I : Synthesis of diethylstilbestrol derivatives as possible differential ligands for estrogen receptor alpha versus beta.

II : Construction and validation of recombinant Adenovirus for the expression of estrogen receptor isoforms.

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Ligands that have differential action on estrogen receptor alpha (ERalpha) and estrogen receptor beta (ERbeta) are of great potential interest for research and for clinical applications. We started a synthesis of symmetric stilbene like molecules, starting with benzil derivatives. Addition of alkyl Grignard reagents to benzil 4,4’-dimethoxybenzil, 4,4’-dimethylbenzil and 4,4’bis(dimethylamino)benzil followed by dehydration of the resulting pinacols produced a mixture of E and Z stilbene-like compounds. Together with the diols intermediates and starting materials, the olefins were evaluated for their estrogenic or antiestrogenic properties by cell transfection using the ER responsive luciferase reporter (4-ERE luc) in human embryonic retinoblast (HER-911) cells. For structure determination of the synthesized 2,3-diphenyl-2-butene stereoisomers, a simple spectroscopic method was applied to determine the geometry of tetrasubstituted alkenes. The observation of the 3J-coupling constants in proton NMR spectra on the 13C satellite signals could confirm a previous misassignment of 2,3-diphenylbutene geometry. Hence, the (E)-isomer showed a 1.5 Hz coupling constant, whereas the (Z) isomer showed a 1.1 Hz coupling constant. Based on this new assignment and a stereospecific preparation, we also proposed a revision concerning the NMR data of 2,3-diphenyl-2,3-butanediol. Partial estrogenic activities were observed when benzil, diphenylbutanediol and a mixture of a 4,4’ dimethoxybenzylbutene and 4,4’ dimethoxybenzylidene were dosed at relatively high concentrations (10^{-5}M and 10^{-4}M ) to transfected HER-911 cells. Since the final aim was to examine whether the newly observed differential estrogenicity would be maintained in primary cells of different kinds, we decided to construct several recombinat adenovectors encoding ERalpha and ERbeta variants. Vectors encoding the following ER forms were prepared: (a) HA-ER_alpha.GFP; (b) HA-ER_beta.GFP; (c) HA-ERbeta(nativecDNA); (d) HA-ERbeta(codon-optimised cDNA). These vectors were necessary to complete the panel of expression vectors to express ERalpha and ERbeta in different stoichiometric ratios. The plasmid vectors and the r-adeno could be assembled and functionally tested.

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