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Preliminary Communications

Synthesis of 2,2,4-trimethyl-1,2-dihydroquinolinyl substituted 1,2,3-triazole derivatives: Their evaluation as potential PDE 4B inhibitors possessing cytotoxic properties against cancer cells

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ABSTRACT

The 2,2,4-trimethyl-1,2-dihydroquinolinyl substituted 1,2,3-triazole derivatives were designed as potential inhibitors of PDE4B. These compounds were synthesized *via* a multi-step sequence consisting of copper-catalyzed azide-alkyne cycloaddition (CuAAC) as a key step in aqueous media. The required alkynes were prepared from nimesulide *via* *N*-propargylation and then nitro group reduction followed by a CAN mediated modified Skraup reaction of the resulting amine. All the synthesized compounds showed PDE4B inhibitory properties *in vitro* at 30 μM with two compounds showing >50% inhibition that were supported by the *in silico* docking results of these compounds at the active site of PDE4B. Three of these PDE4 inhibitors showed promising cytotoxic properties against A549 human lung cancer cells *in vitro* with IC_{50} ~8–9 μM.

1. Introduction

The dysregulation of 3′,5′-cyclic adenosine mono-phosphate (cAMP) metabolism in oncogenesis was speculated more than 50 years ago at the time of discovery of cAMP. In his Noble address, the 1971 noble prize winner in Physiology or Medicine Dr. Earl Sutherland commented that, defective cAMP formation might be involved in the growth of tumors [1]. Indeed, the relevance of disordered cAMP metabolism to the genesis of multiple cancers has been confirmed recently. These studies suggested that the mechanism might involve altered expression and activity of phosphodiesterases (PDEs) [2,3], a superfamily of enzymes that degrade cAMP and cGMP. According to their specificity for cAMP or cGMP, PDEs can be subdivided into 11 different groups or isozymes (PDE1-PDE11) whereas the cAMP specific PDE4 isozymes are encoded by four genes (A–D) that give rise to four isoforms, e.g., PDE4A-D [4]. Since PDE4 specifically is widely expressed in tumor cells hence, inhibition of PDE4 in cancerous cells might be a new therapeutic target for cancer. For example, PDE4 inhibitors have been reported to (i) inhibit brain tumor cell growth [5,6], (ii) reduce proliferation and angiogenesis of lung cancer cell lines[7] and (iii) cause selective apoptosis of malignant cells without affecting the normal cells [8].

Recently, we have synthesized [9] a library of compounds based on a template **A** (Fig. 1) designed by incorporating the structural features of 2,2,4-substituted 1,2-dihydroquinolines and nimesulide [10], a well known anti-inflammatory drug available for patient's use for the treatment of pain. In continuation of this research we became interested in further structural elaboration of **A** that could lead to the identification of PDE4 inhibitors as potential anticancer agents. Accordingly, in view of PDE4 inhibiting [11] and anticancer [12] properties of triazole class of compounds we introduced a 1,2,3-triazole moiety to **A** to design a new template **B** (Fig. 1). Our anticipation that compounds related to **B** may inhibit PDE4 was further supported by the docking studies of a representative molecule e.g. *N*-*m*-tolyl-2-(4-{[*N*-(2,2,4-trimethyl-7-phenoxy-1,2-dihydroquinolin-6-yl)methylsulfonamido]methyl}-1*H*-1,2,3-triazol-1-yl)acetamide (**C**) into PDE4B protein *in silico* which showed good interactions (Glide score −8.24) through three H-bonds with Tyr-233, Hie-234 and Hie-278 residues of PDE4B (Fig. 2). Both, methyl substituted phenyl and the triazole ring were involved in π-π stacking with Tyr-233 and Hie-234 respectively. In addition, a metal coordination was

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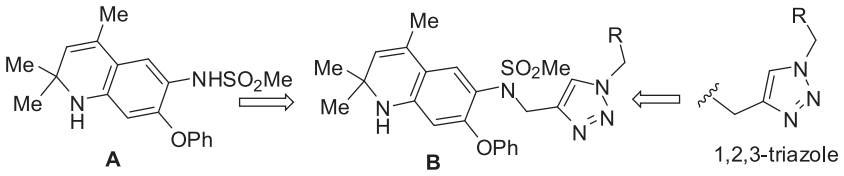


R = quinolin-3-yl, arylcarbamoyl, 4-(methylsulfonamido)arylcarbamoyl etc

Fig. 1. Design of novel and potential PDE4 inhibitors (**B**) as anticancer agents.

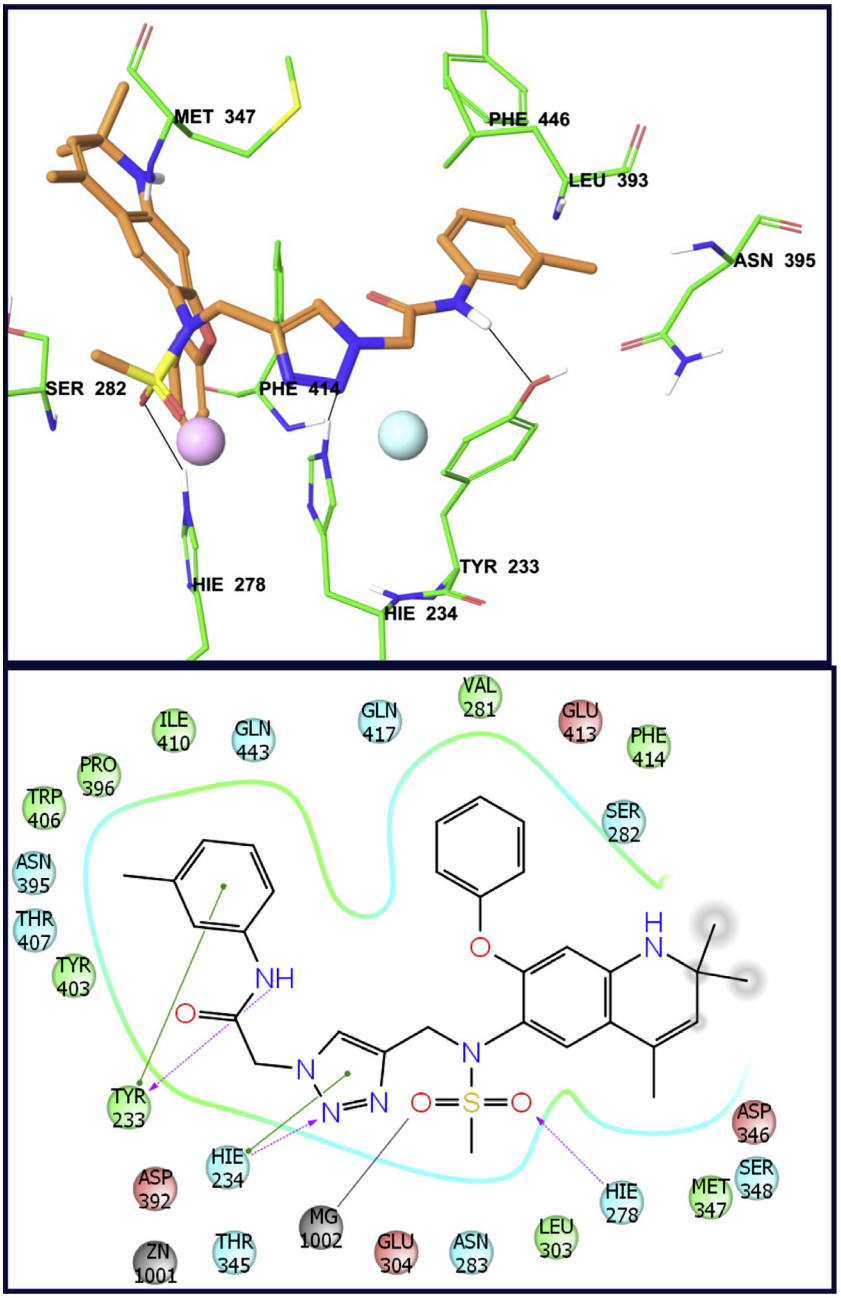


Fig. 2. Binding mode and interactions of molecule **C** at the inhibitor binding site of PDE4B (HIE: Histidine with hydrogen on the epsilon (E) nitrogen).

observed between Mg-1002 and SO_2 group of the molecule **C**. Due to our continuing interest on NCEs related to nimesulide [13–18], and novel PDE4 inhibitors [19,20] we now report the synthesis, *in vitro* PDE4 inhibition and cytotoxic effects of a series of novel 2,2,4-trimethyl-1,2-dihydroquinolinyl substituted 1,2,3-triazole **B** derived from nimesulide. To the best of our knowledge synthesis and pharmacological evaluation of this class of compounds is not known in the literature. Additionally, evaluation of novel PDE4 inhibitors for the potential treatment of cancer is not common in the literature.

2. Materials and methods

2.1. Materials

2.1.1. Cells and reagents

Sf9 cells were obtained from ATCC (Washington, DC, USA) and were routinely maintained in Grace's supplemented medium (Invitrogen) with 10% FBS. cAMP was purchased from SISCO Research Laboratories (Mumbai, India). PDElight HTS cAMP phosphodiesterase assay kit was procured from Lonza (Basel, Switzerland). PDE4B1 clone was procured from OriGene Technologies (Rockville, MD, USA). PDE4D2 enzyme was purchased from BPS Bioscience (San Diego, CA, USA).

2.1.2. General methods

Melting points were determined by open glass capillary method on a Cintex melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACF-300 machine or a Varian 300 or 400 MHz spectrometer using $CDCl_3$ or DMSO-d_6, with reference to tetramethylsilane as an internal reference. Mass spectra were recorded on a Jeol JMC D-300 instrument by using Electron ionization at 70 eV. All reactions were monitored by TLC (thin layer chromatography) on pre-coated silica gel plates. Column chromatography was performed by using silica gel (100–200 mesh, SRL, India) [10–20 times (by weight) of the crude product].

3. Experimental procedure

3.1. General procedure for the synthesis of **5**

A mixture of azide (1 mmol), an appropriate terminal alkyne (1 mmol), copper sulphate pentahydrate (0.25 mmol) and sodium ascorbate (200 mg) in 7:1 aqueous DMF (2 mL) was stirred vigorously for 10–30 min. The progress of the reaction is monitored by checking TLC at a regular interval. After completion of the reaction, the reaction mixture was quenched in crushed ice. The solid separated was filtered, dried and purified by column chromatography on silica gel using chloroform/ethyl acetate to give the desired product.

3.1.1. N-((1-((2-Chloro-6-methoxyquinolin-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-N-(2,2,4-trimethyl-7-phenoxy-1,2-dihydroquinolin-6-yl)methanesulfonamide (**5a**)

Pale yellow solid; yield 95%; mp 94–96 °C; R_f 0.82 ($CHCl_3$: EtOAc 1:1); MS *m/z* 645.2 (M+1, 100%); IR: 3369, 2960, 1613, 1497, 1488, 1336, 1218, 1148. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (d, *J* 7.8 Hz, 1H), 7.80 (s, 1H), 7.71 (s, 1H), 7.32–7.41 (m, 3H), 6.97–7.17 (m, 4H), 6.81 (s, 1H), 5.74 (s, 2H), 5.72 (s, 1H), 5.15 (s, 1H), 4.95 (s, 2H), 3.88 (s, 3H), 3.62 (bs, 1H, NH), 3.04 (s, 3H), 1.70 (s, 3H), 1.20 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.6, 155.6, 154.7, 146.0, 145.6, 144.7, 143.5, 137.1, 130.0, 129.7, 128.3, 127.2, 127.1, 124.2, 124.0, 123.9, 119.7, 116.9, 116.8, 105.1, 101.1, 55.7, 52.3, 51.2, 46.7, 39.4, 31.6, 29.7.

3.1.2. N-((1-((2-Chloro-6-methylquinolin-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-N-(2,2,4-trimethyl-7-phenoxy-1,2-dihydroquinolin-6-yl)methanesulfonamide (**5b**)

Pale yellow solid; yield 86%; mp 102–104 °C; R_f 0.67 ($CHCl_3$: EtOAc 3:1); MS *m/z* 629.2 (M+1, 100%); IR: 3354, 2964, 2919, 1612, 1588, 1488, 1333, 1148. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.90 (d, *J* 7.8 Hz, 1H), 7.76 (s, 1H), 7.73 (s, 1H), 7.59 (dd, *J* 8.2, 2.0 Hz, 1H), 7.48 (s, 1H), 7.34 (d, *J* 7.4 Hz, 1H), 7.33 (d, *J* 7.3 Hz, 1H), 7.15 (d, *J* 7.3 Hz, 1H), 7.02 (d, *J* 8.8 Hz, 2H), 6.81 (s, 1H), 5.74 (s, 2H), 5.72 (s, 1H), 5.15 (s, 1H), 4.92 (s, 2H), 3.63 (bs, 1H, NH), 3.04 (s, 3H), 2.51 (s, 3H), 1.75 (s, 3H), 1.20 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.6, 154.8, 147.9, 146.1, 145.6, 144.7, 137.8, 137.7, 133.5, 130.0, 128.2, 128.0, 127.3, 127.1, 127.0, 126.7, 126.6, 124.2, 123.8, 119.8, 116.9, 116.7, 101.1, 52.3, 51.2, 46.7, 39.4, 31.6, 29.7, 20.2.

3.1.3. N-((1-((2-Chloroquinolin-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-N-(2,2,4-trimethyl-7-phenoxy-1,2-dihydroquinolin-6-yl)methanesulfonamide (**5c**)

Pale yellow solid; yield 84%; mp 180–182 °C; R_f 0.72 ($CHCl_3$: EtOAc 3:1); MS *m/z* 615.2 (M+1, 100%); IR: 3370, 2958, 2921, 2852, 1614, 1487, 1321, 1215. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (d, *J* 7.8 Hz, 1H), 7.78 (s, 1H), 7.77–7.62 (m, 3H), 7.59–7.55 (m, 1H), 7.34 (d, *J* 7.4 Hz, 1H), 7.33 (d, *J* 7.3 Hz, 1H), 7.12 (d, *J* 7.3 Hz, 1H), 6.96 (d, *J* 8.8 Hz, 2H), 6.80 (s, 1H), 5.76 (s, 2H), 5.74 (s, 1H), 5.20 (s, 1H), 4.94 (s, 2H), 3.93 (bs, 1H, NH), 3.04 (s, 3H), 1.75 (s, 3H), 1.20 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.6, 154.8, 148.8, 147.5, 145.7, 144.7, 138.3, 131.2, 130.0 (2C), 128.3, 128.2, 127.8, 127.7, 127.3, 127.1, 127.0, 126.9, 124.3, 123.9, 119.8 (2C), 116.8, 116.7, 101.1, 52.3, 51.2, 46.7, 39.4, 31.6, 29.5.

3.1.4. N-((1-((2-Chloro-7-methylquinolin-3-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)-N-(2,2,4-trimethyl-7-phenoxy-1,2-dihydroquinolin-6-yl)methanesulfonamide (**5d**)

Pale yellow solid; yield 83%; mp 180 °C; R_f 0.64 ($CHCl_3$: EtOAc 3:1); MS *m/z* 629.2 (M+1, 100%); IR: 3369, 2964, 2924, 1612, 1589, 1505, 1488, 1335, 1216, 1149. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83–7.80 (m, 3H), 7.59 (d, *J* 8.2 Hz, 1H), 7.40–7.28 (m, 3H), 7.15 (d, *J* 7.3 Hz, 1H), 7.00 (d, *J* 8.8 Hz, 2H), 6.78 (s, 1H), 5.77 (s, 2H), 5.75 (s, 1H), 5.15 (s, 1H), 4.92 (s, 2H), 3.65 (bs, 1H, NH), 3.04 (s, 3H), 2.51 (s, 3H), 1.75 (s, 3H), 1.20 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.4, 155.8, 155.5, 154.7, 145.4, 144.8, 134.5 (2C), 130.1, 130.0, 129.5 128.3, 127.3, 127.1, 125.2, 124.6, 124.3, 120.2, 120.1, 119.9, 119.8, 116.7, 101.1, 53.4, 52.3, 46.6, 39.5, 31.6, 29.7, 20.9.

3.1.5. N-Phenyl-2-(4-((N-(2,2,4-trimethyl-7-phenoxy-1,2-dihydroquinolin-6-yl)methylsulfonamido)methyl)-1H-1,2,3-triazol-1-yl)acetamide (**5e**)

Off white solid; yield 90%; mp 200–202 °C; R_f 0.54 ($CHCl_3$: EtOAc 1:1); MS *m/z* 573.2 ($M+H^+$, 100%); IR: 3345, 2956, 2922, 2919, 1698, 1488, 1332, 1150. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.87 (s, 1H), 7.41–7.31 (m, 5H), 7.16–6.85 (m, 8H), 6.84 (s, 1H), 5.75 (s, 1H), 5.15 (s, 1H), 5.12 (s, 2H), 4.83 (bs, 1H, NH), 3.05 (s, 3H), 1.79 (s, 3H), 1.25 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.5, 155.9, 155.6, 145.6, 143.9, 138.8, 130.4, 129.3, 127.9, 127.2, 126.8, 125.9, 124.6, 124.2, 120.4, 119.5, 115.7, 115.3, 100.3, 52.6, 51.8, 45.1, 31.9, 18.5.

3.1.6. N-p-Tolyl-2-(4-((N-(2,2,4-trimethyl-7-phenoxy-1,2-dihydroquinolin-6-yl)methylsulfonamido) methyl)-1H-1,2,3-triazol-1-yl)acetamide (**5f**)

Pale yellow solid; yield 88%; mp 98–100 °C; R_f 0.5 ($CHCl_3$: EtOAc 1:1); MS *m/z* 587.3 ($M+H^+$, 100%); IR: 3347, 2921, 2852, 1691, 1610, 1487, 1332, 1213, 1147. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (s, 1H), 7.87 (s, 1H), 7.39–7.30 (m, 5H), 7.14–7.02 (m, 6H), 6.84 (s, 1H), 5.79 (s, 1H), 5.15 (s, 1H), 5.12 (s, 2H), 5.10 (bs, 1H, NH), 3.09 (s, 3H), 2.29 (s, 3H), 1.79 (s, 3H), 1.25 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.5, 155.5, 154.7, 145.4, 144.8, 134.6, 130.0 (2C), 129.5 (2C), 128.3, 127.9, 125.2, 124.6, 124.3, 120.2 (2C), 119.9 (2C), 116.8, 116.7, 116.5, 101.1, 53.4, 52.3, 46.6, 39.5, 31.6 (2C), 29.4.

*3.1.7. N-m-Tolyl-2-(4-((N-(2,2,4-trimethyl-7-phenoxy-1,2-dihydroquinolin-6-yl)methylsulfonamido)methyl)-1H-1,2,3-triazol-1-yl)acetamide (**5g**)*

Pale yellow solid; yield 95%; mp 186–188 °C; R_f 0.59 ($CHCl_3$: EtOAc 1:1); MS *m*/*z* 587.2 ($M+H^+$, 100%); IR: 3347, 2921, 2852, 1691, 1610, 1487, 1332, 1213, 1147. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.01 (s, 1H), 7.98 (s, 1H), 7.20–7.02 (m, 11H), 6.84 (s, 1H), 5.79 (s, 1H), 5.16 (s, 1H), 5.12 (s, 2H), 4.97 (bs, 1H, NH), 3.06 (s, 3H), 2.31 (s, 3H), 1.79 (s, 3H), 1.26 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.1, 155.6, 154.7, 145.4, 144.8, 138.9, 137.0, 130.0, 128.8, 128.3, 127.3, 127.1, 125.7, 125.2, 124.6, 124.3, 120.8, 119.7, 117.2, 116.8, 101.1, 53.4, 52.3, 46.6, 39.5, 31.6, 29.7, 21.4;

*3.1.8. N-(4-Methoxyphenyl)-2-(4-((N-(2,2,4-trimethyl-7-phenoxy-1,2-dihydroquinolin-6-yl)methylsulfonamido) methyl)-1H-1,2,3-triazol-1-yl)acetamide (**5h**)*

Pale yellow solid; yield 95%; mp 88–90 °C; R_f 0.66 ($CHCl_3$: EtOAc 1:1); MS *m*/*z* 603.2 (M+H, 100%); IR: 3341, 2920, 2852, 1694, 1610, 1510, 1487, 1332, 1233, 1215, 1145. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.01 (s, 1H), 7.36–7.31 (m, 8H), 7.18–7.02 (m, 2H), 6.85–6.81 (m, 3H), 5.75 (s, 1H), 5.16 (s, 1H), 5.10 (s, 2H), 4.92 (bs, 1H, NH), 3.78 (s, 3H), 3.05 (s, 3H), 2.18 (s, 3H), 1.25 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.5, 156.8, 155.5, 154.7, 145.4, 144.8, 130.1, 128.3, 127.3, 127.1, 125.2, 124.3, 122.0, 121.9, 119.7, 116.8, 116.7, 114.1, 101.1, 55.5, 53.4, 52.3, 46.6, 39.5, 31.8, 29.4;

*3.1.9. N-(4-(Methylsulfonamido)-3-phenoxyphenyl)-2-(4-((N-(2,2,4-trimethyl-7-phenoxy-1,2-dihydroquinolin-6-yl)methylsulfonamido) methyl)-1H-1,2,3-triazol-1-yl)acetamide (**5i**)*

Off white solid; yield 83%; mp 220–222 °C; R_f 0.52 ($CHCl_3$: EtOAc 1:1); MS *m*/*z* 758.2 ($M+H^+$, 100%); IR: 3341, 2926, 2855, 1700, 1613, 1589, 1487, 1330, 1213, 1147. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.80 (s, 1H), 7.36–7.31 (m, 2H), 7.18–7.02 (m, 15H), 6.85–6.81 (s, 1H), 5.76 (s, 1H), 5.17 (s, 1H), 5.10 (s, 2H), 4.96 (bs, 1H, NH), 3.07 (s, 3H), 2.95 (s, 3H), 2.20 (s, 3H), 1.25 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.6, 156.3, 155.9, 155.6, 151.4, 145.6, 137.8, 130.5, 128.3, 127.9, 127.2, 126.8, 124.6, 124.4, 123.6, 120.4, 119.6, 116.8, 115.7, 115.3, 114.4, 109.3, 100.3, 52.4, 51.8, 46.1, 40.8, 31.9, 18.4.

3.2. In vitro assay of compounds for the inhibition of PDE4B enzyme

3.2.1. PDE4B protein production and purification

PDE4B1 cDNA was sub-cloned into pFAST Bac HTB vector (Invitrogen) and transformed into DH10Bac (Invitrogen) competent cells. Recombinant bacmids were tested for integration by PCR analysis. Sf9 cells were transfected with bacmid using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. Subsequently, P3 viral titer was amplified, cells were infected and 48 h post infection cells were lysed in lysis buffer (50 mM Tris–HCl pH 8.5, 10 mM 2-Mercaptoethanol, 1% protease inhibitor cocktail (Roche), 1% NP40). Recombinant His-tagged PDE4B protein was purified as previously described elsewhere [24]. Briefly, lysate was centrifuged at 10,000 rpm for 10 min at 4 °C and supernatant was collected. Supernatant was mixed with Ni–NTA resin (GE Life Sciences) in a ratio of 4:1 (v/v) and equilibrated with binding buffer (20 mM Tris–HCl pH 8.0, 500 mM-KCl, 5 mM imidazole, 10 mM 2-mercaptoethanol and 10% glycerol) in a ratio of 2:1 (v/v) and mixed gently on rotary shaker for 1 h at 4 °C. After incubation, lysate–Ni–NTA mixture was centrifuged at 4500 rpm for 5 min at 4 °C and the supernatant was collected as the flow-through fraction. Resin was washed twice with wash buffer (20 mM Tris–HCl pH 8.5, 1 M KCl, 10 mM 2-Mercaptoethanol and 10% glycerol). Protein was eluted sequentially twice using elution buffers (Buffer I: 20 mM Tris–HCl pH 8.5, 100 mM KCl, 250 mM imidazole, 10 mM 2-mercaptoethanol, 10% glycerol, Buffer II: 20 mM Tris–HCl pH 8.5, 100 mM KCl, 500 mM imidazole, 10 mM 2-mercaptoethanol, 10% glycerol). Eluates were collected in four fractions and analyzed by SDS–PAGE. Eluates containing PDE4B protein were pooled and stored at −80 °C in 50% glycerol until further use.

3.2.2. PDE4 enzymatic assay

The inhibition of PDE4 enzyme was measured using PDE light HTS cAMP phosphodiesterase assay kit (Lonza) according to manufacturer's recommendations. Briefly, 10 ng of in house purified PDE4B1 enzyme was pre-incubated either with DMSO (vehicle control) or compound for 15 min before incubation with the substrate cAMP (5 μM) for 1 h. The reaction was halted with stop solution and reaction mix was incubated with detection reagent for 10 min in dark. Luminescence values (RLUs) were measured by a Multilabel plate reader (Perklin Elmer 1420 Multilabel counter). The percentage of inhibition was calculated using the following formula:

$$\%\text{inhibition} = \frac{(\text{RLU of vehicle control} - \text{RLU of inhibitior})}{\text{RLU of vehicle control}} \times 100$$

3.3. MTT assay for cytotoxicity

The viability of the cells was assessed by MTT [3,4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, which is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product. Doxorubicin, a known anticancer drug was used as a reference compound in this assay. Cells (1×10^4) were plated in a 96-well plate. After 24 h, they were treated with different concentration (0–10 μM) of different test compounds diluted appropriately with culture media for 48 h. Cells grown in a media containing equivalent amount of DMSO served as positive control and cells in medium without any supplementation were used as negative control. After the treatment, media containing compound were carefully removed by aspiration. 100 μL of 0.4 mg/mL MTT in PBS was added to each well and incubated in the dark for 4 h. 100 μL of DMSO was added to each well and kept in an incubator for 4 h for dissolution of the formed formazan crystals. Amount of formazan was determined by measuring the absorbance at 540 nm using an ELISA plate reader. The data were presented as percent dead cells, whereas absorbance from non-treated control cells was defined as 100% live cells. The percent dead cells was plotted (*Y*-axis) against concentration (*X*-axis) of compounds, where IC_{50} values could be interpolated from the graph.

3.4. Docking studies

3.4.1. Docking method

The docking analysis of molecules was performed using Maestro, version 9.2 [21] implemented from Schrödinger molecular modeling suite. Molecules were sketched in 3D format using build module of maestro and LigPrep module was used to produce low-energy conformers. The structural coordinates of Phosphodiesterase 4B (PDB ID: 1XMY) [22] were obtained from the protein data bank (PDB). PBD protein was prepared by giving preliminary treatment like adding hydrogen, adding missing residues, refining the loop with prime and finally minimized by using OPLS-2005 force field. The grid for molecular docking was generated with bound co-crystallized ligand. Molecules were docked using Glide in extra-precision mode, with up to three poses saved per molecule. The ligands were kept flexible, whereas the receptor was kept rigid throughout the docking studies. The lowest energy conformations were selected and the ligand interactions (H-Bond and Hydrophobic interactions) with target protein were determined.

4. Results and discussion

4.1. Chemistry

The target compounds **5** (or **B**) were synthesized by adopting a multi-step sequence as outlined in Scheme 1. The synthesis of 1,2,3-triazoles are generally carried out by using click chemistry approach which involves copper (I)-catalyzed 1,3-cycloaddition reaction between a terminal alkyne and an azide [23]. Thus, the copper-catalyzed azide-alkyne cycloaddition (CuAAC) in aqueous media was used as a key step in our synthesis. A mixture of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate was used as a precatalyst system which generated the Cu(I) species *in situ*. The required alkyne was prepared from nimesulide (**1**) that was reacted with propargyl bromide to give the compound **2**. The reduction of nitro group of **2** followed by ceric ammonium nitrate (CAN) catalyzed reaction of the resulting amine with acetone afforded the 2,2,4-trimethyl-1,2-dihydroquinoline **3** *via* a modified Skraup reaction [9]. The alkyne (**3**) was then coupled with a number of azides (**4**) in the presence of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate smoothly under mild conditions in aqueous media to afford the desired compound (**5**) in 83–95% yield.

4.2. Pharmacology

To assess their PDE4B inhibitory potential, all the synthesized compounds were tested using an enzyme based *in vitro* assay [24]. A well known PDE4 inhibitor Rolipram was used as a reference compound in this assay. While all these compounds showed significant inhibition of PDE4 when tested at 30 μM (Table 1) two of them e.g. **5e** and **5g** showed promising inhibition (>50%). Notably, nimesulide did not show any inhibition at the same concentration whereas the compound **A** (Fig. 1) was found to be a moderate inhibitor of PDE4 (inhibition <40%). To understand the nature of interactions of this class of heterocycles with the PDE4B protein docking studies were performed using compound **5e** in addition to **5g** (or compound **C** as shown in Fig. 2) that showed ~50% inhibition of PDE4B. The dock scores of compounds **5e** and **5g** after docking into the PDE4B protein are presented in Table 2.

Table 1
Inhibition of PDE4B by compound **5** at 30 μM.

Entry	Compound **5**	Average% inhibition	SD
1	**5a**	38.3	1.3
2	**5b**	39.3	2.3
3	**5c**	37.8	2.6
4	**5d**	36.1	1.9
5	**5e**	52.7	1.2
6	**5f**	43.4	2.1
7	**5g**	58.2	0.9
8	**5h**	46.4	2.5
9	**5i**	34.4	1.2
10	Rolipram	90.2	1.1

SD = standard deviation.

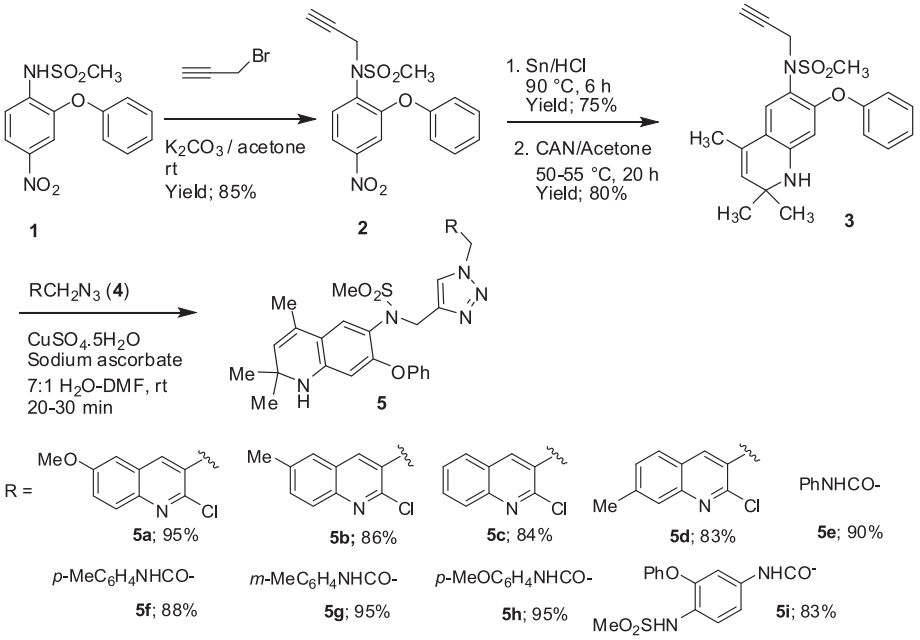
Table 2
Glide score and contributing XP parameters.

Compound code	GScore	LipophilicEvdW	PhobEn	HBond	Electro
5e	−9.16	−5.71	−1.57	−0.67	−1.21
5g	−8.24	−6.33	0.0	−0.52	−1.39

GScore: glide score.
LipophilicEvdW: Chemscore lipophilic pair term and fraction of the total protein–ligand vdw energy.
HBond: Rewards for hydrogen bonding interaction between ligand and protein.
PhobEn: Hydrophobic enclosure reward.
Electro: Electrostatic reward.

The molecular interactions of **5e** are presented in Fig. 3 which indicated that **5e** interacted with Hie-234 through a H-bond and with Phe-446 through π–π stacking.

In view of encouraging PDE4B inhibitory properties of 2,2,4-trimethyl-1,2-dihydroquinolinyl substituted 1,2,3-triazole derivatives we then decided to perform *in vitro* cytotoxic evaluation of these compounds. Being the second leading cause of death worldwide cancer especially lung, prostate, cervical and liver cancers have attracted enormous attention and development of suitable agents to treat these types of cancers is highly desirable. We have used a number of human cancer cell lines e.g. A549 (lung adenocarcinoma epithelial cell line), DU145 (prostate cancer cell line),



Scheme 1. Synthesis of 2,2,4-trimethyl-1,2-dihydroquinolinyl substituted 1,2,3-triazole derivatives **5** (or **B**).

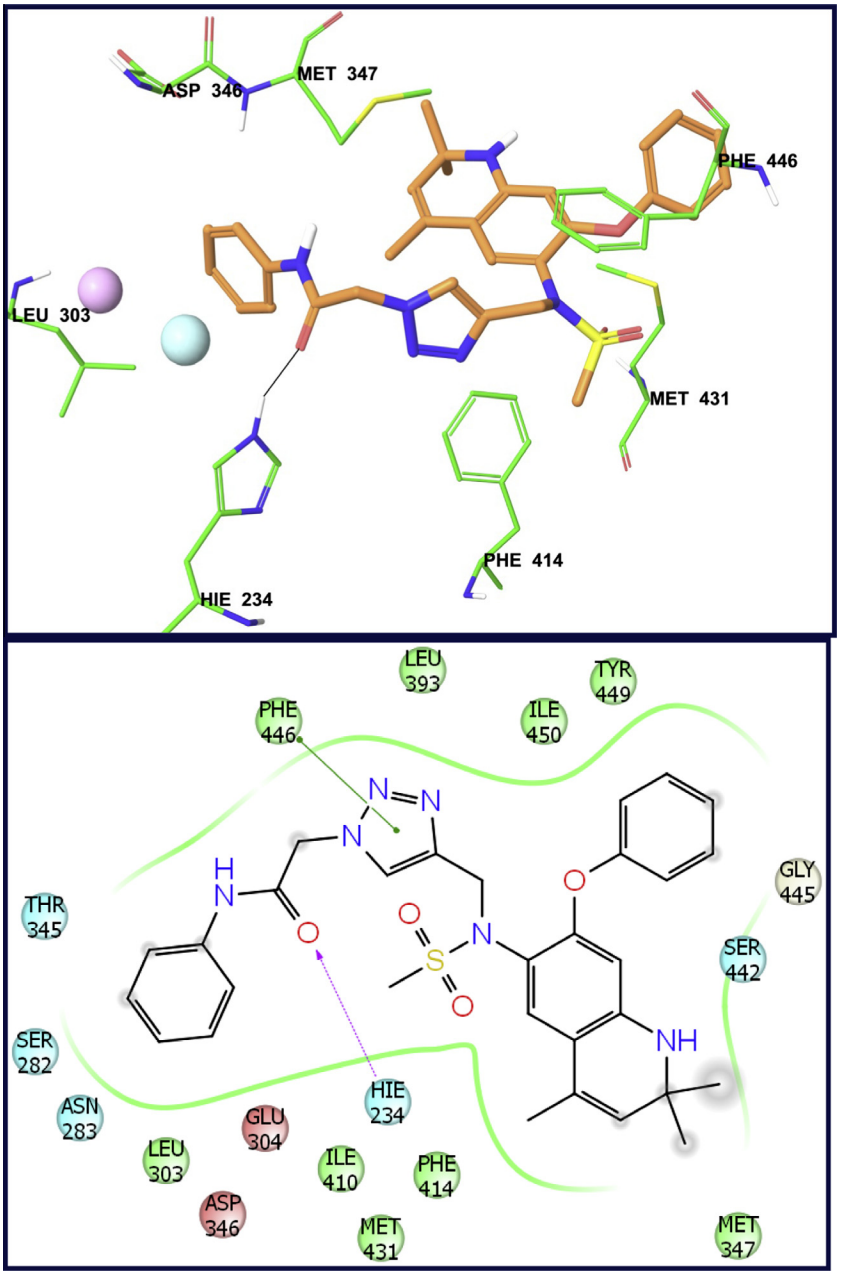


Fig. 3. Binding mode and interactions of molecule **5e** at the inhibitor active site of PDE4B (HIE: Histidine with hydrogen on the epsilon (E) nitrogen).

HeLa (cervical cancer cell line) and HepG2 (hepatocellular liver carcinoma cell line) for our *in vitro* assay. A colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was used to measure the effect of test compounds along with a standard drug doxorubicin on call viability and the corresponding IC_{50} values are presented in Table 3. While all the compounds except **5i** were found to be active against A549 cells, compound **5a**, **5f** and **5g** showed promising effects comparable to that of doxorubicin. The compound **5f** showed activities against HepG2 cells but was 16-fold less potent than doxorubicin. All other compounds were found to be either less active or inactive (e.g. **5b**, **5d** and **5h**). The compound **5i** showed best activities against HeLa cells though found to be 3-fold less potent than doxorubicin. Among other compounds, **5f** and **5g** showed IC_{50} ~14–15 μM against HeLa cells. The compound **5b** and **5f** showed IC_{50} ~10 μM when tested against DU145 cells whereas **5d**, **5h** and **5i** were found to be inactive. Notably, compounds **5e** and **5g** though identified as the most active inhibitors of PDE4 enzyme were not really the most active

Table 3
In vitro evaluation of cytotoxicities of compound **5** against various cancer cell lines.

Entry	Compound **5**	IC_{50} values (μM)			
		A549[a]	HepG2[b]	HeLa[c]	DU145[d]
1	**5a**	8.4 ± 0.11	16.8 ± 0.13	19.2 ± 0.22	13.3 ± 0.22
2	**5b**	11.4 ± 0.12	NA[e]	NA[e]	10.4 ± 0.21
3	**5c**	16.8 ± 0.15	24.5 ± 0.16	35.86 ± 0.21	18.2 ± 0.12
4	**5d**	49.7 ± 0.14	NA[e]	NA[e]	NA[e]
6	**5e**	11.4 ± 0.22	93.1 ± 0.16	24.8 ± 0.21	25.2 ± 0.13
7	**5f**	9.6 ± 0.19	13.1 ± 0.21	14.6 ± 0.18	10.1 ± 0.11
8	**5g**	8.7 ± 0.24	94.5 ± 0.11	15.8 ± 0.32	12.4 ± 0.21
9	**5h**	18.8 ± 0.18	NA[e]	NA[e]	NA[e]
10	**5i**	NA[e]	20.2 ± 0.11	9.7 ± 0.24	NA[e]
11	Doxorubicin	9.7 ± 0.09	0.8 ± 0.10	3.5 ± 0.21	0.5 ± 0.12

[a] A549 – Lung cancer (CCL-185).
[b] DU145 – Prostate cancer (HTB-81).
[c] HeLa – Cervical cancer (CCL-2).
[d] HepG2 – Liver cancer (HB-8065).
[e] NA = Not active.

cytotoxic compounds. Nevertheless, the compound **5g** being promising in both PDE4 and MTT assay (against A549 cells) seemed to have medicinal value. Thus the present class of PDE4 inhibitors has potential for the development of new anticancer agents especially for lung cancer.

5. Conclusions

In conclusion, novel 2,2,4-trimethyl-1,2-dihydroquinolinyl substituted 1,2,3-triazole derivatives were designed as potential inhibitors of PDE4. These compounds were synthesized *via* a multi-step sequence consisting of copper-catalyzed azide-alkyne cycloaddition (CuAAC) as a key step in aqueous DMF. The required alkyne was prepared from nimesulide *via* *N*-propargylation of the methanesulfonamide moiety and then nitro group reduction followed by a CAN mediated modified Skraup reaction of the resulting amine. All the synthesized compounds showed PDE4B inhibitory properties *in vitro* at 30 μM with two compounds e.g. **5e** and **5g** showing >50% inhibition. This observation was supported by the docking studies of these two compounds at the active site of PDE4B enzyme. *In vitro* cytotoxic evaluation of these compounds were also performed using four human cancer cell lines e.g. A549, DU145, HeLa and HepG2 in an MTT assay. Three of these PDE4 inhibitors e.g. **5a**, **5f** and **5g** showed promising cytotoxic properties against A549 human lung cancer cells *in vitro* with IC_{50} ~8–9 μM. The present class of compounds therefore represents new templates for the identification and development of novel PDE4 inhibitors/potential anticancer agents.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2013.12.002.

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