Supporting Information

Rec. Nat. Prod. 13:5 (2019) 390-404

NF-κB Inhibition Activity of Curcumin-Loaded Sterically Stabilized Micelles and Its Up-Regulator Effect on Enhancement of Cytotoxicity of a New Nano-Pirarubicin Formulation in the Treatment of Breast Cancer

Zahra Eskandari^{1,2}, Fatemeh Bahadori^{*2}, Melda Altıkatoglu Yapaoz^{*1}, Vildan Betül Yenigün³, Abdurrahim Kocyigit³ and Hayat Onyuksel⁴

¹Department of Chemistry, Biochemistry Division, Faculty of Sciences and Arts, Yildiz Technical University, Istanbul, Türkiye

²Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, BezmialemVakif University, Istanbul, Türkiye

³Department of Medical Biochemistry, Faculty of Medicine, BezmialemVakif University, Istanbul, Türkiye

⁴Department of Biopharmaceutical Sciences, University of Illinois at Chicago, Chicago, IL 60612, USA

Table of Contents	Page
Table S1: 1H- and 13C-NMR data of Curcumin (400 and 100 MHz, resp., in d-DMSO; d in	2
ppm, J in Hz)	
Figure S1: 1H-NMR Spectrum of Curcumin (400 MHz, d-DMSO)	3
Figure S2: Expanded ¹ H-NMR Spectrum of Curcumin (From 2.90 to 10.50 ppm)	3
Figure S3: Expanded ¹ H-NMR Spectrum of Curcumin (From 5.96 to 7.81 ppm)	4
Figure S4: ¹³ C-NMR (100 MHz, CDCl ₃) Spectrum of Curcumin	4
Figure S5: Expanded ¹³ C-NMR Spectrum of Curcumin (From 95 to 151 ppm)	5
Figure S6: APT Spectrum of Curcumin	5
Figure S7: Expanded APT Spectrum of Curcumin (From 95 to 181 ppm)	6
Figure S8: COSY (400 MHz) Spectrum of Curcumin	6
Figure S9: Expansion of the COSY Spectrum of Curcumin	7
Figure S10: Expansion of the COSY Spectrum of Curcumin	7
Figure S11: HSQC (400 MHz) Spectrum of Curcumin	8
Figure S12: Expansion of the HSQC Spectrum of Curcumin	8
Figure S13: HMBC (400 MHz) Spectrum of Curcumin	9
Figure S14: Expansion of HMBC Spectrum of Curcumin (5.8 to 7.8 and 90-190 ppm)	9
Figure S15: DSC thermogram of A:P-SSM, B:C-SSM and C:free SSM	10
Figure S16: BSA standard curves obtained using Bradford method	11
Table S2: Statistical analysis results of cytotoxicity assays on MCF-7 cells	11

^{*} Corresponding authors: E-Mail: fatemehbahadori@gmail.com ; maltikatoglu@yahoo.com

	δ(Η)	δ(C)	COSY	НМВС
H-1 Enol	6.04	103.95	3,3',4,4'	C-2,3
H-1 B-diketo**	5.95	55.00	3,3',4,4'	C-10,10′
C-2,2' Enol**	-	186.31	-	H-3,3',4,4'
C-2,2' β-diketo**	-	163.00	-	H-4,4',9,9'
H-3,3' Enol**	6.73 (<i>d</i> , <i>J</i> =16)	124.20	1*,4,4', 6*,6'*	C-1, C-2,2',C-5,5'
H-3,3΄ β-diketo**	6.66 (<i>d</i> , <i>J</i> = 16)	123.9	10-10'	C-1, C-2,2',C-4,4'
H-4,4' Enol**	7.53 (<i>d</i> , <i>J</i> =16)	143.81	1*,3,3', 6*,6'* ,9*,9'*,10*,10'*	C-2,2′ ,3,3′,5,5′,6,6′,10,10′
H-4,4΄ β-diketo**	7.59 (<i>d</i> , <i>J</i> = 16)	133.80	3*,3'*	C-6,6′,10,10′
C 5,5'	-	129,46	-	H-3,3', 4,4', 6,6', 9,9'
H-6,6′	7.30 (<i>d</i> , <i>J</i> =2)	114,48	4*,4'*,9*,9'*,10,10'	C-(OCH ₃), 4,4′, 5,5′, 7,7′, 8,8′, 9,9′, 10,10′
C-7,7′	-	151.11	-	H-6,6', 9,9', 10,10'
OCH3 7,7'	3.82	58.80	-	H-6,6′, 9,9′, 10,10′
C-8,8′	-	152.47	-	H-6,6', 9,9', 10,10' C-2.2' β-diketo**
H-9,9′	6.81(<i>d</i> , <i>J</i> =9)	118.83	4*,4'*,6*,6'*,10,10'	C-(OCH ₃), 5,5′, 6,6′, 7,7′, 8,8′
H-10,10 [′]	7,13(<i>dd,J</i> =2,9)	126.21	4*,4′*,6,6′,9,9′,	C-(OCH ₃), 4,4',6,6', 7,7', 8,8', 9,9'
OH 2,2′	9.642(s)	-	-	-

Table S1: ¹H- and ¹³C-NMR data of Curcumin (400 and 100 MHz, resp., in *d*-DMSO; d in ppm, J in Hz)

*: Very weak interaction **: Enol and keto expresses the tautomeric form of the carbons 2 and 2'



Figure S1: ¹H-NMR Spectrum of Curcumin (400 MHz, *d*-DMSO)



Figure S2: Expanded ¹H-NMR Spectrum of Curcumin (From 2.90 to 10.50 ppm)



Figure S3: Expanded ¹H-NMR Spectrum of Curcumin (From 5.96 to 7.81 ppm) CARBON_01



Figure S4:¹³C-NMR (100 MHz, CDCl₃) Spectrum of Curcumin



Figure S6: APT Spectrum of Curcumin





Figure S9: Expansion of the COSY Spectrum of Curcumin



Figure S10: Expansion of the COSY Spectrum of Curcumin



Figure S12: Expansion of the HSQC Spectrum of Curcumin







Figure S14: Expansion of HMBC Spectrum of Curcumin (5.8 to 7.8 and 90-190 ppm)



Figure S15: DSC thermogram of A:P-SSM, B:C-SSM and C:free SSM



Figure S16: BSA standard curves obtained using Bradford method

Table S2: Statistical analysis results of cytotoxicity assays on MCF-7 cells

Oneway

		ANOVA			
DATA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	553.674	2	276.837	.347	.713
Within Groups	11981.107	15	798.740		
Total	12534.781	17			

Post Hoc Tests

Multiple Comparisons								
			M D'''			95% Confidence Interval		
	(I) H	(J) H	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound	
Tukey HSD	24	48	7.50482	16.31707	.891	-34.8783	49.8879	
		72	13.55939	16.31707	.690	-28.8237	55.9425	
	48	24	-7.50482	16.31707	.891	-49.8879	34.8783	
		72	6.05457	16.31707	.927	-36.3285	48.4377	
	72	24	-13.55939	16.31707	.690	-55.9425	28.8237	
		48	-6.05457	16.31707	.927	-48.4377	36.3285	
Scheffe	24	48	7.50482	16.31707	.900	-36.7762	51.7859	
		72	13.55939	16.31707	.714	-30.7217	57.8404	
	48	24	-7.50482	16.31707	.900	-51.7859	36.7762	
		72	6.05457	16.31707	.934	-38.2265	50.3356	
	72	24	-13.55939	16.31707	.714	-57.8404	30.7217	
		48	-6.05457	16.31707	.934	-50.3356	38.2265	

Homogeneous Subsets

			DATA
			Subset for alpha = 0.05
	н	N	1
Tukey HSD ^a	72	6	56.0134
	48	6	62.0679
	24	6	69.5727
	Sig.		.690
Scheffe ^a	72	6	56.0134
1	48	6	62.0679
	24	6	69.5727
	Sig.		.714

Oneway

ANOVA

H24					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5971.692	4	1492.923	2.45 6	.072
Within Groups	15197.613	25	607.905		
Total	21169.305	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable:H24

			Mean Difference			95% Confide	lence Interval	
	(I) MATE	MATE	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound	
Tukey HSD	1	2	-11.57102	14.23499	.924	-53.3773	30.2353	
		3	22.99471	14.23499	.502	-18.8116	64.8010	
		4	20.20082	14.23499	.622	-21.6055	62.0071	
		5	22.67903	14.23499	.515	-19.1273	64.4854	
	2	1	11.57102	14.23499	.924	-30.2353	53.3773	
		3	34.56574	14.23499	.141	-7.2406	76.3721	
		4	31.77184	14.23499	.201	-10.0345	73.5782	
		5	34.25005	14.23499	.147	-7.5563	76.0564	
	3	1	-22.99471	14.23499	.502	-64.8010	18.8116	
		2	-34.56574	14.23499	.141	-76.3721	7.2406	
		4	-2.79389	14.23499	1.000	-44.6002	39.0124	
		5	31568	14.23499	1.000	-42.1220	41.4906	
	4	1	-20.20082	14.23499	.622	-62.0071	21.6055	
		2	-31.77184	14.23499	.201	-73.5782	10.0345	
		3	2.79389	14.23499	1.000	-39.0124	44.6002	
		5	2.47821	14.23499	1.000	-39.3281	44.2845	
	5	1	-22.67903	14.23499	.515	-64.4854	19.1273	
		2	-34.25005	14.23499	.147	-76.0564	7.5563	

42.1220 39.3281 35.7158 70.2815 67.4876
39.3281 35.7158 70.2815 67.4876
35.7158 70.2815 67.4876
70.2815 67.4876
67.4876
69.9658
58.8578
81.8526
79.0587
81.5369
24.2921
12.7211
44.4929
46.9711
27.0860
15.5150
50.0807
49.7650
24.6078
13.0368
47.6025
44.8086