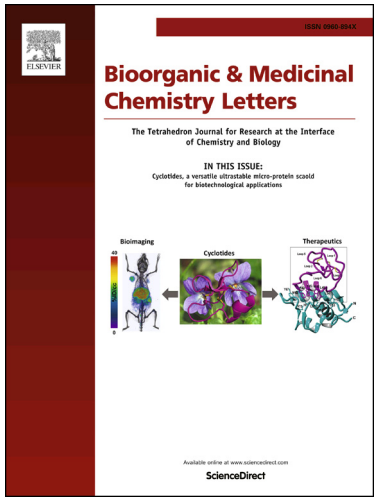

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Synthesis and bioactivity of 3,5-dimethylpyrazole derivatives as potential PDE4 inhibitors

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Abstract:

A series of 3,5-dimethylpyrazole derivatives containing 5-phenyl-2-furan moiety were designed and synthesized as phosphodiesterase type 4 (PDE4) inhibitors. Bioassay results showed that the title compounds exhibited considerable inhibitory activity against PDE4B and blockade of LPS-induced TNFα release. Among the designed compounds, compound **If** showed the best inhibitory activity against PDE4B with the IC_{50} value of 1.7 μM, which also showed good *in vivo* activity in animal models of asthma/COPD and sepsis induced by LPS. The primary structure–activity relationship (SAR) study and docking results suggested that introduction of the substituent groups to the phenyl ring at the *para*-position, especially methoxy group, was helpful to enhance inhibitory activity against PDE4B.

Keywords: 3,5-dimethylpyrazole derivatives; synthesis; PDE4 inhibitor; SAR; molecular simulation

Cyclic nucleotide phosphodiesterases (PDEs), which include 11 diverse families according to their structures and properties, are essential in the metabolism of the secondary signal messengers, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate(cGMP). [1,2] It is also well known that cAMP, specifically hydrolyzed by the PDE4, regulates the function of airway smooth muscle, inflammatory cells, and immune cells. [3,4] When PDE4 is inhibited, the accumulation of cAMP leads to activating the cascades of specific protein phosphorylation, which contributes to lots of functional responses such as suppression of TNFα production. Therefore, PDE4 inhibitors are an important type of anti-inflammatory drugs for the treatment of asthma and chronic obstructive pulmonary disease (COPD), which have been a major public health burden worldwide. [5-7]

Since the discovery of rolipram and piclamilast (Fig. 1),[3,8] the first and second representative drug of diether derivatives of catechol class, which is an important type of selective PDE4 inhibitors, [9,10] PDE4 inhibitors have been extensively studied in the past few years. It was reported the 4-(3,4-dialkoxyphenyl) moiety (Fig. 1) was essential for PDE4 inhibition where the catechol ether oxygens played an important role in binding to the enzyme. [7] 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. [11] What is more, replacement of the amide portion of SCH 365351 with either a five-membered heterocyclic oxazole moiety or furan ring is helpful to enhance potent PDE4 inhibitory activity. [11-14]

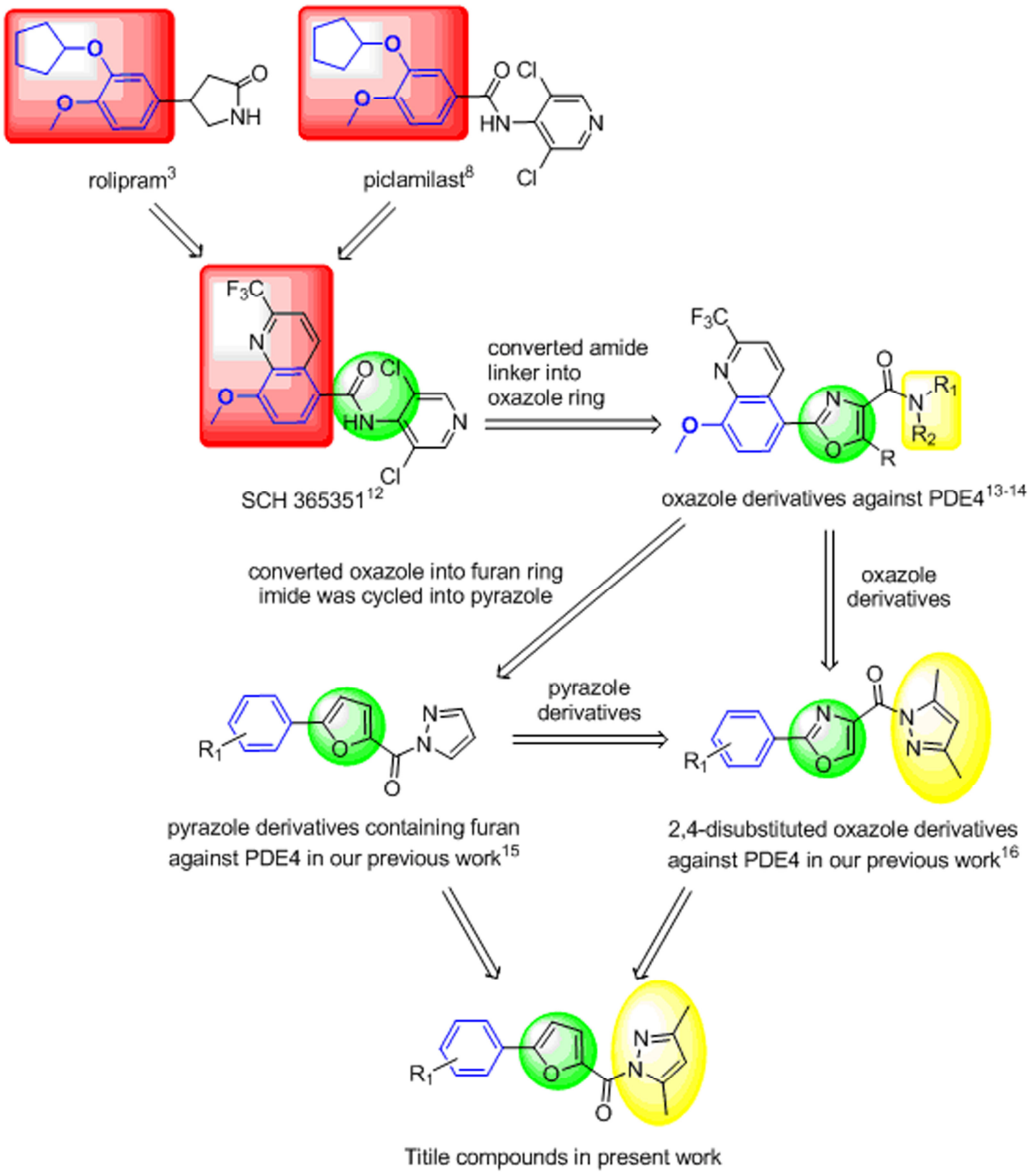
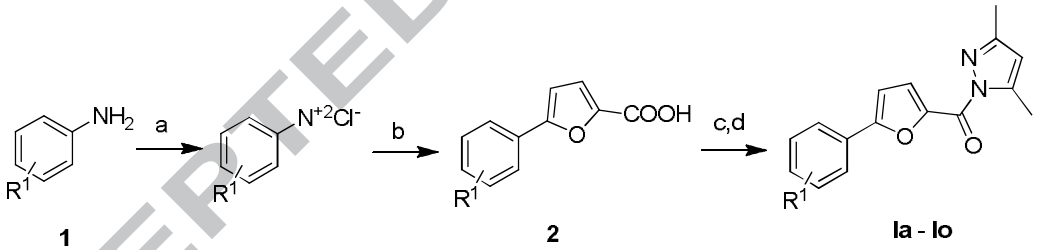


Figure 1. The designed strategy for the title compounds.

In our previous work, we found two series of pyrazole derivatives exhibited significant PDE4B inhibitory activity (Fig. 1).[15-16] The primary SARs demonstrated that the five-membered heterocyclic moiety was essential for the bioactivity through integral hydrogen bonds and a π-π stacking interaction. However, it is interesting that we can not find the π-π stacking interaction in furan-pyrazole moiety like found in oxazole-pyrazole moiety. Moreover, the π-π stacking interaction enhanced when the

pyrazole ring was substituted to 3,5-dimethylpyrazole ring. Based on these results, in order to obtain the novel PDE4B inhibitors and get the further relationship between the bioactivities and the primary SARs, 3,5-dimethylpyrazole derivatives **Ia**-**Io** containing furan moiety as bioisosteric surrogates of oxazole moiety were further designed and synthesized (Fig. 1).

The synthetic route of title compounds **Ia**-**Io** was shown in Scheme 1. The key intermediate **2** was synthesized from substituted aniline by Meerwein arylation reaction according to our previous procedure.[17,18] Treatment of 5-substituted phenyl-2-furan carboxylic acid **2** with thionyl chloride in anhydrous toluene under reflux provided the 5-phenyl-2-furancarbonyl chloride, then followed by treatment with 3,5-dimethyl-pyrazole in anhydrous dichloromethane afforded the compounds **Ia**-**Io** (see the Supplementary data for the details).



Scheme 1. The synthetic route of the title compounds **Ia**-**Io**. Reagents and conditions: (a) $NaNO_2$, hydrochloric acid, 0–5 ^oC, 3 h; (b) furoic acid, $CuCl_2$ (cat.), acetone–H_2O, rt, 5 h; (40–65%, two steps) (c) $SOCl_2$, anhydrous toluene, reflux, 3 h; (d) 3,5-dimethylpyrazole, anhydrous dichloromethane, reflux, 4 h (75–91%, two steps). R^1 = **Ia**: H, **Ib**: 4-Cl, **Ic**: 4-F, **Id**: 4-NO_2, **Ie**: 4-CH_3, **If**: 4-OCH_3, **Ig**: 4-Br, **Ih**: 3-Cl, **Ii**: 3-F, **Ij**: 3-NO_2, **Ik**: 2-Cl, **Il**: 2-F, **Im**: 2-NO_2, **In**: 2,4-di-F, **Io**: 2,6-di-F.

All the title compounds **Ia**-**Io** were evaluated for their inhibition against PDE4B

with rolipram as the reference drug. As a standard, compounds with IC_{50} values ≤ 20 μM against PDE4B, were tested for their inhibition against PDE4D. The results, expressed as IC_{50}, are summarized in Table 1. What is more, the inhibition of blockade of LPS-induced TNF-α release was also listed in Table 1.

Table 1. Impact on enzymatic potency (PDE4) and inhibition of TNF-*α* release from human blood mononuclear cells stimulated with lipopolysaccharide [a]

Compds.	R^1	PDE4B IC_{50} (μM)	PDE4D IC_{50} (μM)	TNF*α* IC_{50} (μM)
Ia	H	70.3±4.2	NT[b]	178.5±7.9
Ib	4-Cl	3.9±0.5	26.4±1.2	25.1±1.1
Ic	4-F	7.2±0.5	49.3±1.8	24.3±1.5
Id	4-NO_2	15.4±1.5	87.3±3.4	41.7±2.3
Ie	4-CH_3	8.4±0.4	52.9±2.4	37.1±1.8
If	4-OCH_3	1.7±0.3	9.6±0.7	10.4±0.8
Ig	4-Br	51.1±1.9	NT[b]	154.2±6.3
Ih	3-Cl	20.9±1.1	NT[b]	58.4±2.0
Ii	3-F	20.2±1.2	NT[b]	70.8±2.6
Ij	3-NO_2	35.9±2.8	NT[b]	69.3±3.1
Ik	2-Cl	52.4±2.1	NT[b]	112.3±5.7
Il	2-F	45.3±1.8	NT[b]	98.2±4.1
Im	2-NO_2	62.7±2.9	NT[b]	168.2±6.8
In	2,4-di-F	3.2±0.4	19.6±1.1	19.2±0.8
Io	2,6-di-F	14.5±1.2	88.2±2.8	88.7±3.2
rolipram		1.9±0.4	2.5±0.6	16.5±1.0

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

As shown in Table 1, all the compounds except compounds **Ia**, **Ig, Ik** and **Im** exhibited moderate to good inhibitory activity against PDE4B with the IC_{50} range of

1.0-50 μM. Among these derivatives, compound **If** displayed the strongest inhibition activity (IC_{50} = 1.7 μM) as well as good selectivity against PDE4B toward PDE4D (selective index = 5.6). Compounds **Ib**, **If** and **In** showed the similar inhibition against PDE4B as the positive drug rolipram, but exhibited over 5.6-fold higher selectivity rations for PDE4B over PDE4D than rolipram, confirming that 3,5-dimethylpyrazole derivatives containing furan moiety was appropriate for obtaining the new PDE4B inhibitors with high affinity and selectivity.

Introduction of different substituents at the 2, 3 or 4-position of the benzene ring resulted in the improvement of inhibitory activity against PDE4B since compound **Ia** (R^1 = H) showed the poorest activity, maybe due to increasing the affinity to specific areas in PDE4B enzyme. It was noted that both the substituent positions on the phenyl ring and the substituent electronegativity seemed to have a significant impact on potency. In analyzing the inhibitory activity of compounds **Ib**-**Ig**, in which the structural variations were made in the para-position of the phenyl ring. It was observed that substitution in the phenyl ring was beneficial to enhance inhibition against PDE4B. The attachment of electron donor groups, such as methoxy (**If**, IC_{50} = 1.7 μM) and methyl (**Ie**, IC_{50} = 8.4 μM), to the phenyl ring at the para-position led to the remarkable increased activity when compared to **Ia**. The same tendency was also observed when different halogens were attached to the phenyl ring. However, this increase **Ig** (R^1 = 4-Br, IC_{50} = 51.1 μM) was not as significant as the other two halogens, such as **Ic** (R^1 = 4-F, IC_{50} = 7.2 μM) and **Ib** (R^1 = 4-Cl, IC_{50} = 3.9 μM), respectively. Compound **Id** (R^1 =4-NO_2, IC_{50} = 15.4 μM), which have

electron-withdrawing group NO_2, also demonstrated increased inhibitory activity against PDE4B, although with slightly less intensity than the halogens analogues (**Ib** and **Ic**). Thus, all derivatives containing either electron donating groups or electron withdrawing groups were more active than the unsubstituted analogue **Ia**, revealing the importance of this structural modification at the para-position for inhibition against PDE4B.

We also analyzed the effect of binding both halogens such as Cl as well as F atoms, and NO_2 group in the other two positions of the phenyl ring. It was found that the *meta*-substitution generated the more active derivatives such as **Ih** and **Ii** than the *ortho*-substitution compounds in this study. In general, the activity in the order of compounds **Ib >Ih > Ik** or **Id >Ij > Im** suggests that the attachment of halogens or NO_2 group to the phenyl ring at the *para*-position is superior to the other kinds of substitution position. Moreover, di-substitutions were also evaluated in the phenyl ring by halogen atoms, deriving compounds **In** (R^1 = 2,4-di-F), and **Io** (R^1 = 2,6-di-F). These compounds were more active than compound **Ia**. Especially, **In** as one of the most potential in the series with an IC_{50} = 3.2 μM, displayed higher selective index than the reference drug rolipram.

The ability of all the title compounds **I** to inhibit the release of HM-TNFα was consistent well with their relative ability to inhibit PDE4B.The compound with 4-methoxy substituted group (**If**) was the most potent among all the derivatives assessed in this study, approximately 1.6 times more active than rolipram.

LPS induced sepsis model for the measurement of TNF-α inhibition (in *Swiss*

Albino mice) and neutrophilia inhibition for asthma and COPD (in *Sprague Dawley* rats) with selected *in vitro* active compounds **If** and **In** were performed. The details such as oral dosage and number of animals grouped for the experiments were listed in Table 2. The results showed that compound **In** demonstrated better inhibitory activity against TNF-α release (43.1%) and LPS induced neutrophilia inhibition (36.5%) than the positive control rolipram (41.7% and 32.8%) and compound **In** (29.6% and 24.2%).

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

Compds.	R^1	*Swiss Albino* mice (n = 6)		*Sprague Dawley* rats (n = 6)	
		Does (mg/kg, po)	TNF-α Inhibition (%)	Does (mg/kg, po)	LPS induced neutrophilia (% inhibition)
If	4-OCH_3	10	43.1±0.3[a]	10	36.5±0.5[a']
In	2.4-di-F	10	29.6±0.4[c]	10	24.2±0.4[c']
rolipram		10	41.7±0.3[b]	10	32.8±0.6[b']

The letters a-c' denoted the results of difference significance analysis. Means followed by the same letter within the same column are not significantly different ($p \geq 0.05$, Fisher's LSD multiple comparison test).

The binding of title compounds to the PDE4 structure was explored in order to consider the inhibitory activity. The bioassay results showed that compound **If** has the best activity among all the title compounds. Therefore, docking simulation of compound **If** at PDE4B (PDB ID: 1XMY) and PDE4D (PDB ID: 1Q9M) was conducted using Surflex-Dock in Sybyl 8.0 (see the Supplementary data for the methods),[19,20] and the docking contour maps were shown in Figure 2 and Figure 3.

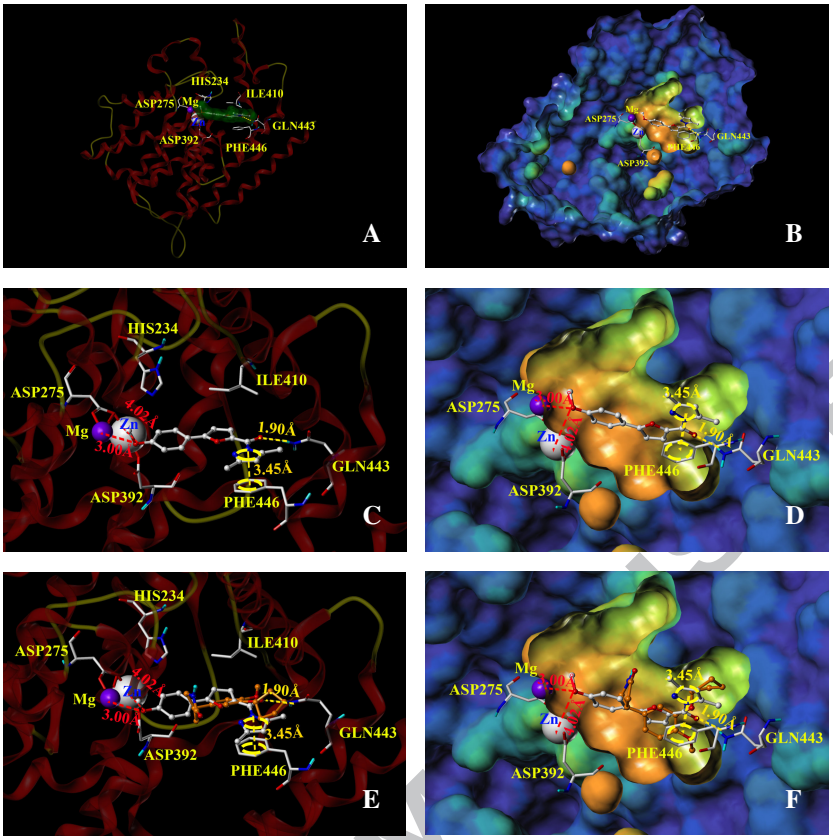


Figure 2. Model of PDE4 and docking of compounds **If** (A, B). The entire PDE4B structure (N-terminal domain, a catalytic domain and C-terminal domain) bound to **If** (C, D). The catalytic domain bound to **If** (E, F). The catalytic domain bound to **If** overlaid with rolipram (orange).

The docking orientation revealed that the five-membered 3,5-dimethylpyrazole moiety as the pivotal pharmacophore formed integral hydrogen bonds with the conserved glutamine residue (Gln443) (Fig. 2) and the heterocyclic ring was evidently positioned between the phenylalanine (Phe446) and isoleucine (Ile410) (Fig. 2C), which formed the cavity accommodating the hydrophobic moiety of compounds **If**. In compound **If**, the oxygen atom of carbonyl group with Gln443 (1.90 Å, Fig. 2C and 2D), which played the same role as the oxygen atoms of methoxy group and

cyclopentyloxy group in rolipram, respectively (Fig. 2E and 2F). Compared to our previous work[15], we found obvious π-π stacking interaction between the furan-3,5-dimethylpyrazole moiety of compound **If** and the benzene ring (3.45 Å, Fig. 2C and 2D) in the phenylalanine (Phe446). The para-methoxy group formed coordinate bond with the Zn^{2+} (4.02 Å, **If**, Fig. 2C) and Mg^{2+} (3.00 Å, **If**, Fig. 2C and 2D) cations and the remainder of the molecule was displayed to extend into the catalytic domain in close to both the Zn^{2+} and Mg^{2+} cations (Fig. 2C and 2D). The formation of hydrogen bonds, π-π stacking interaction and the hydrophobic interactions in the ligand–receptor complex was important for the binding affinity, which could block the access of cAMP to the catalytic domain and avoid the hydrolysis of cAMP, which formed the foundation for inhibition of PDE4.

We also superposed the structures of PDE4B (Fig. 3, green) and PDE4D (Fig. 3, purple) with compound **If**. The metal cations in PDE4B (Fig. 3A) were Mg^{2+} and Zn^{2+}, but which were two Zn^{2+} cations in PDE4D (Fig. 3B). Meanwhile there was a lack of a hydrophobic pocket in PDE4D compared with PDE4B. All the results could be the reasons to the selectivity of the title compounds interacted with the different subtypes of the enzyme.

In summary, the design and synthesis of 3,5-dimethylpyrazole derivatives containing 5-phenyl-2-furan moiety as novel PDE4B inhibitors were reported. Their bioactivity against phosphodiesterase type 4 and TNF-α were evaluated. Compound **If** showed the best inhibitory activity against PDE4B and blockade of LPS-induced TNF-α release among all the title compounds. The primary structure-activity

relationship study and docking results suggested the effect of all the title compounds on inhibition against PDE4B related to both the substituent types and the substituent positions on the phenyl ring. All derivatives containing either electron donating groups or electron withdrawing groups were more active than the unsubstituted analogue. Introduction of substituent group to the phenyl ring at the *para*-position, especially methoxy group, was helpful to enhance inhibitory activity against PDE4B, which was consistent well with the observed docking simulation.

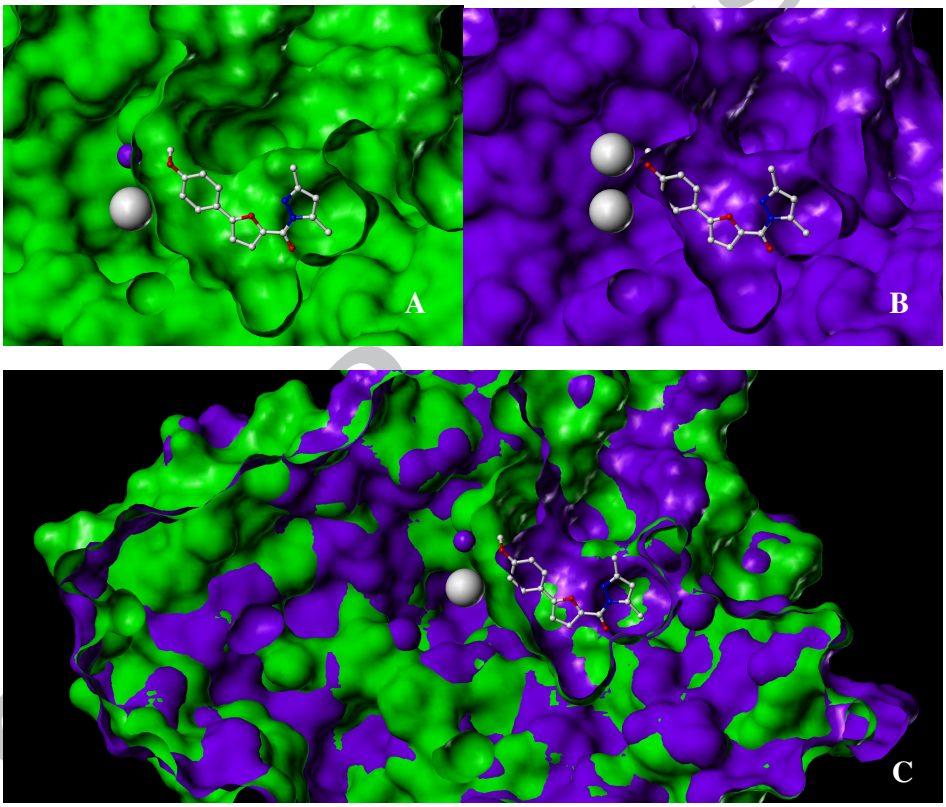


Figure 3. Docking study of compound **If** with PDE4B (green, A), PDE4D (purple, B), the overlapping of PDE4B and PDE4D (C)

Acknowledgements

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Graphical Abstract

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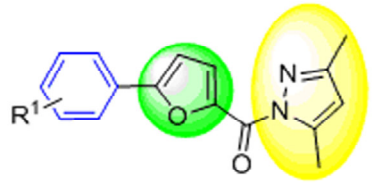

Titile compounds in present work

If (R^1 = 4-OCH_3)
IC_{50} = 1.7 μM (PDE4B)
IC_{50} = 10.4 μM (TNFα)