Supplementary Materials

Isolation of Nocuolin A and synthesis of new oxadiazine derivatives. Design, synthesis, molecular docking, apoptotic evaluation and cathepsin B inhibition

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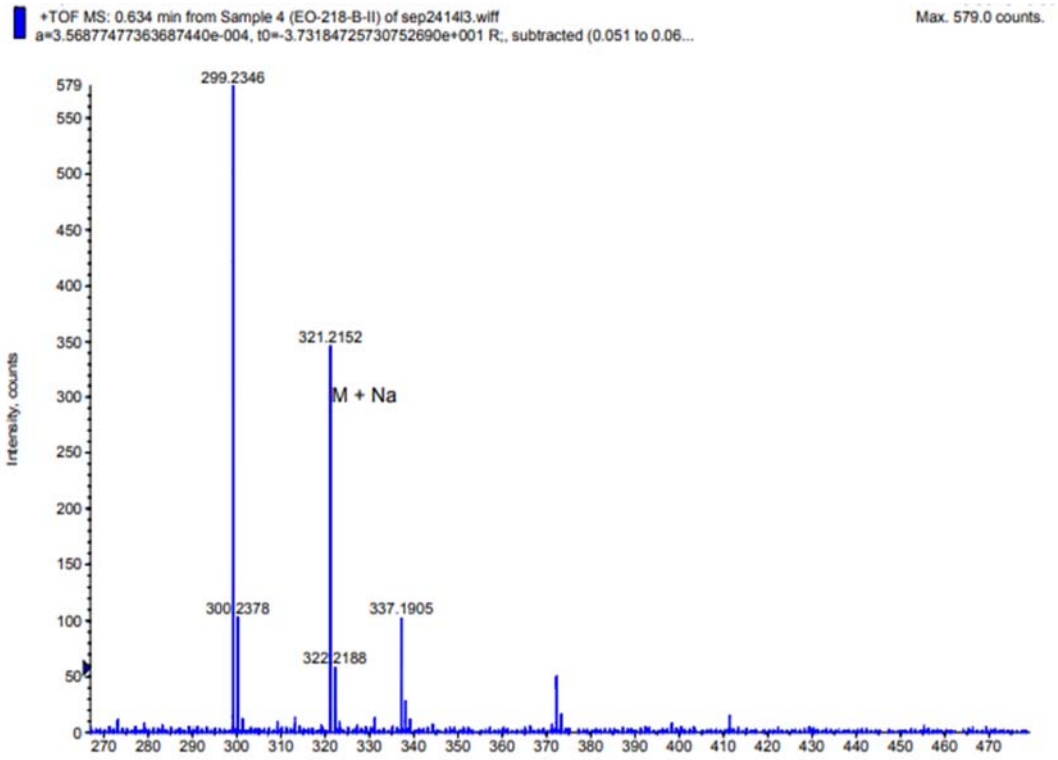
- **Figure S24**. DEPT-135 spectrum of 4-{3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropoxy}-4-oxobutanoic acid (**3**) in $CDCl_3$ 75 MHz.
- **Figure S25**. ^{1}H-^{1}H COSY spectrum of 4-{3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropoxy}-4-oxobutanoic acid (**3**) in $CDCl_3$.
- **Figure S26**. ^{1}H-^{13}C HSQC spectrum of 4-{3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropoxy}-4-oxobutanoic acid (**3**) in $CDCl_3$.
- **Figure S27**. ^{1}H-^{13}C HMBC spectrum of 4-{3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropoxy}-4-oxobutanoic acid (**3**) in $CDCl_3$.

- Structural elucidation of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**).

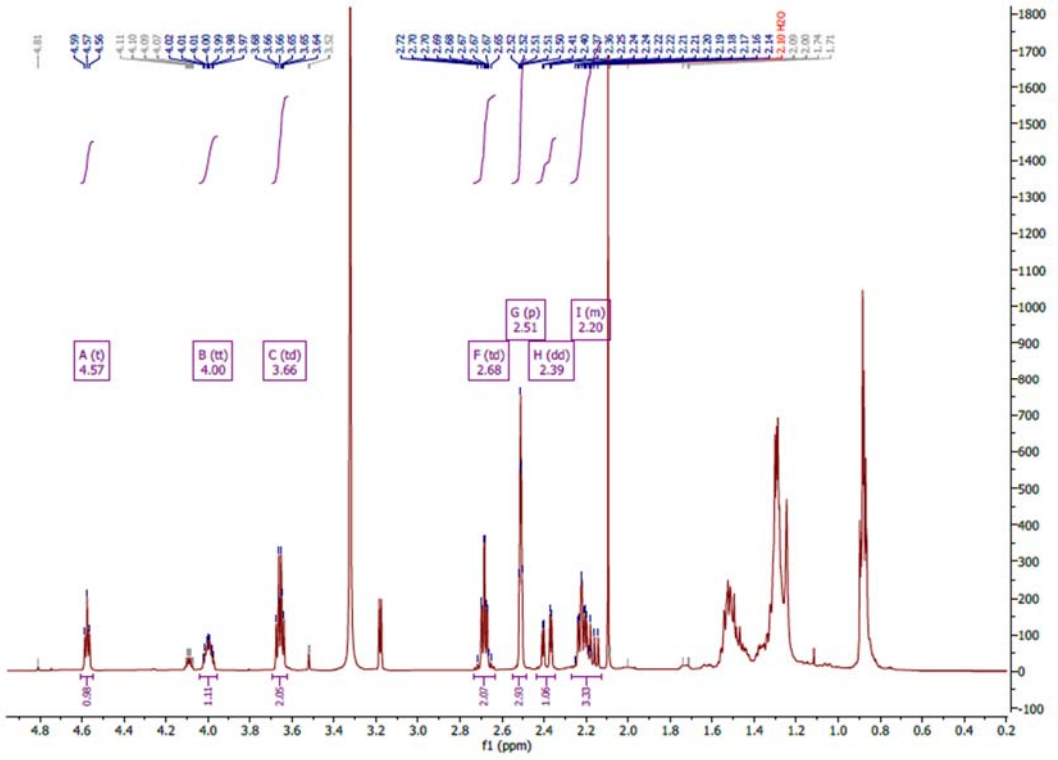
Compound **1** was isolated as a colourless viscous oil, with $C_{16}H_{30}N_2O_3$ as a molecular formula based on the $[M + Na]^+$ ion peak at *m*/*z* 321.2152 (calcd. for $C_{16}H_{30}NaN_2O_3$ 321.2149) obtained by HRESIMS (Figure S1). ^{1}H NMR (Figure S2) in DMSO-d_6 showed the presence of the hydroxyl proton at δ_H 4.57 (t, *J* = 5.4 Hz) which coupled by a ^{1}H-^{1}H COSY correlation with methylene protons at δ_H 3.66 (td, *J* = 6.6 and 5.4 Hz, H-3'') and δ_C 57.4 determined by ^{13}C NMR (Figure S3) and DEPT-135 (Figure S4) spectra. The ^{1}H-^{1}H COSY (Figure S5) and ^{1}H-^{13}C HSQC (Figure S6) spectra showed that the methylene CH_2-3'' was correlated with the methylene CH_2-2'' at δ_H 2.69 (t, *J* = 6.6 Hz, 1H, Ha-2''), 2.68 (t, *J* = 6.6 Hz, 1H, Hb-2'') and δ_C 37.3. The methylene CH_2-2'' and CH_2-3'' of spin system A were interconnected to the quaternary carbon at δ_C 165.6 (C-1'') as shown by the ^{1}H-^{13}C HMBC spectrum (Figure S7) which suggested an amide bond (N-CO-CH_2-CH_2OH). Other two spin systems in the ^{1}H NMR spectrum could be identified in the ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra as follows: CH_3-CH_2-CH_2-CH_2-CH_2 (C-1'/C-5', spin system B) and CH_3-CH_2-CH_2-CH_2-CH_2-CHR-CH_2 (C-5/C-11, spin system C). The spin system B and C were seen as interconnected via attachment to the quaternary carbon at δ_C 150.2 (C-4) by ^{1}H-^{13}C HMBC correlations between C-4 with methylene protons H-5 at δ_H 2.39 (dd, *J* = 18.3 and 3.8 Hz, H-5a), 2.17 (dd, *J* = 18.3 and 8.8 Hz, H-5b) and with methylene protons H-1' at δ_H 2.23 (t, *J* = 7.3 Hz, H-1'a), 2.22 (t, *J* = 7.3 Hz, H-1'b). The correlation between H-1' with a nitrogen atom at δ_N 302.6 which was observed in the ^{1}H-^{15}N HMBC (Figure S8) showed the presence of an imine bond between C-4 and N-3 atoms. Finally, the signals at δ_H 4.00 (dddd, *J* = 8.8, 7.8, 4.8 and 3.8 Hz) and δ_C 75.3 were assigned to methane oxygenated CH-6, using the ^{1}H-^{1}H COSY correlations with methylene protons CH_2-5 and CH_2-7 at δ_H 1.54 (m, H-7a), 1.48 (m, H-7b) and δ_C 33.7 (C-7). All assignments of ^{1}H and ^{13}C NMR in DMSO-d_6 of the compound are shown in Table S1.

- **Table S1.** ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC-TOCSY connectivity and most representatives ^{1}H-^{13}C HMBC connectivity for 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in DMSO-d_6.

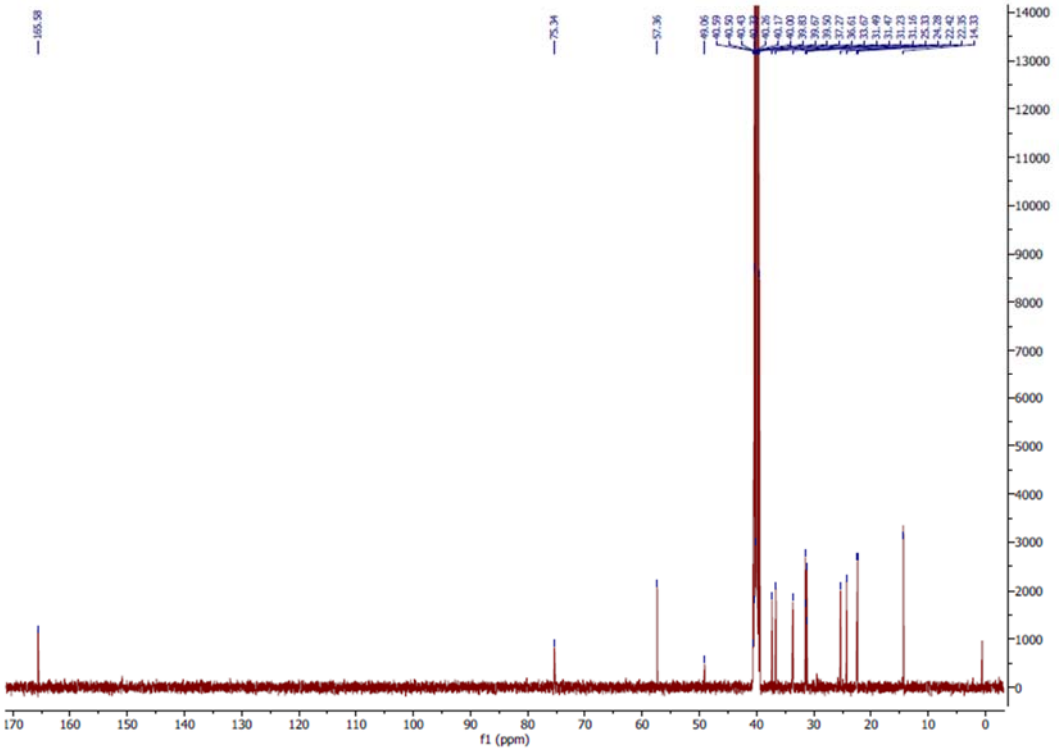
No	δ_C, DEPT-135	δ_H, m (*J*, Hz)	^{1}H-^{1}H COSY	^{1}H-^{13}C HMBC
4	150.8			5, 1'
5	31.5, CH_2	2.39, dd (18.3, 3.8)	5, 6	4, 6, 7
		2.17, dd (18.3, 8.8)	5, 6	4, 6, 7
6	75.3, CH	4.00, dddd (8.8, 7.8, 4.8, 3.8)	5, 7	5
7	33.7, CH_2	1.54, m	6, 8	5
		1.48, m		
8	24.3, CH_2	1.47, m	9, 7	
		1.36, m		
9	31.5, CH_2	1.27, m	8, 10	11
10	22.6, CH_2	1.30, m	9, 11	11
11	14.3, CH_3	0.87, t (6.8)	10	9, 10
1'	36.3, CH_2	2.23, t (7.3)	2'	4, 2'
		2.22, t (7.3)		
2'	25.3, CH_2	1.53, m	1', 3'	1'
3'	31.2, CH_2	1.29, m	2', 4'	5'
4'	22.4, CH_2	1.30, m	3', 5'	5'
5'	14.3, CH_3	0.88, t (7.3)	4'	3', 4'
1''	165.6			2'', 3''
2''	37.3, CH_2	2.69, m (6.6)	3''	1'', 3'', OH
		2.68, m (6.6)		
3''	57.4, CH_2	3.66, td (6.6, 5.4)	2'', OH	1'', 2'', OH
OH		4.57, t (5.4)	3''	2'', 3''



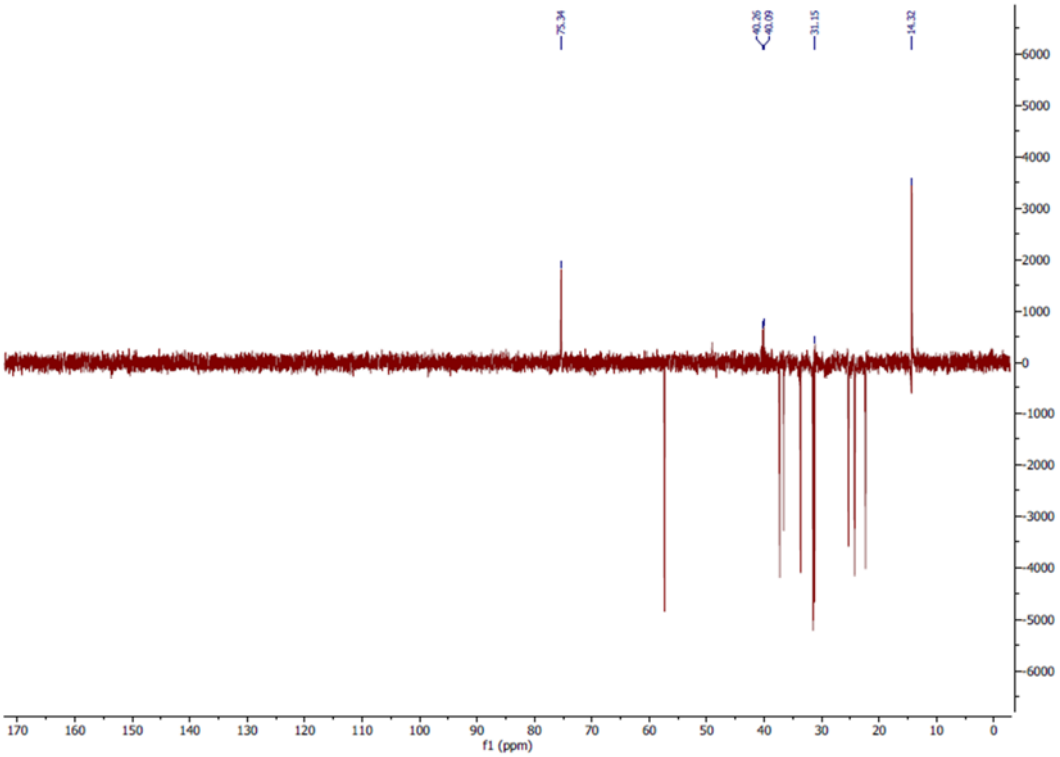
- **Figure S1**. HRESIMS spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**).



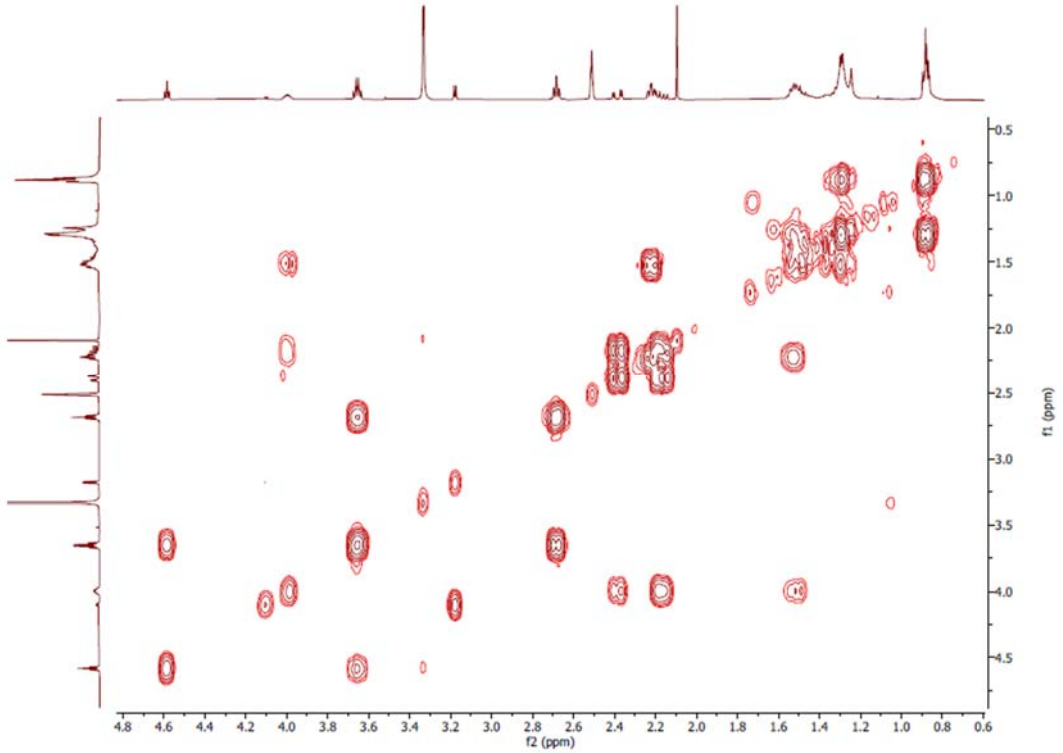
- **Figure S2**. [1]H NMR spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in DMSO 500 MHz.



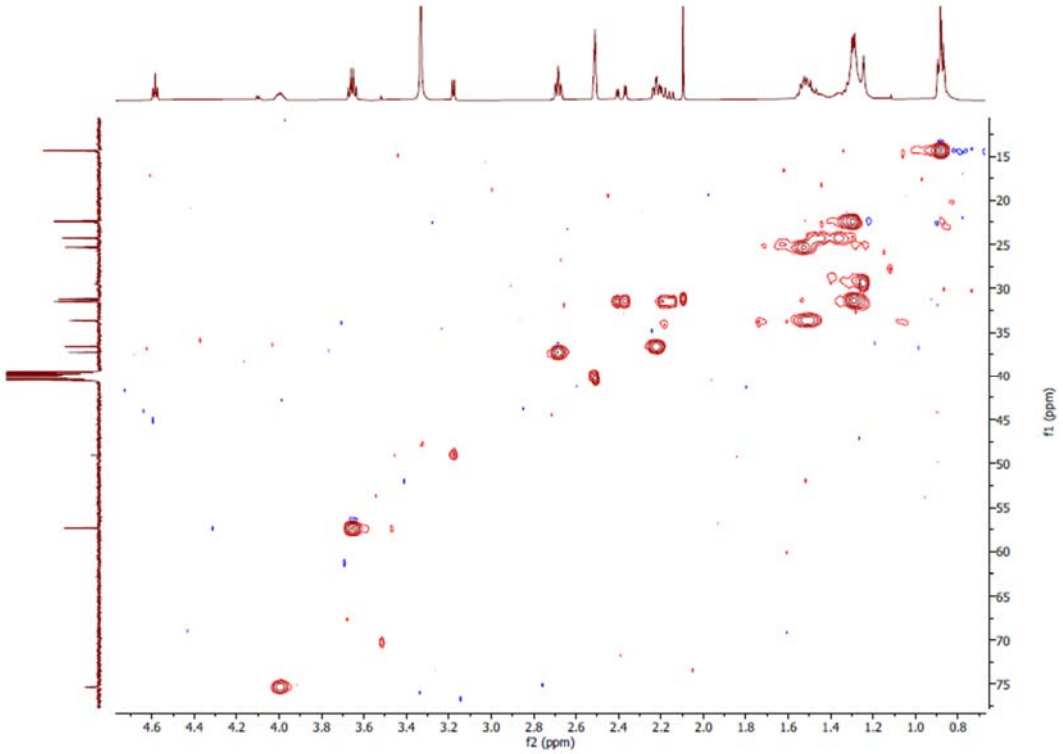
- **Figure S3**. ^{13}C NMR spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in DMSO 175 MHz.



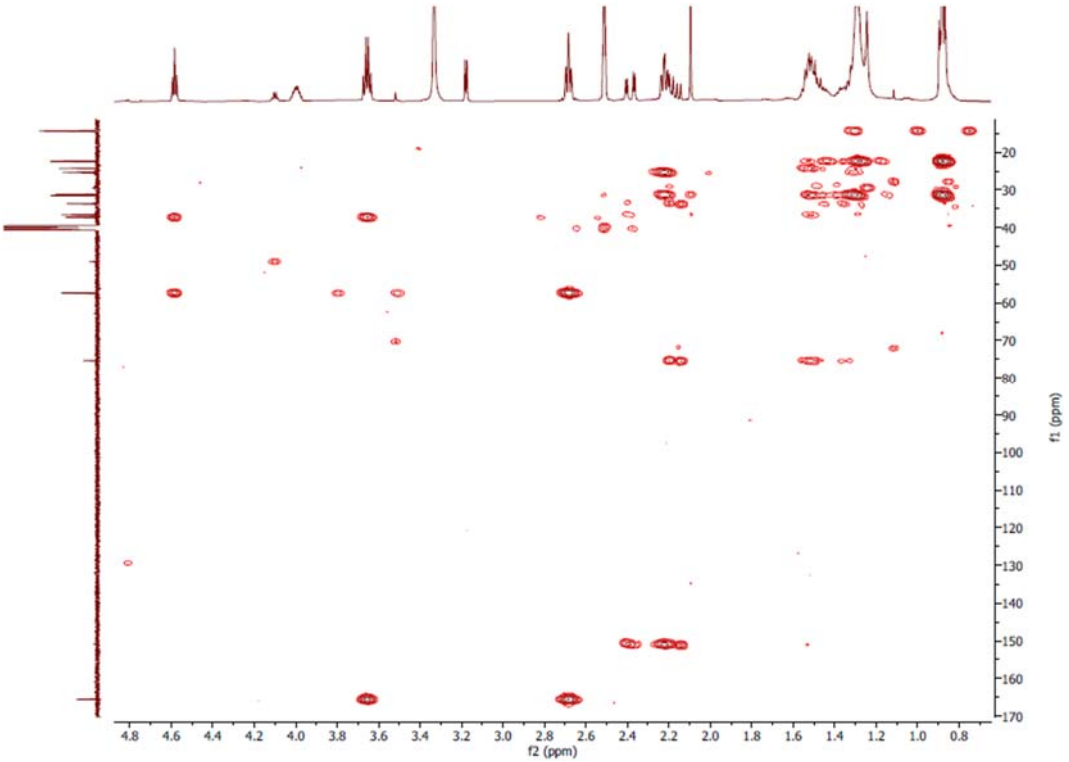
- **Figure S4**. DEPT-135 spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in DMSO 175 MHz.



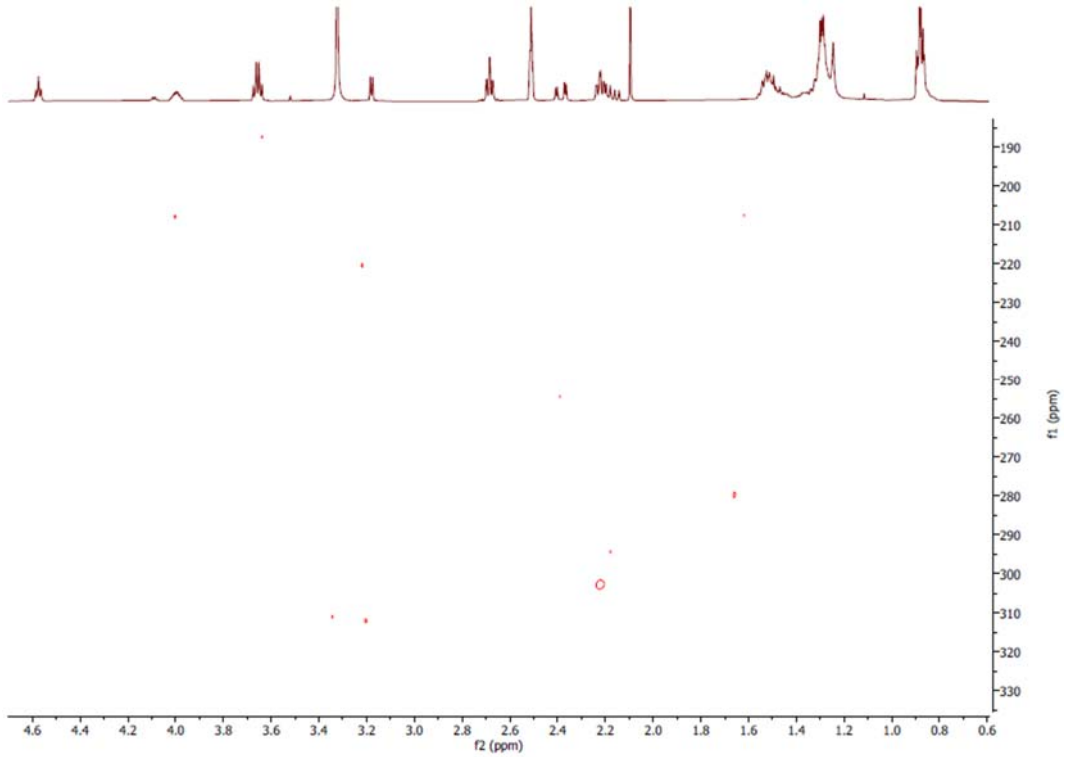
- **Figure S5**. ^{1}H-^{1}H COSY spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in DMSO.



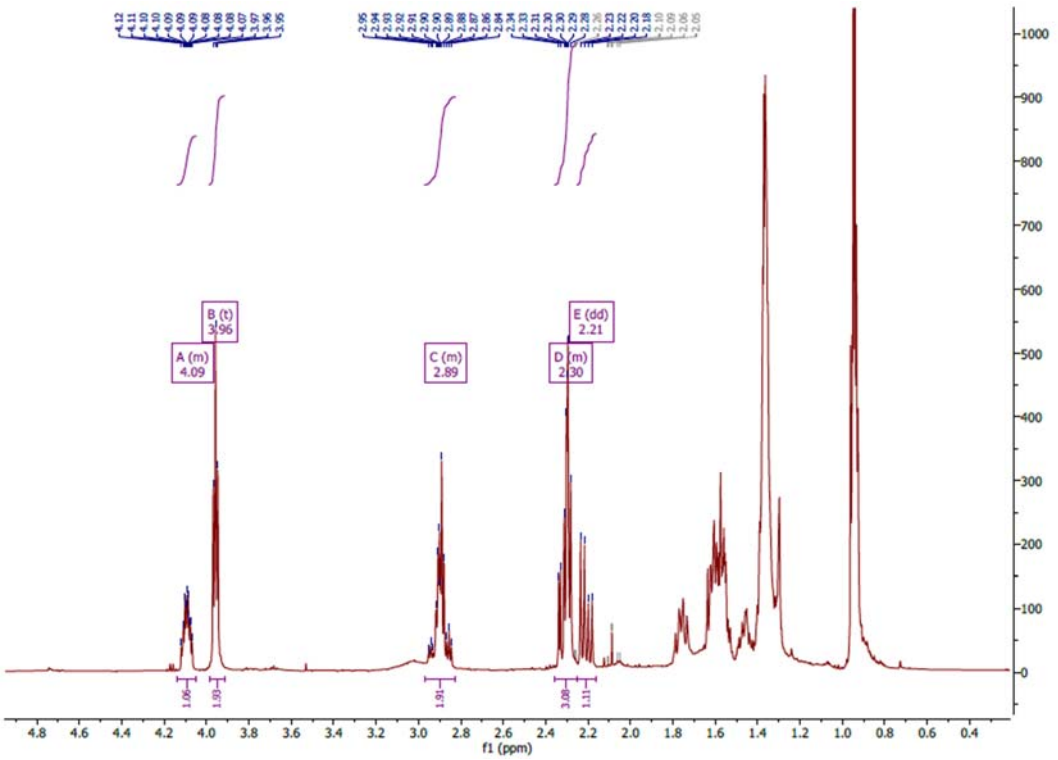
- **Figure S6**. ^{1}H-^{13}C HSQC spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in DMSO.



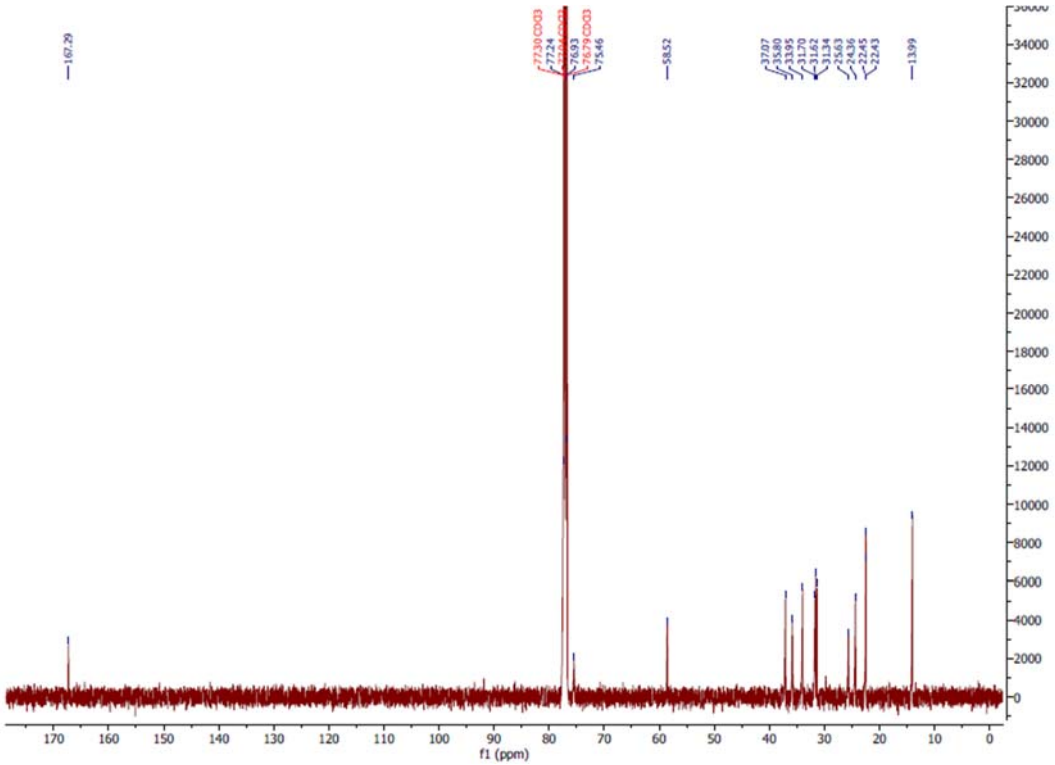
- **Figure S7**. ^{1}H-^{13}C HMBC spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in DMSO.



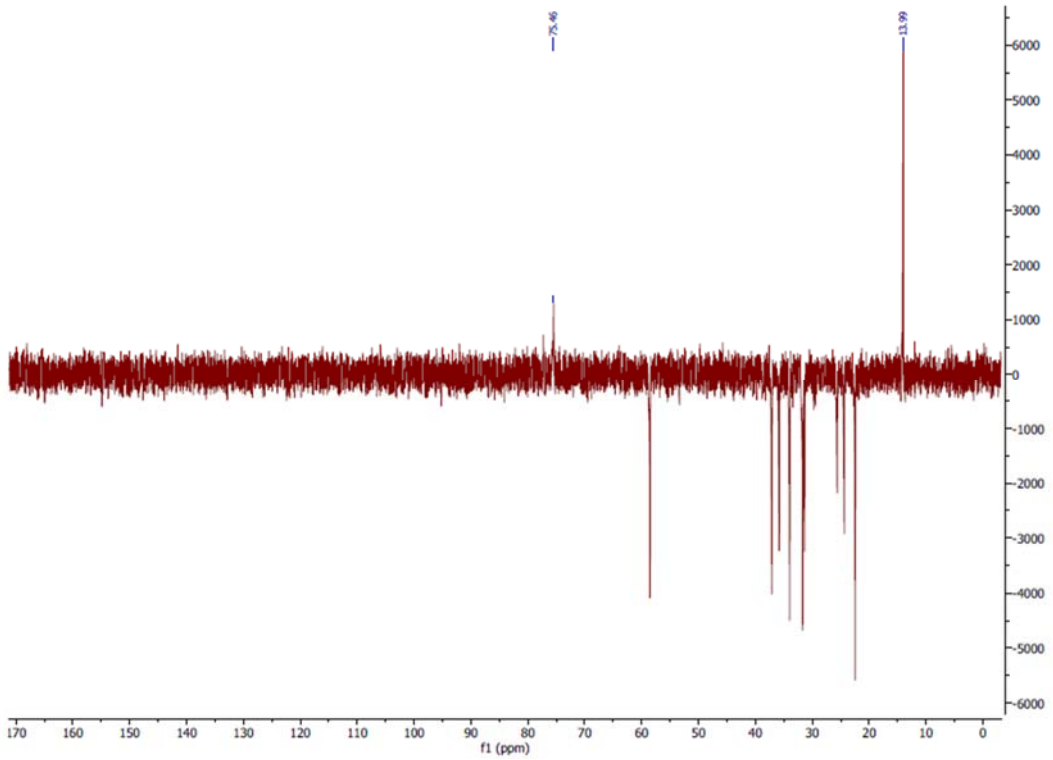
- **Figure S8**. ^{1}H-^{15}N HMBC spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in DMSO.



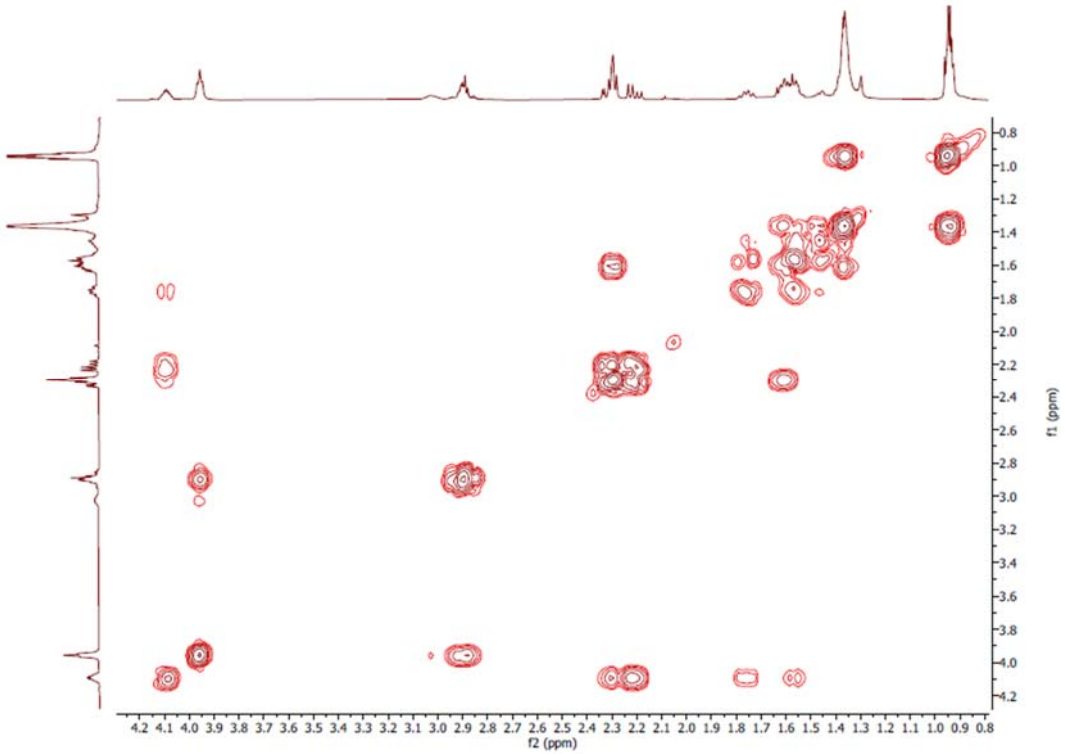
- **Figure S9**. ^{1}H NMR spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in $CDCl_3$ 500 MHz.



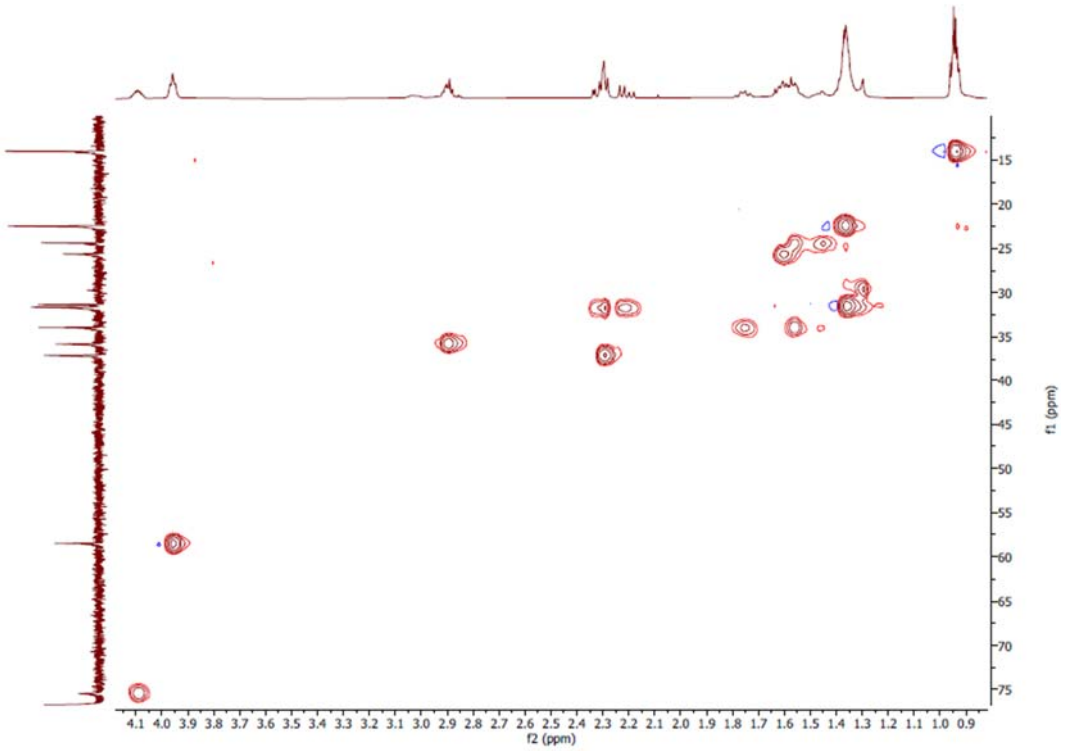
- **Figure S10**. ^{13}C NMR spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in $CDCl_3$ 175 MHz.



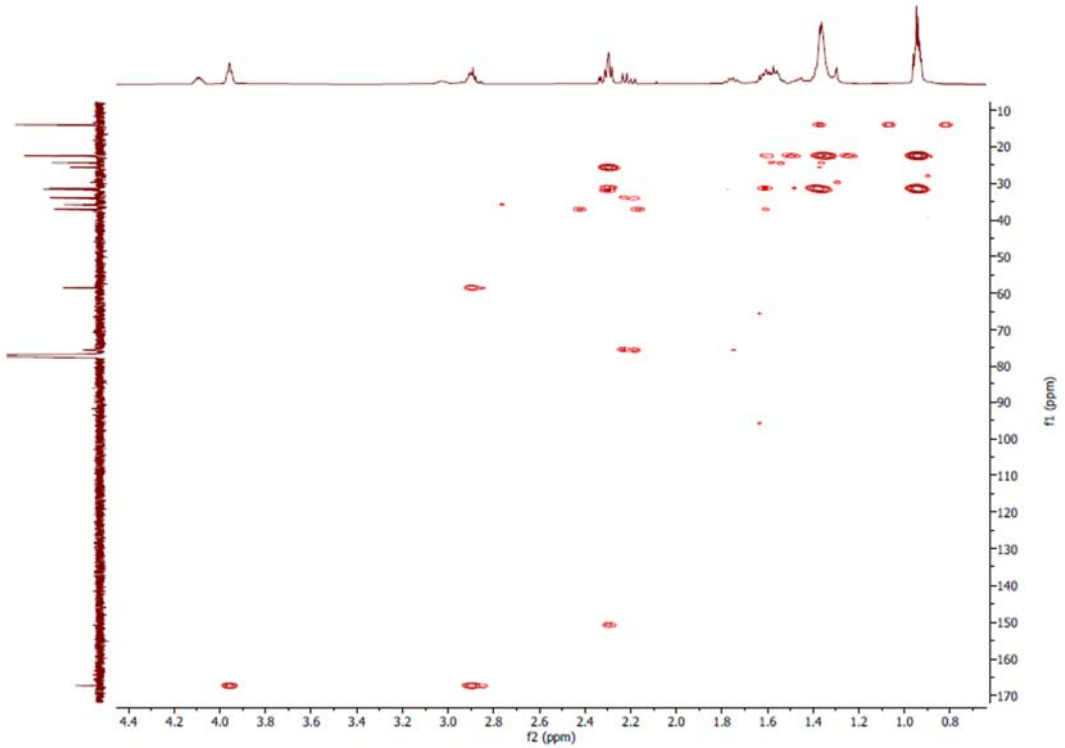
- **Figure S11**. DEPT-135 spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in $CDCl_3$ 175 MHz.



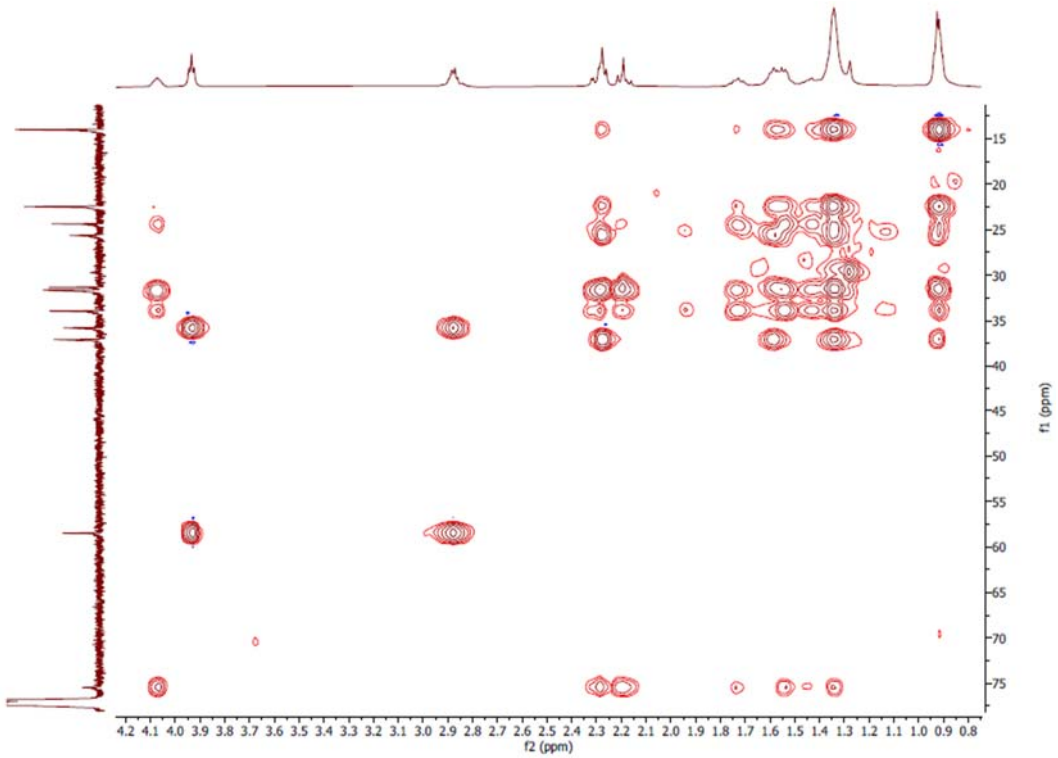
- **Figure S12**. ^{1}H-^{1}H COSY spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in $CDCl_3$.



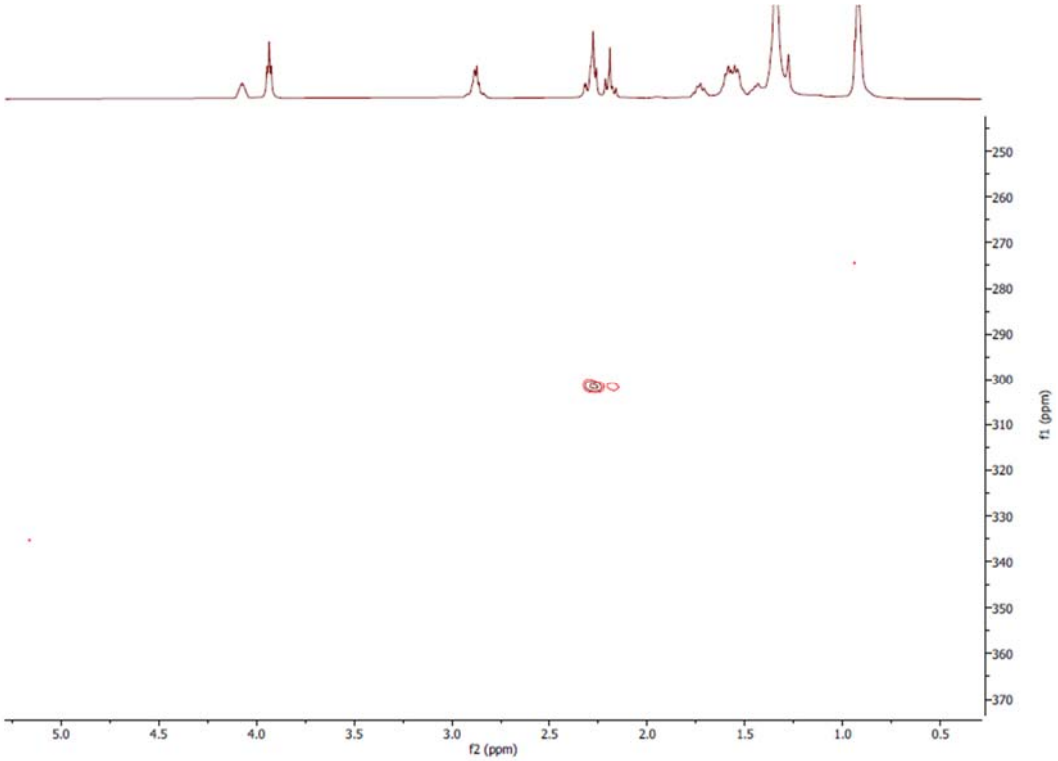
- **Figure S13**. ^{1}H-^{13}C HSQC spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in $CDCl_3$.



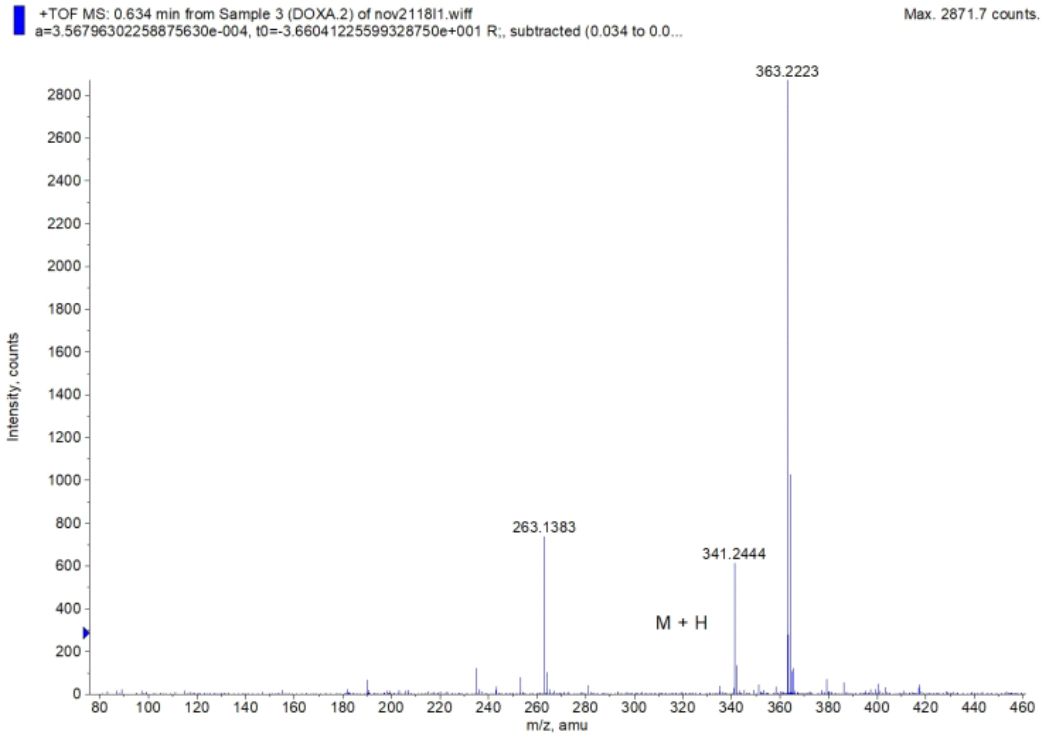
- **Figure S14**. ^{1}H-^{13}C HMBC spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in $CDCl_3$.



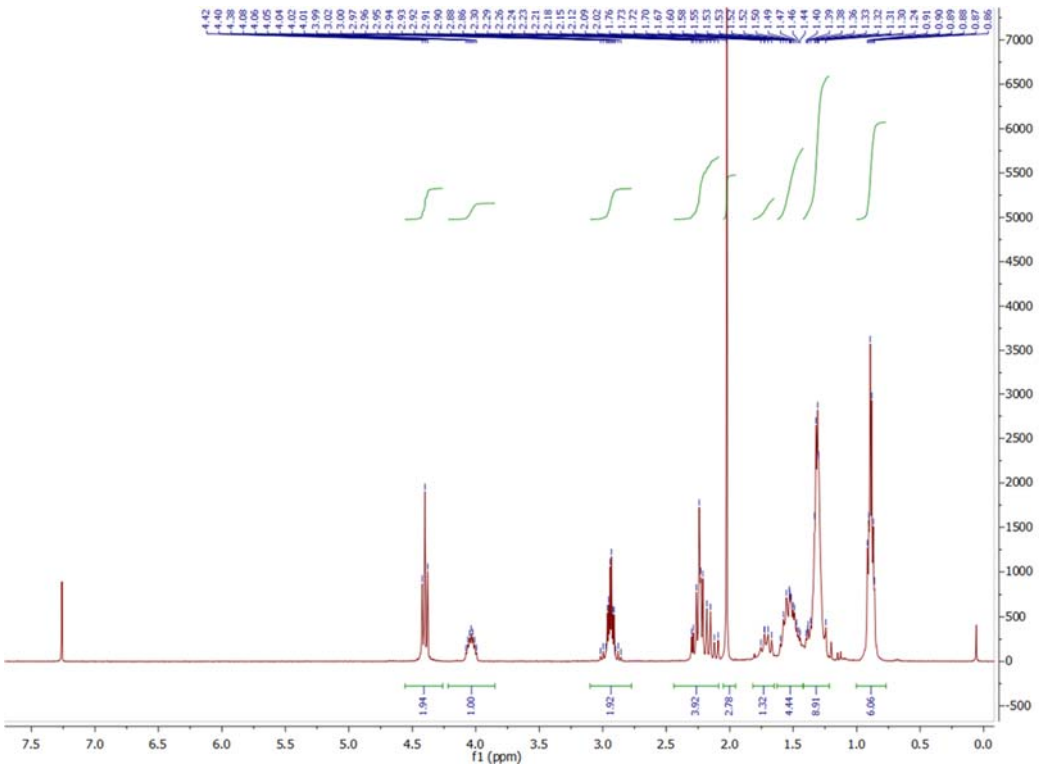
- **Figure S15**. ^{1}H-^{13}C HSQC-TOCSY spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in $CDCl_3$.



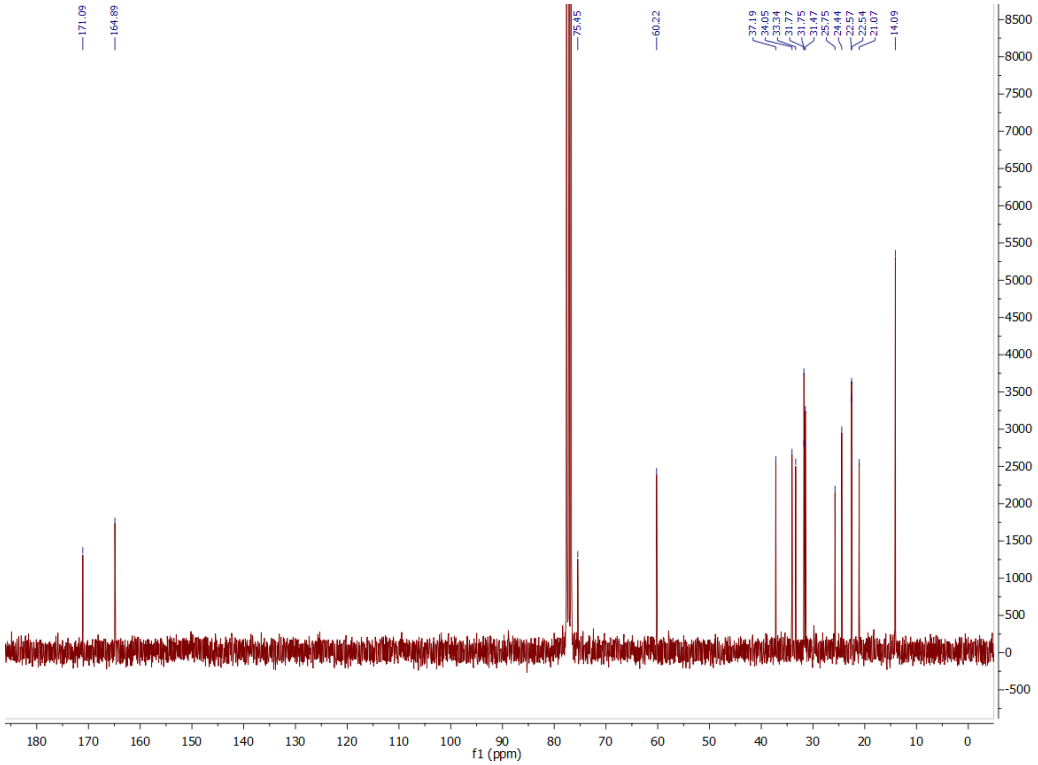
- **Figure S16**. ^{1}H-^{15}N HMBC spectrum of 1-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-hydroxypropan-1-one (**1**) in $CDCl_3$.



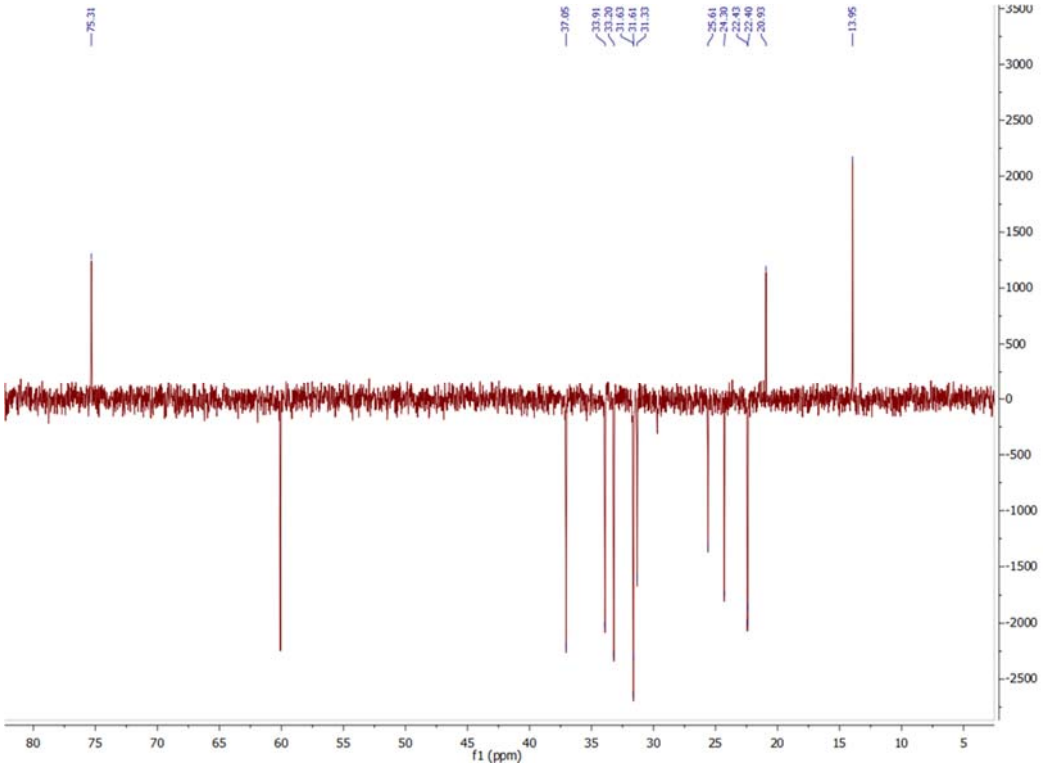
- **Figure S17**. HRESIMS spectrum of 3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropyl acetate (**2**).



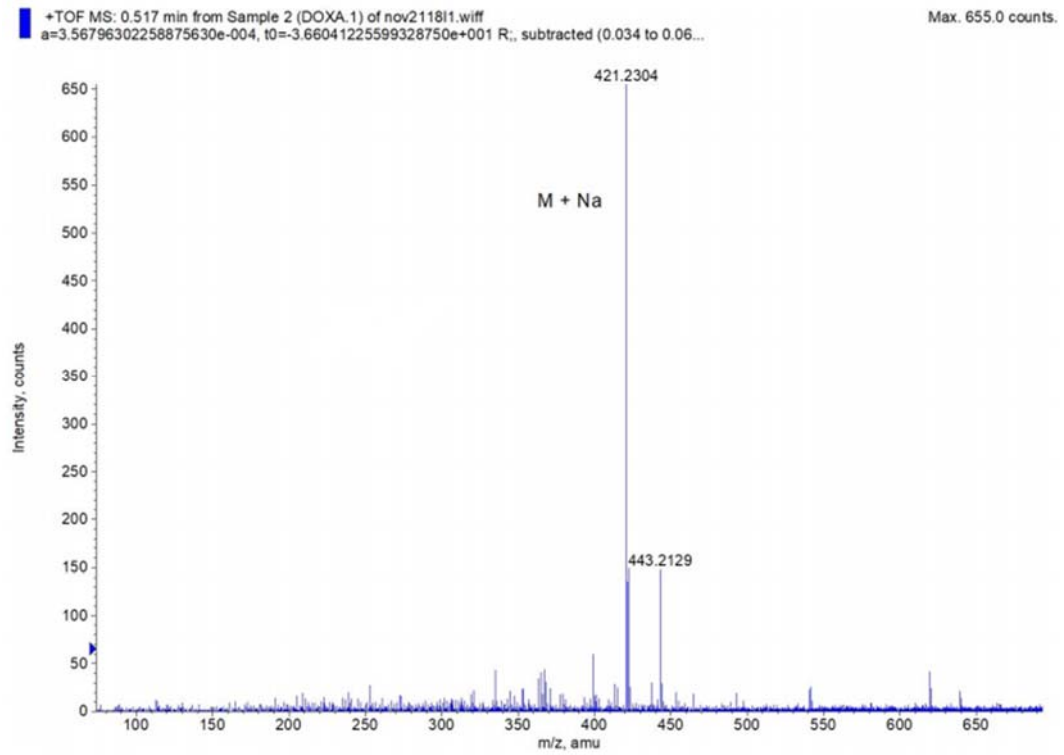
- **Figure S18**. ^{1}H NMR spectrum of 3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropyl acetate (**2**) in $CDCl_3$ 300 MHz.



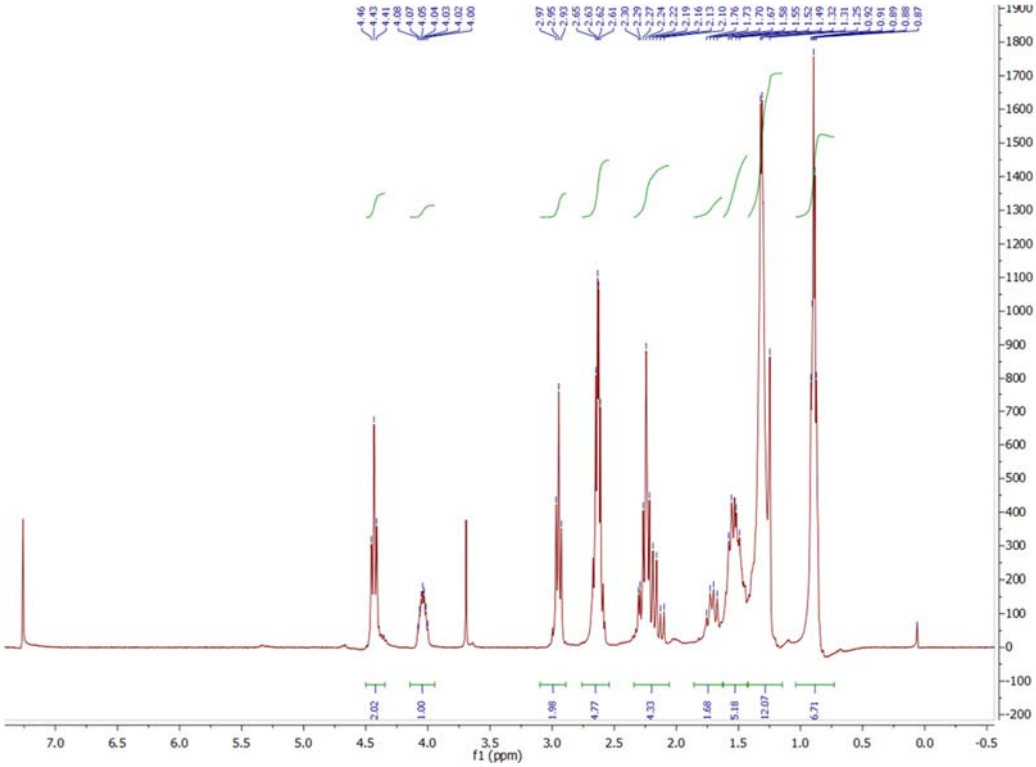
- **Figure S19**. ^{13}C NMR spectrum of 3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropyl acetate (**2**) in $CDCl_3$ 75 MHz.



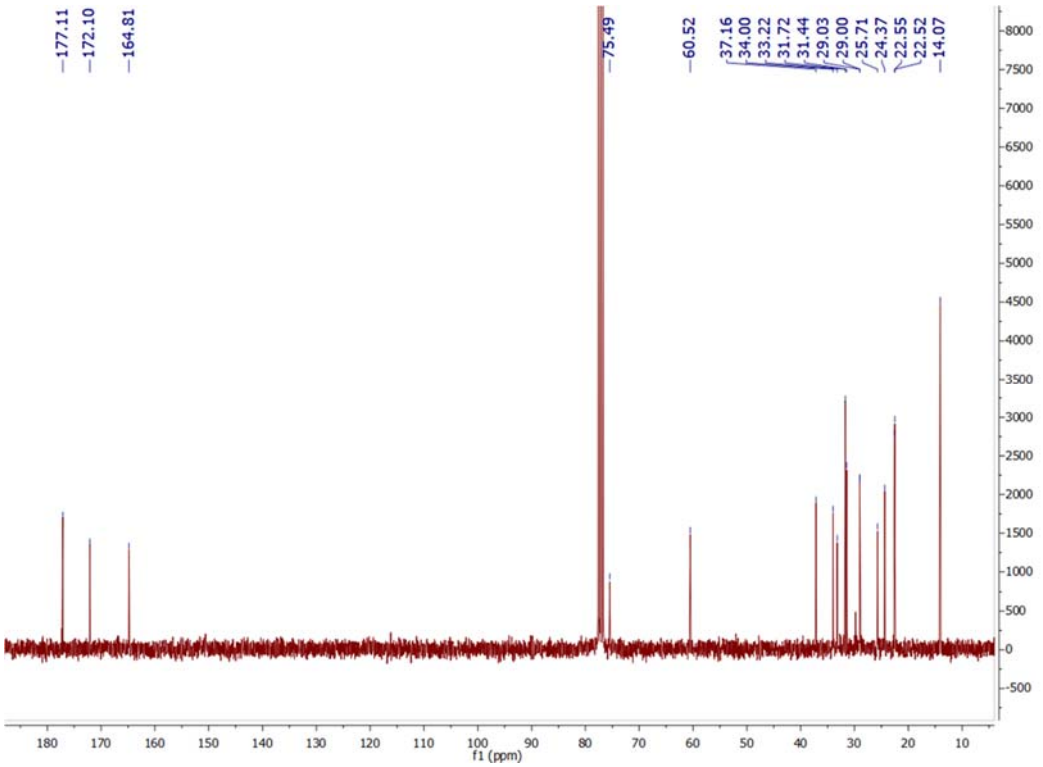
- **Figure S20**. DEPT-135 spectrum of 3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropyl acetate (**2**) in $CDCl_3$ 75 MHz.



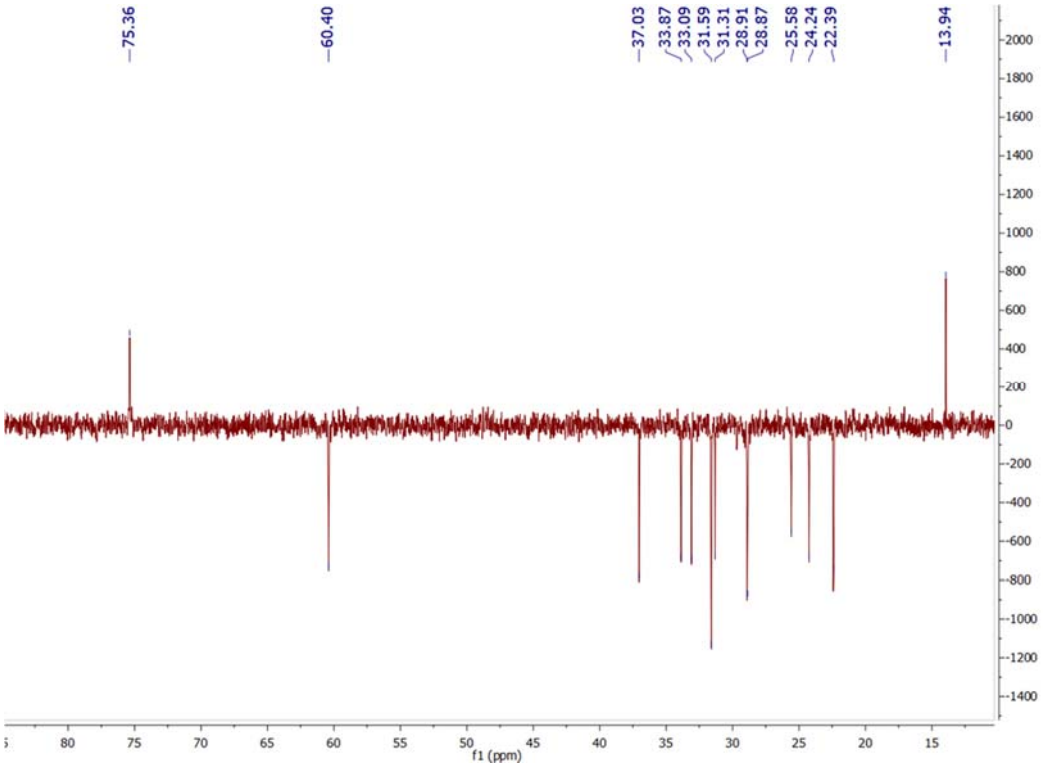
- **Figure S21**. HRESIMS spectrum of 4-{3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropoxy}-4-oxobutanoic acid (**3**).



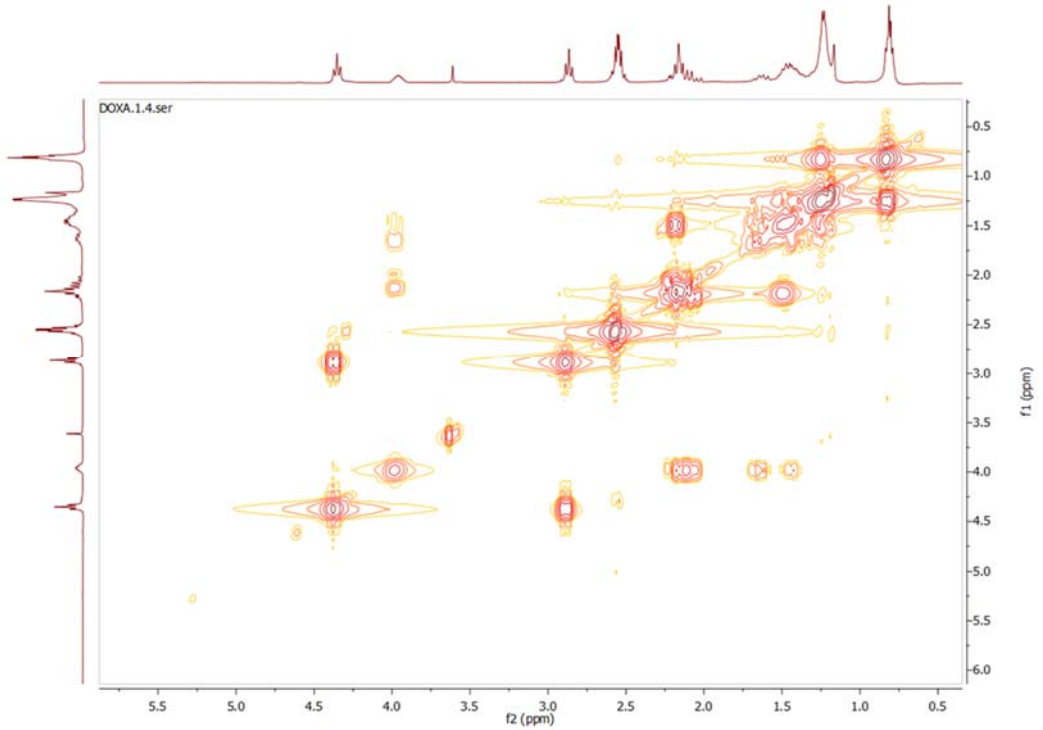
- **Figure S22**. ^{1}H NMR spectrum of 4-{3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropoxy}-4-oxobutanoic acid (**3**) in $CDCl_3$ 300 MHz.



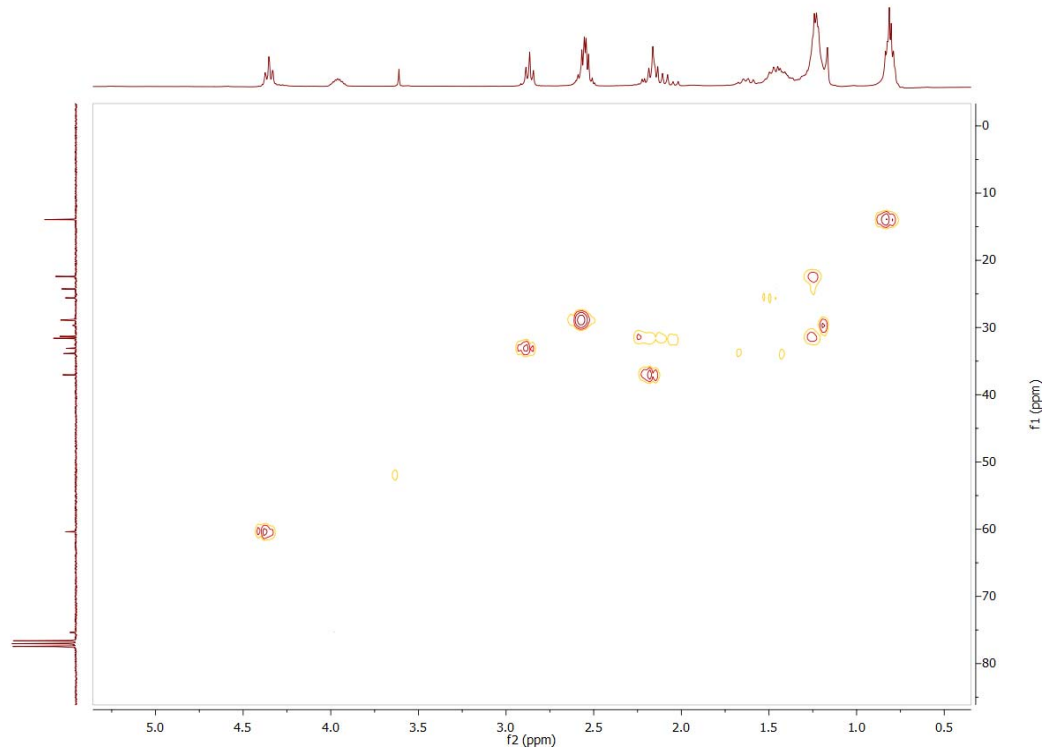
- **Figure S23**. ^{13}C NMR spectrum of 4-{3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropoxy}-4-oxobutanoic acid (**3**) in $CDCl_3$ 75 MHz.



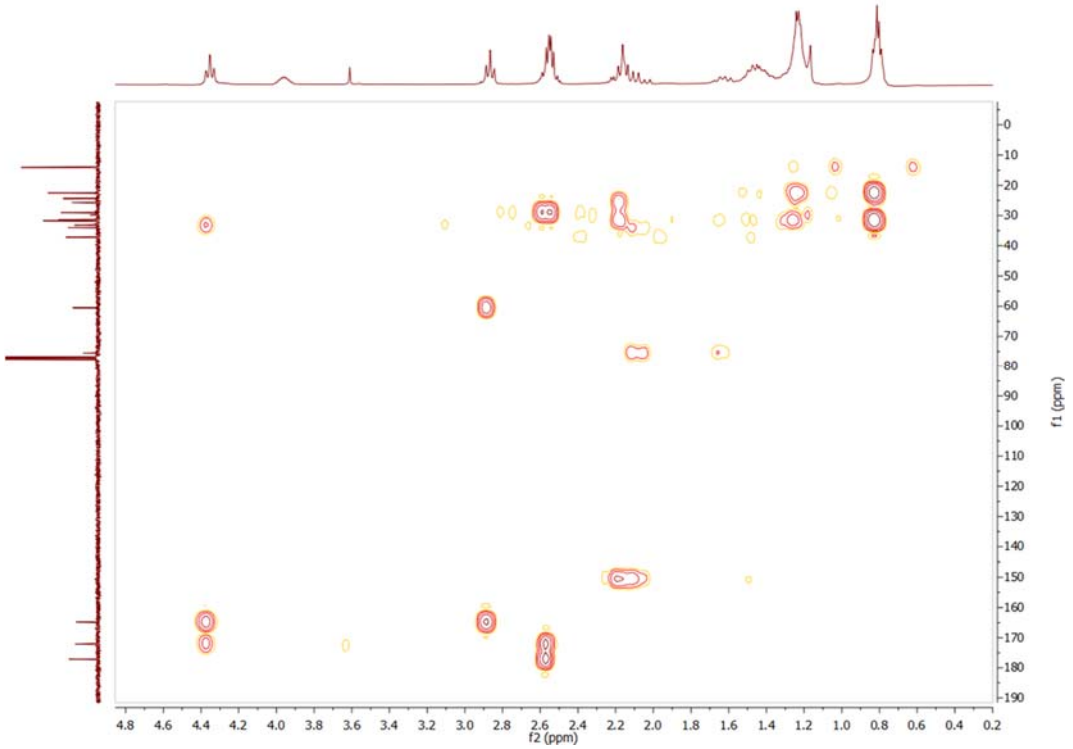
- **Figure S24**. DEPT-135 spectrum of 4-{3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropoxy}-4-oxobutanoic acid (**3**) in $CDCl_3$ 75 MHz.



- **Figure S25**. ^{1}H-^{1}H COSY spectrum of 4-{3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropoxy}-4-oxobutanoic acid (**3**) in $CDCl_3$.



- **Figure S26**. ^{1}H-^{13}C HSQC spectrum of 4-{3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropoxy}-4-oxobutanoic acid (**3**) in $CDCl_3$.



- **Figure S27**. ^{1}H-^{13}C HMBC spectrum of 4-{3-[(6*R*)-5,6-Dihydro-4,6-dipentyl-2*H*-1,2,3-oxadiazin-2-yl]-3-oxopropoxy}-4-oxobutanoic acid (**3**) in $CDCl_3$.