Supplementary Information

Design, Synthesis and biological evaluation of novel spiro[indole-pyridothiazine] analogs as antiproliferative agents

Kapil Arya,* Pooja Tomar and Jyoti Singh

[a]*Department of M.Tech. Chemical Synthesis and Process Technologies,*
University of Delhi, Delhi-110007, India
Email:aryakapil2001@yahoo.com

Experimental Section :

KB, HCT-8, MCF-7 Cell lines were cultured in DMEM with 10% FCS, CAKI-1 was cultured in Mc Coy's Medium with 10% FCS and A-549 was cultured in RPMI with 10% FCS and 1% v/v antibiotic. The proliferation of epiderimoid carcinoma of nasopharynx (KB), lung carcinoma (A-549), ileocecal carcinoma (HCT-8), renal cancer (CAKI-1) and breast cancer (MCF-7) cells can be assayed at different time intervals besides 24 h. DMSO was used for preparation of stock solutions (50 μM) of test compounds and stored at −20°C. These concentrated solutions were added immediately to cell culture walls on the day of experimentation. The final DMSO concentration was 0.1% on each well and it showed no interference with the biological activities tested. Tested samples at pre-set concentrations were added to 6 wells with 5-fluorouracil co-assayed as a positive reference. The absorbance was measured at 490 nm with a microtiter plate reader.

In vitro cell viability assay—MTT assay: The MTT assay followed established literatures[34-36]. 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyl tetrazolium bromide was used as an indicator of metabolically active cells. Known number of KB, HCT-8, MCF-7, CAKI-1 or A-549 cells were transferred into 96-well microtiter plates in a volume of 200 μl of culture medium and incubated for 48 h before addition of test compound. Cells were then exposed to known concentrations of the compound to be tested (100 μM expressed as final concentration) for 24 h at 37°C. After drug exposure, the culture medium was removed and 100 μl of MTT reagent (diluted in culture medium, 1 mg/ml) was added. After incubating for 4 h, the MTT/medium was removed and DMSO (100 μl) was added to dissolve the formazan crystals. Absorbance of the colored solution was measured on a microtiter plate reader using a test wavelength of 490 nm. The data represented the mean of three experiments in triplicate. Results were evaluated by comparing the absorbance of the walls containing compound treated cells with the absorbance of wells containing 0.1% DMSO alone (solvent control).

Synthesis

All of the chemicals used in the synthesis were purchased from Sigma-Aldrich and were used as received. Thin layer chromatography (Merck TLC silica gel 60) was used to monitor the progress of the reactions. The compounds were purified by silica gel column (60-120 mesh).Ultrasonication was performed in a GEX750-5C ultrasonic processor equipped with a 3 × 140 mm probe that was immersed directly into the reaction mixture. The operating frequency was 24 kHz and the output power was 0–750 W through manual adjustment. The temperature was controlled by a Buchi B-491 water bath at 25 ± 1 °C and reaction was carried out at 95 °C. IR spectra (KBr) were recorded on a Shimadzu FT IR-8400S spectrophotometer and ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-400, at 400 and 100 MHz respectively, using TMS as in internal standard. High-resolution mass spectra (HRMS) were obtained on a Finnigan MAT 8200 system using sector double focus and an electron-impact source with an ionizing voltage of 70 eV. DIPMS (direct insertion probe mass spectrum) values are reported in m/z. Elemental

analysis were performed on Carlo Erba Model EA-1108 elemental analyser. ZSM-5 zeolite was obtained from Zeolyst International, The Netherlands, having SiO_2/Al_2O_3 ratio 50 with BET surface area 452 m^2 g^{-1} and pore volume 0.38 cm^3 g^{-1}. Isatin derivatives[37] and Brønsted acidic ionic liquid with different inorganic anions of the type BF_4^-, PF_6^-, $PTSA^-$ were prepared by method reported in literature.[38] Analytical high performance liquid chromatography (HPLC) was performed on Varian 210 and eluting with a hexane/*i*PrOH solution (**Figure 1-15**).

General procedure for the synthesis of spiro [indole-pyrido-thiazine]derivatives

All these compounds (**4a-o**) were prepared by literature method[17] and characterized spectroscopically. In a typical reaction conditions, An equimolar mixture (1.0 mmol) of N-substituted indole 2,3-dione (1), amine (2) and 2-mercaptonicotinic acid (3) was sonicated under ultrasound irradiation in the presence of a catalytic amount of ZSM-5- ($[MIM]^+BF_4^-$) (1.5 g) at 95 °C in H_2O (5.0 ml). The mixture was irradiated for the period indicated in Table 1. The completion of the reaction was monitored by TLC (n-hexane– EtOAc, 6 : 4). After the completion of the reaction, the flask was detached from the probe and the content was transferred into a beaker. The formed product was filtered and washed with water to afford the corresponding pure product **4**, with no need for further recrystallization. The pure products were characterized by spectral data (^{1}H NMR , ^{13}C NMR, HRMS and GC Mass). The spectral data for some products are given below.

1-Methyl-3'-(4-nitrophenyl) spiro [3H-indole-3,2'-[4H]pyrido[3,2-e]-1,3-thiazine]-2,4'(1H) diones] (**4k**) : IR (KBr): 2950-2870, 1715, 1680, 1620, 1430 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 2.55 (3H, s, CH_3), 8.60-7.75 (11H, m, Ar-H and pyridine H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): 34.3, 68.4, 109.3, 111.7, 115.8, 124.3, 131.4, 137.5, 142.8, 150.3, 155.6, 165.3, 172.8 ppm; EI-MS (m/z,%) : 418 $[M]^+$; Anal. Calcd. for $C_{21}H_{14}N_4O_4S$: C, 60.28; H, 3.37; N, 13.39; S,7.66. Found: C, 60.32; H, 3.38; N, 13.41; S, 7.66.

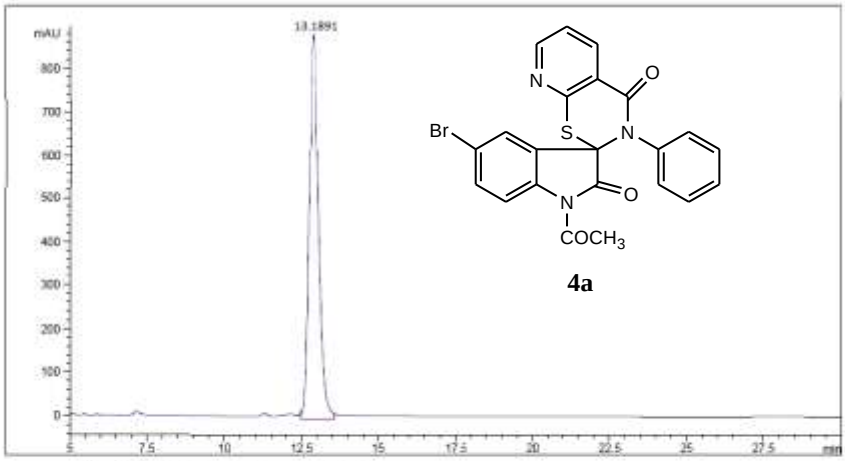
1-Acetyl-3'-(4-nitrophenyl) spiro [3H-indole-3,2'-[4H]pyrido[3,2-e]-1,3-thiazine]-2,4'(1H) diones] (**4l**) : IR (KBr): 2965-2880, 1730, 1710, 1680, 1620, 1420 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 2.76 (3H,s,$COCH_3$), 8.62-7.45 (11H, m, Ar-H and pyridine H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): 37.3,68.4, 104.5, 108.5, 112.7, 117.3, 126.9, 131.7, 139.3, 145.6, 154.9, 160.7, 168.5, 175.3 ppm; EI-MS (m/z,%) : 446$[M]^+$; Anal. Calcd. for $C_{22}H_{14}N_4O_5S$: C, 59.19; H, 3.16; N, 12.55; S,7.18. Found: C, 59.15; H, 3.15; N, 12.53; S, 7.19.

1-Methyl-5-bromo-3'-(4-chlorophenyl) spiro [3H-indole-3,2'-[4H]pyrido[3,2-e]-1,3-thiazine]-2,4'(1H) diones] (**4m**) : IR (KBr): 2940-2860, 1720, 1680, 1610, 780, 720 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 2.84 (3H, s, CH_3), 8.60-7.75 (10H, m, Ar-H and pyridine H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): 31.7, 65.6, 108.4, 112.4, 118.7, 125.3, 130.6, 136.2, 145.3, 149.6, 154.3, 168.7, 176.2 ppm; EI-MS (m/z,%) : 486 $[M+2]^+$, 484 $[M]^+$; Anal. Calcd. for $C_{21}H_{13}BrClN_3O_2S$: C, 51.82; H, 2.69; N, 8.63; S,6.59. Found: C, 51.85; H, 2.67; N, 8.64; S, 6.60.

1-Acetyl-5-bromo-3'-(4-chlorophenyl) spiro [3H-indole-3,2'-[4H]pyrido[3,2-e]-1,3-thiazine]-2,4'(1H) diones] (**4n**) : IR (KBr): 2960-2870, 1725, 1715, 1690, 1610, 770, 710 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 2.84 (3H,s,$COCH_3$), 8.56-7.52 (10H, m, Ar-H and pyridine H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): 35.2, 64.9, 105.3, 107.8, 113.4, 119.5, 123.2, 128.3, 134.6, 140.4, 147.2, 155.8, 161.3, 167.5, 174.6 ppm; EI-MS (m/z,%) : 514 $[M+2]^+$, 512 $[M]^+$; Anal. Calcd. for $C_{22}H_{13}BrClN_3O_3S$: C, 51.33; H, 2.55; N, 8.16; S,6.23. Found: C, 51.36; H, 2.56; N, 8.15; S, 6.22.

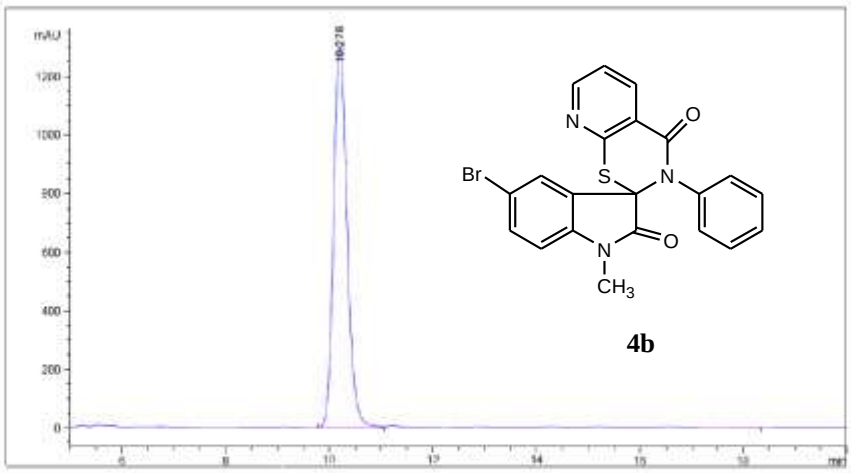
1-Methyl-3'-(4-bromophenyl) spiro [3H-indole-3,2'-[4H]pyrido[3,2-e]-1,3-thiazine]-2,4'(1H) diones] (**4o**) : IR (KBr): 2955-2870, 1715, 1670, 1620, 710 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 2.80 (3H, s, CH_3), 8.66-7.84 (11H, m, Ar-H and pyridine H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): 37.2, 71.3, 107.4, 111.3, 117.2, 123.6, 131.9, 138.7, 142.3, 150.4, 155.7, 166.9, 173.7 ppm; EI-MS (m/z,%) : 453 $[M+2]^+$, 451 $[M]^+$; Anal. Calcd. for $C_{21}H_{14}BrN_3O_2S$: C, 55.76; H, 3.12; N, 9.29; S,7.09. Found: C, 55.79; H, 3.13; N, 9.28; S, 7.10.

HPLC Chromatogram of Synthesized compounds (4a-o)



4a

Peak #	RetTime [min]	Area [mAU*s]	Area %
1	13.1891	1.85684	100.0000
Totals :		1.85684	100.0000



4b

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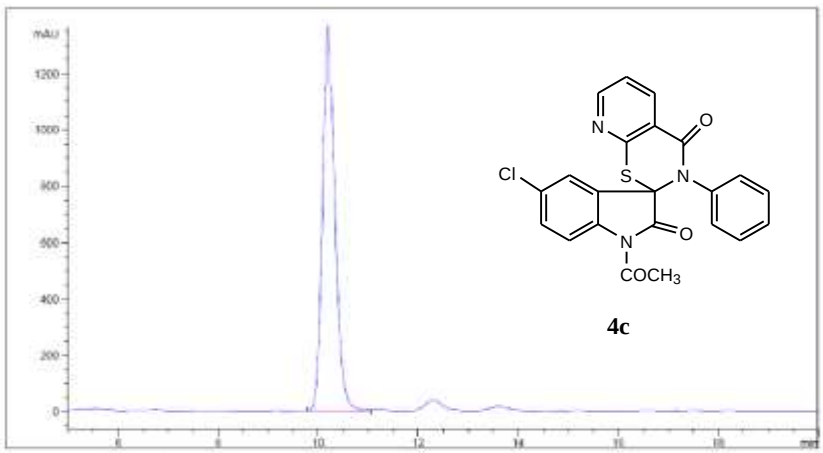
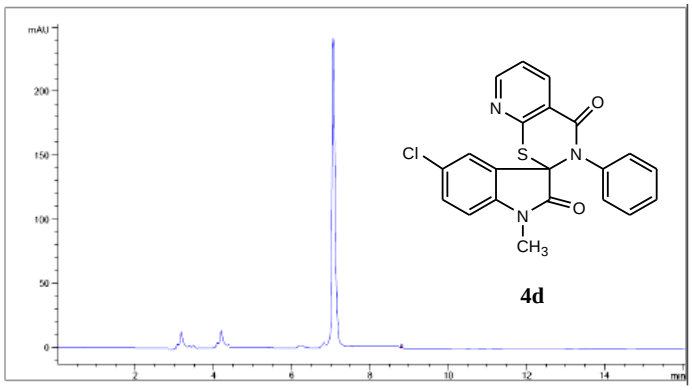
1	10.278	2.35027e4	100.0



4c

Peak #	RetTime [min]	Area [mAU*s]	Area %
1	10.2745	1.37472	99.999



4d

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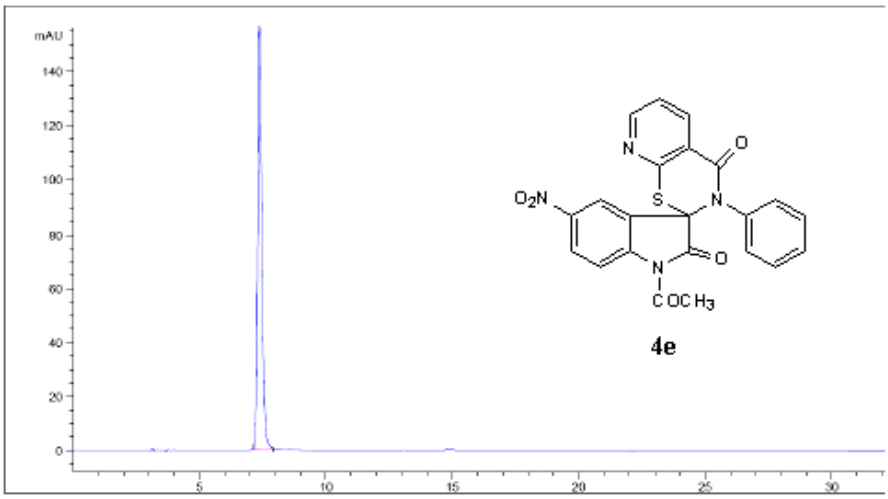
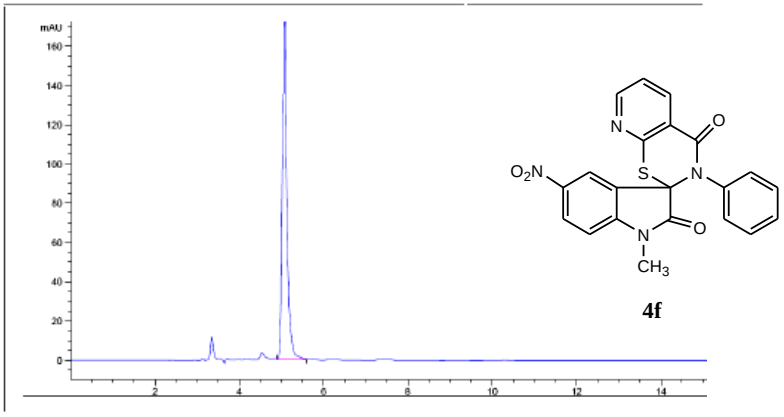
Totals :		2070.4654	100.0000



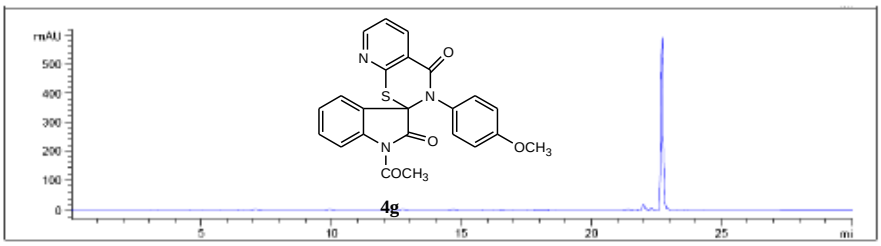
4e

Peak #	RetTime [min]	Area [mAU*s]	Area %
1	7.4176	1710.4193	100.0000
Totals :		1710.4193	



4f

Peak #	RetTime [min]	Area [mAU*s]	Area %
1	5.041	1217.46675	100.0000



4g

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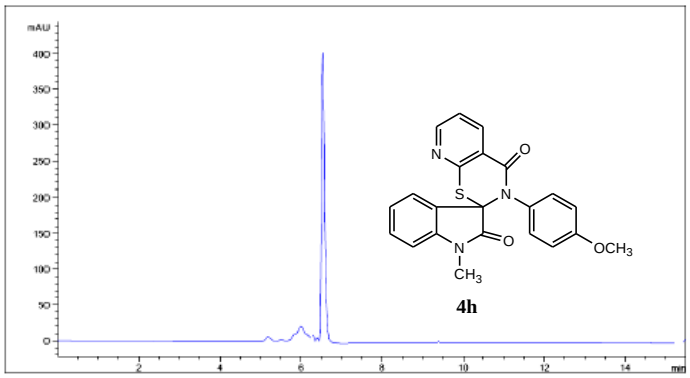
1	21.466	4810.79355	100.0000



4h

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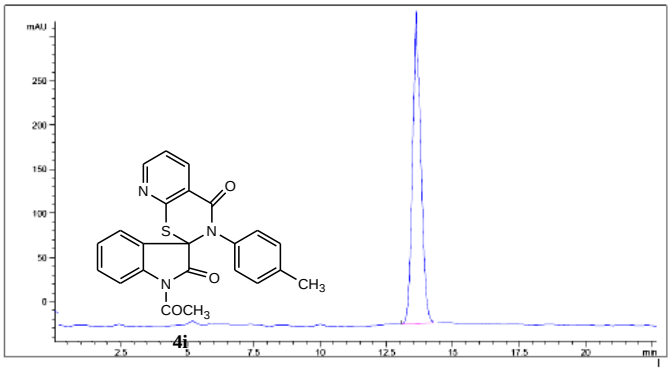
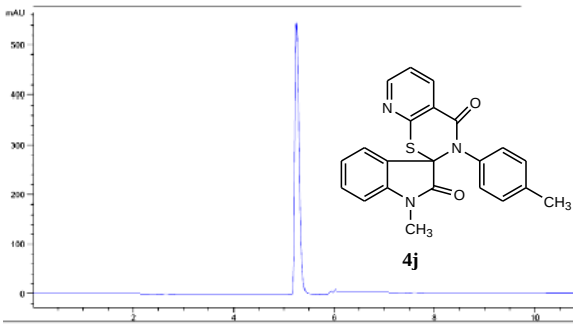


4i

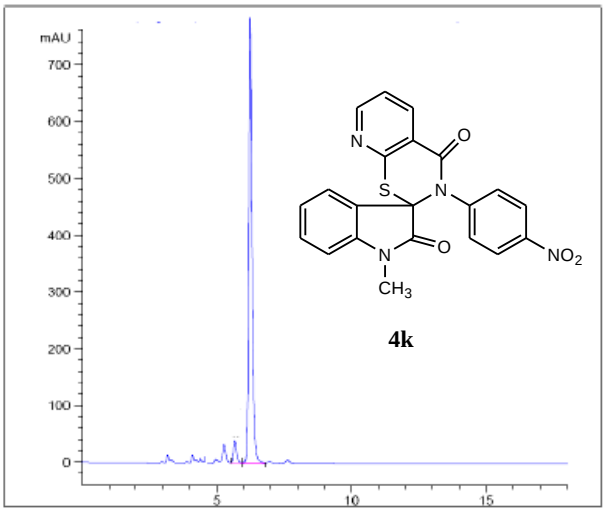
Peak #	RetTime [min]	Area [mAU*s]	Area %
1	13.118	6773.679	100.0000

Totals : 6773.679 100.0000



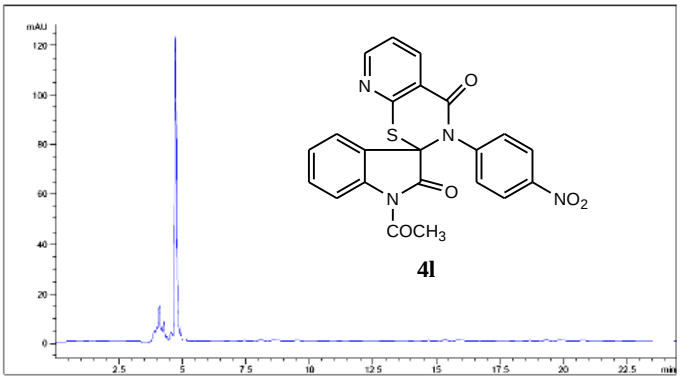
4j

Peak #	RetTime [min]	Area [mAU*s]	Area %
1	5.4118	2006.427	100.00



4k

Peak #	RetTime [min]	Area [mAU*s]	Area %
1	6.279	669.79126	100.000



4l

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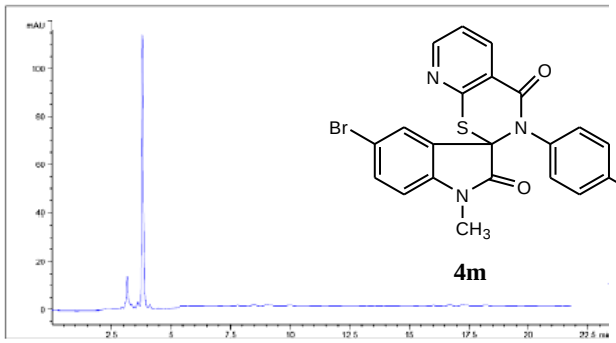
1	4.629	973.3978	100.0



4m

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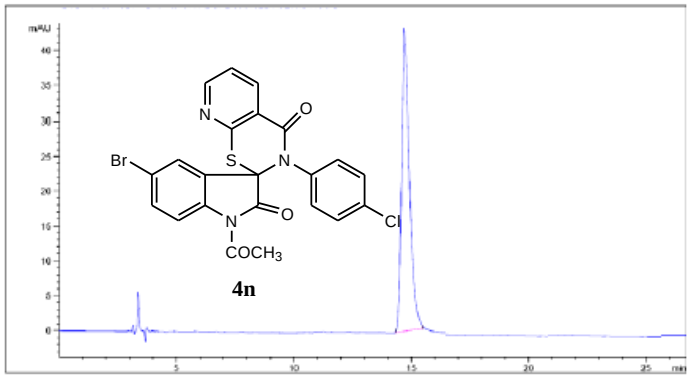
1	13.119	429.9970	100.00



4n

Peak #	RetTime [min]	Area [mAU*s]	Area %
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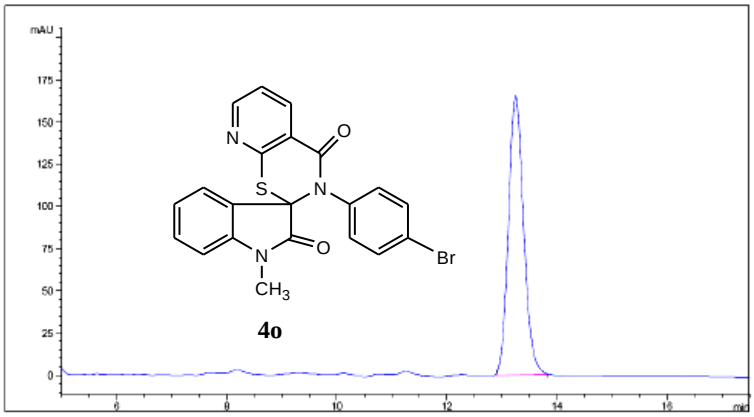

4o

Peak #	RetTime [min]	Area [mAU*s]	Area %
1	13.321	3175.3918	100.0000