

SUPPLEMENTARY DATA

Antitumour and acute toxicity studies of 4-(pyridin-4-yl)-6-(thiophen-2-yl)pyrimidin-2(1H)-one against ehrlich ascites carcinoma and sarcoma-180

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Table S1. *In vivo* anticancer activity of SK-25 against sarcoma-180 (solid)

		Sarcoma-180 (Solid)						
Treatment	Dose (mg/Kg/i.p)	Average body weight (g)				Av.Tumor weights (mg)	%Tumor Growth Inhibition	Mortality
		Day1st	Day 5th	Day 9th	Day 13th			
Control	0.2 ml N.S.	21.58	22.32	23.61	26.02	1217.3±95.92	-	0/10
SK-25	20	21.35	22.27	23.56	23.52	816.78±120.89**	32.90	0/7
SK-25	30	21.15	22.32	23.43	22.64	661.14±57.44***	45.68	0/7
5-FU	22	21.17	22.29	22.36	22.69	594.0±95.90***	51.20	0/7

***P < 0.001, **P < 0.01

Table S2. Hematological values following treatment of SK-25 in EAC tumor (Solid) and Sarcoma-180 (solid) tumor bearing Swiss mice.

Parameters	EAC (Solid) tumor				Sarcoma-180 (Solid) tumor			
	Normal control	5-FU	SK-25 (20 mg/kg)	SK-25 (30 mg/kg)	Normal control	5-FU	SK-25 (20 mg/kg)	SK-25 (30 mg/kg)
WBC ($10^3\mu/L$)	40.48±6.17	31.78±5.86	38.78±6.56	32.48±2.51	42.88±5.47	32.16±4.25	36.72±3.60	34.22±3.28
RBC ($10^6\mu/L$)	9.12±0.27	9.07±0.33	9.15±0.04	8.63±0.43	9.22±0.31	8.77±0.29	8.26±0.69	9.03±0.42
Hemoglobin (g/dL)	13.22±0.26	12.60±0.39	11.96±0.43	12.54±0.54	13.1±0.46	12.72±0.24	11.88±0.89	12.35±0.51
Hematocrit (%)	43.32±1.77	43.14±0.20	41.05±0.93	42.84±1.75	43.32±1.77	41.07±0.82	40.30±2.41	39.66±2.84
MCV (fL)	44.68±1.62	46.92±1.36	45.88±1.64	45.80±1.40	44.81±2.91	47.80±1.63	47.42±0.79	47.82±1.78
MCH (pg)	13.72±0.25	14.04±0.28	14.18±0.10	14.20±0.55	13.7±0.38	14.32±0.18	14.44±0.39	14.66±0.74
MCHC^s (g/dL)	31.08±1.44	30.32±0.66	30.80±0.63	29.48±0.52	31.43±0.99	30.05±0.66	29.92±0.36	29.56±0.52
Lymphocytes (%)	67.7±6.00	58.50±5.99	62.88±3.94	59.66±7.63	66.36±7.56	54.85±4.64	57.25±5.99	48.3±7.73
Neutophils (%)	34.71±4.35	31.9±5.07	33.38±2.32	27.96±2.68	31.9±3.27	33.54±4.07	29.39±2.88	29.33±2.45
Monocytes	5.78±2.20	7.68±1.63	8.7±1.58	9.7±1.58	5.63±0.93	8.38±2.39	8.56±1.66	9.3±1.34
Esinophils (%)	1±0.42	2.38±0.48	1.46±0.90	2.44±0.56	0.9±0.26	2.1±0.40	0.7±0.43	1.95±0.31
Basophils (%)	0.10±0.03	0.12±0.02	0.12±0.02	0.10±0.00	0.16±0.02	0.13±0.11	0.27±0.06	0.21±0.20

Values are mean ± S.E. (n = 5) statistically no significant variation in control when compared with treated groups by applying one way ANOVA followed by Bonferroni test.

Table S3. Mean values of various biochemical parameters measured from serum of after therapy of SK-25 in EAC (Solid) and Sarcoma-180 (Solid) tumor bearing Swiss mice.

Parameter	EAC (Solid) tumor	Sarcoma-180 (Solid) tumor
s		

	Normal control	5-FU	SK-25 (20 mg/Kg)	SK-25 (30 mg/Kg)	Normal control	5-FU	SK-25 (20 mg/Kg)	SK-25 (30 mg/Kg)
Glucose (mg/dl)	85.23 ± 6.23	66.56±6.3 5	78.52±3. 57	88.12±7.2 7	82.38±4.8 6	68.71±5.4 4	81.12±5. 37	87.86±9. 23
SGPT (UL⁻¹)	40.16±5.54	36.62±2.8 3	41.12±2. 50	43.56±3.5 4	40.64±4.2 4	38.66±3.7 5	40.56±3. 87	40.19±2. 04
SGOT (UL⁻¹)	40.88±3.45	34.45±3.4 7	39.88±2. 45	35.60±1.2 5	41.78±2.2 8	36.96±1.3 8	39.98±2. 84	38.27±3. 11
Alkaline Phosphat e (UL⁻¹)	41.51±3.72	40.72±5.2 2	41.66±3. 32	41.16±3.3 4	41.3±5.32	40.23±4.3 4	40.25±3. 22	39.87±2. 88
Total Proteins (g/dl)	5.23±0.12	5.21±0.11	5.43±0.1 2	5.95±0.11	5.39±0.12	5.46±0.15	5.77±0.1 1	6.15±0.1 2
Urea(mg/ dl)	44.25±4.69	39.44±0.3 5	48.22±1. 37	47.61±3.1 7	43.25±2.4 8	36.44±2.4 9	44.44±1. 25	40.22±3. 21
Uric Acid (mg/dl)	5.32±0.53	6.54±0.11	6.36±0.8 6	7.48±0.53	6.12±0.43	6.62±0.53	6.21±0.5 9	7.12±0.5 8
Creatinin (mg/dl)	0.39±0.01	0.37±0.01	0.35±0.0 1	0.34±0.01	0.38±0.01	0.39±0.01	0.40±0.0 1	0.41±0.0 1
Triglycerid es	108.10±10. 86	118.31±6. 24	98.12±5. 86	94.18±10. 86	109.09±9. 82	116.38±8. 46	99.11±4. 86	95.43±3. 51
Cholester ol (mg/dl)	81.32±3.21	85.6±4.44	77.3±4.1 8	72.8±9.42	82±3.25	86.3±6.12	77.2±3.7 8	70.7±9.5 8

Values are mean ± S.E. (n= 5), no statistically significant variation in control when compared with treated groups by applying one way ANOVA followed by Bonferroni test.

Table S4. General manifestation and behavioral examination of SK-25 treated mice in acute toxicity assay.

Observations	After administration			
	4h	24h	7 th day	14 th day
Fur and Skin	N	N	N	N
Eyes	N	N	N	N

Mucous membrane	N	N	N	N
Salivation	N	N	N	N
Behavioral patterns	N	N	N	N
Respiratory patterns	N	N	N	N
Lethargy	N	N	N	N
Sleep	N	N	N	N
Tremors	NO	NO	NO	NO
Diarrhea	NO	NO	NO	NO
Convulsions	NO	NO	NO	NO
Abdominal contortions	NO	NO	NO	NO
Coma	NO	NO	NO	NO

N: Normal, NO: Not Observed

Table S5. Effects of SK-25 on body weight, food intake and mortality in acute toxicity assay.

Parameters	SK-25 as a single dose					
	Days	Control	5 mg	50 mg	300 mg	1000 mg
Food Intake (g)	7	6.77 ± 0.47	7.01 ± 0.31	6.33 ± 0.49	6.78 ± 0.40	6.41 ± 0.43
	14	7.04 ± 0.38	7.07 ± 0.47	6.96 ± 0.41	6.60 ± 0.33	7.36 ± 0.23
Body weight (g)	0	22.28 ± 0.57	22.53 ± 0.44	22.43 ± 0.40	22.03 ± 0.53	22.66 ± 0.28
	7	22.81 ± 0.49	23.27 ± 0.40	23.02 ± 0.32	22.52 ± 0.22	23.95 ± 0.41
	14	23.75 ± 0.52	23.71 ± 0.48	23.33 ± 0.21	23.27 ± 0.25	24.25 ± 0.52
Mortality	14	0/6	0/6	0/6	0/6	0/6

Table S6. Gross pathological findings of SK-25 in acute toxicity assay at 1000 mg/kg as a single dose.

Dose (mg/kg)	Animal	Fate	Gross pathological findings	
			External	Internal
SK-25 5mg/kg	1	TS	NAD	NAD
SK-25 5mg/kg	2	TS	NAD	NAD
SK-25 5mg/kg	3	TS	NAD	NAD
SK-25 5mg/kg	4	TS	NAD	NAD
SK-25 5mg/kg	5	TS	NAD	NAD

SK-25 5mg/kg	6	TS	NAD	NAD
SK-25 50mg/kg	7	TS	NAD	NAD
SK-25 50mg/kg	8	TS	NAD	NAD
SK-25 50mg/kg	9	TS	NAD	NAD
SK-25 50mg/kg	10	TS	NAD	NAD
SK-25 50mg/kg	11	TS	NAD	NAD
SK-25 50mg/kg	12	TS	NAD	NAD
SK-25 300mg/kg	13	TS	NAD	NAD
SK-25 300mg/kg	14	TS	NAD	NAD
SK-25 300mg/kg	15	TS	NAD	NAD
SK-25 300mg/kg	16	TS	NAD	NAD
SK-25 300mg/kg	17	TS	NAD	NAD
SK-25 300mg/kg	18	TS	NAD	NAD
SK-25 1000mg/kg	19	TS	NAD	NAD
SK-25 1000mg/kg	20	TS	NAD	NAD
SK-25 1000mg/kg	21	TS	NAD	NAD
SK-25 1000mg/kg	22	TS	NAD	NAD
SK-25 1000mg/kg	23	TS	NAD	NAD
SK-25 1000mg/kg	24	TS	NAD	NAD

TS: Terminal Sacrifice, NAD: No Abnormalities Detected

Table S7. Computed parameters for DMPK parameters.^a

	SK-25
molecule	
#stars	0
#amine	0
#amidine	0
#acid	0
#amide	0
#rotor	1
#rtvFG	0
CNS	0
mol_MW	255.294
dipole	9.893
SASA	501.212
FOSA	0

FISA	117.935
PISA	331.745
WPSA	51.532
volume	813.487
donorHB	1
accptHB	4.5
dip ² /V	0.1203072
ACxDN ^{.5} /SA	0.0089782
glob	0.840835
QPpolrz	28.928
QPlogPC16	9.36
QPlogPoct	15.261
QPlogPw	9.679
QPlogPo/w	2.278
QPlogS	-3.917
CIQPlogS	-3.653
QPlogHERG	-5.69
QPPCaco	754.262
QPlogBB	-0.482
QPPMDCK	698.647
QPlogKp	-2.428
IP(eV)	9.244
EA(eV)	1.475
#metab	1
QPlogKhsa	-0.062
HumanOralAbsorption	3
PercentHumanOralAbsorption	91.786
SAfluorine	0
SAamideO	0
PSA	64.023
#NandO	4
RuleOfFive	0
#ringatoms	17
#in34	0
#in56	17
#noncon	0
#nonHatm	18
RuleOfThree	0
Jm	0.115

^aA description of each parameter and the recommended ranges for 95% of known drugs is available on the QikProp user manual (http://gohom.win/ManualHom/Schrodinger/Schrodinger_2015-2_docs/qikprop/qikprop_user_manual.pdf)