opnMe by Boehringer Ingelheim

AMPK activator

BI-9774

Boehringer Ingelheim

Table of contents

Summary

BI-9774 is a highly potent and well characterized AMPK activator.

Chemical Structure

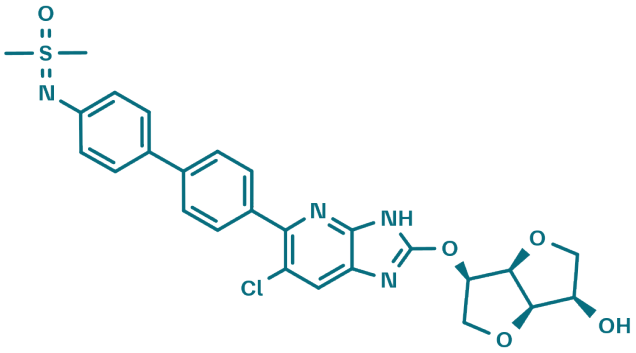


Figure 1: 2D structure of BI-9774, an AMPK activator

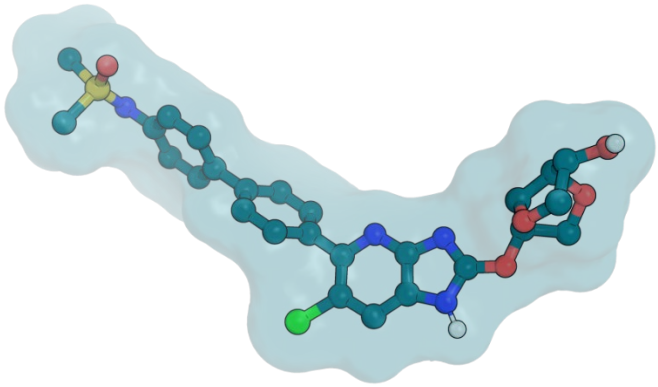


Figure 2: BI-9774, 3D conformation, as observed in a binding model of the complex with the kinase domain of AMPK

Highlights

BI-9774 is a highly potent and well characterized AMPK activator which can be used for *in vitro* and *in vivo* studies.

Target information

Sensing and regulating the cellular energy status in response to environmental and/or nutritional stress is highly important and AMP-activated protein kinase (AMPK) is a major contributor for this task. Cellular energy depletion leads to the activation of AMPK thereby inhibiting ATP consuming and upregulating ATP generating pathways.

AMPK, a Ser/Thr protein kinase, is a heterotrimeric enzyme complex composed of a catalytic α-subunit (two isoforms) together with a β-scaffold subunit (two isoforms) and a γ-regulatory subunit (three isoforms):

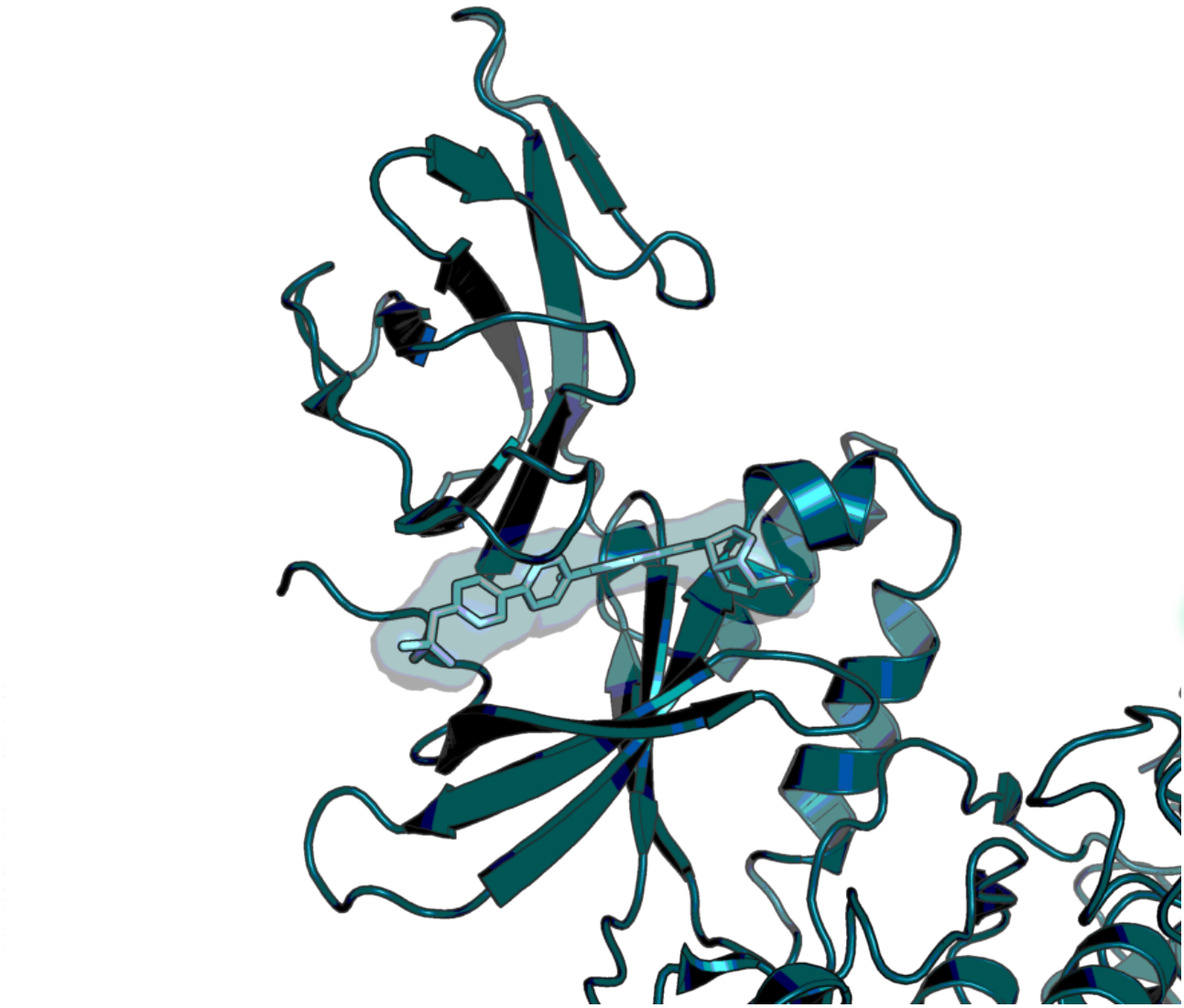


Figure 3: Structure of human full-length AMPK complex (α2β1γ1) with an activator (Xiao et. al, 2013[1])

An AMPK activator mimics the activation of the enzyme by a low energy status of the cell and changes metabolism from ATP consuming anabolic pathways to ATP-producing catabolic pathways. Therefore, an AMPK activator inhibits gluconeogenesis and fatty acid synthesis in liver and increases glucose uptake and fatty acid oxidation in skeletal muscle. The coordinated effects on glucose/fat metabolism should reduce plasma glucose and plasma lipids. Furthermore, an AMPK activator is expected to induce cardioprotective (e.g. ATP producing) pathways in cardiomyocytes.

In vitro activity

BI-9774 displays an EC_{50} of 64 nM (E_{max} = 590 %) in an ADP-Glo kinase assay with human AMPK ($\alpha_1\beta_1\gamma_1$) and an EC_{50} of 8 nM (E_{max} = 168 nM %) in a cellular GLUT4 translocation assay.

PROBE NAME	BI-9774
MW [Da, free base][a]	541.0
Human AMPK $\alpha_1\beta_1\gamma_1$ (EC_{50}/E_{max})[b]	64 nM / 560 %CTL
Human AMPK $\alpha_2\beta_1\gamma_1$ (EC_{50}/E_{max})[b]	88 nM / 580 %CTL
Human AMPK $\alpha_2\beta_2\gamma_2$ (EC_{50}/E_{max})[b]	15 nM / 262 %CTL
Human AMPK $\alpha_2\beta_2\gamma_3$ (EC_{50}/E_{max})[b]	24 nM / 396 %CTL
Human AMPK $\alpha_1\beta_1\gamma_3$ (EC_{50}/E_{max})[b]	84 nM / 427 %CTL
Human AMPK $\alpha_1\beta_2\gamma_2$ (EC_{50}/E_{max})[b]	41 nM / 148 %CTL
Human AMPK $\alpha_1\beta_2\gamma_3$ (EC_{50}/E_{max})[b]	12 nM / 222 %CTL
GLUT4 transl. (L6 cells rat) (EC_{50}/E_{max})[c]	8 nM / 168 %CTL

[a] For the salt form you will get, please refer to the label on the vial and for the molecular weight of the salt, please refer to the FAQs

[b] AMPK Kinase Assay: Recombinant human AMPK (containing subunits alpha, beta and gamma) was obtained from a baculovirus expression system. The activity of the AMPK protein was determined using the ADP Glo® Luminescence Kinase test (Promega; V9103X). In this homogeneous test, the amount of ADP remaining after the kinase reaction is quantified by a luciferin-luciferase reaction using luminescence. The luminescence signal obtained correlates with the amount of ADP resulting from the kinase reaction and thus correlates with the activity of the protein kinase.

[c] Measurement of GLUT4myc translocation in L6 myoblasts: The amount of myc-tagged GLUT4 at the surface of intact cells is measured by an antibody-coupled colorimetric assay after the induced translocation of GLUT4 from an intracellular storage pool to the plasma membrane. A stimulator of GLUT4 translocation will give values above 100% CTL.

In vitro DMPK and CMC parameters

PROBE NAME	BI-9774
logD @ pH 11	0.9
Solubility @ pH 6.8 [µg/mL]	1
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	17
Caco-2 efflux ratio	4
MDCK permeability P_{appAB} @ 1µM [10^{-6} cm/s]	0.05
MDCK efflux ratio	68
Microsomal stability (human/mouse/rat) [% QH]	<23 / <23 / <22
Hepatocyte stability (human/mouse/rat) [% QH]	1 / 25 / 3
Plasma Protein Binding (human/mouse/rat) [%]	98.6 / 98.7 / 98.7
hERG [inh. % @ 10 µM]	9
CYP 3A4 (IC_{50}) [µM]	>50
CYP 2C8 (IC_{50}) [µM]	>50
CYP 2C9 (IC_{50}) [µM]	>50
CYP 2C19 (IC_{50}) [µM]	>50
CYP 2D6 (IC_{50}) [µM]	>50

In vivo DMPK parameters

BI-9774	MOUSE	RAT	MINIPIG
Clearance [$\%Q_H$][a]	17	14	4[b]
Mean residence time after *i.v.* dose [h]	1.5	3.8	5.8
t_{max} [h]	0.9	3.3	4.0
C_{max} [nM]	50	74	111
F [%]	34	38	16
Vss [L/kg]	1.4	2.3	0.6

[a] *i.v./p.o.* dose: 0.54/5.4 mg/kg
[b] *i.v./p.o.* dose: 0.27/2.7 mg/kg

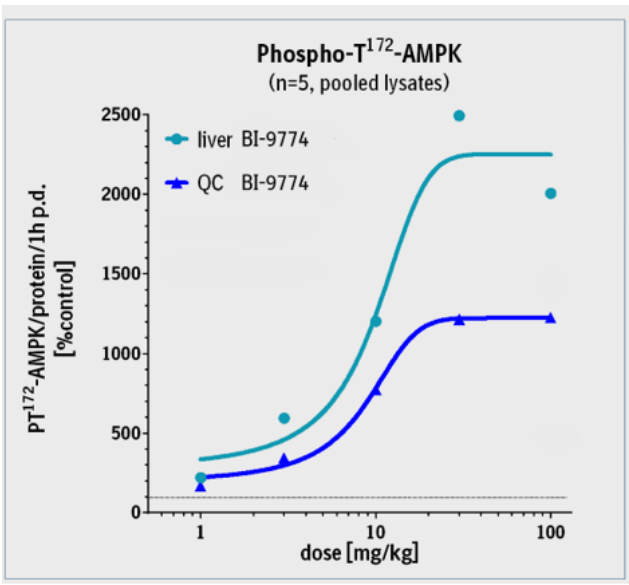
In vivo pharmacology

The AMPK activator BI-9774 showed dose dependent activation of BI-9774 with a maximal activation 10/20-fold. The activation was mainly driven by free concentration of BI-9774.

The molecule shows anti-diabetic efficacy in diabetic rodents. It also acutely improved glucose tolerance, increased muscle glucose uptake and lactate and glycogen in rodents. BI-9774 subchronically reduced blood glucose, HbA1c and body weight in ZDF rats.

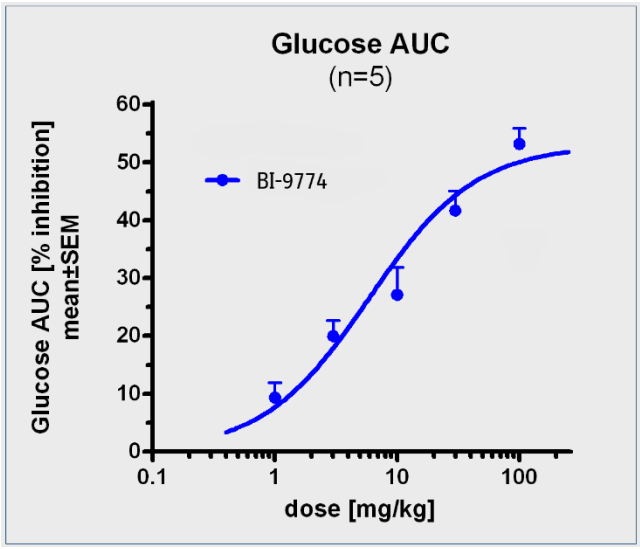
An acute cardio protection in I/R rat model could be shown.

BI-9774 showed reduction in inotropism and heart rate in acute cardiovascular studies in anesthetized rats. Heart weight and heart glycogen increased in HanWistar rats treated for 2 glucose tolerance weeks with BI-9774.



	BI-9774	
Dose (mg/kg)	nM @ 1h	Free conc. / Glut4 EC_{50}
1	203	0.3
3	488	0.8
10	2898	4.7
30	8422	14
100	17080	28

Figure 4: Target engagement measurement in rat (HanWistar): PhosphoT172 AMPK in liver, skeletal muscle. Dose dependent activation by BI-9774, max. activation 10/20-fold.



	BI-9774	
Dose (mg/kg)	nM @ 4h	Free conc. / Glut4 EC_{50}
1	100	0.2
3	432	0.7
10	1461	2.4
30	4582	7.4
100	11750	19

Figure 5: Acute efficacy in rat (HanWistar): Dose-dependent glucose lowering of BI-9774 was seen in non-diabetic rats.

Summary Antidiabetic profile:

	ACUTE GTT	SKELETAL MUSCLE	SUBCHRONIC STUDY
BI-9774	oGTT↑ in normoglycemic rodents and mZDF rats	Acute glucose uptake↑, lactate↑, glycogen↑	Blood glucose↓, moderate HbA1c↓ and BW↓ in ZDF rats (6 weeks)

Summary Safety profile:

	RODENTS	ECG	CARDIAC FUNCTION
BI-9774	HW= and H glycogen↑ in ZDF rats (6 weeks); HW↑ and H glycogen↑ in HW rats (2 weeks)	No WPW syndrome	HR↓ and EDP↑ in ZDF and HW rats, transient effect in dogs

Summary Cardiovascular safety:

Acute cardiovascular safety (anesthetized Wistar Rats), subchronic target related cardiovascular safety anesthetized Wistar and ZDF rats):

	Exp model	Heart rate	AP	PP	Ino-tropism	PR	QRS	QTc	Tot. Plasma conc.@ 1st effect (i.e. Inotrop.)	Free conc. / $\alpha_1\beta_1g_1$ EC_{50}
BI-9774	Subacute anesth. W Rats, 3 d, p.o.								2.2 µM	
	Subchronic anesth. W rats, 2 weeks, p.o.	↓↓			↓				0.1 µM	
	Subchronic anesth. ZDF rats, 17 d, p.o.	↓↓↓	↓		↓				0.1 µM	
	Acute anesth. Wistar rats, i.v.	↓↓	- DAP only	↓	↓↓↓		-		0.2 µM (@1st effect), i.e. 2.6 nM fu	0.29
	Subchronic anesth. ZDF rats, 6 weeks, p.o.	↓↓↓	↓		↓				0.065 µM	

***AP-arterial pressure, PP-pulsed pressure, PR, QRS, QT – EKG peaks**

Selectivity

BI-9774 activates all human AMPK isoforms tested (see table above) as well as rat AMPK ($\alpha_1\beta_1\gamma_1$) with EC_{50} values below 100 nM. It is selective at 10µM on the SafetyScreen44™ and Invitrogen® panels. However, in the Invitrogen kinase panel, inhibits MYLK with an IC_{50} of 170 nM (Grempler et.al., 2018).

SELECTIVITY DATA AVAILABLE	BI-9774
SafetyScreen44™ with kind of support of eurofins	Yes
Invitrogen®	Yes
DiscoverX®	No
Dundee	No

Co-crystal structure of the Boehringer Ingelheim BI-9774 and the target protein

The X-ray crystal structure of target in complex with BI-9774 was not determined.

Reference molecule(s)

MK-8722 is another pan-AMPK activator available.

Summary

BI-9774 is a highly potent and well characterized AMPK activator.

Supplementary data

2D Selectivity data can be downloaded free of charge from opnMe.

References

1. Xiao B., Sanders M. J., Carmena D., Bright N. J., Haire L. F., Underwood E., Patel B. R., Heath R. B., Walker P. A., Hallen S., Giordanetto F., Martin S. R., Carling D., Gamblin S. J. Structural basis of AMPK regulation by small molecule activators *Nature Communications* **2013**, *4*, 3017**.** DOI: 10.1038/ncomms4017, PubMed.

2. Feng D., Biftu T., Romero F. A., Kekec A., Dropinski J., Kassick A., Xu S., Kurtz M. M., Gollapudi A., Shao Q., Yang X., Lu K., Zhou G., Kemp D., Myers R. W., Guan H. P., Trujillo M. E., Li C., Weber A., Sebhat I. K. Discovery of MK-8722: A Systemic, Direct Pan-Activator of AMP-Activated Protein Kinase *ACS Medicinal Chemistry Letters* **2018,** *9* (1), 39-44. DOI:10.1021/acsmedchemlett.7b00417, PubMed.

3. Myers R. W., Guan H. P., Ehrhart J., Petrov A., Prahalada S., Tozzo E., Yang X., Kurtz M. M., Trujillo M., Trotter D. G., Feng D., Xu S., Eiermann G., Holahan M. A., Rubins D., Conarello S., Niu X., Souza S. C., Miller C., Liu J., Lu K., Feng W., Li Y., Painter R. E., Milligan J. A., He H., Liu F., Ogawa A., Wisniewski D., Rohm R. J., Wang L., Bunzel M., Qian Y., Zhu W., Wang H., Bennet B., Scheuch L. L., Fernandez G. E., Li C., Klimas M., Zhou G., van Heek M., Biftu T., Weber A., Kelley D. E., Thornberry N., Erion M. D., Kemp D. M., Sebhat I. K. Systemic pan-AMPK activator MK-8722 improves glucose homeostasis but induces cardiac hypertrophy *Science* **2017,** 357, 507–511. DOI:10.1126/science.aah5582, PubMed.

4. Grempler R., Wolff M., Simon E., Schmid R., Eisele C., Rieber K., Fischer E., Mettel S., Gabrielyan O., Delic D., Luippold G., & Redeman N. Discovery and translation of a target engagement marker for AMP-activated protein kinase (AMPK) *PLoS ONE* **2018,** 13(5): e0197849. DOI:10.1371/journal.pone.0197849, PubMed.

5. Gollner A., Heine C., Hofbauer K. S. Kinase Degraders, Activators and Inhibitors: Highlights and Synthesis Routes to the Chemical Probes on opnMe.com, Part 1 *ChemMedChem* **2023,** 18, e202300031. DOI:https://doi.org/10.1002/cmdc.202300031, PubMed.