Chen, L., Pernazza, D., Scott, L.M., Lawrence, H.R., Ren, Y., Luo, Y., Wu, X., Sung, S., Guida, W.C., Sebti, S.M., Lawrence, N.J., and Wu, J.

Inhibition of cellular Shp2 activity by a methyl ester analog of SPI-112

Supplementary Information

1. Chemical synthesis of SPI-112Me

The methyl ester **1** (SPI-112Me) was prepared using the procedure similar to that described earlier [1]. The sulfonamide **3** was prepared from the sulfonyl chloride **2** and used without purification. When the reaction of **3** was performed on a small scale (0.5 g), the product carboxylic acid was isolated as a single geometric isomer (previously assigned as the *Z*-isomer). Attempts to prepare the reaction at larger scales in the microwave reactor led to significant contamination of the product with the *E*-isomer). Esterification of the acid **4** gave the methyl ester **1** that was 99.0% pure as measured by HPLC.

2,3-Dioxoindoline-5-sulfonyl chloride 2
Using the procedure described by Lee et al. [2] from isatin-5-sulfonic acid sodium salt dihydrate (Aldrich) (4.25 g) gave **2** (7.14 g, 40%).

2,3-Dioxo-2,3-dihydro-1*H*-indole-5-sulfonic acid 4-fluorobenzylamide (3).
Diisopropylethylamine (DIPEA) (6.03 mL, 34.9 mmol) and 4-fluorobenzylamine (2.80 g, 22.35 mmol) were added to a solution of the sulfonyl chloride **2** (4.25 g, 17.25 mmol) in anhydrous THF (120 mL) at 0 °C under inert atmosphere. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The resulting mixture was poured into water (100 mL) and extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried (Na_2SO_4) and evaporated to provide **3** (7.14 g) as a yellow-orange solid. This crude product was used without further purification.

(*Z*)-3-(2-(5-(*N*-(4-Fluorobenzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzoic acid (4). A mixture of crude **3** (0.5 g) and 3-hydrazinobenzoic acid (0.23 g, 1.5 mmol) in ethanol (5.0 mL) were heated in a microwave reactor (Biotage Initiator) for 10 minutes at 100 °C. The yellow solid that formed was isolated by filtration on a sintered funnel to provide the product (0.44 g). Under these optimized reaction conditions, approximately 3% of the *E* isomer was observed by ^{1}H NMR. The process was repeated 3 times with the same amounts (exceeding the indicated amounts led to product contaminated with 10% of the *E* isomer). The combined yield of **4** was 1.77 g (78% from **2**). The analytical data of **4** (^{1}H NMR, ^{13}C NMR, LC-MS) agrees with that previously reported [1].

(*Z*)-3-(2-(5-(*N*-(4-Fluorobenzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzoic acid methyl ester (1). Thionyl chloride (0.3 mL) was carefully added, dropwise and at 0 °C, to a mixture of **4** (120 mg, 0.25 mmol) and methanol (1.5 mL) in a microwave tube (CEM, 5 mL size) equipped with a stirring bar. After the addition was completed, the tube was capped and heated in a CEM Discover microwave (MaxPower mode; run time: 5 minutes; hold time: 30 minutes; max pressure: 300 psi; stirring: on) at 85 °C for 30 minutes. The crude product which precipitated was collected by filtration (sintered funnel) and washed with methanol (2.0 mL).

(NB: metallic spatulas or other metallic tools should be avoided until all the thionyl chloride has been removed). The process was repeated 8 times and the products combined and re-dissolved in 100 mL THF. Removal of THF under vacuum afforded the ester **1** (922 mg, 85%) as a yellow solid; HPLC purity: 99.0 % (C18 column; 20% water in MeOH, flow rate 1.0 mL/min, t_R = 4.6 min). The analytical data of **1** (^{1}H NMR, ^{13}C NMR, LC-MS) agrees with that previously reported [1].

(*Z*)-3-(2-(5-(*N*-(4-Fluorobenzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzoic acid –*N*-methylglucamine salt (SPI-112 meglumine salt): The *N*-methylglucamine (meglumine) salt of acid 4 (SPI-112) was prepared using a procedure similar to one described earlier [3]. To a suspension of the acid 4 (50 mg, 0.106 mmol) in anhydrous THF (10.0 mL) was added a solution of N-methylglucamine (20.7 mg, 0.106 mmol) in anhydrous methanol (2.0 mL) under argon, and the resulting mixture was stirred at room temperature overnight. Then the solvent was evaporated *in vacuo* and the residue was dried for 5 hours under high vacuum to give the salt (72mg) in quantitative yield. ^{1}H NMR spectrum confirmed the formation of the meglumine salt with >95% purity. ^{1}H NMR (400 MHz, CD_3OD) δ 8.06 (br s, 1H), 7.97 (d, *J* = 1.8 Hz, 1H), 7.68 (dd, *J* = 8.3, 1.8 Hz, 2H), 7.46 (d, *J* = 9.0 Hz, 1H), 7.40 (d, *J* = 7.7 Hz, 1H), 7.24 (dd, *J* = 8.5, 5.5 Hz, 2H), 7.02 (d, *J* = 8.2 Hz, 1H), 6.94 (t, *J* = 8.8 Hz, 2H), 4.06 (s, 2H), 4.05 – 3.99 (m, 1H), 3.82 (dd, *J* = 4.7, 1.6 Hz, 1H), 3.78 (dd, *J* = 11.0, 3.1 Hz, 1H), 3.73 – 3.60 (m, 3H), 3.13 (s, 1H), 3.12 (d, *J* = 2.8 Hz, 1H), 2.68 (s, 3H).

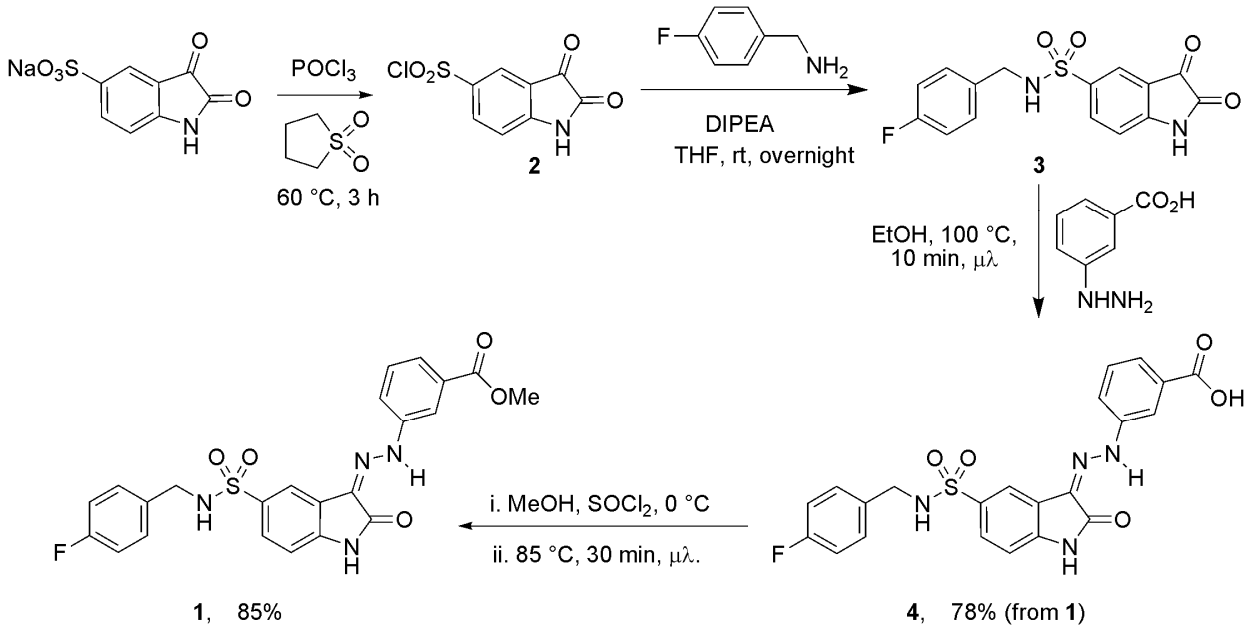
References

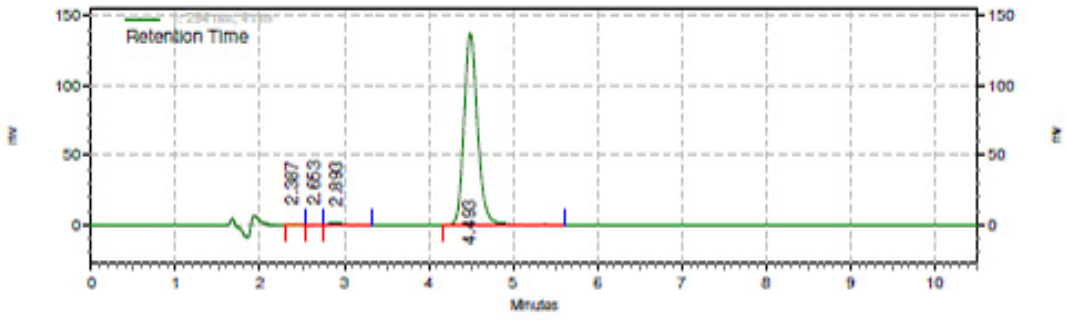
[1] Lawrence HR, Pireddu R, Chen L, Luo Y, Sung SS, Szymanski AM, et al. Inhibitors of Src homology-2 domain containing protein tyrosine phosphatase-2 (Shp2) based on oxindole scaffolds. J Med Chem 2008;51:4948-56.

[2] Lee D, Long SA, Murray JH, Adams JL, Nuttall ME, Nadeau DP, et al. Potent and selective nonpeptide inhibitors of caspases 3 and 7. J Med Chem 2001;44:2015-26.

[3] Gobert RP, van den Eijnden M, Szyndralewiez C, Jorand-Lebrun C, Swinnen D, Chen L, et al. GLEPP1/protein-tyrosine phosphatase phi inhibitors block chemotaxis in vitro and in vivo and improve murine ulcerative colitis. J Biol Chem 2009;284:11385-95.

Scheme 1: Synthetic scheme for the preparation of ester **1**.





1: 254 nm, 4 nm Results

Retention Time	Area	Area %
2.387	2763	0.18
2.653	962	0.06
2.893	11527	0.75
4.493	1530312	99.01
Totals		
	1545564	100.00

HPLC data for 1. Column: Restek Ultra C18 5μM 150mM x 4.6mM; Method: 20% water in MeOH, flow rate 1.0 mL/min, t_R = 4.6 min. Detector: Jasco MD-2010 *plus* Multiwavelength.

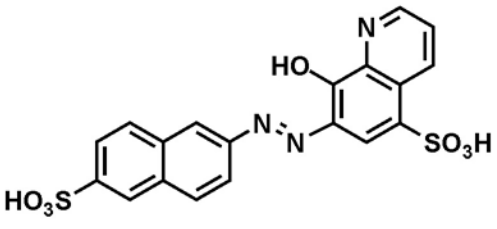
2. HPLC and LC/MS detection of SPI-112

Reference samples and equipment

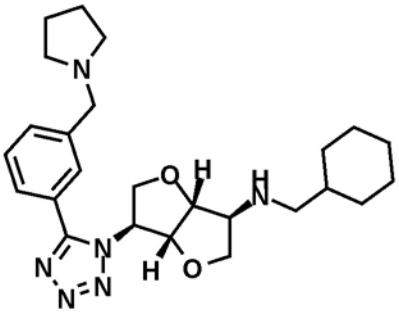
The SPI-112 and SPI-112Me samples (25 μM) were prepared in 20 mM Tris:HCl (pH 7.4) from 100 mM stock in DMSO. The SPI-112 HPLC reference (method: 0.1% diethylamine, 16.5% acetonitrile in water, flow rate 1.0 ml/min, t_R = 7.0 min) and the SPI-112Me HPLC reference (method: 20% water in MeOH, flow rate 1.0 ml/min, t_R = 4.2 min) were obtained with a HPLC equipped with Jasco MD-2010 detector *plus* Multiwavelength (254 nm wavelength) using a Restek Ultra C18 5μM 150mM x 4.6mM column. LC/MS analysis was performed using Agilent 1200 Series LC/6210 ESI-TOF MS and the Analyst QS software.

Detection of SPI-112 in cell sample

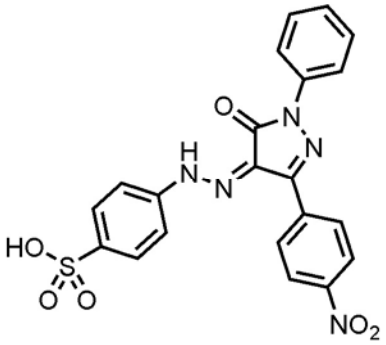
TF-1/Shp2E76K cells (9×10^7) were treated with 25 μM SPI-112Me and cells were disrupted by sonication in 20 mM Tris;HCl (pH7.4). The aqueous insoluble cell material was extracted with 0.6 ml DMSO. The DMSO extract was dried under vacuum and the residue was re-dissolved in 0.1 ml DMSO. After mixing with 20 mM Tris:HCl (75 μl), the sample was centrifuged (3500 rpm, 4 min) and the supernatant was analyzed by HPLC. Both SPI-112Me (method: 20% water in MeOH, flow rate 1.0 ml/min, t_R = 4.1 min) and SPI-112 (method: 0.1% diethylamine, 16.5% acetonitrile in water, flow rate 1.0 ml/min, t_R = 7.1 min) were detected (sFig. 2 and data not shown). LC/MS analysis of the same material confirmed the presence of SPI-112 [ESI–; calculated mass for $C_{22}H_{16}FSN_4O_5$ (M-H): 467.08309; measured mass: 467.08250; error (ppm): -1.26] (sFig. 3).



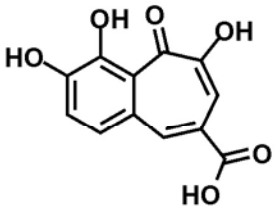
NSC-87877
(Shp2 IC_{50}: 0.32 μM)



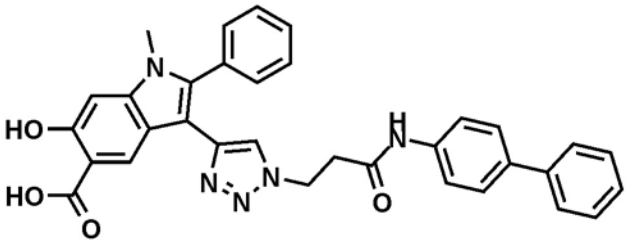
Furanofuran 2a
(Shp2 IC_{50}: 2.5 μM)



PHPS1
(Shp2 IC_{50}: 2.1 μM)

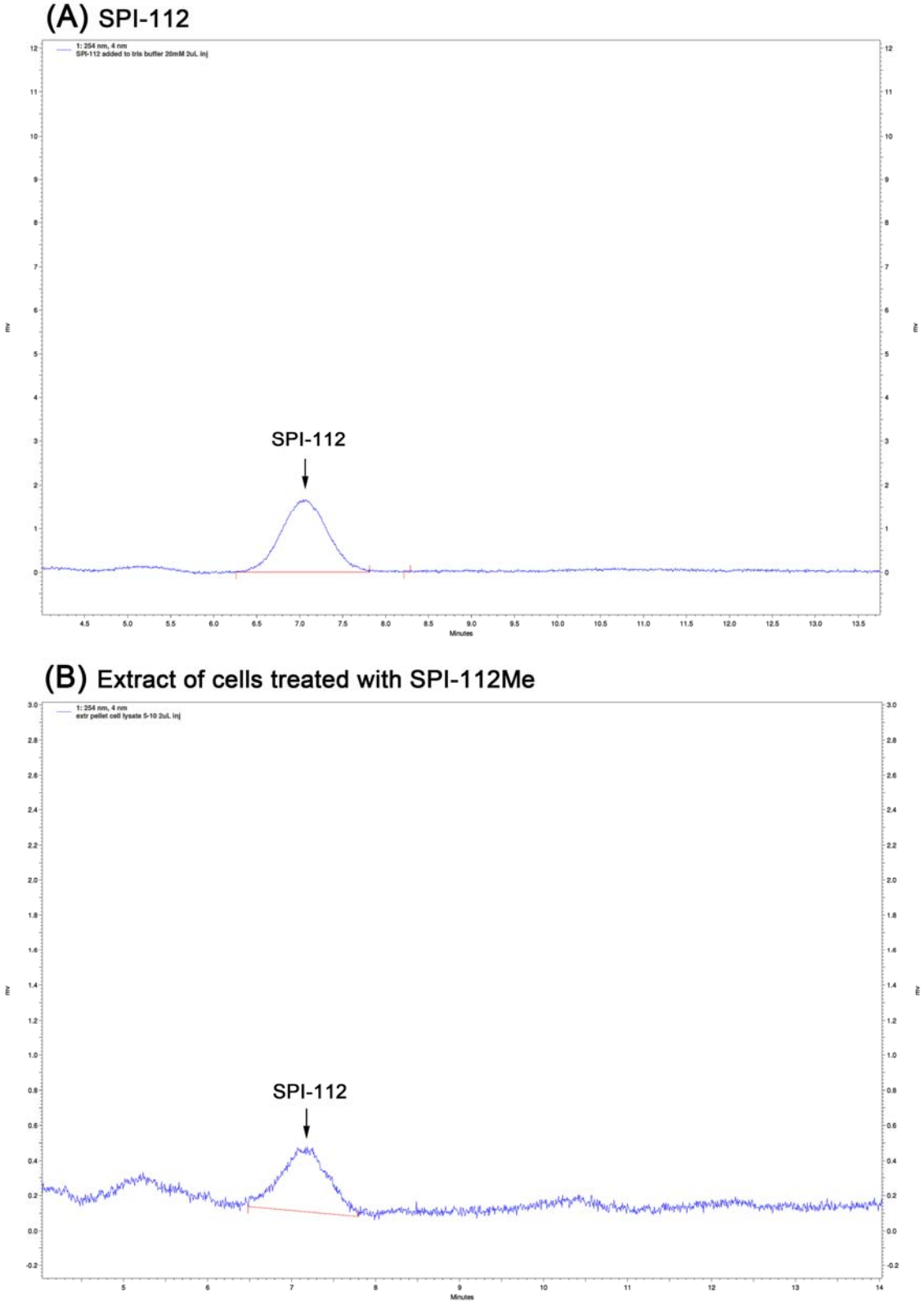


DCA
(Shp2 IC_{50}: 2.1 μM)

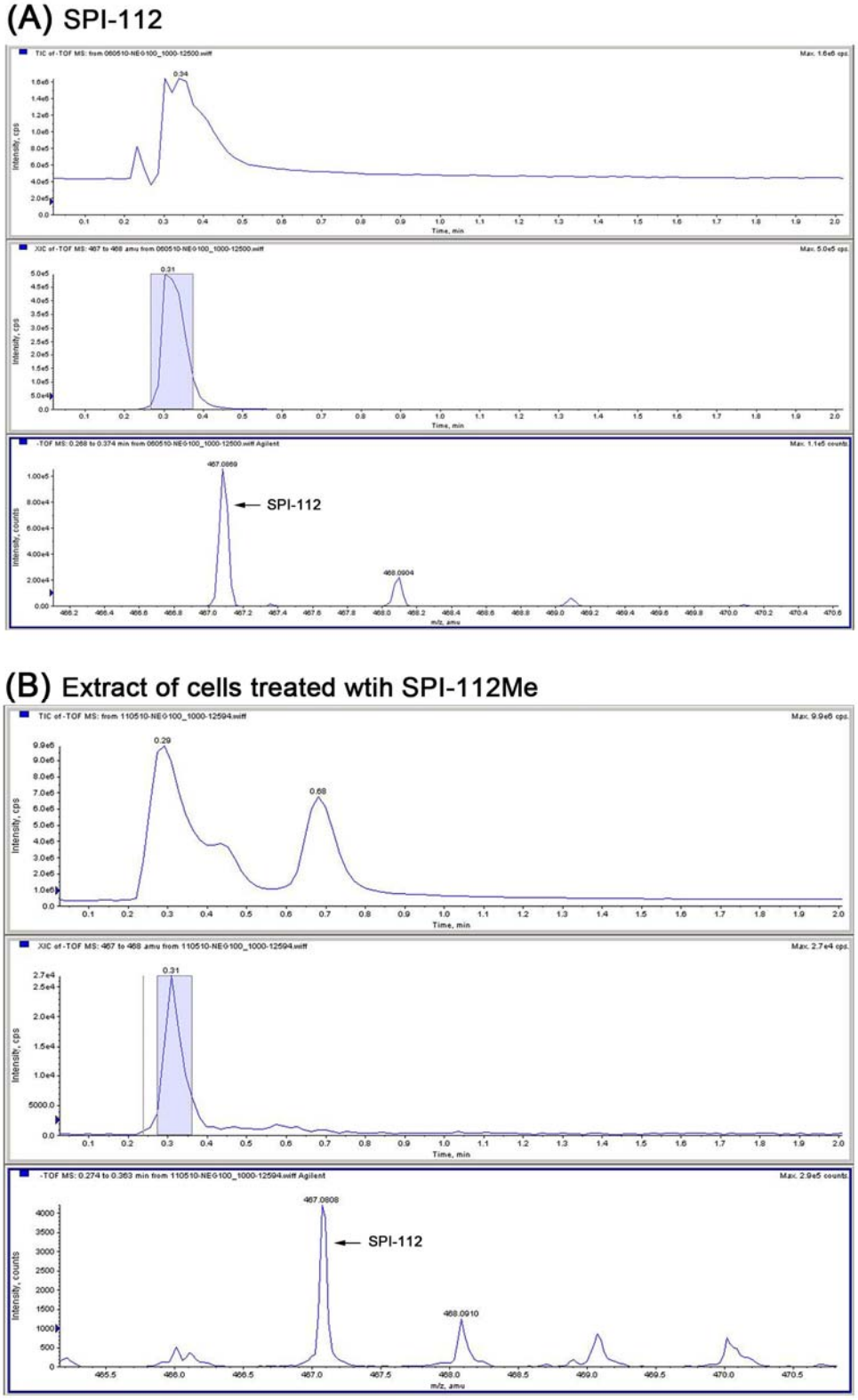


II-B08
(Shp2 IC_{50}: 5.5 μM)

sFig. 1. Chemical structures of reported Shp2 PTP inhibitors.



sFig. 2. HPLC analysis of SPI-112. SPI-112 reference (A) or extract of cells treated with SPI-112Me was analyzed by HPLC.



sFig. 3. TOF LC/MS analysis of SPI-112. SPI-112 (A) or extract of cells treated with SPI-112Me (B) was analyzed by LC/MS with the Analyst QS software.