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# Discovery and Preclinical Characterization of GSK1278863 (daprodustat), A Small Molecule Hypoxia Inducible Factor (HIF)-Prolyl Hydroxylase Inhibitor for Anemia

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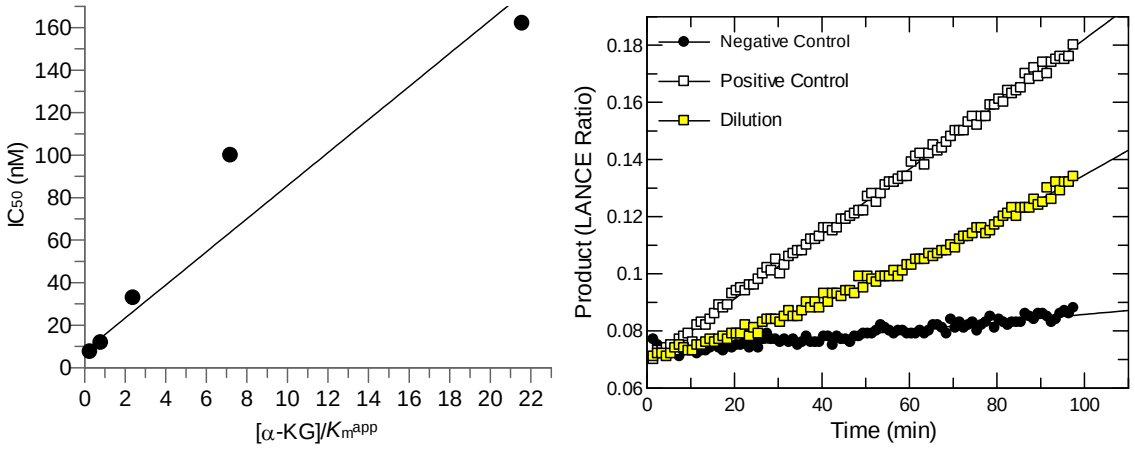
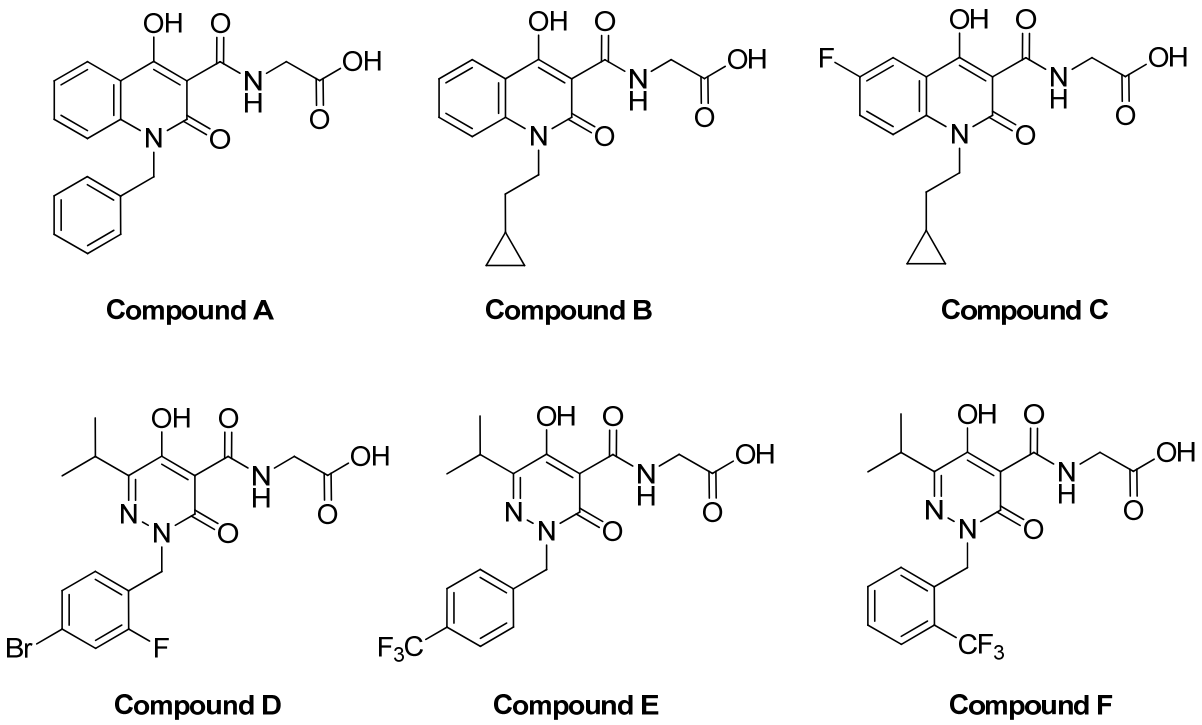



**Supplementary Figure 1**: Mode of inhibition against PHD2 (left) where GSK1278863 $IC_{50}$ values were plotted as a function of [α-KG]/$K_m^{app}$ and fit to an equation for competitive inhibition. Determination of the dissociation half-life value ($t_{1/2}$) for GSK1278863 from PHD2 by rapid dilution method (right). The yellow squares show the enzyme activity after dilution and was fit to an equation to determine the observed rate of recovery ($k_{obs}$). Control reactions at 10x $IC_{50}$ (filled diamonds) and 0.1x $IC_{50}$ (open squares) represent enzyme activity prior to dilution and after dilution if the compound was rapidly reversible.




**Supplementary Figure 2**: Structures of Compounds A-F. Prepared as described in (Chai et al., 2007), (Shaw et al., 2008)

**Supplementary Table 1:** Mean oral pharmacokinetic parameters of GSK1278863in the dog[a]

| Dose (mg/kg) | Version, formulation | $C_{max}$ (μg/mL) | $AUC_{0-24h}$ (μg.h/mL) | $AUC_{0-inf}$ (μg.h/mL) | $DNAUC_{0-inf}$ [b] (μg.h/mL/mg/kg) | Oral F (%) |
|---|---|---|---|---|---|---|
| 3.2[c] | Free acid, solution | 2.54 ± 0.09 | 21.45 ± 4.60 | 28.38 ± 6.80 | 8.76 | 46± 6 |
| 5[d-f] | Free acid, capsule | 2.72 ± 0.25 | 24.09 | 24.65 ± 4.86 | 4.90 | ~25 |

[a]Mean and standard deviation of parameter, where appropriate (n = 3). [b] Dose-normalized AUC(0-inf). [c]Solution in 2% DMSO, 20% (w/v) Captisol® in water, pH~8.0. [d]Crystalline free acid in gelatin capsule. [e]Non-crossover bioavailability estimation. [f]Average estimation from n=2 dogs since data from 24 h sample not available for third dog.

**Supplementary Table 2**: Mean oral pharmacokinetic parameters of GSK1278863 in the mouse

| **Dose (mg/kg)** | **$C_{max}$ (μg/mL)** | **Tmax (hours)** | **$AUC_{0\text{-}24h}$ (μg.h/mL)** | **$DNAUC_{0\text{-}24h}$[b] (μg.h/mL/mg/kg)** |
|---|---|---|---|---|
| 1.9[a,c] | 4.86 | 1.0 | 22.12 | 11.64 |
| 3.9[a,d] | 5.60 | 2.0 | 71.43 | 18.31 |
| 30[e-g] | 31.50 | 6.0 | 427.27 | 14.24 |

[a]Composite sampling design (n = 3 animals per time point) in male CD-1 mice. [b]Dose-normalized AUC(0-24 h). [c]Solution in 2% DMSO, 20% (w/v) Captisol® in water, pH ~ 7-7.5. [d]Solution in 50% PEG-500, 10% ethanol and 40% of 40% (w/v) Encapsin in water. [e]Composite sampling design (n=3 animals per time point) in female B6D2F1/Crl mice. [f]Suspension in 1% methylcellulose. [g]Animals were not fasted overnight prior to dosing.