APPENDIX

Appendix A

List of chemicals and reagents used in this study

Name of Chemicals	Company
Absolute ethanol	E.Merck, Germany
Acetic acid	E.Merck, Germany
Acrylamide	Sigma-Aldrich, USA
Acrylamide (Eastman)	Sigma-Aldrich, USA
Agarose	GIBCO, USA
Amido black B	Sigma-Aldrich, USA
Ammonium persulfate	Sigma-Aldrich, USA
Bis (Estaman)	Sigma-Aldrich, USA
Bovine serum albumin	PIERCE, USA
Bromphenol blue	Sigma-Aldrich, USA
Coomassie brillant blue R-250	Sigma-Aldrich, USA
Coomasie® Plus Protein Assay Reagent	PIERCE, USA
Copper sulfate	E.Merck, Germany
Dibasic sodium phosphate	Sigma-Aldrich, USA
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich, USA
Dulbecco's modified eagle medium	GIBCO, USA
Fetal calf serum	Seromed, USA
Folin&clocalteu's phenol reagent	E.Merck, Germany
Glycerol	Sigma-Aldrich, USA
Glycine	Sigma-Aldrich, USA
HEPES	Sigma-Aldrich, USA
High range molecular weight marker	BIO-RAD, USA
HRP-conjugated goat anti-mouse IgG	Sigma-Aldrich, USA
Hydrochloric acid	E.Merck, Germany

Hanks' balanced salt solution	GIBCO, USA
Magnesium chloride	Sigma-Aldrich, USA
Mercaptoethanol	Sigma-Aldrich, USA
Methanol	E.Merck, Germany
Mouse monoclonal anti P-glycoprotein clone F4	Sigma-Aldrich, USA
Monobasic sodium phosphate	Sigma-Aldrich, USA
MTT Thiazolyl blue	Sigma-Aldrich, USA
Penicillin-streptomycin	GIBCO, USA
Phenylmethyl sulfonyl fluoride (PMSF)	Sigma-Aldrich, USA
Potassium chloride	Sigma-Aldrich, USA
Potassium phosphate	Sigma-Aldrich, USA
POPOP	Sigma-Aldrich, USA
PPO	Sigma-Aldrich, USA
2-propanol	E.Merck, Germany
Rhodamine123	Sigma-Aldrich, USA
SDS-PAGE standard broad range	Sigma-Aldrich, USA
Skim milk	Difco, USA
Sodium carbonate	Sigma-Aldrich, USA
Sodium chloride	Sigma-Aldrich, USA
Sodium dodecyl sulfate	Sigma-Aldrich, USA
Sodium potassium tartrate	Sigma-Aldrich, USA
Sodium hydroxide	Sigma-Aldrich, USA
SuperSignal®West Pico Chemiluminescent substrate	PIERCE, USA
Tris (hydroxymetry) aminomethane	Sigma-Aldrich, USA
Tween 20	Sigma-Aldrich, USA
Verapamil	Sigma-Aldrich, USA
Vinblastine sulphate salt	Sigma-Aldrich, USA
[G- ^{3}H] vinblastine sulphate	Amersham, UK

Appendix B

List of instruments used in this study

Instrument	Company
Analytical balance AC 100	Satorious
Autoclave	Tomy autoclave SS-240
Automatic pipette	GIBCO
β counter (liquid scintillation counter)	Pharmacia
Carbondioxide incubator	Forma Scientific
Deionizedd water machine	Barnstead
Distilled water machine	Hamilton
ECL-hyper film	Amersham
Flow cytometer	Becton-Dickinson
Freezer (-80 °C)	Forma scientific
Freezer (-20 °C)	Sanyo
Gel doc	BIO-RAD
Glassware	Pyrex
Hood	British Klocker Switchgear
Hot air oven	Haraeus
Inverted microscope	Nikon
Larminar flow biological cabinet	NUAIR2000 Fembrook Lane Plymouth, MN55447
Light microscope	Olympia Tokyo
Liquid nitrogen tank	Taylor-wharton
Magnetic stirrer	Sybron / Thermolyne
Microcentrifuge, bench-topped	Clay
Mini protein II slab gel	BIO-RAD
Pasture pipette	Pyrex
pH meter	Hanna Instruments 8417

Power supply	E-C Appraratus corporation
Refrigerator	Sanyo, Hitachi
Serological pipette	Pyrex
Shaker bath	Unitronic 320 OR
Slab gel dryer	Savant
Spectrophotometer	MILTON ROY spectonic 1001 plus
25 or 75 cm^3 T-flask	Nunc
Trans-blot® electrophoretic tranfer cell	BIO-RAD
Ultracentrifuge	Ivan Sorval Inc.,USA
Vertical gel electrophoresis apparatus	Life Technologies ™
Vortex	Scientific industries
Water bath	GFL 1083

Appendix C

Preparation of some reagents and buffers

Human cervical carcinoma cell culture

1. Incomplete DMEM medium with phenol red

DMEM	1	package (13.5 g)
HEPES	3.57	g
$NaHCO_3$	3.7	g
0.34% 2-mercaptoethanol	1.0	ml
Deionized distilled water	800	ml

Adjust pH to 7.2-7.4 then adjust volume to 1,000 ml and sterilize by suction filter (membrane pore size 0.2 μm)

2. Completed DMEM medium with phenol red

Incomplete DMEM medium	89.5	ml
Fetal calf serum	10	ml
Pen/strep	0.5	ml

Stored at 4°C.

3. Incomplete DMEM medium without phenol red

DMEM	1	package (13.5 g)
HEPES	3.57	g
$NaHCO_3$	3.7	g
0.34% 2-mercaptoethanol	1.0	ml
Deionized distilled water	800	ml

Adjust pH to 7.2-7.4 then adjust volume to 1,000 ml and sterilize by suction filter (membrane pore size 0.2 μm)

4. Completed DMEM medium without phenol red

Incomplete DMEM medium	89.5	ml
Fetal calf serum	10	ml
Pen/strep	0.5	ml

5. Freezing solution

Fetal calf serum	9.2	ml
DMSO	0.8	ml

Stored at 4°C.

Cell survival measurement

1. MTT stock dye solution

MTT	1.0	g
PBS pH 7.4	200	ml

After dissolve MTT dye, filtrate any nonsoluble powder by filtration with membrane filter pore size 0.2 μm, collecte in dark container.

2. Phosphate buffer saline (PBS) pH 7.4

KH_2PO_4	0.24	g
Na_2HPO_4	1.44	g
NaCl	8.0	g
KCl	0.2	g

Dissolve in 800 ml deionized distilled water, adjusted pH to 7.4 then top up to 1,000 ml. Sterilize by autoclave.

Rhodamine123 accumulation and efflux

1. Rhodamine123 (1 mg/ ml)

Rhodamine123	0.001	g
DMSO	1	ml

Stored at -20 °C.

2. Verapamil stock (50 mg/ml)

Verapamil	0.05	g
DMSO	1	ml

Stored at 4 °C.

3. Hanks' balanced salt solution (HBSS) without phenol red and sodium bicarbonate.

HBSS powder	9.7	g/ package
$NaHCO_3$	0.35	g
HEPES	2.603	ml
0.34% 2-mercaptoethanol	1.0	ml
Deionized distilled water	800	ml

Adjust pH to 7.2-7.4 then adjust volume to 1,000 ml and sterilize by suction filter (membrane pore size 0.2 μm)

4. Completed HBSS without phenol red

Incomplete HBSS	90	ml
Fetal calf serum	10	ml

Stored at 4°C.

Radiolabeled drug accumulation and efflux

1. 3N Sodium hydroxide

NaOH	12	g
Deionized distilled water	100	ml

2. 6N Hydrochloric acid

12N HCl was diluted in deionized distilled water to 6 N.

3. Tripop scintillation cocktail

PPO	10	g
POPOP	0.25	g
Toluene	2.5	l

Plasma membrane preparation

1. Hypotonic buffer

1 M Tris-HCl pH 7.4	0.5	ml
4.2 M $MgCl_2$	18	μl
KCl	0.0373	g

100 mM PMSF	1	ml

Deionized distilled water was top up to 50 ml

2. Laemmli buffer

1.0 M Tris-HCl, pH 6.8	0.5	ml
Glycerol	1.0	ml
10% SDS	2.0	ml
Deionized distilled water	6.25	ml

Protein determination

1. Reagent A

2% (w/v) Na_2CO_3 in 0.1 N NaOH

NaOH	2	g
Na_2CO_3	10	g
Deionized distilled water	500	ml

2. Reagent B

Part A; $CuSO_4$	0.5	g
Distilled water	50	ml
Part B; Na-K Tatrate	1	g
Deionized distilled water	50	ml

Before using 0.5 ml of part A and B were mixed with the final concentration 0.5%(w/v) $CuSO_4.5H_2O$ and 1%$NaKC_4H_4O_6.2H_2O$ (Na-K Tatrate).

3. Reagent C

Working solution was freshly prepared by mixing reagent A 50 ml and reagent B ratio 50:1.

4. Folin-ciocalteau phenol reagent 1N

Folin-ciocalteau phenol reagent 2N was diluted in deionized distilled water to 1 N.

SDS-PAGE analysis

1. Stock solution A : separating gel buffer 1.5 mM Tris HCl, pH 8.8

Tris base	18.15	g

Deionized distilled water	80	ml

Adjust pH to 8.8 then adjust volume to 100 ml and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.2 μm, collect in dark container.

2. Stock solution C: stock acrylamide solution (30% T, 2.7%)

Acrylamide	29.2	g
Bis (Estaman)	0.8	g
Deionized distilled water	70	ml

Adjust volume to 100 ml and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.2 μm, collect in dark container.

3. Stock solution D : stacking gel buffer 0.5 mM Tris HCl pH 6.8

Tris base	6.05	g
Deionized distilled water	70	ml

Adjust pH to 6.8 then adjust volume to 100 ml and filtrate any nonsoluble powder by filtration with membrane filter pore size 0.2 μm, collect in dark container.

4. Stock ammonium persulfate solution (10% w/v APS in deionized water)

Ammonium persulfate	0.1	g
Deionized distilled water	1	ml

5. Electrode buffer

Tris-base	3.0	g
Glycine	14.4	g
SDS	1.0	g

Dissolve in deionized water 1,000 ml then filtrate by suction filter and store at 4 °C.

6. 5X nonreducing buffer

1.0 M Tris-HCl pH6.8	0.625	ml
Glycerol	1.0	ml
1% Bromphenol blue	0.125	ml

Adjust volume to 10 ml with distilled water.

7. 5X reducing buffer

5X nonreducing buffer	475	μl
2-mercaptoethanol	25	μl

8. High range molecular weigh marker

Marker	1	µl
5X reducing buffer	19	µl

9. Coomassie blue

Coomassie blue	0.25	g
Methanol	20	ml
Acetic acid	10	ml

Deionized water was top up to 100 ml.

10. Coomassie blue destaining solution

Methanol	100	ml
Acetic acid	50	ml

Deionized water was top up to 500 ml

11. Stock 10% SDS solution

SDS	0.2	ml
Deionized distilled water	1	ml

12. Separating gel 7.5%

Deionized distilled water	2.425	ml
Tris-HCl, pH 8.8 (solution A)	1.25	ml
10% SDS	50	µl
Acrylamide/Bis (solution C)	1.25	ml
10% APS	25	µl
TEMED	2.5	µl

13. Stacking gel 4%

Deionized distilled water	3.05	ml
Tris-HCl, pH 6.8 (solution D)	1.25	ml
10% SDS	50	µl
Acrylamide/Bis (solution C)	0.65	ml
10% APS	25	µl
TEMED	5	µl

Proetin Western blot analysis

1. Bloting buffer

Tris-base	3.03	g
Glycine	14.4	g
Methanol	200	ml

Dissolve in deionized distilled water 1,000 ml then filtrate by filtration and store at 4 °C.

2. Amido black

Amido black	0.25	g
Isopropanol	62.5	ml
Acetic acid	25.0	ml

Top up with deionized distilled water to 250 ml.

3. PBS, pH7.4

Na_2HPO_4	1.3	g
NaH_2PO_4	0.204	g
NaCl	7.28	g

Adjust pH to 7.4 then adjust volume to 1,000 ml and sterilize by filtration.

4. Amido black destaining solution

Isopropanol	125	ml
Acetic acid	50	ml

Top up with deionized water to 250 ml.

5. Blocking reagent

Skim milk	5	g
Anti foam	20	µl

Dissolve in PBS, pH 7.4.

6. Washing buffer

PBS pH 7.4	500	ml
Tween 20	500	µl

7. Film developer (Kodak)

Part A	2.99	g
Part B	21.8	g

Part C	0.7246	g

Top up with deionized water to 250 ml. Store at 4 °C in dark.

8. Film fixer (Kodak)

Part A	50	ml
Part B	10	ml

Top up with deionized water to 250 ml. Store at 4 °C in dark.

VITA

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