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Research paper

Synthesis and bioactivity of phenyl substituted furan and oxazole carboxylic acid derivatives as potential PDE4 inhibitors

Yinuo Lin [a, 1], Wasim Ahmed [a, 1], Min He [a, 1], Xuwen Xiang [a], Riyuan Tang [b, c, **], Zi-Ning Cui [a, b, *]

[a] State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Integrative Microbiology Research Centre, Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou, 510642, China
[b] Guangdong Laboratory for Lingnan Modern Agriculture, Guangzhou, 510642, China
[c] Department of Applied Chemistry, College of Materials and Energy, South China Agricultural University, Guangzhou 510642, China

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ABSTRACT

In this present study, a series of 5-phenyl-2-furan and 4-phenyl-2-oxazole derivatives were designed and synthesized as phosphodiesterase type 4 (PDE4) inhibitors. *In vitro* results showed that the synthesized compounds exhibited considerable inhibitory activity against PDE4B and blockade of LPS-induced TNF-α release. Among the designed compounds, Compound **5j** exhibited lower IC_{50} value (1.4 μM) against PDE4 than parent rolipram (2.0 μM) in *in vitro* enzyme assay, which also displayed good *in vivo* activity in animal models of asthma/COPD and sepsis induced by LPS. Docking results suggested that introduction of methoxy group at para-position of phenyl ring, demonstrated good interaction with metal binding pocket domain of PDE4B, which was helpful to enhance inhibitory activity.

1. Introduction

Physiological processes of animals, plants and microbes are regulated by secondary messengers. Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) plays a cardinal regulatory role in airway epithelium, inflammatory cells, airway smooth muscle cells and immune cells in animal secondary messengers. Balance of these two important messengers (cAMP and cGMP) are disrupted by the group of enzymes called cyclic nucleotide phosphodiesterases (PDEs) [1,2]. These groups of enzymes consist of 11 contrasting families, segregated on the basis of their structures and properties during catalysis of secondary messengers [3,4]. Out of these 11 different members, PDE4 is considered as one of the most important enzyme for targeting the cAMP, and the presence of PDE4 is also reported in immune cells. PDE4 members target the catalysis of cAMP by hydrolysis of 3′-phosphodiester bond resulting in an inactive 5′-monophosphate [5] and thus inhibition of the PDE4 is contemplated an important enzymatic aspect to control it. PDE4 inhibition accelerates the increasing amount of cAMP which is helpful for physiological processes like airway muscle relaxation as well to stop the activation of proinflammatory cell activation. Hence, there is an acute need to develop potential inhibitor of PDE4 that can act as anti-inflammatory agent.

These types of chemical compounds can be further used for control of disease like asthma and pulmonary diseases [6,7]. In former times, researchers reported the development of novel inhibitors (rolipram and piclamilast) as anti-inflammatory drugs in 1990s (Fig. 1). Inhibition of PDE4 elevates the cellular cAMP levels, due to which the activation of specific phosphorylation cascade, that inhibit the release of tumor necrosis factors-α (TNF-α), activation of inflammatory cells and cytokines (interleukin-2, interleukin-12 and leukotriene B4).

* Corresponding author. State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Integrative Microbiology Research Centre, Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou, 510642, China..
** Corresponding author. Guangdong Laboratory for Lingnan Modern Agriculture, Guangzhou, 510642, China.
E-mail addresses: rytang@scau.edu.cn (R. Tang), ziningcui@scau.edu.cn (Z.-N. Cui).
[1] These authors contributed equally to this paper.

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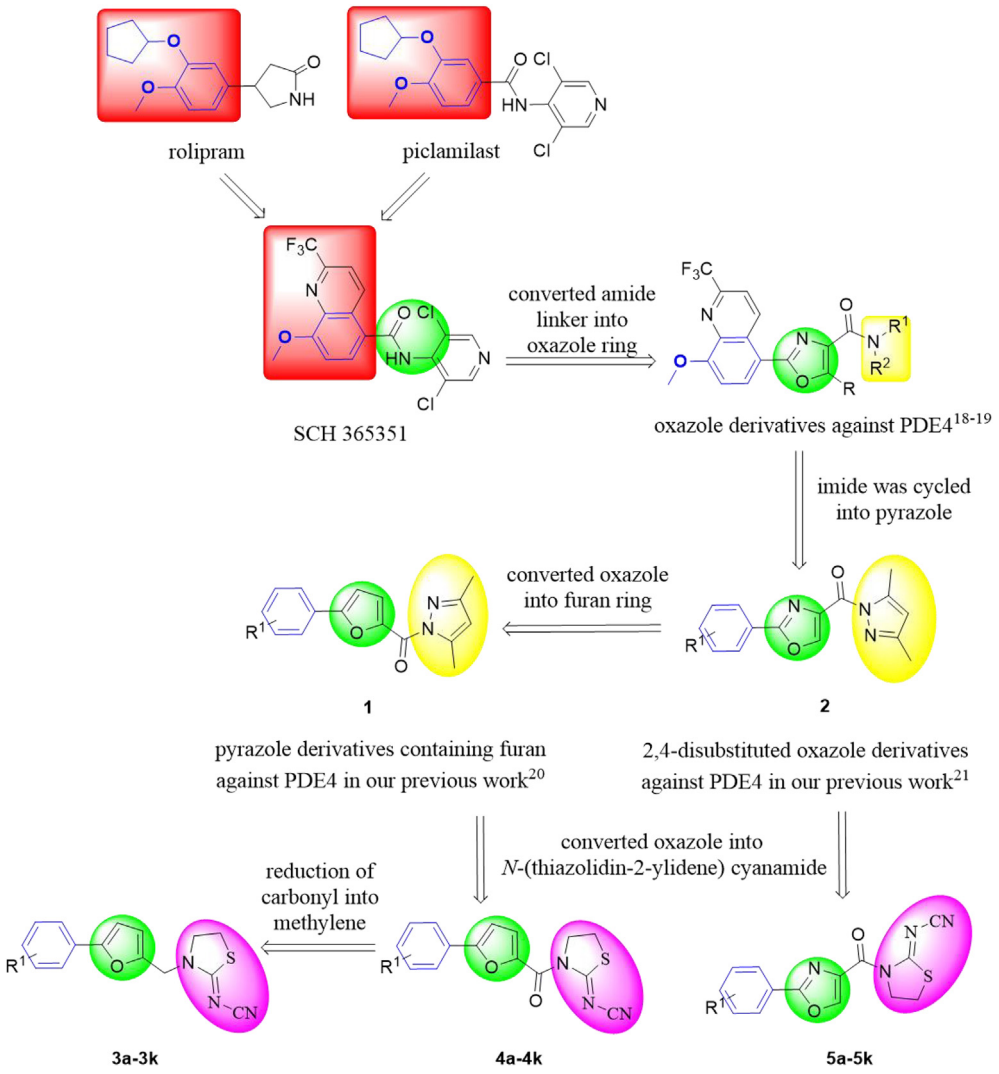


Fig. 1. The designed strategy for the title compounds [18–21].

Rolipram and piclamilast are well known for their high inhibitory activity against PDE4 and good curative effects, however, treatment with rolipram can cause nausea, sweating and other unbearable side effects for patients, as a structural analogue, piclamilast also contains aforementioned side effects, which limits their application. Hence, we have been focusing on the construction of new PDE4 inhibitor skeletons. As shown in our previous reports [8], 4-(3,4-dialkoxyphenyl) moiety was essential for PDE4 inhibition where the catechol ether oxygens played an important role in binding to the enzyme. Further study shows that the modification of the 4-(3,4-dialkoxyphenyl) moiety to the 8-methoxyquinoline-5-carboxamides (such as SCH 365351) could enhance the PDE4 inhibitory activity. Replacement of the amide moiety of SCH 365351 with five-membered heterocyclic rings (oxazole or furan ring) and the cyclization of imide moiety into pyrazole ring are both helpful to enhance PDE4 inhibitory activity. Further investigation found that simplification from 8-methoxyquinoline moiety to benzene ring was also beneficial to the enhancement of PDE4 inhibitory activity. Based on our previous findings described above, compounds **1** (pyrazole derivative containing furan ring) and **2** (pyrazole derivative containing oxazole ring) was designed to show significant PDE4 inhibitor activity [8,9] (Fig. 1). With these two skeletons in hand, we wish to disclose here design and synthesis of three novel series of compounds **3** (2-alkyl-5-phenyl furan derivative), **4** (2-carbonyl-5-phenyl furan derivative) and **5** (2-phenyl-4-carbonyl oxazole derivative) and speculated the effect of new introduced heterocyclic *N*-(thiazolidin-2-ylidene) cyanamide in the design. As demonstrating in our previous research, five membered heterocyclic moiety, forming hydrophobic interaction with protein, was essential for the bioactivity. A series of designed molecules derived from furan and oxazole scaffolds that are further inflated with superior PDE4 activity and selectively conducted in wet lab experiments and supported with the significant *in vivo* efficacy than rolipram.

2. Material and methods

2.1. General

Mass spectra were checked with a Bruker APEX IV spectrometer (Bruker, Fallanden, Switzerland). ^{1}H NMR and ^{13}C NMR spectra were measured on Bruker DPX600 (Bruker, Fallanden,

Switzerland), while tetramethylsilane was used as an internal standard. Melting points were recorded with a Cole-Parmer melting point apparatus (ColeParmer, Vernon Hills, Illinois, USA). Elemental analyses were performed on a Vario EL elemental analyzer. Analytical thin-layer chromatography was carried out on silica gel 60 F254 plates. The promoter activity of *hpa1* was checked by a FACS-Caliber flow cytometer (CytoFLEX USA). RNA concentration and purity were monitored using the Nanovue UV-Vs spectrophotometer (GE Healthcare Bio-Science, Sweden). The cDNA levels were quantified by Applied Biosystems 7500 Real-Time PCR System (Thermo,USA). The growth rates were recorded using a Bioscreen (Bioscreen, Finland).

2.2. Synthesis of title compounds **3**, **4** and **5**

2.2.1. General procedure for the synthesis of title compounds **3a-3k**

According to the reported literature [10a–c], Intermediates 5-substituted phenyl-2-furoic acid **7a-7k** were prepared from substituted aniline by Meerwein arylation reaction, and the corresponding 5-phenylfuran-2-carbonyl chloride were prepare using $SOCl_2$ as solvent and reactant. A solution of 5-phenylfuran-2-carbonyl chloride (5.0 mmol, 1 equiv) was prepared in dry THF and cooled to −10 °C under nitrogen atmosphere. Sodium borohydride (6.0 mmol, 1.2 equiv) was added to the THF solution and the reaction mixture was stirred for 2 h at 0 °C. The reaction was quenched by adding 10% aqueous ammonium chloride solution. THF was distilled off and the residue was diluted by adding chloroform and water. The product was extracted with three portions of chloroform and the organic layers were combined, dried over anhydrous $MgSO_4$, filtered and concentrated to get the crude alcohol. It was purified by column chromatography (silica gel, hexane and chloroform as eluents) to isolate a colorless liquid (80% yield).

A mixture of (5-phenylfuran-2-yl) methanol (4.0 mmol) in dry dichloromethane (20 mL) and pyridine (4.0 mmol) was cooled in an ice bath. Solution of thionyl chloride (15.0 mmol) in dry dichloromethane (10 mL) was added under N_2 atmosphere, at such a rate to keep the temperature between −10 and 0 °C. After complete addition, the reaction mixture was stirred at room temperature for 2 h. Ice was added and the reaction mixture was stirred for further 5 min. A small amount of $NaHCO_3$ was added to adjust pH 6.0. The organic layer was separated and dried over $MgSO_4$. Filtration and evaporation of the solvent gave 2-(chloromethyl)-5-phenylfuran (40% yield).

2-cyanoimino-1,3-thiazolidine (2.0 mmol) and potassium carbonate (3.0 mmol) were dissolved in 5 mL tetrahydrofuran and stirred at room temperature. To the solution, 2-(chloromethyl)-5-phenylfuran in acetonitrile was added dropwise. The reaction mixture was refluxed for 3 h. After the completion of the reaction, the solvent was evaporated under reduced pressure, water was added, and the mixture was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel chromatography to give the corresponding product in 64% yield.

2.2.2. General procedure for the synthesis of title compounds **4a-4l**

Intermediates 5-substituted phenyl-2-furoic acids **7a-7l** were prepared from substituted aniline by Meerwein arylation reaction according to the reported literature [10a–c].

Thionyl chloride (15 mL) was added to 0.01 mol of 5-substituted phenyl-2-furoic acid. The mixture was refluxed at 80 °C for 3 h. The reaction was monitored by periodic thin layer chromatography (TLC). When the reaction was completed, excess of thionyl chloride was removed under reduced pressure. The crude product was dissolved in 20 mL of anhydrous acetonitrile and added to the 10 mL of acetonitrile solution containing 2 mmol of 2-cyanoimino-1,3-thiazolidine and 2 mmol of K_2CO_3. The mixture was stirred for 3–6 h at 75 °C, then acetonitrile was removed under reduced pressure. An excess of water was added and the reaction mixture was extracted three times with dichloromethane. After that, it was washed successively with 10% HCl, 10% $NaHCO_3$ and water, dried over anhydrous $MgSO_4$. The product was purified by column chromatography (40 × 250 mm) on silica gel using dichloromethane and methanol (v/v 95:5) as the eluent to yield the title compounds.

2.2.3. General procedure for the synthesis of title compound **5a-5k**

Intermediates ethyl 2-phenyloxazole-4-carboxylate were prepared following the reported literature [11], followed by the hydrolysis of ester group to get 2-substituted phenyl-4-oxazole carboxylic acids as described below.

Ethyl 2-phenyloxazole-4-carboxylate was dissolved in THF-H_2O (1:1, 20 mL) and 2 M aqueous NaOH (2 equiv.) solution was added dropwise at 0 °C. Reaction mixture was stirred at room temperature for 2–4 h and monitored by TLC. Upon completion of reaction, THF was removed in vacuo and aqueous layer was washed with ethyl acetate and pH of aqueous layer was adjusted to 2 by the slow addition of 2 M aqueous HCl at 0 °C, which gives the precipitate at this stage. Solid was filtered, washed with water and dried in *vacuo* and used for the next step without further purification.

Mixture of 2-phenyloxazole-4-carboxylic acid (1 equiv.) and thionyl chloride (5 mL) was refluxed for 1 h under nitrogen atmosphere. Reaction was cooled to room temperature and excess of thionyl chloride was removed under *vacuum*. Crude mass was dissolved in acetonitrile, followed by the addition of K_2CO_3 (3 equiv.) and 2-cyanoimino-1,3-thiazolidine (1 equiv.) at room temperature. Reaction mixture was stirred for 3–6 h at room temperature and monitored by TLC. Upon completion of reaction, it was diluted with water and organic compounds were extracted with dichloromethane. Organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Product was purified by silica gel (200–300 mesh) column chromatography using methanol/DCM solvents as an eluent to give the title compound **5** in moderate to good yield.

2.3. Bioassay

2.3.1. Assay of human PDE4 activity

Enzyme PDE4 was isolated from the sample as previously described method [10a–c,12]. The enzyme was prepared from U937 cells which was derived from human monocytes, and was stored at −20 °C after preparation. Dilution of enzyme was done with triple distilled water containing bovine serum albumin and measurement of PDE4 activity was performed using this stored enzyme. The substrate solution was prepared by adding [^{3}H] cAMP (300,000 dpm (5000 Bq)/assay) and 100 μmol/L cAMP solution to 100 mmol/L Tris–HCl (pH 8.0) containing 5 mmol/L ethylene glycol-bis (*β*-aminoethyl ether) and *O,O′*-bis(2-aminoethyl) ethyleneglycol- *N,N,N′,N′*-tetraacetic acid. The substrate solution was mixed with the enzyme solution containing a test compound dissolved in DMSO, and incubation was done for 30 min at 30 °C. Assays were performed in duplicate at different concentrations of each test compound.

2.3.2. Assay of TNF-a release

The blood is mixed with saline at a ratio of 1:1, and the peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats using Lymphoprep tubes [13]. The PBMCs were suspended in RPMI 1640 with 0.5% human serum albumin, pen/strep, and 2 mM L-glutamine at 5 × 10^5 cells/mL. The cells were pre-incubated with

the test compounds in 96-well plates for 30 min and stimulated for 18 h with 1 mg/mL lipopolysaccharide. TNF-α concentration in the supernatants was measured by homogeneous time-resolved fluorescence resonance (TR-FRET). The assay is quantified by measuring fluorescence at 665 nm (proportional to TNF-α concentration) and 620 nm (control). Results are expressed as IC_{50} values (μM).

2.3.3. LPS induced sepsis for measurement of TNF-a inhibition in mice

The LPS induced sepsis model in mice was performed following the literature [14]. Female Swiss albino mice were selected according to the body weights, which were equivalent within each group. The mice were fasted for 20 h with free access to water and dosed for oral administration (po) with the test compounds suspended in vehicle containing 0.5% Tween 80 in 0.25% sodium salt of carboxymethyl cellulose. The control mice were performed the vehicle alone. After 30 min of oral dosing, the mice were injected into intraperitoneal cavity with 500 μg of lipopolysaccharide (*Escherichia coli*, LPS: B4 from Sigma) in phosphate buffer. Then the mice were bled via retro-orbital sinus puncture after 90 min of LPS administration. Serum samples were collected by centrifuging the blood samples at 4000 rpm for 20 min, which were stored overnight at 4 °C. Immediately, the serum samples were checked for TNF-α levels using commercial mouse TNF-α ELISA kit (Amersham Biosciences) and assay was carried out following the manufacturer instruction.

2.3.4. LPS induced neutrophilia model for asthma and COPD

LPS induced neutrophilia in Sprague Dawley rats was assessed according to the described protocol [15]. Male Sprague Dawley rats were acclimatized to laboratory conditions for one week prior to the experiment. According to the body weight, the rats were distributed to various groups randomly. Except normal group, all the rats were exposed to 100 μg/mL lipopolysaccharide (*Escherichia coli*, LPS: B4 from Sigma) for 40 min. The rats were dosed for oral administration (po) with the test compounds suspended in the vehicle containing 0.25% sodium salt of carboxymethylcellulose before half an hour of LPS exposure. Bronchoalveolar lavage (BAL) was performed 6 h after LPS exposure, total cell count and DLC (differential leukocyte count) were checked and compared with control.

2.4. Molecular docking

Molecular docking was performed on Surflex-Dock module of Sybyl 8.0 to know the exact active site of amino acids [10a–c,16]. Crystal structure of PDE4B (PDB ID:1XMY) obtained from Protein Date Bank was used as the receptor for molecular docking study. The 3D structure of compounds **3j**, **4j** and **5j** was drawn and optimized with SYBYL package. The docking procedure was started with the protomol generation, which was created using a ligand-based approach (native ligand for PDE4B structure). Proto threshold was set to 0.5 and proto bloat was kept at 0 as a default parameter. For docking, max conformation and max rotation values were 20 and 100, respectively. Pre-dock and post-dock energy minimization methods were also applied. Docking results were compared by the total score values. The pose with the higher total-score value was considered as the best one. After the end of molecular docking, the interactions of the docked domain with ligand were analyzed.

3. Result and discussion

3.1. Chemistry

Based on our previous research work, we have designed three new and novel modified structures, 2,5-disubstituted furan (**3** and **4**) and 2,4-disubstituted oxazole (**5**) as shown in Fig. 1. We wish to analyze the effect of different heterocyclic rings, therefore thiozolidine ring was selected to attach with furan or oxazole moieties, the effect of the reduction of amide to amine was also planned to analyze. Introduction of a heterocyclic ring 2-cyanoimino-1,3-thiazolidine was considered to be a new parameter of analysis.

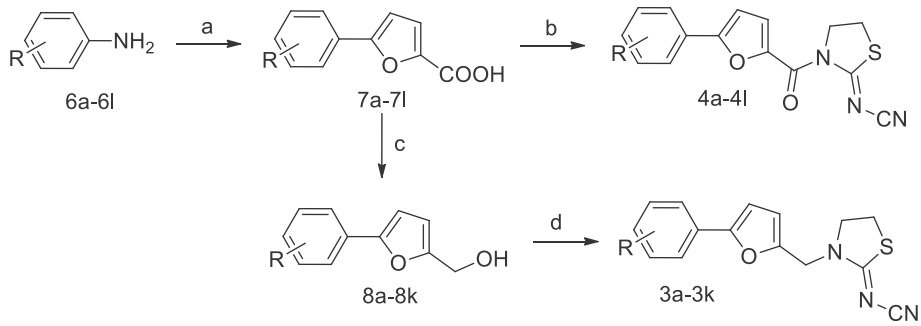
In order to prepare the title compounds **3** and **4**, intermediate 5-substituted phenyl-2-furoic acids **7a-7l** were synthesized (Meerwein arylation) following our previous reports [10a–c] (Scheme 1). Obtained carboxylic acids was refluxed in $SOCl_2$ for 1 h to form corresponding acid chlorides, which was coupled with *N*-(thiazolidin-2-ylidene) cyanamide in the presence of base K_2CO_3 in acetonitrile to furnish the title compounds **4a-4l** (Scheme 1). In order to probe the influence of amine group instead of amide, structure **3** was designed which is the reduced form of amide **4**. To synthesize alcohol precursor **8a-8k,** reduction of carboxylic acid **7a-7k** was first performed with lithium aluminium hydride, but due to low yield of the products, different methods were explored. Hence, carboxylic acid was converted into its corresponding acid chloride first by treatment with thionyl chloride and then it was reduced with sodium borohydride in THF/DMF solvent at −10 to 0 °C. Alcohol was obtained in moderate to good yield by applying this reduction method. In order to couple this key intermediate with *N*-(thiazolidin-2-ylidene) cyanamide, alcohol was transformed into the corresponding chloride by reacting with thionyl chloride, and then nucleophilic substitution with *N*-(thiazolidin-2-ylidene) cyanamide in presence of K_2CO_3 in acetonitrile, title compounds **3a-3k** were obtained. (Scheme 1).

Following the reported literature, carboxylic acid precursors of title compounds **5a-5k** were obtained by treating corresponding substituted formamides **9a-9k** with ethyl 3-bromopyruvate [11], followed by the hydrolysis of esters with sodium hydroxide. With the carboxylic acid in hand, it was planned to couple with *N*-(thiazolidin-2-ylidene) cyanamide, hence, different coupling methods were tried such as i. EDCI, HOBt; ii. DCC, DMAP, but the yield was not satisfactory. The best result was obtained by treatment of carboxylic acids with thionyl chloride and then coupled with *N*-(thiazolidin-2-ylidene) cyanamide (Scheme 2).

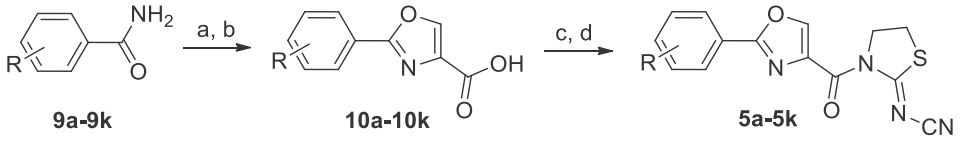
All the new compounds were characterized by ^1H, ^{13}C NMR and mass spectroscopy. The structures of compounds **3i**, **4l** and **5e** were confirmed by X-ray single crystal diffraction and shown in Fig. 2. (See the supporting information data for details).

3.2. Biological evaluation and SAR studies

The *in vitro* activity of the inhibition of PDE4 and blockade of LPS induced TNF-α in human blood monolayer cells were listed in Table 1. Rolipram was used as positive marker during experiment. It was observed that inhibition activity was highly influenced by the substitution at different position of phenyl ring. Compounds with chloro substitution were found to be more active than unsubstituted for PDE4B inhibition. Among the chloro substitution at different position, para substituted compound **3d** (IC_{50} = 15.7 μM) was found to be more effective than **3b** (IC_{50} = 101.6 μM) and **3c** (IC_{50} = 62.8 μM). On comparison with same substituted at series **4** and **5**, compound **5d** (IC_{50} = 3.6 μM) showed better activity. It was observed that chloro substituents compounds showed better activity (TNF-α inhibition) than unsubstituted. Among these chloro substituted compounds, para substituted compound **5d** showed



Scheme 1. Synthetic route to title compounds **3** and **4.** Reagents and conditions: (a) i. $NaNO_2$, HCl; ii. furan-2-carboxylic acid, cat. $CuCl_2$, acetone, H_2O; (b) i. $SOCl_2$, reflux, 1h; ii. K_2CO_3, *N*-(thiazolidin-2-ylidene) cyanamide, CH_3CN, rt, 3–6 h; (c) i. $SOCl_2$, reflux, 1h; ii. $NaBH_4$, THF, DMF, −10 to 0 °C; (d) i. $SOCl_2$, Py, DCM, −10 to −5 °C; ii. K_2CO_3, *N*-(thiazolidin-2-ylidene) cyanamide, CH_3CN, rt, 3–6 h.



Scheme 2. Synthetic route to title compounds **5a-5k**. Reagents and conditions: (a) i. ethyl 3-bromopyruvate, $NaHCO_3$, THF, 60 °C, 23 h; ii. $(CF_3CO)_2O$, THF, rt, 12 h; (b) 2 M aq. NaOH, THF, H_2O, 0 °C to rt, 2–4 h; (c) i. $SOCl_2$, reflux, 1 h; ii. K_2CO_3, *N*-(thiazolidin-2-ylidene) cyanamide, CH_3CN, rt, 3–6 h.

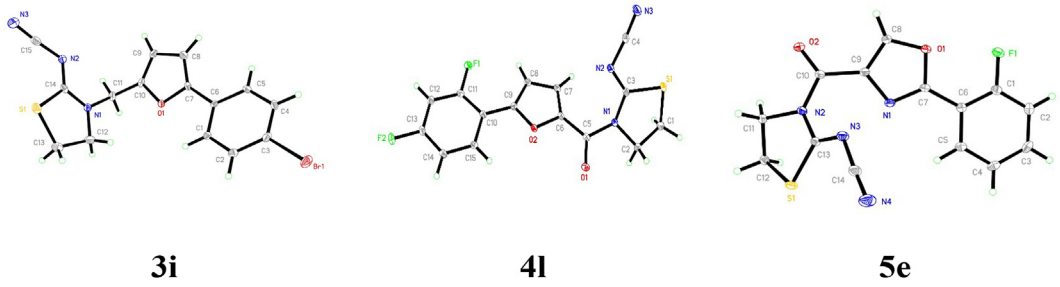


Fig. 2. Single crystal structures of compounds **3i**, **4l** and **5e**.

better results. Same pattern was observed for the fluoro substituted compounds **3g** (IC_{50} = 18.9 μM) and little variation was found in comparison with *para* chloro substituted compound (**3d).** When the substituent was changed to bromo at para position, inhibition activity was further decreased (**3i**: IC_{50} = 52.7 μM). In comparison with series **3**, **4** and **5**, compound **5i** (IC_{50} = 46.7 μM) exhibit better activity than **3i** (IC_{50} = 52.7 μM) and **4i** (IC_{50} = 49.6 μM). Inhibition of TNF-α also followed the similar pattern and **5i** (IC_{50} = 131.2 μM) showed better activity than **3i** (IC_{50} = 156.2 μM) and **4i** (IC_{50} = 138.9 μM). 2, 6-Difluoro substituent was also analyzed and found that **5k** (IC_{50} = 16.3 μM) revealed modest activity against PDE4B in comparison with **3k** (IC_{50} = 45.7 μM) and **4k** (IC_{50} = 22.1 μM), also the same pattern was observed against TNF-α (**5k**: IC_{50} = 49.3 μM, **4k**: IC_{50} = 60.1 μM, **3k**: IC_{50} = 82.3 μM). When the substituent at para position was changed from electron withdrawing to electron donating group, significance change was observed against PDE4B. IC_{50} values of **5h** was found 9.2 μM in comparison to **3h** and **4h** (IC_{50} = 20.1 μM and 12.5 μM respectively). Among the *para* substitutions, methoxy substituted (electron donating group) compound was found to be more promising. IC_{50} value of compound **5j** was found 1.4 μM which was better than **3j** (IC_{50} = 9.6 μM) and **4j** (IC_{50} = 2.8 μM). Also the inhibition activity against TNF-α for compound **5j** (IC_{50} = 11.8 μM) was better than any other compound of series **3** and **4**. We also speculated the selected *in vitro* active compounds for their PDE4B selectivity over PDE4D and it was concluded that compounds with all three series had obvious selectivity towards PDE4B. Compound **5j** showed better selectivity (PDE4D/B = 11.21) than reference rolipram and also with other tested compounds. Selected *in vitro* active compounds **3j**, **4j** and **5j** were tested for LPS induced sepsis model for the measurement of TNF-α inhibition (in female Swiss Albino mice) and neutrophilia inhibition for asthma and COPD (in male Sprague Dawley rats). Table 2 demonstrated the results of experiment including details such as oral dosages and number of animals grouped. The results showed that compound **5j** revealed better inhibitory activity against TNF-α release (52.6%) and LPS induced neutrophilia inhibition (42.1%) than the positive control rolipram (45.3% and 36.8%), compound **3j** (31.2% and 28.7%) and **4j** (40.5% and 33.8%).

Table 1
Inhibition (IC_{50}, μM) of enzymatic potency (PDE4B and PDE4D) and TNF-α release from human blood mononuclear cells stimulated with lipopolysaccharide [a].

Compd.	R	PDE4B IC_{50} (μM)	PDE4D	Selectivity (D/B)	TNF-α IC_{50} (μM)
3a	H	162.5 ± 5.8	NT[b]	–	198.6 ± 6.4
3b	2-Cl	101.6 ± 3.9	NT[b]	–	149.1 ± 4.2
3c	3-Cl	62.8 ± 2.9	NT[b]	–	89.7 ± 2.8
3d	4-Cl	15.7 ± 0.9	58.3 ± 1.2	3.71	44.2 ± 1.5
3e	2-F	85.6 ± 2.8	NT[b]	–	121.4 ± 3.7
3f	3-F	59.7 ± 2.1	NT[b]	–	89.5 ± 3.1
3g	4-F	18.9 ± 1.1	110.5 ± 3.1	5.85	45.8 ± 1.8
3h	4-CH_3	20.1 ± 1.1	121.5 ± 4.2	6.04	53.9 ± 1.8
3i	4-Br	52.7 ± 1.8	NT[b]	–	156.2 ± 6.6
3j	4-OCH_3	9.6 ± 0.8	32.8 ± 1.2	3.42	36.8 ± 1.5
3k	2,6-di-F	45.7 ± 1.4	159.6 ± 4.8	3.49	82.3 ± 2.5
4a	H	111.3 ± 4.5	NT[b]	–	169.5 ± 3.9
4b	2-Cl	62.8 ± 2.4	NT[b]	–	122.5 ± 3.7
4c	3-Cl	29.4 ± 1.5	99.8 ± 3.3	3.39	74.3 ± 1.9
4d	4-Cl	6.6 ± 1.0	26.4 ± 1.7	4.00	35.6 ± 1.0
4e	2-F	62.4 ± 1.2	NT[b]	–	109.8 ± 3.1
4f	3-F	37.8 ± 0.9	NT[b]	–	72.6 ± 2.5
4g	4-F	11.3 ± 0.7	55.7 ± 1.8	4.93	29.8 ± 0.9
4h	4-CH_3	12.5 ± 1.1	54.3 ± 2.1	4.34	41.3 ± 1.8
4i	4-Br	49.6 ± 1.2	NT[b]	–	138.9 ± 4.4
4j	4-OCH_3	2.8 ± 0.6	18.7 ± 1.0	6.68	15.3 ± 0.9
4k	2,6-di-F	22.1 ± 1.1	88.6 ± 1.9	4.01	60.1 ± 1.9
5a	H	71.2 ± 2.9	NT[b]	–	141.5 ± 5.7
5b	2-Cl	40.3 ± 1.8	NT[b]	–	101.4 ± 4.2
5c	3-Cl	18.1 ± 0.9	102.5 ± 3.1	5.66	62.5 ± 2.1
5d	4-Cl	3.6 ± 0.6	26.8 ± 1.1	7.44	27.2 ± 1.2
5e	2-F	52.8 ± 1.4	NT[b]	–	92.8 ± 3.7
5f	3-F	27.6 ± 1.0	NT[b]	–	64.3 ± 3.1
5g	4-F	6.8 ± 0.8	45.9 ± 1.3	6.75	22.1 ± 1.0
5h	4-CH_3	9.2 ± 0.7	66.9 ± 1.8	7.27	35.2 ± 1.4
5i	4-Br	46.7 ± 1.4	NT[b]	–	131.2 ± 5.2
5j	4-OCH_3	1.4 ± 0.3	15.7 ± 0.8	11.21	11.8 ± 0.6
5k	2,6-di-F	16.3 ± 1.0	98.7 ± 2.5	6.06	49.3 ± 1.8
rolipram		2.0 ± 0.3	2.8 ± 0.7	1.40	18.9 ± 0.9

[a] Results are the average of at least three assays.
[b] NT, not tested.

Table 2
LPS induced TNF-α in SA mice and neutrophil influx in BALF of SD rats.

Compd.	R	*Swiss Albino* mice (n = 6)		*Sprague Dawley* rats (n = 6)	
		Does(mg/kg, po)	TNF-á Inhibition (%)	Does(mg/kg, po)	LPS induced neutrophilia (% inhibition)
3j	4-OCH_3	10	31.2	10	28.7
4j	4-OCH_3	10	40.5	10	33.8
5j	4-OCH_3	10	52.6	10	42.1
rolipram		10	45.3	10	36.8

3.3. Docking results

The binding mode of the synthesized active compounds at the PDE4B cavity was simulated with docking analysis by using Surflex-Dock in Sybyl 8.0 software Fig. 3. For the docking tasks, crystal structures of the PDE4B with (*R*)-rolipram was employed (PDB ID: 1XMY, 2.4 Å resolution) [17]. In docking studies, it was observed that 4-methoxy phenyl ring of the compounds extended into the metal binding pocket domain, thiazolidine ring into Q switch and P clamp pocket [17]. Amide moiety of compound **4j** exhibited H-bonding interaction with the conserved glutamine (Gln443) residue which was involved in bidentate H-bonding with −CO- at 1.7 Å and 2.63 Å respectively Fig. 3C and 3D. Similarly, compound **5j** exhibited same interaction with the conserved Gln443 residue at 1.87 Å and 3.13 Å respectively Fig. 3E and 3F. Fig. 3 (A and B) demonstrated that reduction of the amide bond (compound **3j**) decreased its ability in establishing crucial interactions with Gln443, and only unveiled hydrophobic interaction between thiazolidine ring and phenylalanine (Phe 446) at a distance of 3.07 Å.

Further, compound **5j** also depicted more stability within the binding pocket due to the hydrophobic interaction formed between Phe 446 and thiazolidine ring of the compound. Figure 4 G and 4 H revealed that compound **4j** form more stable interaction with PDE4B compare with rolipram by extra coordinate bonds. Oxygen of *p*-methoxy group constituted coordinate bonds with the Zn^{2+} (**3j**: 4.56 Å, **4j**: 4.55 Å and **5j**: 3.40 Å, Fig. 4) and Mg^{2+} (**3j**: 2.45 Å **4j**: 3.46 Å and **5j**: 3.31 Å, Fig. 3) cations.

4. Summary

In the continuation of our effort towards the finding of new PDE4 inhibitors, herein, we designed and synthesized three novel series of compounds containing 2,5-disubstituted furan (**3** and **4**) and 2,4-disubstituted oxazole (**5**) moieties. Synthesized compounds were evaluated for *in vitro* activity against phosphodiesterase type 4 and TNF-α. Compounds **3j**, **4j** and **5j** were found to show moderate to good inhibitory activity against PDE4 and TNF-α. Further these compounds were tested for *in vivo* activity in animal

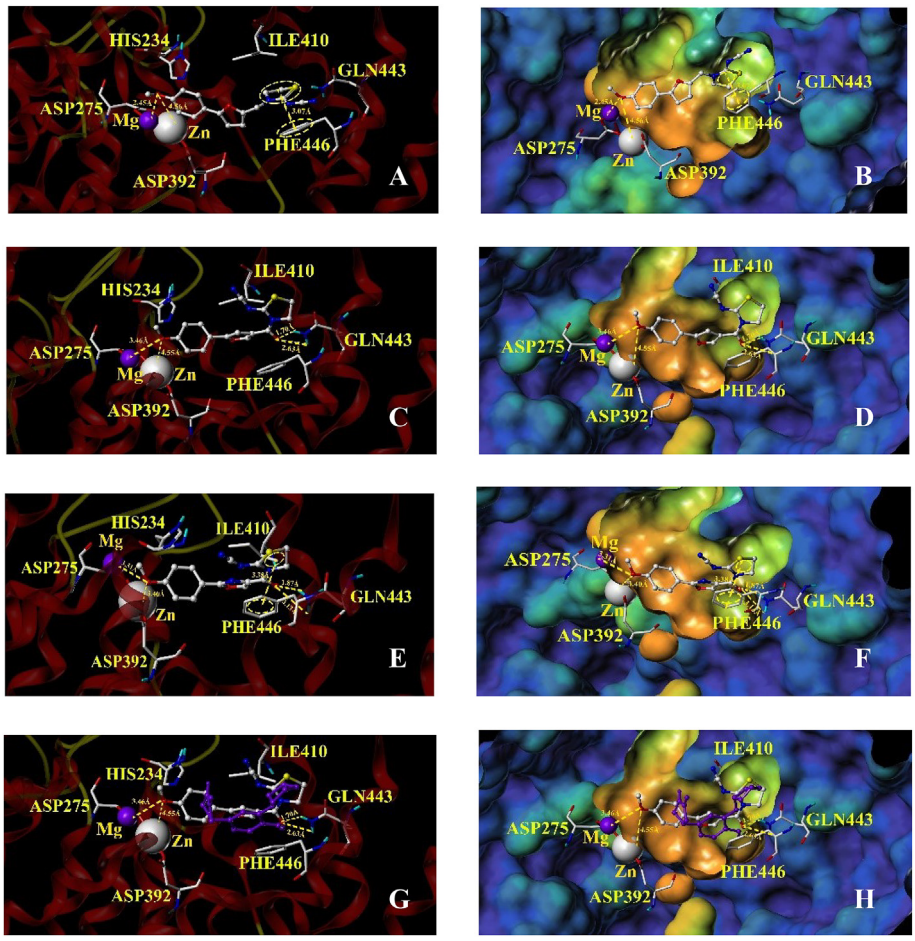


Fig. 3. Interaction of PDE4B in complex with compound **3j** (A, B), compound **4j** (C, D) and compound **5j** (E, F). The catalytic domain bound to **4j** overlaid with rolipram (violet color) (G, H). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

models of asthma/COPD and sepsis induced by LPS in which compound **5j** displayed good *in vivo* activity in animal models. A primary SAR study revealed that incorporation of 2-cyanoimino-1,3-thiazolidine ring was effective and favored the inhibitory activity and substituent at *para* position of phenyl ring in the molecule had an important effect of inhibitory and it can be concluded that the effect of substitutions at para position in the structures were found to be crucial. The docking results demonstrated that title compounds interacted well with PDE4B protein by intermolecular hydrogen bonding (−CO−N, Gln443), hydrophobic interaction (thiazolidine Phe 446) and the metal coordination in the ligand-receptor complex (*p*-OMe, Zn^{2+} and Mg^{2+}). We believe that such efforts will be beneficent in future towards the development of advanced and effective PDE4 inhibitors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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