







climacteric maladies by non-feminizing estrogens will also be unlikely.

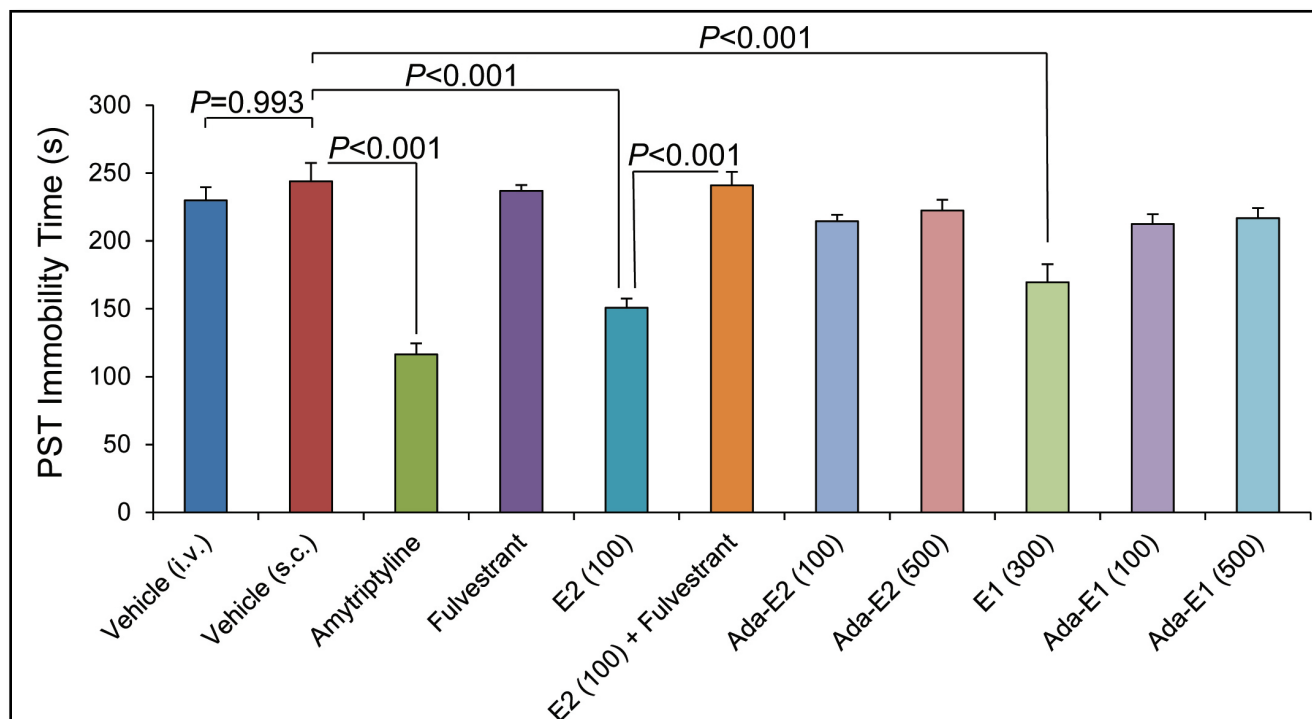
**Table 1.** Physicochemical properties, drug-likeness, estrogen receptor (ER) binding affinities and antioxidant properties of human estrogens and their 2-adamantyl-substituted derivatives.

Compound	Molecular Mass	logP	logS	Drug-likeness Score <sup>a</sup>	ER-binding Affinity <sup>b</sup> (IC <sub>50</sub> , nM)		Antioxidant Potency (IC <sub>50</sub> , μM) <sup>c</sup>
					ERα	ERβ	
E2	272.1	3.88	-4.02	0.09	1.3	0.7	11.8 ± 1.6
E1	270.0	4.02	-4.07	-0.41	6.1	3.5	17.7 ± 2.3
Ada-E2	406.2	6.01	-6.73	-1.23	>10,000	>10,000	1.5 ± 0.1
Ada-E1	404.2	6.15	-6.78	-1.72	>10,000	>10,000	5.3 ± 1.2

<sup>a</sup> Drug-likeness scores, along with logP and logS values, were calculated through the Osiris Property Explorer [26]. Negative values indicate unfavorable drug-likeness.

<sup>b</sup> Measured by competitive radioligand-binding assay [21]

<sup>c</sup> Determined experimentally by the FTC method [12,22]



**Figure 2.** Evaluation of E2, E1, Ada-E2 and Ada-E1 (doses given in parentheses as μg/kg body weight) for antidepressant-like activity in the PST using OVX young adult CD1 mice. Displayed data are means ± SEM (N/group=6 except for s.c. vehicle, where N/group was 12). One-way ANOVA:  $F_{(10,61)}=15.0, P<0.001$ .

**CONCLUSION**

In conclusion, our lead evaluation has confirmed both genomic and non-genomic mechanisms are required for broad-spectrum estrogen neuro-protection and treatment of

menopausal symptoms. Therefore, non-feminizing estrogens are not suitable to fulfill their overall premise. In addition, our analyses have revealed serious shortcomings regarding pharmaceutically important properties and drug-likeness of prototypical lead compounds. On the other hand, our

recently published brain-selective estrogen therapy promises to provide full benefits of the hormone's activity through both genomic and non-genomic mechanisms with a concomitant improvement in drug-like properties and, also, fully avoiding peripheral impacts that leads to feminizing effects [25]. Consequently, the creation of novel non-feminizing estrogens as lead compounds has lost its impetus in the context of drug discovery and development.

## ACKNOWLEDGMENT

The authors are grateful for the financial support by the National Institutes of Health (AG031535 to LP, AG031421 to KPT) and the Robert A. Welch Foundation (endowment BK-0031 to LP).

## REFERENCES

- Spence RD, Voskuhl RR (2012) Neuroprotective effects of estrogens and androgens in CNS inflammation and neurodegeneration. *Front Neuroendocrinol* 33: 105-115.
- Guo JB, Duckles SP, Weiss JH, Li XJ, Krause DN (2012) 17 $\beta$ -estradiol prevents cell death and mitochondrial dysfunction by an estrogen receptor-dependent mechanism in astrocytes after oxygen-glucose deprivation/reperfusion. *Free Rad Biol Med* 52: 2151-2160.
- Prokai L, Simpkins JW (2007) Structure-non-genomic neuroprotection relationship of estrogens and estrogen-derived compounds. *Pharmacol Ther* 114: 1-12.
- Braun CMJ, Roberge C (2014) Gender-related protection from or vulnerability to severe CNS diseases: Gonado-structural and/or gonado-activational? A meta-analysis of relevant epidemiological studies. *Int J Dev Neurosci* 38: 36-51.
- Nelson HD (2008) Menopause. *Lancet* 371: 760-770.
- Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, et al. (2002) Risks and benefits of estrogen plus progestin in healthy postmenopausal women. Principal results from the Women's Health Initiative randomized controlled trial. *JAMA* 288: 321-333.
- Prentice RL, Anderson GL (2008) The women's health initiative: Lessons learned. *Ann Rev Public Health* 29: 131-150.
- Zhang FG, Chen YM, Pisha E, Shen L, Xiong YS, et al. (1999) The major metabolite of equilin, 4-hydroxyequilin, autoxidizes to an o-quinone which isomerizes to the potent cytotoxin 4-hydroxyequilin-o-quinone. *Chem Res Toxicol* 12: 204-213.
- Prokai-Tatrai K, Prokai L (2005) Impact of metabolism on the safety of estrogen therapy. *Ann NY Acad Sci* 1052: 243-257.
- Honjo H, Iwasa K, Fushiki S, Hosoda T, Tatsumi H, et al. (2003) Estrogen and non-feminizing estrogen for Alzheimer's disease. *Endocrine J* 50: 361-367.
- Xia S, Cai ZY, Thio LL, Kim-Han JS, Dugan LL, et al. (2002) The estrogen receptor is not essential for all estrogen neuroprotection: New evidence from a new analog. *Neurobiol Dis* 9: 282-293.
- Prokai L, Rivera-Portalatin NM, Prokai-Tatrai K (2013) Quantitative structure-activity relationships predicting the antioxidant potency of 17 $\beta$ -estradiol-related polycyclic phenols to inhibit lipid peroxidation. *Int J Mol Sci* 14: 1443-1454.
- Liu R, Yang SH, Perez E, Yi KD, Wu SS, et al. (2002) Neuroprotective effects of a novel non-receptor-binding estrogen analogue: *In vitro* and *in vivo* analysis. *Stroke* 33: 2485-2491.
- Walf AA, Paris JJ, Rhodes ME, Simpkins FW, Frye CA (2011) Divergent mechanisms for trophic actions of estrogens in the brain and peripheral tissues. *Brain Res* 1379: 119-136.
- Yi KD, Perez E, Yang, SH, Liu R, Covey DF, et al. (2011) The assessment of non-feminizing estrogens for use in neuroprotection. *Brain Res* 1379: 61-70.
- Petrone AB, Gatson JW, Simpkins JF, Reed MN (2014) Non-feminizing estrogens: A novel neuroprotective therapy. *Mol Cell Endocrinol* 389: 40-47.
- Österlund MK (2010) Underlying mechanisms mediating the antidepressant effects of estrogens. *Biochem Biophys Acta* 1800: 1136-1144.
- Bekku N, Yoshimura H (2005) Animal model of menopausal depressive-like state in female mice: prolongation of immobility time in the forced swimming test following ovariectomy. *Psychopharmacol* 183: 300-307.
- Schmidt PJ, Ben Dor R, Martinez PE, Guerrieri GM, Harsh VL, et al. (2015) Effects of estradiol withdrawal on mood in women with past perimenopausal depression. A randomized clinical trial. *JAMA Psychiatry* 72: 714-726.
- Lunn WHW, Farkas E (1968) The adamantyl carbonium ion as a dehydrogenating agent, its reactions with estrone. *Tetrahedron* 24: 6773-6776.
- Prokai L, Prokai-Tatrai K, Perjesi P, Zharikova AD, Perez EJ et al. (2003) Quinol-based cyclic antioxidant mechanism in estrogen neuroprotection. *Proc Nat Acad Sci U S A* 100: 11741-11746.
- Kikuzaki H, Nakatani N (1993) Antioxidant effects of some ginger constituents. *J Food Sci* 58: 1407-1410.

23. Prokai-Tatrai K, Hua X, Nguyen V, Szarka S, Blazics B, Prokai L, et al. (2013) 17 $\beta$ -estradiol eye drops protect the retinal ganglion cell layer and preserve visual function in an *in vivo* model of glaucoma. *Mol Pharmaceutics* 10: 3253-3261.
24. Prokai-Tatrai K, Szarka S, Nguyen, V, Sahyouni F, Walker C, et al. (2012) "All in the mind"? Brain-targeting chemical delivery system of 17 $\beta$ -estradiol (Estredox) produces significant uterotrophic side effect. *Pharm Anal Acta* S7: 10.4172/2153-2435.
25. Prokai L, Nguyen V, Szarka S, Garg P, Sabnis G, et al. (2015) The prodrug DHED selectively delivers 17 $\beta$ -estradiol to the brain for treating estrogen-responsive disorders. *Sci Trans Med* 7: 297ra113 2.
26. Sander T (2016) Osiris property explorer.
27. Hann, MM (2011) Molecular obesity, potency and other addictions in drug discovery. *Med Chem Commun* 2: 349-355.
28. Prokai L, Prokai-Tatrai K (2011) Quinol compound and pharmaceutical composition. US Patent 7,534,779 B2.
29. Opas EE, Gentile MA, Kimmel DB, Rodan GA, Schmidt A (2006) Estrogenic control of thermoregulation in ER $\alpha$ KO and ER $\beta$ KO mice. *Maturitas* 53: 210-216.