

Figure S1. Knockdown efficiency of siRNA of DHRS11 and AKR1C3 in C4-2 (A) and 22Rv1 cells (B, C). The cells were transfected with universal negative siRNA (Neg), DHRS11 siRNA (si-1 and si-2) or AKR1C3 siRNA (si-3 and si-4) and cultured for 48h. The mRNA expression levels of DHRS11 and AKR1C3 in the cells were analyzed by RT-qPCR. $**p < 0.01$ significantly different from the cells treated with Neg.

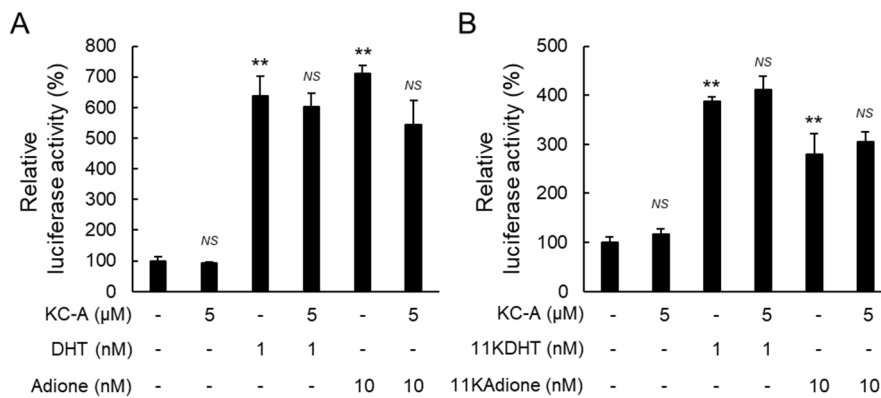


Figure S2. Luciferase activity in AR EcoScreen GR KO cells. The luciferase activities were evaluated by *in vitro* reporter gene assays using the AR-EcoScreen GR KO cells. Relative luciferase activity was presented as the relative ratio of firefly luciferase activity using Steady-Glo luciferase assay kit to the cell numbers calculated by alamar blue assay. The cells were pretreated for 2 h without or with 5 μ M KC-A, and then cultured for 24 h in the absence or presence of 1 nM DHT, 10 nM Adione (A), 1 nM 11KDHT or 10 nM 11KAdione (B). $**p < 0.01$, $NS p > 0.05$, significantly different from the control cells treated with DMSO alone. $NS p > 0.05$, significantly different from the cells treated with DHT, Adione, 11KDHT or 11KAdione alone.

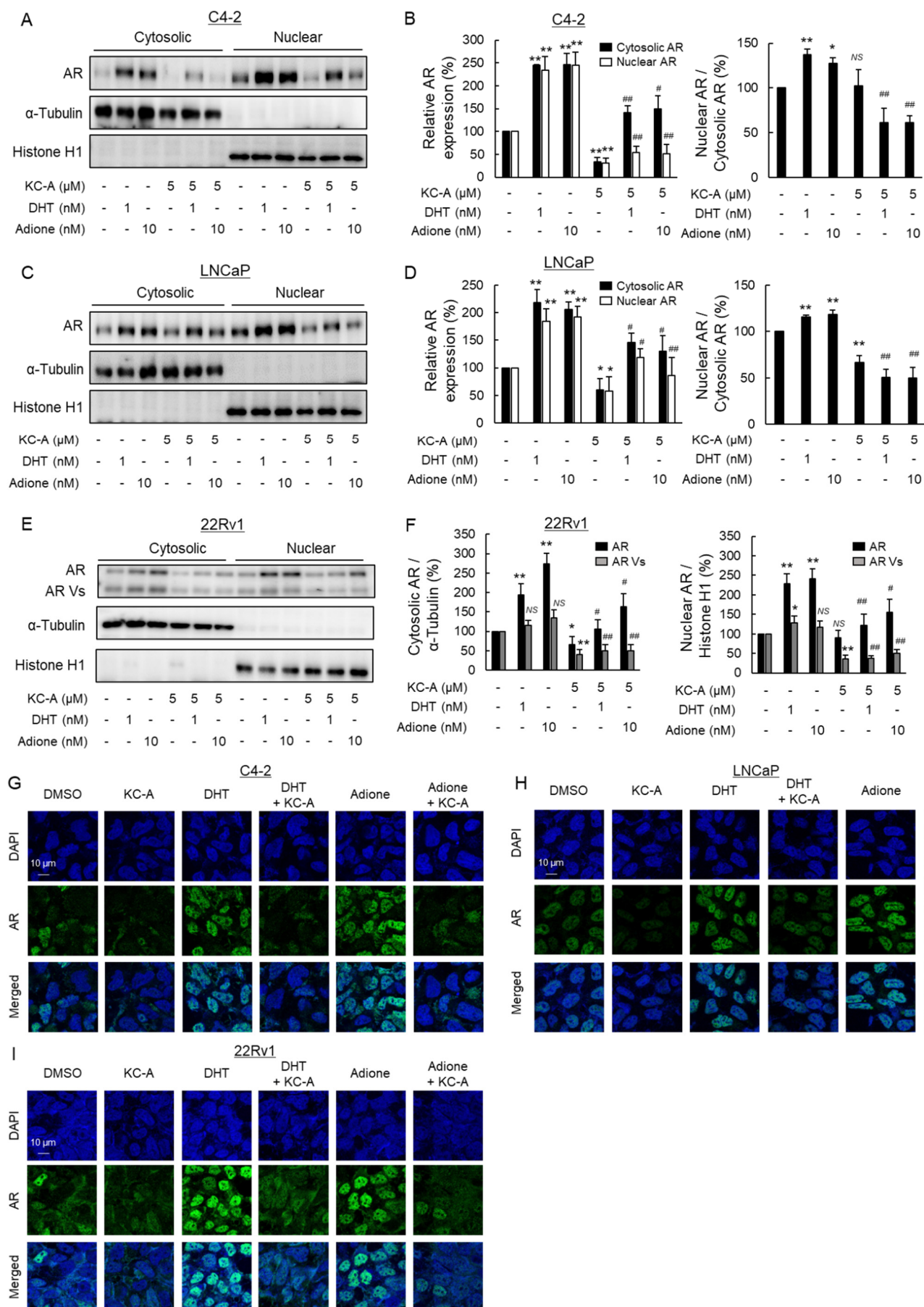


Figure S3. Effects of KC-A on androgens-induced AR signaling in C4-2, LNCaP and 22Rv1 cells. The cells were pretreated for 2 h without or with 5 μ M KC-A, and then cultured for 24 h in the absence or presence of 1 nM DHT or 10 nM Adione in the cells. (A-F) Nuclear expression of AR and AR Vs. The nuclei of the cells were fractionated from the cytosol by using LysoPure Nuclear and Cytoplasmic Extractor Kit, as described in the Experimental Section and analyzed by immunoblotting. The protein levels are expressed as a percentage of the band density relative to those in the control cells treated with DMSO alone. $**p < 0.01$, $*p < 0.05$, $NS\ p > 0.05$ significantly different from the cells treated with DMSO alone. $**p < 0.01$, $*p < 0.05$ significantly different from the cells treated with 1 nM DHT or 10 nM Adione alone. (G-I) Immunofluorescence staining. The cells were stained with the anti-AR antibody (green) and DAPI (blue). Merged images were also shown. Scale bar indicates 10 μ m.

Figure S4-1 The ^1H NMR spectrum of KC-A (1) in $\text{DMSO-}d_6$ (500 MHz)

Figure S4-2 The ^{13}C NMR spectrum of KC-A (1) in $\text{DMSO-}d_6$ (125MHz)

Figure S4-3 The DQF-COSY spectrum of KC-A (1) in $\text{DMSO-}d_6$

Figure S4-4 The HMQC spectrum of KC-A (1) in $\text{DMSO-}d_6$

Figure S4-5 The HMBC spectrum of KC-A (1) in $\text{DMSO-}d_6$

Figure S4-6 The IR spectrum of KC-A (1)

Figure S4-7 The UV spectrum of KC-A (1) in MeOH

Figure S4-8 The HR-ESI-MS spectrum of KC-A (1)

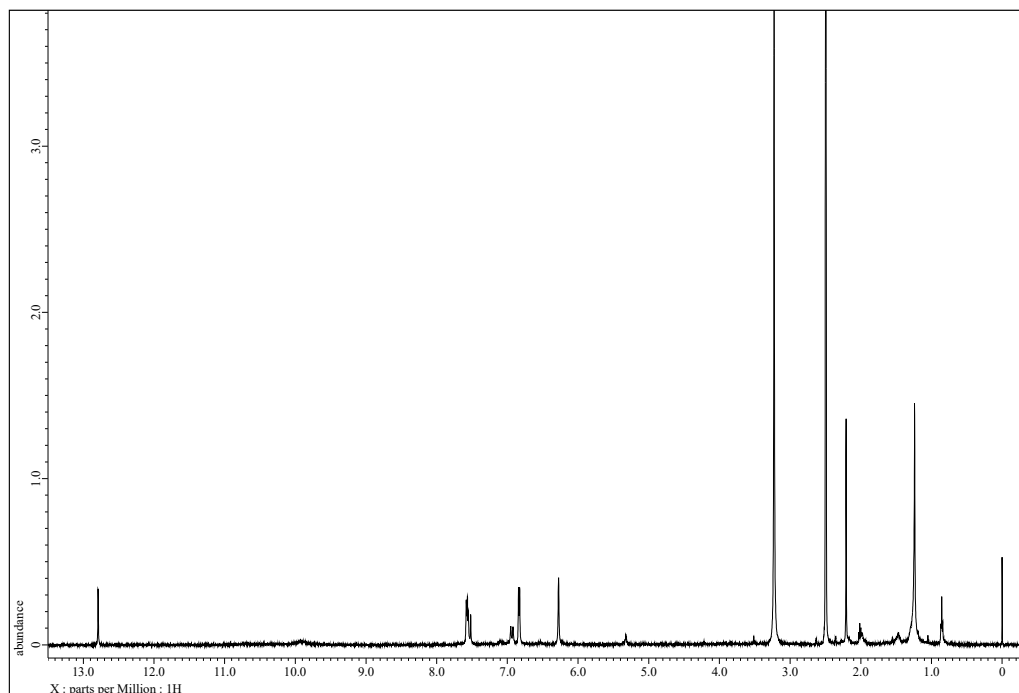


Figure S4-1 The ^1H NMR spectrum of KC-A in $\text{DMSO-}d_6$ (500 MHz)

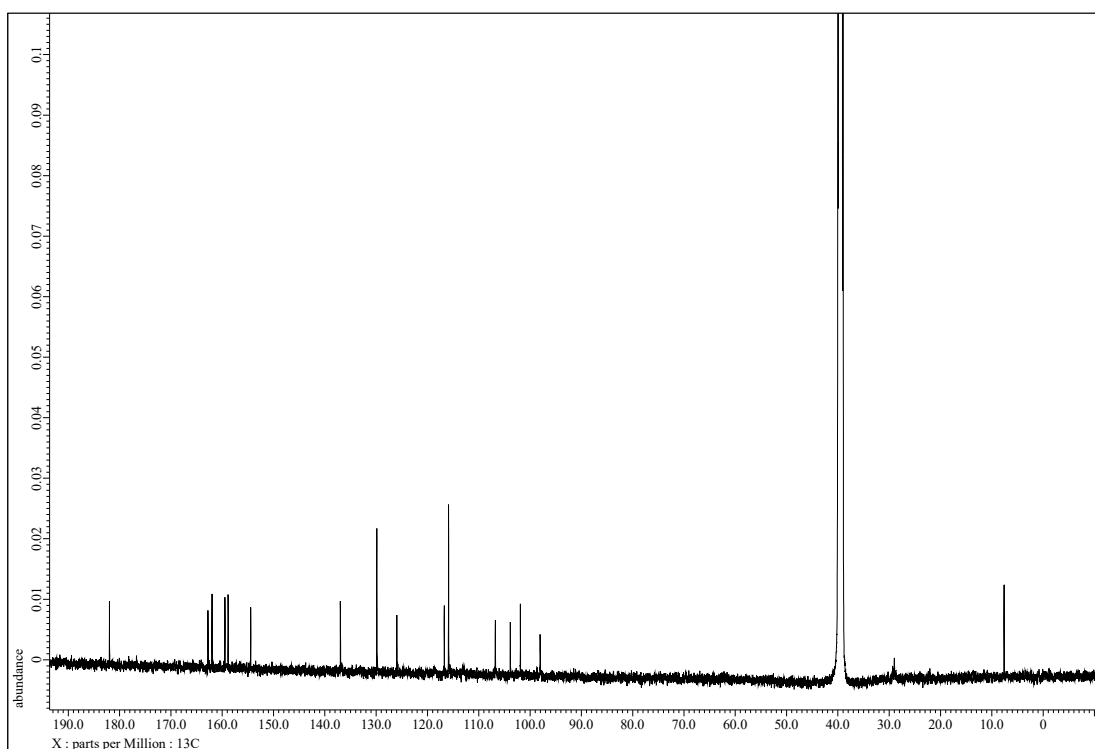


Figure S4-2 The ^{13}C NMR spectrum of KC-A in $\text{DMSO-}d_6$ (125MHz)

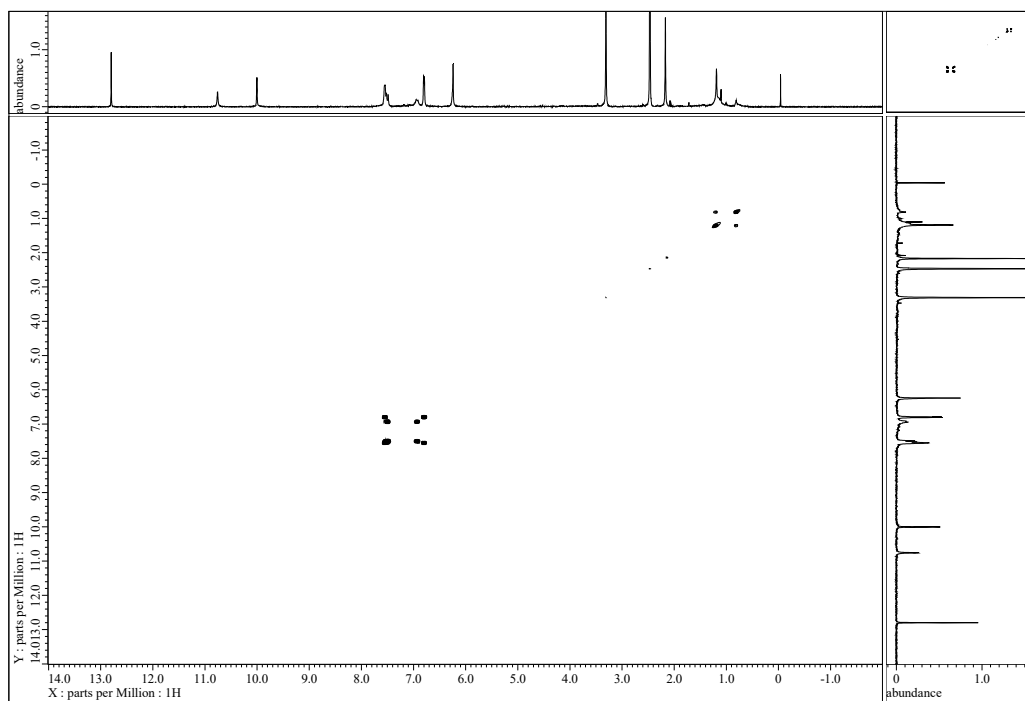


Figure S4-3 The DQF-COSY spectrum of KC-A in $\text{DMSO-}d_6$ (500 MHz)

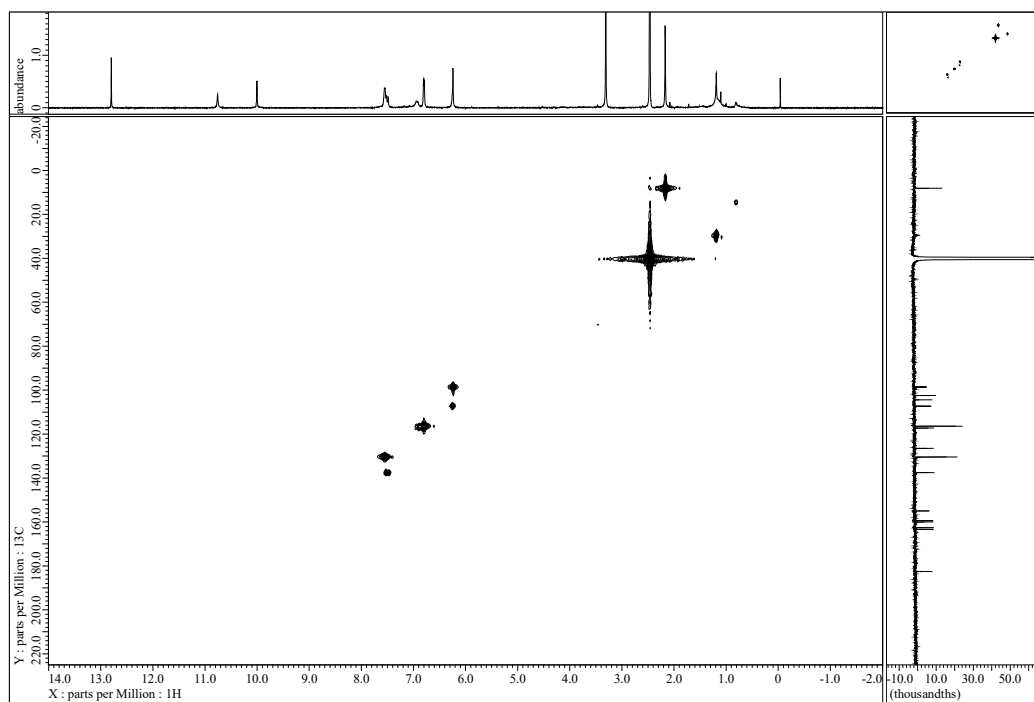


Figure S4-4 The HMQC spectrum of KC-A (**1**) in DMSO-*d*₆

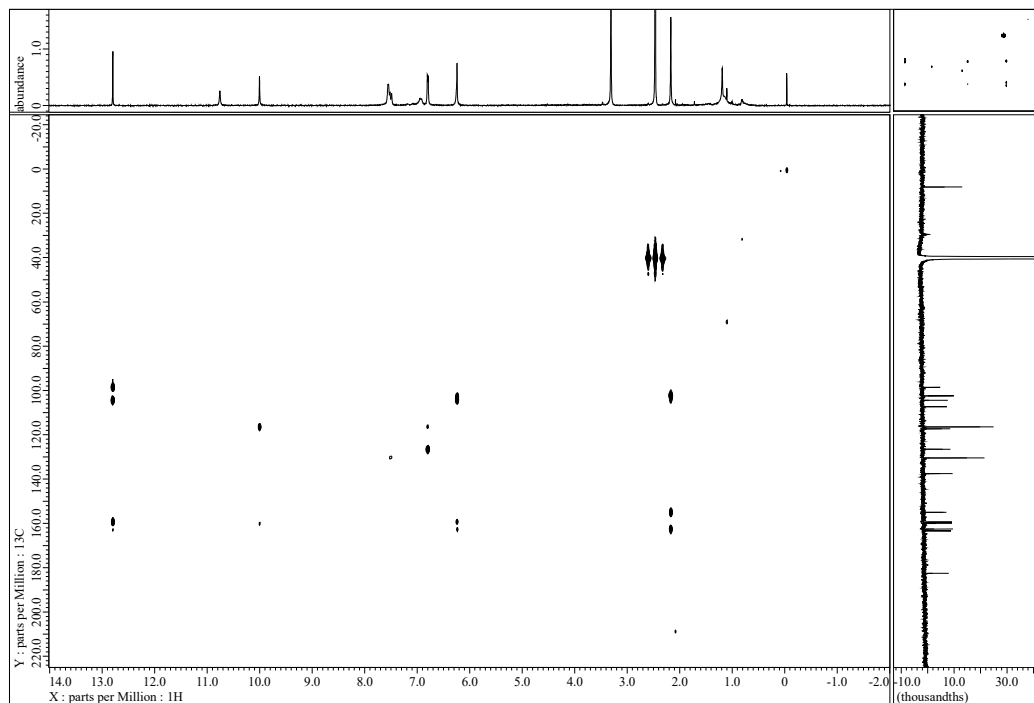


Figure S4-5 The HMBC spectrum of KC-A in DMSO-*d*₆

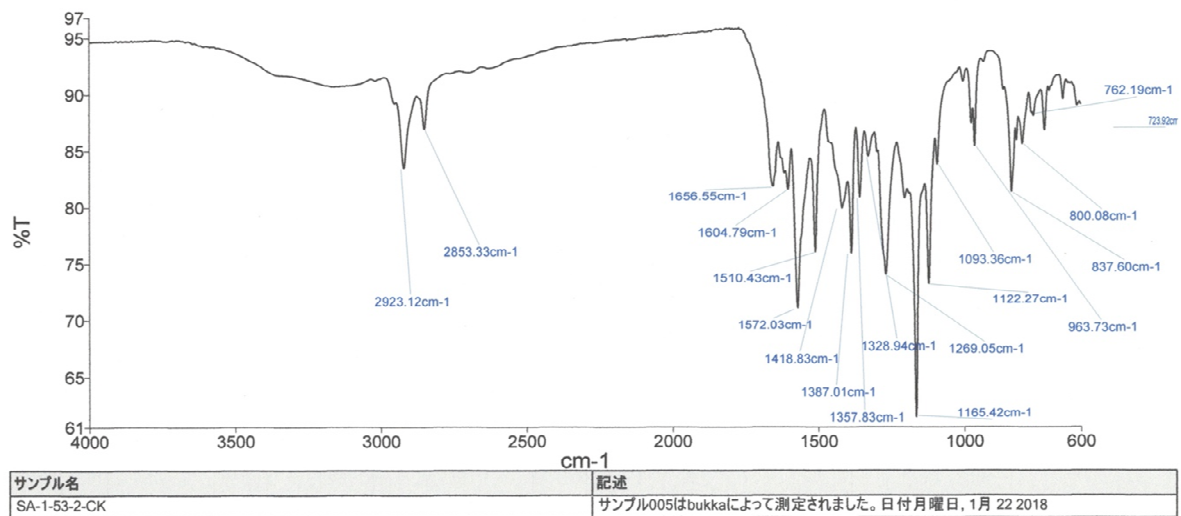
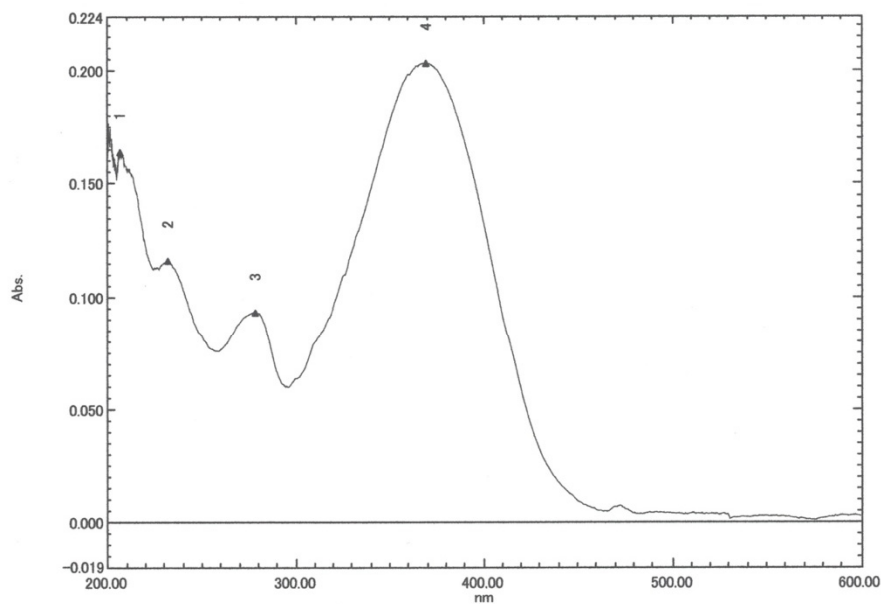


Figure S4-6 The IR spectrum of KC-A

データセット SA-1-53-2-CK - RawData



[測定プロパティ]
 波長範囲(nm): 200.00 ~ 600.00
 スキャンスピード: 高速
 サンプルングビッチ: 0.1
 オート サンプルングビッチ: 無効
 測定モード: シングル

No.	波長 (nm)	吸光度	説明
1	206.50	0.164	
2	232.30	0.116	
3	278.60	0.093	
4	369.10	0.203	
5			

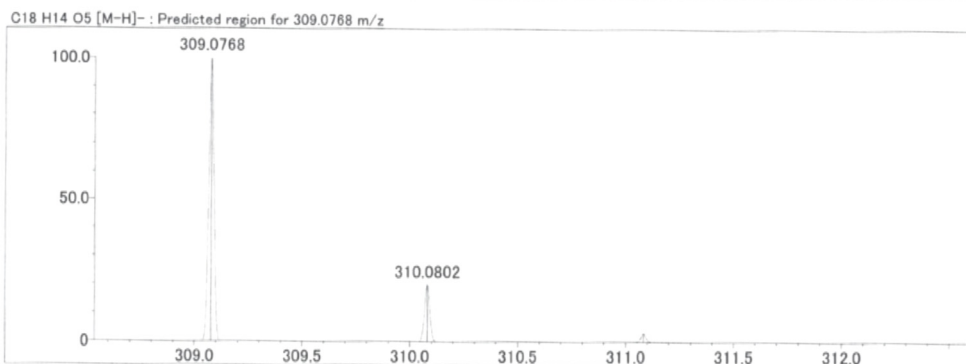
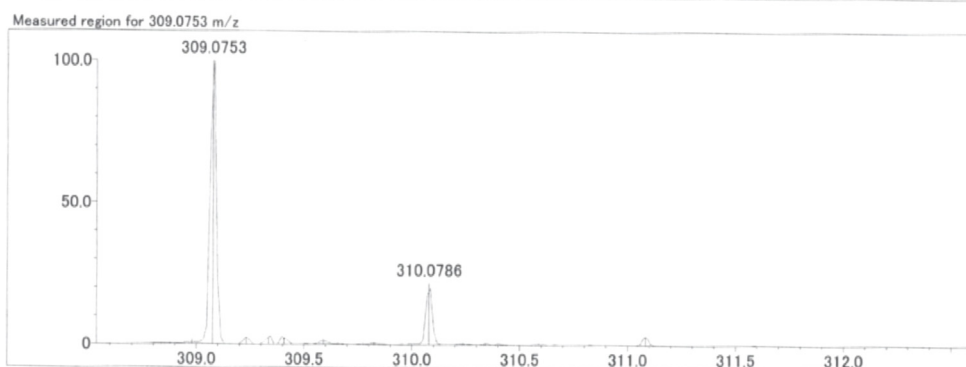
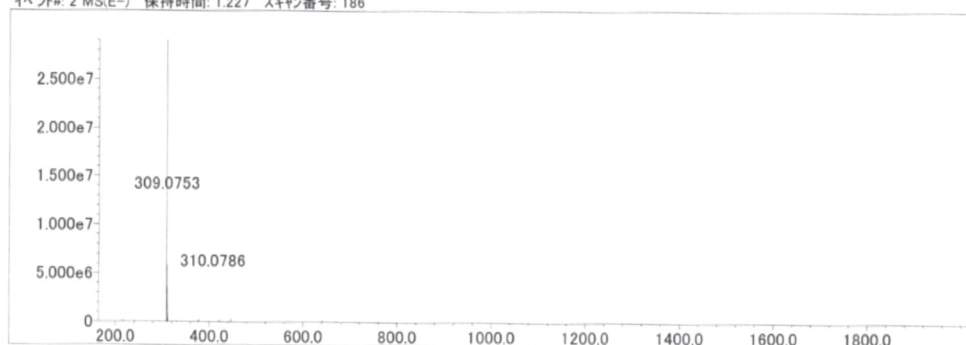
Figure S4-7 The UV spectrum of KC-A in MeOH

Data File: C:\LabSolutions\Data\SYOYAKU\ayabe\20171114\SA-1-53-2-CK.lcd

Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Elmt	Val.	Min	Max	Use Adduct
H	1	10	100	F	1	0	0	Cl	1	0	0	10B	3	0	0	H
B	3	0	0	Na	1	0	0	K	1	0	0					HCOO
C	4	10	100	Si	4	0	0	Zn	2	0	0					CH3COO
N	3	0	0	P	3	0	0	Br	1	0	0					Cl
O	2	4	100	S	2	0	0	I	3	0	0					2H

Error Margin (mDa): 5.0
DBE Range: not fixed
Electron Ions: odd
HC Ratio: unlimited
Apply N Rule: yes
Use MSn Info: no
Max Isotopes: all
Isotope RI (%): 1.00
Isotope Res: 10000
MSn Iso RI (%): 75.00
MSn Logic Mode: AND
Max Results: 100

イベント#: 2 MS(E-) 保持時間: 1.227 スキャン番号: 186



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	75.73	C18 H14 O5	[M-H]-	309.0753	309.0768	-1.5	-4.85	83.80	12.0

Figure S4-8 The HR-ESI-MS spectrum of KC-A

Table S1. Sequences of siRNAs against DHRS11 and AKR1C3.

	Sense (5' --- 3')	Antisense (5' --- 3')
Universal negative siRNA	UUCUCCGAACGUGUCACGUdTdT	ACGUGACACGUUCGGAGAAdTdT
DHRS11 siRNA-1 (si-1)	CACAAUUCGCCUCAAACUdTdT	AGUUUGAAGGCGAAUUGUGdtdC
DHRS11 siRNA-2 (si-2)	CUUAUAUCUGUGUUGUUAUdTdT	AUAACAACACAGAUUAUAAGdGdT
AKR1C3 siRNA-1 (si-3)	UCUCCACUAUUUUAACAGUdTdT	ACUGUUAAAAUAGUGGAGAdTdT
AKR1C3 siRNA-2 (si-4)	GGUGAGGAACUUUCACCAAdTdT	UUGGUGAAAGUUCCUCACCCdTdT

Table S2. Primers used in the RT-qPCR analysis.

	Forward primer (5' --- 3')	Reverse primer (5' --- 3')
PSA	CGCAAGTTCACCCTCAGAAGGT	GACGTGATACCTTGAAGCACACC
TMPRSS2	CCTCTAACTGGTGTGATGGCGT	TGCCAGGACTTCCTCTGAGATG
AR	ATGGTGAGCAGAGTGCCCTATC	ATGGTCCCTGGCAGTCTCCAAA
AR V7	CCATCTTGTCGTCTTCGAAATGTTA	TTTGAATGAGGCAAGTCAGCCTTTCT
DHRS11	CACCAGTGGTTGGAAGGACA	GTCGTGGAGTTTGAAGGCGA
AKR1C3	TCAAAGCTTTGGTCCACTTTTCA	CCATTTTCATCTGTTGGTGAAAG
β -Actin	CCACGAAACTACCTTCAAC	GATCTTCATTGTGCTGGG