SEMAX — PHE-4 → 4-FLUORO-PHENYLALANINE (4F-PHE) SUBSTITUTION; PARA-FLUORINE ON THE AROMATIC RING OF THE CONSERVED HIS-PHE-ARG-TRP-DERIVED PHE PHARMACOPHORE RESIDUE

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PROMISING COGNITIVE

PHE-4 → 4-FLUORO-PHENYLALANINE (4F-PHE) SUBSTITUTION; PARA-FLUORINE ON THE AROMATIC RING OF THE CONSERVED HIS-PHE-ARG-TRP-DERIVED PHE PHARMACOPHORE RESIDUE

MELANOCORTIN RECEPTOR 4

AVERAGE CONFIDENCE	PTM / IPTM	VERDICT
82.7%	0.843 / 0.957	PROMISING
TARGET	**UNIPROT**	**BINDING PROBABILITY**
Melanocortin receptor 4	P32245	—

TLDR

Fold №24 applies a para-fluoro phenylalanine substitution at position 4 of Semax (MEHFPGP → MEH[4F-F]PGP), targeting MC4R aromatic pocket selectivity. While the structural prediction metrics were technically adequate (pLDDT 0.83, pTM 0.84), the fold is DISCARDED on biological grounds: the literature reveals Semax lacks the canonical Arg-Trp (HFRW) dipeptide required for meaningful MC4R occupancy, making subtype selectivity optimization premature. The modification hypothesis is scientifically coherent in principle but built on an unvalidated receptor engagement premise that the evidence does not support.

EXECUTIVE SUMMARY

Semax Phe-4 → 4F-Phe: pLDDT 0.83, structurally confident — but DISCARDED. Semax lacks the canonical Arg-Trp MCR pharmacophore; no published MC4R binding data exists for this scaffold. Selectivity optimization is premature without affinity evidence.

DETAILED ANALYSIS

Semax (MEHFPGP) is a synthetic heptapeptide derived from the ACTH(4-7) fragment, extended with a C-terminal Pro-Gly-Pro tail. Its neuroprotective and nootropic profile is well-established across stroke, spinal cord injury, neuroinflammation, and neurotrophic factor upregulation contexts. Crucially, however, its pharmacological mechanisms are pleiotropic — encompassing BDNF/TrkB upregulation, monoaminergic modulation, copper chelation via the Met-Glu-His triad, USP18-mediated ubiquitination effects, and anti-inflammatory cytokine regulation — with no published study directly demonstrating binding to or functional agonism at any specific melanocortin receptor subtype (MC1R–MC5R).

The modification hypothesis for Fold №24 targets MC4R selectivity. Phe-4 in Semax corresponds to the Phe residue of the canonical His-Phe-Arg-Trp (HFRW) melanocortin pharmacophore, the core tetrapeptide responsible for high-affinity MCR binding across α-MSH and ACTH analogs. The hypothesis proposes that para-fluorination of this residue would perturb the aromatic ring quadrupole moment and modestly shift lipophilicity, exploiting subtle differences in transmembrane pocket residue identity between MC4R and other MCR subtypes (notably MC4R Ile-194 vs. MC1R Met-128) to bias receptor engagement toward MC4R.

The structural prediction pipeline returned metrics that would ordinarily warrant attention: pLDDT 0.83, pTM 0.84, and a notably high ipTM of 0.96. These numbers suggest the prediction algorithm found a confident, geometrically stable pose for the modified heptapeptide. The heuristic peptide profile assigns low aggregation propensity (0.0), a stability score of 0.80, moderate BBB penetration estimate (0.46), and a short estimated half-life of 15–45 minutes — consistent with the unprotected native Semax profile and not dramatically altered by the single ring fluorination.

Despite these structurally adequate metrics, the DISCARDED verdict is scientifically justified on biological grounds. The fundamental problem is that Semax lacks the Arg and Trp residues (positions 8-9 of ACTH) that constitute the complete HFRW pharmacophore. Semax retains only the HF dyad. The literature consensus across melanocortin receptor structural biology is that the full HFRW tetrapeptide — particularly the Arg and Trp residues — is required for high-affinity MC4R engagement. Optimizing subtype selectivity through Phe-4 fluorination is therefore premature if basal MC4R affinity of the Semax scaffold has not been established.

There is no published Ki, IC50, or Kd for Semax at any MCR subtype, and the C-terminal Pro-Gly-Pro extension (which is the key divergence from canonical ACTH/α-MSH analogs) may impose conformational constraints that further preclude classical HFRW docking geometry.

The literature does offer indirect support for a melanocortinergic component to Semax's activity — Eremin et al. (2005) explicitly invokes the anatomical and functional links between melanocortinergic and monoaminergic systems to contextualize Semax's dopaminergic effects — but this indirect inference is a far cry from receptor occupancy data. The copper-chelation literature (two recent studies on the MEH coordination motif) actually provides one useful insight: Phe-4 is not a primary coordination residue for copper, suggesting the position is chemically permissive for substitution without abolishing that particular activity. However, this permissiveness does not translate into evidence of MCR engagement.

In the context of the lab's recent fold history, this fold is notably distinct in its selectivity orientation. Fold №1 explored N-terminal acetylation of Semax (refined, pLDDT 0.80), a metabolic stability modification that does not presuppose a specific receptor mechanism. Folds №8 and №18 on the structurally homologous Selank peptide pursued C-terminal amidation and N-methylation respectively, both targeting proteolytic resistance with more tractable mechanistic premises. The selectivity-through-fluorination approach of Fold №24 represents the most pharmacologically ambitious hypothesis in this cognitive peptide series — and precisely because it builds on an unvalidated mechanistic foundation, the structural confidence numbers cannot rescue it from a DISCARDED verdict.

The negative result here is genuinely informative: it demarcates the boundary of what the current prediction framework can usefully evaluate for Semax. Structural predictions require a defensible receptor-ligand model, and that model requires at least indirect evidence of receptor engagement. Future work should either establish Semax MCR binding affinity empirically, or pivot the selectivity hypothesis to a target better supported by the existing Semax mechanistic literature — BDNF/TrkB pathway modulation, copper coordination geometry tuning, or DPP-IV resistance (as explored in adjacent folds) are all better-anchored premises for in silico exploration.

RESEARCH BRIEF

FOLD №24 — SEMAX PHE-4 → 4-FLUORO-PHE: MC4R SELECTIVITY TUNING

Verdict: DISCARDED | Peptide: Semax (MEHFPGP) | Class: Cognitive | Target: MC4R

MECHANISM OF ACTION (BACKGROUND)

Semax (Met-Glu-His-Phe-Pro-Gly-Pro) is a synthetic heptapeptide derived from the ACTH(4-7) fragment with a C-terminal Pro-Gly-Pro extension, originally developed as a neuroprotective and nootropic agent. Its documented mechanisms are pleiotropic: upregulation of BDNF and NGF via TrkB/C signaling, modulation of dopaminergic and serotonergic neurotransmission, copper chelation through the Met-Glu-His N-terminal triad, anti-inflammatory cytokine regulation, and recently described effects on the USP18/ubiquitination pathway. Its structural heritage from ACTH(4-10) implies a melanocortinergic lineage — the His-Phe dyad (positions 3-4) is a remnant of the canonical His-Phe-Arg-Trp (HFRW) pharmacophore central to MC receptor binding across α-MSH and ACTH analogs — but **no published study directly demonstrates Semax binding to, or functional agonism at, any MC receptor subtype (MC1R–MC5R)** at defined pharmacological concentrations.

Critically, Semax lacks the Arg and Trp residues of the HFRW tetrapeptide that are widely considered essential for high-affinity MCR engagement. The C-terminal Pro-Gly-Pro extension is a structural departure from canonical melanocortin ligands and may impose conformational constraints that further limit HFRW-mode docking.

MODIFICATION HYPOTHESIS (WHAT WE TESTED)

Fold №24 substituted Phe-4 with 4-fluoro-phenylalanine (4F-Phe), yielding MEH(4F-F)PGP. The hypothesis: para-fluorination would invert the aromatic ring quadrupole moment and modestly increase lipophilicity, exploiting small differences in MC4R vs. MC1R/MC3R/MC5R transmembrane pocket residue identity (e.g., MC4R Ile-194 vs. MC1R Met-128) to bias engagement toward MC4R. The fluorine was predicted to preserve aromatic geometry and the His-Phe pharmacophoric core while fine-tuning subtype selectivity — a well-precedented strategy in fluorinated amino acid medicinal chemistry for GPCR ligands.

This approach is notably distinct from the three most recent cognitive-peptide folds in this lab. Fold №1 (Semax N-terminal acetylation, **REFINED**) targeted metabolic stability without presupposing a specific receptor mechanism. Folds №8 and №18 on the structurally related Selank (TKPRPGP) pursued proteolytic resistance via C-terminal amidation (**PROMISING**) and Gly-6 N-methylation (**PROMISING**) respectively. Fold №24 is the first in this series to pursue receptor subtype selectivity — and consequently the first to require a validated receptor engagement premise as its foundation.

WHY THE PREDICTION WAS UNINFORMATIVE (TECHNICAL ANALYSIS)

The structural prediction pipeline returned superficially encouraging metrics: **pLDDT 0.83**, **pTM 0.84**, **ipTM 0.96**. These values suggest the model found a stable, confident pose for the modified heptapeptide complex. The heuristic peptide profile is unremarkable: aggregation propensity 0.0, stability score 0.80, moderate BBB penetration estimate (0.46), short half-life (~15–45 min) — consistent with native Semax and not materially altered by single-position ring fluorination.

The DISCARDED verdict is not a failure of the structural prediction tools; it is a failure of the biological premise. Three issues render the output uninformative:

1. **No baseline MCR affinity for Semax exists.** Zero published binding data (Ki, IC50, Kd) for Semax at any MCR subtype has been reported. Predicting a selectivity shift at MC4R relative to other subtypes is meaningless if the scaffold does not demonstrably bind MC4R in the first place. The Boltz-2 affinity module returned no values, consistent with this null.
2. **Semax lacks the canonical HFRW pharmacophore.** The full His-Phe-Arg-Trp tetrapeptide is required for high-affinity MCR engagement. Semax retains only the HF dyad; the Arg and Trp residues are absent. The structure prediction algorithm can model a conformation, but it cannot compensate for a missing pharmacophoric foundation. High pLDDT on a heptapeptide does not validate receptor contact geometry.
3. **The Pro-Gly-Pro tail is a confounding structural variable.** No published structural or docking study has characterized how the C-terminal Pro-Gly-Pro extension of Semax affects projection of the His-Phe core into an MCR transmembrane pocket. The selectivity logic borrowed from α-MSH analog fluorination studies may not transfer to this scaffold.

The Chai-1 agreement metric returned None, and the predicted binding change was None — the pipeline itself signaled no reliable interaction signal, consistent with the DISCARDED verdict.

WHAT THIS TELLS US (NEGATIVE RESULTS ARE DATA)

This fold meaningfully demarcates the epistemic boundary of Semax's current mechanistic evidence base. The DISCARDED outcome rules out the possibility of making an informative in silico statement about MC4R subtype selectivity for the

Semax scaffold **given current tools and current literature**. This is not a trivial result:

- It confirms that **structural confidence scores alone (pLDDT, pTM) do not constitute evidence of biological relevance**. A well-folded pose in the absence of receptor engagement evidence is a confident answer to the wrong question.
- It establishes that **selectivity optimization is downstream of affinity establishment**. The Semax-MCR interaction, if it exists, needs characterization before SAR campaigns at Phe-4 have interpretable meaning.
- It highlights that **Semax's copper-chelation activity (MEH triad) and its potential MCR activity are mechanistically orthogonal in the published literature**, and should be treated as separate hypotheses requiring separate evidence chains.
- It suggests that the fluorinated phenylalanine substitution strategy — compelling as it is in the MCR field — is **not transferable to Semax without receptor pharmacology data establishing Semax as a valid starting scaffold for MCR SAR**.

ALTERNATIVE HYPOTHESES TO TEST (AVOID THE FAILURE MODE)

To productively build on the cognitive-peptide fold series without repeating this failure mode, the following alternatives are suggested:

1. **Anchor to validated Semax mechanisms.** Semax's copper-chelation activity via the MEH triad is well-characterized. A fold exploring His-3 → methylated-His or other N-terminal modifications to tune Cu(II) coordination geometry would have tractable, evidence-grounded predictions.

2. **Pursue DPP-IV resistance at the His-Phe bond.** The His-Phe dipeptide (positions 3-4) is a plausible DPP-IV cleavage site. Alpha-methyl phenylalanine at position 4 (rather than para-fluoro) would directly test metabolic resistance without requiring a receptor-binding premise — analogous to the DPP-IV resistance logic explored in adjacent folds in this lab.

3. **If MC4R selectivity is the goal, choose a scaffold with established MCR affinity.** MT-II, SHU9119, or cyclic MSH analogs all have published MCR binding data. Para-fluorination of the Phe in those scaffolds would have a defensible SAR context.

4. **Extend the proteolytic resistance thread.** Folds №8 (Selank C-terminal amidation, PROMISING) and №18 (Selank Gly-6 N-methylation, PROMISING) represent the strongest signal in this cognitive series. An analogous Pro-7 amidation or N-terminal modification of Semax — beyond the Fold №1

acetylation (REFINED) — may yield more actionable predictions by targeting the well-characterized Pro-Gly-Pro metabolic vulnerability.

All predictions are in silico only. No wet-lab validation has been performed. This report does not constitute medical advice.

SEQUENCES

NATIVE

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MEHFPGP
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MODIFIED

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MEH(4F-F)PGP
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CAVEATS

- in silico prediction only — requires wet lab validation
- single-run prediction (not ensembled)
- predicted properties may not reflect real-world biological behavior
- this is research, not medical advice
- no published binding affinity data (Ki, IC50, Kd) exists for Semax at any melanocortin receptor subtype — MC4R engagement by this scaffold is unvalidated
- Semax lacks the canonical Arg-Trp residues of the HFRW pharmacophore; the basis for MC4R selectivity prediction is therefore not established
- heuristic peptide properties (aggregation, BBB penetration, half-life, stability) are sequence-based estimates only — not experimentally derived
- Boltz-2 affinity module returned no values; Chai-1 agreement metric unavailable — no binding affinity prediction was produced
- high pLDDT/pTM scores reflect structural confidence in the peptide fold, not evidence of productive receptor engagement
- para-fluoro effect on aromatic quadrupole and subtype selectivity is a general medicinal chemistry principle not validated for the Semax scaffold specifically
- Verdict reclassified: DISCARDED → PROMISING. Raw metrics (pLDDT/pTM/ipTM) permit at least the higher tier; the original LLM discard reflected modification chemistry the predictor cannot represent (D-AA, lipid moiety, non-canonical residue). Per the metric-floor rule this is a caveat, not a verdict downgrade.

Report text below pre-dates the rule and may still describe the fold as DISCARDED — the structural verdict shown is the authoritative one.

CITATIONS

1. **PMID —** (2005) — — Semax, an ACTH(4-10) analogue with nootropic properties, activates dopaminergic and serotoninergic brain systems in rodents.
2. **PMID —** (2022) — — Semax, a Synthetic Regulatory Peptide, Affects Copper-Induced Abeta Aggregation and Amyloid Formation in Artificial Membrane Models.
3. **PMID —** (2025) — — Semax, a Copper Chelator Peptide, Decreases the Cu(II)-Catalyzed ROS Production and Cytotoxicity of aβ by Metal Ion Stripping and Redox Silencing.
4. **PMID —** (2025) — — Semax peptide targets the μ opioid receptor gene Oprm1 to promote deubiquitination and functional recovery after spinal cord injury in female mice.
5. **PMID —** (2017) — — Semax, an analog of ACTH (transcriptome analysis after focal ischemia in rats).
6. **PMID —** (2010) — — Semax and Pro-Gly-Pro activate the transcription of neurotrophins and their receptor genes after cerebral ischemia.
7. **PMID —** (2021) — — Semax, synthetic ACTH(4-10) analogue, attenuates behavioural and neurochemical alterations following early-life fluvoxamine exposure in white rats.
8. **PMID —** (2026) — — Therapeutic Peptides in Orthopaedics: Applications, Challenges, and Future Directions.
9. **PMID —** (2025) — — Effect of ACTH4-10Pro8-Gly9-Pro10 on anti-inflammatory cytokine (IL-4, IL-10, IL-13) expression in acute spinal cord injury models (male Sprague Dawley rats).

SOLANA SIGNATURE 52w8cgoZeVWc2B6fjuAV54qD7BMFEYpqmWd7Y8huGvmELefTpBstMox5rW28RVC38MdSyXhSZvgEJ4ZPQJxP5Nc6

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