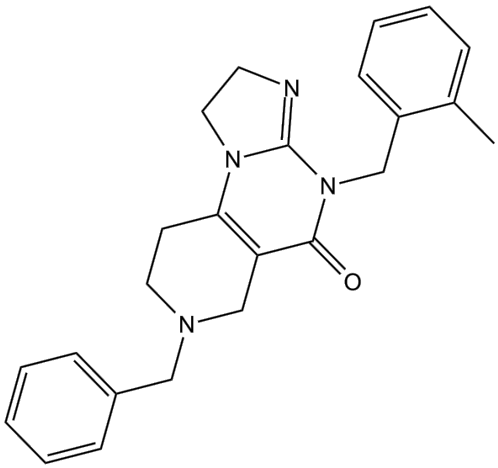
Product Data Sheet

Product Name: ONC201
Cat. No.: GC11711

Chemical Properties

Cas. No.	1616632-77-9		
Chemical Name	7-benzyl-4-(2-methylbenzyl)-1,2,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(4H)-one		
SMILES	CC1=CC=CC=C1CN(C2=NCCN32)C(C(C4)=C3CCN4CC5=CC=CC=C5)=O		
Formula	$C_{24}H_{26}N_4O$	M.Wt	386.49
Solubility	DMSO : ≥100 mg/mL ；Water : Insoluble	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		

Structure



Protocol

Cell experiment [1]:

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Cell lines	HCT116 human colon cancer cells
Preparation Method	HCT116 human colon cancer cells were treated with or without 5μM ONC201 for 72h, floating and adherent cells were analyzed on a cytometer. For surface TRAIL experiments, adherent cells were harvested by brief trypsinization, fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min, incubated with an anti-TRAIL antibody at 1:250 overnight, washed and incubated with anti-rabbit Alexa Fluor 488 for 30 min, and analyzed. Cells were gated on forward and side scatter to eliminate debris and dead cells from the analysis. Surface TRAIL data are expressed as median fluorescence intensity relative to that of control samples unless indicated otherwise. Surface DR5 was analyzed similarly with an antibody. For sub-G1 content and cell cycle profile analysis, all cells were pelleted and ethanol-fixed, followed by staining with propidium iodide in the presence of RNase. Cell viability assays were carried out in 96-well black-walled clear-bottom plates with CellTiter-Glo according to the manufacturer's protocols.
Reaction Conditions	5μM; 72h
Applications	ONC201 exposure causes a dose-dependent increase in TRAIL mRNA and induces TRAIL protein localization on the cell surface of HCT116 human colon cancer cells in a p53-independent manner, while also inducing TRAIL-mediated apoptosis.

Animal experiment [2]:

Animal models	C57Bl/6J mice

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Preparation Method	Under brief anesthesia, male or female C57Bl/6J mice were injected with freshly harvested KPC tumor pieces (2-3mm) subcutaneously into the right hind flank. Volumes of outgrowing tumors were evaluated using the formula: volume = width^2 × length × ½. When tumors reached a volume of approximately 100mm ^{3}, about 9–12 days post-tumor inoculation, animals were injected intravenously (i.v.) with equivalent volumes of solvent alone and served as vehicle-treated controls (vehicle, n=5). Additional groups were injected with L_GEM (20mg/kg), ONC201 (15mg/kg), or their combination three times a week for two weeks. During the treatment, mice were monitored for body weight and tumor volume. Tumor volumes were measured on alternate days and plotted over time. Mice were sacrificed after the treatment regimen or when they became moribund before the tumor volume reached 2000mm^3. At the end of each experiment, blood samples, tumors, and other major organs were removed from the animals of all groups for further analysis.
Dosage form	15mg/kg; three times a week for two weeks; i.v.
Applications	Combinated treatment of ONC201and L_GEM reduced the tumor size, inhibited the expression of p-ERK and p-AKT and upregulated the expression of cleaved Caspase 3 of tumor tissues in KPC tumor-bearing syngeneic mice.

Caution: Product has not been fully validated for medical applications. For research use only.
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References:
[1] Allen, J. E., Krigsfeld, G., Mayes, P. A., Patel, L., Dicker, D. T., Patel, A. S., Dolloff, N. G., Messaris, E., Scata, K. A., Wang, W., Zhou, J. Y., Wu, G. S., & El-Deiry, W. S. (2013). Dual inactivation of Akt and ERK by TIC10 signals Foxo3a nuclear translocation, TRAIL gene induction, and potent antitumor effects. *Science translational medicine*, *5*(171), 171ra17.
[2] Kumar, V., Sethi, B., Staller, D. W., Shrestha, P., & Mahato, R. I. (2024). Gemcitabine elaidate and ONC201 combination therapy for inhibiting pancreatic cancer in a KRAS mutated syngeneic mouse model. *Cell death discovery*, *10*(1), 158.

Background

ONC201 is an orally active small molecule compound belonging to the imipridone class and acts as a selective antagonist of the dopamine D2/3 receptors (DRD2/3)[1]. DRD2/3, which are G protein-coupled receptors (GPCRs), are overexpressed in various tumor cells[2]. ONC201 induces tumor cell apoptosis by antagonizing DRD2/3 and activating the ClpP protease, and further enhances its antitumor effects by activating the immune system. This multi-target mechanism of action enables ONC201 to exhibit extensive anti-tumor activity in various tumor types[3]. ONC201 is commonly used in cancer research, particularly in the study of various solid tumors and hematological malignancies[4][5].

In vitro,ONC201 (5μM, 72h) exposure causes a dose-dependent increase in TRAIL mRNA and induces TRAIL protein localization on the cell surface of HCT116 human colon cancer cells in a p53-independent manner, while also inducing TRAIL-mediated apoptosis[6]. Combination of L_GEM and ONC201(200nM,72h) arrests MIA PaCa-2 cells in the G2 phase and induces apoptosis[7].

In vivo, combinated treatment of ONC201(15mg/kg; three times a week for two weeks) and L_GEM reduced the tumor size, inhibited the expression of p-ERK and p-AKT and upregulated the expression of cleaved Caspase 3 of tumor tissues in KPC tumor-bearing syngeneic mice[7].

References:
[1] Stein, M. N., Bertino, J. R., Kaufman, H. L., Mayer, T., Moss, R., Silk, A., Chan, N., Malhotra, J., Rodriguez, L., Aisner, J., Aiken, R. D., Haffty, B. G., DiPaola, R. S., Saunders, T., Zloza, A., Damare,

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S., Beckett, Y., Yu, B., Najmi, S., Gabel, C., … Mehnert, J. M. (2017). First-in-Human Clinical Trial of Oral ONC201 in Patients with Refractory Solid Tumors. *Clinical cancer research : an official journal of the American Association for Cancer Research*, *23*(15), 4163–4169.
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[3] Prabhu, V. V., Morrow, S., Rahman Kawakibi, A., Zhou, L., Ralff, M., Ray, J., Jhaveri, A., Ferrarini, I., Lee, Y., Parker, C., Zhang, Y., Borsuk, R., Chang, W. I., Honeyman, J. N., Tavora, F., Carneiro, B., Raufi, A., Huntington, K., Carlsen, L., Louie, A., … El-Deiry, W. S. (2020). ONC201 and imipridones: Anti-cancer compounds with clinical efficacy. *Neoplasia (New York, N.Y.)*, *22*(12), 725–744.
[4] Allen, J. E., Prabhu, V. V., Talekar, M., van den Heuvel, A. P., Lim, B., Dicker, D. T., Fritz, J. L., Beck, A., & El-Deiry, W. S. (2015). Genetic and Pharmacological Screens Converge in Identifying FLIP, BCL2, and IAP Proteins as Key Regulators of Sensitivity to the TRAIL-Inducing Anticancer Agent ONC201/TIC10. *Cancer research*, *75*(8), 1668–1674.
[5] Ishizawa, J., Kojima, K., Chachad, D., Ruvolo, P., Ruvolo, V., Jacamo, R. O., Borthakur, G., Mu, H., Zeng, Z., Tabe, Y., Allen, J. E., Wang, Z., Ma, W., Lee, H. C., Orlowski, R., Sarbassov, dosD., Lorenzi, P. L., Huang, X., Neelapu, S. S., McDonnell, T., … Andreeff, M. (2016). ATF4 induction through an atypical integrated stress response to ONC201 triggers p53-independent apoptosis in hematological malignancies. *Science signaling*, *9*(415), ra17.
[6] Allen, J. E., Krigsfeld, G., Mayes, P. A., Patel, L., Dicker, D. T., Patel, A. S., Dolloff, N. G., Messaris, E., Scata, K. A., Wang, W., Zhou, J. Y., Wu, G. S., & El-Deiry, W. S. (2013). Dual inactivation of Akt and ERK by TIC10 signals Foxo3a nuclear translocation, TRAIL gene induction, and potent antitumor effects. *Science translational medicine*, *5*(171), 171ra17.
[7] Kumar, V., Sethi, B., Staller, D. W., Shrestha, P., & Mahato, R. I. (2024). Gemcitabine elaidate and ONC201 combination therapy for inhibiting pancreatic cancer in a KRAS mutated syngeneic mouse model. *Cell death discovery*, *10*(1), 158.

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