Supplemental Information

Optimization of a Small Tropomyosin-related Kinase B (TrkB) Agonist 7,8-dihydroxyflavone Active in Mouse Models of Depression

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Contents of SI:

Supportive Text of Molecular Modeling:

Molecular modeling for 7,8-dihydroxyflavone docking to TrkB receptor extracellular domain

Some molecular modeling study using TrkA intracellular domain and SAR of NGF antagonists have been recently reported [1-3]. A homology model of the entire TrkB extracellular domain (ECD) was prepared by comparative modeling, showing that the TrkB overall structure is similar to TrkA, with a leucine-rich repeat (LRR) (with two cysteine-rich caps: NT and CT) orientated vertically to the first Ig domain (Ig 1) (Supplemental Figure 1A). The inter-domain interactions that limit hinge flexibilities in TrkA are generally preserved in TrkB. While the surface of the modeled TrkB structure has limited propensity to accommodate a 7,8-dihydroxyflavone-sized compound in depth, visualizing the structure suggests a large cavity between the NT cap and the first repeat of the LRR domain, located on the back or the convex surface, of the LRR domain. Indeed, prediction of the 7,8-dihydroxyflavone-binding mode to TrkB by a maximum-entropy optimization-based docking method [4] showed that 80% of the top solutions were converged to this pocket, suggesting that 7,8-dihydroxyflavone inserts into this pocket with the carbonyl group pointing to the deepest recess (Supplemental Figure 1B). In this model, the rotatable benzene ring of 7,8-dihydroxyflavone would form hydrophobic interactions with TrkB Phe55 and Pro56 at the edge of the pocket. The higher-affinity derivatives of 7,8-dihydroxyflavone with modifications at the benzene ring and the 7,8-sites would also be compatible with the putative binding pocket, as these sites are located near the mouth of the pocket (Supplemental Figure 1C). The figure and the coordinates for the modeled compound **13** in complex with TrkB are shown in Supplemental Figure 1D. Clearly, compound **13** docks in the same pocket with the added groups face outside, so the replacement of two hydroxyl groups with imidazole ring does not

significantly alter the docking pattern. Although this putative binding pocket presents good chemistry for 7,8-dihydroxyflavone, its validity still needs to be tested experimentally, as does the potential model of neurotrophin-independent TrkB activation utilizing this pocket.

References:

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Supplemental Table 1. Summary of positive control for microsomal stability screening

Compound	Test conc (μM)	Test species	Mean remaining Parent with NADPH (%)	Mean remaining parent NADPH-free (%)	comment
Verapamil	1	Human	6.1	94	High metabolized control
Warfarin	1	Human	86.2	97.3	Low metabolized control

The experimental conditions for the ADMET are described at www.apredica.com

Supplemental Table 2. Summary of positive control for reactive metabolite identification

Compound	Scan	Potential Reactive Metabolites Identified	m/z[b]	Comment
Ticlopidine	Precursor	Yes	585	Positive Control
	Neutral loss	Yes	587	Positive Control

The experimental conditions for the ADMET are described at www.apredica.com

Supplemental Table 3. Summary of positive control for CYP screening

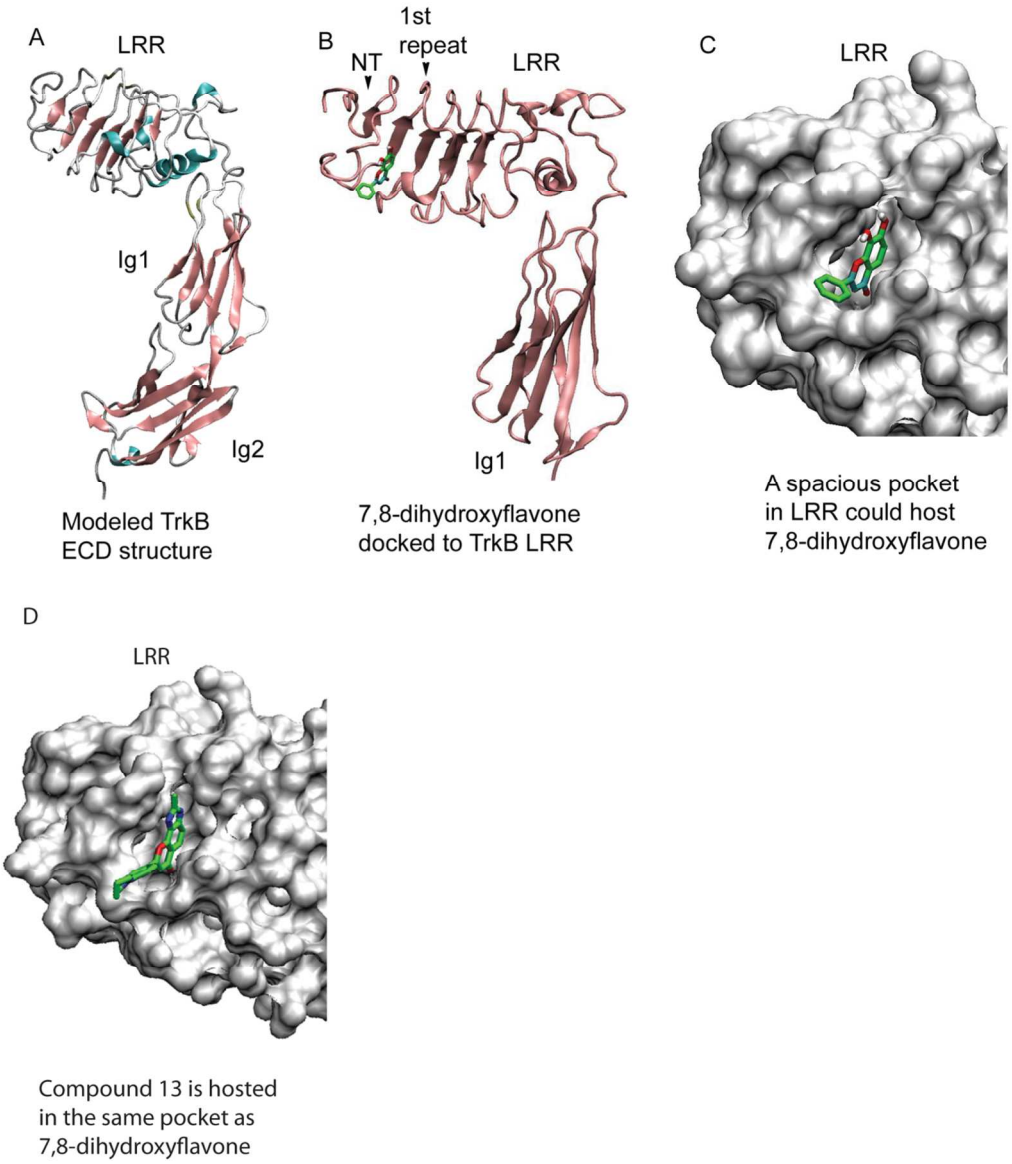
	CYP1A2	CYP3A4-Modaxolam	CYP3A4-Testoterone	CYP2C9	CYP2C19	CYP2D6
Controls	α-Naptho-flavone	Ketoconazole	Ketoconazole	Sulphaphenzaole	Ticlopidine	Quinidine
IC_{50}(μM)	0.03	0.2	0.04	0.16	6.8	0.1

The experimental conditions for the ADMET are described at www.apredica.com

Supplemental Table 4. Summary of positive control for plasma protein binding

Compound	Test conc (μM)	Test species	Fu_{plasma} (%)	Mean plasma fraction bound (%)	Recovery (%)	Binding classification
Propranolol	5 μM	Human	16	84	70.1	Low binding control
Warfarin	5 μM	Human	0.4	99.6	92.2	High binding control

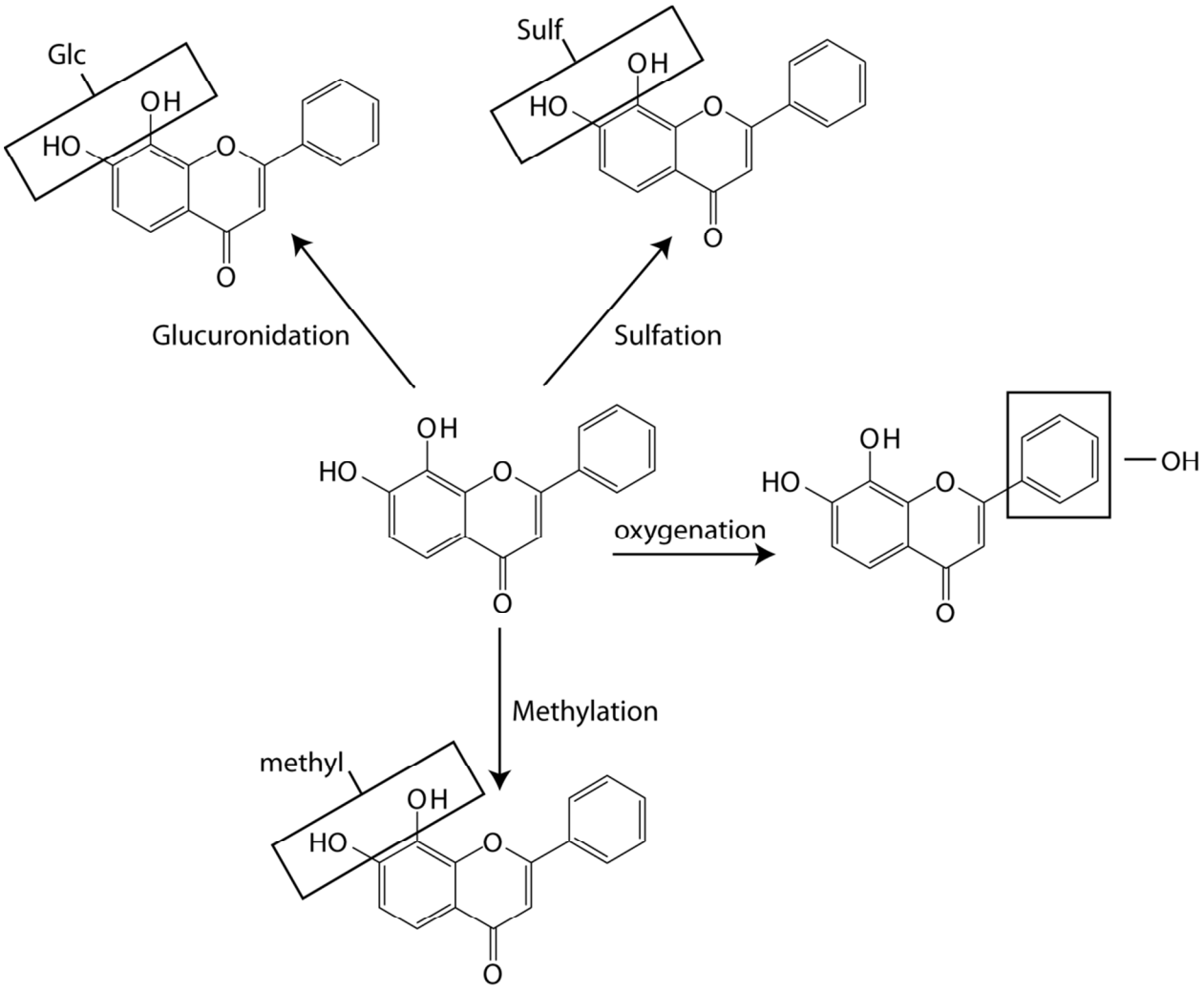
The experimental conditions for the ADMET are described at www.apredica.com



Supplemental Figure 1. Homology model of the TrkB extracellular domain (ECD) and the docking of 7,8-dihydroxyflavone to TrkB ECD.

A, Homology model of TrkB ECD, consisting of the Leucine-rich repeat (LRR) domain capped with N-terminal (NT) and C-terminal (CT) cysteine-rich sequences, and two immunoglobulin(Ig)-like domains (Ig1 and Ig2). Coloring scheme: pink for strands, cyan for helices, and silver for connecting segments. B, Docking model between 7,8-dihydroxyflavone and the LRR-Ig1 domains of TrkB. In this model 7,8-dihydroxyflavone is docked to the convex

surface of the TrkB LRR domain, sandwiched between NT and the first repeat. 7,8-dihydroxyflavone is shown as bonds with green for carbon atoms and red for oxygen atoms; TrkB is shown as ribbons. C, The large pocket in the TrkB LRR domain is spacious enough for 7,8-dihydroxyflavone, and the orientation of 7,8-dihydroxyflavone in the pocket is compatible with derivation at both the 7,8-sites and the remote benzene ring. 7,8-dihydroxyflavone is shown as bonds and TrkB LRR as silver surface. D, Compound **13** docks the same pocket of TrkB as 7,8-dihydroxyflavone with the imidazole ring faces outside.



Supplemental Figure 2. 7,8-dihydroxyflavone is metabolized by glucuronidation, sulfation and methylation.