Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3983–3987

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Benzyl Vinylogous Amide Substituted Aryldihydropyridazinones and Aryldimethylpyrazolones as Potent and Selective PDE3B Inhibitors

Scott D. Edmondson,[a,*] Anthony Mastracchio,[a] Jiafang He,[a] Christine C. Chung,[b] Michael J. Forrest,[c] Scott Hofsess,[c] Euan MacIntyre,[c] Joseph Metzger,[c] Naphtali O'Connor,[a] Kajal Patel,[c] Xinchun Tong,[a] Michael R. Tota,[b] Lex H. T. Van der Ploeg,[b] Jeff P. Varnerin,[b] Michael H. Fisher,[a] Matthew J. Wyvratt,[a] Ann E. Weber[a] and Emma R. Parmee[a]

[a]*Department of Medicinal Chemistry, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Metabolic Disorders, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA*
[c]*Department of Pharmacology, Merck & Co., Inc., PO Box 2000, Rahway, NJ 07065, USA*

Received 27 June 2003; revised 14 August 2003; accepted 27 August 2003

Abstract—Aryldihydropyridazinones and aryldimethylpyrazolones with 2-benzyl vinylogous amide substituents have been identified as potent PDE3B subtype selective inhibitors. Dihydropyridazinone **8a** (PDE3B $IC_{50}=0.19$ nM, 3A $IC_{50}=1.3$ nM) was selected for in vivo evaluation of lipolysis induction, metabolic rate increase, and cardiovascular effects.
© 2003 Elsevier Ltd. All rights reserved.

Cyclic nucleotide phosphodiesterases (PDEs) are responsible for catalyzing the hydrolysis and deactivation of secondary cellular messengers' cAMP and cGMP. The 11 isozymes in this gene family are responsible for regulating a number of physiologically relevant functions via downstream signaling cascades.[1] PDE3 inhibitors have been investigated clinically for their cardiotonic properties (vasodilation and inotropic activity) and antithrombotic properties.[2] Research in this field has led to the discovery of some well described isozyme selective PDE3 inhibitors like milrinone (**1**),[3] cilostamide (**2**),[4] SK&F 95654 (**3**),[5] and NSP-513 (**4**).[6]

To date, two highly homologous subtypes of PDE3 have been identified and each of these subtypes is localized in different tissues.[7] PDE3A was identified in vascular smooth muscle tissue and is located primarily in the heart and blood vessels. On the other hand, the PDE3B subtype is found most prominently in brown and white adipocytes. In addition to cardiotonic effects, inhibitors of PDE3 have been reported to stimulate lipolysis in adipocytes.[8] A potent subtype inhibitor of PDE3B would be an excellent tool to gauge the effects of PDE3B inhibition on lipolysis and whether such changes subsequently evoke an increase in metabolic rate. Such an inhibitor might find applications for the treatment of obesity. To date, limited work has surfaced describing potent PDE3B inhibitors.[8a,9] Consequently, we set out to identify a series of potent inhibitors that are selective for PDE3B over 3A (Fig. 1).

Many known PDE3 inhibitors (e.g., **1**, **3**, and **4**) possess a pyridazinone core that is important for selectivity over the other PDE isozymes (i.e., PDE2, PDE4, PDE5, PDE7).[10] Unfortunately, few known PDE3 inhibitors have been tested against the PDE3B subtype. Two inhibitors that have been tested against PDE3B are milrinone (**1**) and cilostamide (**2**). Although **1** is less potent against PDE3B than **2**, it is about two-fold selective for PDE3B over 3A (Table 1).[8a] Dihydropyridazinones such as **3** and **4** are reported to be more potent PDE3 inhibitors than **1**, but no reports exist of this structural motif with respect to inhibition against the 3B subtype. We hoped to take advantage of the

*Corresponding author. Tel.: +1-732-594-0287; fax: +1-732-594-5350; e-mail: scott_edmondson@merck.com

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.08.056

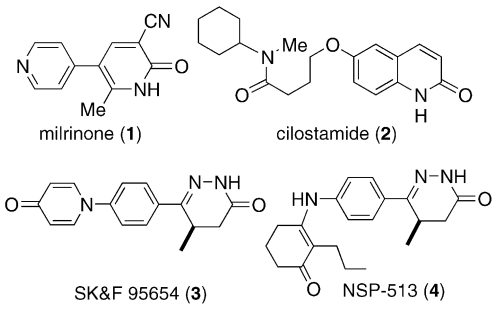
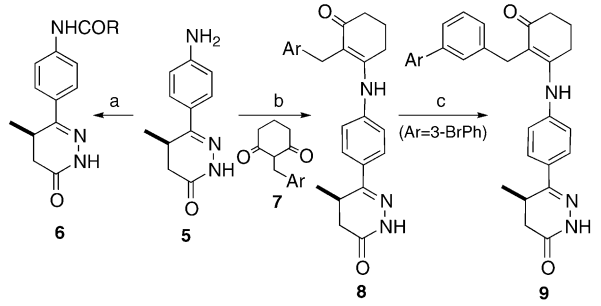


Figure 1. Known PDE3 inhibitors.

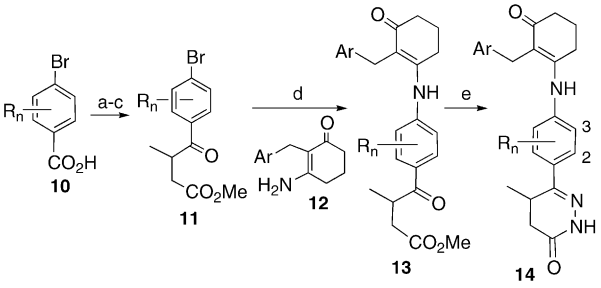
enhanced potency displayed by some of these inhibitors in our search for potent PDE3B inhibitors. Consequently, we decided to initiate a more comprehensive investigation of substituents on the dihydropyridazinone core.

Acylations of aniline **5**[11] under standard conditions afforded **6** in good yields (Scheme 1). Substituted benzyl vinylogous amides **8** were formed by heating **5** with β-substituted 1,3-cyclohexanediones **7**[12] (Scheme 1). Suzuki couplings with derivatives of **8** (Ar = 3-BrPh) afforded biaryl analogues such as **9**.

In order to access analogues with substitution on the central phenyl ring, a different synthetic route was utilized (Scheme 2). A series of benzoic acids (**10**) were converted to the corresponding Weinreb amides then alkylated with ethyl Grignard. Enolates of the resulting ethyl ketones were then alkylated with methyl bromoacetate to afford **11**. Bromide **11** underwent palladium



Scheme 1. Synthesis of substituted dihydropyridazinones: (a) Et_3N, RCOCl, DCM; (b) *p*-TsOH (cat), tol, **7**, DMSO, Δ; (c) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3 (aq), EtOH, dioxane, Δ.



Scheme 2. Synthesis of central-ring substituted dihydropyridazinones **14**: (a) MeONHMe–HCl, HOBt, EDC, DIEA, DCM; (b) EtMgCl, THF, 0 °C; (c) LHMDS, $BrCH_2CO_2Me$, THF, −78 °C; (d) 2-(2-Me_2NPh)PhP(*c*-hex)$_2$, $Pd_2(dba)_3$, **12**, THF, Cs_2CO_3, Δ; (e) NH_2NH_2, H_2O, MeOH, Δ.

mediated Buchwald-type coupling with **12** to afford vinylogous amides **13**.[13] These β-ketoesters were exposed to hot methanolic hydrazine to produce dihydropyridazinones **14** in good yields.

Encouragingly, the acylated dihydropyridazinones **6** are uniformly more potent at PDE3B than **1** and they maintain selectivity for 3B over 3A (Table 1).[14] The simple Cbz derivative **6d** is especially potent at PDE3B with an $IC_{50} = 1.0$ nM. Indeed this compound is 8.5 times more potent than the corresponding ethyl carbamate **6c**. We reasoned that the distance between the aryl groups in **6d** might be important for improving PDE3B potency. In order to apply this to the more potent vinylogous amide structure class, we replaced the propyl side chain of **4** with a benzyl group to give **8a**. We were pleased to find that **8a** is significantly more potent against PDE3B than **6d**, with a potency in the *picomolar* range ($IC_{50} = 0.19$ nM). This compound is 46 times more potent against PDE3B than **8b**, supporting the hypothesis that the pendant aryl group is important for potency. We were also pleased to observe that **8a** is 7-fold selective for PDE3B over 3A. Importantly, the *S* enantiomer of **8a** was tested and found to be more than 20 times less potent at PDE3B.[15]

Substitution on the pendant phenyl ring has little effect on PDE3B potency with most compounds retaining IC_{50}'s in the subnanomolar range (Table 2). While 4-substitution on this ring typically reduces PDE3B potency (**14h**), sterically demanding groups at the 3-position afford the most potent PDE3B inhibitors to date (i.e., biphenyl derivatives **9a** $IC_{50} = 0.068$ and **9b** $IC_{50} = 0.049$ nM). Regardless of substitution around the pendant phenyl ring, 3A/3B selectivity remains comparable or inferior to the parent compound **8a**.

Substitution on the central phenyl ring had a more dramatic effect on the potency and selectivity of PDE3B inhibitors (Table 2). Larger groups at the 2-position (e.g., **14i** and **14n**) result in a marked reduction of potency at PDE3B, but they retain or even improve the 3A/3B subtype selectivity. On the other hand, fluorine substituents on the central phenyl ring result in compounds with subnanomolar potency against PDE3B and a comparable selectivity profile to that of **8a**.

Table 1. Inhibition of PDE3A and PDE3B by dihydropyridazinones. All compounds are >94% ee (R) configuration except **6d**

Entry	R =	PDE3B IC_{50} (nM)[a]	PDE3A IC_{50} (nM)[a]
1[b]	—	280	440
2[b]	—	18	16
6a	Me	37	96
6b	2-CF_3Ph	5.0	15
6c	OEt	8.5	24
6d[c]	OBn	1.0	2.9
8a	Ph	0.19	1.3
8b	H	8.8	44

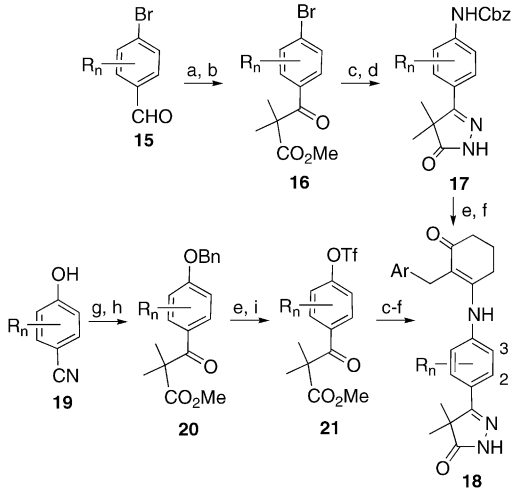
[a]Values are means of two or more experiments.
[b]These values are cited from ref 8a.
[c]Racemic.

4,4-Dimethylpyrazolones have been reported as ring-contracted mimics of dihydropyridazinone PDE3 inhibitors.[16] Using chemistry illustrated in Scheme 3, we next examined a series of dimethylpyrazolone vinylogous amides with respect to PDE3B potency and subtype selectivity. The addition of methyl isobutyrate enolate to substituted bromobenzaldehydes **15** followed by oxidation of the resulting benzylic alcohol afforded β-ketoesters **16**. Buchwald coupling of **16** with benzyl carbamate[17] followed by cyclization gave the dimethylpyrazolone **17**. Deprotection to form the aniline was followed by condensation with **7** to afford the desired vinylogous amides **18**. Alternatively, benzyl protection of phenol **19** followed by zinc promoted addition of ethyl 2-bromoisobutyrate to the nitrile afforded β-ketoester **20**. Deprotection followed by triflate formation generated **21**, which was converted to **18** following the previous sequence.

Table 2. Inhibition of PDE3A and PDE3B by dihydropyridazinones

Entry	Ar=	R=	PDE3B IC_{50} (nM)[a]	PDE3A IC_{50} (nM)[a]
14a[b]	2-MeOPh	H	0.13	0.39
14b[b]	2-CF_3Ph	H	0.35	0.83
14c[b]	3-FPh	H	0.70	2.8
14d[b]	3-BrPh	H	0.30	0.94
14e[b]	3-IPh	H	0.11	0.33
14f[b]	3-NO_2Ph	H	0.94	3.1
9a[b]	3-Thiophene	H	0.068	0.11
9b[b]	3-NO_2Ph	H	0.049	0.11
14g[b]	4-MeOPh	H	0.70	2.3
14h[b]	4-NO_2Ph	H	5.5	24
14i[c]	Ph	2-Me	2.6	22
14j[c]	Ph	3-Me	0.61	3.7
14k[c]	Ph	2-Et	21	101
14l[c]	Ph	2-F	0.24	1.6
14m[c]	Ph	3-F	0.26	1.2
14n[c]	Ph	2-Cl	4.8	46
14o[c]	Ph	2,5-F_2	0.62	1.6

[a]Values are means of two or more experiments.
[b]>94% ee (*R*).
[c]Racemic.



Scheme 3. Synthesis of dimethylpyrazolones **18**: (a) Me_2CHCO_2Me, LDA, THF, −78 °C; (b) Dess–Martin periodinane, DCM; (c) Xantphos, $Pd_2(dba)_3$, THF, Cs_2CO_3, $CbzNH_2$, Δ; (d) NH_2NH_2, EtOH, Δ; (e) $Pd(OH)_2$, H_2, EtOH; (f) **7**, toluene, TsOH, DMSO, Δ; (g) BnBr, THF, NaH, Bu_4NI; (h) Me_2CBrCO_2Et, Zn, THF, Δ then HCl, H_2O; (i) 2-$PyrNTf_2$, THF, KHMDS, −78 °C.

Although the pyrazolone vinylogous amides are generally less potent than the dihydropyridazinone analogues (Table 3), they still maintain potencies against PDE3B in the low nanomolar range. Furthermore, this series is more selective for 3B over 3A. Entry **18f** is the most selective PDE3B subtype inhibitor yet reported, with a 3A/3B selectivity of 33 and an IC_{50} = 4.5 nM against PDE3B. Potency against PDE3B could be enhanced by halide substitutions on the *ortho* position of the pendant phenyl ring, such as the 2,6-dichloride **18h** which exhibits a potency in the subnanomolar range (IC_{50} = 0.13 nM). Although analogues with sterically demanding substituents on the central phenyl ring are typically less potent at PDE3B, they retain much of the 3A/3B selectivity. On the other hand, fluoride substitution on the central phenyl ring appears to *increase* PDE3B potency, with 2,3-difluorophenyl analogue **18n** exhibiting an excellent overall in vitro profile (PDE3B IC_{50} = 0.33 nM, 3A/3B = 23).

When dosed in the rat (0.5 mg/kg iv), the half-life of **8a** was 1.0 h with a clearance of 6.8 mL/min/kg. Oral administration of **8a** (1.0 mg/kg) led to good overall blood levels (AUC_{norm} = 3.6 μM h) and an oral bioavailability of 61%. In order to test the effect of a potent PDE3B inhibitor on lipolysis and metabolic rate, analogue **8a** was selected as a benchmark for further biological evaluation.

To determine whether **8a** would evoke lipolysis in vivo, anesthetized rats were administered the compound in increasing doses via an intravenous infusion. Elevation of blood glycerol levels compared to vehicle was measured at 60 min to provide an index of lipolytic activity. We were pleased to find that **8a** caused a dose dependent increase in glycerol levels, with an ED_{50} = 0.60 mg/kg. Over a 90 min time period, the increase in plasma glycerol after administration of **8a** was time dependent and observed to be maximum at 60 min post dose (1.0 mg/kg iv).

Table 3. Inhibition of PDE3 subtypes by vinylogous amide pyrazolones

Entry	Ar=	R=	PDE3B IC_{50} (nM)[a]	PDE3A IC_{50} (nM)[a]
18a	Ph	H	10	53
18b	Ph	2-Me	4.8	38
18c	3-CNPh	2-Me	10	130
18d	Ph	2-F	2.5	20
18e	3-CNPh	2-F	3.2	69
18f	3-NO_2Ph	2-F	4.5	150
18g	2-ClPh	2-F	0.53	7.2
18h	2,6-Cl_2Ph	2-F	0.13	2.2
18i	2-FPh	2-F	1.1	16
18j	3-FPh	2-F	5.6	43
18k	4-FPh	2-F	3.1	63
18l	3-NO_2Ph	2-Cl	30	550
18m	2-ClPh	2-Cl	6.3	77
18n	3-CNPh	2,3-F_2	0.33	7.6

[a]Values are means of at least two experiments.

The observed increase of plasma glycerol indicates an increase of lipolysis leading to higher blood levels of glycerol and free fatty acids. Since free fatty acids undergo thermogenic oxidation in brown adipose tissue (where PDE3B is localized), we next sought to measure increases in metabolic rate after exposure to **8a**. We thus administered **8a** orally to male rats in increasing doses (1, 3, and 10 mg/kg) and measured percentage increases in thermogenesis compared to the predose metabolic rate. The potent PDE3B inhibitor **8a** showed a significant increase in metabolic rate at 3.0 and 10.0 mg/kg (14 and 21%, respectively) over an 8-h period post dosing.

In order to determine the effects of **8a** on cardiovascular activity, we administered this compound intravenously to anesthetized rats in increasing doses (0.01 to 0.1 mg/kg) and measured blood pressure over 60 min. A rapid (earliest time point = 5 min) and sustained (60 min = duration of study) decrease in mean arterial pressure was observed in response to **8a**. The maximal decrease in blood pressure was 40%, obtained at 0.1 mg/kg, 60 min post dosing and a 20% decrease was observed at 0.02 mg/kg. These cardiovascular effects are likely due to the off target PDE3A inhibition, which is known to induce vasodilation and is localized primarily in blood vessels and heart. Nevertheless, it is also possible that PDE3B inhibition could lead to the above effects.

In summary, a series of potent and subtype selective 2-benzylvinylogous amide derived PDE3B inhibitors were prepared and tested in vitro. Analysis of a series of dihydropyridazinones led to the discovery of the most potent PDE3B inhibitors yet reported (**9a** and **9b**). Investigations into a series of dimethylpyrazolones led to the discovery of several potent PDE3B inhibitors that are the most subtype selective compounds reported to date (e.g., **18f**, 3A/3B = 33 and **18n**, 3A/3B = 23). Benchmark compound **8a** was then shown to stimulate lipolysis in adipocytes with an $ED_{50} = 0.6$ mg/kg. Orally administered **8a** increased metabolic rate in the rat at 3 and 10 mg/kg. Finally, **8a** showed a significant lowering of blood pressure in the rat at doses as low as 0.01 mg/kg. The results described above suggest that an orally active, potent, and *selective* PDE3B inhibitor may be therapeutically useful for the treatment of obesity. Nevertheless, a compound substantially more selective than **8a** will be necessary to ultimately determine if hypotension is an effect of PDE3B inhibition.

Acknowledgements

We thank Dr. Steven L. Colletti and Mr. Richard Berger for experimental procedures helpful for the conversion of **10** to **11**.

References and Notes

1. (a) Soderling, S. H.; Beavo, J. A. *Curr. Opin. Cell Biol.* **2000**, *12*, 174. (b) Beavo, J. A. *Phsyiol. Rev.* **1995**, *75*, 725. (c) Weishaar, R. E.; Cain, M. H.; Bristol, J. A. *J. Med. Chem.* **1985**, *28*, 537.

2. (a) Manganiello, V. C.; Taira, M.; Degerman, E.; Belfrage, P. *Cell. Signal.* **1995**, *7*, 445. (b) Alvarez, R.; Banerjee, G. L.; Bruno, J. J.; Jones, G. H.; Littschwager, K.; Strosberg, A. M.; Venuti, M. C. *Mol. Pharmacol.* **1986**, *29*, 554.

3. For a lead reference describing this compound, see: Jaski, B. E.; Filer, M. A.; Wright, R. F.; Braunwald, E.; Colucci, W. S. *J. Clin. Invest.* **1985**, *75*, 643.

4. Nishi, T.; Tabusa, F.; Tanaka, T.; Ueda, H.; Shimizu, T.; Kanabe, T.; Kimura, Y.; Nakagawa, K. *Chem. Pharm. Bull.* **1983**, *31*, 852.

5. For a lead reference, see: Murray, K. J.; Eden, R. J.; Dolan, J. S.; Grimsditch, D. C.; Stutchbury, C. A.; Patel, B.; Knowles, A.; Worby, A.; Lynham, J. A.; Coates, W. J. *Br. J. Pharmacol.* **1992**, *107*, 463.

6. Hirose, H.; Mashiko, S.; Kimura, T.; Ishida, F.; Mochizuki, N.; Nishibe, T.; Nishikibe, M. J. *Cardiovasc. Pharm.* **2000**, *35*, 586.

7. (a) Maurice, D. H.; Liu, H. *Br. J. Pharmacol.* **1998**, *125*, 1501. (b) Degerman, E.; Belfrage, P.; Manganiello, V. C. *J. Biol. Chem.* **1997**, *272*, 6823. (c) Reinhardt, R. R.; Chin, E.; Zhou, J.; Taira, M.; Murata, T.; Manganiello, V. C.; Bondy, C. A. *J. Clin. Invest.* **1995**, *95*, 1528. (d) Taira, M.; Hockman, S. C.; Calvo, J. C.; Taira, M.; Belfrage, P.; Manganiello, V. C. *J. Biol. Chem.* **1993**, *268*, 18573. (e) Meacci, E.; Taira, M.; Moos, M.; Smith, C. J.; Movesian, M. A.; Degerman, E.; Belfrage, P.; Manganiello, V. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3721.

8. For a lead reference, see: (a) Snyder, P. B. *Emerg. Ther. Targets* **1999**, *3*, 587. (b) Ekolm, D.; Hemmer, B.; Gao, G.; Vergelli, M.; Martin, R.; Manganiello, V. C. *J. Immunol.* **1997**, *20*, 1529. (c) Murata, T.; Taira, M.; Manganiello, V. C. *FEBS Lett.* **1996**, *390*, 29.

9. (a) Leroy, M.-J.; Degerman, E.; Taira, M.; Murata, T.; Wang, L. H.; Movsesian, M. A.; Meacci, E.; Mangianello, V. C. *Biochemistry* **1996**, *35*, 10194. (b) Snyder, P. B.; Beaton, G.; Rueter, J. K.; Fanning, D. L.; Warren, S. D.; Hadidaruah, S. S. W.O. Patent 070,469, 2002.

10. (a) Mertens, A.; Friebe, W.-G.; Muller-Beckmann, B.; Kampe, W.; Kling, L.; von der Saal, W. *J. Med. Chem.* **1990**, *33*, 2870. (b) Coates, W. J.; Prain, H. D.; Reeves, M. L.; Warrington, B. H. *J. Med. Chem.* **1990**, *33*, 1735. (c) Moos, W. H.; Humblet, C. C.; Sircar, I.; Rithner, C.; Weishaar, R. E.; Bristol, J. A.; McPhail, A. T. *J. Med. Chem.* **1987**, *30*, 1963. (d) Sircar, I.; Weishaar, R. E.; Kobylarz, D.; Moos, W. H.; Bristol, J. A. *J. Med. Chem.* **1987**, *30*, 1955.

11. Owings, F. F.; Fox, M.; Kowalski, C. J.; Baine, N. H. *J. Org. Chem.* **1991**, *56*, 1963.

12. Rajamannar, T.; Palani, N.; Balasubramanian, K. K. *Syn. Commun.* **1993**, *23*, 3095.

13. Edmondson, S. D.; Mastracchio, A.; Parmee, E. R. *Org. Lett.* **2000**, *2*, 1109.

14. The catalytic regions of PDE3B and PDE3A were expressed as soluble proteins in *Escherichia coli*. The human PDE3B gene fragment was inserted into a pET30a vector (Novagen) such that an S-tag and a poly-histidine tag would be added to the N-terminus of the protein starting at amino acid 387. The human PDE3A gene fragment was inserted into a pET23a vector such that a T7 tag would be added to the N-terminus of PDE3A starting at amino acid 388. The proteins were expressed in *E. coli* BL21 (DE3) pLysS following an IPTG induction. The soluble protein extract from *E. coli* was precipitated by ammonium sulfate (50% saturation) and resuspended in an equivalent volume of buffer (20 mM Tris, pH 7.4, 75 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT, 0.5 mM EDTA, 1:1000 poly-histidine tagged protein protease inhibitors (Sigma-Aldrich), and 35% ethylene glycol). After an overnight dialysis against the above buffer, the sample was purified over DEAE Sepharose FF (40–70 mg protein/mL resin, Amersham

Biosciences) equilibrated in the same buffer. The column was washed with buffer plus an additional 70 mM NaCl and eluted with a 70–250 mM NaCl gradient. The partially purified PDE3A and PDE3B was stored at −80 °C until used.

PDE3 enzyme activity was determined using the phosphodiesterase [^{3}H]cAMP SPA enzyme assay (Amersham Life Science). The assay buffer was 50 mM Tris pH 7.5, 8.3 mM $MgCl_2$, 1.7 mM EGTA and 10 nM [^{3}H] cAMP (~40 Ci/mmol). The reactions were conducted in a total volume of 100 μL at room temperature for 2–15 min. Data was analyzed in Graph Pad Prism software.

15. The *S* enantiomer of **8a** was 5.1 nM at PDE3B and 26 nM at 3A.
16. Sircar, I.; Morrison, G. C.; Burke, S. E.; Skeean, R.; Weishaar, R. E. *J. Med. Chem.* **1987**, *30*, 1724.
17. Yin, J.; Buchwald, S. L. *Org. Lett.* **2000**, *2*, 1101.