Development of New Inhibitors of HDAC 1-3 Enzymes Aided by *In Silico* Design Strategies

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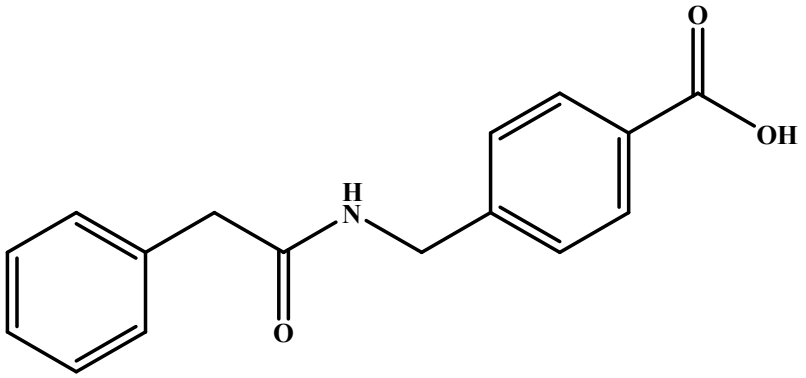
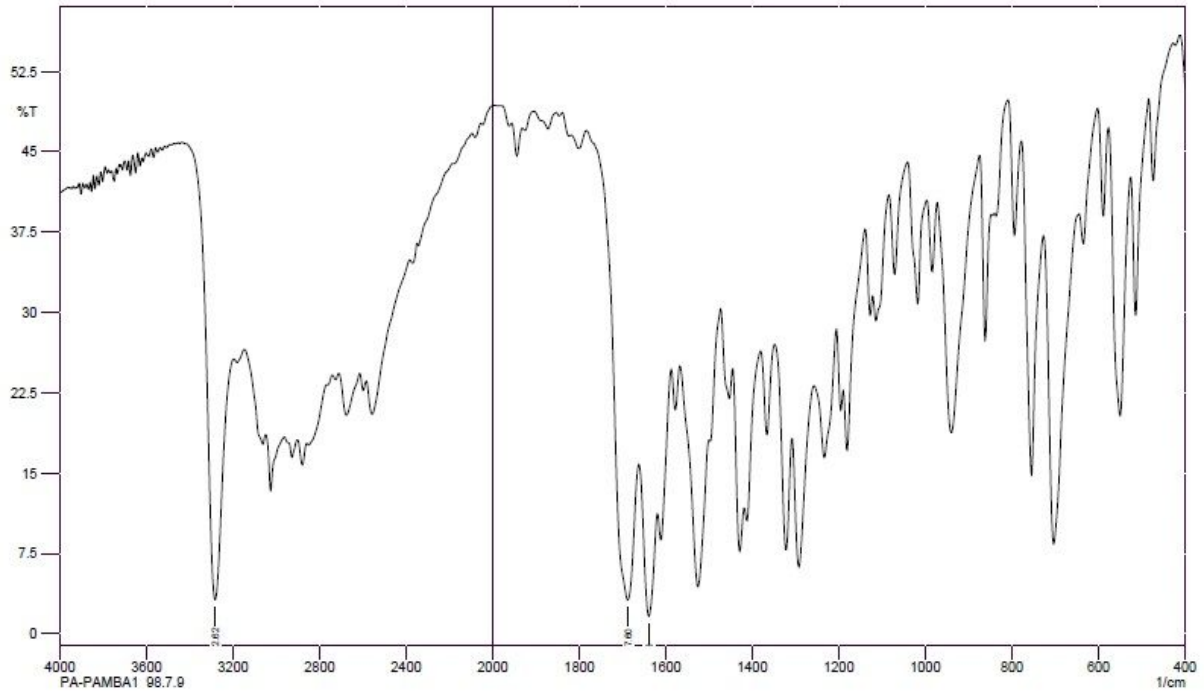
IR spectra of 8a and 8b:

8a: 4-(Phenylacetylamino-methyl)-benzoic acid

White powder; m.p: 204-208 ˚C; yield: 97%; IR(KBr): 3285, 1687, 1639 cm^{-1}.

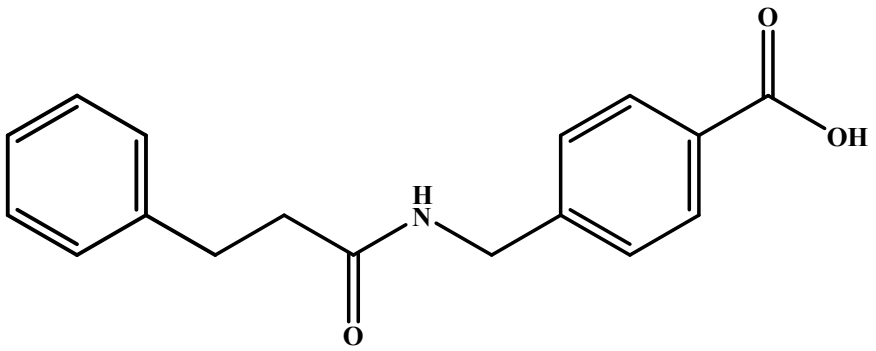


IR spectra:

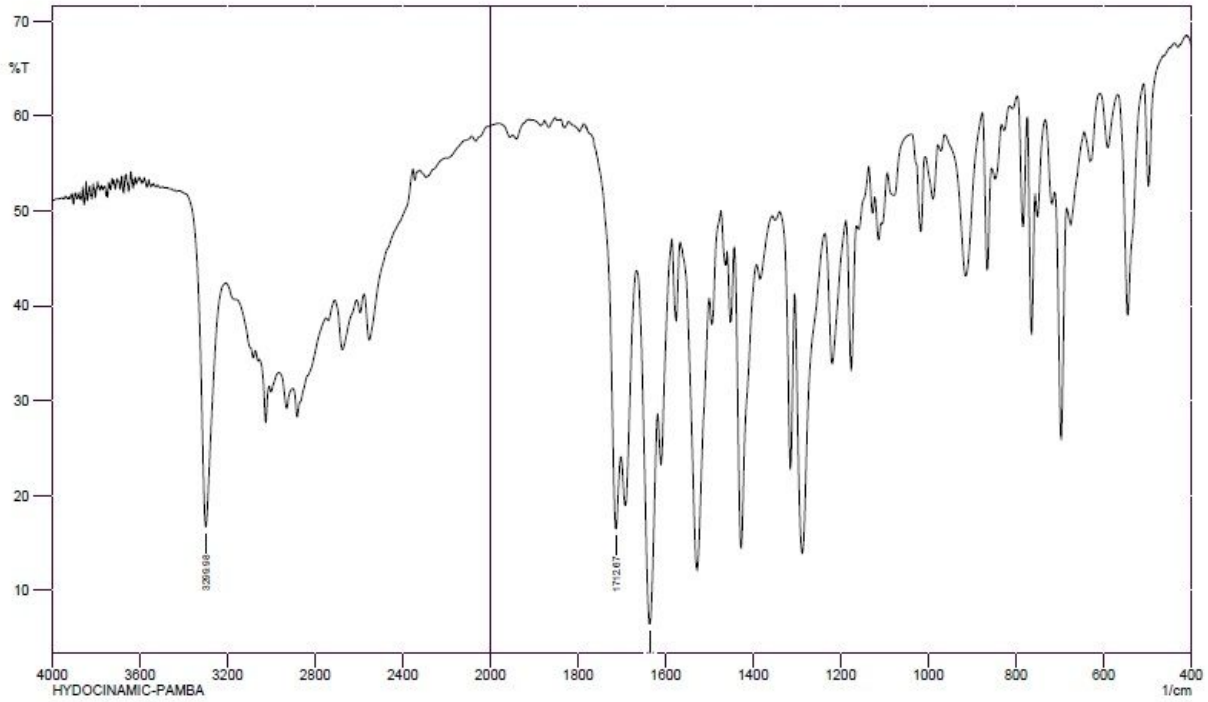


8b: 4-[(3-Phenyl-propionylamino)-methyl]-benzoic acid

White powder; m.p: 198-201 ˚C; yield: 97%; IR(KBr): 3299, 1712, 1635 cm^{-1}.



IR spectra: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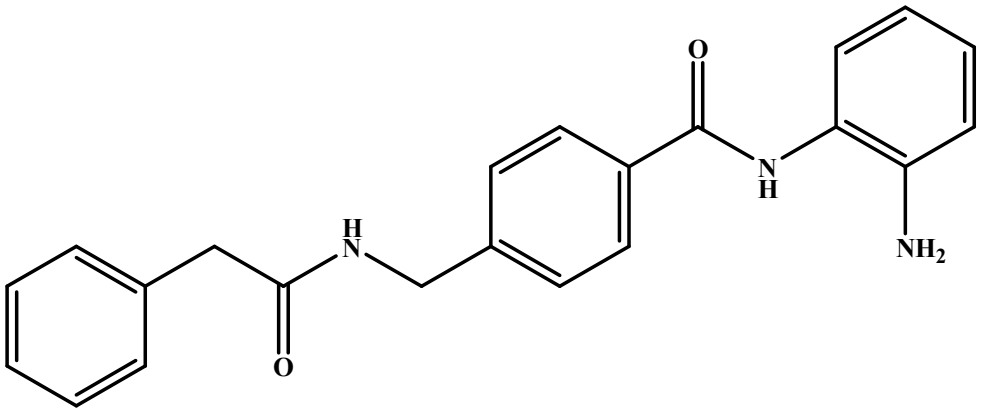
%T
60
50
40
30
20
10
3299.68
1712.67
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm
HYDOCINAMIC-PAMBA

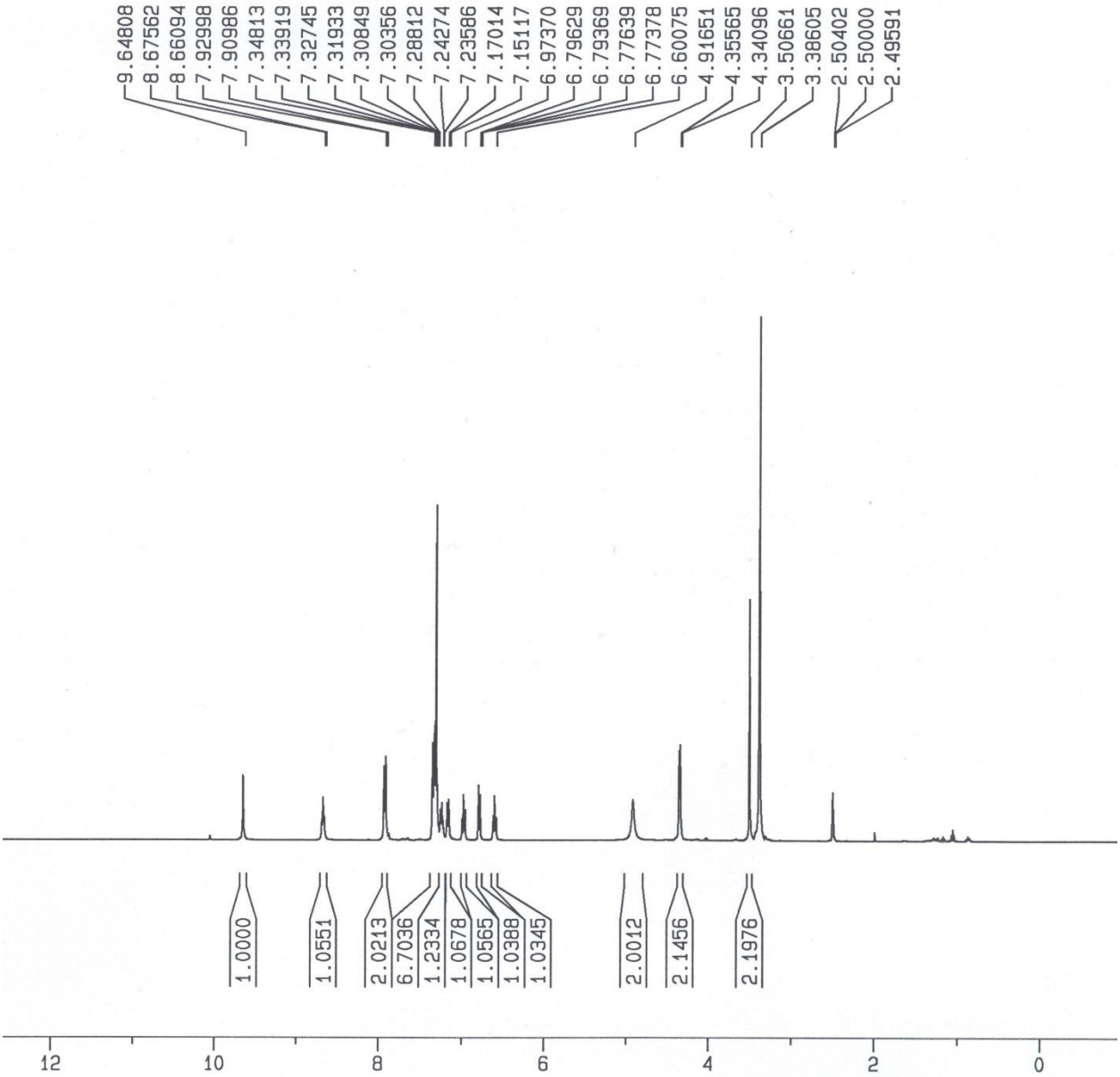
IR, 1H NMR, 13C NMR, Mass, CHNS, HPTLC spectra of 3a and 3b:

3a: N-(2-Amino-phenyl)-4-(phenylacetylamino-methyl)-benzamide

White powder; yield: 88%; m.p: 221-223 ˚C; IR (KBr): 3479, 3384, 3259, 1656, 1637 cm^{-1}; ^{1}HNMR (500 MHz, DMSO-d_6) d (ppm): 3.50 (s, 2H, CH_2), 4.34-4.35 (d, J=7.34, 2H, CH_2), 4.91 (s, 2H, NH_2), 6.60 (t, J=10, 1H-aromatic, CH), 6.77-6.79 (d, J=11.25, 1H-aromatic, CH), 6.97 (t, J= 10.21, 1H-aromatic, CH), 7.15-7.17 (d, J=9.48, 1H-aromatic, CH), 7.22-7.34 (m, 7H-aromatic, CH), 7.90-7.92 (d, J=10.06, 2H-aromatic, CH), 8.67 (t, J=7.34, 1H, NH), 9.64 (s, 1H, NH); ^{13}CNMR (500 MHz, DMSO-d_6): 41.920, 42.392, 116.148, 116.3287, 123.344, 126.402, 126.467, 126.681, 126.868, 127.798, 128.240, 129.010, 133.092, 136.345, 143.083, 165.080, 170.266; Anal Calcd for $C_{22}H_{21}N_3O_2$: C, 73.52%; H, 5.89%; N, 11.69%. Found: C, 73.36%, H, 5.28%, N; 11.19%; Purity: 100% (by HPTLC).



1HNMR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7.23586
7.17014
7.15117
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6.79629
6.79369
6.77639
6.77378
6.60075
4.91651
4.35565
4.34096
3.50661
3.38605
2.50402
2.50000
2.49591
1.0000
1.0551
2.0213
6.7036
1.2334
1.0678
1.0565
1.0388
1.0345
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2.1976
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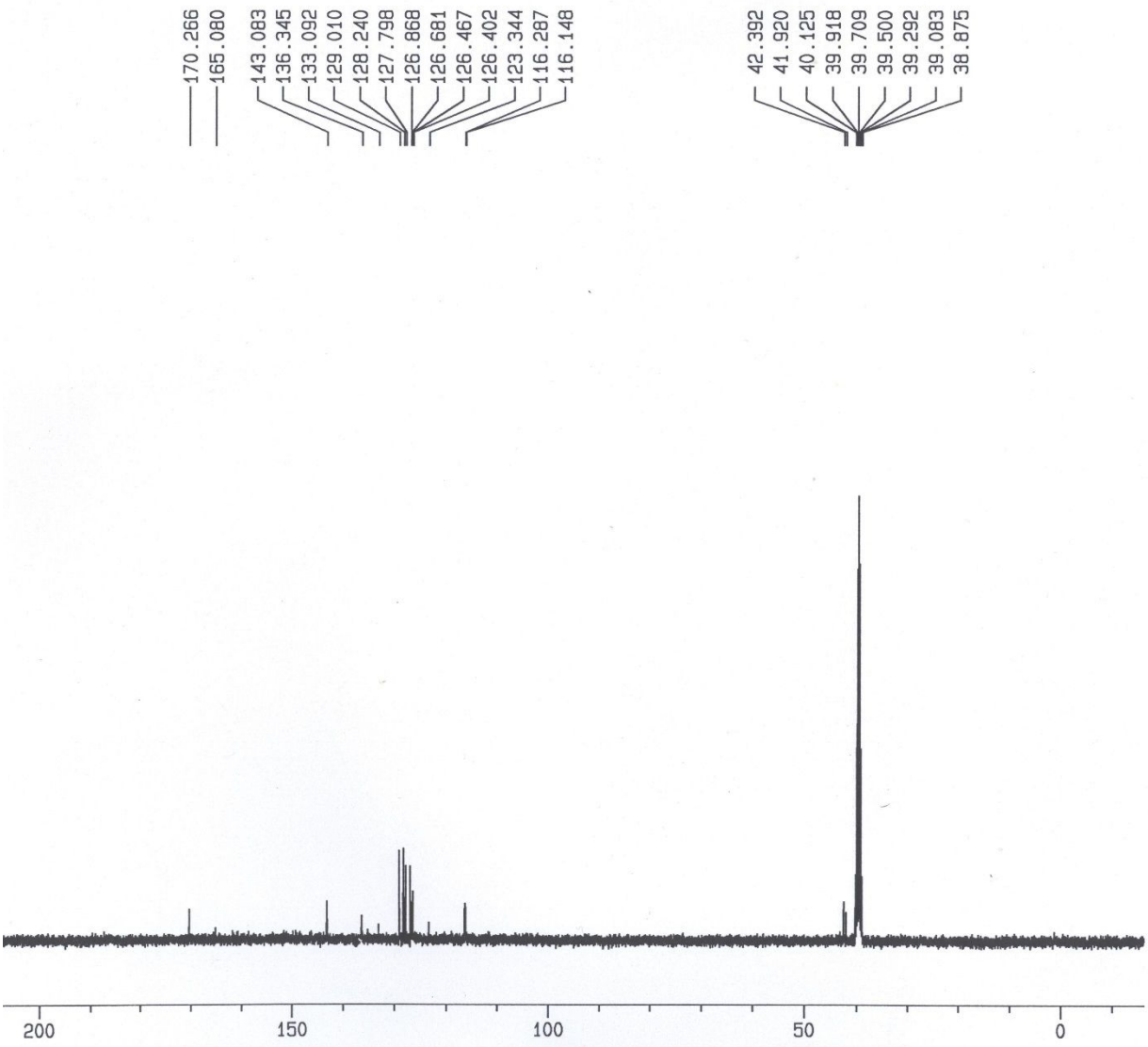
10
8
6
4
2
0

^{13}CNMR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123.344
116.287
116.148
42.392
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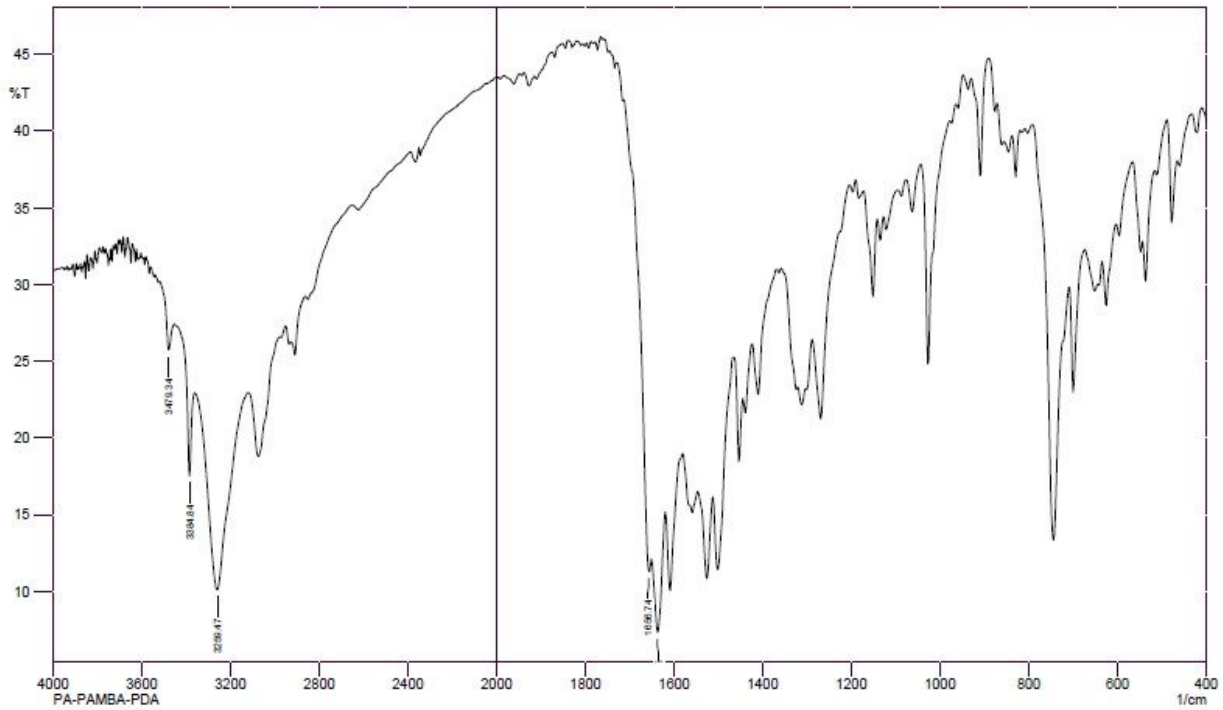
0

IR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1800
1600
1400
1200
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800
600
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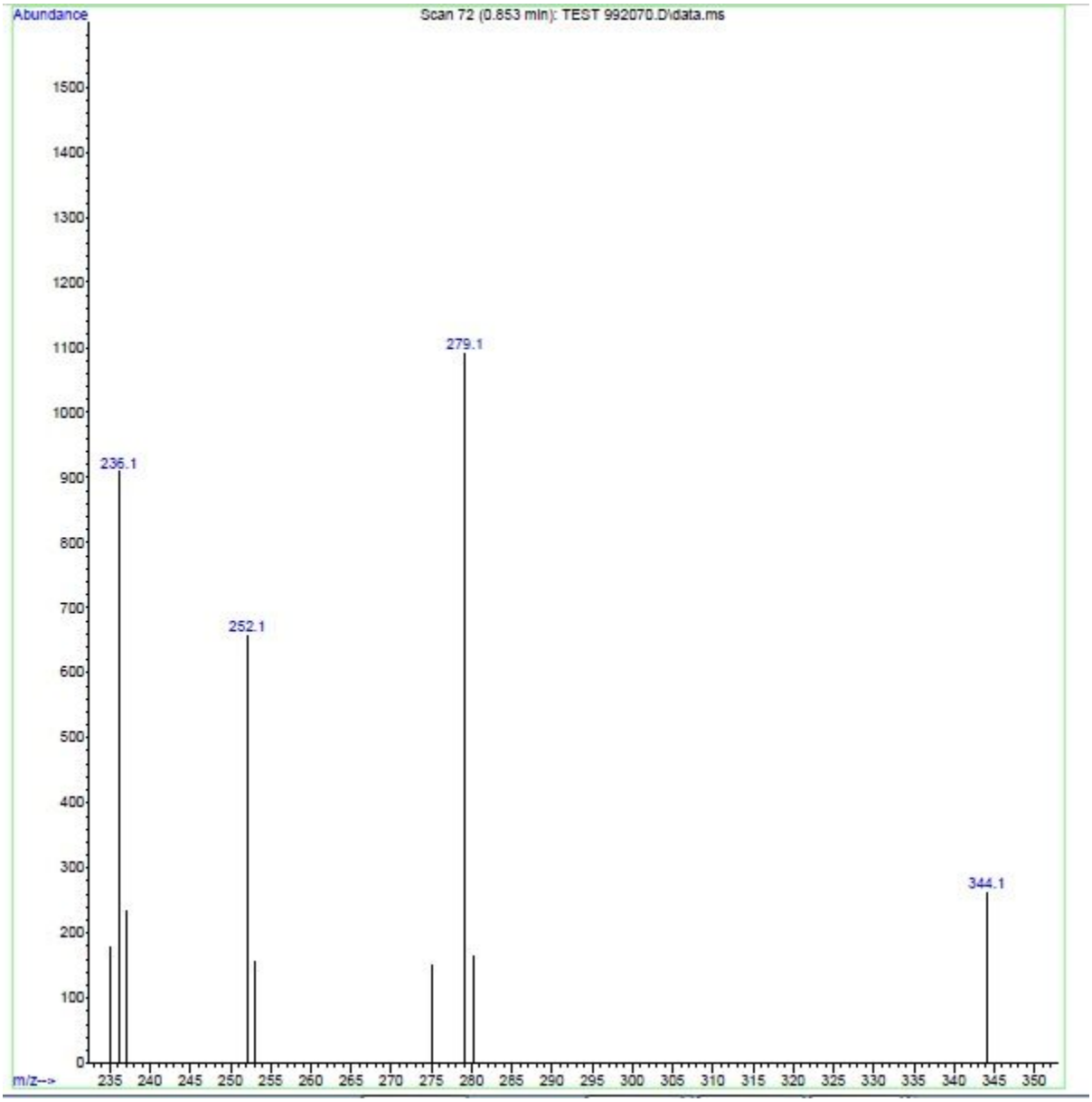
PA-PAMBA-PDA
1/cm

Mass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1200
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900
800
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400
300
200
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0
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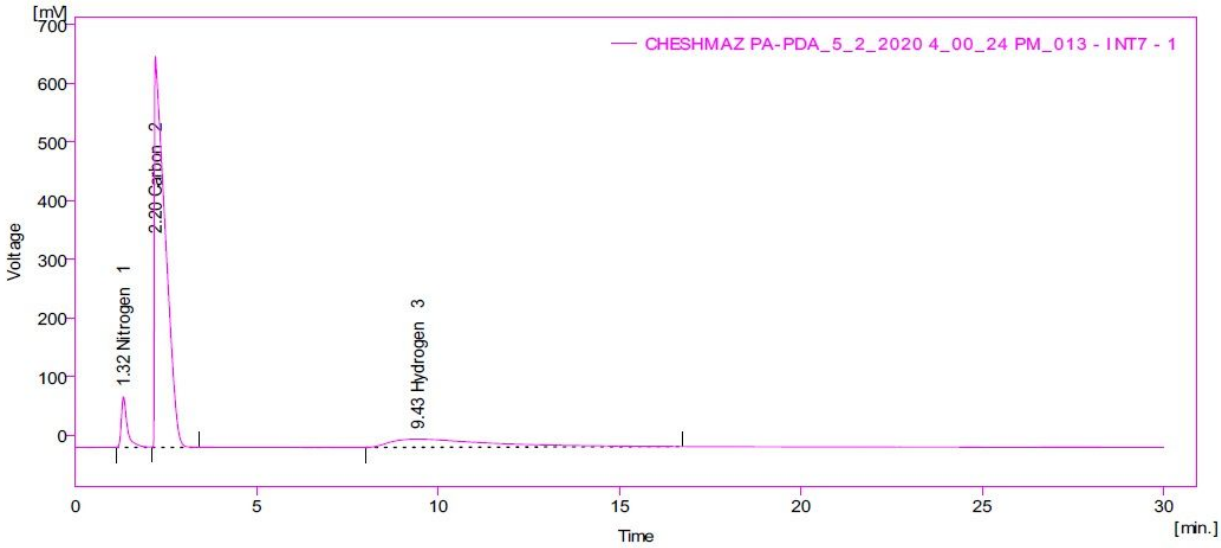
236.1
252.1
344.1
m/z-->
235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350

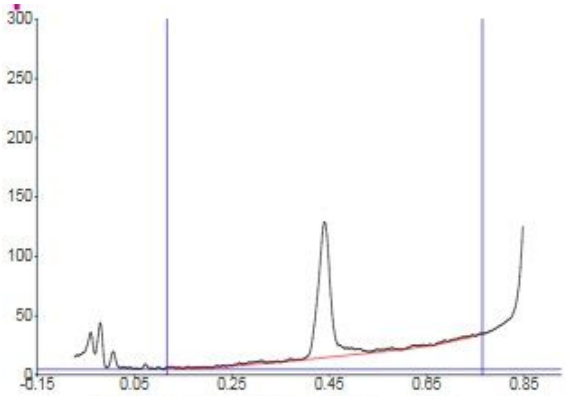
CHN

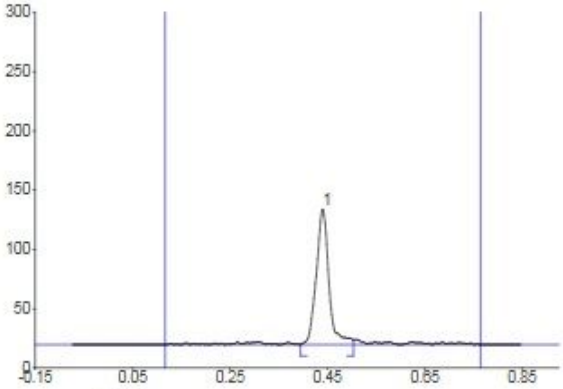


Result Table (ESTD - CHESHMAZ PA-PDA_5_2_2020 4_00_24 PM_013 - INT7 - 1)

	Reten. Time [min]	Response	Weight [mg]	Weight [%]	Peak Type	Element Name	Carbon Response Ratio
1	1.320	992.894	0.273	11.19	Ordnr	Nitrogen	0.073
2	2.200	13615.550	1.792	73.36	Ordnr	Carbon	1.000
3	9.433	2730.009	0.129	5.28	Ordnr	Hydrogen	0.201
	Total		2.443	89.84			

HPTLC

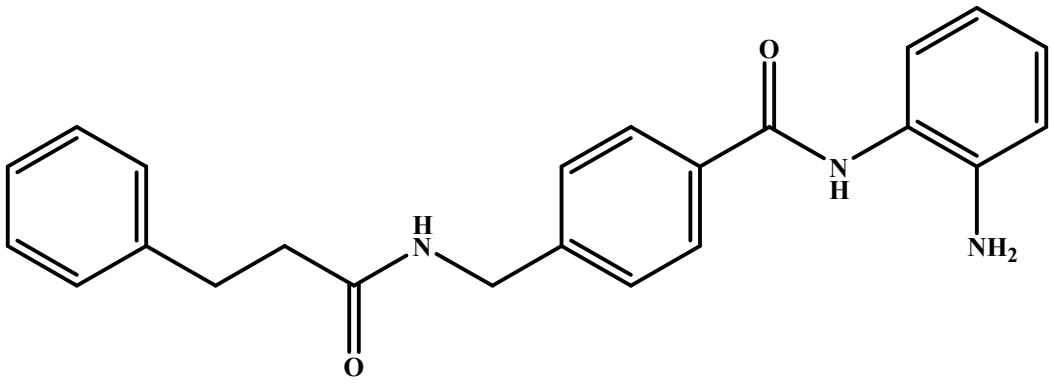




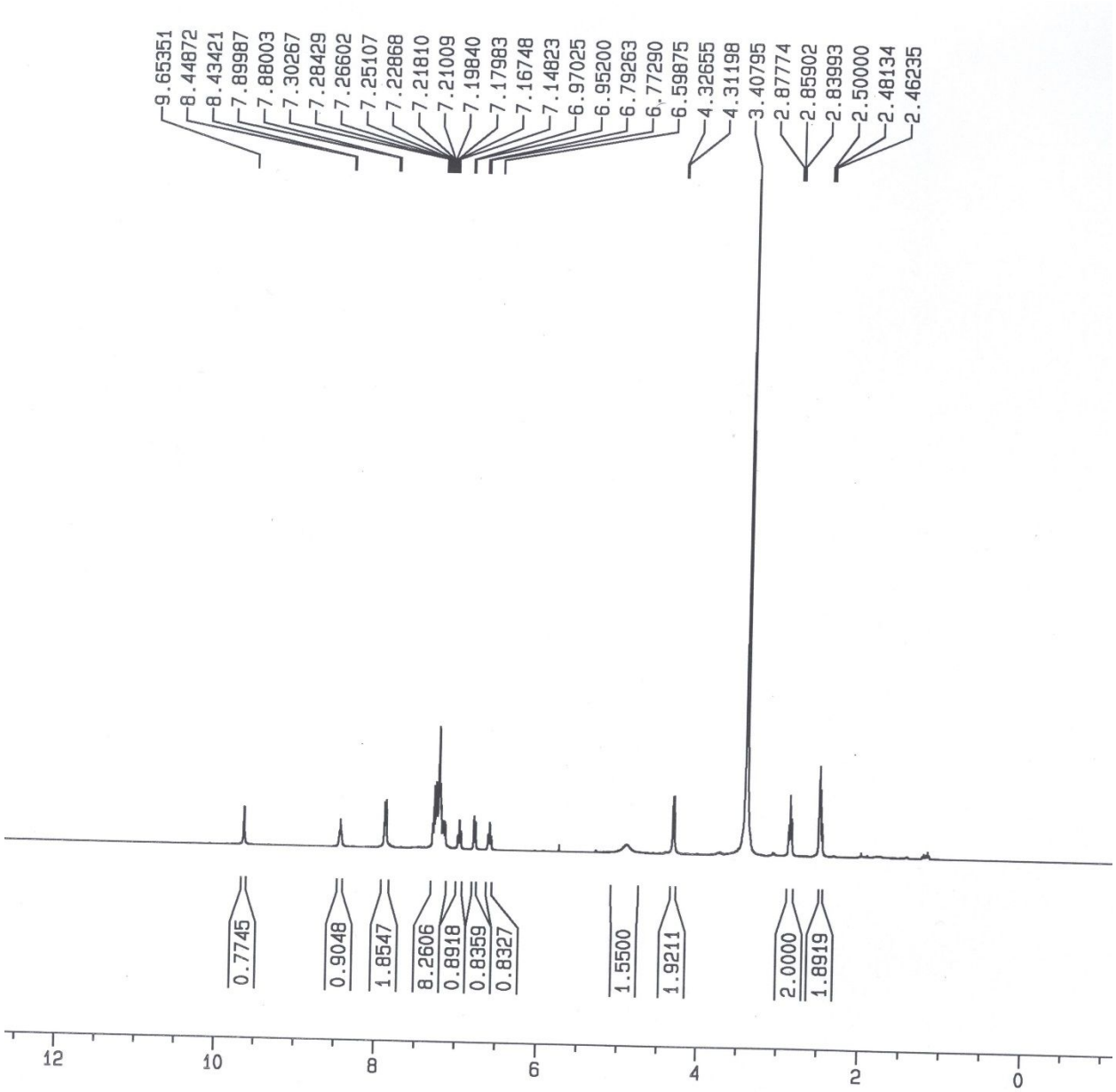
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.39	0.2	0.44	114.4	100.00	0.50	4.1	2245.0	100.00	unknown *

3b: N-(2-Amino-phenyl)-4-[(3-phenyl-propionylamino)-methyl]-benzamide

Yellow powder; yield: 77%; m.p: 161-164 ˚C (decompose); IR (KBr): 3467, 3380, 3255, 1647, 1629 cm^{-1}; ^{1}HNMR (500 MHz, DMSO-d_6) d (ppm): 2.46-2.50 (t, J=9.41, 2H, CH_2), 2.83-2.87 (t, J=9.45, 2H, CH_2), 4.31-4.32 (d, J=7.28, 2H, CH_2), 4.90 (s, 2H, NH_2), 6.58-6.61 (t, J=9.28, 1H-aromatic, CH), 6.77-6.79 (d, J=9.86, 1H-aromatic, CH), 6.95-6.98 (t, J= 9.83, 1H-aromatic, CH), 7.14-7.30 (m, 8H-aromatic, CH), 7.90-7.92 (d, J=9.92, 2H-aromatic, CH), 8.43-8.46 (t, J=7.305, 1H, NH), 9.65 (s, 1H, NH); ; ^{13}CNMR (500 MHz, DMSO-d_6): 31.280, 37.0075, 41.7557, 116.210, 116.352, 123.385, 125.995, 126.555, 126.779, 126.848, 127.095, 127.805, 128.353, 128.373, 133.017, 141.260, 143.194, 165.180, 171.254 ; Anal Calcd for $C_{23}H_{23}N_3O_2$: C, 73.97%; H, 6.21%; N, 11.25%. Found: C, 73.89 %; H, 5.92%; N, 10.33%; HRMS (m/z): Calcd: $[M+H]^+$: 373.18, found: 373.3; Purity: 100% (by HPTLC).



^{1}HNMR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0.9048
1.8547
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0.8918
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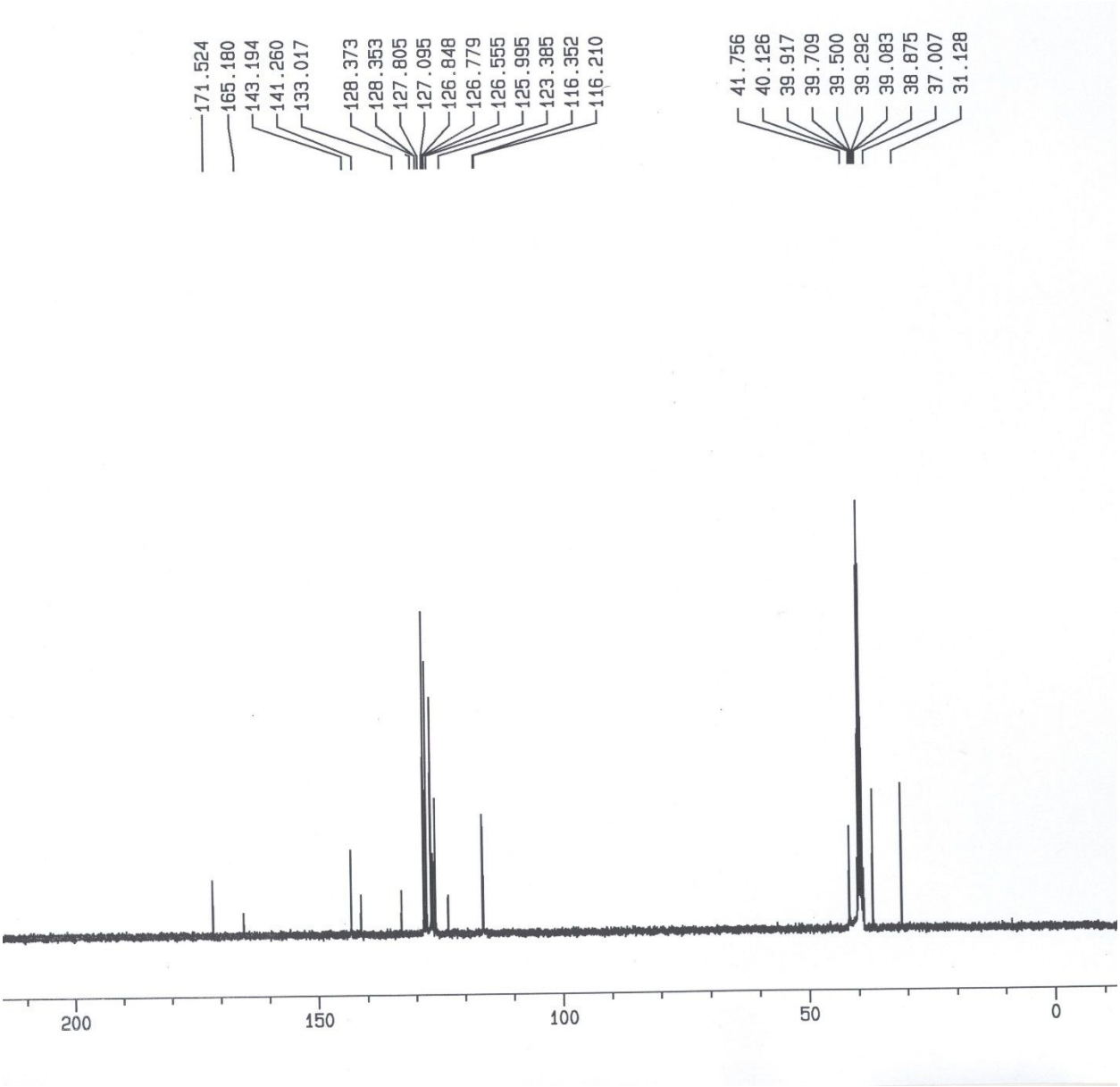
^{13}CNMR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116.352
116.210
41.756
40.126
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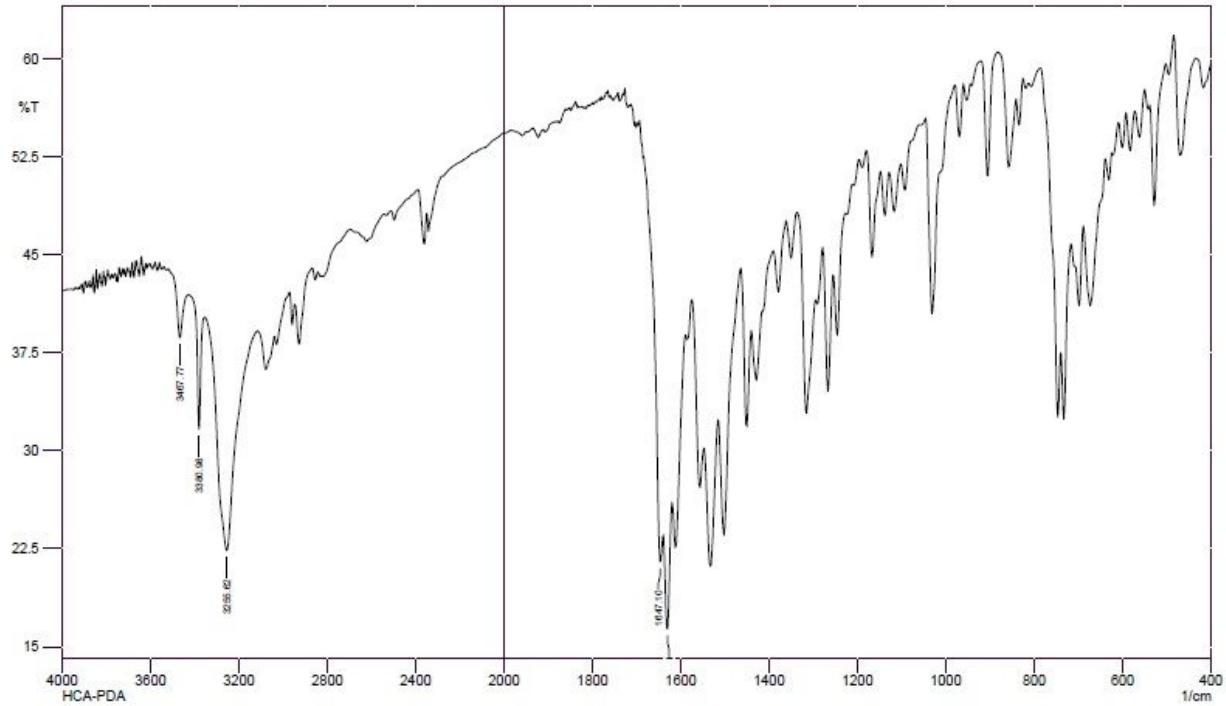
38.875
37.007
31.128
200
150
100
50
0

IR



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HCA-PDA

No. of Scans;
Resolution;
Apodization;

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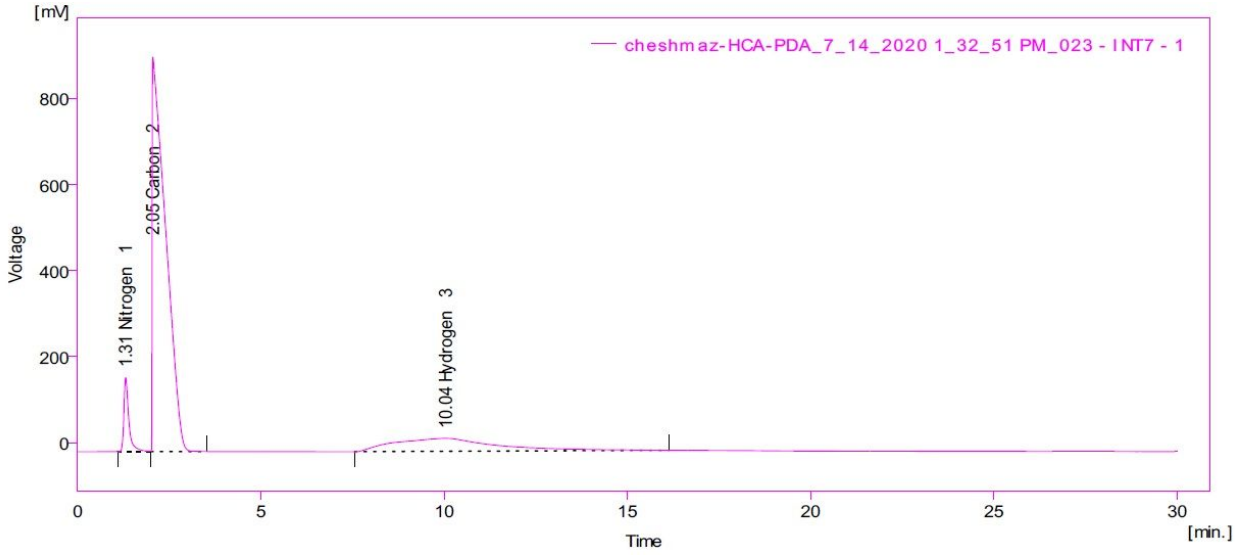
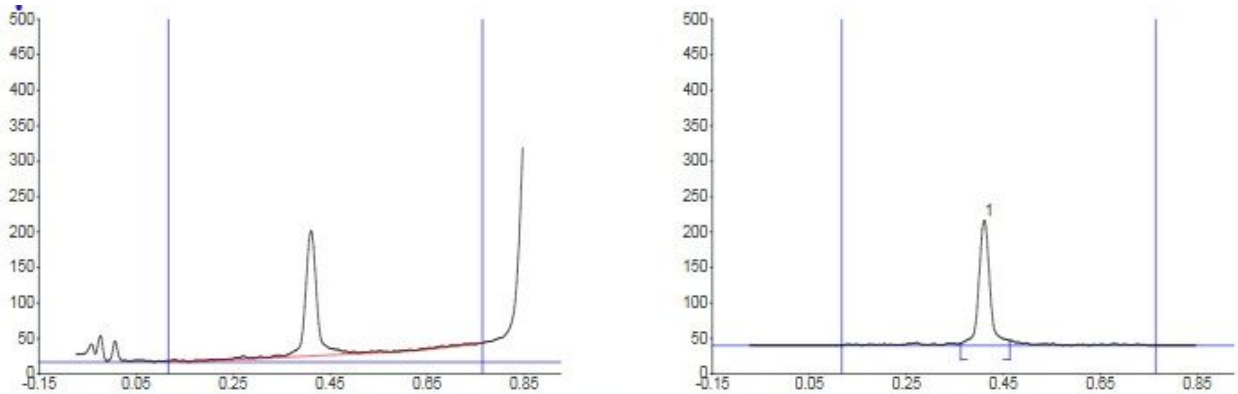
CHN



Result Table (ESTD - cheshmaz-HCA-PDA_7_14_2020 1_32_51 PM_023 - INT7 - 1)

	Reten. Time [min]	Response	Weight [mg]	Weight [%]	Peak Type	Element Name	Carbon Response Ratio
1	1.307	1617.317	0.472	10.33	Ordnr	Nitrogen	0.070
2	2.050	23254.839	3.377	73.89	Ordnr	Carbon	1.000
3	10.040	5880.133	0.271	5.92	Ordnr	Hydrogen	0.253
	Total		4.570	90.13			

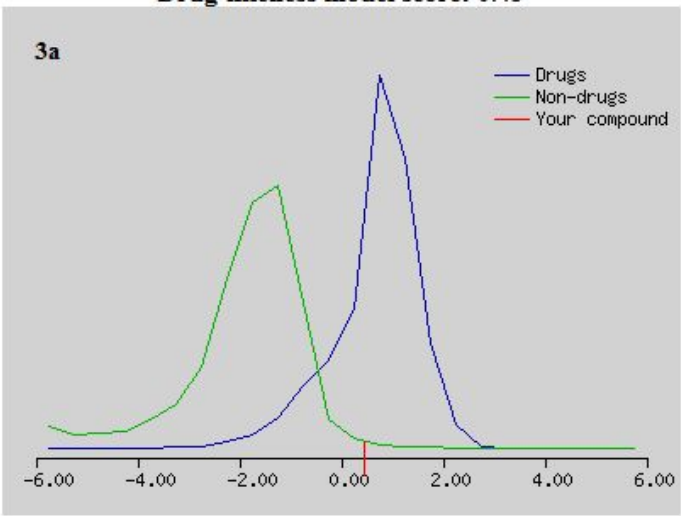
HPTLC



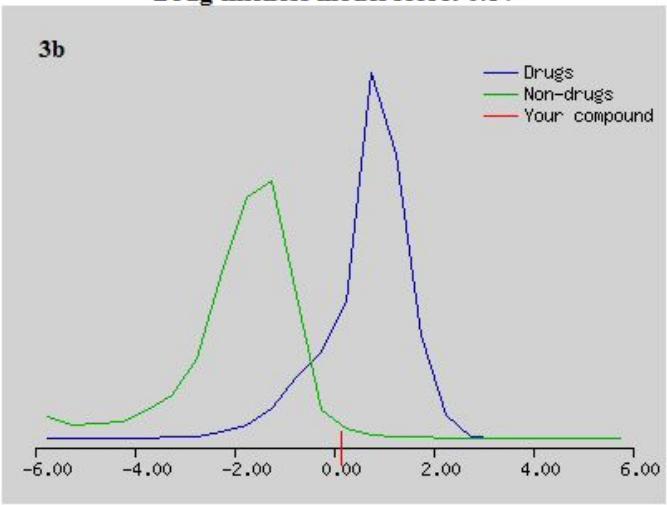
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.36	3.3	0.41	176.7	100.00	0.46	7.1	3109.9	100.00	unknown *

Druglikeness score of 3a, 3b and Ms-275:

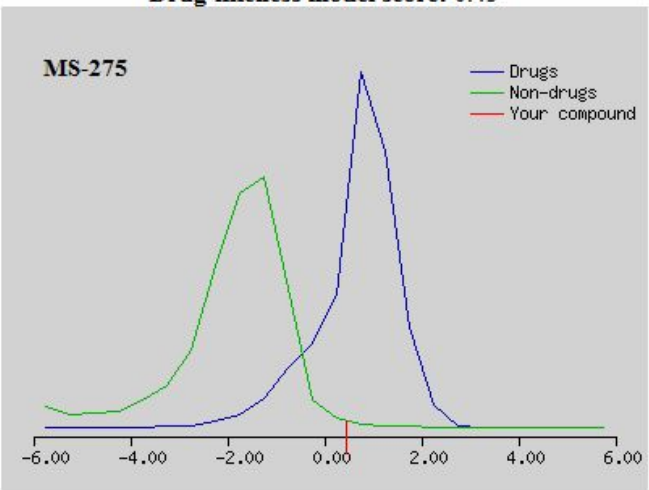
Drug-likeness model score: 0.45



Drug-likeness model score: 0.14



Drug-likeness model score: 0.45



Docking of 3a, 3b and MS-275 on HDAC1-3

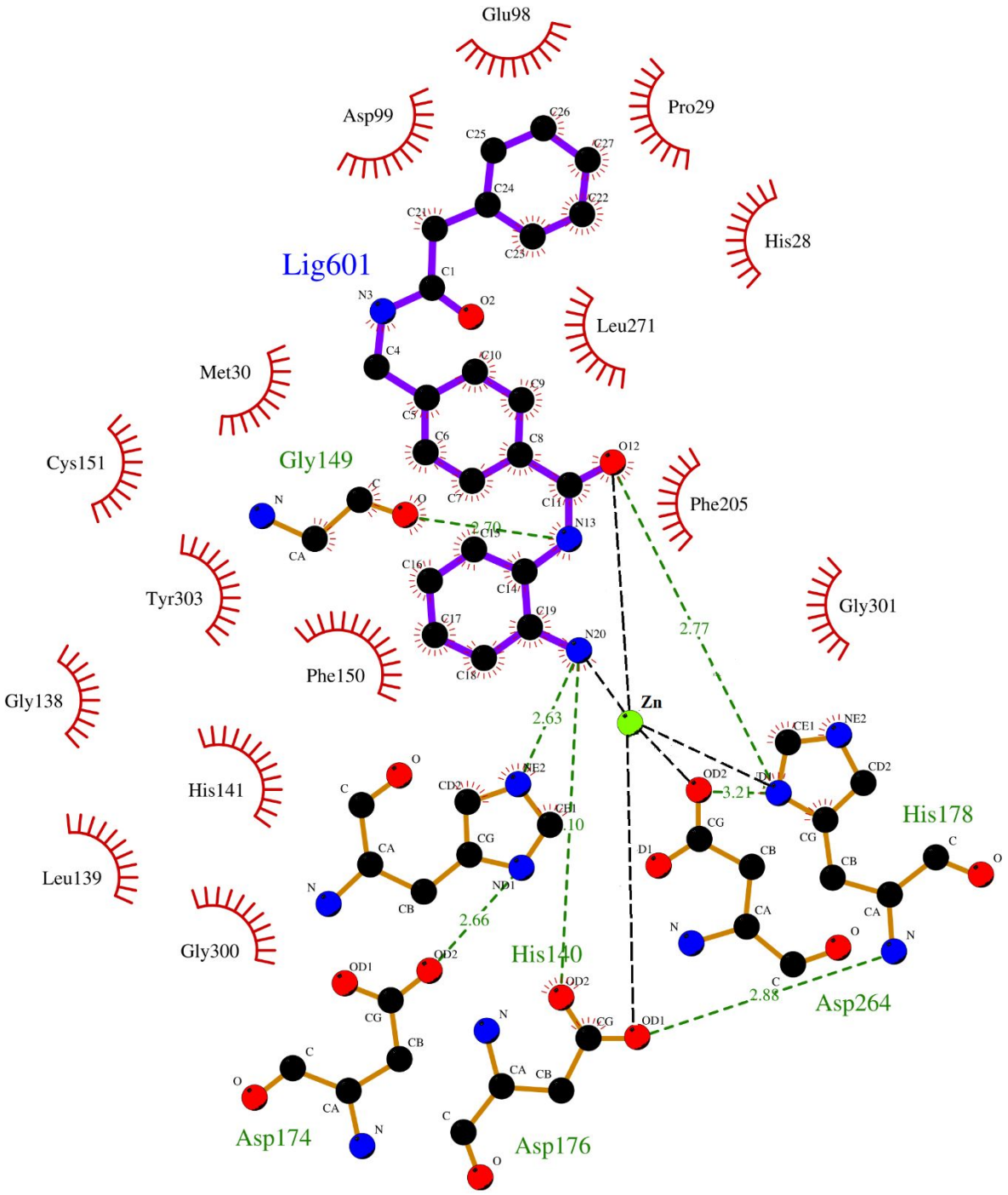


Figure S1. 2D diagram of docking of 3a to HDAC1.

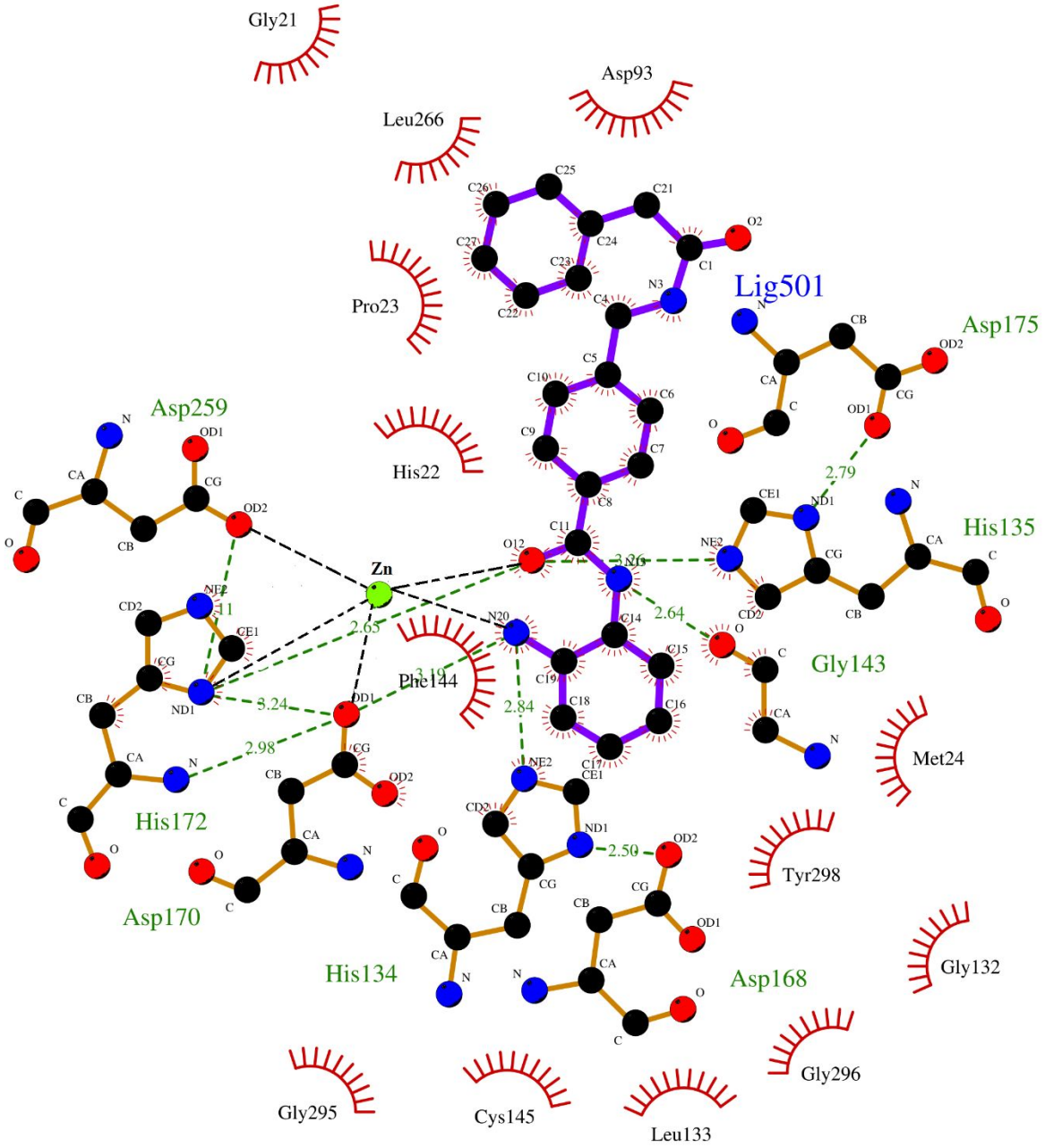


Figure S2. 2D diagram of docking of 3a to HDAC3.

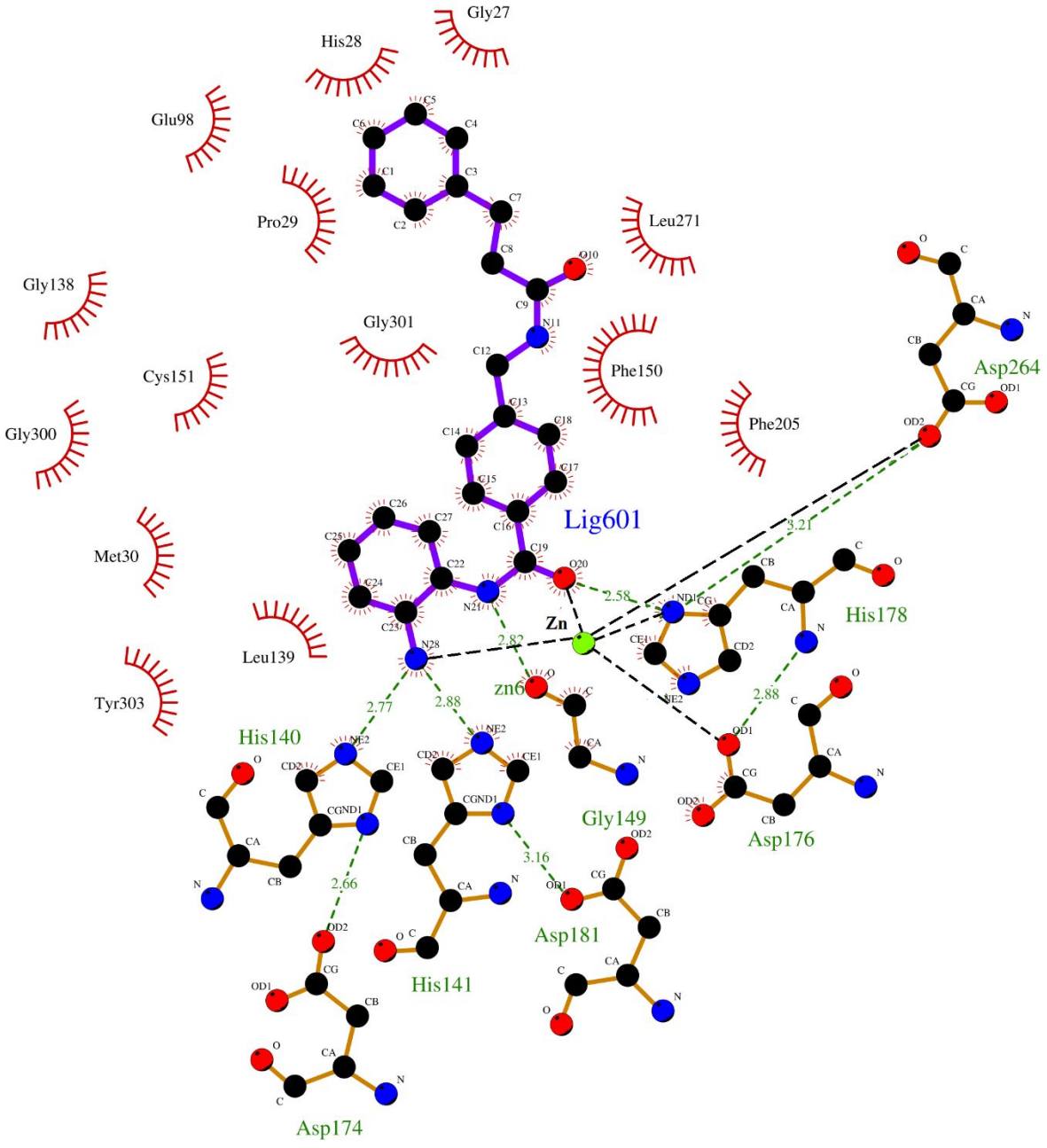


Figure S3. 2D diagram of docking of 3b to HDAC1.

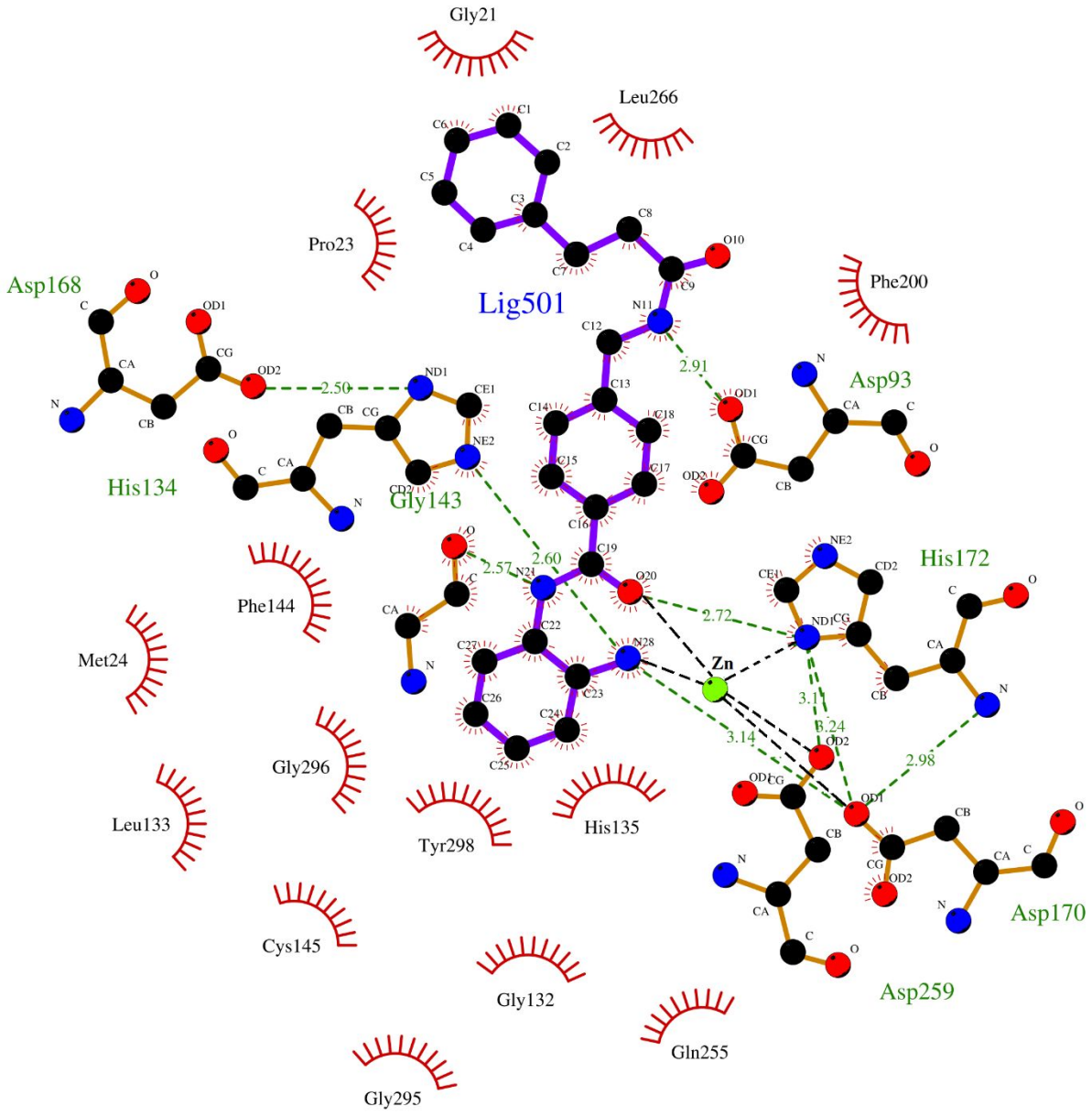


Figure S4. 2D diagram of docking of 3b to HDAC3.

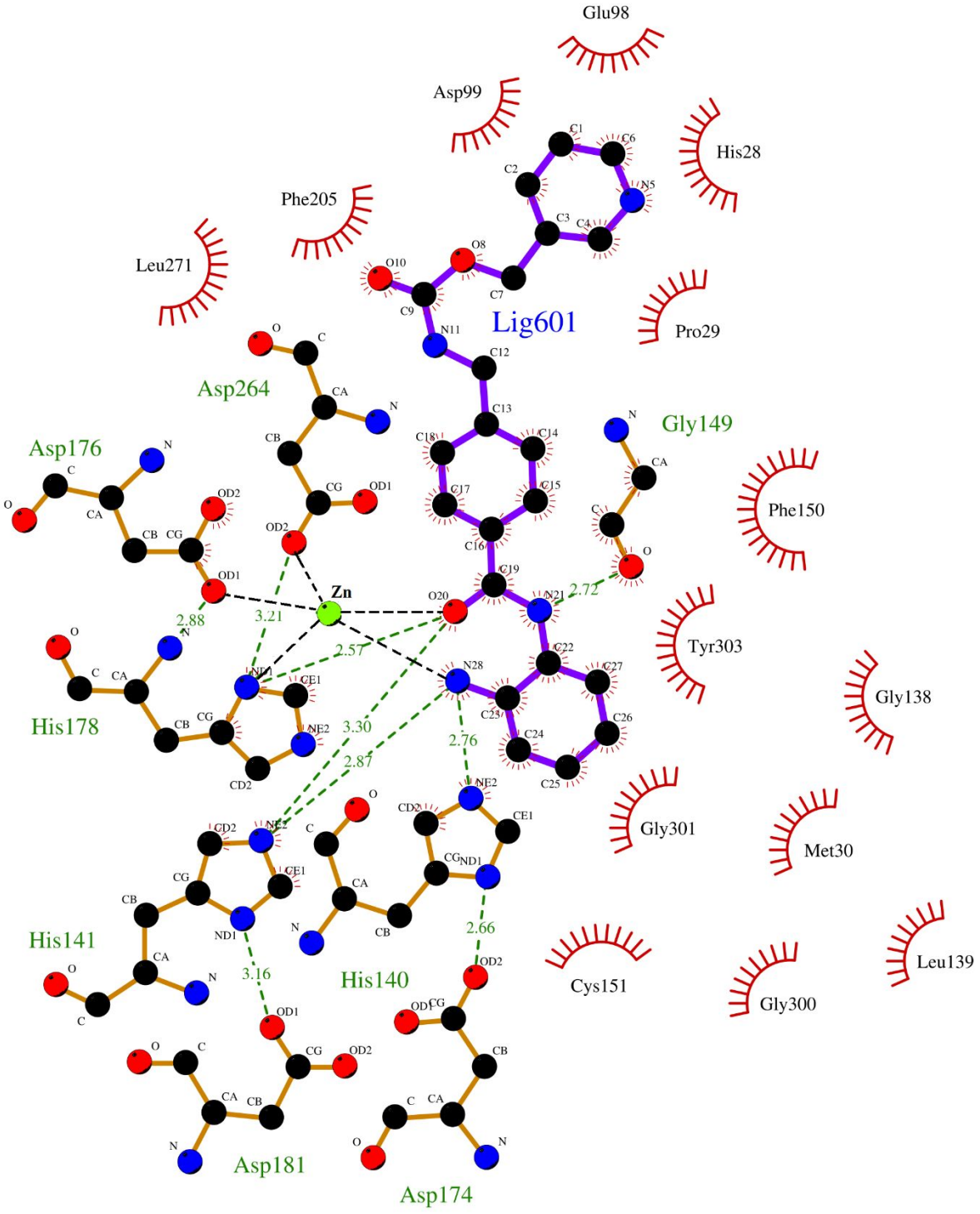


Figure S5. 2D diagram of docking of MS_275 to HDAC1.

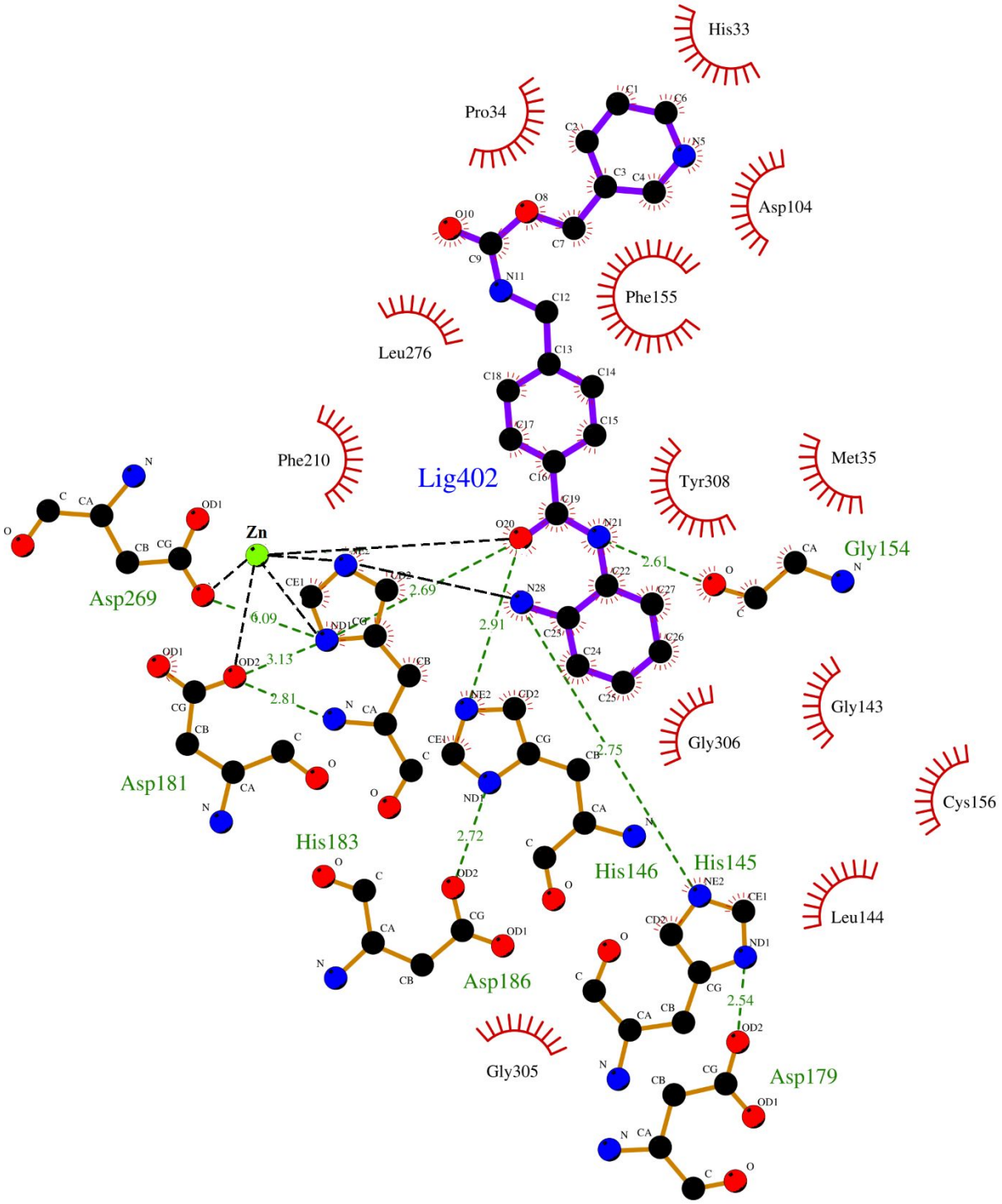


Figure S6. 2D diagram of docking of MS_275 to HDAC2.

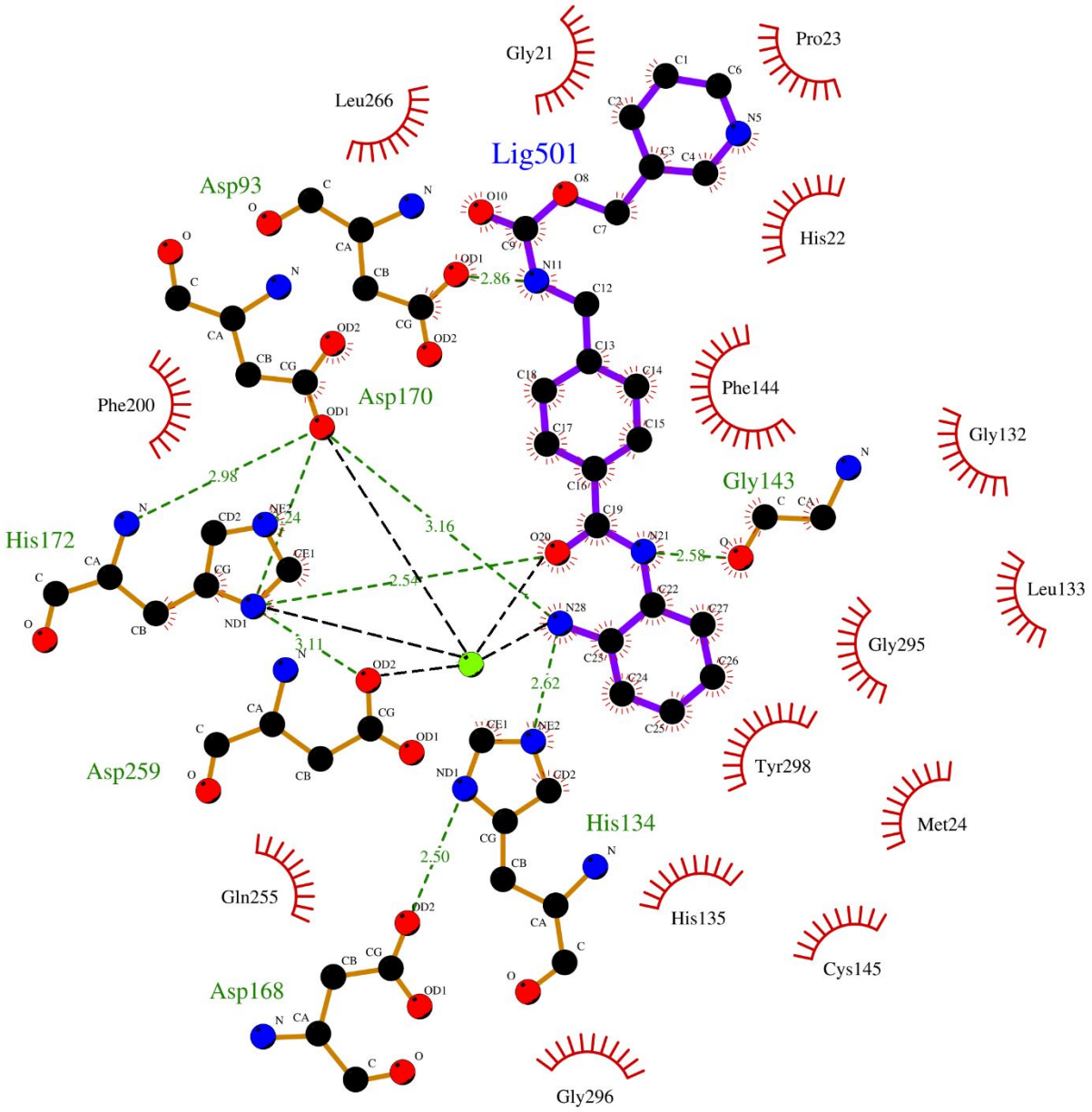


Figure S7. 2D diagram of docking of MS_275 to HDAC3.

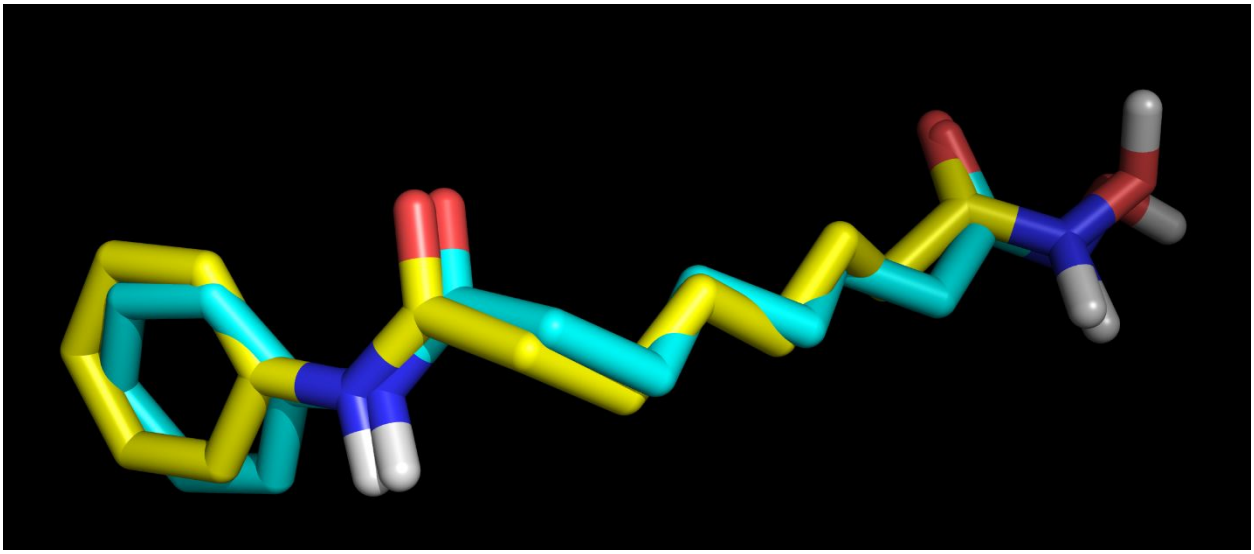


Figure S8. Superimposition of crystalized and docked SAHA.