SUPPORTING INFORMATION

MANUMYCIN-A IS A POTENT INHIBITOR OF MAMMALIAN THIOREDOXIN REDUCTASE-1 (TRXR-1)

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General: Manumycin A, deoxymanumycin A and dihydromanumycin A were purchased from Adipogen Life Sciences and used without further purification. Man-A and its derivatives were analyzed by HPLC (LiChrospher 100 RP-8, 5 µm column, 4.0 x 250 mm. 50:50 acetonitrile:water, 0.1% TFA, 1 mL/min) with UV detection (Man A 345 nm, dihydro-Man A 345 nm, deoxy-Man A 398 nm). Man A dihydro-Man A and deoxy-Man A were found to be 97%, 92% and 87% pure respectively. Man A and its derivatives were analyzed by HESI MS (Bruker Evo-Q Elite Triple-Quadrupole) operating in positive ion mode and the anticipated *m/z* for M+1 was observed in all cases (551.6, 553.6 and 535.6 for Man A, dihydro-Man A and deoxy-Man A respectively). Cytochrome c was purchased from Lee biochemical and superoxide dismutase was purchased from MP Biomedicals. Enzyme assay kit (fluorescent TrxR/Trx) and rat recombinant TrxR-1 were purchased from Cayman Chemical. All other reagents were purchased from Sigma-Aldrich or Fisher Scientific and used without further purification. The TrxR/Trx assay was performed according to the kit manufacturer's instructions with noted exceptions below. UV/Visible and fluorescence measurements were performed in 384 well microplates using a Synergy® 2 (BioTek Instrument, Inc.) or an Infinite® M1000 PRO (Tecan Group Ltd.) microplate reader. All assays were performed in triplicate. Results are presented as an average of three trials ±standard deviation. Error limits on graphs represent the standard deviation of three trials.

TrxR/Trx inhibition assay. The assay was performed in 384 well black flat bottom plate in a final volume of 100 µL. Rat TrxR-1 (10 µL, 1 µM, 0.2 mg/mL BSA, 50 mM Tris-Cl, 1 mM EDTA pH 7.5) was pre-reduced with NADPH (5 µL, 4mg/mL) in the presence of Man-A (in 25% DMSO) final concentrations of 0, 100, 250,750, and 1000 nM. After 30 minutes, human Trx (10 µL, 1 µM) was added and incubated for an additional 30 minutes. Fluorescent substrate (20 µL, 0.4 mg/ml, eosin labeled bovine insulin) was added to the enzyme/Man-A solution (80 µL) to initiate the reaction.. The fluorescence was monitored at λex/λem= 520nm/545 nm every 5 minutes for 1 hour.

DTNB reduction assay. (Method A) *Pre-incubation with Man-A*. The assay was performed in 384 well black flat bottom plate in a final volume of 100 µL. Rat recombinant TrxR-1 (100 µL, 80 nM) was reduced with NADPH (100 µL, 0.1 mM) in 50 mM Tris-HCl, 1 mM EDTA pH 7.5 for 30 minutes at room temperature. Man-A (or its derivatives in 25% DMSO) was added for final concentrations of 0, 5, 50, 500, 1800, 5000 nM. The mixture was incubated for additional sixty minutes at room temperature. DTNB (20 µL, 10 mM) in 0.1 M sodium phosphate and 1 mM EDTA, pH 8.0 was added to initiate the reaction. The reduction of DTNB was monitored at 412 nm every 5 minutes for 1 hour. (Method B) *Without pre- incubation with Man-A*. The assay was performed as described above except that Man-A and DTNB were added simultaneously after NADPH reduction of TrxR. All result are expressed as percent of control at termination (60 minutes).

Time course inhibition of DTNB reduction by TrxR. The assay was performed in 384 well black flat bottom plate in a final volume of 100 µL. Rat recombinant TrxR-1 (100 µL, 80 nM) was reduced with NADPH (100 µL, 0.1 mM) in 50 mM Tris-HCl, 1 mM EDTA pH 7.5 for 30 minutes at room temperature. Man-A (in 25% DMSO) was added for final concentrations of 1 µM and incubated for 15, 10, 5 and 2 min after which DTNB (20 µL, 10 mM) in 0.1 M sodium phosphate and 1 mM EDTA, pH 8.0 was added to initiate the reaction. The reduction of DTNB was monitored at 412 nm every 5 minutes for 1 hour.

Irreversible inhibition of TrxR by Man-A. In order to determine the reversibility of inhibition by Man-A, TrxR-1 (80 µL, 80 nM) was reduced with 0.1 mM NADPH (50 mM Tris-HCl, 1 mM EDTA pH 7.5) for 30 minutes. The reduced TrxR was divided into three samples viz. control (no Man-A), Man-A and Man-A not subjected to gel filtration. A fourth sample of TrxR (80 nM) was not reduced with NADPH. A solution of Man-A (in 25% DMSO) was added to the TrxR samples (280 µL) for final concentrations of TrxR (5.2 nM) and Man-A (2 µM).The control sample was substituted with equal volume of 25% DMSO instead of Man-A solution. Samples were incubated for 1 hour at ambient temperature, after which they were passed through Micro Bio-spin P-6 gel column of MW limit of 6000 (Bio-Rad). One sample containing Man A was not passed through the gel filtration column. All samples (280 µL) were re-reduced with (20 µL, 0.1 mM) NADPH for 30 minutes. Finally, the DTNB assay was performed as described above by adding 20 µL of 10 mM DTNB to 80 µL of reaction mixture and the absorbance was monitored at 412 nm every 5 minutes for 1 hour.

Reaction of Sel-green probe with Seleno-*L*-cysteine. A stock solution of seleno-*L*-cystine (500 µL, 156 µM) was reduced with 1.5 equivalent of immobilized TCEP (>8 µmol/mL) for 1 hour. After reduction, the immobilized TCEP was removed by centrifugation at 14, 000 X *g*, for 10 minutes. The reduced *L*-selenocystiene (20 µL, 312 µM) was incubated with Man-A, deoxy-Man-A or dihydro-Man-A (10 µL, 625 µM) for 1 hour. Sel-green (20 µL, 100 µM) was added and fluorescence monitored at λex/λem= 370 nm/510 nm every 5 minutes for 1 hour.

NADPH consumption by TrxR in the presence of Man-A on. The assay was performed in 384 well black flat bottom plate in a final volume of 100 µL. Rat recombinant TrxR-1 (10 µL, 2 µM) and Man-A (2.5 µL, 2.5 mM) was added in the final volume of 100 µL in assay

buffer (50 mM Tris-HCl, 1 mM EDTA pH 7.5). NADPH (7.5 µL, 2.68 mM) was added and the absorbance was monitored at 340 nm for 30 minutes.

Superoxide radical anion production by TrxR in the presence of Man A. The NADPH oxidase activity induced by Man-A was determined by monitoring the reduction of cytochrome c. The assay was performed in 384 well black flat bottom plate in a final volume of 100 µL. Cytochrome c (10 µL, 1 µM) NADPH (7.5 µL, 2.68 mM), Man-A (2.5 µL, 2.5 mM/DMSO) or 2.5 µL DMSO were combined. The reaction was initiated by addition of rat TrxR-1 (10 µL, 1 µM). The production of superoxide was confirmed by addition of 6 units SOD/well in parallel samples. SOD is used to quench superoxide generated by TrxR. The reduction of cytochrome C was measured at 550 nm.

Preparation of cell lysate for biochemical assays. The human lymphoblast cell line (GM02152) was purchased from Coriell institute. Cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 15 % bovine serum (FBS) and 2 µM sodium selenite and were maintained in a humidified incubator at 37°C and 5% CO_2 until they reach log phase.
For TrxR/Trx assay GM02152 cells were seeded in 75 cm^2 flasks containing growth medium. The cells were harvested by centrifugation (720 X *g*, 4 minutes) when log phase was reached. The cells were lysed by addition of 100 µL lysis buffer (10 mM Tris-HCl, 200 mM KCl, 2 mM EDTA, 40% glycerol, 0.2% TritonX-100, pH 7.5) by rotation at 4°C for 2 hours. Cellular debris was removed by centrifugation at 17000 X *g* at 4°C for 30 minutes. Protein concentration of the lysate was determined by the Bradford method using Coomassie protein assay reagent in triplicate. Typical concentration of the lysate ranged from 10-15 mg/ml.

TrxR/Trx inhibition assay with cell lysate. The assay was performed in 384 well black flat bottom plate in a final volume of. 100 µL. Cell lysate (15.6 µg in lysis buffer) was reduced with NADPH (5 µL, 4 mg/ml). Man-A (in 25% DMSO) was added to a final concentration of 1 µM. After 30 minutes, human Trx (10 µL, 1 µM) was added and incubated for an additional 30 minutes. Fluorescent substrate (20 µL, 0.4 mg/ml, eosin labeled bovine insulin) was added to the enzyme/Man-A solution (80 µL) to initiate the reaction.. The fluorescence was monitored at λex/λem= 520nm/545 nm every 5 minutes for 1 hour.

LIST OF ABBREVIATION

Man A	Manumycin A
Deoxy-Man A	Deoxymanumycin A
Dihyro-Man A	Dihydromanumycin A
FTase	Farnesyl transferase
TrxR1	Mammalian cytosolic thioredoxin reductase 1
NADPH	Nicotinamide adenine dinucleotide phosphate
SecTRAP	Selenium compromised thioredoxin reductase apoptotic proteins
Trx	Thioredoxin
FAD	Flavin adenine dinucleotide
ROS	Reactive oxygen species
DTNB	5,5'-dithio-bis-(2-nitrobenzoic acid)
amu	atomic mass unit
SOD	Superoxide dismutase
TCEP	Tris(2-carboxyethyl)phosphine
Tris	*tris*(hydroxymethyl)aminomethane
MW	Molecular weight
BSA	Bovine serum albumin

DMSO	Dimethyl sulfoxide
GM02152 cells	Human lymphoblast cell line