EGFR inhibitor | BI-8128

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Summary

BI-8128 is a reversible and highly selective, fourth generation EGFR inhibitor suitable for *in vitro* and *in vivo* studies with potent activity against the primary oncogenic EGFR variants del19 and L858R as well as the acquired EGFR resistance mutations T790M and C797S.

Chemical Structure

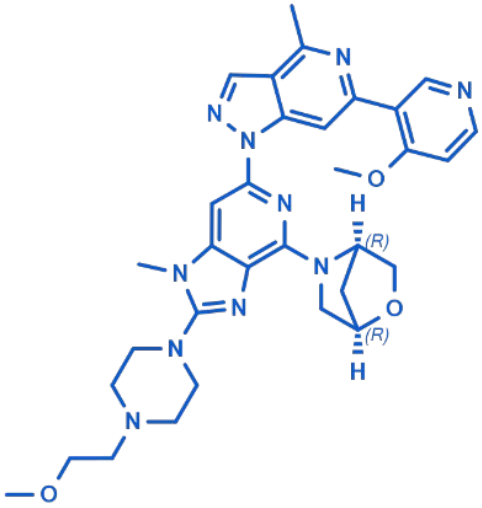

Figure 1: 2D structure of BI-8128, an orally bioavailable EGFR 4th generation inhibitor

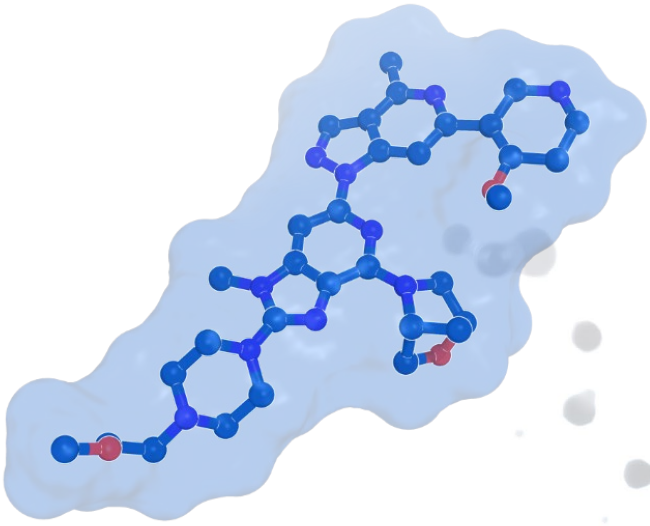

Figure 2: BI-8128, low energy 3D conformation, based on the structure of a complex of EGFR with an inhibitor that is structurally related to BI-8128 (see Figure 3).

Highlights

BI-8128 is a reversible and potent, orally bioavailable fourth generation epidermal growth factor receptor (EGFR) inhibitor, suitable for *in vitro* and *in vivo* studies. EGFR is one of the most common oncogenic driver genes in non-small cell lung cancer (NSCLC). BI-8128 potently inhibits the two major primary oncogenic EGFR variants del19 and L858R, in the presence and absence of the acquired EGFR resistance mutations T790M and C797S, while sparing wild-type EGFR.

Target information

EGFR is a transmembrane protein whose tyrosine kinase activity is activated upon binding of members of the EGF family of ligands, and which is involved in several intracellular signaling pathways, including MAPK, Akt and JNK. EGFR mutations are common drivers in NSCLC, and are present in 10–15% of Caucasian and up to 50% of Asian patients, with a higher incidence especially in non-smokers or former light smokers[1].

Deletions in exon 19 (del19 or E19del) and L858R point mutations in exon 21 represent the vast majority of EGFR-activating mutations[4]. First and second generation EGFR-tyrosine kinase inhibitors (TKIs) markedly improved survival and clinical outcomes in patients harboring EGFR-activating mutations[2-10]. However, most patients eventually developed drug resistance, resulting in disease progression. More than 50% of acquired resistance to the first-generation EGFR-TKIs is caused by the T790M mutation, a threonine to methionine substitution at amino acid position 790 in exon 20 of the EGFR gene[11,12]. This mutation hampers the binding of first generation reversible EGFR-TKIs to the kinase ATP-binding site of EGFR, leading to drug resistance. To overcome the secondary EGFR T790M mutation, third-generation EGFR-TKIs were developed. Osimertinib is an oral, highly potent, irreversible third-generation EGFR-TKI that selectively targets EGFR-activating mutations as well as T790M resistance mutation[13]. Predictably however, resistance to covalent inhibitors has also emerged, in the form of the C797S mutation, which substitutes the reactive cysteine to a serine at amino acid position 797 in exon 20 of the EGFR gene.

BI-8128 is a novel fourth generation EGFR-TKI, which is highly potent on the primary activating EGFR variants del19 and L858R and inhibits both variants also in the presence of the acquired EGFR resistance mutations T790M and/or C797S. Furthermore, the compound is suitable for *in vitro* and *in vivo* studies.

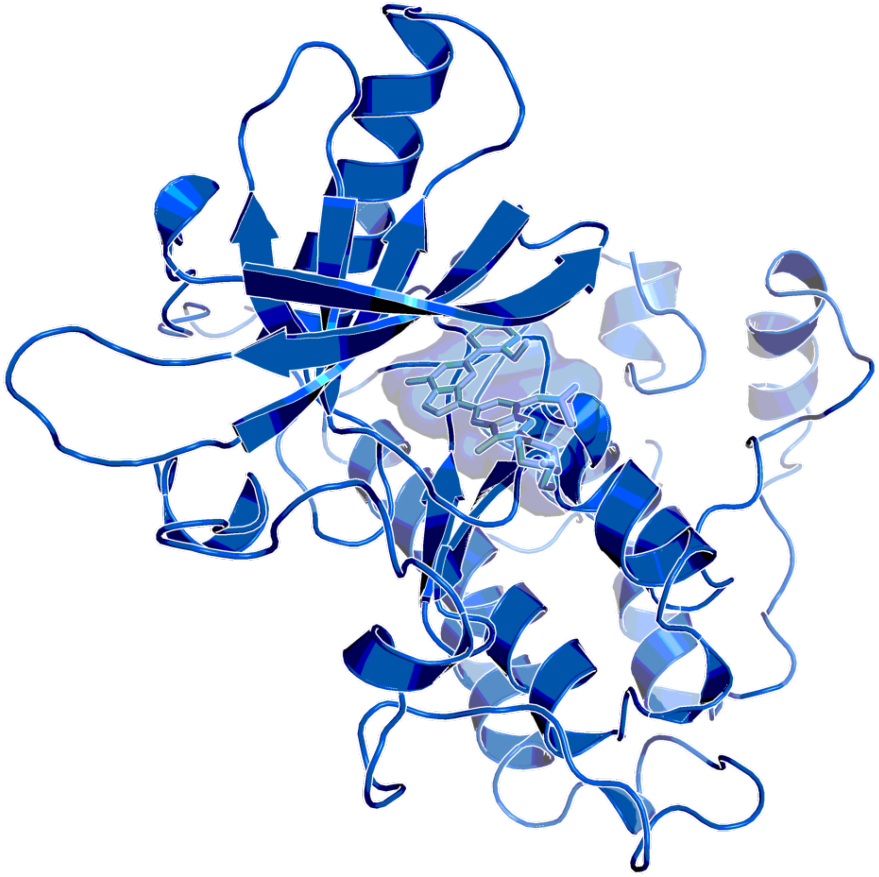

Figure 3: Model of the 3D structure of EGFR in complex with BI-8128, based on an in-house X-ray structure with a highly related inhibitor.

In vitro activity

BI-8128 was profiled for potency and selectivity in engineered Ba/F3 and solid cancer cell lines *in vitro* (see table below for data). BI-8128 demonstrated potent, low nanomolar anti-proliferative activity in Ba/F3 cells that are dependent on the activity of human EGFR variants del19, del19 T790M C797S, L858R, and L858R T790M C797S. An EGFR-independent Ba/F3 cell line and a Ba/F3 clone dependent on EGFR wild-type signaling was only inhibited at much higher concentrations indicating low off-target cytotoxicity and sparing of EGFR wild-type activity. A parental human PC-9 NSCLC cell line (native EGFR del19 genotype), an engineered PC-9 C797S variant (EGFR del19 C797S genotype) and a double resistance mutation containing engineered PC-9 T790M C797S variant (EGFR del19 T790M C797S) showed potent growth inhibition by BI-8128 in the low double digit nanomolar range. In line with the proliferation data, BI-8128 reduced the pharmacodynamic phospho-EGFR biomarker in the low nanomolar range in PC-9 variants with and without the resistance mutations T790M and C797S. BI-8128 inhibited A431 cell whose proliferation depends on EGFR wild-type

signaling with markedly reduced potency compared to cell models harboring oncogenic EGFR variants demonstrating EGFR wild-sparing activity of the compound. Further cellular profiling data (not shown) demonstrate activity of BI-8128 also on the following clinically detected acquired resistance mutations emerging upon treatment with third-generation EGFR inhibitor treatment: C797G, C797N, L792H, L792F, L792Y (but not on L718Q). The third-generation EGFR inhibitor and reference compound osimertinib showed severely impaired or no inhibitory activity in cell models harboring the EGFR C797S mutation, while potently inhibiting EGFR del19 and EGFR L858R cell models.

PROBE NAME / REFERENCE COMPOUND	BI-8128	OSIMERTINIB
MW [Da]	610.7	499.6
Ba/F3 parental + IL-3 (EGFR-independent)[a]	3558	1008
Ba/F3 EGFR wild-type + EGF ligand, proliferation, (IC_{50}) [nM][a]	318	56
Ba/F3 EGFR del19, proliferation, (IC_{50}) [nM][a]	1.7	0.7
Ba/F3 EGFR del19 T790M C797S, proliferation, (IC_{50}) [nM][a]	3.3	635
Ba/F3 EGFR L858R, proliferation, (IC_{50}) [nM][a]	9.6	1.6
Ba/F3 EGFR L858R T790M C797S, proliferation, (IC_{50}) [nM][a]	16	588
A549[b]	1615	>1000
A431 (EGFR wild-type amplification), proliferation, (IC_{50}) [nM][b]	492	112
PC-9 parental (EGFR del19 genotype), proliferation, (IC_{50}) [nM][b]	16	n.d.
PC-9 C797S (EGFR del19 C797S genotype), proliferation, (IC_{50}) [nM][b]	21	>1000
PC-9 T790M C797S (EGFR del19 T790M C797S genotype), proliferation, (IC_{50}) [nM][b]	33	>1000

PC-9 parental (EGFR del19 genotype), phospho-EGFR, (IC_{50}) [nM][c]	9.6	3.3
PC-9 C797S (EGFR del19 C797S genotype), phospho-EGFR, (IC_{50}) [nM][c]	19.4	>5000
PC-9 T790M C797S (EGFR del19 T790M C797S genotype), phospho-EGFR, (IC_{50}) [nM][c]	1.4	>1000

[a]Ba/F3 cell engineering and cellular proliferation assays were conducted according to the experimental protocol described in Reference 14.

[b]Solid cancer cell line engineering and cellular proliferation assays (A549, PC-9 variants and A431) were conducted according to the experimental protocol described in Reference 14.

[c]Phospho-EGFR inhibition assays were performed by treating the indicated cell lines with a concentration range of BI-8128 or osimertinib for 6 hours followed by cell lysis and phospho-EGFR quantification by semi-quantitative capillary western blotting.

In vitro DMPK and CMC parameters

BI-8128 displays good overall DMPK properties. Good solubility was identified at different pHs. *In vitro* hepatocyte stability data is in accordance with blood clearance data in animal models. Acceptable inhibition properties on a set of CYP-isoforms were also identified.

PROBE NAME	BI-8128
logP pH = 9.0	3.3
Solubility @ pH 6.8 [µg/ml]	101
Caco2 permeability P_{app}a-b/b-a @ 1µM [10^{-6} cm/s]	Low recovery
Caco2 efflux ration	-
MDCK permeability P_{app}a-b/b-a @ 1µM [10^{-6} cm/s]	Low recovery

MDCK efflux ratio	-
Microsomal stability (human/mouse/rat) [% Q_H]	48/<24.2/39
Hepatocyte stability (human/mouse/rat) [% Q_H]	56/31/22
Plasma protein binding (human/mouse/rat) [%]	97.6/98.1/96.0
Blood-Plasma Ratio (human/rat/dog)	1.1/1.05/1.35
hERG [inh. % @ 1 µM]	9.5
CYP 3A4 (IC_{50}) [µM]	6.1
CYP 2C8 (IC_{50}) [µM]	>50
CYP 2C9 (IC_{50}) [µM]	>50
CYP 2C19 (IC_{50}) [µM]	30.6
CYP 2D6 (IC_{50}) [µM]	>50

In vivo DMPK parameters

For *in vivo* studies BI-8128 was formulated in 0.5% natrosol (acidified with HCl) for oral administration as well as. 25% HP-β-CD for intravenous studies. BI-8128 shows low to moderate clearance, a medium volume of distribution and high bioavailability in animal models such as mouse and rat. Efflux at the blood-brain barrier assessed in mice revealed BI-8128 to have good brain-penetrating potential showing a $k_{p,\,uu,\,brain}$ of 0.25.

BI-8128	MOUSE	RAT
Dose *i.v./p.o.* [mg/kg]	1/10	1/10
Plasma Clearance [% Q_H]	29	52

Blood Clearance [% Q_H]		50
Mean residence time after iv dose [h]	2.5	3.8
t_{max} [h]	2	4
C_{max} [nM]	1200	437
F [%]	100	57
V_{ss} [l/kg]	3.9	8.4
$K_{p, uu, brain}$ (6 h post dose 10 mg/kg *p.o.*)	4	

In vivo pharmacology

The in vivo efficacy of BI-8128 was investigated using the EGFR-mutation positive human NSCLC cell line PC-9 subcutaneously implanted as xenograft models in mice. The compound was formulated in acidified 0.5% natrosol. Oral administration of BI-8128 at a dose of 25 mg/kg bid (6 hours dosing interval between two daily doses) resulted in deep tumor regressions in a parental PC-9 xenograft model (EGFR del19 genotype), as in an engineered PC-9 C797S xenograft model (EGFR del19 C797S genotype) and in an engineered PC-9 T790M C797S xenograft model (EGFR del19 T790M C797S genotype). Please refer to Figure 4 below for experimental data, efficacy of additionally tested doses and mouse body weight across dose groups). Assessment of the pharmacodynamic biomarker phospho-EGFR in PC-9 T790M C797S xenograft tumors revealed a larger than five-fold reduction in the level of the biomarker at the end of a daily dosing cycle (24h post first daily dose) at doses of 25 mg/kg or above bid suggesting potent and inhibition of oncogenic EGFR del19 co-harboring both resistance mutations in tumors. Treatment of the PC-9 C797S and PC-9 T790M C797S models with 25 mg/kg qd osimertinib had no effect on tumor growth. In summary, oral administration of BI-8128 at a dose of 25 mg/kg bid induces tumor regressions in an isogenic series of PC-9 xenograft models with and without the resistance mutations T790M and C797S.

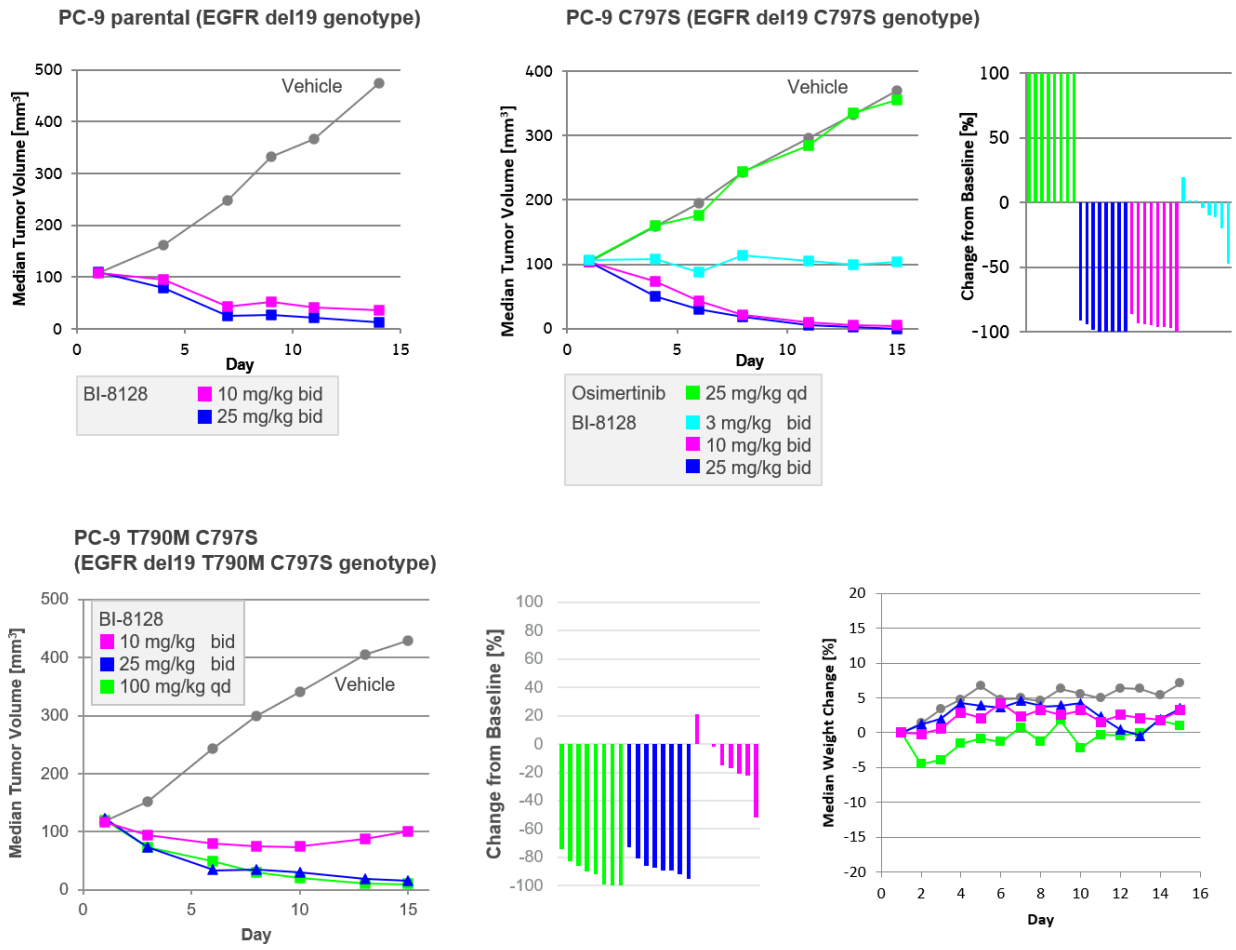

Figure 4: *In vivo* efficacy of BI-8128 in subcutaneously implanted xenograft experiments employing an isogenic series of PC-9 NSCLC models with the indicated EGFR genotypes.

Negative control

There is no negative control available

Selectivity

SELECTIVITY DATA AVAILABLE	BI-8128
SafetyScreen44™ with kind support of eurofins	Yes
Invitrogen®	Yes
DiscoverX®	No

BI-8128 was tested on 205 targets in a selectivity panel and showed selectivity for 196 targets (≤ 50% inhibition @ 10 µM). In 13 assays (PDE4D2, M1/H, ALPHA1AH, M4/H, LCK_CE, HERG, M3/H, ADENOSINETRANSPORTER, PKCALPHA(H)@CE) the compound showed inhibition between 51-100% @ 10µM.

Reference molecule(s)

The third generation EGFR inhibitor osimertinib is commercially available.

Supplementary data

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

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