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Discovery of new inhibitor for PDE3 by virtual screening

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ABSTRACT

In this work, we tried to find a new scaffold for a PDE3 using virtual screening for the obesity treatment. We first analyzed structural features for the known PDE3 inhibitors based on the PDE3B-ligand complex structure, and then carried out a docking study based on PDE3B 3D structure. We obtained a compound as potent PDE3 inhibitor stimulating lipolysis in murine adipocytes and human adipocytes.

Obesity has been seriously recognized as a major public health problem in industrialized and even in developing countries because the metabolic deregulation by an excess of fat mass can sequentially lead to complications including diabetes mellitus, coronary heart disease and hypertension. Main strategy for the treatment of obesity is the pharmaceutical targeting of lipolysis. In adipocytes, the stimulation of triglyceride (TAG) hydrolysis to free fatty acid (FFA) and glycerol can diminish the fat stores and combat obesity.[1,2]

The adipocyte lipolysis can be controlled by several molecules via complicated signaling pathways. In the lipolytic cascade, catecholamine-induced activation of adrenergic receptors can subsequently stimulate the increase of intracellular cyclic AMP (cAMP) by adenylyl cyclase and further promote the activation of cAMP-dependent protein kinase A (PKA) which subsequently induces the activation of hormone-sensitive lipase (HSL) and results in the hydrolysis of TAG into diacylglycerol and monoacylglycerol that is finally hydrolyzed into FFA and glycerol.[3] The lipolysis can be negatively regulated by the enzyme, phosphodiesterase (PDE) 3 that is a major isoforms among 11 isozymes in the PDE family and is activated by insulin in adipocytes.[4,5] Since insulin-induced phosphorylation and activation of PDE3 leads to increased hydrolysis of cAMP, decreased activity of PKA and thereby an inhibition in PKA-dependent activation of HSL and lipolysis, PDE3 in adipocytes has been considered to be play a critical role in the antilipolytic action of insulin.[6] Furthermore, the decreased adipocyte size, enhanced catecholamine-stimulated lipolysis and blocked insulin inhibition of catecholamine-stimulated lipolysis have been shown in adipocytes of PDE3 knockout mice and these results shown the critical role of PDE3 in the regulation of lipolysis in adipocyte function.[7]

Therefore, the stimulation of lipolysis by inhibiting PDE3 activity has been suggested to be a useful means for the treatment of obesity. In fact, inhibitors of PDE3 have been reported to stimulate lipolysis in adipocytes[8] and further study on crystal structure of human PDE3B has allowed or the design of more potent and selective PDE3 inhibitors.[9] In this study, the structure-based studies for PDE3 inhibitors was performed and further its enzymatic activity assay and cell-based lipolysis assay were carried out for evaluating those lipolytic activities.

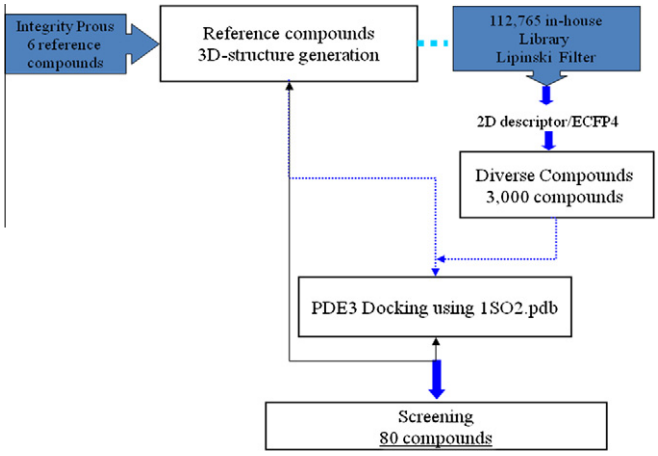

Figure 1. The workflow of virtual screening for finding PDE3 inhibitors.

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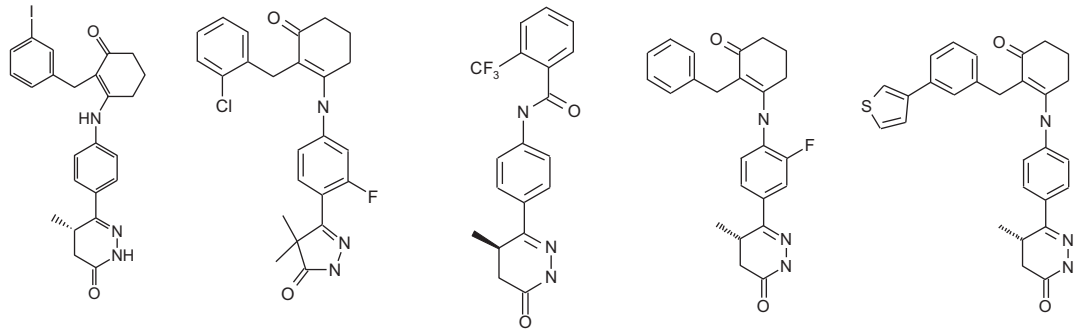
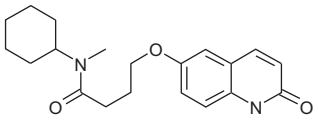
Table 1
The Dock Score and structures of six reference compounds obtained from the known inhibitors

Compound	Dock Score (kcal/mol)
1 (ligand of 1SO2)	65.46
2	60.27
3	64.38–
4	51.84
5	91.70
6 (Cilostamide)	47.07



com1[a] com2[a] com3[a] com4[a] com5[a]



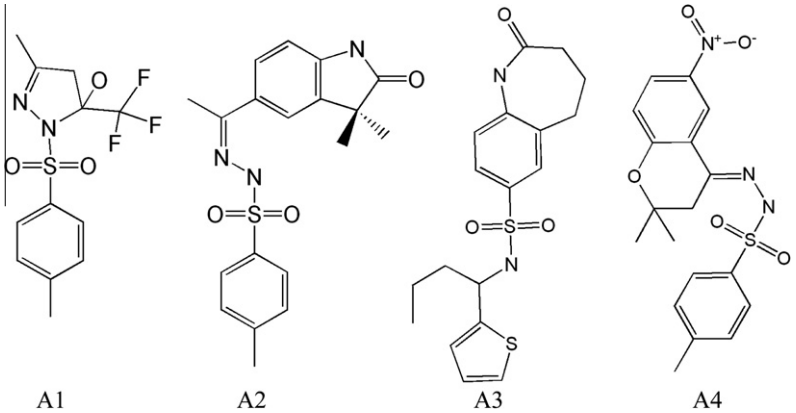
com6[b]

[a] See Ref. 13.
[b] See Ref. 14.

Table 2
Effect of PDE inhibitors on lipolysis in murine C3H/10T1/2 adipocytes and human subcutaneous adipocytes

	C3H10T1/2 adipocytes lipolysis activity at 10 μM (% control) [a]	C3H10T1/2 adipocytes lipolysis activity (EC_{50})	Human adipocytes lipolysis activity at 10 μM (% control)
Isoprotenol[b]	291.30	19.00 nM	214.39
HL-1-87c	227.17	10.40 nM	143.43
Cilostamide	247.36	9.10 nM	192.40
A1	171.30	6.80 nM	116.67
A2	191.70	8.90 nM	171.50
A3	131.90	–	130.20
A4	168.40	5.20 nM	116.67

[a] Values represent the glycerol concentration in the culture supernatant and are expressed as percentages of vehicle control.
[b] Isoproterenol is used as a positive control for screening pharmaceuticals that regulate free fatty acid release from adipocytes.



A1 A2 A3 A4

Figure 2. Structures of candidates compounds (compounds **1**–**4**).

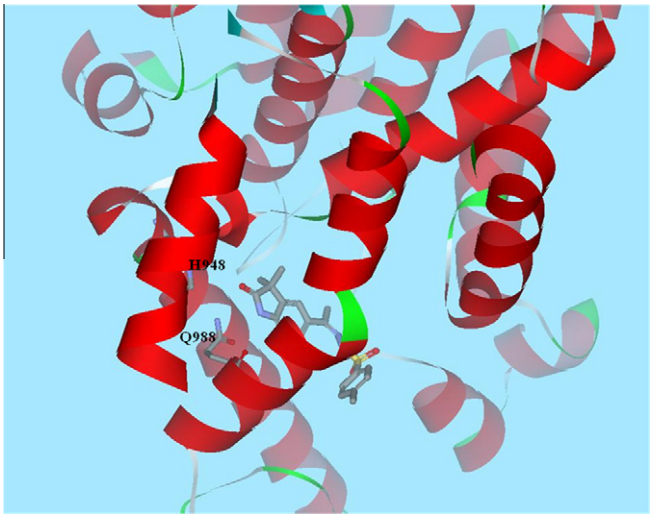


Figure 3. The docking mode for compound **A2**.

3D structures of PDE3B-ligand complex were built using a co-crystal structure, PDB entry; 1SO2.[10] Conversely, to prepare the 3D structures of the selected compounds, the molecular modeling software package Accelrys Discovery Studio2.1 was used. Partial atomic charges were calculated by the Gasteiger–Huckel method and energy minimizations were carried out using the CHARMM force field[11] with a distance-dependent dielectric and the Powell conjugate gradient algorithm.

Table 3
Docking results

	Dock Score (kcal/mol)
Cilostamide	51.78
A1	49.37
A2	52.28
A3	49.17
A4	44.80

For docking by LigandFit[12] interfaced with Accelrys Discovery Studio2.0, NH_2 group of side chain in Q988 residue was defined as the hydrogen donor interaction site and the default parameters used. The predicted docking mode for ligand of 1SO2 was well generated showing RMSD value, 0.3 Å, compared the crystallographic structure. Through the docking study for ligand of 1SO2, Dock Score were 65.45 kcal/mol. Also, we used the known PDE3B inihibitors[13,14] for deciding the criteria of compounds selection among the diverse 3000 compounds as shown in Figure 1. The results of docking for the known compounds are shown in Table 1. According to the docking results for the known compounds, we finally filtered 80 compounds among 313 compounds having value of more than 44.5 kcal/mol, and then examined the inhibition on lipolysis in murine C3H/10T1/2 adipocytes[15] and human subcutaneous adipocytes.[16] Isoproterenol, a positive control, induced lipolysis[17] in murine C3H/10T1/2 adipocytes (291.3% of vehicle control) and

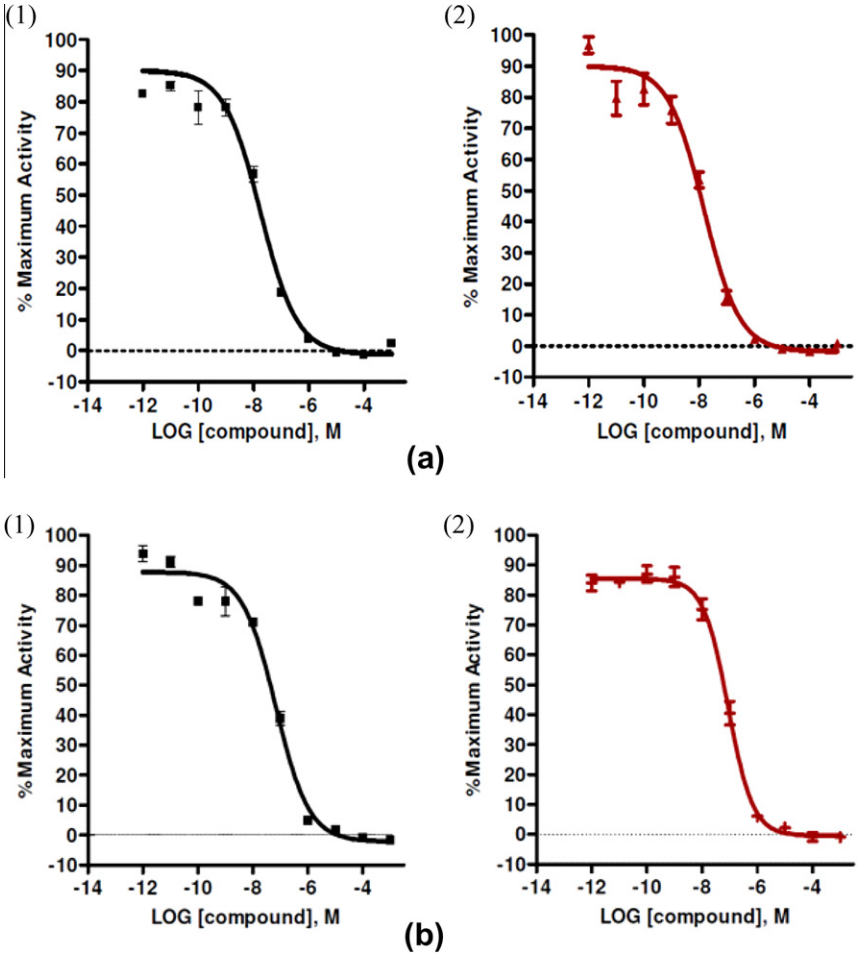


Figure 4. PDE 3A (a) and B (b) inhibitory activity of cilostamide (1) and compound **2A** (2).

human adipocytes (169.2% of vehicle control). Cilostamide, a selective PDE3 inhibitor, stimulated lipolysis in murine C3H/10T1/2 adipocytes (247.4% of vehicle control) and human adipocytes (156.0% of vehicle control), as shown in Table 2. Through the lipolysis assay of human and murine adipocytes experiments, we obtained four compounds to be expected as PDE 3 inhibitor, as shown in Table 2. In Figures 2 and 3 and Table 3, we reported structures, docking mode and Dock Score of the obtained four compounds. As expected, the four compounds have favorable docking score and docking mode. Among them, compound **A1** stimulated lipolysis in murine C3H/10T1/2 adipocytes (171.3% of vehicle control) and weakly stimulated lipolysis in human adipocytes. Compound **A2** also stimulated lipolysis in murine adipocytes and human adipocytes. Compound **A3** weakly stimulated lipolysis in murine adipocytes and human adipocytes. Compound **A4** stimulated lipolysis in murine C3H/10T1/2 adipocytes (168.4% of vehicle control) but had no effect in human adipocytes (Supplementary data showed the adipogenesis effect in 3T3-L1 cells and the adipogenesis related gene expression effect reporting compound **A2** has more potent adipogenesis effect than cilostamide).

Also, we carried out PDE3A and PDE3B enzymatic assays.[18] Cilostamide was used as a reference compound. The results for PDE 3 activities are shown in Figure 4. Among 4 compounds, compound **A2** exhibited the strongest inhibitory activity for PDE3. When we evaluated the IC_{50} value of compound **2A** compared to cilostamide (IC_{50} = 18.1 nM) for PDE3A activity, compound **A2** (IC_{50} = 14.8 nM) has better inhibitory activity for PDE3A. On the other hand, for PDE3B activity, compound **A2** (IC_{50} = 88.40 nM) has less inhibitory activity for PDE3B compared to cilostamide (IC_{50} = 69.07 nM). PDE3 activity assay was performed in triplicate (Caliper Life Sciences, ML). We verified that our new scaffold, compound **2A**, showed a strong activity for PDE3 shown in Figure 4.

In this study, we obtained new scaffold as the early hit compound of PDE3 for the obesity treatment using virtual screening. Further optimization of compound **2A** and the other hit compounds is now underway and will be reported.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.120.

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15. C3H/10T1/2 (ATCC CCL-226, mouse embryo cells) were grown and maintained in Dulbecco′s modified Eagle′s medium (DMEM) high glucose containing 10% fetal bovine serum and 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) in a 5% CO_2 environment. C3H/10T1/2 cells differentiated to mature adipocytes by the same medium containing 20 μg/ml insulin, 0.5 mM isobutylmethylxanthine (IBMX) and 1 μM dexamethasone for four days, were replaced with medium containing 20 μg/ml insulin for three days, and then cultured for two days in culture medium.
16. Human subcutaneous preadipocytes (Zen-Bio, Inc., ZBM-1) were grown and maintained in Preadipocyte medium [DMEM/Ham′s F-12 (1:1, vol/vol), 10% fetal calf serum, 15 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B] (Zen-Bio, Inc., #PM-1) in a 5% CO_2 environment. Human subcutaneous preadipocytes differentiated to mature adipocytes by Adipocyte Differentiation Medium [Adipocyte medium, 0.25 mM IBMX, and a proprietary γ PPAR agonist (10 μM)] (Zen-Bio, Inc., #DM-2) for seven days, and then were replaced with fresh Adipocyte medium [DMEM/Ham′s F-12 (1:1, vol/vol), 3% fetal calf serum, 1 μM dexamethasone, 100 nM human insulin, 33 μM D-biotin, 17 μM Na-pantothenate, 15 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B] (Zen-Bio, Inc., #AM-1) for seven days.
17. For lipolysis assay, differentiated C3H/10T1/2 cells (5×10^4 cells/well in a 24-well plate) or human adipocytes (2.5×10^4 cells/well in a 48-well plate) were incubated with compounds for 24 h at 37 °C and then glycerol assay was carried out. Cultured supernatants (25 μl) and 100 μl of free glycerol assay reagent (Cayman Chemical Company, MI) were mixed in a 96-well plate. After 15 min incubation at room temperature, the absorbance at 540 nm was measured with fluorescence plate reader (Bio-Tek Instruments, Inc., Winooski, VT). Absolute glycerol concentrations were calculated from a standard curve. Means and SEM are expressed as a percentage of the vehicle control.
18. PDE 3B assays were performed in a reaction medium containing iFluor 488-AHC-cAMP, human recombinant PDE3B (BPS Bioscience Inc., San Diego, CA), 0.002% Brij, MgCl2, DTT and DMSO or compounds. This PDE3B activity assay was carried out in Caliper Life Sciences.