6,7-Dihydro-4*H*-indolones: Synthesis and Biological Properties

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Syntheses of 6,7-dihydro-4*H*-indolones **6** and **10** by a selective *Birch* reduction of the benzene ring of the indole system are described. The antiestrogen zindoxifene and the 2-phenylindole **2** as well as **6** and **10** were tested for their relative binding affinities at the androgen receptor as well as for antiandrogenic and estrogenic properties. Both compounds **6** and **10** showed potent indirect antiandrogenic activity which were similar to those of the 2-phenylindoles zindoxifene and **2**.

6,7-Dihydro-4*H*-indolone: Synthese und biologische Eigenschaften

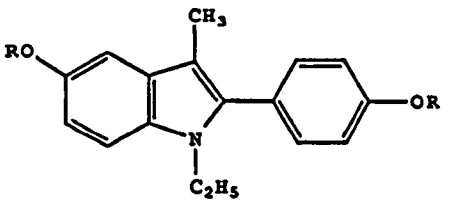
Die Synthese der 6,7-Dihydro-4*H*-indolone **6** und **10** durch selektive *Birch*-Reduktion des Benzolringes des Indolsystems werden beschrieben. Das Antiöstrogen Zindoxifen und das 2-Phenylindol **2** sowie **6** und **10** wurden auf ihre relative Bindungsaffinität zum Androgenrezeptor sowie antiandrogene und östrogene Aktivität untersucht. Beide Verbindungen **6** und **10** zeigten ausgeprägte indirekte antiandrogene Eigenschaften, die im Bereich der der 2-Phenylindole Zindoxifen und **2** lagen.

The main structural difference between estradiol and testosterone lies in ring A of the steroid system. For binding to the estrogen receptor (ER), a functional group with proton donor characteristics (phenolic OH-group) is essential[1].

Many steroidal and nonsteroidal estrogens like estradiol, diethylstilbestrol (DES) or hexestrol (HES) as well as many nonsteroidal antiestrogens like hydroxytamoxifen or zindoxifene thus possess a hydroxylated phenyl ring.

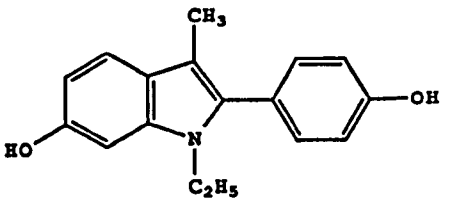
In contrast to estrogens a functional group with proton acceptor characteristics (keto group) is necessary for compounds with the ability to bind to the androgen receptor (AR)[1]. Endogenous androgens like testosterone (T) and dihydrotestosterone (DHT) as well as the steroidal antiandrogen cyproterone acetate (CPA) contain a keto-group in position 3 of ring A.

We have shown[2] that hydrogenation of one of the phenyl rings of hexestrol to a cyclohexenone system led to compounds with increased AR binding affinity and interesting biological properties.



zindoxifene R = Ac

1 R = H



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As the antiestrogen zindoxifene, a 2-phenylindole derivative, showed very promising tumor inhibitory effects against a variety of prostatic carcinoma tumor models[3,4], we investigated the effect of the reduction of the benzene moiety of this indole to a cyclohexenone system with regard to AR affinity, antiandrogenic and estrogenic properties.

In addition to zindoxifene (OH-group in 5-position) an additional phenylindole[5] **2**, having a hydroxy group in position 6 of the indole moiety, with a very high ER affinity (RBA = 33) was selected for *Birch* reduction to see how the position of the keto-group affects AR affinity.

Chemistry

Powerful reduction systems such as alkali metals in liquid ammonia are capable of reducing indoles. Ring selectivity can be controlled by the presence or absence of a proton source like methanol. The presence of excess methanol leads to the preferential reduction of the benzene ring. If methanol is omitted, usually the heterocyclic pyrrole ring is reduced[6,7].

In the class of the antiestrogenic 2-phenylindoles selectivity of reduction is complicated by the presence of a second aromatic system. Aromatic carbocycles like fluorene containing both a methoxy and a hydroxy group are only reduced in the methoxy substituted moiety[8]. In the same way, treatment of compound **5** (with an acetoxy group in the 2-phenyl moiety and a 5-methoxy group in the indole moiety) with lithium in liquid ammonia in the presence of methanol yielded the 4,7-dihydroderivative of **5**. This enol ether was hydrolized to the corresponding 6,7-dihydro-4*H*-indol-5-one **6**.

N-Substitution of 2-(4-methoxyphenyl)-3-methyl-5-methoxyindole was accomplished by treatment with NaH in dimethylformamide followed by addition of C_2H_5I to give **3**. Selective demethylation of the 4-methoxy group in the 2-phenyl moiety of **3** was readily effected with NaSEt in dimethylformamide. The monohydroxyphenylindole **4** was separated from oxidation products after conversion to the acetate **5** using acetic anhydride in pyridine followed by chromatographic isolation. Birch reduction of **5** was

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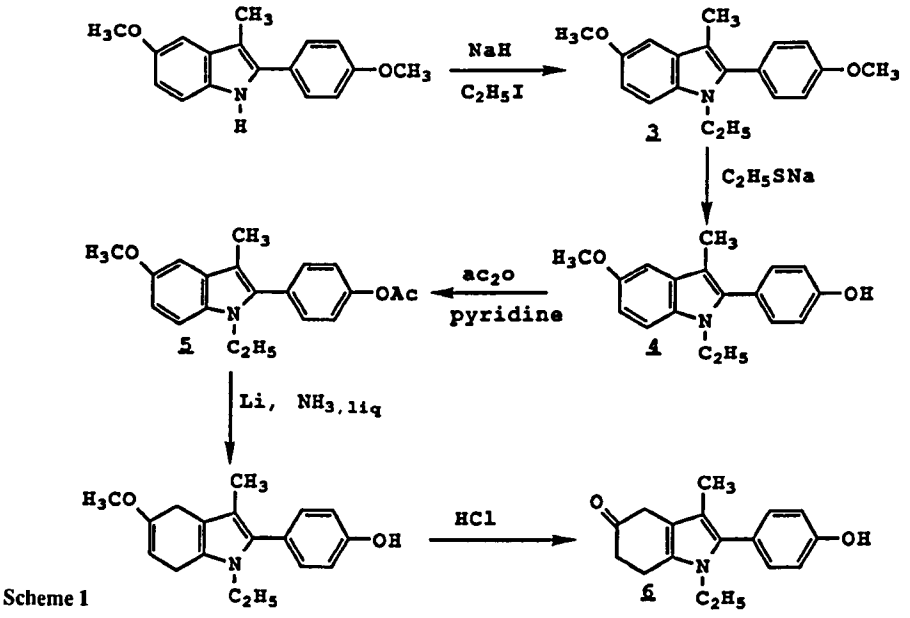
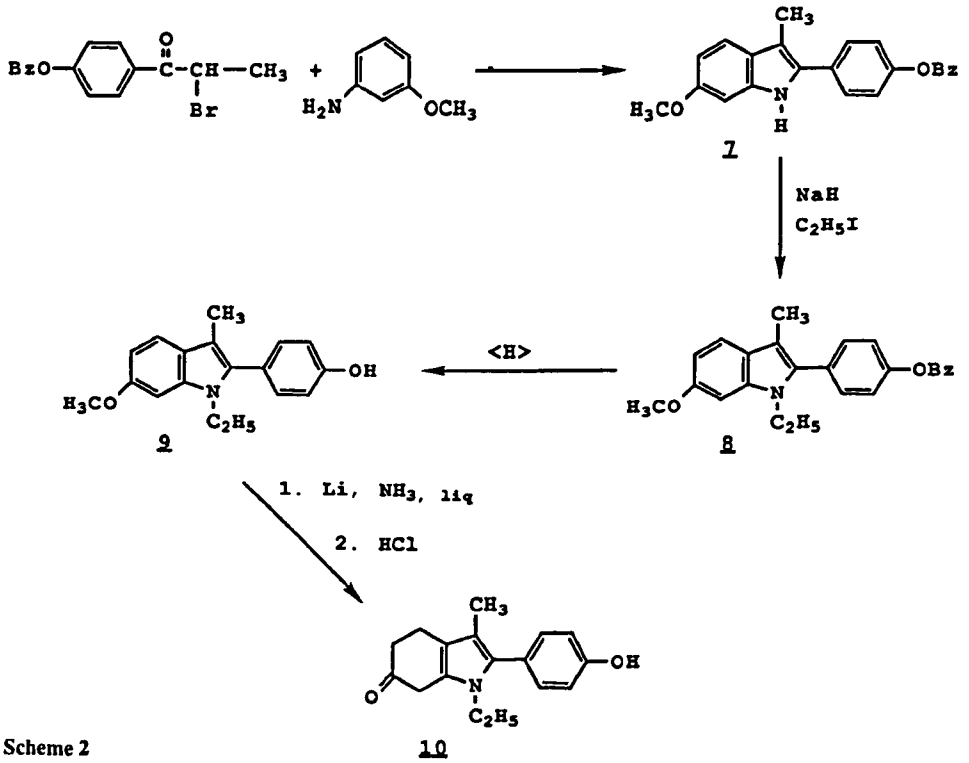


Scheme 1



Scheme 2

achieved by using a 17-fold excess of Li in liquid NH_3 in the presence of MeOH as proton donator and THF as cosolvent. Hydrolysis of the enol ether with 6N HCl in CH_2Cl_2 yielded the indolone **6**.

For synthesis of the indolone **10** the starting 1*H*-2-phenylindole **7** had to be prepared. Reaction of *m*-anisidine with 2-bromo-4'-benzyloxy-propiophenone afforded the 1*H*-2-(4-benzyloxyphenyl)indole **7**. N-substitution was accomplished by deprotonation with NaH in DMF and subsequent addition of C_2H_5I. Debenzylation of **8** was readily effected by hydrogenolysis in the presence of Pd/C (5%). In this case acetylation for further purification was not necessary. The monohydroxyphenylindole **9** was reduced by Li in liquid NH_3 to the corresponding enol ether and hydrolized to the indolone **10** under the conditions described for compound **5**.

Comparison of the NMR-spectra of the intermediate 4,7-dihydroindoles of **6** and **10** with the spectrum of 4,7-dihydro-1-methyl-5-methoxy-indole[7] showed the same typical signals for the vinylic and aliphatic protons. The identity of the hydrolized indolones **6** and **10** was established by ^{1}H-NMR spectroscopy and elemental analysis.

Biological properties

In a first test, the relative binding affinities (RBA) to the androgen and estrogen receptor were determined. Although compound **6** and **10** are more similar to T respectively DHT than the unreduced 2-phenylindoles by containing a cyclohexenone system, both compounds had no measurable affinity to the androgen receptor. On the other side, the cyclohexenone system led to a dramatic decrease in the RBA values to the estrogen receptor compared to the parent 2-phenylindoles **1** and **2**. **6** showed a 50 times lower ER binding affinity than the corresponding 2-phenylindole **1** (Table 1).

Table 1: Affinity of **1**, **2**, **6**, and **10** to the androgen receptor (AR) and the estrogen receptor (ER)

	RBA[a]	
	AR	ER
1	<0.01	9.5
2	-	33.0
6	<0.01	0.2
8	<0.01	-

a) RBA = ratio of the molar concentration of testosterone (AR), estradiol (ER) and inhibitor required to decrease radioligand by 50%, times 100. RBA of testosterone and estradiol = 100 by definition.

As neither **6** nor **10** showed any measurable affinity for the androgen receptor, it is unlikely that these compounds act directly as antiandrogens by competing with endogenous T for the AR. An indirect antiandrogenic effect, however, can also be exerted by decreasing the luteinizing hormone release from the pituitary and thus the T-production in the testes[9].

At a dose of 1000 µg both compounds **6** and **10** led to a strong reduction of the seminal vesicle weights in intact mature mice (Table 2). The indirect antiandrogenic properties of **6** and **10** at the 1000 µg dose were similar to those of zindoxifene (131 µg 53%; 1310 µg 68% inhibition) in the five day antiandrogenic assay[3].

Table 2: Effect of **6** and **10** on the seminal vesicle weights of intact mature mice

	Dose[a] (µg)	Inhibition (%)
6	100	26
	1000	57
10	1000	41

a) Dose/animal per day, compounds were administered daily for nine days sc.

The strong antiandrogenic effect of both compounds **6** and **10** in the seminal vesicle weight test made it interesting to compare the estrogenic activity of **6** and **10** with those of the respective 2-phenylindoles. Surprisingly **6** as well as **10** had a significant effect on the growth of the uterus of immature mice. In the low doses of 5 and 25 µg, **6** showed a weak but significant estrogenic activity. The 100 µg dose led to an increase of the estrogenicity to 73% of the estrone standard. Already at the 25 µg dose **10** reached about half of the activity of the estrone standard. However, no further increase of the estrogenic properties was observed at the 100 µg dose. Although the reduction of the benzene ring of the indole caused a drastic change in structure, **6** almost retained the estrogenic activity of the parent 2-phenylindole zindoxifene.

Table 3: Estrogenic effect of zindoxifene, **2**, **6**, and **10** in the immature mouse uterine weight test

	Dose[a]	Estrogenic Effect[b] (%)
zindoxifene	5	44
	25	47
	125	68
2	5	93
	25	100
	125	95
6	5	23*
	25	20*
	100	73*
10	25	42
	100	54*

a) dose/animal per day; dissolved in olive oil, administered subcutaneously on three consecutive days.

b) % of estrone standard; 0.4 µg for **6** and **10**; 0.1 µg for zindoxifene and **2**.

* Significant ($p < 0.01$).

The results obtained clearly show that the reduction of 2-phenylindoles to 6,7-dihydro-4*H*-indolones does not lead to compounds with high binding affinity to the androgen receptor in spite of the presence of a keto-group. Although the binding affinities for the ER are strongly decreased, the estrogenic properties of **5** and **10** are almost unchanged as compared to the parent 2-phenylindoles. Therefore, the indirect antiandrogenic activity of **6** and **10** is most probably due to their estrogenic properties.

Consequently, our aim to obtain new compounds with AR affinity and thus direct antiandrogenic activity accompanied by decreased estrogenicity compared to zindoxifene and **2** was not fulfilled by this approach.

Experimental Part

Mp: Büchi 510 apparatus (uncorr.).- ^{1}H-NMR Spectra: Varian EM 360 L.- IR-Spectra: Beckman Acculab 7.- Elemental Analyses: Mikroanalyt. Laboratorium, Univ. Regensburg.

1-Ethyl-2-(4-methoxyphenyl)-5-methoxy-3-methylindole (**3**)

A solution of 17.7 mmol (5.00 g) of 1*H*-2-(4-methoxyphenyl)-3-methyl-5-methoxyindole in 30 ml dry DMF was added slowly to a stirred mixture of 28 mmol (0.84 g) of NaH (80% oil dispersion) in 50 ml dry DMF with cooling. After stirring for 45 min at 0°C, 20 mmol (3.11 g) of C_2H_5I in 20 ml dry DMF was added at 0°C. After stirring for 30 min at 0°C and 2 h at room temp. the excess of NaH was decomposed by dropwise addition of water. After addition of Et_2O, the org. layer was washed with brine and dried ($MgSO_4$). The residue obtained by evaporation of the solvent was crystallized from EtOH. Yield 90%, mp. 113°C (Lit.[5]: 111-119°C), $C_{19}H_{21}NO_2$ (295.4).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.18 (t, J = 7 Hz, 3H, CH_2-CH_3); 2.21 (s, 3H, CH_3); 3.88 and 3.93 (s, 6H, OCH_3); 4.05 (q, J = 7 Hz, 2H, CH_2-CH_3); 6.85-7.47 (m, 7 H, aromat. H).

1-Ethyl-2-(4-benzyloxyphenyl)-6-methoxy-3-methylindole (**8**)

Yield 75%, mp. 99°C, $C_{25}H_{25}NO_2$ (371.5) Calcd. C 80.8 H 6.78 N 3.8 Found C 80.3 H 6.68 N 3.5.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.17 (t, J = 7 Hz, 3H, CH_2-CH_3); 2.18 (s, 3H, CH_3); 3.90 (s, 3H, OCH_3); 4.02 (q, J = 7 Hz, CH_2-CH_3); 5.15 (s, 2H, OCH_2-Ph); 6.80-7.58 (m, 12 H, aromat. H).

1-Ethyl-2-(4-hydroxyphenyl)-3-methyl-5-methoxyindole (**4**)

Under N_2, 60 mmol (3.75 g) of 2-mercaptoethanol in 10 ml dry DMF was added to a suspension of 90 mmol (2.1 g) of NaH (80% oil dispersion) in 20 ml dry DMF and stirred for 30 min. Then, a solution of 6.80 mmol (2.0 g) of **3** in dry DMF was added and heated for 2 h at 150°C (bath temp.). The mixture was poured into ice water, acidified with 3N HCl and extracted with ethyl acetate. The combined org. layers were washed with brine and dried ($MgSO_4$). After evaporation of the solvent the residue was crystallized from EtOH. Yield 20%, mp. 121-123°C (Lit.[10]: 134-135°C).- $C_{18}H_{19}NO_2$ (281.4).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.12 (t, J = 7 Hz, 3H, CH_2-CH_3); 2.13 (s, 3H, CH_3); 3.85 (s, 3H, OCH_3); 3.98 (q, J = 7 Hz, 2H, CH_2-CH_3); 5.40 (s, 1H, OH); 6.78-7.28 (m, 7H, aromat. H).

2-(4-Acetoxyphenyl)-1-ethyl-5-methoxy-3-methylindole (**5**)

16 mmol (4.50 g) of **4** were treated with a mixture of 25 mmol (2.36 ml) of acetic anhydride and 25 mmol (2.02 ml) of pyridine and stirred overnight. The mixture was poured onto ice and extracted with Et_2O. The org. layer was washed with 3N HCl and brine and dried ($MgSO_4$). The residue obtained by evaporation of the solvent was chromatographed over SiO_2 with CH_2Cl_2. The product was recrystallized from EtOH. Yield 70%, mp. 102-104°C (Lit.[10]: 93-94°C).- $C_{20}H_{21}NO_3$ (323.4).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.20 (t, J = 7 Hz, 3H, CH_2-CH_3); 2.25 (s, 3H, CH_3); 2.37 (s, 3H, $OCOCH_3$); 3.90 (s, 3H, OCH_3); 4.08 (q, J = 7 Hz, 2H, CH_2-CH_3); 6.92-7.58 (m, 7H, aromat. H).

1-Ethyl-2-(4-hydroxyphenyl)-3-methyl-6,7-dihydro-4H-indol-5-one (**6**)

A solution of 5 mmol (1.40 g) of **5** in 6 ml dry MeOH in 60 ml liquid ammonia was treated with 85 mg-atom (0.59 g) of Li-granula in portions. After each addition the blue colour should disappear. Stirring was continued for 1 h at -70°C and then for 1 h at room temp. The excess of Li was decomposed by 2 ml MeOH. After evaporation of the ammonia, water, and if necessary 3N HCl, was added to give a clear solution. The mixture was extracted with ether. The combined org. phases were washed with water to remove excess of MeOH.- After evaporation of the ether, the residue was dissolved in 5 ml CH_2Cl_2 and cooled to 0°C. Ice cold 6N HCl (5 ml) was added slowly, followed by stirring for 30 min at room temp. and 45 min at 40°C. The mixture was treated with water and extracted with ethyl acetate. The org. phase was washed with saturated $NaHCO_3$ solution and dried (Na_2SO_4). After evaporation of the CH_2Cl_2 the residue was purified by column chromatography (SiO_2; CH_2Cl_2:EtOAc, 8:2). Yield 37%, mp. 164-166°C.- $C_{17}H_{19}NO_2$ x H_2O (287.4) Calcd. C 71.1 H 7.37 N 4.9 Found C 70.3 H 7.26 N 4.3.- IR (KBr): 1715 cm^{-1} (C=O).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.12 (t, J = 7 Hz, 3H, CH_2-CH_3); 1.88 (s, 3H, CH_3); 2.72-3.15 (m, 4H, CH_2); 3.42 (s, 2H, CH_2); 3.80 (q, J = 7 Hz, 2H, CH_2-CH_3); 6.97 and 7.25 (AB, J_{AB} = 9 Hz, 4H, aromat. H).

1-Ethyl-2-(4-hydroxyphenyl)-3-methyl-6,7-dihydro-4H-indol-6-one (**10**)

Yield 15%, mp. 174-179°C.- $C_{17}H_{19}NO_2$·H_2O (287.4) Calcd. C 71.1 H 7.37 N 4.9 Found C 71.0 H 7.26 N 4.3.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.06 (t, J = 7 Hz, 3H, CH_2-CH_3); 1.91 (s, 3H, CH_3); 2.60-2.85 (m, 4H, CH_2); 3.44 (s, 2H, C(O)-CH_2); 3.65 (q, J = 7 Hz, 2H, CH_2-CH_3); 6.82 and 7.05 (AB, J_{AB} = 9 Hz, 4H); 8.03 (s, 1H, OH).

2-(4-Benzyloxyphenyl)-6-methoxy-3-methylindole (**7**)

A solution of 35 mmol (11.17 g) of 2-bromo-(4-benzyloxy)-propiophenone in 25 ml xylene was added slowly to a boiling mixture of 120 mmol (15.00 g) of *m*-anisidine and 35 ml of *N,N*-dimethylaniline with stirring. The mixture was kept at 170°C bath temp. for 4 h. After cooling, ethyl acetate was added and the mixture extracted with 3N HCl. The aqueous layer was extracted several times with ethyl acetate. After washing with 3N HCl and brine, the org. layer was dried ($MgSO_4$). The product was obtained after evaporation of the solvents and crystallization from EtOH. Yield 44%, mp. 170-172°C.- $C_{23}H_{21}NO_2$ (343.4) Calcd. C 80.4 H 6.16 N 4.1 Found C 79.1 H 6.15 N 3.8.- ^{1}H-NMR (d_6-Benzol): δ (ppm) = 2.43 (s, 3H, CH_3); 3.69 (s, 3H, OCH_3); 4.98 (s, 2H, CH_2); 6.84-7.70 (m, 12 H, aromat. H); 9.57 (s, 1H, NH).

1-Ethyl-2-(4-hydroxyphenyl)-6-methoxy-3-methylindole (**9**)

0.25 g Pd/C (5%) was added to a solution of 7.5 mmol (2.79 g) of **8** in 200 ml EtOH. The suspension was shaken under H_2 at room temp. until no more H_2 was taken up. The mixture was filtered and EtOH was removed. The crude product was recrystallized from EtOH. Yield 93%, mp. 123-128°C.- $C_{18}H_{19}NO_2$ (281.4) Calcd. C 76.8 H 6.81 N 5.0 Found C 75.6 H 6.65 N 4.84.- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.16 (t, J = 7 Hz, 3H, CH_2-CH_3); 2.15 (s, 3H, CH_3); 3.84 (s, 3H, OCH_3); 3.93 (q, J = 7 Hz, CH_2-CH_3); 6.63-7.45 (m, 12 H, aromat. H).

Binding affinities for the androgen and estrogen receptor

The relative binding affinities were determined by the dextran coated charcoal method as described with some modifications[11]. 100 µl aliquots of calf uterine cytosol were incubated with 100 µl (1 nM) [^{3}H]-mibolerone (AR) for 2 h, [^{3}H]-estradiol (ER) for 16 h in the presence of varying concentrations of the test compounds (five or six concentrations). Incubations were stopped by adding dextran-coated charcoal. After centrifugation, the radioactivity of a 100 µl supernatant aliquot was counted. The percentage bound radioligand was plotted *vs.* the concentration of unlabelled test compounds. From this plot, the molar concentrations of unlabelled standards and of test compounds reducing radioligand binding by 50% were determined.

Antiandrogenic activity on intact mature male mice

Mature male mice (23-25 g; 6-7 mice/group from Ivanovas, Kisslegg, FRG) were used. The test compounds dissolved in olive oil were injected for 9 consecutive days[11]. 24 h after the last injection, blood was collected by cardiac puncture under ether anesthesia, seminal vesicles were removed, dissected free from adhering tissue, dried overnight at 100°C and weighed.

Estrogenic activity

The uterotrophic activity in the immature mouse uterine weight test was determined as previously described[12].

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