

Supplementary information

Fragment-based design of *Mycobacterium tuberculosis* InhA inhibitors.

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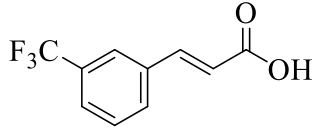
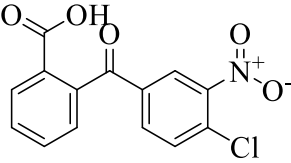
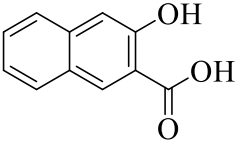
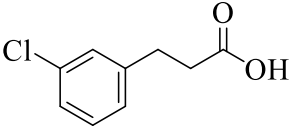
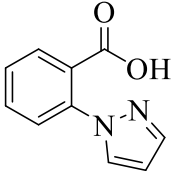
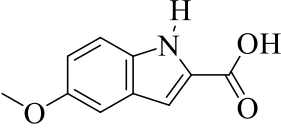
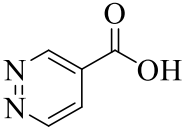
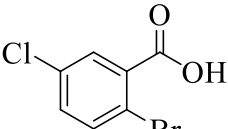
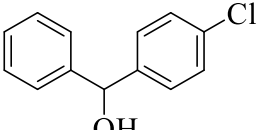
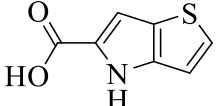
^a Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, United Kingdom.

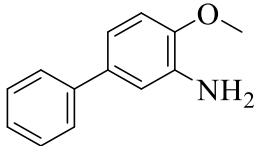
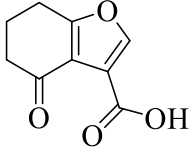
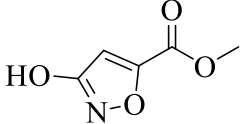
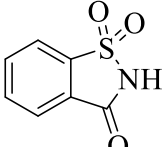
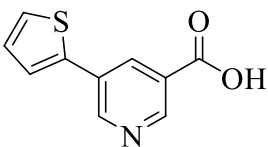
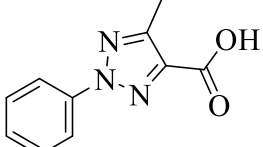
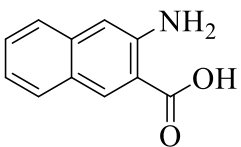
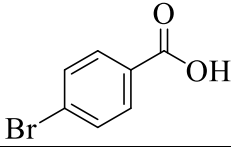
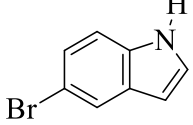
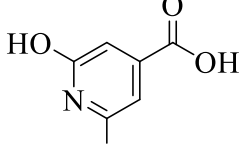
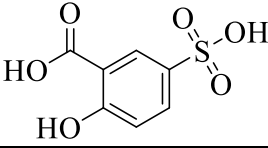
^b Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge, CB2 1GA, United Kingdom.

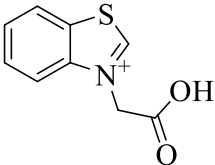
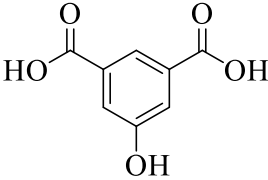
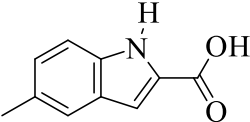
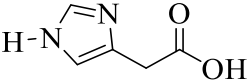
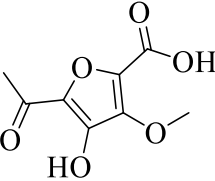
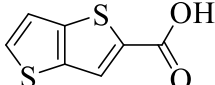
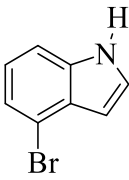
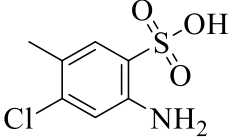
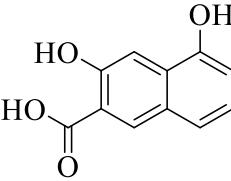
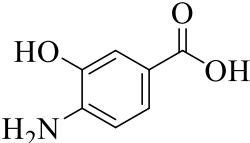
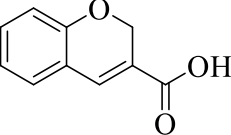
^c Department of Biochemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, 84215, Bratislava, Slovakia

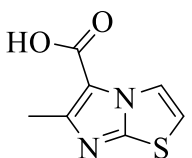
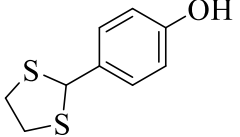
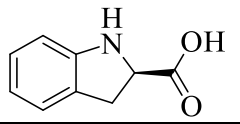
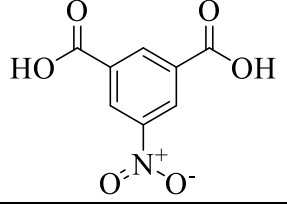
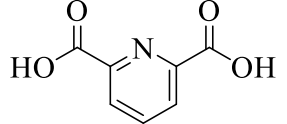
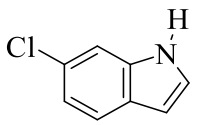
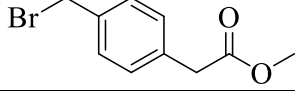
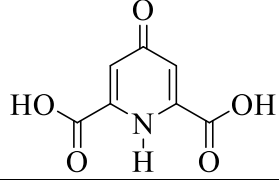
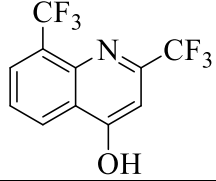
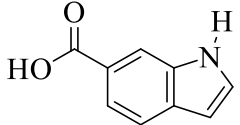
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Table S1: Fragment hits identified by thermal shift.

	Chemical Structure	ΔT_m ($^{\circ}\text{C}$)	Confirmed by ligand-based NMR*	X-ray crystal structure
Fragment 1		+ 3.2	Yes	Yes
Fragment 2		+ 4.2	Yes	Yes
Fragment 3		+ 4.2	Yes	Yes
Fragment 4		+ 3.0	Yes	Yes
Fragment 5		+ 6.0	Yes	Yes
		+ 3.8	Yes	No
		+ 4.1	No	No
		+ 3.4	Yes	No
		+ 6.8	No	No
		+ 6.4	No	No

	+ 3.4	No	No
	+ 3.4	No	No
	+ 3.0	No	No
	+ 3.4	No	No
	+ 5.8	Yes	No
	+ 3.1	Yes	No
	+ 6.1	Yes	No
	+ 3.2	Yes	No
	+ 4.6	Yes	No
	+ 8.3	No	No
	+ 8.0	No	No

		+ 8.7	No	No
		+ 9.0	Yes	No
		+ 4.3	Yes	No
		+ 4.3	No	No
		+ 6.0	No	No
		+ 5.2	Yes	No
		+ 4.9	Yes	No
		+ 3.2	No	No
		+ 6.2	Yes	No
		+ 3.2	No	No
		+ 3.4	Yes	No

	+ 4.1	No	No
	+ 11.0	No	No
	+ 4.5	No	No
	+ 9.7	No	No
	+ 9.7	No	No
	+ 3.3	No	No
	+ 3.6	No	No
	+ 10.4	No	No
	+ 6.0	No	No
	+ 3.3	No	No

*Fragments were only considered confirmed hits by NMR if they showed interactions with InhA in at least two ligand-based NMR techniques (CMPG, STD and WaterLOGSY)

Table S2: X-ray crystallography data collection and final refinement statistics

Ligand#	Fragment 1	Fragment 2	Fragment 3
PDB ID	6SQ5	6SQ7	6SQ9
Data collection*			
Space group	<i>P6₂22</i>	<i>P6₂22</i>	<i>P6₂22</i>
Cell parameters:			
a [Å]	97.53	97.52	97.40
b [Å]	97.53	97.52	97.40
c [Å]	140.23	140.18	140.69
$\alpha/\beta/\gamma$ [°]	90/90/120	90/90/120	90/90/120
Resolution range [Å]	140.23 – 1.84 (2.05 – 1.84)	84.46 – 1.76 (1.96 – 1.76)	84.35 – 1.75 (2.02 – 1.75)
No. of observations			
total	680038 (192608)	762097 (213551)	1022057 (369232)
unique	35046 (9753)	39915 (11123)	40716 (13985)
R_{merge}	0.061 (0.952)	0.065 (0.644)	0.084 (0.649)
$I/\sigma(I)$	26.4 (3.8)	24.1 (3.8)	22.7 (4.8)
CC(1/2)	0.999 (0.952)	0.999 (0.78)	0.999 (0.987)
Completeness [%]	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
Multiplicity	19.4 (19.7)	19.1 (19.2)	25.1 (26.4)
Refinement			
Refinement program	PHENIX	PHENIX	PHENIX
Resolution [Å]	72.35 – 1.84	84.46 – 1.76	84.35 – 1.75
No. reflections	34995	39876	40667
$R_{\text{work}}/R_{\text{free}}$ [%]	16.3/18.2	15.6/16.4	16.8/17.9
RMS deviations			
Bonds [Å]	0.006	0.006	0.006
Angles [°]	0.826	0.947	0.880
Ramachandran			
Favoured [%]	96	96	96
Outliers [%]	0	0	0.4

* Parameters shown in brackets are for the highest resolution shown

Ligand#	Fragment 4	Fragment 5	Compound 23
PDB ID	6SQB	6SQD	6SQL
Data collection*			
Space group	<i>P</i> 6 ₂ 22	<i>P</i> 6 ₂ 22	<i>P</i> 6 ₂ 22
Cell parameters:			
a [Å]	97.83	97.61	97.92
b [Å]	97.83	97.61	97.92
c [Å]	140.59	139.46	139.95
α/β/γ [°]	90/90/120	90/90/120	90/90/120
Resolution range [Å]	84.72–1.77 (2.05 – 1.77)	84.53 – 1.72 (1.81 – 1.72)	84.81 – 2.35 (2.48 – 2.35)
No. of observations			
total	1025427 (365696)	804481 (107937)	311279 (47644)
unique	39125 (13427)	42463 (6110)	16974 (2437)
R _{merge}	0.084 (0.758)	0.058 (0.737)	0.354 (2.238)
I/σ(I)	28.2 (4.8)	24.8 (3.0)	11.9 (3.8)
CC(1/2)	0.999 (0.970)	0.999 (0.962)	0.989 (0.900)
Completeness [%]	100.0 (100.0)	100.0 (100.0)	99.0 (99.9)
Multiplicity	26.2 (27.2)	18.9 (17.7)	18.3 (19.6)
Refinement			
Refinement program	PHENIX	PHENIX	PHENIX
Resolution [Å]	84.72 – 1.77	84.53 – 1.72	84.81 – 2.35
No. reflections	39077	42210	17420
R _{work} /R _{free} [%]	15.6/17.4	16.0/18.0	16.2/20.4
RMS deviations			
Bonds [Å]	0.007	0.007	0.007
Angles [°]	0.866	0.907	0.883
Ramachandran			
Favoured [%]	96	96	97
Outliers [%]	0	0	0.4

* Parameters shown in brackets are for the highest resolution shell

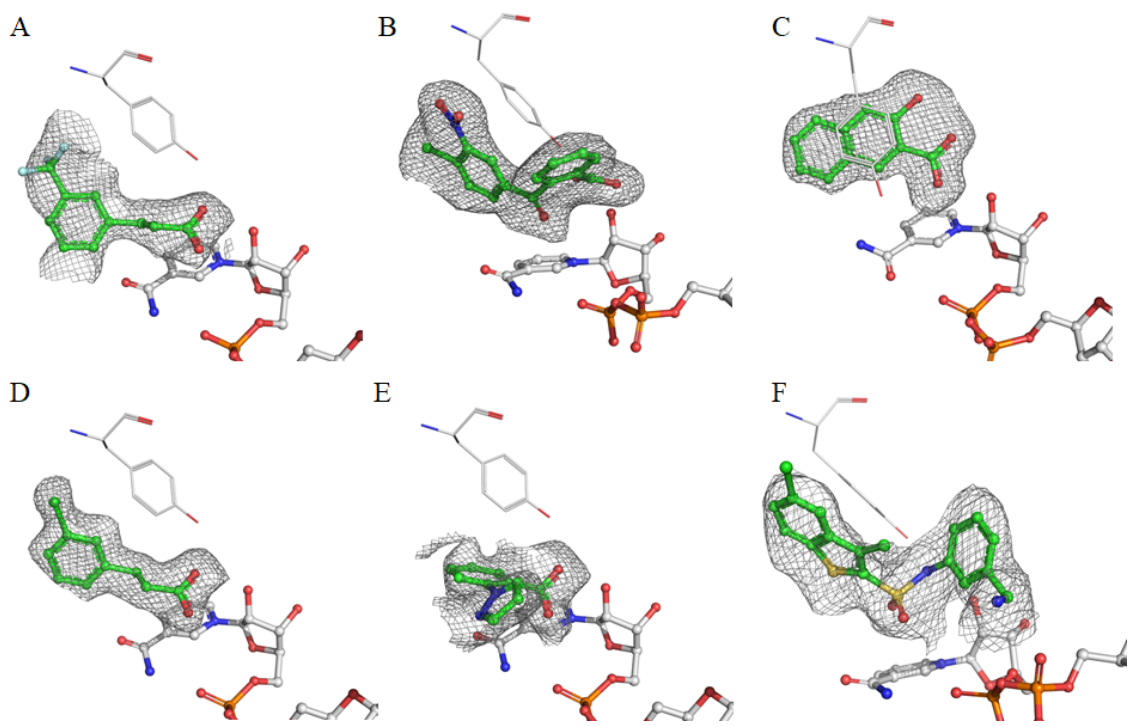


Figure S1: Fo-Fc “Omit” map of compounds **1** (A), **2** (B), **3** (C), **4** (D), **5** (E) and **23** (F).

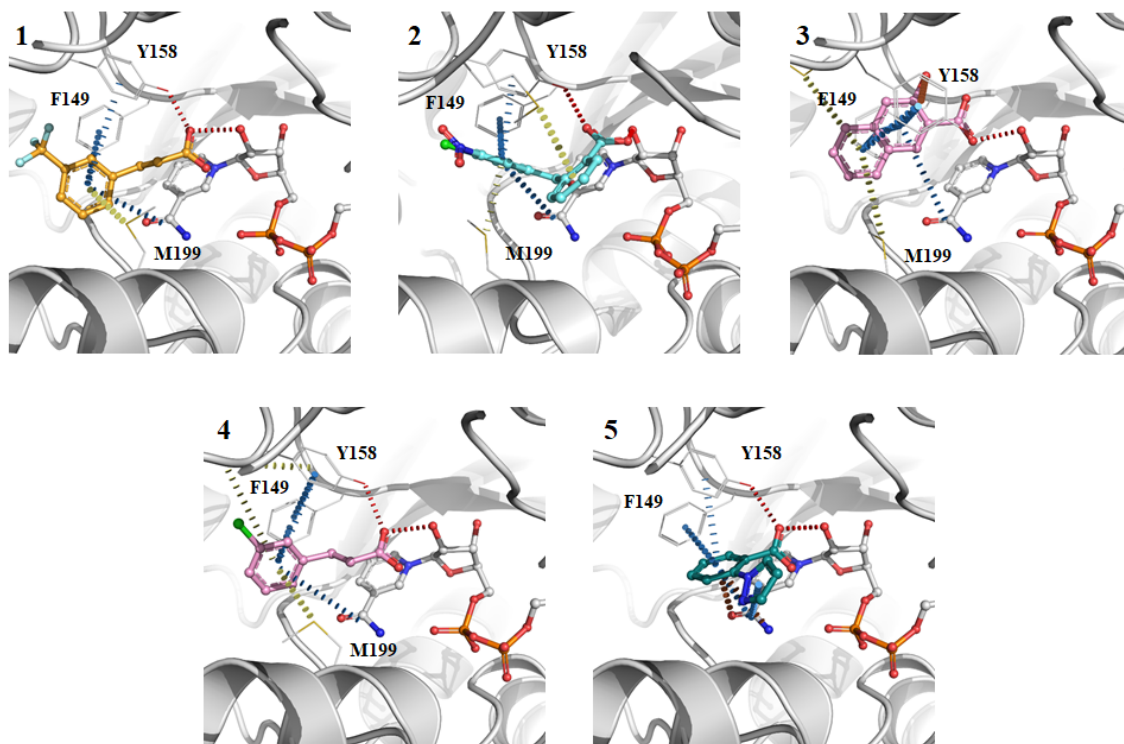


Figure S2: X-ray crystal structures of the fragment hits showing interaction maps between ligand and InhA-NAD⁺. NAD⁺ is shown in white while each fragment is shown in a different colour. Red disks represent hydrogen bonds, blue disks depict π - π interactions, yellow disks sulphur- π , brown disks donor- π interactions. Interactions were calculated using Intermezzo plugin for Pymol (Ochoa B., *et al.* unpublished). Carbonyl groups of fragments **1**, **4** and **5** occupy the same area of the active site. Y158 adopts an “in” conformation in all structures except for **3** where it adopts a new previously unseen conformation.

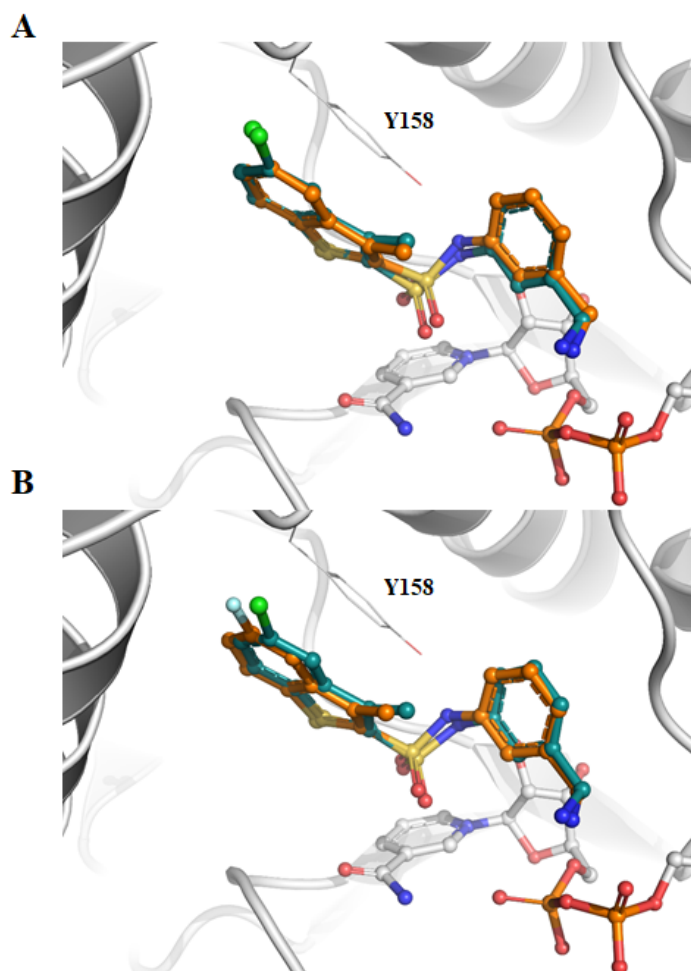
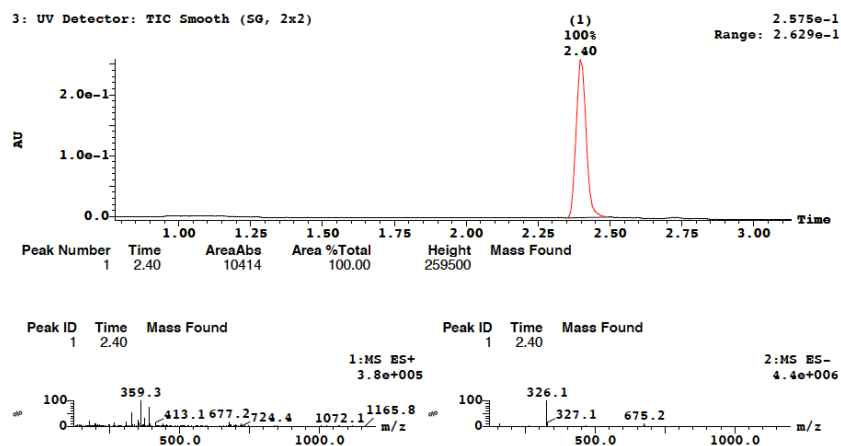


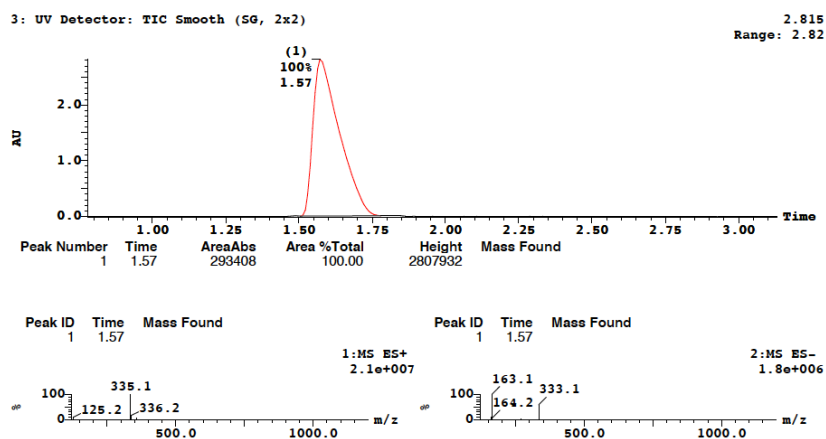
Figure S3: Docking poses of compound **23** (a) and **24** (b), both in orange superposed to the X-ray crystal structure of compound **23** in teal. The compounds **23** and **24** were docked into the structure for fragment **1** (PDB code 6SQ5).

Figure S4: LCMS data for the compounds screened against InhA

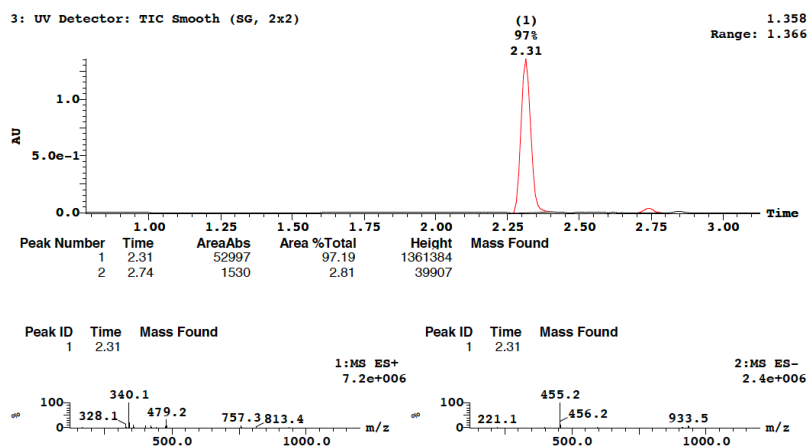
Compound 6



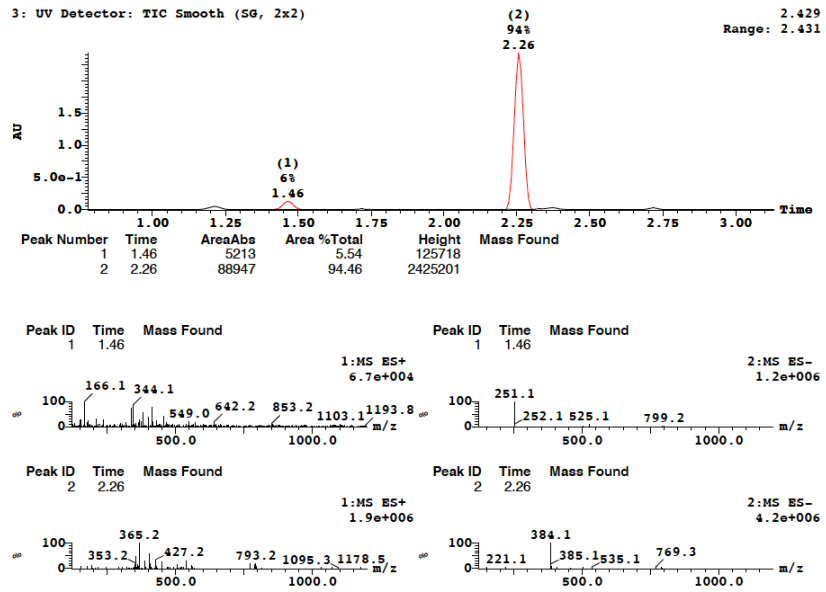
Compound 7



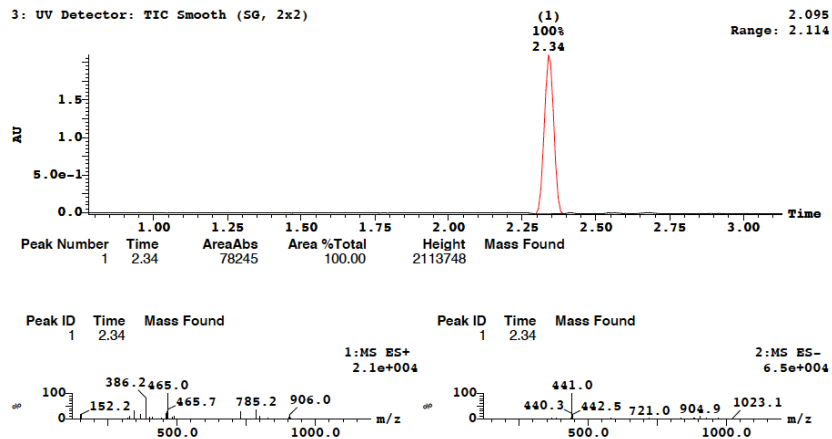
Compound 8



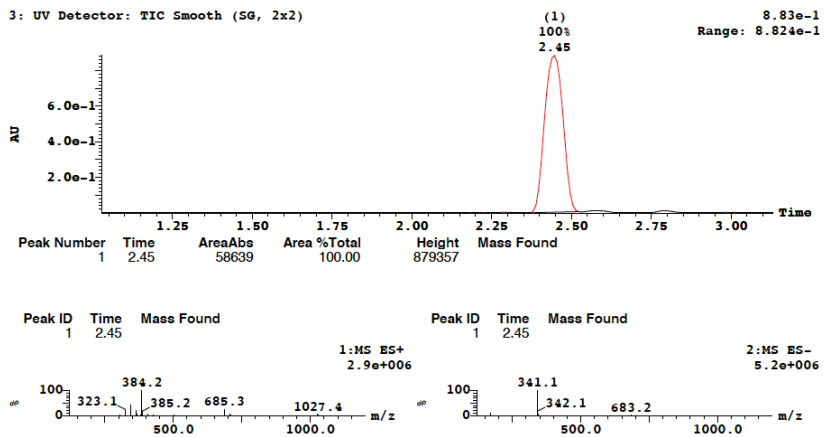
Compound 9



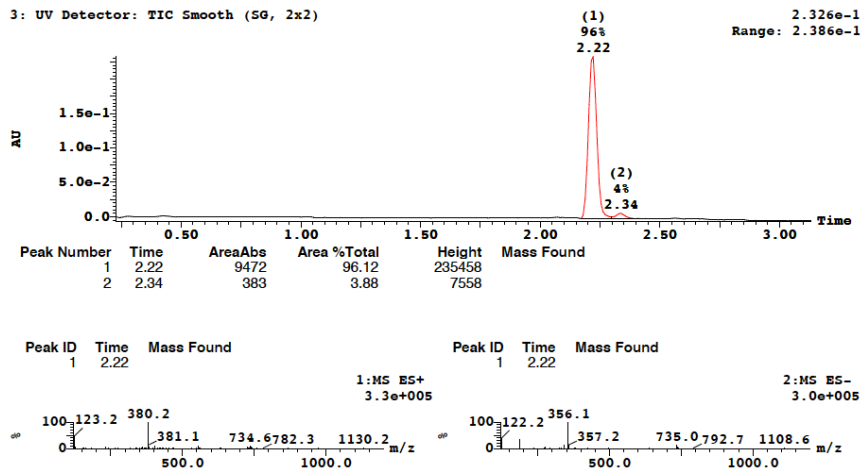
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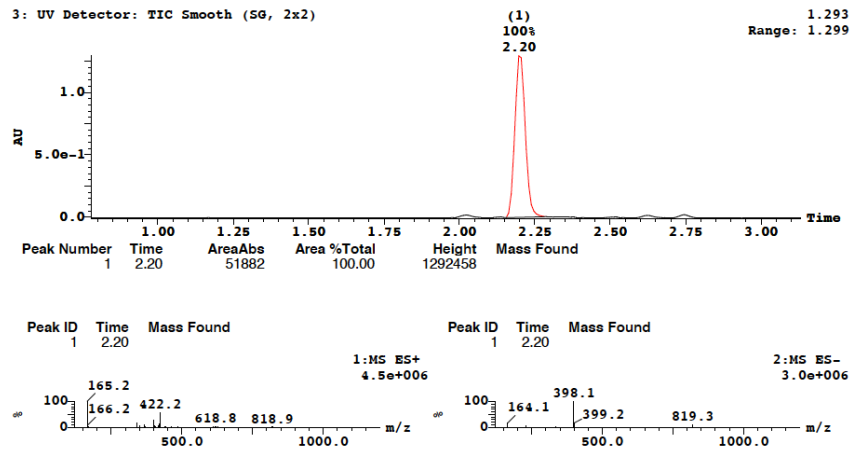
Compound 11



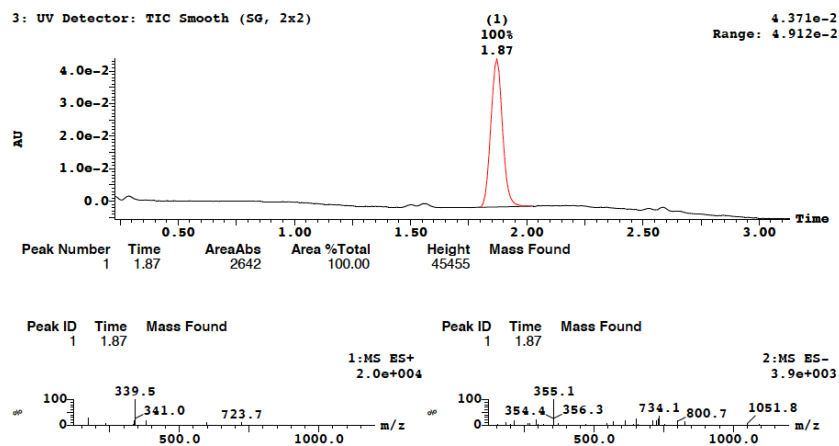
Compound 12



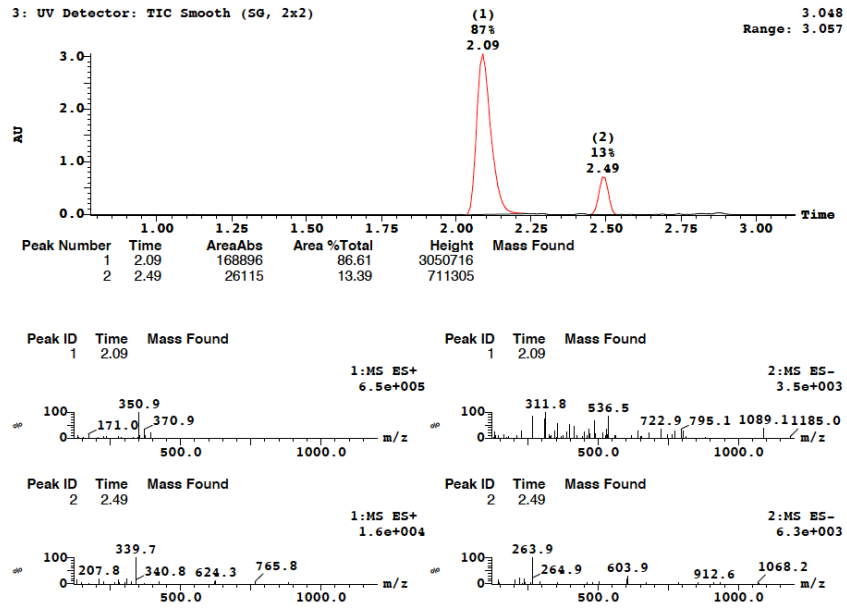
Compound 13



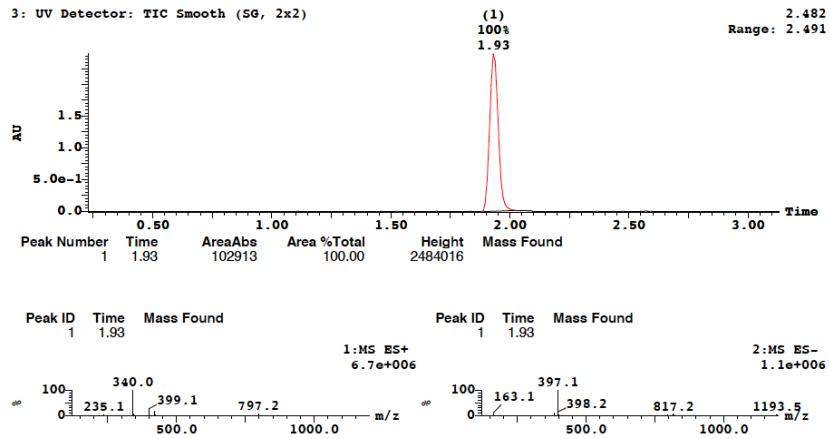
Compound 14



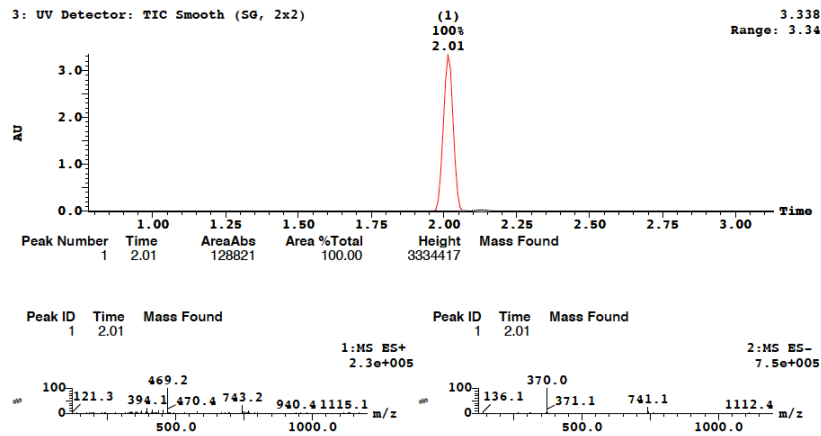
Compound 15



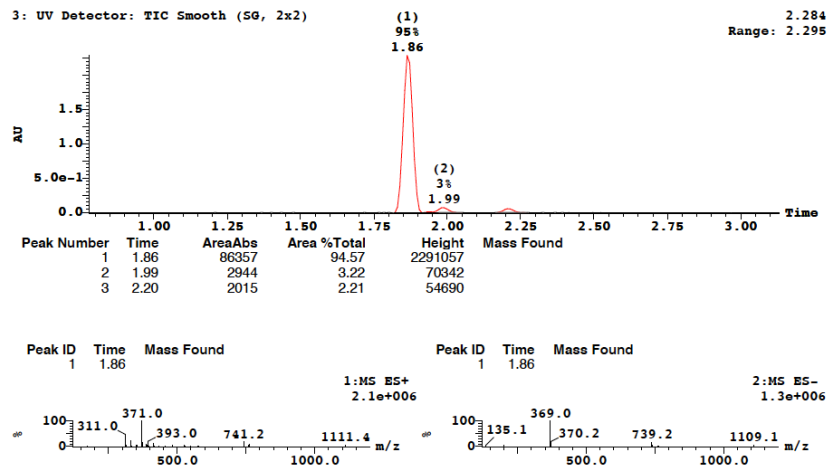
Compound 16



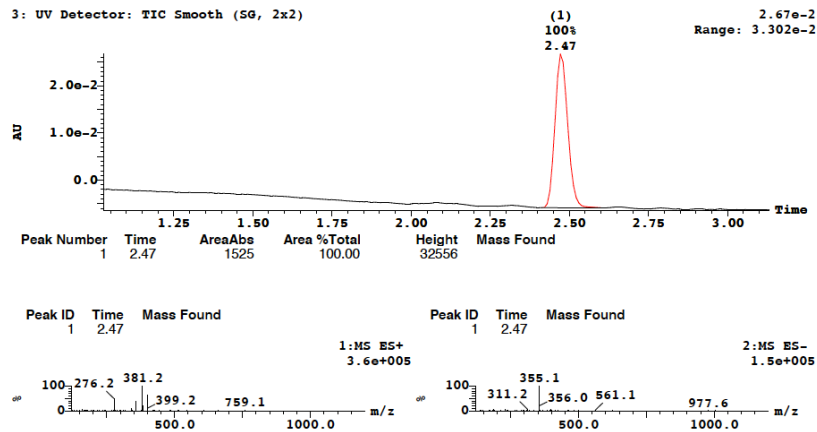
Compound 17



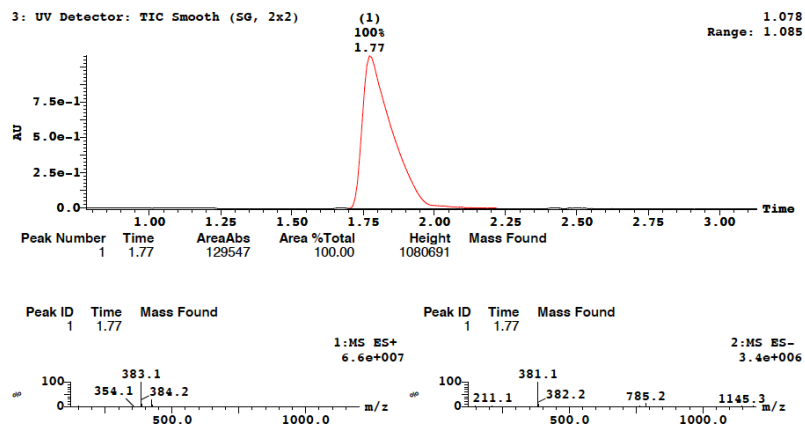
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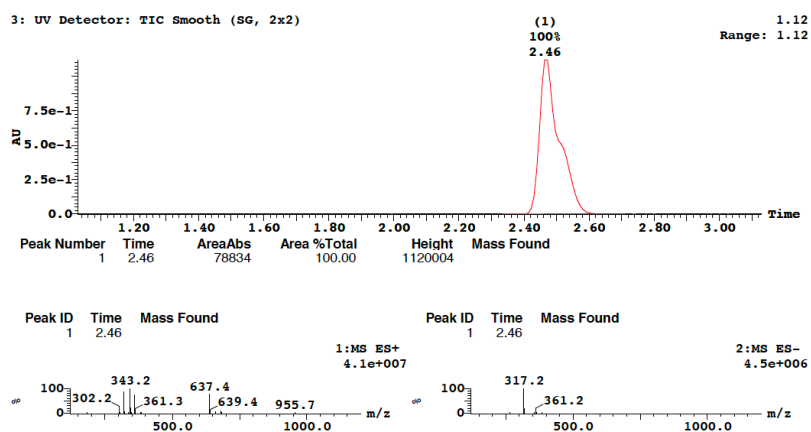
Compound 19



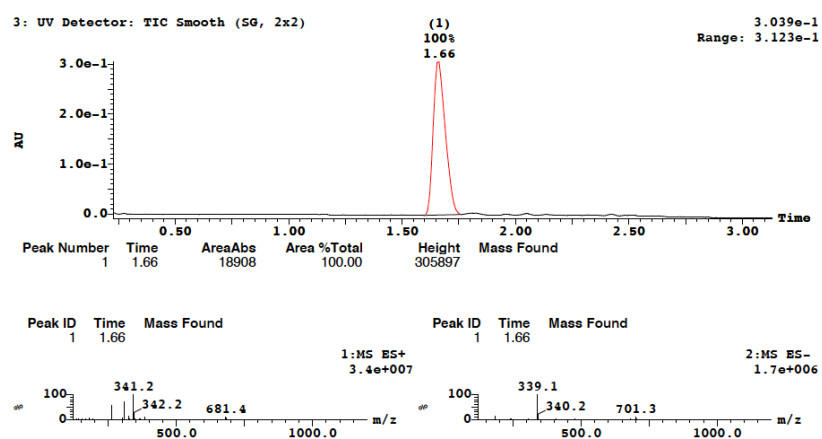
Compound 20



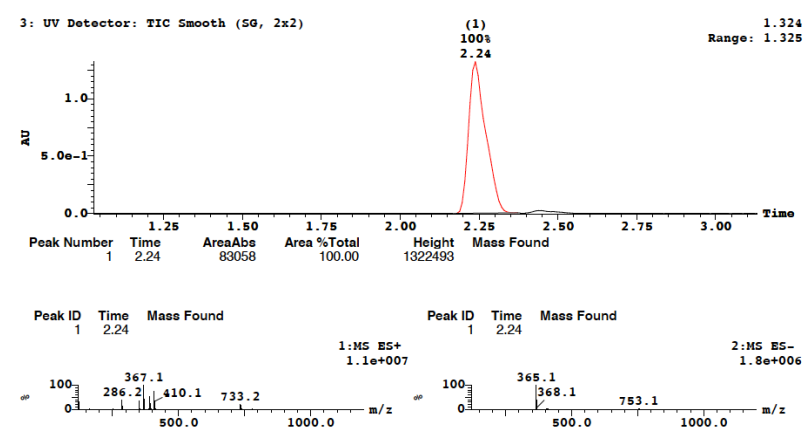
Compound 21



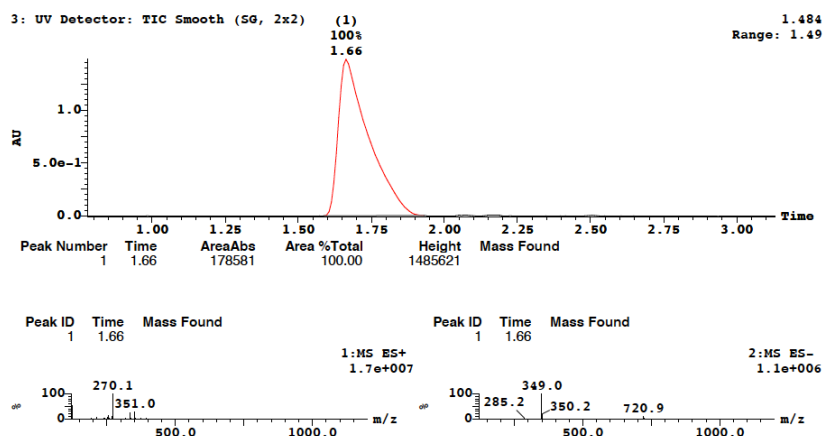
Compound 22



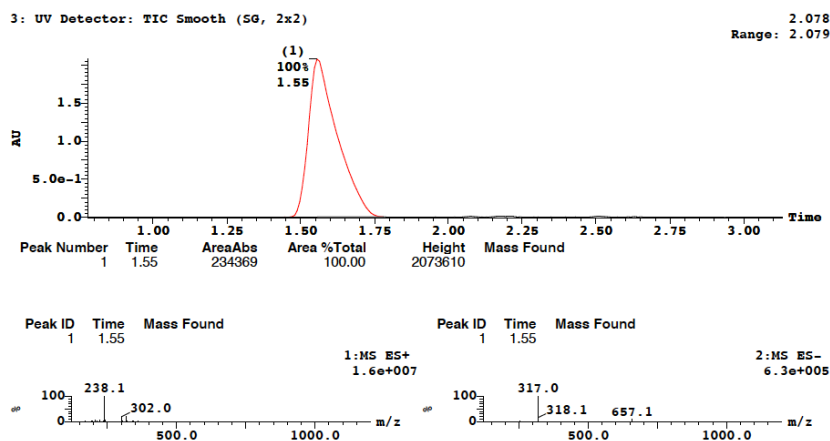
Compound 23



Compound 24



Compound 25



Compound 26

