The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

Small Molecule Radiotracers for PET Imaging of PD-L1 with Copper-64

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

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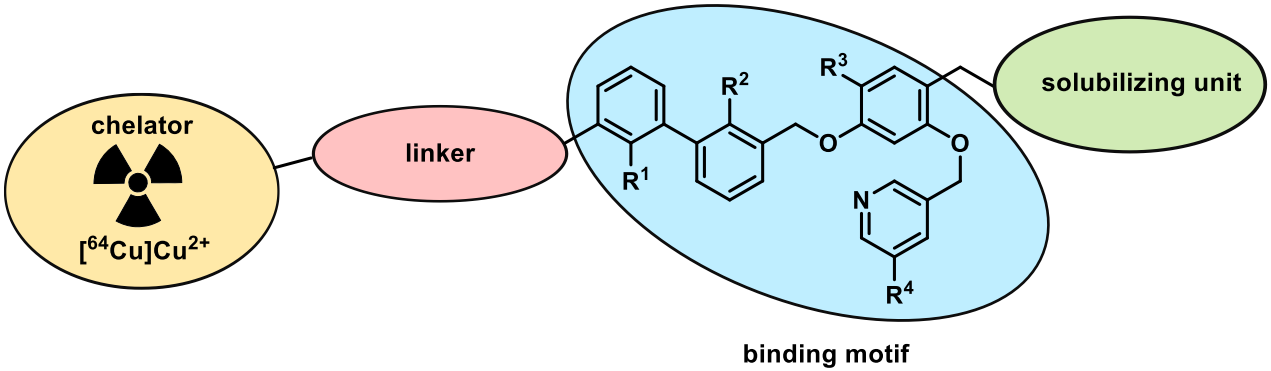

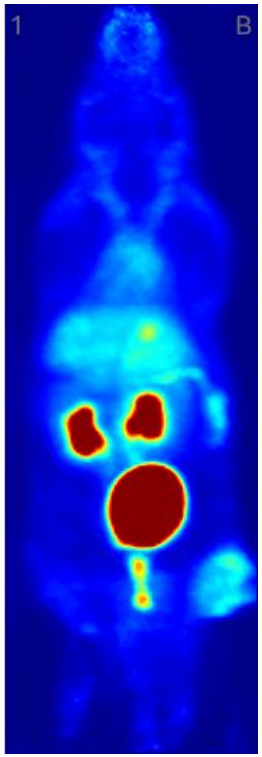

- **15 PD-L1 PET-radiotracers**
- **Convergent synthesis**
- ***In vitro* and *in vivo* evaluation**

R^1 = Me, H
R^2 = Me, Br
R^3 = Cl, Br, I
R^4 = CN, SO_2Me

up to four solubilizing units: SO_3H, PO_3H_2
six linker structures
DOTA and NODAGA conjugates

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The programmed cell death ligand (PD-L1) is expressed on a number of different tumor entities and inhibits the immune response through binding to PD-1 on T-cells. Immune checkpoint inhibitors (ICI) prevent this blockade and thus can reactivate an immune response. However, only about 30% of the patients respond to an ICI monotherapy. Therefore, clinicians are in need for a non-invasive PET/SPECT radioligand for patient stratification and therapy monitoring.

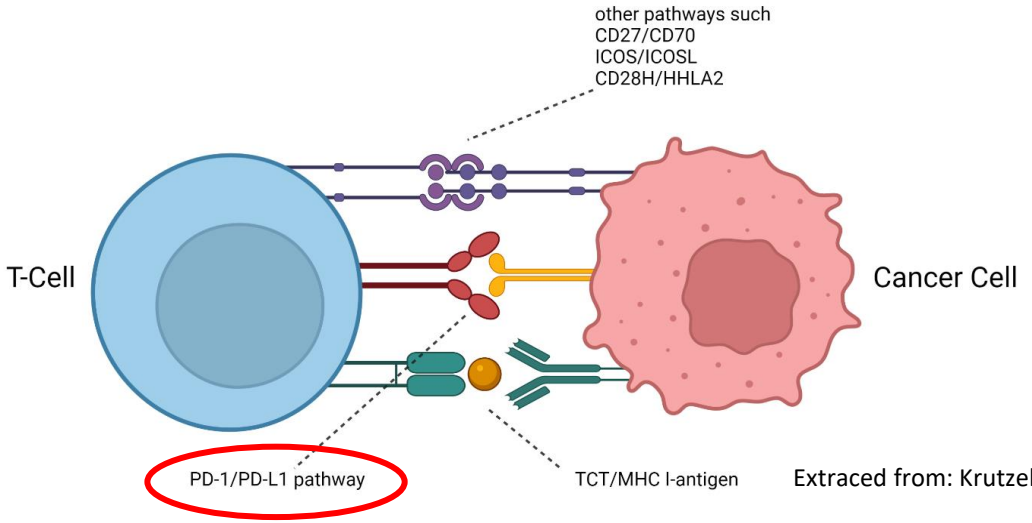
Based on the structures of non-peptidic PD-L1 inhibitors, six different radiotracers were synthesized and radiolabelled with $[^{64}Cu]Cu^{2+}$ (HZDR, 30 MeV TR-FLEX cyclotron). Binding affinities were determined on PC3 cells stably overexpressing hPD-L1. For *in vivo* studies, qualitative PET/CT imaging experiments (nanoSCAN PET/CT, Mediso) were performed in NMRI-FoxN1-nude mice bearing PC3-hPD-L1 and mock xenograted tumors.

Two PD-L1 inhibitors were modified with strongly water-soluble acid groups, hydrophilic linker units and a NODAGA-chelator resulting in six different radioligands. The log(D) values of the copper-64 labelled radiotracers were between –3.17 and –4.15 and binding affinities ranged between 80.5 and 533 nM. Depending on the number and the pattern of sulfonate and phosphonate groups, *in vivo* experiments showed drastically different pharmacokinetic profiles. The radiotracer with one sulfonate and phosphonate group and the most hydrophobic linker exhibited a short circulation time, renal clearance, good tumor uptake (SUV_{max} = 3.5) and a distinct contrast between the hPD-L1 and the mock tumor.

In conclusion one PD-L1 radiotracer showed a promising pharmacokinetic profile, which is currently further modified to improve the binding affinity and tumor uptake.

Keywords: copper-64; PD-L1; positron emission tomography (PET); radiotracers; structure-activity relationship (SAR)

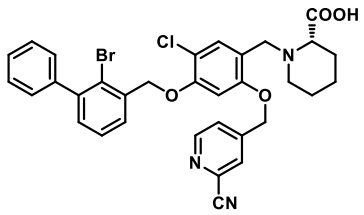
Introduction



Extraced from: Krutzek *et al.*, Pharmaceuticals, **2022**

- Programmed cell death-ligand 1 (PD-L1) is upregulated e.g. in lung, colorectal and ovarian cancer
- PD-1/PD-L1 immune checkpoint blockade allows immune evasion of cancer cells
- Immune checkpoint inhibitor (ICI) monotherapy: only ca. 30% response rate
- Clinical need for a diagnostic tool
- Molecular imaging techniques (PET, SPECT) can address heterogenous PD-L1 expression

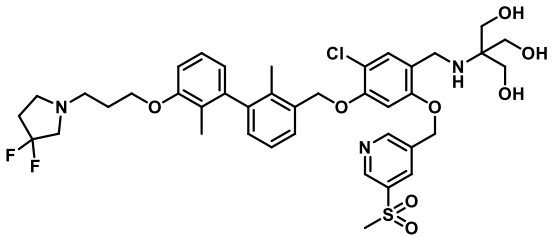
Results & Discussion



1[1]

IC_{50} = 80 x 10^{-6} nM

clog *P* = 5.67

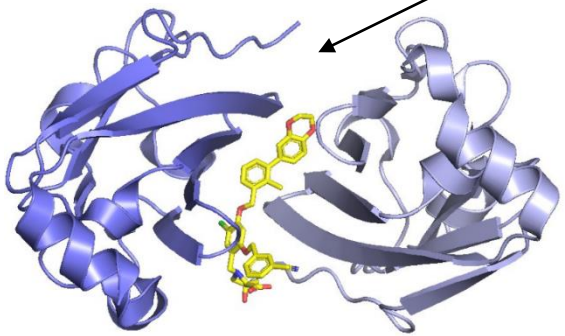


2[2]

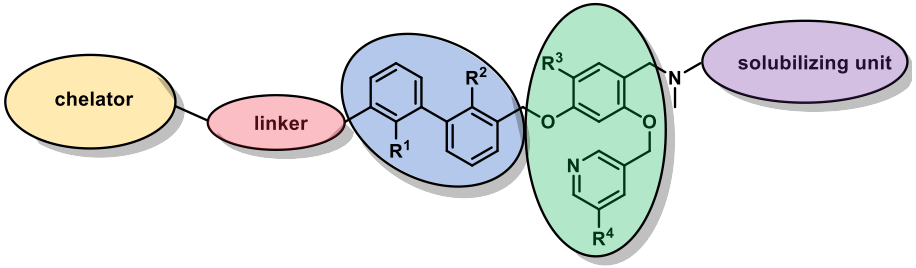
IC_{50} = 0.5 nM

clog *P* = 7.79

attachment site for radiolabel



crystal structure of dimeric PD-L1 with **1** (PDB code: 6R3K)

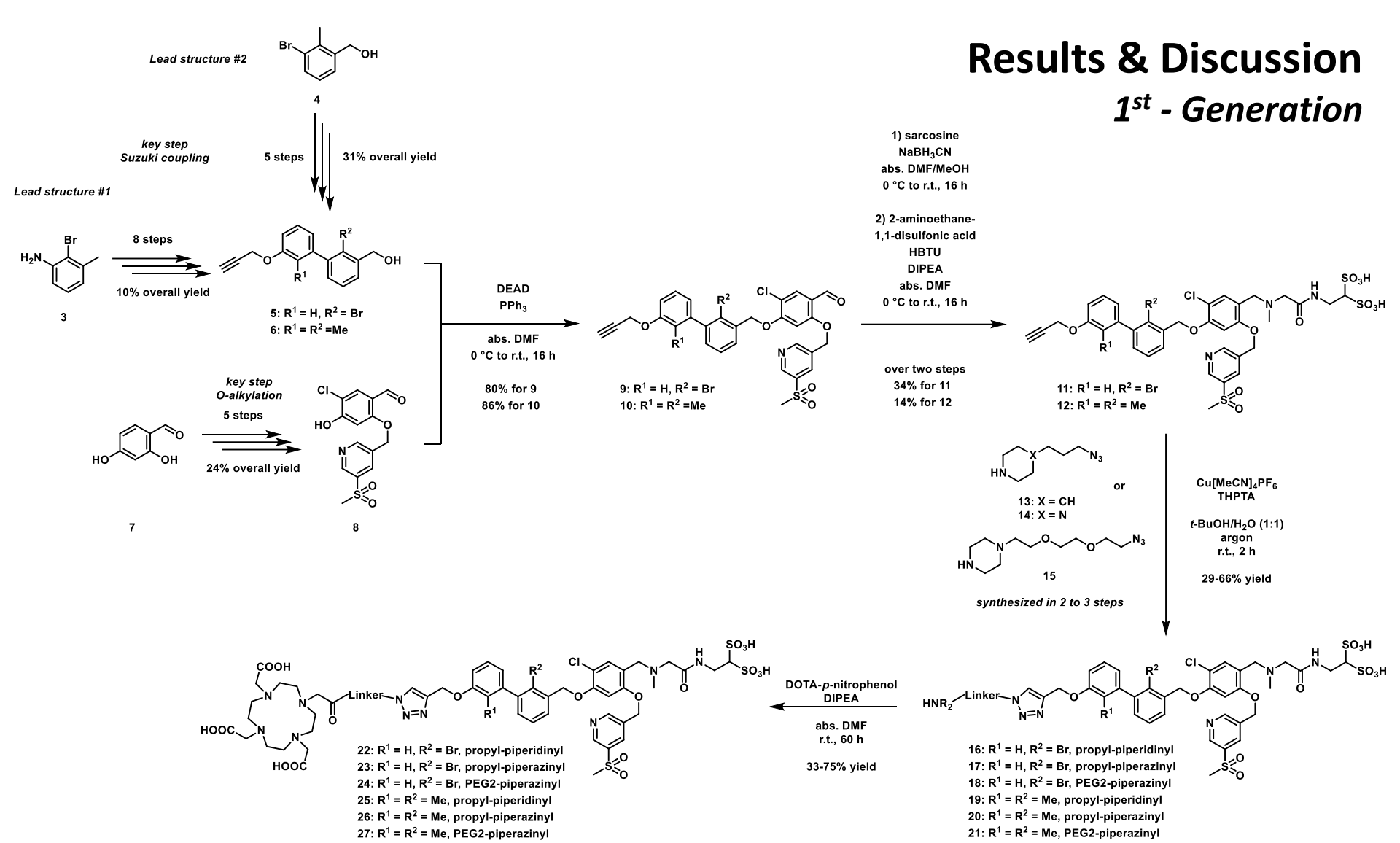


introduction of more hydrophilic groups

[1] Feng *et al.* WO2017202276 **2017**.
[2] Yeung *et al.* WO2017066227 A1 **2017**.
[3] S. Stadlbauer, F. Krutzek, K. Kopka, patent application EP21212444.0, **2021**.

Results & Discussion

1st - Generation



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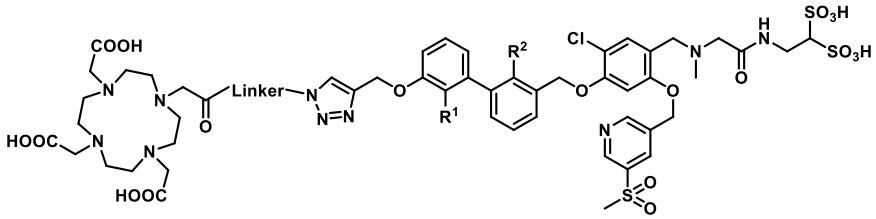
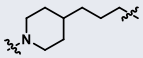
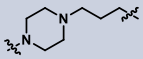
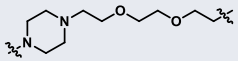
Results & Discussion

1st - Generation



Linker	R^1 = H, R^2 = Br				R^1 = R^2 = Me			
	Compound	K_D [nM]	B_{max} [pmol/mg]	Log $D_{7.4}$	Compound	K_D [nM]	B_{max} [pmol/mg]	Log $D_{7.4}$
	[^{64}Cu]Cu-**22**	585 ± 53.0	11.7 ± 0.77	–2.73 ± 0.04	[^{64}Cu]Cu-**25**	123 ± 17.3	13.5 ± 1.38	–2.75 ± 0.04
	[^{64}Cu]Cu-**23**	487 ± 58.6	8.91 ± 0.63	–3.03 ± 0.05	[^{64}Cu]Cu-**26**	59.9 ± 6.05	5.43 ± 0.63	–3.14 ± 0.02
	[^{64}Cu]Cu-**24**	351 ± 8.31	5.85 ± 1.72	–3.47 ± 0.10	[^{64}Cu]Cu-**27**	71.3 ± 13.2	7.04 ± 0.63	–3.50 ± 0.01

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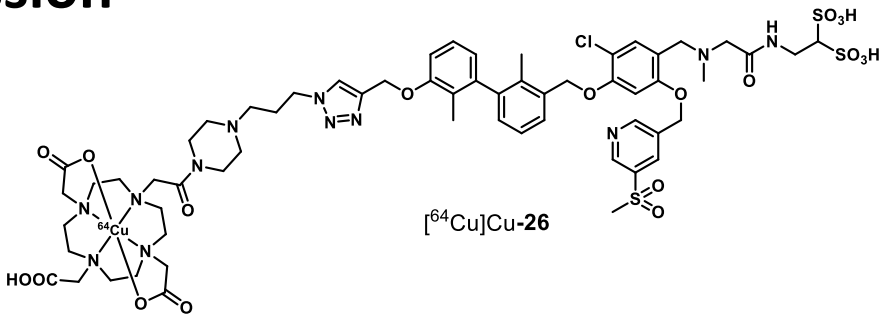

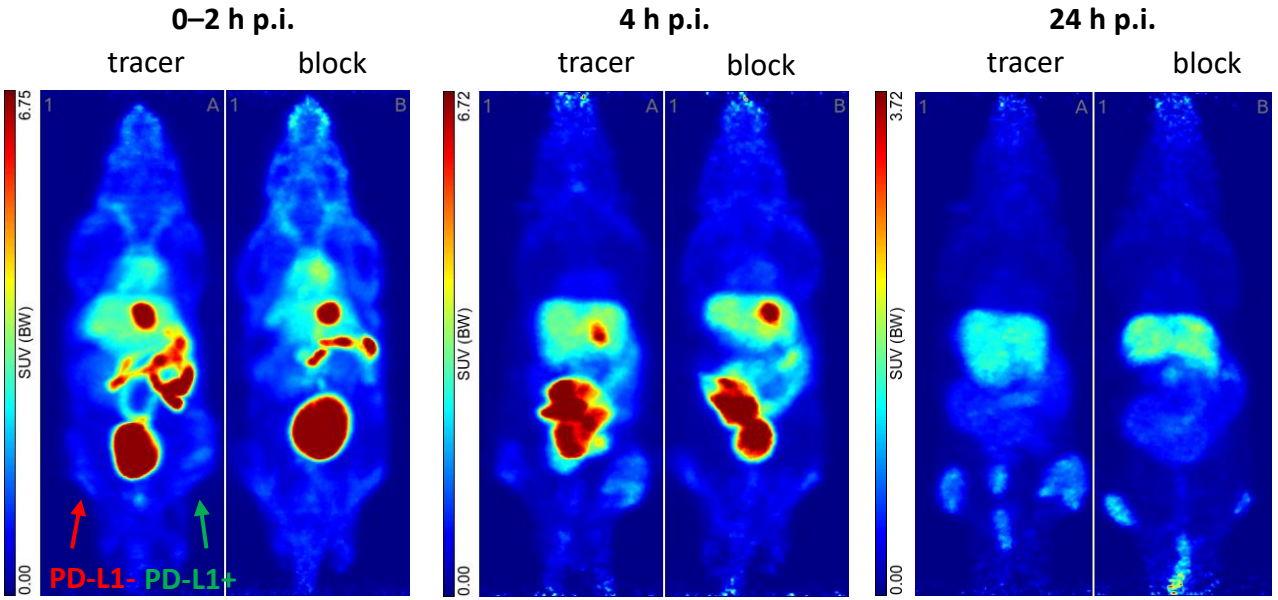

- NMRI-FoxN1 mice bearing PC3-hPD-L1 xenografted tumors
- Block: 100 nmol **26**
- left thigh: PD-L1-
  right thigh: PD-L1+

→ Unusual high circulation time for small molecule-based radiotracers (albumin binding)

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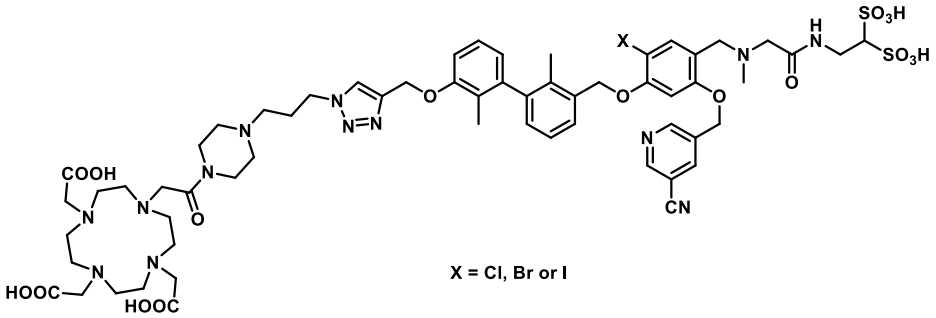
Results & Discussion

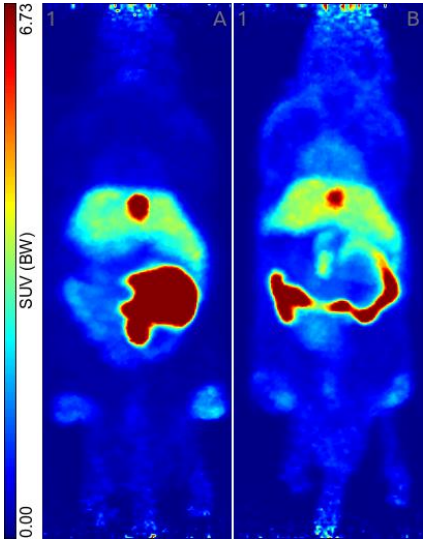
2nd - Generation



Halogen	Compound	K_D [nM]	B_{max} [pmol/mg]	Log $D_{7.4}$
Cl	[^{64}Cu]Cu-**27**	128 ± 17.2	20.9 ± 2.82	–2.99 ± 0.02
Br	[^{64}Cu]Cu-**28**	135 ± 3.37	44.4 ± 3.24	–3.48 ± 0.03
I	[^{64}Cu]Cu-**29**	300 ± 26.8	49.6 ± 1.78	–2.89 ± 0.02

4 h p.i.

tracer | Block (100 nmol **28**)



→ Higher B_{max} values result in improved tumor uptake despite higher K_D values

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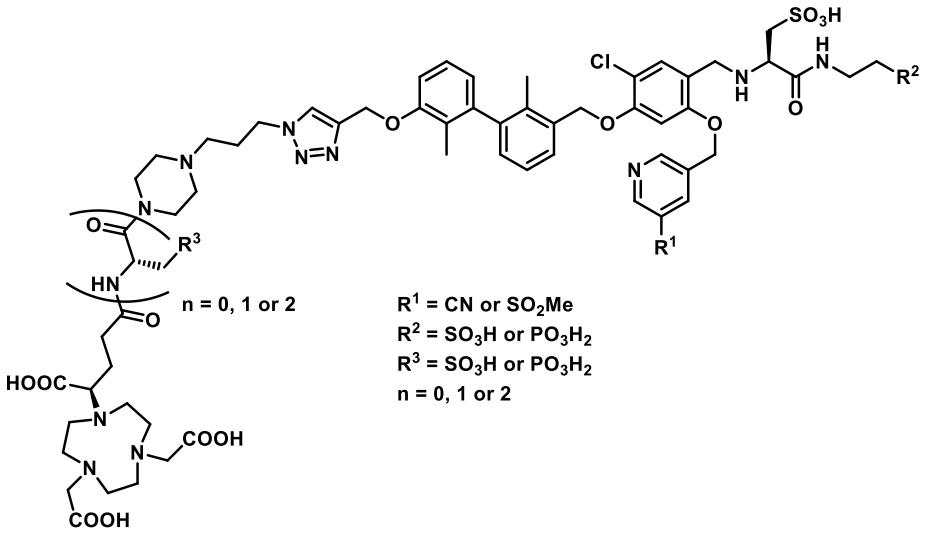
Results & Discussion

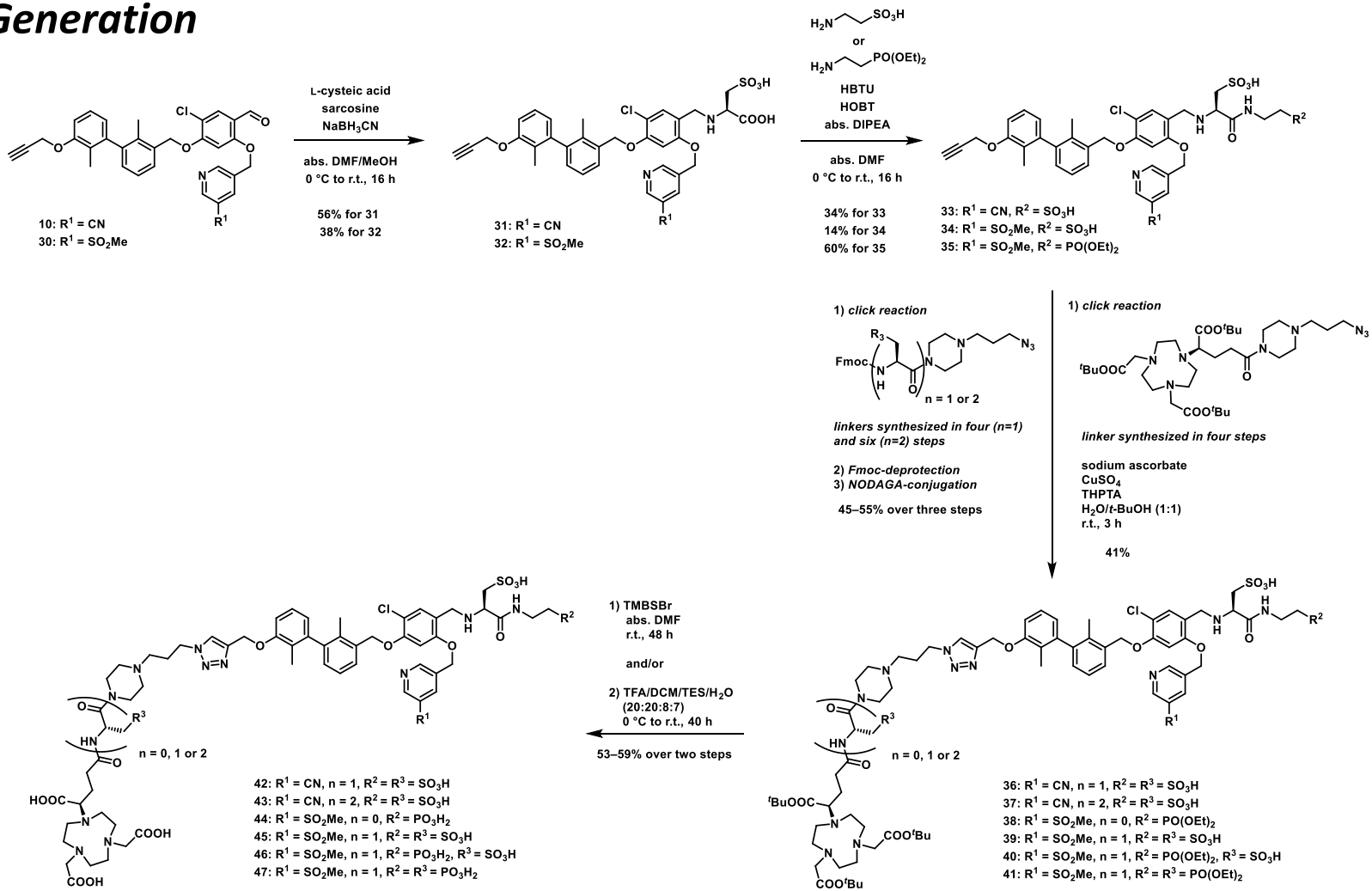
3rd - Generation



- NODAGA- instead of DOTA-chelator to avoid transchelation of copper-64
- Introduction of more hydrophilic moieties
- Partial replacement of sulfonic acids with phosphonic acids to reduce albumin binding

Results & Discussion

3rd - Generation



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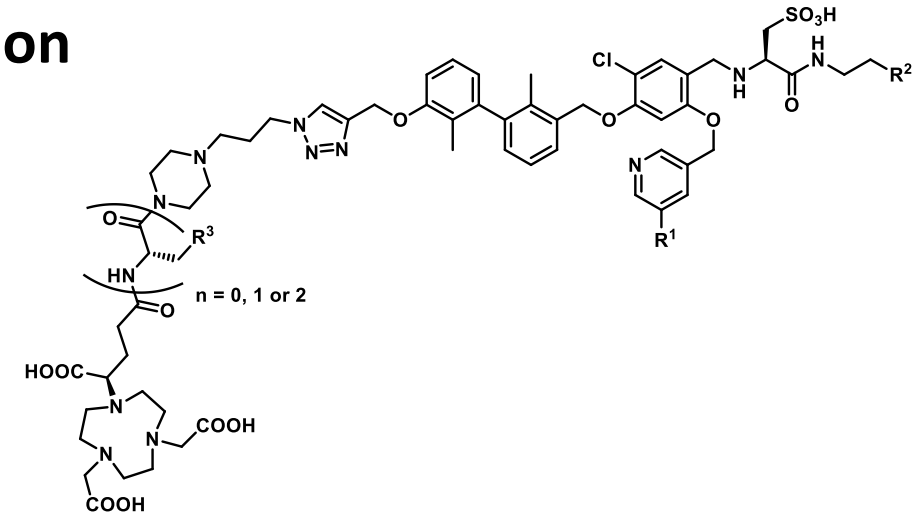
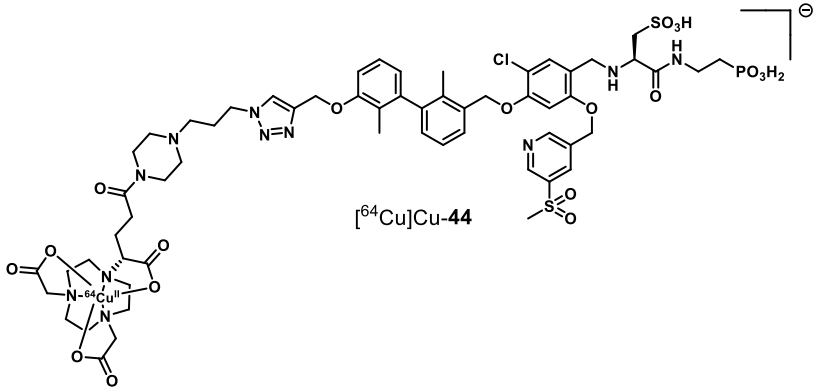
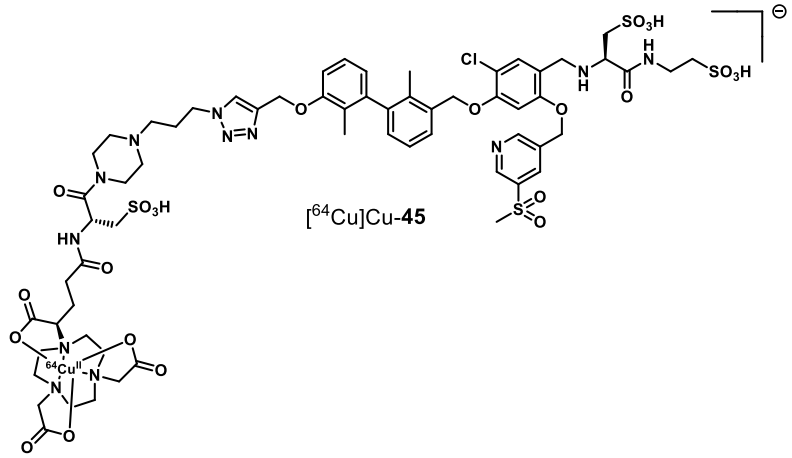


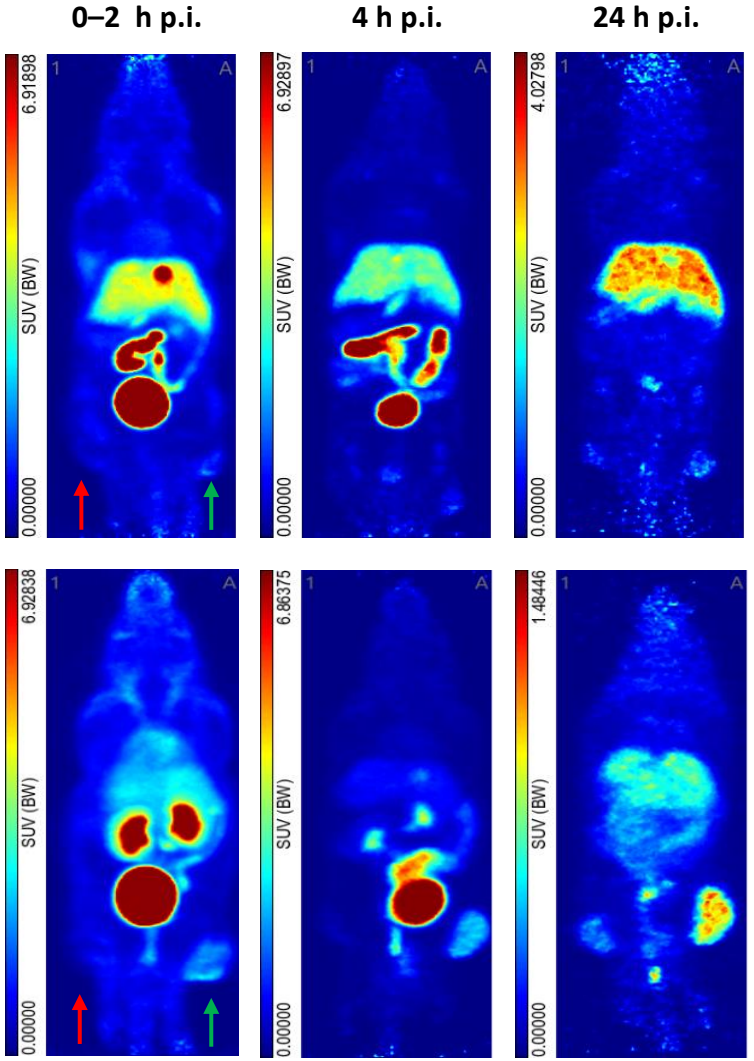
Compound	R^1	R^2	R^3	n	K_D [nM]	Log $D_{7.4}$
[^{64}Cu]Cu-**42**	CN	SO_3H	SO_3H	1	80.5 ± 5.02	–4.00 ± 0.14
[^{64}Cu]Cu-**43**	CN	SO_3H	SO_3H	2	199 ± 23.8	–4.15 ± 0.09
[^{64}Cu]Cu-**44**	SO_2Me	PO_3H_2	-	0	82.4 ± 7.42	–3.17 ± 0.02
[^{64}Cu]Cu-**45**	SO_2Me	SO_3H	SO_3H	1	93.7 ± 10.8	–3.80 ± 0.02
[^{64}Cu]Cu-**46**	SO_2Me	PO_3H_2	SO_3H	1	112 ± 11.0	–3.81 ± 0.08
[^{64}Cu]Cu-**47**	SO_2Me	PO_3H_2	PO_3H_2	1	532 ± 77.0	–4.28 ± 0.08

Results & Discussion

3rd - Generation







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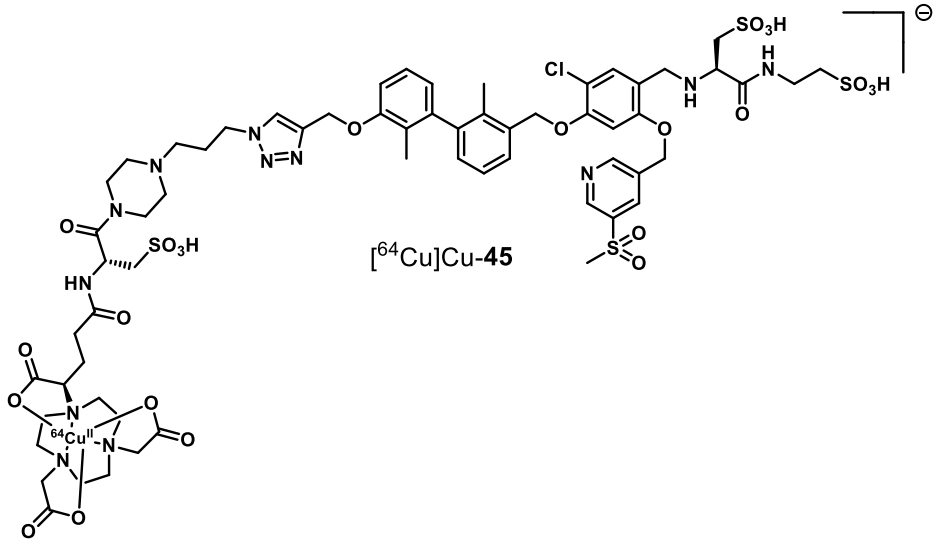
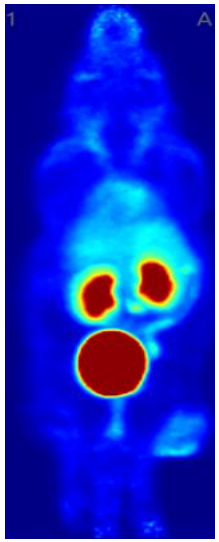
Conclusion

- 15 Small molecule-based PD-L1 radiotracers synthesized so far
- Highest determined binding affinity approx. 60 nM
- Hydrophilic compounds with log $D_{7.4}$ values between –2.73 and –4.28
- [^{64}Cu]Cu-**45** with renal clearance and good tumor uptake (SUV_{max} = 3.5)
- Specificity proven with PD-L1 negative tumors and blocking experiments
- Structural modifications to further improve tumor uptake





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Acknowledgments

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