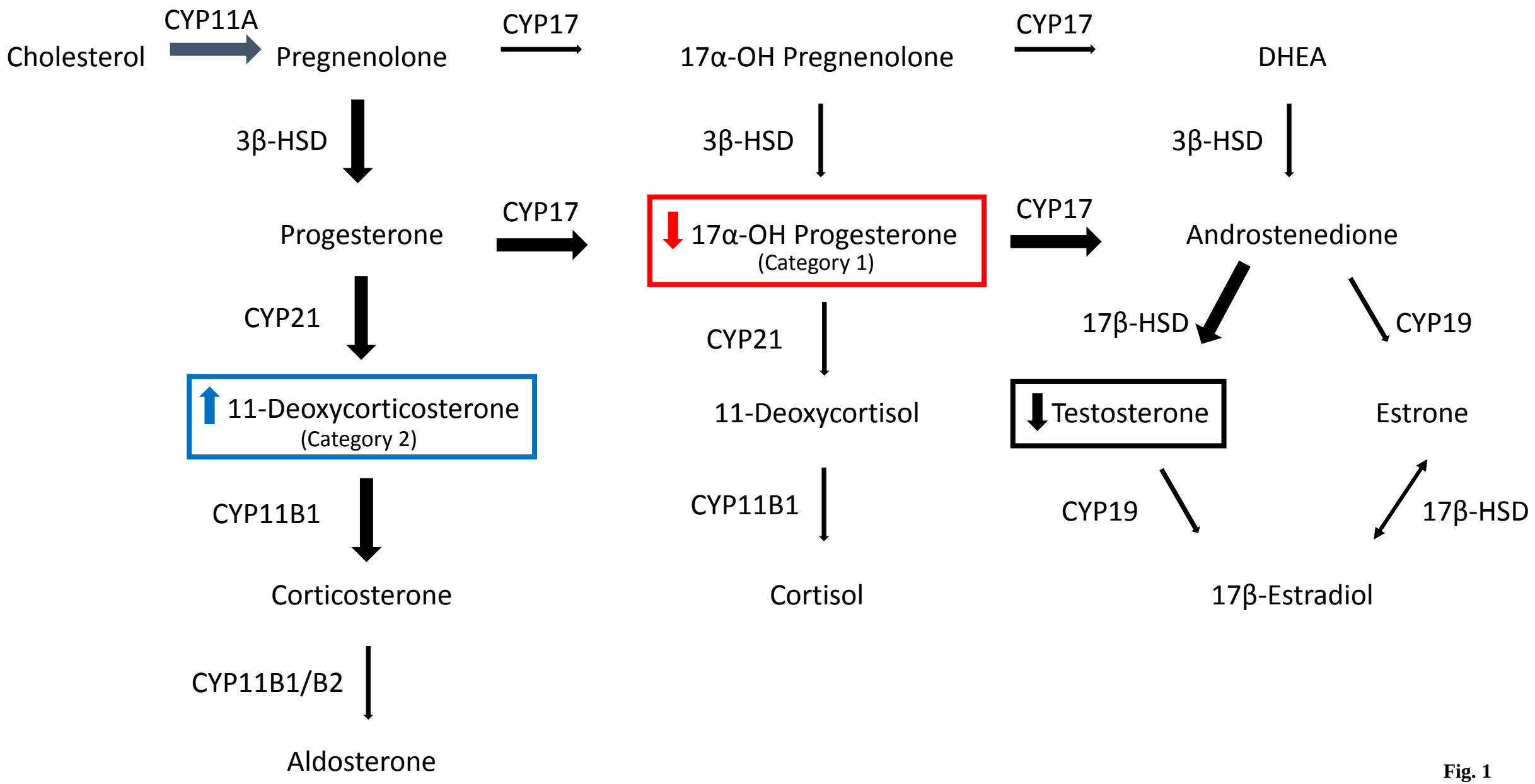
Cholesterol
CYP11A
Pregnenolone
CYP17
17α-OH Pregnenolone
CYP17
DHEA
3β-HSD
3β-HSD
3β-HSD
Progesterone
CYP17
17α-OH Progesterone
(Category 1)
CYP17
Androstenedione
CYP21
CYP21
17β-HSD
CYP19
11-Deoxycorticosterone
(Category 2)
11-Deoxycortisol
Testosterone
Estrone
CYP11B1
CYP11B1
CYP19
17β-HSD
Corticosterone
Cortisol
17β-Estradiol
CYP11B1/B2
Aldosterone

Fig. 1

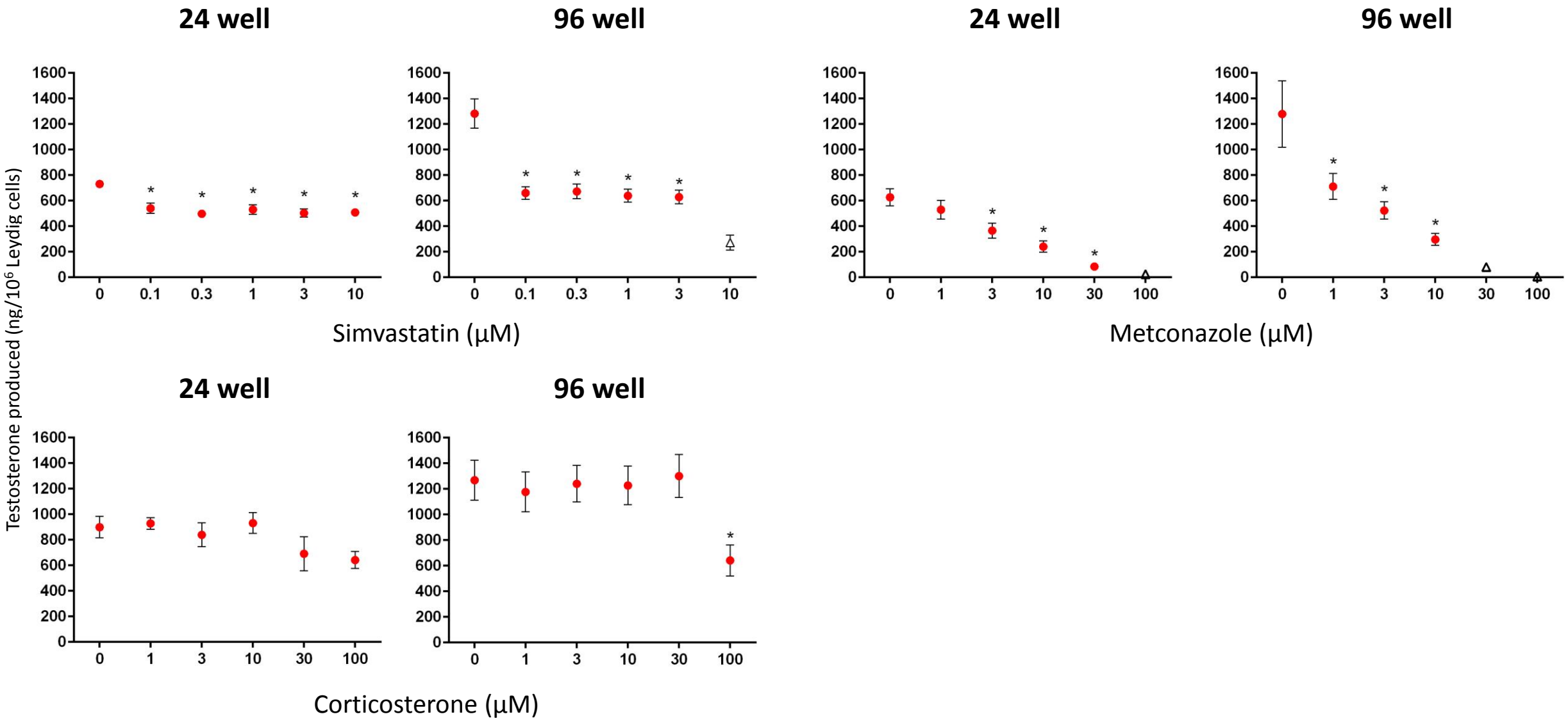
24 well
96 well
24 well
96 well
Testosterone produced (ng/10⁶ Leydig cells)
1600
1400
1200
1000
800
600
400
200
0
0
0.1
0.3
1
3
10
30
100
Simvastatin (µM)
Metconazole (µM)
24 well
96 well
Corticosterone (µM)

Fig. 2

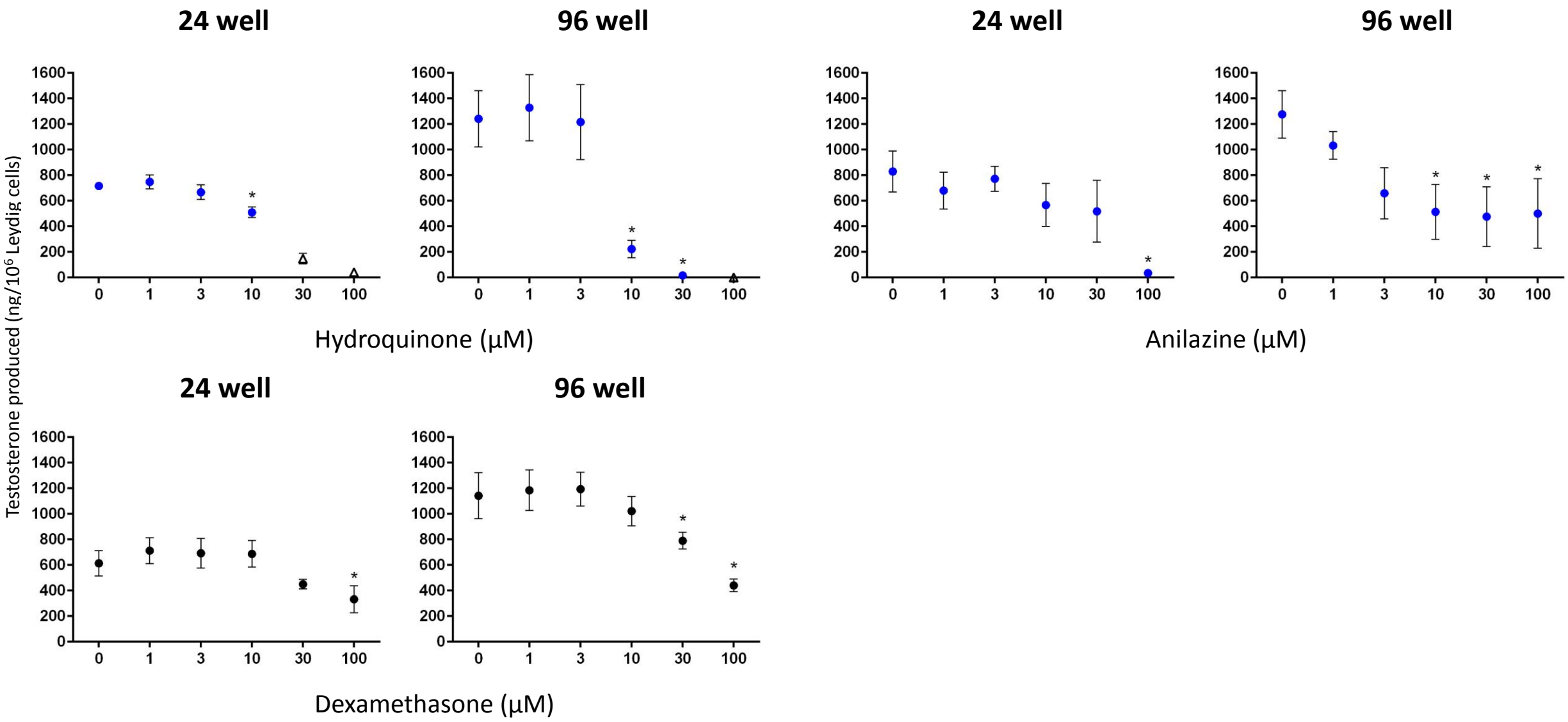
24 well
96 well
24 well
96 well
Testosterone produced (ng/10⁶ Leydig cells)
1600
1400
1200
1000
800
600
400
200
0
0
1
3
10
30
100
*
Hydroquinone (µM)
Anilazine (µM)
24 well
96 well
Dexamethasone (µM)

Fig. 3

Table 1: HTS H295R AC50 (µM) values for 17α-hydroxyprogesterone and 11-deoxycorticosterone showing that responders in the 96 well format of the Leydig cell assay have AC50 values < 3 µM.

Category 1: AC50 for 17α-hydroxyprogesterone in the HTS H295R assay

Responders	AC 50 (µM)	Non-responders	AC 50 (µM)
metconazole	0.09	permethrin	9.99
cycloheximide	0.10	esfenvalerate	27.50
tamoxifen citrate	0.14		
17α-ethynylestradiol	0.33		
3,4,4'-trichlorocarbanilide	0.37		
flusilazole	0.38		
simvastatin	0.72		
flufenoxuron	1.09		
finasteride	1.13		
triphenyl phosphate	1.81		
spirodiclofen	2.01		
*corticosterone**	*44.16*		

Category 2: AC50 for 11-deoxycorticosterone in the HTS H295R assay

Responders	AC 50 (µM)	Non-responders	AC 50 (µM)
1,4 dihydroxy-2-napthoic acid	0.43	prednisone	39.51
dimethipin	1.59		
4-chloro-o-phenylenediamine	2.69		
anilazine	2.84		
hydroquinone	3.20		

* corticosterone does not follow < 3 µM pattern

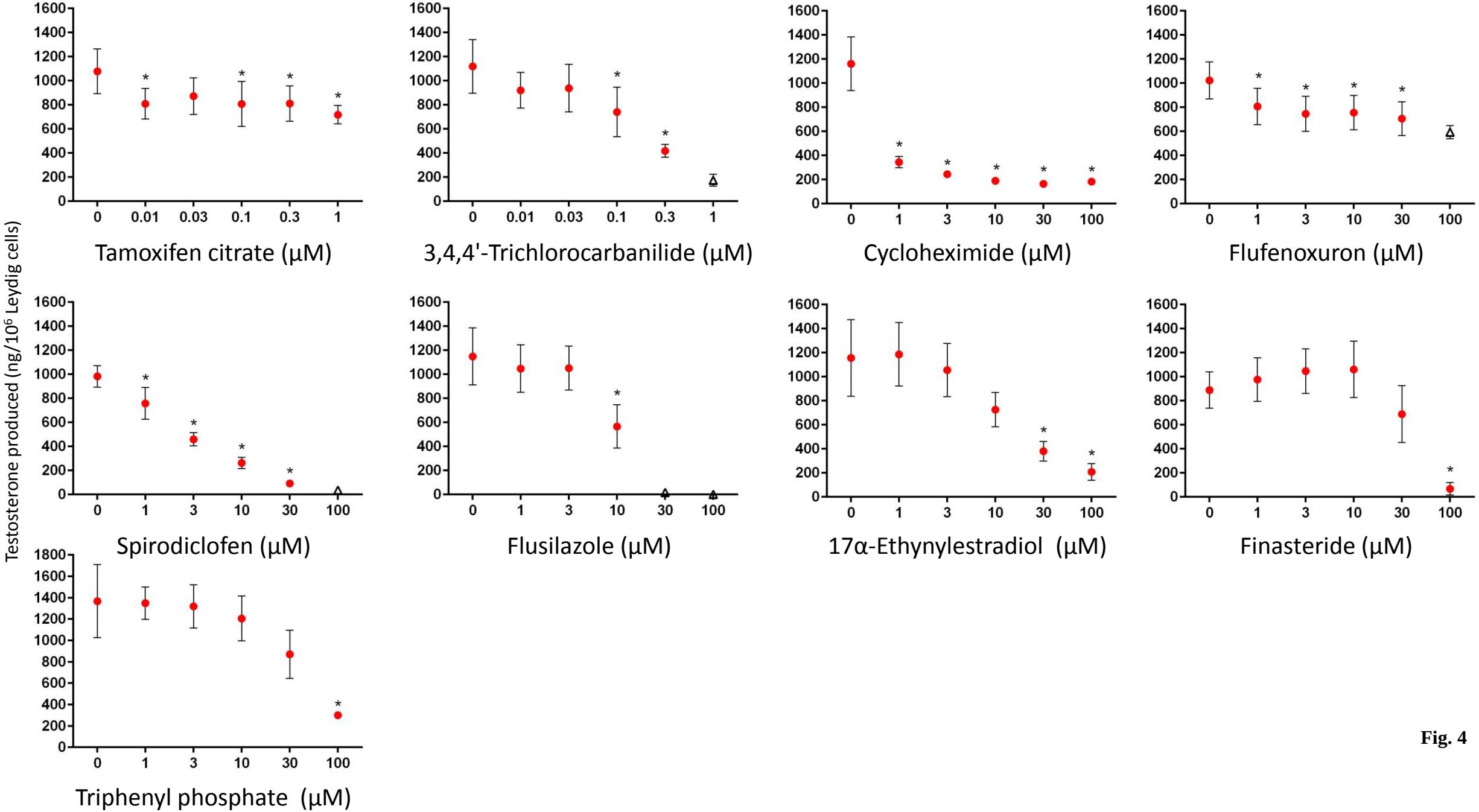
Testosterone produced (ng/10⁶ Leydig cells)
Tamoxifen citrate (µM)
3,4,4'-Trichlorocarbanilide (µM)
Cycloheximide (µM)
Flufenoxuron (µM)
Spirodiclofen (µM)
Flusilazole (µM)
17α-Ethynylestradiol (µM)
Finasteride (µM)
Triphenyl phosphate (µM)

Fig. 4

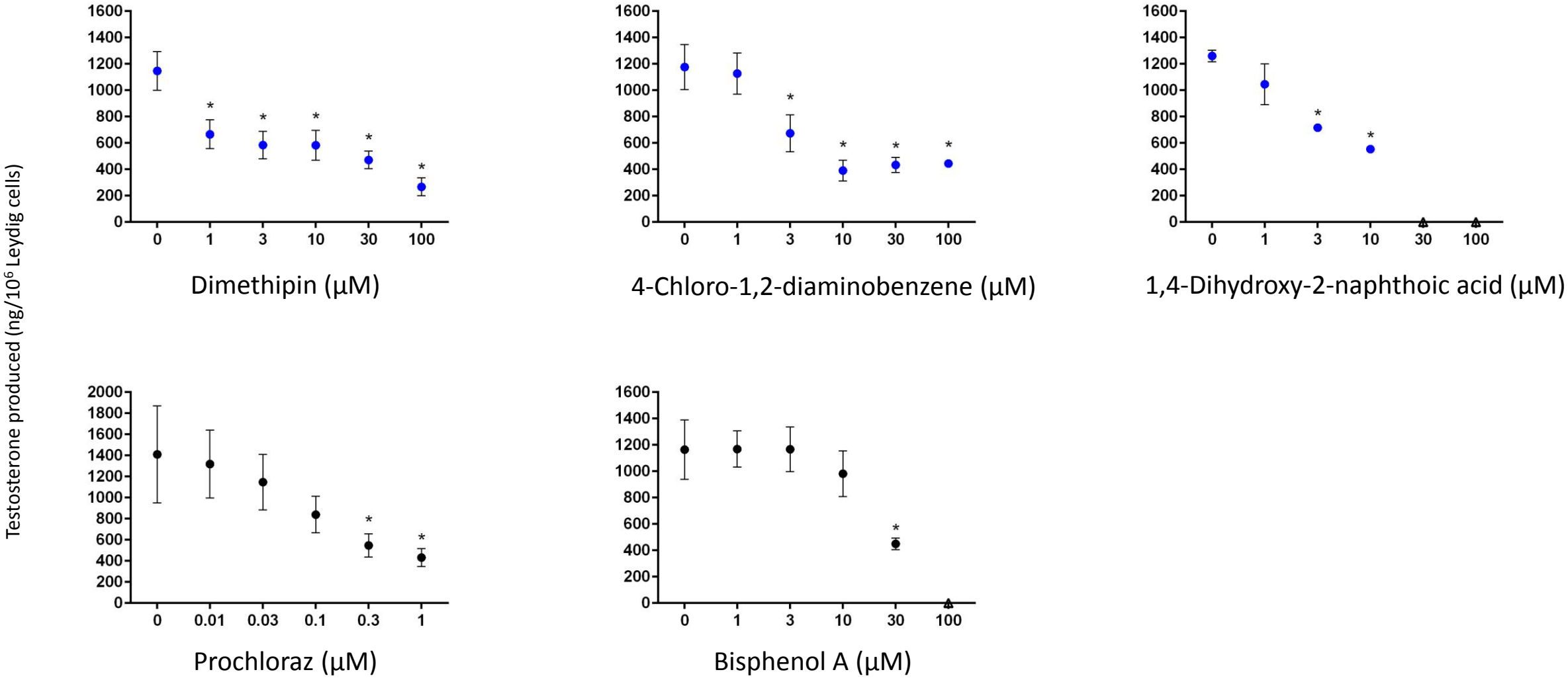
Testosterone produced (ng/10⁶ Leydig cells)
Dimethipin (µM)
4-Chloro-1,2-diaminobenzene (µM)
1,4-Dihydroxy-2-naphthoic acid (µM)
Prochloraz (µM)
Bisphenol A (µM)

Fig. 5

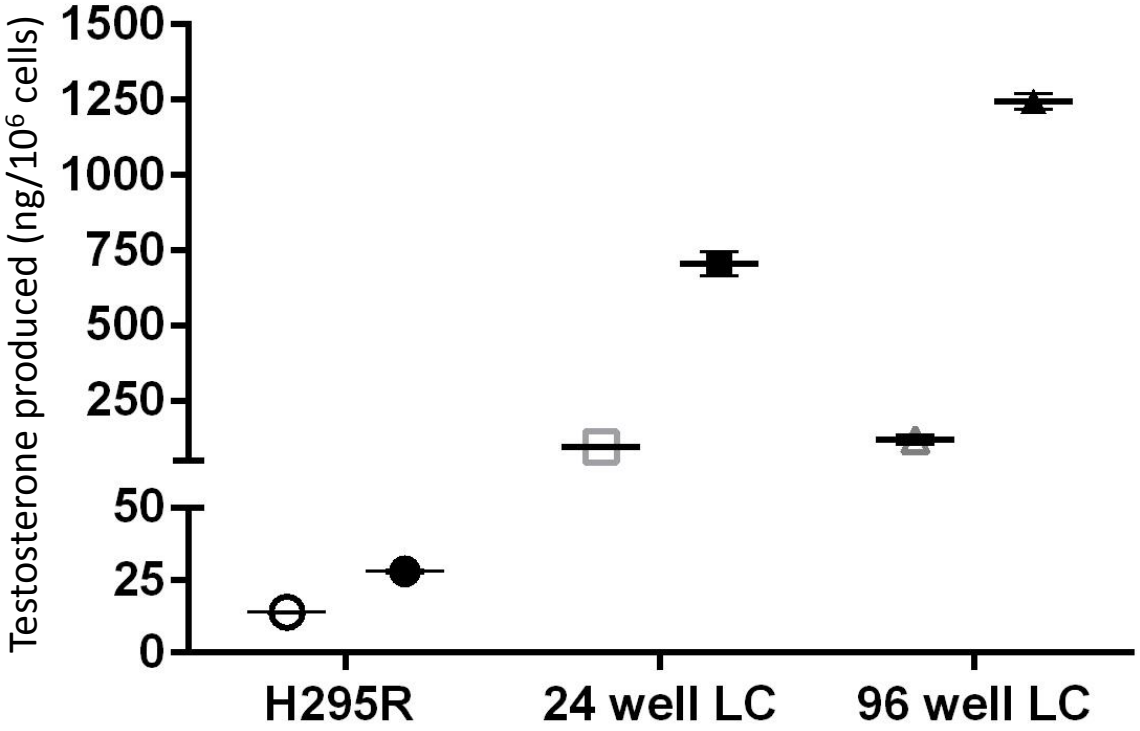
Testosterone produced (ng/10^6 cells)
1500
1250
1000
750
500
250
50
25
0
H295R
24 well LC
96 well LC

Fig. 6

Supplementary Table 1: Chemical categorization, identification, CAS number, purity, and source.

Category based upon H295R substrates	Chemical Name	CAS#	Purity	Source
x	Bisphenol A	80-05-7	99.9	Sigma-Aldrich
x	Prochloraz	67747-09-5	99.1	Sigma-Aldrich
x	Dexamethasone	50-02-2	99.2	Sigma-Aldrich
1	Corticosterone	50-22-6	> 98.5	Sigma-Aldrich
1	Cycloheximide	66-81-9	98	EMD Millipore
1	Esfenvalerate	66230-04-4	99.5	Sigma-Aldrich
1	17α-Ethynylestradiol	57-63-6	99.8	Sigma-Aldrich
1	Finasteride	98319-26-7	99.6	Sigma-Aldrich
1	Flufenoxuron	101463-69-8	98.1	Sigma-Aldrich
1	Flusilazole	85509-19-9	99.8	Sigma-Aldrich
1	Metconazole	125116-23-6	99.4	Sigma-Aldrich
1	Permethrin	52645-53-1	98.8 (isomer mix)	Sigma-Aldrich
1	Simvastatin	79902-63-9	99	Sigma-Aldrich
1	Spirodiclofen	148477-71-8	99.5	Sigma-Aldrich
1	Tamoxifen citrate	54965-24-1	99.7	United States Pharmacopeia
1	3,4,4'-Trichlorocarbanilide	101-20-2	98.9	Aldrich
1	Triphenyl phosphate	115-86-6	99.9	Aldrich
2	Anilazine	101-05-3	99.9	Sigma-Aldrich
2	4-Chloro-1,2-diaminobenzene	95-83-0	98.4	Aldrich
2	1,4-Dihydroxy-2-naphthoic acid	31519-22-9	99.3	Aldrich
2	Dimethipin	55290-64-7	99	Supelco Analytical
2	Hydroquinone	123-31-9	100.2	Sigma-Aldrich
2	Prednisone	53-03-2	99.8	Sigma-Aldrich