Product Datasheet

Technical Data

Formula	$C_{24}H_{25}ClFN_5O_3.2C_4H_4O_4$					
Molecular Weight	717.18	**CAS No.**	850140-73-7			
Solubility (25°C)*	In vitro	DMSO	100 mg/mL (139.43 mM)			
		Water	Insoluble			
		Ethanol	Insoluble			
	In vivo (Add solvents to the product individually and in order)	Oral Administration	Homogeneous suspension	CMC-NA	≥5mg/ml	Taking the 1 mL working solution as an example, add 5 mg of this product to 1 ml of CMC-Na solution, mix evenly to obtain a homogeneous suspension with a final concentration of 5 mg/ml.

* <1 mg/ml means slightly soluble or insoluble.
* Please note that Capsid Bio tests the solubility of all compounds in-house, and the actual solubility may differ slightly from published values. This is normal and is due to slight batch-to-batch variations.
* Room temperature shipping (Stability testing shows this product can be shipped at 2-8°C for storage purposes.)

Preparing Stock Solutions

Concentration \ Volume / Mass	1 mg	5 mg	10 mg
1 mM	1.3944 mL	6.9718 mL	13.9435 mL
5 mM	0.2789 mL	1.3944 mL	2.7887 mL
10 mM	0.1394 mL	0.6972 mL	1.3944 mL
50 mM	0.0279 mL	0.1394 mL	0.2789 mL

Biological Activity

Description	Afatinib Dimaleate irreversibly inhibits **EGFR/HER2** including **EGFR(wt)**, **EGFR(L858R)**, EGFR(L858R/T790M) and HER2 with IC50 of 0.5 nM, 0.4 nM, 10 nM and 14 nM, respectively; 100-fold more active against Gefitinib-resistant L858R-T790M EGFR mutant. Afatinib (BIBW2992) Dimaleate induces **autophagy**.

Targets

EGFR (L858R) [1] (Cell-free assay)	EGFR (wt) [1] (Cell-free assay)	EGFR (L858R/T790M) [1] (Cell-free assay)	HER2 [1] (Cell-free assay)
0.4 nM	0.5 nM	10 nM	14 nM

In vitro	BIBW2992 is more effective than erlotinib, gefitinib, or lapatinib in inhibiting survival of lung cancer cell lines harboring wild-type (H1666) or L858R/T790M (NCI-H1975) EGFR. BIBW2992 is similarly effective against NSCLC lines expressing HER2 776insV (NCI-H1781) or EGFR E746_A750del (HCC827), but shows no activity toward A549 cells, which express wild-type EGFR and HER2.[1] Afatinib enhances cytotoxicity of topotecan and mitoxantrone to SP cells, and increases the apoptosis induced by topotecan and mitoxantrone in SP cells.[2]
In vivo	In the MDA-MB-453 xenograft model, BIBW2992 (20 mg/kg, p.o.) results in dramatic tumor regression with a cumulative treated/control tumor volume ratio (T/C ratio) of 2%, and downregulation of EGFR and AKT phosphorylation.[1] In A7, A431, FaDu, UT-SCC-14 and UT-SCC-15 xenograft models, application of BIBW 2992 (30 mg/kg, p.o.) leads to a significant prolongation of tumour growth time.[3] In HER2-amplified xenograft medel, Afatinib (30 mg/kg, p.o.) results a markedly inhibition in tumor growth and a significant improvement in the duration of overall survival.[4] In HER2-positive gastric cancer NCI-N87 xenograft, afatinib (25 mg/kg, p.o.) leads to dramatic tumor volume regression within 4 d and near-complete tumor resolution after 21 d of treatment.[5]

Protocol (from reference)

Kinase Assay:[1]

- **In vitro kinase activity assay**

  EGFR kinase: Each 100 μL enzyme reaction contained 10 μL of inhibitor in 50% Me2SO, 20 μL of substrate solution (200 mM HEPES pH 7.4, 50 mM Mg-acetate, 2.5 mg/mL poly (EY), 5 μg/mL bio-pEY) and 20 μL enzyme preparation. The enzymatic reaction is started by addition of 50 μL of a 100 μM ATP solution made in 10 mM MgCl2. Assays are carried out at room temperature for 30 min and terminated by the addition of 50 μL of stop solution (250 mM EDTA in 20 mM HEPES pH 7.4). 100 μL are transferred to a streptavidin coated microtiterplate, after an incubation time of 60 min at room temperature the plate is washed with 200 μL of wash solution (50 mM Tris, 0.05% Tween20). A 100 μL aliquot of a HRPO- labeled anti-PY antibody (PY20H Anti-Ptyr:HRP) 250 ng/mL are added to the wells. After 60 min of incubation, the plate is washed three times with a 200 μL wash solution. The samples are then developed with a 100μL TMB Peroxidase Solution (A:B= 1:1). The reaction is stopped after 10 min. The plate is transferred to an ELISA reader and extinction is measured at OD450nm. HER2-IC enzyme: Enzyme activity is assayed in the presence or absence of serial inhibitor dilutions performed in 50 % Me2SO. Each 100 μL reaction contains similar components as described for EGFR kinase assay with addition of 1000 μM Na3VO4. The enzymatic reaction is started by addition of 50μL of 500 μM ATP solution made in 10 mM Mg-acetate. The dilution of the enzyme is set so that incorporation of phosphate into bio-pEY is linear with respect to time and amount of enzyme. The enzyme preparation is diluted in 20 mM HEPES pH 7.4, 130 mM NaCl, 0.05% Triton X-100, 1 mM DTT and 10% glycerol. Assays are carried out at room temperature for 30 min and terminated by the addition of 50 μL of stop solution. Src kinase assays: Each 100 μL reaction contained 10 μL of inhibitor in 50 % Me2SO, 20μL of enzyme preparation, 20 μL of substrate solution supplemented with 1000 μM Na3VO4. The enzymatic reaction is started by addition of 50 μL of a 1000 μM ATP solution made in 10 mM Mg-acetate. BIRK kinase assay: 250 mM Tris pH 7.4, 10mM DTT, 2.5 mg/mL poly(EY), 5 mg/mL bio-pEY is used as substrate solution and enzymatic reaction is started by addition of 50 μL of a 2 mM ATP solution made in 8 mM MnCl2, 20 mM Mg-acetate. VEGF2 and HGFR kinase assays: Assays are carried out at room temperature for 20 minutes and terminated by the addition of 10 μL of 5 % H3PO4. The precipitate is then trapped onto GF/B filters using a 96 well filter mate universal harvester. After extensive washing the filter plate is dried for 1 h at 50°C, sealed and incorporated radioactivity is determined by scintillation counting using a TopCount™ or a Microbeta b counter™.

Cell Assay:[2]

- **Cell lines**

  SP and NSP cell lines

- **Concentrations**

1 μM

- **Incubation Time**
  48 h
- **Method**
  Cytotoxicity is determined using MTT assay. The IC 50 value is defined as the drug concentration resulting in 50% cell death. Both the fitted sigmoidal dose response curve and IC50 are calculated by Bliss method.

Animal Study:[4]

- **Animal Models**
  SCID mice harbouring ARK2 xenografts
- **Dosages**
  25 mg/kg
- **Administration**
  p.o.

References

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2748240/ https://pubmed.ncbi.nlm.nih.gov/24972892/ https://pubmed.ncbi.nlm.nih.gov/25444177/

+ Expand

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SHIPPING & STORAGE

Capsid Bio products are transported at room temperature. If you receive the product at room temperature, please rest assured, the Capsid Bio Quality Inspection Department has conducted experiments to verify that the normal temperature placement of one month will not affect the biological activity of powder products. After collecting, please store the product according to the requirements described in the datasheet. Most Capsid Bio products are stable under the recommended conditions.

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EU Headquarters
Gajeva ulica 28, HR-10000 Zagreb, Croatia

US Headquarters
12222 Merit Dr STE 130 Dallas, Texas, 75251

UK Headquarters
121 ABILITY HOUSE, BROOKER ROAD, EN9 1JH, ESSEX

Orders: Sales@capsidbio.com
Enquiries: info@capsidbio.com
www.capsidbio.com