opnMe by Boehringer Ingelheim

RSK Inhibitor

BIX 02565

Boehringer Ingelheim

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Summary

BIX 02565 is a highly potent nanomolar inhibitor of the ribosomal S6 kinase (RSK) isoforms. It has been extensively characterized on a standardized kinase panel, proving to have a relatively high selectivity. Further to this, it demonstrates inhibition of adrenergic receptor subtypes and the imidazoline I_2 receptor. In an animal model, BIX 02565 showed dose-dependent decrease in mean arterial pressure accompanied by bradycardia. BIX 02565 makes it potentially difficult to distinguish efficacy as a result of off-target vasodilatation from inhibition of RSK2.

Chemical Structure

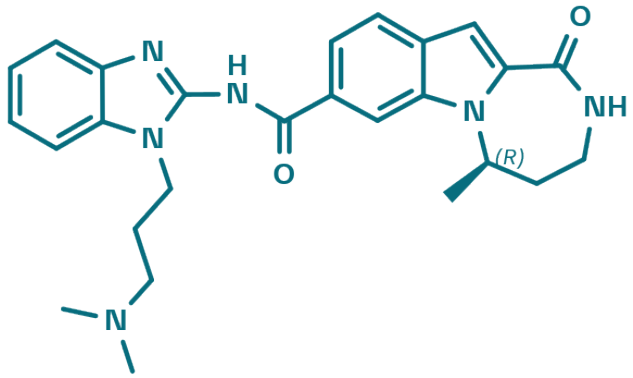

Figure 1: 2D structure of BIX 02565, a RSK inhibitor

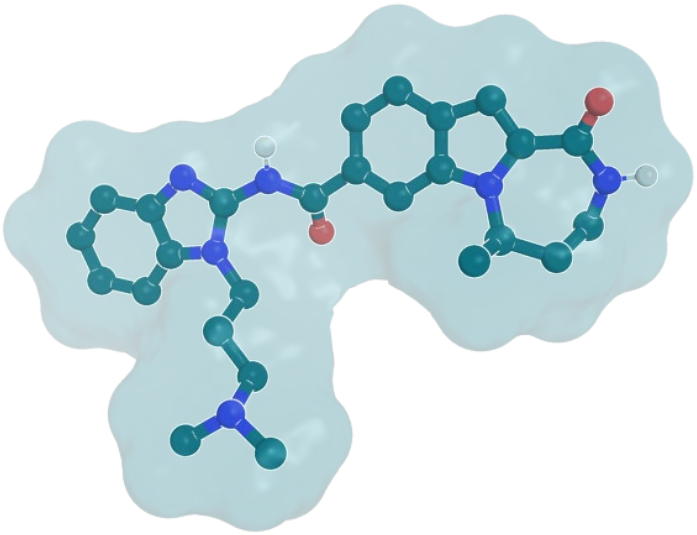

Figure 2: BIX 02565, 3D conformation

Highlights

BIX 02565 has been characterized as a highly potent inhibitor of the N-terminal kinase domain of the three RSK isoforms expressed in cardiac cells. It showed the best combination of potency, selectivity, and solubility among a panel of molecules and is well suited for both *in vitro* and *in vivo* experiments. Generated against human RSK, BIX 02565 shows cross-reactivity to mouse and rat RSK. The overall balanced profile makes it an attractive compound to study the role of RSK kinase.

Target information

The p90 ribosomal s6 kinases (RSKs) are a group of serine/threonine kinases that are constituents of the AGC subfamily in the human kinome. The RSK isoforms are activated by growth factors, cytokines, peptide hormones and neurotransmitters that stimulate the Ras-ERK pathway. RSK regulates numerous biological processes through its phosphorylation of cellular substrates. One important cardiovascular target of RSK is the Na+/H+ exchanger isoform 1 (NHE1). RSK has also been reported to regulate PKC and ROS mediated phosphorylation of cardiac troponin I and to induce pro-renin converting enzyme in ischemia and diabetic cardiomyopathy. RSK is implied in regulation of cardiac cells and there are scientific data that support the notion of a potential role in heart failure secondary to myocardial infarction.

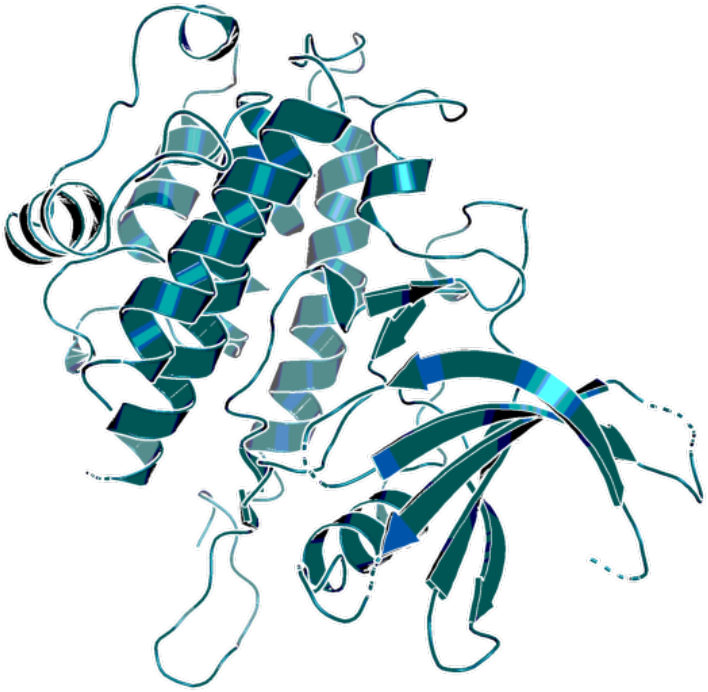

Figure 3: Structure of the Human Ribosomal protein S6 kinase (PDB Code: 2WKN).

In vitro activity

BIX 02565 inhibits RSK kinases in nanomolar range and inhibition of adrenergic receptor subtypes (α_{1A}, α_{2A}, α_{1B} and β_2) and the Imidazoline I_2 (IC_{50} values between 0.052 and 1.820 µM).

PROBE NAME / NEGATIVE CONTROL	BIX 02565
MW [Da, free base][a]	458.6
RSK1 (IC_{50}) [nM]	3
RSK2 (IC_{50}) [nM]	1
RSK3 (IC_{50}) [nM]	1
HLR-CREB (IC_{50}) [nM][b]	20
pNHE1 (DC_{50}) [nM][b]	70
Adrenergic α_{1A} [µM]	0.91
Adrenergic α_{2A} [µM]	1.42
Adrenergic α_{1B} [µM]	0.052
Adrenergic β_2 [µM]	1.82
Imidazoline I_2 [µM]	0.097

[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] Assay conditions[1-3]

In vitro DMPK and CMC parameters

PROBE NAME	BIX 02565
logP @ pH 11	3.39
Solubility @ pH 4.5 / 7.4 [µg/mL]	>45 / 26
Caco-2 permeability AB @ pH 7.4 [*10^{-6} cm/s]	4.4
Caco-2 efflux ratio	16.6
Microsomal stability (human/rat) [% Q_H]	<30 / 10
Hepatocyte stability (human/rat) [% Q_H]	58 / 45

Plasma Protein Binding (human/rat) [%]	94 / 90
PAMPA [10^{-6} cm/sec]	43.5
hERG [inh. % @ 10 µM]	54.8
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

BIX 02565	RAT
Clearance [% Q_H][a]	75
Mean residence time after *i.v.* dose [h][a]	4.9
C_{max} [nM][b]	6550
F [%][b]	100
V_{ss} [L/kg][a]	15

[a] *i.v.* dose. 1 mg/kg
[b] *p.o.* dose: 100 mg/kg

In vivo pharmacology

In telemetry-instrumented rats, BIX 02565 elicits concentration-dependent decreases in MAP after each dose. BIX 02565 produces concentration-dependent relaxation *ex vivo* in the phenylephrine-constricted rat aortic ring. Subsequently, BIX 02565 is infused in the anesthetized rat in a low-dose and high-dose series of continuous infusions to test the effect of compound on hemodynamics *in vivo*. Nevertheless, the off-target pharmacology of BIX

02565 made it potentially difficult to distinguish efficacy as a result of off-target vasodilatation from inhibition of RSK2[3].

Selectivity

BIX 02565 was profiled against the Invitrogen kinase panel (229 kinases), and dose-response was obtained for each kinase with inhibition > 50% at 3 µM. Kinase inhibition > 80% at 3 µM is predictive of an IC_{50} of 1 µM or below. Kinases outside the RSK family (IC_{50} [nM]): LRRK2 (16), PRKD1 (35), CLK2 (112), PRKD2 (139), RET (161), PRKD3 (219), FGFR2 (320), CLK1 (512), FLT3 (714), PDGFRa (956).

SELECTIVITY DATA AVAILABLE	BIX 02565
SafetyScreen44™ with kind support of eurofins	Yes
Invitrogen®	Yes
DiscoverX®	Yes
Dundee	No

Co-crystal structure of the Boehringer Ingelheim probe compound and the target protein

A RSK2 homology model was created based on the publicly available crystal structure of RSK1 (pdb: 2z7r)[2].

Reference molecule(s)

BI-D1870, RMM-46 – Calbiochem

Supplementary data

2D structure files can be downloaded free of charge from opnMe.

References

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