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„Synthesis of phosphorylated aza- and oxoheterocycles and their application in obtaining compounds with cytotoxic activity".

The main goal of my research was to develop a method for the synthesis of various phosphorylated azaheterocycles, such as pyrazolones, pyridopyrimidinones or pyrazolepyrimidinones, and oxoheterocycles, such as isoxazolones and chromenones. In general, obtained phosphorylated heterocycles were transformed into 2-methylidene-1-oxoheterocycles in two-step reaction sequence: Michael addition of Grignard reagents or reduction of double bond conjugated with carbonyl group and Horner-Wadsworth-Emmons (HWE) reaction with formaldehyde. Obtained α-methylidenelactams and α-methylidenelactones were then tested for their cytotoxic activity.

Starting ethyl 2-acyl-2-diethoxyphosphorylacetates were synthesised using modified literature procedure and fully characterized. Next, 2-acyl-2-diethoxyphosphorylacetates were reacted with various nitrogen- and oxygen-containing nucleophiles.

Arylhydrazines were the first class of the effective nucleophiles. It was necessary to elaborate two different synthetic protocols. Due to the fact that obtained 3-substituted 4-diethoxyphosphorylpyrazol-5-ones were unreactive, neither in Michael addition of Grignard reagents, nor in the reduction of double bond they were *N*-methylated producing 5-substituted 4-diethoxyphosphoryl-1-methyl-2,3-dihydro-1*H*-pyrazol-3-ones, which were then reduced to pyrazolidinones and finally transformed into 5-substituted 4-methylidenepyrazolidin-3-ones in HWE reaction.

2-Acylo-2-diethoxyphosphorylacetates were also reacted with hydroxylamine hydrochloride giving 3-substituted 4-diethoxyphosphoryloisoxazol-5-ones. Unfortunately, this reaction was efficient only for alkanoylacetates. 2-Acetyl-2-diethoxyphosphorylacetate was also reacted with methylhydroxylamine hydrochloride yielding 4-diethoxyphosphoryl-2,3-dimethyl-2,5-dihydroisoxazol-5-one. Unfortunately, this reaction was not general and other 2-acyl-2-diethoxyphosphorylacetates were unreactive. Further attempts to transform 3-substituted 4-diethoxyphosphoryloisoxazol-5-ones into HWE reagents failed.

The other class of the effective nucleophiles were 3-aminopyrazoles. Their reactions with 2-acyl-2-diethoxyphosphorylacetates gave variously substituted at C-2 and C-5 6-diethoxyphosphoryl-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-7-ones in high yields. Unfortunately, similarly to previously obtained phosphorylated heterocycles both Michael addition of Grignard reagents and reduction of double bond were unsuccessful. Assuming that the reason for these failures is presence of the free amino group N-butylation of 6-diethoxyphosphoryl-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-7-ones was performed giving 4-butyl-6-diethoxyphosphoryl-7-dihydropyrazolo[1,5-*a*]pyrimidin-7-ones. In all cases there were also a small amount of O-butylation product, but after optimization of the reaction conditions towards N-butylation both N- and O-butylated products were isolated and fully characterised. 4-Butyl-6-diethoxyphosphoryl-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-7-ones were then subjected to the reduction of the double bond conjugated with carbonyl group leading to 2,5-disubstituted *trans*-4-butyl-6-diethoxyphosphoryl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pirymidyn-7-ones

which were olefinated with formaldehyde producing 6-methylidene-5,6-dihydropyrazolo[1,5-*a*]pirymidyn-7(4*H*)-ones in good yields.

2-Aminopyrazoles and phenoles were unreactive with 2-acyl-2-diethoxyphosphorylacetates. Therefore, it was necessary to convert them into ethyl 3-methoxy-2-diethoxyphosphoryl-2-alkenoates.

Firstly, ethyl 3-methoxy-2-diethoxyphosphoryl-2-alkenoates were successfully reacted with 2-aminopyridines. Series of 2-substituted 3-diethoxyphosphoryl-4*H*-pyrido[1,2-*a*]pirymidyn-4-ones was obtained in good yields. Unfortunately, all attempts to perform Michael addition of Grignard reagents or reduction of double bond conjugated with carbonyl group failed. Interestingly, when 3-diethoxyphosphoryl-2-methyl-4*H*-pyrido[1,2-*a*]pirymidyn-4-one was hydrogenated reduction of the pyridine ring occurred.

3-Methoxy-2-diethoxyphosphoryl-2-alkenoates were also reacted with phenols leading to various 4-substituted 3-diethoxyphosphorylchromen-2-ones which were next converted into 4,4-disubstituted chromenones via Michael addition of Grignard reagents. These HWE reagents were finally reacted with formaldehyde giving differently functionalized 4,4-disubstituted 3-methylidenechromen-2-ones in high yields.

This work also presents synthesis of non-racemic 4,4-disubstituted 3-methylidenechromen-2-ones using 1,1'-binaphto-2,2'-dioxophosphoryl group as the chiral auxiliary. The first attempts were based on the transformation of the diethoxyphosphoryl group of 3-diethoxyphosforylochromenone into 3-dichlorophosphoryl group followed by conversion into 1,1'-binaphto-2,2'-dioxophosphoryl group in reaction with (*R*)-binaphtol. Unfortunately, the latter one was unsuccessful. For this reason it was necessary to synthesize (*R*)-3-(1,1'-binaphto-2,2'-dioxyphosphoryl)chromen-2-one starting from ethyl (*R*)-1,1'-binapht-2,2'-dioxophosphorylacetate. This acetate was obtained in good yield modifying the literature procedures. Next, obtained acetate was transformed into (*R*)-3-(1,1'-binaphto-2,2'-dioxyphosphoryl)chromen-2-one by acetylation, O-methylation and reaction with 3-methoxyphenol or 3,5-dimethoxyphenol. Resulting two (*R*)-3-(1,1'-binaphto-2,2'-dioxophosphoryl)chromen-2-ones were then reacted with Grignard reagents yielding 4,4-disubstituted (*R*)-3-(1,1'-binaphto-2,2'-dioxophosphoryl)-3,4-dihydro-2*H*-chromen-2-ones as a mixture of diastereoisomers. Their chromatographic separation failed but, luckily, the dominant diastereoisomers were isolated for some adducts by crystallization. Absolute configurations of two of them were established by X-ray crystallography. 4,4-Disubstituted (*R*)-3-(1,1'-binaphto-2,2'-dioxophosphoryl)-3,4-dihydro-2*H*-chromen-2-ones were finally reacted with formaldehyde. This transformation was successful for some chromenones giving (R)-4,4-disubstituted 3-methyliden-3,4-dihydro-2*H*-chromen-2-ones with good enantiomeric excess from 68% to 99%.

All of the obtained 2-methylidene-1-oxoheterocycles were finally tested *in vitro* against several cancer cell lines: HL-60, NALM-6 and MCF-7. 5-Substituted 4-methylidene-2-phenyl-1-methylpyrazolidin-3-ones turned out to be the least active or inactive at all. IC_{50} values for these compounds were from 24,4 to 750 μM. 4,4-Disubstituted 3-methylidene-3,4-dihydro-2*H*-chromen-2-ones proved to be the most active compounds with IC_{50} values below 1 μM in some cases. These chromenones are now subjected to more in-depth biological studies.

Selected 4,4-disubstituted 3-methylidene-3,4-dihydro-2*H*-chromen-2-ones were also tested against healthy humans cells (HUVEC). In most cases cytotoxicity of the tested compounds was comparable or 2-4 times higher than toxicity towards normal cells. However, two chromenones (4-ethyl-5,7-dimethoxy-3-methylidene-4-methyl-3,4-dihydro-2*H*-chromen-2-one and 4-ethyl-4-methyl-3-methylidene-3,4-dihydro-2*H*-benzo[*h*]chromen-2-one) displayed much better therapeutic index of 10 and 13, respectively, against NALM-6 cells.

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