Supplemental Material

Metabolism of SKLB-TB1001, a potent Anti-tuberculosis Agent, in Animals

Running title: *In Vivo* Metabolism of SKLB-TB1001

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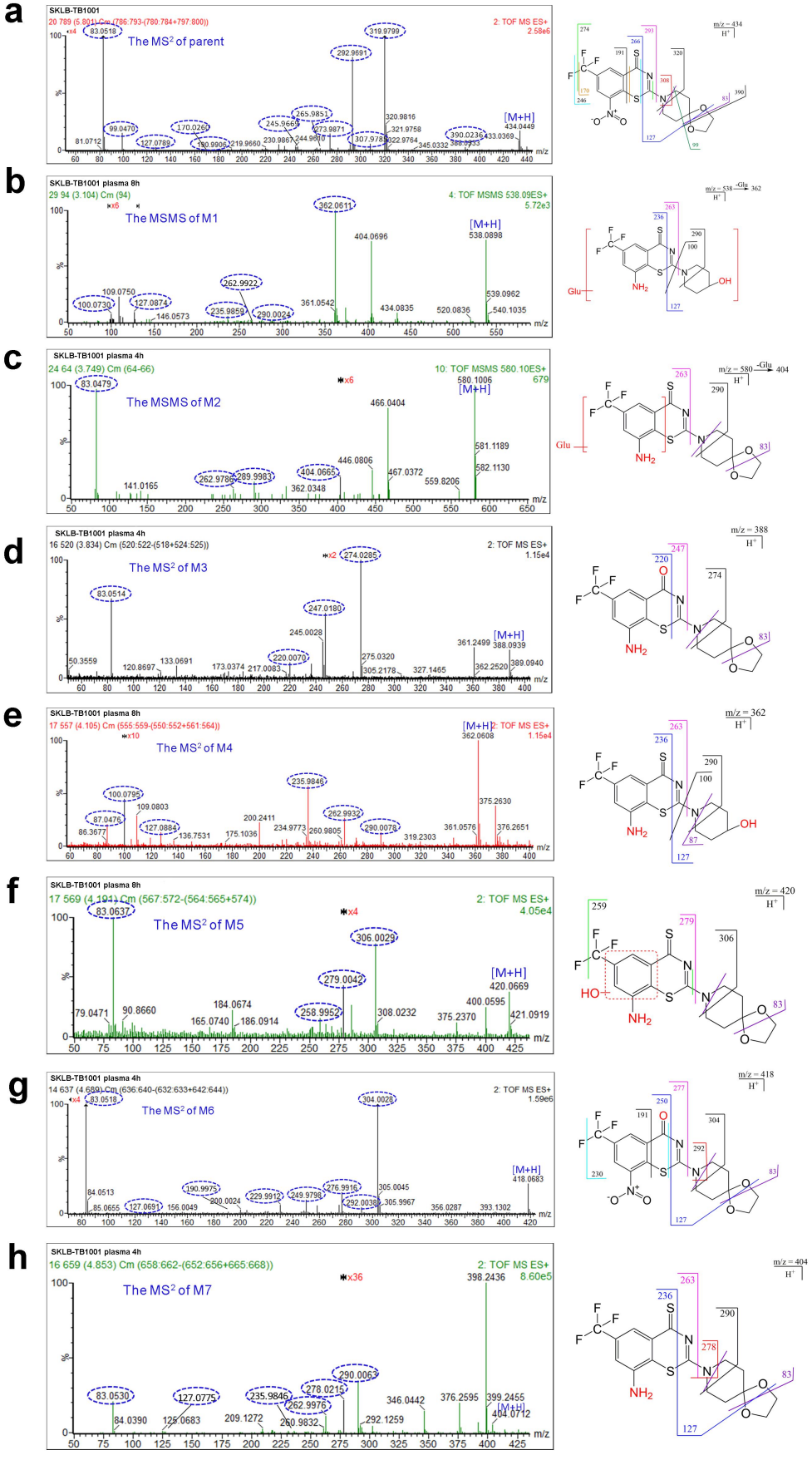
a
SKLB-TB1001
The MS² of parent
[M+H]
b
SKLB-TB1001 plasma 8h
The MSMS of M1
[M+H]
c
SKLB-TB1001 plasma 4h
The MSMS of M2
[M+H]
d
SKLB-TB1001 plasma 4h
The MS² of M3
[M+H]
e
SKLB-TB1001 plasma 8h
The MS² of M4
[M+H]
f
SKLB-TB1001 plasma 8h
The MS² of M5
[M+H]
g
SKLB-TB1001 plasma 4h
The MS² of M6
[M+H]
h
SKLB-TB1001 plasma 4h
The MS² of M7
[M+H]

FIG S1 a. MS fragmentation assignment of SKLB-TB1001; b. M1 was identified as glucuronidation product of M4 (nitro reduction and O-dealkylation product of SKLB-TB1001); c. M2 was identified as glucuronidation product of M7 (nitro reduction product of SKLB-TB1001); d. M3 was identified as a product with replacement of sulfur by oxygen and nitro reduction of SKLB-TB1001; e. M4 was identified as O-dealkylation product of M7 (nitro reduction product of SKLB-TB1001); f. M5 was identified as the nitro reduction and hydroxylation product of SKLB-TB1001; j. M6 was identified as a product with replacement of sulfur by oxygen of SKLB-TB1001; h. M7 was identified as nitro reduction of SKLB-TB1001

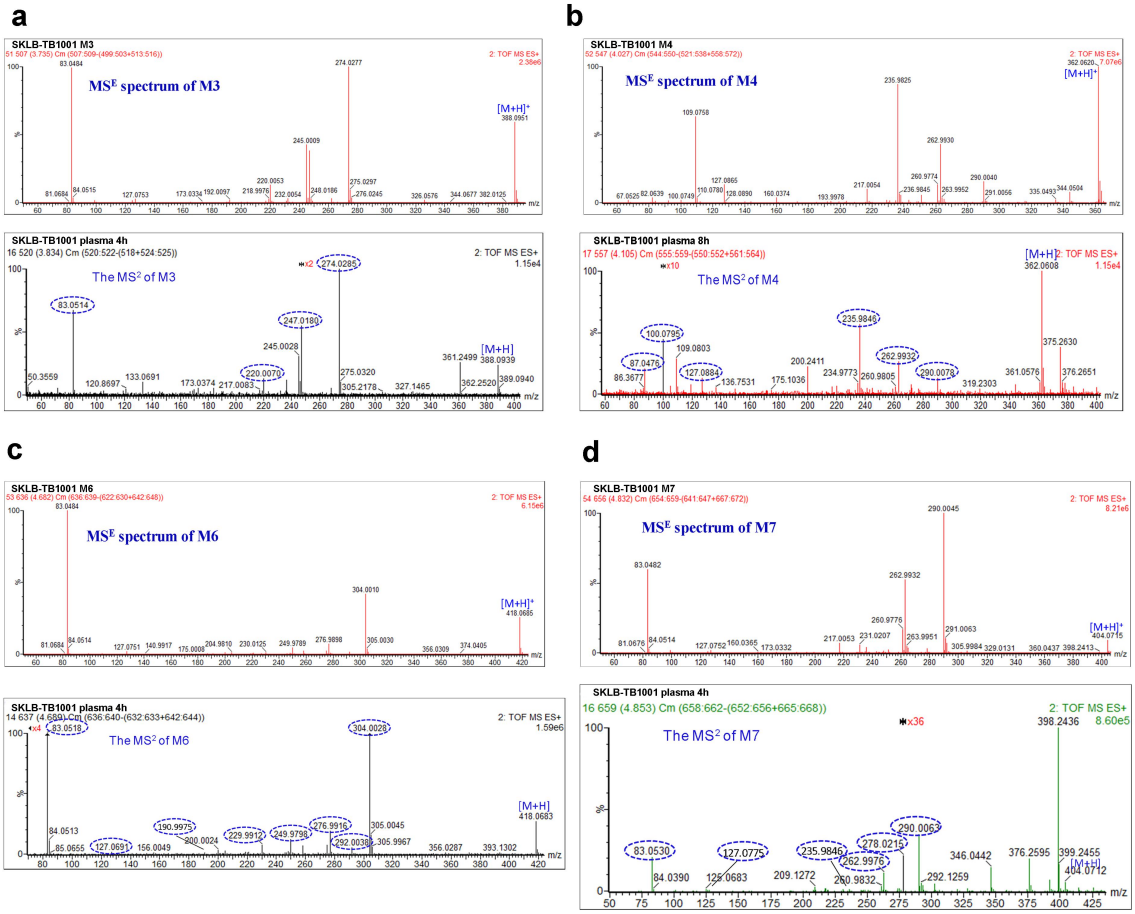

FIG S2 MS^E spectrum of M3, M4, M6, M7 in synthesized standard and in mice plasma

Table S1 Individual and mean Lung[a] to plasma ratio concentration-time data of SKLB-TB1001 after PO dose at 50 mg/kg in male CD1 mice

Lung to plasma ratio								
Dose (mg/kg)	Dose route	Sampling time (hr)	lung/plasma Individual			Mean	SD	CV(%)
50	PO	0.25	0.216	0.264	0.283	0.255	0.0346	13.6
		4	0.374	0.429	0.293	0.365	0.0686	18.8
		8	0.494	0.345	0.289	0.376	0.106	28.2
		24	NA	NA	NA	NA	NA	NA

[a] Lung tissue was homogenized with 3 volumes (v/w) of homogenizing solution (PBS, pH7.4) for 2 min.

NA: Not available.

Table S2 Individual and mean Lung to plasma ratio concentration-time data of M6 after PO dose at 50 mg/kg in male CD1 mice

Lung to plasma ratio								
Dose (mg/kg)	Dose route	Sampling Time(hr)	lung/plasma Individual			Mean	SD	CV(%)
50	PO	0.25	NA	NA	0.960	0.960	NA	NA
		4	NA	NA	NA	NA	NA	NA
		8	NA	NA	NA	NA	NA	NA
		24	NA	NA	NA	NA	NA	NA

Table S3 HPLC condition

Gradient Program:

Time(min)	Flow Rate(μl/min)	A (%)	B (%)
0.00	400	98	2
0.34	400	98	2
8.00	400	5	95
9.00	400	5	95
9.10	400	98	2
10.00	400	98	2

Column: Xbridge Acquity UPLC®BEH C18 (2.1×50 mm, 1.7 μm)

Mobile Phase: A (H2O with 0.1% formic acid)

B (ACN with 0.1% formic acid)

Table S4 MS condition

UPLC-UV-G2-S Q-Tof: MS^E Centroid ESI (+)

Scan Mode: MS^E Centroid
Source
Capillary (KV): 3.00(+)
Sampling Cone: 40
Source Offset: 80
Temperature (℃)
Source:120
Desolvation: 350
Gas Flows (L/h)
Cone Gas: 50
Desolvation Gas: 600