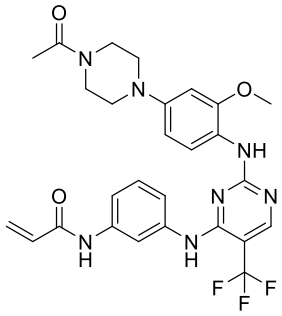
Product data sheet

MedKoo Cat#: 205796 Name: Rociletinib CAS#: 1374640-70-6 (free base) Chemical Formula: $C_{27}H_{28}F_3N_7O_3$ Exact Mass: 555.22057 Molecular Weight: 555.55	
Product supplied as:	Powder
Purity (by HPLC):	≥ 98%
Shipping conditions	Ambient temperature
Storage conditions:	Powder: -20°C 3 years; 4°C 2 years.
	In solvent: -80°C 3 months; -20°C 2 weeks.

1. Product description:
Rociletinib, also known as AVL-301 and CO1686, is an orally available small molecule, irreversible inhibitor of epidermal growth factor receptor (EGFR) with potential antineoplastic activity. EGFR inhibitor CO-1686 binds to and inhibits mutant forms of EGFR, including T790M, thereby leading to cell death of resistant tumor cells. Compared to other EGFR inhibitors, CO-1686 inhibits T790M, a secondary acquired resistance mutation, as well as other mutant EGFRs and may have therapeutic benefits in tumors with T790M-mediated resistance to other EGFR tyrosine kinase inhibitors.

2. CoA, QC data, SDS, and handling instruction
SDS and handling instruction, CoA with copies of QC data (NMR, HPLC and MS analytical spectra) can be downloaded from the product web page under "QC And Documents" section. Note: copies of analytical spectra may not be available if the product is being supplied by MedKoo partners. Whether the product was made by MedKoo or provided by its partners, the quality is 100% guaranteed.

3. Solubility data

Solvent	Max Conc. mg/mL	Max Conc. mM
DMSO	43	77.40

4. Stock solution preparation table:

Concentration / Solvent Volume / Mass	1 mg	5 mg	10 mg
1 mM	1.80 mL	9.00 mL	18.00 mL
5 mM	0.36 mL	1.80 mL	3.60 mL
10 mM	0.18 mL	0.90 mL	1.80 mL
50 mM	0.04 mL	0.18 mL	0.36 mL

5. Molarity Calculator, Reconstitution Calculator, Dilution Calculator
Please refer the product web page under section of "Calculator"

6. Recommended literature which reported protocols for in vitro and in vivo study
In vitro study

1. Walter AO, Sjin RT, Haringsma HJ, Ohashi K, Sun J, Lee K, Dubrovskiy A, Labenski M, Zhu Z, Wang Z, Sheets M, St Martin T, Karp R, van Kalken D, Chaturvedi P, Niu D, Nacht M, Petter RC, Westlin W, Lin K, Jaw-Tsai S, Raponi M, Van Dyke T, Etter J, Weaver Z, Pao W, Singh J, Simmons AD, Harding TC, Allen A. Discovery of a mutant-selective covalent inhibitor of EGFR that overcomes T790M-mediated resistance in NSCLC. Cancer Discov. 2013 Dec;3(12):1404-15. doi: 10.1158/2159-8290.CD-13-0314. Epub 2013 Sep 24. PMID: 24065731; PMCID: PMC4048995.

In vivo study

1. Walter AO, Sjin RT, Haringsma HJ, Ohashi K, Sun J, Lee K, Dubrovskiy A, Labenski M, Zhu Z, Wang Z, Sheets M, St Martin T, Karp R, van Kalken D, Chaturvedi P, Niu D, Nacht M, Petter RC, Westlin W, Lin K, Jaw-Tsai S, Raponi M, Van Dyke T, Etter J, Weaver Z, Pao W, Singh J, Simmons AD, Harding TC, Allen A. Discovery of a mutant-selective covalent inhibitor of EGFR that overcomes T790M-mediated resistance in NSCLC. Cancer Discov. 2013 Dec;3(12):1404-15. doi: 10.1158/2159-8290.CD-13-0314. Epub 2013 Sep 24. PMID: 24065731; PMCID: PMC4048995.

7. Bioactivity

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Biological target:

Rociletinib (CO-1686, AVL-301) is an irreversible, mutant-selective EGFR inhibitor with Ki of 21.5 nM and 303.3 nM for EGFRL858R/T790M and EGFRWT in cell-free assays, respectively.

In vitro activity

Selectivity and activity of CO-1686 against cells expressing EGFR mutations was demonstrated in a panel of cell lines (Fig. 2A and SI Table 2). The effect of CO-1686 treatment on cell growth was determined in 4 NSCLC cell lines expressing mutant EGFR (HCC827, PC9, HCC827-EPR and NCI-H1975) and in 3 cell lines expressing WT EGFR (A431, NCI-H1299 and NCI-H358). HCC827 and PC9 cell lines both harbor the EGFR delE746-A750 activating mutation in exon 19. HCC827-EPR is a resistant clone of HCC827 that acquired T790M in response to continuous exposure to erlotinib and the MET inhibitor PHA-665,752. NCI-H1975 is another T790M-positive cell line that harbors the EGFR L858R/T790M double mutation. CO-1686 potently inhibited proliferation in the mutant-EGFR NSCLC cells with GI50 values ranging from 7 – 32 nM. In comparison, the GI50 value for A431 cells, an epidermoid cell line that is driven by amplified WT EGFR, was 547 nM. Two cell lines expressing WT EGFR in the presence of an N- or KRAS mutation (NCI-H1299 and NCI-H358, respectively) were inhibited by CO-1686 at a concentration of 4275 and 1806 nM, respectively. Similar results were obtained when determining effects of CO-1686 on EGFR signaling by immunoblot analysis in the WT-driven A431 cells compared to the EGFR mutant cells. IC50 values for inhibition of EGFR phosphorylation were above 2000 nM in the three WT EGFR expressing cells, while CO-1686 inhibited p-EGFR with IC50 values ranging from 62 – 187 nM in the mutant-EGFR expressing cells (SI Table 2) confirming the mutant-selective properties of CO-1686. CO-1686 inhibits cell proliferation and EGFR phosphorylation equally in the parental HCC827 (EGFR del19) as well as the erlotinib-resistant HCC827-EPR (del19/T790M) clone. Treatment with CO-1686 induces apoptosis in both cell lines as demonstrated by an increase in cleaved PARP and BimEL protein (Fig. 2B), irrespective of the T790M status. Erlotinib on the other hand has no effect in the T790M-positive HCC827-EPR cells (Fig. 2B). Additionally, we treated PC-9/ER (del19/T790M) and H3255/XLR cells (L858R/T790M) with erlotinib and CO-1686 in standard growth inhibition assays. Both are polyclonal populations of cells that acquired T790M in response to continuous exposure to EGFR TKIs. Again, CO-1686 was superior to erlotinib in inhibiting the growth of these cells (SI Fig. 2A, B). Collectively, the EGFR signaling and cell growth/apoptosis data indicate that CO-1686 selectively and potently affects cells harboring activating EGFR mutations as well as the T790M resistance mutation and has minimal activity in cells expressing WT EGFR.

Reference: Cancer Discov. 2013 Dec;3(12):1404-15. https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/24065731/

In vivo activity

Initial CO-1686 pharmacokinetics were evaluated in female NCRnu.nu mice (n=3/gp) following IV and oral delivery (SI Fig. 4A, B). CO-1686 displayed high oral bioavailability (65%) and a relatively long half-life of 2.6 hours when dosed at 20 mg/kg. Tumor-bearing mice were dosed orally once daily with CO-1686 as single agent and its effect on tumor growth was determined in several EGFR dependent xenograft models (Fig. 3A–C). Continuous oral dosing of CO-1686 causes dose-dependent and significant tumor growth inhibition in all EGFR-mutant models examined. At 100 mg/kg/day CO-1686 caused tumor regressions in two erlotinib-resistant models expressing the L858R/T790M EGFR mutation, the NCI-H1975 cell line xenograft (Fig. 3A) and the patient-derived lung tumor xenograft (PDX) LUM1868 (Fig. 3B), while erlotinib had no inhibitory effect on tumor growth (Fig. 3A, B). The second generation EGFR TKI afatinib reduced tumor growth in NCI-H1975 EGFR T790M xenograft model (Fig. 3A) but to a lesser extent than observed with CO-1686 ($P < 0.01$). Anti-tumor activity of CO-1686 in the HCC827 xenograft model that expresses the exon del19 activating EGFR mutation was comparable to erlotinib and afatinib (Fig. 3C). Exploration of different oral dosing schedules demonstrated that in the NCI-H1975 model CO-1686 caused tumor regressions either given as 100 mg/kg once daily (QD) or as 50 mg/kg twice daily (BID), with no significant alterations in body weight with either dosing schedule (SI Fig. 5A, B). However, the BID CO-1686 administration schedule was statistically superior to QD day 15 post-dosing ($P < 0.01$) and was therefore chosen as the optimal dosing regimen (SI Fig. 5A).

Reference: Cancer Discov. 2013 Dec;3(12):1404-15. https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/24065731/