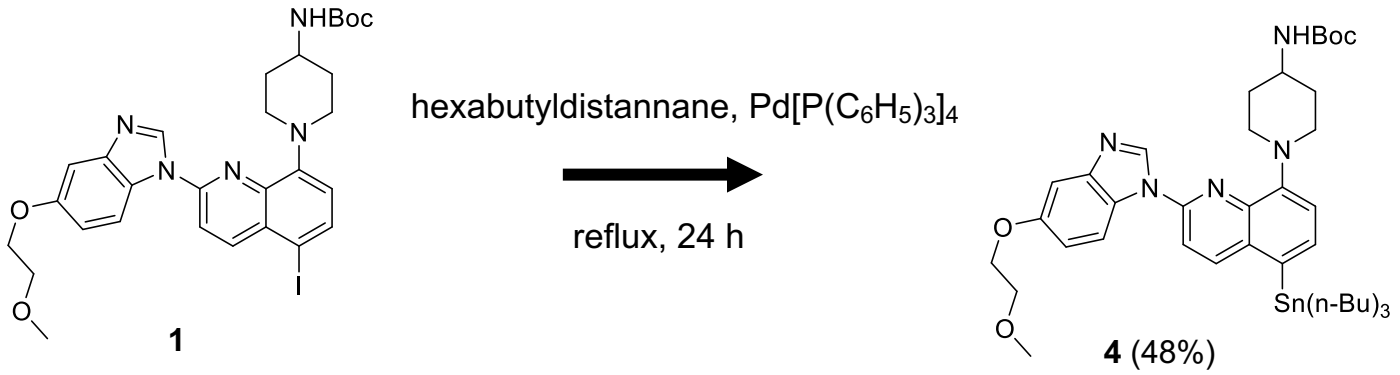



2. Synthesis of BrB-IQP (**5**)

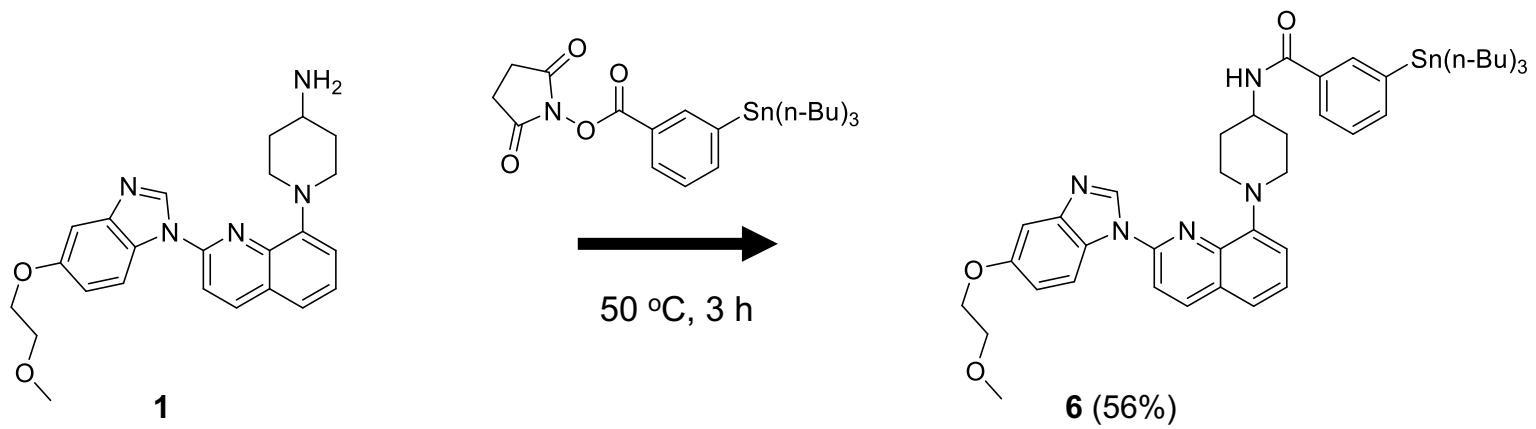


# Synthesis of precursors

1. Synthesis of tin-IQP-Boc (**4**)

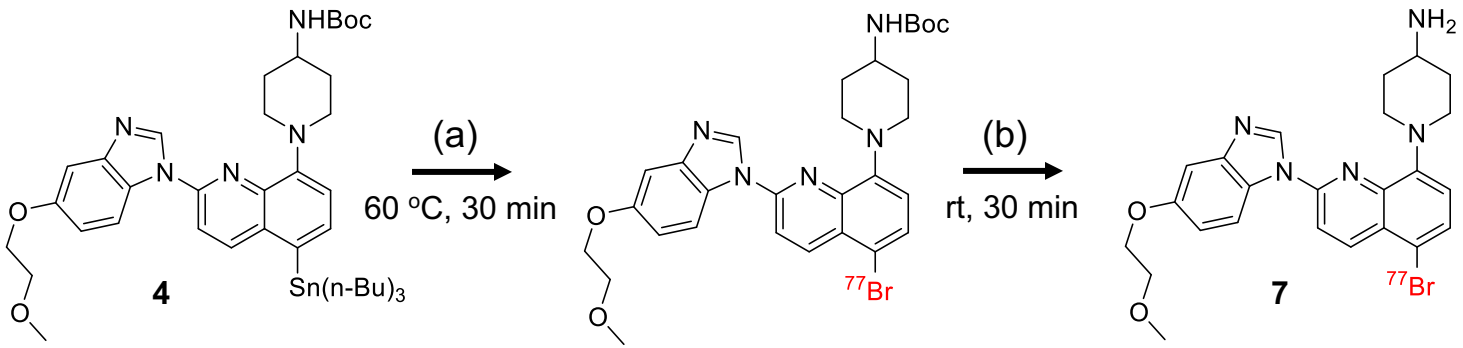



2. Synthesis of ATE-IQP (**6**)



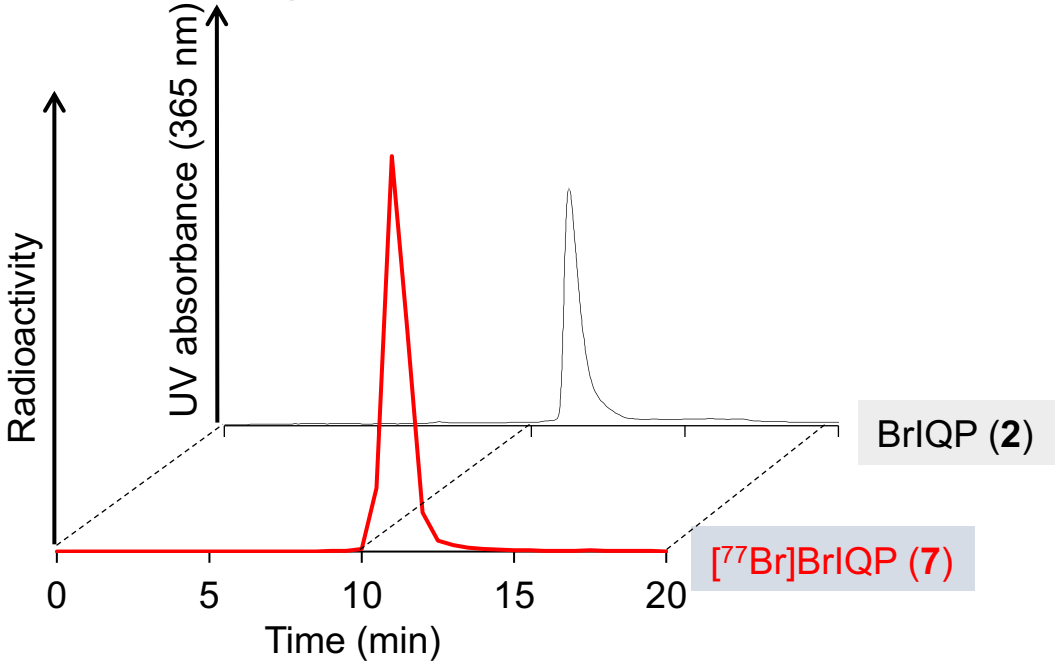
# Preparation of [$^{77}$Br]BrIQP

## Radiolabeling




(a) [$^{77}$Br]Br$^-$, NCS, acetic acid (b) TFA

## Chromatograms of [$^{77}$Br]BrIQP and BrIQP



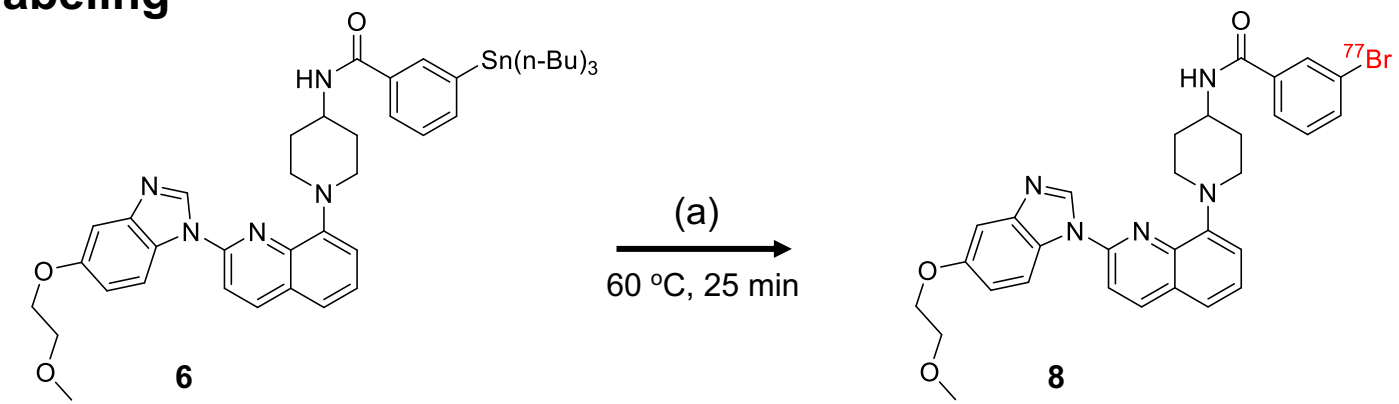

**Radiochemical yield: 95.0%**

**Radiochemical purity: 99.3%**

HPLC condition:
Column: 5C$_{18}$-MS-II (4.6 ID x 150 mm)
Mobile phase: (B) 70 – 90%, 0 – 20 min
A) 0.05% TEA in water
B) 0.05% TEA in MeOH
Flow rate: 1.0 mL / min
Temperature: 40 °C

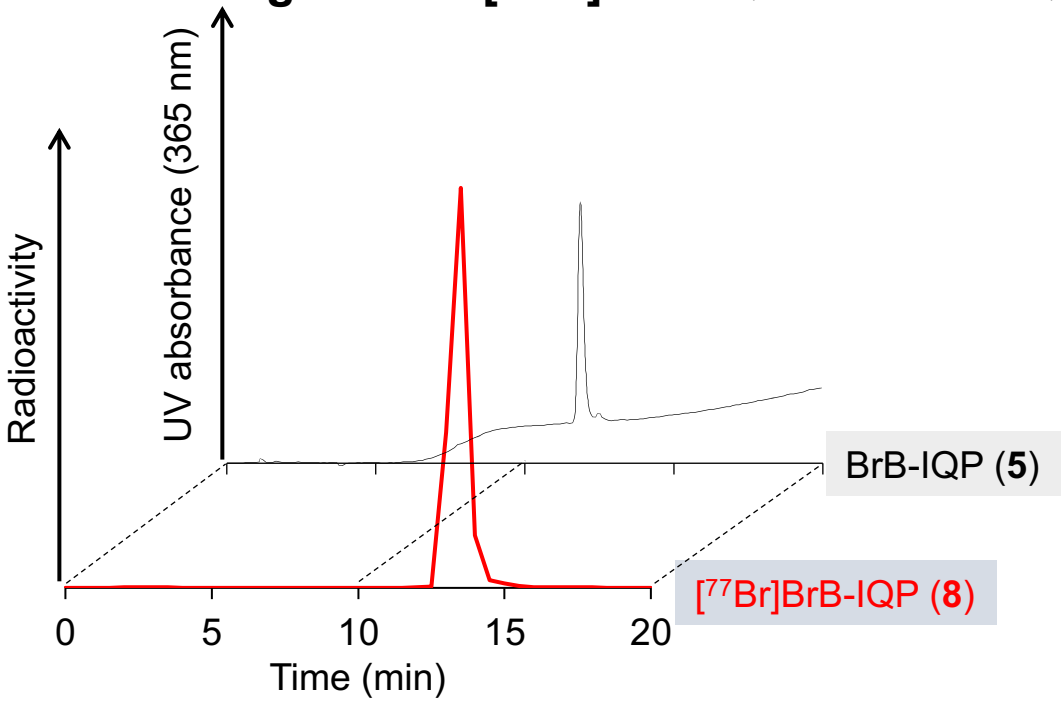
# Preparation of [$^{77}$Br]BrB-IQP

## Radiolabeling




(a) [$^{77}$Br]Br$^{-}$, NCS, acetic acid

## Chromatograms of [$^{77}$Br]BrB-IQP and BrB-IQP




**Radiochemical yield: 83.0%**

**Radiochemical purity: 99.0%**

HPLC condition:
Column: 5C$_{18}$-MS-II (4.6 ID x 150 mm)
Mobile phase: (B) 70 – 90%, 0 – 20 min
A) 0.05% TEA in water
B) 0.05% TEA in MeOH
Flow rate: 1.0 mL / min
Temperature: 40 °C

# Partition coefficient and stability test

- Partition coefficient of radiotracers

| Radiotracer | Log *P* value |
|---|---|
| [$^{77}$Br]BrIQP | 2.36 ± 0.06 |
| [$^{125}$I]IIQP | 2.71 ± 0.03 |
| [$^{77}$Br]BrB-IQP | 3.02 ± 0.04 |
| [$^{125}$I]IB-IQP | 3.19 ± 0.17 |

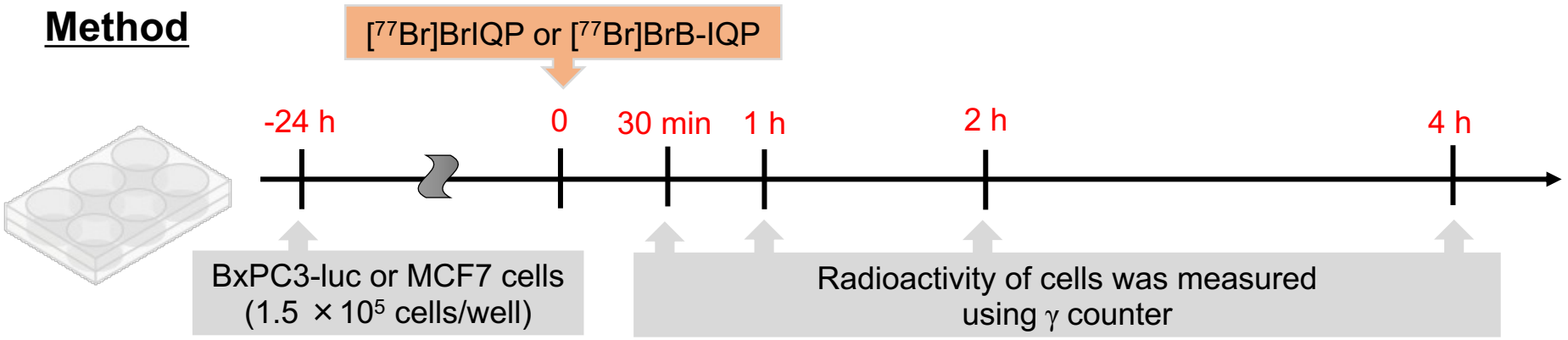
- Stability of radiotracers

| Radiotracer | Purity after 24 h (%) |
|---|---|
| [$^{77}$Br]BrIQP | 93.6 ± 0.90 |
| [$^{77}$Br]BrB-IQP | 93.0 ± 0.60 |

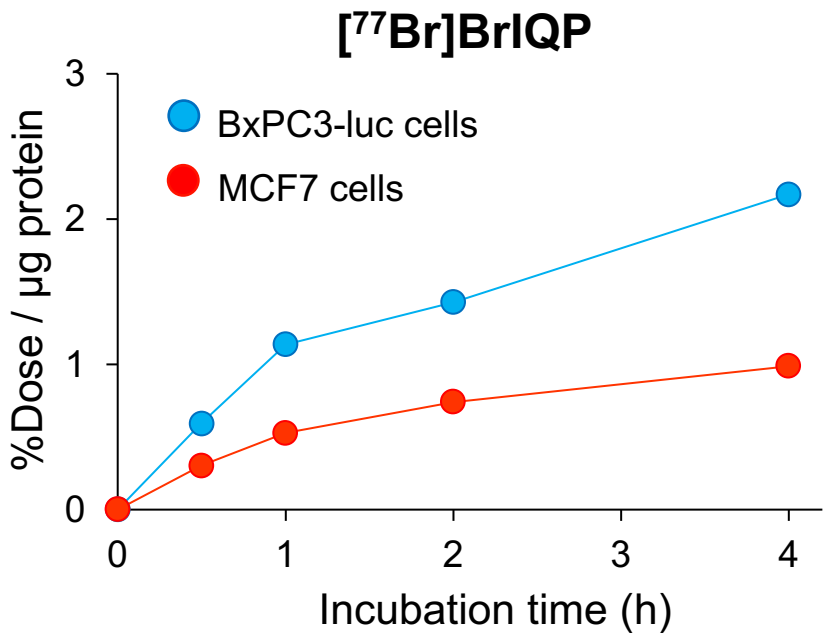
[$^{77}$Br]BrIQP and [$^{77}$Br]BrB-IQP were stable in 0.1 M PBS pH 7.4 (37$^{o}$C)

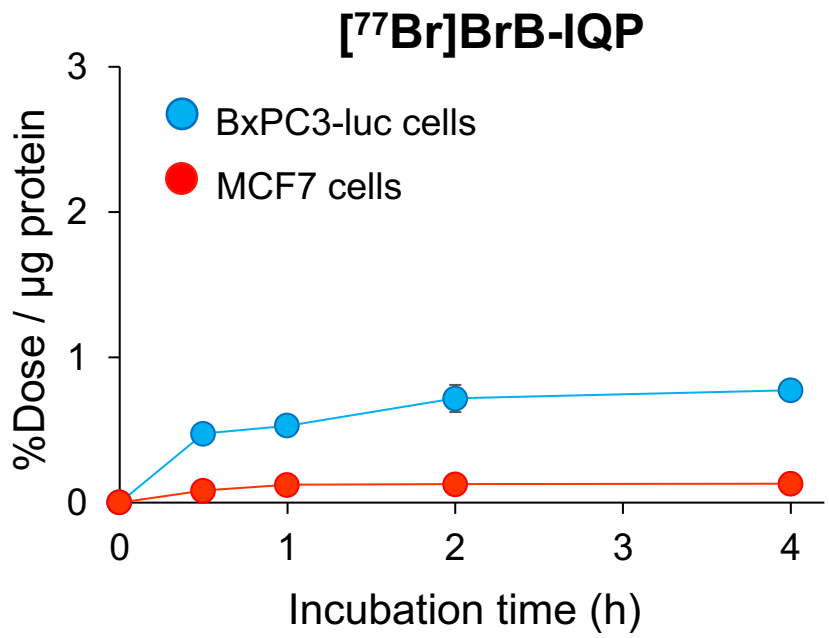
# Cell uptake study

## Method



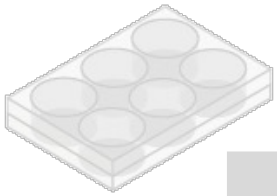

## Results



# *In vitro* blocking study

## Method



IQP, BrIQP, or BrB-IQP

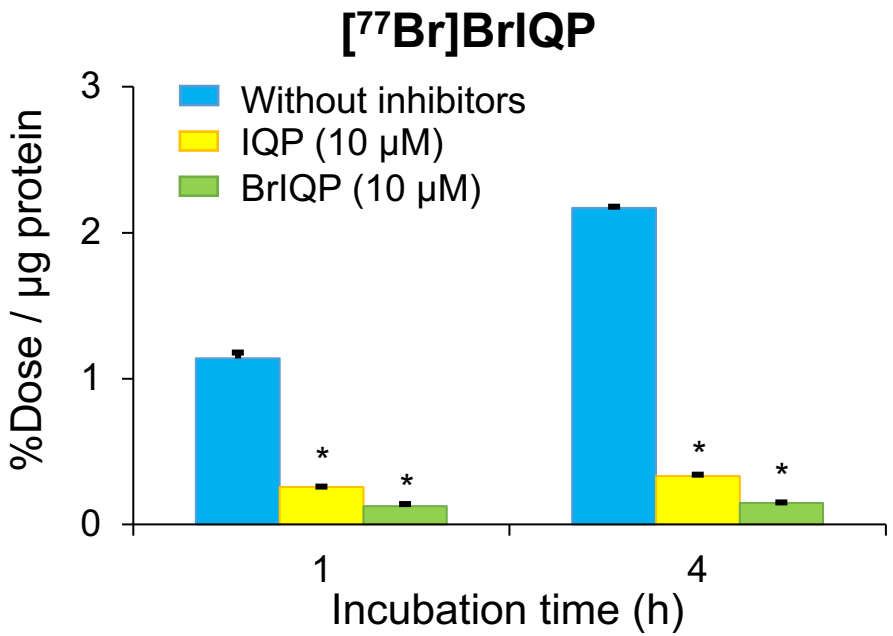
[$^{77}$Br]BrIQP or [$^{77}$Br]BrB-IQP

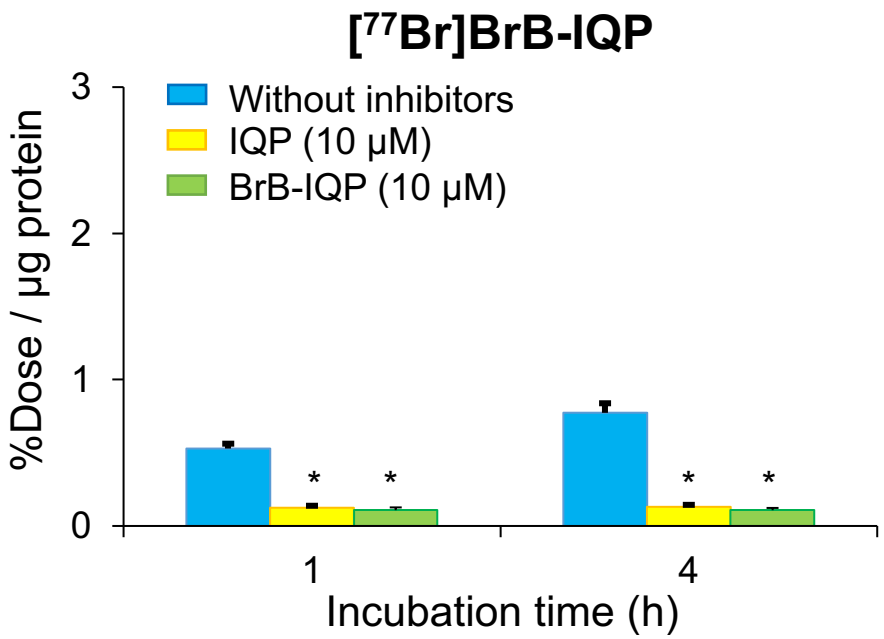
-24 h | -15 min | 0 | 1 h | 4 h

BxPC3-luc cells ($1.5 \times 10^5$ cells/well)

Radioactivity of cells was measured using γ counter

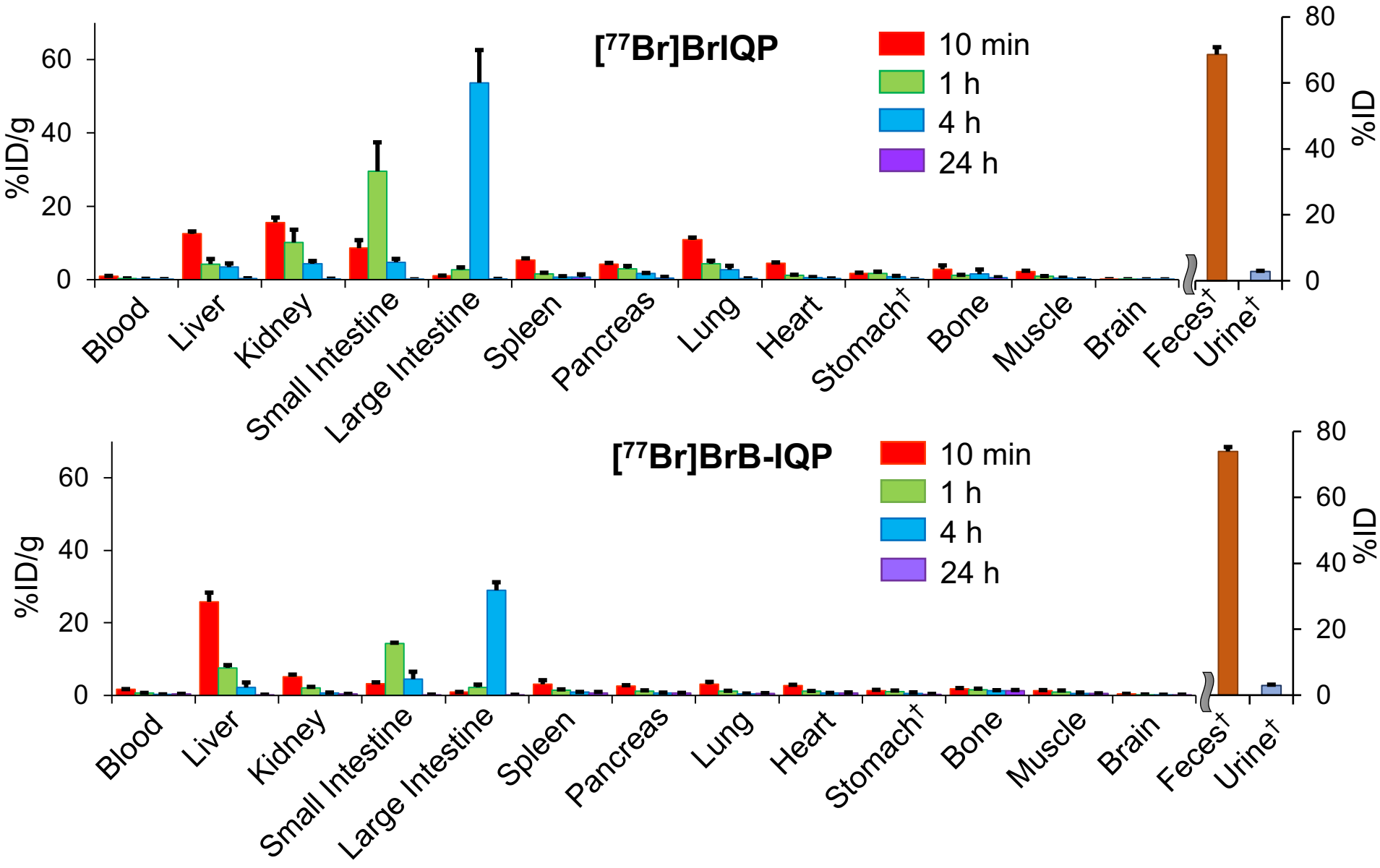
## Results




* $p < 0.01$ vs. [$^{77}$Br]BrIQP or [$^{77}$Br]BrB-IQP

# Biodistribution in normal mice




†expressed as %ID

# Tumor uptake of [$^{77}$Br]BrIQP and [$^{77}$Br]BrB-IQP in tumor-bearing mice

## Method

[$^{77}$Br]BrIQP or [$^{77}$Br]BrB-IQP

-2 wk | 0 | 1 h

Inoculation of BxPC3-luc cells into nude mice (sc) (5 x $10^6$ cells/mouse)

sacrifice

Control group

IQP (i.p.) (40 mg/Kg)

[$^{77}$Br]BrIQP

-2 wk | -1 h | 0 | 1 h

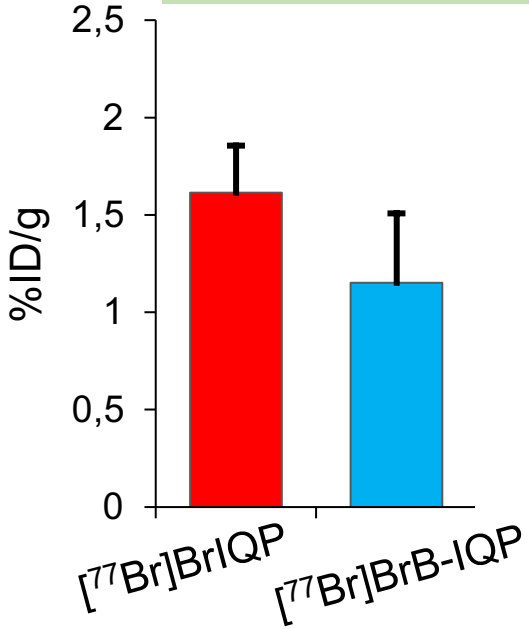
Inoculation of BxPC3-luc cells into nude mice (sc) (5 x $10^6$ cells/mouse)

sacrifice

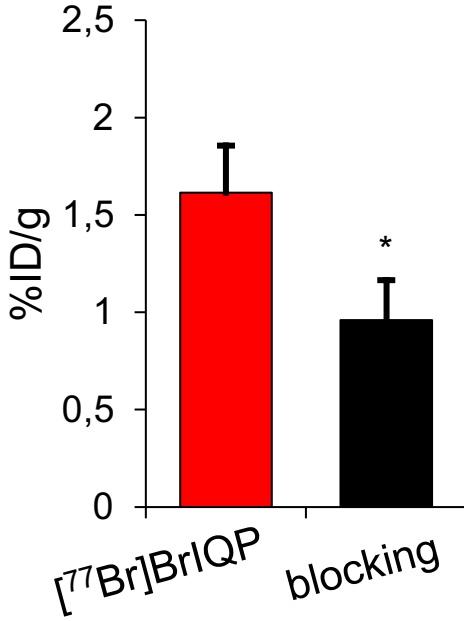
Blocking group

## Results

### Tumor uptake



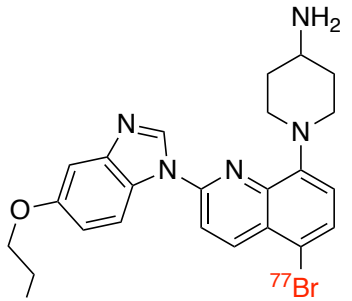

### *In vivo* blocking studies



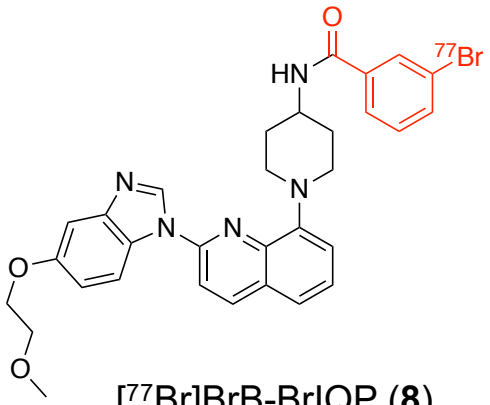

*$p < 0.05$

# Conclusion

1. [$^{77}$Br]BrIQP and [$^{77}$Br]BrB-IQP were easily prepared using a bromodestannylation reaction under non-carrier added condition with high radiochemical yields (95.0 and 83.0%, respectively) and high purities (>99%).




[$^{77}$Br]BrIQP (**7**)




[$^{77}$Br]BrB-BrIQP (**8**)

2. The method can be used to introduce $^{76}$Br into IQP instead of $^{77}$Br.

3. [$^{76}$Br]BrIQP could be more promising as an imaging probe for PET than [$^{76}$Br]BrB-IQP.

# Cell viability

## Method

**IQP** or **BrIQP** or **BrB-IQP**

-24 hr

0 hr

72 hr

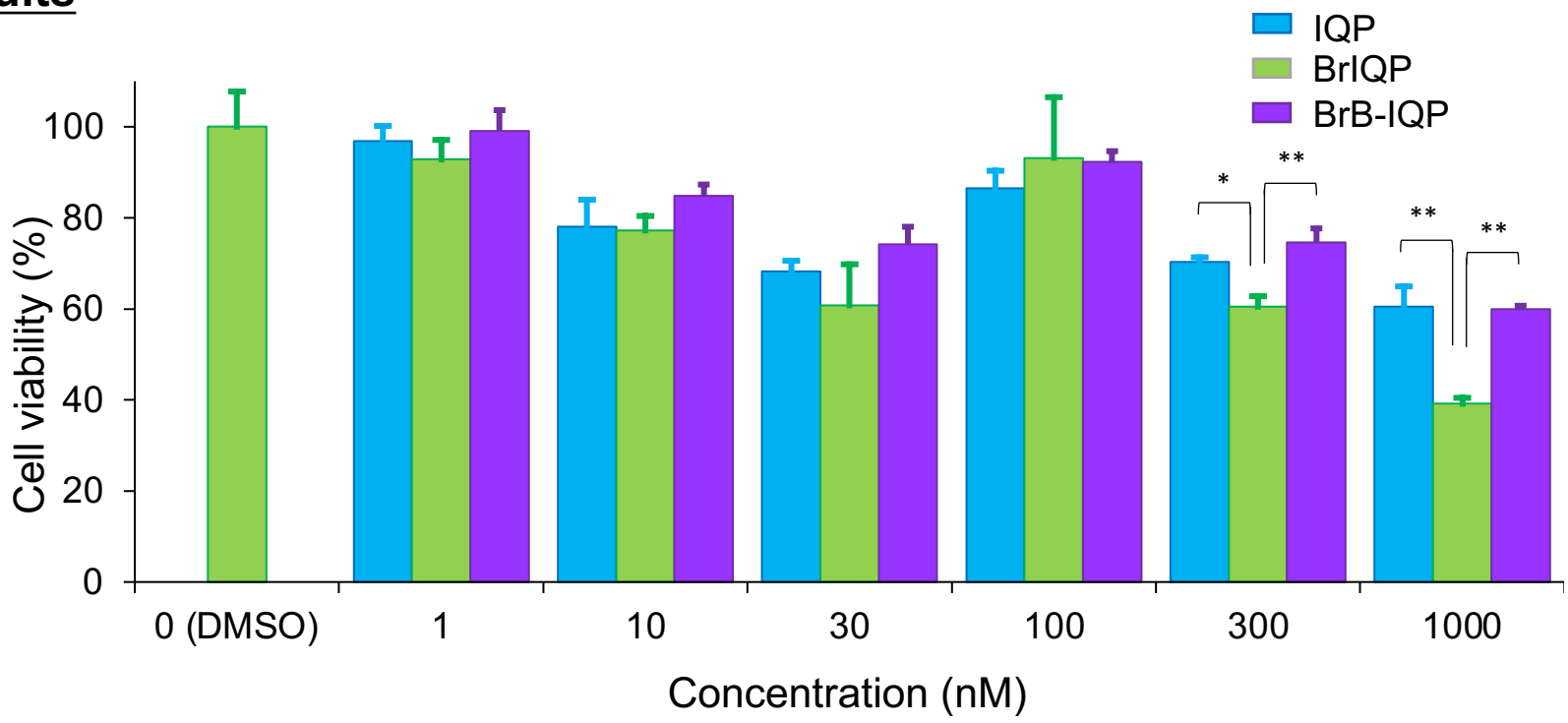
30 min

TR-PCT1 (5 × $10^3$ cells/well)

WST-8 reagent

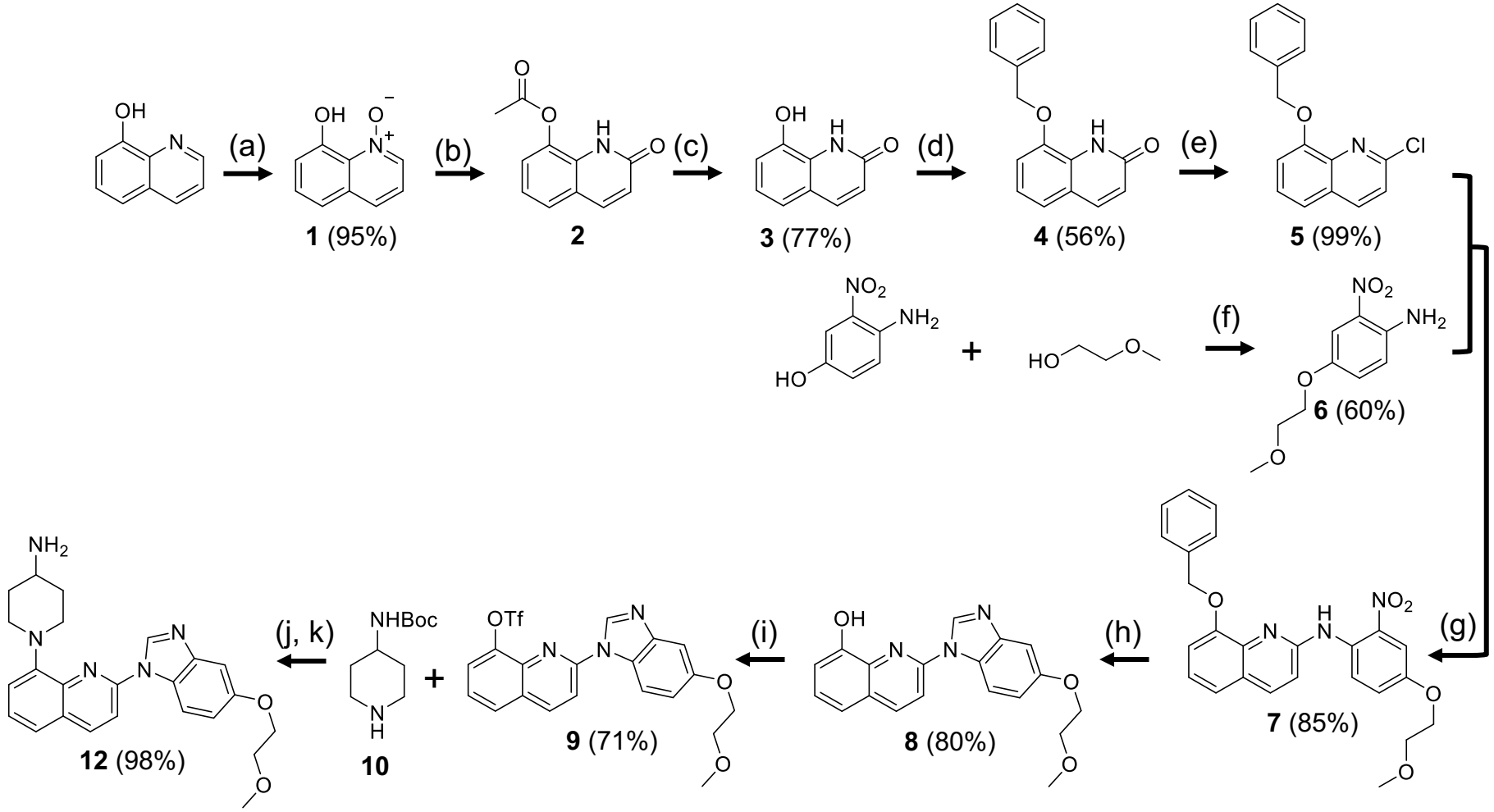
Optical density

## Results




$^{*}p < 0.01$, $^{**}p < 0.001$

# Synthesis of IQP




(a) sodium tungstate dihydrate, hydrogen peroxide, 75 °C, 5 h (b) acetic anhydrate, 100 °C, 7.5 h (c) HCl, 95 °C, 2 h (d) benzyl bromide, potassium carbonate, reflux, 6 h (e) $POCl_3$, reflux, 16 h (f) MsCl, cesium carbonate, reflux, 3 d (g) palladium acetate, DIPHOS, cesium carbonate, phenyl boronic acid, 115 °C, 3 d (h) palladium chloride, triethylamine (TEA), formic acid, formamidine acetate, reflux, overnight (i) *N*-phenyl-bis(trifluoromethanesulfonimide), rt, 2 d (j) tris(dibenzylideneacetone)dipalladium(0), cesium carbonate, rac-BINAP, reflux, 3 d (k) TFA, rt, 15 min.

*European patent specification.* ***2006****; EP 1 235825 B1*
*Chem Med Chem.* ***2014****; 9(10): 2360-70*