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DOCTORAL DISSERTATION ABSTRACT

„Stereoselective synthesis of 3-methylidenedihydroquinolin-2(1*H*)-ones and 5-methylidenedihydrouracils with cytotoxic activity"

Heterocycles containing uracyl or quinolin-2-one skeletons display many valuable biological properties. Uracyl is the pyrimidine derivative that occurs naturally as one of the four RNA nucleobases. It is used in the synthesis of biologically active enzyme inhibitors, nucleosides, and oligonucleotides. In the quest for new drugs uracil seems to be the privileged structure. Its cytotoxic activity is one of the most widely reported. For example, 5-fluorouracil is the approved drug in cancer treatment. Also its derivatives, such as tegafur, independently or in combination with cisplatin, are widely used in anticancer therapies. Uracil derivatives are also very potent antiviral, antibacterial, insecticidal and herbicidal agents. Among biologically active quinolin-2-ones Aripiprazole is a neuroleptic drug used in the treatment of schizophrenia and bipolar disorder. Rebamipide is used for mucosal protection, healing of gastroduodenal ulcers, and treatment of gastritis. Repirinast is an antihistamine.

A very special group of natural and synthetic heterocycles are α-alkylidene-γ- and δ-lactones and lactams accommodating very characteristic *exo*-alkylidene moiety conjugated with carbonyl group. Presence of this moiety is believed to be crucial for their biological activity. It is generally accepted that they can act as Michael acceptors and react with bionucleophiles, especially cysteine mercapto groups or free intracellular glutathione. It explains their wide and often strong biological activity.

It is possible that many yet unexamined heterocycles containing an *exo*-alkylidene moiety conjugated with carbonyl group are likely to possess anticancer activity. Therefore, combining this pharmacophoric unit with other heterocyclic skeletons which also display desirable cytotoxic effect can enhance biological activity and/or improve therapeutic index of these molecular hybrids.

Professor Janecki's group has been developing synthesis of various phosphorylated aza- and oxaheterocycles. Among them phosphorylated tetrahydrofuranones, pyrolidinones, isoxasolidinones, pyrazolones, pyridazones, chromenones and quinolin-4-ones can be found. These compounds were transformed into α-alkylidene-γ and δ-lactones utilizing approach based on Horner-Wadsworth-Emmons (HWE) methodology. The olefination of aldehydes using corresponding dialkoxyphosphoryl aza- and oxaheterocycles gives access to compounds with *exo*-alkylidene moiety conjugated with carbonyl group. Many compounds obtained in his laboratory were tested for their cytotoxic activity against several human cancer cell lines and it is worth to stress that many of the tested compounds revealed very promising cytotoxic activity (IC_{50} < 1 μM).

The main goal of the presented dissertation was developement of the efficient syntheses of new uracil and quinolin-2-one derivatives on both, racemic as well as enantiomeric level. The heterocyclic structures were expected to possess enhanced anticancer potential.

The first task was the effective synthesis of 1-alkyl-3-diethoxyphosphoryldihydroquinolin-2-ones and 1,3-substituted 5-diethoxyphosphoryluracils by utilizing corresponding diethoxyphosphorylacetamides. Then it was planned to transform them into 1,4-disubstituted 3-methylidenedihydroquinolin-2(1*H*)-ones and 1,3,6-trisubstituted 5-methylideneuracils.

The next task was the enantioselective synthesis of the series of 1,4-disubstituted 3-methylidene-dihydroquinolin-2(1*H*)-ones and 1,3,6-trisubstituted 5-methylidenedihydrouracils. The planned strategy was based on chiral auxiliary approach. It was intended to transform diethoxyphosphoryl group of 1-alkyl-3-diethoxyphosphoryldihydroquinolin-2-ones and 1,3-substituted 5-diethoxy-phosphoryluracils into bis(1-phenylethylamine)phosphoryl groups by utilization of commercially available (*R*)- and (*S*)-1-phenyethylamine. It would led to enantiomerically pure phosphonamides.

It is worth to stress that all the planned goals were accomplished. The effective racemic and scalemic syntheses of the new molecular hybrids - 1,4-disubstituted 3-methylidenedihydroquinolin-2(1*H*)-ones and 1,3,6-trisubstituted 5-methylideneuracils - were developed.

The key intermediates in the synthesis of 1,4-disubstituted 3-methylidenedihydroquinolin-2(1*H*)-ones, 1-alkyl-3-diethoxyphosphoryldihydroquinolin-2-ones, were obtained by two methods. In both methods modified procedures described in the literature for analogous compounds were utilized. In the first method *o*-aminobenzaldehyde was condensed with diethoxyphosphorylacetyl chloride, which led to diethoxyphosphorylacetamide that was subjected to intramolecular cyclization in the presence of piperidine. This way 3-diethoxyphosphoryloquinolin-2-one was obtained in combined 44% yield. This product was also obtained by Knoevenagel condensation of *o*-nitrobenzaldehyde with ethyl diethoxyphosphorylacetate followed by the reduction of nitro group in obtained this way ethyl 2-diethoxyphosphoryl-3-(2-nitrophenyl)acrylate and subsequent spontaneous intramolecular cyclization. Combined yield of this method was 78%. 3-Diethoxyphosphorylodihydroquinolin-2-on was next reacted with alkyl halides in alkaline conditions and this way four 1-alkyl-3-diethoxyphosphoryldihydroquinolin-2-ones were obtained in good yields (59-70%). Michael addition of Grignard reagents to 1-alkyl-3-diethoxyphosphorylquinolin-2-ones gave access to 1,4-disubstituted 3-methylidenequinolin-2-ones in moderate to good yields (21-91%). Finally these compounds were used in the effective olefination of formaldehyde using HWE methodology to give fourteen 1,4-disubstituted 3-methylidenedihydroquinolin-2(1*H*)-ones (yields 73-96%). These compounds were then tested *in vitro* against three human cancer cell lines: leukemia NALM-6 and HL-60 as well as breast cancer MCF-7. In general, cytotoxicities of all these compounds are moderate or low, with IC_{50} values ranging from 40,2 to 405 μM. Research summarized in this paragraph are described in the article **M. Pięta**, J. Kędzia, A. Janecka, D. K. Pomorska, M. Różalski, U. Krajewska, T. Janecki, "Novel synthesis and cytotoxic activity of 1,4-disubstituted 3-methylidene-3,4-dihydroquinolin-2(1H)-ones" RSC Advances, 2015, 5, 78324-78335.

To obtain 1,3-disubstituted 5-diethoxyphosphoryluracils which are key intermediates in the synthesis of 1,3-di- and 1,3,6-trisubstituted 5-methylidenedihydrouracils, new methodology was developed in which *N*-substituted diethoxyphosphorylacetamides were utilized. After condensation of these amides with DMF-DMA and reaction with primary aliphatic and aromatic amines, *N,N'*-disubstituted 3-amine-2-diethoxyphosphorylopropeneamides were obtained in good yields (50-82%). These compounds were effectively cyclized with phosgene in optimized conditions to give expected 1,3-disubstituted 5-diethoxyphosphoryluracils (yields 68-90%). These key intermediates were next transformed utilizing two reaction pathways. In the first one, 1,3-disubstituted 5-diethoxy-phosphoryluracils were subjected to reduction of the carbon-carbon double bond to give 1,3-disubstituted 5-diethoxyphosphoryldihydrouracils (yields 81-90%) followed by HWE olefination of formaldehyde (yields 45-95%), what gave fourteen 1,3-disubstituted 5-methylidenedihydrouracils. In the second reaction path 1,3-disubstituted 5-diethoxyphosphoryluracils were transformed *via* addition of Grignard reagents into 1,3,6-trisubstituted 5-diethoxyphosphoryluracils in high yields (75-96%). Finally HWE olefination of formaldehyde gave efficiently sixteen 1,3,6-trisubstituted 5-methylidenedihydrouracils (yields 61-97%). Both series of final products were tested *in vitro* against three human cancer cell lines: leukemia NALM-6 and HL-60 as well as breast cancer MCF-7. In general, cytotoxicities of all these compounds are high or moderate, with IC_{50} values ranging from 0,19 to 61,58 μM. Part of the results summarized in this paragraph are published in the article

M. Pięta, J. Kędzia, T. Janecki "An efficient synthesis of 1,3-disubstituted 5-diethoxy-phosphoryluracils" *Tetrahedron Lett*. **2015**, *56*, 1891–1893.

The next goal was to synthetize non-racemic 1,4-disubstituted 3-methylidenedihydroquinolin-2(1*H*)-ones. Therefore, 1-alkyl-3-diethoxyphosphoryldiquinolin-2-ones were transformed into chiral 3-({bis[(1-phenylethyl)amine]}phosphoryl)dihydroquinolin-2-ones. In the reaction of these substrates with phosphorus pentachloride corresponding phosphonic acid dichlorides were obtained which were next condensed with enantiomerically pure (*R*)- and (*S*)-1-phenylethylamine to give (*R,R*)- and (*S,S*)-3-({bis[(1-phenylethyl)-amine]}phosphoryl)dihydroquinolin-2-ones in 44-82% yields. Then the addition of Grignard reagents to these compounds was performed in optimized conditions. This way 1,4-disubstituted 3-({bis[(1-phenylethyl)amine]}phosphoryl)tetrahydroquinolin-2-ones with diastereoizomeric ratios ranging from 71:29 to 92:8 were obtained. After purification and separation using column chromatography enriched mixtures were obtained with dr from 94:6 to 99:1. These mixtures were than subjected to HWE reactions with formaldehyde and sixteen 1,4-disubstituted 3-methylidenedihydroquinolin-2(1*H*)-ones with high enantiomeric excesses ranging from 90 to 99% and in 62-96% yields were obtained. Research summarized in this paragraph are the subject of the article **M. Pięta**, J. Kędzia, J. Wojciechowski, T. Janecki „Asymmetric synthesis of 1,4-disubstituted 3-methylidenedihydroquinolin-2(1*H*)-ones" Tetr. Asymmetry **2017**, *28*, 567-576.

The final task was the synthesis of non-racemic 1,3,6-trisubstituted 5-methylidenedihydrouracils. 3-(4-Bromophenyl)-5-diethoxyphosphoryl-1-ethyluracil was chosen as starting material and transformed into chiral (*R,R*)- and (*S,S*)-5-({bis[(1-phenylethyl)amine]}phosphoryl)uracil. In this synthesis 3-(4-bromophenyl)-5-diethoxyphosphoryl-1-ethyluracil was subjected to the reaction with trimethylsilyl bromide and then methanolysis to give corresponding phosphonic acid. This acid in the reaction with oxalyl chloride gave phosphonic acid dichloride and subequent condensation with enantiomerically pure (*R*)- and (*S*)-1-phenylethylamine gave (*R,R*)- and (*S,S*)-5-({bis[(1-phenylethyl)amine]}phosphoryl)-dihydrouracils in high combined yields (85%). Addition of Grignard reagents to these compounds yielded 6-alkyl- or 6-aryl-3-({bis[(1-phenylethyl)amine]}phosphoryl)-3-(4-bromophenyl)-1-ethyldihydrouracils with diastereoizomeric ratios ranging from 73:10:17 to 91:9. After purification and separation using column chromatography enriched mixtures were obtained with dr from 93:0:7 to >99:1 and in 50-60% yields. These mixtures were than subjected to HWE reaction with formaldehyde and eight 6-alkyl- or 6-aryl-3-(4-bromophenyl)-1-ethyl-5-methylidenedihydrouracils with high enantiomeric excesses (>99%) and good yields (51-89%) were obtained.

Both non-racemic series - 1,4-disubstituted 3-methylidenedihydroquinolin-2(1*H*)-ones and 1,3,6-trisubstituted 5-methylidenedihydrouracils - were tested *in vitro* against three human cancer cell lines: leukemia NALM-6 and HL-60 as well as breast cancer MCF-7. In the majority of pairs of enantiomers cytotoxic activities were similar. Only in a few pairs one enantiomer was twice our fourfold more active than the another.