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Der Pharma Chemica, 2015, 7(2):77-85
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Biological activity of drug like small molecules based on quinoxaline containing amino substitution at C-2

K. Raghavendra Rao[a,b], Akula Raghunadh[a], Dipak Kalita[a], Eppakayala Laxminarayana[b], Manojit Pal[c] and Suresh Babu Meruva*[a]

[a]*Technology Development Centre, Custom Pharmaceutical Services, Dr. Reddy's Laboratories Ltd, Miyapur, Hyderabad, India*
[b]*Department of Physics and Chemistry, Mahatma Gandhi Institute of Technology, Chaitanya Bharati, Gandipet, Hyderabad, A.P, India*
[c]*Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad, India*

ABSTRACT

A series of novel "drug like" small molecules based quinoxaline containing amino substitution at C-2were synthesized. The strategy used is simple, efficient and afforded good yields of quinoxaline derivatives. The biological activity of these quinoxaline derivatives for PDE4B enzyme inhibition with reference to rolipram have been reported.

Keywords: quinoxaline, Rolipram, PDE4B enzyme.

INTRODUCTION

Quinoxaline derivatives possessing a piperizine ring at C-2 have shown remarkable 5-HT1A agonistic and 5-HT3 antagonistic activities as is exemplified by the discovery of TZB-30878 (**A**) as an orally bioavailable agent for irritable bowel syndrome (Fig. 1) [1]. 2-Amino substituted quinoxalines (**B**, Fig. 1) on the other hand have been reported as interleukin-8 (IL-8) receptor antagonists for the potential treatment of chemokine-mediated diseases [2]. These observations and our interest on quinoxalines [3-7] prompted us to establish the biological activity of novel quinoxaline derivatives (**3**, **4**,**5** and **6**) containing amino substitution at C-2 (Fig. 2). The key intermediate (**7**) required for the synthesis of these derivatives has been established as per the literature conditions (Scheme 1). In continuation of our interest in bioactive molecules [8-16] we now report our results on the biological activity for the compounds generated based on the scaffolds **3-6**which were synthesized by using efficient chemistry methodologies (Scheme 2).

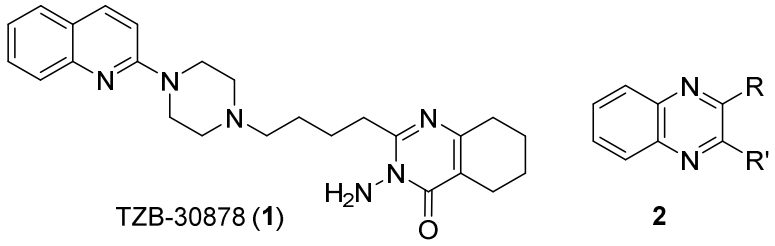


Fig. 1. Examples of biologically active 2-amino substituted quinoline / quinoxaline derivatives

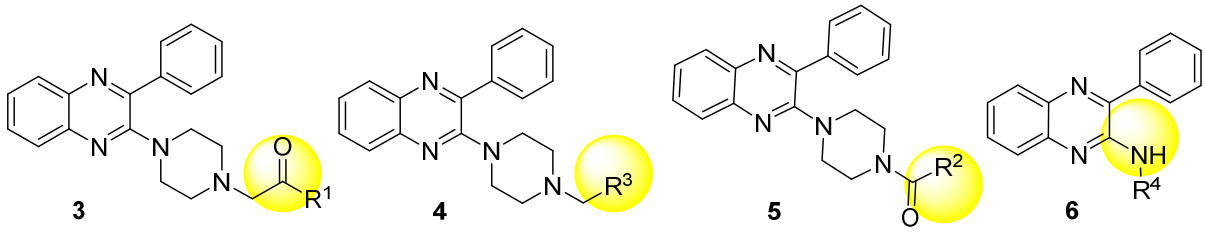
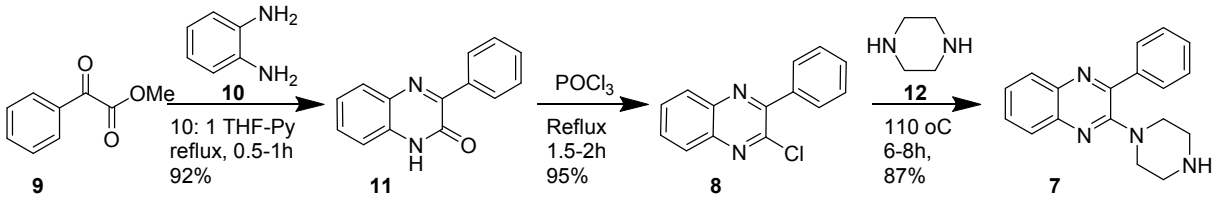
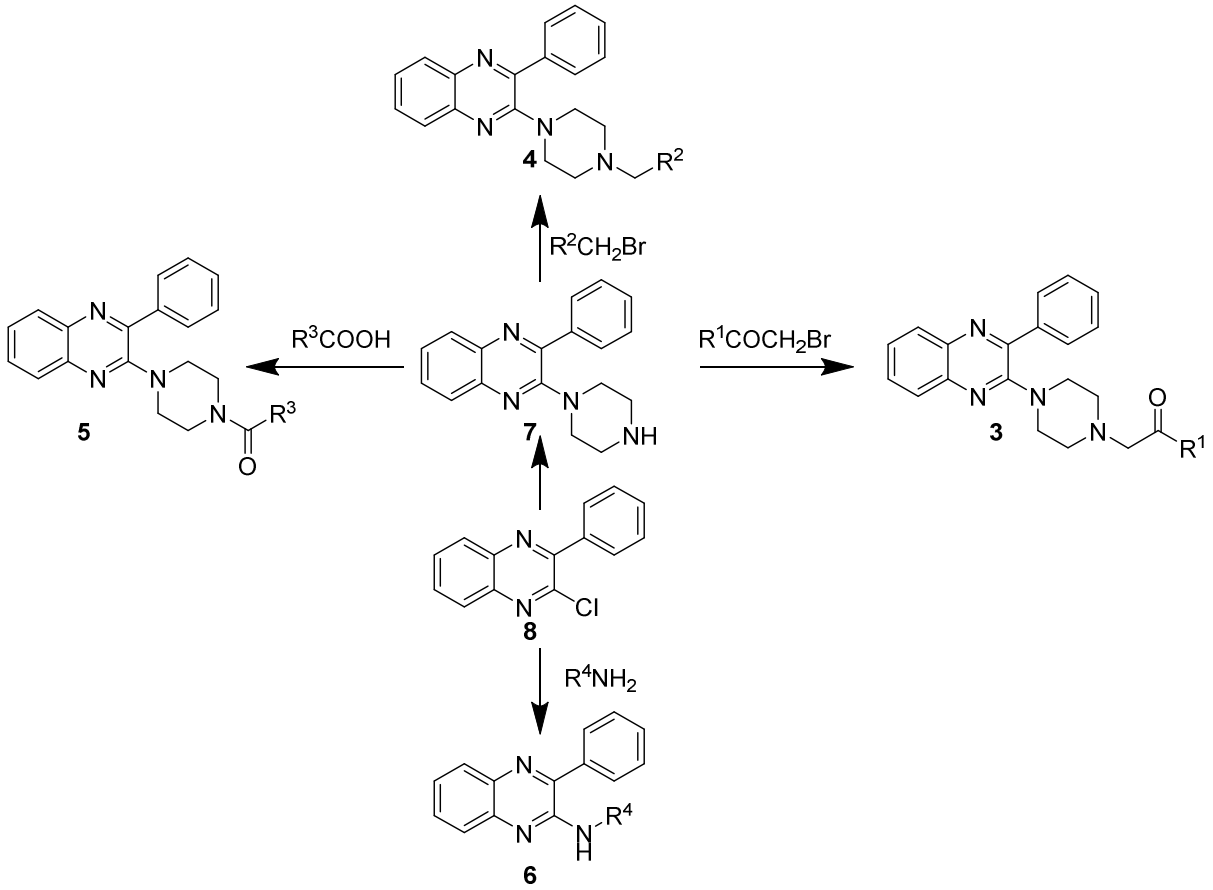


Fig. 2. Novel quinoxaline derivatives (C-F) containing amino substitution at C-2



Scheme1.Synthesisof 7



Scheme-2. Synthesis of compounds 3-6

RESULTS AND DISCUSSION

The methodology utilized for the synthesis of these scaffolds have been recently reported.[17] In further to that we intended to study the biological importance of these compounds. Therefore herewith we have reported the biological activityof substituted quinoxaline derivatives. The synthetic outcome of our methodology resulted in four different substituted 2-amino quinoxalines such as compounds from **13** to **18** are 4-phenacyl derivative of 2-piperzine quinoxalines. Similarly another sequence of derivatives are synthesized with amide functionality, compounds **19** to **22** such as acyclic, substituted phenyl and furan derivatives. In addition to that further synthesized substituted phenyl (**23** to **25**) and benzyl derivatives (**26** to **31**) of 2-amino quinoxalines in overall excellent yields.

PDE4B protein production and purification

PDE4B1 cDNA was sub-cloned into pFAST Bac HTB vector (Invitrogen) and transformed into DH10Bac (Invitrogen) competent cells. Recombinant bacmids were tested for integration by PCR analysis. Sf9 cells were transfected with bacmid using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. Subsequently, P3 viral titer was amplified, cells were infected and 48 h post infection cells were lysed in lysis buffer (50 mM Tris-HCl pH 8.5, 10 mM 2-mercaptoethanol, 1 % protease inhibitor cocktail (Roche), 1 % NP40). Recombinant His-tagged PDE4B protein was purified as previously described elsewhere. 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 ºC. After incubation, lysate-Ni-NTA mixture was centrifuged at 4,500 rpm for 5 min at 4 ºC 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 ºC in 50% glycerol until further use.

PDE4 enzymatic assay

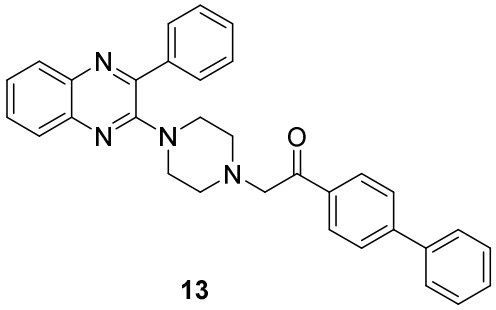
The inhibition of PDE4 enzyme was measured using PDElight HTS cAMP phosphodiesterase assay kit (Lonza) according to manufacturer's recommendations. Briefly, 10 ng of in house purified PDE4B1 or 0.5 ng commercially procured PDE4D2 enzyme was pre-incubated either with DMSO (vehicle control) or compound for 15 min before incubation with the substrate cAMP (5 µM) for 1 hour. The reaction was halted with stock solution and reaction mix was incubated with detection reagent for 10 minutes in dark. Dose response studies were performed at 13 different concentrations ranging from 200 µM to 0.001 µM. Luminescence values (RLUs) were measured by a Multilabel Plate Reader (PerklinElmer 1420 Multilabel Counter). The percentage of inhibition was calculated using the following formula and the IC_{50} values were determined by a nonlinear regression analysis from dose response curve using Graphpad Prism software (San Diego, U.S.A). IC_{50} values are presented as mean ± SD.

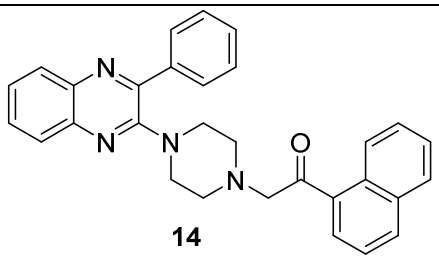
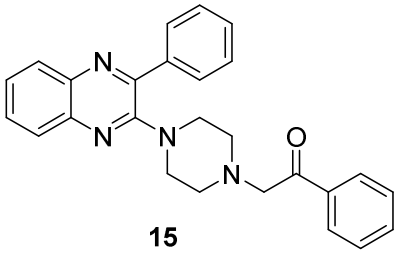
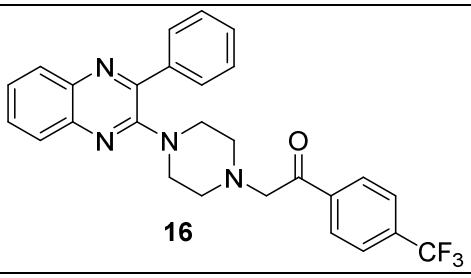
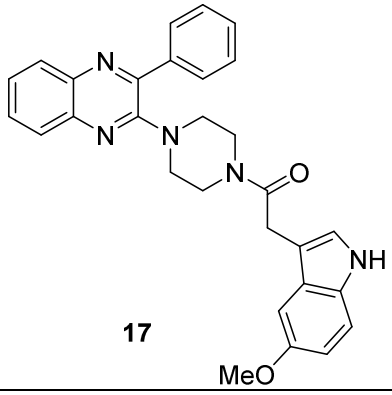
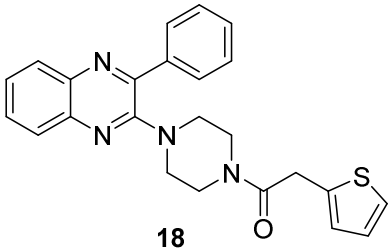
$$\%\ inhibition = \frac{(RLU of vehicle control - RLU of inhibitior)}{RLU of vehicle control} X\ 100$$

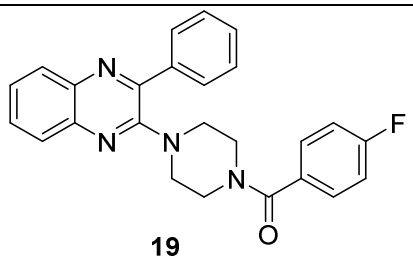
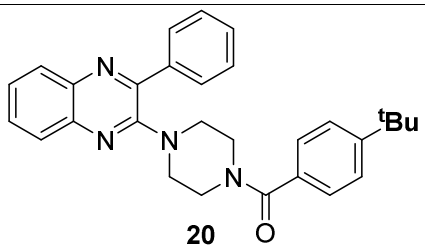
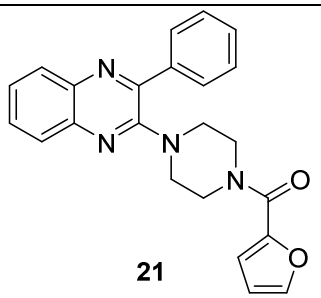
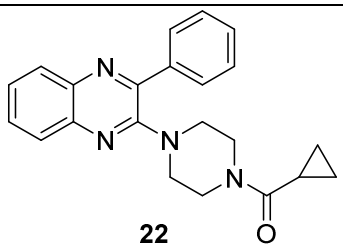
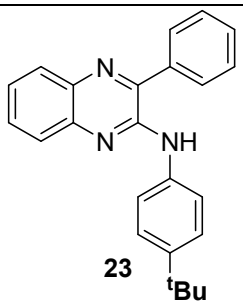
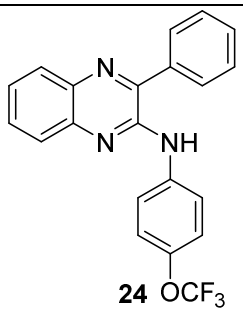
The synthesized substituted quinoxaline derivatives were tested for their PDE4B inhibitory potential *in vitro* at 30 μM using PDE4B enzyme and rolipram as a reference compound. Rolipram, a selective phosphodiesterase-4 inhibitor is a potential antidepressant drug in the early 1990s.[18] It served as a prototype molecule for several companies' drug discovery and development efforts.[19] Rolipram was discontinued after clinical trials, it showed that its therapeutic range was too narrow; it could not be dosed at high enough levels to be effective without causing significant gastrointestinal side effects. It continues to be used in research as a well-characterized PDE4 inhibitor. It has been used in studies to understand whether PDE4 enzyme inhibition could be useful in autoimmune diseases.[20]

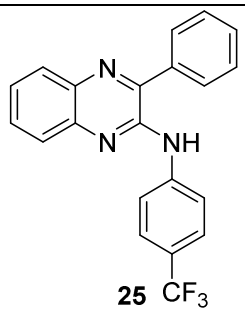
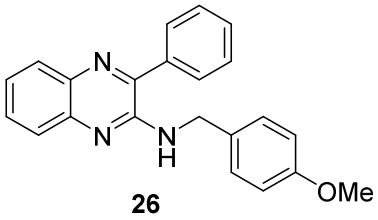
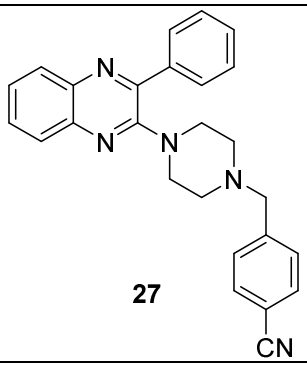
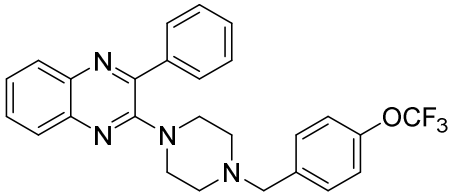
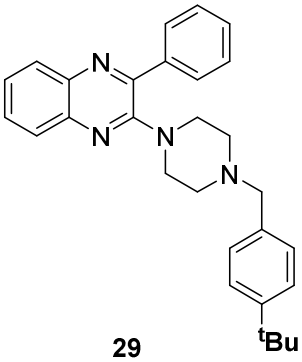
As part of SAR studies the synthesized substituted quinoxalines such as, biphenyl (**13**), naphthyl (**14**), phenyl (**15**), 4-trifluoro phenyl (**16**), substituted 3-indolyl and thiophene (**17**). Among these derivatives **16** was showed marginally high PDE4B inhibition. Compounds **19** to **23, 25, 27, 29** and **30** were showed moderate PDE4B inhibition. Whereas compounds **13, 14** and **26** were showed less inhibition. However **24**, **28** and **31** were showed comparatively high PDE4B inhibition (Table 1).

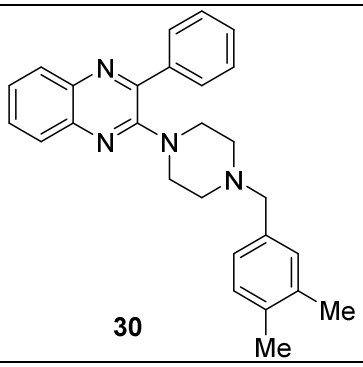
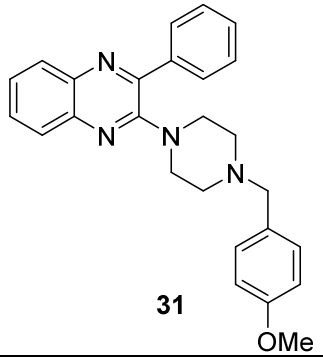
Table1. PDE4B enzyme inhibition of substituted quinoxalines

S. No.	Compound	Percentage of inhibition of PDE4B enzyme (%)
1	 **13**	11

2	 **14**	15
3	 **15**	35
4	 **16**	48
5	 **17**	26
6	 **18**	27

7	 **19**	43
8	 **20**	39
9	 **21**	37
10	 **22**	41
11	 **23**	37
12	 **24**	52

13	 **25**	33
14	 **26**	17
15	 **27**	37
16	 **28**	56
17	 **29**	33

18	 **30**	46
19	 **31**	51

CONCLUSION

In conclusion, we have explored quinoxalineas a template for the generation of a library of novel “drug like” small molecules. The strategy used for the present synthesis is simple, efficient and afforded good yields of desired quinoxaline derivatives. It is therefore amenable for the generation of diversity based small molecules related to quinoxaline framework of potential pharmacological interest. As expected the high PDE4B inhibition of these derivatives and observed few derivatives has showed moderate to good PDE4B inhibition.

Acknowledgement
Authors would like tothankDr. Reddy’s Laboratories Ltd. and also thankful to analytical department for providing analytical support.

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