Potent and selective indol-4-yl pyrazolo[1,5-*a*]pyrimidine-derived PI3K*δ* inhibitors as potential candidates for treatment of COPD and asthma.

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INTRODUCTION

Asthma is a long-term inflammatory disorder affecting children and adults. More than 300 milion people suffer from it worldwide. Although corticosteroids are the mainstay of asthma treatment, they possess severe side-effects. Due to considerable differences in response to available treatment options in the asthmatic population, which includes patients suffering from steroid resistant asthma, novel therapies are needed [1].
Phosphoinositide 3-kinase *δ* (PI3K*δ*), the member of the class I PI3K family, is very important signaling molecule which regulates differentiation, proliferation, migration and survival of immune cells [2,3]. Different PI3K isoforms regulate distinct cellular events, enabling the therapeutic opportunities treatment inflammatory and autoimmune diseases including asthma or COPD [4,6]. PI3K*δ*, an attractive target for asthma treatment in patients suffering from asthma resistant to currently available therapies. However, many known PI3K inhibitors have adverse effects [6]. This drives sciencists to search for safer compounds.

SYNTHESIS

We conducted nine step synthesis starting from simple reagents like benzyl alcohol and ethyl-bromoacetate. Further steps led to obtaining amino-pyrazole ring, then condensing it with diethyl malonate to pyrazolo-[1,5-a]pyrimidine ring. After chlorination, the substitution with morpholine occurs selectively on chlorine atom in position 7. Chlorine from position 5 was substitued in Suzuki reaction. Subsequent steps were: removal of benzyl protecting group and oxidatiing alcohol to aldehyde which was substrate for final compounds obtained in reductive amination reaction.

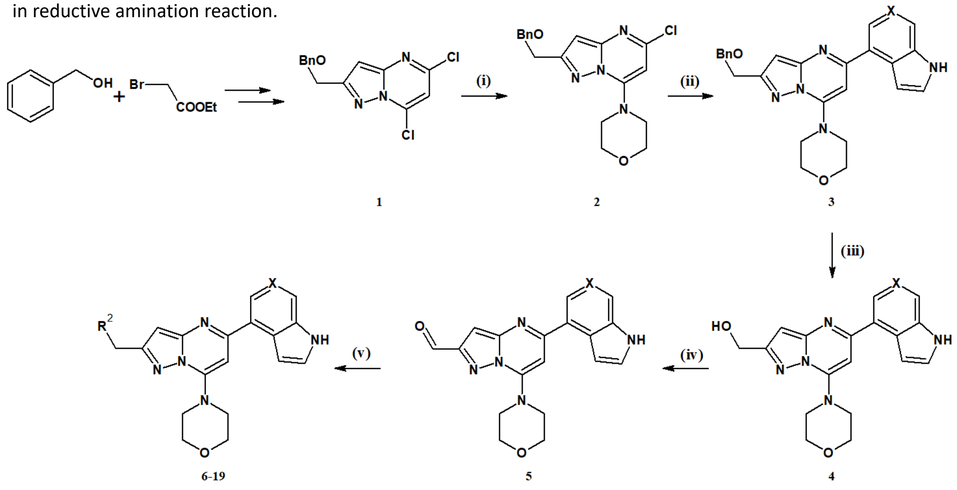


Figure 1. Synthesis of 5-(indol-4-yl)pyrazolo[1,5-*a*]pyrimidine derivatives. (i) morpholine, K_2CO_3, acetone, RT, 1.5 h, 92%; (ii) X=C:indole-4-boronic acid pinacol ester/ X=N: 6-azaindole, tetrakis(triphenylphosphino)palladium(0), 2M aq Na_2CO_3, DME, reflux, 16h, 83%; (iii) H_2, 10%Pd/C, DMF/EtOH, 60°C, 24 h, 66%; (iv) Dess-Martin reagent, DMF, RT, 1h, 78%; (v) amine, sodium triacetoxyborohydride, DCM, RT, 2h, 25-93%.

RESULTS

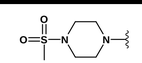
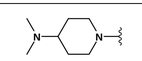
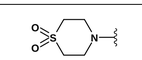
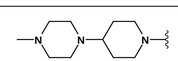
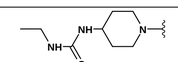
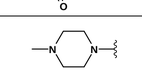
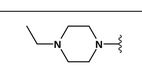
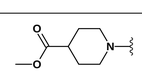
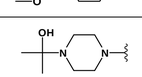
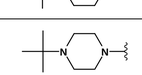
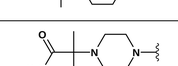
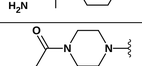
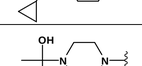
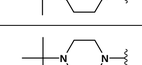
Compound	R^1	R^2	IC50 PI3Kδ [nM]	IC50 PI3Kα [nM]	α/δ
6			402	2 351	5.8
7			37	6 380	172
8			266	8 650	33
9			52	15 630	301
10			360	14 200	39
11			193	42 400	220
12			43	34 300	798
13			138	8 460	61
14			13	15 820	1 217
15			6.6	12 470	1 889
16			41	17 740	433
17			22	1 270	58
18			28	3 650	130
19			2.8	2 670	954

Table 1. IC_{50} values of synthesized compounds. IC_{50} values were determined as mean based on two independent experiments.

We have synthesized new indole and 6-azaindole derivatives. They were tested for activity against PI3K*δ* and PI3K*α* to compare their selectivity (Table 1). Compounds **15** and **19** differing only by nitrogen atom in position 6 of indole ring turned out to be most promising. Their activity against *β* and *γ* isoform was measured additionary to confirm their selectivity. The compunds **15** and **19** showed strong inhibitory activity in B cell proliferation assay (20nM, 19nM respectively). The results of the metabolic stability studies in murine and human microsomes and the kinetic solubility of compounds **15** and **19** are shown in Table 2. Compound **19** has better kinetic solubility and metabolic stability in microsomes than compound **15**.

Compound	IC_{50} PI3Kδ [nM]	Selectivity α/δ	Selectivity β/δ	Selectivity γ/δ	IC_{50} CD19 [nM]	Solubility* [μM]	MLM $t_{1/2}$ [min]	MLM Cl [ml x min^{-1} x mg^{-1}]	HLM $t_{1/2}$ [min]	HLM Cl [ml x min^{-1} x mg^{-1}]
15	6.6	1 889	829	> 9 091	20	444	126	13.7	76	22.8
19	2.8	954	7 714	12 286	19	> 500	198	7.0	370	3.7

Table 2. Selected properties of compounds 15 and 19. *Kinetic solubility at pH 7.4 buffer.

CONCLUSIONS

We developed new small molecule inhibitors based on indol-4-yl pyrazolo[1,5-a]pyrimidine derivatives with IC_{50} values in the low nanomolar range (2.8 - 402nM) and with high selectivity against PI3K*δ*. The most potent compound (**19**) with good metabolic stability was chosen as candidate for pre-clinical studies as dry powder inhalation in asthma treatment [7].

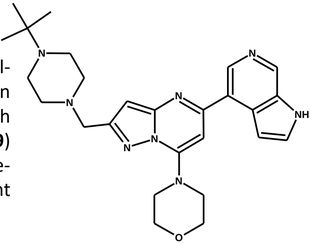


Figure 2. Compound **19**

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