Novel benzimidazole-derived PI3Kδ inhibitors as highly potent drugs candidates for SLE and other inflammatory and autoimmune diseases.

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INTRODUCTION

PI3K (Phosphoinositide 3-kinase) is the family of lipid kinases that participates in many key cellular processes like proliferation, growing up, migration, cytokines production, and apoptosis. These proteins are highly expressed in many human cells, such as leukocytes, which have implications for the body's immune response. They are involved in metabolism regulation, normal embryogenesis, or maintaining glucose homeostasis. Inhibition of PI3K (especially the first class which contains four subunits: α, β, γ, δ) is regarded to be very attractive as a potential mechanism for the treatment of many diseases like SLE (Systemic Lupus Erythematosus), MS (Multiple Sclerosis), IBD (Inflammatory Bowel Disease) and other inflammatory and autoimmune diseases [1-4]. In this work, we present a series of new, very potent, active, and selective small molecule inhibitors based on the structure of appropriately substituted 5-(2-difluoromethylobenzimidazo-1-yl)pyrazolo[1,5-*a*]pyrimidine [5] as highly potent drug candidates.

SYNTHESIS

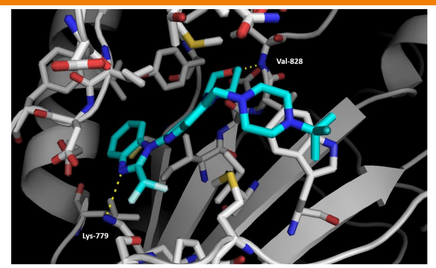


Figure 1. Example of 3D modeling binding mode of CPL302415 – binding with amino acids.

In our research, it was reported that modifications of benzimidazoles groups and amine subunits play a crucial role in the activity and selectivity of PI3Kδ inhibitors. We focused on the pyrazolo[1,5-*a*]pyrimidine core. It was investigated that there are two very important interactions. The first one is the binding between the oxygen atom of the morpholine ring and *Val-828*. The second bond is formed between the nitrogen atom at the third position of benzimidazole derivative (especially 2-(difluoromethyl)-1*H*-benzimidazole) and *Lys-779*. (*Fig.1*).

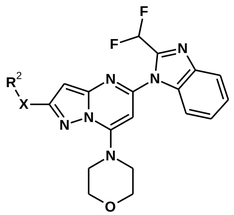
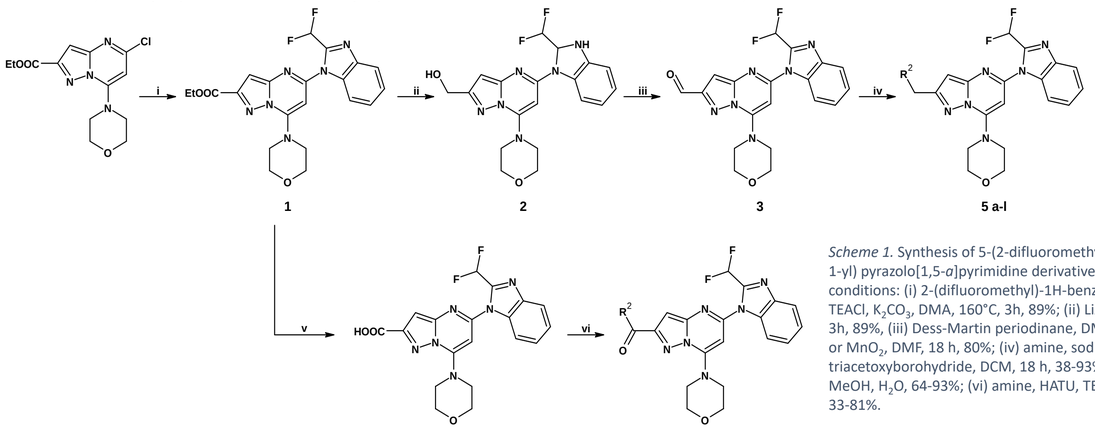


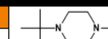
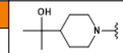
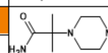
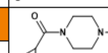
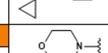
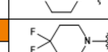
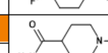
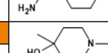
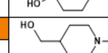
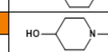
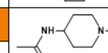
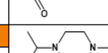
Figure 2. General structure of pyrazolo[1,5-*a*]pyrimidine derivatives.

A new family of substituted pyrazolo[1,5-*a*]pyrimidines (*Fig.2*) was prepared in multi-step synthesis included the hydrolysis reaction, oxygenation reaction, or reductive amination. Compounds **(5a – 5l)** were obtained after four-step synthesis from 5-chloro-7-(morpholin-4-yl)pyrazolo[1,5-*a*]pyrimidine-2-carboxylate**.** Compounds **(6a – 6l) (**replacement of the (-CH_2) group with a (-CO) group in position no.2 of pyrazolo[1,5-*a*]pyrimidine derivatives) were prepared after three-step synthesis from the same substrate (*Scheme 1*).



Scheme 1. Synthesis of 5-(2-difluoromethylobenzimidazo-1-yl) pyrazolo[1,5-*a*]pyrimidine derivatives. Reagents and conditions: (i) 2-(difluoromethyl)-1H-benzimidazole, TEACl, K_2CO_3, DMA, 160°C, 3h, 89%; (ii) $LiAlH_4$, THF, 0°C, 3h, 89%, (iii) Dess-Martin periodinane, DMF, RT, 2h, 95% or MnO_2, DMF, 18 h, 80%; (iv) amine, sodium triacetoxyborohydride, DCM, 18 h, 38-93%; (v) LiOH, MeOH, H_2O, 64-93%; (vi) amine, HATU, TEA, DCM, RT, 2 h, 33-81%.

RESULTS

Compound	R^2	X	IC_{50} PI3K$d\delta$ [nM]	IC_{50} PI3Kα [nM]	IC_{50} PI3Kγ [nM]	α/δ	γ/δ
5 a		CH_2	18	1 428	16 904	79	939
6 a		CO	84	12 481	48 777	148	580
5 b		CH_2	52	1 729	6 347	33	122
6 b		CO	101	4 863	2 483	48	25
5 c		CH_2	31	624	2 197	20	71
6 c		CO	74	n/a	3 593	n/a	52
5 d		CH_2	24	73	156	3.0	6.5
6 d		CO	63	n/a	3 831	0.0	61
5 e		CH_2	135	498	1 380	3.7	10
6 e		CO	226	2 385	8 536	11	38
5 f		CH_2	443	721	1 709	1.6	3.6
6 f		CO	801	4 561	35 750	5.7	45
5 g		CH_2	240	1 259	1 855	5.2	7.7
6 g		CO	314	3 127	2 793	10	8.9
5 h		CH_2	615	1 852	7 553	3.0	12
6 h		CO	213	5 408	1 818	25	8.5
5 i		CH_2	593	1 988	8 318	3.4	14
6 i		CO	90	2 354	1 782	26	20
5 j		CH_2	561	1 570	4 317	2.8	7.7
6 j		CO	195	797	2 081	4.1	10.7
5 k		CH_2	445	n/a	1 503	0.0	3.4
6 k		CO	141	1 476	1 689	10	12
5 l		CH_2	38	n/a	13 330	0.0	350
6 l		CO	768	n/a	11 397	n/a	15

The library of active compounds was obtained. It was observed that the most promising are piperazine- and piperidine- derivatives (position *R2*) (*Fig.2*). Compounds with satisfactory parameters were selected from the entire library. Nine final structures with an IC_{50} value of less than 100 nM were obtained (Tab.1). The most promising compound (**CPL302415**) is 1-{2-[(4-*tert*-butylpiperazin-1-yl)methyl]-7-(morpholin-4-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl} -2-(difluoromethyl)-1*H*-benzimidazole (*Fig.3*) with potent activity (IC50 = 18 nM), selectivity (PI3Kα/PI3Kδ = 79; PI3Kγ/PI3Kδ = 939) and promising others parameters (Tab.2.)

Solubility*	>500 µM	**PAMPA**	13.3 x 10^{-6} cm/s
MiceLM t $_{1/2}$	378 min	**PPB Human**	79 %
MiceLM CL	3.7 mL x min^{-1}x mg^{-1}	**PPB Monkey**	81 %
HumanLM t $_{1/2}$	145 min	**PPB Mice**	83 %
HumanLM CL	9.6 mL x min^{-1}x mg^{-1}	**PPB Rat**	82 %

Table 2. CPL302415 parametres, * KINETIC solubility pH 7.4.

Table 1. Obtained, selected PI3K inhibitors – pyrazolo[1,5-*a*]pyrimidine derivatives; n/a – data not available.

CONCLUSIONS

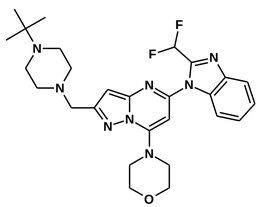


Figure 3. Structure of *CPL302415*.

In our work, we synthesized a library of very active benzimidazole derivatives (PI3Kδ IC_{50} = 0.02 – 0.80 µM). Many substituted pyrazolo[1,5-*a*]pyrimidines were prepared with different amine groups in multi-step synthesis. Then we developed the process of lead compound(s) synthesis to select the preclinical candidate for SLE. Molecular modeling studies explain the interaction of active structures in the PI3Kδ ATP binding site. Pyrazolo[1,5-*a*]pyrimidine with morpholine at position 7, 2-(difluoromethyl)benzimidazole at position 5 and N-*tert*-butylamine as amine proved to be the most potent structure (IC_{50} = 18 nm). Moreover, this structure shows good selectivity to other PI3K isoforms (PI3Kα/PI3Kδ = 79; PI3Kγ/PI3Kδ = 939) and promising physical properties. That lead compound (**CPL302415**) (*Fig.3*) is subjected to further biological, toxicological, and pharmacokinetic studies.

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[5] – EP3277687B1; "7-(Morpholin-4-yl)pyrazole[1,5-*a*]pyrimidine derivatives which are useful for the treatment of immune or inflammatory diseases or cancer".

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