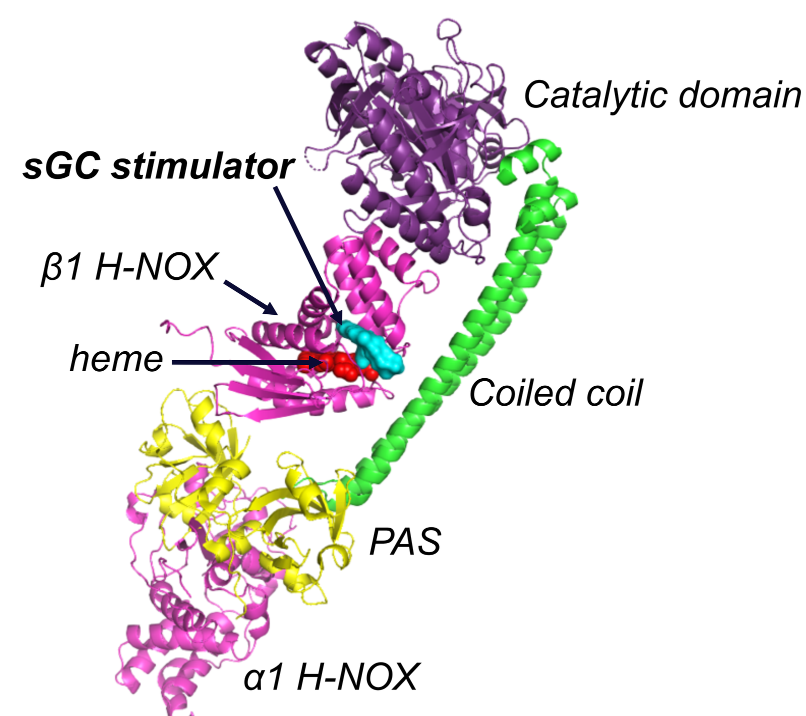
Development of a Soluble Guanylate Cyclase Radioligand Binding Assay Using [^{3}H]-Praliciguat

Marco M. Kessler, Deborah F. Dodge, Daniel P. Zimmer, Joon Jung, Paul A. Renhowe, John R. Hadcock
Cyclerion Therapeutics, 301 Binney Street, Cambridge, MA 02142

Introduction

Soluble guanylate cyclase (sGC) stimulators are a class of small molecule agonists that bind to sGC and act in synergy with nitric oxide (NO) to increase production of cGMP from GTP. sGC stimulators have been characterized for their ability to stimulate cGMP production in cellular and purified enzyme assays, but a robust assay for determining relative binding affinities of sGC stimulators has been lacking. Praliciguat is an investigational, oral, once-daily sGC stimulator.

Structural model of stimulator bound to sGC



Presented by Jung et al., 8th International Conference on cGMP (2017)

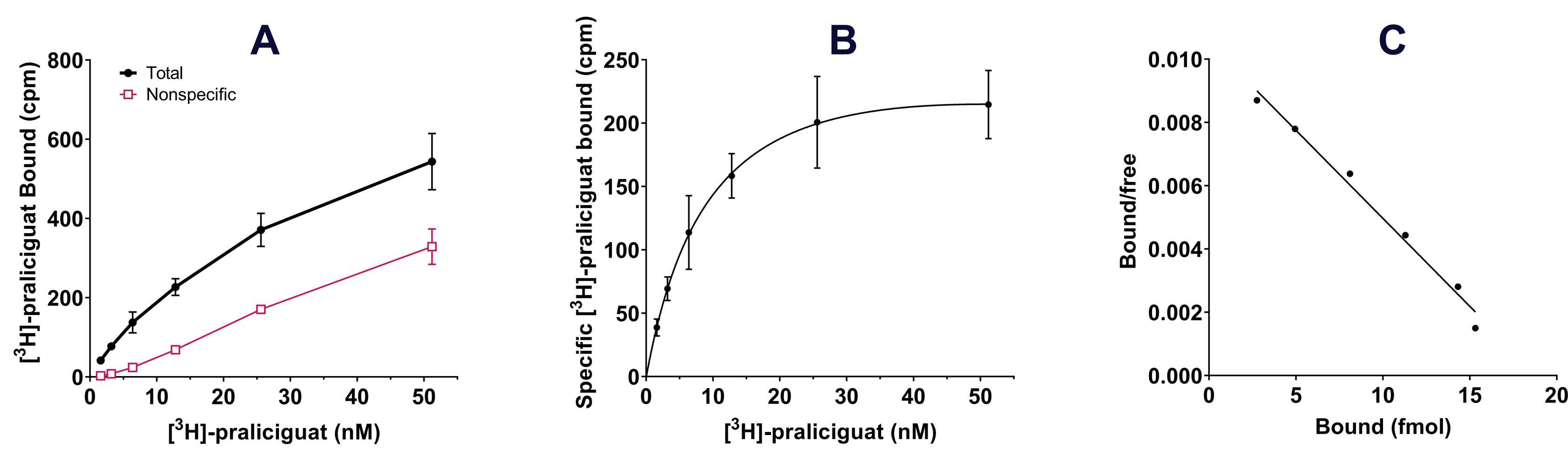
Methods

We adapted a binding assay using [^{3}H]-praliciguat radioligand and size-exclusion chromatography to analyze the binding of praliciguat to purified human recombinant sGC. [^{3}H]-praliciguat was custom synthesized by American Radiolabeled Chemicals (St. Louis, MO) with a specific activity of 14 Ci/mmol. NO was provided as DETA-NO. The binding assay was used to explore cofactors required for binding and was further developed as a competitive binding assay.

Results

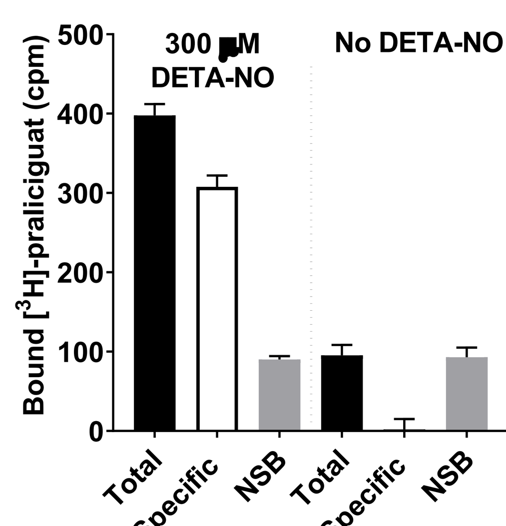
[^{3}H]-Praliciguat bound to human recombinant sGC with high affinity

- Saturable binding with 6-9 nM K_D
- No cooperativity, single binding site (linear Scatchard)



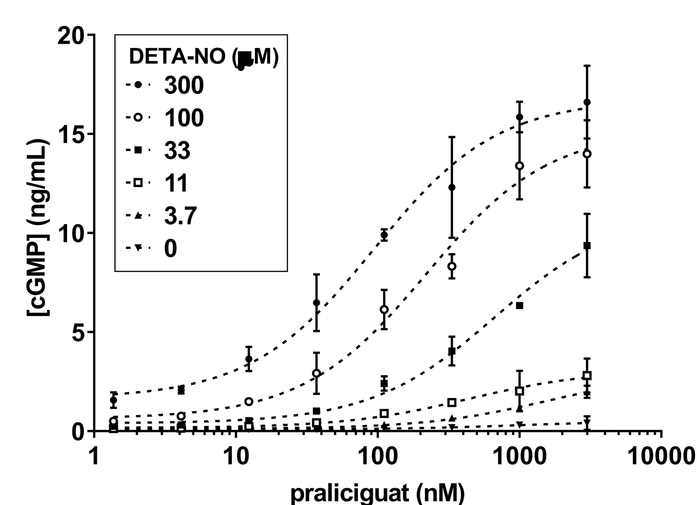
Binding conditions: 40 ng sGC (1.3 nM), 0.3 mM DETA-NO, 0.4 mM GTP, 1.6-51.2 nM [^{3}H]-praliciguat (n=3 replicates). **A.** [^{3}H]-praliciguat, in the presence (Nonspecific) and absence (Total) of 1 µM praliciguat for 22 h at 37°C. **B.** Specific saturation binding curve yield a 6.9 nM K_D and a Hill slope of 1.1. **C.** Scatchard plot resulted in a 9 nM K_D. Under conditions similar to those used for binding, sGC was fully active with a cGMP-forming activity of 10.8 µmol cGMP/min/mg protein (assay conditions: 40 ng sGC, 0.3 mM DETA-NO, 3 mM $MgCl_2$, 0.4 mM GTP, 2 mM DTT, 0.025% BSA; 30 min at room temperature).

Praliciguat binding required NO



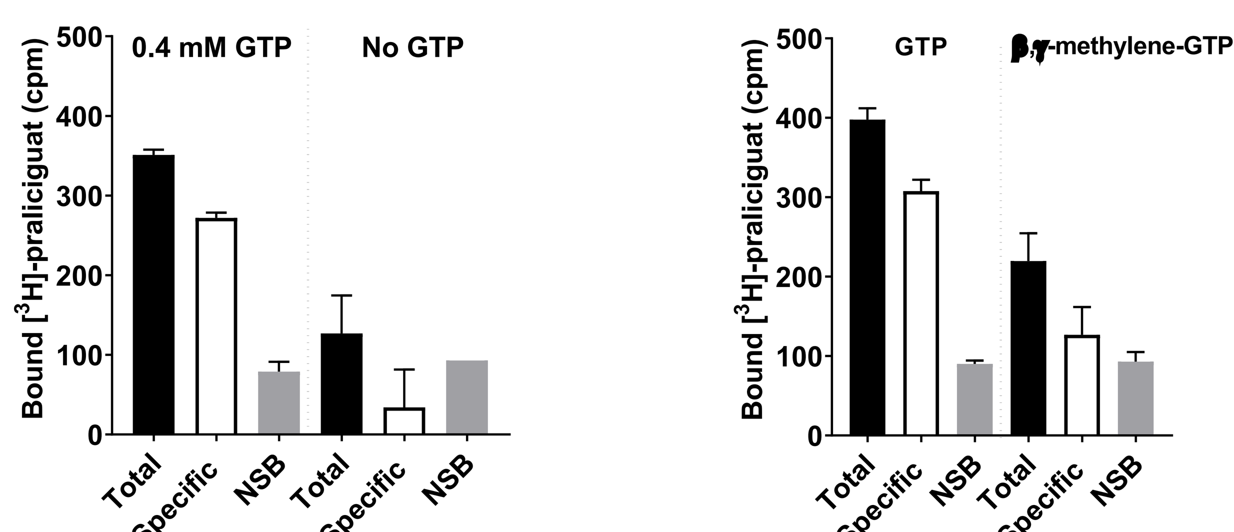
Binding of [^{3}H]-praliciguat to sGC in the presence and absence of DETA-NO

Praliciguat was synergistic with NO in the sGC activity assay



Praliciguat concentration response in human recombinant sGC activity assay under increasing concentrations of DETA-NO (0 µM to 300 µM).

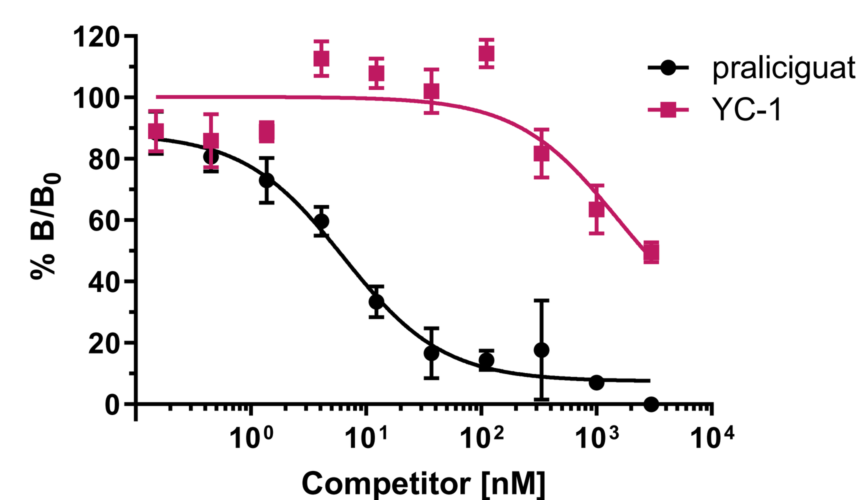
Praliciguat binding was greater with GTP



Binding of [^{3}H]-praliciguat to sGC in the presence and absence of GTP and nonhydrolyzable GTP analogs (0.4 mM).

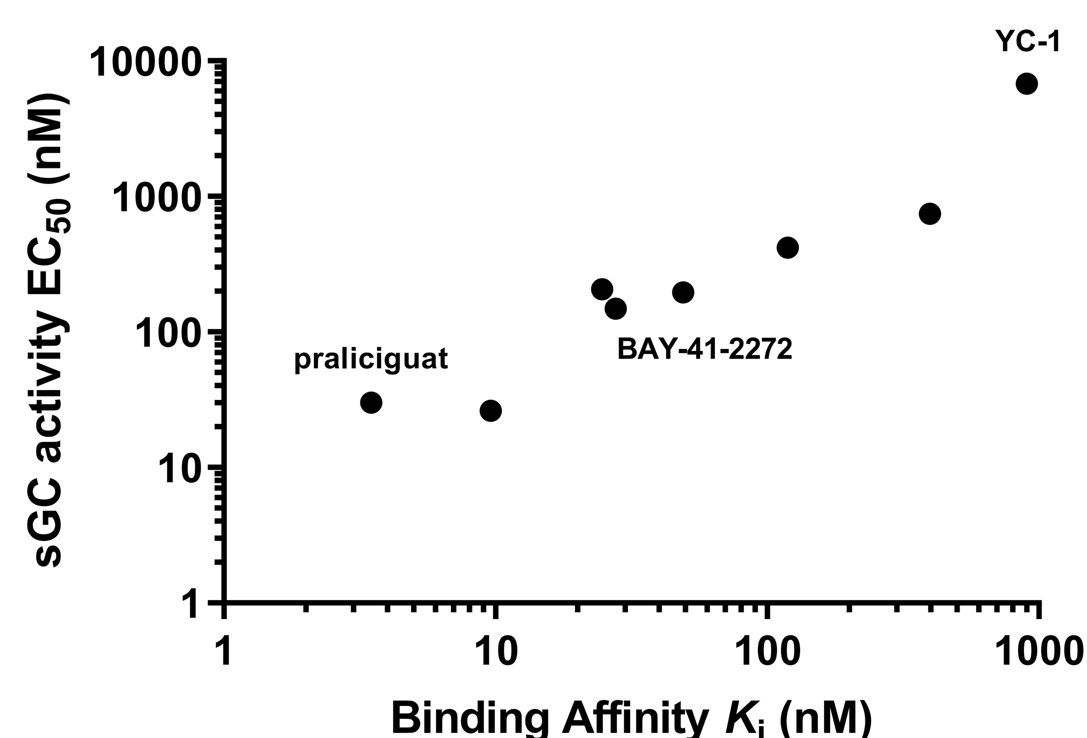
Results (continued)

Competitive radioligand binding



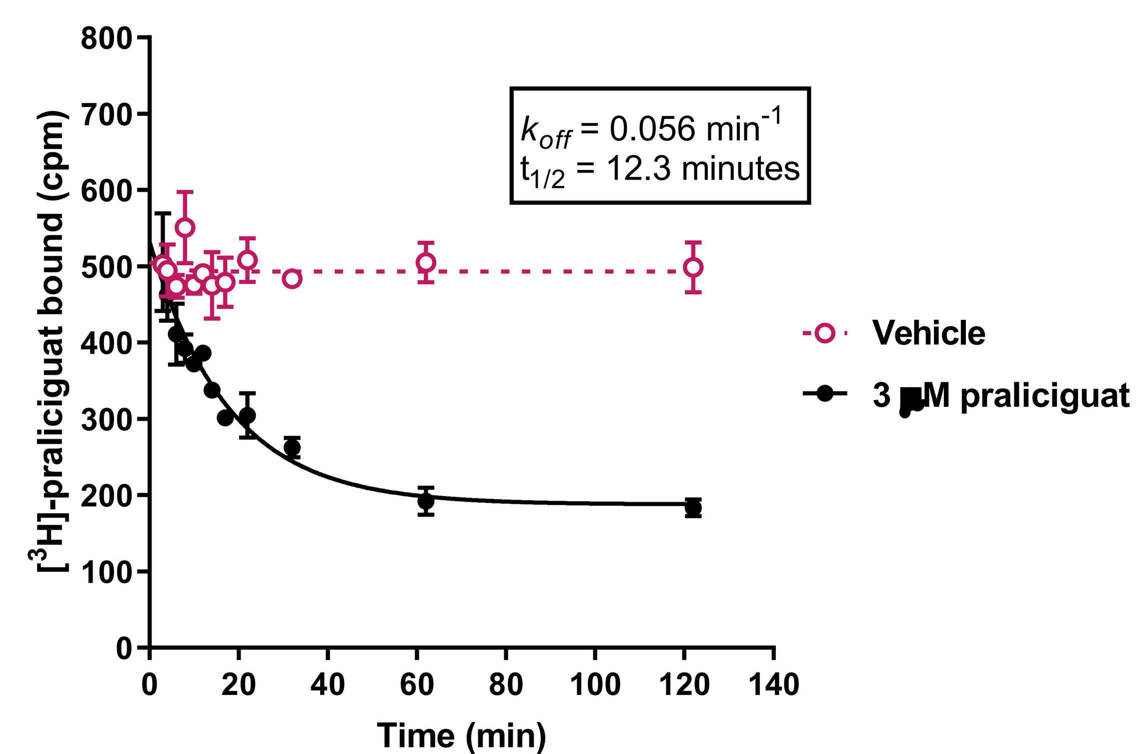
Competitive radioligand-binding curve using [^{3}H]-praliciguat. Human recombinant sGC was incubated with [^{3}H]-praliciguat and increasing concentrations of unlabeled praliciguat or the sGC stimulator YC-1 for 1.5 h at 37°C. sGC was isolated using gel filtration chromatography and radioactivity was quantitated in a liquid scintillation counter. The K_i for praliciguat was 3.64 ± 0.26 nM (n=4 experiments). %B/B0 was calculated as bound radioactivity in the presence of competitor relative to bound radioactivity determined with no competitor.

Binding affinity correlated with potency in cGMP assay



Correlation between relative binding affinities (K_i) of different sGC stimulators in the competitive radioligand binding assay and potency (EC_{50}) in the human sGC activity assay performed in the presence of 30 µM DETA-NO.

In a kinetic assay, the dissociation half-life of praliciguat from sGC was 12.3 minutes



Kinetics of [^{3}H]-praliciguat dissociation from sGC. Human recombinant sGC was incubated with [^{3}H]-praliciguat for 1 h at 37°C followed by challenge with either vehicle (DMSO) or 3 µM praliciguat. [^{3}H]-Praliciguat bound to sGC was isolated at different times after the challenge using gel filtration chromatography and quantitated in a liquid scintillation counter. The calculated association rate (assuming K_D=9 nM and k_{off}=0.056 min^{-1}) was 6.2 X 10^6 M^{-1} min^{-1}.

Conclusions

- Praliciguat bound with high affinity to a single binding site on sGC with no observed cooperativity.
- In the binding assay, praliciguat binding required NO and was greater in the presence of the sGC substrate, GTP.
- Our observations are consistent with the role of praliciguat as an allosteric modulator of sGC, potentiating the activity of NO.
- This binding assay can be used to explore binding kinetics and may be a useful tool for identification and characterization of sGC stimulators with novel binding and kinetic properties.