FLUORO-CLOMIPHENE AND ITS SYNTHETIC PRECURSORS: SYNTHESIS AND RECEPTOR BINDING

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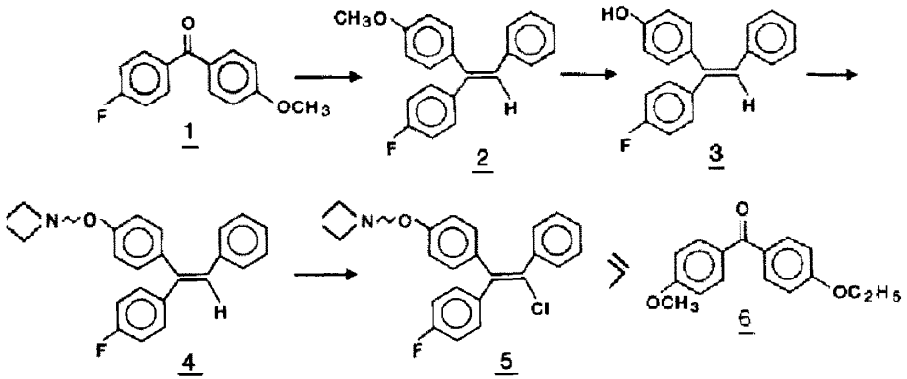
ABSTRACT

In an attempt to synthesize compounds with selective estrogen-receptor binding, fluoro- and amino-clomiphene were totally synthesized from benzyl chloride, and their estrogenic/antiestrogenic activity as well as that of some of their chemical intermediates was evaluated. The triazene prepared from the amino-clomiphene was converted into fluoro-clomiphene with 39% yield. In the uterotropic test, both amino- and fluoro-clomiphene exerted mild equipotent estrogenic activity, with minimal saturation doses being 50 and 100 μg/rat/day for three days. In the receptor binding test both derivatives demonstrated similar displacement, with an $A_{50\%}$ value in the 10^{-5}M range, as compared to 10^{-6}M for clomiphene and 10^{-9}M for diethyl-stilbestrol. This synthesis may be useful for the preparation of ^{18}F-labeled clomiphene for biodistribution studies.

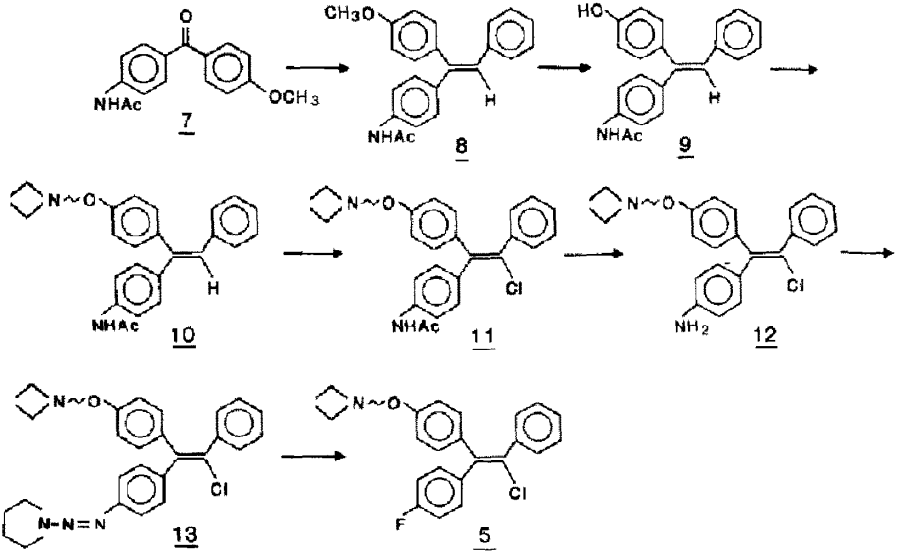
INTRODUCTION

Of the triphenylethylene antiestrogens reported in the literature, two have so far reached the clinic: tamoxifen and clomiphene. Both drugs bind with high affinity to estrogen-receptor systems of target cells (1) and to antiestrogen-specific binding sites, existing in the cytosols of estrogen-receptor positive tissues, i.e., mammary and uterus (2). In a previous publication we have described the total synthesis of amino-tamoxifen, which could be quickly diazotized into fluoro-tamoxifen (3). In the present paper we suggest the same approach of diazotization of amino-clomiphene to yield fluoro-clomiphene. We report here the total synthesis, as well as receptor binding affinity and uterotropic activity of

Scheme I



Scheme II



amino-clomiphene, fluoro-clomiphene and their chemical intermediates, and suggest a procedure for the diazotization, which may be utilized for attaching a fluorine or a radiofluorine (^{18}F) atom to the clomiphene molecule.

EXPERIMENTAL

Elemental analyses were done at The Hebrew University of Jerusalem Microanalytical Laboratory. Melting points were taken on a Fischer-Johns melting point apparatus and are uncorrected. UV spectra were determined in ethanol with a Gilford 2400-S spectrophotometer. IR spectra were obtained with a Perkin-Elmer 157G spectrophotometer. ^1H NMR spectra were recorded on a Bruker WH-300 pulsed FT spectrophotometer, in $CDCl_3$, unless otherwise indicated. Chemical shifts are reported in ppm downfield from internal TMS. Electron impact mass spectra were recorded on an MAT 311 instrument.

Chromatography columns were packed with Merck 35-70 silica gel or dry silica (Woelm-Pharma) and eluted successively with hexane, hexane-CH_2Cl_2, CH_2Cl_2 and CH_2Cl_2-CH_3OH. Solvents obtained from Frutarom (Israel) were used without further purification. TLC was taken on Merck silica gel GF_{254} plates (0.25 mm thickness). In this study, "work up" means diluting with H_2O, extracting with $CHCl_3$, drying the organic phase with $MgSO_4$, filtering and drying.

1-(4-Fluorophenyl)-1-(4-methoxyphenyl)-2-phenylethylene (2)

Benzylmagnesium chloride (200 mmol) was prepared from 25 g of benzylchloride and 4.6 g of Mg in 250 mL dry ether. Ketone 1 (9.2 g, 40 mM) was added as solid in portions. A slight reaction occurred. After 5 h reflux, the reaction product was decomposed on conc. HCl + ice, extracted with ether and worked up as usual. The oil was dissolved in 50 mL acetic acid and 10 mL H_2SO_4 and stirred for 3 h at room temperature. After treatment with water and work up, the resulting dark oil was chromatographed on silica gel. Elution with 33% CH_2Cl_2 in hexane gave 4.4 g, 36%, of white viscous oil, which solidified into a sticky solid, mp 60-65°C, on long standing. TLC showed a mixture of E:Z isomers. Rf (CH_2Cl_2:Hexane - 1:4) = 0.5, 0.45. IR_{neat} - 2950, 1600 cm^{-1}. UV - 230 (25,700), 244 sh (21,200), 303 (25,000), mμ. ^1H NMR $CDCl_3$ - 7.26-6.75 (14H, m), 3.77, 3.75 (3H, s, OCH_3, 3:2 Z:E). Anal. Calc. for $C_{21}H_{17}FO$: C=82.89,

H=5.59, F=6.25. Found: C=82.72, H=5.40, F=5.57. MS - 304 (M^+, 100%), 289 (M-CH_3, 20%), 273 (M-OCH_3, 23%), 271 (M-OCH_3-2H, 22%), 259 (22%), 246 (29%), 233 (19%), 209 (17%), 196 (C_6H_5F-C≡ C-C_6H_5, 28%), 183 (18%), 165(38%).

1-(4-Fluorophenyl)-1-(4-hydroxyphenyl)-2-phenylethylene (3)

Compound 2 (4.2 g, 14 mM) was dissolved in 100 mL CH_2Cl_2, and 5 mL BBr_3 was added dropwise at room temperature. A slight reaction occurred and the color turned green. After 1 h no starting material was detected by TLC and the reaction was decomposed on ice and worked up. Chromatography gave 3.7 g, 92% yield, white viscous oil, which did not solidify. Rf CH_2Cl_2 = 0.6. IR_{neat} - 3500-3200, 3050, 1600 cm^{-1}. UV - 246 (10,300), 307 (12,100), mμ. ^1H NMR $CDCl_3$ -7.16-6.5 (14H, m). Anal. Calc. for $C_{20}H_{15}FO$: C=82.75, H=5.17, F=6.55. Found: C=82.89, H=5.30, F=6.25.

1-(4-Fluorophenyl)-1-[4-[2-(N,N-diethylamino)ethoxy]phenyl]-2-phenylethylene (4)

To fluorophenol 3 (3.5 g, 12 mM) and 2.6 g, 15 mM, 2-diethylaminoethyl chloride hydrochloride in 100 mL ethanol was added 1.8 g KOH, and the suspension stirred and refluxed for 3 h. It was cooled, diluted with water and extracted with $CHCl_3$. Chromatography gave 0.5 g starting material and 2.8 g 4, oily solid. Rf (CH_2Cl_2: CH_3OH -9:1) = 0.5. Yield 60%, 69%. IR $CHCl_3$ - 3400, 2950, 1600 cm^{-1}. UV - 243 (10,600), 304 (12,100), mμ. ^1H NMR - 1.08, 1.07 (6H, 2t, $NCH_2\underline{CH_3}$), 2.64 (4H, m, $N\underline{CH_2}CH_3$), 2.88 (2H, m, $N\underline{CH_2}CH_2O$), 4.05, 4.04 (2H, 2t, $NCH_2\underline{CH_2}O$), 6.82-7.40 (14H, m, aromatic and vinylic). ^1H NMR shows 1:1 E:Z mixture. Anal. Calc. for $C_{26}H_{28}FNO$: C=80.20, H=7.20, N=3.60. Found: C=80.31, H=7.27, N=3.45.

Fluoro-clomiphene (5)

Compound 4 (2.5 g, 6.4 mM) was dissolved in 50 mL $CHCl_3$, and 50 mL $CHCl_3$ containing 0.7 g Cl_2 was added. After 1 h at room temperature and 1 h reflux it was made basic, extracted with $CHCl_3$ and chromatographed on silica gel. A white solid (1.7 g) was obtained. Yield 65%, mp 80-85°C. Rf (CH_2Cl_2: CH_3OH - 9:1) = 0.6. IR $CHCl_3$ - 3400, 2900, 1605 cm^{-1}. UV - 236 (14,600), 286 (4,600), 298 sh (4,000), mμ. ^1H NMR - 1.17, 1.15 (6H, 2 overlapping t, $N(CH_2\underline{CH_3})_2$), 2.86, 2.84 (4H, 2 overlapping q, $N(\underline{CH_2}CH_3)_2$), 3.11 (2H, t, J=6.0 cps, $OCH_2\underline{CH_2}N$, Z), 3.17 (2H, t, J=6.0 cps, $OCH_2\underline{CH_2}N$, E), 4.18 (2H, t, J=6.0 cps, $O\underline{CH_2}CH_2N$, Z),

4.26 (2H, t, J=6.0 cps, $O\underline{CH_2}CH_2N$, E) 7.45-6.70 (13H, m). E:Z ratio 6:4. Anal. Calc. for $C_{26}H_{27}ClFNO$: C=73.67, H=6.38. Found: C=73.81, H=6.87. MS -396, 394 ($M-C_2H_5$), 359 ($M-C_2H_5$-Cl), 342, 304, 286, 100(B), 86 m/e.

4-Methoxy-4'-ethoxybenzophenone (6)

In an attempt to use Horner-Wittig reaction instead of Grignard reaction, olefin 2 was not obtained. The product was identified as 4-methoxy-4'-ethoxybenzophenone 6. The reaction mechanism is not clear. To 5.75 g, 25 mM, 4-fluoro-4'-methoxy-benzophenone 1 and 7.3 g, 32 mM, $(OC_2H_5)_2P(O)-CH_2C_6H_5$ in 250 mL dry THF, under N_2, was added 1.7 g, 35 mM, 50% NaH. The reaction was stirred and refluxed for 4 h and worked up as usual. Chromatography on silica gel gave 2.5 g recovered starting material 1 followed by white solid, 1.1 g, mp 105°C. Yield 17% (30% with recovered starting material). Anal. Calc. for $C_{16}H_{16}O_3$: C=75.00, H=6.25. Found: C=74.60, H=6.31, F=0.00. MS - 256 (M^+, 76%), 227 ($M-C_2H_5$, 8%), 225 ($M-OCH_3$, 10%), 211 ($M-OC_2H_5$, 10%), 199 ($M-C_2H_5$-CO, 3%), 197 ($M-OCH_3$-CO, 4%), 149 ($M-OCH_3.C_6H_4$, 61%), 135 ($M-OC_2H_5.C_6H_4$, 100%), 121 ($C_6H_4OC_2H_5^+$, 40%), 107 ($C_6H_4OCH_3^+$, 8%), m/e. IR $CHCl_3$ - 1640, 1600, 1585 cm^{-1}. UV 225 (15,300), 295 (21,840), mμ. ^1H NMR - 1.40 (3H, t, J=7.0 cps, OC_2H_5), 3.73 (3H, s, OCH_3), 3.96 (2H, q, J=7.0 cps, OC_2H_5), 6.70, 7.50 (8H, ABq, J_{AB}=8 cps).

1-(4-Acetylaminophenyl)-1-(4-methoxyphenyl)-2-phenylethylene (8)

A - Benzylmagnesium chloride 165 mM was prepared from 21.5 g benzyl chloride and 4 g magnesium in 150 mL dry ether, and 9 g, 33 mM ketone 7 (4) dissolved in 300 mL dry THF was added during 15 min. There was a slight reaction and a precipitate was formed. After 5 h reflux the reaction was decomposed on conc. HCl + ice, extracted with ether and worked up. The red sticky solid consisted of the alcohol and olefin with some starting material. Standing at room temperature for a few days or absorption on silica gel overnight caused dehydration of the alcohol to the olefin. Chromatography on silica gel yielded 5.5 g light-yellow solid, mp 160-175°C, yield 48%. Rf (3% CH_3OH in CH_2Cl_2) = 0.5. IR $CHCl_3$ -3420, 3300, 2900, 1690, 1600 cm^{-1}. UV - 228 (18,200), 265 (21,800), 315 (19,600), mμ. ^1H NMR - 2.16 (3H, s, NHAc, E), 2.17 (3H, s, NHAc, Z), 3.80 (3H, s, OCH_3, Z), 3.82 (3H, s, OCH_3, E), 6.84-7.48 (13H, m, aromatic protons), 7.64 (1H, s, vinylic proton), 6:4 Z:E mixture. Anal. Calc. for $C_{23}H_{21}NO_2$: C=80.46, H=6.12, N=4.08. Found: C=80.16, H=5.86, N=3.99. MS -343

(M^+, 100%), 287 ($M-CH_2CO-CH_3$, 12%), 283 (15%), 281 (14), 259 (15%), 236 ($M-C_6H_5OCH_3$, 16%), 209 ($M-C_6H_5NHAc$, 6%) m/e.

B - Reaction of 24 g $TiCl_3$-$LiAlH_4$ with 6 g; 22 mM, ketone 7 and 3 g, 29 mM benzaldehyde in THF gave 2.4 g olefin 8, 31% yield, 55:45 Z:E mixture.

1-(4-Acetylaminophenyl)-1-(4-hydroxyphenyl)-2-phenylethylene (9)

Compound 8 (5.5 g, 16 mM) was dissolved in 100 mL CH_2Cl_2, and 5 mL BBr_3 was added dropwise at room temperature. A strong reaction occurred. After 1 h no starting material was detected and the reaction was decomposed on ice-water and worked up to give a white solid, mp 80-95°C, 4.0 g, yield 76%. Rf (3% CH_3OH in CH_2Cl_2) = 0.1. IR $CDCl_3$ -3400, 2950, 1685 cm^{-1}. UV 229 (15,000), 265 (16,900), 318 (16,100), mμ. ^1H NMR - 2.19, 2.20 (3H, s, NHAc, 1:1 E:Z), 6.74-7.65 (14H, m). MS -329 (M^+, 100%), 313 (12%), 287 ($M-CH_2CO$, 49%), 270 (11%), 250 (8%), 239 (6%), 195 (14%), 165 (10%) m/e. Anal. Calc. for $C_{22}H_{19}NO_2$: C=80.24, H=5.77, N=4.25. Found: C=79.96, H=5.67, N=4.46.

1-(4-Acetylaminophenyl)-1-[4-[2-(N,N-diethylamino)ethoxy]phenyl]-2-phenylethylene (10)

Compound 9 (3.7 g, 11 mM) and 1.5 g, 27 mM, KOH were dissolved in 30 mL ethanol. After stirring for 20 min at room temperature 2.6 g, 15 mM of 2-diethylaminoethylchloride hydrochloride and 100 mL toluene were added and the reaction was refluxed for 3 h. Work up and chromatography on silica gel gave 1.5 g, 31%, light-yellow oily solid, mp 70-90°C. Rf (CH_2Cl_2:CH_3OH - 9:1) = 0.4. IR $CHCl_3$ - 3400, 3300, 2900, 1685, 1600 cm^{-1}. UV - 267 (21,200), 315 (15,150), mμ. ^1H NMR - 1.07, 1.09 (6H, 2t, J=7.0 cps, $NCH_2\underline{CH_3}$, Z and E), 2.16, 2.17 (3H, 2s, NHAc, E and Z), 2.66, 2.67 (4H, 2q, J=7.0 cps, $N\underline{CH_2}CH_3$, Z and E), 2.89, 2.90 (2H, 2t, J=6.2 cps, $N-\underline{CH_2}CH_2O$, Z and E), 4.05, 4.06 (2H, 2t, J=6.2 cps, $NCH_2\underline{CH_2}O$, Z and E), 6.80-7.50 (13H, m, aromatic protons), 7.80 (1H, br s, vinylic proton). MS - 428 (M^+, 3%), 328 ($M-(Et)_2\overset{+}{N}H-CH=CH_2$, 3%), 286 (M-100-$CH_2CO$, 4%) 100 ($Et_2\overset{+}{N}H-CH=CH_2$, 28%), 86 ($Et_2\overset{+}{N}=CH_2$, 100%), m/e. Anal. Calc. for $C_{28}H_{32}N_2O_2$: C=78.50, H=7.47, N=6.54. Found: C=78.20, H=7.10, N=6.82.

1-(4-Acetylaminophenyl)-1- [4-[2-(N,N'-diethylamino)ethoxy]phenyl] -2-phenyl-2-chloroethylene. Acetylamino-clomiphene (11)

Compound 10 (1.4 g, 3.5 mM) was dissolved in 40 mL CH_2Cl_2 and a solution of 0.5 g, 7 mM, Cl_2 in 10 mL CCl_4 was added. After the strong reaction subsided it was stirred 3 h at room temperature, made basic and worked up as usual. Chromatography gave 0.5 g slightly yellow semi-solid, 33%. Rf (CH_2Cl_2:CH_3OH-9:1) = 0.5. IR $CHCl_3$ - 3400, 2900, 1690, 1600 cm^{-1}. UV - 258 (19000), 305 sh (14,500), mμ. ^1H NMR-$CDCl_3$ - 1.05 (E), 1.09 (Z) (6H, 2t, J=7.0 cps, $NCH_2\underline{CH_3}$), 2.18 (Z), 2.25 (E) (3H, 2s, NHAc), 2.64 (E), 2.68 (Z) (4H, 2 overlapping q, J=7.0 cps, $N\underline{CH_2}CH_3$), 2.84 (E), 2.93 (Z) (2H, 2t, J=6.0 cps, $N\underline{CH_2}CH_2O$), 3.97 (E), 4.10 (Z) (2H, 2t, J=6.0 cps, $NCH_2\underline{CH_2}O$), 6.60-7.50 (13H, m, aromatic protons). MS-464, 462 (M^+, ^{37}Cl,^{35}Cl, 3%, 8%), 422, 420 (M-ketene, 2%, 7%), 342 (M-Cl-Et_2NCH_2, 25%), 149 (32%), 100 ($Et_2\overset{+}{N}H$-CH=CH_2, 35%), 86 ($Et_2\overset{+}{N}$=CH_2, 100%), m/e. Anal. Calc. for $C_{28}H_{31}N_2O_2Cl$: C=72.64, H=6.70, N=6.05, Cl=7.67. Found: C=72.39, H=6.75, N=6.09, Cl=7.60. NMR shows Z:E ratio of 55:45.

1-(4-Aminophenyl)-1- [4- [2-(N,N-diethylamino)ethoxy]phenyl] -2-phenyl-2-chloroethylene. Amino-clomiphene (12)

Acetylamino-clomiphene 11 (1.5 g, 3.2 mM) was dissolved in 25 mL methanol, 25 mL 4N HCl was added and it was heated at 100°C for 1 h, cooled, made basic, and extracted with CH_2Cl_2. After usual work-up and chromatography on silica gel 0.72 g, 53% semisolid oil was obtained. Compound 12 is light-sensitive and should be kept in the dark. UV - 230 (18,000), 250 (15,300), 295 (9,400), 345 (4,900), mμ. ^1H NMR $CDCL_3$ - 1.07 (E), 1.10 (Z) (64, 2t, J=7.0 cps, $NCH_2\underline{CH_3}$), 2.63 (E), 2.67 (Z) (4H, 2q, J=7.0 cps, $N\underline{CH_2}$ CH_3), 2.87 (E), 2.93 (Z) (2H, 2t, J=6.0 cps, $N\underline{CH_2}CH_2O$), 4.00 (E), 4.12 (Z) (2H, t, J=6.0 cps), $NCH_2\underline{CH_2}O$), 6.45-7.40 (13H, m, aromatic protons). E:Z ratios 1:1. Anal. Calc. for $C_{26}H_{29}N_2OCl$: C=74.19, H=6.89, N=6.66, Cl=8.44. Found: C=74.22, H=6.92, N=6.51, Cl=8.13. MS - 422, 420 (M^+, ^{37}Cl, ^{35}Cl, 2%, 7%), 300 (M-Cl-Et_2NCH_2, 32%), 100 (37%), 86 (100%).

1-[4-[3',3'-(1,5-Pentadiyl)triazine]phenyl]-1-[4-[2-(N,N-diethylamino)ethoxy]phenyl]-2-phenyl-2-chloroethylene (13)

To 0.8 g, 2 mM, amino-clomiphene 12 in 50 mL 6N HCl, cooled in ice, was added 0.2 g $NaNO_2$. After 0.5 h stirring in the cold it was filtered, 0.6 mL piperidine added, and the reaction stirred for 1 h at room temperature, made

basic and worked up. Chromatography gave pale-yellow semisolid, 0.55 g, 56%. UV - 229 (17,200), 300 sh (16,800), 345 (19,200), mμ. ^{1}H NMR $CDCl_3$ -1.05 (6H, 2 overlapping t, J=7.0, $NCH_2\underline{CH_3}$), 1.81 (6H, narrow m, piperidino protons), 2.65 (E), 2.69 (Z) (4H, 2q, J=7.0 cps, $N\underline{CH_2}CH_3$), 2.89 (E), 2.94 (Z) (2H, 2t, J=6.0 cps, $N\underline{CH_2}CH_2O$), 3.84 (4H, m, piperidino protons), 4.03 (E), 4.12 (Z) (2H, t, J=6.0 cps, $NCH_2\underline{CH_2}O$), 6.40-7.90 (13H, m). Anal. Calc. for $C_{31}H_{37}N_4OCl$: C=72.02, H=7.16, N=10.84, Cl=6.87. Found: C=72.10, H=7.24, N=10.61, Cl=6.67.

Fluoro-clomiphene 5 from triazene 13

To 0.5 g, 1 mM, triazene 13 in 15 mL benzene was added 10 mL HF (40%). The reaction was stirred at room temperature for 0.5 h and at 50°C for 15 min. It was poured on ice and worked up. Chromatography gave 0.15 g, 39%, fluoro-clomiphene 5, identified by NMR, IR and TLC.

Cytosol preparation and receptor-binding studies. Dextran-coated charcoal was used to measure the number of estrogen receptors and the dissociation constants of the test compounds in rat uterine cytosol, by competition binding, as described in our previous publication (3). Uterine cytosol was prepared from 21- to 25-day-old Sabra rats, and 10 nM estradiol was used as a tracer.

Uterotropic assay in Sabra rats. Clomiphene, amino-clomiphene, fluoro-clomiphene and some of their synthetic precursors were examined for their uterotropic activity in 21-day-old immature female Sabra rats. The rats were randomized 6-8 per group, and each compound was injected separately, s.c., on three consecutive days. On the fourth day the rats were killed by cervical dislocation, their uteri removed, stripped of adhering tissue and weighed.

Stock solutions of estradiol and hexestrol were prepared as described (3). Stock solutions of clomiphene (1.0 mg/mL), amino-clomiphene (10 mg/mL) and fluoro-clomiphene (2.5 mg/mL) were prepared in propyleneglycol and diluted with it to the desired concentrations. Daily doses of clomiphene were 0.1, 1.0, 10.0 or 50.0 μg/0.1 mL. Daily doses of amino-clomiphene were 1.0, 10.0, or 50.0 μg/0.1 mL and 200 μg/0.2 mL. Fluoro-clomiphene was administered daily at 10.0, 50.0 or 100 μg/0.1 mL and 500 μg/0.2 mL.

RESULTS AND DISCUSSION

Chemical syntheses. The total syntheses of both amino-clomiphene and fluoro-clomiphene are given in Schemes I and II. It is noteworthy that intermediate 8 is synthesized in a higher yield (48%) by the Grignard reaction than by the $TiCl_3$-$LiAlH_4$ reagent (31%). An attempt to prepare 2 by the Horner-Wittig phosphonate reaction led unexpectedly to 6, with substitution of the fluorine atom; BBr_3 led to isomerization and to an E:Z mixture.

Biological activity. All test compounds - clomiphene, amino-clomiphene, fluoro-clomiphene and their synthetic precursors - demonstrate an increase in rat uterine wet weight (Table 1), suggesting that in this particular test they exert some degree of estrogenic activity. Our results confirm that, in terms of uterine wet weight, estradiol exerts a significantly lower activity than hexestrol does, and therefore the test compounds differ in their comparison from the two controls used: while in comparison with estradiol, all clomiphene derivatives were fully agonistic, in comparison with the hexestrol they were only partial agonists. When the test compounds were tested in combination with either a saturation or an unsaturation dose of estradiol or hexestrol, their antiestrogenic property was extenuated (results not shown). The hexestrol dose which allows expression of the activity of the test compounds is 0.1 μg/rat/day. In this test in our Sabra strain rats (descendants of the Wistar strain), the order of activity is: amino-clomiphene (the strongest inhibitor) > fluoro-clomiphene > clomiphene.

Clomiphene citrate has been used clinically for the induction of ovulation since 1962, in an E : Z isomer mixture (6:4). In vivo studies suggest that it acts as both an estrogen agonist and antagonist, depending on the species, organ, tissue and cell type (4,5). It has been established that in some rat strains and in some other species clomiphene demonstrates mixed estrogenic-antiestrogenic properties. When administered simultaneously with estradiol, clomiphene inhibits

Table 1. Uterotropic Effect of Clomiphene, Fluoro-Clomiphene and Six Chemical Intermediates as Compared to that of Estradiol and Hexestrol (Numbers refer to Schemes I and II)

Compound	Minimal Saturation Dose (μg/rat/day)	Maximal Uterine Wet Weight (mg)	Percent of the Control Effect
Saline	*	29.7 ± 2.2	100
Estradiol	0.2	73.4 ± 5.6	233
Hexestrol	0.1	61.9 ± 2.7	306
Clomiphene	10.0	72.3 ± 2.5	226
5 Fluoro-clomiphene	100.0	71.3 ± 2.0	237
12 Amino-clomiphene	50.0	65.0 ± 2.0	217
3 (Scheme I)	250.0	89.7 ± 2.4	128
4 (Scheme I)	250.0	71.8 ± 4.5	102
9 (Scheme II)	50.0	42.2 ± 4.0	60
10 (Scheme II)	250.0	62.3 ± 4.3	88
11 (Scheme II)	50.0	51.8 ± 1.4	74

* Saline dose was 0.2 mL.

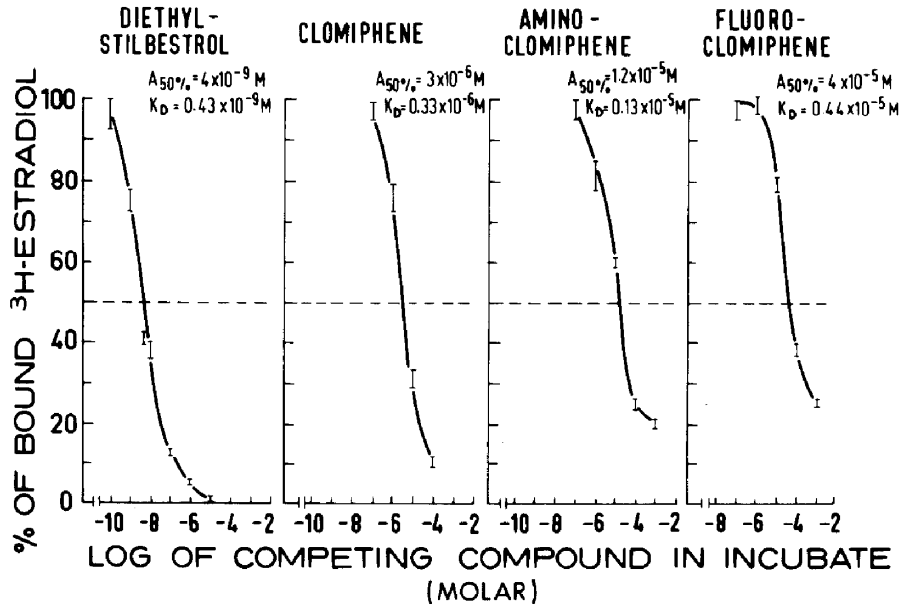


Fig. 1. Competitive inhibition of ^{3}H-estradiol binding to rat uterine cytosol by diethyl-stilbestrol, clomiphene, amino-clomiphene and fluoro-clomiphene.

the uterine growth caused by estradiol, up to its own basal level (5-7). If administered alone, clomiphene stimulates uterine growth, but it demonstrates lower potency and lower intrinsic activity (6). The uterotropic effects of all three test compounds were similar to that of estradiol itself, and, as the uterotropic effects of hexestrol were significantly higher than that of estradiol - the antiestrogen effect of the test compounds could only be demonstrated when hexestrol was used as agonist, at a saturation dose of 0.1 μg/rat/day. The stimulation of uterine epithelial hypertrophy is shared by all triphenylethylene derivatives (8). Both isomers of clomiphene (E and Z) demonstrate antagonistic properties on the mammary gland (9). Species specificity for clomiphene is remarkable, but it should be noted that in the human clomiphene is primarily an estrogen antagonist, as it inhibits most of the known effects of estradiol in women (10). It is widely accepted today that the E isomer of clomiphene is anti-estrogenic in the human, while the Z isomer is mildly estrogenic (11,12). Our studies were all performed with an E:Z mixture of about 6:4.

In our in vitro studies all test compounds inhibited the binding of ^{3}H-estradiol to the rat uterine cytosol receptors, and this inhibition was dose-dependent. The overall order of potency was clomiphene > fluoro-clomiphene > amino-clomiphene, with clomiphene activity 50% inhibition ($A_{50\%}$) of ^{3}H-estradiol binding at a concentration of 3×10^{-6}M. For comparison, the $A_{50\%}$ of diethyl-stilbestrol is 4×10^{-9}M, and its K_D value is 0.43×10^{-9}M (Fig. 1). The formula for calculating the K_D values is given elsewhere (3). These results (for clomiphene) are in line with other in vitro studies reported in the literature, where it has been demonstrated that clomiphene blocks the binding of ^{3}H-estradiol to the estrogen receptors, by competitive inhibition. The findings by Skidmore et al (13), that K_D for the binding of E and Z isomers to the estrogen receptors varies from 4 to 250 nM, respectively, agree with the known divergence of affinity for these isomers to

the estradiol receptors. Relative association constants from competitive binding assays obtained by Sasson and Notides (14) were 1.8% of that of estradiol for Zuclomiphene and 0.09% for Enclomiphene, i.e., $A_{50\%}$ of $8x10^{-7}$ for the Z and $2x10^{-5}$M for the E isomer, values close to those obtained in our laboratory for their mixture. In light of these results it seems that fluoro-clomiphene may be a candidate for radiofluorination.

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