Drugs of the Future 2006, 31(3): 207-229

CCC: 0377-8282/2006
DOI: 10.1358/dof.2006.031.03.966246

Review Article

Phosphodiesterase 7 (PDE7) as a therapeutic target

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Abstract

The concept that targeting phosphodiesterase type 4 (PDE4) with small-molecule inhibitors could lead to the discovery of novel, steroid-sparing compounds with utility in treating a multitude of diseases associated with chronic inflammation has gained general acceptance over the last 20 years. A fundamental obstacle that has yet to be overcome is to develop compounds that dissociate beneficial from adverse events. Unfortunately, both of these activities of PDE4 inhibitors represent an extension of their pharmacology and improving the therapeutic ratio has proved to be a major challenge. Several strategies have been considered with some degree of success, but compounds with an optimal pharmacophore that have been validated in human subjects have not yet been reported. An alternative approach is to inhibit other cAMP PDE families that are also expressed in immune and proinflammatory cells, in the hope that the beneficial activity can be retained in the absence of side effects. One such candidate is PDE7. In this article we review the literature on PDE7 and explore the possibility that selective small-molecule inhibitors of this enzyme family could provide a novel approach to treat a variety of immunological and immunodeficiency conditions.

Introduction

An enzyme that hydrolyzes the 3'-ribose phosphate bond of cAMP to the catalytically inactive 5'-adenosine monophosphate was identified more than 40 years ago (1). Since that time, 11 molecularly, biochemically and immunologically distinct enzyme families –collectively known as phosphodiesterases (PDE)– that selectively degrade cyclic purine nucleotides have unequivocally been identified (2-6). Phosphodiesterases that act on cyclic pyrimidine monophosphates have also been discovered, but they have received relatively little attention and, instead, most investigators have focused on those enzymes that hydrolyze cAMP and cGMP, for which functionally important second messenger roles have been firmly established.

Phosphodiesterases have proven to be very "drugable" targets, with compounds on the market or in late-stage clinical development for a variety of diseases. Theophylline was probably the first PDE inhibitor to be used clinically for the treatment of asthma, where it acts as a bronchodilator. However, the recent appreciation of PDE heterogeneity subsequently led to the synthesis of highly selective inhibitors, which have demonstrated efficacy in treating a variety of disorders, including intermittent claudication (PDE3) (7), chronic inflammation (PDE4) (8-10), male erectile dysfunction (PDE5) (11) and pulmonary hypertension (PDE5) (12). Furthermore, the identification of at least 11 distinct families of PDE isozymes raises the prospect that other PDEs may in the future be exploited to therapeutic advantage.

In this article we explore the possibility that PDE7 may represent a novel therapeutic target for the treatment of a host of immunological and immunodeficiency conditions that could include asthma, chronic obstructive pulmonary disease (COPD), Crohn's disease, myasthenia gravis, atopic dermatitis, psoriasis, systemic lupus erythematosus, rheumatoid arthritis, diabetes and multiple sclerosis, where the proliferation and/or differentiation of T-lymphocytes is implicated in disease pathogenesis.

Discovery and characteristics of PDE7

In 1993, Micheali *et al.* (13) reported the development of a highly sensitive functional screen for the isolation of

cDNAs that encode cAMP PDEs, by complementation of defects in a strain of the yeast *Saccharomyces cerevisiae* that lacks both endogenous cAMP PDE genes. Three groups of cDNAs were isolated from a human glioblastoma cDNA library using this technology. Two of those genes were closely related to the *Drosophila* "dunce" cAMP PDE (*i.e.*, PDE4-like), while the third encoded an enzyme that readily degraded cAMP but with characteristics distinct from all other known PDEs. At that time, the new PDE was given the name high-affinity cAMP-specific phosphodiesterase 1 (HCP-1). Primary sequence analysis established that HCP-1 shared significant homology with a stretch of 300 *C*-terminal amino acids that constitute the catalytic domain of other mammalian cAMP PDEs (13). Within this highly conserved region, HCP-1 exhibited the highest and lowest degree of homology to PDE4 (35% identity; 51% similarity) and PDE2 (24% identity; 37% similarity) family members, respectively. However, given that the homology in the catalytic core between PDE4 variants varies between 85% and 95% and that cAMP hydrolysis by HCP-1 was insensitive to the selective PDE4 inhibitors Ro-20-1724 and rolipram, it was concluded that the novel enzyme represented the first member of a previously unknown cAMP PDE family and was designated PDE7 (13). It is noteworthy that the discovery of PDE7 coincided with a report published at the same time of an atypical cAMP hydrolytic activity (called JK-21) in several human T-cell lines, including Jurkat, MOLT-4, HPB-ALL and HUT-78 (14), which is now known to be a PDE7 family member, most likely PDE7A1 (15) (see below for description of PDE7 gene products).

PDE7 isogenes

Two genes (*PDE7A*, *PDE7B*) have been identified in mice, rats and humans that encode PDE7 isozymes (13, 16-19). Using fluorescence *in situ* hybridization, Han *et al.* (20) localized murine *PDE7A* to the proximal region of chromosome 3 between the genes that encode interleukin (IL)-7 and corticotropin-releasing hormone (CRH). This area of murine chromosome 3 is syntenic with the 8q region of human chromosome 13, where human *PDE7A* is located (20, 21). Currently, no diseases or mutations have been mapped to the vicinity of the *PDE7A* locus. Nevertheless, the localization of this gene in humans should permit in the future the detection of novel mutations or pathologies that are related to defects in PDE7A catalytic activity (20).

Human and murine *PDE7B* map to chromosomes 6q23-q24 and 10, respectively (17). Interestingly, a human gene, *EPM2A* (epilepsy, progressive myoclonic epilepsy, type 2 gene alpha), encoding a protein tyrosine phosphatase, is related to Lafora's disease, which is a progressive myoclonus epilepsy that has been identified at 6q24 (22). Given that a loss of function of the heteromultimeric cAMP-regulated K_M channels, which are composed of subunits encoded by the *KCNQ2* and *KCNQ3* genes, may lead to electrical hyperexcitability in the CNS and predispose to benign familial neonatal convulsions –an autosomal dominant epilepsy of young children (23)–, the linkage of *PDE7B* and *EPM2A* is of interest. Indeed, cAMP-elevating drugs may prove beneficial in this form of epilepsy (23).

Gene structure, variants and post-transcriptional regulation of *PDE7A*

Figure 1 shows the genomic organization of *HSPDE7A*. The coding region of this gene spans 124 kb and contains 14 exons (24). Screening of murine and human skeletal muscle DNA libraries has identified three splice variants (*PDE7A1, PDE7A2, PDE7A3*) derived from the same gene. Spicing occurs at the 5'-end of the mRNA and the intron/exon boundaries have been confirmed by isolation and sequencing of human genomic clones (15, 25). *HSPDE7A2* begins at exon 2, which is spliced out in the other two isoforms (Figs. 1 and 2) (24), whereas *PDE7A3* diverges from *PDE7A1* at G^{415} and has a short and unique *C*-terminus that is truncated at residue 424 (Figs. 1 and 2). All three variants contain a potential cAMP-dependent protein kinase (PKA) phosphorylation site (S^{84} in *PDE7A1* and *PDE7A3*; S^{58} in *PDE7A2*) (25, 26). In addition, *PDE7A1* and *PDE7A3* feature a repeat PKA pseudosubstrate binding site sequence (RRGAIS) at their *N*-termini, which is found only once in *PDE7A2* (Fig. 2) (25, 26). These may be potentially important findings, as they imply that the activity of PDE7A isozymes may be regulated post-transcriptionally by cAMP (see below).

Translation in humans of *PDE7A1, PDE7A2* and *PDE7A3* mRNA transcripts yields proteins that are composed of 482, 456 and 424 amino acids, respectively (Fig. 2). On SDS polyacrylamide gels, the three splice variants migrate as 55-57- (7A1), 50-52- (7A2) and 50-kDa (7A3) proteins, which are similar to the weights predicted from their amino acid composition (55,506, 52,726 and 48,828 Da, respectively). The *N*-terminus of *PDE7A1* and *PDE7A3* is rich in proline, serine and positively charged amino acids, whereas the same region of *PDE7A2* is hydrophobic (15, 25, 26) and features potential myristoylation (G^2) and palmitoylation (C^8) sites that may dictate intracellular localization (Fig. 2). Indeed, following subcellular fractionation of a variety of tissues, *PDE7A2* has been found only in the particulate fraction, consistent with the hydrophobicity of its *N*-terminus; in contrast, *PDE7A1* is predominantly cytosolic (15, 26). The subcellular distribution of *PDE7A3* has not been investigated but it shares the same *N*-terminus as *PDE7A1* and could localize to soluble cellular structures.

Regulation of *PDE7A1* expression

Despite the large number of PDE genes that have been discovered (23 at the time of writing; 6), the identification and characterization of promoter regions have been poorly studied and are restricted to surprisingly few genes, such as *PDE3B* (27, 28), *PDE4A* (29, 30) *PDE4D* (31, 32), *PDE5A* (33-36) and *PDE6A* (37). In 2003,

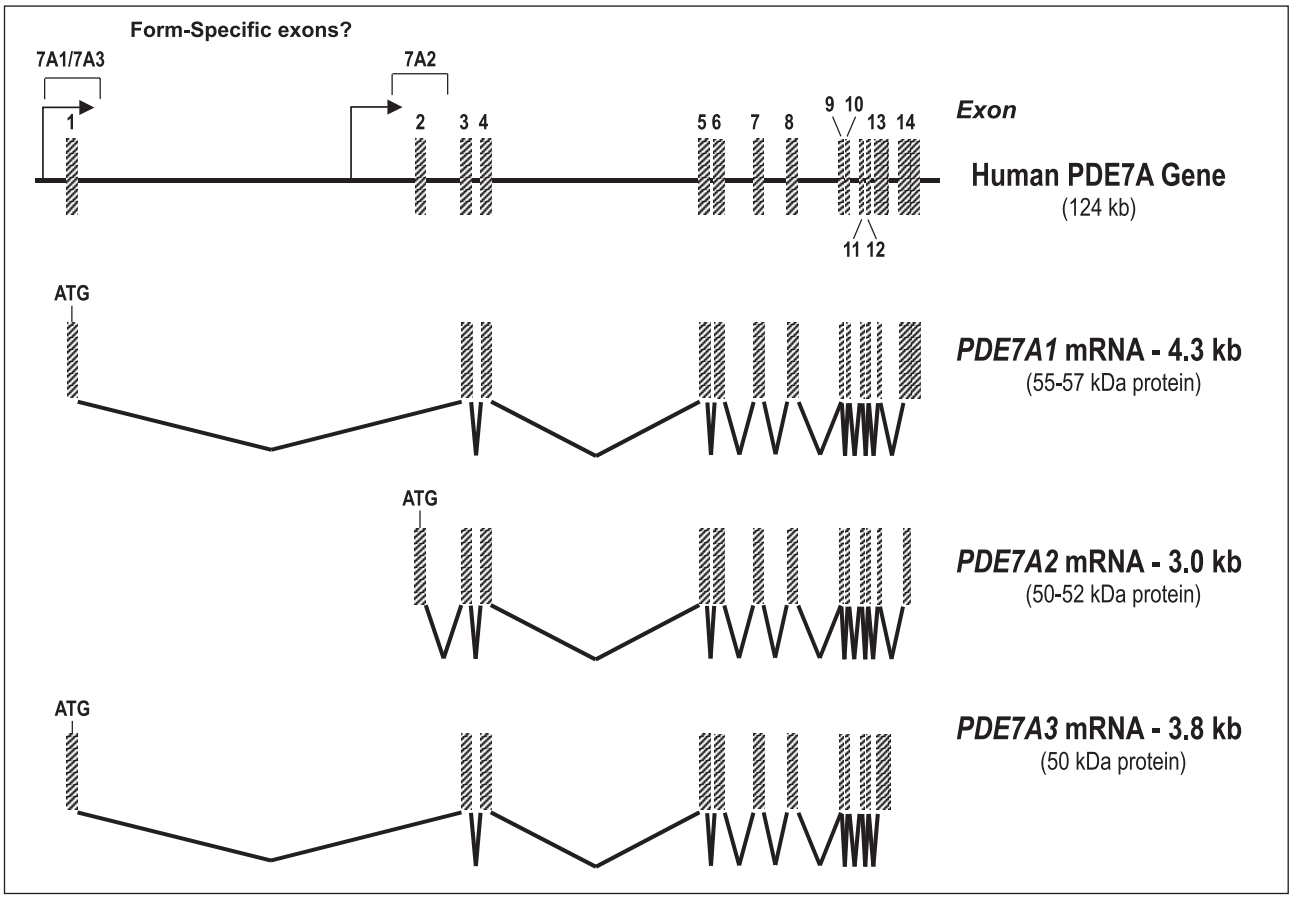


Fig. 1. Schematic representation of the structure of the 142-kb *PDE7A* locus and mRNA transcripts derived from two potential transcriptional units. Exons are numbered and presented as hatched vertical bars; introns are drawn as solid lines. The electrophoretic mobility of the proteins encoded by each mRNA is shown in parentheses below the name of each variant. Modified from Ref. 24.

Torras-Llort and Azorin extended this list of genes to include human *PDE7A,* with emphasis on the *PDE7A1* promoter (24).

Consistent with the longest reported *HSPDE7A1* cDNA deposited with the GenBank database (accession number L12052), Torras-Llort and Azorin (24) confirmed in 2003 the presence of an unusually short 5'-untranslated region of only 50 bp in length. In addition, the 5'-region immediately flanking the mapped +1 position was shown to contain several features that are indicative of a *cis*-regulatory domain, including genomic sequences that are rich in the CpG pattern. These so-called "CpG islands" are important because they are resistant to methylation, a covalent modification that normally promotes transcriptional silencing, and tend to be associated with genes that are frequently induced. Indeed, transient transfection of Jurkat T-cells with a 2907-bp fragment upstream of the *PDE7A* transcriptional start site was found to have strong promoter activity that was reduced by 40% following deletion of 1.5 kb of sequence to –1425. A larger deletion to –988 had no further impact on promoter activity, whereas the sequence –2900 to –1116 by itself was inert, indicating that the proximal region up to –988 retains most of the promoter activity. Significantly, 5'-deletion to position –560, which eliminates part of the CpG island, abolished promoter activity (24).

Within the 5'-half of the CpG island are putative binding domains for a number of transcription factors that may regulate *HSPDE7A* expression. These include three cAMP response elements (CRE), and sites for stimulatory protein-1 (Sp-1), nuclear factor-κB (NF-κB), nuclear factor of activated T-cells-1 (NF-AT-1) and E-twenty six-2 (Ets-2) (24). Thus, it is likely that *PDE7A* is regulated by multiple transcription factors in addition to CRE-binding protein (CREB). Torras-Llort and Azorin (24) have reported that a 1.4-kb promoter construct to position –1400 contains all three CREs and is induced and repressed, respectively, in Jurkat T-cells following co-transfection with a plasmid overexpressing CREB or KCREB, a dominant negative mutant form of CREB. However, while these data imply that *HSPDE7A* is probably regulated by CREB, neither dibutyryl-cAMP nor forskolin activated the promoter construct (24). Similarly, overexpression of the catalytic subunit of the α-isoform of protein kinase A (PKA) in Jurkat T-cells transfected with the –998-kb or –1.4-kb promoter constructs was inactive (24). These results are consistent with a lack of effect of 8-Br-cAMP and the β_2-adrenoceptor agonist fenoterol on PDE7A

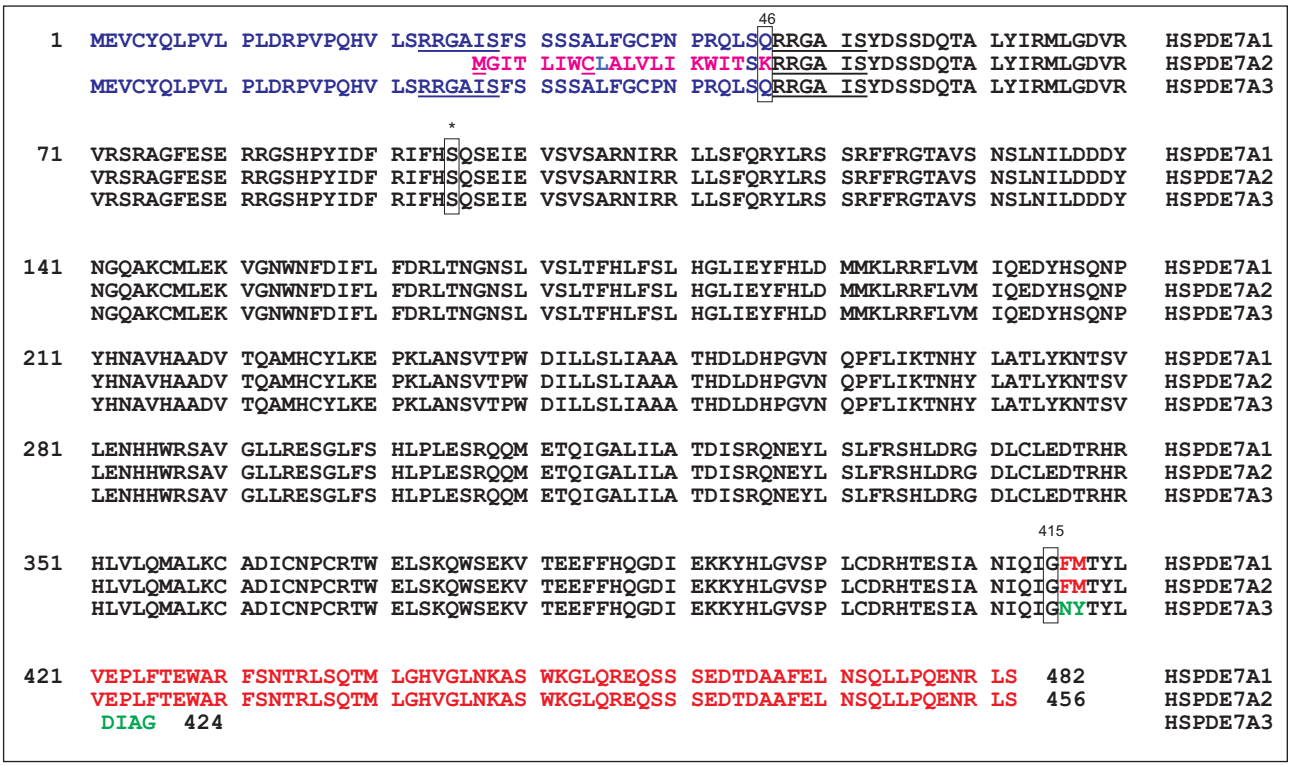


Fig. 2. Amino acid sequence alignment of the open reading frames of HSPDE7A isozymes. The unbroken sequence of 370 amino acids common to all three variants is shown in black (Q^{46} to G^{415} in PDE7A1 numbering). Amino acids shown in blue are common to the *N*-terminus of PDE7A1 and PDE7A3 isoforms, whereas those highlighted in purple are unique to PDE7A2, which is also shorter by 26 amino acids as it starts at exon 2. The *C*-termini of PDE7A1 and PDE7A2 are identical (shown in red), but in PDE7A3 the sequence is unique (shown in green) and truncated at G^{424}. All three proteins present a potential PKA phosphorylation site at S^{84} in PDE7A1 numbering (identified by an asterix). PDE7A1 and PDE7A3 also feature repeat PKA pseudosubstrate binding sites (RRGAIS; blue underline) at their *N*-termini. A single RRGAIS is found within the *N*-terminus of PDE7A2 3' to the unique region, which features potential sites of myristoylation (G^2) and palmitoylation (C^8) (purple underline).

expression in human T-lymphocytes (38), and have led to the suggestion that the *PDE7A1* promoter is constitutively activated by CREB, such that cAMP-elevating drugs have no effect on transcription (24). Indeed, all three of the CREs showed strong dimethyl sulfate (DMS) footprints, indicating that they are constitutively occupied, most likely by CREB. It is noteworthy that this finding in T-lymphocytes may be cell type-specific, as agents that elevate cAMP in B-cells effectively increase PDE7A expression (39).

In contrast, the –2.9-kb construct was induced by the phorbol diester phorbol myristate acetate (PMA), implicating conventional (α, β_1, β_2) or novel (δ, ε, η, θ, μ) protein kinase C (PKC) isoforms in regulating the expression of *HSPDE7A1.* Consistent with this finding is the increased (~ 7-fold) expression of PDE7A1 in human monocytes following long-term treatment (24 h) with PMA (Fig. 3; Smith, S.J., Giembycz, M.A., unpublished data). The induction of the *PDE7A1* promoter by PMA in Jurkat T-cells is associated mainly with the CpG island, and this may reflect the participation of other transcription factors, such as Ets-2, NF-AT-1 and NF-κB, which bind to the promoter and are activated by PKC (40, 41). Upregulation of PDE7A by PMA may be relevant to the mechanisms that perpetuate chronic inflammation in diseases such as asthma and COPD, as many proinflammatory chemokines and cytokines are known to signal, in part, through PKC (42-44). However, it is interesting that the steady-state levels of PDE7A1 and PDE7A2 mRNA in human umbilical vein endothelial cells are reduced in a time-dependent manner following their exposure to tumor necrosis factor-α (TNF-α) (45) demonstrating that cytokines can also repress PDE7A and/or reduce mRNA stability. Whether a commensurate reduction in protein expression accompanied the drop in mRNA in these cells was unexplored.

Regulation of PDE7A has also been explored in human peripheral blood $CD4^+$ T-lymphocytes that lack CD25 (IL-2 receptor) and human leukocyte antigen (HLA)-DR (46). Co-ligation of CD3 and CD28 with activating antibodies leads to increased expression of PDE7A1 at the mRNA and protein level (25, 46). However, it is important to qualify these data, as this population of cells accounts for a small fraction of the total $CD4^+$ T-cell pool and represents a quiescent or nonproliferating phenotype. Indeed, $CD4^+/CD25^+/HLA\text{-}DR^+$ cells express, constitutively, high levels of PDE7A1 mRNA and protein and no further increase is seen in response to anti-CD3/anti-CD28

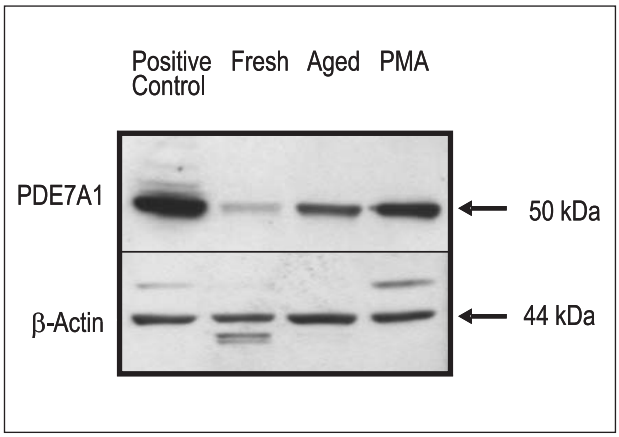


Fig. 3. Effect of aging monocytes and PMA on the expression of HSPDE7A1. Cells were cultured for 24 h in RPMI or exposed to PMA (1 μM) and then subjected to Western blotting using a rabbit anti-PDE7A antibody. HUT-78 cells were used as a positive control.

antibodies (25, 47, 48). The signaling mechanism(s) of PDE7A induction has not been examined.

Regulation of *PDE7A2* and *PDE7A3* expression

The splice variants of *PDE7A* are differentially expressed across some cells and tissues. Thus, *PDE7A1* and *PDE7A3* are prominent in cells of the lung and immune system (25, 48), whereas skeletal muscle, heart and kidney are rich sources of *PDE7A2* (15, 26). To account for this tissue-specific expression, it is likely that the *PDE7A* gene is subject to complex regulatory control. Rapid amplification of cDNA ends (RACE) using primers complementary to sequences within the first exon that are common to both *PDE7A1* and *PDE7A3* produces a single product, indicating that transcription of these splice variants is under the control of the same promoter (24, 25). In contrast, the transcription start site of *PDE7A2* maps to exon 2 of *PDE7A* (26), which is located ~ 50 kb away from the +1 position of *PDE7A1*. Thus, *PDE7A2* may be regulated by a second promoter located in the first intron (see Fig. 1). This differential control of gene expression is not a peculiar finding and is shared by other PDE genes, including *PDE4D*, where an intronic promoter regulates the expression of PDE4D1 and PDE4D2, so-called “short forms” of the enzyme (31). At the time of writing, there was no further information on the regulation or expression of *PDE7A2*.

Activating antibodies directed against CD3 and CD28 have been reported to upregulate *PDE7A3* in $CD25^{-}$/$HLA\text{-}DR^{-}/CD4^{+}$ T-lymphocytes in a manner that is reminiscent of *PDE7A1* (25). However, although transcription of both splice variants is probably driven by the same promoter (24), the appearance of mRNA transcripts followed distinct kinetics, with *PDE7A1* being upregulated quickly relative to *PDE7A3* ($t_{1/2}$ approximately 30 min and 4 h, respectively) (25), which may relate to differences in mRNA stability.

PDE7B variants and their regulation

Using a bioinformatics approach to identify novel PDEs, Sasaki *et al.* in 2000 isolated a cAMP PDE cDNA from human caudate nucleus (17). Initially, an expressed sequence tag (EST) database of a murine mammary gland cDNA library was searched using the amino acid sequence of *HSPDE7A* as a query. This procedure identified one clone that was similar but not identical to the *N*-terminus of *PDE7A.* A human cDNA that corresponded to the murine EST orthologue was then cloned by PCR and the sequence extended by 3'- and 5'-RACE with human caudate nucleus cDNA as a template (17). The open reading frame (ORF) of the cDNA encoded a 450-amino-acid protein with a predicted molecular mass of 51,836 Da. Within the catalytic domain, this PDE showed the highest degree of homology (67.1%) to HSPDE7A. As PDEs with greater than 65% homology within their catalytic core are normally considered members of the same family, this new enzyme was named PDE7B. Independent identification of HSPDE7B was reported by Gardner *et al*. (49) later in 2000.

Mouse and rat orthologues of PDE7B have also been found (16, 19, 49). The murine protein is composed of 446 amino acids and has a predicted molecular weight of 51,308 Da. Interestingly, HSPDE7B has a different *C*-terminus to the orthologous murine protein, suggesting that the *PDE7B* gene may be subject to alternative mRNA splicing (17). Indeed, Northern analyses have detected transcripts of 6.2 and 3.1 kb in a number of human tissues, which is consistent with this hypothesis (19), although at the time of writing, no further information on human *PDE7B* multiplicity was available. However, three rat cDNAs encoding distinct proteins of 446, 359 and 459 amino acids that correspond to RNPDE7B1 (51,476 Da), RNPDE7B2 (41,457 Da) and RNPDE7B3 (52,681 Da), respectively, have been identified (19) (Fig. 4).

Like PDE7A, putative PKA phosphorylation sites (RRGSY) have been identified in PDE7B (17, 19). In the rat proteins, a PKA consensus sequence was found at the *N*- and *C*-terminus of both PDE7B1 (S^{45} and S^{426}) and PDE7B3 (S^{58} and S^{439}), whereas in PDE7B2 only a single site (S^{339}) at the *C*-terminus was found (19). All of the RNPDE7B splice variants are serine phosphorylated by PKA *in vitro* and in intact cell systems, which presumably has an impact on enzyme activity (19). In RNPDE7B1 and RNPDE7B3, PKA promotes serine phosphorylation at RRGSY sites at both the *N*- and *C*-termini (19).

Regulation of *PDE7B1* expression

No information is currently available on the regulation of the human *PDE7B* gene, although data are available for *RNPDE7B1*. 5'-RACE experiments have localized the transcription start site within *RNPDE7B1* to a position 350 bp upstream of the first methionine (50). Using the on-line database TFSEARCH, Sasaki *et al.* (50) identified several putative motifs for transcription factors in the *PDE7B1* promoter, including a canonical CRE (at –166 bp) and

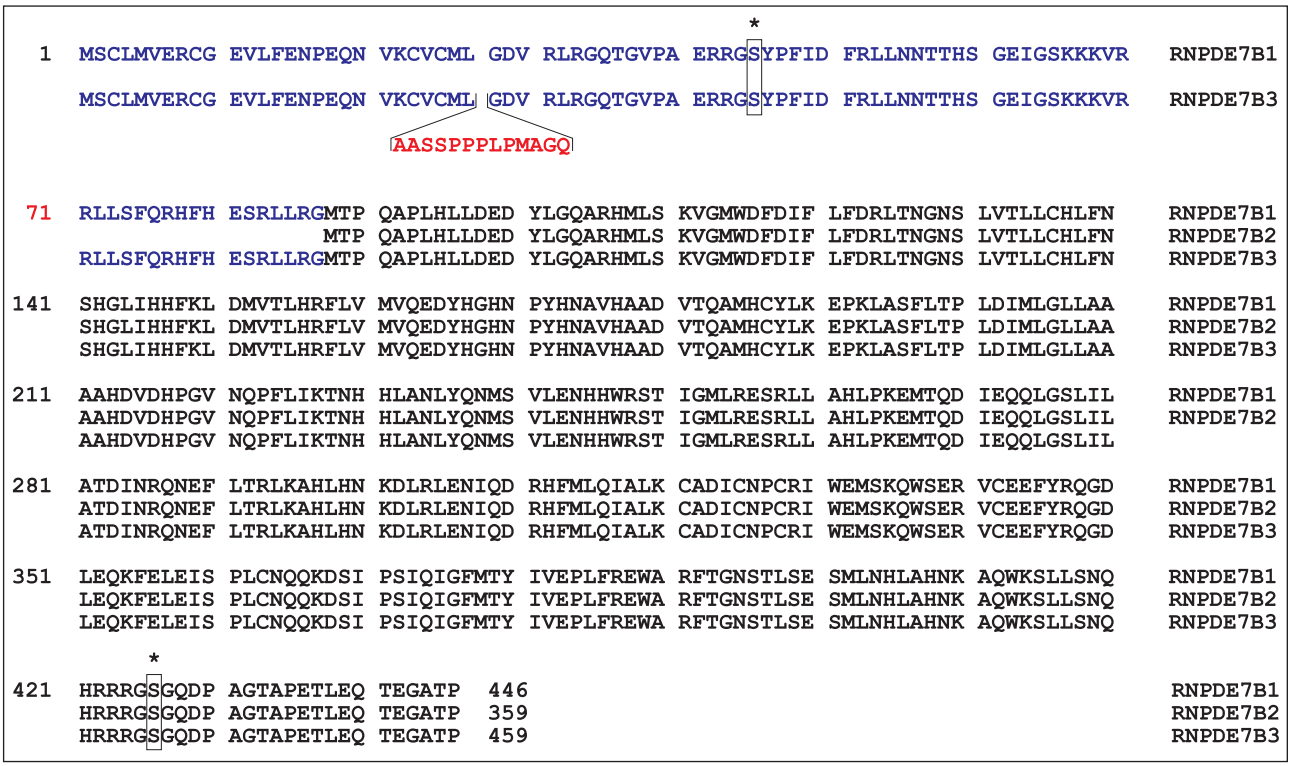


Fig. 4. Amino acid sequence alignment of the open reading frames of RNPDE7B isozymes. The unbroken sequence of 360 amino acids common to all three variants is shown in black (M^{86} to P^{446} in PDE7B1 numbering). Amino acids shown in blue are common to the *N*-terminus of PDE7B1 and PDE7B3. These residues are absent in RNPDE7B2, which is shorter by 87 amino acids. PDE7B3 features an additional 13 amino acid residues within the *N*-terminal region of PDE7B1 (shown in red). The *C*-termini of PDE7B1, PDE7B2 and PDE7B2 are identical. All three proteins feature potential PKA phosphorylation sites (RRGSY), which are identified by an asterix. In PDE7B1 and PDE7B3, these are found in both the *N*- and *C*-termini, whereas in PDE7B2 only a site at the *C*-terminus was found.

sites for activator protein-1 (AP-1; at –1014 bp), myeloid zinc finger protein-1 (MZF-1; between –1249 and –1027 bp) and sex region Y (SRY; at –715 bp). Stimulation with forskolin of reporter constructs harboring the full-length (–1418 bp) and deletion mutants of the rat *PDE7B* promoter upstream of a luciferase gene activated all constructs that contained a CRE, but not those in which the CRE was deleted (50). Moreover, nucleotide substitutions within the CRE site abolished the ability of forskolin to transactivate the luciferase gene, indicating that gene expression was driven by a CRE-dependent mechanism (50).

Evidence of cAMP-dependent induction of *PDE7B* has also been reported in intact cells. Sasaki *et al.* found that dopamine and the selective dopamine D1 receptor agonist SKF-82958 increased mRNA levels in rat striatal neurons by 60-70% at 6 h, which then declined to baseline by 12 h (50). This effect was abolished by Sch-23390, but not by raclopride, dopamine D1- and D2-selective antagonists, respectively (50). Actinomycin D also prevented *PDE7B* induction, indicating a need for transcription rather than stabilization of pre-existing mRNA. The dopamine D1 receptor is known to positively couple to adenylyl cyclase via Gs, and a role for cAMP in the increase in PDE7B mRNA was further supported by the findings that the effect of dopamine was enhanced by 3-isobutyl-1-methylxanthine (IBMX) and mimicked by forskolin and 8-Br-cAMP. Furthermore, the singular effect of all cAMP-elevating agents on *PDE7B* expression was abolished by the nonselective PKA inhibitor H-89. Using primers specific for all three *PDE7B* splice variants, Sasaki *et al*. (50) also showed that the increase in PDE7B mRNA evoked by dopamine and forskolin was due entirely to the PDE7B1 transcript.

Tissue distribution of PDE7 isoforms

PDE7A and PDE7B are not distributed equally and are thus believed to subserve nonoverlapping, tissue-specific functions. Thus, while PDE7A is abundantly expressed in the lungs, hematopoietic cells, placenta, Leydig cells, spleen, human cavernous smooth muscle, collecting tubules of the kidney and the adrenal glands (both cortex and medulla), high levels of PDE7B are detected in the pancreas, heart, thyroid and skeletal muscles (16, 17, 48, 49, 51-53). However, PDE7A and PDE7B mRNA are also co-localized in some tissues such as osteoblasts (54) and particular regions of the brain that include several cortical areas, the dentate gyrus, most components of the olfactory system, the striatum, many thalamic nuclei and pyramidal cells of the hippocampus (Table I) (52, 55). In contrast, there are a number of structures where coincident expression is not seen. Thus,

Table I: Estimated densities of PDE7A and PDE7B mRNA transcripts in different regions of the rat brain.

Brain area	PDE7A	PDE7B
Cortex		
Parietal cortex	++	++
Frontal cortex	++	++
Cingulate cortex	++	++
Retrospenial cortex	+/++	–/+
Entorhinal cortex	++	+/++
Olfactory system		
Olfactory bulb	+++	+
Anterior olfactory nucleus	++	+
Olfactory tubercle	++	++++
Piriform cortex	+++	++
Islands of Calleja	+/++	++
Islands of Calleja, major island	+/++	+++
Basal ganglia and related areas		
Caudate putamen	++	+++
Accumbens	–	++/+++
Substantia nigra	++	–
Limbic areas		
Ammon's horn		
CA1 (pyramidal cell layer)	++	+/++
CA2 (pyramidal cell layer)	++/+++	+
CA3 (pyramidal cell layer)	++/+++	+
Dentate gyrus	+++	+++
Subiculum	++	–/+
Pre-, parasubiculum	++	ND
Amygdala	++	+
Lateral septal nucleus	+	–
Medial septal nucleus	+	–
Thalamus and hypothalamus		
Medial habenular nucleus	++/+++	++
Lateral habenular nucleus	+/++	–/+
Anterodorsal thalamic nucleus	ND	+++
Ventroposterior thalamic nucleus	+/++	+++
Laterodorsal thalamic nucleus	+/++	+++
Mediodorsal thalamic nucleus	+/++	+++
Posterior thalamic nucleus group	+	+++
Zona incerta	++	–
Reticular thalamic nucleus	+	–
Ventromedial hypothalamus	+	+
Dorsomedial hypothalamus	+	–
Arcuate nucleus	+	+
Periventricular hypothalamus	–	+
Medial geniculate nucleus	+/++	++/+++
Internal capsule	+/++	–
Brainstem		
Red nucleus	++	+
Superior colliculus	+/++	–/+
Pontine nucleus	++/+++	–/+
Ventral tegmental nucleus	++	–
Accessory facial nucleus	++	–
Facial nucleus	+	–
Dorsal cochlear nucleus	+/++	–
Nucleus of the solitary tract	+/++	+/++
Hypoglossal nucleus	++	–
Prepositus hypoglossal nucleus	+/++	–
Cuneate nucleus	++	–
Dorsal motor nucleus of vagus	–	++/+++
Lateral reticular nucleus	+	–

Continuation

Table I (cont.): Estimated densities of PDE7A and PDE7B mRNA transcripts in different regions of the rat brain.

Brain area	PDE7A	PDE7B
Brainstem		
Gracile nucleus	++	–
Inferior olive	+/++	–
Cerebellum		
Molecular layer	–	–
Granular layer	++/+++	+
Purkinje cell layer	+++	++
White matter	–	–
Cerebellar nucleus	+	–/+
Circumventricular organs		
Choroid plexus	++/+++	–
Subfornical organ	–	+
Area postrema	+	++/+++

Data taken from Refs. 52 and 55. The levels of hybridization signals are indicated by "+++" very strong; "++" moderate; "+" low; "–/+" very low; and "–" not detected. ND, not determined.

Table II: Expression of PDE7A1 and PDE7A2 in human proinflammatory and immune cells.

Cell type	mRNA		Protein	
	HSPDE7A1	HSPDE7A2	HSPDE7A1	HSPDE7A2
Human primary cells				
$CD4^+$ T-Lymphocytes	+	+	+	–
$CD8^+$ T-Lymphocytes	+	+	+	–
B-Lymphocytes	+*		+	ND
Neutrophils	+	+	+*	
Alveolar macrophages	+	+	+*	
Monocytes	+	+	+	–
Eosinophils	ND	ND	+	–
Bronchial epithelial cells	+*		ND	
Lung mast cells	+*		–	
Lung basophils	+*		–	
Airways smooth muscle cells	+	+	+	–
Vascular smooth muscle cells	+	+	+	–
Vascular endothelial cells	+	+	ND	ND
Human cell lines				
HUT-78 (T-cell)	+	+	+	–
Jurkat (T-cell)	+	+	+	–
16-HBE14 (epithelial cell)	+	+	+	–
NCIH292 (epithelial cell)	+*		ND	ND
BEAS-2B (epithelial cell)	+	+	+	–
U-937 (monocyte)	+	+	+	–
MRC-5 (fibroblast)	+	+	+	–
AML14.3D10 (eosinophil)	+	+	+	–
Jiyoye (B-cell)	+	ND	–	ND

+ Enzyme detected; – enzyme not detected; ND, not determined; *isozyme not determined. Data taken from Ref. 48.

while PDE7A mRNA is present in many brainstem nuclei, PDE7B is essentially absent. Conversely, strong hybridization signals for PDE7B, but not PDE7A, mRNA are found in the accumbens and the dorsal motor nucleus of the vagus (Table I) (52, 55).

The functions of PDE7A and PDE7B in central and peripheral tissues are obscure, although several possibilities have been proposed. The presence of PDE7A in Leydig cells, the adrenal glands and hematopoietic cells could imply a role in regulating testosterone production, catecholamine release and responses relating to the immune system (discussed below), respectively (55). Similarly, the high expression of PDE7B mRNA in the limbic system may indicate a role of this isozyme family in learning, memory and synaptic plasticity, whereas the presence of these isoforms in the subfornical organ and area postrema could point to involvement in drinking behavior and emesis, respectively (55). However, until highly selective PDE7 inhibitors become widely available that also discriminate between PDE7A and PDE7B, we can only speculate on the responses regulated by this relatively new family of PDEs.

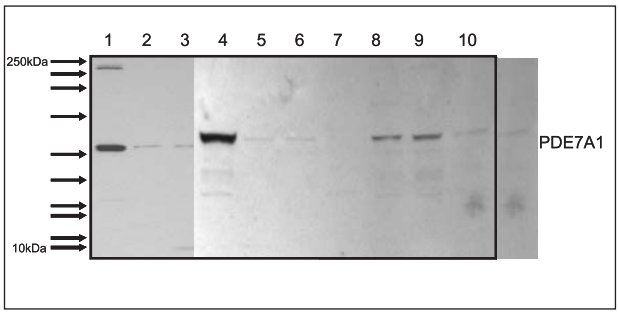


Fig. 5. Western blot analysis of PDE7A1 expression in human immune and proinflammatory cells. Key: 1, HUT-78 cells; 2, Fibroblasts; 3, Lung macrophages; 4, CD4+ T-lymphocytes; 5, BEAS-2B epithelial cells; 6, Airways smooth muscle cells; 7, Eosinophils; 8, Neutrophils; 9, CD8+ T-lymphocytes; 10, Monocytes. Data from Ref. 48.

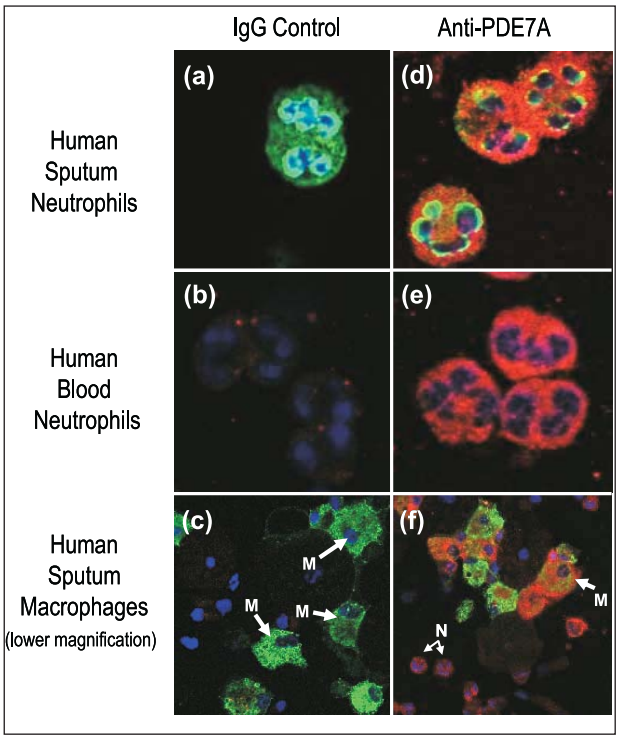


Fig. 6. Immunoconfocal identification of PDE7A in human neutrophils and macrophages taken from a normal subject. Cell preparations from blood and induced sputum were stained with a rabbit IgG control antibody (panels **a**, **b** and **c**) and an anti-PDE7A antibody (panels **d**, **e** and **f**). Neutrophils and macrophages from sputum were also counterstained with antibodies directed against human neutrophil elastase (**a** and **d**) and CD68 (**c** and **f**), respectively. PDE7A+ cells are stained red while elastase+ and CD68+ cells stain green. Blue staining indicates cell nuclei. M, macrophage; N, neutrophil.

PDE7A *splice variants*

The finding of PDE7A in the lungs and hematopoietic cells (see Table II) implies that these isozymes may play an important role in regulating many cAMP-dependent processes related to the immune system. Indeed, all human proinflammatory and immune cells (primary and cell lines) that have been studied express mRNA for PDE7A1 and PDE7A2 at an approximate ratio of 4:1. However, irrespective of the method used for detection (*i.e.*, Western blotting, immunocytochemistry, confocal microscopy), PDE7A2 has never been found at the protein level despite unequivocal identification of PCR products corresponding to this transcript. This finding is not peculiar to PDE7A. In human mononuclear cells, several of the PDE4D splice variants are expressed only at the mRNA level (47, 56). Whether this is due to translational repression or a low translation rate of these particular mRNA transcripts, instability of the active enzymes or to the fact that the proteins are expressed at very low but nevertheless functional levels is unexplored. It is noteworthy that neither Western blotting nor immunoconfocal microscopy has found protein for PDE7A2 even in response to agents, such as PMA, that markedly upregulate PDE7A1 (see Fig. 3).

Of the immunocompetent/proinflammatory cells that have been studied, $CD4^+$ and $CD8^+$ T-lymphocytes express relatively high levels of PDE7A1 that are readily detected by RT-PCR and Western blotting (Table II, Fig. 5) (47, 48, 57). Human airways smooth muscle cells, blood monocytes and lung macrophages, together with several cell lines including HUT-78 (T-cell) and BEAS-2B (epithelial), are also $PDE7A1^+$ under the same experimental conditions (Table II) (48). In contrast, Western blotting has failed to detect PDE7A1 in human neutrophils (Fig. 5), although the protein is clearly visualized using immunoconfocal laser microscopy (Fig. 6) (48). This more sensitive technique has also been employed to determine the expression profile of PDE7A in cells such as macrophages present in sputum and bronchoalveolar lavage fluid (Fig. 6). The inability to detect PDE7A1 in neutrophils by Western blotting suggests that the concentration of this enzyme is lower than that found in the other cell types examined. Although immunoconfocal laser microscopy cannot distinguish between the PDE7A splice variants, much of the staining is cytosolic (Fig. 6), indicating that PDE7A1 (and/or PDE7A3, see below) may be the most abundant isoform expressed.

Very little is known about PDE7A3. Studies by Glavas *et al*. (25) identified this splice variant in human $CD4^+$ T-lymphocytes after co-stimulation with anti-CD3/CD28 antibodies, but its distribution in other cells is unknown. Given that *PDE7A3* and *PDE7A1* are probably regulated by the same promoter (24), it is possible that the expression pattern of both transcripts is similar.

PDE7B *splice variants*

In the rat, mRNA transcripts for PDE7B1 have been detected in the heart, brain, spleen, lungs, skeletal muscle and kidneys (19). PDE7B3 has also been found in the heart, lungs and skeletal muscle, although at lower levels, whereas PDE7B2 mRNA is seemingly confined to the testes (19). It has been estimated that the testes contain

more PDE7B2 mRNA than PDE7B1 and PDE7B3 expressed in the brain and other peripheral tissues (19). *In situ* hybridization of PDE7B transcripts in the testis has found strong labeling in the seminiferous tubules, in pachytene spermatocytes during meiotic prophase and in meiotic germ cells (19). No information is currently available on the subcellular distribution of PDE7B splice variants, although differences in the *N*-terminus of these proteins are likely to determine, at least in part, their intracellular location (*cf*. PDE7A).

Enzymology of PDE7

PDE7A

PDE7A encodes cAMP-specific PDEs that are insensitive (IC_{50} > 100 µM) to cGMP and standard inhibitors of PDE2 (EHNA), PDE3 (amrinone, siguazodan, enoximone), PDE4 (rolipram, Ro-20-1724) and PDE5 (zaprinast, sildenafil) (13, 16, 58, 59). In contrast, PDE7A1 is inhibited by the generally nonselective compound IBMX, with an IC_{50} of 5-53 µM (the variation in potency probably relates to the different substrate concentrations [50 nM to 1 µM] adopted across experiments) (39, 58-60). The sensitivity of PDE7A2 and PDE7A3 to inhibition has not been investigated, but as IBMX is a catalytic site inhibitor, similar K_i values are predicted.

The hydrolysis of cAMP by recombinant human PDE7A1 expressed in yeast (*S. cerevisiae*), bacteria (*Escherichia coli*) or baculovirus-infected Sf9 (*Spodoptera fugiperda*) cells follows simple Michaelis-Menten kinetics, with K_m values of between 10 and 530 nM reported (13, 15, 26, 58-61). The 50-fold discrepancy in the affinity of cAMP is currently unexplained, but may be due to the nature of the expression system, mode of purification and the fact that in one study, an *N*-terminally truncated enzyme ($PDE7A1_{147\text{-}482\text{-}HIS}$) was used (58). Indeed, purification of $PDE7A1_{147\text{-}482\text{-}HIS}$ engineered in *E. coli* by metal chelate or ion exchange chromatography yielded proteins for which cAMP had a K_m of 100 and 500 nM, respectively (58). These values are somewhat different from the affinity derived from unpurified preparations of the same enzyme (K_m = 70-200 nM).

The hydrolysis of cAMP by PDE7A2 also adheres to simple Michaelis-Menten behavior. However, both the K_m (~100 nM) and V_{max} are approximately 2-fold lower than for PDE7A1 (26). Thus, alternate mRNA splicing has little effect on the kinetics of cAMP hydrolysis catalyzed by PDE7A variants but as discussed above, profoundly influences their subcellular distribution. A kinetic analysis of PDE7A3 has not yet been described.

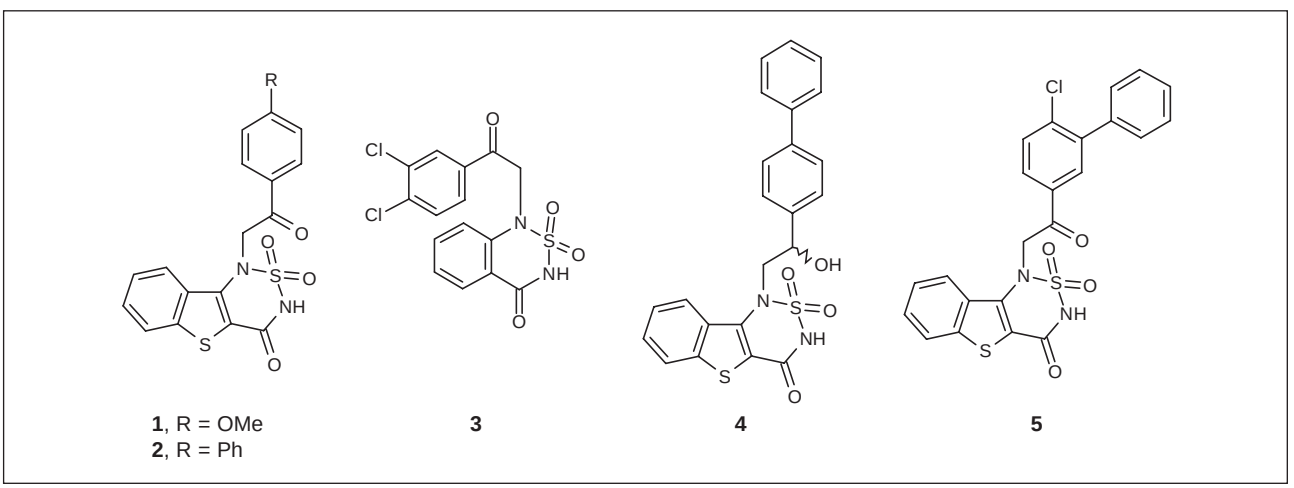
PDE7B

PDE7B also encodes cAMP-specific PDEs that are insensitive (IC_{50} > 50 µM) to cGMP and standard inhibitors of PDE2 (EHNA), PDE3 (milrinone, enoximone), PDE4 (rolipram, Ro-20-1724) and PDE5 (zaprinast, sildenafil) (16, 17, 19, 49). Nevertheless, HSPDE7B, MMPDE7B and RNPDE7B are inhibited by IBMX (IC_{50} = 2-10 µM), dipyridamole (IC_{50} = 0.5-9 µM) and the dual PDE1/5 inhibitor Sch-51866 (IC_{50} = 1-44 µM) (16, 17, 19, 49). The splice variants of rat PDE7B are equally susceptible to inhibition by IBMX, dipyridamole and Sch-51886 at concentrations in the low micromolar range (19).

The hydrolysis of cAMP by recombinant human PDE7B expressed in Sf9 and COS-7 cells follows simple Michaelis-Menten kinetics, with K_m values of 130 and 200 nM, respectively (17, 49). Similarly, cAMP is hydrolyzed by recombinant murine PDE7B (K_m = 30-100 nM) expressed in Sf9 cells (16, 49) and RNPDE7B1 (K_m = 50 nM), RNPDE7B2 (K_m = 70 nM) and RNPDE7B3 (K_m = 50 nM) expressed in COS-7 cells, which also conforms to Michaelis-Menten behavior with comparable affinities (19).

Selective PDE7 inhibitors

Despite the discovery of PDE7A more than 13 years ago, there are surprisingly few reports of selective



1, R = OMe
2, R = Ph

3 **4** **5**

Fig. 7. Benzo- and benzothienothiadiazine-based PDE7 inhibitors.

Table III: Potency and selectivity profile of some structurally dissimilar PDE7 inhibitors.

Compound	PDE7A (IC_{50}, μM)	PDE4 (IC_{50}, μM)	PDE4/PDE7A ratio
1	11	30	2.7
2	25	>20	-
3	8	19	2.4
6	0.36	-	-
7	0.026	62	238
11	1.3	10%	-
12	2.3	42%	-
13	4.2	20%	-
14	0.010	0.550	55
15	0.011	0.160	15
16	0.006	4.8	800
17	0.030	3.2	107
18	0.005	6.0	1200
19	1.5	57	38
20	0.140	7.5	54
21	0.160	17	106
22	0.090	19.3	214
23	0.068	34.3	504
24	0.030	8.8	293
25	0.065	33.3	512
26	0.061	11.9	195
27	0.0039	92.3	23,667
28	0.027	>101	> 3741
29	0.085	40.8	480
30	0.052	20	385
31	0.170	-	-
32	0.066	-	-
33	0.014	-	-
34	0.016	1.42	89
35	0.021	1.63	78
36	0.026	1.59	62
37	0.024	1.5	63
38	0.019	1.46	77
39	0.046	15.0	326
40	0.055	12.2	222
41	0.038	7.35	193
42	0.120	-	-
43	0.032	-	-
48	0.003	3.0	1000
49	0.060	3.2	53
50	1.7	0.00013	0.000076

inhibitors. However, since 2000, examples of compounds with PDE7-inhibitory activity have emerged and some of these have acceptable selectivity for both *in vitro* and *in vivo* studies. None of the compounds described in the literature and in the following sections are reported to discriminate between PDE7A and PDE7B (62, 63-65).

Benzo- and benzothienothiadiazine derivatives

Martinez *et al.* (66) were the first to describe two series of lead compounds (**1-5**; Fig. 7) that inhibited recombinant human PDE7A expressed in yeast, but none was selective for PDE7A over other cAMP PDE isozymes (Table III). Nevertheless, compounds **1**, **2** and **3** were considered the best for lead optimization (Fig. 7). Indeed, a 3-dimensional quantitative structure-activity relationship (SAR) study using comparative molecular field analysis of 19 thiadiazine derivatives has allowed the design, *in silico*, of compounds (**4**, **5**) with theoretically enhanced PDE7A-inhibitory activity (67).

Sulfonamide derivatives

Benzenesulfonamides with PDE7-inhibitory activity were claimed in a patent filed by GlaxoSmithKline (SmithKline Beecham) in 2002 (68). One of these (**6;** Fig. 8, Table III) has an IC_{50} of 360 nM and is 10-fold selective over other PDE isozyme families (69). A more potent and selective compound, BRL-50481 (**7**; Fig. 8), was described by Smith *et al*. (59). This derivative is > 200-fold selective for recombinant human PDE7A1 expressed in baculovirus-infected Sf9 cells over PDE1-5 (Table III)

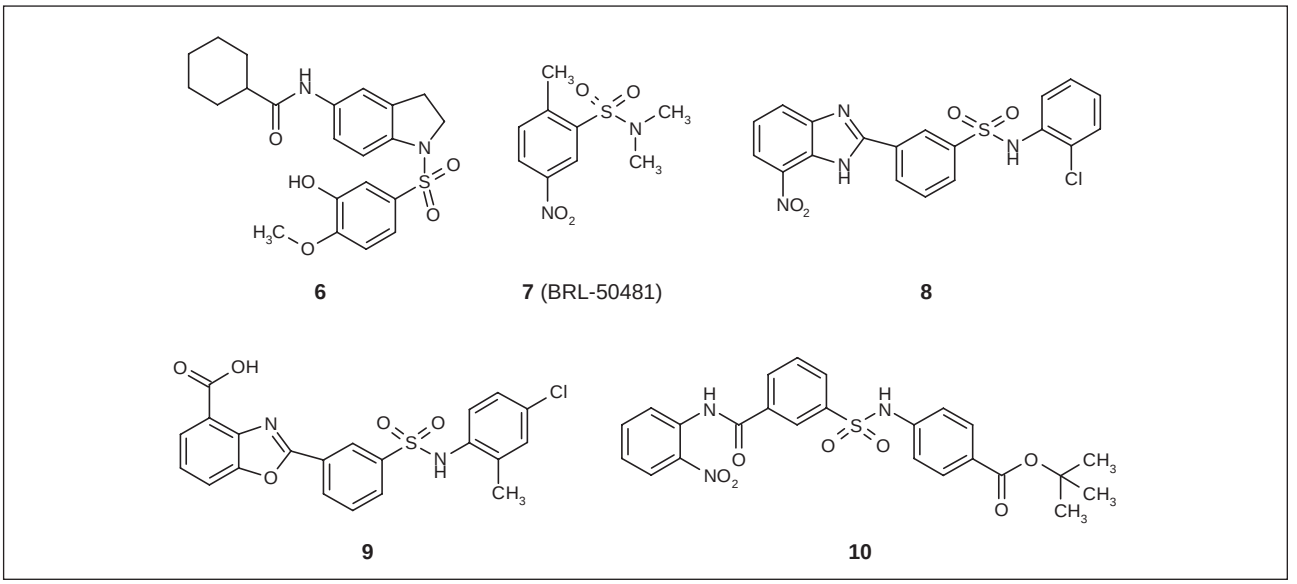


Fig. 8. Sulfonamide PDE7 inhibitors.

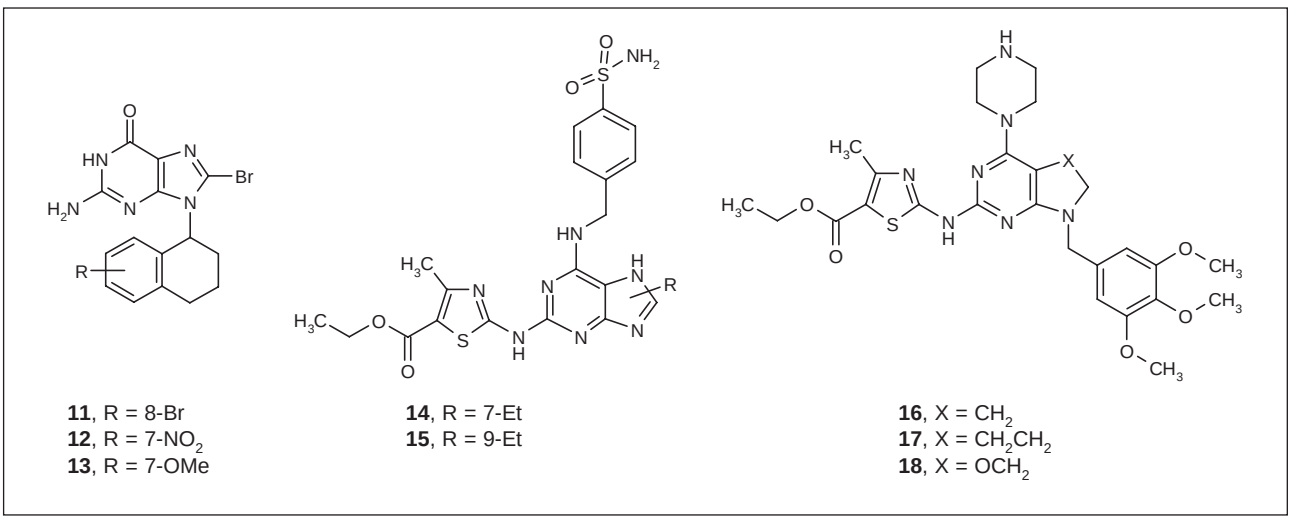


Fig. 9. Purine and pyrimidine derivatives as PDE7 inhibitors.

and is a purely competitive inhibitor (with respect to substrate) of the enzyme, with a K_i of 180 nM. Two other related series of PDE7A inhibitors with submicromolar potency are claimed by Celltech Chiroscience (Celltech, now part of UCB) (70, 71). These are 3-substituted derivatives of *N*-phenylbenzenesulfonamide (*e.g.*, **8-10;** Fig. 8), but to the authors' knowledge, no enzymology has been published (72).

Purine and pyrimidine nucleotide analogues

Chemists from the former Celltech Chiroscience found that certain analogues of guanine were selective inhibitors of PDE7A (**11-13;** Fig. 9) (73, 74). In particular, incorporation of an 8-bromo-substituted tetralin ring at position 9 of the guanine template produced **11**, which has an IC_{50} against PDE7A isolated from HUT-78 T-cells of 1.3 µM, with weak activity against PDE3 and PDE4 (Table III). Assuming simple kinetics of inhibition, this compound should be 50-100-fold selective for PDE7A over PDE4.

Screening of the Bristol-Myers Squibb compound collection also resulted in the identification of purine analogues with inhibitory activity against PDE7 expressed in the HUT-78 T-cell line, which was used as a surrogate for recombinant human PDE7A1 (75-78). Compound **14** (Fig. 9) is a potent inhibitor of PDE7A (IC_{50} = 10 nM), although its selectivity over PDE2, PDE4 and PDE5 is relatively weak (45-, 16- and 13-fold, respectively) and it is thus unsuitable for *in vitro* pharmacological testing (Table III) (75). At pH 6.5, compound **14** and related analogues are also very poorly soluble in aqueous media (< 5 µg/ml)

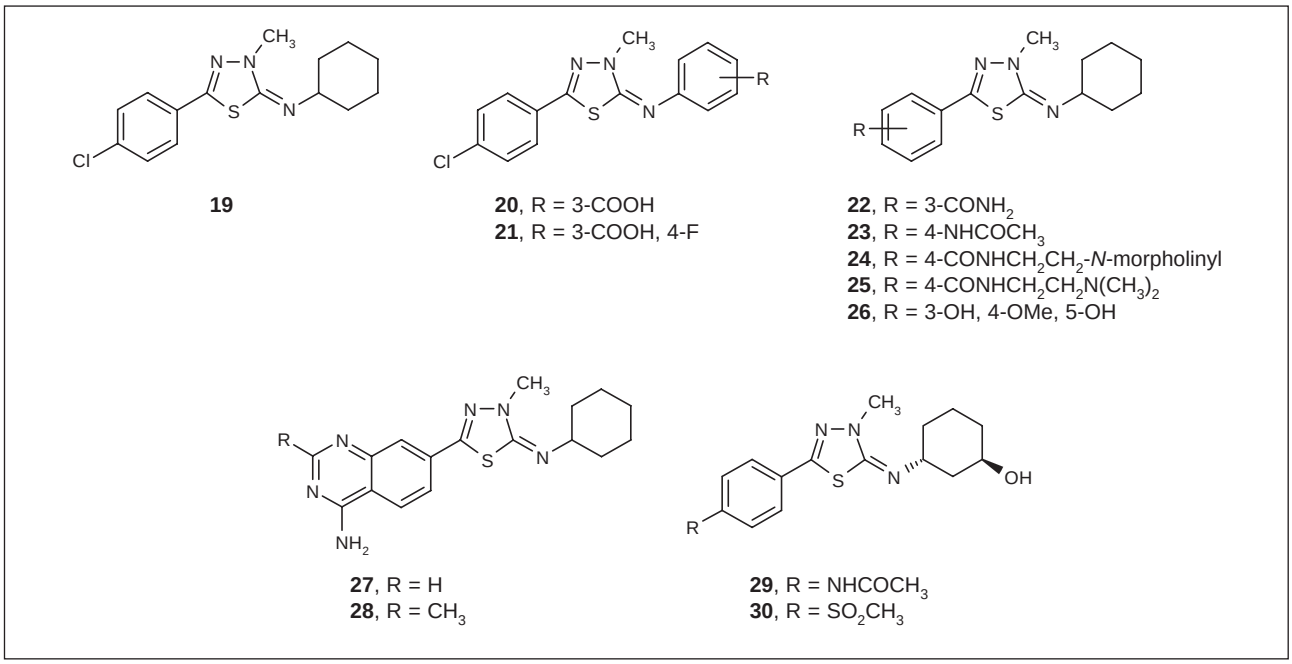


Fig. 10. PDE7 inhibitors based on the 1,3,4-thiadiazole template.

and an appropriate formulation for intravenous dosing was not identified (75). Changing the position of the ethyl substituent on the purine nucleus from position 7 to position 9 (**15**; Fig. 9) had little effect on potency (IC_{50} = 11 nM) but increased the PDE4/PDE7 ratio from 16 to 50 (Table III); however, the relative selectivity against PDE1, PDE2, PDE3 and PDE5 was uniformly reduced (75).

Bristol-Myers Squibb has also reported the inhibitory effect against PDE7 of fused pyrimidine-based compounds (76, 78). Thus, compounds **16-18** (Fig. 9) showed comparable potency against PDE7 when compared to their purine counterparts, but were considerably more selective for PDE7 than PDE4 (Table III) (76). Moreover, although these compounds are also poorly soluble in aqueous media at pH 6.5, their membrane permeability, measured by the parallel artificial membrane permeation assay (PAMPA) described by Kansy *et al.* (79), is significantly better (> 100 nm/s) than that of the purine analogues, which should permit effective gastrointestinal absorption and evaluation *in vivo* (76).

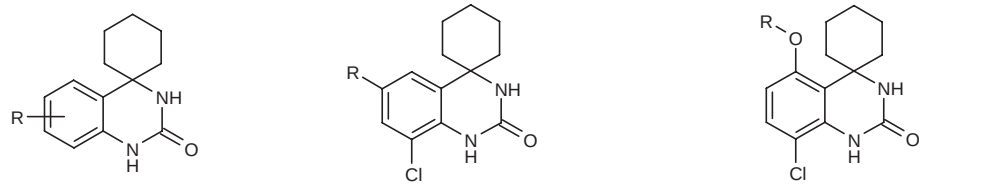
Thiadiazole derivatives

High-throughput screening of the Pfizer (Warner-Lambert) compound collection identified **19** (Fig. 10) as a relatively weak inhibitor of PDE7A1 with 38-fold selectivity over PDE4D3 (Table III) (62). SAR studies around the 1,3,4-thiadiazole template led subsequently to the discovery of several potent and selective inhibitors of PDE7A1 (**20-30**; Fig. 10) (62, 80). Replacement of the cyclohexyl moiety in **19** with phenyl and substitution at the 3-position with a carboxylic acid function to form **20** resulted in a more potent (IC_{50} = 140 nM) PDE7 inhibitor with improved selectivity over PDE4D3 (152-fold). Further substitution of the same phenyl ring in **20** with fluorine at position 4 to give the 2-fluorobenzoic acid derivative (**21**) increased selectivity further (247-fold *vs*. PDE4D3), although potency was unaffected (Table III).

SAR studies around the phenyl function in **19** also resulted in compounds with improved potency and selectivity for PDE7A1 (62). Replacement of the 4-chloro moiety with amide (**22-25**) at the 3- or 4-position, or a combination of hydroxyl and *O*-methoxy functions (**26**), resulted in compounds with IC_{50} values between 30 and 90 nM and that were 213-, 503-, 292-, 512- and 195-fold, respectively, selective for PDE7A1 over PDE4D3 (Table III). A profound further increase in potency and PDE7A1 selectivity was achieved by preparing 4-aminoquinazoline derivatives (**27, 28**), which are structural analogues of the adenine part of cAMP (62). PDE7A inhibitors of this structural class have also been claimed by Bristol-Myers Squibb (81). In particular, the 4-amino-2-methylquinazoline derivative (**27**) inhibits PDE7A1 with an IC_{50} of 3.9 nM and has a PDE4D3/PDE7A1 selectivity ratio of over 23,000 (Table III) (62). Optimization around the 1,3,4-thiadiazole template towards orally bioavailable compounds that retain inhibitory potency and selectivity for PDE7A1 has been achieved by substitution around the cyclohexyl and phenyl functions in **19** (Table III). Two such compounds**, 29** and **30** (Fig. 10), are orally bioavailable in the rat (76% and 100%, respectively), with a plasma half life of ~2.5 h (63).

Spiroquinazolinones

Pfizer (Warner-Lambert) has also described a new family of spiroquinazolinones (**31-41**; Fig. 11, Table III) that are potent and selective inhibitors of PDE7A (64, 65,

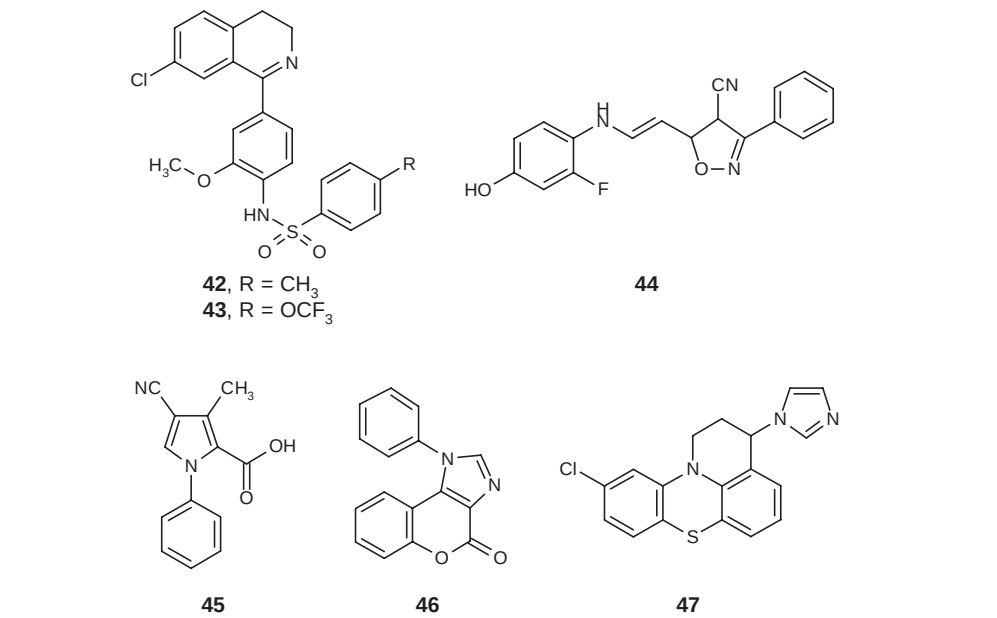


31, R = 8-Cl
32, R = 8-Br
33, R = 5,8-DiCl
34, R = 6-Ph, 8-Cl

35, R = *p*-C_6H_4-COHN-$(CH_2)_2$-$N(CH_3)_2$
36, R = *m*-C_6H_4-COHN-$(CH_2)_2$-$N(CH_3)_2$
37, R = *p*-C_6H_4-COHN-$(CH_2)_3$-$N(CH_3)_2$
38, R = *m*-C_6H_4-COHN-$(CH_2)_3$-$N(CH_3)_2$

39, R = CH_2-COOH
40, R = CH_2-CH_2-OH
41, R = CH_2-CH_2-(4-morpholino)

Fig. 11. Spiroquinazolinone derivatives as PDE7 inhibitors.



42, R = CH_3
43, R = OCF_3

44

45 **46** **47**

Fig. 12. PDE7 inhibitors of other structural classes, including dihydroisoquinoline (**42**, **43**) dihydroisoxazole (**44**), pyrrole (**45**) and imidazole (**46**, **47**) derivatives.

82). High-throughput screening of the company's compound collection identified **31**, which has submicromolar potency against full-length HSPDE7A1 expressed in Sf9 cells and was selected for lead optimization (Table III) (65). Subsequent SAR studies showed that the nature of the substituent at position 8 of the aromatic ring had a significant impact on activity. Thus, replacement of the chlorine moiety with bromine increased potency 2.5-fold (**32**), whereas disubstitution to produce the 5,8-dichloro- and 6-phenyl, 8-chloro derivatives (**33, 34**) yielded the most potent compounds, with IC_{50} values of 14 and 16 nM, respectively (65). Further modifications of the 6-phenyl ring with ionizable functions were also performed to improve solubility. In particular, retaining the chloro moiety at position 8 and substituting the aromatic ring at the *para*- or *meta*-position with *N*-[2-(dimethylamino)alkyl]-benzamido functions produced compounds (**35-38**) that had greater solubility in aqueous media (30-70 µg/ml at pH 7.4) and were reasonably selective (60-80-fold) for PDE7A1 over PDE4D3 (65). Efforts to produce compounds with suitable pharmacokinetic characteristics for *in vivo* studies were also based on replacement of the 5-chloro substituent in **33** (which is very hydrophobic) with carboxylic acid, alcohol or morpholine functions to form **39**, **40** and **41**, respectively (64). Two of these compounds (**40, 41**) are orally bioavailable in the rat (21% and 27%, respectively) and have an *in vivo* half life of ~1.5 h.

PDE7 inhibitors of other structural classes

Derivatives of several additional structural classes (**42-47**; Fig. 12) are claimed in the patent literature to be PDE7 inhibitors, including dihydroisoquinolines (Byk Gulden [Altana]; *e.g.,* **42, 43**) (83, 84), dihydroisoxazoles, pyrroles and imidazoles (Merck KGaA; *e.g.*, **44-47**) (85-

88), pyrazolopyrimidinones and imidazotriazinones (Daiichi Suntory) (89, 90), 4-aminothieno[2,3-*d*]pyrimidine-6-carbonitriles (Almirall Prodesfarma) (91) and (4,2-disubstituted-thiazol-5-yl)amines (Bristol-Myers Squibb) (92). Other PDE7A inhibitors cited in the peer-reviewed literature include Icos's IC-242 (39) and the potent BMS-586353 from Bristol-Myers Squibb (93), which have IC_{50} values of 370 and 8 nM, respectively. Both of these molecules are cell-permeant and show good selectivity (> 200-fold) for PDE7A over all other PDEs tested. Their structures have not been disclosed.

PDE7 inhibitors in immunological and immunodeficiency disorders

The advent of selective PDE7 inhibitors has recently allowed an assessment of the functional role of PDE7 in several cells and tissues. While the possible therapeutic benefit of blocking PDE7B is largely unexplored and remains speculative, the selective targeting of PDE7A could have application in a number of immunological disorders, including asthma, COPD, Crohn's disease, myasthenia gravis, atopic dermatitis, psoriasis, systemic lupus erythematosis, rheumatoid arthritis, diabetes and multiple sclerosis. Indeed, excitement in this area was initially fueled by a report published in 1999 that IL-2 production by, and the subsequent proliferation of, anti-CD3/anti-CD28-stimulated human T-lymphocytes was associated with induction of *PDE7A1*. Significantly, delivery to these cells of antisense oligonucleotides directed against PDE7A mRNA transcripts prevented these responses in a PKA-sensitive manner (Fig. 13) (25, 46). The results described by Li *et al*. (46) are supported by one set of studies conducted in mice deficient in the *PDE7A* gene (94). Thus, the ability of anti-CD3 antibodies to promote the proliferation of splenocytes and purified T-lymphocytes was reduced in *PDE7A*$^{-/-}$ mice by 66% and 60%, respectively, relative to their wild-type counterparts (Fig. 14a); in contrast, cell proliferation evoked by a combination of ionomycin and phorbol ester was unaffected by *PDE7A* gene deletion (94). Similarly, as shown in Figures 15 and 16, the number of interferon gamma$^+$/CD3$^+$ T-cells harvested from mice lacking the *PDE7A* gene and their ability to release interferon gamma in response to anti-CD3 antibodies both *in vitro* and *ex vivo* were significantly lower when compared to wild-type mice (the percentage of T-cells from wild-type animals expressing interferon gamma amounted to only 1-4% of the total population). Taken together, these data implicate PDE7A in the regulation of murine T-cell proliferation and Th1 cytokine generation.

Additional, albeit indirect, evidence linking T-cell proliferation and PDE7A was provided by Nakata *et al.* (95). These investigators found that T-2585, a highly potent PDE4 inhibitor, suppressed T-cell proliferation and cytokine synthesis at high concentrations where PDE7A was also inhibited, whereas piclamilast (RP-73401), a PDE4 inhibitor without activity at PDE7A, was inactive (95). However, the results of this latter study are unusual

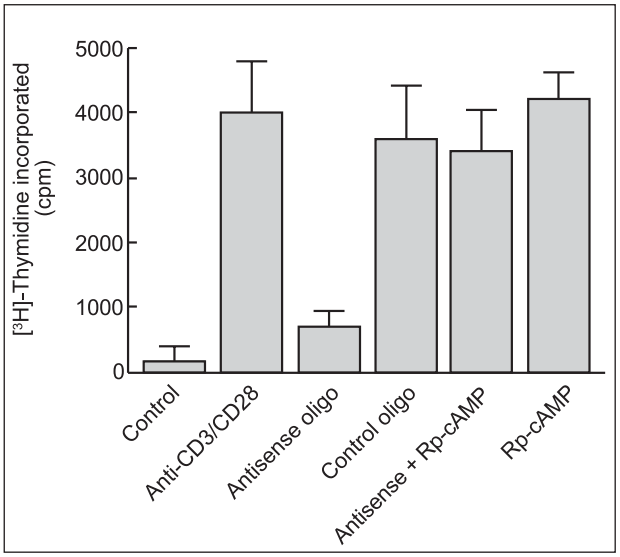


Fig. 13. Suppression of anti-CD3/anti-CD28-induced human T-cell proliferation by antisense oligonucleotides directed against PDE7A and reversal by the PKA inhibitor Rp-cAMP. CD4$^+$ T-cells were stimulated with anti-CD3/anti-CD28 (both 0.2 ng/ml) in the absence and presence of antisense or control oligonucleotides. In some experiments, Rp-cAMP was added at the same time as the antisense oligonucleotide or together with the activating antibodies only. The amount of [^{3}H]-thymidine incorporated into DNA was used as an index of proliferation. Data redrawn from Ref. 46.

in that they failed to confirm that selective inhibition of PDE4 is antimitogenic (47, 96-101) and must be interpreted with caution. Indeed, subsequent investigations with small-molecule inhibitors selective for PDE7A have not corroborated these findings. Thus, BRL-50481 (**7**; Fig. 8) has no effect on IL-15-induced proliferation of human CD8$^+$ T-lymphocytes (59). Similarly, in another study where *PDE7A*$^{-/-}$ mice were used, T-cell proliferation and Th1 (IL-2, interferon gamma, TNF-α) and Th2 (IL-4, IL-5, IL-13) cytokine production evoked by ligation of CD3/CD28 were preserved (Figs. 14b and 17) (93). The same result was found using wild-type mice treated with BMS-586353, a highly potent PDE7 inhibitor (93).

It is unclear why the results of Smith *et al*. (59) and Yang *et al*. (93) do not concur with data reported by Li *et al*. (46) or Michaeli (94), but they are unlikely to be species-related or to a redundant mechanism in mice that compensates for the deficiency in *PDE7A*. Two additional possibilities may account for some of the discrepancies. In the study reported by Smith *et al*. (59), CD8$^+$ T-lymphocytes were isolated from other leukocytes by negative immunoselection using a mixture of antibodies against CD11b, CD16, CD19, CD36, CD56 and CD4. Although the same methodology was used by Li *et al.* (46), antibodies against CD25 and HLA-DR were also employed, which will remove all activated and proliferating T-cells. Thus, it is possible that naïve T-cells are regulated differently by PDE7A when compared to their activated and proliferating counterparts. Alternatively, the

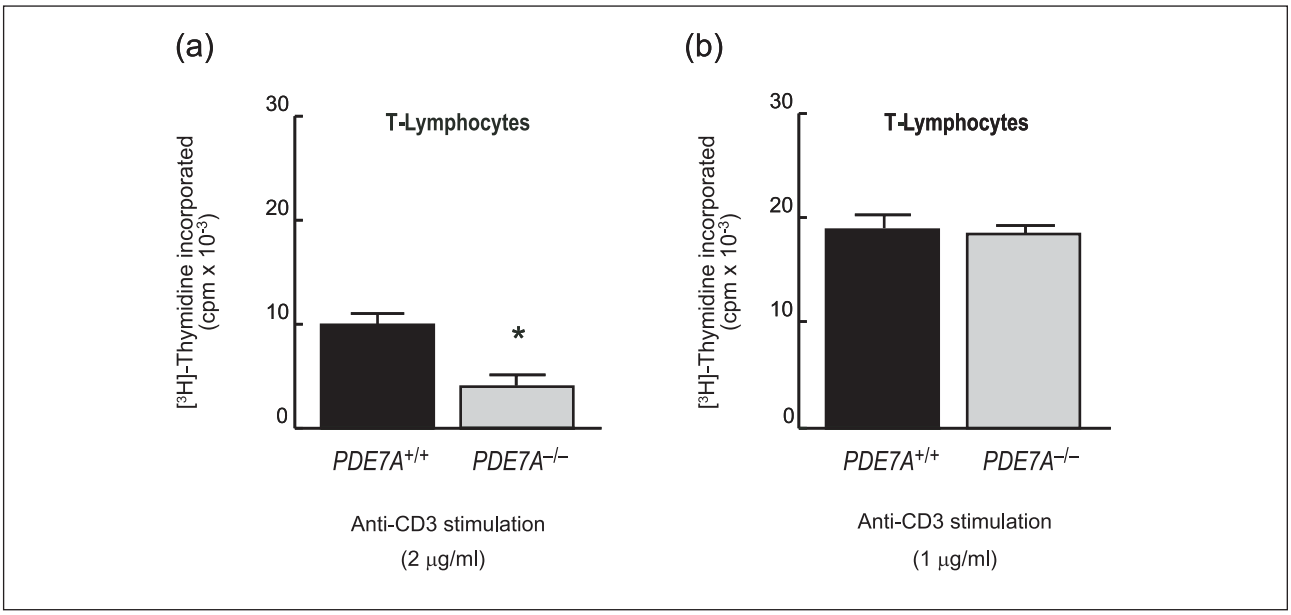


Fig. 14. Effect of an anti-CD3 antibody on the *in vitro* proliferation of T-lymphocytes harvested from wild-type and *PDE7A* knockout mice. Panels (**a**) and (**b**) show the results of studies reported by Michaeli (94) and Yang *et al*. (93), respectively. Note the marked discrepancy between the two studies. **p* < 0.05, significantly different from wild-type animals.

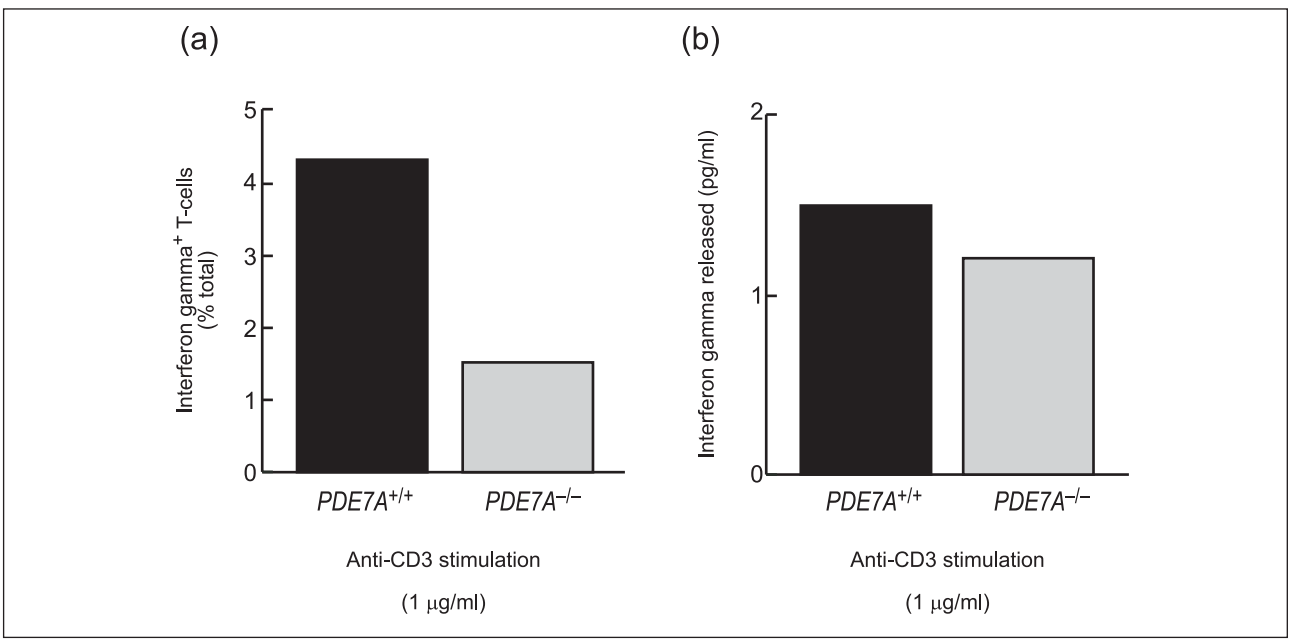


Fig. 15. Effect of an anti-CD3 antibody on the number of interferon gamma$^+$/CD4$^+$ T-lymphocytes (**a**) and the release of interferon gamma (**b**) from these cells harvested from wild-type and *PDE7A* knockout mice. Data taken from Michaeli (94).

use of naked antisense oligonucleotides, as used by Li *et al*. (46), may not have specifically targeted the mRNA of interest, or alternatively, and may have evoked toxic effects that were sequence-nonspecific (102). The divergence in data between the two studies conducted with *PDE7*$^{-/-}$ mice has not been explained.

Although inhibition of PDE7A with BRL-50481 does not attenuate the proliferation of T-cells *per se*, it significantly augments the antimitogenic (Fig. 18) and cAMP-elevating activities of rolipram (59). Similarly, the suppression by PDE4 inhibitors of TNF-α release from lipopolysaccharide (LPS)-stimulated human blood monocytes and lung macrophages is significantly enhanced by BRL-50481 (Fig. 19) (59). Collectively, these data are reminiscent of the behavior of PDE3 inhibitors in human T-cells (47) and demonstrate that PDE7A can regulate

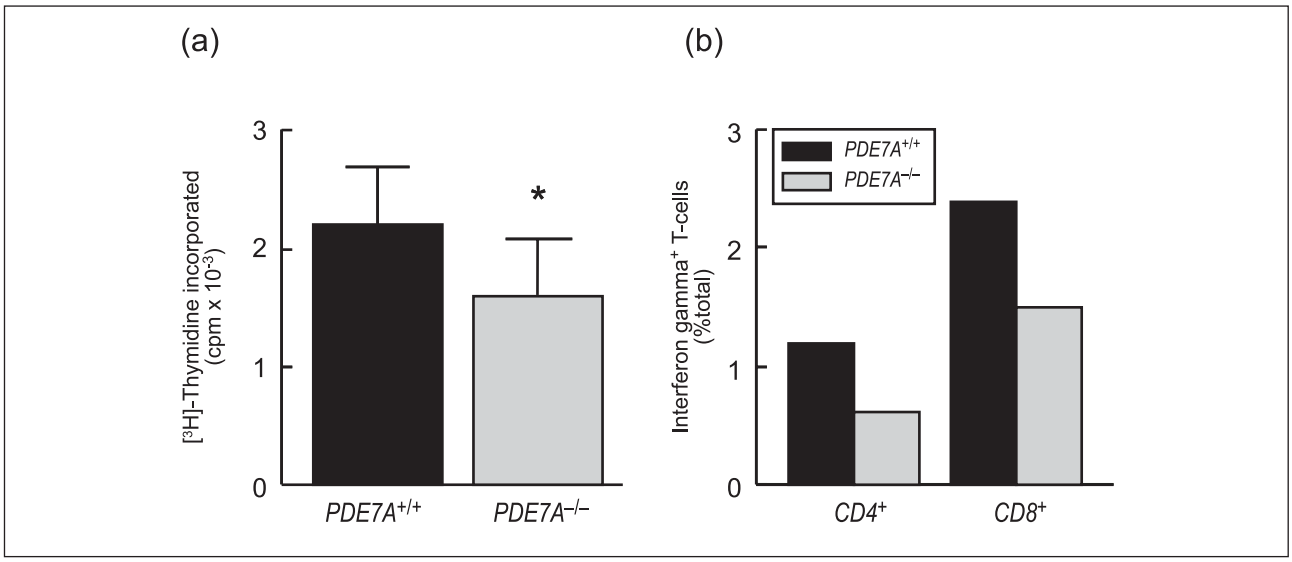


Fig. 16. Effect of an anti-CD3 antibody given intravenously to wild-type and *PDE7A* knockout mice on the *ex vivo* proliferation of (**a**) and the number of interferon gamma$^+$ T-lymphocytes (**b**). Data taken from Michaeli (94).

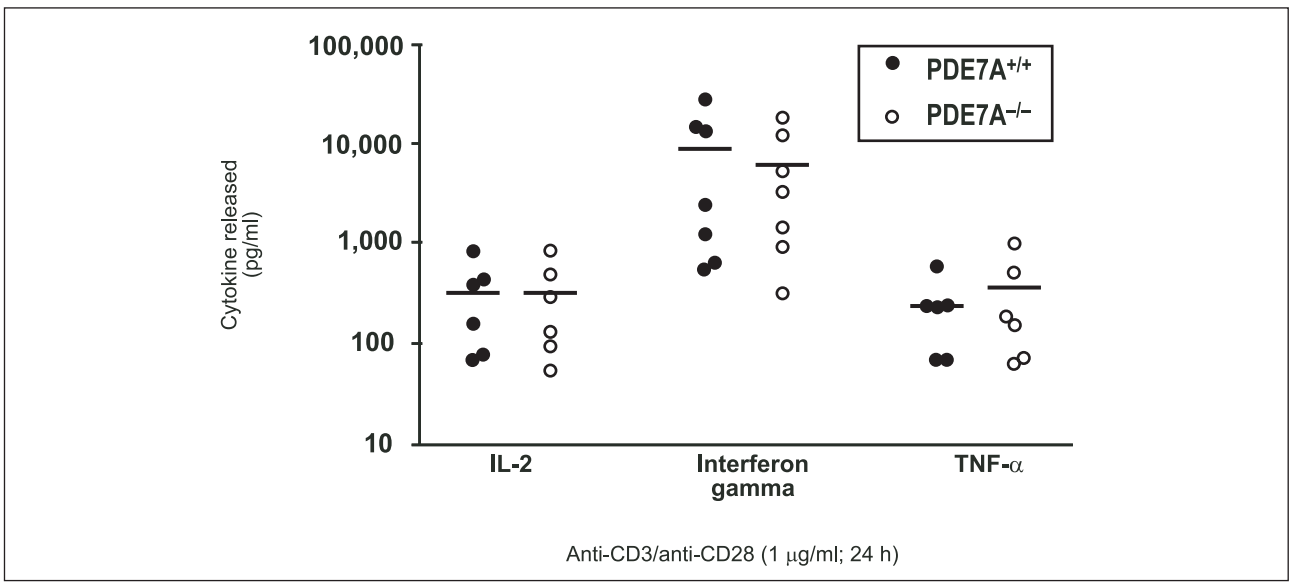


Fig. 17. Effect of anti-CD3/anti-CD28 on the release of IL-2, interferon gamma and TNF-α from T-lymphocytes harvested from wild-type and *PDE7A* knockout mice. Data redrawn from Yang *et al*. (93).

proinflammatory and immune cell function provided PDE4 is inhibited concomitantly (see below).

Of potential interest is the report by Smith *et al.* in 2004 that human monocytes cultured in RPMI-1640 for 24 h showed upregulation of PDE7A1 (see Fig. 3) and conferred functional sensitivity to BRL-50481 (*i.e*., LPS-induced TNF-α release was significantly inhibited) (59). Moreover, in monocytes in which PDE7A1 was upregulated, the inhibition of TNF-α release evoked by rolipram and other cAMP-elevating agents was enhanced in a purely additive manner. These data imply that PDE7A inhibitors alone may regulate the responsiveness of monocytes and possibly other proinflammatory and immune cells under circumstances where PDE7A is highly expressed, such as in chronic inflammation. In this respect, many chemokines and cytokines relevant to the pathogenesis of chronic inflammatory diseases signal, in part, through a PKC-dependent mechanism (42-44), and it is known that the human PDE7A1 promoter is activated by phorbol esters (24). Moreover, as discussed above, PDE7A1 expression in human monocytes treated with PMA is significantly increased relative to time-matched control cells (see Fig. 3).

Although inhibition of PDE7A has no demonstrable antiinflammatory activity under normal conditions, *PDE7A*$^{-/-}$ mice respond to immunization (with keyhole

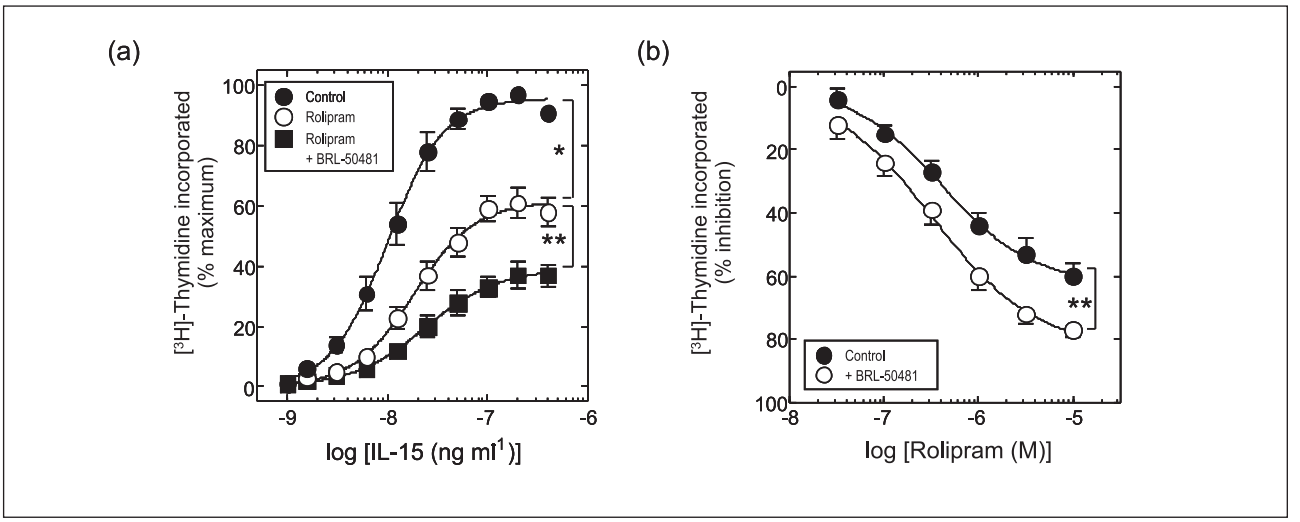


Fig. 18. Effect of BRL-50481 on IL-15-induced proliferation of human CD8+ T-lymphocytes. In panel (**a**) the effect of rolipram (10 μM) and rolipram and BRL-50481 (30 μM) in combination is shown on IL-15-induced [^{3}H]-thymidine uptake. Panel (**b**) shows the antimitogenic action of rolipram in the absence and presence of BRL-50481 (30 μM) on IL-15 (100 ng/ml)-induced [^{3}H]-thymidine uptake. BRL-50481 (30 μM) *per se* did not suppress IL-15-induced proliferation. Data redrawn from Ref. 59. *$p < 0.05$, significant inhibition of proliferation; ** $p < 0.05$, significant inhibition of proliferation over the effect of rolipram alone.

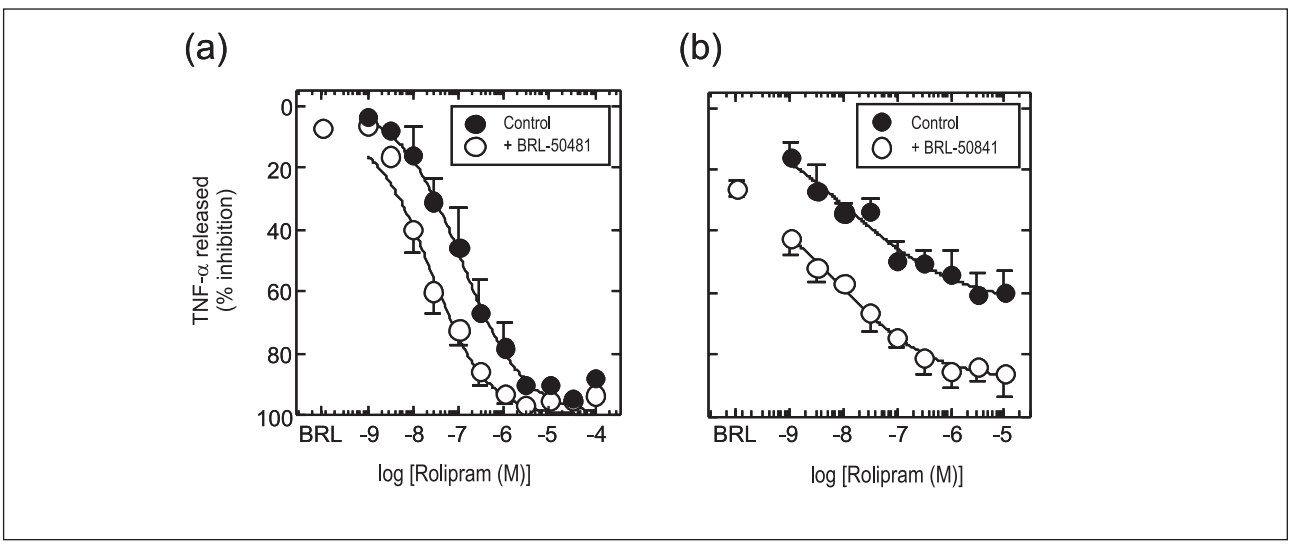


Fig. 19. Effect of BRL-50481 on the inhibition by rolipram of TNF-α release from human monocytes. Freshly isolated cells (panel **a**) or cells aged in culture for 24 h (panel **b**) were pretreated (30 min) with rolipram in the absence and presence of BRL-50481 (30 μM). Lipopolysaccharide (LPS; 3 ng/ml) was then added and the TNF-α elaborated in the culture medium was determined 24 h later by ELISA. Data redrawn from Ref 59.

limpet hemocyanin) with a significantly enhanced antibody response when compared to wild-type animals, indicating a possible role of this enzyme in B-cell function (93).

Concluding remarks

The ubiquitous distribution of PDE7A across human immune and proinflammatory cells has provided impetus for the design of selective small-molecule inhibitors for evaluation of their potential as novel antiinflammatory drugs. However, of the limited studies thus far reported, inhibitors of PDE7A are remarkably inactive *in vitro* and *in vivo* on functional responses (*e.g.,* T-cell proliferation, cytokine output) that may be considered proinflammatory. At best, they interact synergistically (normally additively) with PDE4 inhibitors and, in this respect, their behavior is reminiscent of PDE3 inhibitors in the same experimental settings. Nevertheless, PDE7A may play prominent functional roles under circumstances where PDE7A is upregulated or in responses that have not been rigorously investigated, such as antibody production. The possibility

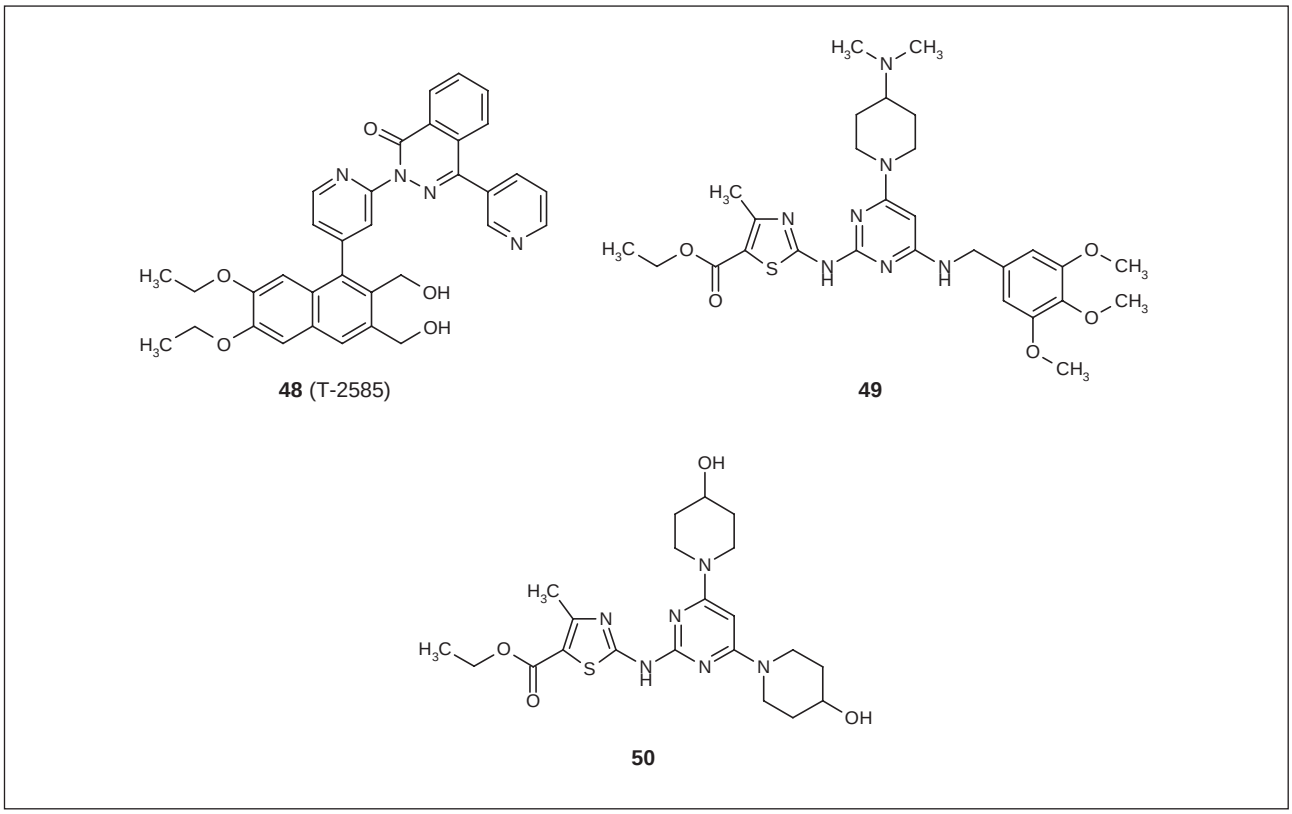


Fig. 20. Dual inhibitors of PDE4 and PDE7.

that greater efficacy could be achieved with hybrid inhibitors of PDE7 and PDE4 (**48-50**; Fig. 20, Table III) is also a concept worthy of investigation and is the focus of several recent patent filings (87, 88, 103, 104). Examples of such compounds include **48** (T-2585) and **49**, although they are more selective (1,000- and 53-fold, respectively) for PDE7 over PDE4. Another compound, **50** (105), can also be considered to be a dual selective inhibitor. T-2585 inhibits PDE4 with exquisite potency (IC_{50} = 130 pM) and also inhibits PDE7 in the low micromolar range (IC_{50} = 1.7 μM). While T-2585 preferentially blocks PDE4 over PDE7 (Table III), studies by Nakata *et al*. have provided data that this compound effectively attenuates T-cell proliferation in the low micromolar range under conditions where a highly selective PDE4 inhibitor devoid of activity against PDE7 was only weakly active (see above) (95).

Finally, it is appropriate to acknowledge that the expression of PDE7 across a variety of brain structures could compromise the beneficial activity of selective inhibitors that are able to penetrate the blood-brain barrier (18, 52, 106). In addition, compounds that inhibit both PDE7A and PDE7B, which probably include all of those listed in Table III, may be of particular concern, as high levels of PDE7B mRNA are expressed in the area postrema (and adjacent nucleus tractus solitarius) of the rat brain (52, 55), which lies outside the blood-brain barrier. If the same holds true in the human brain, then additional caution is warranted, as these structures are intimately involved in regulating emesis (107, 108) and are targets for the vomiting-inducing effects of PDE4 inhibitors (109). Thus, PDE7 inhibitors that do not discriminate between the two gene families may evoke the same or similar adverse events that have plagued the development of PDE4 inhibitors. This may be a particular problem with dual PDE4/PDE7 inhibitors if simultaneous inhibition of these enzymes in the area postrema results in a synergistic activation of those mechanisms that promote vomiting.

In conclusion, results from studies on the role of PDE7 in models of inflammation have thus far been disappointing. However, too few *in vitro* and *in vivo* studies have been conducted with selective inhibitors to allow an objective assessment of whether PDE7A represents a viable target for the development of a new generation of immunomodulatory compounds for asthma, COPD and related T-cell-mediated pathologies. The recent identification of highly selective PDE7 inhibitors, some of which are orally bioavailable and have acceptable pharmacokinetics for *in vivo* studies, should, in the near future, indicate whether PDE7 can be exploited to therapeutic advantage.

Acknowledgements

M.A.G. is an Alberta Heritage Senior Scholar and is funded by the Canadian Institutes of Health Research. S.J.S. is supported and funded by GlaxoSmithKline (Stevenage, U.K.).

References

1. Butcher, R.W., Sutherland, E.W. *Adenosine 3',5'-phosphate in biological materials. I. Purification and properties of cyclic 3',5'-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3',5'-phosphate in human urine*. J Biol Chem 1962, 237: 1244-50.

2. Soderling, S.H., Bayuga, S.J., Beavo, J.A. *Identification and characterization of a novel family of cyclic nucleotide phosphodiesterases*. J Biol Chem 1998, 273: 15553-8.

3. Beavo, J.A. *Multiple isozymes of cyclic nucleotide phosphodiesterase*. Adv Second Messenger Phosphoprotein Res 1988, 22: 1-38.

4. Beavo, J.A. *Cyclic nucleotide phosphodiesterases: Functional implications of multiple isoforms*. Physiol Rev 1995, 75: 725-48.

5. Conti, M., Jin, S.L. *The molecular biology of cyclic nucleotide phosphodiesterases*. Prog Nucleic Acid Res Mol Biol 1999, 63: 1-38.

6. Lugnier, C. *Cyclic nucleotide phosphodiesterase (PDE) superfamily: A new target for the development of specific therapeutic agents*. Pharmacol Ther 2005, 109: 366-98.

7. Liu, Y., Shakur, Y., Yoshitake, M., Kambayashi, J.J. *Cilostazol (Pletal): A dual inhibitor of cyclic nucleotide phosphodiesterase type 3 and adenosine uptake*. Cardiovasc Drug Rev 2001, 19: 369-86.

8. Giembycz, M.A. *Cilomilast: A second generation phosphodiesterase 4 inhibitor for asthma and chronic obstructive pulmonary disease*. Expert Opin Invest Drugs 2001, 10: 1361-79.

9. Baeumer, W., Szelenyi, I., Kietzmann, M. *Cilomilast, an orally active phosphodiesterase 4 inhibitor for the treatment of COPD*. Exp Opin Clin Immunol 2005, 1: 27-36.

10. Dyke, H.J., Montana, J.G. *Update on the therapeutic potential of PDE4 inhibitors*. Expert Opin Invest Drugs 2002, 11: 1-13.

11. Rotella, D.P. *Phosphodiesterase 5 inhibitors: Current status and potential applications*. Nat Rev Drug Discov 2002, 1: 674-82.

12. Galie, N., Ghofrani, H.A., Torbicki, A. et al. *Sildenafil citrate therapy for pulmonary arterial hypertension*. New Engl J Med 2005, 353: 2148-57.

13. Michaeli, T., Bloom, T.J., Martins, T. et al. *Isolation and characterization of a previously undetected human cAMP phosphodiesterase by complementation of cAMP phosphodiesterase-deficient Saccharomyces cerevisiae*. J Biol Chem 1993, 268: 12925-32.

14. Ichimura, M., Kase, H. *A new cyclic nucleotide phosphodiesterase isozyme expressed in the T-lymphocyte cell lines*. Biochem Biophys Res Commun 1993, 193: 985-90.

15. Bloom, T.J., Beavo, J.A. *Identification and tissue-specific expression of PDE7 phosphodiesterase splice variants*. Proc Natl Acad Sci USA 1996, 93: 14188-92.

16. Hetman, J.M., Soderling, S.H., Glavas, N.A., Beavo, J.A. *Cloning and characterization of PDE7B, a cAMP-specific phosphodiesterase*. Proc Natl Acad Sci USA 2000, 97: 472-6.

17. Sasaki, T., Kotera, J., Yuasa, K., Omori, K. *Identification of human PDE7B, a cAMP-specific phosphodiesterase*. Biochem Biophys Res Commun 2000, 271: 575-83.

18. Hoffmann, R., Abdel'Al, S., Engels, P. *Differential distribution of rat PDE-7 mRNA in embryonic and adult rat brain*. Cell Biochem Biophys 1998, 28: 103-13.

19. Sasaki, T., Kotera, J., Omori, K. *Novel alternative splice variants of rat phosphodiesterase 7B showing unique tissue-specific expression and phosphorylation*. Biochem J 2002, 361: 211-20.

20. Han, P., Fletcher, C.F., Copeland, N.G., Jenkins, N.A., Yaremko, L.M., Michaeli, T. *Assignment of the mouse Pde7A gene to the proximal region of chromosome 3 and of the human PDE7A gene to chromosome 8q13*. Genomics 1998, 48: 275-6.

21. Milatovich, A., Bolger, G., Michaeli, T., Francke, U. *Chromosome localizations of genes for five cAMP-specific phosphodiesterases in man and mouse*. Somat Cell Mol Genet 1994, 20: 75-86.

22. Minassian, B.A., Lee, J.R., Herbrick, J.A. et al. *Mutations in a gene encoding a novel protein tyrosine phosphatase cause progressive myoclonus epilepsy*. Nat Genet 1998, 20: 171-4.

23. Schroeder, B.C., Kubisch, C., Stein, V., Jentsch, T.J. *Moderate loss of function of cyclic-AMP-modulated KCNQ2/KCNQ3 K^+ channels causes epilepsy*. Nature 1998, 396: 687-90.

24. Torras-Llort, M., Azorin, F. *Functional characterization of the human phosphodiesterase 7A1 promoter*. Biochem J 2003, 373: 835-43.

25. Glavas, N.A., Ostenson, C., Schaefer, J.B., Vasta, V., Beavo, J.A. *T cell activation up-regulates cyclic nucleotide phosphodiesterases 8A1 and 7A3*. Proc Natl Acad Sci USA 2001, 98: 6319-24.

26. Han, P., Zhu, X., Michaeli, T. *Alternative splicing of the high affinity cAMP-specific phosphodiesterase (PDE7A) mRNA in human skeletal muscle and heart*. J Biol Chem 1997, 272: 16152-7.

27. Kasuya, J., Liang, S.J., Goko, H. et al. *Cardiac type cGMP-inhibited phosphodiesterase (PDE3A) gene structure: Similarity and difference to adipocyte type PDE3B gene*. Biochem Biophys Res Commun 2000, 268: 827-34.

28. Niiya, T., Osawa, H., Onuma, H. et al. *Activation of mouse phosphodiesterase 3B gene promoter by adipocyte differentiation in 3T3-L1 cells*. FEBS Lett 2001, 505: 136-40.

29. Rena, G., Begg, F., Ross, A. et al. *Molecular cloning, genomic positioning, promoter identification, and characterization of the novel cyclic AMP-specific phosphodiesterase PDE4A10*. Mol Pharmacol 2001, 59: 996-1011.

30. Olsen, A.E., Bolger, G.B. *Physical mapping and promoter structure of the murine cAMP-specific phosphodiesterase PDE4A gene*. Mamm Genome 2000, 11: 41-5.

31. Vicini, E., Conti, M. *Characterization of an intronic promoter of a cyclic adenosine 3',5'-monophosphate (cAMP)-specific phosphodiesterase gene that confers hormone and cAMP inducibility*. Mol Endocrinol 1997, 11: 839-50.

32. Le Jeune, I.R., Shepherd, M., Van Heeke, G., Houslay, M.D., Hall, I.P. *Cyclic AMP-dependent transcriptional up-regulation of phosphodiesterase 4D5 in human airway smooth muscle cells. Identification and characterization of a novel PDE4D5 promoter*. J Biol Chem 2002, 277: 35980-9.

33. Lin, C.S., Chow, S., Lau, A., Tu, R., Lue, T.F. *Human PDE5A gene encodes three PDE5 isoforms from two alternate promoters*. Int J Impot Res 2002, 14: 15-24.

34. Lin, C.S., Chow, S., Lau, A., Tu, R., Lue, T.F. *Regulation of human PDE5A2 intronic promoter by cAMP and cGMP: Identification of a critical Sp1-binding site*. Biochem Biophys Res Commun 2001, 280: 693-9.

35. Lin, C.S., Chow, S., Lau, A., Tu, R., Lue, T.F. *Identification and regulation of human PDE5A gene promoter*. Biochem Biophys Res Commun 2001, 280: 684-92.

36. Lin, C.S., Lau, A., Tu, R., Lue, T.F. *Identification of three alternative first exons and an intronic promoter of human PDE5A gene*. Biochem Biophys Res Commun 2000, 268: 596-602.

37. Taylor, R.E., Shows, K.H., Zhao, Y., Pittler, S.J. *A PDE6A promoter fragment directs transcription predominantly in the photoreceptor*. Biochem Biophys Res Commun 2001, 282: 543-7.

38. Seybold, J., Newton, R., Wright, L. et al. *Induction of phosphodiesterases 3B, 4A4, 4D1, 4D2, and 4D3 in Jurkat T-cells and in human peripheral blood T-lymphocytes by 8-bromo-cAMP and Gs-coupled receptor agonists. Potential role in α2-adrenoreceptor desensitization*. J Biol Chem 1998, 273: 20575-88.

39. Lee, R., Wolda, S., Moon, E., Esselstyn, J., Hertel, C., Lerner, A. *PDE7A is expressed in human B-lymphocytes and is up-regulated by elevation of intracellular cAMP*. Cell Signal 2002, 14: 277-84.

40. Graef, I.A., Chen, F., Crabtree, G.R. *NFAT signaling in vertebrate development*. Curr Opin Genet Dev 2001, 11: 505-12.

41. Macleod, K., Leprince, D., Stehelin, D. *The ets gene family*. Trends Biochem Sci 1992, 17: 251-6.

42. Kontny, E., Kurowska, M., Szczepanska, K., Maslinski, W. *Rottlerin, a PKC isozyme-selective inhibitor, affects signaling events and cytokine production in human monocytes*. J Leukoc Biol 2000, 67: 249-58.

43. New, D.C., Wong, Y.H. *CC chemokine receptor-coupled signalling pathways*. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) 2003, 35: 779-88.

44. Johnson, Z., Power, C.A., Weiss, C. et al. *Chemokine inhibition - Why, when, where, which and how?* Biochem Soc Trans 2004, 32: 366-77.

45. Miro, X., Casacuberta, J.M., Gutierrez-Lopez, M.D., de Landazuri, M.O., Puigdomenech, P. *Phosphodiesterases 4D and 7A splice variants in the response of HUVEC cells to TNFα*. Biochem Biophys Res Commun 2000, 274: 415-21.

46. Li, L., Yee, C., Beavo, J.A. *CD3- and CD28-dependent induction of PDE7 required for T cell activation*. Science 1999, 283: 848-51.

47. Giembycz, M.A., Corrigan, C.J., Seybold, J., Newton, R., Barnes, P.J. *Identification of cyclic AMP phosphodiesterases 3, 4 and 7 in human CD4$^+$ and CD8$^+$ T-lymphocytes: Role in regulating proliferation and the biosynthesis of interleukin-2*. Br J Pharmacol 1996, 118: 1945-58.

48. Smith, S.J., Brookes-Fazakerley, S., Donnelly, L.E., Barnes, P.J., Barnette, M.S., Giembycz, M.A. *Ubiquitous expression of phosphodiesterase 7A in human proinflammatory and immune cells*. Am J Physiol Lung Cell Mol Physiol 2003, 284: L279-89.

49. Gardner, C., Robas, N., Cawkill, D., Fidock, M. *Cloning and characterization of the human and mouse PDE7B, a novel cAMP-specific cyclic nucleotide phosphodiesterase*. Biochem Biophys Res Commun 2000, 272: 186-92.

50. Sasaki, T., Kotera, J., Omori, K. *Transcriptional activation of phosphodiesterase 7B1 by dopamine D1 receptor stimulation through the cyclic AMP/cyclic AMP-dependent protein kinase/cyclic AMP-response element binding protein pathway in primary striatal neurons*. J Neurochem 2004, 89: 474-83.

51. Barber, R., Baillie, G.S., Bergmann, R. et al. *Differential expression of PDE4 cyclic AMP phosphodiesterase isoforms in inflammatory cells of smokers with COPD, smokers without COPD, and non-smokers*. Am J Physiol Lung Cell Mol Physiol 2004, 287: L332-43.

52. Miro, X., Perez-Torres, S., Palacios, J.M., Puigdomenech, P., Mengod, G. *Differential distribution of cAMP-specific phosphodiesterase 7A mRNA in rat brain and peripheral organs*. Synapse 2001, 40: 201-14.

53. Kuthe, A., Wiedenroth, A., Magert, H.J. et al. *Expression of different phosphodiesterase genes in human cavernous smooth muscle*. J Urol 2001, 165: 280-3.

54. Ahlstrom, M., Pekkinen, M., Huttunen, M., Lamberg-Allardt, C. *Cyclic nucleotide phosphodiesterases (PDEs) in human osteoblastic cells; the effect of PDE inhibition on cAMP accumulation*. Cell Mol Biol Lett 2005, 10: 305-19.

55. Reyes-Irisarri, E., Perez-Torres, S., Mengod, G. *Neuronal expression of cAMP-specific phosphodiesterase 7B mRNA in the rat brain*. Neuroscience 2005, 132: 1173-85.

56. Nemoz, G., Zhang, R., Sette, C., Conti, M. *Identification of cyclic AMP-phosphodiesterase variants from the PDE4D gene expressed in human peripheral mononuclear cells*. FEBS Lett 1996, 384: 97-102.

57. Secchiero, P., Zella, D., Curreli, S. et al. *Pivotal role of cyclic nucleoside phosphodiesterase 4 in Tat-mediated CD4$^+$ T cell hyperactivation and HIV type 1 replication*. Proc Natl Acad Sci USA 2000, 97: 14620-5.

58. Richter, W., Hermsdorf, T., Kronbach, T., Dettmer, D. *Refolding and purification of recombinant human PDE7A expressed in Escherichia coli as inclusion bodies*. Protein Exp Purif 2002, 25: 138-48.

59. Smith, S.J., Cieslinski, L.B., Newton, R. et al. *Discovery of BRL 50481 [3-(N,N-dimethylsulfonamido)-4-methylnitrobenzene), a selective inhibitor of phosphodiesterase 7: In vitro studies in human monocytes, lung macrophage and CD8$^+$ T-lymphocytes*. Mol Pharmacol 2004, 66: 1679-89.

60. Wang, P., Wu, P., Egan, R.W., Billah, M.M. *Cloning, characterization, and tissue distribution of mouse phosphodiesterase 7A1*. Biochem Biophys Res Commun 2000, 276: 1271-7.

61. Wang, H., Liu, Y., Chen, Y., Robinson, H., Ke, H. *Multiple elements jointly determine inhibitor selectivity of cyclic nucleotide phosphodiesterases 4 and 7*. J Biol Chem 2005, 280: 30949-55.

62. Vergne, F., Bernardelli, P., Lorthiois, E. et al. *Discovery of thiadiazoles as a novel structural class of potent and selective PDE7 inhibitors. Part 1: Design, synthesis and structure-activity relationship studies*. Bioorg Med Chem Lett 2004, 14: 4607-13.

63. Vergne, F., Bernardelli, P., Lorthiois, E. et al. *Discovery of thiadiazoles as a novel structural class of potent and selective*

PDE7 inhibitors. Part 2: Metabolism-directed optimization studies towards orally bioavailable derivatives. Bioorg Med Chem Lett 2004, 14: 4615-21.

64. Bernardelli, P., Lorthiois, E., Vergne, F. et al. *Spiroquinazolinones as novel, potent, and selective PDE7 inhibitors. Part 2: Optimization of 5,8-disubstituted derivatives*. Bioorg Med Chem Lett 2004, 14: 4627-31.

65. Lorthiois, E., Bernardelli, P., Vergne, F. et al. *Spiroquinazolinones as novel, potent, and selective PDE7 inhibitors. Part 1*. Bioorg Med Chem Lett 2004, 14: 4623-6.

66. Martinez, A., Castro, A., Gil, C. et al. *Benzyl derivatives of 2,1,3-benzo- and benzothieno[3,2-a]thiadiazine 2,2-dioxides: First phosphodiesterase 7 inhibitors*. J Med Chem 2000, 43: 683-9.

67. Castro, A., Abasolo, M.I., Gil, C., Segarra, V., Martinez, A. *CoMFA of benzyl derivatives of 2,1,3-benzo and benzothieno[3,2-alpha]thiadiazine 2,2-dioxides: Clues for the design of phosphodiesterase 7 inhibitors*. Eur J Med Chem 2001, 36: 333-8.

68. Aubart, K.M., Christensen, S.B., Leber, J.D. (SmithKline Beecham Corp.) *Compounds and their use as PDE inhibitors*. US 6617357.

69. Castro, A., Jerez, M.J., Gil, C., Martinez, A. *Cyclic nucleotide phosphodiesterases and their role in immunomodulatory responses: Advances in the development of specific phosphodiesterase inhibitors*. Med Res Rev 2005, 25: 229-44.

70. Haughan, A.F., Lowe, C., Buckley, G.M. et al. (Celltech Chiroscience, Ltd.) *Sulphonamide derivatives*. WO 0198274.

71. Lowe, C., Dyke, H., Haughan, A.F., Galvin, F.C.A., Morgan, T., Picken, C.L. (Darwin Discovery, Ltd.) *Heterobiarylsulphonamides and their use as PDE7 inhibitors*. WO 0174786.

72. Editorial 1. *PDE7 Inhibitors*. Exp Opin Ther Patents 2004, 12: 601-3.

73. Barnes, M.J., Cooper, N., Davenport, R.J. et al. *Synthesis and structure-activity relationships of guanine analogues as phosphodiesterase 7 (PDE7) inhibitors*. Bioorg Med Chem Lett 2001, 11: 1081-3.

74. Dyke, H., Haughan, A.F., Lowe, C. et al. (Darwin Discovery, Ltd.) *9-(1,2,3,4-Tetrahydronaphthalen-1-yl)-1,9-dihydropurin-6-one derivatives as PDE7 inhibitors*. WO 0068230.

75. Pitts, W.J., Vaccaro, W., Huynh, T. et al. *Identification of purine inhibitors of phosphodiesterase 7 (PDE7)*. Bioorg Med Chem Lett 2004, 14: 2955-8.

76. Kempson, J., Pitts, W.J., Barbosa, J. et al. *Fused pyrimidine based inhibitors of phosphodiesterase 7 (PDE7): Synthesis and initial structure-activity relationships*. Bioorg Med Chem Lett 2005, 15: 1829-33.

77. Vaccaro, W., Roberge, J.Y., Leftheris, K., Pitts, W.J., Barbosa, J. (Bristol-Myers Squibb Co.) *Purine inhibitors of phosphodiesterase*. WO 02102314.

78. Guo, J., Barbosa, J., Pitts, W.J., Carlsen, M., Quesnelle, C., Dodier, M. (Bristol-Myers Squibb Co.) *Pyrimidine inhibitors of phosphodiesterase (PDE) 7*. WO 02102313.

79. Kansy, M., Senner, F., Gubernator, K. *Physicochemical high throughput screening: Parallel artificial membrane permeation assay in the description of passive absorption processes*. J Med Chem 1998, 41: 1007-10.

80. Vergne, F., Ducrot, P., Adrainjara, C., Bernardelli, P., Lorthiois, E. (Warner-Lambert Co.) *New thiadiazoles and oxadiazoles and their use as phosphodiesterase-7 inhibitors*. EP 1326853.

81. Pitts, W.J., Barbosa, J. (Bristol-Myers Squibb Co.) *Quinazoline and pyrido[2,3-d]pyrimidine inhibitors of phosphodiesterase (PDE) 7*. WO 02102315.

82. Bernardelli, P., Ducrot, P., Lorthiois, E., Vergne, F. (Warner-Lambert Co.) *New spirotricyclic derivatives and their use as phosphodiesterase-7 inhibitors*. WO 02074754.

83. Bundschuh, D.S., Kley, H.-P., Steinhilber, W. (Byk Gulden Lomberg Chemische Fabrik) *(Dihydro)isoquinolines derivatives as phosphodiesterase inhibitors*. WO 0240450.

84. Steinhilber, W. (Byk Gulden Lomberg Chemische Fabrik) *Dihydroisoquinolines as novel phosphodiesterase inhibitors*. WO 0240449.

85. Eggenweiler, H.-M., Rochus, J., Wolf, M., Gassen, M., Poschke, O. (Merck Patent GmbH) *Imidazole derivatives as phosphodiesterase VII inhibitors*. WO 0129049.

86. Eggenweiler, H.-M., Rochus, J., Wolf, M., Gassen, M., Poschke, O. (Merck Patent GmbH) *Imidazole compounds used as phosphodiesterase inhibitors*. WO 0136425.

87. Eggenweiler, H.-M., Jonas, R., Wolf, M., Gassen, M., Welge, T. (Merck Patent GmbH) *Imidazole compounds used as phosphodiesterase VII inhibitors*. WO 0136425.

88. Eggenweiler, H.-M., Ackerman, K.-A., Jonas, R., Wolf, M., Gassen, M., Welge, T. (Merck Patent GmbH) *Imidazopyridine derivatives as phosphodiesterase VII inhibitors*. US 6613778.

89. Inoue, H., Murafuji, H., Hayashi, Y. (Daiichi Suntory Pharma Co., Ltd.) *Imidazotriazinone derivatives as PDE7 (phosphodiesterase 7) inhibitors*. WO 2004111053.

90. Inoue, H., Murafuji, H., Hayashi, Y. (Daiichi Suntory Pharma Co., Ltd.) *Pyrazolopyrimidone derivatives having PDE7-inhibitory activity*. EP 1454897.

91. Terricabras-Belart, E., Segarra-Matamoros, V.M., Alvarez-Builla Gomez, J., Vaquero Lopez, J.J., Minguez Ortega, J.M. (Almirall Prodesfarma SA) *4-Aminothieno[2,3-d]pyrimidine-6-carbonitrile derivatives as PDE7 inhibitors*. WO 2004065391.

92. Vergne, F., Bernardelli, P., Lorthiois, E., Ducrot, P. (Bristol-Myers Squibb Co.) *(4,2-Disubstituted-thiazol-5-yl)amine compounds as PDE7 inhibitors*. WO 03082839.

93. Yang, G., McIntyre, K.W., Townsend, R.M. et al. *Phosphodiesterase 7A-deficient mice have functional T cells*. J Immunol 2003, 171: 6414-20.

94. Michaeli, T. (Albert Einstein College of Medicine of Yeshiva University) *Transgenic animal having a disrupted PDE7A gene and used thereof*. US 6740793.

95. Nakata, A., Ogawa, K., Sasaki, T. et al. *Potential role of phosphodiesterase 7 in human T cell function: Comparative effects of two phosphodiesterase inhibitors*. Clin Exp Immunol 2002, 128: 460-6.

96. Essayan, D.M., Kagey-Sobotka, A., Lichtenstein, L.M., Huang, S.K. *Differential regulation of human antigen-specific Th1 and Th2 lymphocyte responses by isozyme selective cyclic nucleotide phosphodiesterase inhibitors*. J Pharmacol Exp Ther 1997, 282: 505-12.

97. Landells, L.J., Szilagy, C.M., Jones, N.A. et al. *Identification and quantification of phosphodiesterase 4 subtypes in CD4 and CD8 lymphocytes from healthy and asthmatic subjects*. Br J Pharmacol 2001, 133: 722-9.

98. Staples, K.J., Bergmann, M., Tomita, K. et al. *Adenosine 3',5'-cyclic monophosphate (cAMP)-dependent inhibition of IL-5 from human T lymphocytes is not mediated by the cAMP-dependent protein kinase A*. J Immunol 2001, 167: 2074-80.

99. Jimenez, J.L., Punzon, C., Navarro, J., Munoz-Fernandez, M.A., Fresno, M. *Phosphodiesterase 4 inhibitors prevent cytokine secretion by T lymphocytes by inhibiting nuclear factor-κB and nuclear factor of activated T cells activation*. J Pharmacol Exp Ther 2001, 299: 753-9.

100. Averill, L.E., Stein, R.L., Kammer, G.M. *Control of human T-lymphocyte interleukin-2 production by a cAMP-dependent pathway*. Cell Immunol 1988, 115: 88-99.

101. Robicsek, S.A., Blanchard, D.K., Djeu, J.Y., Krzanowski, J.J., Szentivanyi, A., Polson, J.B. *Multiple high-affinity cAMP-phosphodiesterases in human T-lymphocytes*. Biochem Pharmacol 1991, 42: 869-77.

102. Stein, C.A. *The experimental use of antisense oligonucleotides: A guide for the perplexed*. J Clin Invest 2001, 108: 641-4.

103. Hatzelmann, A., Marx, D., Steinhilber, W. (Altana Pharma AG) *Phthalazinone derivatives useful as PDE4/7 inhibitors*. WO 02085906.

104. Pitts, W.J., Watson, A.J., Dodd, J.H. (Bristol-Myers Squibb Co.) *Dual inhibitors of PDE7 and PDE4*. WO 02088079.

105. Ukita, T., Sugahara, M., Terakawa, Y. et al. *Novel, potent, and selective phosphodiesterase-4 inhibitors as antiasthmatic agents: Synthesis and biological activities of a series of 1-pyridylnaphthalene derivatives*. J Med Chem 1999, 42: 1088-99.

106. Perez-Torres, S., Cortes, R., Tolnay, M., Probst, A., Palacios, J.M., Mengod, G. *Alterations on phosphodiesterase type 7 and 8 isozyme mRNA expression in Alzheimer's disease brains examined by in situ hybridization*. Exp Neurol 2003, 182: 322-34.

107. Miller, A.D. *Central mechanisms of vomiting*. Dig Dis Sci 1999, 44: 39S-43S.

108. Miller, A.D., Leslie, R.A. *The area postrema and vomiting*. Front Neuroendocrinol 1994, 15: 301-20.

109. Robichaud, A., Tattersall, F.D., Choudhury, I., Rodger, I.W. *Emesis induced by inhibitors of type IV cyclic nucleotide phosphodiesterase (PDE IV) in the ferret*. Neuropharmacology 1999, 38: 289-97.