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APPENDICES

APPENDIX A

PREPARATION OF REAGENTS

Storage buffer

50 mM Tris HCl, 150 mM NaCl, 0.01 mM EDTA Triplex II®, 2 mM DTT, 50%v/v glycerol and 0.1% w/v TritonX-100.

5X TBE buffer + 0.5% SDS

To make 1 liter of 5X TBE buffer + 0.5% SDS, 53.91 gm Tris base (890 mM), 27.51 gm boric acid (890 mM), 2.92 gm EDTA Triplex II® (20 mM) and 5.00 gm SDS (0.5%) were dissolved with continuously stirring. The solution was adjusted to 1,000 ml with deionized distilled water. The final 1X TBE buffer + 0.1% SDS was 5 fold dilution of the concentrated stock.

10X TE buffer

To make 100 ml of 1X TE buffer, 1.21 gm Tris base (100 mM) and 0.29 gm EDTA Triplex II® (10 mM), were dissolved with continuously stirring. The solution was adjusted to 100 ml with deionized distilled water. The final 1X TE buffer is 10 fold dilution of the concentrated stock.

TEN buffer

To make 100 ml of 1X TEN buffer, 1.17 gm NaCl (0.2 M) was dissolved with 1X TE buffer and adjusted to 100 ml.

Loading buffer

2% SDS, 0.01% bromophenol blue, 15% glycerol and adjusted volume by 1X TBE buffer.

Hoechst 33258 stock solution

10 mg/ml Hoechst 33258 was dissolved in deionized distilled water and stored at -20°C for 6 month.

Hoechst 33342 stock solution

10 mg/ml Hoechst 33342 was dissolved in deionized distilled water and stored at -20°C for 6 month.

Proteinase K

1 mg/ml proteinase K was dissolved in 1X TE buffer and store in 4°C for 1 month.

APPENDIX B

TABLES OF EXPERIMENTAL RESULTS

Table 9 The fluorescence intensity of dyes in various conditions

Dyes	Conc.	Fluorescence Intensity			
		Buffer of dye solution	Enzyme reaction buffer	Supercoiled DNA	Relaxed DNA
H 33258	12.5 μg/ml in TE buffer (n = 3)	5083±558	5222±388	5512±369	5260±368
	12.5 μg/ml in TEN buffer (n = 3)	10894±118	11459±126	12137±175	12164±105
	1 μg/ml in TEN buffer (n = 2)	3350±85	3222±454	8269±230	8696±514
H 33342	12.5 μg/ml in TE buffer (n = 3)	2604±394	1894±107	2419±36	2394±71
	12.5 μg/ml in TEN buffer (n = 3)	11894±1873	12014±1360	12131±1257[b]	12931±1589[b]
	1 μg/ml in TEN buffer (n = 2)	2284±100	1752±69[a]	7619±257[b]	8426±148[b,c]
Picogreen	1:400 (n = 3)	1331±55	1369±78	10148±631	5637±236*
Sybr Green I	1:10,000 (n = 3)	9577±203	8775±126	255433±9426	221332±6154*

Each value represented the mean value with S.E.M. of two or three independent experiments. a, b, c and asterisk refer significant differences from supercoiled DNA, buffer of dye solution, enzyme reaction buffer of H 33258 or H 33342 and supercoiled DNA of Picogreen or Sybr Green I, respectively. Student's *t*-test was used for the comparison of two mean values, and statistical significance was taken as $p < 0.01$.

Table 10 The fluorescence intensity of supercoiled and relaxed DNA determined by Picogreen as increasing DNA concentration

DNA conc.(ng/ml)	Fluorescence Intensity				
	10	50	100	500	1000
Supercoiled DNA	1952±13	4346±103	6149±144	16239±211	26325±44
Relaxed DNA	1633±51	2703±57	4255±183	11323±295	16979±296

Each value represented the mean value with S.E.M. of four replicates.

Table 11 The band intensity, the fluorescence intensity and the percentage of relaxtion activity as function of time of incubation

Time of Incubation	Band Intensity	Fluorescence Intensity	% Relaxation	
			Gel-based assay	Fluorescence microplate assay
0	205.79±3.10	11812±958	0	0
1	203.52±1.69	11065±858	2.26	10.9
2	202.97±0.58	10233±934	2.81	23.1
3	201.33±2.15	9200±1021	4.44	38.3
4	198.52±1.51	8944±1063	7.24	42.0
5	194.89±0.76	8138±1252	10.9	53.8
10	176.58±4.02	6589±728	29.1	76.6
25	140.69±6.18	5196±370	64.9	97.0
50	105.49±6.83	4990±372	100	100

Each value represented the mean value with S.E.M. of three replicates.

Table 12 The percentage of inhibition of camptothecin

Concentration (μM)	% Inhibition	
	Gel-based assay	Fluorescence microplate assay
0.1	16.9±11.9	9.81±6.74
0.5	26.1±16.4	16.8±7.39
1	31.3±13.0	19.1±9.23
5	70.6±13.5	62.3±16.1
10	76.7±10.4	65.1±15.1
25	86.7±7.34	89.2±25.2
50	91.4±10.4	74.2±16.0

Each value represented the mean value with S.E.M. of four independent experiments.

Table 13 The percentage of inhibition of heparin

Concentration (μg/ml)	% Inhibition	
	Gel-based assay	Fluorescence microplate assay
0.1	-2.24±5.25	4.07±3.55
1	29.1±20.7	14.1±3.64
10	103±9.56	66.4±11.0
50	109±6.47	115±10.8
100	104±1.73	116±13.6

Each value represented the mean value with S.E.M. of three independent experiments.

Table 14 The band intensity and the fluorescence intensity as a result of enzyme reaction incubating with quercetin

	Band Intensity	Fluorescence Intensity
Supercoiled DNA (used as control)	150.9±27.7	13001±1860
Supercoiled DNA + 1,000 μM quercetin	121.1±18.0	1735±293*
Relaxed DNA (used as control)	104.4±29.6	7003±624
Relaxed DNA + 1,000 μM quercetin	89.98±20.1	3596±783*
5% DMSO	114.8±27.5	7111±816
1 μM quercetin	141.6±21.1	82911146
5 μM quercetin	138.5±21.7	8328±1045
50 μM quercetin	134.7±22.1	7990±1183
100 μM quercetin	143.0±18.5	8398±1265
250 μM quercetin	139.4±18.5	8274±1129
500 μM quercetin	152.2±21.4	6007±1276
1,000 μM quercetin	147.5±19.2	3390±806

Each value represented the mean value with S.E.M. of four independent experiments. Asterisks refer significant differences from controls (supercoiled DNA control and relaxed DNA control). Student's *t*-test was used for the comparison of two mean values, and statistical significance was taken as $p < 0.01$.

Table 15 The percentage of inhibition of menadione

Concentration (μM)	% Inhibition	
	Gel-based assay	Fluorescence microplate assay
0.5	-0.27±0.38	-11.1±1.08
5	2.58±1.19	-7.66±5.04
25	30.4±20.6	-0.33±25.4
50	64.9±21.6	48.0±13.2
250	91.0±10.9	100±50.5
500	81.2±4.20	98.7±0.42

Each value represented the mean value with S.E.M. of two independent experiments.

Table 16 The fluorescence intensity of Picogreen as a result of enzyme reaction incubating with topoisomerase II inhibitors

	Fluorescence Intensity
Supercoiled DNA	7073±347
Supercoiled DNA + 1 mM etoposide	6766±73
Supercoiled DNA + 20 μM ellipticine	5572±162*
Relaxed DNA	4206±42
5% DMSO	4300±32
50 μM camptothecin	6214±313
1 mM etoposide	4107±27
20 μM ellipticine	4979±94*
Enzyme reaction buffer	1053±349

Each value represented the mean value with S.E.M. of three replicates. Asterisks refer significant differences from supercoiled DNA. Student's *t*-test was used for the comparison of two mean values, and statistical significance was taken as $p < 0.01$.

Table 17 The fluorescence intensity of Picogreen as a result of enzyme reaction incubating with selected unknown compounds

	Fluorescence Intensity
Supercoiled DNA	12483±8235
Supercoiled DNA + 100 μM vitexin	7965±589
Supercoiled DNA + 100 μM chelidonine	7242±1835
Sum supercoiled DNA[a] (used as control)	9230±2575
Supercoiled DNA + 100 μM sanguinarine	3377±144*
Supercoiled DNA + 100 μM chelerythrine	4837±44*
Relaxed DNA	5039±823
5% DMSO	4034±63
Sum relaxed DNA[b]	4537±432
50 μM camptothecin	5302±217
50 μM vitexin	4722±110
50 μM chelidonine	4928±1054
50 μM sanguinarine	4257±65
50 μM chelerythrine	5567±251

[a]sum raw data of supercoiled DNA, supercoiled DNA with vitexin and supercoiled DNA with chelidonine

[b]sum raw data of relaxed DNA and 5% DMSO

Each value represented the mean value with S.E.M. of three replicates. Asterisks refer significant differences from control. Student's *t*-test was used for the comparison of two mean values, and statistical significance was taken as $p < 0.01$.

Table 18 The relative fluorescence of Picogreen among stop processes

Stop process	Relative Fluorescence (pH 12/pH 7.4)			
	Supercoiled DNA	Relaxed DNA	5% DMSO	50 μM camtothecin
Cool at 4°C	0.6330±0.0990	1.253±0.0753	1.229±0.1042	0.9523±0.1875
Heat at 95°C for 1 min	0.5874±0.0221	1.016±0.0588	1.025±0.0656	0.7002±0.0539
10%SDS/ 1 mg/ml proteinase K	0.7817±0.1395	1.339±0.1391	1.250±0.3313	0.9683±0.2160

Each value represented the mean value with S.E.M. of three replicates. Asterisks refer significant differences from control.

Table 19 The relative fluorescence of Picogreen among various enzyme amounts

	Relative Fluorescence (pH 12/pH 7.4)
Supercoiled DNA	0.7632±0.1606
Topoisomerase I 0.25 unit	1.347±0.1943
Topoisomerase I 1 unit	1.316±0.1434
Topoisomerase I 5 unit	1.331±0.0741

Each value represented the mean value with S.E.M. of three replicates. Asterisks refer significant differences from control.

Table 20 The fluorescence intensity, the relative fluorescence and the percentage of enzyme activity as function of time of incubation

Time of Incubation	Fluorescence Intensity		Relative Fluorescence	% Conversion
	Before added NaOH	After added NaOH		
0	5166±318	5213±414	1.009±0.0417	0
1	4946±249	4678±414	0.9503±0.0961	-9.95
2	4640±176	4689±303	1.013±0.0696	0.718
3	4916±599	4514±366	0.9267±0.0367	-14.0
4	4351±274	4602±261	1.066±0.0885	9.79
5	4099±206	4463±410	1.093±0.1026	14.4
10	3567±280	4508±325	1.273±0.0962	45.2
25	3191±193	4488±286	1.408±0.0571	68.3
50	2989±194	4680±360	1.594±0.2163	100

Each value represented the mean value with S.E.M. of three replicates. Asterisks refer significant differences from control.

Table 21 The percentage of inhibition of camptothecin and menadione

Camtothecin (n = 4)		Menadione (n = 3)	
Concentration (μM)	**% inhibition**	**Concentration (μM)**	**% inhibition**
0.1	-4.80±9.36	0.5	-10.6±8.06
0.5	4.49±6.37	5	-0.43±11.3
1	21.1±10.6	25	19.5±8.07
5	46.8±22.9	50	59.5±5.22
10	69.7±11.4	250	88.0±8.44
25	83.1±9.67	500	106±17.7
50	85.4±3.14	-	-

Each value represented the mean value with S.E.M. of three or four experiment. Asterisks refer significant differences from control.

VITAE

Mr Pratchaya Jetinai was born on March 14, 1978 in Ubon Ratchathanee province, Thailand. He was graduated in Bachelor of Science in Pharmaceutical Sciences in 2000 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University. He worked as a pharmacist at pharmacy department of Chulalongkorn Memorial Hospital for three years before coming to study Biomedicinal Chemistry Program.