

ABSTRACT

Objectives: CA IX is upregulated in cancer in response to hypoxia making it an attractive molecular target for radiopharmaceutical development. A series of small molecule benzenesulfonamide (BzSA) based compounds incorporating novel tridentate chelates for labeling with the M(CO)₃ core (M = Re or ^{99m}Tc), were synthesized and evaluated as inhibitors of CA IX.

Methods: CA IX inhibitors were synthesized starting with a BzSA tethered via an ethylene linker to bis(pyridyl)amine or functionalized bis(imidazolyl)amine chelators. Rhenium complexes were tested at 1-10,000 nM for inhibition of CA IX and CA II via 4-nitrophenylacetate hydrolysis. ^{99m}Tc compounds were studied *in vivo* in nude mice bearing HeLa xenografts. Specific tumor localization was determined by co-injection with 10 mg/kg acetazolamide (AZO).

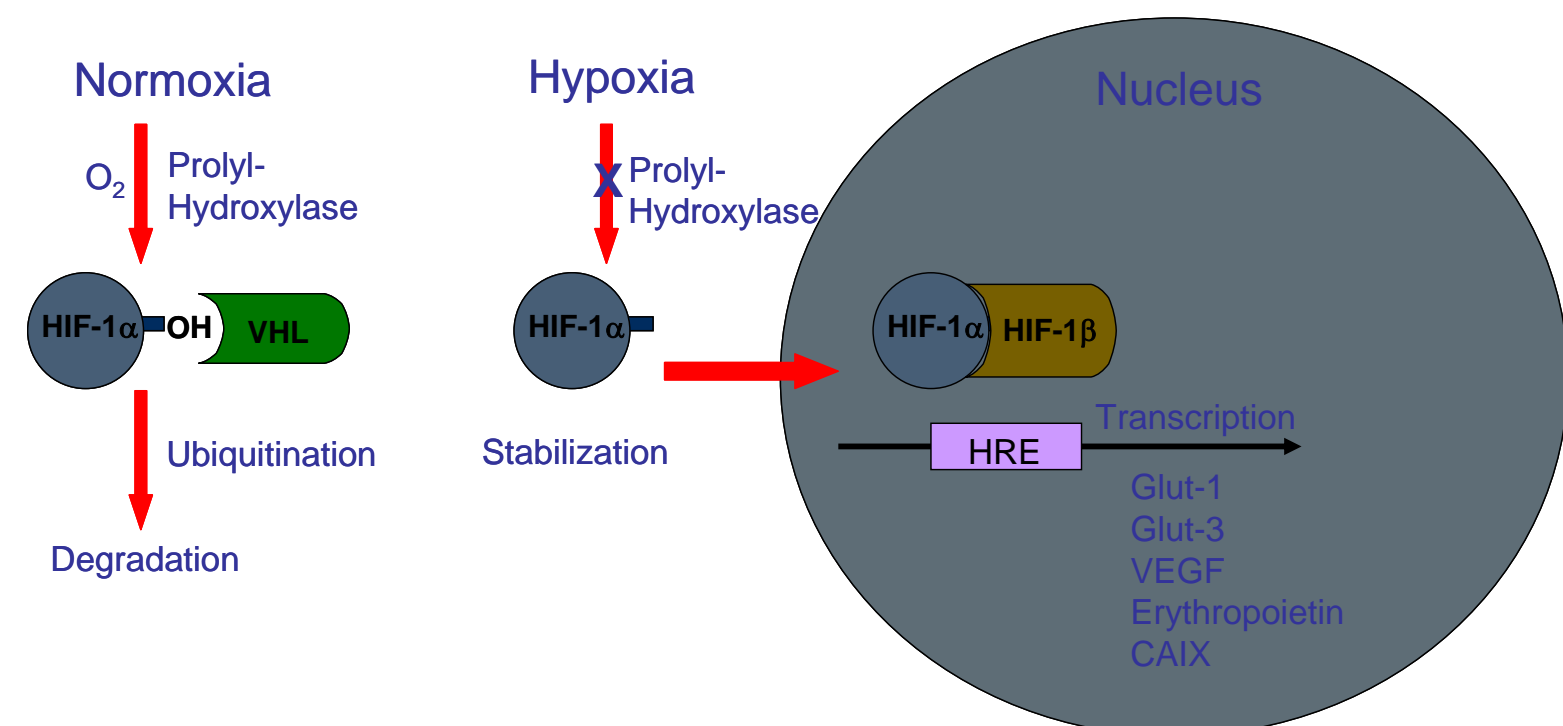
Results: BzSA analogs containing novel chelators were prepared: 4-(2-((X, Y)amino)ethyl)benzenesulfonamide, where X = Y = (pyridin-2-ylmethyl) (1), X = (pyridin-2-ylmethyl), Y = carboxymethyl (2), X = Y = (1-(carboxymethyl)-1H-imidazol-2-yl)methyl (3), and X = Y = (1-(2-(bis(carboxymethyl)amino)-2-oxoethyl)-1H-imidazol-2-yl)methyl (4). High affinity binding to CA IX was observed (IC₅₀ = 23-93 nM, 2 < 1 < 3 < 4) with approximately 10-fold selectivity for CA IX over CA II. In HeLa xenograft mice, tumor uptake ranged from 0.23-2.6 %ID/g with 4 > 3 > 2 > 1 at 1 h. Specificity for carbonic anhydrases was confirmed by competition with AZO.

Conclusions: A series of BzSA analogs containing novel chelates were shown to bind to CA IX with high affinity and selectivity over CA II. The uptake of ^{99m}Tc analogs in HeLa xenografts was specific to carbonic anhydrases. These novel compounds may be exploited to significantly impact the current paradigm for diagnosis, staging, treatment selection and therapy of solid tumors.

INTRODUCTION

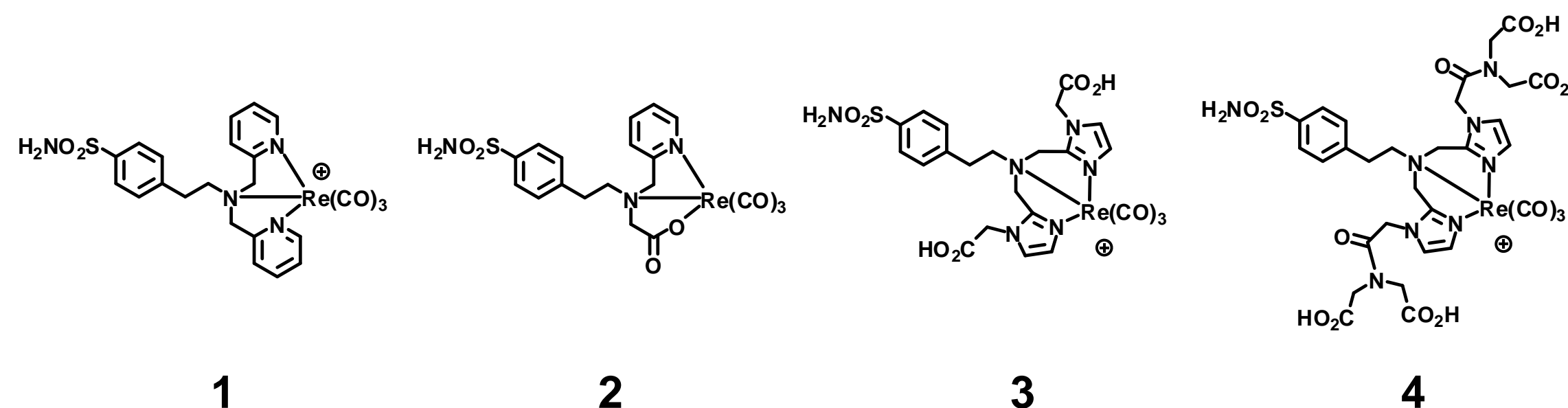
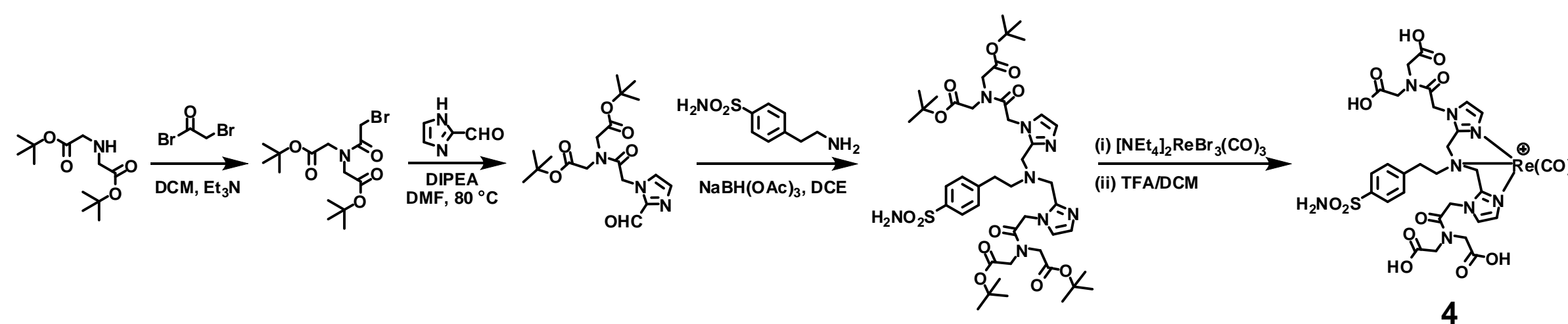
CA IX

- Catalyzes the reaction CO₂ + H₂O ↔ H⁺ + HCO₃⁻
- Regulates pH in extracellular space
- Transcriptionally activated by HIF1α
- Upregulated in response to hypoxia
- Overexpressed in many cancers including: renal cell, cervical, breast, and esophageal
- Constitutively expressed in renal cell carcinoma

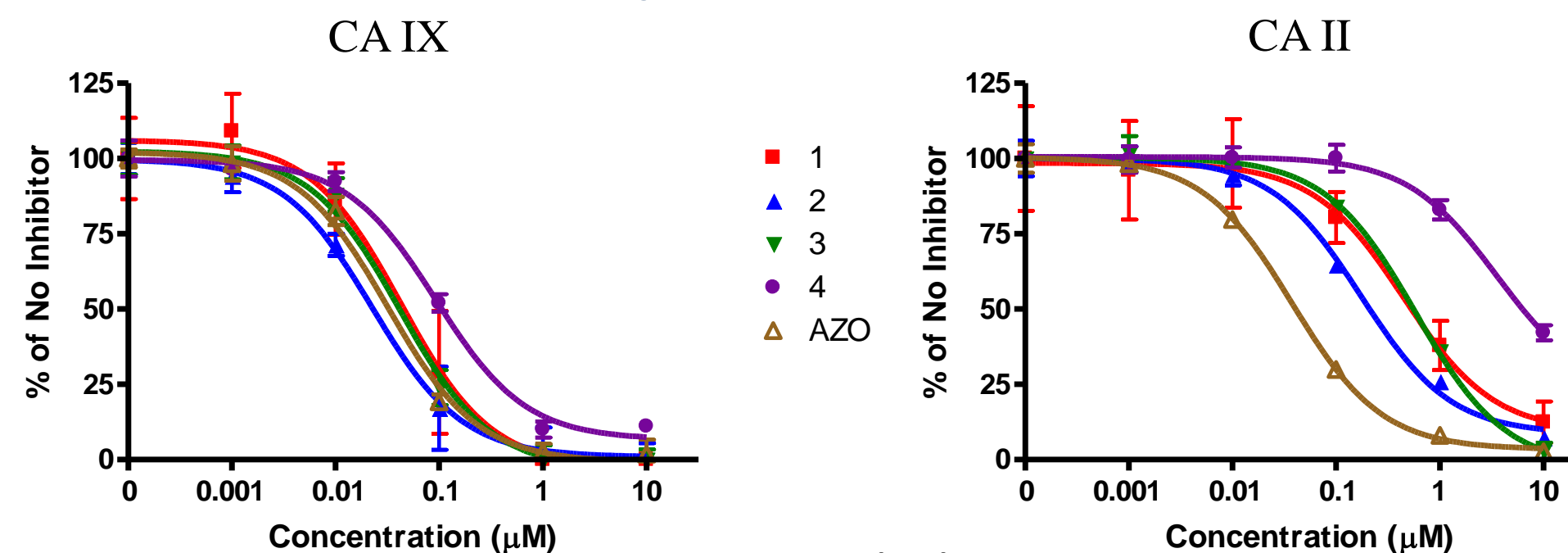


RESULTS

Synthesis of BzSA



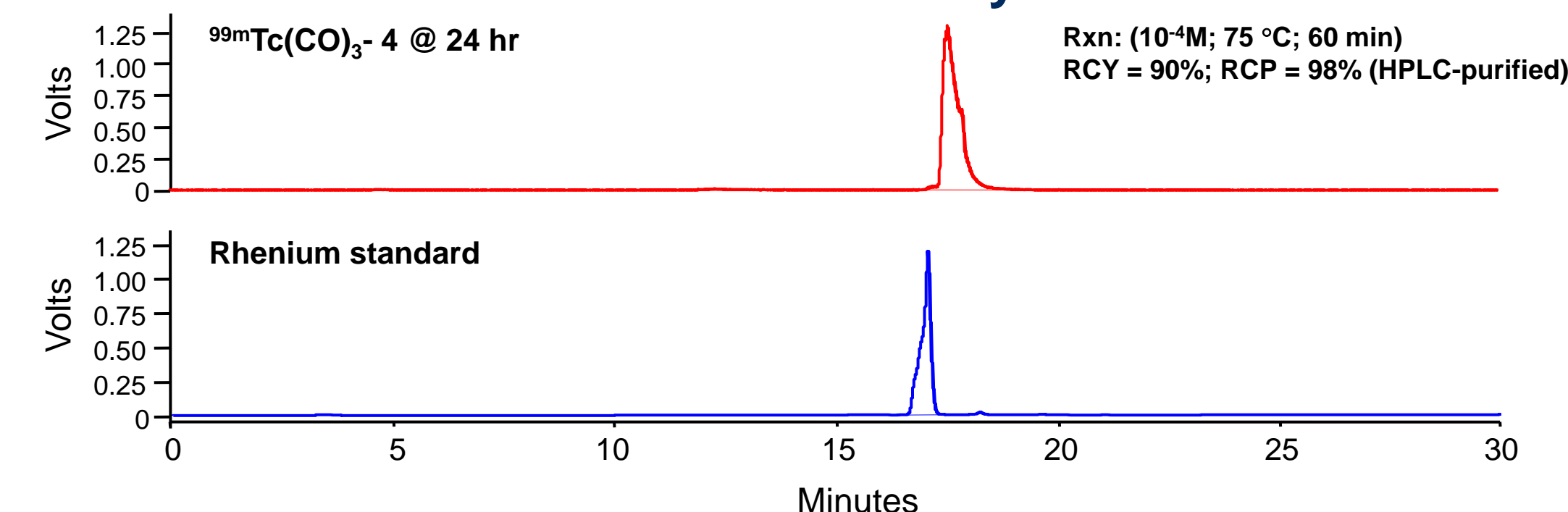
Enzyme Inhibition



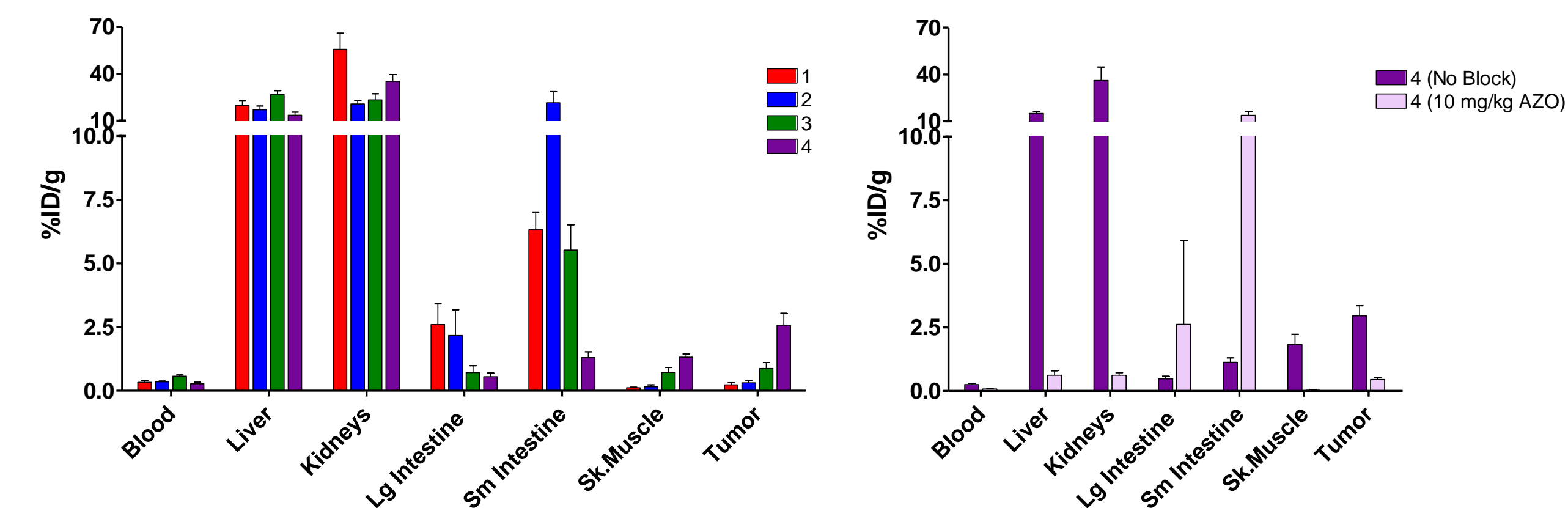
Compound	IC ₅₀ (nM)	
	CA IX	CA II
1	40	455
2	23	170
3	43	579
4	93	3577
AZO	35	44

Test compounds (1-10,000 nM) were incubated with recombinant human CA II (20 nM) or CA IX (67 nM) and 4-NPA (6.7 mM), and the change in absorbance at 405 nm was monitored. IC₅₀ values were calculated with GraphPad Prism software using non-linear regression analysis. CA IX is extracellular, CA II is intracellular. Acetazolamide (AZO) was used as a control.

Radiochemistry



Tissue Distribution



CA IX inhibitors were radiolabeled with ^{99m}Tc and injected into HeLa tumor bearing mice (n=5) and sacrificed at 1 hr post injection (left). Mice were co-injected with ^{99m}Tc-4 and 10 mg/kg of the carbonic anhydrase inhibitor, AZO (right).

CONCLUSIONS

- Benzenesulfonamide analogs containing novel tridentate chelators for Re/Tc(CO)₃ were successfully synthesized.
- The compounds were successfully prepared as the Re(CO)₃ and ^{99m}Tc(CO)₃ complexes and displayed high affinity and selectivity for CA IX.
- Tissue distribution studies showed acetazolamide sensitive uptake in CA IX expressing HeLa tumors; indicating that the binding was related to CA IX expression.
- These compounds may permit the ability to detect tumor hypoxia, via CA IX, allowing more appropriate treatments that are less influenced by tumor oxygen content and potentially lead to improved outcomes.