Electronic Supporting Information

A Natural Product-Like Inhibitor of NEDD8-Activating Enzyme

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Experimental section

Materials and cell lines. The natural product and natural product-like compound collection, which includes compound **1** and the other 11 tested compounds, was obtained from AnalytiCon Discovery (Potsdam, Germany). The average purity of the tested compounds was >88%. This database is publicly available and can be accessed free of charge. NEDD8 Conjugation Initiation Kit and anti-Ubc12 rabbit polyclonal antibody was obtained from Boston BioChem (Cambridge, MA, USA). Caco-2 cells were provided by Professor Y.C. Cheng (Department of Pharmacology, Yale University School of Medicine). Cells were cultured in Minimum Essential Media containing 10% fetal bovine serum and were incubated at 37 °C/5% CO_2.

NAE activity assay. This assay was performed using the NEDD8 Conjugation Initiation Kit according to the instructions of the manufacturer (Boston BioChem). Briefly, NAE was incubated with Ubc12, NEDD8 and the indicated concentrations of **1** in HEPES reaction buffer for 10 min. The reaction was initiated by the addition of Mg^{2+} and ATP, and the mixture was incubated at room temperature for 60 min. The reaction was quenched by the addition of EDTA, and was electrophoresed under non-reducing conditions on a 12% SDS-PAGE gel. Ubc12 levels were determined by western blot analysis.

Cellular activity. Caco-2 cells were exposed to the indicated concentrations of **1** or DMSO overnight. Cells were washed three times with ice-cold PBS, resuspended in RIPA lysis buffer, and incubated on ice for 30 min. Cell debris was removed by centrifugation at 14,000 rpm for 30 min at 4 °C, and the protein concentration of the supernatant was determined with Bio-Rad protein assay dye reagent (Bio-Rad). Equal protein amounts were electrophoresed under non-reducing conditions on a 12% SDS-PAGE and subjected to western blot analysis.

Western blot analysis. Protein samples were transferred to a PVDF membrane. The membrane was blocked with 5% milk for 1 h at room temperature, and probed with anti-Ubc12 or anti-β-actin antibody in 5% milk overnight at 4 °C. The membrane was washed with PBS/0.05% Tween 20 (PBST) and incubated with horseradish peroxide-conjugated secondary antibody in 5% milk for 1.5 h at room temperature. Protein bands were detected using enhanced chemiluminescence as specified by the manufacturer (ECL, Amersham).

Kinase assay. Kinase screening assay was performed by Caliper Life Sciences Inc.

using the Caliper LabChip technology and a 12-sipper chip. Compound **1** was tested at 20 μM against 11 kinases in duplicate.

Molecular modeling. A natural product or natural product-like chemical library containing over 20,000 compounds was screened *in silico*. Molecular docking was performed by using the ICM-Pro 3.6-1d program (Molsoft).[1] According to the ICM method, the molecular system was described by using internal coordinates as variables. Energy calculations were based on the ECEPP/3 force field with a distance-dependent dielectric constant. The biased probability Monte Carlo (BPMC) minimization procedure was used for global energy optimization. The BPMC global-energy-optimization method consists of 1) a random conformation change of the free variables according to a predefined continuous probability distribution; 2) local-energy minimization of analytical differentiable terms; 3) calculation of the complete energy including nondifferentiable terms such as entropy and solvation energy; 4) acceptance or rejection of the total energy based on the Metropolis criterion and return to step (1). The binding between the small molecules and NAE-NEDD8 were evaluated with a full-atom ICM ligand binding score from a multireceptor screening benchmark as a compromise between approximated Gibbs free energy of binding and numerical errors. The score was calculated by:

$$S_{\text{bind}} = E_{\text{int}} + T\Delta S_{\text{Tor}} + E_{\text{vw}} + \alpha_1 E_{\text{el}} + \alpha_2 E_{\text{hb}} + \alpha_3 E_{\text{hp}} + \alpha_4 E_{\text{sf}}$$

where E_{vw}, E_{el}, E_{hb}, E_{hp}, and E_{sf} are van der Waals, electrostatic, hydrogen bonding, and nonpolar and polar atom solvation energy differences between bound and unbound states, respectively. E_{int} is the ligand internal strain, ΔS_{Tor} is its conformational entropy loss upon binding, and T = 300 K, and α_i are ligand- and receptor independent constants. The initial model of NAE was built from the X-ray crystal structure of the quaternary APPBP1-UBA3-NEDD8-ATP complex (PDB: 1R4N),[1] according to a previously reported procedure.[2] Hydrogen and missing heavy atoms were added to the receptor structure followed by local minimization by using the conjugate gradient algorithm and analytical derivatives in the internal coordinates. In the docking analysis, the binding site was assigned across the entire structure of the protein complex. Each compound was assigned the MMFF[3] force field atom types and charges and was then subjected to Cartesian minimization. The ICM docking was performed to find the most favorable orientation. The resulting trajectories of the complex between the small molecules and protein complex were energy minimized, and the interaction energies were computed. Each compound was docked three times and the minimum of the three scores was used. The 12 highest scoring compounds were utilized for biological testing without further selection.

References

1 M. Totrov, R. Abagyan, *Proteins Suppl*. **1997**, *29*, 215.
2 H. Walden, M. S. Podgorski, D. T. Huang, D. W. Miller, R. J. Howard, D. L. Minor Jr., J. M. Holton, B. A. Schulman, *Mol. Cell.* **2003**, *12*, 1427–1437.
3 Halgren, T. Merck molecular force field I-V. J. Comput. Chem. 1995, 17, 490–641.

Table S1. Percentage inhibition values of kinase activity by compound **1** (20 μM).

Kinase	**% Inhibition**
AKT1	16.5
EGFR	2
IKKb	–2
JAK2	5
JAK3	9.5
JNK2	5.5
MAPK1	0
MAPK14	–0.5
MAPK3	5.5
PI3Ka	59
PKCa	–85.5