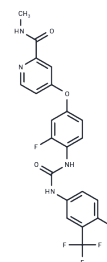


## Regorafenib

## Chemical Properties

CAS No. :	755037-03-7
Formula:	C <sub>21</sub> H <sub>15</sub> ClF <sub>4</sub> N <sub>4</sub> O <sub>3</sub>
Molecular Weight:	482.82
Storage:	Powder: -20°C for 3 years   In solvent: -80°C for 1 year Actual storage temperature shall be subject to the COA.



## Biological Description

Description	Regorafenib (BAY 73-4506) is an orally active, multi-targeted receptor tyrosine kinase inhibitor that inhibits RET, C-RAF, VEGFR2, c-Kit, VEGFR1, and PDGFR $\beta$ , exhibiting both antitumor and anti-angiogenic activity.
Targets(IC50)	Raf,FGFR,c-RET,Autophagy,c-Kit,PDGFR,Tie-2,VEGFR
In vitro	<p><b>METHODS:</b> Human hepatocellular carcinoma cells Hep3B, PLC/PRF/5 and HepG2 were treated with Regorafenib (0-10 <math>\mu</math>M) for 72 h. Cell viability was measured by MTT.</p> <p><b>RESULTS:</b> Regorafenib concentration-dependently decreased the viability of Hep3B cells with an IC50 of about 5 <math>\mu</math>M. PLC/PRF/5 cells were similarly sensitized to Hep3B cells. However, HepG2 cells were more sensitive, with an IC50 of approximately 1 <math>\mu</math>M. [1]</p> <p><b>METHODS:</b> Tumor cells NIH-3T3/VEGFR2, CHO/TIE2, HAoSMC/PDGFR-<math>\beta</math> and MCF-7/FGFR were treated with Regorafenib (10-3000 nM) for 1 h. The expression levels of the target proteins were detected by Western Blot.</p> <p><b>RESULTS:</b> Regorafenib inhibited p-VEGFR2, p-TIE2, p-PDGFR-<math>\beta</math> and p-FGFR. [2]</p>
In vivo	<p><b>METHODS:</b> To test the antitumor activity in vivo, Regorafenib (3-100 mg/kg) was orally administered to NCr nu/nu mice bearing tumors Colo-205 or MDA-MB-231 once daily for nine days.</p> <p><b>RESULTS:</b> Regorafenib inhibited tumor growth. In the Colo-205 model, Regorafenib at a dose of 10 mg/kg resulted in a TGI of 75% on day 14. In the MDA-MB-231 model, Regorafenib was highly effective at doses as low as 3 mg/kg, resulting in a significant TGI of 81%. [2]</p> <p><b>METHODS:</b> To assay antitumor activity in vivo, Regorafenib (3-10 mg/kg) was administered orally to NMRI nu/nu mice harboring tumors HT-29 or MDA-MB-231 once daily for twenty-seven days.</p> <p><b>RESULTS:</b> Regorafenib dose-dependently inhibited HT-29 and MDA-MB-231 tumor growth. [3]</p>
Kinase Assay	In vitro assays using recombinant VEGFR2 (murine aa785-aa1367), VEGFR3 (murine aa818-aa1363), PDGFR-b (aa561-aa1106), RAF-1 (aa305-aa648) and BRAFV600E (aa409-aa765) kinase domains were performed as previously described. Initial in vitro kinase inhibition profiling was performed at a fixed 1 $\mu$ M compound concentration under Millipore standard conditions [10 $\mu$ M adenosine-50'-triphosphate (ATP) concentration]. Inhibitory concentration of 50% (IC50) values were determined from selected responding kinases, e.g., VEGFR1 and RET. TIE2 kinase inhibition was measured with a homogeneous time-resolved fluorescence (HTRF) assay using a recombinant

Kinase Assay	fusion protein of glutathione-S-transferase, the intracellular domain of TIE2 and the peptide biotin-Ahx-EPKDDAYPLYSDFG as substrate.
Cell Research	Each cell line was seeded at $0.3 \times 10^5$ cells/2ml of DMEM containing 10% FBS in 35 mm tissue culture dishes. The cells were incubated for 24 h to allow attachment, and then the medium was replaced by fresh culture medium containing Regorafenib at increasing concentrations (1 $\mu$ M, 2.5 $\mu$ M, 5 $\mu$ M, 7.5 $\mu$ M and 10 $\mu$ M). In these experimental conditions, the cells were allowed to grow for 72 or 96 h. Time-course experiments on Hep3B cells were performed with 7.5 $\mu$ M of Regorafenib at short (15, 60, 180 min.), middle (24, 48, 72 and 96 h) or long times (up to seven days). When the cells were treated for long times the drug was replaced with a fresh one. Each experiment included a control with the equivalent concentration of DMSO (solvent control) as the one used for adding Regorafenib. Each experiment was performed in triplicate and repeated 3 times. Subsequent analyses were performed at specific Regorafenib concentrations and incubation times [2].
Animal Research	Female athymic NCr nu/nu mice, kept in accordance with Federal guidelines, were subcutaneously inoculated with $5 \times 10^6$ Colo-205 or MDAMB-231 cells or implanted with 1 mm <sup>3</sup> 786-O tumor fragments. When tumors reached a volume of ~100 mm <sup>3</sup> , regorafenib or vehicle control was administered orally qd $\times$ 21 in the 786-O model, and qd $\times$ 9 in the Colo-205 and MDA-MB231 models, respectively, at doses of 100, 30, 10, and 3 mg/kg. Paclitaxel was administered intravenously at 10 mg/kg in ethanol/Cremophor ELV/saline (12.5%/12.5%/75%) every 2 days $\times$ 5. Tumor size (volume) was estimated twice weekly ( $l \times w^2$ )/2, and the percentage of tumor growth inhibition (TGI) was obtained from terminal tumor weights (1-T/C100). Mice were weighed every other day starting from the first day of treatment. The general health status of the mice was monitored daily [1].

### Solubility Information

Solubility	DMSO: 126.3 mg/mL (261.59 mM),Sonication is recommended. Ethanol: < 1 mg/mL (insoluble or slightly soluble), H2O: < 1 mg/mL (insoluble or slightly soluble), (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 9 mg/mL (18.64 mM),Suspension. <i>Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.</i>

## Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.0712 mL	10.3558 mL	20.7117 mL
5 mM	0.4142 mL	2.0712 mL	4.1423 mL
10 mM	0.2071 mL	1.0356 mL	2.0712 mL
50 mM	0.0414 mL	0.2071 mL	0.4142 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

## Reference

- Carr BI, et al. Fluoro-Sorafenib (Regorafenib) effects on hepatoma cells: growth inhibition, quiescence, and recovery. *J Cell Physiol.* 2013 Feb;228(2):292-7.
- Huang M, Chen M, Qi M, et al. Perivascular cell-derived extracellular vesicles stimulate colorectal cancer revascularization after withdrawal of antiangiogenic drugs. *Journal of Extracellular Vesicles.* 2021, 10(7): e12096.
- Duan F, Huang R, Zhang F, et al. Biphasic modulation of insulin signaling enables highly efficient hematopoietic differentiation from human pluripotent stem cells. *Stem Cell Research & Therapy.* 2018 Jul 27;9(1):205
- Wilhelm SM, et al. Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. *Int J Cancer.* 2011 Jul 1;129(1):245-55.
- Zopf D, et al. Pharmacologic activity and pharmacokinetics of metabolites of regorafenib in preclinical models. *Cancer Med.* 2016 Nov;5(11):3176-3185.
- Wang C, Huang M, Lin Y, et al. ENO2-derived phosphoenolpyruvate functions as an endogenous inhibitor of HDAC1 and confers resistance to antiangiogenic therapy. *Nature Metabolism.* 2023: 1-22.
- Breitenecker K, Hedrich V, Pupp F, et al. Synergism of the receptor tyrosine kinase Axl with ErbB receptors mediates resistance to regorafenib in hepatocellular carcinoma. *Frontiers in Oncology.* 2023, 13: 1238883.
- Duan F, Huang R, Zhang F, et al. Biphasic modulation of insulin signaling enables highly efficient hematopoietic differentiation from human pluripotent stem cells[J]. *Stem cell research & therapy.* 2018 Jul 27;9(1):205.

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