VILNIUS UNIVERSITY
CPST INSTITUTE OF CHEMISTRY

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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF PYRROLO[3,2-*d*]PYRIMIDINES AND THEIR STRUCTURAL ANALOGUES

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The official discussion will be held on 12 a. m. January 21, 2011 at the meeting of the Scientific board at Inorganic chemistry lecture hall of the Faculty of Chemistry of Vilnius University.

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VILNIAUS UNIVERSITETAS
FTMC CHEMIJOS INSTITUTAS

Erika Jonutė

PIROLO[3,2-*d*]PIRIMIDINŲ BEI JŲ STRUKTŪRINIŲ ANALOGŲ SINTEZĖ IR PRIEŠVĖŽINIS AKTYVUMAS

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Introduction

Cancer is one of the most topical diseases of society. At present cancer is usually treated by radiotherapy and chemotherapy. Cancer cells are developing from the normal ones, so for a long time it was not easy to identify the possible site of action of anticancer drugs. For this reason, a majority of traditional anti-cancer drugs (DNA-intercalators, DNA-alkylating agents, etc) kill not only cancer cells, but normal-cells as well. Thus, the effectiveness of such drugs can be monitored only if the malignant cells grow and multiply much faster than healthy, which, unfortunately, is not always valid for all cancer strains. For this reason, traditional anticancer drugs always cause side effects, mainly - weakening of the patient's immune system. At the end of the XX century a new era of chemotherapy, based on cell chemistry and biochemistry, began. In recent years numerous genes and proteins that are causally involved in the initiation and progression of cancer have been identified. Based on these discoveries, has been the possibility to develop new drugs, which blocked certain process of cancer cells. In that case are suppressed division and growth of cells. These anticancer drugs are usable for targeted therapy. Theory of cancer biology explained that one of the ways to overcome this disease is the suppressing proliferation of cancer cells.

The pyrrolo[3,2-*d*]pyrimidine heterocyclic framework constitutes the basis of an important class of compounds possessing remarkable biological activities. These compounds are 9-deazaanalogues of biogenic purines and have been reported to be inhibitors of purine nucleoside phosphorylase and thymidylate synthase; in addition to antagonists of the neuropeptide Y5, and the A1 and A2 adenosine receptors.

The main aims of present investigation were to synthesize a variety of pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides and their structural analogues; to study chemical properties of the title compounds and to evaluate their antiprofilerative activity together with structure-activity relationships.

The main results obtained in this work are as follows:

It was found, that 6-arylethynyl-5-nitropyrimidines and 2-arylethynyl-3-nitropyridines undergo pyridine-catalysed intramolecular cyclization reactions to form pyrrolo[3,2-*d*]pyrimidine 5-oxides and pyrrolo[3,2-*b*]pyridin-3-one 1-oxides, respectively. On the other hand, 1,2-alkoxy-5-phenylethynyl-4-nitrobenzenes underwent cycloizomerization reaction to 3*H*-indol-3-one 1-oxides only in the presence of transition metal salts.

Moreover, the triple bond of 6-arylethynyl-5-nitropyrimidines is easily attacked by primary and secondary amines form *syn-* (in the case of secondary amines) or *anti*-addition (in the case of primary amines) products.

A novel, simple and high-yielding synthetic method of pyrrolo[3,2-d]pyrimidine framework *via* one-pot reaction of 2,4-disubstituted 6-arylethynyl-5-nitropyrimidines with secondary amines, followed by reductive cyclization has been developed.

It was found, that reduction of pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides and pyrrolo[3,2-*b*]pyridin-3-one 1-oxides led to the formation of the corresponding 5*H*-pyrrolo[3,2-*d*]pyrimidin-7-oles and 1*H*-pyrrolo[3,2-*b*]pyridin-3-oles.

A relatively short and efficient synthetic method of preparing 2,4-disubstituted 6-phenylpyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides through one-pot oxidation/substitution of methylthio group at the 2nd position of the pyrrolo[3,2-*d*]pyrimidine heterosystem was developed.

In vitro antiproliferative activities of synthesized compounds were examined in the human solid tumor cell lines A2780, HBL-100, HeLa, SW1573, T-47D and WiDr.[a] The in vitro experiments show that the most active compounds are disubstituted 6-phenylpyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides containing *N*-alkylamino or *N,N*-dialkylamino substituents in the 2nd position of the pyrrolo[3,2-*d*]pyrimidine heterosystem. Cell cycle studies demonstrate arrest in the G_2/M phase when the breast and lung cancer cells were exposed to the most active compound.

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2. SYNTHESIS AND CHEMICAL PROPERTIES OF PYRROLO[3,2-*d*]PYRIMIDINE AND THEIR STRUCTURAL ANALOGUES

2.1. Sonogashira coupling reaction between 5-nitro-6-chloropyrimidines and 3-nitro-2-chloropyridines and arylacetylenes

The 2,4-disubstituted 6-arylethynyl-5-nitropyrimidines (**2a-n**) were synthesized by the classical Sonogashira coupling reaction of the corresponding 2,4-disubstituted 6-chloro-5-nitropyrimidines (**1a-n)** with arylacetylenes (Table 1). The reaction was carried out in dry triethylamine under argon atmosphere in the presence of catalytic amounts of $PdCl_2(PPh_3)_2$ and CuI:

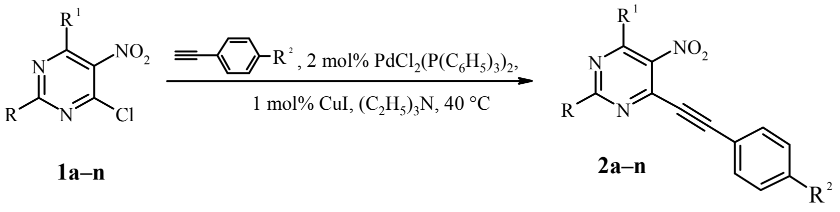
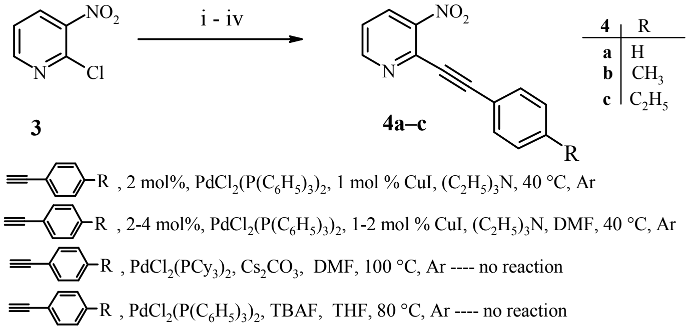


Table 1. Data of synthesis of 2,4-disubstituted 6-arylethynyl-5-nitropyrimidines **2a–n** from 2,4-disubstituted 6-chloro-5-nitropyrimidines **1a–n**

Starting compound	R	R^1	R^2	Product	Yield, %
1a	H	NH_2	H	**2a**	75
1b	SCH_3	NH_2	H	**2b**	70
1c	SCH_3	NH_2	CH_3	**2c**	75
1d	SCH_3	NH_2	C_2H_5	**2d**	70
1e	SCH_3	$NH(CH_2)_2N(CH_2)_4O$	H	**2e**	67
1f	SCH_3	$NHCH_2C_6H_5$	H	**2f**	50
1g	SCH_3	$N(CH_2)_4$	H	**2g**	70
1h	SCH_3	$N(CH_3)C_6H_5$	H	**2h**	67
1j	H	$NHCH_2C_6H_5$	H	**2j**	45
1k	H	$N(CH_2)_4O$	H	**2k**	60
1l	H	$N(CH_2)_5$	H	**2l**	77
1m	H	$N(CH_2)_4NCH_3$	H	**2m**	40
1n	H	$N(C_2H_5)_2$	H	**2n**	45

The coupling reaction of 2-chloro-3-nitropyrimidine (**3**) with phenylacetylene under the same conditions led to the formation of 2-phenylethynyl-3-nitropiridine (**4a**) in moderate yield (45 %). We observed more smooth reaction in triethylamine and dimethylformamide mixture (2:1) and the product was isolated in better yield (61–65 %).

However, it should be noted, that 2-chloro-3-nitropyridine was not as active in Sonagashira coupling reactions as 6-chloro-5-nitropyrimidines. The introduction of electron-donating groups (methyl and ethyl) in arylacetylene component, added some difficulties to the reaction. Therefore coupling reactions with 4-methyl- and 4-ethylphenylacetylenes under standart conditions gave only undefined reaction mixtures. We solved this problem by increasing amounts of catalysts (reaction conditions ii).



3 **4a–c**

4	R
a	H
b	CH_3
c	C_2H_5

i - R , 2 mol%, $PdCl_2(P(C_6H_5)_3)_2$, 1 mol % CuI, $(C_2H_5)_3N$, 40 °C, Ar

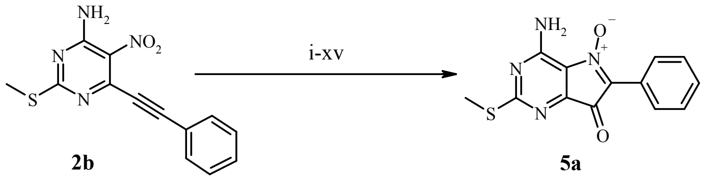
ii- R , 2-4 mol%, $PdCl_2(P(C_6H_5)_3)_2$, 1-2 mol % CuI, $(C_2H_5)_3N$, DMF, 40 °C, Ar

iii- R , $PdCl_2(PCy_3)_2$, Cs_2CO_3, DMF, 100 °C, Ar ---- no reaction

iv- R , $PdCl_2(P(C_6H_5)_3)_2$, TBAF, THF, 80 °C, Ar ---- no reaction

2.2. Synthesis of pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides and their structural analogues through intramolecular cyclization reaction of the corresponding arylethynylnitroarenes

2,4-disubstituted 5-nitro-6-phenylethynylpyrimidines (**2b–d**) could be converted to the 2-methylthio-6-phenylpyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides (**5a–c**) during refluxing in dry pyridine. The latter methodology has been developed in our laboratory earlier. This way was simple and rapid, but purification of the obtained products was complicated in some cases. To avoid that difficulty and to increase the yields of the compounds **5a–c**, we decided to develop a new method for the cyclization of 5-nitro-6-phenylethynylpyrimidines into pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides. For this purpose the cyclization reaction of **2b** was studied under different conditions. Various initiators: nitrosobenzene in different solvents, pyridine, concentrated sulphuric acid, tetrabutylammonium fluoride (TBAF), UV light and transition metal salts were used (Table 2).



2b **5a**

Table 2. Formation of 4-amino-2-methylthio-6-phenylpyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (**5a**) from 4-amino-2-methylthio-5-nitro-6-phenylethynylpyrimidine (**2b**) under different conditions.

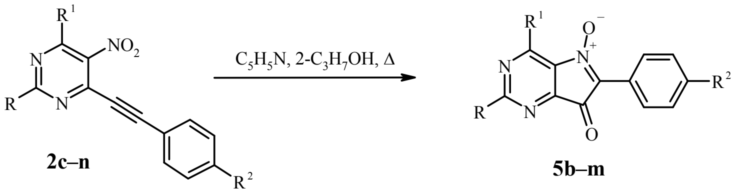
Method	Initiator	Solvent	Time, hours	Yield, %
i[a]	C_5H_5N		0,5	75
ii[b]	H_2SO_4 (konc.)		4	no reaction
iii[c]	TBAF	THF	48	no reaction
iv[c]	UV	$CHCl_3$	48	no reaction
v[a]	C_6H_5NO	*o*-xylene	2	52
vi[a]	C_6H_5NO	CH_2Cl_2	18 – 20	50
vii[a]	C_6H_5NO	CH_3OH	4	40
viii[a]	C_6H_5NO	C_2H_5OH	2	80
ix[a]	C_6H_5NO	2- C_3H_7OH	0,5	91
x[a]	C_6H_5NO	1- C_4H_9OH	0,5	75
xi[a]	C_6H_5NO	$C_6H_5CH_3$	0,7	70
xii[a]	C_5H_5N	2- C_3H_7OH	0,5	95
xiii[a]	$AgNO_3$	CH_2Cl_2	48	no reaction
xiv[a]	CuI	CH_2Cl_2	48	no reaction
xv[a]	$AuCl_3$	CH_2Cl_2	48	no reaction

a – reactions were performed at reflux temperature, b – reaction was performed at 0 °C, c – reaction was performed at room temperature

The data obtained indicate that only pyridine and nitrosobezene initiated the transformation of **2b** into 4-amino-2-methylthio-6-phenylpyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (**5a**). Transition metals salts, UV light, TBAF and cold concentrated sulphuric acid had no success. In these cases after the work-up of the reaction mixtures the initial compound **2b** was recovered.

Cyclization of **2b** occurred using a catalytic amount of freshly-prepared nitrosobenzene in different solvents at reflux. It was found that the fastest cyclization and the highest yield of **5a** was achieved in boiling 2-propanol. Moreover, it was established that cyclization of **2b** also proceeds using a catalytic amount of pyridine in boiling 2-propanol. The latter method of cyclization of 5-nitro-6-phenylethynylpyrimidines into pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides seemed to be the shortest and the most efficient.

Thus, the 4-substituted 2-methylthio-6-phenylpyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides (**5b–m**) were prepared via pyridine initiated cyclization of 4-substituted 5-nitro-6-phenylethynylpyrimidines (**2c–n**). Reactions were performed in boiling 2-propanol for 30 minutes.

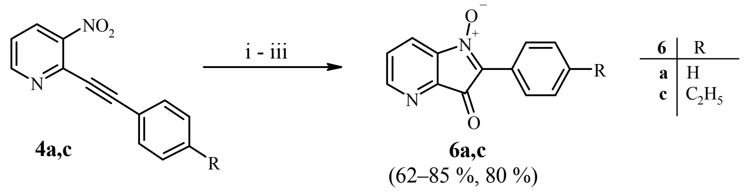


2c–n **5b–m**

Table 3. Data of synthesis of 2,4-disubstituted 6-phenylpyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides **5b–m** from 2,4-disubstituted 6-chloro-5-nitro-6-phenylethynylpyrimidines **2c–n**

Starting compound	R	R^1	R^2	Product	Yield, %
2c	SCH_3	NH_2	CH_3	**5b**	91
2d	SCH_3	NH_2	C_2H_5	**5c**	87
2e	SCH_3	$NH(CH_2)_2N(CH_2)_4O$	H	**5d**	85
2f	SCH_3	$NHCH_2C_6H_5$	H	**5e**	75
2g	SCH_3	$N(CH_2)_4$	H	**5f**	85
2h	SCH_3	$N(CH_3)C_6H_5$	H	**5g**	87
2j	H	$NHCH_2C_6H_5$	H	**5h**	95
2k	H	$N(CH_2)_4O$	H	**5j**	80
2l	H	$N(CH_2)_5$	H	**5k**	75
2m	H	$N(CH_2)_4NCH_3$	H	**5l**	98
2n	H	$N(CH_2CH_3)_2$	H	**5m**	80

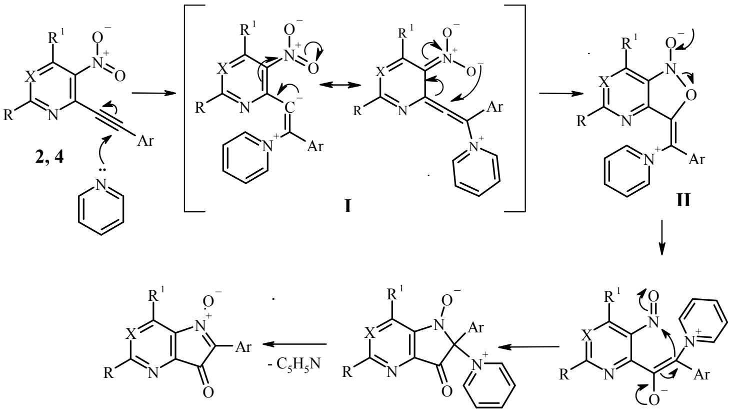
On the other hand, the cyclization reaction of 2-arylethynyl-3-nitropyridines **4** was not so smooth. First of all, the catalytic amount of pyridine in boling 2-propanol seemed to be not the best condition for cycloisomerization (the reaction was incomplete by TLC after 24 h. of heating). However, when 2-arylethynyl-3-nitropyridines (**4a,c**) were refluxed in dry piridine cycloizomerisation occurred in 4 hours.



4a,c **6a,c**
(62–85 %, 80 %)

i: C_5H_5N, 2-C_3H_7OH, Δ
ii: C_5H_5N, Δ
iii: $AuCl_3$, CH_2CH_2, r. t. ---- no reaction

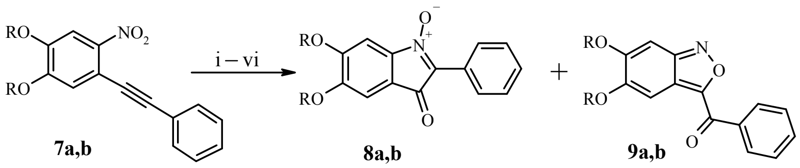
We speculate, that pyridine catalyzes the cyclization of starting compounds **2** and **4** by a mechanism depicted in the following scheme. First of all, pyridine attacks the triple bond via conjugated addition reaction and zwiterionic intermediates **I** are formed. The next steps could be electrocyclic ring closure (intermediates **II**), N—O bond cleavage (intermediates **III**) and recyclization reaction followed by an elimination of pyridine molecule to give **5**, **6**.



X = C, N
R = H, SCH_3
R^1 = *N*-alkylamino, *N,N*-dialkylamino, *N,N*-alkylphenylamino

Finally, we studied the intramolecular cyclization of 1,2-dialkoxy-5-phenylethynyl-4-nitrobenzenes (**7a,b**). It is noteworthy, that the use of pyridine did not initiate the reaction at all. After the prolonged heating of compound **7a** in dry pyridine, no changes of the starting material were observed by TLC. This result can be easily explained by the fact, that triple bonds in starting compounds are electron rich due to neighbouring aromatic ring with electron donating methoxy groups, so nucleophilic activation becomes impossible. So we decided to take advantage of transition-metal salts catalytic potency. When 5 mol % of gold (III) chloride was added to the solution of the starting compound **7a** in dry dichloromethane, the quick and selective conversion of the starting material was observed by TLC. While CF_3CO_2Ag in boiling dichloromethane provided a slightly slower conversion of **7a**, CF_3CO_2Ag, CuI and $FeCl_3$ in dichloromethane at room temperature as well as $AgNO_3$ in boiling dichloromethane proved to be far less effective (the reaction was slow and only traces of products were observed by TLC). Moreover, it was found, the the desired 2-phenyl-3*H*-indol-3-one 1-oxides (**8a,b**) were not the only products in the present reaction. Small amounts of antrhranyls **9a,b** were formed as side reaction products. Therefore the cycloizomerization of 1,2-dialkoxy-5-phenylethynyl-4-nitrobenzenes in the presence of transition metal salts is not regioselective.

Moreover, it should be noted that transition metal salts did not initiate the cycloizomerization of 6-arylethynyl-5-nitropyrimidines **2** and 2-arylethynyl-3-nitropyridines **4**. We believe, that the reason of latter result is presenting of electron-withdrawing heterocycles, that makes triple bond electron poor, and the complexation of metal ion with π-electrons is not so effective.



7a,b **8a,b** **9a,b**

i: Py, Δ —— no reaction
ii: CuI/CH_2Cl_2
iii: $AuCl_3/CH_2Cl_2$
iv: $FeCl_3/CH_2Cl_2$
v: $AgNO_3$ /CH_2Cl_2
vi: CF_3CO_2Ag /CH_2Cl_2

7, 8, 9 a R = CH_3,
7, 8, 9 b R—R = CH_2—CH_2

In conclusion, it was found that pyridine initiated cycloizomerization reactions of 6-phenylethynyl-5-nitropyrimidines and 2-phenylethynyl-3-nitropyridines to the corresponding pyrrolo[3,2-*d*]-pyrimidin-7-one 5-oxides and pyrrolo[3,2-*b*]pyridin-3-one 1-oxides. On the other hand the cycloisomerization of 1,2-dialkoxy-5-phenylethynyl-4-nitrobenzenes was successful only in the presence of transition metal salts.

2.3 Study on the reactions of 2,4-disubstituted 6-phenylethynyl-5-nitropyrimidines and 2-phenylethynyl-3-nitropyridines with amines.

In the previous chapter it was shown that 6-arylethynyl-5-nitropyrimidines **2** and 2-arylethynyl-3-nitropyridines **4** in the presence of pyridine undergo smooth intramolecular cyclization to give pyrrolo[3,2-*d*]pyrimidine-5-oxides **5** and pyrrolo[3,2-*b*]pyridine-5-oxides **6**. Encouraged by aforesaid results we decided to investigate the behavior of the title compounds in the presence of different amines. When 4-amino-5-nitro-6-phenylethynylpyrimidine (**2b**) was treated with another tertiary amine – triethylamine no reaction was observed.

In the presence of catalytic amount of secondary amines reaction conversion of 5-nitro-6-phenylethynylpyrimidines **2a**,**b** was incomplete and formation of traces of intense red or orange products was observed by TLC.

Performing reaction of **2a**,**b** with an equivalent amount of selected secondary amines in dichloromethane at room temperature furnished compounds **10a–c**. Neither ^{13}C-NMR nor IR spectra of **10a–c** showed the presence of C≡C or CO groups in the molecules. In the ^{1}H-NMR spectra new singlet at 6.46–6.59 ppm due to vinylic CH along with signals of the corresponding amine was observed. These data indicated that addition reaction of amines to the triple bond took place. It is noteworthy that, in the ^{1}H-NMR spectra of **10a–c**, singlets of H and SCH_3 in position 2 of the pyrimidine ring were observed in an upfield region than usual (7.59 ppm for C2-H and 1.58–1.69 ppm for C2-SCH_3, respectively).

Slow crystallization of **10c** from dichloromethane provided single crystals suitable for the X-ray crystallographic analysis, which enabled the outcome of the reaction to be elucidated unambiguously (Figure 1). Moreover, the crystallographic data of **10c** showed that in the solid state the molecule adopted a conformation in which the benzene ring is turned out of plane of the pyrimidine ring and C(11)H=C(12)-N(13) moiety. The torsion C(11)-C(12)-C(18)-C(23) was found to be 84.85^{o}. Thus, the hydrogen at C-2 of the pyrimidine ring is constrained above the benzene ring.

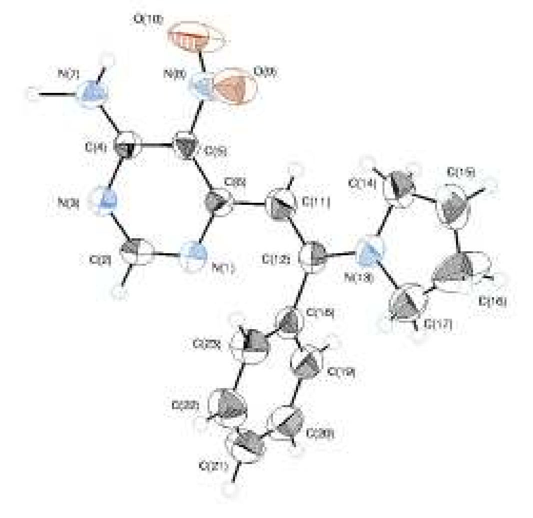
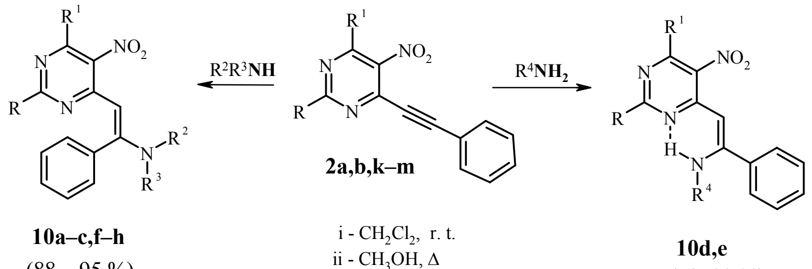


Figure 1. ORTEP drawing of compound **10c**.

As a consequence upfield shift of C2-H and C2-SCH$_3$ signals in the ^{1}H-NMR spectra results from shielding effect of benzene ring on these protons. Thus, these data indicate that compounds formed in the reaction of **2a,b** with secondary amines were the corresponding 4-amino-5-nitro-6-[(*E*)-2-phenyl-2-(1-dialkylamino)ethenyl]pyrimidines **(10a–c)**.

Analogous reactions with a primary amine - propylamine were much more slower. Full conversion of compounds **2a,b** at room temperature was achieved only in about 48 hours. ^{1}H-NMR, ^{13}C-NMR and IR spectra of the obtained products showed that in the case of propylamine addition reaction to C≡C bond took place again. Singlets for C2-H and C2-SCH$_3$ of the pyrimidine ring in the ^{1}H-NMR spectra of **10d,e** were observed in ordinary positions for these groups – 8.21 ppm and 2.57 ppm, respectively. Probably, in compounds **10d,e** benzene ring is directed away from the pyrimidine ring and shielding effect of benzene ring in the ^{1}H-NMR spectra for protons at C2 of the pyrimidine ring is absent. This can be realized if addition reaction of primary amines to the triple bond of **2a,b** proceeds with the formation of *anti*-addition products **10d,e**.



10a–c,f–h
(88 – 95 %)

2a,b,k–m

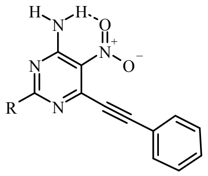
i - CH_2Cl_2, r. t.
ii - CH_3OH, Δ

10d,e
(78, 92 %)

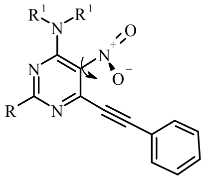
Table 4. Data of reactions 2,4-disubstituted 6-phenylethynyl-5-nitropyrimidines **2a,b,k-m** with amines

Starting compound	R	R^1	NR^{2}R^3	NHR^4	Conditons	Product
2b	SCH_3	NH_2	$N(CH_2)_5$	---	i	**10a**
2b	SCH_3	NH_2	$N(CH_2)_4$	---		**10b**
2a	H	NH_2	$N(CH_2)_4$	---		**10c**
2b	SCH_3	NH_2	---	NHC_3H_7		**10d**
2a	H	NH_2	---	NHC_3H_7		**10e**
2k	H	$N(CH_2)_4O$	$N(CH_2)_5$	---	ii	**10f**
2l	H	$N(CH_2)_5$	$N(CH_2)_5$	---		**10g**
2m	H	$N(CH_2)_4NCH_3$	$N(CH_2)_5$	---		**10h**

Reactions of 4-dialkylamino-5-nitro-6-phenylethynylpyrimidines **2k–m** with secondary amines were very slow and inefficient. A trace amounts of enamines **10f–h** was observed only by TLC. We speculate, that due to bulky *N,N*-dialkylamino substituent, nitro group is turned out the plane of the pyrimidine ring and therefore only inductive effect of the nitro group realises and the activation of the tripple bond is not so effective:



2a,b

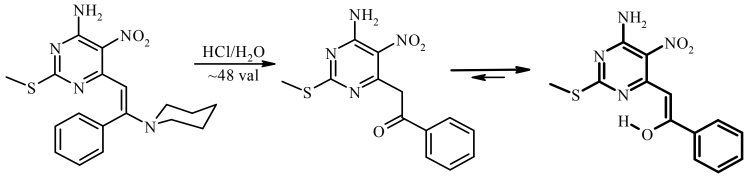


2k–m

However in protic solvent boiling methanole the addition of secondary amines to tripple bond of 4-dialkylamino-5-nitro-6-phenylethynylpyrimidines **2k–m** was complete after 1.5–2 hours.

Enamine **10a** smoothly underwent acidic hydrolysis. The hydrolysis product **11** exists in two tautomeric forms (**11a** and **11b**) in the solutions (observed by ^{1}H-NMR). It should be noted, that predominant form is enol **11b** (60 % in dimethylsulfoxide and

73 % in chloroform solutions). The ratio of the form does not depend on the concentration of compound in the NMR sample.



10a **11a** **11b**

Solvent	**11a**	**11b**
$CDCl_3$	27 %	73 %
DMSO-d_6	40 %	60 %

Next, we studied the reaction of 5-nitro-6-phenylethynylpyrimidine **4a** with amines. The latter compound was not so reactive towards nucleophiles – the reactions took several days to complete and moreover in all cases the mixture of different products was formed (observed by TLC). In the reaction of 5-nitro-6-phenylethynylpyrimidine **4a** with pyrrolidine, we observed that the desired product **12** was extremely unstable and reactive towards water in small amounts presented in solvent. Thus after the work-up red crystalline product **13** was isolated. Crystallographic data of compound **13** showed, that after the reaction of starting compound **4a** with pyrrolidine, the smooth hydrolysis reaction took place and α-[(3-nitro-2-pyridinyl)methylene]benzenemethanol formed.

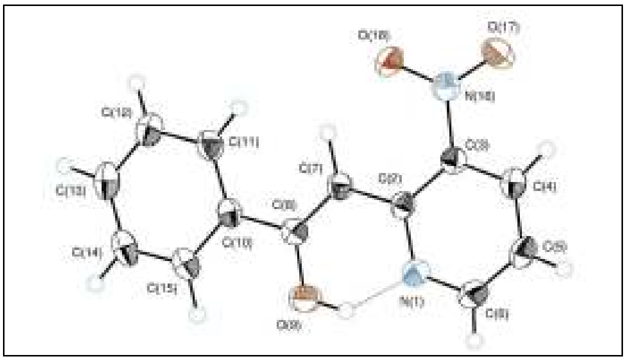
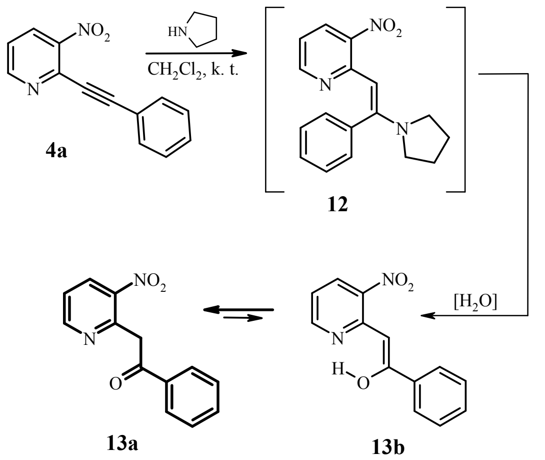


Figure 2. ORTEP view of compound **13.**

Analogously as in the case of compound **11**, α-[(3-nitro-2-pyridinyl)-methylene]benzenemethanol **13** exists in two tautomeric forms in solutions. However after the performing NMR analysis of compound **13** in dimethylsuldoxide or chloroform solutions, it was found, that contrariwise to compound **11**, predominant tautomeric form of **13** is ketone **13a** (82 % and 74 %, respectively). Interestingly, that when we dissolved compound **13** in deuterated methanol and immediately performed NMR analysis, we found, that in methanole solution there are only one tautomeric form of **13**, namely enol

13b. We were surprised by these results and therefore we performed several NMR measurements after different times after preparations of solutions.



Solvent	**13a**	**13b**
CDCl$_3$	74 %	26 %
DMSO-d_6	82 %	18 %
CD$_3$OD	0 %[a]; 70 %[b]; 79 %[c]	100 %[a]; 30 %[b]; 21 %[c]

a –immediately after the preparation of solution, b – in 6 days, c – in 14 days

Thus, we found, that in aprotonic solvents (chloroform and dimethylsulfoxide) the tautomeric equilibration takes place immediately after the preparation of solutions (no changes of the ratio of tautomers were observed after the several hours or days). On the other hand, the tautomeric equilibration of **13** in protic solvent methanol takes place very slowly. As it is seen in Figure 3, there is only enol form **13b** in methanol immediately after the preparation of solution (^{1}H-NMR spectrum **a**). After 6 days (^{1}H-NMR spectrum **b**), there are two tautomeric forms **13a** and **13b** in 70 and 30 ratio, respectively. After 14 days, tautomeric equilibration finished and in the ^{1}H NMR spectrum **c** we can see two tautomeric forms **13a** and **13b** in 79 and 21 ratio. We believe, that slower tautomeric equilibration in protic solvent takes place due to protonation of carbonyl group.

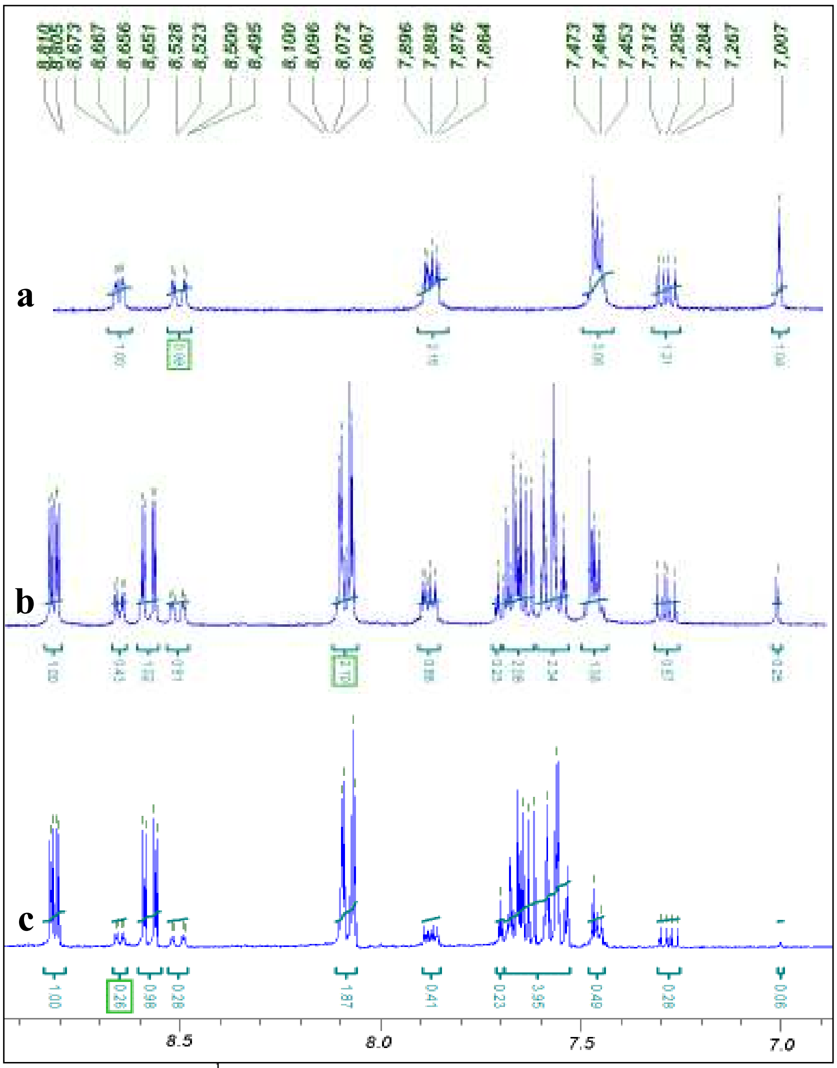
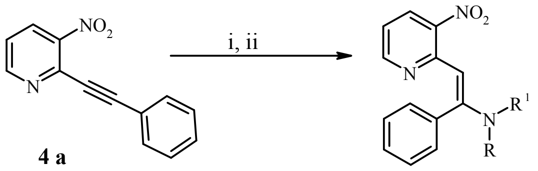


Figure 3. Fragment of ^{1}H NMR spectrum of **13** in methanol (CD_3OD). a– immediately after the preparation of solution, b – after 6 days, c – after 14 days.

The reaction 2-phenylethynyl-3-nitropyridine **4a** with secondary amines was successful and faster when we used solvent free conditions or performed reaction in boiling methanol:



4 a

14a,b

i - RR^1NH, r. t.
ii - RR^1NH, CH_3OH, Δ

Conditions	NRR^1	Product	Yield, %
i	$N(C_2H_5)_2$	**14a**	43
ii			75
ii	$N(CH_2)_5$	**14b**	68

Compounds **14a,b** are unstable and underwent hydrolysis reaction just exposed to air.

2.4 Efficient One-Pot Synthesis of 6-Phenylpyrrolo[3,2-d]pyrimidines from 6-Phenylethynyl-5-nitropyrimidines.

As the reactions of 6-phenylethynyl-5-nitropyrimidines **2** with secondary amines were fast and high-yielding, we decide to study the possibility of straightforward one-pot synthesis of the pyrrolo[3,2-*d*]pyrimidine framework from 6-phenylethynyl-5-nitropyrimidines *via* reductive cyclization of intermediate enamines **10**.

Our initial studies were aimed at finding optimal conditions for the amine-mediated reductive cyclization of the 6-phenylethynyl-5-nitropyrimidines **2**. Our investigation began with 4-amino-5-nitro-6-phenylethynylpyrimidine **2b**, the corresponding amine (1 equiv.) under the reductive conditions. The best results were obtained using secondary amines, such as diethylamine or piperidine in methanol and performing reduction by hydrogen in the presence of 10 % palladium on charcoal.

Moreover, using of primary amines (benzylamine) resulted in longer reaction times. The latter result can be explained by much more slower reaction of starting compounds **2b** with benzylamine and also by stabilization of intermediate enamines by intramolecular hydrogen bond between NH moiety and nitrogen of the pyrimidine ring. It is noteworthy, that better yields of final product have been achieved when the starting material was refluxed with an equivalent of secondary amine in methanol for 10–15 min, and after the formation of enamine **10** was completed (observed by TLC as deep-red spot) the nitrogroup was subsequently reduced in the same flask by hydrogen, using 10 % Pd/C at the room temperature. Upon reduction, the intermediate **15** rapidly cyclized *via* intramolecular 1,5-electrocyclic reaction to give the target pyrrolo[3,2-*d*]pyrimidine **16a**. It should be noted, that we did not find any evidence about the reduction of intermediate enamines C=C bound. Also, it is important to start the reduction of the nitro group only after the complete formation of enamine **10**. Otherwise, the formation of 2,5-

diamino-6-phenylethynylpyrimidine **17** stops the reaction in the first step, because of inactivation of the triple bond by electron-donating 5-amino group.

So, according to the present methodology, we have prepared 2,4-disubstituted 6-phenylpyrrolo[3,2-*d*]pyrimidines **16** via consequent conjugative addition of secondary amine to 6-phenylethynyl-5-nitropyrimidines **2** and reductive cyclization reaction of intermediate enamines **10**.

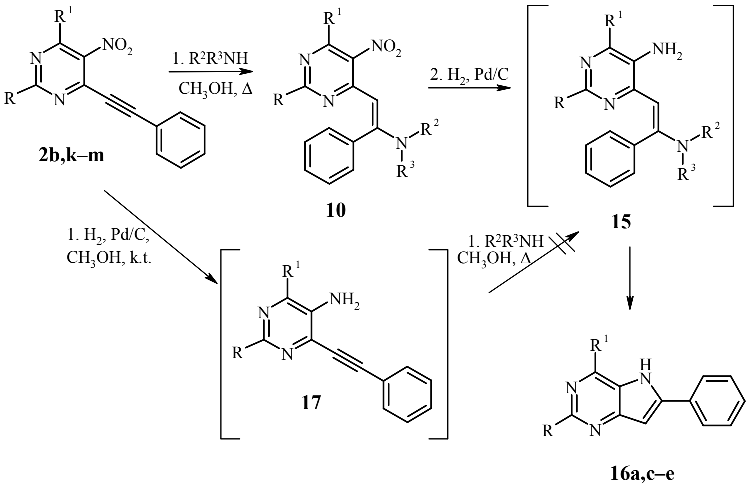


Table 5. Data of synthesis of 6-phenylpyrrolo[3,2-*d*]pyrimidines **16a,c–e** from 6-phenylethynylpyrimidine **2b,k–m**

Starting compound	R	R^1	NR^{2}R^3	Product	Yield, %
2b	SCH_3	NH_2	$N(CH_2)_5$	**16a**	90
			$N(C_2H_5)_2$		82
			$NHCH_2C_6H_5$		75
2k	H	$N(CH_2)_4O$	$N(C_2H_5)_2$	**16c**	88
			$NHCH_2C_6H_5$		62
2l	H	$N(CH_2)_5$	$N(C_2H_5)_2$	**16d**	91
2m	H	$N(CH_2)_4NCH_3$	$N(C_2H_5)_2$	**16e**	85

In conclusion, we have developed a novel, simple and high-yielding synthetic method of pyrrolo[3,2-*d*]pyrimidine framework via one-pot reaction of 2,4-disubstituted 6-phenylethynyl-5-nitropyrimidines with secondary amines, followed by reductive cyclization. We believe that the present methodology extends promise for the convenient synthetic protocol for the preparation of pyrrolo[3,2-*d*]pyrimidine derivatives of biological interest.

2.5. Modification of pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides and their structural analogues

The next step of synthetic approach to pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides was modification of the 2nd of the pyrrolo[3,2-*d*]pyrimidine system. Substitution of methylthio moiety at the 2nd position of pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides by nitrogen nucleophiles was exceedingly difficult. No reaction took place when compound **5a** was treated with secondary amine piperidine in dimethylsulfoxide at an elevated temperature for 24 hours. To avoid such difficulties the synthetic route was redesigned to employ another method, which is based on oxidation of the methylthio moiety followed by nucleophilic substitution reaction. Performing an oxidation of compound **5a** by a slight excess of *m*-chloroperbenzoic acid (*m*CPBA) in dichloromethane at room temperature for 1–2 hours led to formation of 2-methylsulfinyl derivative **18**. Nucleophilic substitution of the 2-methylsulfinyl group by various amines underwent easily and rapidly, so it could be achieved at room temperature in dimethylsulfoxide solution within 3 hours. On the other hand, the synthetic route could be realized in a one-pot method. Thus, reaction of 2-methylthiopyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides **5a,b,e,f** with *m*CPBA in dichloromethane at room temperature, followed after 1–2 hours by treatment of the reaction mixture with 3 equivalents of different amines for 3 hours provided the corresponding 2,4-disubstituted pyrrolo[3,2-d]pyrimidin-7-one 5-oxides (**19a–s**).

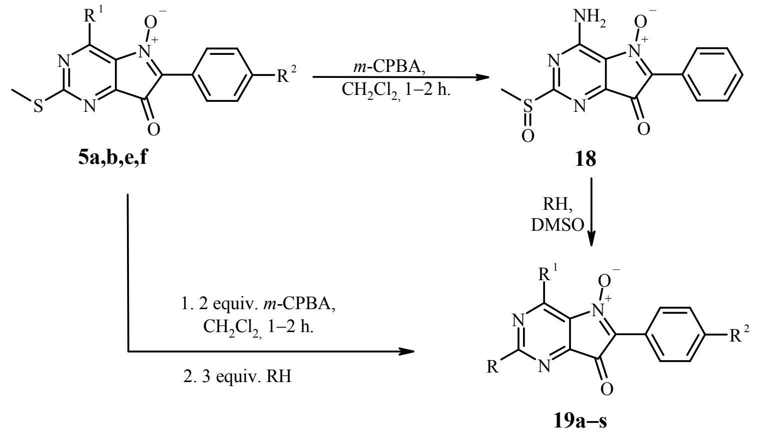
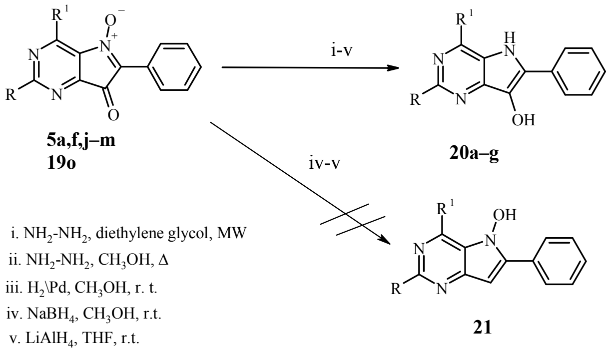


Table 6. Data of synthesis of 2,4-disubstituted 6-arylpyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide **19a–s** from 2-methylthiopyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides (**5a,b,e,f**)

Starting compound	R^1	R^2	R	Product	Yield, %
5a	NH_2	H	NHC_3H_7	**19a**	71, 80
5a	NH_2	H	$NHCH_2C_6H_5$	**19b**	74, 78
5a	NH_2	H	$N(CH_2)_4O$	**19c**	70, 83
5a	NH_2	H	$N(CH_2)_5$	**19d**	75, 79
5a	NH_2	H	$NH(CH_2)_2N(CH_2)_4O$	**19e**	72, 75
5a	NH_2	H	$NHCH_2CH{=}CH_2$	**19f**	75
5a	NH_2	H	$NHCH_2CO_2CH_3$	**19g**	52
5e	$NHCH_2C_6H_5$	H	$NHCH_2C_6H_5$	**19h**	60
5a	NH_2	H	$NH(CH_2)_2C_6H_5$	**19j**	80
5a	NH_2	H	$NH(CH_2)_2N(CH_2)_4$	**19k**	79
5a	NH_2	H	$NH(CH_2)_3N(CH_3)_2$	**19l**	69
5f	$N(CH_2)_4$	H	$N(CH_2)_4O$	**19m**	63
5a	NH_2	H	$N(CH_2)_4NCH_3$	**19n**	85
5a	NH_2	H	$N(CH_2)_6$	**19o**	92
5b	NH_2	CH_3	$N(CH_2)_6$	**19p**	90
5f	$N(CH_2)_4$	H	$N(CH_2)_6$	**19m**	80

So, a relatively short and efficient synthetic method of preparing 2,4-disubstituted 6-phenylpyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides through one-pot oxidation/substitution of methylthio group at the 2nd position of the pyrrolo[3,2-*d*]pyrimidine heterosystem was developed.

On the other hand, it should be possible to modify the pyrrole ring of pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides and of pyrrolo[3,2-*b*]pyridin-3-one 1-oxides. Therefore we decided to synthesize reduced derivatives of latter compounds. It should be noted that using different reductants: hydrazine hydrate in methanol or diethyleneglycol, hydrogen and Pd/C as catalyst, sodium borohydride in methanol or lithium aluminum hydride in THF, led to the reduction of pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides **5** and **19** and give the 5*H*-pyrrolo[3,2-*d*]pyrimidin-7-oles (**20**) and no *N*-hydroxy derivatives **21** (which could be expected from the literature results) were formed.

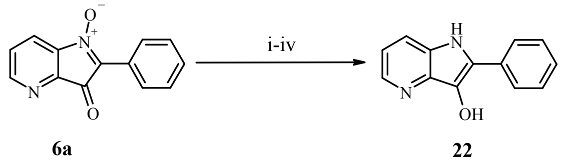


i. NH_2-NH_2, diethylene glycol, MW
ii. NH_2-NH_2, CH_3OH, Δ
iii. H_2\Pd, CH_3OH, r. t.
iv. $NaBH_4$, CH_3OH, r.t.
v. $LiAlH_4$, THF, r.t.

Table 7. Data of reduction of pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides **5** and **19**

Starting compound	R	R^1	Product	Yield, %
5a	SCH_3	NH_2	**20a**	54 – 92
5f	SCH_3	$N(CH_2)_4$	**20b**	85
5j	H	$N(CH_2)_4O$	**20c**	90
5k	H	$N(CH_2)_5$	**20d**	89
5l	H	$N(CH_2)_4NCH_3$	**20e**	76
5m	H	$N(C_2C_5)_2$	**20f**	64
19o	$N(CH_2)_6$	NH_2	**20g**	87

Analogously, 2-phenylpyrrolo[3,2-*b*]pyridin-3-one 1-oxide (**6a**) during reduction reaction formed the corresponding 2-phenyl-1*H*-pyrrolo[3,2-*b*]pyridin-3-ole (**22**).



i. NH_2-NH_2, diethylene glycol, MW
ii. NH_2-NH_2, CH_3OH, Δ
iii. H_2\Pd, CH_3OH, r. t.
iv. $NaBH_4$, CH_3OH, r.t.

3. ANTIPROLIFERATIVE ACTIVITY OF PYRROLO[3,2-*d*]PYRIMIDIN-7-ONE 5-OXIDES, THEIR STRUCTURAL ANALOGUES AND REDUCED DERIVATIVES

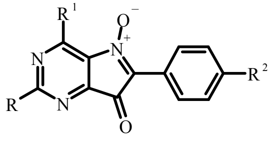
Anticancer activity of synthesized compounds was studied in Spain, La Laguna University, under the guidance of Dr. José M. Pardon.

The antiproliferative profile of the 2,4-disubstituted pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides and their derivatives was evaluated in vitro against a panel of six human solid tumor cell lines: A2780 (ovarian), HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast) and WiDr (colon). The *in vitro* activity was evaluated with the National Cancer Institute (NCI) protocol after 48h of drug exposure using the sulforhodamine B (SRB) assay.

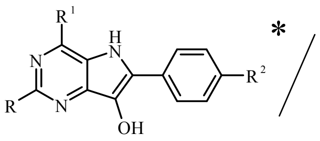
The sensitivities expressed as GI_{50} (50% growth inhibition activity) are listed in Table 3. In addition to the antitumor activity, the lipophilicity (Clog*P*) of the compounds was evaluated by in silico calculation based on their chemical structure. Clog*P* values were calculated to correlate lipophilicity with antitumor activity. The Clog*P* values for the compounds reported in this study are in the range -1.1 to 3.2. Taken as a whole, lipophilicity is not sufficient to explain the observed differences in growth inhibition.

The GI_{50} values allow classifying the 2,4-disubstituted pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides in two groups. The first group is comprised of pyrrolo[3,2-*d*]pyrimidines (**5**) and (**18**). These compounds showed overall a modest antiproliferative activity with GI_{50} values higher than 26 μM. From this series, only three compounds were active against all cell lines; the derivatives **5m**, **5j** and **5k**, with GI_{50} values in the range 15-38 μM.

The second group is formed by all derivatives bearing *N*-alkyl or *N,N*-dialkylamino **19** substituents at C-2 position of the pyrrolo[3,2-*d*]pyrimidine heterocyclic framework. This set of compounds exhibited the best results. The vast majority of the compounds were able to induce antiproliferative effects in all cell lines. Only derivatives **19j** and **19n** showed inactive against one or two of the cell lines. The most potent derivatives were compounds **19f**, **19d**, **19o** and **19p**, bearing allylamino, piperidino and azepanyl substituents, respectively. Their antiproliferative activity was similar against the six cell lines, showing GI_{50} values within the range 0,35-9,1 μM. This is a remarkable effect, since the general observation for conventional antitumor drugs is that WiDr, T-47D and HeLa cancer cells are more drug resistant than A2780 and HBL-100 cancer cells.



5, 18, 19



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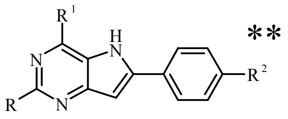

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Table 8. *In vitro* antiproliferative activity (GI_{50}, μM) of 2,4-disubstituted 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides

Comp.	**R**	**R^1**	**R^2**	**Human solid tumor cell line**					
				A2780	**HBL-100**	**HeLa**	**SW1573**	**T-47D**	**WiDr**
5h	H	$NHCH_2C_6H_5$	H	---	---	**28**	**17**	**19**	**14**
5m 20f	H	$N(C_2H_5)_2$	H	---	**19** 21*	**15** 16*	**26** 41*	**71** >100*	**99** >100*
5j 20c 16b	H	$N(CH_2)_4O$	H	---	**18** 19* 37**	**25** 22* 24**	**19** 32* 32**	**>100** 56* 56**	**>100** >100* >100**
5l 16d	H	$N(CH_2)_4NCH_3$	H	---	**>100** 79**	**>100** >100**	**>100** >100**	**>100** 51**	**>100** 43**
5k	H	$N(CH_2)_5$	H	**15**	**24**	**38**	**19**	**87**	**>100**
5a	SCH_3	NH_2	H	**5,9** 5,4*	**7,4** 7,4*	**38** 7,8*	**8,9** 7,4*	**6,8** 6,9*	**6,0** 5,9*
5b	SCH_3	NH_2	CH_3	**93**	**28**	**>100**	**31**	**>100**	**>100**
5c	SCH_3	NH_2	C_2H_5	**54**	**37**	**80**	**37**	**43**	**53**
5e	SCH_3	$NHCH_2C_6H_5$	H	**59**	**27**	**36**	**26**	**81**	**79**
5d	SCH_3	$NHCH_2CH_2N(CH_2)_4O$	H	**>100**	**24**	**>100**	**>100**	**>100**	**>100**
5f 20b	SCH_3	$N(CH_2)_4$	H	**>100**	**>100** >100*	**>100** >100*	**>100** >100*	**>100**	**>100** >100*
18	$SOCH_3$	NH_2	H	**79**	**32**	**>100**	**>100**	**>100**	**>100**

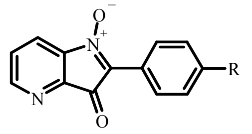
Table 8. *In vitro* antiproliferative activity (GI$_{50}$, μM) of 2,4-disubstituted 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides (continuation)

Comp.	**R**	**R^1**	**R^2**	**Human solid tumor cell line**					
				A2780	**HBL-100**	**HeLa**	**SW1573**	**T-47D**	**WiDr**
19f	$NHCH_2CH=CH_2$	NH_2	H	**1,2**	**2,7**	**7,6**	**6,8**	**2,8**	**4,7**
19g	$NHCH_2CO_2CH_3$	NH_2	H	**1,9**	**2,8**	**19**	**12**	**14**	**18**
19b	$NHCH_2C_6H_5$	NH_2	H	**3,7**	**5,1**	**29**	**30**	**4,0**	**24**
19j	$NHCH_2CH_2C_6H_5$	NH_2	H	**37**	**17**	**>100**	**22**	**41**	**71**
19k	$NHCH_2CH_2N(CH_2)_4$	NH_2	H	**2,6**	**1,8**	**16**	**6,2**	**13**	**14**
19e	$NHCH_2CH_2N(CH_2)_4O$	NH_2	H	**2,9**	**3,4**	**15**	**14**	**17**	**16**
19l	$NHCH_2CH_2CH_2N(CH_3)_2$	NH_2	H	**2,3**	**2,0**	**20**	**2,1**	**16**	**19**
19c	$N(CH_2)_4O$	NH_2	H	**37**	**22**	**38**	**27**	**7,5**	**20**
19n	$N(CH_2)_4NCH_3$	NH_2	H	**24**	**18**	**>100**	**23**	**>100**	**46**
19d	$N(CH_2)_5$	NH_2	H	**2,9**	**1,7**	**3,5**	**3,3**	**2,7**	**3,7**
19o	$N(CH_2)_6$	NH_2	H	**1,8**	**1,4**	**2,0**	**1,3**	**0,35**	**1,8**
19p 20g	$N(CH_2)_6$	NH_2	CH_3	**4,7**	**4,7** 12*	**9,1** 8,2*	**4,6** 15*	**4,8**	**4,9** 2,3*

From the biological activity data, some structure activity relationships can be inferred. The presence at C-2 position of the pyrimidine ring of hydrogen **5h,m,j,l,k**, methylthio **5a-f** or methylsulfonyl **18** moieties resulted in modest or inactive compounds. Neither the alkyl substituent at the phenyl group (R^2) nor the amines at C-4 position of the pyrimidine ring (R^1) appear to be crucial for the modulation of the antiproliferative activity. In contrast, the *N*-alkylamino or *N,N*-dialkylamino substituents at C-2 position induced an enhancement of the biological activity. Overall, the derivatization at C-2 of the pyrimidine ring with phenylethylamino (**19j**), morpholino (**19c**) or *N*-methylpiperazino (**19n**) moieties produced a decrease of the biological activity. The amines that led to the most potent derivatives were allylamine (**19f**), piperidine (**19d**) and azepane (**19o,p**).

Moreover, was found, that pyrrolo[3,2-*b*]pyridin-3-one 1-oxide and 3*H*-indole-3-one 1-oxide showed overall a modest antiproliferative activity. In addition, the reduction of the C=O and N^+-O groups reduced the antitumor activity of pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides. GI_{50} value of pyrrolo[3,2-*d*]pyrimidine **20** and **16** are shown in table 8.

The antiproliferative activity results of the pyrrolo[3,2-*b*]pyridin-3-onų 1-oxides and 3*H*- indol-3-one 1-oxides showed, that obtained derivatives have low influence on tumor cells (Table 9 and Table 10)



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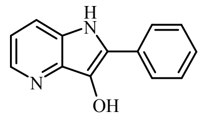

22 *

Table 9. *In vitro* antiproliferative activity (GI_{50}, μM) of pyrrolo[3,2-*b*]pyridin-3-one 1-oxide **6** and their analogue **22**

Comp.	**R**	**Human solid tumor cell line**					
		A2780	**HBL-100**	**HeLa**	**SW1573**	**T-47D**	**WiDr**
6a 22	H	**33** 23*	**40** 61*	**29** 53*	**24** 39*	>100 >100*	**86** >100*
6b	C_2H_5	**15**	**43**	**14**	**40**	**22**	**>100**

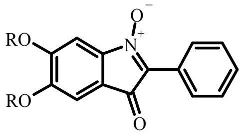

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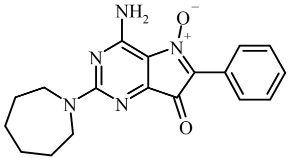
Table 10. *In vitro* antiproliferative activity (GI_{50}, μM) of 3*H*-indol-3-one 1-oxide

Comp.	**R**	**R**	**Human solid tumor cell line**					
			A2780	**HBL-100**	**HeLa**	**SW1573**	**T-47D**	**WiDr**
154a	CH_3	CH_3	---	>100	>100	>100	>100	>100
154b	$CH_2 – CH_2$		---	26	32	32	30	>100

The observation that the major part of the *N*-alkylamino or *N,N*-dialkylamino derivatives **19** evaluated in this study present antiproliferative activity lead us to consider the 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide as a privileged structure with the substituents on the pyrimidine ring (R and R^1) modulating the biological activity.



19o

Moreover, cell cycle phase distribution was studied by flow cytometry to determine if cell growth inhibition involved cell cycle changes. For these studies we selected **19o**, the most active compound from the series.

The effect on the cell cycle was investigated after 24 h exposure. Cells were exposed to compound **19o** at three different drug concentrations: 2, 5 and 10 μM. The drug doses were chosen based on two premises. On the one hand, the GI_{50} values against each cell line. On the other hand, the sensitivity of the cell line to drug treatment, since at higher drug doses large cell death prevents examination of the cell cycle phase distribution. Control cells were incubated in the absence of test drug. The results are shown in Figure 4.

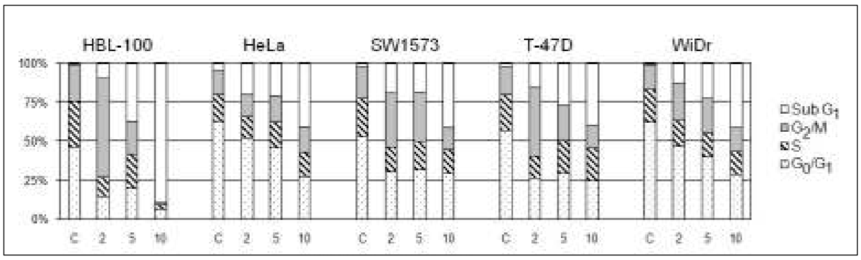


Figure 4. Cell cycle phase distribution in untreated cells (C) and cells treated with compound **19o** for 24 h at 2, 5 and 10 μM.

Overall, cell cycle distributions of samples collected from control and treated cells show in all cell lines an increase of the sub G_0 compartment in a dose dependent manner. The results indicate that compound **19o** produces net cell killing. However, the data revealed a diverse pattern of sensitivity between HBL-100 cells and the remaining cell lines HeLa, SW1573, T-47D and WiDr. In this particular context, HBL-100 cells are more sensitive to compound **19o** as observed in the increased sub G_0 compartment at 5 and 10 μM. Additionally, a clear cell cycle arrest in the G_2/M phase was observed for the breast cancer cell lines HBL-100 and T-47D, and for the lung cancer cells SW1573 when exposed at 2 μM of **19o** (Figure 5). This increase was concomitant with a decrease in both the G_1 and S compartments. On the contrary, cell cycle arrest was not apparent in HeLa and WiDr cells.

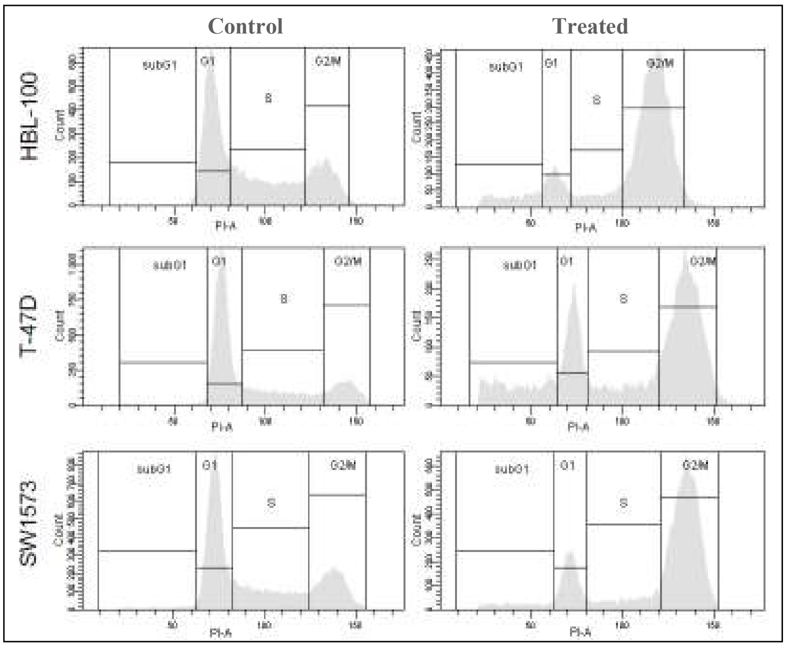


Figure 5. Cell cycle phase distribution in control and treated HBL-100, T-47D, and SW1573 cells, after 24 h exposure to compound **19o** at 2 µM.

Cell cycle studies demonstrate arrest in the G_2/M phase when the breast and lung cancer cells were exposed to compound **19o**. The title products appear as good lead molecules for the development of novel antitumor agents.

Conclusions

1. It was found that 6-arylethynyl-5-nitropyrimidines and 2-arylethynyl-3-nitropyridines undergo pyridine-catalysed intramolecular cyclization reactions to form pyrrolo[3,2-*d*]pyrimidine 5-oxides and pyrrolo[3,2-*b*]pyridin-3-one 1-oxides, respectively. On the other hand, 1,2-alkoxy-5-phenylethynyl-4-nitrobenzenes underwent cycloizomerization reaction to 3*H*-indol-3-one 1-oxides only in the presence of transition metal salts.

2. It was found that the triple bond of 6-phenylethynyl-5-nitropyrimidines is easily attacked by primary and secondary amines to form *syn*- (in the case of secondary amines piperidine and pyrolidine) or *anti*-addition (in the case of primary amine propylamine) products. It was observed that 2-phenylethynyl-3-nitropyridines and 4-dialkylamino-6-arylethynyl-5-nitropyrimidines are less reactive towards nucleophilic reagents.

3. 6-[(*E*)-2-fenil-2-(1-piperidin)ethenyl]-5-nitropyrimidines and *N,N*-dialkyl-α-[(3-nitro-2-pyridinyl)methylene]benzenemethanamines underwent a smooth hydrolysis to 6-[(*Z*)-2-phenyl-2-hydroxyethenyl]-5-nitropyrimidines and α-[(3-nitro-2-pyridinyl)methylene]benzenemethanol.

4. It was found that in solutions [(*Z*)-2-phenyl-2-hydroxyethenyl]-5-nitropyrimidines and α-[(3-nitro-2-pyridinyl)methylene]benzenemethanol exist in two tautomeric forms. It is noteworthy, that the major tautomeric forms of [(*Z*)-2-phenyl-2-hydroxyethenyl]-5-nitropyrimidines are enols and the major tautomeric form of α-[(3-nitro-2-pyridinyl)methylene]benzenemethanol is ketone.

5. A novel, simple and high-yielding synthetic method of pyrrolo[3,2-d]pyrimidine framework *via* one-pot reaction of 2,4-disubstituted 6-phenylethynyl-5-nitropyrimidines with secondary amines, followed by reductive cyclization has been developed.

6. A relatively short and efficient synthetic method of preparing 2,4-disubstituted 6-arylpyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides through one-pot oxidation/substitution of methylthio group at the 2nd position of the pyrrolo[3,2-*d*]pyrimidine heterosystem was developed.

7. It was found that reduction of pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides and pyrrolo[3,2-*b*]pyridin-3-one 1-oxides led to the formation of the corresponding 5*H*-pyrrolo[3,2-*d*]pyrimidin-7-oles and 1*H*-pyrrolo[3,2-*b*]pyridin-3-oles.

8. After the evaluation of antiprofilerative activity of all synthesized compounds (in vitro experiments in the human solid tumor cell lines A2780, HBL-100, HeLa, SW1573, T-47D and WiDr) it was found that the most active compounds are disubstituted 6-phenylpyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides, containing *N*-alkylamino or *N,N*-dialkylamino substituents in position 2 of the pyrrolo[3,2-*d*]pyrimidine heterosystem. Cell cycle studies demonstrate arrest in the G_2/M phase when the breast and lung cancer cells were exposed to the most active compound.

LIST OF ORIGINAL PUBLICATIONS

Papers:

1. I. Cikotienė, E. Pudziuvelytė, A. Brukstus; “Efficient One-Pot Synthesis of 6-Arylpyrrolo[3,2-*d*]pyrimidines from 6-Arylethynyl-5-nitropyrimidines”, *Synlett*, **2010**, 7, 1107.

2. E. Pudziuvelyte, C. Ríos-Luci, L. G. León, I. Cikotiene and J. M. Padrón, “Synthesis and Antiproliferative Activity of 2,4-disubstituted 6-Aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-Oxides”, *Bioorg. Med. Chem.*, **2009**, 17, 4955.

3. I. Cikotiene, E. Pudziuvelyte, A. Brukstus; “Synthesis of 2,4-disubstituted 6-Phenyl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-Oxides”, *J. Heterocycl. Chem.,* **2008,** 45, 1615.

4. I. Cikotiene, E. Pudziuvelyte, A. Brukstus; “Unexpected reactions of 4-amino-2-methylsulfonyl-5-nitro-6-phenylethynylpyrimidine with nucleophiles”, *Chem. Heterocycl. Comp.*, **2008**, 44, 762.

5. I. Cikotiene, E. Pudziuvelyte, A. Brukstus, S. Tumkevicius; “Study on the Reactions of 4-Amino-5-nitro-6-phenylethynylpyrimidines with Amines and Thiols”, *Tetrahedron*, **2007**, 63, 8145.

Abstracts of the international conferences:

1. I. Cikotienė, R. Buksnaitienė, E. Pudziuvelytė, I. Zutautaitė, R. Sazinas, D. Motiejaitis and A. Brukstus; Synthesis of different heterocycles via transformations of functionally substituted alkynes, Tarptautinės konferencijos „*24th European colloqium on Heterocyclic Chemistry*“ pranešimų medžiaga, **2010**, Austrija, Vienna, OP – 8;.

2. I. Cikotiene, R. Buksnaitiene, E. Pudziuvelyte, C. Ríos-Luci, L. G. León, J. M. Padrón, V. Lather and M. X. Fernandes, „Intramolecular cyclizations of some alkynylazines and synthesis of novel antitumour compounds“, Tarptautinės mokslinės konferencijos „*13th Blue Danube Symposium on Heterocyclic Chemistry*“ pranešimų medžiaga, **2009**, Slovėnija, Bled, Oral Communication 4, p. 48;

3. I. Cikotiene, E. Pudziuvelyte, M. Petkute, A. Brukstus, S. Tumkevicius; Novel use of 5-nitropirimidines in synthesis of pirimidine nucleus containing heterocycles, Tarptautinės mokslinės konferencijos „*12th Blue Danube Symposium on Heterocyclic Chemistry*“ pranešimų medžiaga, **2007**, Vengrija, Tihany, PO 15, p. 64;

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1. E. Pudžiuvelytė, I. Čikotienė, Efektyvi 6-arilpirolo[3,2-d]pirimidinų sintezė iš 6-ariletinil-5-nitropirimidinų, mokslinės konferencijos „Organinė chemija“ pranešimų medžiaga, **2010**, Kaunas, p. 34.

2. E. Pudžiuvelytė, C. Ríos-Luci, L. G. León, I. Čikotienė and J. M. Padrón, 2,4-Dipakeistų 6-aril-7-okso-7H-pirolo[3,2-d]pirimidin-5-oksidų sintezė ir priešvėžinio

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3. I. Cikotiene, E. Pudziuvelyte, M. Petkute, A. Brukstus, S. Tumkevicius; Synthesis of Pirimidine Moiety Containing Heterocycles via Transformations Of 4-Substituted-5-nitropirimidines under non-Reductive Conditions, Lietuvos 8-osios chemikų konferencijos „Chemija 2007“ pranešimų tezės, **2007**, Vilnius, p. 66;

Pirolo[3,2-*d*]pirimidinų bei jų struktūrinių analogų sintezė ir priešvėžinis aktyvumas

Santrauka

Vėžys viena aktualiausių sveikatos problemų, kurios gydymui dažnai naudojama radioterapija ir chemoterapija. Ši liga išsivysto, kai sutrinka normalus ląstelių dalijimosi procesas. Ilgą laiką nebuvo aiškūs priešvėžinių vaistų veikimo principai, todėl dažnai tradiciniai citotoksiniai vaistai (DNR-interkaliatoriai, DNR alkilinantys agentai ir t.t.) buvo neefektyvūs, nes jų poveikis buvo stebimas ne tik vėžinėms, bet ir sveikoms ląstelėms. Tobulėjant molekuliniams metodams, kuriais galima nustatyti konkrečias vėžį sukeliančias priežastis, buvo siekiama vėžio gydymą pakreipti kita linkme. XX a. pabaigoje atsirado naujas chemoterapinis gydymo metodas, paremtas ląstelių chemija ir biochemija. Be to, per paskutinius metus buvo nustatyta daug genų ir baltymų, kurie yra atsakingi už vėžio inicijavimą ir progresavimą. Remiantis šiais pasiekimaisi atsirado galimybė sukurti vaistus, užblokuojančius tam tikrus navikinėse ląstelėse vykstančius procesus, taip sustabdant vėžinės ląstelės dalijimąsi ir augimą. Tokie vaistai yra naudojami taikinių terapijoje. Pasidomėjus vėžinių susirgimų biologija, paaiškėjo, kad vienas iš būdų įveikti šią ligą - sustabdyti vėžinių ląstelių dauginimosi procesus.

Pirolo[3,2-*d*]pirimidinų heterociklinės sistemos dariniai yra svarbūs dėl vertingų biologinių savybių. Šiai junginių klasei priskiriami purinų 9-deazaanalogai, kurie yra purino nukleozidų fosforilazės ir timidilatsintazės inhibitoriai, neuropeptidinio Y5 ir A1, A2 adenozino receptorių antagonistai.

Jau keletą metų mūsų laboratorijoje dirbama su pirolo[3,2-*d*]pirimidinais ir jų struktūriniais analogais, bet jų biologinis aktyvumas nebuvo tirtas ir įvertintas. Tik remiantis literatūros duomenimis, buvo galima nuspėti, kaip šie junginiai elgsis biologinėse sistemose ir kokie veiksniai ar struktūros modifikavimo ypatumai gali turėti tam įtakos. Atsiradusi galimybė ištirti mūsų susintetintus junginius *in vitro* vėžinių ląstelių grupėse ir gauti pirmieji teigiami rezultatai paskatino dar labiau domėtis šiais junginiais. Atsižvelgiant į tai buvo suformuluotas *šio darbo tikslas* susintetinti pirolo[3,2-*d*]pirimidinus bei jų struktūrinius analogus, siekiant įvertinti jų priešvėžinio aktyvumo priklausomybę nuo cheminės struktūros.

Šio darbo metu vykdant pirolo[3,2-*d*]pirimidin-7-onų 5-oksidų ir jų struktūrinių analogų (pirolo[3,2-*b*]piridin-3-onų 1-oksidų bei 3*H*-indol-3-onų 1-oksidų) intramolekulinės ciklizacijos reakcijas nustatyta, kad 6-feniletinil-5-nitropirimidinų ir 2-feniletinil-3-nitropiridinų ciklizaciją puikiai inicijuoja piridinas, o 5-feniletinil-1,2-alkoksi-4-nitrobenzenai į atitinkamus 3*H*-indol-3-onų 1-oksidus persigrupuoja tik veikiant pereinamųjų metalų druskoms.

Pastebėta, kad 2,4-dipakeistų 6-feniletinil-5-nitropirimidinų trigubasis ryšys yra aktyvus reakcijose su pirminiais ir antriniais aminais. Piperidinas ir prolidinas susidaro *sin*-, o propilaminas – *anti*-jungimosi produktus. Be to, nustatyta, kad 2-feniletinil-3-nitropiridinas ir 4-dialkilamino-6-feniletinil-5-nitropirimidinai lėtai reaguoja net su antriniais aminais.

Hidrolizuojant 6-[(*E*)-2-fenil-2-(1-piperidin)etenil]-5-nitropirimidiną ir *N,N*-dialkyl-α-[(3-nitro-2-piridinil)methylene]benzenemetanaminus buvo gauti 6-[(*Z*)-2-fenil-2-hidroksietenil]-5-nitropirimidinas ir α-[(3-nitro-2-piridinil)metilene]benzenemetanolis.

Be to, atlikus minėtų hidrolizės produktų ^{1}H-BMR analizę paaiškėjo, kad 6-[(*Z*)-2-fenil-2-hidroksietenil]-5-nitropirimidino ir 2-[(*Z*)-2-fenil-2-hidroksietenil]-3-nitropiridino tirpaluose nusistovi dviejų tautomerų pusiausvyra. Pažymėtina tai, kad 6-[(*Z*)-2-fenil-2-hidroksietenil]-5-nitropirimidino tirpale vyrauja enolinė, 2-[(*Z*)-2-fenil-2-hidroksietenil]-3-nitropiridino – ketoninė forma.

Pasiūlytas naujas, paprastas ir efektyvus pirolo[3,2-*d*]pirimidinų sintezės būdas, kai iškart po 2,4-dipakeistų 6-feniletinil-5-nitropirimidinų reakcijos su aminais, atliekama ciklizacija redukcinėmis sąlygomis.

Rastas greitas ir efektyvus 2,4-dipakeistų 6-aril-7*H*-pirolo[3,2-*d*]pirimidin-7-onų 5-oksidų sintezės būdas, kai oksiduojant ir pakeičiant metiltiogrupę, į antrą pirolo[3,2-*d*]pirimidino heterosistemos padėtį įvedami *N*-alkilamino ir *N,N*-dialkilamino pakaitai.

Redukuojant 2,4-dipakeistus 6-fenil-7*H*-pirolo[3,2-*d*]pirimidin-7-onų 5-oksidus ir 2-fenil-3*H*-pirolo[3,2-*b*]piridin-3-ono 1-oksidą greitai ir geromis išeigomis sisidarė 6-fenil-5*H*-pirolo[3,2-*d*]pirimidin-7-oliai ir 2-fenil-1*H*-pirolo[3,2-*b*]piridin-3-olis.
Remiantis susintetintų junginių priešvėžinio aktyvumo (*in vitro* eksperimentų A2780, HBL-100, HeLa, SW1573, T-47D and WiDr ląstelių grupėse) duomenimis nustatyta, kad aktyviausi junginiai yra 2,4-dipakeisti 6-aril-7*H*-pirolo[3,2-*d*]pirimidin-7-onų 5-oksidai, kurie pirolo[3,2-*d*]pirimidino heterosistemos antroje padėtyje turi *N*-alkilamino arba *N,N*-dialkilamino pakaitus. Be to, ląstelės ciklo sutrikdymo tyrimas parodė, kad krūties ir plaučių vėžio ląsteles paveikus 4-amino-2-azepanil-6-fenil-7*H*-pirolo[3,2-*d*]pirimidin-7-ono 5-oksidu, ląstelių ciklas sustabdomas G_2/M fazėje.

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