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Synthesis and evaluation of PI3Kγ enzyme inhibitory activity of Novel (1H-pyrazol-4-yl)methanamines

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ABSTRACT
A series of (1H-pyrazol-4-yl)methanamines have been synthesized and then were evaluated for the PI3Kγ enzyme inhibitory potential. Minor modification of the initially synthesized molecules offered significant improvement of the inhibitory potential from 36% to 73%. Some selected compounds were then subjected to the *in silico* study for understanding the most likely interactions to aid the future researches focusing on discovering novel inhibitors against the PI3Kγ enzyme.

Introduction

PI3Kγ isozymes constitute the only class IB PI3K, which is activated by G-protein coupled receptors thereby being involved in a lot of cellular signaling processes. They are predominantly expressed in hematopoietic cells and have been studied intensively in the context of immune-mediated diseases [1-2]. These isozymes are also involved in the production of reactive oxygen species by neutrophils [3] and play a non-redundant function in neutrophil, monocyte/macrophage, and T cell chemotaxis in vitro and in vivo [4-5]. PI3Kγ isozymes mediate adenosine-induced degranulation in systemic anaphylaxis [6]. PI3Kγ deficiency and PI3Kγ inhibitors offer protection in preclinical models of lupus, inflammatory arthritis and multiple sclerosis [7-9].

Being highly expressed in cardiac myocytes PI3Kγ enzymes play important roles in cardiac physiology, both as a kinase and as an adapter regulating cAMP-mediated signaling cascades [10-13]. These preferential signaling through these class IB PI3K isoforms in leucocytes has prompted considerable pharmaceutical interests [14-15]. Accordingly we have been encouraged to run extensive researches aiming to discover new inhibitors against this PI3Kγ isozyme. Already we have published the inhibition of PI3K gamma enzyme by novel phenylpyrazoles [16]. From the observation from those new phenylpyrazoles, we have been interested to synthesize and evaluate (1H-pyrazol-4-yl)methanamines for the PI3Kγ inhibitory potency and the observations have been detailed in this report.

Materials and methods

General

All the chemical and reagents used in this project were

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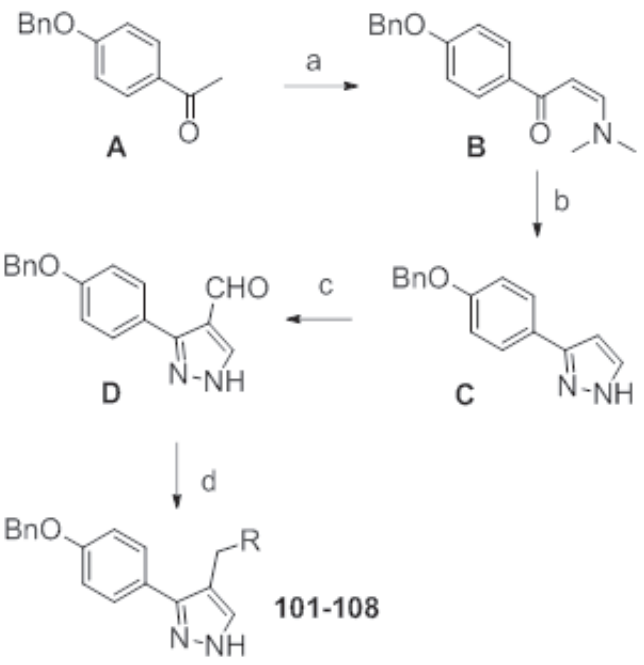
collected from suppliers like, Sigma-Aldrich, TCI, Fluka, Alfa Aesar, Dae Jung, etc. The 1H NMR spectra were recorded in $CDCl_3$, CD_3OD or Acetone-*d6* using TMS as internal standard with Varian 300 MHz high resolution NMR spectrometer. Multiplicities were abbreviated as follows: singlet (s), doublet (d), triplet (t), multiplet (m), and broad (br). The reactions were monitored by using the TLC Silica Gel 60 F254 glass plates collected from MERK, Germany.

Chemistry

General procedure for the synthesis of 3-(4-(benzyloxy) phenyl)-^{1}H-pyrazole-4-carbaldehyde (**D**). The reported compounds were prepared by following reported methods [17-20] with minor variations. The overall steps undertaken have been shown in scheme 1. The commercially available 4-benzyloxybenzophenone, **A**, was treated with DMF-DMA in toluene under refluxing condition for 16 h to get compound **B**, which on further treatment with hydrazine and then formylation by $POCl_3$ and DMF gave the compound **D**.



Reagents and conditions: a. N,N-dimethylformamaide dimethyl acetal (DMF-DMA), Toluene, reflux, 16 h; b. Hydrazine.H_2O, EtOH, 70^{o}C, 2 h; c. $POCl_3$, DMF, RT, 12 h; d. Respective amine, $NaBH(OAc)_3$, AcOH, Dichloromethane, RT, 8 h.

Scheme 1. Protocol for synthesis of compounds **101-108**

General procedure for the synthesis of compounds ***101-108***. To the solution of 3-(4-(benzyloxy)phenyl)-1H-pyrazole-4-carbaldehyde (556 mg, 2 mmol) and amine (2.6 mmol) in 1,2-dichloroethane (3 ml) were added sodium triacetoxyborohydride (665 mg, 3 mmol) and acetic acid (2 mmol) and the resulting mixture was stirred at room temperature for 8 hours. At the end of the reaction, ethylacetate:water extraction followed by silica gel flash column chromatography using Hexane and 20-80% of Ethylacetate as the eluent gave the products ***101-108*** in 57-71% yield.

4-((3-(4-(benzyloxy)phenyl)-1H-pyrazol-4-yl)methyl) morpholine **(101)**: 1H NMR (CDCl3, 300 MHz) δ 2.46 (br s, 4 H), 3.44 (br s, 2 H), 3.69 (br s, 4 H), 5.08 (s, 2 H), 6.99 (d, J = 8.4 Hz, 2 H), 7.33 – 7.49 (m, 6 H), 7.64 (d, J = 8.4 Hz, 2 H). Yield: 63%

1-((3-(4-(benzyloxy)phenyl)-1H-pyrazol-4-yl)methyl) piperazine **(102)**: 1H NMR (CDCl3, 300 MHz) δ 2.77 (br s, 4 H), 3.31 (br s, 4 H), 3.52 (br s, 2 H), 5.15 (s, 2 H), 7.09 (m, 2 H), 7.31 – 7.51 (m, 5 H), 7.64 (s, 1 H), 7.83 (m, 2 H). Yield: 59%

4-((3-(4-(benzyloxy)phenyl)-1H-pyrazol-4-yl)methyl)-N-methyl piperazine-1-carboxamide **(103)**: 1H NMR (CD3OD, 300 MHz) δ 2.75 (br s, 4 H), 3.00 (s, 3 H), 3.90 (m, 6 H), 5.13 (s, 2 H), 7.10 (d, J = 8.7 Hz, 2 H), 7.30 – 7.45 (m, 5 H), 7.56 (m, 2 H), 7.72 (s, 1 H). Yield: 70%

Methyl-2-((3-(4-(benzyloxy)phenyl)-1H-pyrazol-4-yl) methylamino)acetate **(104)**: 1H NMR (Acetone-d6, 300 MHz) δ 3.43 (s, 2 H), 3.66 (s, 3 H), 3.77 (s, 2 H), 5.17 (s, 2 H), 7.08 (m, 2 H), 7.31–7.43 (m, 3 H), 7.49 (m, 2 H), 7.01 (s, 1H), 7.84 (m, 2 H). Yield: 71%

N-((3-(4-(benzyloxy)phenyl)-1H-pyrazol-4-yl)methyl)-3-nitrobenzenamine **(105)**: 1H NMR (CDCl3, 300 MHz) δ 4.19 (br s, 1 H), 4.29 (s, 2 H), 5.09 (s, 2 H), 7.01 (m, 2 H), 7.24–7.59 (m, 11 H). Yield: 62%

N-((3-(4-(benzyloxy)phenyl)-1H-pyrazol-4-yl)methyl)-4-nitrobenzenamine **(106)**: 1H NMR (CDCl3, 300 MHz) δ 4.44 (m, 2 H), 5.16 (s, 2 H), 6.57 (br s, 1 H), 6.71 (m, 2 H), 7.00 (m, 2 H), 7.32 – 7.42 (m, 3 H), 7.49 (m, 2 H), 7.62 (m, 2 H), 7.12 s, 1 H), 8.04 (m, 2 H). Yield: 57%

N-((3-(4-(benzyloxy)phenyl)-1H-pyrazol-4-yl)methyl) pyridin-3-amine **(107)**: 1H NMR (CDCl3, 300 MHz) δ 4.27 (s, 2 H), 5.09 (s, 2 H), 6.88 (m, 1 H), 7.09 (m, 3 H), 7.29 - 7.53 (m, 6 H), 7.63 (s, 1 H), 8.00 (m, 1 H), 8.06 (m, 1 H). Yield: 72%

Ethyl3-((3-(4-(benzyloxy)phenyl)-1H-pyrazol-4-yl) methylamino)benzoate **(108)**: 1H NMR (CDCl3, 300 MHz) δ 2.82 (m, 5 H), 4.31 (s, 2 H), 5.16 (s, 2 H), 6.95 (m, 1 H), 7.08 (d, J = 8.4 Hz, 2 H), 7.20 – 7.42 (m, 6 H), 7.49 (m, 2 H), 7.67 (m, 3 H). Yield: 69%

Evaluation of the PI3Kγ inhibitory property

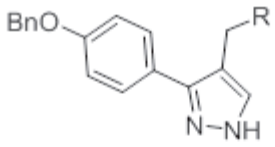
The synthesized compounds were evaluated for the inhibitory potency against the PI3Kγ isozyme according to the 'Millipore protocol for the PI3 Kinase Activity Assay' using 10 µmole dose of the ATP in vitro. The observations

have been mentioned in table 1.

Enzyme assay results were further evaluated by adopting the molecular modeling technology where compounds **101**, **102** and **108** were docked in the receptor site of PI3Kγ isozyme by using Autodock vina [21] software. The necessary enzyme pdb file, 2CHW, was collected from online source and then was used for this docking study.

Results and Discussion

Initially we have synthesized compound **101** and **102** and then evaluated those for the PI3Kγ isozyme inhibitory property. At 10 micromolar concentration both of them showed similar inhibitory potential (36% and 37% inhibition, respectively) thereby indicating a pharmacophoric characteristics (Table 1). Thus we have been interested to synthesize more compounds afterwards. In case of compound **103**, the inhibitory potential was increased to 52%. The similar observation (54% inhibition) was found from compound **104**. Both the compounds were having relatively longer terminal polar groups, urea and ester functionalities, respectively.



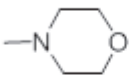
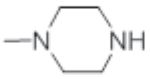
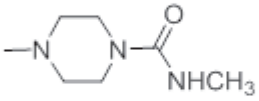
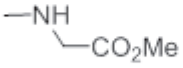
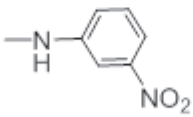
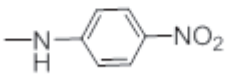
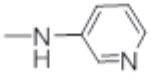
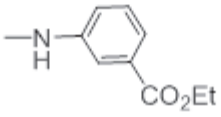
Compound	R	% inhibition (at 10 micromole)
101		36
102		37
103		52
104		54
105		53
106		61
107		51
108		73

Table 1. Percentage inhibition against PI3 kinase gamma isozymes as shown by the (1H-pyrazol-4-yl) methanamines

From the above observations we became interested to introduce aromatic ring with polar substituents to increase the length of the compound and accordingly synthesized compounds **105**, **106** and **107**. Though the nitrobenzene group showed slight increase, the heterocyclic pyridine ring did not show any improvement (51%). Thus it appeared that the polar group should be slightly distally located. Thus we have synthesized compound **108** and while testing this compound the activity was significantly increased (73% inhibition against the PI3Kγ isozyme).

Thus all of the synthesized (1H-pyrazol-4-yl)methanamine derivatives were showing moderate to good levels of inhibitory potential against the PI3Kγ isozyme in this enzyme based assay.

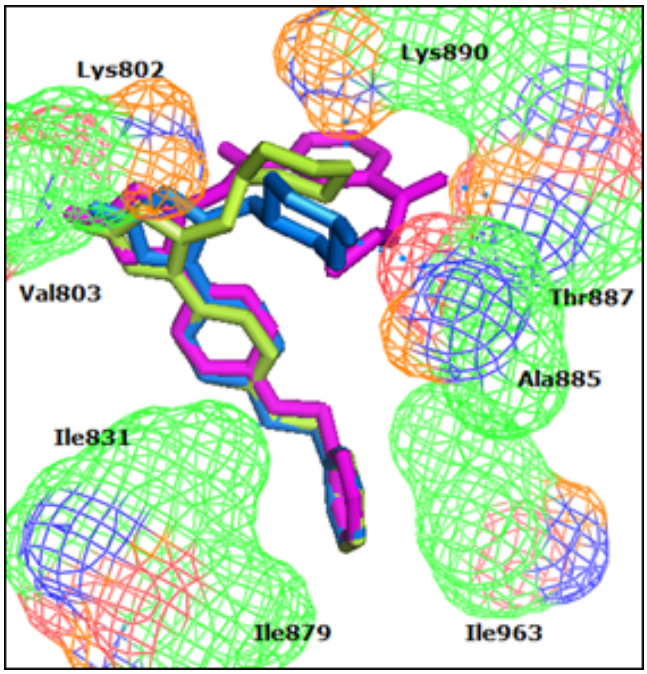


Figure 1 Orientation of compounds **101** (green), **102** (cyan) and **108** (magenta) as observed from the in silico study. Polar interactions have been shown by cyan dotted lines.

For searching the binding modes, compounds **101**, **102** and **108** compounds were docked individually and the highest affinity binding modes were superimposed to compare their binding patterns (Figures 1). All the compound were aligned ensuring similar access to the non-polar space surrounded by three isoleucine residues, Ile963, Ile879 and Ile831. At the same time they had more or less similar access to the backbone CONH of Lys802 and Val803 by their pyrrole moieties. But the differences were in the positioning of their aminosubstituents. Compound **101**, colored by green, was approaching to the side-chain of Lys890. Compound **102**, colored by cyan, by acting as the hydrogen bond donor, approached towards the carbonyl oxygen of Ala885 backbone. But while considering **108**, colored by magenta, with the relatively distal carbonyl oxygen on the ester functionality, approached towards Thr887 backbone NH group. These variations in the alignments may appear as the cause of the relatively higher binding potential of **108.**

Conclusions

Consistent PI3Kγ inhibitory potential was observed from the enzyme assay of these molecules in our study. Thus (1H-pyrazol-4-yl)methanamine moiety appears as an interesting pharmacophore having the potential to be exploited for development of novel PI3Kγ inhibitory scaffold. This is ongoing in our laboratory and will be reported in time.

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