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Pyrazole substituted resorcinol derivatives with PI3Kγ inhibitory potential

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ABSTRACT

Phosphoinositide 3-kinase gamma (PI3Kγ) enzymes play significant roles in inflammatory cell recruitment to tumors and accordingly a lot of studies have targeted development of small molecule inhibitors against these enzymes for managing various chronic inflammatory disorders. In this study, a number of pyrazole substituted resorcinol derivatives have been synthesized in the laboratory and then were evaluated for the inhibitory potential against the gamma isozyme. Highest inhibitory potential was observed from the introduction of a non-polar phenyl or methyl benzyl substitution (66.4% and 59.5%) on the OH group of the resorcinol. Addition of relatively polar moiety resulted in decrease in the inhibitory potential and lowest inhibition was observed from 4-pyridyl methyl and 2-morpholino ethyl moieties (23.6% and 24.5% inhibition respectively). The results were encouraging due to remarkable inhibition showed by these compounds against the PI3K enzyme. Thus, the scaffold appears as interesting pharmacophore suitable for further development.

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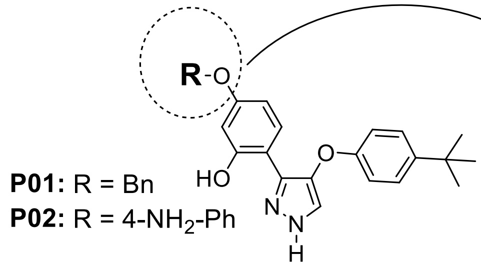
1 INTRODUCTION

The phosphoinositide 3-kinases (PI3Ks) are the enzymes from the family of lipid kinases. These enzymes play important roles in intracellular signaling of diverse tyrosine kinase receptors and G-protein–coupled receptors (Rommel *et al.*, 2007; Vanhaesebroeck *et al.*, 2010; Okkenhaug, 2013 and Winkler *et al.*, 2013). Various biological roles of these isoforms have been thought in a variety of inflammatory processes and hematologic malignancies. Accordingly, selective inhibition of these kinases has been developed targeting inflammatory disorders. Evidence suggests that the PI3Kγ isoform especially plays important roles in inflammatory cell recruitment to tumors or tumor inflammation. These types of activities are conducive to angiogenesis, tumor growth, and localized immunosuppression (Schmid *et al.*, 2011 and Joshi *et al.*, 2014). Small-molecule inhibitors of PI3Kγ isoforms have been proved to suppress the inflammatory process and the associated tumor growth (Schmid *et al.*, 2011 and Joshi *et al.*, 2014).

The importance of PI3Kγ isozyme in the chronic pathological conditions in the human life led to the development of suitable small molecule inhibitors of this enzyme in laboratory. Accordingly, the scaffolds (**P01** and **P02**) have been reported (Sukumar *et al.*, 2013 and Sukumar *et al.*, 2016) to possess the PI3Kγ isozyme inhibitory activity.



The observed activity of these **P01** and **P02** led to subsequent structure-activity-relationship (SAR) study of the scaffold by varying the 'R' group by introducing additional groups (**Ar**) and the observations have been reported here.

2 MATERIAL AND METHODS

2.1 Chemicals

All of the necessary chemicals, reagents and catalysts were purchased from either Sigma-Aldrich (USA) or TCI (Japan) depending upon availability. Various necessary acids, bases and solvents were collected from Duksan Pure Chemicals Co. Ltd. or Daejung Chemicals, Aldrich Chemical Co., or Sigma-Aldrich Ltd. The various gases like, argon, nitrogen and hydrogen were supplied by Daesung Industrial gases.

2.2 Equipment

Usual reactions have been run by the magnetic stirrers from Heidolph, Corning, or Radleys Discovery Technologies. Buchi rotary evaporator system, Julabo Cooling system and Edwards vacuum pump were used for evaporation and drying purposes. For monitoring the reactions, the MERK KGaA 60 F_{254} silica gel plates were used, whereas and flash column chromatography by silica gels (particle size: 38-75 μm) collected from MERK.

2.3 Characterization

The ^{1}H proton NMR spectra were recorded from Varian 300 MHz or Bruker 300 MHz NMR spectrometer using $CDCl_3$ as solvents and TMS as internal standard. Multiplicities were abbreviated as follows: singlet (s), doublet (d), triplet (t), multiplet (m), and broad (br).

Experimental

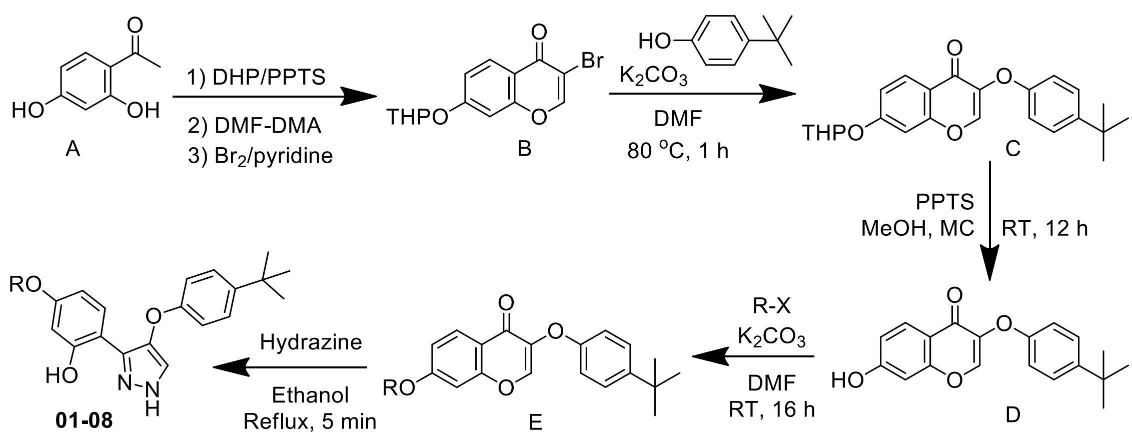


Fig. 1: Synthesis of various pyrazole substituted resorcinol derivatives

For synthesizing (Fig.1) the O-protected bromo derivative 4H-chromen-4-one (**B**), 1-(2,4-dihydroxyphenyl)ethanone (**A**) was treated first with 3,4-dihydro-2*H*-pyran (DHP) and pyridinium *p*-toluene-4-sulphonate (PPTS), then DMF-DMA and then Bromine and Pyridine. On treatment with the 4-tertiary butyl phenol, this bromo chromen offered the phenoxy derivative, **C**, which on subsequent O-deprotection and treatment with various aromatic halides gave the intermediate chromen, **E**. In the final stage, the phenoxy chromen was treated with hydrazine to get the desired various Pyrazole substituted Resorcinol derivatives, **01-08**.

2.4 Synthesis of 3-bromo-7-((tetrahydro-2*H*-pyran-2-yl)oxy)-4*H*-chromen-4-one (B)

For synthesizing the chromen-4-ones published methods (Manna *et al.,* 2005; Cardenas *et al.,* 2006 and Bodendiek *et al.,* 2009) were applied with minor modifications. A solution of 3,4-dihydro-2*H*-pyran (DHP) (32.8 mmol) in CH_2Cl_2 (30 mL) was added drop-wise to a solution of 1-(2,4-dihydroxyphenyl)ethanone (**A**) (11 mmol) and pyridinium *p*-toluene-4-sulphonate (PPTS) (98 mg) at rt and then were stirred for 4 hours. After subsequent addition of saturated $NaHCO_3$ solution the mixture was extracted with ethyl acetate, dried with $MgSO_4$, filtered and concentrated under reduced pressure. After dilution with hexane and addition of *N,N*-dimethylformamide dimethyl acetal (16.5 mmol), the resulting mixture was refluxed for 3 hours and then was subjected to evaporation of volatiles. The resultant solid was dissolved in $CHCl_3$ (30 mL) and successively treated with pyridine (11 mmol) and Br_2 (22 mmol) for 12 hours. Saturated aqueous $Na_2S_2O_3$ solution was then added and stirring continued for 30 min. The mixture was then extracted with ethyl acetate, dried with $MgSO_4$, filtered and concentrated to get the crude product which was purified by flash column chromatography using hexane: ethyl acetate system to get the desired 3-bromo-7-((tetrahydro-2*H*-pyran-2-yl)oxy)-4*H*-chromen-4-one **(B)**, 87% yield.

2.5 Synthesis of 3-(4-(*tert*-butyl)phenoxy)-7-((tetrahydro-2*H*-pyran-2-yl)oxy)-4*H*-chromen-4-one (C)

Compound **C** was synthesized by following the reported method (Bradbury *et al.,* 2004) with minor modifications. A mixture of 4-*tert*-butylphenol (1.0 mmol), 3-bromo-7-((tetrahydro-2*H*-pyran-2-yl)oxy)-4*H*-chromen-4-one (**B**) (1.0 mmol) and potassium carbonate (2.0 mmol) in *N,N*- dimethylformamide was stirred at 80 °c for 1 hour. After cooling to room temperature, aqueous ammonium chloride solution was added, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The resulting solid was collected by filtration, washed with dichloromethane, and dried *in vacuo* to get the 3-(4-(*tert*-butyl)phenoxy)-7-((tetrahydro-2*H*-pyran-2-yl)oxy)-4*H*-chromen-4-one (**C**), 76% yield.

2.6 Synthesis of 3-(4-(*tert*-butyl)phenoxy)-7-hydroxy-4*H*-chromen-4-one (D)

THP de-protection was done by following the reported (Baroudi *et al.*, 2010) method. The mixture of 3-(4-(*tert*-butyl)phenoxy)-7-((tetrahydro-2*H*-pyran-2-yl)oxy)-4*H*-chromen-4-one (**C**) (1.22 mmol) and pyridinium *p*-toluene-4-sulphonate (PPTS) (0.12 mmol) in 15 mL of dichloromethane/MeOH (50:50) was stirred at room temperature for 12 hours. After subsequent evaporation of solvent under reduced pressure, the reaction mixture was diluted with dichloromethane and then washed with water and brine and water again. The organic layer was dried by sodium sulphate, filtered and concentrated under reduced pressure to get the crude product which was then purified by flash column chromatography using increasing polarity gradients of hexane:ethyl acetate system to get the desired 3-(4-(*tert*-butyl)phenoxy)-7-hydroxy-4*H*-chromen-4-one (**D**), 79 % yield.

2.7 General procedure for synthesizing E by coupling 3-(4-(*tert*-butyl)phenoxy)-7-hydroxy-4*H*-chromen-4-one (C) with various aromatic halides

A mixture (Bradbury *et al.*, 2004) of 3-(4-(*tert*-butyl)phenoxy)-7-hydroxy-4*H*-chromen-4-one (**D**) (1.0 mmol), desired aromatic halide (1.0 mmol) and potassium carbonate (2.0 mmol) in *N,N*-dimethylformamide was stirred at RT for 12 hours. At the end of the reaction, aqueous ammonium chloride solution was added and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The resulting solid was collected by filtration, and dried *in vacuo* to get the crude product (**E**), which was then purified by flash column chromatography using increasing polarity gradients of hexane and ethyl acetate (74-86% yield).

2.8 General procedure for synthesizing pyrazole substituted resorcinol derivatives (01-08)

To the solutions of various **E** (2.3 mmol) in ethanol (Marie-Claude *et al.*, 1991), hydrazine hydrate (2.5

mmol) dissolved in ethanol was added slowly. After completion of addition, the mixture was refluxed for 5-10 minutes. The solution became clear and the solid precipitated out was collected by filtration, washed with ethanol and dried to get the expected
3-(4-(benzyloxy)phenyl)-4-(4-*tert*-butylbenzyl)-
-1*H*-pyrazole (**01-08**) (78-93% yield).

2.9 Spectral data of the synthesized compounds

2-(4-(4-*tert*-Butylphenoxy)-1*H*-pyrazol-3-yl)-5-(4-methoxybenzyl-oxy)phenol (01): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.29 (s, 9 H), 4.95 (s, 2 H), 6.46 (m, 1 H), 6.64 (s, 1H), 6.90 (m, 4H), 7.34 (m, 5 H), 7.85 (d, *J* = 8.7 Hz, 1 H), 10.86 (s, 1H)

2-(4-(4-*tert*-Butylphenoxy)-1*H*-pyrazol-3-yl)-5-(pyridin-4-ylmethoxy)phenol (02): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30 (s, 9 H), 5.08 (s, 2 H), 6.46 (m, 1 H), 6.60 (s, 1 H), 6.98 (m, 2 H), 7.33 (m, 4 H), 7.43 (s, 1 H), 7.88 (m, 1 H), 8.59 (s, 1 H), 10.88 (s, 1 H)

2-(4-(4-*tert*-Butylphenoxy)-1*H*-pyrazol-3-yl)-5-(3-methylbenzyloxy)phenol (03): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.28 (s, 9 H), 2.33 (s, 3 H), 4.98 (s, 2 H), 6.47 (m, 1 H), 6.66 (s, 1 H), 7.09-7.32 (m, 6 H), 7.85 (d, *J* = 8.7 Hz, 1 H), 10.13 (br s, 1 H), 10.99 (br s, 1 H)

2-(4-(4-*tert*-Butylphenoxy)-1*H*-pyrazol-3-yl)-5-(2-morpholinoethoxy)phenol (04): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30 (s, 9 H), 2.57 (m, 4 H), 2.79 (m, 2 H), 3.73 (m, 4 H), 4.12 (m, 2 H), 6.40 (m, 1 H), 6.56 (s, 1 H), 6.98 (d, *J* = 8.7 Hz, 2 H), 7.30 (m, 2 H), 7.42 (s, 1 H), 7.85 (d, *J* = 8.7 Hz, 1 H), 10.82 (br s, 1 H)

2-(4-(4-*tert*-Butylphenoxy)-1*H*-pyrazol-3-yl)-5-phenoxyphenol (05): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30 (s, 9 H), 6.50 (m, 1 H), 6.64 (m, 1 H), 6.95-7.12 (m, 5 H), 7.29-7.35 (m, 4 H), 7.43 (s, 1 H), 7.90 (d, *J* = 8.7 Hz, 1 H), 9.88 (br s, 1 H), 10.87 (br s, 1 H).

2-(4-(4-*tert*-Butylphenoxy)-1*H*-pyrazol-3-yl)-5-(4-nitrophenoxy)phenol (06): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.31 (s, 9 H), 6.58 (m, 1 H), 6.75 (m, 1 H), 6.90-6.93 (m, 2 H), 6.99-7.08 (m, 3 H), 7.33-7.36 (m, 2 H), 7.47 (s, 1 H), 8.03 (d, *J* = 7.8 Hz, 1 H), 8.16-8.21 (m, 2 H)

2-(4-(4-*tert*-Butylphenoxy)-1*H*-pyrazol-3-yl)-5-(2-nitrophenoxy)phenol (07): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30 (s, 9 H), 6.53 (m, 1 H), 6.67 (m, 1 H), 6.96-6.99 (m, 2 H), 7.11 (d, *J* = 8.1 Hz, 1 H), 7.20 (d, *J* = 8.1 Hz, 1 H), 7.31-7.34 (m, 2 H), 7.48-7.53 (m, 2 H), 7.94-7.98 (m, 2 H), 10.01 (br s, 1 H), 11.02 (br s, 1 H)

***N*-(4-(4-(4-(4-*tert*-Butylphenoxy)-1*H*-pyrazol-3-yl)-3-hydroxyphenoxy)phenyl)-*N*-(methyl sulfonyl)methanesulfonamide (08):** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30 (s, 9 H), 3.39 (s, 6 H), 6.54 (d, *J* = 8.7 Hz, 1 H), 6.70 (s, 1 H), 6.98 (d, *J* = 8.4 Hz, 2 H), 7.05 (d, *J* = 8.4 Hz, 2 H), 7.26-7.34 (m, 4 H), 7.44 (s, 1 H), 7.98 (d, *J* = 8.4 Hz, 1 H), 11.02 (br s, 1 H)

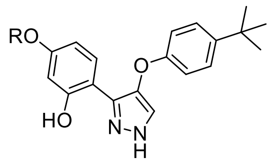
2.10 Observation of the *in vitro* inhibitory activity against the PI3Kγ isozyme

The synthesized resorcinol derivatives were evaluated for the inhibitory potency against the PI3Kγ isozyme. This biological evaluation was done from Millipore England according to their protocol through application of 10 micro molar doses *in vitro*.

3 RESULTS AND DISCUSSION

While observing the inhibitory potential after making benzyl substitution on the remote OH group of the resorcinol moiety as shown in Fig.2 (compounds **01**-**03**), the non-polar group (**01** and **03**) was found to show more potent inhibitory potency against the PI3Kγ isozyme. The comparatively polar pyridine moiety of **02** was remarkably less potent with only 23.6% inhibition. Even the methyl substitution offered higher potency (compound **03**) compared to the methoxy substitution (compound **01**). The observation was further justified by the introduction of more polar morpholine moiety (compound **04**) where the inhibitory potency was reduced by approximately 60% while compared to methyl benzyl moiety (**03**).

Observation was made by introduction of just the phenyl group (compound **05**) in place of benzylic moiety. This change resulted in further higher inhibition (66.4%) as shown in Fig.2. In the further study, introduction of nitro group on the phenyl ring was tried. Though the inhibitory potential was reduced remarkably (compound **06** and **07**), the para substitution was found to show greater reduction in the potency than the ortho substitution. This may have been linked to the unavailability of sufficient space in the binding site. Similar observation was also found by introduction of the dimethanesulfonamide substitution at the para position of the phenyl moiety (compound **08.** Fig.2).



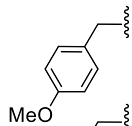
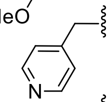
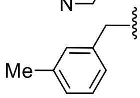
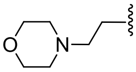
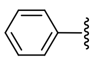
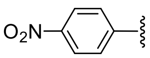
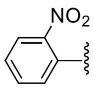
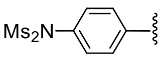
Sample No.	R	% inhibition (@ 10 micromolar)
01		50.3
02		23.6
03		59.5
04		24.5
05		66.4
06		34.2
07		47.1
08		42.9

Fig. 2: Percentage of inhibition against PI3Kγ isozymes as shown by the pyrazole substituted resorcinol derivatives

4 CONCLUSION

The synthesized pyrazole substituted resorcinol derivatives have been found to show promising inhibitory potential against the PI3Kγ isozymes. There was interesting correlation of the structure change and the activity change. This can be considered for further structure-activity-relationship study targeting the development of small molecule as the PI3Kγ inhibitor.

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