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Synthesis of novel 8-nitro-substituted 1,3-benzothiazin-4-ones

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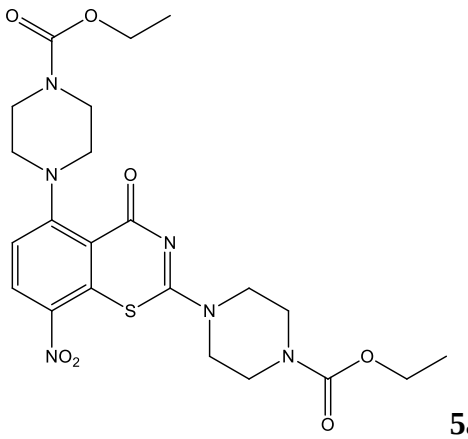
Experimental

Unless otherwise indicated, all common reagents and solvents were used from commercial suppliers without further purification. Melting points were measured on the instrument Boetius. The ^{1}H NMR (400.13 MHz) and ^{19}F NMR (376.45 MHz) spectra were obtained on a Bruker Avance II DMX400 spectrometer in DMSO-d_6. The ^{1}H NMR experiments were carried out using trimethylsilane as the internal standard and the ^{19}F NMR spectra were referenced to $CFCl_3$ (C_6F_6 was used as secondary reference, δ_F –162.9 ppm), liquid ammonium was use as a standard for ^{15}N NMR spectra. ^{13}C (125.7 MHz) NMR spectra were recorded on the AVANCE-500 spectrometer in DMSO-d_6 solution. Mass spectra were recorded on the SHIMADZU GCMS-QP2010 Ultra instrument with electron ionization (EI) of the sample. Microanalyses (C, H, N) were performed using the Perkin–Elmer 2400 elemental analyzer.

2,5-Bis(4-ethoxycarbonylpiperazin-1-yl)-8-nitro-1,3-benzothiazin-4-one (5a). Thionyl chloride (0.8 ml, 11 mmol) and one drop of DMF were added to the solution of 2,6-difluoro-3-nitrobenzoic acid **2** (1.39 g, 6.85 mmol) in absolute toluene (5 ml). The mixture was stirred at 95 ºC for 2.5 h, and then the excess of thionyl chloride was removed *in vacuo*. A solution of ammonium thiocyanate (0.52 g, 6.85 mmol) in acetonitrile (10 ml) was added to the obtained toluene solution of 2,6-difluoro-3-nitrobenzoyl chloride. The mixture was stirred at 40 °C for 5 min, the precipitate of NH_4Cl was filtered off, and ethyl 1-piperazinecarboxylate (2 ml, 13.7 mmol) was added to the solution of 2,6-difluoro-3-nitrobenzoyl isothiocyanate. The mixture was stirred at room temperature for 3 h, then concentrated *in vacuo*. The residue was triturated with the mixture of hexane (5 ml) and ethanol (0.2 ml), washed with water (8 ml) and recrystallized from ethanol. Yield 2.8 g (79%), mp 147-149 ºC. ^{1}H NMR: 1.20 (t, 6H, 2CH_3, ^{3}J = 7.1 Hz), 3.42-3.46 (m, 4H, EtOOC-N(CH_2)$_2$), 3.50-3.56 (m, 8H, 2N(CH_2)$_2$), 3.79-3.83 (m, 4H, EtOOC-N(CH_2)$_2$), 4.08 (q, 4H, 2OCH_2, ^{3}J = 7.0 Hz), 7.18 (d, 1H, H-6, ^{3}J = 9.7 Hz), 8.32 (d, 1H, H-7, ^{3}J = 9.8 Hz). ^{19}F NMR: signals are absent. ^{15}N NMR: 80.71 (2 NCOOEt), 92.58 (1 N(CH_2)$_2$), 110.08 (1 N(CH_2)$_2$), 365.96 (NO_2). Mass-spectrum, *m/z* (I_{rel}, %): 520 $[M]^+$ (14), 504 (14), 503 (36), 433 (11), 432 (33), 431 (100), 430 (12), 419 (23), 418 (70), 414 (20), 407 (16), 406 (49), 405 (44), 393 (23), 392 (92), 391 (24), 390 (70), 376 (11), 346 (13), 320 (11), 290 (11), 249 (15), 236 (12), 231 (10), 201 (10), 138 (15), 137 (14), 128 (26), 116 (12), 110 (15), 83 (14), 70 (24), 69 (21), 68 (11), 56 (87), 55 (16), 44 (19), 43 (13), 42 (36). Found, %: C 50.73, H 5.46, N 16.15. $C_{22}H_{28}N_6O_7S$. Calculated, %: C 50.76, H 5.42, N 16.14.



5a

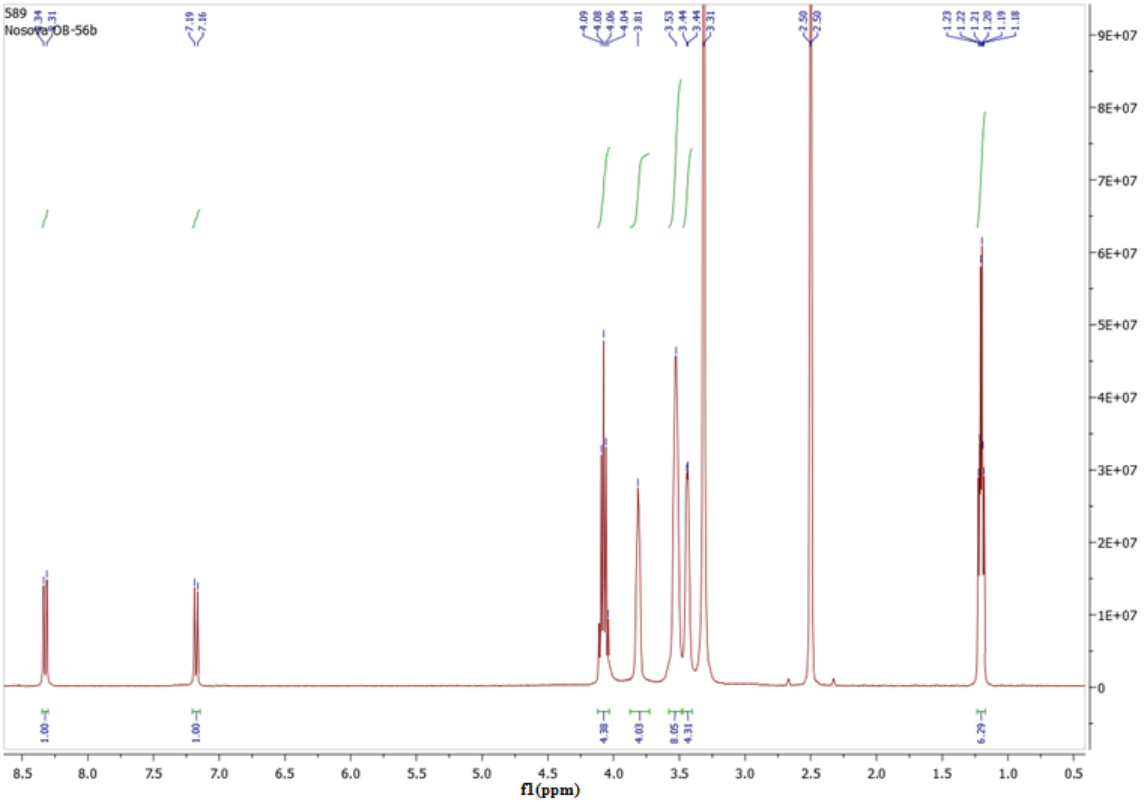


Figure S1. NMR ^{1}H spectrum of benzothiazinone **5a**.

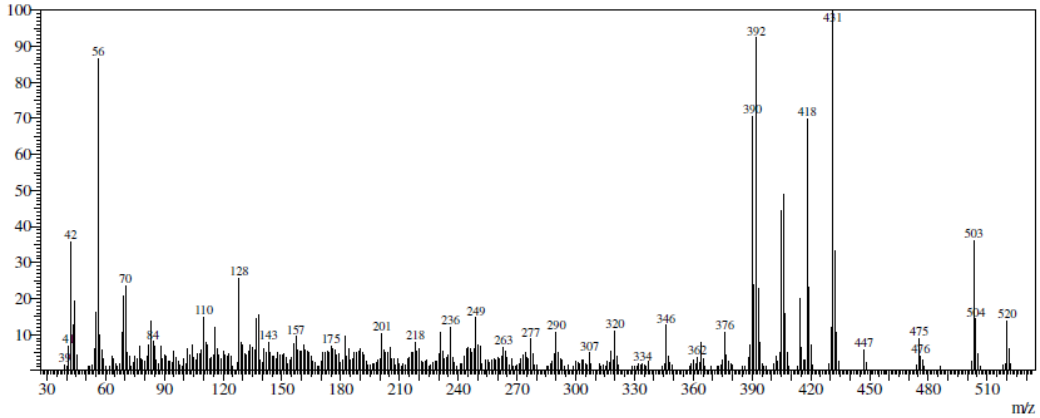


Figure S2. Mass-spectrum of benzothiazinone **5a**.

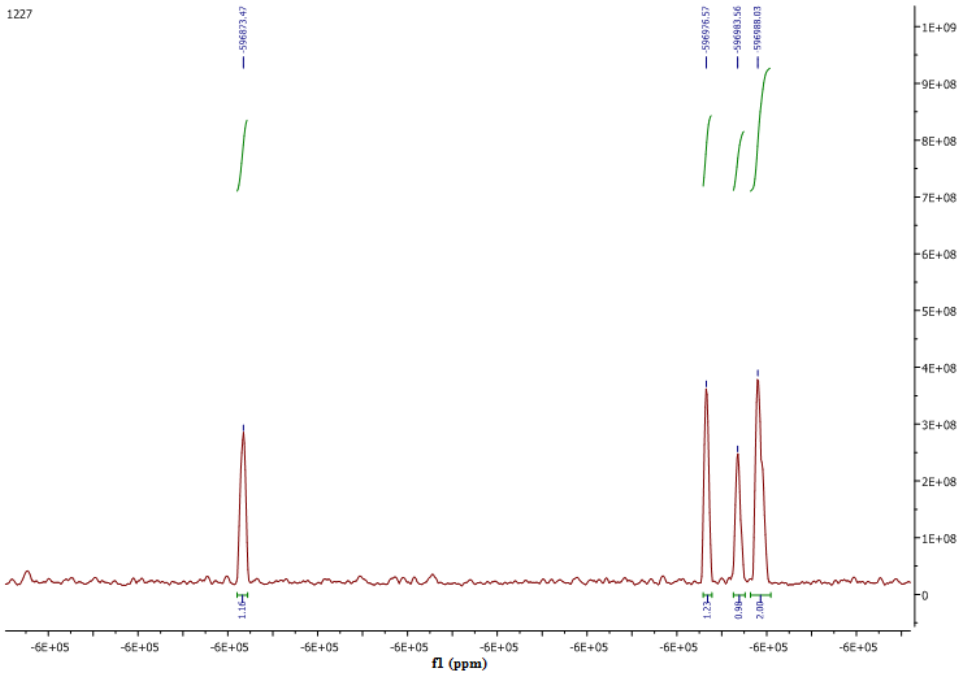


Figure S3. NMR ^{15}N spectrum of benzothiazinone **5a**.

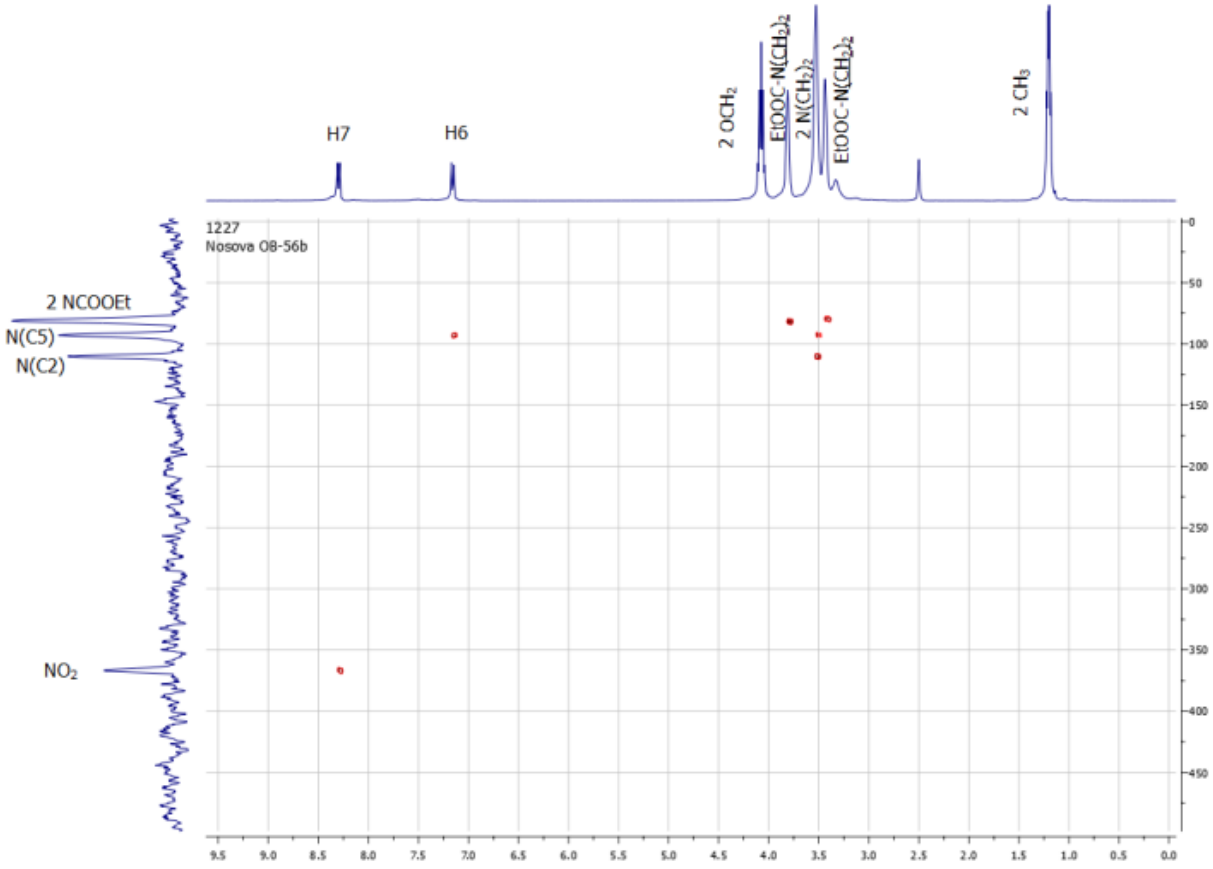


Figure S4. HMBC ^{1}H-^{15}N spectrum of benzothiazinone **5a**.

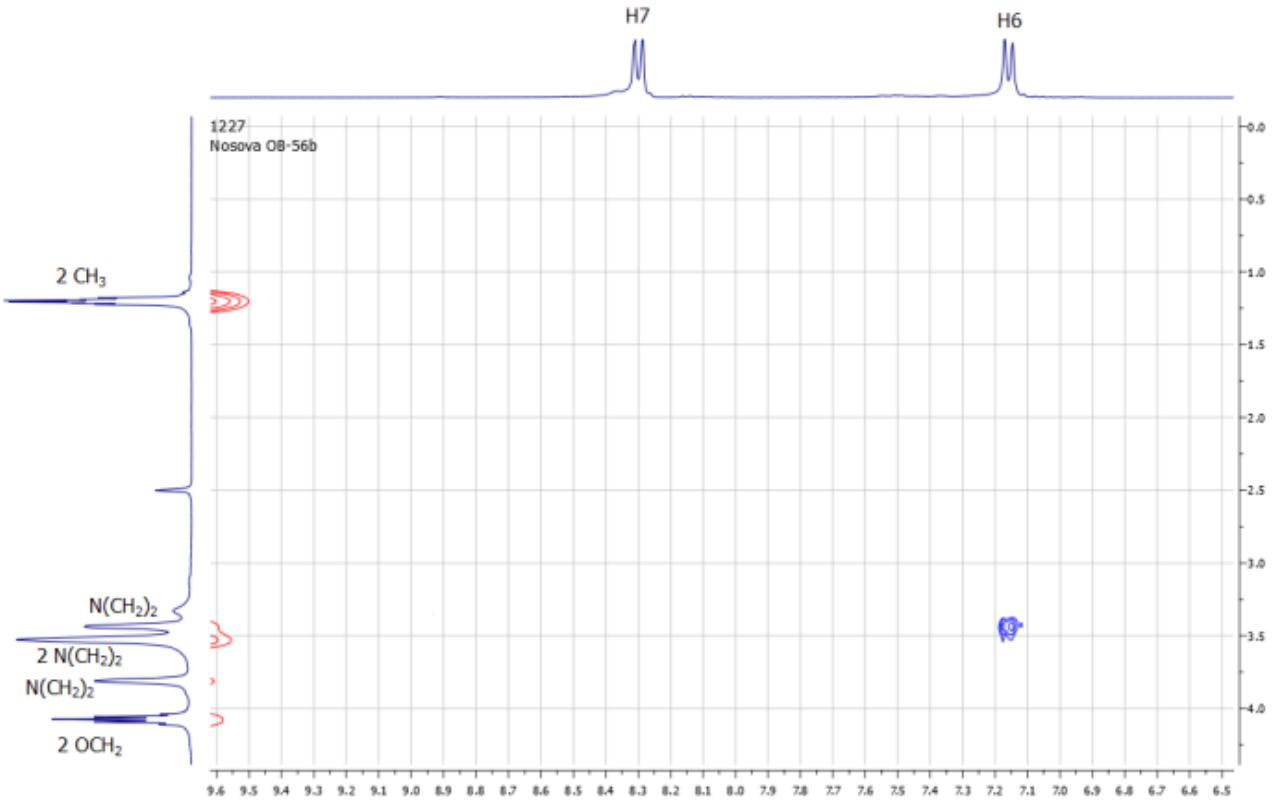
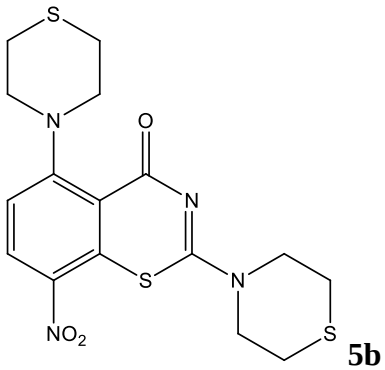


Figure S5. NOESY spectrum of benzothiazinone **5a**.

Compounds **5b-f** were obtained similarly.

2,5-Bis(thiomorpholin-4-yl)-8-nitro-1,3-benzothiazin-4-one (5b). After the completing of reaction the precipitate formed was filtered off, washed with water and recrystallized from ethanol. Yield 2.3 g (82%), mp 234-236 ºC. ^{1}H NMR: 2.73-2.77 (m, 8H, S(CH$_2$)$_2$), 3.62-3.64 (m, 4H, N(CH$_2$)$_2$), 4.05-4.09 (m, 4H, N(CH$_2$)$_2$), 7.20 (d, 1H, H-6, ^{3}J = 9.7 Hz), 8.30 (d, 1H, H-7, ^{3}J = 9.7 Hz). ^{19}F NMR: signals are absent. Mass-spectrum, *m/z* (I_{rel}, %): 410 $[M]^+$ (23), 377 (20), 349 (15), 339 (10), 338 (18), 337 (100), 137 (12), 129 (16), 69 (13). Found, %: C 46.79, H 4.45, N 13.64. $C_{16}H_{18}N_4O_3S_3$. Calculated, %: C 46.81, H 4.42, N 13.65.



5b

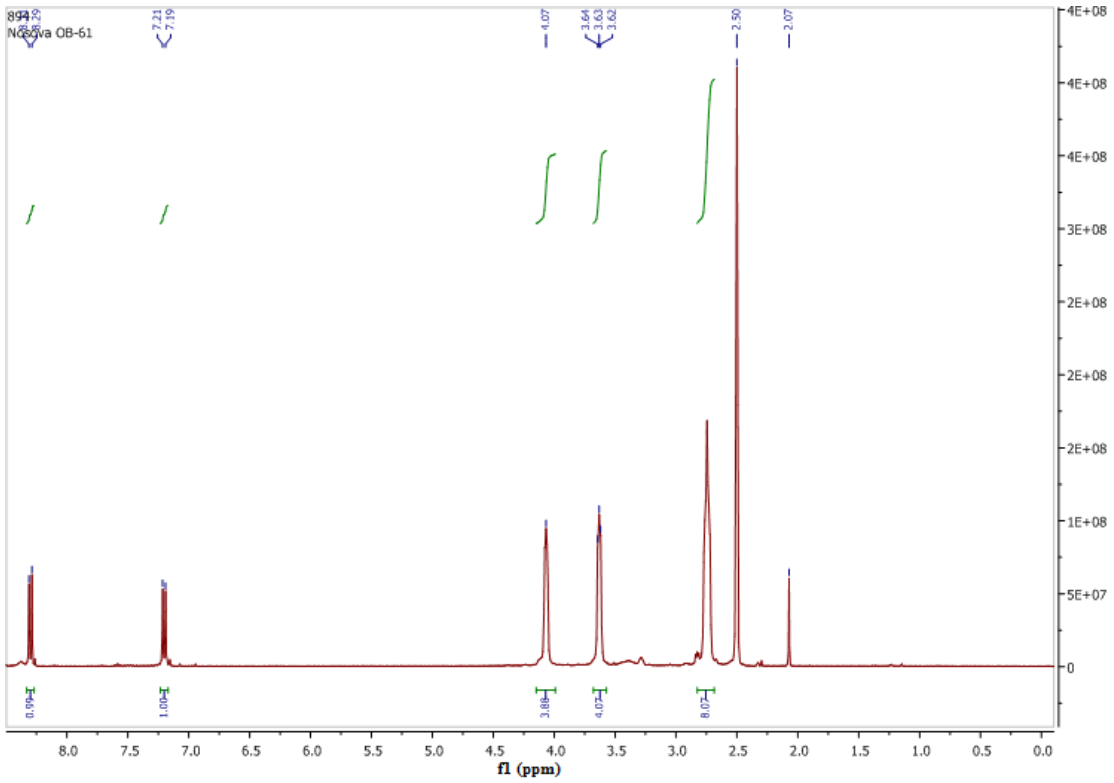


Figure S6. NMR ^{1}H spectrum of benzothiazinone **5b**.

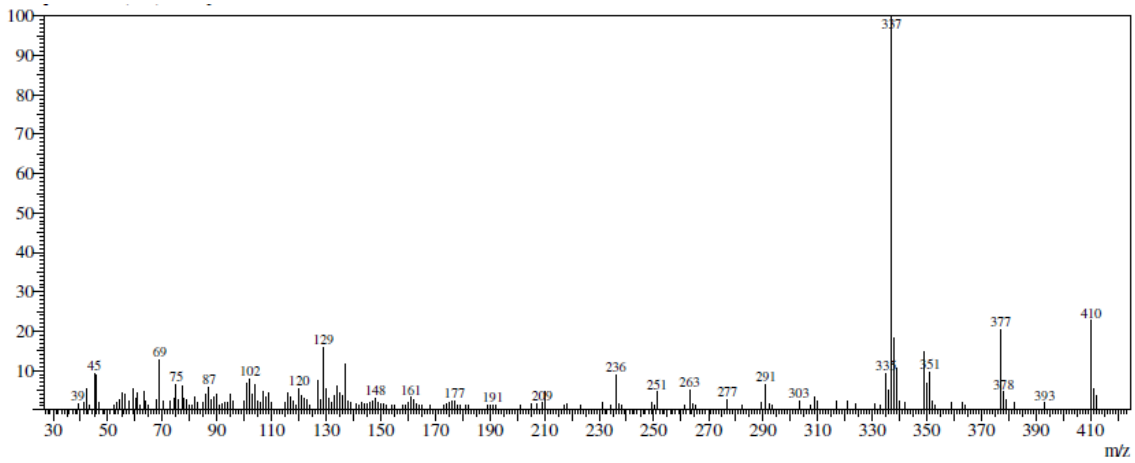


Figure S7. Mass-spectrum of benzothiazinone **5b**.

2,5-Bis(morpholin-4-yl)-8-nitro-1,3-benzothiazin-4-one (5c). After the completing of reaction the precipitate formed was filtered off, washed with water and recrystallized from ethanol. Yield 1.8 g (70 %), mp 255-266 °C. ^{1}H NMR: 3.39-3.41 (m, 4H, $N(CH_2)_2$), 3.71-3.73 (m, 8H, $2O(CH_2)_2$), 3.80-3.82 (m, 4H, $N(CH_2)_2$), 7.17 (d, 1H, H-6, ^{3}J = 9.7 Hz), 8.29 (d, 1H, H^{7}, ^{3}J = 9.6 Hz). ^{19}F NMR: signals are absent. ^{13}C NMR: 44.9 (NCH_2), 49.7 (NCH_2), 64.5 (OCH_2), 64.6 (OCH_2), 111.1, 113.3, 128.1, 131.1, 131.9, 153.7, 159.6, 167.9. Mass-spectrum, *m/z* (I_{rel}, %): 378 $[M]^{+}$ (19), 362 (11), 361 (38), 360 (99), 359 (15), 347 (15), 335 (32), 334 (22), 321 (22), 320 (24), 319 (83), 318 (11), 305 (13), 292 (12), 250 (12), 236 (27), 234 (12), 182 (11), 143 (10), 138 (11), 137 (100), 136 (13), 135 (17), 134 (22), 133 (15), 131 (10), 130 (18), 123 (14), 122 (11), 121 (12),

120 (19), 118 (12), 117 (15), 116 (13), 113 (31), 109 (29), 108 (15), 107 (21), 106 (10), 105 (11), 104 (19), 103 (13), 102 (13), 95 (16), 91 (10), 90 (16), 89 (11), 86 (39), 83 (11), 82 (25), 78 (11), 77 (36), 76 (11), 75 (26), 70 (14), 69 (42), 63 (22), 58 (10), 56 (30), 55 (24), 54 (14), 45 (18), 43 (11), 42 (32), 41 (14). Found, %: C 50.76, H 4.82, N 14.80. $C_{16}H_{18}N_4O_5S$. Calculated, %: C 50.78, H 4.79, N 14.81.

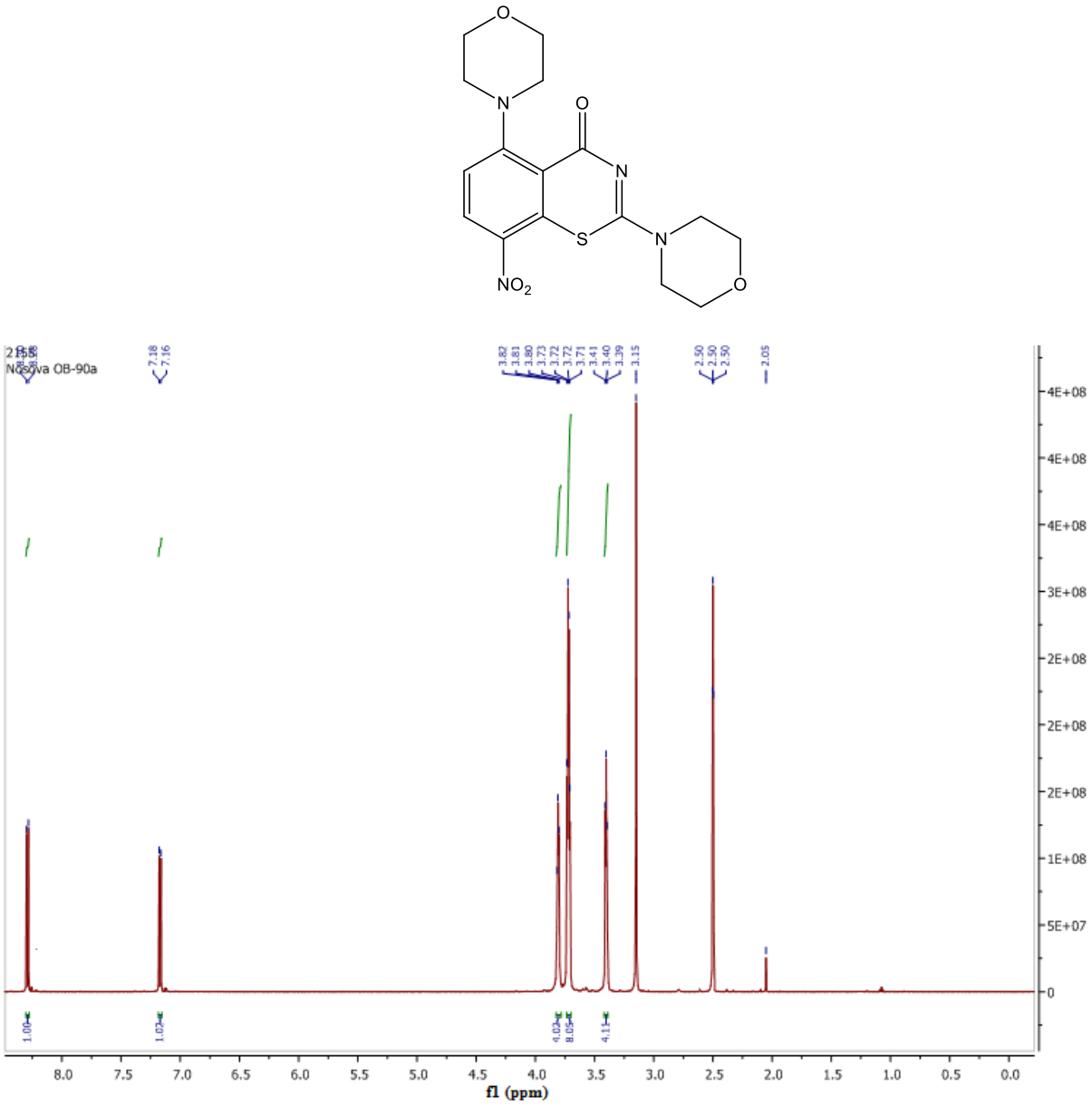


Figure S8. NMR ^{1}H spectrum of benzothiazinone **5c**.

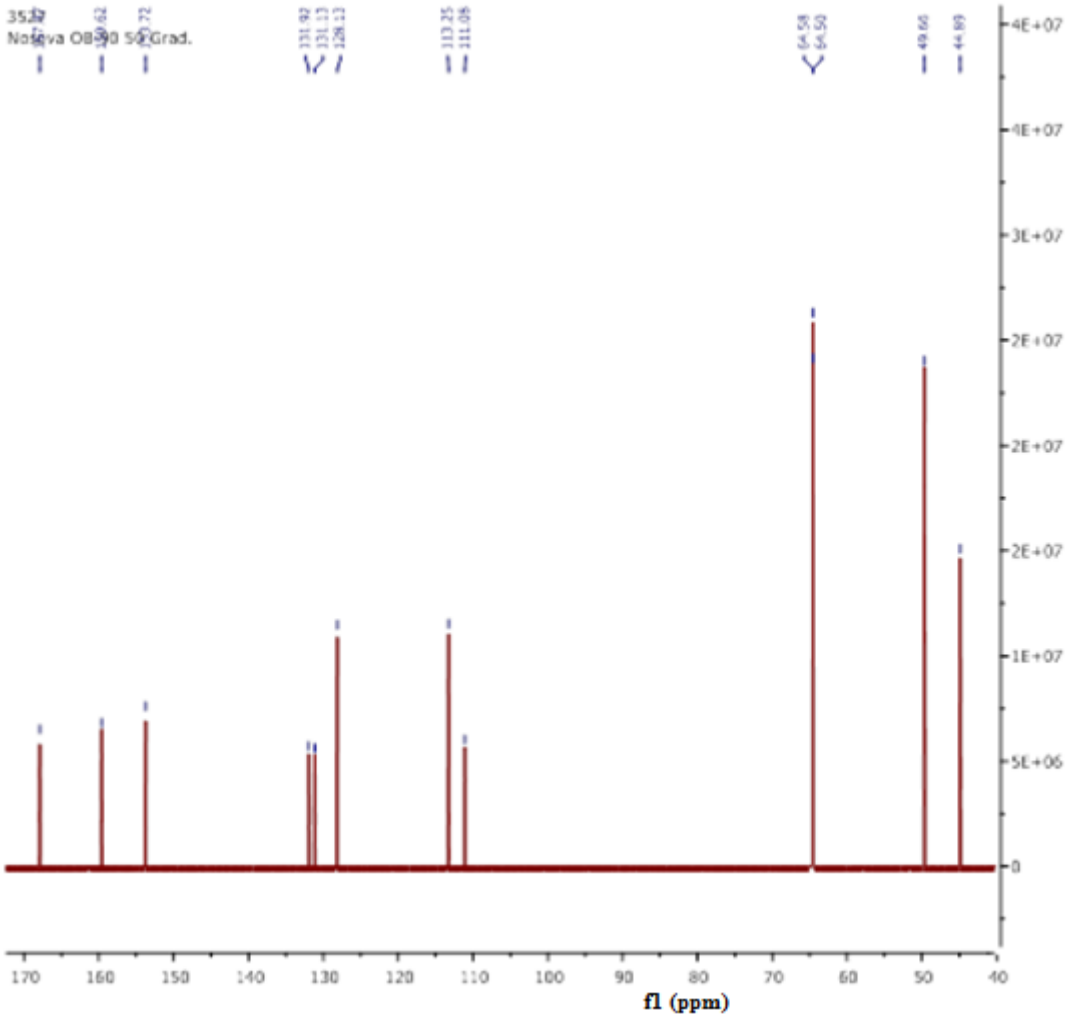


Figure S9. NMR ^{13}C spectrum of benzothiazinone **5c**.

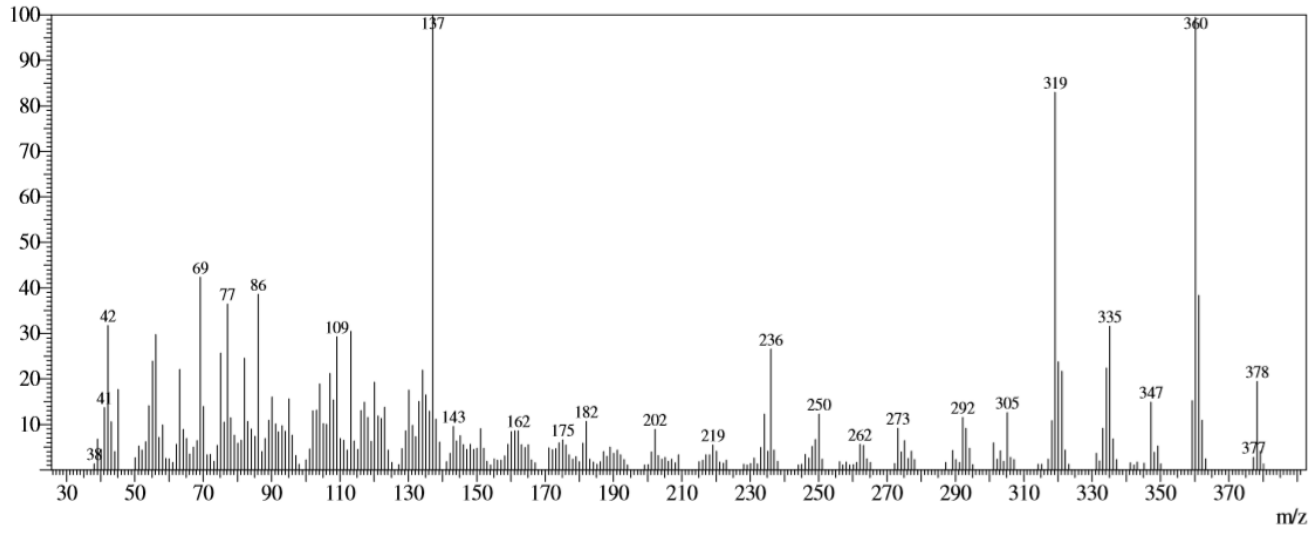
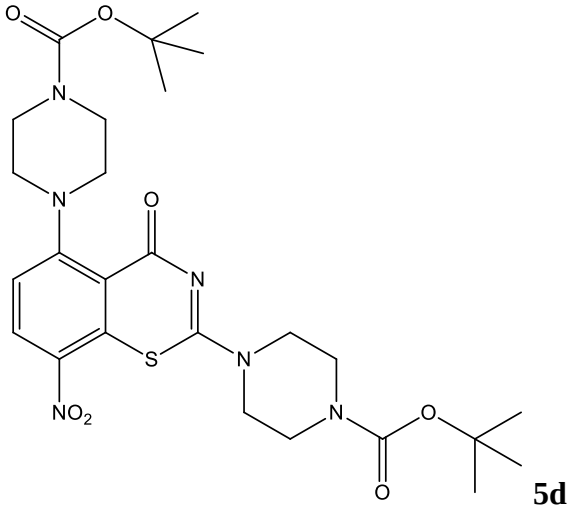


Figure S10. Mass-spectrum of benzothiazinone **5c**.

2,5-Bis(4-*tert*-butoxycarbonylpiperazin-1-yl)-8-nitro-1,3-benzothiazin-4-one (5d). After the completing the reaction, the precipitate formed was filtered off, washed with water and recrystallized from ethanol. Yield 3.6 g (91 %), mp 234-236 ºC. ^1H NMR: 1.44 (s, 9H, $C(CH_3)_3$),

1.45 (s, 9H, $C(CH_3)_3$), 3.39-3.44 (m, 4H, $N(CH_2)_2$), 3.48-3.51 (m, 8H, 2 $C(O)N(CH_2)_2$), 3.81-3.83 (m, 4H, $N(CH_2)_2$), 7.16 (d, 1H, H-6, ^3J = 9.7 Hz), 8.31 (d, 1H, H-7, ^3J = 9.6 Hz). ^{19}F NMR: signals are absent. ^{13}C NMR: 28.0 (CH_3), 30.6 (CMe_3), 45.3 (NCH_2), 49.7 (NCH_2), 79.2 (NCH_2), 79.4 (NCH_2), 111.8, 114.1, 129.2, 132.0, 133.0, 153.7, 153.9, 154.7, 160.5, 168.8. Mass-spectrum, *m/z* (I_{rel}, %): 576 $[M]^+$ (1), 459 (13), 404 (10), 403 (24), 390 (11), 364 (14), 359 (13), 346 (12), 320 (15), 69 (11), 57 (100), 56 (60), 55 (15), 44 (20), 42 (16), 41 (52), 39 (17). Found, %: C 54.18, H 6.28, N 14.54. $C_{26}H_{36}N_6O_7S$. Calculated, %: C 54.15, H 6.29, N 14.57.



5d

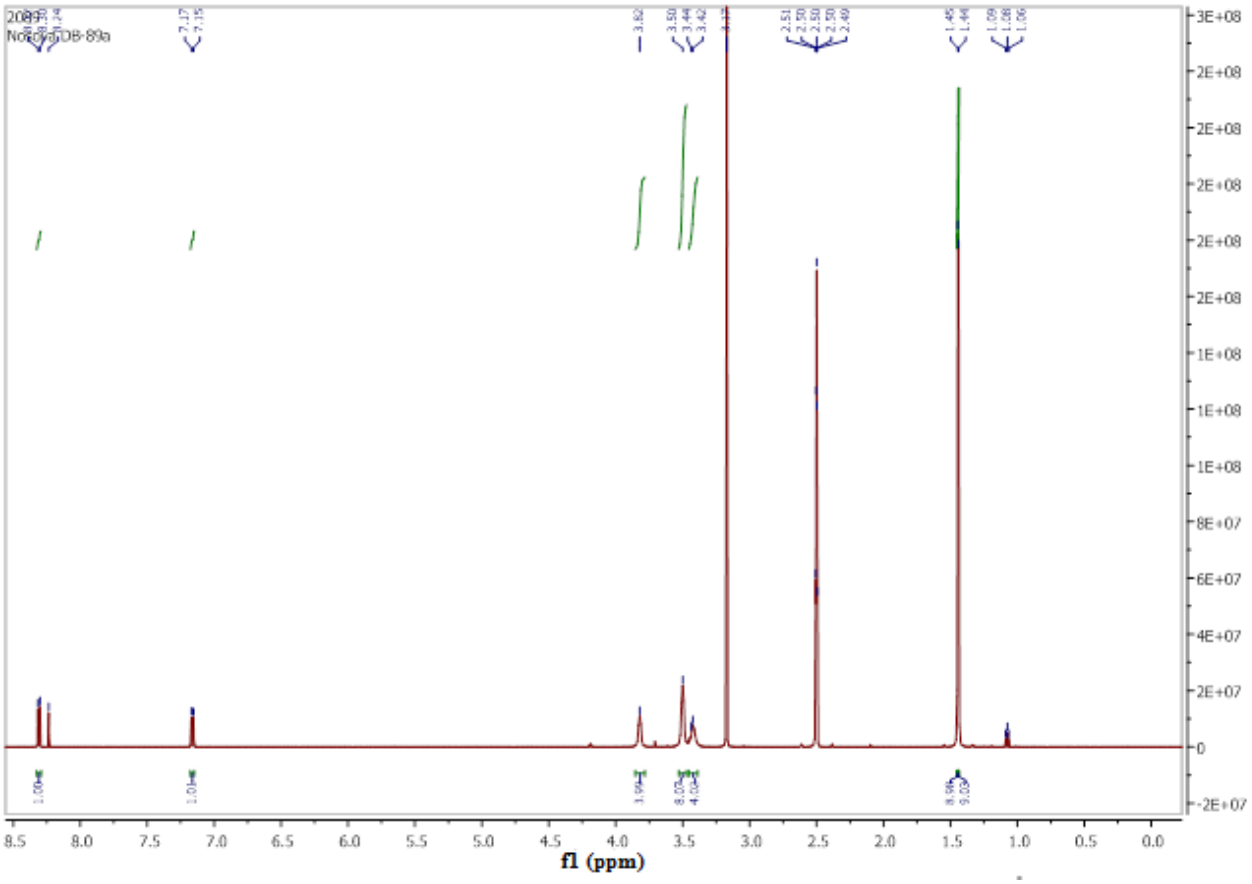


Figure S11. NMR ^1H spectrum of benzothiazinone **5d**.

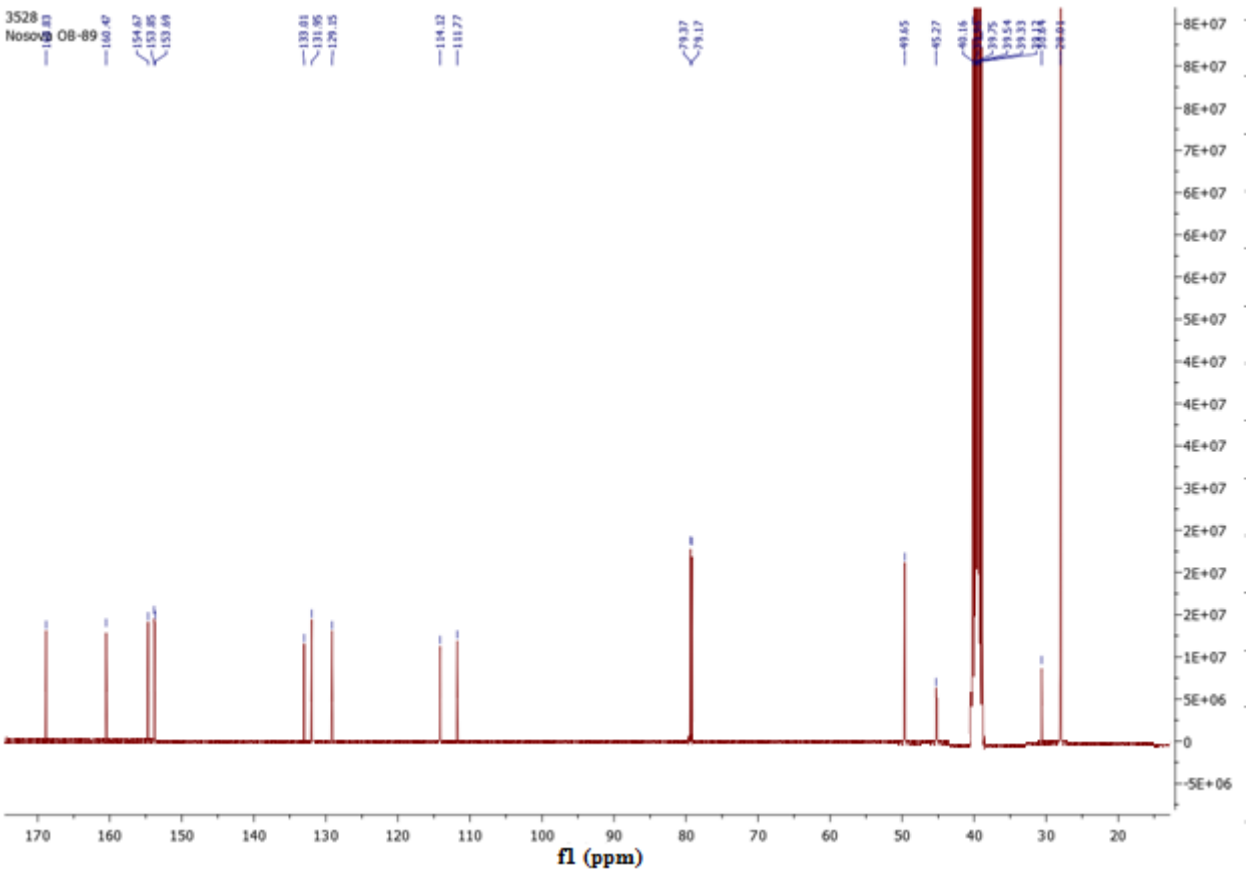


Figure S12. NMR ^{13}C spectrum of benzothiazinone **5d**.

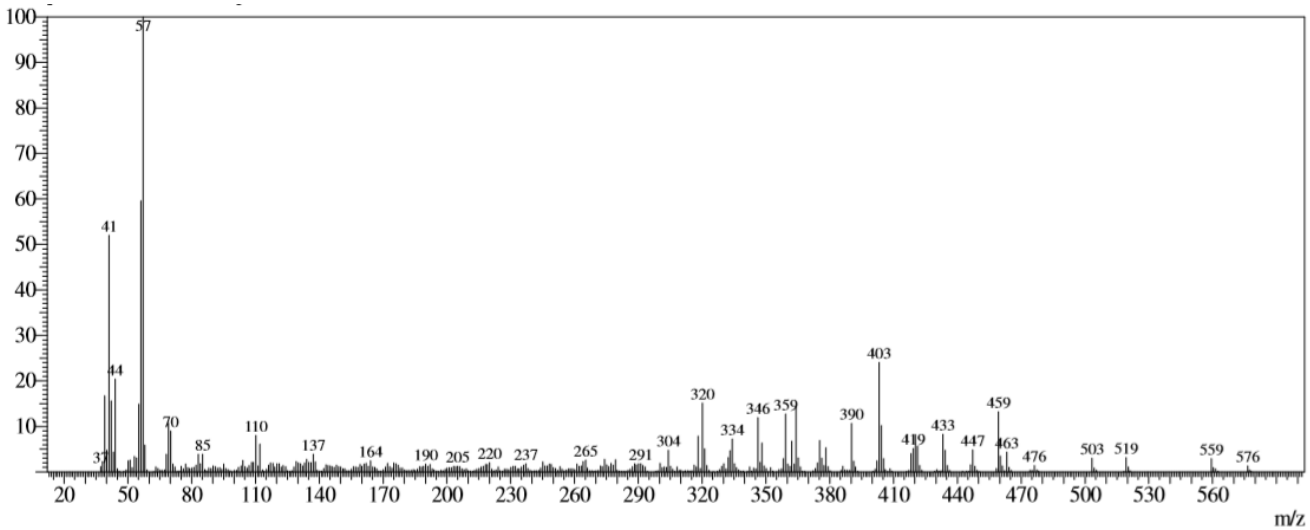
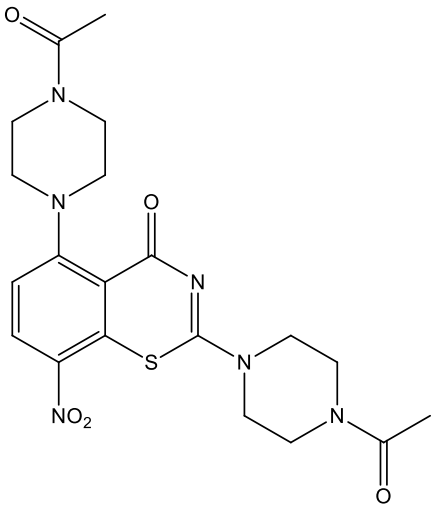


Figure S13. Mass-spectrum of benzothiazinone **5d**.

2,5-Bis(4-acetylpiperazin-1-yl)-8-nitro-1,3-benzothiazin-4-one (5e). After the completing the reaction, the mixture was concentrated *in vacuo*, the residue was triturated with diethyl ether, washed with water and recrystallized from ethanol. Yield 2.7 g (85 %), mp 204-206 ºC. ^{1}H NMR: 2.03 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 3.44-3.48 (m, 4H, $N(CH_2)_2$), 3.57-3.64 (m, 8H, $2OCN(CH_2)_2$), 3.77-3.79 (m, 2H, NCH_2), 3.83-3.85 (m, 2H, NCH_2), 7.18 (d, 1H, H-6, ^3J = 9.6 Hz), 8.31 (d, 1H,

H-7, ^{3}J = 9.6 Hz). ^{19}F NMR: signals are absent. ^{13}C NMR: 21.2 (CH_3), 44.4 (NCH_2), 44.7 (NCH_2), 45.2 (NCH_2), 45.5 (NCH_2), 49.2 (C(O)N<u>C</u>H$_2$), 50.0 (C(O)N<u>C</u>H$_2$), 111.5, 113.9, 129.1, 131.8, 133.1, 154.6, 160.5, 168.5, 168.7, 168.8. Mass-spectrum, *m/z* (I_{rel}, %): 460 $[M]^+$ (2), 443 (22), 112 (18), 56 (33), 43 (100), 42 (14). Found, %: C 52.18, H 5.28, N 18.29. $C_{20}H_{24}N_6O_5S$. Calculated, %: C 52.16, H 5.25, N 18.25.



5e

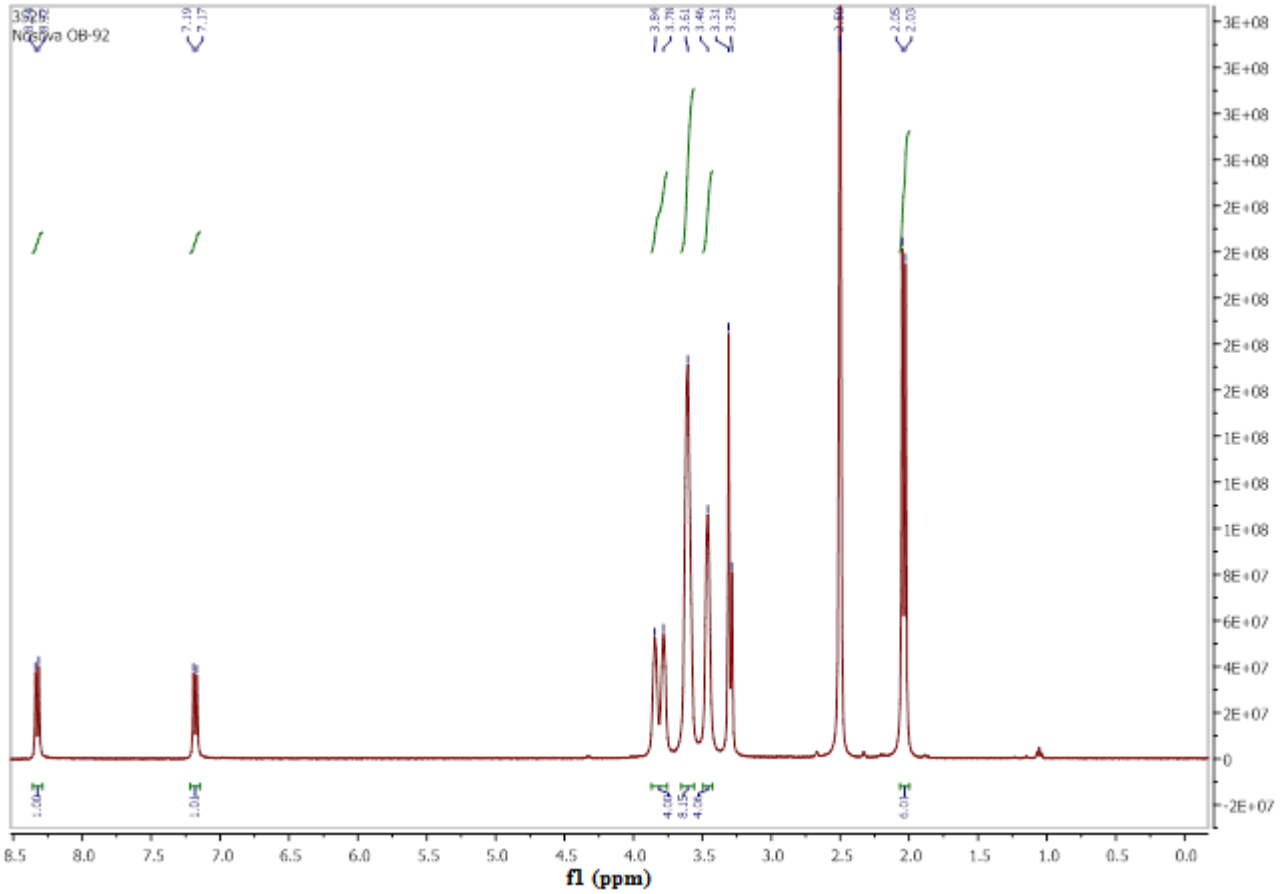


Figure S14. NMR ^{1}H spectrum of benzothiazinone **5e**.

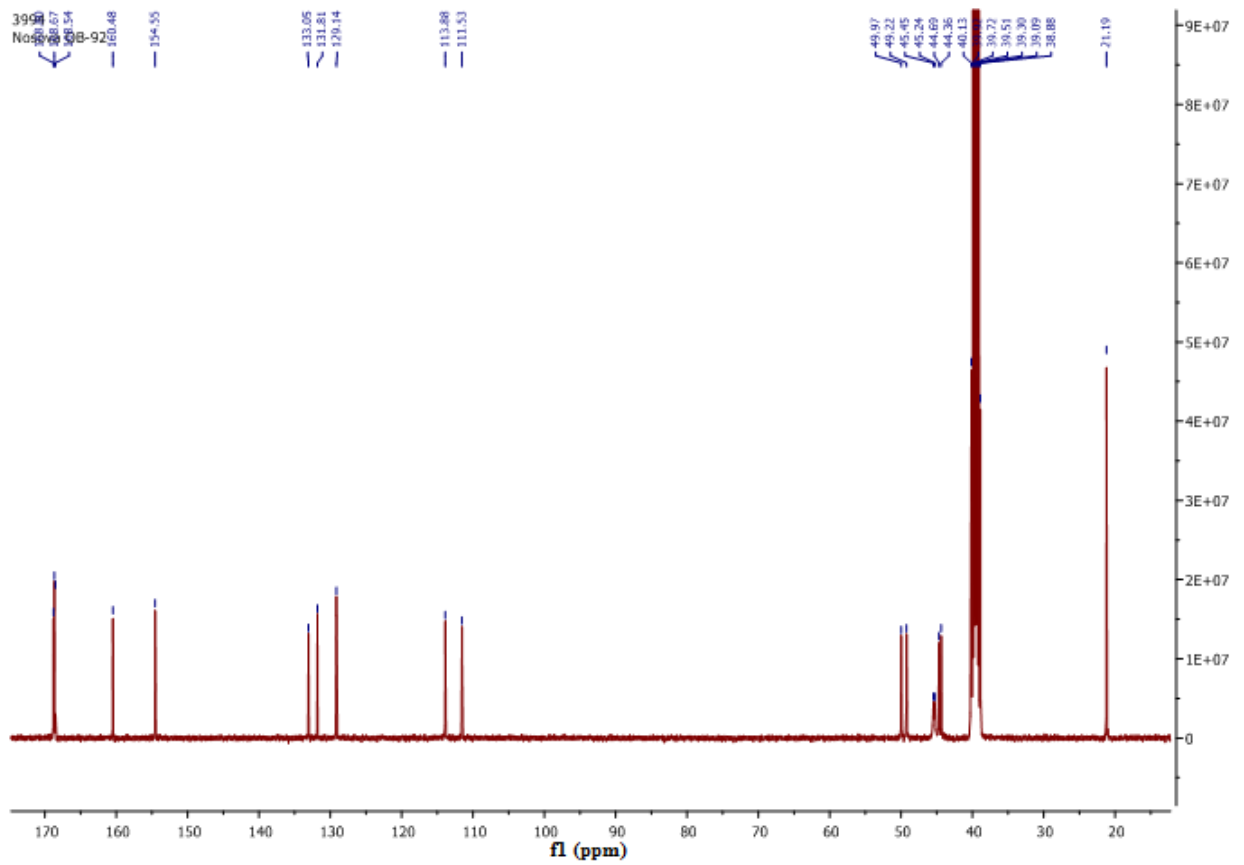


Figure S15. NMR ^{13}C spectrum of benzothiazinone **5e**.

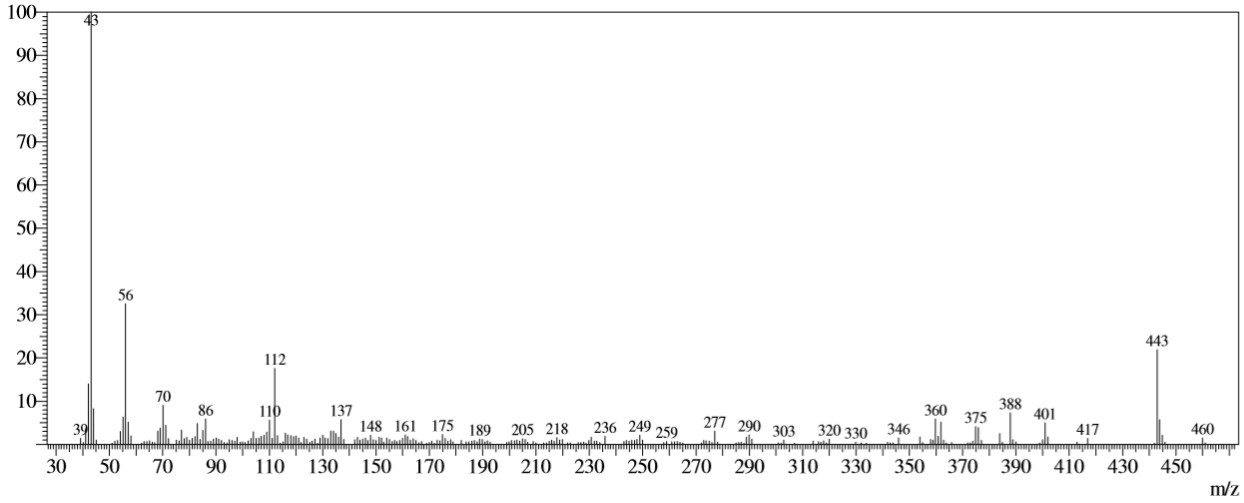
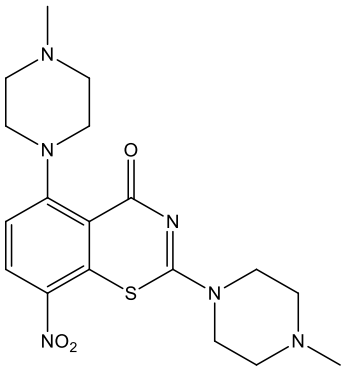


Figure S16. Mass-spectrum of benzothiazinone **5e**.

2,5-Bis(4-methylpiperazin-1-yl)-8-nitro-1,3-benzothiazin-4-one (5f). After the completing the reaction, the mixture was concentrated *in vacuo*, the residue was triturated with diethyl ether, washed with water and recrystallized from ethanol. Yield 2.1 g (76 %), mp 189-191 ºC. ^1H NMR: 2.22 (s, 6H, $2CH_3$), 2.41-2.45 (m, 4H, $N(CH_2)_2$), 3.30-3.32 (m, 4H, $N(CH_2)_2$), 3.38-3.40 (m, 4H, $N(CH_2)_2$), 3.77 (m, 4H, $N(CH_2)_2$), 7.21 (d, 1H, H-6, ^3J = 9.0 Hz), 8.27 (d, 1H, H-7, ^3J

= 8.1 Hz). ^{19}F NMR: signals are absent. ^{13}C NMR: 45.4 (NCH_2), 45.5 (NCH_2), 50.2 (CH_3), 53.9 (NCH_2), 54.2 (NCH_2), 111.8, 114.4, 129.1, 131.6, 133.2, 154.6, 160.2, 169.9. Mass-spectrum, *m/z* (I_{rel}, %): 405 $[M]^+$ (1), 347 (18), 335 (19), 334 (100), 290 (15), 277 (10), 83 (31), 71 (12), 70 (88), 58 (11), 56 (13), 43 (32), 42 (30). Found, %: C 53.41, H 5.73, N 20.72. $C_{18}H_{24}N_6O_3S$. Calculated, %: C 53.45, H 5.98, N 20.78.



5f

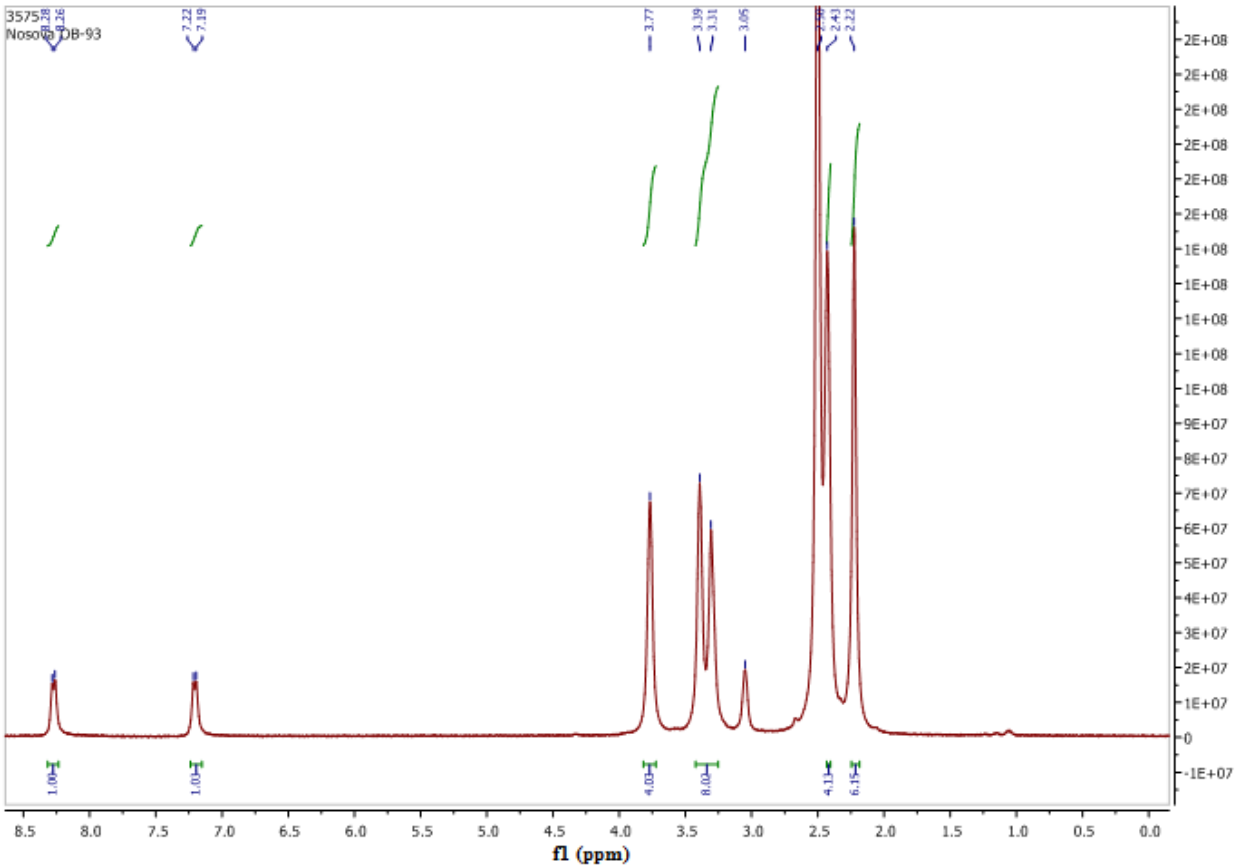


Figure S17. NMR ^{1}H spectrum of benzothiazinone **5f**.

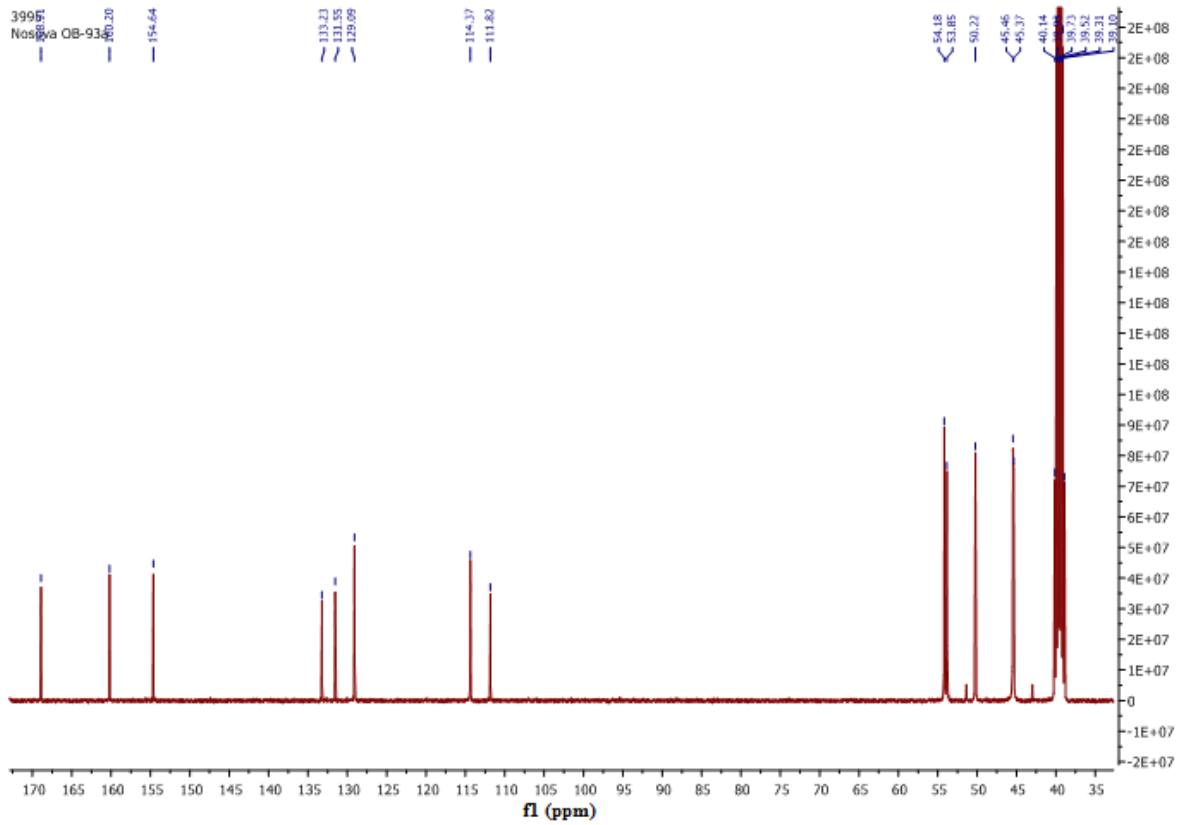


Figure S18. NMR ^{13}C spectrum of benzothiazinone **5f**.

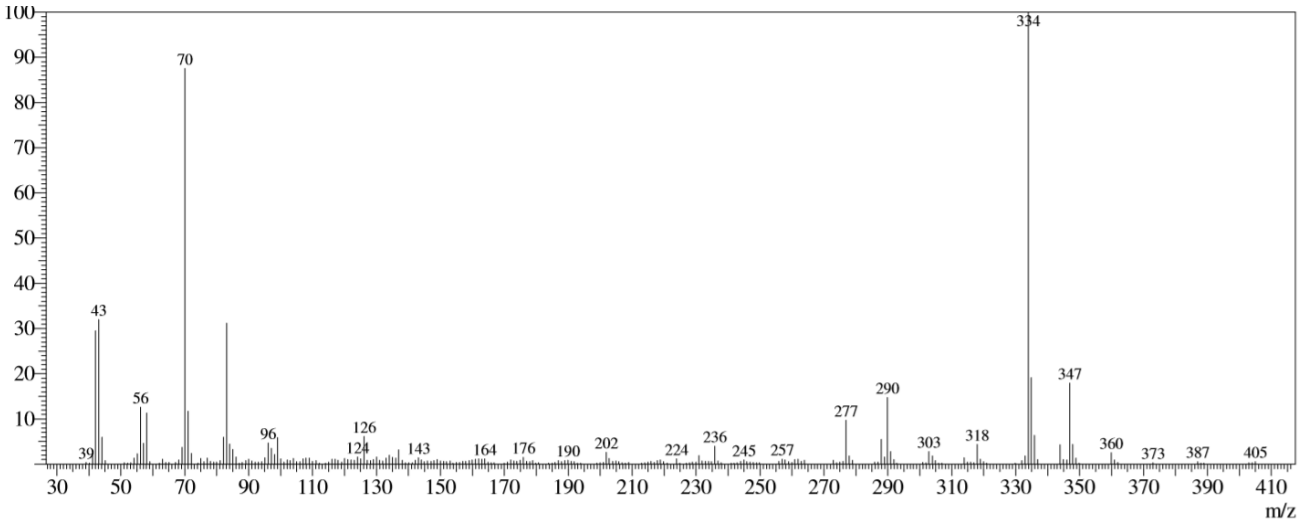
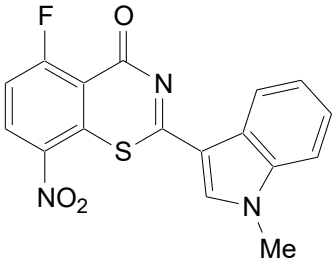


Figure S19. Mass-spectrum of benzothiazinone **5f**.

5-Fluoro-8-nitro-2-(1-methylindol-3-yl)-1,3-benzothiazin-4-one (7). To a toluene solution of 2,6-difluoro-3-nitrobenzoyl chloride (0.88 ml, 6.26 mmol), a solution of ammonium thiocyanate (0.476 g, 6.26 mmol) in acetonitrile (10 ml) was added. The mixture was stirred at 40 °C for 5 min, the precipitate of NH_4Cl was filtered off, and 1-methylindol (1.2 ml, 9.39 mmol) was added to the solution of 2,6-difluoro-3-nitrobenzoyl isothiocyanate. The mixture was stirred at room temperature

for 3 h, the precipitate was filtered off and washed with hot ethanol (10 ml). Yield 1.9 g (84 %), mp 288-290 ºC. ^{1}H NMR: 3.99 (s, 3H, CH_3), 7.33-7.37 (m, 2H, C_6H_4), 7.57-7.61 (m, 2H, C_6H_4), 8.48-8.51 (m, 1H, H-6 or H-7), 8.68 (s, 1H, H-2'), 8.69-8.72 (m, 1H, H-6 or H-7). ^{19}F NMR: -98.73 (dd, 1F, $^{3}J_{FH}$ 10.1 Hz, $^{4}J_{FH}$ 3.9 Hz). Mass-spectrum, *m/z* (I_{rel}, %): 355 $[M]^+$ (16), 157 (11), 156 (100), 155 (23). Found, %: C 57.51, H 2.80, N 11.79. $C_{17}H_{10}FN_3O_3S$. Calculated, %: C 57.46, H 2.84, N 11.83.



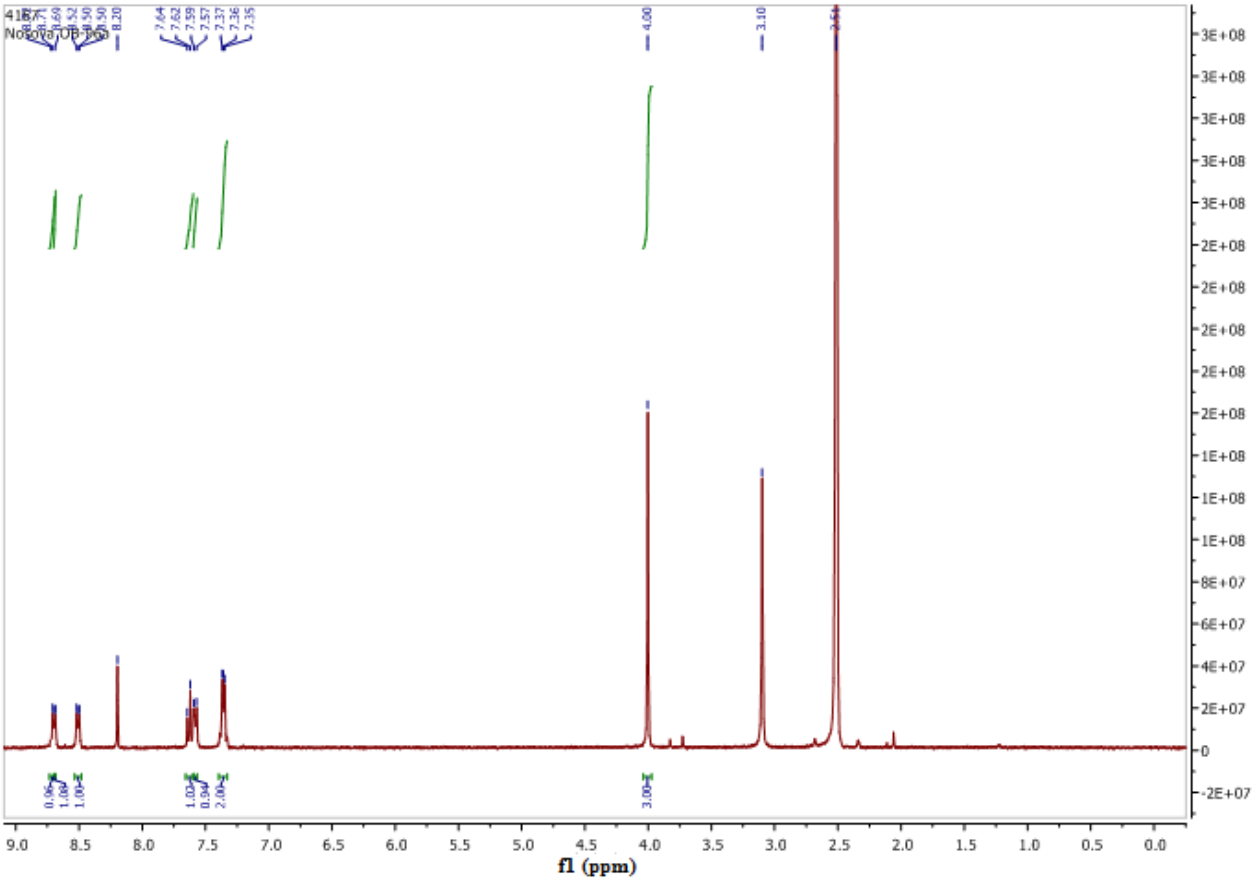


Figure S20. NMR ^{1}H spectrum of benzothiazinone **7**.

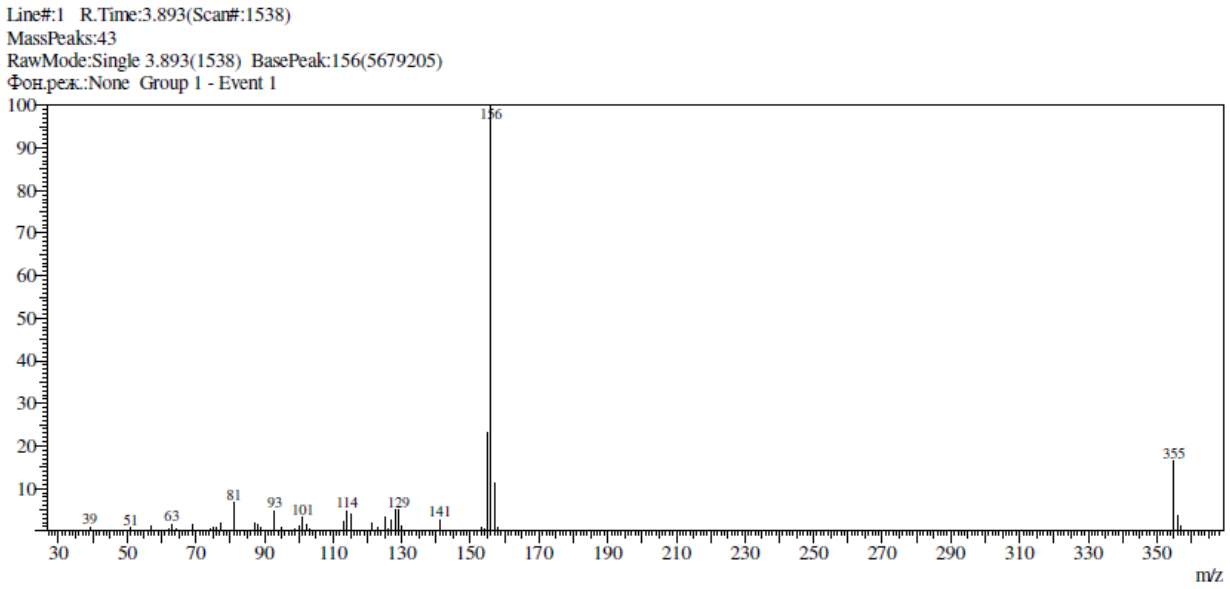


Figure S21. Mass-spectrum of benzothiazinone **7**.

The procedure of screening towards micobacteria

The culture of *M. tuberculosis* $H_{37}R_v$ was grown to an optical density of OD_{600} = 1.0, after which it was inoculated into each well of the plate (final concentration of 1×105 cells per ml) containing 100 µl of Middlebrook medium with growth supplement ADC and Tween 80. Each of the test compounds at a starting concentration of 64 µg ml^{-1} was added to the top row of the plate wells, followed by a series of consecutive double dilutions for each of the test compounds (32; 16; 8; 4; 2; 1; 0.5 µg ml^{-1}); isoniazide was used as a positive control. The plate was incubated for 6 days in a static mode at 37 °C, then 2.5 µl of sterile 0.1% resazurine solution (to a final concentration of 0.0025% w/v) was added to each well and incubated for another 16-18 h under similar conditions, after which a change in color from blue to pink was visually detected. The MIC value was taken to be equal to the minimum concentration of the test compound at which no change in color from blue to pink occurred. MIC values were determined in three independent experiments.