**SOLVENT & SOLUBILITY**

<table>
<thead>
<tr>
<th></th>
<th>In Vitro</th>
<th></th>
<th>In Vivo</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMSO</td>
<td>100 mg/mL (180.34 mM; Need ultrasonic)</td>
<td>1. Add each solvent one by one: <strong>10% DMSO &gt;&gt; 40% PEG300 &gt;&gt; 5% Tween-80 &gt;&gt; 45% saline</strong></td>
<td>Solubility: 2.5 mg/mL (4.51 mM); Suspended solution; Need ultrasonic</td>
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<tr>
<td></td>
<td>DMSO</td>
<td>100 mg/mL (180.34 mM; Need ultrasonic)</td>
<td>2. Add each solvent one by one: <strong>10% DMSO &gt;&gt; 90% corn oil</strong></td>
<td>Solubility: ≥ 2.5 mg/mL (4.51 mM); Clear solution</td>
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<tr>
<td></td>
<td>Solvent</td>
<td>Mass</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Concentration</td>
<td>1 mg</td>
<td>5 mg</td>
<td>10 mg</td>
</tr>
<tr>
<td></td>
<td>1 mM</td>
<td>1.8034 mL</td>
<td>9.0168 mL</td>
<td>18.0336 mL</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>0.3607 mL</td>
<td>1.8034 mL</td>
<td>3.6067 mL</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>0.1803 mL</td>
<td>0.9017 mL</td>
<td>1.8034 mL</td>
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</tbody>
</table>

Please refer to the solubility information to select the appropriate solvent.

**BIOLOGICAL ACTIVITY**

**Description**

TAK-632 is a potent pan-RAF inhibitor with IC₅₀ of 1.4, 2.4 and 8.3 nM for CRAF, BRAF<sup>V600E</sup>, BRAF<sup>WT</sup>, respectively.

**IC₅₀ & Target**

IC₅₀: 1.4 nM (C-RAF), 2.4 nM (BRAF<sup>V600E</sup>), 8.3 nM (BRAF<sup>WT</sup>), 66 nM (Aurora B), 160 nM (VEGFR)<sup>[1]</sup>

**In Vitro**

TAK-632 inhibits PDGFRβ, FGFR3, GSK3β, CDK2, P38α, PDGFRα, TIE2, and CDK1 with a range of IC₅₀ values from 120-790 nM. CHK1, IKKβ, and MEK1 are inhibited over an IC₅₀ range of 1400-1700 nM. With 1 h of preincubation time, TAK-632 inhibits BRAF and CRAF in an ATP competitive manner (at low ATP concentrations BRAF IC₅₀: 15 nM, CRAF: 8.1 nM). The respective biochemical activity of TAK-632 against BRAF and CRAF reduces to IC₅₀ values of 58 nM and 62 nM at high ATP concentrations. TAK-632 demonstrates strong inhibition of pMEK and pERK in HMVII cells with IC₅₀
values of 49 nM and 50 nM, respectively[1]. TAK-632 shows strong antiproliferative effects both in A375 and SK-MEL-2 cells ($GI_{50}$ of 40-190 nM in A375 cells and $GI_{50}$ of 190-250 nM in SK-MEL-2 cells)[2].

**In Vivo**

TAK-632 demonstrates dramatically improved solubility (740 µg/mL) in pH 6.8 phosphate buffer and exhibits significant oral absorption (at a dose of 25 mg/kg, AUC, 32.47 µg h/mL; F, 51.7%) in rats. In a dog PK study, 10 mg/kg administration of TAK-632 also shows superior oral bioavailability (F: 108%). Oral single administration of TAK-632 inhibits pERK in tumors at 8 h after its administration over a dose range of 1.9-24.1 mg/kg. In particular, 9.7-24.1 mg/kg dosing with TAK-632 strongly inhibits pERK levels to 11% of the control. TAK-632 exhibits dose-dependent antitumor efficacy without severe body weight reduction over a dose range of 3.9-24.1 mg/kg. Significant tumor regression is observed at 9.7 mg/kg and 24.1 mg/kg (T/C=−2.1% and −12.1%, respectively)[1]. TAK-632 exhibits potent antitumor efficacy when orally administered at 60 mg/kg once daily (T/C=37%, P<0.001) or at 120 mg/kg once daily (T/C=29%, P<0.001) for 21 days without severe toxicity in NRAS-mutant melanoma using a SK-MEL-2 xenograft model[2].

**PROTOCOL**

**Kinase Assay [2]**

Immunoprecipitated BRAF or CRAF is incubated with recombinant inactive MEK (K97R) at 30°C for 30 minutes in kinase reaction buffer containing ATP/Mg$^{2+}$. RAS/RAF wild-type (A431, CsFb, and HeLa), KRAS-mutant (A549, HCT-116, and Mia PaCa-2), and NRAS-mutant melanoma (GAK, HMV-II, and SK-MEL-2) cells are treated with TAK-632 (0, 0.32, 1.6, 8, 40, 200, 1000 and 5000 nM) at the indicated concentrations for 2 hours. Cell lysates are analyzed by Western blot analysis[2].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**Cell Assay [2]**

Cell viability is assessed (3 replicates) using the Sulforhodamine B assay or by the CellTiter-Glo luminescent cell viability assay. The concentrations of TAK-632 that produced 50% growth inhibition ($GI_{50}$) are calculated using PCP software. The combination index (CI) is calculated using CalcuSyn software. To investigate the antiproliferative activity of TAK-632, we performed proliferation assays in various cell lines harboring mutated BRAF, NRAS, or KRAS. HMV-II, SK-MEL-2, or A375 cells are cotreated with TAK-632 and TAK-733 at the indicated concentrations for 72 hours. Cell viability is measured. The CI value at EC$_{50}$ is calculated. A375 cells stably expressing NRAS$^{Q61K}$ or $\Delta N$-BRAF are cotreated with TAK-632 and TAK-733 at the indicated concentrations for 72 hours. Cell viability is measured. The CI value at EC$_{50}$ is calculated[2].

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**Animal Administration [2]**

Mice[2]

The xenograft-implanted nude mice are used. Mice bearing SK-MEL-2 xenografts are treated once daily for 21 consecutive days with vehicle or TAK-632 at the indicated concentrations (10 mice per each treatment group). Day 0 indicates the beginning of treatment. Tumors are measured twice a week. Mice bearing SK-MEL-2 xenografts are treated once daily (QD) for 3 days with vehicle, TAK-632 at 60 mg/kg (60 mpk), or TAK-632 at 120 mg/kg (120 mpk). Tumor xenografts are obtained at indicated time points after the final treatment and analyzed by Western blot analysis. Individual blots with dividing lines are combined from a single electrophoresis gel. Bars represent densitometric analysis of phospho-ERK, normalized to vehicle-treated control (mean±SD).

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REFERENCES
