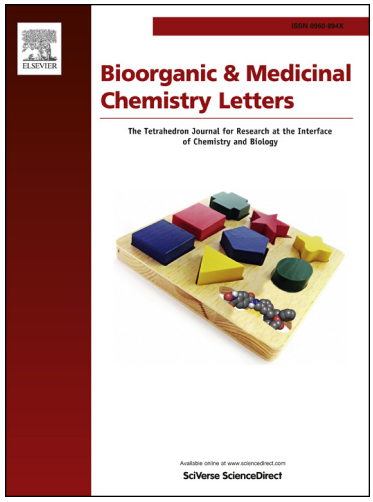

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Synthesis and biological evaluation of nimesulide based new class of triazole derivatives as potential PDE 4B inhibitors against cancer cells

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Abstract: A new class of 1,2,3-triazol derivatives derived from nimesulide was designed as potential inhibitors of PDE4B. Synthesis of these compounds was carried out *via* a multi-step sequence consisting of copper-catalyzed azide-alkyne cycloaddition (CuAAC) as a key step in aqueous media. The required azide was prepared *via* the reaction of aryl amine (obtained from nimesulide) with α-chloroacetyl chloride followed by displacing the α-chloro group by an azide. Some of the synthesized compounds showed encouraging PDE4B inhibitory properties *in vitro* i.e. > 50% inhibition at 30 μM that were supported by the docking studies of these compounds at the active site of PDE4B enzyme (dock scores ~ -28.6 for a representative compound). Two of these PDE4 inhibitors showed promising cytotoxic properties against HCT-15 human colon cancer cells *in vitro* with IC_{50} ~ 21-22 µg/mL.

Keywords: nimesulide, 1,2,3-triazole, cycloaddition, PDE4B, cytotoxic activities

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The design and construction of compound library based on novel "drug like" molecules *via* structural modifications of a known drug by taking advantages of modern synthetic methodologies is the central focus of current medicinal chemistry and new drug discovery. Due to its simplicity the use of "click chemistry" has attracted particular attention[1] for this purpose.

Cancer being the second leading cause of death[2] worldwide after cardiovascular diseases requires urgent attention and thus discovery and development of suitable agents possessing novel mechanism of action is highly desirable to treat various types of cancer. Recently, studies have indicated that phosphodiesterases 4 (PDE4, a member of super family of enzymes e.g. PDEs)[3] that degrade cyclic adenosine monophosphate (cAMP) specifically is widely expressed in tumor cells. Thus, inhibition of PDE4 in cancerous cells can be a new therapeutic target for cancer. This is exemplified by the reports on ability of PDE4 inhibitors to (i) inhibit brain tumor cell growth,[4,5] (ii) reduce proliferation and angiogenesis of lung cancer cell lines[6] and (iii) cause selective apoptosis of malignant cells without affecting the normal cells.[7]

Nimesulide[8] (Fig. 1), a well known non-steroidal anti-inflammatory drug (NSAID) is presently available in several countries for the treatment of acute pain, the symptomatic treatment of osteoarthritis and primary dysmenorrhoea in adolescents and adults above 12 years old. It is a cycloxygenase-2 (COX-2) inhibitor possessing analgesic and antipyretic properties and shows a fast onset of action due to its multi-factorial mode of action. However, nimesulide has been withdrawn from the market in many countries due to concerns about the risk of its hepatotoxicity.[9] Studies have indicated that the observed hepatotoxicity of nimesulide can be linked to its uncoupling effects on mitochondria *via* a protonopheretic mechanism and oxidation of mitochondrial NADH and NADPH.[10] Since the chemical reduction of nitro group of nimesulide to amine completely suppressed the above mitochondrial responses hence it is reasonable to use the reduced form i.e. the corresponding amine derived from nimesulide for the design of new chemical entities. In view of reported anticancer properties[11] of nimesulide and anti-inflammatory activities 1,2,3-triazoles[12] we anticipated that combining the structural features of nimesulide and 1,2,3-triazole in a single molecule (Fig. 1) may afford novel template **A** for the design and synthesis of new anticancer agents that may inhibit PDE4. This was supported by the docking studies of a representative molecule e.g. 2-(4-((4-chlorophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-N-(4-(methylsulfonamido)-3-phenoxyphenyl)acetamide (**B**) into PDE4B protein *in silico* which showed strong interactions (Glide score -26.18) involving $-OCH_2-$ and $-NHSO_2Me$ groups of **B** with the Gln443 and Asn283 residues of PDE4B respectively (Fig. 2). Due to our continuing interest in the structural modifications of nimesulide,[13-18] we now report the synthesis, *in vitro* PDE4 inhibition[19] and cytotoxic effects of a series of novel 1,2,3-triazoles **A** derived from nimesulide. To the best of our knowledge synthesis and pharmacological evaluation of 1,2,3-triazoles derived from nimesulide is not known in the literature. Additionally, evaluation of novel PDE4 inhibitors for the potential treatment of cancer is not common in the literature.

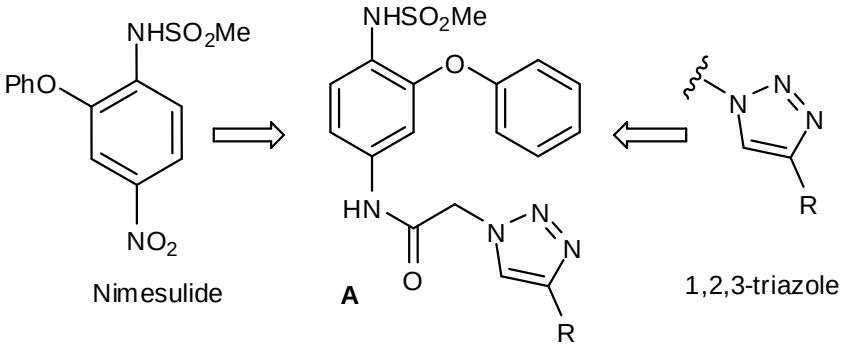
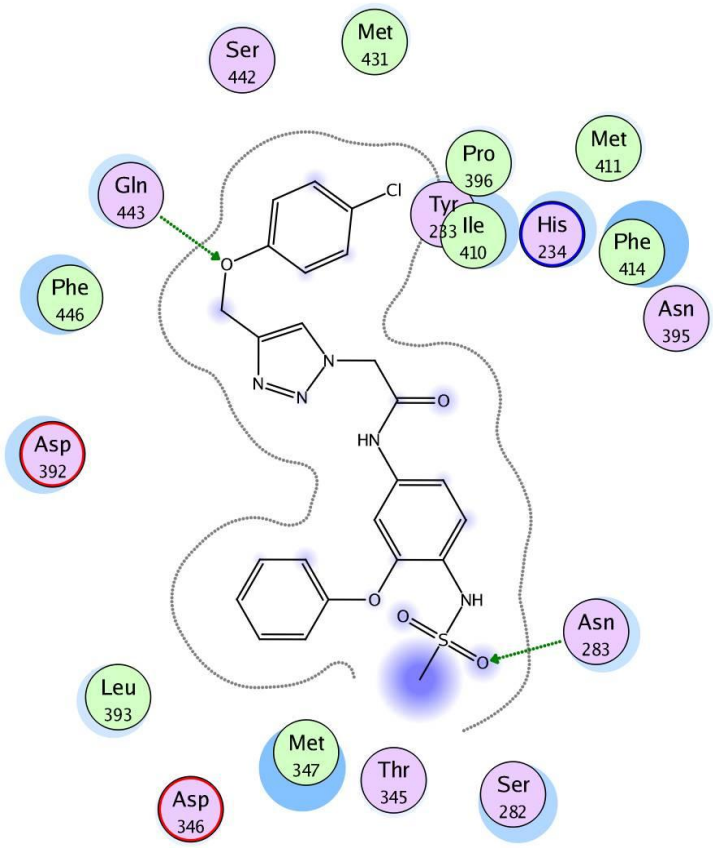


Fig. 1. Design of novel and potential anticancer agents based on nimesulide and 1,2,3-triazole



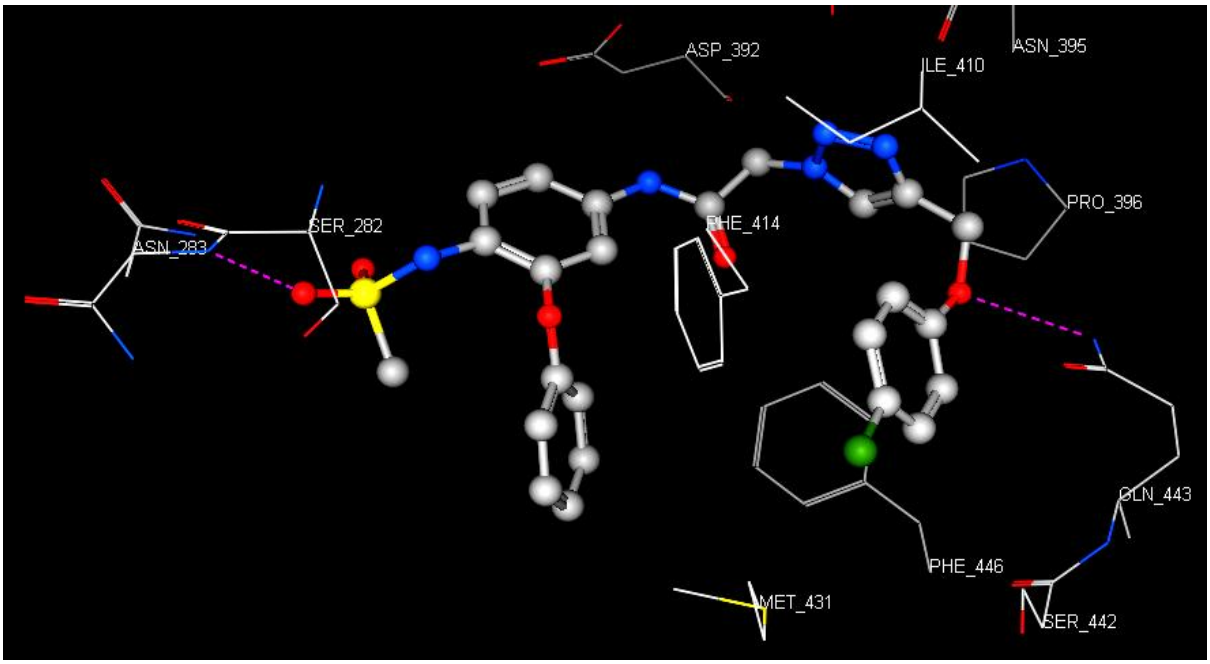
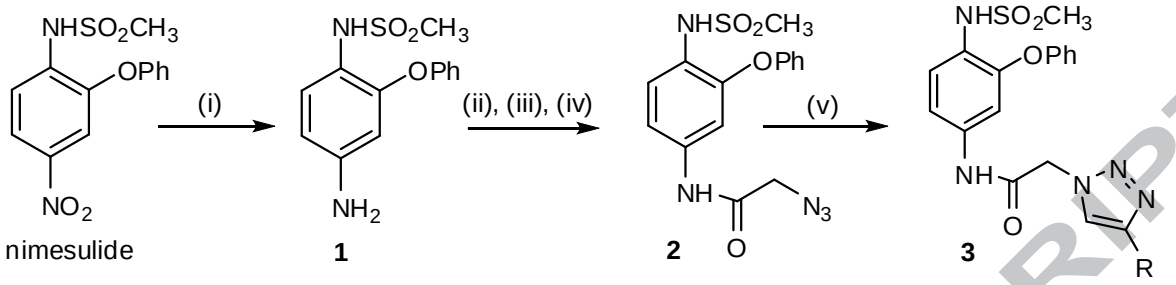


Fig. 2. Binding of designed molecule **B** in the PDE4B pocket

The synthesis of our target compounds **3** (or **A**) was carried out by adopting a multi-step sequence as outlined in Scheme 1. The 1,2,3-triazoles are generally synthesized by using click chemistry approach that involves copper (I)-catalyzed 1,3-cycloaddition reaction between a terminal alkyne and an azide.[1] Thus, the copper-catalyzed azide-alkyne cycloaddition (CuAAC) in aqueous media was used as a key step in our synthesis. A mixture of $CuSO_4$ and sodium ascorbate was used as a precatalyst system which generated the Cu(I) species *in situ*. The required azide was prepared[20] from nimesulide that was reduced to amine (**1**) which on reaction with 2-chloroacetyl chloride afforded the corresponding chloro derivative. Treating this chloro compound with KI followed by sodium azide (**1**) provided the corresponding azide derivative (**2**). The azide (**2**) was then coupled with a variety of terminal alkynes (**2a-i**) in the presence of $CuSO_4$ and sodium ascorbate smoothly under mild conditions in water to afford the desired 1,2,3-triazoles (**3**). All these compounds were well characterized by spectral (NMR, IR and MS) and analytical data. For example the appearance of two singlets near δ 5.2 & 5.1 ppm in the ^{1}H NMR spectrum of **3b** (see ESI) indicated the presence of two methylene groups. The methyl protons of $NHSO_2Me$ group appeared as a singlet at δ 3.0 ppm. The NH hydrogens appeared as two singlets at δ 10.5 and 9.3 ppm that disappeared during D_2O exchange experiment. As expected from its molecular structure, a total of 20 signals appeared for twenty chemically nonequivalent carbon atoms when broad band decoupled ^{13}CNMR spectrum of **3b** (Fig. 3) was recorded. The peak at δ 164.2 ppm confirmed amide C=O group. Additionally, 16 out of 20 signals were assigned for different aromatic carbons which include both quaternary (C) and methine (CH) carbon atom. The methyl carbon of $-NHSO_2Me$ appeared at δ 29.3 ppm. In order to assign the chemical shift values correctly DEPT spectrum was also recorded (Fig. 3). This showed signals at (i) δ 60.6 and 51.8 ppm (inverted peaks) due to the presence of two CH_2 (ii) δ 29.3 ppm due to a methyl and (iii) δ 129.8, 129.2, 127.6, 125.9, 123.8, 120.5, 119.2, 114.3, 113.7 and 108.4 ppm because of ten CH groups. Additionally, the signals for six aromatic quaternary

carbons and one C=O group appeared in the broad band decoupled spectrum disappeared completely in the DEPT spectrum.



R (time; % yield)
3a: Ph (50 min; 86)
3b: $PhOCH_2$ (1h; 83)
3c: $C_{10}H_7OCH_2$ (2h; 97)
3d: *p*-$CH_3C_6H_4$ (2h; 83)
3e; *p*-$CH_3C_6H_4OCH_2$ (10 min; 71)
3f; *p*-$ClC_6H_4OCH_2$ (10 min; 83)
3g; *p*-Cl(*m*-CH_3) $C_6H_3OCH_2$ (10 min; 85)
3h; *o*-$ClC_6H_4OCH_2$ (10 min; 95)
3i; *p*-$NO_2C_6H_4OCH_2$ (30 min; 85)

Scheme 1. Reagents and conditions: (i) Sn/HCl (ii) $ClCH_2COCl$, Et_3N, $CHCl_3$, room temp, 1.5 h, 85%; (iii) KI, acetone, room temp, 3h, 91%; (iv) NaN_3, acetone, room temp, 6h, 90%; (v) R-C≡CH, $CuSO_4$, Na-ascorbate, H_2O, room temp, 10 min-2h.

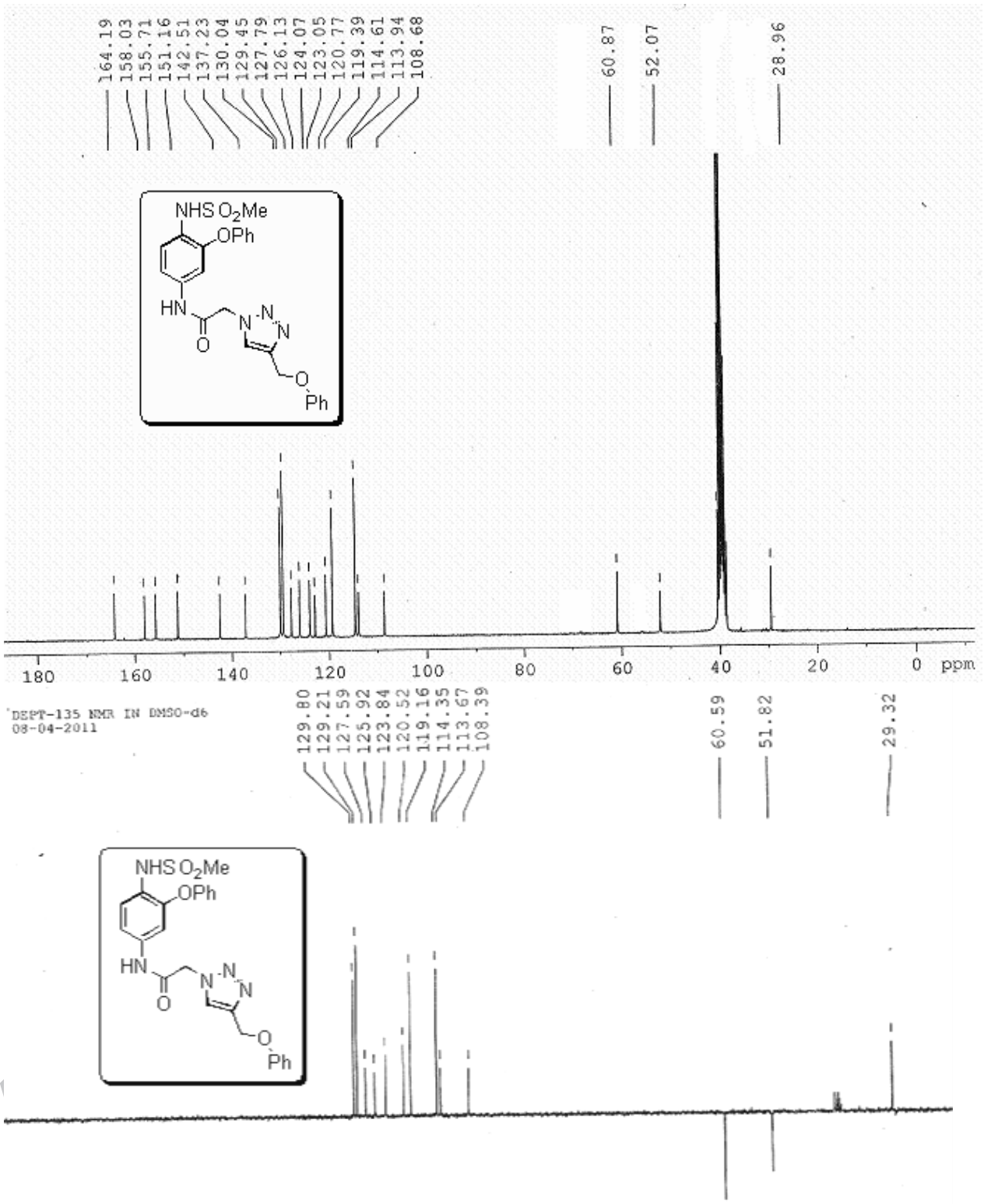


Fig. 3. ^{1}H and ^{13}C NMR spectra of compound **3b** in DMSO-d_6.

All the 1,2,3-triazoles synthesized were assessed for their PDE4B inhibitory potential using an enzyme based assay.[21] Rolipram was used as a reference compound in this assay. A number of compounds e.g. **3a-c**, **3e** and **3f** showed significant inhibition (> 50%) of PDE4 when tested at 30 μM (Table 1) whereas nimesulide did not show any inhibition at the same concentration. To understand the nature of interactions of this class of heterocycles with the PDE4B protein docking studies were performed using selected compounds e.g. **3a, 3c, 3e, 3f** and **3i** that showed ~ 50% inhibition of PDE4B. The dock scores and molecular interactions of compound **3** after docking into the PDE4B protein are presented in Table 2. It is evident that the compound **3a** showed highest dock score which is in agreement with its in vitro data generated using the enzyme assay. Additionally, all these compounds showed similar docking score which are also in agreement with their PDE4B inhibition data (Table 1). While all these 1,2,3-triazoles interacted with the same and /or similar amino acid residues at the active site of the PDE4B protein their orientation of interactions were largely found to be somewhat dissimilar depending on the substituents present on the triazole ring (Fig. 2, 4 and 5, see also Fig. S-1 and S-2 in the ESI). All these molecules showed common interactions with Gln443 of PDE4B protein similar to Rolipram (see Fig S-3 in the ESI). In general, the moieties or substitutents that participated simultaneously or individually in the interactions include the triazole ring, central benzene ring, the -$NHSO_2$- group, -CH_2O- moiety etc. In a dose response study the compound **3a** showed dose dependent inhibition of PDE4B with IC_{50} value of 4.92 ± 0.53 µM.

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

Entry	Compound **3**	Average % Inhibition	SD
1.	**3a**	64.09	5.20
2.	**3b**	51.17	1.31
3.	**3c**	59.69	5.61
4.	**3d**	36.01	2.32
5.	**3e**	56.67	1.94
6.	**3f**	51.32	2.01
7.	**3g**	32.91	0.88
8.	**3h**	42.53	1.90
9.	**3i**	47.11	2.43
10.	Rolipram	94.12	1.01

SD = standard deviation

Table 2. Dock scores and summary of molecular interactions of compound **3** after docking into PDE4B

Compound **3**	Dock scores	**S**ummary of interactions
3a	-28.62	Gln443, His234,His278
3c	-26.62	Gln443, Asn283
3e	-24.92	Gln443
3f	-26.18	Gln443, Asn283
3i	-25.16	Gln443

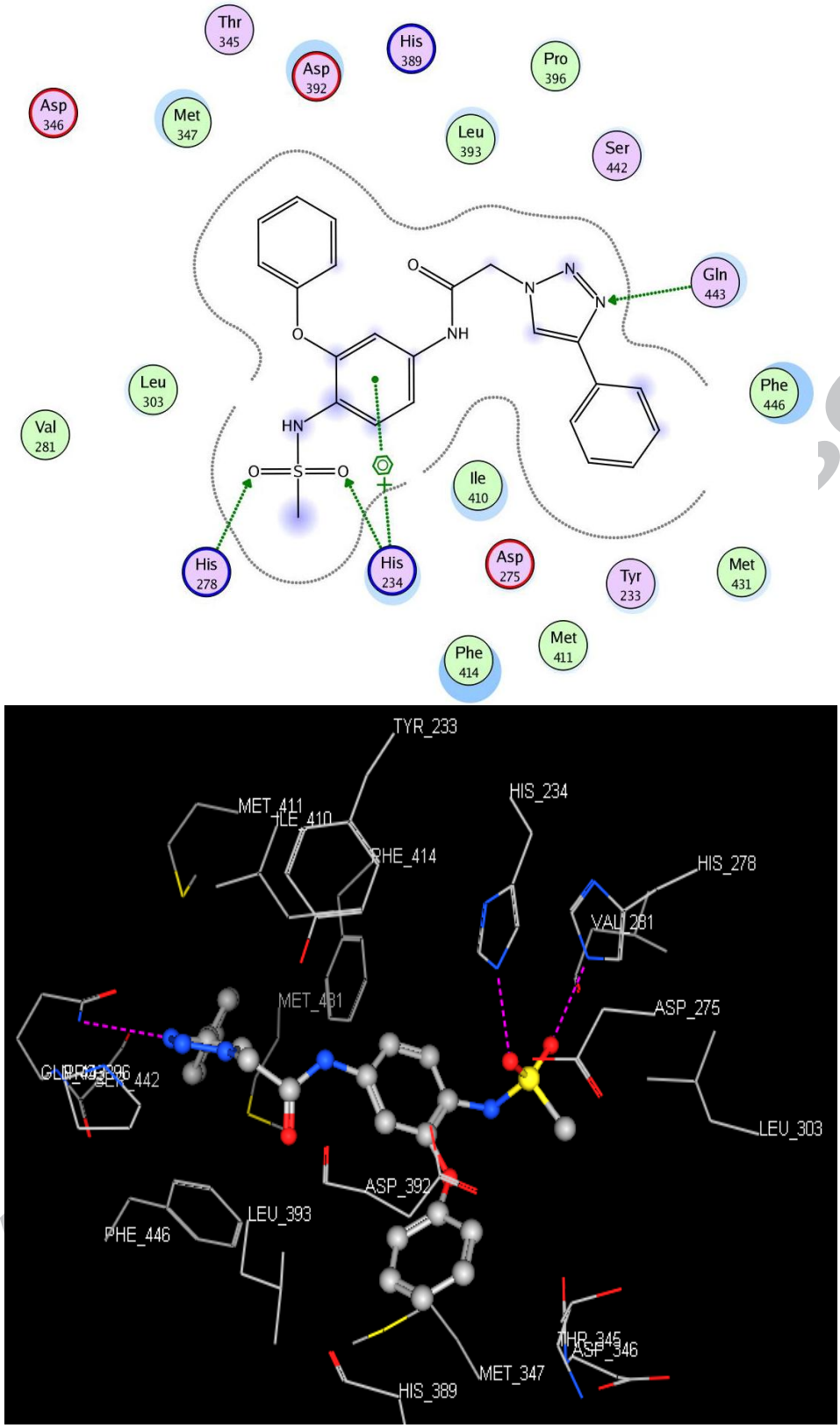


Fig. 4. Docking of **3a** at the active site of PDE4B.

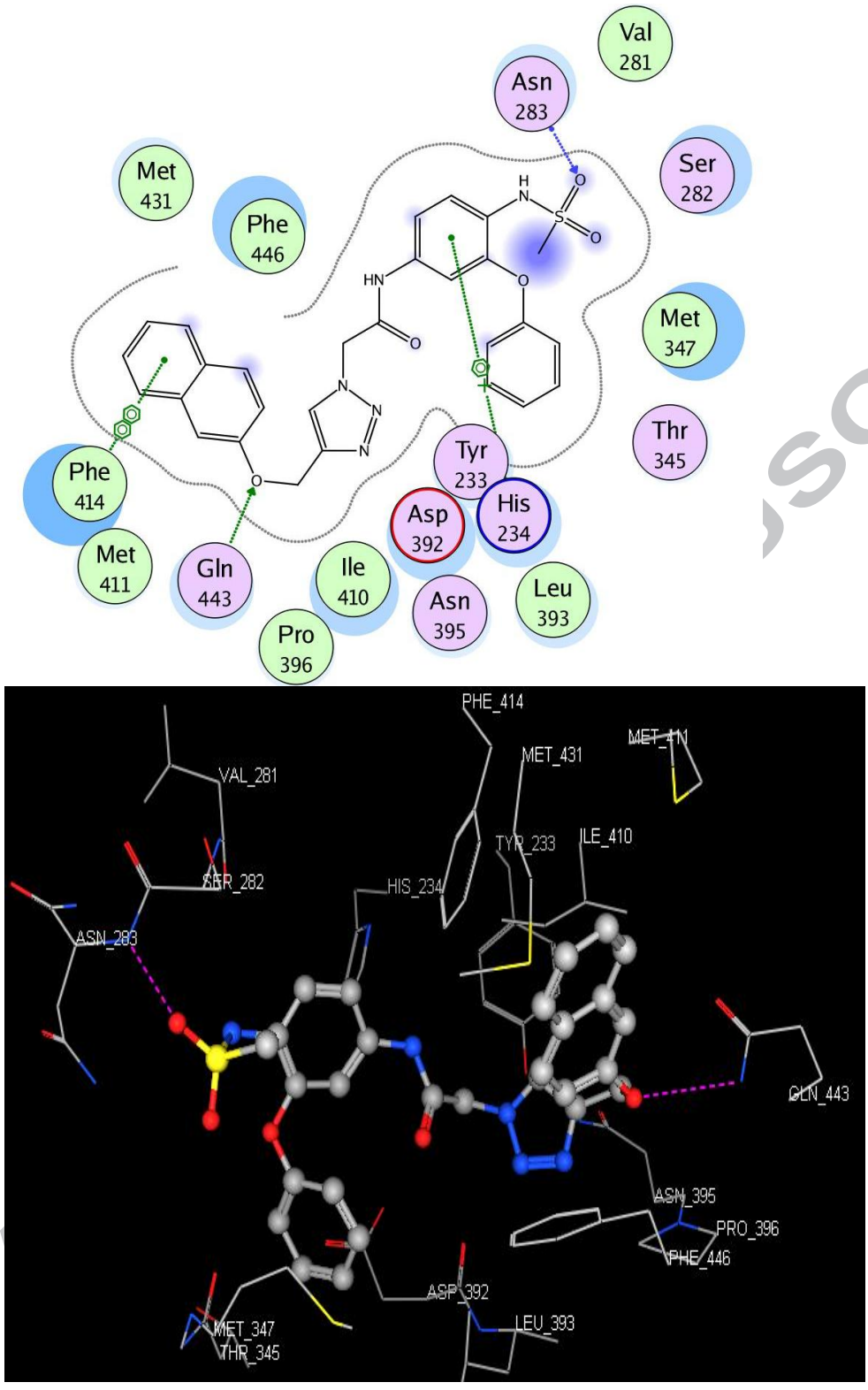


Fig. 5. Docking of **3c** at the active site of PDE4B.

Having identified a number of 1,2,3-triazoles (**3**) as novel inhibitors of PDE4B we performed *in vitro* cytotoxic evaluation of some of these compounds using human colon cancer cell line HCT-15.[22] Colon (or colorectal) cancer is widespread in the Western world and is the focus of our current research. Moreover, recent study suggested that PDE4B is upregulated by oncogenic KRAS, and inhibition of PDE4 catalytic activity by rolipram can induce both epithelial cell polarity and luminal apoptosis in human colorectal cancer (CRC) HCT116 cells.[23] The test compounds were examined for their ability to inhibit the cancerous cells (HCT-15) based on an MTT assay. Doxorubicin, a known anthracycline antibiotic was used as a reference compound in our assay. All these compounds were tested at five different concentrations e.g. 5, 10, 15, 20 and 25μg/mL and the percentage of cell death measured for each compound along with IC_{50} values of active compounds are summarized in Table 3. It is evident that **3a** and **3d** showed good activities especially at higher dose. Both these compounds showed IC_{50} values in the range 21-22 μg/mL (Table 3) compared to doxorubicin's IC_{50} value of 50 μg/mL (0.09 μM). Thus the present 1,2,3-triazole based PDE4 inhibitors have potential for the development of new anticancer agents especially for colon cancer.

Table 3. The *in vitro* anticancer properties of some of the 1,2,3-triazoles (**3**) synthesized.

Entry	Compounds	% of cell death at various concentrations					IC_{50} (μg/mL)
		5 μg /mL	10 μg /mL	15 μg /mL	20 μg /mL	25 μg /mL	
1	**3a**	10.8	15.2	31.0	42.7	60.1	22.4
2	**3c**	10.6	14.3	21.1	25.3	33.6	n.d.
3	**3d**	37.0	40.2	45.6	49.6	53.0	21.0

All the values are the average of the experiments done in triplicates. The cell line used is HCT-15 human colon cancer cell line. Doxorubicin [IC_{50} = 50 μg /mL (0.09 μM)] was used as a reference compound. n.d. = not done.

In conclusion, novel 1,2,3-triazole derivatives derived from nimesulide were designed as potential inhibitors of PDE4. Synthesis of these compounds was carried out *via* a multi-step sequence consisting of copper-catalyzed azide-alkyne cycloaddition (CuAAC) as a key step in aqueous media. The required azide was prepared *via* the reaction of aryl amine (obtained from nimesulide) with α-chloroacetyl chloride followed by displacing the α-chloro group by an azide. All the 1,2,3-triazole derivatives were prepared in 71-97% yield. Some of the synthesized compounds showed encouraging PDE4B inhibitory properties *in vitro* i.e. > 50% inhibition at 30 μM that were supported by the docking studies of these compounds at the active site of PDE4B enzyme. All these compounds showed dock scores in the range of -28.6 to -24.9. Two of these PDE4 inhibitors i.e. **3a** and **3d** showed promising cytotoxic properties against HCT-15 human colon cancer cells *in vitro* with IC_{50} ~ 21-22 μg/mL. Thus, the present 1,2,3-triazole class of compounds represent new templates for the identification and development of novel PDE4 inhibitors / potential anti-cancer agents.

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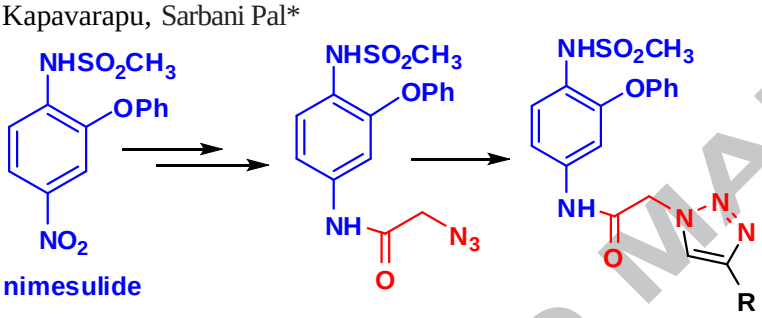

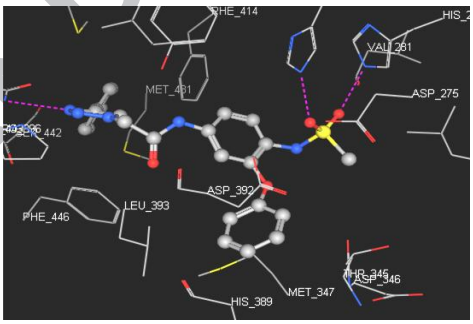

Binding mode with PDE4B (R = Ph)