Supporting Information to

Synthesis and biological evaluation of NMDI-14 derivatives as anti-mesothelioma agents

Hong Nhung Nguyen,[1] Koya Suzuki,[2] Yasuaki Kimura,[1] Takatsugu Hirokawa,[3,4]
Yuko Murakami-Tonami,[2] and Hiroshi Abe[1,5]

[1]*Graduate School of Science, Nagoya University Furo-cho, Chikusa-Ku, Nagoya, Aichi 464-8602 (Japan)*

[2] *Juntendo University, Graduate School of Medicine 2-1-1, Hongo, Bunkyo-ku, Tokyo, 113-8421 (Japan)*

[3]*Molecular Profiling Research Center for Drug Discovery, AIST 2-4-7 Aomi, Koto-ku, Tokyo, 135-0064 (Japan)*

[4]*Department of Chemical Biology, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba Ibaraki, 305-8575 (Japan)*

[5]*CREST Japan Science and Technology Agency, Tokyo 102-0076 (Japan)*

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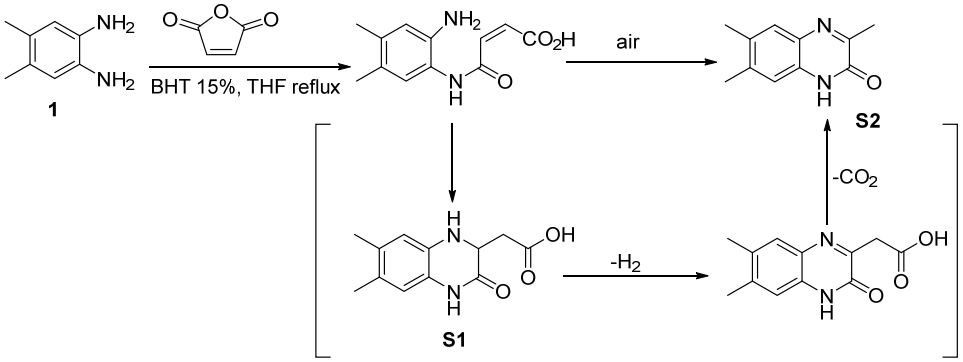

Scheme S1 Reaction of 4,5-dimethylphenyl-1,2-diamine **1** with maleic anhydride afforded undesired decarboxylated **S2** instead of the target **S1**.

Docking Simulation of NMDI14

Our approach to docking NMDI14 molecule to the potential binding sites of SMG7 utilized four main steps: (1) structure preparation of SMG7 for docking, (2) binding site prediction, (3) docking of NMDI14 molecule, and (4) post-docking processing using clustering, re-scoring according to the calculated binding free energy. The human SMG7 structure was obtained from Protein Data Bank (chain A of 1YA0) [1] and refined for docking simulations using the Protein Preparation Wizard[2] Script within Maestro. For NMDI14 molecule, ionization and energy minimization were performed by the OPLS3 force field in the LigPrep Script in the Maestro (Schrödinger, LLC, New York, NY, USA). These minimized structures were employed as input structures for docking simulations. Next, putative druggable sites on NMDI14 were detected and represented by small dummy atoms using the grid-based energy calculation[3,4] incorporated in the SiteMap program (Schrödinger, LLC, New York, NY, USA). As a result, we obtained a druggable binding region previously reported by Martin *et al.*[5] Docking simulations were performed using the Glide[6,7] SP docking program (Schrödinger, LLC, New York, NY, USA). Up to 100 docking poses of NMDI14 molecule were generated in a grid box defined by a potential binding site position from previous step. After the docking simulations were completed, 14 representative poses of cluster center from 100 poses on a potential binding site were selected by clustering analysis with average linkage method using Conformer Cluster Script in the Maestro. Finally, ligand binding free energy of the representative poses was calculated using the MM-GBSA (Schrödinger, LLC, New York, NY, USA).

Biological Evaluation

Cell culture and establishment of stable cell lines

Stable LATS1 and LATS2 double knockdown in human mesothelial cell line (HOMC-D4) was established by induction of shRNA, which was encapsulated in lentivirus. Each shRNA targeting human LATS1 (5'-AACATTAGTGTACCTGGACTG-3', 5'-ACTTTGCCGAGGACCCGAA-3') and LATS2 (5'-GTTCGGACCTTATCAGAAA-3') were incorporated into a pLKO.1 puro vector. A non-target (NT) shRNA (SHC002V) was purchased from Merck. Lentiviral particles were generated according to the Merck Lentiviral transduction protocol using 293T. After infection, cells were selected by puromycin treatment. HOMC-D4 cells were grown in RPMI, supplemented with 10% FBS (10% FBS-RPMI).

Drug treatment and cell viability assay

NMDI14 (Calbiochem, Merck), Compound **8b, 8c, 8d, 8e** and **8f** were dissolved in DMSO and diluted by 10% FBS-RPMI. NT cells and LATS1/2 double knockdown cells seeded at a density of 1.5 $\times 10^3$ cells/well in 96 well culture plate. After 24 hr incubation, a series of drugs applied each well. After 72 hr incubation, drug treated cells were harvested for qPCR and were performed cell viability assay, respectively. Cell viability assay was performed using by a Cell Counting Kit-8 (Dojindo Molecular Technologies, Inc.) following the manufacturer's instructions. The absorbance was measured using spectrometer at 450- 630 nm and obtained value was normalized to no drug treated one.

Quantitative Real-Time PCR

The total RNA was isolated using ISOSPIN (NIPPON GENE), and cDNA was synthesized using ReverTraAce (TOYOBO) according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was performed using KAPA SYBR FAST qPCR Kit (Merck), and 7900HT Fast Real-Time PCR System (Applied Biosystems). The oligonucleotide sequences targeting human GAS5 (forward: GCACCTTATGGACAGTTG, reverse: GGAGCAGAACCATTAAGC), human GAPDH (forward: ATCATCCCTGCCTCTACTGG, reverse: CCCTCCGACGCCTGCTTCAC) for qRT-PCR were synthesized by Eurofins genomics.

References

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