Discovery, Properties and Structural Biology Appraisal of TES-1025 as the First In Class Potent and Selective ACMSD Inhibitor

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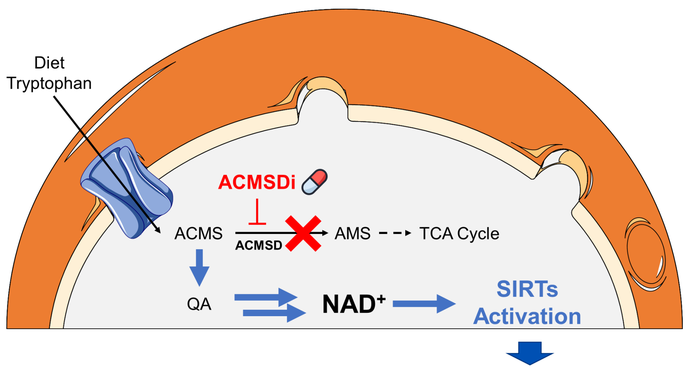
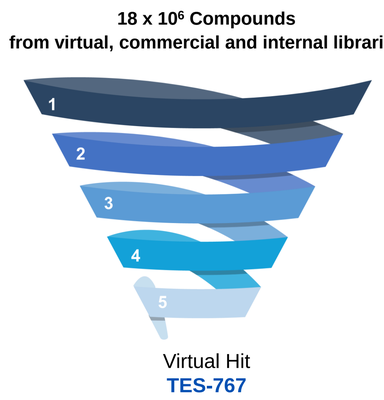
Introduction

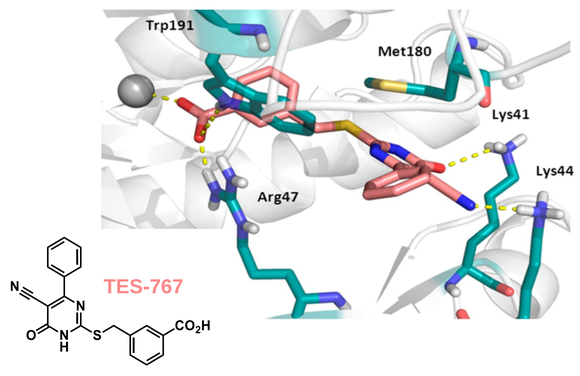


α-Amino-β-Carboxymuconic-ε-Semialdehyde Decarboxylase (ACMSD) is an enzyme of the kynurenine pathway of tryptophan (Trp) metabolism [1] and represents a branch point in the *de novo* NAD^+ biosynthesis directing the conversion of Trp to NAD^+ [2]. **ACMSD** is mainly expressed in **kidneys** and **liver** where **NAD^+** is selectively synthesised *de novo* from dietary Trp [3-4]. ACMSD inhibition increases intracellular NAD^+ levels, activates **sirtuins** and protects *in-vivo* liver and kidneys from diseases associated with NAD^+ depletion, such as Non-alcoholic Steatohepatitis (NASH) and Acute Kidney Injury (AKI), respectively [5-6].

ACMS: aminocarboxymuconic semialdehyde; AMS: aminomuconic semialdehyde; QA: quinolinic acid; TCA: tricarboxylic acid.

Structure-Based Drug Discovery (SBDD) – Hit Identification and Validation





TES-767	
MW	363.3
*h*ACMSD IC_{50} (μM)	0.05
Solubility (μM, PBS)	491
PAMPA-HDM (log(cm/s))	-6.5
Mouse Micr. Stability t ½ (min)	>120
LE	0.48

A. Virtual Screening

1. Pharmacophore model on *h*ACMSD/DHAP and PDC co-crystals (PDB: 2WM1 and 4IH3).
2. Molecular docking (Glide SP) of commercial, virtual and internal libraries.
3. Clustering and visual inspection.
4. Biochemical screening for human ACMSD inhibition (400 cpds).
5. Virtual Hit identification **TES-767**.

B. TES-767 Binding Mode Analysis

TES-767 docking pose. The Zn^{2+} metal ion is displayed as a grey ball.

- Ionic interactions of the *m*-carboxylate group with the Zn^{2+} atom, Trp191 and Arg47 residues.
- H-bond interactions (yellow dashes) of 1,6-dihydropyrimidine nucleus with Lys41 and Lys44.
- Hydrophobic interactions with Met180.

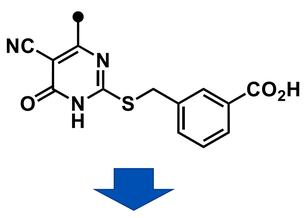
C. TES-767 Profile

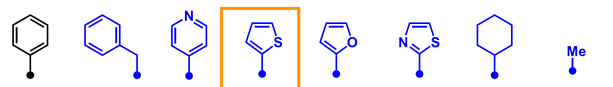
- Nanomolar biochemical potency.
- High aqueous solubility.
- Low passive permeability.
- High *in-vitro* metabolic stability in mouse microsomes.
- Optimal Ligand Efficiency (LE).

SAR Investigation on:

- Pharmacophoric *m*-carboxyl group.
- Phenyl ring replacement.

Medicinal Chemistry – Hit to Lead Optimisation





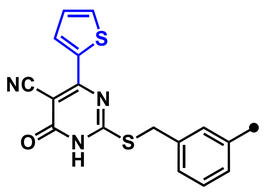
TES-Code	767	1	2	968	4	5	6	7
*h*ACMSD IC_{50} (μM)	0.05	0.851	0.083	0.012	0.066	0.049	0.077	1.74

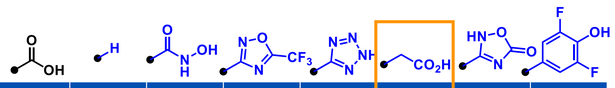
D. Phenyl Ring Exploration

Hydrophobic interactions are preferred, with 5-membered aromatic rings favoured with respect to aliphatic ones.

- Phenyl to benzyl (**1**) = 17-fold drop in potency.
- Phenyl to cyclohexyl (**6**) = slight decrease of potency.
- Phenyl to methyl (**7**) = 35-fold potency drop.
- Phenyl to 2-thiophen (**TES-968**) = 5-fold increase of potency.

TES-968 was selected as a follow-up compound.



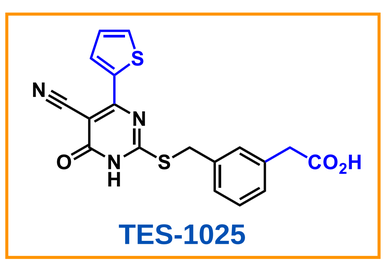


TES-Code	968	8	9	10	11	1025	12	13
*h*ACMSD IC_{50} (μM)	0.012	4.45	1.74	2.65	0.005	0.013	0.025	0.040
pKa (Sirius T3)	3.99	-	-	-	4.07	4.39	5.9	7.0

E. Carboxylate Group Exploration

- Carboxyl group removal (**8**) = 370-fold potency loss.
- Neutral Zn^{2+} chelator groups hydroxamate (**9**) and trifluoromethyl oxadiazole (**10**) = about 100-fold less potent.
- Carboxyl group to tetrazole (**11**) = single digit nanomolar compound.
- Carboxyl group elongation (**TES-1025**) = starting potency preserved.
- Inhibitory potency showed a positive correlation trend with group acidity (pKa) and charge distribution.

TES-1025 was selected as the best compromise of properties among all the bioisosteres explored.



TES-1025			
MW	383.4	PAMPA-HDM (log(cm/s))	-6.0
*h*ACMSD IC_{50} (μM)	0.013	Mouse Micr. Stability t ½ (min)	>120
*h*ACMSD K_i (μM)	0.00085	*h*ERG (10 μM)	Not Active
Solubility (μM, PBS)	> 500	LE	0.58

F. TES-1025 Biochemical and *in-vitro* ADME Profile:

- Nanomolar binding potency.
- Picomolar value of apparent *Ki* with a competitive mechanism of action.
- High solubility and moderate passive permeability.
- High *in-vitro* metabolic stability in mouse microsomes.
- Optimal safety profile (*h*ERG).
- Increased LE to 0.58.

Structural Biology

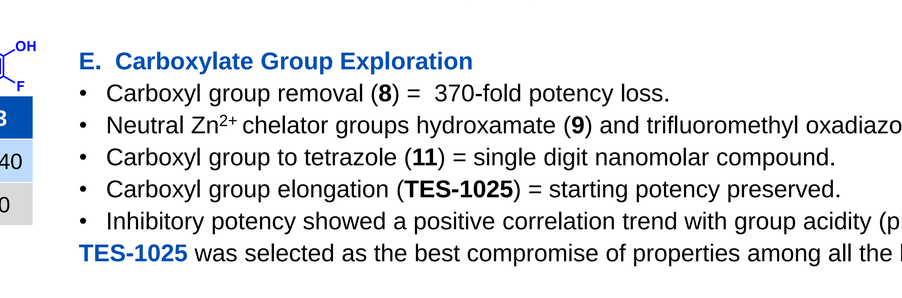
G. Human ACMSD/TES-1025 X-ray Complex

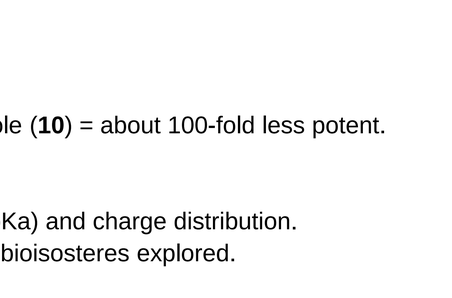
- Complex resolution 2.5 Å.
- Functional dimeric structure confirmed (dark blue monomer A, light blue monomer B) with full sites occupancy by TES-1025.
- Active site located at the C-terminal opening of the β-barrel and characterised by a Zn^{2+} metal ion coordinated with His6, His8, His174, Asp291 and a conserved water molecule.

TES-1025 Interactions:

- The carboxyl group coordinates the Zn^{2+} metal ion and makes H-bonds with Trp-191, while interacts with Asp291 through a water molecule.
- The phenyl ring makes π-π stacking interactions with Trp191.
- The pyrimidine carbonyl group interacts with Trp188 and Arg243 of chain B.
- The pyrimidine nitrogen makes charge-charge interactions with a positively charged nitrogen of Arg47.
- The 2-thienyl ring fits into a hydrophobic cavity generated by Trp176, Phe46, Met180 and Trp191.

X-ray structure of dimeric human ACMSD with TES-1025 (PDB: 7PWY):





TES-1025 stabilises the homodimeric inactive complex of *h*ACMSD by engaging a unique specific pattern of interactions with catalytic amino acid residues of both chain A and B of the enzyme dimer. This strong network of interactions confirms the high affinity and potency displayed by the ligand [7].

Conclusions

Applying a SBDD approach followed by a focused medicinal chemistry campaign, we discovered TES-1025 as the first in class potent and selective ACMSD inhibitor [6]. We have previously reported that ACMSD inhibition by TES-1025 induces protective effects in preclinical models of liver and kidney diseases associated with NAD^+ depletion such as AKI and NASH [5]. Here we report the structural basis of ligand-protein interactions with TES-1025 shedding light on the mechanism of inhibition by this class of compounds. A competitive binding mode was experimentally confirmed by kinetic analysis, showing a sub-nanomolar *Ki*, whilst co-crystal X-ray studies revealed an unforeseen network of interactions that lock TES-1025 within the active site of the protein and illustrate the principles of the inhibitory mechanism of TES-1025 [7].
Overall, these findings provide structural information regarding the mechanism of inhibition exerted by this novel class of compounds and consolidate the rationale for the therapeutic use of TES-1025 as an ACMSD inhibitor.

References: [1] Schwarcz, R. Pellicciari, R., *J. Pharmacol. Exp. Ther.* **2002**, *303*, 1-10; [2] Fukuoka, S. I. et al., *J. Biol. Chem.* **2002**, *277*, 35162–35167; [3] Liu, L. et al., *Cell Metab*. **2018**, *27*, 1067-1080; [4] Yoshino, J., *Trends Endocrinol. Metab*. **2019**, *30*, 229-232; [5] Katsyuba, E. et al., *Nature*, **2018**, *563*, 354-359; [6] Pellicciari, R. et al., *J. Med. Chem.* **2018**, *61*, 745-759; [7] Cianci, M. et al., *Front. Mol. Biosci.* **2022**, *9*, 834700.

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