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Technical Information about ESI-09

Membrane-permeant inhibitor of Epac 1 and Epac 2

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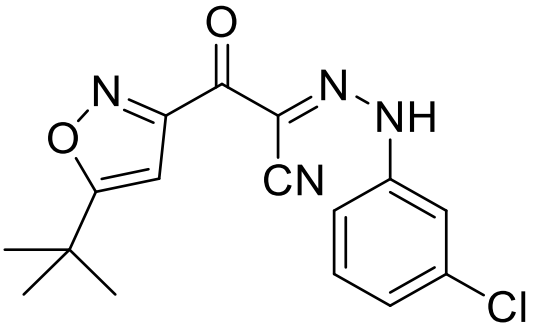

Abbreviation: **ESI-09**

Formula	CAS No.	Molecular Weight	UV	BIOLOG Cat. No.
$C_{16}H_{15}ClN_4O_2$	[263707-16-0]	330.8	λ_{max} 384 nm / ε 25300 / pH 7	B 133

Name: 3- [5- (tert.- Butyl)isoxazol- 3- yl]- 2- [2- (3- chlorophenyl)hydrazono]- 3- oxopropanenitrile

Description / Properties: According to Almahariq et al. ESI-09 is a competitive, membrane-permeant inhibitor of both exchange proteins activated directly by cyclic AMP, Epac 1 and Epac 2. ESI-09 displays *in vitro* IC_{50} values of 3.2 µM for Epac 1 and 1.4 µM for Epac 2 with 25 µM cAMP as competing activator (Almahariq et al. 2013). It displays an at least 100-fold selectivity for Epac proteins compared to cyclic AMP-dependent protein kinase I and II in *in vitro* assays (Almahariq et al. 2013). In a recent report it was suggested that general protein denaturing properties are the molecular basis for the inhibitory action of ESI-09, thus raising concerns about the selectivities for Epac 1 and Epac 2 (Rehmann 2013).

Specification: Crystallized or lyophilized solid. **Please keep in mind that equal concentrations of the compound may look different in volume due to sensitivity of the lyophilized form to humidity. The compound can even contract to small droplets.** Normally the product is located in the conical bottom of the tube. Micromolar quantities are determined by weight.

Purity: Typical analysis is better than 95% (HPLC / UV / 270 nm). The product is not sterile and has not been tested for endotoxins.

Stability and Storage: ESI-09 is chemically rather stable and does not need special care during handling or shipment. Nevertheless, we recommend that the compound should be stored in the freezer (- 20° Celsius necessary, - 80° recommended), for longer storage periods preferably in freeze-dried form.

Toxicity and Safety: Please keep in mind, that the *in vivo* properties of this compound are not sufficiently characterized up to now. Avoid skin contact or ingestion and allow only trained personnel to handle the product.
Our products are designed, developed and sold for research purposes only! They are intended for *in vitro* and nonhuman *in vivo* laboratory applications. Any other use requires approval of health authorities.
Not for drug, household or related uses!

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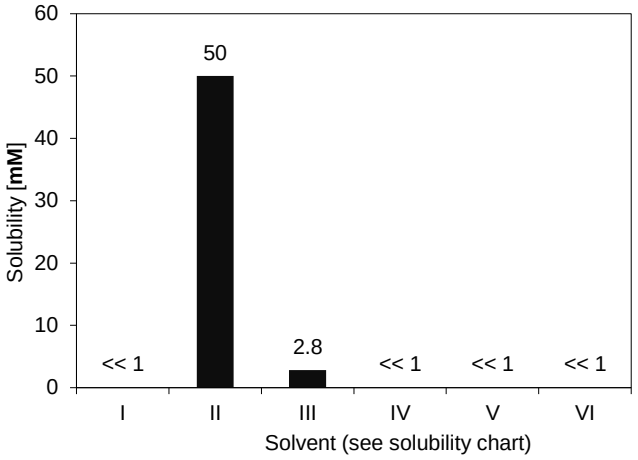
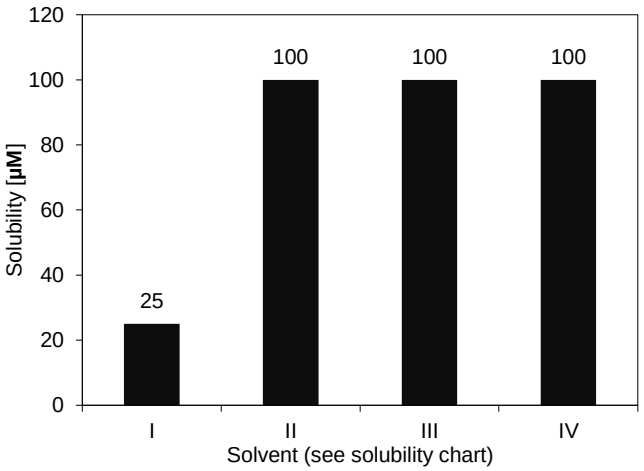
Solubility: Detailed information on the solubility of ESI-09 in water and various buffers are listed in the solubility chart below. Concentrations have been determined at ambient temperature and can be considered as concentrations usually obtainable, however, slight batch-to-batch variations cannot be ruled out.

No.	Solvent	Solubility [mM]
I	H_2O	<< 1
II	DMSO	50
III	Ethanol 96%	2.8
IV	PBS, pH 7.4	<< 1
V	25 mM Hepes/NaOH, pH 7.2	<< 1
VI	25 mM Tris/HCl, pH 7.4	<< 1



Due to its rather high lipophilicity, the solubility of ESI-09 in water or buffers is limited. We suggest to first use a small amount of DMSO for dissolution, and to dilute with DMSO and water or buffer down to the final concentration to be used in the biological assay (see below for corresponding protocols). Please rinse tube walls carefully and preferably use ultrasonic or vortex to achieve total and uniform mixing. When opening the tube please make sure that no substance is lost within the cap.

No.	Solvent	Solubility [µM]
I	H_2O (1% DMSO)	25
II	PBS, pH 7.4 (1% DMSO)	100
III	25 mM Hepes/NaOH, pH 7.2 (1% DMSO)	100
IV	25 mM Tris/HCl, pH 7.4 (1% DMSO)	100



Preparation of a 25 µM solution in water (1% DMSO):

Add 100 µL DMSO to a 5 µmol vial of ESI-09 to achieve a 50 mM solution. Use 5 µL of this solution and dilute with 95 µL DMSO to get a 2.5 mM solution. Addition of 990 µL water to 10 µL of this 2.5 mM DMSO solution will result in a 25 µM solution in water (1% DMSO).

Preparation of a 100 µM solution in various buffers (1% DMSO):

Add 100 µL DMSO to a 5 µmol vial of ESI-09 to achieve a 50 mM solution. Use 20 µL of this solution and dilute with 80 µL DMSO to get a 10 mM solution. Addition of 990 µL buffer (PBS, Hepes, Tris) to 10 µL of this 10 mM DMSO solution will result in a 100 µM solution in buffer (1% DMSO).

Selected References for ESI-09:

Singhmar, P.; Huo, X.J.; Li, Y.; Dougherty, P.M.; Mei, F.; Cheng, X.; Heijen, C.J.; Kavelaars, A., *Pain*, **159**, 884 - 893 (2018): "Orally Active Epac Inhibitor Reverses Mechanical Allodynia and loss of Intraepidermal Nerve Fibers in a Mouse Model of Chemotherapy-induced Peripheral Neuropathy"

Parnell, E.; Palmer, T.M.; Yarwood, S.J., *Trends Pharmacol. Sci.*, **36**, 203 - 214 (2015): "The Future of EPAC-targeted Therapies: Agonism versus Antagonism"

Rehmann, H., *Sci. Rep.*, 3:3032 (2013): "Epac-Inhibitors: Facts and Artefacts"

Almahariq, M.; Tsalkova, T.; Mei, F.C.; Chen, H.; Zhou, J.; Sastry, S.K.; Schwede, F.; Cheng, X., *Mol. Pharmacol.*, **83**, 122 - 128 (2013): "A Novel EPAC-Specific Inhibitor Suppresses Pancreatic Cancer Cell Migration and Invasion"

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