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Development of Stable, Orally Bioavailable, Small-Molecule Positive Modulators of HGF/MET Signaling for the Treatment of Cognitive Impairment

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CONCLUSIONS

1. The design and synthesis of novel HGF/MET positive modulators produced a series of active small molecules with favorable physiochemical properties
2. Several compounds, including the two example compounds (compounds 2 and 6), were orally bioavailable and distributed efficiently to the brain
3. Oral administration of compound 2 or 6 in a chemically induced model of spatial memory deficit significantly restored cognitive performance

KEY TAKEAWAY

Novel, orally bioavailable small-molecule HGF/MET positive modulators were distributed efficiently to the brain and rescued spatial memory deficits; based on these promising preclinical results, these compounds are being developed as potential therapeutic agents for Alzheimer's disease and other neurological disorders

Acknowledgments

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Disclosures

Robert Taylor, Doug Boatman, Jewel Johnston, Maya Kneip, and Kevin Church are employees and stockholders of Athira Pharma, Inc.

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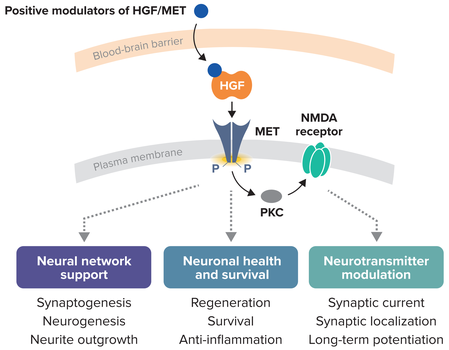
INTRODUCTION

Figure 1. Positive modulators of the HGF/MET pathway may promote regenerative processes and inhibit inflammation



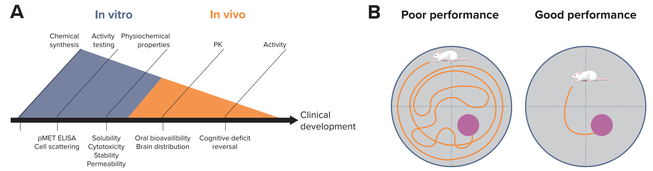
- HGF/MET signaling promotes neuroprotective, neurotrophic, and anti-inflammatory mechanisms[1-4]
- MET expression is reduced in neurodegenerative disorders such as Alzheimer's disease[5]
- Based on 3-dimensional conformation modeling of first-generation HGF/MET modulators, a series of cyclized compounds were generated, with a focus on increasing oral bioavailability while reducing both molecular weight and rotatable bonds
- A screening funnel was developed to evaluate the in vitro activity, physiochemical properties, oral brain distribution, and in vivo activity of the resulting compound series
- Two example compounds that are orally bioavailable, brain penetrant, and capable of reversing chemically induced spatial memory deficits are reported

OBJECTIVE

To develop novel, small-molecule positive modulators of the HGF/MET neurotrophic system that are orally bioavailable, can cross the blood-brain barrier, and improve cognitive performance in a rodent model of spatial memory deficit

METHODS

Figure 2. A series of cyclized compounds based on modeling of first-generation HGF/MET positive modulators were processed through a screening funnel to identify compounds with sufficient activity, stability, bioavailability, and in vivo activity to proceed to clinical development



(**A**) Each stage of the screening process proceeded stepwise to identify activity, stability, physiochemical properties, PK, and in vivo activity.
(**B**) Efficacy was assessed through a behavioral assay using the MWM.

In Vitro

- Small batches of chemicals were synthesized in solution-phase chemistry, and molecules were characterized by HPLC and NMR
  - Physiochemical properties were calculated by use of DataWarrior (OpenMolecules)
- MET activation was assessed using a pMET sandwich ELISA kit (Cell Signaling Technologies) in HEK-293 cells that were incubated for 15 minutes with various small molecules and a subthreshold dose of HGF (1 ng/mL)
- Cell scattering was assayed by imaging MDCK cells – labeled with a nonspecific membrane stain (WGA-488) – treated for 24 hours with small molecules and a subthreshold dose of HGF (5 ng/mL)
- Solubility, stability, and permeability were also assessed (**Supplemental Methods, QR code**)

In Vivo

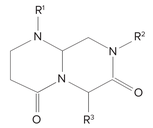
- PK was assessed in Sprague Dawley rats that received small molecules (IV or PO); bioavailability was assessed by comparing the AUC of PK curves
- Brain distribution was assessed after IV administration of novel small molecules
  - After 15 minutes, animals were anesthetized by use of isoflurane and perfused with PBS before dissection and homogenization of target tissue
  - Test compounds were quantified by detection via LC-MS/MS
  - Predicted unbound brain exposure was calculated under consideration of dose normalization, nonspecific protein binding, oral bioavailability, and brain distribution
- By use of the MWM, spatial memory performance was evaluated in rats
  - Behavioral data were assessed 5 times on each of 8 consecutive days
  - The time to find the submerged escape platform (escape latency) was recorded for each trial
  - 60 minutes before testing, rats received test compounds (indicated dose, PO)
  - 20 minutes before testing, rats received a cholinergic transmission inhibitor (scopolamine, 3 mg/kg IP) to induce an amnesic state

RESULTS

Figure 3. Several compounds were identified for further development based on in vitro activity and favorable physiochemical properties

Compound	R1	R2	R3	pMET ELISA	Cell scattering	Solubility	Stability	Permeability
2			CH3					
3			CH3					
6			CH3					
8			CH3					
12			CH3					
30			CH3					
32			CH3					
35			CH3					
49								
50								

R region key

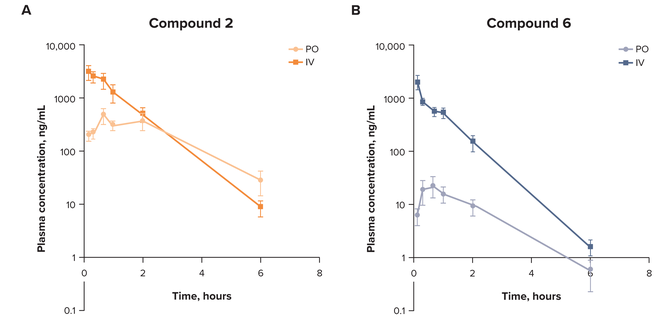


Acceptable
Unacceptable
Not tested

Of 74 compounds, 10 are highlighted to exemplify the various R regions (R1, R2, and R3) explored in this set of cyclized compounds. Each compound's performance in a series of assays designed to assess activity and drug-like properties was used to determine whether they were acceptable (turquoise) or unacceptable (grey) for ongoing development.[a] White indicates that the compound was not tested in the indicated assay. Based on these results, a series of compounds were selected for further development. Here we present example data related to compound 2 and compound 6 (indicated by orange and blue boxes).

[a]Development thresholds are as follows: pMET ELISA, statistically significant augmentation of pMET above HGF 1 ng/mL alone; cell scattering, statistically significant augmentation of colony-scattering behavior above HGF 5 ng/mL alone; solubility, aqueous solubility ≥300 uM; stability, >50% compound remaining after 4 hours in the following solutions: simulated gastric fluid, simulated intestinal fluid, rat plasma, and human plasma; permeability, greater than 2×10^{-6} cm/s as measured in the parallel artificial membrane permeability assay.

Figure 4. Tested compounds were orally bioavailable



Plasma concentration of (**A**) compound 2 and (**B**) compound 6 over time after PO or IV dosing.
Data are mean ± SEM (n = 8 animals).

- Example compounds (compound 2 and compound 6) were rapidly distributed and cleared from the plasma when administered IV; both compounds exhibited slower distribution with oral administration. Other tested compounds (not shown) also had similar bioavailability results

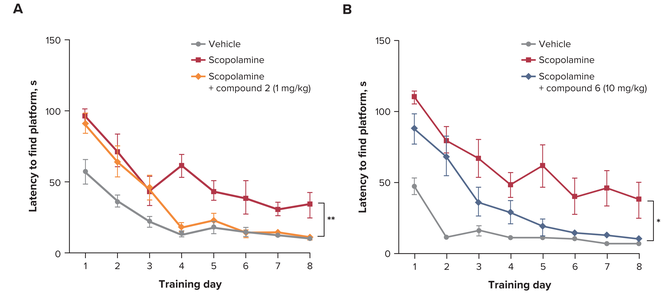
Table 1. Modeling of unbound concentration of compounds in the brain suggested that the cyclized compounds can achieve therapeutic doses with oral dosing

	Compound 2 Mean ± SEM, nM	Compound 6 Mean ± SEM, nM
Whole brain	8.40 ± 1.43	1.02 ± 0.22
Hippocampus	7.26 ± 0.98	0.90 ± 0.19
Cerebellum	6.78 ± 2.19	1.05 ± 0.25
Cortex	9.10 ± 1.66	1.20 ± 0.22

Calculated brain exposure after 1 mg/kg dose PO.

- Compound 2 and compound 6 both distributed to all evaluated regions of the brain. Dose modeling indicated that lower doses of compound 2 will likely be needed to reach neuroactive exposures

Figure 5. Novel compounds restored spatial memory performance in the MWM



Both example compounds demonstrate rescue of scopolamine-induced spatial memory deficits, with (**A**) compound 2 and (**B**) compound 6 showing significant restoration of spatial memory performance (2-way ANOVA).
Data are mean ± SEM (n = 8 animals).
*P = 0.033; **P = 0.004.

Abbreviations **ANOVA**, analysis of variance; **AUC**, area under the curve; **ELISA**, enzyme-linked immunosorbent assay; **HEK-293**, human embryonic kidney 293; **HGF**, hepatocyte growth factor; **HPLC**, high-performance liquid chromatography; **IP**, intraperitoneally; **IV**, intravenously; **LC-MS/MS**, liquid chromatography with tandem mass spectrometry; **MDCK**, Madin-Darby canine kidney; **MWM**, Morris water maze; **NMDA**, *N*-methyl-D-aspartate; **NMR**, nuclear magnetic resonance; **P**, phosphorylation; **PBS**, phosphate-buffered saline; **PK**, pharmacokinetics; **PKC**, protein kinase C; **pMET**, phosphorylation of MET; **PO**, orally; **SEM**, standard error of the mean.

References **1.** Ebens A et al. *Neuron.* 1996;17:1157-1172. **2.** Maina F, Klein R. *Nat Neurosci.* 1999;2:213-217. **3.** Shang J et al. *J Neurosci Res.* 2011;89:86-95. **4.** Nakamura T, Mizuno S. *Proc Jpn Acad Ser B Phys Biol Sci.* 2010;86:588-610. **5.** Hamasaki H et al. *Neuropathology.* 2014;34:284-290.

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Poster #122

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Supplemental methods

Solubility

- Solubility was assessed by turbidity assay measuring optical clarity of aqueous solutions at 620 nM
- Compounds were considered soluble in the absence of measurable turbidity at a concentration of 300 µM

Stability

- Compound stability was assessed by quantification of remaining compound following 4-hour incubation in simulated digestive fluids and blood plasma from rats and humans
- Compounds were considered stable if at least 50% remained at the end of a 4-hour incubation period in all fluid categories

Permeability

- Membrane permeability was predicted using a parallel artificial membrane permeability assay (PAMPA, Corning Cat#353015) using manufacturer's instructions
- Permeability measurements of Pe > 2E-6 cm/s were considered acceptable for further development