Spiro Thiochromene-Oxindoles as novel Anti-Inflammatory Agents: Design, sustainable synthesis, in-vitro and in-silico evaluations.

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Supporting Information

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Chemistry

General information

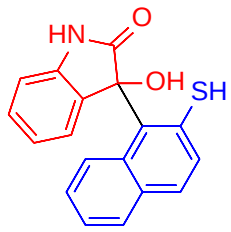
All 1,3-diketones, isatin/5-Bromoisatin, and 2-naphthalenethiol were procured from commercial suppliers and used without further purification. The progress of the reaction was monitored by Thin Layer Chromatography (TLC) on Merck-made silica gel 60-F-254 aluminum plates. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol resonance -400 instrument using DMSO-d6 as solvent and a Bruker resonance-500 instrument using $CDCl_3$ as solvent. The melting points were determined in open capillary tubes and were uncorrected. FTIR spectra were recorded using a KBr disc on a Bruker ALPHA analyzer. Mass spectra were recorded on a Xevo G2-S Q Tof (waters, USA) Mass spectrometer (direct mass ESI-APCI). The purity of all final compounds (≥95%) was established by High Performance Liquid Chromatography (HPLC).

General procedure for the synthesis of intermediate 2’

In a 25 mL round-bottomed flask, a mixture of Isatin (1.0 mmol) and 2-naphthalenethiol (1.0 mmol) was refluxed using water as the solvent (5 mL) for 4 hours. The progression of the reaction was monitored by TLC (Ethylacetate/hexane 2:8). After completion of the reaction,

10 mL water was added, and the solid product was isolated by filtration. Subsequently, the product was washed with water many times and dried.

3-Hydroxy-3-(2-mercaptonaphthalen-1-yl)indolin-2-one (2')

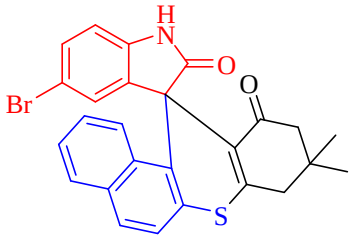


The title compound was prepared following the general procedure using Isatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and providing a cream color solid. Yield: 0.2895g, 94%; R_f = 0.680 (Ethylacetate/hexane 2:8) M.P.: 150-152ºC ^{1}H-NMR (400 MHz, $CDCl_3$ /TMS, δ PPM): 6.99-8.43 (m, -NH, 10H aromatic hydrogens), 4.88 (s, 1H), 4.34 (s, 1H); ^{13}C {^{1}H} NMR (400 MHz, $CDCL_3$): δ 170.8 147.9 136.8 132.6 130.6 129.7 128.8 127.7 126.4 125.1 124.6 123.9 120.4 112.5 109.5 85.2; HRMS (ESI) m/z :[M+H]+ calcd for $C_{18}H_{13}NO_2S+H^+$ 308.0701 found 308.0712.

General procedure for the synthesis of compounds 4 (a-l)

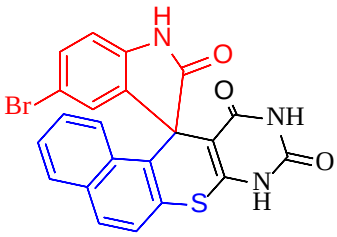
In a 25 mL round-bottomed flask, a mixture of Isatin/5-bromoisatin (1.0 mmol), 2-naphthalenethiol (1.0 mmol), and 1,3-diketones (1.0 mmol) was refluxed using water as the solvent (5mL) for the indicated time. The progression of the reaction was monitored by TLC (Ethylacetate/hexane 2:8). After completion of the reaction, an additional 10 mL of water was added, and the solid product was isolated by filtration. Subsequently, the product was washed with water many times. The pure product (racemic mixture) was thus, obtained after drying and did not require further purification steps or the addition of any organic solvent.

5'-Bromo-9,9-dimethyl-9,10-dihydrospiro[benzo[a]thioxanthene-12,3'-indoline]-2',11(8H)-dione (4a)



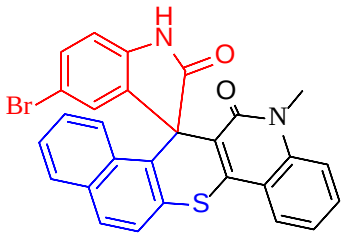
The title compound was prepared following the general procedure using 5-bromoisatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and dimedone (1.0 mmol, 0.1400g) providing cream color solid. Yield: 0.4508g, 92%; R_f = 0.706 (Ethylacetate/hexane 2:8) M.P.: 160-162ºC IR (ν cm^{-1} KBr): 1716 (C=O group), 1582 (-CONH group), 1242 (C-S-C cyclic thioether) ^{1}H-NMR (400 MHz, $CDCl_3$ /TMS, δ PPM): 6.73-7.99 (m, -NH, 9H aromatic hydrogens), 2.46-2.57 (s ,2H), 2.15-2.27 (s, 2H), 1.06-1.12(s, 6H); ^{13}C {^{1}H} NMR (400 MHz, $CDCl_3$): δ 195.6 178.5 164.0 141.6 135.7 134.4 133.6 132.6 129.1 127.9 127.6 126.8 126.7 125.8 125.6 114.5 113.3 110.9 51.0 45.9 41.3 32.1 28.8 27.6; HRMS (ESI) m/z :[M+H]+ calcd for $C_{26}H_{20}BrNO_2S+H^+$ 490.0476 found 490.0484; Found 97.5% purity by HPLC. HPLC Condition: 1260 Infinity II with VWD, Eclipse C18 column, 4.6 x 250 mm, 5 microns, CH_3CN/H_2O 70:30, λ = 254 nm.

5'-Bromospiro[benzo[5,6]thiochromeno[2,3-d]pyrimidine-12,3'-indoline]-2',9,11(8H,10H)-trione (4b)



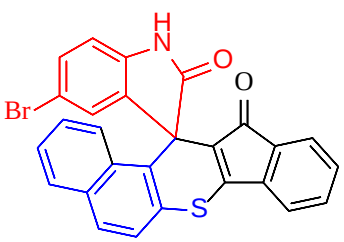
The title compound was prepared following the general procedure using 5-bromoisatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and barbituric acid (1.0 mmol, 0.1280g) providing violet color solid. Yield: 0.3918g, 82%; R_f = 0.682 (Ethylacetate/hexane 2:8) M.P.: 124-126ºC IR (ν cm^{-1} KBr): 1575 (-CONH group), 1258 (C-S-C cyclic thioether) ^{1}H-NMR (500 MHz, $CDCl_3$ /TMS, δ PPM): 7.44-8.00 (m, 3H, -NH, 10H aromatic hydrogens); ^{13}C {^{1}H} NMR (500 MHz, $CDCl_3$): δ 170.9 162.2 150.4 141.9 134.4 133.6 132.6 129.1 127.9 127.6 126.8 126.7 126.3 125.8 118.2 110.5 99.5 58.1; HRMS (ESI) m/z :[M +H]+ calcd for $C_{22}H_{12}N_3O_3S+H^+$ 477.9861 found 477.9868; Found 100% purity by HPLC. HPLC Condition: 1260 Infinity II with VWD, Eclipse C18 column, 4.6 x 250 mm, 5 microns, CH_3CN/H_2O 70:30, λ = 254 nm.

5'-Bromo-5-methylspiro[benzo[5,6]thiochromeno[3,2-c]quinoline-7,3'-indoline]-2',6(5H)-dione (4c)



The title compound was prepared following the general procedure using 5-bromoisatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and 4-hydroxy-1-methyl-2(1H)-quinolone (1.0 mmol, 0.1751g) providing coral pink color solid. Yield: 0.3902g, 79%; R_f = 0.683(Ethylacetate/hexane 2:8) M.P.: 174-176ºC IR (ν cm^{-1} KBr): 1649 (-CONH group), 1247 (C-S-C cyclic thioether) ^{1}H-NMR (500 MHz, $CDCl_3$ /TMS, δ PPM): 7.45-7.98 (m, -NH, 13H aromatic hydrogens), 3.57 (s, 3H); ^{13}C {^{1}H} NMR (500 MHz, $CDCl_3$): δ 184.7 160.4 149.4 141.0 135.6 133.6 129.1 127.9 127.6 126.8 126.7 126.4 113.6 108.3 68.0 28.4; HRMS (ESI) m/z :[M+H]+ calcd for $C_{28}H_{17}BrN_2O_2S+H^+$ 525.0272 found 525.0278; Found 100% purity by HPLC. HPLC Condition: 1260 Infinity II with VWD, Eclipse C18 column, 4.6 x 250 mm, 5 microns, CH_3CN/H_2O 70:30, λ = 254 nm.

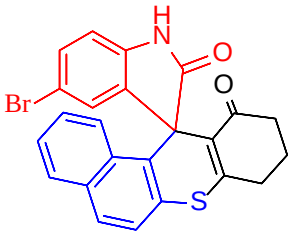
5'-Bromo-12H-spiro[benzo[f]indeno[1,2-b]thiochromene-13,3'-indoline]-2',12-dione (4d)



The title compound was prepared following the general procedure using 5-bromoisatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and 1,3-indanedione (1.0 mmol, 0.1461g) providing dark brown color solid. Yield: 0.4364g, 88%; R_f = 0.703

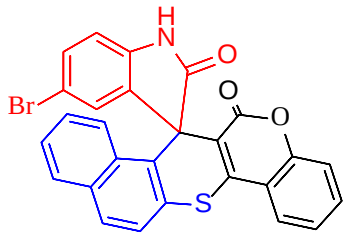
(Ethylacetate/hexane 2:8) M.P.: 162-164ºC IR (ν cm^{-1} KBr): 1722 (C=O group) 1674 (-CONH group), 1223 (C-S-C cyclic thioether) ^{1}H-NMR (500 MHz, $CDCl_3$ /TMS, δ PPM): 7.41-7.99 (m, -NH, 13H aromatic hydrogens); ^{13}C {^{1}H} NMR (500 MHz, $CDCl_3$): δ 186.4 172.6 154.1 140.9 134.4 133.6 133.2 129.1 128.0 127.9 127.6 127.3 126.8 126.7 125.8 113.8 46.1; HRMS (ESI) m/z :[M+H]+ calcd for $C_{27}H_{14}BrNO_2S+H^+$ 496.0007 found 496.0015; Found 100% purity by HPLC. HPLC Condition: 1260 Infinity II with VWD, Eclipse C18 column, 4.6 x 250 mm, 5 microns, CH_3CN/H_2O 70:30, λ = 254 nm.

5'-Bromo-9,10-dihydrospiro[benzo[a]thioxanthene-12,3'-indoline]-2',11(8H)-dione (4e)



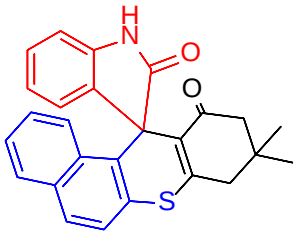
The title compound was prepared following the general procedure using 5-bromoisatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and 1,3-cyclohexanedione (1.0 mmol, 0.1120g) providing off-white color solid. Yield: 0.3334g, 72%; R_f = 0.683 (Ethylacetate/hexane 2:8) M.P.: 114-116ºC IR (ν cm^{-1} KBr): 1719 (C=O group) 1657 (-CONH group), 1246 (C-S-C cyclic thioether) ^{1}H-NMR (500 MHz, $CDCl_3$ /TMS, δ PPM): 7.45-7.98 (m, -NH, 9H aromatic hydrogens), 3.69 (2H) 2.74 (2H) 1.25 (2H); ^{13}C {^{1}H} NMR (500 MHz, $CDCl_3$): δ 195.4 180.3 162.5 155.5 140.9 137.1 134.4 133.6 132.6 129.8 129.1 127.9 127.6 126.8 126.7 126.3 125.8 119.3 115.1 106.0 44.1 28.2 26.7 18.5; HRMS (ESI) m/z :[M+H]+ calcd for $C_{24}H_{16}BrNO_2S+H^+$ 463.0242 found 463.0250; Found 99.5% purity by HPLC. HPLC Condition: 1260 Infinity II with VWD, Eclipse C18 column, 4.6 x 250 mm, 5 microns, CH_3CN/H_2O 70:30, λ = 254 nm.

5'-Bromo-6H-Spiro [benzo[5,6]thiochromeno[3,2-c]chromene-7,3'-indoline]-2',6-dione (4f)



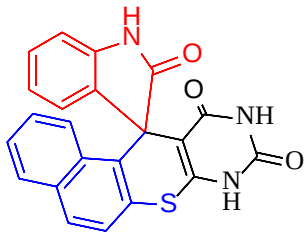
The title compound was prepared following the general procedure using 5-bromoisatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and 4-hydroxycoumarin (1.0 mmol, 0.1621g) providing rust brown color solid. Yield: 0.3898g, 76%; R_f = 0.706 (Ethylacetate/hexane 2:8) M.P.: 182-184ºC IR (ν cm^{-1} KBr): 1738 (-COO group), 1659 (-CONH group), 1219 (C-O-C cyclic ether) ^{1}H-NMR (500 MHz, $CDCl_3$ /TMS, δ PPM): 7.44-8.00 (m, -NH, 14H aromatic hydrogens); ^{13}C {^{1}H} NMR (500 MHz, $CDCl_3$): δ 175.6 164.9 144.3 134.4 133.6 132.6 129.1 127.9 127.6 126.8 126.3 125.8 118.2 117.5 115.0 102.5 64.8 ; HRMS (ESI) m/z :[M+H]+ calcd for $C_{27}H_{14}BrNO_3S+H^+$ 511.9956 found 511.9962; Found 100% purity by HPLC. HPLC Condition: 1260 Infinity II with VWD, Eclipse C18 column, 4.6 x 250 mm, 5 microns, CH3CN/H2O 70:30, λ = 254 nm.

9,9-Dimethyl-9,10-dihydrospiro[benzo[a]thioxanthene-12,3'-indoline]-2',11(8H)-dione (4g)



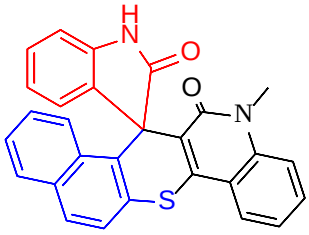
The title compound was prepared following the general procedure using Isatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and dimedone (1.0 mmol, 0.1400g) providing cream color solid. Yield: 0.3586g, 87%; R_f = 0.707 (Ethylacetate/hexane 2:8) M.P.: 152-154ºC IR (ν cm^{-1} KBr): 1711 (C=O group), 1592 (-CONH group), 1249 (C-S-C cyclic thioether) ^{1}H-NMR (400 MHz, $CDCl_3$ /TMS, δ PPM): 7.42-7.98 (m, -NH, 10H aromatic hydrogens), 3.73 (s, 2H), 2.05 (s, 2H), 1.26(s, 6H); ^{13}C {^{1}H} NMR (400 MHz, $CDCl_3$): δ 203.2, 182.2 173.8 167.4 134.3 133.5 132.6 129.1 127.9 127.6 126.3 125.7 58.6 48.0 37.2 32.0 29.8; HRMS (ESI) m/z :[M+H]+ calcd for $C_{26}H_{21}NO_2S+H^+$ 412.1371 found 412.1379; Found 100% purity by HPLC. HPLC Condition: 1260 Infinity II with VWD, Eclipse C18 column, 4.6 x 250 mm, 5 microns, CH_3CN/H_2O 70:30, λ = 254 nm.

Spiro[benzo[5,6]thiochromeno[2,3-d]pyrimidine-12,3'-indoline]-2',9,11(8H,10H)-trione (4h)



The title compound was prepared following the general procedure using Isatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and barbituric acid (1.0 mmol, 0.1280g) providing charcoal gray color solid. Yield: 0.3680g, 92%; R_f = 0.680 (Ethylacetate/hexane 2:8) M.P.: 104-106ºC IR (ν cm^{-1} KBr): 1575 (-CONH group), 1258 (C-S-C cyclic thioether) ^{1}H-NMR (500 MHz, $CDCl_3$ /TMS, δ PPM): 7.44-8.00 (m, 3H, -NH, 10H aromatic hydrogens); ^{13}C {^{1}H} NMR (500 MHz, $CDCl_3$): δ 168.0 159.1 142.9 138.6 134.4 133.6 129.1 128.5 127.6 127.3 126.4 126.1 125.8 60.3; HRMS (ESI) m/z :[M+H]+ calcd for $C_{22}H_{13}N3O_3S+H^+$ 400.0756 found 400.0762; Found 99.4% purity by HPLC. HPLC Condition: 1260 Infinity II with VWD, Eclipse C18 column, 4.6 x 250 mm, 5 microns, CH3CN/H2O 70:30, λ = 254 nm.

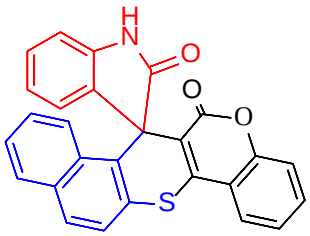
5-Methylspiro[benzo[5,6]thiochromeno[3,2-c]quinoline-7,3'-indoline]-2',6(5H)-dione (4i)



The title compound was prepared following the general procedure using Isatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and 4-hydroxy-1-methyl-2(1H)-quinolone (1.0 mmol, 0.1751g) providing beige color solid. Yield: 0.3901g, 87%; R_f = 0.705 (Ethylacetate/hexane 2:8) M.P.: 192-194ºC IR (ν cm^{-1} KBr): 1647 (-CONH group), 1252 (C-S-C cyclic thioether) ^{1}H-NMR (500 MHz, $CDCl_3$ /TMS, δ PPM): 7.46-7.98 (m, -NH, 14H aromatic hydrogens), 3.62 (s, 3H); ^{13}C {^{1}H} NMR (500 MHz, $CDCl_3$): δ 182.4 165.1 153.8

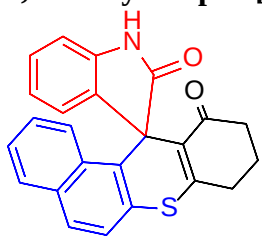
134.2 133.5 132.5 128.9 127.7 127.4 126.7 126.2 125.7 47.8 38.6; HRMS (ESI) m/z :[M+H]+ calcd for $C_{22}H_{13}N_3O_3S+H^+$ 447.1167 found 447.1171; Found 100% purity by HPLC. HPLC Condition: 1260 Infinity II with VWD, Eclipse C18 column, 4.6 x 250 mm, 5 microns, CH_3CN/H_2O 70:30, λ = 254 nm.

6H-spiro[benzo[5,6]thiochromeno[3,2-c]chromene-7,3'-indoline]-2',6-dione (4j)



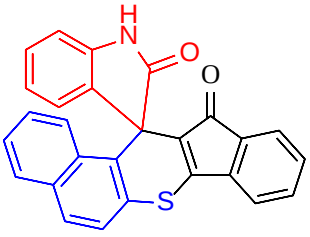
The title compound was prepared following the general procedure using Isatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and 4-hydroxycoumarin (1.0 mmol, 0.1621g) providing sand color solid. Yield: 0.3993g, 92%; R_f = 0.703 (Ethylacetate/hexane 2:8) M.P.: 152-154ºC IR (ν cm^{-1} KBr): 1734 (-COO group), 1652 (-CONH group), 1214 (C-O-C cyclic ether) ^{1}H-NMR (500 MHz, $CDCl_3$ /TMS, δ PPM): 7.40-7.99 (m, -NH, 14H aromatic hydrogens); ^{13}C {^{1}H} NMR (500 MHz, $CDCl_3$): δ 173.0 169.6 140.9 138.3 138.1 134.4 133.6 132.5 129.1 127.9 127.6 126.8 126.7 125.8 113.1 51.1; HRMS (ESI) m/z :[M+H] + calcd for $C_{27}H_{15}NO_3S+H^+$ 434.0851 found 434.0859; Found 100% purity by HPLC. HPLC Condition: 1260 Infinity II with VWD, Eclipse C18 column, 4.6 x 250 mm, 5 microns, CH_3CN/H_2O 70:30, λ = 254 nm.

9,10-Dihydrospiro[benzo[a]thioxanthene-12,3'-indoline]-2',11(8H)-dione (4k)



The title compound was prepared following the general procedure using Isatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and 1,3-cyclohexanedione (1.0 mmol, 0.1120g) providing black color solid. Yield: 0.3221g, 83%; R_f = 0.681 (Ethylacetate/hexane 2:8) M.P.: 172-174ºC IR (ν cm^{-1} KBr): 1718 (C=O group) 1654 (-CONH group), 1242 (C-S-C cyclic thioether) ^{1}H-NMR (500 MHz, $CDCl_3$ /TMS, δ PPM): 7.46-7.99 (m, -NH, 10H aromatic hydrogens), 2.76 (2H) 2.32 (2H) 2.02 (2H); ^{13}C {^{1}H} NMR (500 MHz, $CDCl_3$): δ 198.9 166.9 154.2 147.5 134.4 133.6 132.6 129.9 127.9 127.6 126.8 126.7 125.7 52.4 37.3 27.7 20.1; HRMS (ESI) m/z :[M+H]+ calcd for $C_{24}H_{17}NO_2S+H^+$ 384.1058 found 384.1064; Found 99.03% purity by HPLC. HPLC Condition: 1260 Infinity II with VWD, Eclipse C18 column, 4.6 x 250 mm, 5 microns, CH_3CN/H_2O 70:30, λ = 254 nm.

12H-spiro[benzo[f]indeno[1,2-b]thiochromene-13,3'-indoline]-2',12-dione (4l)

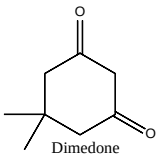
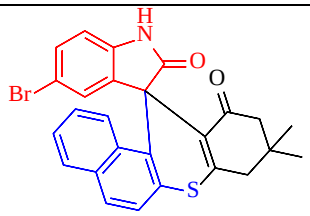
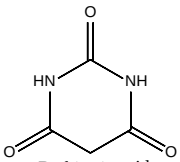
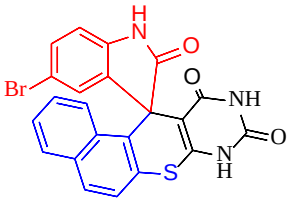
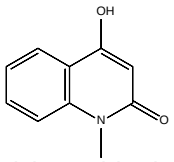
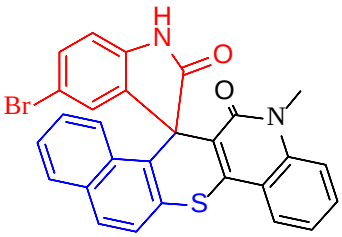
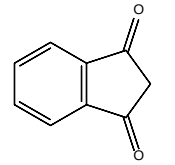
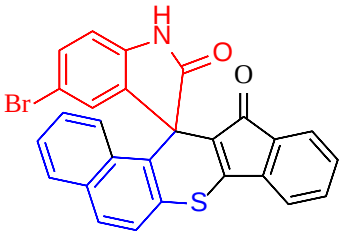


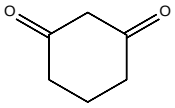
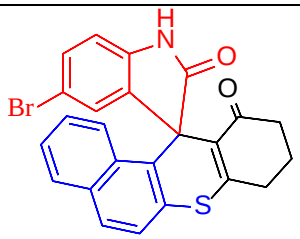
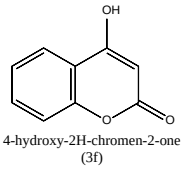
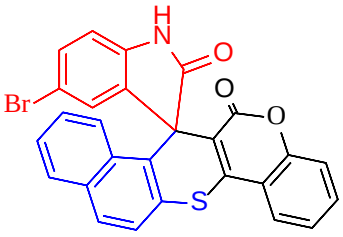
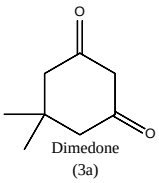
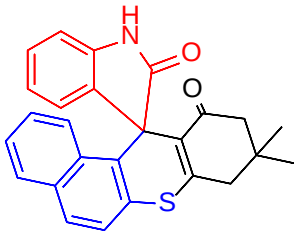
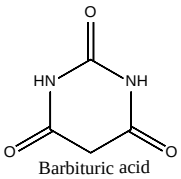
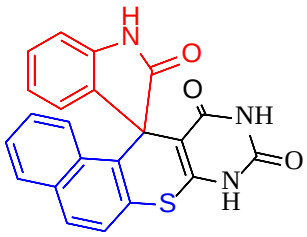
The title compound was prepared following the general procedure using Isatin (1.0 mmol, 0.1470 g), 2-naphthalenethiol (1.0 mmol, 0.1602g), and 1,3-indanedione (1.0 mmol, 0.1461g) providing charcoal color solid. Yield: 0.3762g, 90%; R_f = 0.701 (Ethylacetate/hexane 2:8)

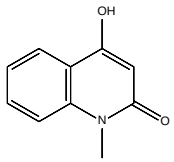
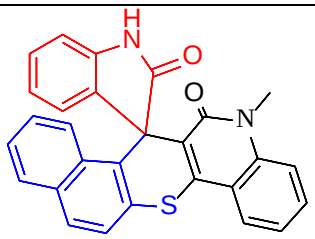
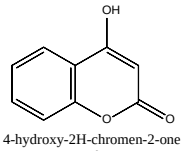
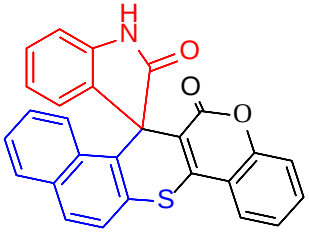
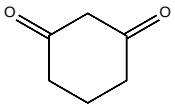
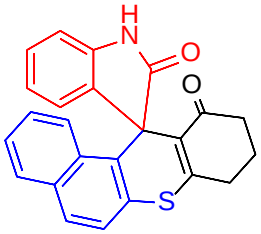
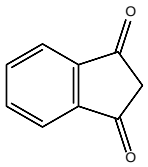
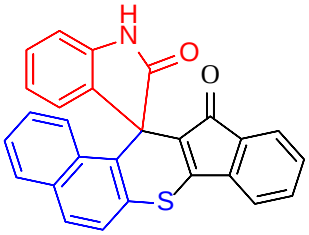
M.P.: 162-164°C IR (ν cm^{-1} KBr): 1716 (C=O group) 1667 (-CONH group), 1216 (C-S-C cyclic thioether) ^{1}H-NMR (500 MHz, $CDCl_3$ /TMS, δ PPM): 6.75-7.92 (m, -NH, 14H aromatic hydrogens); ^{13}C {^{1}H} NMR (500 MHz, $CDCl_3$): δ 187.9 175.9 160.7 142.5 136.1 136.0 135.7 134.4 133.6 133.2 132.6 129.6 129.1 127.9 127.9 127.6 126.8 126.7 125.8 123.5 123.3 110.3 52.4; HRMS (ESI) m/z :[M+H]+ calcd for $C_{27}H_{15}NO_2S+H^+$ 418.0902 found 418.0909; Found 100% purity by HPLC. HPLC Condition: 1260 Infinity II with VWD, Eclipse C18 column, 4.6 x 250 mm, 5 microns, CH_3CN/H_2O 70:30, λ = 254 nm.

Generalization of protocol, reaction conditions, and physical properties of compounds 4 (a-l)

Table 1. Generalization of protocol, reaction conditions[a], and physical properties of compounds 4 (a-l)

Compounds	1,3-dione	Products	Color	Time (hours)	Yield (%)[b]	M.P. (^{0}C)
4a	 Dimedone (3a)	 5'-bromo-9,9-dimethyl-9,10-dihydrospiro[benzo[*a*]thioxanthene-12,3'-indoline]-2',11(8*H*)-dione	Cream	7	92	160-162
4b	 Barbituric acid (3b)	 5'-bromospiro[benzo[5,6]thiochromeno[2,3-*d*]pyrimidine-12,3'-indoline]-2',9,11(8*H*,10*H*)-trione	Violet	6	82	124-126
4c	 4-hydroxy-1-methylquinolin-2(1H)-one (3c)	 5'-bromo-5-methylspiro[benzo[5,6]thiochromeno[3,2-*c*]quinoline-7,3'-indoline]-2',6(5*H*)-dione	Coral pink	6	79	174-176
4d	 1H-indene-1,3(2H)-dione (3d)	 5'-bromo-12*H*-spiro[benzo[*f*]indeno[1,2-*b*]thiochromene-13,3'-indoline]-2',12-dione	Dark brown	6	88	160-162

4e	 Cyclohexane-1,3-dione (3e)	 5'-bromo-9,10-dihydrospiro[benzo[^{a}]thioxanthene-12,3'-indoline]-2',11(8H)-dione	Off white	7	72	114-116
4f	 4-hydroxy-2H-chromen-2-one (3f)	 5'-bromo-6H^{-}spiro[benzo[5,6]thiochromeno[3,2-^{c}]chromene-7,3'-indoline]-2',6-dione	Rust brown	6	76	182-184
4g	 Dimedone (3a)	 9,9-dimethyl-9,10-dihydrospiro[benzo[^{a}]thioxanthene-12,3'-indoline]-2',11(8H)-dione	Cream	6	87	152-154
4h	 Barbituric acid (3b)	 spiro[benzo[5,6]thiochromeno[2,3-^{d}]pyrimidine-12,3'-indoline]-2',9,11(8H,10H)-trione	Charcoal gray	4.5	92	124-126

4i	 4-hydroxy-1-methylquinolin-2(1H)-one (3c)	 5-methylspiro[benzo[5,6]thiochromeno[3,2-*c*]quinoline-7,3'-indoline]-2',6(5*H*)-dione	Beige	5	87	192-194
4j	 4-hydroxy-2H-chromen-2-one (3f)	 6*H*-spiro[benzo[5,6]thiochromeno[3,2-*c*]chromene-7,3'-indoline]-2',6-dione	Sand	6	92	152-154
4k	 Cyclohexane-1,3-dione (3e)	 9,10-dihydrospiro[benzo[*a*]thioxanthene-12,3'-indoline]-2',11(8*H*)-dione	Black	6	83	172-174
4l	 1H-indene-1,3(2H)-dione (3d)	 12*H*-spiro[benzo[*f*]indeno[1,2-*b*]thiochromene-13,3'-indoline]-2',12-dione	Charcoal	6	90	162-164

[a]Reaction conditions: Isatin/5-bromoisatin (1.0 mmol), 2-naphthalenethiol (1.0 mmol), and 1,3-diketones (1.0 mmol) refluxed in 5 mL water using 28 mol% of taurine. [b]Isolated yields.

In-vitro anti-inflammatory activity

The impact of the sample on heat-induced bovine serum albumin (BSA) denaturation assay was assessed using a method outlined by Chandra et al.[1] with minor adjustments. The reaction mixtures comprised varying concentrations (50-250 µg/mL) of the sample and the reference drug diclofenac sodium, an NSAID, along with 1% w/v BSA and phosphate-buffered saline (PBS, pH 6.4) separately, while PBS served as the control. The reaction mixtures were

incubated at 37 °C for 20 min, followed by an increase in temperature to maintain the samples at 70 °C for 5 min. After cooling, turbidity was measured at 660 nm using a UV-visible spectrophotometer. The experiment was conducted in triplicate for precision. The control represents 100% protein denaturation. Diclofenac sodium was used as a control (Standard). The percentage inhibition of BSA denaturation was calculated as follows:

$$\%\ \boldsymbol{Inhibition\ of\ BSA\ denaturation} = \mathbf{100} * \left[\mathbf{1} - \left(\frac{\boldsymbol{A2}}{\boldsymbol{A1}}\right)\right]$$

Where A1 = absorbance of the control, and A2 = absorbance of the test sample.

The IC_{50} values were determined using the lowess curves and cubic spline curves to fit the data and were generated with GraphPad Prism software (version 10.3.1).

In silico studies for the anti-inflammatory activity

Molecular docking studies

Docking simulations were performed by using AutoDock VINA integrated into the PyRx 0.8 [2] virtual screening tool to identify compounds with high binding affinity. Insilco docking simulation studies to evaluate the molecular interactions of 4a-4l compounds were done with COX1 (PDB ID: 3KK6, co-crystallized ligand inhibitor: Celecoxib) [3], COX-2 (PDB ID:5KIR, co-crystallized with Rofecoxib). [4] Protein structure was processed to ensure an optimized structure for docking studies and it was executed with the UCSF Chimera Dock Prep module that includes the following steps: elimination of water molecules and other ligands, addition of missing atoms and residues, energy minimization and assigning charges and polar hydrogens and then converted to the pdbqt format. The 2D structure of the ligands was drawn with ChemDraw software and the structures were optimized through energy minimization with MMFF94 force field parameters and conjugate gradient algorithm using the Open Babel module of PyRx and eventually converted the ligands to the AutoDock compatible pdbqt format to carry out docking exploration. Post-docking analysis and visualization of binding poses and molecular interactions were done with BIOVIA Discovery Studio 2021 and Chimera X tools. [5] Autodock Vina grid box was created around the antagonist active site with the following details of the vina search space with the following coordinates (*Å*), X: -33.259, Y: 43.443, Z: -5.595 for COX-1, X: 23.606, Y: 1.782, Z: 34.5785 for COX-2. The validation process of the docking program involved the meticulous redocking of Celecoxib into its co-crystallized site. The remarkably low Root Mean Square Deviation (RMSD) value of 0.64 Å, observed between the docked and native poses, serves as a robust indicator of the program's efficacy in accurately predicting the binding pose of Celecoxib. This outcome underscores the program's proficiency in faithfully reproducing the spatial arrangement of Celecoxib within its intended binding site, affirming the reliability and precision of the docking algorithm.

Table 2. Molecular interaction summary of compounds with COX-1

Compounds	Binding Energy (K.cal/mol)	Interacting Amino acids*	Nature of interactions

4a	-6.4	**ASN515**, HIS95, PHE91, PRO514, HIS90, THR94, SER516, HIS513	H-bond, alkyl, π-alkyl, π-sulfur, van der waals
4b	-6.8	**ASN515**, PHE356, HIS95, TYR355, SER516, THR94, HIS90, GLY354, GLN192	H-bond, π-alkyl, π- π T shaped, π-π stacked, halogen, carbon-hydrogen bond, van der waals
4c	-6.9	**ASN515**, PHE356, HIS95, TYR355, PRO514, SER353, THR94, HIS513, HIS90, SER516, GLN912, GLY354, ARG97	H-bond, π-alkyl, π- π T shaped, π-π stacked, π-sigma, halogen, carbon-hydrogen bond, van der waals
4d	-7.1	**ASN515**, THR94, HIS95, PRO514, PHE91, SER516, GLY354, PHE356	H-bond, π-alkyl, π- π T shaped, π-sigma, carbon-hydrogen bond, van der waals
4e	-6.1	HIS513, PHE91, HIS95, PRO514, SER87, PHE88, HIS90, THR94, ASN515	alkyl, π-alkyl, π- π T shaped, π-π stacked, π-sulfur, carbon-hydrogen bond, van der waals
4f	-7.3	**GLY354**, PHE356, ASN515, THR94, GLN192, PRO191, SER516, HIS90, HIS513, PRO514, HIS95	H-bond, π- π T shaped, π-sigma, carbon-hydrogen bond, van der waals
4g	-6.7	**ASN515**, PRO514, PHE356, GLY96, ARG97, THR94, GLY354	H-bond, π-alkyl, van der waals
4h	-6.5	**ASN515**, PHE356, HIS95, SER516, THR94, TYR355, GLY354, GLN192	H-bond, π- π T shaped, π-π stacked, carbon-hydrogen bond, van der waals
4i	-7.7	**ASN515**, PHE356, PRO514, SER353, TYR355, GLY354, GLN192, THR94, SER516, HIS90, HIS513, HIS95	H-bond, π-alkyl, π- π T shaped, π-π stacked, π-sigma, amide-π stacked, carbon-hydrogen bond, van der waals
4j	-7.2	**ARG120**, TYR355, VAL349, MET113, VAL116, ILE523, ALA527, LEU531, ILE345, LEU534, LEU359, SER530, VAL344, LEU112, LEU357, ILE89	H-bond, π-alkyl, π- π T shaped, π-π stacked, π-sigma, π-sulfur, carbon-hydrogen bond, van der waals
4k	-6.8	**ASN515**, HIS581, PHE356, GLN351, GLY354, THR94, PRO191, GLN192, GLY193, ASP584	H-bond, π-alkyl, π- π T shaped, van der waals
4l	-7.3	**ASN515**, THR94, HIS95, PRO514, PHE356, PHE91, SER516, GLY354	H-bond, π-alkyl, π- π T shaped, π-sigma, carbon-hydrogen bond, van der waals

Celecoxib	-10.5	**ARG120**, **GLY526**, SER353, LEU352, ALA527, VAL349, TYR355, VAL116, ILE517, PHE518, ILE523, TRP387, PHE381, MET522, TYR385, PHE529, LEU384, SER530, LEU531, LEU359, HIS90, SER516, GLN192	H-bond, alkyl, π-alkyl, π-sulfur, π-sigma, amide-π stacked, carbon-hydrogen bond, van der waals

*H-bond forming residues colored in green.

Table 3. Molecular interaction summary of compounds with COX-2

Compounds	Binding Energy (K.cal/mol)	Interacting Amino acids*	Nature of interactions
4a	-7.9	**ARG120**, TYR355, GLY526, ALA527, LEU531, VAL349, LEU359, VAL523, LEU352, PHE518, SER353, ASN350, LEU534, VAL116, GLU524, ARG513, HIS90, ILE517, ALA516, GLN192, MET522	H-bond, alkyl, π-alkyl, π- π T shaped, amide- π stacked, carbon hydrogen bond, van der waals
4b	-8.4	**TYR355**, **SER353**, PHE518, MET522, LEU352, VAL349, LEU531, ALA527, LEU384, VAL523, TRP387, GLY526, ARG120, ARG513, HIS90, GLN192, ALA516, ILE517	H-bond, alkyl, π-alkyl, π- π T shaped, halogen, carbon hydrogen bond, van der waals
4c	-7.3	**HIS95**, PRO514, HIS90, ALA516, ASP515, TYR91, THR94, GLY354, GLN192, ARG513	H-bond, alkyl, π-alkyl, π-anion, carbon hydrogen bond, van der waals
4d	-6.9	HIS95, ASP515, PRO514, HIS356, PRO191, GLN192, GLY354, THR94, ALA516, HIS90, TYR91	π-alkyl, π-anion, π- π T shaped, van der waals
4e	-8.9	**ALA527**, **VAL523**, PHE518, VAL349, LEU531, LEU352, TYR355, LEU359, MET522, GLY526, GLU524, ARG120, PRO528, VAL116, ASN350, ARG513, HIS90, SER353, GLY354, GLN192, ALA516, ILE517, TRP387, TYR385, TYR348, LEU534	H-bond, alkyl, π-alkyl, π- sulphur, carbon hydrogen bond, van der waals
4f	-7.5	**ASP515**, HIS95, HIS356, LYS97, THR94, PHE96, PRO514, ALA516, GLN192, GLY354, PRO191	H-bond, π- π T shaped, van der waals
4g	-7.6	**GLY354**, ASP515, HIS95, PRO514, ALA516, TYR91, HIS356, THR94, HIS90, TYR355, SER353, ILE517, GLN192, PRO191	H-bond, π-alkyl, π-anion, π- π T shaped, van der waals
4h	-8.6	**ALA527**, **SER353**, LEU352, SER530, LEU531, VAL349, VAL523, PHE518, LEU534, TYR348, TYR385, TRP387, ILE517, GLN192, ALA516, HIS90, LEU359, ASN350, TYR355, VAL116, ARG120	H-bond, π-alkyl, π-sigma, π- sulphur, amide- π stacked, carbon hydrogen bond, van der waals

4i	-7.8	**ALA527**, TYR355, SER530, LEU352, PHE518, HIS90, LEU359, LEU531, VAL349, ALA516, VAL523, SER353, PRO528, ARG120, VAL116, GLY354, ARG513, GLN192, ILE517, TRP387, TYR348, TYR385, LEU534	H-bond, π-alkyl, π-sigma, π- sulphur, π- π T shaped, amide- π stacked, π-lone pair, carbon hydrogen bond, van der waals
4j	-7.9	**ALA527,** TYR355, SER530, PHE518, LEU352, ALA516, VAL349, LEU531, LEU359, SER353, VAL523, HIS90, GLN192, ILE517, TRP387, TYR385, TYR348, LEU534, ASN350, VAL116, ARG120	H-bond, π-alkyl, π-sigma, π- sulphur, π- π Tshaped, amide- π stacked, carbon hydrogen bond, van der waals
4k	-8.7	**ALA527**, **VAL523**, SER530, LEU531, VAL349, LEU352, PHE518, LEU534, TYR348, TYR385, TRP387, ILE517, GLN192, ALA516, HIS90, LEU359, GLY354, SRG513, SER353, TYR355, VAL116, ARG120, GLY526	H-bond, alkyl, π-alkyl, amide- π stacked, π-sulphur, carbon hydrogen bond, van der waals
4l	-7.6	**GLY354**, HIS95, ASP115, PRO514, ALA516, PRO191, GLN192, HIS356, THR94, TYR355, SER353, HIS90, ILE517, TYR91	H-bond, π-alkyl, π- π T shaped, π-anion, van der waals
Celecoxib	-11.6	**ARG513**, **LEU352**, **GLN192**, **PHE518**, **ILE517**, SER353, TRP387, TYR385, ALA527, LEU359, TYR355, LEU531, VAL116, VAL349, VAL523, HIS90, ARG120, ALA516, GLY354, MET522, GLY526, LEU384	H-bond, alkyl, π-alkyl, π-sigma, carbon hydrogen bond, van der waals

*H-bond forming residues colored in green.

ADMET studies

To evaluate the pharmacokinetic properties of our compounds, we performed an in-silico assessment using online servers such as pkCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction) [6] and SwissADME (http://www.swissadme.ch/). [7] These platforms analyze the pharmacokinetic profiles of top compounds, focusing on aspects such as water solubility, toxicity, metabolism, distribution, and absorption. They predict pharmacokinetic parameters based on the structural similarity of compounds with known ADMET features. These machine learning-based tools are highly regarded for their reliability and accuracy in ADMET prediction. SWISS ADME webserver had been used for the evaluation of physico-chemical and drug-likeliness properties of compounds which are found to be promising in the *in vitro* and *in-silico* assessments.

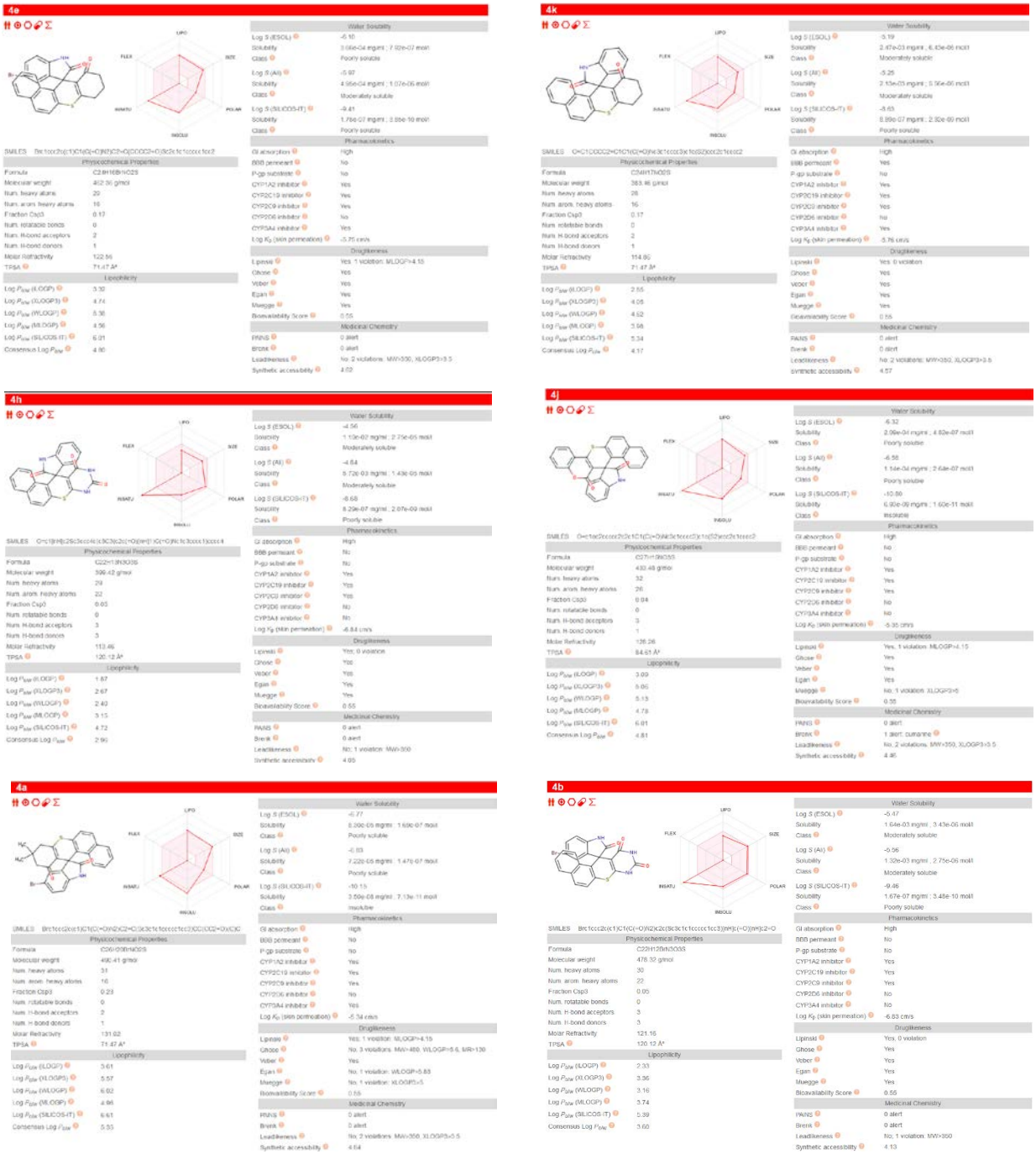
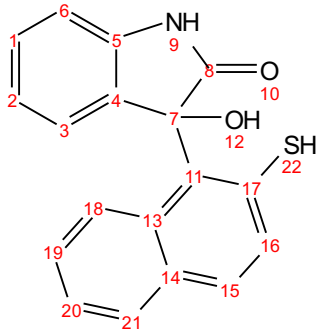


Figure 1. ADMET results for top compounds 4e, 4k, 4h, 4j, 4a, 4b.

^{1}H NMR, ^{13}C NMR, Mass Spectra, and HPLC traces of synthesized compounds.

16-12-2022.3.fid
manju- intermed -1H-CDCL3

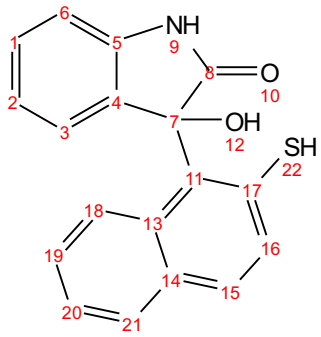
15



^{1}H NMR of 2' (500 MHz, $CDCl_3$)

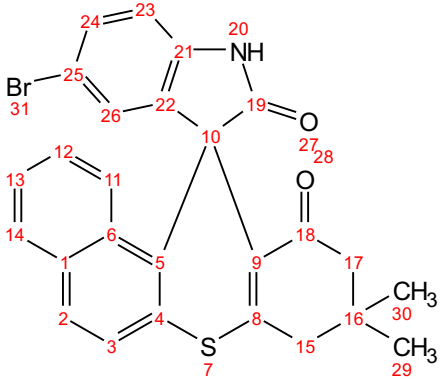
16-12-2022.4.fid

manju-- intermed -13c -CDCL3



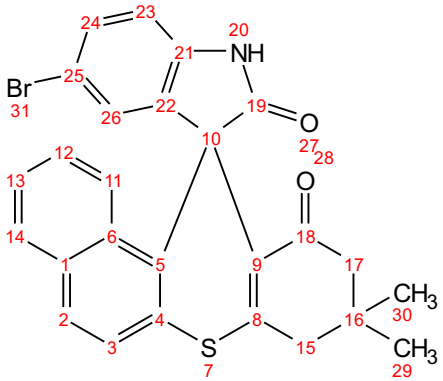
^{13}C {H} NMR of 2' (500 MHz, $CDCl_3$)

1H.9.fid
IT-8_1H_CDCl3_08-03-2023



^{1}H NMR of 4a (500 MHz, $CDCl_3$)

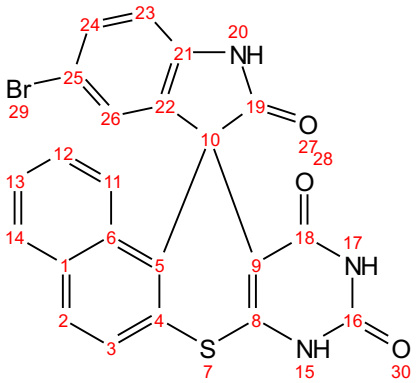
13C.10.fid
IT-8_13C_CDCl3_08-03-2023



^{13}C {H} NMR of 4a (500 MHz, $CDCl_3$)

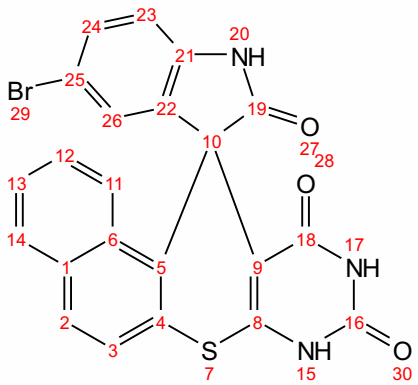
f1 (ppm)

1H.1.fid
IT-9_1H_CDCl3_09-03-2023



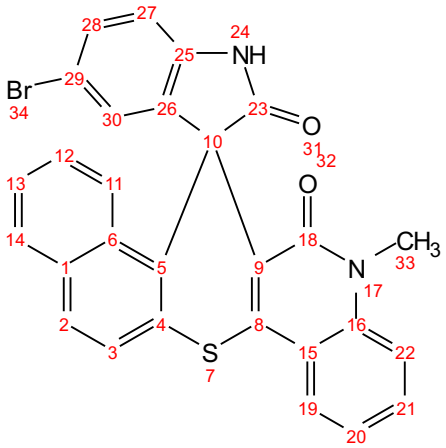
^{1}H NMR of 4b (500 MHz, $CDCl_3$)

13C.2.fid
IT-9_13C_CDCl3_10-12-2024



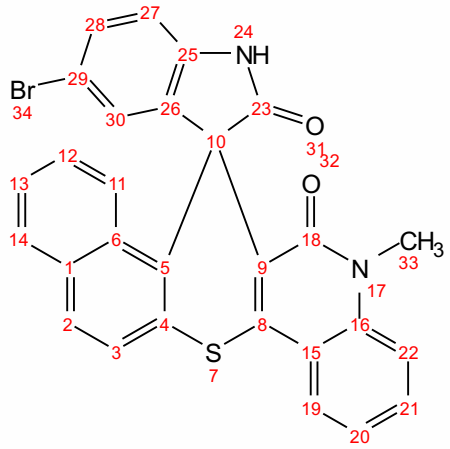
^{13}C {H} NMR of 4b (500 MHz, $CDCl_3$)

1H.3.fid
IT-10_1H_CDCl3_09-03-2023



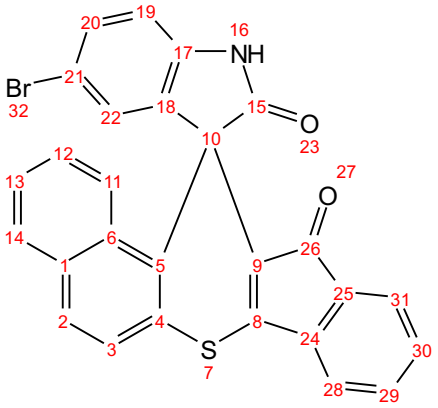
^{1}H NMR of 4c (500 MHz, $CDCl_3$)

13C.4.fid
IT-10_13C_CDCl3_10-12-2024



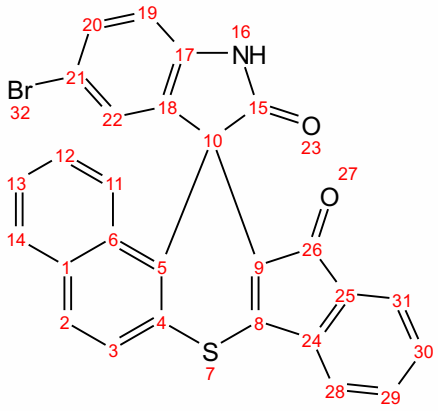
^{13}C {H} NMR of 4c (500 MHz, $CDCl_3$)

1H.5.fid
IT-11_1H_CDCl3_09-03-2023



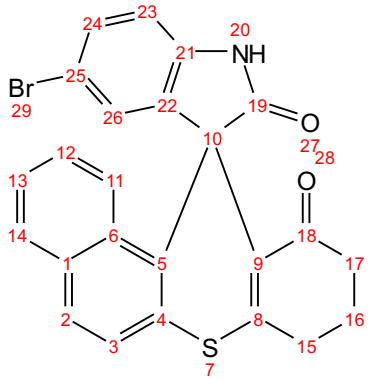
^{1}H NMR of 4d (500 MHz, $CDCl_3$)

13C.6.fid
IT-11_13C_CDCl3_10-12-2024



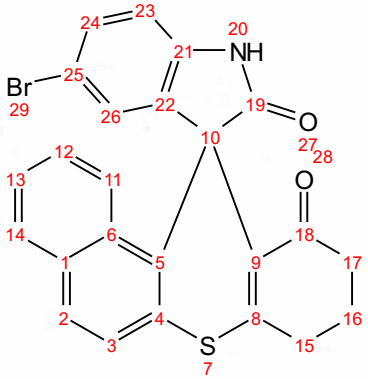
^{13}C {H} NMR of 4d (500 MHz, $CDCl_3$)

1H.7.fid
IT-12_1H_CDCl3_09-03-2023



^{1}H NMR of 4e (500 MHz, $CDCl_3$)

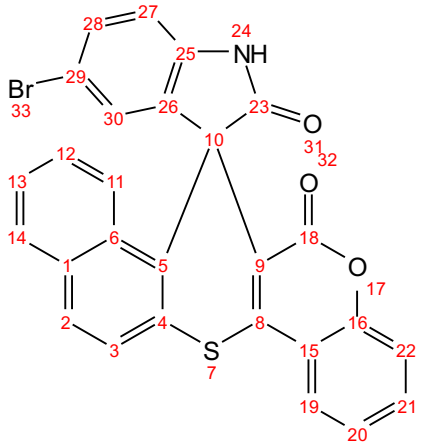
13C.8.fid
IT-12_13C_CDCl3_10-12-2024



^{13}C {H} NMR of 4e (500 MHz, $CDCl_3$)

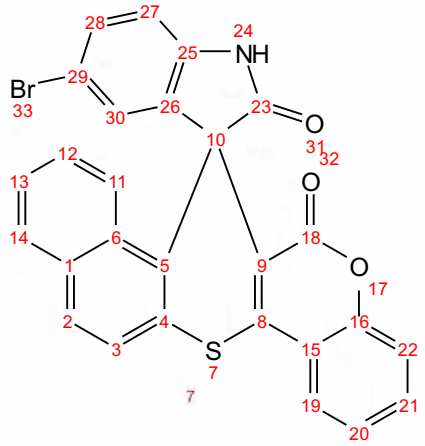
f1 (ppm)

1H.9.fid
IT-13_1H_CDCl3_09-03-2023



^{1}H NMR of 4f (500 MHz, $CDCl_3$)

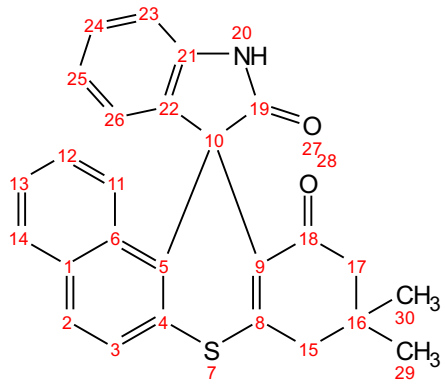
13C.10.fid
IT-13_13c_CDCl3_10-12-2024



^{13}C {H} NMR of 4f (500 MHz, $CDCl_3$)

f1 (ppm)

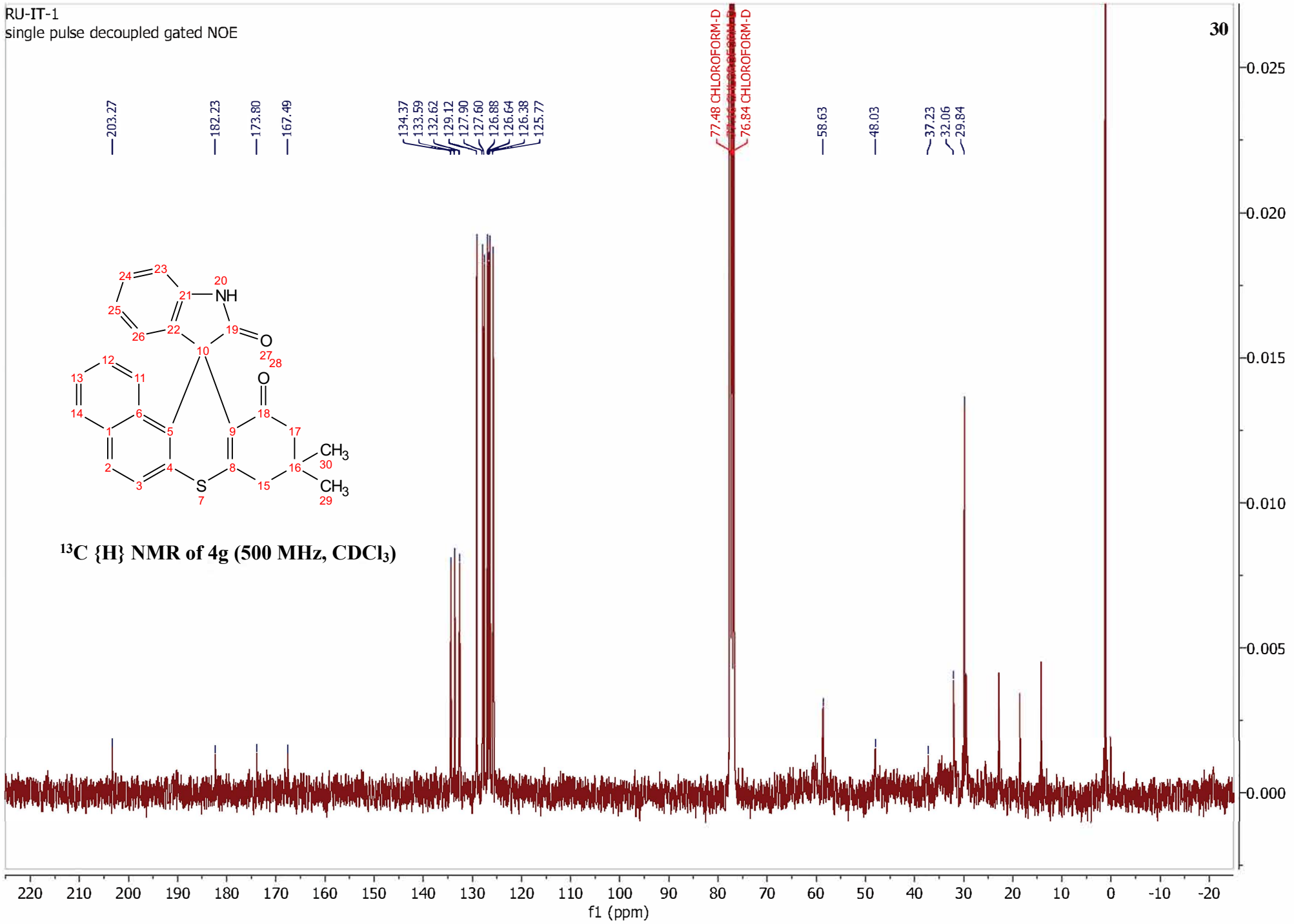
RU-IT-1
single_pulse



^{1}H NMR of 4g (500 MHz, $CDCl_3$)

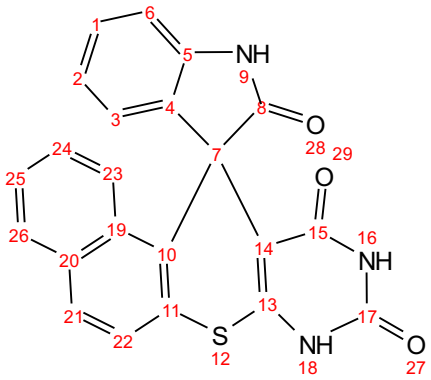
RU-IT-1
single pulse decoupled gated NOE

30



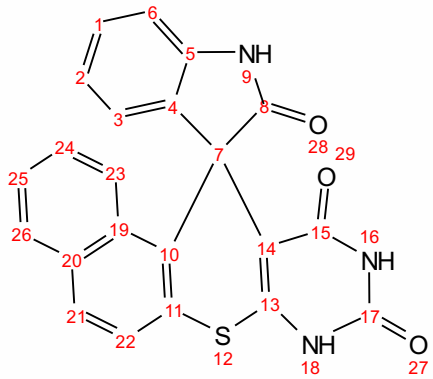
^{13}C {H} NMR of 4g (500 MHz, $CDCl_3$)

16-12-2022.1.fid
hj-sk-- it-2 -1H-CDCL3



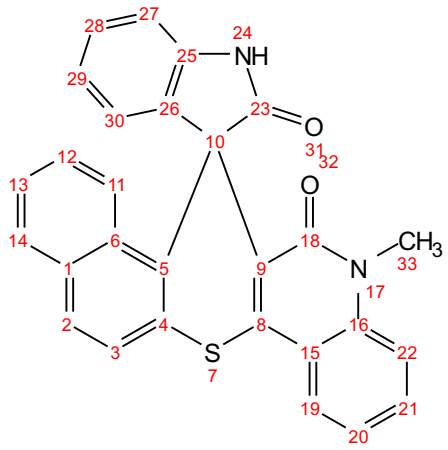
^{1}H NMR of 4h (500 MHz, $CDCl_3$)

16-12-2022.2.fid
hj-sk-- it-2 -13c H-CDCL3



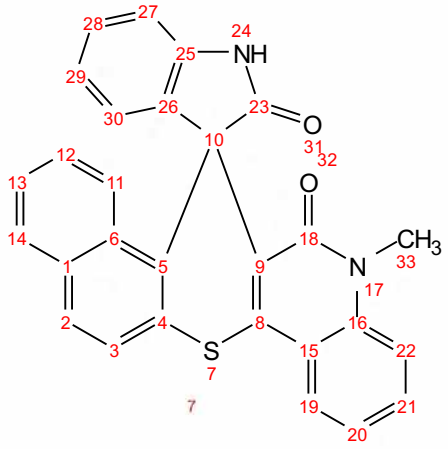
^{13}C {H} NMR of 4h (500 MHz, $CDCl_3$)

1H.1.fid
IT-3_1H_CDCl3_08-03-2023



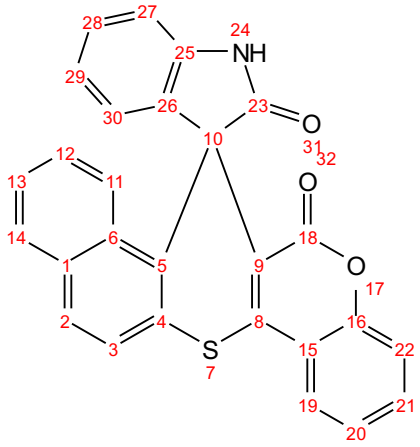
^{1}H NMR of 4i (500 MHz, $CDCl_3$)

13C.2.fid
IT-3_13C_CDCl3_10-12-2024



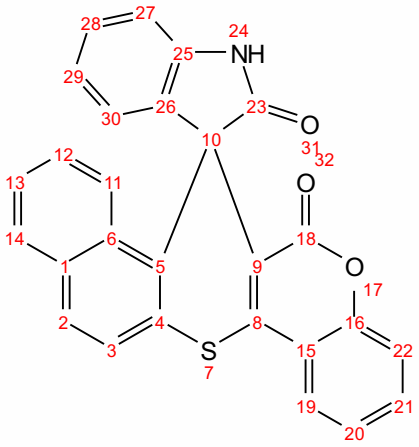
^{13}C {H} NMR of 4i (500 MHz, $CDCl_3$)

1H.4.fid
IT-5_1H_CDCl3_08-03-2023



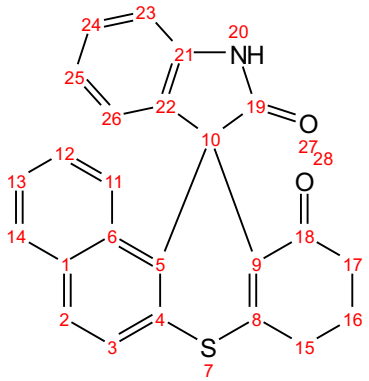
^{1}H NMR of 4j (500 MHz, $CDCl_3$)

13C.3.fid
IT-5_13C_CDCl3_10-12-2024



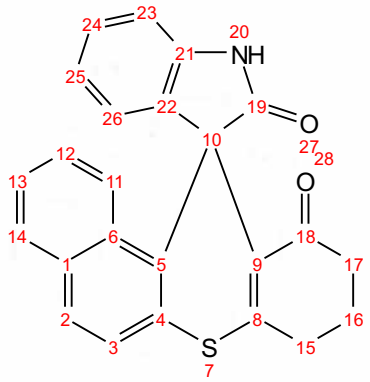
^{13}C {H} NMR of 4j (500 MHz, $CDCl_3$)

1H.5.fid
IT-6_1H_CDCl3_08-03-2023



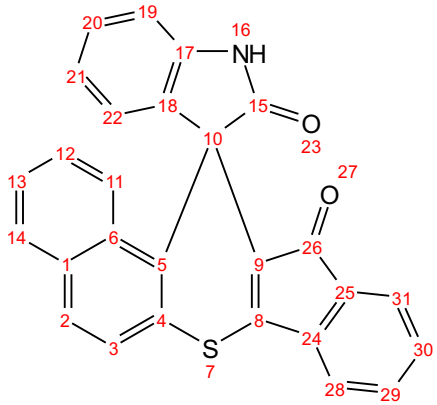
^{1}H NMR of 4k (500 MHz, $CDCl_3$)

13C.6.fid
IT-6_13C_CDCl3_10-12-2024



^{13}C {H} NMR of 4k (500 MHz, $CDCl_3$)

1H.7.fid
IT-7_1H_CDCl3_08-03-2023

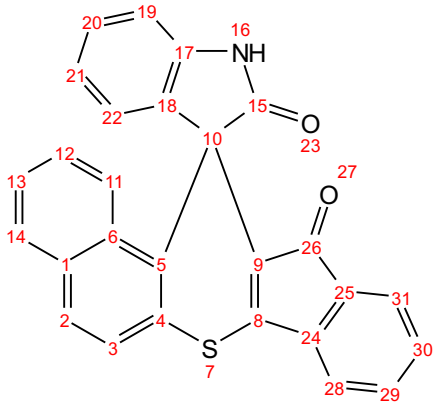


^{1}H NMR of 4l (500 MHz, $CDCl_3$)

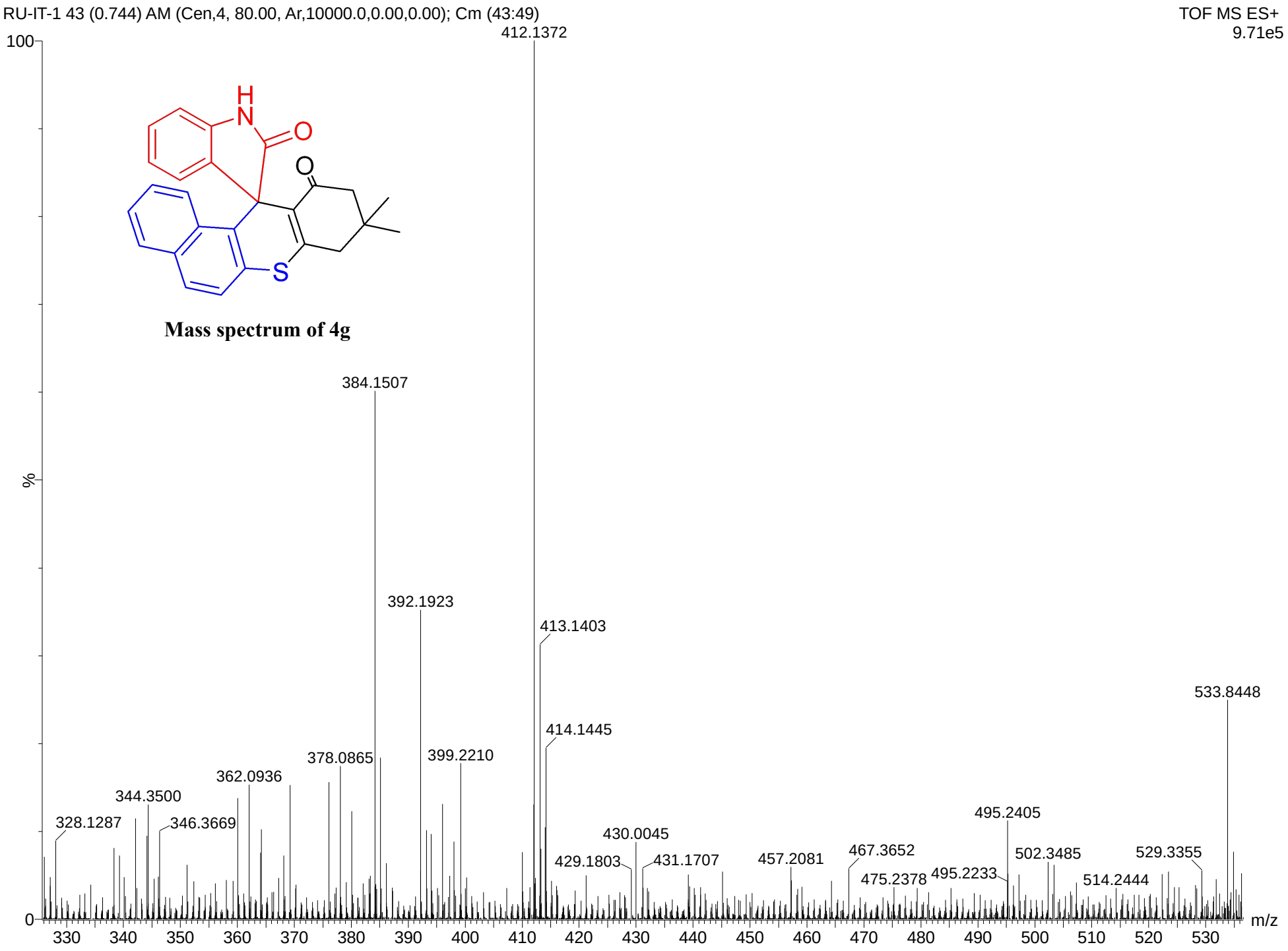
f1 (ppm)

13C.8.fid
IT-7_13C_CDCl3_08-03-2023

40



^{13}C {H} NMR of 4l (500 MHz, $CDCl_3$)

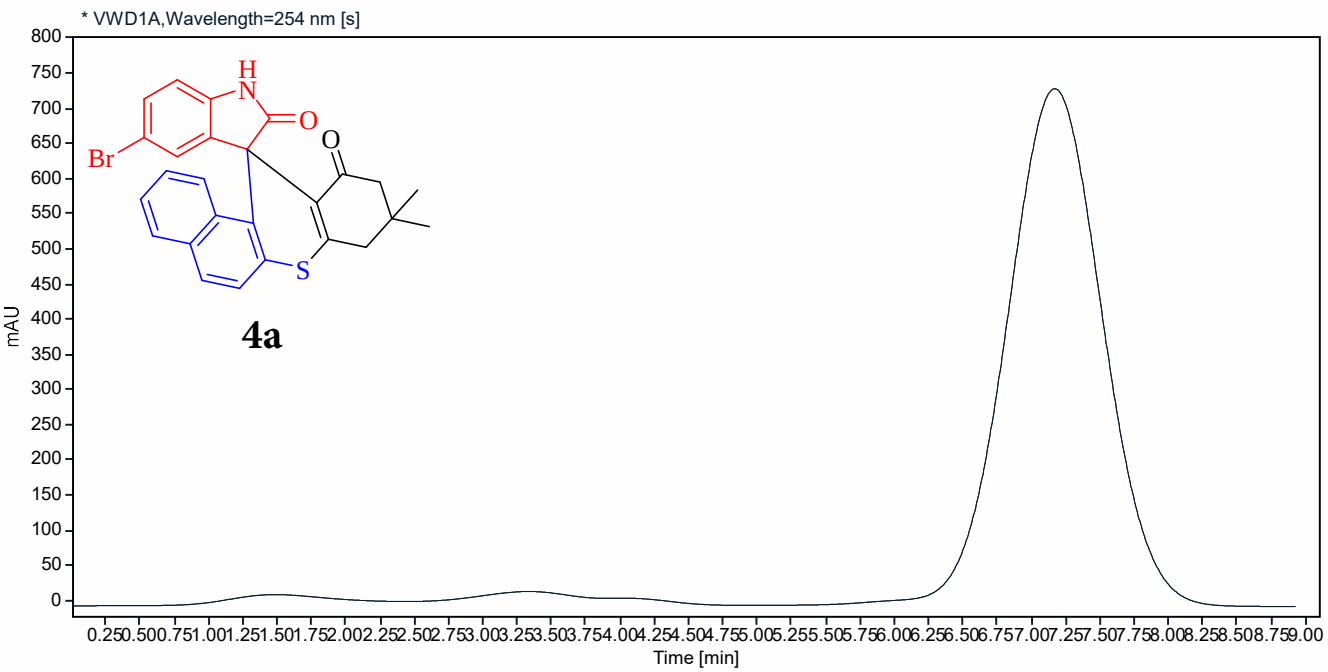


Mass spectrum of 4g

Single Injection Report

Agilent

Sequence Acquired Date	2024-10-03 12:34:56+05:30
Sample Name	4A
Injection Volume	15.000
Injection Acq Method Name	03-10-2024.amx
Sequence Name	SingleSample
Sample Vial Number	P1-A1



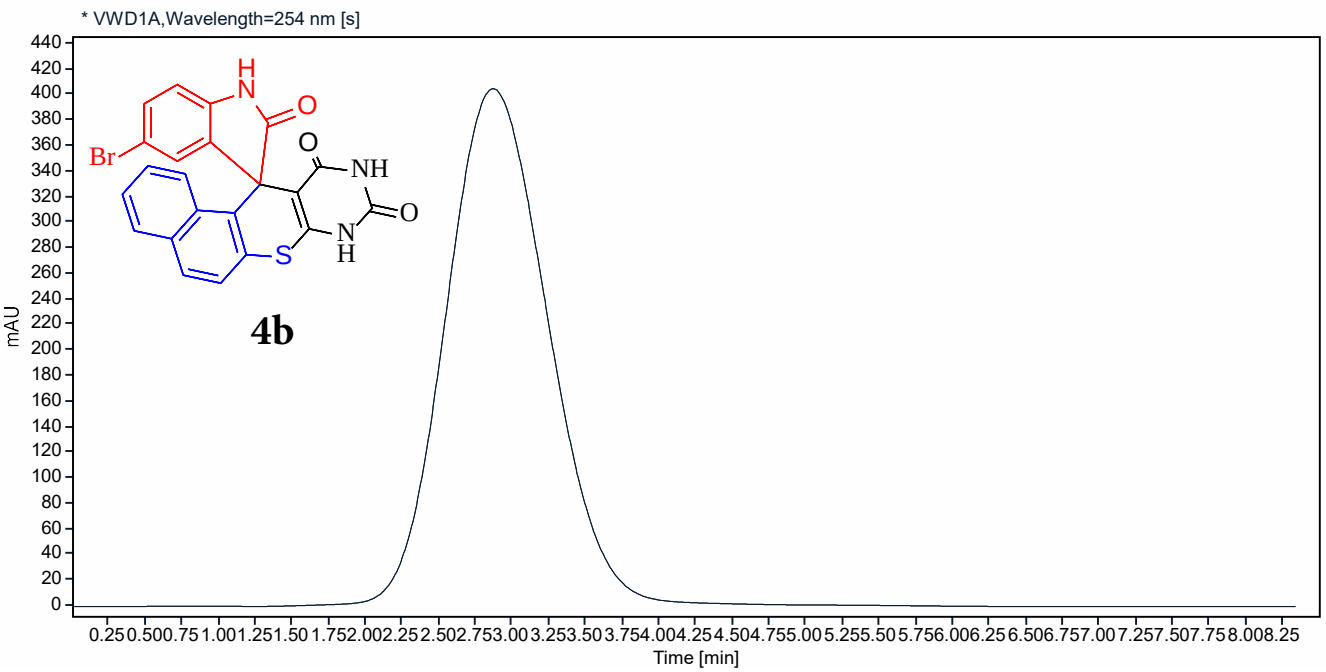
Signal: * VWD1A,Wavelength=254 nm [s]

RT [min]	Type	Width [min]	Area	Height	Area%
1.478	MM m	1.2565	440.0839	10.9952	1.2270
3.328	MM m	1.3831	451.3692	11.5377	1.2584
7.164	MM m	2.3413	34976.2459	730.8566	97.5146
		Sum	**35867.6990**		

Single Injection Report

Agilent

Sequence Acquired Date	2024-10-03 12:45:07+05:30
Sample Name	4B
Injection Volume	15.000
Injection Acq Method Name	03-10-2024.amx
Sequence Name	SingleSample
Sample Vial Number	P1-A2



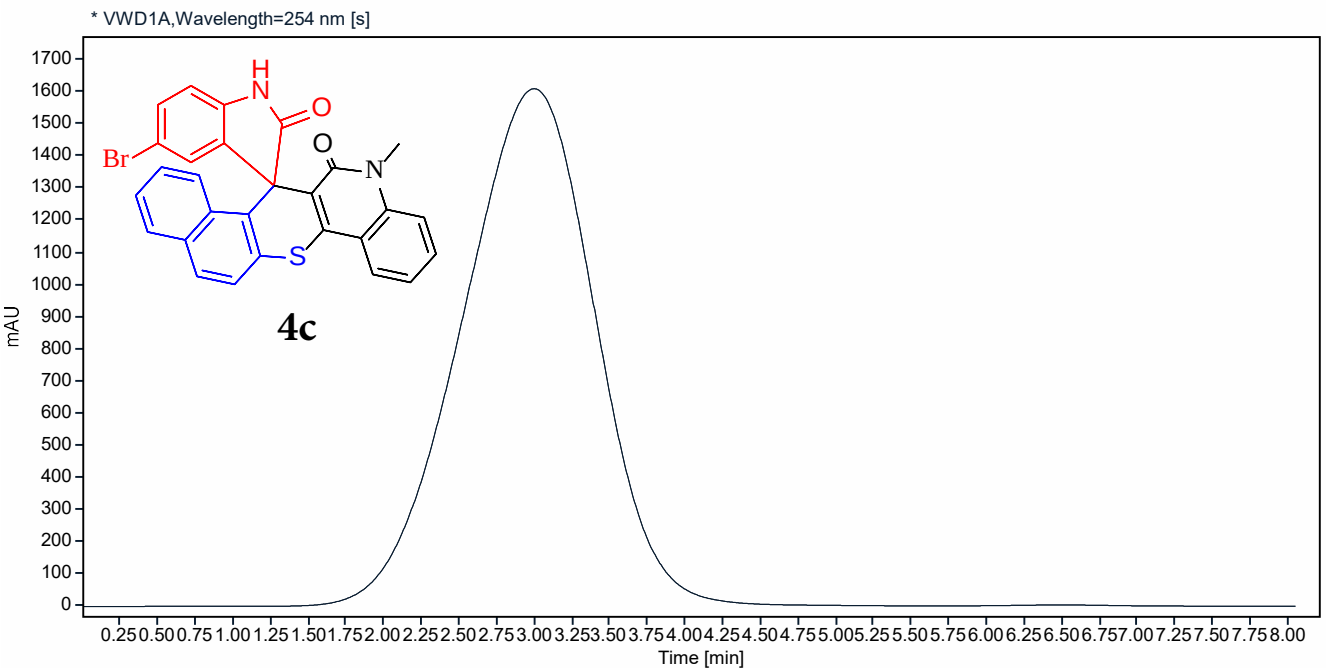
Signal: * VWD1A,Wavelength=254 nm [s]

RT [min]	Type	Width [min]	Area	Height	Area%
2.867	MM m	2.2041	19130.0073	402.1882	100.0000
		Sum	**19130.0073**		

Single Injection Report

Agilent

Sequence Acquired Date	2024-10-03 12:55:46+05:30
Sample Name	4C
Injection Volume	15.000
Injection Acq Method Name	03-10-2024.amx
Sequence Name	SingleSample
Sample Vial Number	P1-A3



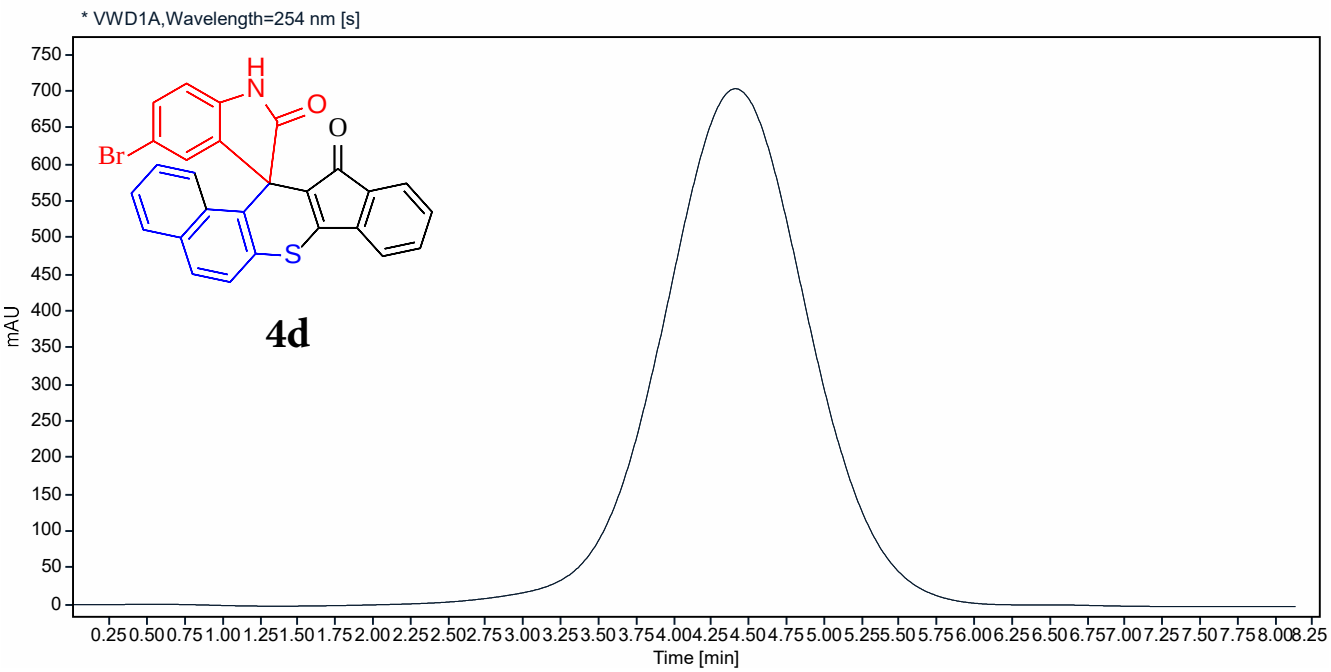
Signal: * VWD1A,Wavelength=254 nm [s]

RT [min]	Type	Width [min]	Area	Height	Area%
2.994	MM m	2.5789	96522.6799	1596.1788	100.0000
		Sum	**96522.6799**		

Single Injection Report

Agilent

Sequence Acquired Date	2024-10-03 13:08:19+05:30
Sample Name	4D
Injection Volume	15.000
Injection Acq Method Name	03-10-2024.amx
Sequence Name	SingleSample
Sample Vial Number	P1-A4



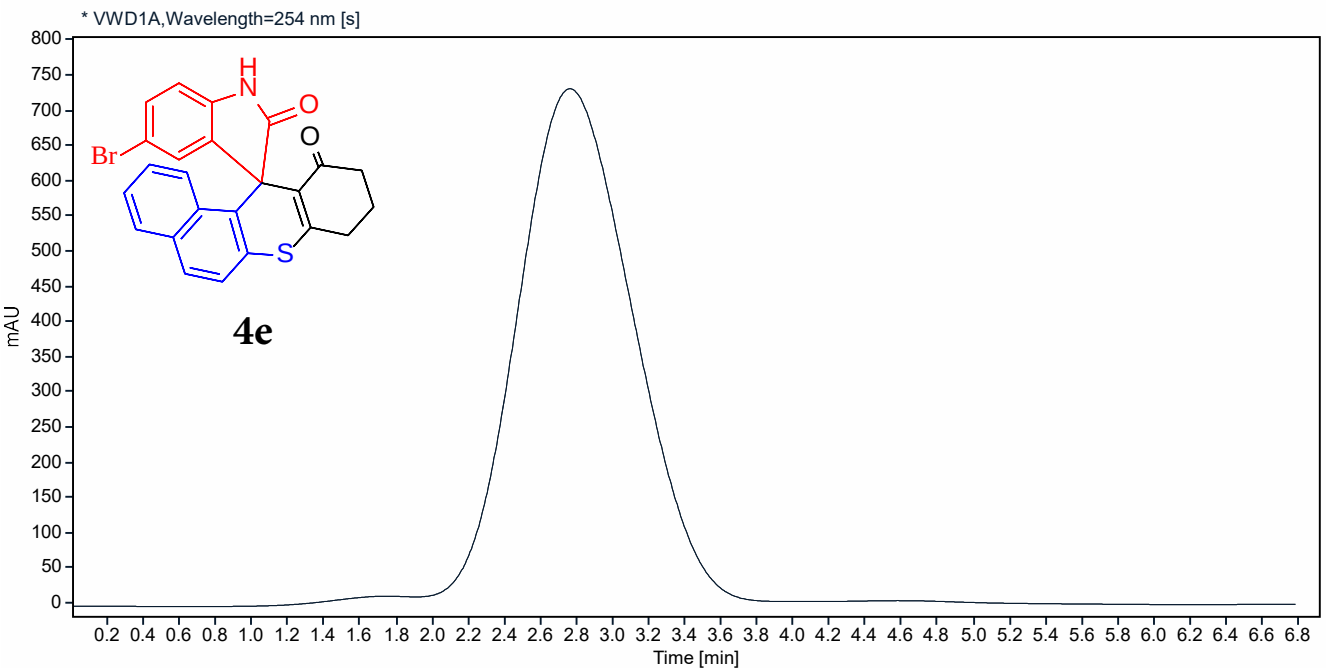
Signal: * VWD1A,Wavelength=254 nm [s]

RT [min]	Type	Width [min]	Area	Height	Area%
4.405	MM m	3.2144	46283.2595	696.9527	100.0000
		Sum	**46283.2595**		

Single Injection Report

Agilent

Sequence Acquired Date	2024-10-03 13:19:57+05:30
Sample Name	4E
Injection Volume	15.000
Injection Acq Method Name	03-10-2024.amx
Sequence Name	SingleSample
Sample Vial Number	P1-A5



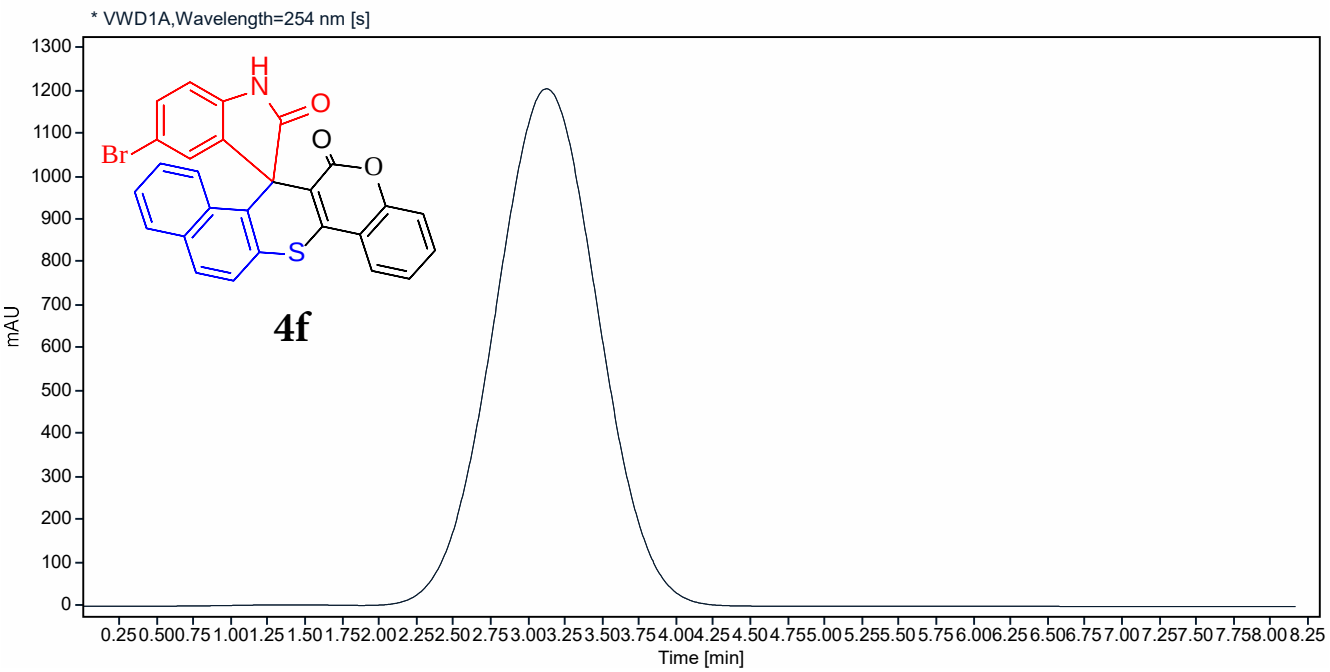
Signal: * VWD1A,Wavelength=254 nm [s]

RT [min]	Type	Width [min]	Area	Height	Area%
1.745	MM m	0.6753	74.0004	2.7842	0.2350
2.759	MM m	1.7449	31330.3940	721.8420	99.5137
4.575	MM m	1.2981	79.1187	2.3323	0.2513
		Sum	**31483.5131**		

Single Injection Report

Agilent

Sequence Acquired Date	2024-10-03 13:29:33+05:30
Sample Name	4F.
Injection Volume	15.000
Injection Acq Method Name	03-10-2024.amx
Sequence Name	SingleSample
Sample Vial Number	P1-A6



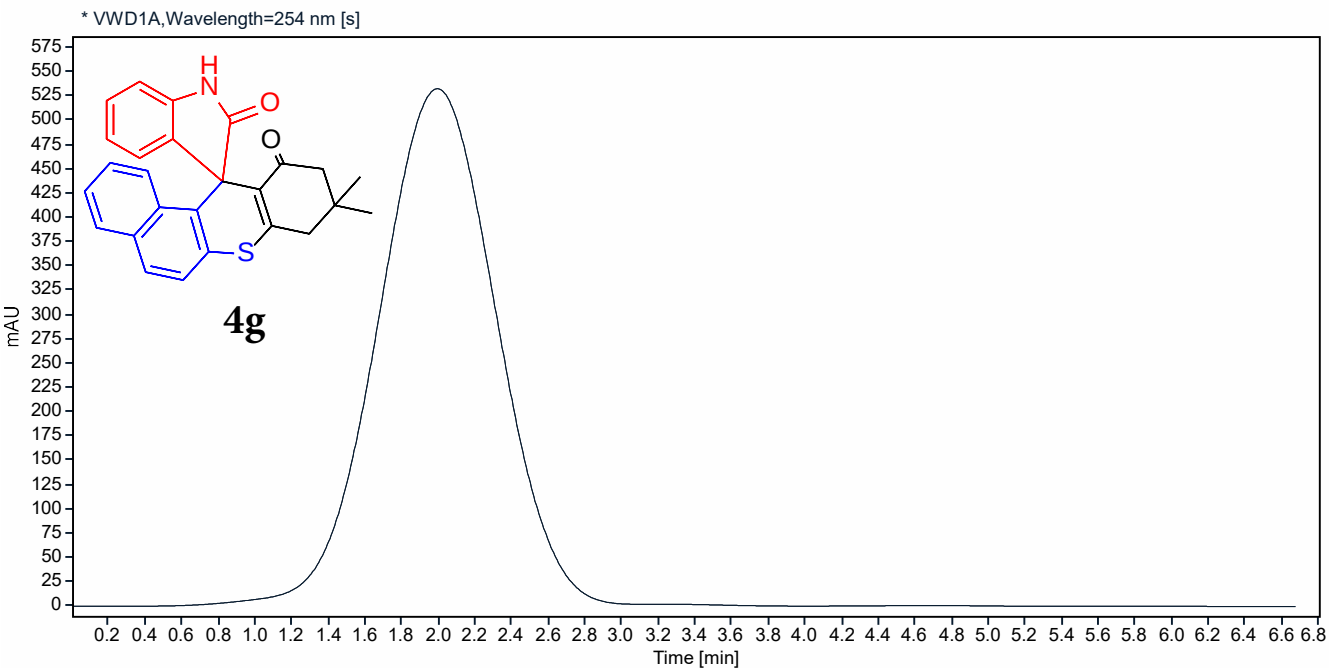
Signal: * VWD1A,Wavelength=254 nm [s]

RT [min]	Type	Width [min]	Area	Height	Area%
3.120	MM m	2.1103	57799.5557	1200.4883	100.0000
		Sum	**57799.5557**		

Single Injection Report

Agilent

Sequence Acquired Date	2024-10-03 13:38:30+05:30
Sample Name	4G
Injection Volume	15.000
Injection Acq Method Name	03-10-2024.amx
Sequence Name	SingleSample
Sample Vial Number	P1-A7



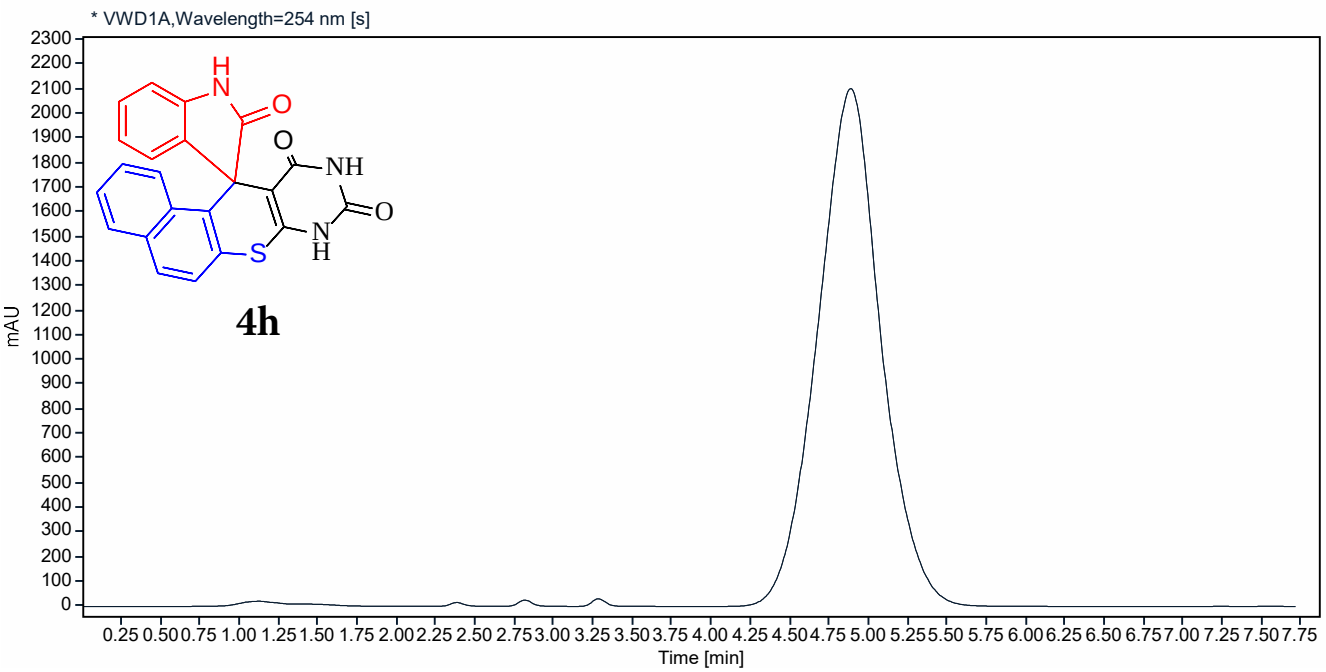
Signal: * VWD1A,Wavelength=254 nm [s]

RT [min]	Type	Width [min]	Area	Height	Area%
1.991	MM m	1.9202	23109.4087	527.1856	100.0000
		Sum	**23109.4087**		

Single Injection Report

Agilent

Sequence Acquired Date	2024-10-03 13:47:22+05:30
Sample Name	4H
Injection Volume	15.000
Injection Acq Method Name	03-10-2024.amx
Sequence Name	SingleSample
Sample Vial Number	P1-A8



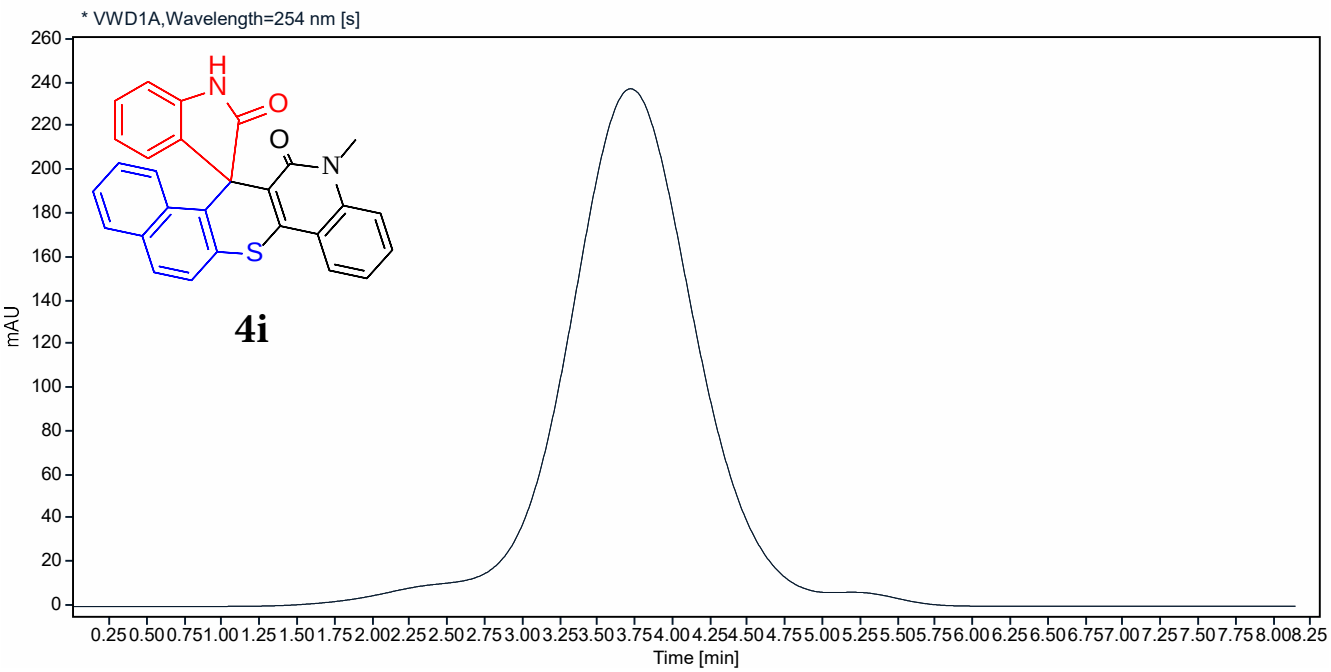
Signal: * VWD1A,Wavelength=254 nm [s]

RT [min]	Type	Width [min]	Area	Height	Area%
2.380	MM m	0.1601	65.9579	13.2093	0.1123
2.812	MM m	0.1819	124.2314	23.0790	0.2116
3.279	MM m	0.2220	170.6674	29.6818	0.2906
4.876	MM m	2.2354	58358.5572	1201.3952	99.3855
		Sum	**58719.4140**		

Single Injection Report

Agilent

Sequence Acquired Date	2024-10-03 13:58:00+05:30
Sample Name	4I
Injection Volume	15.000
Injection Acq Method Name	03-10-2024.amx
Sequence Name	SingleSample
Sample Vial Number	P1-A9



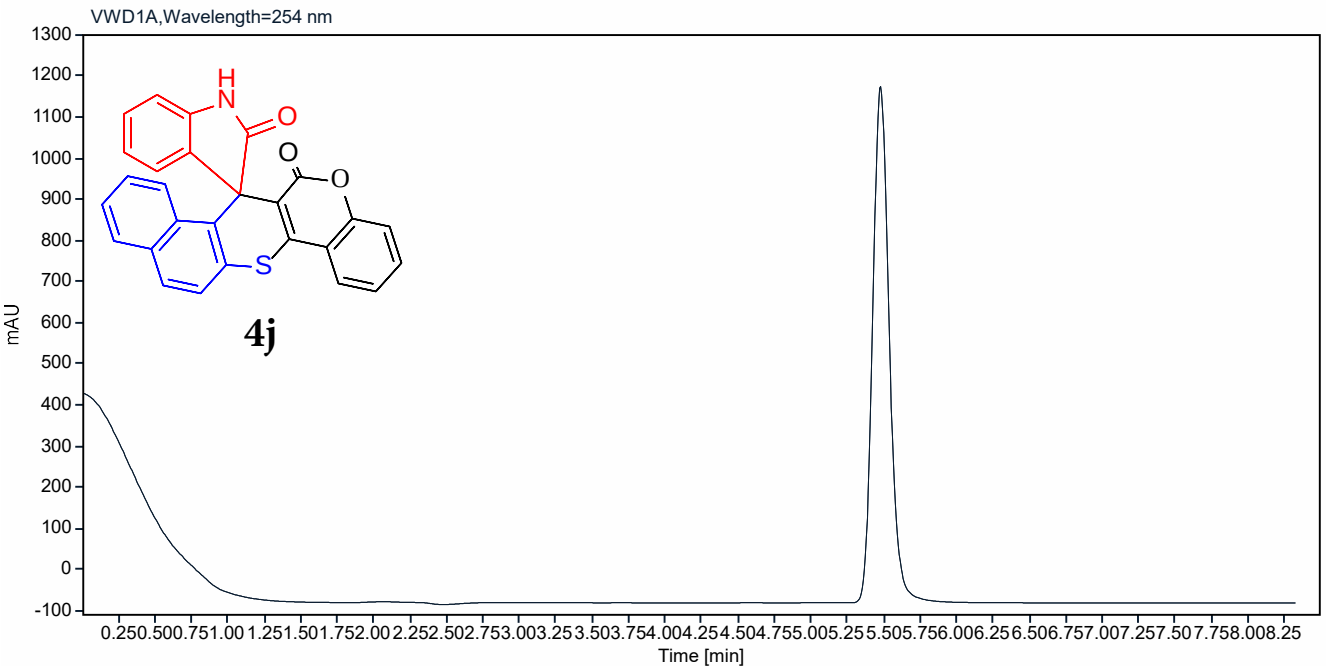
Signal: * VWD1A,Wavelength=254 nm [s]

RT [min]	Type	Width [min]	Area	Height	Area%
3.716	MM m	2.4849	12594.5137	228.7684	100.0000
		Sum	**12594.5137**		

Single Injection Report

Agilent

Sequence Acquired Date	2024-10-03 14:11:11+05:30
Sample Name	4J
Injection Volume	15.000
Injection Acq Method Name	03-10-2024.amx
Sequence Name	SingleSample
Sample Vial Number	P1-A10



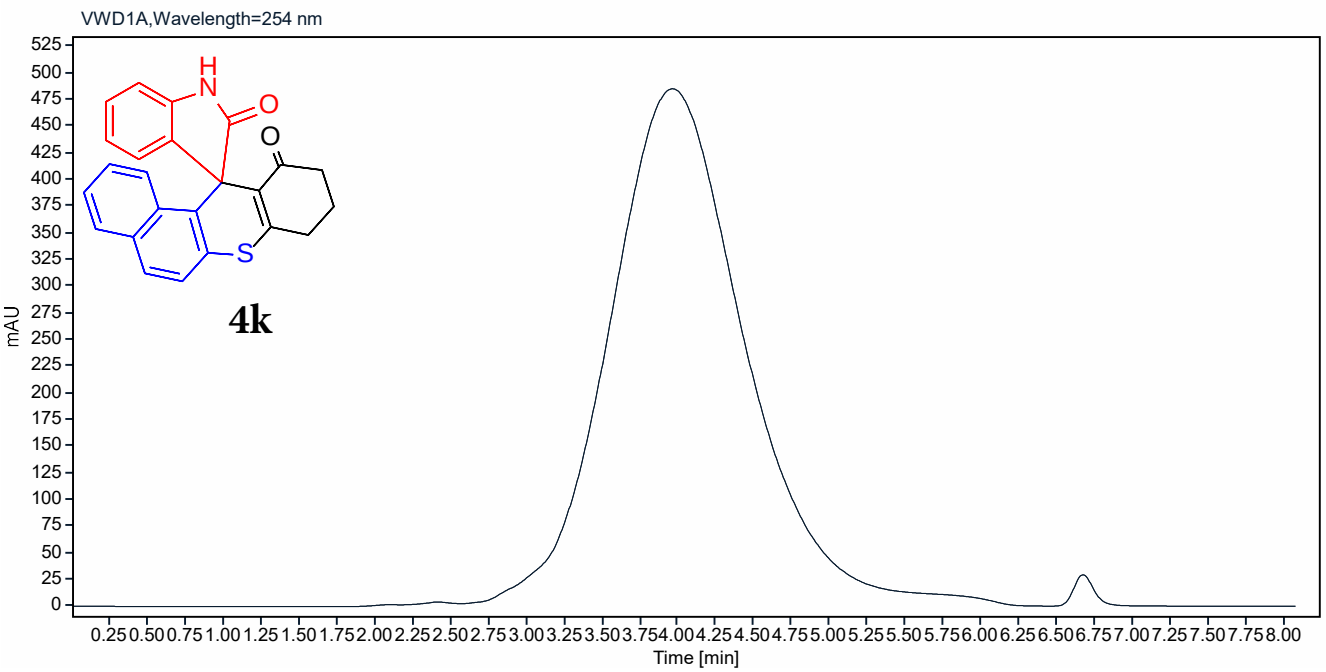
Signal: VWD1A,Wavelength=254 nm

RT [min]	Type	Width [min]	Area	Height	Area%
5.472	MM m	0.5147	9725.1919	1248.5674	100.0000
		Sum	**9725.1919**		

Single Injection Report

Agilent

Sequence Acquired Date	2024-10-03 14:23:44+05:30
Sample Name	4K
Injection Volume	15.000
Injection Acq Method Name	03-10-2024.amx
Sequence Name	SingleSample
Sample Vial Number	P1-A11



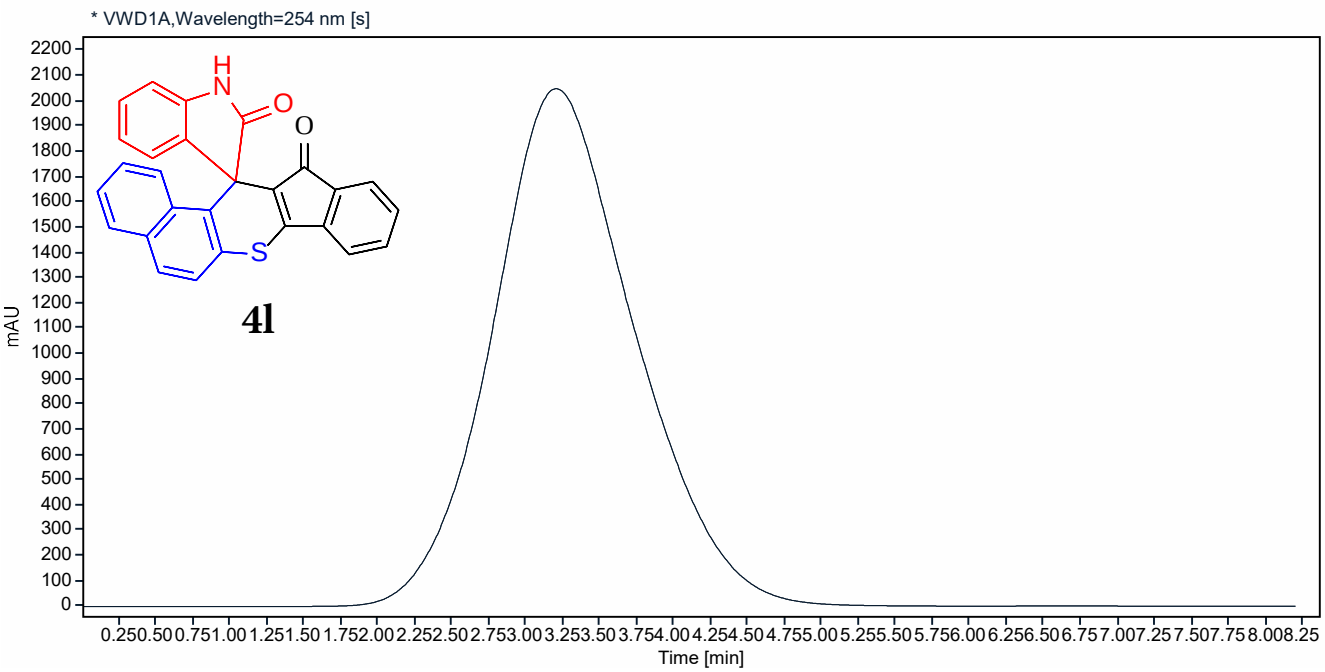
Signal: VWD1A,Wavelength=254 nm

RT [min]	Type	Width [min]	Area	Height	Area%
3.960	MM m	2.4462	27334.2916	470.4968	99.0386
6.669	MM m	0.4581	265.3455	29.1157	0.9614
		Sum	**27599.6371**		

Single Injection Report

Agilent

Sequence Acquired Date	2024-10-03 14:32:27+05:30
Sample Name	4L
Injection Volume	15.000
Injection Acq Method Name	03-10-2024.amx
Sequence Name	SingleSample
Sample Vial Number	P1-B1



Signal: * VWD1A,Wavelength=254 nm [s]

RT [min]	Type	Width [min]	Area	Height	Area%
3.203	MM m	2.9416	134201.3558	2036.1647	100.0000
		Sum	**134201.3558**		

References

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