pharmaceutics

Supplementary Materials: Microscale Thermophoresis as a Screening Tool to Predict Melanin Binding of Drugs

Laura Hellinen, Sina Bahrpeyma, Anna-Kaisa Rimpelä, Marja Hagström, Mika Reinisalo and Arto Urtti

The (microscale thermophoresis) MST traces are presented in the Supplementary Figure S1 below. The time regions from which the changes in the F_{norm} (and consequently binding affinity) were determined are displayed in the Table S1. The cold region was from -1.00 to 0 seconds (i.e. the cold region started one second before infrared laser was switched on to which the cold region ends).

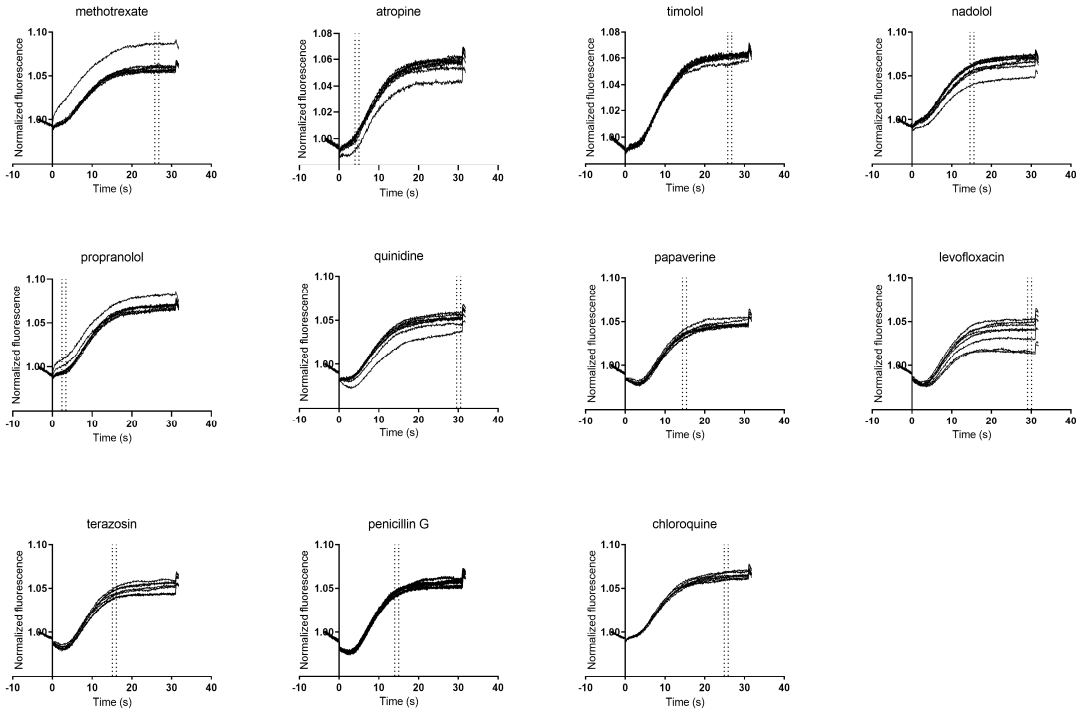

Figure S1. Microscale thermophoresis trace curves showing normalized fluorescence during the experiment and the time region which was used to determine the K_d.

Table S1. The hot regions in the MST curves used to determine the K_d values.

Compound	MST hot region (s) used to determine F_{norm}
timolol	25.8-26.8
nadolol	14.6-15.6
atropine	4.0-5.0
quinidine	29.6-30.6
chloroquine	24.9-25.9
penicillin G	14.0-15.0
terazosin	15.1-16.1
papaverin	14.4-15.4
levofloxacin	29.1-30.1

K_d determination strategies. The K_d values were tested in both initial fluorescence and the above described MST modes within the MO Affinity Analysis Software (Fig. S1, Table S1) and the K_d values obtained with each of these modes are described in the Table S2 below. Since the user manual incorporated into the software (Nanopedia) advices to utilize the initial fluorescence mode to determine K_d if ligand-induced fluorescence changes are observed, the initial fluorescence mode was used whenever fitting with raw fluorescence counts was successful. The fitting of the two modes are the same, but in the case of initial fluorescence mode, the raw fluorescence counts measured before the heating were used instead of normalized fluorescence values (F_{norm}). In the case of levofloxacin, the K_d fitting with initial fluorescence was unsuccessful with 60 % excitation, even though trend towards ligand-induced fluorescence change could be visually seen. The fitting with initial fluorescence was successful once higher excitation power (80 %) was used and the K_d is within the same range than the value obtained with the MST mode using 60 % excitation.

Table S2. K_d values determined with MST (F_{norm}) or initial fluorescence modes.

Compound	MST mode		Initial fluorescence mode	
	K_d (μM)	**K_d confidence[1] (μM)**	**K_d (μM)**	**K_d confidence[1] (μM)**
atropine	>900	1300	Failed	-
diclofenac	Failed	-	Failed	-
methotrexate*	>10 000	200 000	3 500	13 000
timolol	260	130	Failed	-
nadolol*	260	52	200	33
propranolol*	230	124	80	92
quinidine*	370	370	214	29
papaverine*	93	150	28	23
pazopanib*	Failed	-	7.8	1100
levofloxacin	47	40	[2]Failed/54	[2]Failed/10
chloroquine	0.8	7.7	Failed	-
penicillin G	3.2	7.6	Failed	-
terazosin*	96	193	29	28

The K_d values obtained with the initial fluorescence mode were utilized for compounds marked with*
[1]K_d confidences were obtained within the MO Affinity Analysis Software. With the confidence of 68 %, the K_d is in the reported range. [2]Unsuccessful with 60 % excitation power, reported value gained with 80 % excitation.

The target (melanin) occupation (fraction (%) bound values) in each of the studied ligand concentration are presented in the Figure S2 below. The categorization of the compounds into low (K_d >650 μM) intermediate (K_d 65-650 μM), high (K_d 6.5-65 μM) and extreme (K_d < 6.5 μM) melanin-binders were based on the predicted ligand binding in human RPE-choroid in vivo (fraction (%) bound of ligand; low (>10%), intermediate (1-10 %), high (0.1-1 %) and extreme (< 0.1 %).

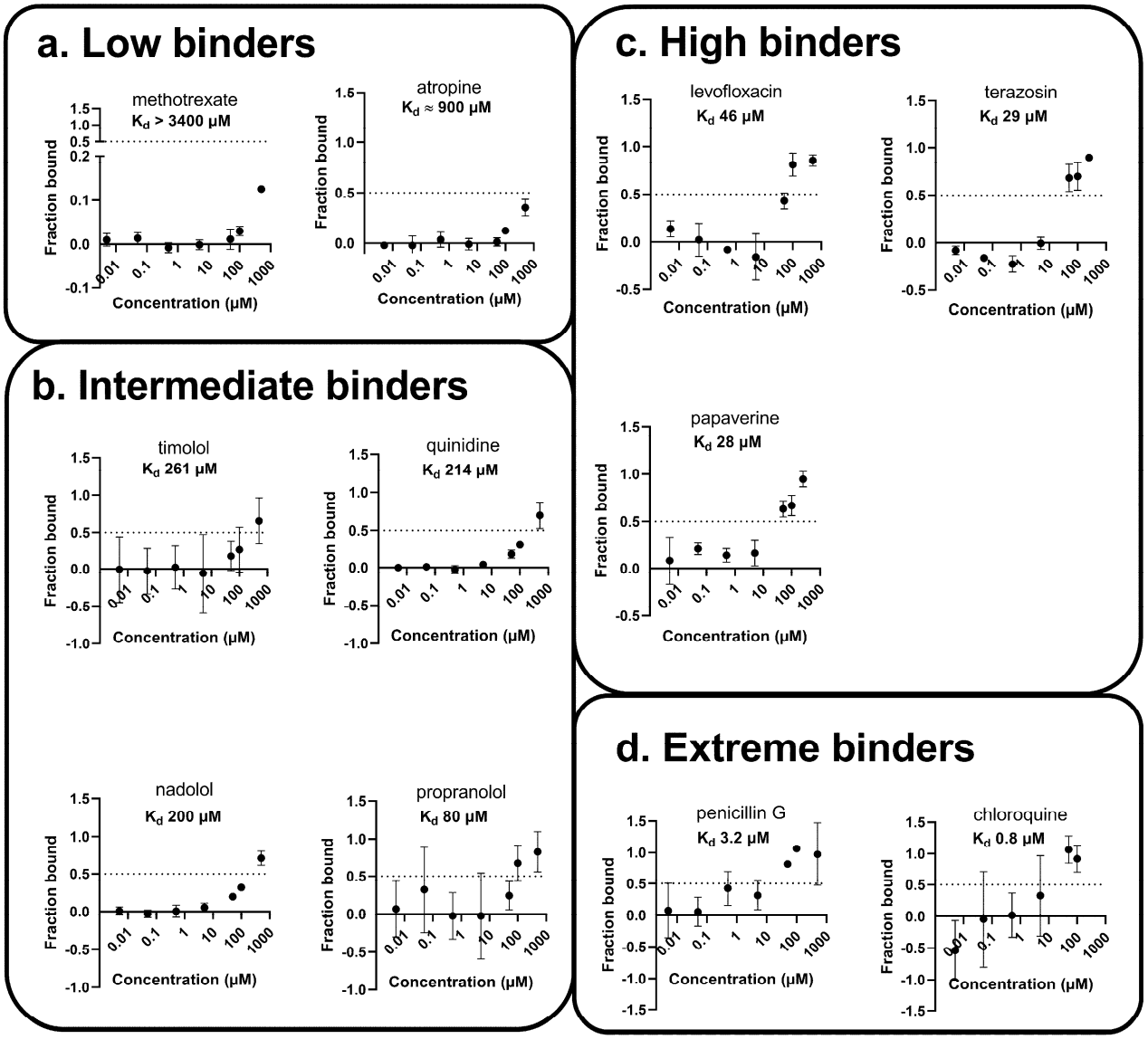

Figure S2. MST was able to distinguish compounds with different binding affinities towards melanin. Fraction of melanin in bound state at studied range of concentration according to MST analysis for (A) low binders, (B) intermediate melanin binders, (C) high binders, and (D) extreme binders.

Table S3. Mass spectrometric conditions used in the compound quantification.

Compound	Precursor m/z	Fragment m/z	Collision Energy V	Ionization
terazosin	388.27	290.20	24	ESI+
terazosin	388.27	247.14	32	ESI+
terazosin D8	396.26	298.25	28	ESI+
terazosin D8	396.26	251.80	34	ESI+
papaverine	340.18	202.08	26	ESI+
papaverine	340.18	171.08	38	ESI+
papaverine D3	343.24	205.14	26	ESI+
papaverine D3	343.24	174.13	42	ESI+
levofloxacin	362.11	318.21	18	ESI+
levofloxacin	362.11	261.14	26	ESI+
levofloxacin D8	370.24	326.33	20	ESI+
levofloxacin D8	370.24	265.28	28	ESI+