Electronic supplementary Information

2-Anilinonicotinyl linked 1,3,4-oxadiazole derivatives: Synthesis, antitumour activity and inhibition of tubulin polymerization

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General. (**A**) **Chemistry.** All chemicals and reagents were purchased from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 GF-254, and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H and ^{13}C spectra were recorded Bruker UXNMR/XWIN-NMR (300 MHz) or Varian VXR-Unity (400 MHz) instruments. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. Coupling constants are reported in Hertz (Hz) ESI spectra were recorded on Micro mass, Quattro LC using ESI^{+} software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. EI mass spectra were recorded on a VG-7070H Micromass mass spectrometer at 200 ^{o}C, 70eV, with a trap current of 200 μA and 4kV of acceleration voltage. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. IR spectra (KBr) were measured with a Thermo Nicolet Nexus 670 Spectrometer (ν in cm^{-1}). Melting points were determined with an Electro thermal melting point apparatus, and are uncorrected. Purity was evaluated by analytical HPLC using waters 515 pump coupled to a waters 2487 dual λ Absorbance UV detector. Mobile phase A: NH_4OAc (5mM buffer with HOAc, pH 5.5)-CH_3CN (95:5, v/v); mobile phase B: H_2O-CH_3CN (5:95, v/v). A linear gradient was run from 10 to 90 % B in 10 min, hold 90 % B 8 min. UV detection at 254 nm. Column used was C18 Spherisorb with 5 μm particle size. Flow rate: 1 mL/min: injection volume: 20μL. retention times are given in minutes at 220 or 254 nm.

(**B**) **Biology.**

(**a**) **Cell lines:** A549 (Human lung cancer cells) was obtained from ATCC, USA. A549 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen), supplemented with 10% fetal calf serum and 100 U/mL Pencillin and 100mg/mL streptomycin sulfate (Sigma). The cells were passaged and maintained at 37 ^{o}C in a humidified atmosphere containing 5% CO_2.

(b) ***In vitro*** **antitumour screening**: The synthesized compounds (**5a**–**m**) have been evaluated for their *in vitro* cytotoxicity in five different human cancer cell lines (A549,

HeLa, HepG2, DU-145 and MDA-MB-231). A protocol of 48 h continuous drug exposure has been used and a sulforhodamine B (SRB) protein assay has been employed to estimate cell viability or growth as described earlier. The cell lines were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 2mM L-glutamine and were seeded into 96-well plates in 100 µL at plating densities depending on the doubling time of individual cell lines. The plates were incubated at 37^{o} C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs. Aliquots of 3 µL of the drug dilutions were added to cells resulting in the required final drug concentrations. For each compound three concentrations (0.1, 1 and 10 µM) were evaluated and each were done in triplicate wells. Plates were incubated further for 48 h and assay was terminated by the addition of 50 µL of cold trichloro acetic acid (TCA) (final concentration, 10% TCA) and incubated for 60 min at 4 ^{o}C. The plates were washed five times with water and air-dried. Sulforhodamine B (SRB) solution (50 µL) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 min at room temperature. The residual dye was removed by washing five times with 1% acetic acid. The plates were air-dried. Bound stain was subsequently eluted with 10 mM Trizma base, and the absorbance was read on an ELISA plate reader at a wavelength of 560 nm. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. The above determinations were repeated three times. Percentage growth was expressed as the (ratio of average absorbance of the test well to the average absorbance of the control wells) 100. Growth inhibition of 50% (GI_{50}) was calculated from [(T0 - T)/(C - T0)] * 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Where, T0 = Optical density at time zero, OD of control = C, and OD of test growth in the presence of drug = T.

(c) Tubulin polymerization assay: An *in vitro* assay for monitoring the time dependent polymerization of tubulin to microtubules was performed employing a fluorescence-based tubulin polymerization assay kit (BK011, Cytoskeleton, Inc.) according to the manufacturer's protocol.The reaction mixture in a final volume of 10 mL in PEM buffer (80 MM PIPES, 0.5 mm EGTA, 2MM $MgCl_2$, Ph 6.9) contained, 2 mg/mL bovine brain tubulin, the tubulin 10 µM, fluorescent reporter, 1 mM GTP in the presence or absence

of test compounds **5a**–**m** (3μM) at 37 ^{o}C. Tubulin polymerization was followed by monitoring the fluorescence -enhancement due to the incorporation of a fluorescence reporter into microtubules as polymerization proceeds. Fluorescence emission at 420 nm (excitation wavelength is 360 nm) was measured for 1h at 1 min interval in a multimode plate reader (Varioskan, Thermo scientific Inc.). Nocodazole was used as a positive control under similar experimental conditions.

(d) Cell cycle analysis: Human lung cancer cell line (A549) in 6 well plates were incubated for 48 h in the presence or absence of tested compounds **5a–m** (2 μM) and nocodazole (1 μM). Cells were harvested with Trypsin-EDTA, fixed with ice-cold 70% ethanol at 4 ^{o}C for 30 min, ethanol was removed by centrifugation and cells were stained with 250 μL of DNA staining solution [10 mg of Propidium Iodide (PI), 0.1mg of trisodium citrate, and 0.03 ml of Triton X-100 were dissolved in 100 mL of sterile water] at room temperature for 30 min in the dark. The DNA contents of 20,000 events were measured by flow cytometry (DAKO CYTOMATION, Beckman Coulter, Brea, CA). Histograms were analyzed using Summit Software.

(e)Analysis of soluble versus polymerized tubulin in cells: Cells were plated in 24-well dishes, grown to 60-80% confluency, and treated with either no drug or varying concentrations (0.1, 1 and 10 μM) of **5m** drug alone, nocodozole and taxol (at 1 μM concentration) were used as positive and negative controls. Cells were incubated with drug for 24 h, later the media was removed, cells were rinsed in 1X PBS at 22 °C, harvested at the same temperature in lysis buffer containing 0.1 M Pipes, 1 mM EGTA, 1 mM $MgSO_4$, 30% glycerol, 5% DMSO, 5 mM GTP, 0.125% NP-40, and protease inhibitors, including aprotinin [200 units/mL], pH 6.9 and then centrifuged at 15000*g* at 22 °C for 30 min in an Sorvall Legendmicro 21R model temperature controlled centrifuge (Thermo scientific), to separate polymerized (P) from soluble (S) tubulin. Pellets of polymerized "P" tubulin were resuspended in a volume of lysis buffer equal to the soluble "S" fraction, and resolved in 7%SDS/PAGE as described earlier. After transfer to NC membrane immunoblotting was performed with mouse anti α-tubulin antibody [DMIA, Sigma, St. Louis, MO], followed by an FITC-conjugated secondary antibody (Sigma). The blot was imaged using Phosphor imager (Fugifilm, Japan).

Quantitative analysis of the soluble and polymer fractions was done by densitometry using Gene-box (Syngene).

(f) Caspase assay: A549 cells were plated in 6 well plates, grown to 60-80% confluence, and treated with either no drug or different concentrations (0.25, 1.25 and 2.5 μM) of test compound **5m**. Nocodozole was used as positive control. After 24 hours cells were collected by scraping and washed with PBS, centrifuged to collect pellet. The cells were lysed in 200 μL of 1X lysis buffer bypurging at least through an insulin syringe followed by incubation on ice for 10-20 min. The lysate was centrifuged at 13,200 rpm for 20 min at 4 °C and the odtained clear supernatant was used for caspase activity measurements employing AMC-conjugated substrates for caspase 9 and caspase 3 as described earlier

Table 2. Cell cycle distribution of compounds **5a–m** treated A549 cells at 2 μM concentrations.

Compound	Cell cycle distribution (%)		
	G1	S	G2/M
Control	78.44 ± 0.6	10.28 ±0.19	10.78 ±0.25
5a	72.5 ±0.57	14.71 ±0.12	12.54 ±0.36
5b	75.52 ±0.27	12.64 ±0.45	11.4 ±0.42
5c	74.43 ±0.09	13.71 ±0.03	11.56 ±0.5
5d	72.64 ±0.23	14.58 ±0.43	13.45 ±0.3
5e	72.57 ±0.46	14.51 ±0.23	13.51 ±0.64
5f	68.48 ±0.52	17.49 ±0.09	14.5 ±0.69
5g	76.46 ±0.36	11.59 ±0.45	11.51 ±0.24
5h	75.33 ±0.25	12.6 ±0.5	12.53 ±0.42
5i	79.61 ±0.53	10.55 ±0.17	9.43 ±0.5
5j	81.49 ±0.25	12.58 ±0.57	5.44 ±0.33
5k	80.36 ±0.21	12.63 ±0.43	7.29 ±0.21
5l	73.51 ±0.44	14.56 ±0.27	12.38 ±0.5
5m	65.5 ±0.26	15.52 ±0.33	18.86 ±0.07
Nocodazole(1μM)	55.42 ±0.42	25.67 ±0.22	18.72 ±0.04

Cells were treated with compounds at a final conc of 2 μM for 48h. Following the termination of incubation, cells were fixed, stained with propidium iodide and analzed by FACS as mentioned in the "experimental section"

Data indicates mean±S.D of three separate experiments

Table 3. Dose dependent increase of cells treated with **5m** in G2/M phase.

5m Concentration	% of cells at G2/M
0	13 ±0.31
1	20 ±0.36
2	24 ±0.47
4	29 ±0.28

Data indicates mean±S.D of three separate experiments

Spectral Data and Procedure of Compounds (8a–d, 9a–d,10 a–m and **5a–m).**

Synthesis of Ethyl 2-(4-fluoroanilino) nicotinate (8a)

The compound 2-chloro nicotino ethylester **6** (185 mg, 1 mmol) and 4-fluoro aniline **7a** (111 mg, 1 mmol) were taken in ethylene glycol and refluxed at 160 ^{o}C for 6 h. After completion of the reaction as confirmed by TLC, the reaction mixture was cooled and extracted in ethyl acetate (4x25 mL) from the aqueous layer and concentrated in vacuo. The crude product was further purified by column chromatography using 60-120 silica gel (ethyl acetate/hexane, 1:9) to afford compound **8a** as a pale yellow solid, yield 195 mg, 75%; mp 67–69 °C. ^{1}H NMR ($CDCl_3$+DMSO-d_6, 200 MHz): δ 10.19 (bs, 1H, –N**H**), 8.32 (dd, 1H, *J* = 4.7, 2.0 Hz, Pyridine-**H**), 8.22 (dd, 1H, *J* = 8.0, 2.0 Hz, Pyridine-**H**), 7.65 (m, 2H, Ar**H**), 7.00 (m, 2H, Ar**H**), 6.70 (dd, 1H, *J* = 7.4, 4.7 Hz, Pyridine-**H**), 4.40 (q, 2H, *J* = 7.4 Hz, –OC$\mathbf{H}_2$), 1.44 (t, 3H, *J* = 7.4 Hz, –C$\mathbf{H}_3$); MS (EI): *m/z* 260 M^+.

Ethyl 2-(4-chloroanilino) nicotinate (8b)

The title compound was prepared according to the method described for compound **8a**, employing compound **6** (185 mg, 1 mmol) and 4-chloro aniline (**7b**, 128 mg, 1 mmol) to obtain the pure product as a pale yellow solid, yield 199 mg, 72%; mp 98–100 ^{o}C. ^{1}H NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 10.26 (bs, 1H, –N**H**), 8.34 (q, 1H, *J* = 2.7 Hz, Pyridine-**H**), 8.20 (dd, 1H, *J* = 8.3, 2.7 Hz, Pyridine-**H**), 7.67 (d, 2H, *J* = 9.1 Hz, Ar**H**),

7.24 (d, 2H, *J* = 9.1 Hz, Ar**H**), 6.70 (dd, 1H, *J* = 7.6, 4.5 Hz, Pyridine-**H**), 4.38 (q, 2H, *J* = 7.5 Hz,–OC**H**$_2$), 1.42 (t, 3H, *J* = 7.5 Hz, –C**H**$_3$); MS (EI): *m/z* 276 M^+.

Ethyl 2-(3,4,5 trifluoroanilino) nicotinate (8c)

The title compound was prepared according to the method described for compound **8a**, employing compound **6** (185 mg, 1 mmol) and 3,4,5-trifluoro aniline (**7c**, 147 mg, 1 mmol) to obtain the pure product as a pale yellow solid, yield 225 mg, 76%; mp 106–108 ^oC. ^1H NMR ($CDCl_3$, 500 MHz): δ 10.33 (bs, 1H, –N**H**), 8.39 (dd, 1H, *J* = 4.5, 2.2 Hz, Pyridine-**H**), 8.25 (dd, 1H, *J* = 8.3, 2.2 Hz, Pyridine-**H**), 7.47 (m, 2H, Ar**H**), 6.79 (q, 1H, *J* = 8.3, 5.3 Hz, Pyridine-**H**), 4.38 (q, 2H, *J* = 7.5 Hz, –OC**H**$_2$), 1.42 (t, 3H, *J* = 7.5 Hz, –C**H**$_3$); MS (EI): *m/z* 296 M^+.

Ethyl 2-(3,4,5 trimethoxy anilino) nicotinate (8d)

The title compound was prepared according to the method described for compound **8a**, employing compound **6** (185 mg, 1 mmol) and 3,4,5 trimethoxy aniline (**7d**, 183 mg, 1 mmol) to obtain the pure product as a pale yellow solid, yield 236 mg, 71%; mp 143–145 ^oC. ^1H NMR ($CDCl_3$, 500 MHz): δ 10.14 (bs, 1H, –N**H**), 8.37 (dd, 1H, *J* = 5.3, 2.3 Hz, Pyridine-**H**), 8.25 (dd, 1H, *J* = 7.6, 2.3 Hz, Pyridine-**H**), 7.00 (s, 2H, Ar**H**), 6.71 (dd, 1H, *J* = 8.3, 5.3 Hz, Pyridine-**H**), 4.38 (q, 2H, *J* = 7.5 Hz, –OC**H**$_2$), 3.88 (s, 6H, 2×–OC**H**$_3$), 3.83 (s, 3H, –OC**H**$_3$), 1.43 (t, 3H, *J* = 7.5 Hz, –C**H**$_3$); MS (EI): *m/z* 332 M^+.

2-(4-Fluoroanilino)-3-pyridinecarbohydrazide (9a)

The compound **8a** (260 mg, 1 mmol) and hydrazine hydrate (0.25 mL, 5 mmol) were refluxed in ethanol (10 mL) for 2 h. After completion of reaction as indicated by TLC, the reaction mixture was cooled and left for overnight, crystal were obtained, filtered and washed with ethanol to obtain pure compound **9a** as a yellow crystalline needles, yield 180 mg 73%; mp 159–161 ^oC. ^1H NMR ($CDCl_3$+DMSO-d_6, 200 MHz): δ 10.20 (bs, 1H, –N**H**), 8.22 (dd, 1H, *J* = 5.0, 1.4 Hz, Pyridine-**H**), 7.99 (dd, 1H, *J* = 7.9, 1.4 Hz, Pyridine-**H**), 7.66 (dd, 2H, *J* = 9.3, 5.0 Hz, Ar**H**), 6.96 (m, 2H, Ar**H**), 6.70 (dd, 1H, *J* = 7.9, 5.0 Hz, Pyridine-**H**); MS (EI): *m/z* 246 M^+.

2-(4-Chloroanilino)-3-pyridinecarbohydrazide (9b)

The title compound was prepared according to the method described for compound **9a**, employing compound **8b** (277 mg, 1 mmol) and hydrazine hydrate (0.25 mL, 5 mmol) to obtain pure product **9b** as a yellow crystalline needle, yield 183 mg, 70%; mp 195–197 ^{o}C. ^{1}H NMR ($CDCl_3$+DMSO-d_6, 200 MHz): δ 10.10 (s, 1H, –N**H**), 8.25 (dd, 1H, *J* = 4.5, 1.5 Hz, Pyridine-**H**), 7.79 (dd, , 1H, *J* = 7.4, 1.5 Hz, Pyridine-**H**), 7.69 (d, 2H, *J* = 8.9 Hz, Ar**H**), 7.21 (d, 2H, *J* = 8.9 Hz, Ar**H**), 6.71 (dd, 1H, *J* = 7.4, 5.2 Hz, Pyridine-**H**); MS (EI): *m/z* 262 M^+.

2-(3,4,5-trifluoroanilino)-3-pyridinecarbohydrazide (9c)

The title compound was prepared according to the method described for compound **9a**, employing compound **8c** (296 mg, 1 mmol) and hydrazine hydrate (0.25 mL, 5 mmol) to obtain the pure product **9c** as a yellow crystalline needle, yield 197 mg, 70%; mp 149–151 ^{o}C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.25 (bs, 1H, –N**H**), 8.34 (dd, 1H, *J* = 4.9, 2.0 Hz, Pyridine-**H**), 8.20 (dd, 1H, *J* = 8.5, 2.0 Hz, Pyridine-**H**), 7.40 (m, 2H, Ar**H**), 6.79 (q, 1H, *J* = 8.3, 5.0 Hz, Pyridine-**H**), 4.35 (bs, 2H, –N**H**$_2$); MS (EI): *m/z* 282 M^+.

2-(3,4,5-trimethoxy anilino)-3-pyridinecarbohydrazide (9d)

The title compound was prepared according to the method described for compound **9a**, employing compound **8d** (332 mg, 1 mmol) and hydrazine hydrate (0.25 mL, 5 mmol) to obtain the pure product **9d** as a yellow crystalline needle, yield 238 mg, 75%; mp 164–167 ^{o}C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.67 (bs, 1H, –N**H**), 10.06 (bs, 1H, –N**H**), 8.29 (dd, 1H, *J* = 4.3, 1.5 Hz, Pyridine-**H**), 7.99 (dd, 1H, *J* = 7.6, 1.1 Hz, Pyridine-**H**), 7.05 (s, 2H, Ar**H**), 6.81 (dd, 1H, *J* = 7.6, 4.9 Hz, Pyridine-**H**), 4.60 (bs, 2H, –N**H**$_2$), 3.77 (s, 6H, 2×–OC**H**$_3$), 3.61 (s, 3H, –OC**H**$_3$); MS (EI): *m/z* 318 M^+.

N-(4-chlorophenyl)-2-(2-(4-fluorophenylamino)nicotinoyl)hydrazinecarbothioamide (10a)

To a solution of 2-(4-fluoroanilino)-3-pyridinecarbohydrazide (**9a,** 246 mg, 1 mmol) in benzene (10 mL), 4-chloro phenylthioisocyanate (184 mg, 1.2 mmol) was added slowly at 0 ^{o}C. The resulting mixture was stirred at room temperature for 8 h and then cooled to 0 ^{o}C for 1 h, the crystalline solid thus obtained was filtered and washed with benzene to furnish pure compound **10a** as a yellow solid, yield 299 mg, 72%; mp 203–205 ^{o}C. ^{1}H

NMR (DMSO-d_6, 300 MHz): δ 10.92 (bs, 1H, –N**H**), 10.82 (bs, 1H, –N**H**), 9.90 (s, 2H, Ar**H**), 8.44 (d, 1H, *J* = 4.3 Hz, Pyridine-**H**), 8.27 (d, 1H, *J* = 7.4 Hz, Pyridine-**H**), 7.88–7.73 (m, 2H, Ar**H**), 7.61–7.20 (m, 4H, Ar**H**), 7.03 (dd, 1H, *J* = 7.6, 4.7 Hz, Pyridine-**H**); MS (ESI): *m/z* 416 $(M+1)^+$.

2-(2-(4-fluorophenylamino)nicotinoyl)-N-(4-nitrophenyl)hydrazine carbothioamide (10b)

The title compound was prepared according to the method described for compound **10a**, employing compound **9a (**246 mg, 1 mmol) and 4-nitro phenylthioisocyanate (200 mg, 1.2 mmol) to obtain the pure product **10b** as a yellow solid, yield 298 mg, 70%; mp 234–236 ^oC. ^1H NMR (DMSO-d_6, 300 MHz): δ 11.02 (bs, 1H, –N**H**), 10.32 (bs, 1H, –N**H**), 8.32 (d, 1H, *J* = 3.2 Hz, Pyridine-**H**), 8.00 (d, 1H, *J* = 7.6 Hz, Pyridine-**H**), 7.74 (d, 2H, *J* = 8.5 Hz, Ar**H**), 7.64 (d, 2H, *J* = 8.5 Hz, Ar**H**), 7.40 (d, 2H, *J* = 8.9 Hz, Ar**H**), 7.34 (d, 2H, *J* = 8.9 Hz, Ar**H**), 7.03 (dd, 1H, *J* = 7.6, 4.7 Hz, Pyridine-**H**); MS (ESI): *m/z* 427 $(M+1)^+$.

2-(2-(4-fluorophenylamino)nicotinoyl)-N-(3,4,5-trimethoxyphenyl)hydrazinecarbo thioamide (10c)

The title compound was prepared according to the method described for compound **10a**, employing compound **9a (**246 mg, 1 mmol) and 3,4,5-trimethoxy phenylthioisocyanate (251 mg, 1.2 mmol) to obtain the pure product **10c** as a yellow solid, yield 298 mg, 70%; mp 194–196^oC. ^1H NMR (DMSO-d_6, 300 MHz): δ 10.85 (bs, 1H, –N**H**), 10.35 (bs, 1H, –N**H**), 8.53 (dd, 1H, *J* = 4.7, 2.0 Hz, Pyridine-**H**), 8.23 (d, 1H, *J* = 7.9, 2.0 Hz, Pyridine-**H**), 7.84 (d, 2H, *J* = 8.2 Hz, Ar**H**), 7.46 (d, 2H, *J* = 8.2 Hz, Ar**H**), 7.03 (dd, 1H, *J* = 7.9, 4.7 Hz, Pyridine-**H**), 6.92 (s, 2H, Ar**H**), 3.80 (s, 6H, 2×–OC**H**$_3$), 3.66 (s, 3H, –OC**H**$_3$)
MS (ESI): *m/z* 472 $(M+1)^+$.

2-(2-(4-chlorophenylamino)nicotinoyl)-N-phenyl hydrazine carbothioamide (10d)

The title compound was prepared according to the method described for compound **10a**, employing compound **9b (**263 mg, 1 mmol) and phenyl thioisocyanate (143 mg, 1.2 mmol) to obtain the pure product **10d** as a yellow solid, yield 294 mg, 74%; mp 228–230 ^oC. ^1H NMR (DMSO-d_6, 300 MHz): δ 11.02 (bs, 1H, –N**H**), 10.26 (bs, 1H, –N**H**), 8.34 (d, 1H, *J* = 3.0 Hz, Pyridine-**H**), 8.00 (d, 1H, *J* = 7.1 Hz, Pyridine-**H**), 7.74 (d, 2H, *J* = 7.9

Hz, Ar**H**), 7.64 (d, 2H, *J* = 7.9 Hz, Ar**H**), 7.40 (d, 2H, *J* = 8.5 Hz, Ar**H**), 7.34 (d, 2H, *J* = 8.5 Hz, Ar**H**), 6.98 (dd, 1H, *J* = 7.1, 3.2 Hz, Pyridine-**H**); MS (ESI): *m/z* 398 $(M+1)^+$.

N-(4-chlorophenyl)-2-(2-(4-chlorophenylamino)nicotinoyl)hydrazine carbo thioamide (10e)

The title compound was prepared according to the method described for compound **10a**, employing compound **9b (**263 mg, 1 mmol) and 4-chloro phenylthioisocyanate (184 mg, 1.2 mmol) to obtain the pure product **10e** as a yellow solid, yield 302 mg, 70%; mp 189–191 ^oC. ^1H NMR (DMSO-d_6, 300 MHz): δ 11.05 (bs, 1H, –N**H**), 10.41 (bs, 1H, –N**H**), 8.40 (d, 1H, *J* = 3.2 Hz, Pyridine-**H**), 8.06 (d, 1H, *J* = 7.3 Hz, Pyridine-**H**), 7.80 (d, 2H, *J* = 8.2 Hz, Ar**H**), 7.63 (d, 2H, *J* = 7.8 Hz, Ar**H**), 7.38 (d, 2H, *J* = 8.2 Hz, Ar**H**), 7.00 (d, 2H, *J* =7.80 Hz, Ar**H**), 6.94 (dd, 1H, *J* = 7.3, 3.2 Hz, Pyridine-**H**); MS (ESI): *m/z* 433 $(M+1)^+$.

N-(4-chlorophenyl)-2-(2-(3,4,5-trimethoxyphenylamino)nicotinoyl)hydrazine carbothioamide (10f)

The title compound was prepared according to the method described for compound **10a**, employing compound **9b (**263 mg, 1 mmol) and 3,4,5-trimethoxy phenylthioisocyanate (251 mg, 1.2 mmol) to obtain the pure product **10f** as a yellow solid, yield 375 mg, 77%; mp 232–234 ^oC.^1H NMR (DMSO-d_6, 300 MHz): δ 10.90 (bs, 1H, –N**H**), 10.40 (bs, 1H, –N**H**), 8.34 (dd, 1H, *J* = 4.9, 3.0 Hz, Pyridine-**H**), 8.02 (d, 1H, *J* = 7.7, 1.3 Hz, Pyridine-**H**), 7.80 (d, 2H, *J* = 9.1 Hz, Ar**H**), 7.38 (d, 2H, *J* = 8.2 Hz, Ar**H**), 7.03 (dd, 1H, *J* = 7.6, 4.9 Hz, Pyridine-**H**), 6.90 (s, 2H, Ar**H**), 3.78 (s, 6H, 2×–OC**H**$_3$), 3.62 (s, 3H, –OC**H**$_3$); MS (ESI): *m/z* 488 $(M+1)^+$.

N-phenyl-2-(2-(3,4,5-trifluorophenylamino)nicotinoyl)hydrazine carbothioamide (10g)

The title compound was prepared according to the method described for compound **10a**, employing compound **9c (**282 mg, 1 mmol) and phenyl thioisocyanate (143 mg, 1.2 mmol) to obtain the pure product **10g** as a yellow solid, yield 304 mg, 73%; mp 196–198 ^oC. ^1H NMR (DMSO-d_6, 300 MHz): δ 10.85 (bs, 1H, –N**H**), 10.20 (bs, 1H, –N**H**), 8.46 (d, 1H, *J* =7.0 Hz, Pyridine-**H**), 8.36–8.13 (m, 2H, Ar**H**), 8.02 (d, 1H, *J* =4.0 Hz,

Pyridine-**H**), 7.84–7.72 (m, 2H, Ar**H**), 7.65–7.52 (m, 3H, Ar**H**), 6.99 (dd, 1H, *J* = 7.2, 4.0 Hz, Pyridine-**H**); MS (ESI): *m/z* 418 $(M+1)^+$.

N-(4-chlorophenyl)-2-(2-(3,4,5-trifluorophenylamino)nicotinoyl)hydrazinecarbothioamide (10h)

The title compound was prepared according to the method described for compound **10a**, employing compound **9c (**282 mg, 1 mmol) and 4-chloro phenylthioisocyanate (184 mg, 1.2 mmol) to obtain the pure product **10h** as a yellow solid, yield 361 mg**,** 80%; mp 176–178 ^oC. ^1H NMR (DMSO-d_6, 300 MHz): δ 10.81 (bs, 1H, –N**H**), 9.84 (bs, 1H, –N**H**), 8.40–8.15 (m, 2H, Pyridine-**H,** Ar**H**), 7.82–7.64 (m, 2H, Ar**H**), 7.54–7.38 (m, 2H, Ar**H**), 7.14 (t, 2H, *J* = 8.3 Hz, Ar**H**), 6.90 (dd, 1H, *J* = 9.1, 6.0 Hz, Pyridine-**H**); MS (ESI): *m/z* 452 $(M+1)^+$.

N-(4-nitrophenyl)-2-(2-(3,4,5-trifluorophenylamino) nicotinoyl)hydrazine carbothioamide (10i)

The title compound was prepared according to the method described for compound **10a**, employing compound **9c (**282 mg, 1 mmol) and 4-nitro phenylthioisocyanate (200 mg, 1.2 mmol) to obtain the pure product **10i** as a yellow solid, yield 323 mg, 70%; mp 218–220 ^oC. ^1H NMR (DMSO-d_6, 300 MHz): δ 10.99 (s, 1H, –N**H**), 10.81 (s, 1H, –N**H**), 10.20 (s, 1H, –N**H**), 8.46 (d, 1H, *J* = 3.6 Hz, Pyridine-**H**), 8.34–8.13 (m, 3H, Pyridine-**H**, Ar**H**), 8.00–7.66 (m, 4H, Ar**H**), 7.05 (dd, 1H, *J* = 7.7, 4.9 Hz, Pyridine-**H**); MS (ESI): *m/z* 463 $(M+1)^+$.

N-phenyl-2-(2-(3,4,5-trimethoxyphenylamino)nicotinoyl)hydrazine carbothioamide (10j)

The title compound was prepared according to the method described for compound **10a**, employing compound **9d (**318 mg, 1 mmol) and phenyl thioisocyanate (143 mg, 1.2 mmol) to obtain the pure product **10j** as a yellow solid, yield 340 mg, 75%; mp 204–206 ^oC. ^1H NMR (DMSO-d_6, 300 MHz): δ 10.90 (bs, 1H, –N**H**), 10.59 (bs, 1H, –N**H**), 8.40 (d, 1H, *J* = 4.5 Hz, Pyridine-**H**), 8.24 (d, 1H, *J* = 7.2 Hz, Pyridine-**H**), 7.35 (d, 2H**,** *J* = 8.0 Hz, Ar**H**), 7.28–7.20 (m, 3H, Ar**H**), 7.12 (s, 2H, Ar**H**), 6.90 (dd, 1H, *J* = 7.2, 4.5 Hz, Pyridine-**H**), 3.77 (s, 6H, 2×–OC**H**$_3$), 3.60 (s, 3H, –OC**H**$_3$); MS (ESI): *m/z* 454 $(M+1)^+$.

2-(2-(4-chlorophenylamino)nicotinoyl)-N-(3,4,5-trimethoxyphenyl) hydrazine carbothioamide (10k)

The title compound was prepared according to the method described for compound **10a**, employing compound **9d** (318 mg, 1 mmol) and 4-chloro phenylthioisocyanate (184 mg, 1.2 mmol) to obtain the pure product **10k** as a yellow solid, yield 341 mg, 70%; mp 105–106 ^{o}C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.82 (bs, 1H, –N**H**), 10.60 (bs, 1H, –N**H**), 9.92 (s, 1H**,** –NH), 8.37 (d, 1H, *J* = 4.0 Hz, Pyridine-**H**), 8.22 (d, 1H, *J* = 7.4 Hz, Pyridine-**H**), 7.48 (d, 1H**,** *J* = 5.9 Hz, Ar**H**), 7.39 (d, 2H**,** *J* = 8.5 Hz, Ar**H**)**,** 7.09 (s, 2H**,** Ar**H**), 6.89 (dd, 1H, *J* = 7.6, 4.7 Hz, Pyridine-**H**), 3.77 (s, 6H, 2×–OC**H**$_3$), 3.61 (s, 3H, –OC**H**$_3$); MS (ESI): *m/z* 488 $(M+1)^+$.

N-(4-nitrophenyl)-2-(2-(3,4,5-trimethoxyphenylamino)nicotinoyl)hydrazine carbothioamide (10l)

The title compound was prepared according to the method described for compound **10a**, employing compound **9d (**318 mg, 1 mmol) and 4-nitro phenylthioisocyanate (200 mg, 1.2 mmol) to obtain the pure product **10l** as a yellow solid, yield 339 mg, 68%; mp 210–211 ^{o}C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.92 (bs, 1H, –N**H**), 10.62 (s, 1H, –N**H**), 10.19 (s, 1H, –N**H**), 8.39 (s, 1H, Pyridine-**H**), 8.34–8.11 (m, 3H, Pyridine-**H,** Ar**H**), 8.02–7.76 (m, 2H**,** Ar**H**), 7.19–7.00 (m, 2H**,** Ar**H**), 6.90 (dd, 1H, *J* = 7.7, 4.9 Hz, Pyridine-**H**), 3.77 (s, 6H, 2×–OC**H**$_3$), 3.62 (s, 3H, –OC**H**$_3$); MS (ESI): *m/z* 499 $(M+1)^+$.

N-(3,4,5-trimethoxyphenyl)-2-(2-(3,4,5-trimethoxyphenylamino)nicotinoyl) hydrazine carbothioamide (10m)

The title compound was prepared according to the method described for compound **10a**, employing compound **9d (**318 mg, 1 mmol) and 3,4,5-trimethoxy phenylthioisocyanate (251 mg, 1.2 mmol) to obtain the pure product **10m** as a yellow solid, yield 380 mg**,** 70%; mp 183–185 ^{o}C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.57 (s, 1H, –N**H**), 9.62 (s, 1H, –N**H**), 9.45 (s, 1H, –N**H**), 8.26 (d, *J* = 3.4 Hz, 1H, Pyridine-**H**), 8.17 (d, *J* = 7.4 Hz, 1H, Pyridine-**H**), 7.01 (s, 2H**,** Ar**H**)**,** 6.98 (s, 2H**,** Ar**H**), 6.73 (dd, *J* = 7.4, 4.7 Hz, 1H, Pyridine-**H**), 3.85–3.73 (m, 12H, 4×–OC**H**$_3$), 3.72–3.62 (m, 6H, 2× –OC**H**$_3$); MS (ESI): *m/z* 544 $(M+1)^+$.

N-(4-chlorophenyl)-5-(2-(4-fluorophenylamino)pyridin-3-yl)-1,3,4-oxadiazol-2-amine (5a)

To a stirred solution of compound **10a (**416 mg, 1 mmol) in THF were added tosyl chloride (229 mg, 1.2 mmol) and pyridine (0.17 mL, 2.2 mmol) and reflux for 2-3 h. To the reaction mixture 2N HCl was added and extracted into ethyl acetate and further purified by column chromatography using ethyl acetate/hexane (1:1) as eluent to afford pure compound **5a** as a colorless solid, yield 294 mg, 77%; mp 267–269 ^{o}C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.05 (bs, 1H, –N**H**), 9.96 (bs, 1H, –N**H**), 8.34 (d, 1H, *J* = 2.7 Hz, Pyridine-**H**), 8.03 (d, 1H, *J* = 7.2 Hz, Pyridine-**H**), 7.77 (dd, 2H, *J* = 8.1, 4.5 Hz, Ar**H**), 7.67 (d, 2H, *J* = 8.1 Hz, Ar**H**), 7.44 (d, 2H, *J* = 8.9 Hz, Ar**H**), 7.19 (t, 2H, *J* = 8.9 Hz, Ar**H**), 7.01 (dd, 1H, *J* = 7.2, 4.5 Hz, Pyridine-**H**); ^{13}C NMR (DMSO-d_6, 300 MHz): 159.5, 150.7, 146.2, 144.4, 139.1, 136.4, 132.5, 127.3, 122.1, 116.7, 115.9, 115.6, 114.2, 111.5, 103.6; HPLC purity: t_R 13.16 min (98.0 %); MS (ESI): *m/z* 382 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{19}H_{14}ClFN_5O$, calcd 382.0862, found 382.0871 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 3421, 3313, 2854, 1679, 1626, 1590, 1501, 1457, 1224, 1095, 1067, 1021, 829, 759, 549.

5-(2-(4-fluorophenylamino)pyridin-3-yl)-N-(4-nitrophenyl)-1,3,4-oxadiazol-2-amine (5b)

The title compound was prepared according to the method described for compound **5a**, employing compound **10b (**426 mg, 1 mmol), tosyl chloride (229 mg, 1.2 mmol) and pyridine (0.17 mL, 2.2 mmol) to obtain the pure product **5b** as a colorless solid, yield 274 mg, 70%; mp 325 ^{o}C charred. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.02 (bs, 1H, –N**H**), 10.08 (bs, 1H, –N**H**), 8.38 (d, 1H, *J* = 5.1 Hz, Pyridine-**H**), 8.05 (d, 1H, *J* = 8.1 Hz, Pyridine-**H**), 7.82 (d, 2H, *J* = 8.8 Hz, Ar**H**), 7.67 (d, 2H, *J* = 8.8, Ar**H**), 7.44 (d, 2H, *J* = 8.8 Hz, Ar**H**), 7.40 (d, 2H, *J* = 8.8 Hz, Ar**H**), 7.05 (dd, 1H, *J* = 7.3, 5.1 Hz, Pyridine-**H**); ^{13}C NMR (DMSO-d_6, 300 MHz): 158.7, 150.4, 146.5, 144.1, 140.1, 136.7, 135.9, 132.7, 122.7, 116.5, 115.8, 115.2, 114.3, 112.2, 104.6; HPLC purity: t_R 12.40 min (95.6 %); MS (ESI): *m/z* 393 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{19}H_{14}FN_6O_3$, calcd 393.1113, found 393.1111 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 3447, 3316, 1660, 1593, 1505, 1334, 1106, 1063, 1030, 840, 762.

5-(2-(4-fluorophenylamino)pyridin-3-yl)-N-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol -2-amine (5c)

The title compound was prepared according to the method described for compound **5a**, employing compound **10c (**471 mg, 1 mmol), tosyl chloride (229 mg, 1.2 mmol) and pyridine (0.17 mL, 2.2 mmol) to obtain the pure product **5c** as a colorless solid, yield 319 mg, 73%; mp 269–270 ^{o}C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.80 (bs, 1H, –N**H**), 9.94 (bs, 1H, –N**H**), 8.34 (dd, 1H, *J* = 4.7, 1.7 Hz, Pyridine–**H**), 8.03 (dd, 1H, *J* = 7.7, 1.7 Hz, Pyridine-**H**), 7.77 (dd, 2H, *J* = 9.1, 4.9 Hz, Ar**H**), 7.20 (t, 2H, *J* = 8.9 Hz, Ar**H**), 7.05–6.96 (m, 3H, Ar**H**, Pyridine-**H**), 3.80 (s, 6H, 2×–OC**H**$_3$), 3.63 (s, 3H, –OC**H**$_3$); ^{13}C NMR (DMSO-d_6, 300 MHz): 159.2, 153.0, 151.3, 148.0, 145.2, 139.0, 136.9, 134.4, 129.1, 118.3, 117.6, 116.1, 113.2,106.5, 96.6, 59.30, 55.20; HPLC purity: t_R 12.93 min (95.5 %); MS (ESI): *m/z* 438 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{22}H_{21}N_5O_4F$, calcd 438.1577, found 438.1578 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 3447, 3290, 2937, 1636, 1596, 1540, 1506, 1455, 1426, 1226, 1127, 1035, 1002, 823, 757.

5-(2-(4-chlorophenylamino)pyridin-3-yl)-N-phenyl-1,3,4-oxadiazol-2-amine (5d)

The title compound was prepared according to the method described for compound **5a**, employing compound **10d** (398 mg, 1 mmol), tosyl chloride (229 mg, 1.2 mmol) and pyridine (0.17 mL, 2.2 mmol) to obtain the pure product **5d** as a colorless solid, yield 266 mg, 73%; mp 284–286 ^{o}C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.03 (bs, 1H, –N**H**), 10.07 (s, 1H, –N**H**), 8.36 (dd, 1H, *J* = 5.1, 1.5 Hz, Pyridine-**H**), 8.02 (dd, 1H, *J* = 7.3, 2.2 Hz, Pyridine-**H**), 7.80 (d, 2H, *J* = 8.8 Hz, Ar**H**), 7.65 (d, 2H, *J* = 8.8 Hz, Ar**H**), 7.42 (d, 2H, *J* = 8.5 Hz, Ar**H**), 7.38 (d, 2H, *J* = 8.8 Hz, Ar**H**), 7.02 (dd, 1H, *J* = 7.3, 5.1 Hz, Pyridine-**H**); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 157.5, 155.1, 149.9, 147.9, 137.2, 135.5, 133.4, 127.1, 126.8, 124.6, 124.5, 119.6, 117.2, 112.7, 101.7; HPLC purity: t_R 15.32 min (96.4 %); MS (ESI): *m/z* 364 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{19}H_{15}ClN_5O$, calcd 364.0960, found 364.0965 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 3321, 3216, 2853, 1671, 1632, 1581, 1536, 1492, 1457, 1094, 1023, 821, 759, 629.

N-(4-chlorophenyl)-5-(2-(4-chlorophenylamino)pyridin-3-yl)-1,3,4-oxadiazol-2-amine (5e)

The title compound was prepared according to the method described for compound **5a**, employing compound **10e (**432 mg, 1 mmol), tosyl chloride (229 mg, 1.2 mmol) and

pyridine (0.17 mL, 2.2 mmol) to obtain the pure product **5e** as a colorless solid, yield 397 mg, 75%; mp 255–256 ^{o}C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.90 (s, 1H, –N**H**), 10.12 (s, 1H, –N**H**), 8.37 (d, 1H, *J* = 3.4 Hz, Pyridine-**H**), 8.05 (d, 1H, *J* = 7.6 Hz, Pyridine-**H**), 7.82 (d, 2H, *J* = 8.5 Hz, Ar**H**), 7.64 (d, 2H, *J* = 7.5 Hz, Ar**H**), 7.40 (d, 3H, *J* = 8.5 Hz, Ar**H**), 7.04 (t, 2H, *J* = 6.6 Hz, Ar**H**, Pyridine-**H**); ^{13}C NMR (DMSO-d_6, 300 MHz): 158.1, 155.8, 146.4, 138.2, 134.6, 128.2, 123.2, 119.0, 117.5, 112.3, 106.3; HPLC purity: t_R 13.47 min (97.8 %); MS (ESI): *m/z* 398 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{19}H_{14}Cl_2N_5O$, calcd 398.0566, found 398.0575 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}):3318, 3136, 2925, 1671, 1493, 1456, 1084, 1024, 823, 752, 689.

5-(2-(4-chlorophenylamino)pyridin-3-yl)-N-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-amine (5f)

The title compound was prepared according to the method described for compound **5a**, employing compound **10f (**488 mg, 1 mmol), tosyl chloride (229 mg, 1.2 mmol) and pyridine (0.17 mL, 2.2 mmol) to obtain the pure product **5f** as a colorless solid, yield 344 mg, 76 %; mp 284–286 ^{o}C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.80 (bs, 1H, –N**H**), 10.07 (bs, 1H, –N**H**), 8.38 (dd, 1H, *J* = 4.9, 1.3 Hz, Pyridine-**H**), 8.04 (dd, 1H, *J* = 7.7, 1.3 Hz, Pyridine-**H**), 7.83 (d, 2H, *J* = 8.9 Hz, Ar**H**), 7.40 (d, 2H, *J* = 8.7 Hz, Ar**H**), 7.06 (dd, 1H, *J* = 7.6, 4.9 Hz, Pyridine-**H**), 7.01 (bs, 2H, Ar**H**), 3.80 (s, 6H, 2×–OC**H**$_3$), 3.64 (s, 3H, –OC**H**$_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): 159.2, 156.3, 153.1, 152.0, 151.8, 138.7, 136.8, 136.4, 136.2, 135.4, 134.4, 128.6, 121.4, 114.7, 95.2, 60.10, 55.73; HPLC purity: t_R 14.40 min (95.6 %); MS (ESI): *m/z* 454 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{22}H_{21}ClN_5O_4$, calcd 454.1278, found 454.1282 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 3312, 3216, 2936, 1632, 1591, 1537, 1509, 1488, 1452, 1233, 1126, 1005, 825, 757.

N-phenyl-5-(2-(3,4,5-trifluorophenylamino)pyridin-3-yl)-1,3,4-oxadiazol-2-amine (5g)

The title compound was prepared according to the method described for compound **5a**, employing compound **10g (**417 mg, 1 mmo), tosyl chloride (229 mg, 1.2 mmol) and pyridine (0.17 mL, 2.2 mmol) to obtain the pure product **5g** as a colorless solid, yield 314 mg, 82%; mp 248–250 ^{o}C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.89 (s, 1H, –N**H**), 10.13

(s, 1H, –N**H**), 8.41 (dd, 1H, *J* = 5.1, 1.5 Hz, Pyridine-**H**), 8.06 (dd, 1H, *J* = 7.3, 1.7 Hz, Pyridine-**H**), 7.79 (dd, 2H, *J* = 10.3, 5.9 Hz, Ar**H**), 7.63 (d, 2H, *J* = 8.1 Hz, Ar**H**), 7.47–7.25 (m, 3H, Ar**H**), 7.11 (dd, 1H, *J* = 7.3, 4.4 Hz, Pyridine-**H**), 7.05 (t, 1H, *J* = 7.3,Hz, Ar**H**); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 75 MHz): 159.3, 156.1, 150.9, 149.4, 138.2, 135.5, 129.3, 129.1, 127.8, 122.2, 117.2, 115.5, 104.0, 103.9, 103.6; HPLC purity: t_R 12.95 min (95.6 %); MS (ESI): *m/z* 384 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{19}H_{13}F_3N_5O$, calcd 384.1072, found 384.1056 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 3448, 2856, 1676, 1645, 1595, 1523, 1448, 1337, 1242, 1150, 1113, 1047, 845, 794, 754, 626.

N-(4-chlorophenyl)-5-(2-(3,4,5-trifluorophenylamino)pyridin-3-yl)-1,3,4-oxadiazol-2-amine (5h)

The title compound was prepared according to the method described for compound **5a**, employing compound **10h (**452 mg, 1 mmol), tosyl chloride (229 mg, 1.2 mmol) and pyridine (0.17 mL, 2.2 mmol) to obtain the pure product **5h** as a colorless solid, yield 300 mg, 72%; mp 288–290 ^{o}C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.06 (bs, 1H, –N**H**), 10.10 (bs, 1H, –N**H**), 8.41 (d, 1H *J* = 3.4 Hz, Pyridine-**H**), 8.05 (d, 1H, *J* = 7.6 Hz, Pyridine-**H**), 7.87–7.71 (m, 2H, Ar**H**), 7.64 (d, 2H, *J* = 7.9 Hz, Ar**H**), 7.44 (d, 2H, *J* = 8.3 Hz, Ar**H**), 7.18–7.05 (m, 1H, Pyridine-**H**); ^{13}C NMR (TFA-*d*, 75 MHz,): 159.7, 156.9, 155.7, 153.7, 153.5, 151.2, 147.3, 142.5, 138.7, 132.9, 126.8, 122.6, 117.4, 115.0, 111.3; HPLC purity: t_R 13.47 min (95.3 %); MS (ESI): *m/z* 418 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{19}H_{12}ClF_3N_5O$, calcd 418.0679, found 418.0682 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 3326, 2928, 1689, 1525, 1449, 128, 1046, 814, 758, 627.

N-(4-nitrophenyl)-5-(2-(3,4,5-trifluorophenylamino)pyridin-3-yl)-1,3,4-oxadiazol-2-amine (5i)

The title compound was prepared according to the method described for compound **5a**, employing compound **10i (**462 mg, 1 mmol), tosyl chloride (229 mg, 1.2 mmol) and pyridine (0.17 mL, 2.2 mmol) to obtain the pure product **5i** as a colorless solid, yield 328 mg, 76 %; mp 299–301 ^{o}C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.78 (s, 1H, –N**H**), 10.07 (bs, 1H, –N**H**), 8.45 (dd, 1H, *J* = 4.5, 1.8 Hz, Pyridine-**H**), 8.31 (d, 2H, *J* = 8.9 Hz, Ar**H**), 8.11 (dd, 1H, *J* = 8.1, 1.8 Hz, Pyridine-**H**), 7.88-7.76 (m, 4H, Ar**H**), 7.14 (dd, 1H, *J* = 7.2, 4.5 Hz, Pyridine-**H**); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 75 MHz,): 158.4, 157.2, 151.7, 149.9,

144.3, 141.2, 135.4, 125.2, 121.8, 121.7, 116.9, 115.2, 114.9, 114.0, 102.5; HPLC purity: t_R 13.37 min (96.4 %); MS (ESI): *m/z* 429 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{19}H_{12}F_3N_6O_3$, calcd 429.0925, found 429.0922 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 3325, 2860, 1679, 1599, 1523, 1444, 1333, 1254, 1228, 1043, 794, 760.

N-phenyl-5-(2-(3,4,5-trimethoxyphenylamino)pyridin-3-yl)-1,3,4-oxadiazol-2-amine (5j)

The title compound was prepared according to the method described for compound **5a**, employing compound **10j (**453 mg, 1 mmol), tosyl chloride (229 mg, 1.2 mmol) and pyridine (0.17 mL, 2.2 mmol) to obtain the pure product **5j** as a colorless solid, yield 306 mg, 85%; mp 247–248 ^oC. ^1H NMR (DMSO-d_6, 300 MHz): δ 10.86 (bs, 1H, –N**H**), 9.94 (bs, 1H, –N**H**), 8.37 (dd, 1H, *J* = 4.7, 1.5 Hz, Pyridine-**H**), 8.04 (dd, 1H, *J* = 7.6, 1.3 Hz, Pyridine-**H**), 7.65 (d, 2H, *J* = 7.7 Hz, Ar**H**), 7.39 (t, 2H, *J* = 7.9 Hz, Ar**H**), 7.13 (s, 2H, Ar**H**), 7.09–6.96 (m, 2H, Pyridine-**H,** Ar**H**), 3.81 (s, 6H, 2×–OC**H**$_3$), 3.65 (s, 3H, –OC**H**$_3$);. ^{13}C NMR (DMSO-d_6, 75 MHz): 159.2, 155.1, 152.8, 151.7, 149.7, 138.7, 135.9, 135.2, 132.9, 129.0, 122.1, 117.2, 114.0, 102.9, 97.95, 60.04, 55.77; HPLC purity: t_R 11.83 min (97.5 %); MS (ESI): *m/z* 420 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{22}H_{21}N_5O_4Na$, calcd 442.1491, found 442.1478 (M+Na); IR (KBr) (ν_{max}/cm^{-1}): 3323, 2936, 1669, 1632, 1588, 1503, 1453, 1234, 1131, 1032, 1004, 835, 798, 758, 681.

N-(4-chlorophenyl)-5-(2-(3,4,5-trimethoxyphenylamino)pyridin-3-yl)-1,3,4-oxadiazol -2-amine (5k)

The title compound was prepared according to the method described for compound **5a**, employing compound **10k (**488 mg, 1 mmol), tosyl chloride (229 mg, 1.2 mmol) and pyridine (0.17 mL, 2.2 mmol) to obtain the pure product **5k** as a colorless solid, yield 372 mg, 82%; mp 214–216 ^oC. ^1H NMR (DMSO-d_6, 300 MHz): δ 11.07 (bs, 1H, –N**H**), 9.92 (bs, 1H, –N**H**), 8.35 (bs, 1H, –N**H**), 8.00 (d, 1H, *J* = 6.8 Hz, Pyridine-**H**), 7.66 (d, 2H *J* = 7.9 Hz, Pyridine-**H,** Ar**H**), 7.43 (d, 2H, *J* = 7.9 Hz, Ar**H**), 7.11 (s, 2H, Ar**H**), 6.98 (t, 1H, *J* = 5.3 Hz, Pyridine-**H**), 3.79 (s, 6H, 2×–OC**H**$_3$), 3.64 (s, 3H, –OC**H**$_3$); HPLC purity: t_R 12.50 min (96.1 %); MS (ESI): *m/z* 454 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{22}H_{21}ClN_5O_4$, calcd 454.1282, found 454.1283 $(M+1)^+$.^{13}C NMR (DMSO-d_6, 75 MHz): 159.4, 157.1, 153.3, 152.1, 150.1, 137.7, 136.3, 135.8, 133.5, 129.4, 126.3, 119.2, 114.5, 103.4, 98.45,

60.60, 56.26 ; IR (KBr) (ν_{max}/cm^{-1}): 3333, 2937, 1682, 1584, 1500, 1455, 1232, 1127, 1005, 831, 756, 652.

N-(4-nitrophenyl)-5-(2-(3,4,5-trimethoxyphenylamino)pyridin-3-yl)-1,3,4-oxadiazol-2-amine (5l)

The title compound was prepared according to the method described for compound **5a**, employing compound **10l (**498 mg, 1 mmol), tosyl chloride (229 mg, 1.2 mmol) and pyridine (0.17 mL, 2.2 mmol) to obtain the pure product **5l** as a white colorless, yield 362 mg 78%; mp 268–270 ^{o}C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.72 (bs, 1H, –N**H**), 9.89 (bs, 1H, –N**H**), 8.39 (dd, *J* = 3.2, 1.6 Hz, 1H, Pyridine-**H**), 8.32 (d, *J* = 7.9 Hz, 2H, Ar**H**), 8.07 (d, *J* = 7.1 Hz, 1H, Pyridine-**H**), 7.86 (d, *J* = 8.7 Hz, 2H, Ar**H**), 7.12 (s, 2H, Ar**H**), 7.02 (dd, *J* = 7.9, 4.7 Hz, 1H, Pyridine-**H**), 3.81 (s, 6H, 2×–OC**H**$_3$), 3.65 (s, 3H, –OC**H**$_3$); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 75 MHz): 156.7, 155.6, 151.1, 150.2, 148.2, 142.6, 139.7, 134.1, 133.6, 131.4, 123.4, 115.2, 112.0, 100.9, 96.27, 58.40, 54.06.; HPLC purity: t_R 12.03 min (95.8 %); MS (ESI): *m/z* 465 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{22}H_{20}N_6O_6Na$, calcd 487.1337, found 487.1342 (M+Na).; IR (KBr) (ν_{max}/cm^{-1}): 3533, 3421, 2936, 1585, 1505, 1446, 1324, 1235, 1123, 837, 760.

N-(3,4,5-trimethoxyphenyl)-5-(2-(3,4,5-trimethoxyphenylamino)pyridin-3-yl)-1,3,4-oxadiazol-2-amine (5m)

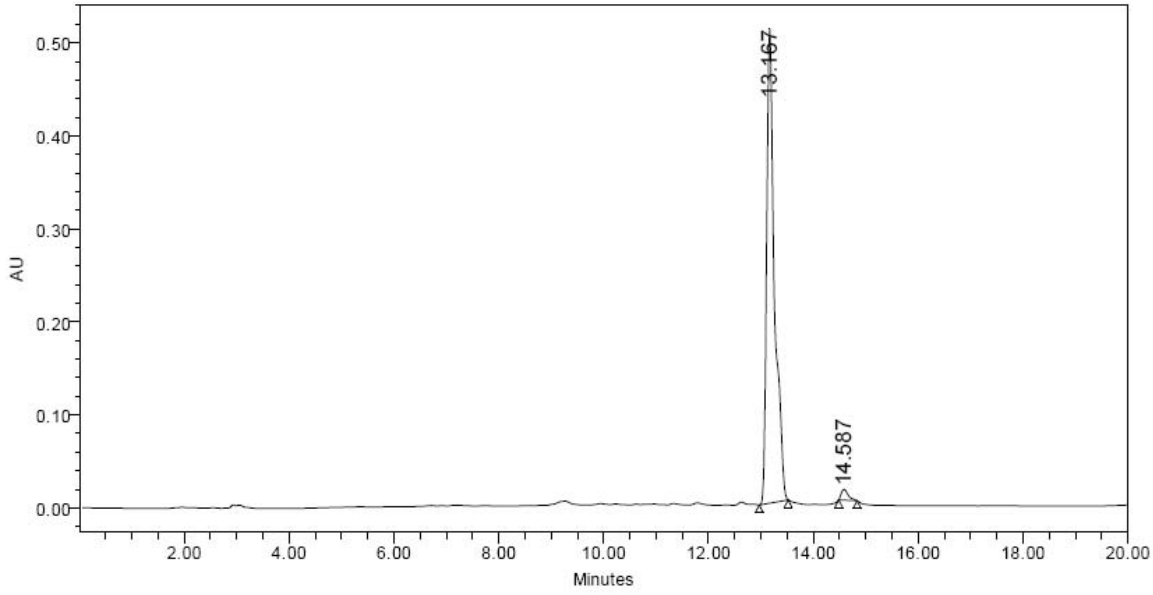
The title compound was prepared according to the method described for compound **5a**, employing compound **10m (**544 mg,1 mmol), tosyl chloride (229 mg, 1.2 mmol) and pyridine (0.17 mL, 2.2 mmol) to obtain the pure product **5m** as a off-white solid, yield 407 mg 80 %; mp 208–210 ^{o}C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.34 (bs, 1H, –N**H**), 10.07 (s, 1H, –N**H**), 8.17 (d, 1H, *J* = 4.7 Hz, Pyridine-**H**), 8.07 (d, 1H, *J* = 7.6 Hz, Pyridine-**H**), 6.96-6.87 (m, 4H, Ar**H**), 6.87 (m, 1H, Pyridine-**H**), 3.80 (s, 12H, 4×–OC**H**$_3$), 3.73 (s, 3H, –OC**H**$_3$), 3.69 (s, 3H, –OC**H**$_3$); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 75 MHz): 158.3, 154.7, 153.1, 151.9, 149.8, 133.1, 133.0, 132.9, 126.9, 126.7, 112.3, 103., 102.9, 98.16, 94.20, 59.19, 54.70; HPLC purity: t_R 11.33 min (98.8 %); MS (ESI): *m/z* 510 $(M+1)^+$; HRMS (ESI *m/z*) for $C_{25}H_{28}N_5O_7$, calcd 510.1989, found 510.1978 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 3419, 3316, 2922, 1627, 1594, 1531, 1492, 1441, 1318, 1248, 1084, 823, 692, 758, 61

Empower 2 software

Default Individual Report

SAMPLE INFORMATION

Sample Name:	5a	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxa_grad_DMSO
Vial:	4	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	kamal
Injection Volume:	20.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
Date Acquired:	1/5/2011 2:23:55 AM IST		
Date Processed:	1/13/2011 4:46:06 AM IST		



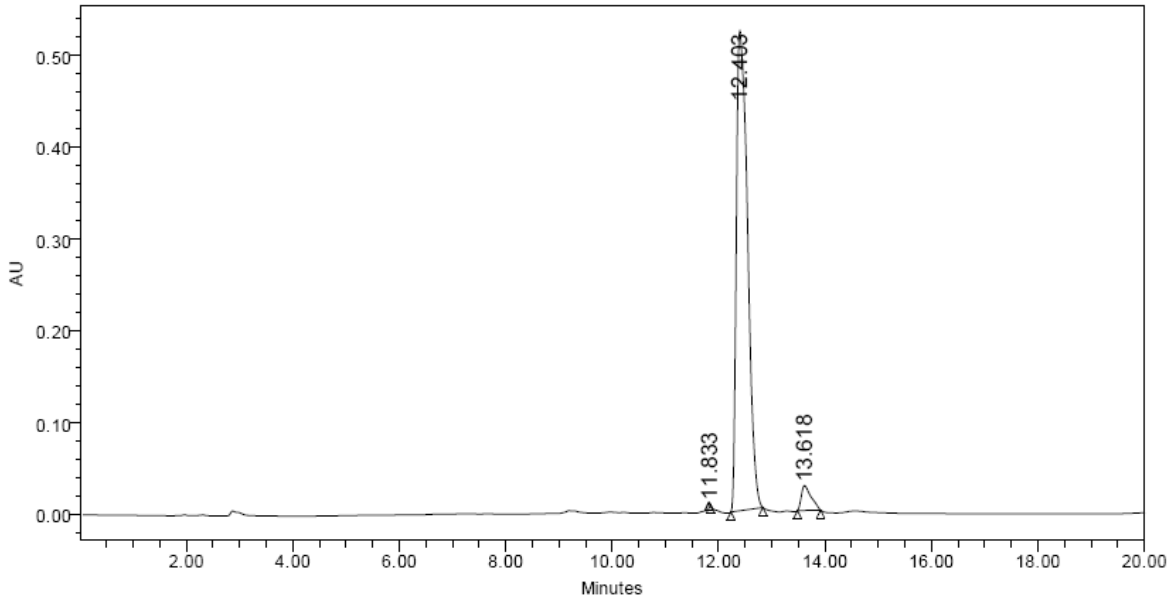
	RT	Area	% Area	Height
1	13.167	5289096	98.06	508851
2	14.587	104656	1.94	11557

Empower 2 software

Default Individual Report

SAMPLE INFORMATION

Sample Name:	5b	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxa_grad_DMSO
Vial:	5	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	kamal
Injection Volume:	20.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
Date Acquired:	1/5/2011 2:45:27 AM IST		
Date Processed:	1/13/2011 4:47:18 AM IST		



	RT	Area	% Area	Height
1	11.833	625	0.01	625
2	12.403	7648683	95.67	523741
3	13.618	345572	4.32	27246

Empower2 software

Default Individual Report

SAMPLE INFORMATION

Sample Name:	5c	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxadiazoles_gradient
Vial:	7	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	Default2
Injection Volume:	10.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
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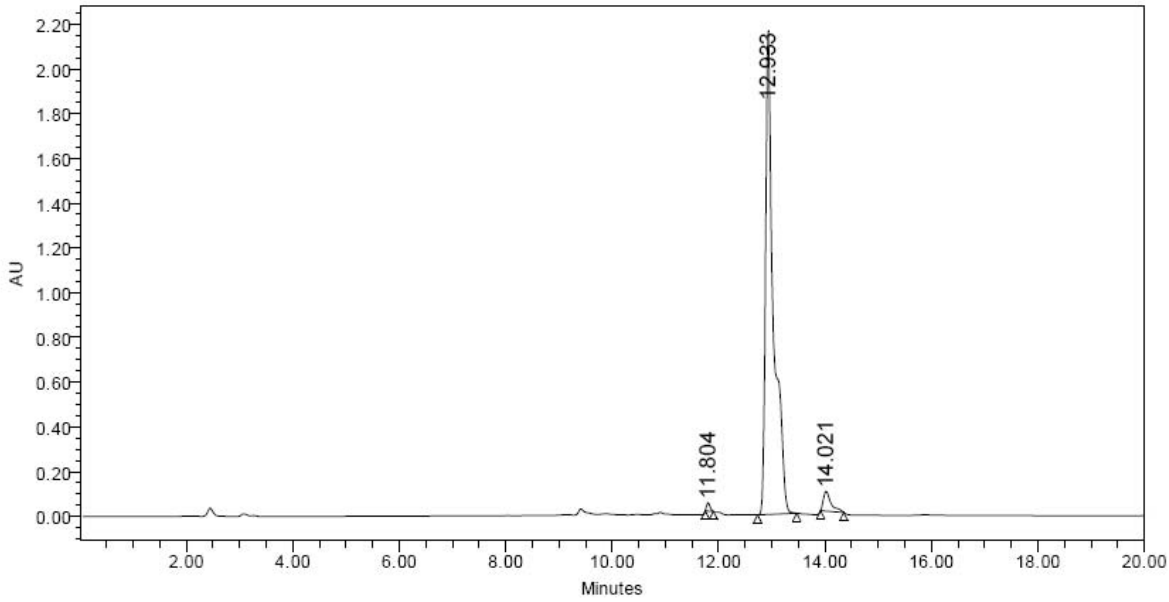
Date Processed:	1/13/2011 4:40:43 AM IST		



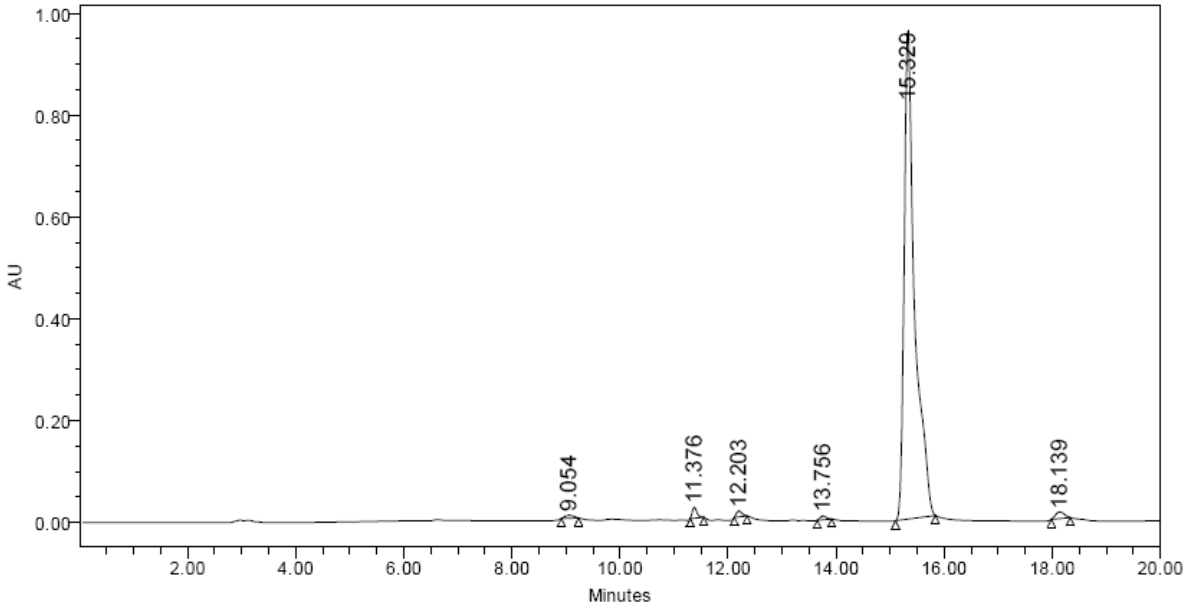
	RT	Area	% Area	Height
1	11.804	167903	0.71	37285
2	12.933	22538777	95.50	2153077
3	14.021	893131	3.78	89020

Empower 2 software

Default Individual Report

SAMPLE INFORMATION

Sample Name:	5d	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxa
Vial:	11	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	kamal
Injection Volume:	20.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
Date Acquired:	1/5/2011 5:56:02 AM IST		
Date Processed:	1/13/2011 4:54:04 AM IST		



	RT	Area	% Area	Height
1	9.054	63085	0.47	5554
2	11.376	131402	0.98	22022
3	12.203	83651	0.62	11374
4	13.756	60778	0.45	7686
5	15.329	12971821	96.40	960659
6	18.139	145853	1.08	12984

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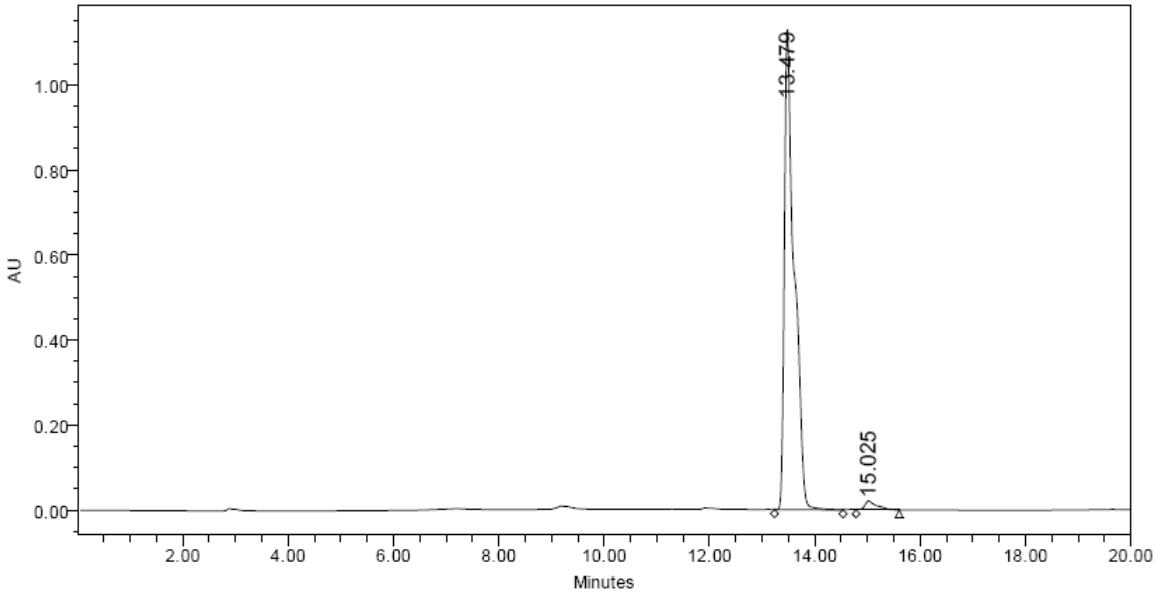
Default Individual Report

SAMPLE INFORMATION

Sample Name:	5e	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxa_rep
Vial:	2	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
Date Acquired:	1/11/2011 5:52:49 AM IST		
Date Processed:	1/13/2011 4:07:54 AM IST		



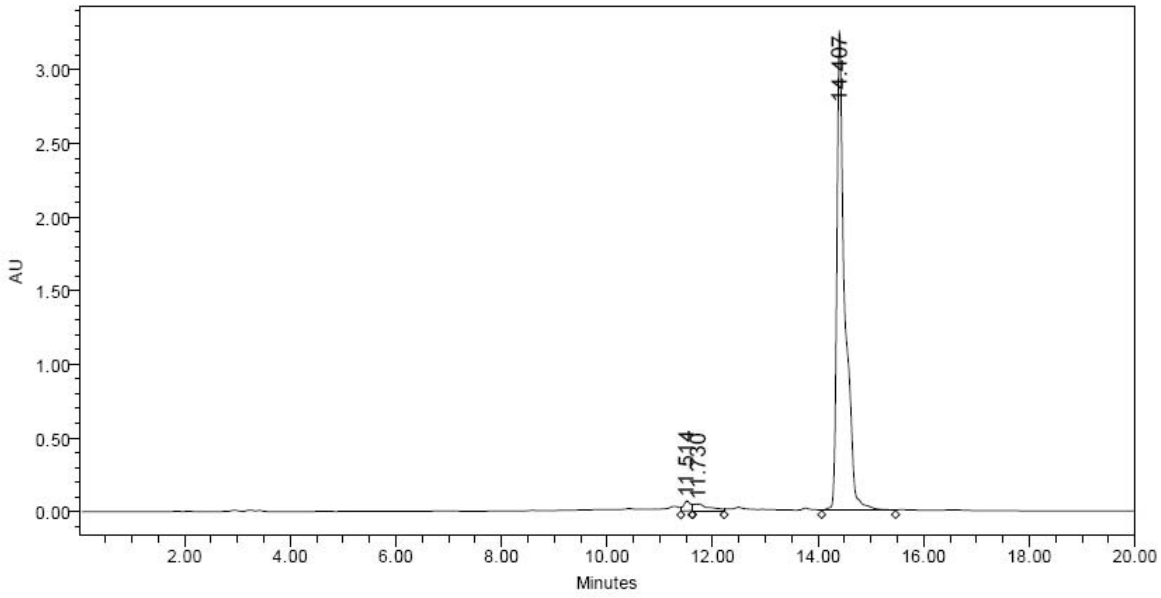
	RT	Area	% Area	Height
1	13.479	14670692	97.81	1129925
2	15.025	328304	2.19	20839

Empower 2 software

Default Individual Report

SAMPLE INFORMATION

Sample Name:	5f	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxa_gradient
Vial:	1	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	Default2
Injection Volume:	10.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
Date Acquired:	1/4/2011 2:07:27 AM IST		
Date Processed:	1/13/2011 4:35:40 AM IST		



	RT	Area	% Area	Height
1	11.514	613088	1.67	66249
2	11.730	971971	2.65	46335
3	14.407	35136942	95.68	3269263

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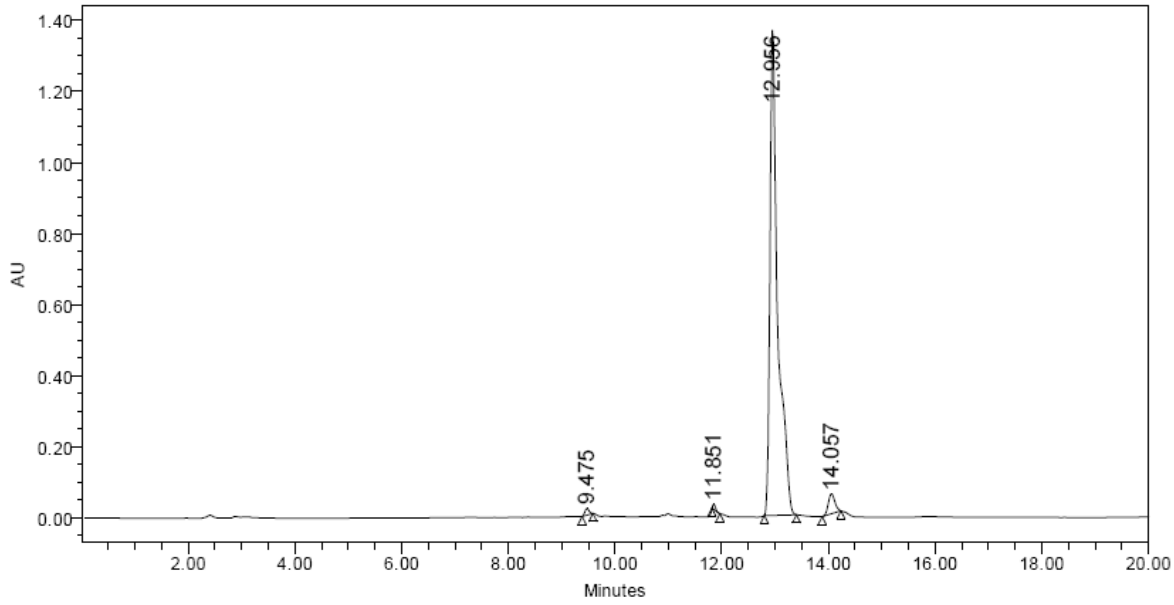
Default Individual Report

SAMPLE INFORMATION

Sample Name:	5g	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxa
Vial:	13	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	kamal
Injection Volume:	20.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
Date Acquired:	1/5/2011 6:38:47 AM IST		
Date Processed:	1/13/2011 5:04:34 AM IST		



	RT	Area	% Area	Height
1	9.475	102354	0.70	18537
2	11.851	53734	0.37	15080
3	12.956	13915648	95.63	1367266
4	14.057	479567	3.30	56250

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Default Individual Report

SAMPLE INFORMATION

Sample Name:	5h	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxa
Vial:	8	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	kamal
Injection Volume:	20.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
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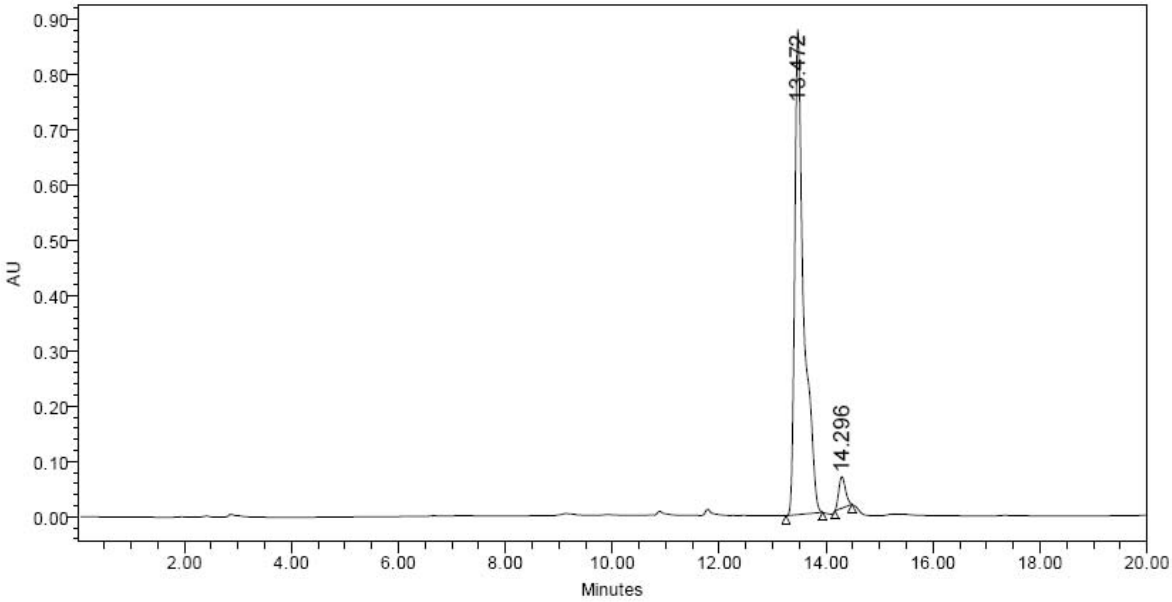
Date Processed:	1/13/2011 5:14:18 AM IST		



	RT	Area	% Area	Height
1	13.472	10358679	95.36	878391
2	14.296	503697	4.64	57200

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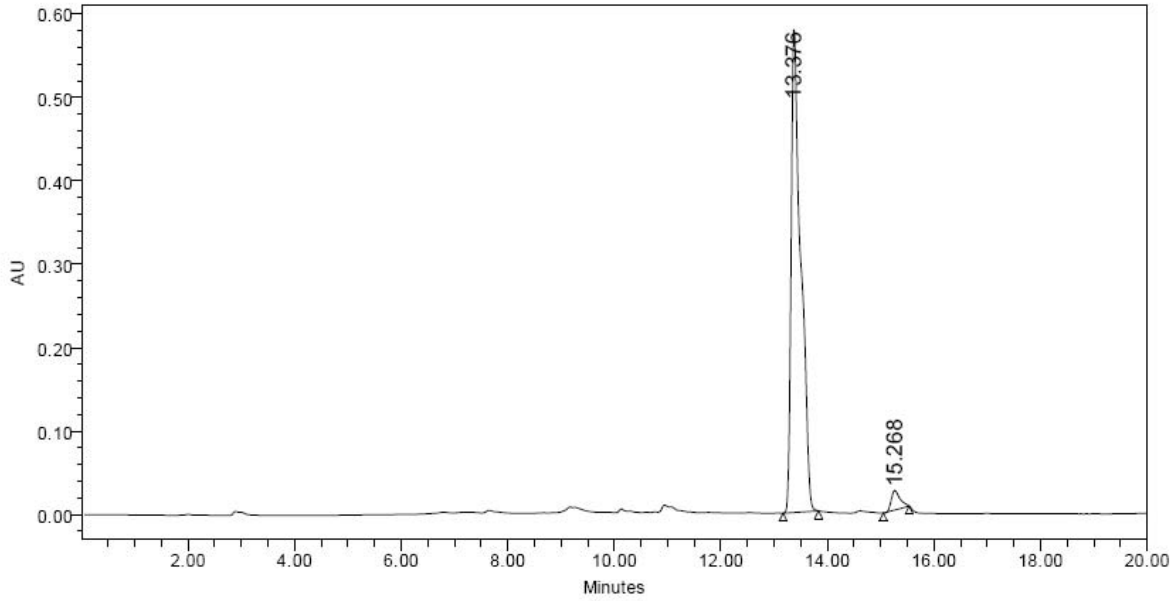
Default Individual Report

SAMPLE INFORMATION

Sample Name:	5i	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxa_rep
Vial:	5	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	Default
Injection Volume:	20.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
Date Acquired:	1/11/2011 6:58:35 AM IST		
Date Processed:	1/13/2011 4:22:08 AM IST		



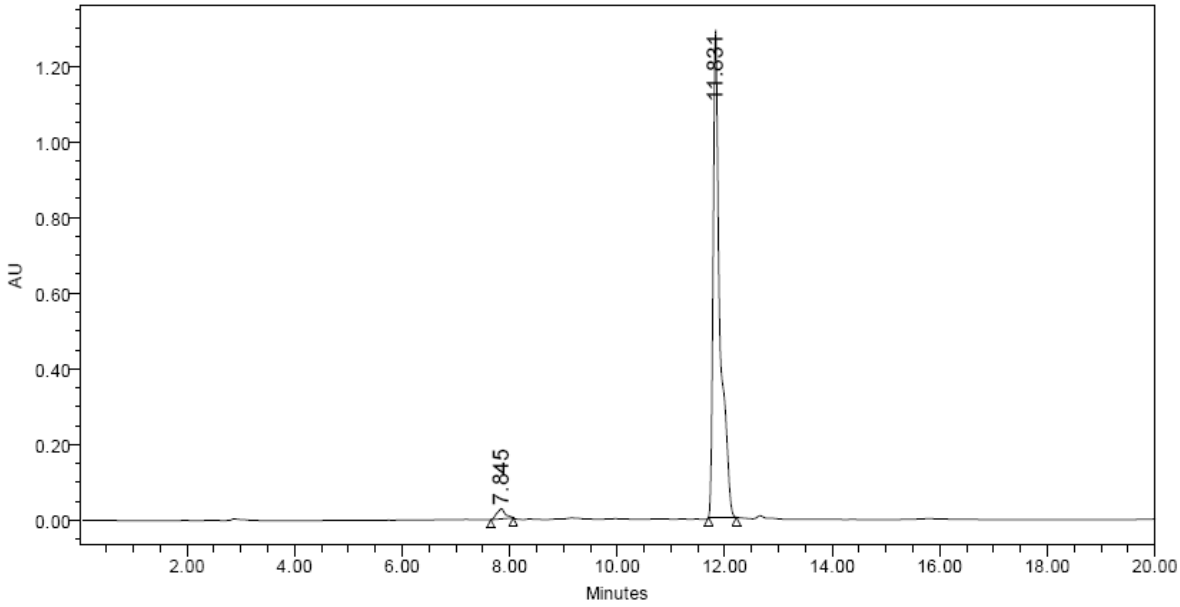
	RT	Area	% Area	Height
1	13.376	7255665	96.43	584210
2	15.268	268479	3.57	23416

Empower 2 software

Default Individual Report

SAMPLE INFORMATION

Sample Name:	5j	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxa
Vial:	10	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	kamal
Injection Volume:	20.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
Date Acquired:	1/5/2011 5:21:54 AM IST		
Date Processed:	1/13/2011 4:50:11 AM IST		



	RT	Area	% Area	Height
1	7.845	284834	2.43	26923
2	11.831	11418941	97.57	1280963

Empower 2 software

Default Individual Report

SAMPLE INFORMATION

Sample Name:	5k	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxa_rep
Vial:	7	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	Default1
Injection Volume:	20.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
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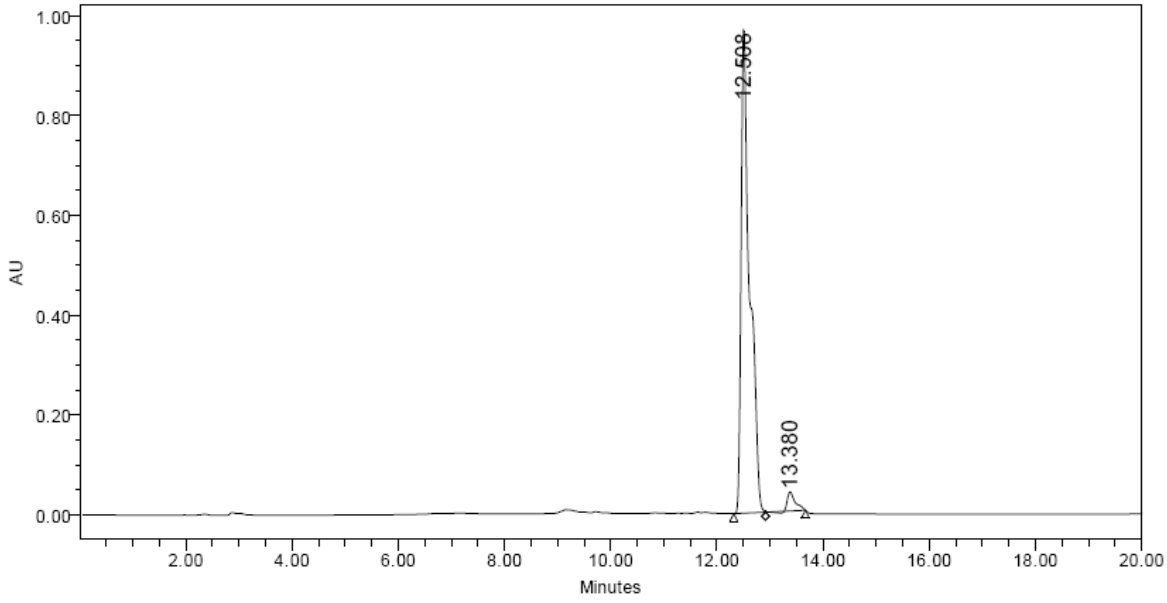

	RT	Area	% Area	Height
1	12.508	10907686	96.17	977656
2	13.380	434438	3.83	38240

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Default Individual Report

SAMPLE INFORMATION

Sample Name:	5l	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxa
Vial:	14	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	kamal
Injection Volume:	20.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
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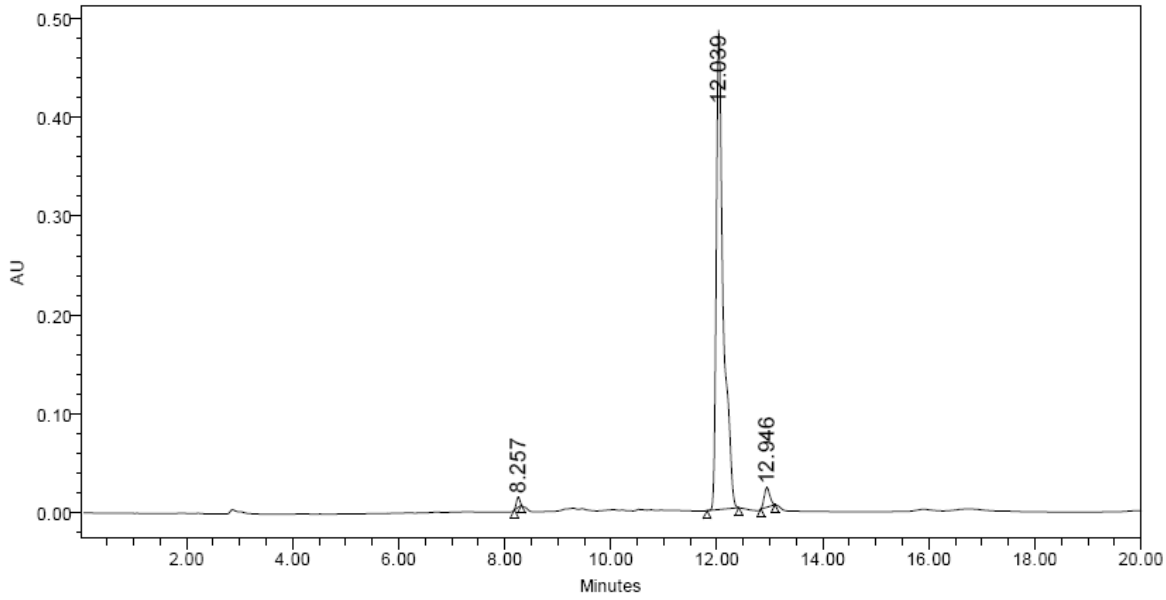


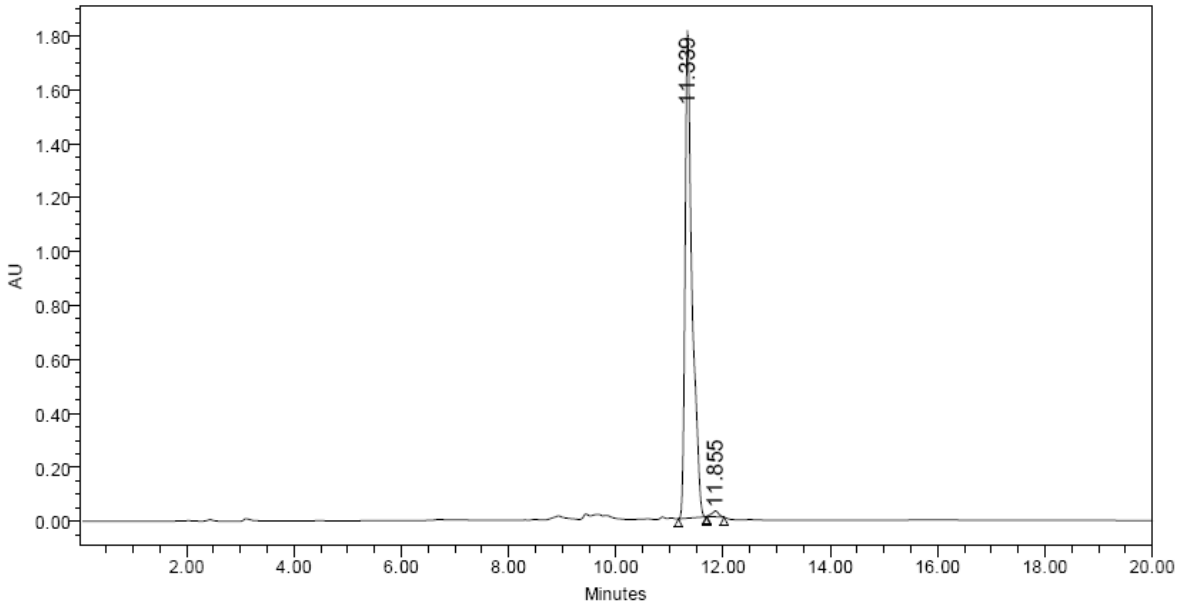
	RT	Area	% Area	Height
1	8.257	44831	0.97	10218
2	12.039	4445360	95.89	485324
3	12.946	145708	3.14	19780

Empower 2 software

Default Individual Report

SAMPLE INFORMATION

Sample Name:	5m	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	Oxadiazoles_gradient
Vial:	1	Acq. Method Set:	Kamals
Injection #:	1	Processing Method:	Default1
Injection Volume:	10.00 ul	Channel Name:	2487Channel 2
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	254 nm
Date Acquired:	1/4/2011 3:52:49 AM IST		
Date Processed:	1/13/2011 4:27:00 AM IST		



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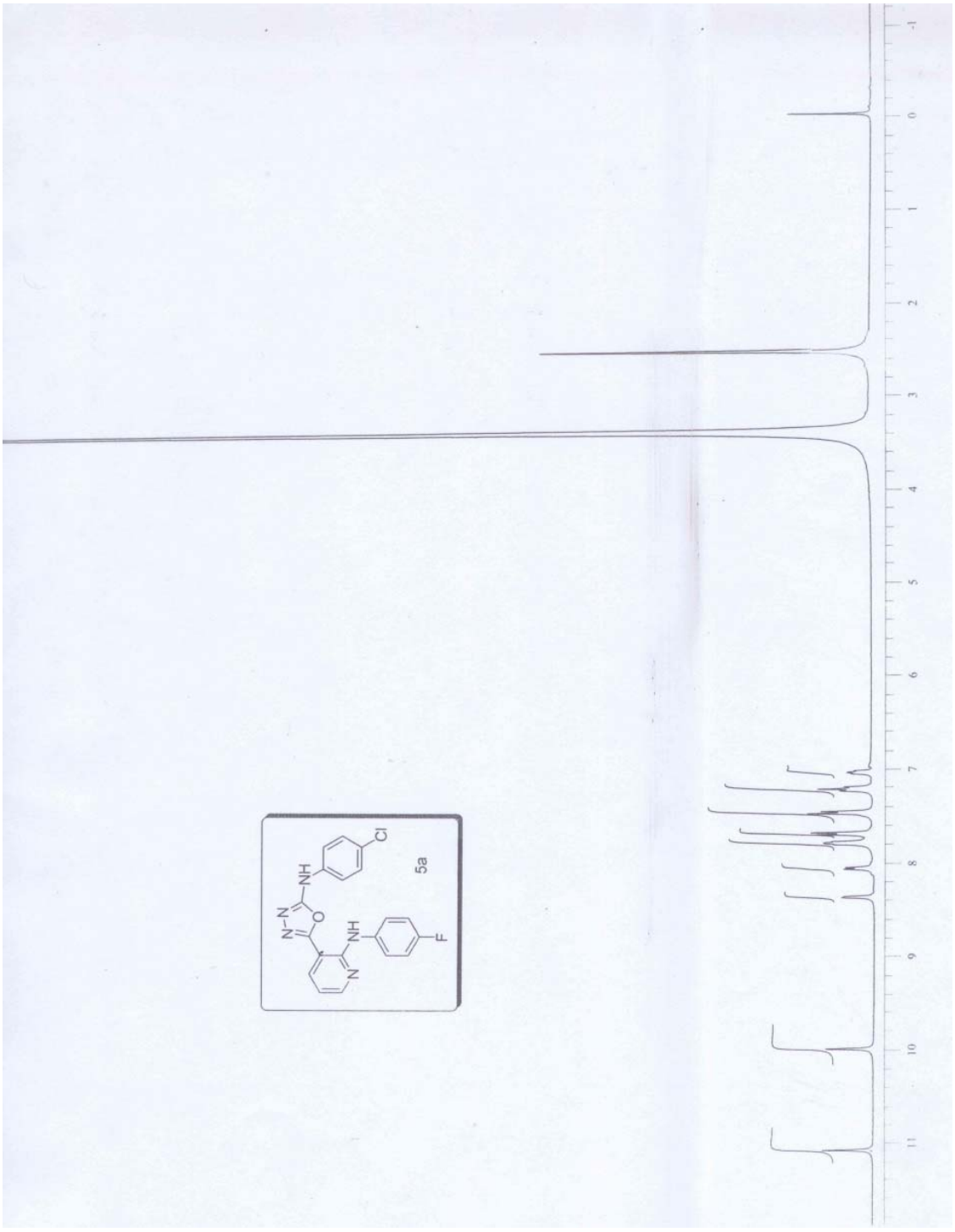
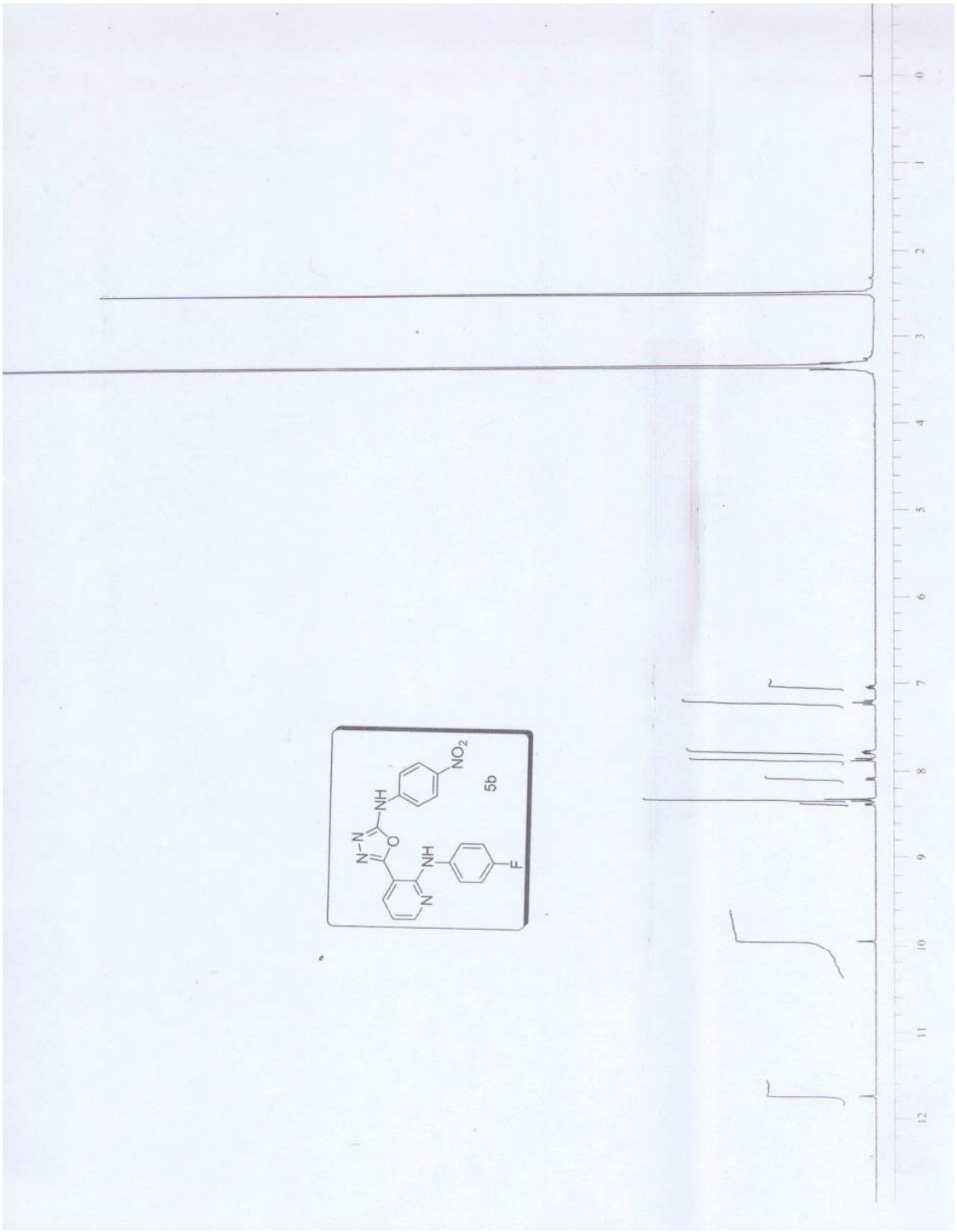
1	11.339	16514627	98.83	1809653
2	11.855	195505	1.17	21831


Cl
NH
N
N
O
NH
F
N
5a


NO2
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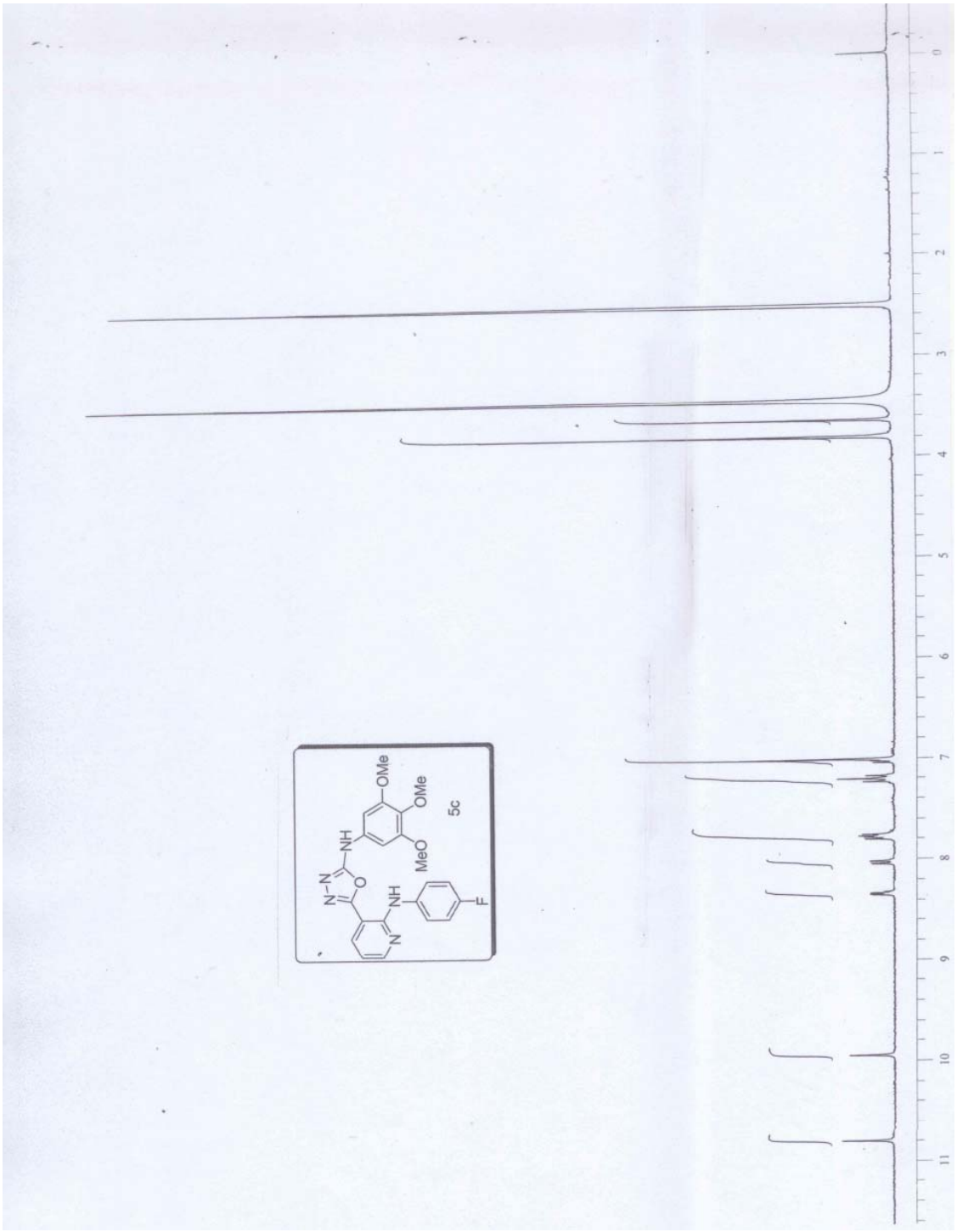
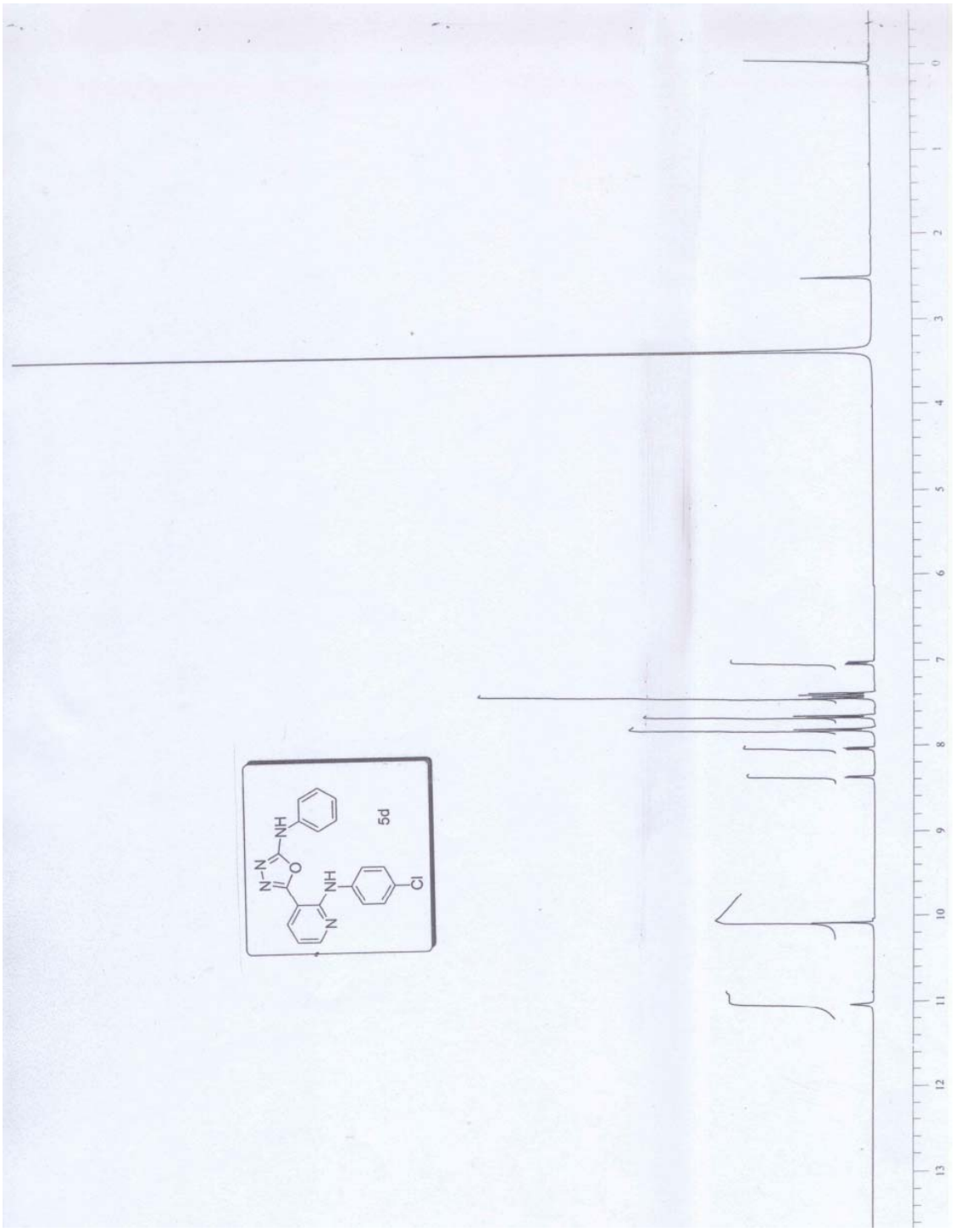
N
N
O
NH
F
N
5b


OMe
OMe
MeO
NH
N
O
N
NH
N
F
5c


5d
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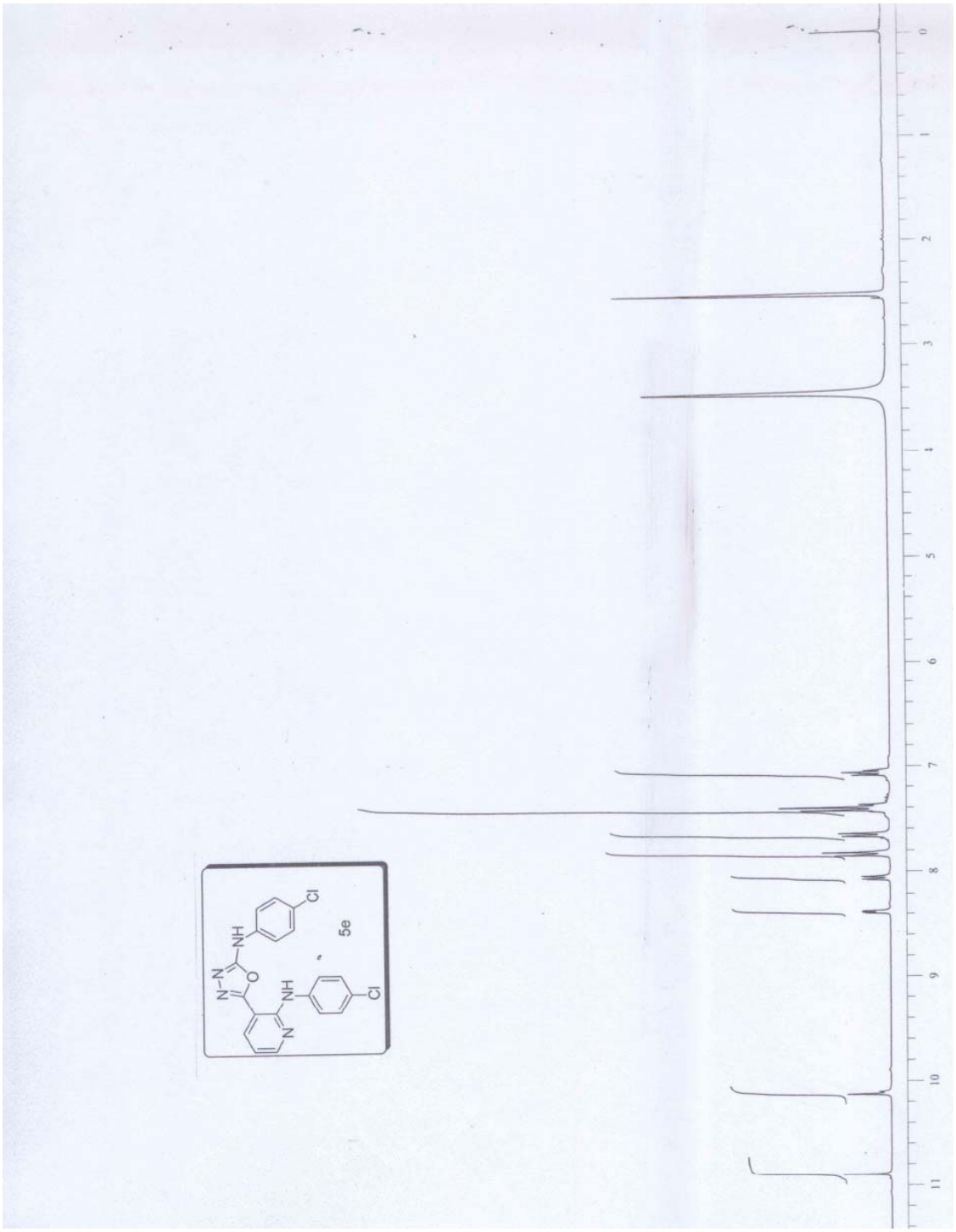
N
O
N
NH
Cl
N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Cl
NH
N
O
N
NH
Cl
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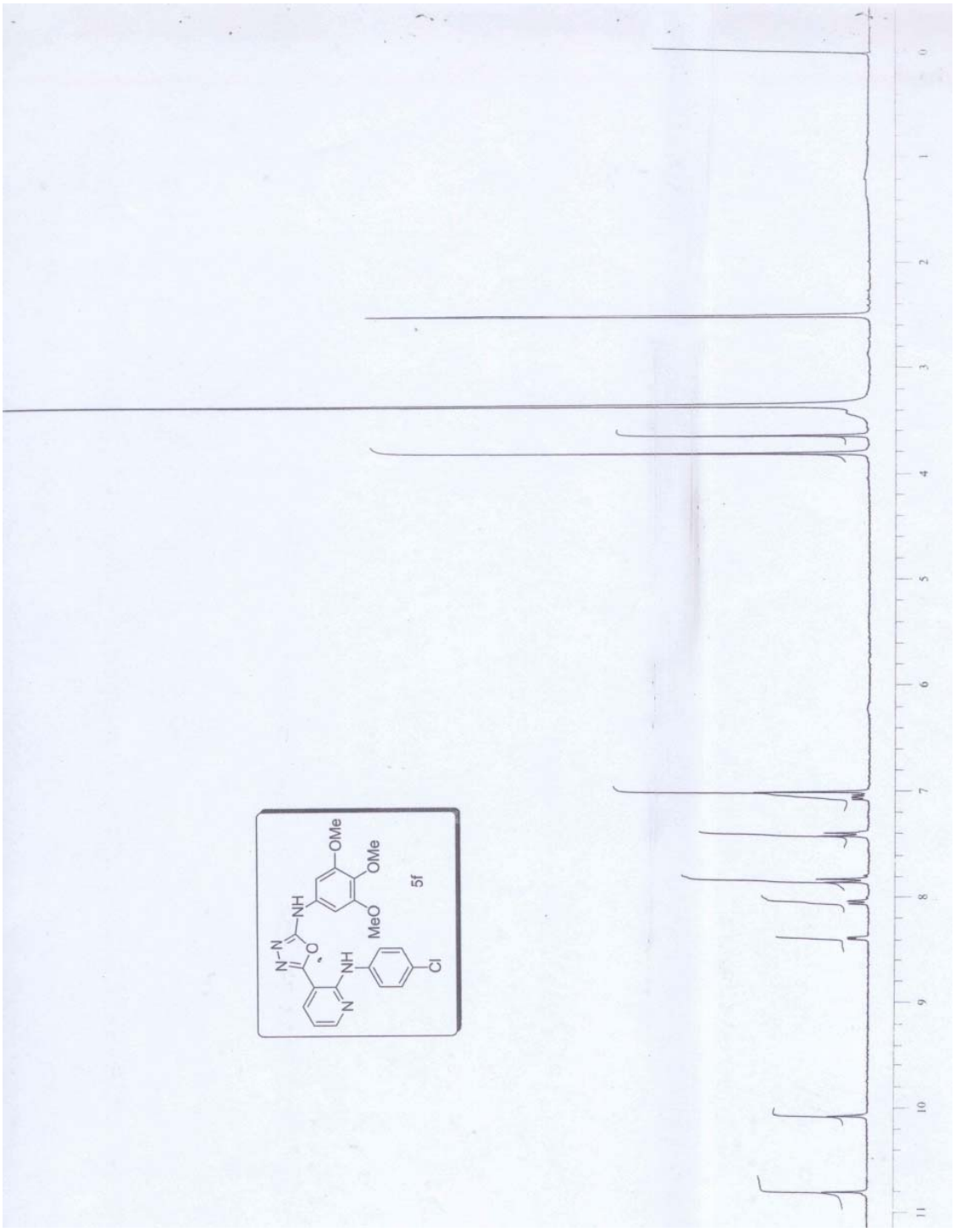
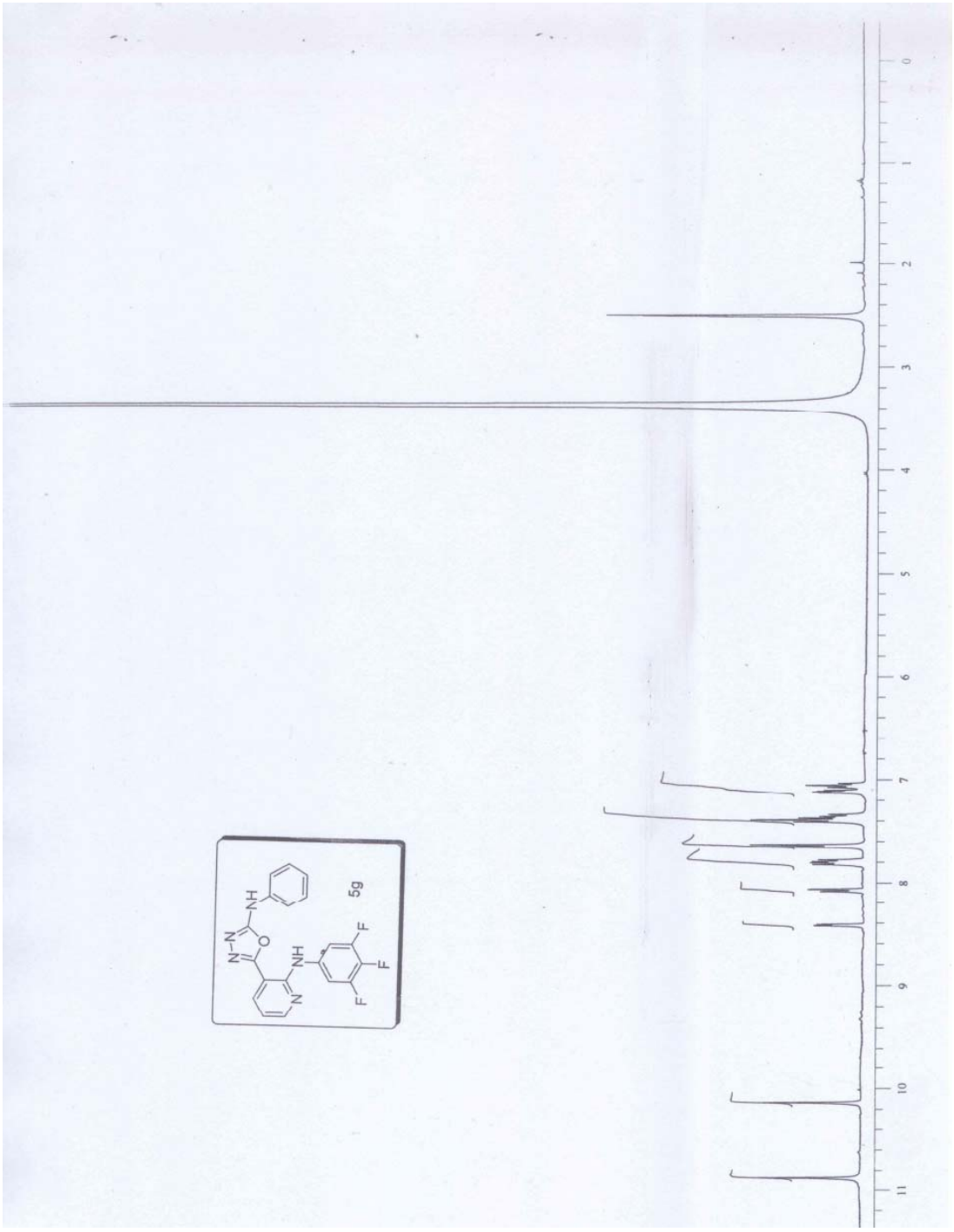

OMe
OMe
MeO
NH
NH
Cl
5f


5g
NH
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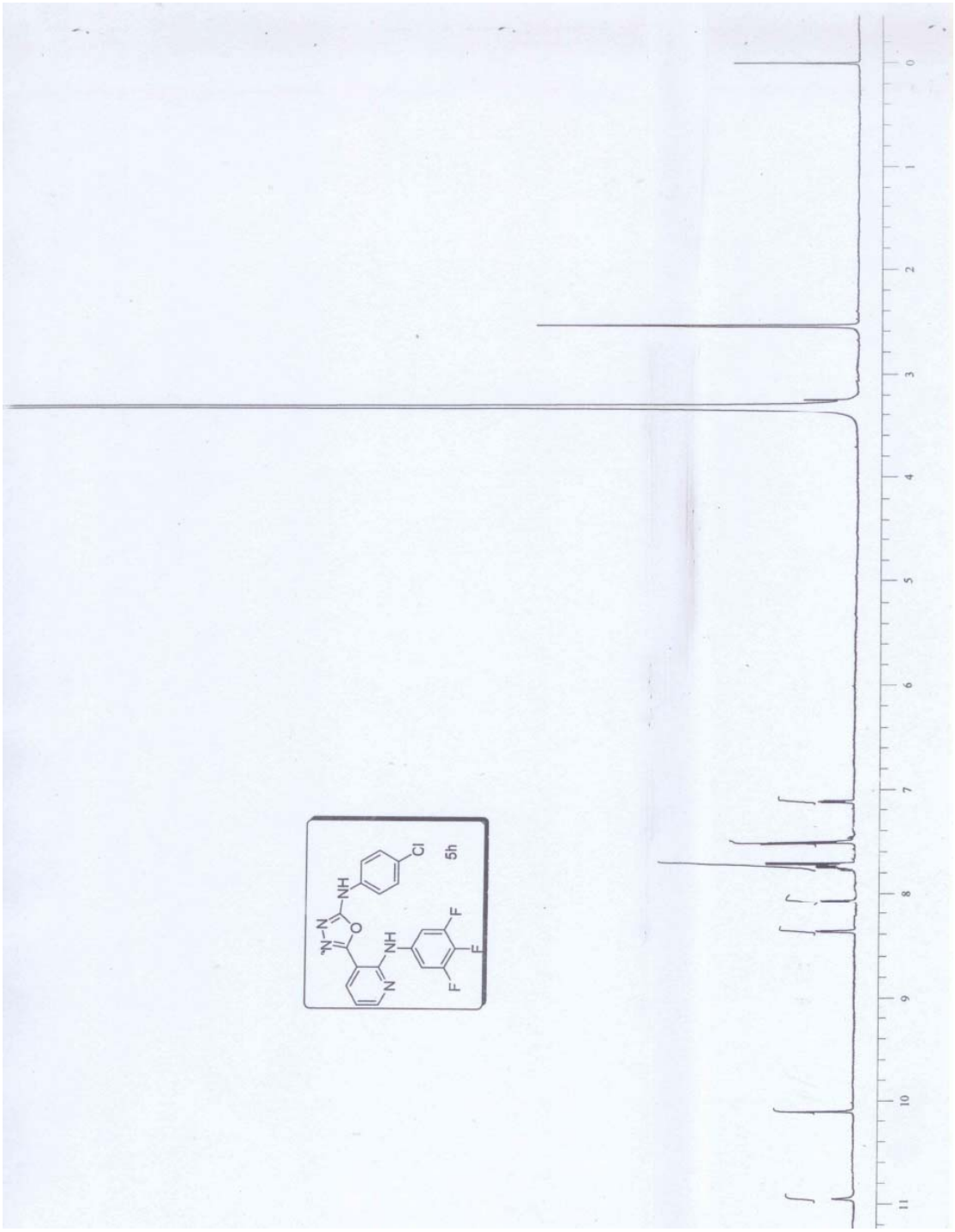
O
N
NH
N
F
F
F
0
1
2
3
4
5
6
7
8
9
10
11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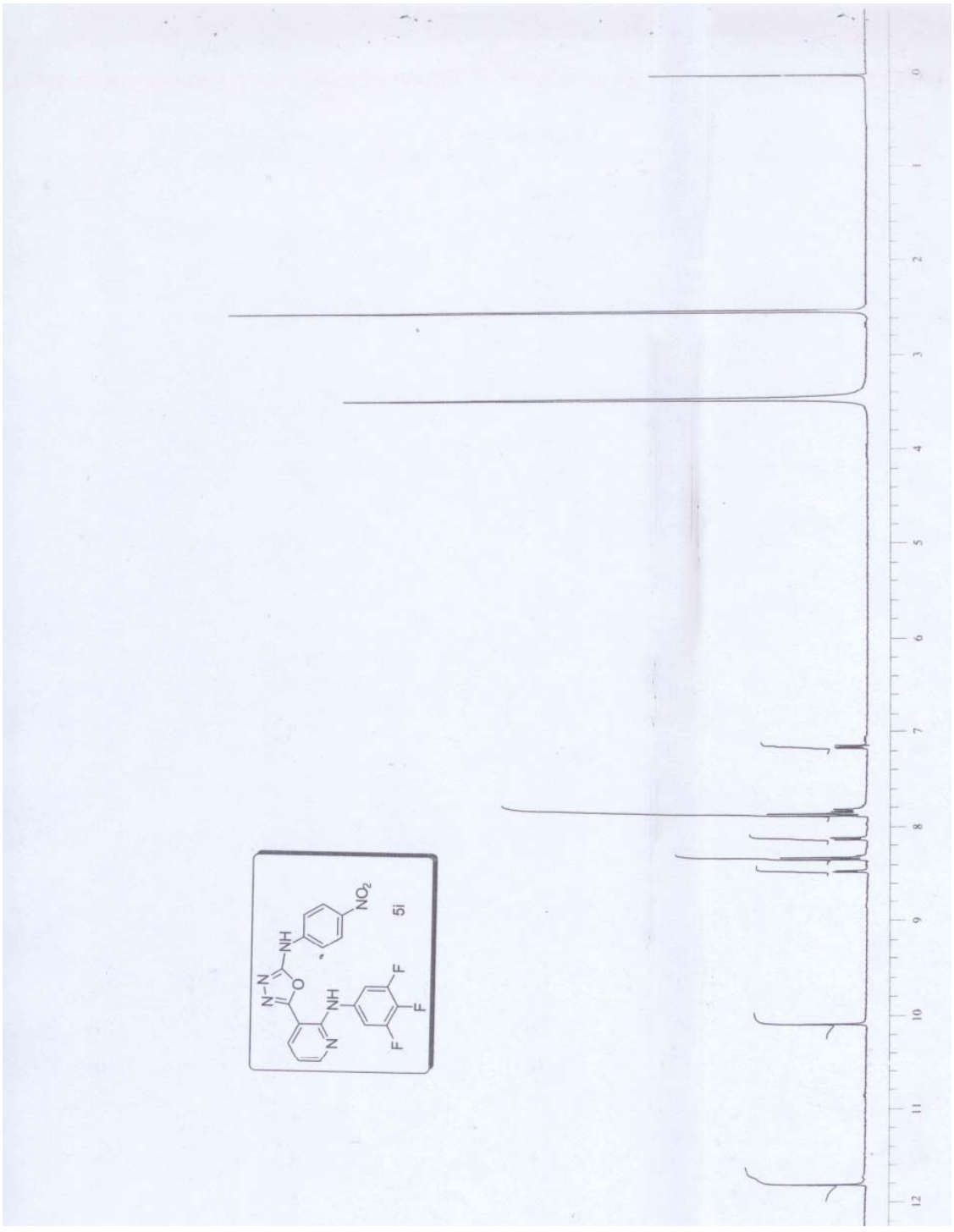
NH
F
F
F
N


5i
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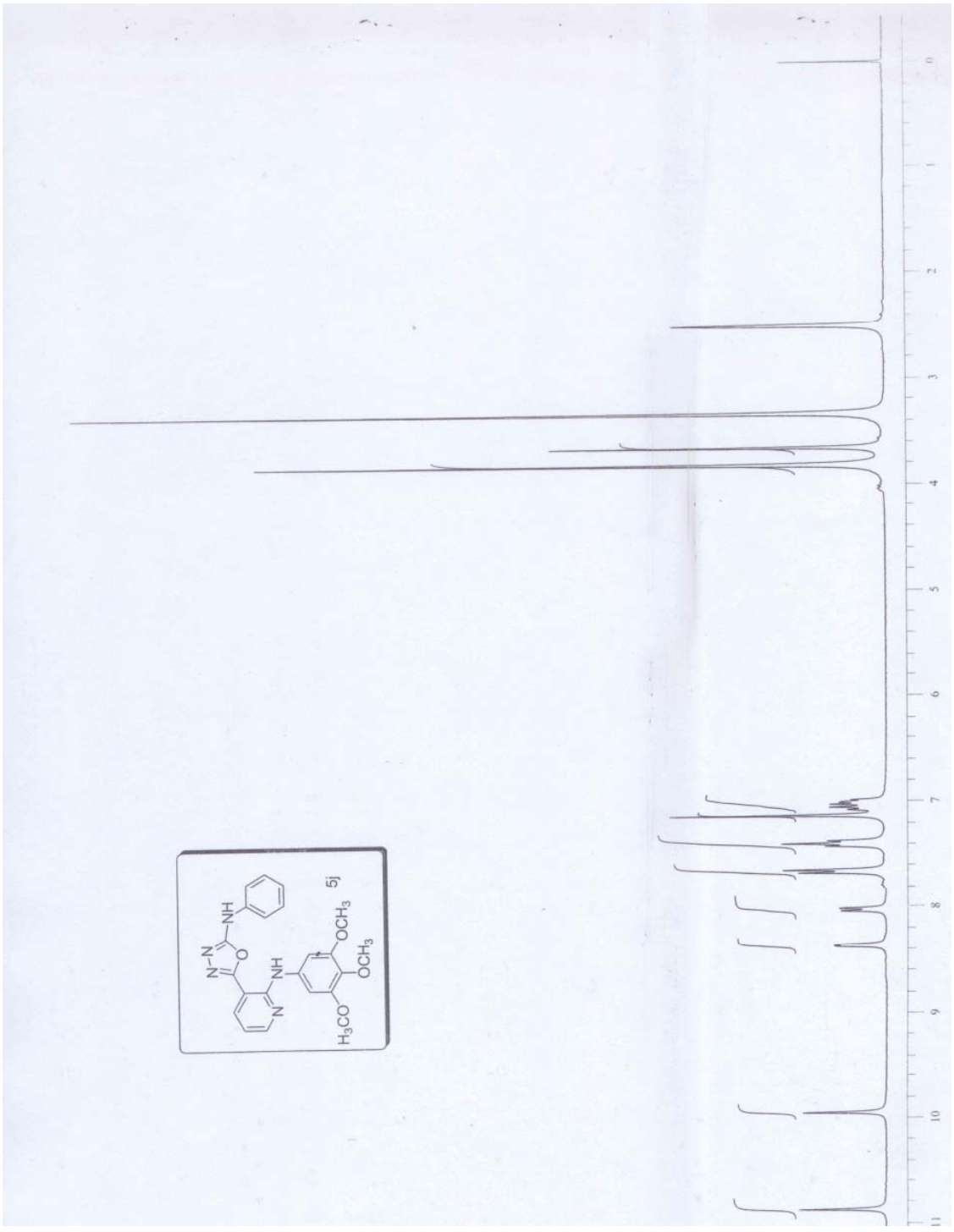
O
NH
F
F
F
N


5j
NH
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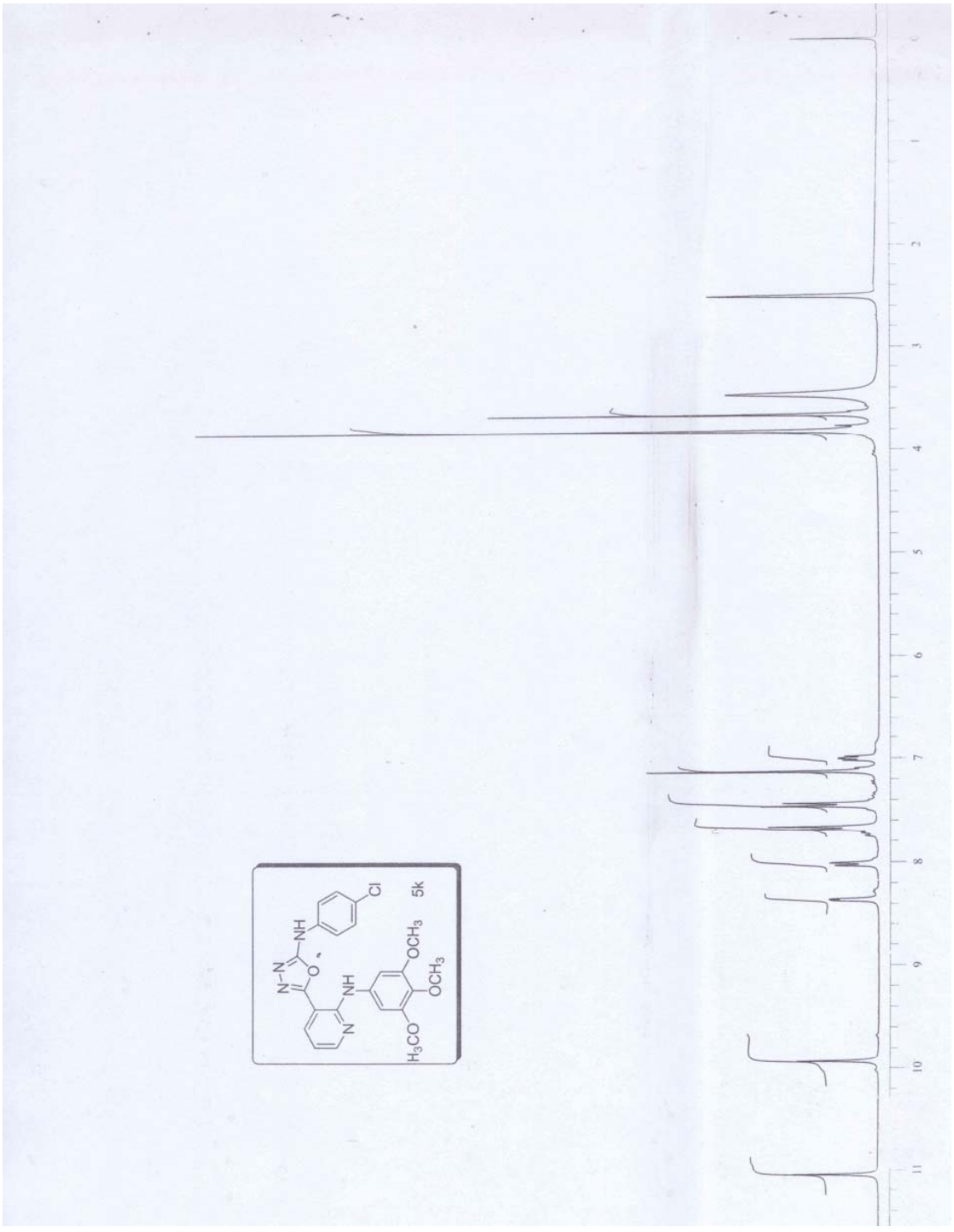
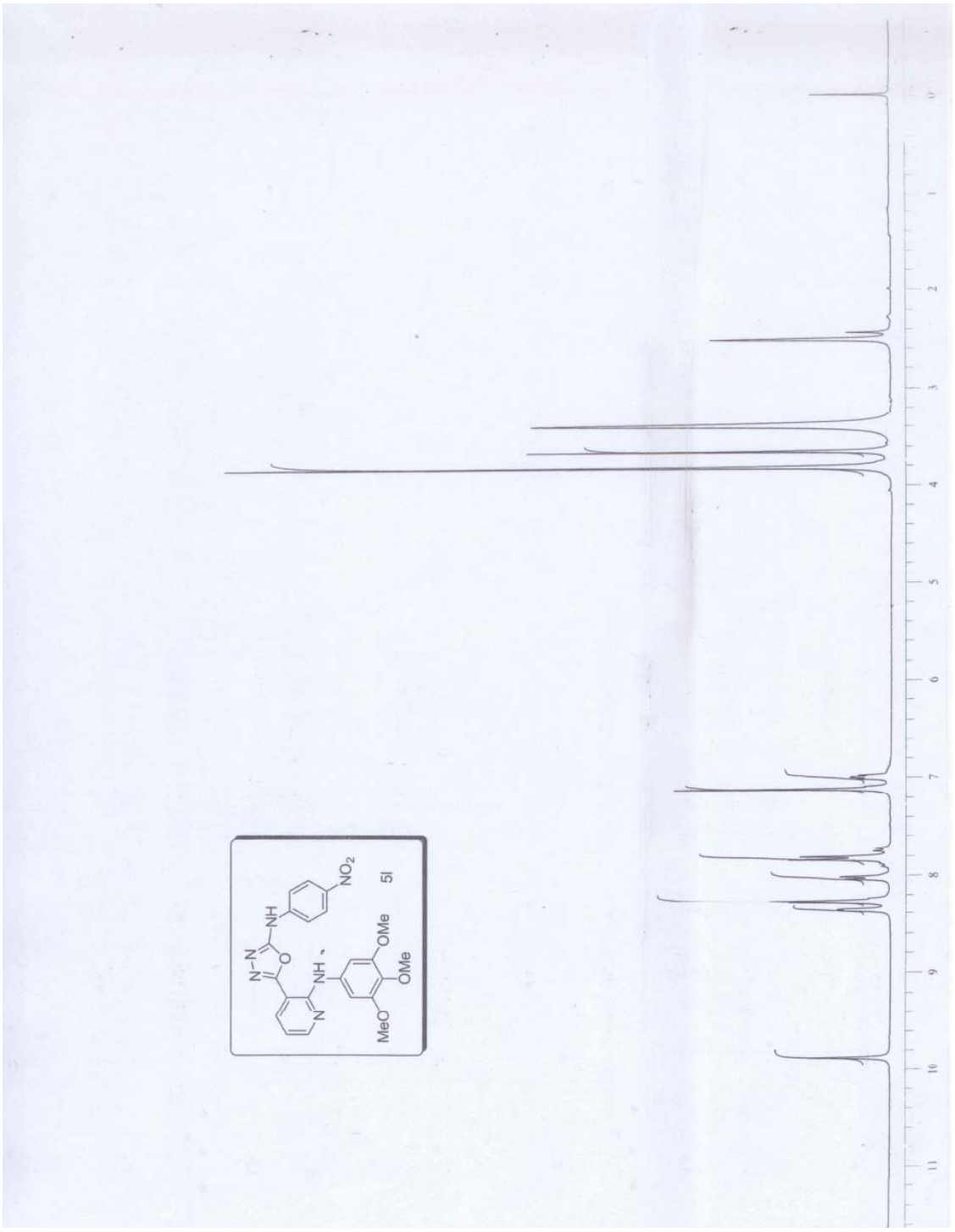
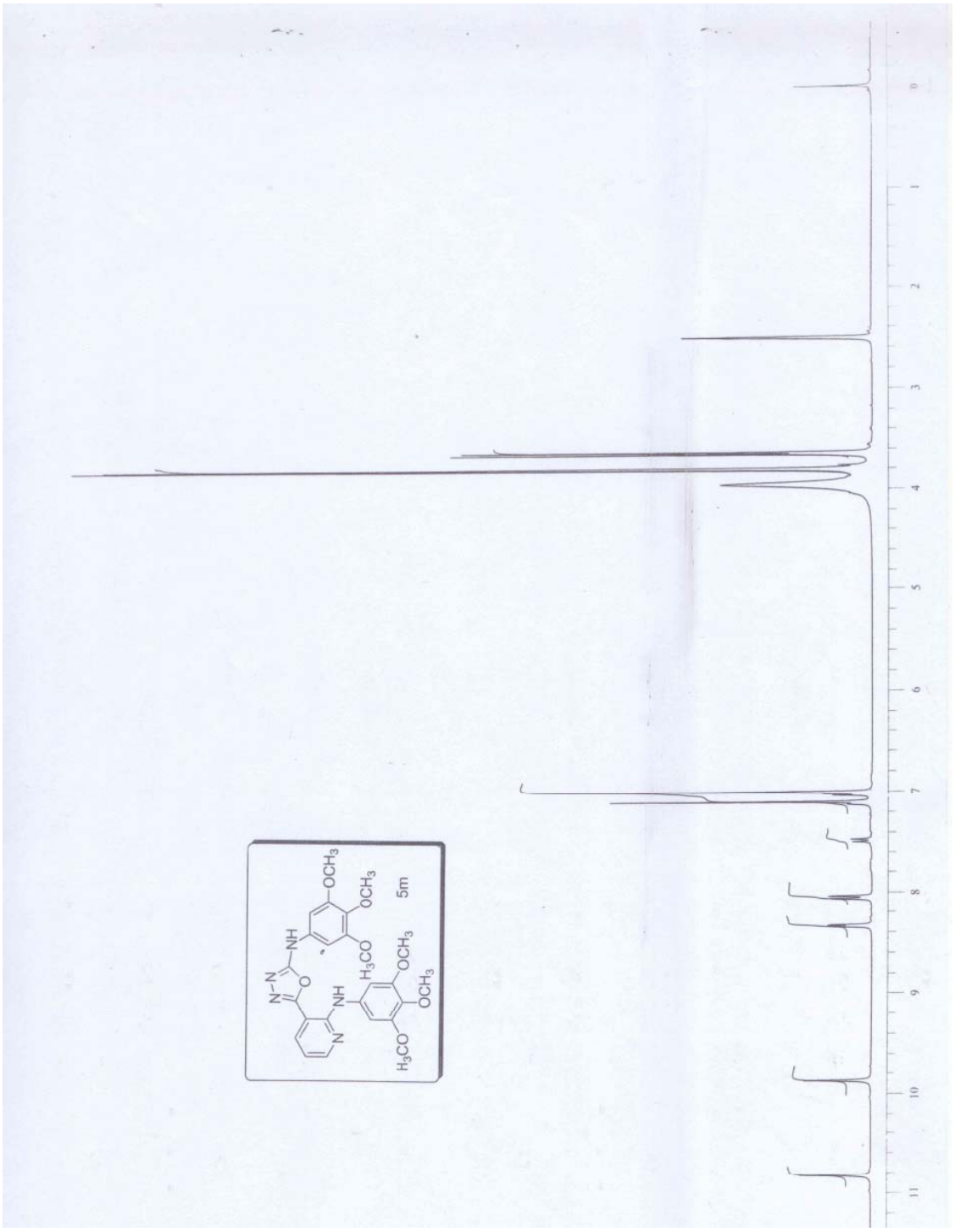
N
O
NH
N
OCH3
OCH3
H3CO


5k
Cl
NH
N
N
O
NH
N
OCH3
OCH3
H3CO


5l
NO2
NH
N
O
NH
OMe
OMe
MeO
N


OCH3
OCH3
5m
NH
H3CO
OCH3
N
N
O
NH
OCH3
N
H3CO