Supporting Information

Structure Activity Relationship of Heterocyclic P2Y$_{14}$ Receptor Antagonists: Removal of the Zwitterionic Character with Piperidine Bioisosteres

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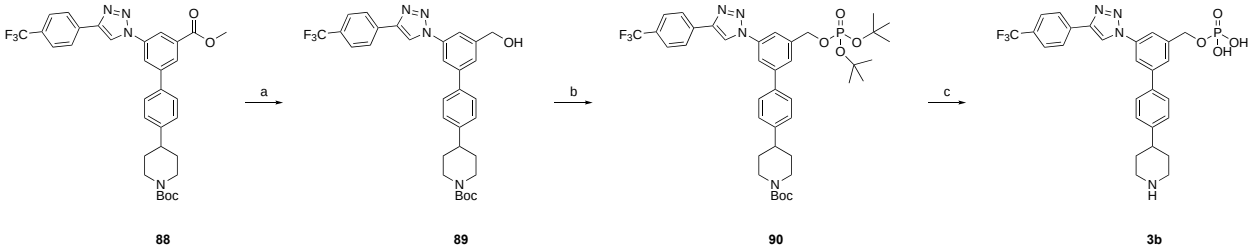
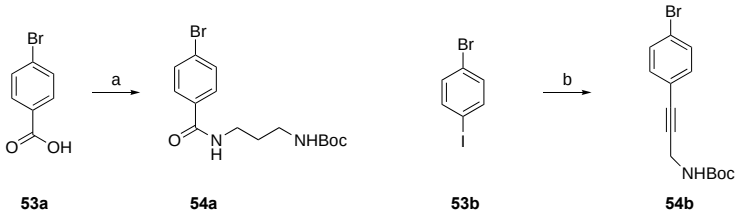


Scheme S1. Reagents and Conditions: (a) LAH, THF, 0 °C, 1 h, 45%; (b) di-*tert*-butyl *N*,*N*-diethylphosphoramidite, tetrazole, THF, rt, 1 h, then MCPBA, -78 °C, 84%; (c) TFA:THF = 2:1, rt, 2 h, 69%. See the main text for the procedures.



Scheme S2. Reagents and conditions: a) Boc-propylene-diamine, HATU, DIPEA, DMF, rt, 2 h, 31%; b) $PdCl_2(PPh_3)_2$, copper iodide, THF, triethylamine, *N*-Boc-propargylamine, rt, 94%. See the main text for the procedures.

Molecular modeling

Table S1. Inter-replicate average RMSD, Interaction Energy (as sum of electrostatic and van der Waals), Weighted Dynamic Scoring Function (wDSF), percentage of hydrogen bonds of Lys77$^{2.60}$, Tyr102$^{3.33}$ and Lys277$^{7.35}$. Compounds with wDSF/time lower than compound **3a** (reference) and IC_{50} lower than 1 μM are highlighted in green and considered true positives. False positives (wDSF lower than **3a**, but IC_{50} higher than 1 μM) are highlighted in red, false negatives (wDSF higher than **3a**, but IC_{50} lower than 1 μM) in orange, and not synthesized compounds in gray. See Figure S1 for structures.

Compound	Average values among 3 replicates						IC_{50} (μM)
	RMSD (Å)	Interaction Energy (kcal/mol)	Slope wDSF	Lys77$^{2.60}$ (% hbonds)	Tyr102$^{3.33}$ (% hbonds)	Lys277$^{7.35}$ (% hbonds)	
1a	1.90	-201.46	-99.63	63.89	26.98	83.20	0.00796
3a	3.01	-173.21	-70.55	91.24	18.91	75.69	0.0317
7	3.25	-171.93	-50.64	88.04	74.31	66.91	1.48
8	2.22	-177.83	-71.17	83.20	48.07	64.22	0.811
9	2.94	-179.27	-53.88	65.91	31.47	65.16	1.86
10	2.98	-176.40	-52.43	83.67	7.02	81.89	3.16
11	2.00	-235.43	-120.39	92.96	77.49	73.24	0.197
13	4.30	-223.25	-37.41	1.62	66.31	48.44	0.632
14	2.37	-250.33	-107.21	82.93	60.16	77.18	0.588
15	3.05	-173.01	-53.41	86.53	54.38	74.64	>10
16	7.05	-148.38	-20.43	0.00	16.18	36.64	1.29
17	3.66	-177.61	-48.05	84.71	88.02	82.80	17.8
18	1.94	-200.75	-96.58	54.27	87.71	79.47	0.292
19	2.20	-243.49	-109.20	37.02	70.24	77.80	0.308
21	4.04	-175.87	-42.88	7.76	45.36	76.89	9.22
22	1.92	-263.37	-118.51	87.47	66.82	82.38	14.1
23	2.09	-210.72	-91.24	88.60	83.27	80.53	8.91
25	2.34	-223.24	-77.24	67.96	38.36	76.64	2.00
27	2.32	-204.06	-99.71	87.62	80.47	79.22	2.86
28	2.90	-140.71	-48.10	87.71	80.42	29.00	2.23
29	2.23	-206.94	-99.22	92.73	88.18	77.42	0.296
30	2.75	-209.82	-64.94	0.02	14.64	77.71	0.389
31-tau1	5.92	-211.06	-32.84	0.00	47.29	51.09	0.895
31-tau2	1.51	-207.34	-134.67	95.56	1.69	69.44	0.895
piperazine 6	2.15	-212.78	-107.65	87.02	73.80	80.24	0.233[a]
oxadiazole, 91	2.92	-180.32	-63.42	70.04	52.24	81.11	-
4-hydroxy, 92	2.15	-217.61	-93.32	84.51	74.04	48.33	-
azido, 93	-	-	-	-	-	-	-

[a]Reported in Jung, Y. H. et al. Exploration of alternative scaffolds for $P2Y_{14}$ receptor antagonists containing a biaryl core. *J. Med. Chem.* **2020**, *63*, 9563–9589.

Table S2. Intra-replicate average RMSD, Interaction Energy (as sum of electrostatic and van der Waals), Weighted Dynamic Scoring Function (wDSF), percentage of hydrogen bonds of $Lys77^{2.60}$, $Tyr102^{3.33}$ and $Lys277^{7.35}$, for compounds **29**, **32** and **33**.

Compound		**Average values within each replicate**					
		RMSD (Å)	**Interaction Energy (kcal/mol)**	**Slope wDSF**	**$Lys77^{2.60}$ (% hbonds)**	**$Tyr102^{3.33}$ (% hbonds)**	**$Lys277^{7.35}$ (% hbonds)**
29	**run1**	1.99	-210.51	-117.02	93.60	89.60	74.53
	run2	2.39	-204.78	-92.71	90.93	84.47	76.80
	run3	2.30	-205.52	-87.92	93.67	90.47	80.93
	average	2.23	-206.94	-99.22	92.73	88.18	77.42
32	**run1**	2.23	-196.17	-89.77	89.00	75.33	26.00
	run2	2.12	-220.47	-107.67	74.00	86.87	80.13
	run3	1.85	-217.20	-104.66	69.27	79.60	81.00
	average	2.07	-211.28	-100.70	77.42	80.60	62.38
33	**run1**	2.76	-151.94	-58.53	94.00	67.47	0.73
	run2	7.31	-155.58	-11.72	0.00	50.60	1.20
	run3	3.51	-138.78	-23.08	85.87	59.93	1.47
	average	4.53	-148.77	-31.11	59.96	59.33	1.13

Table S3. Reversal of CCI-induced mechano-allodynia in the mouse by P2Y$_{14}$R antagonists.

Compound (10 μmol/kg, i.p.)	**Effects at 30 min (%Reversal±SD)**	**Effects at 1 h (%Reversal±SD)**	**Effects at 2 h (%Reversal±SD)**	**Effects at 3 h (%Reversal±SD)**	**Effects at 5 h (%Reversal±SD)**	**IC_{50} ($mP2Y_{14}$, nM)**	**MW (D)**
1a[a]	87.9±12.9	100±0.0	96.4±6.2	78.6±9.4	21.3±14.1	21.6±7.0	475.5
1b[a]	83.3±9.6	100±0.0	100±0.0	92.4±8.7	40.7±7.5	29.7±9.3	517.6
3a[a]	52.4±9.6	67.4±7.0	57.2±13.3	31.9±16.8	12.7±13.2	142±58	492.5
32	41.5±13.4	71.0±17.4	34.9±18.4	15.7±14.4	0.0±0.0	18.6±8.4	489.5

a Data published in Mufti et al.[1] IC_{50} values are from the fluorescent binding assay, as described in the main text.

1. Mufti, F.; Jung, Y. H.; Giancotti, L. A.; Yu, J.; Chen, Z..; Phung, N. B.; Jacobson, K. A.; Salvemini, D. P2Y$_{14}$ receptor antagonists reverse chronic neuropathic pain in a mouse model. *ACS Med. Chem. Lett.* **2020**, *11*, 1281−1286.

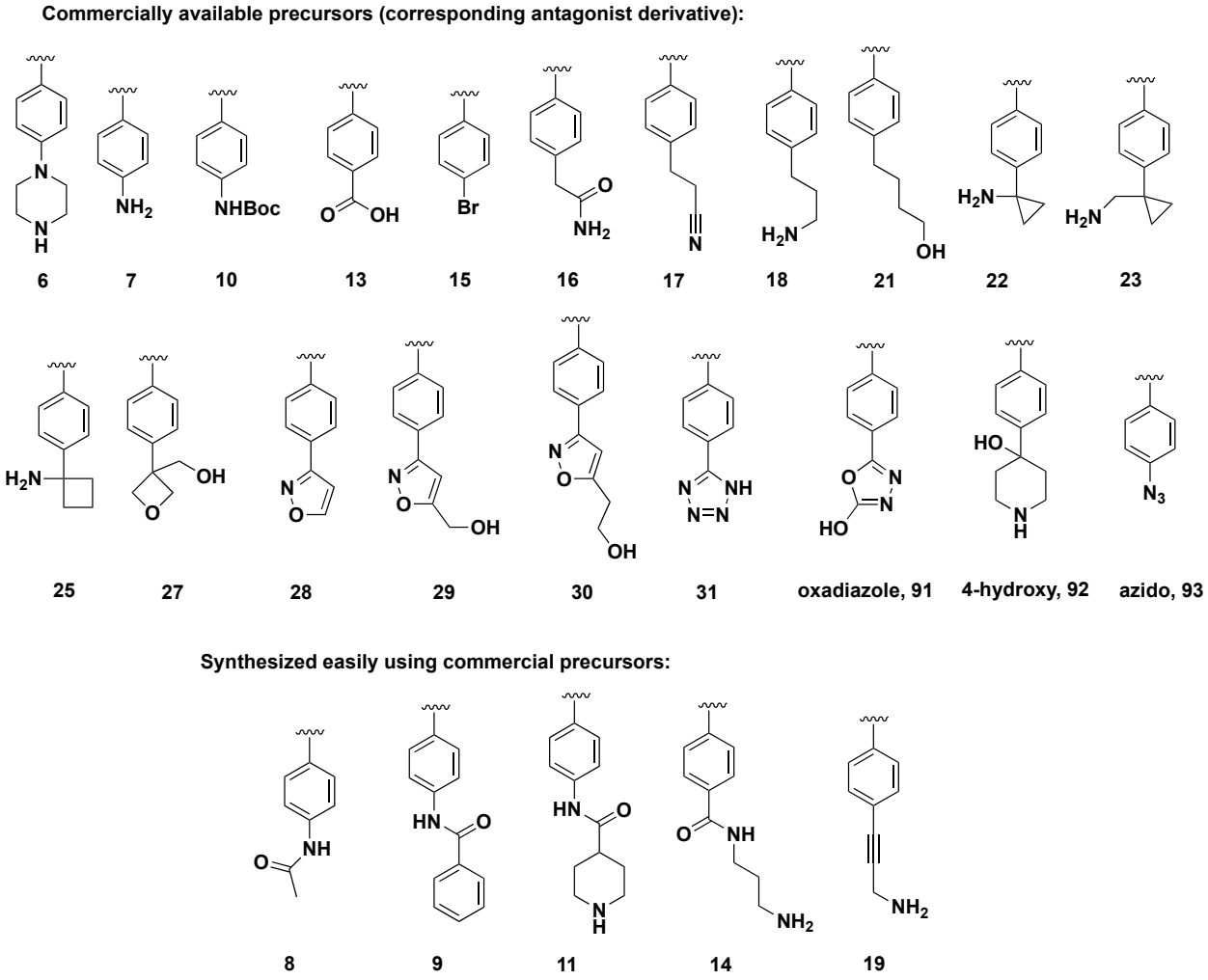


Figure S1. R-groups corresponding to 20 commercially available bromo-aryl precursors and 5 R-groups easily obtainable by chemical synthesis starting from commercial precursors. These R-groups were used to build a library of compounds of the triazole series, which was submitted to molecular modeling evaluation to prioritize the synthesis. The compound bearing the azido substituent was not subjected to MD simulations because of lack of parameters for the azido group.

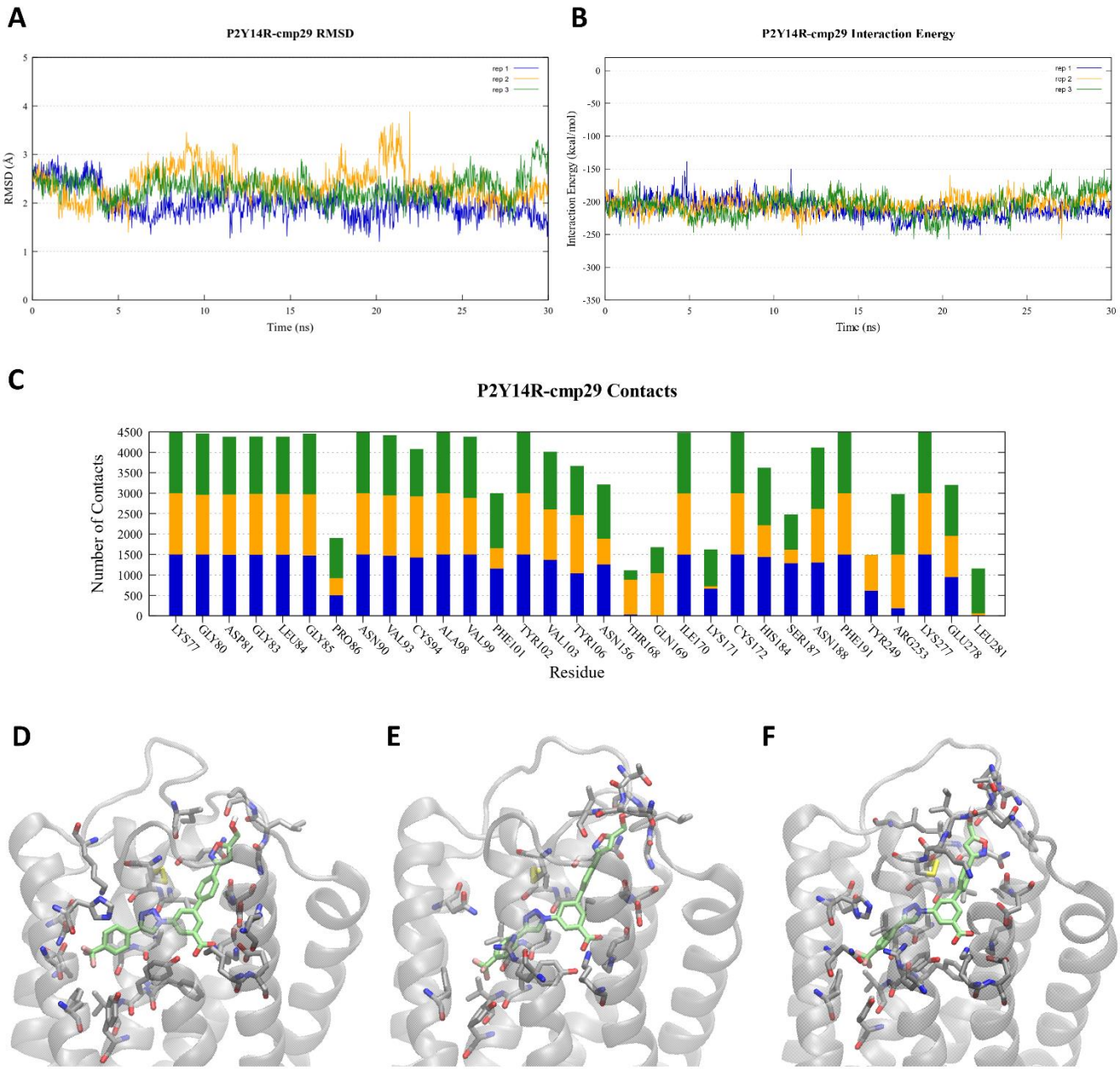


Figure S2. Results of the MD simulations of the complex between hP2Y$_{14}$R and compound **29**. **A)** RMSD of ligand heavy atoms relative to the docking pose, after superposition of the protein Cα atoms to the starting position. **B)** Ligand-receptor interaction energy (as sum of electrostatic and van der Waals components). **C)** Number of contacts (distance ≤ 4 Å) between ligand and receptor residues. Residues in contact with the ligand for more than half of the simulation in at least one replicate are reported. A stride of 20 ps was employed, so a maximum of 1500 contacts (frames) can be observed for each replicate. **D, E** and **F)** Last frame of replicates 1-2-3. The receptor is reported in gray and the ligand in lime.

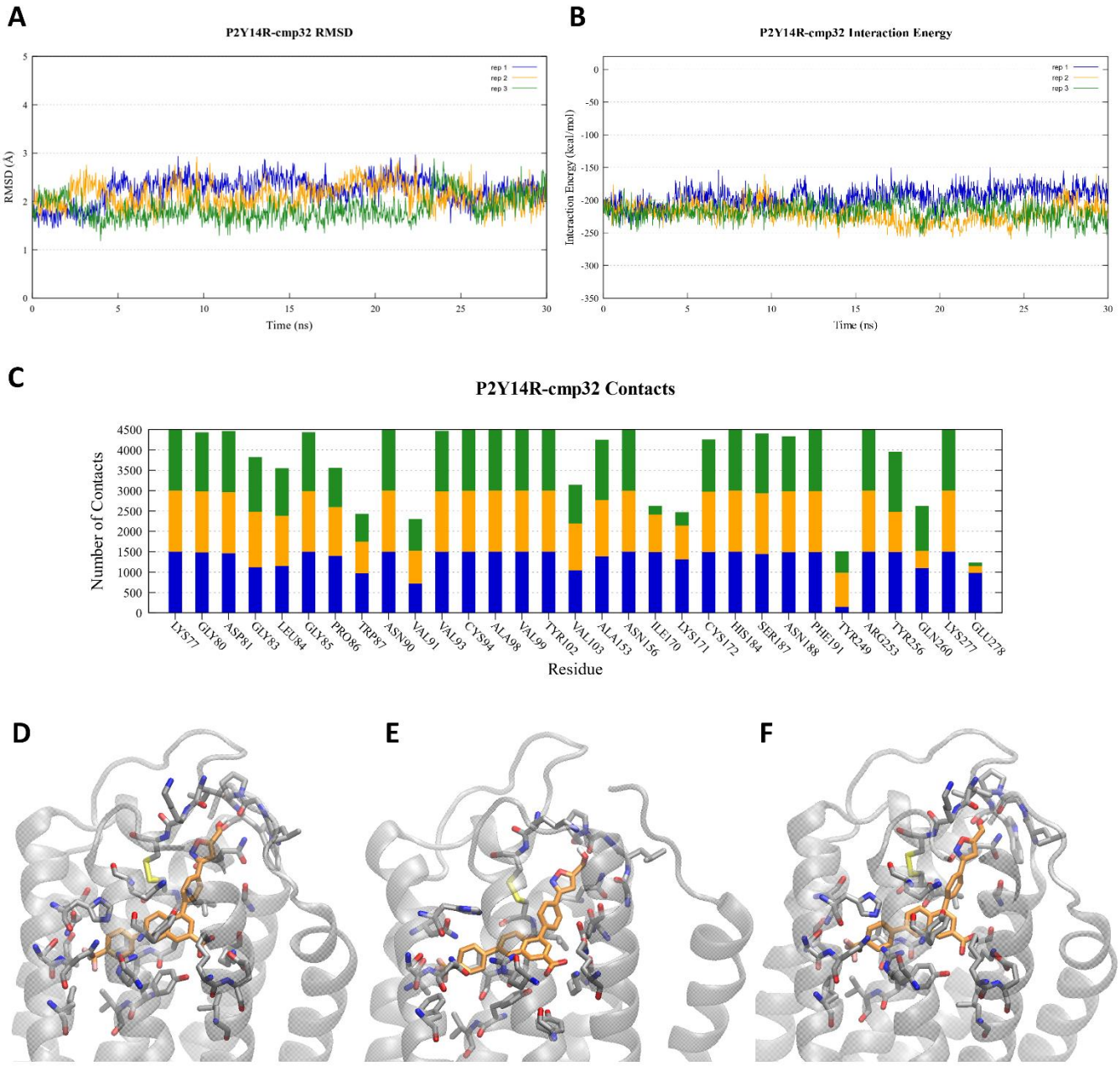


Figure S3. Results of the MD simulations of the complex between hP2Y$_{14}$R and compound **32**. **A**) RMSD of ligand heavy atoms relative to the docking pose, after superposition of the protein Cα atoms to the starting position. **B**) Ligand-receptor interaction energy (as sum of electrostatic and van der Waals components). **C**) Number of contacts (distance ≤4 Å) between ligand and receptor residues. Residues in contact with the ligand for more than half of the simulation in at least one replicate are reported. A stride of 20 ps was employed, so a maximum of 1500 contacts (frames) can be observed for each replicate. **D**, **E** and **F**) Last frame of replicates 1-2-3. The receptor is reported in gray and the ligand in orange.

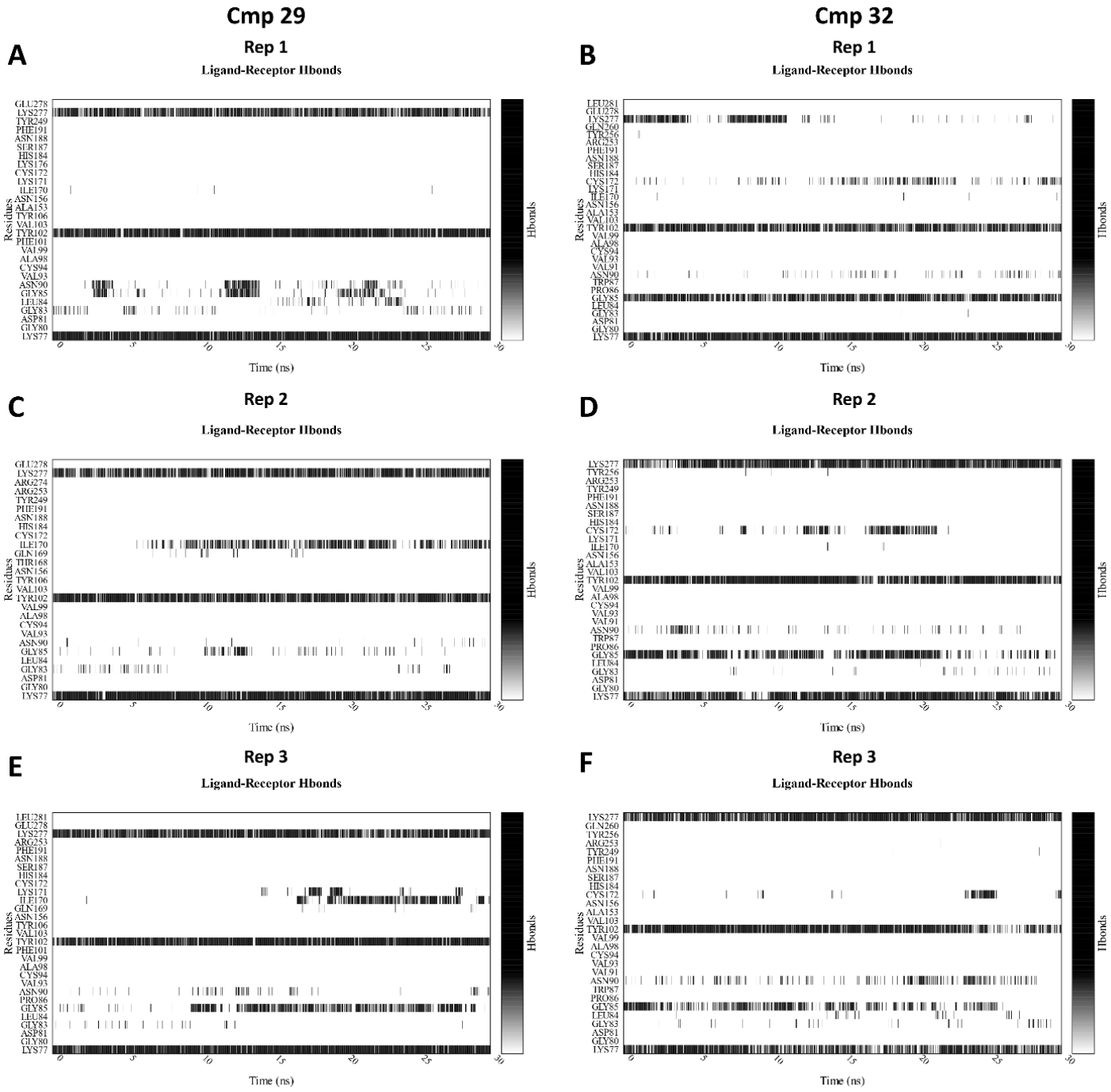


Figure S4. Ligand-receptor hydrogen bonds during 30 ns MD simulations. Residues that are in contact (distance ≤ 4 Å) with the ligand for more than half of the simulation are shown. The panels on the left (**A**, **C** and **E**) are relative to compound **29** (replicates 1, 2 and 3), while panels on the right (**B**, **D** and **F**) are relative to compound **32** (replicates 1, 2 and 3).

Figure S4

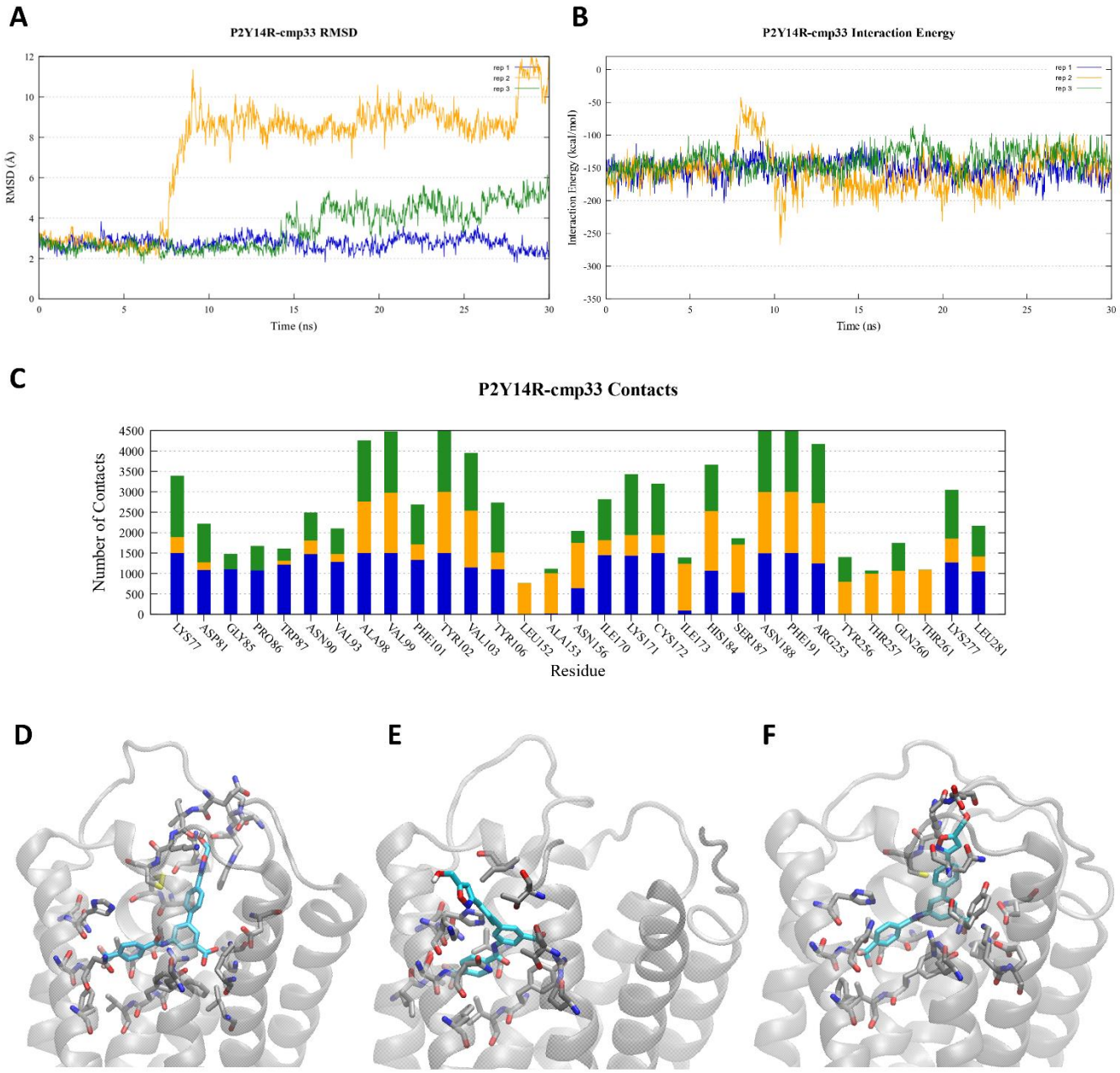


Figure S5. Results of the MD simulations of the complex between hP2Y$_{14}$R and compound **33**. **A**) RMSD of ligand heavy atoms relative to the docking pose, after superposition of the protein Cα atoms to the starting position. **B**) Ligand-receptor interaction energy (as sum of electrostatic and van der Waals components). **C**) Number of contacts (distance ≤4 Å) between ligand and receptor residues. Residues in contact with the ligand for more than half of the simulation in at least one replicate are reported. A stride of 20 ps was employed, so a maximum of 1500 contacts (frames) can be observed for each replicate. **D**, **E** and **F**) Last frame of replicates 1-2-3. The receptor is reported in gray and the ligand in cyan.

Off-target activity:

Determined by the the Psychoactive Drug Screening Program (PDSP)

We thank Dr. Bryan L. Roth (Univ. North Carolina at Chapel Hill) and National Institute of Mental Health's Psychoactive Drug Screening Program (Contract # HHSN-271-2008-00025-C) for screening data. Reference: Besnard, J.; Ruda, G. F.; Setola, V.; Abecassis, K.; Rodriguiz, R. M.; Huang, X. P.; Norval, S.; Sassano, M. F.; Shin, A. I.; Webster, L. A.; Simeons, F. R.; Stojanovski, L.; Prat, A.; Seidah, N. G.; Constam, D. B.; Bickerton, G. R.; Read, K. D.; Wetsel, W. C.; Gilbert, I. H.; Roth, B. L.; Hopkins, A. L. Automated design of ligands to polypharmacological profiles. *Nature* **2012**, *492*, 215–220.

Procedures: https://pdsp.unc.edu/pdspweb/content/UNC-CH%20Protocol%20Book.pdf

Unless noted in the text, no significant interactions (<50% inhibition at 10 μM) for any of the nucleosides were found at the following sites (human unless noted): $5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, $5HT_{1E}$, $5HT_{2A}$, $5HT_{2B}$, $5HT_{2C}$, $5HT_3$, $5HT_{5A}$, $5HT_6$, $5HT_7$, α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, β_3, BZP rat brain site, D_1, D_2, D_3, D_4, D_5, $GABA_A$, H_1, H_2, H_3, H_4, M_1, M_2, M_5, δ-opioid receptor (DOR), κ-opioid receptor (KOR), μ-opioid receptor (MOR), σ_1, σ_2, DAT, NET, SERT. K_i values in μM, or % inhibition at 10 μM, are given. Representative curves are shown in Fig. S6.

Results shown as: receptor, K_i, μM or % inhibition at 10 μM.

1a, PPTN (PDSP 37482): (in earlier paper)[1,2]

1b, *N*-Ac-PPTN (PDSP 55252): (in earlier paper)[2]

1c, *N*-formyl-PPTN (PDSP 58298): $5HT_{1D}$ 81%; α_{1B}, 2.65; α_{2A} 3.97; α_{2B} 82%; α_{2C} 3.93; D_1 0.52, D_5 1.44; σ_1 0.759, σ_2 84%, H_4, 1.70.

1d, *N*-CF_3CO-PPTN: not submitted.

3a, MRS4217 (PDSP 37481): (in earlier paper)[1,2]

6, MRS4544 (PDSP 53888): (in earlier paper)[2]

11, MRS4681 (PDSP 55946): σ_1, 2.33±0.15; DOR, 2.0±0.38.

13, MRS4678 (PDSP 57280): none detected.

16, MRS4594 (PDSP 53890): σ_2 0.931±0.388; TSPO, 2.81±0.18.

18, MRS4635 (PDSP 55841): none detected.

19, MRS4698 (PDSP 56449): none detected.

29, MRS4586 (PDSP 53889): DOR, 6.9±1.9; H_1, 2.65±0.74; TSPO, 0.751±0.101.

30, MRS4702 (PDSP 57282): DOR, 2.15, TSPO, 1.93.

31, MRS4683 (PDSP 57281): DOR, 3.30±1.76; σ_2, 2.26±1.28.

32, MRS4654 (PDSP 56758): σ_1 2.68±0.21; σ_2 4.78±0.99; TSPO 4.63±0.87.

37a, MRS4543 (PDSP 57573): $5HT_{1D}$, 2.39; $5HT_{1B}$, 5.57; $5HT_{5A}$, 2.04; $5HT_{7A}$, 2.22; D_1 1.22; D_3, 0.84; D_5, 3.01; H_2, 54%; α_{1A}, 1.23; α_{1B}, 2.52; α_{2B}, 1.81; α_{2C}, 0.790; σ_1, 0.133; σ_2, 0.23; M_5, 2.23; β_2, 4.18; β_3, 1.24; DAT 0.165; SERT, 1.80; NET, 0.469.

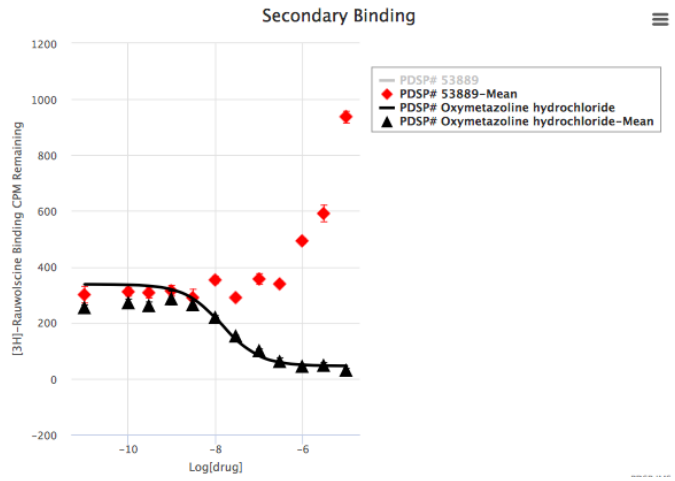
37c, MRS4741 (PDSP 57574): σ_2, 0.585±0.125.

References:

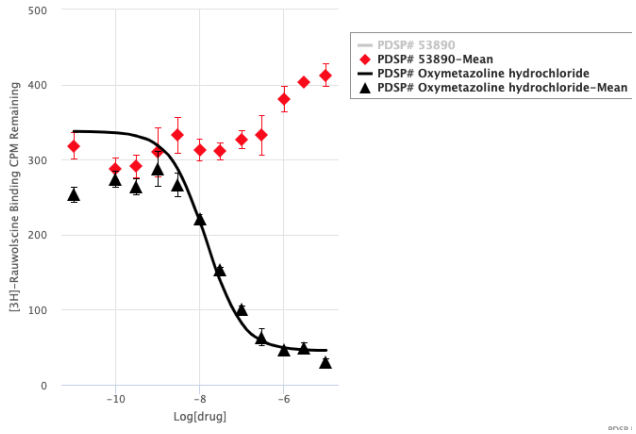
1. Yu, J.; Ciancetta, A.; Dudas, S.; Duca, S.; Lottermoser, J.; Jacobson, K.A. Structure-guided modification of heterocyclic antagonists of the $P2Y_{14}$ receptor. *J. Med. Chem.* **2018**, *61*, 4860−4882, doi: 10.1021/acs.jmedchem.8b00168.
2. Jung, Y. H.; Yu, J.; Wen, Z.; Salmaso, V.; Phung, N. B.; Karcz, T.; Chen, Z.; Duca, S.; Bennett, J. M.; Dudas, S.; Cook, D. N.; Salvemini, D.; Gao, Z. G.; Jacobson, K. A. Exploration of alternative scaffolds for $P2Y_{14}$ receptor antagonists containing a biaryl core. *J. Med. Chem.* **2020**, *63*, 9563–9589.

Figure S6. Binding curves for selected off-target assays performed by the PDSP. A. Binding enhancement at α_{2A}R by compound **29**. B. Binding enhancement at α_{2A}R by compound **16**. C. TSPO binding inhibition by compound **30**.

A



B



C

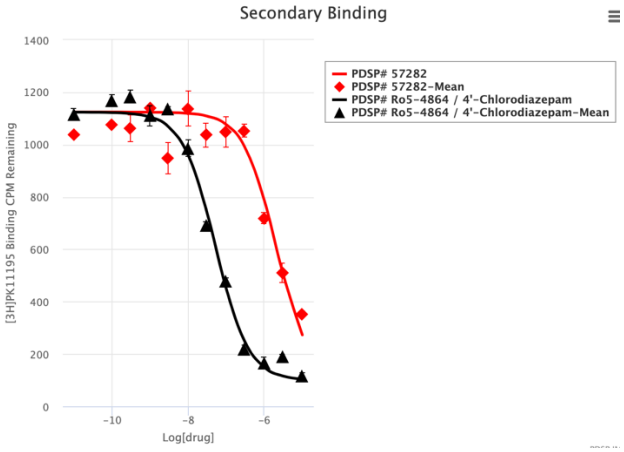


Figure S7A. Hydrolytic stability of the prodrug **37a** in the absence of PLE. HPLC traces of **3a** (active drug alone) and **37a** (prodrug alone), and of **37a** following incubation at pH 7.4 and 37 °C for 24 h.

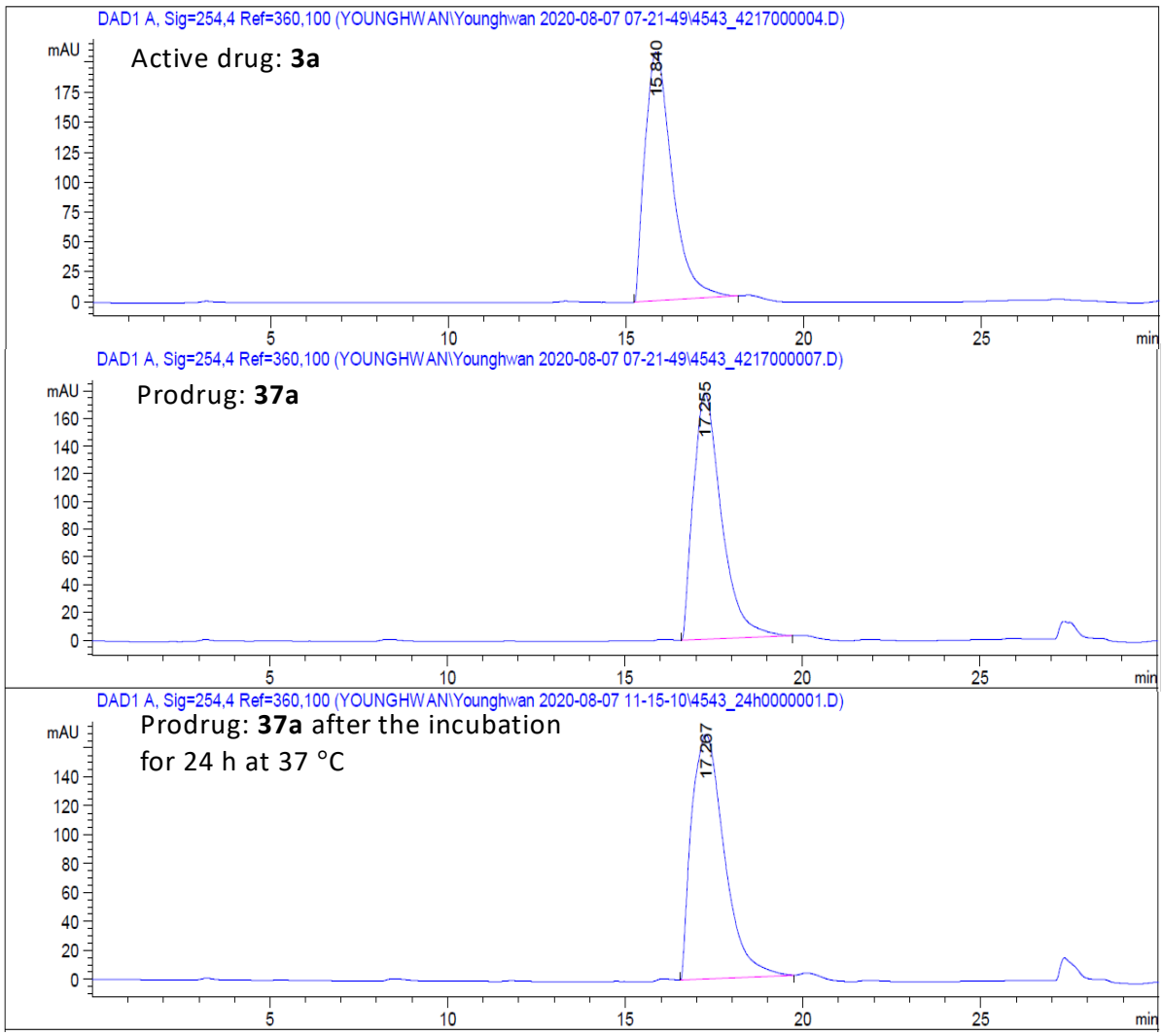


Figure S7B. Enzymatic hydrolysis of the prodrug **37a** to produce active drug **3a**. HPLC traces of **37a** after the reaction with PLE for 5, 35, 65, and 120 min at 37 °C.

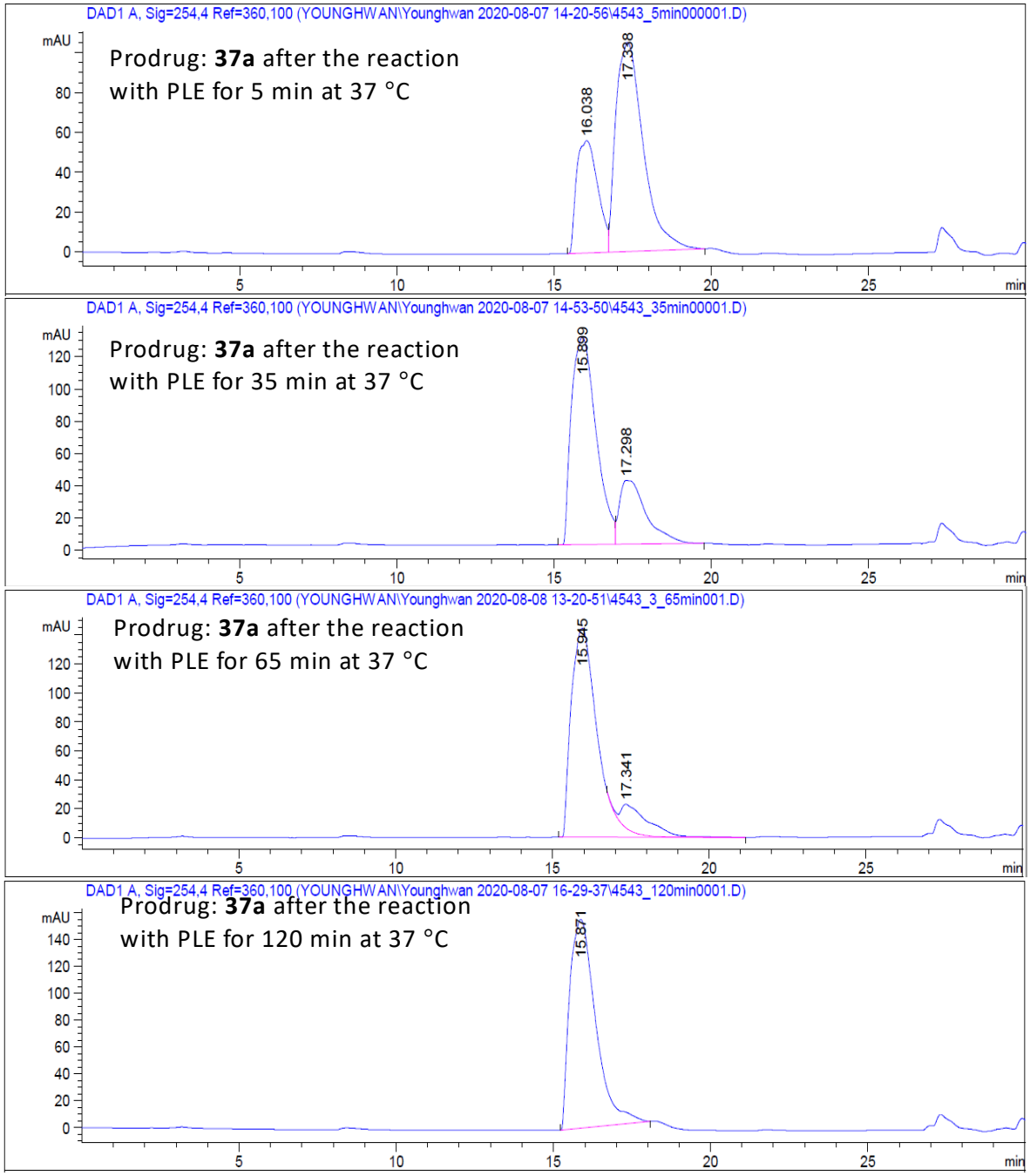


Figure S7C. Enzymatic hydrolysis of the prodrug **37a** to produce active drug **3a**. The half-life of **37a** by the reaction with PLE was 20.20 ± 1.85 min (Mean ± SEM).

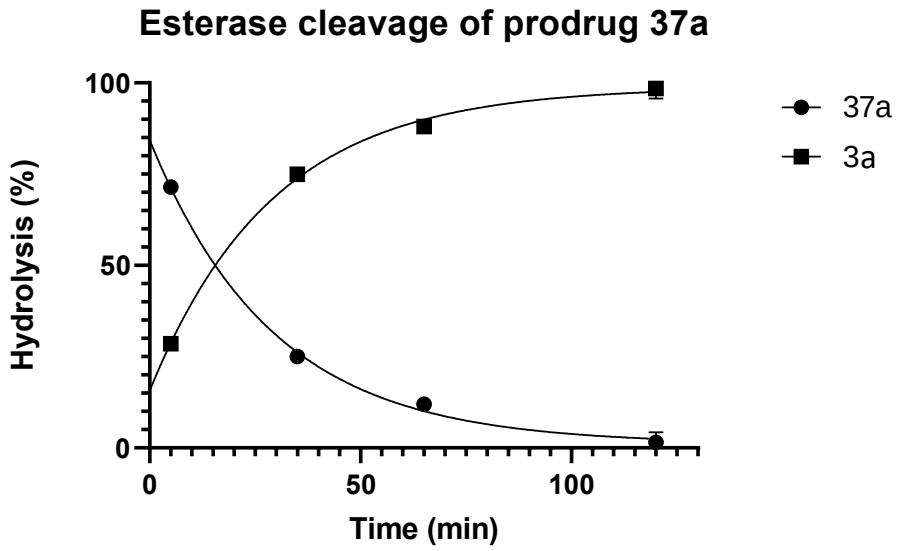


Figure S8A. Hydrolytic stability of the prodrug **37b** in the absence of PLE. HPLC traces of **32** (active drug alone) and **37b** (prodrug alone), and of **37b** following incubation at pH 7.4 and 37 °C for 24 h.

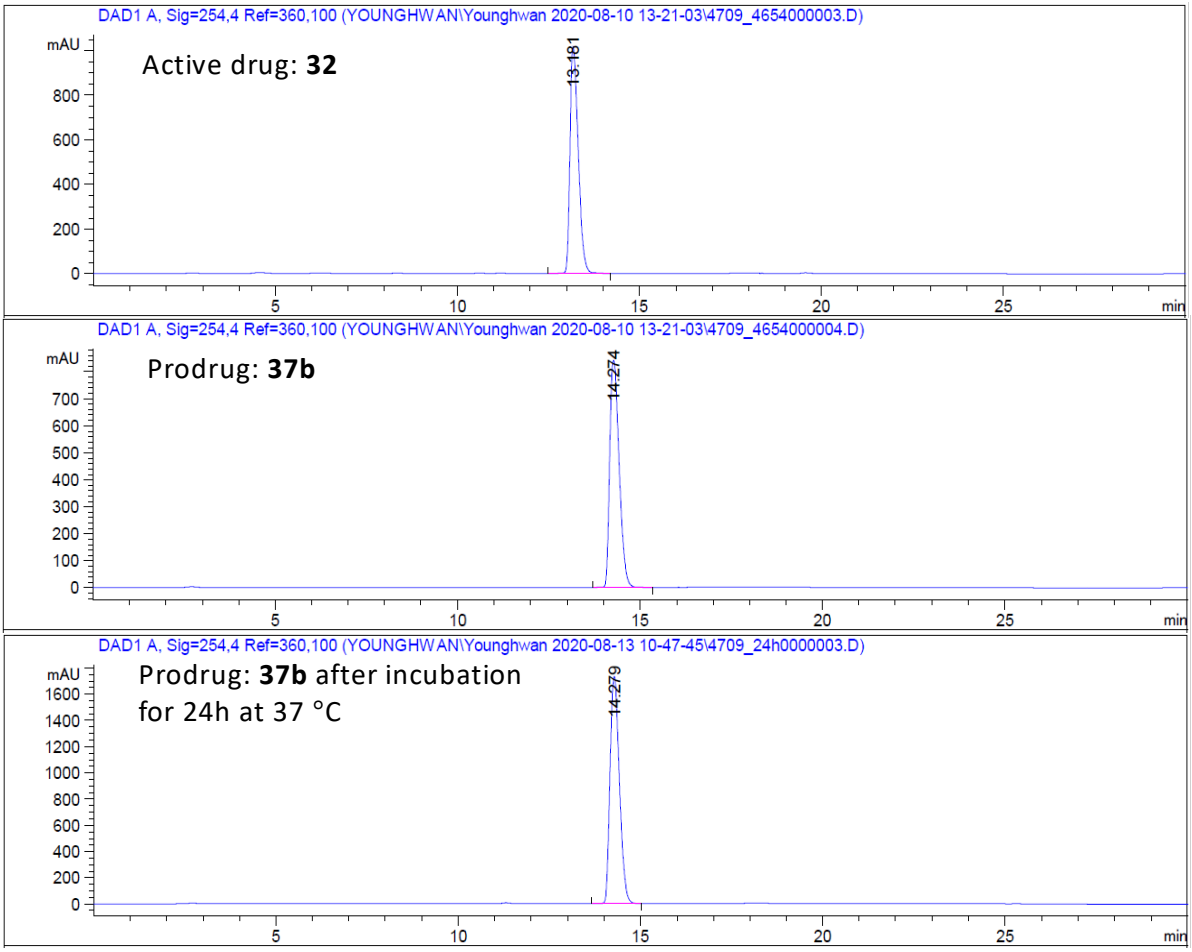


Figure S8B. Enzymatic hydrolysis of the prodrug **37b**. HPLC traces of **37b** after the reaction with PLE for 0, 2, 6, 24, 48, and 72 h at 37 °C.

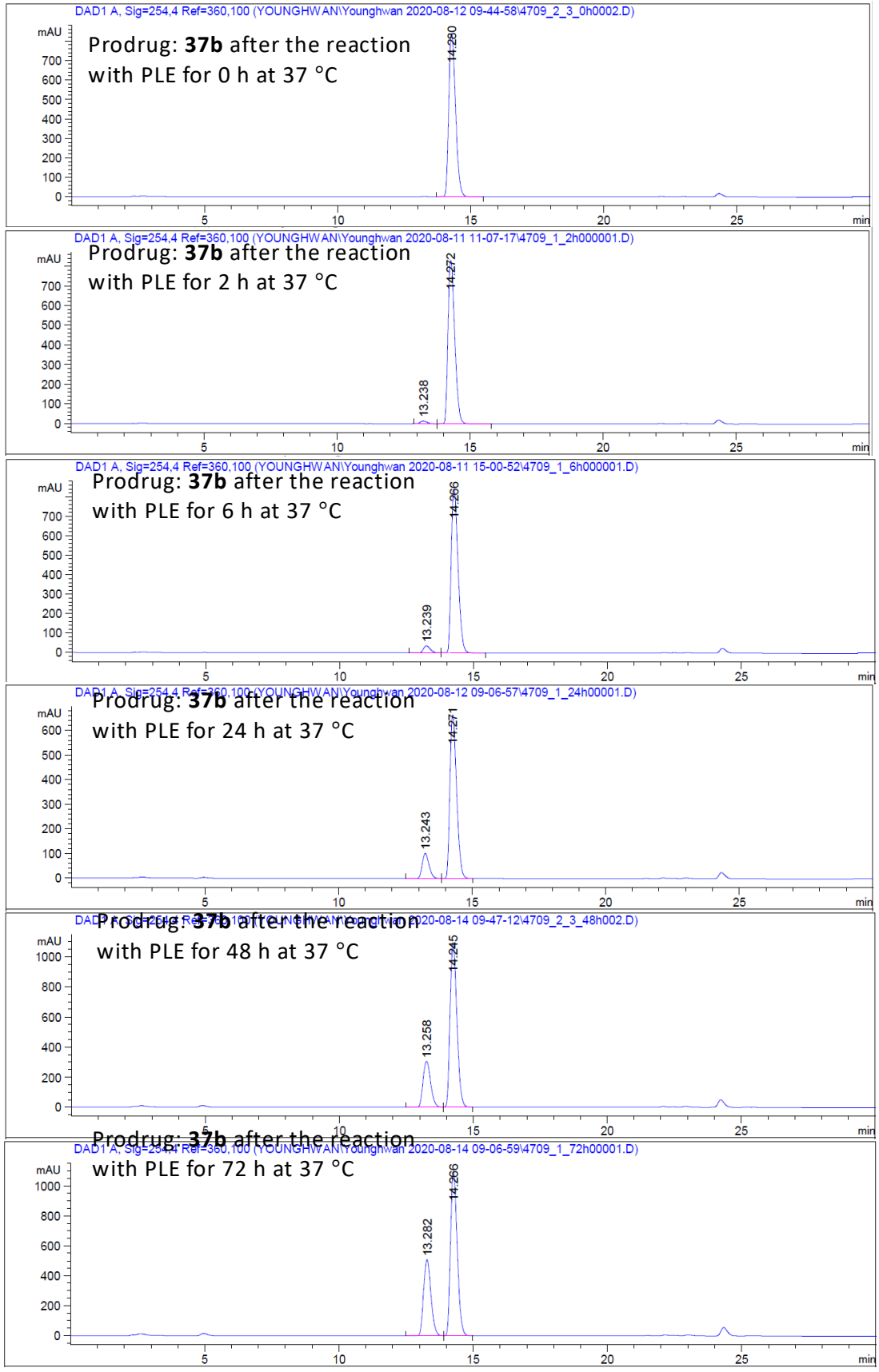


Figure S8C. Enzymatic hydrolysis of the prodrug **37b**. Mean ± SD is shown.

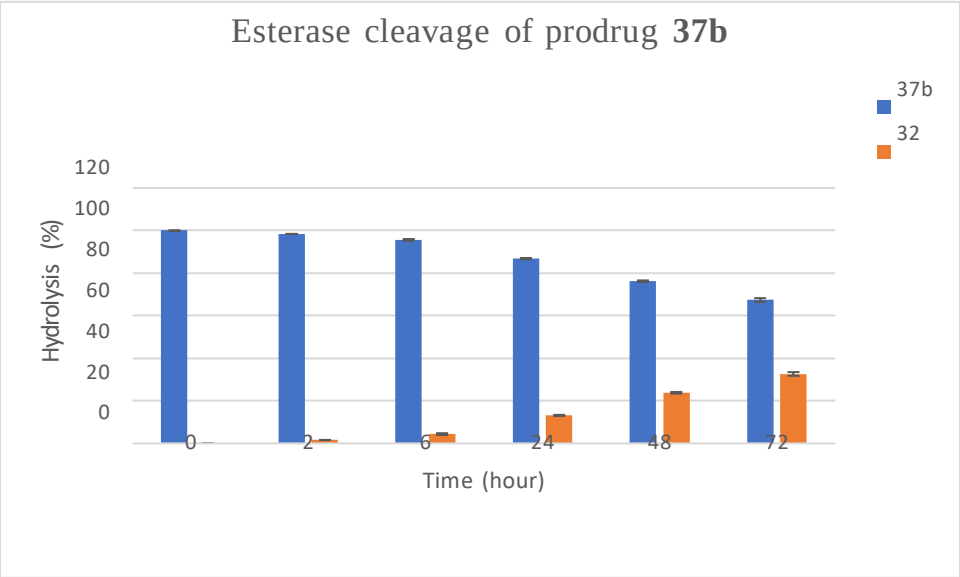


Figure S9A. Hydrolytic stability of the prodrug **37c** in the absence of PLE. HPLC traces of **1b** (active drug alone) and **37c** (prodrug alone), and of **37c** following incubation at pH 7.4 and 37 °C for 24 h.

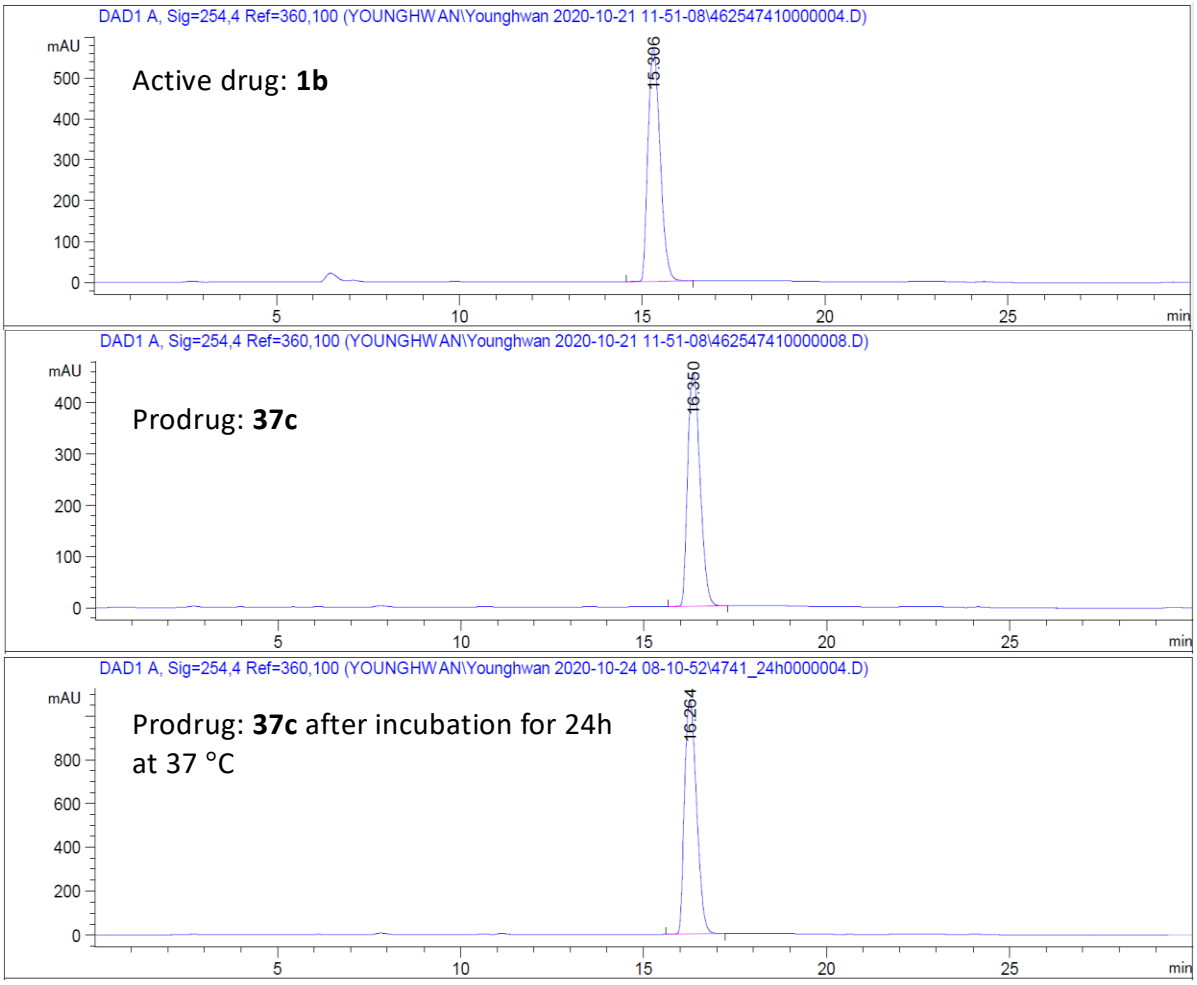


Figure S9B. Enzymatic hydrolysis of the prodrug **37c**. HPLC traces of **37c** after the reaction with PLE for 0, 2, 6, 24, 48, and 72 h at 37 °C.

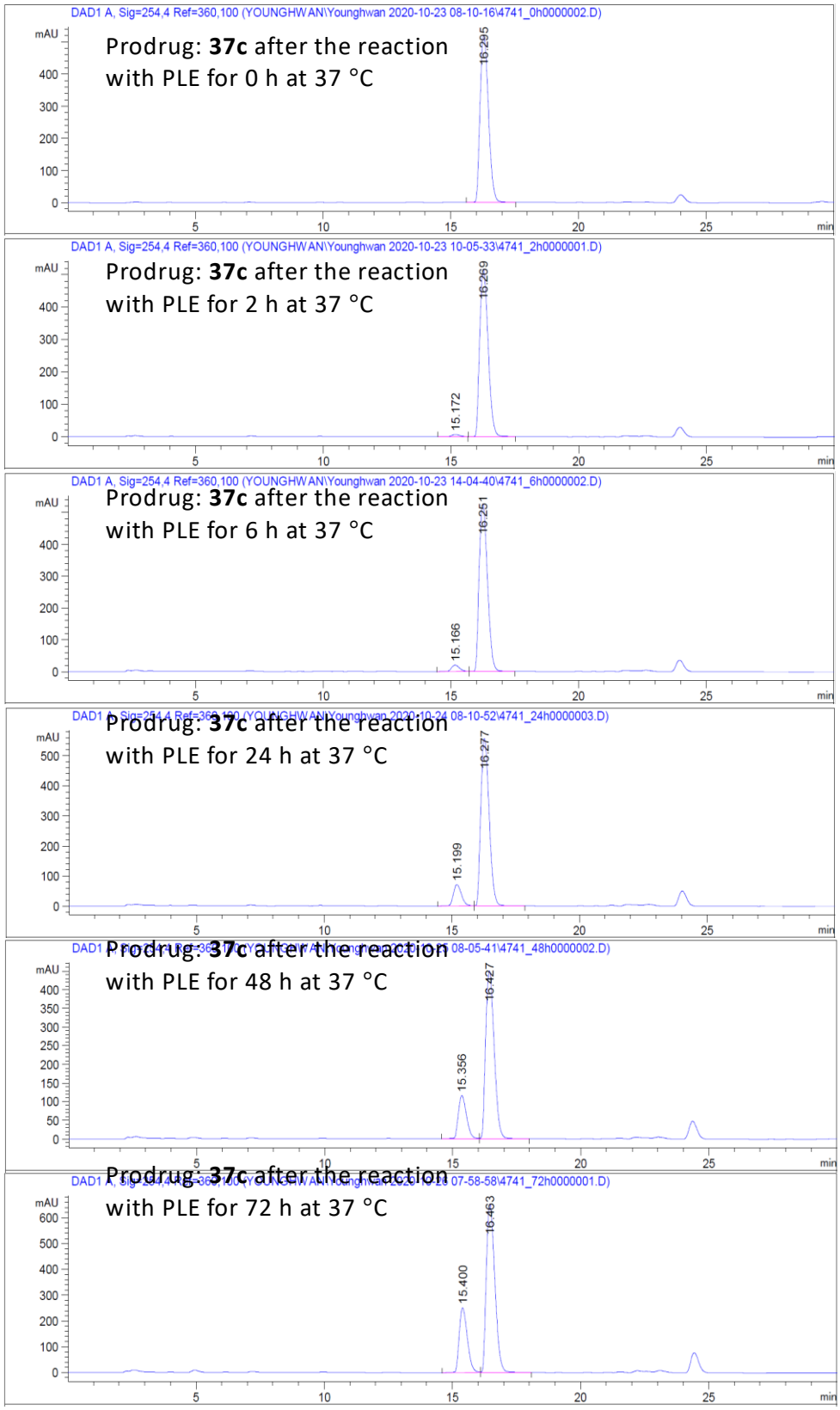
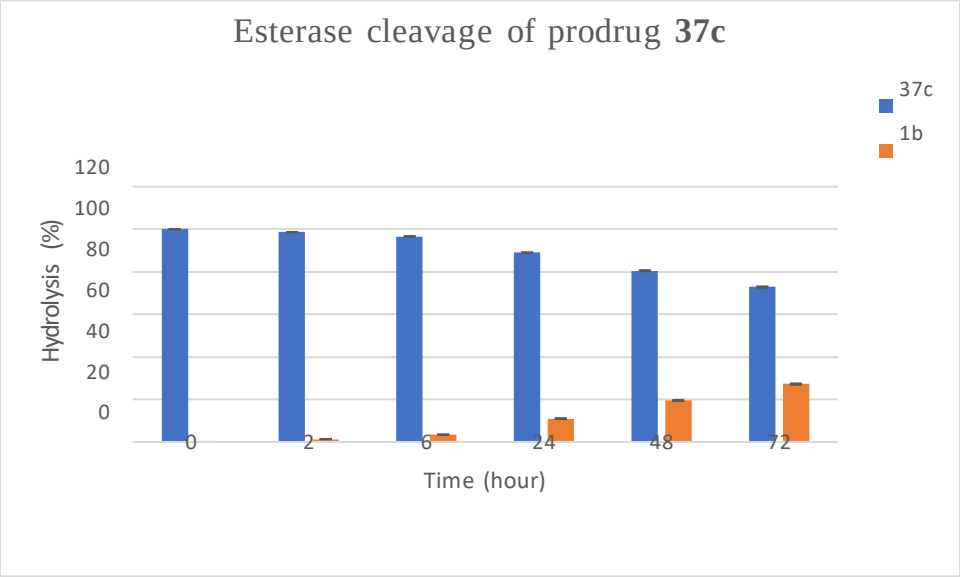
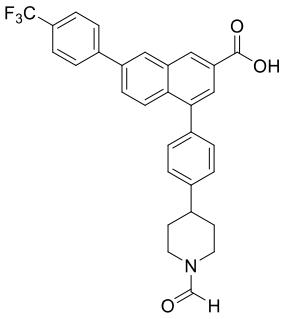


Figure S9C. Enzymatic hydrolysis of the prodrug **37c**. Mean ± SD is shown.



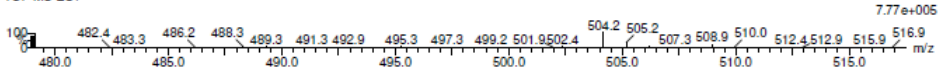
4-(4-(1-Formylpiperidin-4-yl)phenyl)-7-(4-(trifluoromethyl)phenyl)-2-naphthoic acid (**1c**).



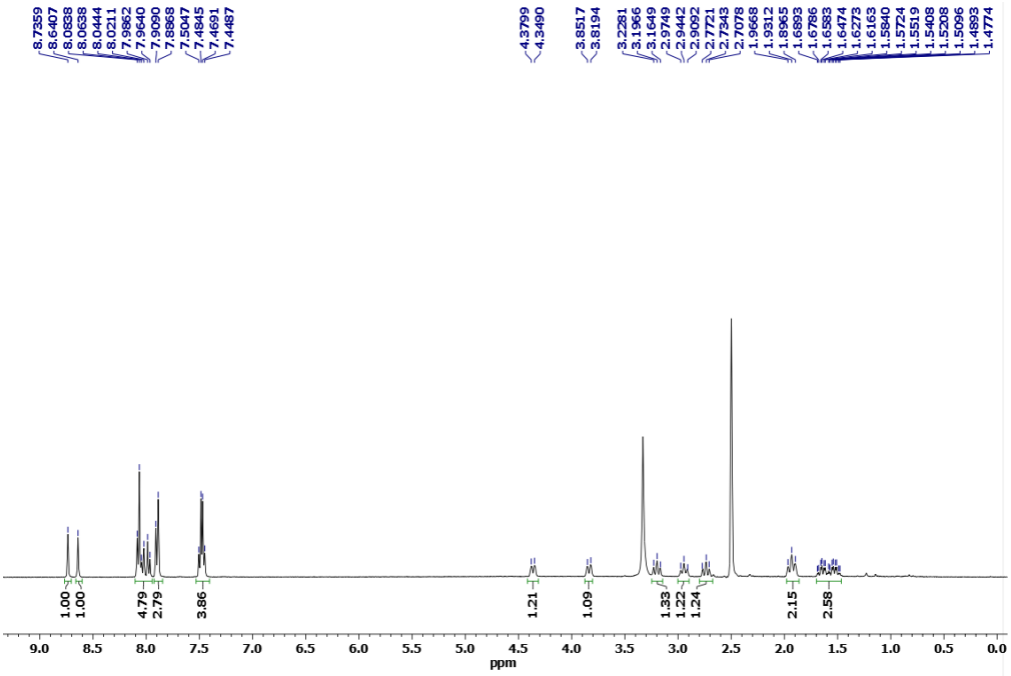
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

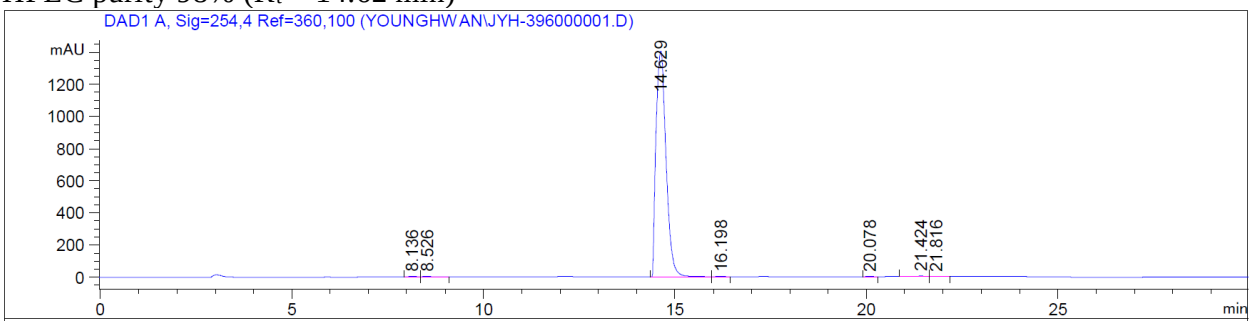
Monoisotopic Mass, Even Electron Ions
55 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 1-1 O: 0-10 F: 3-3
JYH-30OCT20-396-1 202 (3.434) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



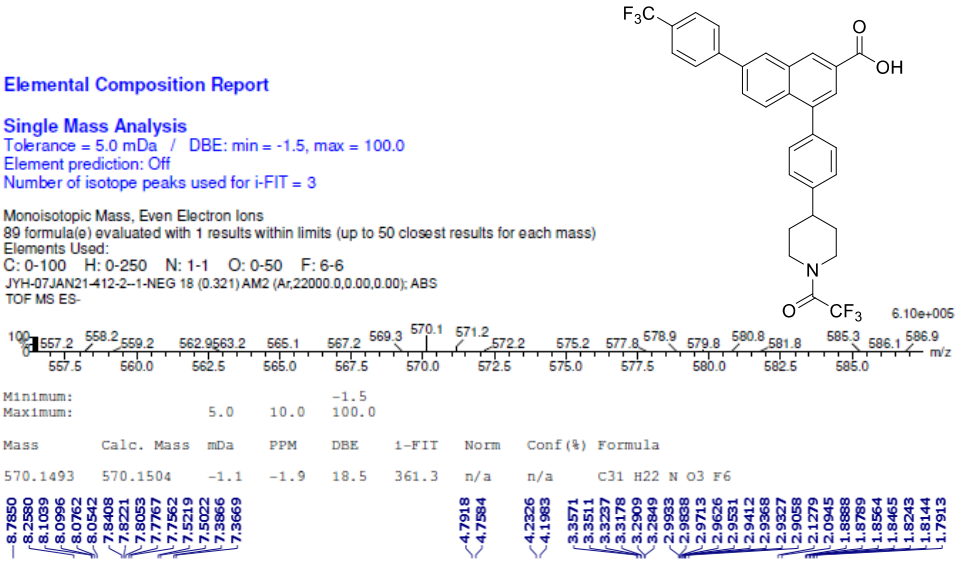
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
504.1792	504.1787	0.5	1.0	17.5	377.0	n/a	n/a	C30 H25 N O3 F3

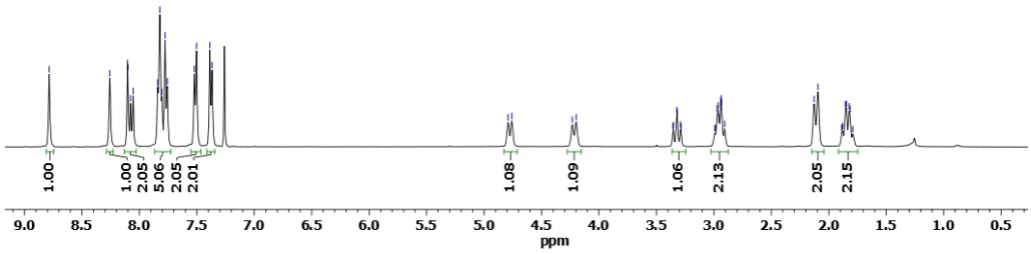


HPLC purity 98% (R_t = 14.62 min)

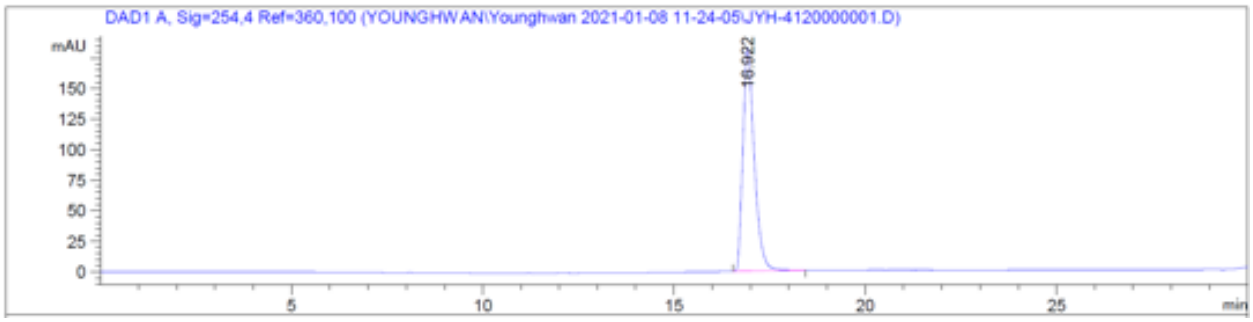


4-(4-(1-(2,2,2-Trifluoroacetyl)piperidin-4-yl)phenyl)-7-(4-(trifluoromethyl)phenyl)-2-naphthoic acid (**1d**).

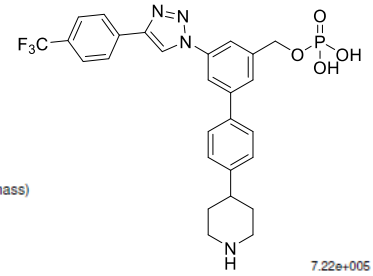




HPLC purity 99% (R_t = 16.92 min)



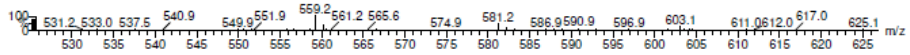
(4'-(Piperidin-4-yl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-yl)methyl dihydrogen phosphate (**3b**).



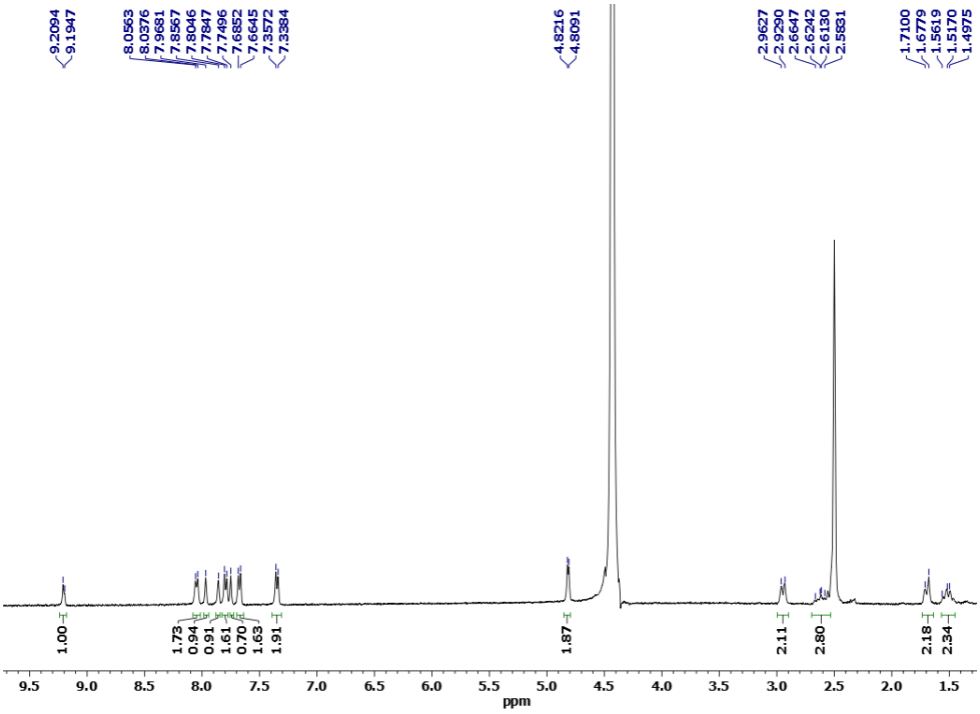
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

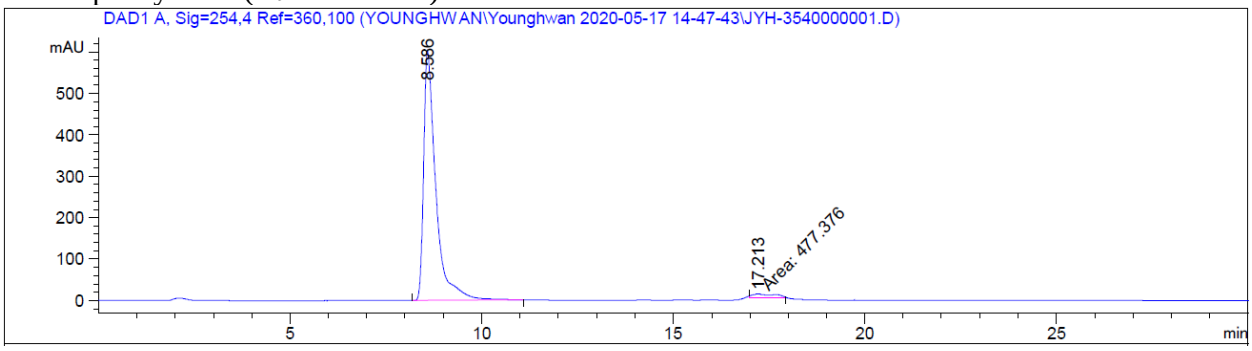
Monoisotopic Mass, Even Electron Ions
98 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-60 F: 3-3 P: 1-1
JYH-19MAY20-354-HCL 169 (2.876) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



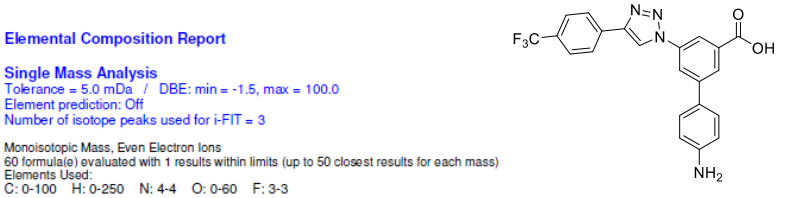
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
559.1716	559.1722	-0.6	-1.1	15.5	404.5	n/a	n/a	C27 H27 N4 O4 F3 P

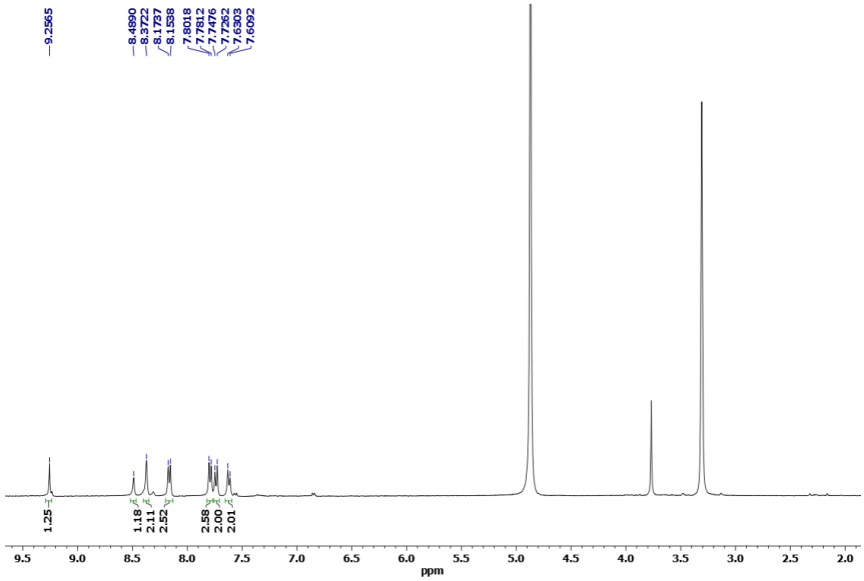


HPLC purity 96% (R_t = 8.58 min)

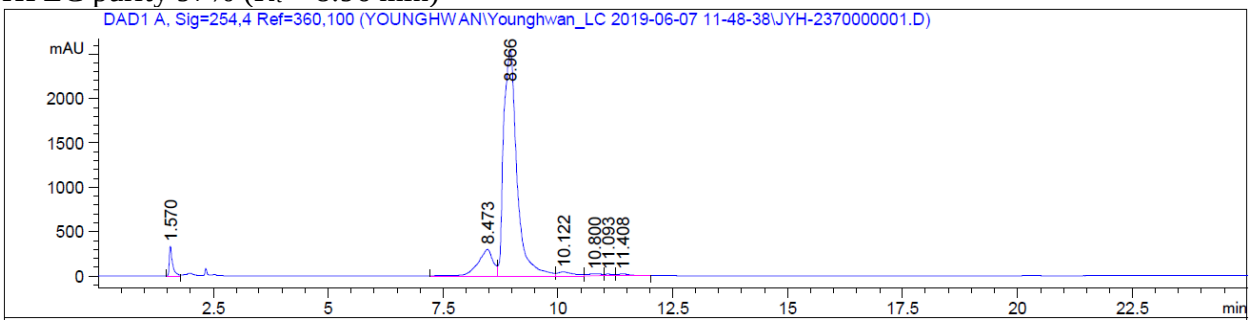


Amino-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**7**).

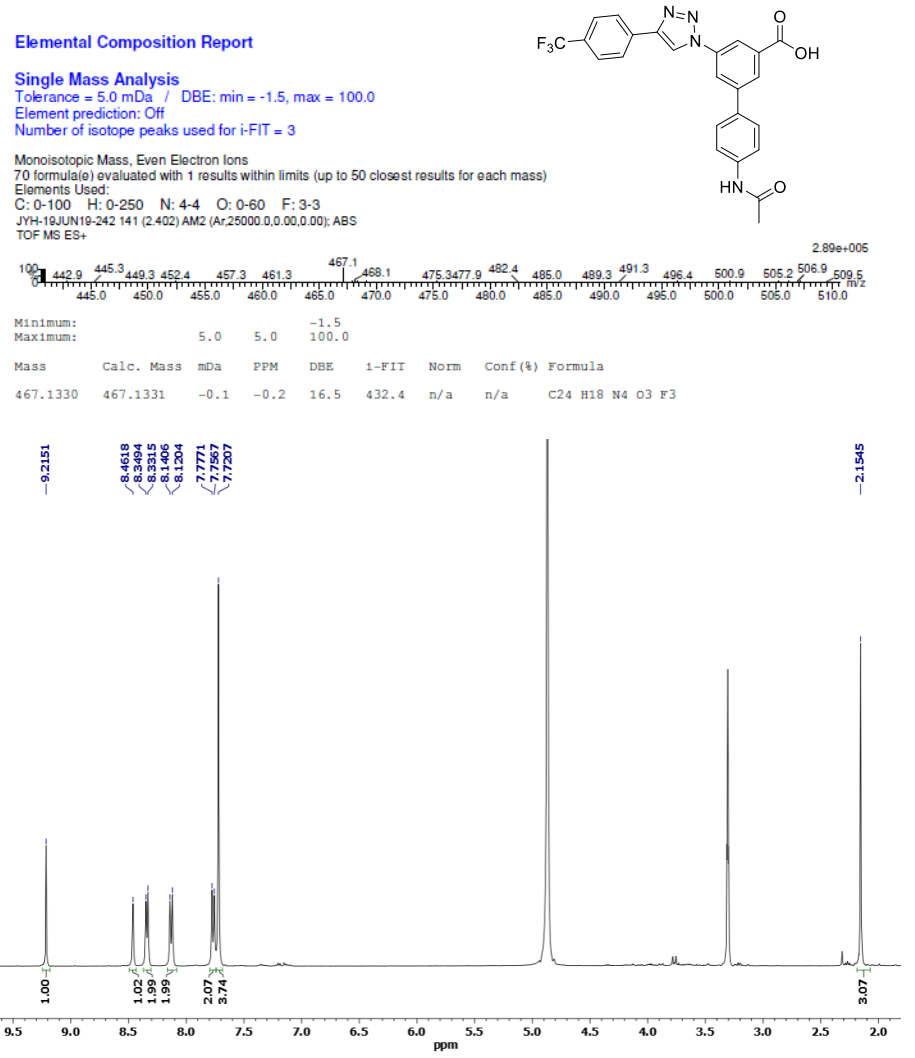




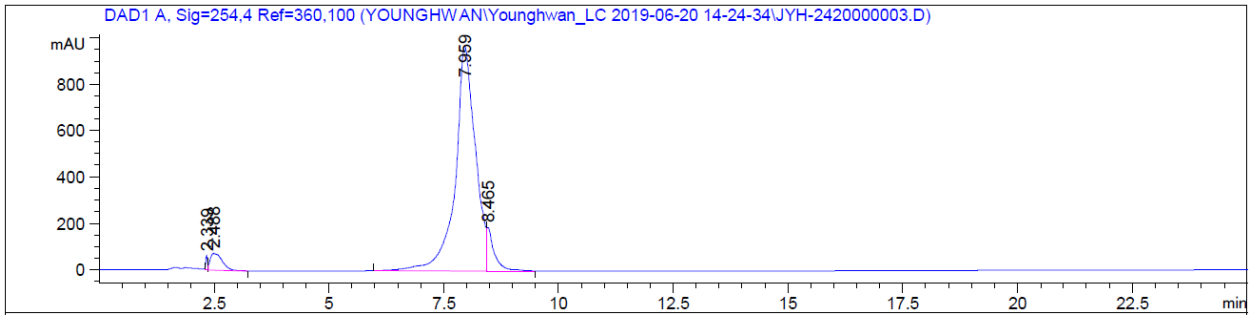
HPLC purity 97% (R_t = 8.96 min)



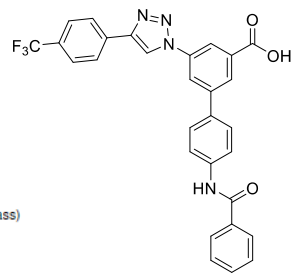
4'-Acetamido-5-(4-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**8**).



HPLC purity 99% (R_t = 7.95 min)



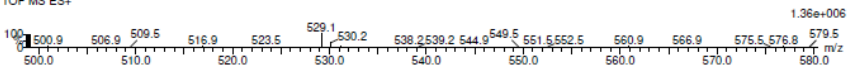
4'-Benzamido-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**9**).



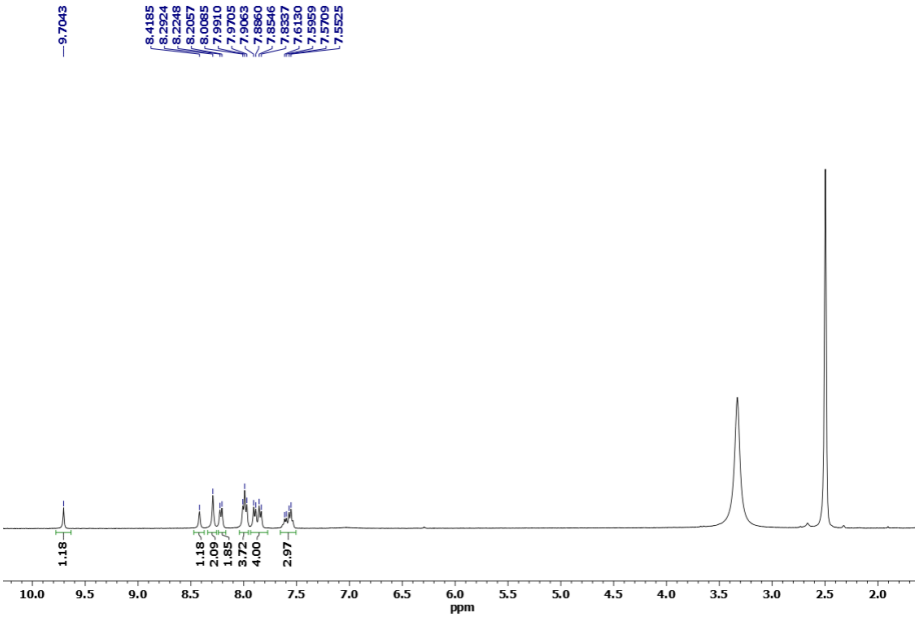
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

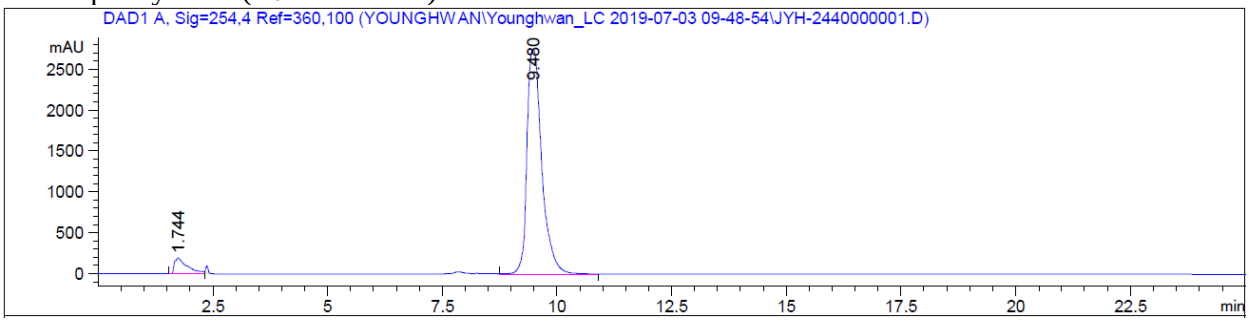
Monoisotopic Mass, Even Electron Ions
96 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-60 F: 3-3
JYH-27JUN19-244-HPLC 152 (2.588) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



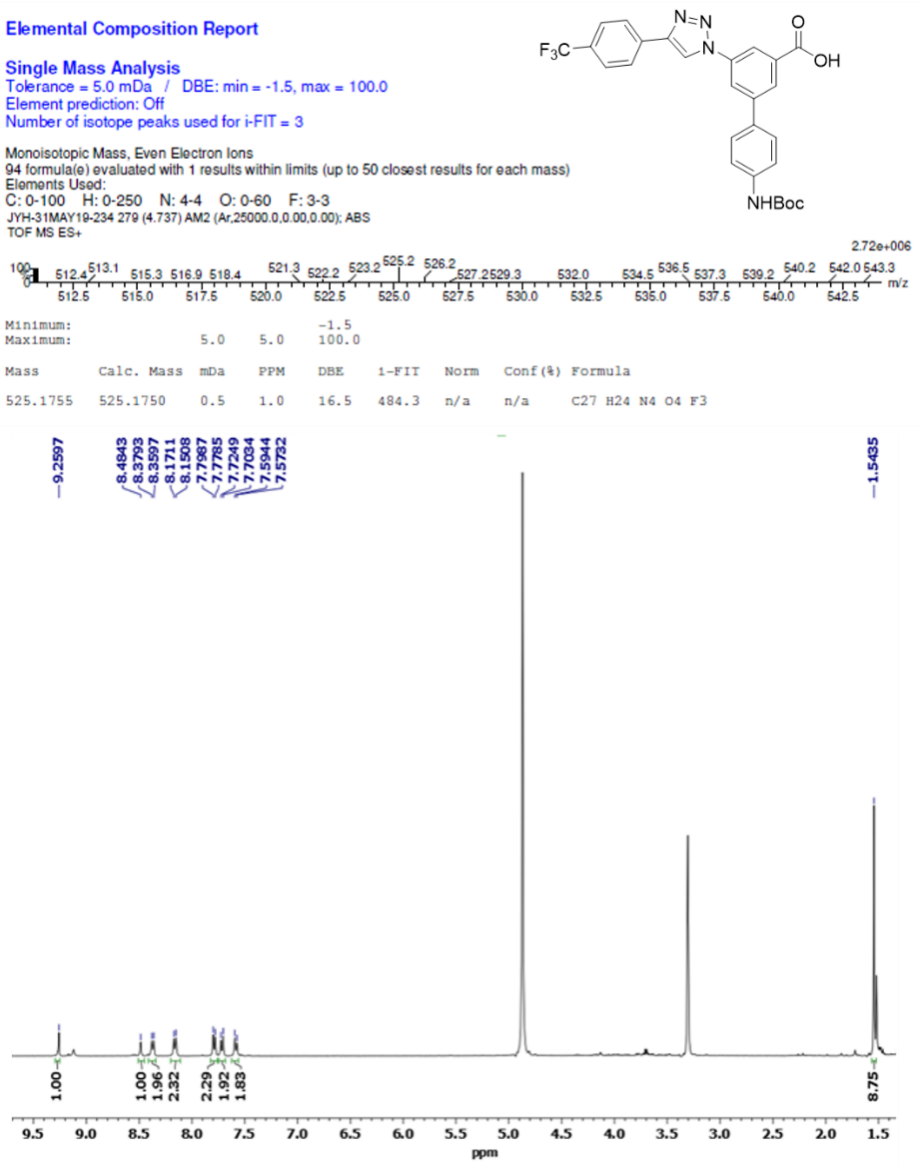
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
529.1489	529.1488	0.1	0.2	20.5	408.2	0.000	99.98	C29 H20 N4 O3 F3
	529.1452	3.7	7.0	-1.5	416.5	8.327	0.02	C11 H28 N4 O16 F3



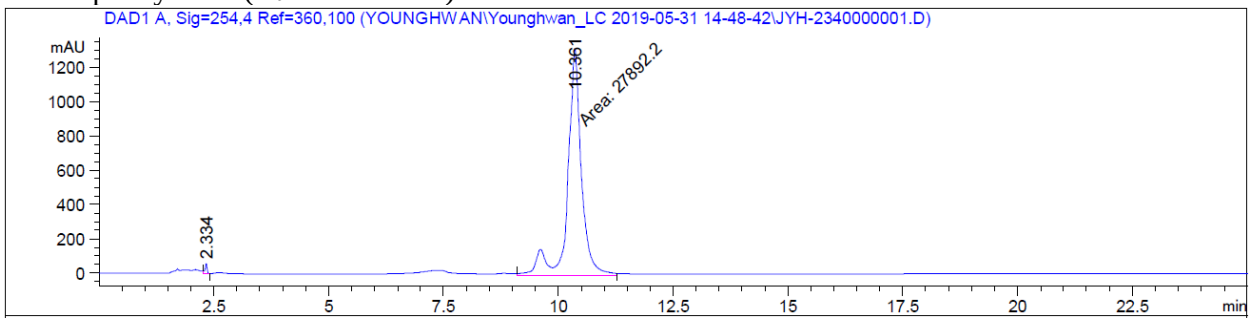
HPLC purity 99% (R_t = 9.48 min)



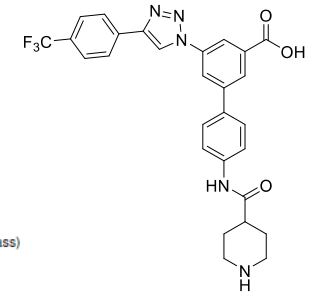
4'-((*tert*-Butoxycarbonyl)amino)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**10**).

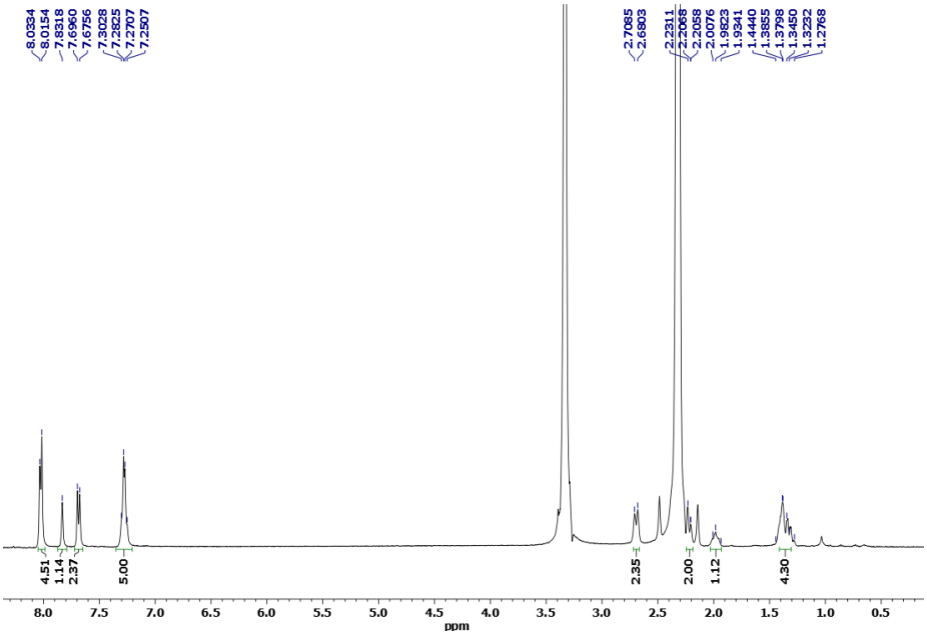


HPLC purity 99% (R_t = 10.36 min)

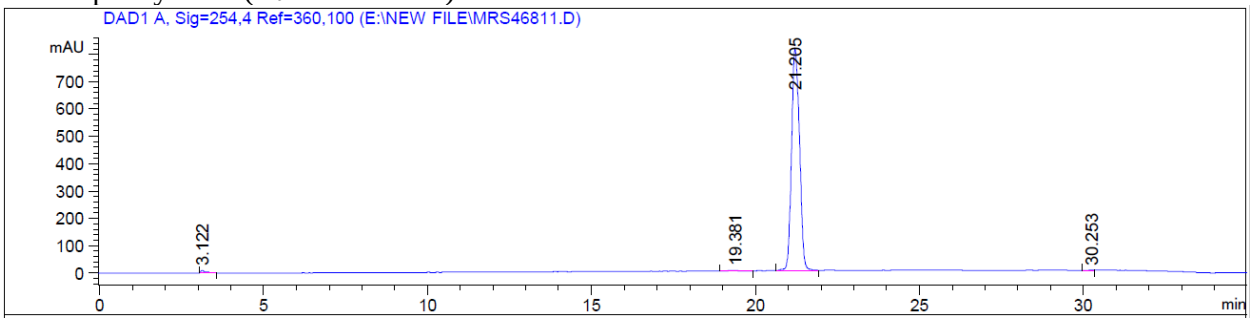


4’-(Piperidine-4-carboxamido)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**11**).

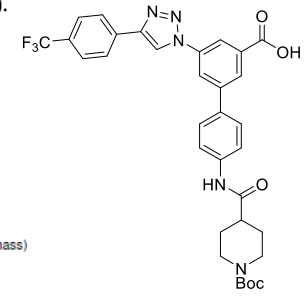


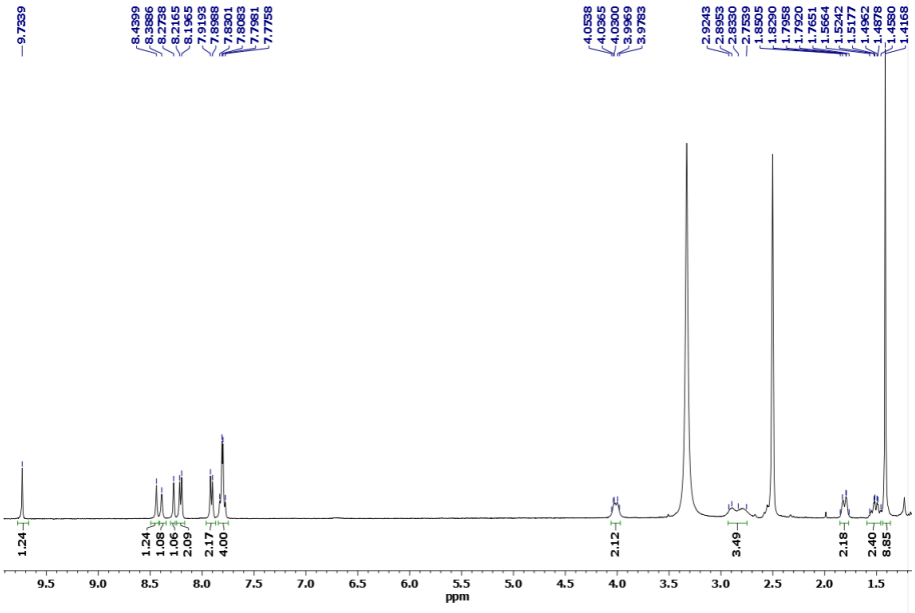


HPLC purity 99% (R_t = 21.20 min)

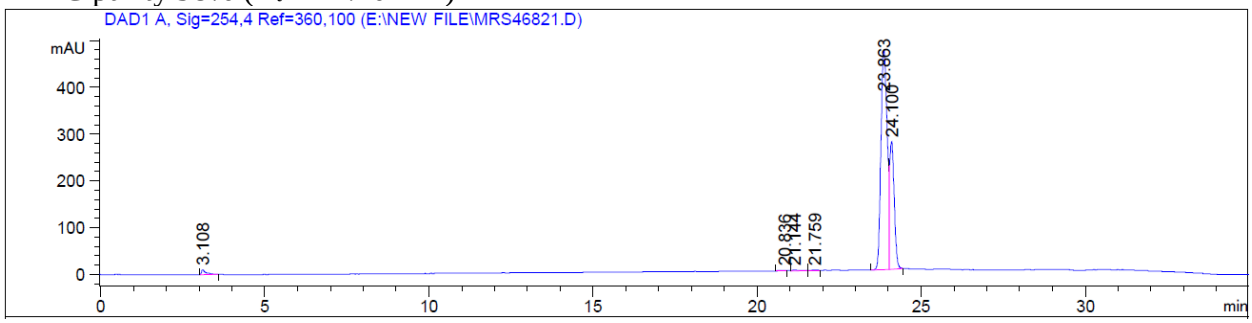


4'-(1-(*tert*-Butoxycarbonyl)piperidine-4-carboxamido)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**12**).

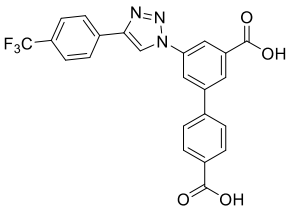




HPLC purity 98% (R_t = 24.10 min)



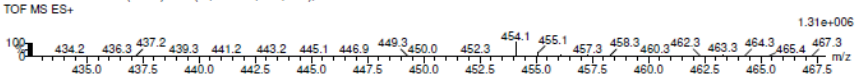
5-(4-(4-(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3,4'-dicarboxylic acid (**13**)



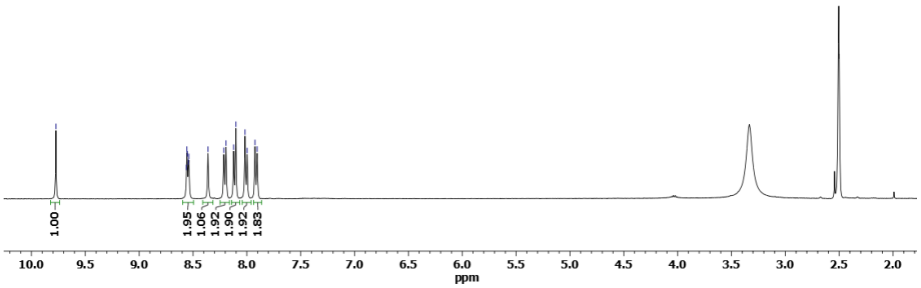
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

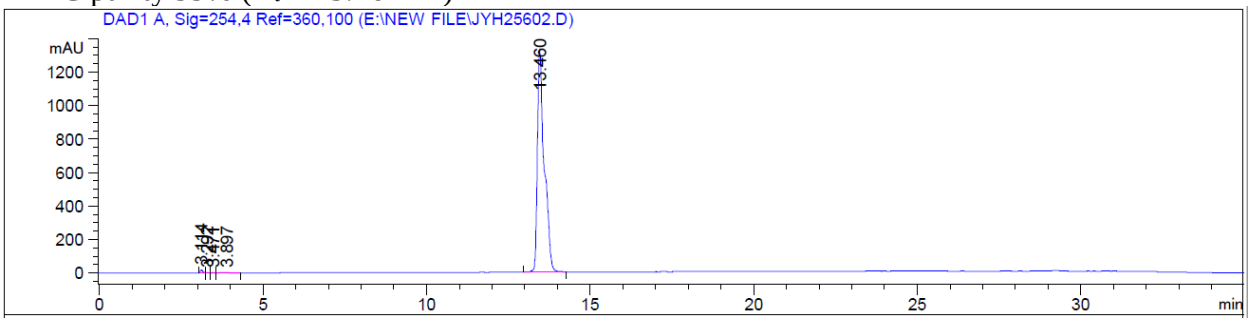
Monoisotopic Mass, Even Electron Ions
74 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 3-3 O: 0-60 F: 3-3
JYH-31JUL19-256 146 (2.486) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



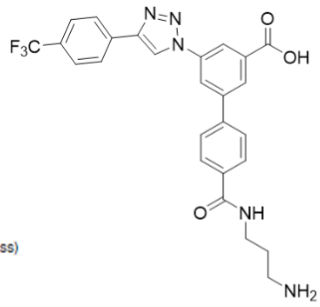
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
454.1017	454.1015	0.2	0.4	16.5	439.7	n/a	n/a	C23 H15 N3 O4 F3



HPLC purity 99% (R_t = 13.46 min)



4'-((3-Aminopropyl)carbamoyl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**14**)

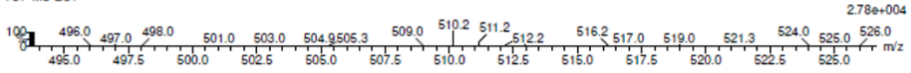


Elemental Composition Report

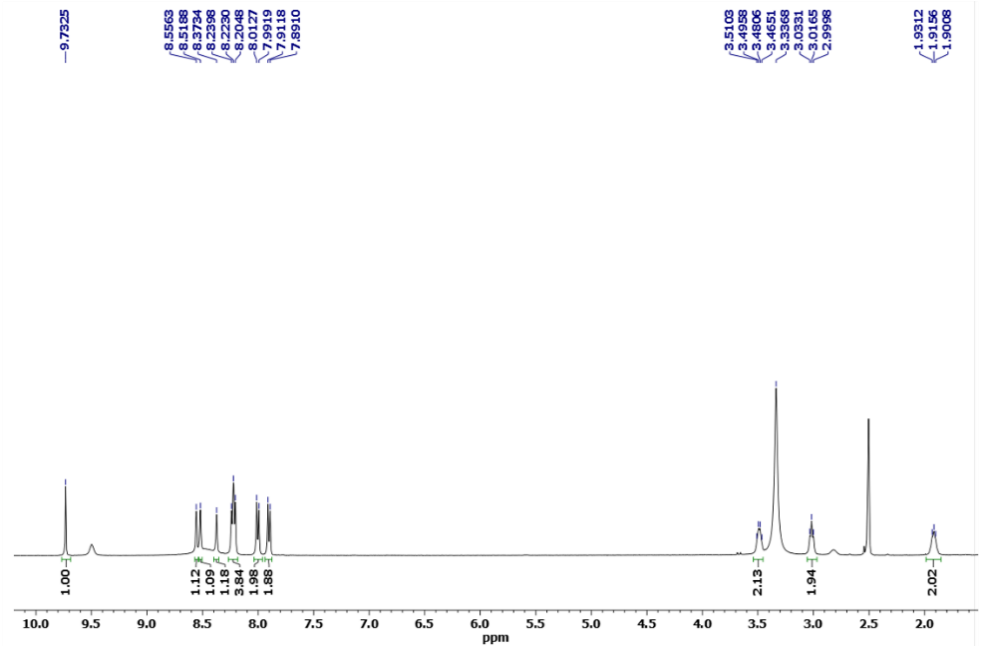
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
86 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 5-5 O: 0-60 F: 3-3
JYH-26JUL19-255-2 146 (2.487) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+

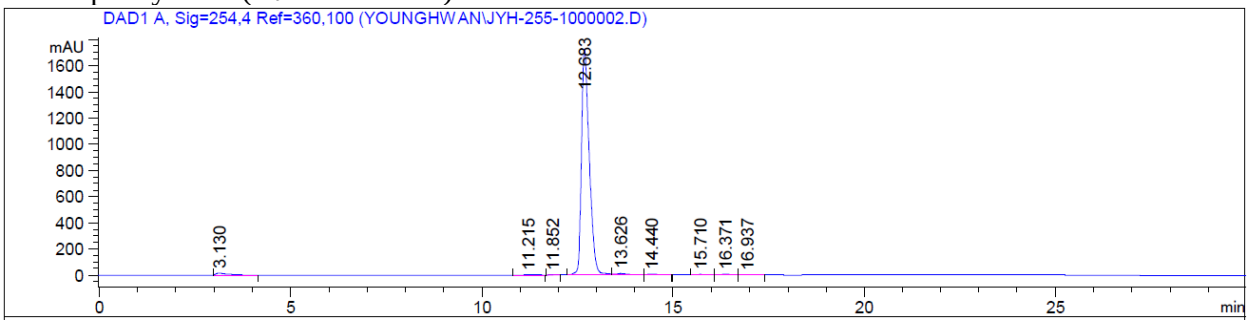
2.78e+004



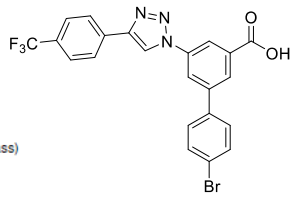
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
510.1754	510.1753	0.1	0.2	16.5	257.6	n/a	n/a	C26 H23 N5 O3 F3



HPLC purity 97% (R_t = 12.68 min)



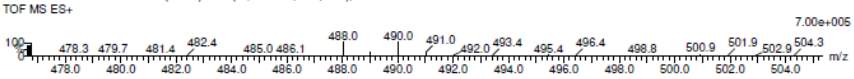
4'-Bromo-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**15**)



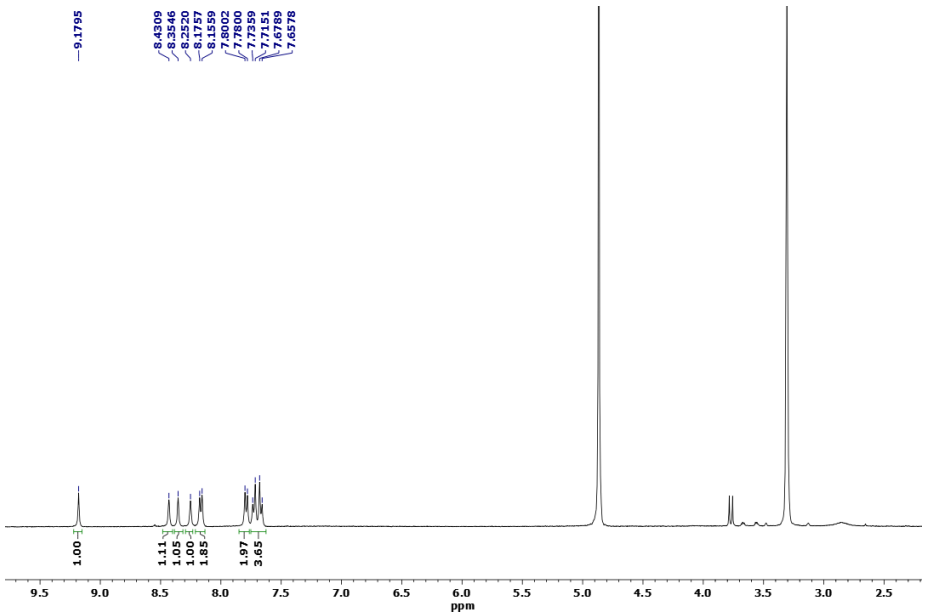
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

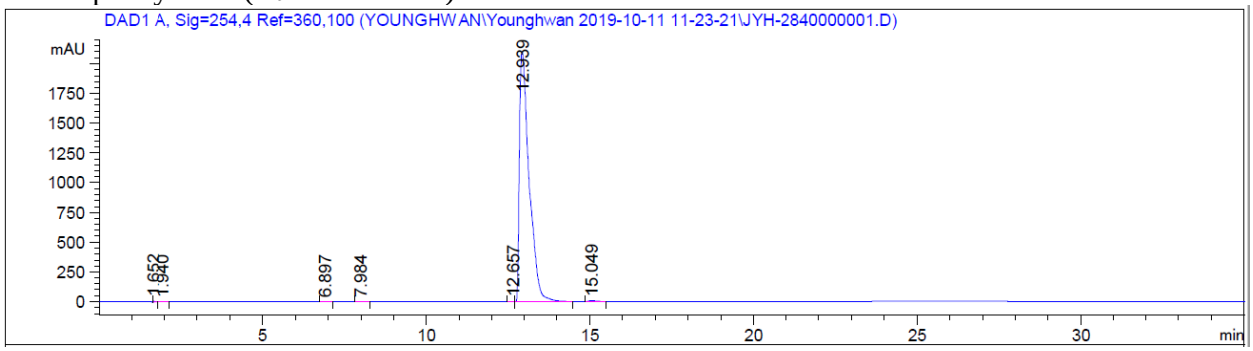
Monoisotopic Mass, Even Electron Ions
53 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 3-3 O: 0-60 F: 3-3 79Br: 1-1
JYH-08OCT19-284-HPLC 205 (3.485) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



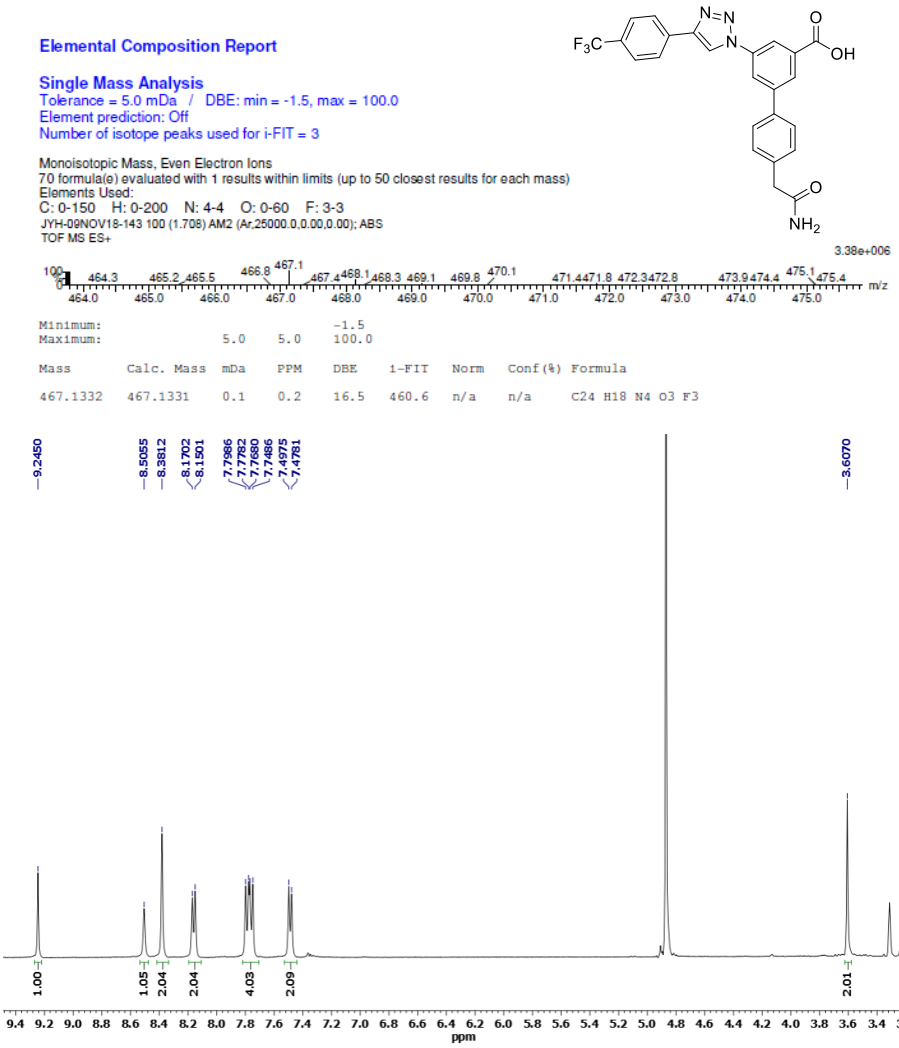
Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%) Formula
488.0214	488.0221	-0.7	-1.4	15.5	481.8	n/a	n/a C22 H14 N3 O2 F3 79Br



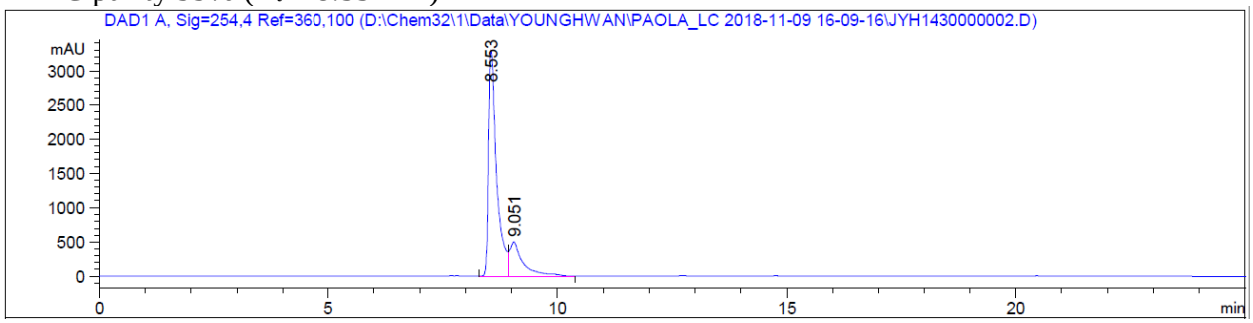
HPLC purity 99% (R_t = 12.93 min)



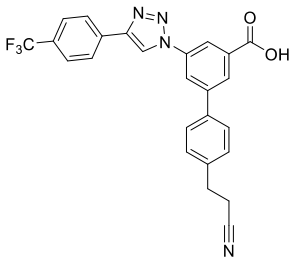
4'-(2-Amino-2-oxoethyl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**16**).



HPLC purity 99% (R_t = 8.55 min)



4'-(2-Cyanoethyl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**17**).



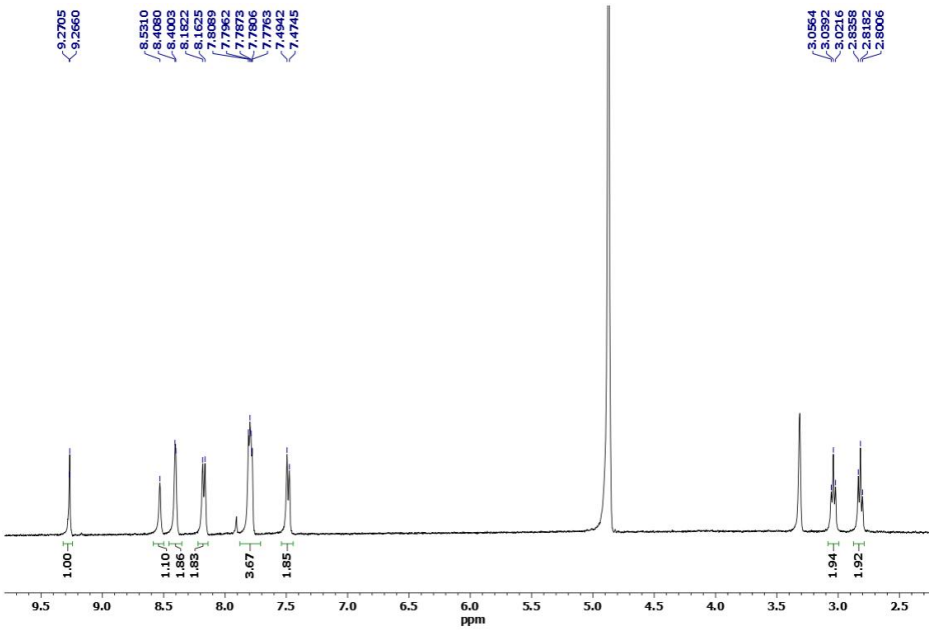
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

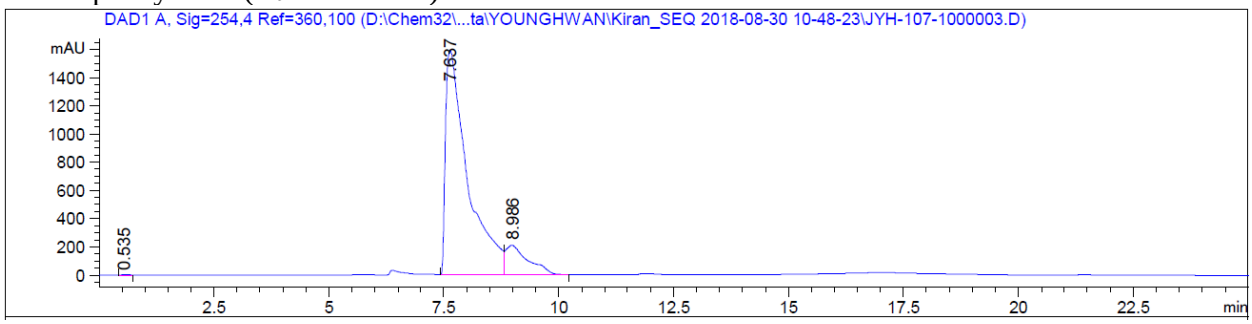
Monoisotopic Mass, Even Electron Ions
68 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 4-4 O: 0-40 F: 3-3
JYH-30AUG18-107-2-1 89 (1.522) AM2 (Ar,42000.0,0.00,0.00); ABS
TOF MS ES+

1.26e+005

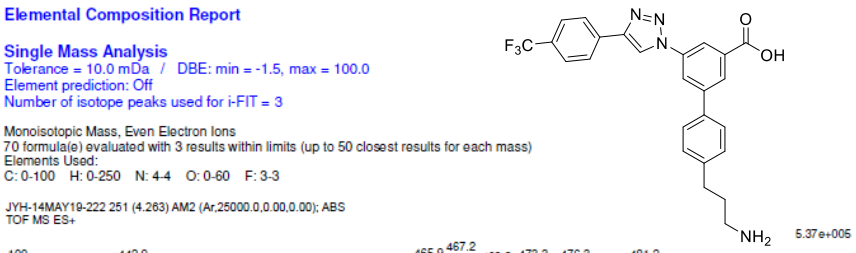
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
463.1381	463.1382	-0.1	-0.2	17.5	41.9	n/a	n/a	C25 H18 N4 O2 F3

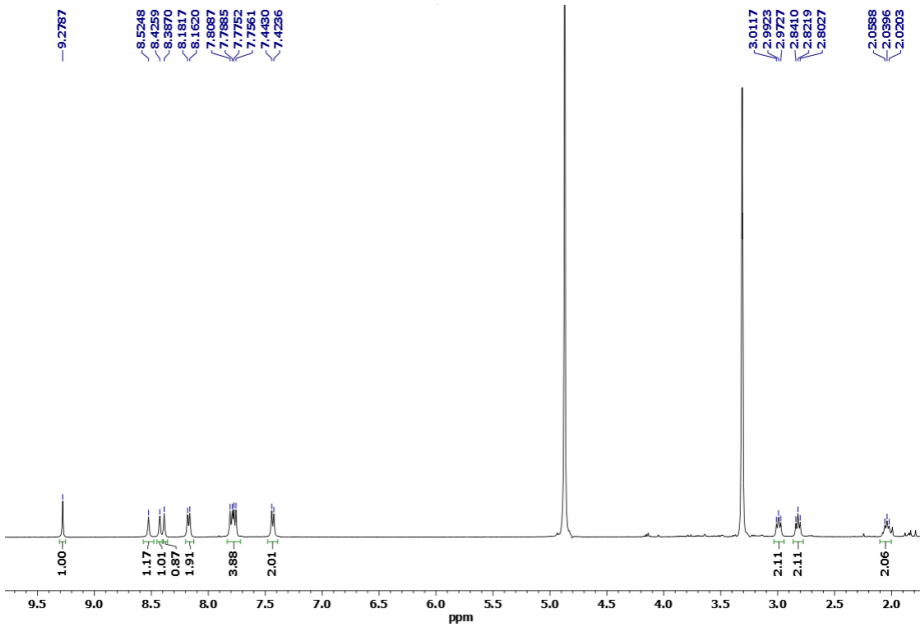


HPLC purity 99% (R_t = 7.63 min)

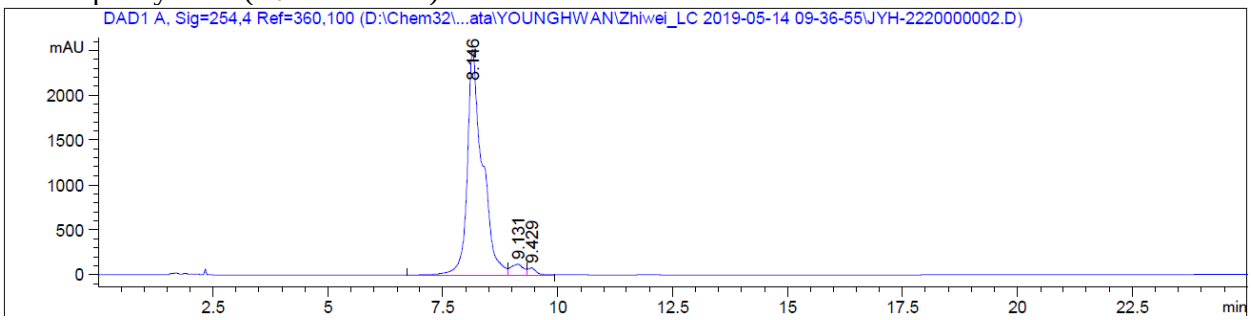


4'-(3-Aminopropyl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**18**).

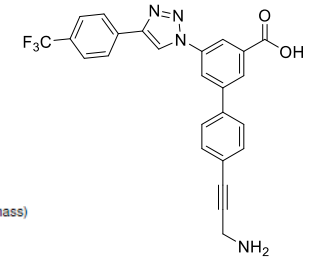


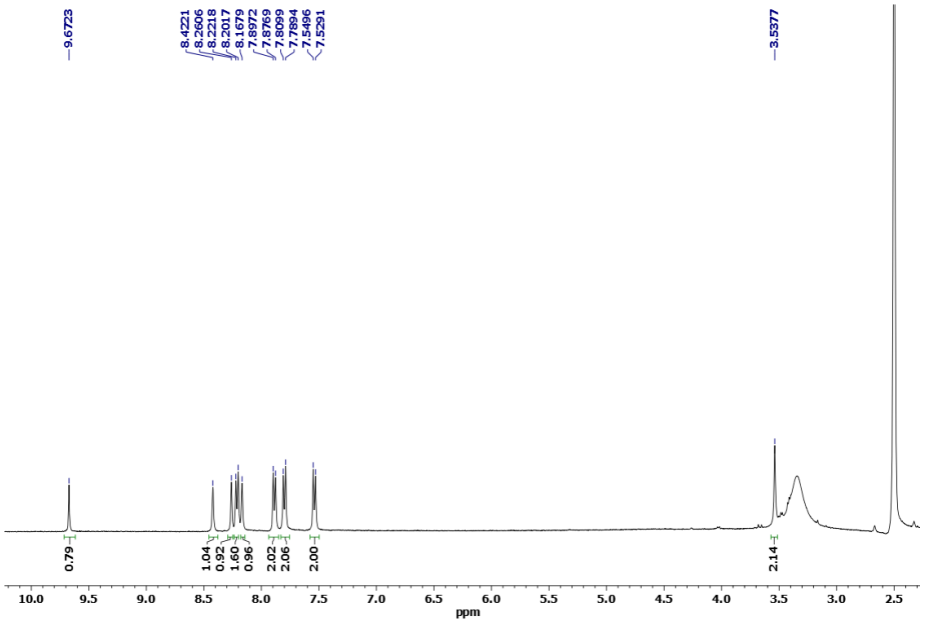


HPLC purity 98% (R_t = 8.14 min)

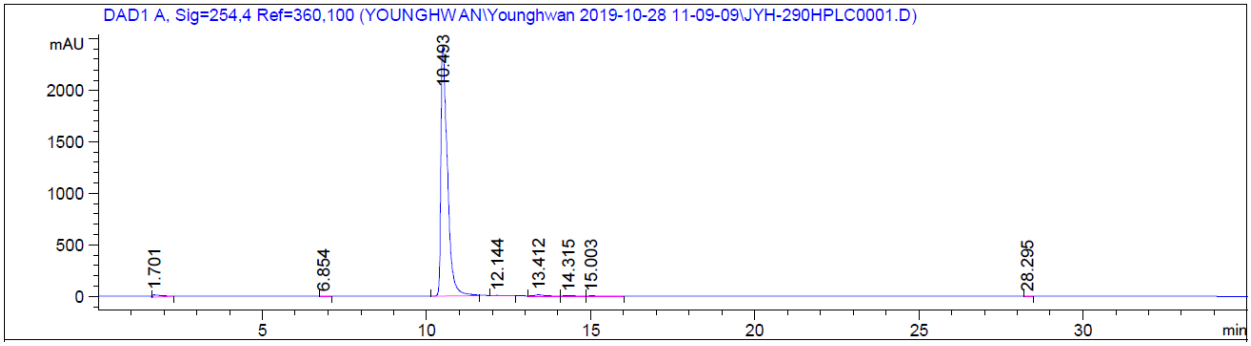


4'-(3-Aminoprop-1-yn-1-yl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**19**)

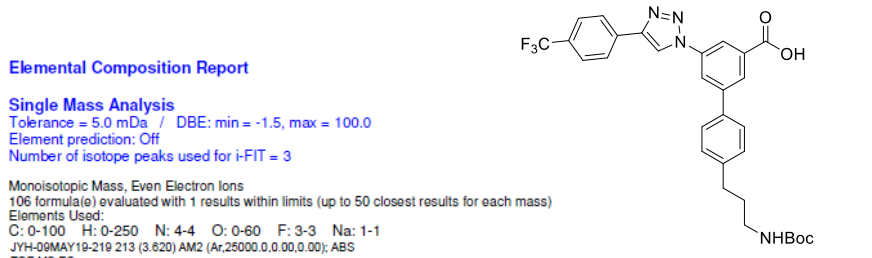


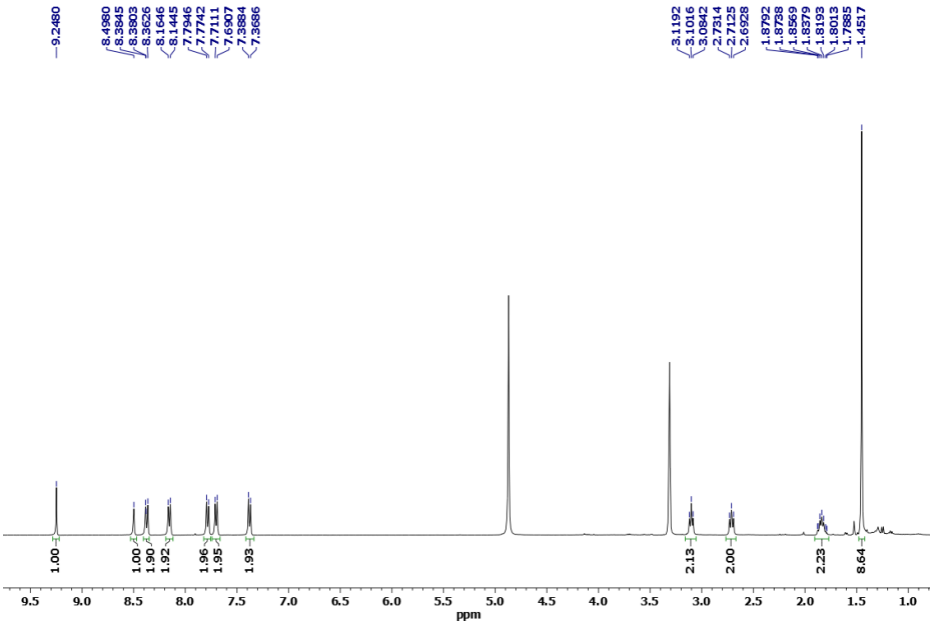


HPLC purity 97% (R_t = 10.49 min)

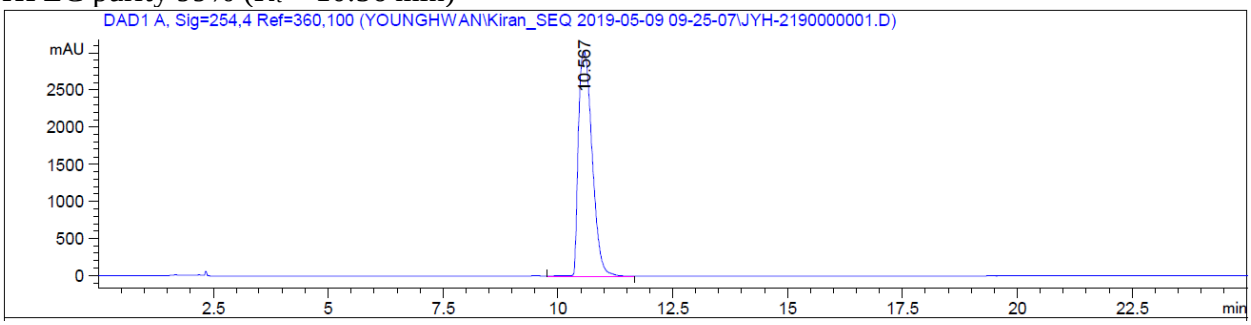


4'-(3-((*tert*-Butoxycarbonyl)amino)propyl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**20**).

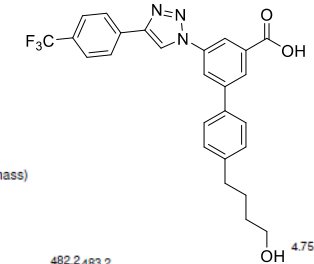




HPLC purity 99% (R_t = 10.56 min)



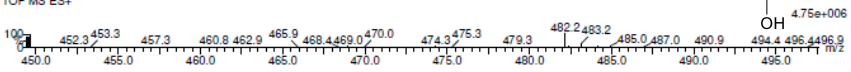
4'-(4-Hydroxybutyl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**21**).



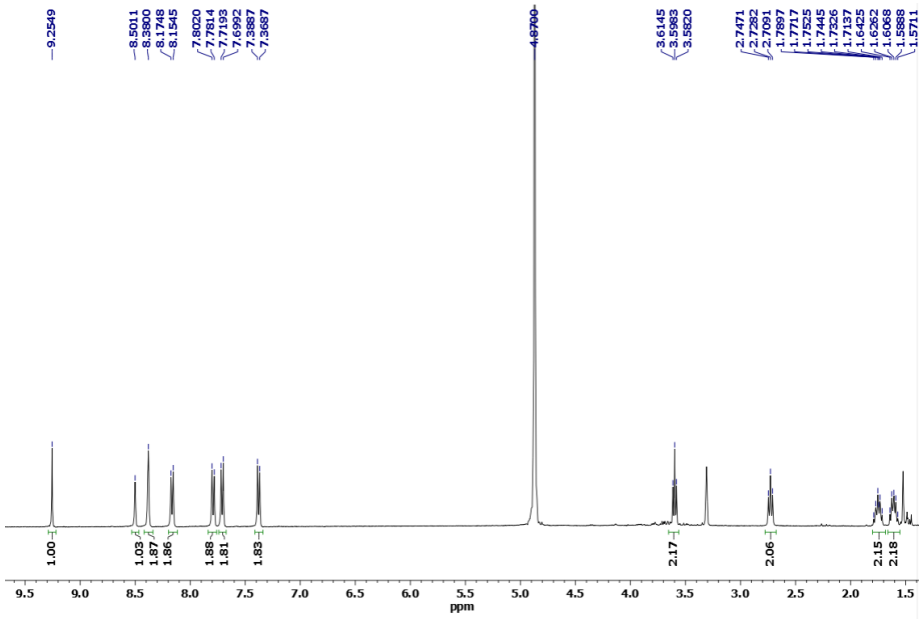
Elemental Composition Report

Single Mass Analysis
Tolerance = 20.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

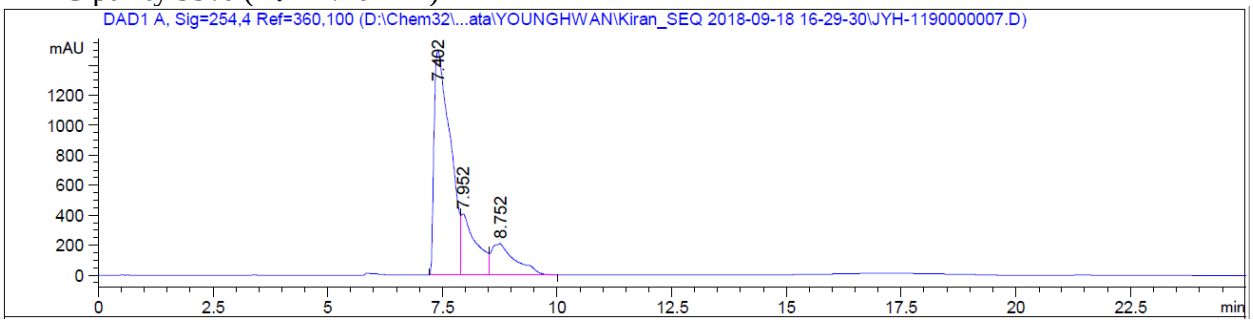
Monoisotopic Mass, Even Electron Ions
83 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-125 H: 0-200 N: 3-3 O: 0-30 F: 3-3
JYH-07OCT18-119 379 (6.428) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



		mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
Minimum:				-1.5				
Maximum:		20.0	5.0	100.0				
Mass	Calc. Mass							
482.1694	482.1692	0.2	0.4	15.5	491.6	0.154	85.69	C26 H23 N3 O3 F3
	482.1750	-5.6	-11.6	6.5	495.4	3.945	1.93	C19 H27 N3 O8 F3
	482.1598	9.6	19.9	2.5	499.3	7.810	0.04	C15 H27 N3 O11 F3
	482.1844	-15.0	-31.1	19.5	497.6	6.141	0.22	C30 H23 N3 F3
	482.1539	15.5	32.1	11.5	493.6	2.111	12.11	C22 H23 N3 O6 F3



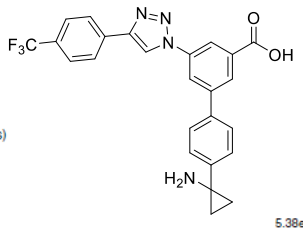
HPLC purity 99% (R_t = 7.40 min)



4'-(1-Aminocyclopropyl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**22**).

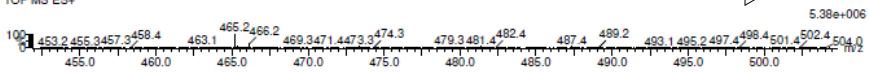
Elemental Composition Report

Single Mass Analysis
Tolerance = 10.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

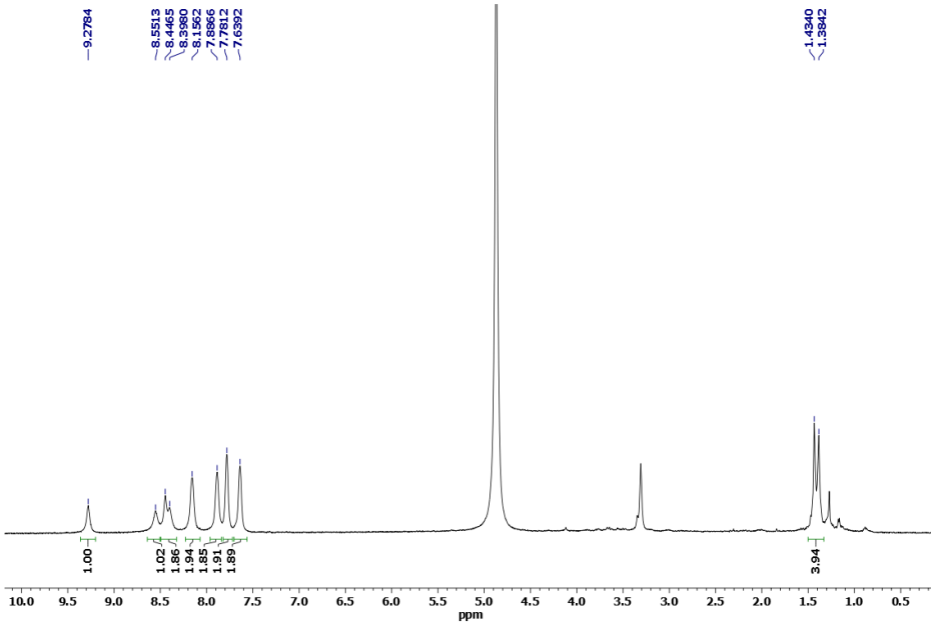


Monoisotopic Mass, Even Electron Ions
73 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-125 H: 0-200 N: 4-4 O: 0-60 F: 3-3

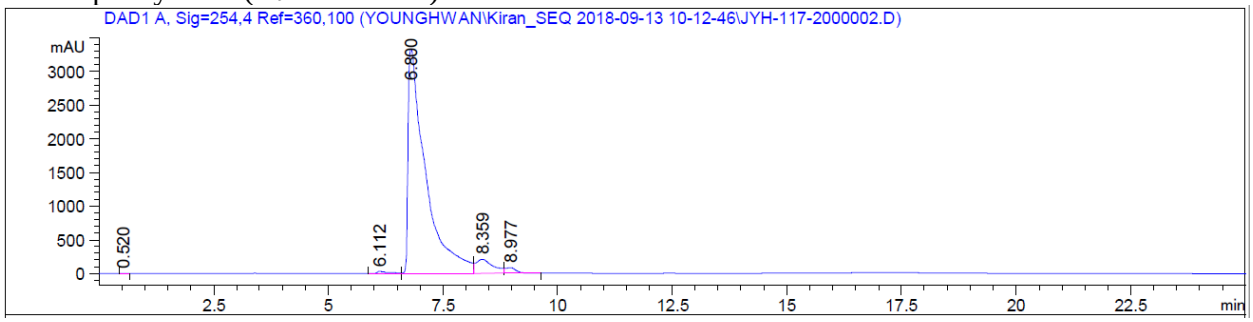
JYH-14SEP18-117-2-1 205 (3.485) AM2 (Ar,42000.0,0.00,0.00); ABS
TOF MS ES+



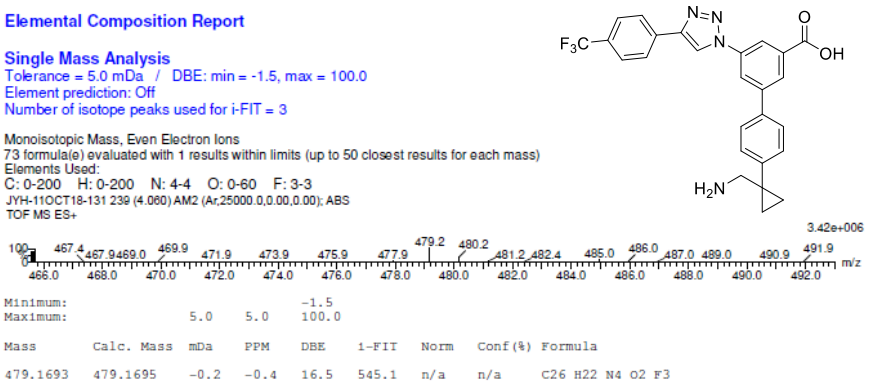
Minimum:				-1.5				
Maximum:		10.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
465.1539	465.1538	0.1	0.2	16.5	701.1	0.000	99.98	C25 H20 N4 O2 F3
	465.1597	-5.8	-12.5	7.5	709.7	8.584	0.02	C18 H24 N4 O7 F3
	465.1445	9.4	20.2	3.5	712.8	11.656	0.00	C14 H24 N4 O10 F3

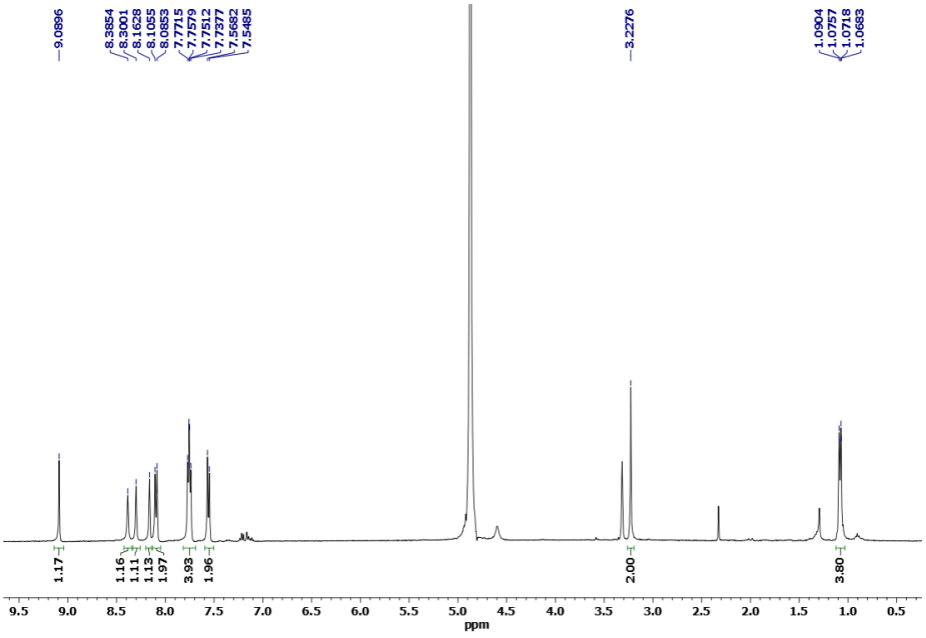


HPLC purity 98% (R_t = 6.80 min)

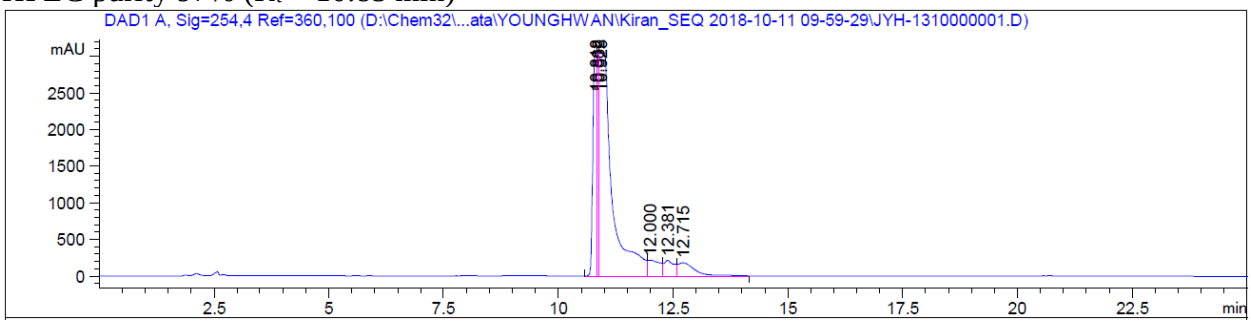


4'-(1-(Aminomethyl)cyclopropyl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**23**).

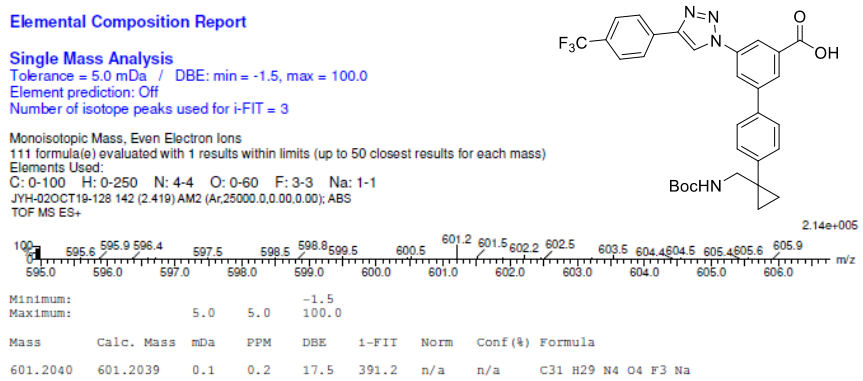


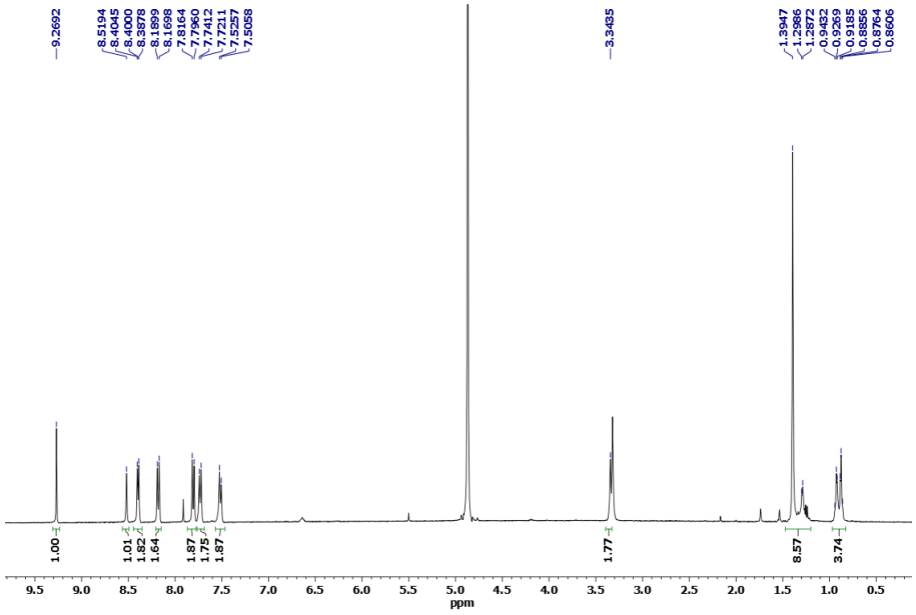


HPLC purity 97% (R_t = 10.85 min)

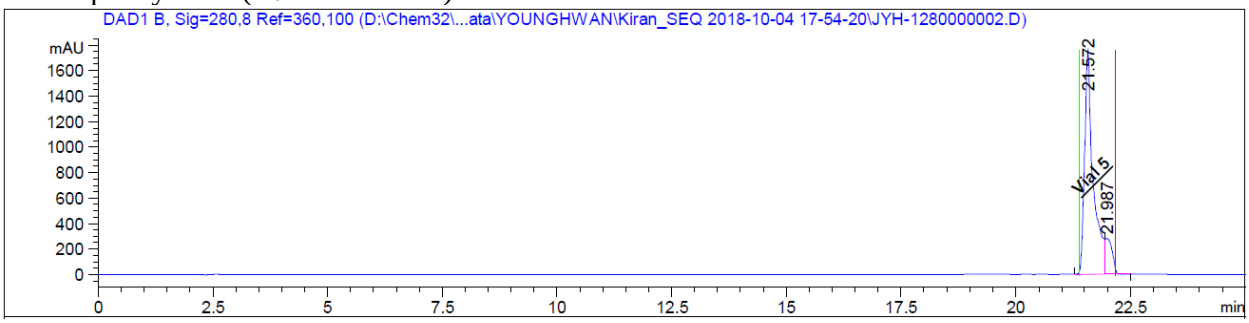


4'-(1-(((*tert*-Butoxycarbonyl)amino)methyl)cyclopropyl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**24**).

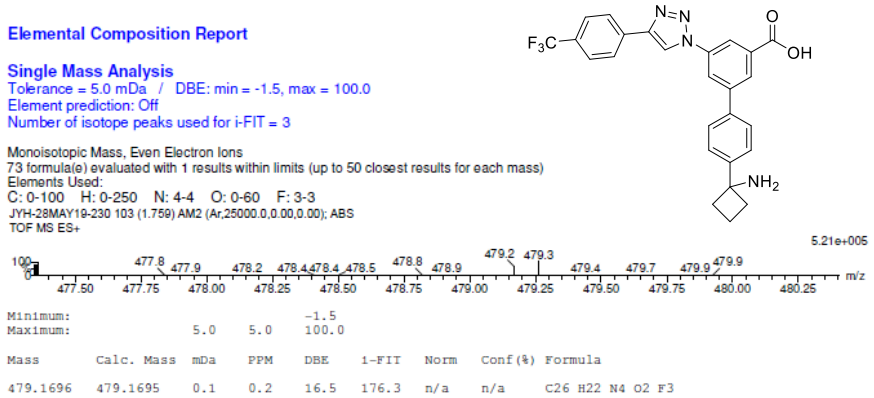


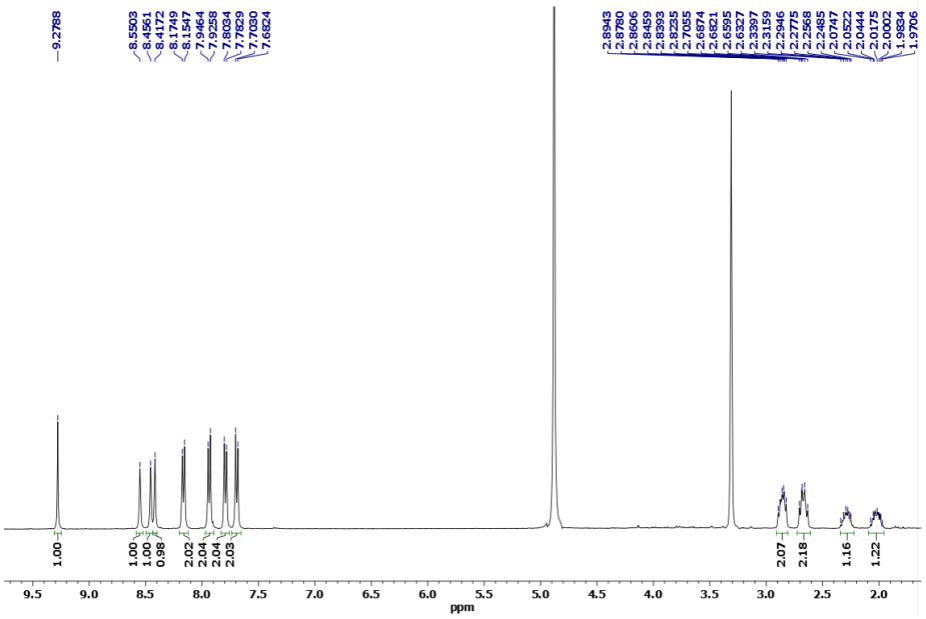


HPLC purity 99% (R_t = 21.57 min)

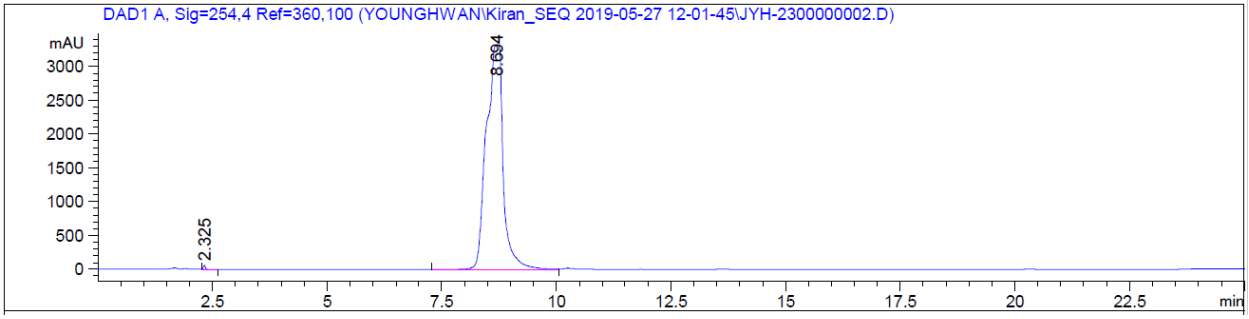


4'-(1-Aminocyclobutyl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**25**).

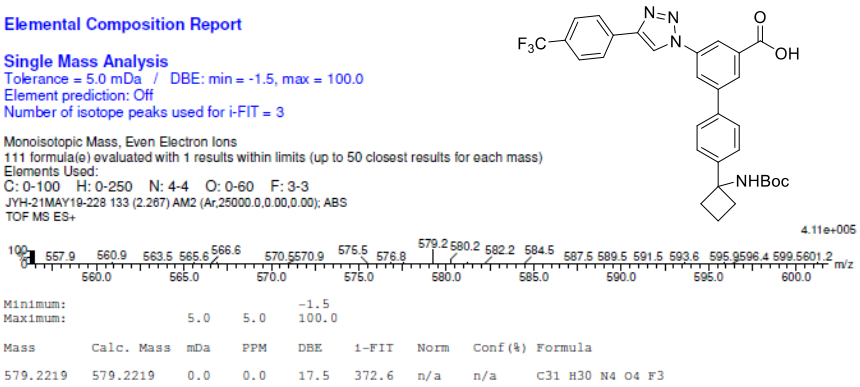


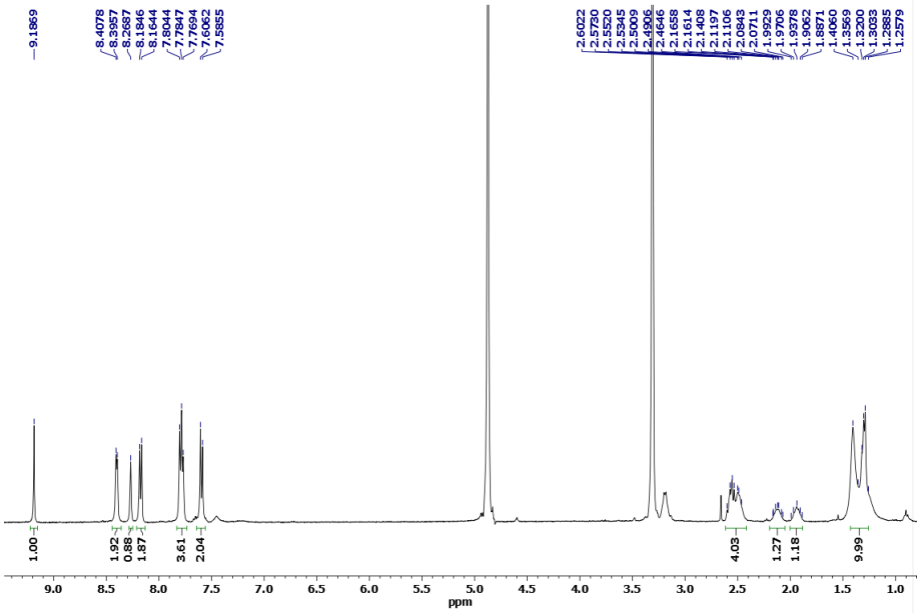


HPLC purity 99% (R_t = 8.69 min)

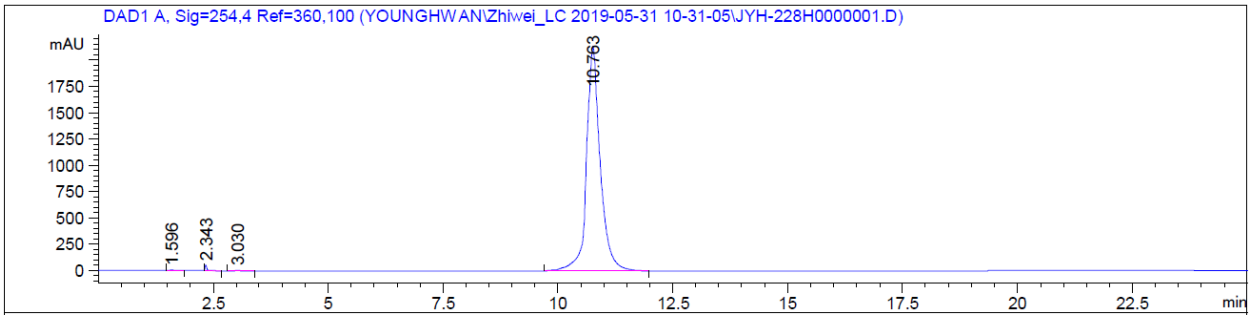


4'-(1-((*tert*-Butoxycarbonyl)amino)cyclobutyl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**26**).

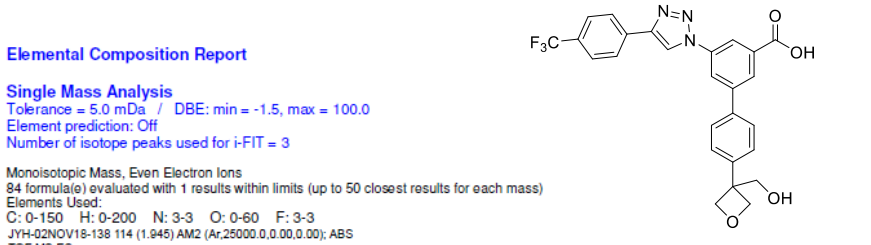


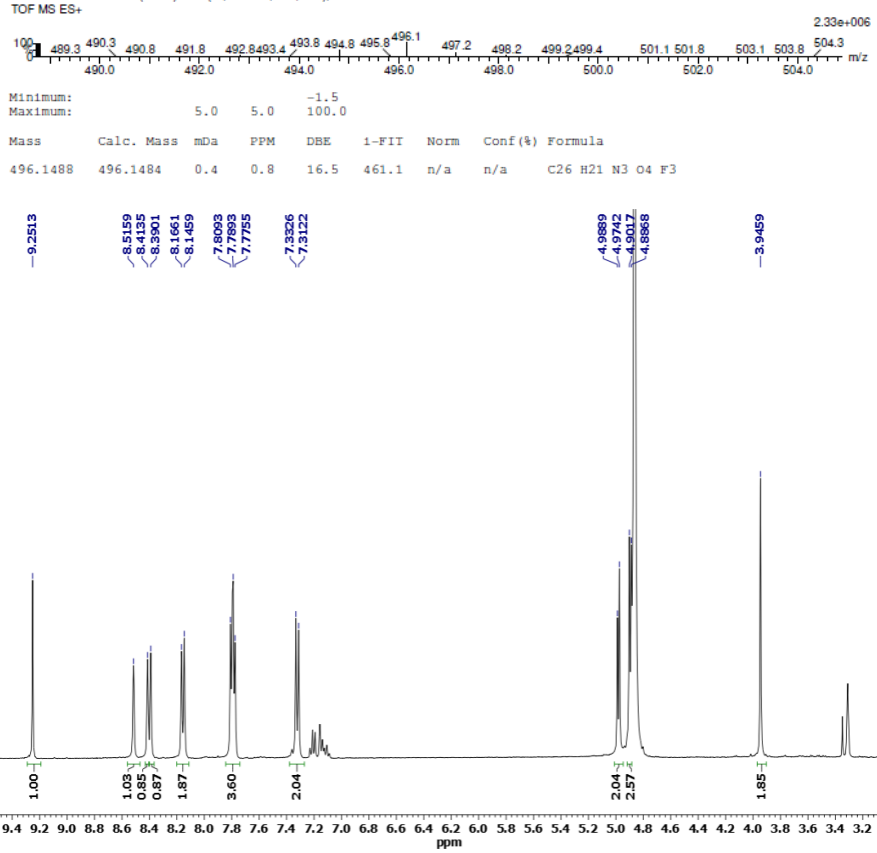


HPLC purity 99% (R_t = 10.76 min)

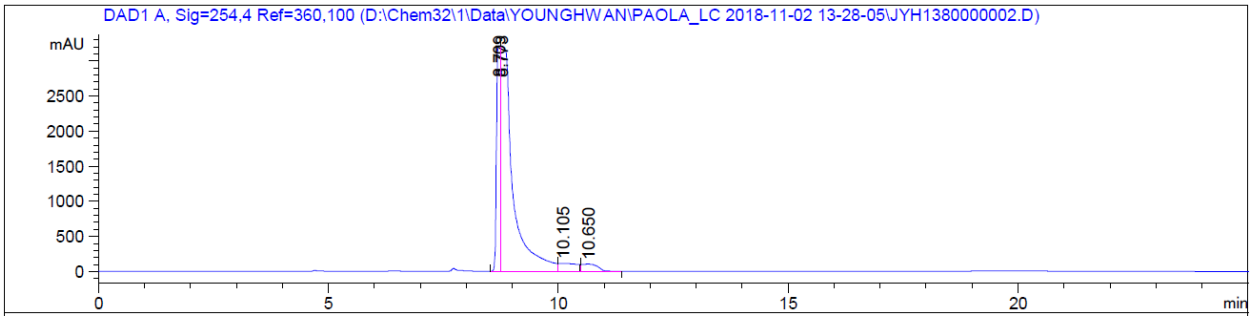


4'-(3-(Hydroxymethyl)oxetan-3-yl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**27**).

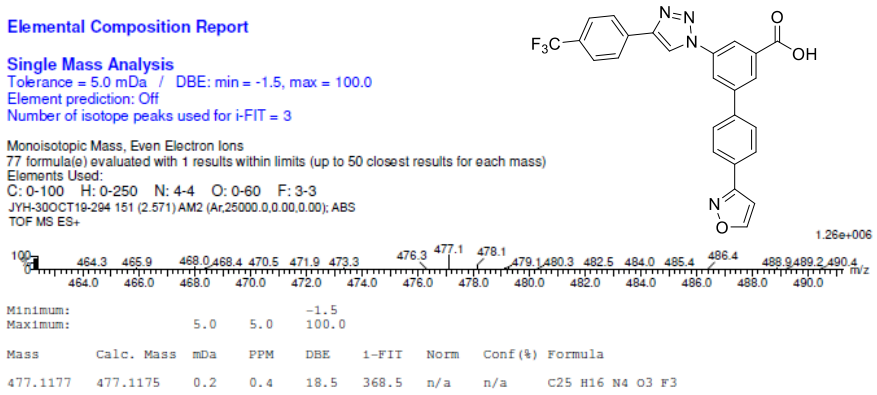


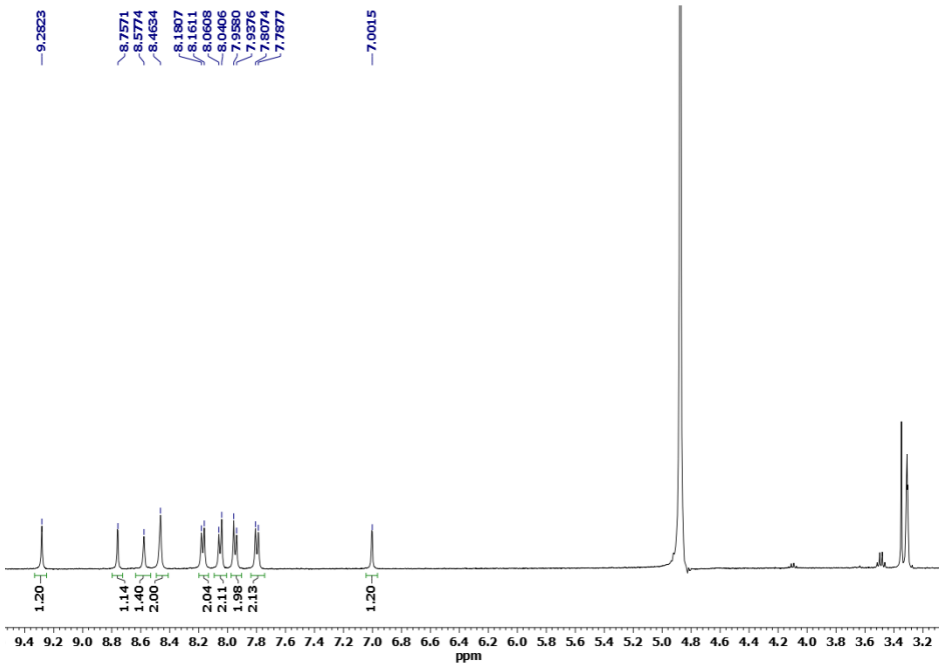


HPLC purity 99% (R_t = 8.71 min)

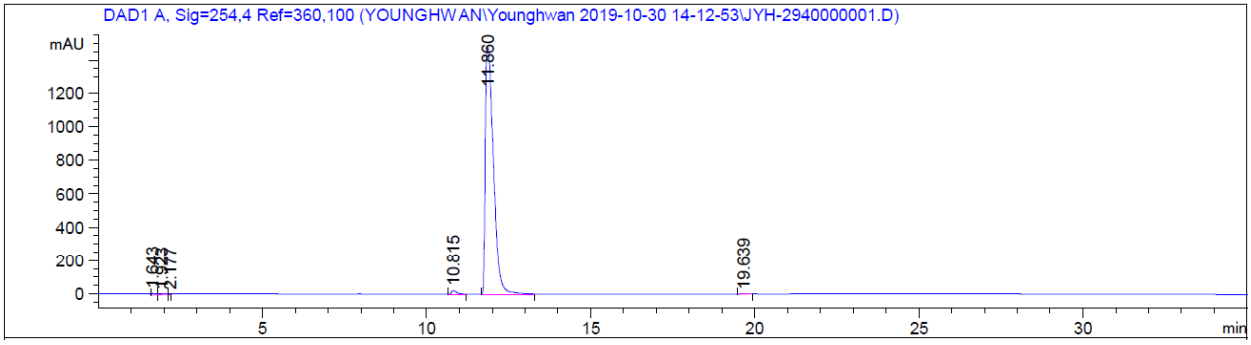


4'-(Isoxazol-3-yl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**28**).

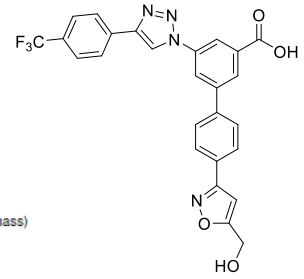


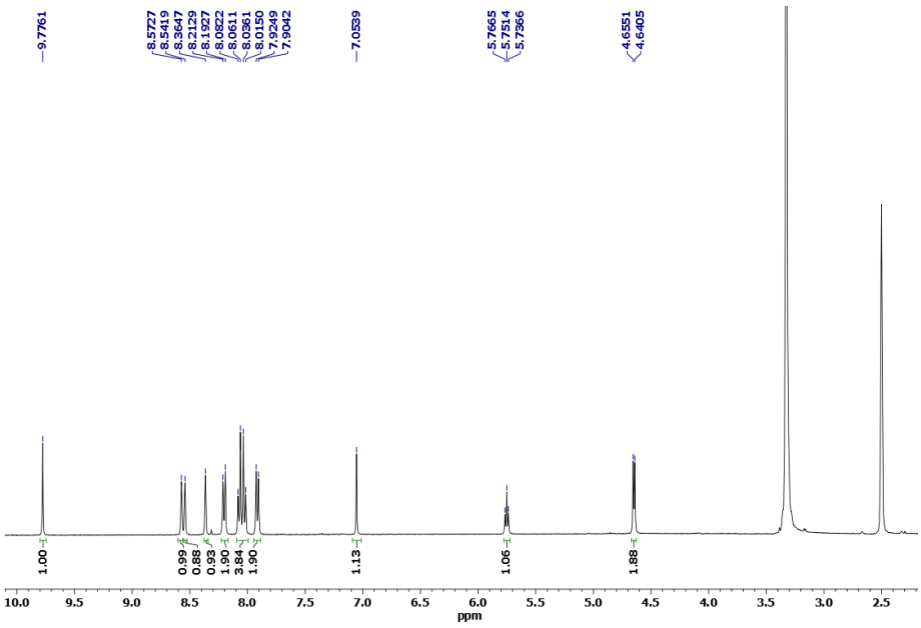


HPLC purity 98% (R_t = 11.86 min)

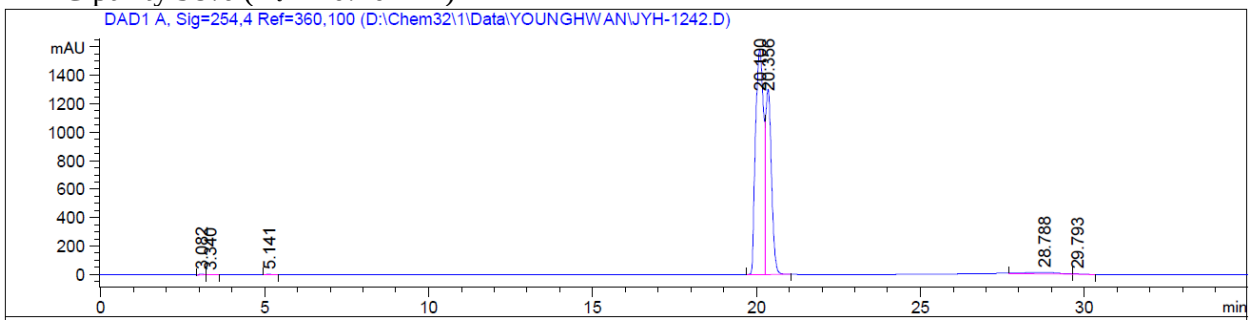


4'-(5-(Hydroxymethyl)isoxazol-3-yl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**29**).

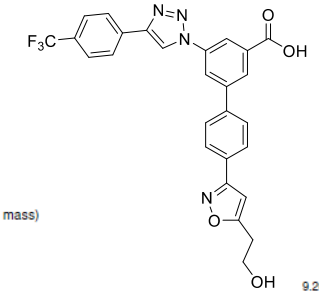




HPLC purity 98% (R_t = 20.10 min)



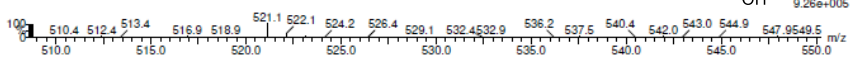
4'-(5-(2-Hydroxyethyl)isoxazol-3-yl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**30**).



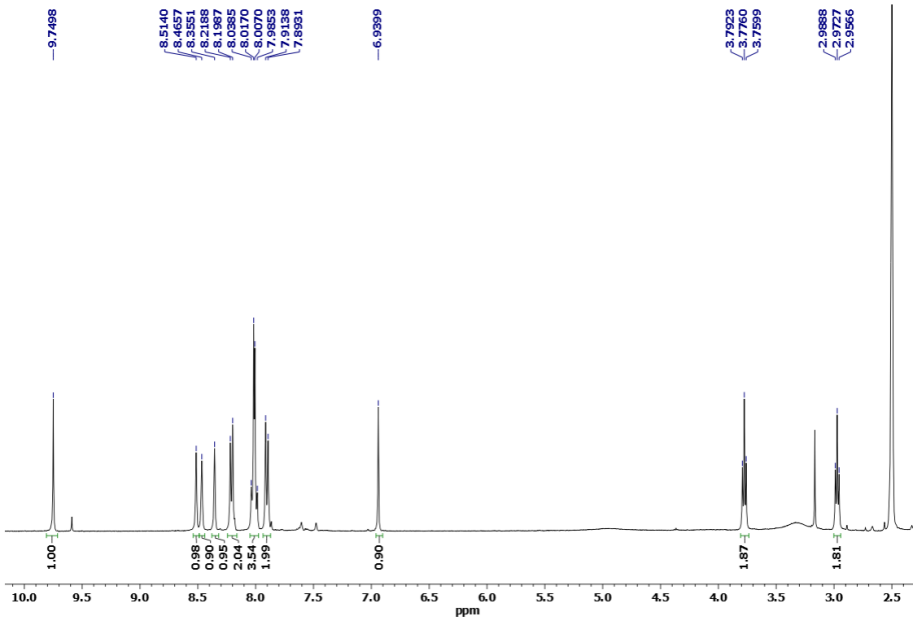
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

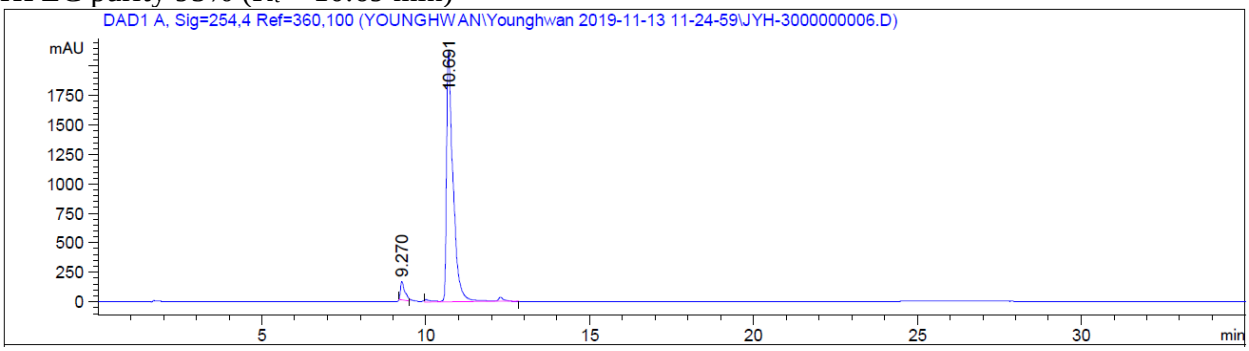
Monoisotopic Mass, Even Electron Ions
93 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 4-4 O: 0-60 F: 3-3
JYH-12NOV19-300 126 (2.148) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



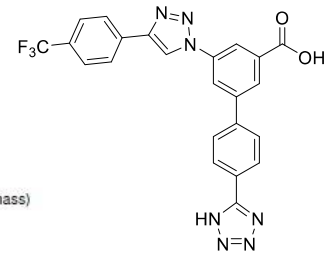
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
521.1435	521.1437	-0.2	-0.4	18.5	401.0	n/a	n/a	C27 H20 N4 O4 F3



HPLC purity 95% (R_t = 10.69 min)



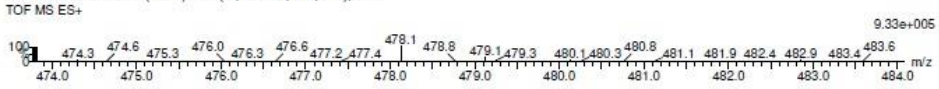
4'-(1*H*-Tetrazol-5-yl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylic acid (**31**).



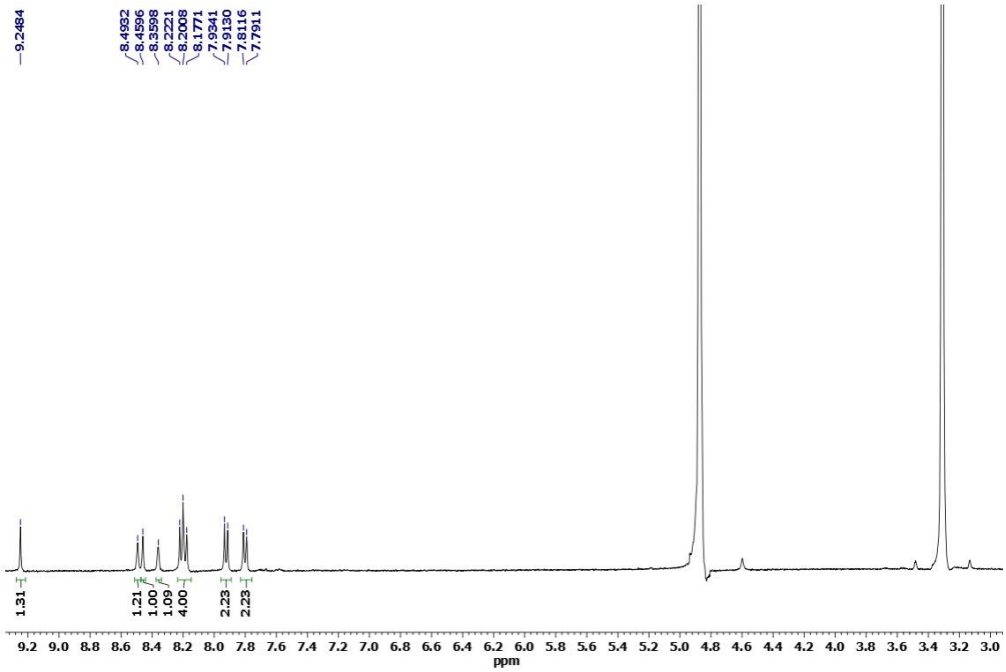
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

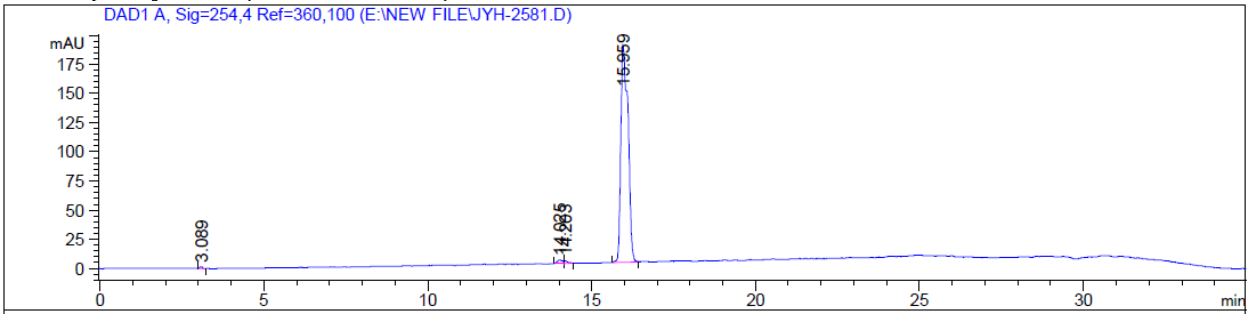
Monoisotopic Mass, Even Electron Ions
70 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 7-7 O: 0-60 F: 3-3
JYH-13AUG19-258 222 (3.772) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



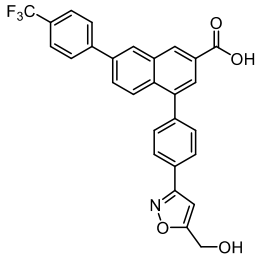
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
478.1242	478.1239	0.3	0.6	18.5	377.1	n/a	n/a	C23 H15 N7 O2 F3



HPLC purity 98% (R_t = 15.95 min)



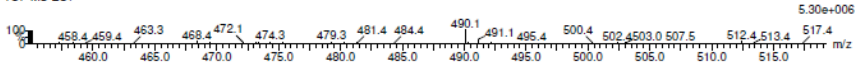
4-(4-(5-(Hydroxymethyl)isoxazol-3-yl)phenyl)-7-(4-(trifluoromethyl)phenyl)-2-naphthoic acid (**32**).



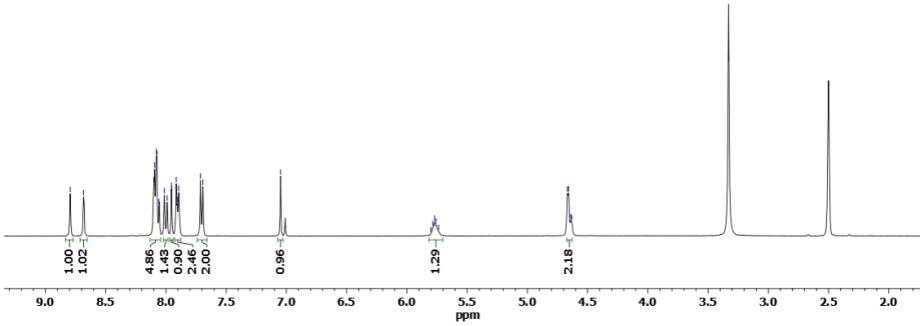
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

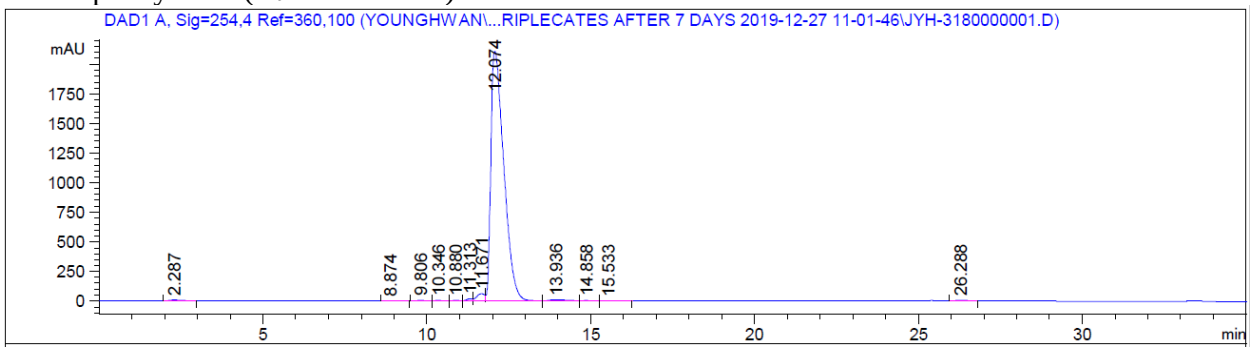
Monoisotopic Mass, Even Electron Ions
94 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 1-1 O: 0-60 F: 3-3
JYH-02JAN20-318 368 (6.242) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



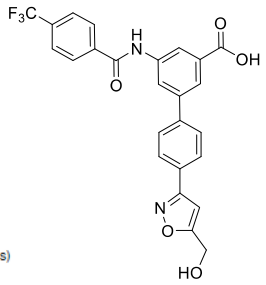
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
490.1269	490.1266	0.3	0.6	18.5	657.9	n/a	n/a	C28 H19 N O4 F3



HPLC purity 98% (R_t = 12.07 min)



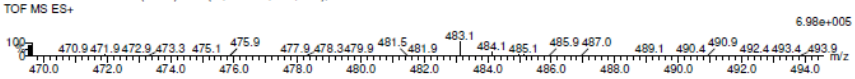
4'-(5-(Hydroxymethyl)isoxazol-3-yl)-5-(4-(trifluoromethyl)benzamido)-[1,1'-biphenyl]-3-carboxylic acid (**33**)



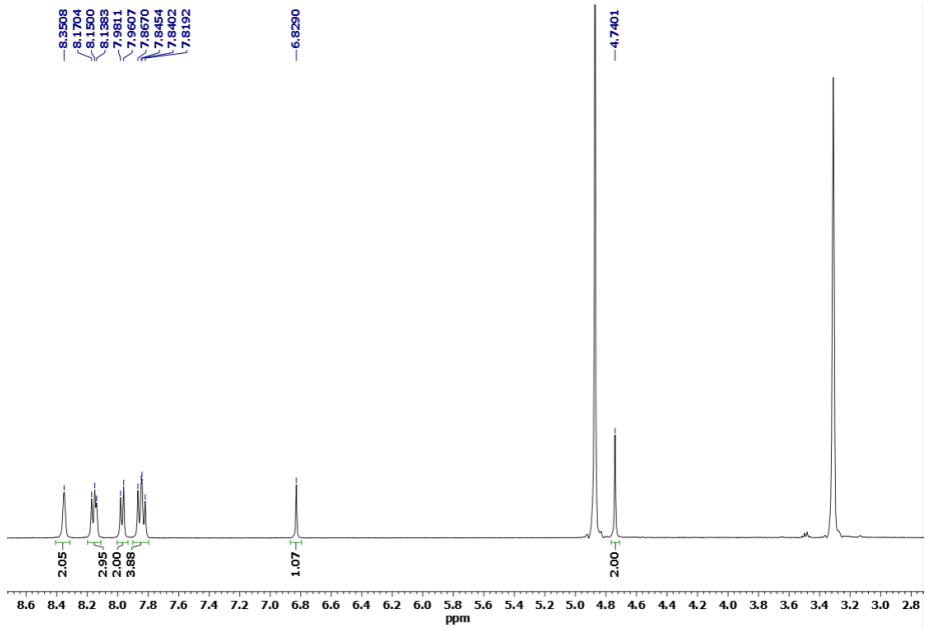
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

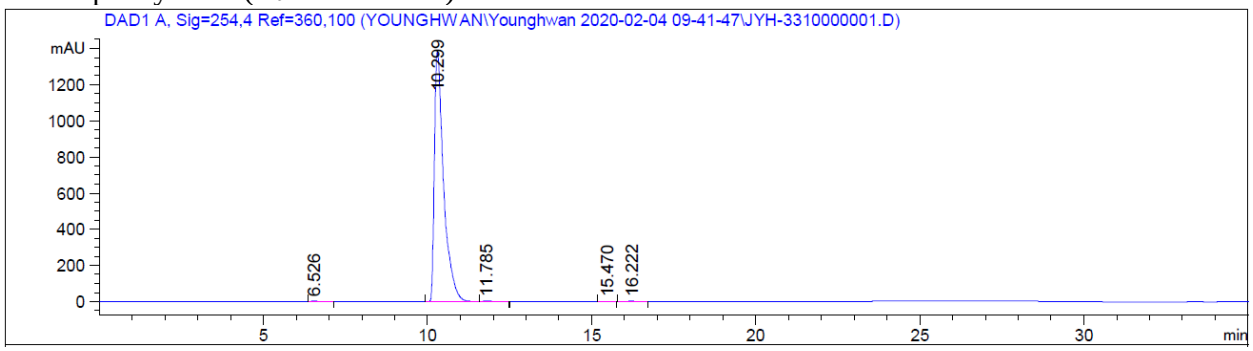
Monoisotopic Mass, Even Electron Ions
81 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 2-2 O: 0-60 F: 3-3
JYH-04FEB20-331-P 180 (3.062) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



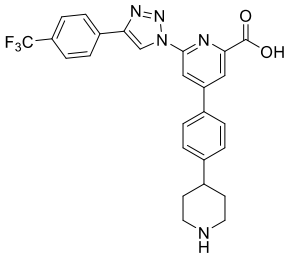
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
483.1170	483.1168	0.2	0.4	16.5	414.1	n/a	n/a	C25 H18 N2 O5 F3



HPLC purity 99% (R_t = 10.29 min)



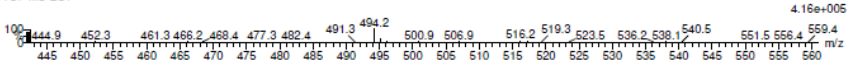
4-(4-(Piperidin-4-yl)phenyl)-6-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)picolinic acid (**34**).



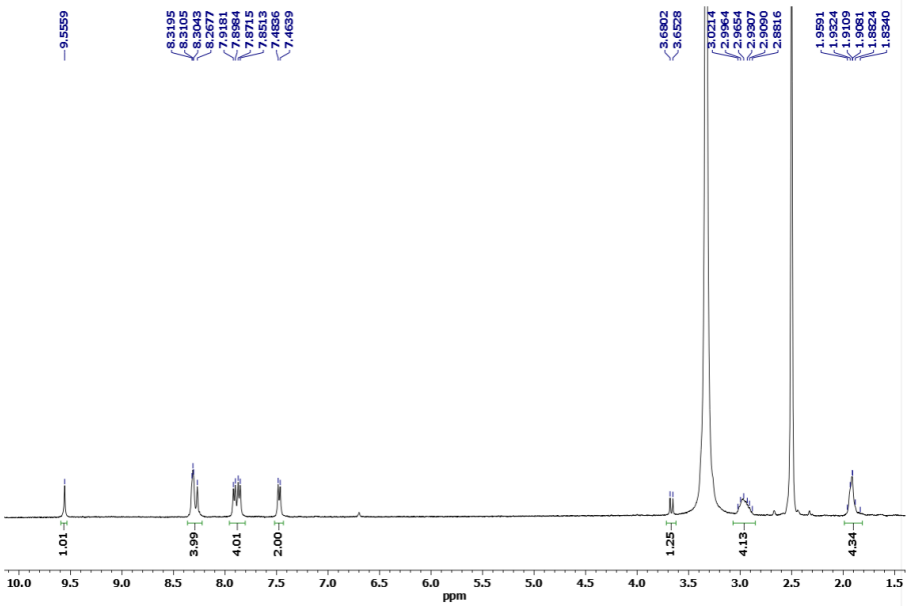
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

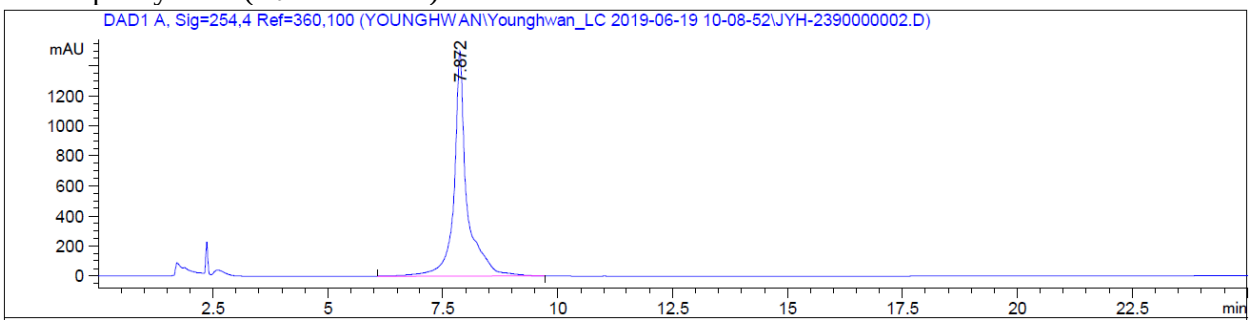
Monoisotopic Mass, Even Electron Ions
80 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 5-5 O: 0-60 F: 3-3
JYH-14JUN19-239-HPLC 108 (1.844) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



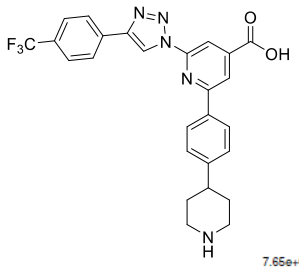
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
494.1805	494.1804	0.1	0.2	16.5	415.3	n/a	n/a	C26 H23 N5 O2 F3



HPLC purity 99% (R_t = 7.87 min)



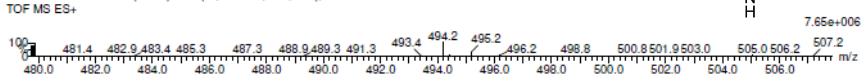
2-(4-(Piperidin-4-yl)phenyl)-6-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)isonicotinic acid (**35**).



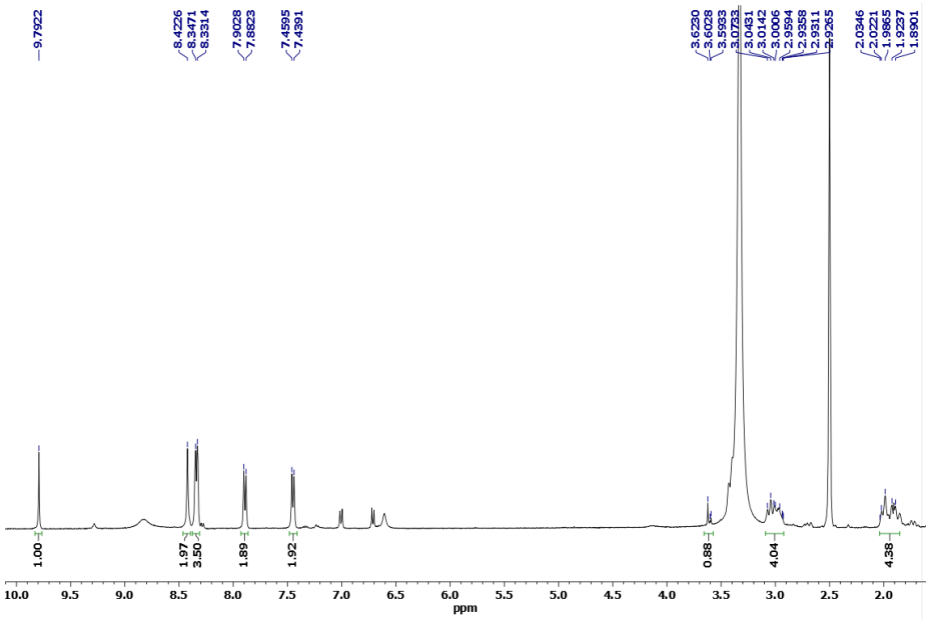
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

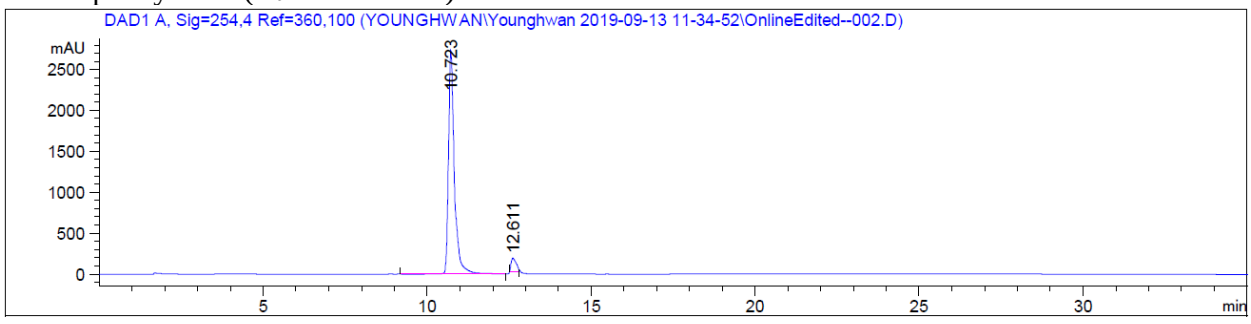
Monoisotopic Mass, Even Electron Ions
80 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 5-5 O: 0-60 F: 3-3
JYH-13SEP19-267 234 (3.975) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



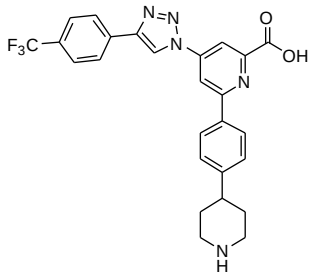
Minimum:				−1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
494.1805	494.1804	0.1	0.2	16.5	513.8	n/a	n/a	C26 H23 N5 O2 F3



HPLC purity 95% (R_t = 10.72 min)



6-(4-(Piperidin-4-yl)phenyl)-4-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)picolinic acid (**36**).

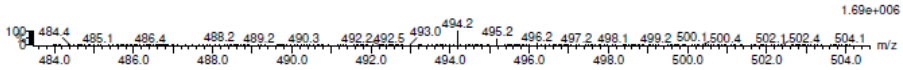


Elemental Composition Report

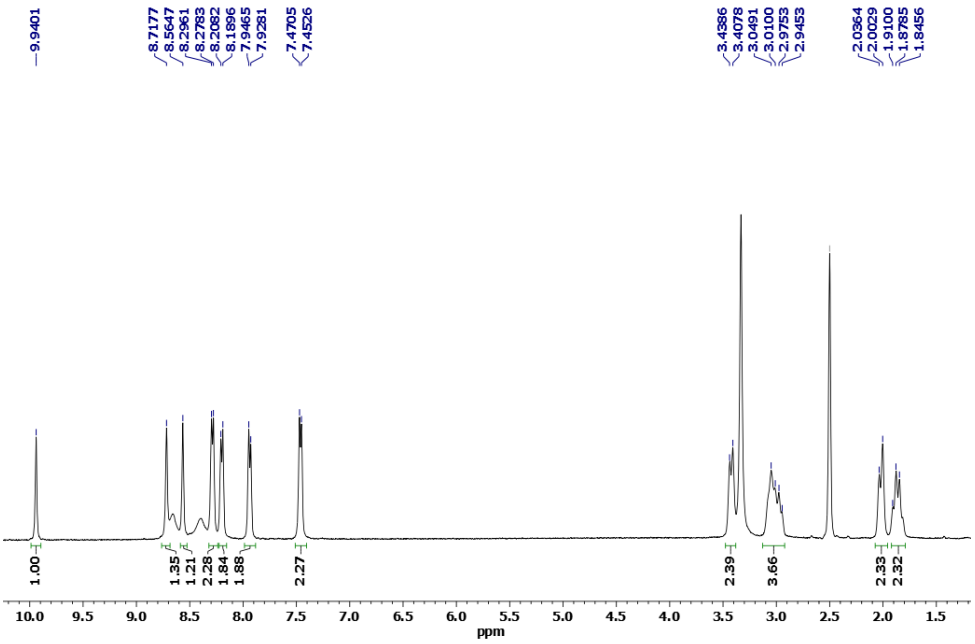
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
80 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 5-5 O: 0-60 F: 3-3

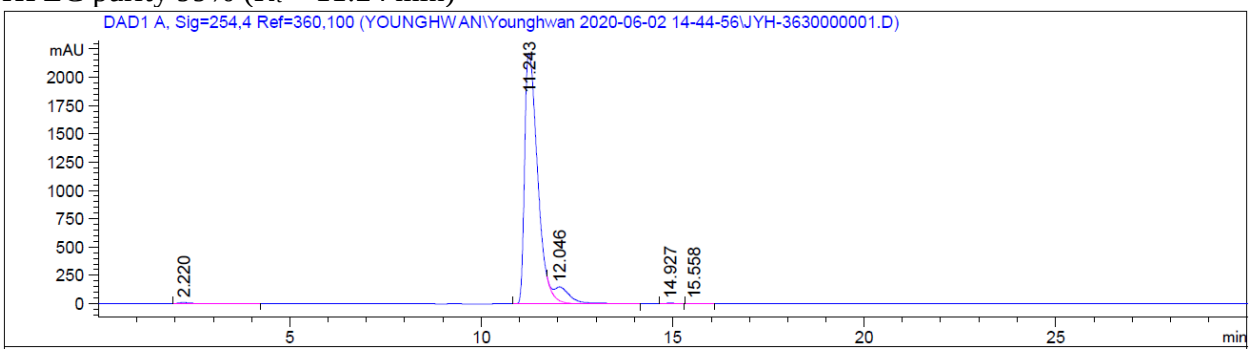
JYH-05JUN20-363 208 (3.535) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



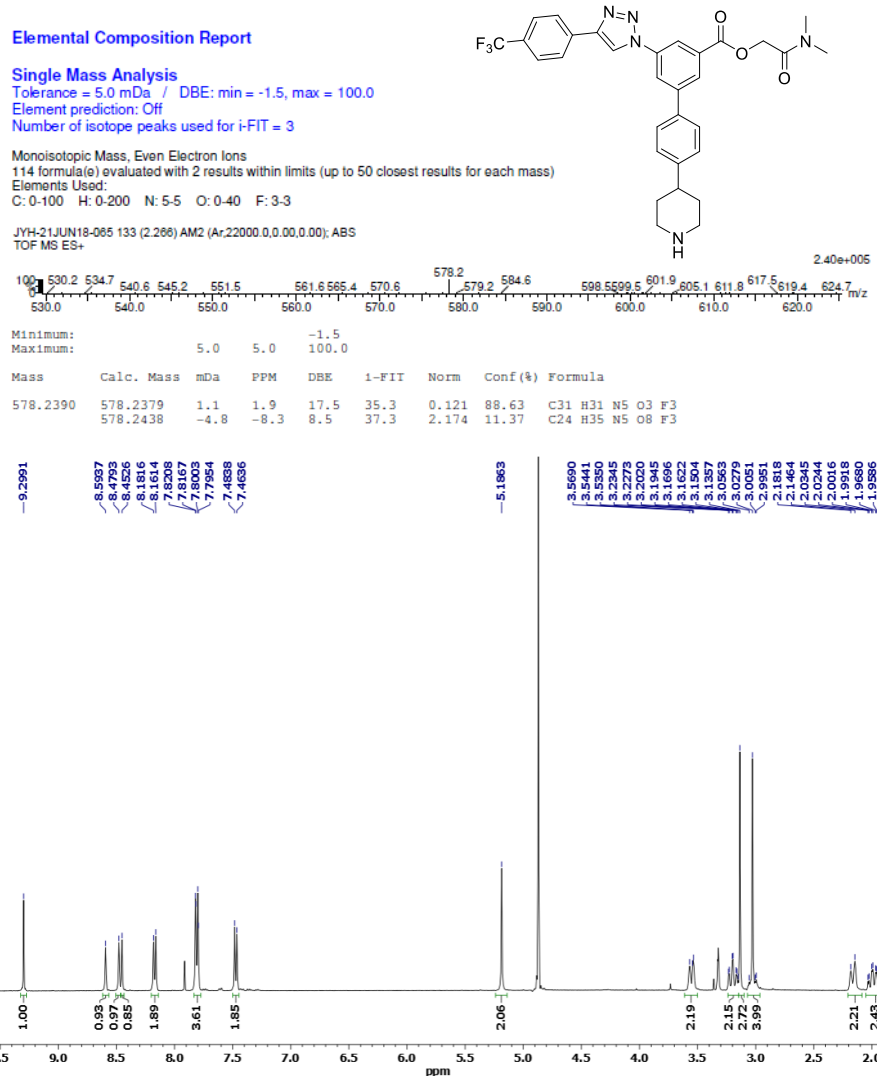
Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
494.1808	494.1804	0.4	0.8	16.5	445.1	n/a	n/a	C26 H23 N5 O2 F3



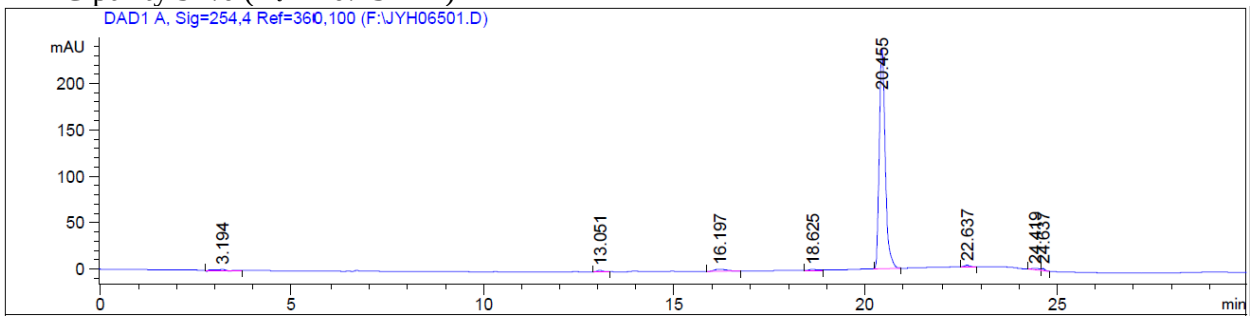
HPLC purity 99% (R_t = 11.24 min)



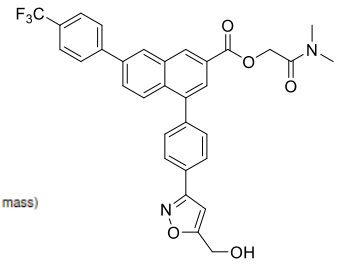
2-(Dimethylamino)-2-oxoethyl 4'-(piperidin-4-yl)-5-(4-(4-(trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-3-carboxylate (**37a**).



HPLC purity 97% (R_t = 20.45 min)



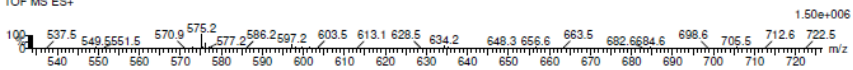
2-(Dimethylamino)-2-oxoethyl 4-(4-(5-(hydroxymethyl)isoxazol-3-yl)phenyl)-7-(4-(trifluoromethyl)phenyl)-2-naphthoate (**37b**).



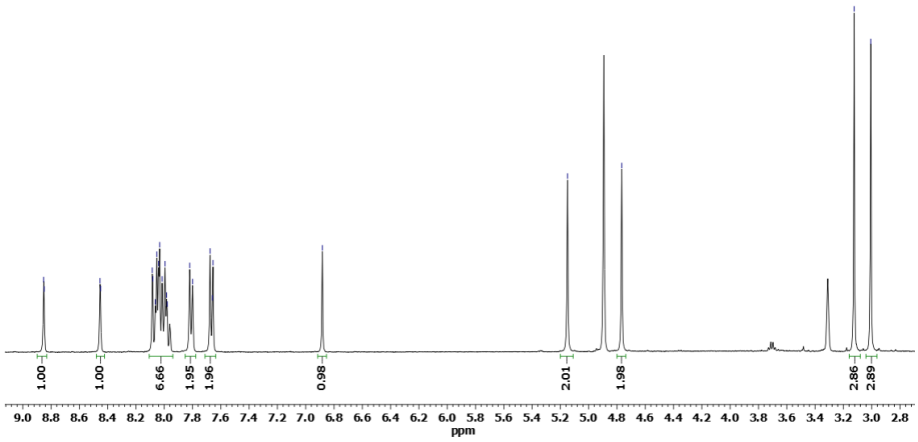
Elemental Composition Report

Single Mass Analysis
Tolerance = 8.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

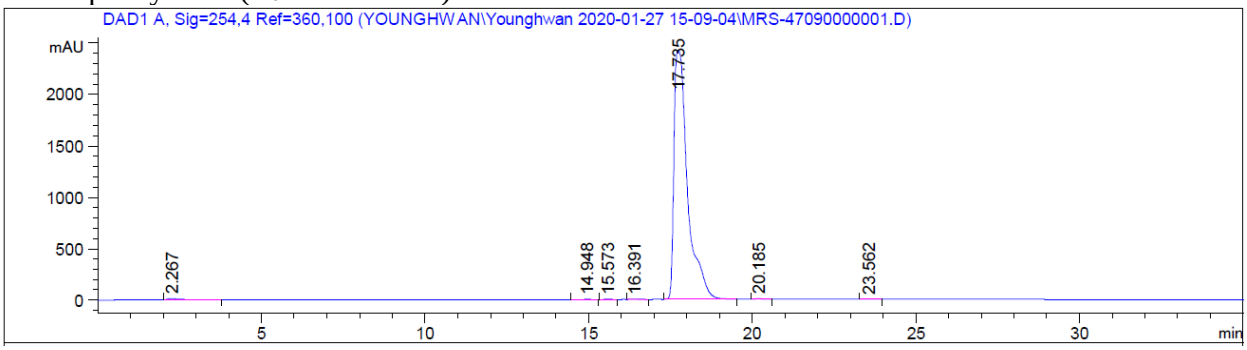
Monoisotopic Mass, Even Electron Ions
116 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 2-2 O: 0-60 F: 3-3
JYH-23JAN20-328-1 91 (1.556) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



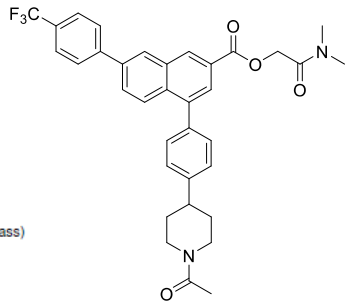
Minimum:				-1.5				
Maximum:		8.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
575.1786	575.1794	-0.8	-1.4	19.5	412.8	0.033	96.75	C32 H26 N2 O5 F3
	575.1735	5.1	8.9	28.5	417.9	5.120	0.60	C39 H22 N2 F3
	575.1853	-6.7	-11.6	10.5	416.4	3.631	2.65	C25 H30 N2 O10 F3



HPLC purity 99% (R_t = 17.73 min)



2-(Dimethylamino)-2-oxoethyl 4-(4-(1-acetylpiperidin-4-yl)phenyl)-7-(4-(trifluoromethyl)phenyl)-2-naphthoate (**37c**).

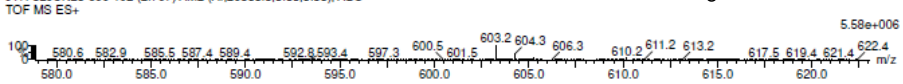


Elemental Composition Report

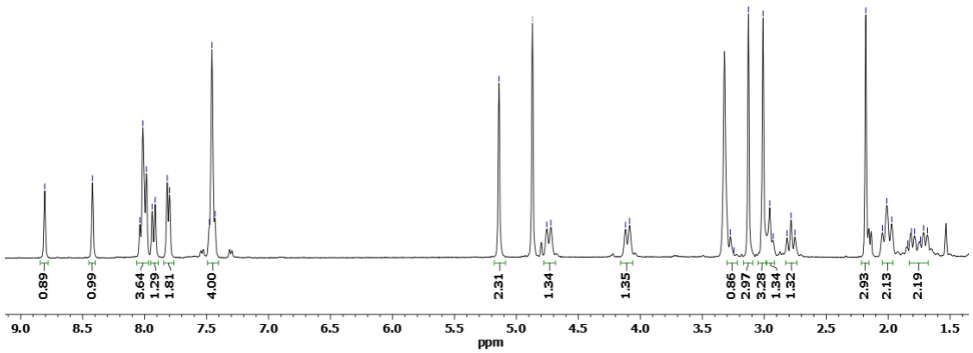
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
129 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-250 N: 2-2 O: 0-60 F: 3-3

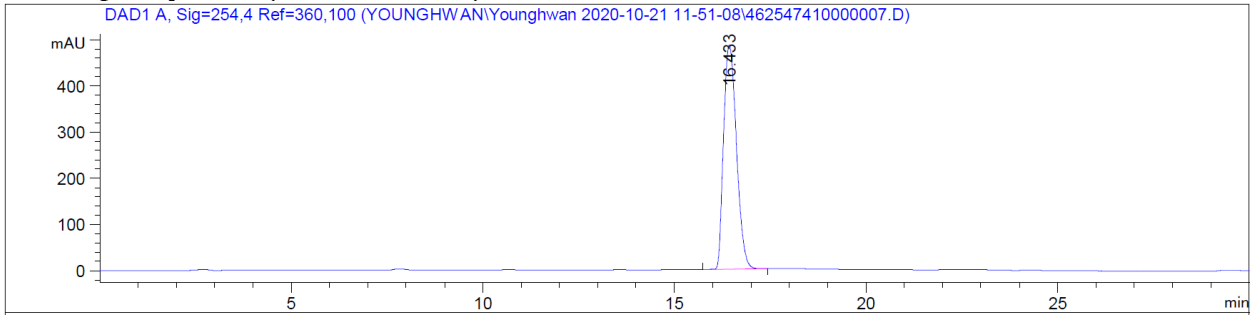
JYH-02JUN20-359 162 (2.757) AM2 (Ar,25000.0,0.00,0.00); ABS
TOF MS ES+



Minimum:				-1.5				
Maximum:		5.0	5.0	100.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
603.2479	603.2471	0.8	1.3	18.5	547.9	n/a	n/a	C35 H34 N2 O4 F3



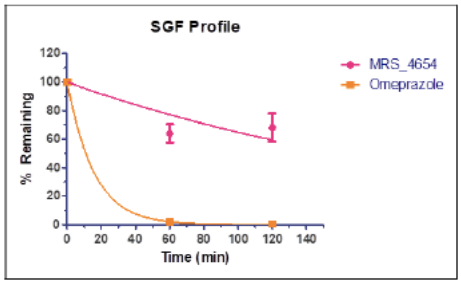
HPLC purity 99% (R_t = 16.43 min)



ADMET properties of compound 32 (MRS4654). Determined by Jai Research Foundation (JRF), Department of Toxicology, Valvada - 396 105, Dist. Valsad, Gujarat, India; Protocol RES 1-02-26140 (MRS4654).

Simulated gastric fluid (SGF)

SGF Results				
Compound	**Replicate**	**% Remaining in 120 min**	**Avg % Remaining (120 min)**	**Half-life (min)**
32	1	69.02	68.13	160.60
	2	77.48		
	3	57.89		
Omeprazole	1	0.00	0.46	10.84
	2	0.00		
	3	1.37		



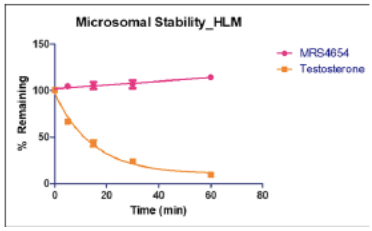
Simulated intestinal fluid (SIF)

SIF Results				
Compound	**Replicate**	**% Remaining in 120 min**	**Avg % Remaining (120 min)**	**Half-life (min)**
32	1	130.73	114.41	NA
	2	107.29		
	3	105.20		
Verapamil	1	130.85	125.65	NA
	2	120.47		
	3	125.62		

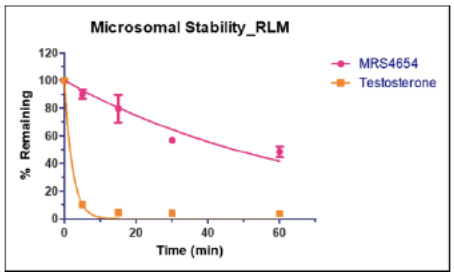
NA=Not Applicable

Liver microsome stability (human, rat, mouse)

Microsomal stability Results HLM					
Compound	**% Remaining (60 min)**	**Half life (min)**	**Avg Half life (min)**	**CL_{int} (µL/min/mg of protein)**	**Avg CL_{int} (µL/min/mg of protein)**
32-Set 1	112.60	NA	Stable	NA	NA
32-Set 2	115.70	NA		NA	
Testosterone-Set 1	9.12	9.43	10.13	44.11	41.25
Testosterone-Set 2	9.34	10.83		38.39	

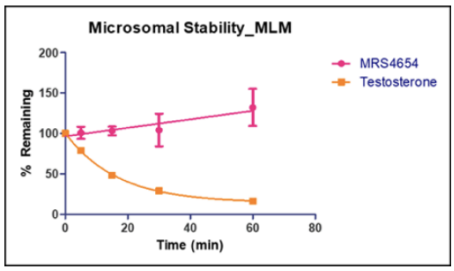


Microsomal stability Results RLM					
Compound	**% Remaining (60 min)**	**Half-life (min)**	**Avg Half-life (min)**	**CL_{int} (µL/min/mg of protein)**	**Avg CL_{int} (µL/min/mg of protein)**
32-Set 1	51.19	52.34	47.68	7.94	8.80
32-Set 2	45.68	43.02		9.67	
Testosterone-Set 1	3.65	1.43	1.52	290.16	273.89
Testosterone-Set 2	3.83	1.61		257.62	



Microsomal stability Results MLM					
Compound	**% Remaining (60 min)**	**Half life (min)**	**Avg Half life (min)**	**CL_{int} (µL/min/mg of protein)**	**Avg CL_{int} (µL/min/mg of protein)**
32-Set 1	148.46	NA	Stable	NA	NA
32-Set 2	116.07	NA		NA	
Testosterone-Set 1	16.24	11.28	11.58	36.86	35.95
Testosterone-Set 2	16.64	11.87		35.03	

NA= Not Applicable



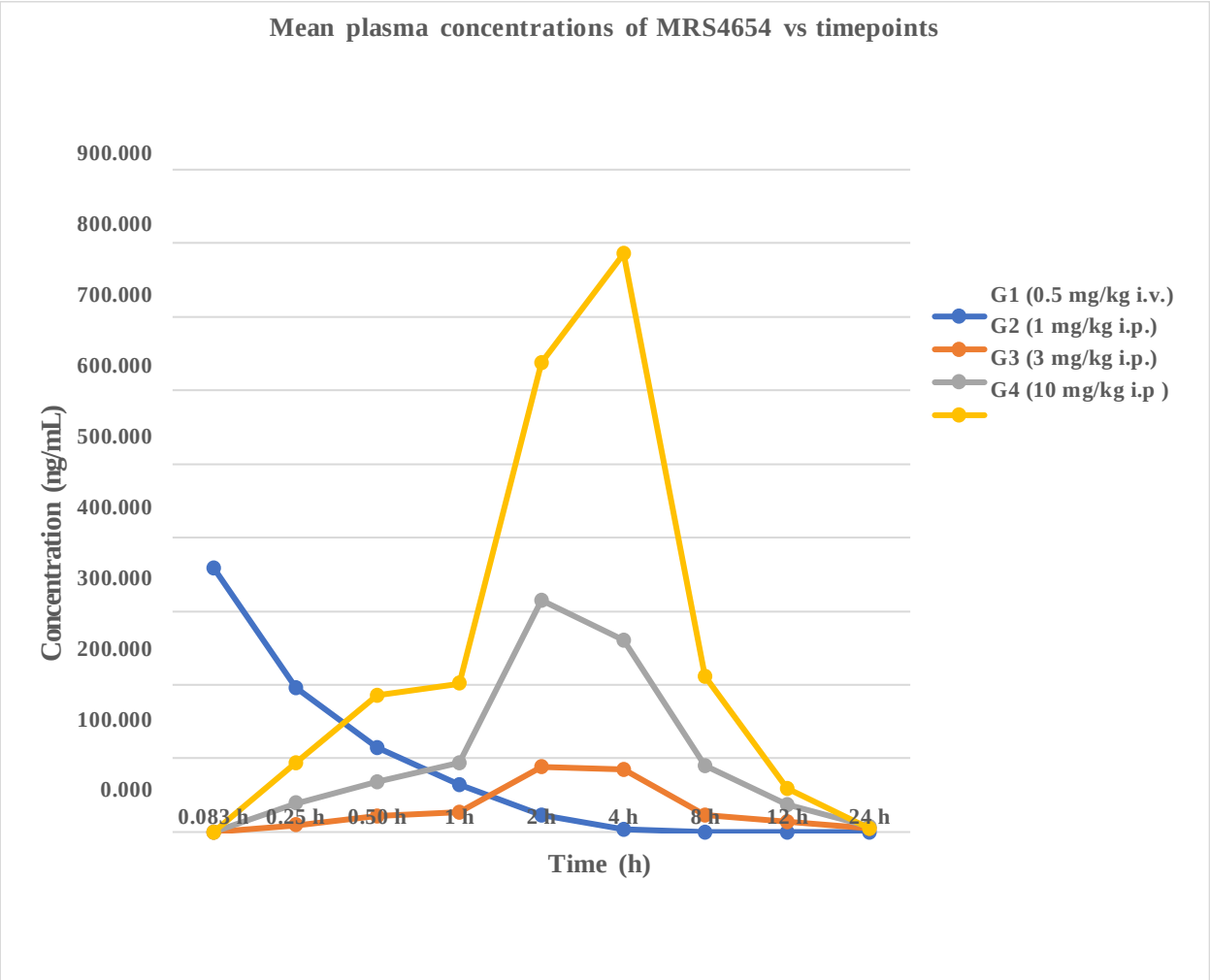
Caco2 cell permeability

Compound Name	Average Values					
	Papp (10^{-6} cm/sec)		Efflux Ratio	A to B % Recovery	B to A % Recovery	Classification
	Apical to Basal	Basal to Apical				
32	0.00	1.32	NC	10.39	58.12	LOW
Digoxin	2.12	11.77	6.38	91.00	96.23	MEDIUM
Propranolol	25.07	21.74	0.86	68.10	86.70	HIGH
Atenolol	0.45	0.30	0.66	83.58	86.95	LOW

NC: denotes Not calculated; as very low Papp in A-B and B-A.
Efflux ratio > 2 is Efflux indicated

Result of permeability Papp (10^{-6}cm/s)	Range
<1.5	Low permeable
1.5 to 10	Medium permeable
>10	High permeable

Figure S10. Time (h) – mean plasma concentration (ng/mL) graph for compound 32.



Patch clamp hERG assay

ScreenPatch® Assay (SyncroPatch® 384PE Based Assay), performed by Charles River Cleveland, Inc., 14656 Neo Parkway, Cleveland, OH 44128 USA.
Procedure: Cells were cultured in Ham's F-12 supplemented with 10% fetal bovine serum, 100 U/mL penicillin G sodium, 100 µg/mL streptomycin sulfate and 400 µg/mL Zeocin. Before testing, cells in culture dishes were rinsed with Hank's Balanced Salt Solution, detached with Accutase. Immediately before use in the SyncroPatch® 384PE system, the cells were washed in HB-PS to remove the Accutase and re-suspended in 20 mL of HB-PS.
The test article effects were evaluated using SyncroPatch® 384PE systems (SP384PE; Nanion Technologies, Livingston, NJ). HEPES-buffered intracellular solution (Charles River proprietary) for whole cell recordings was loaded into the intracellular compartment of the SP384PE. Extracellular buffer (HB-PS) and Cell suspension (in HB-PS) were pipetted into the extracellular compartment of the SP384PE chip. After establishment of a whole-cell configuration, membrane currents were recorded using patch clamp amplifier in the SP384PE system. Test article (TA) concentrations were applied to naïve cells (n = 4, where n = replicates/ concentration). Each application consisted of addition of 40 µL of 2X concentrated test article solution to the total 80 µL of final volume of the extracellular well of the SP384PE chip. Duration of exposure to each compound concentration was five (5) minutes.
hERG Test Voltage Protocol. hERG current was measured using stimulus voltage patterns with fixed amplitudes: activation pre-pulse (TP1) to +40 mV for 2 s and test pulse (TP2) to -40 mV for 2 s from a holding potential of -80 mV. hERG current was measured as the outward peak current at TP2 (tail current). The stimulation was repeated with 0.1 Hz frequency during 2 min as baseline and 5 min after TA application. The control inhibitor Cisapride showed an IC_{50} value of 0.019 µM.

Test Article ID	Test Conc., ∝M	Mean, %	SEM	N	IC_{50}/(EC_{50}), ∝M
1a	0.01	-0.4	1.0	4	>30
	0.03	1.4	5.7	4	
	0.1	1.1	5.0	4	
	0.3	1.2	2.2	4	
	1	1.8	4.3	4	
	3	-1.8	2.9	3	
	10	-3.0	2.8	4	
	30	-10.8	0.7	3	
32	0.01	4.0	5.7	4	(>30)
	0.03	4.9	6.7	4	
	0.1	0.0	3.0	4	
	0.3	-4.8	3.7	4	
	1	0.9	2.5	4	
	3	-19.0	5.6	4	
	10	-31.4	4.3	4	
	30	-28.1	3.8	4	
1b	0.01	1.7	3.4	4	(16.032)
	0.03	1.6	8.0	4	
	0.1	-1.3	2.9	4	
	0.3	8.5	4.7	4	
	1	-5.0	4.3	4	
	3	-19.3	5.2	4	
	10	-42.1	2.5	4	
	30	-79.0	5.4	4