Electronic Supplementary Material (ESI) for Food & Function

Modelling a possible bioactivity of ellagitannin-derived metabolites. *In silico* tools to evaluate their potential xenoestrogenic behavior.

Luca Dellafiora[1], Pedro Mena[2,3], Pietro Cozzini[1], Furio Brighenti[3], Daniele Del Rio[3]

[1]Molecular Modeling Laboratory, Department of Food Science, University of Parma, 43125 Parma, Italy

[2]Department of Food Science and Technology, CEBAS-CSIC, P.O. Box 164, E-30100, Espinardo, Murcia, Spain.

[3]The φ[2] Laboratory of Phytochemicals in Physiology, Human Nutrition Unit, Department of Food Science, University of Parma, 43125 Parma, Italy.

Correspondence to:

Daniele Del Rio, The φ[2] Laboratory of Phytochemicals in Physiology, Human Nutrition Unit, Department of Food Science, University of Parma, via Volturno 39, 43125 Parma, Italy. Phone: +39-0521-033830. Fax: +39-0521-033832. E-mail: daniele.delrio@unipr.it

and

Pietro Cozzini, Molecular Modeling Laboratory, Department of Food Science, University of Parma, Parco Area delle Scienze 95/A, 43125 Parma, Italy. Phone: +39-0521-905669. Fax: +39-0521-905556. E-mail: pietro.cozzini@unipr.it

Table S1.- Chemicals contained in the training-set of the procedure used (19 active and 16 inactive compounds)

TRAINING SET			
Compound	**α-dependent agonistic activity**	**Experimental evidences**	
11β-ethyl-EE [a)]	Yes	$LogEC_{50}$ = -11.2	RRA = 1.778
17α-iodovinyl-E2 [a)]	Yes	$LogEC_{50}$ = -9.9	RRA = 0.120
17α-progesterone [a)]	No	$LogEC_{50}$ = > -5	RRA = 0.000
3α-OH-11β-ethyl-NET [a)]	Yes	$LogEC_{50}$ = -9	RRA = 0.013
3α-OH-5β-hydrogen-7α-methyl-NET [a)]	Yes	$LogEC_{50}$ = -7.8	RRA = 0.001
3β-OH-11-methylen-NET [a)]	Yes	$LogEC_{50}$ = -9.5	RRA = 0.044
5α-hydrogen-nandrolone [a)]	No	$LogEC_{50}$ = -6.5	RRA = 0.000
7α-methyl-nandrolone [a)]	No	$LogEC_{50}$ = -7.6	RRA = 0.000
17α-estradiol [a)]	Yes	$LogEC_{50}$ = -8.8	RRA = 0.012
5α-hydrogen-T (DHT) [a)]	No	$LogEC_{50}$ = > -6	RRA = 0.000
Deoxynivalenol (DON) [b)]	No	No interactions with ERs	
E2 [a)]	Yes	$LogEC_{50}$ = -10.8	RRA = 1.000
EE [a)]	Yes	$LogEC_{50}$ = -11.1	RRA = 1.860
Estriol [a)]	Yes	$LogEC_{50}$ = -9.9	RRA = 0.035
HT-2 toxin [b)]	No	No interactions with ERs	
Nafoxidine [a)]	No	$LogEC_{50}$ = > -5	RRA = 0.000
Ochratoxin A (OTA) [c)]	No	No interactions with ERs	
Resveratrol 3-O-Glucuronide [d)]	No	No reported ERs-mediated activity	
Resveratrol 3-O-Sulfate [d)]	No	Only acts as antagonist	
T-2 toxin [b)]	No	No interactions with ERs	
Coumestrol [i)]	Yes	Appreciable agonistic activity	
RU58668 [l)]	No	Pure antiestrogen	
ICI182780 [a)]	No	$LogEC_{50}$ = > -5	RRA = 0.000
Genistein [i)]	Yes	Appreciable agonistic activity	
Daidzein [i)]	Yes	Appreciable agonistic activity	
Tamoxifen [a)]	No	$LogEC_{50}$ = > -5	RRA = 0.000
Testosterone [a)]	No	$LogEC_{50}$ = > -5	RRA = 0.000
ZOM-1[e)]	No	No interactions with ERs	
α-Zearalanol [f)]	Yes	Agonistic activity is reported	
β-Zearalanol [f)]	Yes	Agonistic activity is reported	
α-Zearalenol [g)]	Yes	Agonistic activity is reported	
β-Zearalenol [g)]	Yes	Agonistic activity is reported	
Zearalenone [fg)]	Yes	Agonistic activity is reported	
Urolithin A [h)]	Yes	Agonistic activity is reported	
Urolithin B [h)]	Yes	Agonistic activity is reported	

a) “Comparison of *In Vitro* and *In Vivo* Screening Models for Androgenic and Estrogenic Activities” (Sonnenveld et al., 2006). b) “An *in vitro* investigation of endocrine disrupting effects of trichothecenes deoxynivalenol (DON), T-2 and HT-2 toxins” (Ndossi et al., 2012). c) “Endocrine disrupting effects of ochratoxin A at the level of nuclear receptor activation and steroidogenesis” (Frizzel et al., 2013). d) “Anti-estrogenic activity of a human resveratrol metabolite” (Ruotolo et al., 2013). e) “Cleavage of zearalenone by *Trichosporon mycotoxinivorans* to a novel non estrogenic metabolite” (Vekiru et al., 2010). f) “Endocrine disrupting effects of zearalenone, alpha- and beta-zearalenol at the level of nuclear receptor binding and steroidogenesis” (Frizzel et al., 2011). g) “Investigations on cellular proliferation induced by zearalenone and its derivatives in relation to the estrogenic parameters” (Minervini et al., 2005). h). “Urolithins, ellagic acid-derived metabolites produced by human colonic microflora, exhibit estrogenic and antiestrogenic activities” (Larrosa et al., 2006). i). “Comparative study on the nuclear hormone receptor activity of various phytochemicals and their metabolites by reporter gene assays using Chinese hamster ovary cells” (Takeuchi et al., 2009). l). “Basic guide to the mechanisms of antiestrogen action.” (MacGregor and Jordan, 1998).
Abbreviations: E2, EE, NET and T stay for 17-β-estradiol, 17-α-ethinyl-E2, noretisterone and testosterone, respectively.

Table S2.- Output of SBVS upon training-set. Compounds having LDA-R higher than 0.1 should be considered "potentially active".

	Compound	Experimental agonistic activity	SBVS results	
			LDA-R	Predicted Activity
1	11β-ethyl-EE	+	2.126	Potentially active
2	17α-iodovinyl-E2	+	1.873	Potentially active
3	17α-progesterone	–	-2.735	Potentially inactive
4	3α-OH-11β-ethyl-NET	+	1.555	Potentially active
5	3α-OH-5β-hydrogen-7α-methyl-NET	+	1.237	Potentially active
6	3β-OH-11-methylen-NET	+	0.637	Potentially active
7	5α-hydrogen-nandrolone	–	-0.325	Potentially inactive
8	7α-methyl-nandrolone	–	-0.026	Potentially inactive
9	17α-estradiol	+	2.351	Potentially active
10	5α-hydrogen-T (DHT)	–	-0.225	Potentially inactive
11	Deoxynivalenol (DON)	–	-0.883	Potentially inactive
12	E2	+	1.713	Potentially active
13	EE	+	2.087	Potentially active
14	Estriol	+	1.106	Potentially active
15	HT-2 toxin	–	-0.108	Potentially inactive
16	Nafoxidine	–	-3.499	Potentially inactive
17	Ochratoxin A (OTA)	–	-2.716	Potentially inactive
18	Resveratrol 3-O-Glucuronide	–	-2.811	Potentially inactive
19	Resveratrol 3-O-Sulfate	–	-1.109	Potentially inactive
20	T-2 toxin	–	-0.186	Potentially inactive
21	Coumestrol	+	1.914	Potentially active
22	RU58668	–	-0.465	Potentially inactive
23	ICI182780	–	-1.631	Potentially inactive
24	Genistein	+	1.596	Potentially active
25	Daidzein	+	1.885	Potentially active
26	Tamoxifen	–	-2.204	Potentially inactive
27	Testosterone	–	0.032	Potentially inactive
28	ZOM-1	–	-2.466	Potentially inactive
29	α-Zearalanol	+	1.782	Potentially active
30	β-Zearalanol	+	1.255	Potentially active
31	α-Zearalenol	+	2.667	Potentially active
32	β-Zearalenol	+	1.112	Potentially active
33	Zearalenone	+	1.675	Potentially active
34	Urolithin A	+	1.669	Potentially active
35	Urolithin B	+	1.649	Potentially active

Table S3.- Docking results of "potentially active" compounds identified by SBVS from training-set. Only positive HSs have been recorded.

Compound		alpha ER LBD	
		HINT score	Predicted interaction
1	11β-ethyl-EE	1780	Positive
2	17α-iodovinyl-E2	1680	Positive
3	3α-OH-11β-ethyl-NET	810	Positive
4	3α-OH-5β-hydrogen-7α-methyl-NET	540	Positive
5	3β-OH-11-methylen-NET	850	Positive
6	17α-estradiol	1040	Positive
7	E2	1500	Positive
8	EE	1600	Positive
9	Estriol	1170	Positive
10	Coumestrol	1040	Positive
11	Genistein	760	Positive
12	Daidzein	1120	Positive
13	α-Zearalanol	780	Positive
14	β-Zearalanol	570	Positive
15	α-Zearalenol	750	Positive
16	β-Zearalenol	670	Positive
17	Zearalenone	700	Positive
18	Urolithin A	850	Positive
19	Urolithin B	1300	Positive

33 **Table S4.- Chemicals contained in the test-used for procedure validation**

TEST SET	
Compound	**Reported Agonistic activity**
Enterolactone [b)]	Yes (both isoform)
Formonetin [b)]	Yes (both isoform)
Biochanin A [b)]	Yes (both isoform)
Equol [a,b)]	Yes (both isoform)
O-Desmethylangolensin [b)]	Yes (both isoform)
Secoisolariciresinol [c)]	no
Epigallocatechin gallate [b)]	no
Flavone [a)]	no
Guggulsterone [a)]	no
trans-resveratrol [a,b)]	Yes (only beta)

a) "Screening of synthetic and plant-derived compounds for (anti) estrogenic and (anti) androgenic activities" (Bovee et al., 2008). b) "Comparative Study on the Nuclear Hormone Receptor Activity of Various Phytochemicals and Their Metabolites by Reporter Gene Assays Using Chinese Hamster Ovary Cells" (Takeuchi et al., 2009). c) "Lignans as food constituents with estrogen and antiestrogen activity" (Aehle et al., 2011).

34

Table S5.- Output of SBVS upon test-set. Compounds having LDA-R higher than 0.1 should be considered "potentially active".

	Compound	Experimental agonistic activity	SBVS results	
			LDA-R	Predicted Activity
1	Enterolactone	+	1.433	Active
2	Formonetin	+	1.121	Active
3	Biochanin A	+	1.408	Active
4	Equol	+	2.079	Active
5	O-Desmethylangolensin	+	2.274	Active
6	Secoisolariciresinol [a)]	–	0.530	Active
7	Epigallocatechin gallate [a)]	–	1.397	Active
8	Flavone	–	-0.930	Inactive
9	Guggulsterone	–	-2.773	Inactive
10	*trans*-resveratrol [a)]	+ in βER and – in αER	2.076	Active

a) wrongly predicted.

Table S6.- . Docking results of "potentially active" compounds identified by SBVS from test-set. Some errors have been corrected.

	Compound	Alpha-ER LBD		Beta-ER LBD	
		HINT score	Predicted Interaction	HINT score	Predicted Interaction
1	Enterolactone	1150	Positive	700	Positive
2	Formonetin	1370	Positive	1270	Positive
3	Biochanin A	1010	Positive	750	Positive
4	Equol	1560	Positive	1080	Positive
5	O-Desmethylangolensin	880	Positive	700	Positive
6	Secoisolariciresinol [a)]	-180	Negative	-400	Negative
7	Epigallocatechin gallate [a)]	-1200	Negative	-1800	Negative
8	*trans*-resveratrol	540	Positive [b)]	600	Positive

a) corrected error. b) residual error.