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Identification of potent pyrimidine inhibitors of phosphodiesterase 7 (PDE7) and their ability to inhibit T cell proliferation

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ABSTRACT

A series of pyrimidine based inhibitors of PDE7 are discussed. The synthesis, structure–activity relationships (SAR) and selectivity against several other PDE family members as well as activity in T cells are presented. These compounds were found to have effects on T cell proliferation, however it is not clear whether the mechanism is related to PDE7 inhibition.

Phosphodiesterases (PDEs) play a critical role in various biological processes by hydrolyzing the key second messengers cAMP and cGMP to the corresponding 5′-monophosphate nucleotides. In the immune system, cAMP is the primary regulatory cyclic nucleotide and it is believed that cAMP broadly suppresses the functions of immune and inflammatory cells. The reduction of cAMP levels is mediated principally by the action of cell-specific phosphodiesterases (PDEs) and as such, an approach to sustain cAMP levels through PDE-inhibition would provide a strategy to treat a variety of immune and inflammatory diseases.[1]

A functional role of PDE7A in activation and/or proliferation of T cells has been reported.[2] Resting T lymphocytes express mainly PDE3 and PDE4. However, upon activation, T cells dramatically up-regulate PDE7A1 and appear to principally rely on this isozyme for regulation of cAMP levels. Suppression of PDE7 up-regulation by anti-sense oligonucleotides inhibited T cell proliferation and decreased IL-2 production, and maintained elevated levels of intracellular cAMP in CD3xCD28 stimulated T cells. PDE7A3, a splice variant of PDE7A1, is also reported to be up-regulated in activated $CD4^+$ T cells.[3] This expression profile suggests inhibitors of PDE7A would have broad application as an immunosuppressant. To this end, several groups have reported the preparation of potent inhibitors of PDE7[4] with optimization of pharmacokinetic properties.[5] We recently reported chemistry efforts around our purine-based deck hit.[6] However, since our report that PDE7A deficient mice show no deficiencies in T cell function,[7] the identification of a small molecule inhibitor of PDE7 has continued to be of interest in order to complete our understanding of the relationship between PDE7 inhibition and T cell function. Recently there have been reports of selective PDE7 inhibitors which have failed to demonstrate significant effects on T cells.[8] We report herein on selective inhibitors of PDE7 which were found to suppress T cell proliferation and evidence which suggests that this activity is not related to PDE7 inhibition.

Our initial SAR focused on bicyclic systems such as **1a** and **1b**. In an effort to understand the minimal phamacophore we prepared pyrimidine **2a**. This compound displayed a 22-fold loss in PDE7 potency compared to **1a**. However we postulated that addition of a second group at the 6-position would increase the potency against PDE7 similar to that observed with the constrained analog **1b** (Fig. 1).

The synthesis of pyrimidine analogues **2b–m** is shown in Scheme 1. Thiazole guanidine **5** was prepared through the condensation of 2-imino-4-thiobiurea **3** with 2-chloroacetoacetate **4** in the presence of pyridine. Guanidine **5** was condensed with commercially available diethyl malonate under basic conditions in refluxing ethanol to give the desired pyrimidone **6** in ~100% yield. Dichloropyrimidine **7** was formed in 80–95% yield after treatment of **6** with $POCl_3$. The chlorine atom at the 4 position of dichloropyrimidine could be readily displaced with amines to produce intermediate **8**. Subjecting intermediate **8** to a second displacement with a variety of amines at higher temperature using a microwave reactor produced compounds **2b–m.**

In vitro data for the diaminopyrimidine series (**2b–m**) is presented in Table 1. We were gratified to find that compared to

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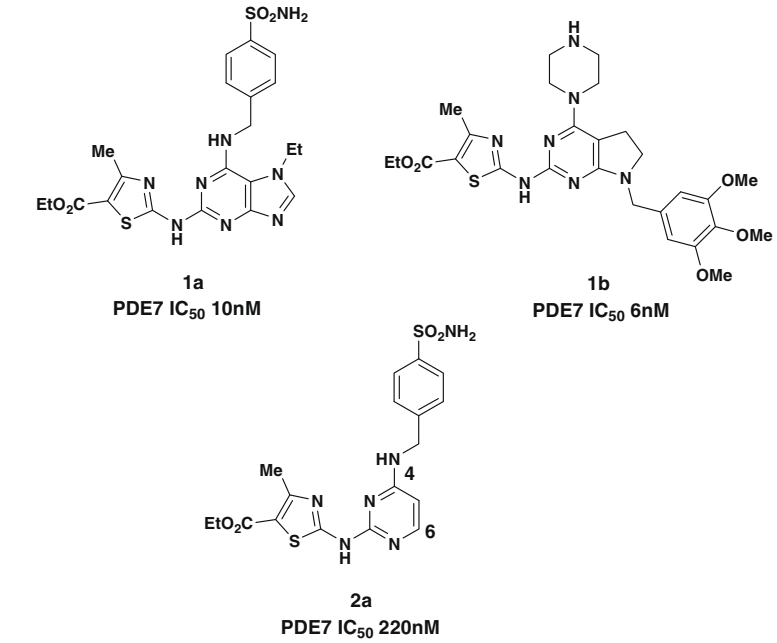
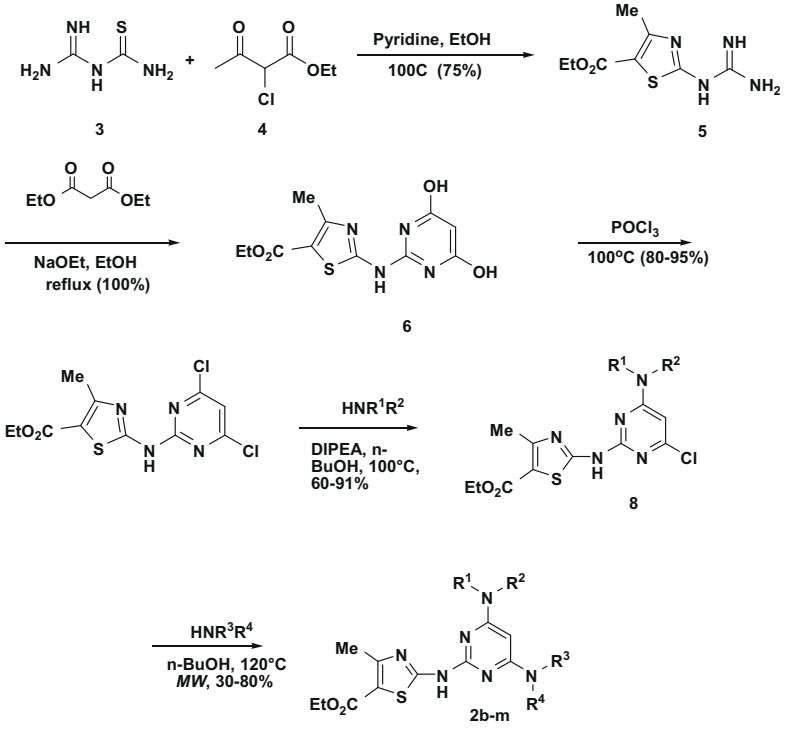
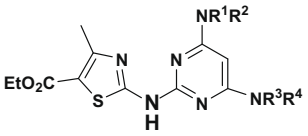


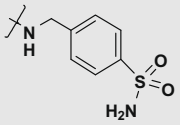
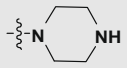
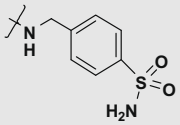
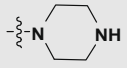
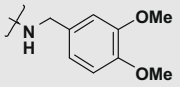
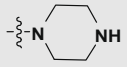
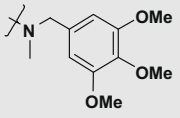
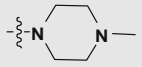
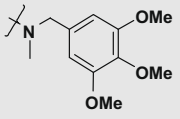
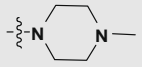
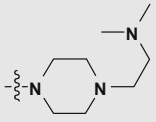
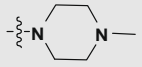
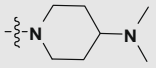
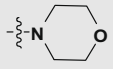
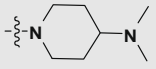
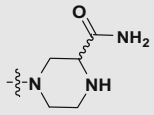
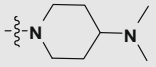
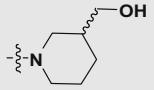
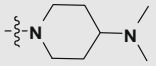
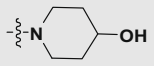
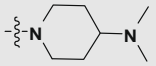
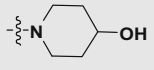
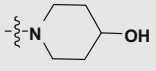
Figure 1. Proposed target **2b**–**m**.



Scheme 1.

Table 1



Compd	–NR^{1}R^2	–N R^{3}R^4	PDE7 IC$_{50}$ (μM)	PDE1 IC$_{50}$ (μM)	PDE3 IC$_{50}$ (μM)	PDE4 IC$_{50}$ (μM)	PDE5 IC$_{50}$ (μM)	T cell IC$_{50}$ (μM)	Cytotoxicity IC$_{50}$ (μM)
2b			0.10	>10	>50	0.030	0.065	0.50	>25
2c			0.010	4.1	2.8	0.22	0.072	7.2	>25
2d			0.031	>10	8.0	2.3	0.32	1.2	12
2e			0.010	7.9	22	3.2	7.6	0.45	14
2f			0.039	4.1	27	5.1	5.2	0.69	>25
2g			0.056	27	19	9.4	>10	2.2	15
2h			0.083	>50	46	>10	>10	2.5	15
2i			0.13	15	13	9.5	>10	2.0	>25
2j			0.082	23	>50	8.0	6.5	3.1	>25
2k			0.12	11	>50	9.3	>10	5.0	>25
2l			0.076	23	>50	>10	>10	1.5	>25
2m			0.063	4.9	20	3.2	0.70	0.12	>25

2a all compounds with a second substituent showed improved potency against PDE7. Compounds **2e** and **1b** which contain the same substitution pattern in different chemotypes were essentially equipotent. This compound also possessed excellent selectivity against PDE1 and PDE3-5. Interestingly, we observed the phenyl ring was not a requirement for PDE7 activity (**2g–m**). Compounds 2c–l all had excellent aqueous solubility at acidic pH, and had aqueous solubility in the range of >20 μg/mL <100 μg/mL at pH 6.5. In general CACO2 permeability for these compounds was high (data not shown) suggesting these compounds had acceptable

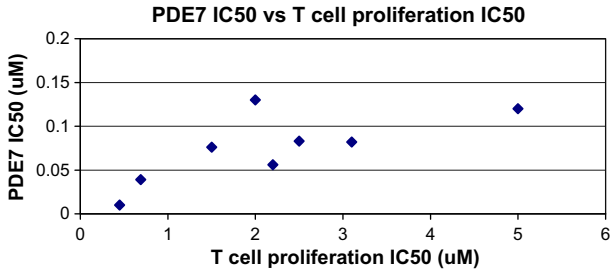


Figure 2.

biopharmaceutical properties.[9] Compound **2m** also maintained comparable aqueous solubility (20 μg/ml at pH 6.5) even in the absence of a basic amine. The compounds displayed PDE7 IC_{50}'s comparable to our earlier reported series together with excellent PDE family selectivity profiles (e.g., **2e–l**).

Since the compounds had sufficient solubility they were examined in an in vitro T cell proliferation assay.[10] Compounds **2b–m** were found to inhibit T cell proliferation in a range of IC_{50}'s from 0.12 μM to 7.2 μM. A graph of the PDE7 IC_{50}'s versus the T cell proliferation IC_{50}'s for the most selective compounds (**2e–l**) suggests a rough correlation, although the data set is limited (Fig. 2). The inhibition of T cell proliferation by these compounds is surprising since we had seen minimal effects on T cell proliferation between wild type and PDE7 KO animals. This discrepancy could result from a number of potential compensatory mechanisms in KO animals. Alternatively the reports of selective PDE7 inhibitors which are devoid of effects in T cells could result from a lack of cell penetration. In an effort to put our results in context we examined compound **2m** in a proliferation assay using splenocytes derived from both wild type and PDE7 KO animals. The percent inhibition values obtained with compound **2m** for both wild type and PDE7 KO animals were virtually identical (wild type IC_{50} = 0.21 μM, PDE7 KO IC_{50} = 0.15 μM). This result suggests that the inhibition reported in Table 1 is not directly related to inhibition of PDE7. There have been reports in the literature of dual inhibitors of PDE4 and PDE7 showing greater potency in assays than might be expected from inhibition of a single PDE.[11] It is not clear that this is the case for **2 m** since the effects observed in cells are at a level below the enzyme IC_{50} for PDE4. Further results from this lab regarding the activity of these and similar compounds will be presented in due course.

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10. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood by density gradient centrifugation over Lymphoprep, 1.077. Cells were plated into 96 well U-bottom plates at 2.5×10^5 cells/well in 10% FBS RPMI 1640 (Life Technologies/Gibco-BRL) containing 10 μg/ml anti-CD3 (G19-4, Bristol-Myers Squibb P.R.I., Princeton, NJ) and 1 μg/ml anti-CD28 (9.3, Bristol-Myers Squibb P.R.I.) in the presence and absence of inhibitors. DMSO (used as a solvent for inhibitors) was added to the medium at 0.2% final concentration. The total volume per well was 200 μl. Cells were incubated at 37C 5% CO_2 for 3 days, at which time 0.5 μCi of 3*H*-thymidine was added to each well. Six hours following the addition of 3*H*-thymidine, the plates were harvested onto filter plates, 30 μl EcoLite scintillant (ICN, Costa Mesa, CA) was added per well, and plates read on a Top Count-NXT scintillation counter.
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