CALBIOCHEM®

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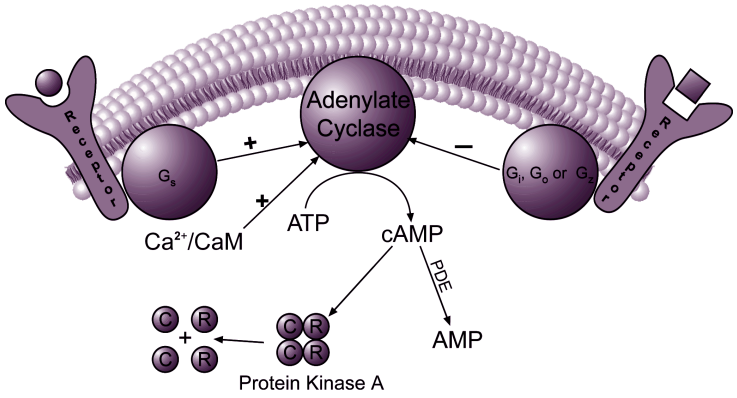
ACTIVATORS AND INHIBITORS OF ADENYLATE CYCLASE

Transmembrane receptors of various hormones are coupled to adenylate cyclase (AC) via heterotrimeric G-proteins. Ligand binding to the receptor changes the receptor conformation, allowing it to associate with a G-protein. This results in the activation of the specific G-protein via exchange of GTP for GDP bound to the α-subunit of the G-protein. The activated G-protein in turn activates AC resulting in the conversion of ATP to cAMP. cAMP then acts to regulate a wide variety of cellular processes. AC can couple with both the stimulatory and the inhibitory G-proteins (G_s and G_i, respectively). Interaction with G_s stimulates its activity and interaction with G_i inhibits its enzymatic activity.

AC is composed of two cytoplasmic domains, and two membrane-spanning domains, each of which contains six transmembrane spans. The amino acid sequence of each cytoplasmic domain, which is thought to contain a nucleotide (ATP) binding site, is well-conserved among the various subtypes. Although ACs can exist in both particulate and soluble forms, the particulate form is more prevalent in mammals.

Based on the conservation of their catalytic domains three classes of ACs have been cloned and are described as class I-AC from Enterobacteria; class II-''toxic'' ACs, including calmodulin (CaM)-activated enzymes from *Bordetella pertussis* and *Bacillus anthracis*; and class III-AC homologues from bacteria to human that include nine isoforms found in mammals, designated AC-1 to AC-9. These nine isoforms are stimulated by the α-subunit of G_s-protein and by forskolin. ACs are also capable of receiving signals from a variety of other sources, such as G_i-α, protein kinase A, protein kinase C, CaM kinase, and Ca^{2+}/CaM. Hormonal activation of CaM-dependent adenylate cyclase occurs at very low Ca^{2+} levels. The activity of AC is inhibited by high levels of Ca^{2+}, which also activate CaM-dependent phosphodiesterase.



Toxins that modulate adenylate cyclase activity via ADP-ribosylation of G-proteins

The α subunit of some G-proteins contains sites for modification by cholera toxin or pertussis toxin. Using NAD as the donor, these toxins catalyze the covalent incorporation of ADP-ribose into the G-protein α-subunit. Pertussis toxin catalyzes the ADP-ribosylation of G_i at a site that impairs the ability of the heterotrimeric G-proteins to interact with receptors. Cholera toxin ADP-ribosylates G_s in a manner that stabilizes the GTP-bound form resulting in persistent activation. CALBIOCHEM® is pleased to offer both holotoxins and purified toxin subunits.

Ref.: Nowak, J.Z., and Zawilska, J.B. 1999. *Postepy. Hig. Med. Dosw.* **53,**147; Sunahara, R.K., et al. 1996. *Annu. Rev. Pharmacol. Toxicol.* **36,** 461; MacNeil, S., et al. 1985. *Cell Calcium* **6,** 213; Stiles, G.L. 1989. *J. Cardiovasc. Pharmacol.* **14** (Suppl 5), S1.

Product	Cat. No.	M.W.	Comments
Cholera Toxin, *Vibrio cholerae*, Type Inaba 569B	227035	84,000	ADP-ribosylates G_s causing persistent activation of adenylate cyclase.
Cholera Toxin, *Vibrio cholerae*, Type Inaba 569B, Azide Free	227036	84,000	Formulated without azide for use in tissue culture.
Cholera Toxin A Subunit	227037	28,000	Portion of the holotoxin responsible for activating adenylate cyclase via ADP-ribosylation of G_s.
Cholera Toxin B Subunit	227039	55,000	Portion of cholera toxin responsible for binding to the GM_1 ganglioside receptor on the cell surface.
Cholera Toxin B Subunit, Peroxidase Conjugate	227041	—	Suitable for the demonstration of dendritic branching in retrogradely labeled neurons.
Pertussis Toxin, *Bordetella pertussis*	516560	105,000	Catalyzes the ADP-ribosylation of G_i, uncoupling G_i from receptors.
Pertussis Toxin A Protomer	516854	28,000	Enzymatic component of the holotoxin which contains both the NAD-glycohydrolase and the ADP-ribosyltransferase activities.
Pertussis Toxin B Oligomer	516852	4 subunits	Portion of holotoxin responsible for binding to cell surfaces and facilitating the entry of the A protomer.

Ligands Which Modulate Adenylate Cyclase Activity via G-protein Coupled Receptors

Product	Cat. No.	Effect on Adenylate Cyclase	Receptor	Ref.
Adenosine	1160	Inhibitor/ Activator	A_1/ A_2	1
Adenosine, N^6-Cyclohexyl-	116830	Inhibitor	Adenosine A_1	2
Angiotensin II	05-23-0101	Inhibitor	Angiotensin II	3
Carbacyclin	212402	Activator	PGI_2	4,5
Dopamine	4000	Activator/ Inhibitor	D_1/D_2	6
Endothelin 1	05-23-3800	Activator/ Inhibitor	ET_A/ ET_B	7
L-Epinephrine	3249	Activator/ Inhibitor	β_1, β_2/ α_2	8
L-(–)-Epinephrine-(+)-bitartrate	324900	Activator/ Inhibitor	β_1, β_2/ α_2	8
Glucagon	05-23-2700	Activator	Glucagon	9
Isoproterenol, HCI	420355	Activator	β Adrenergic	8
(±)-Octopamine, HCI	494420	Activator	$Octopamine_2$	10
PACAP 27 Amide	05-23-2151	Activator	PACAP I and II	11,12
PACAP 38	05-23-2150	Activator	PACAP I and II	12,13
Parathyroid Hormone 1-34	Several	Activator	PTH	14
Prostaglandin D_2	538909	Activator	PGD_2	15
Prostaglandin E_1	538903	Activator	PGE_1	16
Prostaglandin E_2	538904	Activator/ Inhibitor	EP_2/ EP_3	15,17
Prostaglandin I_2	538925	Activator	PGI_2	18
[Arg^8]-Vasopressin	05-23-0150	Activator	Vasopressin	19
[Lys^8]-Vasopressin	05-23-0153	Activator	Vasopressin	19

References:

1. Ramkumar, V., et al. 1991. *Mol. Pharmacol.* **40**, 639.
2. Ishikawa, A., et al. 1994. *Neuroscience Lett.* **171**, 129.
3. Baukal, A.J., et al. 1994. *J. Biol. Chem.* **269**, 24546.
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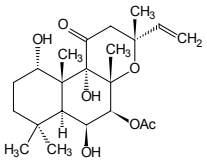
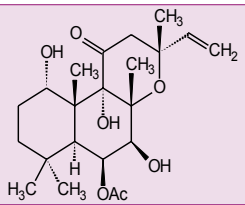
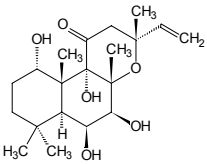
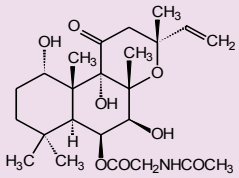
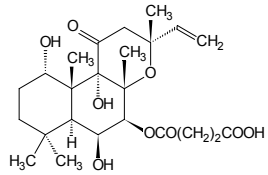
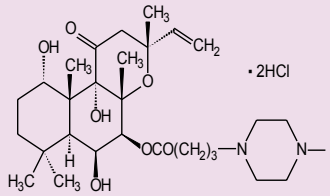
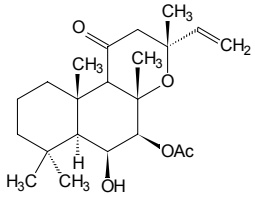
19. Gorbulev, V., et al. 1993. *Eur. J. Biochem.* **215**, 1.

Forskolin and Forskolin Analogs - Unique Activators of Adenylate Cyclase

Forskolin, a diterpene, is a potent activator of adenylate cyclase that has been used extensively to increase intracellular cAMP levels and to elicit cAMP-dependent physiological responses. This activation is believed to occur via its interaction with the catalytic subunit of adenylate cyclase. Although low levels of forskolin directly elicit small increases in cAMP levels, they greatly potentiate hormonal activation of adenylate cyclase in a number of intact cells. Forskolin, thus, is an invaluable tool for studying the role of cAMP in physiological responses to hormones. More recently, forskolin has also been shown to inhibit a number of membrane transport proteins and channel proteins through a mechanism that does not involve the production of cAMP. Forskolin does not affect the activity of guanylate cyclase or cyclic nucleotide phosphodiesterases.

Product	Cat. No.	Structure	Comments	Ref.
Forskolin	344270		Rapid and reversible activator of adenylate cyclase. EC_{50} = 5 - 10 µM in rat cerebral cortical membranes.	1
Forskolin, 6-Acetyl-7-deacetyl-	344271		Less potent derivative of parent compound. EC_{50} = 40 µM in rat cerebral cortical membranes.	2
Forskolin, 7-Deacetyl-	344274		Less potent derivative of parent compound. EC_{50} = 20 µM in rat cerebral cortical membranes.	2
Forskolin, 7-Deacetyl-6-(N-acetylglycyl)-	344272		Chemically modified forskolin with greater stability and water solubility. Exhibits about 80% of the stimulatory activity of its parent compound. Reported to reduce cAMP-sensitive K^+ current.	3
Forskolin, 7-Deacetyl-7-O-hemisuccinyl-	344275		Can be readily activated and used for preparation of probes and affinity supports. Free carboxyl group can be readily coupled to aminoethyl-agarose via its N-hydroxy-succinimide ester. The immobilized derivative can be used to purify adenylate cyclase.	4
Forskolin, 7-Deacetyl-7-(O-N-methylpiperazino)-γ-butryl-Dihydrochlonde	344273		Water-soluble (20 µM) forskolin derivative which activates adenylate cyclase in rat brain membrane preparations (EC_{50} = 13 µM).	5
Forskolin, 1,9-Dideoxy-	344277		Naturally-occurring analog of forskolin which does not stimulate adenylate cyclase (EC_{50} >1 mM). For use as a negative control.	6

References:

1. Seamon, K.B., et al. 1981. *Proc. Natl. Acad. Sci. USA* **78**, 3363.
2. Seamon, K.B., et al. 1983. *J. Med. Chem.* **26**, 436.
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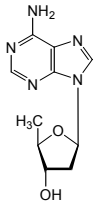
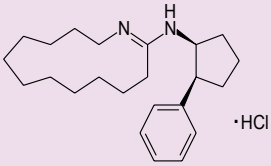
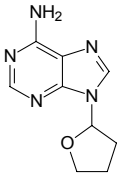
6. Seamon, K.B., et al. 1984. *Proc. Natl. Acad. Sci. USA* **81**, 5081.

Products which Inhibit Adenylate Cyclase by Non-Receptor-Dependent Mechanisms

Product	Cat. No.	Structure	Comments	Ref.
2',5'-Dideoxyadenosine	288104		A cell-permeable adenosine analog that binds to the "P-site" adenosine receptor located on the catalytic subunit of adenylate cyclase. Blocks parathyroid hormone-induced cAMP production (IC_{50} = 100 µM).	1,2
MDL-12,330A, Hydrochloride	444200		Cell-permeable. Irreversibly inhibits adenylate cyclase in rat liver membranes (IC_{50}= 250 µM).	3
SQ 22536	568500		Cell-permeable. Blocks parathyroid hormone-induced cAMP production (IC_{50} = 200 µM).	1

References:

1. Reid, I.R., et al. 1990. *Amer. J. Physiol.* **258**, E708.
2. Williams, M. 1987. *Annu. Rev. Pharmacol. Toxicol.* **27**, 315.
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Inhibitors of cAMP Phosphodiesterases (PDE IV).

The hydrolysis of cAMP and cGMP is catalyzed by many cyclic nucleotide phosphodiesterases (PDE). These enzymes are classified based on their substrate specificity and on how they are regulated by calmodulin and cGMP. PDE inhibitors are often used in combination with adenylate cyclase activators to produce greater cAMP levels than may be achieved by using either class of reagents alone.

Product	Cat. No.	Target PDE family	Comments
Denbufylline	253500	Selective	A xanthine derivative that acts as a selective inhibitor of cAMP-specific phosphodiesterase (K_i ~ 1 µM).
Etazolate, HCl (SQ20009)	331500	Selective	A selective cAMP-specific phosphodiesterase inhibitor (IC_{50} = 2 µM).
IBMX (3-Isobutyl-1-methylxanthine)	410957	Non-selective	A non-specific inhibitor of cAMP and cGMP phosphodiesterases (IC_{50} = 2 - 50 µM). Also acts as an adenosine receptor antagonist.
Ro-20-1724	557502	Selective	Selective inhibitor of cAMP-specific phosphodiesterase IC_{50} = 2 µM).
Rolipram	557330	Selective	Selective competitive inhibitor of cAMP-specific phosphodiesterase (IC_{50} = 800 nM). A rolipram-insensitive PDE IV subtype is also known to exist.

Please call our Technical Service Department or your local sales office for more information on these products.

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