Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
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Quinolone-1-(2*H*)-ones as Hedgehog Signalling Pathway Inhibitors

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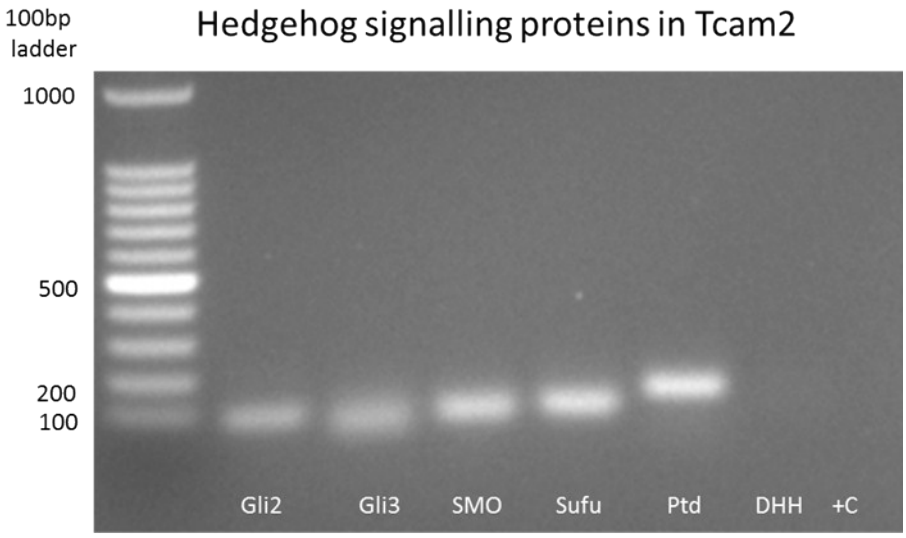
Electronic supporting information

Contents

TCAM-2 cells express the HSP

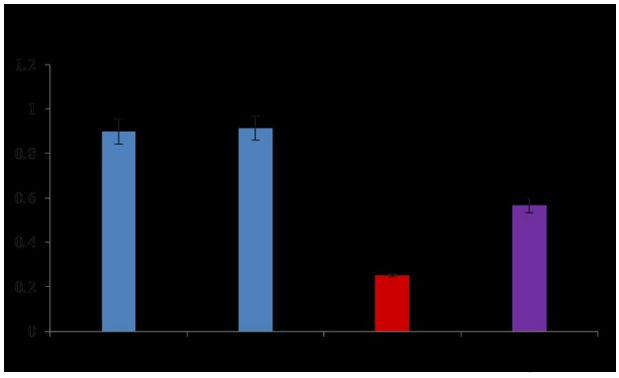
Table 1. TCAM-2 cell line expresses the HSP's components, including Ptch1, Sufu, Smo, and Gli2,3

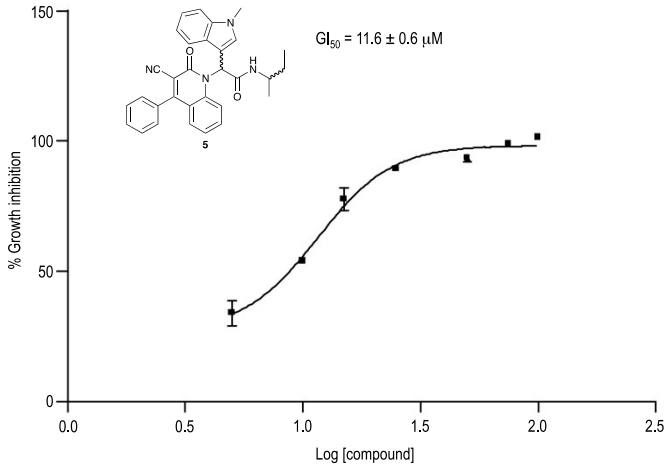


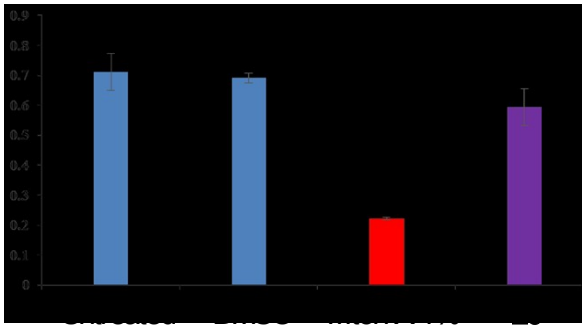
HH PCR products from Tcam2 cDNA using Real Time primers

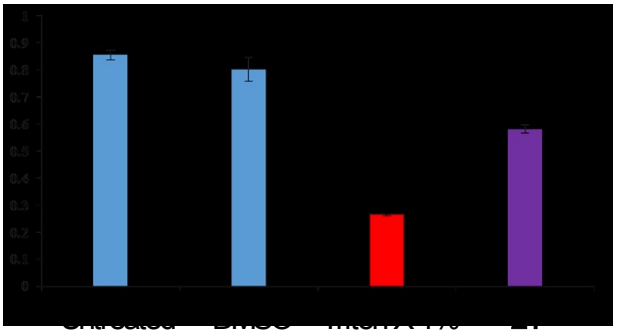
Cytotoxicity of indole analogues against TCAM-2 cells

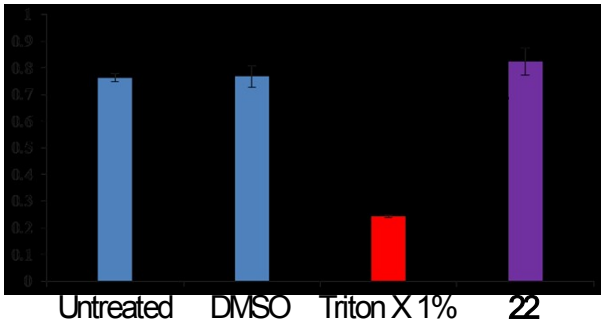
Table 4. Cytotoxicity of analogues **5, 20-30** at 10 µM concentration against TCAM- 2 cell line was measured after 72 hours of incubation, with DMSO and Triton X (1%) as controls. Only compounds **5**, **23**, and **24** expressed a growth inhibition greater than 50% and were further investigated for their dose responses to generate the GI_{50} (n=2)

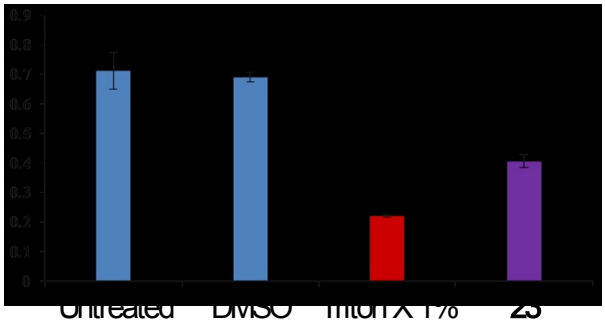









Untreated
DMSO
Triton X 1%
22


Untreated
DMSO
Triton X 1%
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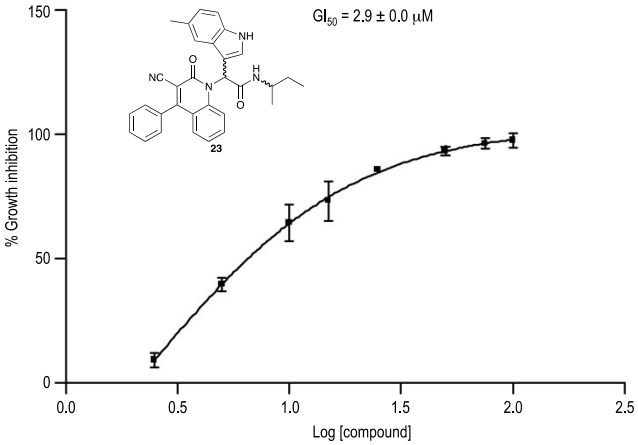
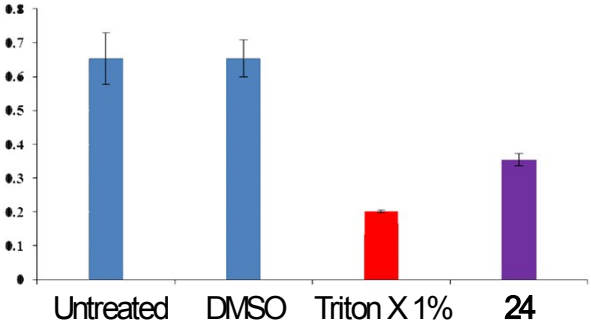

GI50 = 2.9 ± 0.0 μM
% Growth inhibition
Log [compound]
23


Untreated
DMSO
Triton X 1%
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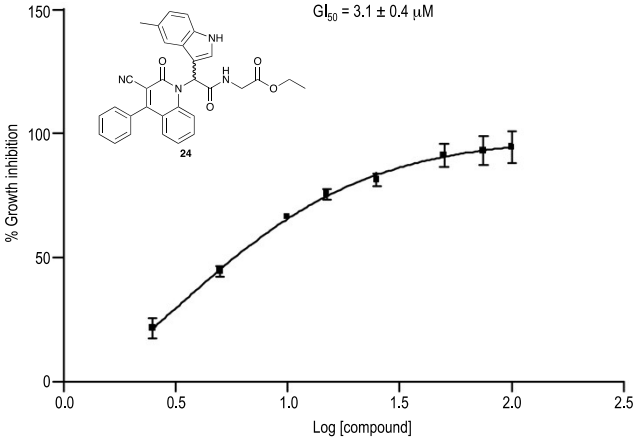
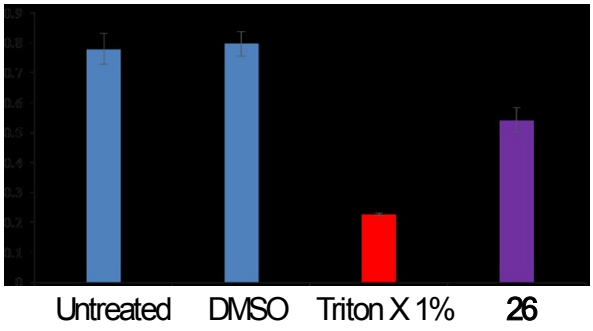

GI50 = 3.1 ± 0.4 μM
% Growth inhibition
Log [compound]
24


Untreated
DMSO
Triton X 1%
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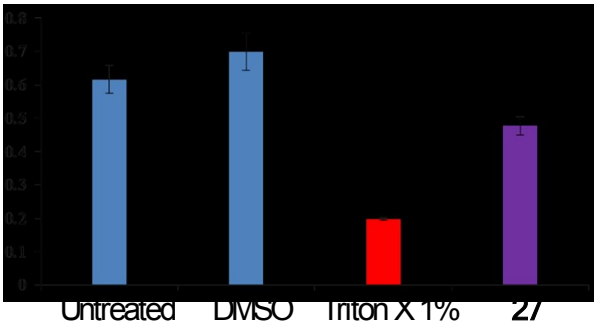
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DMSO
Triton X 1%
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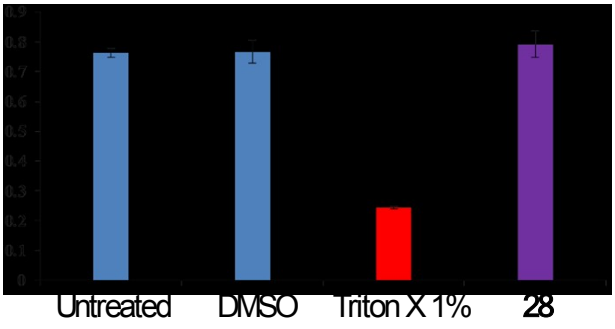
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DMSO
Triton X 1%
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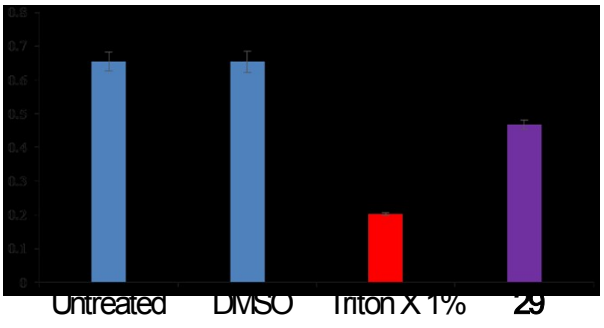
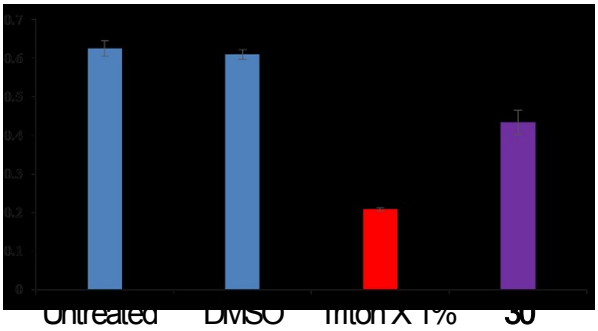
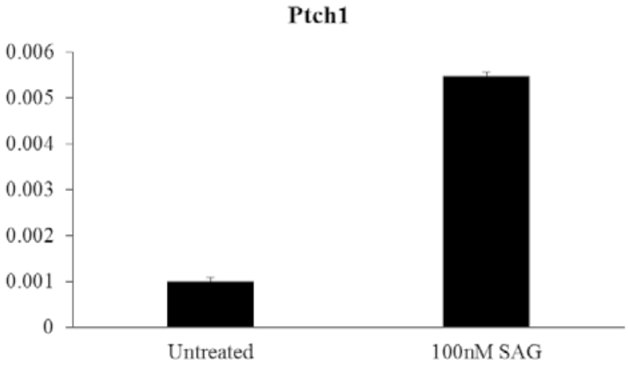
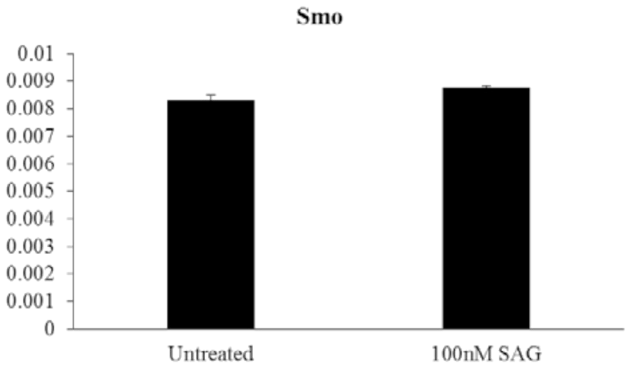
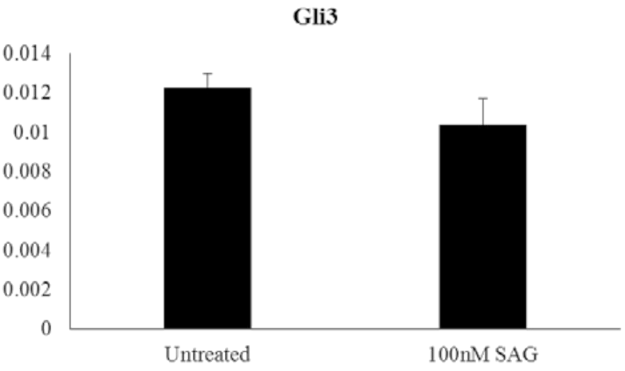

Untreated
DMSO
Triton X 1%
29

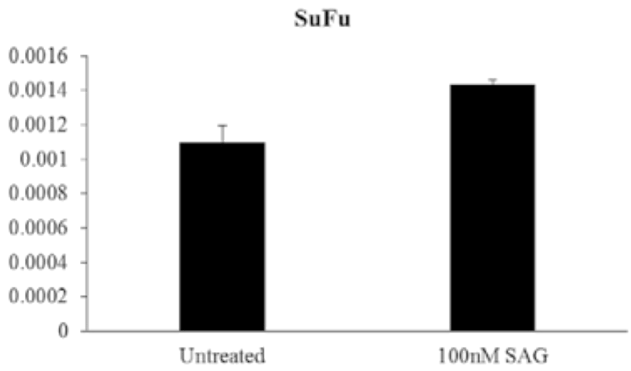


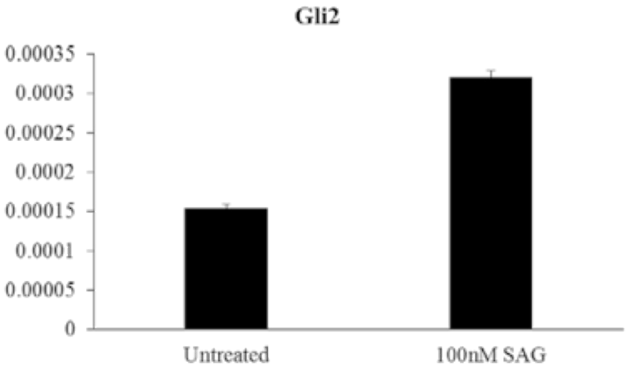
Expression of *Ptch1*, *Sufu*, *Smo*, *Gli2* and *Gli3* in Shh LIGHT2 cell line activated by 100 nM SAG





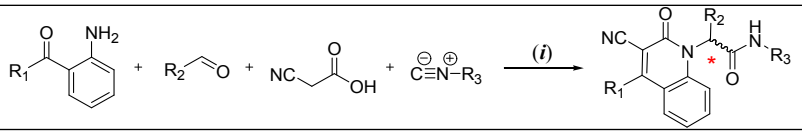


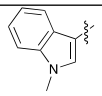
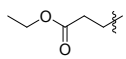
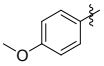
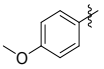
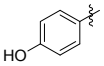
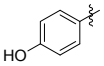
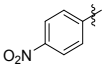
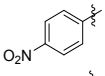
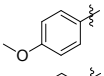
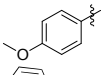
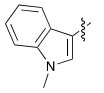
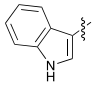


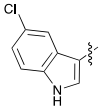
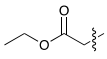
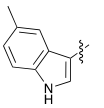
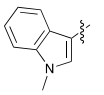


Synthesis of quinolone-1-(2H)-ones 20-30 via a Ugi-Knoevenagel reaction

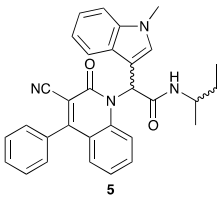
Appendix for Table 2 & 4. Isolated yields of quinolin-1-(2*H*)-ones (**5**, **10**–**30**) (the Ugi 4CR products) and structural differentiations using the ^{1}H NMR, IR spectra and base cations in HRMS $[M+H]^+$ and $[M-H]^-$. ***Reagents and conditions:*** (***i***) MeOH, rt, 24 h.



Compound	R_1	R_2	R_3	Yield %	^{1}H of CH* (ppm)	υ_{CN} (cm^{-1})	Mass Peak (*m/z*)
5				38	6.94 – 6.88 (m)	2227	502.2369 $[M+H]^+$
10				25	6.85 (bs)	2230	513.1455 $[M-H]^-$
11				56	6.95 (s)	2229	498.1483 $[M-H]^-$
12				68	6.94 – 6.88 (m)	2228	480.2207 $[M+H]^+$
13				37	6.92 (d)	2229	480.1636 $[M-H]^-$
14				71	6.74 (d)	2231	464.2052 $[M-H]^-$
15				66	6.69 (d)	2230	402.1895 $[M-H]^-$
16				26	7.29 – 7.18 (m)	2227	431.1798 $[M-H]^-$
17				49	6.25 (s)	2225	473.2267 $[M-H]^-$
18				68	6.94 – 6.84 (m)	2227	458.2522 $[M-H]^-$
19				49	7.1 (bs)	2228	418.1481 $[M-H]^-$
20				13	7.39 – 7.43 (m)	2229	463.2104 $[M+H]^+$
21				11	7.46 − 7.54 (m)	2229	489.2284 $[M+H]^+$
22				36	7.15 – 7.20 (m)	2235	489.2284 $[M+H]^+$
23				46	7.41 – 7.47 (m)	2236	503.2444 $[M+H]^+$
24				34	7.55 − 7.59 (m)	2232	519.2026 $[M+H]^+$
25				46	7.65 – 7.67 (m)	2236	505.1869 $[M+H]^+$
26				26	7.57 – 7.60 (m)	2226	519.2027 $[M+H]^+$
27				50	7.38 – 7.41 (m)	2232	287 $[M+ACN+2H]^{2+}$

28				33	7.53 – 7.58 (m)	2236	539.1481 $[M+H]^+$
29				47	7.40 – 7.43 (m)	2228	489.2283 $[M+H]^+$
30				26	7.40 (s)	2229	489.2287 $[M+H]^+$

2-(3-cyano-2-oxo-4-methylquinolin-1-(2*H*)-yl)-2-(1-methyl-1*H*-indol-3-yl)-N-(pentan-2-yl)acetamide (**5**)



5

Yield**:** 13%; MP: 243-245 ºC

IR (ν_{max}/cm^{-1}): 3246 (NH), 3083 (CH), 2972 (CH), 2229 (CN), 1637 (CO).

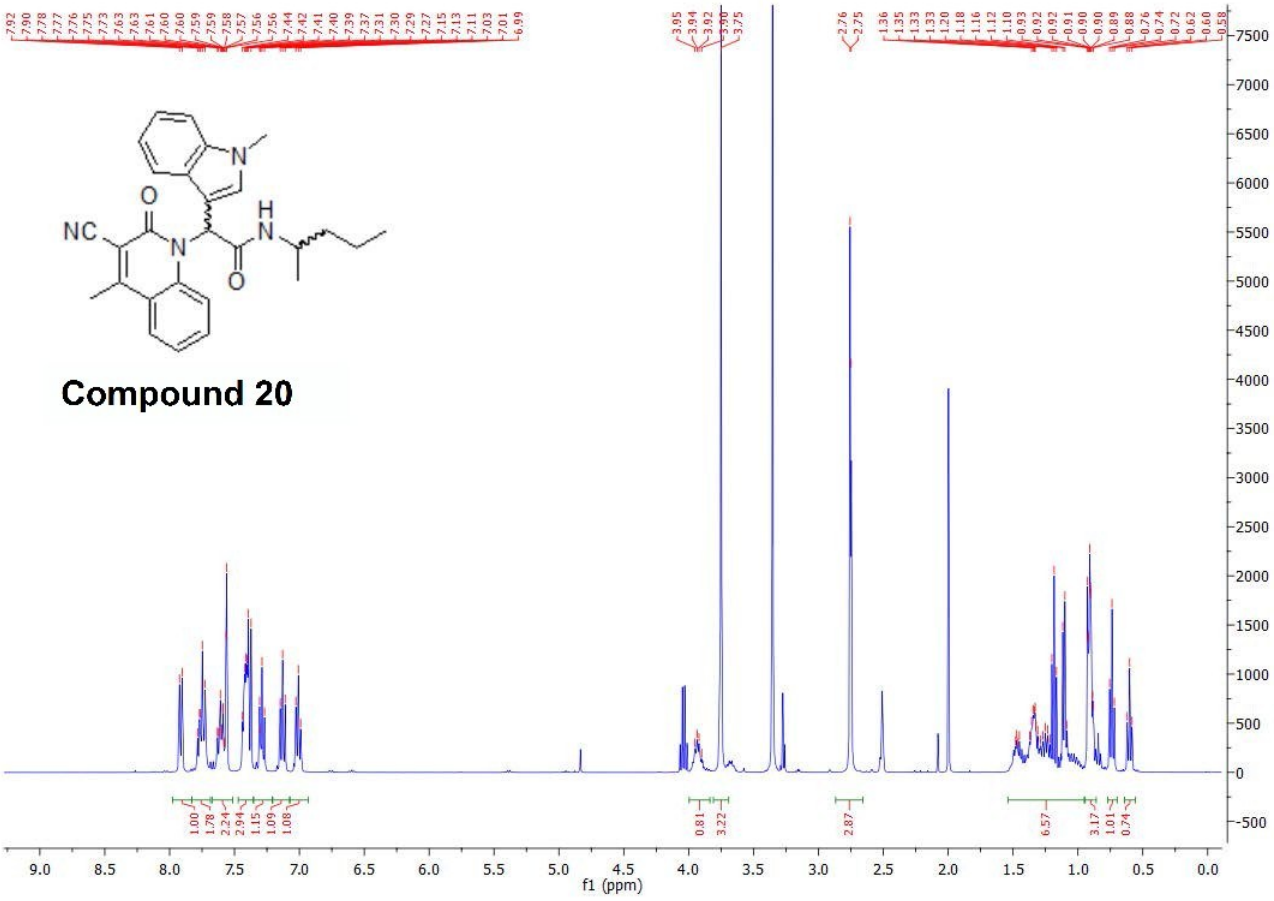
The ^{1}H NMR displays a mixture of isomers, with the ratio 1.35 : 1.0 calculated at 0.74 and 0.60 ppm, respectively. ^{1}H NMR spectra are reported as a whole without splitting due to the complex overlapping. All peaks detected in ^{13}C NMR are reported.

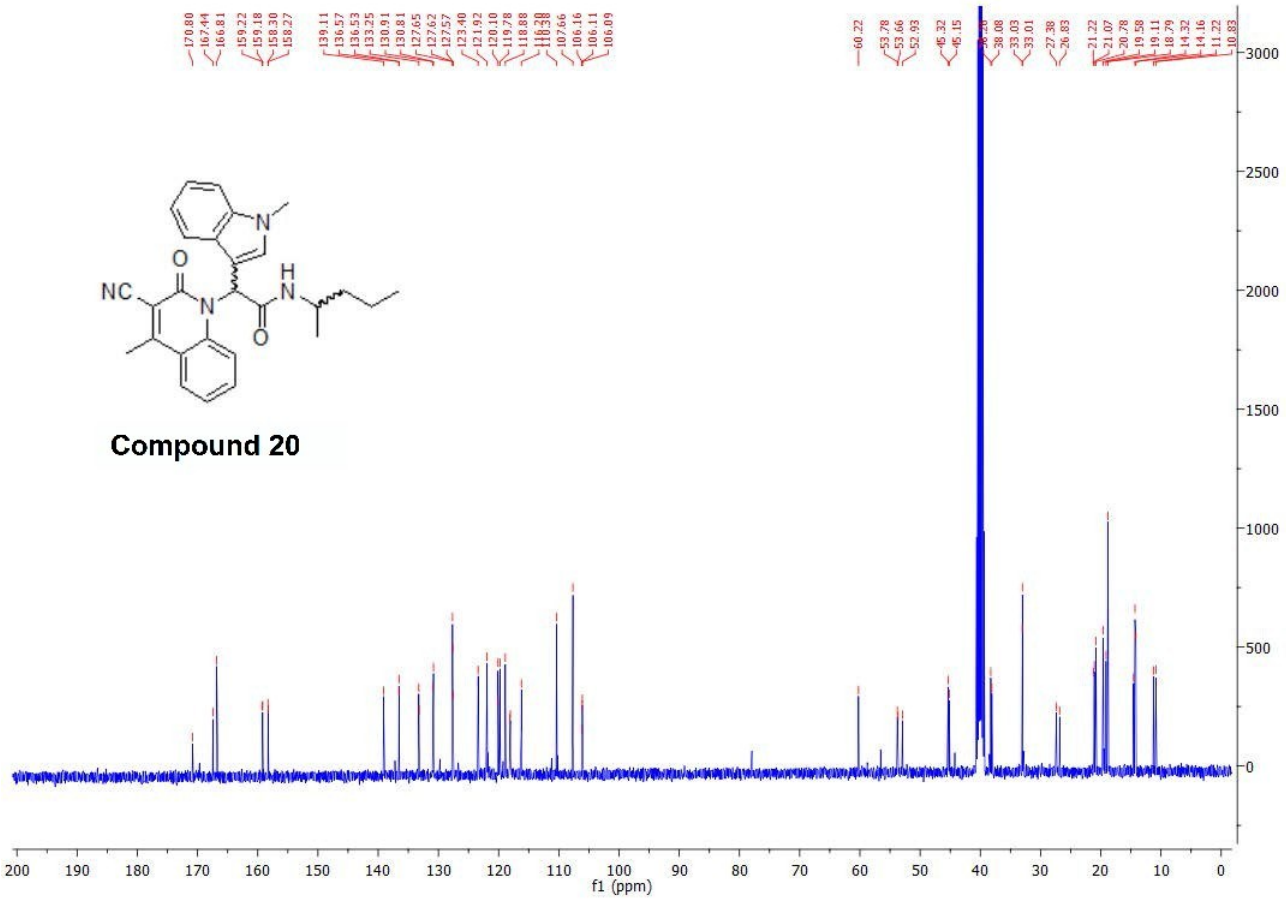
^{1}H NMR (400 MHz, DMSO) δ 7.91 (d, *J* = 8.2 Hz, 1H), 7.83 – 7.69 (m, 2H), 7.67 – 7.51 (m, 2H), 7.47 – 7.35 (m, 3H), 7.29 (dd, *J* = 9.8, 5.4 Hz, 1H), 7.13 (t, *J* = 7.6 Hz, 1H), 7.01 (t, *J* = 7.4 Hz, 1H), 3.98-3.86 (m, 1H), 3.75 (s, 3H), 2.75 (d, *J* = 3.2 Hz, 3H), 1.54 – 1.15 (m, 4H), 0.93-0.87 (m, 3H), 0.77-0.56 (m, 2H).

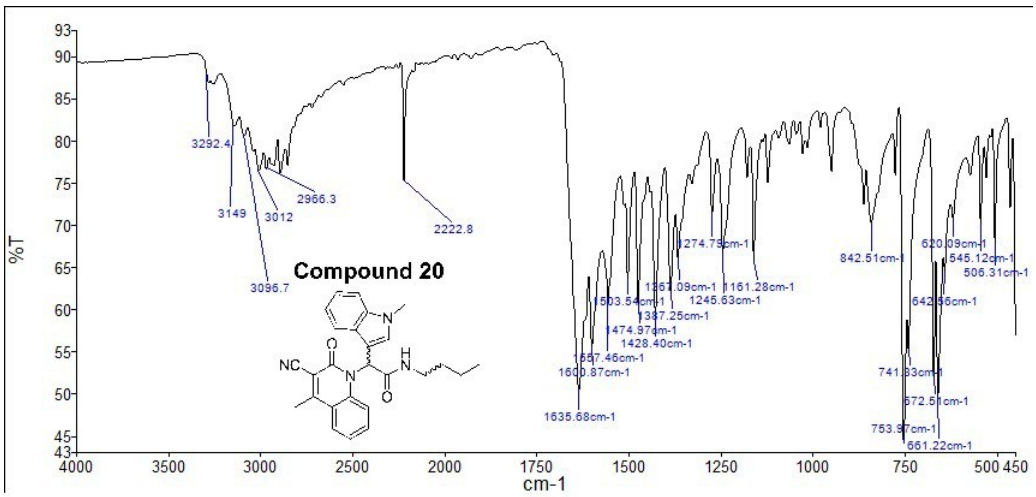
^{13}C NMR (101 MHz, DMSO-*d6*) δ 167.4, 166.8, 159.2, 159.2, 158.3, 158.3, 139.1, 136.6, 136.5, 133.3, 133.2, 130.9, 130.81, 127.7, 127.6, 127.6, 123.4, 121.9, 120.1, 120.1, 119. 8, 118.9, 118.1, 118.1, 116.2, 110.4, 107.7, 106.2, 106.1, 106.1, 60.2, 53.8, 53.7, 52.9, 45.3, 45.2, 38.3, 38.0, 33.0 (Cx2), 27.4, 26.8, 21.2, 21.1, 20.8, 19.6, 19.1, 18.8, 14.6, 14.3, 14.2, 11.2, 10.8

RP-HPLC Alltima™ C18 5 µm 150 mm x 4.6 mm, 10–100% B in 15 min, Rt min = 7.07, 93 %

LRMS (ESI-) m/z 440, 520 [M+DMSO+2H]$^{+}$, 100%. HRMS (ES+) for $C_{27}H_{28}N_4O_2Na$; calculated 463.2110, found 463.2104.




Compound 20
f1 (ppm)


Compound 20
%T
cm-1
3292.4
3149
3096.7
3012
2966.3
2222.8
1635.68cm-1
1600.87cm-1
1557.46cm-1
1503.54cm-1
1474.97cm-1
1428.40cm-1
1387.25cm-1
1361.09cm-1
1274.79cm-1
1245.63cm-1
1161.28cm-1
842.51cm-1
753.97cm-1
741.83cm-1
672.51cm-1
661.22cm-1
642.56cm-1
620.09cm-1
545.12cm-1
506.31cm-1

==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

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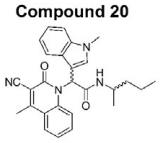
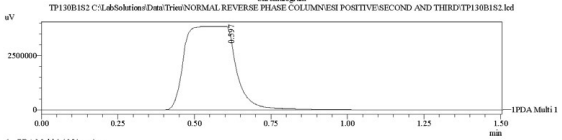
Sample Type	: Unknown
Level#	: 0
Sample Name	: TP130B1S2
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 43
Injection Volume	: 5
Data File	: TP130B1S2.lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Kelly\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/21/2015 11:51:18 AM

Compound 20



Chromatogram

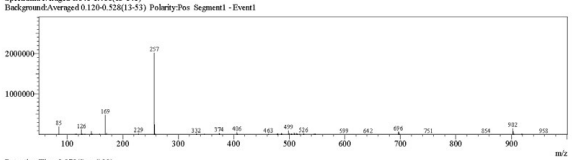
TP130B1S2 C:\LabSolutions\Data\Trieu\NORMAL REVERSE PHASE COLUMN\ESI POSITIVE\SECOND AND THIRD\TP130B1S2.lcd



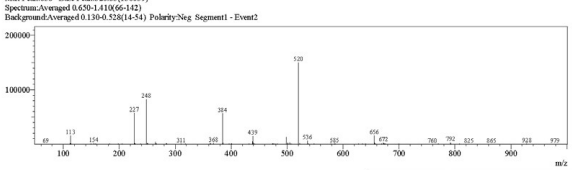
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.880(Scan#:89)
Mrc Peak:630 Base Peak:256.80(2022293)
Spectrum:Averaged 0.640-1.400(65-141)
Background:Averaged 0.120-0.528(13-53) Polarity:Pos Segment1 - Event1



Retention Time:0.870(Scan#:88)
Mrc Peak:658 Base Peak:520.35(150059)
Spectrum:Averaged 0.650-1.410(66-142)
Background:Averaged 0.130-0.528(14-54) Polarity:Neg Segment1 - Event2



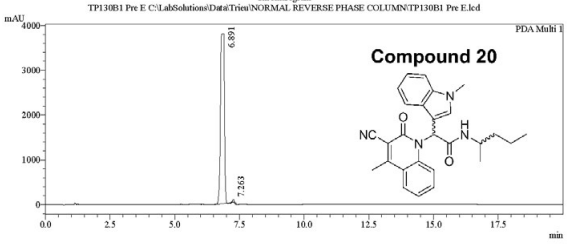
==== Shimadzu LCMSsolution Analysis Report ====

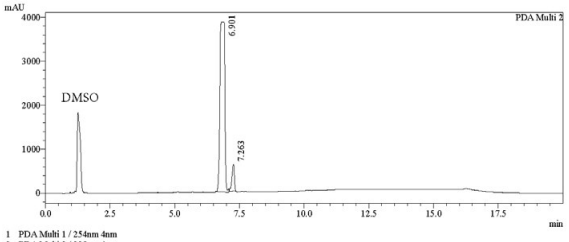
Acquired by	: Admin
Sample Name	: TP130B1 Pre E
Sample ID	:
Vail #	: 43
Injection Volume	: 30 uL
Data File Name	: TP130B1 Pre E.lcd
Method File Name	: Econosphere C18 EPS 5u lot 50195421 part 70070 150mm id 4.6mm.lcm
Batch File Name	: 2015 Ugi Knoevenagel products continue.lcb
Report File Name	: DefaultLCMS.lcr
Data Acquired	: 10/20/2015 3:15:53 PM
Data Processed	: 10/20/2015 3:42:46 PM

<Chromatogram>

Chromatogram

TP130B1 Pre E C:\LabSolutions\Data\Trieu\NORMAL REVERSE PHASE COLUMN\TP130B1 Pre E.lcd





1 PDA Multi 1 / 254nm 4nm
2 PDA Multi 2 / 220nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.891	39983729	3798093	99.645	98.361
2	7.263	142482	63301	0.355	1.639
Total		40126210	3861394	100.000	100.000

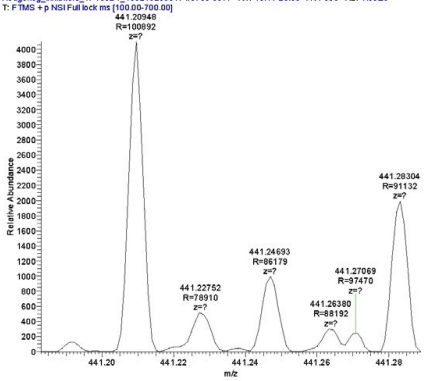
PeakTable

PDA Ch2 220nm 4nm

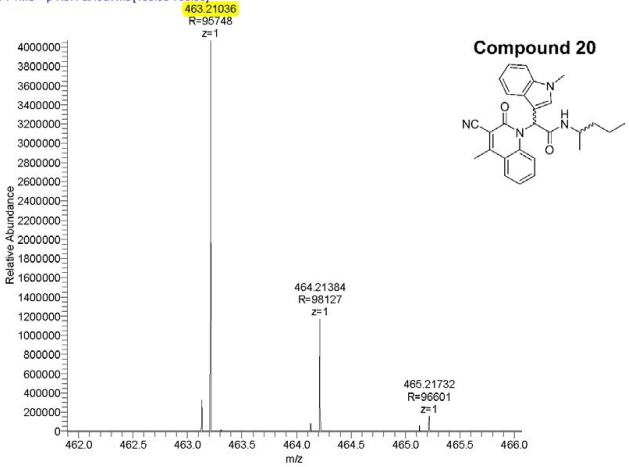
Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.901	50404388	3863757	92.871	94.834
2	7.263	3868877	610468	7.129	5.166
Total		54273265	4474225	100.000	100.000

Compound	Chemical Formula	Predicted monoisotopic neutral MW	Predicted MH+	MH+ Measured	Main MS Ions	Main MS/MS Fragments
TP130B1	C27H28N4O2	440.2212	441.2285	n/a	463.21036(+Na) 257.16466 (fragment)	257.1652 207.5034

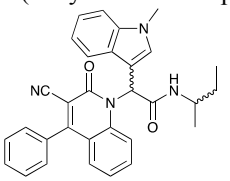
Hedgehog_Inhibitors_TP130B1_160216233017 #3763-6677 RT: 13.14-23.65 AV: 530 NL: 4.09E3
T: FTMS + p NSI Full lock ms [100.00-700.00]



Hedgehog_Inhibitors_TP130B1_160216233017 #5361-5648 RT: 18.73-19.71 AV: 48 NL: 4.07E6
T: FTMS + p NSI Full lock ms [100.00-700.00]



2-(3-Cyano-2-oxo-4-phenylquinolin-1-(2H)-yl)-2-(1H-indol-3-yl)-N-(pentan-2-yl)acetamide (**21**)



Yield: 70mg, 11 %; **MP** 182-183 ºC

IR (υ_{max}/cm^{-1}) 3420 (NH), 2229 (CN), 1678 (CONH), 1646 (CON)

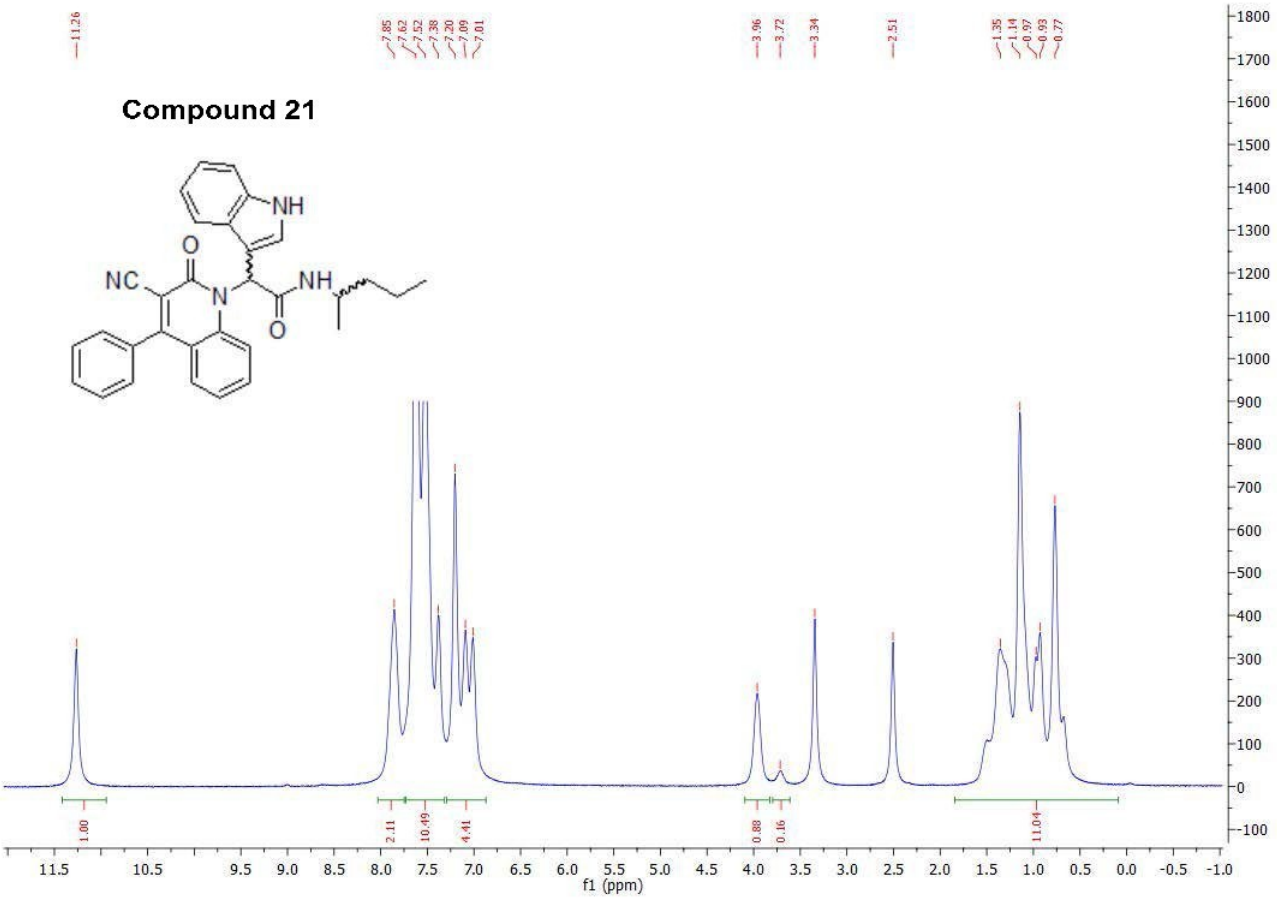
The ^{1}HNMR displays a mixture of isomers, with the ratio 5.5 : 1.0 calculated at 3.96 and 3.72 ppm, respectively. The ^{1}HNMR is reported as a whole without splitting due to the complex overlapping. All peaks detected in the ^{13}C NMR spectrum are reported.

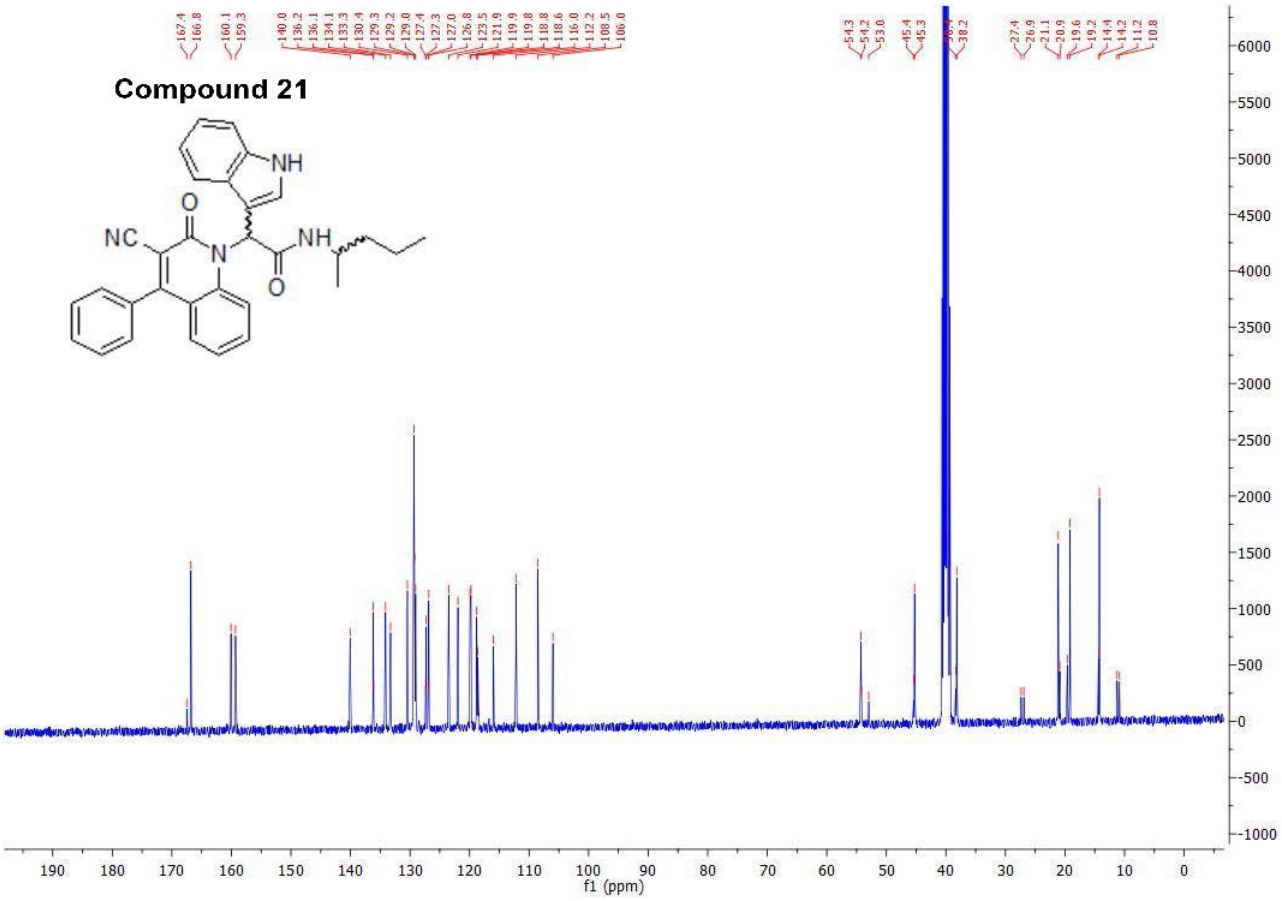
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.26 (s, 1H), 7.85 (s, 2H), 7.73 – 7.32 (m, 10H), 7.29 – 6.87 (m, 4H), 3.96 (s, 1H), 1.84 – 0.09 (m, 11H).

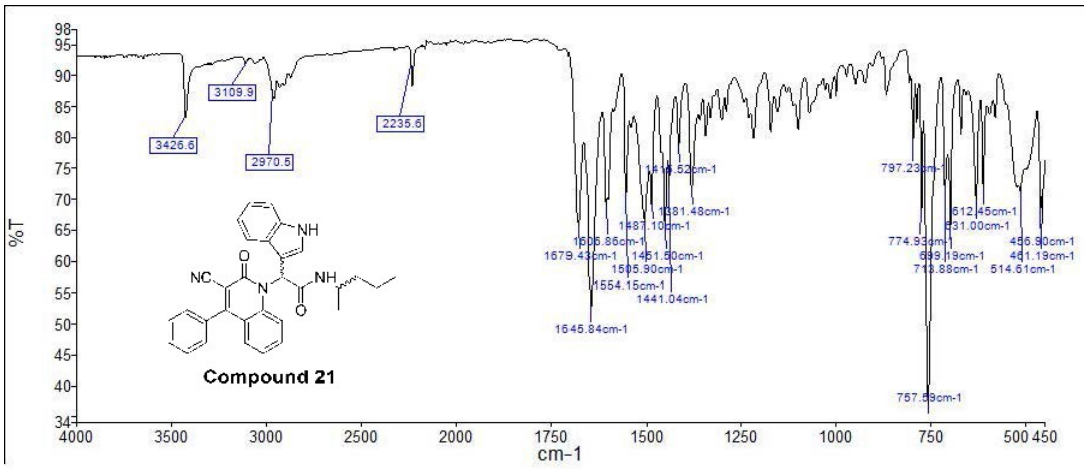
^{13}C NMR (101 MHz, DMSO) δ 167.4, 166.8, 160.1, 159.3, 140.1, 136.2, 136.1, 134.1, 133.3, 130.4, 129.3, 129.2, 129.1, 127.4, 127.3, 127.0, 126.9, 123.5, 122.0, 119.9, 119.8, 118.8, 118.6, 116.0, 112.2, 108.5, 106.0, 54.3, 54.2, 53.0, 45.4, 45.3, 38.4, 38.2, 27.4, 26.9, 21.1, 20.9, 19.6, 19.2, 14.4, 14.2, 11.3, 10.8.

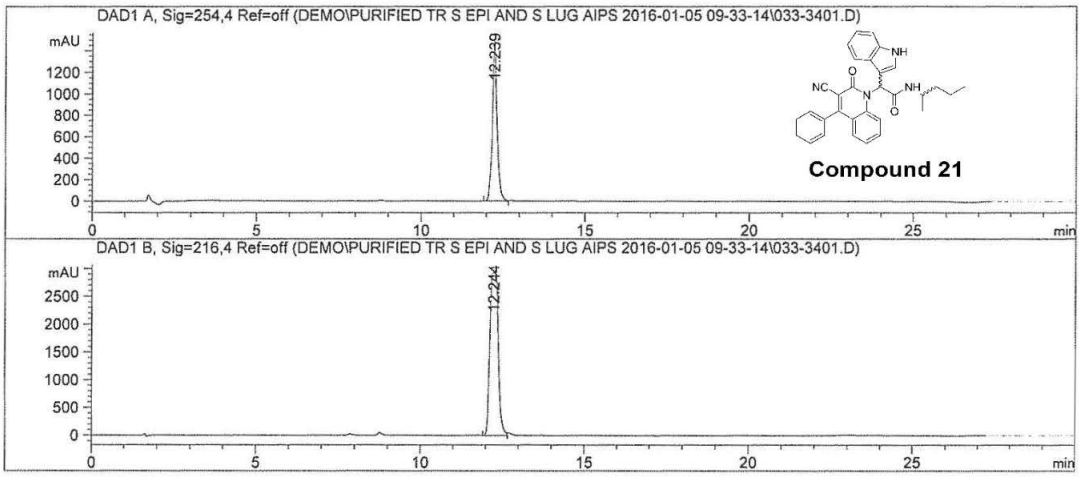
RP-HPLC Phenomenex Onyx™ Monolithic C18 5 µm 100 mm x 4 mm, 10–100% B in 15 min, Rt min = 12.24, 100 %

LRMS (ESI+) m/z 488, 489; $[M+H]^+$, 40%. HRMS (ES+) for $C_{31}H_{28}N_4O_2$; calculated 489.2285, found 489.2284.




Compound 21
NH
O
NC
N
NH
O
f1 (ppm)


3426.6
3109.9
2970.5
2235.6
1645.84cm-1
1554.15cm-1
1505.90cm-1
1487.10cm-1
1451.50cm-1
1441.04cm-1
1381.48cm-1
797.23cm-1
757.59cm-1
774.93cm-1
699.19cm-1
713.88cm-1
514.61cm-1
NH
NC
N
NH
O
Compound 21
%T
cm-1




```
=====================================================================
                    Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #    [min]        [min]   [mAU*s]     [mAU]      %
----|-------|----|-------|----------|----------|--------|
   1  12.239 BV    0.1407 1.49408e4  1493.16357 100.0000

Totals :                  1.49408e4  1493.16357


Signal 2: DAD1 B, Sig=216,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #    [min]        [min]   [mAU*s]     [mAU]      %
----|-------|----|-------|----------|----------|--------|
   1  12.244 BV    0.2858 5.21904e4  2930.83984 100.0000
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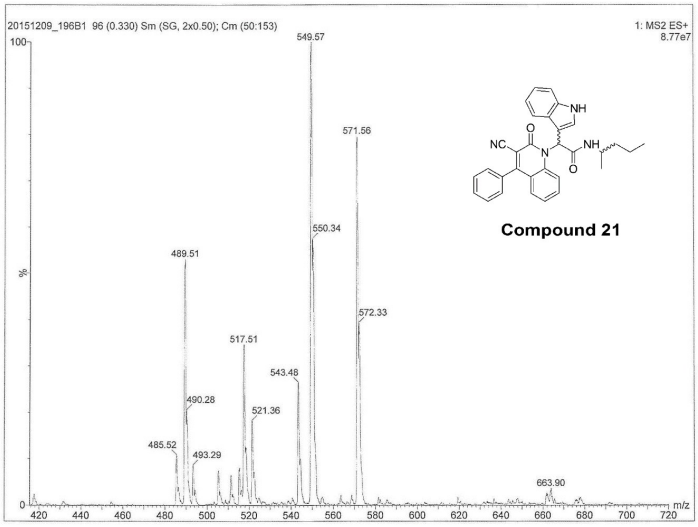
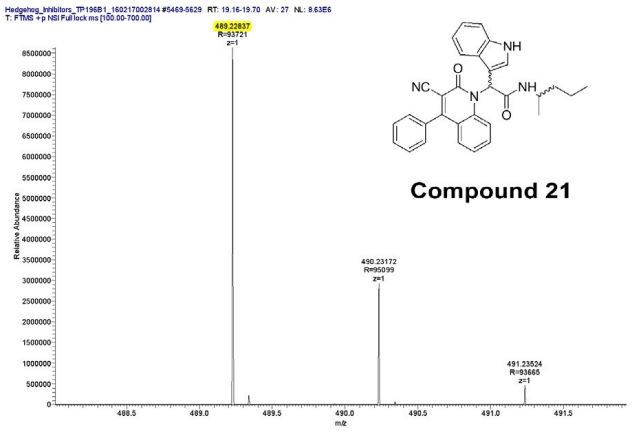
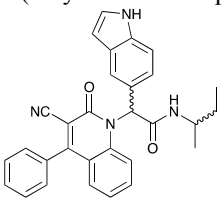


Compound	Chemical Formula	Predicted monoisotopic neutral MW	Predicted MH+	MH+ Measured	Main MS Ions	Main MS/MS Fragments
TP 196B1	C31H28N4O2	488.2212	489.2285	489.2284	243.14903 (fragment) 489.2284	243.1486 86.0970 489.3408



2-(3-cyano-2-oxo-4-phenylquinolin-1(2H)-yl)-2-(1H-indol-5-yl)-N-(pentan-2-yl)acetamide (**22**)



Yield 36%; MP 271-272 ºC

IR (υ_{max}/cm^{-1}) 3403 (NH), 3338 (NH), 2956 (CH), 2235(CN), 1647 (CO).

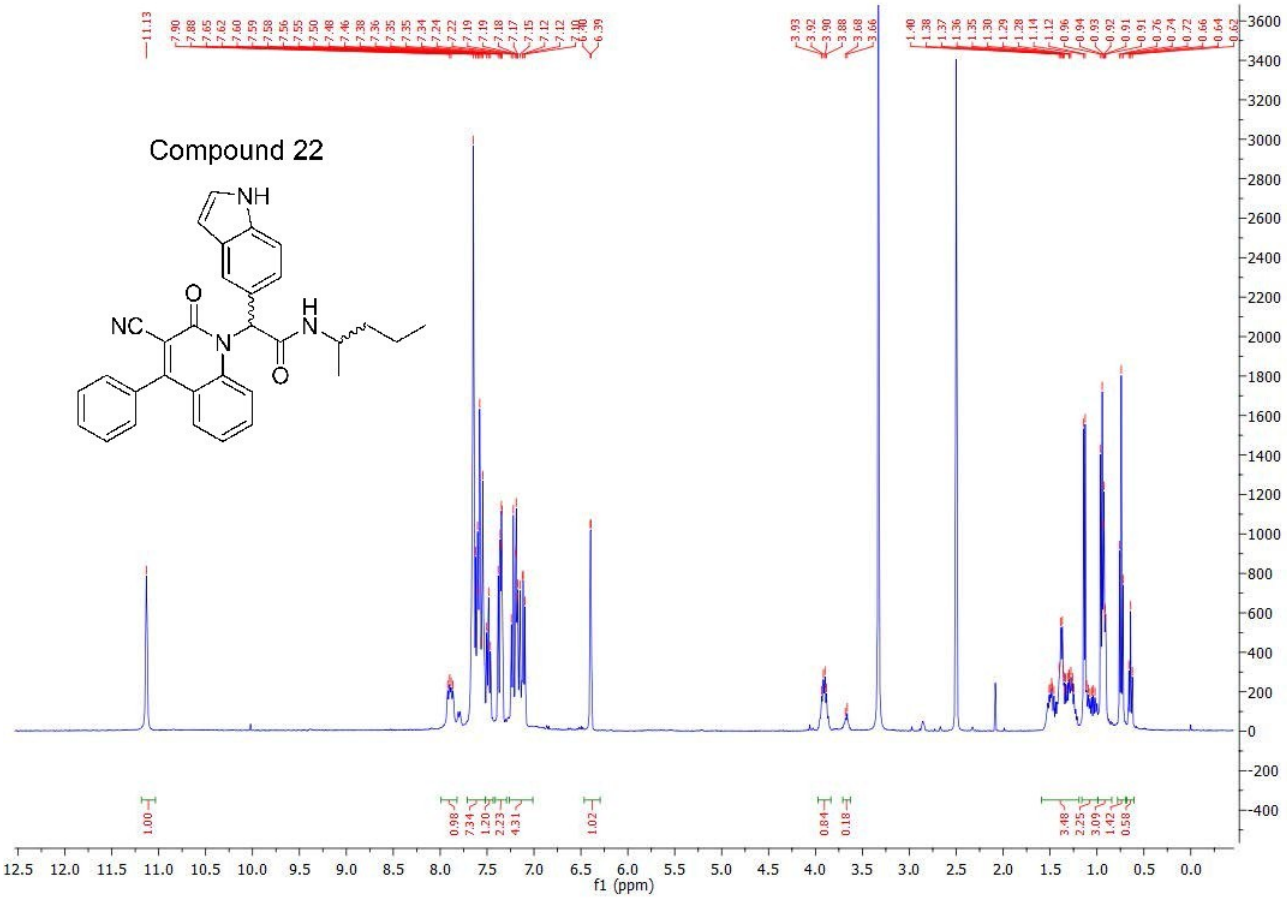
The ^{1}H NMR displays a mixture of isomers, with the ratio 2.45 : 1.0 calculated at 0.74 and 0.64 ppm, respectively. ^{1}H NMR is reported as a whole without splitting due to the complex overlapping. All peaks detected in the ^{13}C NMR spectrum are reported.

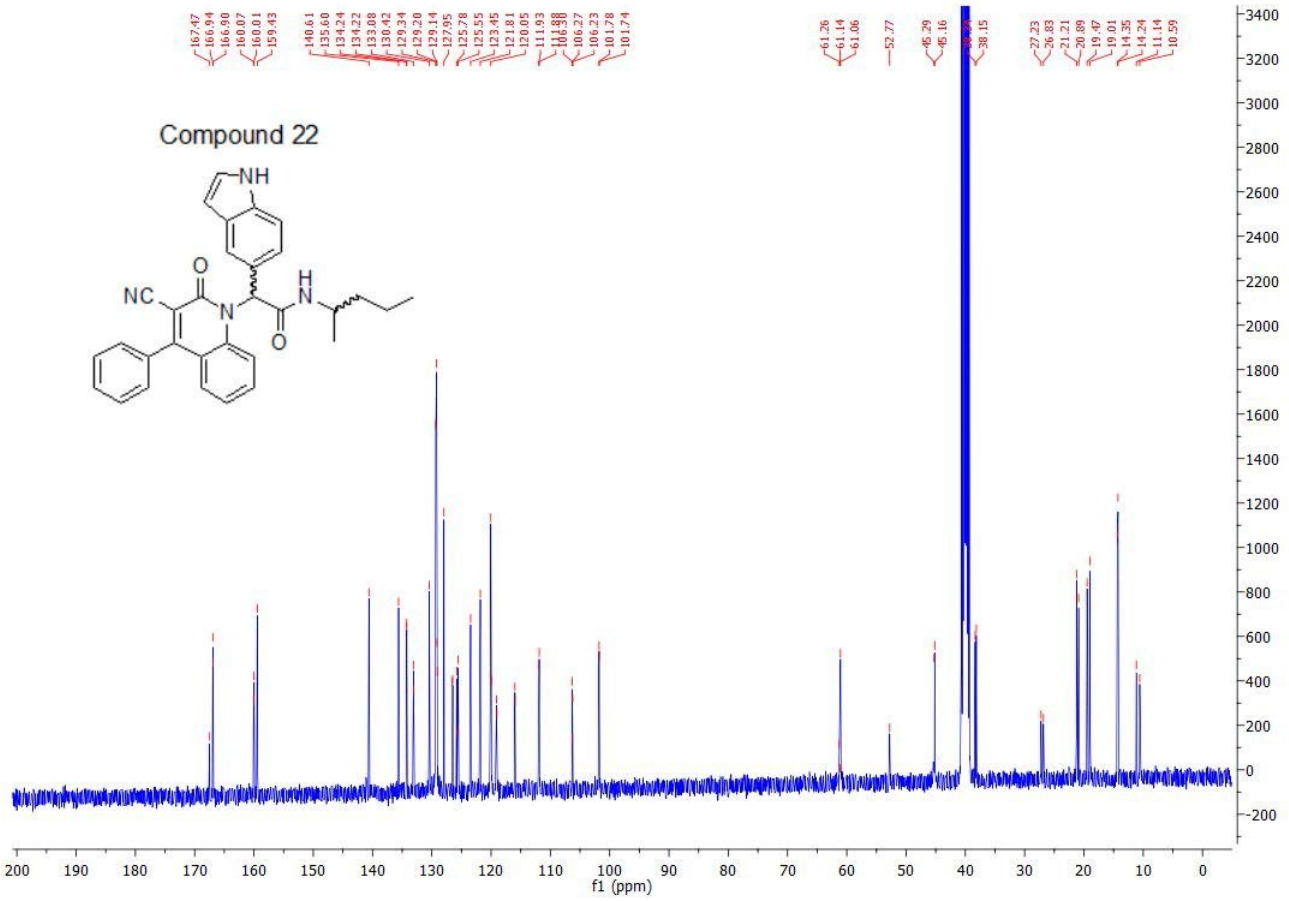
^{1}H NMR (400 MHz, DMSO) δ 11.13 (s, 1H), 7.89 (dd, *J* = 14.3, 8.1 Hz, 1H), 7.71 – 7.52 (m, 7H), 7.52 – 7.44 (m, 1H), 7.41 – 7.29 (m, 2H), 7.26 – 7.01 (m, 4H), 6.40 (d, *J* = 1.8 Hz, 1H), 3.91 (dd, *J* = 13.4, 7.0 Hz, 1H), 1.59 – 1.19 (m, 3H), 1.16 – 0.99 (m, 2H), 0.99 – 0.84 (m, 3H), 0.81-0.55 (m, 2H).

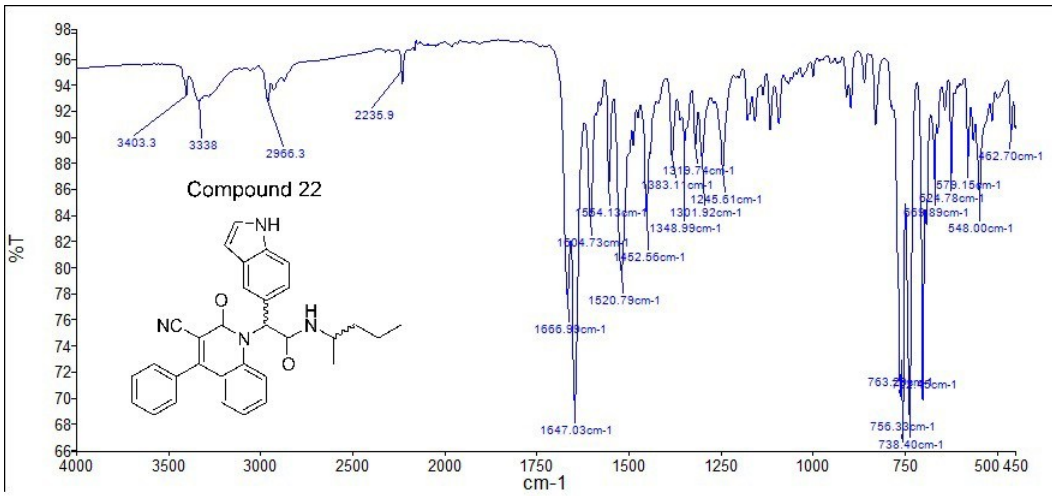
^{13}C NMR (101 MHz, DMSO-*d6*) δ 167.5, 167.0, 166.9, 160.1, 160.0, 159.4, 140.6, 135.6, 134.2, 134.2, 133.1, 133.0, 130.4, 129.3 (Cx2), 129.2 (Cx2), 129.1, 129.1, 128.0, 126.5, 126.5, 125.8, 125.7, 125.6, 123.5, 121.8, 120.1, 120.1, 120.0, 119.1, 119.0, 116.0, 116.0, 111.9, 111.9, 106.3, 106.3, 106.2, 101.8, 101.7, 61.3, 61.1, 61.1, 52.8, 45.3, 45.2, 38.3, 38.2, 27.2, 26.8, 21.2, 20.9, 19.5, 19.0, 14.4, 14.3, 11.1, 10.6

RP-HPLC Alltima™ C18 5u 150 mm x4.6 mm, 10–100% B in 15 min, Rt min= 7.07, 98.6%

LRMS (ESI-) m/z - 488, 520 [M+CH_3OH-H] 95%. HRMS (ES+) for $C_{31}H_{28}N_4O_2$; calculated 489.2285, found 489.2284




Compound 22
f1 (ppm)


Compound 22
%T
cm-1
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3338
2966.3
2235.9
1647.03cm-1
1666.99cm-1
1604.73cm-1
1584.13cm-1
1520.79cm-1
1452.56cm-1
1348.99cm-1
1301.92cm-1
1245.61cm-1
1383.11cm-1
1319.74cm-1
756.33cm-1
738.40cm-1
548.00cm-1
524.78cm-1
579.15cm-1
462.70cm-1

==== Shimadzu LCMSsolution Data Report ====

==== Shimadzu LCMSsolution Analysis Report ====

Acquired by	: Admin
Sample Name	: TP126B1 Old
Sample ID	:
Vail #	: 53
Injection Volume	: 30 uL
Data File Name	: TP126B1 Old .lcd
Method File Name	: Econosphere C18 EPS 5u lot 50195421 part 70070 150mm id 4.6mm.lcm
Batch File Name	: 2015 Ugi Knoevenagel products continue.lcb
Report File Name	: DefaultLCMS.lcr
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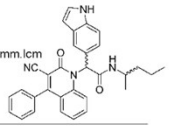
Data Processed	: 10/15/2015 10:07:01 AM

Compound 22



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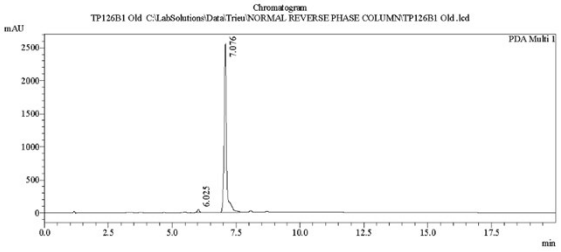
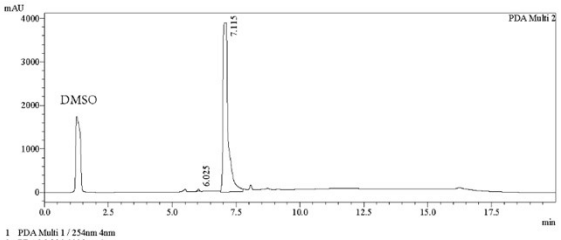




1 PDA Multi 1 / 254nm 4nm
2 PDA Multi 2 / 220nm 4nm

PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.025	224446	44876	1.347	1.724
2	7.076	16434733	2558207	98.653	98.276
Total		16659179	2603083	100.000	100.000

PeakTable

PDA Ch2 220nm 4nm

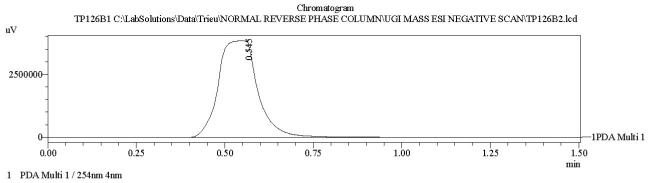
Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.025	218499	45903	0.403	1.166
2	7.115	54050292	3891227	99.597	98.834
Total		54268791	3937130	100.000	100.000

==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

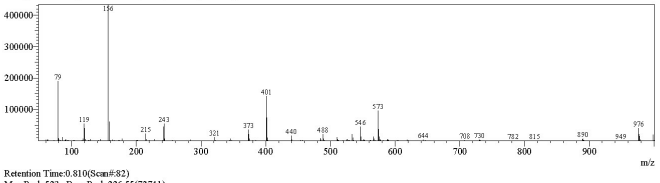
Acquired by	: Admin
Date Acquired	: 7/23/2015 6:26:49 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: TP126B1
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 59
Injection Volume	: 5
Data File	: TP126B2.lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Kelly\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/23/2015 6:28:20 PM



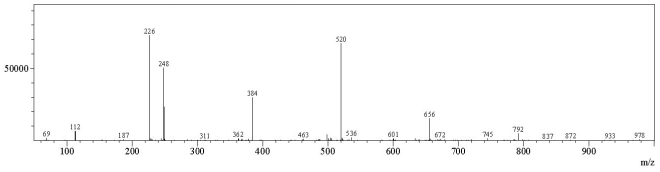
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

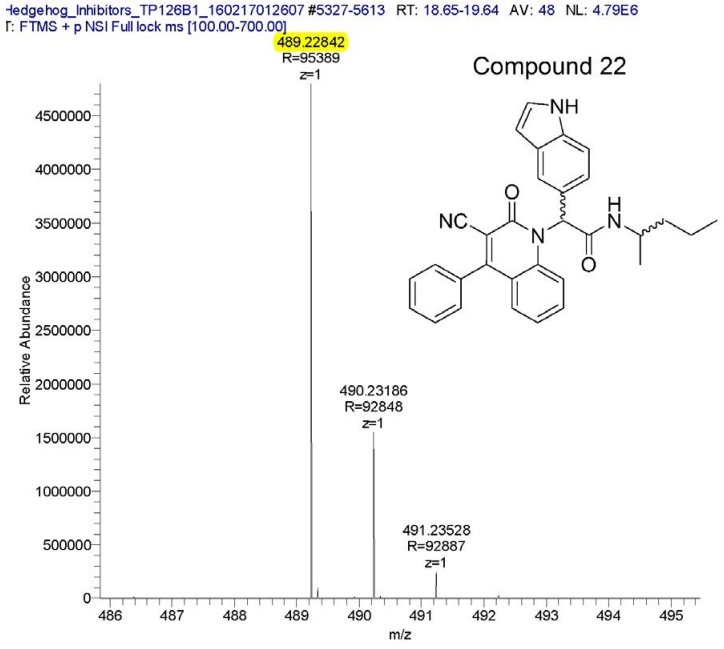
Retention Time:0.820(Scan#:83)
Max Peak:535 Base Peak:156.55(669410)
Spectrum:Averaged 0.620-1.300(63-131)
Background:Averaged 0.100-0.454(11-47) Polarity:Pos Segment1 - Event1



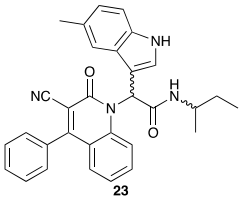
Retention Time:0.810(Scan#:82)
Max Peak:523 Base Peak:226.55(72741)
Spectrum:Averaged 0.630-1.310(64-132)
Background:Averaged 0.110-0.454(12-48) Polarity:Neg Segment1 - Event2



Compound	Chemical Formula	Predicted monoisotopic neutral MW	Predicted MH+	MH+ Measured	Main MS Ions	Main MS/MS Fragments
TP126B1	C31H28N4O2	488.2212	489.2285	489.2284	402.1253 243.14907 (fragment) 489.2284	402.1245 374.1294 215.1574



2-(3-cyano-2-oxo-4-phenylquinolin-1(2*H*)-yl)-2-(5-methyl-1*H*-indole-3-yl)-*N*-(pentan-2-yl)acetamide (**23**)



Yield: 46%; **MP** 178-180 ºC

IR (υ_{max}/cm^{-1}): 3427 (bp NH), 2962 (CH), 2236 (CN), 1645 (CON);

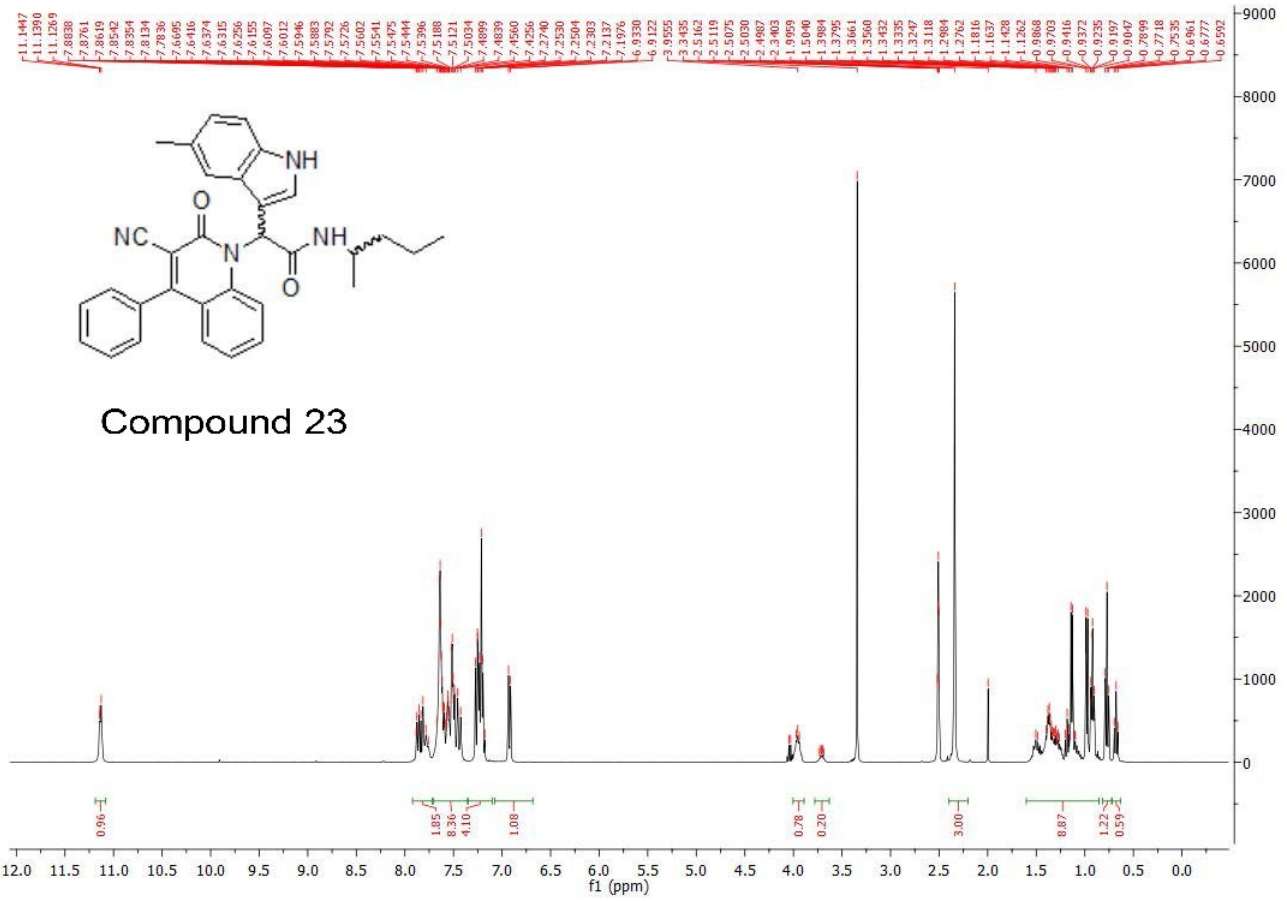
The ^{1}H NMR displays a mixture of isomers, with the ratio 2.1 : 1.0 calculated at 0.77 and 0.68 ppm, respectively. ^{1}H NMR spectral data is reported as a whole without splitting due to the complex overlapping. All peaks detected in ^{13}C NMR are reported.

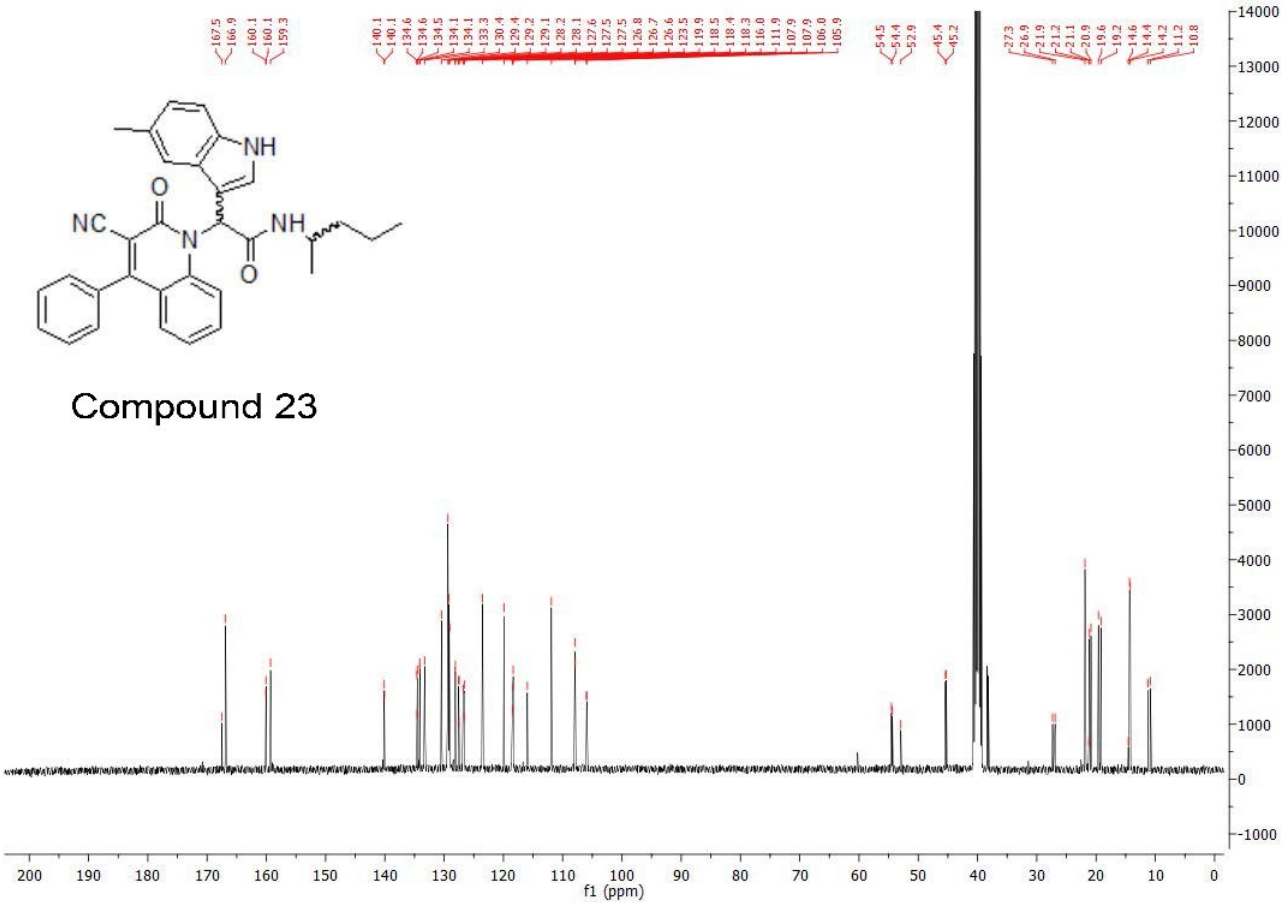
^{1}H NMR (400 MHz, DMSO) δ 11.13 (d, *J* = 4.9 Hz, 1H), 7.90 – 7.37 (m, 10H), 7.29-7.16 (m, 4H), 6.92 (d, *J* = 8.3 Hz, 1H), 4.03 – 3.87 (m, 1H), 2.34 (s, 3H), 1.57 – 1.20 (m, 3H), 1.20 – 0.86 (m, 5H), 0.82-0.60 (m, 3H).

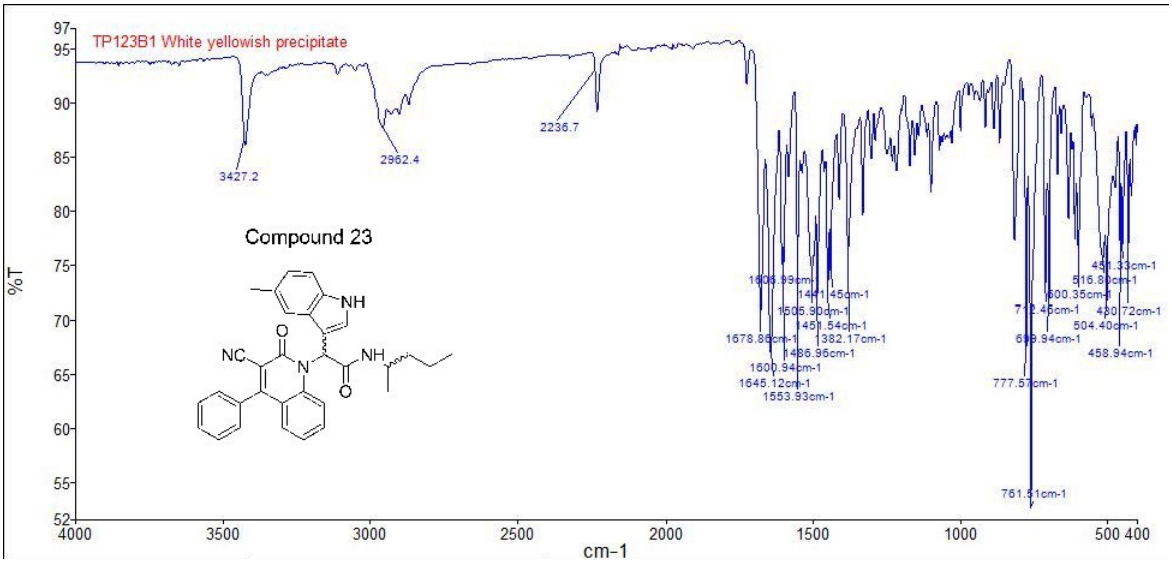
^{13}C NMR (101 MHz, DMSO-*d6*) δ 167.5, 166.9, 160.1, 160.1, 159.3, 140.1, 140.1, 134.6, 134.6, 134.5, 134.1, 133.3, 130.4, 129.4, 129.2, 129.1, 128.2, 128.1, 127.5, 127.5, 126.8, 126.6, 123.5, 119.9, 118.5, 118.4, 118.3, 116.0, 111.9, 107.9, 107.9, 106.0, 105.9, 54.5, 54.4, 52.9, 45.4, 45.2, 38.4, 38.2, 27.3, 26.9, 21.9, 21.1, 20.9, 19.6, 19.2, 14.4, 14.2, 11.2, 10.8;

RP-HPLC Alltima™ C18 5 μ 150 mm x 4.6 mm, 10–100% B in 15 min, Rt min=10.89, 100%;

LRMS (ESI-) m/z 502, 521 $[M+NH_4]^+$ 40%. HRMS (ES+) for $C_{32}H_{30}N_4O_2$; calculated 503.2442, found 503.2444.




Compound 23
f1 (ppm)


TP123B1 White yellowish precipitate
3427.2
2962.4
2236.7
Compound 23
%T
cm-1

<Sample Information>

Sample Name	: TP123B1 E		
Sample ID	: TP123B1 E		
Data Filename	: TP123B1 E.lcd		
Method Filename	: 30-100 over 15 mins.lcm		
Batch Filename	: TP174-176B3 10-100 over 15mins.lcb		
Vial #	: 1-10	Sample Type	: Unknown
Injection Volume	: 15 uL		
Date Acquired	: 8/08/2014 1:33:34 PM	Acquired by	: System Administrator
Date Processed	: 8/08/2014 2:03:35 PM	Processed by	: System Administrator

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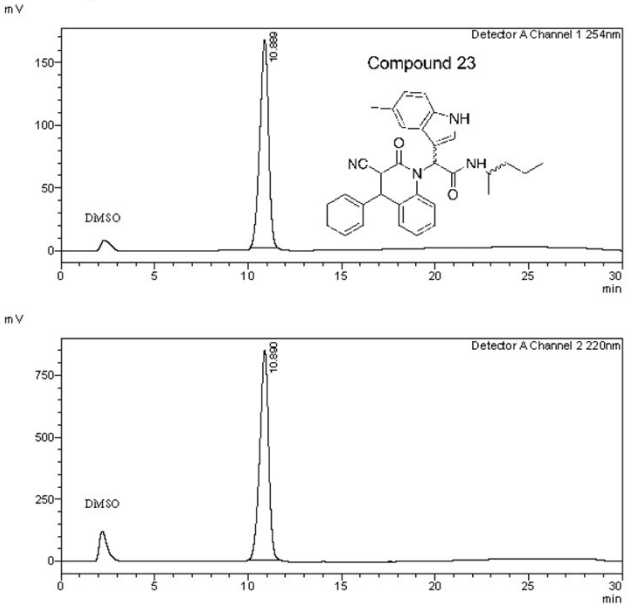


<Peak Table>

Detector A Channel 1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	10.889	5320260	165568	100.000		M	
Total		5320260	165568				

Detector A Channel 2 220nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	10.890	27640600	846427	100.000		M	
Total		27640600	846427				

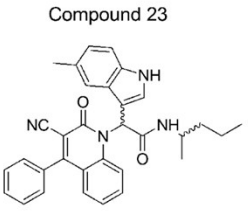
C:\LabSolutions\Data\Project1\TRIEU\TP123B1 E.lcd

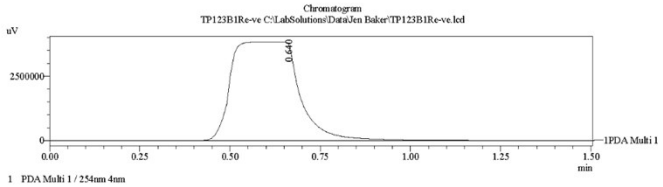
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

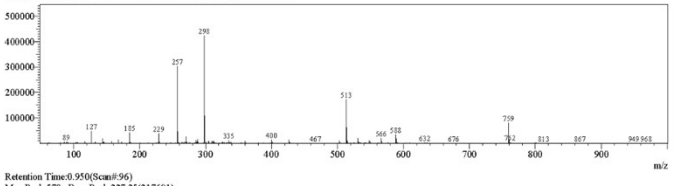
Acquired by	: Admin
Date Acquired	: 10/13/2015 12:57:05 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: TP123B1Re-ve
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 30
Injection Volume	: 10
Data File	: TP123B1Re-ve.lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Jen Baker\FIA-ESI_Scan(-).lcm
Report Format	: Default.LCMS.lcr
Tuning File	: C:\LabSolutions\Data\AutoTuning\Autotune_ESI_26AUG15.lct
Processed by	: Admin
Modified Date	: 10/13/2015 12:58:37 PM



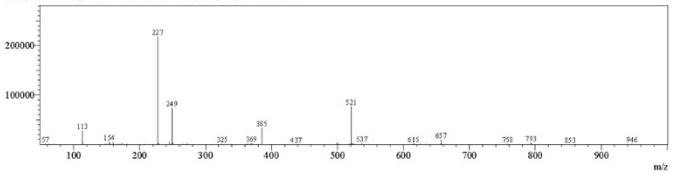


<Spectrum>

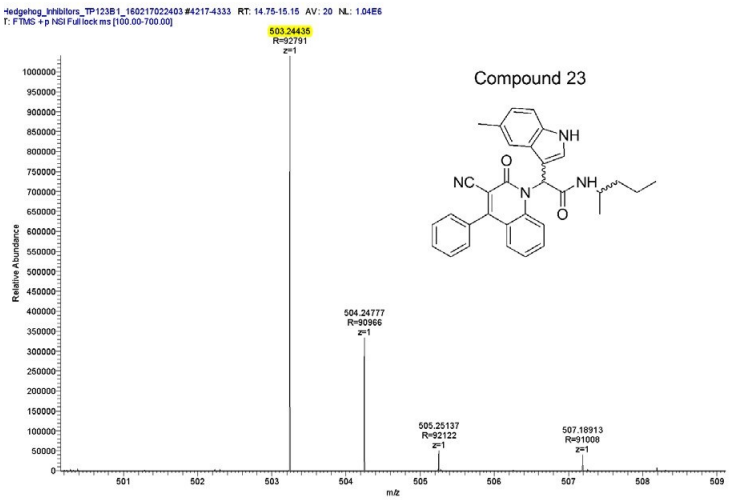
Retention Time:0.620(Scan#:63)
Max Peak:593 Base Peak:298.10(423998)
Spectrum:Averaged 0.620-1.400(63-141)
Background:Averaged 0.020-0.606(3-61) Polarity:Pos Segment1 - Event1



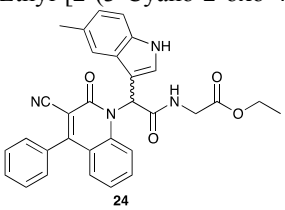
Retention Time:0.950(Scan#:96)
Max Peak:570 Base Peak:227.25(217681)
Spectrum:Averaged 0.630-1.410(64-142)
Background:Averaged 0.030-0.606(4-62) Polarity:Neg Segment1 - Event2



Compound	Chemical Formula	Predicted monoisotopic neutral MW	Predicted MH+	MH+ Measured	Main MS Ions	Main MS/MS Fragments
TP123B1	C32H30N4O2	502.2369	503.2442	503.2444	257.16473 (fragment) 247.0866 (fragment)	257.1653 86.0971 144.0811



Ethyl-[2-(3-Cyano-2-oxo-4-phenyl-2*H*-quinolin-1-yl)-2-(5-methyl-1*H*-indol-3-yl)-acetamido]-acetate (**24**)



24

Yield 34.8%; MP 199-200 ºC

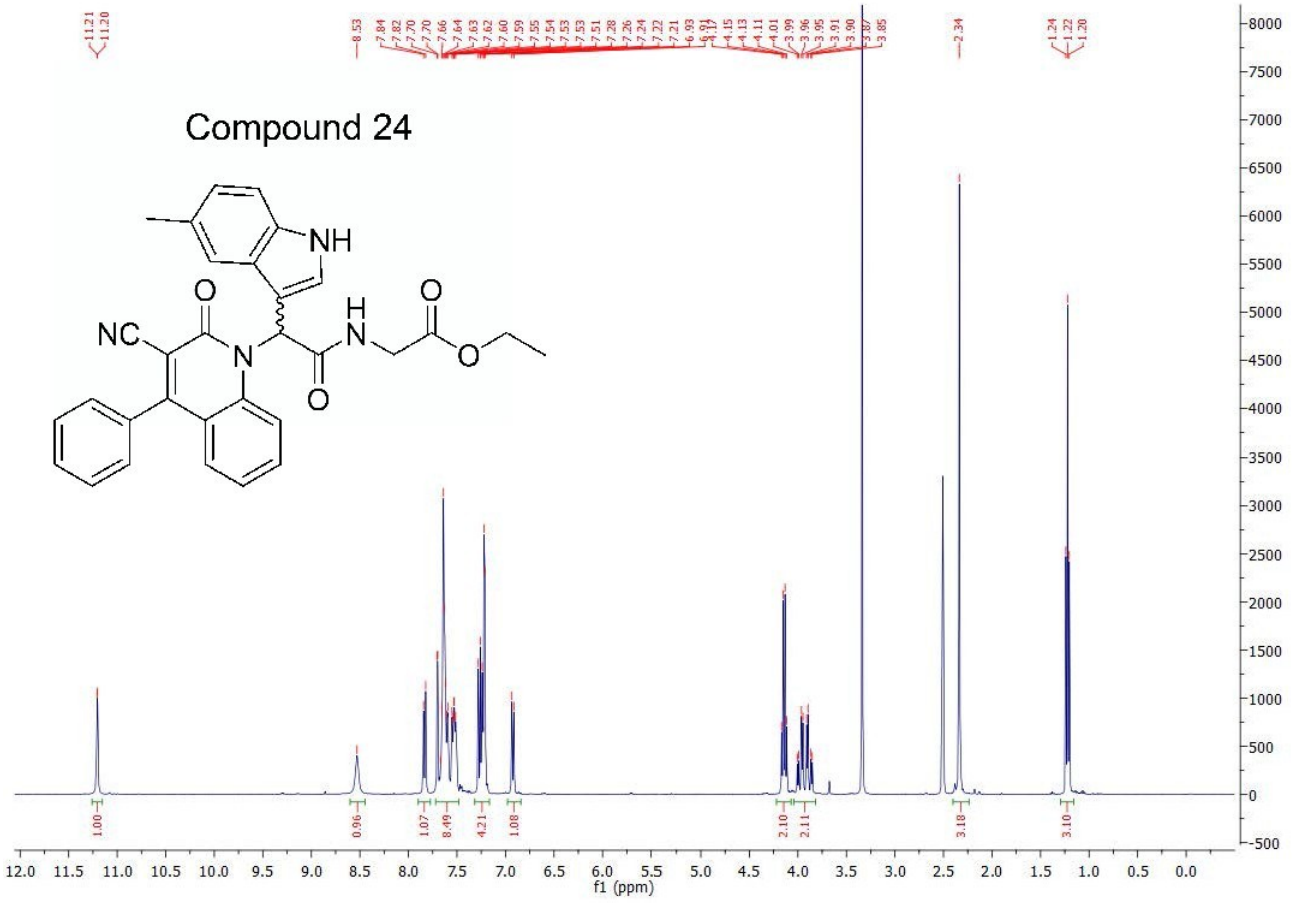
IR (ν_{max}/cm^{-1}): 3423 (NH), 3410 (NH), 2232 (CN), 1731 (COO), 1673 (CON);

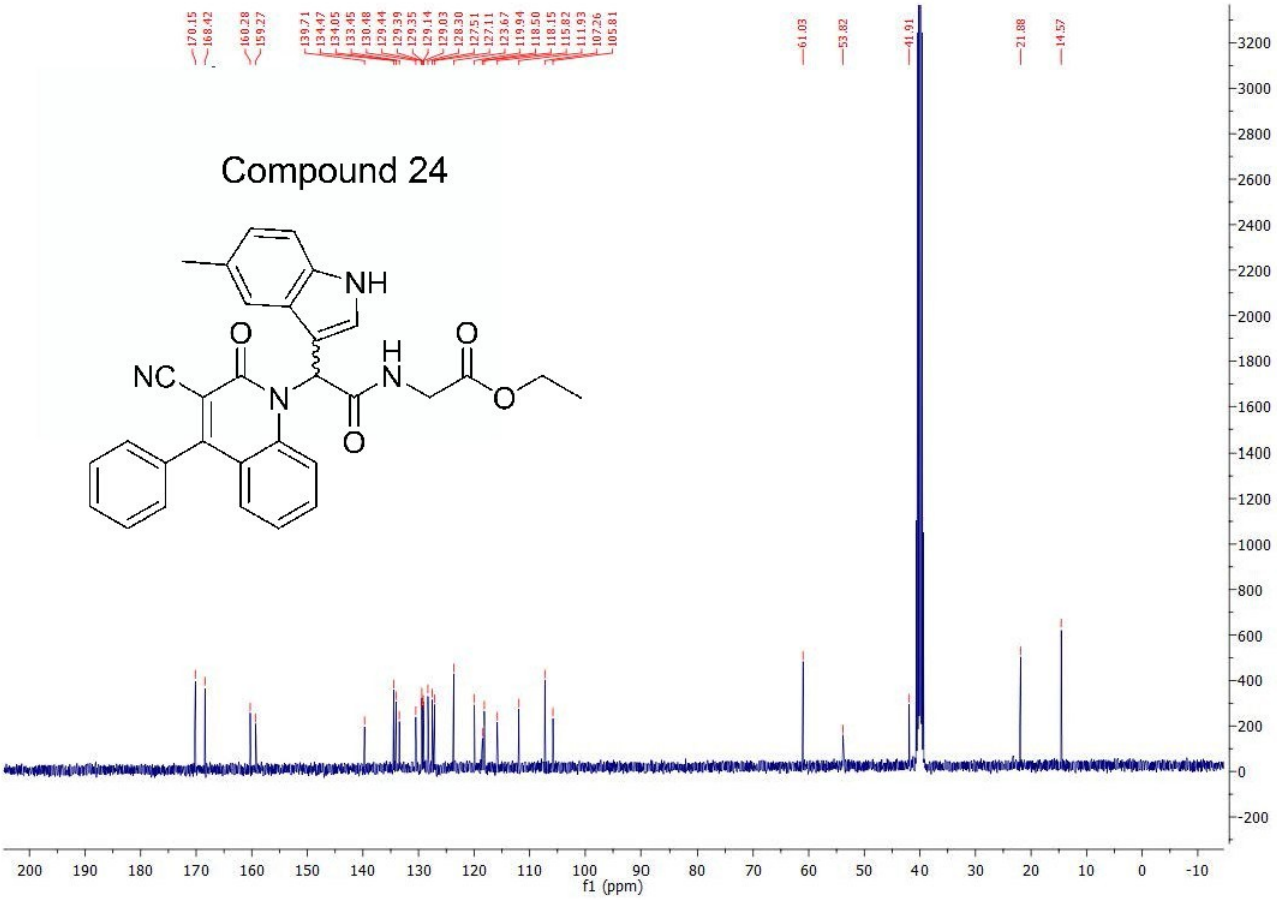
^{1}H NMR (400 MHz, DMSO) δ 11.21 (d, *J* = 1.8 Hz, 1H), 8.53 (s, 1H), 7.83 (d, *J* = 8.7 Hz, 1H), 7.72 – 7.48 (m, 8H), 7.32 – 7.17 (m, 4H), 6.92 (d, *J* = 8.3 Hz, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 4.02-3.84 (m, 2H), 2.34 (s, 3H), 1.22 (t, *J* = 7.1 Hz, 3H).

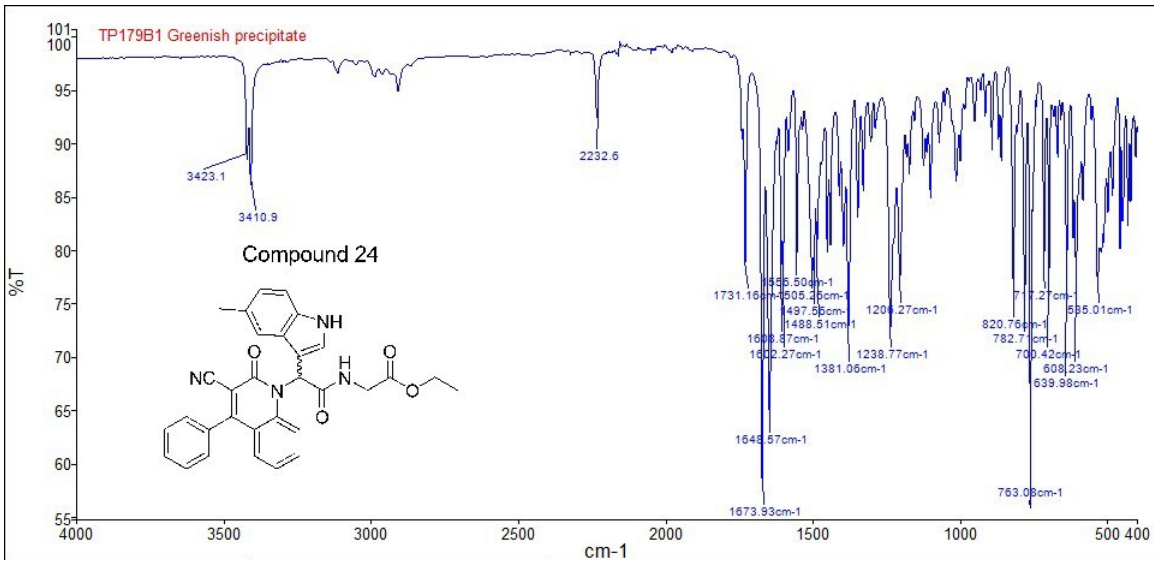
^{13}C NMR (101 MHz, DMSO-*d6*) δ 170.2, 168.4, 160.3, 159.3, 139.7, 134.5, 134.1, 133.5, 130.5, 129.4, 129.4, 129.4, 129.1, 129.0, 128.3, 127.5, 127.1, 123.7 (Cx2), 119.9, 118.5, 118.2, 115.8, 111.9, 107.3, 105.8, 61.0, 53.8, 41.9, 21.9, 14.6.

RP-HPLC Alltima™ C18 5u 150 mm x4.6 mm, 10–100% B in 15 min, Rt min=13.72, 97.9% (254nm and 220nm)

LRMS (ESI+) m/z518, 541 [M+Na-H]$^{+}$ 60%. HRMS (ES+) for $C_{31}H_{26}N_4O_4$; calculated 519.2027, found 519.2026




Compound 24
NH
O
NC
N
H
N
O
O
O
170.15
168.42
160.28
159.27
139.71
134.47
134.05
133.45
130.48
129.44
129.39
129.35
129.14
129.03
128.30
127.51
127.11
123.67
119.94
118.50
118.15
115.82
111.93
107.26
105.81
61.03
53.82
41.91
21.88
14.57
f1 (ppm)


TP179B1 Greenish precipitate
Compound 24
NH
NC
3423.1
3410.9
2232.6
1731.16cm-1
1673.93cm-1
1648.57cm-1
1381.06cm-1
1238.77cm-1
1204.27cm-1
763.08cm-1
%T
cm-1

<Sample Information>

Sample Name	: TP179B1		
Sample ID	: TP179B1		
Data Filename	: TP179B1.lcd		
Method Filename	: 10-100 over 15 mins.lcm		
Batch Filename	: TRIEU Second Third Generation and New pro.lcb		
Vial #	: 1-17	Sample Type	: Unknown
Injection Volume	: 30 uL		
Date Acquired	: 8/09/2014 2:39:27 PM	Acquired by	: System Administrator
Date Processed	: 8/09/2014 3:09:29 PM	Processed by	: System Administrator

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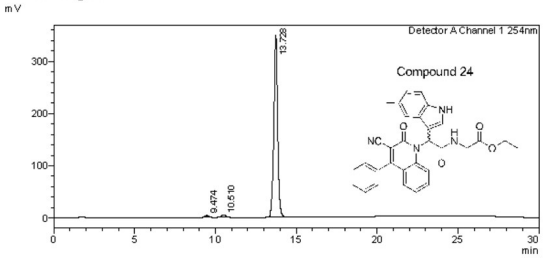

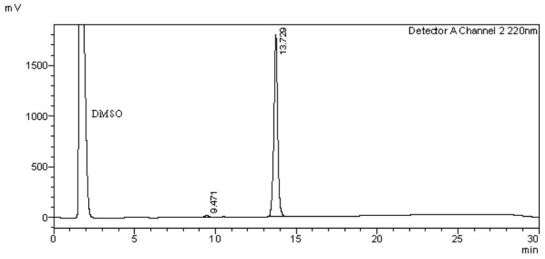

<Peak Table>

Detector A Channel 1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	9.474	50354	3410	0.891		M	
2	10.510	63506	3798	1.124		M	
3	13.728	5535547	348160	97.985		M	
Total		5649407	355368				

Detector A Channel 2 220nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	9.471	275545	15893	0.956		M	
2	13.729	28561970	1790048	99.044		M	
Total		28837515	1805941				

==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

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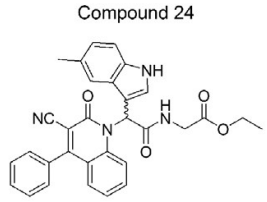
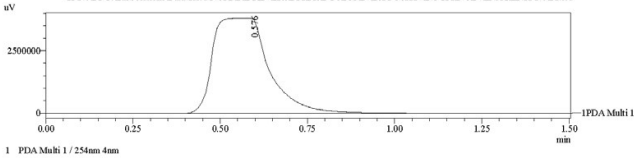
Date Acquired	: 7/24/2015 9:57:16 AM
Sample Type	: Unknown
Level#	: 0
Sample Name	: TP179B1
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 52
Injection Volume	: 15
Data File	: TP179B2.lcd
Method File	: FIA-ESI_Scan(+).lcm
Original Method	: C:\LabSolutions\Data\Trieu\Mass spec files\FIA-ESI_Scan(+).lcm
Report Format	: Default.LCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/24/2015 9:58:48 AM



Chromatogram

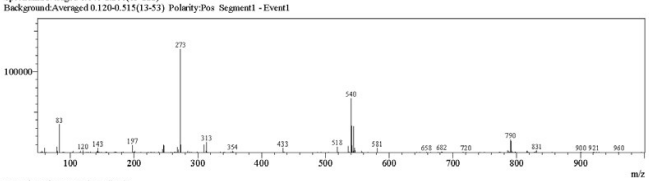
TP179B1 C:\LabSolutions\Data\Trieu\NORMAL REVERSE PHASE COLUMN\ESI POSITIVE\UGI KNOEVENAGEL\TP179B2.lcd



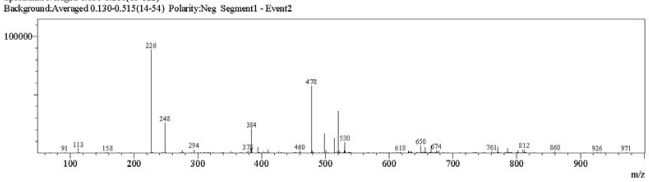
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.980(Scan#:99)
Max Peak:323 Base Peak:272.70(128022)
Spectrum:Averaged 0.640-1.200(65-121)
Background:Averaged 0.120-0.515(13-53) Polarity:Pos Segment1 - Event1

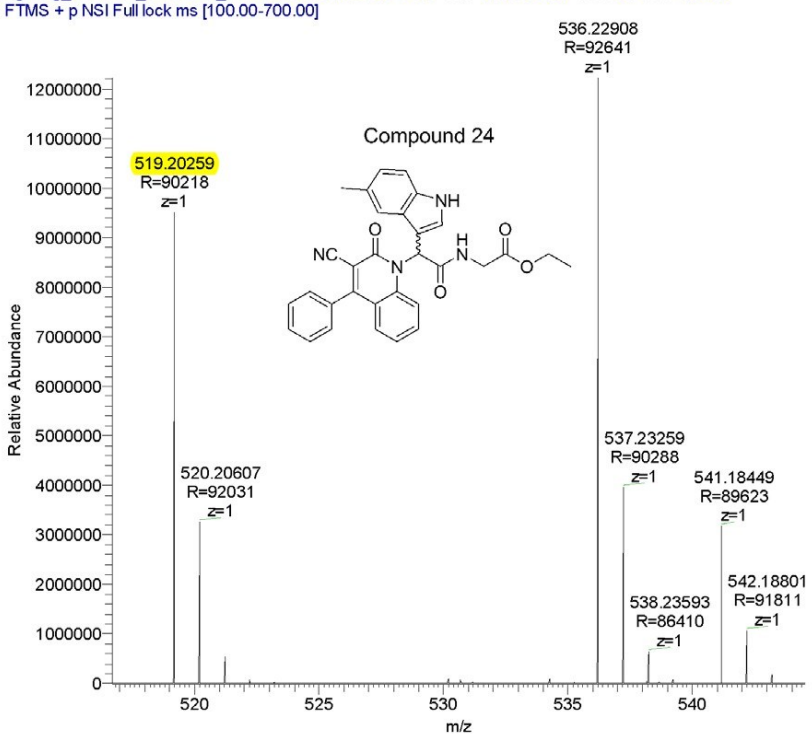


Retention Time:0.850(Scan#:86)
Max Peak:512 Base Peak:226.50(88595)
Spectrum:Averaged 0.650-1.210(66-122)
Background:Averaged 0.130-0.515(14-54) Polarity:Neg Segment1 - Event2

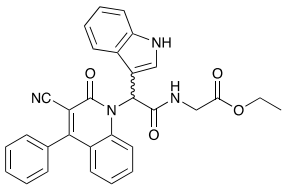


Compound	Chemical Formula	Predicted monoisotopic neutral MW	Predicted MH+	MH+ Measured	Main MS Ions	Main MS/MS Fragments
TP179B1	C31H26N4O4	518.1954	519.2027	519.2026	273.12305 (fragment) 536.22908(+NH4) 519.2026	273.1237 245.1288

Hedgehog_Inhibitors_TP179B1_160217032158 #5152-5295 RT: 18.01-18.49 AV: 24 NL: 1.22E7
T: FTMS + p NSI Full lock ms [100.00-700.00]



Ethyl-[2-(3-Cyano-2-oxo-4-phenyl-2H-quinolin-1-yl)-2-(1H-indol-3-yl)-acetamido]-acetate (**25**)



Yield: 32%; MP 179.3-180.5 ºC;

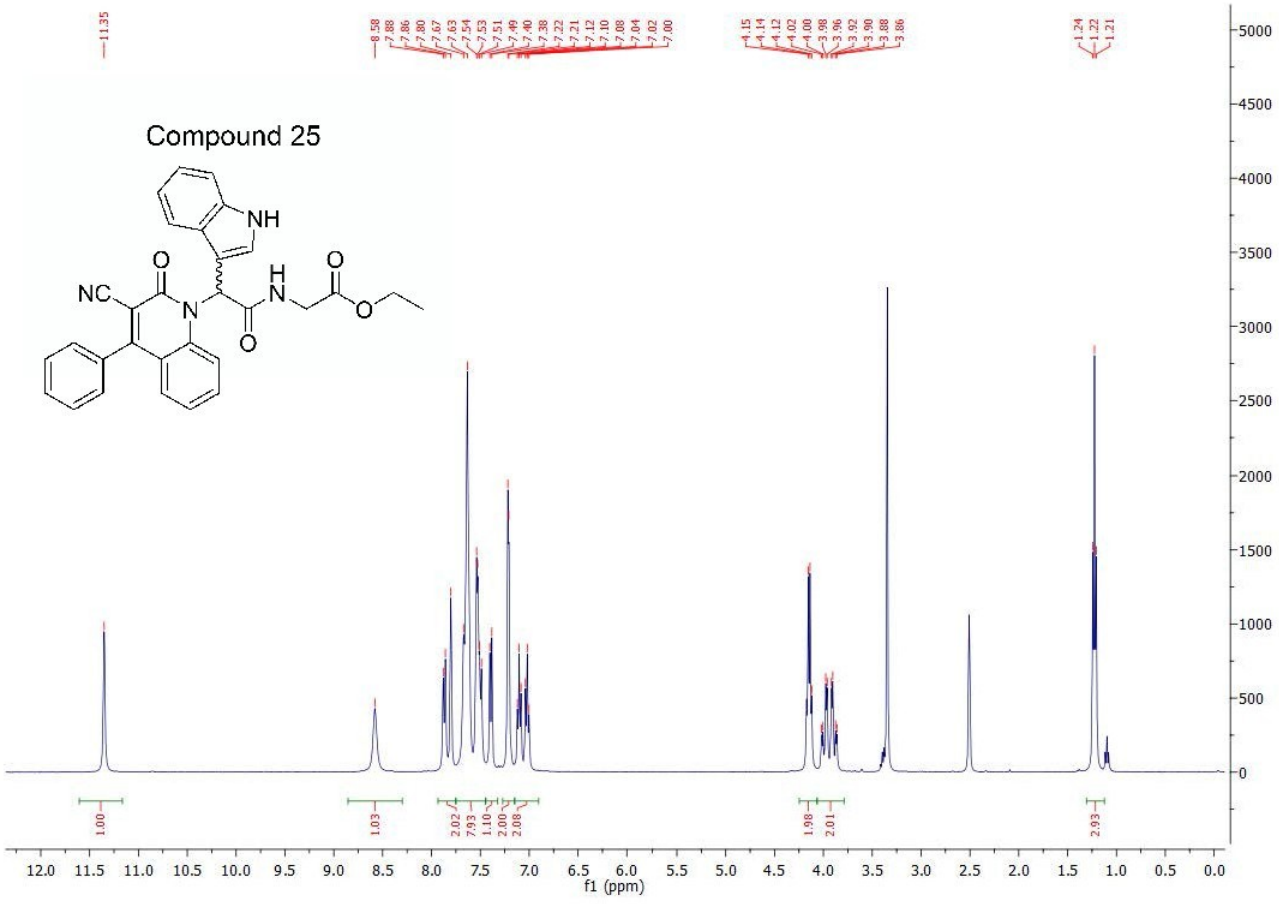
IR (υ_{max}/cm^{-1}) 3420 (NH), 2236 (CN), 1737 (COO), 1686 (CONH), 1646 (CON)

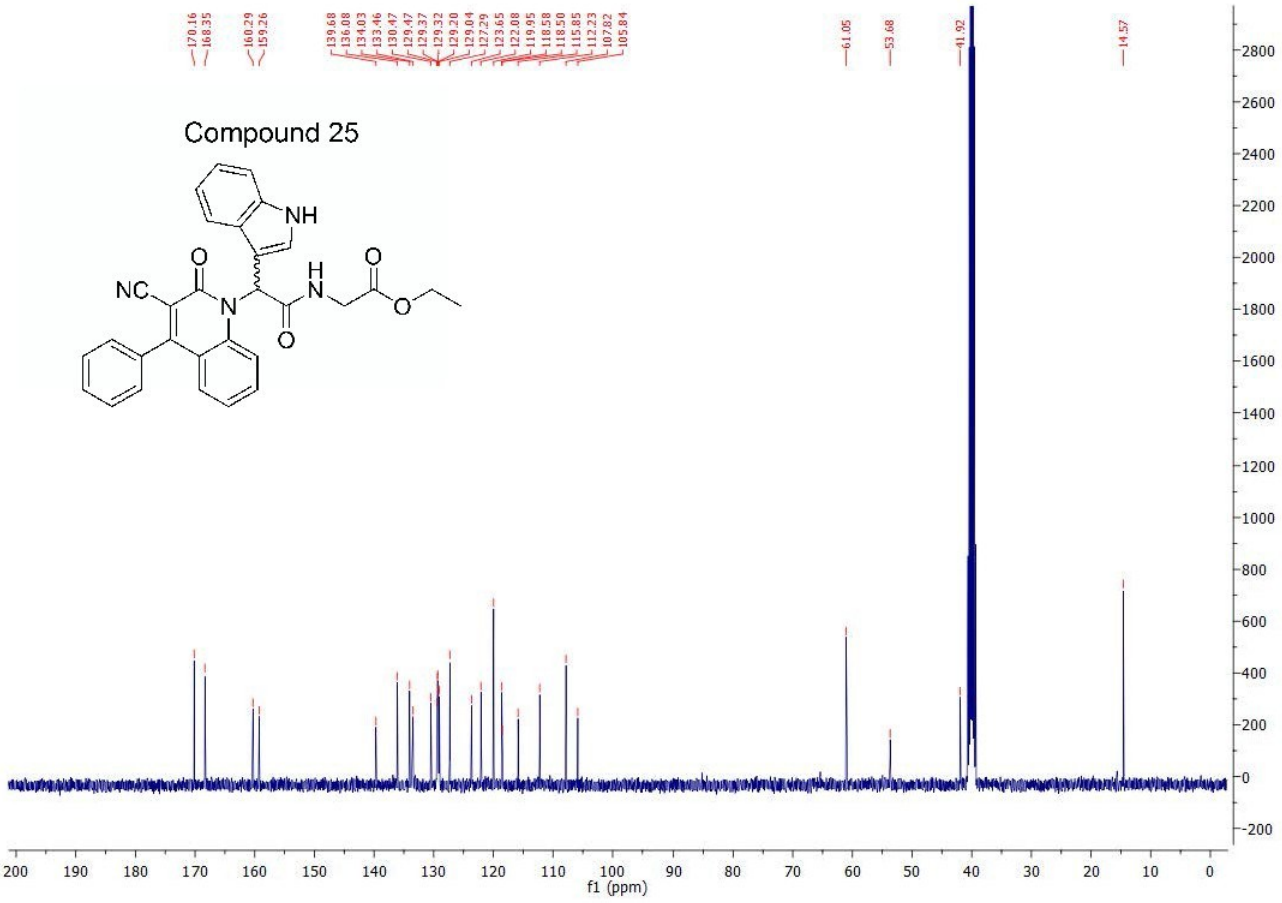
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.35 (s, 1H), 8.58 (s, 1H), 7.93 – 7.75 (m, 2H), 7.75-7.45 (m, 8H), 7.39 (d, J = 8.0 Hz, 1H), 7.21 (d, J = 3.7 Hz, 2H), 7.15-6.91 (m, 2H), 4.25 – 4.06 (m, 2H), 4.04-3.80 (m, 2H), 1.22 (t, J = 7.0 Hz, 3H).

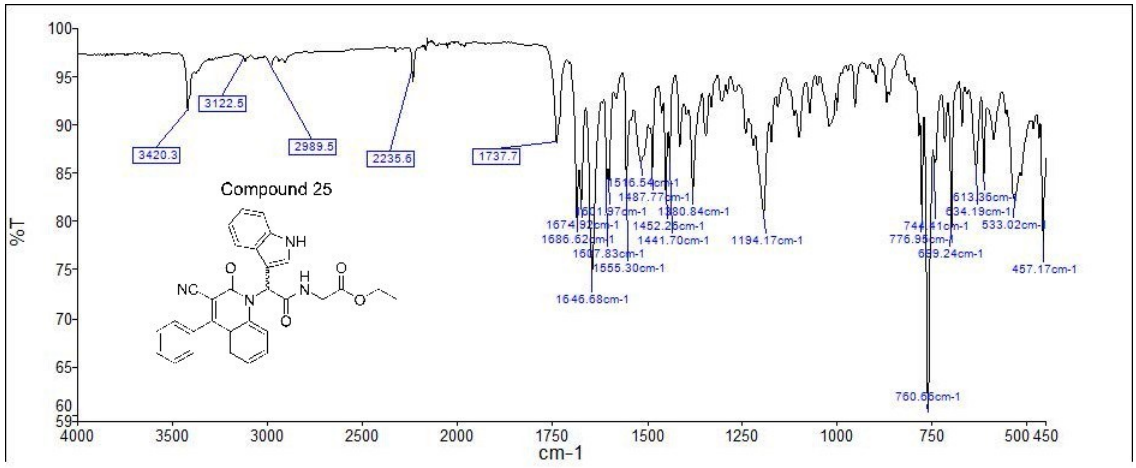
^{13}C NMR (101 MHz, DMSO-*d6*) δ 170.2, 168.4, 160.3, 159.3, 139.7, 136.1, 134.0, 133.5, 130.5, 129.5, 129.4 (Cx2), 129.3 (Cx2), 129.2, 129.0, 127.3, 123.7, 122.1, 120.0 (Cx2), 118.6, 118.5, 115.9, 112.2, 107.8, 105.8, 61.1, 53.7 41.9, 14.6.

RP-HPLC Phenomenex Onyx™ Monolithic C18 5 µm 100 mm x 4 mm, 10–100% B in 15 min, Rt min = 11.09, 100%.

LRMS (ESI+) m/z 504, 505 [M+H]$^{+}$, 100%. HRMS (ES+) for $C_{30}H_{24}N_4O_4$; calculated 505.1870, found 505.1869.




Compound 25
NH
NC
O
N
H
N
O
O
O
170.16
168.35
160.29
159.26
139.68
136.08
134.03
133.46
130.47
129.47
129.37
129.32
129.20
129.04
127.29
123.65
122.08
119.95
118.58
118.50
115.85
112.23
107.82
105.84
61.05
53.68
41.92
14.57
f1 (ppm)


Compound 25
3420.3
3122.5
2989.5
2235.6
1737.7
1646.68cm-1
1555.30cm-1
1194.17cm-1
760.65cm-1
457.17cm-1
%T
cm-1

Data File C:\CHEM32\...EMO\PURIFIED TR S EPI AND S LUG AIPS 2016-01-05 09-33-14\034-3501.D
Sample Name: TP186B5

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Acq. Operator   : Simi120102015                Seq. Line :  35
Acq. Instrument : LC1260                       Location : Vial 34
Injection Date  : 1/6/2016 3:19:44 AM             Inj :  1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\DEMO\PURIFIED TR S EPI AND S LUG AIPS 2016-01-05 09-
                  33-14\10 TO 100 OV 15MIN 10UL.M
Last changed    : 12/10/2015 4:12:43 PM by Simi120102015
Analysis Method : C:\CHEM32\1\DATA\DEMO\PURIFIED TR S EPI AND S LUG AIPS 2016-01-05 09-
                  33-14\10 TO 100 OV 15MIN 20UL.M (Sequence Method)
Last changed    : 1/6/2016 10:52:32 AM by Simi120102015
                  (modified after loading)
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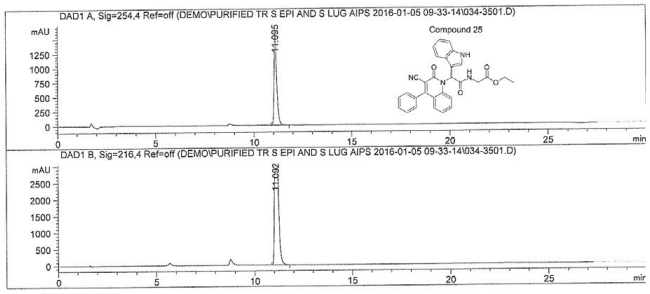



```
                        Area Percent Report

Sorted By             :      Signal
Multiplier:                  :      1.0000
Dilution:                    :      1.0000
Use Multiplier & Dilution Factor with ISTDs

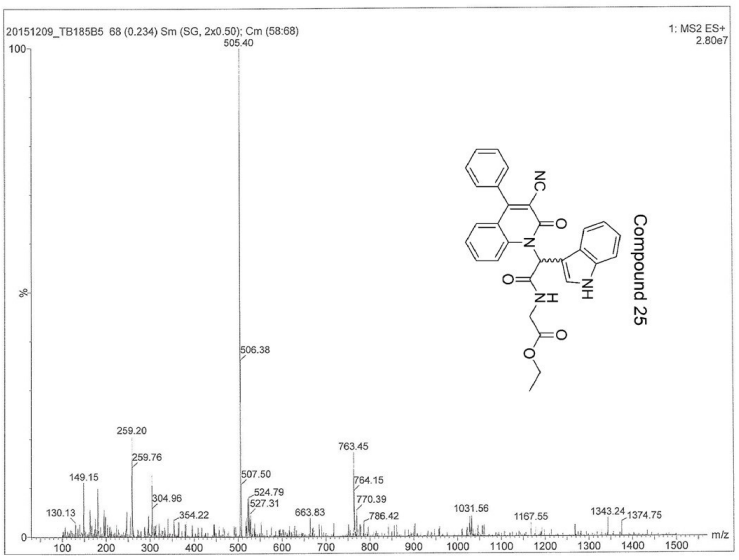

Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]      [mAU]      %
----|-------|----|-------|----------|----------|--------|
   1  11.095 BB    0.1291 1.49871e4  1664.42249 100.0000

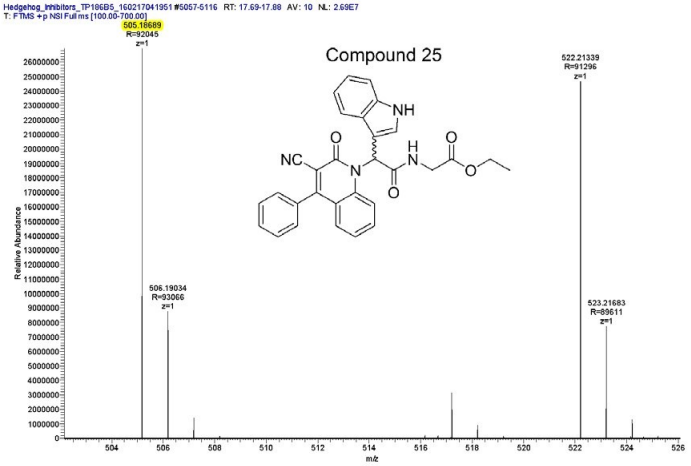
Totals :                  1.49871e4  1664.42249


Signal 2: DAD1 B, Sig=216,4 Ref=off

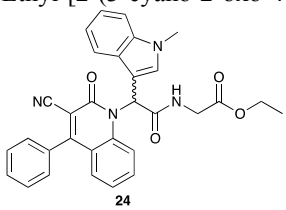
Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]      [mAU]      %
----|-------|----|-------|----------|----------|--------|
   1  11.092 BV    0.2252 4.76297e4  2936.25610 100.0000
```

Compound	Chemical Formula	Predicted monoisotopic neutral MW	Predicted MH+	MH+ Measured	Main MS Ions	Main MS/MS Fragments
TP 186B5	C30H24N4O4	504.1797	505.1870	505.1869	259.1074 (fragment) 505.1867 522.21336 (+NH4)	259.1080 231.1131



Ethyl-[2-(3-cyano-2-oxo-4-phenyl-2*H*-quinolin-1-yl)-2-(1-methylindole-3-yl)-acetamido]-acetate (**26**)



24

Yield: 27%; **MP** 209-211 ºC

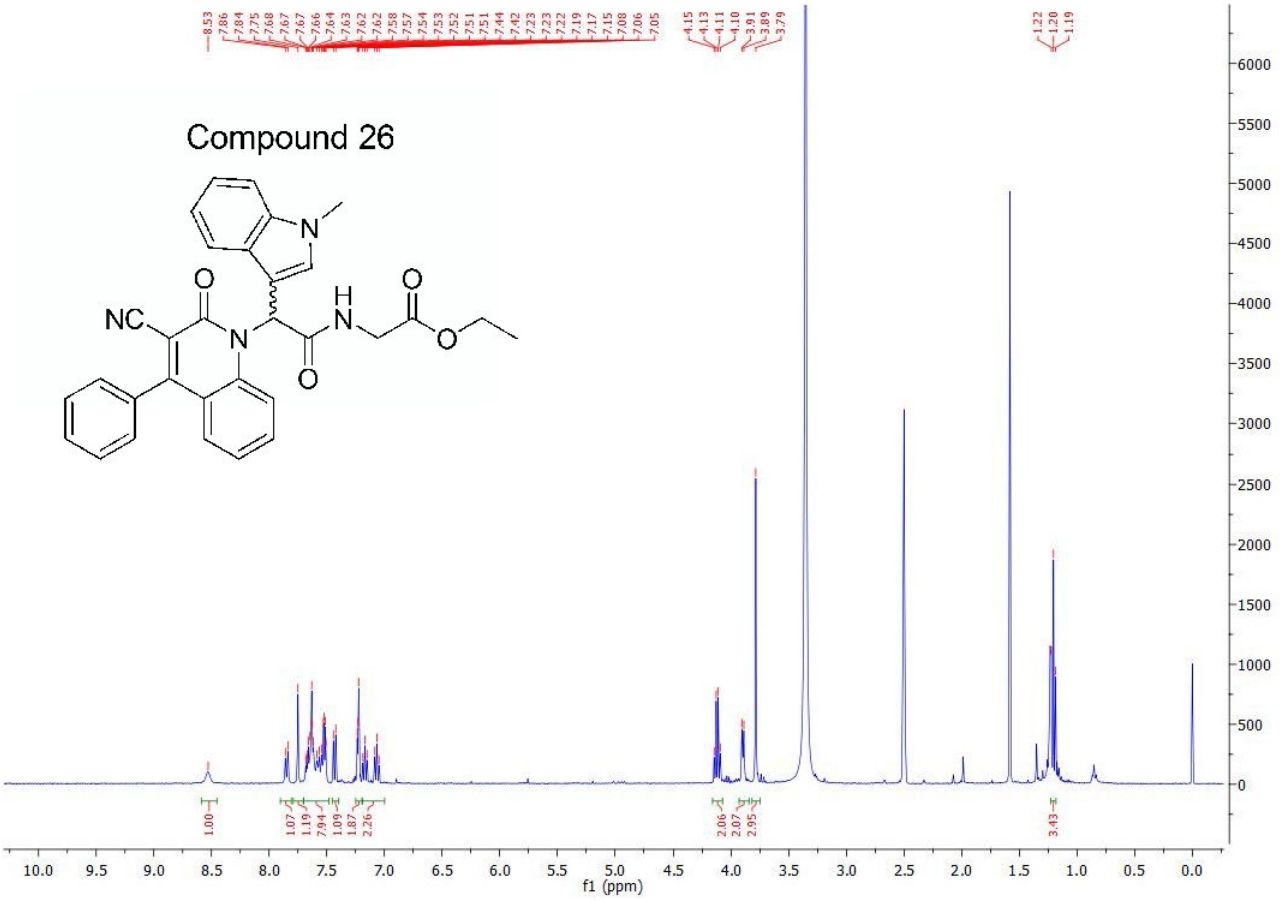
IR (u/cm^{-1}) 3422 (NH), 2920 (CH), 2229 (CN), 1743 (COO), 1639 (CON).

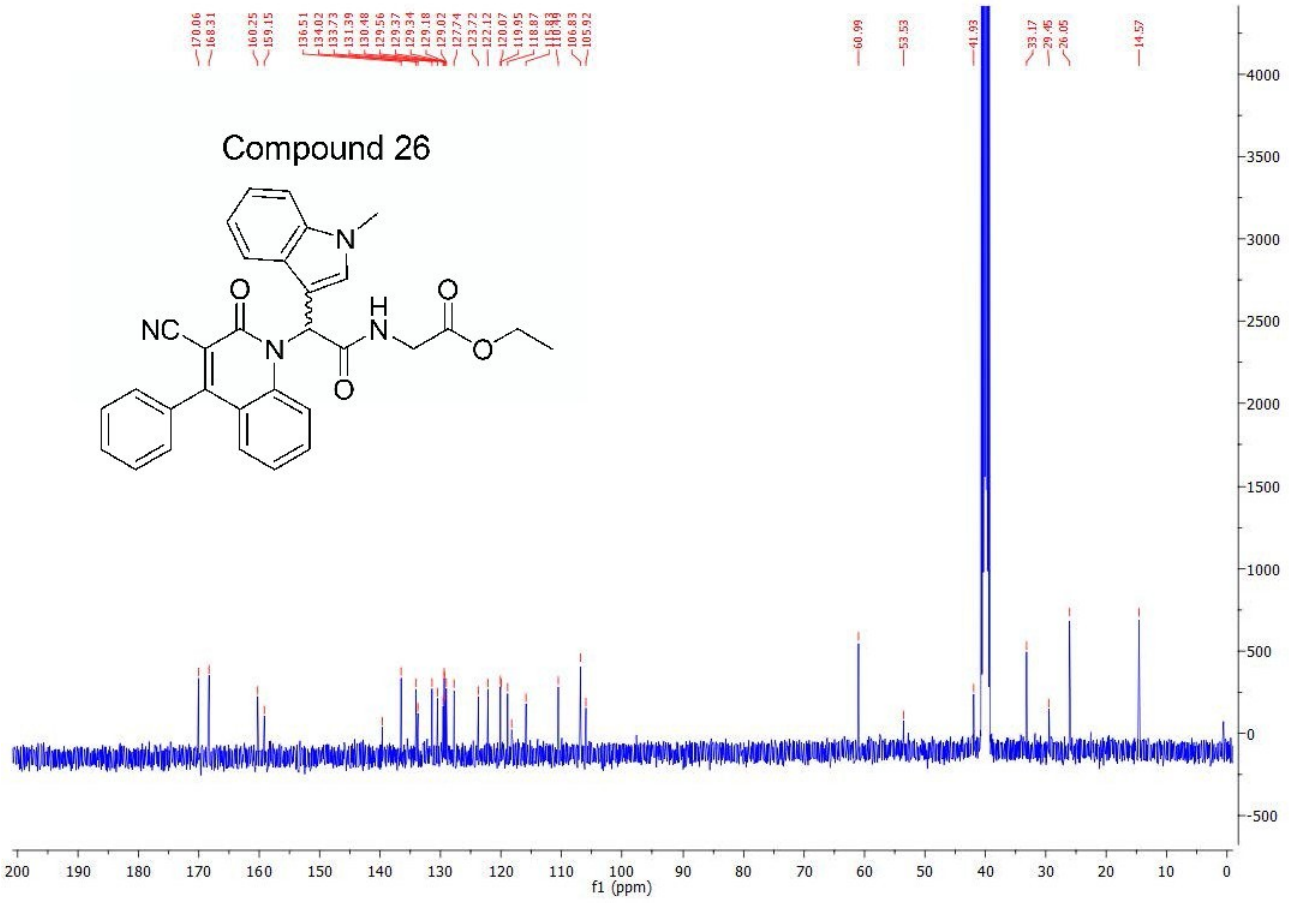
^{1}H NMR (400 MHz, DMSO) δ 8.53 (bs, 1H), 7.85 (d, J = 8.8 Hz, 1H), 7.75 (s, 1H), 7.70 – 7.48 (m, 7H), 7.43 (d, J = 8.2 Hz, 1H), 7.25 – 7.19 (m, 2H), 7.17 (t, *J* = 7.2 Hz, 1H), 7.06 (t, *J* = 7.2 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.90 (d, J = 6.6 Hz, 2H), 3.79 (s, 3H), 1.20 (t, J = 7.1 Hz, 3H).

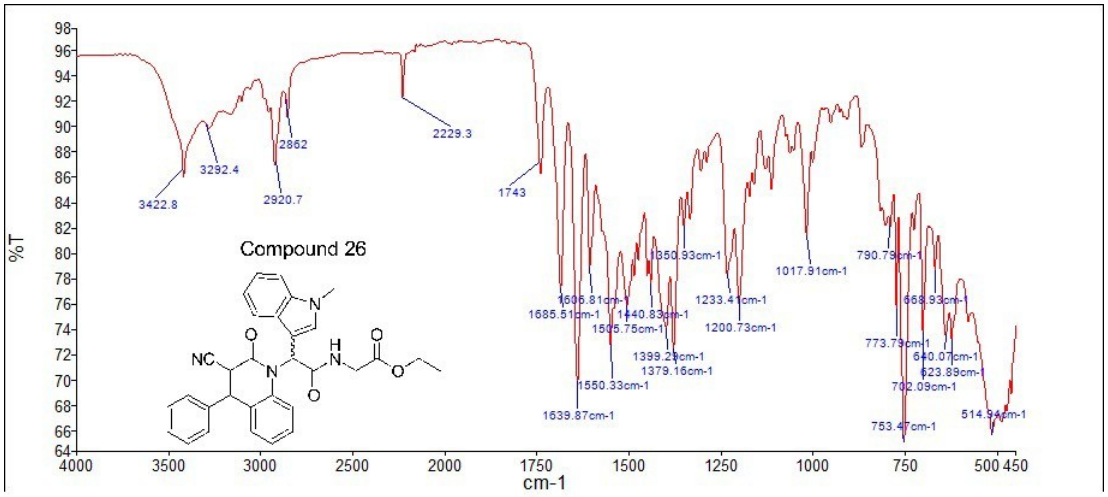
^{13}C NMR (101 MHz, DMSO-*d6*) δ 170.1, 168.3, 160.3, 159.2, 139.6, 136.5, 134.0, 133.7, 131.4, 130.5, 129.5, 129.4 (Cx2), 129.2, 129.0, 127.7, 123.7, 122.1, 120.1, 120.0, 118.9, 118.2, 115.9, 110.5, 106.8, 105.9, 105.9, 61.0, 41.9, 33.2, 14.6.

RP-HPLC Alltima™ C18 5mm 150 mm x4.6 mm, 10–100% B in 15 min Rt min=14.26, 98.10%

LRMS (ESI-) m/z 518, 540 [M+ Na-H]$^{+}$, 100%. HRMS (ES+) for $C_{31}H_{26}N_4O_4$; calculated 519.2027, found 519.2027




Compound 26
f1 (ppm)


Compound 26
%T
cm-1
3422.8
3292.4
2920.7
2862
2229.3
1743
1685.51cm-1
1639.87cm-1
1550.33cm-1
1379.16cm-1
1017.91cm-1
790.79cm-1
773.79cm-1
753.47cm-1
623.89cm-1
514.94cm-1

<Sample Information>

Sample Name	: TP151B1		
Sample ID	: TP151B1		
Data Filename	: TP151B1.lcd		
Method Filename	: 10-100 over 15 mins.lcm		
Batch Filename	: TRIEU Second Third Generation and New pro.lcb		
Vial #	: 1-20	Sample Type	: Unknown
Injection Volume	: 30 uL		
Date Acquired	: 8/09/2014 4:10:39 PM	Acquired by	: System Administrator
Date Processed	: 8/09/2014 4:40:41 PM	Processed by	: System Administrator

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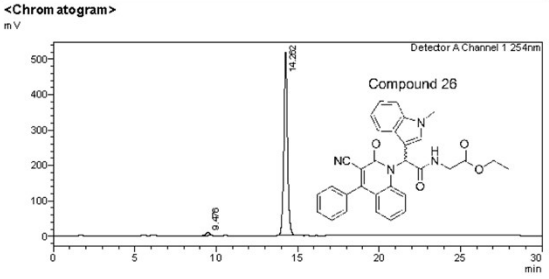

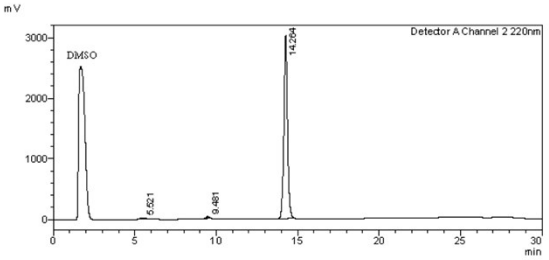

<Peak Table>

Detector A Channel 1 254nm

C:\LabSolutions\Data\Project1\TRIEU\TP151B1.lcd

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	9.476	152369	9271	1.856		M	
2	14.262	8055967	516482	98.144		M	
Total		8208335	525753				

Detector A Channel 2 220nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.521	466598	20316	0.980		M	
2	9.481	434968	31398	0.913		M	
3	14.264	46731373	3017885	98.107		M	
Total		47632939	3069600				

==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

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Date Acquired	: 7/23/2015 6:19:19 PM
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Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 56
Injection Volume	: 5
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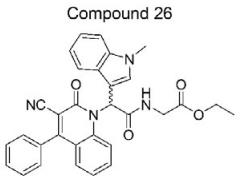
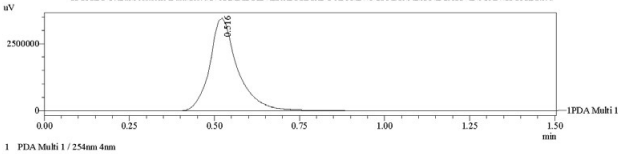
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Kelly\FIA-ESI_Scan(-).lcm
Report Format	: Default.LCMS.lsr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/23/2015 6:20:50 PM



Chromatogram

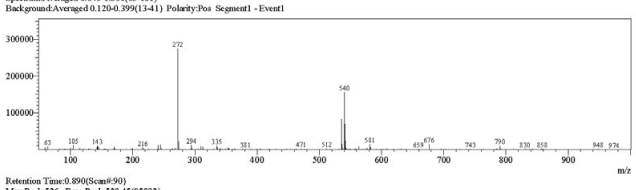
TP151B1 C:\LabSolutions\Data\Trieu\NORMAL REVERSE PHASE COLUMN\UGI MASS ESI NEGATIVE SCAN\TP151B2.lcd



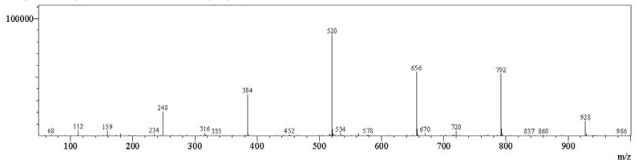
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

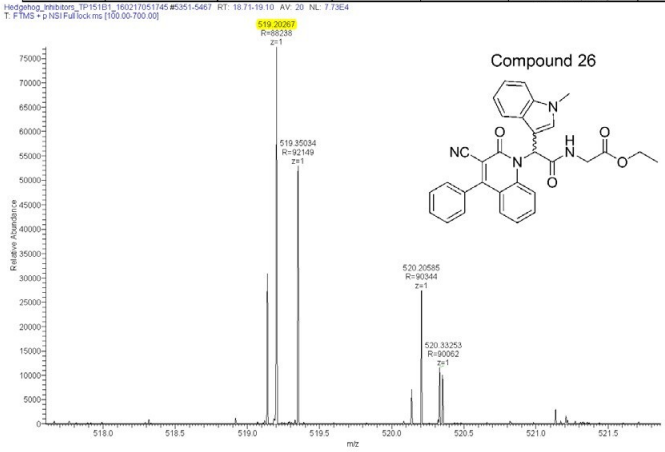
Retention Time:0.800(Scan#:81)
Max Peak:652 Base Peak:272.55(274674)
Spectrum:Averaged 0.640-1.300(65-131)
Background:Averaged 0.120-0.399(13-41) Polarity:Pos Segment1 - Event1

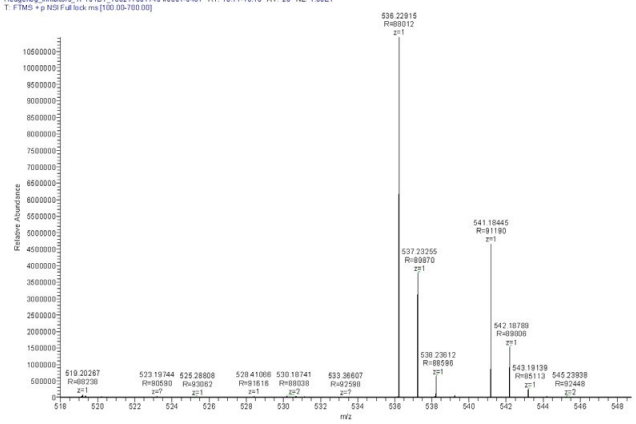


Retention Time:0.890(Scan#:90)
Max Peak:526 Base Peak:520.45(85932)
Spectrum:Averaged 0.650-1.310(66-132)
Background:Averaged 0.130-0.399(14-42) Polarity:Neg Segment1 - Event2

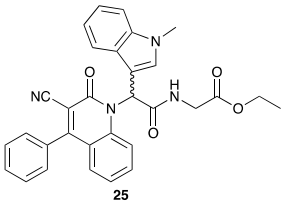


Compound	Chemical Formula	Predicted monoisotopic neutral MW	Predicted MH+	MH+ Measured	Main MS Ions	Main MS/MS Fragments
TP151B1	C31H26N4O4	518.1949	519.2027	519.2027*	273.1231 (fragment) 536.2292 (+Na) 541.1845 (+NH4)	273.1235





Ethyl-3-[2-(3-cyano-2-oxo-4-phenyl-2H-quinolin-1-yl)-2-(1-methyl-1H- indol-3-yl)-acetylamino]-propionate (27)



Yield: 50%; **M P:** 267-268 ºC

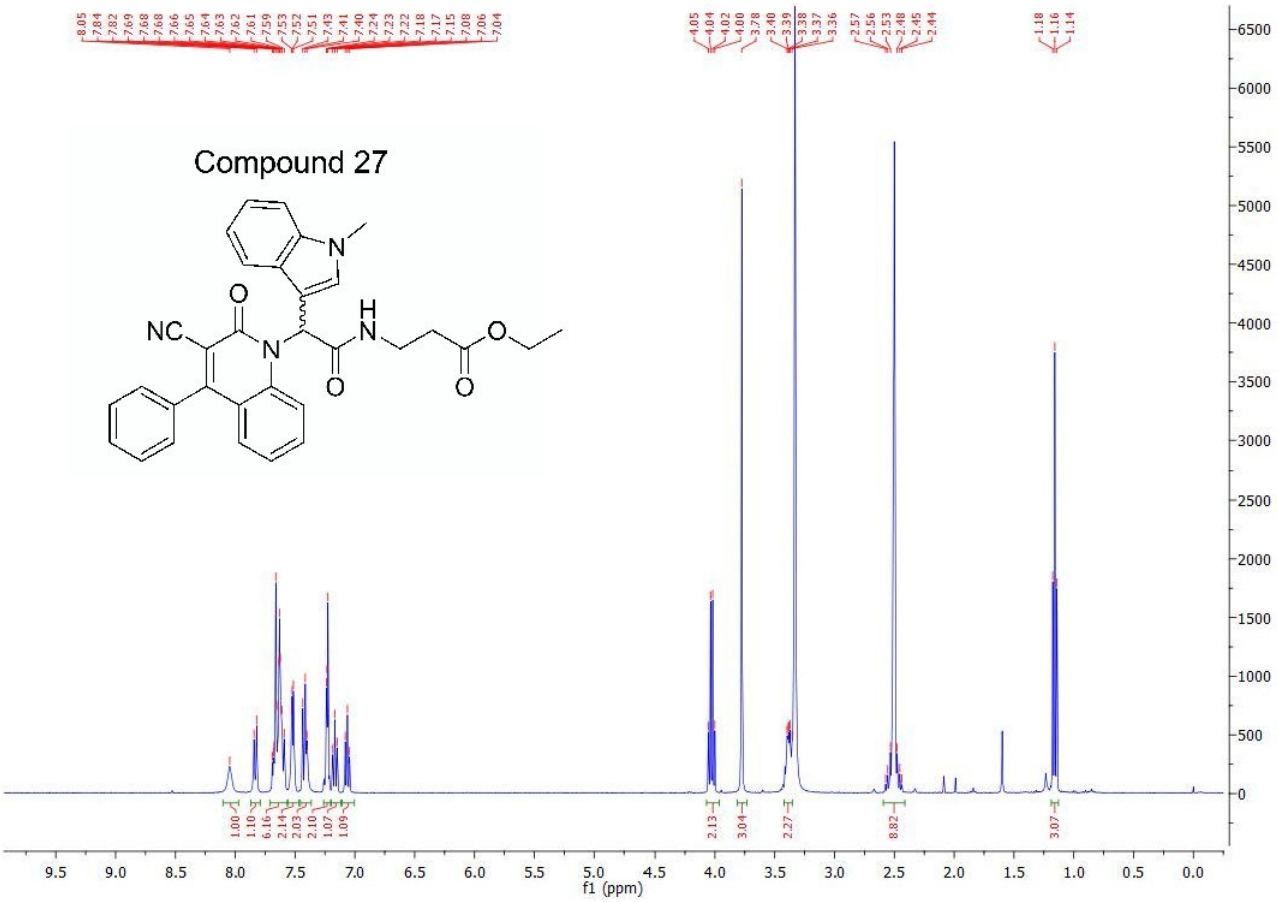
IR (νmax/cm$^{-1)}$ 3410 (NH), 2232(CN), 1710(COO), 1686(CON)

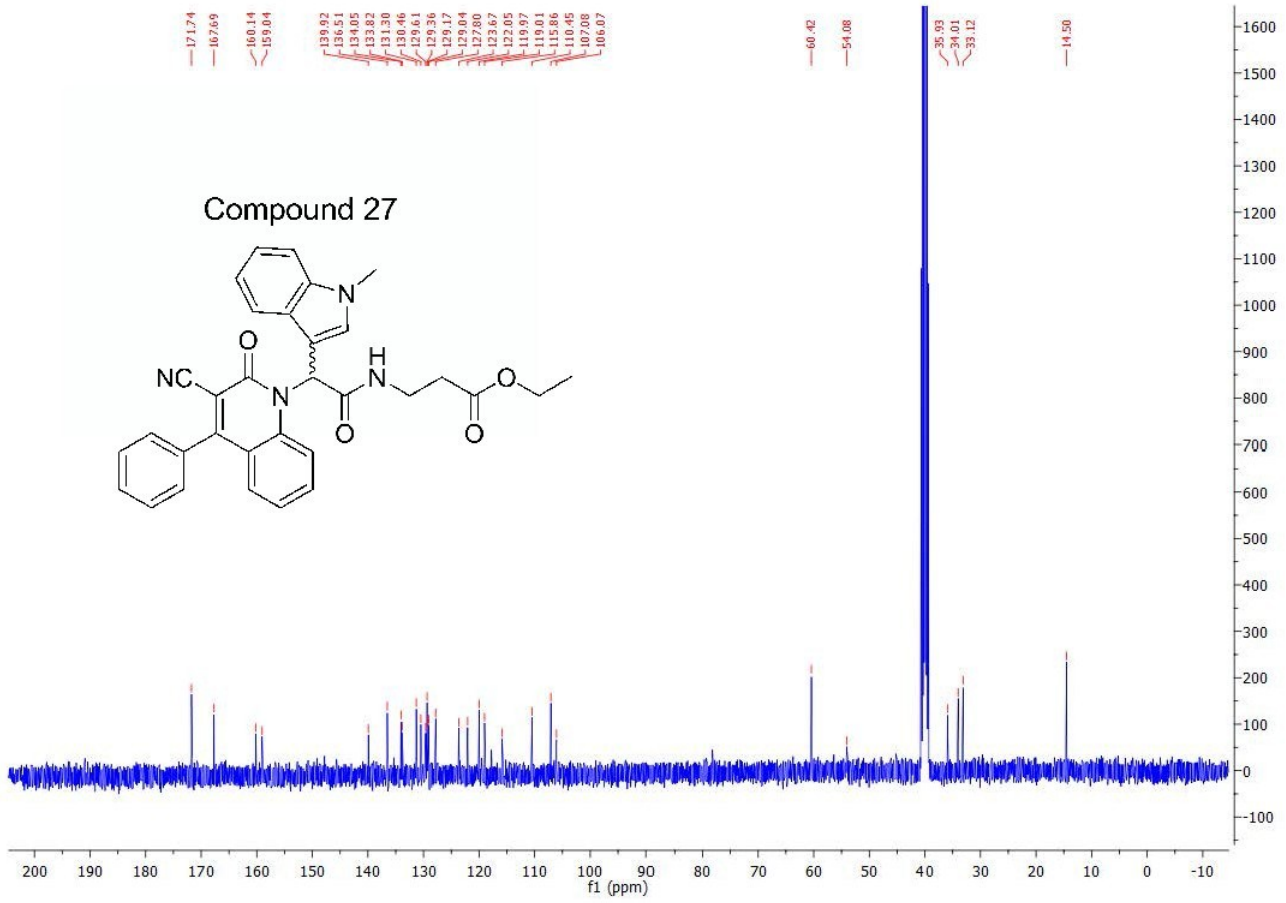
^{1}H NMR (400 MHz, DMSO) δ 8.05 (bs, 1H), 7.83 (d, J = 8.7 Hz, 1H), 7.71 – 7.56 (m, 6H), 7.56 – 7.47 (m, 2H), 7.45-7.38 (m, 2H), 7.26 – 7.20 (m, 2H), 7.17 (t, J = 7.2 Hz, 1H), 7.06 (t, J = 7.2 Hz, 1H), 4.03 (q, J = 7.1 Hz, 2H), 3.78 (s, 3H), 3.42 – 3.35 (m, 2H), 2.57-2.44 (m, 2H), 1.16 (t, J = 7.1 Hz, 3H).

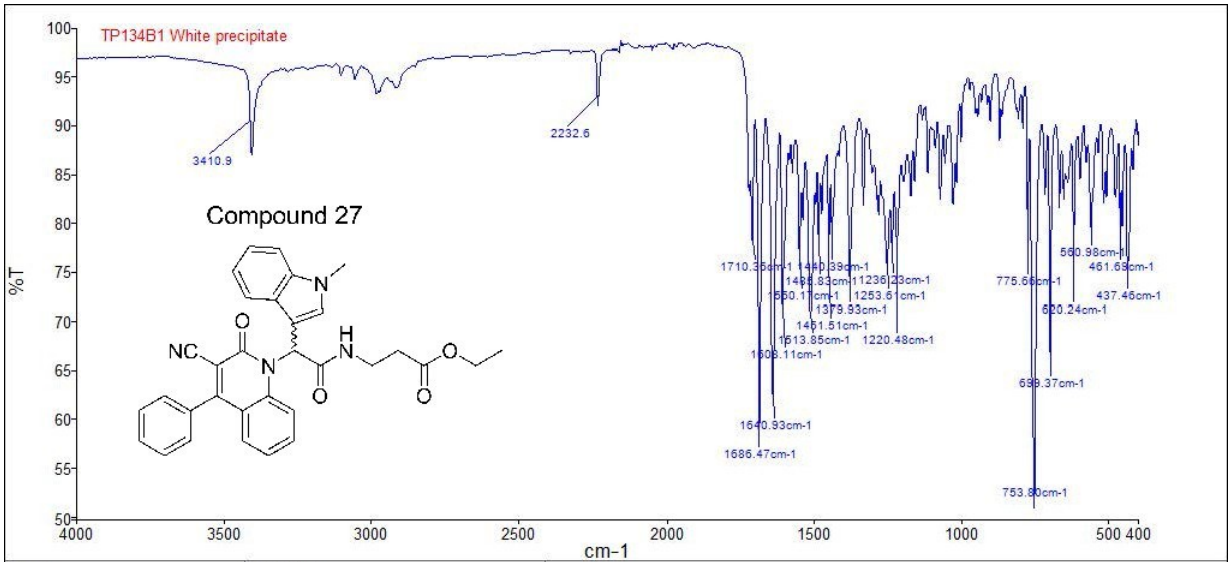
^{13}C NMR (101 MHz, DMSO-*d6*) δ 171.7, 167.7, 160.1, 159.0, 139.9, 136.5, 134.1, 133.8, 131.3, 130.5, 129.6, 129.4, 129.2, 129.0, 127.8, 123. 7, 122.1, 120.0, 119.9, 119.0, 117.8, 115.9, 110. 5, 107.1, 106.1, 60.4, 54.1, 35.9, 34.0, 33.1, 14.5

RP-HPLC Alltima™ C18 5u 150 mm x4.6 mm, 10–100% B in 15 min, Rt min=14.46, 95.8%

LRMS (ESI+) m/z 532, 287 $[M+ACN+ 2H]^{2+}$ 100%. HRMS (ES+) for $C_{16}H_{11}N_2O^+$ (main fragment); calculated 247.087, found 247.0865.




Compound 27
f1 (ppm)


TP134B1 White precipitate
Compound 27
%T
cm-1

<Sample Information>

Sample Name	: TP134B1		
Sample ID	: TP134B1		
Data Filename	: TP134B1.lcd		
Method Filename	: 10-100 over 15 mins.lcm		
Batch Filename	: TRIEU Second Third Generation and New pro.lcb		
Vial #	: 1-29	Sample Type	: Unknown
Injection Volume	: 30 uL		
Date Acquired	: 8/09/2014 8:44:24 PM	Acquired by	: System Administrator
Date Processed	: 8/09/2014 9:14:26 PM	Processed by	: System Administrator

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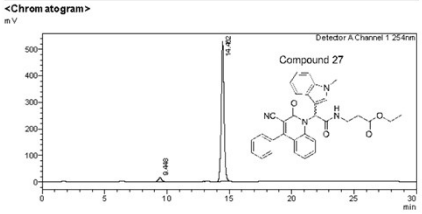

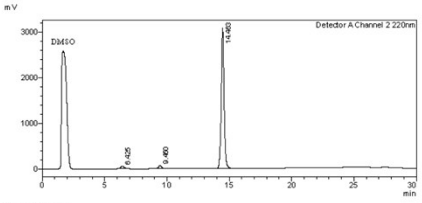

<Peak Table>

Detector A Channel 1 254nm

C:\LabSolutions\Data\Project1\TRIEU\TP134B1.lcd

20/10/2014 1:31:23 PM Page 2 / 2

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	9.448	276842	16046	3.236		M	
2	14.462	8277664	527462	96.764		M	
Total		8554507	543498				

Detector A Channel 2 220nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	6.425	1115783	49239	2.232		M	
2	9.450	980758	61730	1.962		M	
3	14.463	47898085	3070407	95.806		M	
Total		49994626	3181376				

==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

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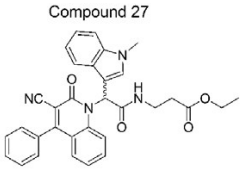
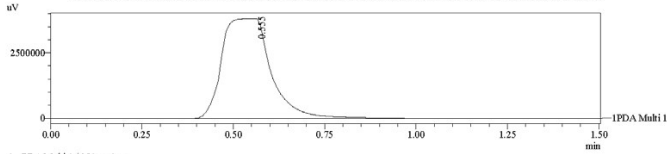
Date Acquired	: 7/24/2015 9:33:05 AM
Sample Type	: Unknown
Level#	: 0
Sample Name	: TP134B1
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 51
Injection Volume	: 5
Data File	: TP134B2.lcd
Method File	: FIA-ESI_Scan(+).lcm
Original Method	: C:\LabSolutions\Data\Trieu\Mass spec files\FIA-ESI_Scan(+).lcm
Report Format	: Default.LCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/24/2015 9:34:36 AM



Chromatogram

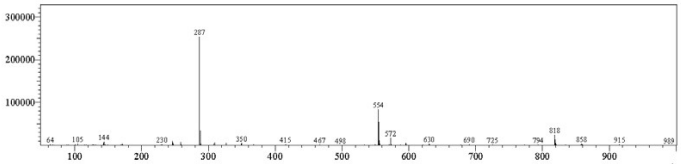
TP134B1 C:\LabSolutions\Data\Trieu\NORMAL REVERSE PHASE COLUMN\ESI POSITIVE\UGI KNOEVENAGEL\TP134B2.lcd



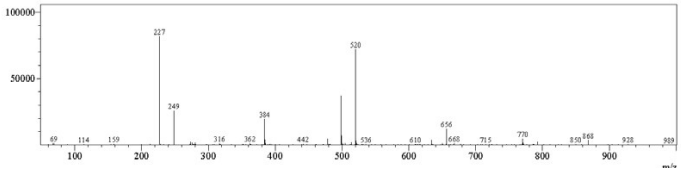
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

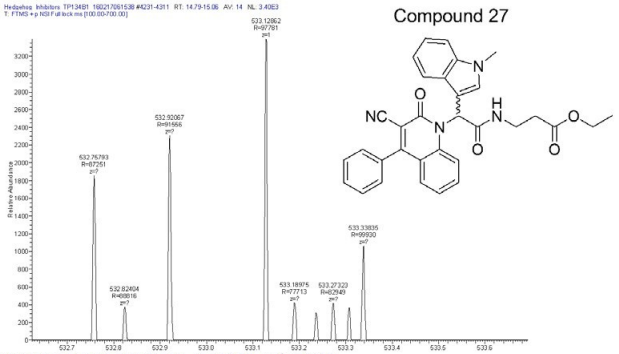
Retention Time:0.900(Scan#:91)
Max Peak:331 Base Peak:286.65(253957)
Spectrum:Averaged 0.600-1.160(61-117)
Background:Averaged 0.140-0.493(15-51) Polarity:Pos Segment1 - Event1

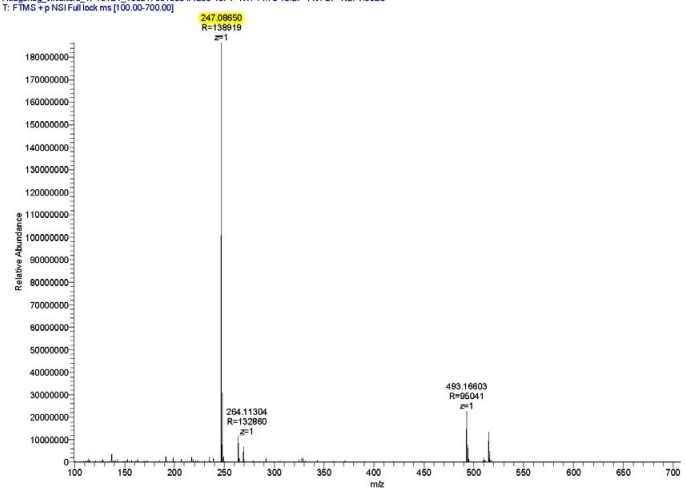


Retention Time:0.810(Scan#:82)
Max Peak:522 Base Peak:226.60(81618)
Spectrum:Averaged 0.610-1.170(62-118)
Background:Averaged 0.150-0.493(16-52) Polarity:Neg Segment1 - Event2

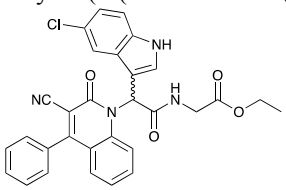


Compound	Chemical Formula	Predicted monoisotopic neutral MW	Predicted MH+	MH+ Measured	Main MS Ions	Main MS/MS Fragments
TP134B1	C32H28N4O4	532.2111	533.2183	n/a	247.0865	247.087^





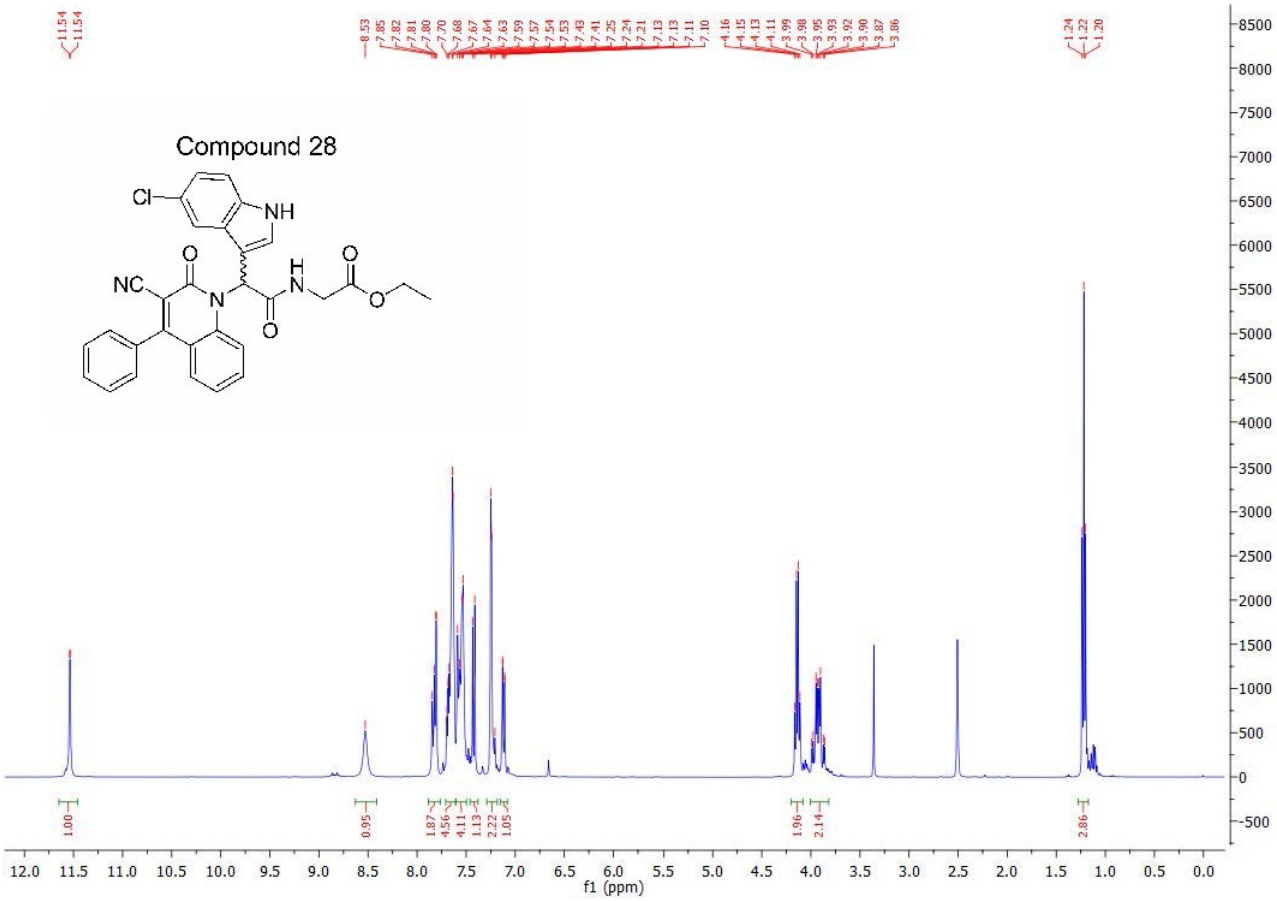
Ethyl-2-(2-(5-chloroindole(1*H*)-3-yl)-2-(3-cyano-2-oxo-4-phenyl-1(2H)-quinolin-yl)-acetamido)-acetate (**28**)

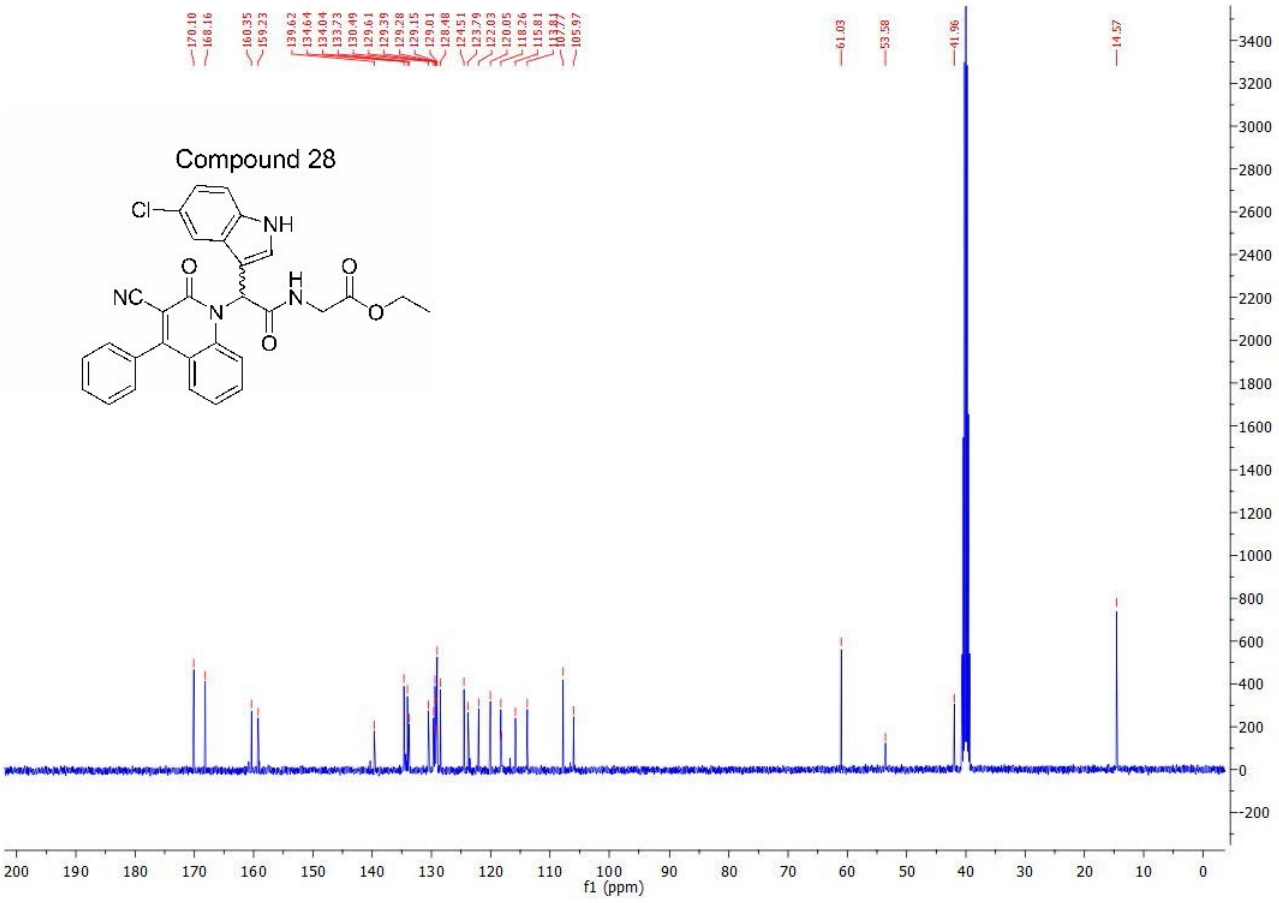


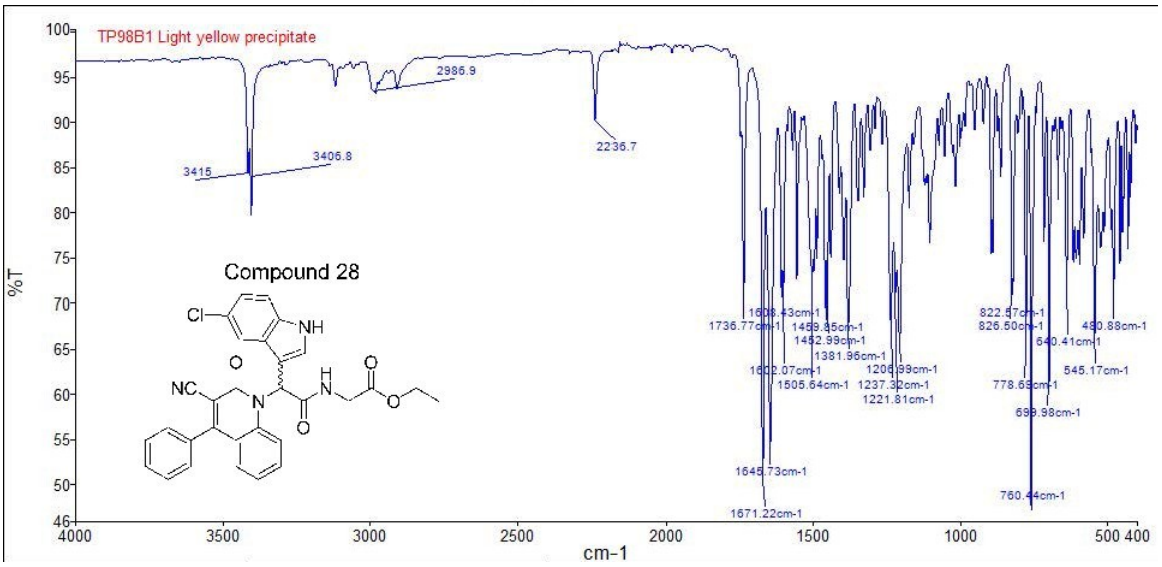
Yield: 33%; **MP:** 201-203 °C
IR (νmax/cm^{-1}): 3415 (NH), 3406 (NH), 2236 (CN), 1736 (COO), 1671 (CON)

^{1}H NMR (400 MHz, DMSO) δ 11.54 (d, *J* = 1.4 Hz, 1H), 8.53 (s, 1H), 7.82 (dd, *J* = 11.9, 5.5 Hz, 2H), 7.71 – 7.61 (m, 4H), 7.61-7.5 (m, 4H), 7.42 (d, *J* = 8.6 Hz, 1H), 7.29 – 7.18 (m, 2H), 7.12 (dd, J = 8.6, 1.7 Hz, 1H), 4.14 (q, *J* = 7.0 Hz, 2H), 3.93 (qd, *J* = 17.2, 5.8 Hz, 2H), 1.22 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-*d6*) δ 170.1, 168.2, 160.4, 159.2, 139.6, 134.6, 134.0, 133.7, 130.5, 129.6(Cx2), 129.4, 129.2, 129.0 (Cx2), 128.5, 124.5, 123.8, 122.0, 120.1, 118.3 (Cx2), 118.2, 115.8, 113.8, 107.8, 106.0, 61.0, 42.0, 14.6
RP-HPLC Alltima™ C18 5µm 150 mm x4.6 mm, 10–100% B in 15 min, Rt min=14.07, 99.4%
LRMS (ESI+) m/z 538, 292 $[M+2Na]^{2+}$, 60%. HRMS for $C_{30}H_{23}ClN_4O_4$; calculated 539.1481, found 539.1481




Compound 28
Cl
NH
NC
O
N
H
N
O
O
O
170.10
168.16
160.35
159.23
139.62
134.64
134.04
133.73
130.49
129.61
129.39
129.28
129.15
129.01
128.48
124.51
123.79
122.03
120.05
118.26
115.81
105.97
61.03
53.58
41.96
14.57
f1 (ppm)


TP98B1 Light yellow precipitate
Compound 28
3415
3406.8
2986.9
2236.7
1736.77cm-1
1645.73cm-1
1671.22cm-1
1505.64cm-1
1452.99cm-1
1381.96cm-1
1237.32cm-1
1221.81cm-1
826.50cm-1
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699.98cm-1
760.44cm-1
545.17cm-1
%T
cm-1

<Sample Information>

Sample Name	: TP98B1		
Sample ID	: TP98B1		
Data Filename	: TP98B1.lcd		
Method Filename	: 10-100 over 15 mins.lcm		
Batch Filename	: TRIEU Second Third Generation and New pro.lcb		
Vial #	: 1-24	Sample Type	: Unknown
Injection Volume	: 30 uL		
Date Acquired	: 8/09/2014 6:12:18 PM	Acquired by	: System Administrator
Date Processed	: 8/09/2014 6:42:19 PM	Processed by	: System Administrator

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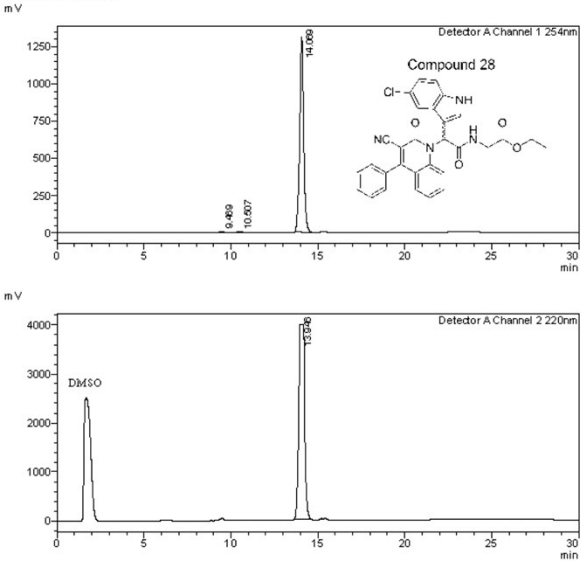

<Peak Table>

Detector A Channel 1 254nm

20/10/2014 1:40:45 PM

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2	10.507	52847	4013	0.252		M	
3	14.069	20821958	1310947	99.408		M	
Total		20945959	1321448				

Detector A Channel 2 220nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	13.946	93602997	3968571	100.000		M	
Total		93602997	3968571				

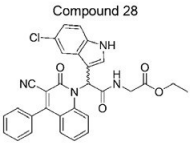
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

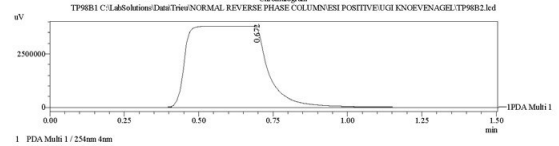
Acquired by	: Admin
Date Acquired	: 7/24/2015 10:02:15 AM
Sample Type	: Unknown
Level#	: 0
Sample Name	: TP98B1
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 57
Injection Volume	: 15
Data File	: TP98B2.lcd
Method File	: FIA-ESI_Scan(+).lcm
Original Method	: C:\LabSolutions\Data\Trieu\Mass spec files\FIA-ESI_Scan(+).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/24/2015 10:03:47 AM

Compound 28



Chromatogram

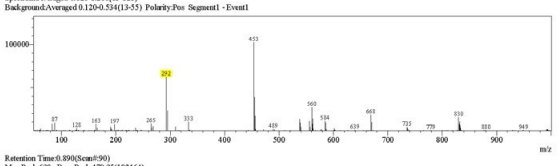
TP98B1 C:\LabSolutions\Data\Trieu\NORMAL REVERSE PHASE COLUMN\ESI POSITIVE\UGI KNOEVENAGEL\TP98B2.lcd



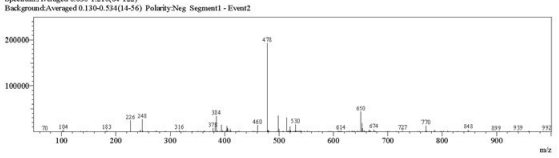
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

Retention Time:0.880(Scan#:89)
Max Peak:304 Base Peak:453.55(102202)
Spectrum:Averaged 0.620-1.200(61-121)
Background:Averaged 0.120-0.534(13-55) Polarity:Pos Segment1 - Event1

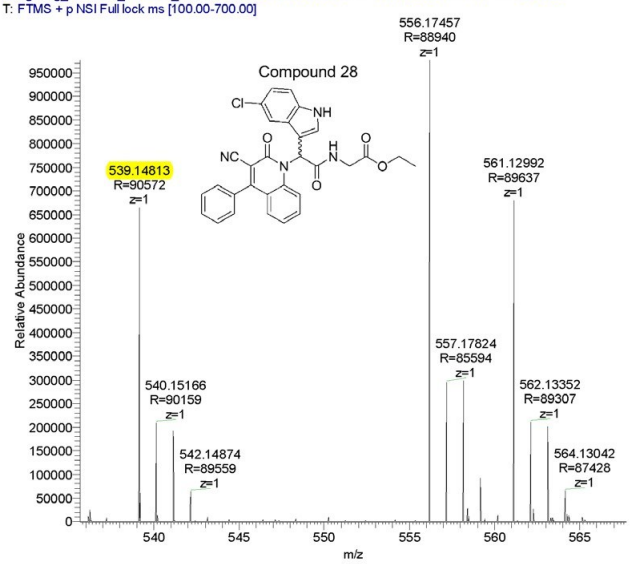


Retention Time:0.890(Scan#:90)
Max Peak:639 Base Peak:478.35(192464)
Spectrum:Averaged 0.630-1.210(64-122)
Background:Averaged 0.130-0.534(14-56) Polarity:Neg Segment1 - Event2

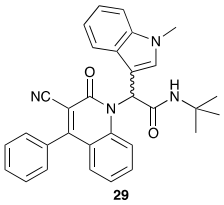


Compound	Chemical Formula	Predicted monoisotopic neutral MW	Predicted MH+	MH+ Measured	Main MS Ions	Main MS/MS Fragments
TP98B1	C30H23ClN4O4	538.1408	539.1481	539.1481	539.1481 556.17458 (+NH4)	293.0692 265.0742

Hedgehog_Inhibitors_TP98B1_160217071332 #5060-5428 RT: 17.70-18.96 AV: 61 NL: 9.76E5
T: FTMS + p NSI Full lock ms [100.00-700.00]



N-tert-Butyl-2-(3-cyano-2-oxo-4-phenyl-2*H*-quinolin-1-yl)-2-(5-methyl-1*H*-indol-3-yl)-acetamide (**29**)



Yield: 48% **MP:** 196-198 ºC

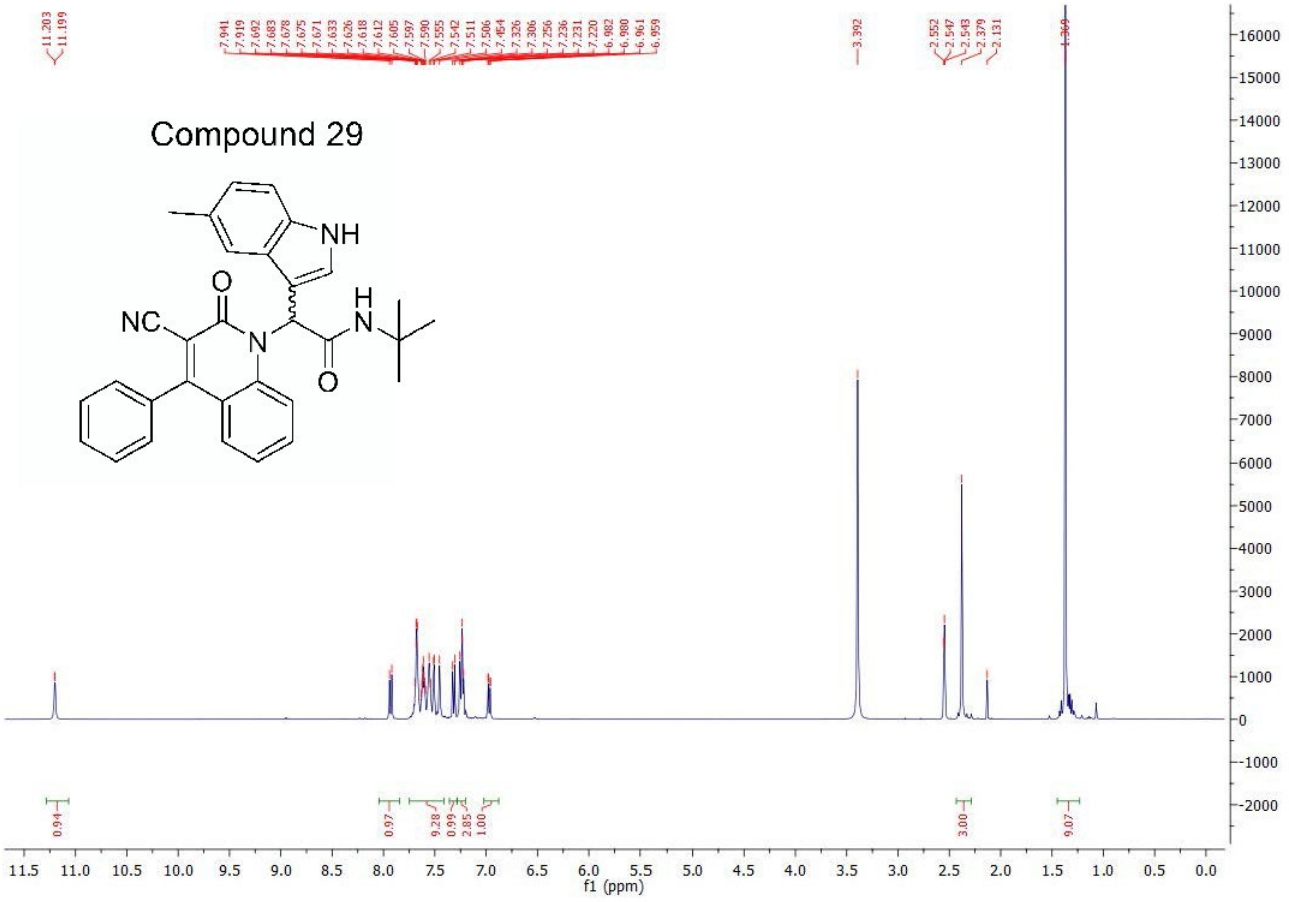
IR (υ_{max}/cm^{-1}) 3427 (NH), 2978 (CH), 2228 (CN), 1650 (CON)

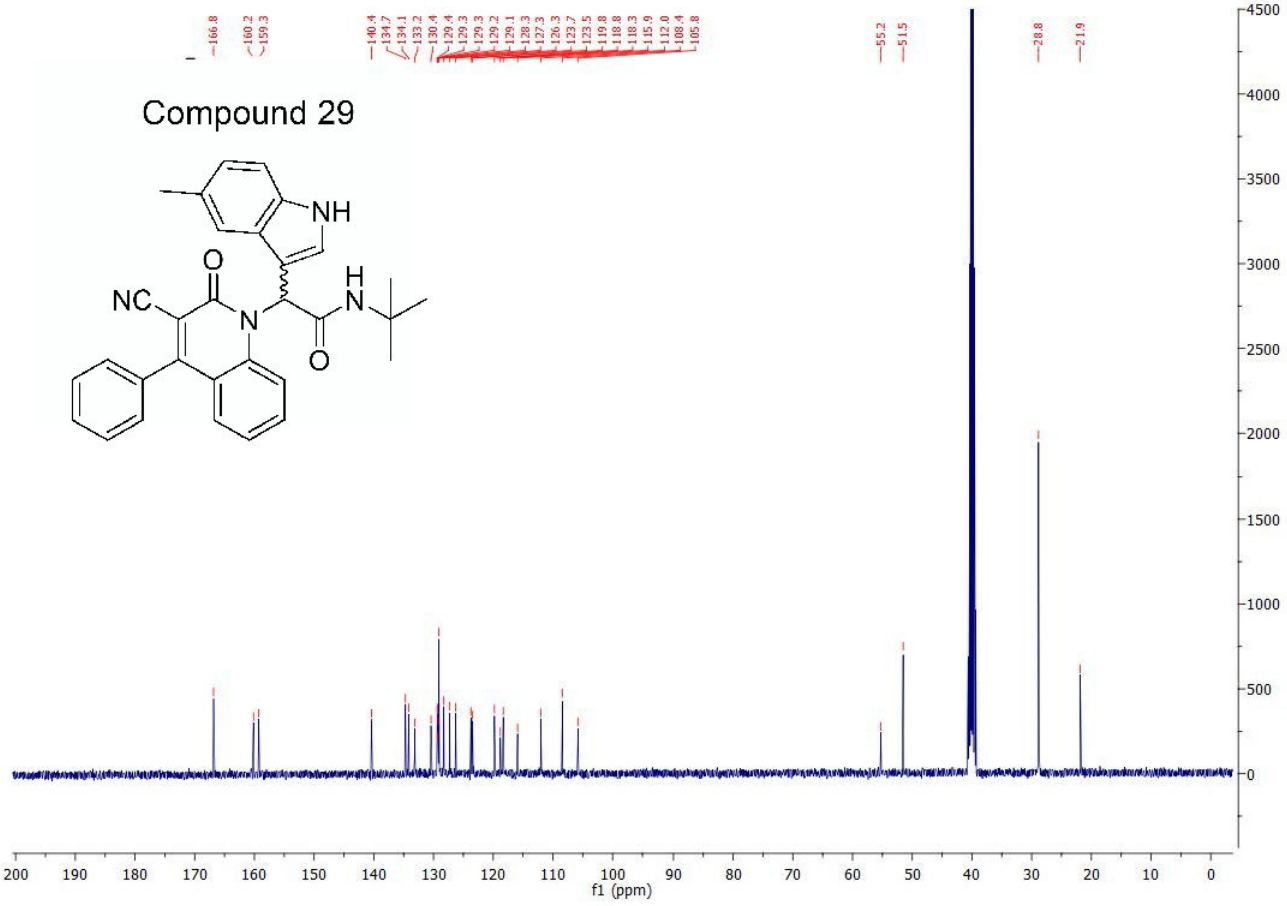
^{1}H NMR (400 MHz, DMSO) δ 11.13 (d, *J* = 4.9 Hz, 1H), 7.90 – 7.37 (m, 10H), 7.29-7.16 (m, 4H), 6.92 (d, *J* = 8.3 Hz, 1H), 4.03 – 3.87 (m, 1H), 2.34 (s, 3H), 1.57 – 1.20 (m, 3H), 1.20 – 0.86 (m, 5H), 0.82-0.60 (m, 3H).

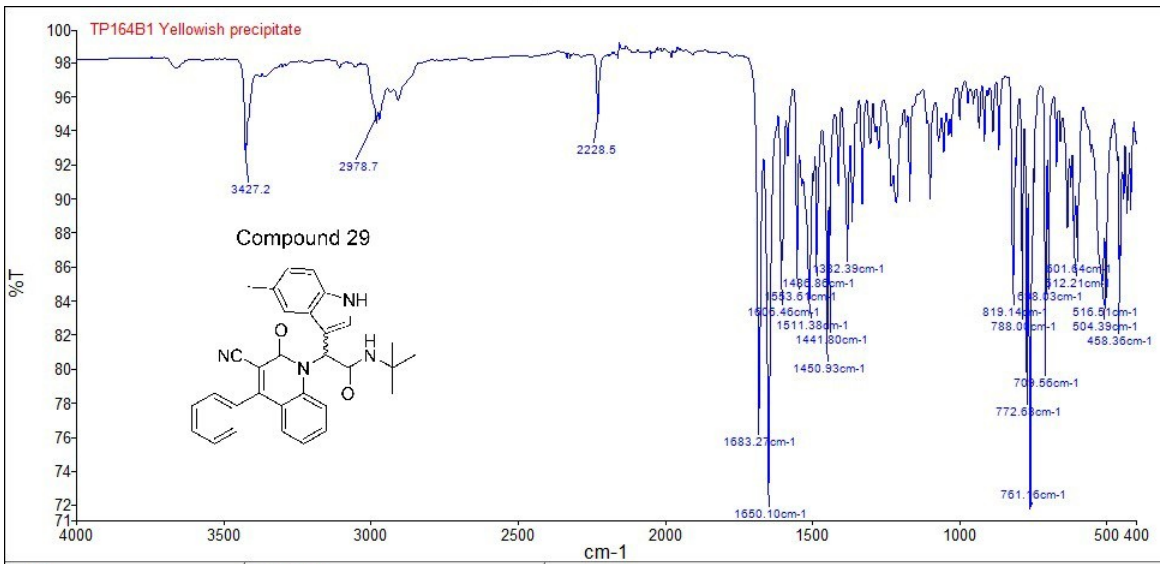
^{13}C NMR (101 MHz, DMSO) δ 167.5, 166.9, 160.1, 160.1, 159.3, 140.1, 140.1, 134.6, 134.6, 134.5, 134.1, 133.3, 130.4, 129.4, 129.2, 129.1, 128.2, 128.1, 127.5, 127.5, 126.8, 126.6, 23.5, 19.9, 118.48, 118.4, 118.3, 116.0, 111.9, 107.9, 107.9, 106.0, 105.9, 54.5, 54.4, 52.9, 45.4, 45.2, 38.4, 38.2, 27.3, 26.9, 21.9, 21.1, 20.9, 19.6, 19.2, 14.4, 14.2, 11.2, 10.8.

RP-HPLC Alltima™ C18 5u 150 mm x4.6 mm, 10–100% B in 15 min, Rt min=14.59, 95.3%

LRMS (ESI-) m/z 488, 243 $[M-2H]^{2+}$, 90%. HRMS for $C_{31}H_{28}N_4O_2$; calculated 489.2285, found 489.2283




Compound 29
NH
O
NC
N
H
N
O
f1 (ppm)


TP164B1 Yellowish precipitate
Compound 29
%T
cm-1
3427.2
2978.7
2228.5
1683.27cm-1
1650.10cm-1
1450.93cm-1
761.16cm-1

[redacted]

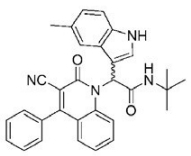
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

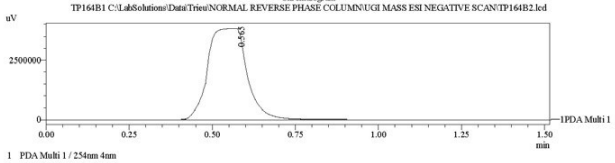
Acquired by	: Admin
Date Acquired	: 7/23/2015 6:24:19 PM
Sample Type	: Unknown
Level#	: 0
Sample Name	: TP164B1
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 58
Injection Volume	: 5
Data File	: TP164B2.lcd
Method File	: FIA-ESI_Scan(-).lcm
Original Method	: C:\LabSolutions\Data\Kelly\FIA-ESI_Scan(-).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/23/2015 6:25:50 PM

Compound 29



Chromatogram

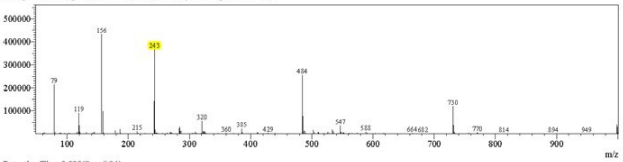
TP164B1 C:\LabSolutions\Data\Trieu\NORMAL REVERSE PHASE COLUMN\UGI MASS ESI NEGATIVE SCAN\TP164B2.lcd



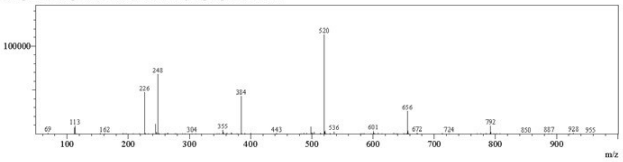
1 PDA Multi 1 / 254nm 4nm

<Spectrum>

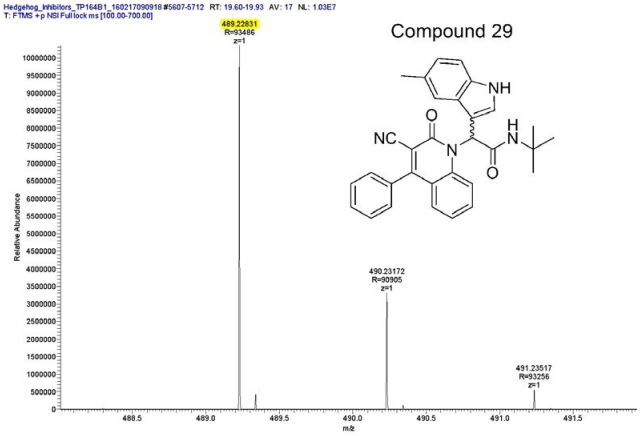
Retention Time:0.840(Scan#:85)
Max Peak:554 Base Peak:156.50(432178)
Spectrum:Averaged 0.640-1.280(65-129)
Background:Averaged 0.180-0.560(19-57) Polarity:Pos Segment1 - Event1



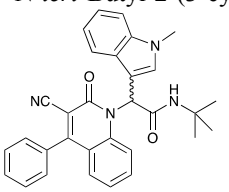
Retention Time:0.830(Scan#:84)
Max Peak:589 Base Peak:520.30(113099)
Spectrum:Averaged 0.650-1.290(66-130)
Background:Averaged 0.190-0.560(20-58) Polarity:Neg Segment1 - Event2



Compound	Chemical Formula	Predicted monoisotopic neutral MW	Predicted MH+	MH+ Measured	Main MS Ions	Main MS/MS Fragments
TP164B1	C31H28N4O2	488.2212	489.2285	489.2283	243.149 (fragment) 489.2283	243.1496 187.0870 489.3408



N-*tert*-Butyl-2-(3-cyano-2-oxo-4-phenyl-2H-quinolin-1-yl)-2-(1-methyl-1H-indole-3-yl)-acetamide (**30**)



MP: 232-234ºC

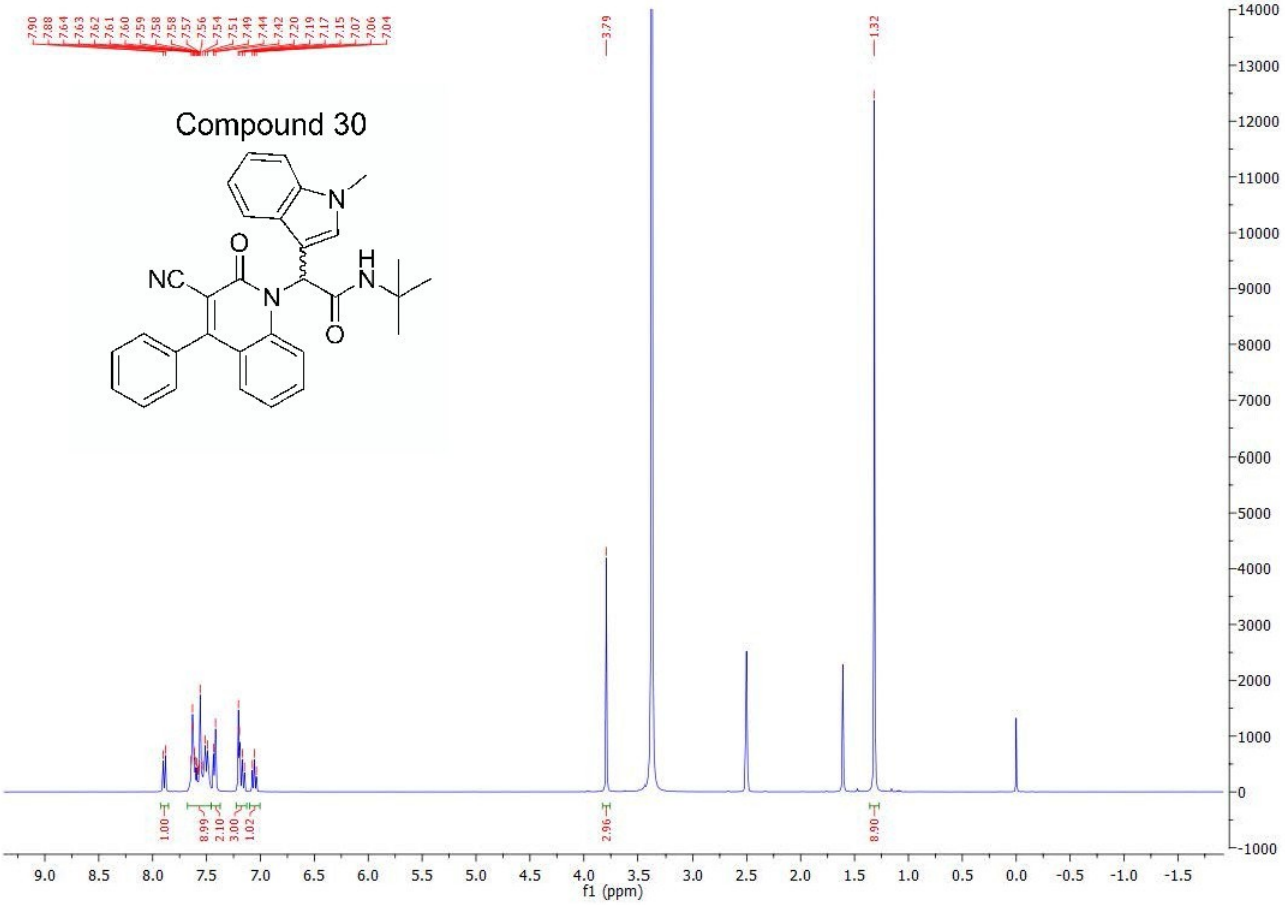
IR (ν_{max}/cm^{-1}): 3357 (NH), 2979 (CH), 2229 (CN), 1650 (CO).

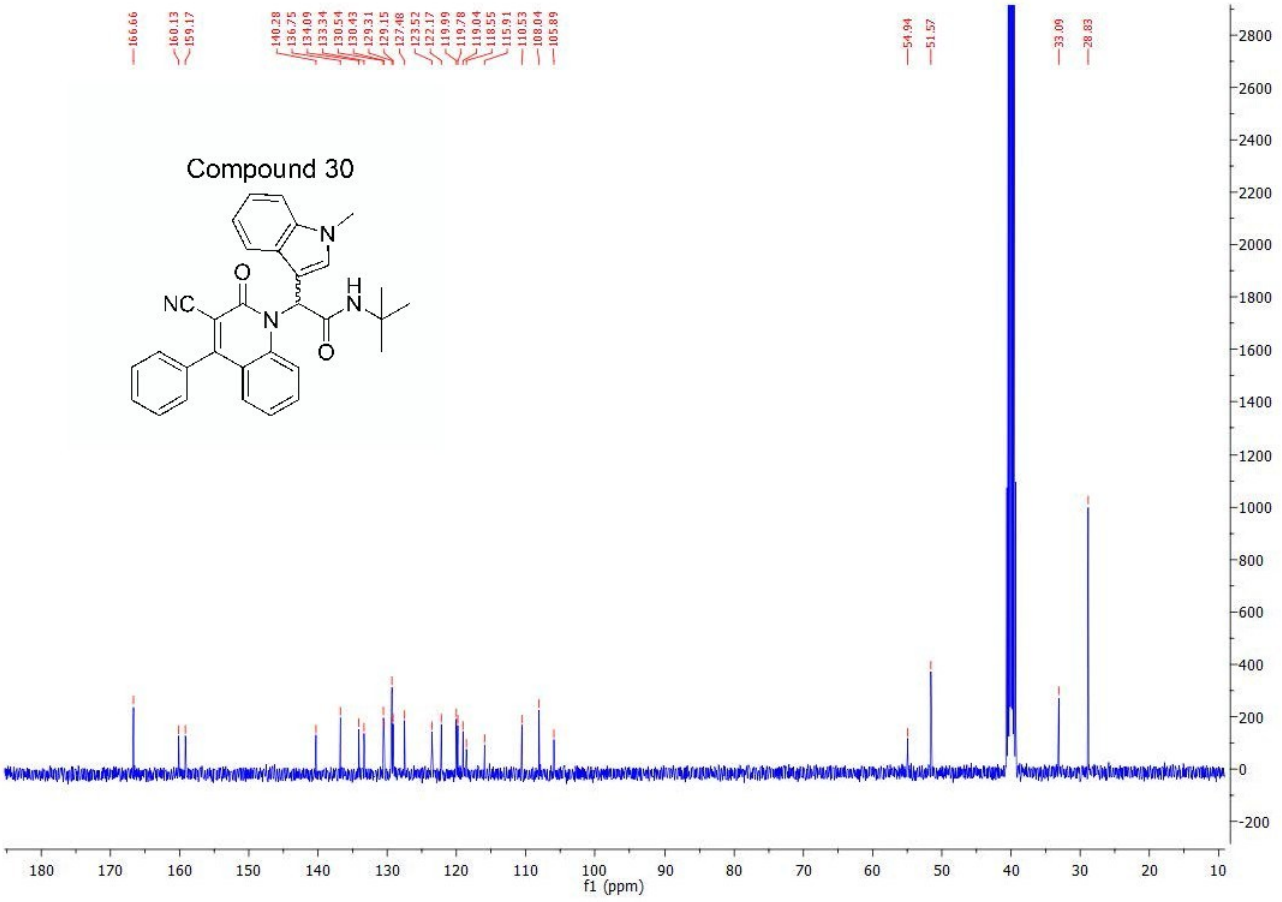
^{1}H NMR (400 MHz, DMSO) δ 7.89 (d, *J* = 8.8 Hz, 1H), 7.68 – 7.46 (m, 9H), 7.43 (d, *J* = 7.8 Hz, 2H), 7.22-7.13 (m, 3H), 7.06 (t, *J* = 7.4 Hz, 1H), 3.79 (s, 3H), 1.32 (s, 9H).

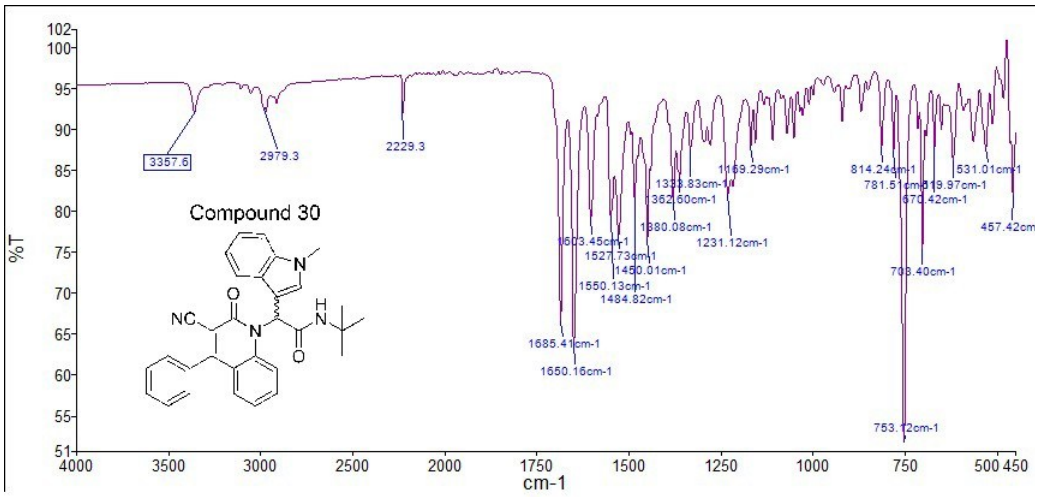
^{13}C NMR (101 MHz, DMSO): δ 136.8, 134.1, 133.3, 130.54, 130.4, 129.3 (Cx3), 129.2 (Cx2), 127.5, 123.5, 122.2, 120.0, 119.8, 119.0, 118.6, 115.9, 110.5, 108.0, 105.9, 54.9, 51.6, 33.1, 28.8 (Cx3)

RP-HPLC Alltima™ C18 5u 150 mm x4.6 mm, 10–100% B in 15 min, Rt min= 7.03, 96.7%

LRMS (ESI+) m/z 488, 243 $[M-2H]^{2+}$, 100%. HRMS (ES+) for $C_{31}H_{28}N_4O_2$; calculated 489.2285, found 489.2287



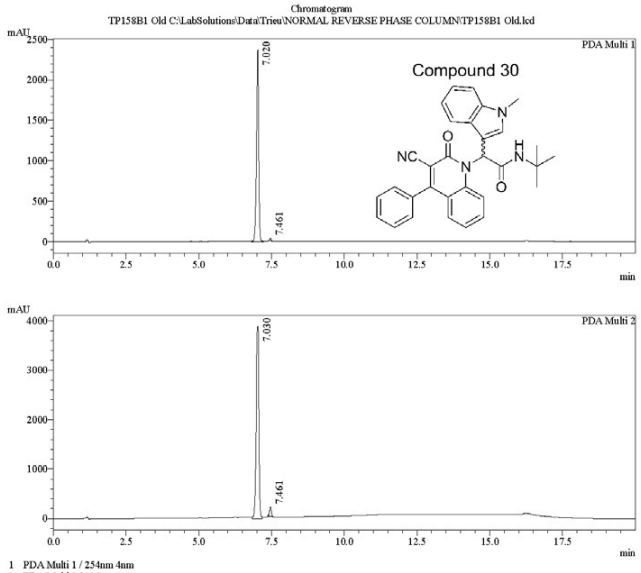

Compound 30
166.66
160.13
159.17
140.28
136.75
134.09
133.34
130.54
130.43
129.31
129.15
127.48
123.52
122.17
119.99
119.78
119.04
118.55
115.91
110.53
108.04
105.89
54.94
51.57
33.09
28.83
f1 (ppm)


Compound 30
%T
3357.6
2979.3
2229.3
1685.41cm-1
1650.16cm-1
1603.45cm-1
1550.13cm-1
1527.73cm-1
1484.82cm-1
1450.01cm-1
1380.08cm-1
1362.60cm-1
1333.83cm-1
1231.12cm-1
1169.29cm-1
814.24cm-1
781.51cm-1
753.12cm-1
719.97cm-1
670.42cm-1
703.40cm-1
531.01cm-1
457.42cm
cm-1

==== Shimadzu LCMSsolution Analysis Report ====

Acquired by	: Admin
Sample Name	: TP158B1 Old
Sample ID	:
Vail #	: 56
Injection Volume	: 30 uL
Data File Name	: TP158B1 Old.lcd
Method File Name	: Econosphere C18 EPS 5u lot 50195421 part 70070 150mm id 4.6mm.lcm
Batch File Name	: 2015 Ugi Knoevenagel products continue.lcb
Report File Name	: DefaultLCMS.lcr
Data Acquired	: 9/16/2015 1:49:14 PM
Data Processed	: 10/15/2015 11:30:54 AM

<Chromatogram>



PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.020	12152785	2372981	99.066	98.712
2	7.461	114628	30955	0.934	1.288
Total		12267413	2403936	100.000	100.000

PeakTable

PDA Ch2 220nm 4nm

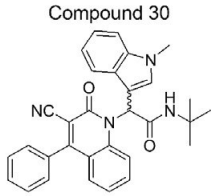
Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.030	25077202	3887009	96.782	95.000
2	7.461	833821	204563	3.218	5.000
Total		25911023	4091572	100.000	100.000

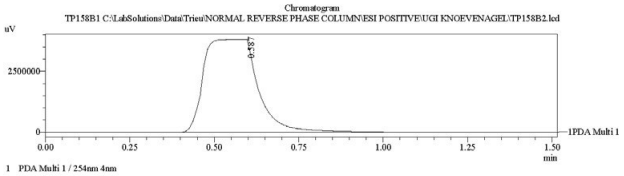
==== Shimadzu LCMSsolution Data Report ====

<Chromatogram>

Sample Information

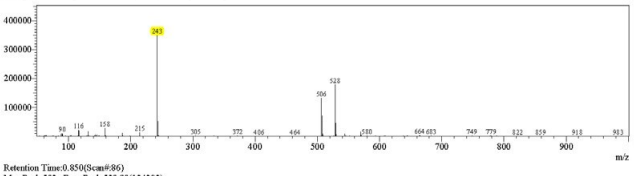
Acquired by	: Admin
Date Acquired	: 7/24/2015 9:38:01 AM
Sample Type	: Unknown
Level#	: 0
Sample Name	: TP158B1
Sample ID	:
ISTD Amount	: (Level1 Conc.)
Sample Amount	: 1
Dilution Factor	: 1
Tray#	: 1
Vial#	: 54
Injection Volume	: 5
Data File	: TP158B2.lcd
Method File	: FIA-ESI_Scan(+).lcm
Original Method	: C:\LabSolutions\Data\Trieu\Mass spec files\FIA-ESI_Scan(+).lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\LCsolution\Log\Tuning\Autotune_030908.lct
Processed by	: Admin
Modified Date	: 7/24/2015 9:39:34 AM



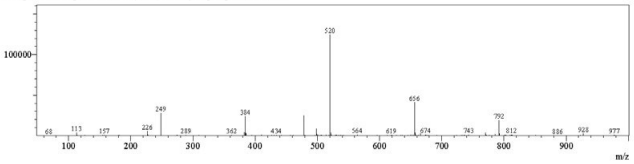


<Spectrum>

Retention Time:0.880(Scan#:89)
Max Peak:311 Base Peak:242.65(349222)
Spectrum:Averaged 0.620-1.280(63-129)
Background:Averaged 0.200-0.509(21-51) Polarity:Pos Segment1 - Event1



Retention Time:0.850(Scan#:86)
Max Peak:502 Base Peak:520.30(124282)
Spectrum:Averaged 0.630-1.290(64-130)
Background:Averaged 0.210-0.509(22-52) Polarity:Neg Segment1 - Event2



Compound	Chemical Formula	Predicted monoisotopic neutral MW	Predicted MH+	MH+ Measured	Main MS Ions	Main MS/MS Fragments
TP158B1	C31H28N4O2	488.2212	489.2285	489.2287*	243.14917 (fragment)	243.149^