Electronic supplementary materials *Mendeleev Commun.*, 2025, **35**, 444–446

Isoxazole-linked 7-hydroxycoumarin–2,6-dimethylheptane conjugates as inhibitors of TDP1 enzyme

Dmitry O. Tsypyshev, Alexandra L. Zakharenko, Maria V. Podurina, Tatyana M. Khomenko, Evgeniy V. Kondrashov, Olga I. Lavrik, Konstantin P. Volcho and Nariman F. Salakhutdinov

Table of content

S1. Experimental. S1

S2. ^{1}H and ^{13}C NMR spectra of compounds **12, 14 - 17**. S4

S3. 2D NMR spectra of compound **15** S14

S4. HRMS spectra of compounds **12, 14-17**. S18

References S23

S1. Experimental.

Reagents and solvents were purchased from commercial suppliers (Sigma-Aldrich, Acros, Japan) and used as received. GC-MS: Agilent 7890A gas chromatograph equipped with an Agilent 5975C quadrupole mass spectrometer as a detector; quartz column HP-5MS (copolymer 5%–diphenyl–95%–dimethylsiloxane) of length 30 m, internal diameter 0.25 mm and stationary phase film thickness 0.25 μm. Optical rotation: polAAr 3005 spectrometer. ^{1}H and ^{1}C NMR: Bruker Avance—II 400 apparatus at 400.10 MHz (^{1}H) and 101.01 MHz (^{13}C), J in Hz; structure was determined by analyzing the ^{1}H NMR spectra, including ^{1}H–^{1}H double resonance spectra and ^{1}H–^{1}H 2D homonuclear correlation (COSY, NOESY), J-modulated ^{13}C NMR spectra (JMOD), and ^{13}C–^{1}H 2D heteronuclear correlation with one-bond (C–H COSY, ^{1}J(C,H) = 160 Hz, HSQC, ^{1}J(C,H) = 145 Hz) and long-range spin–spin coupling constants (C–H COSY, ^{1}J(C,H) = 160 Hz, HSQC, ^{1}J(C,H) = 145 Hz). HR-MS: DFS Thermo Scientific spectrometer in a full scan mode (15–500 m/z, 70 eV electron impact ionization, direct sample administration).

Spectral and analytical investigations were carried out at the Collective Chemical Service Center of the Siberian Branch of the Russian Academy of Sciences. All product yields are given for pure compounds purified by recrystallization from ethanol or isolated by column chromatography (SiO_2; 60–200 μ; Macherey-Nagel). The purity of the target compounds was determined by GC-MS methods. All of the target compounds reported in this paper had a purity of no less than 95%.

Syntheses of coumarins **6-8** were carried out from resorcinol **2** and appropriate β-keto esters **3-5**, in accordance with [S1]. Concentrated H_2SO_4 (5 mL, 94 mmol) was added dropwise to cooled (0–5 °C) solution of resorcinol (47.5 mmol) and appropriate β-keto ester (47.5 mmol) in dry ethanol (12.5 mL) with vigorous stirring. The mixture was stirred until it congealed, left overnight at room temperature, and poured into ice water (150 ml). The resulting solid was filtered off and crystallized from ethanol–water (75%). The yields of products **6**, **7** and **8** were 70%, 72%, and 70%, respectively.

To synthesize the 3,7-dimethyloctanal **10**, a solution of 10 mmol of alcohol **9** in 10 mL of dry CH_2Cl_2 was added while stirring to a suspension of pyridinium

chlorochromate (15 mmol) in 40 mL of dry CH_2Cl_2 at 20 °C under argon [S2]. Stirring was continued for 2 h. The mixture was diluted with 100 mL of diethyl ether and filtered through silica gel. The residue on the filter was washed with 100 mL of diethyl ether, and the filtrate was evaporated. The yield of the compound **10** was 94%.

To obtain oxime **11**, to a vigorously stirred solution of hydroxylamine hydrochloride (20 mmol) in water (10 mL), in which **10** (16 mmol) was suspended or dissolved, a solution of sodium bicarbonate (20 mmol) in water (15 mL) was added, and the mixture was stirred and heated for another 5 hours [S3]. The organic layer thus formed was separated, and the aqueous layer was extracted with ether and combined with the organic layer. This was dried with sodium sulfate and evaporated. The yield of compound **11** was 74%.

To obtain **12**, to a solution of oxime **11** (6.89 mmol) in 5 mL of 2,3-dichloropropene, 1 drop of pyridine is added, followed by N-chlorosuccinimide (7.58 mmol) under stirring. Dissolution of NCS occurs followed by precipitation of succinimide. The mixture is stirred at room temperature for 30 min and then heated to 40°C for 12 hours. Then, a solution of triethylamine (15.5 mmol) in 1 mL of 2,3-dichloropropene is added at room temperature and vigorous stirring [S4]. Heating and formation of a precipitate occurs. The reaction mixture is stirred for 1 hour, the solvent is distilled off *in vacuo*, the target compound is separated from the resulting residue by column chromatography using chloroform as an eluent. The yield of compound **12** was 97%.

5-(Chloromethyl)-3-(2,6-dimethylheptyl)isoxazole (**12**) Yield: 97%. Yellow liquid. HRMS: found: m/z=228.1154 $[M\text{-}CH_3]^+$, calcd: 228.1150 $(C_{12}H_{19}ON^{35}Cl)^+$. ^1H NMR (400 MHz, $CDCl_3$, δ) 0.83 (dd, J = 0.7, 6.6 Hz, 6H-C(1, 11)), 0.88 (d, J = 6.7 Hz, 3H-C(12)), 1.09 - 1.33 (m, 6H-C(3, 4, 5)), 1.49 (dp, J = 6.6, 13.3 Hz, 1H-C(2)), 1.77 (tdt, J = 4.4, 6.2, 7.8 Hz, 1H-C(6)), 2.44 (dd, J = 8.1, 14.3 Hz, 1H-C(7a)), 2.61 (dd, J = 6.0, 14.3 Hz, 1H-C(7b)), 4.55 (s, 2H-C(15)), 6.12 (s, 1H-C(13)). ^{13}C NMR (101 MHz, $CDCl_3$, δ) 19.34 (q, C-11), 22.41 (q, C-10), 22.52 (q, C-12), 24.54 (t, C-7), 27.77 (d, C-9), 32.47 (d, C-5), 33.14 (t, C-4), 34.43 (t, C-13), 36.68 (t, C-8), 38.89 (t, C-6), 103.65 (d, C-2), 163.32 (s, C-3), 166.84 (s, C-1).

To obtain isoxazoles **14-17**, a vial was loaded with 5 mL of acetone, 0.62 mmol of 5-chloromethylisoxazole **12**, 0.41 mmol of the corresponding hydroxycoumarin, and 1 mmol of potassium carbonate. The vial was put into an Anton Paar Monowave 50 reactor, and the reaction mixture was stirred for 3.5 hours at 125°C with the resulting pressure of 6 atmospheres [S5]. After completion, the organic layer was washed with water, the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated. The resulting product was purified using column chromatography on SiO_2, using hexane-ethyl acetate mixture with EtOAc concentration gradually increasing from 0% to 15%. The yields of **14, 15**, **16** and **17** were 40%, 33%, 45% and 32%, respectively.

3-((3-(2,6-Dimethylheptyl)isoxazol-5-yl)methoxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (**14**). Yield: 40%. Solid, m.p. 86.4°C. HRMS: found: m/z=423.2412 $[M]^+$, calcd: m/z= 423.2404 $(C_{26}H_{33}O_4N)^+$. ^1H NMR (400 MHz, $CDCl_3$, δ) 0.80 (dd, J = 1.4, 6.6 Hz, 6H-C(24, 26)), 0.86 (d, J = 6.7 Hz, 3H-C(25)), 1.04 – 1.32 (m, 6H-C(20,21,22)), 1.45 (dp, J = 6.7, 13.3 Hz, 1H-C(19)), 1.69 - 1.82 (m, 5H-C(23, 4, 5)), 2.43 (dd, J = 8.2, 14.3 Hz, 1H-C(18a)), 2.48 (t, J = 5.9 Hz, 2H-C(3)), 2.60 (dd, J = 5.9, 14.3 Hz, 1H-C(18b)), 2.67 (t, J = 5.2 Hz, 2H-C(6)), 5.11 (s, 2H-C(14)), 6.17 (s, 1H-C(16)), 6.78 (d, J = 2.6 Hz, 1H-C(12)), 6.83 (dd, J = 2.6, 8.8 Hz, 1H-C(10)), 7.41 (d, J =

8.8 Hz, 1H-C(9)). ^{13}C NMR (101 MHz, $CDCl_3$, δ) 19.30 (q, C-25), 21.11 (t, C-4), 21.40 (t, C-5), 22.33 (q, C-24), 22.44 (q, C-26), 23.64 (t, C-3), 24.46 (t, C-21), 24.98 (t, C-6), 27.69 (d, C-23), 32.40 (d, C-19), 33.05 (t, C-18), 36.63 (t, C-22), 38.84 (t, C-20), 61.25 (t, C-14), 101.52 (d, C-12), 103.68 (d, C-16), 111.85 (d, C-10), 114.39 (s, C-8), 120.94 (s, C-2), 124.17 (d, C-9), 146.82 (s, C-7), 153.07 (s, C-13), 159.17 (s, C-1), 161.60 (s, C-11), 163.08 (s, C-17), 166.12 (s, C-15).

7-((3-(2,6-Dimethylheptyl)isoxazol-5-yl)methoxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**15**). Yield: 33%. Solid, m.p. 70.2°C. HRMS: found: m/z=409.2246 $[M]^+$, calcd: m/z= 409.2248 $(C_{25}H_{31}O_4N)^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ) 0.82 (dd, J = 0.9, 6.6 Hz, 6H-C(23, 25)), 0.87 (d, J = 6.7 Hz, 3H-C(24)), 1.03 - 1.34 (m, 6H-C(19,20,21)), 1.47 (dp, J = 6.6, 13.2 Hz, 1H-C(18)), 1.71 - 1.82 (m, 1H-C(22)), 2.16 (p, J = 7.5 Hz, 2H-C(4)), 2.45 (dd, J = 8.1, 14.3 Hz, 1H-C(17a)), 2.62 (dd, J = 6.0, 14.3 Hz, 1H-C(17b)), 2.85 (tt, J = 1.9, 7.3 Hz, 2H-C(3)), 3.00 (tt, J = 1.8, 8.4 Hz, 2H-C(5)), 5.11 - 5.17 (m, 2H-C(13)), 6.18 (s, 1H-C(15)), 6.85 - 6.88 (m, 2H-C(9, 11)), 7.29 - 7.35 (m, 1H-C(8)). ^{13}C NMR (101 MHz, $CDCl_3$, δ) 19.33 (q, C-24), 22.34 (t, C-4), 22.38 (q, C-23), 22.49 (q, C-25), 24.52 (t, C-20), 27.74 (d, C-22), 30.23 (t, C-3), 31.88 (t, C-5), 32.46 (d, C-18), 33.09 (t, C-17), 36.67 (t, C-21), 38.87 (t, C-19), 61.32 (t, C-13), 101.71 (d, C-11), 103.75 (d, C-15), 112.13 (d, C-9), 113.09 (s, C-7), 125.03 (s, C-2), 125.69 (d, C-8), 155.36 (s, C-12), 155.92 (s, C-6), 159.76 (s, C-10), 160.06 (s, C-1), 163.17 (s, C-16), 166.06 (s, C-14).

7-((3-(2,6-Dimethylheptyl)isoxazol-5-yl)methoxy)-4-phenyl-2H-chromen-2-one (**16**). Yield: 45%. Amorphous compound. HRMS: found: m/z=445.2250 $[M]^+$, calcd: m/z= 445.2248 $(C_{28}H_{31}O_4N)^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ) 0.82 (d, J = 6.6 Hz, 6H-C(26, 28)), 0.88 (d, J = 6.6 Hz, 3H-C(27)), 1.06 - 1.34 (m, 6H-C(22,23,24)), 1.42 - 1.53 (m, 1H-C(21)), 1.78 (hept, J = 7.4 Hz, 1H-C(25)), 2.46 (dd, J = 8.1, 14.3 Hz, 1H-C(20a)), 2.63 (dd, J = 5.9, 14.3 Hz, 1H-C(20b)), 5.17 (s, 2H-C(16)), 6.20 (s, 1H-C(18)), 6.22 (s, 1H-C(2)), 6.83 (dd, J = 2.6, 8.9 Hz, 1H-C(6)), 6.93 (d, J = 2.5 Hz, 1H-C(8)), 7.37 - 7.43 (m, 3H-C(5, 11, 15)), 7.46 - 7.52 (m, 3H-C(12, 13, 14)). 13C NMR (101 MHz, CDCl3, δ) 19.35 (q, C-27), 22.41 (q, C-26), 22.52 (q, C-28), 24.54 (t, C-23), 27.76 (d, C-25), 32.48 (d, C-21), 33.11 (t, C-20), 36.68 (t, C-24), 38.88 (t, C-22), 61.33 (t, C-16), 102.00 (d, C-8), 103.85 (d, C-18), 112.26 (d, C-2), 112.30 (d, C-6), 113.19 (s, C-4), 128.14 (d, C-5), 128.20 (d, C-11, C-15), 128.73 (d, C-12, C-14), 129.55 (d, C-13), 135.15 (s, C-10), 155.46 (s, C-3), 155.60 (s, C-9), 160.55 (s, C-1), 160.81 (s, C-7), 163.22 (s, C-19), 165.86 (s, C-17).

7-((3-(2,6-Dimethylheptyl)isoxazol-5-yl)methoxy)-4-methyl-2H-chromen-2-one (**17**). Yield: 32%. Solid, m.p. 40.5°C with subsequent decomposition. HRMS: found: m/z=383.2085 $[M]^+$, calcd: m/z= 383.2091 $(C_{23}H_{29}O_4N)^+$. ^{1}H NMR (400 MHz, $CDCl_3$, δ) 0.82 (d, J = 6.7 Hz, 6H-C(22, 23)), 0.88 (d, J = 6.7 Hz, 3H-C(21)), 1.08 – 1.34 (m, 2H-C(17,18,19)), 1.47 (dp, J = 6.7, 13.3 Hz, 1H-C(16)), 1.78 (dq, J = 6.8, 13.3 Hz, 1H-C(20)), 2.36 (s, 3H-C(10)), 2.45 (dd, J = 8.1, 14.3 Hz, 1H-C(15a)), 2.62 (dd, J = 6.0, 14.3 Hz, 1H-C(15b)), 5.15 (s, 2H-C(11)), 6.11 (s, 1H-C(2)), 6.18 (s, 1H-C(13)), 6.84 (d, J = 2.6 Hz, 1H-C(8)), 6.89 (dd, J = 2.6, 8.8 Hz, 1H-C(6)), 7.49 (d, J = 8.8 Hz, 1H-C(5)). ^{13}C NMR (101 MHz, $CDCl_3$, δ) 18.42 (q, C-10), 19.30 (q, C-22), 22.33 (q, C-21), 22.43 (q, C-23), 24.46 (t, C-18), 27.69 (d, C-20), 32.41 (d, C-16), 33.06 (t, C-15), 36.64 (t, C-19), 38.84 (t, C-17), 61.31 (t, C-11), 101.81 (d, C-8), 103.74 (d, C-13), 112.16 (d, C-2), 112.35 (d, C-6), 114.23 (s, C-4), 125.62 (d, C-5), 152.10 (s, C-3), 154.88 (s, C-9), 160.40 (s, C-1), 160.70 (s, C-7), 163.12 (s, C-14), 165.91 (s, C-12).

S2. ^{1}H and ^{13}C NMR spectra of compounds 12, 14 - 17.

Fig. S1. ^{1}H NMR spectrum of Compound **12**

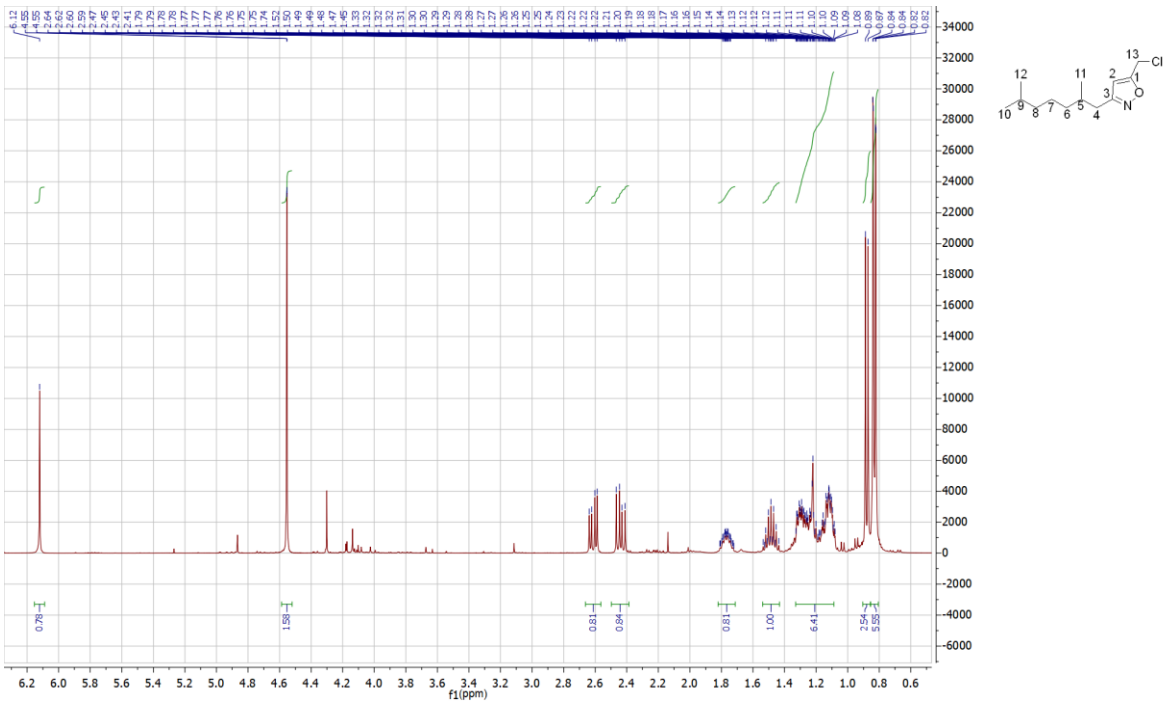


Fig. S2. ^{13}C NMR spectrum of Compound **12**

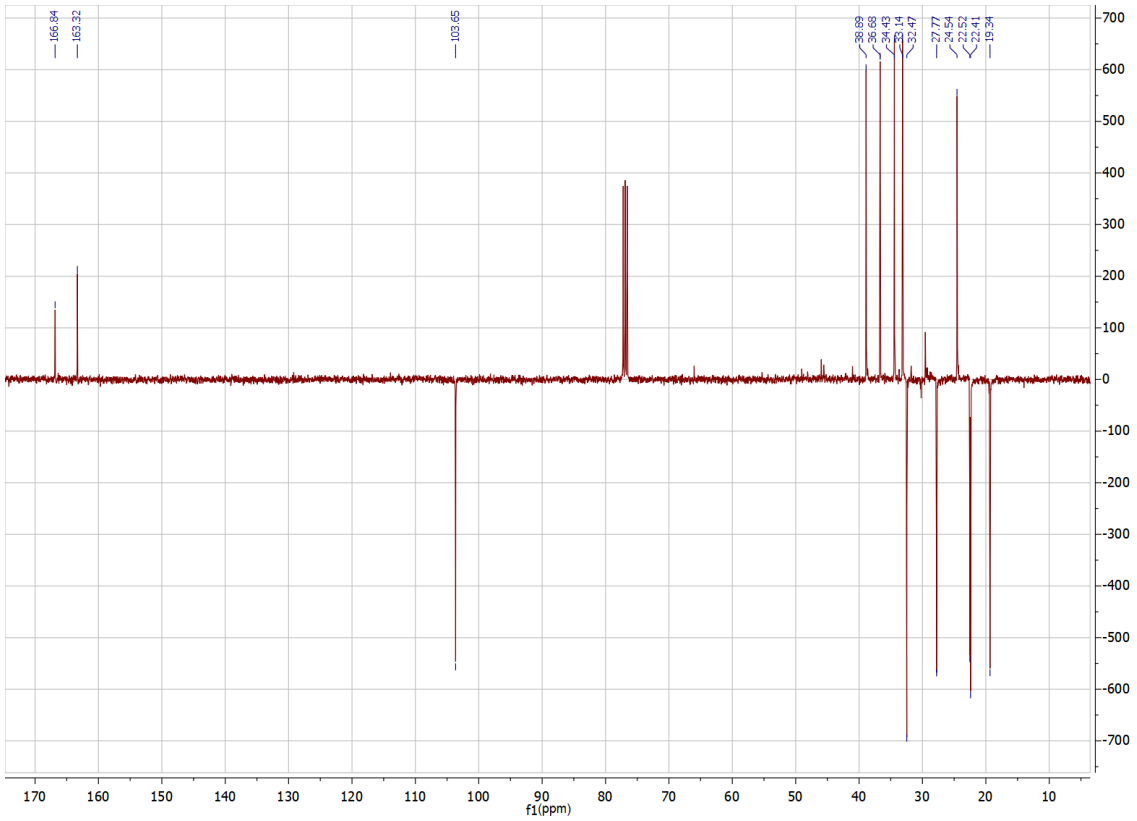


Fig. S3. ^{1}H NMR spectrum of Compound **14**

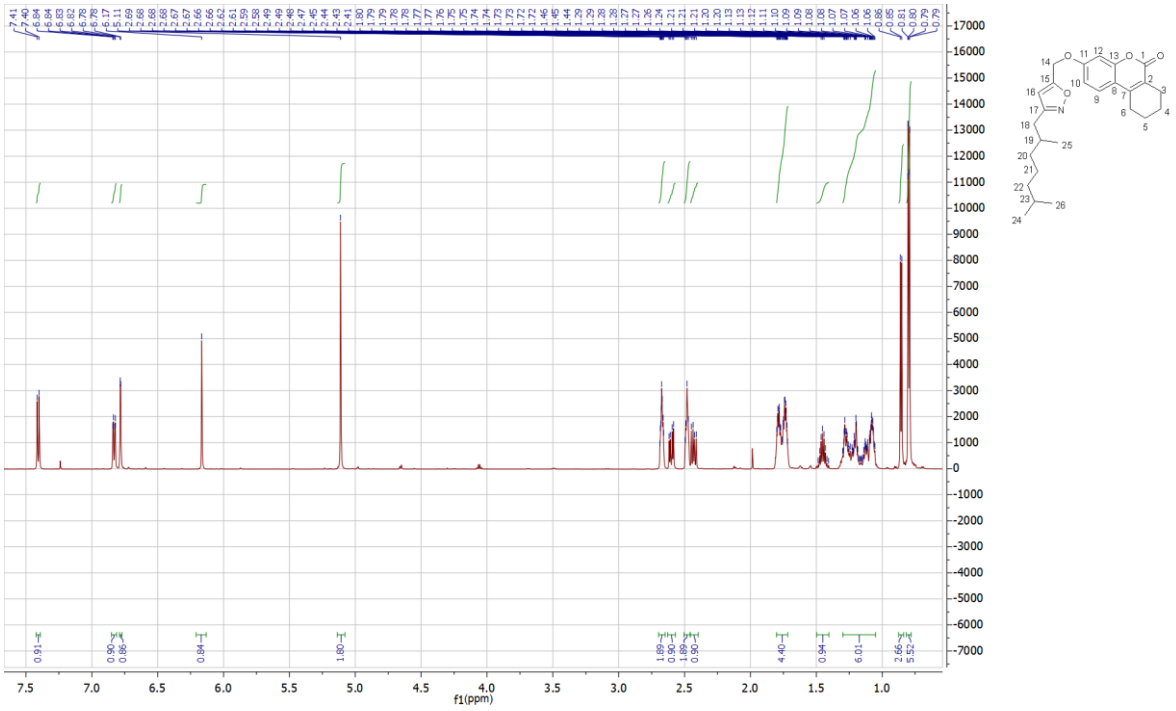


Fig. S4. ^{13}C NMR spectrum of Compound **14**

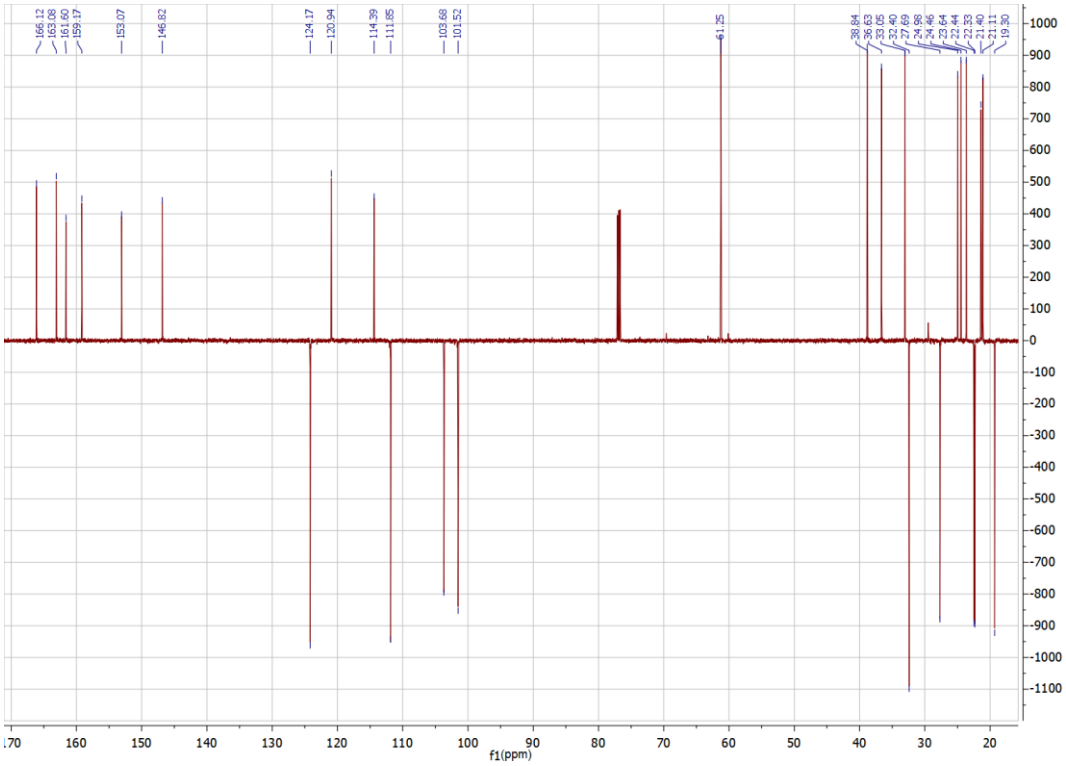


Fig. S5. ^{1}H NMR spectrum of Compound **15**

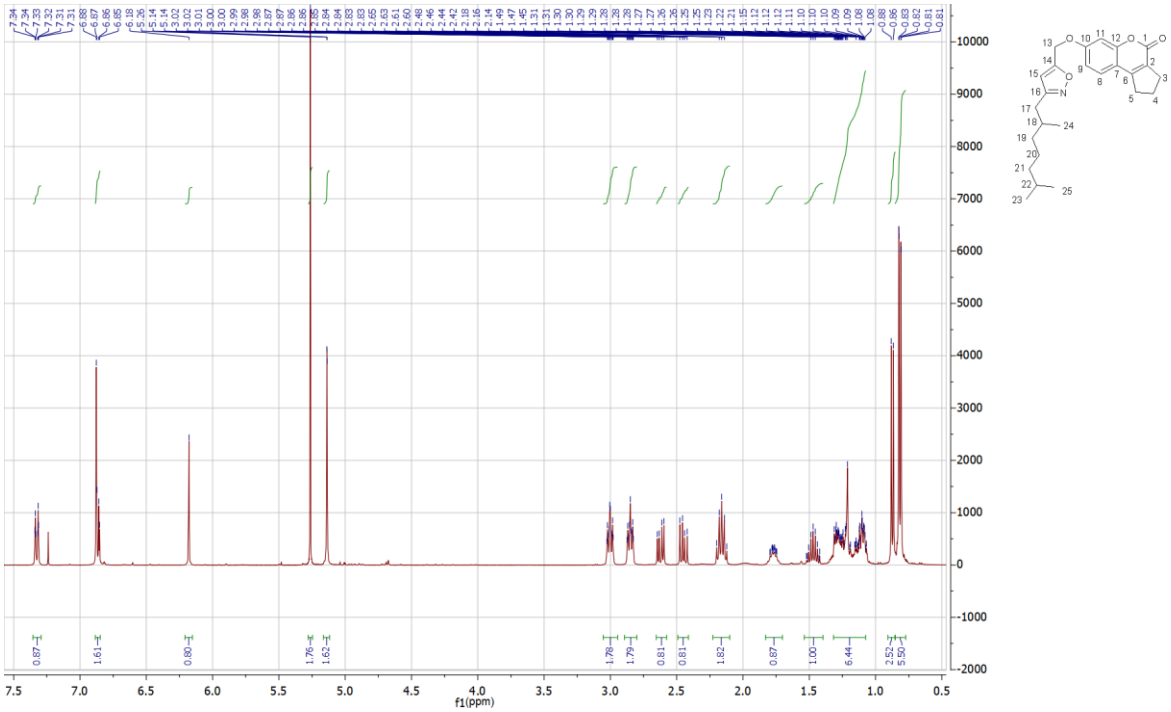


Fig. S6. ^{13}C NMR spectrum of Compound **15**

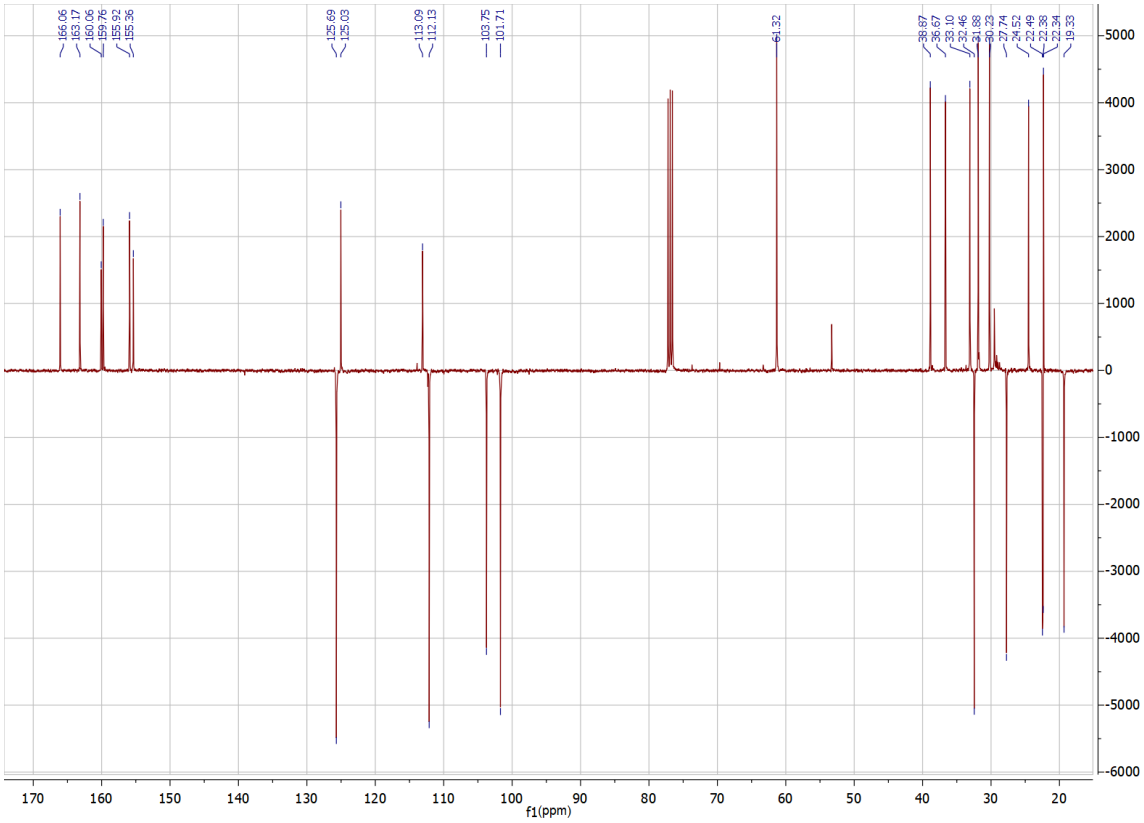


Fig. S7. ^{1}H NMR spectrum of Compound **16**

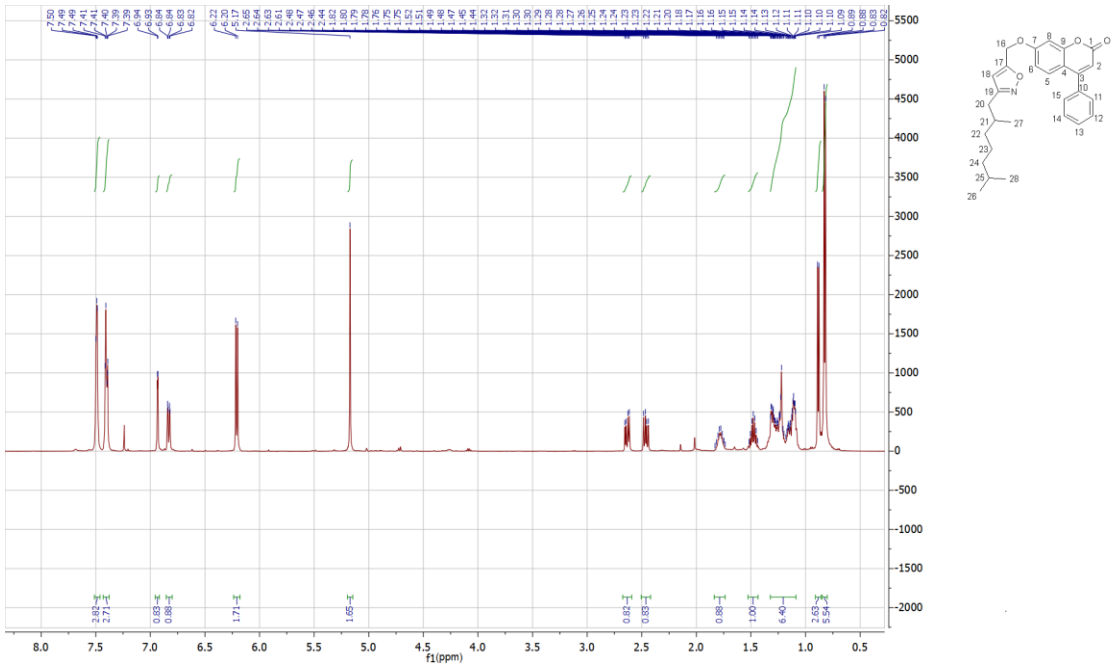


Fig. S8. ^{13}C NMR spectrum of Compound **16**

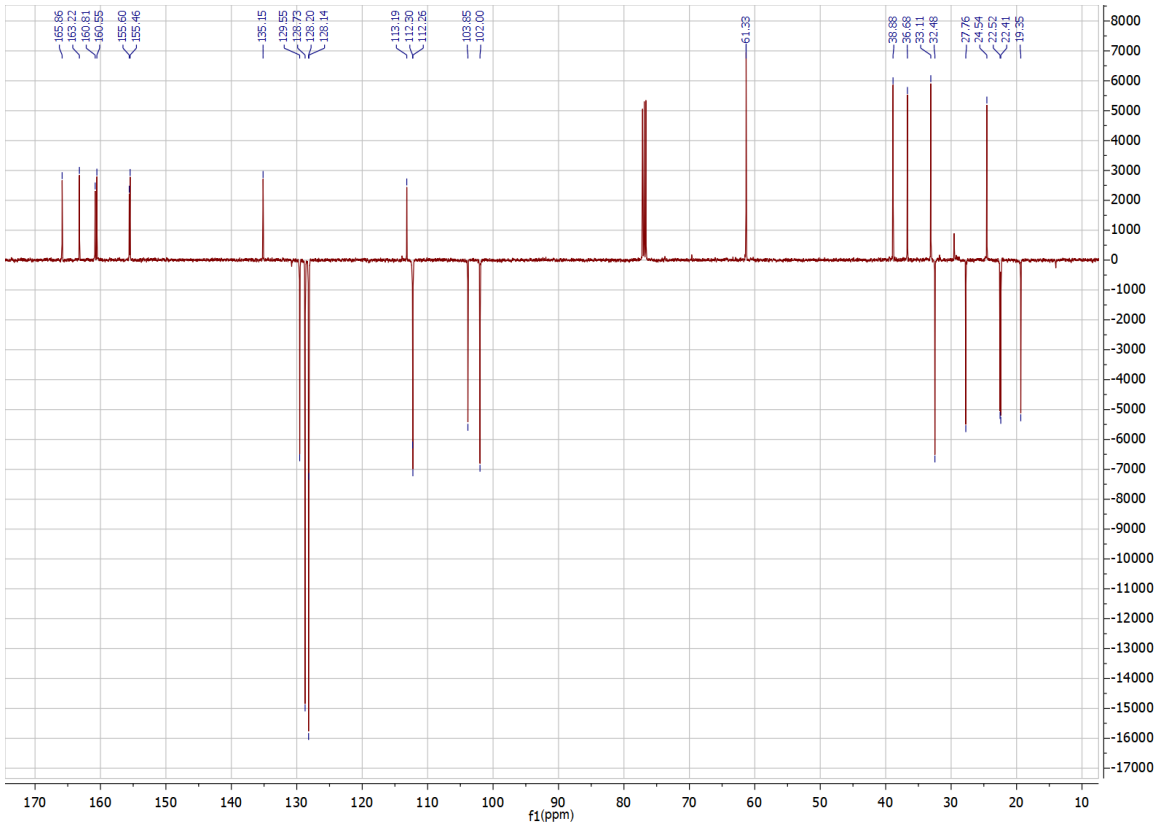


Fig. S9. ^{1}H NMR spectrum of Compound **17**

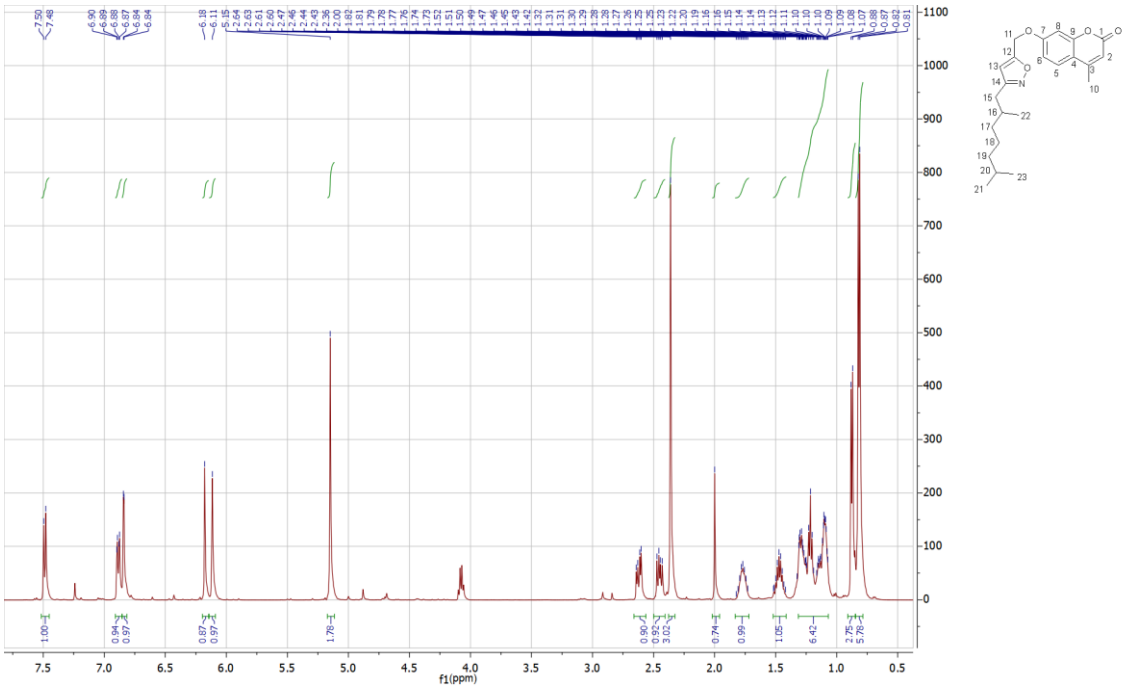
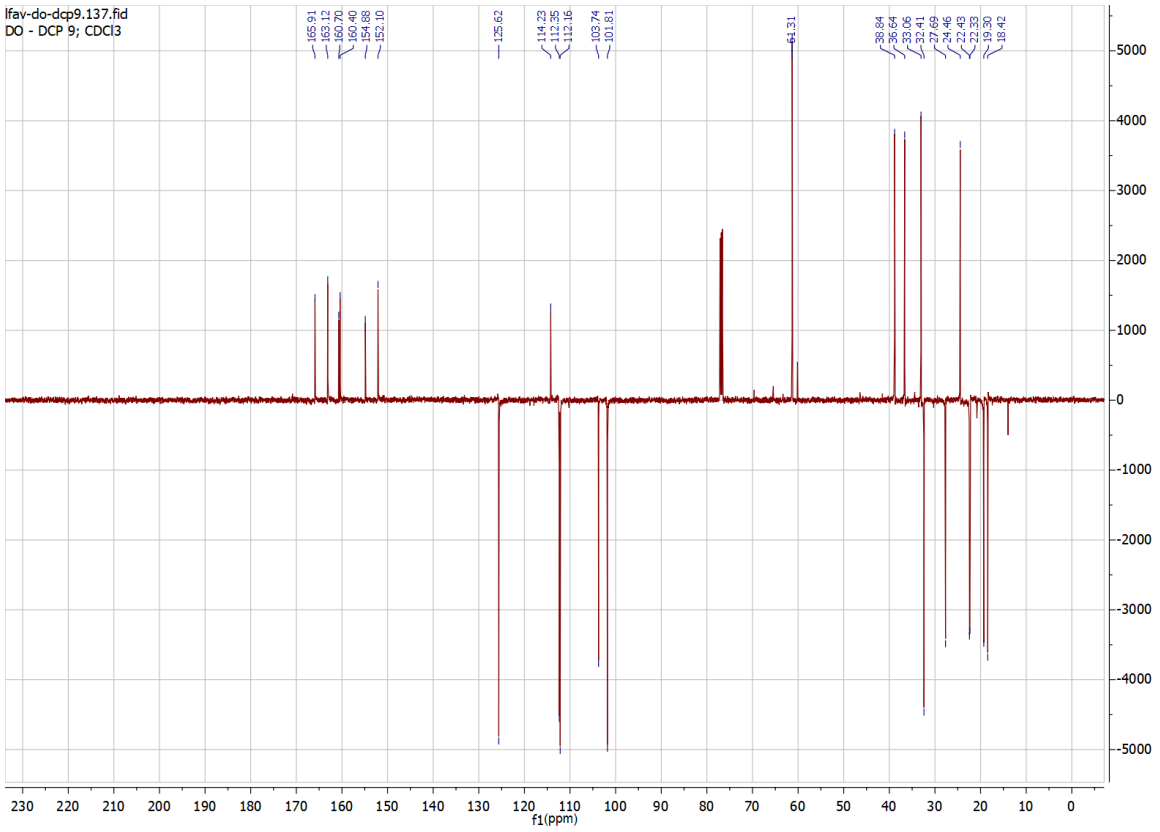


Fig. S10. ^{13}C NMR spectrum of Compound **17**



S3. 2D NMR spectra of the compound 15

Fig. S11. COSY NMR spectrum of Compound **15**

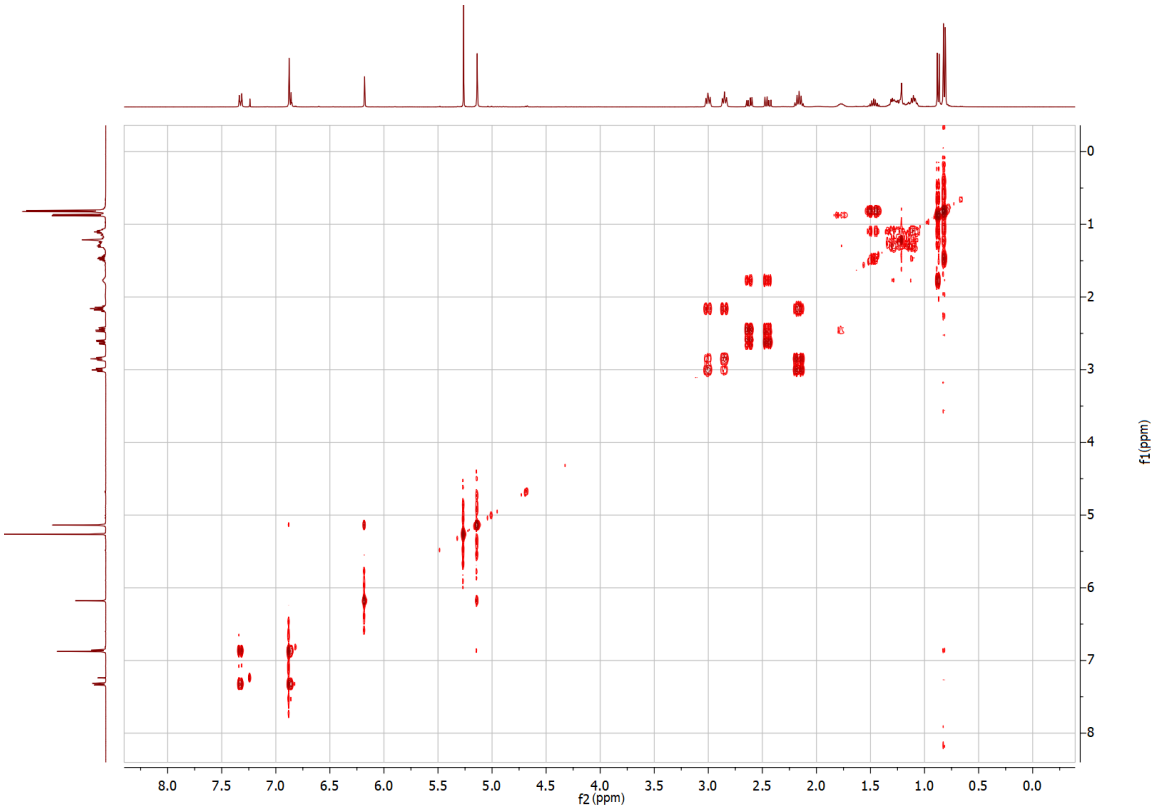


Fig. S12. NOESY NMR spectrum of Compound **15**

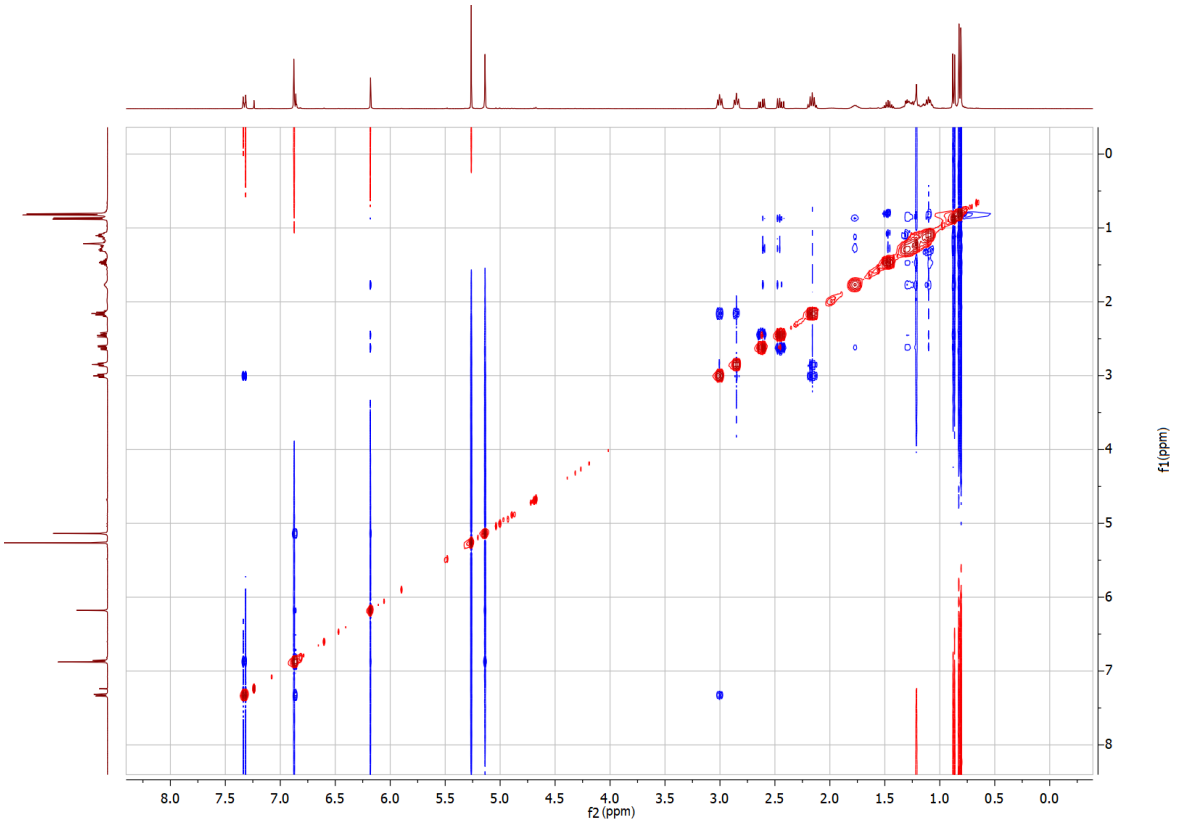


Fig. S13. HSQC NMR spectrum of Compound **15**

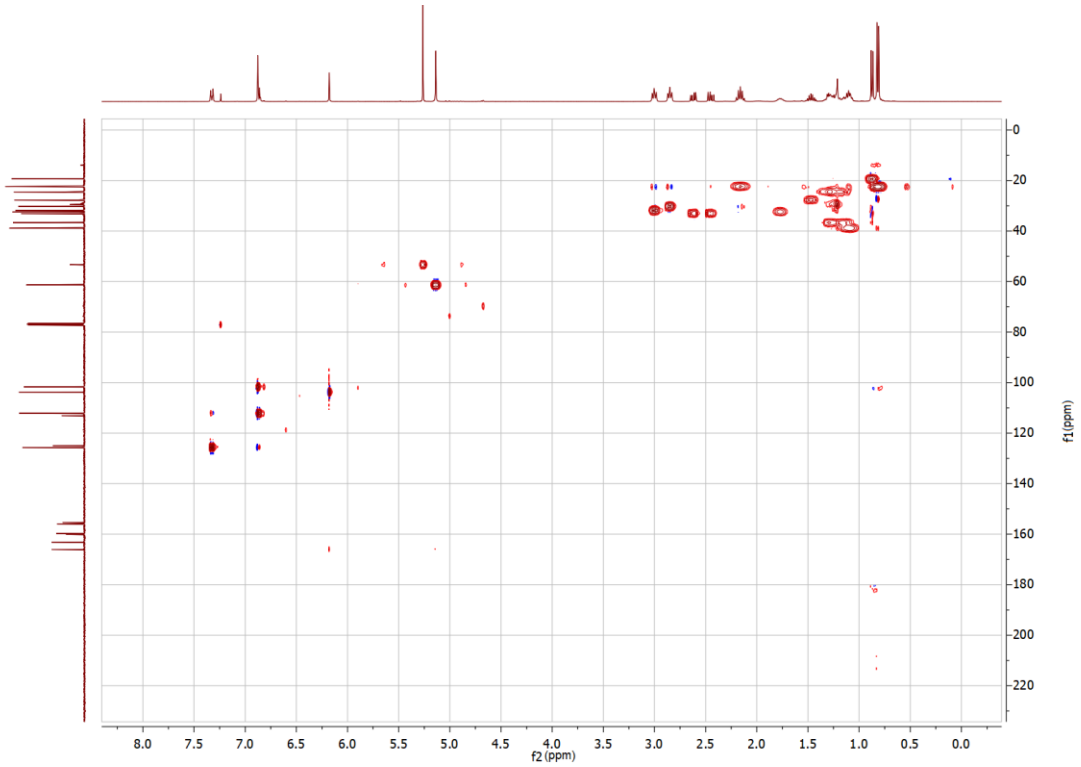
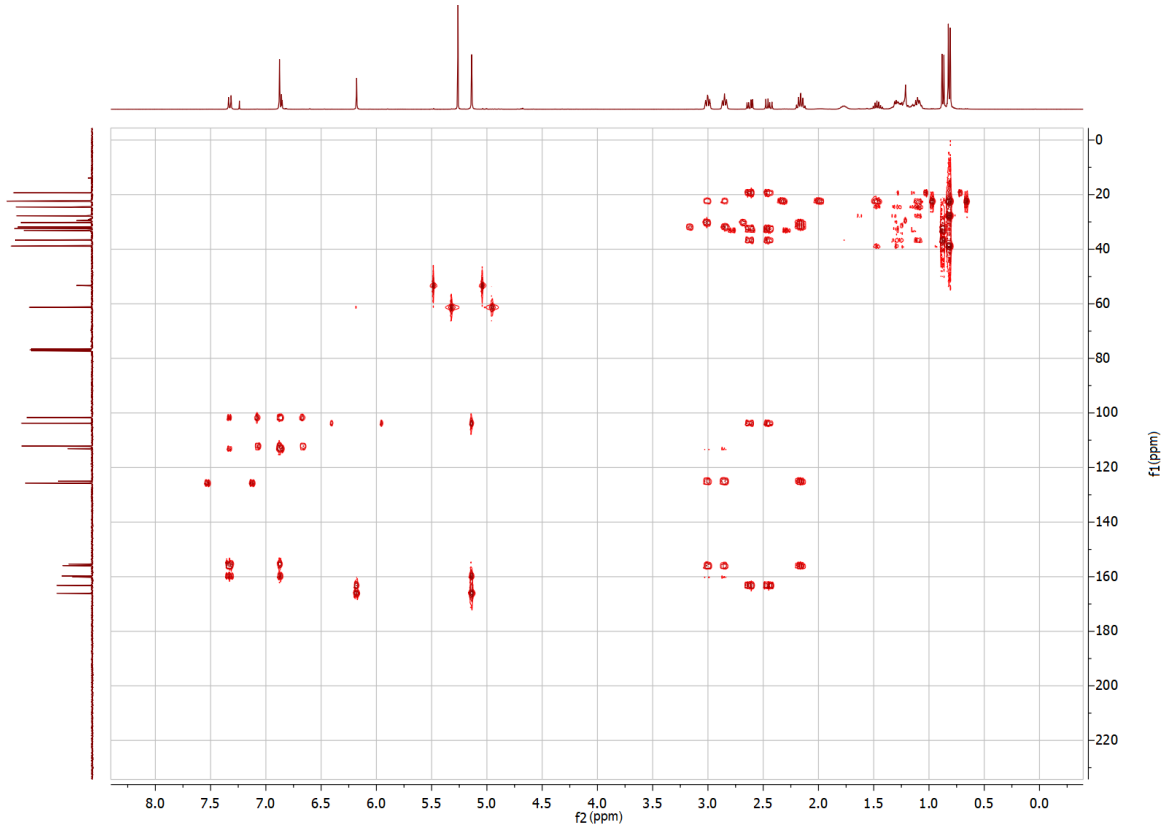


Fig. S14. HMBC NMR spectrum of Compound **15**



S4. HRMS spectra of compounds 12, 14-17.

Fig. S15. HRMS spectrum of Compound **12**

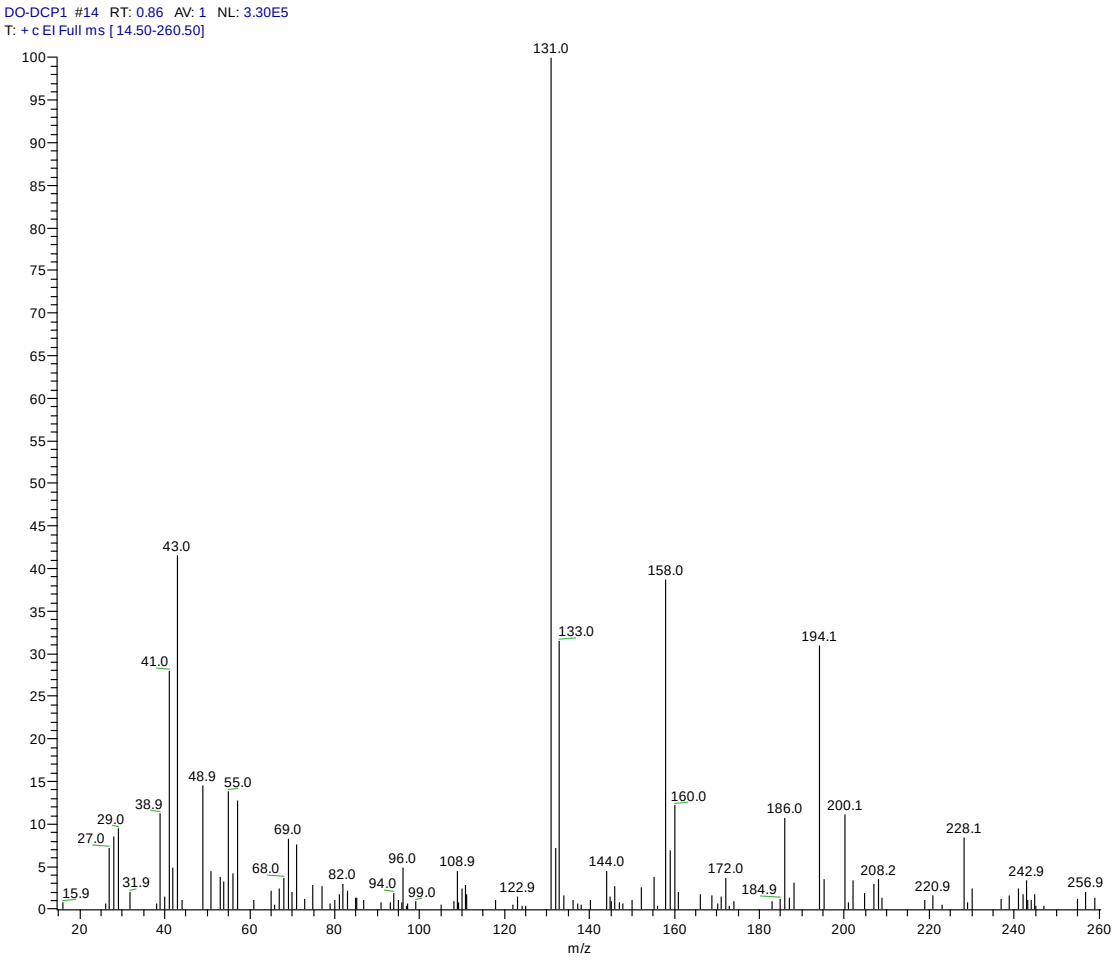


Fig. S16. HRMS spectrum of Compound **14**

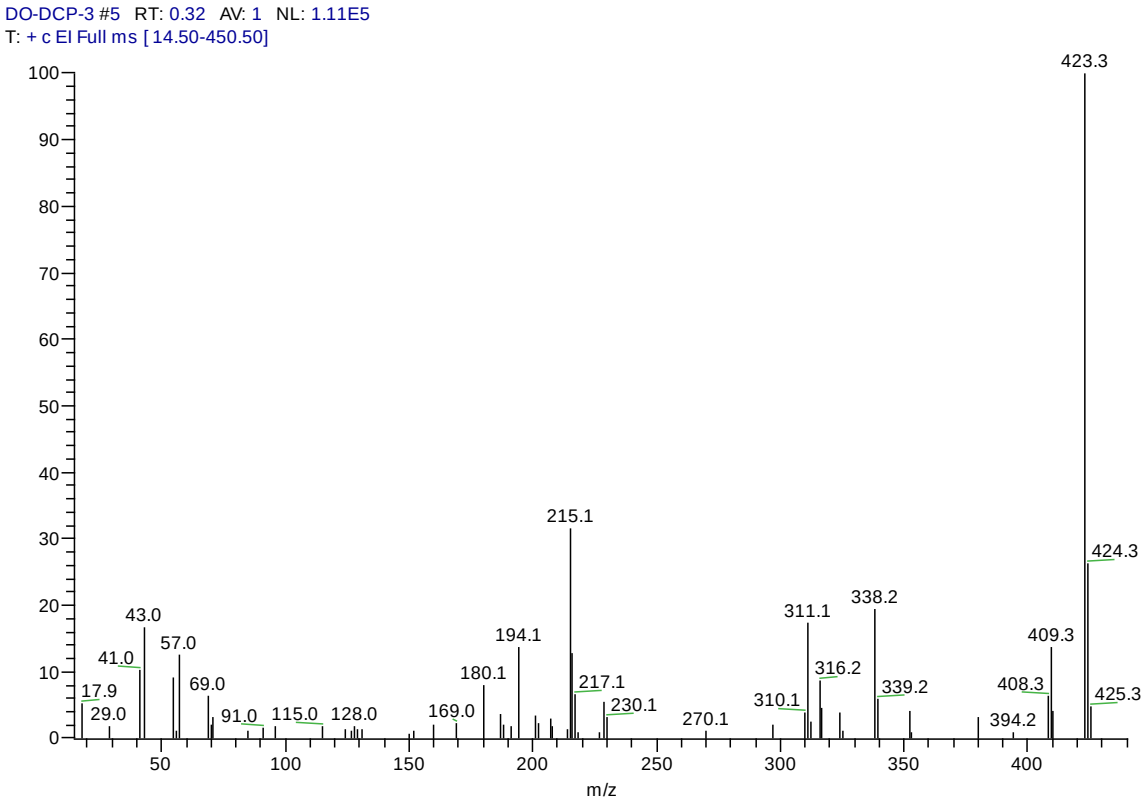


Fig. S17. HRMS spectrum of Compound **15**

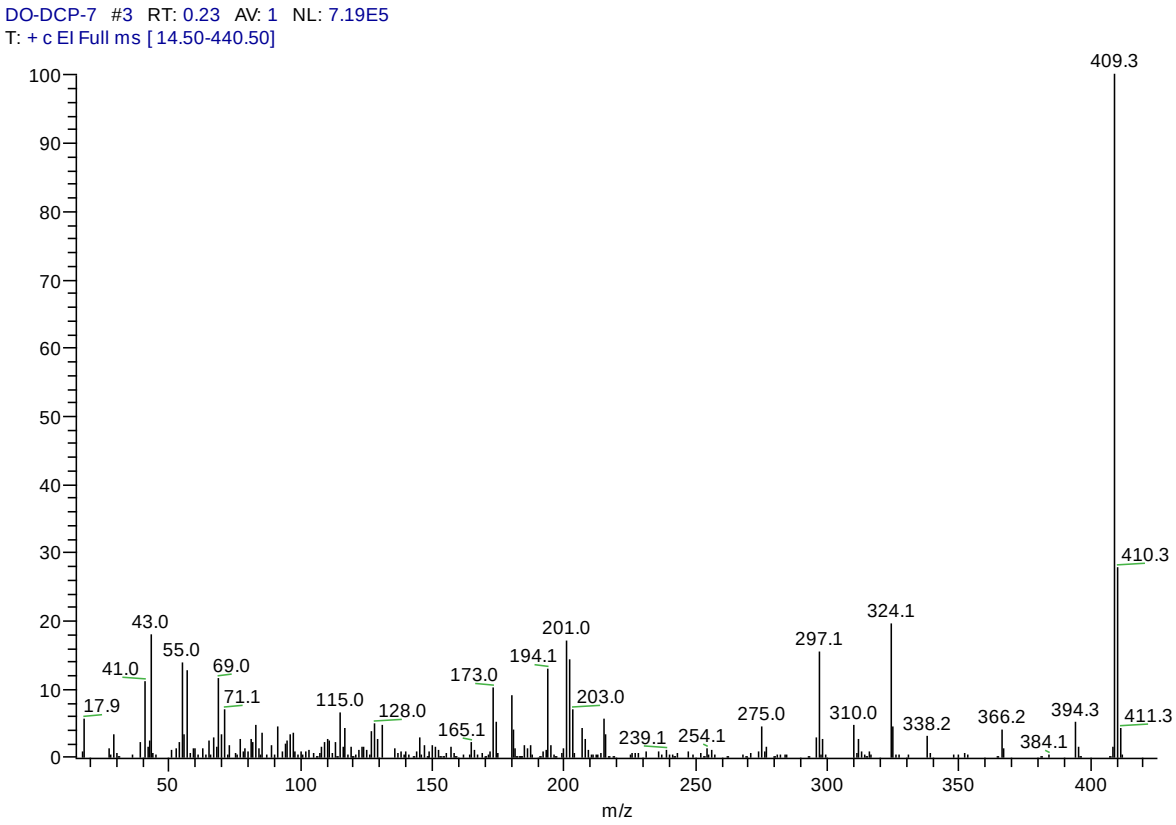


Fig. S18. HRMS spectrum of Compound **16**

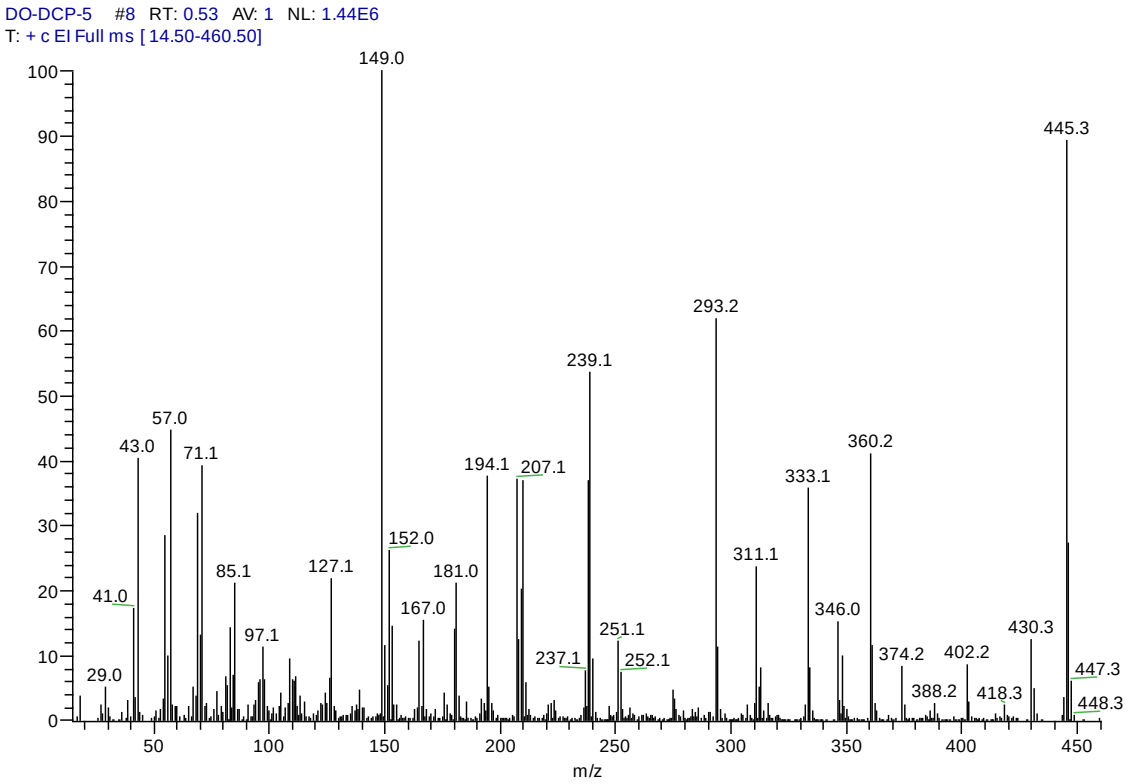
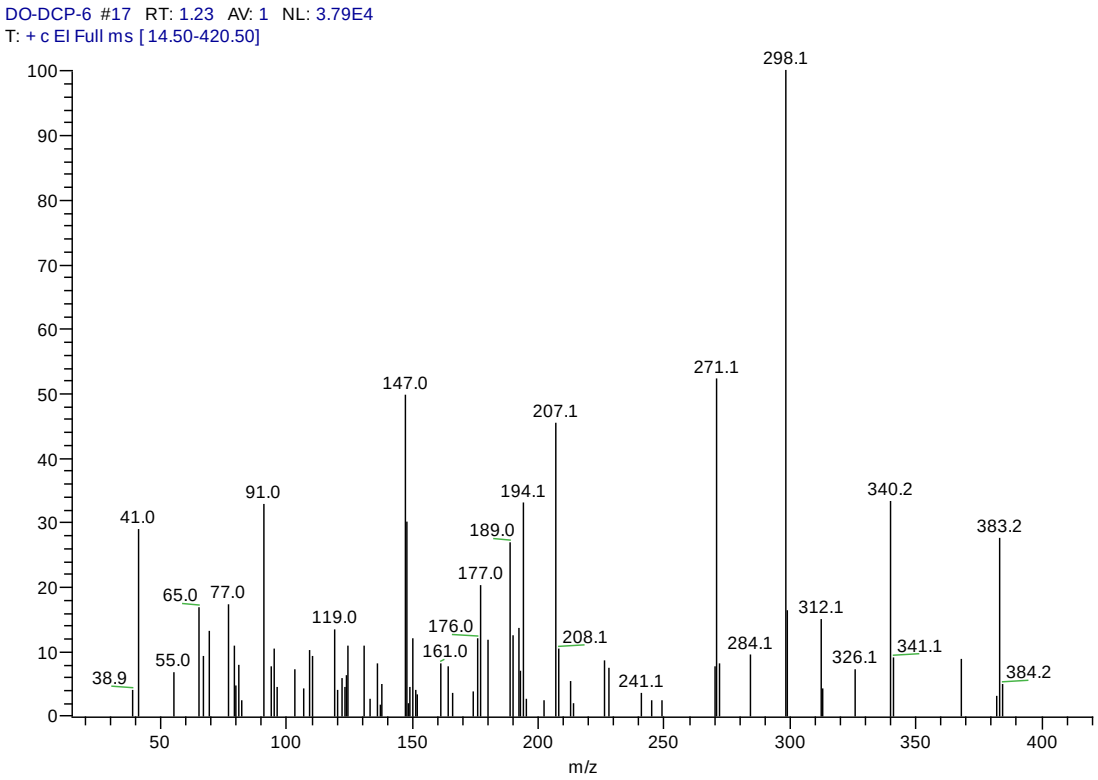


Fig. S19. HRMS spectrum of Compound **17**



References

S1. Ya. L. Garazd, T. N. Panteleimonova, M. M. Garazd and V. P. Khilya, *Chem. Nat. Compd.*, 2003, **39**, 330; https://doi.org/10.1023/B:CONC.0000003410.74701.dc.

S2. V. N. Odinokov, G. Yu. Ishmuratov, R. Ya. Kharisov, S. I. Lomakina and G. A. Tolstikov, *Russ. Chem. Bull.*, 1989, **38**, 1768; https://doi.org/10.1007/BF00956974.

S3. R. T. Dahill, Jr., *US Patent 3637533A*, 1972; https://patents.google.com/patent/US3637533A/en.

S4. E. V. Kondrashov and N. S. Shatokhina, *Chem. Heterocycl. Compd*., 2019, **55**, 1228; https://doi.org/10.1007/s10593-019-02606-2.

S5. C. Gnerre, M. Catto, F. Leonetti, P. Weber, P.-A. Carrupt, C. Altomare, A. Carotti and B. Testa, *J. Med. Chem.*, 2000, **43**, 4747; https://doi.org/10.1021/jm001028o.