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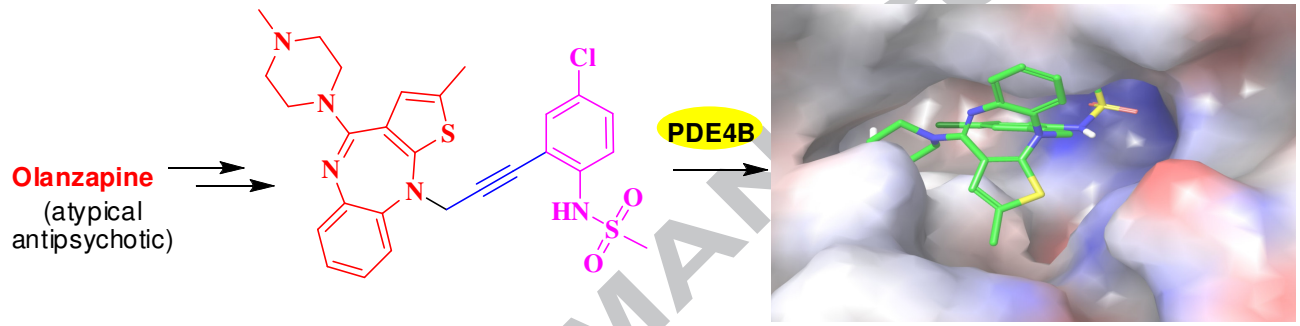

Synthesis of *N*-(3-arylprop-2-ynyl)substituted olanzapine derivatives as potential inhibitors of PDE4B

P. Vijaya Babu,[a,b] Dhilli Rao Gorja,[a,b] Chandana Lakshmi T. Meda,[a] Girdhar Singh Deora,[c] Sunder Kumar Kolli,[d] Kishore V. L. Parsa,[a] K. Mukkanti,[b] Manojit Pal[a,*]

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Abstract: The linkage between dopamine D2 receptors and PDE activity *via* cAMP prompted us to design a series of novel *N*-(3-arylprop-2-ynyl)substituted olanzapine derivatives as potential inhibitors of PDE4B. The target compounds were conveniently prepared by using a simple and inexpensive method involving Pd/C-mediated C-C bond forming reaction under Sonogashira conditions. A number of compounds were synthesized by using this strategy in good yields. Some of the compounds showed promising inhibition of PDE4B when tested *in vitro* that was supported by the docking studies.

Keywords: olanzapine, alkyne, PDE4, docking

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Schizophrenia being a complex neuropsychiatric disorder is characterized by abnormalities in the perception of reality[1] and affects approximately 1% of the adult population worldwide.[2,3] The antipsychotic medications are the mainstay of treatment for most schizophrenic patients and can be classified into two major classes e.g. conventional (typical) and novel (atypical) neuroleptic drugs. These drugs mainly suppress dopamine and sometimes serotonin receptor activities. Clozapine that belongs to the atypical class is a debenzepine based antipsychotic and was found to be superior over other common antipsychotics.[4] Its binding to the D-2, D-4, 5-HT_{2A} and 5-HT_{2C} along with D-1, α-1, α-2, M-1 and H-1 receptors was found to be beneficial.[5] A structurally close analogue of clozapine called olanzapine (zyprexa) belonging to the thienobenzodiazepine class has also been developed and found to possess similar pharmacological properties.[6] While useful in treating some aspects of schizophrenia recent studies have suggested that currently available antipsychotic medications still possess considerable limitations. One of the many drug strategies that have been proposed in recent years is based on the fact that members of the phosphodiesterase (PDE) gene family may play a role in the treatment of schizophrenia.[7a] For example antipsychotic drugs are known to function through antagonism (blockade) of D2 dopamine receptors which in turn causes an increase level of cAMP (Fig. 1). This is also the outcome of inhibition of PDEs, a family of enzymes that degrade cyclic nucleotides (Fig. 1). This linkage between dopamine D2 receptors and PDE activity *via* cAMP is the basis for a possible therapeutic potential for PDE inhibitors in schizophrenia.[7b] Indeed, increasing evidences suggest that inhibition of PDE especially PDE4 and PDE10 can be beneficial for treating the positive, negative or cognitive symptoms associated with schizophrenia.[7]

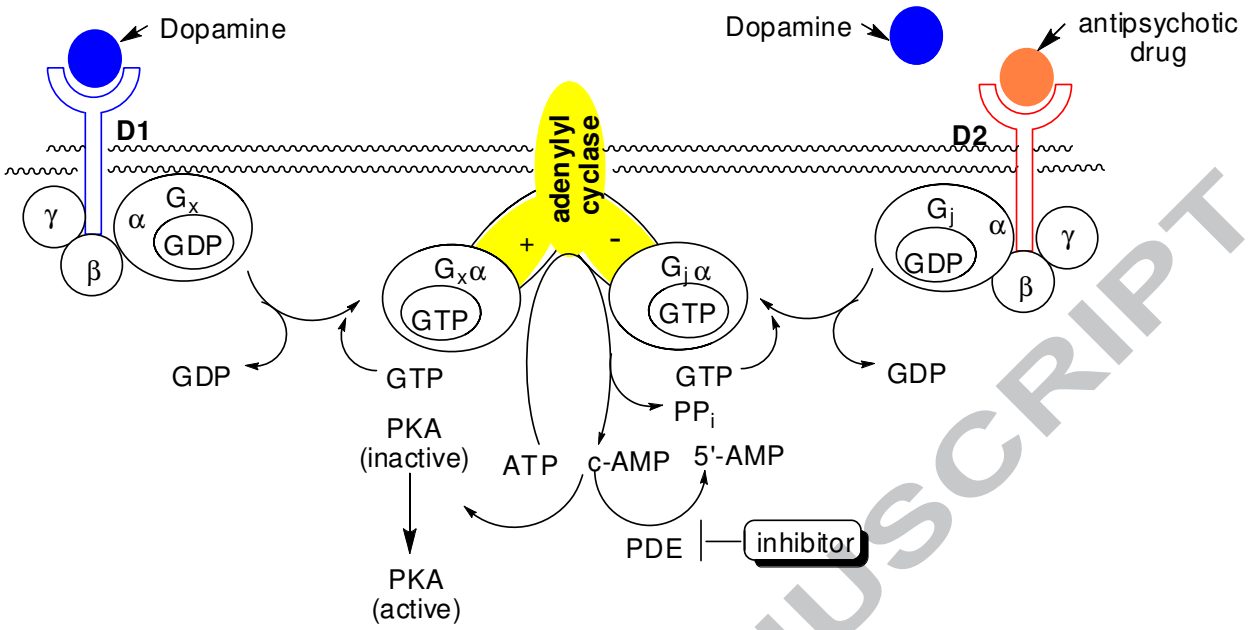


Fig. 1. Increase of cAMP levels by antipsychotic medication and inhibition of PDE:[7b] The activity of enzyme adenylyl cyclase (AC) is increased by G protein-coupled D1-type dopamine receptors and decreased by D2 dopamine receptors. The antipsychotic drugs (believed to be antagonist of the D2 dopamine receptor) increase AC activity and the AC converts ATP to cAMP. The cAMP levels are controlled by PDEs *via* degrading the cAMP molecule to 5′-AMP. Inhibition of PDE activity by its inhibitors increases cAMP levels.

Baesd on these observations and the fact that atypical antipsychotics including olanzapine (A, Fig. 2) have been used as a first line therapy to treat schizophrenia, we decided to focus on the identification of new PDE4 inhibitors *via* structural modifications of olanzapine.[8] A diverse class of compounds has been explored for the discovery of novel PDE4 inhibitors.[9] For example, alkyne derivatives **B** (Fig. 2) has been reported as inhibitors of PDE4.[10] Thus, we hypothesized that combining some of the structural features of **A** and **B** in a single molecule may lead to a new class of compound **C** (Figure 1) which may be explored for the identification of novel PDE4 inhibitors[11] thereby potential treatment of schizophrenia. We initially became interested in the synthesis of **C** and subsequent evaluation of their PDE4 inhibiting properties *in vitro*.

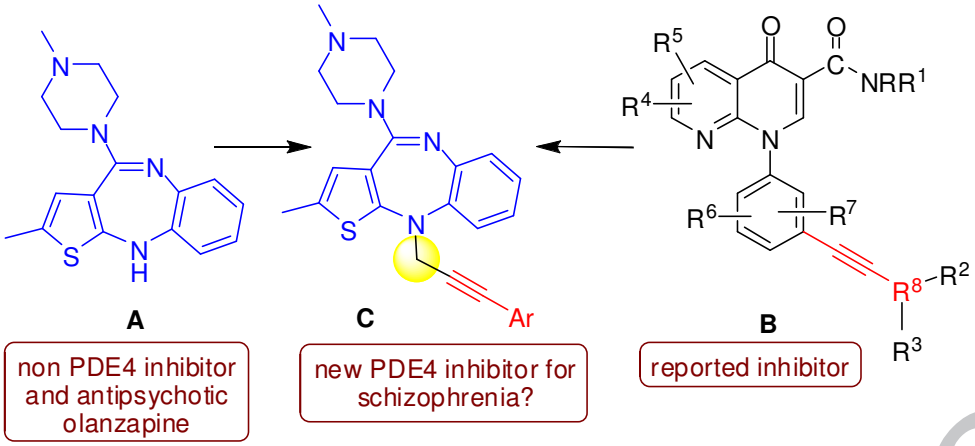
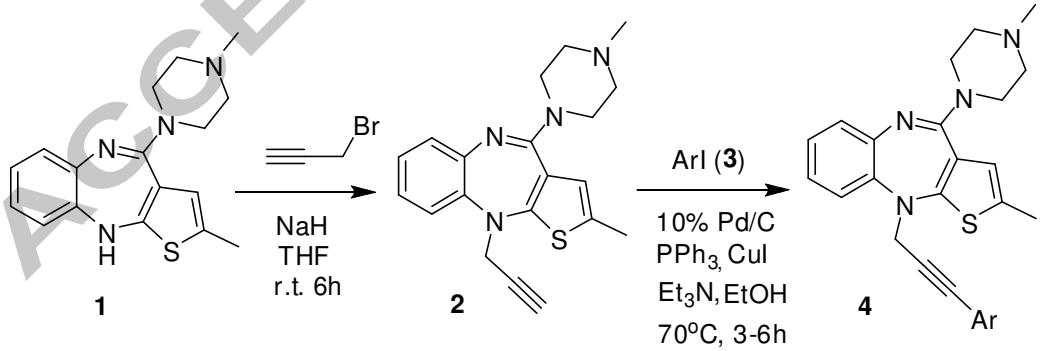


Fig. 2. Design of new PDE4 inhibitors (**C**) based on olazapione **A** and reported inhibitors **B**.

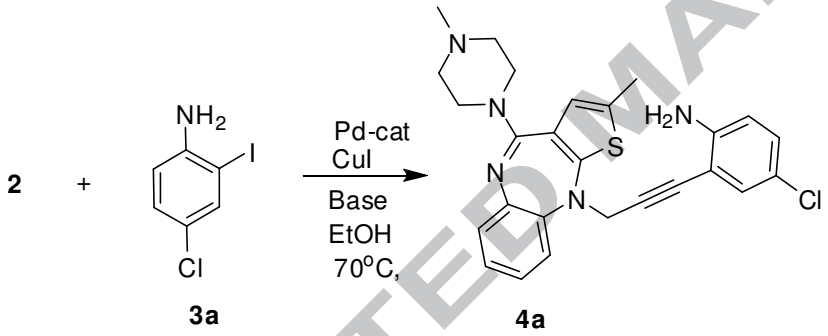
The designed target compounds were conveniently prepared by using a Pd/C-mediated C-C bond forming reaction under Sonogashira type coupling conditions.[12] The use of Pd/C–CuI–PPh_3 as an alternative but inexpensive catalyst system for Sonogashira type coupling has gained considerable interest.[12] The use of Pd/C as a catalyst offers several advantages. For example, Pd/C is stable, easy to handle and can be stored for a long period of time without taking any extra precautions. Additionally, it can be separated easily from the product *via* simple filtration and is recyclable. More importantly, in view of the use of Pd/C for hydrogenation in industrial scale for over more than 100 years the Pd/C mediated coupling reactions are amenable for large scale preparation. Thus, due to the simplicity, advantages and versatility of the Pd/C mediated C-C bond forming reactions we decided to explore this strategy for the synthesis of compound **C** (or **4**) as shown in Scheme 1.



Scheme 1. Synthesis of *N*-(3-phenylprop-2-ynyl)substituted olanzapine derivatives **4**.

The key starting material i.e. the terminal alkyne **2** was synthesized by treating olanzapine (**1**) with propargyl bromide in the presence of NaH in THF (Scheme 1).[13] Initially, the alkyne **2** was reacted with 4-chloro-2-iodoaniline **3a** in the presence of 10%Pd/C-PPh_3-CuI and Et_3N in EtOH at 70°C for 6 h (entry 1, Table 1). The desired product **4a** was isolated in 90% yield. An increase in reaction time did not improve the product yield (entry 2, Table 1) whereas change of base from Et_3N to K_2CO_3 suppressed the product formation significantly (entry 3, Table 1). The reaction also did not afford **4a** in good yield in the absence of Pd/C or CuI indicating their key role in the present reaction (entry 4 and 5, Table 1). The use of other Pd-catalyst e.g. $Pd(PPh_3)_2Cl_2$ was also examined (entry 5, Table 1). While the reaction proceeded in this case the yield of product **4a** was inferior to that of entry 1. Thus the reaction condition of entry 1 was chosen for the further study.

Table 1. Effect of reaction conditions on coupling of terminal alkyne **2** with **3a**

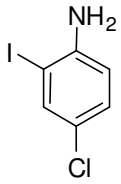
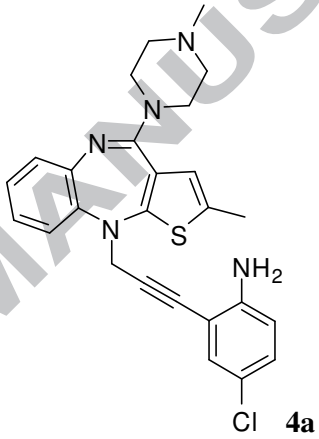
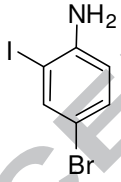
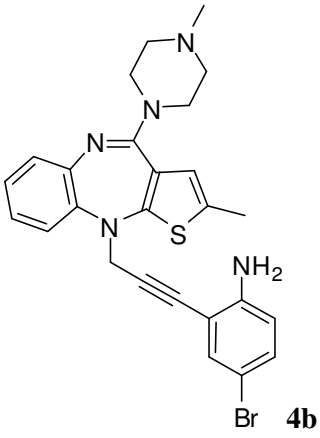


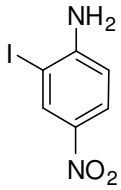
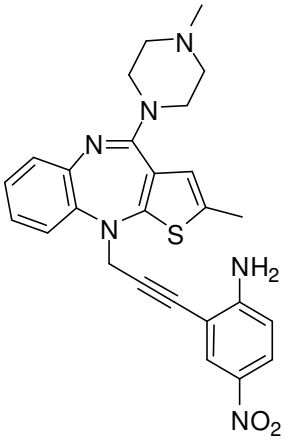
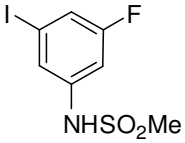
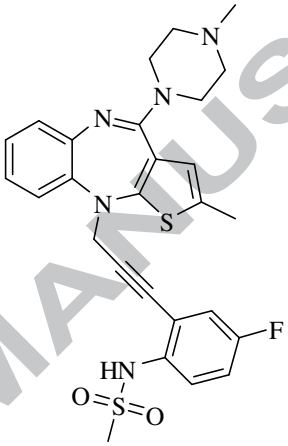
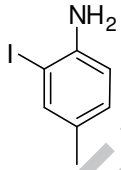
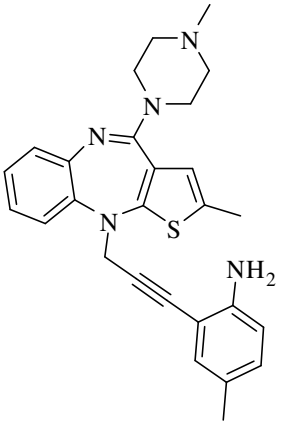
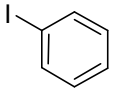
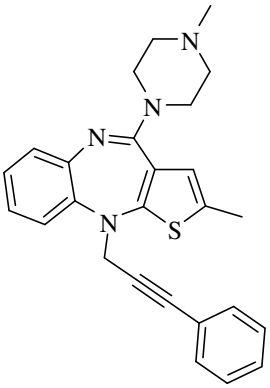
Entry	Pd-catalysts	Base	Time (h)	Yield [b] (%)
1	10% Pd/C–PPh_3	Et_3N	6	90
2	10% Pd/C–PPh_3	Et_3N	8	89
3	10% Pd/C–PPh_3	K_2CO_3	10	10
4	PPh_3[c]	Et_3N	10	9
5	10% Pd/C–PPh_3[d]	Et_3N	8	10
6	$Pd(PPh_3)_2Cl_2$	Et_3N	6	60

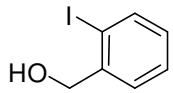
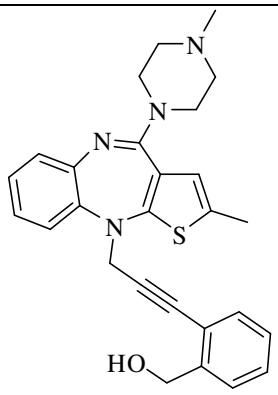
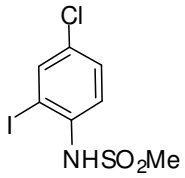
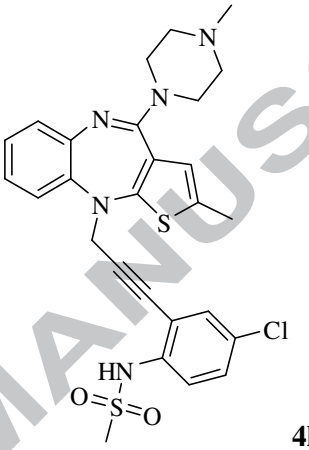
[a]All reactions were carried out using **2** (1 equiv), alkyne **3a** (1 equiv), a Pd-catalyst (0.016 equiv), PPh_3 (0.125 equiv), CuI (0.02 equiv), and a base (2 equiv) in EtOH (5.0 mL), at 70 °C. [b]Isolated yield. [c]The reaction was carried out without Pd/C. [d]The reaction was carried out without CuI.

We then prepared a small library of compounds using the optimized conditions (Table 2). A number of iodoarenes (**3**) were coupled with the alkyne **2** in the presence of 10%Pd/C-PPh_3-CuI to give the desired products **4** in acceptable to good yield.[14] Notably, a shorter reaction time was applied in the case of iodoarene **3d** and **3h** to avoid the further intramolecular cyclization of the alkyne **4d** and **4h** generated *in situ*.[8]

Table 2. Pd/C-mediated synthesis of *N*-(3-arylprop-2-ynyl)substituted olanzapine derivatives (**4**).[a]

Entry	Iodoarene (**3**)	Time (h)	Product (**4**)	Yield[b] (%)
1	 **3a**	6	 **4a**	90
2	 **3b**	6	 **4b**	85

3	 **3c**	6	 **4c**	93
4	 **3d**	3	 **4d**	90
5	 **3e**	5.5	 **4e**	92
6	 **3f**	5	 **4f**	85

7	 **3g**	6	 **4g**	80
8	 **3h**	3	 **4h**	78

[a]All reactions were carried out using **2** (1 equiv.), alkyne **3** (1 equiv.), 10% Pd/C (0.016 equiv.), PPh_3 (0.125 equiv.), CuI (0.02 equiv.), and Et_3N (2 equiv.) in EtOH (5.0 mL), at 70 °C. [b]Isolated yield.

The PDE4B inhibitory potential of all the synthesized compounds were assessed *in vitro* at 30 μM by using a PDE4B enzyme assay[15] (Table 3). Rolipram[16] a well known PDE4 inhibitor was used as a reference compound in this assay. Compounds that showed inhibition greater than 50% were considered as promising inhibitors. Accordingly, compounds **4c, 4d, 4g** and **4h** were identified as hit molecules and **4h** that showed ~ 63% inhibition was found to be the best. While rolipram showed ~ 88% inhibition the parent compound olanzapine however did not show any PDE4 inhibitory properties. It is therefore evident from Table 3 that a non PDE4 inhibitor olanzapine has been converted into PDE inhibitors *via* introducing an appropriate 3-arylprop-2-ynyl moiety at its central ring nitrogen. It is also evident that an anilide moiety attached to the alkyne group played a key role in the inhibitory activities e.g. **4g** and **4h**. In a dose response study the compound **4h** showed inhibitory activities across all the dose tested (31, 23, 15 and 14% at 10, 3, 1 and 0.3 μM) indicating its potential as a PDE4

inhibitor.

Table 3. Inhibition of PDE4B by compound **4** at 30 uM.

Entry	Compounds	Average % inhibition	SD
1.	**4a**	27.21	1.72
2.	**4b**	22.57	2.10
3.	**4c**	50.15	1.82
4.	**4d**	60.56	2.43
5.	**4e**	28.43	3.12
6.	**4f**	26.68	2.90
7.	**4g**	59.52	0.87
8.	**4h**	63.11	1.09
9.	Rolipram	88.01	0.09

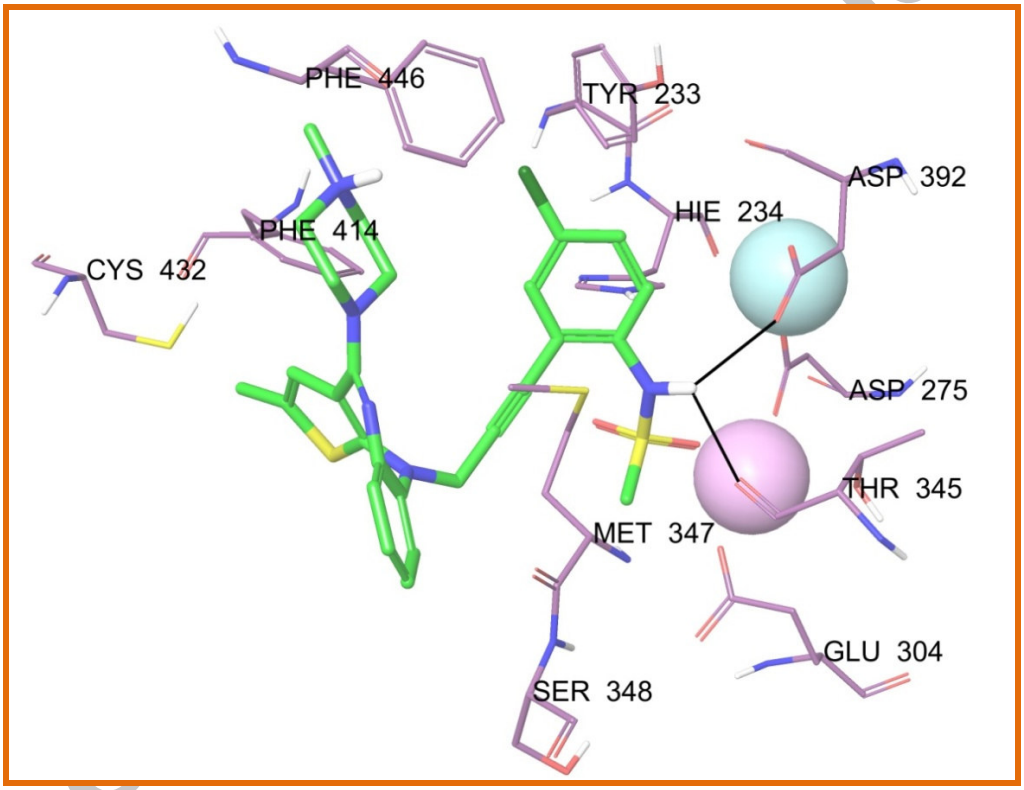
SD = standard deviation

We then performed PDE4B docking studies using compounds **4c, 4g,** and **4h** to understand the nature of interactions of these molecules with this protien *in silico*. The docking analysis of these molecules was carried out using Maestro, version 9.2[17] implemented from Schrödinger molecular modeling suite. The structural coordinates of Phosphodiesterase 4B (PDB ID: 1XMY)[18] were obtained from the protein data bank (PDB). The GLIDE scores obtained after docking of these molecules with PDE4B protein are summarized in Table 4. The data shown in Table 4 clearly suggests that these molecules bind well with the PDE4B whereas the compound **4h** is best among them. Thus, the sulfonamide NH of **4h** participated in two H-bond interactions with Asp 392 and Thr 345 whereas the benzene ring of the benzenesulfonamide moiety participated in a π-π stacking with Hie 234 (Fig. 3, For binding orientation of **4h** at the inhibitor binding pocket of PDE4B, see: Fig. S-4 and S-5, ESI). A H-bond interaction with Tyr 233 and two π-π stacking with Tyr 233 and Phe 446 were observed in case of **4g** (see Fig. S-2, ESI). The amino group of **4c** showed one H-bonding with Asp 392 of PDE4B (see Fig. S-3, ESI).

Table 4: Glide score and contributing XP parameters.

Compound	GScore	LipophilicEvdW	PhobEn	HBond	Electro
4h	-8.36	-5.62	-1.34	-0.89	-0.34
4g	-8.11	-5.51	-1.62	-0.70	-0.28
4c	-5.89	-6.03	-1.37	-0.32	-0.82
Rolipram	-11.09	-4.65	-1.98	-1.49	-0.51

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



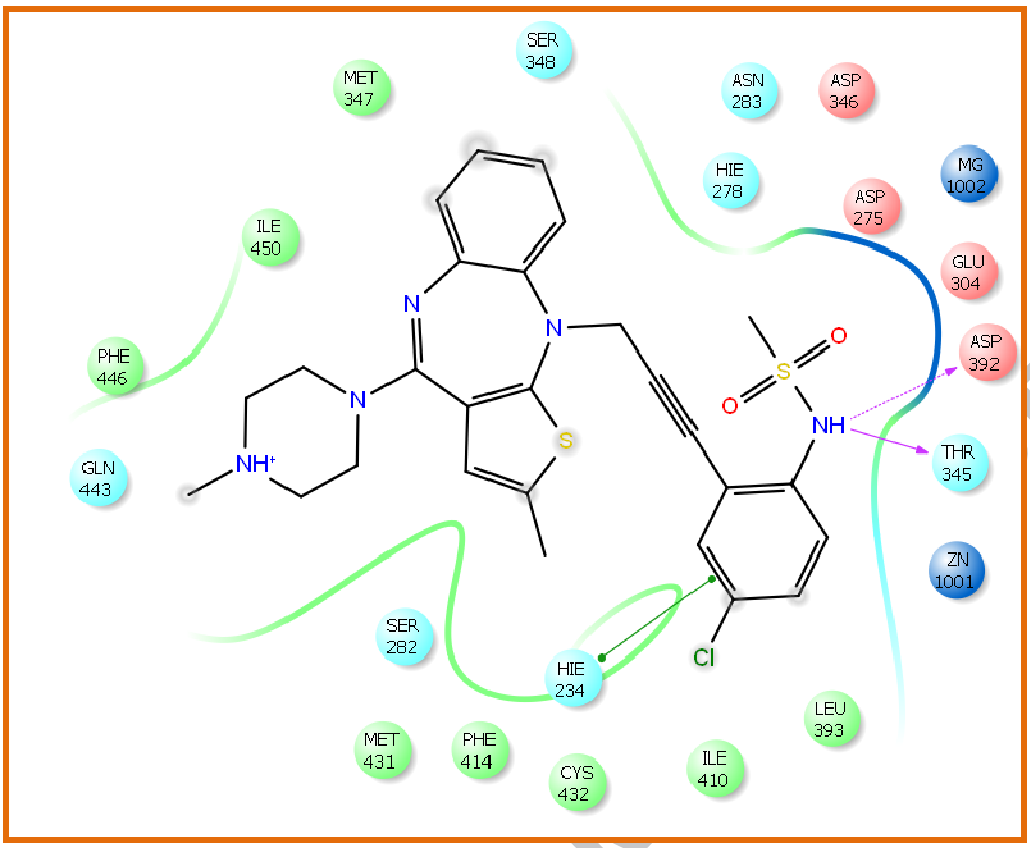


Fig. 3. Binding mode and interactions of **4h** at the inhibitor binding site of PDE4B.

In conclusion, for the first time *N*-(3-arylprop-2-ynyl)substituted olanzapine derivatives have been designed and synthesized by using Pd/C-Cu mediated C-C bond forming reaction. One compound showed PDE4B inhibitory properties *in vitro* and good interactions with PDE4B protein *in silico*. Since linkage between dopamine D2 receptors and PDE activity *via* cAMP can be the basis for a possible therapeutic potential for PDE inhibitors in schizophrenia, the present class of olanzapine derivatives has medicinal value. The strategy presented here has potential for the discovery of novel PDE4 inhibitors.

Acknowledgements

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14. General procedure for the preparation of compound **4**: A mixture of compound **2** (1.0 equiv), 10% Pd/C (0.016 equiv), PPh_3 (0.125 equiv), CuI (0.02 equiv), and triethylamine (2 equiv) in ethanol (5 mL) was stirred at 25–30 °C for 30 min under nitrogen. To this was added iodoarene (**3**) (1.0 equiv), and the mixture was initially stirred at room temperature for 1 h and then at 70 °C for the time indicated in Table 2. After completion of the reaction, the mixture was cooled to room temperature, diluted with EtOAc (50 mL), and filtered through Celite. The organic layers were collected, combined, washed with water (3 × 30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under low vacuum. The crude residue was purified by column chromatography on silica gel using methanol/dichloromethane to afford the desired product. Compound **4a**: brown solid; mp 198-200 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25-7.20 (m, 1H), 7.11-6.98 (m, 4H), 6.97-6.90 (m, 1H), 6.59 (d, J = 8.4 Hz, 1H), 6.32 (s, 1H, H-3), 4.54 (bs, 2H, -CH_2C≡), 3.74-3.60 (m, 4H, 2CH_2), 2.75-2.67 (m, 2H, CH_2), 2.63-2.56 (m, 2H, CH_2), 2.41 (s, 3H, Me), 2.35 (s, 3H, Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.0, 154.2, 145.9, 144.5, 142.6, 132.4, 132.1, 130.6, 127.5, 126.9, 124.9, 123.8, 121.1, 118.1, 115.8, 114.4, 107.3, 89.8 (-C≡), 83.6 (-C≡), 54.4 (CH_2), 45.9 (CH_2), 42.3 (CH_2), 31.5 (CH_2), 29.6 (NMe), 20.1 (CH_2), 15.8 (Me); IR (KBr): 2930, 2853, 2257 (-C≡C-), 1590, 1421, 1248 cm^{-1}; m/z (CI): 475.4 (M+1, 100%); HPLC: 95.3%; column: X Bridge C-18 75*4.6mm, 3.5µm, mobile phase A: 0.1% formic acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/10, 0.5/10, 4/95, 10/95, 10.5/10, 12/10; flow rate: 1.0 mL/min; UV 230 nm, retention time 3.2 min. Compound **4b**: light brown solid; mp 182-184 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (d, J = 2.4 Hz, 1H, ArH), 7.24-7.16 (m, 2H, ArH), 7.11-6.97 (m, 3H, ArH), 6.56 (d, J = 8.4 Hz, 1H, ArH), 6.35 (s, 1H, H-3 (ArH)), 4.55 (bs, 2H, -CH_2C≡), 4.15 (s, 2H, NH_2), 3.65 (s, 4H, 2CH_2), 2.71-2.52 (m, 4H, 2CH_2), 2.42 (s, 3H, Me), 2.38 (s, 3H, Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.1, 153.9, 147.2, 144.3, 144.3, 142.7, 137.0, 134.0, 132.5, 127.6, 125.0, 123.7, 121.2, 117.9, 115.7, 109.0, 108.0, 91.2 (-C≡), 82.1 (-C≡), 54.7 (CH_2), 46.1 (CH_2), 42.1 (CH_2), 34.6 (CH_2), 29.6 (NMe), 22.6 (CH_2), 15.8 (Me); IR (KBr): 2926, 2848, 2794, 2253 (-C≡C-), 1579, 1145 cm^{-1}; m/z (CI): 521.4 (M+1, 100%),

HPLC: 96.8%; column: Symmetry C-18 75 x 4.6 mm 3.5µ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: ACN (gradient) (T/%B): 0/10, 0.5/10, 4/95, 10/95, 10.5/10, 12/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 3.3 min. Compound **4h**: light yellow solid; mp 204-206 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (s, 1H, ArH), 7.51 (s, 1H, ArH), 7.42-7.34 (m, 1H, ArH), 7.20-7.09 (m, 4H, ArH), 6.34 (s, 1H, H-3 (ArH)), 4.51 (bs, 2H, -CH_2C≡), 4.25-4.03 (m, 3H, CH_2 & NH), 3.94-3.79 (m, 2H, CH_2), 3.26-3.11 (m, 2H, CH_2), 3.04 (s, 3H, Me), 3.0-2.93 (m, 2H, CH_2), 2.67 (s, 3H, Me), 2.38 (s, 3H, Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.7, 139.2, 138.4, 133.9, 133.2, 132.5, 131.5, 127.6, 125.3, 124.2, 123.5, 121.1, 120.7, 120.3, 118.2, 114.0, 112.6, 86.2 (-C≡), 84.9 (-C≡), 53.7 (CH_2), 44.5 (CH_2), 42.2 (CH_2), 40.2 (CH_2), 31.9 (NMe), 29.7 ($MeSO_2$), 22.7 (CH_2), 15.8 (Me); IR (KBr): 2920, 2843, 2228 (-C≡C-), 1605, 1583, 1495, 1260 cm^{-1}; m/z (CI): 553.8 (M +1, 100%); HPLC: 95.8%; Column: Symmetry C-18 75*4.6 mm 3.5µm, mobile phase A: 0.1% TFA in water, mobile phase B: CH_3CN (gradient) T/B%: 0/10, 0.5/10, 6/95, 10/95, 10.5/10, 12/10; flow rate: 1.0 mL/min; UV 260 nm, retention time 4.0 min.

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