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Identification of purine inhibitors of phosphodiesterase 7 (PDE7)

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Abstract—A series of purine based inhibitors of PDE7 has been derived from screening lead **1a**. The synthesis, structure–activity relationships (SAR), and selectivity against several other PDE family members are described.

Phosphodiesterases (PDEs) hydrolyze the second messenger molecules cAMP and cGMP to affect cellular signaling. At least 11 families of PDE's exist, some of which (PDE3, 4, 7, and 8) are specific for cAMP, and others (PDE5, 6, and 9) for cGMP. Additional family members (PDE1, 2, 10, and 11) have dual specificity. PDE's are involved in a myriad of important physiological functions and as such continue to be a major target for pharmacological intervention on the part of the pharmaceutical industry.[1] A recent publication demonstrated a role for PDE7A in the activation and/or proliferation of T cells.[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 antisense oligonucleotides inhibited proliferation and IL-2 production, and maintained elevated levels of intracellular cAMP in CD3xCD28 stimulated T cells. PDE7A has also been demonstrated to be up-regulated in human B-lymphocytes.[3] PDE7A3, a splice variant of PDE7A1 is also reported to be upregulated in activated $CD4^+$ T cells.[4] This expression profile suggests inhibitors of PDE7A would have broad application as an immunosuppressant. Inhibition of PDE7 has been suggested to be of use in chronic obstructive pulmonary disease and asthma.[5] Additionally PDE7A1 and PDE7A2 have been shown to have significant expression in rat brain although no function has been ascribed to their presence in that tissue.[6]

PDE7B, the second member of the PDE7 family, shares 70% amino acid homology with PDE7A in the catalytic domain. It is expressed in a wide variety of tissues and has been shown to discriminate among several general PDE inhibitors.[7,8]

To date there have been a few low micromolar inhibitors of PDE7 described in the general literature,[9,10] including one guanine system.[11] There has also been a report of PDE7 inhibitors, which have an effect in T cells although these compounds are not entirely selective for PDE7.[12]

Our initial approach to develop inhibitors of PDE7 was prompted by the discovery of lead **1a** from the BMS compound collection (see Fig. 1). The SPA screening assay utilized a whole cell lysate of the Hut78 T cell line as a surrogate for the use of recombinant PDE7A1.[13] A representative panel of known PDE inhibitors were

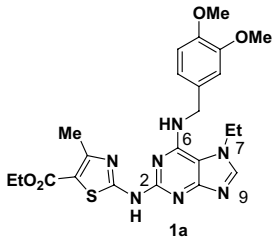

Figure 1. Screening lead **1a**.

Keywords: Phosphodiesterase 7; PDE7; Inhibitor.
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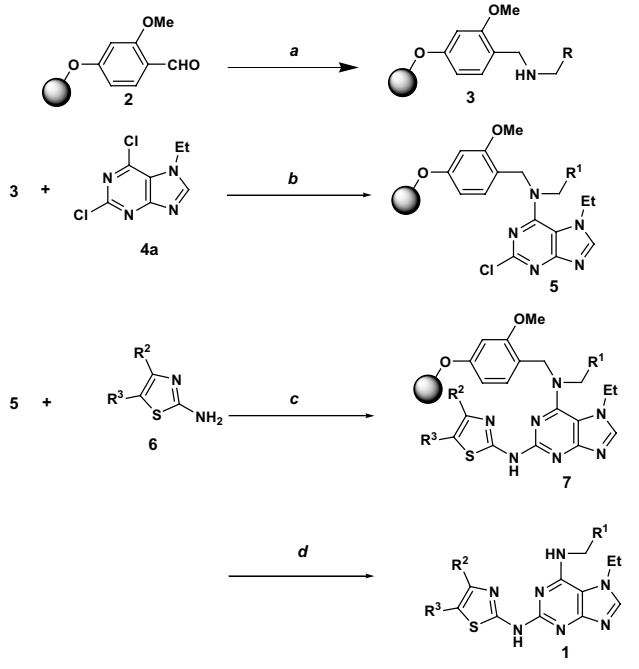
Table 1

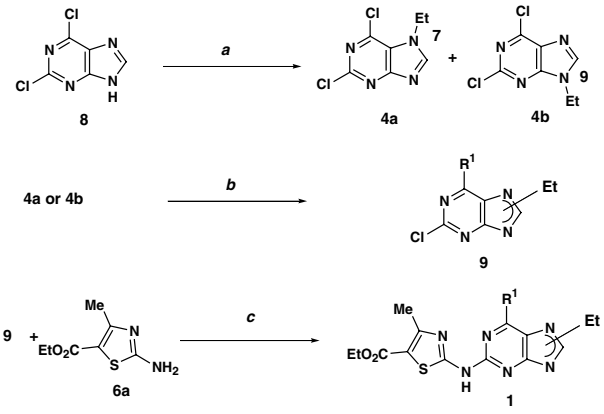
	PDE1 IC_{50} (μM) (PDE1/PDE7)	PDE2 IC_{50} (μM) (PDE2/PDE7)	PDE3 IC_{50} (μM) (PDE3/PDE7)	PDE4 IC_{50} (μM) (PDE4/PDE7)	PDE5 IC_{50} (μM) (PDE5/PDE7)	PDE7 IC_{50} (μM)
Zardaverine	>10	>10	0.11	0.21	>10	>10
8-Methoxy IBMX	1.80	>10	>10	>10	3.02	>10
Cilostamide	>10	>10	0.01	>10	7.15	>10
Rolipram	>10	>10	>10	0.74	>10	>10
Sildenafil	>10	>10	7.0	4.6	0.011	>10
1a	0.22 (1.5)	1.7 (11)	0.58 (3.8)	1.1 (7.3)	0.27 (1.8)	0.15

examined for inhibition of PDE activity in this cell line (see Table 1). Of the PDE inhibitors examined, none demonstrated activity less than 10 μM against PDE7 in our assay. In contrast lead compound **1a** had a PDE7 IC_{50} of 0.15 μM. The selectivity against other PDEs was poor, ranging between 1.5- and 11-fold. This lack of selectivity is interesting. The compound inhibited recombinant PDE7 in a competitive fashion with respect to cAMP (data not shown). One might expect a submicromolar active site inhibitor to discriminate between PDE's to a greater degree with respect to preferred substrate (i.e., cAMP or cGMP) or level of active site homology, however for this lead (**1a**) this was not the case. A focused chemistry effort was initiated to determine if the potency and selectivity could be improved.

One advantage to this lead series was that it had been prepared via a solid-phase synthetic route, which would allow rapid expansion of some SAR. The solid-phase chemistry involved reaction of SASRIN aldehyde resin 2, with the desired amine under reductive amination conditions. Reaction with the appropriate 2,6-dichloro-



Scheme 1. Reagents and conditions: (a) SASRIN aldehyde resin, amine, $ZnCl_2$, BH_3·pyridine, THF, rt, 16 h; (b) Hunigs base, NMP, 85 °C, 16 h; (c) $Pd_2(dba)_3$, (+)-TolBINAP, *t*-BuONa, dioxane/toluene, 110 °C, 16 h; (d) TFA, dichloromethane.



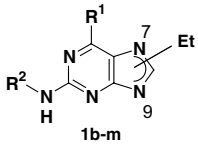
Scheme 2. Reagents and conditions: (a) Na_2CO_3, EtI, acetone, reflux 3 h (total yield 72–80%); (b) Hunigs base, amine, NMP, 85 °C, 16 h (85–93%); or NaH, and arylsulfonamide (40–45%) or alcohol (50–65%); (c) $Pd_2(dba)_3$, 2-(di-*t*-butylphosphino)biphenyl, anhyd K_3PO_4, DMA, 110 °C, 2–4 h (35–51%).

7-ethylpurine, provided the 6-substituted purine **5**. Amination at the 2-position was accomplished using Buchwald conditions.[14] The final products **1** were obtained by cleavage from the resin using TFA (Scheme 1).

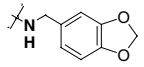
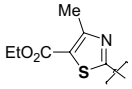
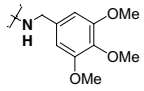
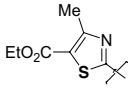
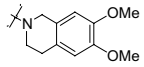
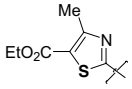
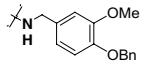
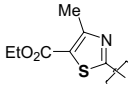
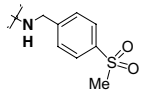
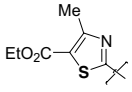
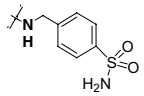
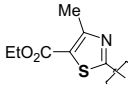
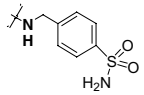
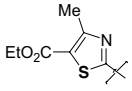
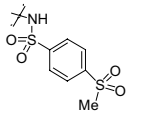
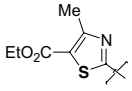
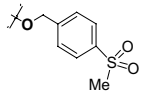
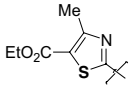
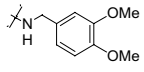
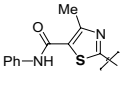
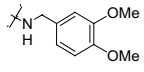
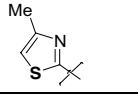
In order to obtain compounds with greater diversity at the 6-, and 7/9-positions on the purine ring, and avoid complications with solid phase TFA deprotection, solution phase chemistry was employed as depicted in Scheme 2. 2,6-Dichloropurine (**8**) was alkylated with ethyl iodide to provide of **4a** and **4b** in approximately a 1 to 4 ratio. After separation by flash column chromatography, the chlorine at the 6-position of the purine could be readily displaced with amines to produce intermediate **9**. Alternatively the chlorine could be displaced with the sodium salt of either sulfonamides or alcohols to produce intermediate **9**. Thiazole **6a** was originally coupled to **9** in low (12%) yield under the Buchwald conditions utilized for the resin bound synthesis. A wide variety of conditions were examined in an effort to improve the coupling yield with this amine. The use of a new ligand[15] for palladium with potassium phosphate as the base in DMA improved the reproducibility and yields to a range of 35–50%. The analogues prepared were examined for PDE inhibition.

The SAR for the inhibition of PDE catalytic activity are summarized in Table 2. Low inhibitor selectivity between PDE7 and PDE4 might be anticipated based on several considerations. PDE4 was originally classified as a high affinity cAMP specific enzyme based on a K_m for

Table 2. SAR of **1b–l** with respect to PDE inhibition, PDE#/PDE7 represents IC_{50} ratio



1b-m

Compd	R^1	R^2	Et position	PDE7 IC_{50} μM	PDE1/ PDE7	PDE2/ PDE7	PDE3/ PDE7	PDE4/ PDE7	PDE5/ PDE7
1b			7	0.11	19	NT	11	NT	0.38
1c			7	0.10	1.7	14	33	24	15
1d			7	0.15	2.8	3.6	17	1.3	4.6
1e			7	0.94	1.3	2.3	2.5	3.8	2.9
1f			7	0.055	18	25	130	22	0.90
1g			7	0.011	86	86	270	50	1.0
1h			9	0.010	200	45	>5000	16	13
1i			9	1.6	NT	NT	0.11	0.046	0.55
1j			9	0.37	NT	NT	21	NT	4.1
1k			7	0.82	12	11	12	7.0	1.1
1l			7	15% @ 1 μM	NT	NT	NT	NT	NT

cAMP of approximately 4 μM. The K_m for cAMP for PDE7 has been determined to be 0.2 μM. Additionally the K_m for cAMP for PDE8 is reported to be even lower (0.05 μM).[16] Another selectivity consideration is the degree of homology in the enzyme active site among the PDE family members (see Fig. 2). It is interesting to note that the compounds reported here generally demonstrated low selectivity against PDE5 despite this enzymes high K_m for cAMP (150 μM) and low homology to PDE7.

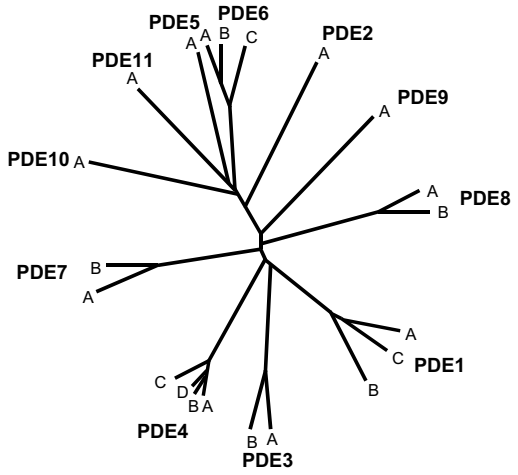


Figure 2. Dendrogram of PDE active site homology.[18]

Cursory examination of the lead structures **1a** and the analogues **1b** through **1e** suggests similarity with the 3,4-dialkoxyphenyl motif observed in many PDE4 inhibitors.[17] A logical approach to improve selectivity against PDE4 would be to vary R^1.

A significant (16- to 50-fold) improvement in PDE4 selectivity and an increase in PDE7 potency was observed when the phenyl substitution pattern was changed in analogues **1f**–**h**. These analogues also demonstrated improved selectivity against PDE1, PDE2, and PDE3. Analogue **1h** demonstrated a significantly improved selectivity against PDE3 when compared to the 7-ethyl analogue **1g**. Additionally **1h** was approximately 100-fold selective against PDE6 (data not shown) and greater than 1000-fold selective against PDE8 (data not shown). Based on the selectivity data presented it is difficult to draw any correlation between the degree of PDE homology and inhibitor selectivity for this set of compounds. Unfortunately **1g** and **1h** have an aqueous solubility at pH 6.5 of less than 5 μg/mL. A suitable formulation for iv dosing could not be identified. Oral dosing of **1h** at 100MPK as a suspension produced peak plasma concentrations of less than 150 nM. A sulfonamide or oxygen linker were tolerated (**1i**, **1j**). Analogue **1l**, which does not contain an ester (e.g., **1a**) or amide (**1k**) is significantly less potent. Additional SAR studies on the thiazole are needed to better understand the observed loss in potency.

In summary we have reported the identification of a potent purine based inhibitor of PDE7 (**1a**) by screening the BMS compound collection. An exploratory chemistry effort demonstrated that the potency and PDE selectivity could be improved (e.g., **1h**). Future reports from our laboratory will describe efforts to further improve the selectivity of these lead compounds for PDE7 over other PDE family members and address issues such as solubility, which will permit the in vivo evaluation of PDE7 inhibitors.

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13. Inhibition of PDE activity in Hut78 cell lysate was determined using an SPA specific for cAMP (Amersham Pharmacia Biotech, Buckinghamshire, UK) according to the manufacturers instructions with minor modifications. Enzyme assays were performed at room temperature in the presence of 50 mM Tris–HCl, pH 7.5, containing 8.3 mM $MgCl_2$, 1.7 mM EGTA, and 0.5 mg/mL BSA. Each assay was performed in a 100 μL reaction volume in 96 well microtitre plates containing the above buffer, 0.3 μL of Hut78 cell lysate treated with 2 μM Zardaverine to inhibit PDE3 and PDE4, 0.05 μCi of [5′,8-^{3}H] Adenosine 3′,5′-cyclic phosphate as an ammonium salt for 20 min. The reaction was terminated by the addition of 50 μL PDE SPA beads (1 mg) water with 10 mM cold cAMP (Sigma, St. Louis MO). The reaction mix was allowed to settle for 20 min before counting in a Top Count-NXT scintillation counter (Packard BioScience, Meriden, CT). For selectivity studies, the assay was essentially unchanged except that ^{3}H-cyclic GMP was used as the substrate for PDE1, PDE5, and PDE6. The following PDEs/activators and enzyme sources were used: PDE1, bovine (Sigma St. Louis), calmodulin; PDE2, rat kidney, cGMP; PDE3, human platelet; PDE4, rat kidney; PDE5, human platelet, and PDE6, bovine retina.
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