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Novel imidazophenoxazine-4-sulfonamides: Their synthesis and evaluation as potential inhibitors of PDE4

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ABSTRACT

A number of novel imidazophenoxazine-4-sulfonamides have been designed as potential inhibitors of PDE4. All these compounds were readily prepared via an elegant multi-step method involving the initial construction of 1-nitro-10*H*-phenoxazine ring and then fused imidazole ring as key steps. Some of these compounds showed promising PDE4B and D inhibition when tested in vitro and good interactions with these proteins in silico. Three of these compounds showed dose dependent inhibition of PDE4B with IC_{50} value of 3.31 ± 0.62, 1.23 ± 0.18 and 0.53 ± 0.18 μM.

1. Introduction

Due to their interesting pharmacological properties, compounds containing the phenoxazine framework (**A**, Fig. 1) have attracted considerable attention in medicinal chemistry. A number of compounds based on **A** have been reported to be potent anti-proliferative agents.[1] For example 5*H*-pyrido[2,3-*a*]phenoxazin-5-one (**B**, Fig. 1) that belongs to this class has shown promising anti-proliferative activities when tested against human neoplastic cell lines.[2] A phenoxazine based dual PPAR agonist for example, DRF 2725 (**C**, Fig. 1) has shown potent antihyperglycemic and lipid modulating properties.[3] In view of the pharmacological importance of phenoxazine derivatives and our long standing interest in identification of novel phosphodiesterase 4 (PDE4) inhibitors[4] we became interested in assessing PDE4 inhibiting properties of small molecules based on phenoxazine fused with an imidazole ring for example, imidazo[4,5,1-*kl*]phenoxazine. 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. PDE4 exists in four different isoforms for example, PDE4A, B, C and D and plays a key role in the hydrolysis of cAMP to AMP.[5] Thus, inhibition of PDE4 results in elevated levels of cAMP in the airway tissues and cells thereby suppressing inflammatory cell functions. 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 the first-generation PDE4 inhibitor showed adverse effects such as nausea and vomiting.[5] More recently, cardiovascular effects of PDE4 inhibitors have been reported.[6] While these dose-limiting side effects were reduced by second-generation inhibitors like cilomilast[7a] (Ariflo) and roflumilast, their therapeutic index has delayed market launch so far. While roflumilast (Daxas®, Nycomed) has been launched in Europe for the treatment of COPD recently it is however, necessary to devote a continuing effort in exploring new class of compounds for their PDE4 inhibitory potential. Additionally, the improvement of fasting blood glucose and hemoglobin A1C levels shown by roflumilast during its clinical studies in patients with type 2 diabetes[7b] and the recent report[7c] of resveratrol-PDE link have generated renewed interest in the discovery and development of new PDE4 inhibitor. The design of our target compounds (**E**, Fig. 2) was performed by incorporating a phenoxazine ring (**A**) into the benzo[*d*]imidazol based known inhibitors[8a] of PDE4 (**D**, Fig. 2). A sulfonamide group was introduced at C-4 of the resulting imidazo[4,5,1-*kl*]phenoxazine moiety with the anticipation that this group might induce anti-inflammatory[8b] as well as favorable drug like properties[8c] within the molecule. Herein we report imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide as a new template for

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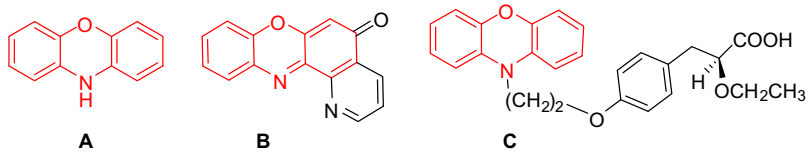


Figure 1. The phenoxazine framework (**A**) and related bioactive molecules **B** and **C**.

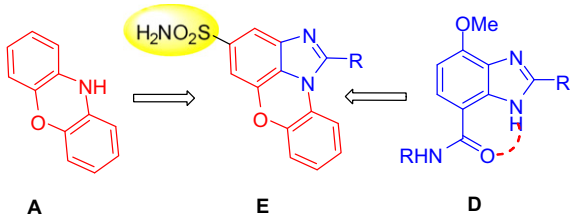


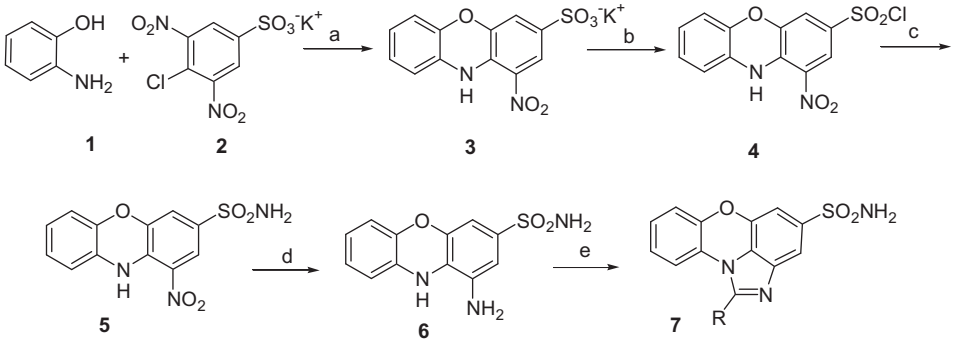
Figure 2. Design of imidazophenoxazine based new inhibitors (**E**) of PDE4.

the discovery of novel inhibitors of PDE4. To the best of our knowledge this is the first example of disclosing PDE4 inhibitors based on this pharmacophore.

2. Results and discussion

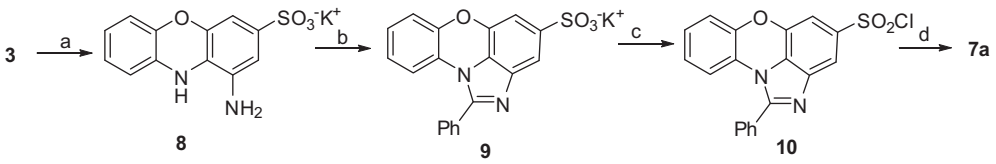
2.1. Chemistry

While several synthesis[9] of substituted phenoxazines have been attempted earlier incorporation of two substituents like nitro and sulfonic acid in a single step followed by subsequent functional group modifications however remained unexplored. Recently, the synthesis of phenoxazine derivatives starting from 2-aminophenol and substituted difluorobenzene has been reported.[10] Introduction of sulfonic acid group at C-3 position of the phenoxazine nucleus led to the construction of *p*-aminobenzenesulfonic acid pharmacophore in phenoxazine nucleus. It was reported[11] that the synthetic applications of phenoxazine appeared to be problematic due to the insoluble nature of the molecule. Therefore it was planned to introduce a nitro group at an initial stage to increase the solubility. The synthon potassium-4-chloro-3,5-dinitrobenzene sulfonate,[12] used to build heterocyclic nuclei in the earlier work[13] has been used in the present synthesis. Thus synthesis of our target compounds that is, 1-aryl imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamides (**7**) was carried out starting from potassium salt of 1-nitro-10*H*-phenoxazine-3-sulfonate (**3**)[14] (Scheme 1). Previously, there was only one report[15] in which 1-amino phenoxazine was treated with formic acid to construct the imidazo fused phenoxazine ring. The key starting material **3** prepared (Scheme 1) by the condensation of 2-aminophenol (**1**) with potassium salt of 4-chloro-3,5-dinitrobenzenesulfonate (**2**) was treated with excess of phosphorous oxychloride in a 1:7 molar ratio to get the corresponding sulfonyl chloride (**4**). This was subsequently treated with aqueous ammonia in THF to afford the 1-nitro-10*H*-phenoxazine-3-sulfonamide (**5**). On reduction with Raney Ni the compound **5** afforded 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) which finally treated with a range of aromatic aldehydes to give the target compounds **7** (Scheme 1).



	R	% yield
7a	phenyl	64
7b	3-methoxy4-hydroxy phenyl	53
7c	3-hydroxy phenyl	60
7d	4- methoxy phenyl	52
7e	4-hydroxy phenyl	60
7f	2-hydroxy phenyl	60
7g	4-diethylamino-2-hydroxyphenyl	50
7h	3-nitrophenyl	71
7i	2-bromophenyl	60
7j	3-bromophenyl	60
7k	2-chlorophenyl	70
7l	5-bromo-2-fluorophenyl	53
7m	2,3-dichlorophenyl	52
7n	4-chlorophenyl	70
7o	4-dimethylaminophenyl	51
7p	2,6-dichlorophenyl	64

Scheme 1. Reagents and conditions: (a) NaOH, EtOH, 3 h, 83%; (b) $POCl_3$, reflux, 3 h, 84%; (c) aq ammonia, THF, 0 °C, 30 min, 80%; (d) Raney Ni, NH_2NH_2, MeOH, reflux, 30 min, 85%; (e) RCHO, DMF, 100 °C, 48 h.



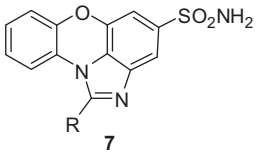
Scheme 2. Reagents and conditions: (a) Raney Ni, NH_2NH_2, MeOH, reflux, 30 min, 77%; (b) PhCHO, DMF, 100 °C, 24 h, 52%; (c) $POCl_3$, reflux, 3 h; (d) aq ammonia, THF, 0 °C, 30 min, 50%.

One of the target compounds **7a** was also prepared via an alternative route (Scheme 2). Thus, the compound **3** was reduced to the potassium salt of 1-amino-10*H*-phenoxazine-3-sulfonate (**8**) using Raney Ni, which was treated with benzaldehyde to give the potassium salt of 1-phenylimidazo[4,5,1-*kl*]phenoxazine-4-sulfonates (**9**). On treating with $POCl_3$ the compound **9** provided the corresponding chloro derivative **10** which on treatment with aqueous ammonia in THF afforded the compound **7a**.[16]

2.2. Pharmacology

Having synthesized a range of 1-substituted imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamides many of these compounds were tested initially for their PDE4B inhibitory properties in vitro at 30 μM using PDE4B enzyme assay[17] (Table 1). Rolipram[18] was used as a reference compound in this assay. Except **7g** (along with **7m** and **7o**) all other compounds showed significant inhibition of PDE4B and compounds **7f, 7j** and **7l** showed superior inhibitions in compared to other molecules when tested at 30 μM (Table 1). The initial compound **7a** showed good inhibition of PDE4B which though was not affected by the addition of OH and OMe substituents to the C-1 benzene ring for example, compound **7b** (Table 1, entry 2) but improved upon addition of *m*-OH group to the C-1 benzene ring for example, compound **7c** (Table 1). However, a *p*-$MeOC_6H_4$ moiety at C-1 decreased the activity for example, compound **7d** (Table 1, entry 4) whereas a *p*-HOC_6H_4 or *o*-HOC_6H_4 moiety at C-1 restored the activity for example, compound **7e** and **7f** (Table 1, entries 5 and 6). A bulkier group that is, *p*-(Et_2N)-*o*-$(HO)C_6H_3$ at C-1 was not tolerated for example, compound **7g** (Table 1, entry 7) and a *m*-$NO_2C_6H_4$ moiety at C-1 decreased the activity (Table 1, entry 8). Interestingly, both the *o*-BrC_6H_4 and *m*-BrC_6H_4 group at C-1 was well tolerated for example, compounds **7i** and **7j** (Table 1, entries 9 and 10). While *o*-ClC_6H_4 group at C-1 decreased the activity marginally for example, compound **7k** (Table 1, entry 11) a 2-F-5-BrC_6H_3 or *p*-ClC_6H_4 or 2,6-dichloro phenyl at the same position was well tolerated (Table 1, entries 12–14). Based on their promising inhibitory properties dose response studies were carried out using most active compounds **7f**, **7j** and **7l**. All of them showed dose dependent inhibition of PDE4 with IC_{50} value of 3.31 ± 0.62, 1.23 ± 0.18 and 0.53 ± 0.18 μM, respectively (Figs. 3–5) in compared to rolipram's IC_{50} value of 0.941 ± 0.24 μM (Fig. 6). Thus the compound **7l** was identified as the most potent compound in this series. To assess the other subtype inhibitory potential of **7** few selected compounds were tested against PDE4D when compounds **7f**, **7g**, **7h**, and **7l** showed 88.4%, 16.9%, 68.6%, and 85.3% inhibition, respectively.

Table 1
Inhibition of PDE4B by compound **7** at 30 μM



Entry	Compounds	R =	Average % inhibition	SD
1	**7a**	Phenyl	79.65	1.16
2	**7b**	3-Methoxy4-hydroxy phenyl	82.43	1.48
3	**7c**	3-Hydroxy phenyl	87.58	0.54
4	**7d**	4-Methoxy phenyl	66.53	4.16
5	**7e**	4-Hydroxy phenyl	88.80	0.92
6	**7f**	2-Hydroxy phenyl	89.28	3.31
7	**7g**	4-Diethylamino-2-hydroxyphenyl	19.66	1.43
8	**7h**	3-Nitrophenyl	66.81	2.56
9	**7i**	2-Bromophenyl	81.02	1.17
10	**7j**	3-Bromophenyl	89.69	1.23
11	**7k**	2-Chlorophenyl	76.32	0.86
12	**7l**	5-Bromo-2-fluorophenyl	92.86	0.53
13	**7n**	4-Chlorophenyl	80.35	1.32
14	**7p**	2,6-Dichlorophenyl	80.23	2.15

SD = standard deviation.

2.3. Docking studies

In order to understand the nature of interactions of these molecules with PDE4B docking studies were carried out using compounds **7f, 7j** and **7l**. 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 interaction of compound **7f** with the PDE4B protein (Fig. 7) was mainly contributed by (i) a H-bonding between the amino group of **7f** and His-278, (ii) a H-bonding between the hydroxyl group of **7f** and Asp-392 and (iii) two Π–Π stacking interactions between the benzoimidazole moiety and His234 of the protein. Similarly, the interaction of compound **7j** with the PDE4B protein (Fig. 8) was contributed by (i) H-bonding between the NH_2-group of **7j** and Thr-345 as well as Asp-392, (ii) Π–Π stacking interaction between the central 1,4-oxazine ring and tyrosine (Tyr233) and (iii) Π–Π stacking interaction between the phenyl group of **7j** and phenylalanine (Phe446). The interaction of compound **7l** with the PDE4B protein (Fig. 9) was mainly contributed by (i) a H-bonding between the amino group of **7l** and Asp-275 and (ii) Π–Π stacking interaction between aromatic ring systems of **7l** and Tyr233, His234 and Phe446. Overall, the present imidazophenoxazine-4-sulfonamides showed good interactions

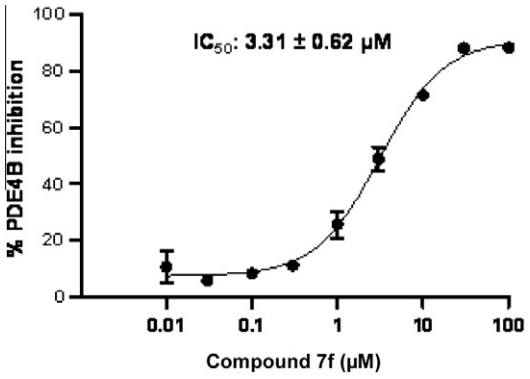


Figure 3. Dose dependent inhibition of PDE4B by compound **7f**.

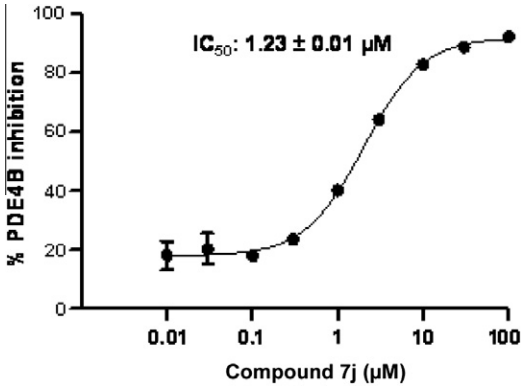


Figure 4. Dose dependent inhibition of PDE4B by compound **7j**.

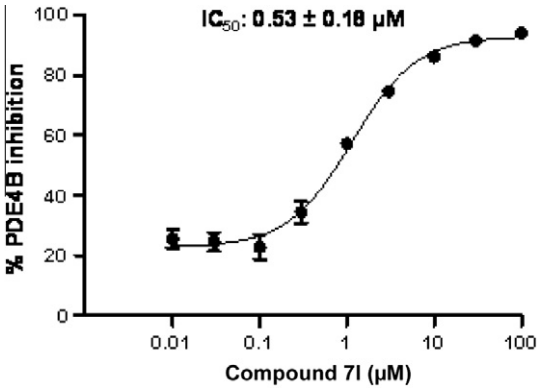


Figure 5. Dose dependent inhibition of PDE4B by compound **7l**.

with PDE4B protein where the central phenoxazine ring and sulfonamide moiety played a key role in these interactions. Notably, the observed dissimilar orientation of binding of **7f** compared to **7j** and **7l** was possibly aided by the strong H-bonding between the hydroxyl group of **7f** and Asp-392 of the PDE4B protein. Docking of all these molecules into the PDE4D suggested that they bind well with this protein (see Supplementary data) which correlated the results of their PDE4D inhibitions in vitro. Thus, the amino group of sulfonamide moiety of **7f** interacted with Asp-201 and 318 and the hydroxyl group of **7f** formed an H-bond with Asn 321. Aromatic rings of molecules **7j** participated in Π–Π stacking with Tyr-159, His-160 and Phe-372. The amino group of same molecule formed a hydrogen bond with Thr-271. In case of molecule **7l**

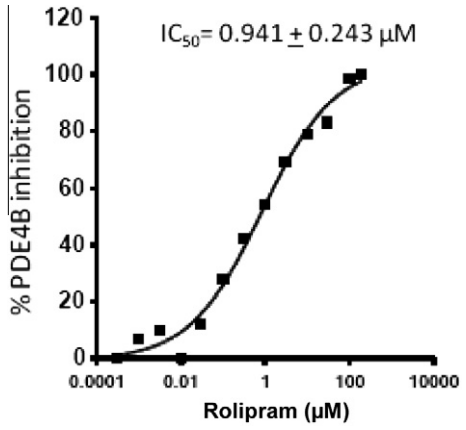


Figure 6. Dose dependent inhibition of PDE4B by rolipram.

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

Entry	Compound	Dock score	E-1[a]	E-2[b]	E-3[c]	E-4[d]
1	**7f**	−6.18	−2.95	−1.4	−0.59	−1.05
2	**7j**	−7.84	−3.82	0	−2.88	−0.97
3	**7l**	−8.19	−3.99	−0.22	−2.82	−0.88

[a] Chemscore lipophilic pair term and fraction of the total protein–ligand vdw energy reward.
[b] Hydrophobic enclosure reward.
[c] Electrostatic reward.
[d] Rewards for hydrogen bonding interaction between ligand and protein.

Π–Π stacking with Tyr-159 and hydrogen bonding with Asp-318 and Thr-271 was observed (see Figs. in Supplementary data).

3. Conclusions

In conclusion, a number of novel imidazophenoxazine-4-sulfonamides have been designed and synthesized as potential inhibitors of PDE4. All these compounds were readily prepared via a multistep method starting from potassium-4-chloro-3,5-dinitro benzene sulfonate involving the construction of 1-nitro-10*H*-phenoxazine ring and then fused imidazole ring as key steps. Some of these compounds showed promising PDE4B and D inhibition when tested in vitro and good interactions with these proteins in silico. The docking studies indicated that the central phenoxazine ring and sulfonamide moiety played a key role in these interactions. In a dose response study three compounds showed dose dependent inhibition of PDE4B with IC_{50} value of 3.31 ± 0.62, 1.23 ± 0.18 and 0.53 ± 0.18 μM. Overall, the imidazophenoxazine-4-sulfonamide framework presented here could be a new template for the identification of small molecule based novel inhibitors of PDE4.

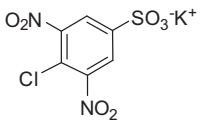
4. Experimental

4.1. Chemistry

4.1.1. General methods

Unless stated otherwise, reactions were performed under nitrogen atmosphere. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (60–120 mesh) using hexane, ethyl acetate, dichloromethane, methanol. ^{1}H NMR and ^{13}C NMR spectra were determined in DMSO-d_6 solution by using 400 and 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer. Melting points were determined using melting point apparatus and are uncorrected. MS spectra were obtained on a mass spectrometer.

4.1.2. Potassium-4-chloro-3,5-dinitrobenzenesulfonate (2)



Chlorobenzene (50 ml) was added to a mixture of fuming sulfuric acid (260 ml) and sulfuric acid (60 ml) at 70 °C. The mixture was stirred at same temperature for 1 h. Potassium nitrate (50 g) was added portion wise to the reaction mixture for about 15 min, and then fuming sulfuric acid (130 ml) was added followed by 2 more

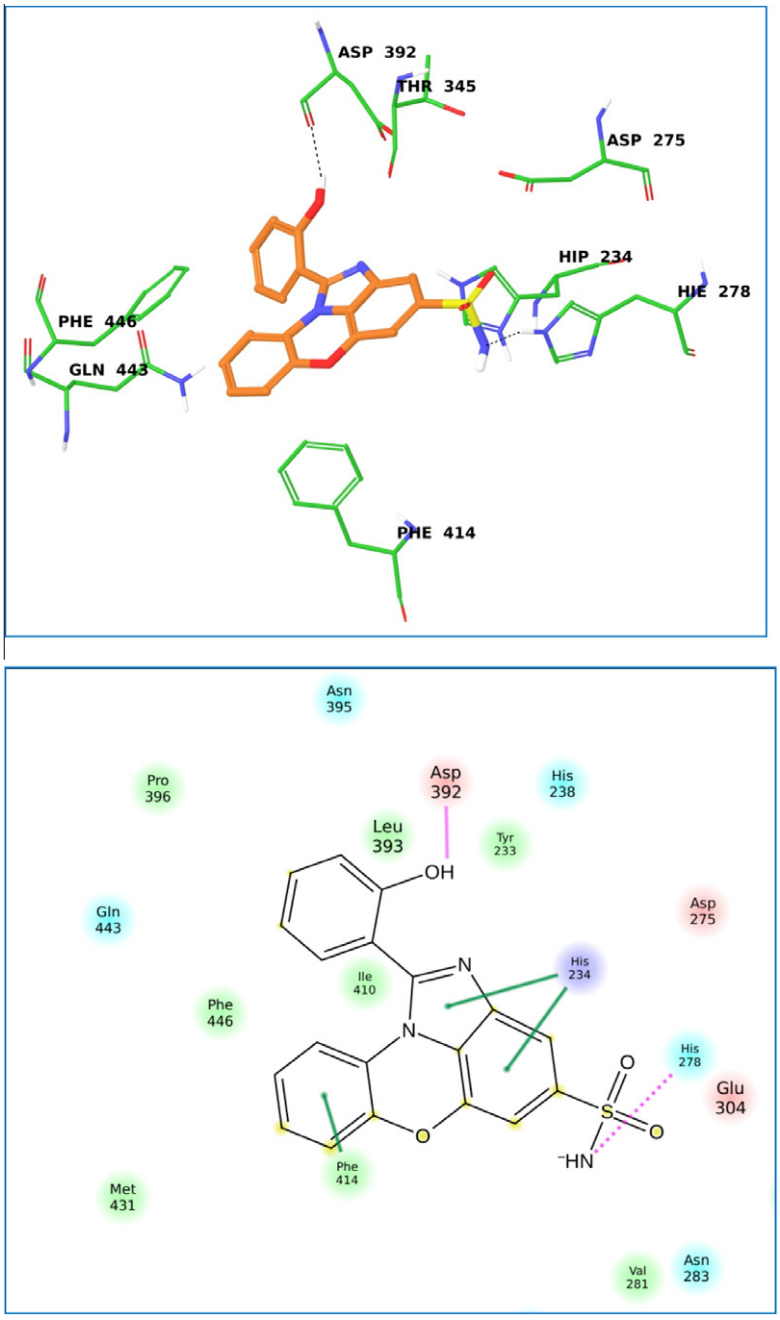
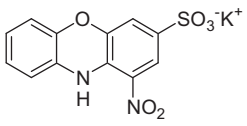


Figure 7. Docking of **7f** at the active site of PDE4B.

portions of potassium nitrate (50 + 50 g) portion wise. The reaction mass was stirred at 130 °C for 1 h, cooled to room temperature, poured in excess crushed ice and allowed to stand for 12 h. The solid was filtered and washed with cold water (3 × 200 ml), dried and washed three times with hot toluene (3 × 200 ml) and dried, yield 56% (80 g) of pure product; mp 298–300 °C (lit.[12] 290 °C).

4.1.3. Potassium-1-nitro-10*H*-phenoxazine-3-sulfonate (3)



To a solution of sodium hydroxide (7.5 g, 0.187 mol) in ethanol (500 ml) was added 2-aminophenol (17 g, 0.156 mol) and potassium-4-chloro-3,5-dinitrobenzenesulfonate (50 g, 0.156 mol). The reaction mixture was refluxed for 1 h. Then a solution of sodium hydroxide (3.75 g) in water (5 ml) was added to the reaction mass and refluxed for two more hours. Completion of the reaction was monitored by TLC. The reaction mass was cooled to room temperature and the solid was filtered and washed twice with ethanol (2 × 200 ml) and dried, yield 83% (45 g); mp >300 °C; *m*/*z* (CI) 307 (M-K, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.45 (s, 1H, NH), 7.73 (s, 1H), 7.20 (dd, J = 2 and 6.8 Hz, 1H,), 7.01–6.9 (m, 1H), 6.94–6.36 (m, 3H); IR (KBr, cm^{-1}) ν_{max}: 3341, 1531, 1203, 1052, 745.

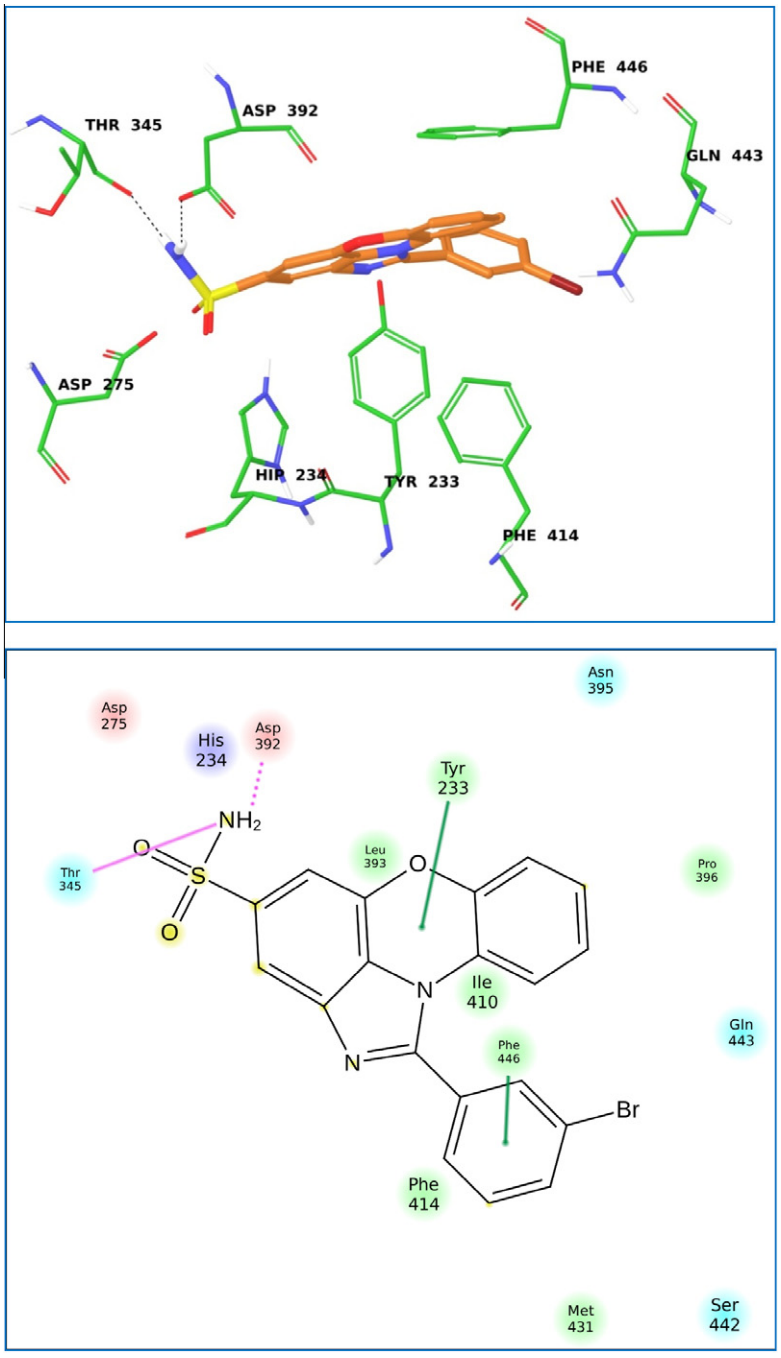
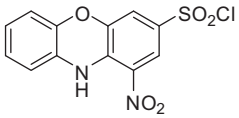


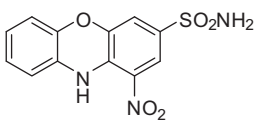
Figure 8. Docking of **7j** at the active site of PDE4B.

4.1.4. 1-Nitro-10*H*-phenoxazine-3-sulfonylchloride (4)



Potassium-1-nitro-10*H*-phenoxazine-3-sulfonate (10 g) in $POCl_3$ (70 ml) was refluxed for 3 h. Completion of the reaction was monitored by TLC. The excess of $POCl_3$ was removed by distillation, the crude cherry red coloured solid was poured in crushed ice and filtered, washed with water, cold methanol and dried, and yield 8 g (84%); mp 204 °C (decomposition) and the product was immediately used for the next step.

4.1.5. 1-Nitro-10*H*-phenoxazine-3-sulfonamide (5)



To a solution of aqueous ammonia (10 ml) in tetrahydrofuran (50 ml) was added 1-nitro-10*H*-phenoxazine-3-sulfonylchloride

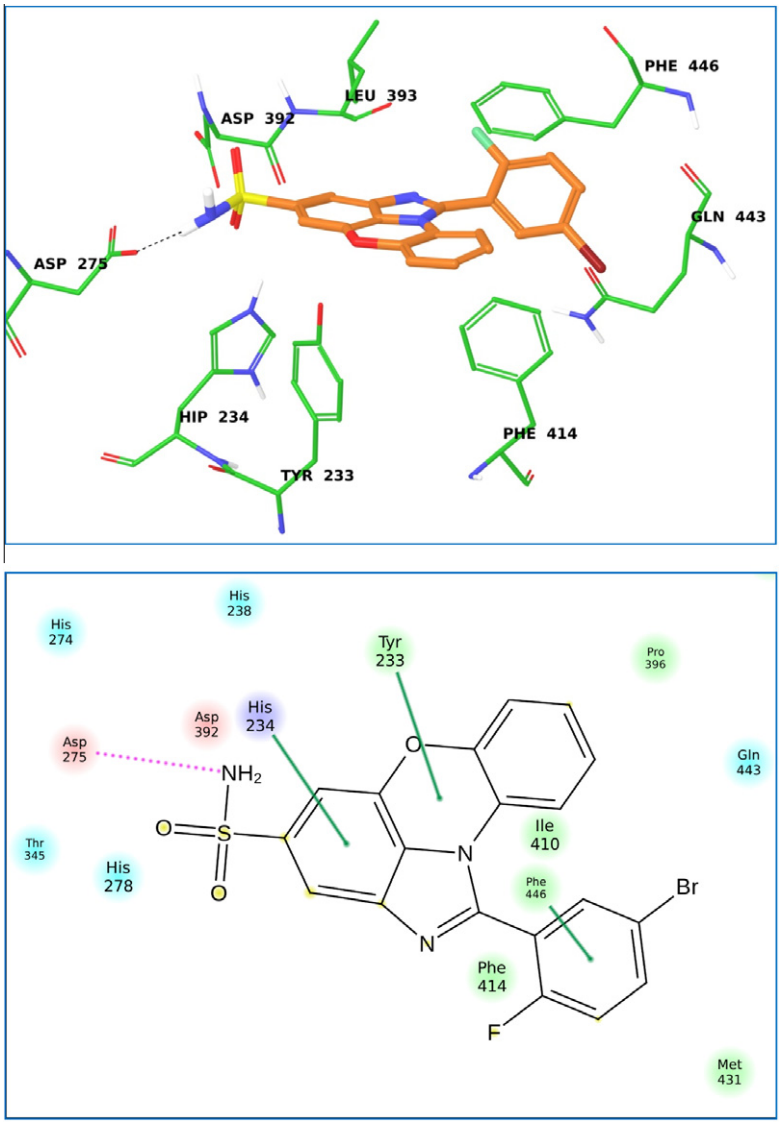
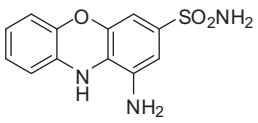


Figure 9. Docking of **7l** at the active site of PDE4B.

(8 g) at 0 °C and the resulting reaction mixture was stirred for 30 min at same temperature. Completion of the reaction was monitored by TLC. The excess of solvent was removed by distillation under reduced pressure and the crude solid was diluted with water and extracted with ethyl acetate (300 ml). The organic layer was collected, washed with water (300 ml), dilute HCl (100 ml), water (100 ml) and saturated sodium chloride solution (100 ml) and dried over anhydrous sodium sulfate. The organic layer was filtered and concentrated under reduced pressure, yield 80% (6.0 g); mp 282–283 °C; *m*/*z* (CI) 306 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (s, 1H), 7.91 (s, 1H), 7.43 (s, 2H), 7.20 (dd, J = 2.2 and 8.0 Hz, 1H), 7.12 (s, 1H), 6.89–6.72 (m, 3H); IR (KBr, cm^{-1}) υ_{max} 3388, 3335, 3255, 1575, 1536, 1506, 1406, 1337, 1291, 1226, 1146, 1093, 936, 876, 756

4.1.6. 1-Amino-10*H*-phenoxazine-3-sulfonamide (6)

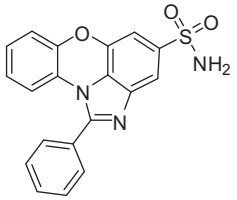


To a suspension of 1-nitro-10*H*-phenoxazine-3-sulfonamide (**5**) (3.9 g) in methanol was added Raney Ni (2.4 g) and hydrazine hydrate (4 ml). The resulting reaction mixture was refluxed for 30 min. Completion of the reaction was monitored by TLC. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure, yield 85% (3.0 g); off white solid; mp 176 °C (decomposition); *m*/*z* (CI) 276 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (s, 1H), 7.01 (s, 2H), 6.77–6.75 (m, 1H), 6.74 (s, 1H), 6.70 (d, J = 4 Hz, 2H), 6.55–6.53 (m, 1H), 6.37 (s, 1H), 5.12 (s, 2H); IR (KBr, cm^{-1}) υ_{max} 3385, 3316, 3268, 1605, 1583, 1522, 1504, 1451, 1424, 1337, 1315, 1278, 1235, 1145, 1113, 1080, 1038, 752.

4.1.7. General procedure for the synthesis of 1-(aryl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamides (7)

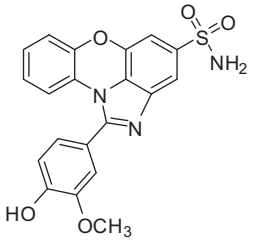
To a solution of 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**, 7 mmol) in dimethylformamide (40 ml) was added aryl aldehyde (10 mmol). The resulting reaction mixture was stirred at 100 °C for 48 h. Completion of the reaction was monitored by TLC. Then the reaction mass was cooled to room temperature and poured in cold water and the solid was filtered and dried, purified by column chromatography using 3% methanol in dichloromethane.

4.1.8. 1-Phenylimidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7a)



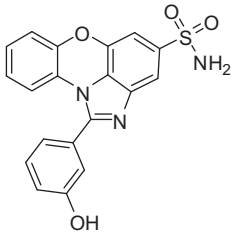
Compound **7a** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.4 g, 5 mmol) and benzaldehyde (0.64 g, 6 mmol) in DMF according to the general procedure as an ash coloured solid; yield 64% (1.2 g); mp 316–317 °C; *m*/*z* (CI) 364 (M+1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (d, J = 6.8 Hz, 2H), 7.68–7.62 (m, 4H), 7.39 (s, 2H), 7.27 (d, J = 8 Hz, 1H), 7.18 (t, J = 8 Hz, 1H,), 7.13 (s, 1H), 6.94 (t, J = 8 Hz, 1H), 6.89 (d, J = 8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.2, 144.8, 141.7, 140.8, 130.8, 130.2, 129.5, 129.0, 128.8, 127.6, 126.1, 124.9, 124.4, 118.5, 116.1, 111.2, 102.4; IR (KBr, cm^{-1}) υ_{max} 3344, 3024, 1662, 1494, 1454, 1380, 1336, 1299, 1267, 1148, 1096, 751.

4.1.9. 1-(4-Hydroxy-3-methoxyphenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7b)



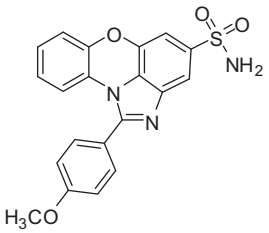
Compound **7b** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.4 g, 5 mmol) and 4-hydroxy-3-methoxybenzaldehyde (1.12 g, 7.5 mmol) in DMF according to the general procedure as an off white solid, yield 53% (1.23 g); mp 294–296 °C; *m*/*z* (CI) 408 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s, 1H), 7.64 (s, 1H), 7.38 (s, 2H), 7.34 (d, J = 2 Hz, 1H), 7.26–7.16 (m, 3H), 7.114 (s, 1H), 7.075–6.981 (m, 3H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.8, 148.9, 147.6, 144.9, 141.8, 141.6, 140.8, 127.5, 126.1, 125.2, 124.5, 123.5, 122.7, 120.6, 118.4, 116.4, 115.6, 113.4, 111.8, 102.3, 55.8; IR (KBr, cm^{-1}) υ_{max} 3343, 3064, 1662, 1499, 1459, 1424, 1328, 1303, 1235, 1151, 1032, 936, 875, 787, 760.

4.1.10. 1-(3-Hydroxyphenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7c)



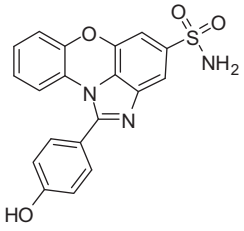
Compound **7c** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.4 g, 5 mmol) and 3-hydroxy benzaldehyde (0.98 g, 8.1 mmol) in DMF according to the general procedure as an off white solid, yield 60% (1.2 g); mp 273–276 °C; *m*/*z* (CI) 378 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, 1H, OH), 7.66 (s, 1H), 7.43 (t, J = 8.0 Hz, 1H,), 7.38 (s, 2H, SO_2NH_2), 7.26 (d, J = 7.6 Hz, 1H), 7.19–7.12 (m, 4H), 7.05 (dd, J = 1.6 and 8.0 Hz, 1H,), 6.98 (m, 2H); IR (KBr, cm^{-1}) υ_{max} 3324, 3246, 3115, 1662, 1582, 1493, 1453, 1382, 1308, 1266, 1163,1151, 1102, 755.

4.1.11. 1-(4-Methoxyphenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7d)



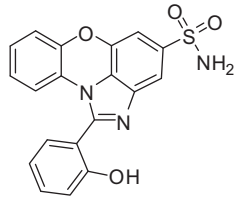
Compound **7d** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (2.0 g, 7.2 mmol) and 4-methoxybenzaldehyde (1.5 g, 10 mmol) in DMF according to the general procedure as an off white solid; yield 52% (1.5 g); mp 302–303 °C; *m*/*z* (CI) 392 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (d, J = 8.8 Hz, 2H), 7.66 (s, 1H), 7.39 (s, 2H, SO_2NH_2), 7.23 (d, J = 8.0 Hz, 1H), 7.18–7.13 (m, 3H), 7.12 (s, 1H), 6.99–6.93 (m, 2H), 3.89 (3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.0, 150.4, 145.0, 141.9, 141.6, 140.9, 131.1, 127.6, 126.2, 125.1, 124.5, 122.2, 118.5, 116.2, 114.3, 111.0, 102.3, 55.4; IR (KBr, cm^{-1}) υ_{max} 3385, 3116, 1664, 1613, 1584, 1495, 1378, 1301, 1229, 1208, 1128, 1079, 750.

4.1.12. 1-(4-Hydroxyphenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7e)



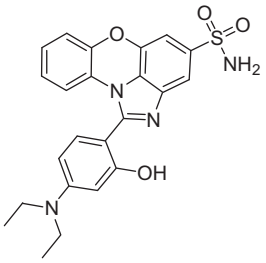
Compound **7e** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.5 g, 5.4 mmol) and 4-hydroxybenzaldehyde (0.99 g, 8.1 mmol) in DMF according to the general procedure as an off white solid, yield 60% (1.2 g); mp 323–325 °C; *m*/*z* (CI) 378 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (s, 1H), 7.63–7.60 (m, 3H), 7.37 (s, 2H), 7.26–7.18 (m, 2H), 7.10 (s, 1H), 7.02–6.97 (m, 4H); IR (KBr, cm^{-1}) υ_{max} 3323, 3243, 3117, 1663, 1588, 1497, 1452, 1382, 1308, 1266, 1151, 1102, 795.

4.1.13. 1-(2-Hydroxyphenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7f)



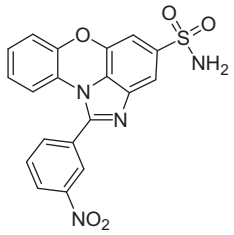
Compound **7f** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.4 g, 5.4 mmol) and 2-hydroxybenzaldehyde (0.98 g, 8.1 mmol) in DMF according to the general procedure as an off white solid, yield 60% (1.2 g); mp 308–310 °C; *m*/*z* (CI) 378 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 7.66 (s, 1H), 7.53–7.50 (m, 2H), 7.38 (s, 2H), 7.25–7.23 (m, 1H), 7.18–6.95 (m, 5H), 6.82 (d, J = 7.6 Hz, 1H); IR (KBr, cm^{-1}) υ_{max} 3329, 3236, 3119, 1661, 1572, 1463, 1463, 1392, 1318, 1266, 1151, 1102, 1023, 962, 853,789, 744, 667.

4.1.14. 1-(4-(Diethylamino)-2-hydroxyphenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7g)



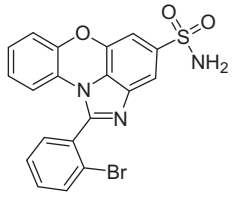
Compound **7g** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.5 g, 5.4 mmol) and 4-diethylamino-2-hydroxybenzaldehyde (1.2 g, 6.4 mmol) in DMF according to the general procedure as an off white solid; yield 50% (1.2 g); mp 280–282 °C; *m*/*z* (CI) 449 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (1H), 7.65 (s, 1H), 7.35 (s, 2H), 7.31 (d, *J* = 7.6 Hz, 1H), 7.19–7.12 (m, 4H), 6.51–6.4 (m, 3H), 3.4 (q, *J* = 7.2 Hz, 4H), 1.0 (t, *J* = 7.2 Hz, 6H); IR (KBr, cm^{-1}) υ_{max} 3332, 3233, 3117, 1661, 1562, 1473, 1389, 1321, 1257, 1151, 1102, 1015, 755.

4.1.15. 1-(3-Nitrophenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7h)



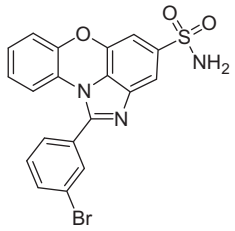
Compound **7h** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.45 g, 5.2 mmol) and 3-nitrobenzaldehyde (1.18 g, 7.8 mmol) in DMF according to the general procedure as an off white solid, yield 71% (1.5 g); mp 332–335 °C; *m*/*z* (CI) 407 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.67 (s, 1H), 8.52 (d, *J* = 9.6 Hz, 1H), 8.29 (d, *J* = 8.0 Hz, 1H), 7.94 (t, *J* = 8.0 Hz, 1H), 7.70 (s, 1H), 7.41 (s, 2H), 7.30–7.17 (m, 3H), 6.97–6.89 (m, 2H); IR (KBr, cm^{-1}) υ_{max} 3344, 3024, 1654, 1540, 1491, 1350, 1304, 1195, 1077, 1036, 734.

4.1.16. 1-(2-Bromophenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7i)



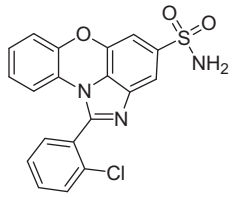
Compound **7i** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.5 g, 5.4 mmol) and 2-bromobenzaldehyde (1.2 g, 6.4 mmol) in DMF according to the general procedure as an off white solid, yield 60% (1.4 g); mp 310–312 °C; *m*/*z* (CI) 440 (M-2, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94–7.92 (m, 1H), 7.80–7.77 (m, 1H), 7.71–7.66 (m, 3H), 7.40 (s, 2H), 7.29–7.27 (m, 1H), 7.21–7.16 (m, 2H), 6.95 (t, *J* = 8.0 Hz, 1H), 6.41 (d, *J* = 8.0 Hz, 1H); IR (KBr, cm^{-1}) υ_{max} 3343, 3069, 1662, 1483, 1476, 1339, 1234, 1179, 1137, 1085, 847, 766, 647.

4.1.17. 1-(3-Bromophenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7j)



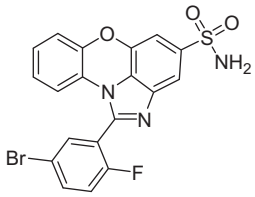
Compound **7j** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.5 g, 5.4 mmol) and 3-bromobenzaldehyde (1.5 g, 8.1 mmol) in DMF according to the general procedure as an off white solid; yield 60% (1.4 g); mp 310–312 °C; *m*/*z* (CI) 440 (M-2, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (s, 1H), 7.49 (t, *J* = 8.8 Hz, 1H,), 7.34 (s, 2H), 7.24 (d, *J* = 8.8 Hz, 1H,), 7.12–7.08 (m, 4H), 7.05 (d, *J* = 8.0 Hz, 1H), 6.98 (m, 2H); IR (KBr, cm^{-1}) υ_{max} 3342, 3029, 1663, 1483, 1455, 1307, 1262, 1192, 1136, 1083, 837, 756, 657.

4.1.18. 1-(2-Chlorophenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7k)



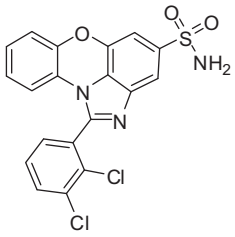
Compound **7k** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.5 g, 5.4 mmol) and 2-chlorobenzaldehyde (1.14 g, 8.1 mmol) in DMF according to the general procedure as an off white solid; yield 70% (1.4 g); mp 327–330 °C; *m*/*z* (CI) 396 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82–7.73 (m, 3H), 7.71 (s, 1H), 7.66–7.64 (m, 1H), 7.40 (s, 2H), 7.29–7.21 (m, 1H), 7.21–7.19 (m, 2H), 6.96 (t, *J* = 8.0 Hz, 1H), 6.45 (d, *J* = 7.8 Hz, 1H); IR (KBr, cm^{-1}) υ_{max} 3342, 3029, 1663, 1483, 1455, 1307, 1262, 1192, 1136, 1083, 837, 756, 657.

4.1.19. 1-(5-Bromo-2-fluorophenyl)imidazo[4,5,1-kl]phenoxazine-4-sulfonamide (7l)



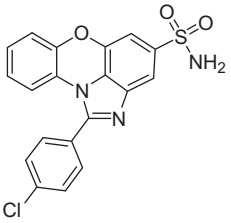
Compound **7l** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.4 g, 5 mmol) and 5-bromo-2-florobenzaldehyde(1.12 g, 7.5 mmol) in DMF according to the general procedure as a light brown solid; yield 53% (1.23 g); mp 315–317 °C; *m*/*z* (CI) 458 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07–7.99 (m, 1H), 7.99–7.96 (m, 1H), 7.71 (s, 1H), 7.55 (t, *J* = 9.6 Hz, 1H), 7.42–7.31 (m, 2H), 7.29–7.21 (m, 2H), 7.17 (s, 1H), 7.04 (t, *J* = 7.2 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 1H); IR (KBr, cm^{-1}) υ_{max} 3342, 3032, 1665, 1497, 1459, 1329, 1301, 1225, 1188, 1080, 1038, 737, 690, 679.

4.1.20. 1-(2,3-Dichlorophenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7m)



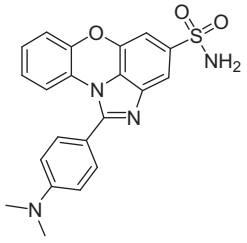
Compound **7m** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.5 g, 5.4 mmol) and 2,3-dichlorobenzaldehyde (1.42 g, 8.1 mmol) in DMF according to the general procedure as an ash colour solid, yield 52% (1.2 g); mp 316–320 °C; *m*/*z* (CI) 430 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (s, 1H), 7.38 (s, 2H), 7.32 (s, 1H), 7.30–7.25 (m, 3H), 7.15 (s, 1H), 7.01–6.92 (m, 3H); IR (KBr, cm^{-1}) υ_{max} 3344, 3024, 1662, 1499, 1484, 1380, 1336, 1299, 1267, 1158, 1096, 955, 821, 785, 725.

4.1.21. 1-(4-Chlorophenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7n)



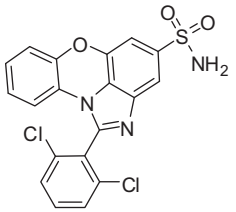
Compound **7n** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.5 g, 5.4 mmol) and 4-chlorobenzaldehyde (1.140 g, 8.1 mmol) in DMF according to the general procedure as an off white solid; yield 70% (1.4 g); mp 335–337 °C; *m*/*z* (CI) 396 (M+1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86–7.84 (m, 2H), 7.73–7.67 (m, 3H), 7.39 (s, 2H), 7.29–7.27 (m, 1H), 7.20 (t, *J* = 7.2 Hz, 1H), 7.14 (s, 1H), 7.00 (t, *J* = 8.2 Hz, 1H), 6.90 (d, *J* = 7.6 Hz, 1H); IR (KBr, cm^{-1}) υ_{max} 3344,, 3037, 1663, 1483, 1455, 1307, 1262, 1192, 1136, 756.

4.1.22. 1-(4-Dimethylaminophenyl)imidazo [4,5,1-*kl*]phenoxazine-4-sulfonamide (7o)



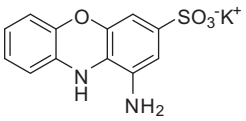
Compound **7o** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.4 g, 5 mmol) and 4-diethylaminobenzaldehyde (1.56 g, 10 mmol) in DMF according to the general procedure as an off white solid; yield 51% (1.5 g); mp 260–262 °C; *m*/*z* (CI) 405 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.62–7.60 (m, 3H), 7.36 (s, 2H), 7.26–7.24 (m, 1H), 7.19–7.16 (m, 2H), 7.09 (s, 1H), 7.02–6.98 (m, 1H), 6.88 (d, *J* = 8.2 Hz, 2H), 3.04 (s, 6H); IR (KBr, cm^{-1}) υ_{max} 3332, 3233, 3117, 1662, 1562, 1473, 1389, 1321, 1257, 1151, 1102, 1090, 1015, 928, 866, 765, 684.

4.1.23. 1-(2,6-Dichlorophenyl)imidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7p)



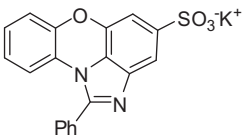
Compound **7p** was prepared by using 1-amino-10*H*-phenoxazine-3-sulfonamide (**6**) (1.5 g, 5.4 mmol) and 2,6-dichlorobenzaldehyde (1.13 g, 6.4 mmol) in DMF according to the general procedure as an off white solid; yield 64% (1.5 g); mp 288–290 °C; *m*/*z* (CI) 430 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.62–7.60 (m, 2H), 7.36 (s, 2H), 7.26–7.24 (m, 1H), 7.19–7.16 (m, 2H), 7.09 (s, 1H), 7.00 (t, *J* = 7.6 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 2H); IR (KBr, cm^{-1}) υ_{max} 3344, 3024, 1662, 1494, 1454, 1380, 1336, 1299, 1267, 1148, 1096, 823, 765, 741,720.

4.1.24. Potassium-1-amino-10*H*-phenoxazine-3-sulfonate (8)



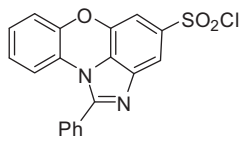
To a solution of potassium 1-nitro-10*H*-phenoxazine-3-sulfonate (**3**) (5 g) in methanol (200 ml) was added Raney Ni (5 g) and hydrazine hydrate (2 ml). The resulting mixture was stirred for 30 min at 65 °C. Completion of the reaction was monitored by TLC. The catalyst was removed by filtration under reduced pressure and concentrated under reduce pressure obtained as a light ash colour solid; yield 77% (3.50 g); mp >300 °C; *m*/*z* (CI) 315 (M-1, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 (s, 1H), 6.82–6.65 (m, 1H), 6.64–6.45 (m, 4H), 6.21(s, 1H), 4.78 (s, 2H); IR (KBr, cm^{-1}) υ_{max} 3338, 1611, 1500, 1442, 1417, 1329, 1188, 1108, 1085, 1044, 848.

4.1.25. Potassium-1-phenylimidazo[4,5,1-*kl*]phenoxazine-4-sulfonate (9)



To a solution of potassium-1-amino-10*H*-phenoxazine-3-sulfonate (**8**) (3.0 g, 9.43 mmol) in DMF (80 ml) was added benzaldehyde (1.0 g, 9.43 mmol) and the resulting reaction mixture was stirred for 24 h at 100 °C. Completion of the reaction was monitored by TLC. The solvent was removed from the reaction under reduced pressure, then the crude solid was triturated with ethyl acetate, filtered and purified by column chromatography; yield 52% (2 g), mp >300 °C; *m*/*z* (CI) 365 (M-K, 100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (d, 2H, *J* = 7.9 Hz), 7.64–7.59 (m, 3H), 7.42 (s, 1H), 7.22 (d, 1H, *J* = 8.0 Hz), 7.12 (t, 1H, *J* = 8.0 Hz), 6.91–6.83 (m, 3H); IR (KBr, cm^{-1}) υ_{max} 3492, 1661, 1494, 1455, 1302, 1195, 1078, 1039, 700.

4.1.26. 1-Phenylimidazo[4,5,1-*kl*]phenoxazine-4-sulfonylchloride (10)



Potassium-1-phenylimidazo[4,5,1-*kl*]phenoxazine-4-sulfonate (**9**) (2 g) was added to $POCl_3$ (14 ml) and refluxed for 3 h. Completion of the reaction was monitored by TLC. Excess of $POCl_3$ was removed by distillation and the crude solid was poured in excess crushed ice and the solid was filtered and used immediately for the next step.

4.1.27. Alternative preparation of 1-Phenylimidazo[4,5,1-*kl*]phenoxazine-4-sulfonamide (7a)

To a solution of aqueous ammonia (5 ml) in tetrahydrofuran (20 ml) was added 1-phenylimidazo [4,5,1-*kl*]phenoxazine-4-sulfonylchloride (**10**) at 0 °C. The resulting reaction mixture was stirred for 30 min at same temperature. Completion of the reaction was monitored by TLC and the excess solvent was removed under reduced pressure, the crude solid was acidified with dilute hydrochloric acid and the solid was filtered and dried. Compound is matching with the prepared by another method, yield 50% (0.8 g).

5. Pharmacology

5.1. Materials and methods

5.1.1. 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).

5.1.2. 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.[17] 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.

5.1.3. PDE4 enzymatic assay

The inhibition of PDE4 enzyme was measured using PDElight HTS cAMP phosphodiesterase assay kit (Lonza) according to manufacturer's recommendations. Briefly, 10 ng of in house purified PDE4B1 or 0.5 ng commercially procured PDE4D2 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. Dose response studies were performed at 9 different concentrations ranging from 100 μM to 0.01 μM. 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 and the IC_{50} values were determined by a nonlinear regression analysis from dose response curve using Graphpad Prism software (San Diego, USA). IC_{50} values are expressed as mean ± SD.

$$\%\text{Inhibition} = [(\text{RLU of vehicle control} - \text{RLU of inhibitor})/(\text{RLU of vehicle control})] \times 100$$

6. Docking study

6.1. With PDE4B

Docking simulations of molecules were performed using Schrodinger software suite (Maestro, version 9.2).[19] The Protein (PDE4B) for docking studies was retrieved from protein data bank with PDB ID: 3O0J.[20] The 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 search grid was generated by picking the co-crystal ligands and extended up to 20 Å. The hydroxyl groups of search area were kept flexible during grid generation process.

6.2. With PDE4D

Docking simulations of molecules were performed using Schrodinger software suite (Maestro, version 9.2).[19] The Protein coordinates (PDE4D) for docking studies was retrieved from protein data bank with PDB ID: 1XOR.[20] The 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 search grid was generated by picking the co-crystal ligands and extended up to 20 Å. The hydroxyl groups of search area were kept flexible during grid generation process.

All molecules were minimized by using MacroModel[21] application. Molecules were docked by using glide XP (extra precision) docking mode.[22] We performed flexible docking by allowing sample ring conformations and sample nitrogens to move to possible extent in docking. The docking results are shown in tables and figures.

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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.bmc.2013.01.023.

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