Mangostanaxanthone IV ameliorates streptozotocin-induced neuro-inflammation, amyloid deposition, and tau hyperphosphorylation via modulating PI3K/Akt/GSK-3β pathway

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Methodology:

Induction of SAD

To prevent cerebral vein penetration, the freehand ICV technique indicated by Pelleymounter and colleagues [1,2] and modified by Warnock [3] was used in the present study. The needle was introduced at the following co-ordinates from bregma; 1 mm mediolateral, -0.1 mm anteroposterior, and -3 mm dorsoventral. Mice started to exhibit normal behavior 1 min after the injection [4].

Morris water maze (MWM) test

The MWM test traces the animals' learning and visuospatial cognitive functions [5]. A large stainless steel circular pool (150 cm in diameter and 60 cm in height) was used in the present study. It was divided arbitrarily into four equal quadrants with the help of two perpendicular threads. A platform (10 cm width, 28 cm in height), painted in black, was constantly put inside the target quadrant of this pool. The pool was half filled with water which was made opaque using dye so that the platform becomes invisible. The procedure was carried out on five successive days [6]. Each mouse was subjected to two consecutive trials on the first 4 days of the

test, with an interval of at least 15 min between the trials. The maximum time for each trial was 120 s. If the animal managed to find the hidden platform within that time, it was kept there for extra 20 s before being removed. The mouse that failed to find the hidden platform during the designated time was gently guided onto the platform and kept there for 20 s. The mean escape latency (MEL) time which is the time taken by each mouse to find the hidden platform, was recorded for each mouse throughout each of the trials executed over the four testing days and was considered as an acquisition index [7]. On the test day, the time consumed by each mouse in the target quadrant where the hidden platform was previously positioned, was recorded as memory indicator [7,8].

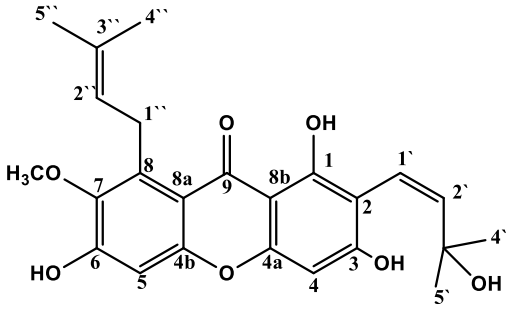
Estimation of biochemical parameters

Mouse ELISA kits were used for the measurement of TNF-α (Cusabio, Wuhan, China) as well as GSH, MDA, H_2O_2, IL-6, and NADPH oxidase brain contents (My Bio Source, CA, USA). The assessment was performed according to the instructions supplied in each kit.

Characterization of isolated compound

Mangostanaxanthone IV**:** Yellow crystals, mp 219 ^{o}C; $[\alpha]_D^{25}$ +14.1 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 248 (4.56), 318 (4.32), 356 (3.98) nm; IR (KBr disc) ν_{max} 3486, 2947, 1644, 1611, 1580, 1052 cm^{-1}; NMR spectral data, see Table 1; HR ESIMS *m*/*z* 427.1759 $[M+H]^+$ (calcd for $C_{24}H_{29}O_7$, 427.1757).

Table S1: NMR data of compounds **Mangostanaxanthone IV (**MX-IV**)** ($CDCl_3$, 850 and 214 MHz).



	MX-IV		
No.	**δ_H (mult., *J* (Hz)**	**δ_C (mult.)**	**HMBC**
1	-	157.9 s	-
2	-	104.5 s	-
3	-	159.9 s	-
4	6.20 s	94.2 d	2, 3, 4a, 8b
4a	-	156.3 s	-
4b	-	155.7 s	-
5	6.82 s	101.7 d	6, 7, 4b, 8a
6	-	154.7 s	-
7	-	142.7 s	-
8	-	137.0 s	-
8a	-	112.2 s	-
8b	-	103.7 s	-
9	-	182.0 s	-
1-OH	13.70 s	-	1, 8b
3-OCH_3	-	-	-
7-OCH_3	3.79 s	62.0 q	7
1`	6.72 d (9.6)	115.7 d	2, 3, 2`, 3`, 4`, 5`
2`	5.57 d (9.6)	127.2 d	1, 2, 3`, 4`, 5`
3`	-	77.9 C	-
4`	1.46 s	28.3 q	2`, 3`, 5`
5`	1.46 s	28.3 q	2`, 3`, 4`
1``	4.08 d (7.8)	26.6 t	7, 8, 8a, 2``, 3``
2``	5.25 tq (7.8, 1.8)	123.1 d	8, 4``, 5``
3``	-	132.0 s	-
4``	1.68 s	25.8 q	2``, 3``
5``	1.82 s	18.2 q	2``, 3``

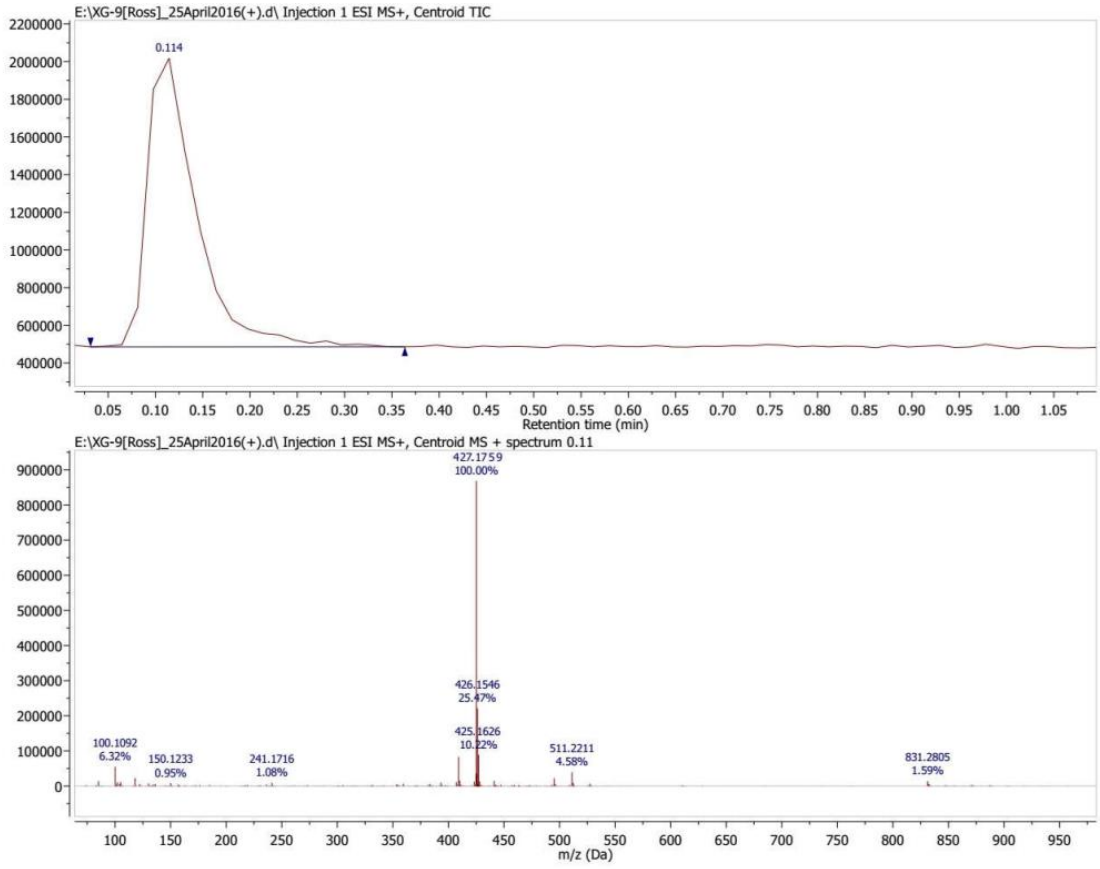


Figure S1: High resolution mass spectra of compound MX-IV

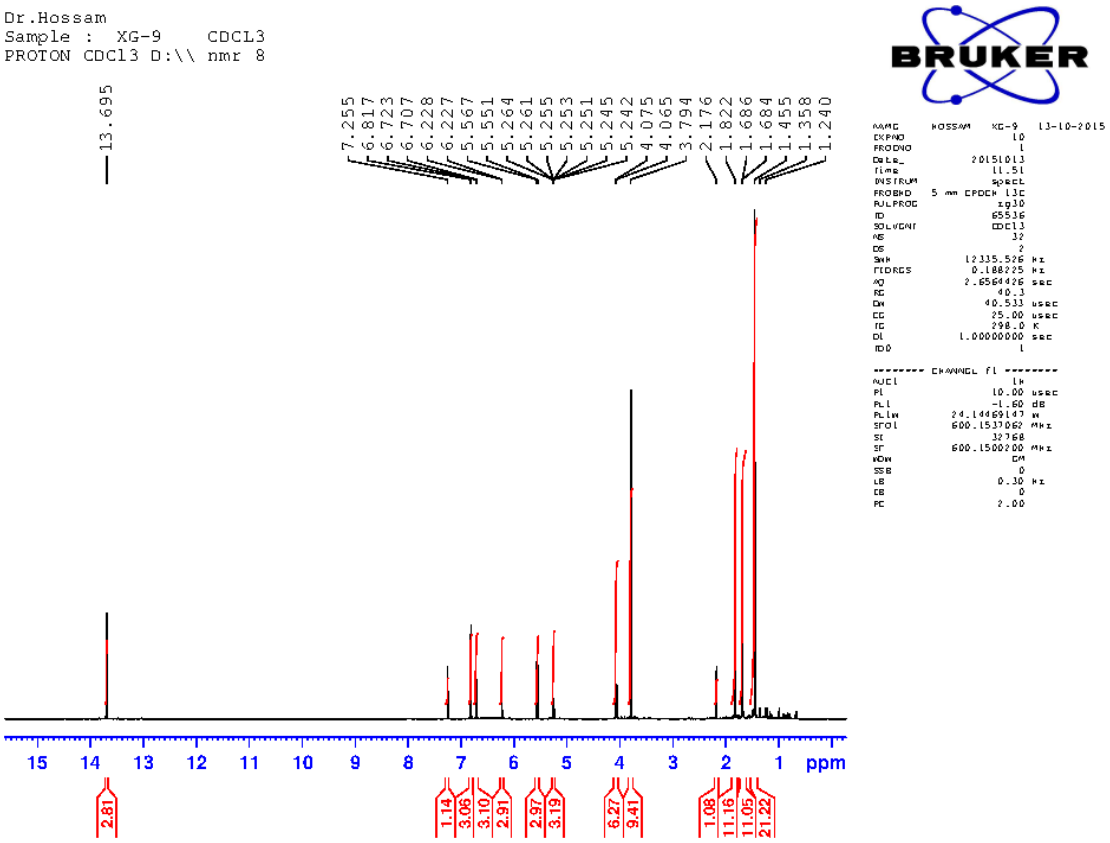


Figure S2: ^{1}H NMR spectra of compound MX-IV in $CDCl_3$

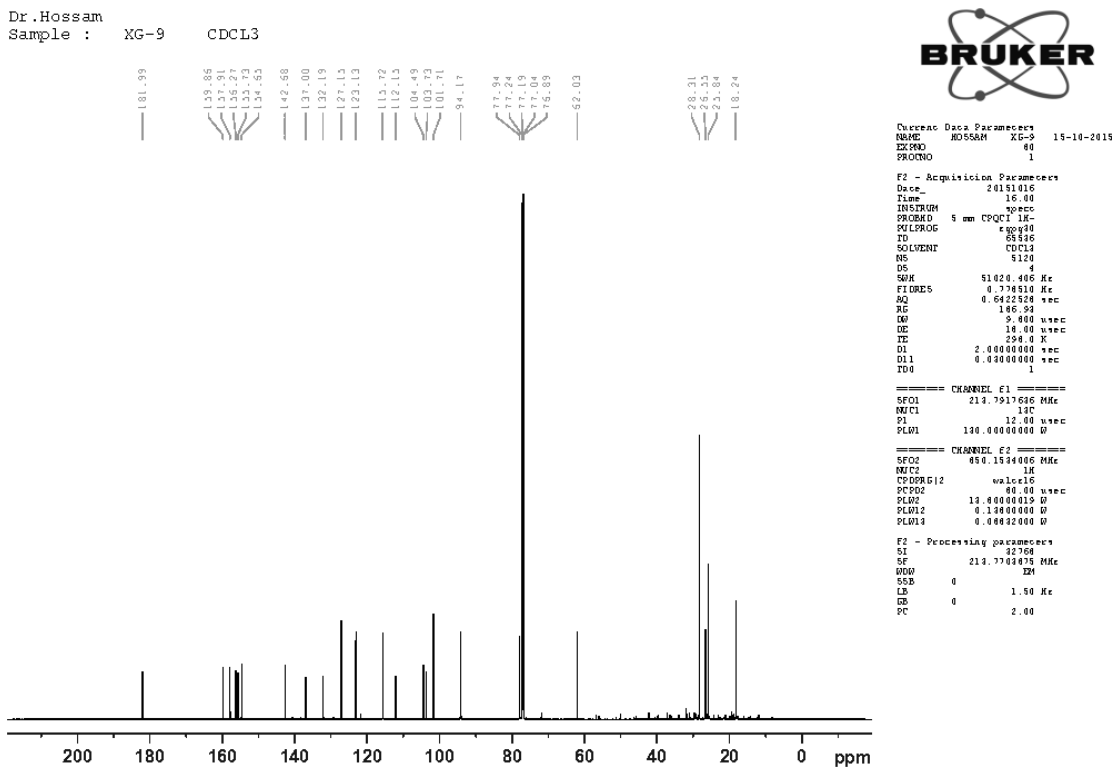


Figure S3: ^{13}C NMR spectra of compound MX-IV in $CDCl_3$

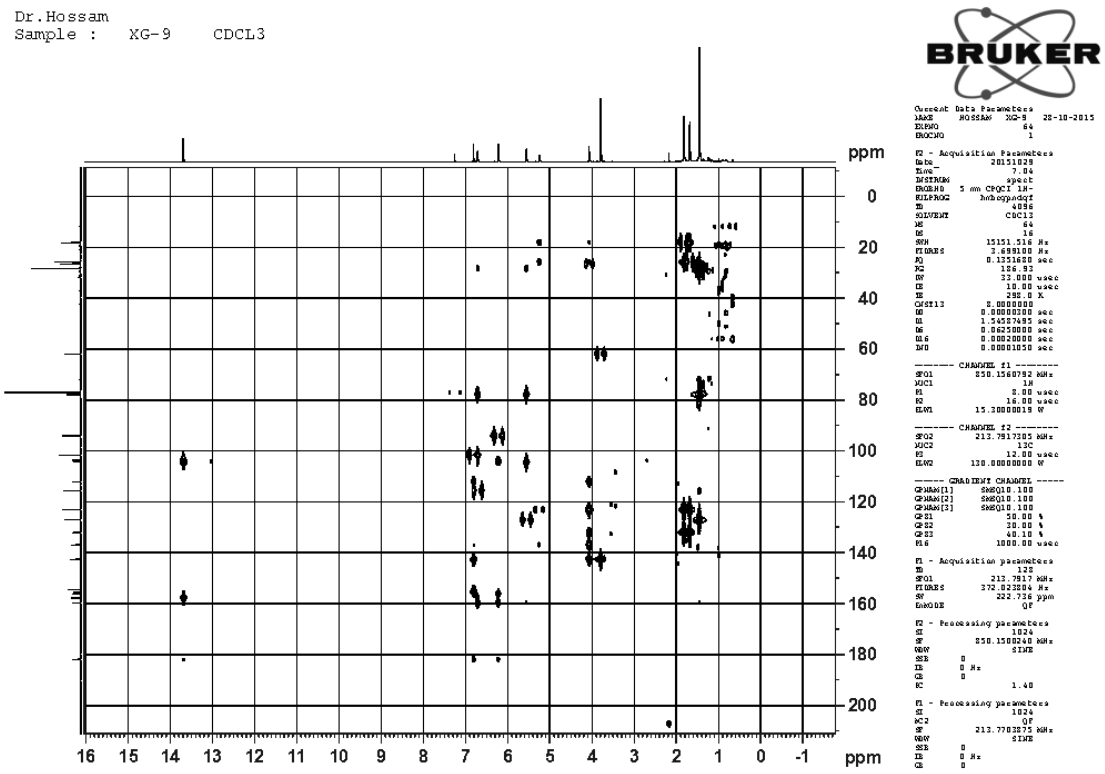


Figure S4: HMBC spectra of compound MX-IV in $CDCl_3$

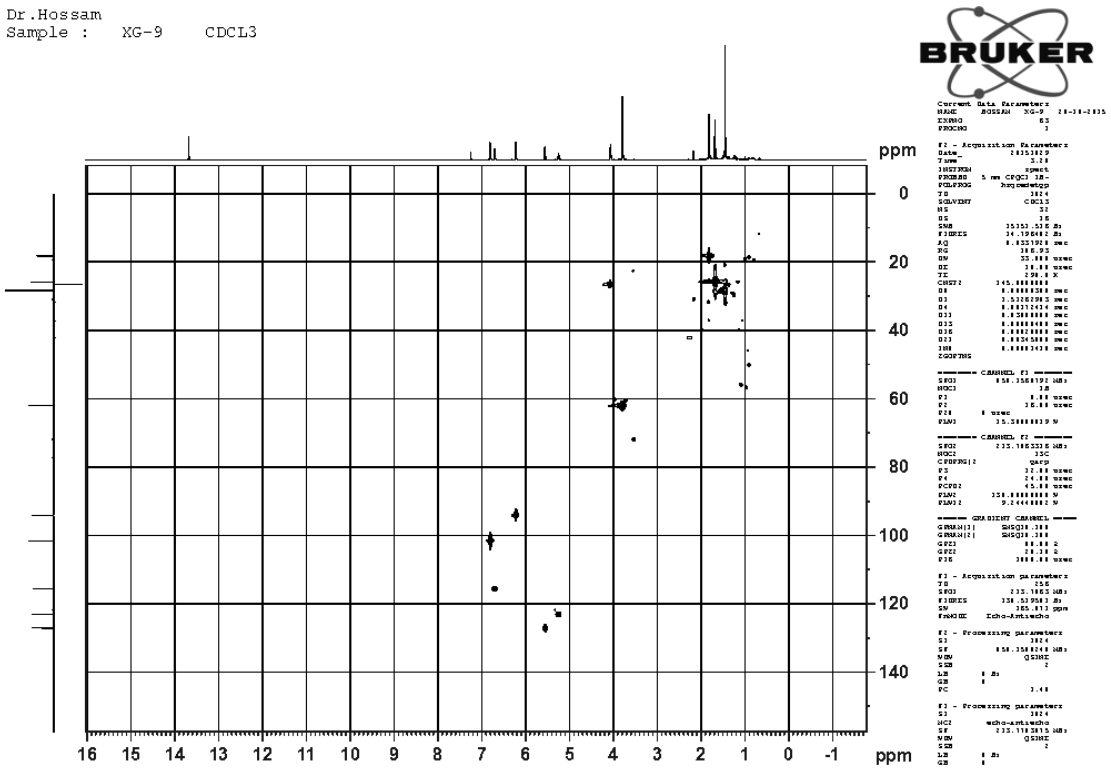


Figure S5: HSQC spectra of compound MX-IV in $CDCl_3$

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