Synthesis and Biological Evaluation of Novel 2,6,9-Trisubstituted 8-Azapurines

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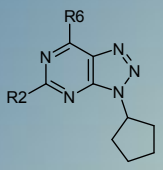
Introduction

Cyclin-dependent kinases (CDKs) are key elements of the cell cycle regulation being considered to be potential targets for novel anti-proliferative drugs[1]. Among them, 2,6,9-trisubstituted purines are the typical example of small molecule inhibitors of CDKs, and their detailed systemic SAR screening lead to discovery of Roscovitine which is in the 2nd phase of preclinical development for the treatment of non- small cell lung carcinoma and nasopharyngeal cancer[1]. Recently, a series of novel extremely potent C6-heterobiarylmethylaminopurines, possesing strong anti-cancer activity, was developed[2]. The success of novel purine anti-cancer substances has prompt attemps to develop related CDK inhibitors by three different approaches. The first one optimizes various substituents at the purine moiety, the second one involves purine bioisosteres as a basic scaffold, and finally, third possibility uses a combination of two above mentioned approaches[1,4]. We decided for the last approach and chose 8-azapurine (1,2,3-triazolo[4,5-d]pyrimidine) scaffold, as an important and well known purine bioisostere [1,3,4], which was supposed to be a useful tool for the preparation of new interesting small molecules interacting with various molecular targets [3].

Here we describe a series of novel 2,6,9-trisubstituted 8-azapurines with fixed N_9 position substituted by cyclopentyl moiety (fig. 1). Selected molecules were screened both for CDK2 inhibitory activity, and cytoxicity on selected cancer cell lines.



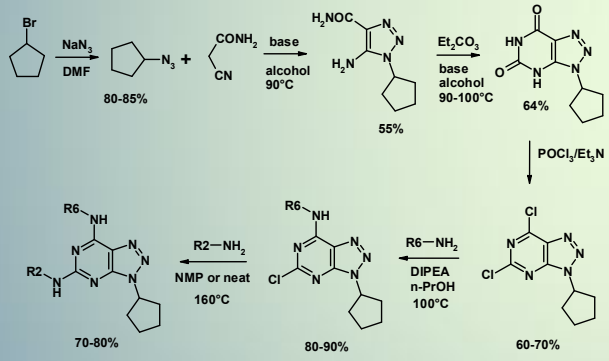
R2 = *trans*-4-aminocyclohexylamino
R6 = heterobiarylmethylamino or arylamino

Fig. 1. Structure of 2,6,9-trisubstituted 8-azapurines

Chemistry

The synthesis started from commercially available bromocyclopentane and sodium azide to give cyclopentyl azide, which was further reacted with cyanoacetamide *via* 1,3-dipolar cycloaddition, according to modified method reported in the literature [4,5]. The resulting 4-amino-5-carboxamido-1-cyclopentyl-1,2,3-triazole was then reacted with diethyl carbonate under strong basic conditions to 8-azaxanthine, which was further chlorinated with phenylphosphoryl chloride or phosphoryl chloride at presence of suitable base [4]. Thus obtained key intermediate - 2,6-dichloro-9-cylopentyl-8-azapurine - was undergone to aromatic nucleophilic substitution at C_6 position with appropriate aryl amine or heterobiarylmethyl amine, and then at C_2 position with various amines [4]. Among them, 1,4-*trans*-diaminocyclohexane, appeared to be the most suitable pharmacophore. Three key reaction steps: 1.st cyclization – formation of substituted 1,2,3-triazole ring, 2.nd cyclization – ring closure of pyrimidine moiety, and subsequent chlorination of substituted 8-azaxanthine with phosphoryl chloride, were optimized. We tried a usage of several type of bases, various temperatures and reaction times and the most successful procedure for both cyclization methods involves the usage of potassium tert. butoxide as a strong base in tert. butanol at 90 – 100 °C (pressure conditions – autoclave or glass presssure tube); reaction time 21 hours. The most effective method for the chlorination of 8-azaxanthine was boiling (105 °C) phosphoryl chloride (25 ekviv.) at presence of auxiliary base – triethylamine (2 ekviv.); reaction time 4 hours. Using this procedure we reached 70 % isolation yield of key intermediate: 2,6-dichloro-9-cyclopentyl-8-azapurine. The alternative chlorination agens was phenylphosphoryl chloride without presence of base.The main advantage of the synthesis is no need of chromatographic purification; a crystallization of raw intermediates including final products is usually sufficient. Moreover, the suggested synthetic protocol can be easily up-scaled to the semi-pilot or pilot plant production.

Scheme 1. Synthesis of 2,6,9-trisubstituted 8-azapurines



Tab. 1. Cyclization I (optimization study)

Base	Solvent	Temp. [°C]	Reaction time [hrs]	Yield (isol.) [%]
K_2CO_3	MeOH	68	21	0
MeONa	MeOH	68	21	0
MeONa	MeOH	90	21	8
EtONa	EtOH	90	21	39
t-BuOK	t-BuOH	90	21	55

Tab. 2. Cylization II (optimization study)

Base	EtONa					MeOK	t-BuOK
Ekv. base	4.6	3.0	4.5	5.0	5.0	5.0	5.0
Ekv. Et_2CO_3	1.5	2.0	2.0	2.0	3.0	3.0	3.0
Solvent	EtOH	EtOH	EtOH	EtOH	EtOH	MeOH	MeOH
Temp. [°C]	78	85-95	85-90	85-90	92	92	100
Reaction time [hrs]	4.5	4.5	21	21	21	21	21
Yield isol.[%]	0	0	35	44	35	0	64

Tab. 3. Biological activity of final compounds

Cpd	R2	R6	IC_{50} [μM] CDK2	Cytotoxicity K 562	MCF 7
I			1.1	2.2	2.9
II			1.5	2.5	4.8
III			12.6	14.1	26.1
IV			9.3	3.3	43.8
Rosco -vitine			0.17	45.5	12.3

Results

Novel prepared 8-azapurines I – IV are weak CDK2 inhibitors (IC_{50} in μmol values) in comparison with parent purine molecules (nanomolar activities) but some of them possess relatively strong cytotoxicity (>10 times more active than roscovitine), especially on leukemic cell lines. This fact could be explained by interactions of 8-azapurines with other molecular targets responsible for cell survival. The presence of nitrogen instead of carbon at C8 purine position probably cause an unfavourable interaction with CDK active site *via* hydrogen bond interactions due to lack of proton[1], but on the other hand, the advantage of nitrogen atom presence may be a blockage of potential metabolic oxidative deactivation pathway *via* xanthine-oxidase which is typical for purines[6].

Conclusions

- A series of novel 2,6,9-trisubstituted 8-azapurines were prepared and screened for CDK2 inhibitory activity and cytotoxicity on two selected cancer cell lines.
- An optimized and simple synthetic protocol for the construction of 8-azapurine scaffold with potential possibility of up-scaling is introduced.
- An optimized method of chlorination of N9-substituted 8-azaxanthines is performed.
- Final molecules are weak CDK2 inhibitors but some of them have relative strong cytotoxic activity.

References

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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ