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In-vivo effective Dibenzo[b,d]furan-1-yl-thiazoles as Novel PDE-4 Inhibitors

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A B S T R A C T

Herein we report the synthesis, PDE-4B and TNF-α inhibitory activities of a few Dibenzo[b,d]furan-1-yl-thiazole derivatives. The hydroxycyclohexanol amide derivatives **14**, **18**, **24**, **29**, **31** and **33** exhibited promising *invitro* PDE-4B and TNF-α inhibitory activities. Compound **24** showed good systemic availability in preclinical animal models and was also found to be non-toxic (exploratory mutagenicity test). Further it exhibited promising results in *invivo* Asthma / COPD and Uveitis models.

Key Words: PDE-4 inhibitors; TNF-α; Dibenzofuran.

1. Introduction

The cyclic nucleotide phosphodiesterases (PDEs) are a family of enzymes that selectively catalyze the hydrolysis of 3' cyclicphosphate bonds of the cyclic adenosine monophosphate (cAMP) and/ or cyclic guanosine monophosphate (cGMP) which in turn act as key regulators of many important physiological processes. There are more than 200 splice isoforms for 21 human PDE genes that encode for the 12 PDE isoenzymes by variation in their amino acid sequence. The sequence identity between different families is only 30-40%, but these isozymes differ enough in their kinetic and physical characteristics, substrate selectivity, tissue distribution and sub-

cellular localization [1-4]. Many cell types express more than one PDE and distribution of isoenzymes between the cells varies markedly.

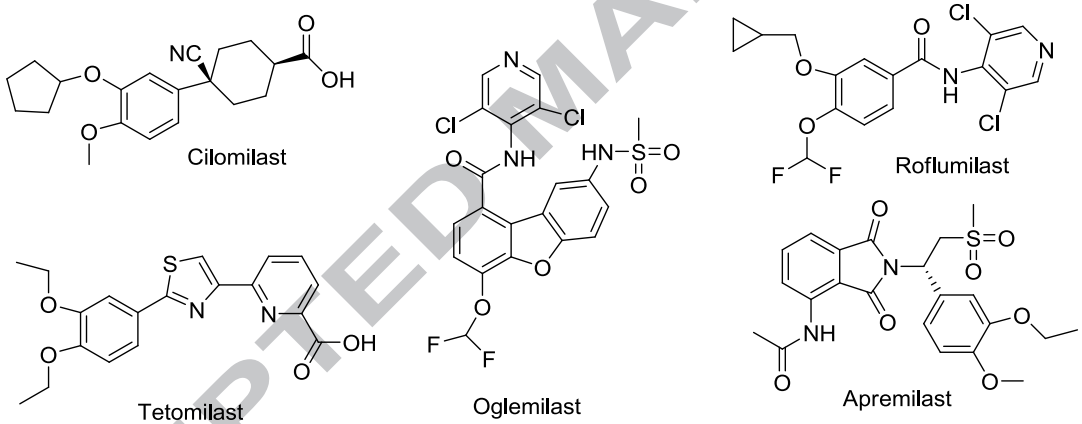
Phosphodiesterase type 4 (PDE-4) is a cAMP-specific and Ca^{2+} independent enzyme and hydrolyses cAMP in mast cells, basophils, eosinophils, monocytes and lymphocytes. The human recombinant PDE-4 exists in four isoforms A, B, C and D. Isoforms A, B, and D are expressed in many inflammatory cells such as eosinophils, basophils, neutrophils, monocytes, mast cells, macrophages, T cells, CD^{4+}, CD^{8+}, B cells, various smooth muscle cells, epithelial cells, endothelial cells and fibroblasts. PDE-4C enzyme is mainly found in testis, skeletal muscle, the central nervous system (CNS) and human fetal lung [5, 6].

Tumor necrosis factor (TNF-α), a cell signaling protein involved in systemic inflammation, is one of the cytokines that makes the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by many other cell types such as CD^{4+} lymphocytes, NK cells, neutrophils, mast cells, eosinophils and neurons. It has been well demonstrated that TNF-α production in pro-inflammatory cells becomes attenuated by an elevation of intracellular cyclic adenosine 3',5'-monophosphate (cAMP). This second messenger is regulated by the PDE family of enzymes [7, 8]. Excessive or unregulated TNF-α production has been implicated in mediating or exacerbating a number of undesirable physiological conditions in diseases including osteoarthritis and other arthritic conditions septic shock, endotoxic shock and respiratory distress syndrome. Since TNF-α also participates in the onset and progress of autoimmune diseases, PDE-4 inhibitors may find utility as therapeutic agents for rheumatoid arthritis, multiple sclerosis and Crohn's disease [9, 10].

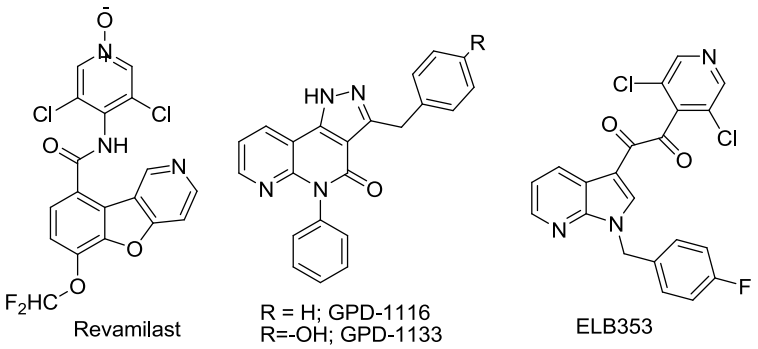
Inhibition of the PDE-4 in these cells effectively elevates the intracellular cAMP levels, thereby activating specific protein phosphorylation cascades and regulating cellular functions. This in turn inhibits the release of inflammatory mediators such as TNF-α, interleukin-2 (IL-2), interleukin-12 (IL-12). The association between cAMP elevation in inflammatory cells with airway smooth muscle relaxation and inhibition of mediator release has led to widespread interest in the design of PDE-4 inhibitors [11, 12]. Unfortunately, clinical utility of several PDE-4 inhibitors is limited due to their undesirable side effect profile which includes

nausea, emesis and gastric acid secretion due to action on PDE-4 in parietal cells in the gut [13, 14].

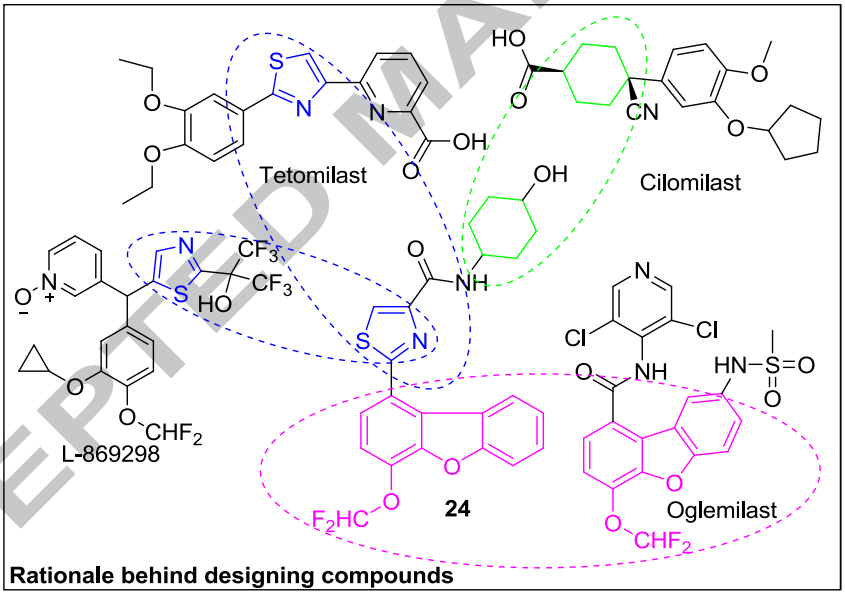
At present the most advanced PDE-4 inhibitor compounds are Roflumilast (Nycomed/Forest/Tanabe) [15-17] and Cilomilast (GlaxoSmithKline) [18, 19], which have been studied quite extensively. Roflumilast got EU and FDA approval for COPD after completion of four Phase III trials world wide. Another PDE-4 inhibitor of interest which has reached (Phase III) clinical trials is Tetomilast (Otsuka) [20] and is reported to be in Phase III for the treatment of Crohn's disease and ulcerative colitis [21]. Apremilast (Celgene) [22-25] is a selective PDE-4 inhibitor with additional activities inhibiting cytokines. Initially it was developed for asthma and COPD and is now approved for psoriasis and other chronic inflammatory diseases. Oglemilast (Glenmark/Forest/Teijin) [25, 26] a potent PDE-4 inhibitor which entered Phase IIb clinical trials for asthma and COPD is now discontinued.



Other compounds of interest are Revamilast (Glenmark), MEM 1414 (Memory pharmaceuticals) which have entered Phase II clinical trials in the field of inflammation. ELB353 (Biotie Therapies) exhibits good efficacy profile inhibiting LPS-induced pulmonary neutrophilia in rats similar to that of Roflumilast. This compound completed Phase I clinical trials with better adverse effect profile compared to other competitors. Furthermore GPD 1116 (ASKA Pharma) and its metabolite 1133 exhibited interesting preclinical results [25, 27-29].



Rationale of design: In the literature thiazole bearing compounds are found to inhibit both PDE-IV and TNF-α enzymes [30-32]. Tetomilast, a PDE-IV inhibitor from Otsuka contains thiazole moiety in it [20]. As part of our program for development of PDE-4 inhibitors in the field of inflammation, we came up with a new series of dibenzofuran (Oglemilast/ Revamilast fragment) fused thiazole (Tetomilast fragment) compounds carrying cyclohexyl (Cilomilast fragment) derivatives.



2. Results and discussion

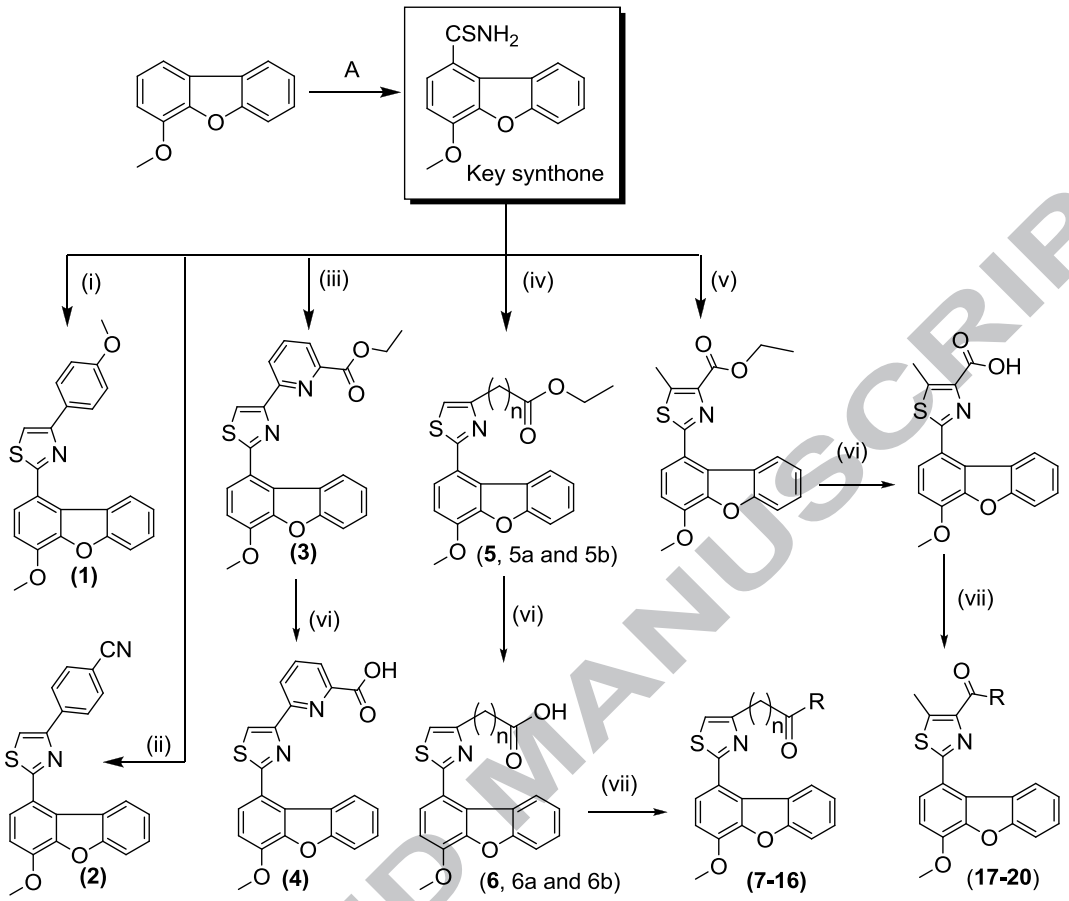
2.1 Chemistry

To begin with the key synthon thioamides, were synthesised by following either of two routes. Route-I, where direct installation of desired thioamide group on dibenzofuran was carried out using potassium thiocyanate in acidic medium (yield, 70-80%). Route-II was followed for the compounds bearing a substitution at R_3 position of second phenyl ring of dibenzofuran moiety. The desired starting carboxylic acids for this route were prepared using literature procedure [33] and converted to corresponding thioamides in three steps. Amidation of carboxylic acid

was performed using aqueous ammonia followed by treatment with TFAA to get corresponding nitrile compounds. Reaction of phosphorous pentasulphide with these nitrile compounds gave the key thioamides.

Thiazole moiety on dibenzofuran ring was constructed by reacting α-bromoketones such as 2-bromo-1-(4-methoxyphenyl)ethanone, 4-(2-bromoacetyl)benzonitrile and ethyl 6-(2-bromoacetyl)picolinate with the thioamides in presence of sodium bicarbonate in ethanol at room temperature (RT) (compounds **1**-**3**), yield 30-46%. Esters of compounds **6**, **25** and **28** were synthesised using corresponding α-bromoketones (ethyl 3-bromo-2-oxopropanoate/ ethyl 5-bromo-4-oxopentanoate/ ethyl 4-bromo-3-oxobutanoate) and thioamides in dimethoxy ethane and water at 80 °C (yield 50-73%), whereas, esters of **17** and **32** were synthesised using ethyl 3-bromo-2-oxobutanoate with corresponding thioamide in presence of potassium carbonate in ethanol and acetone at 80 °C (yield 10-15%). Ester bearing compounds were hydrolysed to corresponding acids using potassium hydroxide in ethanol - water mixture at RT to get compounds **4**, **6**, **25**, **28** and **32** (yield 45-75%). Some of these acids were made into amides **7**-**24**, **26**, **27**, **29**-**31, 33** & **34** using various aliphatic, alicyclic and aromatic amines in presence of a coupling agent [34].

Route - I

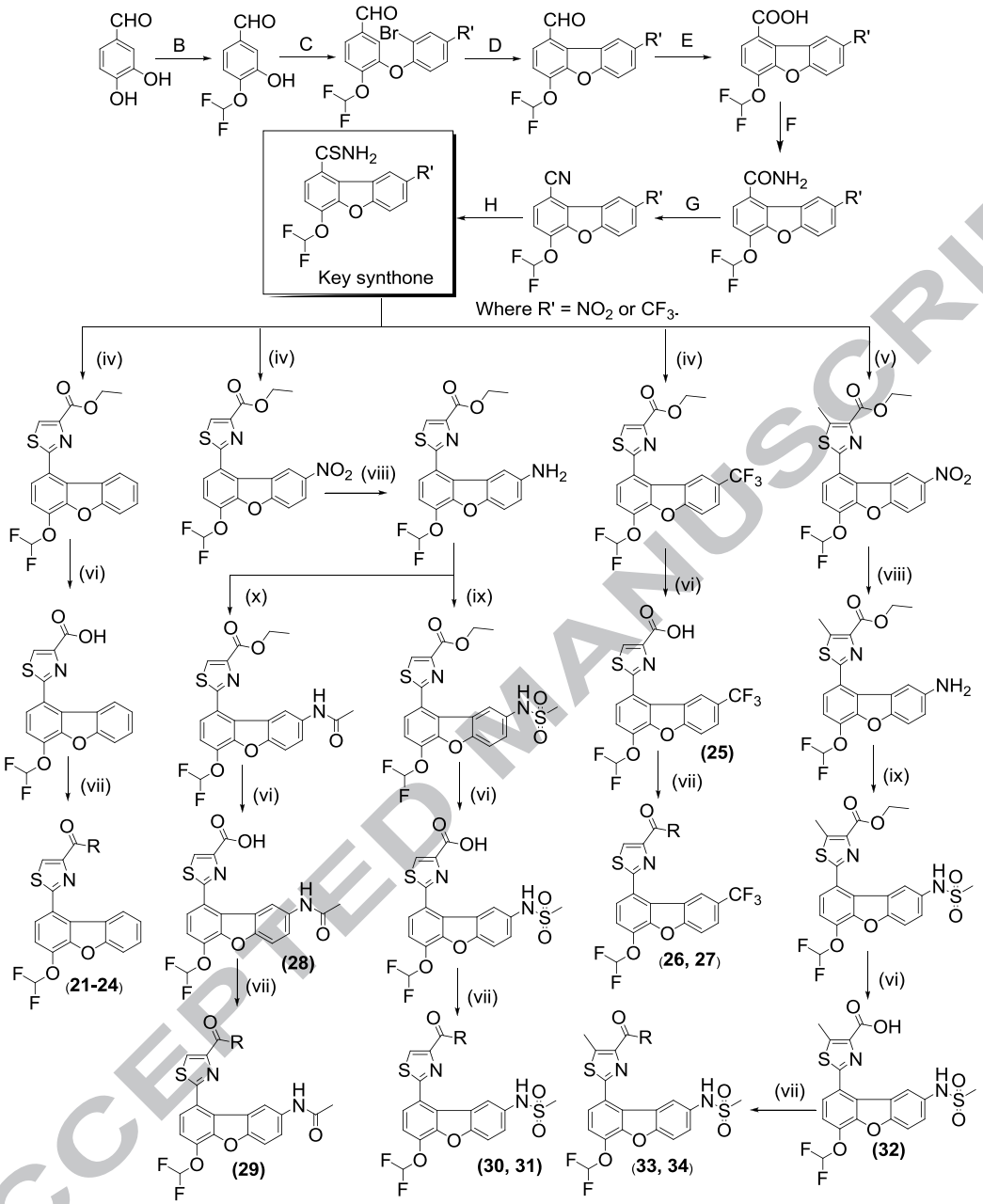


(**6**) n=0; (**6a**) n=2;(**6b**) n=1; **(7)** R = Aniline, n=0; **(8)** R = p-Chloroaniline, n=0; **(9)** R = -NH_2, n=0;
(10) R = 4-amino-3,5-dichloropyridine, n=0; **(11)** R = 2-(piperazin-1-yl)pyrimidine, n=0;
(12) R = 1-(pyridin-2-yl)piperazine, n=0; **(13)** R = morpholin-4-amine, n=0;
(14) R = 4-aminocyclohexanol, n=0; **(15)** R = 4-amino-3,5-dichloropyridine, n=2;
(16) R = 4-amino-3,5-dichloropyridine, n=1; **(17)** R = Ethanolamine; **(18)** R = 4-aminocyclohexanol;
(19) R = Cyclopentylamine; **(20)** R = 4-amino-3,5-dichloropyridine.

Reagents and Reaction conditions :

A) Methane sulphonic acid addition at 0-5 °C, potassium thiocyanate, RT, over night stirring, yield 70-80%;
i) 2-bromo-1-(4-methoxyphenyl)ethanone, $NaHCO_3$, ethanol, RT, over night stirring, yield 40%;
ii) 4-(2-bromoacetyl)benzonitrile, $NaHCO_3$, ethanol, RT, over night stirring, yield 46%;
iii) Ethyl 6-(2-bromoacetyl)picolinate, $NaHCO_3$, ethanol, RT, over night stirring, yield 30%;
iv) Corresponding bromoester (ethyl 3-bromo-2-oxopropanoate/ ethyl 5-bromo-4-oxopentanoate/ ethyl 4-bromo-3-oxobutanoate), Diemthoxy ethane (DME), water, 80 °C, 4 h, yield 50-75%;
v)) Ethyl 3-bromo-2-oxobutanoate, K_2CO_3, ethanol, 80 °C, 2 h, yield 15%;
vi) KOH, ethanol, water, RT, 2 h.; yield 40 - 70%;
vii) Corresponding Amine, EDCI, HOBt, DMAP, DIPEA and DMF; RT, 10-25 h., yield 20-84%.

Route - II



(21) R = 4-amino-3,5-dichloropyridine, **(22)** R = 2,2,2-trifluoroethanamine, **(23)** R = *O*-methylhydroxylamine, **(24)** R = 4-aminocyclohexanol, **(26)** R = 4-aminocyclohexano, **(27)** R = piperidin-4-ol, (**29**) R = 4-aminocyclohexanol, **(30)** R = 4-amino-3,5-dichloropyridine, **(31)** R = 4-aminocyclohexanol, **(33)** R = 4-aminocyclohexanol, **(34)** R = *O*-methylhydroxylamine,

Reagents and Reaction conditions :

For the steps B, C, D and E followed procedures given in ref. 33.
F) Ethylchloroformate, TEA, aq. ammonia, THF, stirring at 0-5 °C, 2 h. over night stirring at RT, yield 55-70%;
G) Trifluoroacetic anhydride, TEA, MDC, 0-5 °C to RT, 2 h., yield 70-80%;
H) P_2S_5, ethanol, 80 °C, 7 h., yield 85-90%;
iv) Ethyl 3-bromo-2-oxopropanoate, corresponding thioamide (4-(difluoromethoxy)dibenzo[*b*,*d*]furan-1-carbothioamide/ 4-(difluoromethoxy)-8-nitrodibenzo[*b*,*d*]furan-1-carbothioamide/ 4-(difluoromethoxy)-8-(trifluoromethyl)dibenzo[*b*,*d*]furan-1-carbothioamide, Diemthoxy ethane (DME), water, 80 °C, 2 h, yield 48%;
v) Ethyl 3-bromo-2-oxobutanoate, K_2CO_3, ethanol, 80 °C, 2 h, yield 18%;
vi) KOH, ethanol, water, RT, 2 h.; yield 40 - 70%;
vii) Corresponding Amine, EDCI, HOBt, DMAP, DIPEA and DMF; RT, 10-25 h., yield 20-84%;
viii) 10%Pd on carbon (wet 50%), H_2- 3Kg pressure, 2 h., yield 80%;
ix) Methane sulfonyl chloride, Et_3N or pyridine, Dichloromethane, 4 h, yield 70-80%;
x) Acetyl chloride, Et_3N, Dichloromethane, 4 h, yield 70-80%.

2.2Biology

2.2.1 *In-vitro* experiments:

The synthesised Dibenzo[b,d]furan-1-yl thiazole derivatives were evaluated for their ability to inhibit the PDE-4B enzyme (purified human enzyme) and inhibition of production of TNF-α in human Peripheral Blood Mononuclear cells (PBMC). Interesting compounds were tested for their IC_{50} values and are summarized in Table-I & II**.** Apremilast and Roflumilast were used as positive controls. GRC 3886 which belongs to dibenzofuran class of compounds inhibited the PDE-4 enzyme at 1.4nM and TNFα at 190nM [25].

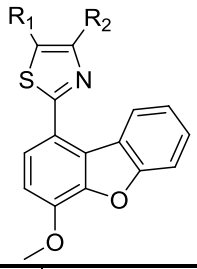
Compounds **1-4** (compound **4** is with tetomilast side chain), where R_2 is an aryl group connected directly to thiazole ring fused to dibenzofuran moiety exhibited low to moderate PDE-4B inhibition (5 - 19.4%) at 100 nM concentration. Compound **5** where R_2 is a simple ethyl ester retained moderate PDE-4B inhibition (17.6%) and interestingly its acid **6** demonstrated a two fold increased activity (36.0%) at 100 nM and three fold activity (80.4%) at 1000 nM concentration.

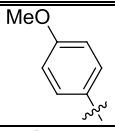
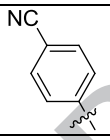
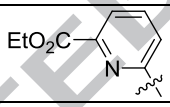
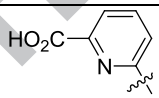
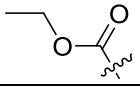
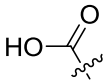
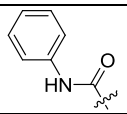
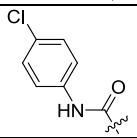
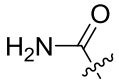
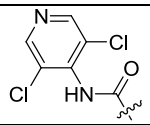
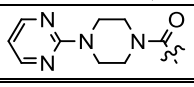
The carboxylic group of compound **6** was coupled with various aromatic, alicyclic and aliphatic amines to prepare corresponding amides. Among all the aromatic amides, compound **10** where R_2 is N-(3,5-dichloropyridin-4-yl)acetamide, a side chain of Oglemilast/ Roflumilast exhibited PDE-4B enzyme inhibitory activity of 70.3% at 100 nM concentration (IC_{50} 48.4 nM) which is almost two folds more active than the parent acid **6**. A significant decrease in the activity was observed with lengthening of carbon chain of these amides **15 (**8.9%) and **16** (32.7%) at 100 nM concentration. Replacement of aromatic amides with alicyclic amides increased the enzyme inhibitory activities substantially. Particularly 4-hydroxycyclohexanol amides **14** and **18** exhibited PDE-4B enzyme inhibitory activity of 71.8% (IC_{50} 49.5 nM) and 62.6% (IC_{50} 43.8 nM). No significant change in inhibitory activity was observed for substituting R_1 = H or R_1 = CH_3 on the thiazole ring.

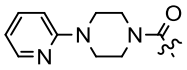
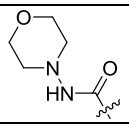
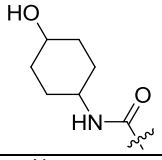
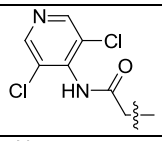
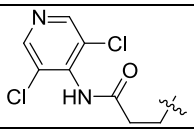
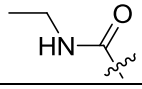
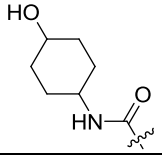
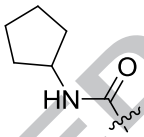
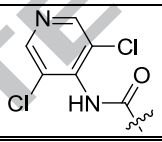
All the interesting compounds (includes all the cyclohexanolamides and few selective compounds based on its side chain) were tested for inhibition of production of TNF-α (IC_{50}) in human Peripheral Blood Mononuclear Cells (PBMC). Except compound **26**, all the cyclohexanol amides **14** (37.7 nM), **18** (27.7 nM), **24** (31 nM), **29** (39.6 nM), **31** (18.4 nM) and **33** (54.4 nM) showed the inhibitory activity (IC_{50}) at nano molar concentrations (nM) whereas all other tested compounds **9 (**0.386 μM), **10**

(1.75 μM), **16** (2.78 μM) and **21** (2.21 μM) exhibited the TNF-α inhibitory activity at micro molar concentrations (μM).

Table-I: PDE-IVB and TNF-α inhibitory data of compounds **1-20**

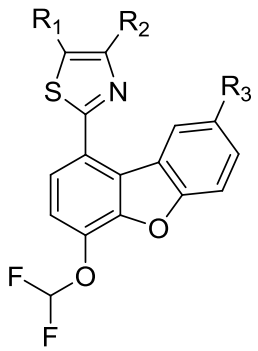


S.NO	R_1	R_2	PDE-4 inhibition *nM* (human PDE-4B)			TNF-α inhibition *nM* (human PBMC)		
			1000	100	IC_{50}	1000	100	IC_{50}
1.	H		41.5	19.4	ND	ND	ND	ND
2.	H		15.8	5.2	ND	ND	ND	ND
3.	H		15.6	5.8	ND	ND	ND	ND
4.	H		26.4	18.2	ND	ND	ND	ND
5.	H		24.4	17.6	ND	ND	ND	ND
6.	H		80.4	36.0	ND	ND	ND	ND
7.	H		7.4	2.4	ND	ND	ND	ND
8.	H		5.2	1.2	ND	15.81	2.98	ND
9.	H		43.8	15.4	ND	69.8	35.5	385.7
10.	H		92	70.3	48.4	34.2	ND	1753
11.	H		59.3	25.8	ND	ND	ND	ND

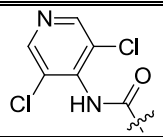
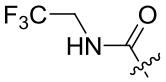
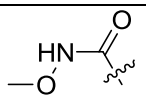
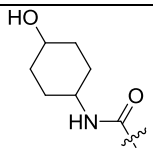
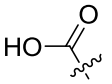
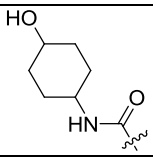
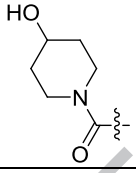
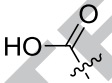
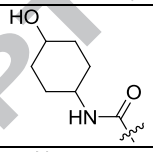
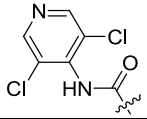
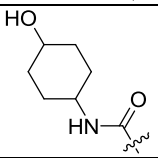
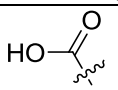
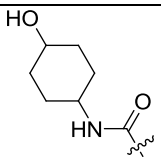
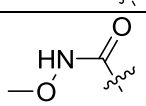
12.	**H**		**52.9**	**21.9**	**ND**	**ND**	**ND**	**ND**
13.	**H**		**ND**	**39.6**	**ND**	**22**	**ND**	**ND**
14.	**H**		**ND**	**71.8**	**49.5**	**ND**	**63.2**	**37.7**
15.	**H**		**38.5**	**8.9**	**ND**	**ND**	**ND**	**ND**
16.	**H**		**87.8**	**32.7**	**ND**	**21**	**8**	**2780**
17.	**-CH_3**		**ND**	**24.6**	**ND**	**23.8**	**9.9**	**ND**
18.	**-CH_3**		**ND**	**62.6**	**43.8**	**103.1**	**82.7**	**27.7**
19.	**-CH_3**		**ND**	**1.6**	**ND**	**39.1**	**21.9**	**ND**
20.	**-CH_3**		**ND**	**11.1**	**ND**	**9.9**	**0.9**	**ND**

*ND: Not done

Table-II: PDE-IVB and TNF-α inhibitory data of compounds **21 - 34**



S.NO	R_1	R_2	R_3	PDE-4 inhibition *nM* (human PDE-4B)	TNF-α inhibition *nM* (human PBMC)

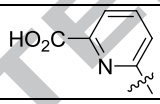
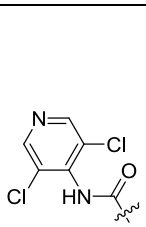
				1000	100	IC_{50}	1000	100	IC_{50}
21.	**H**		**H**	**88.6**	**42.3**	**127.1**	**28.3**	**14.5**	**2208**
22.	**H**		**H**	**ND**	**ND**	**ND**	**31.9**	**26.7**	**ND**
23.	**H**		**H**	**ND**	**35.7**	**ND**	**ND**	**8.5**	**ND**
24.	**H**		**H**	**ND**	**65.3**	**63.5**	**93.8**	**77.7**	**31**
25.	**H**		**CF_3**	**ND**	**59.3**	**ND**	**ND**	**-4.2**	**ND**
26.	**H**		**CF_3**	**ND**	**51.5**	**ND**	**ND**	**17.2**	**ND**
27.	**H**		**CF_3**	**ND**	**27.4**	**ND**	**ND**	**14**	**ND**
28.	**H**		**NHCOMe**	**ND**	**56.5**	**ND**	**ND**	**15.5**	**ND**
29.	**H**		**NHCOMe**	**ND**	**69**	**40.4**	**91.9**	**83.4**	**39.6**
30.	**H**		**$NHSO_2Me$**	**ND**	**43.4**	**ND**	**ND**	**25.1**	**ND**
31.	**H**		**$NHSO_2Me$**	**ND**	**67**	**31.4**	**97.8**	**78.6**	**18.4**
32.	**CH_3**		**$NHSO_2Me$**	**ND**	**62.6**	**ND**	**ND**	**ND**	**ND**
33.	**CH_3**		**$NHSO_2Me$**	**ND**	**67.6**	**24.7**	**92.8**	**61.8**	**54.4**
34.	**CH_3**		**$NHSO_2Me$**	**ND**	**81.2**	**ND**	**ND**	**35.4**	**ND**

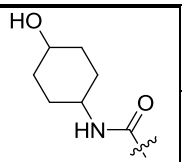
Apremilast	**ND**	**ND**	**74**	**ND**	**ND**	**77**
Roflumilast	**ND**	**ND**	**0.2**	**ND**	**ND**	**4.3**
GRC 3886 (lit. value)			**1.4**			**190**

*ND: Not done

In Table-III & Figure-I interesting compounds have been grouped with varying substitution at R_2 position and their activities for easy reference. This table includes the compounds bring the side chains selected from some of the standard PDE-4 inhibitors viz. Tetomilast (pyridyl carboxylic acid), Rouflumilst/ Oglemilast (dichloropyridyl amide) and Cilomilast (cyclohexane).

Table-III: PDE-IVB and TNF-α inhibitory data of Interesting compounds

S.NO (As allotted in Table-I and II)	**R**	**R_1**	**R_2**	**R_3**	**PDE-4 inhibition *nM* (human PDE-4B)**		**TNF-α inhibition *nM* (human PBMC)**	
					100	**IC_{50}**	**100**	**IC_{50}**
Tetomilast side chain bearing (at R_2) compound:								
4.	$-OCH_3$	-H		-H	**18.2**	**ND**	**ND**	**ND**
Roflumilast / Oglemilast side chain bearing (at R_2) compounds:								
10.	$-OCH_3$	-H		-H	**70.3**	**48.4**	**ND**	**1753**
20.	$-OCH_3$	$-CH_3$		-H	**11.1**	**ND**	**0.9**	**ND**
21.	$-OCHF_2$	-H		-H	**42.3**	**127.1**	**14.5**	**2208**
30.	$-OCHF_2$	-H		$-NHSO_2Me$	**43.4**	**ND**	**25.1**	**ND**
Cyclohexanol side chain bearing (at R_2) compounds:								
14.	$-OCH_3$	-H		-H	**71.8**	**49.5**	**63.2**	**37.7**
18.	$-OCH_3$	$-CH_3$		-H	**62.6**	**43.8**	**82.7**	**27.7**
24.	$-OCHF_2$	-H		-H	**65.3**	**63.5**	**77.7**	**31**

26.	-$OCHF_2$	-H		-CF_3	**51.5**	**ND**	**17.2**	**ND**
29.	-$OCHF_2$	-H		-NHCOMe	**69**	**40.4**	**83.4**	**39.6**
31.	-$OCHF_2$	-H		-$NHSO_2Me$	**66.9**	**31.4**	**78.6**	**18.4**
33.	-$OCHF_2$	-CH_3		-$NHSO_2Me$	**67.6**	**24.7**	**61.8**	**54.4**

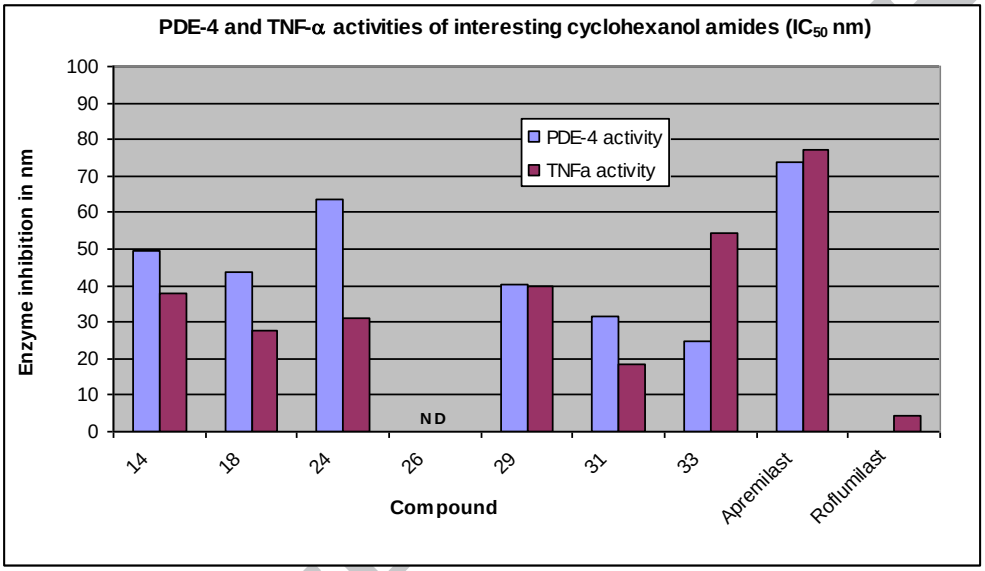


Figure-I: *Invitro* enzyme inhibitory activities of interesting compounds

Mutagenicity Test – Exploratory

Compound **24** was subjected to *Salmonella typhimurium* reverse mutation assay and found it is non-mutagenic and non-cytotoxic up to 5mg/plate (Table-IV).

Table-IV: Mutagenicity test results of compound **24**

Strains used	TA98 and TA100
Dose Concentrations (mg/plate)	0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5 & 5
Metabolic activation	Rat liver S9 fraction
Result	Non-mutagenic and non-cytotoxic up to 5 mg/plate
	Ames (TA 98 & TA 100) - Negative

2.2.2 *In-vivo* experiments:

In the process of lead compound identification a few *invitro* active molecules were subjected to following preliminary *invivo* experiments:

Exp 1. Asthma and COPD - Rat model

Exp 2. Estimation of TNF-α - Mice model

Further compound **24** was explored with the following other four *in-vivo* experiments.

Exp 3. Pharmoco Kinetics (PK) - Rat model

Exp 4. LPS induced total cell count - Rat model

Exp 5. LPS induced Uveitis - Rat model and

Exp 1: LPS induced Neutrophil influx in BALF of SD rats for asthma and COPD

LPS induced neutrophilia for asthma and COPD was performed in male *Sprague Dawley* rats with selected *in-vitro* active compounds, the details such as oral dosage, LPS induced neutrophilia (% of inhibition) and number of animals grouped for the experiments are tabulated in Table-V.

Table-V: LPS induced Neutrophil influx in BALF of SD rats after 6 h (n = 8)

Compound No.	Dose (mg/kg)	LPS induced neutrophilia (% inhibition)
24	10	58
29	5	35.7
	10	51.2
33	10	19.3
Roflumilast	10	73.2

Exp 2: LPS induced sepsis model in mice for the measurement of TNF-α

The LPS induced sepsis model was performed in female *Swiss Albino* mice for the measurement of TNF-α. The fasted mice were dosed orally with the test compound at 10 mg/kg body weight; the percentage of inhibition was tabulated in Table-VI. Out of the test compounds **10 (**37.8%); **14** (46.7%) and **23** (40.7%) exhibited similar activity though they showed different *invitro* potency, whereas compound **24** efficiently inhibited 82.2% of the enzyme at much lower concentration (3mg/kg body weight).

Table-VI: LPS induced TNF-α in Swiss Mice (n = 6)

Compound No.	Dose (mg/kg, p.o.)	TNF-α Inhibition (%)
10	10	37.8
14	10	46.7

23	10	40.7
24	0.3	16.4
	1	34.2
	3	82.2
Roflumilast	3	59.2
GRC 3886	1	53.9
	10	72.5

Exp 3: Pharmacokinetic parameters of compound 24 in male wistar rats

Following a single dose oral and intravenous administration, the plasma concentration of compound **24** declined in a time dependent manner, with the pharmacokinetic parameters adequately described by non-compartmental analysis against plasma concentration versus time data. Compound **24** is absorbed slowly and peak concentrations were reached 4.8 h post-dose, when studied at pharmacological dose in rats (Table-VII). Volume of distribution after intravenous administration was about 0.94 L/kg and it was about 1.5 times of the total body water in rats, indicating that it may be distributed in extra cellular as well as intracellular fluid. Clearance after intravenous administration of compound **24** in rat was 0.35 L/h/kg (Figure-II**)**. The Clearance was low in rat which was about 10% of the rat liver blood flow. Compound **24** has a terminal half-life of about 1.9 h in rats. The oral bioavailability of compound **24** was studied in rat at 10 mg/kg and the absolute oral bioavailability is about 42%.

Table-VII : Pharmacokinetic parameters of compound **24** in male wistar rats

Parameters	**Unit**	**Estimates**	
		Oral	**I.V**
Dose	mg/kg	10	5
T_{max}	hr	4.8	-
C_{max}	mg/L	1.4	-
Co	mg/L	-	4
AUC_{last}	hr*mg/L	11.5	13.7
Vd	L/kg	-	1
CL	L/hr/kg	-	0.4
$t_{1/2}$	Hr	5.0	1.9
F	%	42	

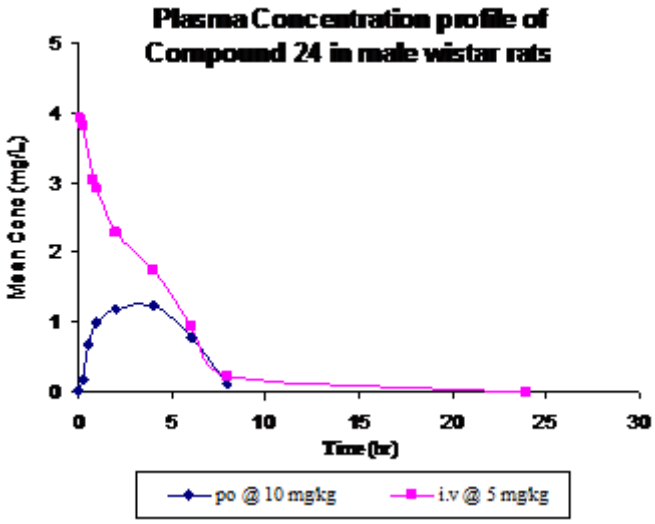


Figure-II. Mean plasma concentration profile of Compound 24 (oral and intravenous administration) in male wistar rats

Exp 4: LPS induced Total cell count in BALF of SD rats

A single dose oral administration of compound **24** at 10 mg/kg concentration produced inhibition of 75.2% on par with the internal standard Roflumilast which showed the inhibition of 73.2% of LPS induced total cells BALF of male SD rats after 6 h. The explorative dose response studies of the compound produced inhibition of 52.8, 34.6, 60.7, 70.9, 75.2 and 78.7% at concentrations 0.1, 0.3, 1, 3, 10 and 30 mg/kg respectively (Figure-III**)**. Flattening of inhibition was observed from 3mg/kg body weight onwards and maximum inhibition (78.7%) was observed at 30mg/kg weight.

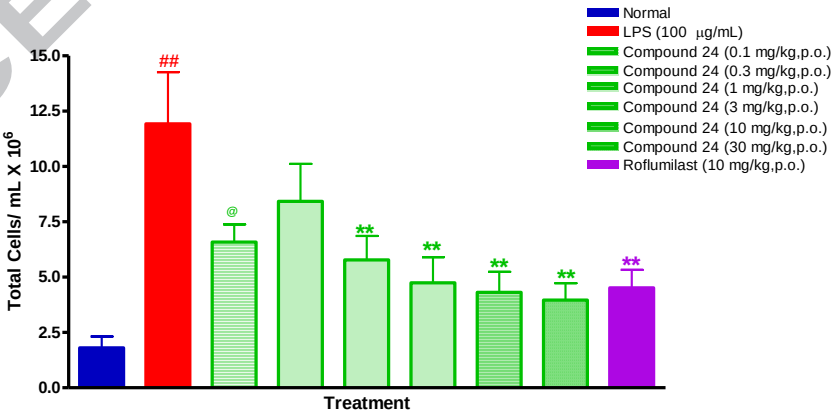


Figure-III. Compound 24 - LPS induced Total cell count in BALF of SD rats

Exp 5: LPS induced Uveitis in Female Wistar Rats

Endotoxin-induced uveitis (EIU) was induced in male Wistar rats by a footpad injection of lipopolysaccharide (LPS). LPS 250 µg/animal was injected to both left

and right hind paw (each hind paw receives 125 µg). After 24 hr of LPS injection, the animal was injected orally with the Compound **24** @ 0.1, 0.5, 1 and 3 mg/kg and Dexamethsone @ 10 µg/10 µL/eye. One hr after drug administration, aqueous humor was collected from both eyes and total number of infiltrating cells was counted using a hemacytometer and the percentage of inhibition was calculated. Compound **24** produced 43.6, 54.4, 90.3, and 96.9 % inhibition at 0.1, 0.5, 1 and 3 mg/kg respectively whereas Dexamethasone @ 10 µg/10µL/eye showed 79.1 % inhibition (Figure- IV). The number of infiltrating cells in the anterior segment of the eye was reduced in rats treated with Compound **24** (**p<0.01) when compared with that with LPS and vehicle treatment (n=10). The results showed the total cell number in aqueous humor was significantly reduced at the oral dose of 1 mg/kg (p<0.01) itself and there was only slight increase in the response at 3 mg/kg. Preliminary result of this study shows that oral administration of Compound **24**, at the dose of 1 mg/kg, produces anti-inflammatory effects against LPS-induced ocular inflammation.

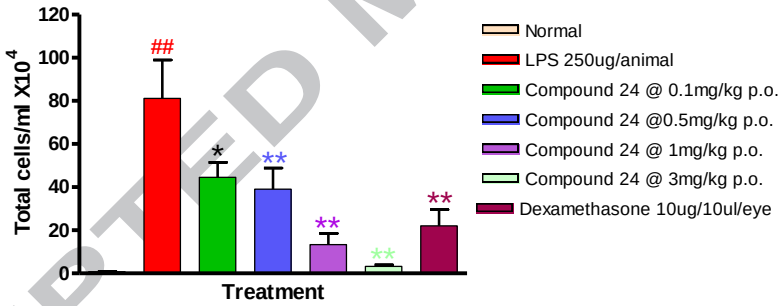


Figure-IV. Compound 24 - LPS induced Uveitis in female wistar rats

3. Conclusion

Thirty four new Dibenzo[b,d]furan-1-yl-thiazole derivatives (**1**-**34**) were synthesised and tested their PDE-4B and TNF-α inhibitory activities. The promising *invitro* potent compounds were screened for their metabolic stability in mouse liver microsomes (MLM), human liver microsomes (HLM) and they exhibited moderate to good stability in both microsomes (data not shared). The *invivo* PK experiments (both oral and intravenous) on compound **24** shows good systemic availability. Hence this compound was screened for Asthma/ COPD and TNF-α inhibition in mice and the results are encouraging. Moreover, the results from LPS induced Uveitis on this compound **24** were found to be interesting and the exploratory mutagenicity test

reveals that the compound is non toxic. Further developmental studies on this compound and the backup (**31)** are underway.

Experimental

4.1. Chemistry

Starting materials were procured from Sigma Aldrich and Johnson Matthey, Alfa aesar (lab grade) and are used as such without further purification. Penultimate compounds were purified by silica gel Column chromatography (silica gel 60-100 mesh, E. Merck). All the solvents were from commercial sources and are dried according to standard procedures. Reaction progress was monitored by thin layer chromatography (TLC) silica on aluminium sheet obtained from Merck. Melting points were recorded on a Buchi apparatus and are uncorrected. IR spectra (KBr pellets) were recorded on a JASCO spectrometer and frequencies were expressed in cm^{-1}. Mass spectra (GC/MS) were recorded on Agilent MSD VL mass spectrometer and M+1 of the compounds were expressed in %. ^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer in $CDCl_3$ or DMSO-d_6 solution using TMS as an internal standard and the chemical shifts are reported in ppm (δ). Coupling constants (*J*) are expressed in Hz.

4.2 Preparation of key synthon thioamides:

Two routes were followed to synthesise the thioamides, Route-I where R_3 is only hydrogen and Route-II for compounds substituted with either of -CF_3 or -NO_2 group. The nitro group was further reduced to amine as depicted in the schemes described.

Route – I: Synthesis of 4-methoxy dibenzo[b,d]furan-1-carbothioamide:

To a mixture of 4-methoxy dibenzo[b,d]furan (1 g, 5.05 mmol) in methane sulphonic acid (11 mL), was added potassium thiocyanate (1 g, 10.29 mmol) at 0-5 °C and the reaction mass was stirred at RT. The reaction mixture was poured into crushed ice and the solid obtained was filtered. It was triturated with n-hexane and dried to get a pale brown coloured solid (0.9 g). Yield - 70%; TLC - R_f 0.25 (EtOAc: n-Hexane, 3:7); HPLC purity - 91.4%; ^{1}H-NMR (400MHz, $CDCl_3$): δ 4.08 (s, 3H, -OCH_3), 6.97 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.28 (m, 1H, Ar-H), 7.35 (m, 1H, Ar-H), 7.65 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.69 (d, *J* = 7.8Hz, 1H, Ar-H), 7.94 (d, *J* = 8.0Hz, 1H, Ar-H), 8.31 (d, *J* = 8.2 Hz, 1H, Ar-H); Mass m/z: 258.1 (M+1).

Route – II: General procedure for the thioamides with a substitution of –CF_3 and –NO_2 at R_3.

Step1: Synthesis of 4-(difluoromethoxy)dibenzo[b,d]furan-1-carboxylic acid:

Followed the procedure from reference 33.

Step 2: Synthesis of 4-(difluoromethoxy)dibenzo[b,d]furan-1-carboxamide

To a solution of 4-(difluoromethoxy)dibenzo[b,d]furan-1-carboxylic acid (8.7 mmol) in THF (5 mL), was added triethylamine (21.5 mmol) followed by ethylchloroformate (41.9 mmol) at 0-5 °C and the reaction mass was stirred at the same temperature for 2 h. Aqueous ammonia (5 mL) was added and the mass was kept stirring overnight at RT. The reaction mixture was poured into crushed ice and the solid settled was filtered. It was triturated with n-hexane and dried to furnish the amide. Yield: 55 - 70%; TLC - R_f 0.45 to 0.55 (Chloroform: methanol, 9:1); HPLC purity: 90 - 95%.

Step 3: Synthesis of 4-(difluoromethoxy)dibenzo[b,d]furan-1-carbonitrile:

To a solution of 4-(difluoromethoxy)dibenzo[b,d]furan-1-carboxamide (0.34 mmol) in DCM (5 mL), was added triethylamine (1.1 mmol) followed by trifluoroacetic anhydride (1.1 mmol) at 0-5 °C and the reaction mass was stirred at RT for 2 h. The reaction mixture was poured into cold water and extracted with MDC (2x25 mL). Organic layer was further washed with brine and dried over anhydrous sodium sulphate. The organic layer was concentrated under reduced pressure to give the desired nitrile compound. Yield: 70- 85%; TLC - Rf 0.31 (EtOAc: n-Hexane, 3:7); HPLC purity: 70 - 80%.

Step 4: Synthesis of 4-(difluoromethoxy)dibenzo[b,d]furan-1-carbothioamide:

Phosphorous pentasulfide (49 mmol) in ethanol (10 mL) was refluxed for 30 minutes and 4-(difluoromethoxy)dibenzo[b,d]furan-1-carbonitrile (R' = -CF_3 or -NO_2, 9.8 mmol) was added and refluxing was continued for 6 h. The reaction mixture was concentrated under reduced pressure, cold water (50 mL) was added and extracted with EtOAc (2x 50 mL). Organic layer was washed with brine and dried over sodium sulphate and concentrated under reduced pressure to get the corresponding thioamide. Yield: 50-70%; TLC - R_f 0.25 (EtOAc: n-Hexane, 3:7); HPLC purity: 85 - 90%.

4.3 Synthesis of 2-(4-methoxydibenzo[b,d]furan-1-yl)-4-(4-methoxyphenyl) thiazole (1):

General procedure-A: To a stirred solution of 4-methoxydibenzo[b,d]furan-1-carbothioamide (50 mg, 0.194 mmol) in ethanol (5 mL) was added sodium bicarbonate (15 mg, 0.179 mmol) followed by 2-bromo-1-(4-methoxyphenyl)ethanone (45 mg, 0.196 mmol) and the mixture was stirred at RT for 1 h. Subsequently the reaction mixture was poured into cold water (25 mL) and the obtained precipitate was filtered to get a peach coloured solid (30mg), Yield - 40%; TLC - R_f 0.62 (EtOAc : Hexane 3:7); HPLC purity - 98.6%; M.P.153-156 °C; ^{1}H-NMR ($CDCl_3$): δ 3.88 (s, 3H, -OCH_3), 4.13 (s, 3H, -OCH_3), 7.00-7.06 (m, 3H, Ar-H), 7.28-7.32 (m, 3H, Ar-H), 7.46-7.52 (m, 2H, Ar-H), 7.63-7.67 (m, 2H, Ar-H), 8.02 (d, *J* = 8.72 Hz, 2H, Ar-H), 8.83 (d, *J* = 7.96 Hz, 1H, Ar-H); Mass m/z: 388.1 (M+1).

4.4 Synthesis of 4-(2-(4-methoxydibenzo[b,d]furan-1-yl)thiazol-4-yl) benzonitrile (2):

Prepared by General procedure-A using 4-(2-bromoacetyl)benzonitrile in the reaction. Pale pink coloured solid, Yield - 50%; TLC - R_f 0.32 (EtOAc : n-Hexane, 3:7); HPLC purity - 94.8%; M.P. 212-215 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.15 (s, 3H, -OCH_3), 7.07 (d, *J* = 8.44 Hz, 1H, Ar-H), 7.30 (d, *J* = 7.84 Hz, 1H, Ar-H), 7.51-7.55 (m, 1H, Ar-H), 7.64-7.70 (m, 2H, Ar-H), 7.76-7.81 (m, 3H, Ar-H), 8.19 (d, *J* = 8.28 Hz, 2H, Ar-H), 8.72 (d, *J* = 8 Hz, 1H, Ar-H); Mass m/z: 383.1 (M+1).

4.5 Synthesis of ethyl 6-(2-(4-methoxydibenzo[b,d]furan-1-yl)thiazol-4-yl) picolinate (3):

To a stirred solution of 4-methoxydibenzo[b,d]furan-1-carbothioamide (140 mg, 0.544 mmol) in ethanol (5 mL) was added sodium bicarbonate (60 mg, 0.714 mmol) followed by ethyl 6-(2-bromoacetyl)picolinate (150 mg, 0.551 mmol) and the mixture was stirred at RT for 20 h. Subsequently the reaction mixture was poured into cold water (25 mL) and extracted with ethyl acetate (2x25 mL). Organic layer was dried over anhydrous sodium sulphate, filtered and evaporated at reduced pressure, which was further purified by column chromatography using a gradient of EtOAc in hexane (0-6%) to get a pale pink colour solid (0.065 g); Yield - 30%; TLC - R_f 0.5 (EtOAc: n-Hexane, 3:7); HPLC purity - 98.1%; M.P - N/R; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.48-1.51 (m, 3H, -CH_3), 4.14 (s, 3H, -OCH_3), 4.50-4.55 (m, 2H, -OCH_2), 7.07 (d, *J* = 8.32 Hz, 1H, Ar-H), 7.49-7.53 (m, 1H, Ar-H), 7.65-7.69 (m, 2H, Ar-H), 7.94-7.98 (m, 1H, Ar-H), 8.09 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.42 (s, 1H, Ar-H), 8.47 (d, *J* = 7.76 Hz, 1H, Ar-H), 8.73 (d, *J* = 7.76 Hz, 1H, Ar-H); Mass m/z: 431.1 (M+1).

4.6 Synthesis of ethyl 2-(4-methoxydibenzo[b,d]furan-1-yl)thiazole-4-carboxylate (5):

General procedure-B: To a stirred solution of 4-methoxydibenzo[b,d]furan-1-carbothioamide (0.584 mmol) in dimethoxyethane and water (2:1) was added ethyl 3-bromo-2-oxopropanoate (0.717 mmol) at RT. After 10 minutes, the reaction mixture was refluxed at 80 °C for 2 h, subsequently it was poured into cold water (25 mL) and the precipitate obtained was filtered to get white solid, Yield - 73%; TLC - R_f 0.53 (EtOAc : n-Hexane, 3:7); HPLC purity - 97.4%; M.P. 151-154 °C; ^{1}H-NMR ($CDCl_3$): δ 1.50-1.53 (m, 3H, -OCH_2CH_3), 4.13 (s, 3H, -OCH_3), 4.48-4.53 (m, 2H, -CH_2), 7.04 (d, J = 8.4 Hz, 1H, Ar-H), 7.35-7.39 (m, 1H, Ar-H), 7.50-7.55 (m, 1H, Ar-H), 7.65-7.69 (m, 2H, Ar-H), 8.24 (s, 1H, Ar-H), 9.20 (d, J = 8 Hz, 1H, Ar-H); Mass m/z: 354.1 (M+1).

4.7 Synthesis of ethyl 3-(2-(4-methoxydibenzo[b,d]furan-1-yl)thiazol-4-yl) propanoate (5a):

Prepared by General procedure-B using ethyl 5-bromo-4-oxopentanoate in place of ethyl 3-bromo-2-oxopropanoate, white solid, Yield - 68%. Mass m/z: 382.1 (M+1).

4.8 Synthesis of ethyl 2-(2-(4-methoxydibenzo[b,d]furan-1-yl)thiazol-4-yl)acetate (5b):

Prepared by General procedure-B using ethyl 4-bromo-2-oxobutanoate in place of ethyl 3-bromo-2-oxopropanoate. White solid, Yield - 53%. Mass m/z: 368.1 (M+1).

4.9 Synthesis of ethyl 2-(4-methoxydibenzo[b,d]furan-1-yl)-5-methylthiazole-4-carboxylate (5c):

General procedure-C: To a stirred solution of 4-methoxydibenzo[b,d]furan-1-carbothioamide (1.0 mmol) in ethanol (5 mL) was added potassium carbonate (1.5 mmol) followed by 3-bromo-2-oxobutanoate (1.2 mmol) at RT. The reaction mixture was refluxed at 80°C for 2 h, subsequently it was poured into cold water (25 mL) and the precipitate obtained was filtered to get a peach coloured solid, Yield -10%; Mass m/z: 340.0 (M+1).

4.10 Synthesis of ethyl 2-(4-difluoromethoxydibenzo[b,d]furan-1-yl)-5-methylthiazole-4-carboxylate:

Prepared by General procedure-C using 4-difluoromethoxydibenzo[b,d]furan-1-carbothioamide in place of 4-methoxydibenzo[b,d]furan-1-carbothioamide to get peach coloured solid. Yield - 15%. Mass m/z: 404.09 (M+1).

4.11 General procedure for the hydrolysis of esters to acids (4, 6 Carboxylic acids of 15-24, 25, 28 and 32)

4.11.1 Synthesis of 6-(2-(4-methoxydibenzo[b,d]furan-1-yl)thiazol-4-yl)picolinic acid (4)

General procedure-D: To a slurry of ethyl 6-(2-(4-methoxy dibenzo[b,d]furan-1-yl)thiazol-4-yl) picolinate (50 mg, 0.116 mmol) in ethanol (5 mL) was added potassium hydroxide (30 mg, 0.535 mmol) followed by water (0.5 mL) and the mass was stirred at RT for 2 h. Subsequently the reaction mixture was poured into cold water, acidified with 1N HCl to a pH of 3-4; and the precipitated solid was filtered and triturated with ether followed by n-hexane to give a off-white solid; Yield - 42%; TLC - R_f 0.23 ($CHCl_3$: MeOH, 8.5:1.5); HPLC purity- 96%; M.P. N/R; ^{1}H-NMR (400MHz, $CDCl_3$): δ 4.15 (s, 3H, -OCH_3), 7.09 (d, J = 8.4 Hz, 1H, Ar-H), 7.26-7.32 (m, 1H, Ar-H), 7.51-7.55 (m, 1H, Ar-H), 7.67-7.71 (m, 2H, Ar-H), 8.08-8.12 (m, 1H, Ar-H), 8.24 (d, J = 6.56 Hz, 2H, Ar-H), 8.56 (d, J = 7.84 Hz, 1H, Ar-H), 8.75 (d, J = 7.92 Hz, 1H, Ar-H); Mass m/z: 403.1(M+1).

4.11.2 2-(4-Methoxydibenzo[b,d]furan-1-yl)-1,3-thiazole-4-carboxylic acid (6)

Off-white solid, Yield - 70%; TLC - R_f 0.38 ($CHCl_3$: MeOH, 9:1); HPLC purity - 98.5%; M.P. 228-230 °C ; ^{1}H-NMR (DMSO-d_6): δ 4.08 (s, 3H, -OCH_3), 7.33-7.39 (m, 2H, Ar-H), 7.58-7.62 (m, 1H, Ar-H), 7.78 (d, J = 8.28 Hz, 1H, Ar-H), 7.85 (d, J = 8.44 Hz, 1H, Ar-H), 8.58 (s, 1H, Ar-H), 9.27 (d, J = 7.96 Hz, 1H, Ar-H); Mass m/z: 326.0 (M+1).

Followed the same procedure in synthesising the other acids 6a and 6b.

4.11.3 2-[4-(Difluoromethoxy)-8-(trifluoromethyl)dibenzo[b,d]furan-1-yl]-1,3-thiazole-4-carboxylic acid (25)

Pale brown coloured solid, Yield - 76%; TLC - R_f 0.41 (CH_2Cl_2 : MeOH, 9:1); HPLC purity - 94.1%; M.P. 246-248 °C; ^{1}H-NMR (DMSO-d_6): δ 7.42-7.59 (m, 2H, Ar-H), 8.02-8.09 (m, 3H, Ar-H), 8.69 (s, 1H, Ar-H), 10.02 (s, 1H, Ar-H), 13.38 (s, 1H, -COOH); Mass m/z: 430.0 (M+1).

4.11.4 2-[8-(Acetylamino)-4-(difluoromethoxy)dibenzo[b,d]furan-1-yl]-1,3-thiazole-4-carboxylic acid (28)

Off-white solid, Yield - 77%; TLC - R_f 0.29 (CH_2Cl_2 : MeOH, 8:2); HPLC purity – 96.8%; M.P. 301.5-303.5 °C; ^{1}H-NMR (DMSO-d_6): δ 2.03 (s, 3H, -$NHCOCH_3$), 7.37-7.56 (m, 2H, Ar-H), 7.74-7.78 (m, 3H, Ar-H), 8.62 (s, 1H, Ar-H), 8.78 (s, 1H, Ar-H), 9.90 (s, 1H); Mass m/z: 417.0 (M-1).

4.11.5 2-(4-(Difluoromethoxy)-8-(methylsulfonamido)dibenzo[b,d]furan-1-yl)-5-methyl-1,3-thiazole-4-carboxylic acid (32)

Off-white solid, Yield - 45%; TLC - R_f 0.49 (CH_2Cl_2 : MeOH, 9:1). HPLC purity - 94.7%; M.P. 278-283 °C; ^{1}H -NMR (DMSO-d_6): δ 2.84 (s, 3H, -CH_3), 2.97 (s, 3H, -SO_2CH_3), 7.37-7.56 (m, 3H, Ar-H), 7.74 (d, *J* = 8.52 Hz, 1H, Ar-H), 7.83 (d, *J* = 8.84 Hz, 1H, Ar-H), 8.74 (d, *J* = 2.16 Hz, 1H, Ar-H), 9.61 (s, 1H, Ar-H), 13.14 (s, 1H, -COOH); Mass m.z: 469.0 (M+1).

4.12 General procedure for the Synthesis of amides (7-24, 26, 27, 29-31 & 32).

Acid compound (**6, 6a, 6b, 6c** or **25** or **28** or **32**; 1.0 mmol), EDCI (1.0 mmol), HOBt (1.0 mmol), DMAP (0.5 mmol) and DIPEA (5 mmol) were taken in DMF (4 mL for each 100 mg of acid), stirred for 20 minutes and cooled to 0 °C. Corresponding amine (1.2 mmol) was added and the temperature was brought to RT. The reaction mixture was stirred till completion of the reaction, cold water was added and the precipitate settled was filtered. If the precipitate could not be filtered, it was treated with MDC (3x 25 mL) and water (30 mL). The organic layer was separated, washed with brine solution, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The resulting crude product was purified by column chromatography over silica gel using MDC/MeOH solvent combinations (up to 10% MeOH in MDC) to get the corresponding title compounds.

4.12.1 2-(4-Methoxydibenzo[b,d]furan-1-yl)-N-phenyl-1,3-thiazole-4-carboxamide (7)

Pale yellow coloured solid, Yield - 31%; TLC - R_f 0.53 (EtOAc : n-Hexane, 3:7); HPLC purity - 99.2%; M.P. 206-208 °C; ^{1}H-NMR ($CDCl_3$): δ 4.16 (s, 3H, -OCH_3), 7.09 (d, *J* = 8.36 Hz, 1H, Ar-H), 7.14-7.18 (m, 1H, Ar-H), 7.31-7.41 (m, 3H, Ar-H), 7.53-7.57 (m, 1H, Ar-H), 7.62 (d, *J* = 8.32 Hz, 1H, Ar-H), 7.71-7.74 (m, 3H, Ar-H), 8.32 (s, 1H, Ar-H), 8.52 (d, *J* = 8 MHz, 1H, Ar-H), 9.39 (s, 1H, Ar-H); Mass m/z: 401.1 (M+1).

4.12.2 N-(4-Chlorophenyl)-2-(4-methoxydibenzo[b,d]furan-1-yl)-1,3-thiazole-4-carboxamide (8)

Off-white solid, Yield - 20%; TLC - R_f 0.6 (EtOAc : n-Hexane, 3:7); HPLC purity - 98.4%; M.P. 225-228 °C; ^{1}H-NMR ($CDCl_3$): δ 4.16 (s, 3H, -OCH_3), 7.09 (d, *J*

= 8.4 Hz, 1H, Ar-H), 7.26-7.35 (m, 3H, Ar-H), 7.54 (d, *J* = 7.24 Hz, 1H, Ar-H), 7.57 (m, 1H, Ar-H), 7.61 (d, *J* = 8.36 Hz, 2H, Ar-H), 7.66-7.73 (m, 1H, Ar-H), 8.32 (s, 1H, Ar-H), 8.47 (d, *J* = 8.4 Hz, 1H, Ar-H), 9.39 (s, 1H, Ar-H); Mass m/z: 435.0 (M+1)

4.12.3 2-(4-Methoxydibenzo[b,d]furan-1-yl)-1,3-thiazole-4-carboxamide (9)

Off-white solid, Yield - 35%; TLC - R_f 0.53 (EtOAc : n-Hexane, 7:3); HPLC purity – 92.1%; M.P. 213-215 °C; ^{1}H-NMR (DMSO-d_6): δ 4.08 (s, 3H, -OCH_3), 7.32-7.37 (m, 2H, Ar-H), 7.59 (s, 1H, Ar-H), 7.73-7.80 (m, 4H, Ar-H), 8.43 (s, 1H, Ar-H), 8.61 (s, 1H, Ar-H); Mass m/z: 325.0 (M+1)

4.12.4 N-(3,5-Dichloropyridin-4-yl)-2-(4-methoxydibenzo[b,d]furan-1-yl)-1,3-thiazole-4-carboxamide (10)

White solid, Yield - 46%; TLC - R_f 0.61 (EtOAc : n-Hexane, 1:1); HPLC purity – 96.5%; M.P. 233-238 °C; ^{1}H-NMR ($CDCl_3$): δ 4.14 (s, 3H, -OCH_3), 7.09 (d, *J* = 8.44 Hz, 1H), 7.27-7.34 (m, 1H, Ar-H), 7.49-7.55 (m, 1H, Ar-H), 7.61 (d, *J* = 8.32 Hz, 1H, Ar-H), 7.64-7.70 (m, 1H, Ar-H), 8.41 (s, 1H, Ar-H), 8.45-8.51 (m, 1H, Ar-H), 8.61 (d, *J* = 7.28 Hz, 2H, Ar-H), 9.17 (s, 1H, -NH); Mass m/z: 470.0 (M+).

4.12.5 (2-(4-Methoxydibenzo[b,d]furan-1-yl)-1,3-thiazol-4-yl)(4-(pyrimidin-2-yl)piperazin-1-yl)methanone (11)

Off-white solid, Yield - 25%; TLC - R_f 0.61 (EtOAc : n-Hexane, 7:3); HPLC purity – 99.7%; M.P. 177-180 °C; ^{1}H-NMR (DMSO-d_6): δ 3.82-3.86 (m, 8H, - piperdine CH_2), 4.08 (s, 3H), 6.67-6.71 (m, 1H, Ar-H), 7.34-7.38 (m, 2H, Ar-H), 7.58-7.61 (m, 1H, Ar-H), 7.78 (d, *J* = 8.16 Hz, 1H, Ar-H), 7.83 (d, *J* = 8.52 Hz, 1H, Ar-H), 8.30 (s, 1H, Ar-H), 8.37 (d, *J* = 4.64 Hz, 2H, Ar-H), 8.87 (d, *J* = 8.04 Hz, 1H, Ar-H); Mass m/z: 472.1 (M+1).

4.12.6 (2-(4-Methoxydibenzo[b,d]furan-1-yl)-1,3-thiazol-4-yl)(4-(pyridin-2-yl)piperazin-1-yl)methanone (12)

Off-white solid, Yield - 25%; TLC - R_f 0.61 (EtOAc : n-Hexane, 7:3); HPLC purity - 96.5%; M.P. N/R; ^{1}H-NMR (DMSO-d_6) δ 3.54-3.86 (m, 8H, - piperdine CH_2), 4.08 (s, 3H, -OCH_3), 6.65-6.68 (m, 1H, Ar-H), 6.84 (d, *J* = 8.56 Hz, 1H, Ar-H), 7.34-7.39 (m, 2H, Ar-H), 7.52-7.60 (m, 2H, Ar-H), 7.77-7.84 (m, 2H, Ar-H), 8.11 (d, *J* = 3.28 Hz, 1H, Ar-H), 8.30 (s, 1H, Ar-H), 8.88 (d, *J* = 7.88 Hz, 1H, Ar-H); Mass m/z: 472.1 (M+1).

4.12.7 2-(4-Methoxydibenzo[b,d]furan-1-yl)-N-morpholino-1,3-thiazole-4-carboxamide (13)

Green coloured solid, Yield - 28%; TLC - R_f 0.23 (EtOAc : n-Hexane, 7:3); HPLC purity - 99.9%; M.P. 205-209 °C; ^{1}H-NMR ($CDCl_3$): δ 2.98 (s, 4H, -NCH_2CH_2), 3.90 (s, 4H, -OCH_2CH_2), 4.14 (s, 3H, -OCH_3), 7.07 (d, *J* = 8.36 Hz, 1H, Ar-H), 7.29 (d, *J* = 7.52 Hz, 1H, Ar-H), 7.52-7.60 (m, 2H, Ar-H), 7.70 (d, *J* = 7.96 Hz, 1H), 8.23 (s, 1H, Ar-H), 8.28 (s, 1H, Ar-H), 8.46 (d, *J* = 7.88 Hz, 1H, Ar-H); Mass m/z: 410.0 (M+1).

4.12.8 N-(4-Hydroxycyclohexyl)-2-(4-methoxydibenzo[b,d]furan-1-yl)-1,3-thiazole-4-carboxamide (14)

Off-white solid, Yield -28%; TLC - R_f 0.43 (CH_2Cl_2 : MeOH, 9.4:0.6); HPLC purity - 98.3%; M.P. 211-213 °C; ^{1}H-NMR ($CDCl_3$): δ 1.33-1.48 (m, 6H, -CH_2), 2.02-2.04 (m, 2H, -CH_2), 2.14-2.17 (m, 2H, -CH_2), 3.41-3.47 (m, 1H, -CH), 3.82-3.85 (m, 1H, -CH), 4.14 (s, 3H, -OCH_3), 7.07 (d, *J* = 8.36 Hz, 1H, Ar-H), 7.35 (d, *J* = 8.68 Hz, 1H, Ar-H), 7.51-7.55 (m, 1H, Ar-H), 7.59 (d, *J* = 8.32 Hz, 1H, Ar-H), 7.69 (d, *J* = 8.28 Hz, 1H, Ar-H), 8.19 (s, 1H, Ar-H), 8.46 (d, *J* = 8 Hz, 1H, Ar-H); Mass m/z: 423.1 (M+1).

4.12.9 N-(3,5-Dichloropyridin-4-yl)-2-(2-(4-methoxydibenzo[b,d]furan-1-yl)-1,3-thiazol-4-yl)acetamide (15)

Pale brown coloured solid, Yield - 25%; TLC - R_f 0.69 (EtOAc : n-Hexane, 1:1); HPLC purity - 94.5%; M.P. 236-240 °C; ^{1}H-NMR ($CDCl_3$): δ 4.13-4.18 (m, 5H, CH_2,-OCH_3), 7.06 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.19-7.23 (m, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.45-7.49 (m, 1H, Ar-H), 7.60 (d, *J* = 8.36 Hz, 1H, Ar-H), 7.65 (d, *J* = 8.32 Hz, 1H, Ar-H), 8.38 (s, 2H, Ar-H), 8.51 (d, *J* = 8 Hz, 1H, Ar-H), 9.50 (s, 1H, -NHCO); Mass m/z: 484.0 (M+).

4.12.10 N-(3,5-Dichloropyridin-4-yl)-3-(2-(4-methoxydibenzo[b,d]furan-1-yl)-1,3-thiazol-4-yl)propanamide (16)

Pale pink coloured solid, Yield - 41%; TLC - R_f 0.48 (EtOAc : n-Hexane, 1:1); HPLC purity - 91.2%; M.P. N/R; ^{1}H-NMR ($CDCl_3$): δ 2.99-3.02 (m, 2H, -CH_2), 3.38-3.41 (m, 2H, -CH_2CO), 4.13 (s, 3H, Ar-H), 7.04-7.15 (m, 3H, Ar-H), 7.56 (d, *J* = 8.32 Hz, 2H, Ar-H), 8.28 (s, 2H, Ar-H), 8.33 (d, *J* = 7.92 Hz, 1H, Ar-H), 9.36 (s, 1H, -NH); Mass m/z: 498.0 (M+).

4.12.11 N-Ethyl-2-(4-methoxydibenzo[b,]furan-1-yl]-5-methyl-1,3-thiazole-4-carboxamide (17)

Off-white solid, Yield - 21%; TLC - R_f 0.45 (EtOAc : n-Hexane, 3:7); HPLC purity - 97.5%; M.P. 146-150 °C; ^{1}H-NMR ($CDCl_3$): δ 1.17-1.21 (m, 3H, -

NCH_2CH_3), 2.88 (s, 3H. $-CH_3$), 3.39-3.46 (m, 2H, $-CH_3$), 4.06 (s, 3H, $-OCH_3$), 6.97 (d, *J* = 8.36 Hz, 1H, Ar-H), 7.19-7.23 (m, 1H, Ar-H), 7.42-7.49 (m, 3H, Ar-H), 7.61 (d, *J* = 8.28 Hz, 1H, Ar-H), 8.39 (d, *J* = 7.96 Hz, 1H, Ar-H); Mass m/z: 367.1 (M+1).

4.12.12 N-(4-Hydroxycyclohexyl)-2-(4-methoxydibenzo[b,d]furan-1-yl)-5-methyl-1,3-thiazole-4-carboxamide (18)

Off-white solid, Yield - 26%; TLC - R_f 0.53 (CH_2Cl_2 : MeOH, 9.5:0.5); HPLC purity - 96.1%; M.P. 216-220 °C; ^{1}H-NMR (DMSO-d_6): δ 1.34-1.42 (m, 4H, aliphatic CH_2), 1.83-1.90 (m, 4H, aliphatic CH_2), 2.83 (s, 3H, $-CH_3$), 3.33-3.38 (m, 1H, -NHCH), 3.71-3.89 (m, 1H, -OHCH), 4.06 (s, 3H, $-OCH_3$), 4.57 (d, *J* = 4.22 Hz 1H), 7.30-7.35 (m, 2H, Ar-H), 7.58-7.62 (m, 1H, Ar-H), 7.67 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.78-7.85 (m, 2H, Ar-H), 8.62 (d, *J* = 8 Hz, 1H, Ar-H); Mass m/z: 437.1 (M+1).

4.12.13 N-Cyclopentyl-2-(4-methoxydibenzo[b,d]furan-1-yl)-5-methyl-1,3-thiazole-4-carboxamide (19)

Off-white solid, Yield - 35%; TLC - R_f 0.54 (EtOAc : n-Hexane, 3:7); HPLC purity - 98.8%; M.P. 147-150 °C; ^{1}H-NMR ($CDCl_3$): δ 1.51-1.54 (m, 2H, aliphatic CH_2), 1.62-1.73 (m, 4H, aliphatic CH_2), 2.06-2.10 (m, 2H, aliphatic CH_2), 2.95 (s, 3H, CH_3), 4.13 (s, 3H, $-OCH_3$), 4.41-4.45 (m, 1H, -CH), 7.04 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.52-7.57 (m, 3H, Ar-H), 7.68 (d, *J* = 8.24 Hz, 1H, Ar-H), 8.54 (d, *J* = 7.04 Hz, 1H, Ar-H); Mass m/z: 407.1 (M+1).

4.12.14 N-(3,5-Dichloropyridin-4-yl)-2-(4-methoxydibenzo[b,d]furan-1-yl)-5-methyl-1,3-thiazole-4-carboxamide (20)

Off-white solid, Yield - 24%; TLC - R_f 0.51 (EtOAc : n-Hexane, 4:6); HPLC purity - 92.2%; M.P. 229-231 °C; ^{1}H-NMR (DMSO-d_6): δ 2.87 (s, 3H, $-CH_3$), 4.08 (s, 3H, $-OCH_3$), 7.30-7.36 (m, 2H, Ar-H), 7.57-7.61 (m, 1H, Ar-H), 7.70 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.78 (d, *J* = 8.5 Hz, 1H, Ar-H), 8.63 (d, *J* = 8 Hz, 1H, Ar-H), 8.76 (s, 2H, Ar-H), 10.44 (s, 1H, -CONH); Mass m/z: 484 (M+).

4.12.15 N-(3,5-dichloropyridin-4-yl)-2-(4-(difluoromethoxy)dibenzo[b,d]furan-1-yl)-1,3-thiazole-4-carboxamide (21)

Pale pink coloured solid, Yield - 26%; TLC - R_f 0.65 (EtOAc : n-Hexane, 1:1); HPLC purity - 95.6%; M.P. 179-182 °C; ^{1}H-NMR (DMSO-d_6): δ 7.26-7.34 (m, 1H, Ar-H), 7.41 (d, *J* = 8 Hz, 1H, Ar-H), 7.55-7.61 (m, 2H, Ar-H), 7.66-7.71 (m, 1H, Ar-H), 8.44 (d, *J* = 7.8 Hz, 1H, Ar-H), 8.48 (s, 1H, Ar-H), 8.60 (s, 2H, Ar-H), 9.14 (s, 1H, Ar-H); Mass m/z: 506 (M+1).

4.12.16 2-((4-Difluoromethoxy)dibenzo[b,d]furan-1-yl)-N-(2,2,2-trifluoroethyl)-1,3-thiazole-4-carboxamide (22)

Off-white solid, Yield - 32%; TLC - R_f 0.41 (EtOAc : n-Hexane, 3:7); HPLC purity - 98.6%; M.P. 234-236 °C; ^{1}H-NMR ($CDCl_3$): δ 4.14-4.17 (m, 2H, -$CH_2CH_2CF_3$), 6.74-7.10 (m, 1H, Ar-H), 7.31-7.35 (m, 1H, Ar-H), 7.39 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.56-7.61 (m, 2H, Ar-H), 7.68-7.73 (m, 2H, Ar-H), 8.36 (d, *J* = 10.16 Hz 2H, Ar-H); Mass m/z: 443 (M+1).

4.12.17 2-((4-Difluoromethoxy)dibenzo[b,d]furan-1-yl)-N-methoxy-1,3-thiazole-4-carboxamide (23)

Off-white solid, Yield - 21%; TLC - R_f 0.39 (EtOAc : n-Hexane, 1:1); HPLC purity - 98.2%; M.P. 162-164 °C; ^{1}H-NMR ($CDCl_3$): δ 3.95 (s, 3H, -OCH_3), 6.74-7.10 (m, 1H, Ar-H), 7.33-7.39 (m, 2H, Ar-H), 7.55-7.60 (m, 2H, Ar-H), 7.69 (d, *J* = 8.32 Hz, 1H, Ar-H), 8.28 (d, *J* = 7.92 Hz, 1H, Ar-H), 8.37 (s, 1H, Ar-H), 9.77 (s, 1H, Ar-H); Mass m/z: 391 (M+1).

4.12.18 2-((4-Difluoromethoxy)dibenzo[b,d]furan-1-yl)-N-(4-hydroxycyclohexyl)-1,3-thiazole-4-carboxamide (24)

Off-white solid, Yield - 41%; TLC - R_f 0.49 (CH_2Cl_2 : MeOH, 9.4:0.6); HPLC purity - 96.5%; M.P. 160-163 °C; ^{1}H-NMR ($CDCl_3$): δ 1.26-1.52 (m, 4H, Aliphatic CH_2), 2.04 (d, *J* = 9.12 Hz, 2H, Aliphatic CH_2), 2.16 (d, *J* = 9.52 Hz, 2H, Aliphatic CH_2), 3.55-3.71 (m, 1H, -NHCH), 3.97-4.09 (m, 1H, -OHCH), 6.73-7.09 (m, 1H, Ar-H), 7.30 (d, *J* = 7.22 Hz, 1H, Ar-H), 7.38 (d, *J* = 8.36 Hz, 1H, Ar-H), 7.56-7.60 (m, 2H, Ar-H), 7.70 (d, *J* = 8.24 Hz, 1H, Ar-H), 8.26 (s, 1H, Ar-H), 8.40 (d, *J* = 8 Hz, 1H, Ar-H); Mass m/z: 459 (M+1).

4.12.19 2-(4-(Difluoromethoxy)-8-(trifluoromethyl)dibenzo[b,d]furan-1-yl)-N-(4-hydroxycyclohexyl)-1,3-thiazole-4-carboxamide (26)

Pale brown coloured solid, Yield - 77%; TLC - R_f 0.46 (CH_2Cl_2 : MeOH, 9:1); HPLC purity - 95.4%; M.P. 217-219 °C; ^{1}H-NMR (DMSO-d_6): δ 1.25-1.31 (m, 2H, aliphatic CH_2), 1.36-1.42 (m, 2H, aliphatic CH_2), 1.84 (d, *J* = 10.24 Hz, 4H, aliphatic CH_2), 3.33-3.38 (m, 1H, -NHCH), 3.72-3.82 (m, 1H, -OHCH), 4.57 (d, *J* = 4.32 Hz 1H, -OH), 7.42-7.67 (m, 1H, Ar-H), 7.92 (d, *J* = 8.52 Hz, 1H, Ar-H), 8.02 (d, *J* = 8.84 Hz, 1H, Ar-H), 8.07 (d, *J* = 7.96 Hz, 1H, Ar-H), 8.12 (d, *J* = 8.64 Hz, 1H, Ar-H), 8.52 (s, 1H, Ar-H), 9.06 (s, 1H, Ar-H); Mass m/z: 527 (M+1).

4.12.20 (2-(4-(Difluoromethoxy)-8-(trifluoromethyl)dibenzo[b,d]furan-1-yl)-1,3-thiazol-4-yl)(4-hydroxypiperidin-1-yl)methanone (27)

Pale brown coloured solid, Yield - 73%; TLC - R_f 0.47 (CH_2Cl_2 : MeOH, 9:1); HPLC purity - 95.9%; M.P. 212-214 °C; ^{1}H-NMR ($CDCl_3$): δ 1.21-1.23 (m, 1H), 1.24-1.30 (m, 1H), 1.42-1.48 (m, 2H), 1.60-1.65 (m, 1H, aliphatic CH_2), 1.80-1.85 (m, 1H), 3.74-3.83 (m, 2H), 4.01-4.05 (m, 1H), 4.78 (d, *J* = 3.52 Hz, 1H, -OH), 7.42-7.67 (m, 2H, Ar-H), 8.03 (d, *J* = 8.44 Hz, 2H, Ar-H), 8.11 (d, *J* = 8.6 Hz, 1H, Ar-H), 8.31 (s, 1H, Ar-H), 9.49 (s, 1H, Ar-H); Mass m/z: 513 (M+1).

4.12.21 2-(8-Acetamido-4-(difluoromethoxy)dibenzo[b,d]furan-1-yl)-N-(4-hydroxy cyclohexyl)-1,3-thiazole-4-carboxamide (29)

Off-white solid, Yield - 32%; TLC - R_f 0.57 (CH_2Cl_2 : MeOH, 8:2); HPLC purity - 99.1%; M.P. 225-227 °C; ^{1}H-NMR (DMSO-d_6): δ 1.23-1.31 (m, 2H), 1.40-1.51 (m, 2H), 1.78-1.88 (m, 4H), 2.05 (s, 3H, -$COCH_3$), 3.82-3.85 (m, 2H), 4.57 (d, *J* = 4.32 Hz, 1H, -OH), 7.52-7.55 (m, 1H, Ar-H), 7.73-7.80 (m, 4H, Ar-H), 8.50 (s, 1H, Ar-H), 8.69 (s, 1H, Ar-H), 10.12 (s, 1H, -CONH); Mass m/z: 516 (M+1).

4.12.22 N-(3,5-Dichloropyridin-4-yl)-2-(4-(difluoromethoxy)-8-(methyl sulfonamido) dibenzo[b,d]furan-1-yl)-1,3-thiazole-4-carboxamide (30)

Off-white solid, Yield - 22%; TLC - R_f 0.44 (EtOAc : n-Hexane, 6:4); HPLC purity - 98.9%; M.P. 237-240 °C; ^{1}H-NMR (DMSO-d_6): δ 2.87 (s, 3H, -SO_2CH_3), 7.53-7.68 (m, 3H, Ar-H), 7.81-7.85 (m, 2H, Ar-H), 8.16 (s, 1H, Ar-H), 8.74 (s, 2H, Ar-H), 8.81 (s, 1H, Ar-H), 9.63 (s, 1H, -NH), 10.42 (s, 1H, -NH); Mass m/z: 599 (M+).

4.12.23 2-(4-(Difluoromethoxy)-(methylsulfonamido)dibenzo[b,d]furan-1-yl)-N-(4-hydroxycyclohexyl)-1,3thiazole-4-carboxamide (31)

Yellow coloured solid, Yield - 84%; TLC - R_f 0.37 ($CHCl_3$: MeOH, 9:1); HPLC purity - 97%; M.P. N/R; ^{1}H-NMR ($CDCl_3$): δ 1.24-1.27 (m, 2H), 1.45-1.48 (m, 2H), 1.70-1.91 (m, 4H), 2.91 (s, 3H, -CH_3), 3.32-3.36 (m, 1H), 3.72-3.92 (m, 1H), 4.54 (d, *J* = 4.16 Hz, 1H, -OH), 7.49-7.57 (m, 3H, Ar-H), 7.77 (d, *J* = 8.44 Hz, 1H, Ar-H), 7.81-7.86 (m, 2H, Ar-H), 8.22 (s, 1H, Ar-H), 8.51 (s, 1H, Ar-H), 9.74 (s, 1H, -CONH); Mass m/z: 552 (M+1).

4.12.24 2-(4-(Difluoromethoxy)-8-(methylsulfonamido)dibenzo[b,d]furan-1-yl)-N-(4-hydroxycyclohexyl)-5-methyl-1,3thiazole-4-carboxamide (33)

Off-white solid, Yield - 66%; TLC - R_f 0.59 (CH_2Cl_2 : MeOH, 9:1); HPLC purity - 97%; M.P. 244-246 °C; ^{1}H-NMR (DMSO-d_6): δ 1.33-1.53 (m, 4H, aliphatic CH_2), 1.80-1.88 (m, 4H, aliphatic CH_2), 2.58 (s, 3H, -CH_3), 2.88 (s, 3H, -$NHCOCH_3$),

3.33-3.40 (m, 1H, -CH), 3.72-3.85 (m, 1H,-CH), 4.53 (d, *J* = 3.6 Hz, 1H, -OH), 7.48-7.55 (m, 2H, Ar-H), 7.67-7.72 (m, 2H, Ar-H), 7.84 (d, *J* = 8.88 Hz, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 9.76 (s, 1H, -NHCO); Mass m/z: 564 (M-1).

4.12.25 2-(4-(Difluoromethoxy)-8-(methylsulfonamido)dibenzo[b,d]furan-1-yl)-N-methoxy-5-methyl-1,3-thiazole-4-carboxamide (34)

Off-white solid, Yield - 22%; TLC - R_f 0.56 (EtOAc : n-Hexane, 8:2); HPLC purity - 95.5%; M.P. N/R; ^{1}H-NMR (DMSO-d_6): δ 2.87 (s, 3H, -CH_3), 2.94 (s, 3H, -SO_2CH_3), 3.70 (s, 3H, -$NHOCH_3$), 7.37-7.55 (m, 4H, Ar-H), 7.68-7.84 (m, 1H, Ar-H), 8.23 (s, 1H, Ar-H), 9.76 (s, 1H, -NH), 11.48 (s, 1H, -NH); Mass m/z: 498 (M+1)

4.2 <u>Biology</u>

4.2.1 Phosphodiesterase- 4 screening assay: PDE-4 (Phosphodiesterase type IV) enzymes convert cyclic AMP (cAMP) into AMP. The assay is performed to determine the effect of test compounds on the inhibition of purified human PDE-4B enzyme. It involves the detection of the tritiated AMP (product) using SPA beads known as yittrium silicate. The linear AMP binds preferentially to SPA yittrium silicate beads compared to cyclic nucleotides in the presence of zinc sulphate. The binding of the radiolabelled product to the bead brings the isotope into close proximity to allow radiation from the tritium to excite the scintillant within the bead to emit light. The unbound radiolabel is not close enough to allow this energy transfer and the light emitted due to the binding of tritiated AMP is detected as cpm.

<u>PDE Activity (SPA Based Assay Protocol)</u>

PDE-4B2 activity was inhibited by the compounds according to the invention in a modified SPA (scintillation proximity assay) test, supplied by GE Healthcare Life Sciences (see procedural instructions "Phosphodiesterase [^{3}H]-cAMP SPA enzyme assay, code TRKQ 7090"), carried out in 96-well microtitre plates. The test volume is 100 µL and contains 50 mM Tris buffer (pH 7.4), 8.3 mM Mg^{2+} in the presence of inhibitor or test compound, and containing PDE-4B2 enzyme (sufficient to ensure that 10-20% of the cAMP is converted under the said experimental conditions). The final concentration of DMSO in the assays (1% v/v) does not substantially affect the activity of the PDEs investigated. After a pre-incubation of 5 minutes at 37 °C the reaction is started by adding the substrate (cAMP; 0.5 µM cAMP, including about 50,000 cpm of [^{3}H]-cAMP) and the assays are incubated for a further 10 minutes; after that they are stopped by adding SPA beads containing 18 mM $ZnSO_4$ (50 µL). After the beads have been sedimented (>30 minutes) the microtitre plates are analyzed

in a Microplate luminescence detection device (TopCount* NXT; PerkinElmer Life Sciences). Where, the signal in the absence of enzyme is defined as the background. 100% activity was defined as the signal detected in the presence of enzyme and DMSO with the backdground subtracted. The corresponding IC_{50} values of the compounds for the inhibition of PDE-4B2 activity are determined from the concentration-effect curves by means of non-linear regression fit of the standard 4-parameter/multiple binding sites equation from an eight- to ten-point titration. Representative results of PDE-4B inhibition are shown in the Table-I at 100 *nM* [35, 36].

4.2.2 *In-vitro* measurement of Tumor Necrosis Factor Alpha (TNF-α): This assay determines the effect of test compounds on the production of TNF-α in human Peripheral Blood Mononuclear Cells (PBMC). Compounds were tested for their ability to inhibit the activity of TNF-α in human PBMC. PBMC were isolated from blood (of healthy volunteers) using BD Vacutainer CPT^{TM} (Cell preparation tube, BD Bio Science) and suspended in RPMI medium [37]. The test compounds were pre-incubated with PBMC (0.5million/incubation well) for 15 minutes at 37 ^{o}C and then stimulated with Lipopolysaccharide (*Escherichia coli*: B4; 1 µg/mL) for 18 hours at 37 ^{o}C in 5% CO_2. The levels of TNF-α in the cell culture medium were estimated using enzyme-linked immunosorbent assay performed in a 96 well format as per the procedure of the manufacturer (Cayman Chemical, Ann Arbor, USA). Representative results of TNF-α inhibition are shown in the Table-I.

4.2.3 LPS induced neutrophilia model for Asthma and COPD: LPS induced neutrophilia in Sprague Dawley rats was performed using the protocol described [38]. Male Sprague Dawley rats were acclimatized to laboratory conditions five to seven days prior to the start of the experiment. They were randomly distributed to various groups based on body weight. Except normal group all the animals were exposed to LPS 100 µg/mL for 40 minutes. The rats were dosed with the test compound suspended in the vehicle containing 0.25 % carboxymethylcellulose before half an hour of LPS exposure. BAL was performed 6 h after LPS exposure, total cell count and DLC was done and compared with control and the standard drug. Percentage Inhibition for neutrophilia was calculated and is shown in Table-II.

4.2.4 LPS induced sepsis for measurement of TNF-α inhibition in mice: The LPS induced sepsis model in mice was performed as described by Les sekut et al. [39].

Female Swiss albino mice were selected and the body weights were equivalent within each group. The mice were fasted for 20 h with free access to water. The mice were dosed orally with the test compound suspended in vehicle containing 0.5% Tween 80 in 0.25% Carboxy-methylcellulose sodium salt. The control mice were administered the vehicle alone. After 30 minutes of oral dosing, mice were injected with 500 µg of Lipopolysaccharide (*Escherichia coli*, LPS: B4 from Sigma) in phosphate buffer saline solution into the intraperitoneal cavity of the mice. After 90 minutes of LPS administration mice were bled via retro-orbital sinus puncture. Blood samples were stored overnight at 4 °C. Serum samples were collected by centrifuging the samples at 4000 rpm for 15 minutes at 4 °C. Immediately the serum samples were analysed for TNF-α levels using commercially available mouse TNF-α ELISA kit (Amersham Biosciences) and assay was performed accordingly to the manufacturer instruction. Representative results of TNF-α inhibition are shown in the Table-III.

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ABBREVIATIONS USED

PDE: phosphodiesterase; TNF-α: tumor necrosis factor; *nm*: nanomolar; IL: interleukin; μM: @ at; micromolar; PBMC: peripheral blood mononuclear cells; LPS: lipopolysaccharides; COPD: chronic obstructive pulmonary disease; CC: column chromatography; HCl: hydrochloric acid; h.: hour; RT: room temperature; M.P: melting point; ND: Not done; cpm: counts per minute; $CHCl_3$: chloroform; MDC/ CH_2Cl_2: methylene dichloride; MeOH: methanol; EtOAc: ethylacetate; HOBt: hydroxybenzotriazole; DMAP: dimethylaminopyridine; DMF: dimethylformamide; DIPEA: diisopropylethylamine; Exp: Experiment; cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate (cGMP); cmp: cytidine-5'-mono-phosphate; SPA: Scintillation proximity assay.

Graphical Abstract

In-vivo effective Dibenzo[b,d]furan-1-yl-thiazoles as Novel PDE-4 Inhibitors

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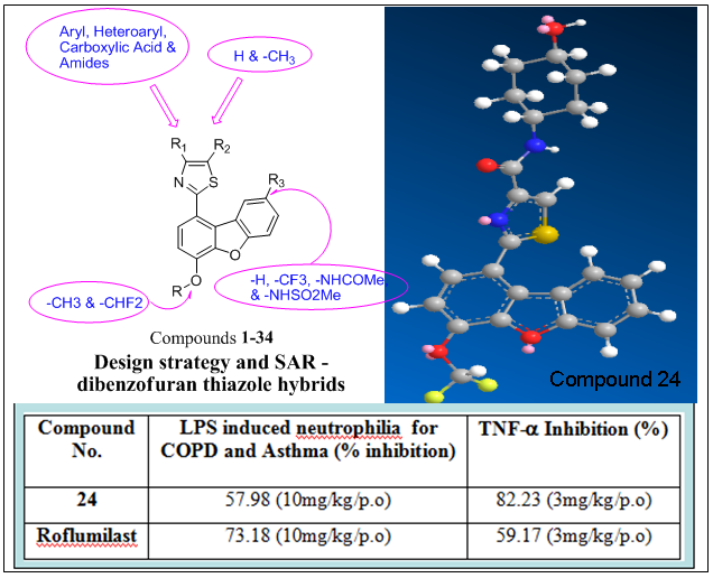

Compound No.	LPS induced neutrophilia for COPD and Asthma (% inhibition)	TNF-α Inhibition (%)
24	57.98 (10mg/kg/p.o)	82.23 (3mg/kg/p.o)
Roflumilast	73.18 (10mg/kg/p.o)	59.17 (3mg/kg/p.o)

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