Product Data Sheet

NK-252

Cat. No.: HY-19734 CAS No.: 1414963-82-8 Molecular Formula: $C_{13}H_{11}N_5O_3$ Molecular Weight: 285.26 Target: Keap1-Nrf2

Pathway: NF-κΒ

Storage: Powder -20°C 3 years

4°C 2 years

In solvent -80°C 2 years

> -20°C 1 year

SOLVENT & SOLUBILITY

In Vitro DMSO: $\geq 29 \text{ mg/mL} (101.66 \text{ mM})$

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Mass Concentration	1 mg	5 mg	10 mg
	1 mM	3.5056 mL	17.5279 mL	35.0557 mL
	5 mM	0.7011 mL	3.5056 mL	7.0111 mL
	10 mM	0.3506 mL	1.7528 mL	3.5056 mL

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

In Vivo

- 1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.08 mg/mL (7.29 mM); Clear solution
- 2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.08 mg/mL (7.29 mM); Clear solution
- 3. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.08 mg/mL (7.29 mM); Clear solution

BIOLOGICAL ACTIVITY

Description	NK-252 is a potential Nrf2 activator, which exhibits a great Nrf2-activating potential.	
IC ₅₀ & Target	Nrf2 ^[1]	
In Vitro	The luciferase activity in Huh-7.5 cells treated with Oltipraz (OPZ) or NK-252 shows activation of the NAD(P)H quinone oxidoreductase 1 (NQO1)-ARE in a dose-dependent manner. NK-252 displays this effect with higher potency than OPZ based	

on the fact that the EC2 value (concentration for a 2-fold induction above background), calculated with linear extrapolation from the values above and below the induction threshold, is 20.8 μ M for OPZ and 1.36 μ M for NK-252. NK-252 has potential as an Nrf2 activator in hepatic cells. Prototypical Nrf2 activators that include OPZ have been reported to protect microglial cells from H₂O₂-induced cytotoxicity. The protective effects of OPZ and NK-252 are examined against H₂O₂-induced cytotoxicity using Huh-7 cells to evaluate their antioxidant properties. The cells treated with OPZ or NK-252 show increased resistance to H₂O₂-induced cytotoxicity compared with control cells^[1].

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

In Vivo

Rats on a choline-deficient L-amino acid–defined (CDAA) diet given OPZ or NK-252 display decreased fibrosis scores compared with CDAA control rats, with median scores of 3, corresponding to bridging fibrosis. CDAA control rats display approximately 20-fold augmentation of the liver fibrosis area compared with rats fed a normal control diet (naive) (14.7 and 0.72%, respectively). This augmentation is also drastically reduced by administration of OPZ or NK-252 (5.80% for OPZ, 6.20% for NK-252_low, and 4.97% for NK-252_high). The effects of NK-252 on both fibrosis score and fibrosis area are dose-dependent^[1]. NK-252 alone has no antitumour effect in P388/S- and P388/VCR-mice. The combination therapy of Etoposide with NK-252 administered p.o. significantly increases the life-span of mice inoculated i.p. with P388/S compared with the corresponding therapeutic effects with Etoposide alone. The combination therapy with Etoposide and NK-252 significantly increases the life-span of mice inoculated i.p. with P388/VCR compared with the corresponding survival time with Etoposide alone^[2].

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PROTOCOL

Cell Assay [1]

The Huh-7.5 cells, a subline derived from Huh-7 cells, are transfected with ARE/pGL4.32 by lipofectamine LTX. The stable clonal transfectant is isolated by selection in hygromycin B (0.1 mg/mL). Cells derived from stable clones are transfected with control or Nrf2 small interfering RNA by lipofectamine RNAiMAX (30 hours), then treated with OPZ, NK-252 (0.1-30 μ M, 16 hours) , or DMSO alone (control). The luciferase activity values are measured using the Steady-Glo Luciferase Assay System^[1].

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Animal Administration [1][2]

Rats^[1]

Six-week-old male Fischer 344 rats are randomly divided into four compound administration groups and four control groups. Compound administration groups of rats fed a CDAA diet receive oral administration as follows: 1) OPZ from 1 week after feeding at a dose of 60 mg/kg once daily for 9 weeks (CDAA+OPZ group; N=8), 2) NK-252 from 1 week after feeding at a dose of 20 mg/kg once daily for 9 weeks (CDAA+NK-252_low group; N=8), 3) NK-252 from 1 week after feeding at a dose of 60 mg/kg once daily for 9 weeks (CDAA+NK-252_high group; N=8), or 4) NK-252 from 6 weeks after feeding at a dose of 60 mg/kg once daily for 4 weeks (CDAA+NK-252_delayed administration: DA group; N=7). Two control groups of rats are fed a CDAA diet for 6 or 10 weeks (pre-CDAA control or CDAA control group; N=9 each), and the other two control groups of rats are fed standard rodent chow (CRF-1) for 6 or 10 weeks (prenaive or naive; N=3 each). Laparotomy and blood sampling are performed under isoflurane anesthesia. After blood sampling, rats are euthanized by exsanguination under isoflurane anesthesia, and the livers are immediately extirpated. [2] Mice[2]

Six- to 8-week-old male BALB/c x DBA/2 F_1 (hereafter called CD2 F_1) mice weighing 22 to 26 g are used. Male CD2 F_1 mice are inoculated i.p. with 10^6 cells of P388/S and P388/VCR cell line on day 0. Each group consist of six mice. NK-250 and NK-252 (100, 300, and 1000 mg/kg) are given p.o. daily from day 1 to 5. Mean survival days and the range of survival days are analysed.

 $\label{eq:mce} \mbox{MCE has not independently confirmed the accuracy of these methods. They are for reference only.}$

CUSTOMER VALIDATION

• Cell Metab. 2023 Oct 3;35(10):1688-1703.e10.

- Cell Death Dis. 2020 Jul 30;11(7):607.
- Phytomedicine. 2024 Feb 1, 155401.
- iScience. 2020 Apr.
- Aging. 2020 Nov 7;12(21):21161-21185.

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REFERENCES

[1]. Shimozono R et al. Nrf2 activators attenuate the progression of nonalcoholic steatohepatitis-related fibrosis in a dietary rat model. Mol Pharmacol. 2013 Jul, 84(1):62-70

[2]. Kiue A, et al. Enhancement of antitumour activity of etoposide by dihydropyridines on drug-sensitive and drug-resistant leukaemia in mice. Br J Cancer. 1991 Aug;64(2):221-6.

Caution: Product has not been fully validated for medical applications. For research use only.

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