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Design and synthesis of 4-alkynyl pyrazoles as inhibitors of PDE4: A practical access via Pd/C–Cu catalysis

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ABSTRACT

The design and synthesis of 4-alkynyl pyrazole derivatives has led to the identification of new class of PDE4 inhibitors. All these compounds were accessed for the first time via a facile Pd/C–CuI–PPh_3 mediated C–C bond forming reaction between an appropriate pyrazole iodide and various terminal alkynes. In vitro PDE4B inhibitory properties and molecular modeling studies of some of the compounds synthesized indicated that 4-alkynyl pyrazole could be a promising template for the discovery of novel PDE4 inhibitors.

Pyrazole represents an important class of nitrogen-containing heterocycles and compounds containing pyrazole framework display numerous pharmacological properties.[1] This is exemplified by the identification of cyclooxygenase-2 (COX-2) inhibitors[2] and cannabinoid Type 1 receptor antagonists.[3] Moreover, *O*-[(benzyl- or benzoylpyrazolyl)propynyl]oximes of *N*-methylpiperidinone, 3-tropinone, and 3-quinuclidinone (**I**) (Fig. 1) has been reported as useful structures for new muscarinic agents of potential application in Alzheimer's disease.[4] We have a long standing interest in pyrazole derivatives[5] of potential pharmacological significance. In view of promising biological activities of alkynes **I** and as part of our ongoing program on identification of novel PDE-4/TNF-α inhibitors[6] we became interested in assessing PDE-4 inhibiting properties of small molecules[7] based on 4-alkynyl substituted pyrazole. These molecules (**C**) were derived from a known inhibitor Ibudilast[8] (**A**) via (**B**) following a relevant drug design approach (Fig. 1). Herein we report 4-alkynyl substituted pyrazole as a new template for the discovery of novel inhibitors of PDE4. The synthesis of library of small molecules based on 4-alkynyl substituted pyrazole (**C**) was carried out using a Pd/C-mediated C–C bond forming reactions.

A number of methods have been reported for the synthesis of alkynyl substituted pyrazole framework and most of them are based on Sonogashira coupling of iodo pyrazole with terminal alkynes.[4,9] Many of these methods have been developed recently[9c–e] and usually involve the use of $(PPh_3)_2PdCl_2$ as a catalyst. The use of Pd/C–CuI–PPh_3 as a less expensive catalyst system for efficient Sonogashira coupling has been explored earlier.[10] The catalyst Pd/C is stable and easy to handle as well as separable from the product.

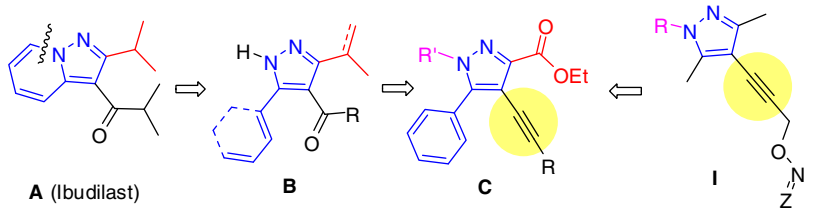

Figure 1. The known bioactive pyrazole (**I**) and the design of new PDE4 inhibitors (**C**) based on 4-alkynyl substituted pyrazole.

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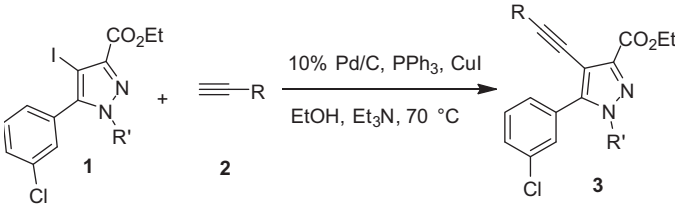


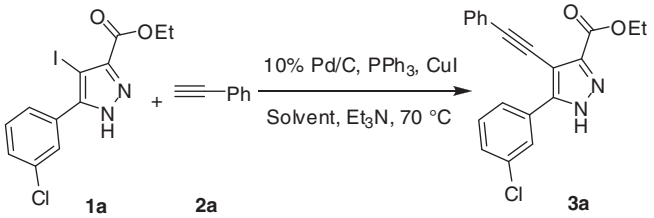
Scheme 1. Pd/C-mediated synthesis of 4-alkynyl substituted pyrazoles.

Moreover, the catalyst can be recycled.[10] Due to our continuing interest in Pd/C-mediated alkynylation of aryl and heteroaryl halides we decided to explore the Pd/C based methodology for the synthesis of our target compounds that is, 4-alkynyl pyrazoles as shown in Scheme 1. To the best of our knowledge alkynylation of pyrazole under Pd/C–Cu catalysis is not common in the literature.

The key starting material that is, 5-(3-chlorophenyl)-4-iodo-1*H*-pyrazole-3-carboxylate (**1a**) required for our study, was prepared (Scheme 2) from ethyl 3-chloroacetophenone (**4**) which was converted to ethyl 4-(3-chlorophenyl)-2,4-dioxobutanoate (**5**). The compound **5** on treatment with hydrazine provided 5-(3-chlorophenyl)-1*H*-pyrazole-3-carboxylate[11] (**6**) which was iodinated[12] to give the iodo derivative **1a**. The methylation of **1a** provided another starting compound **1b**.

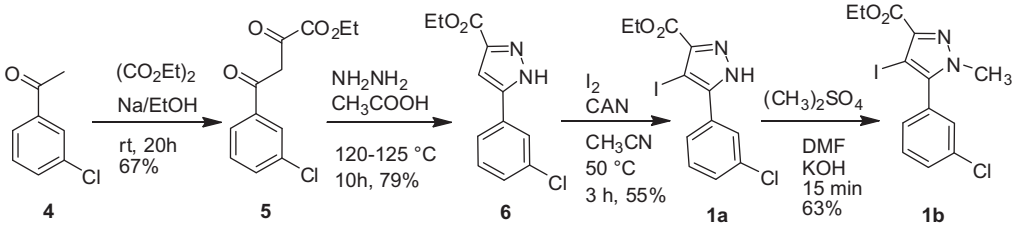
All the terminal alkynes used are commercially available. Initially, we chose to examine the coupling reaction of iodo compound **1a** with phenylacetylene (**2a**) in the presence of 10% Pd/C (0.026 equiv), PPh_3 (0.20 equiv), CuI (0.05 equiv), and triethylamine (3.0 equiv) in various solvents. The corresponding results are summarized in Table 1. The reaction was initially carried out in ethanol for 8 h and the desired product that is, ethyl 5-(3-chlo-

Table 1
Effect of solvents on Pd/C-mediated reaction of 5-(3-chlorophenyl)-4-iodo-1*H*-pyrazole-3-carboxylate (**1a**) with phenylaetylene[a] (**2a**)



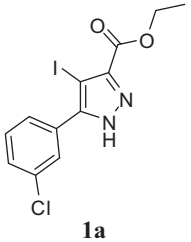
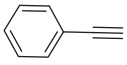
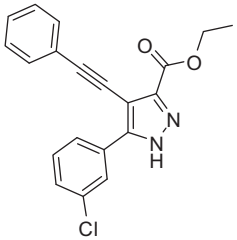
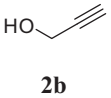
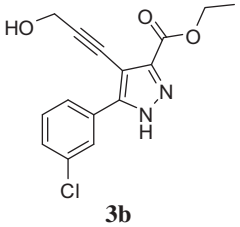
Entry	Solvent	Time (h)	% Yield[b]
1	EtOH	8	85
2	EtOH	16	80
3	MeOH	8	80
4	MeCN	10	70
5	1,4-Dioxane	8	65
6	DMF	8	65

[a] All the reactions were carried out by using **1a** (1.0 equiv), **2a** (1.5 equiv), 10% Pd/C (0.026 equiv), PPh_3 (0.20 equiv), CuI (0.05 equiv), and Et_3N (3.0 equiv) at 70 °C under a nitrogen atmosphere.
[b] Isolated yields.



Scheme 2. Preparation of iodo pyrazoles (**1**).

Table 2
Pd/C-mediated synthesis of 4-alkynyl substituted pyrazoles (**3**)[a]

Entry	Iodopyrazole (**1**)	Alkynes (**2**)	Products[b] (**3**)	% Yield[c]
1	 **1a**	 **2a**	 **3a**	85
2	**1a**	 **2b**	 **3b**	82

(*continued on next page*)

Table 2 (*continued*)

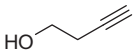
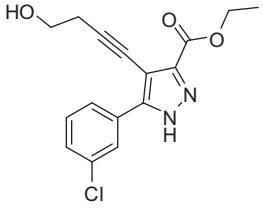
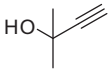
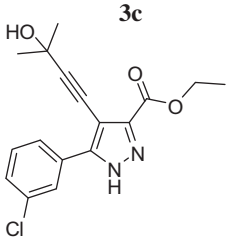
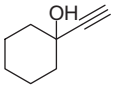
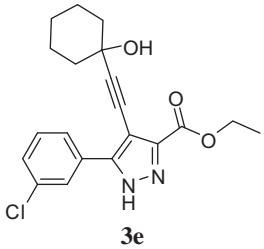
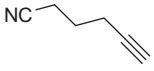
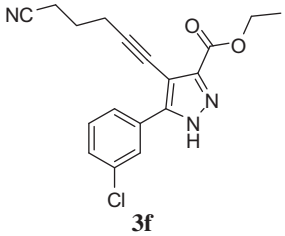
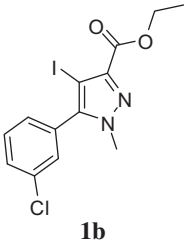
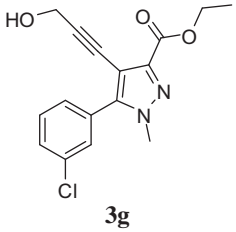
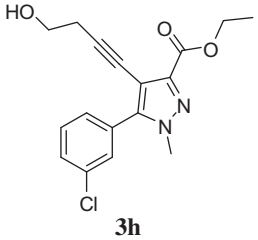
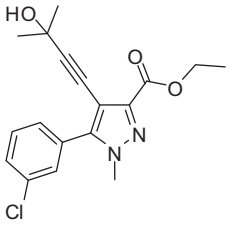
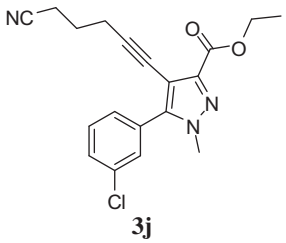
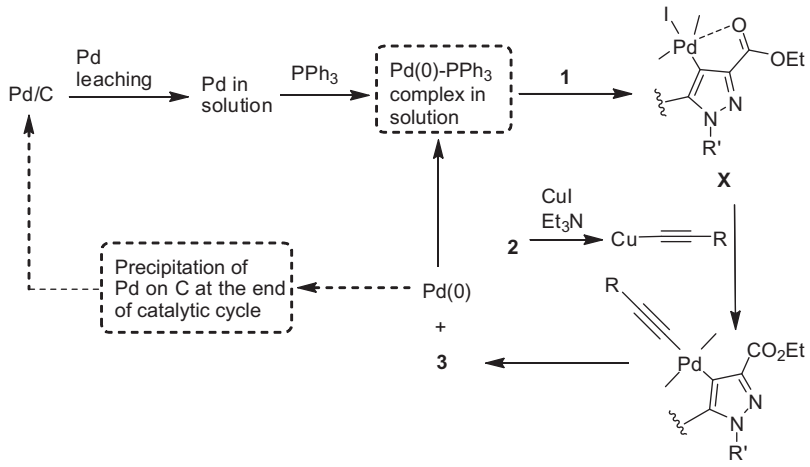
Entry	Iodopyrazole (**1**)	Alkynes (**2**)	Products[b] (**3**)	% Yield[c]
3	**1a**	 **2c**	 **3c**	83
4	**1a**	 **2d**	 **3d**	83
5	**1a**	 **2e**	 **3e**	80
6	**1a**	 **2f**	 **3f**	82
7	 **1b**	**2b**	 **3g**	83
8	**1b**	**2c**	 **3h**	80
9	**1b**	**2d**	 **3i**	83

Table 2 (*continued*)

Entry	Iodopyrazole (**1**)	Alkynes (**2**)	Products[b] (**3**)	% Yield[c]
10	**1b**	**2f**	 **3j**	83

[a] All the reactions were carried out by using **1** (1.0 equiv), **2** (1.5 equiv), 10% Pd/C (0.026 equiv), PPh_3 (0.20 equiv), CuI (0.05 equiv), and Et_3N (3 equiv) in EtOH at 70 °C for 8–9 h.
[b] Identified by ^1H NMR, IR, and MS.
[c] Isolated yields.



Scheme 3. Plausible mechanism of Pd/C-mediated alkynylation of 5-(3-chlorophenyl)-4-iodo-1*H*-pyrazole-3-carboxylate (**1**).

Table 3
Inhibition of PDE4B by compound **3** at 30 μM

Entry	Compounds	Average % inhibition	SD
1	**3a**	70.0	1.0
2	**3b**	68.1	3.1
3	**3c**	65.1	2.5
4	**3d**	70.0	2.8
5	**3e**	21.1	4.5
6	**3f**	71.1	2.2
7	**3g**	69.1	1.9
8	**3h**	59.2	2.3
9	**3i**	67.3	1.3
10	**3j**	73.4	3.6

SD = standard deviation.

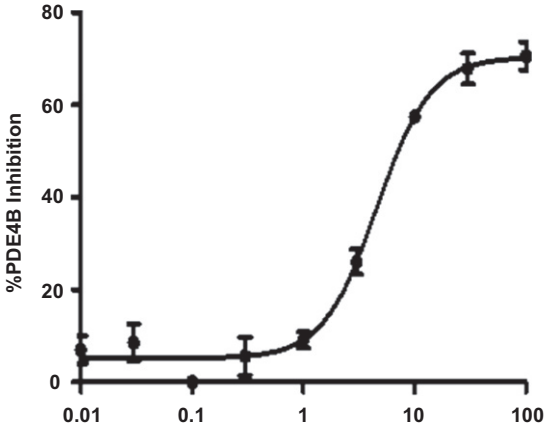


Figure 2. Dose dependent inhibition of PDE4B by **3j**.

rophenyl)-4-(phenylethynyl)-1*H*-pyrazole-3-carboxylate (**3a**) was isolated in 85% yield (Table 1, entry 1). The reaction proceeded well in EtOH via a C–C bond forming reaction to give the alkynylated product. The increase of reaction time did not improve the product yield of **3a** further (Table 1, entry 2). The use of other solvents such as MeOH, MeCN, 1,4-dioxane and DMF was examined and found to be effective in terms of product yields (Table 1, entries 3–6). Since the best result was achieved by using EtOH as a solvent hence all other studies were carried out using EtOH.

Having established the optimum reaction conditions for the preparation of **3a** we then used this methodology for the preparation of a library of compounds related to **3a**. Thus, a variety of commercially available terminal alkynes were employed under the reaction conditions presented in entry 1 of Table 1 and the results are summarized in Table 2.

As evident from Table 2 that the reaction proceeded well with both aliphatic (Table 2, entries 2–10) and aromatic alkynes (Table 2, entry 1). All the reactions were generally completed within 8–9 h irrespective of the nature of substituents present in the terminal alkynes (**2**) affording the desired products (**3a–j**) in good to excellent yields.

Mechanistically, the alkynylation of iodopyrazole derivative **1** proceeds via generation of an active Pd(0) species in situ that

Table 4
Docking scores and binding energy of compounds after docking with PDE4B

Compound	Docking score	Binding energy (kcal/mol)
3a	−8.70	−2345.97
3b	−8.12	−2338.44
3d	−8.14	−2346.08

undergoes oxidative addition with **1** to give the organo-Pd(II) species **X** (Scheme 3). The active Pd(0) species is generated from the minor portion of the bound palladium (Pd/C) via a Pd leaching process into the solution.[10] The leached Pd then becomes an active species by interacting with phosphine ligands that is, a dissolved Pd(0)–PPh_3 complex actually catalyzes the C–C bond forming reaction in solution. At the end of the reaction re-precipitation of Pd occurs on the surface of the charcoal. Once generated, the organo-Pd(II) species **X** then facilitates the stepwise formation of C–C bond via (i) trans organometallation with copper acetylide generated in situ from CuI and terminal alkyne followed by (ii) reductive elimination of Pd(0) to afford the 4-alkynylpyrazole derivatives (**3**). The iodide displacement step was perhaps aided by an intramolecular coordination of the neighboring carbonyl oxygen to the palladium center of **X**.

All the compounds synthesized were tested for their PDE4B inhibitory potential in vitro.[13] In inflammatory and immune cells, the inhibition of cellular responses, including the production and/or release of proinflammatory mediators, cytokines, and active oxygen species, is associated with elevated levels of cAMP. Since PDE4 (that exists in four different isoforms e.g., PDE4A, B, C and D) plays a key role in the hydrolysis of cAMP to AMP[7] hence inhibition of PDE4 should result in elevated levels of cAMP in the airway tissues and cells thereby suppressing inflammatory cell function. This is supported by the fact that PDE4 inhibitors have been found to be beneficial for the treatment of inflammatory and immunological diseases including asthma and chronic obstructive pulmonary disease (COPD). Notably, rolipram[14] the first-generation PDE4 inhibitor showed adverse effects such as nausea and vomiting. More recently, cardiovascular effects of

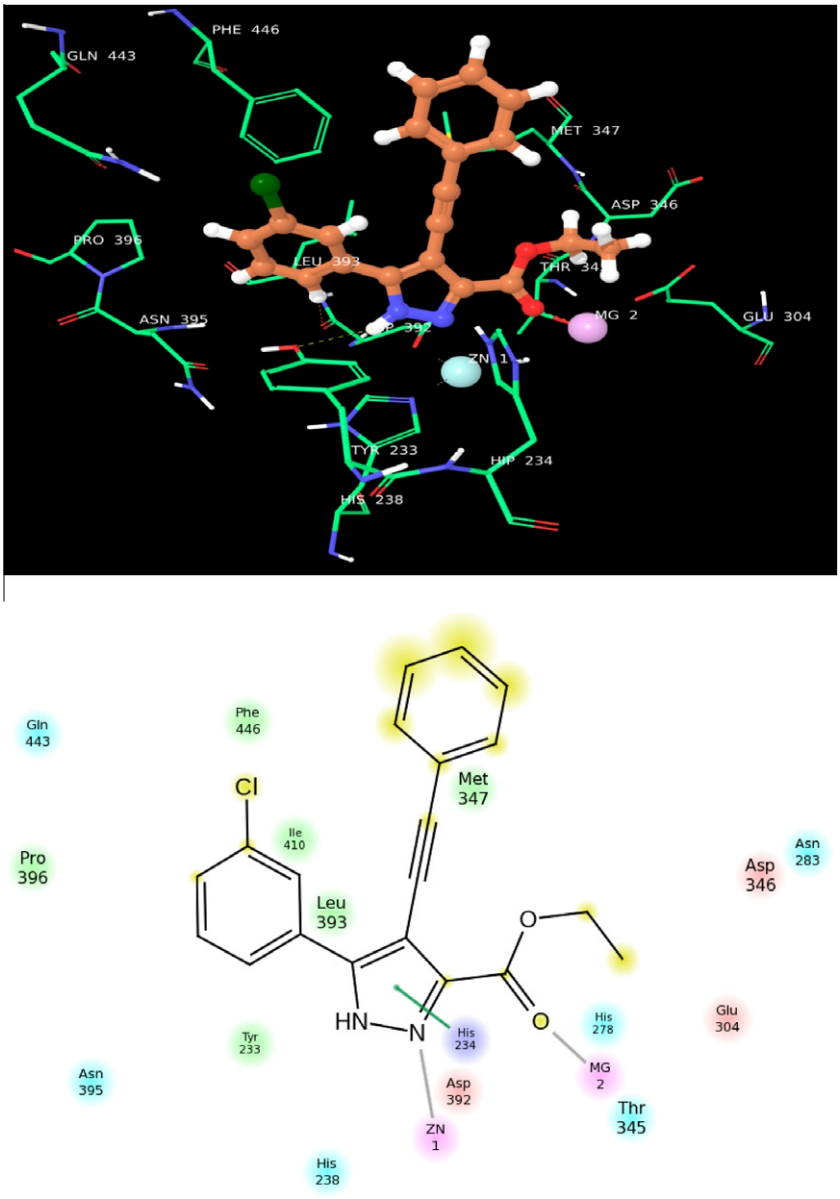


Figure 3. Docking of **3a** at the active site of PDE4B.

PDE4 inhibitors have been reported.[15] While these dose-limiting side effects were reduced by second-generation inhibitors like cilomilast[16] (Ariflo) and roflumilast, their therapeutic index has delayed market launch so far. It is therefore necessary to devote a continuing effort in exploring new class of compounds for their PDE4 inhibitory potential. Moreover, identification of novel small molecules possessing balanced inhibitory properties could be useful in minimizing the adverse side effects. Accordingly, our compounds were evaluated against PDE4B by using PDE4B enzyme isolated from *Sf*9 cells.[13] Rolipram, a well known inhibitor of PDE4 was used as a reference compound. Most the compounds except **3e** showed significant inhibition of PDE4B in compared to other molecules when tested at 30 μM (Table 3). A dose response study was carried out using a representative compound **3j** which showed dose dependent inhibition of PDE4B (Fig. 2). Since inhibition of PDE4D subtype is linked to the emetic response[17] hence some of the compounds were evaluated for their PDE4B selectivity over PDE4D. Accordingly compounds **3a**, **3d**, **3f** and **3j** that showed PDE4B inhibition >70% (Table 3) were tested against PDE4D using commercially procured PDE4D enzyme. All these compounds showed 20–30% inhibition at 30 μM indicating their ~twofold selectivity towards PDE4B. Nevertheless, to understand the nature of interactions of present 4-alkynyl substituted pyrazole derivatives (**3**) with PDE4B docking studies were carried out using few representative compounds for example, **3a, 3b** and **3d**. The docking score and binding energy obtained after docking of these molecules with PDE4B protein is summarized in Table 4. The data shown in Table 4 clearly suggests that these molecules bind well with PDE4B. The interaction of compound **3a** with the PDE4B protein (Fig. 3) was mainly contributed by a H-bonding between the pyrazole nitrogen and OH of tyrosine (Tyr233), π–π stacking interaction between pyrazole of **3a** and histidine (His234), coordinate interaction between Mg ion and the ester carbonyl group and coordinate interaction between Zn ion and pyrazole nitrogen.

Similarly, the interaction of compound **3b** with the PDE4B protein (Fig. 4) was contributed by H-bonding between pyrazole nitrogen and OH of tyrosine (Tyr233), π–π stacking interaction between pyrazole and histidine (His234) and coordinate interaction between Mg ion and the ester carbonyl group of **3b**. The interaction of compound **3d** with the PDE4B protein (Fig. 5) was mainly contributed by a π–π stacking interaction between pyrazole and histidine (His234) and the coordinate interaction between Mg ion and the ester carbonyl group of **3d**. Overall, the present 4-alkynyl pyrazoles[18] showed good interactions with PDE4B protein where the

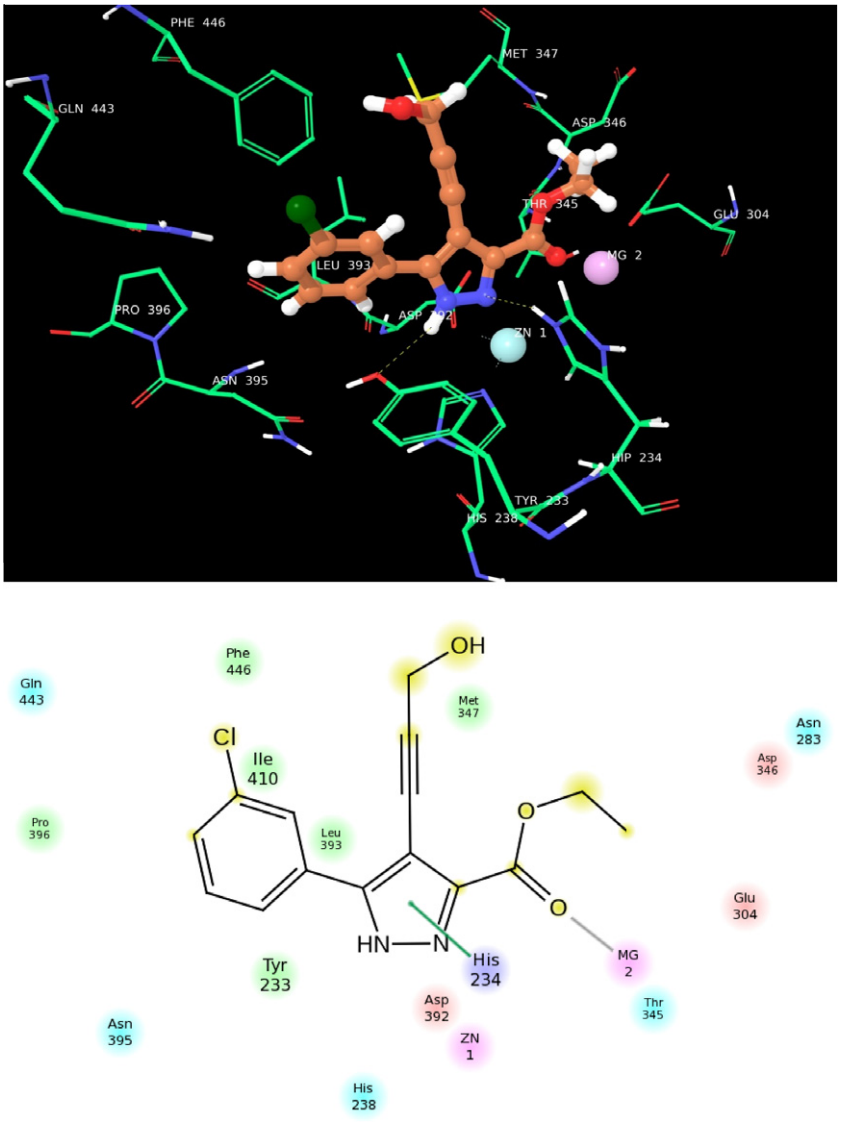


Figure 4. Docking of **3b** at the active site of PDE4B.

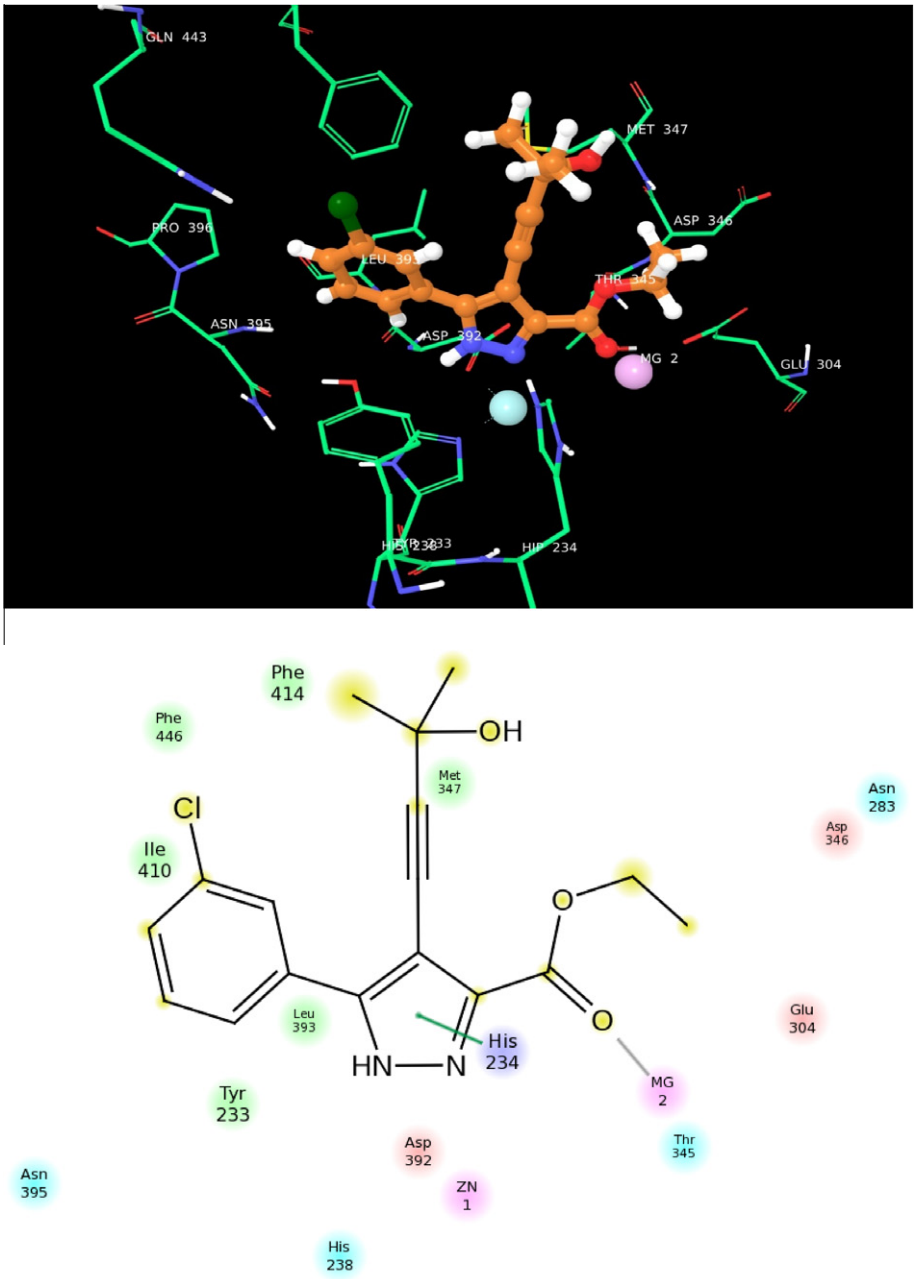


Figure 5. Docking of **3d** at the active site of PDE4B.

central pyrazole ring and its ester moiety played a key role while the alkyne side chain attached to the pyrazole ring was extended into a hydrophobic pocket. All these compounds also showed a coordinate interaction with both metals Zn and Mg. Finally, all ligands occupied enough space with lot of hydrophobic interaction contributing to their activity.

In conclusion, new 4-alkynyl substituted pyrazoles have been designed and accessed via a facile and inexpensive methodology based on Pd/C-mediated coupling of an appropriate iodide with terminal alkynes. All the compounds synthesized were tested for PDE4B inhibitory properties in vitro and many of them showed significant inhibition. The docking results suggested that these molecules interact well with PDE4B protein where the central pyrazole ring and its ester moiety played a key role while the alkyne side chain was extended into a hydrophobic pocket. Overall, 4-alkynyl pyrazole has been identified as a new template for the identification of small molecule based novel inhibitors of PDE4.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.02.008.

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