Supporting Information

Synthesis and Evaluation of ^{18}F-Labeled 2-Phenylbenzothiazoles as Positron Emission Tomography Imaging Agents for Amyloid Plaques in Alzheimer's Disease

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1. Experimental details for compound 1b, 1c, 1d, 2b, 2c, 2d, 3c, 3d, 3e, 3f and 3h

N-3',5'-dimethoxyphenyl-4-fluorobenzamide (1b). 1b was synthesized following method A starting from 3,5-dimethoxyaniline (12.26 g, 0.080 mol) and 4-fluorobenzoyl chloride (12.68 g, 0.080 mol) in pyridine (60 ml). This yielded 20.83 g of **1b** as a white solid (0.076 mol, 95%). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 3.74 (s, 6H, 2CH_3O), δ 6.27 (s, 1H, 4'-H), δ 7.08 (d, 2H, ^4J = 1.8 Hz, 2'-H 6'-H), δ 7.37 (t, 2H, ^3J = 8.6 Hz, 3-H 5-H), δ 8.02 (dd, 2H, ^3J = 8.4 Hz, ^4J = 3.2 Hz, 2-H 6-H), δ 10.18 (s, 1H, NH-CO). Accurate MS ES$^+$ m/z [M+H]$^+$ 276.1030 (calculated for $C_{15}H_{14}NO_3F$ 276.1031). Mp: 116.4 – 117.0 °C.

N-4'-methoxyphenyl-4-nitrobenzamide (1c). 1c was synthesized following method A starting from p-anisidine (12.32 g, 0.100 mol) and 4-nitrobenzoyl chloride (18.56 g, 0.100 mol) in pyridine (60 ml) and refluxing for 2 h. This yielded 15.21 g of **1c** as a green solid (0.056 mol, 56%). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 3.76 (s, 3H, CH_3O), δ 6.95 (d, 2H, ^3J = 8.9 Hz, 3'-H 5'-H), δ 7.68 (d, 2H, ^3J = 8.9 Hz, 2'-H 6'-H), δ 8.18 (d, 2H, ^3J = 8.7 Hz, 2-H 6-H), δ 8.36 (d, 2H, ^3J = 8.6 Hz, 3-H 5-H), δ 10.43 (s, 1H, NH-CO). Accurate MS ES$^+$ m/z [M+H]$^+$ 273.0869 (calculated for $C_{14}H_{12}N_2O_4$ 273.0870). Mp: 193.2 – 194.3 °C.

N-4'-methylphenyl-4-nitrobenzamide (1d). 1d was synthesized following method A starting from p-toluidine (10.72 g, 0.100 mol) and 4-nitrobenzoyl chloride (18.56 g, 0.100 mol) in pyridine (150 ml) and refluxing for 2 h. Crystallization from methanol yielded 22.18 g of **1d** as yellow needles (0.086 mol, 86%). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 2.30 (s, 3H, CH_3), δ 7.18 (d, 2H, ^3J = 8.4 Hz, 3'-H 5'-H), δ 7.67 (d, 2H, ^3J = 8.4 Hz, 2'-H 6'-H), δ 8.18 (d, 2H, ^3J = 8.8 Hz, 2-H 6-H), δ 8.37 (d, 2H, ^3J = 8.8 Hz, 3-H 5-H), δ 10.48 (s, 1H, NH-CO).

Accurate MS ES$^+$ m/z $[M+H]^+$ 257.0903 (calculated for $C_{14}H_{12}N_2O_3$ 257.0921). Mp: 196.4 – 197.1 °C.

N-3',5'-dimethoxyphenyl-4-fluorothiobenzamide (2b). **2b** was synthesized following method B starting from **1b** (2.04 g, 0.0074 mol) and Lawesson's reagent (1.69 g, 0.0042 mol) in chlorobenzene (10 ml). After refluxing for 3 h, chlorobenzene was evaporated and the residue was purified with silica column chromatography using dichloromethane as eluent to yield 0.764 g of **2b** as a yellow solid (0.0026 mol, 37%). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 3.75 (s, 6H, 2CH_3O), δ 6.44 (s, 1H, 4'-H), δ 7.17 (d, 2H, ^4J = 1.8 Hz, 2'-H 6'-H), δ 7.29 (t, 2H, ^3J = 8.9 Hz, 3-H 5-H), δ 7.87 (dd, 2H, ^3J = 8.6 Hz, ^4J = 3.0 Hz, 2-H 6-H), δ 11.69 (s, 1H, NH-CS). Accurate MS ES$^+$ m/z $[M+H]^+$ 292.0790 (calculated for $C_{15}H_{14}NO_2FS$ 292.0802). Mp: 105.2 – 105.4 °C.

N-4'-methoxyphenyl-4-nitrothiobenzamide (2c). **2c** was synthesized following method B starting from **1c** (14.43 g, 0.053 mol) and Lawesson's reagent (12.94 g, 0.032 mol) in 1,4-dioxane (120 ml). Crystallization from methanol yielded 13.78 g of **2c** as brown crystals (0.048 mol, 91%). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 3.78 (s, 3H, CH_3O), δ 7.02 (d, 2H, ^3J = 8.9 Hz, 3'-H 5'-H), δ 7.77 (d, 2H, ^3J = 8.9 Hz, 2'-H 6'-H), δ 7.99 (d, 2H, ^3J = 8.7 Hz, 2-H 6-H), δ 8.30 (d, 2H, ^3J = 8.7 Hz, 3-H 5-H), δ 11.98 (s, 1H, NH-CS). Accurate MS ES$^+$ m/z $[M+H]^+$ 289.0652 (calculated for $C_{14}H_{12}N_2O_3S$ 289.0641). Mp: 174.5 – 175.1 °C.

N-4'-methylphenyl-4-nitrothiobenzamide (2d). **2d** was synthesized following method B starting from **1d** (19.22 g, 0.075 mol) and Lawesson's reagent (18.20 g, 0.045 mol) in 1,4-

dioxane (120 ml). Crystallisation from methanol yielded 19.46 g of **2d** as yellow crystals (0.071 mol, 95%).^{1}H-NMR (DMSO-d_6, 200 MHz): δ 2.33 (s, 3H, CH_3), δ 7.26 (d, 2H, ^3J = 8.4 Hz, 3'-H 5'-H), δ 7.76 (d, 2H, ^3J = 8.4 Hz, 2'-H 6'-H), δ 8.00 (d, 2H, ^3J = 8.8 Hz, 2-H 6-H), δ 8.30 (d, 2H, ^3J = 8.8 Hz, 3-H 5-H), δ 12.06 (s, 1H, NH-CS). Accurate MS ES$^+$ m/z $[M+H]^+$ 273.0683 (calculated for $C_{14}H_{12}N_2O_2S$ 273.0692). Mp: 195.3 – 196.7 °C.

5,7-Dimethoxy-2-(4'-fluorophenyl)-1,3-benzothiazole (3c). 3c was synthesized following method C starting from **2b** (0.583 g, 0.0020 mol) and potassium ferricyanide (5.27 g, 0.016 mol). The residue was purified with silica column chromatography using a mixture of dichloromethane and hexane (90:10 V/V) as eluent to yield 0.268 g of **3c** as a white solid (0.0009 mol, 45%). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 3.90 (s, 3H, 5-CH_3O), δ 3.96 (s, 3H, 7-CH_3O), δ 6.50 (d, 1H, ^4J = 1.8 Hz, 6-H), δ 7.17 (t, 2H, ^3J = 8.7 Hz, 3'-H 5'-H), δ 7.18 (d, 1H, ^4J = 2.0 Hz, 4-H), δ 8.06 (dd, 2H, ^3J = 8.4 Hz, ^4J = 3.6 Hz, 2'-H 6'-H). Accurate MS ES$^+$ m/z $[M+H]^+$ 290.0625 (calculated for $C_{15}H_{12}NO_2FS$ 290.0645). Mp: 189.7 – 190.5 °C.

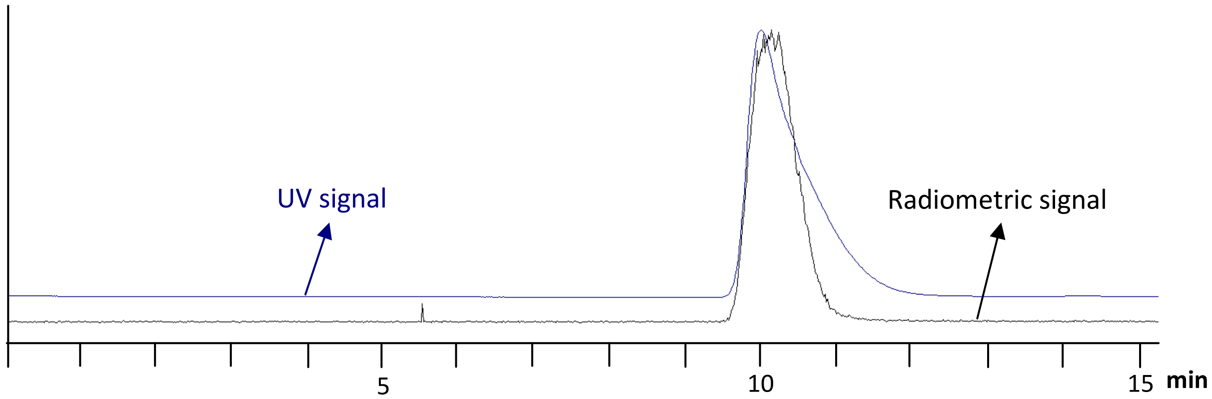
5,7-Dihydroxy-2-(4'-fluorophenyl)-1,3-benzothiazole (3d). 3d was synthesized following method D starting from **3c** (0.50 g, 0.0017 mol), using 9.9 ml BBr_3 and stirring for 12 h. The residue was purified with silica column chromatography using gradient mixtures of hexane and ethylacetate (up to 30%) as eluent to yield 0.384 g of **3d** as a white solid (0.0015 mol, 88%). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 6.45 (d, 1H, ^4J = 1.8 Hz, 6-H), δ 6.89 (d, 1H, ^4J = 1.8 Hz, 4-H), δ 7.38 (t, 2H, ^3J = 8.8 Hz, 3'-H 5'-H), δ 8.09 (dd, 2H, ^3J = 8.7 Hz, ^4J = 5.2 Hz, 2'-H 6'-H), δ 9.58 (s, 1H, 5-OH), δ 10.48 (s, 1H, 7-OH). Accurate MS ES$^+$ m/z $[M+H]^+$ 262.0330 (calculated for $C_{13}H_8NO_2FS$ 262.0333). Mp: 275.0 – 276.5 °C.

6-Methoxy-2-(4'-nitrophenyl)-1,3-benzothiazole (3e). 3e was synthesized following method C starting from **2c** (12.97 g, 0.045 mol) and potassium ferricyanide (59.27 g, 0.180 mol). The residue was purified with silica column chromatography using dichloromethane as eluent to yield 3.92 g of **3e** as a yellow solid (0.014 mol, 31%). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 3.92 (s, 3H, CH_3O), δ 7.15 (dd, 1H, ^3J = 9.2 Hz, ^4J = 2.6 Hz, 5-H), δ 7.38 (d, 1H, ^4J = 2.6 Hz, 7-H), δ 8.00 (d, 1H, ^3J = 8.6 Hz, 4-H), δ 8.20 (dd, 2H, ^3J = 6.8 Hz, ^4J = 2.0 Hz, 2'-H 6'-H), δ 8.33 (dd, 2H, ^3J = 7.0 Hz, ^4J = 2.2 Hz, 3'-H 5'-H). Accurate MS ES$^+$ m/z $[M+H]^+$ 287.0488 (calculated for $C_{14}H_{10}N_2O_3S$ 287.0485). Mp: 203.6 – 204.7 °C.

6-Hydroxy-2-(4'-nitrophenyl)-1,3-benzothiazole (3f). 3f was synthesized following method D starting from **3e** (3.15 g, 0.011 mol) and using 0.025 mol BBr_3. The reaction was stirred overnight and the residue was purified with silica column chromatography using dichloromethane as eluent to yield 1.83 g of **3f** as a yellow solid (0.0067 mol, 61%). ^{1}H-NMR (DMSO-*d*$_6$, 200 MHz): δ 7.05 (dd, 1H, ^3J = 8.8 Hz, ^4J = 1.8 Hz, 5-H), δ 7.47 (d, 1H, ^4J = 2.6 Hz, 7-H), δ 7.93 (d, 1H, ^3J = 9.0 Hz, 4-H), δ 8.24 (d, 2H, ^3J = 8.8 Hz, 2'-H 6'-H), δ 8.36 (d, 2H, ^3J = 8.8 Hz, 3'-H 5'-H), δ 10.10 (s, 1H, 6-OH). Accurate MS ES$^+$ m/z $[M+H]^+$ 273.0350 (calculated for $C_{13}H_8N_2O_3S$ 273.0328). Mp: 253.4 – 254.2 °C.

6-Methyl-2-(4'-nitrophenyl)-1,3-benzothiazole (3h). 3h was synthesized following method C starting from **2d** (16.34 g, 0.060 mol) and potassium ferricyanide (65.85 g, 0.200 mol). Crystallisation from ethyl acetate yielded 9.47 g of **3h** as yellow crystals (0.035 mol, 58%). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.53 (s, 3H, CH_3), δ 7.36 (d, 2H, ^3J = 5.6 Hz, 3'-H 5'-H), δ 7.74 (s, 1H, 5-H), δ 8.00 (d, 1H, ^3J = 5.6 Hz, 7-H), δ 8.24 (d, 2H, ^3J = 9.0 Hz, 4-H), δ 8.34 (d, 2H, ^3J = 8.9 Hz, 2'-H 6'-H). Accurate MS ES$^+$ m/z $[M+H]^+$ 271.0537 (calculated for $C_{14}H_{10}N_2O_2S$ 271.0536). Mp: 212.9 – 214.6 °C.

2. Quality control HPLC chromatograms of [^{18}F]5



Quality control HPLC chromatograms of **[^{18}F]5** coinjected with authentic non-radioactive **5**. Stationary phase: XTerra RP C18 (3.5 µm, 3.0 mm x 100 mm); Mobile phase: 0.05 M ammonium acetate and ethanol/THF (75:25) (45:55 V/V); Flow rate: 0.3 ml/min; Detection: UV 234 nm.

3. Purity of key target compounds together with HPLC chromatograms

HPLC method:

Stationary phase: XTerra^{TM} RP C_{18} column (5 µm, 4.6 mm x 250 mm; Waters)

Mobile phase:

METHOD 1:

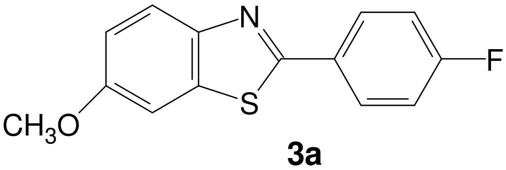
	NH_4OAc 0.05 M	**Acetonitrile**
0 min	95	5
20 min	5	95
25 min	5	95
30 min	95	5

METHOD 2: 50% NH_4OAc 0.05M and 50% EtOH/THF (75/25)

Flow rate: 1 mL/min

UV-wavelength: 254 nm

Compound	**Method**	**Retention time (RT, min)**	**Purity (%)**
3a	2	20.63	98.66
3b	1	20.82	99.36
3d	1	18.00	96.83
3e	2	25.20	97.56
3g	1	22.67	98.51
3h	1	23.88	96.69
5	1	24.25	98.03
8	1	16.37	96.38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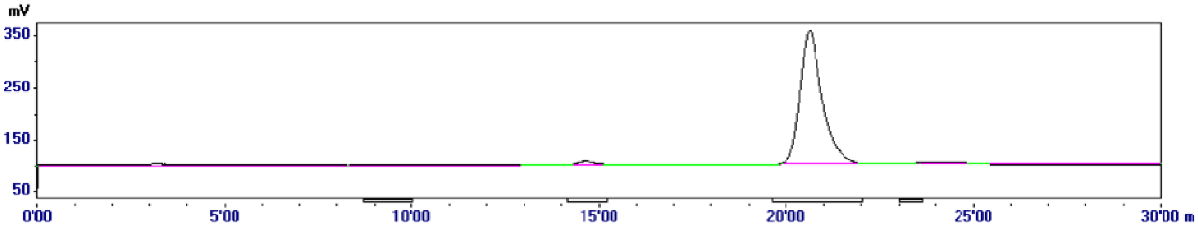
3a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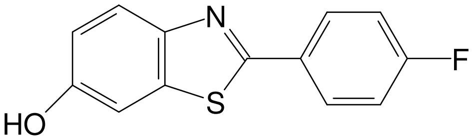
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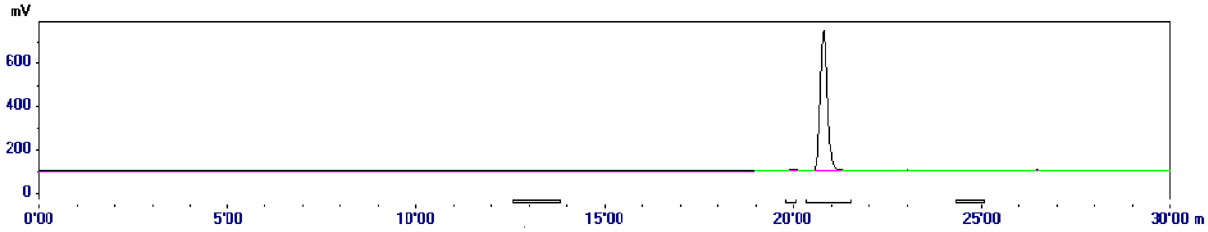
S
F
HO

3b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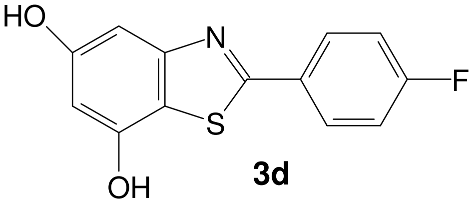
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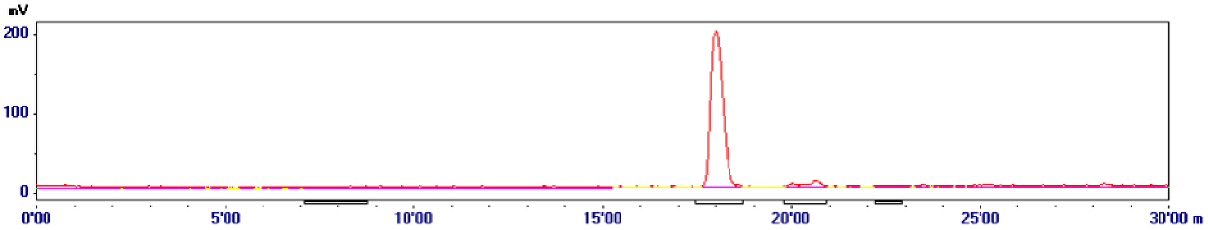
S
F
OH

3d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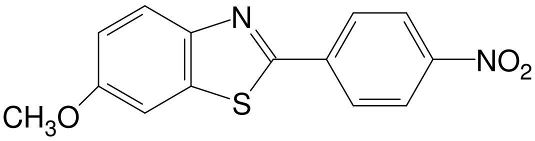
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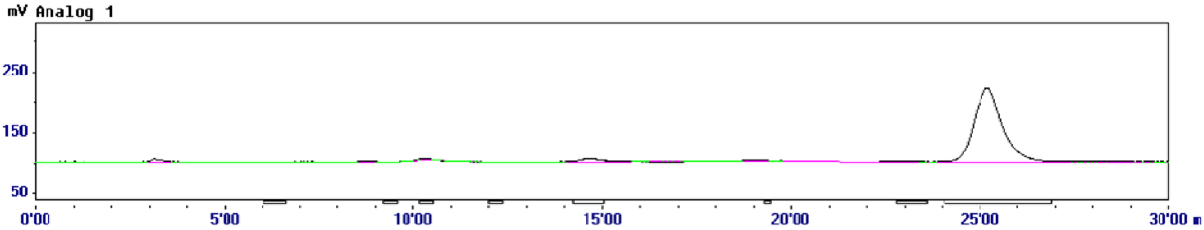
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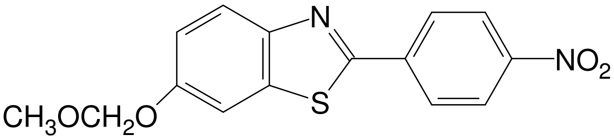
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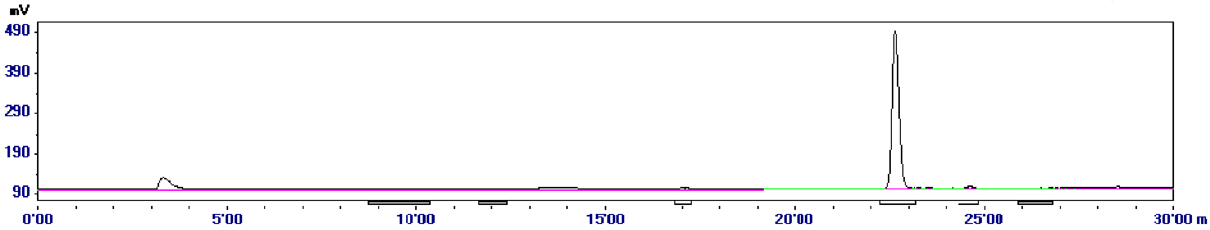
3g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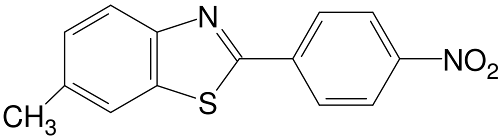
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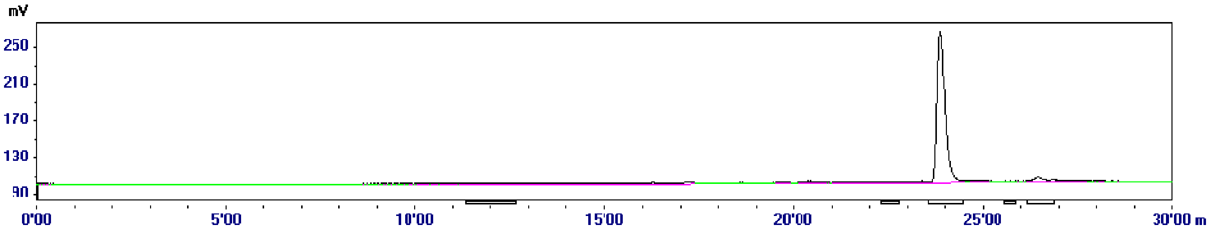
3h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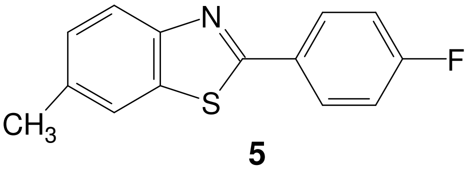
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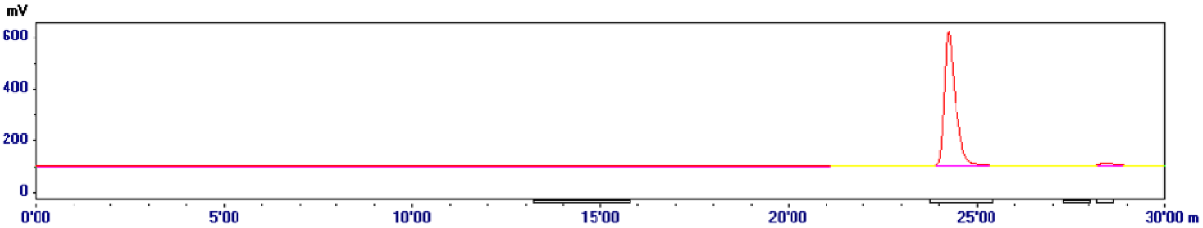
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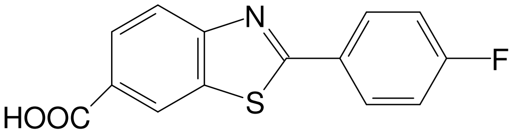
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F
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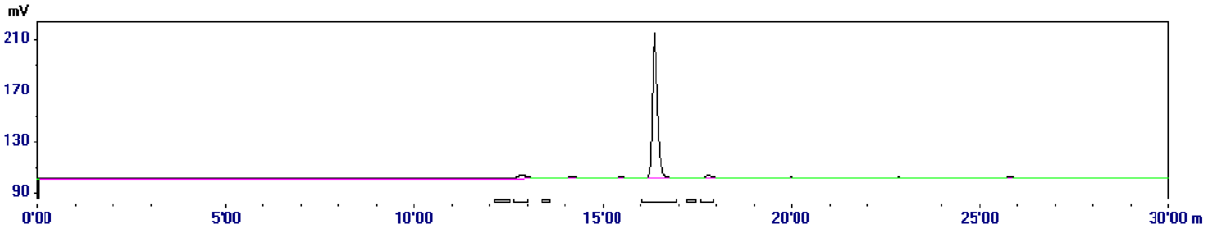
8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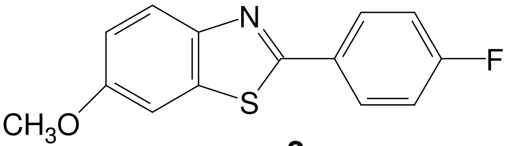
4. ^{1}H-NMR spectra of key target compounds



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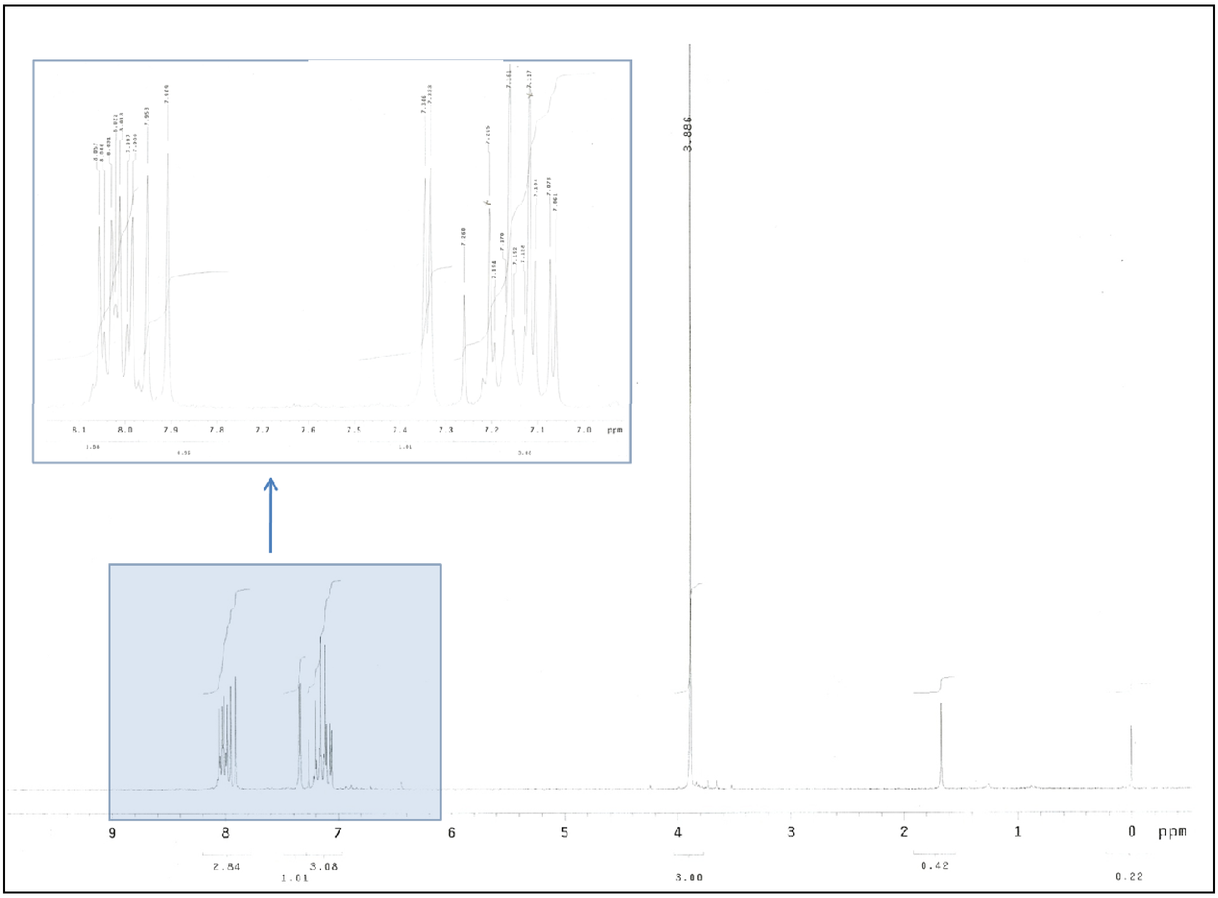

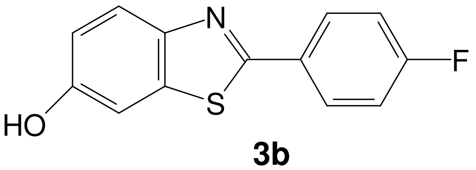

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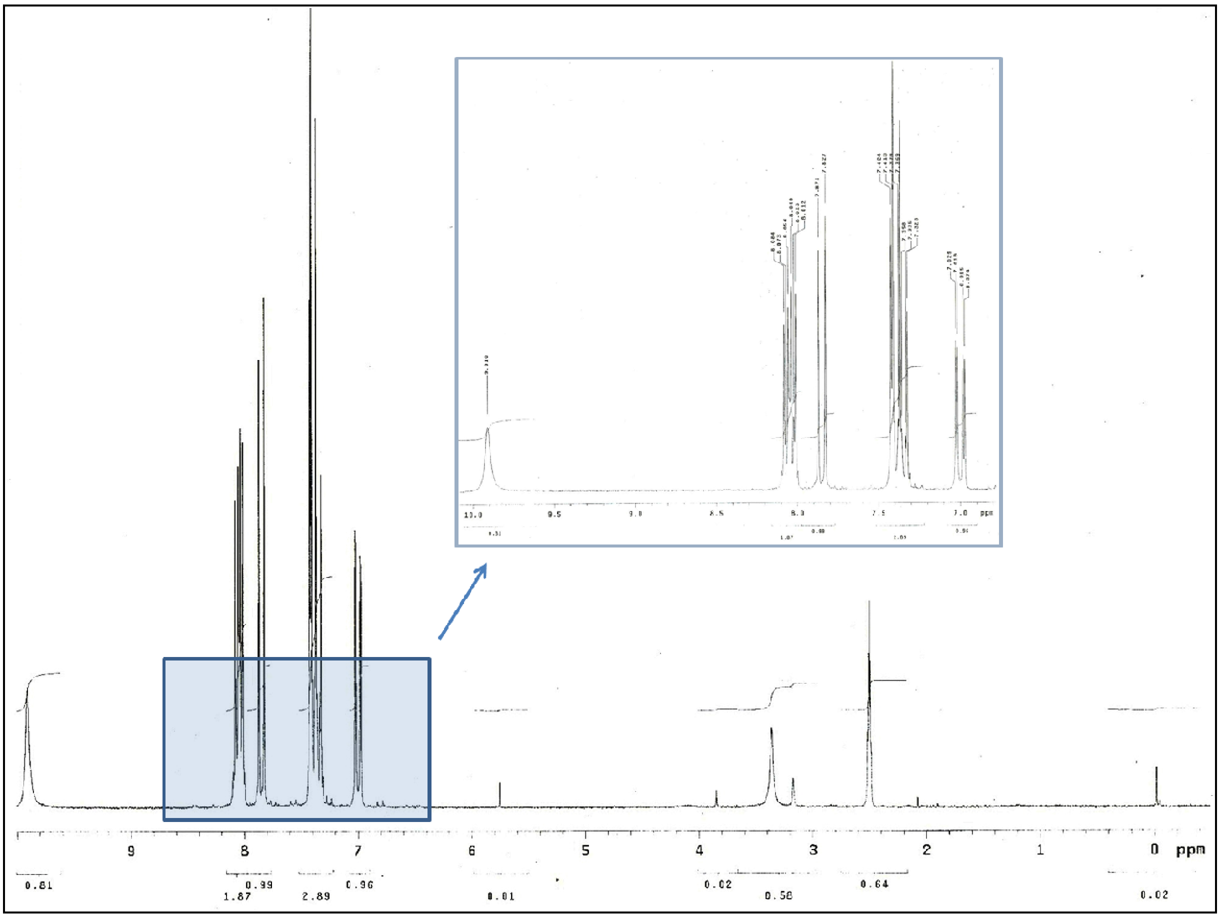

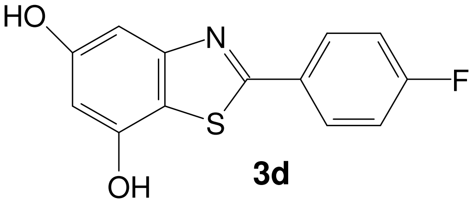
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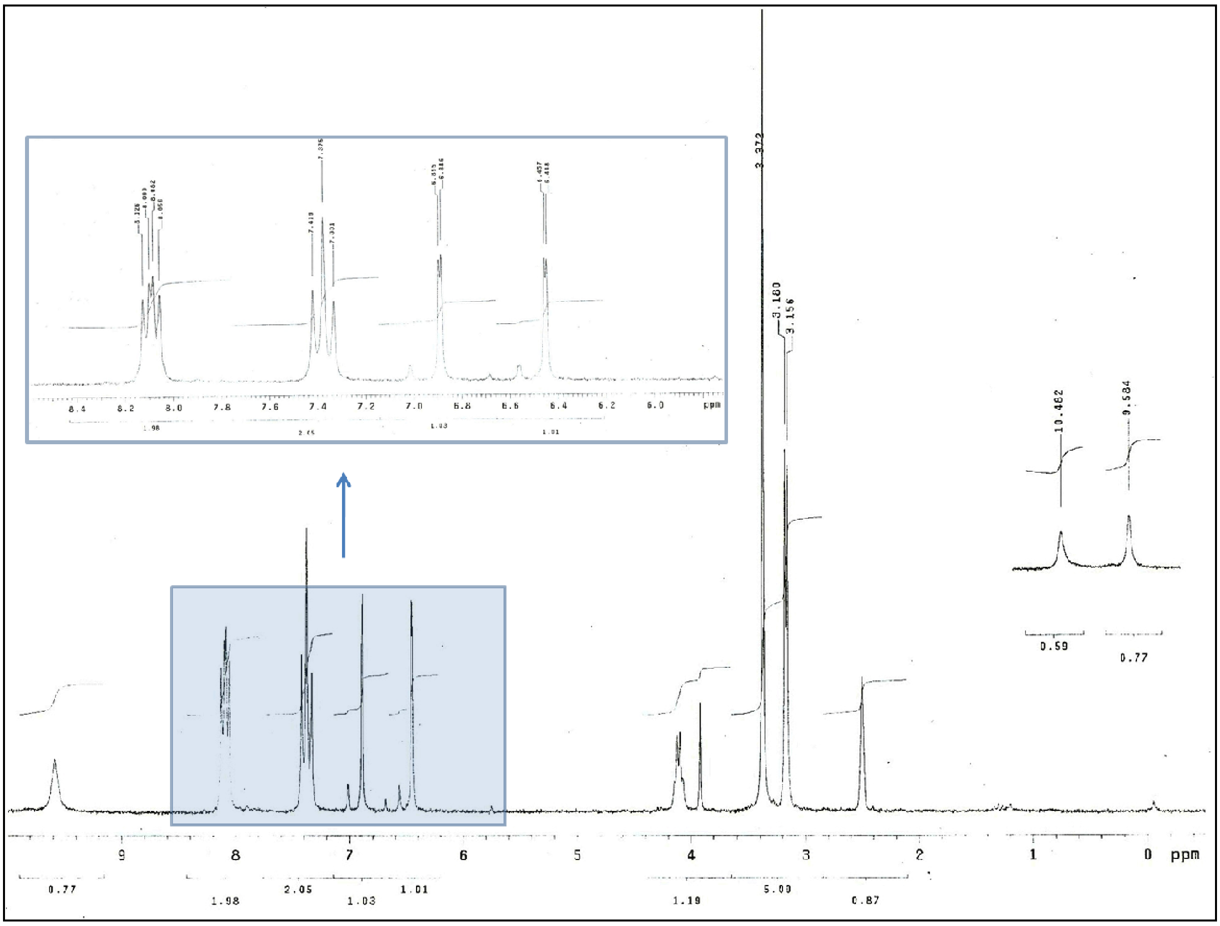
3d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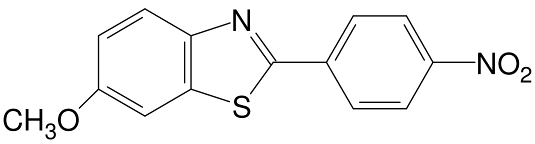
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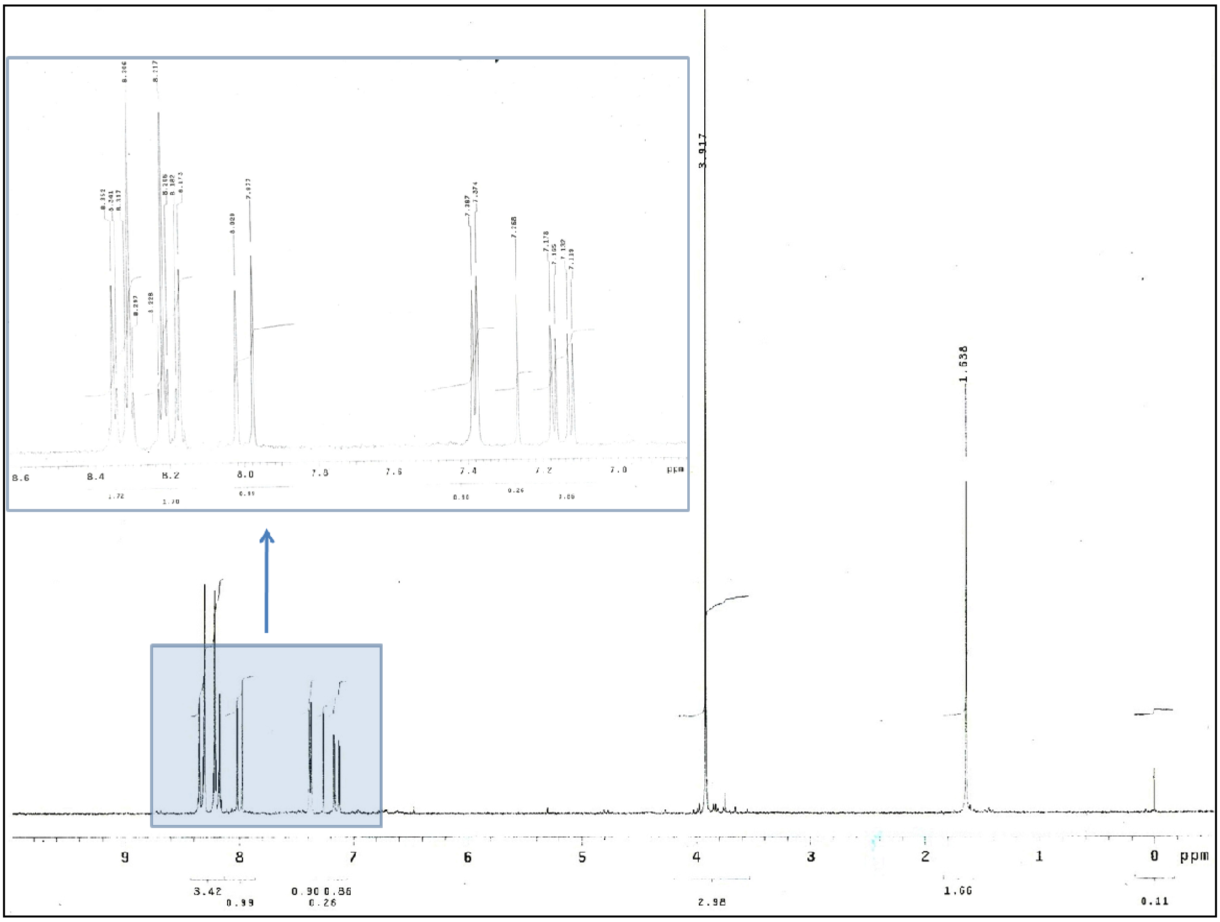
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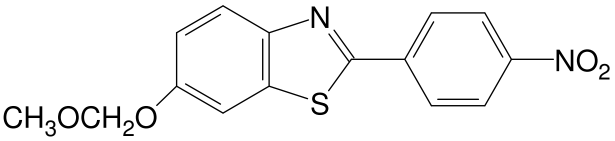
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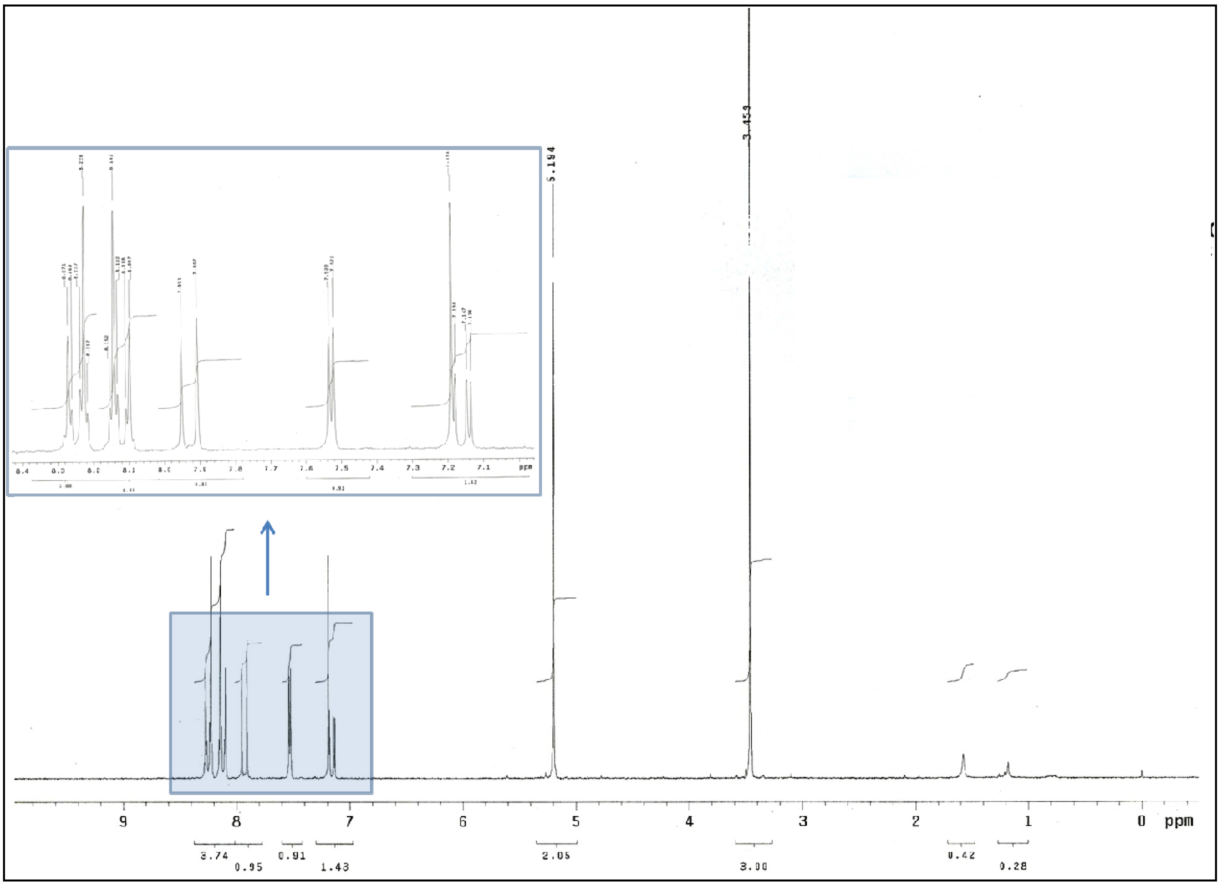
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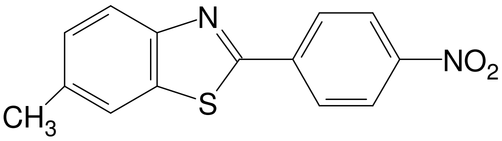
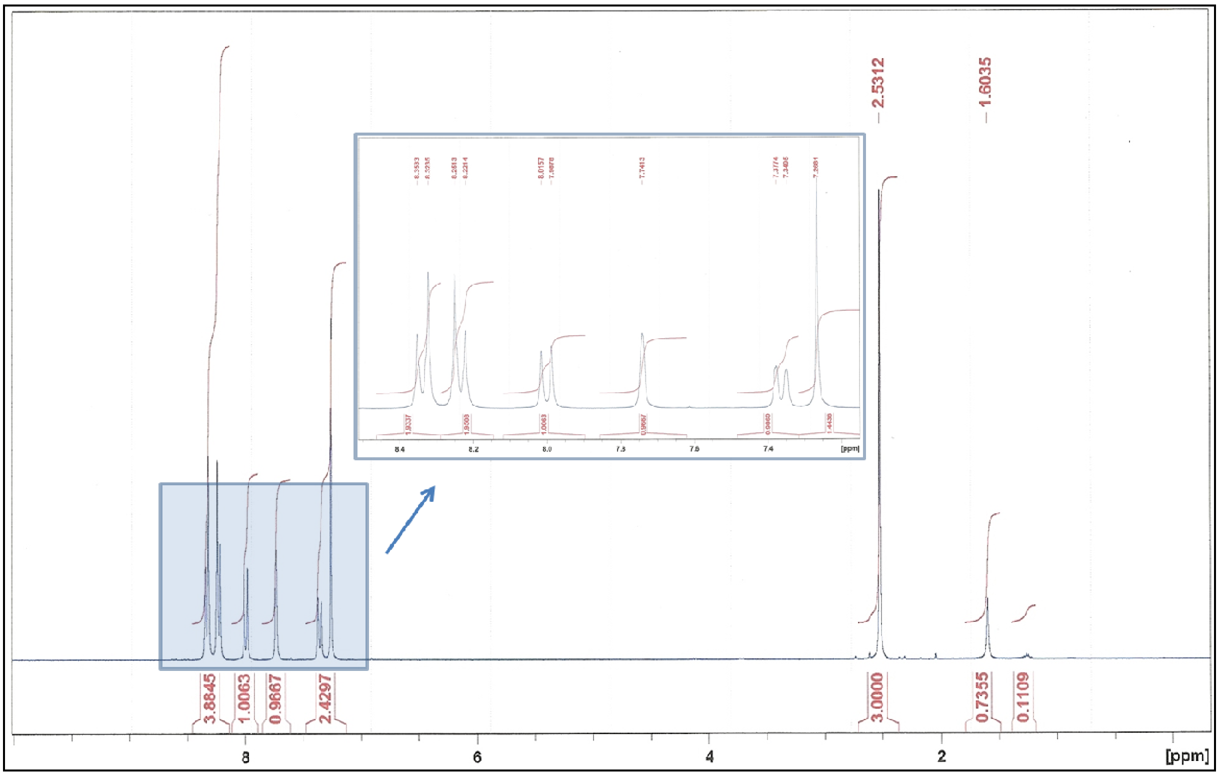
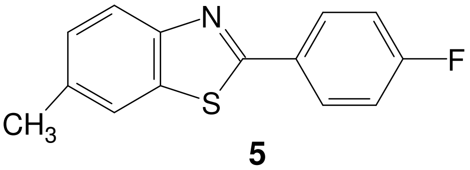
2
1
0
ppm
8.74
0.95
0.91
1.43
2.05
3.00
0.42
0.28

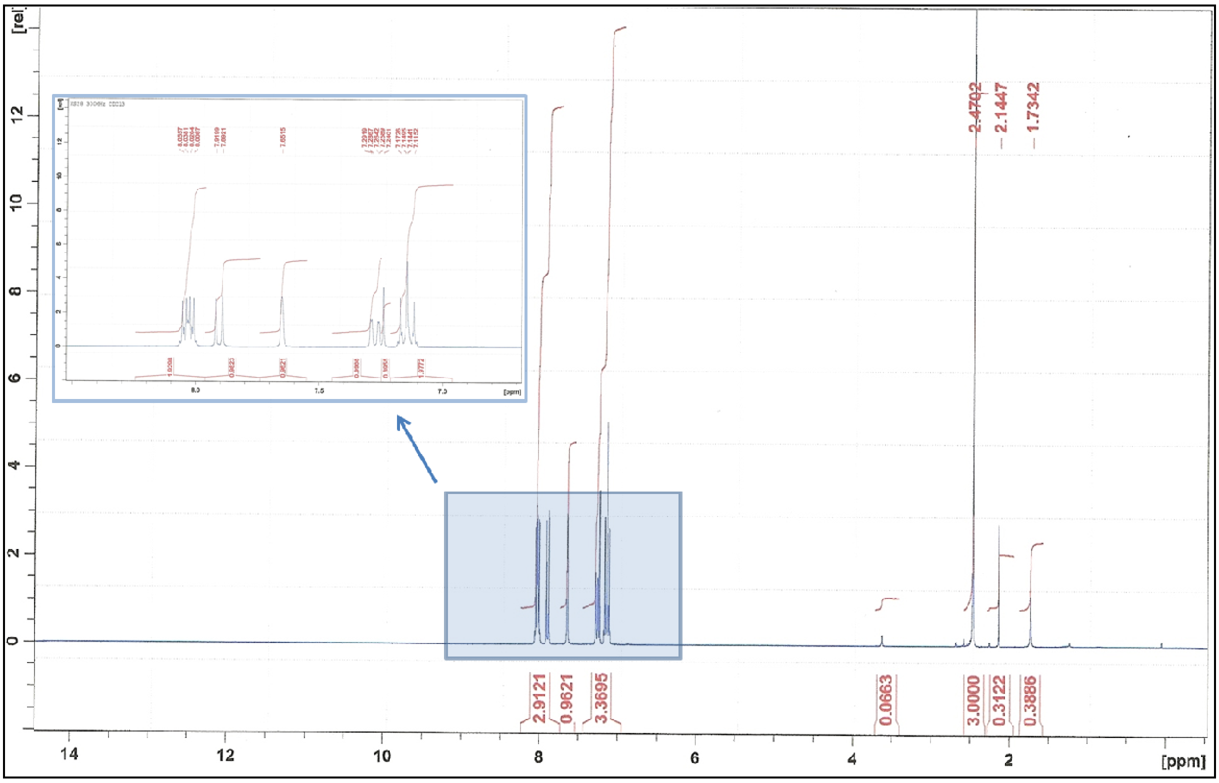

N
S
CH3
NO2

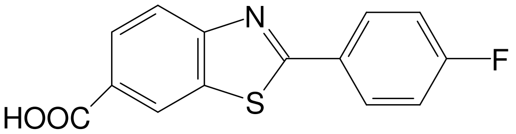
3h


2.5312
1.6035
8.3533
8.3235
8.2513
8.2214
8.0157
7.9878
7.7413
7.3774
7.3495
7.2661
8.4
8.2
8.0
7.8
7.6
7.4
[ppm]
3.8845
1.0063
0.9667
2.4297
3.0000
0.7355
0.1109
8
6
4
2
[ppm]

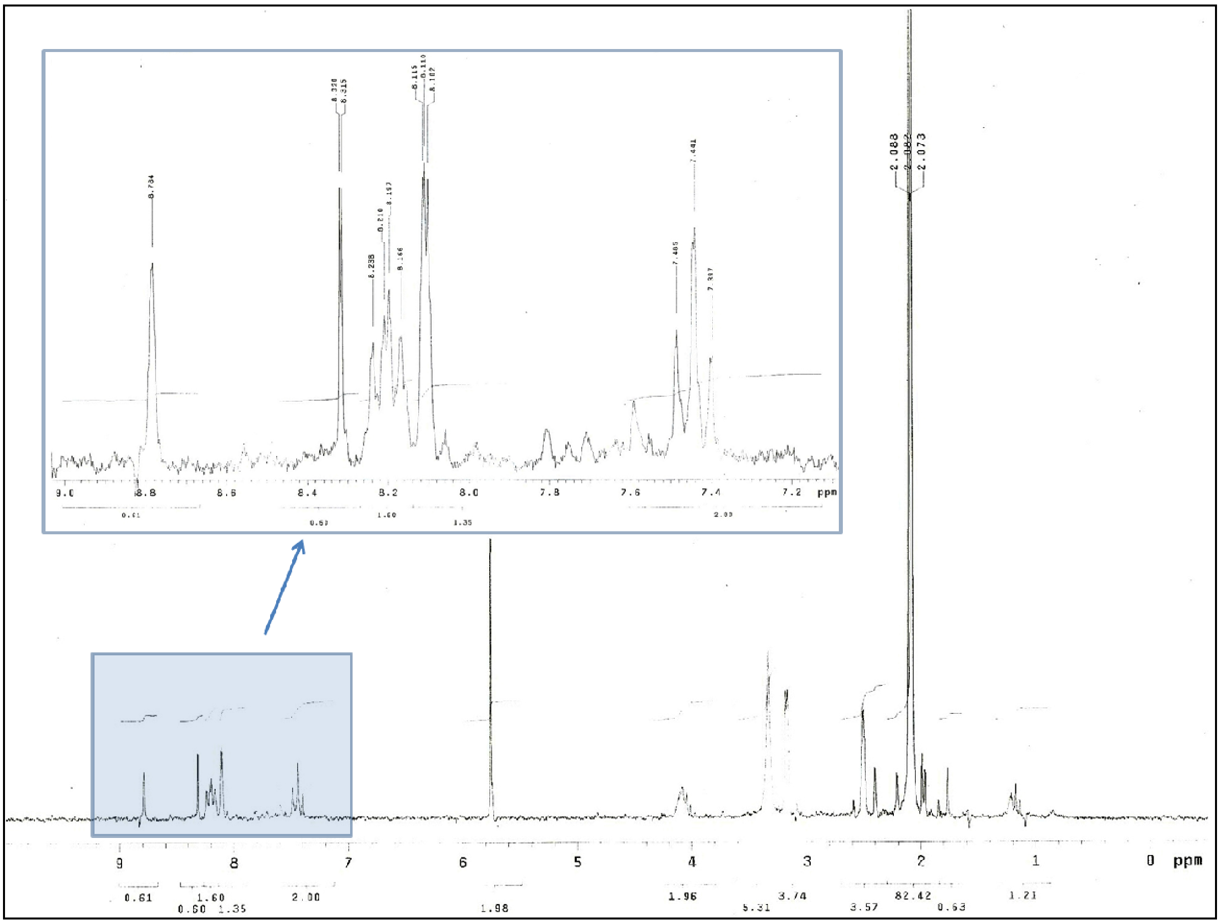


5


[rel]
12
10
8
6
4
2
0
2.4702
2.1447
1.7342
2.9121
0.9621
3.3695
0.0663
3.0000
0.3122
0.3886
14
12
10
8
6
4
2
[ppm]
8.0
7.5
7.0
[ppm]

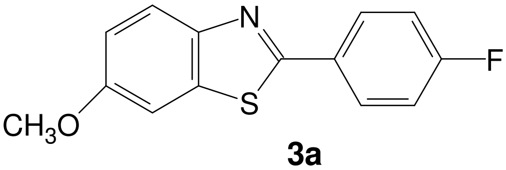

N
S
F
HOOC

8

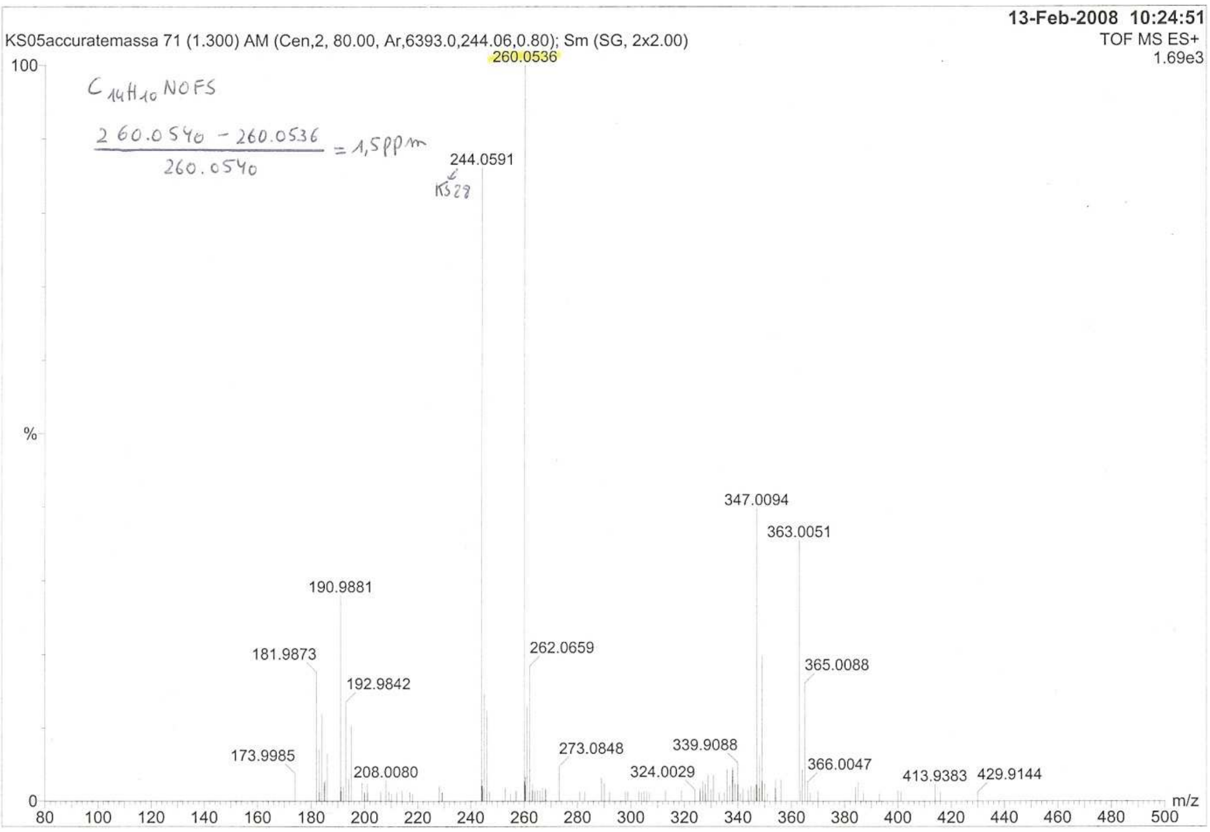

8.784
8.328
8.315
8.238
8.210
8.197
8.166
8.115
8.110
8.102
7.485
7.441
7.397
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
ppm
0.61
0.60
1.60
1.35
2.00
2.088
2.082
2.073
9
8
7
6
5
4
3
2
1
0
ppm
0.61
1.60
0.60
1.35
2.00
1.88
1.96
5.31
3.74
3.57
82.42
0.63
1.21

5. MS spectra of key target compounds

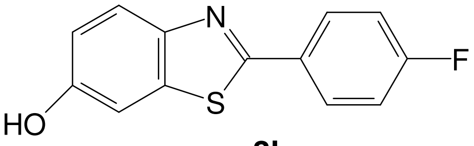
Exact mass measurement was performed on a time-of-flight mass spectrometer (LCT, Micromass, Manchester, UK) equipped with an orthogonal electrospray ionization interface, operated in positive mode (ES$^+$). Accurate mass determination was done by coinfusion with a compound with known mass as an internal calibration standard (lock mass).



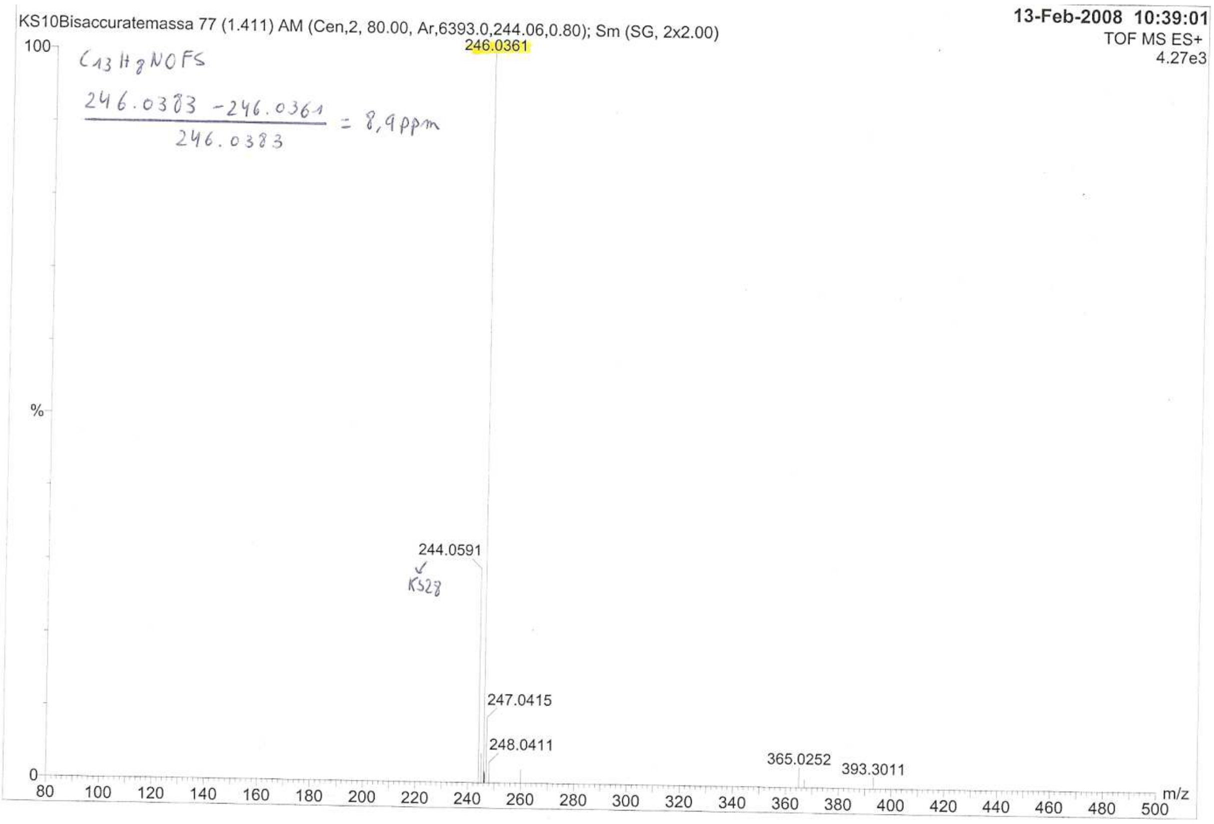
3a



Accurate MS ES$^+$ m/z $[M+H]^+$ 260.0536 (calculated for $C_{14}H_{10}NOFS$ 260.0540). Lock mass: $C_{14}H_{10}FNS$ 244.0591.



3b

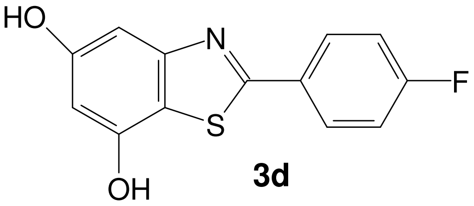


$C_{13}H_8NOFS$

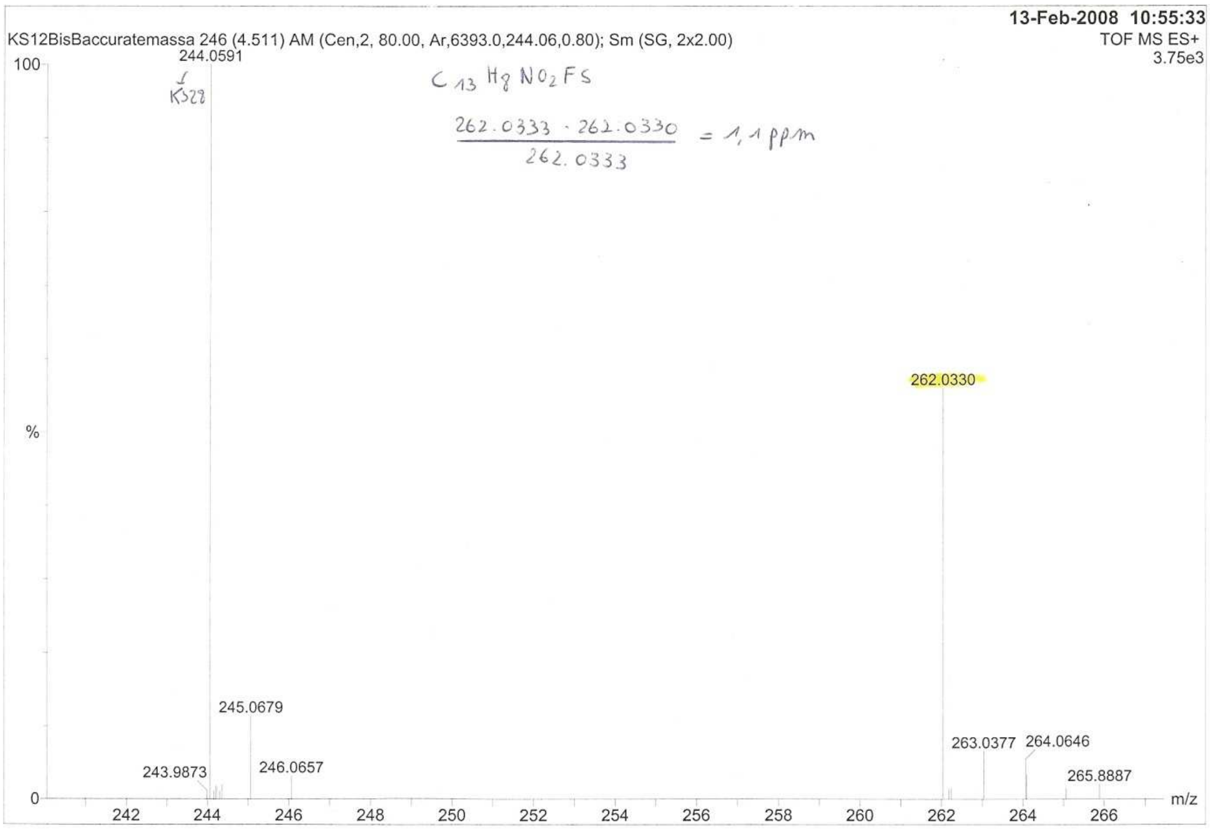
$\frac{246.0383 - 246.0361}{246.0383} = 8,9\,ppm$

✓ KS28

Accurate MS ES$^+$ m/z [M+H]$^+$ 246.0361 (calculated for $C_{13}H_8NOFS$ 246.0383). Lock mass: $C_{14}H_{10}FNS$ 244.0591.



3d

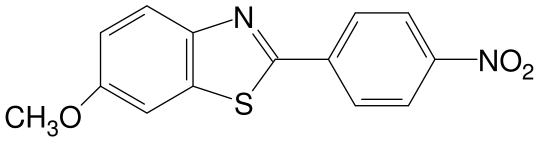


✓ KS28

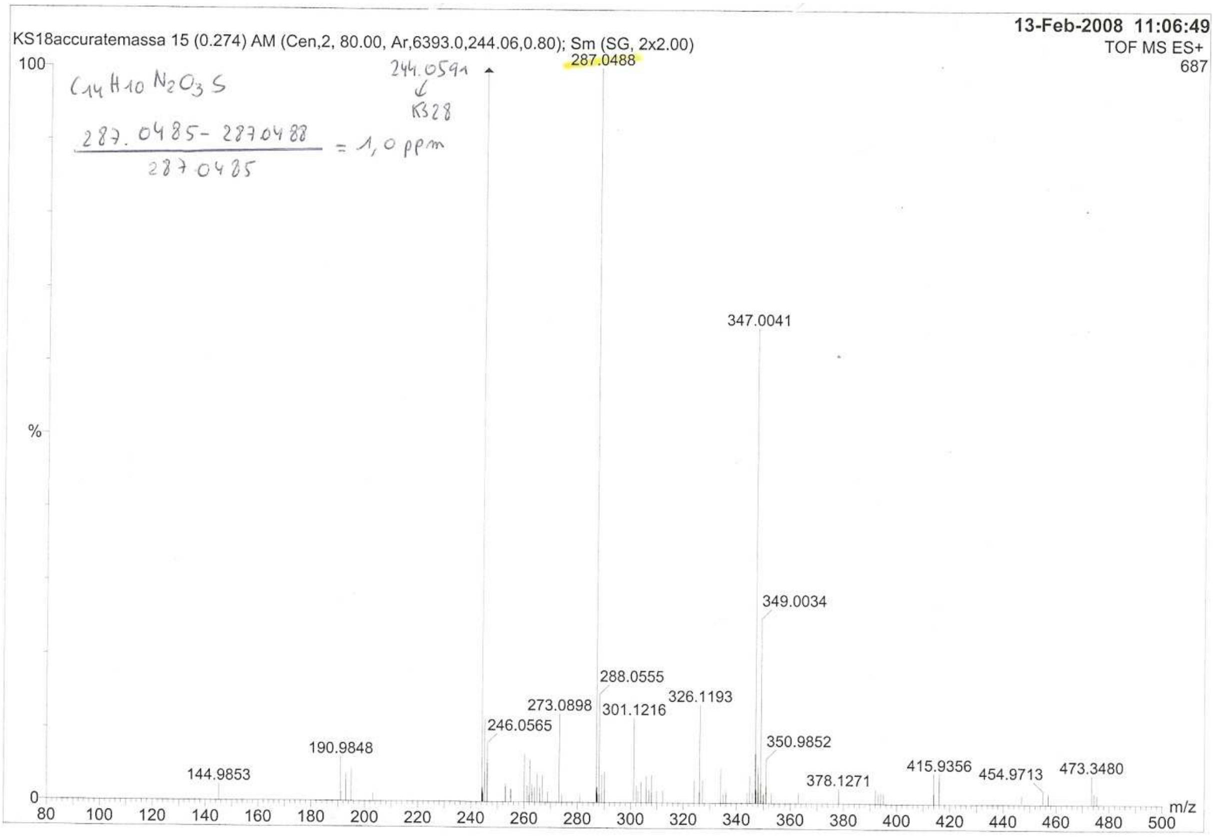
$C_{13}H_8NO_2FS$

$\frac{262.0333 - 262.0330}{262.0333} = 1{,}1$ ppm

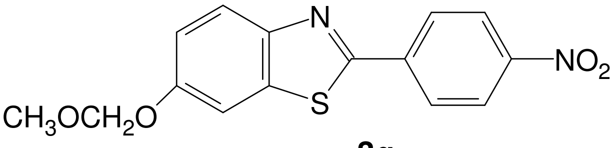
Accurate MS ES$^+$ m/z $[M+H]^+$ 262.0330 (calculated for $C_{13}H_8NO_2FS$ 262.0333). Lock mass: $C_{14}H_{10}FNS$ 244.0591.



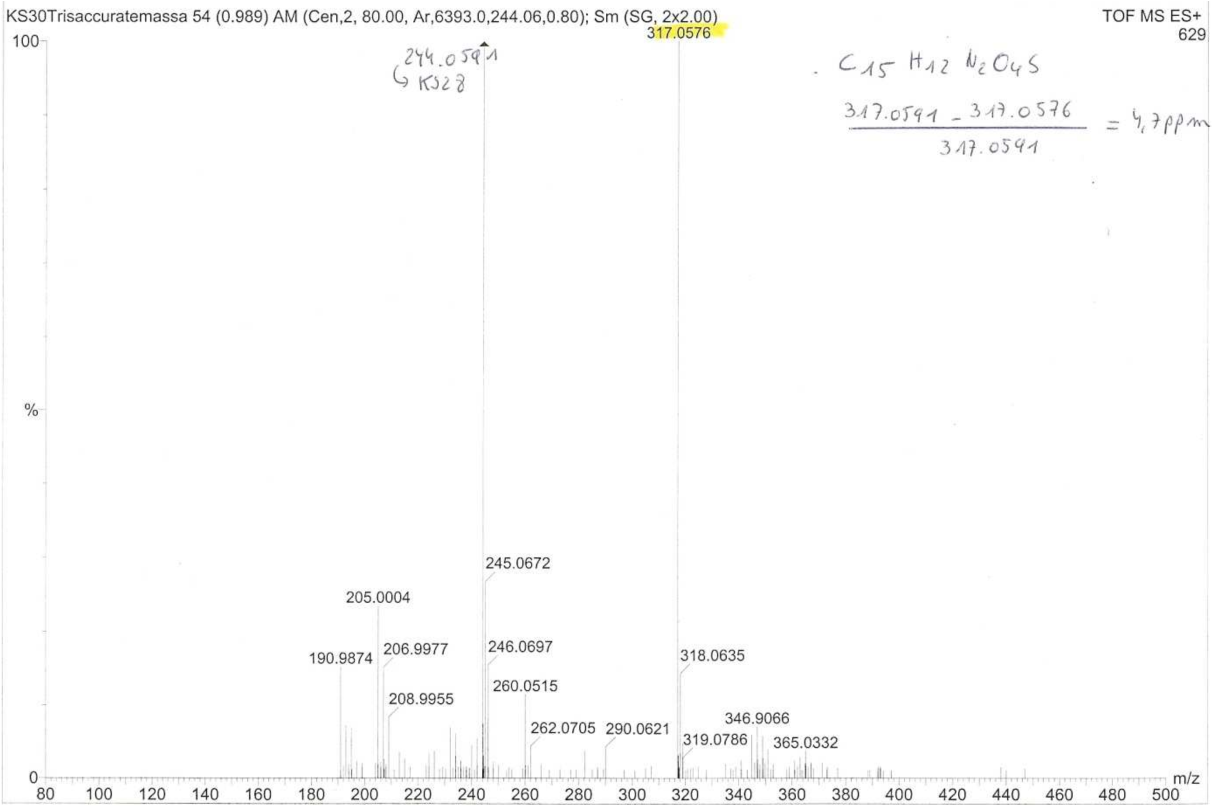
3e



Accurate MS ES$^+$ m/z $[M+H]^+$ 287.0488 (calculated for $C_{14}H_{10}N_2O_3S$ 287.0485). Lock mass: $C_{14}H_{10}FNS$ 244.0591.



3g

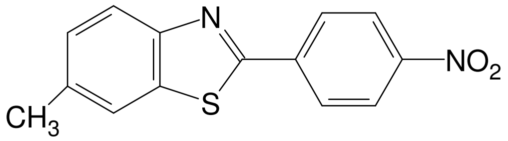


244.0591
↳ KS28

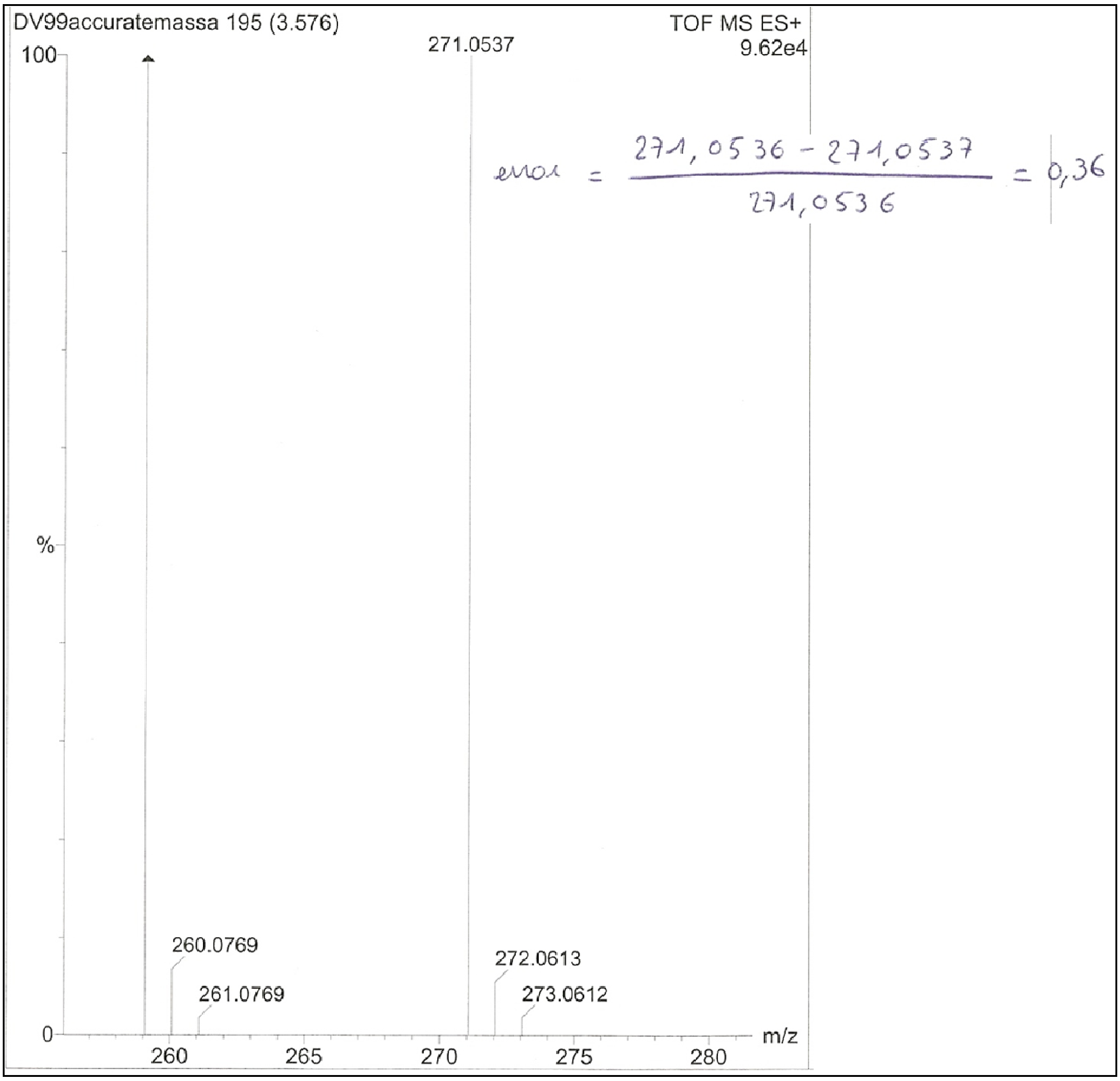
. $C_{15}H_{12}N_2O_4S$

$$\frac{317.0591 - 317.0576}{317.0591} = 4{,}7\,ppm$$

Accurate MS ES$^+$ m/z [M+H]$^+$ 317.0576 (calculated for $C_{15}H_{12}N_2O_4S$ 317.0591). Lock mass: $C_{14}H_{10}FNS$ 244.0591.

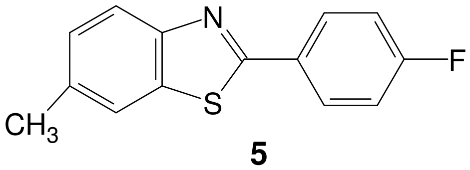


3h

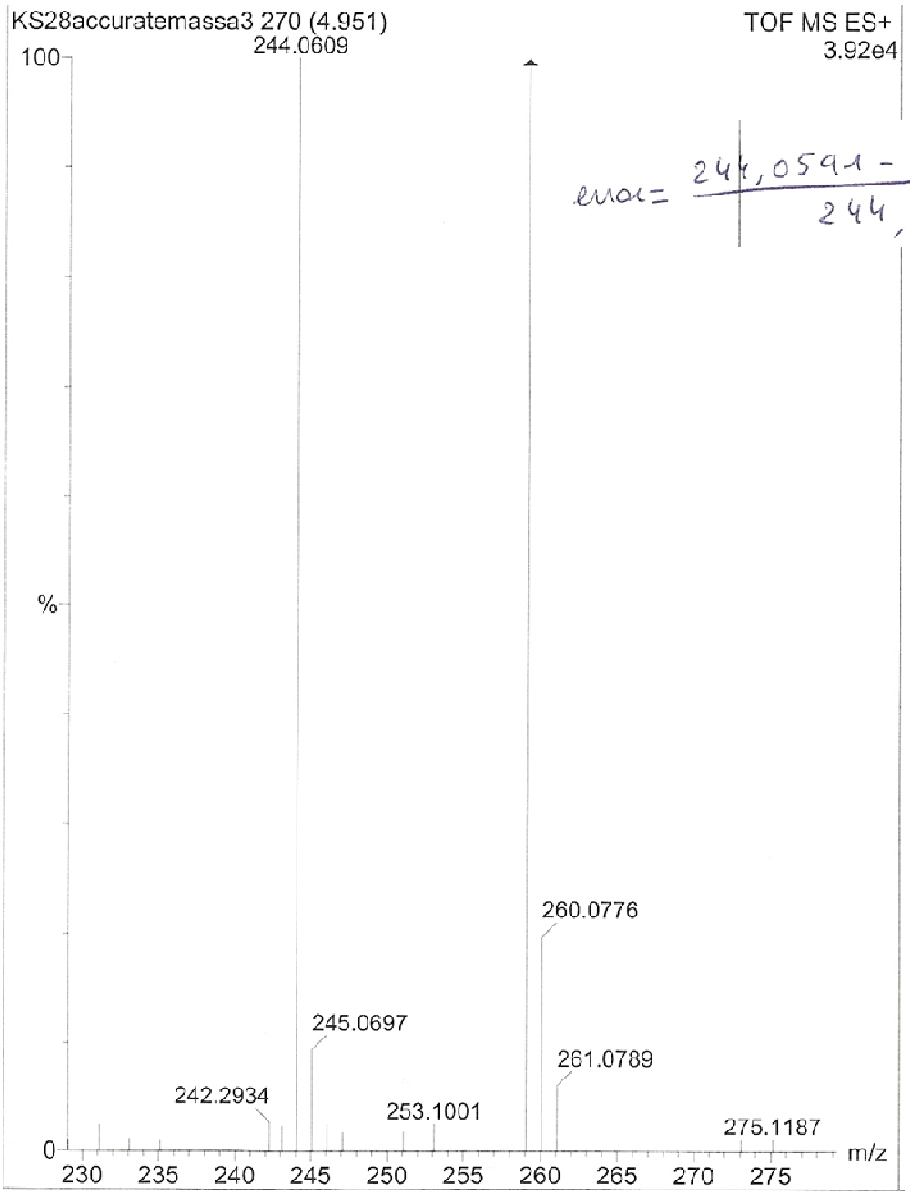


$$error = \frac{271,0536 - 271,0537}{271,0536} = 0,36$$

Accurate MS ES$^+$ m/z [M+H]$^+$ 271.0537 (calculated for $C_{14}H_{10}N_2O_2S$ 271.0536). Lock mass: $C_{14}H_{11}FN_2S$ 259.3271.

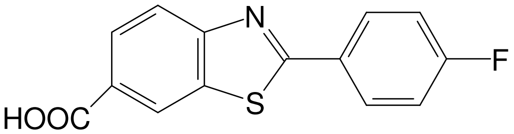


5

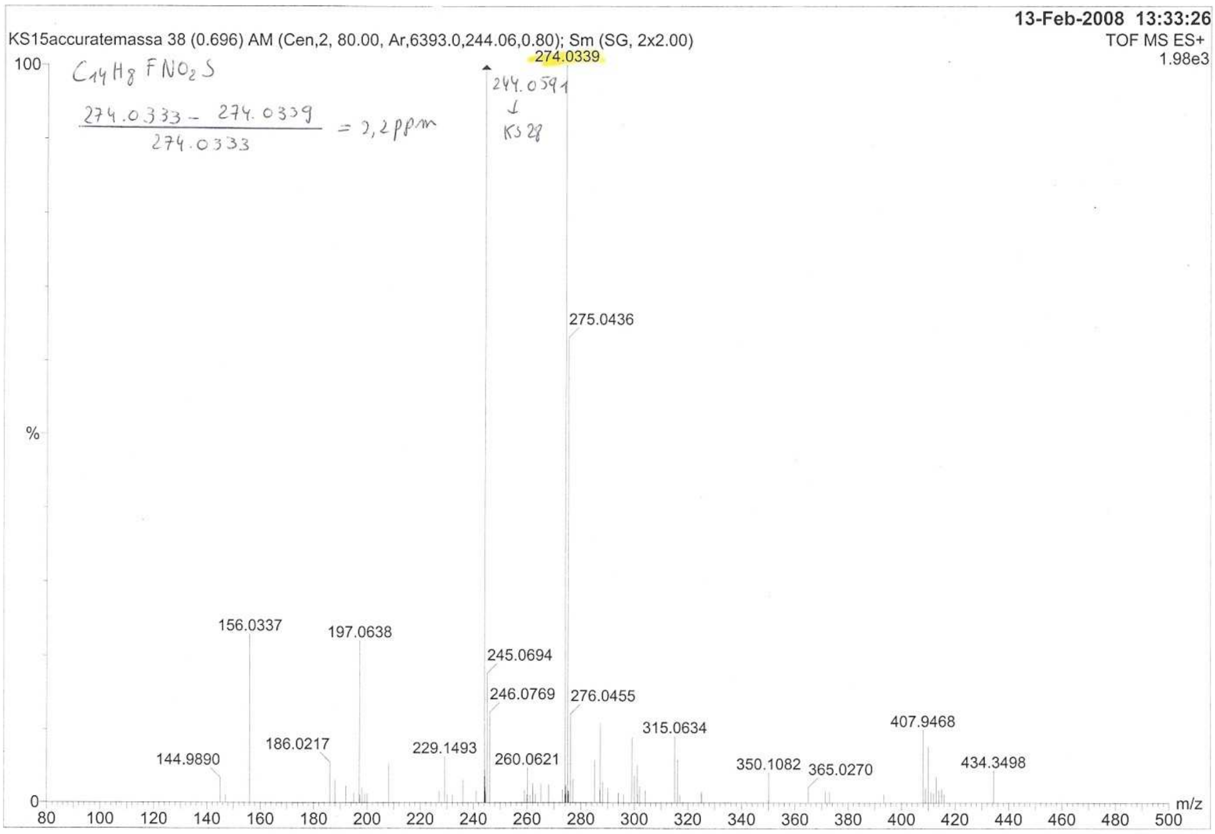


$$\text{erro} = \frac{244{,}0591 - 244{,}0609}{244{,}0591} = 7{,}4$$

Accurate MS ES$^+$ m/z $[M+H]^+$ 244.0498 (calculated for $C_{14}H_{10}FNS$ 244.0591). Lock mass: $C_{14}H_{11}FN_2S$ 259.3271.



8



Accurate MS ES$^+$ m/z [M+H]$^+$ 274.0339 (calculated for $C_{14}H_8FNO_2S$ 274.0333). Lock mass: $C_{14}H_{10}FNS$ 244.0591.