Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Target-Based Whole-Cell Screening by ^{1}H NMR Spectroscopy**

Junhe Ma, Qing Cao, Sarah M. McLeod, Keith Ferguson, Ning Gao, Alexander L. Breeze, and Jun Hu**

anie_201410701_sm_miscellaneous_information.pdf

CONTENTS:

1. Supporting figure and table

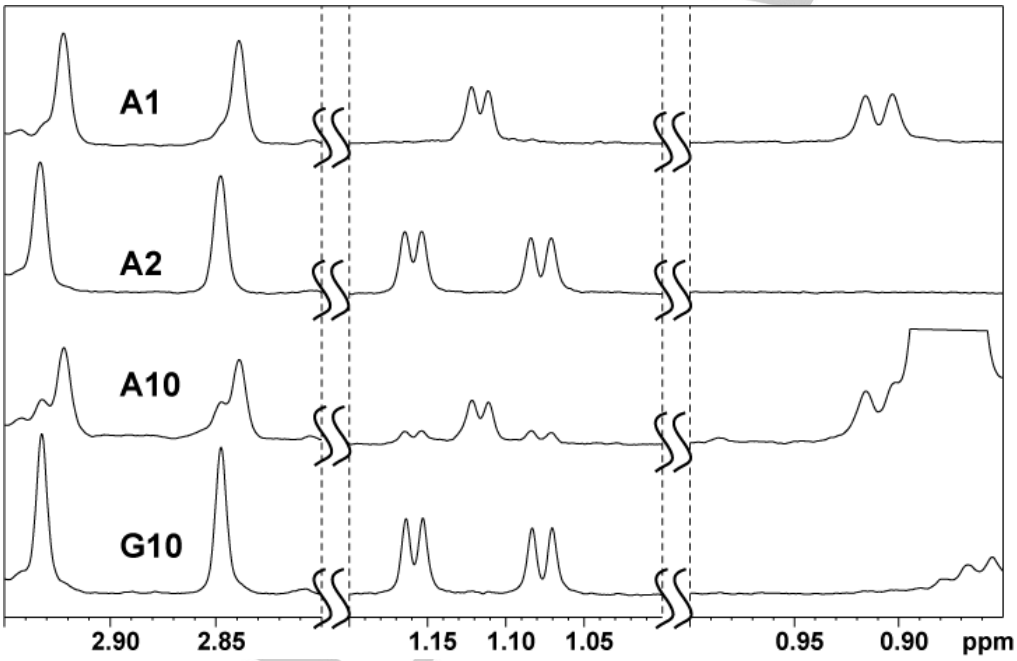
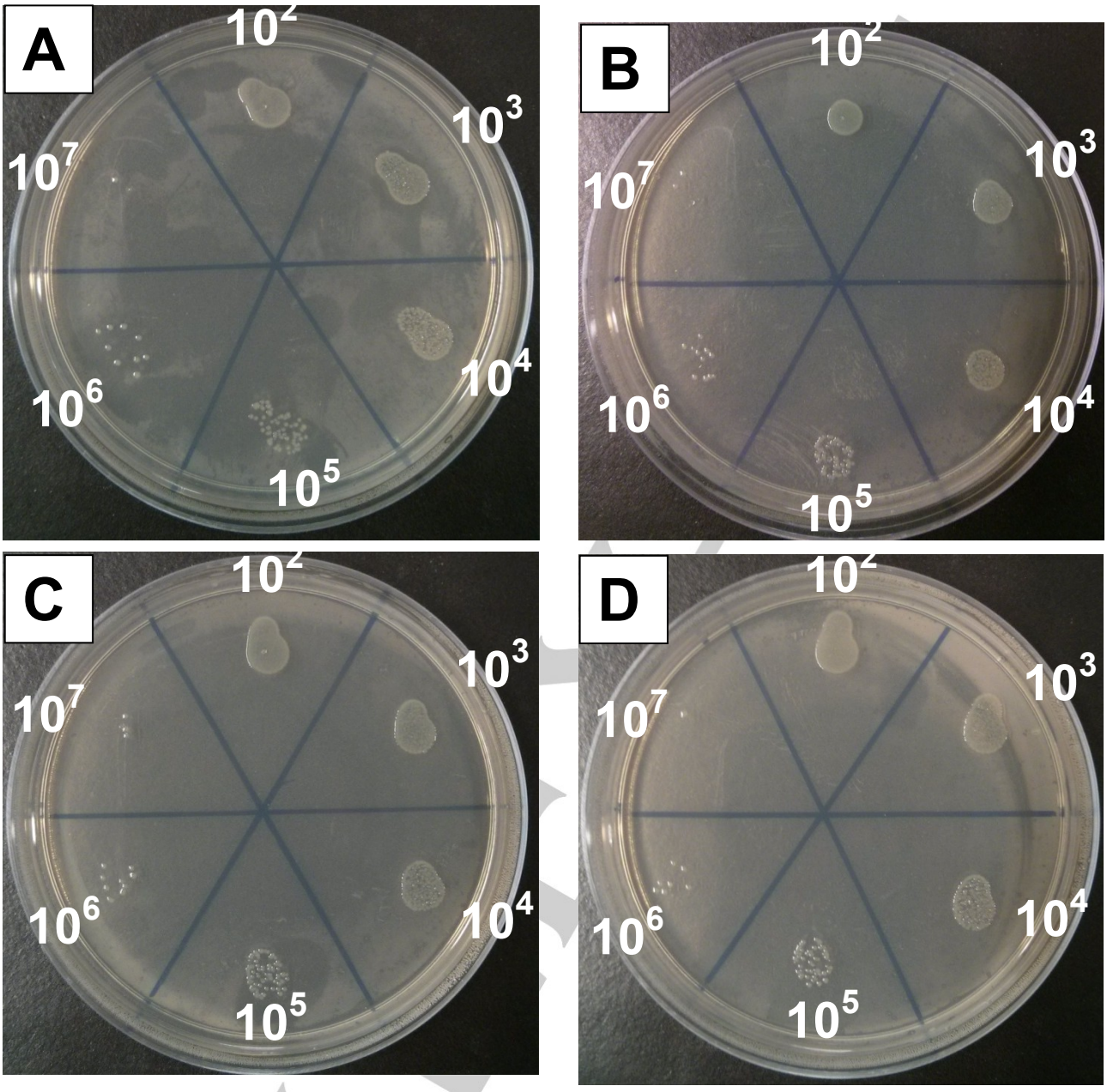


Figure S1. NMR signals of wells A1, A2 A10 and G10, showing the nitrogen attached methyl proton signals (2.95~2.80 ppm) and carbon attached methyl proton signals (1.20 ~ 1.00 ppm and 0.96~0.85 ppm).

[a] Dr. J. Ma, Q.Cao, N. Gao, Dr. J. Hu
Discovery Sciences, AstraZeneca Boston
[b] Dr. S. McLeod, K. Ferguson
Infection Innovative Medicines, AstraZeneca Boston
Waltham, Massachusetts 02451(USA)
E-mail: jun.hu2@astrazeneca.com
[c, d] Prof. A. L. Breeze
Astbury Centre for Structural Molecular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, LS2 9JT, United Kingdom
A.L.Breeze@leeds.ac.uk


A
10^2
10^3
10^7
10^4
10^6
10^5
B
10^2
10^3
10^7
10^6
10^4
10^5
C
10^2
10^3
10^7
10^6
10^4
10^5
D
10^2
10^3
10^7
10^6
10^4
10^5

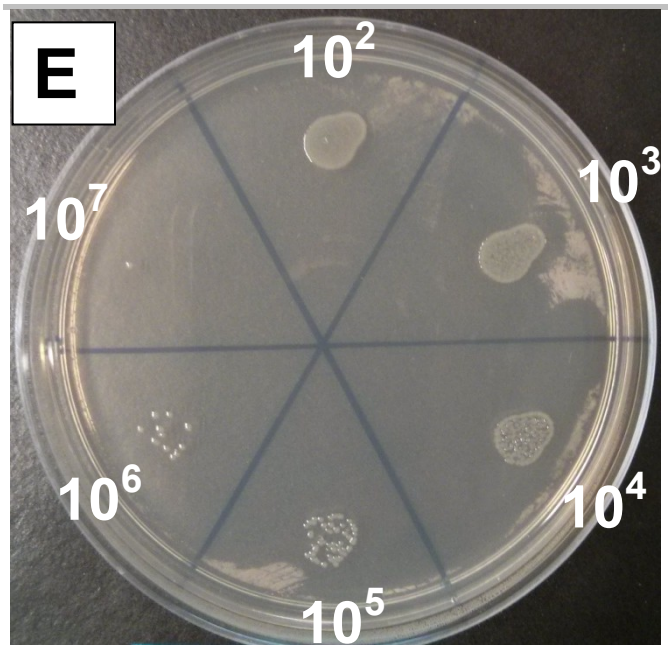


Figure S1. Plating colony tests to examine NDM-1 *E. coli* cell viability after treatment with screening compounds. All NDM-1 *E. coli* cells in these tests were from the same batch. The fold of dilution is labeled on each section of the LB/kanamycin plates. NDM-1 *E. coli* cells treated with DMSO were plated on plate A as reference. Cells with the addition of compounds from screening wells A5, B6 and C10 were plated on plate B, C and D, respectively. Cells with the addition of 5 different compounds (100 μM each) were plated on plate E. The section labeled with 10^6 fold dilution is used for the study of cell viability. There are 9, 11, 9, 8 and 11 single colonies on plate A, B C, D and E, respectively. The data demonstrate that cells were still alive after treatment with 0.5 mM screening compound or a combination of 5 compounds. The effects on cell viability after treatment with compounds are minimal.

Table S1. Percentage of inhibition of screening compounds calculated based on the hydrolyzed meropenem signal intensity (same order as in the 96-well plate in Figure 3)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	99.9	2.0	9.3	73.0	1.2	23.0	63.2	64.4	-0.6	-0.8	99.9
B	25.1	86.9	7.1	8.7	7.0	93.0	90.2	11.6	-1.5	6.4	33.4	62.4
C	12.4	-2.0	9.0	12.1	12.9	6.1	6.2	39.3	11.3	31.1	37.0	58.3
D	99.9	-1.9	3.4	60.2	99.8	9.0	16.0	11.8	10.6	3.6	85.5	27.7
E	-1.0	99.9	5.2	4.8	1.3	2.0	81.0	0.8	1.2	-1.6	-1.7	4.0
F	-1.6	-4.1	-3.3	-1.1	-3.6	-0.9	-1.1	27.9	-0.8	-1.5	-0.4	-0.7
G	0.1	-3.1	-3.5	0.5	99.7	99.3	94.9	-1.3	80.2	98.9	90.3	95.2
H	-1.3	-0.6	99.2	2.9	-1.1	-2.1	-1.5	0.1	53.7	-0.8	99.5	-0.2

2. Materials and methods

2-1. Reagents

Meropenem was purchased from Sigma-Aldrich Corporation. All inhibitors were either purchased from Sigma-Aldrich Corporation or from AstraZeneca in-house compound libraries. The compounds were dissolved in the deuterated DMSO as 100 mM stock solution.

The NDM-1 *Escherichia coli* cell strain was obtained following the previous protocol.[1]

2-2. Percentage of inhibition

Percentage of inhibition is calculated based on the intensity of product signal in the presence of compounds. The equation for the calculation is defined as following: [2]

Percentage inhibition = $(1- (I_0 – I_{EDTA}) / (I_{DMSO} - I_{EDTA})) \times 100\%$

where, I_0 is the intensity of the product signal in each well. I_{EDTA} is the intensity in the presence of EDTA. I_{DMSO} is the intensity in the presence of DMSO.

2-3. 96-well screening plate preparation

The 96-well plate for screening was prepared as following.
Step 1: 2.5 µl of 100 mM compounds, plus two d6-DMSO and EDTA controls, were deposited in each well of a 96-well plate.
Step 2: 275 µl fresh living NDM-1 *Escherichia coli* cells (OD_{600} = 0.25) in phosphate buffer (50 mM, pH 7.2) were added into the compound solution by multi-pipette and incubated at room temperature for at least half an hour.
Step 3: 10 µl of 5 mM meropenem was added into the mixture to allow the reaction to occur. In the mean time a NMR sample with the same condition of well A1 was prepared and put into the magnet to monitor the reaction.
Step 4: Once the reaction was half completed, 10 µl of 0.5 M EDTA was added to quench the reaction. After 30 minutes, the 96-well plate was transferred into a Bruker automatic Tecan/SampleRail system and each sample was mixed with 200 µl additional phoshpate buffer (50 mM, pH 7.2) before NMR measurement.

2-4. ^{1}H NMR measurements and analyses

All spectra were acquired on a Bruker 600MHz spectrometer with a TXI probe equipped with cryogenically cooled transmit/receive coils and a pulsed-field z-gradient coil. For 1D ^{1}H NMR experiments, a sweep width of 9615.385 Hz was used. Water suppression was achieved by means of the excitation sculpting scheme and the water-selective 180° Sinc shaped pulse was 3 ms long.[3] The free induction decay was collected in 32K data points. A 1 Hz line broadening function was applied during the Fourier transformation. All the spectra were acquired with a fixed number of scans of 64. The temperature was kept constant at 25 °C if not noted otherwise. NMR spectra were processed and analyzed with Topspin 3.2.

References:

(1) J. Ma; S. McLeod, K. MacCormack, S. Sriram, N. Gao, A. L. Breeze, J. Hu, *Angew. Chem. Int. Ed. Engl.* **2014**, *53,* 2130-2133.

(2) M. E. Kovach, P. H. Elzer, D. S. Hill, G. T. Robertson, M. A. Farris, R. M. 2nd Roop, K. M. Peterson, *Gene* **1995**, *166*, 175-176.

(3) K. S. Scott, J. Keeler, T. L. Hwang, A. J. Shaka, *J. Am. Chem. Soc.* **1995**, *117*, 4199-4200.