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Conformationally restricted novel pyrazole derivatives: Synthesis of 1,8-disubstituted 5,5-dimethyl-4,5-dihydro-1*H*-benzo[g]indazoles as a new class of PDE4 inhibitors

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ABSTRACT

A number of novel 1,8-disubstituted 5,5-dimethyl-4,5-dihydro-1*H*-benzo[g]indazoles based on a conformationally restricted pyrazole framework have been designed as potential inhibitors of PDE4. All these compounds were readily prepared by using simple chemistry strategy. The in vitro PDE4B inhibitory properties and molecular modeling studies of some of the compounds synthesized along with the X-ray single crystal data of a representative compound is presented.

The 4,5-dihydro-1*H*-benzo[g]indazole framework (**A**, Fig. 1), found to be integral part of several bioactive agents has attracted particular interest in the area of medicinal chemistry and new drug discovery. For example, compounds based on **A** have been reported to be effective ligands for cannabinoid receptors[1a] whereas the lead molecule PHA-408 (**B**, Fig. 1) that belongs to this class has been identified as a highly selective, ATP-competitive and tight-binding inhibitor of nuclear factor κ-B kinase.[1b] The 3-(1-piperazinyl)-4,5-dihydro-1*H*-benzo[g]indazoles (**C**, Fig. 1) have been reported as high affinity ligands for the human dopamine D4 receptor with improved selectivity over ion channels.[1c] Being a conformationally restricted form of 5-phenyl-1*H*-pyrazole the 4,5-dihydro-1*H*-benzo[g]indazole framework **A** attracted our attention due to our longstanding interest in pyrazole derivatives.[2a–e] Moreover,

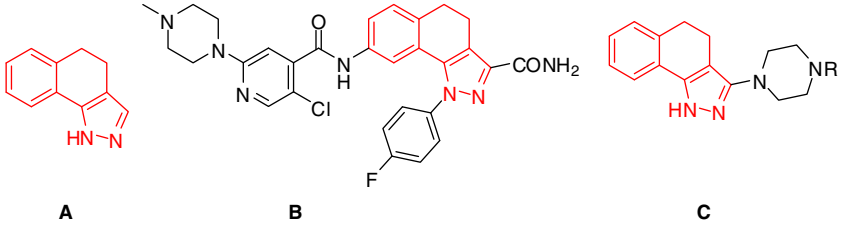

Figure 1. The 4,5-dihydro-1*H*-benzo[g]indazole framework (**A**) and related bioactive molecules **B** and **C**.

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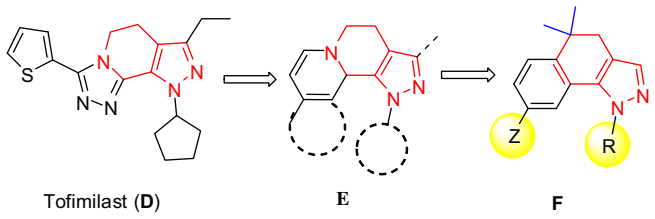
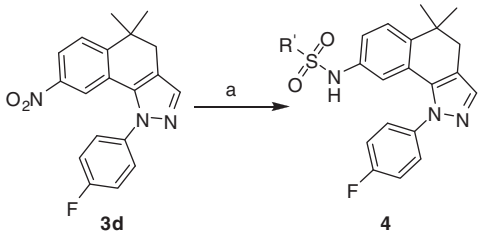
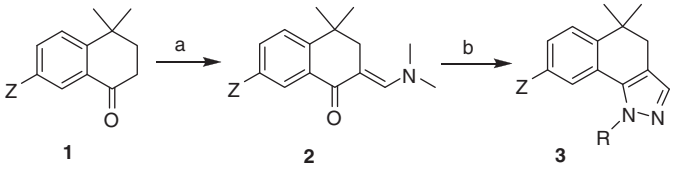


Figure 2. Tofimilast (**D**) and design of novel PDE4 inhibitors (**F**).



	R	% yield
4a	methyl	61
4b	phenyl	58

Scheme 2. Reagents and condition: (a) (i) 10% Pd/C, EtOAc, H_2, room temp, 12 h; (ii) $R'SO_2Cl$, pyridine, CH_2Cl_2, rt, 12 h.



	Z	R	% yield
3a	NO_2	phenyl	82
3b	NO_2	4-methylphenyl	81
3c	NO_2	2,3-dimethylphenyl	83
3d	NO_2	4-fluorophenyl	84
3e	NO_2	4-chlorophenyl	84
3f	NO_2	4-bromophenyl	82
3g	NO_2	4-trifluoromethylphenyl	80
3h	Br	phenyl	82
3i	Br	4-methylphenyl	83
3j	Br	4-fluorophenyl	85
3k	Br	4-chlorophenyl	82
3l	Br	4-bromophenyl	83
3m	Br	4-trifluoromethylphenyl	83
3n	Br	*i*-propyl	82
3o	Br	*t*-butyl	85

Scheme 1. Reagents and condition: (a) DMF-DMA, 105–110 °C, 2.0 h; (b) $RNHNH_2$ HCl, EtOH, 75–80 °C, 2.0 h.

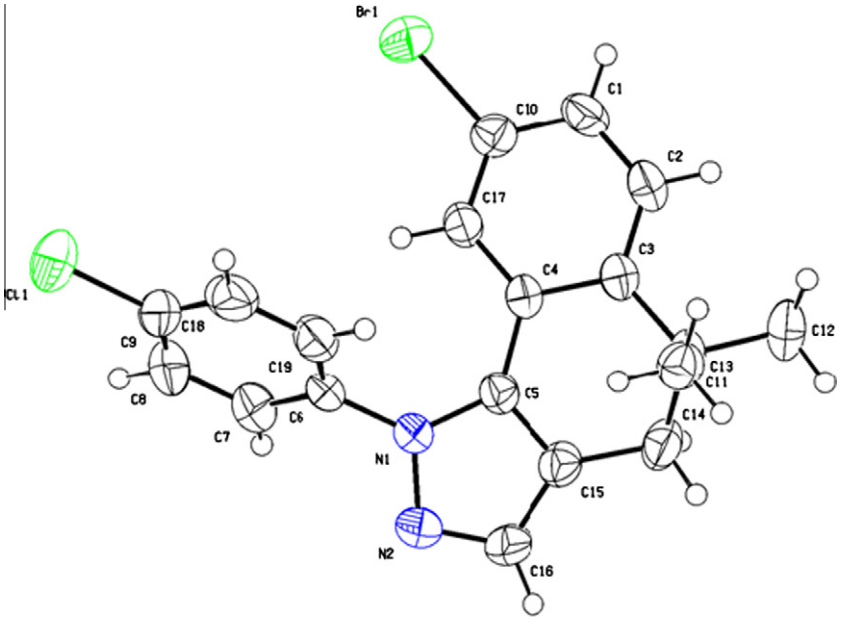


Figure 3. ORTEP representation of the **3k** (Thermal ellipsoids are drawn at 50% probability level).

Table 1
Inhibition of PDE4B by compound **3** at 30 μM

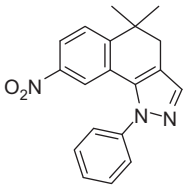
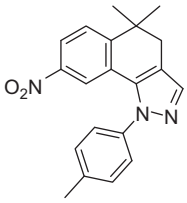
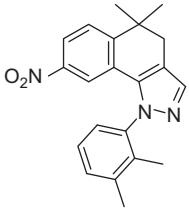
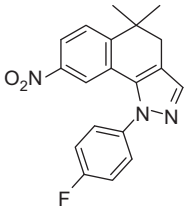
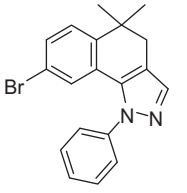
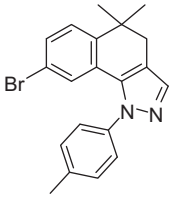
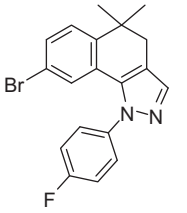
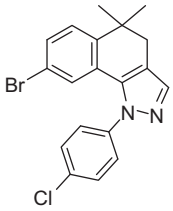
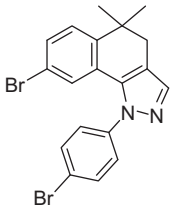
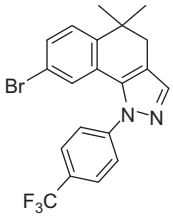
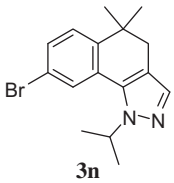
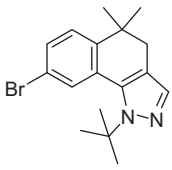
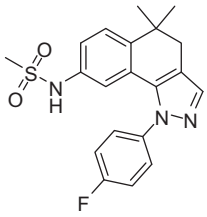
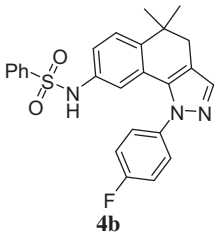
Entry	Compounds	Average% inhibition	SD
1	 **3a**	72.13	5.44
2	 **3b**	68.22	4.93
3	 **3c**	72.74	0.54
4	 **3d**	66.81	4.16
5	 **3h**	76.24	6.28
6	 **3i**	64.89	5.69
7	 **3j**	72.22	6.43

Table 1 (*continued*)

Entry	Compounds	Average% inhibition	SD
8	 **3k**	68.06	5.76
9	 **3l**	60.34	5.07
10	 **3m**	42.25	5.56
11	 **3n**	56.80	3.09
12	 **3o**	71.41	2.36
13	 **4a**	75.08	3.37
14	 **4b**	75.59	3.90

SD = standard deviation.

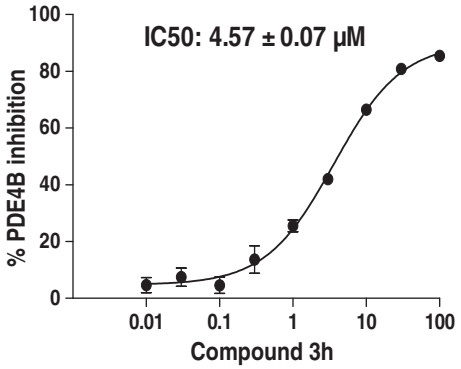


Figure 4. The IC_{50} value of compound **3h**.

conformationally restricted pyrazole derivatives have been explored for the identification of phosphodiesterase 4 (PDE4) inhibitors[3] as is exemplified by the discovery and development of tofimilast[4] (**D**, Fig. 2) for inhaled administration in asthma and chronic obstructive pulmonary diseases (COPD). Notably, COPD and asthma, a major public health burden worldwide, are reported to be causing the deaths of more than 250,000 people every year according to the WHO statistics (2007). In our effort[2b,f–h] towards the identification of novel inhibitors of PDE4 we became interested

Table 2
Glide scores and other parameters of compounds after docking with PDE4B

Entry	Compounds	Glide scores (Kcal/mol)	E-1[a] (Kcal/mol)	E-2[b] (Kcal/mol)	E-3[c] (Kcal/mol)
1	**3a**	−7.00	−4.65	−1.66	−0.03
2	**3c**	−6.84	−4.75	−1.71	−0.04
3	**3h**	−7.72	−5.15	−1.73	−0.11
4	**3j**	−7.48	−5.38	−1.73	−0.06

[a] E-1 = chemscore lipophilic term and fraction of total Van der Waals energy.
[b] E-2 = hydrophobic reward.
[c] E-3 = electrostatic reward.

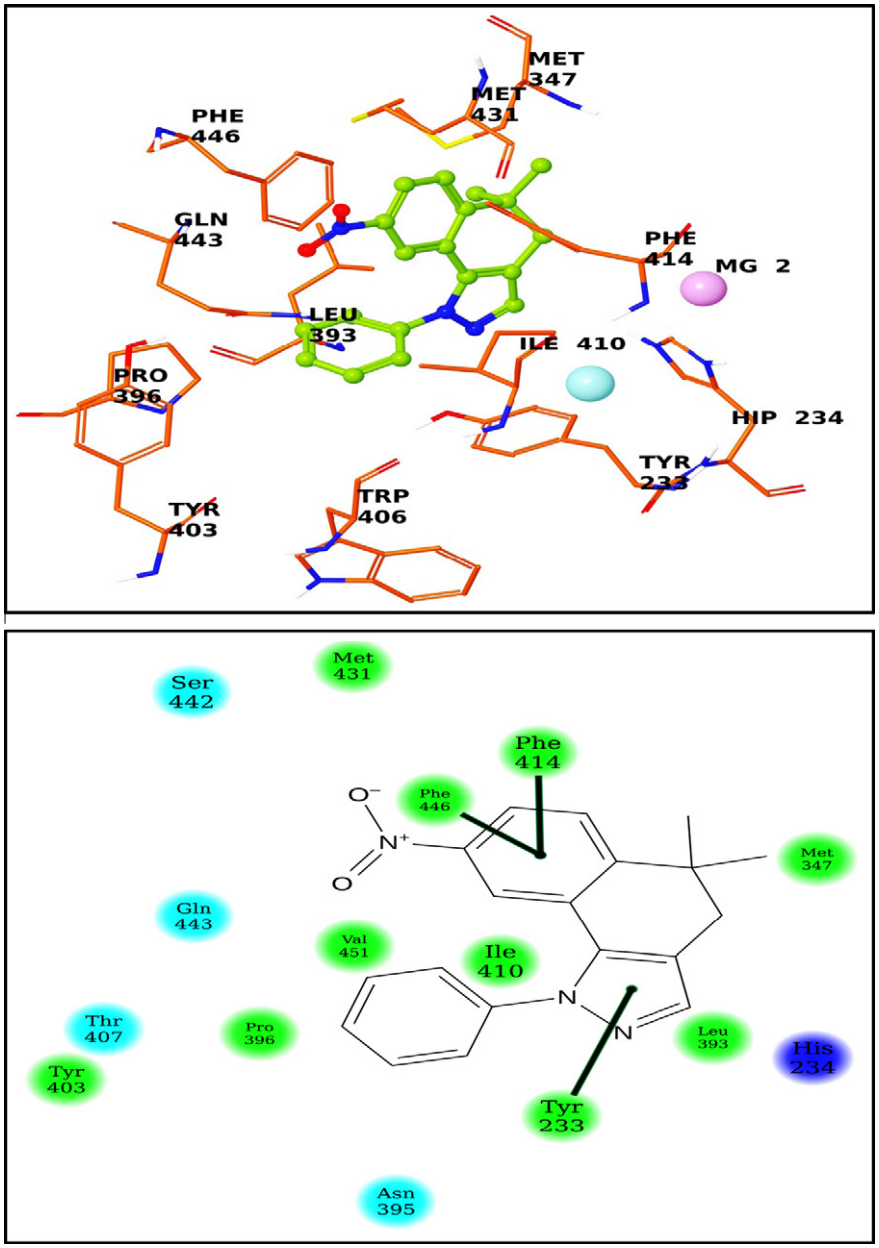


Figure 5. Docking of **3a** at the active site of PDE4B.

in evaluating PDE4 inhibitory properties of compound **F** designed based on the framework **A**, compound **B** and more importantly tofimilast **D** (Fig. 2). The potent inhibition of TNF-α and PDE4 shown by the compound **B** and **D** respectively prompted us to explore **F**. The structure of compound **F** was arrived via **E** by modifying the core structure of **D**. The substituents Z and R were introduced to the basic framework of **F** for the generation of diversity-based library of small molecules to be evaluated in the subsequent in vitro study.

While a number of literature methods are known[5] for the construction of the 4,5-dihydro-1*H*-benzo[*g*]indazole ring, the synthesis of compounds represented by **F** are, however, not common. To the best of our knowledge, only one method has been reported for the preparation of a similar class of compounds in 1959[6] via a multi-step process that involved preparation and subsequent rearrangement of 2-benzal-4,4-dimethyl-1-tetralone oxide to 2-benzoyl-4,4-dimethyl-1-tetralone followed by reaction with an appropriate hydrazine. We adopted a similar but much shorter as well as simpler strategy to prepare our target compounds as shown in Scheme 1. The key starting material, that is, 7-substituted-4,4-dimethyl-3,4-dihydronaphthalen-1(2*H*)-one (**1**) required for our synthesis was readily prepared via the reaction of 4,4-dimethyl-3,4-dihydronaphthalen-1(2*H*)-one with NBS[7] or HNO_3.[8] Treating the compound **1** with dimethylformamide dimethyl acetal (DMF-DMA) afforded the corresponding enaminoketone **2** which on condensation with a variety of hydrazines in ethanol provided the target compound **3** (or **F**). A range of 5,5-dimethyl-1,8-disubstituted-4,5-dihydro-1*H*-benzo[*g*]indazole derivatives were prepared by using this methodology. Further, the compound **3d** was converted to the corresponding sulfonamide derivatives (**4**) via reduction of the nitro group followed by treating the resultant amine with $R'SO_2Cl$ (Scheme 2). Nevertheless, all the compounds synthesized were well characterized by spectral (NMR, MS and IR) data. Additionally, the molecular structure of a representative compound **3k** was established unambiguously by single crystal X-ray diffraction (Fig. 3).[9]

Having prepared a range of compounds based on 4,5-dihydro-1*H*-benzo[*g*]indazole scaffold we then tested PDE4 inhibitory properties of some of these compounds in vitro.[11] The isozyme PDE4, one of the eleven PDE enzyme families[10] exists in four different

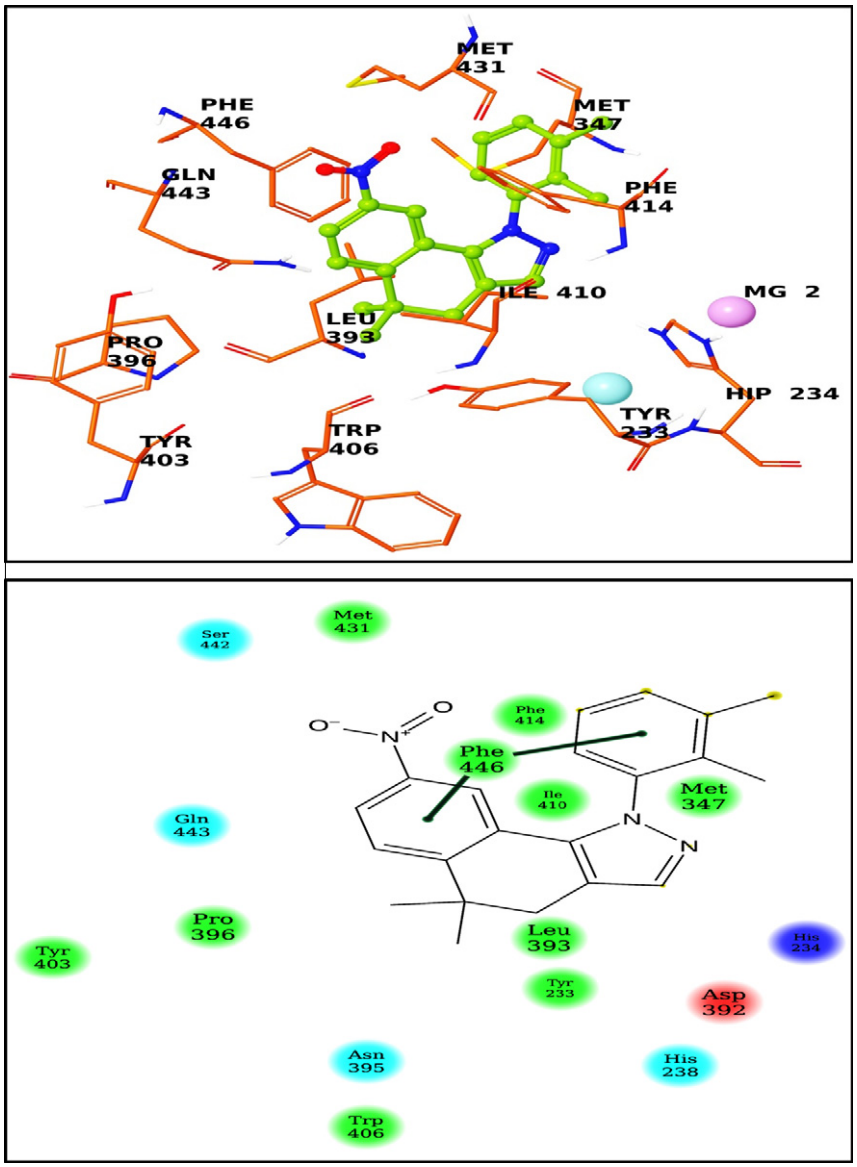


Figure 6. Docking of **3c** at the active site of PDE4B.

isoforms, for example, PDE4A, B, C and D and is responsible for the regulation of intracellular levels of cyclic adenosine monophosphate (cAMP). Indeed, PDE4 is specific for the hydrolysis of cAMP to AMP in mast cells, basophils, eosinophiles, monocytes, and lymphocytes as well as areas in the brain and airway smooth muscles. The elevated levels of cAMP is linked with the inhibition of cellular responses including the production and/or inhibition of proinflammatory mediators, cytokines and active oxygen species in inflammatory and immune cells. Thus, in order to suppress the inflammatory cell function it is therefore necessary to increase the intracellular concentration of cAMP in the airway and tissue cells. This can be achieved by inhibiting PDE4 and inhibitors of PDE4 therefore are beneficial for the treatment of inflammatory and immunological diseases including asthma and COPD. The first-generation PDE4 inhibitor rolipram[12] however showed dose-limiting side effects, for example, nausea and vomiting. While these side effects were reduced by second-generation inhibitors like cilomilast[13] (Ariflo) and roflumilast, their therapeutic index has delayed the market launch of these drugs. Based on the reports that PDE4B subtype is linked to inflammatory cell regulation[14] while the PDE4D subtype is implied in the emetic response[15] efforts have been devoted towards the identification of PDE4B inhibitors for the potential treatment of asthma and COPD. We initially evaluated the PDE4B inhibitory properties of the compounds synthesized in vitro at 30 μM using PDE4B enzyme assay[11] and the data generated for active compounds are summarized in Table 1.

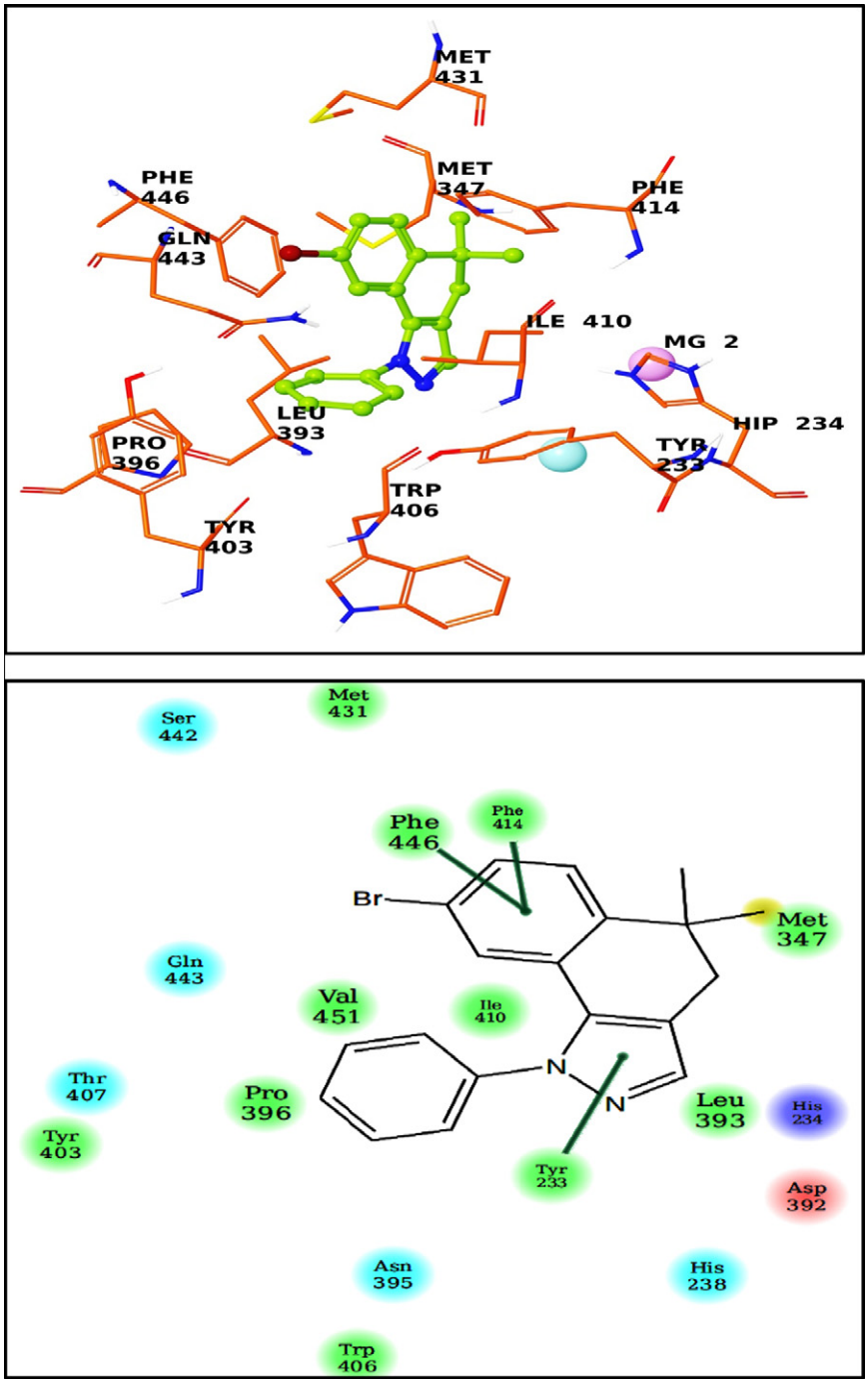


Figure 7. Docking of **3h** at the active site of PDE4B.

Rolipram[12] was used as a reference compound in this assay. Most of the compounds except **3m** showed significant inhibition of PDE4B when tested at 30 μM (Table 1). In a dose–response study compound **3h** showed dose dependent inhibition of PDE4B with an IC_{50} value of 4.57 ± 0.07 μM (Fig. 4). To assess the PDE4D inhibitory potential of **3** and **4** few selected compounds were tested against this enzyme when compound **3a**, **3c**, **3h**, **3j** and **3o** showed 55.8%, 54.9%, 50.0%, 65.4% and 41.7% inhibition, respectively. A recent study have demonstrated that inhibition of PDE4D by allosteric inhibitors (maximum inhibition, I_{max} 80–90%) did not cause emetic side effects raising a possibility that PDE4B inhibitors with partial but not complete inhibition of PDE4D (I_{max} of ~60–80%) could be beneficial for the treatment of COPD and asthma without causing emetic side effects.[16] Thus, the present class of compounds that showed a general tendency towards selective inhibition of PDE4B over PDE4D may have medicinal value.

In order to understand the nature of interactions of these molecules with PDE4B docking studies were carried out using compounds **3a, 3c, 3h** and **3j**. The xp (extra precision) docking was performed for all the molecules using glide module of Schrödinger 2011. The glide scores and other parameters obtained after docking of these molecules into the PDE4B protein are summarized in Table 2. The data shown in Table 2 suggests that these molecules bind well with PDE4B. The individual interaction of compound **3a** and **3h** with the PDE4B protein (Figs. 5 and 7) was mainly contributed by π–π stacking interaction between (i) the benzene ring of benzo[*g*]indazole of **3a** and phenylalanine (Phe 414 and 446), and (ii) the pyrazole moiety and tyrosine (Tyr 233). Whereas π–π stacking interaction of two benzene rings of 1-phenyl benzo[*g*]indazole moiety with phenylalanine (Phe 446) of PDE4B protein was observed in case of **3c** (Fig. 6). Similarly, π–π stacking interaction of the benzene ring of benzo[*g*]indazole

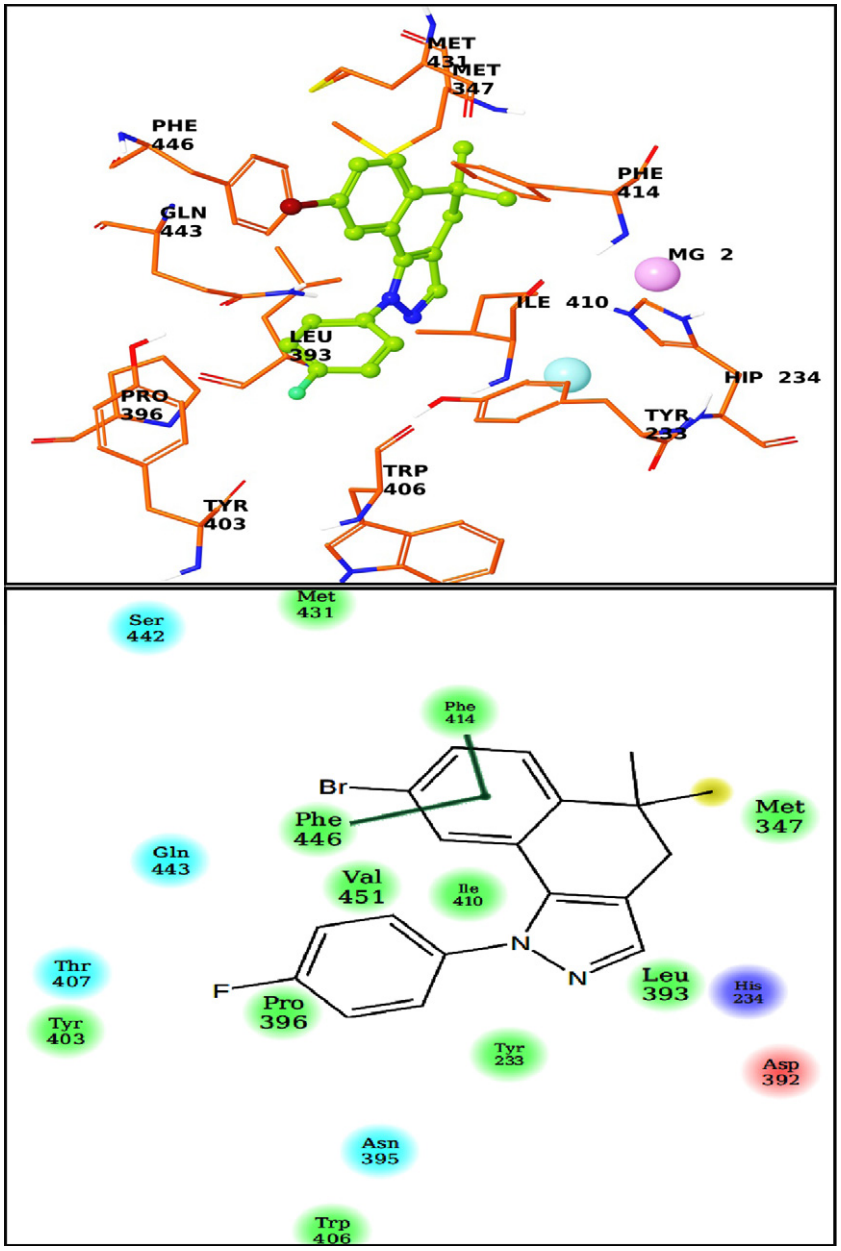


Figure 8. Docking of **3j** at the active site of PDE4B.

moiety and phenylalanine (Phe 414 and 446) was observed in case of **3j** (Fig. 8). Overall, all four molecules showed nearly same binding orientations with more lipophilic vdw energy for **3h** and **3j** compared to **3a** and **3c** whereas more electrostatic reward was observed for **3h** over **3j**. Nevertheless, 5,5-dimethyl-1,8-disubstituted-4,5-dihydro-1*H*-benzo[*g*]indazoles designed based on a conformationally restricted pyrazole framework showed promising PDE4B inhibitory properties in vitro and good interactions with PDE4B protein in silico.

In conclusion, a series of novel compounds based on a conformationally restricted pyrazole framework have been designed as potential inhibitors of PDE4. All these compounds were readily prepared by using simple chemistry strategy and several of them showed promising PDE4B inhibitory properties in vitro. Some of them showed good interactions with PDE4B protein in silico. The benzo[*g*]indazole framework presented here therefore has potential for the discovery and development of novel PDE4 inhibitors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.029.

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