Salicylic acid conjugate of telmisartan inhibits CHIKV infection and inflammation

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Supporting Information

1. Supplementary methods

SM1. Determination of oral acute toxicity of DDABT1

The starting dose was 50mg/kg body weight. DDABT1 was mixed with 1% CMC to achieve the desired concentration. All animals received a single dose of DDABT1 by oral route after being fasted for approximately 15 to 16h, but with free access to water. A dose volume of 10mL/kg was used. Food was supplied approximately 3h to 4h after administration of DDABT1. The study was repeated with higher doses (300mg/kg and 2000mg/kg) and the results were summarized in **Table S1**.

SM2. Anti-inflammatory properties of DDABT1

SM2.1 Acute anti-inflammatory properties of DDABT1

The Wistar rats (180 – 220g) were used in this study. For this, six groups (n=6) were treated with vehicle, diclofenac (10 mg/kg or 0.033mmol/kg), SA (10 mg/kg or 0.724mmol/kg p.o.), TM (10 mg/kg or 0.0194mmol/kg p.o.), combination (SA 0.724mmol/kg + TM 0.0194mmol/kg p.o.) and DDABT1 (12 mg/kg or 0.0194mmol/kg p.o.) 1h prior to carrageenan injection. About 0.1mL of 1% carrageenan was injected into the sub plantar tissue of the left hind paw of each rat. Swellings of feet were measured at 1, 2, 3 and 4h using water plethysmometer. The mean changes in injected paw volume to initial paw volume were calculated. The increase in the volume of paw following inflammation/edema of the control group of animals (no drug treatment) was assumed as 100%. Accordingly, the percentage of inhibition of paw-edema volume was determined using the following equation. Percent inhibition of paw edema = $(M_c\text{-}M_t/M_c)$ 100, Where, M_c is the measure of the increase in rat

paw-edema in the control group of animals (inflammation); M_t is the measure of the increase in rat paw- edema of the drug-treated group.

SM2.2 Sub-acute anti-inflammatory activity analysis

The region below axilla of rats was shaved and cleaned with 70% ethanol**.** Each sterile cotton pellets weighing 10±1mg was implanted subcutaneously (S.C.), in this region under light ether anaesthesia. The animals were divided into six groups (n=6). Control group of animals received only a vehicle, while the standard group of animals received diclofenac (0.033mmol/kg p.o.). Similarly, test group-1 and test group-2 of animals received SA and TM at a dose of 0.724mmol/kg and 0.0194mmol/kg respectively. Simultaneously, test group-3 animal received SA and TM in combination (0.724+0.0194 mmol/kg), and finally animal of test group-4 received DDABT1 at a dose of 0.0194mmol/kg. The drugs were given once daily for 7 days. On the 8th day, the animals were anesthetized with diethyl ether and the pellets were removed carefully and freed from extraneous tissues. The pellets were weighed for wet weight and then dried in an oven at 60°C until a constant weight was obtained. The measure of exudate formation = wet weight of pellet - dry weight of the pellet. The measure of granuloma tissue formation = dry weight - the initial dry weight of the pellet (10 mg).

SM2.3 Chronic anti-inflammatory activity of DDABT1

Animals were grouped and pre-treated with standard and test compounds as described earlier. Arthritis was induced in rats by the intraplantar injection of 0.1mL of CFA in the left hind paw. The paw diameters of all the animal groups were measured by the Vernier caliper (Mitutoyo, Japan) at day 0 before CFA injections and thereafter on 7, 14, 21 and 28 days after the injection of CFA. The animals were treated with diclofenac, SA, SA+TM and DDABT1 once daily

during the observation periods. Blood sample (about 3 mL) was obtained by cardiac puncture on the 29th day and mixed with 3.8% sodium citrate solution in the proportion of four parts of blood to one part of the citrate solution. The erythrocyte sedimentation rate (ESR) was measured by Westergren's method [1] whereas; serum Rheumatoid Factor (RF) estimation was carried out by turbidimetry method [2]. Further, the arthritic limbs of animals were dissected and stored in 10% formalin until radiographic analysis.

2. Supplementary Figures

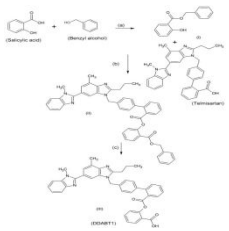

Figure S1. Synthesis of 2-((4'-((1,7'-dimethyl-2'-propyl-1*H*,3'*H*-[2,5'-bibenzoimidazol]-3'-yl)methyl)-[1,1'-biphenyl]-2-carbonyl)oxy)benzoic acid (DDABT1).a) Sulfuric acid was added to a mixture of benzyl alcohol, SA, and refluxed for 9 h to obtain product I; b) Benzyl salicylate was mixed with DCC and DCM(0°C, 1 h). TM mixed with DCM and DMAP was

added to this and stirred under an N_2 atmosphere for the synthesis of product II; c) This was dissolved in methanol, following the addition of tert-butanol and palladium on carbon, stirred under the hydrogen atmosphere (6 h) to yield DDABT1(III).

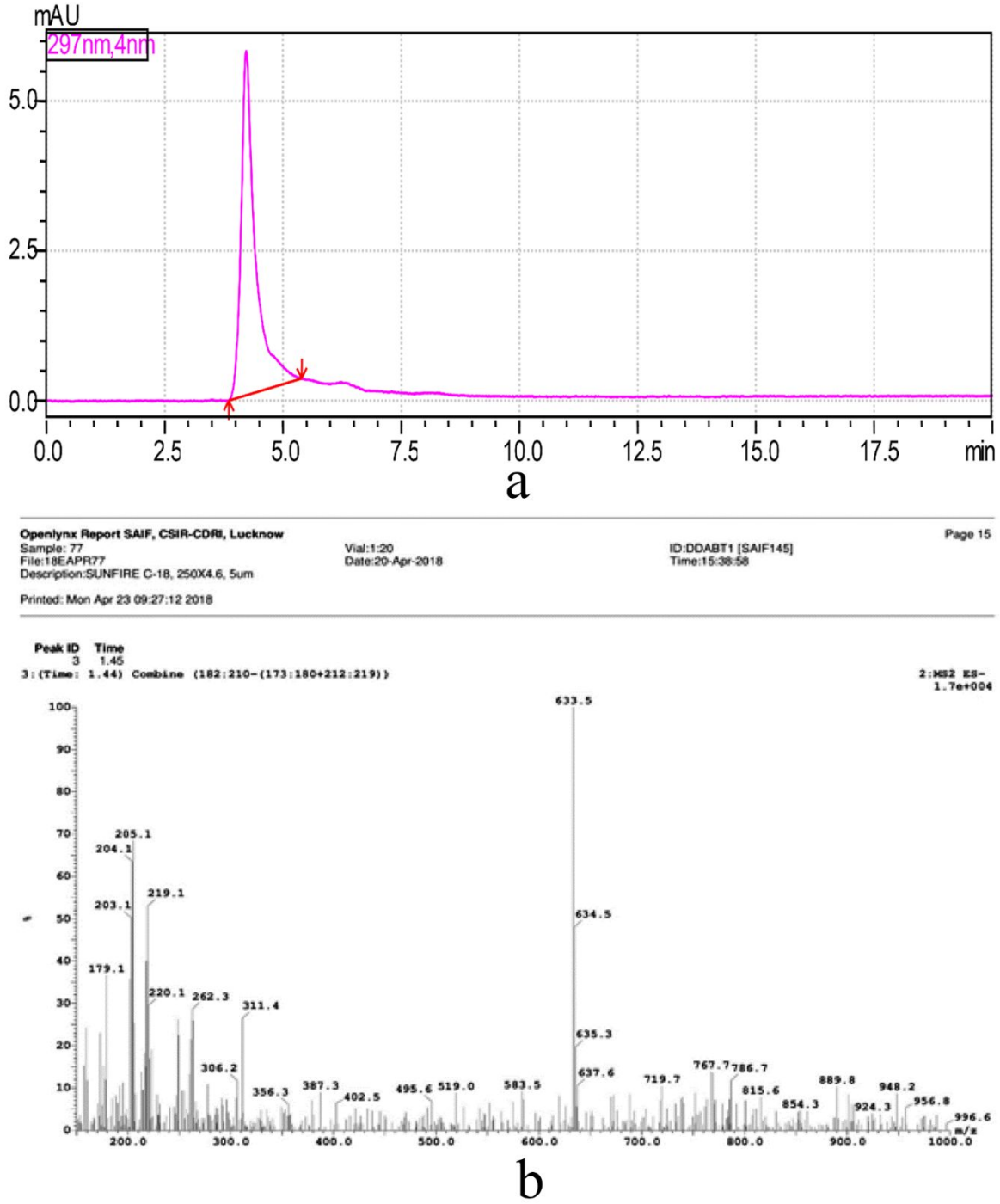

Figure S2. a) HPLC chromatogram of DDABT1.Using a reversed-phase HPLC method with methanol as the mobile phase (flow rate of 0.25mL/min), DDABT1 (4.225min retention time)

was monitored at 297nm for 20 min. **b)** Mass spectrum of DDABT1. The mass spectrum was recorded with the ESI technique in the 6520 Q-TOF Mass spectrometer

3. Supplementary tables

Table S1: Acute oral toxicity of DDABT1 in rats

Step	**Dose (mg/kg)**	**Number of Animals**	**Moribund/ dead Animals**	**Subsequent dead animal**
1	50	3	0	0
2	50	3	0	0
3	300	3	0	0
4	300	3	0	0
5	2000	3	0	0
6	2000	3	0	0

Table S2. Comparison between DDABT1 and TM.

Table (A) shows the comparison between the viral titer of DDABT1 (100 μM) and TM (50 μM). Table (B) shows the comparison between the fold change of E2 and nsP2 genes of CHIKV by DDABT1 (100 μM) and TM (100 μM). Table (C) shows the comparison between relative band intensities of E2 and nsP2 proteins of CHIKV when treated with DDABT1 (100 μM) and TM (100 μM) respectively. Table (D) shows the comparison between the time of addition of DDABT1, telmisartan, and ribavirin.

(A)

Percentage of viral titer							
	0hpi	2hpi	4hpi	6hpi	8hpi	10hpi	12hpi
DDABT1	1.315789	2.631579	6.578947	15.78947	15.78947	34.21053	42.10526
TM[1]	5.317688	10.26447	12.5666	10.26447	13.9046	18.83563	24.2567
RB[1]	12.5666	14.62611	20.84111	31.238	42.31603	66.71717	95.06698

(B)

Relative band intensities (Western Blot)		
Viral proteins	DDABT1 (100 μM)	TM (100 μM)[3]
nsP2	30%	6.50%
E2	5%	1.69%

(C)

Fold change (qRT-PCR)		
Viral genes	DDABT1 (100 μM)	TM (100 μM)[3]
E1	17.38059	20.38985
nsP2	4.425	6.550762

(D)

Treatment	Percentage of viral titer	
	DDABT1 (100 μM)	TM (50 μM)[3]
Pre	97%	60%
During	102%	90%
Post	25%	42%

Table S3. CHIKV Primers used in the study

Sl no.	Gene	Primer name	Sequences
1	Envelope 1	CL11F	5’-TGCCGTCACAGTTAAGGACG-3’
2		CL12R	5’-CCTCGCATGACATGTCCG-3’
3	nsP2	QNSP2F	5’-GACCCGTGGATAAAGACGCT-3’
4		QNSP2R	5’-CCCCGCTGTTTCGAGGATAG-3’
5	GAPDH	GAPDHF	5’-CAAGGTCATCCATGACAACTTTG-3’
6		GAPDHR	5’-GTCCACCACCCTGTTGCTGTAG-3’

References

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