5 Summary

In this work, a series of novel hydroxylated stilbene derivatives and 1,2-diaryl-1,2-diazidoethanes as potential anticancer agents were synthesized and investigated for their estrogenic / antiestrogenic effects in the MCF-7-2a cell line with luciferase assays.

One type of the hydroxylated stilbene derivatives was halo-substituted (*E*)-4-hydroxy-4'-methoxystilbene, which was synthesized directly by a crossed reductive coupling of (halo-substituted) 4-hydroxybezaldehyde and 4-methoxybenzaldehyde with low-valent titanium generated from titanium tetrachloride and zinc in tetrahydrofuran. Halo-substituted (*E*)-4,4'-dihydroxystilbene as an other type was prepared by demethylation of a corresponding 4,4'-dimethoxystilbene with boron tribromide in dichloromethane. The above-mentioned reductive coupling reaction starting from a halo-substituted 4-methoxybenzaldehde produced the corresponding symmetric 4,4'-dimethoxystilbene. The other required asymmetric 4,4'-dimethoxystilbenes were obtained via the classic Wittig reaction using aryl aldehyde and benzyltriphenylphosphonium chloride in the presence of sodium methoxide in dry methanol.

All of these hydroxylated stilbene derivatives showed agonist activities in MCF-7-2a cells and were more active than the corresponding dimethoxystilbenes. One para-phenolic hydroxy group in stilbenes was necessary for a full activation of the gene expression. An other para-phenolic hydroxy or a methoxy group was of benefit to estrogenic activities of stilbenes. Appropriate halo-substituents especially ortho-chloro linked to the aromatic ring of stilbenes enhanced their estrogenic potency, but not in all cases. The most active hydroxylated stilbene in both types was 2'-chloro-4,4'-dihydroxystilbene **43** (EC_{50} = 40 nM). Also the 2'-chloro-4-hydroxy-4'-methoxystilbene **34** (EC_{50} = 155 nM) was the most active agonist of the same type. In addition, all the three symmetric halo-substituted 4,4'-dihydroxystilbens **44** (2,2'-F_2, EC_{50} = 79 nM), **49** (2,2',6,6'-Cl_4, EC_{50} = 94 nM) and **46** (2,2'-Cl_2, EC_{50} = 117 nM) were stronger agonists than the others. None of them showed antagonistic effect.

2-Alkyl-1,1,2-triarylethene containing at least one protected phenol group, regarded as one type of the stilbene derivatives, was synthesized in situ by treating 2-alkyldeoxybenzoin with an appropriate Grignard reagent in tetrahydrofuran, followed by direct hydrolysis and dehydration with 47% hydrobromide acid. Alkylation of deoxybenzoin with alkyl halide in the presence of potassium tert-butoxide in tetrahydrofuran gave the necessary 2-alkyldeoxybenzoin. The methyl ether protecting groups of 2-alkyl-1,1,2-triarylethene were cleaved using

boron tribromide to obtain the hydroxy derivatives. Also the crossed reductive coupling of two aryl ketones was used in some cases to prepare 2-alkyl-1,1,2-triarylethenes.

In contrast to the above-described “agonists”, 2-alkyl-1,1,2-triarylethenes were antagonistically active in MCF-7-2a cells. 2-(4-Hydroxyphenyl)-1,1-bis(4-methoxyphenyl)-prop-1-ene (**76**) and 4,8-dibromo-1,1,2-tris(4-hydroxyphenyl)oct-1-ene (**79**) antagonized completely the E2 effect. The antagonist activity of **79** (IC_{50} = 99 nM) was even higher than that of tamoxifen (IC_{50} = 500 nM). None of them exhibited agonist activity in MCF-7-2a cells.

Synthesis of the novel hydroxylated *erythro*(or *meso*)- and *threo*(or *dl*)-1,2-diaryl-1,2-diazidoethanes was performed by treatment of appropriate hydroxylated stilbenes with ICl / NaN_3 in acetonitrile followed by column chromatographic separation, without one diastereomer significantly predominating. More than one molar equivalent of IN_3 (*i. e.* ICl) and the possible by-product iodine were considered to be detrimental to this reaction due to the existence of free phenol group.

By means of this method with two molar equivalents of ICl and NaN_3, two 2-alkyl-1,1,2-tris(4-methoxyphenyl)-1,2-diazidoethanes **96** and **97** were prepared in high yields starting from the corresponding 2-alkyl-1,1,2-tris(4-methoxyphenyl)ethenes.

Most of the *erythro*(or *meso*)- and *threo*(or *dl*)-1,2-diaryl-1,2-diazidoethanes were capable of activating transcription in MCF-7-2a cells and some of them were full-agonists, e. g. **93** (2,2',6'-Cl_3, EC_{50} = 65 nM) and **95 (**2,2',6,6'-Cl_4, EC_{50} = 56 nM). The agonist activities of these compounds had obvious relevance to the phenolic hydroxy and halo-substituents as well as the configuration. The clear tendencies about this were demonstrated in three points. (1) The dihydroxylated compounds were estrogenically more active than the monohydroxylated compounds bearing a further methoxy group; 2) The compounds bearing more halo-substituents on aromatic rings possessed greater estrogenic potencies; 3) The *erythro* (*meso*) isomer was estrogenically more active than the *threo* (*dl*) isomer. Only the dihydroxylated compound **86** without halo-substituents showed a significant antagonist activity (IC_{50} = 626 nM)

Without the phenolic hydroxy group, the new compound **96** exhibited also a significant antiestrogenic effect in MCF-7-2a cells. This indicated a possibility that 2-alkyl-1,1,2-triaryl-1,2-diazidoethane could become a potential true antagonist by modification of the substituents with an enhancer.

Several compounds containing the stilbene structure, the olefin bond of which was integrated to a ring skeleton, were synthesized in an attempt to gain evidence concerning the pharmacologically active skeleton structure. So reductive coupling of the isomerically pure 1,2,4,5-tetraaryl-1,5-pentandione with low-valent titanium afforded the isomerically pure 1,2,3,5-tetraarylcyclopent-1-ene. An other type was triarylfuran derivative containing a diarylimidazole unit prepared by condensation of a side-formylated triarylfuran with a commercially available benzil and ammonium acetate. The side-formylated triarylfuran was synthesized by cyclization of 1,2,4,5-tetraarylpentane-1,5-dione with iron (III) trichloride or iodine in the presence of sodium acetate in acetic acid to 2,3,5-triarylfuran followed by selective formylation with dichloromethyl methyl ether and titanium tetrachloride. Treatment of 1,2-arylethenone with iodomethyl methyl ether and potassium tert-butoxide followed by column chromatographic separation yielded the required isomerically pure 1,2,4,5-tetraaryl-1,5-pentandione.

1,2,3,5-tetraarylcyclopent-1-ene showed neither agonist nor antagonist activities in the luciferase assay. The modification of triarylfuran by combination with a diarylimidazole unit resulted in the loss of agonist activity. The compound **117** activated slightly the luciferase expression (EC_{50} = 1450 nM), while the hydroxylated compound **120** antagonized completely the E2 effect but at higher concentration with an IC_{50} value of 2750 nM. So these compounds were not active enough to act as potential estrogen receptor modulators

This work provided not only a number of novel pharmacologically active compounds, some of which have potentials to function as biological response modifiers and several of which as antiestrogens for the treatment of breast cancer, but also the convenient and practical synthesis methods, which are of usefulness for further investigation. It is also possible that these novel compounds are developed to treat other diseases.