Folded Conformation, Cyclic Pentamer, Nano-Structure and PAD4 Binding Mode of YW3-56

Haimei Zhu,[†] *Yuji Wang,*[†] *Yaonan Wang,*[†] *Shurui Zhao,*[†] *Ming Zhao,**[†] *Lin Gui,*[†] *Wenyun Xu,*[†] *Xiangyun Amy Chen,*[‡] *Yanming Wang,**[‡] *and Shiqi Peng,**[†]

[†]College of Pharmaceutical Sciences, Capital Medical University, Beijing 100069, P.R. China.

[‡]Center for Eukaryotic Gene Regulation, Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802, USA.

Supporting Information

1. Synthetic route

To conveniently obtain YW3-56 (structure see Figure 1) the 4-step route used in the literature was followed (Wang, Y.; Li, P.; Wang, S.; Hu, J.; Chen, X. A.; Wu, J.; Fisher, M.; Oshaben, K.; Zhao, N.; Gu, Y.; Wang, D.; Chen, G.; Wang, Y. Anticancer PAD inhibitors regulate the autophagy flux and the mammalian target of rapamycin complex 1 activity. *J. Biol. Chem.* 2012, 17, 25941-25953), by changing the coupling reagent from HATU and DIPEA of step i to DCC and NMM, changing the reaction solvent from DMF of step i to THF, changing the coupling reagent from HATU and DIPEA of step ii to DCC and NMM, and changing the reaction solvent from DMF of step ii to a mixed solvent of THF and DMF (1:1). These changes benefitted the procedure, improved the purity and did not decrease the yield of YW3-56.

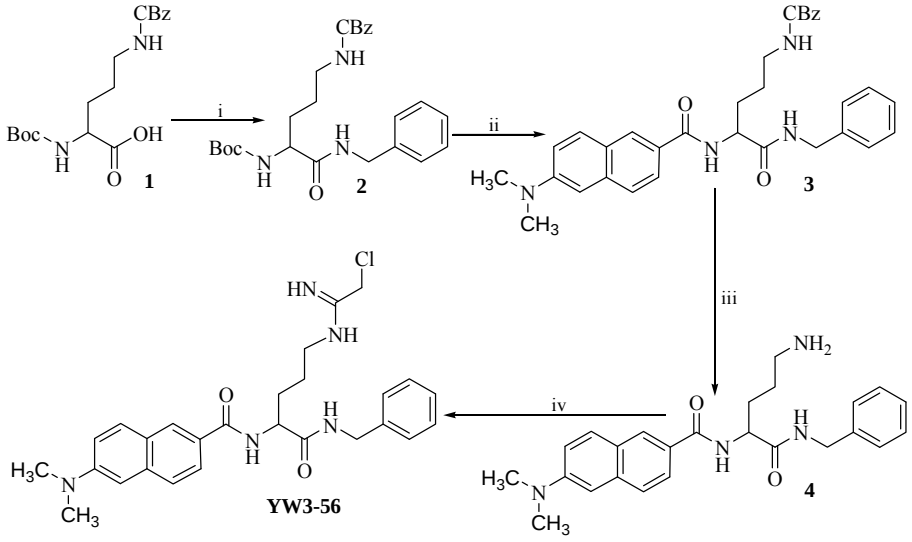
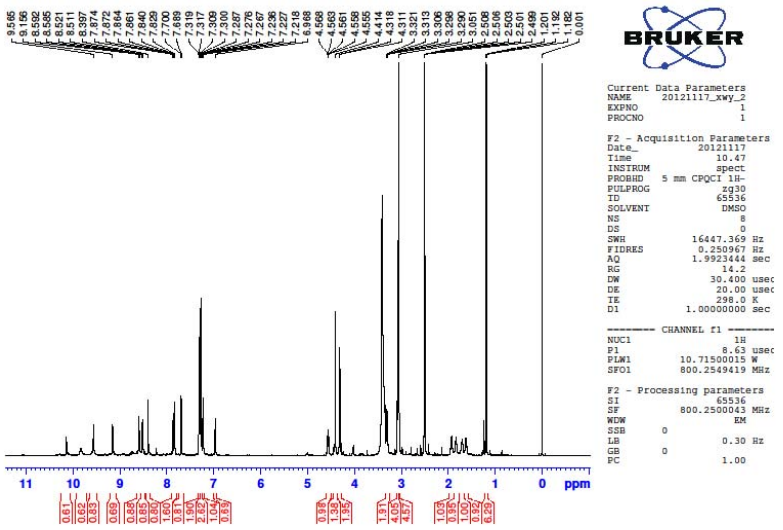


Figure 1. Synthetic route of N-*S*-1-[benzylcarbamoyl-4-(2-chloroacetamidobutyl)]-6-dimethylaminonaphthalene-2-carboxamide (YW3-56). I) Benzyl amine, DCC, NMM and THF; ii) Hydrogen chloride in ethyl acetate HCl/ETOAC; 6-(dimethylamino)-2-naphthoic acid, DCC, NMM, THF/DMF(1:1); iii) H_2, Pd/C (5%) and MeOH; iv) $EtOC(=NH)CH_2Cl$, TEA and MeOH.

2. ^{1}H NMR spectrum measurements

One-dimensional ^{1}H NMR spectra were recorded on a Bruker 800 MHz spectrometer, ~5 mg of YW3-56 in 0.5 mL of deuteron dimethyl sulfoxide (DMSO-d6). The probe temperature was regulated to 298 K. The spectra were recorded using a simple pulse-acquire sequence zg30. Typical acquisition parameters consisted of 64 K points covering a sweep width of 16447 Hz, a pulse width (pw90) of 8.63 μs and a total repetition time of 24 s to ensure full relaxation of the ^{1}H resonances. Digital zero filling to 64 K and a 0.3 Hz exponential function were applied to the FID before Fourier transformation. The resonance at 2.5 ppm due to residual solvents, present as impurities (CD_2HSOCD_2H), tetramethylsilane (TMS) was used as internal reference (Figure 2). Standard absorptive two-dimensional ^{1}H-^{1}H

chemical shift correlation spectra (COSY) were acquired with the same spectrometer (Figure 3). Each spectra consisted of a matrix of 2 K (F2) by 0.5 K (F1) covering a sweep width of 9615.4 Hz. Before Fourier trans- formation, the matrix was zero filled to 1 K by 1 K and standard sinebell apodization functions were applied in both dimensions. Two-dimensional ROESY experiments were acquired in the phase-sensitive mode using the same spectrometer (Figure 4). Each spectra consisted of a matrix of 2 K (F2) by 1 K (F1) covering a sweep width of 9615.4 Hz. Spectra were obtained using spin-lock mixing periods of 200 ms. Before Fourier transformation, the matrix was zero filled to 1 K by 1 K and qsine apodization functions were applied in both dimensions.



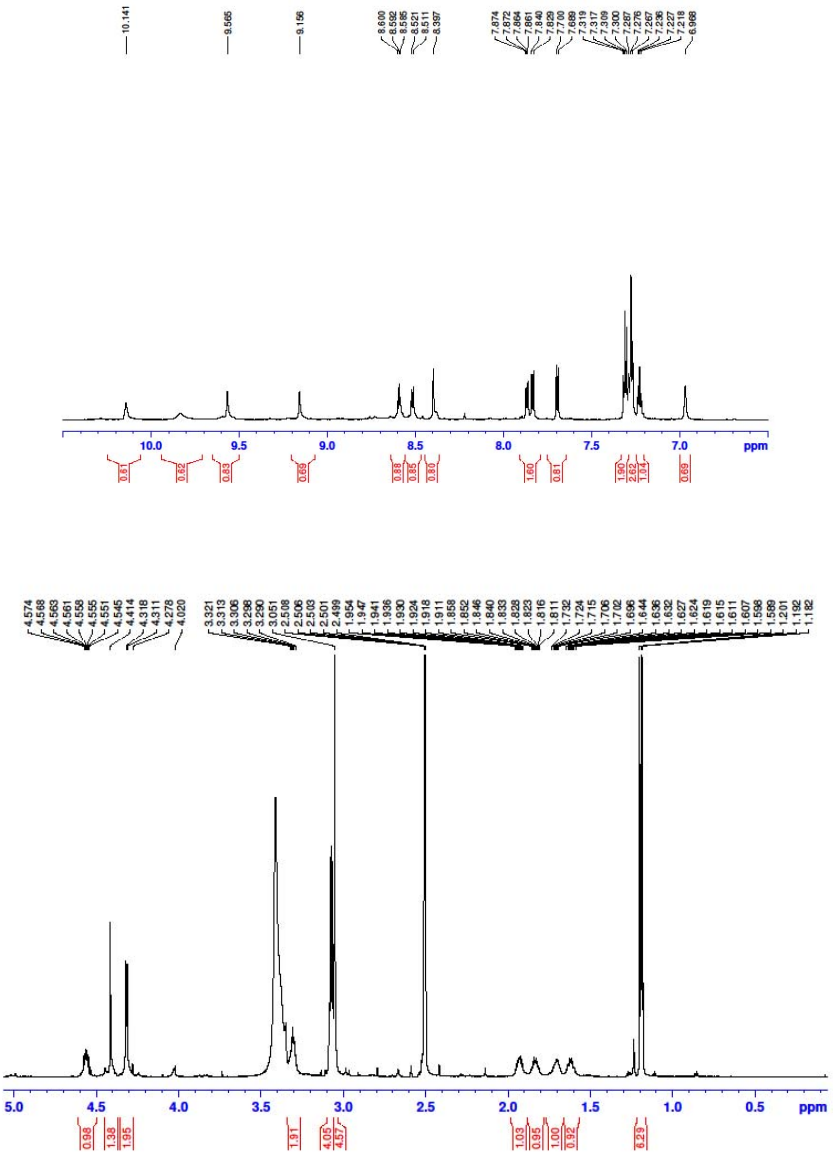
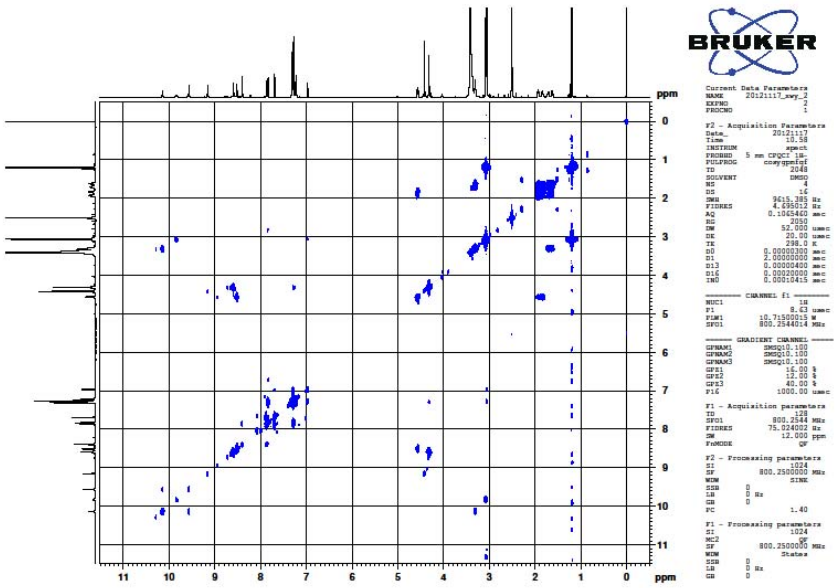
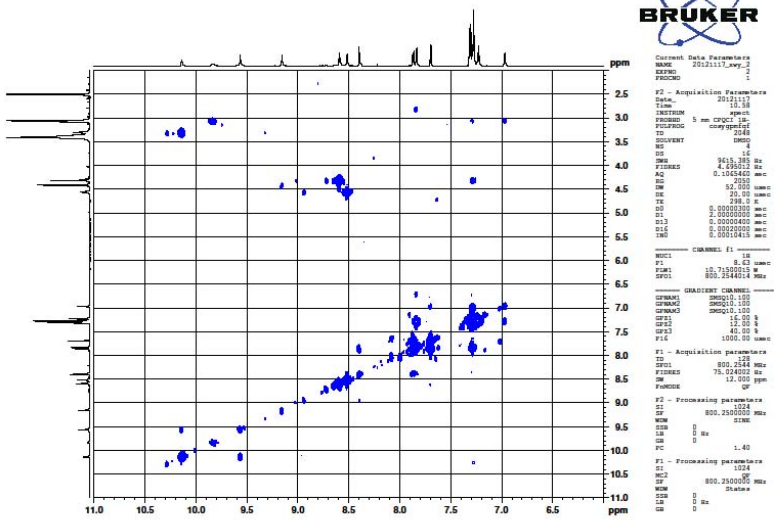


Figure 2. [1]HNMR spectrum of YW3-56 at 800 MHz in deuteron DMSO.


BRUKER
ppm
11 10 9 8 7 6 5 4 3 2 1 0 ppm
Current Data Parameters
NAME 20121117_xwy_2
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20121117
Time 10.58
INSTRUM spect
PROBHD 5 mm CPQCI 1H-
PULPROG cosygpmfqf
TD 2048
SOLVENT DMSO
NS 4
DS 16
SWH 9615.385 Hz
FIDRES 4.695012 Hz
AQ 0.1065460 sec
RG 2050
DW 52.000 usec
DE 20.00 usec
TE 298.0 K
D0 0.00000300 sec
D1 2.00000000 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00010415 sec
CHANNEL f1
NUC1 1H
P1 8.63 usec
PLW1 10.71500015 W
SFO1 800.2544014 MHz
GRADIENT CHANNEL
GPNAM1 SMSQ10.100
GPNAM2 SMSQ10.100
GPNAM3 SMSQ10.100
GPZ1 16.00 %
GPZ2 12.00 %
GPZ3 40.00 %
P16 1000.00 usec
F1 - Acquisition parameters
TD 128
SFO1 800.2544 MHz
FIDRES 75.024002 Hz
SW 12.000 ppm
FnMODE QF
F2 - Processing parameters
SI 1024
SF 800.2500000 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0
PC 1.40
F1 - Processing parameters
SI 1024
MC2 QF
SF 800.2500000 MHz
WDW States
SSB 0
LB 0 Hz
GB 0


BRUKER
ppm
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 ppm
Current Data Parameters
NAME 20121117_xwy_2
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20121117
Time 10.58
INSTRUM spect
PROBHD 5 mm CPQCI 1H-
PULPROG cosygpmfqf
TD 2048
SOLVENT DMSO
NS 4
DS 16
SWH 9615.385 Hz
FIDRES 4.695012 Hz
AQ 0.1065460 sec
RG 2050
DW 52.000 usec
DE 20.00 usec
TE 298.0 K
D0 0.00000300 sec
D1 2.00000000 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00010415 sec
CHANNEL f1
NUC1 1H
P1 8.63 usec
PLW1 10.71500015 W
SFO1 800.2544014 MHz
GRADIENT CHANNEL
GPNAM1 SMSQ10.100
GPNAM2 SMSQ10.100
GPNAM3 SMSQ10.100
GPZ1 16.00 %
GPZ2 12.00 %
GPZ3 40.00 %
P16 1000.00 usec
F1 - Acquisition parameters
TD 128
SFO1 800.2544 MHz
FIDRES 75.024002 Hz
SW 12.000 ppm
FnMODE QF
F2 - Processing parameters
SI 1024
SF 800.2500000 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0
PC 1.40
F1 - Processing parameters
SI 1024
MC2 QF
SF 800.2500000 MHz
WDW States
SSB 0
LB 0 Hz
GB 0

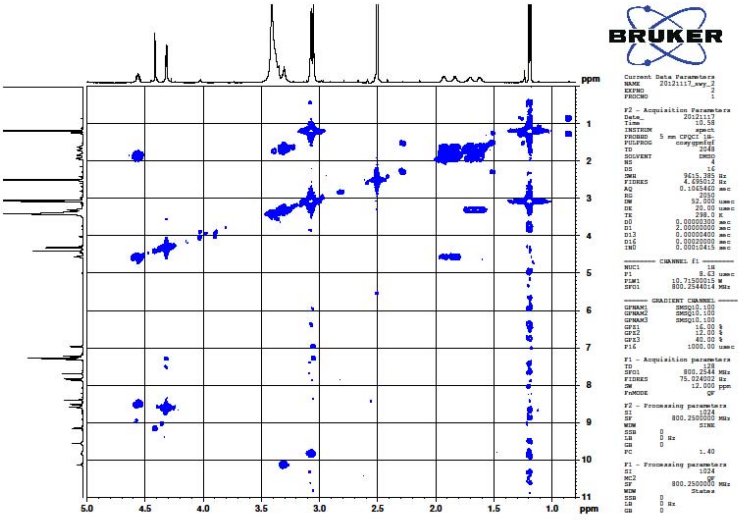
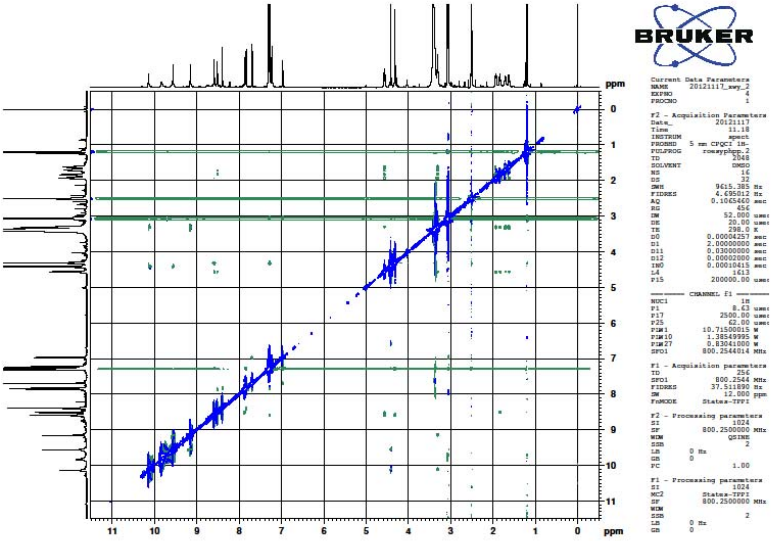


Figure 3. COSY NMR spectrum of YW3-56 at 800 MHz in deuteron DMSO.



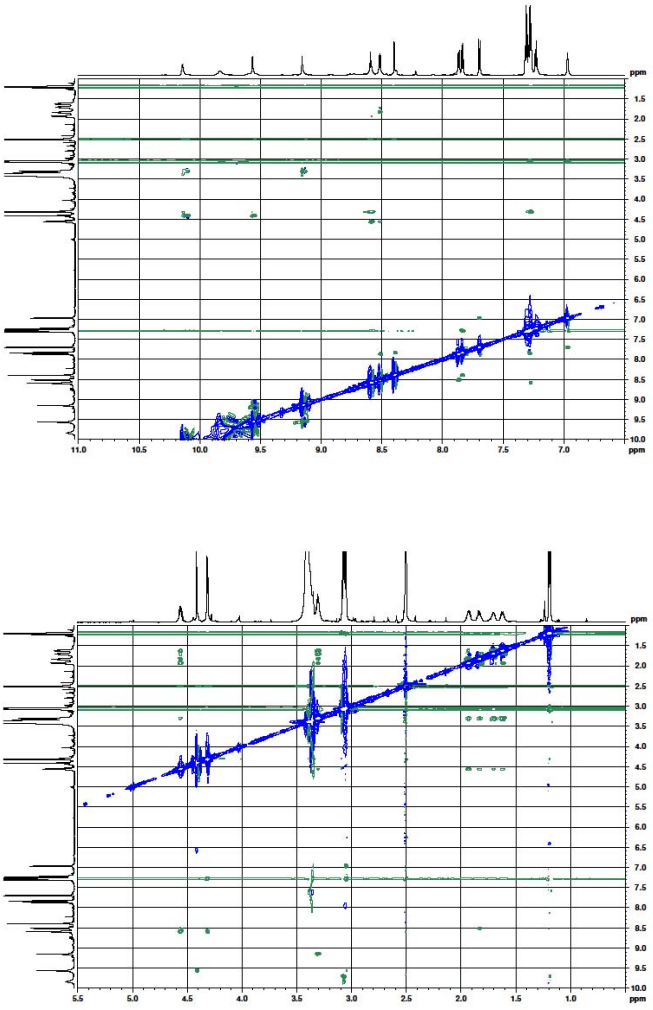


Figure 4. ROESY 2D NMR spectrum of YW3-56 at 800 MHz in deuteron DMSO.

3. Conformation simulation

The computer simulations were carried out in vacuum, water, and DMSO. As seen in Figure 5, the difference between the conformations is negligible.

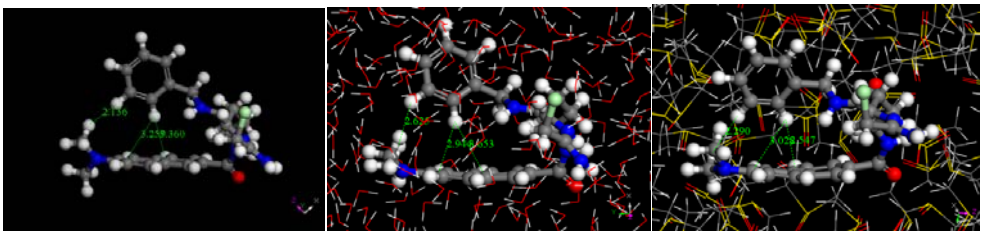


Figure 5. The conformation of YW3-56 sampled in vacuum, water, and DMSO using molecular

dynamics simulations.

4. Another TEM image at 4 ºC kept for one week which covers more large area

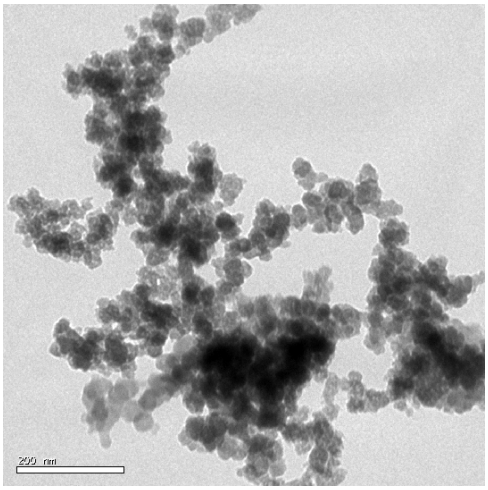


Figure 6. Another TEM image of YW3-56 in ethanol at 4 ºC kept for one week.