

## **Supplemental Information: Colloidal Drug Formulations Can Explain “Bell-Shaped” Concentration-Response Curves**

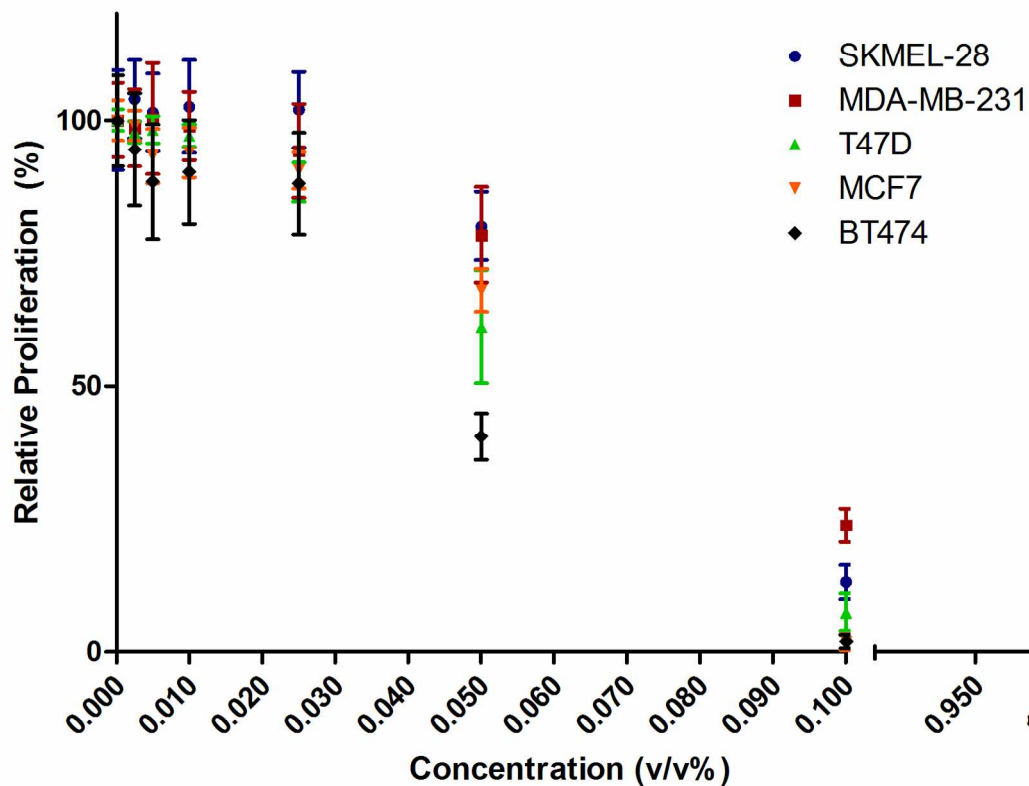
Shawn C. Owen,<sup>†,§</sup> Allison K. Doak,<sup>‡,§</sup> Ahil N. Ganesh,<sup>†</sup> Lyudmila Nedyalkova,<sup>‡</sup> Christopher K. McLaughlin,<sup>†</sup> Brian K. Shoichet<sup>‡,\*</sup> and Molly S. Shoichet<sup>†,\*</sup>

<sup>†</sup> Donnelly Centre, Department of Chemical Engineering & Applied Chemistry, Institute of Biomaterials & Biomedical Engineering, Department of Chemistry, University of Toronto, 160 College Street, Toronto, Ontario M5S3E1

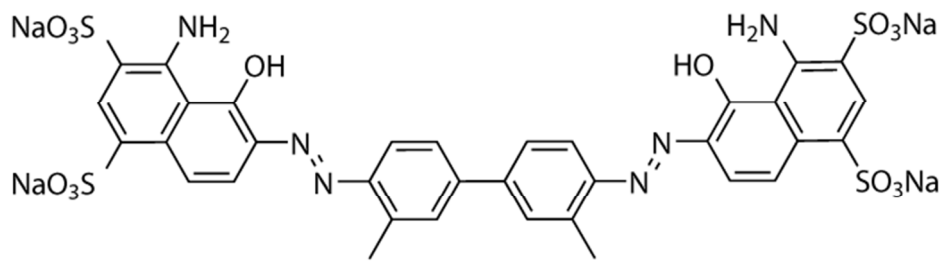
<sup>‡</sup> Department of Pharmaceutical Chemistry, University of California - San Francisco, 1700 4<sup>th</sup> Street, San Francisco, California 94158-2550

§ Denotes equal contribution

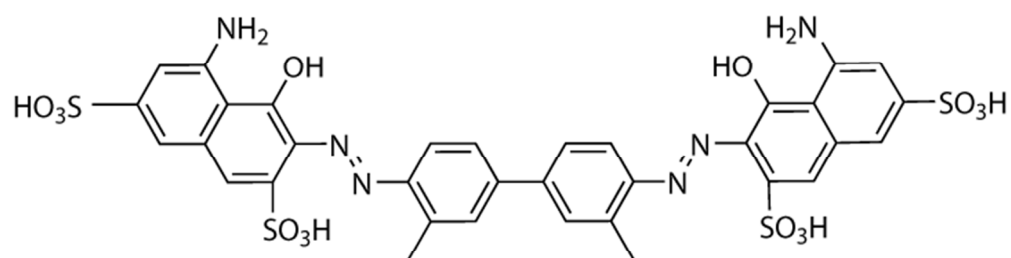
\* To whom correspondence should be addressed. (Brian K. Shoichet – Phone: 415-514-4126. Fax: 415-514-4260. e-mail: [bshoichet@gmail.com](mailto:bshoichet@gmail.com).) (Molly S. Shoichet – Phone: 416-978-1460. Fax: 416-978-4317. e-mail: [molly.shoichet@utoronto.ca](mailto:molly.shoichet@utoronto.ca))



Supplemental Figure S1. Low concentrations of Ultrapure Polysorbate 80 (UP 80) do not significantly inhibit cell proliferation. Surfactants are used to break up colloidal aggregates into monomeric forms. We previously determined that 0.025% Tween 80 does not affect cell viability; however, we have noticed mild toxicity of Tween 80 from different suppliers. As such, we evaluated a purified form of Tween 80, “UP 80,” and tested a range of concentrations to evaluate any effects on cell proliferation. At concentrations  $\leq 0.025\%$  (v/v), UP 80 does not significantly affect cell proliferation.



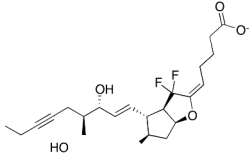
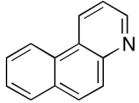
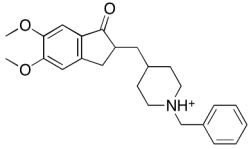
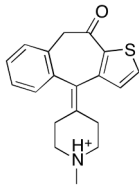
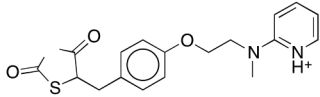
Evans Blue

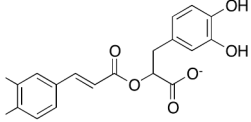
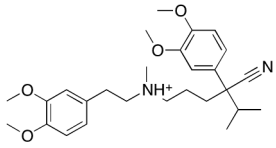


Trypan Blue

Supplemental Figure S2. Evans Blue and Trypan Blue have very similar chemical structures.

Supplementary Table S3. Compounds for which bell-curves have been described in the literature; for these compounds colloid formation is not a likely reason for the loss of activity.

Compound	Bell-Curve Reference
<p>AFP 07</p>  <p>The structure of AFP 07 is a complex molecule featuring a central five-membered ring with an oxygen atom. It has a hydroxyl group (OH) and a hydroxyl group (HO) on adjacent carbons. A side chain contains a double bond and a terminal carboxylate group (COO<sup>-</sup>). Another side chain is a propyl chain with two fluorine atoms (F) on the second and third carbons.</p>	(1)
<p>5,6-benzoquinoline</p>  <p>The structure of 5,6-benzoquinoline is a fused bicyclic system consisting of a benzene ring fused to a quinoline ring.</p>	(2)
<p>Donepezil</p>  <p>The structure of Donepezil is a complex molecule with a central five-membered ring containing a carbonyl group (C=O) and two methoxy groups (OCH<sub>3</sub>). It is connected via a methylene group to a piperidine ring, which is further connected to a benzyl group.</p>	(3)
<p>Ketotifen</p>  <p>The structure of Ketotifen is a complex molecule with a central seven-membered ring containing a sulfur atom and a carbonyl group (C=O). It is connected to a thiophene ring and a piperidine ring.</p>	(4)
<p>Rosiglitazone</p>  <p>The structure of Rosiglitazone is a complex molecule with a central five-membered ring containing a sulfur atom and a carbonyl group (C=O). It is connected to a benzene ring, which is further connected to a piperidine ring.</p>	(5)
<p>Rosmarinic acid</p>	

	(6)
<p>D-verapamil</p> 	(7, 8)

- McCormick, C.; Jones, R. L.; Kennedy, S.; Wadsworth, R. M., Activation of prostanoid EP receptors by prostacyclin analogues in rabbit iliac artery: Implications for anti-restenotic potential. *Eur. J. Pharmacol.* **2010**, *641*, 160-167.
- Cuthbert, A. W., Benzoquinolines and chloride secretion in murine colonic epithelium. *Br. J. Pharmacol.* **2003**, *138*, 1528-1534.
- Solntseva, E. I.; Bukanova, J. V.; Skrebitsky, V. G., Donepezil in low micromolar concentrations modulates voltage-gated potassium currents in pyramidal neurons of rat hippocampus. *Biochem. Biophys. Res. Commun.* **2013**, *430*, 1066-1071.
- Radermecker, M., Inhibition of allergen-mediated histamine-release from human-cells by ketotifen and oxatomide - comparison with other H-1 antihistamines. *Respiration* **1981**, *41*, 45-55.
- Blumentrath, J.; Neye, H.; Verspohl, E. J., Effects of retinoids and thiazolidinediones on proliferation, insulin release, insulin mRNA, GLUT 2 transporter protein and mRNA of INS-1 cells. *Cell Biochem. Funct.* **2001**, *19*, 159-169.
- Zeng, H.; Locatelli, M.; Bardelli, C.; Amoruso, A.; Coisson, J. D.; Travaglia, F.; Arlorio, M.; Brunelleschi, S., Anti-inflammatory properties of clovamide and theobroma cacao phenolic extracts in human monocytes: Evaluation of respiratory burst, cytokine release, NF-kappa B activation, and PPAR gamma Modulation. *J. Agric. Food Chem.* **2011**, *59*, 5342-5350.
- Litman, T.; Nielsen, D.; Skovsgaard, T.; Zeuthen, T.; Stein, W. D., ATPase activity of P-glycoprotein related to emergence of drug resistance in Ehrlich ascites tumor cell lines. *Biochim. Biophys. Acta, Mol. Basis Dis.* **1997**, *1361*, 147-158.
- Glavinas, H.; Mehn, D.; Jani, M.; Oosterhuis, B.; Heredi-Szabo, K.; Krajcsi, P., Utilization of membrane vesicle preparations to study drug-ABC transporter interactions. *Expert Opin. Drug Metab. Toxicol.* **2008**, *4*, 721-732.