Fosgonimeton, a Novel, Small-Molecule Positive Modulator of the HGF/MET System, Is Neuroprotective in Primary Neuron Culture

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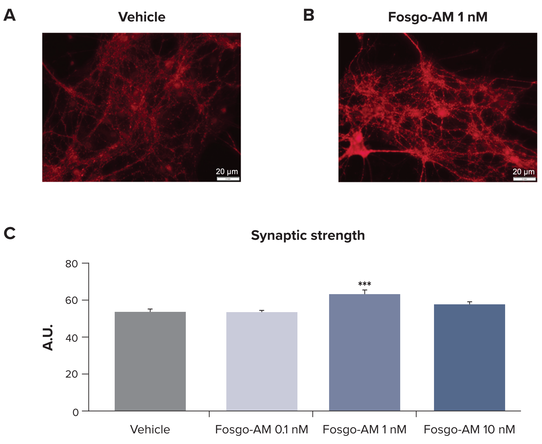
SUPPLEMENTAL INFORMATION

Figure S1. Fosgo-AM increases synaptic strength based on synaptic vesicle density



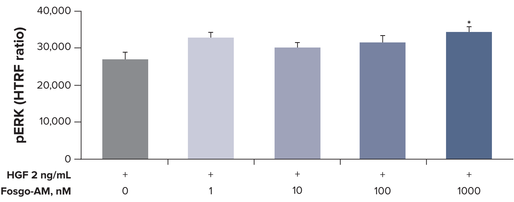
Primary rat hippocampal neurons were cultured and treated with fosgo-AM for 6 days and stained for synaptobrevin II, a marker of synaptic vesicles. Representative images from (**A**) vehicle and (**B**) fosgo-AM 1 nM highlight the effect of fosgo-AM on synaptic strength (relative abundance of presynaptic vesicles per synapse as measured by synaptobrevin II fluorescence intensity). (**C**) Fosgo-AM 1 nM significantly enhanced synaptic strength.

Data presented as mean + SEM.

Statistical significance was determined by 1-way ANOVA with the Dunnett posttest.

***$P < 0.001$ compared with vehicle.

Figure S2. Fosgo-AM enhances ERK phosphorylation



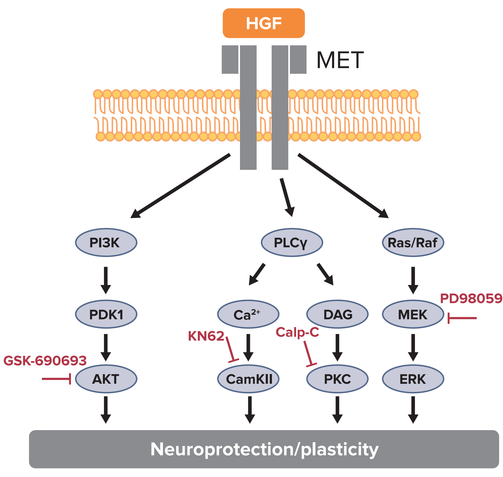
HEK-293 cells were subjected to HTRF with HGF (2 ng/mL) alone or with fosgo-AM (1 nM, 10 nM, 100 nM, or 1 µM). pERK was measured using the advanced phospho-ERK (Thr202/Tyr204) cellular kit (#64AERPEG; CisBio). Treatment with fosgo-AM + HGF in cultured HEK-293 cells led to increased pERK relative to HGF alone (which does not significantly increase pERK at 2 ng/mL; data not shown)

Data presented as mean + SEM.

Statistical significance was determined by 1-way ANOVA with the Dunnett posttest.

*$P < 0.05$ compared with control (grey)

Figure S3. Inhibition of the AKT, CamKII, PKC, and MEK pathways downstream of HGF/MET signaling suggests potential mechanisms for fosgo-AM's neuroprotective effects



Inhibition with GSK-690693, KN62, Calp-C, or PD98059 (red) disrupts downstream elements of the HGF/MET pathway. While inhibition of AKT, PKC, and MEK resulted in disruption of fosgo-AM's neuroprotective effects, inhibition of CamKII did not.

Abbreviations **AKT**, protein kinase B; **ALS**, amyotrophic lateral sclerosis; **Calp-C**, calphostin C; **CamKII**, calcium/calmodulin-dependent protein kinase II; **DAG**, diacylglycerol; **ERK**, extracellular signal-regulated kinase; **fosgo-AM**, active metabolite of fosgonimeton; **HEK-293**, human embryonic kidney 293; **HGF**, hepatocyte growth factor; **HTRF**, homogeneous time-resolved fluorescence; **MEK**, mitogen activated protein kinase; **MET**, mitogen-activated protein kinase kinase; **PDK1**, phosphoinositide-dependent kinase 1; **pERK**, phosphorylation of ERK; **PI3K**, phosphatidyl-inositol 3 kinase; **PKC**, protein kinase C; **PLCγ**, phospholipase Cγ.

Acknowledgments

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Disclosures

Sherif Reda, Jewel Johnston, Robert Taylor, and Kevin Church are employees and stockholders of Athira Pharma, Inc.

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