Supplementary Information

Identification of alkynyl nicotinamide HSN748 as a RET solvent-front mutant inhibitor with intracranial efficacy

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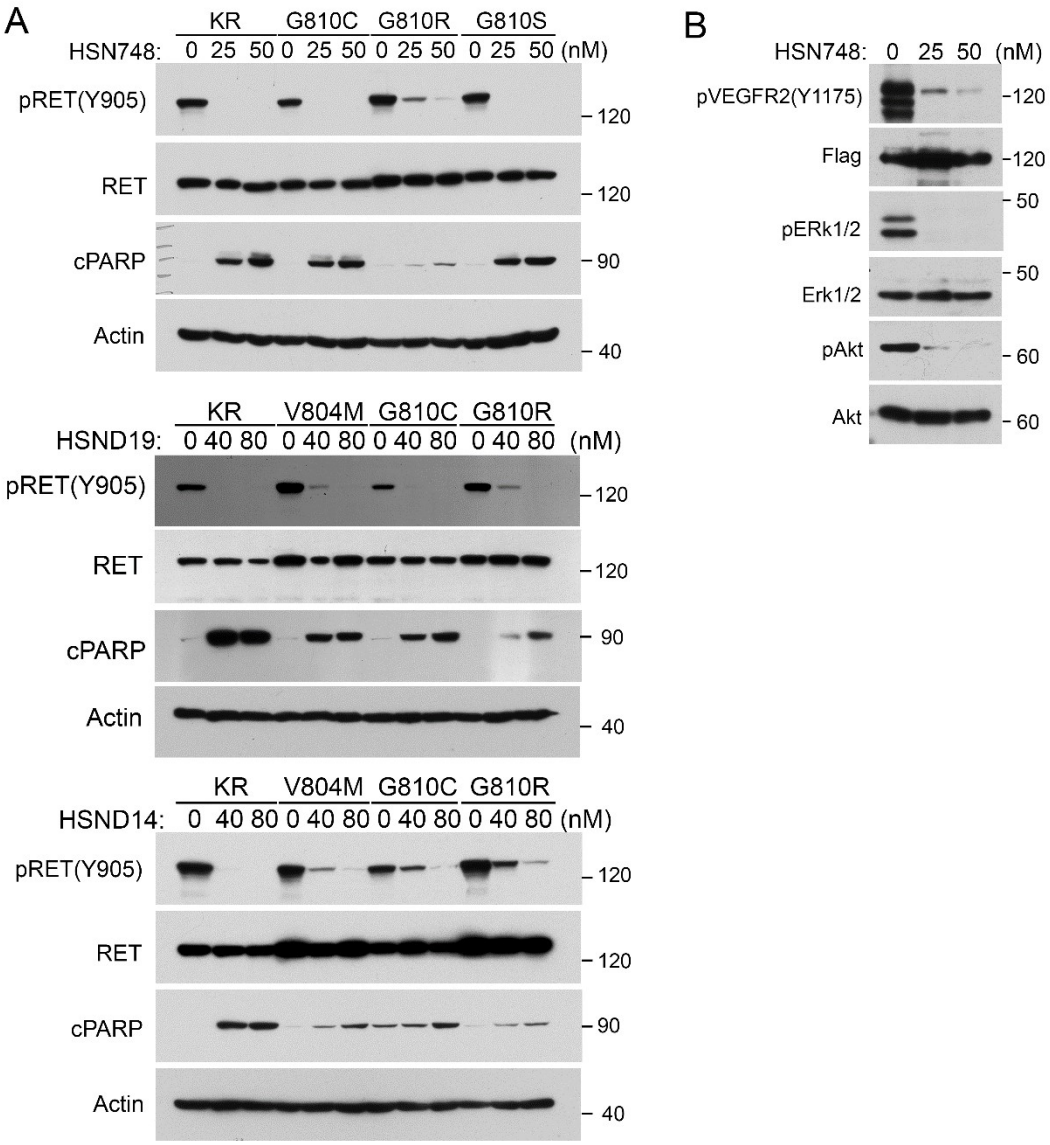

Figure S1. Immunoblotting analyses of cell lysates.
(**A**) Immunoblotting analysis of compounds on inhibition of KR or its mutant kinase activity and induction of apoptosis in B/KR and mutant cells. (**B**) Immunoblotting analysis of HSN748-treated B/TV cells.

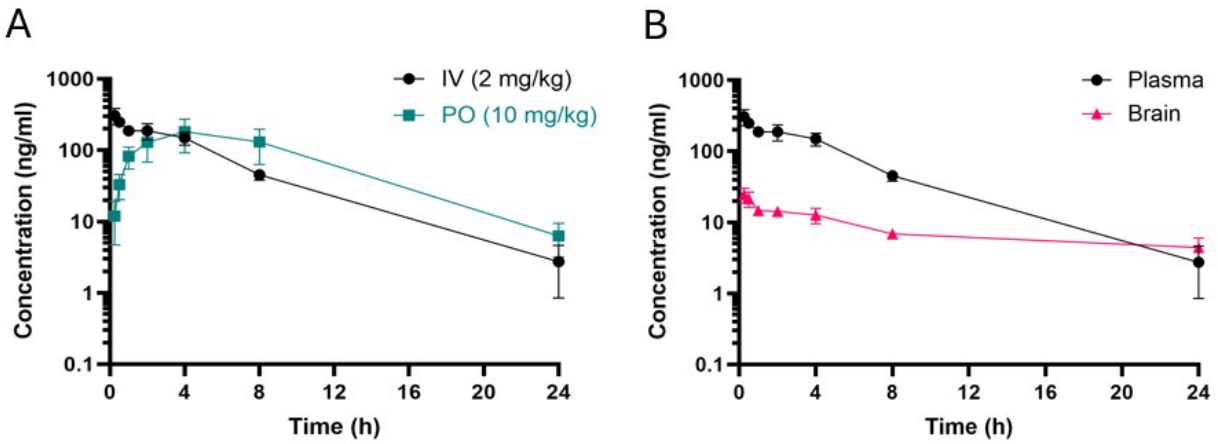

Figure S2. Pharmacokinetics of HSND19 in rats.
(**A**) Plasma concentration-time profiles of HSND19 in rats following an intravenous (IV) dose of 2 mg/kg and an oral (PO) dose of 10 mg/kg. (**B**) Concentration-time profiles of HSND19 in plasma and brain following an IV dose of 2 mg/kg in rats. Each data point represents the mean ± SD from three rats, except for the last time point in the brain profile, where one concentration was below the limit of quantification. The IV plasma profile in panel A is derived from the IV PK study shown in panel B.

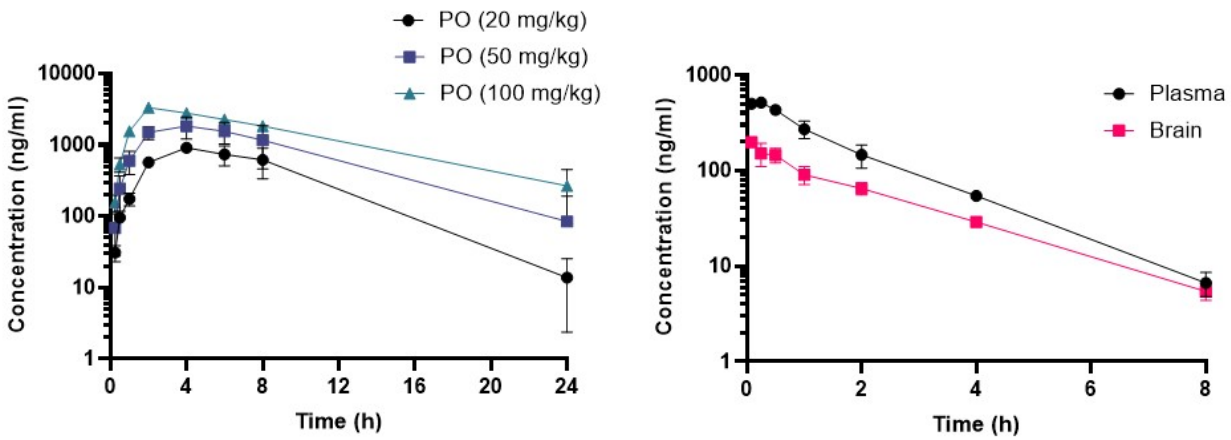

Figure S3. Pharmacokinetics of HSN748 in rats and mice.
(**A**) Plasma concentration-time profiles of HSN748 in rats following oral doses of 20, 50, and 100 mg/kg. (**B**) Concentration-time profiles of HSN748 in plasma and brain following an IV dose of 2 mg/kg in mice. Each data point represents the mean ± SD from three animals.

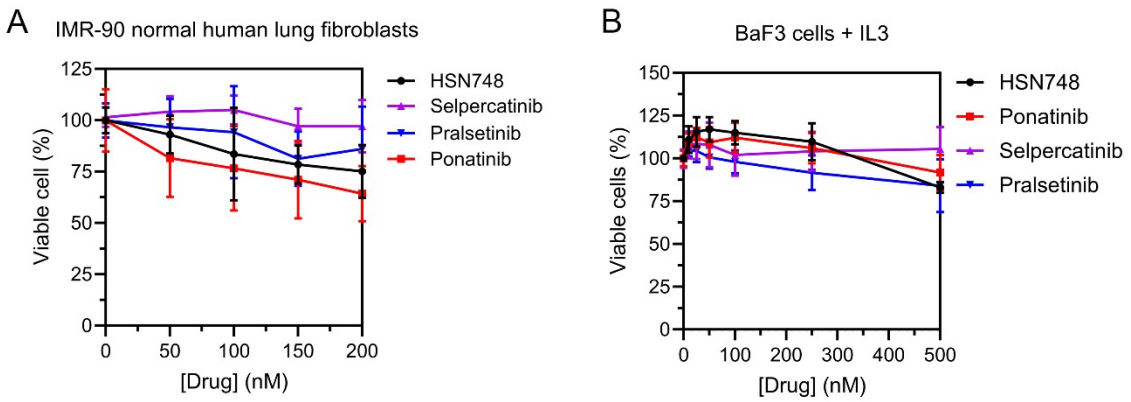

Figure S4. Activity of HSN748 in normal human fibroblasts and IL3-dependent BaF3 cells (**A**) IMR-90 normal human lung fibroblast cells were plated in 96-well plates and treated with indicated drugs for 5 days. (**B**) The non-transformed, IL-3-dependent BaF3 cells were cultured in medium with 0.5 nM mouse IL-3 in 96-well plates and treated as in (**A**) for 3 days. Viable cells were then determined using CellTiter-glo reagent. Data were from two triplicate experiments.

^{1}H and ^{13}C spectra’s

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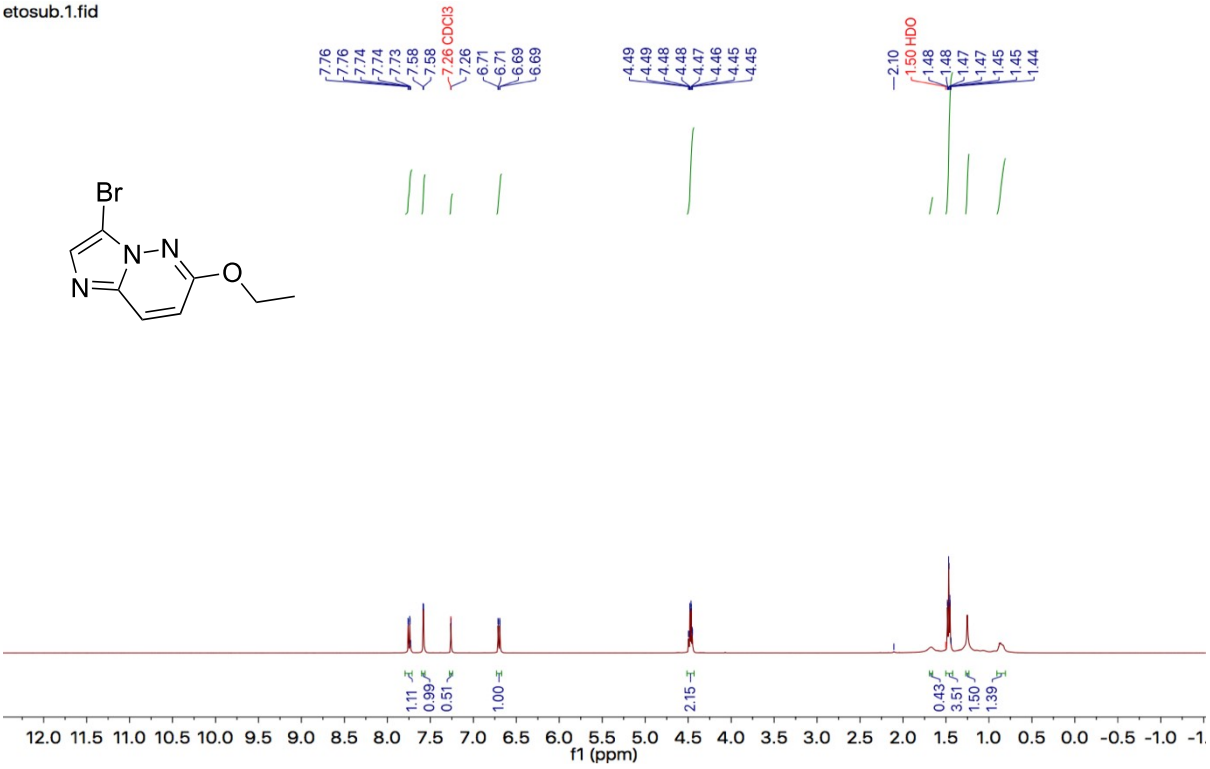

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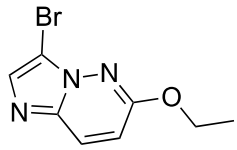

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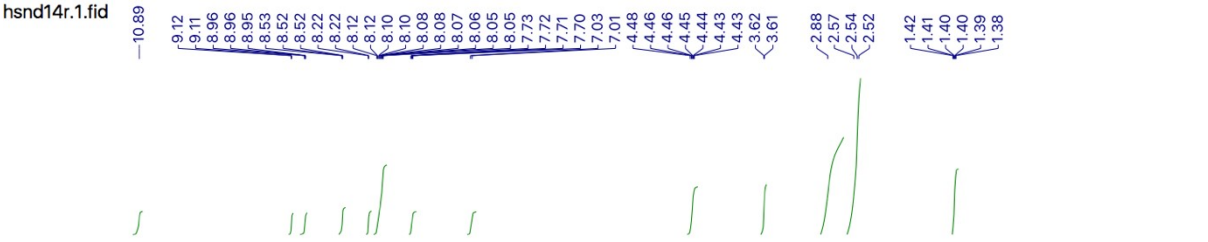

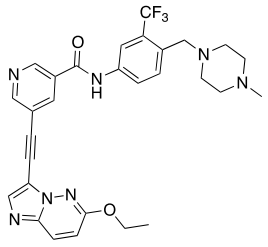

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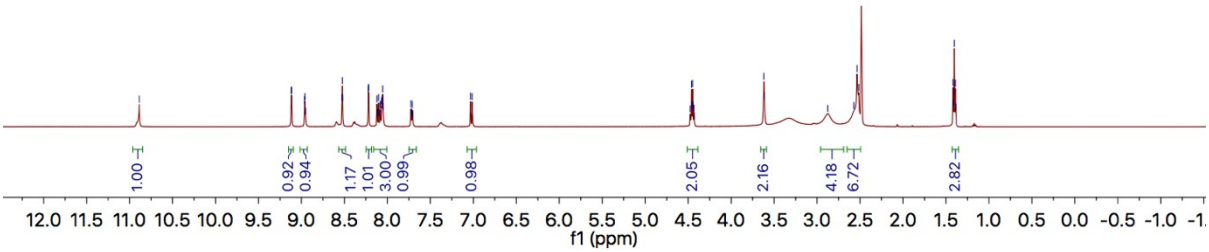

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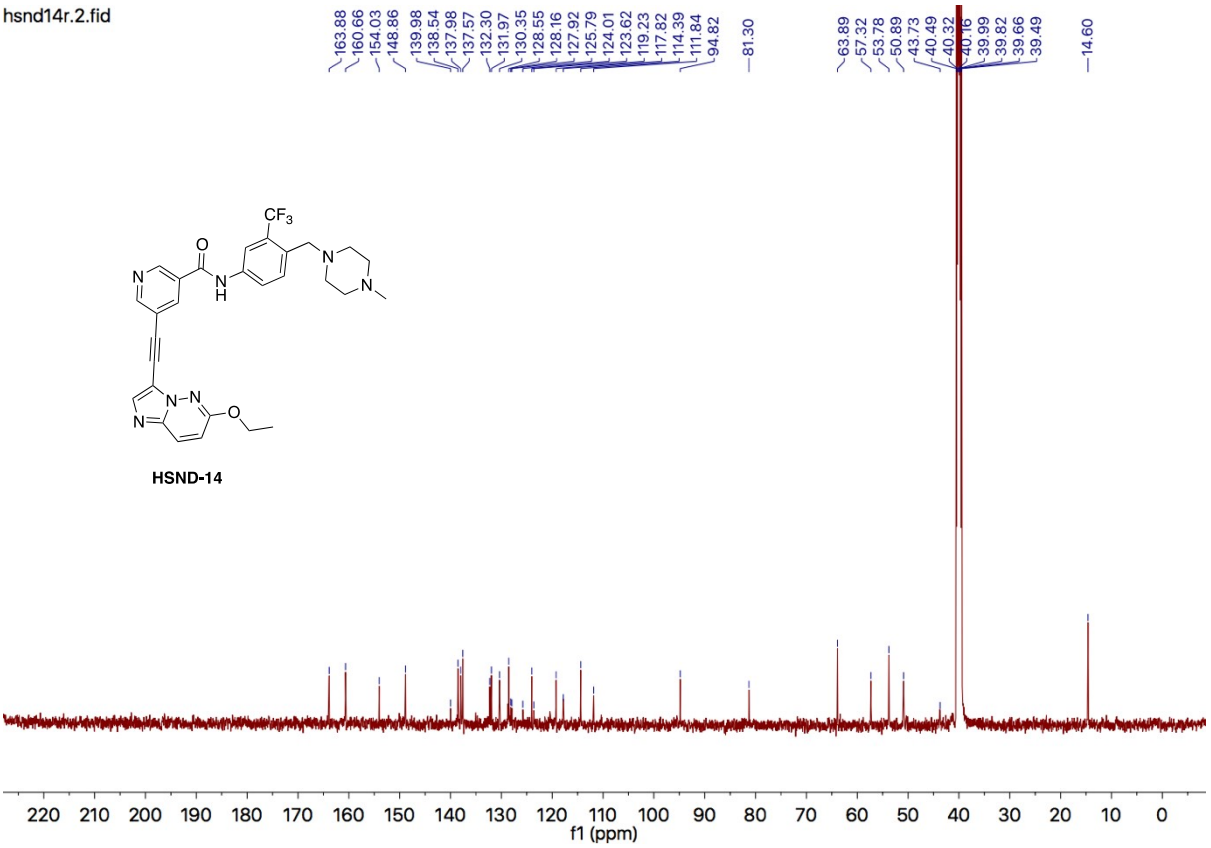

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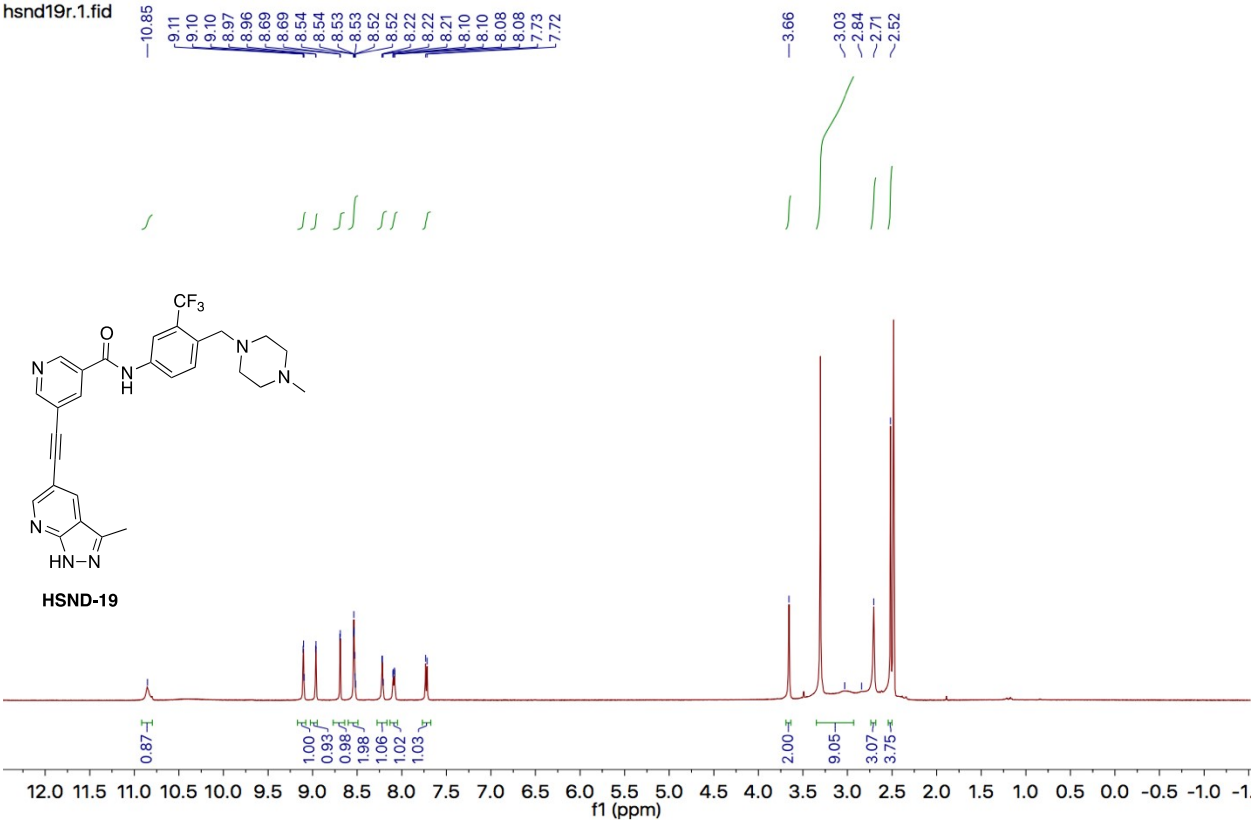

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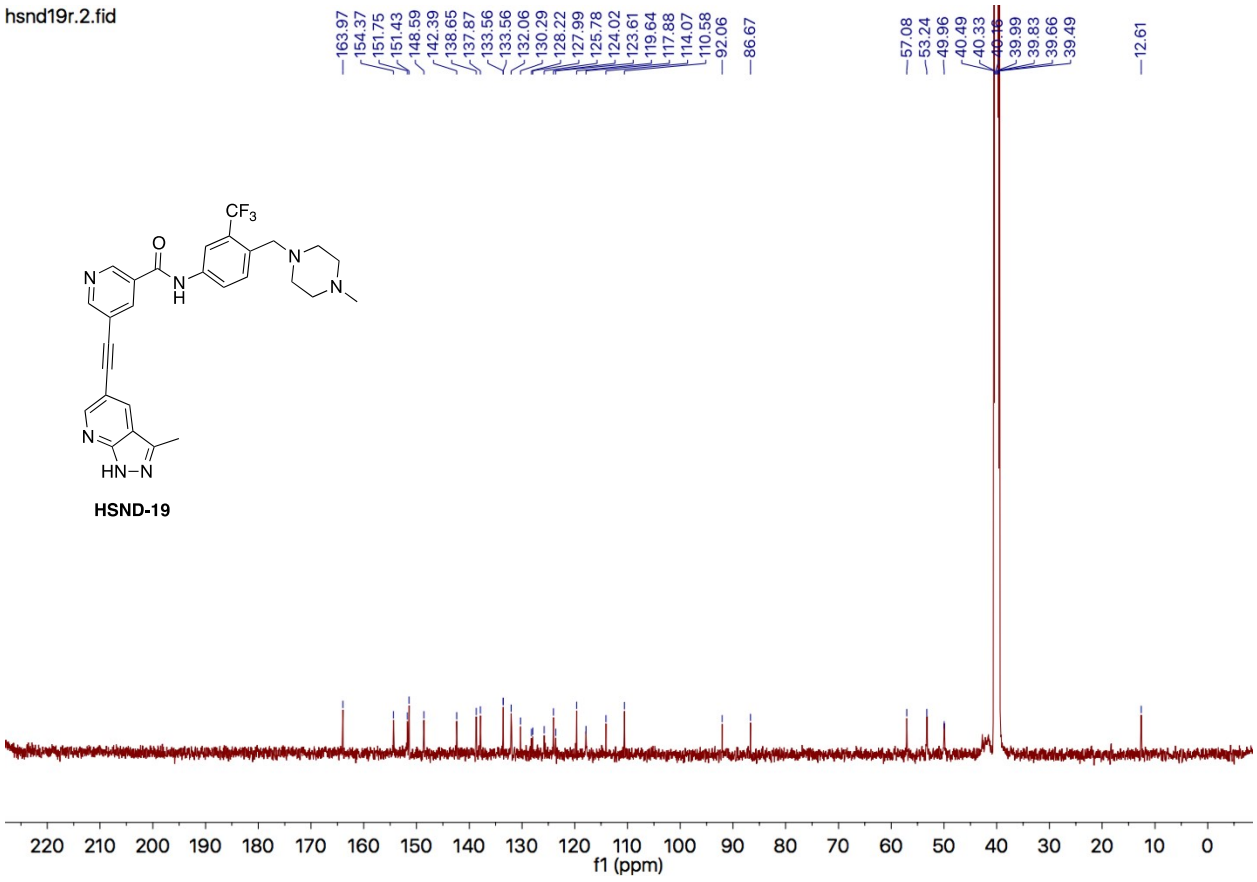

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