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Imidazopyridazinones as novel PDE7 inhibitors: SAR and in vivo studies in Parkinson's disease model

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ABSTRACT

The synthesis and structure-activity relationship studies of a series of compounds from imidazopyridazinone scaffold as PDE7 inhibitors are disclosed. Potent analogs such as compounds **7** (31 nM), **8** (27 nM), and **9** (12 nM) were identified. The PDE selectivity and pharmacokinetic profile of compounds **7**, **8** and **9** are also disclosed. The adequate CNS penetration of compound **7** in mice allowed it to be tested in the MPTP induced PD model and haloperidol induced catalepsy model to probe the differential pharmacology of PDE7 in the striatal pathway.

Phosphodiesterase 7 (PDE7) an enzyme that selectively hydrolyzes cAMP, has been extensively targeted for the treatment of a host of immunological and autoimmune conditions.[1] Recently, interest with PDE7 inhibitors has emerged in the context of Parkinson's disease (PD)[2], considering the expression of PDE7A and 7B, the two isoenzymes of PDE7, in rodent and human brain.[1] The interest was further spurred by the findings that showed PDE7 inhibition can protect dopaminergic neurons against different insults in the lipopolysaccharide rat model of PD.[3,4] Furthermore, the dopamine receptors D1 and D2 signaling[5] has been proposed to be modulated by PDE7 via cAMP levels. It has been shown that PDE7 inhibitor alone or in combination with Levodopa (*l*-dopa) increased neuronal activation and restored paw stride length in MPTP treated mice model.[6] However, sub-optimal potency doses[7] were found to produce maximal efficacy and higher doses were reported to be sub-efficacious.[6] Interestingly, this observation can be correlated with the impact of PDE inhibition in the D1 receptor dependent 'direct' and D2 receptor dependent 'indirect' striatal pathways. Considering this background, our initial goal was to identify potent, selective, CNS-penetrating PDE7 inhibitors to evaluate the potential of PDE7 inhibition as a novel target in the PD therapy.

We have disclosed the structure-activity relationship of isothiazole and isoxazole fused pyrimidones (**II**) inspired from (**I**) as PDE7 inhibitors with adequate CNS penetration.[8] Additionally, we were also keen on exploring structurally diverse CNS penetrating PDE7 inhibitors. Based on previous PDE7 SAR studies,[8,9] we hypothesized that an imidazopyridazinone scaffold (**III**) (Fig. 1) would allow us to explore alternate trajectory for essential/preferred substituents. In this communication we describe the synthesis, SAR, PDE selectivity, and pharmacokinetic profile of a series of compounds from this scaffold. Moreover, we also wish to report our findings of the in vivo experiments in the MPTP treated mice model of PD and in the haloperidol induced catalepsy model.

Synthetic access to imidazopyridazinone (**III**) where R^1 = H, has been previously reported by Gres'ko et. al.[10] We realized that we could access the desired PDE7 SAR by alkylation of compound **3** with various alkyl bromides to provide **4**, at which point the synthesis could proceed to (**III**) (analogs **16**–**19** and **21**) in a manner similar to that previously described (Scheme 1).[10] In the case where R^2 = $-NO_2$, reduction to the amine (**7**)[11] could be effected with iron and ammonium chloride in methanol. Subsequent reaction of the amine (**7**) with phenylchloroformate, followed by treatment with methylamine hydrochloride provided **22**. In the case where R_2 = –CN, the nitrile was readily hydrolyzed to the acid, esterified, reacted with hydrazine hydrate then followed by treatment with triphosgene to form the oxadiazolone analog **23**. In the case where R_2 = methylacrylate, compound **24** could be readily

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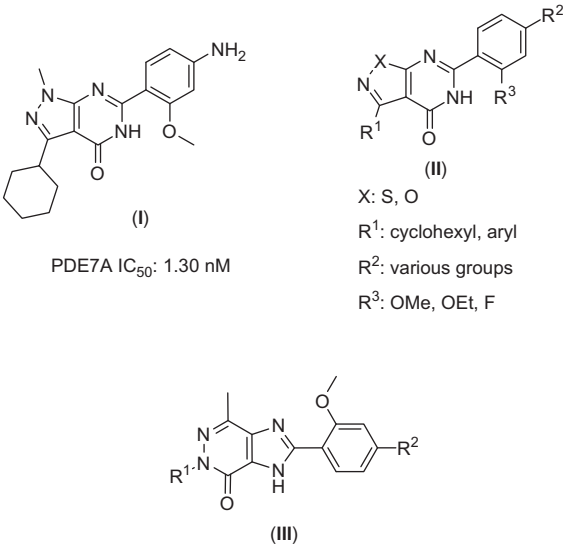
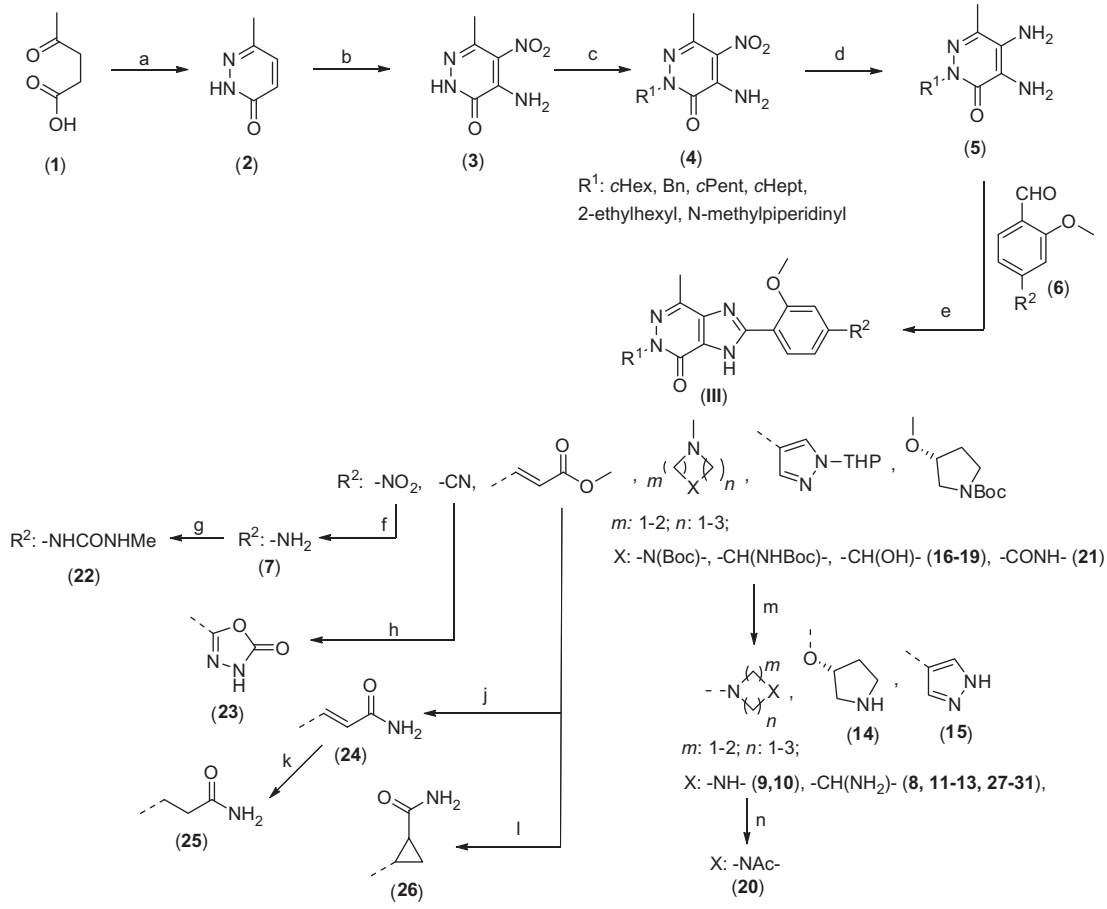


Figure 1. PDE7 inhibitors.

prepared by hydrolysis of the ester followed by coupling with ammonium chloride. Hydrogenation of **24** provided **25**. Alternatively, in the case where R_2 = methylacrylate, reaction with trimethylsulfonium iodide and sodium hydride provided the cyclopropyl ester intermediate. Hydrolysis of the ester followed by coupling with ammonium chloride provided **26**. The removal of the protecting groups (Boc or THP) in **III** with a saturated solution of HCl in ethyl acetate provided the compounds **8**–**15** and **27**–**31**. The synthesis of compound **20** was accomplished upon acylation of compound **9** using acyl chloride.

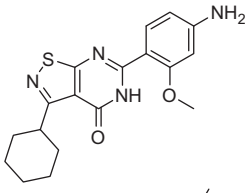
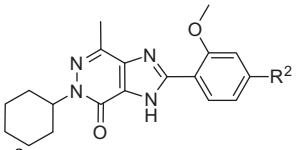
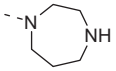
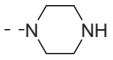
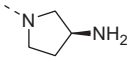
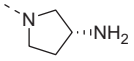
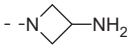
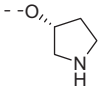
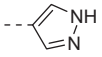
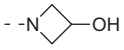
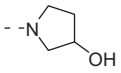
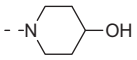
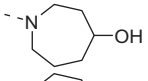
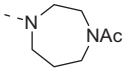
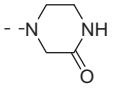
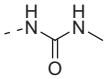
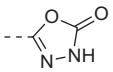
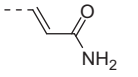
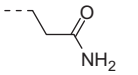
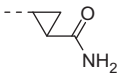
The target compounds were tested for their inhibitory activity at the cloned human recombinant PDE7A, PDE7B and other PDE isozymes following a two step radiometric assay using ^{3}H-cAMP as the radioligand.[12]

We were gratified to find that our hypothesis that the imidazopyridazinone scaffold could provide potent PDE7 inhibitors was correct. In general, the potency of the imidazopyridazinone compounds followed the SAR we had previously observed with our isothiazole and isoxazole fused pyrimidone scaffolds (**II**) (Table 1).[8] Our first compound prepared in this series (**7**) was a potent inhibitor of PDE7 (31 nM) which was about twofold less potent than (**IIa**). Per our expectation, the aminopiperidine derivative (**8**) was equipotent in comparison with compound **7** and an increase in ring size (**9**) resulted twofold increase in potency. However, a surprising fourfold loss in potency was observed with the piperazine derivative (**10**). The isomeric aminopyrrolidines (**11** and **12**), aminoazitidine (**13**), pyrrolidine ether (**14**) and the pyrazole (**15**) derivatives were less potent in direct comparison with **9**. At the R^2 position of **III** the use of cyclic amines with a hydroxy substituent as H-bond



Scheme 1. Reagents and conditions: (a) (i) NH_2–NH_2·H_2O, EtOH, reflux, 3 h; (ii) Br_2, AcOH, 90 °C, 6 h; (b) (i) NH_2–NH_2·H_2O,160 °C, 16 h; (ii) conc. HNO_3, conc. H_2SO_4, 0 °C to rt, 3 h; (iii) conc. H_2SO_4, 60 °C, 5 h; (c) R^1-Br, K_2CO_3, DMF, 110 °C, 16 h; (d) Fe, AcOH, reflux, 12 h; (e) aldehyde (**6**)[8], sulfur, xylene, 160 °C, 2 h; (f) Fe, NH_4Cl, MeOH, rt, 2 h; (g) (i) PhOCOCl, Et_3N, THF, rt, 1 h; (ii) $MeNH_2$·HCl, DMSO, 50 °C, 5 h; (h) (i) NaOH, MeOH, rt, 18 h; (ii) conc. H_2SO_4, EtOH, reflux, 4 h; (iii) NH_2–NH_2·H_2O, EtOH, reflux, 3 h; (iv) triphosgene, THF, reflux, 2 h; (j) (i) LiOH, MeOH–THF–H_2O, rt, 10 h; (ii) NH_4Cl, PyBOP, DIPEA, DMF, rt, 12 h; (k) Pd/C, H_2, MeOH, 40 psi, 2 h; (l) (i) trimethylsulphonium iodide, NaH, DMSO, rt, 18 h; (ii) LiOH, THF–MeOH–H_2O, rt, 2 h; (iii) NH_4Cl, PyBOP, DIPEA, DMF, rt, 12 h; (m) HCl in EtOAc, rt, 4 h; (n) AcCl, Et_3N, CH_2Cl_2, rt, 1 h.

Table 1
SAR of the R^2 position of Imidazopyridazinone

Example		PDE7A IC_{50} (nM) ±SD[a]
IIa		14 ± 4.5
III		
	R^2	
7		31 ± 4.09
8		27 ± 7.64
9		12 ± 1.75
10		104 ± 25.67
11		80 ± 14.09
12		77 ± 10.34
13		73 ± 7.59
14		55 ± 10.87
15		45 ± 10.73
16		53 ± 14.03
17		71 ± 22.99
18		81 ± 19.07
19		33 ± 7.17
20		32 ± 7.17
21		18 ± 2.81
22		24 ± 6.74
23		68 ± 17.98
24		89 ± 15.40
25		93 ± 12.67
26		3 ± 10.98

[a] IC_{50} values are means of three experiments.

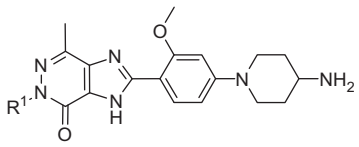
donor were well tolerated (**16**–**19**) and the azepinol derivative (**19**) provided a potent PDE7 inhibitor. The use of H-bond acceptor was also tolerated as evident from the activity of the compound **20**. Since both H-bond donors and acceptors were well tolerated, we prepared examples which were capable of providing both interactions (**21**–**26**) and observed that both piperazinone (**21**) and urea (**22**) were the most potent among the analogs prepared. However, the oxadiazolone derivative (**23**) was fourfold less potent than **21**. Among the acyclic amide derivatives (**24**–**26**), the cyclopropyl amide **26** was most potent. Therefore, the H-bond donor and/or acceptor groups were able to provide potent PDE7 inhibitors in the imidazopyridazinone scaffold.

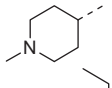
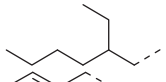
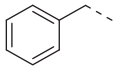
Based on our prior experience in designing PDE7 inhibitors we conducted a limited SAR effort at the R^1 position of **III** (Table 2). As expected the cycloalkyl analogs (**8**, **27**, **28**) were the most potent among the analogs prepared.

The PDE selectivity profile of representative compounds **7**, **8** and **9** presented in Table 3 suggests that the compounds were non-selective within PDE7 isoforms. The compounds were inactive against other PDE isoforms except PDE4 where the compounds have shown >50% inhibition at 10 μM of test concentration. Therefore, the PDE4 IC_{50}'s of compounds **7** and **9** were determined (6.55 μM and 2.9 μM, respectively) and it was confirmed that the compounds were having reasonable selectivity against this enzyme in comparison with PDE7A (>200 fold) and PDE7B (>150 fold).

Next, we attempted to identify a tool compound with reasonable brain penetration, suitable for the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treated mice model of PD. Therefore, we further evaluated the pharmacokinetic profile of compounds **7**, **8** and **9** (Table 4). The compounds were relatively less stable in mouse liver microsomes and more importantly only compound **7** had shown reasonable brain concentrations upon 10 mg/kg i.p. administration in mice. Thus we decided to use compound **7** as our tool molecule for in vivo evaluation. The mouse

Table 2
SAR of the R^1 position of imidazopyridazinone



Example	R^1	PDE7A % Inh. @ 1 μM[a]	PDE7A IC_{50} (nM) ±SD[b]
8		96%	27 ± 7.64
27		89%	ND
28		92%	75 ± 15.23
29		7%	ND
30		6%	ND
31		13%	ND

ND: Not determined.
[a] % Inhibition values are means of two experiments.
[b] IC_{50} values are means of three experiments.

Table 3
PDE selectivity profile

Compound	IC_{50} (nM)		% Inhibition at 10 μM[c]								
	PDE7A ±SD[a]	PDE7B[b]	PDE 1A	PDE 2A	PDE 3A	PDE 4D	PDE 5A	PDE 8A1	PDE 9A2	PDE 10A	PDE 11A
7	31 ± 4.09	43	37	29	6	86[d]	3	0	9	23	10
8	27 ± 7.64	11	16	40	2	81	32	6	9	40	44
9	12 ± 1.75	19	33	28	4	90[e]	3	19	12	2	24

[a] IC_{50} values are means of three experiments.
[b] IC_{50} values are means of two experiments.
[c] % Inhibition values are means of two experiments.
[d] IC_{50} 6.55 μM.
[e] IC_{50} 2.9 μM.

Table 4
Liver microsomal stability and pharmacokinetic profile of compounds **7**, **8** and **9**

Compound	Metabolic stability (% remaining)[a]			Male C57 Mouse PK (10 mg/kg i.p.)[b]					
	HLM	RLM	MLM	C_{brain} (ng/g)[c]	C_{plasma} (ng/mL)[c]	C_{brain}/ C_{plasma}	C_{max} (ng/mL)	AUC (ng.h/mL)	T_{max} (h)
7	43	68	4	1138	699	1.69	1478	792	0.25
8	100	100	44	46	451	0.1	902	2300	4
9	90	40	37	39	446	0.09	570	1374	0.25

[a] Percentage of test compound remaining after 60 min incubation with liver microsomes (human, rat and mouse) at 37 °C.
[b] i.p. formulation: 2.5 μL/mL Tween 80, 0.5% Methyl Cellulose suspension.
[c] Brain and plasma samples were collected 30 mins post i.p. dose.

PDE7A IC_{50} (63 nM)[12] and C57 mouse brain homogenate binding[13] (99.8% bound; 0.2% free) of compound **7** were determined. The pharmacokinetic parameters of compound **7** at various doses were also determined and the results are summarized in Table 5. Based on these results it was evident that at the 10 mg/kg dose upon i.p. administration, the free brain concentration achieved was 10-fold lower than the mouse PDE7A IC_{50} (Table 5). However, free brain concentration achieved at the 30 and 100 mg/kg doses were comparable and many fold higher than the mouse PDE7A IC_{50}, respectively (Table 5).

The in vivo efficacy of compound **7** was evaluated in the acute mice models of MPTP induced PD[14] and a dopamine D2 receptor antagonist, haloperidol induced catalepsy (HIC).[15] The compound **7** was studied over a wide range of doses (1, 3, 10 and 30 mg/kg, i.p.) on rearing behavior in MPTP induced PD in male C57BL/6 mice. Compound **7** produced a statistically insignificant dose dependent increase in rearing behavior at sub-optimal doses (1, 3 and 10 mg/kg, i.p.) and did not produce any efficacy at the highest dose (30 mg/kg, i.p.) tested in the MPTP treated mice (Fig. 2). However, the standard reference treatment *l*-dopa/benserazide (BSZ) produced significant effect on vertical rearing behavior in these mice. In contrast to previous literature evidence,[6] the PDE7 inhibitor (compound **7**) per se did not produce significant efficacy on rearing behavior in MPTP treated mice. However, it may still be possible for PDE7 inhibitors to potentiate the beneficial effect of *l*-dopa by synergizing with the dopamine D1 receptor mediated cAMP signaling in the 'striatal direct pathway'.

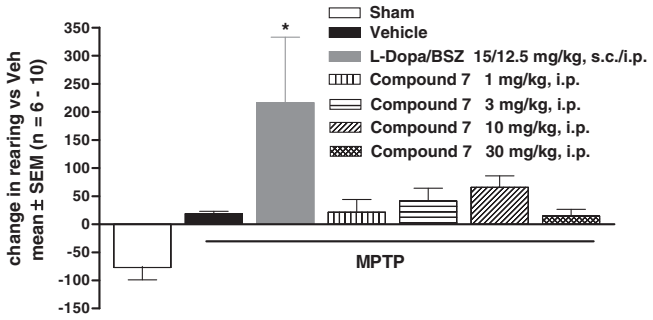


Figure 2. Effect of compound **7** on rearing in MPTP treated male C57BL/6 mice. *p <0.05 versus vehicle by one-way Anova/Tukey's test.

In order to understand the possible reason for efficacy at sub-optimal doses and reduced/lack of efficacy at higher doses as observed from present and previous[6] findings, we further probed the effect of compound **7** alone and in combination with dopamine D2 receptor antagonist, haloperidol in the catalepsy bar test. Dopamine D2 receptors of the 'striatal indirect pathway' coupled to inhibitory G proteins results in an increase of cAMP levels upon blockade by D2 antagonist such as haloperidol, an antipsychotic known for its catalepsy like extra pyramidal (EPS) side effects.[16] Compound **7** produced a degree of catalepsy in mice at a dose of 30 mg/kg (i.p.) but did not show any effect in this model at doses

Table 5
Pharmacokinetic parameters and brain concentration profile of compound **7**

Dose[a]	C_{max} (ng/mL)	AUC (ng.h/mL)	Tmax (h)	C_{plasma} (ng/mL)[b]	C_{brain} (ng/g)[b]	C_{brain}/ C_{plasma}	Estimated Free brain conc.[c]	
							(ng/g)	(nM)
10 mg/kg	1478	792	0.25	699	1138	1.69	2	6
30 mg/kg	6353	5019	0.25	4680	16048	3.43	32	91
100 mg/kg	44185	168407	0.5	44185	134745	3.05	270	763

[a] Male C57 mice were used for the experiments upon i.p. administration; i.p. formulation: 2.5 μL/mL Tween 80, 0.5% Methyl Cellulose suspension.
[b] Brain and plasma samples were collected 30 mins post i.p. dose.
[c] Free brain concentrations were estimated using the mouse brain homogenate binding (99.8% bound; 0.2% free).

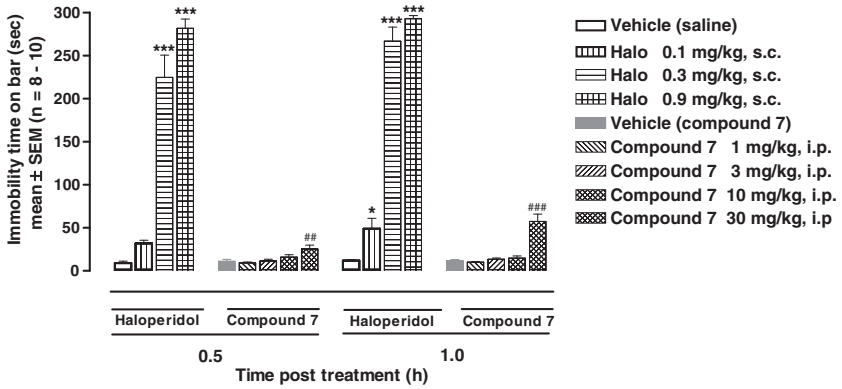


Figure 3. Effect of haloperidol and compound **7** on catalepsy bar test in male C57BL/6 mice. $^{*}p$ <0.05, $^{***}p$ <0.001 versus Naive by one-way Anova/Tukey's test.

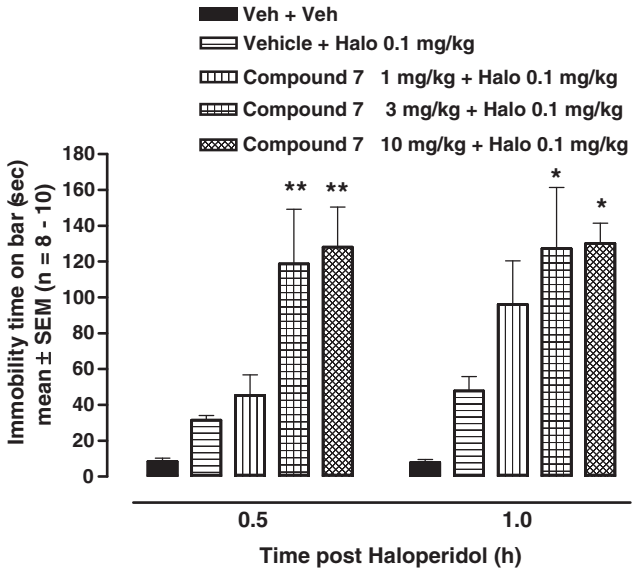


Figure 4. Effect of compound **7** on HIC test in male C57BL/6 mice. $^{*}p$ <0.05, $^{**}p$ <0.01 versus vehicle + Halo by one-way Anova/Tukey's test.

of 1, 3 and 10 mg/kg (i.p.). In contrast, haloperidol (0.1, 0.3 and 0.9 mg/kg, s.c.) produced significant dose dependent catalepsy in mice (Fig. 3). Moreover, pretreatment with sub-optimal potency doses[7] of compound **7** (1, 3, 10 mg/kg) significantly potentiated the cataleptic effect of haloperidol (0.1 mg/kg, s.c.) in a saturating manner (Fig. 4). The sub-optimal potency dose of compound **7** (3 mg/kg, i.p.) in combination with sub-cataleptic dose of haloperidol (0.1 mg/kg, s.c.) also resulted in significant motor deficits in rotarod test in mice (Supplementary Fig. 1).[17] These results suggest that increased cAMP signaling with PDE7 inhibitors in the 'striatal indirect pathway' can potentially produce modest catalepsy. The present result further corroborate previous findings of potentiation of the haloperidol induced catalepsy by PDE inhibitors (PDE4 inhibitor Rolipram, PDE1 inhibitor Vinpocetine) in rodent species.[18] In addition, PDE4 and PDE10 inhibitors which are being developed as potential anti-psychotics are reported to produce modest cataleptic motor deficits in a dose-independent manner.[18] Thus the present results suggest that the differential pharmacology of PDE7 inhibitors through both direct and indirect striatal pathways might be partly responsible for dose dependent self-limiting blunted effects in rodent PD models. Further it can be suggested that even modest cataleptic motor deficit is unwarranted for a potential therapeutic of PD.

In conclusion, we have reported the synthesis and SAR evaluation of imidazopyridazinone as PDE7 inhibitors. The SAR elucidated that various groups with H-bond donor and/or acceptor at the R^2 position of **III** provided potent PDE7 inhibitors. We also identified compound **7** which had adequate CNS penetration upon i.p. administration in mice and that allowed it to be tested in the in vivo models of PD. In the MPTP treated mice model of PD, compound **7** produced a statistically insignificant dose dependent efficacy. However, no effect was observed at the highest dose tested. In addition, compound **7** produced modest catalepsy at the highest dose tested and potentiated the cataleptic and motor deficit effects of haloperidol at sub-optimal doses in mice. Therefore, it could be anticipated that the beneficial effect of PDE7 inhibition to increase the cAMP levels in striatal direct pathway was overshadowed by its effect in the indirect pathway. This differential pharmacology might result in a challenge of titrating an optimal dose for varied human PD population. Therefore detailed preclinical studies are warranted before concluding the potential therapeutic utility of PDE7 inhibitors in PD.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.07.077.

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