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A new therapeutic approach in Parkinson's disease: Some novel quinazoline derivatives as dual selective phosphodiesterase 1 inhibitors and anti-inflammatory agents

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ABSTRACT

The increasing life expectancy in our population makes Parkinson's disease (PD) a growing public health problem. There is a great need to find a way to prevent and delay the disease. It was shown that selective phosphodiesterase 1 (PDE1) inhibitors and anti-inflammatory agents might be effective in treating PD. Therefore, a novel 1,2,9,11-tetrasubstituted-7*H*-thieno[2′,3′:4,5]pyrimido[6,1-*b*]-quinazolin-7-one (**1–15**) and 1,3,10,12-tetrasubstituted-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one (**16–36**) derivatives were synthesized by reported method and investigated for their ability to inhibit PDE1. Most of the synthesized compounds have shown good activity against PDE1 and were less effective than 3-isobutyl-1-methylxanthine. All the compounds were also tested for their in vitro anti-inflammatory activity by carrageenan-induced oedema in rats. In addition, ulcerogenic activity was determined. The combined anti-inflammatory data from in vitro animal model showed that compounds, 9,11-dibromo-1-(2-furyl)-3-(4-tolyl)-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one **23,** 9,11-dibromo-1-(4-methoxyphenyl)-3-phenyl-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one **24,** 9,11-dibromo-1-(4-chlorophenyl)-3-(4-tolyl)-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one **29** and 9-bromo-1-(4-chlorophenyl)-3-(4-tolyl)-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one **36** exhibited even more potent anti-inflammatory activity and low gastric ulceration incidence compare to reference standard Indomethacin. Since compound **23, 24, 29 and 36** exhibits both anti-inflammatory activity and PDE1 inhibition, it needs further detailed studies.

1. Introduction

Parkinson's disease (PD) is a chronic and progressive degenerative disease of the brain that impairs motor control, speech, and other functions.[1] The risk of developing PD is dramatically increased with age, and symptoms often appear after the age of 50. Current research indicating that 1 in 100 individuals over 60 has PD. The role of cAMP in functional and metabolic regulation of the nervous system has been emphasized in a number of studies. Early studies by Nishino et al. highlight a significant decrease in cAMP in patients with PD.[2] It is noteworthy that PDEs are the most important means of inactivating intracellular cAMP in the brain, suggesting that PDE inhibitors present a potentially powerful means to manipulate second messengers involved in learning, memory and mood.[3–6] Furthermore, PDE1A2 is inhibited by some antiparkinson drugs, suggesting a potential role of PDE1 in PD.[7,8]

Markers of neuroinflammation, including activated microglia and increased levels of circulating proinflammatory cytokines, have been observed in the brains and CSF of patients with PD. Yet the link between anti-inflammatory agents and PD in human remains uncertain, despite indications that neuroinflammation may contribute to cell death in the PD brain. Experimental evidence of anti-inflammatory agents such as nonsteroidal anti-inflammatory drugs (NSAIDs) are exerting neuroprotective effects in animal models.[9]

Purine bases and their bioisosteric analogs were widely used building blocks in combinatorial chemistry. Recently a great number of fused pyrimidine derivatives became known as potential drug molecules against various types of diseases. One of the most important compound families are quinazolinones. The quinazolinone moiety is a building block for approximately 150 naturally occurring alkaloids and drugs.[10] The natural quinazolinones and their synthetic analogs possess a variety of biological activities, including PDE inhibitory activity,[11–15] anticonvulsant,[16,17] bronchodilator,[18] anti-inflammatory,[19,20] antimalarial,[21] antituberculous,[22] anti-HIV,[23] narcotic

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antagonist,[24] anti-tumor,[25] tyrosine kinase inhibitor,[26] adenosine antagonist,[27] antimicrobial,[19] etc. Along with these activities, quinazoline derivatives showed a very prominent activity in PD.[28–30] Similarly thienopyrimidine and pyridopyrimidine, the bioisosters of purine bases are known to possess varied pharmacological activities like PDE inhibitory activity,[31–34] antiparkinsonian,[35,36] antimicrobials,[37] analgesic,[38] anti-inflammatory,[39] and many more.

In previous reports we have described numerous quinazolinones and their ability to inhibit cAMP PDE1.[14] Along with potent PDE inhibitory activities, these compounds also showed a very promising anti-inflammatory activity,[19] however these compounds were not fused heterocycles. Among the twenty six compounds, few compounds showed even more potent activities than the theophylline (Fig. 1). As a logical extension to these studies, it has been anticipated to undertake the synthesis of some quinazolinones fused with thienopyrimidine/pyridopyrimidine. Recent studies indicate that Quinazolinones, thienopyrimidines and pyridopyrimidines, bio-isosteres of xanthines, possess a very good PDEs inhibitory activity.

Considering that quinazolines are promising compounds for anti-inflammatory[19,20] and PDE1 inhibitors[11,40] and in the light of aforementioned findings, we aimed to synthesize novel series of 7*H*-thieno[2′,3′:4,5]pyrimido[6,1-*b*]-quinazolin-7-one[41] and 8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-ones (see Fig. 2).[42] Moreover, it was considered of interest to substitute various groups on the thienopyrimidine/pyridopyrimidine nucleus and investigate the influence of such structural variation on the anticipated biological activities. Rutaecarpine, a naturally occurring quinazoline alkaloid, was known to possess various biological activity, especially anti-inflammatory[43] and antiparkinsonian activities.[44] Due to the structural similarities between the title compounds and Rutaecarpine (Fig. 3), it was anticipated that the title compounds would also show anti-inflammatory activity. In addition to the targeted dual PDE1 inhibitory/anti-inflammatory activities, the ulcerogenic toxicity profiles of title compounds were also determined. The preliminary SAR features (based on the results obtained) of these new heterocyclic dual PDE inhibitors/anti-inflammatory compounds are discussed herein.

2. Chemistry

A series of some new 1,2,9,11-tetrasubstituted-7*H*-thieno[2′,3′:-4,5]pyrimido[6,1-*b*]-quinazolin-7-one **1–15** and 1,3,10,12-tetrasubstituted-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-ones **16–36** have been synthesized using appropriate routes. Details of the synthesis and characterization have been well documented.[41,42]

3. Experimental

Locally bred Sprague-Dawley rats of either sex weighing between 120 and 160 g, obtained from National Center for Laboratory Animal Sciences, Hyderabad, India, were used in present study. Animals were kept in wire-mesh cages and maintained under constant environmental conditions [23 ± 2 °C 12-h light]. All animals had free access to standard pellet diet (Hindustan Leaver Ltd., Mumbai) and water, in a constant light-dark cycle. During the course of experiment, the general behavior of animal was normal. All the experimental protocols were approved by the institutional animal ethical committee and experiments were conducted in accordance with the standard guidelines.

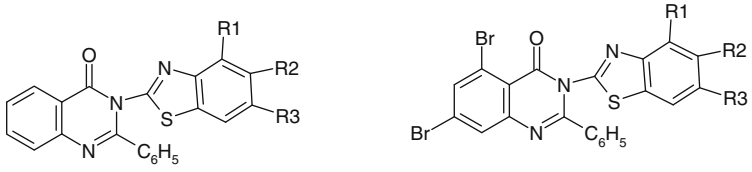
For PDE1 inhibitory activity, Biomol QuantiZyme™ Assay System was purchased from Biomol Research Laboratories. Before performing PDE1 inhibitory activity, it is important to ensure that compounds active in the cyclic nucleotide phosphodiesterase assay do not inhibit the activity of the 5′-nucleotidase. This can be ascertained by using 5′-AMP rather than 3′,5′-cAMP as the substrate and we observed that the title compounds were inactive against 5′-nucleotidase. We have also checked the possible interferences of drugs with dyes fluorescence or absorbance and we did not find any.

3.1. PDE1 inhibitory activity

PDE activity was confirmed by the commercial kit Biomol QuantiZyme™ Assay System. Briefly, in a enzyme precoated 96 well microplate, 1 mU of PDE1 enzyme from bovine brain was incubate with appropriate concentration of compounds (**1–36**), 10 mM of cAMP substrate, 15 μL of assay buffer and 50 kU of 5′ nucleotidase at 37 °C for 60 min. After incubating the plates, the reactions were terminated by addition of 100 μL Biomol Green™ reagent, the wells content was gently shaken and after 30 min the color was read at OD620 nm on a microtiter-plate reader. The 5′AMP release was calculated from: n mol 5′AMP = (OD620 nm − 0.0747)/0.232. The graph was plotted between n mol 5′AMP release and μmol of compounds (**1–36**). The results given in Table 1 are expressed as the concentration of inhibitor giving 50% inhibition of the PDE1 activity. The IC_{50} was determined from a plot of percentage activation versus varying concentration of the inhibitor. To validate enzyme assay method, the IC_{50} value of 3-isobutyl-methylxanthine (IBMX) to inhibit the enzyme activity was also determined as a standard inhibitor.

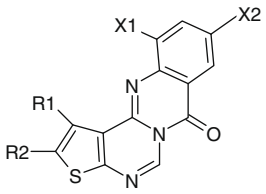
3.2. Anti-inflammatory activity[45]

The Anti-inflammatory activity of the title compounds **1–36** was evaluated by carrageenan-induced rat paw edema model.[45] The test compounds were suspended in 0.5% aqueous carboxy methyl cellulose (CMC) solution and administered orally as well as intra peritoneal to experimental animals (50 mg/kg) 60 min prior the injection of 0.1 ml of freshly prepared solution of carrageenan (1%) in physiological saline solution (154 mM NaCl) into the sub-planter tissue of hind paw of each rat. The same volume of saline solution was injected into hind paw of the control. The volume was measured by water plethysmometer prior to the administration of carrageenan and; 90 min and 180 min after the injection of carrageenan. The increase in volume of the paw was adopted as a measure of oedema. The percentage protection against inflammation was calculated using the formula given below:



Where R1, R2, and R3 = H, CH3,OCH3, OC2H5,Cl,Br and NO2

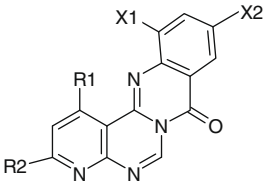
Figure 1. Previously synthesized compounds by our lab having PDE inhibitory, anti-inflammatory and antimicrobial activity.



1: R1= R2= -(CH2-)4; X1=X2=H
2: R1=p-Cl-phenyl; R2= H; X1=X2=H
3: R1=p-Me-phenyl; R2= H; X1=X2=H
4: R1=p-MeO-phenyl; R2= H; X1=X2=H
5: R1=R2= Me; X1=X2=H

6: R1= R2= -(CH2-)4; X1=X2=Br
7: R1=R2= Me; X1=X2=Br
8: R1=p-Cl-phenyl; R2= H; X1=X2=Br
9: R1=p-Me-phenyl; R2= H; X1=X2=Br
10: R1=p-MeO-phenyl; R2= H; X1=X2=Br

11: R1= R2= -(CH2-)4; X1=H;X2=Br
12: R1=R2= Me; X1=H; X2=Br
13: R1=p-Cl-phenyl; R2= H; X1=H; X2=Br
14: R1=p-Me-phenyl; R2= H; X1=H; X2=Br
15: R1=p-MeO-phenyl; R2= H; X1=H; X2=Br



16: R1= 2-furfuryl; R2= 4-tolyl; X1=X2=H
17: R1=p-MeO-phenyl; R2= phenyl; X1=X2=H
18: R1=phenyl; R2= 4-Cl-phenyl; X1=X2=H
19: R1=3-NO2-phenyl; R2= 4-MeO-phenyl; X1=X2=H
20: R1=4-NO2-phenyl; R2= 4-NO2-phenyl; X1=X2=H
21: R1=4-tolyl; R2= phenyl; X1=X2=H
22: R1=4-Cl-phenyl; R2= 4-tolyl; X1=X2=H

23: R1= 2-furfuryl; R2= 4-tolyl; X1=X2=Br
24: R1=p-MeO-phenyl; R2= phenyl; X1=X2=Br
25: R1=phenyl; R2= 4-Cl-phenyl; X1=X2=Br
26: R1=3-NO2-phenyl; R2= 4-MeO-phenyl; X1=X2=Br
27: R1=4-NO2-phenyl; R2= 4-NO2-phenyl; X1=X2=Br
28: R1=4-tolyl; R2= phenyl; X1=X2=Br
29: R1=4-Cl-phenyl; R2= 4-tolyl; X1=X2=Br

30: R1= 2-furfuryl; R2= 4-tolyl; X1=H; X2=Br
31: R1=p-MeO-phenyl; R2= phenyl; X1=H; X2=Br
32: R1=phenyl; R2= 4-Cl-phenyl; X1=H; X2=Br
33: R1=3-NO2-phenyl; R2= 4-MeO-phenyl; X1=H; X2=Br
34: R1=4-NO2-phenyl; R2= 4-NO2-phenyl; X1=H; X2=Br
35: R1=4-tolyl; R2= phenyl; X1=H; X2=Br
36: R1=4-Cl-phenyl; R2= 4-tolyl; X1=H; X2=Br

Figure 2. Compounds **1**–**36** for pharmacological evaluation.

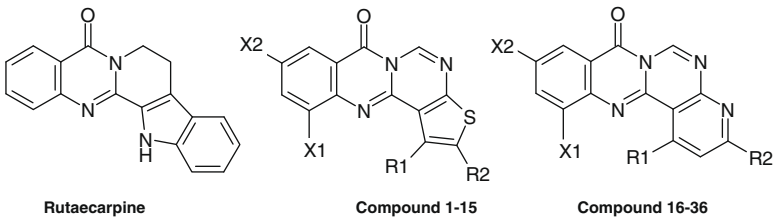


Figure 3. Title compounds, bioisostere of Rutaecarpine.

$(V_C - V_T)/V_C \times 100$ where V_C is the increase in paw volume of control (in the absence of test compound) and V_T is the increase in paw volume after administration of the test compound. The antiedematous effects of the compounds were estimated as percentage inhibition in comparison with control.

3.3. Gastrointestinal ulceration studies[46]

Compounds **1–5, 23, 24, 26, 28, 29, 32, 35** and **36** that exhibited moderate to potent anti-inflammatory profiles in the animal models were also evaluated for their ulcerogenic effects in rat. Rats were fasted for 24 h (with water ad libitum). The test compounds were suspended in a carboxy methyl cellulose vehicle and administered orally by gavage at 200-mg/kg/day dose for 5 days in a volume of 0.5 ml/100 g of body weight. The animals were sacrificed with diethyl ether inhalation, their stomachs removed by cutting along the greater curvature, washed under running water and fixed in 5% formalin solution. The stomachs were then examined for lesions under a dissecting microscope.

4. Pharmacological results and discussion

4.1. Pharmacology

Continuing our studies on quinazolinone derivatives[14,17,19,41,42] that are attractive candidates as PDE1 inhibitors, and anti-inflammatory agents, we have designed a novel thienopyrimidine fused quinazolinones and pyridopyrimidine fused quinazolinones. This dual PDE1 inhibitor/anti-inflammatory offers advantages beyond simple additive effects of administration of the individual agents including providing greater symptomatic efficacy and better utility. The 'message-address' concept of a dual agent could afford proximal inhibition of PDE1 thus keeping ample cAMP concentrations in CNS.

In the pharmacological study, we have investigated PDE inhibitory activity and anti-inflammatory activity as well as the acute ulcerogenicity of potent compounds. Isobutyl methyl xanthine (IBMX) (for PDE1 inhibitory activity) and Indomethacin (for anti-inflammatory and ulcerogenic activity) were used as a reference standard.

4.2. PDE1 inhibitory activity

It was well documented that PDE plays an important role in regulating cAMP and cGMP, and thus become an important site for pharmacological intervention. The identification of drugs that alter the activity of PDE may provide a new approach to altering physiological or pathological processes. PDE mediated process can be effectively inhibited by a number of pharmacological agents of widely different chemical structures. The numerous PDE inhibitors (see Fig. 4) like Vinpocetine (PDE1 inhibitor), Trequinsin (PDE3 inhibitor) and Rolipram (PDE4 inhibitor) showed very good effect

Table 1
The PDE1 inhibitory activity, anti-inflammatory activity and gastric ulceration effect of compounds **1**–**36**

Compound	PDE inhibition IC_{50} (μM) (±SD)	Dose (mg/kg)	Ratio of Ulceratin	Increase in paw edema (ml) ± SEM[b,c] (Inhibition in%)			
				Per oral (po)		Intraperitoneal (ip)	
				90 min	180 min	90 min	180 min
Control	NT[a]	NT[a]	NT[a]	0.92 ± 0.025	0.97 ± 0.029	0.90 ± 0.037	1.00 ± 0.041
1	228 (±30)	50	0/6	0.28 ± 0.019(70)	0.24 ± 0.027(75)	0.26 ± 0.032(71)	0.19 ± 0.039(81)
2	124 (±25)	50	0/6	0.58 ± 0.02(37)	0.46 ± 0.020(53)	0.52 ± 0.03(42)	0.43 ± 0.044(57)
3	230.3 (±35)	50	0/6	0.48 ± 0.031(48)	0.37 ± 0.025(62)	0.44 ± 0.035(51)	0.32 ± 0.04(68)
4	66.9 (±22)	50	0/6	0.40 ± 0.029(57)	0.28 ± 0.03(71)	0.37 ± 0.031(69)	0.26 ± 0.039(74)
5	295.4 (±35)	50	0/6	0.24 ± 0.02(74)	0.16 ± 0.032(84)	0.22 ± 0.03(76)	0.14 ± 0.041(86)
6	104.3 (±20)	50	NT[a]	0.87 ± 0.022(05)	0.82 ± 0.027(15)	0.86 ± 0.033(4)	0.79 ± 0.038(21)
7	95.7 (±17)	50	NT[a]	0.83 ± 0.019(10)	0.76 ± 0.024(22)	0.8 ± 0.037(11)	0.73 ± 0.045(27)
8	83.5 (±15)	50	NT[a]	0.9 ± 0.022(02)	0.87 ± 0.026(10)	0.88 ± 0.032(02)	0.83 ± 0.04(17)
9	61.9 (±11)	50	NT[a]	0.74 ± 0.017(20)	0.65 ± 0.026(33)	0.70 ± 0.035(22)	0.63 ± 0.037(37)
10	60.5 (±10)	50	NT[a]	0.78 ± 0.021(15)	0.69 ± 0.024(29)	0.76 ± 0.033(16)	0.69 ± 0.041(31)
11	128.2 (±23)	50	NT[a]	0.37 ± 0.02(60)	0.31 ± 0.03(68)	0.33 ± 0.038(63)	0.29 ± 0.035(71)
12	145.8 (±25)	50	NT[a]	0.34 ± 0.036(63)	0.28 ± 0.032(71)	0.31 ± 0.034(66)	0.24 ± 0.042(76)
13	90.5 (±12)	50	NT[a]	0.68 ± 0.025(26)	0.54 ± 0.03(44)	0.64 ± 0.03(29)	0.5 ± 0.045(50)
14	85.3 (±19)	50	NT[a]	0.55 ± 0.021(40)	0.47 ± 0.029(52)	0.52 ± 0.035(42)	0.44 ± 0.04(56)
15	82.2 (±15)	50	NT[a]	0.46 ± 0.019(50)	0.40 ± 0.026(59)	0.42 ± 0.032(53)	0.30 ± 0.039(70)
16	56.43 (±12)	50	NT[a]	0.82 ± 0.021(11)	0.76 ± 0.025(22)	0.85 ± 0.037(06)	0.8 ± 0.044(20)
17	72.1 (±15)	50	NT[a]	0.86 ± 0.016(07)	0.80 ± 0.029(18)	0.83 ± 0.034(08)	0.78 ± 0.039(22)
18	80.6 (±20)	50	NT[a]	0.75 ± 0.024(18)	0.72 ± 0.026(26)	0.74 ± 0.036(18)	0.66 ± 0.041(34)
19	39.9 (±13)	50	NT[a]	0.89 ± 0.018(03)	0.88 ± 0.03(09)	0.87 ± 0.034(03)	0.84 ± 0.036(16)
20	83.7 (±16)	50	NT[a]	0.87 ± 0.02(05)	0.86 ± 0.025(11)	0.8 ± 0.03(11)	0.77 ± 0.04(23)
21	91.8 (±22)	50	NT[a]	0.85 ± 0.022(08)	0.82 ± 0.032(15)	0.83 ± 0.034(08)	0.80 ± 0.039(20)
22	98.2 (±24)	50	NT[a]	0.58 ± 0.019(37)	0.49 ± 0.030(49)	0.56 ± 0.036(38)	0.47 ± 0.038(53)
23	65.1 (±13)	50	0/6	0.24 ± 0.021(74)	0.18 ± 0.027(81)	0.21 ± 0.037(77)	0.16 ± 0.04(84)
24	33.7 (±11)	50	0/6	0.22 ± 0.026(76)	0.17 ± 0.0225(82)	0.20 ± 0.035(78)	0.14 ± 0.045(86)
25	78 (±17)	50	NT[a]	0.66 ± 0.022(28)	0.56 ± 0.029(42)	0.63 ± 0.03(30)	0.53 ± 0.042(47)
26	72 (±12)	50	0/6	0.32 ± 0.018(65)	0.25 ± 0.028(74)	0.29 ± 0.032(68)	0.22 ± 0.039(78)
27	72.5 (±15)	50	NT[a]	0.5 ± 0.019(46)	0.45 ± 0.032(54)	0.48 ± 0.035(47)	0.44 ± 0.041(56)
28	52.4 (±17)	50	0/6	0.38 ± 0.022(59)	0.29 ± 0.027(70)	0.34 ± 0.037(62)	0.28 ± 0.038(72)
29	57.6 (±14)	50	1/6	0.26 ± 0.02(72)	0.19 ± 0.03(80)	0.20 ± 0.035(78)	0.17 ± 0.04(83)
30	72.5 (±15)	50	NT[a]	0.70 ± 0.027(13)	0.61 ± 0.021(37)	0.68 ± 0.034(24)	0.59 ± 0.036(41)
31	76.6 (±10)	50	NT[a]	0.68 ± 0.025(26)	0.57 ± 0.025(41)	0.65 ± 0.038(28)	0.56 ± 0.032(44)
32	95.1 (±23)	50	0/6	0.30 ± 0.021(67)	0.22 ± 0.030(77)	0.28 ± 0.039(69)	0.20 ± 0.038(80)
33	100.7 (±20)	50	NT[a]	0.81 ± 0.019(12)	0.73 ± 0.027(25)	0.78 ± 0.04(13)	0.70 ± 0.041(30)
34	101.2 (±22)	50	NT[a]	0.83 ± 0.02(10)	0.76 ± 0.028(22)	0.8 ± 0.036(11)	0.72 ± 0.039(28)
35	78.1 (±17)	50	0/6	0.40 ± 0.021(57)	0.31 ± 0.032(68)	0.36 ± 0.033(60)	0.3 ± 0.048(70)
36	66.4 (±12)	50	1/6	0.18 ± 0.019(80)	0.13 ± 0.03(87)	0.16 ± 0.038(82)	0.11 ± 0.04(89)
IBMX	24.7 (±15)	NT[a]	NT[a]	NT[a]	NT[a]	NT[a]	NT[a]
Indomethacin	NT[a]	50	6/6	0.26 ± 0.029(72)	0.18 ± 0.028(79)	0.22 ± 0.022(76)	0.17 ± 0.02(83)

[a] NT, not tested.
[b] SEM denotes the standard error of the mean.
[c] All data are significantly different from control (P <0.05).

(55–77% Neuroprotection) in PD with maximal effective concentrations of 5–10 μM.[47] So initially the title compounds of this study were evaluated for their ability to inhibit PDE1 enzyme. IBMX was chosen as a standard for comparison in these studies because of its reported non selective PDE inhibitory activity. Inhibition of PDE1 activity was confirmed by using the method of Biomol Quanti-Zyme™ Assay System. The basis for the assay is the cleavage of cAMP by a cyclic nucleotide phosphodiesterase. The 5′-nucleotide released is further cleaved into the nucleoside and phosphate by the enzyme 5′-nucleotidase. The phosphate released due to enzymatic cleavage is quantified using BIOMOL GREEN™ reagent. This assay is less sensitive than radio isotopic PDE assays and likely underestimates the relative inhibitory potencies of the test compounds. The results of this evaluation indicate that the title compounds are significantly less potent than IBMX as inhibitors of PDE.

The concentration that inhibited 50% of the enzymatic activity (IC_{50}), of each compound was determined and reported in Table 1. It has been found that almost all test compounds exhibited marked phosphodiesterase inhibitory activity in biochemical model. However all of them, except compounds **19** and **24** were found

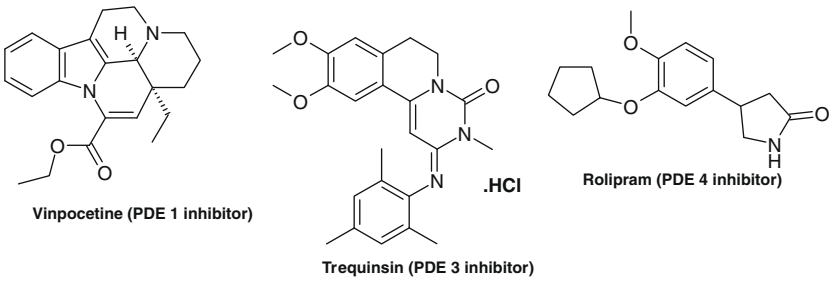


Figure 4. PDE inhibitors showing activity against PD.

to be significantly less active in their potencies when compared with reference standard, IBMX, a non selective PDE inhibitor. The structure activity relationship among each series has been discussed as follows.

4.2.1. SAR studies of 1,2,9,11-tetrasubstituted-7*H*-thieno[2′,3′:4,5]pyrimido[6,1-*b*]-quinazolin-7-one

Structure–activity relationship (SAR) studies indicated that different substitution on the aromatic ring, exerted varied phosphodiesterase inhibitory activity. The electronic nature of the substituent group in aromatic ring led to a significant variation in PDE1 inhibitory activity. For example electronic withdrawing group (mainly bromo substitutions) enhanced the PDE1 inhibitory activity whereas non bromo analogues have shown less activity. The order of activity was dibromo analogues **(6–10) >** monobromo analogues **(11–15) >** non bromo analogues **(1–5)**. The substituent over thiophene nucleus also led to significant variation in PDE1 inhibitory activity. Aromatic substitution in thiophene ring increased the PDE inhibitory activity over the aliphatic substituted compounds. In general the order of the activity was R1 = *p*-methoxy-phenyl; R2 = H > R1 = *p*-methyl-phenyl; R2 = H > R1 = *p*-chlorophenyl; R2 = H > R1 = R2 = methyl > R1 = R2 = –(CH_2)4–. Among this series, 9,11-dibromo-1-(4-methoxyphenyl)-7*H*-thieno[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-7-one **10** was the most potent with IC_{50} value of 60.5 (±10) μM.

4.2.2. SAR studies of 1,3,10,12-tetrasubstituted-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-ones

SAR studies in this series indicated that the R1 and R2 substituent over the pyridine ring, exerted varied PDE1 inhibitory activity. It has been found that PDE1 inhibitory activity increases as R2 = electron releasing groups, whereas if R2 = electron withdrawing groups, then it decreases the PDE inhibitory. The compound with R1 = electron releasing groups was showed more PDE1 inhibitory activity than the compounds with R1 = electron withdrawing groups. This type of effect was mainly observed in compound **16–22.** Among all the compounds in this series, 9,11-dibromo-1-(4-methoxy-phenyl)-3-phenyl-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one **24** and 3-(4-methoxy-phenyl)-1-(3-nitro-phenyl)-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one **19** were the most potent compounds with IC_{50} value of 33.7 (±11) μM and of 39.9 (±13) μM, respectively.

In both series, only two compounds, 9,11-dibromo-1-(4-methoxy-phenyl)-3-phenyl-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one **24** and 3-(4-methoxy-phenyl)-1-(3-nitro-phenyl)-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one **19** [IC_{50} value **24:** 33.7 (±11) μM; **19:** 39.9 (±13) μM] have shown significant PDE inhibitory activity, when compared with standard PDE inhibitor [**IBMX**; IC_{50} = 25 μM].

4.3. Anti-inflammatory activity

4.3.1. Carrageenan-induced paw edema in rats

In PD there is a progressive loss of dopaminergic neurons in the substantia nigra pars compacta. Ever-increasing evidence from human and animal studies has suggested that neuroinflammation is a cause or rather a consequence of neurodegeneration. Growing experimental evidences express that inhibition of the inflammatory response can, in part, prevent degeneration of nigrostriatal dopamine-containing neurons in several animal models of PD. It has been revealed that NSAIDs have neuroprotective properties. This finding proposes that inhibition of inflammation may become a promising therapeutic intervention for PD. In order to search new chemical entity effective against PD, we evaluated the target compounds for their anti-inflammatory activity by using the carrageenan-induced hind paw oedema model in rat.[45] Indomethacin was chosen as a standard for comparison in these studies. The anti-inflammatory activity of the synthesized compounds was studied at 50 mg/kg dose using Indomethacin as reference standard. The results revealed that all the synthesized compounds were exhibited anti-inflammatory activity. However, few of them were found even more potent than the reference standard. The significant reduction of rat paw oedema was observed by most of the test compounds after 180 min compared to control group (Table 1). The comparative anti-inflammatory data (Fig. 5) were clearly indicating that compounds shown higher inhibition when they were administering intraperitoneally. In order to probe structural requirements for optimal anti-inflammatory activity in this series, the substituent attached to the thienopyrimidine, pyridopyrimidine and quinazolinones ring were examined. Although it was difficult to trace a good correlation between chemical structure and anti-inflammatory activity from the result obtained, some preliminary conclusions can be drawn as follows.

4.3.1.1. SAR studies of 1,2,9,11-tetrasubstituted-7*H*-thieno[2′,3′:-4,5]pyrimido[6,1-*b*]-quinazolin-7-one 1–15. According to the results of in vivo experiments, we can conclude that different substitution on the thiophene ring and quinazolinone ring, exerted varied anti-inflammatory activity.

When compared the effect of bromo substituent on the quinazolinone ring of title compounds, it was found that non bromo analogues have shown potent anti-inflammatory activity than the bromo analogues. The order of activity in this series was non-bromo analogues (**1–5**) > monobromo analogues (**11–15**) > dibromo analogues (**6–10**).

When compared the effect of alky and aryl substituent on thiophene ring of the title compounds, it was observed that the alkyl substituent shown more anti-inflammatory activity than the aryl substituent.

Among all the compounds in this series, 1,2-dimethyl-7*H*-thieno[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-7-one **5,** was exhibited more pronounced anti-inflammatory activity than Indomethacin [% inhibition **5 =** 74(90 min), 84 (180 min) when administered orally, 76(90 min), 86 (180 min) administered intraperitoneally and **Indomethacin** = 72(90 min), 79 (180 min) when administered orally, 76(90 min), 83 (180 min) administered intraperitoneally].

4.3.1.2. SAR studies of 1,3,10,12-tetrasubstituted-8*H*-pyrido[2′,-3′:4,5]pyrimido[6,1-*b*]quinazolin-8-ones 16–36. The isosteric replacement of thiophene with pyridine ring in first series, exerted varied anti-inflammatory activity. In general the pyridopyrimidine fused quinazolinone exhibited significantly more potent anti-inflammatory activity than the thienopyrimidine fused quinazolinones.

When compared the effect of bromo substituent on the quinazolinone ring of title compounds, it was found that bromo analogues have shown potent anti-inflammatory activity than the non-bromo analogues. Although, from the result obtained, it was difficult to trace a good correlation between chemical structure (dibromo and monobromo analogues) and anti-inflammatory activity. While considering top 10 potent compounds of this series, it was found that seven potent compounds belonged to monobromo analogues and only three belonged to dibromo analogues. However from the result, it was clearly observed that the most potent compound belonged to dibromo analogues.

Substitutions at pyridine ring (**R1** and **R2**) on the title compounds were examined to define the effect on the anti-inflammatory activity of the title compounds. From Table 1, it was found that, compound 9,11-dibromo-1-(2-furyl)-3-(4-tolyl)-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one **23,** 9,11-dibromo-1-(4-methoxy-phenyl)-3-phenyl-8*H*-pyrido[2',3':4,5]pyrimido[6,1-*b*]quinazolin-8-one **24,** 9,11-dibromo-1-(4-chloro-phenyl)-3-(4-tolyl)-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one **29** and 9-bromo-1-

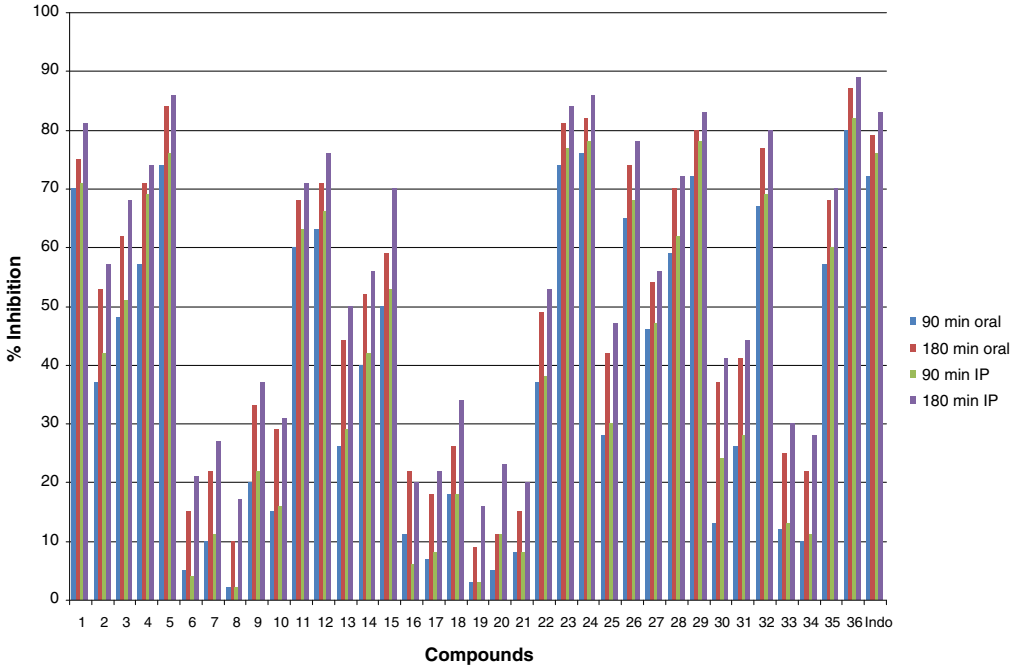


Figure 5. Effect of compounds against carrageenan-induced hind paw edema model at 50 mg/kg dose.

(4-chloro-phenyl)-3-(4-tolyl)-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one **36** were exhibited even more potent anti-inflammatory activity and low gastric ulceration incidence than reference standard Indomethacin [% inhibition **23 =** 74(90 min), 81 (180 min) when administered orally, 77 (90 min), 84 (180 min) administered intraperitoneally; **24 =** 76 (90 min), 82 (180 min) when administered orally, 78 (90 min), 86 (180 min) administered intraperitoneally; **29 =** 72 (90 min), 80 (180 min) when administered orally, 78 (90 min), 83 (180 min) administered intraperitoneally; **36 =** 80 (90 min), 87 (180 min) when administered orally, 82 (90 min), 89 (180 min) administered intraperitoneally and **Indomethacin** = 72 (90 min), 79 (180 min) when administered orally, 76 (90 min), 83 (180 min) administered intraperitoneally].

The most potent compound 9-bromo-1-(4-chloro-phenyl)-3-(4-tolyl)-8*H*-pyrido[2′,3′:4,5]pyrimido[6,1-*b*]quinazolin-8-one **36** consists of R1 = *p*-chlorophenyl and R2 = *p*-tolyl as substituent. Comparing the compounds containing *p*-chlorophenyl group (**18, 22, 25, 29, 32, 36**), it was found that the anti-inflammatory activity get increased when *p*-chlorophenyl group was placed at R1 (**36, 29** and **22**).

In both series, only four compounds, 9,11-dibromo-1-(2-furyl)-3-(4-tolyl)-8*H*-pyrido[2',3':4,5]pyrimido[6,1-*b*]quinazolin-8-one **23,** 9,11-dibromo-1-(4-methoxy-phenyl)-3-phenyl-8*H*-pyrido[2', 3':4,5]pyrimido[6,1-*b*]quinazolin-8-one **24,** 9,11-dibromo-1-(4-chloro-phenyl)-3-(4-tolyl)-8*H*-pyrido[2',3':4,5]pyrimido[6,1-*b*] quinazolin-8-one **29** and 9-bromo-1-(4-chloro-phenyl)-3-(4-tolyl)-8*H*-pyrido[2',3':4,5]pyrimido[6,1-*b*]quinazolin-8-one **36** were exhibited even more potent anti-inflammatory activity and low gastric ulceration incidence than the reference standard Indomethacin.

4.4. Ulcerogenic effects

Compounds with significant anti-inflammatory profile were subjected to ulcerogenic potential test at 200 mg/kg dose level. All the active compounds revealed a superior GI safety profiles with oral dose of 200 mg/kg/day, when compared with reference standard, Indomethacin; which was found to create 100% ulceration under same conditions. Gross observation of the isolated rat stomachs showed a normal stomach texture for all active compounds.

5. Conclusion

In this paper, we are describing the novel thienopyrimidine/pyridopyrimidine fused quinazolinone derivatives as dual PDE1inhibitor/anti-inflammatory. We also demonstrate that all of the title compounds have less ulcer forming property than Indomethacin. The present study indicates that fused quinazolinones are novel inhibitors of PDE1 with good anti-inflammatory activity, and due to these activities their activity against PD is highly probable.

Among both series, pyridopyrimidine fused quinazolinone compounds exhibited even more potent anti-inflammatory activity than the thienopyrimidine fused quinazolinones. This might be due to their more PDE1 inhibitory activity and anticipated (may be) cyclooxygenase II inhibitory activity.

The lack of a correlation between anti-inflammatory activity and PDE1 inhibitory activity both in thienopyrimidine fused quinazolinone and pyridopyrimidine fused quinazolinone series would suggest that the test compound produced anti-inflammatory activity by an alternative mechanism may be by inhibition cyclooxygenase II enzyme (COX-II) (along with PDE1 inhibition). Teismann et al. demonstrate that the COX-II enzyme helps kill neurons in Parkinson's disease and that COX-II inhibitors like Celebrex and Vioxx may be capable to slow cell death in patients.[48] The title compounds **1–36** showed some sort of similarity with the naturally occurring quinazolinocarboline alkaloid **Rutaecarpine** (Fig. 3), which exhibits strong ant-inflammatory activity, with potent and selective COX-II inhibitory activity.[43,49,50] So it will worthwhile if we screen these compounds for their selective COX II inhibitory activity. A selective COX-II inhibitory activity and activity against PD in animal model of potent compounds are under development and will be described later. This work is a further example of searching for good dual PDE1 inhibitor/anti-inflammatory used in against PD.

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