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$InCl_3$ mediated heteroarylation of indoles and their derivatization via C—H activation strategy: Discovery of 2-(1*H*-indol-3-yl)-quinoxaline derivatives as a new class of PDE4B selective inhibitors for arthritis and/or multiple sclerosis

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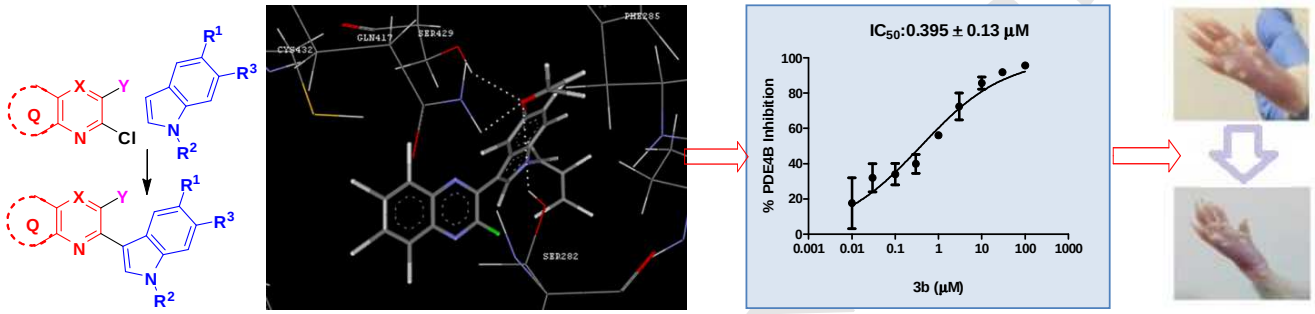

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ABSTRACT

A new class of PDE4 inhibitors were designed and synthesized via the $InCl_3$ mediated heteroarylation of indoles and their further derivatization through the Pd(II)-catalyzed C-H activation strategy. This effort allowed us to discover a series of 2-(1*H*-indol-3-yl)-quinoxaline based inhibitors possessing PDE4B selectivity over PDE4D and PDE4C. One of these compounds i.e. **3b** (PDE4B IC_{50} = 0.39 ± 0.13 μM with ~ 27 and > 250 fold selectivity for PDE4B over PDE4D and C, respectively) showed effects in Zebrafish experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis when dosed at 3, 10 and 30 mg/kg intraperitoneally. Indeed, it halted the progression of the disease across all these doses tested. At an intraperitoneal dose of 30 mg/kg the compound **3b** showed promising effects in adjuvant induced arthritic rats. The compound reduced paw volume, inflammation and pannus formation (in the knee joints) as well as pro-inflammatory gene expression / mRNA levels significantly in arthritic rats. Moreover, this compound was found to be selective towards PDE4 over other families of PDEs *in vitro* and safe when tested for its probable toxicity (e.g. teratogenicity, hepatotoxicity and cardiotoxicity) in Zebrafish.

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1. Introduction

Phosphodiesterase 4 or PDE4, one of the 11 families of PDEs (PDE1-PDE11) [1] is known to be the major PDE enzyme involved in the cAMP-mediated regulation of functional parameters in majority of inflammatory cells (e.g. neutrophils, eosinophils, mast cells, macrophages, and dendritic cells) and structural airway cells (including airway epithelium, endothelium and smooth muscle). Thus, PDE4 selective inhibitors have been reported to be useful for the treatment of psoriasis, psoriatic arthritis,[2] and multiple sclerosis [3] in addition to asthma and chronic obstructive pulmonary disease (COPD) [4]. Notably, psoriatic arthritis (a chronic inflammatory arthritis in the presence of skin and/or nail psoriasis) though currently being managed [5] by the uses of single or combination of drugs chosen from NSAIDs, corticosteroids, DMARDs (such as methotrexate, leflunomide, and sulfasalazine) as well as biological agents (e.g. TNF inhibitors and an anti-IL12/23 inhibitor) some patients do not appear to achieve an adequate clinical response with these treatments. Consequently, there appears to be an unmet need for additional medications in the rheumatologist's armamentarium towards the treatment of psoriatic arthritis. Recently, a PDE4 inhibitor i.e. apremilast has been launched for the treatment of psoriatic arthritis (Fig. 1) [6]. While the first-generation and experimental PDE4 inhibitor rolipram has shown encouraging results in mice models an appropriate and better PDE4 inhibitor is yet to be developed for the potential treatment of multiple sclerosis (MS, a demyelinating autoimmune disease). Notably, roflumilast [7a] (Fig 1) (Daxas®, Nycomed) has been in the European market for the treatment of chronic bronchitis since 2009 and in US market (Daliresp, Forest Lab) for exacerbations during COPD since 2012. The other PDE-4 inhibitors available

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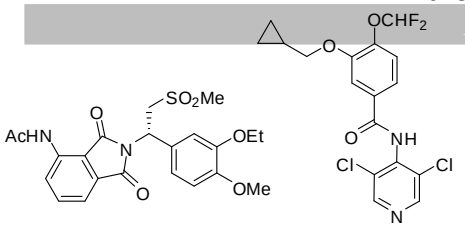

Fig. 1. Marketed PDE4 inhibitors

in the market include crisaborole [7b] and ibudilast [7c,d]. However, all these inhibitors have suffered from some degree of side effects including nausea and emesis [4]. One of the reasons for these observed side effects was thought to be due to the non-selective inhibition of PDE4 isoforms (see the IC_{50} values in Fig. 1). Study have suggested that among the four isoforms e.g. PDE4A, B, C, and D, the inhibition of PDE4B (that plays a key role in inflammatory cell regulation) suppresses TNF-α production via elevation of cAMP levels [8] whereas the inhibition of PDE4D triggers the emetic response [9]. Moreover, PDE4C (not generally found in pro-inflammatory cells) is highly expressed in the CNS where most of the PDE4 inhibitors are believed to promote their above mentioned side effects. Thus, PDE4B selectivity over PDE4D and at the same time minimizing PDE4C inhibition is a promising approach for the development of better PDE4 inhibitors. While, efforts has been devoted to identify inhibitors [9,10,11] possessing selectivity towards PDE4B over PDE4D it is not known if they inhibit PDE4C. Moreover, being a new class of selective inhibitors their potential for the treatment of psoriatic arthritis, and/or multiple sclerosis has not been evaluated. In our earlier effort we identified a novel *N*-indolylmethyl substituted olanzapine based interesting and selective PDE4B inhibitor (**A**, Fig. 2) (IC_{50} ~ 1.1 µM) that

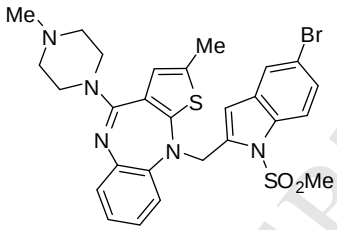

Fig. 2. PDE4 inhibitor (**A**) previously reported by our group [12].

showed >10 fold selectivity towards PDE4B over D, significant inhibition of TNF-α, no major toxicities in RAW 264.7 and HEK 293T cells and no hepatotoxicity and changes in heart rate in zebrafish embryo [12]. While selectivity was achieved but the potency of this inhibitor was not up to our expectation. Therefore identification of a better inhibitor was necessary. With this objective we looked into our previously identified other class of PDE4 inhibitors especially on 2-(1*H*-indol-3-yl)quinoline derivatives **B** (Fig. 3) one of which showed considerable PDE4 inhibitory activity (EC_{50} ~0.89 µM) when tested in a cell based cAMP reporter assay [13]. These findings prompted us to design structurally similar quinoxaline derivatives **C** (Fig. 3) as potential and selective inhibitors of PDE4B. While replacing the quinoline ring of **B** by a quinoxaline moiety was a subtle and obvious modification but fortunately the outcome was positive. Moreover, the substituents on the indole ring too played an important role. It was known that the PDE4B pocket was more hydrophobic in nature and bigger in size than PDE4D [11a, c] hence we aimed to introduce appropriate R^2 group (in **C**, Fig. 3) that preferably contributes towards overall optimal volume and

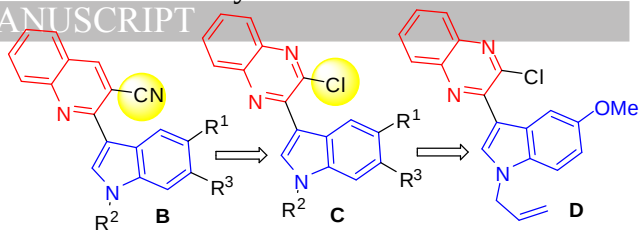

Fig. 3. Reported PDE4 inhibitor **B** and newly designed potential inhibitors **C**.

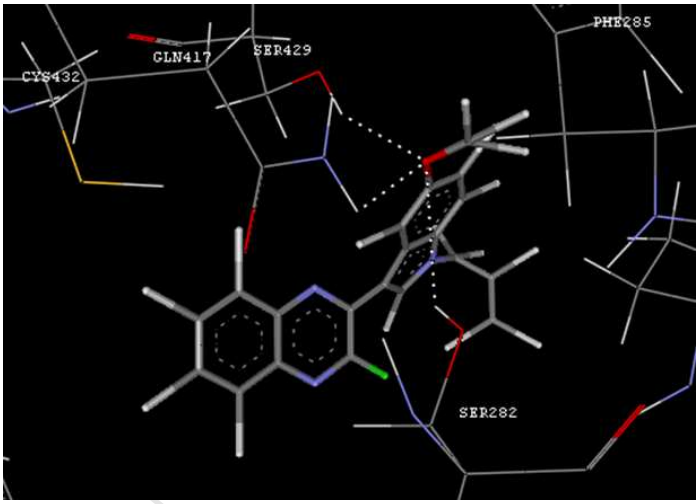

Fig. 4. Docking of **D** into PDE4B (PDB ID: 1XLZ). White dotted lines are H-bond interactions (see also Fig S-1 and S-2 in Suppl. file).

hydrophobicity of the molecule. Our design was further supported by the docking study of a representative molecule **D**. The molecular docking simulations were carried out using GRIP method of docking in Biopredicta module of Vlife MDS (Molecular Design Suite) 4.6. The molecule **D** showed good interactions with PDE4B e.g. (i) H-bond interactions with SER282, SER429 and GLN417 (Fig. 4), (ii) hydrophobic interactions with GLN284, SER282, PHE285, GLU421 and PHE285 and (iii) π-stacking interactions with PHE414 residue of PDE4B (see Fig S-2 in Suppl. file). However it showed only hydrophobic but no H-bond and / or π-interactions with PDE4D and C. Moreover, the docking score indicated possible selectivity of **D** towards PDE4B (-83.64 kcal/mol) over PDE4D (-70.10 kcal/mol) and PDE4C (-60.41 kcal/mol). Notably, docking of a non-selective inhibitor rolipram showed nearly equal interactions with PDE4B (e.g. His234 and Gln443 residues) and PDE4D (e.g. His160 and Gln369 residues) confirming it's lack of selectivity *in silico* too. Herein, we describe the PDE4 inhibitory properties of compounds based on **C** (including **D**, Fig. 3) that allowed us to discover a new class of inhibitors possessing PDE4B selectivity over PDE4D and PDE4C and evaluate their potential for the treatment of psoriatic arthritis and/or multiple sclerosis.

2. Results and discussion

2.1. Chemistry

While methods to introduce aryl or heteroaryl group at C-2 and/or C-3 position of a quinoxaline ring are known in the literature [14] compounds related to **C** were synthesized using an $AlCl_3$ induced C–C bond forming reaction [15] between 2,3-dichloroquinoxaline and indoles (Method 1, Scheme 1) due to the effectiveness of this methodology and easy availability of starting materials. However, the methodology involved the use of a stoichiometric amount of $AlCl_3$ that is prone to generate considerable amount of $Al(OH)_3$ waste during the workup

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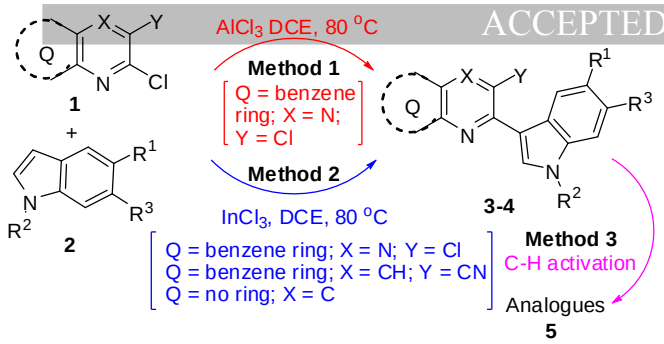
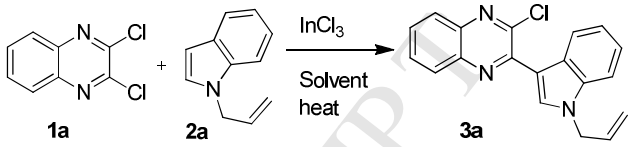


Scheme 1. Reported (Method 1) and current method (Method 2) of heteroarylation of indoles and their derivatization (Method 3).

process (especially during a scale up preparation). Moreover, $AlCl_3$ is not recoverable as well as recyclable. Thus a more environmentally friendly and sustainable method was necessary for the synthesis of target compounds based on **C**. As a less toxic as well as air and water-compatible Lewis acid catalyst $InCl_3$ has been used to catalyze various chemical transformations [16,17] with high regio- and chemoselectivity. We now describe the first $InCl_3$ mediated heteroaryaltion of indoles leading to compounds **3** and **4** (Method 2, Scheme 1) and their further derivatization *via* the direct C-H activation method i.e. the Fujiwara−Moritani reaction [18] (Method 3, Scheme 1).

The $InCl_3$ mediated heteroaryaltion of 2,3-dichloroquinoxaline (**1a**) with 1-allyl-1*H*-indole (**2a**) was examined initially in a variety of solvents to establish the optimized reaction conditions (Table 1). Among the solvents examined 1,2-dichloroethane (DCE) was found to be effective (entry 2, Table 1) when the desired product **3a** was isolated in 92% yield. The reaction also proceeded in other solvents such as acetonitrile (MeCN), dichoromethane (DCM), DMF, toluene, MeOH, PEG-400 and DMSO but afforded **3a** in low or poor yields (Entries 1 and 3-8, Table 1). Notably, the reaction was completed within 0.5 h when performed under ultrasound irradiation (entry 9, Table 1). The reaction however, did not proceed in the absence of $InCl_3$ (entry 6, Table 1) indicating the key role played by the catalyst in the present reaction. Nevertheless, based on above study $InCl_3$ was identified as a new and potential catalyst for the heteroarylation of indoles. It was therefore necessary not only to assess the generality and scope of this C-C bond forming reaction but also its applicability in the synthesis of other analogs of **3a** that were required for conducting pharmacological evaluation as part of the project goal. Accordingly, a large variety of indoles were reacted with **1a** and 2-chloroquinoline-3-carbonitrile (**1b**) (Scheme 2). The reaction proceeded well in all these cases affording the corresponding products (**3**) in good yield (Chart 1, see also Table S-1 in SI). The use of 2-chloronicotinonitrile (**1c**) also afforded the desired products (**4**) in good yields (Scheme 3, Chart 1, see also Table S-2 and Scheme S-1 in SI). Finally, the C-2 alkenylation of indole ring of some of the compounds synthesized was carried out using the C-H activation method (Table 2). The direct C-H bond olefination (the Fujiwara−Moritani reaction) [18] has attracted particular attention in the recent past and a range of directing groups has been reported to aid C(Ar)-H activation including quinolone [19a], pyridine [19b-e] etc. Recently, we have reported quinoxaline as a new directing group for the Pd (or Ru)-catalyzed *ortho* C-H alkenylation of aniline derivatives leading towards the synthesis of alkenyl substituted benzo[4,5]imidazo[1,2-*a*]quinoxalines as inhibitors of PDE4 [20]. This idea (i.e. the usefulness of alkenyl moiety for PDE4 inhibition) and the strategy (i.e. alkenylation *via* C-H activation) was extended in the present case for the synthesis of new indole derivatives (**6**). Thus a range of olefin coupling partners e.g. **5a-c** (Me, Et or t-Bu ester of acrylic acid) were employed to react with a variety of compound **3** in the presence of $Pd(OAc)_2$, $Cu(OAc)_2$ and TFA in 1,4-dioxane to afford the corresponding alkenylated product **6** in good to acceptable yield (Table 2, see also Table S-3 in SI) (for optimization of reaction conditions, see Table S-4 in SI). The compound **7a-b** was obtained *via* hydrolysis of **3t** and **4a** (Chart 1).

Table 1: Effect of conditions on $InCl_3$ catalyzed C-C bond forming reaction between **1a** and **2a**.[a]



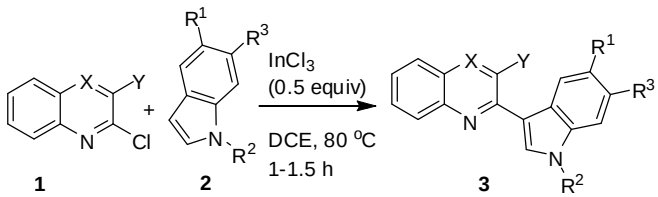
Entry	Solvent	Temp (°C)	Time (h)	%yield[b]
1.	MeCN	60	2.5	64
2.	DCE	80	1.0	92
3.	DCM	60	3.0	73
4.	DMF	100	2.0	26
5.	Toluene	100	4.0	47
6.	MeOH	60	4.5	45
7.	PEG-400	70	4.0	38
8.	DMSO	100	2.0	21
9.	DCE	80	0.5	91[c]
10.	DCE	80	1.0	0[d]

[a]All the reactions are carried out using 2,3-dichloroquinoxaline **1a** (1 mmol) and 1-allyl-1*H*-indole **2a** (1 mmol), in the presence of $InCl_3$ (0.5 mmol) in a solvent (5 mL) under nitrogen.

[b]Isolated yield.

[c]The reaction was carried out under ultrasound using a laboratory ultrasonic bath producing irradiation of 35 kHz.

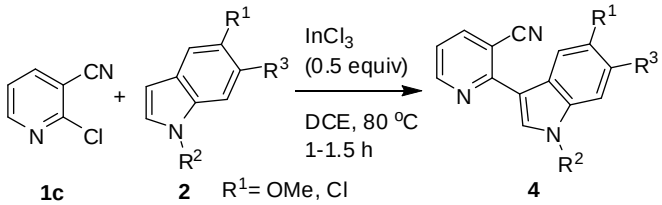
[d]The reaction was performed in the absence of $InCl_3$.



1a; X = N, Y = Cl
1b; X = CH, Y = CN

R^1= OMe, Me, NO_2, Cl. Br, H
R^2 = H, Me, Et, *i*-propyl, allyl, 2-butyl, Bn, $-(CH_2)_2CMe_2Cl$, CH_2CO_2Et, $CH_2CONHPh$, CH_2CO(morpholinyl)
R^3 = Cl

Scheme 2. Synthesis of compound **3**.



R^1= OMe, Cl
R^2 = allyl, CH_2CO_2Et
R^3 = H

Scheme 3. Synthesis of compound **4**.

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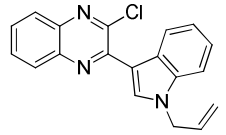
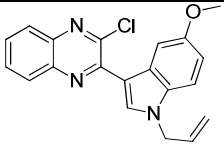
Chart 1. List of compound (**3, 4** and **7**) synthesized.[a,b]


3a (87)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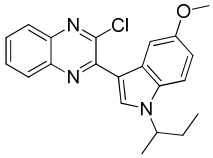
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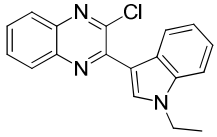
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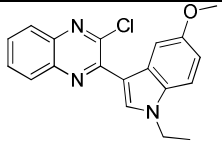
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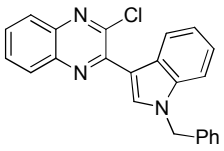
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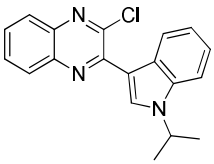
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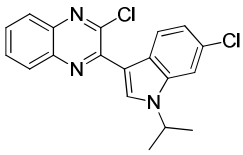
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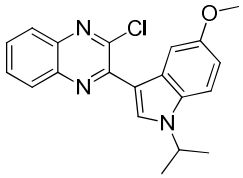
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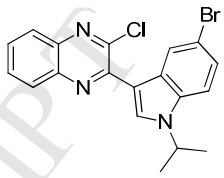
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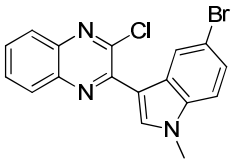
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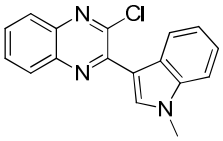
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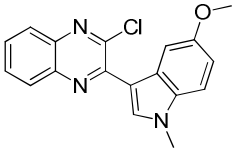
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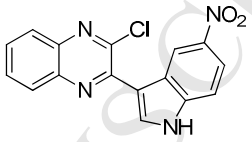
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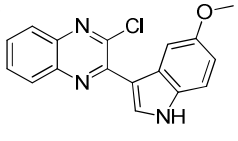
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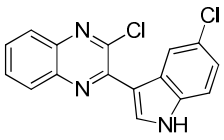
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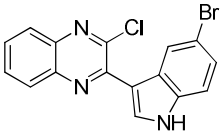
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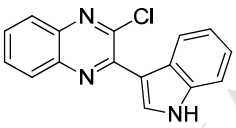
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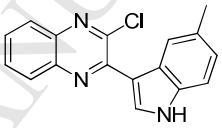
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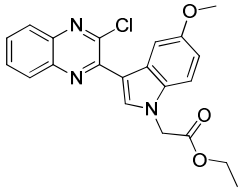
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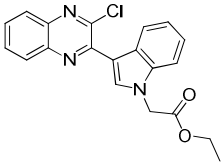
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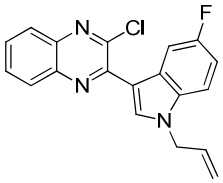
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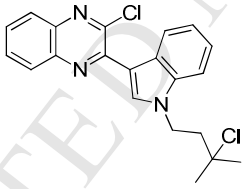
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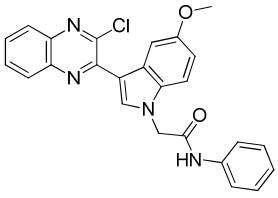
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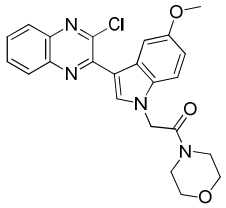
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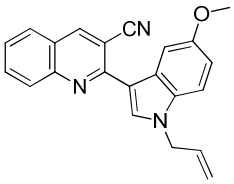
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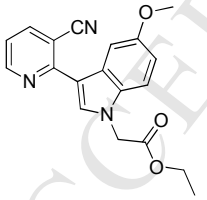
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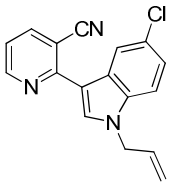
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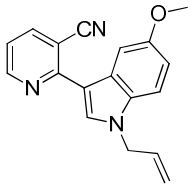
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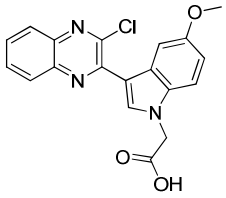
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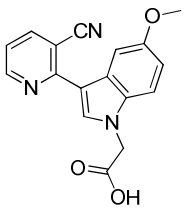
7b (75)[c]

[a]Reaction conditions for the synthesis of compound **3** and **4**: compound **1** (1 mmol), compound **2** (1 mmol) and $InCl_3$ (0.5 mmol) in DCE (5 mL) at 80 °C for 1-1.5h under nitrogen.

[b]Figure in the bracket indicates isolated yield in percentage (%).

[c]Obtained via hydrolysis of **3t** and **4a** respectively using LiOH in THF (see Scheme S-1 in SI).

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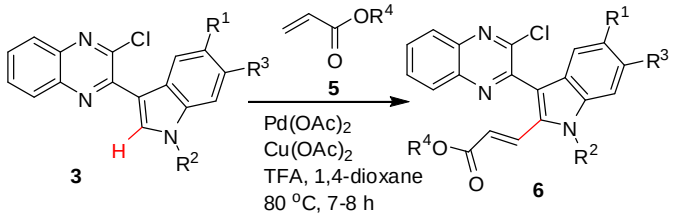
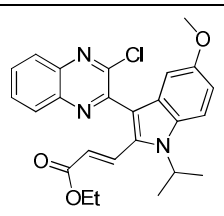
Table 2. Synthesis of compound **6** via C-2 alkenylation of indole ring of **3**.[a,b]



R^1= OMe, Me, Cl. Br, H
R^2= H, Me, *i*-propyl, allyl
R^3= H
R^4 = Me, Et, *t*-Bu



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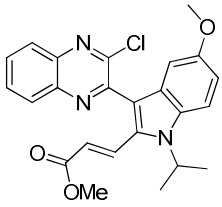

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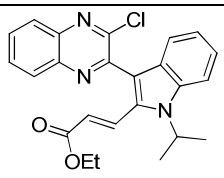

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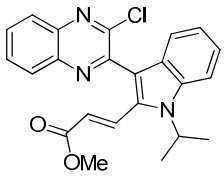

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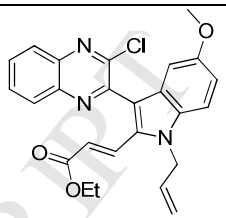

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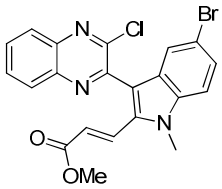

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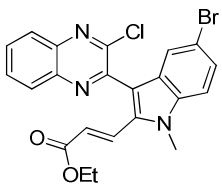

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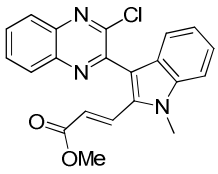

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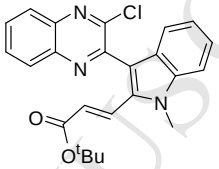

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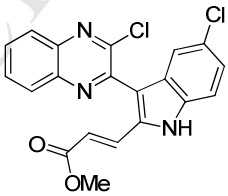

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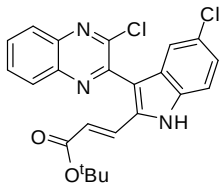

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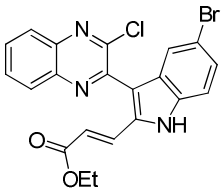

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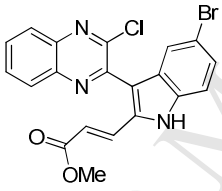

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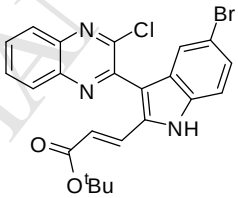

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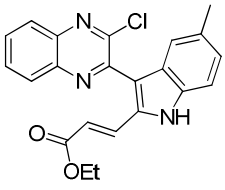

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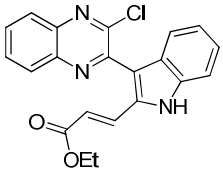

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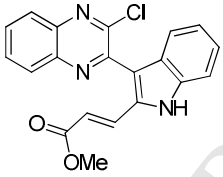
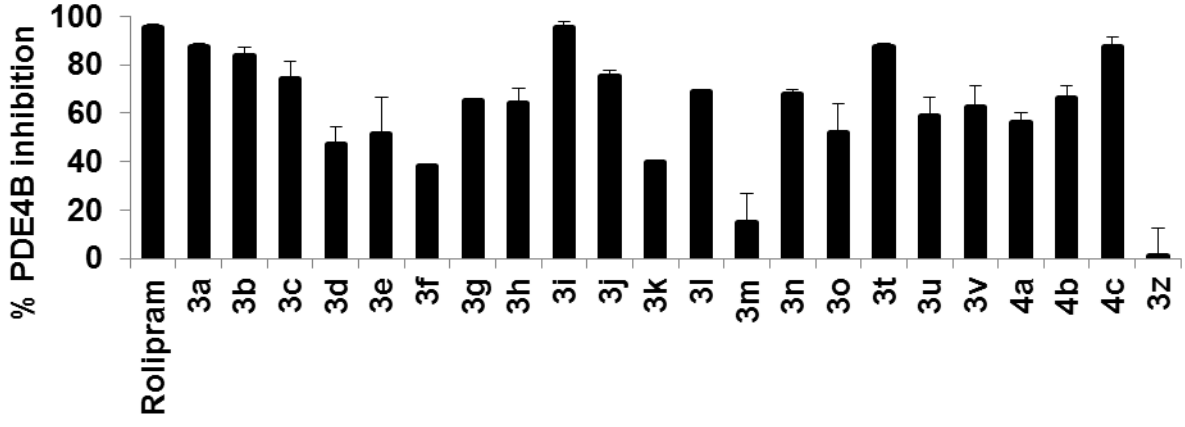


6q (73)

[a]All the reactions were carried out using compound **3** (1 mmol), compound **5** (1.5 mmol), $Pd(OAc)_2$ (5 mol%), $Cu(OAc)_2$ (1 mmol) and TFA (1.2 mmol) in 1,4-dioxane (2.5 mL) at 80 °C for 7-8 h under open air.

[b]Figure in the bracket indicates isolated yield in percentage (%).



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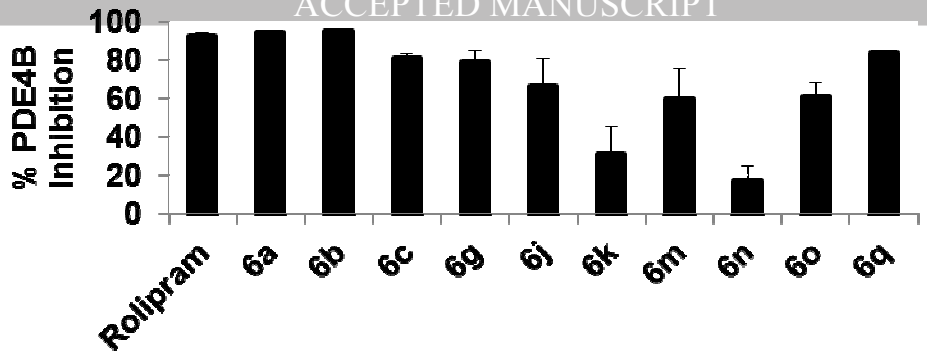

Fig 5. *In vitro* evaluation of compounds against PDE4B at 10 µM.

2.2. *Biology*

Most of the synthesized compounds e.g. **3, 4, 6** and **7** were evaluated for their PDE4B inhibitory properties *in vitro* at 10 µM using an enzyme based assay (Fig. 5) [21]. Rolipram, a well-known inhibitor of PDE4 was used as a reference compound. Compounds that showed > 75% inhibition i.e. **3a-c, 3i, 3j, 3t, 4c, 6a, 6b** and **6q** were taken for concentration dependent studies and their IC_{50} values are presented in Table 3. Some of the remaining compounds either did not show significant inhibition or provided erratic data because of their precipitation in the assay medium during the assay. While drawing the clear SAR (Structure-Activity-Relationship) conclusions from the current series of compounds was somewhat tricky some broad observations noted are summarized in Fig 6. In general the "OMe" group at C-5 of indole ring was beneficial for PDE4B inhibition as well as selectivity. No substituent or a "Br" group at this position contributed to moderate activities. No substituent at C-6 position was favorable. An alkyl chain preferably having three or four-carbon length or a $-CH_2CO_2Et$ group at indole nitrogen was effective compared to no substituent / other substituent at this position. This perhaps contributed towards the effective volume of the molecule to fit better into the PDE4B pocket. Notably, presence of an alkenyl moiety at C-2 of the indole ring did not improve the activity further but maintained a moderate to good activity in several cases. The activity was somewhat influenced by the nature of ester group attached to the alkenyl moiety, e.g. the bulky $-CO_2{}^tBu$ group was not tolerated and the Me-/Et-ester was generally favorable. Generally, the 3-chloro/cyano quinoxaline or pyridine ring at C-3 of indole was effective though the 3-cyano quinoline ring at the same position was found to be ineffective. Based on their promising activity against PDE4B the dose response study against PDE4D was also performed with the compound **3b** and **3t** (Fig 7). Indeed, the compound **3b** and **3t** showed ~ 27 and 72 fold selectivity towards PDE4B over PDE4D, respectively whereas rolipram did not show any selectivity (Table 3). Moreover, these compounds did not inhibit PDE4C significantly (~20-30% inhibition at 100 µM) (Fig. 8) when rolipram inhibited PDE4C by 70% at 10 µM. Thus their selectivity for PDE4B over PDE4C appeared to be > 250 fold. In another study compound **3b** inhibited LPS-induced iNOS (required for the nitric oxide production) and TNF-α expression in macrophages (Fig. 9) indicating its potential for anti-inflammatory effects. The compound **3b** was also tested for its selectivity towards PDE4 over other families of PDEs (e.g. PDE1-PDE11) using an *in vitro* enzymatic assay [22]. Accordingly, the % inhibition shown by **3b** at 10 µM was as follows: 12% (PDE1A1), 23% (PDE1B), 39% (PDE1C), 3% (PDE2A1), 1% (PDE3A), 1% (PDE3B), 40% (PDE4A1A), 36% (PDE4A4B), 2% (PDE5A1), 2% (PDE6C), 3% (PDE7A1), 2% (PDE7B), 3% (PDE8A1), 9% (PDE9A2), 13% (PDE10A1), 15% (PDE10A2), 7% (PDE11A4). This clearly indicated PDE4 selectivity of **3b** over other PDEs.

Table 3. *In vitro* data of most active compounds.

Entry	Compound	IC_{50} (µM)[a]		Selectivity (fold)
		PDE4B	PDE4D	
1	**3a**	4.67±1.07	22.04±4.60	~4.8
2	**3b**	0.39±0.13	10.69±7.23	~27
3	**3c**	1.04±0.38	4.93±3.78	~4.75
4	**3i**	0.44±0.33	5.02±4.18	~11.40
5	**3j**	9.74±3.33	ND	
6	**3t**	0.21±0.06	15.25±2.26	~72
7	**4c**	1.98±0.55	ND	
8	**6a**	5.58±2.26	ND	
9	**6b**	5.97±1.29	ND	
10	**6q**	3.88±0.57	ND	
11	Rolipram	0.94±0.2	0.88±0.3	

[a]Data is represented as mean ± SD of at least 2 independent experiments.

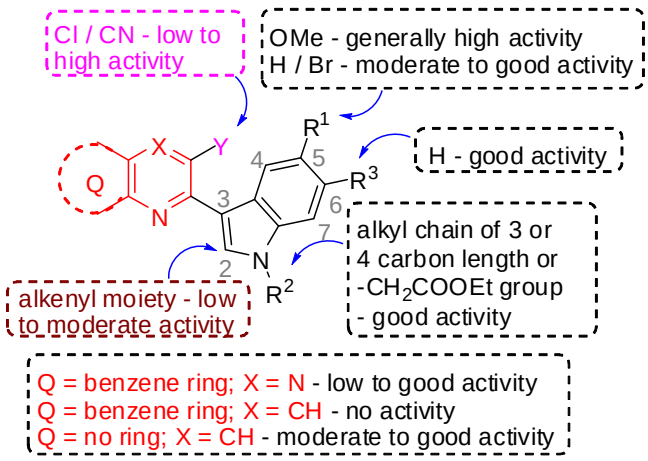

Fig. 6. Summary of SAR for PDE4B inhibitory activities of compound **3**, **4** and **6**.

To gain some preliminary information regarding metabolic stability / PK (pharmacokinetic) properties of compound **3b** the *in vitro* microsomal stability study was performed using the rat liver microsomes. At a concentration of 5 µM the compound **3b**

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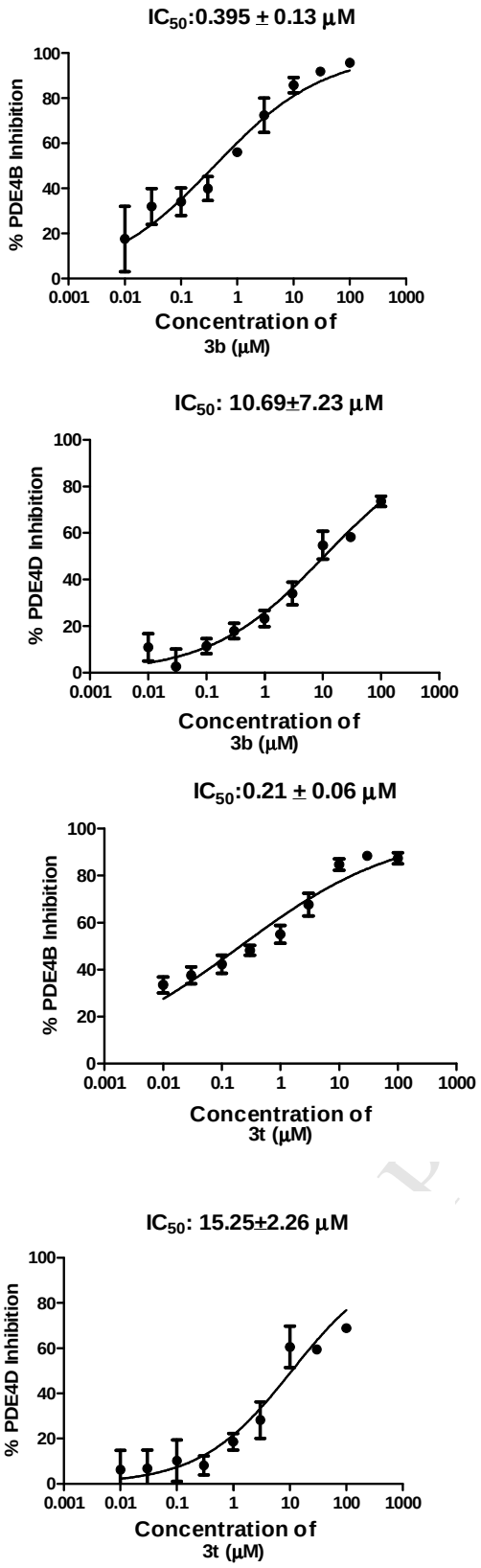


Fig. 7. Concentration dependent study of compound **3b** and **3t** against PDE4B and PDE4D.

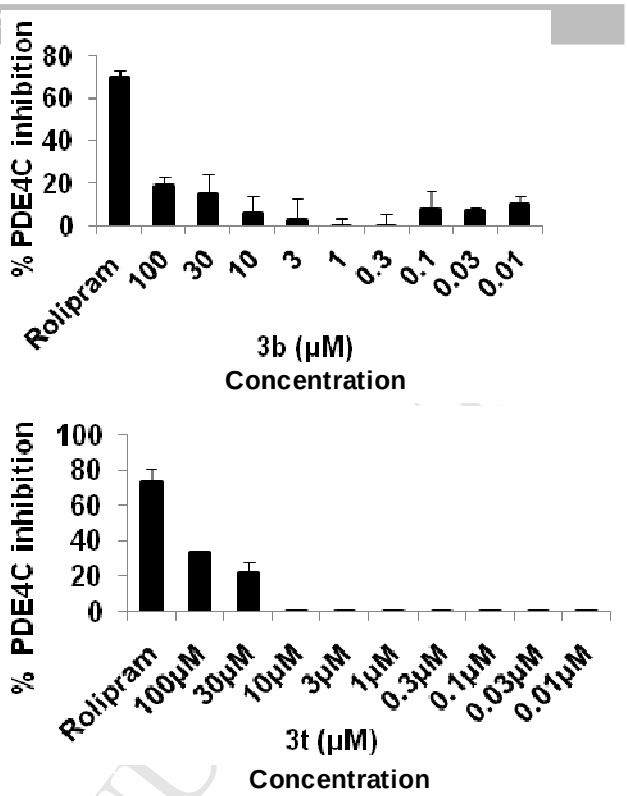
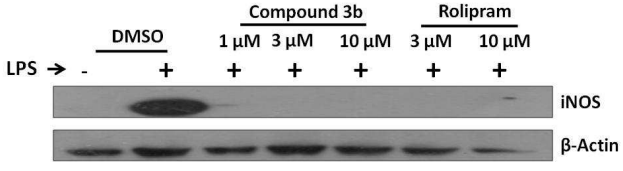


Fig. 8. Concentration dependent study of compound **3b** and **3t** against PDE4C. The reference compound rolipram was used at a concentration of 10 µM.

showed moderate stability after 60 min [i.e. the mean % of **3b** remaining after 60 min compared to 0 min ~ 42.25; half-life ($t_{1/2}$, min) ~ 52.50 and intrinsic clearance (CL_{int}, µL/min/mg) ~ 52.80]. Then a single dose intravenous PK study of compound **3b** was performed in male BALBc mice (dose: 10 mg/kg b.w.). Accordingly, the mean plasma PK parameters were determined as follows: C_{max}(ng/mL) ~ 2850.23; C_0(ng/mL) ~ 5056.31; T_{max} (h) ~ 0.08; AUC_{last}(h*ng/mL) ~ 1091.43; AUC_{inf} (h*ng/mL) ~ 1163.89; AUC_{extrap}(%) ~ 6.23; Vss(L/kg) ~ 4.46; CL(mL/min/kg) ~ 143.20; $T_{1/2}$(h) ~ 0.64; MRT_{last} (h) ~ 0.36.

Having observed promising and interesting *in vitro* activities of compound **3b**, we then evaluated its impact in a Zebrafish model of multiple sclerosis (EAE; experimental autoimmune encephalomyelitis) [23]. Notably, one of us was involved in developing this model as a quick *in vivo* screening model for multiple sclerosis [24]. The EAE was induced by immunization with myelin oligodendrocyte glycoprotein – 35–55 (MOG) where MOG in CFA (complete Freund's adjuvant) was injected subcutaneously (s.c.) in the mid spine regions of zebrafish (using 10 µl bevel-tipped Hamilton syringe with a volume of 5 µl/fish).

(A)



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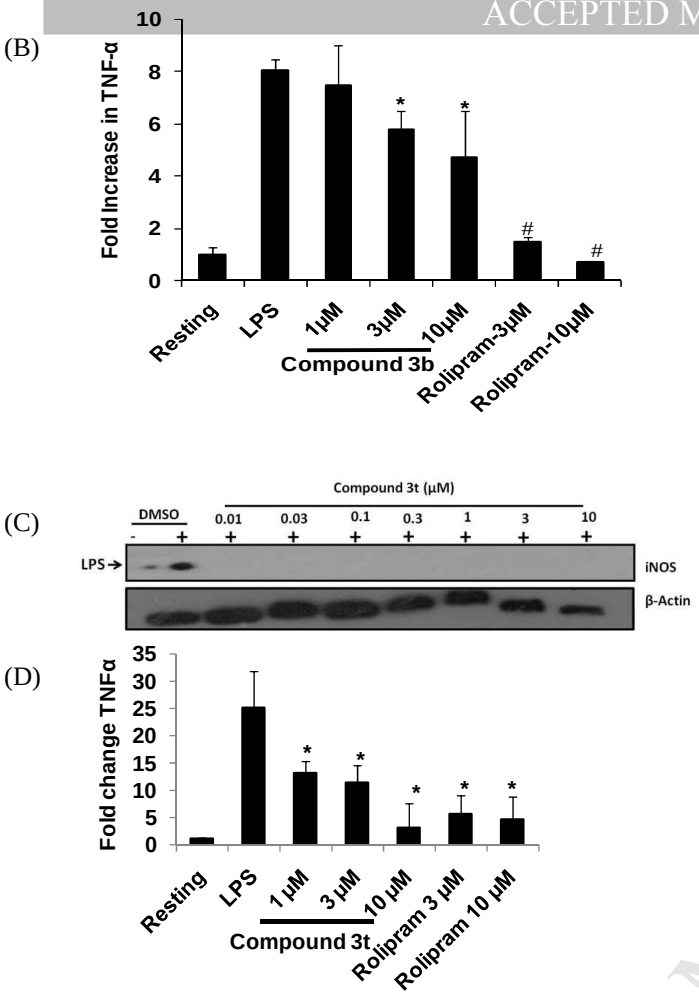


Fig. 9. Compound **3b** abolishes LPS-induced iNOS and TNF-α expression in macrophages. RAW 264.7 cells were incubated with 1, 3 and 10 μM of compound **3b** (A,B) or compound **3t** before stimulation with 1μg/ml of LPS for 10 h and iNOS levels (A,C) and TNF-α mRNA (B,D) were determined by western blotting and qPCR, respectively. Rolipram served as the positive control. Data are represented as mean ±SD. Statistical analysis was performed by unpaired student's t test. *, p <0.05 and #, p<0.001 was considered as significant.

The compound **3b** was administered intraperitoneally at the dose of 3, 10 and 30 mg/kg whereas positive control gilenya (a sphingosine 1 phosphate receptor antagonist) was administered orally at a dose of 1 mg/kg. The effects were evaluated for a period of seven days. While gilenya robustly reduced the lesion score to that of control, **3b** did not reduce the lesion score significantly (Fig. 10). However, it halted the progression of the disease across all the doses tested. We were delighted with this observation as **3b** was the first PDE4B selective inhibitor to show such effects and therefore was of further interest. Next, we evaluated compound **3b** in a rat adjuvant induced arthritis model. At an intraperitoneal dose of 30 mg/kg the compound **3b** significantly reduced paw volume of adjuvant induced arthritic rats similar to that of positive control 2 mg/kg of methotrexate (Fig 11A and Fig 11B). Further, it also reduced pain in arthritic rats as observed by improved reaction time in the hot plate test (Fig 11C). Histopathological analysis of synovial tissues of knee

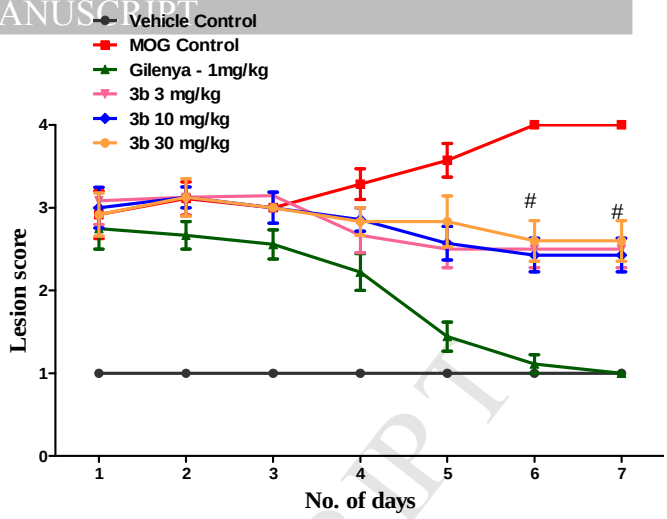
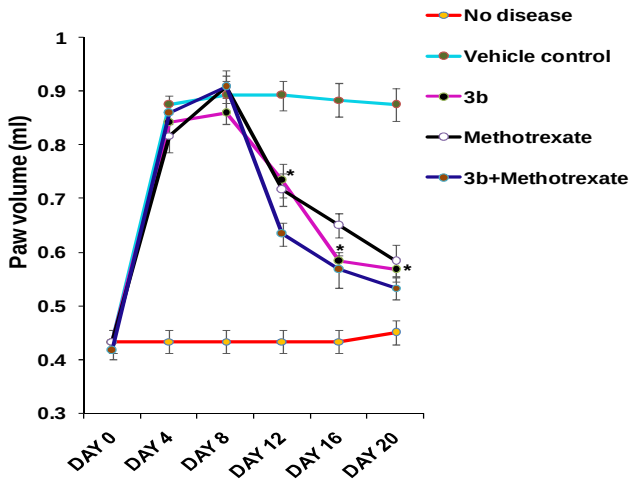


Fig. 10. Effect of compound **3b** in Zebrafish EAE model. Data are represented as mean + SEM. Statistical analysis was performed by Bonferroni multiple comparison post-hoc test. # (compound **3b** treated groups with MOG control), p<0.001 was considered as significant.



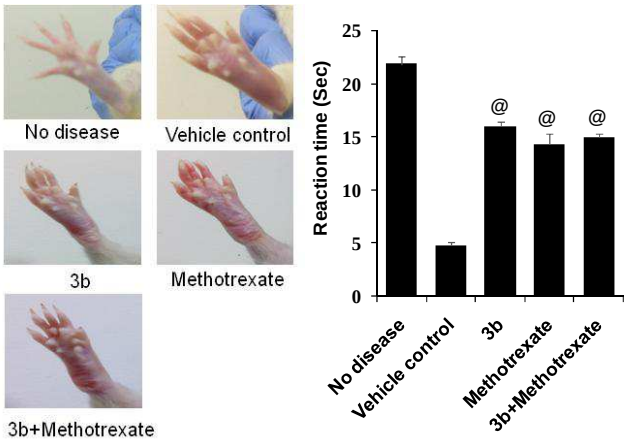


Figure 11. Effect of compound **3b** in adjuvant induced arthritic rats. (A) Paw thickness of adjuvant induced arthritic rats measured by Vernier calipers (B) Images of paw of treated / untreated rats. (C) Reaction time of adjuvant induced arthritic rats assessed in the hot plate test. Data are represented as mean + SEM. Statistical analysis was performed by Bonferroni multiple comparison post-hoc test. * (compound **3b** treated groups with vehicle control), p<0.05, @, p <0.01 was considered as significant.

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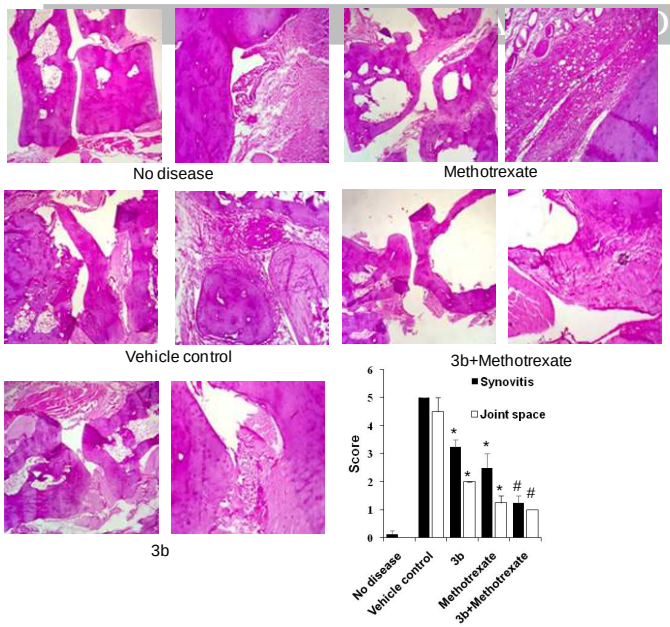


Fig. 12. Compound **3b** reduces inflammation and pannus in synovial tissues of knee joints of adjuvant induced arthritic rats. Histopathological analysis and quantification of lesion scores of inflammation and pannus formation in the knee joints of adjuvant induced arthritic rats. Data are represented as mean + SEM. Statistical analysis was performed by Bonferroni multiple comparison post-hoc test. *, p <0.05 and #, p<0.001 was considered as significant.

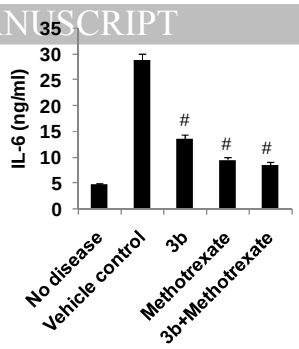
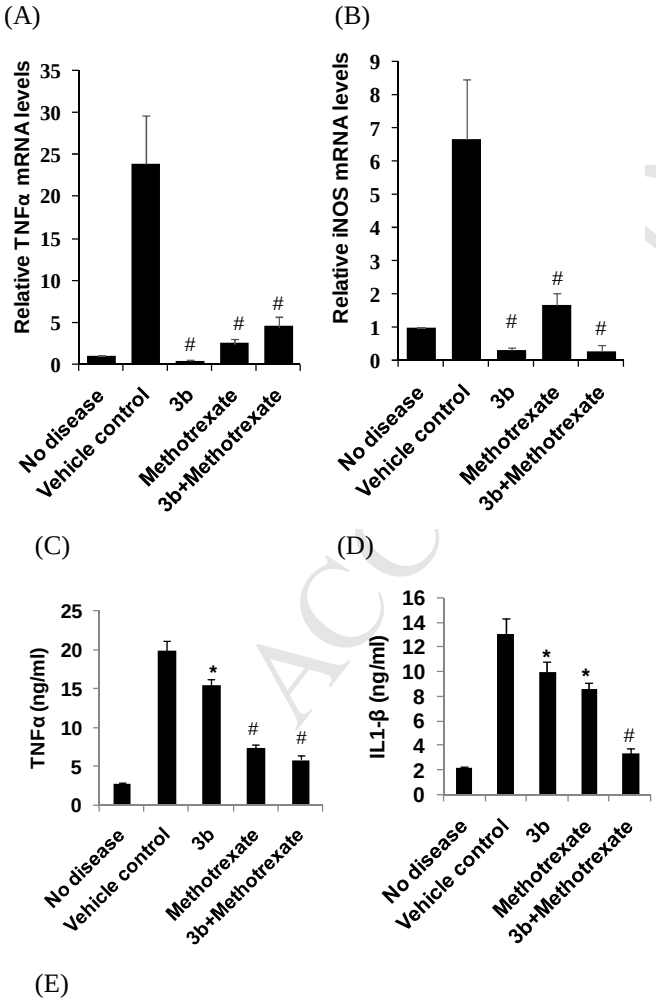


Fig. 13. Compound **3b** reduces pro-inflammatory gene expression in adjuvant induced arthritic rats: qPCR analysis of (A) TNFα, (B) iNOS. Analysis of serum levels of (C) TNFα, (D) IL-1β and (E) IL-6 cytokines in adjuvant induced arthritic rats. Data are represented as mean ± SEM. Statistical analysis was performed by Bonferroni multiple comparison post-hoc test. *, p <0.05 and #, p<0.001 was considered as significant.

joints revealed reduced synovial inflammation (synovitis) and pannus formation in the knee joints of rats treated with compound **3b**. The combination therapy with methotrexate further improved the lesion score (Fig 12). Consistent with reduced synovial inflammation, the gene expression of pro-inflammatory mediators such as TNF-α, IL-1β, IL-6 and iNOS was dampened in compound **3b** treated rats. Further, the mRNA levels of TNFα, IL-1β, IL-6 and iNOS were also robustly decreased in compound **3b** administered rats (Fig. 13).



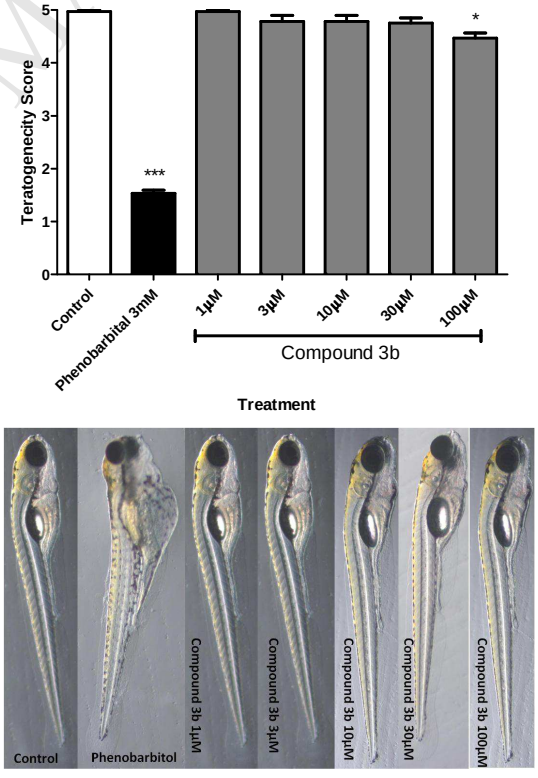


Fig. 14. Compound **3b** does not induce teratogenicity in Zebrafish embryos. Top panel: Teratogenicity scores of compound **3b** and positive control phenobarbitol. Bottom panel: Representative images of teratogenicity assay. Data are represented as mean + SEM. Statistical analysis was performed by Bonferroni multiple comparison post-hoc test. *, p<0.05 was considered as significant.

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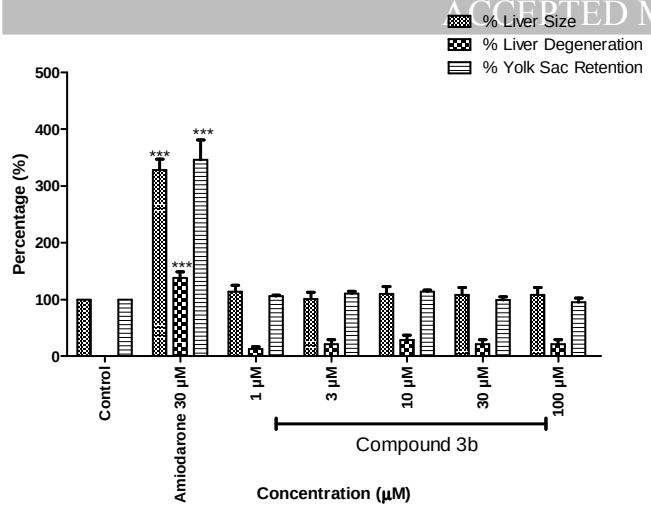

Fig. 15. Compound **3b** does not induce hepatotoxicity in Zebrafish embryos. The above graph represents the qualitative data of % liver size, % liver degeneration & % yolk sac retention in compound 3b treated embryos at different concentrations when compared to positive control amiodarone. Data are represented as mean + SEM. Statistical analysis was performed by Bonferroni multiple comparison post-hoc test. ***, p<0.001 was considered as significant.

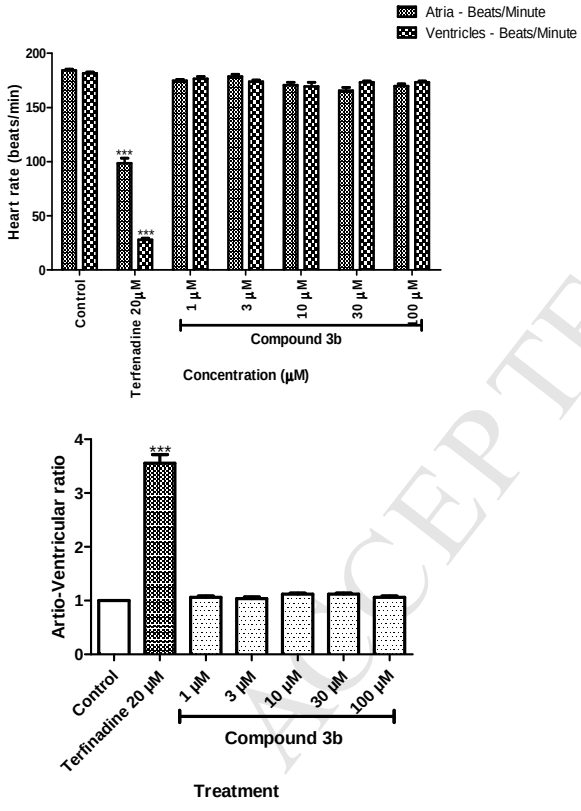

Figure 16. Compound **3b** does not induce cardiac toxicity in Zebrafish embryos. The above graph represents the heart rate (top panel) and atria-ventricular ratio (bottom panel) of compound **3b** treated embryos at different concentrations when compared to positive control terfenadine. Data are represented as mean ± SEM. Statistical analysis was performed by Bonferroni multiple comparison post-hoc test. ***, p<0.001 was considered as significant.

Having found to be active in two *in vivo* models the compound **3b** was then evaluated for its probable toxicity specifically its potential ability to induce teratogenicity, hepatotoxicity and cardiotoxicity in Zebrafish. Methotrexate, phenobarbitol, amiodarone and terfenadine were used individually as a positive control in these assays respectively. It was observed that at the concentration of 1, 3, 10 and 100 µM compound **3b** was safe in all the toxicity assays tested (Fig 14-16; Table 6). Overall, compound **3b** has been identified as a promising candidate for further preclinical development. While the progress of **3b** has been planned it was necessary to identify a backup candidate as part of the drug discovery strategy. Therefore we are focusing on compound **3t** that showed better potency and selectivity compared to **3b** (Table 3).

Table 6. Results of zebrafish embryo toxicity study with toxicological indices and major organs/systems affected in positive control.[a]

	Compound **3b**	Phenobarbital
Test Concentrations (µM)	1, 3, 10, 30, 100	3000
Statistically Significant Toxic Concentration (µM)	100	Positive
No Observed Adverse Effect Level (NOAEL) (µM)	30	Control
Toxicity at MTC (maximum tolerated concentration)		
Body Shape	-	xxx
Somites	-	xxx
Notochord	x	xxx
Tail	-	xxx
Fins	-	xxx
Brain	-	xxx
Upper jaw	x	xxx
Heart	-	xx
Intestine	-	xxx
Lower jaw	x	xx
Liver	-	xxx
Swim Bladder	-	xxx

[a]Significance of sign: - (no effect), x (slightly toxic), xx (moderately toxic), xxx (severely toxic).

3. Conclusion

In conclusion, a new class of PDE4B inhibitors was designed based on the docking of a representative compound into the PDE4B, C and D *in silico*. These 2-(1*H*-indol-3-yl)-quinoxaline based compounds were predicted to be selective towards PDE4B. Their chemical synthesis was carried out via the $InCl_3$ mediated heteroaryaltion of indoles that involved a new C-C bond forming reaction. Further derivatization of these compounds were performed using a Pd(II)-catalyzed C-H activation strategy. A range of target compounds and their analogues were prepared in good yields. Evaluation of PDE4 inhibitory properties of synthesized compounds allowed us to discover inhibitors possessing PDE4B selectivity over PDE4D and PDE4C. One of these compounds i.e. **3b** (PDE4B IC_{50} = 0.39 ± 0.13 µM with ~ 27 and > 250 fold selectivity for PDE4B over PDE4D and C, respectively) showed effects in Zebrafish EAE model of multiple sclerosis when dosed at 3, 10 and 30 mg/kg intraperitoneally. Indeed, it halted the progression of the disease across all these doses tested. At an intraperitoneal dose of 30 mg/kg the compound **3b** showed promising effects in adjuvant induced arthritic rats. Indeed, this compound reduced paw volume, inflammation and pannus formation (in the knee joints) as well as pro-inflammatory gene expression / mRNA levels significantly in

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arthritic rats. Moreover, this compound was found to be selective towards PDE4 over other families of PDEs (e.g. PDE1-PDE11) and safe when tested for its probable toxicity (e.g. teratogenicity, hepatotoxicity and cardiotoxicity) in Zebrafish. Overall, as a new, safe and selective inhibitor of PDE4B and with demonstrated effects in two *in vivo* diseased models the compound **3b** is of further medicinal interest. Moreover, with the demonstrated application of modern chemistry methodologies in accessing NCEs to achieve PDE4B selectivity this research would be of further interest.

4. Experimental section

4.1. Chemistry

4.1.1. General methods

Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), using EtOAc/ *n*-hexane as solvent system and visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or 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), dd (doublet of doublet), td (triplet of doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (*J*) are given in hertz. MS spectra were obtained on a Agilent 6430 series Triple Quard LC-MS / MS spectrometer. Melting points (mp) were determined by using Buchi B-540 melting point apparatus and are uncorrected. Chromatographic purity by HPLC (Agilent 1200 series Chem Station software) was determined by using area normalization method and conditions specified in each case are as follows: column, mobile phase (range used), flow rate, detection wavelength, and retention time.

*4.1.2. General Procedure for the preparation of compound **3** and **4***

A mixture of compound **1** (1.0 equiv), indole **2** (1.0 equiv) and $InCl_3$ (0.5 equiv) in 1,2-dichloroethane (5 mL) was stirred at 80 °C for 1-1.5 h under a nitrogen atmosphere. After completion of the reaction, the mixture was poured into ice-cold water (15 mL), stirred for 10 min and then extracted with ethylacetate (3 x 15 mL). The organic layers were collected, combined, washed with cold water (2 x 20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue obtained was purified by column chromatography using ethylacetate/hexene to give the desired product.

4.1.3. 2-(1-Allyl-1H-indol-3-yl)-3-chloroquinoxaline (**3a**)

Yield: 87%; pale yellow solid; mp: 108-110 °C; R_f = 0.21 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.71-8.68 (m, 1H, ArH), 8.30 (s, 1H, C-2 indolyl H), 8.17 (dd, *J* = 8.4, 0.8 Hz, 1H, ArH), 7.99-7.97 (m, 1H, ArH), 7.78-7.73 (m, 1H, ArH), 7.71-7.67 (m, 1H, ArH), 7.43-7.39 (m, 1H, ArH), 7.36-7.31 (m, 2H, ArH), 6.12-6.02 (m, 1H, =CH), 5.30 (dd, *J* = 10.0, 1.2 Hz, 1H, =CH_2), 5.21 (dd, *J* = 17.2, 1.2 Hz, 1H, =CH_2), 4.88-4.86 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.4, 145.1, 141.0, 138.9, 136.5, 132.5, 132.4, 130.1, 129.2, 128.4, 127.8, 127.7, 123.1, 122.7, 121.7, 118.1, 111.2, 109.9, 49.3 (CH_2); MS (ES mass): 320.0 (M+1); HPLC: 99.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.5 min.

*4.1.4. 2-(1-Allyl-5-methoxy-1H-indol-3-yl)-3-chloroquinoxaline (**3b**)*

Yield: 93%; light yellow solid; mp: 133-135 °C; R_f = 0.24 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.59 (s, 1H, C-2 indolyl H), 8.25 (d, *J* = 2.4 Hz, 1H, ArH), 8.14 (d, *J* = 8.4 Hz, 1H, ArH), 7.97 (d, *J* = 8.0 Hz, 1H, ArH), 7.88-7.82 (m, 1H, ArH), 7.80-7.74 (m, 1H, ArH), 7.47 (d, *J* = 8.4 Hz, 1H, ArH), 6.93 (dd, *J* = 8.8, 2.4 Hz, 1H, ArH), 6.10-6.0 (m, 1H, =CH), 5.20 (dd, *J* = 10.4, 1.6 Hz, 1H, =CH_2), 5.12 (dd, *J* = 17.4, 1.6 Hz, 1H, =CH_2), 4.97 (d, *J* = 5.6 Hz, 2H, CH_2), 3.84 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.7, 148.6, 145.0, 140.9, 138.8, 132.8, 132.5, 131.6, 130.1, 129.1, 128.3, 127.9, 118.1, 116.8, 113.2, 110.7 (2C), 104.5, 55.7 (O<u>Me</u>), 49.5 (NCH_2); MS (ES mass): 350.2 (M+1); HPLC: 99.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.3 min.

*4.1.5. 2-(1-(sec-Butyl)-5-methoxy-1H-indol-3-yl)-3-chloroquinoxaline (**3c**)*

Yield: 91%; pale yellow solid; mp: 110-112 °C; R_f = 0.23 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.53 (s, 1H, C-2 indolyl H), 8.19 (d, *J* = 2.4 Hz, 1H, ArH), 8.13 (d, *J* = 7.6 Hz, 1H, ArH), 7.97 (d, *J* = 7.2 Hz, 1H, ArH), 7.87-7.82 (m, 1H, ArH), 7.79-7.74 (m, 1H, ArH), 7.59 (d, *J* = 8.8 Hz, 1H, ArH), 6.93 (dd, *J* = 9.2, 2.8 Hz, 1H, ArH), 4.66-4.58 (m, 1H, NCH), 3.83 (s, 3H, OCH_3), 1.99-1.85 (m, 2H, CH-C<u>H_2</u>), 1.54 (d, *J* = 6.4 Hz, 3H, CH-C<u>H_3</u>), 0.78 (t, *J* = 7.2 Hz, 3H, CH_2-C<u>H_3</u>); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 155.4, 148.7, 144.7, 140.7, 138.5, 131.6, 131.1, 130.8, 129.8, 128.4, 128.1, 127.9, 112.8, 111.7, 110.0, 104.7, 55.7 (OMe), 53.8 (NCH), 29.6 (CH-<u>CH_2</u>), 20.9 (CH-<u>Me</u>), 10.9 (CH_2-<u>Me</u>); MS (ES mass): 366.0 (M+1); HPLC: 99.6%, Column: Agilent Eclipse Plus C-18 250 * 4.6 mm, 5 µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/5, 3/5, 10/95, 20/95, 22/5, 25/5; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 225.0 nm, retention time 15.6 min.

*4.1.6. 2-Chloro-3-(1-ethyl-1H-indol-3-yl)quinoxaline (**3d**)*

Yield: 82%; pale yellow solid; mp: 90-92 °C; R_f = 0.20 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.72-8.70 (m, 1H, ArH), 8.32 (s, 1H, C-2 indolyl H), 8.15 (d, *J* = 8.0 Hz, 1H, ArH), 7.98 (d, *J* = 8.4 Hz, 1H, ArH), 7.75 (t, *J* = 7.6 Hz, 1H, ArH), 7.68 (t, *J* = 7.2 Hz, 1H, ArH), 7.44-7.42 (m, 1H, ArH), 7.37-7.31 (m, 2H, ArH), 4.32-4.25 (m, 2H, CH_2), 1.58-1.54 (m, 3H, CH_2-C<u>H_3</u>); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.6, 145.0, 141.0, 138.9, 136.1, 131.7, 130.1, 129.1, 128.4, 127.8 (2C), 122.9, 122.8, 121.5, 110.9, 109.6, 41.6 (CH_2), 15.3 (Me) ; MS (ES mass): 308.1 (M+1); HPLC: 99.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 275.0 nm, retention time 4.4 min.

*4.1.7. 2-Chloro-3-(1-ethyl-5-methoxy-1H-indol-3-yl)quinoxaline (**3e**)*

Yield: 86%; pale yellow solid; mp: 126-128 °C; R_f = 0.21 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.60 (s, 1H, C-2 indolyl H), 8.25 (d, *J* = 2.4 Hz, 1H, ArH), 8.13 (d, *J* = 8.0 Hz, 1H, ArH), 7.97 (d, *J* = 8.0 Hz, 1H, ArH), 7.85 (t, *J* = 7.6 Hz, 1H, ArH), 7.76 (t, *J* = 7.6 Hz, 1H, ArH), 7.54 (d, *J* = 8.8 Hz, 1H, ArH), 6.95 (dd, *J* = 8.8, 2.4 Hz, 1H, ArH), 4.34 (q, *J* = 7.2 Hz, 2H, CH_2), 3.84 (s, 3H, OCH_3), 1.42 (t, *J* = 7.2 Hz, 3H,

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CH_2-$C\underline{H_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 155.5, 148.6, 144.5, 140.7, 138.4, 133.7, 131.3, 131.0, 129.7, 128.3, 127.9, 112.8, 111.5, 109.9, 109.7, 105.0, 55.7 (OMe), 41.6 (NCH_2), 15.8 (Me); MS (ES mass): 338.0 (M+1); HPLC: 97.7%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 230.0 nm, retention time 4.3 min.

*4.1.8. 2-(1-Benzyl-1H-indol-3-yl)-3-chloroquinoxaline (**3f**)*

Yield: 78%; pale yellow solid; mp: 128-130 °C; R_f = 0.28 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.73 (d, *J* = 8.0 Hz, 1H, ArH), 8.35 (s, 1H, C-2 indolyl H), 8.01 (d, *J* = 8.0 Hz, 1H, ArH), 7.99 (t, *J* = 7.2 Hz, 1H, ArH), 7.78-7.74 (m, 1H, ArH), 7.71-7.67 (m, 1H, ArH), 7.37-7.30 (m, 5H, ArH), 7.20 (d, *J* = 6.4 Hz, 3H, ArH), 5.44 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.4, 145.1, 141.0, 139.0, 136.6, 136.4, 132.8, 130.1, 129.3 (2C), 128.9 (2C), 128.5, 127.9 (2C), 127.8, 126.8, 123.3, 122.7, 121.8, 111.4, 110.1, 50.6 (CH_2); MS (ES mass): 370.1 (M+1); HPLC: 91.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 245.0 nm, retention time 4.0 min.

*4.1.9. 2-Chloro-3-(1-isopropyl-1H-indol-3-yl)quinoxaline (**3g**)*

Yield: 89%; pale yellow solid; mp: 104-106 °C; R_f = 0.20 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.68-8.66 (m, 1H, ArH), 8.40 (s, 1H, C-2 indolyl H), 8.16-8.14 (m, 1H, ArH), 7.99-7.77 (m, 1H, ArH), 7.77-7.73 (m, 1H, ArH), 7.70-7.66 (m, 1H, ArH), 7.49-7.46 (m, 1H, ArH), 7.36-7.30 (m, 2H, ArH), 4.83-4.76 (m, 1H, NCH), 1.65 (s, 3H, CH_3), 1.63 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.6, 145.1, 141.0, 138.9, 136.0, 130.1, 129.1, 128.5, 128.4, 127.8, 127.7, 122.8, 122.6, 121.6, 111.0, 109.8, 47.7 (NCH), 22.7 (2C, 2Me); MS (ES mass): 322.1 (M+1); HPLC: 99.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 220.0 nm, retention time 4.7 min.

*4.1.10. 2-Chloro-3-(6-chloro-1-isopropyl-1H-indol-3-yl)quinoxaline (**3h**)*

Yield: 87%; light yellow solid; mp: 132-134 °C; R_f = 0.22 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.60 (d, *J* = 8.4 Hz, 1H, ArH), 8.38 (s, 1H, C-2 indolyl H), 8.14 (d, *J* = 8.4 Hz, 1H, ArH), 7.98 (d, *J* = 8.0 Hz, 1H, ArH), 7.77-7.73 (m, 1H, ArH), 7.71-7.61 (m, 1H, ArH), 7.46 (d, *J* = 1.2 Hz, 1H, ArH), 7.28 (d, *J* = 1.2 Hz, 1H, ArH), 4.74-4.67 (m, 1H, NCH), 1.64 (s, 3H, CH_3), 1.63 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.1, 144.9, 140.8, 139.0, 136.5, 130.2, 129.4, 129.0, 128.8, 128.3, 127.9, 126.2, 123.7, 122.1, 111.2, 109.8, 48.0 (NCH), 22.6 (2C, 2Me); MS (ES mass): 355.9 (M+1); HPLC: 99.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 5.3 min.

*4.1.11. 2-Chloro-3-(1-isopropyl-5-methoxy-1H-indol-3-yl)quinoxaline (**3i**)*

Yield: 92%; pale yellow solid; mp: 116-118 °C; R_f = 0.25 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.39 (s, 1H, C-2 indolyl H), 8.26 (d, *J* = 2.8 Hz, 1H, ArH), 8.12-8.10 (m, 1H, ArH), 7.98-7.96 (m, 1H, ArH), 7.76-7.72 (m, 1H, ArH), 7.69-7.65 (m, 1H, ArH), 7.37 (d, *J* = 8.8 Hz, 1H, ArH), 7.00 (dd, *J* = 8.8, 2.4 Hz, 1H, ArH), 4.76-4.69 (m, 1H, NCH), 3.92 (s, 3H, OCH_3), 1.64 (s, 3H,CH_3), 1.62 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.6, 148.7, 145.0, 140.9, 138.8, 131.2, 130.1, 129.0 (2C), 128.3, 128.2, 127.9, 112.9, 110.5, 110.4, 104.5, 55.8 (OMe), 47.9 (NCH), 22.7 (2C, 2Me); MS (ES mass): 352.0 (M+1); HPLC: 99.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.5 min.

*4.1.12. 2-(5-Bromo-1-isopropyl-1H-indol-3-yl)-3-chloroquinoxaline (**3j**)*

Yield: 88%; pale yellow solid; mp: 198-200 °C; R_f = 0.23 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.72 (d, *J* = 2.0 Hz, 1H, ArH), 8.62 (s, 1H, C-2 indolyl H), 8.13-8.11 (m, 1H, ArH), 7.99-7.97 (m, 1H, ArH), 7.88-7.84 (m, 1H, ArH), 7.82-7.78 (m, 1H, ArH), 7.69 (d, *J* = 8.8 Hz, 1H, ArH), 7.42 (dd, *J* = 8.8, 2.0 Hz, 1H, ArH), 4.94-4.87 (m, 1H, NCH), 1.56 (s, 3H, CH_3), 1.55 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 148.1, 144.8, 141.7, 140.6, 138.8, 134.8, 131.3, 131.2, 130.3, 128.5, 128.0, 125.6, 124.8, 114.4, 113.2, 110.1, 48.2 (NCH), 22.6 (2C, 2Me); MS (ES mass): 401.9 (M+3); HPLC: 99.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 5.4 min.

*4.1.13. 2-(5-Bromo-1-methyl-1H-indol-3-yl)-3-chloroquinoxaline (**3k**)*

Yield: 80%; light yellow solid; mp: 137-139 °C; R_f = 0.22 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.86 (s, 1H, ArH), 8.22 (s, 1H, C-2 indolyl H), 8.14 (d, *J* = 8.4 Hz, 1H, ArH), 7.94 (d, *J* = 8.0 Hz, 1H, ArH), 7.75 (t, *J* = 8.4 Hz, 1H, ArH), 7.68 (t, *J* = 8.0 Hz, 1H, ArH), 7.39 (d, *J* = 8.8 Hz, 1H, ArH), 7.20 (d, *J* = 8.8 Hz, 1H, ArH), 3.86 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 147.7, 144.6, 140.8, 138.9, 135.7, 134.1, 130.2, 129.4, 129.0, 128.4, 127.8, 125.9, 125.4, 115.2, 110.9, 110.4, 33.6 (NMe); MS (ES mass): 374.0 (M+3); HPLC: 99.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.8 min.

*4.1.14. 2-Chloro-3-(1-methyl-1H-indol-3-yl)quinoxaline (**3l**)*

Yield: 83%; pale yellow solid; mp: 143-145 °C; R_f = 0.21 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.78-8.65 (m, 1H, ArH), 8.25 (s, 1H, C-2 indolyl H), 8.16-8.14 (m, 1H, ArH), 7.98-7.96 (m, 1H, ArH), 7.76-7.72 (m, 1H, ArH), 7.69-7.65 (m, 1H, ArH), 7.41-7.32 (m, 3H, ArH), 3.91 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.5, 145.0, 141.0, 138.9, 137.1, 133.3, 130.1, 129.1, 128.4, 127.8, 127.6, 123.1, 122.7, 121.6, 110.9, 109.5, 33.4 (NMe); MS (ES mass): 294.0 (M+1); HPLC: 99.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 4.2 min.

*4.1.15. 2-Chloro-3-(5-methoxy-1-methyl-1H-indol-3-yl)quinoxaline (**3m**)*

Yield: 86%; light orange solid; mp: 244-246 °C; R_f = 0.23 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.58 (s, 1H, C-2 indolyl H), 8.26 (d, *J* = 2.4 Hz, 1H, ArH), 8.14 (d, *J* = 8.0 Hz, 1H, ArH), 7.96 (d, *J* = 8.0 Hz, 1H, ArH), 7.86-7.82 (m, 1H, ArH), 7.78-7.74 (m, 1H, ArH), 7.48 (d, *J* = 8.8 Hz, 1H, ArH), 6.96 (dd, *J* = 8.8, 2.4 Hz, 1H, ArH), 3.92 (s, 3H, OCH_3),

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3.85 (s, 3H, NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 155.5, 148.6, 144.4, 140.7, 138.4, 135.2, 132.4, 131.0, 129.7, 128.3, 128.1, 127.9, 112.8, 111.5, 109.5, 104.9, 55.7 (OMe), 33.7 (NMe); MS (ES mass): 324.1.0 (M+1); HPLC: 99.0%, Column: X Bridge C-18 150 * 4.6 mm, 5 µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/10, 3/10, 10/95, 20/95, 22/10, 25/10; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 225.0 nm, retention time 12.0 min.

*4.1.16. 2-Chloro-3-(5-nitro-1H-indol-3-yl)quinoxaline (**3n**)*

Yield: 71%; light brown solid; mp: 306-308 °C; R_f = 0.25 (10% EtOAc/ *n*-hexane); ^1H NMR (400 MHz, DMSO-d_6) δ: 12.53 (s, 1H, NH, D_2O exchangeable), 9.51 (d, *J* = 2.0 Hz, 1H, ArH), 8.77 (d, *J* = 2.8 Hz, 1H, ArH), 8.15-8.12 (m, 2H, ArH), 8.03-8.00 (m, 1H, ArH), 7.93-7.89 (m, 1H, ArH), 7.86-7.82 (m, 1H, ArH), 7.71 (d, *J* = 9.2 Hz, 1H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 147.6, 144.4, 142.3, 140.3, 139.8, 138.8, 134.0, 131.2, 130.4, 128.3, 127.9, 126.2, 119.6, 118.2, 112.9, 112.8; MS (ES mass): 323.1.0 (M-1); HPLC: 98.0%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 220.0 nm, retention time 3.8 min.

*4.1.17. Compound **3o-3s***

These compounds are known and prepared following the reported method [15]. Spectral data of these compounds were compared with the reported data.

*4.1.18. Ethyl-2-(3-(3-chloroquinoxalin-2-yl)-5-methoxy-1H-indol-1-yl)acetate (**3t**)*

Yield: 70%; pale yellow solid; mp: 160-162 °C, R_f =0.27 (10% EtOAc/ *n*-hexane); ^1H NMR (400 MHz, DMSO-d_6) δ: 8.63 (s, 1H, C-2 indolyl H), 8.25 (d, *J* = 2.4 Hz, 1H, ArH), 8.21-8.11 (m, 1H, ArH), 7.98 (dd, *J* = 8.2, 0.94 Hz, 1H, ArH), 7.91-7.83 (m, 1H, ArH), 7.82-7.70 (m, 1H, ArH), 7.43 (d, *J* = 9.2 Hz, 1H, ArH), 6.93 (dd, *J* = 8.8, 2.5 Hz, 1H, ArH), 5.29 (s, 2H, NCH_2), 4.17 (m, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 1.22 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 169.0 (C=O), 155.6, 148.5, 144.5, 140.7, 138.6, 135.4, 132.2, 131.1, 129.0, 128.7, 128.1, 127.0, 112.1, 111.6, 110.5, 105.0, 61.6 (OCH_2), 55.7 (OMe), 48.0 (NCH_2), 14.5 (Me); MS (ES mass): 396.10 (M+1); HPLC: 99.84%, Column: X Bridge C-18 150 * 4.6 mm, 5µm, mobile phase A: 0.1 % HCOOH in water, mobile phase B: CAN (T%B): 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; Flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); retention time 12.9 min.

*4.1.19. Ethyl-2-(3-(3-chloroquinoxalin-2-yl)-1H-indol-1-yl)acetate (**3u**)*

Yield: 70%; pale yellow solid; mp: 194-196 °C, R_f =0.25 (10% EtOAc/ *n*-hexane); ^1H NMR (400 MHz, DMSO-d_6) δ: 8.66 (d, *J* = 3.6 Hz, 2H, ArH), 8.17 (d, *J* = 8.4 Hz, 1H, ArH), 7.99 (d, *J* = 8.4 Hz, 1H, ArH), 7.84 (m, 2H, ArH), 7.53 (d, *J* = 7.2 Hz, 1H, ArH), 7.35-7.18 (m, 2H, ArH), 5.34 (s, 2H, NCH_2), 4.18 (m, 2H, OCH_2), 1.23 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 169.0 (C=O), 148.4, 144.7, 140.7, 138.7, 137.2, 135.1, 131.1, 130.1, 128.5, 128.0, 127.5, 123.4, 122.9, 121.9, 110.9, 110.9, 61.6 (OCH_2), 47.9 (NCH_2), 14.5 (CH_3); MS (ES mass): 366.10 (M+1); HPLC: 98.83%, Column: X Bridge C-18 150 * 4.6 mm, 5µm, mobile phase A: 0.1 % HCOOH in water, mobile phase B: ACN (T%B): 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; Flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); retention time 12.9 min.

*4.1.20. 2-(1-Allyl-5-fluoro-1H-indol-3-yl)-3-chloroquinoxaline (**3v**)*

Yield: 65%; pale yellow solid; mp: 119-121 °C, R_f =0.25 (10% EtOAc/ *n*-hexane); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.44 (dd, *J* = 10.37, 2.54 Hz, 1H, ArH), 8.37 (s, 1H, C-2 indolyl H), 8.17 (dd, *J* = 8.3, 0.9 Hz, 1H, ArH), 7.98 (dd, *J* = 8.2, 1.0 Hz, 1H, ArH), 7.74 (m, 2H, ArH), 7.32 (dd, *J* = 8.9, 4.4 Hz, 1H, ArH), 7.09 (m, 1H, ArH), 6.06 (m, 1H, =CH), 5.26 (m, 2H, $=CH_2$), 4.85 (d, *J* =5.6 Hz, 2H, NCH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.4, 158.01 (C-F *J* = 234.5 Hz), 148.0, 144.68, 140.9, 139.0 (C-F *J* = 192.5 Hz), 133.7, 133.0, 132.3, 130.2, 129.3, 128.4, 128.3, 127.8, 118.4, 111.6, 111.4, 111.2, 111.2 (C-F *J* = 4.5 Hz), 110.7, 110.6 (C-F *J* = 9.8 Hz), 108.4, 108.1 (C-F *J* = 26.2 Hz), 49.6 (NCH_2); MS (ES mass): 338.10 (M+1); HPLC: 99.88%, Column: X Bridge C-18 150 * 4.6 mm, 5µm, mobile phase A: 0.1 % HCOOH in water, mobile phase B: ACN (T%B): 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; Flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); retention time 12.5 min.

*4.1.21. 2-Chloro-3-(1-(3-chloro-3-methylbutyl)-1H-indol-3-yl)quinoxaline (**3w**)*

Yield: 38%; light yellow solid; mp: 208-210 °C; R_f = 0.40 (20% EtOAc-*n*-Hexane); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.69 (dd, *J* = 5.2, 2.0 Hz, 1H, ArH), 8.32 (s, 1H, C-2 indolyl H), 8.16 (dd, *J* = 7.2, 1.2 Hz, 1H, ArH), 7.99 (dd, *J* = 7.2, 1.2 Hz, 1H, ArH), 7.78-7.73 (m, 1H, ArH), 7.71-7.67 (m, 1H, ArH), 7.48 (dd, *J* = 5.6, 1.2 Hz, 1H, ArH), 7.39-7.31 (m, 2H, ArH), 4.55-4.51 (m, 2H, NCH_2), 2.36-2.32 (m, 2H, NCH_2-$C\underline{H}_2$), 1.70 (s, 6H, $(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.4, 145.0, 141.0, 139.0, 136.1, 132.1, 130.1, 129.2, 128.4, 127.8, 127.8, 123.2, 122.8, 121.7, 111.3, 109.6, 68.4 (C-Cl), 45.2 (NCH_2), 43.7 (CH_2), 32.6 (2C, 2Me); MS (ES mass): 384.1 (M+1); HPLC: 99.5%; Column: X BRIDGE C-18 150*4.6 mm, 5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/10, 3/10, 12/95, 23/95, 25/10, 30/10; flow rate: 1.0 mL/min; UV 190-800 nm, retention time 13.4 min.

*4.1.22. 2-(3-(3-Chloroquinoxalin-2-yl)-5-methoxy-1H-indol-1-yl)-N-phenylacetamide (**3x**)*

Yield: 45%; pale yellow solid; mp: 214-216 °C; R_f = 0.48 (30% EtOAc-*n*-Hexane); ^1H NMR (400 MHz, DMSO-d_6) δ: 10.45 (s, 1H, NH, D_2O exchangeable), 8.68 (s, 1H, C-2 indolyl H), 8.28 (d, *J* = 2.0 Hz, 1H, ArH), 8.16 (d, *J* = 8.4 Hz, 1H, ArH), 7.98 (d, *J* = 8.4 Hz, 1H, ArH), 7.86 (t, *J* = 7.2 Hz, 1H, ArH), 7.78 (t, *J* = 7.2 Hz, 1H, ArH), 7.60 (d, *J* = 8.0 Hz, 2H, ArH), 7.46 (d, *J* = 8.8 Hz, 1H, ArH), 7.31 (t, *J* = 7.6 Hz, 2H, ArH), 7.06 (t, *J* = 7.2 Hz, 1H, ArH), 6.94 (dd, *J* = 6.8, 2.0 Hz, 1H, ArH), 5.22 (s, 2H, NCH_2), 3.85 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.9 (C=O), 166.1, 155.6, 148.6, 144.5, 140.7, 139.5, 139.1, 138.5, 135.9, 132.4, 131.2, 129.9, 129.3, 128.4, 128.2, 128.0, 124.0, 119.6, 112.9, 111.5, 110.1, 105.1, 55.7 (OMe), 50.1 (NCH_2); MS (ES mass): 443.10 (M+1); HPLC: 99.6%; Column: X BRIDGE C-18 150*4.6 mm, 5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min; UV 190-800 nm, retention time 11.2 min.

*4.1.23. 2-(3-(3-Chloroquinoxalin-2-yl)-5-methoxy-1H-indol-1-yl)-1-morpholinoethanone (**3y**)*

Yield: 42%; pale yellow solid; mp: 232-234 °C; R_f = 0.42 (30% EtOAc-*n*-Hexane); ^1H NMR (400 MHz, $CDCl_3$) δ: 8.30 (s, 2H, C-2 indolyl H), 8.16-8.11 (m, 1H, ArH), 7.99 (dd, *J* = 7.2, 0.8 Hz, 1H, ArH), 7.79-7.66 (m, 2H, ArH), 7.25 (d, *J* = 7.6 Hz, 1H, ArH), 7.02 (d, *J* = 2.4 Hz, 1H, ArH), 5.02 (s, 2H, NCH_2), 3.94 (s, 3H, OCH_3), 3.73-3.61 (m, 6H, morpholine-O, NCH_2), 3.55-3.47 (m, 2H, morpholine-NCH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.0 (C=O), 155.8, 148.3, 144.9, 140.9, 139.0, 133.1, 131.9, 130.1, 129.3, 128.3, 128.3, 127.9, 113.6, 111.7, 110.0, 105.0, 66.7 (OCH_2), 66.3 (OCH_2), 55.8 (OMe), 48.5 (NCH_2), 45.6 (NCH_2), 42.6 (NCH_2C=O); MS (ES mass): 437.10 (M+1);

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HPLC: 99.3%; Column: X BRIDGE C-18 150*4.6 mm, 5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min; UV 190-800 nm, retention time 10.1 min.

*4.1.24. 2-(1-Allyl-5-methoxy-1H-indol-3-yl)quinoline-3-carbonitrile (**3z**)*

Yield: 65%; Off white solid; mp: 138-140 °C, R_f =0.23 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.06 (s, 1H, ArH), 8.39 (s, 1H, C-2 indolyl H), 8.24 (d, *J* = 2.0 Hz, 1H, ArH), 8.09 (d, *J* = 8.4 Hz, 1H, ArH), 8.01 (d, *J* = 8.0 Hz, 1H, ArH), 7.92 (t, *J* = 7.6 Hz, 1H, ArH), 7.63 (t, *J* = 7.6 Hz, 1H, ArH), 7.47 (d, *J* = 8.8 Hz, 1H, ArH), 6.93 (dd, *J* = 8.8, 2.4 Hz, 1H, ArH), 6.05 (m, 1H, =CH), 5.17 (dd, *J* = 31.7, 13.6 Hz, 2H, $=CH_2$), 4.95 (d, *J* = 5.2 Hz, 2H, NCH_2), 3.84 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 155.4, 152.9, 148.6, 145.6, 134.2, 133.5, 131.9, 131.7, 128.9, 128.7, 127.7, 127.2, 123.8, 119.4, 117.9, 113.0, 112.5, 111.8, 104.9, 103.2, 55.7 (OCH_3), 49.1 (NCH_2); MS (ES mass): 340.10 (M+1); HPLC: 99.40%, Column: X Bridge C-18 150 * 4.6 mm, 5µm, mobile phase A: 0.1 % HCOOH in water, mobile phase B: ACN (T%B): 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; Flow rate: 1.0 mL/min; UV 254.0 nm, Diluent: ACN: WATER (80:20); retention time 11.8 min.

*4.1.25. Ethyl-2-(3-(3-cyanopyridin-2-yl)-5-methoxy-1H-indol-1-yl)acetate (**4a**)*

Yield: 65%; Off white solid; mp: 112-114 °C, R_f =0.25 (20% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.87 (dd, *J* = 4.8, 1.84 Hz, 1H, ArH), 8.22 (s, 1H, C-2 indolyl H), 8.09 (d, *J* = 2.4 Hz, 1H, ArH), 8.05-7.84 (m, 1H, ArH), 7.23-7.08 (m, 2H, ArH), 6.98 (dd, *J* = 8.9, 2.4 Hz, 1H, ArH), 4.91 (s, 2H, NCH_2), 4.23 (m, 2H, OCH_2), 3.90 (s, 3H, OCH_3), 1.23 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.7 (C=O), 156.5, 155.7, 152.5, 141.7, 132.1, 131.5, 127.5, 119.0, 118.8, 113.5, 113.4, 110.0, 104.8, 104.2, 61.9 (OCH_2), 55.9 (OMe), 48.4 (NCH_2), 14.1 (CH_2-Me); MS (ES mass): 336.10 (M+1); HPLC: 99.90%, Column: X Bridge C-18 150 * 4.6 mm, 5µm, mobile phase A: 0.1 % HCOOH in water, mobile phase B: ACN (T%B): 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; Flow rate: 1.0 mL/min; UV 190-800 nm, Diluent: ACN: WATER (80:20); retention time 12.9 min.

*4.1.26. 2-(1-Allyl-5-chloro-1H-indol-3-yl)nicotinonitrile (**4b**)*

Yield: 70%; Off white solid; mp: 74-76 °C; R_f = 0.21 (10% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.92 (d, *J* = 2.8 Hz, 1H, ArH), 8.38 (d, *J* = 10.0 Hz, 2H, ArH), 8.32 (d, *J* = 7.6 Hz, 1H, ArH), 7.60 (d, *J* = 8.4 Hz, 1H, ArH), 7.39 (m, 1H, ArH), 7.31-7.25 (m, 1H, ArH), 6.09-5.99 (m, 1H, =CH), 5.23-5.08 (m, 2H, $=CH_2$), 4.98 (d, *J* = 4.8 Hz, 2H, NCH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 155.5, 153.9, 153.3, 144.5, 142.9, 133.9, 132.4, 127.7, 126.2, 123.8, 123.0, 121.8, 120.2, 118.1, 112.8, 104.1, 49.1 (NCH_2); MS (ES mass): 294.00 (M+1); HPLC: 97.54%, Column: X Bridge C-18 150 * 4.6 mm, 5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: ACN (T%B): 0/10, 3/10, 12/95, 23/95, 25/10, 30/10; Flow rate: 1.0 mL/min; UV 190-800 nm, Diluent: ACN: WATER (80:20); retention time 10.9 min.

*4.1.27. 2-(1-Allyl-5-methoxy-1H-indol-3-yl)nicotinonitrile (**4c**)*

Yield: 65%; Off white solid; mp: 96-98 °C; R_f =0.23 (10% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.89 (d, *J* = 3.2 Hz, 1H, ArH), 8.28 (dd, *J* = 14.4, 7.7 Hz, 2H, ArH), 7.88 (d, *J* = 2.0 Hz, 1H, ArH), 7.45 (d, *J* = 9.2 Hz, 1H, ArH), 7.34 (dd, *J* = 8.0, 5.2 Hz, 1H, ArH), 6.90 (dd, *J* = 8.8, 2.0 Hz, 1H, ArH), 6.03 (m, 1H, =CH), 5.20 (d, *J* = 10.0 Hz, 1H, $=CH_2$), 5.12 (d, *J* = 17.6 Hz, 1H, $=CH_2$), 4.92 (d, *J* = 5.12 Hz, 2H, NCH_2), 3.77 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 156.3, 155.3, 153.3, 142.8, 134.2, 131.9, 131.5, 127.3, 119.8, 119.3, 117.8, 112.8, 112.2, 111.8, 104.6, 103.9, 55.8 (OMe), 49.0 (NCH_2); MS (ES mass): 290.10 (M+1); HPLC: 97.55%, Column: X Bridge C-18 150 * 4.6 mm, 5µm, mobile phase A: 0.1 % HCOOH in water, mobile phase B: ACN (T%B): 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; Flow rate: 1.0 mL/min; UV 190-800 nm, Diluent: ACN: WATER (80:20); retention time 10.6 min.

*4.1.28. Synthesis of compound **7***

To a solution of **3t** or **4a** (1 equiv.) in THF (5 mL) was added LiOH (5 equiv) at the 0 ºC (ice bath) and the mixture was stirred for 15 min at the same temperature. The temperature of the reaction mixture was then allowed to reach room temp and stirring continued for 5-7 h. After completion of the reaction (TLC), the mixture was diluted with cold water (25 mL), washed with EtOAc (2 x 10 mL) and the aqueous layer was collected. The aqueous part was acidified (pH ~ 5-6) using dil HCl solution and the mixture was extracted with EtOAc (3 x 10 mL). The organic layers were collected, combined, washed with water (2 x 15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under low vacuum. The residue obtained was purified by using column chromatography over silica gel (hexane-EtOAc as eluent) to give the desired product.

*4.1.29. 2-(3-(3-Chloroquinoxalin-2-yl)-5-methoxy-1H-indol-1-yl)acetic acid (**7a**)*

Yield: 33%; pale yellow solid; mp: 223-225 °C; R_f = 0.42 (80% EtOAc-*n*-Hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.58 (s, 1H, C-2 indolyl H), 8.28 (d, *J* = 4.0 Hz, 1H, ArH), 8.13 (d, *J* = 8.4 Hz, 1H, ArH), 7.95 (d, *J* = 8.4 Hz, 1H, ArH), 7.86-7.81 (m, 1H, ArH), 7.77-7.72 (m, 1H, ArH), 7.34 (d, *J* = 8.8 Hz, 1H, ArH), 6.90-6.87 (m, 1H, ArH), 4.81 (s, 2H, NCH_2), 3.84 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 155.4 (C=O), 148.7, 144.4, 143.0, 140.7, 139.2, 138.3, 135.7, 132.4, 131.1, 129.6, 128.3, 127.9, 112.5, 111.8, 109.5, 104.9, 55.7 (2C, OMe & NCH_2); MS (ES mass): 368.0 (M+1); HPLC: 98.5%; Column: X BRIDGE C-18 150*4.6 mm, 5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min; UV 190-800 nm, retention time 10.1 min.

*4.1.30. 2-(3-(3-Cyanopyridin-2-yl)-5-methoxy-1H-indol-1-yl)acetic acid (**7b**)*

Yield: 75%; white solid; mp: 217-219 °C; R_f = 0.40 (80% EtOAc-*n*-Hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.61-12.72 (bs, 1H, OH, D_2O Exchangeable), 8.89 (dd, *J* = 2.8, 1.6 Hz, 1H, ArH), 8.28 (dd, *J* = 6.0, 2.0 Hz, 1H, ArH), 8.24 (s, 1H, C-2 indolyl H), 7.88 (d, *J* = 2.40 Hz, 1H, ArH), 7.39-7.33 (m, 2H, ArH), 6.89 (dd, *J* = 6.4, 2.8 Hz, 1H, ArH), 5.11 (s, 2H, NCH_2), 3.77 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.4 (C=O), 156.2, 155.2, 153.3, 142.9, 132.6, 132.5, 127.1, 119.8, 119.1, 112.7, 112.3, 111.6, 104.6, 103.8, 55.8 (OMe), 48.3 (NCH_2); MS (ES mass): 308.1 (M+1); HPLC: 99.0%; column: X BRIDGE C-18 150*4.6 mm, 5µm, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH_3CN (gradient) T/B%: 0/20, 3/20, 10/95, 23/95, 25/20, 30/20; flow rate: 1.0 mL/min; UV190-800 nm, retention time 8.5 min.

*4.1.31. Typical procedure for the preparation of (E)-alkyl 3-(3-(3-chloroquinoxalin-2-yl)-1-alkyl-5-substitited-1H-indol-2-yl)acrylate (**6k**)*

A mixture of 2-(5-bromo-1*H*-indol-3-yl)-3-chloroquinoxaline **3q** (0.280 mmol), ethyl acrylate **5a** (0.421 mmol), $Pd(OAc)_2$ (5 mol%), $Cu(OAc)_2$ (0.280 mmol), and TFA (0.336 mmol) in 1,4-dioxane (2.5 mL) was heated at 80 °C under open air for 7h. The progress of the reaction was monitored by TLC. After completion of the reaction, reaction mixture was cooled to RT, diluted with

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ethyl acetate (15 mL) and passed through celite. The resulting solution was washed with water (3 x 15 mL) followed by brine solution (25 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane to give desired compound **6k**.

*4.1.32. (E)-Ethyl-3-(3-(3-chloroquinoxalin-2-yl)-1-isopropyl-5-methoxy-1H-indol-2-yl)acrylate (**6a**)*

Compound **6a** was synthesized from **3i** following a procedure similar to that of compound **6k;** Yield: 79%; pale yellow solid; mp: 99-101 °C; R_f = 0.27 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11-8.08 (m, 1H, ArH), 8.05-8.02 (m, 1H, ArH), 7.76-7.69 (m, 4H, ArH), 7.43 (d, *J* = 8.8 Hz, 1H, ArH), 7.02 (d, *J* = 8.8 Hz, 1H, ArH), 6.17 (d, *J* = 16.0 Hz, 1H,=CH), 4.76-4.69 (m, 1H, NCH), 3.93 (s, 3H, OCH_3), 3.68 (q, *J* = 7.2 Hz, 2H, OCH_2), 1.62 (s, 3H, CH_3), 1.60 (s, 3H, CH3), 0.73 (t, *J* = 7.2 Hz, 3H, CH_2-C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.9 (C=O), 154.0, 150.4, 148.2, 140.7, 140.5, 139.2, 131.7, 130.0 (2C), 128.9, 128.3, 128.0, 126.5, 121.3, 115.7, 112.1, 111.7, 108.2, 59.5 (OCH_2), 56.6 (OMe), 47.7 (NCH), 22.7 (2C, 2Me) , 13.7 (OCH_2<u>Me</u>); MS (ES mass): 450.0 (M+1); HPLC: 99.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 210.0 nm, retention time 4.0 min.

*4.1.33. (E)-Methyl-3-(3-(3-chloroquinoxalin-2-yl)-1-isopropyl-5-methoxy-1H-indol-2-yl)acrylate (**6b**)*

Compound **6b** was synthesized from **3i** following a procedure similar to that of compound **6k;** Yield: 83%; pale yellow solid; mp: 110-112 °C; R_f = 0.28 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.10-8.04 (m, 2H, ArH), 7.78-7.67 (m, 4H, ArH), 7.44 (d, *J* = 8.8 Hz, 1H, ArH), 7.02 (d, *J* = 8.8 Hz, 1H, ArH), 6.19 (d, *J* = 16.0 Hz, 1H, =CH), 4.80-4.68 (m, 1H, NCH), 3.92 (s, 3H, OCH_3), 3.15 (s, 3H, Ester CH_3), 1.62 (s, 3H, CH_3), 1.60 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.4 (C=O), 154.2, 150.4, 148.2, 140.7, 140.4, 139.5, 131.6, 130.1, 128.9, 128.2, 127.9, 126.7, 120.7, 115.4, 112.0, 111.8, 110.5, 108.2, 56.6 (OMe), 50.8 (Ester O<u>Me</u>), 47.7 (NCH), 22.7 (2C, 2Me); MS (ES mass): 436.0 (M+1); HPLC: 98.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 210.0 nm, retention time 3.9 min.

*4.1.34. (E)-Ethyl-3-(3-(3-chloroquinoxalin-2-yl)-1-isopropyl-1H-indol-2-yl)acrylate (**6c**)*

Compound **6c** was synthesized from **3g** following a procedure similar to that of compound **6k;** Yield: 80%; light yellow solid; mp: 142-144 °C; R_f = 0.25 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09-8.04 (m, 2H, ArH), 7.83-7.75 (m, 4H, ArH), 7.51 (d, *J* = 8.0 Hz, 1H, ArH), 7.45 (d, *J* = 7.2 Hz, 1H, ArH), 7.32 (t, *J* = 7.6 Hz, 1H, ArH), 6.23 (d, *J* = 15.6 Hz, 1H, =CH), 4.83-4.76 (m, 1H, NCH), 3.85 (q, *J* = 7.2 Hz, 2H, OCH_2), 1.63 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 0.83 (t, *J* = 7.2 Hz, 3H, OCH_2-C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.5 (C=O), 149.7, 147.6, 143.6, 140.7, 140.3, 136.5, 130.2 (2C), 128.9, 128.5, 128.1, 128.0, 127.9, 125.6, 122.4, 120.1, 118.3, 111.5, 59.9 (OCH_2), 47.7 (NCH), 22.7 (2C, 2Me), 13.7 (OCH_2<u>Me</u>); MS (ES mass): 420.2 (M+1); HPLC: 99.4%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.0 nm, retention time 4.2 min.

*4.1.35. (E)-Methyl-3-(3-(3-chloroquinoxalin-2-yl)-1-isopropyl-1H-indol-2-yl)acrylate (**6d**)*

Compound **6d** was synthesized from **3g** following a procedure similar to that of compound **6k;** Yield: 77%; light yellow solid; mp: 130-132 °C; R_f = 0.24 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.08-8.05 (m, 2H, ArH), 7.87-7.76 (m, 4H, ArH), 7.52 (d, *J* = 8.4 Hz, 1H, ArH), 7.44 (d, *J* = 7.2 Hz, 1H, ArH), 7.32 (t, *J* = 7.6 Hz, 1H, ArH), 6.22 (d, *J* = 16.0 Hz, 1H, =CH), 4.83-4.76 (m, 1H, NCH), 3.34 (s, 3H, OCH_3), 1.63 (m, 3H, CH_3), 1.62 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.9 (C=O), 149.5, 147.4, 144.1, 140.6, 140.3, 136.5, 130.1 (2C), 128.8, 128.4, 128.0 (2C), 125.6, 122.4, 120.0, 117.7, 111.9, 111.6, 51.0 (OMe), 47.7 (NCH), 22.7 (2C, 2Me); MS (ES mass): 406.2 (M+1); HPLC: 99.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.0 nm, retention time 4.0 min.

*4.1.36. (E)-Ethyl-3-(1-allyl-3-(3-chloroquinoxalin-2-yl)-5-methoxy-1H-indol-2-yl)acrylate (**6e**)*

Compound **6e** was synthesized from **3b** following a procedure similar to that of compound **6k;** Yield: 72%; light yellow solid; mp: 102-104 °C; R_f = 0.28 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11-8.04 (m, 2H, ArH), 7.77-7.70 (m, 3H, ArH), 7.58 (s, 1H, ArH), 7.38 (d, *J* = 8.8 Hz, 1H, ArH), 7.02 (d, *J* = 8.8 Hz, 1H, ArH), 6.14 (d, *J* = 16.4 Hz, 1H, =CH), 6.12-6.00 (m, 1H, =CH allyl), 5.30 (d, *J* = 10.0 Hz, 1H, $=CH_2$), 5.24 (d, *J* = 17.6 Hz, 1H, $=CH_2$), 4.81 (s, 2H, NCH_2), 3.93 (s, 3H, OCH_3), 3.69 (q, *J* = 7.2 Hz, 2H, OCH_2), 0.73 (t, *J* = 7.2 Hz, 3H, CH_2-C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.9 (C=O), 154.2, 150.2, 148.2, 140.7, 140.5, 139.0, 132.5, 132.1, 132.0, 130.2, 130.1, 128.9, 128.0, 126.6, 121.4, 118.3, 115.7, 112.4, 111.9, 108.4, 59.6 (OCH_2), 56.6 (OMe), 49.3 (NCH_2), 13.7 (CH_2-<u>Me</u>); MS (ES mass): 448.2 (M+1); HPLC: 97.7%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.0 nm, retention time 3.9 min.

*4.1.37. (E)-Methyl-3-(5-bromo-3-(3-chloroquinoxalin-2-yl)-1-methyl-1H-indol-2-yl)acrylate (**6f**)*

Compound **6f** was synthesized from **3k** following a procedure similar to that of compound **6k;** Yield: 75%; pale yellow solid; mp: 126-128 °C; R_f = 0.25 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.18-8.16 (m, 1H, ArH), 8.13-8.10 (m, 1H, ArH), 7.88-7.80 (m, 3H, ArH), 7.60 (s, 1H, ArH), 7.44-7.43 (m, 1H, ArH), 7.31 (d, *J* = 8.8 Hz, 1H, ArH), 6.02 (d, *J* = 16.0 Hz, 1H, =CH), 3.94 (s, 3H, NCH_3), 3.72 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.4 (C=O), 148.1, 147.6, 141.1, 141.0, 136.9, 134.4, 132.0, 131.2, 130.6, 129.2, 128.4, 128.2, 127.4, 122.8, 122.0, 114.8, 114.2, 111.4, 51.9 (OMe), 31.4 (NMe); MS (ES mass): 458.1 (M+3); HPLC: 97.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 254.0 nm, retention time 4.2 min.

*4.1.38. (E)-Ethyl-3-(5-bromo-3-(3-chloroquinoxalin-2-yl)-1-methyl-1H-indol-2-yl)acrylate (**6g**)*

Compound **6g** was synthesized from **3k** following a procedure similar to that of compound **6k;** Yield: 66%; pale yellow solid; mp: 125-127 °C; R_f = 0.26 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.18-8.16 (m, 1H, ArH), 8.13-8.10 (m, 1H, ArH), 7.87-7.79 (m, 3H, ArH), 7.60 (s, 1H, ArH), 7.44 (dd, *J* = 8.8, 1.2 Hz, 1H, ArH), 7.31 (d, *J* = 8.8 Hz, 1H, ArH), 6.03 (d, *J* = 16.0 Hz, 1H, =CH), 4.18 (q, *J* = 7.2 Hz, 2H, OCH_2), 3.95 (s,

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3H, NCH_3), 1.25 (t, *J* = 7.2 Hz, 3H, CH_2-$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.0 (C=O), 148.1, 147.6, 141.1 (2C), 136.9, 134.5, 131.8, 131.1, 130.6, 129.2, 128.2, 127.3, 123.1, 122.8, 122.5, 114.7, 114.2, 111.4, 60.8 (OCH_2), 29.6 (NMe), 14.1 (CH_2-$\underline{Me}$); MS (ES mass): 469.8 (M-1); HPLC: 97.6%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 210.0 nm, retention time 4.4 min.

*4.1.39. (E)-Methyl-3-(3-(3-chloroquinoxalin-2-yl)-1-methyl-1H-indol-2-yl)acrylate (**6h**)*

Compound **6h** was synthesized from **3l** following a procedure similar to that of compound **6k;** Yield: 69%; pale yellow solid; mp: 189-191 °C; R_f = 0.24 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.17-8.15 (m, 1H, ArH), 8.13-8.10 (m, 1H, ArH), 7.87-7.82 (m, 3H, ArH), 7.46 (d, *J* = 8.0 Hz, 1H, ArH), 7.37 (d, *J* = 8.4 Hz, 1H, ArH), 7.38-7.34 (m, 1H, ArH),7.18 (t, *J* = 8.0 Hz, 1H, ArH), 6.12 (d, *J* = 16.2 Hz, 1H, =CH), 3.97 (s, 3H, NCH_3), 3.72 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.7 (C=O), 148.9, 147.9, 141.1, 141.0, 138.4, 133.4, 132.5, 131.0, 130.5, 129.2, 128.2, 126.9, 124.6, 121.4, 120.9, 120.5, 115.2, 109.9, 51.8 (OMe), 30.9 (NMe); MS (ES mass): 378.0 (M+1); HPLC: 97.7%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 245.0 nm, retention time 3.9 min.

*4.1.40. (E)-tert-Butyl-3-(3-(3-chloroquinoxalin-2-yl)-1-methyl-1H-indol-2-yl)acrylate (**6i**)*

Compound **6i** was synthesized from **3l** following a procedure similar to that of compound **6k;** Yield: 64%; light yellow solid; mp: 123-125 °C; R_f = 0.26 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.17-8.10 (m, 2H, ArH), 7.83-7.81 (m, 2H, ArH), 7.75 (d, *J* = 16.0 Hz, 1H, =CH), 7.48 (d, *J* = 7.6 Hz, 1H, ArH), 7.42 (s, 1H, ArH), 7.39-7.33 (m, 1H, ArH), 7.18 (s, 1H, ArH), 6.07 (d, *J* = 16.0 Hz, 1H, =CH), 3.97 (s, 3H, NCH_3), 1.44 (s, 9H, $(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.6 (C=O), 148.9, 147.9, 141.1, 140.9, 138.4, 133.8, 131.3, 130.8, 130.4, 129.1, 128.2, 127.9, 126.9, 124.4, 123.2, 121.3, 120.5, 109.9, 80.9 ($O\underline{C}Me_3$), 31.5 (NMe), 28.0 (3C, 3Me); MS (ES mass): 420.2 (M+1); HPLC: 98.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 270.0 nm, retention time 3.4 min.

*4.1.41. (E)-Methyl-3-(5-chloro-3-(3-chloroquinoxalin-2-yl)-1H-indol-2-yl)acrylate (**6j**)*

Compound **6j** was synthesized from **3p** following a procedure similar to that of compound **6k;** Yield: 70%; light yellow solid; mp: 156-158 °C; R_f = 0.28 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.69 (s, 1H, NH, D_2O exchangeable), 8.24-8.18 (m, 1H, ArH), 8.16-8.14 (m, 1H, ArH), 7.90-7.86 (m, 2H, ArH), 7.67 (d, *J* = 16.4 Hz, 1H, =CH), 7.56 (s, 1H, ArH), 7.41 (d, *J* = 8.8 Hz, 1H, ArH), 7.32 (d, *J* = 9.2 Hz, 1H, ArH), 6.35 (d, *J* = 16.4 Hz, 1H, =CH), 3.79 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.7 (C=O), 147.8, 147.9, 140.9, 140.8, 135.7, 134.6, 132.8, 131.8, 131.4, 129.3, 128.3, 128.2, 125.6, 125.2, 120.5, 118.8, 116.4, 113.9, 52.1 (OMe); MS (ES mass): 398.1 (M+1); HPLC: 98.0%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 270.0 nm, retention time 4.4 min.

*4.1.42. (E)-tert-Butyl-3-(5-chloro-3-(3-chloroquinoxalin-2-yl)-1H-indol-2-yl)acrylate (**6k**)*

Yield: 81%; light yellow solid; mp: 129-131 °C; R_f = 0.24 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.75 (s, 1H, NH, D_2O exchangeable), 8.19-8.16 (m, 1H, ArH), 8.13-8.11 (m, 1H, ArH), 7.87-7.83 (m, 2H, ArH), 7.59 (d, *J* = 16.4 Hz, 1H, =CH), 7.54 (s, 1H, ArH), 7.38 (d, *J* = 8.8 Hz, 1H, ArH), 7.30 (d, *J* = 9.2 Hz, 1H, ArH), 6.29 (d, *J* = 16.4 Hz, 1H, =CH), 1.48 (s, 9H, $(CH_3)_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.5 (C=O), 147.9, 147.6, 140.9, 140.8, 135.6, 134.9, 132.0, 131.7, 131.3, 129.3, 128.3, 128.1, 125.5, 125.0, 121.0, 120.6, 116.1, 113.9, 80.6 ($O\underline{C}(Me)_3$), 28.1 (3C, $(Me)_3$); MS (ES mass): 438.2 (M-1); HPLC: 95.5%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 240.0 nm, retention time 4.4 min.

*4.1.43. (E)-Ethyl-3-(5-bromo-3-(3-chloroquinoxalin-2-yl)-1H-indol-2-yl)acrylate (**6l**)*

Compound **6l** was synthesized from **3q** following a procedure similar to that of compound **6k;** Yield: 73%; pale yellow solid; mp: 136-138 °C; R_f = 0.25 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.35 (s, 1H, NH, D_2O exchangeable), 8.17-8.11 (m, 2H, ArH), 7.94 (d, *J* = 3.6 Hz, 2H, ArH), 7.76 (s, 1H, ArH), 7.54-7.39 (m, 3H, ArH), 6.70 (d, *J* = 16.4 Hz, 1H, =CH), 4.13 (q, *J* = 6.8 Hz, 2H, OCH2), 1.20-1.17 (m, 3H, CH_2-$C\underline{H_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.2 (C=O), 147.8, 147.6, 140.5 (2C), 135.9, 134.5, 132.7, 131.8, 131.3, 129.3, 128.9, 128.3, 127.6, 123.5, 119.3, 116.1, 114.3, 113.5, 60.7 (OCH2), 14.5 (CH_2-$\underline{Me}$); MS (ES mass): 458.1 (M+2); HPLC: 97.9%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 245.0 nm, retention time 5.0 min.

*4.1.44. (E)-Methyl-3-(5-bromo-3-(3-chloroquinoxalin-2-yl)-1H-indol-2-yl)acrylate (**6m**)*

Compound **6m** was synthesized from **3q** following a procedure similar to that of compound **6k;** Yield: 77%; light yellow solid; mp: 291-293 °C; R_f = 0.25 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.72 (s, 1H, NH, D_2O exchangeable), 8.20-8.17 (m, 1H, ArH), 8.14-8.12 (m, 1H, ArH), 7.89-7.82 (m, 2H, ArH), 7.70 (s, 1H, ArH), 7.65 (d, *J* = 16.0 Hz, 1H, =CH), 7.43 (dd, *J* = 8.8, 2.0 Hz, 1H, ArH), 7.35 (d, *J* = 8.8 Hz, 1H, ArH), 6.31 (d, *J* = 16.0 Hz, 1H, =CH), 3.77 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.7 (C=O), 147.8, 147.7, 141.0, 140.9, 135.9, 134.4, 132.8, 131.8, 131.3, 129.3, 128.9, 128.3, 127.6, 123.6, 118.9, 116.3, 114.3, 113.5, 52.1 (OMe); MS (ES mass): 444.1 (M+3); HPLC: 95.2%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 245.0 nm, retention time 3.7 min.

*4.1.45. (E)-tert-Butyl-3-(5-bromo-3-(3-chloroquinoxalin-2-yl)-1H-indol-2-yl)acrylate (**6n**)*

Compound **6n** was synthesized from **3q** following a procedure similar to that of compound **6k;** Yield: 80%; light yellow solid; mp: 122-124 °C; R_f = 0.26 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1H, NH, D_2O exchangeable), 8.19-8.17 (m, 1H, ArH), 8.13-8.10 (m, 1H, ArH), 7.87-7.81 (m, 2H, ArH), 7.70 (s, 1H, ArH), 7.59 (d, *J* = 16.0 Hz, 1H, =CH), 7.42 (dd, *J* = 8.8, 1.2 Hz, 1H, ArH), 7.33 (d, *J* = 8.8 Hz, 1H, ArH), 6.29 (d, *J* = 16.0 Hz, 1H, =CH), 1.48 (s, 9H, $(CH_3)_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 165.5 (C=O), 147.9, 147.6, 140.9,

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140.8, 135.9, 134.7, 131.9, 131.7, 131.3, 129.3, 128.8, 128.3, 127.4, 123.6, 121.0, 115.9, 114.2, 113.4, 80.6 (O<u>C</u>Me$_3$), 28.1 (3C, Me$_3$); MS (ES mass): 484.2 (M+1); HPLC: 98.0%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 240.0 nm, retention time 4.5 min.

*4.1.46. (E)-Ethyl-3-(3-(3-chloroquinoxalin-2-yl)-5-methyl-1H-indol-2-yl)acrylate (**6o**)*

Compound **6o** was synthesized from **3r** following a procedure similar to that of compound **6k;** Yield: 66%; light yellow solid; mp: 129-131 °C; R_f = 0.27 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.55 (s, 1H, NH, D_2O exchangeable), 8.22-8.19 (m, 1H, ArH), 8.15-8.13 (m, 1H, ArH), 7.88-7.82 (m, 2H, ArH), 7.68 (d, *J* = 16.0 Hz, 1H, =CH), 7.36 (d, *J* = 8.8 Hz, 2H, ArH), 7.19 (d, *J* = 8.8 Hz, 1H, ArH), 6.29 (d, *J* = 16.0 Hz, 1H, =CH), 4.24 (q, *J* = 7.2 Hz, 2H, OCH_2), 2.43 (s, 3H, CH_3), 1.31 (t, *J* = 7.2 Hz, 3H, CH_2-C<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.3 (C=O), 148.1, 147.8, 141.0 (2C), 135.0, 132.5, 132.4, 130.9 (2C), 130.4, 129.2, 128.1, 127.7, 127.2, 120.5, 117.4, 117.1, 111.0, 60.6 (OCH_2), 29.6 (Me), 14.2 (CH_2-<u>Me</u>); MS (ES mass): 392.1 (M+1); HPLC: 97.3%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 10/95, 10.5/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (80:20); UV 210.0 nm, retention time 3.4 min.

*4.1.47. (E)-Ethyl-3-(3-(3-chloroquinoxalin-2-yl)-1H-indol-2-yl)acrylate (**6p**)*

Compound **6p** was synthesized from **3s** following a procedure similar to that of compound **6k;** Yield: 69%; light yellow solid; mp: 127-129 °C; R_f = 0.28 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.19 (s, 1H, NH, D_2O exchangeable), 8.17-8.12 (m, 2H, ArH), 7.95-7.93 (m, 2H, ArH), 7.57-7.49 (m, 3H, ArH), 7.30 (t, *J* = 7.2 Hz, 1H, ArH), 7.10 (d, *J* = 7.2 Hz, 1H, ArH), 6.73 (d, *J* = 16.0 Hz, 1H, =CH), 4.13 (q, *J* = 7.2 Hz, 2H, OCH_2), 1.20 (t, *J* = 7.2 Hz, 3H, CH_2-C<u>H</u>$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.4 (C=O), 148.4, 147.6, 140.9, 140.7, 137.2, 135.9, 134.5, 133.3, 129.3, 128.3, 127.1, 125.1, 121.3, 118.3, 116.9, 116.1, 113.5, 112.2, 60.5 (OCH_2), 14.5 (CH_2-<u>Me</u>); MS (ES mass): 377.2 (M^+); HPLC: 96.1%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 245.0 nm, retention time 4.0 min.

*4.1.48. (E)-Methyl-3-(3-(3-chloroquinoxalin-2-yl)-1H-indol-2-yl)acrylate (**6q**)*

Compound **6q** was synthesized from **3s** following a procedure similar to that of compound **6k;** Yield: 73%; light yellow solid; mp: 172-174 °C; R_f = 0.26 (10% EtOAc/ *n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.76 (s, 1H, NH, D_2O exchangeable), 8.20-8.17 (m, 1H, ArH), 8.14-8.11 (m, 1H, ArH), 7.87-7.80 (m, 2H, ArH), 7.70 (d, *J* = 16.4 Hz, 1H, =CH), 7.55 (d, *J* = 8.4 Hz, 1H, ArH), 7.46 (d, *J* = 8.0 Hz, 1H, ArH), 7.35 (t, *J* = 8.0 Hz, 1H, ArH), 7.19 (t, *J* = 8.0 Hz, 1H, ArH), 6.35 (d, *J* = 16.4 Hz, 1H, =CH), 3.76 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.8 (C=O), 148.4, 147.7, 140.9, 140.7, 137.3, 133.3, 133.2, 131.7, 131.3, 129.3, 128.3, 127.2, 125.1, 121.3, 121.0, 117.9, 117.0, 112.3, 52.0 (OMe); MS (ES mass): 364.1 (M+1); HPLC: 99.8%, Column: Symmetry C-18 75 * 4.6 mm, 3.5µm, mobile phase A: 0.1 % TFA in water, mobile phase B: CH_3CN (T/%B): 0/20, 0.5/20, 2/95, 8/95, 10/20, 12/20; flow rate: 1.0 mL/min; Diluent: ACN: WATER (90:10); UV 205.0 nm, retention time 3.5 min.

5. Pharmacology

5.1. In vitro assay for PDE4B

5.1.1. Cells and Reagents

The Sf9 cells were obtained from ATCC (Washington D.C., 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

PDE4B 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. *Sf*9 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 in a literature [21]. Briefly, lysate was centrifuged at 10,000 rpm for 10 min at 4 ºC 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 hour at 4˚C. After incubation, lysate-Ni-NTA mixture was centrifuged at 4,500 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. PDE4B enzymatic assay

The inhibition of PDE4B enzyme was measured using PDElight HTS cAMP phosphodiesterase assay kit (Lonza) according to manufacturer's recommendations. Briefly, 10 ng of PDE4B 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 followed by incubation with detection reagent for 10 minutes in dark. Luminescence values (RLUs) were measured by a Multilabel plate reader (Perkin Elmer 1420 Multilabel counter). The percentage of inhibition was calculated using the following formula and IC_{50}s were computed using GraphPad Prism Version 5.04 software.

$$\%\ inhibition = \frac{(RLU\ of\ vehicle\ control - RLU\ of\ inhibitor)}{RLU\ of\ vehicle\ control} X\ 100$$

5.1.4. PDE4D and C enzymatic assay

This assay was performed following a similar method as described above using 0.5 ng commercially procured PDE4D2 or PDE4C enzyme instead of 10 ng of in house purified PDE4B without changing any other factors or conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at xxxxxxxxx

Detailed tables and schemes and details of experimental procedures for (i) docking studies, and (ii) *in vitro* and *in vivo* experiments (file type: word).

Copies of spectra of all target compounds synthesized (file type: PDF).

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Highlights

- 2-(1*H*-Indol-3-yl)-quinoxalines were designed as a new class of PDE4 inhibitors.
- Synthesis involved $InCl_3$ mediated heteroarylation/Pd(II)-catalyzed C-H activation.
- Compound **3b** (PDE4B IC_{50} ~ 0.39 μM) showed PDEs family/PDE4 subtype selectivity.
- **3b** halted progression of the disease in Zebrafish EAE model of multiple sclerosis
- **3b** also showed promising and benefecial effects in adjuvant induced arthritic rats.

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