

FREQUENTLY ASKED QUESTIONS: KINASE INHIBITORS AND ACTIVATORS

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Promega has recently made important additions to its line of highly pure, quality-tested products for cell signaling research. These include the following compounds that are staples in many laboratories engaged in cell signaling research:

- PD 98059 (2'-amino-3'-methoxyflavone), a selective inhibitor of MAP kinase kinase (MEK; [Cat.# V1191](#)).
- SB 203580 (4-[4'-fluorophenyl]-2-[4'-methylsulfinylphenyl]-5-[4'-pyridyl] imidazole), a p38 MAP kinase inhibitor ([Cat.# V1161](#)).
- LY 294002 (2-[4-morpholinyl]-8-phenyl-4H-1-benzopyran-4-one), a phosphatidylinositol 3-kinase (PI 3-kinase) inhibitor ([Cat.# V1201](#)).
- PMA (phorbol 12-myristate 13-acetate), a specific activator of Protein Kinase C (PKC) ([Cat.# V1171](#)).
- 4 α -PMA (4 α -phorbol 12-myristate 13-acetate), an inactive analogue of the PKC activator, PMA ([Cat.# V1181](#)).

Each compound has undergone stringent quality testing to confirm structural integrity and ensure a consistently

high level of purity. These compounds have proven to be extremely useful research. Here we examine questions commonly posed by investigators considering their utility in a variety of experimental applications.

PD 98059

Q: How does PD 98059 differ from U0126, and are there advantages of one of these compounds over the other?

Both PD 98059 ([Cat.# V1191](#)) and U0126 (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio] butadiene; [Cat.# V1121](#)) are MEK inhibitors, and both are useful for probing the role(s) of MEK in various biological processes, particularly those involving mitogen-activated protein kinase (MAP kinase/ERK) activation. Table 1 provides a comparison of the two compounds.

Both inhibitors are noncompetitive with respect to the MEK substrates ATP and ERK (3). Although the binding sites for PD 98059 and U0126 on MEK1 appear to overlap, their mechanisms of action differ (2,3); U0126 is a direct inhibitor of MEK activity, whereas PD 98059 inhibits MEK activation (1–3). In the context of a thorough study, researchers exploit the differences and similarities of the two compounds. For example, if an effect is inhibited by both of these structurally and mechanistically distinct inhibitors, a much stronger argument can be made for MEK playing a role than if only a single inhibitor were used. Furthermore, the selectivity of PD 98059 for MEK1 over MEK2 compared to the lack

Table 1. Comparison of PD 98059 and U0126.

	PD 98059	U0126
Specificity	Inhibits MEK1, an inefficient inhibitor of MEK2 (1,2).	Inhibits MEK1 and MEK2 (3).
Mechanism of Action	Inhibits phosphorylation activation of MEK1 by upstream kinases. Poor inhibitor of phosphorylation-activated MEK1 activity and constitutively active MEK1 mutant (1).	Inhibits phosphorylation-activated MEK1, MEK2, and constitutively active MEK1 and MEK2 mutants (3,4).
Potency (IC50)	For activation of MEK1 by Raf = 5 μ M (1). For activation of MEK2 by Raf = 50 μ M (1). For active MEK1 mutant = 10 μ M (1,3,4). For Raf-activated MEK1 >100 μ M (1,4).	For Raf-activated MEK1,2 = 10 μ M (3,4). For active MEK1 mutant = 0.07 μ M (3,4). For active MEK2 mutant = 0.06 μ M (3).

Table 2. Comparison of LY 294002 and Wortmannin.

	LY 294002	Wortmannin
Specificity	Specific for PI 3-kinase (at 50 μ M does not inhibit PI 4-kinase, DAG-kinase, PKC, PKA, MAPK, S6 kinase, rabbit kidney kinase, EGFR and c-src Y-kinases; 9).	Less specific than LY 294002 (also inhibits PI-4 kinase, MLCK and PLD; 11).
Mechanism of Action	Reversible inhibitor. Competitive for ATP binding on PI 3-kinase (9,10).	Irreversible inhibitor. Reacts covalently with a lysine residue in the ATP binding site of PI 3-kinases (9,10).
Potency (IC50)	Class I PI 3-kinases = 1 μ M (9,11). Class II PI 3-kinases = 19 μ M (11). Class III PI 3-kinases = unknown.	Class I PI 3-kinases = 1–10nM (11). Class II PI 3-kinases = 50–450nM (11). Class III PI 3-kinases = 2–10nM (11).
Stability	Stable in aqueous solution (10).	Unstable in aqueous solution (10).

of preference for either by U0126 can be exploited to probe the relative roles of the two kinases.

SB 203580

Q: What are the properties of SB 203580, and how does it compare to other p38 inhibitors?

SB 203580 (Cat.# V1161) is a pyridinyl imidazole inhibitor of the stress and inflammatory cytokine-activated MAP kinases known variously as p38, p40, stress-activated protein kinase (SAPK), cytokine suppression binding protein (CSBP) or reactivating kinase (RK; 5–7). The p38s consist of α , β , $\beta 2$, γ and δ isoforms. SB 203580 inhibits p38 α , β and $\beta 2$ (not γ and δ) by competing with the substrate, ATP (5,8). While SB 203580 inhibits p38 activity, it does not significantly affect the activation of p38. SB 203580 does not inhibit PKA, PKC, MEKs, MEKs or ERK and JNK MAP kinases (5–7). Most other commercially available p38 inhibitors are pyridinyl imidazoles similar in specificity though typically less potent than SB 203580.

LY 294002

Q: How does LY 294002 differ from Wortmannin, and are there advantages of one of these compounds over the other?

Both LY 294002 (Cat.# V1201) and Wortmannin are inhibitors of the lipid-modifying enzymes known as PI 3-kinases, and both are useful for probing the roles of PI 3- kinases in biological processes (10,11). Table 2 provides a comparison of the two compounds.

Though Wortmannin is more, this advantage is outweighed in many applications by the improved stability and specificity of LY 294002 (9–11). To confirm results obtained with one of the two PI 3-kinase inhibitors, many researchers perform a parallel study with the other.

PMA

Q: There are several commercially available phorbol esters with a wide range of biological activity. What governs the choice of phorbol ester, and what is their mode of action?

Phorbol 12-myristate 13-acetate (PMA, Cat.# V1171), phorbol-12, 13-dibutyrate (PDBu) and phorbol-12, 13-didecanoate (PDD) are commonly used phorbol esters, best known for their capacity to activate group A (α , β I, β II, γ) and group B (δ , ϵ , η , θ) PKCs (12). Phorbol esters mimic diacylglycerol, a natural ligand and activator of PKCs, and most of their effects are primarily attributable to this property (13). The choice of phorbol ester for a particular experimental design is influenced by its biological potency and aqueous solubility. The order of potency of the three mentioned is PDD>PMA>PDBu with aqueous solubility limits of 50nM, 2 μ M and >30 μ M,

respectively (13,14). Structurally, phorbol esters resemble nonionic detergents, and to avoid nonspecific effects attributable to detergent properties, it is desirable to keep their concentrations as low as possible (13). The favorable solubility and potency of PMA make it the most commonly used phorbol ester with a useful working concentration range of 1–100nM. A common alternative name for PMA is 12-O-tetradecanoylphorbol 13-acetate (TPA).

4 α -PMA

Q: Is 4 α -phorbol 12-myristate 13-acetate (4 α -PMA) the negative control of choice in experiments that use phorbol esters?

To distinguish between specific and nonspecific effects, possibly due to detergent-like properties of phorbol esters, a negative control should be chosen that is similar in lipophilicity and aqueous solubility to the active phorbol ester used. 4 α -PMA (Cat.# V1181) is a stereoisomer of PMA that is ineffective at activating PKCs. 4 α -PMA differs from PMA only in the configuration of the hydroxyl group at position 4, and this change does not significantly alter its lipophilicity or aqueous solubility. 4 α -PMA is therefore the negative control of choice for studies that use PMA (15,16). Though researchers frequently match 4 α -PDD with PMA, this is not ideal because 4 α -PDD is considerably more lipophilic and less water-soluble than PMA. 4 α -PDD and 4 α -PDBu are best suited as negative controls in studies that use PDD and PDBu, respectively (15).

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