Supplementary Material

Inhibition of Acetylcholinesterase by Novel Lupinine Derivatives

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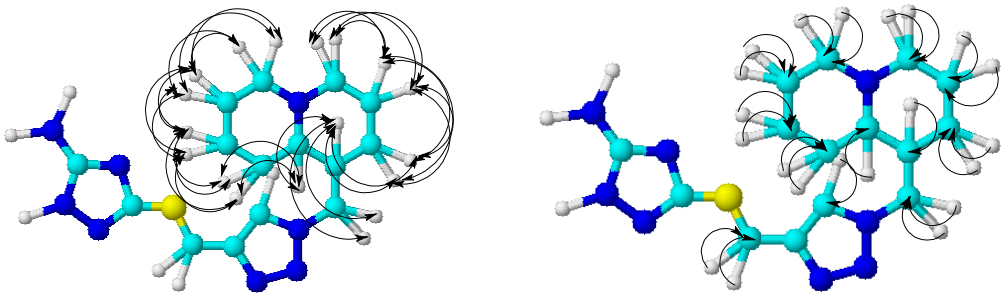


COSY (a) HMQC (b)

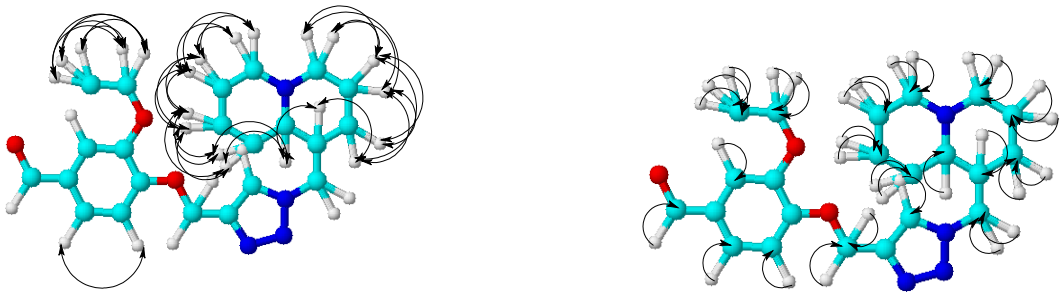
Supplementary Figure S1. Scheme of correlations in the COSY (a) and HMQC (b) spectra of compound 5.

^{1}H-^{1}H COSY spectra of the compound showed the spin-spin couplings through three bonds of the neighboring methine proton of the lupinine cycle with the methylene proton H^{5}-H^{11} (2.05, 4.39 and 4.39, 2.05) and the methylene-methylene protons of the lupinine cycles of $H^{2ax,10ax}$-$H^{2eq,10eq}$ (1.88, 2.73 and 2.73, 1.88) ppm. The heteronuclear couplings of protons with carbon atoms through a single bond were established by ^{1}H-^{13}C HMQC spectroscopy for the following pairs in the compound: H^{3ax}-C^{3} (1.27, 20.64), H^{4ax}-C^{4} (1.39, 29.06), $H^{2ax,10ax}$-$C^{2,10}$ (1.83, 56.82), H^{6}-C^{6} (2.02, 64.17), H^{5}-C^{5} (2.04, 39.27), $H^{2eq,10eq}$-$C^{2,10}$ (2.75, 57.36), H^{11}-C^{11} (4.40, 48.76), H^{17}-C^{17} (4.18, 26.73) and H^{16}-C^{16} (7.89, 124.17) ppm. The heteronuclear couplings of protons with carbon atoms through two or more bonds were determined by ^{1}H-^{13}C HMBC spectroscopy for the following pairs in the compound: H^{17}-C^{16} (4.17, 124.06), H^{17}-C^{15} (4.17, 144.44), H^{17}-C^{19} (4.17, 156.20); H^{11}-C^{21} (7.47, 125.65), H^{21}-C^{19} (7.47, 148.52); H^{16}-C^{16} (8.23, 125.97), H^{16}-C^{5} (4.40, 38.88); H^{16}-C^{16} (7.84, 124.32) and H^{16}-C^{15} (7.84, 144.44) ppm.

COSY (a) HMQC (b)

Supplementary Figure S2. Scheme of correlations in the COSY (a) and HMQC (b) spectra of compound 6.

^{1}H-^{1}H COSY spectra of the compound demonstrated the spin-spin couplings through three bonds of the neighboring methine-methylene groups of H^{5}-H^{11} (2.14, 4.50 and 4.50, 2.14) ppm. The heteronuclear couplings of protons with carbon atoms through a single bond were established by ^{1}H-^{13}C HMQC spectroscopy for the following pairs in the compound: H^{22}-C^{22} (1.02, 16.99), H^{21}-C^{21} (1.51, 23.15), H^{4ax}-C^{4} (1.19, 26.20), H^{4eq}-C^{4} (1.30, 26.30), H^{7ax}-C^{7} (1.45, 29.15), H^{7eq}-C^{7} (1.55, 29.17), $H^{2ax,10ax}$-$C^{2,10}$ (1.91, 57.53), $H^{2eq,10eq}$-$C^{2,10}$ (2.76, 57.53), H^{6}-C^{6} (2.02, 64.40), H^{5}-C^{5} (2.14, 39.43), H^{19}-C^{19} (3.88 and 4.05, 74.36 and 74.54), H^{11}-C^{11} (4.48, 48.48) and H^{16}-C^{16} (7.45, 121.95) ppm. The heteronuclear couplings of protons with carbon atoms through two or more bonds were defined by ^{1}H-^{13}C HMBC spectroscopy for the following pairs in the compound: H^{22}-C^{19} (1.06, 73.35); H^{21}-C^{17} (1.38, 73.58) and H^{21}-C^{15} (1.38, 152.77) ppm.



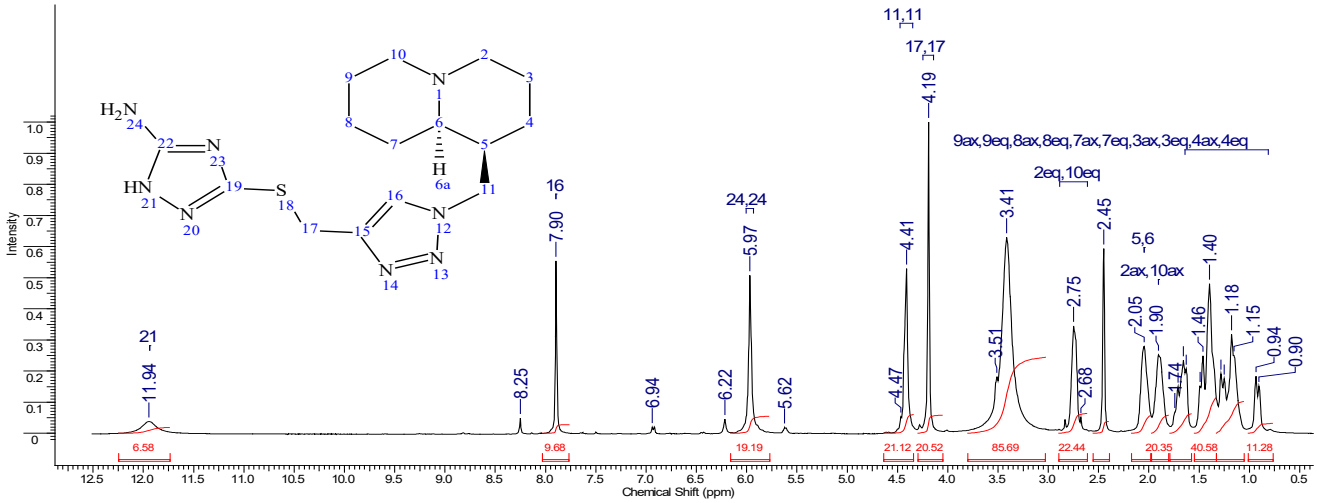
COSY (a) HMQC (b)

Supplementary Figure S3. Scheme of correlations in the COSY (a) and HMQC (b) spectra of compound 7.

^{1}H-^{1}H COSY spectra of the compound showed the spin-spin couplings through three bonds of the neighboring methyl and methylene groups of H^{27}-H^{26} with the cross peak at 1.25, 4.03 and 4.03, 1.25 of the ethoxy fragment of the molecule, methine proton of the lupinine cycle with methylene proton H^{5}-H^{11} (2.07, 4.48 and 4.48, 2.07), methylene-methylene protons of lupinine cycles of $H^{2ax,10ax}$-$H^{2eq,10eq}$ (1.79, 2.68 and 2.68, 1.79) and the aromatic methine protons of H^{24}-H^{23} (7.32, 7.48 and 7.48, 7.32) ppm. The heteronuclear couplings of protons with carbon atoms through a single bond were established by ^{1}H-^{13}C HMQC spectroscopy for the following pairs in the compound: H^{27}-C^{27} (1.25, 15.13), H^{3ax}-C^{3} (1.26, 20.79), H^{4ax}-C^{4} (1.28, 25.98), H^{4eq}-C^{4} (1.48, 25.98), H^{7eq}-C^{7} (1.38, 29.45), $H^{2ax,10ax}$-$C^{2,10}$ (1.78, 57.70), H^{6}-C^{6} (1.92, 64.67), H^{3eq}-C^{3} (1.70, 20.60), H^{5}-C^{5} (2.07, 39.06), $H^{2eq,10eq}$-$C^{2,10}$ (2.68, 57.33), H^{11}-C^{11} (4.48, 48.47), H^{26}-C^{26} (4.01, 64.86), H^{17}-C^{17} (5.21, 62.60), H^{21}-C^{21} (7.32, 111.57), H^{24}-C^{24} (7.31, 113.46), H^{23}-C^{23} (7.46, 126.26) and H^{16}-C^{16} (8.23, 126.26) ppm. The heteronuclear couplings of protons with carbon atoms through two or more bonds were detected by ^{1}H-^{13}C HMBC spectroscopy for the following pairs in the compound: H^{26}-C^{27} (4.00, 15.48); H^{11}-C^{5} (4.49, 39.00); H^{17}-C^{16} (5.22, 125.65), H^{17}-C^{15} (5.22, 142.72), H^{17}-C^{20} (5.22, 153.35); H^{21}-C^{21} (7.47, 125.65), H^{21}-C^{19} (7.47, 148.52); H^{16}-C^{16} (8.23, 125.97), H^{16}-C^{15} (8.23, 142.72); H^{22}-C^{21} (9.77, 111.80); H^{28}-C^{28} (9.77, 192.33) and H^{28}-C^{22} (9.77, 130.80) ppm.

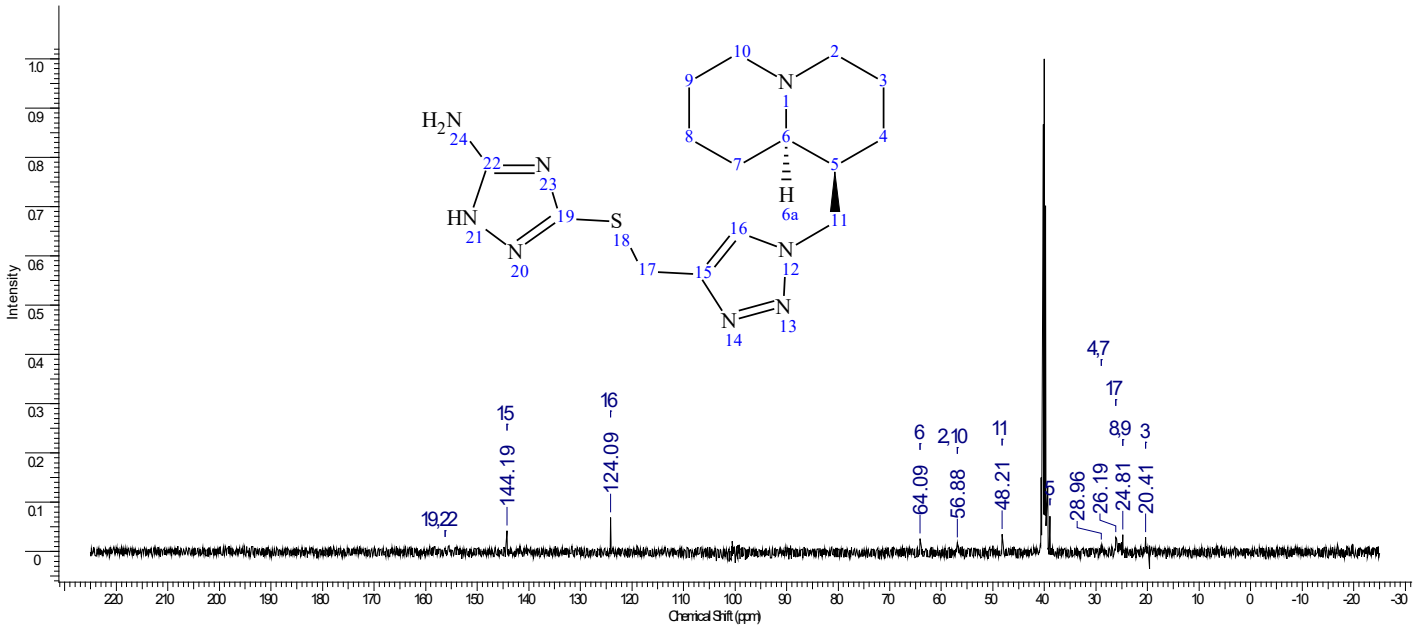
Supplementary Figure S4.1. ^{1}H NMR spectrum of 3-((1-(((1*S*,9a*R*)-octahydro-1*H*-quinolizine-1-yl)methyl)-1*H*-1,2,3-triazole-4-yl)methylthio)-1*H*-1,2,4-triazole-5-amine (5).

^{1}H NMR (399.78 MHz, DMSO-d$_6$)

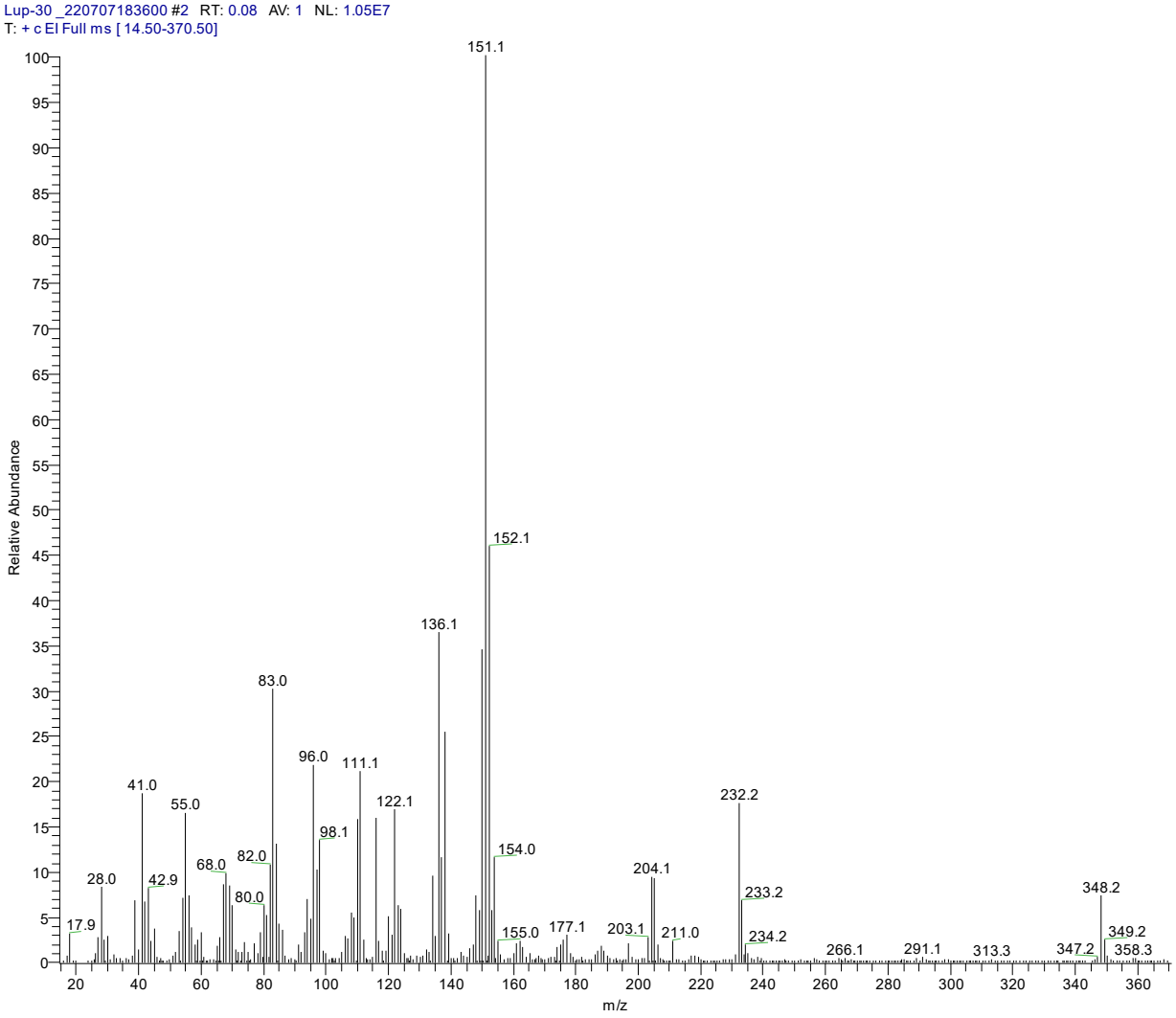


Supplementary Figure S4.2. ^{13}C NMR spectrum of 3-((1-(((1*S*,9a*R*)-octahydro-1*H*-quinolizine-1-yl)methyl)-1*H*-1,2,3-triazole-4-yl)methylthio)-1*H*-1,2,4-triazole-5-amine (5).

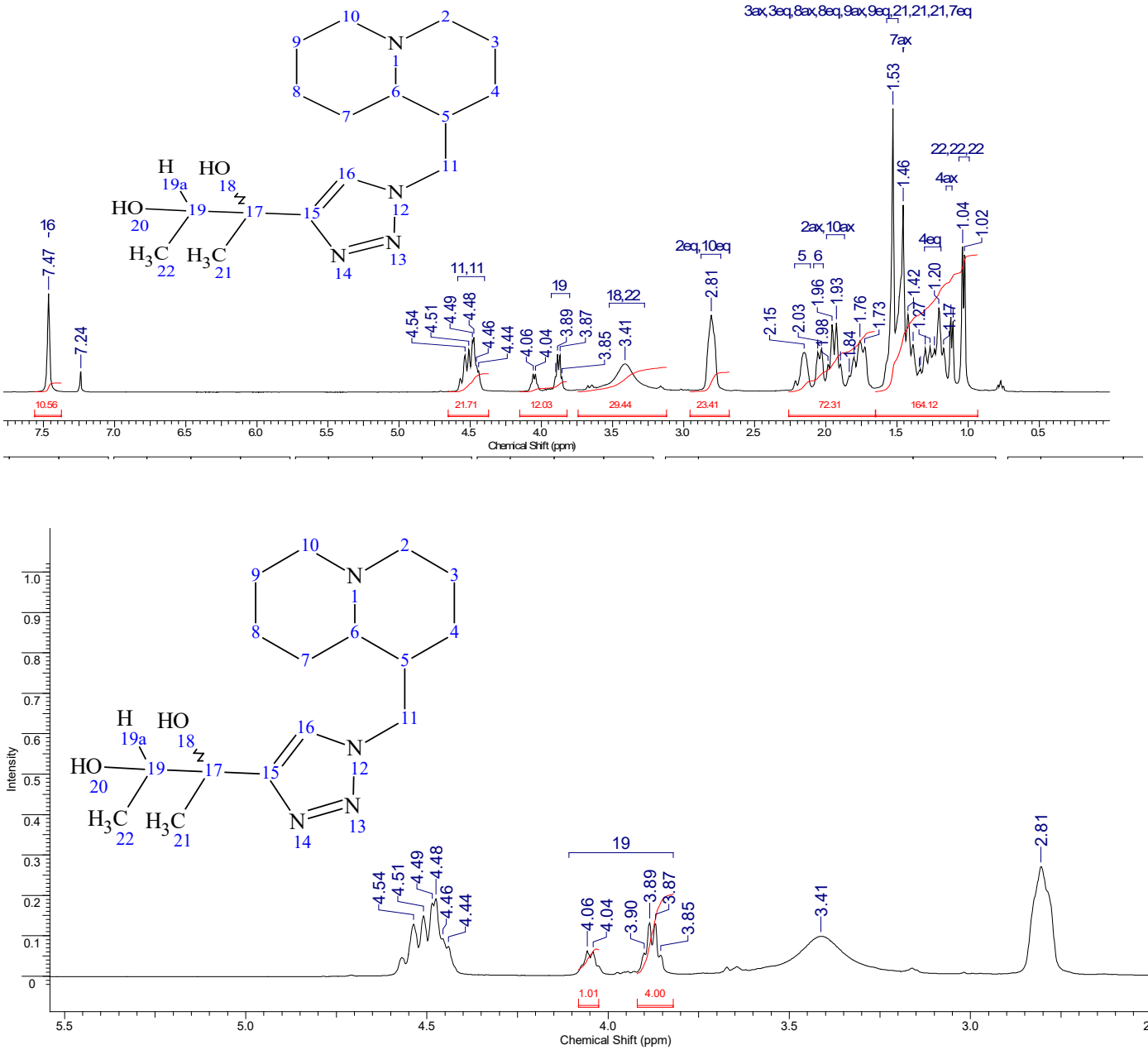
^{13}C NMR (100.53 MHz, DMSO-d$_6$)



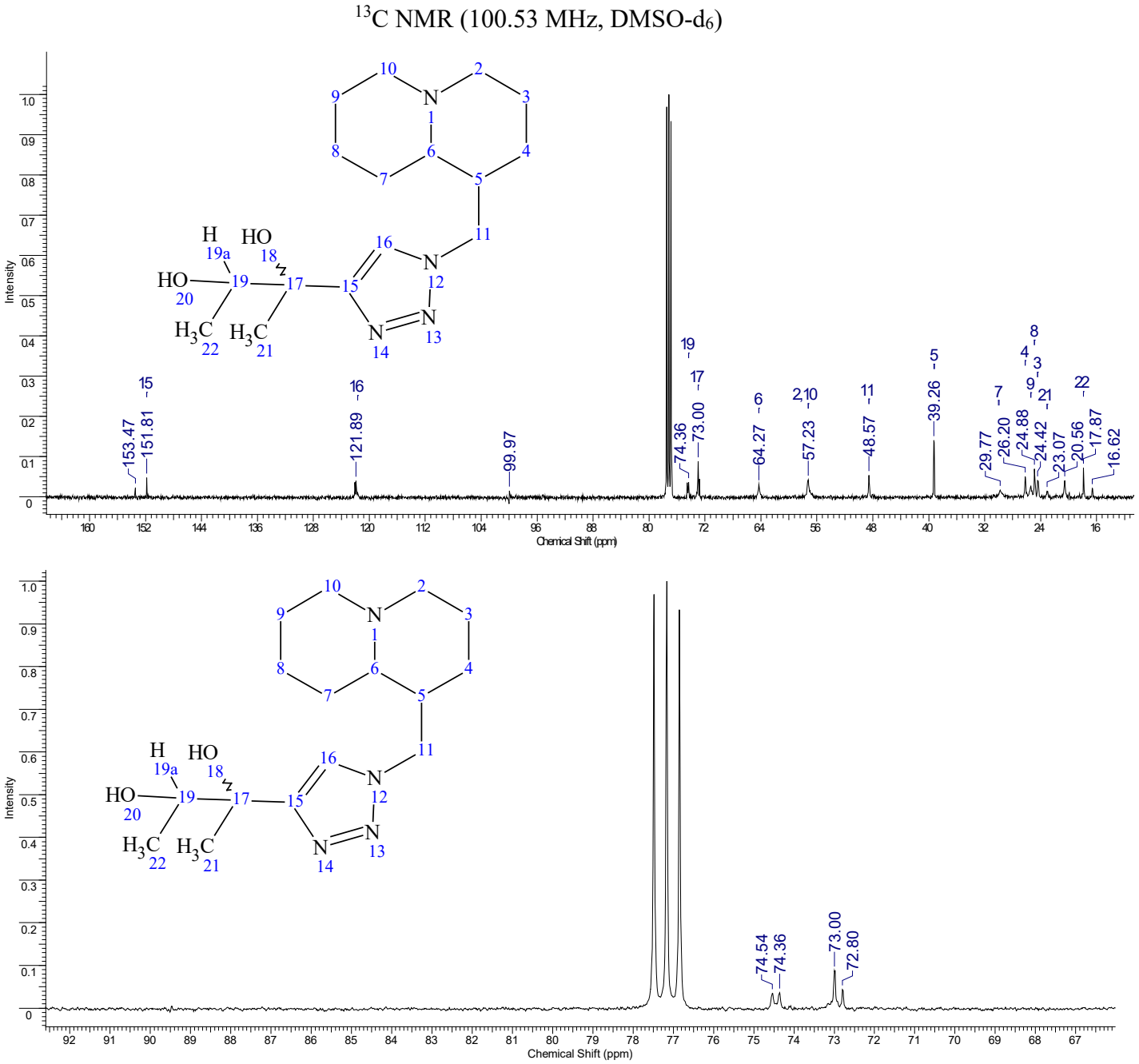
Supplementary Figure S4.3. The mass spectrum of 3-((1-(((1*S*,9a*R*)-octahydro-1*H*-quinolizine-1-yl)methyl)-1*H*-1,2,3-triazole-4-yl)methylthio)-1*H*-1,2,4-triazole-5-amine (5).



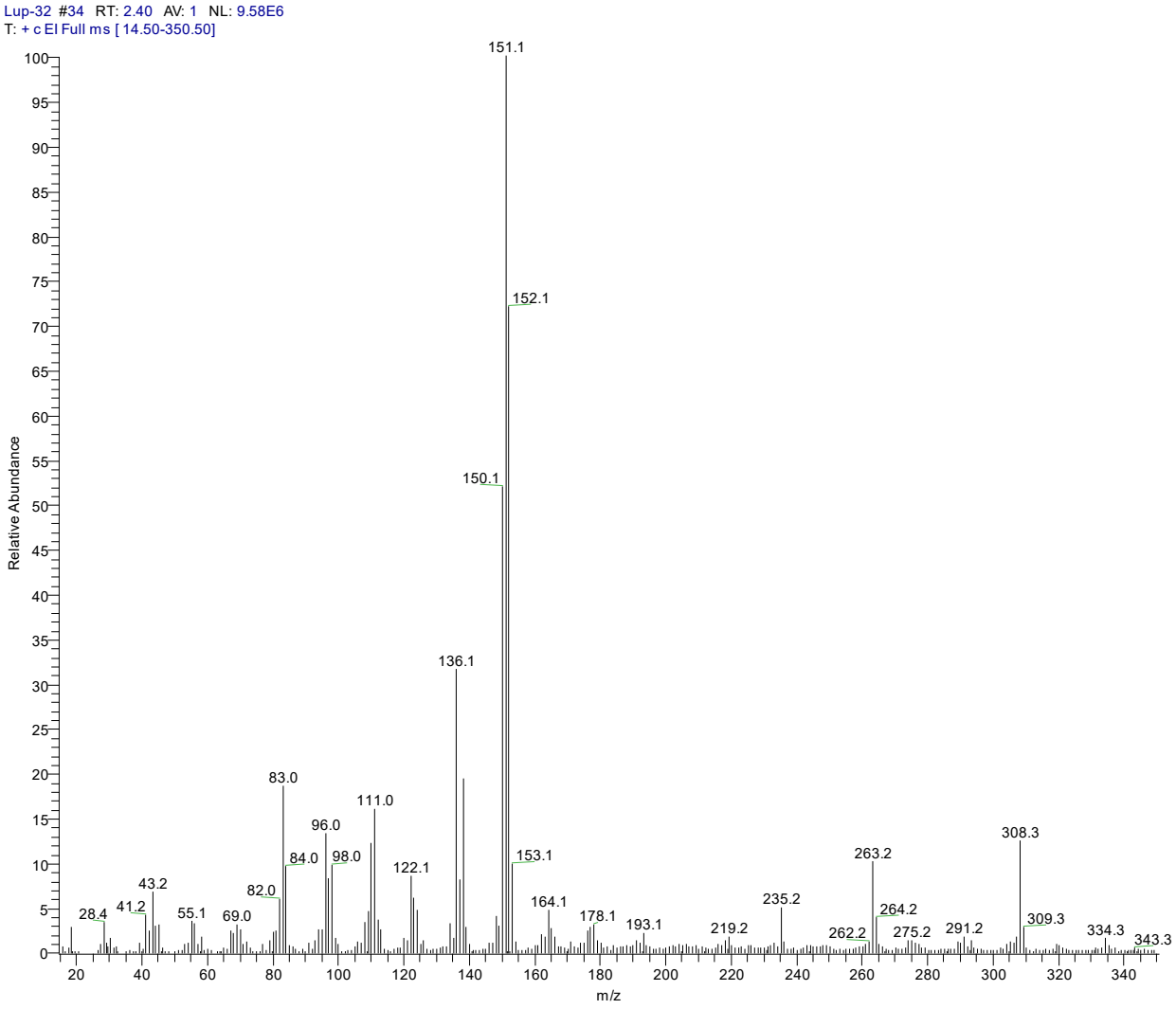
Supplementary Figure S5.1. ^{1}H NMR spectrum of (2*R*,*S*)-2-(1-(((1*S*,9a*R*)-octahydro-1*H*-quinolizine-1-yl)methyl)-1*H*-1,2,3-triazole-4-yl)butane-2,3-diol (6a,b).



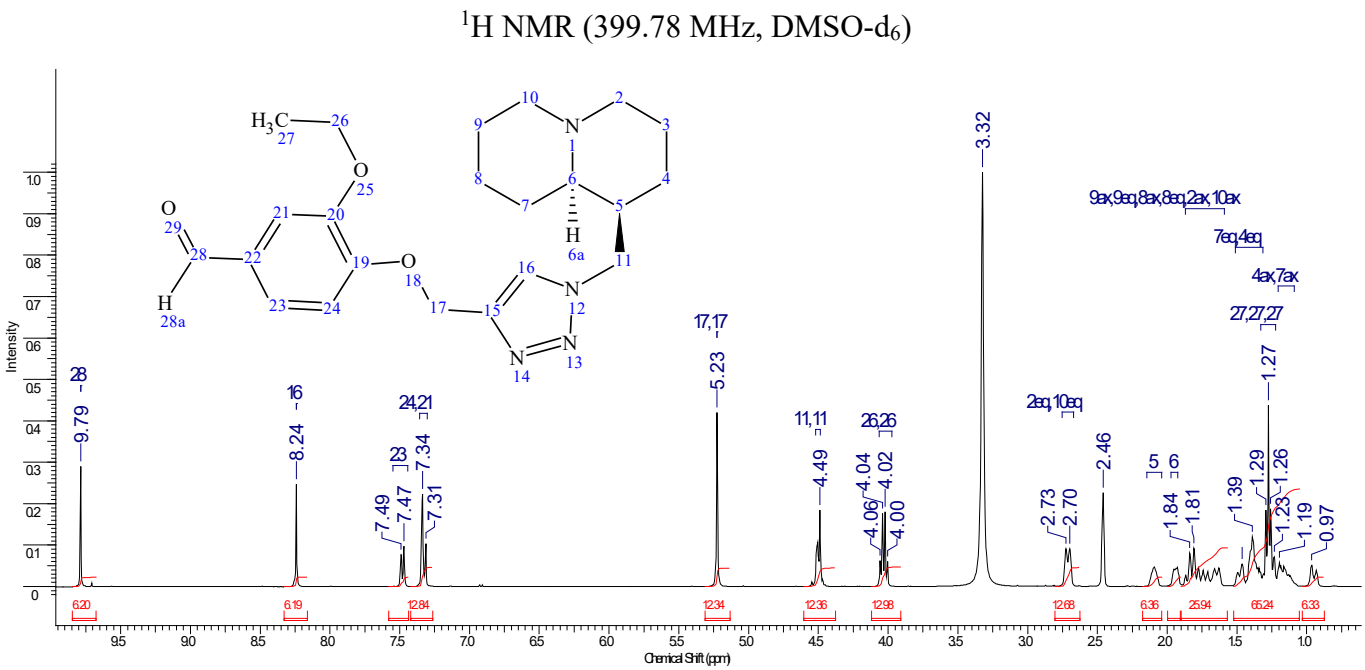
Supplementary Figure S5.2. ^{13}C NMR spectrum of (2*R*,*S*)-2-(1-(((1*S*,9a*R*)-octahydro-1*H*-quinolizine-1-yl)methyl)-1*H*-1,2,3-triazole-4-yl)butane-2,3-diol (6a,b).



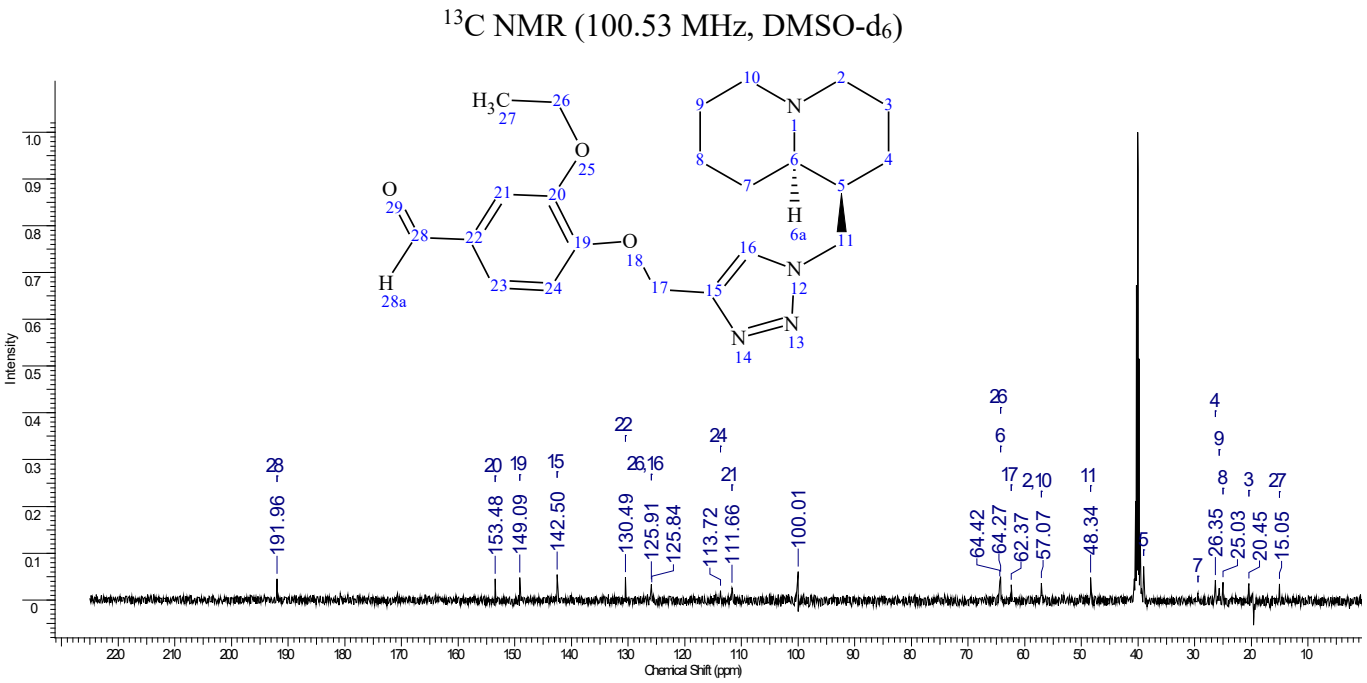
Supplementary Figure S5.3. The mass spectrum of (2*R*,*S*)-2-(1-(((1*S*,9a*R*)-octahydro-1*H*-quinolizine-1-yl)methyl)-1*H*-1,2,3-triazole-4-yl)butane-2,3-diol (6a,b).



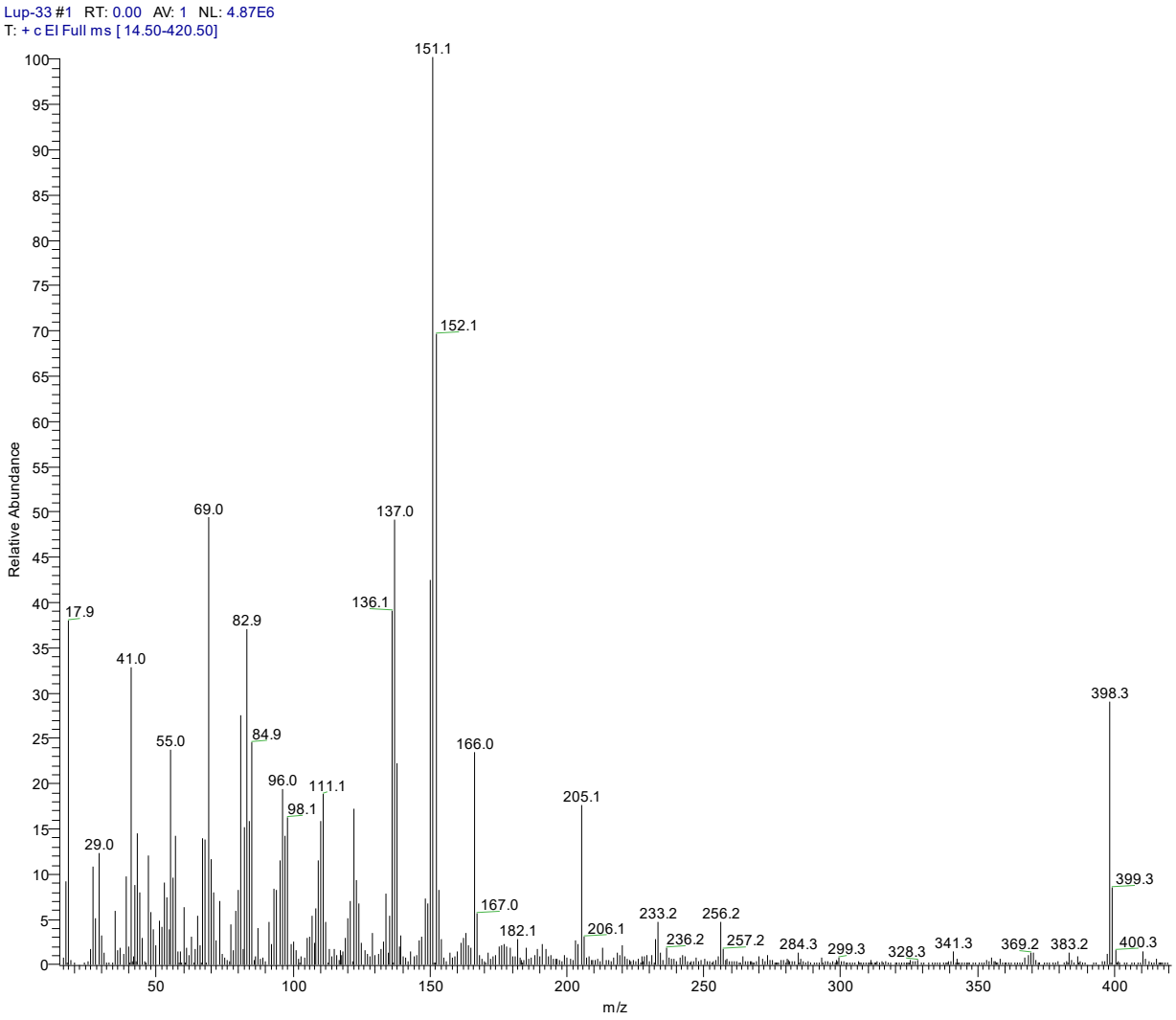
Supplementary Figure S6.1. ^{1}H NMR spectrum of 3-ethoxy-4-((1-(((1*S*,9a*R*)-octahydro-1*H*-quinolizine-1-yl)methyl)-1*H*-1,2,3-triazole-4-yl)methoxy)benzaldehyde (7).



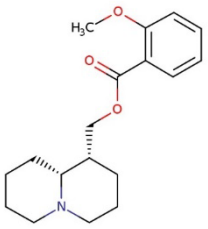
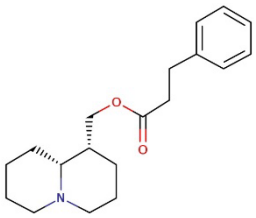
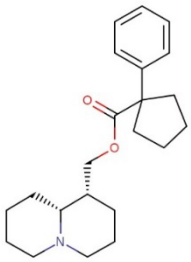
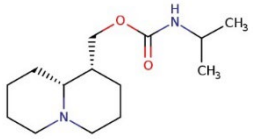
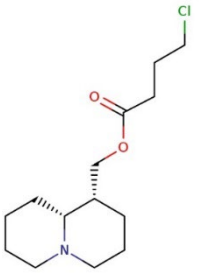
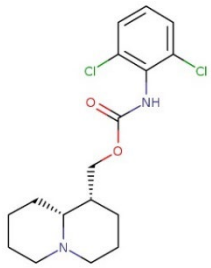
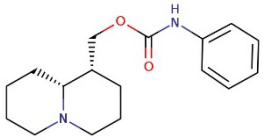
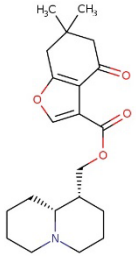
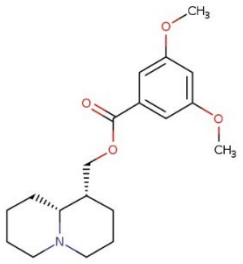
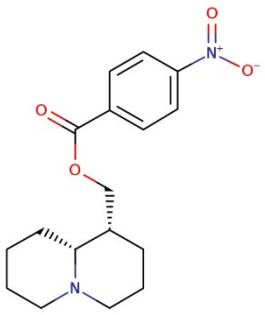
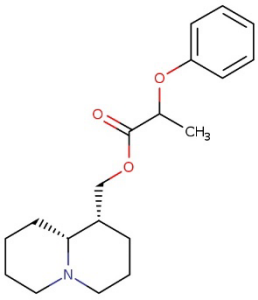
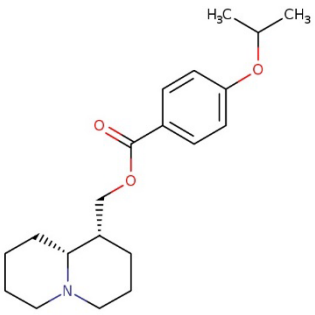
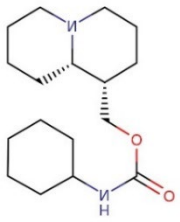
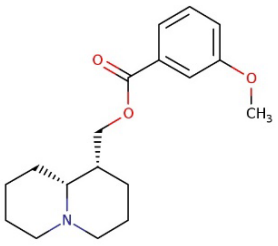
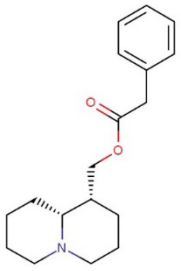
Supplementary Figure S6.2. ^{13}C NMR spectrum of 3-ethoxy-4-((1-(((1*S*,9a*R*)-octahydro-1*H*-quinolizine-1-yl)methyl)-1*H*-1,2,3-triazole-4-yl)methoxy)benzaldehyde (7).



Supplementary Figure S6.3. The mass spectrum of 3-ethoxy-4-((1-(((1*S*,9a*R*)-octahydro-1*H*-quinolizine-1-yl)methyl)-1*H*-1,2,3-triazole-4-yl)methoxy)benzaldehyde (7).



Supplementary Table S1. Chemical structures of lupinine-based esters of different carboxylic acids under investigation (Vitas-M codes are indicated)

 **18** (STOCK1N-00815)	 **19** (STOCK1N-20764)	 **20** (STOCK1N-27463)
 **21** (STOCK1N-08226)	 **22** (STOCK1N-20827)	 **23** (STOCK1N-27506)
 **24** (STOCK1N-09194)	 **25** (STOCK1N-20963)	 **26** (STOCK1N-27613)
 **27** (STOCK1N-11775)	 **28** (STOCK1N-21190)	 **29** (STOCK1N-27767)
 **30** (STOCK1N-11783)		

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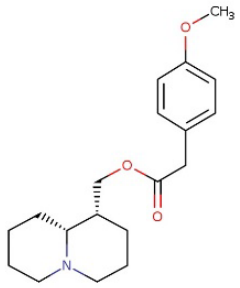
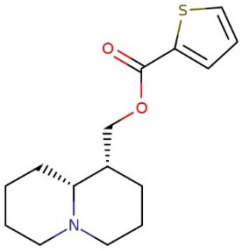
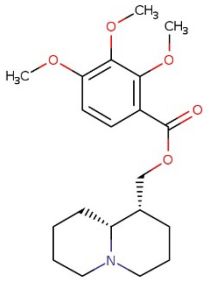
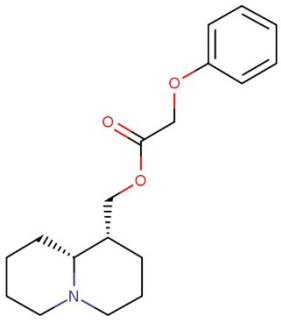
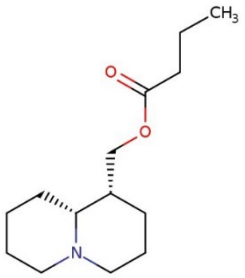
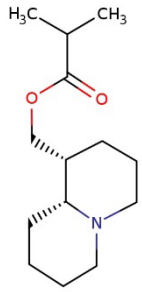
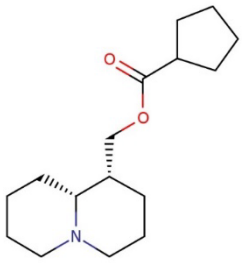
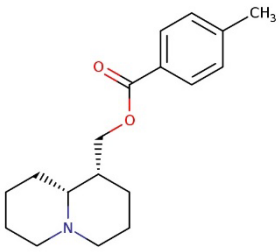
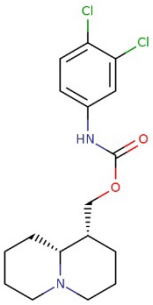
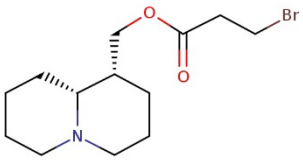
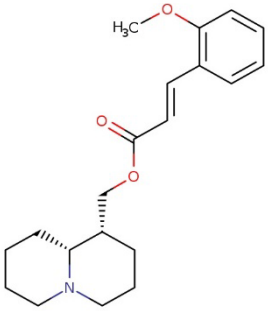
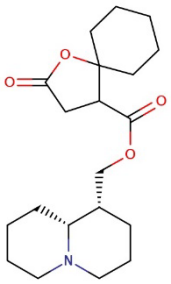
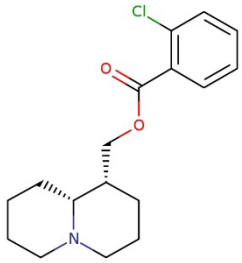
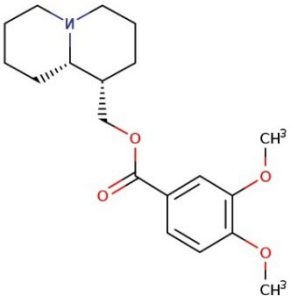
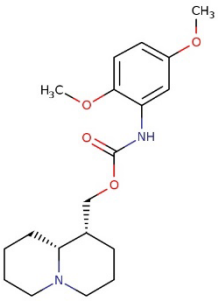
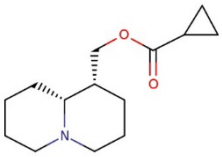
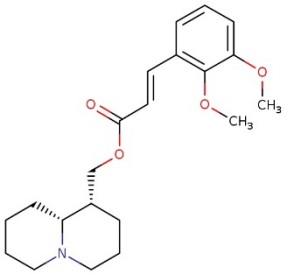
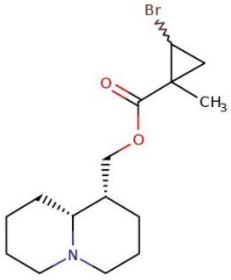
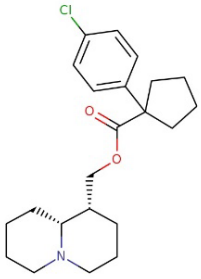
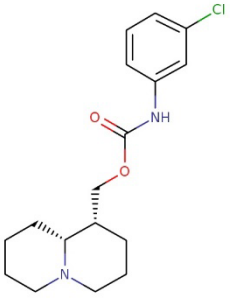
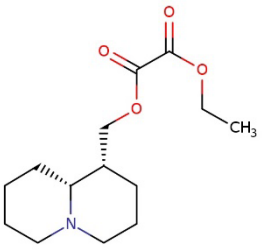
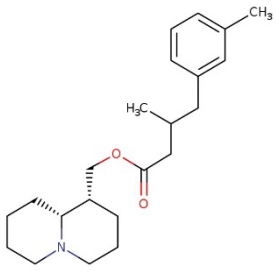
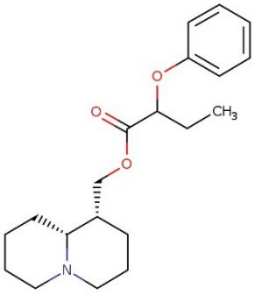
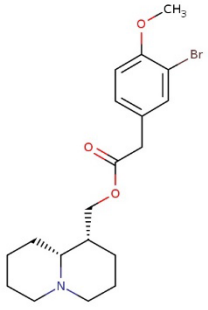
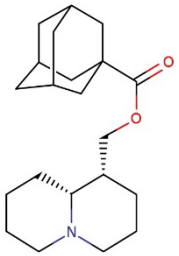
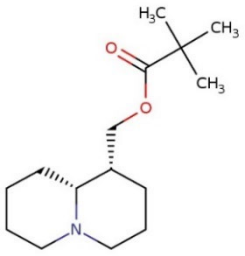
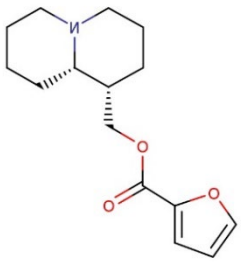
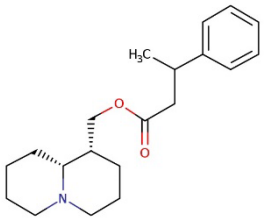
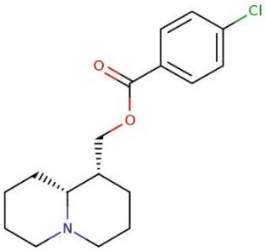
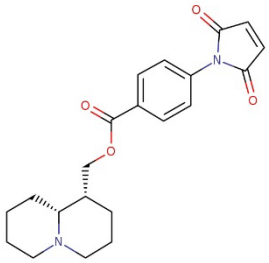
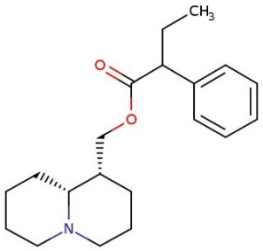
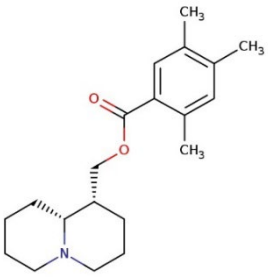
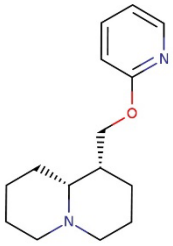
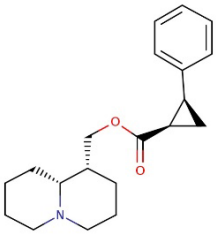
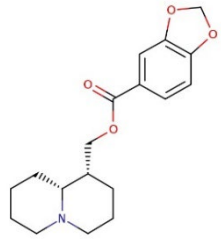
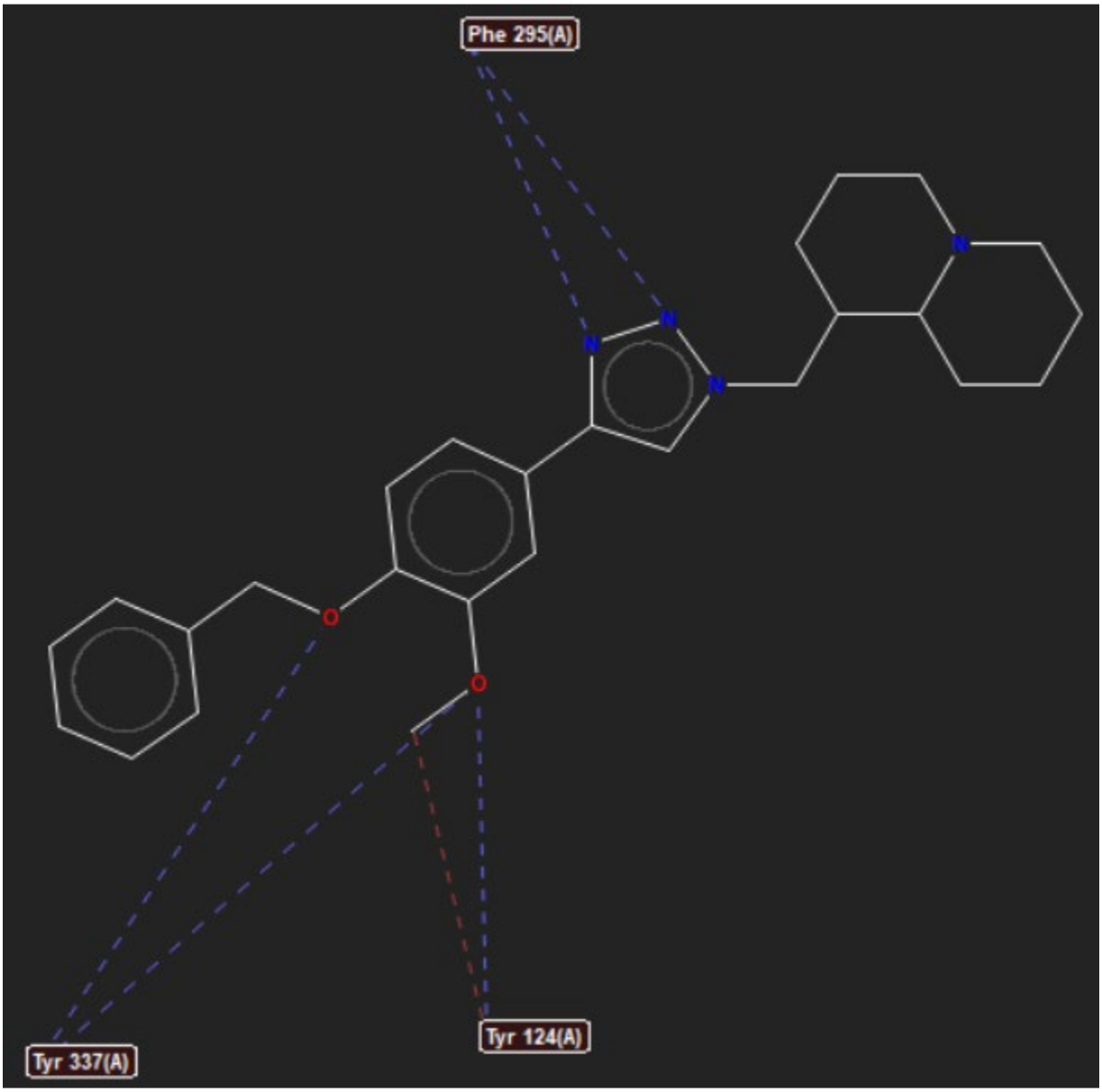
 **33** (STOCK1N-16368)	 **34** (STOCK1N-22221)	 **35** (STOCK1N-28128)
 **36** (STOCK1N-17850)	 **37** (STOCK1N-22377)	 **38** (STOCK1N-28515)
 **39** (STOCK1N-18175)	 **40** (STOCK1N-23538)	 **41** (STOCK1N-29877)
 **42** (STOCK1N-18223)	 **43** (STOCK1N-25368)	 **44** (STOCK1N-30716)

 **45** (STOCK1N-18525)	 **46** (STOCK1N-25694)	 **47** (STOCK1N-32617)
 **48** (STOCK1N-18721)	 **49** (STOCK1N-25897)	 **50** (STOCK1N-41979)
 **51** (STOCK1N-18802)	 **52** (STOCK1N-25964)	 **53** (STOCK1N-42122)
 **54** (STOCK1N-19758)	 **55** (STOCK1N-27190)	 **56** (STOCK1N-42244)
 **57** (STOCK1N-20315)	 **58** (STOCK1N-27196)	 **59** (STOCK1N-42364)

 **60** (STOCK1N-20499)	 **61** (STOCK1N-27242)	 **62** (STOCK1N-43209)
 **63** (STOCK1N-20557)	 **64** (STOCK1N-27249)	 **65** (STOCK1N-43872)
 **66** (STOCK1N-20682)	 **67** (STOCK1N-27417)	

Supplementary Figure S7. 2D diagram of ligand-receptor interactions obtained on docking of compound 15 in AChE. Blue dashed lines – hydrogen bonding interactions. Red dashed line – steric interaction.



Supplementary Table S2. Chemical formulas, selected ADME parameters of the lupinine-based esters calculated with SwissADME web tool, and manually added indicator variable for the quaternary sp^3 carbon atom (Q).

Compound	Formula	MW	N_{rot}	MR	Silicos-IT $LogS_w$	Q
18	$C_{18}H_{25}NO_3$	303.4	5	90.06	-3.98	0
19	$C_{19}H_{27}NO_2$	301.42	6	92.96	-4.66	0
20	$C_{22}H_{31}NO_2$	341.49	5	105.15	-5.21	1
21	$C_{14}H_{26}N_2O_2$	254.37	5	76.47	-2.23	0
22	$C_{14}H_{24}ClNO_2$	273.8	6	78.08	-3.18	0
23	$C_{17}H_{22}Cl_2N_2O_2$	357.27	5	98.09	-5.1	0
24	$C_{17}H_{24}N_2O_2$	288.38	5	88.07	-3.91	0
25	$C_{21}H_{29}NO_4$	359.46	4	103.04	-4.79	0
26	$C_{19}H_{27}NO_4$	333.42	6	96.55	-4.09	0
27	$C_{17}H_{22}N_2O_4$	318.37	5	92.39	-3.22	0
28	$C_{19}H_{27}NO_3$	317.42	6	94.49	-4.02	0
29	$C_{20}H_{29}NO_3$	331.45	6	99.67	-4.4	0
30	$C_{17}H_{30}N_2O_2$	294.43	5	88.78	-2.83	0
31	$C_{18}H_{25}NO_3$	303.4	5	90.06	-3.98	0
32	$C_{18}H_{25}NO_2$	287.4	5	88.15	-4.26	0
33	$C_{19}H_{27}NO_3$	317.42	6	94.64	-4.38	0
34	$C_{15}H_{21}NO_2S$	279.4	4	81.44	-3.13	0
35	$C_{20}H_{29}NO_5$	363.45	7	103.04	-4.2	0
36	$C_{18}H_{25}NO_3$	303.4	6	89.68	-4	0
37	$C_{14}H_{25}NO_2$	239.35	5	73.28	-2.55	0
38	$C_{14}H_{25}NO_2$	239.35	4	73.28	-2.18	0
39	$C_{16}H_{27}NO_2$	265.39	4	80.78	-2.51	0
40	$C_{18}H_{25}NO_2$	287.4	4	88.53	-4.25	0
41	$C_{17}H_{22}Cl_2N_2O_2$	357.27	5	98.09	-5.1	0
42	$C_{13}H_{22}BrNO_2$	304.22	5	76.34	-3	0
43	$C_{20}H_{27}NO_3$	329.43	6	99.77	-4.05	0
44	$C_{20}H_{31}NO_4$	349.46	4	99.22	-3.19	0
45	$C_{17}H_{22}ClNO_2$	307.82	4	88.58	-4.46	0
46	$C_{19}H_{27}NO_4$	333.42	6	96.55	-4.09	0
47	$C_{19}H_{28}N_2O_4$	348.44	7	101.06	-4.13	0
48	$C_{14}H_{23}NO_2$	237.34	4	71.17	-1.95	0
49	$C_{21}H_{29}NO_4$	359.46	7	106.26	-4.16	0
50	$C_{15}H_{24}BrNO_2$	330.26	4	83.58	-3.15	1
51	$C_{22}H_{30}ClNO_2$	375.93	5	110.16	-5.8	1
52	$C_{17}H_{23}ClN_2O_2$	322.83	5	93.08	-4.51	0
53	$C_{14}H_{23}NO_4$	269.34	6	74.56	-1.83	0
54	$C_{22}H_{33}NO_2$	343.5	7	107.54	-5.46	0

55	$C_{20}H_{29}NO_3$	331.45	7	99.29	-4.42	0
56	$C_{19}H_{26}BrNO_3$	396.32	6	102.34	-5.17	0
57	$C_{21}H_{33}NO_2$	331.49	4	100.33	-3.38	1
58	$C_{15}H_{27}NO_2$	253.38	4	77.83	-2.56	1
59	$C_{15}H_{21}NO_3$	263.33	4	75.83	-3.08	0
60	$C_{20}H_{29}NO_2$	315.45	6	97.77	-4.68	0
61	$C_{17}H_{22}ClNO_2$	307.82	4	88.58	-4.46	0
62	$C_{21}H_{24}N_2O_4$	368.43	5	108.14	-3.57	0
63	$C_{20}H_{29}NO_2$	315.45	6	97.77	-4.68	0
64	$C_{20}H_{29}NO_2$	315.45	4	98.47	-5.01	0
65	$C_{15}H_{22}N_2O$	246.35	3	76.57	-3.55	0
66	$C_{20}H_{27}NO_2$	313.43	5	95.65	-4.21	0
67	$C_{18}H_{23}NO_4$	317.38	4	89.63	-3.6	0

Abbreviations: molecular weight (MW), number of rotatable bonds (N_{rot}), molar refraction (MR), and water solubility characteristic (SILICOS-IT Log S_w).

Supplementary Table S3. Experimentally determined, calculated, and LOO-predicted classes for AChE inhibitory activity of the lupinine-based esters of different carboxylic acids.

Compound	Observed	LDA	
		Calculated	LOO-Predicted
18	NA	NA	NA
19	NA	NA	NA
20	NA	NA	NA
21	NA	NA	NA
22	Active	NA	NA
23	NA	Active	Active
24	NA	NA	NA
25	Active	Active	Active
26	NA	NA	NA
27	NA	Active	Active
28	NA	NA	NA
29	NA	Active	Active
30	NA	NA	NA
31	NA	NA	NA
32	NA	NA	NA
33	NA	NA	NA

34	NA	NA	NA
35	NA	Active	Active
36	NA	NA	NA
37	NA	NA	NA
38	NA	NA	NA
39	NA	NA	NA
40	NA	NA	NA
41	Active	Active	NA
42	NA	NA	NA
43	Active	Active	Active
44	Active	Active	Active
45	NA	NA	Active
46	NA	NA	NA
47	NA	NA	Active
48	NA	NA	NA
49	Active	Active	Active
50	NA	NA	NA
51	NA	NA	NA
52	NA	NA	NA
53	NA	NA	NA
54	NA	NA	NA
55	NA	NA	NA
56	NA	Active	Active
57	NA	NA	NA
58	NA	NA	NA
59	NA	NA	NA
60	NA	NA	NA
61	NA	NA	Active
62	NA	Active	Active
63	NA	NA	NA
64	Active	Active	Active
65	NA	NA	NA
66	NA	Active	Active
67	NA	Active	Active