Diversity-Oriented Synthesis Yields A New Drug Lead for Treatment of Chagas Disease.

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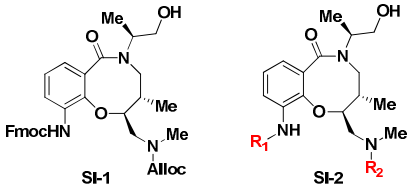
Supporting Information

1. General information for chemistry:

All oxygen and/or moisture sensitive reactions were carried out under N_2 atmosphere in glassware that had been flame-dried under vacuum (~0.5 mm Hg) and purged with N_2 prior to use. All reagents and solvents were purchased from commercial vendors and used as received. NMR spectra were recorded on a Bruker 300 (300 MHz ^{1}H, 75 MHz ^{13}C) or Varian UNITY INOVA 500 (500 MHz 1H, 125 MHz 13C) spectrometer. Proton and carbon chemical shifts are reported in ppm (δ) referenced to the NMR solvent. Data are reported as follows: chemical shifts, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constant(s) in Hz;). Unless otherwise indicated NMR data were collected at 25 °C. Flash chromatography was performed using 40-60 um Silica Gel (60 Å mesh) on a Teledyne Isco Combiflash Rf. Tandem Liquid Chromotography/Mass Spectrometry (LCMS) was performed on a Waters 2795 separations module and 3100 mass detector. Analytical thin layer chromatography (TLC) was performed on EM Reagent 0.25 mm silica gel 60-F plates. Visualization was accomplished with UV light and aqueous potassium permanganate (KMnO4) stain followed by heating. High-resolution mass spectra were obtained at the MIT Mass Spectrometry Facility (Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer). Compound purity and identity were determined by UPLC-MS. Purity was measured by UV absorbance at 210 nm. Identity was determined on a SQ mass spectrometer by positive electrospray ionization. Mobile phase A consisted of either 0.1% ammonium hydroxide or 0.05% trifluoroacetic acid in water, while mobile phase B consisted of either 0.1% ammonium hydroxide or 0.08% trifluoroacetic acid in acetonitrile. The gradient ran from 5% to 95% mobile phase B over 0.8 min at 0.45 mL/min. An Acquity BEH C18, 1.7 um, 1.0x50 mm column was used with column temperature maintained at 65 °C. Compounds were dissolved in DMSO at a nominal concentration of 1 mg/mL, and 0.25 uL of this solution was injected.

2. Synthetic procedures and compound characterization:

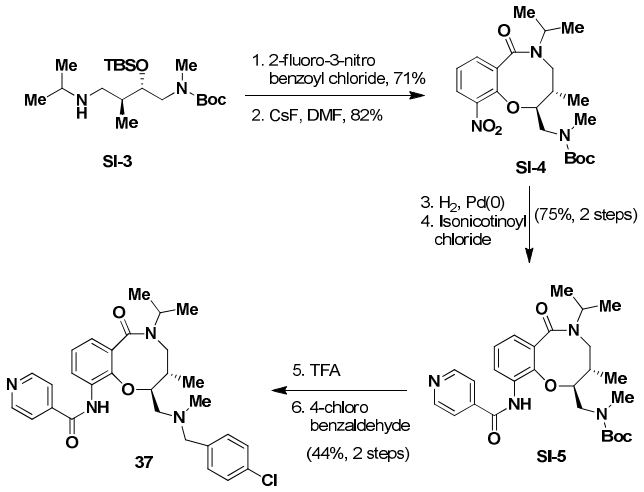
Experimental details of the synthesis of the core **SI-1** and the synthesis of library members of the structure **SI-2** have been described previously.[1]



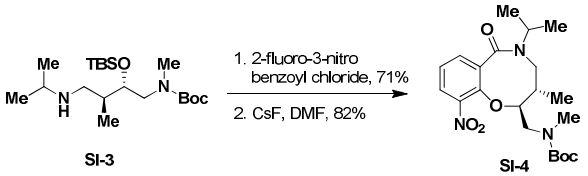
UPLC purity data of all the analogs are shown the Tables S1-S5.

[1] Chou, D. H.; Duvall, J. R.; Gerard, B.; Liu, H.; Pandya, B. A.; Suh, B. C,; Forbeck, E. M.; Faloon, P.; Wagner, B. K.; Marcaurelle, L. A. Synthesis of a novel suppressor of beta-cell apoptosis via diversity-oriented synthesis. *ACS Med. Chem. Lett.* **2011**, 2, 698-702.

Synthesis of a representative isopropyl analog is described below.



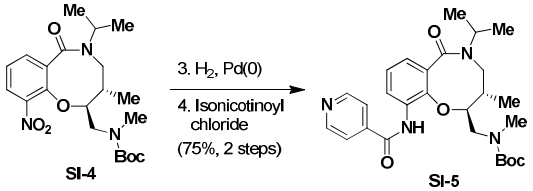
Synthesis of Compound 37:



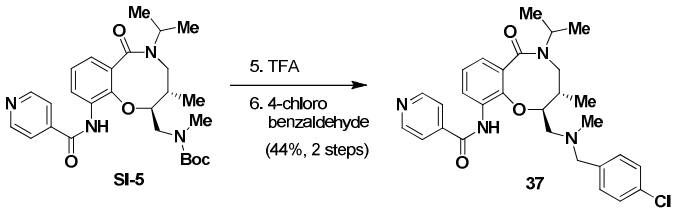
***tert*-Butyl (((*2S*,*3S*)-5-isopropyl-3-methyl-10-nitro-6-oxo-3,4,5,6-tetrahydro-2H-benzo[b][1,5]oxazocin-2-yl)methyl)(methyl)carbamate (SI-4):**

To a solution of *tert*-butyl ((*2S*,*3R*)-2-((tert-butyldimethylsilyl)oxy)-4-(isopropylamino)-3-methyl-4-oxobutyl) (methyl)carbamate **SI-3** (1.9 g, 4.89 mmol) and triethylamine (3.41 ml, 24.44 mmol) in CH_2Cl_2 (49 ml) was added 2-fluoro-3-nitrobenzoyl chloride (0.936 g, 4.6 mmol) at 0 ºC. The reaction mixture was warmed to room temperature and stirred overnight. Water was added and the reaction mixture extracted with CH_2Cl_2. The combined organic extracts were dried (MgSO4), filtered and concentrated. The crude product was purified by column chromatography on silica gel eluting with ethyl acetate/hexanes to deliver the product (1.93 g, 3.47 mmol) in 71% yield.

To a solution of *tert*-butyl ((*2S*,*3R*)-2-((*tert*-butyldimethylsilyl)oxy)-4-(2-fluoro-*N*-isopropyl-3-nitrobenzamido)-3-methyl-4-oxobutyl)(methyl)carbamate (1.9 g, 3.42 mmol) in DMF (68.4 mL) was added cesium fluoride (2.077 g, 13.68 mmol). The resulting suspension was heated at 85 °C for 21.5 h. Additional cesium fluoride (1.04 g, 6.84 mmol) was added to the reaction mixture at the reaction mixture was stirred for 1 h at 85 °C. The reaction mixture was concentrated, taken up in EtOAc, washed with water and the organic layer was dried (MgSO4), filtered and concentrated. The crude product was purified by column chromatography on silica gel eluting with ethyl acetate/hexanes to deliver the product (1.18 g, 3.47 mmol) in 82% yield. MS (ESI) calcd for $C_{21}H_{31}N_3O_6$ $[M+H\text{-}O^tBu]^+$: 366.16 Found: 365.99.



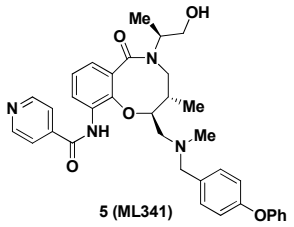
***tert*-butyl (((*2S*,*3S*)-10-(isonicotinamido)-5-isopropyl-3-methyl-6-oxo-3,4,5,6-tetrahydro-2H-benzo[b][1,5]oxazocin-2-yl)methyl)(methyl)carbamate (SI-5):** A solution of *tert*-butyl (((*2S*,*3S*)-5-isopropyl-3-methyl-10-nitro-6-oxo-3,4,5,6-tetrahydro-2H-benzo[b][1,5]oxazocin-2-yl)methyl)(methyl)carbamate **SI-4** (1.40 g, 3.32 mmol) and 10% palladium on carbon in EtOAc (27.7 mL) and MeOH (5.54 mL) was stirred under a hydrogen atmosphere at room temperature for 48 h. The reaction mixture was filtered through Celite and concentrated. The crude material was dissolved in CH_2Cl_2 (10 mL). 2,6-Lutidine (0.446 ml, 3.83 mmol) and isonicotinoyl chloride hydrochloride (0.227 g, 1.277 mmol) were added. The reaction mixture was stirred at room temperature overnight. Water was added, the reaction mixture was extracted with CH_2Cl_2, dried (Na_2SO_4), filtered and concentrated. The crude product was purified by column chromatography on silica gel eluting with ethyl acetate/hexanes to deliver the product (0.238 g, 0.479 mmol) in 75% yield. MS (ESI) calcd for $C_{27}H_{36}N_4O_6$ $[M+H]^+$: 497.27 Found: 497.31.



***N*-((*2S*,*3S*)-2-(((4-chlorobenzyl)(methyl)amino)methyl)-5-isopropyl-3-methyl-6-oxo-3,4,5,6-tetrahydro-2H-benzo[b][1,5]oxazocin-10-yl)isonicotinamide (37):** Trifluoroacetic acid (0.372 ml, 4.83 mmol) was added to a solution of *tert*-butyl (((*2S*,*3S*)-10-(isonicotinamido)-5-isopropyl-3-methyl-6-oxo-3,4,5,6-tetrahydro-2H-benzo[b][1,5]oxazocin-2-yl)methyl)(methyl)carbamate **SI-5** (0.24 g, 0.483 mmol) in CH_2Cl_2 (4 mL) and stirred at room temperature until complete consumption of starting material as evaluated by LC-MS analysis. The reaction was quenched with sodium bicarbonate, extracted with ethyl acetate, dried ($MgSO_4$), filtered and concentrated. The crude product was dissolved in dichloroethane (1.3 mL). 4-Chlorobenzaldehyde (0.018 g, 0.126 mmol) was added and the reaction mixture was stirred for 45 min. Sodium triacetoxyborohydride (0.040 g, 0.189 mmol) was added as a solid, in one portion and the reaction mixture was stirred at room temperature overnight. Additional 4-chlorobenzaldehyde (0.013 ml, 0.126 mmol) and sodium triacetoxyborohydride (0.040 g, 0.189 mmol) were added and stirred for 12 h. The reaction mixture was concentrated and the crude material was dissolved in CH_2Cl_2 (4 mL) and methanol (3 drops). Sodium triacetoxyborohydride (0.040 g, 0.189 mmol) was added as a solid, in one portion and the reaction mixture was stirred at room temperature

overnight. The reaction mixture was quenched with saturated aqueous Rochelle's salt (1 mL) and stirred at 50 °C for 2 h. The reaction mixture was extracted with CH_2Cl_2, dried ($MgSO_4$), filtered and concentrated. The crude product was purified by column chromatography on silica gel eluting with dichloromethane/methanol/triethylamine followed preparative TLC (94% dichloromethane/ 5% methanol/ 1% triethylamine) to deliver the product (14.4 mg, 0.028 mmol) in 44% yield. MS (ESI) calcd for $C_{29}H_{33}ClN_4O_3$ $[M+H]^+$: 521.22 Found: 521.28. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.53 – 12.46 (m, 1H), 10.76 (s, 1H), 8.63 (t, *J* = 6.1 Hz, 3H), 7.56 (d, *J* = 5.0 Hz, 2H), 7.37 – 7.09 (m, 6H), 4.73 (dt, *J* = 13.3, 6.6 Hz, 1H), 3.46 – 3.23 (m, 3H), 3.18 (d, *J* = 14.5 Hz, 1H), 3.08 – 2.91 (m, 1H), 2.82 (dd, *J* = 13.6, 9.9 Hz, 1H), 2.17 (d, *J* = 14.0 Hz, 1.5H), 2.00 (d, *J* = 5.8 Hz, 0.5H), 1.84 – 1.58 (m, 4H), 1.24 (dd, *J* = 16.1, 6.7 Hz, 6H), 0.73 (d, *J* = 6.7 Hz, 3H).

Characterization of Compound 5:



N-((2S,3S)-5-((S)-1-hydroxypropan-2-yl)-3-methyl-2-((methyl(4-phenoxybenzyl)amino) -methyl) -6-oxo-3,4,5,6-tetrahydro-2H-benzo[b][1,5]oxazocin-10-yl)isonicotinamide (5): ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.82 (s, 1H), 8.74 – 8.61 (m, 3H), 7.64 – 7.59 (m, 2H), 7.34 (dd, *J* = 8.6, 7.3 Hz, 2H), 7.29 – 7.25 (m, 2H), 7.21 – 7.08 (m, 3H), 7.05 – 6.94 (m, 4H), 4.04 (ddd, *J* = 11.3, 8.4, 6.5 Hz, 1H), 3.89 (ddd, *J* = 8.1, 6.7, 3.4 Hz, 1H), 3.71 (ddd, *J* = 11.4, 5.9, 3.6 Hz, 1H), 3.59 – 3.39 (m, 2H), 3.33 (d, *J* = 14.1 Hz, 1H), 3.23 – 3.10 (m, 2H), 2.99 – 2.80 (m, 2H), 2.22 (d, *J* = 13.9 Hz, 1H), 1.99 – 1.86 (m, 1H), 1.69 (s, 3H), 1.38 (d, *J* = 6.8 Hz, 3H), 0.72 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3) δ 169.06, 166.60, 157.03, 156.95, 150.50, 144.16, 143.73, 131.89, 131.74, 130.51, 130.46, 129.93, 125.90, 124.12, 123.65, 123.47, 121.97, 119.24, 118.76, 85.80, 64.32, 62.06, 60.83, 57.50, 52.71, 43.69, 35.79, 16.70, 14.02.

3. Materials and methods for biological assays:

Detailed protocols for all the assays are reported in PubChem. See Pubchem AID Nos: 651903 651817, 651845.

Trypsin-EDTA 1X (0.25%, Catalog no. 25200-072) was purchased from Gibco-Invitrogen. Sterile horse serum, from donor herd (only used if appearance of epimastigotes in cultures) was obtained from Sigma (Catalog no. H1270). Sterile, Ca^{2+}/ Mg^{2+}free Phosphate Buffered Saline (PBS) 1X was prepared in house. Nonidet P-40 (NP40, now called Igepal CA 360) was obtained from Fluka (Sigma-Aldrich, St. Louis, MO; Catalog no. 56741) and Gal-Screen Buffer B was obtained from AB Biosciences (CA; Catalog no.T1031).

Cell Lines

The following cell lines were used in this study: LLC-MK2 cells (rhesus monkey kidney epithelial cell line) and NIH/3T3 cells (mouse embryonic fibroblastic cell line) were obtained from ATCC. *T. cruzi* expressing β-galactosidase (*T. cruzi* -β-gal: Tulahuen strain, clone C4)[2] was used.

Media for cell propagation**:** 90% Dulbecco's modified Eagle's medium (DMEM, Mediatech Inc, Manassas, VA; Catalog no. 10-013-CM), 10% heat inactivated fetal bovine serum (FBS, Catalog no.16140-089), and 1% Penicillin-streptomycin-L-glutamine (PSG, Catalog no. 10378-016) were mixed and filtered through a 0.2 micron membrane.

Media for *T. cruzi* culture and assays: 98% DMEM, Phenol Red, 2% FBS, and 1% PSG were mixed and filtered through a 0.2 micron membrane.

Solutions: Gal-Screen. Using a Gal-Screen base kit, Buffer B (Catalog no. T2361) was mixed with 1:25 substrate (Catalog no. T2359).

LLC-MK2 and NIH/3T3 Cell Culture. NIH/3T3 cells were cultivated in DMEM supplemented with 10% FBS and 1% PSG using standard culturing conditions.

Parasite Culture: *T. cruzi* β-gal (Tc).[3]

A T225 flasks was seeded with 4 million LLCMK2 cells in 2% FBS, 1% PSG DMEM for 4-16 hrs. Once mammalian cells adhered to the dish, 8 million tulahuen trypomastigotes are added to the media. After infection, the media is removed at 4 hrs, 72 hrs, and 104 hrs with fresh 2% FBS, 1% PSG DMEM. On days 6 and/or 7, the media is removed from the culture and spun at 2200 RPM in a clinical centrifuge for 10 minutes. The supernatant is aspirated to approximately 15 ml and the tube (with undisturbed pellet) is placed in at 37C, 5%CO2 for a minimum of 4 hrs to allow trypomastigotes. The supernatant containing trypomastigotes is removed without disturbing the pellet, which contains amastigotes and cell debris. Trypomastigote sample is fixed with a final of 8% paraformaldehyde and trypomastigotes are comounted using Nexcelom Cellometer.

4. Multi-mode growth inhibition of *Trypanosoma cruzi.*

The media containing parasites harvested from co-cultured with LLC-MK2 were in 50-ml tubes, and spun for 10 minutes at 2200 rpm. The media was gently aspirated until there was only 15 ml left careful to not touch the pellet, and incubated for 3-5 hours. The NIH/3T3 cells were trypsinized, spun, and resuspended in DMEM, 2% FBS, and 1% PSG, then counted using the Nexcelom Cellometer. The cells were diluted to 166,667 cells/ml, and then added to a flask and plated 5,000 cells/ 30 uL per well using a standard cassette multidrop Combi (Thermo). Then, 100 nL compounds/DMSO were pinned to each well with NIH/3T3 cells, and immediately after

[2]Buckner, F. S.; Verlinde, C. L.; La Flamme, A. C.; Van Voorhis, W. C. Efficient technique for screening drugs for activity against Trypanosoma cruzi using parasites expressing beta-galactosidase. *Antimicrob Agents Chemother* **1996**, *40*, 2592-2597.

[3]Bettiol, E.; Samanovic, M.; Murkin, A.S.; Raper, J.; Buckner, F. Identification of three classes of heteroaromatic compounds with activity against intracellular Trypanosoma cruzi by chemical library screening. *PLoS Negl Trop Dis,* **2009**, *3*, e384.

compound addition, 20 uL/well of parasites (5000 *T. cruzi/* well) were added with a standard cassette multidrop Combi (Thermo) on slow speed. Co-cultured cells were incubated for 4 days (or a minimum of 90 hours). Gal-Screen was prepared with 0.05% NP40, 30 uL per well were dispensed in each well, incubated for 60 minutes, and the luminescence was read using Envision (Perkin-Elmer) at 0.1 sec/well. Amphotericin B or ML164 was used as a control.

5. Cell toxicity: NIH/3T3 (Pubchem AID Nos. 651818, 651844)

For the cell toxicity assay, NIH/3T3 cells were treated similar to experiments above. 5000 NIH/3T3 cells in 30 ul of DMEM with 2% FBS, and 1% PSG of were added to each well. Then, 100 nL compounds/DMSO were pinned to each well with NIH/3T3 cells, and immediately after compound addition, 20 uL/well of media alone were added with a standard cassette multidrop Combi (Thermo) on slow speed. CellTiter-Glo diluted 1:3 in PBS was added to each well (30 uL) were dispensed, incubated for 10 minutes, and the luminescence was read using Envision (Perkin-Elmer) at 0.1 sec/well. Staurosporine was used as a control.

6. Amastigote growth inhibition (Pubchem AID No. 651881, 651890)

Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtitre plates at 2000 cells/well in 100 uL RPMI 1640 medium with 10% FBS and 2 mM l-glutamine. After 24 h the medium was removed and replaced by 100 ul per well containing 5000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene.2 After 48 h the medium was removed from the wells and replaced by 100 µl fresh medium with or without a serial drug dilution of eleven 3-fold dilution steps covering a range from 100 to 0.002 ug/ml. After 96 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterility. Then the substrate CPRG/NP40 (50 µl) was added to all wells. A color reaction developed within 2-6 h and could be read photometrically at 540 nm. Data were analyzed with the graphic program Softmax Pro (Molecular Devices), which calculated IC_{50} values by linear regression[4] from the sigmoidal dose inhibition curves. Benznidazole was used as control.

7. Cell toxicity: L-6 rat myocyte (Pubchem AID No. 651896, 651897)

Assays were performed in 96-well microtiter plates, each well containing 100 ul of RPMI 1640 medium supplemented with 1% L-glutamine (200mM) and 10% FBS, and 4000 L-6 cells[56]. Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 ug/ml were prepared. After 70 hours of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10ul of Alamar Blue was then

[4]Huber, W.; Koella, J.C. A comparison of three methods of estimating EC50 in studies of drug resistance of malaria parasites, Acta *Trop* **1993** 55: 257-261.

[5]Ahmed, S.A.; Gogal, R.M. Jr.; Walsh, J.E. A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H]thymidine incorporation assay. *J Immunol Methods* **1993**, 170, 211-224.

[6]Page, B.; Page, M.; Noel, C.; A new fluorometric assay for cytotoxicity measurements in-vitro. *Int J Oncol* **1993**, *3*, 473-476.

added to each well and the plates incubated for another 2 hours. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. The IC_{50} values were calculated by linear regression[5] from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA). Podophyllotoxine was used as control.

8. Trypanocidal assay (Pubchem AID No. 651877, 651869)

This assay assesses cidal activity of compounds in *T. cruzi* (CA-I/72)-infected Bovine Embryo Skeletal Muscle Cells (BESM). Twenty four hours after infection, infected cells are washed and compounds are added every other day for 20 days (protocol 1) or 29 days (protocol 2). After the initial period, compounds are removed and cells are observed for an additional 38 days (protocol 1) or 37 days (protocol 2) without drug. During which time cultures are monitored for reappearance of *T. cruzi* parasites. This procedure allows us to determine if host cells were effectively cured (i.e., compound is cidal) or if infection still persists (i.e. static activity)[7].

Table S1. SAR of Stereoisomers

Cmpd	*T. cruzi* growth inhibition IC_{50} (nM)		Host Cell Toxicity CC_{50} (nM)		PBS Solubility (μM)	% Plasma protein binding (Human)	UPLC Purity (%)
	Multi-mode	Amastigote	NIH/3T3	L-6			
3	1	24	7,000	14,400	1	99	>99
4	5	22	7,000	13,300	1	99	99
5	1	16	11,200	10,800	1	99	>99
6	2	31	23,000	17,900	2	99	>99
7	450	670	>32,000	43,700	87	95	99
8	23	59	20,000	47,500	93	95	99
9	260	1,200	32,000	52,200	86	95	99
10	16	75	17,000	44,200	98	ND	>99

[7]Doyle, P. S.; Zhou, Y. M.; Hsieh, I.; Greenbaum, D. C.; McKerrow, J. H.; Engel, J. C. The *Trypanosoma cruzi* protease cruzain mediates immune evasion. *PLoS Pathog.* **2011**, *7*, e1002139.

Table S2. SAR by varying secondary amine substituent

Cmpd	*T. cruzi* Growth Inhibition IC_{50} (nM)		Host Cell Toxicity CC_{50} (nM)		PBS Solubility (μM)	% Plasma Protein Binding (Human)	UPLC Purity (%)
	Multi-mode	Amastigote	NIH/3T3	L-6			
5	1	16	11,200	10,800	1	99	>99
11	12	171	>32,000	77,300	100	81	>99
12	>32,000	39,000	>32,000	>100,000	100	42	>99
13	165	ND	>32,000	ND	52	95	>99
14	15	ND	>32,000	ND	47	96	>99
15	1	ND	>32,000	ND	54	97	>99
16	78	ND	>32,000	ND	73	94	>99
17	57	ND	>32,000	ND	70	93	>99
18	127	290	>32,000	>100,000	100	87	>99
19	34	ND	>32,000	ND	66	97	>99
20	1	ND	>32,000	ND	53	97	>99
21	13	422	32,000	42,200	100	ND	>99
22	56	ND	>32,000	ND	71	95	>99
23	1	ND	>32,000	ND	73	97	>99
24	14	ND	>32,000	ND	66	96	>99
25	10	200	15,000	18,900	2	97	>99
26	25	ND	19,000	ND	20	98	99
27	27	ND	>32,000	ND	57	98	>99
28	57	ND	>32,000	ND	36	97	99
29	445	ND	>32,000	ND	27	98	>99
30	<1	83	20,000	22,900	64	97	>99
31	10	ND	>32,000	ND	24	99	>99
32	1	ND	>32,000	ND	43	94	>99
33	50	ND	>32,000	ND	79	95	>99

Table S3. SAR with N-isopropyl substituent'

Cmpd	*T. cruzi* Growth Inhibition IC_{50} (nM)		Host Toxicity CC_{50} (nM)		PBS Solubility (μM)	% Plasma protein binding (Human)	UPLC Purity (%)
	Multi-mode	Amastigote	NIH/3T3	L-6			
34	<1	164	>32,000	50,700	100	97	79
35	45	318	>32,000	47,900	57	97	94
36	2	61	32,000	23,000	44	100	89
37	1	61	32,000	20,800	30	98	99

Table S4. SAR on the amide portion

Cmpd	*T. cruzi* Growth Inhibition IC_{50} (nM)		Host Toxicity CC_{50} (nM)		PBS Solubility (μM)	% Plasma protein binding (Human)	UPLC Purity (%)
	Multi-mode	Amastigote	NIH/3T3	L-6			
38	1	288	26,400	17,700	<1	100	99
39	344	13,400	>32,000	58,200	<1	93	92
40	45	2,100	>32,000	15,400	57	93	93
41	500	6,700	>32,000	55,300	<1	99	91
42	1,000	907	9,000	5,800	39	89	85
43	1,200	1,500	8,600	6,000	65	99	97

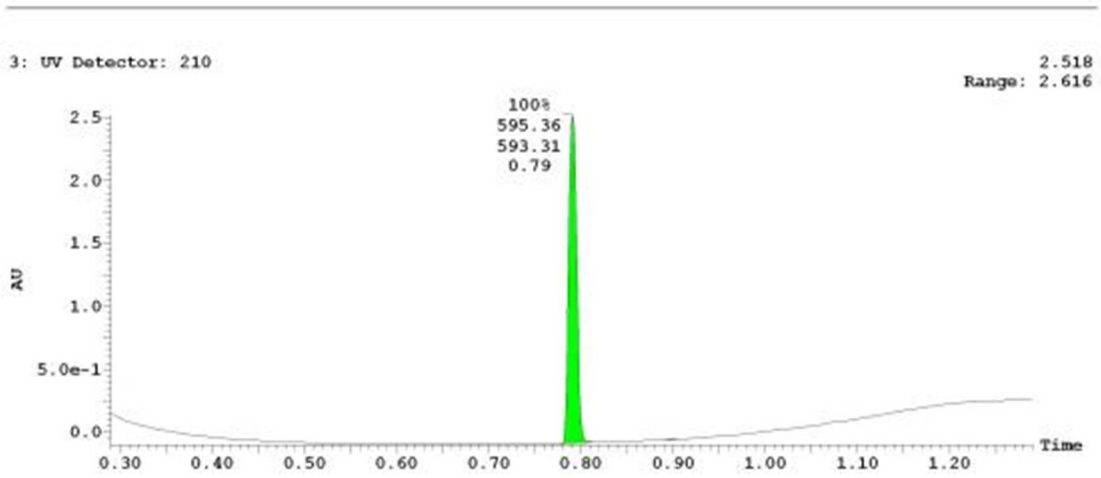
Table S5. Trypanocidal assay results

Cmpd	Dose (nM)	Number of days		Parasite re-emergence	
		During treatment	Post-treatment	During treatment	Post-treatment
5	6,600	20	37	No	No
5	2,200	20	37	No	No
5	740	20	37	No	No
5	250	20	4	No	Yes, after 4 days
5	10,000	29	36	No	No
5	3,300	29	36	No	No
5	1,100	29	36	No	No
5	370	29	36	No	No
5	120	29	36	No	No
5	40	29	36	No	No
3	6,600	20	37	No	No
3	2,200	20	37	No	No
3	740	20	37	No	No
3	250	20	4	No	Yes, after 4 days
3	10,000	29	36	No	No
3	3,300	29	36	No	No
3	1,100	29	36	No	No
3	370	29	36	No	No
3	120	29	36	No	No
3	40	29	36	No	No
4	6,600	20	37	No	No
4	2,200	20	37	No	No
4	740	20	30	No	Yes, after 30 days
4	250	20	4	No	Yes, after 4 days
4	10,000	29	36	No	No
4	3,300	29	36	No	No
4	1,100	29	36	No	No
4	370	29	36	No	No
4	120	29	36	No	No

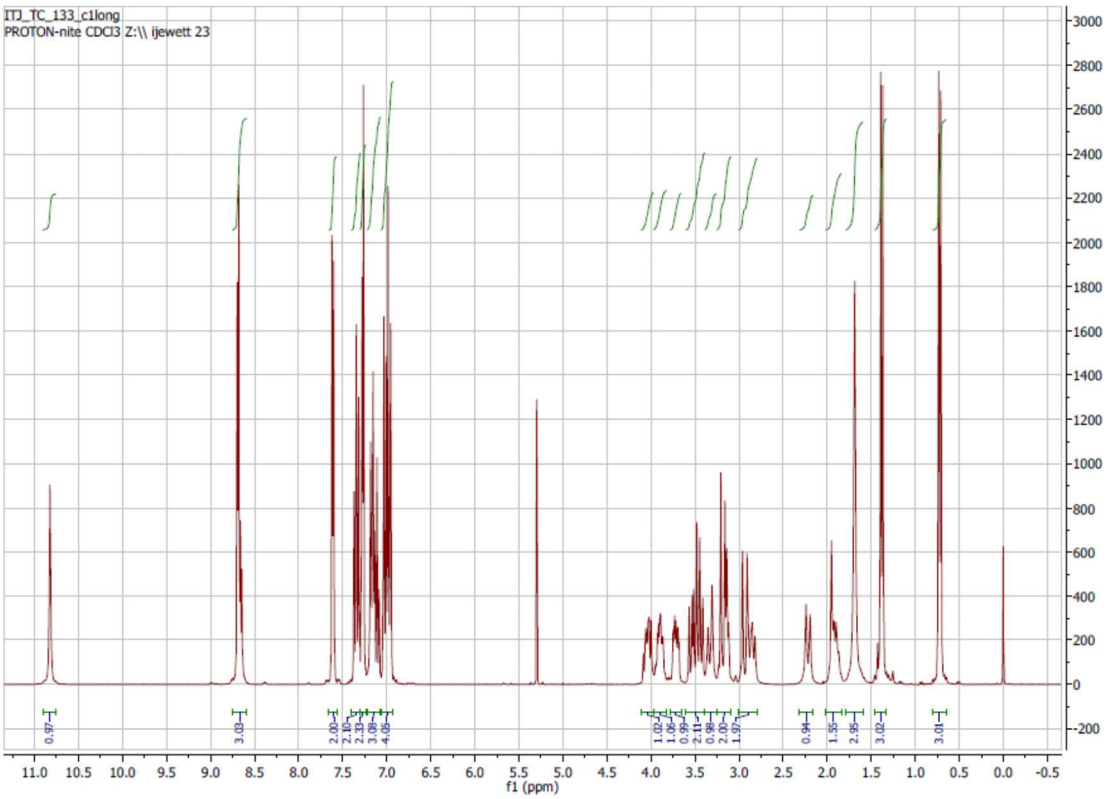
8	6,600	20	37	No	No
8	2,200	20	37	No	No
8	740	20	37	No	No
8	250	20	4	No	Yes, after 4 days
8	10,000	29	36	No	No
8	3,300	29	36	No	No
8	1,100	29	36	No	No
8	370	29	36	No	No
8	120	29	36	No	No
10	6,600	20	37	No	No
10	2,200	20	37	No	No
10	740	20	37	No	No
10	250	20	4	No	Yes, after 4 days
10	10,000	29	36	No	No
10	3,300	29	36	No	No
10	1,100	29	36	No	No
10	370	29	36	No	No
10	120	29	19	No	Yes, after 19 days
10	40	29	8	No	Yes, after 8 days
1	6,600	20	37	No	No
1	2,200	20	6	No	Yes, after 6 days

Appendix S1: Characterization of Compound 5

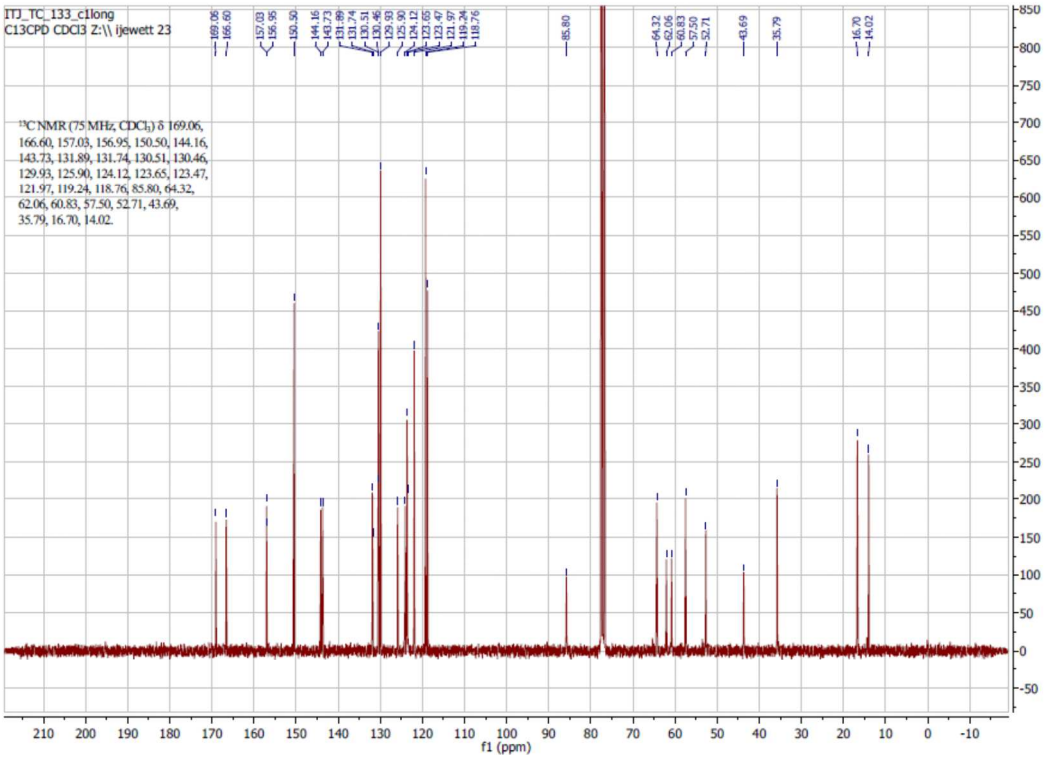
UPLC-MS of Compound 5



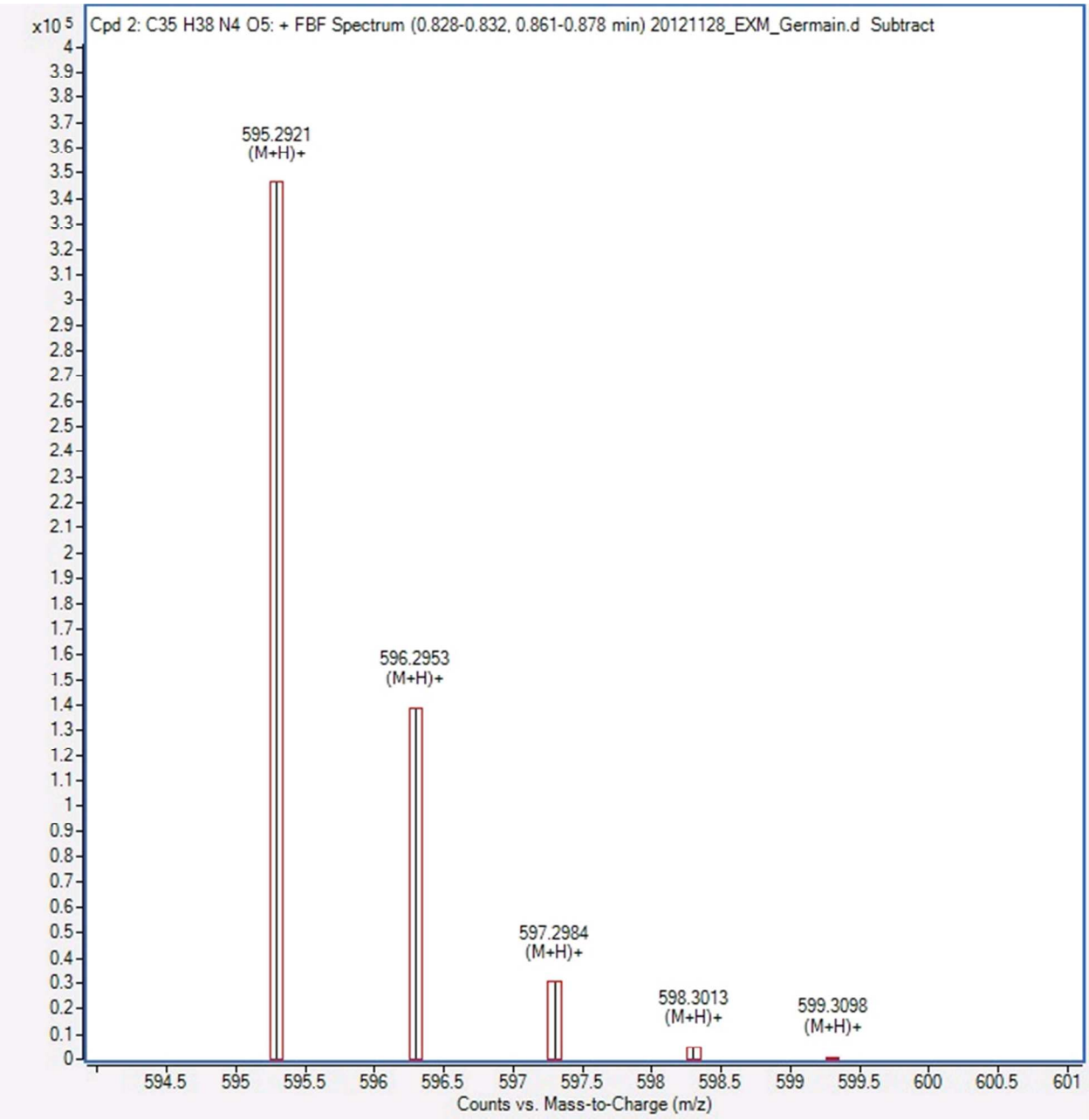
^{1}H NMR (300 MHz, $CDCl_3$) Spectrum of Compound 5



^{13}C NMR (75 MHz, $CDCl_3$) Spectrum of Compound 5

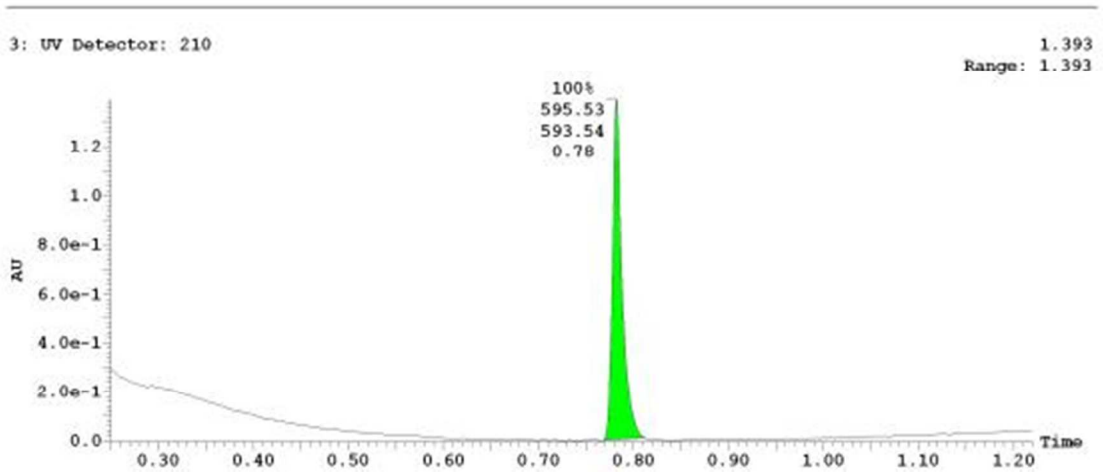


HRMS of Compound 5

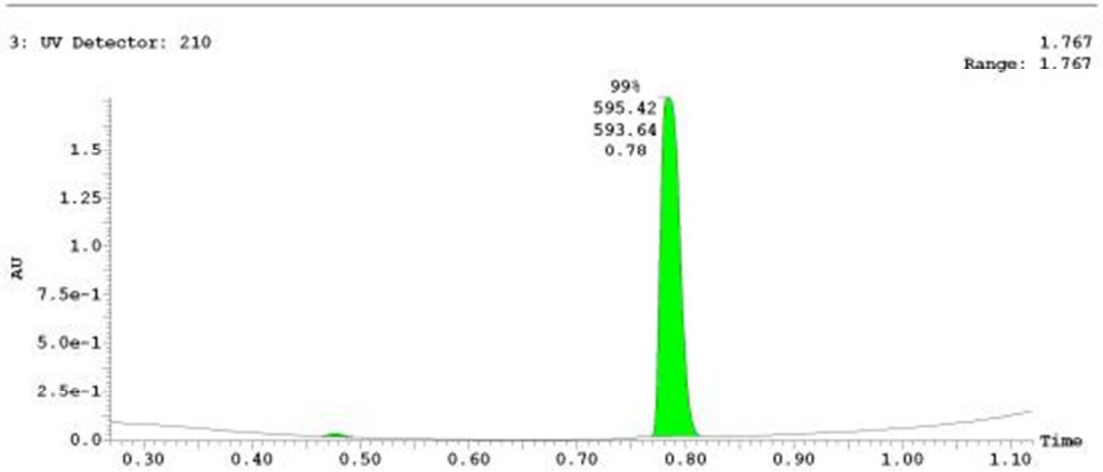


Appendix S2: Select characterization of additional analogs

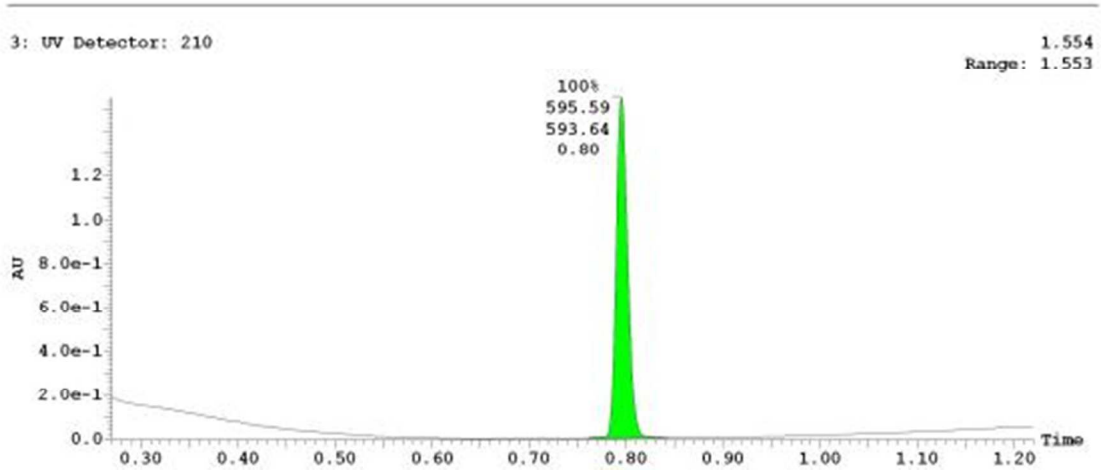
UPLC-MS of Compound 3



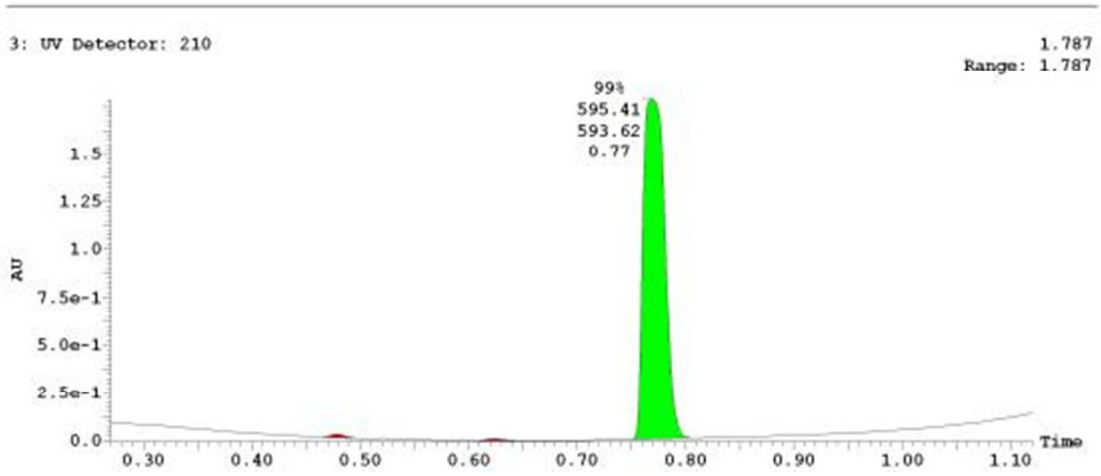
UPLC-MS of Compound 4



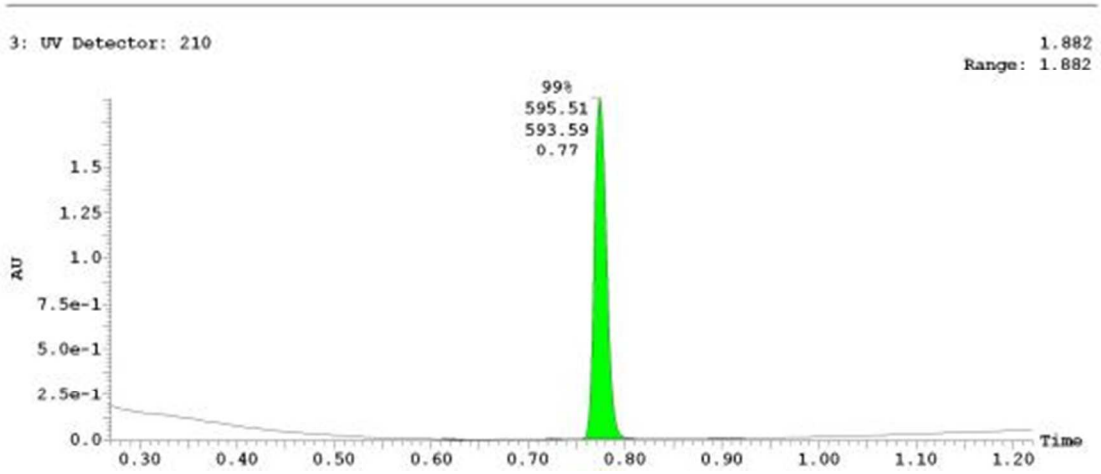
UPLC-MS of Compound 6



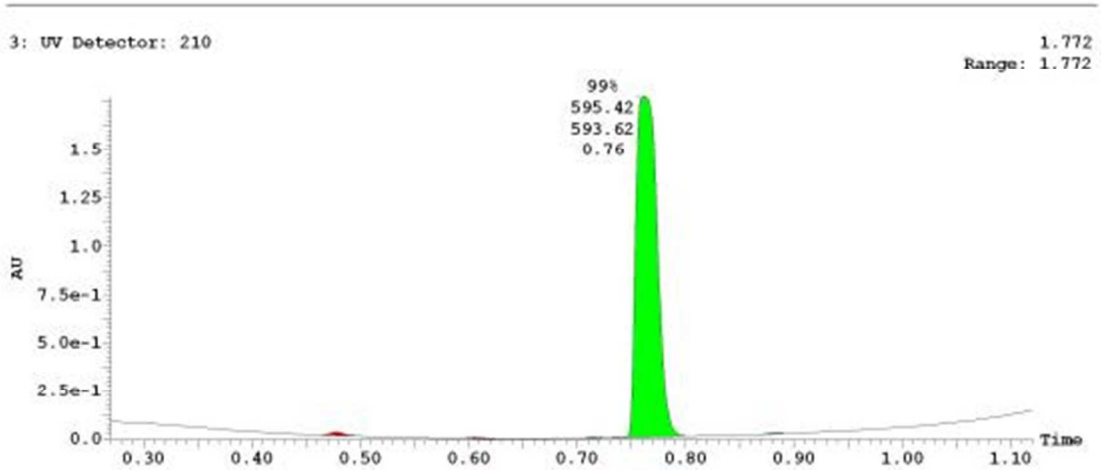
UPLC-MS of Compound 7



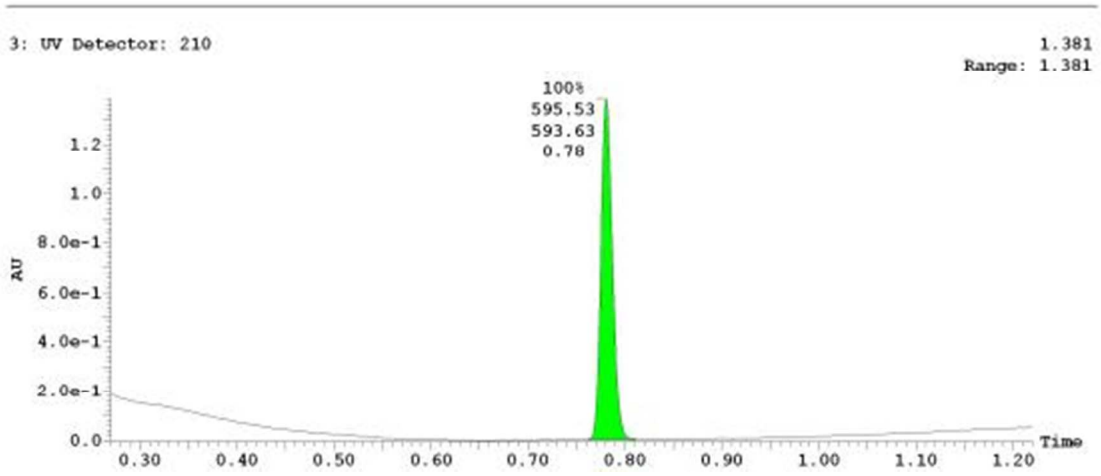
UPLC-MS of Compound 8



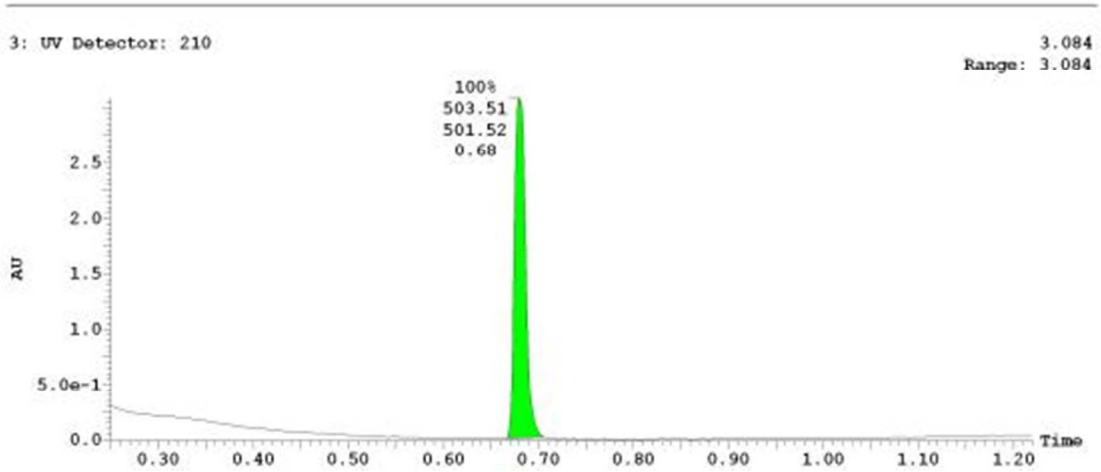
UPLC-MS of Compound 9



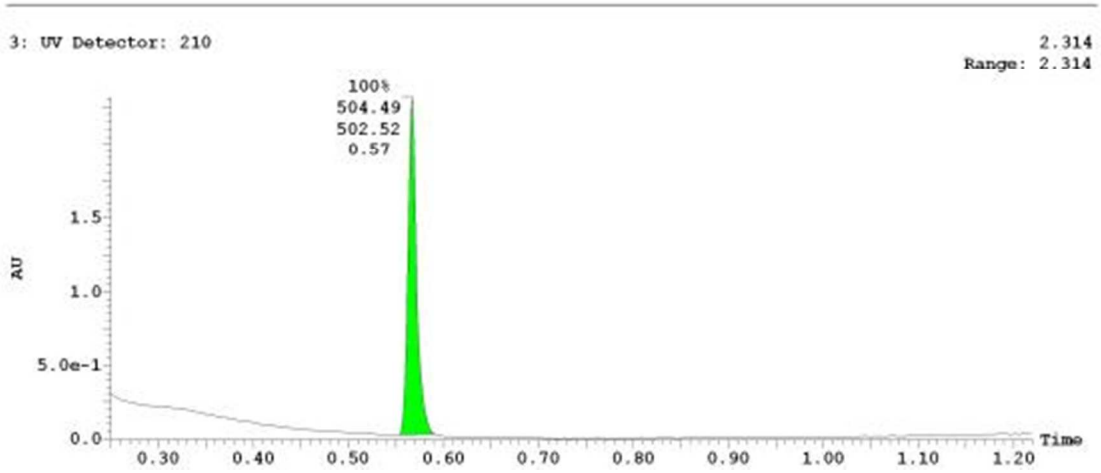
UPLC-MS of Compound 10



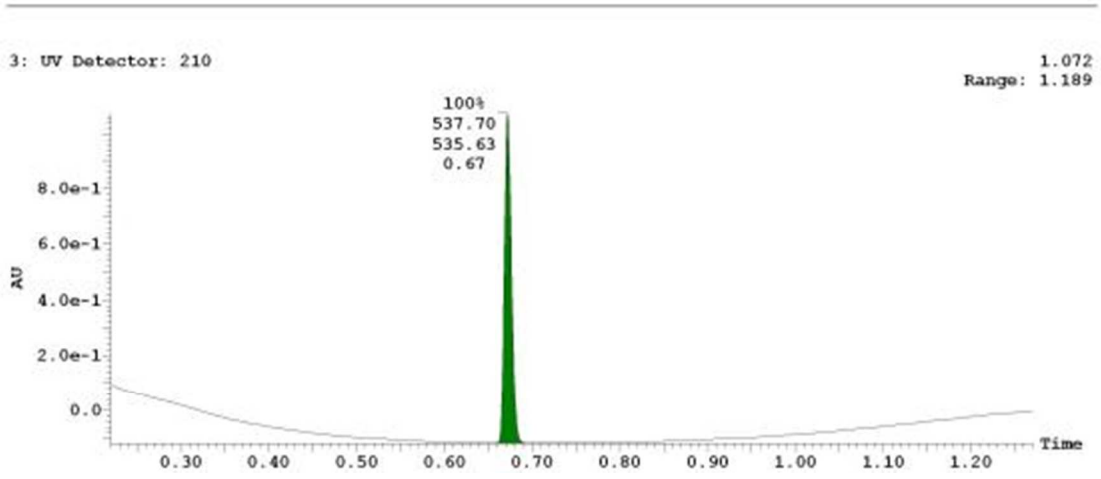
UPLC-MS of Compound 11



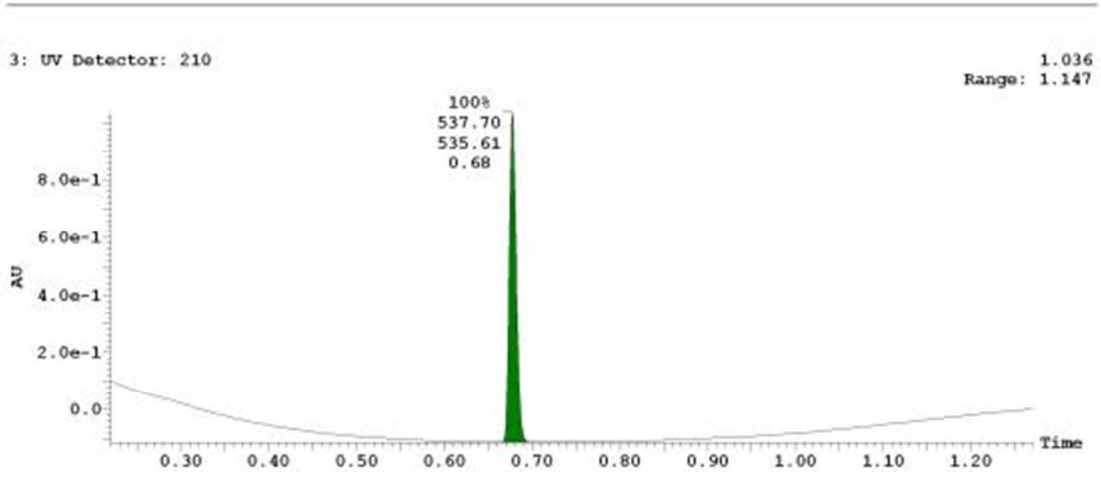
UPLC-MS of Compound 12



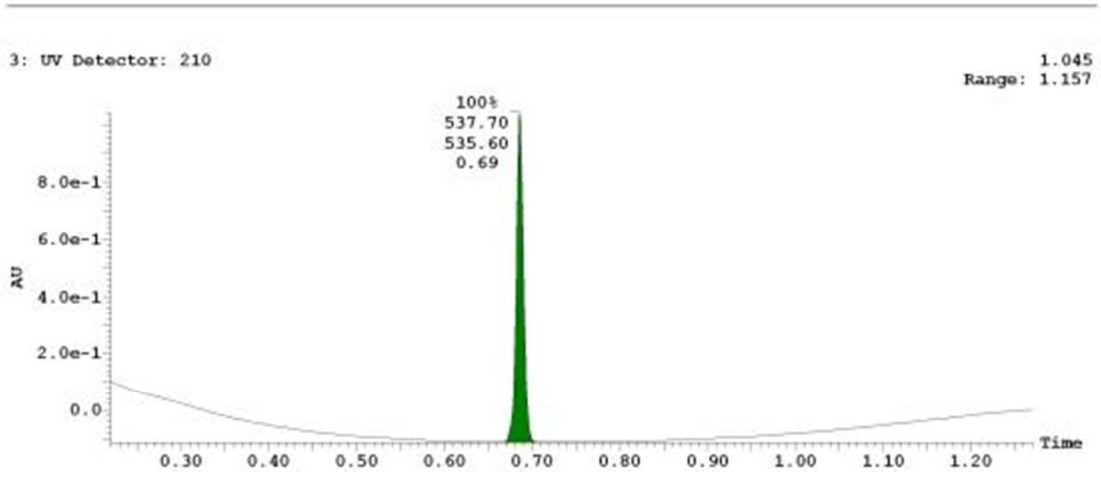
UPLC-MS of Compound 13



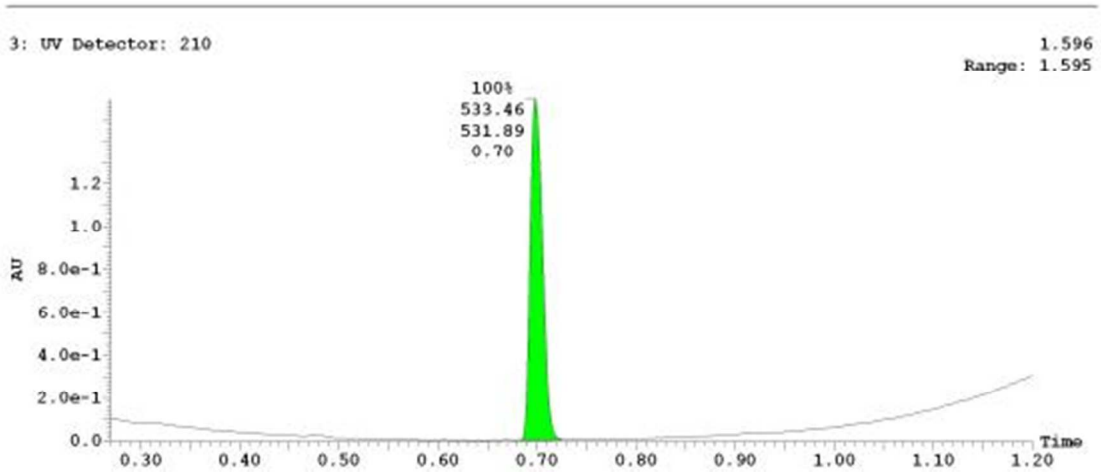
UPLC-MS of Compound 14



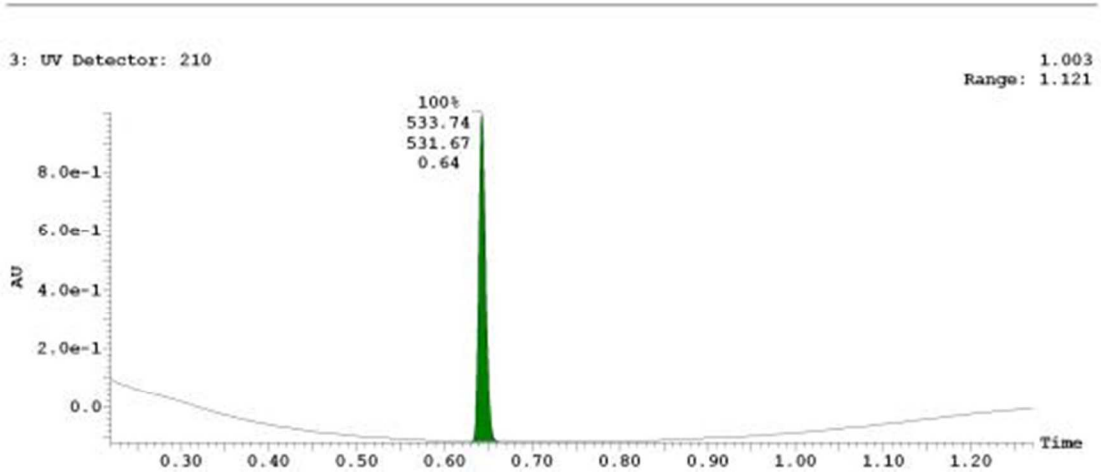
UPLC-MS of Compound 15



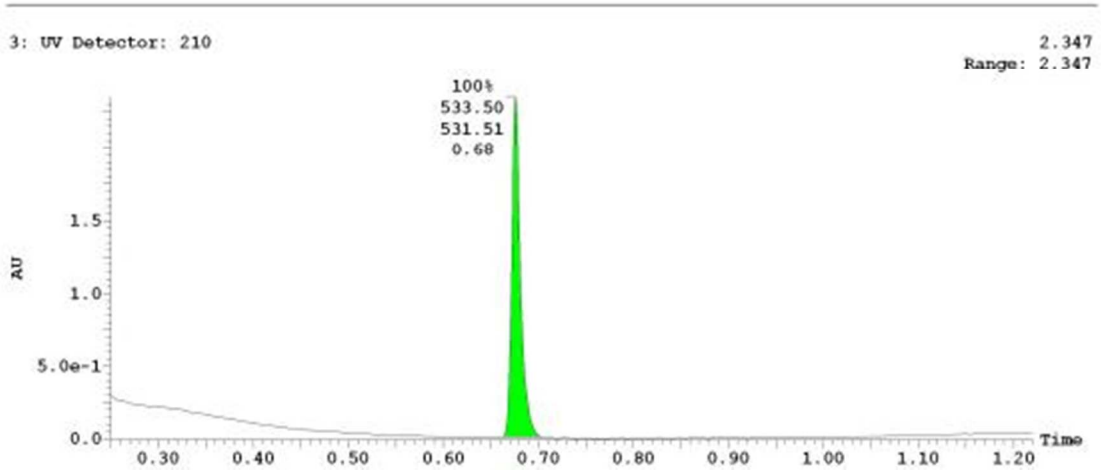
UPLC-MS of Compound 16



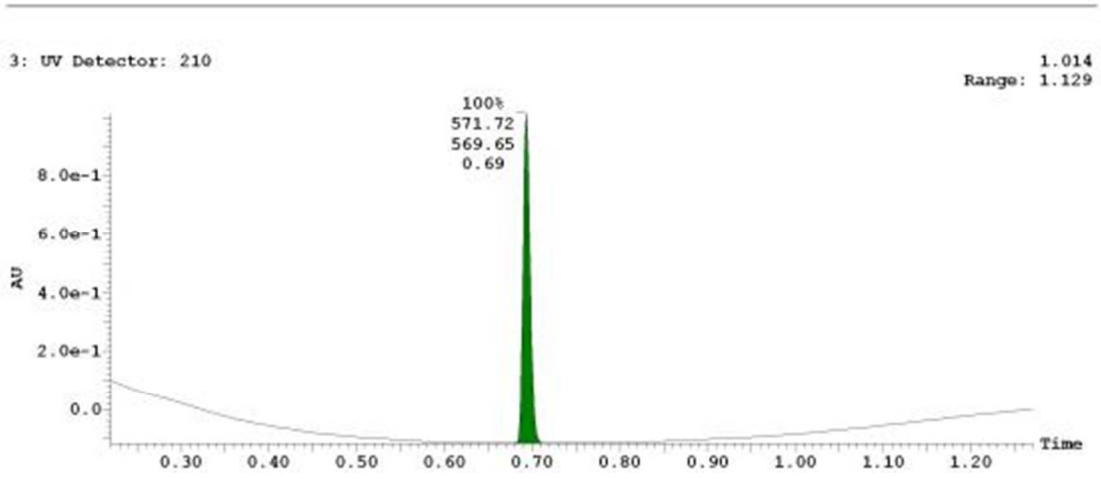
UPLC-MS of Compound 17



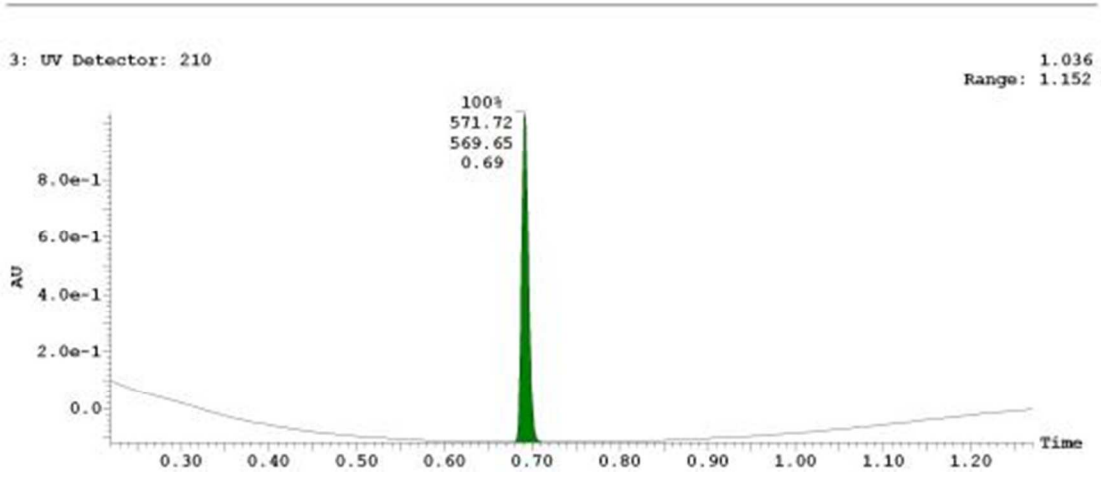
UPLC-MS of Compound 18



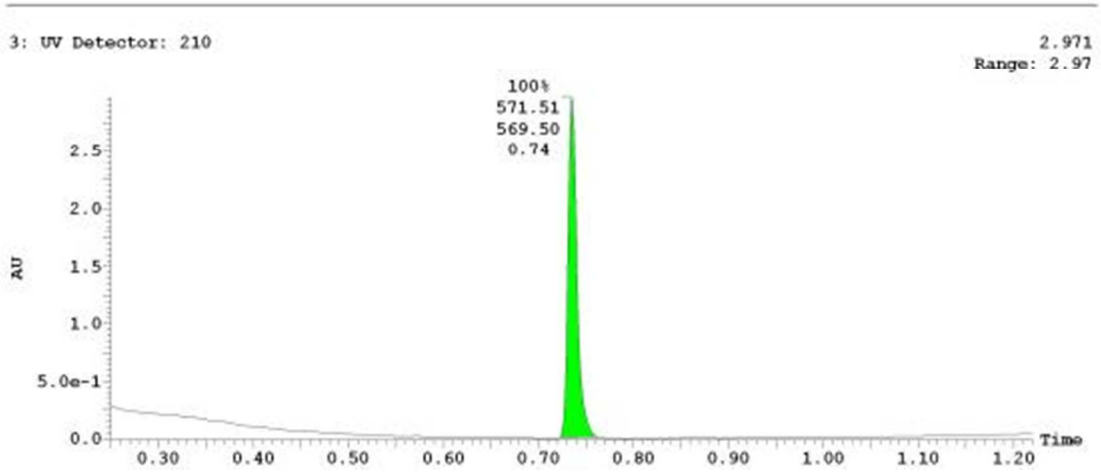
UPLC-MS of Compound 19



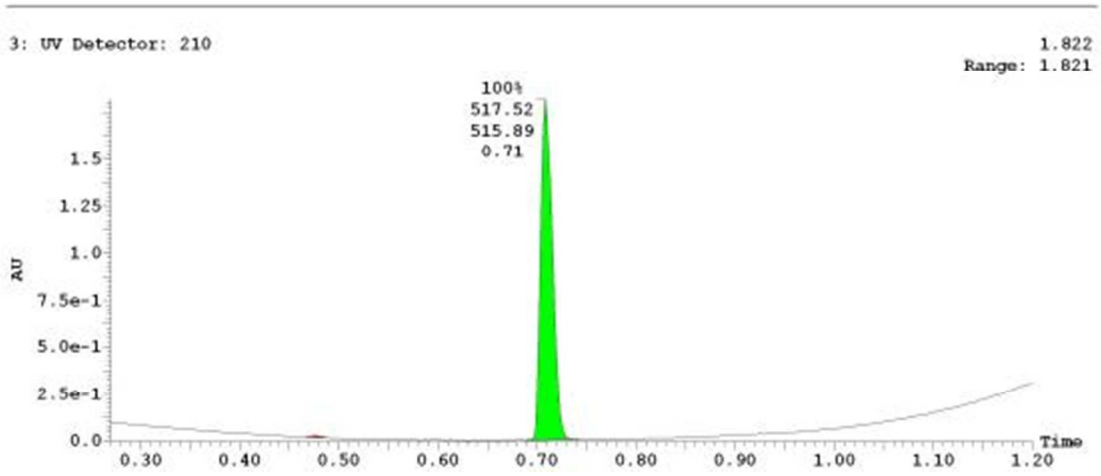
UPLC-MS of Compound 20



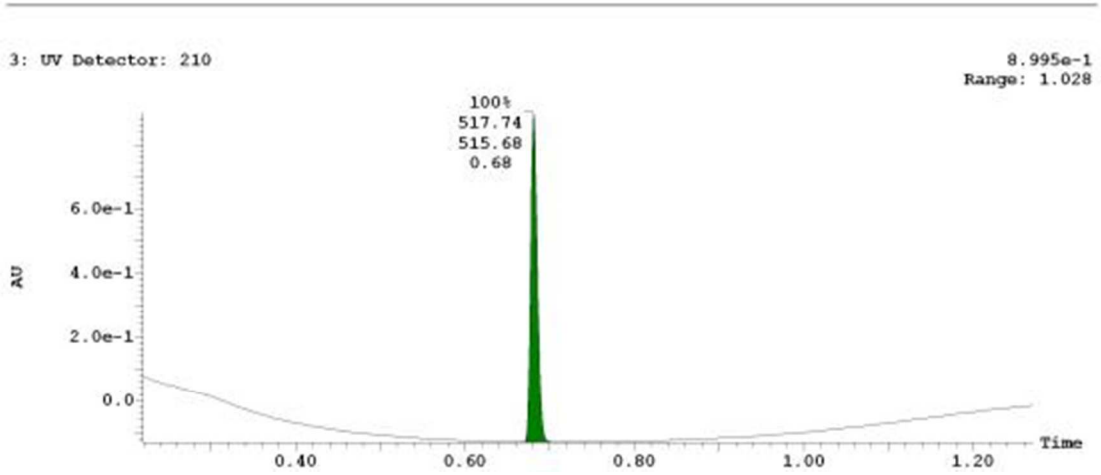
UPLC-MS of Compound 21



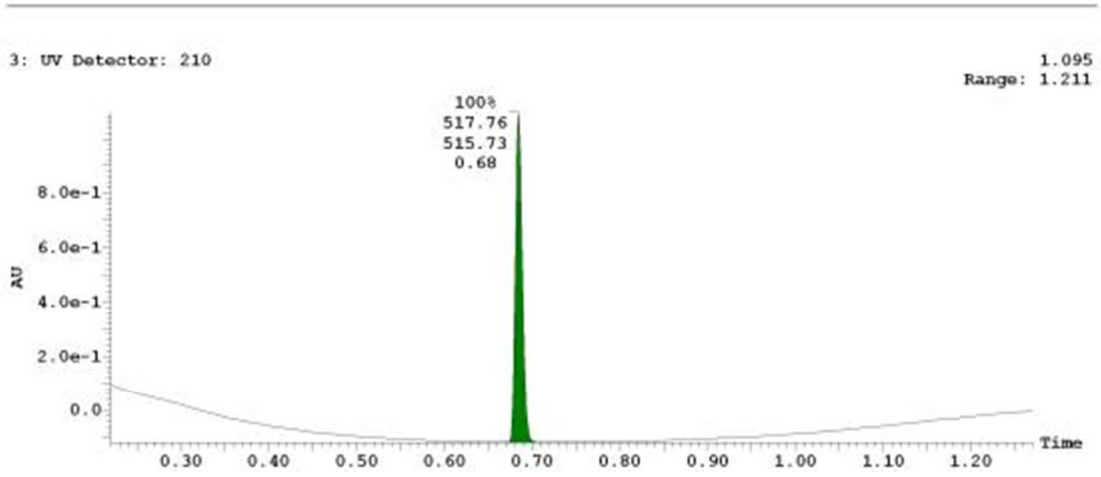
UPLC-MS of Compound 22



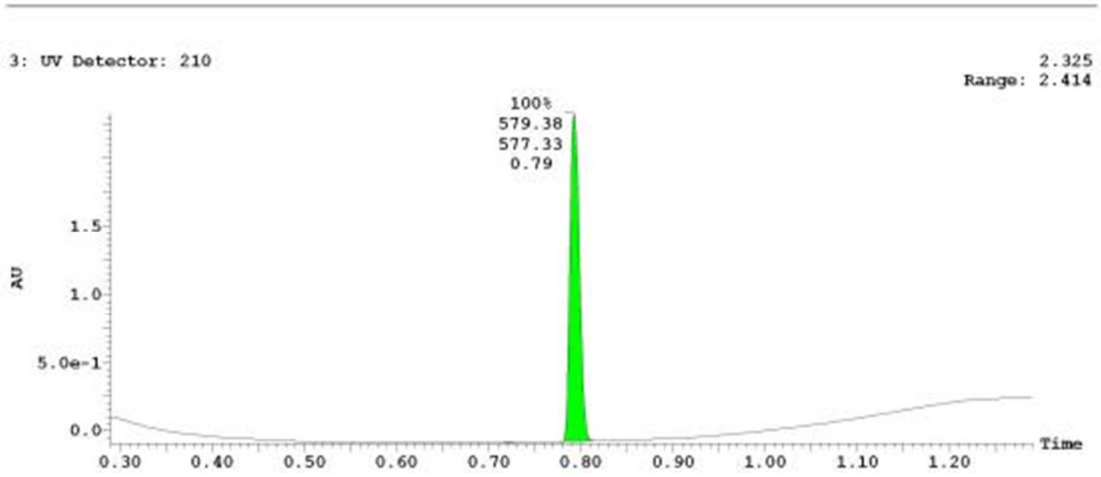
UPLC-MS of Compound 23



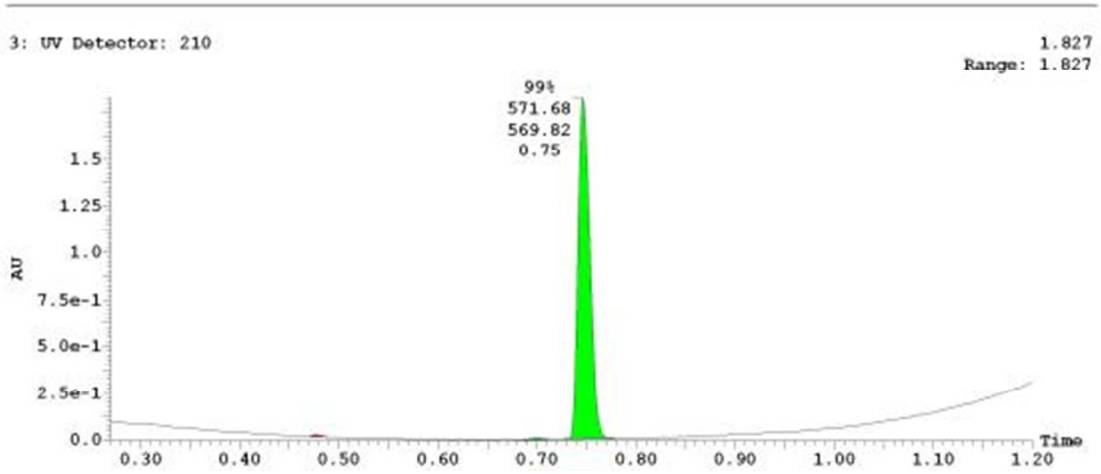
UPLC-MS of Compound 24



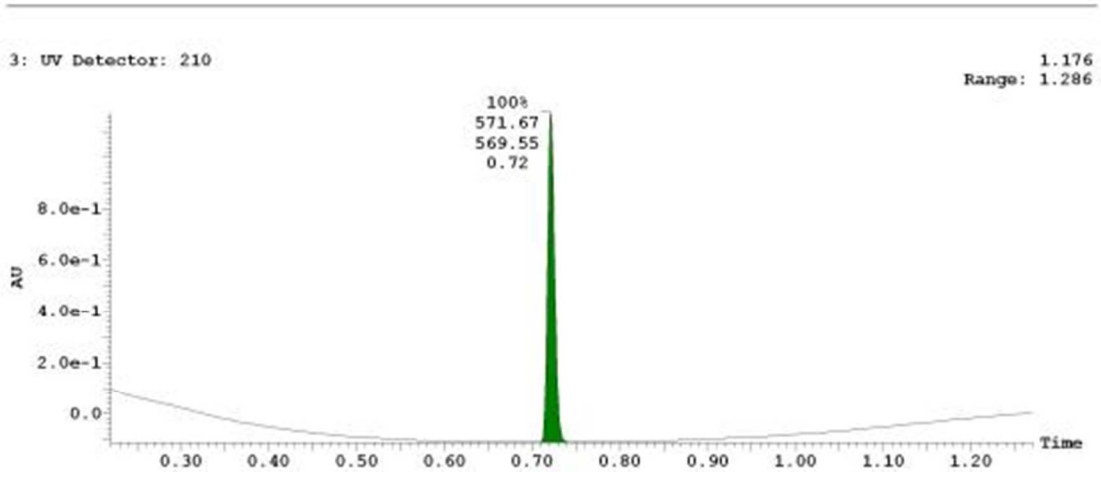
UPLC-MS of Compound 25



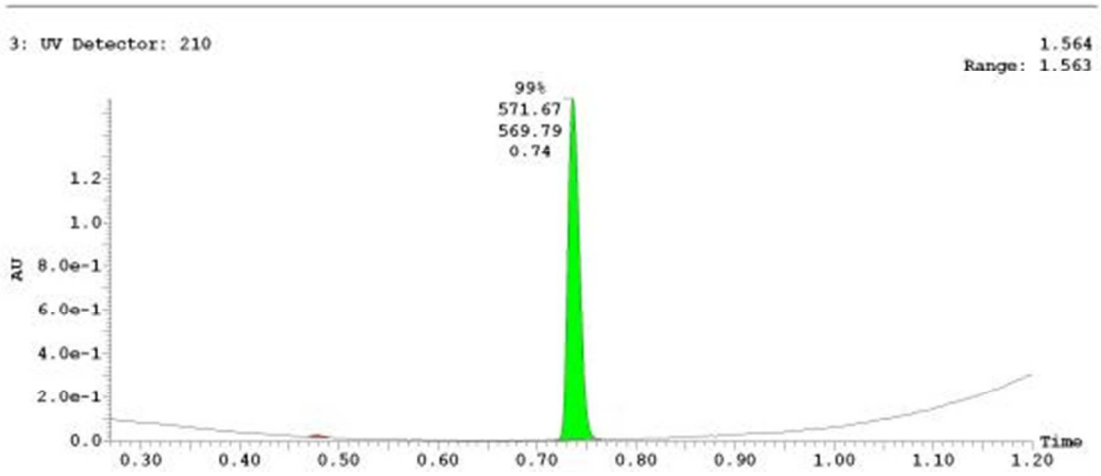
UPLC-MS of Compound 26



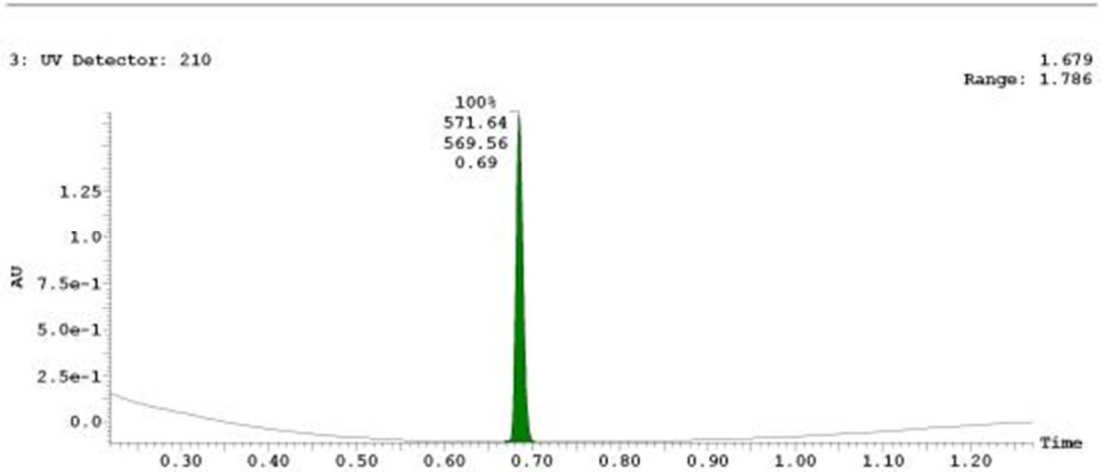
UPLC-MS of Compound 27



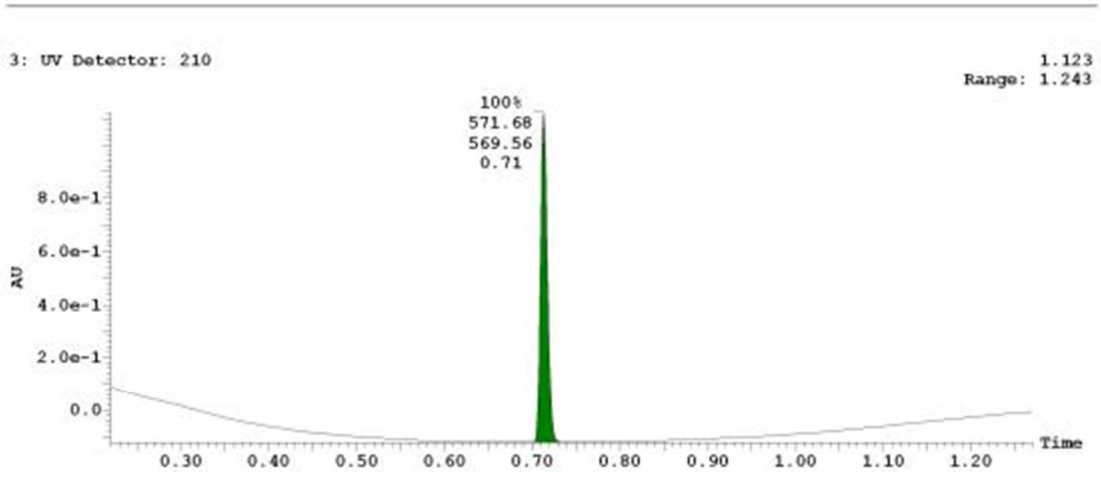
UPLC-MS of Compound 28



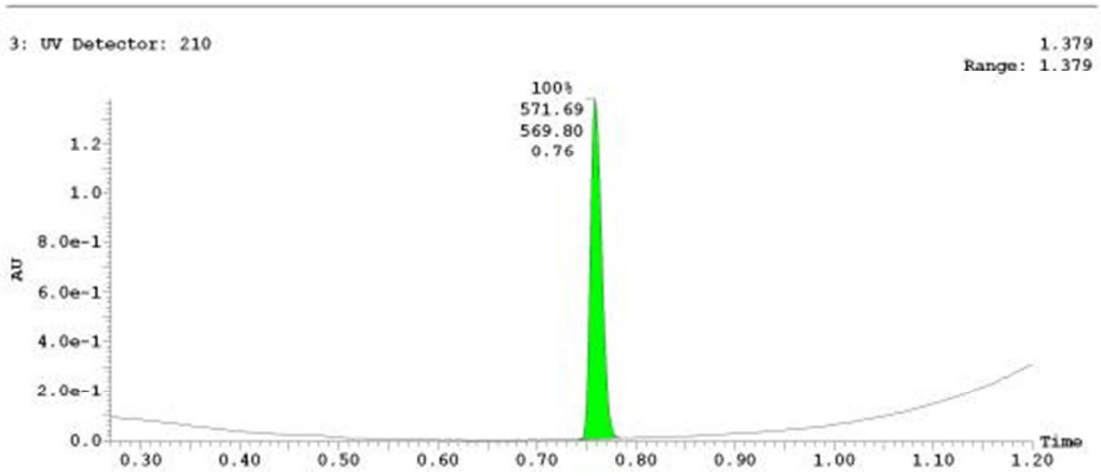
UPLC-MS of Compound 29



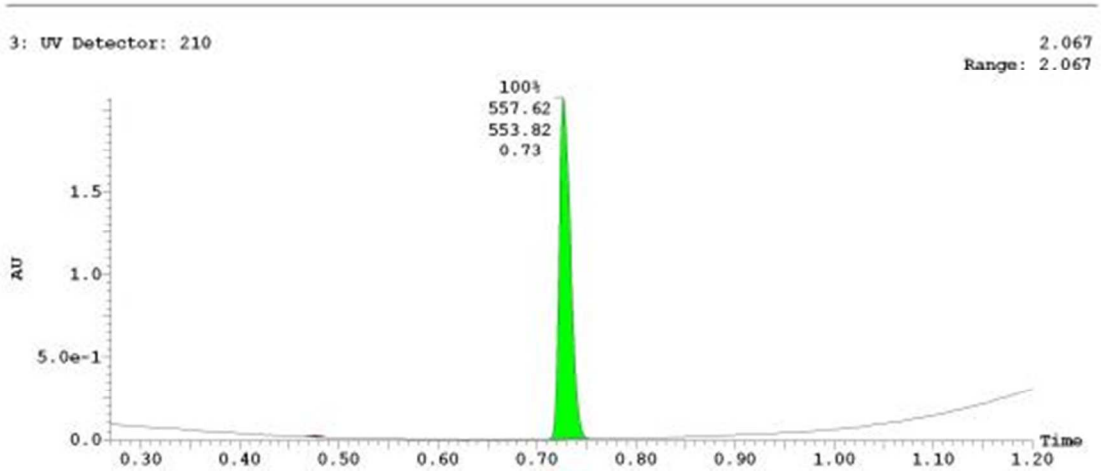
UPLC-MS of Compound 30



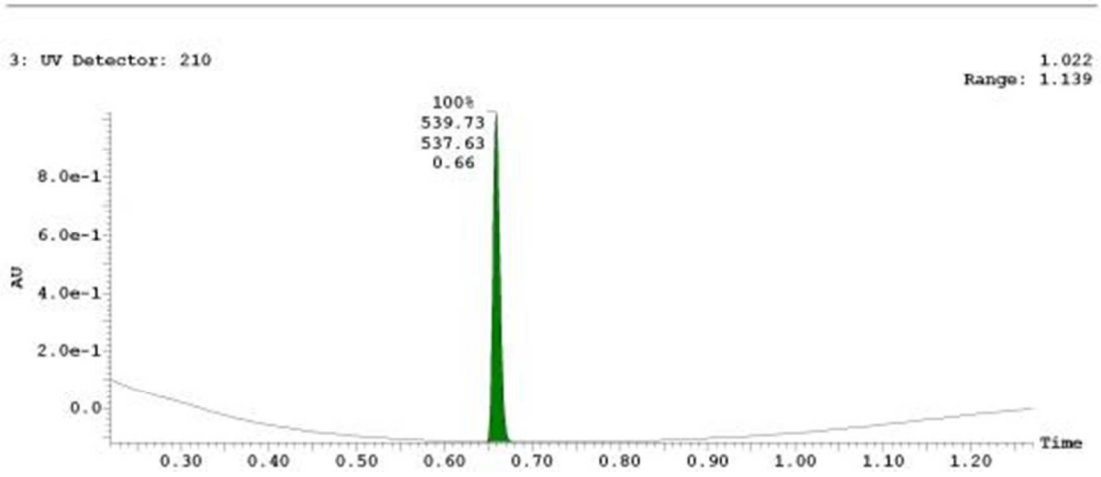
UPLC-MS of Compound 31



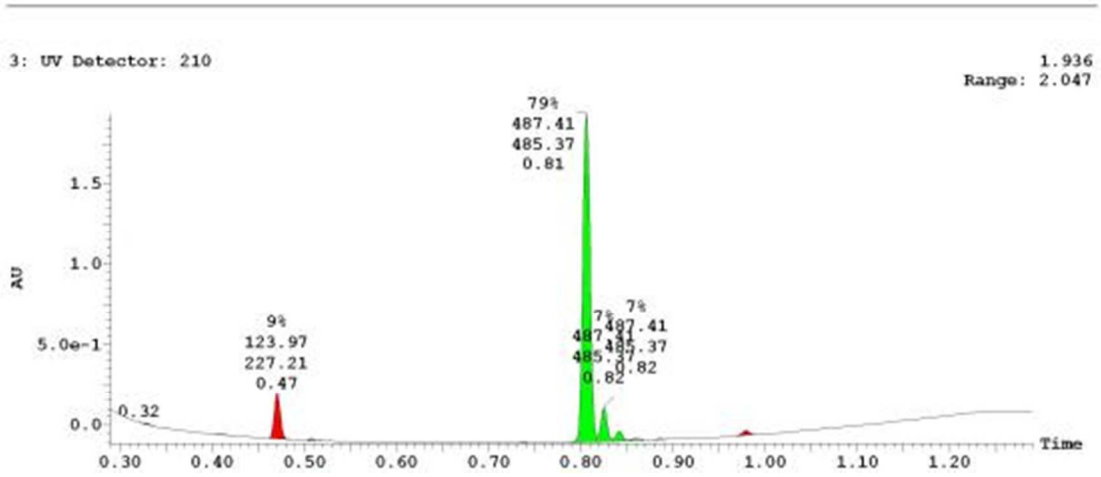
UPLC-MS of Compound 32



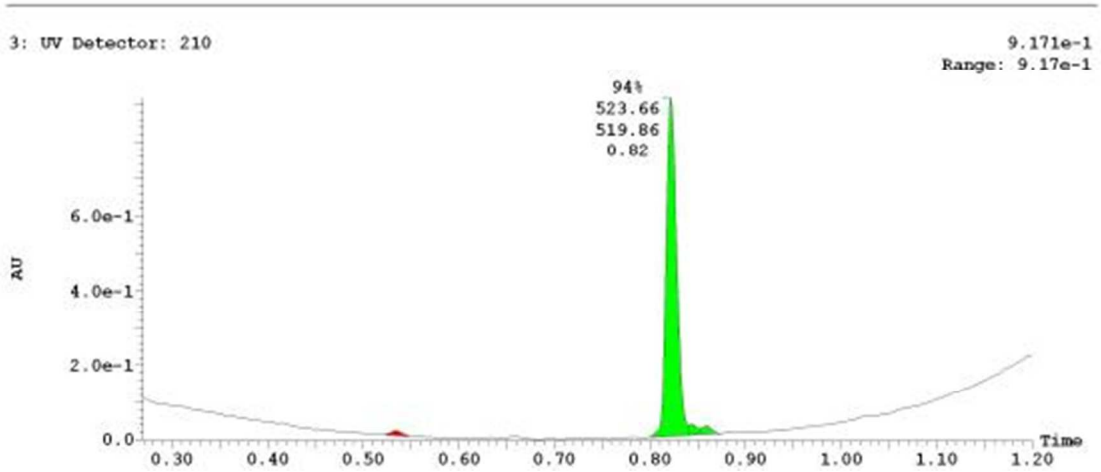
UPLC-MS of Compound 33



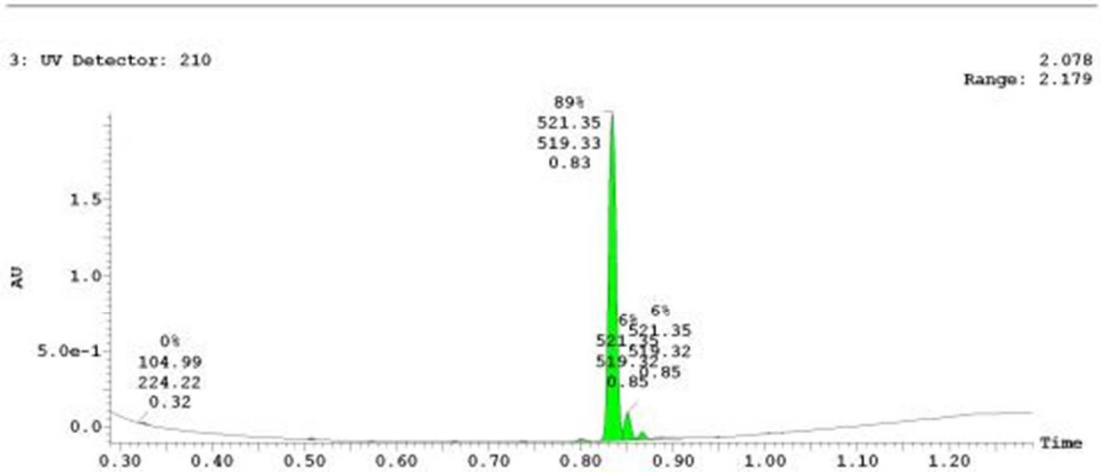
UPLC-MS of Compound 34



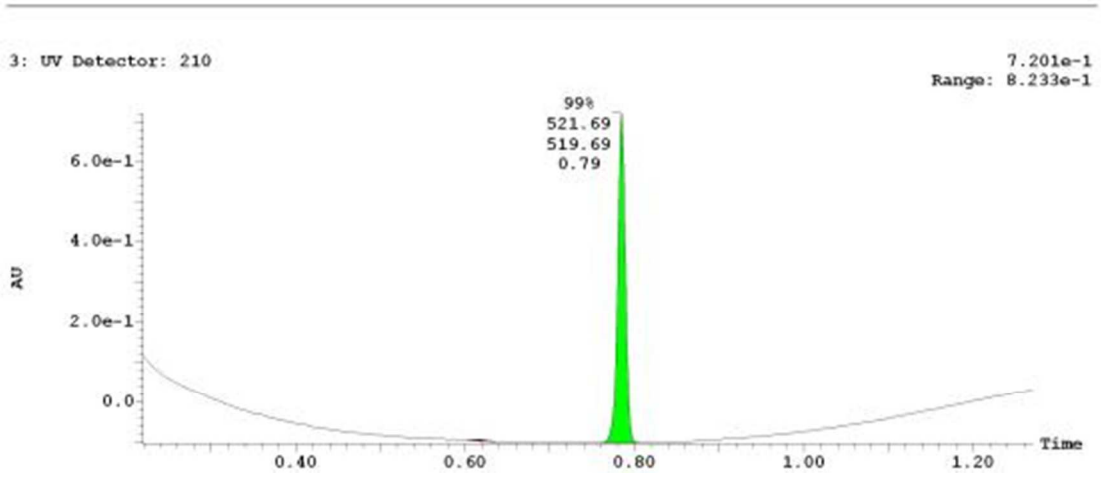
UPLC-MS of Compound 35



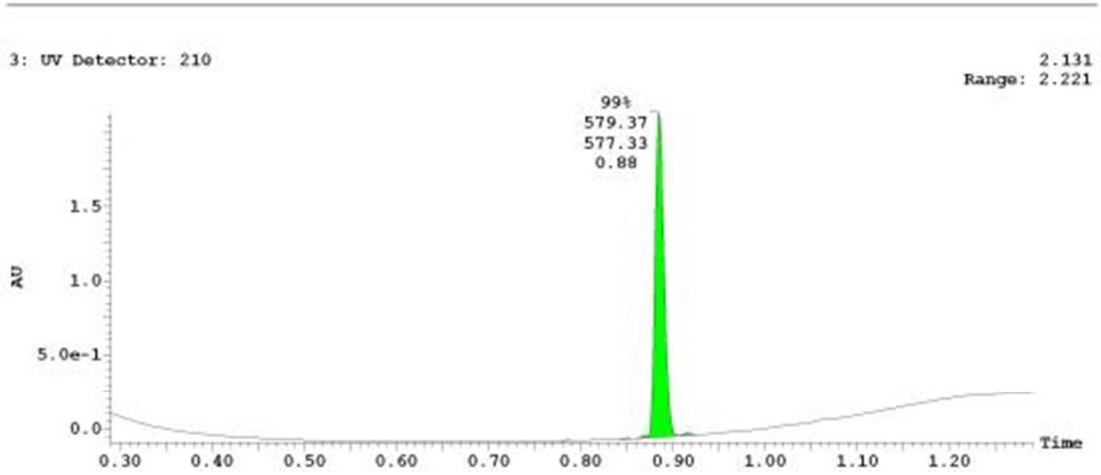
UPLC-MS of Compound 36



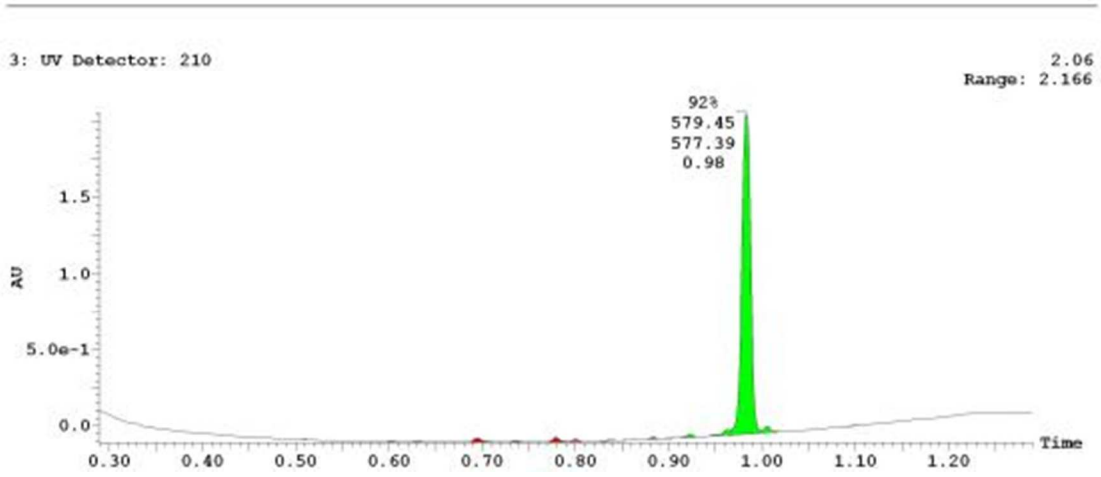
UPLC-MS of Compound 37



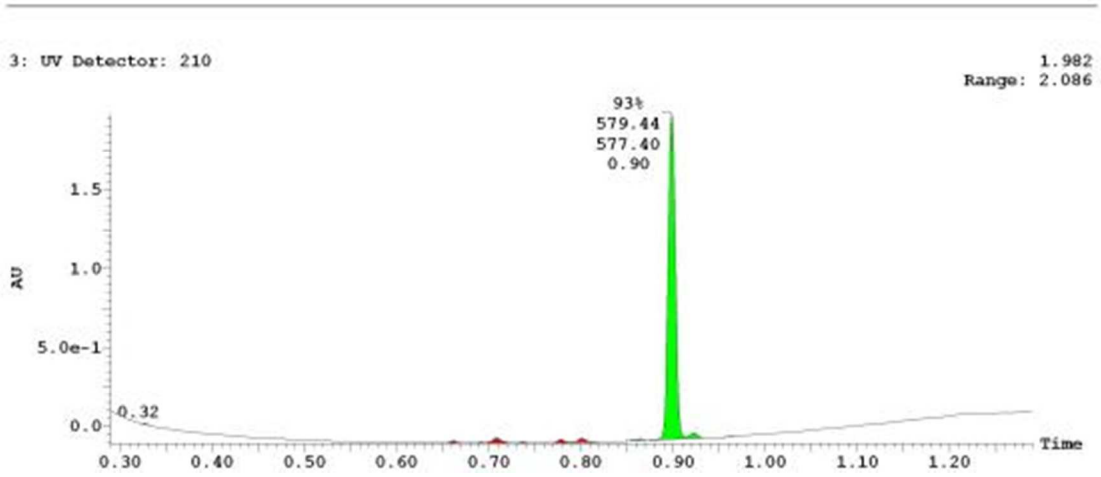
UPLC-MS of Compound 38



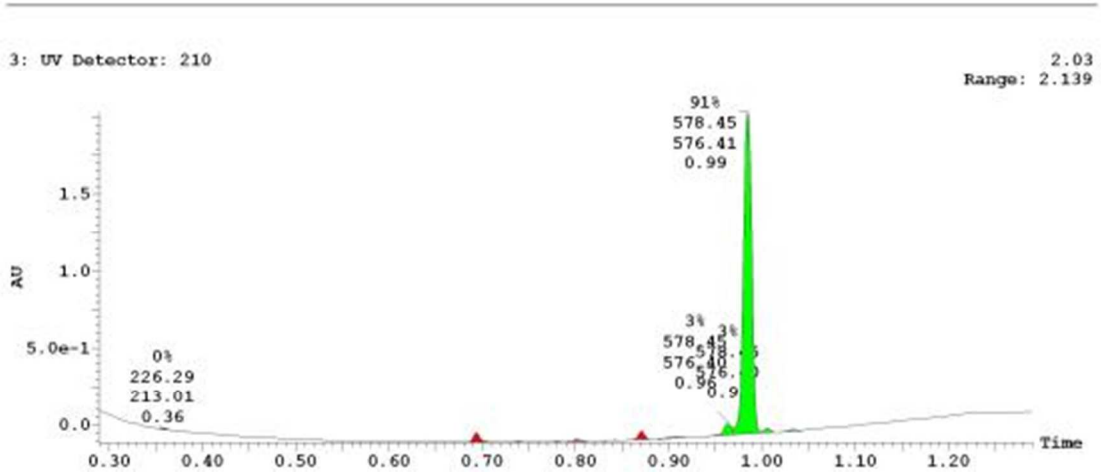
UPLC-MS of Compound 39



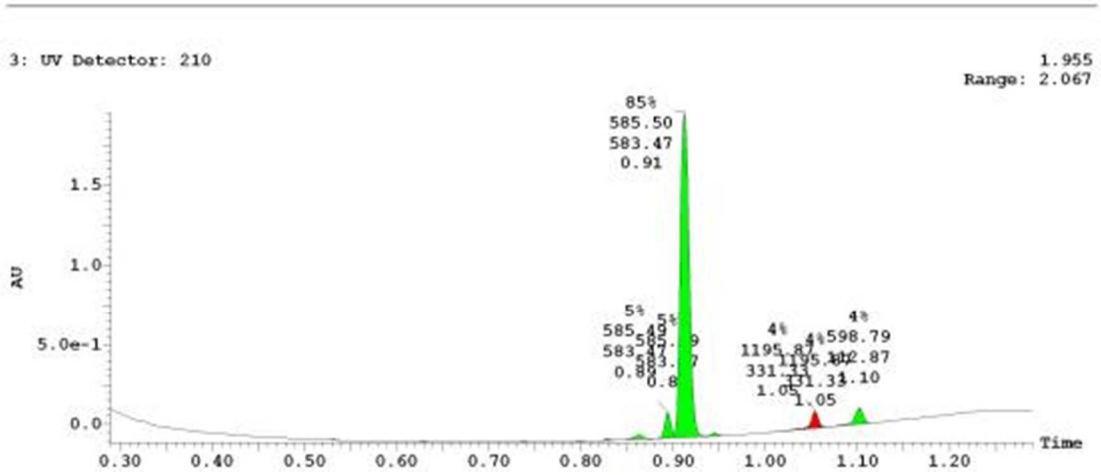
UPLC-MS of Compound 40



UPLC-MS of Compound 41



UPLC-MS of Compound 42



UPLC-MS of Compound 43