Development of a Novel, Potent, and Selective Sialyltransferase Inhibitor for Suppressing Cancer Metastasis

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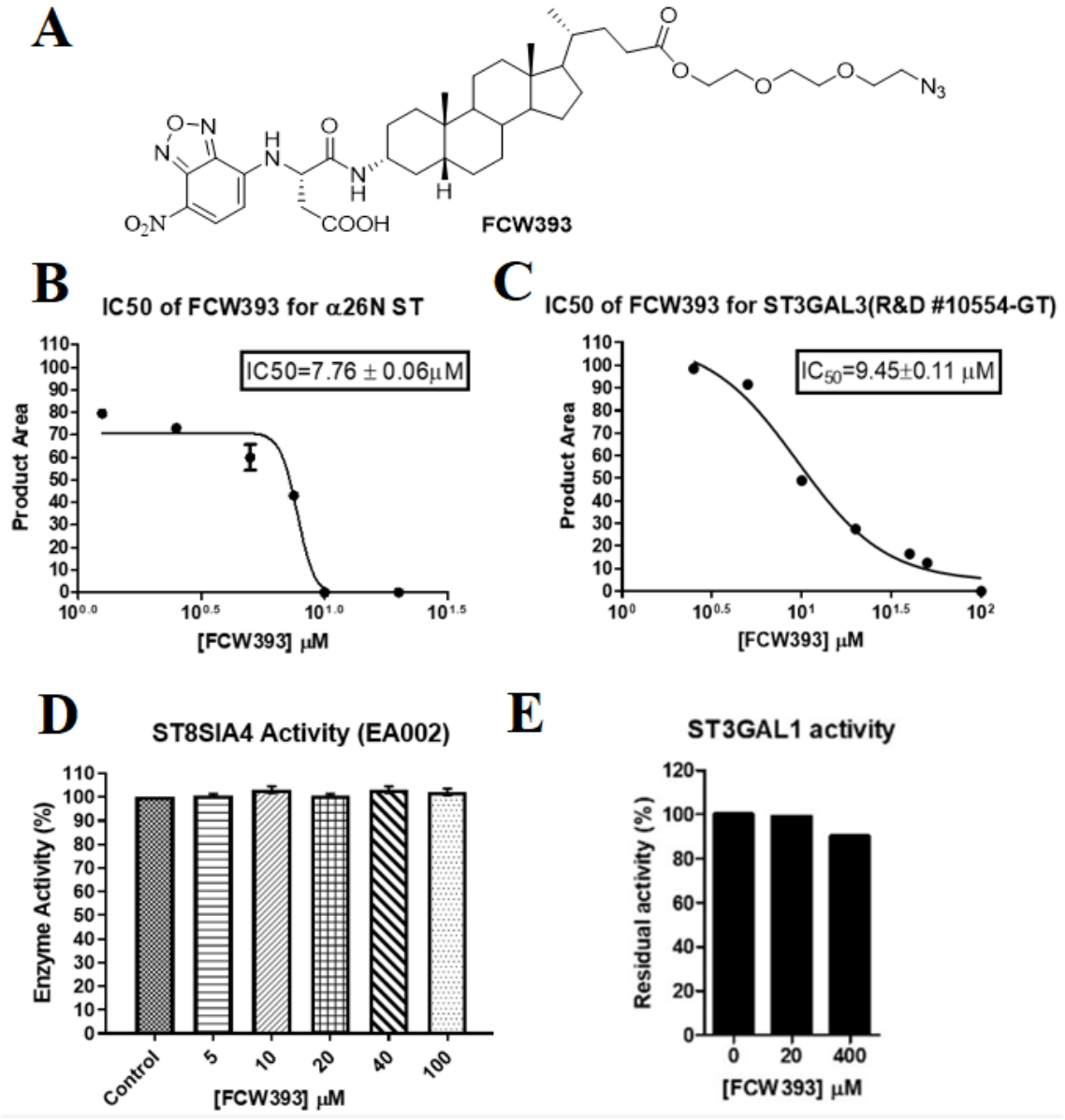
Supplementary Information



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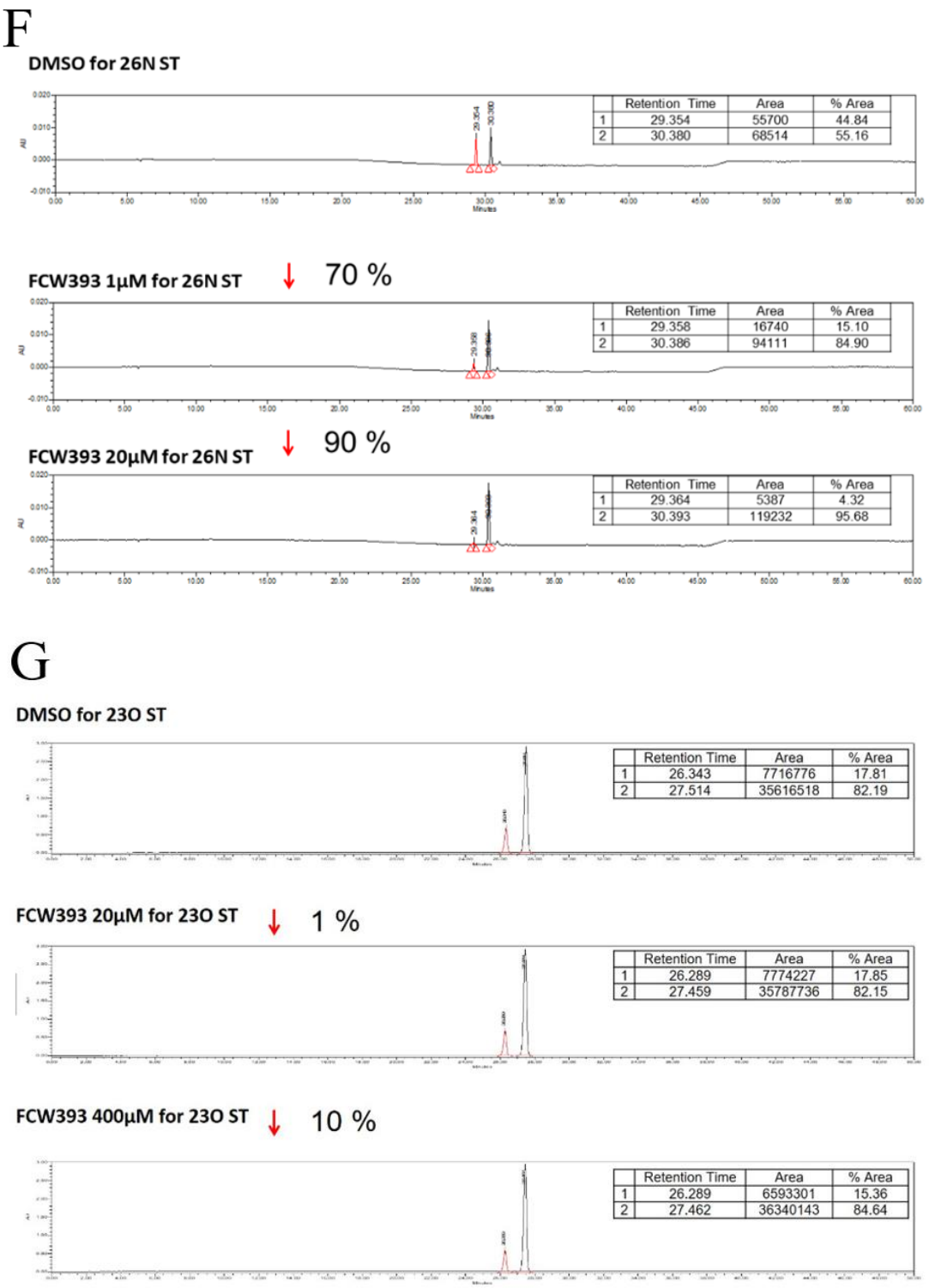


Figure S1. Sialyltransferase inhibition activity of FCW393. (A) Chemical structure of FCW393**;** (B-E) Evaluation of the sialyltransferase inhibition activity of FCW393 on (B) ST6GAL1, (C) ST3GAL3, (D) ST8Sia4 and (E) ST3GAL1 in vitro; (F-G) Representative kinetic data for the inhibition of (F)

ST6GAL1 and (G) ST3GAL1.

The *in vitro* assays for the different ST isozymes were performed as described in the methods section [1]. The data revealed that FCW393 preferentially inhibited both ST6GAL1 (α2,6-*N*-linked sialyltransferase) and ST3GAL3 (α2,3-*N*-linked sialyltransferase) activities rather than ST3GAL1 (α2,3-*O*-linked sialyltransferase) and ST8SIA4 (α2,8-*N*-linked sialyltransferase). Chemical structure of FCW393 is depicted in (A).

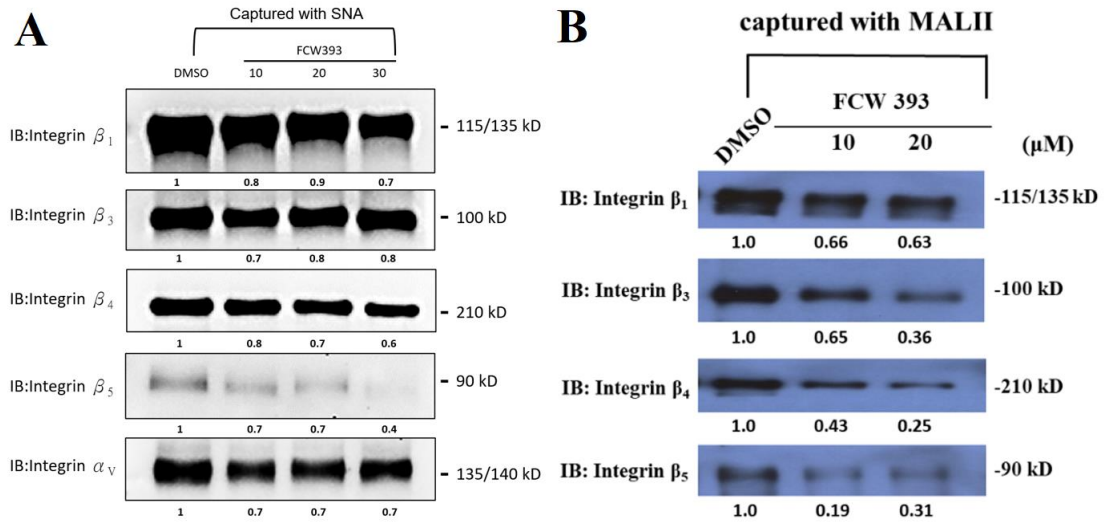


Figure S2. Inhibition of integrin sialylation by FCW393 in MDA-MB-231 cells

MDA-MB-231 cells treated with DMSO, FCW393 were harvested at 48 h after treatment. Cellular proteins were incubated with biotinylated (A) *S. nigra* lectin (SNA) or (B) *M. amurensis* lectin II (MAL II). Streptavidin-agarose beads were added to pull down sialylated proteins and the sialylation status was determined.

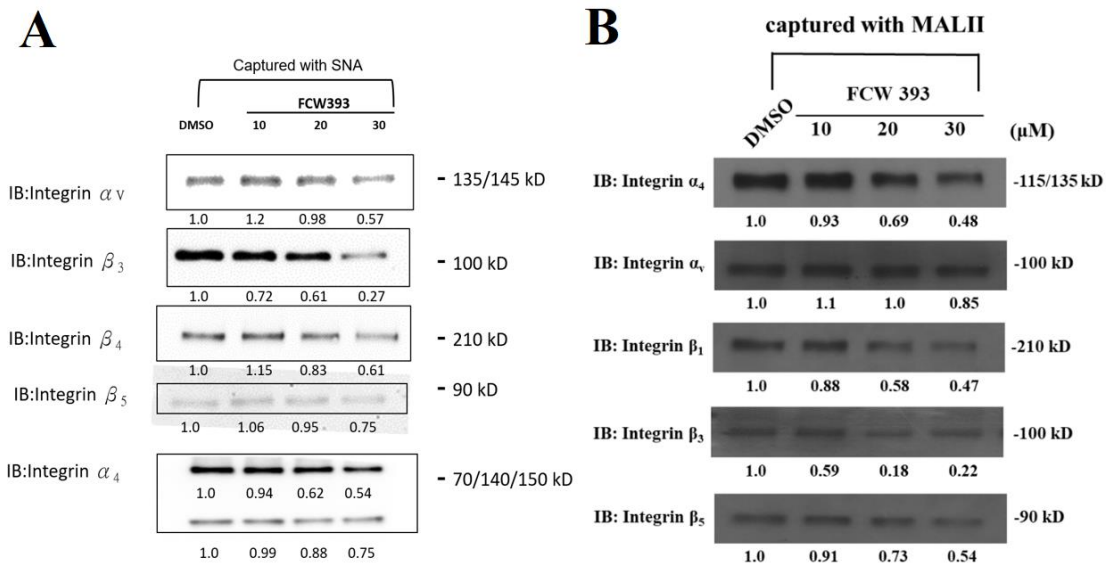


Figure S3. Inhibition of integrin sialylation by FCW393 in B16F10 cells

B16F10 cells treated with DMSO or FCW393 (0–30 μM) were harvested at 48 h after treatment. Cellular proteins were incubated with biotinylated (A) *S. nigra* lectin (SNA) or (B) *M. amurensis* lectin II (MAL II). Streptavidin-agarose beads were added to pull down sialylated proteins and the sialylation status was determined.

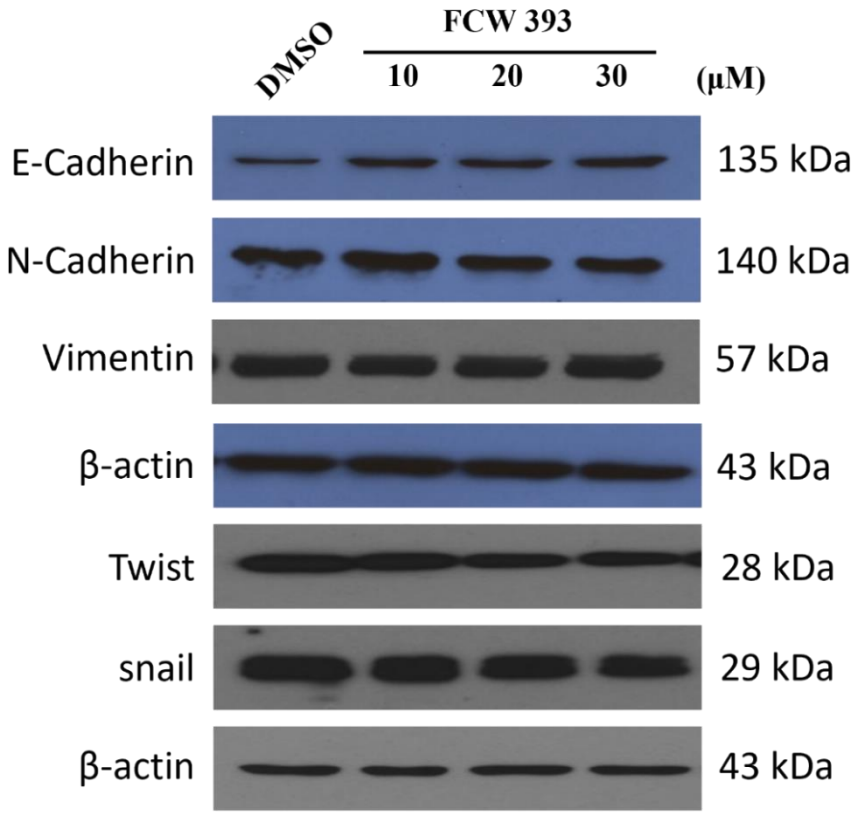


Figure S4. Effect of FCW393 treatment on the expression of EMT signaling in B16F10 melanoma cells

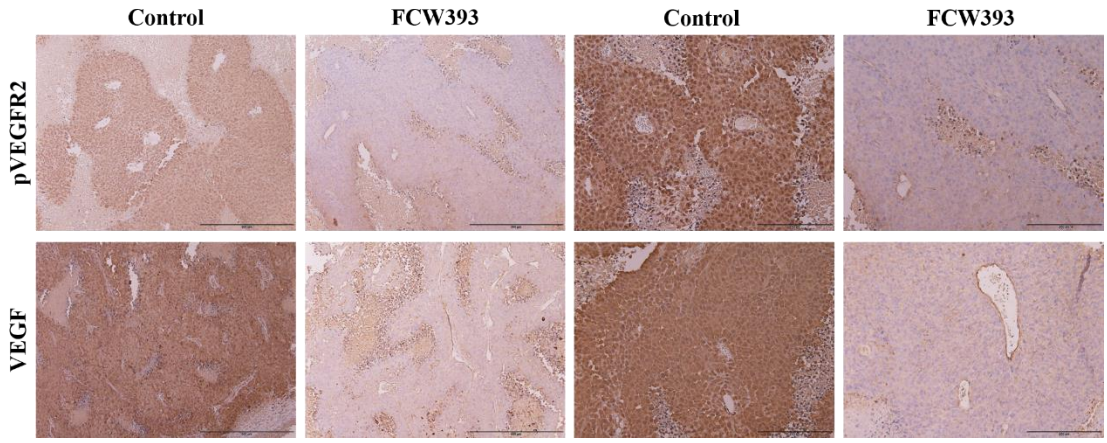


Figure S5. Effect of FCW393 on VEGFR2 and VEGF expression in B16F10 melanoma

After the mice were sacrificed, the melanoma tissues were harvested and examined by immunostaining. Immunohistochemical analysis was used to investigate *p*VEGFR2 (top) and VEGF (bottom) expression level *in vivo*.

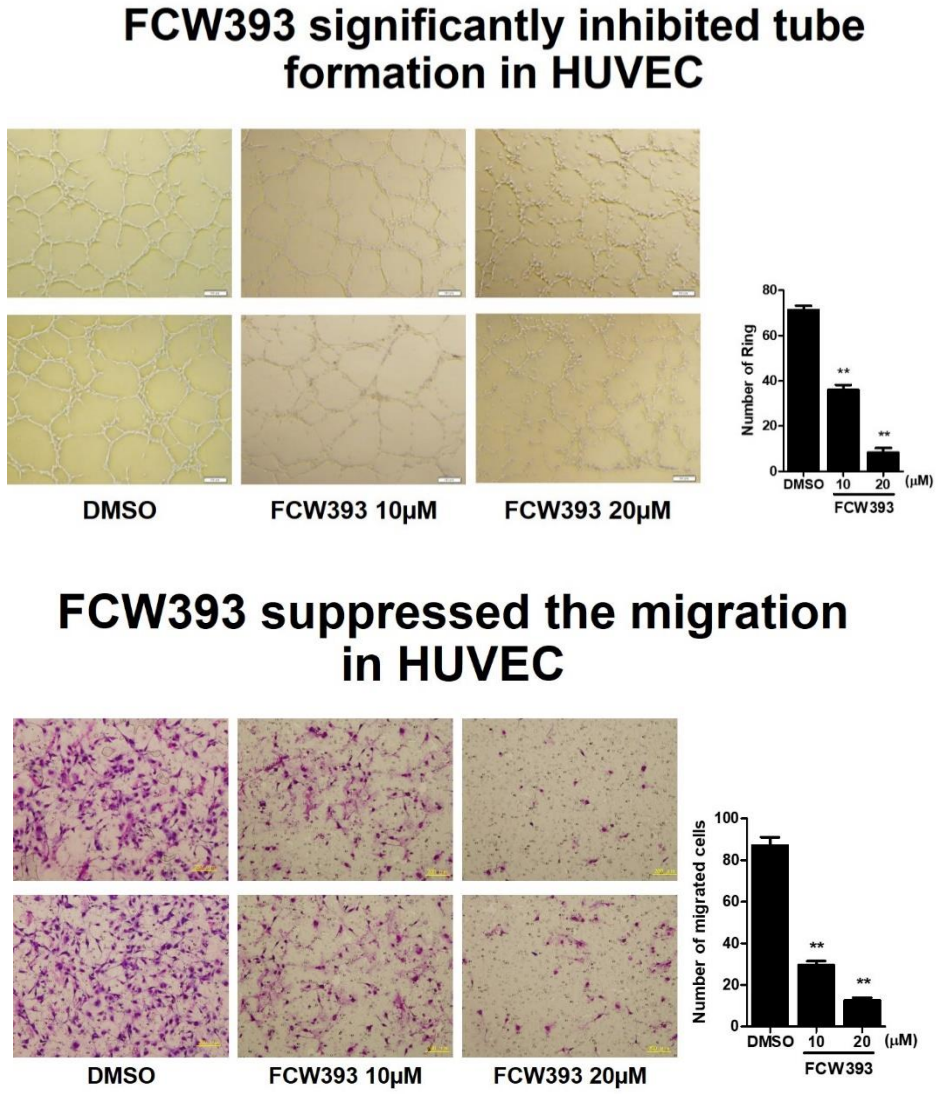


Figure S6. Effect of FCW393 treatment on the migration and tube formation of HUVECs in vitro

The *in vitro* tube formation assay was performed as described previously [2].

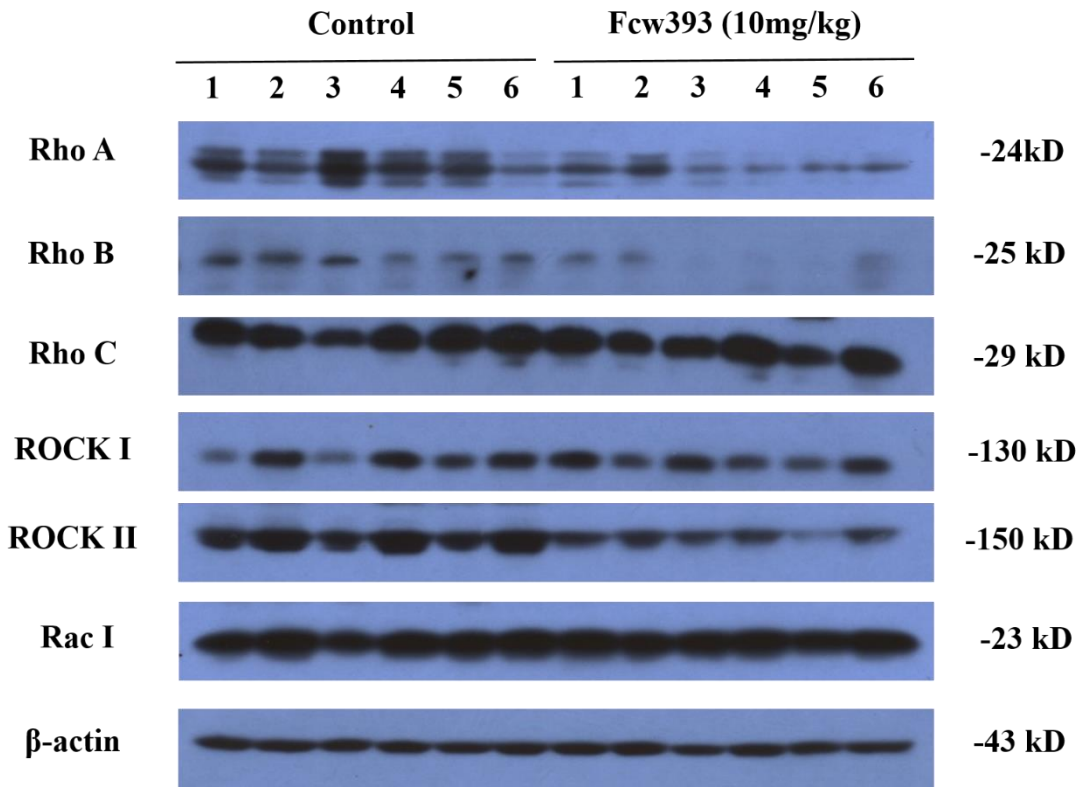


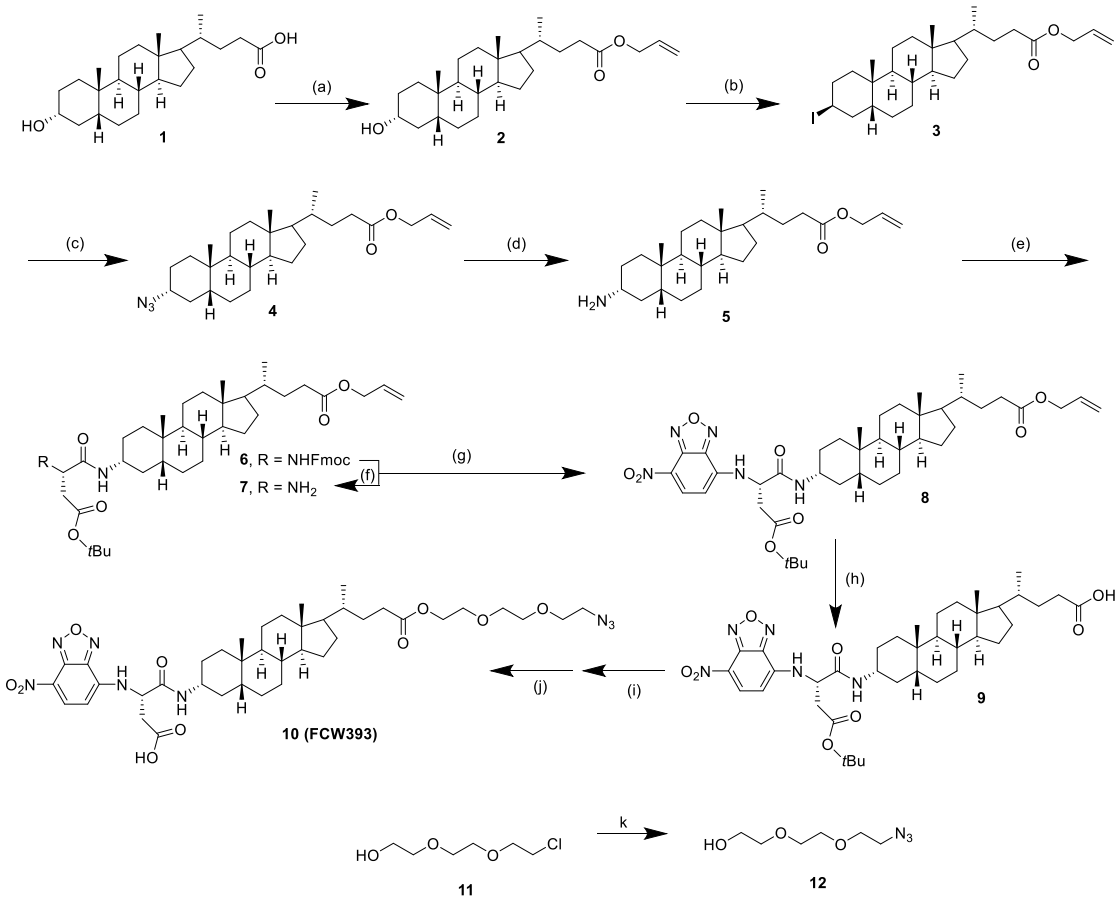
Figure S7. Effect of FCW393 on Rho-ROCK signaling in B16F10 melanoma

After the mice were sacrificed, the tumor proteins were harvested and examined by western blot. The RhoA and RhoB expression levels were inhibited by FCW393 treatment. And the Rho down-stream activation factor ROCK-II were also suppressed by FCW393 treatment (n = 6 in each group).

References:

1. Fu, C.W.; Tsai, H.E.; Chen, W.S.; Chang, T.T.; Chen, C.L.; Hsiao, P.W.; Li, W.S. Sialyltransferase Inhibitors Suppress Breast Cancer Metastasis. *J. Med. Chem.* **2021**, *64*, 527-542.
2. Chen, J.Y.; Tang, Y.A.; Huang, S.M.; Juan, H.F.; Wu, L.W.; Sun, Y.C.; Wang, S.C.; Wu, K.W.; Balraj, G.; Chang, T.T.; Li, W.S.; Cheng, H.C.; Wang, Y.C. A novel sialyltransferase inhibitor suppresses FAK/paxillin signaling and cancer angiogenesis and metastasis pathways. *Cancer Res.* **2011**, *71*, 473–483.

Scheme S1. Synthesis of compound FCW393



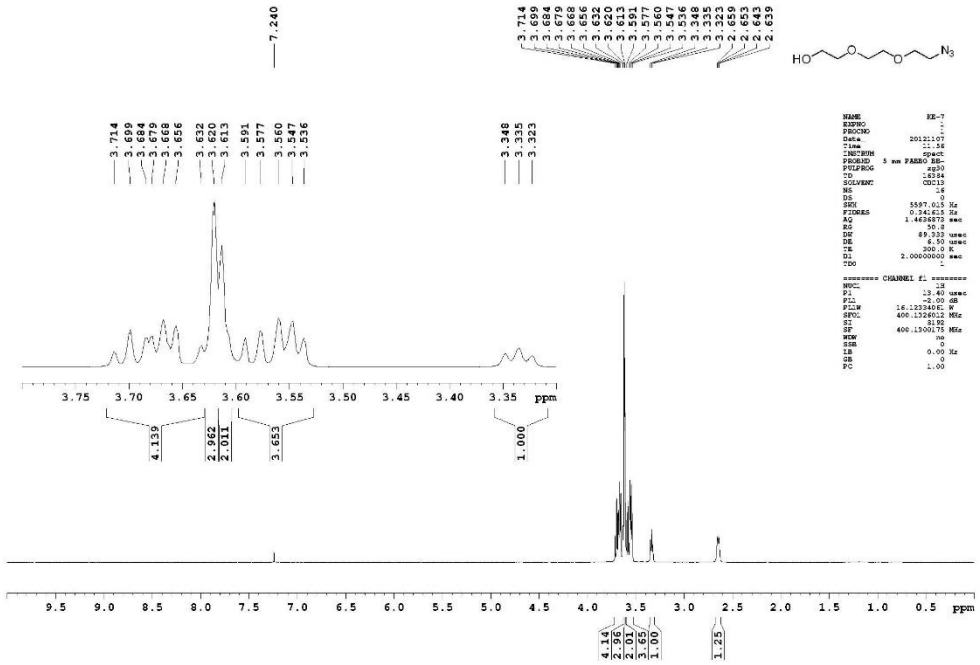
[a] Reagents and conditions: (a) allyl bromide, K_2CO_3, DMF, 100 °C, 12 h; (b) I_2, PPh_3, imidazole, CH_2Cl_2, 0 °C to rt, 12 h, 74 % (two steps); (c) NaN_3, DMF, 100 °C, 3 h, 99 %, (d) PPh_3, THF-H_2O, rt, 12 h, 72 %, (e) Fmoc-L-Asp(O-*t*Bu), HBTU, DIPEA, CH_2Cl_2, rt, 3 h, 93 %, (f) DBU, CH_2Cl_2, rt, 0.5 h, 92 %, (g) NBD-Cl, $NaHCO_3$, THF, EtOH, rt, 2 d, 42 %, (h) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt, 2 h, 95 %, (i) **12**, DCC, DMAP, CH_2Cl_2, rt, 6 h, 72 %, (j) TFA, H_2O, CH_2Cl_2, rt, 3 h, 88 %.

Synthesis of **12**:

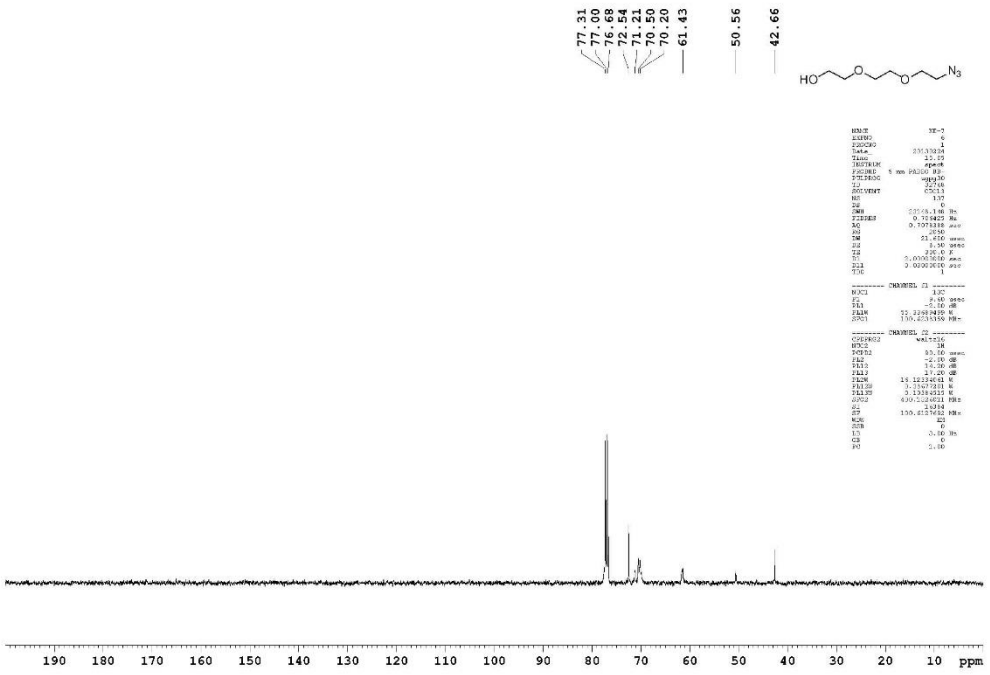
2-(2-(2-Azidoethoxy)ethoxy)ethanol (**12**)

Yield 90 % (colorless oil), ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.71-3.66 (m, 4H), 3.63-3.61 (m, 4H), 3.59-3.54 (m, 3H), 3.34 (t, *J* = 4.8 Hz, 1H), 2.66-2.64 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 72.5, 71.2, 70.5, 70.2, 61.4, 50.6. HRMS calcd for $C_6H_{14}O_3N_3$ $(M + H)^+$, 176.1035; found, 176.1034.

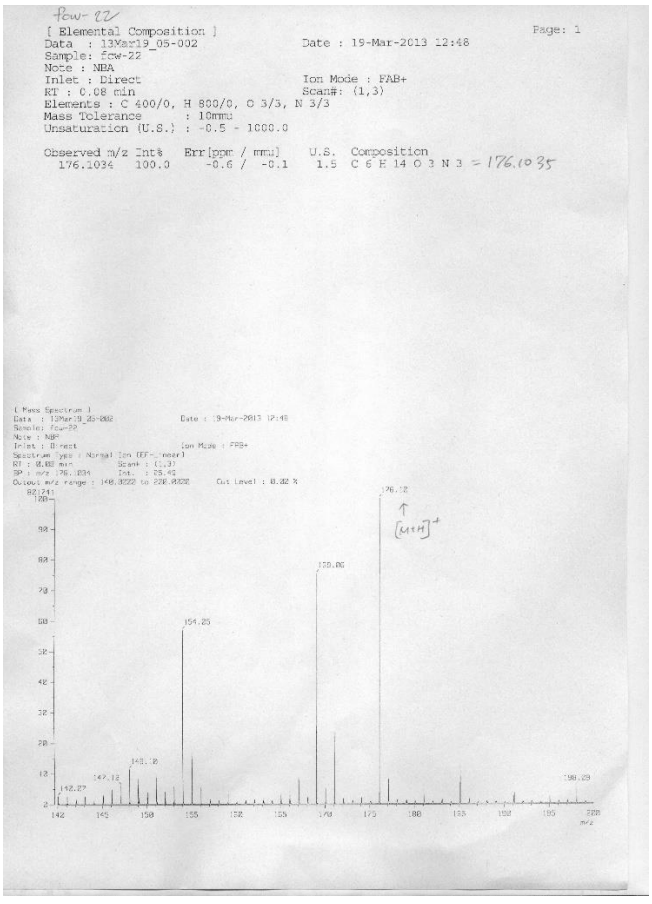
^{1}H- NMR of compound **12**



^{13}C-NMR of compound **12**



HRMS of compound **12**


fcw-22

[Elemental Composition]

Page: 1

Data : 13Mar13_05-002
Date : 19-Mar-2013 12:48
Sample: fcw-22
Note : NBA
Inlet : Direct
Ion Mode : FAB+
RT : 0.08 min
Scan#: (1,3)
Elements : C 400/0, H 800/0, O 3/3, N 3/3
Mass Tolerance : 10mmu
Unsaturation (U.S.) : -0.5 - 1000.0

Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
176.1034	100.0	-0.6 / -0.1	1.5	C 6 H 14 O 3 N 3 = 176.1035

[Mass Spectrum]
Data : 13Mar13_05-002 Date : 19-Mar-2013 12:48
Sample: fcw-22
Note : NBA
Inlet : Direct Ion Mode : FAB+
Spectrum type : Normal Ion (FT-, mass)
RT : 0.08 min Scan# : (1,3)
BP : m/z 176.1034 Int. : 25.42
Output m/z range : 140.0222 to 220.0222 Cut Level : 0.32 %

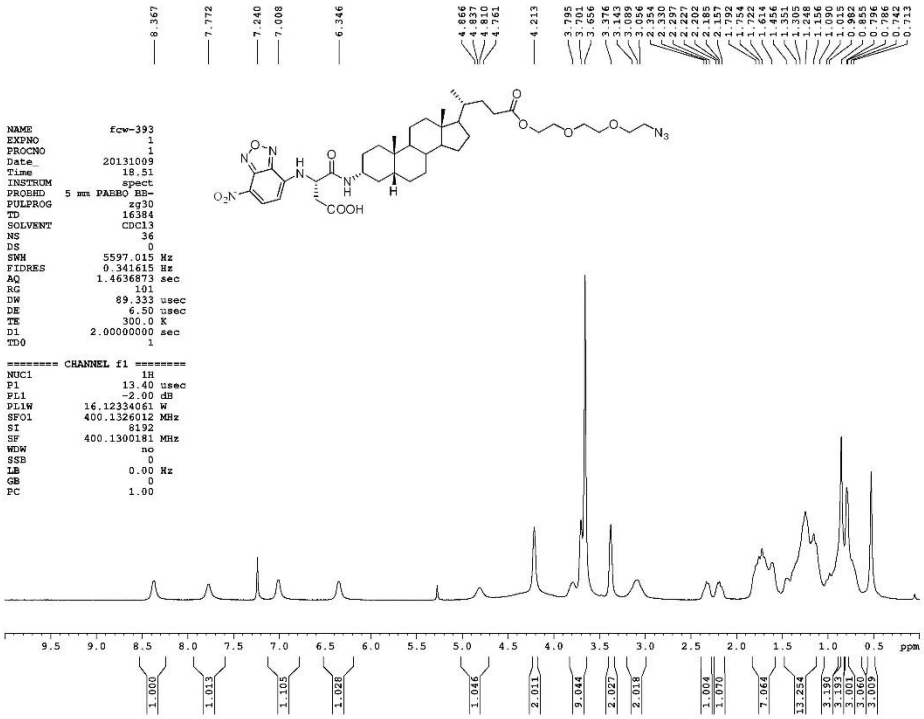
82.241
176.12
[M+H]+
155.05
154.25
143.12
142.12
142.27
198.29
m/z

Synthesis of **FCW393**

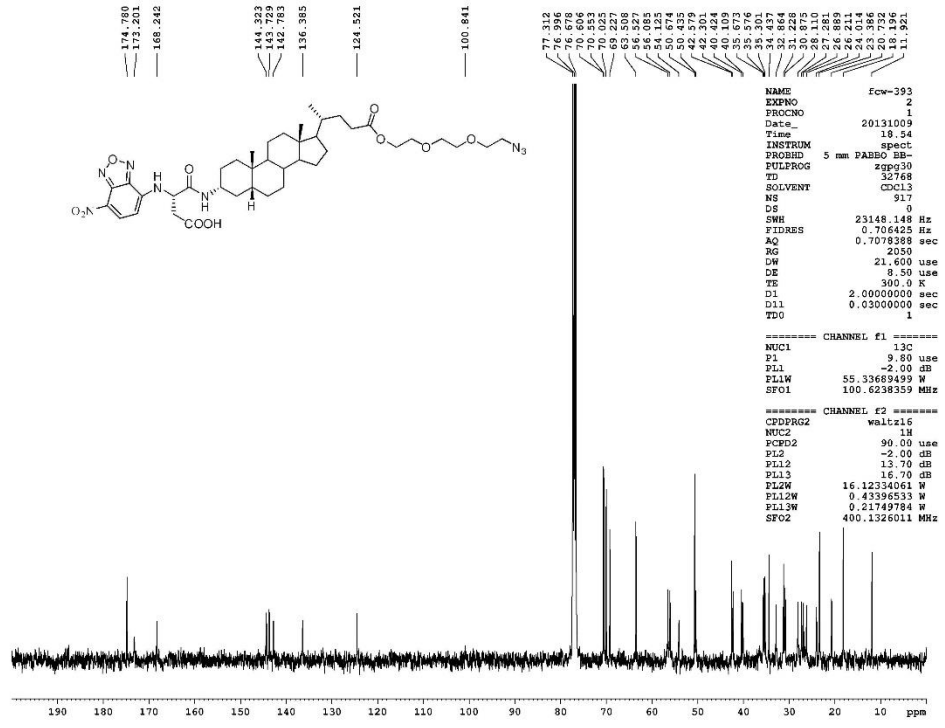
(3S)-4-(((3R,5R,10S,13R)-17-((R)-5-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-5-oxopentan-2-yl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yl)amino)-3-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-4-oxobutanoic acid (**FCW393**)

mp 80-81 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 1H), 7.77 (s, 1H), 7.01 (s, 1H), 6.35 (s, 1H), 4.87-4.76 (m, 1H) 4.21 (s, 2H), 3.80-3.66 (m, 9H), 3.38 (s, 2H), 3.14-3.06 (m, 2H), 2.35-2.30 (m, 1H), 2.23-2.16 (m, 1H), 1.79-1.72 (m, 7H), 1.46-1.09 (m, 13H), 1.02-0.98 (m, 3H), 0.86 (s, 3H), 0.79 (d, *J* = 4.0 Hz, 3H), 0.74-0.67 (m, 3H) 0.53 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.8, 173.2, 168.2, 144.3, 143.7, 142.8, 136.4, 124.5, 100.8, 70.6, 70.5, 70.0, 69.2, 63.5, 56.5, 56.1, 54.1, 50.7, 50.4, 42.6, 42.3, 40.4, 40.1, 35.7, 35.6, 35.3, 34.4, 32.9, 31.2, 30.9, 28.1, 27.3, 26.9, 26.2, 24.0, 23.4, 20.7, 18.2, 11.9. HRMS calcd for $C_{40}H_{58}N_8O_{10}Na$ $(M + Na)^+$, 833.4174; found, 833.4181. HPLC purity: ＞99.5 %.

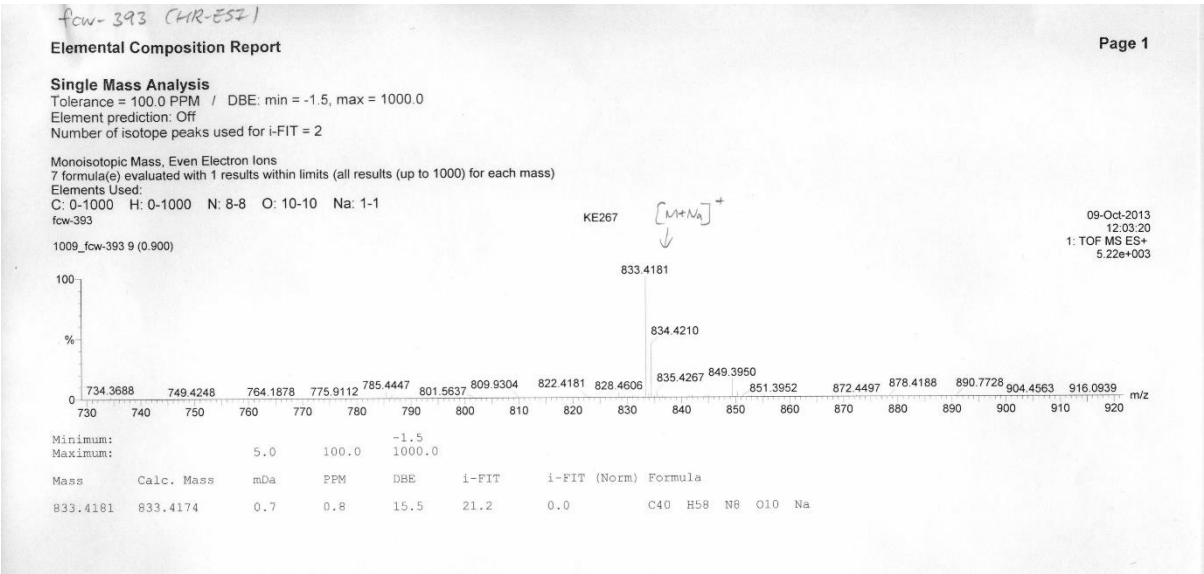
^{1}H- NMR of compound **FCW393**



^{13}C-NMR of compound **FCW393**

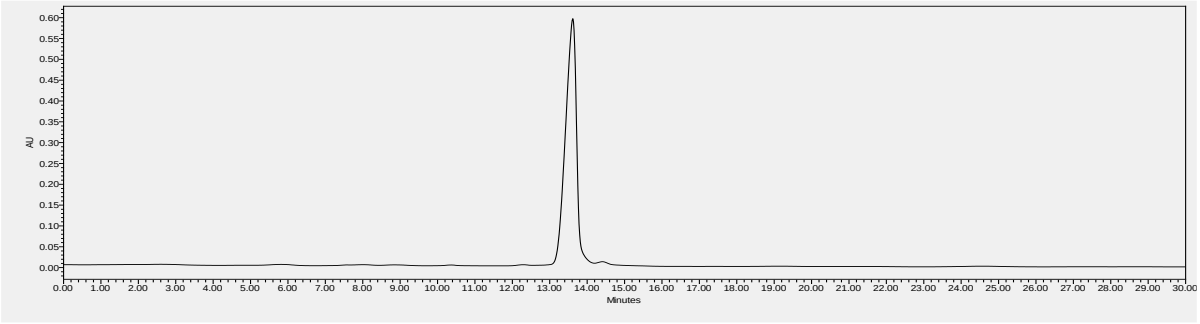


HRMS of compound **FCW393**



HPLC purification of compound **FCW393**

254 nm



Conditions:

A: H_2O + 0.1 % TFA

B: ACN + 0.05 % TFA

Flow A: 0.45 ml/min, Flow B: 1.55 ml/min

Isocratic

Retention time: 13.62 min

Column: Kromasil 300-5C4 column