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Synthesis and effects of oxadiazole derivatives on tyrosinase activity and human SK-MEL-28 malignant melanoma cells

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Supplementary Data

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1. Chemistry

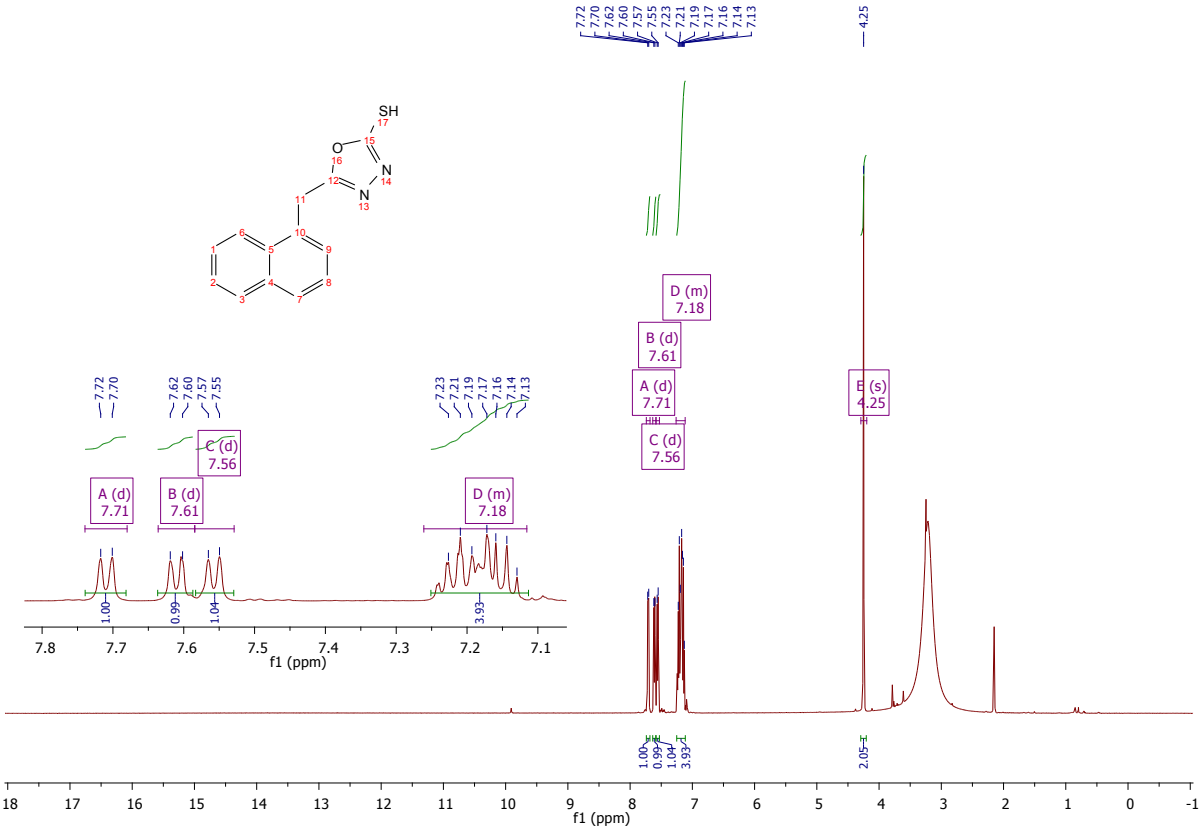
1.1. General information

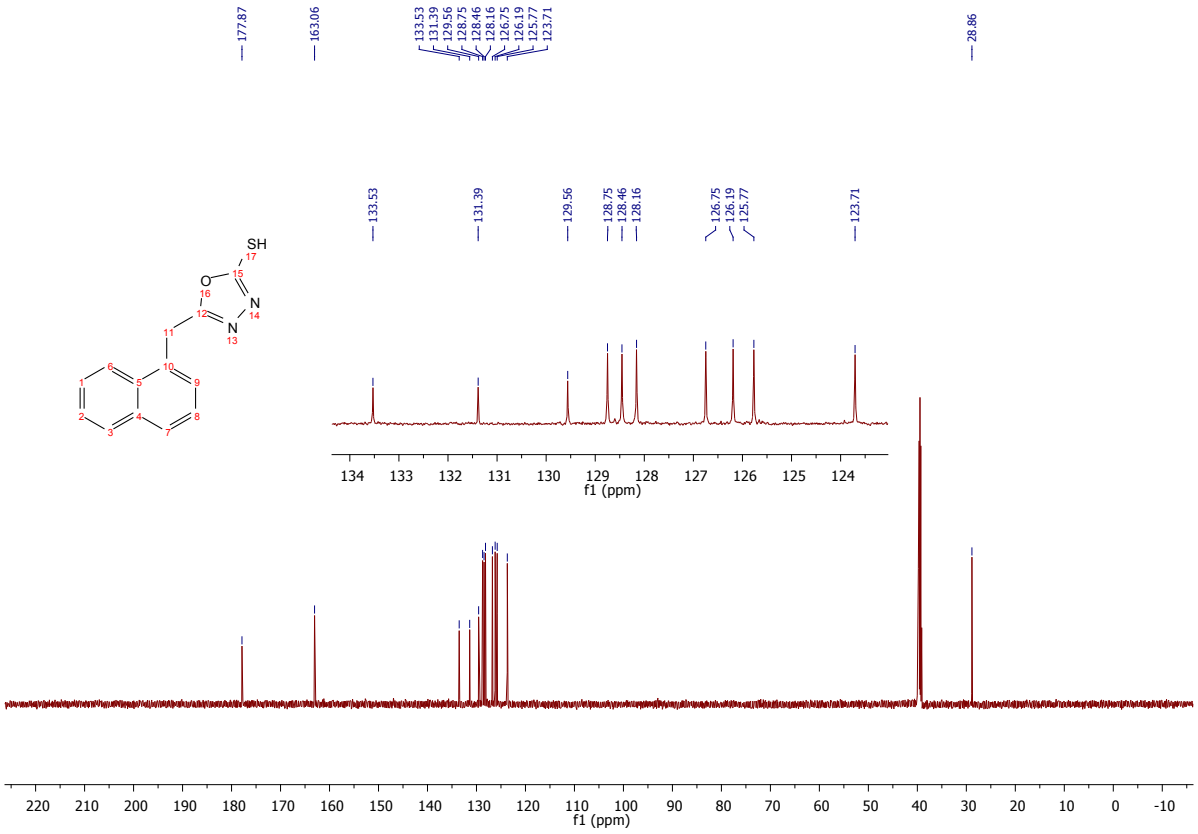
All reagents were purchased from Aldrich and Merck and were used without further purification. All the solvents used in the syntheses were analysis and synthesis grade. The solvents used in spectroscopic measurements were spectroscopic grade. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 500 MHz spectrometer. ESI-HRMS spectra were recorded on a Bruker micrOTOF Mass Spectrometer. Single-crystal X-ray experiment was performed on a Bruker D-QUEST diffractometer (Bruker, AXS Inc., Madison, WI, USA). Melting points were determined on a STUART SMP10 melting point apparatus.

1.2. ^{1}H and ^{13}C NMR

NMR spectra of compound **1**

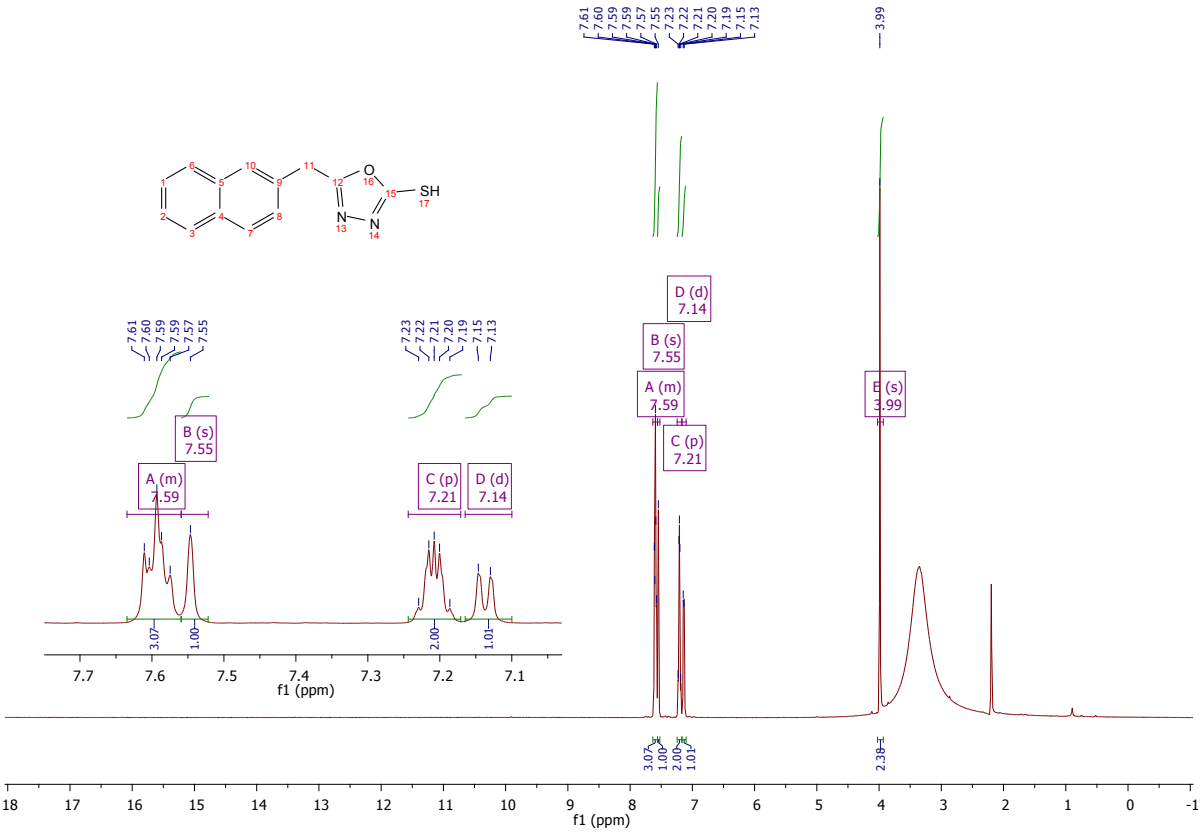
Compound **1**. 5-(naphthalen-1-ylmethyl)-1,3,4-oxadiazole-2-thiol. Brown solid. M.p 172-173°C. ^{1}H NMR (500 MHz, DMSO) δ 7.71 (d, *J* = 8.2 Hz, 1H), 7.61 (d, *J* = 8.7 Hz, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.26 – 7.12 (m, 4H), 4.25 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 177.87, 163.06, 133.53, 131.39, 129.56, 128.75, 128.46, 128.16, 126.75, 126.19, 125.77, 123.71, 28.86. ESI-HRMS: ($C_{13}H_{10}N_2OS$) calc. [M+H] 243.3040, found 243.3029.

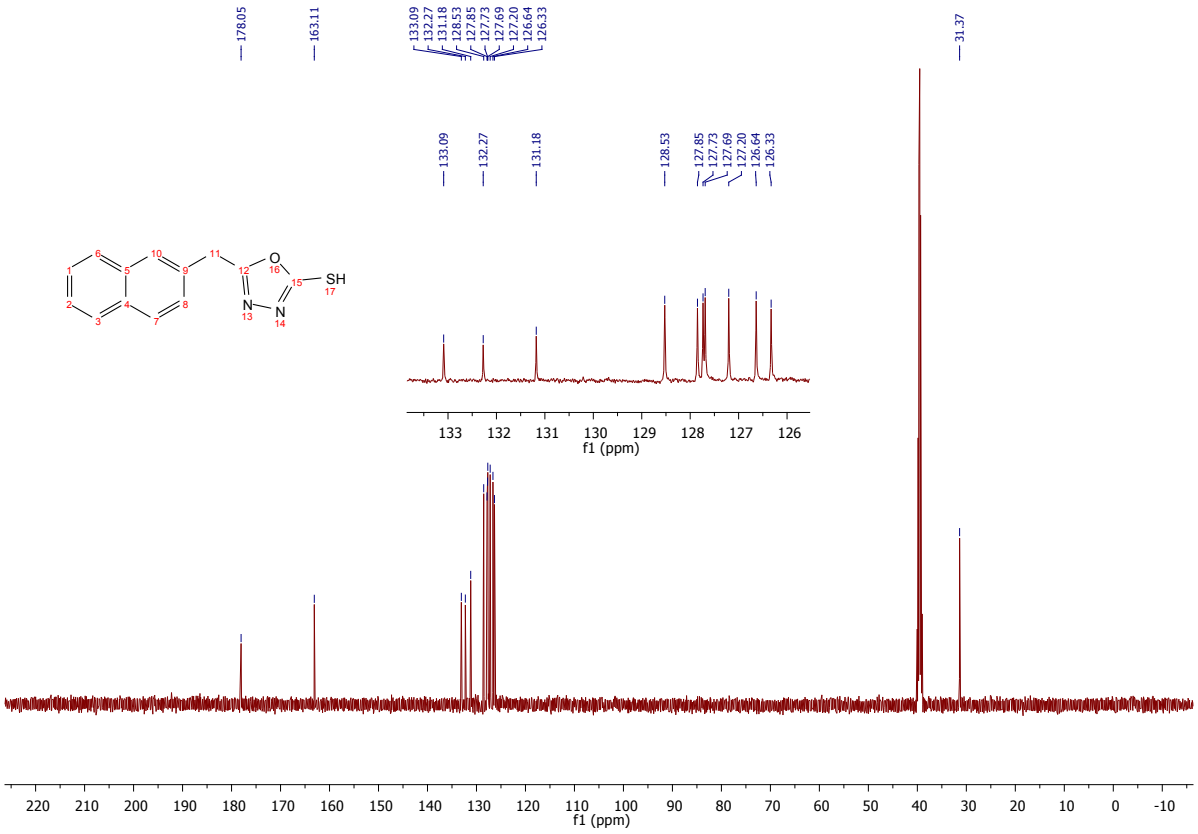



177.87
163.06
133.53
131.39
129.56
128.75
128.46
128.16
126.75
126.19
125.77
123.71
28.86
SH
17
O
15
16
N
14
12
N
13
11
1
6
5
10
9
2
4
8
3
7
f1 (ppm)
f1 (ppm)

NMR spectra of compound **2**

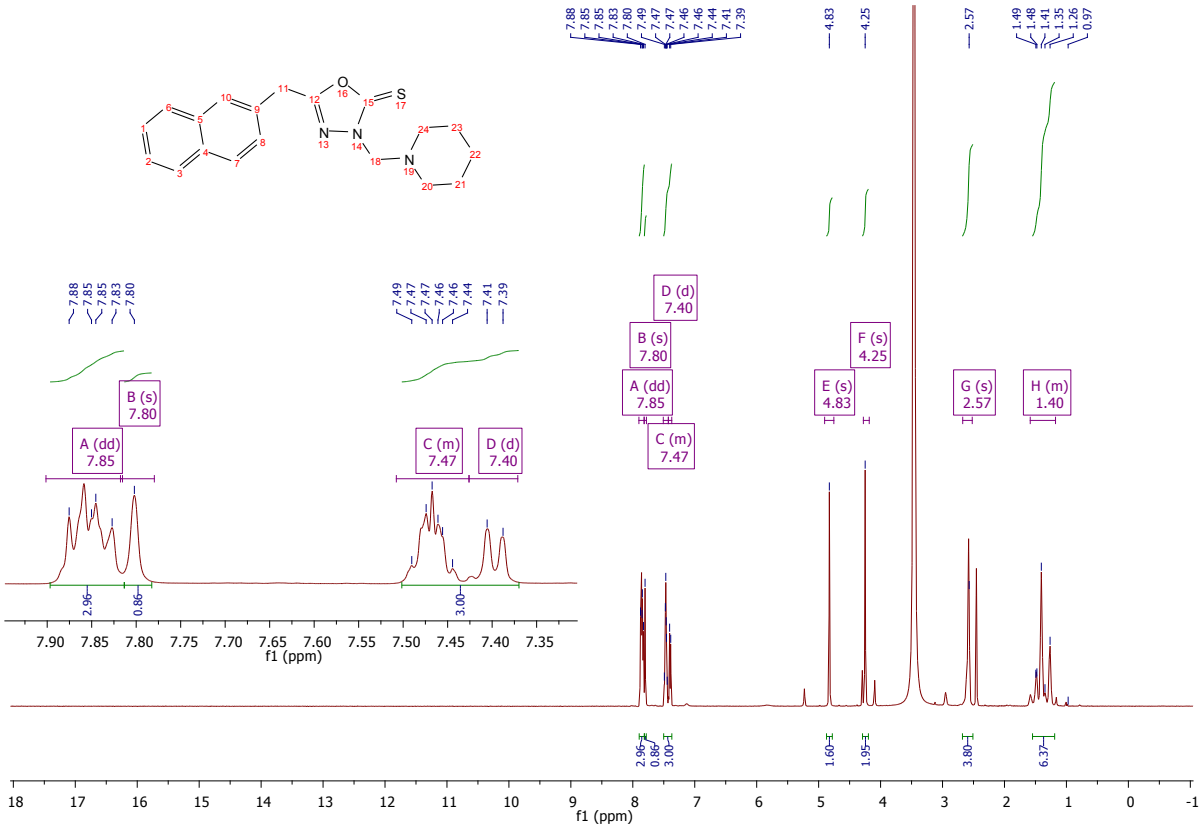
Compound **2**. 5-(naphthalen-2-ylmethyl)-1,3,4-oxadiazole-2-thiol. Pale yellow solid. M.p 138-139°C. ^{1}H NMR (500 MHz, DMSO) δ 7.61, 7.60, 7.59, 7.59, 7.57, 7.55, 7.23, 7.22, 7.21, 7.20, 7.19, 7.15, 7.13, 3.99. ^{13}C NMR (126 MHz, DMSO) δ 178.05, 163.11, 133.09, 132.27, 131.18, 128.53, 127.85, 127.73, 127.69, 127.20, 126.64, 126.33, 31.37. ESI-HRMS: ($C_{13}H_{10}N_2OS$) calc. [M+H] 243.3040, found 243.3033.

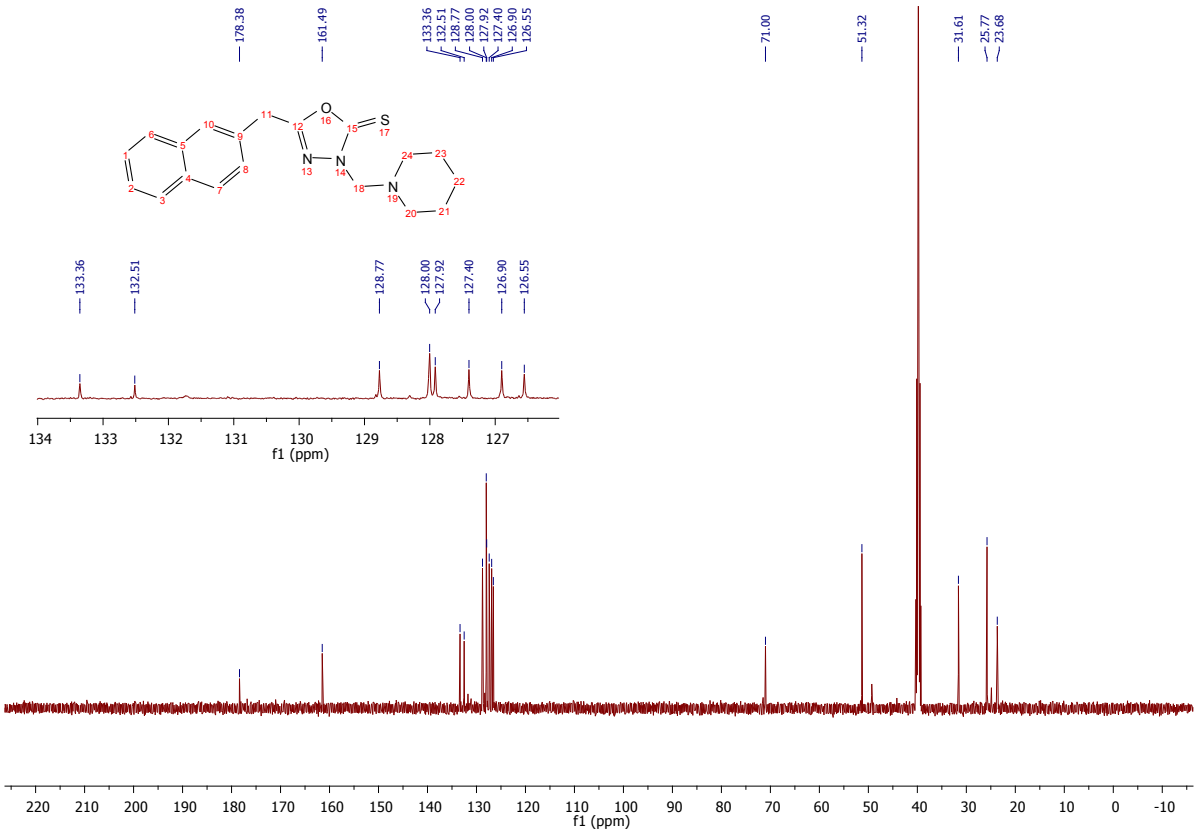



178.05
163.11
133.09
132.27
131.18
128.53
127.85
127.73
127.69
127.20
126.64
126.33
31.37
SH
f1 (ppm)
f1 (ppm)

NMR spectra of compound **3**

Compound **3**. 5-(naphthalen-2-ylmethyl)-3-(piperidin-1-ylmethyl)-1,3,4-oxadiazole-2(3H)-thione. colorless rod crystals. M.p 98-99°C. ^{1}H NMR (500 MHz, dmso) δ 7.85 (dd, *J* = 13.3, 10.9 Hz, 1H), 7.80 (s, 1H), 7.51 – 7.43 (m, 1H), 7.40 (d, *J* = 8.9 Hz, 1H), 4.83 (s, 1H), 4.25 (s, 1H), 2.57 (s, 1H), 1.58 – 1.18 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 178.38, 161.49, 133.36, 132.51, 128.77, 128.00, 127.92, 127.40, 126.90, 126.55, 71.00, 51.32, 31.61, 25.77, 23.68. ESI-HRMS: ($C_{19}H_{21}N_3OS$) calc. [M+H] 340.4650, found 340.4642.




178.38
161.49
133.36
132.51
128.77
128.00
127.92
127.40
126.90
126.55
71.00
51.32
31.61
25.77
23.68
O
S
N
N
N
f1 (ppm)
f1 (ppm)

1.3 X-ray Structural Characterization of Compound 3

Table 1. Crystal data and structure refinement for compound **3**.

Identification code	boly341_0m	
Empirical formula	C19 H21 N3 O S	
Formula weight	339.45	
Temperature	301(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P -1	
Unit cell dimensions	a = 7.0419(4) Å	α = 85.744 (2)°.
	b = 8.0935(5) Å	β = 89.568 (2)°.
	c = 15.4614(8) Å	γ = 80.461 (2)°.
Volume	866.61(9) $Å^3$	
Z	2	
Density (calculated)	1.301 Mg/m^3	
Absorption coefficient	0.197 mm^{-1}	
F(000)	360	
Crystal size	0.440 x 0.420 x 0.130 mm^3	
Theta range for data collection	3.211 to 28.371°.	
Index ranges	-9<=h<=9, -10<=k<=10, -20<=l<=20	
Reflections collected	52192	
Independent reflections	4332 [R(int) = 0.0609]	
Completeness to theta = 25.242°	99.8 %	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4332 / 0 / 217	
Goodness-of-fit on F^2	1.109	
Final R indices [I>2sigma(I)]	R1 = 0.0575, wR2 = 0.1094	
R indices (all data)	R1 = 0.0940, wR2 = 0.1240	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.197 and -0.286 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for compound **3**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
S(1)	2794(1)	3290(1)	104(1)	48(1)
O(1)	2153(2)	6381(2)	-663(1)	37(1)
N(1)	2673(2)	6381(2)	699(1)	35(1)
N(2)	2347(2)	8056(2)	384(1)	38(1)
N(3)	1737(2)	6374(2)	2189(1)	37(1)
C(1)	4783(3)	9871(2)	-1693(1)	48(1)
C(2)	6142(3)	9795(2)	-2316(1)	50(1)
C(3)	5866(3)	9113(2)	-3108(1)	44(1)
C(4)	7263(3)	8996(3)	-3776(2)	60(1)
C(5)	6945(4)	8318(3)	-4529(2)	70(1)
C(6)	5228(4)	7724(3)	-4657(2)	71(1)
C(7)	3851(4)	7819(3)	-4038(1)	61(1)
C(8)	4130(3)	8519(2)	-3249(1)	44(1)
C(9)	2727(3)	8630(2)	-2581(1)	44(1)
C(10)	3038(3)	9288(2)	-1820(1)	40(1)
C(11)	1565(3)	9381(2)	-1102(1)	49(1)
C(12)	2034(2)	7987(2)	-422(1)	36(1)
C(13)	2553(2)	5349(2)	73(1)	34(1)
C(14)	3284(3)	5920(2)	1591(1)	40(1)
C(15)	210(3)	5374(3)	2098(1)	45(1)
C(16)	-1430(3)	5864(3)	2708(1)	55(1)
C(17)	-702(4)	5664(3)	3637(1)	65(1)
C(18)	927(4)	6654(4)	3717(1)	73(1)
C(19)	2501(3)	6148(3)	3073(1)	51(1)

Table 3. Bond lengths [Å] and angles [°] for compound **3**.

S(1)-C(13)	1.6439(18)
O(1)-C(13)	1.363(2)
O(1)-C(12)	1.368(2)
N(1)-C(13)	1.337(2)
N(1)-N(2)	1.389(2)
N(1)-C(14)	1.451(2)
N(2)-C(12)	1.274(2)
N(3)-C(14)	1.442(2)
N(3)-C(19)	1.461(2)
N(3)-C(15)	1.462(2)
C(1)-C(2)	1.351(3)
C(1)-C(10)	1.407(3)
C(1)-H(1)	0.9300
C(2)-C(3)	1.407(3)
C(2)-H(2)	0.9300
C(3)-C(8)	1.410(3)
C(3)-C(4)	1.419(3)
C(4)-C(5)	1.358(3)
C(4)-H(4)	0.9300
C(5)-C(6)	1.394(4)
C(5)-H(5)	0.9300
C(6)-C(7)	1.354(3)
C(6)-H(6)	0.9300
C(7)-C(8)	1.412(3)
C(7)-H(7)	0.9300
C(8)-C(9)	1.421(3)
C(9)-C(10)	1.361(3)
C(9)-H(9)	0.9300
C(10)-C(11)	1.511(3)
C(11)-C(12)	1.481(3)
C(11)-H(11A)	0.9700
C(11)-H(11B)	0.9700
C(14)-H(14A)	0.9700
C(14)-H(14B)	0.9700
C(15)-C(16)	1.506(3)
C(15)-H(15A)	0.9700
C(15)-H(15B)	0.9700
C(16)-C(17)	1.516(3)
C(16)-H(16A)	0.9700
C(16)-H(16B)	0.9700
C(17)-C(18)	1.514(3)
C(17)-H(17A)	0.9700
C(17)-H(17B)	0.9700
C(18)-C(19)	1.510(3)
C(18)-H(18A)	0.9700
C(18)-H(18B)	0.9700
C(19)-H(19A)	0.9700
C(19)-H(19B)	0.9700
C(13)-O(1)-C(12)	106.35(13)
C(13)-N(1)-N(2)	111.95(14)
C(13)-N(1)-C(14)	127.37(15)
N(2)-N(1)-C(14)	120.30(14)
C(12)-N(2)-N(1)	103.51(14)
C(14)-N(3)-C(19)	109.20(14)
C(14)-N(3)-C(15)	111.12(14)

C(19)-N(3)-C(15)	110.66(14)
C(2)-C(1)-C(10)	121.02(18)
C(2)-C(1)-H(1)	119.5
C(10)-C(1)-H(1)	119.5
C(1)-C(2)-C(3)	121.02(19)
C(1)-C(2)-H(2)	119.5
C(3)-C(2)-H(2)	119.5
C(2)-C(3)-C(8)	118.97(18)
C(2)-C(3)-C(4)	122.85(19)
C(8)-C(3)-C(4)	118.18(19)
C(5)-C(4)-C(3)	121.0(2)
C(5)-C(4)-H(4)	119.5
C(3)-C(4)-H(4)	119.5
C(4)-C(5)-C(6)	120.3(2)
C(4)-C(5)-H(5)	119.9
C(6)-C(5)-H(5)	119.9
C(7)-C(6)-C(5)	120.7(2)
C(7)-C(6)-H(6)	119.7
C(5)-C(6)-H(6)	119.7
C(6)-C(7)-C(8)	120.6(2)
C(6)-C(7)-H(7)	119.7
C(8)-C(7)-H(7)	119.7
C(3)-C(8)-C(7)	119.24(19)
C(3)-C(8)-C(9)	118.45(17)
C(7)-C(8)-C(9)	122.31(19)
C(10)-C(9)-C(8)	121.29(18)
C(10)-C(9)-H(9)	119.4
C(8)-C(9)-H(9)	119.4
C(9)-C(10)-C(1)	119.25(18)
C(9)-C(10)-C(11)	121.47(18)
C(1)-C(10)-C(11)	119.27(17)
C(12)-C(11)-C(10)	112.19(15)
C(12)-C(11)-H(11A)	109.2
C(10)-C(11)-H(11A)	109.2
C(12)-C(11)-H(11B)	109.2
C(10)-C(11)-H(11B)	109.2
H(11A)-C(11)-H(11B)	107.9
N(2)-C(12)-O(1)	113.27(15)
N(2)-C(12)-C(11)	128.97(17)
O(1)-C(12)-C(11)	117.76(15)
N(1)-C(13)-O(1)	104.91(14)
N(1)-C(13)-S(1)	131.15(14)
O(1)-C(13)-S(1)	123.94(12)
N(3)-C(14)-N(1)	111.47(14)
N(3)-C(14)-H(14A)	109.3
N(1)-C(14)-H(14A)	109.3
N(3)-C(14)-H(14B)	109.3
N(1)-C(14)-H(14B)	109.3
H(14A)-C(14)-H(14B)	108.0
N(3)-C(15)-C(16)	111.46(16)
N(3)-C(15)-H(15A)	109.3
C(16)-C(15)-H(15A)	109.3
N(3)-C(15)-H(15B)	109.3
C(16)-C(15)-H(15B)	109.3
H(15A)-C(15)-H(15B)	108.0
C(15)-C(16)-C(17)	110.11(18)
C(15)-C(16)-H(16A)	109.6

C(17)-C(16)-H(16A)	109.6
C(15)-C(16)-H(16B)	109.6
C(17)-C(16)-H(16B)	109.6
H(16A)-C(16)-H(16B)	108.2
C(18)-C(17)-C(16)	109.60(18)
C(18)-C(17)-H(17A)	109.7
C(16)-C(17)-H(17A)	109.7
C(18)-C(17)-H(17B)	109.7
C(16)-C(17)-H(17B)	109.7
H(17A)-C(17)-H(17B)	108.2
C(19)-C(18)-C(17)	111.00(18)
C(19)-C(18)-H(18A)	109.4
C(17)-C(18)-H(18A)	109.4
C(19)-C(18)-H(18B)	109.4
C(17)-C(18)-H(18B)	109.4
H(18A)-C(18)-H(18B)	108.0
N(3)-C(19)-C(18)	110.72(18)
N(3)-C(19)-H(19A)	109.5
C(18)-C(19)-H(19A)	109.5
N(3)-C(19)-H(19B)	109.5
C(18)-C(19)-H(19B)	109.5
H(19A)-C(19)-H(19B)	108.1

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for compound **3**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
S(1)	44(1)	40(1)	61(1)	-10(1)	6(1)	-7(1)
O(1)	35(1)	43(1)	34(1)	-10(1)	3(1)	-7(1)
N(1)	33(1)	41(1)	33(1)	-7(1)	3(1)	-7(1)
N(2)	36(1)	40(1)	39(1)	-8(1)	4(1)	-4(1)
N(3)	36(1)	46(1)	31(1)	-5(1)	0(1)	-12(1)
C(1)	56(1)	41(1)	46(1)	-4(1)	-9(1)	-8(1)
C(2)	47(1)	46(1)	60(1)	-1(1)	-9(1)	-14(1)
C(3)	48(1)	36(1)	47(1)	4(1)	-1(1)	-6(1)
C(4)	56(1)	54(1)	67(2)	6(1)	8(1)	-9(1)
C(5)	84(2)	68(2)	54(1)	-1(1)	22(1)	-5(1)
C(6)	93(2)	77(2)	44(1)	-11(1)	6(1)	-13(1)
C(7)	74(2)	65(1)	47(1)	-9(1)	-4(1)	-19(1)
C(8)	53(1)	38(1)	41(1)	0(1)	-3(1)	-8(1)
C(9)	45(1)	43(1)	45(1)	-1(1)	-5(1)	-10(1)
C(10)	45(1)	31(1)	40(1)	2(1)	-3(1)	0(1)
C(11)	49(1)	45(1)	47(1)	-3(1)	0(1)	8(1)
C(12)	30(1)	39(1)	40(1)	-9(1)	5(1)	-3(1)
C(13)	23(1)	43(1)	37(1)	-9(1)	6(1)	-6(1)
C(14)	33(1)	52(1)	36(1)	-4(1)	-3(1)	-8(1)
C(15)	40(1)	57(1)	42(1)	-8(1)	0(1)	-17(1)
C(16)	41(1)	57(1)	68(1)	-3(1)	11(1)	-11(1)
C(17)	73(2)	73(2)	53(1)	-14(1)	26(1)	-27(1)
C(18)	96(2)	95(2)	42(1)	-25(1)	19(1)	-46(2)
C(19)	58(1)	66(1)	35(1)	-3(1)	-4(1)	-27(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for compound **3**.

	x	y	z	U(eq)
H(1)	5003	10316	-1173	57
H(2)	7278	10200	-2221	60
H(4)	8414	9389	-3698	72
H(5)	7876	8250	-4960	84
H(6)	5026	7257	-5174	85
H(7)	2712	7419	-4134	73
H(9)	1574	8245	-2665	53
H(11A)	1499	10443	-841	58
H(11B)	309	9345	-1346	58
H(14A)	3730	4718	1664	48
H(14B)	4352	6481	1721	48
H(15A)	733	4193	2218	54
H(15B)	-268	5532	1506	54
H(16A)	-2402	5160	2646	66
H(16B)	-2017	7022	2565	66
H(17A)	-1742	6068	4022	77
H(17B)	-250	4486	3804	77
H(18A)	434	7844	3616	88
H(18B)	1453	6457	4301	88
H(19A)	3504	6826	3122	61
H(19B)	3067	4980	3204	61

Table 6. Torsion angles [°] for compound **3**.

C(13)-N(1)-N(2)-C(12)	0.17(18)
C(14)-N(1)-N(2)-C(12)	173.64(14)
C(10)-C(1)-C(2)-C(3)	-0.7(3)
C(1)-C(2)-C(3)-C(8)	0.6(3)
C(1)-C(2)-C(3)-C(4)	-179.43(19)
C(2)-C(3)-C(4)-C(5)	179.6(2)
C(8)-C(3)-C(4)-C(5)	-0.5(3)
C(3)-C(4)-C(5)-C(6)	0.1(4)
C(4)-C(5)-C(6)-C(7)	0.3(4)
C(5)-C(6)-C(7)-C(8)	-0.1(4)
C(2)-C(3)-C(8)-C(7)	-179.42(19)
C(4)-C(3)-C(8)-C(7)	0.7(3)
C(2)-C(3)-C(8)-C(9)	-0.2(3)
C(4)-C(3)-C(8)-C(9)	179.88(17)
C(6)-C(7)-C(8)-C(3)	-0.3(3)
C(6)-C(7)-C(8)-C(9)	-179.5(2)
C(3)-C(8)-C(9)-C(10)	-0.1(3)
C(7)-C(8)-C(9)-C(10)	179.05(19)
C(8)-C(9)-C(10)-C(1)	0.1(3)
C(8)-C(9)-C(10)-C(11)	-178.91(16)
C(2)-C(1)-C(10)-C(9)	0.4(3)
C(2)-C(1)-C(10)-C(11)	179.39(17)
C(9)-C(10)-C(11)-C(12)	98.9(2)
C(1)-C(10)-C(11)-C(12)	-80.0(2)
N(1)-N(2)-C(12)-O(1)	-0.69(18)
N(1)-N(2)-C(12)-C(11)	178.92(17)
C(13)-O(1)-C(12)-N(2)	0.95(18)
C(13)-O(1)-C(12)-C(11)	-178.70(15)
C(10)-C(11)-C(12)-N(2)	120.3(2)
C(10)-C(11)-C(12)-O(1)	-60.1(2)
N(2)-N(1)-C(13)-O(1)	0.39(17)
C(14)-N(1)-C(13)-O(1)	-172.51(14)
N(2)-N(1)-C(13)-S(1)	-179.34(13)
C(14)-N(1)-C(13)-S(1)	7.8(3)
C(12)-O(1)-C(13)-N(1)	-0.76(16)
C(12)-O(1)-C(13)-S(1)	179.00(12)
C(19)-N(3)-C(14)-N(1)	-170.51(15)
C(15)-N(3)-C(14)-N(1)	67.1(2)
C(13)-N(1)-C(14)-N(3)	-115.43(18)
N(2)-N(1)-C(14)-N(3)	72.19(19)
C(14)-N(3)-C(15)-C(16)	-178.89(16)
C(19)-N(3)-C(15)-C(16)	59.6(2)
N(3)-C(15)-C(16)-C(17)	-57.6(2)
C(15)-C(16)-C(17)-C(18)	54.7(3)
C(16)-C(17)-C(18)-C(19)	-54.9(3)
C(14)-N(3)-C(19)-C(18)	178.60(17)
C(15)-N(3)-C(19)-C(18)	-58.8(2)
C(17)-C(18)-C(19)-N(3)	57.1(3)

Symmetry transformations used to generate equivalent atoms:

Table 7. Hydrogen bonds for compound **3** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)

2. Biology

2.1 Lineweaver-Burk plots for Kojic Acid and Rhodanine

Figure 1 Lineweaver-Burk plots for inhibition of kojic acid on the oxidation of L-DOPA by mushroom tyrosinase. Concentrations of kojic acid for curves 0-4 were 0, 5, 10, 15, and 20 μM, respectively. The inset represents the secondary plot of slope or Y-intercept versus the kojic acid concentration for determining the K_I and K_{IS}. The line was drawn using linear least square fit.

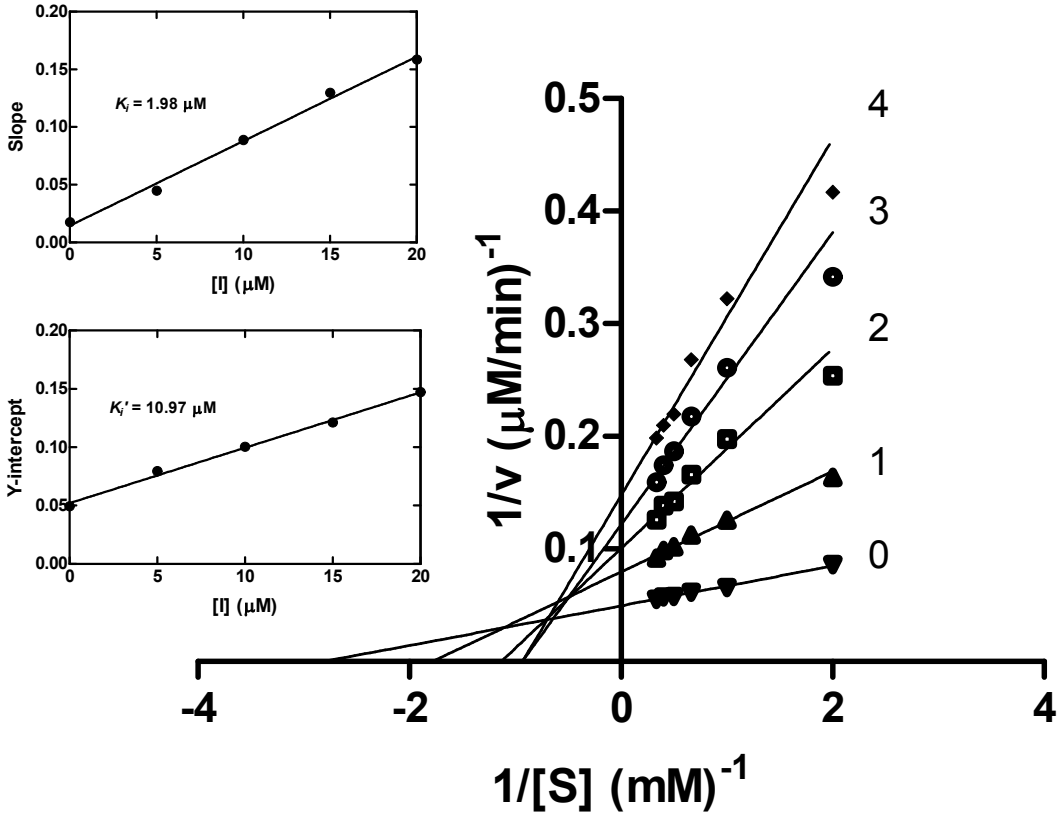
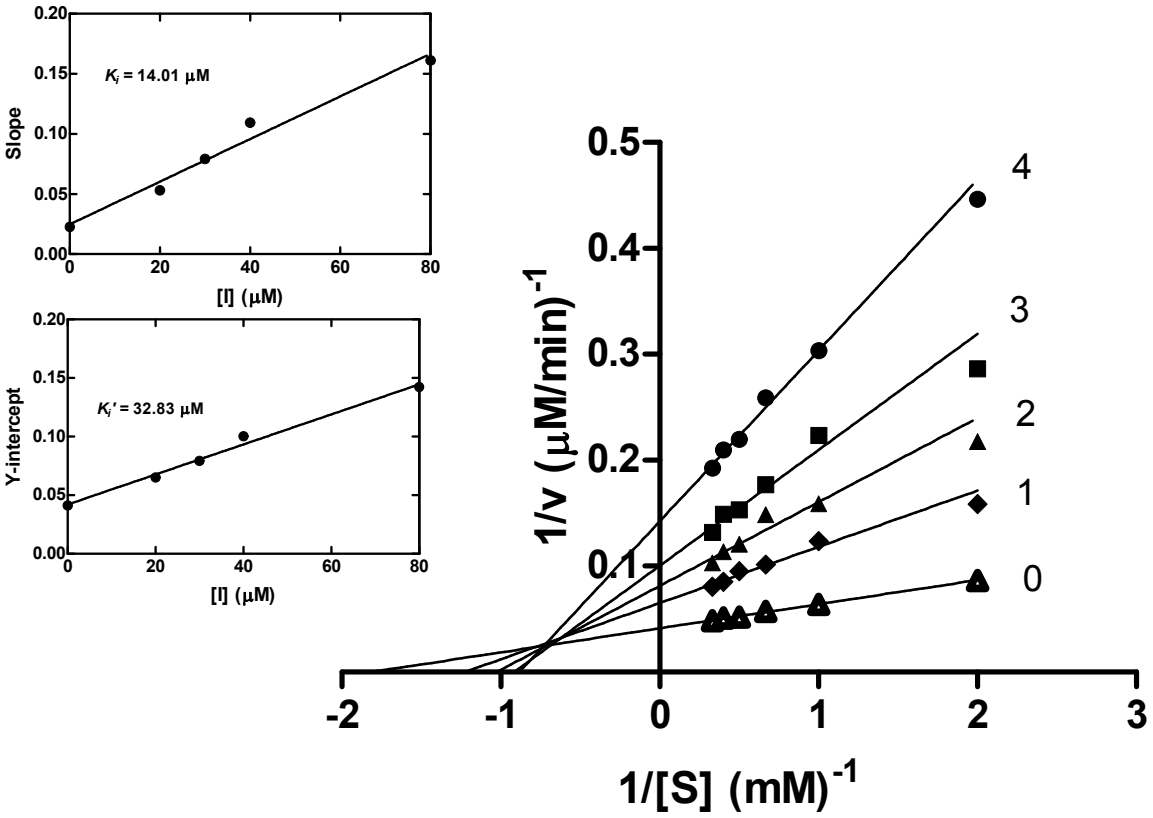


Figure 2 Lineweaver-Burk plots for inhibition of rhodanine on the oxidation of L-DOPA by mushroom tyrosinase. Concentrations of rhodanine for curves 0-4 were 0, 20, 30, 40, and 80 μM, respectively. The inset represents the secondary plot of slope or Y-intercept versus the rhodanine concentration for determining the K_I and K_{IS}. The line was drawn using linear least square fit.



3. Computational studies

3.1 Molecular docking and dynamics simulation

Fig. 3. RMSD plots of tyrosinase backbone in complex with compound **2** (black line) and compound **2** (red line) as a function of simulation time.

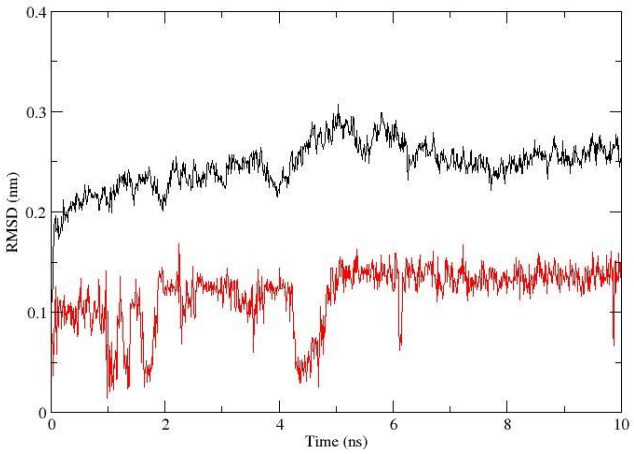


Fig. 4. RMSF plot of selected tyrosinase residues in complex with compound **2** during the final 5 ns of simulation.