

Supporting Information

Discovery of GBA-16-24 as a Highly Potent, Selective ATR inhibitor for the Treatment of FLT3-mutated Acute Myeloid Leukemia

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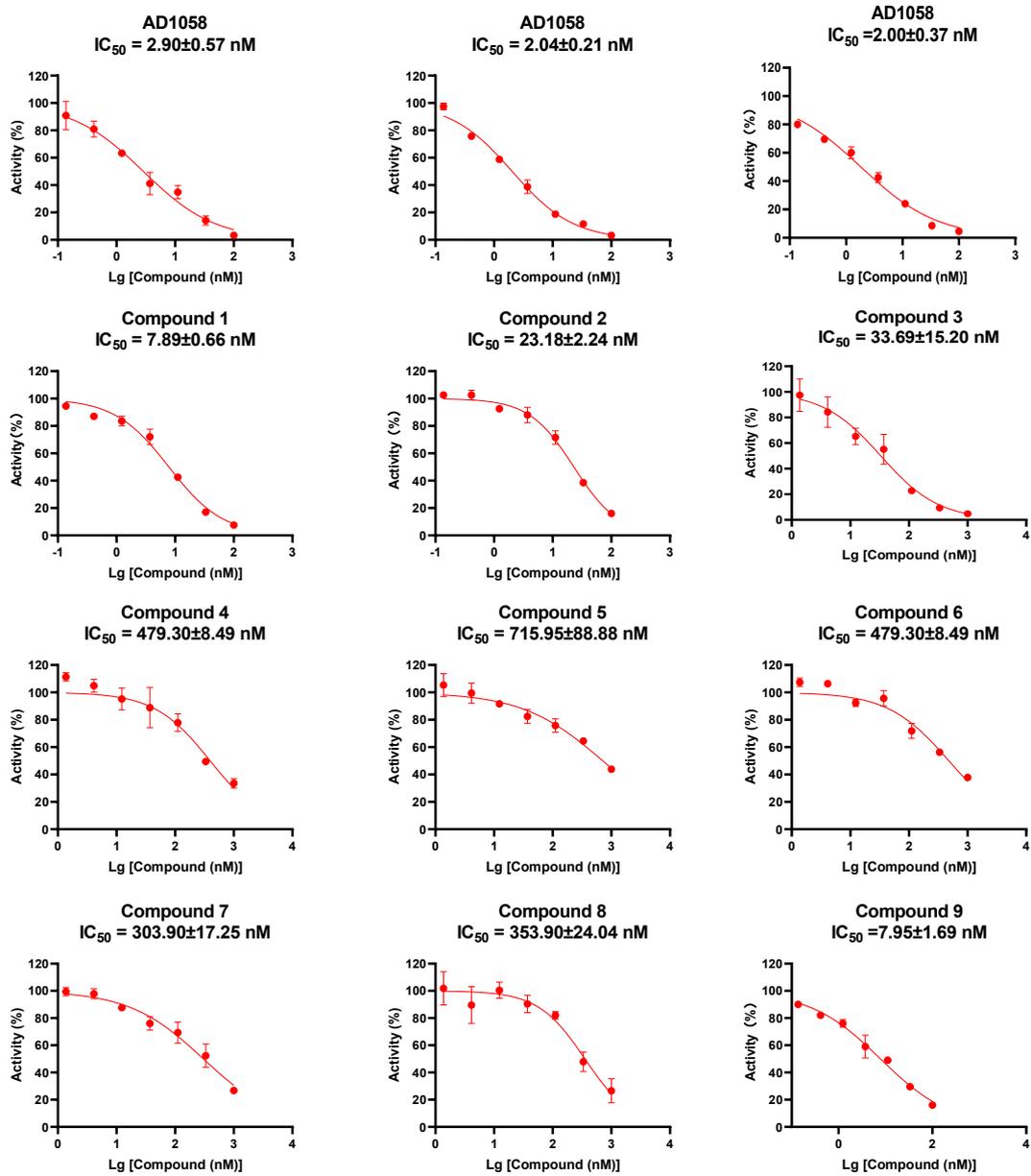
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jli@simm.ac.cn, wangmingliang@simm.ac.cn, jiangkailong@zidd.ac.cn.

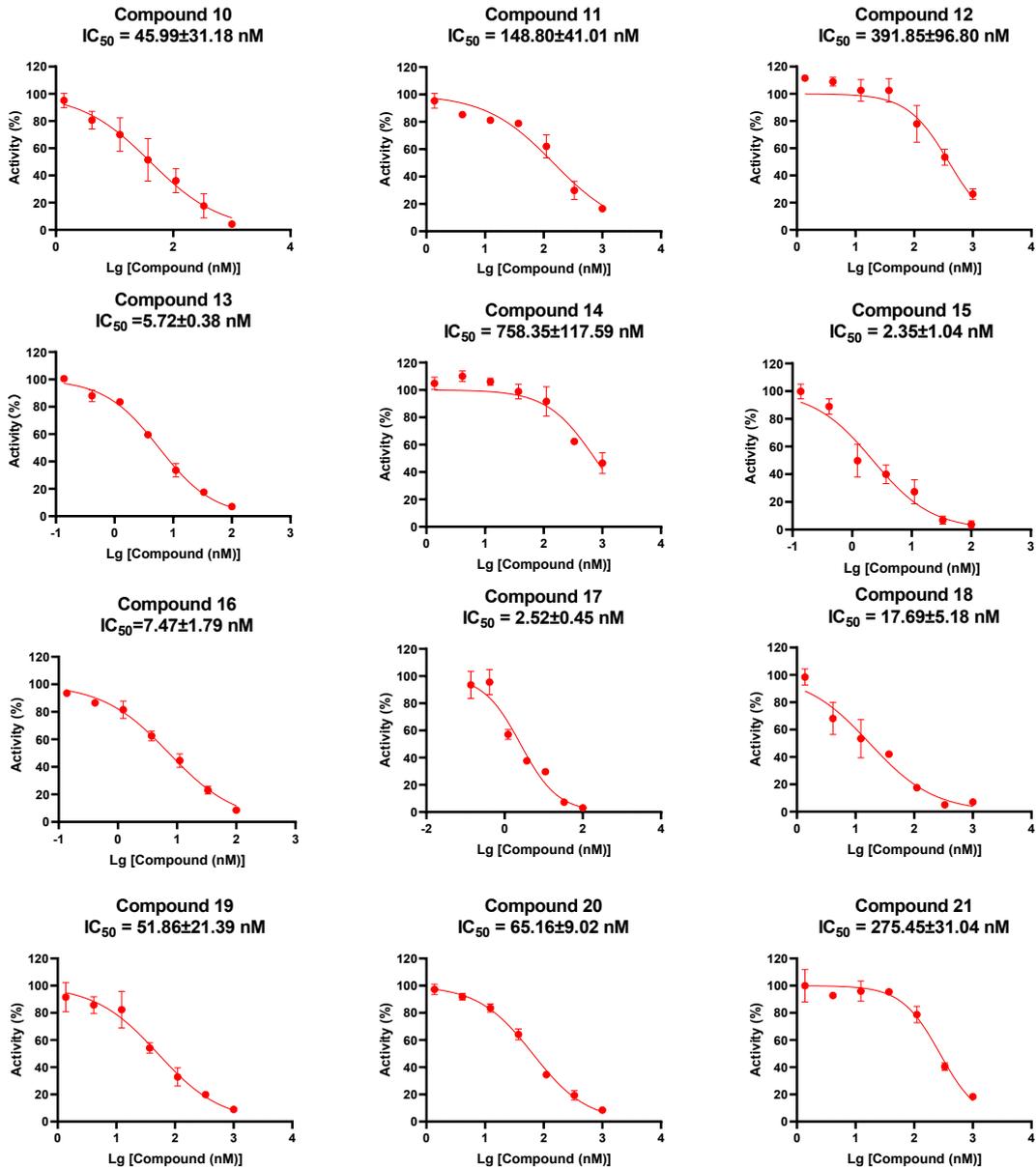
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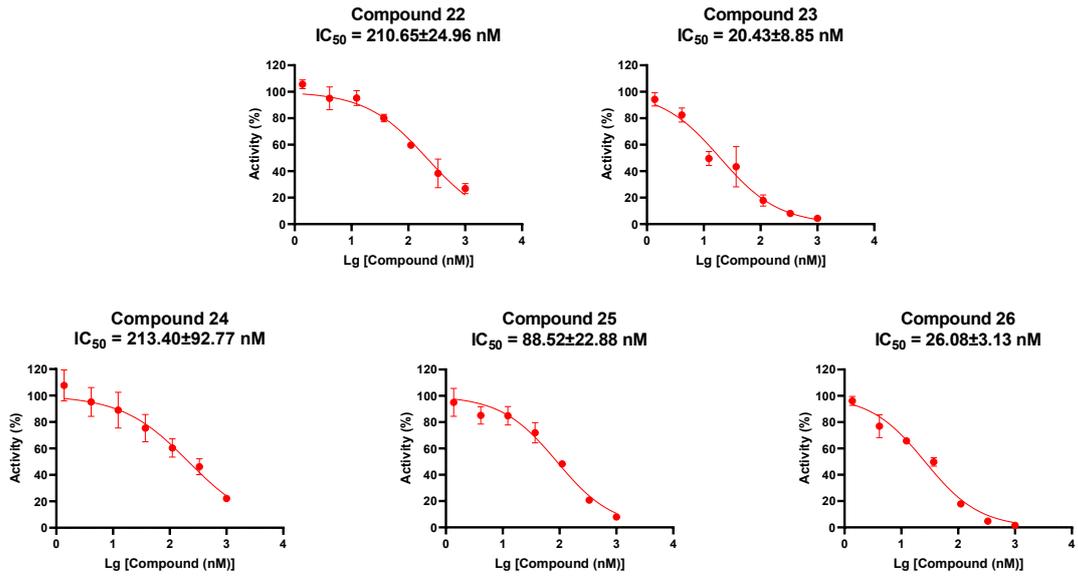
1. Dose–response curves for *in vitro* ATR enzyme assay



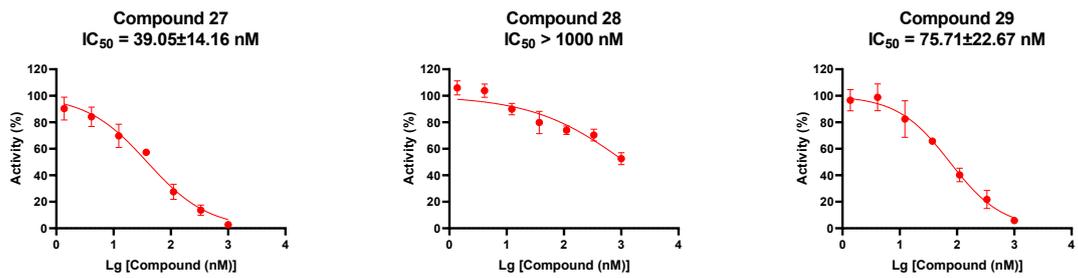
Supplementary Figure 1 Dose–response curves for *In vitro* ATR enzyme assay of AD1058 and compounds 1-9 (Table 1).



Supplementary Figure 2 Dose–response curves for In vitro ATR enzyme assay of compounds 10-21 (Table 2).

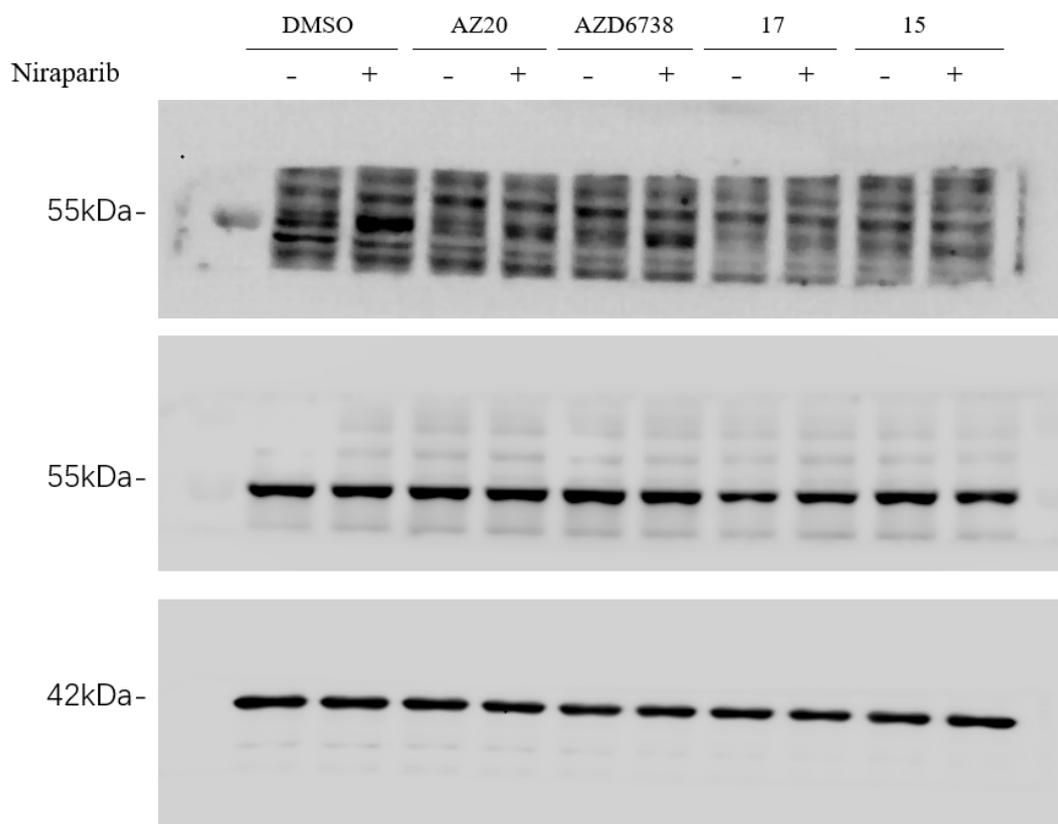


Supplementary Figure 3 Dose–response curves for in vitro ATR enzyme assay of compounds 22-26 (Table 3).



Supplementary Figure 4 Dose–response curves for in vitro ATR enzyme assay of compounds 27-29 (Table 4).

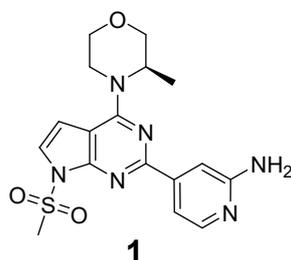
2. Raw data from western blotting



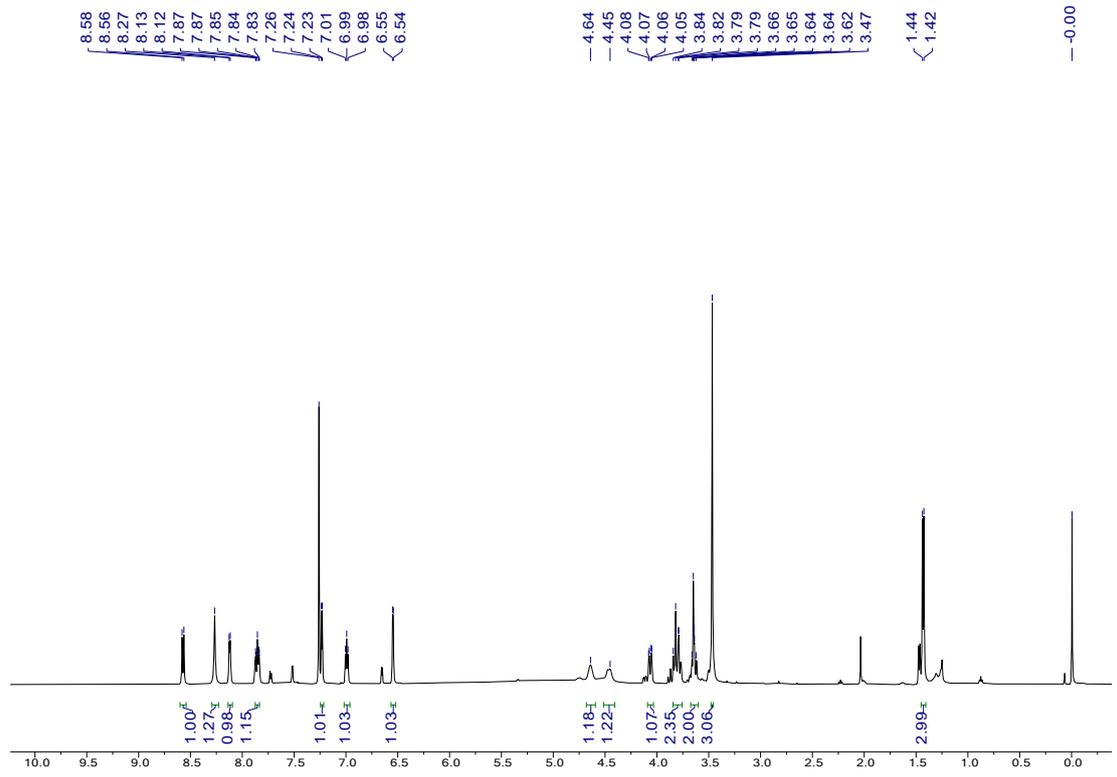
Supplementary Figure 5 Raw data from western blotting (Fig. 3).

3. $^1\text{H}/^{13}\text{C}$ NMR, HRMS spectra of compounds

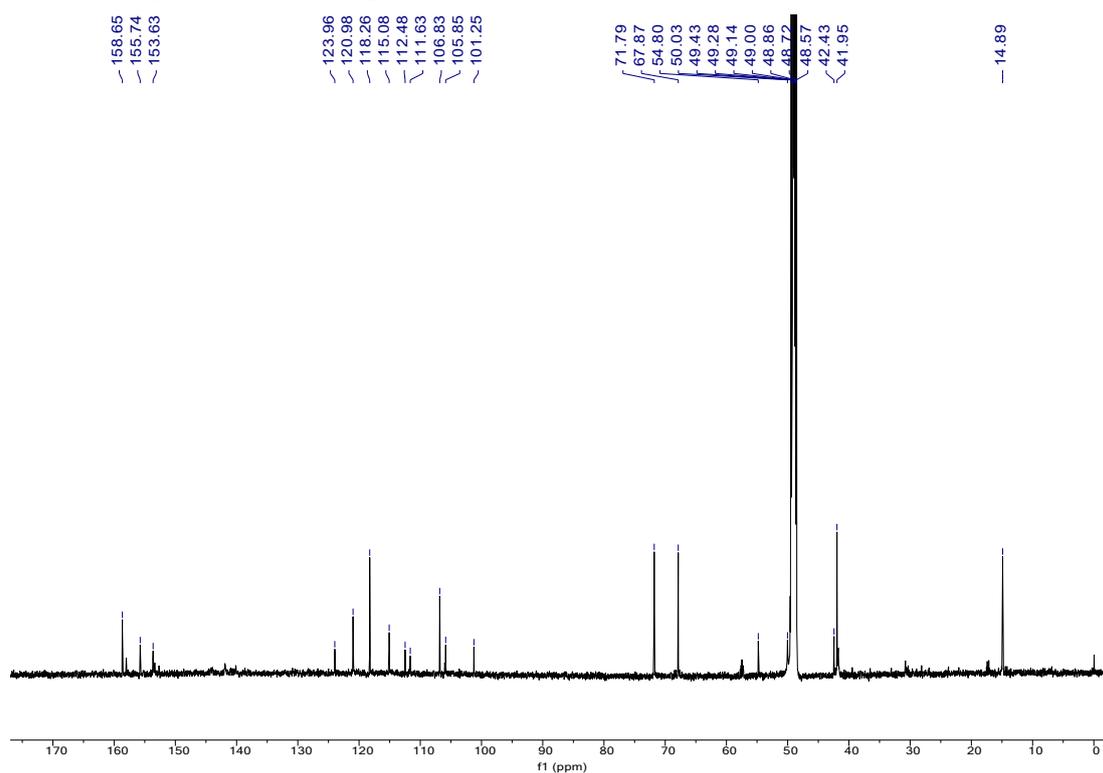
(*R*)-4-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)pyridin-2-amine (1)



^1H NMR spectrum for compound **1**

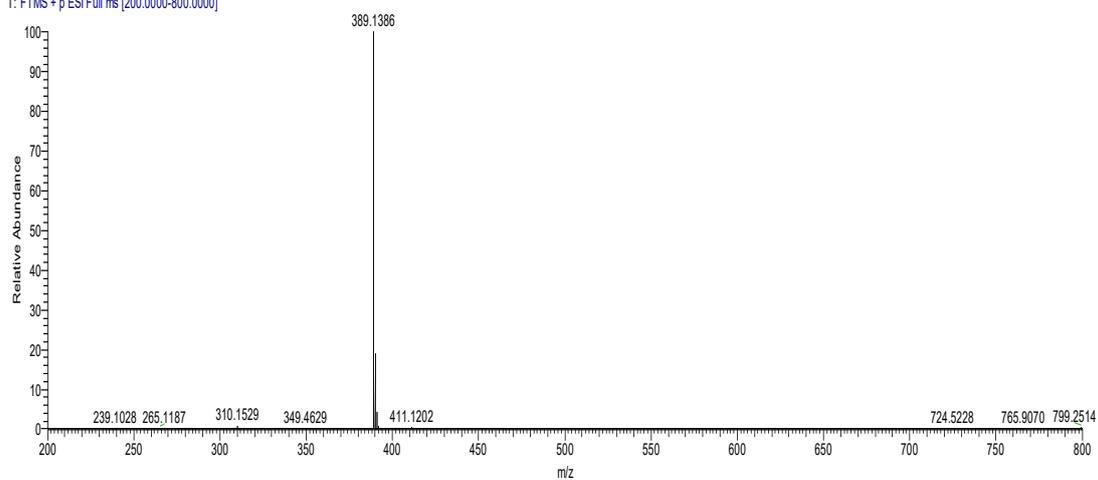


¹³C NMR spectrum for compound 1

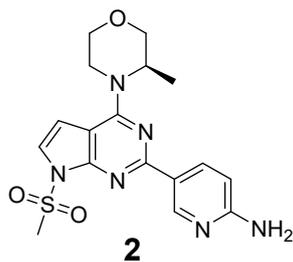


HRMS spectrum for compound 1

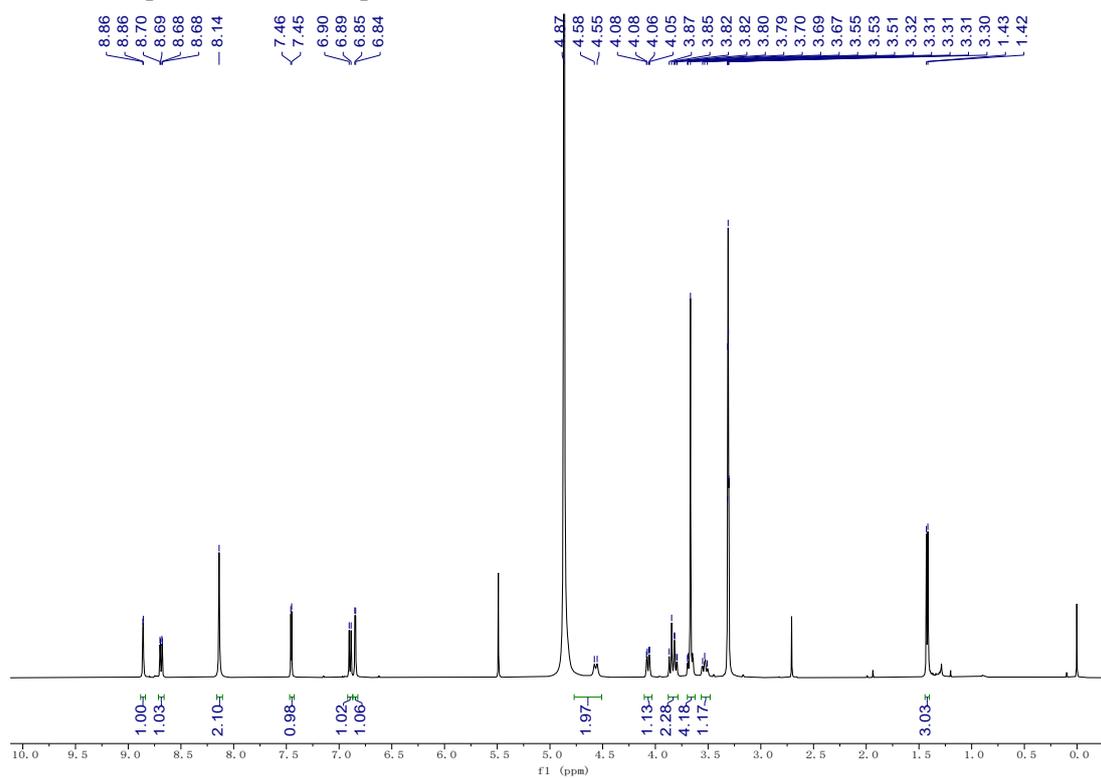
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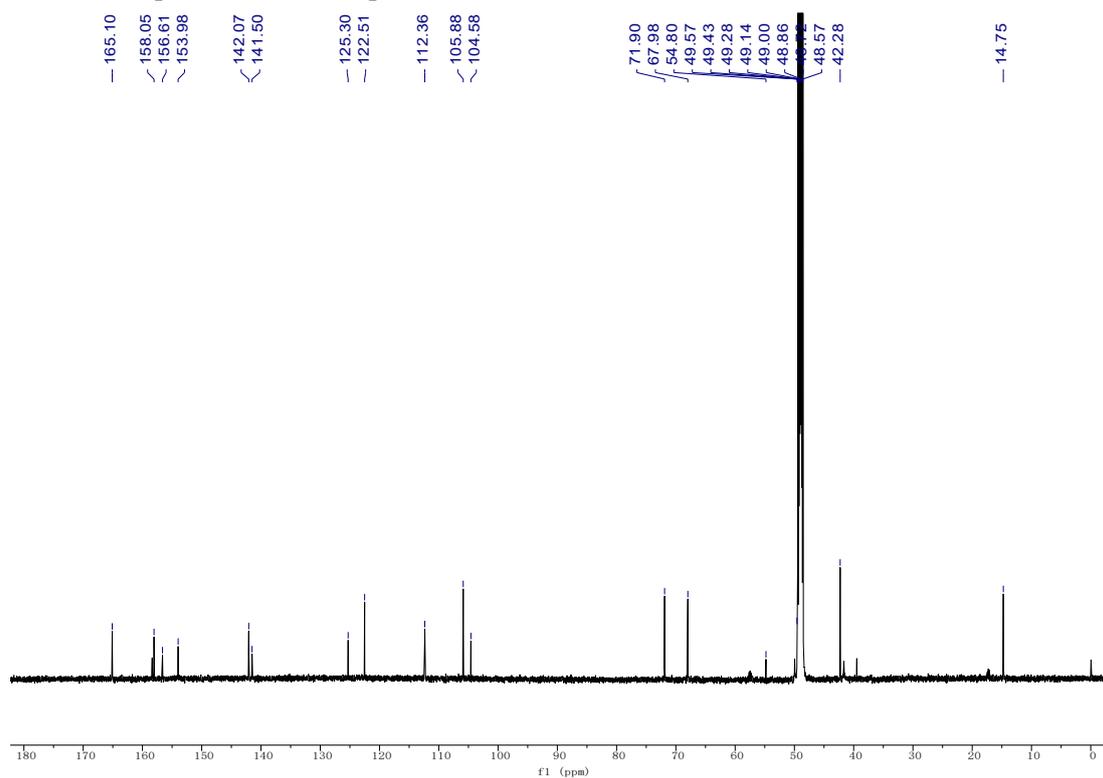
(R)-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)pyridin-2-amine (2)



¹H NMR spectrum for compound 2

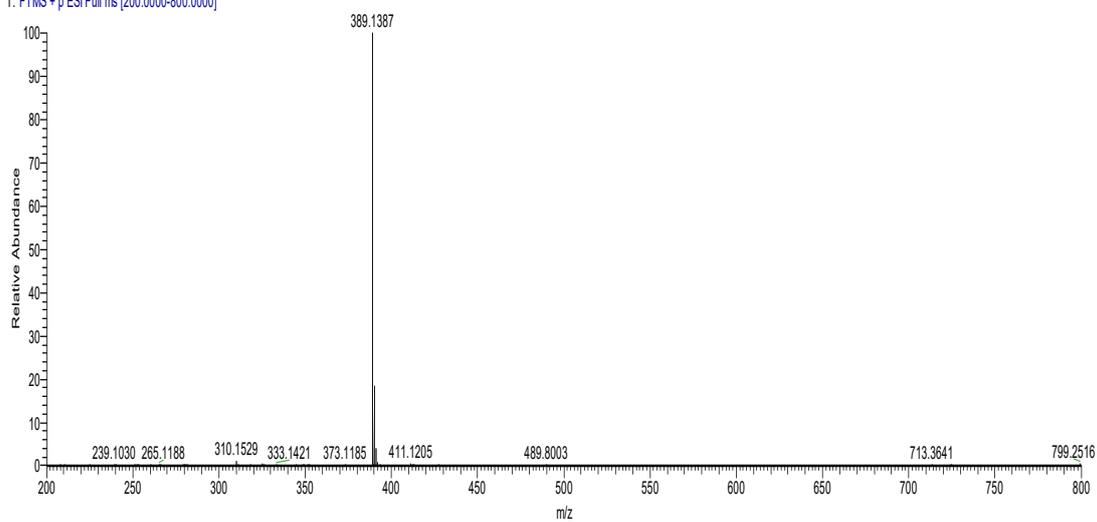


¹³C NMR spectrum for compound 2

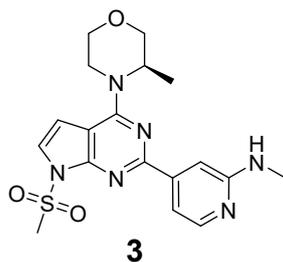


HRMS spectrum for compound 2

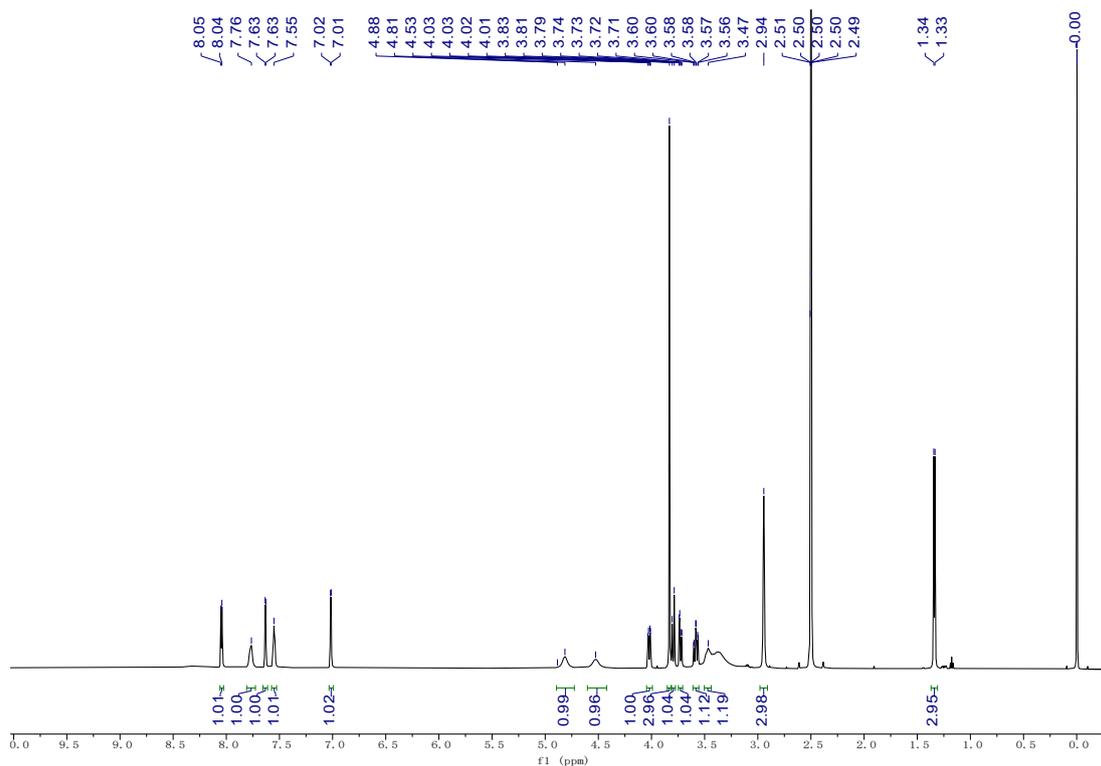
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T: FTMS + p ESI Full ms [200.0000-800.0000]



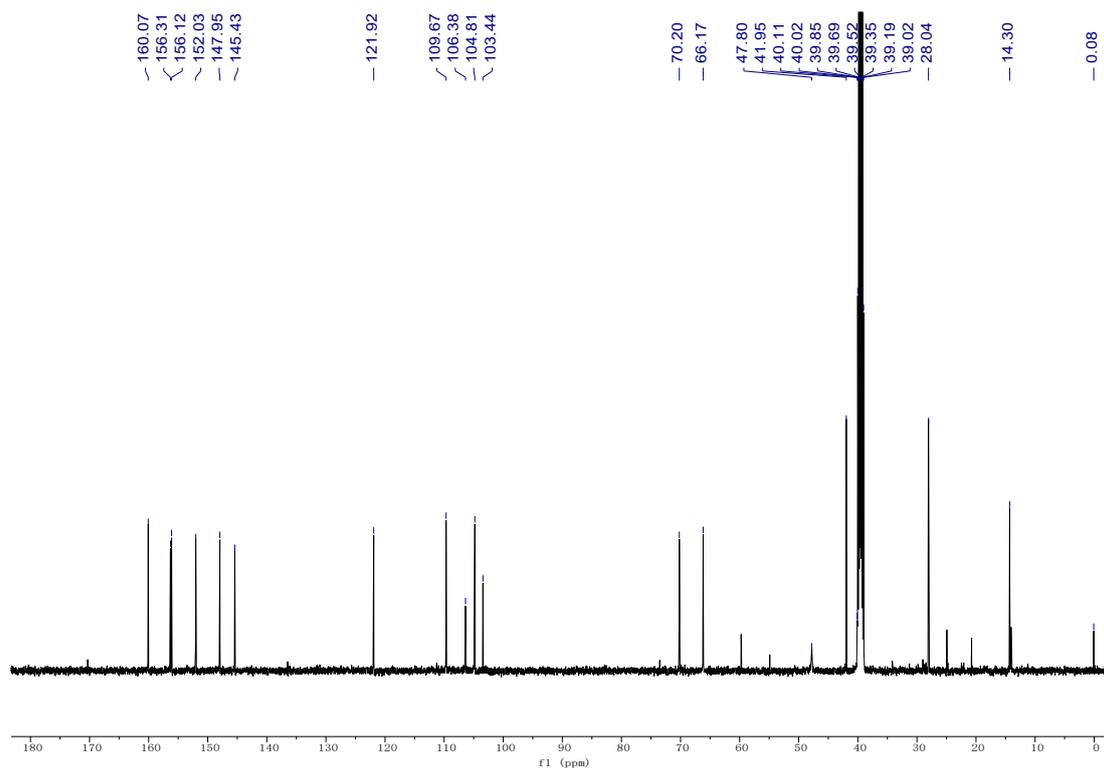
(R)-N-methyl-4-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)pyridin-2-amine (3)



¹H NMR spectrum for compound 3

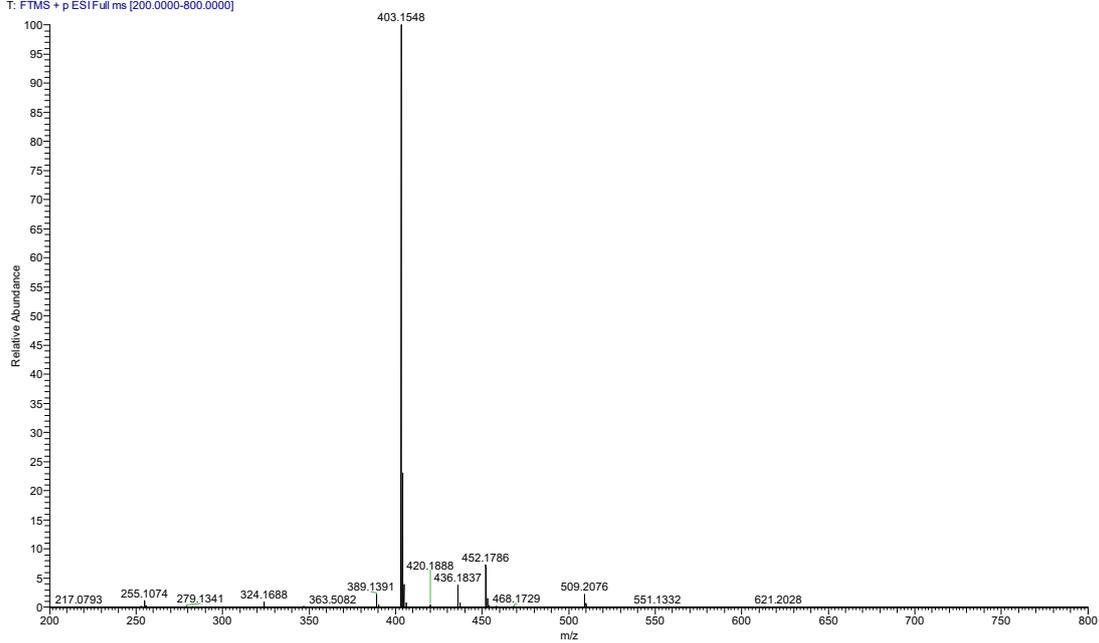


¹³C NMR spectrum for compound 3

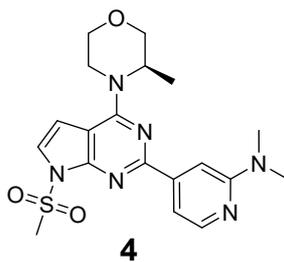


HMRS spectrum for compound 3

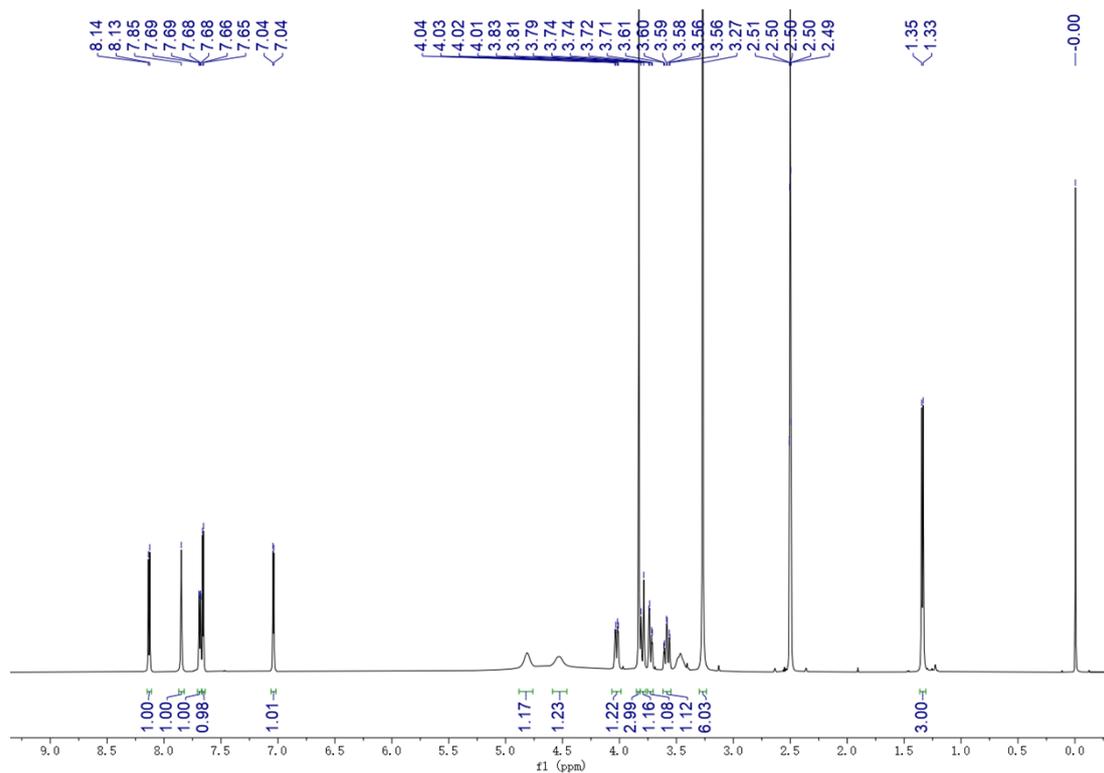
GBA-16-16 #900 RT: 4.01 AV: 1 NL: 2.44E9
T: FTMS + p ESIFull ms [200.0000-800.0000]



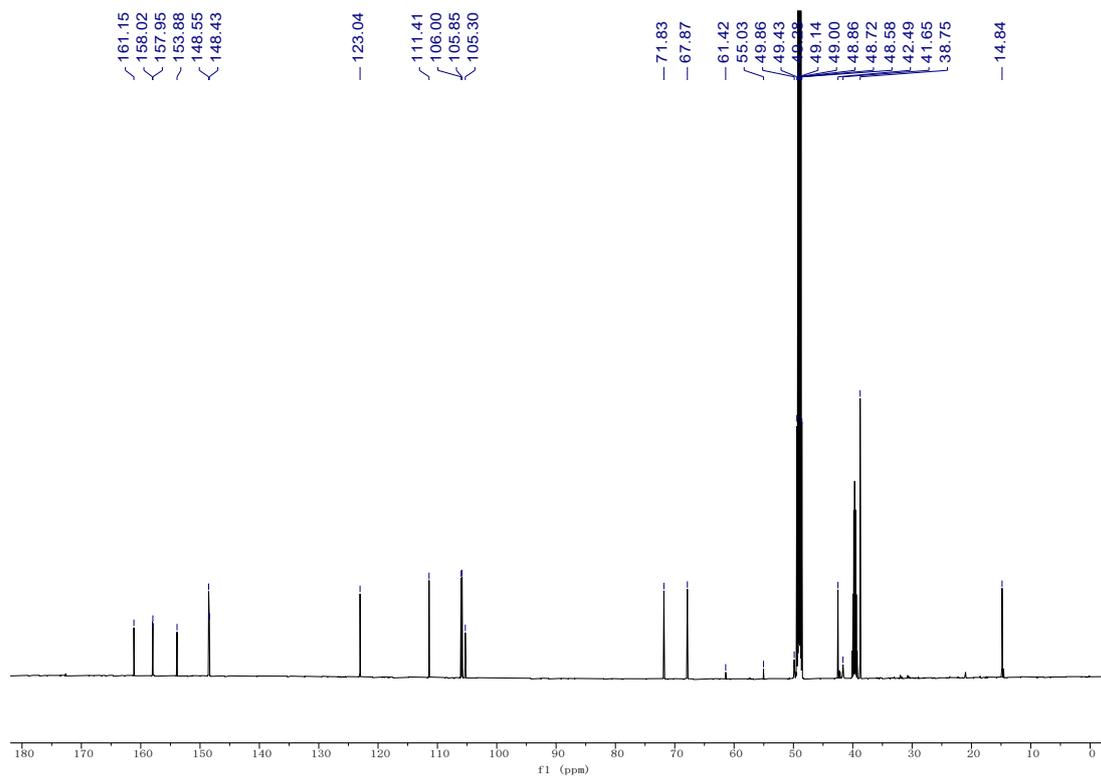
(R)-N,N-dimethyl-4-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)pyridin-2-amine (4)



¹H NMR spectrum for compound 4

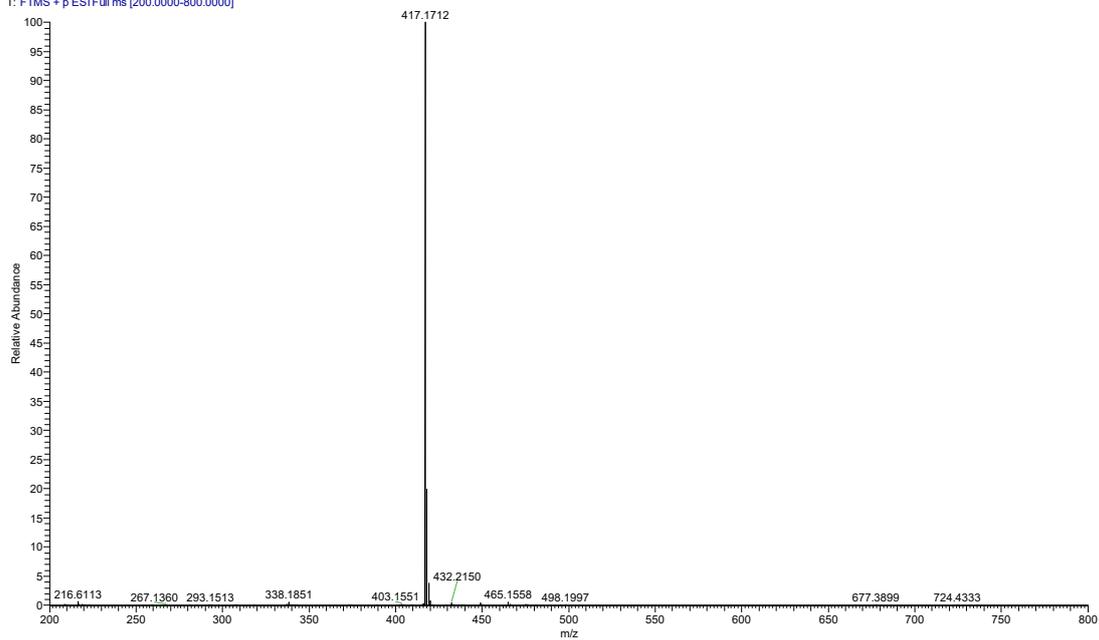


¹³C NMR spectrum for compound 4

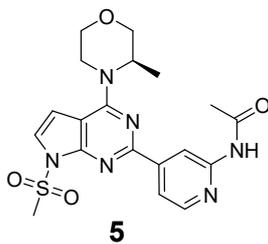


HMRS spectrum for compound 4

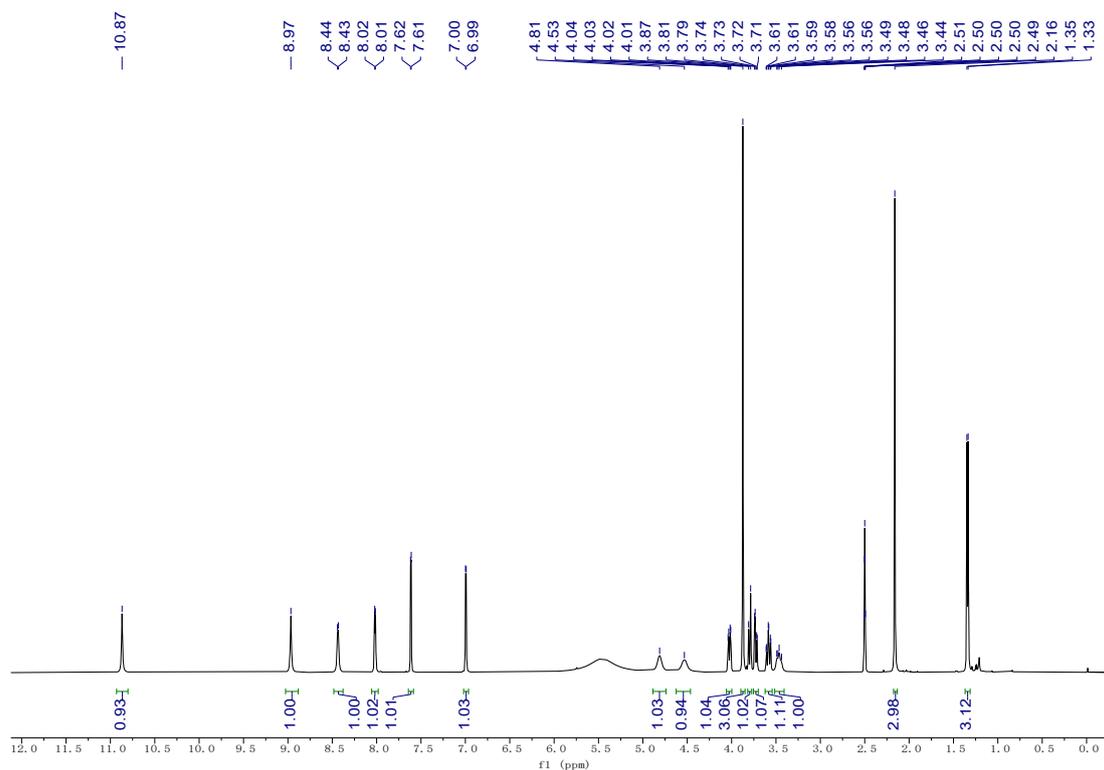
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T: FTMS + p ESI Full ms [200.0000-800.0000]



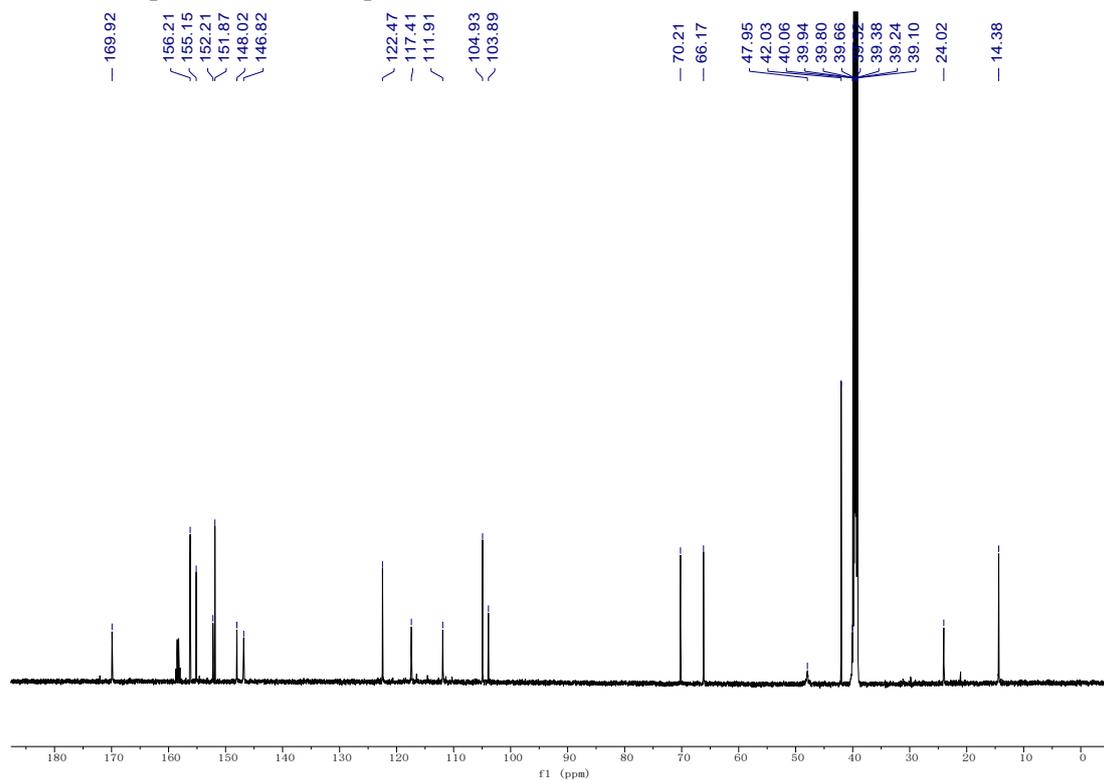
(R)-N-(4-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)pyridin-2-yl)acetamide (5)



¹H NMR spectrum for compound 5

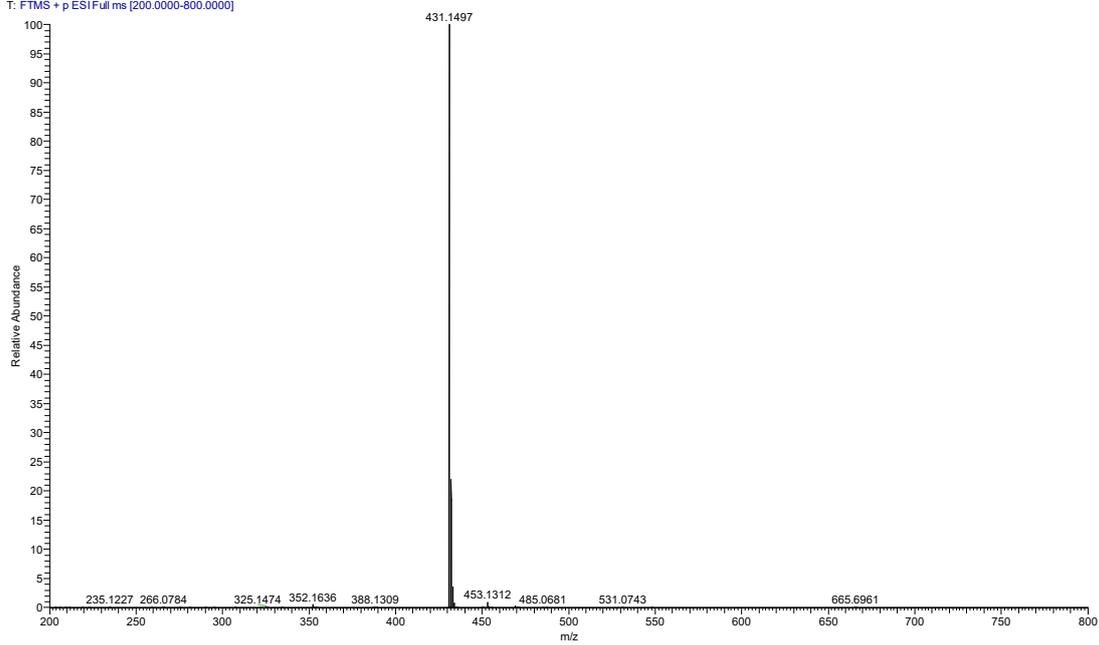


¹³C NMR spectrum for compound 5

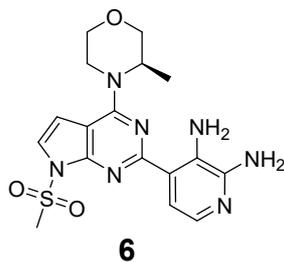


HRMS spectrum for compound 5

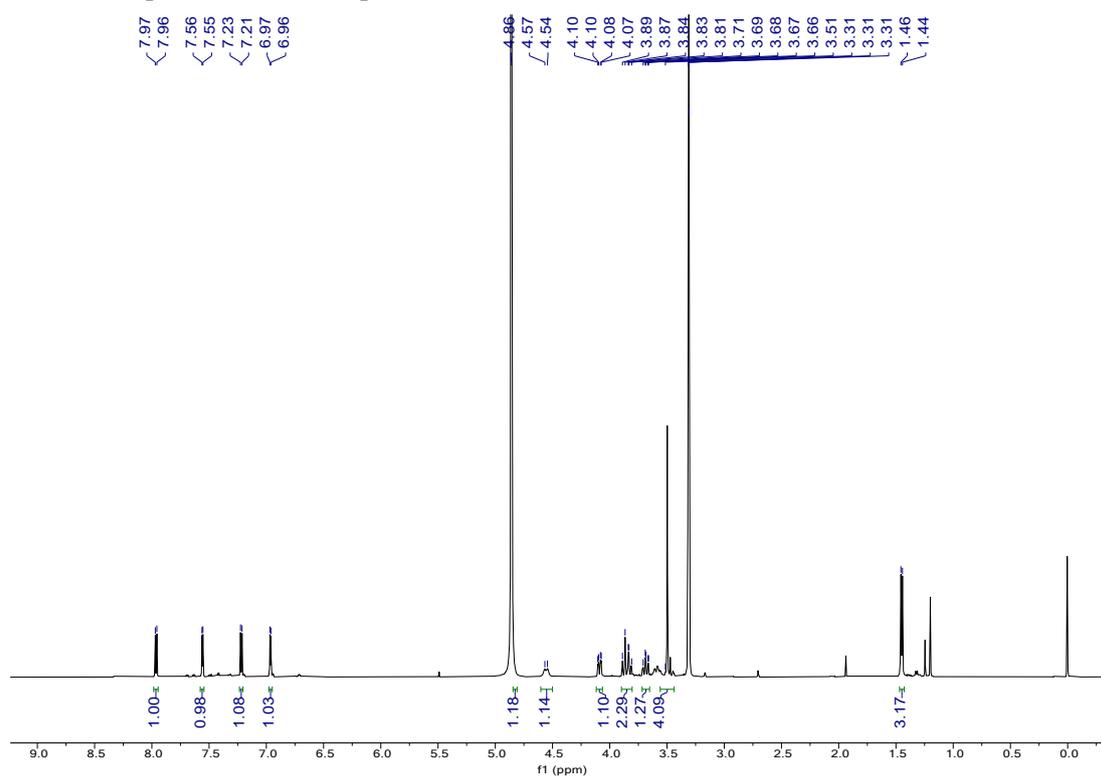
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T: FTMS + p ESIFull.ms [200.0000-800.0000]



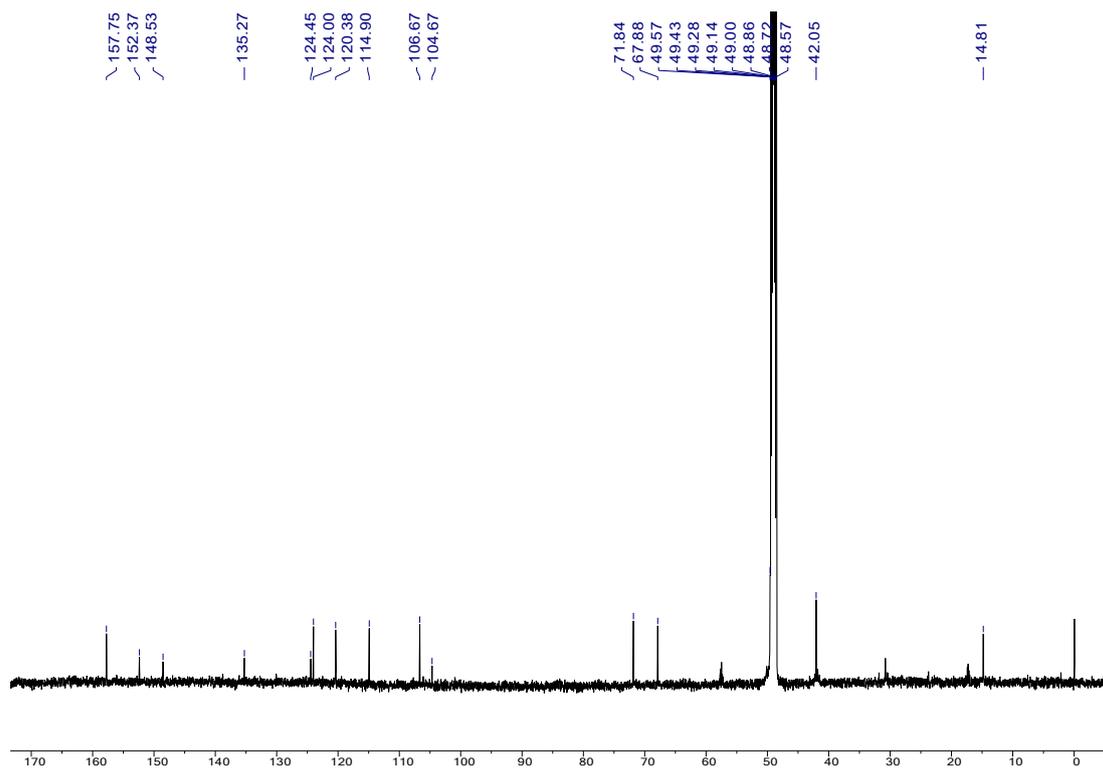
(R)-4-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)pyridine-2,3-diamine (6)



¹H NMR spectrum for compound 6

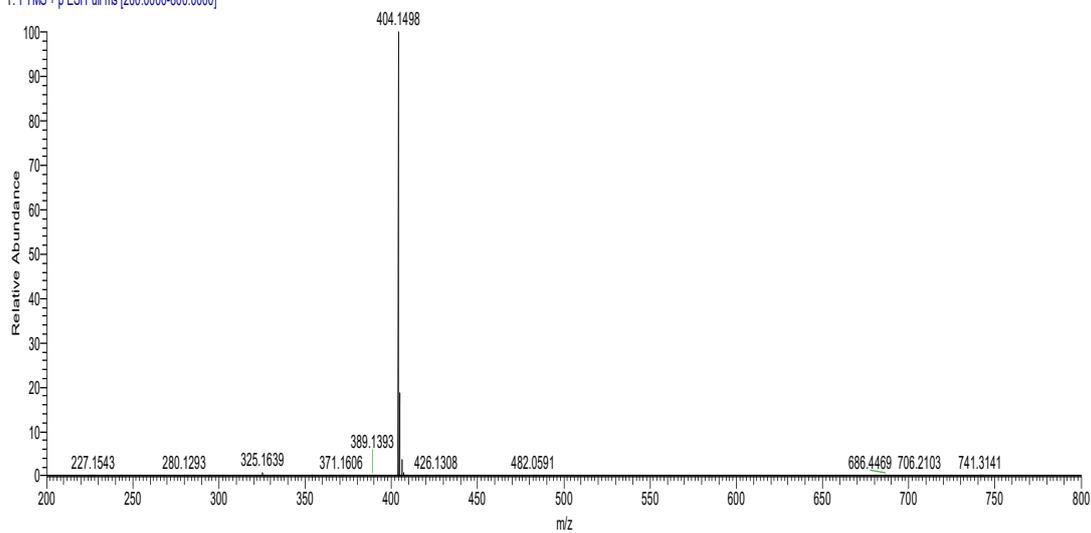


¹³C NMR spectrum for compound 6

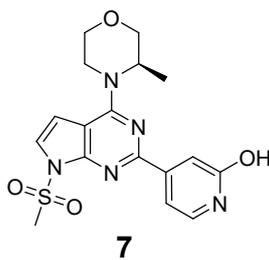


HRMS spectrum for compound 6

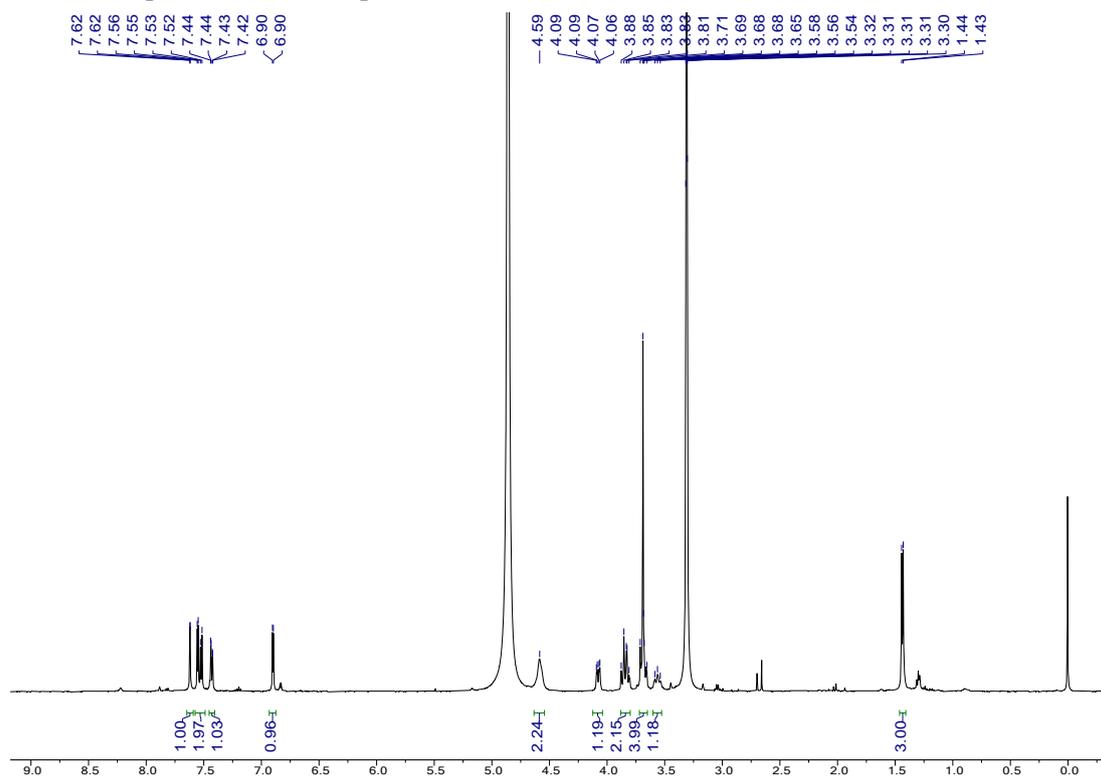
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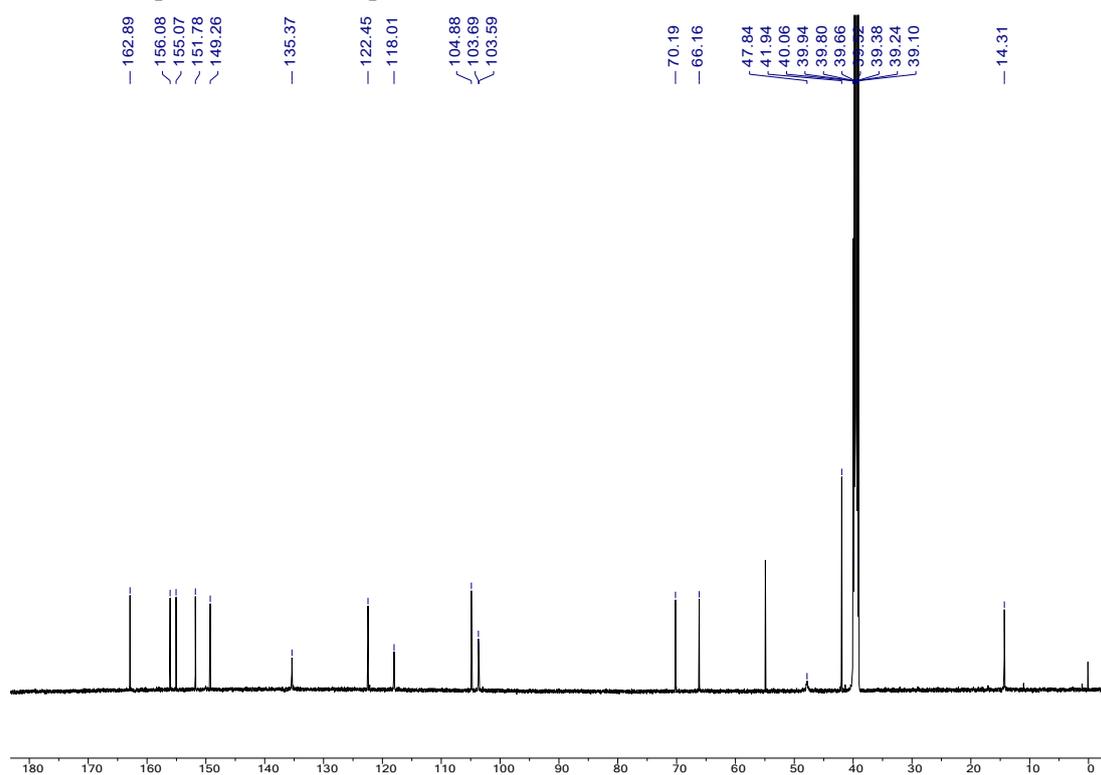
(R)-4-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)pyridin-2-ol (7)



¹H NMR spectrum for compound 7

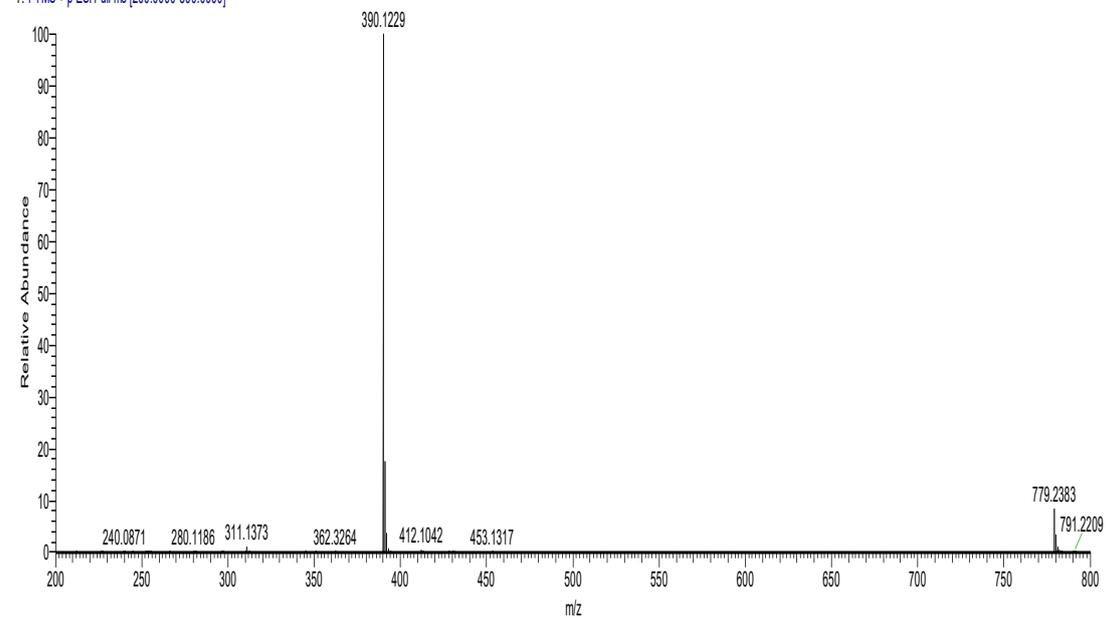


¹³C NMR spectrum for compound 7

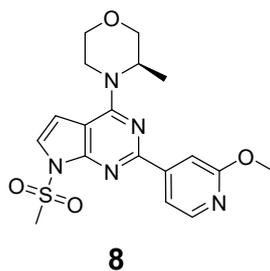


HRMS spectrum for compound 7

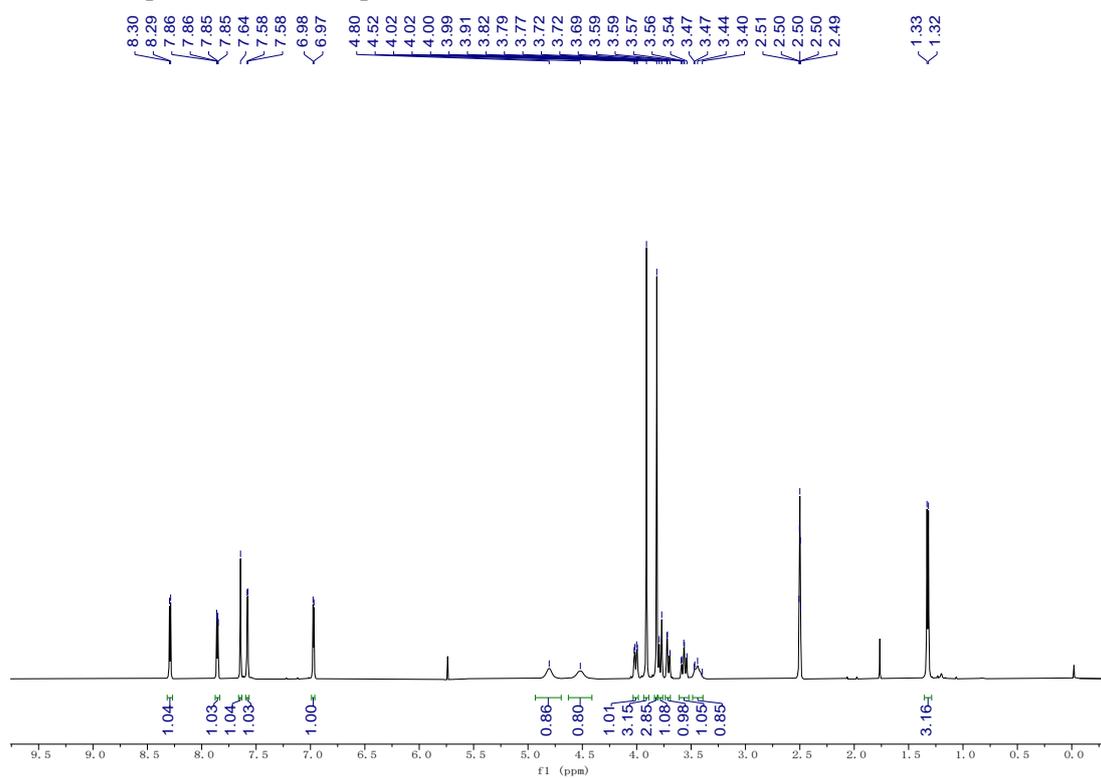
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(R)-4-(2-(3-methoxyphenyl)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-3-methylmorpholine (8)

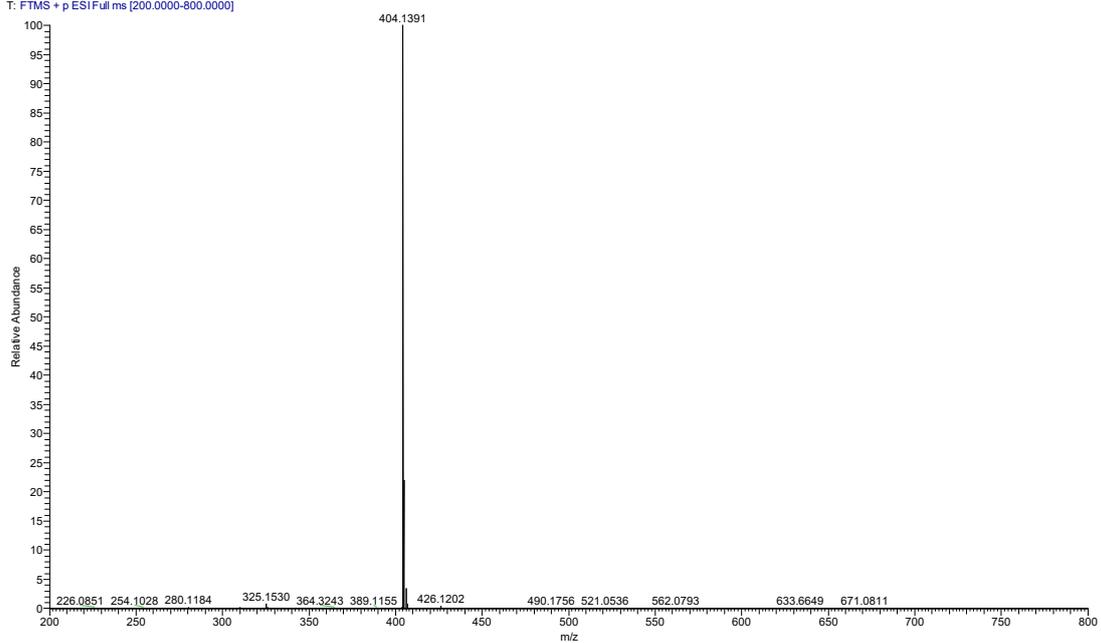


¹H NMR spectrum for compound 8

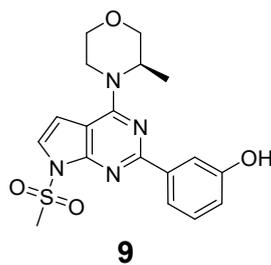


HRMS spectrum for compound 8

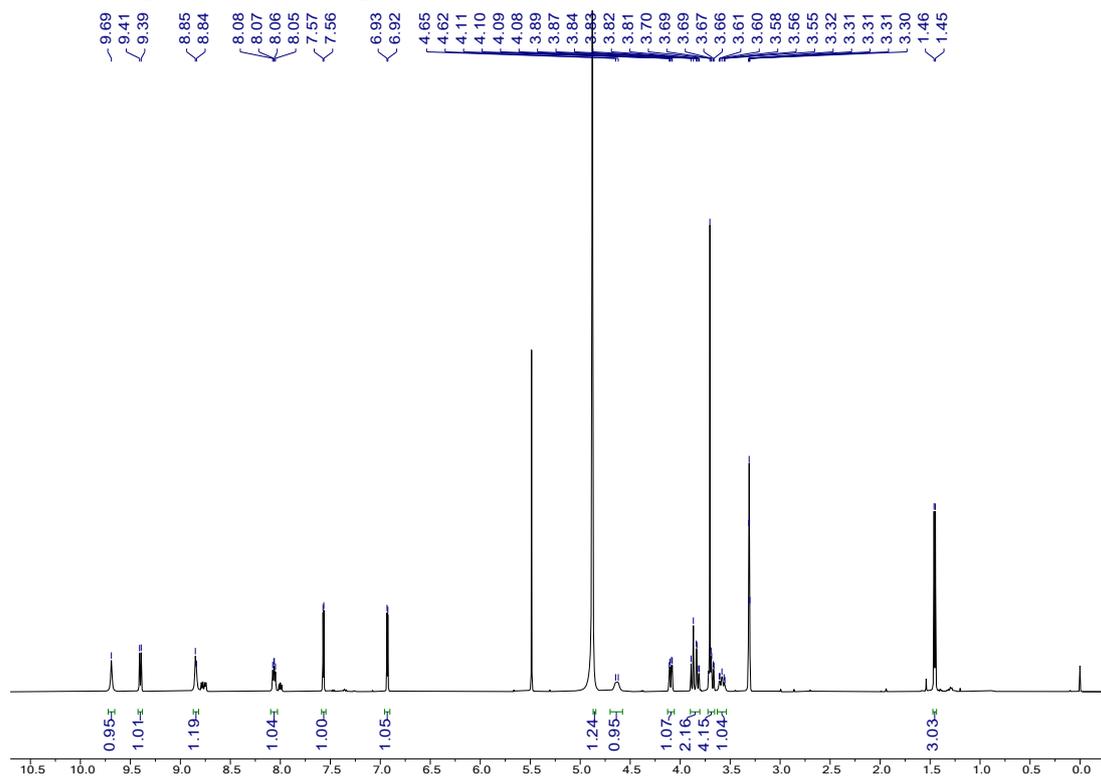
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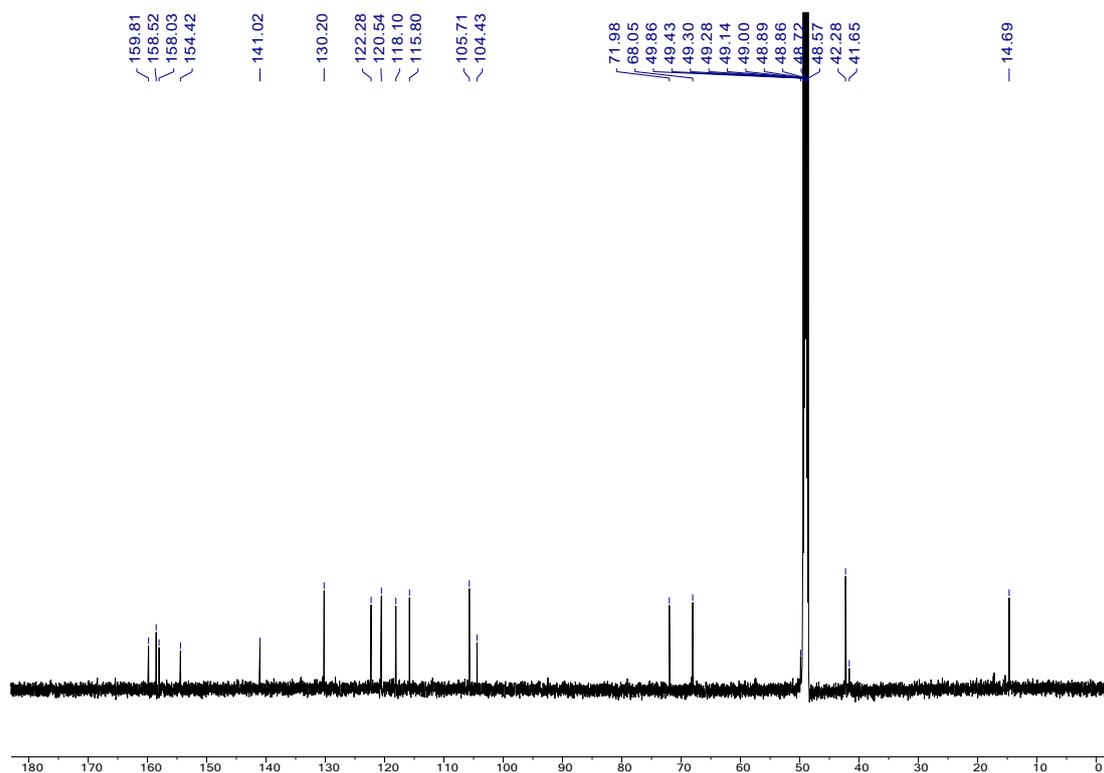
(R)-3-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)phenol(9)



¹H NMR spectrum for compound 9

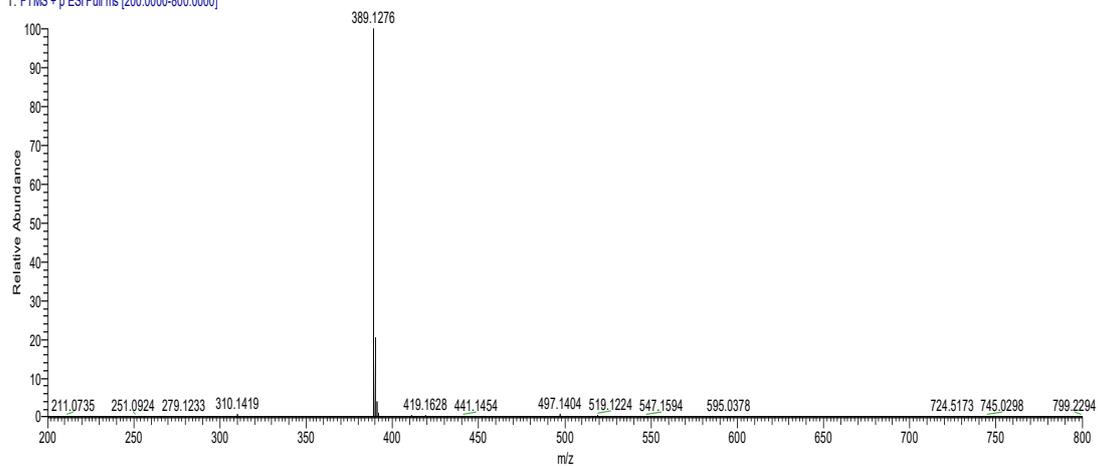


¹³C NMR spectrum for compound **9**

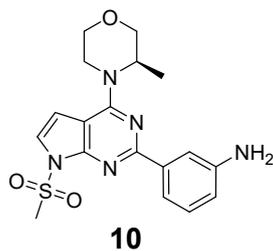


HRMS spectrum for compound **9**

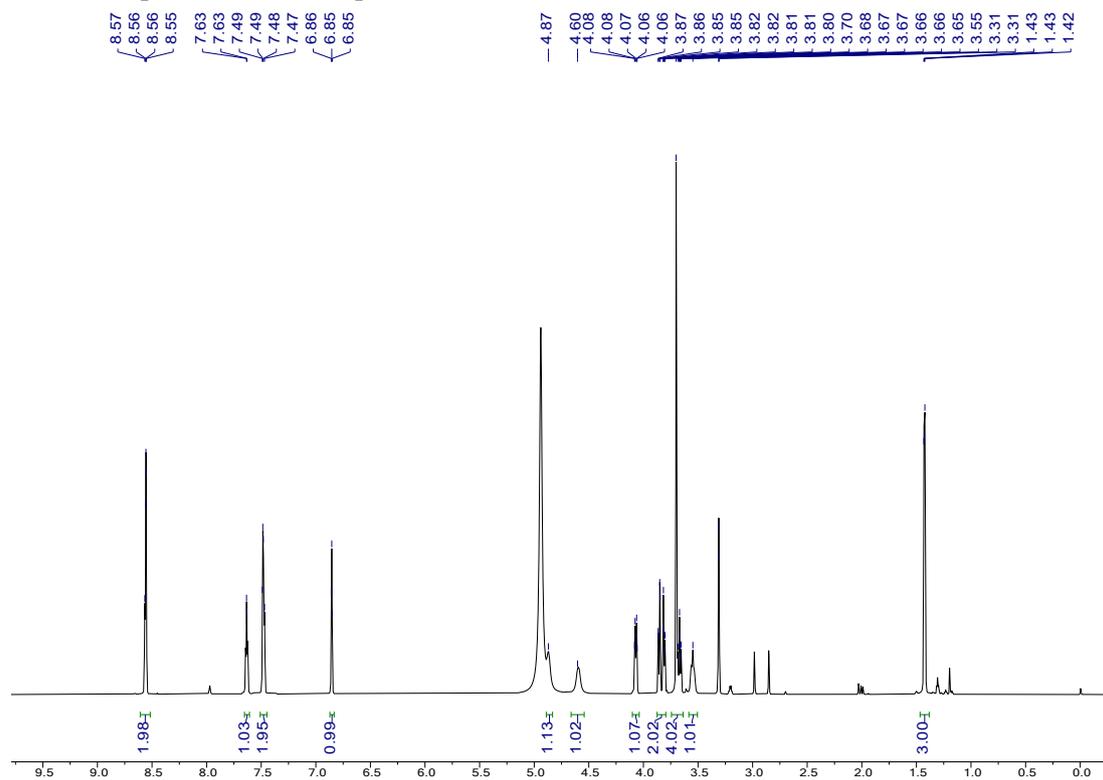
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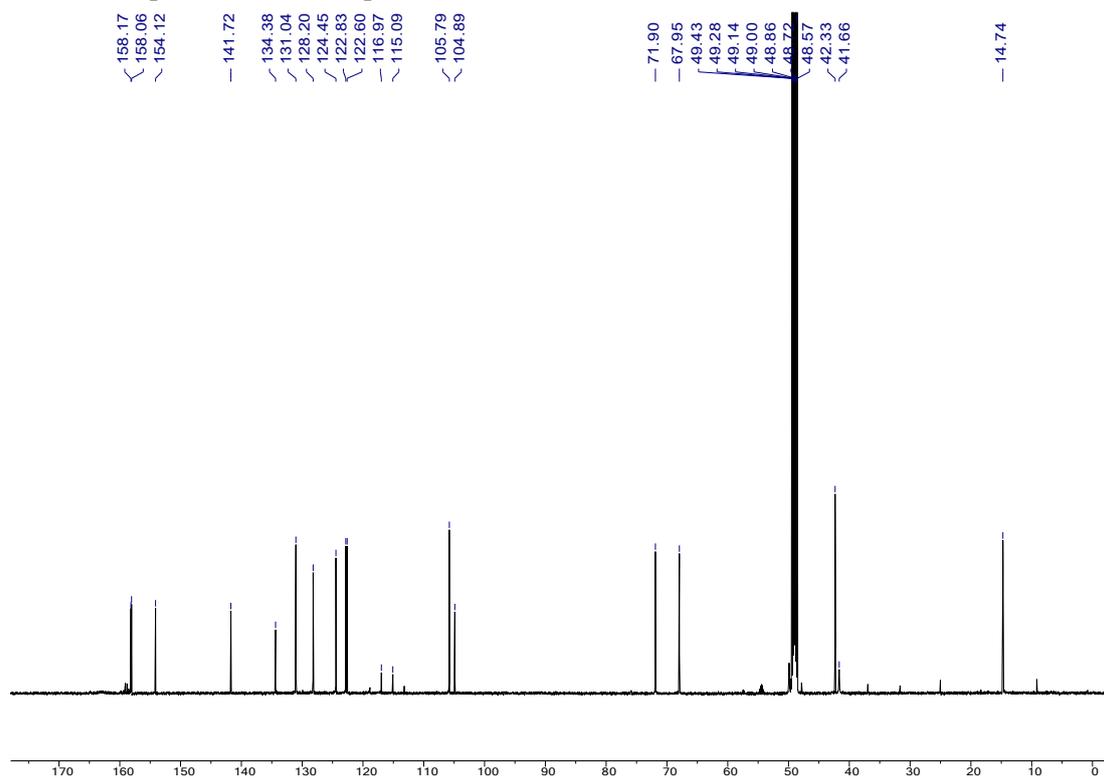
(R)-3-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)aniline
(10)



¹H NMR spectrum for compound **10**

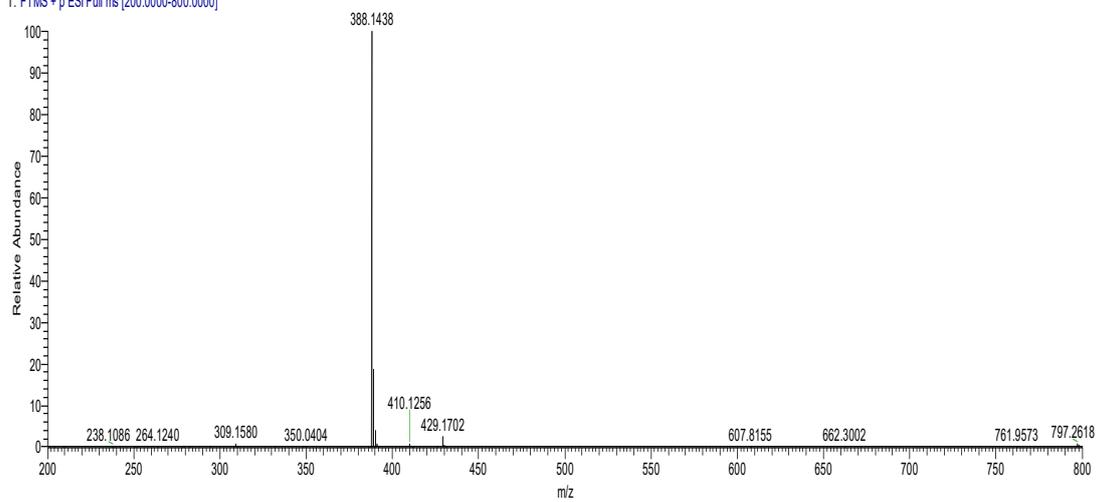


¹³C NMR spectrum for compound 10

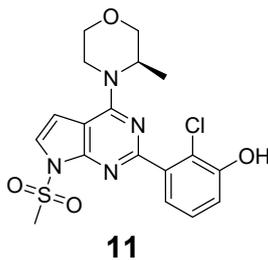


HRMS spectrum for compound 10

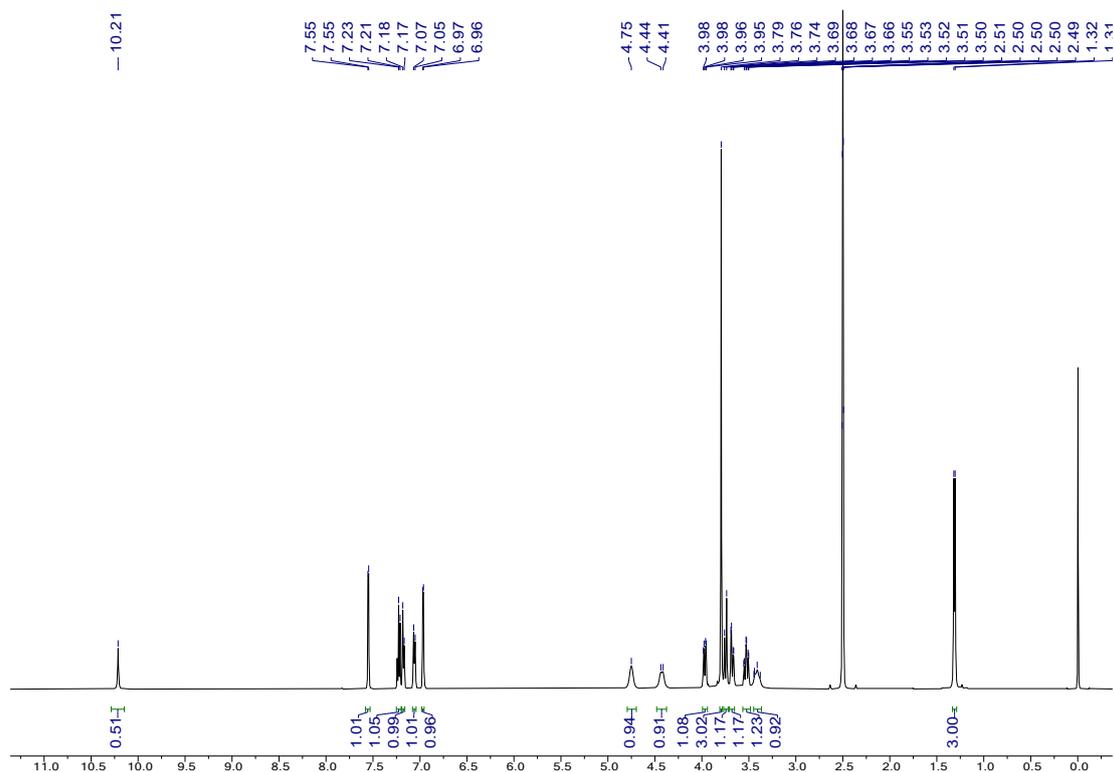
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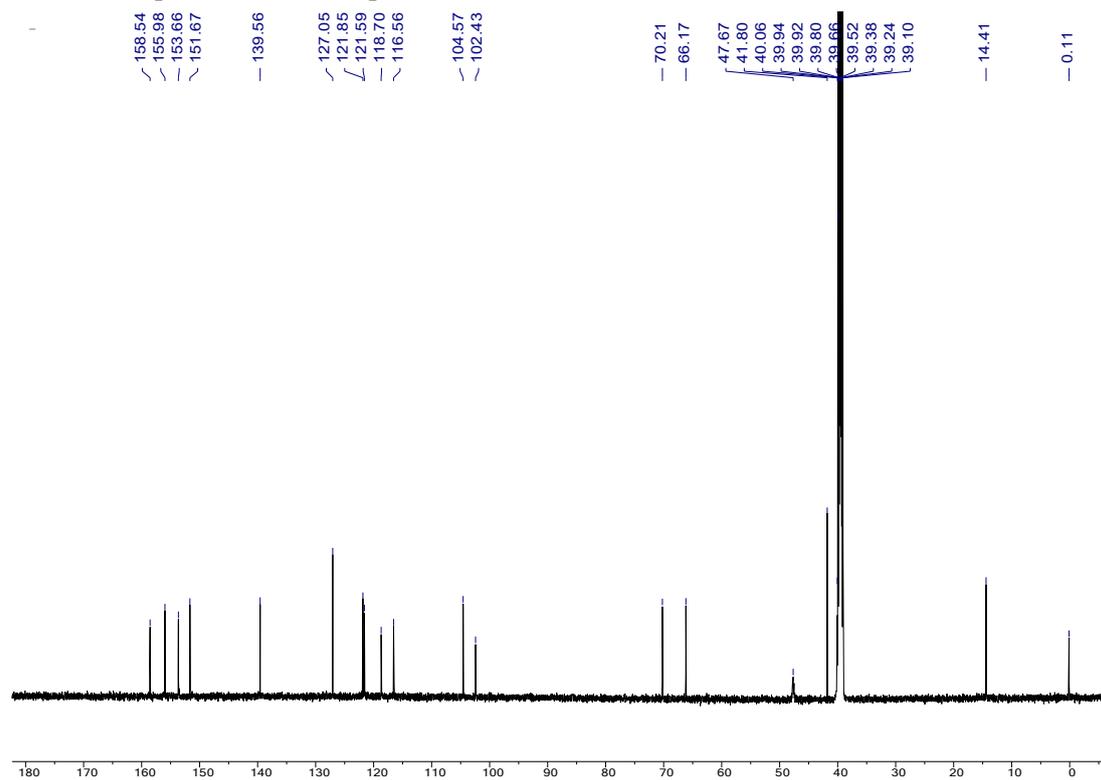
(R)-2-chloro-3-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)phenol (11)



¹H NMR spectrum for compound **11**

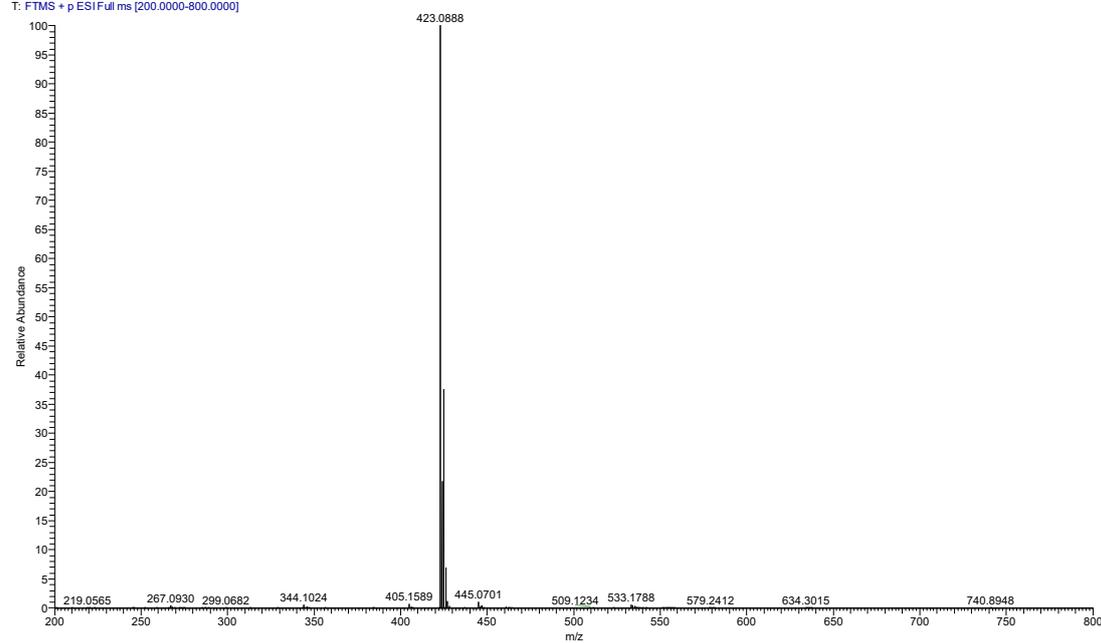


¹³C NMR spectrum for compound 11

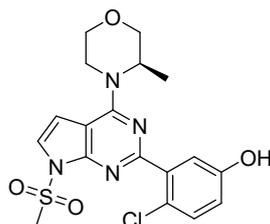


HRMS spectrum for compound 11

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T: FTMS + p ESI Full ms [200.0000-800.0000]

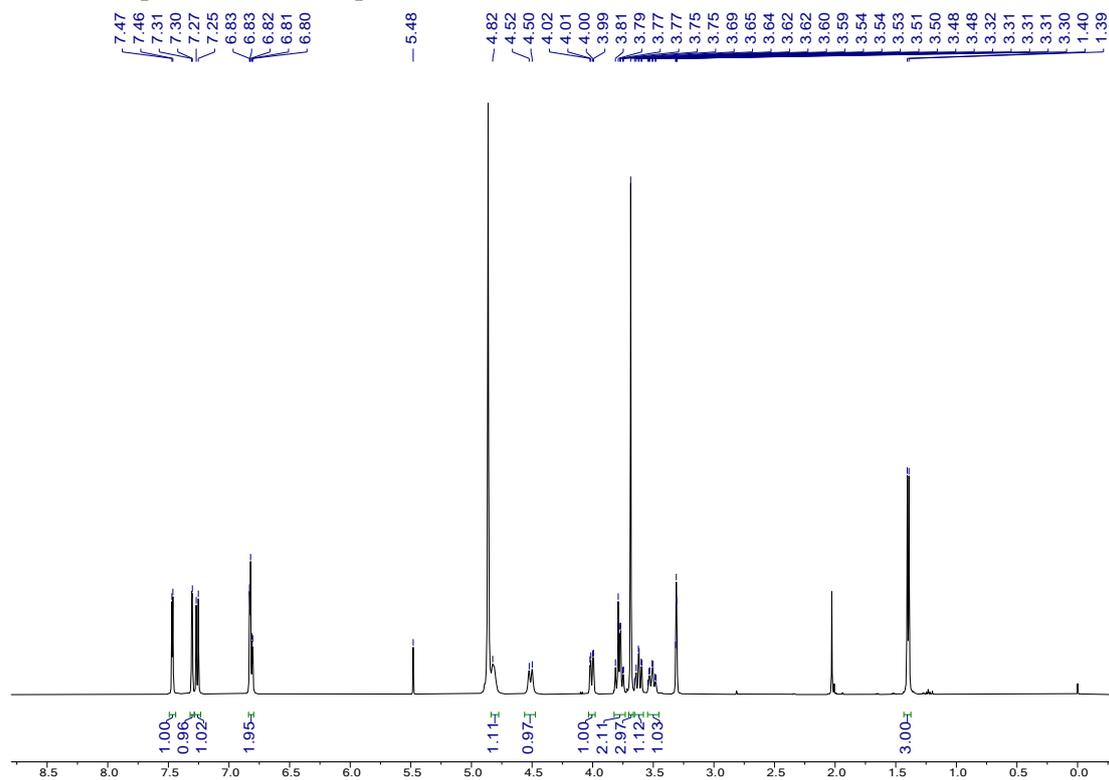


(R)-4-chloro-3-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)phenol (12)

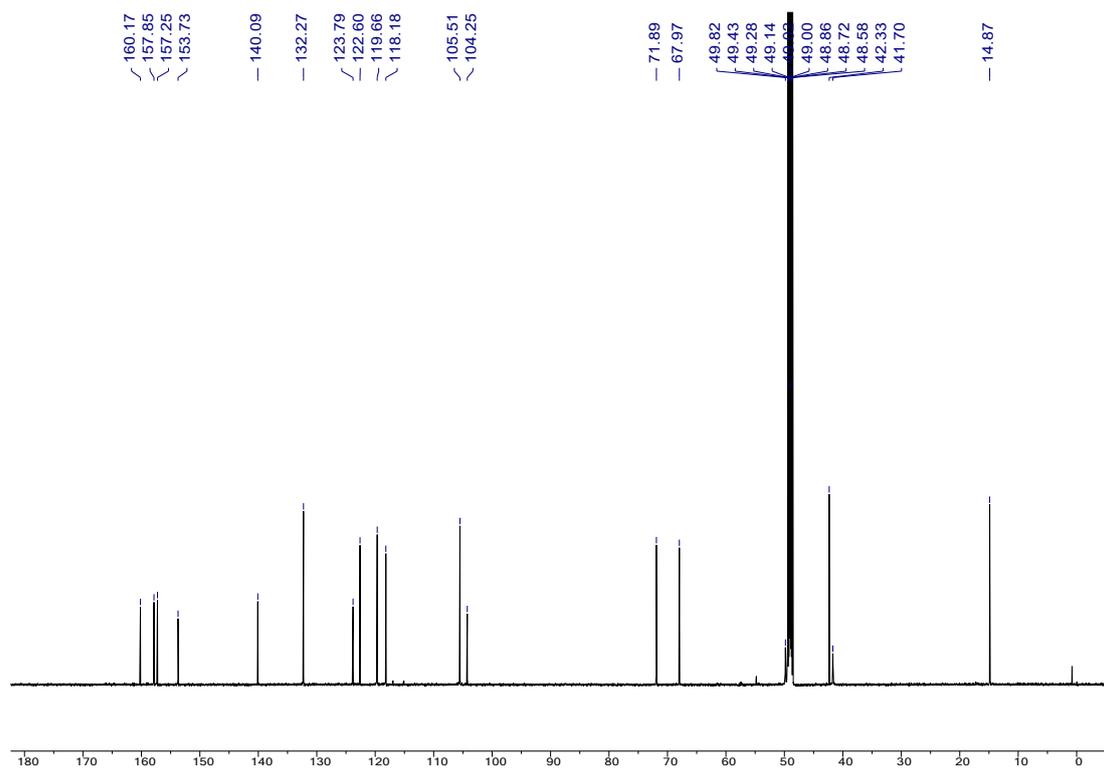


12

¹H NMR spectrum for compound 12

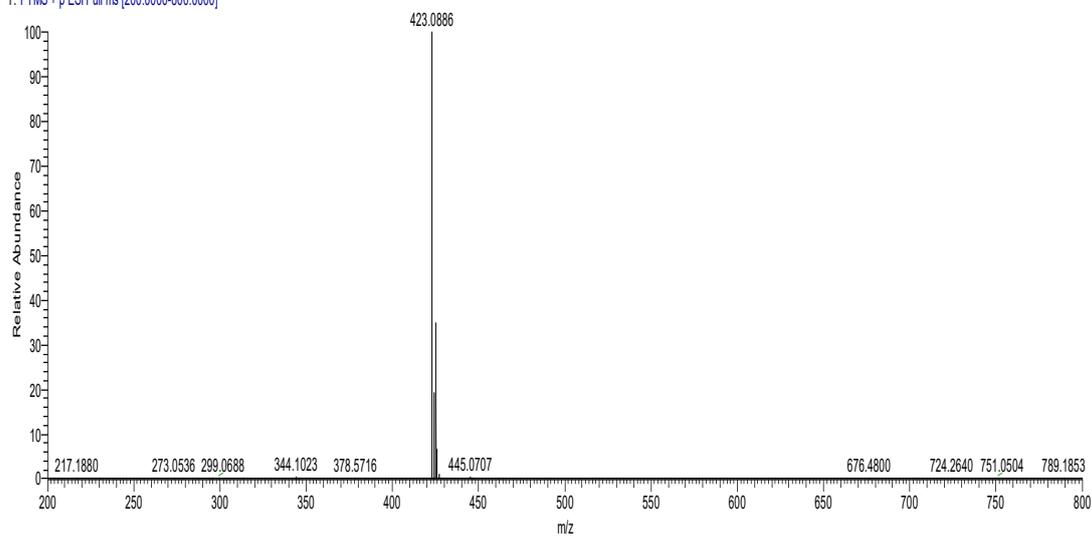


¹³C NMR spectrum for compound 12

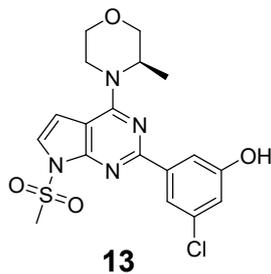


HRMS spectrum for compound 12

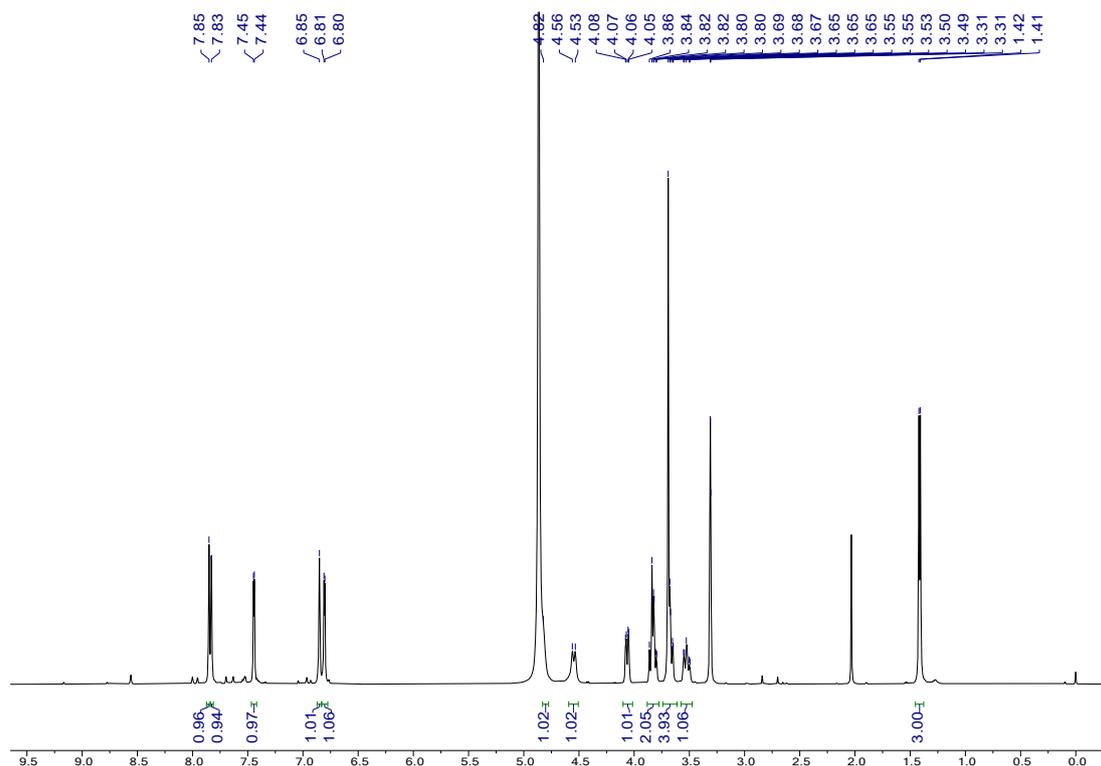
A13 #1231 RT: 5.49 AV: 1 NL: 6.36E9
T: FTMS + p ESI Full ms [200.0000-800.0000]



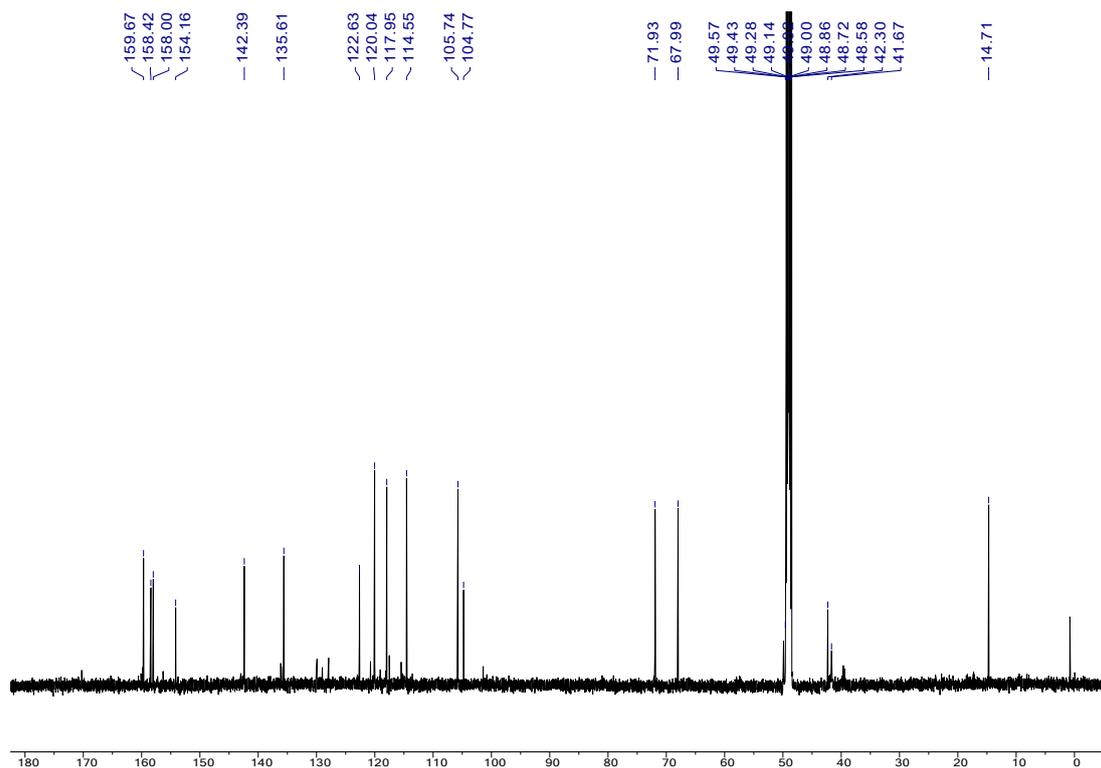
(R)-3-chloro-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)phenol (13)



¹H NMR spectrum for compound **13**

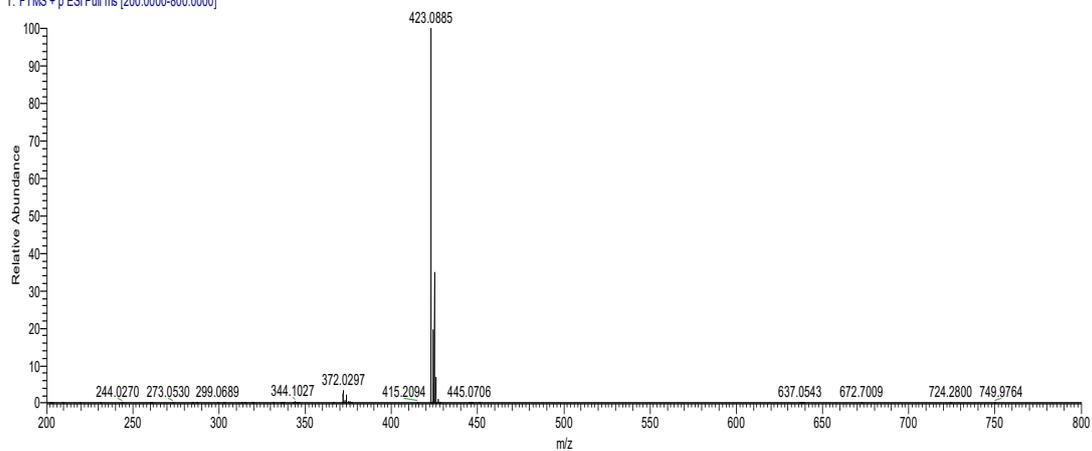


¹³C NMR spectrum for compound 13

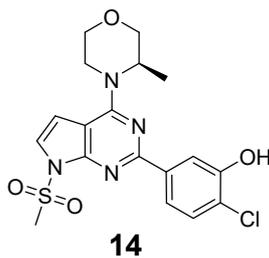


HRMS spectrum for compound 13

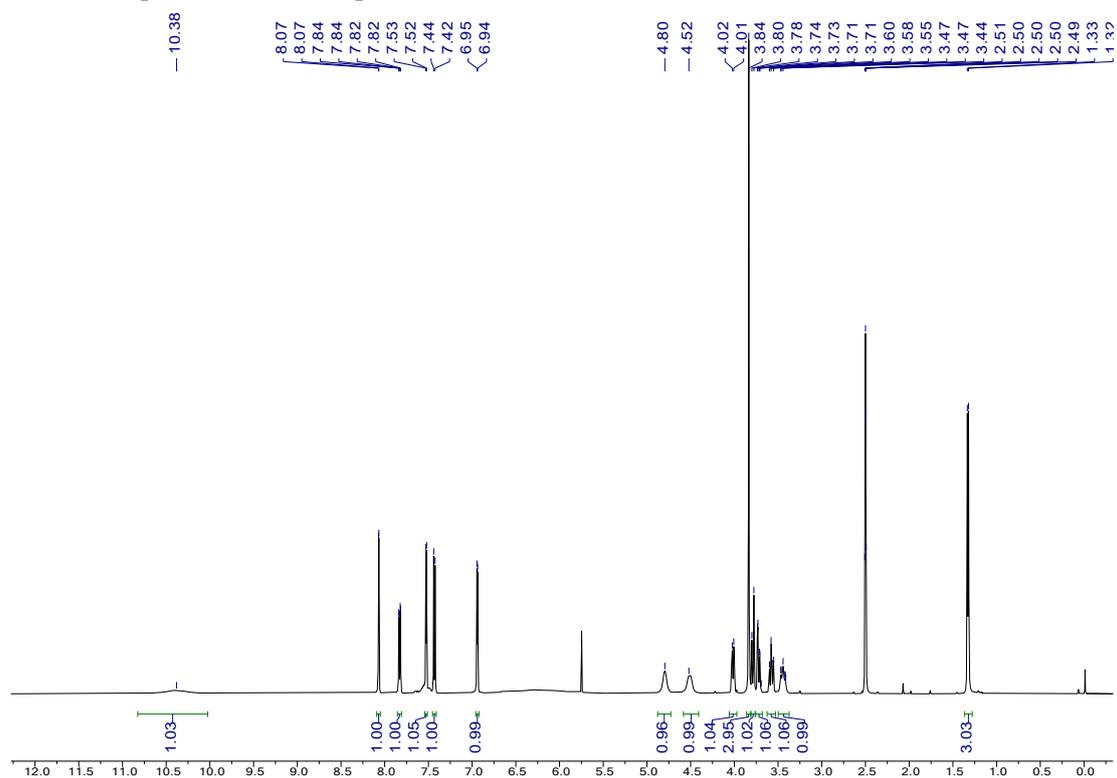
A12#1278 RT: 5.70 AV: 1 NL: 2.47E9
T: FTMS+p ESI Full ms [200.0000-800.0000]



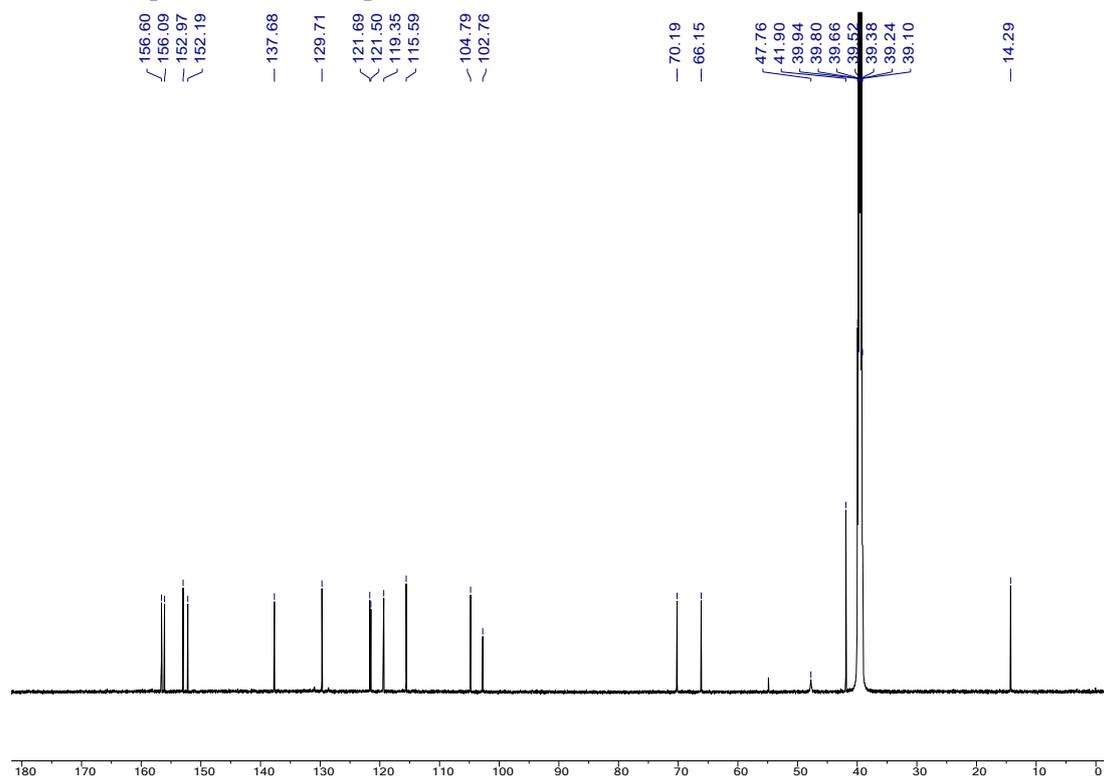
(R)-2-chloro-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)phenol (14)



¹H NMR spectrum for compound 14

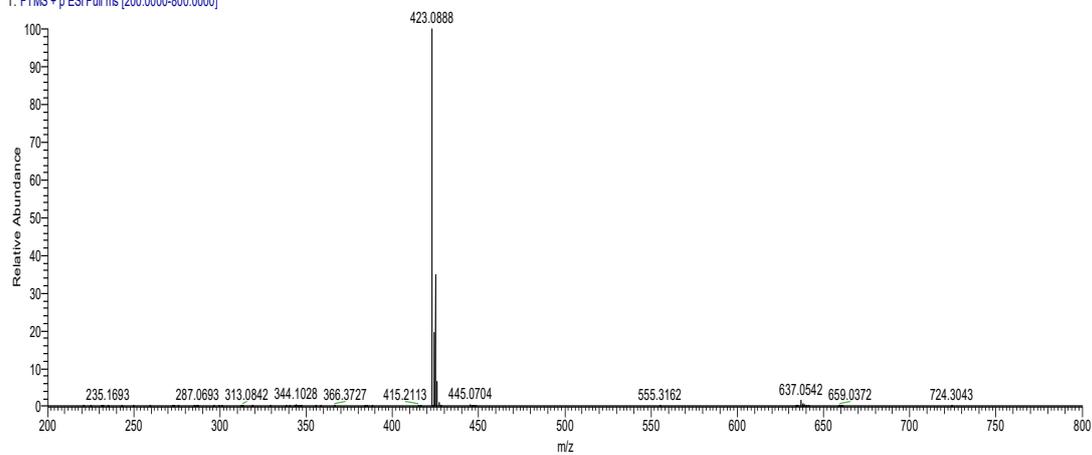


¹³C NMR spectrum for compound 14

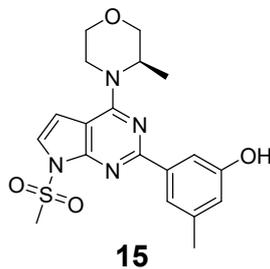


HRMS spectrum for compound 14

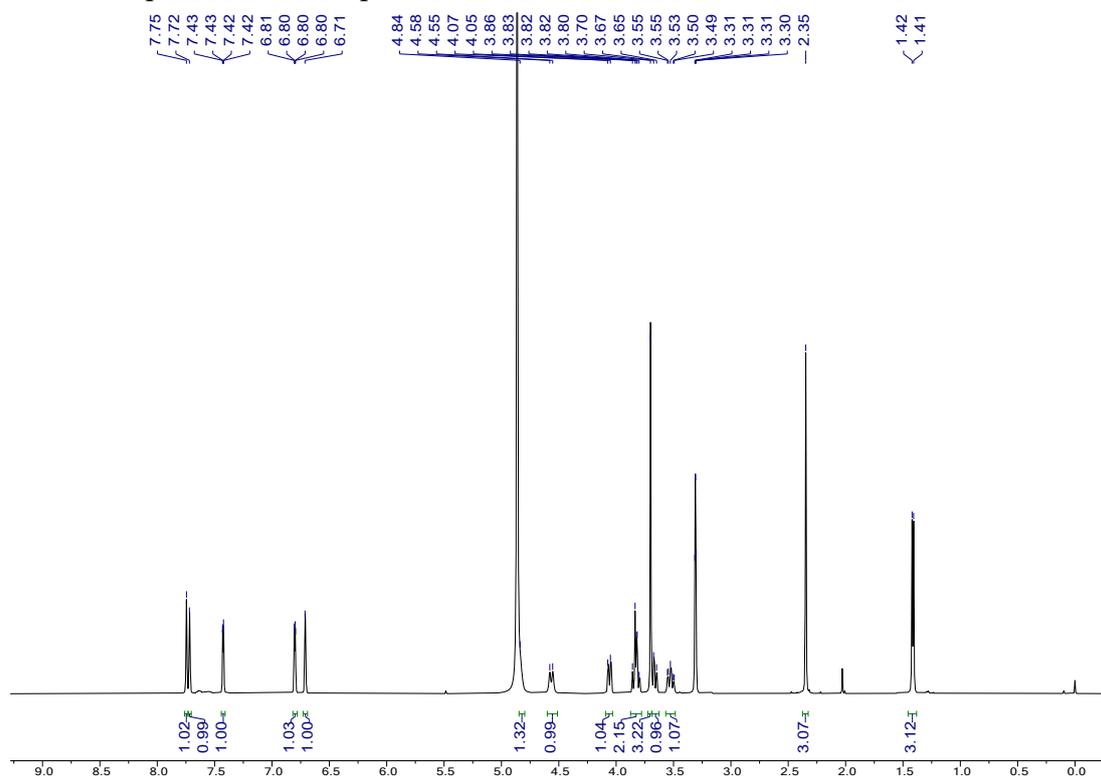
A11#1274 RT: 5.68 AV: 1 NL: 1.57E9
T: FTMS + p ESI Full ms [200.0000-800.0000]



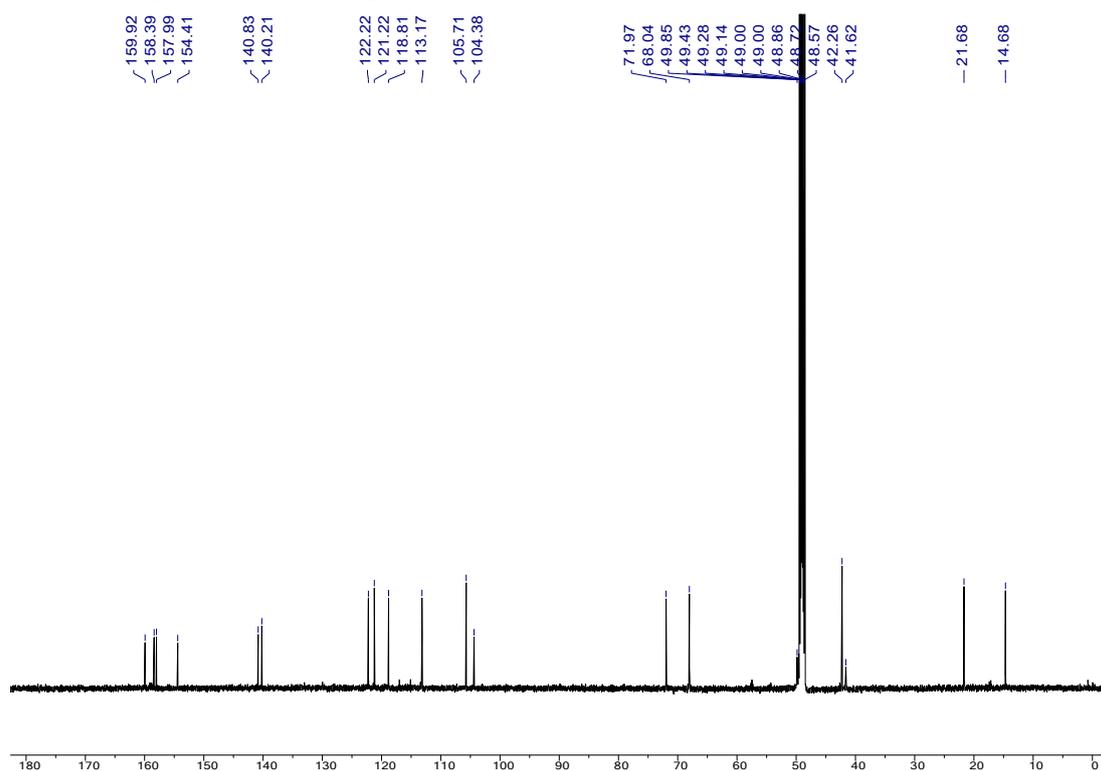
(R)-3-methyl-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)phenol (15)



¹H NMR spectrum for compound 15

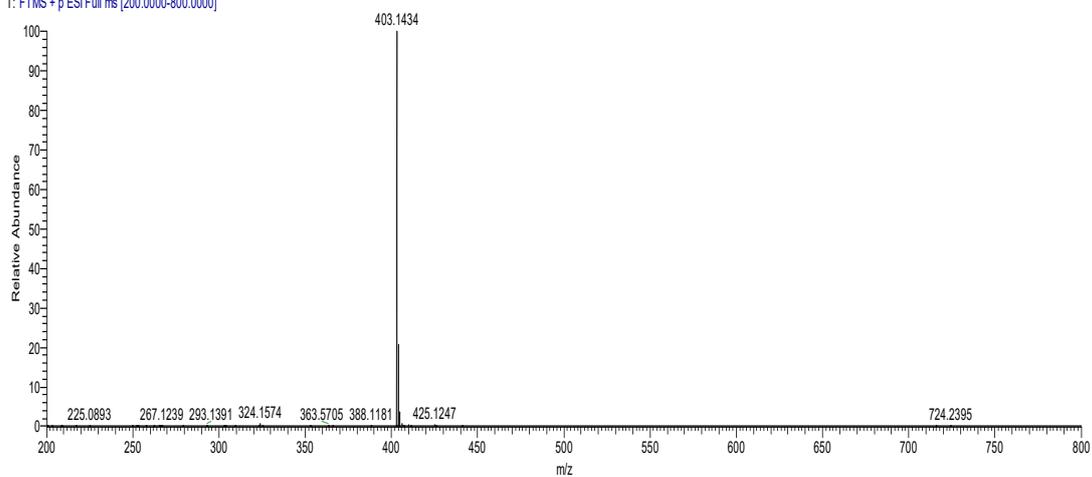


¹³C NMR spectrum for compound 15

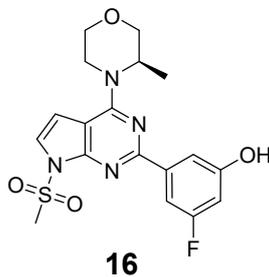


HRMS spectrum for compound 15

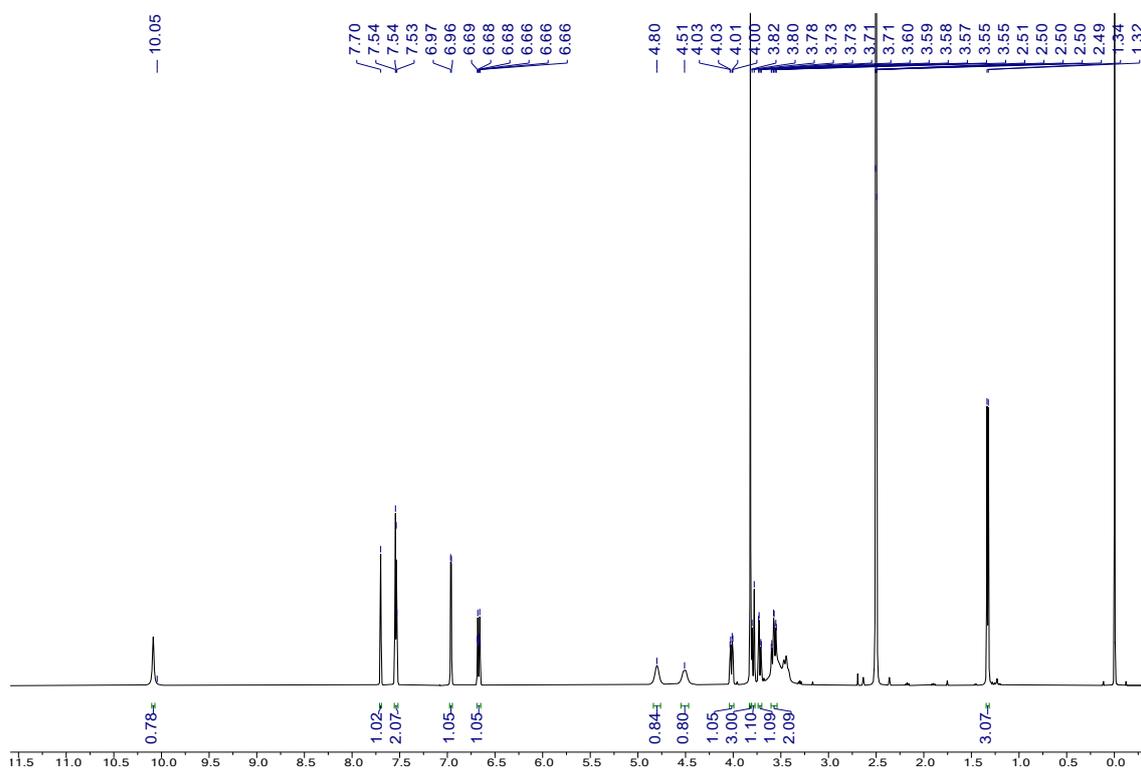
A16#1243 RT: 5.55 AV: 1 NL: 7.43E9
T: FTMS+p ESI Full ms [200.0000-800.0000]



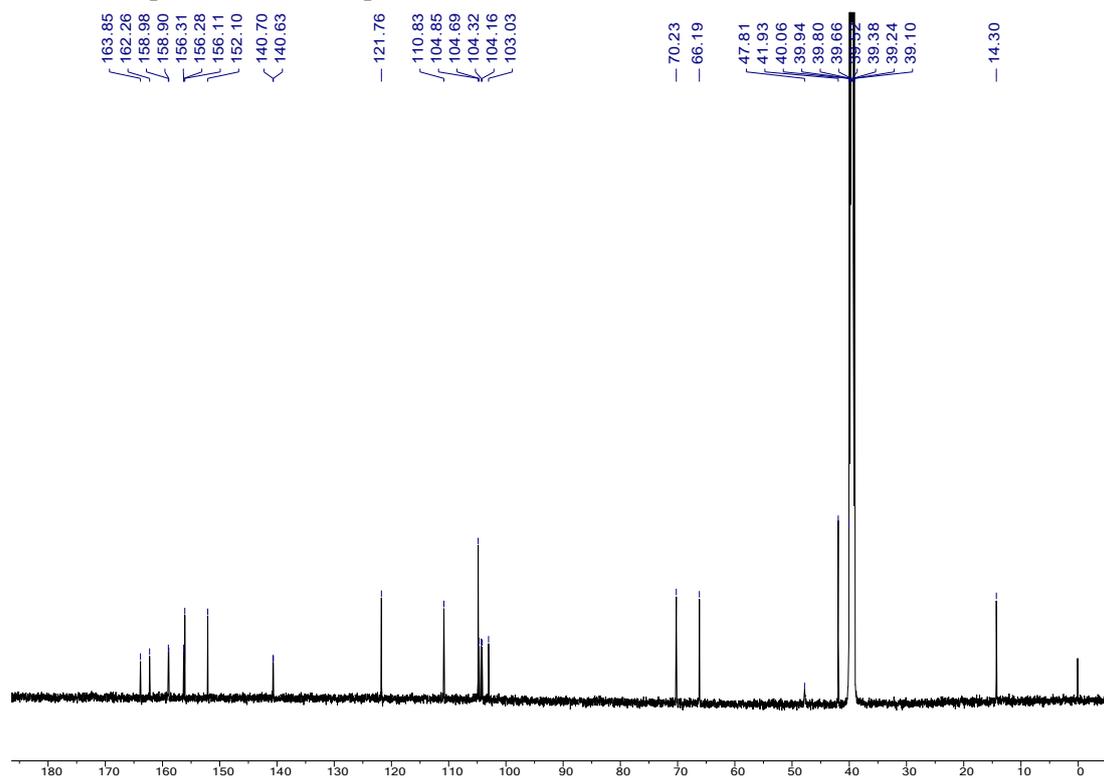
(R)-3-fluoro-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)phenol (16)



¹H NMR spectrum for compound 16

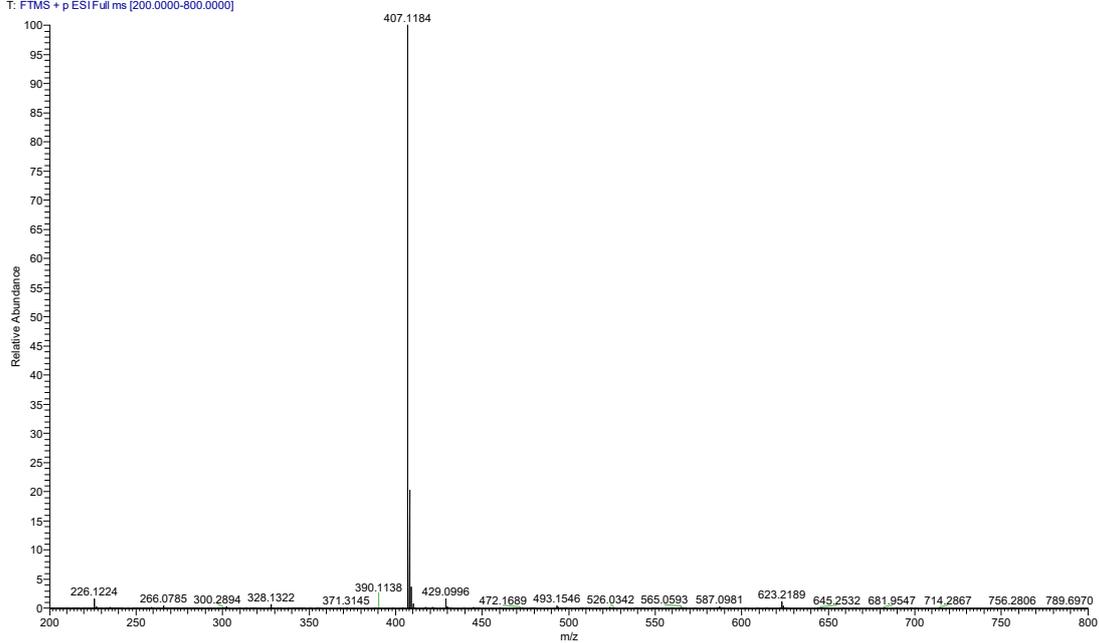


¹³C NMR spectrum for compound 16

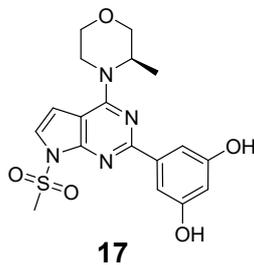


HRMS spectrum for compound 16

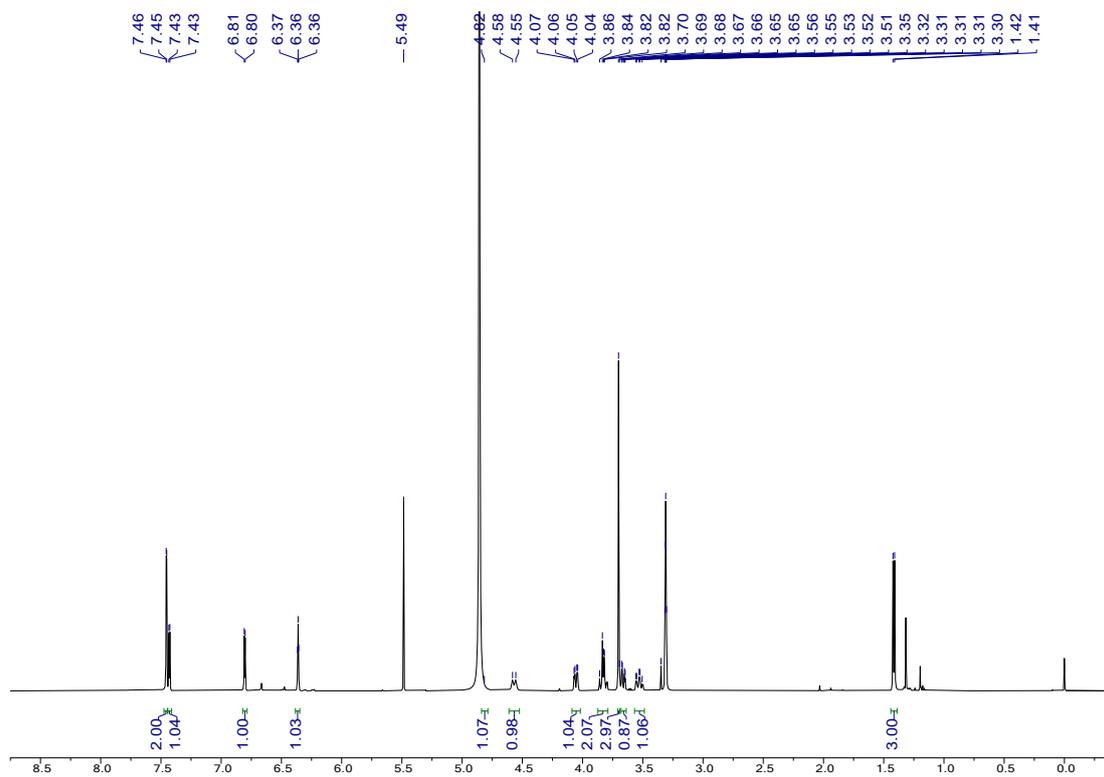
GBA-16-18 #1179 RT: 5.26 AV: 1 NL: 3.41E8
T: FTMS + p ESI Full ms [200.0000-800.0000]



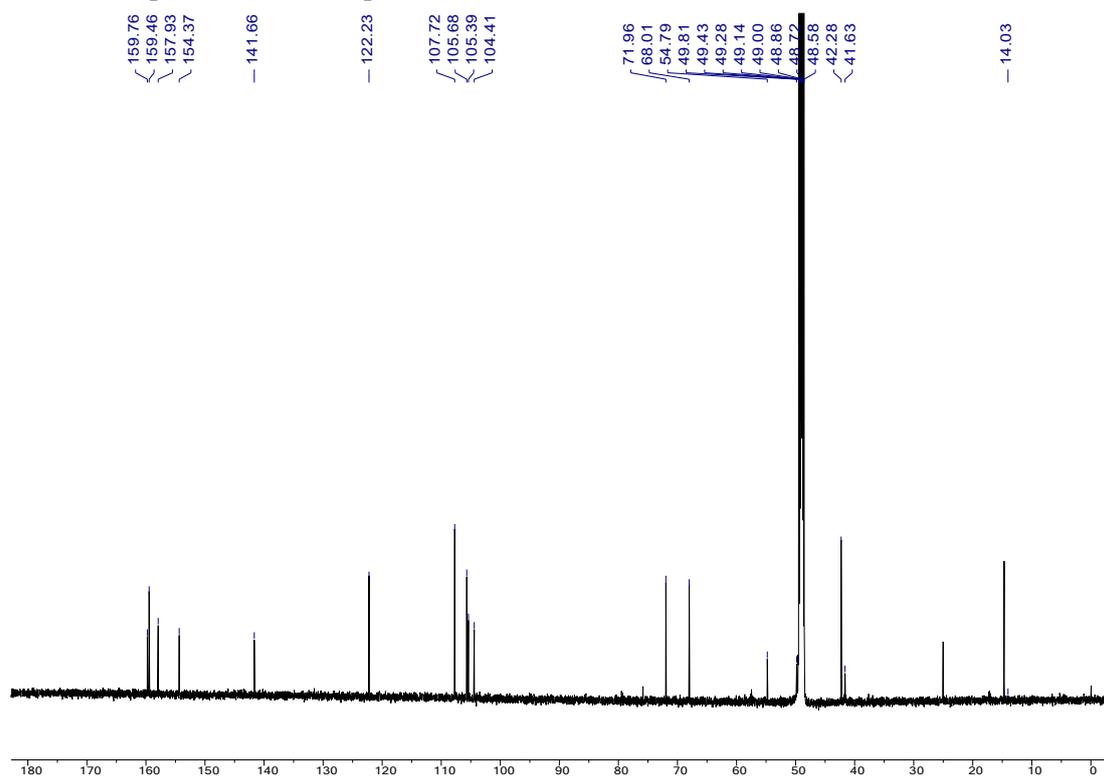
(R)-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)benzene-1,3-diol (17)



¹H NMR spectrum for compound 17

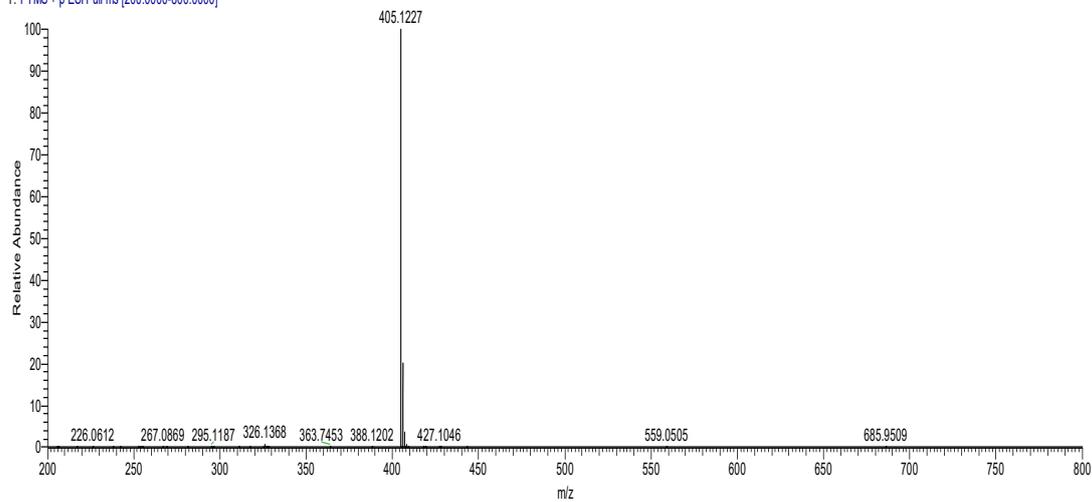


¹³C NMR spectrum for compound 17

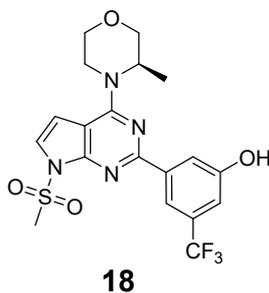


HRMS spectrum for compound 17

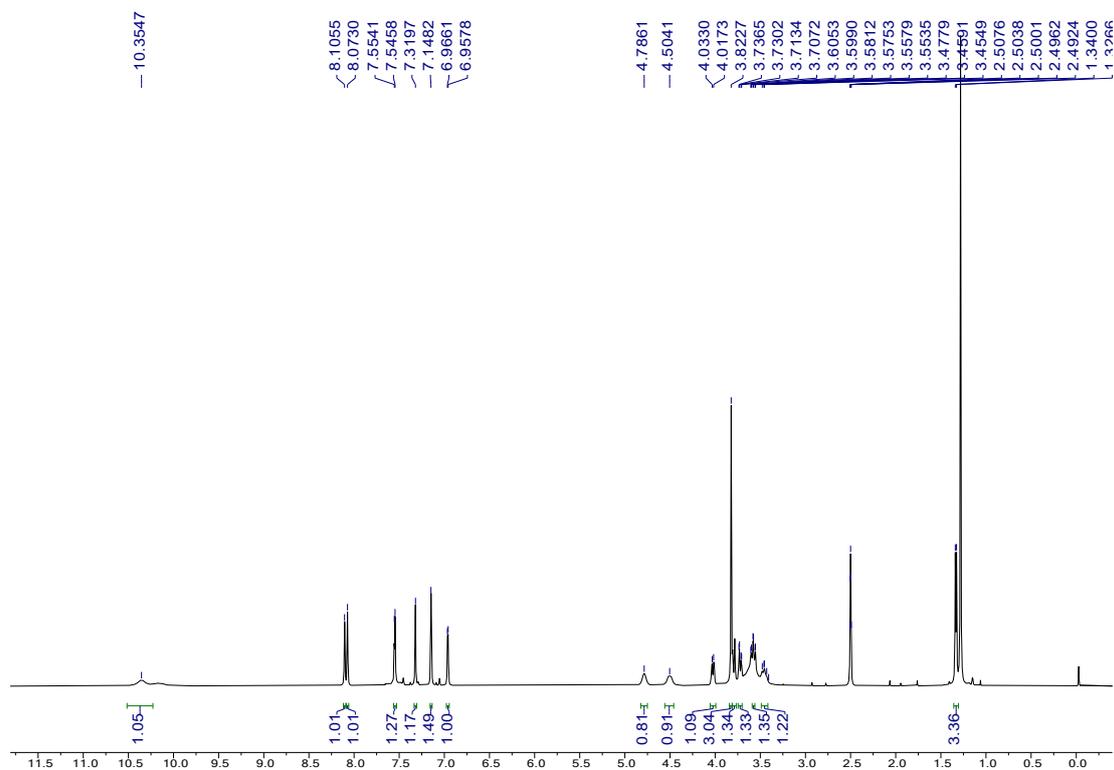
A18 #1144 RT: 5.10 AV: 1 NL: 4.92E9
T: FTMS + p ESI Full ms [200.0000-800.0000]



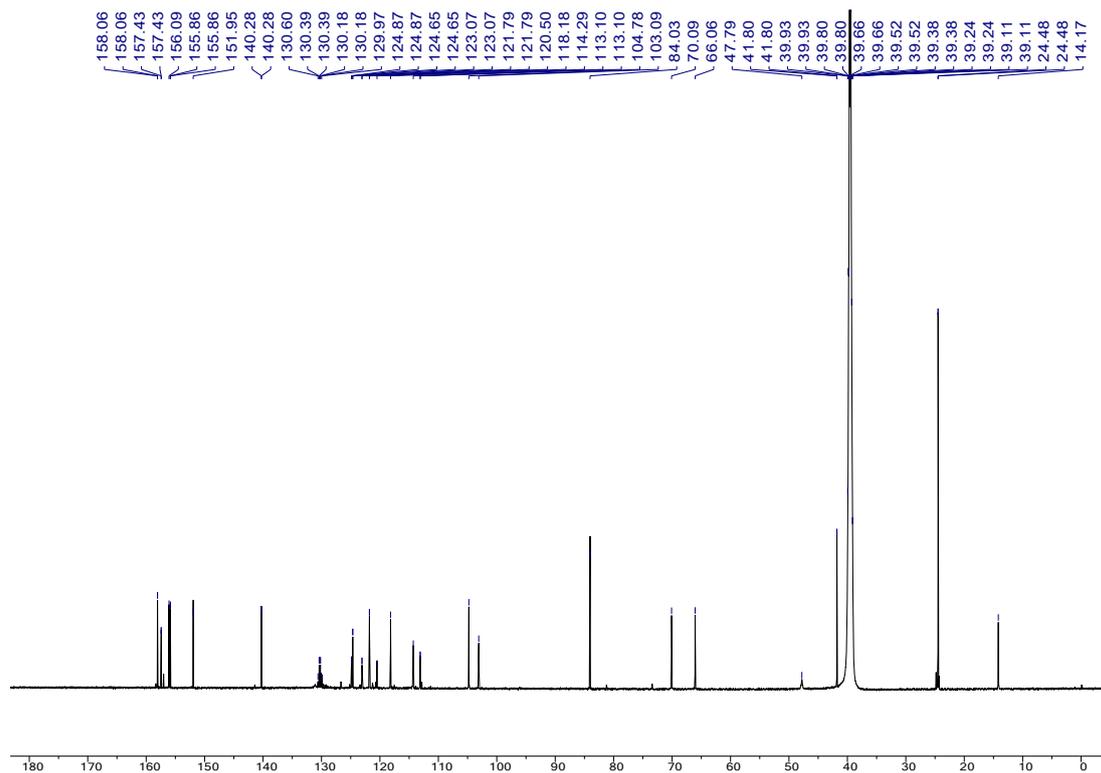
(R)-3-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)-5-(trifluoromethyl)phenol (18)



¹H NMR spectrum for compound 18

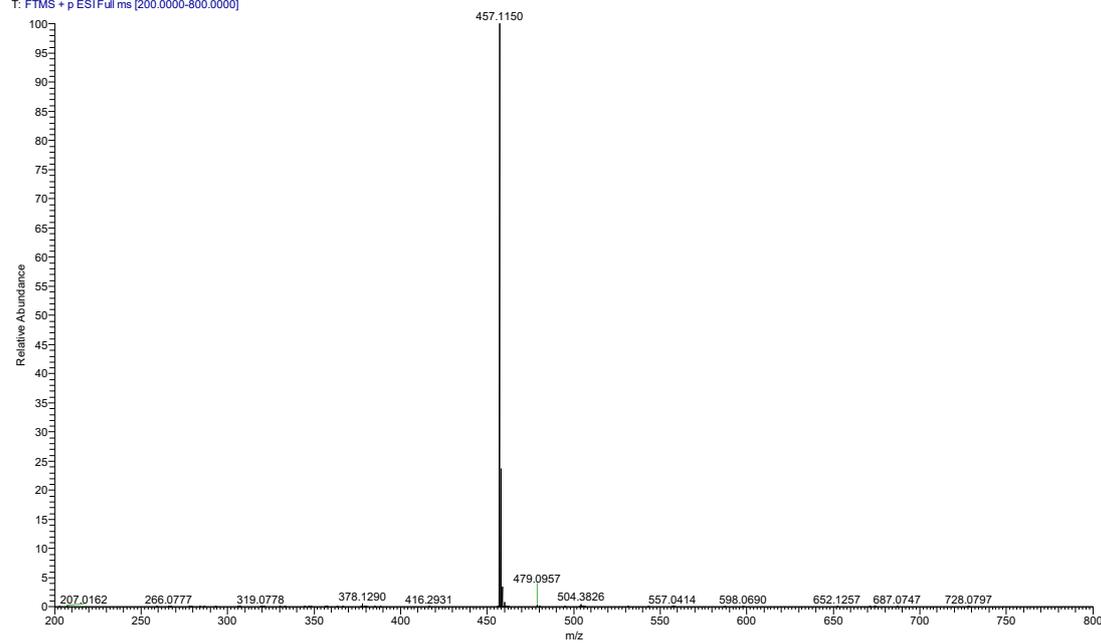


¹³C NMR spectrum for compound 18

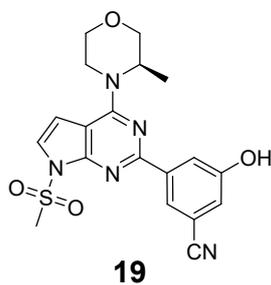


HRMS spectrum for compound 18

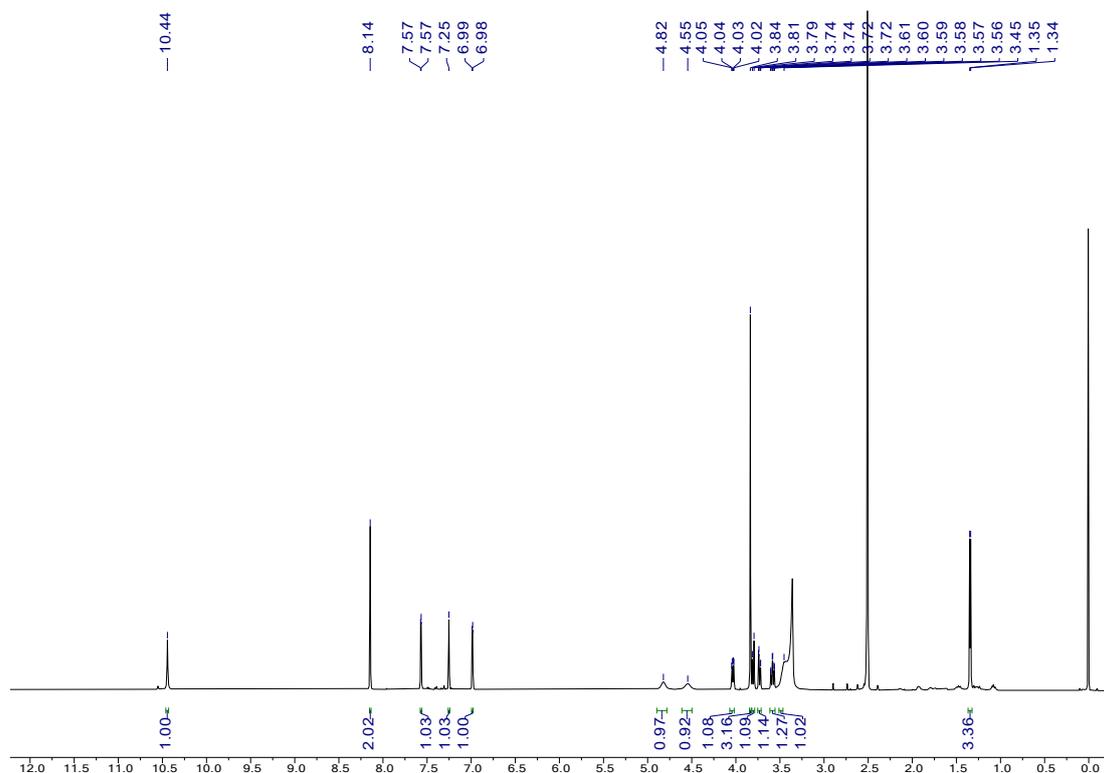
GBA-16-21 #1240 RT: 5.53 AV: 1 NL: 4.55E9
T: FTMS + p ESI Full ms [200.0000-800.0000]



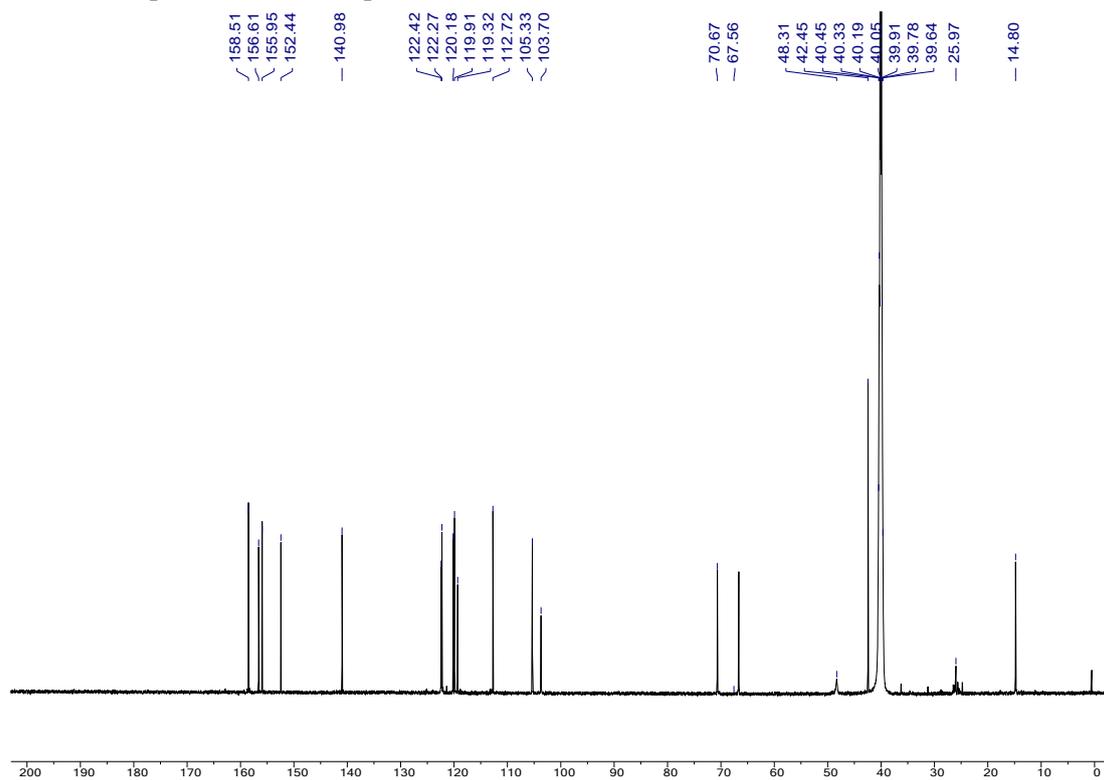
(R)-3-hydroxy-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)benzonitrile (19)



¹H NMR spectrum for compound 19

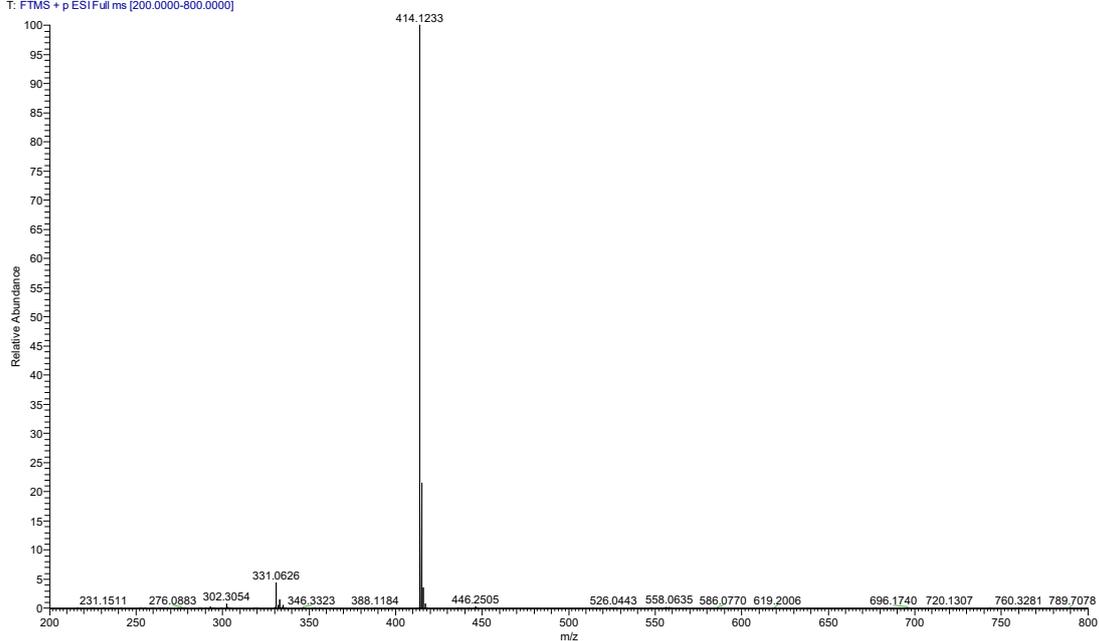


¹³C NMR spectrum for compound 19

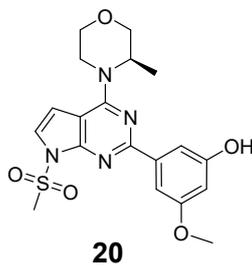


HRMS spectrum for compound 19

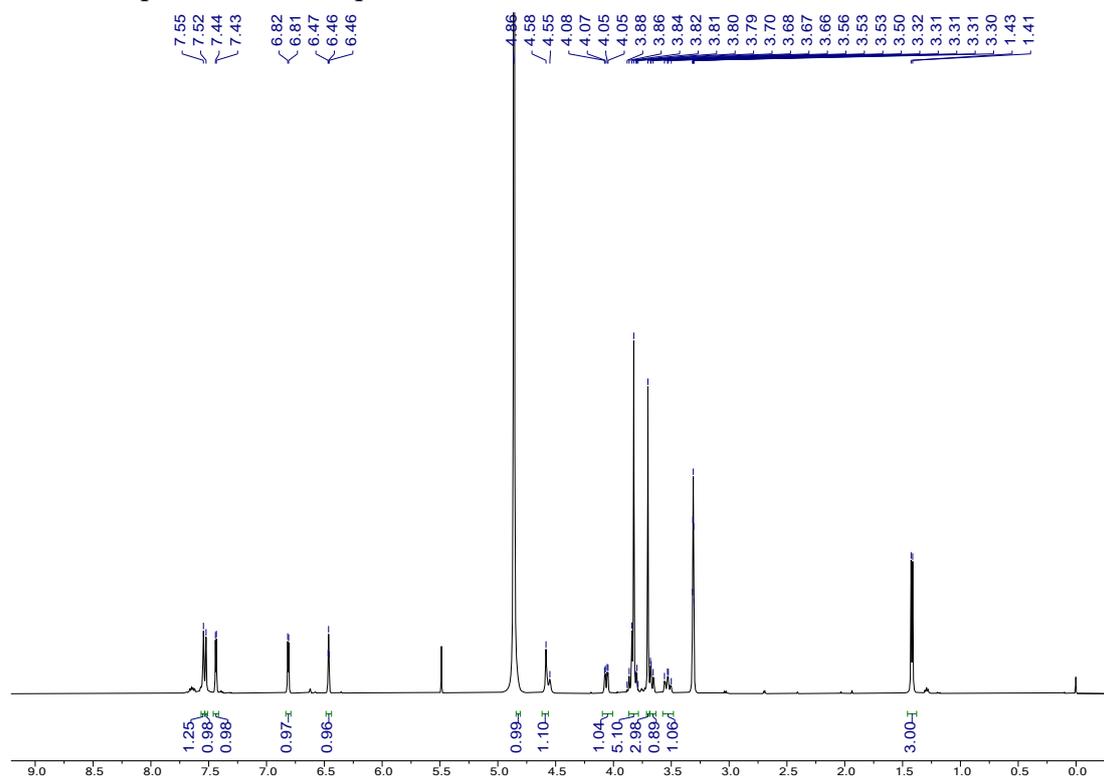
GBA-16-20 #1157 RT: 5.16 AV: 1 NL: 3.51E9
T: FTMS + p ESI Full ms [200.0000-800.0000]



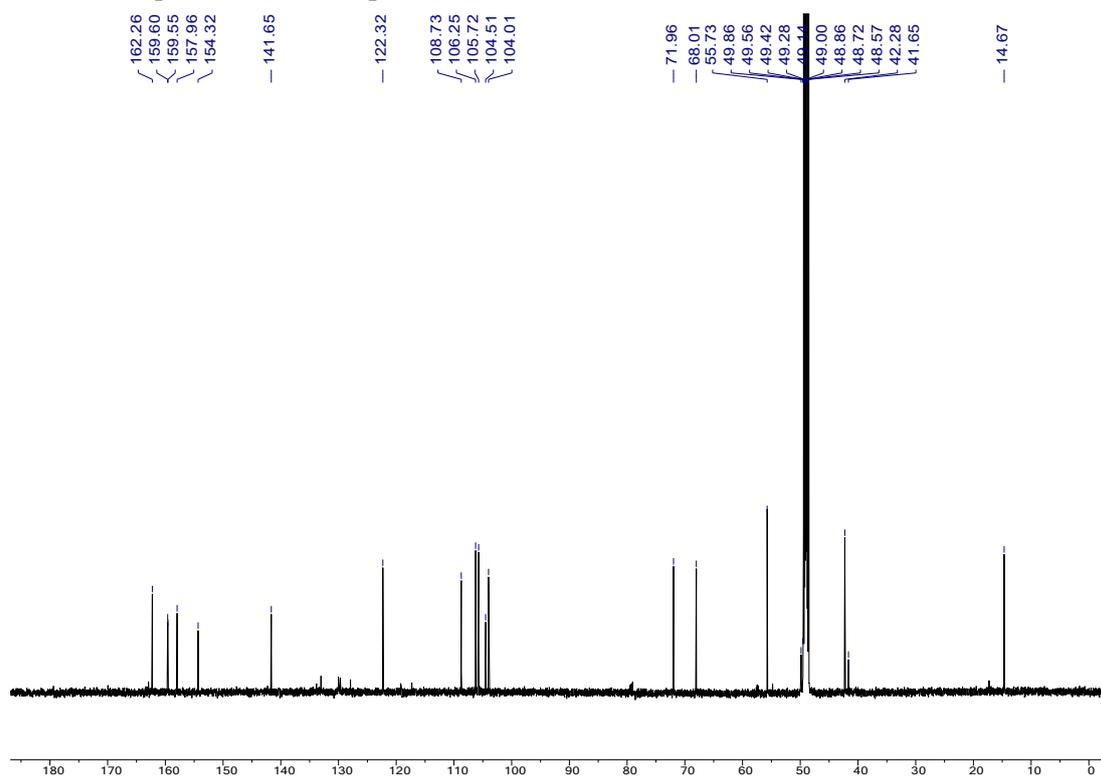
(R)-3-methoxy-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)phenol (20)



¹H NMR spectrum for compound 20

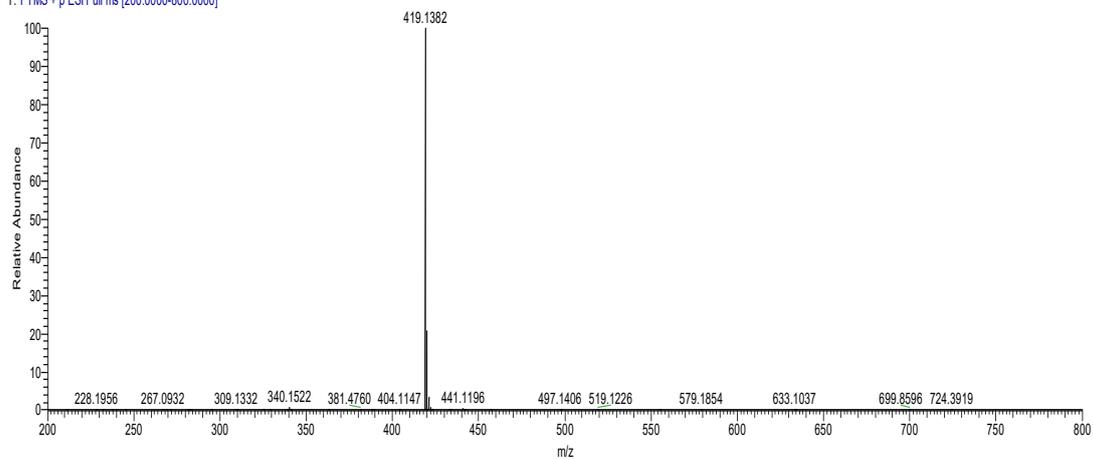


¹³C NMR spectrum for compound 20

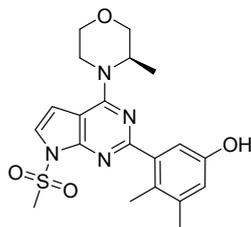


HRMS spectrum for compound 20

A17 #1220 RT: 5.44 AV: 1 NL: 4.55E9
T: FTMS + p ESI Full ms [200.0000-800.0000]

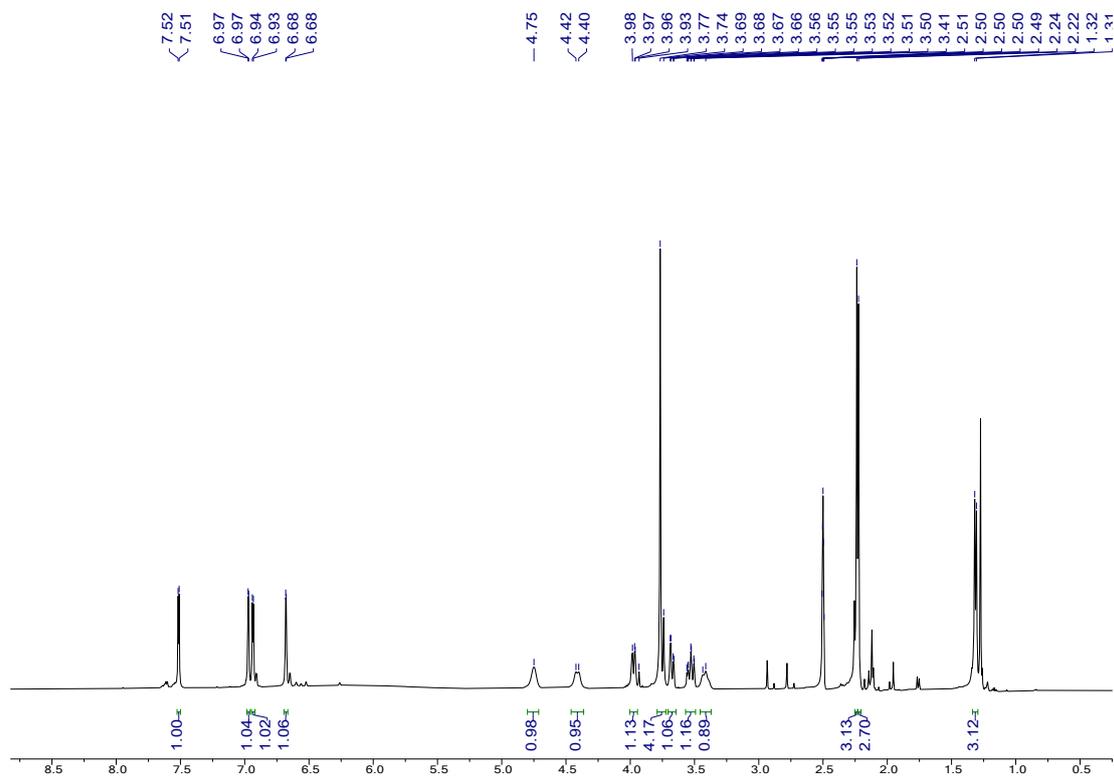


(R)-3,4-dimethyl-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)phenol (21)

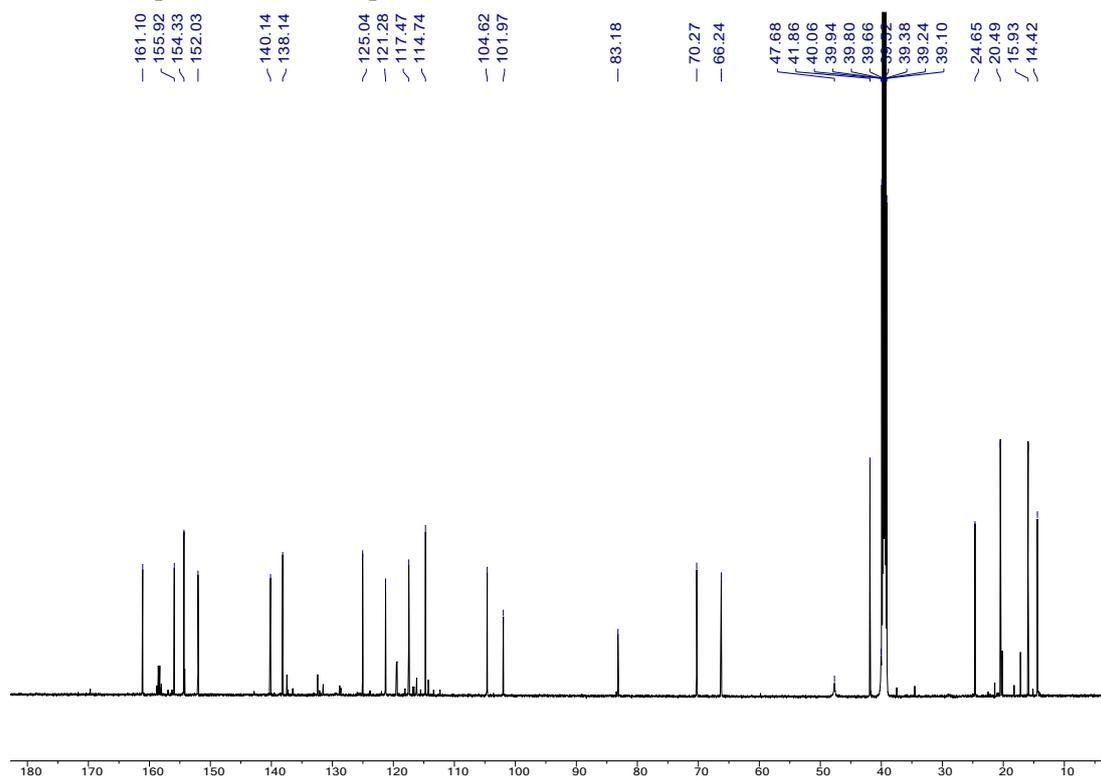


21

¹H NMR spectrum for compound **21**

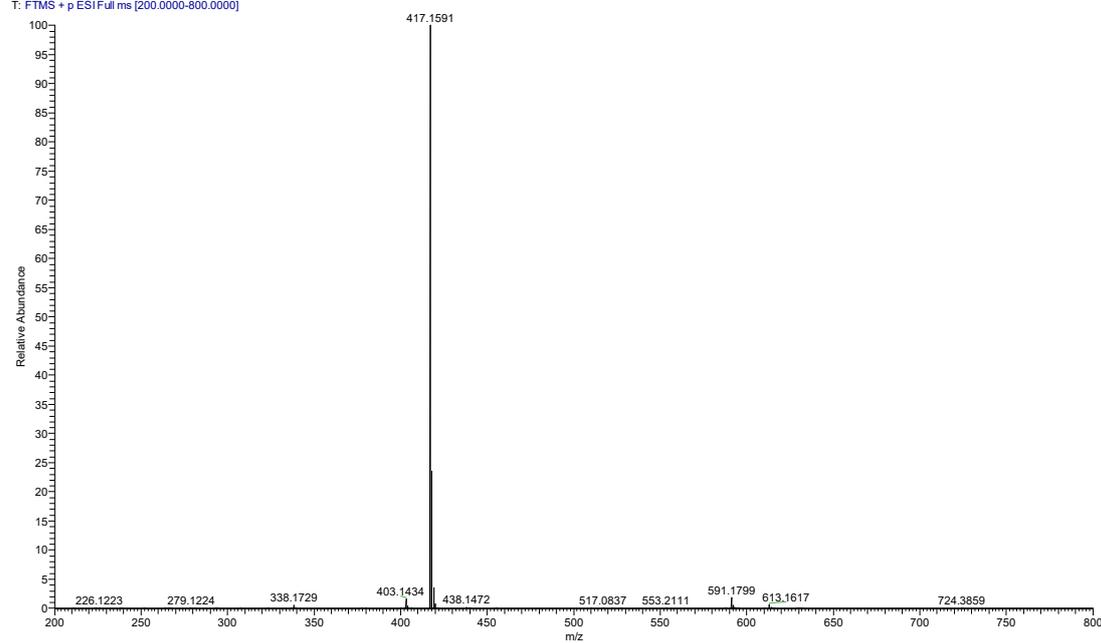


¹³C NMR spectrum for compound 21

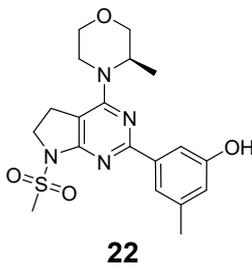


HRMS spectrum for compound 21

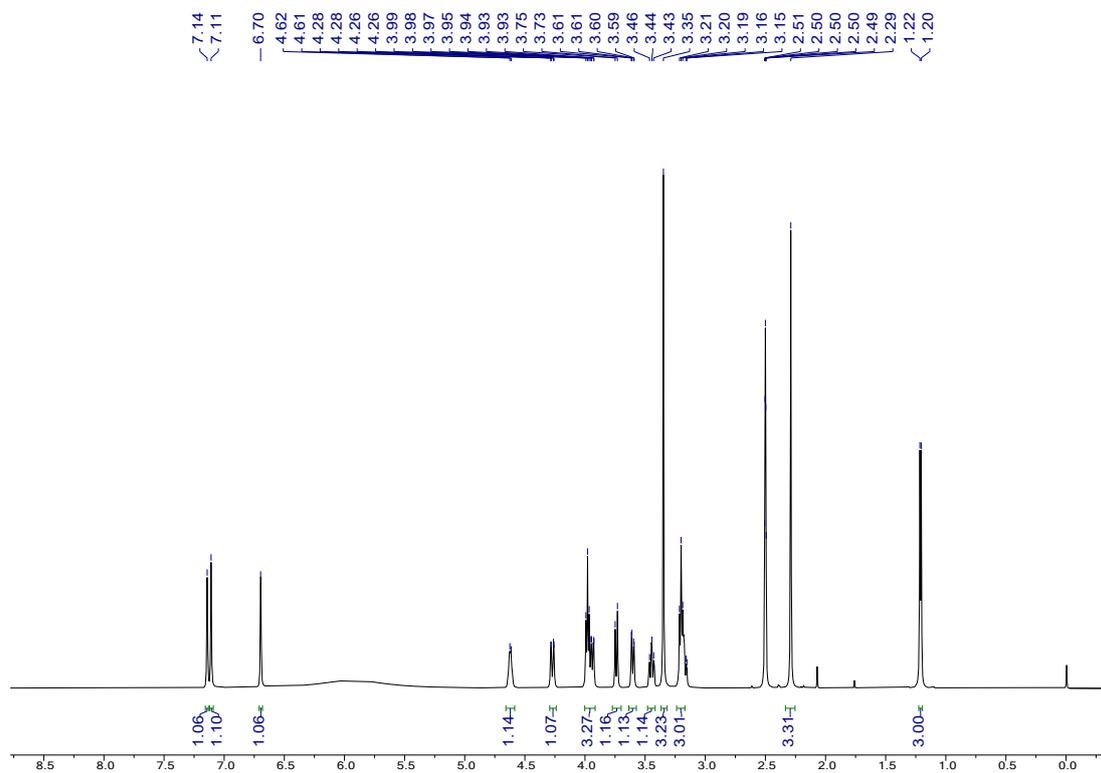
GBA-16-19#1179 RT: 5.26 AV: 1 NL: 8.93E9
T: FTMS + p ESI Full ms [200.0000-800.0000]



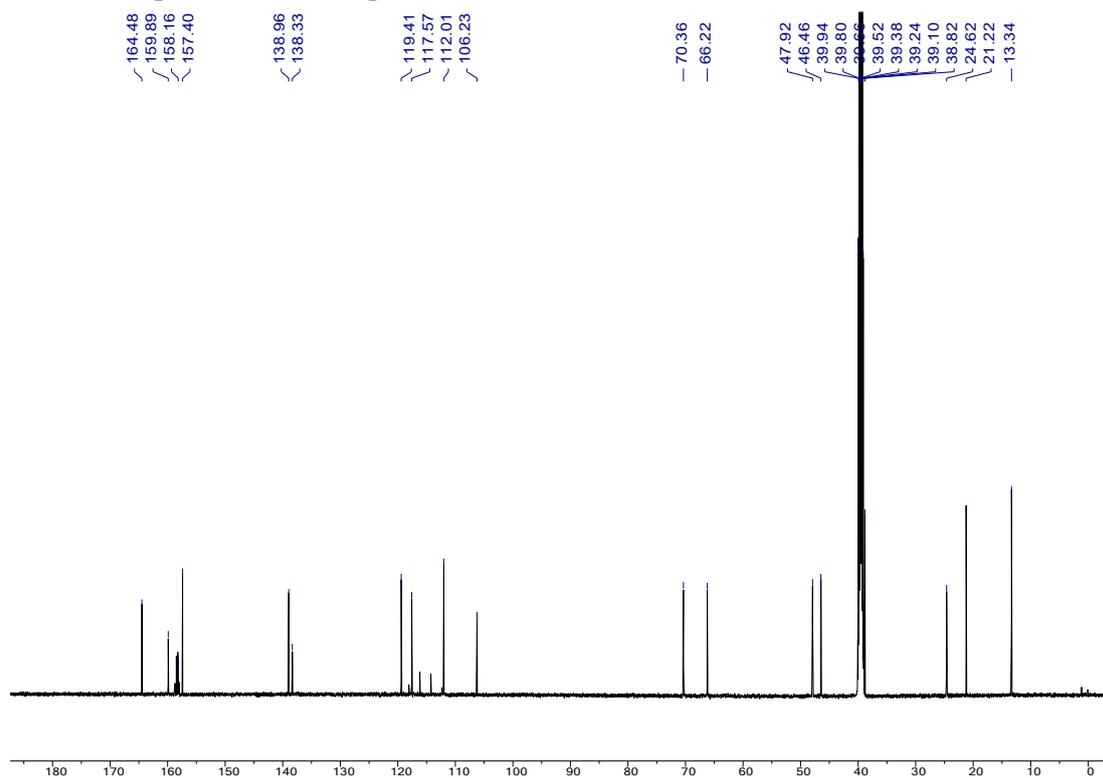
(R)-3-methyl-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-2-yl)phenol (22)



¹H NMR spectrum for compound 22

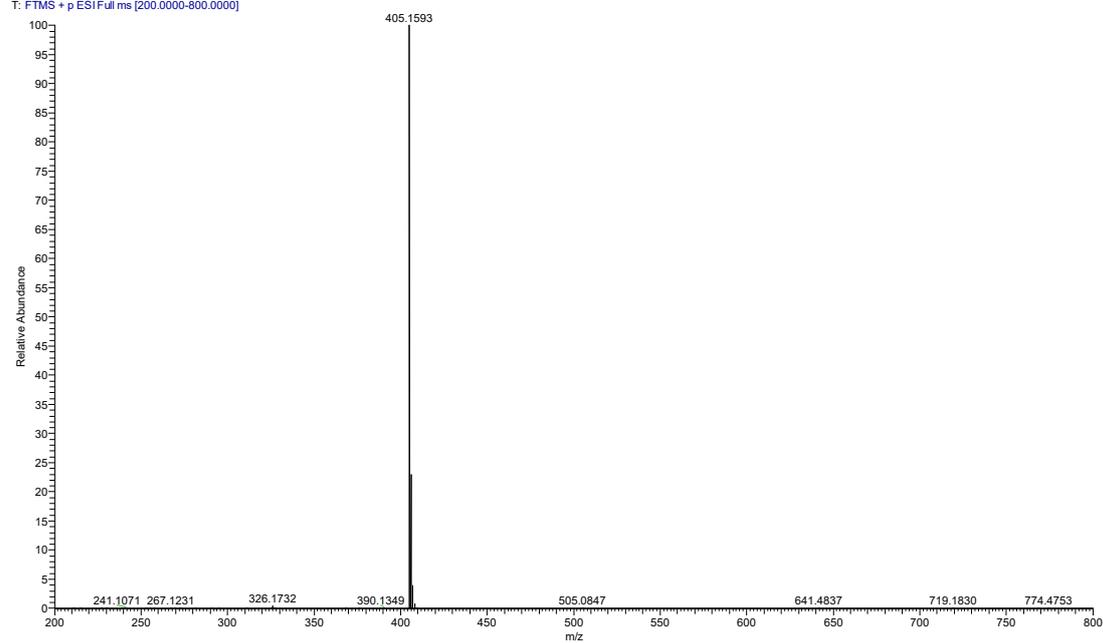


¹³C NMR spectrum for compound 22

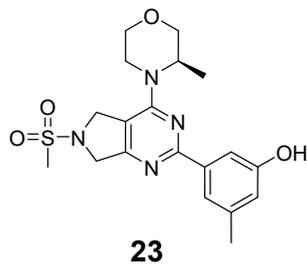


HRMS spectrum for compound 22

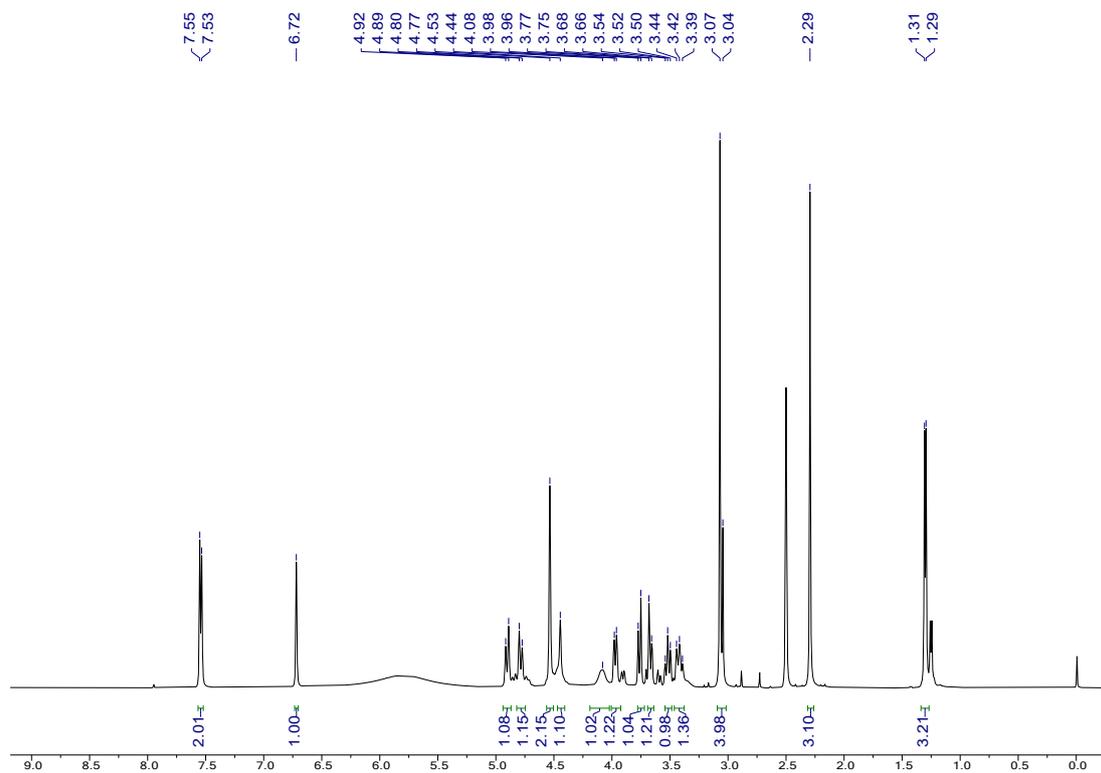
GBA-16-31 #1133 RT: 5.05 AV: 1 NL: 1.43E10
T: FTMS + p ESI Full ms [200.0000-800.0000]



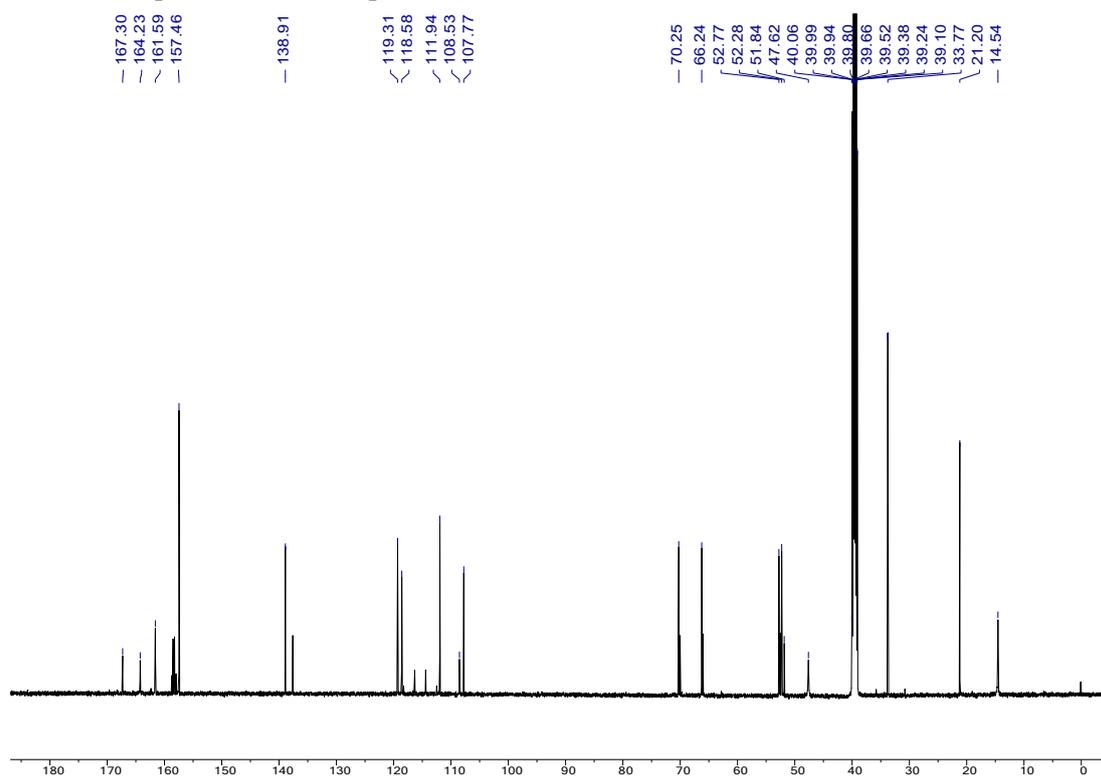
(R)-3-methyl-5-(4-(3-methylmorpholino)-6-(methylsulfonyl)-6,7-dihydro-5H-pyrrolo[3,4-d]pyrimidin-2-yl)phenol(23)



¹H NMR spectrum for compound 23

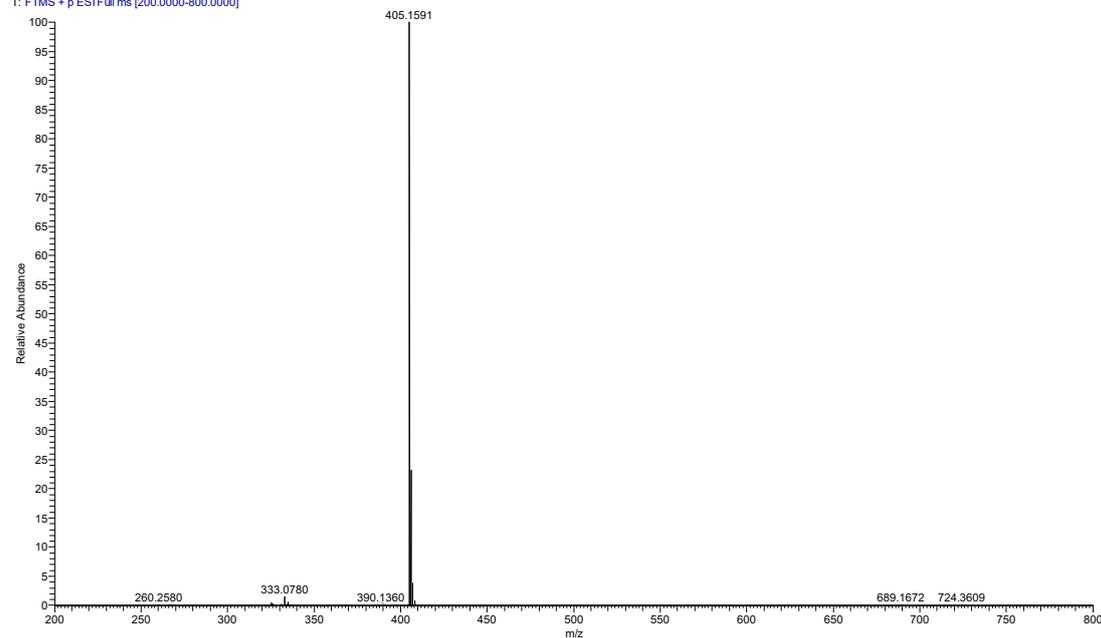


¹³C NMR spectrum for compound 23

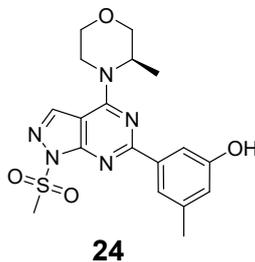


HRMS spectrum for compound 23

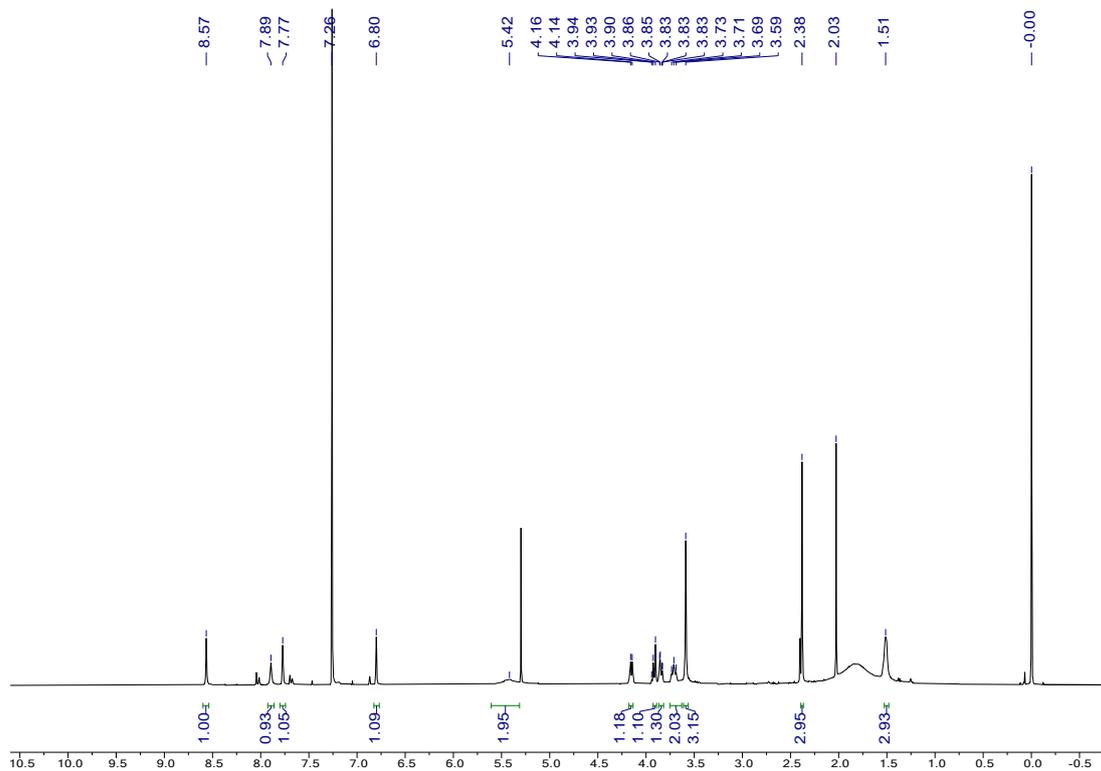
GBA-16-29 #1054 RT: 4.70 AV: 1 NL: 5.96E9
T: FTMS + p ESI Full ms [200.0000-800.0000]



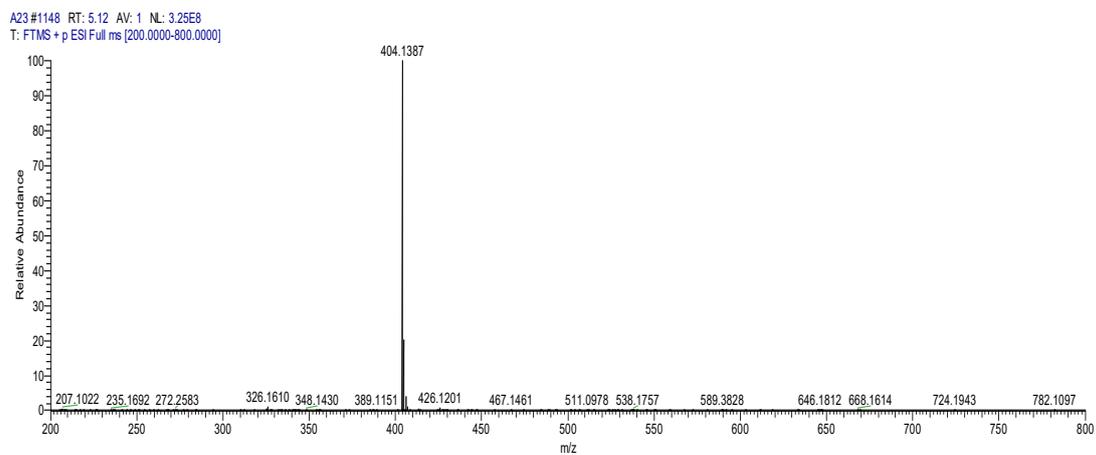
(R)-3-methyl-5-(4-(3-methylmorpholino)-1-(methylsulfonyl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)phenol (24)



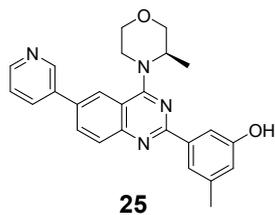
¹H NMR spectrum for compound 24



HRMS spectrum for compound 24

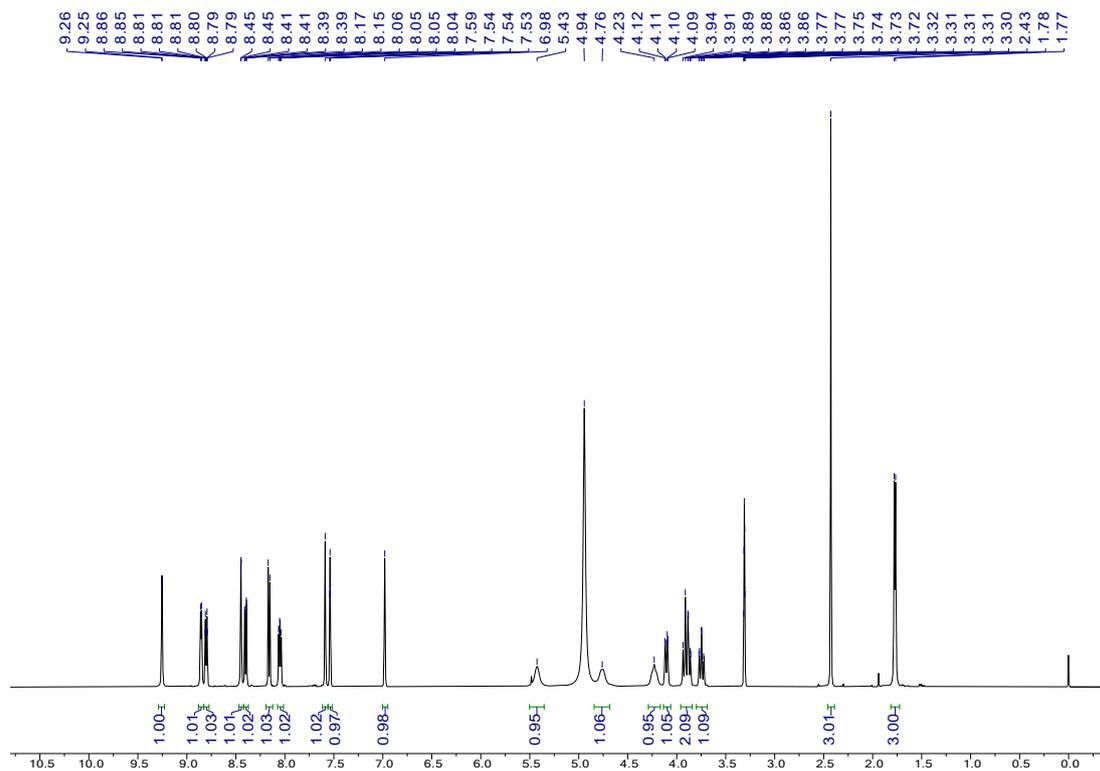


(R)-3-methyl-5-(4-(3-methylmorpholino)-6-(pyridin-3-yl)quinazolin-2-yl)phenol (25)

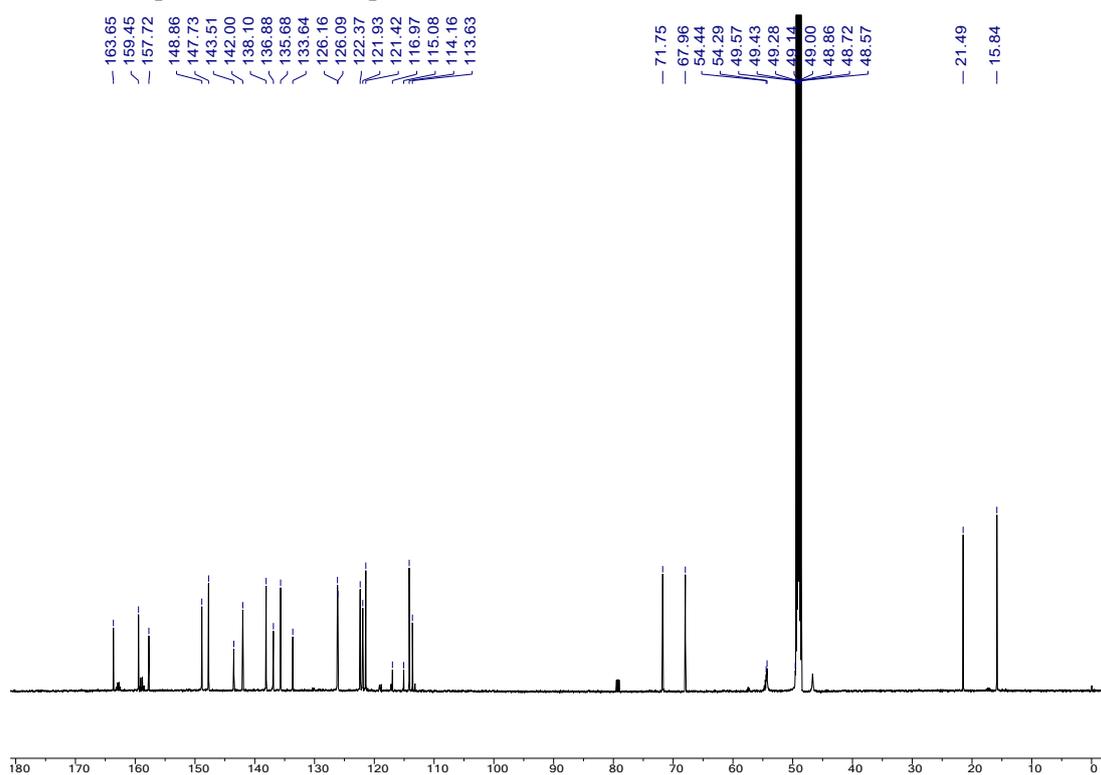


25

¹H NMR spectrum for compound 25

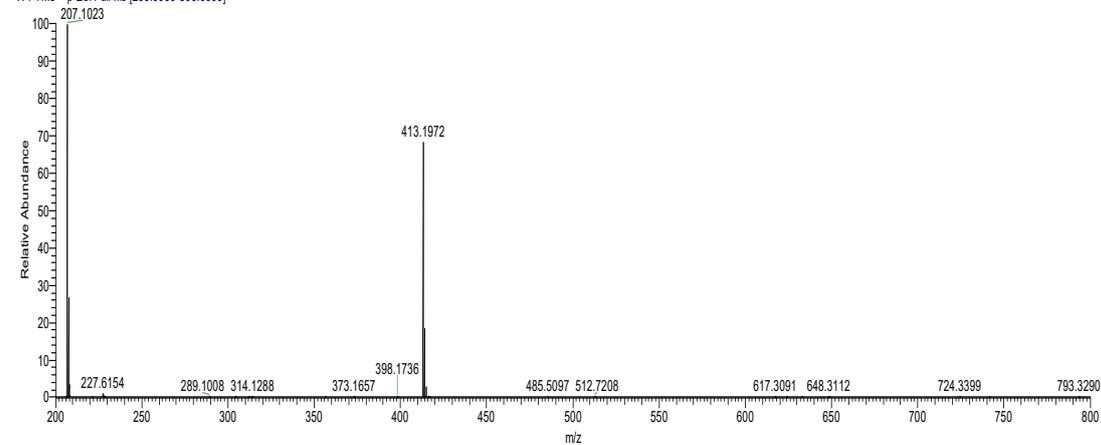


¹³C NMR spectrum for compound 25

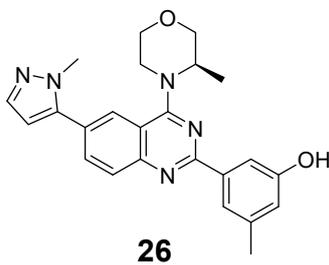


HRMS spectrum for compound 25

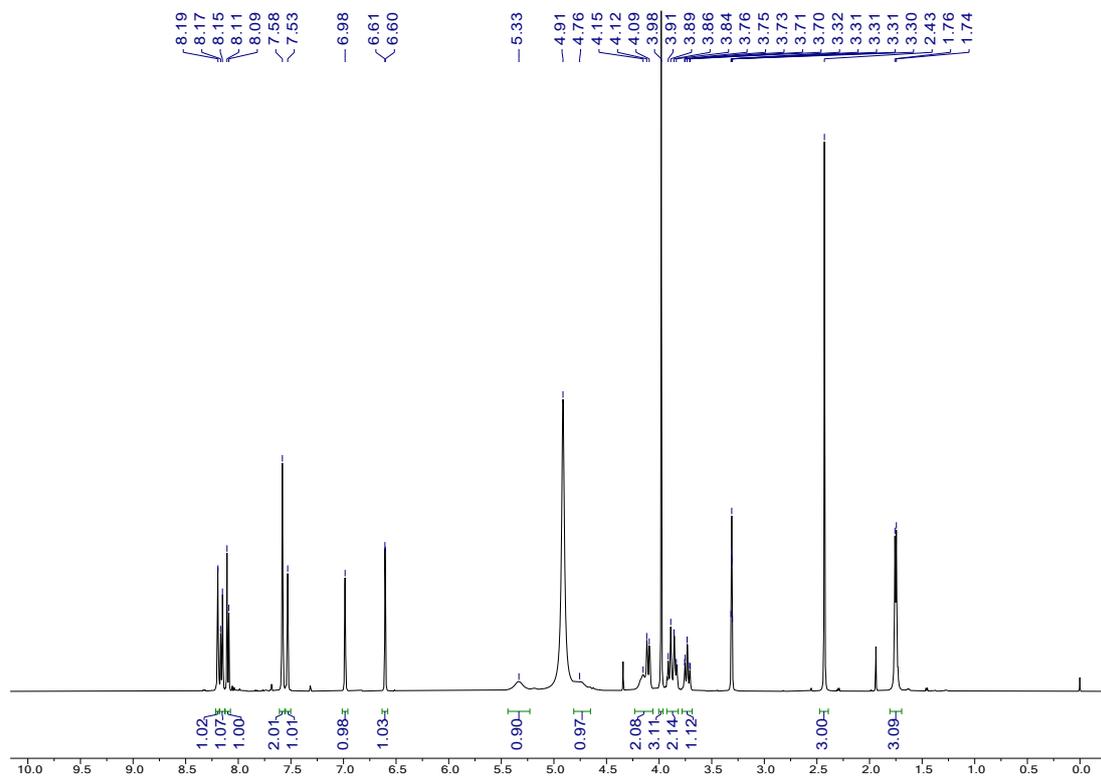
A26 #1123 RT: 5.01 AV: 1 NL: 3.60E9
T: FTMS + p ESI Full ms [200.0000-800.0000]



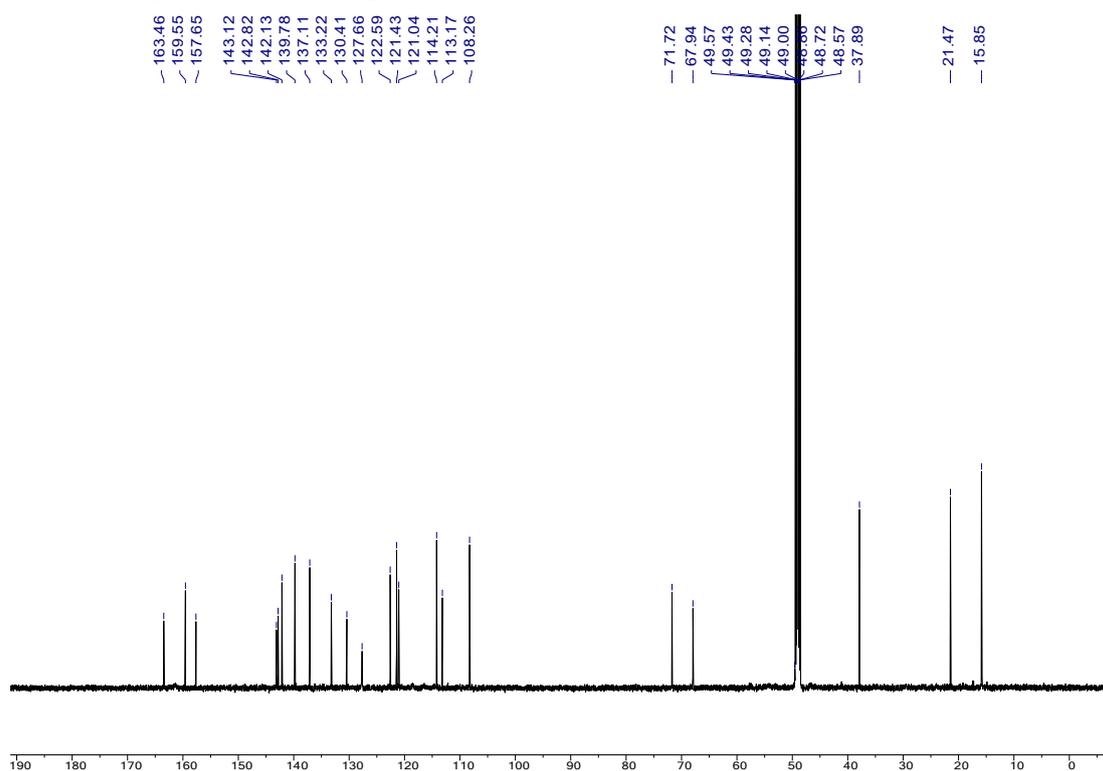
(R)-3-methyl-5-(6-(1-methyl-1H-pyrazol-5-yl)-4-(3-methylmorpholino)quinazolin-2-yl)phenol (26)



¹H NMR spectrum for compound 26

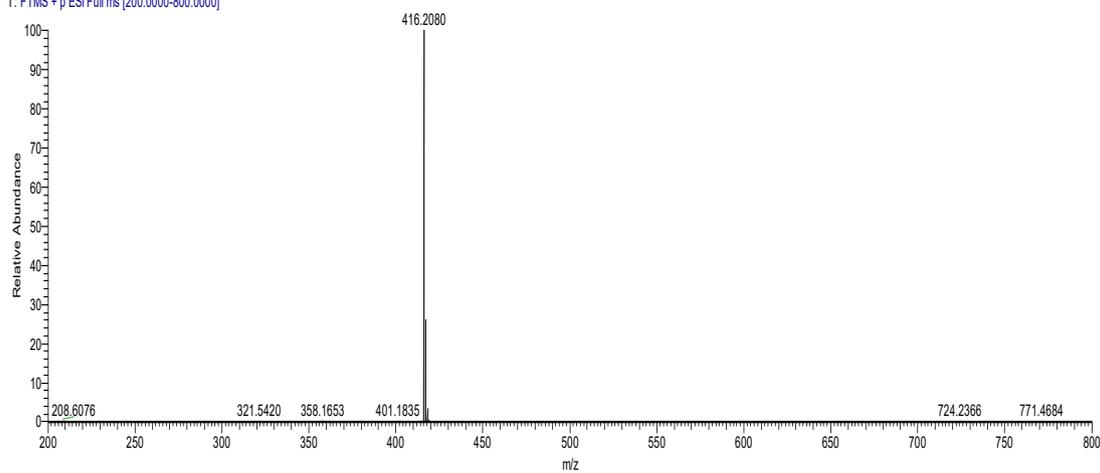


¹³C NMR spectrum for compound 26

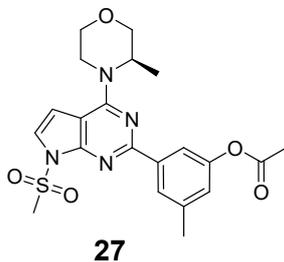


HRMS spectrum for compound 26

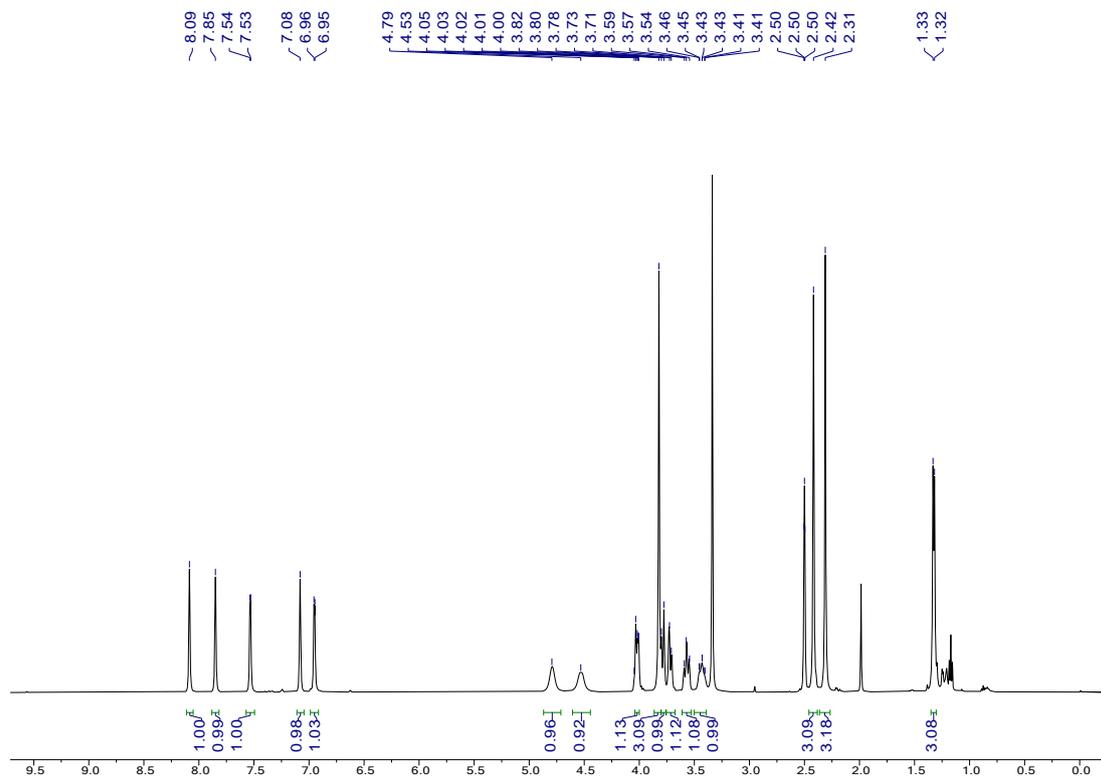
A25 #1157 RT: 5.17 AV: 1 NL: 5.21E9
T: FTMS + p ESI Full ms [200.0000-800.0000]



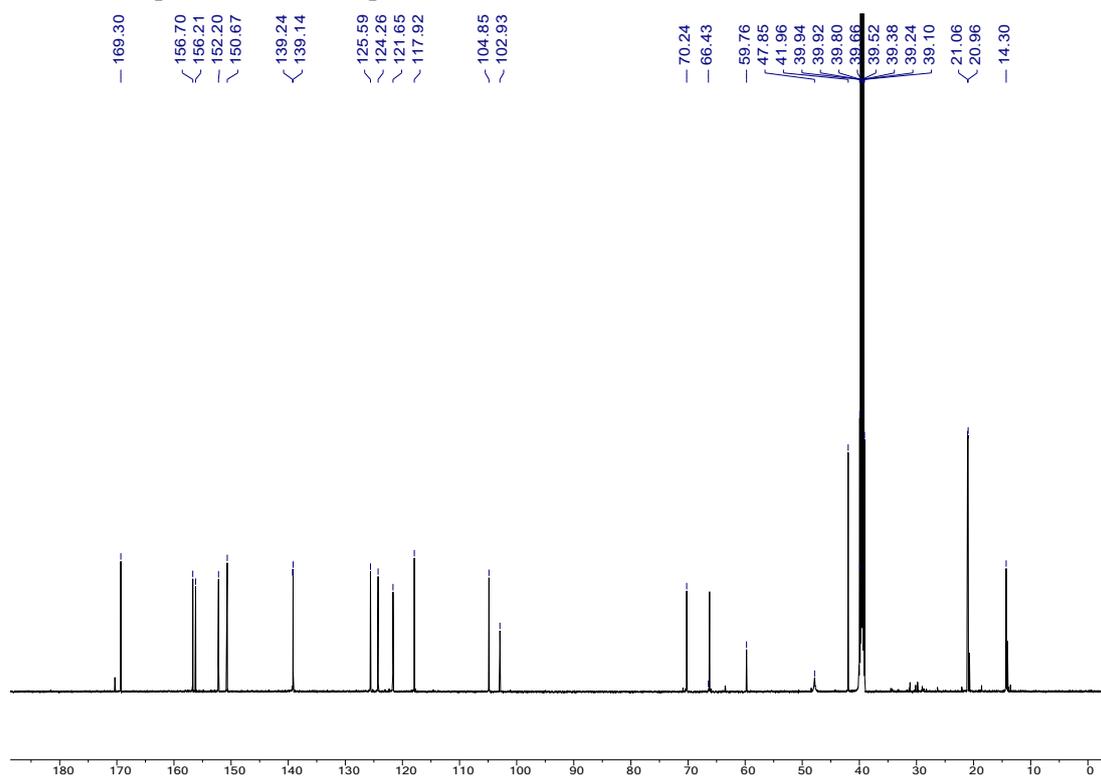
(R)-3-methyl-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)phenyl acetate (27)



¹H NMR spectrum for compound 27

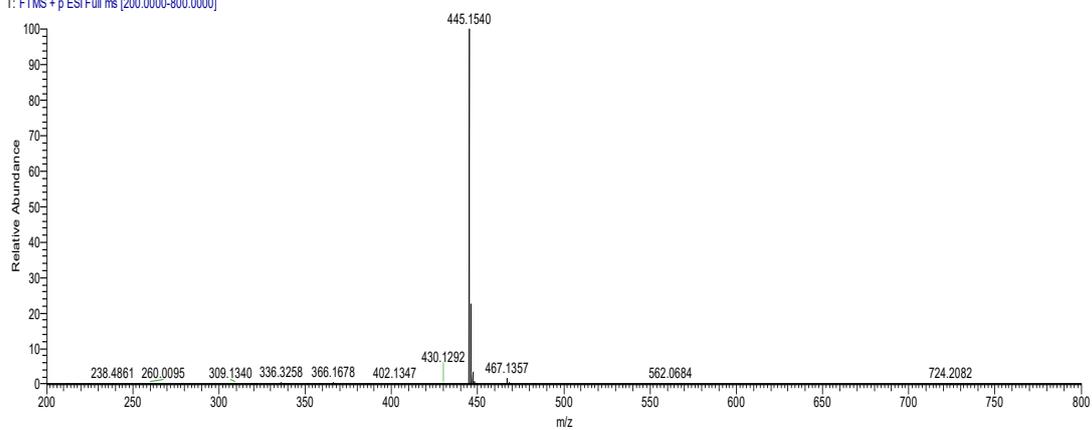


¹³C NMR spectrum for compound 27

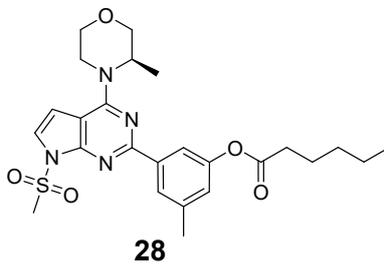


HRMS spectrum for compound 27

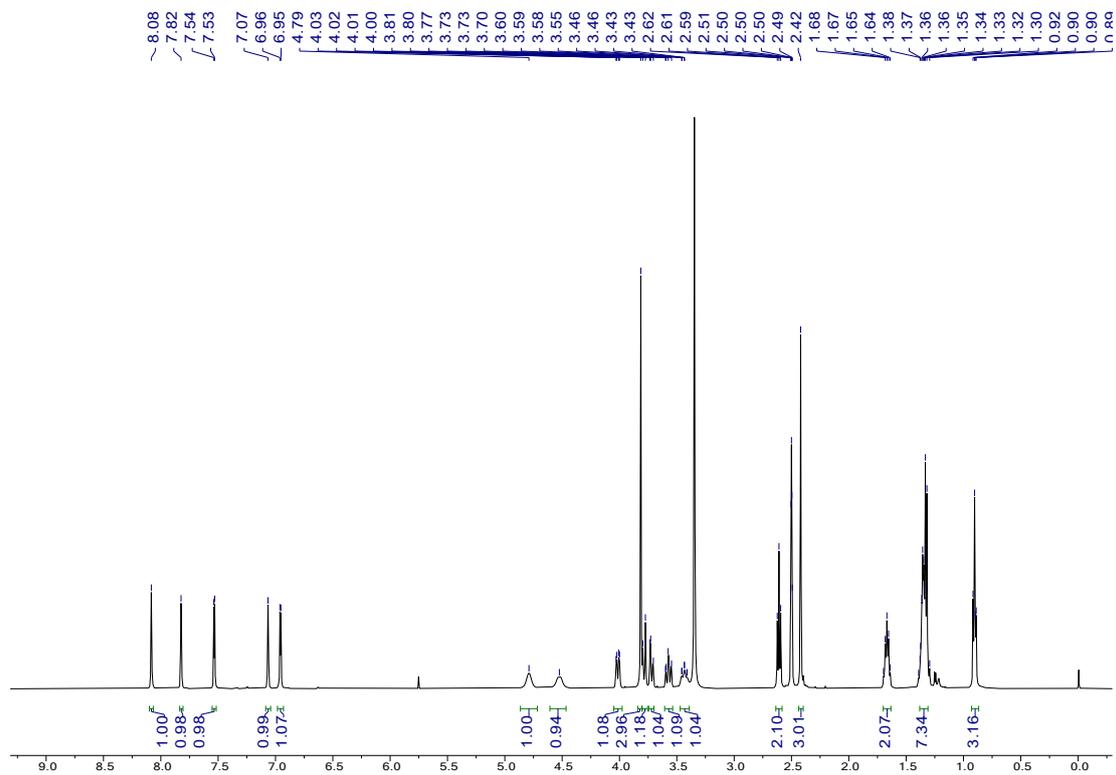
A20 #1308 RT: 5.84 AV: 1 NL: 3.76E9
T: FTMS + p ESI Full ms [200.0000-800.0000]



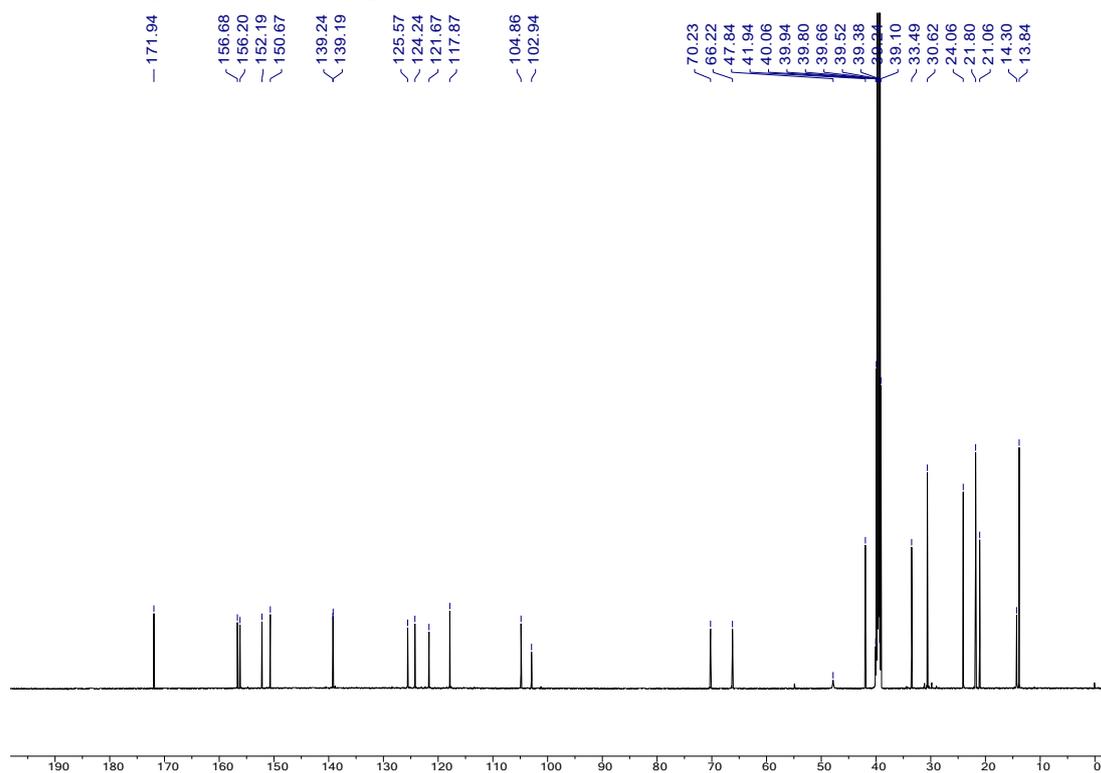
(R)-3-methyl-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)phenyl hexanoate (28)



¹H NMR spectrum for compound 28

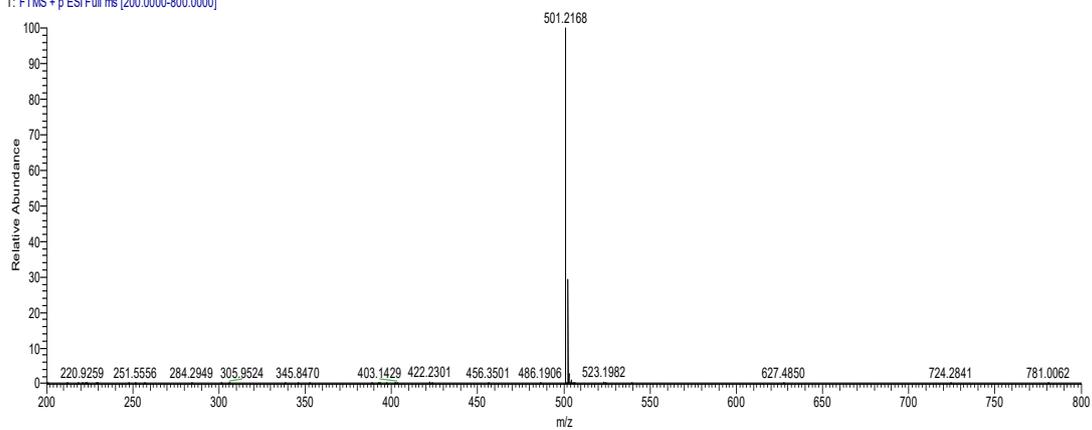


¹³C NMR spectrum for compound 28

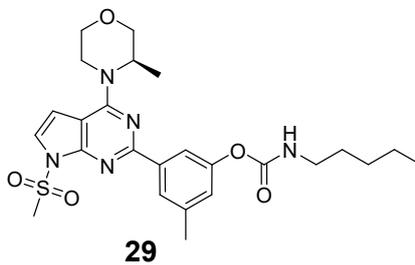


HRMS spectrum for compound 28

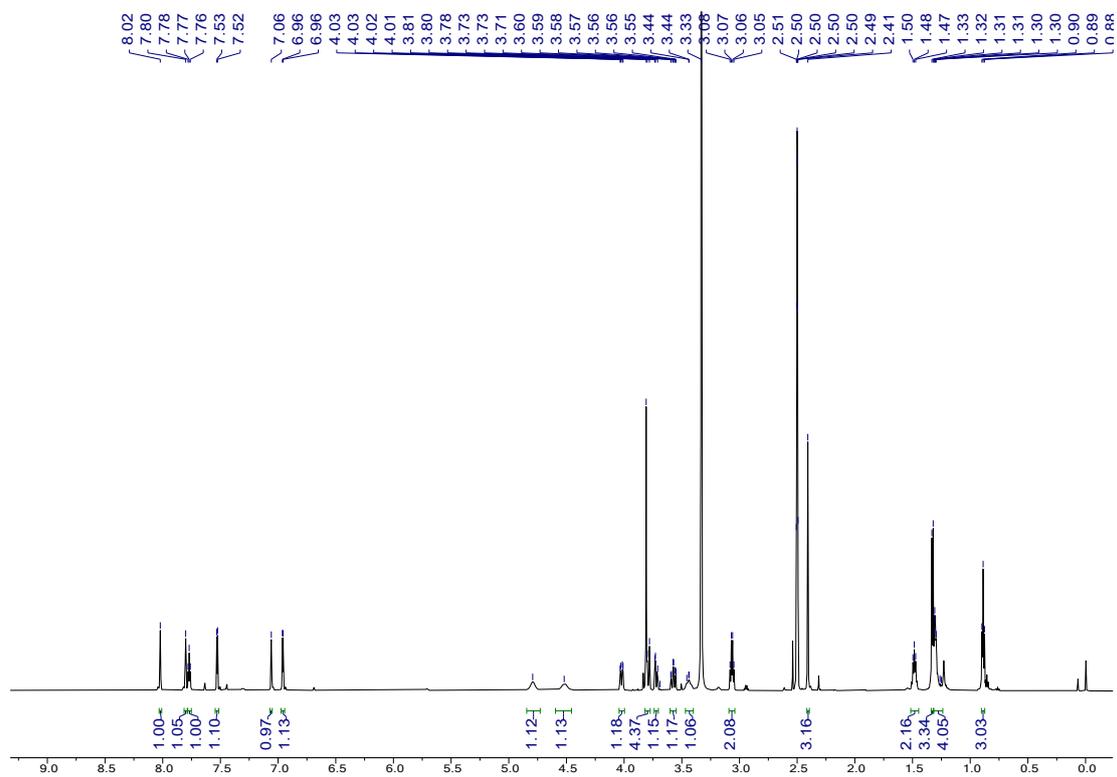
A21 #1462 RT: 6.52 AV: 1 NL: 3.78E9
T: FTMS+p ESI Full ms [200.0000-800.0000]



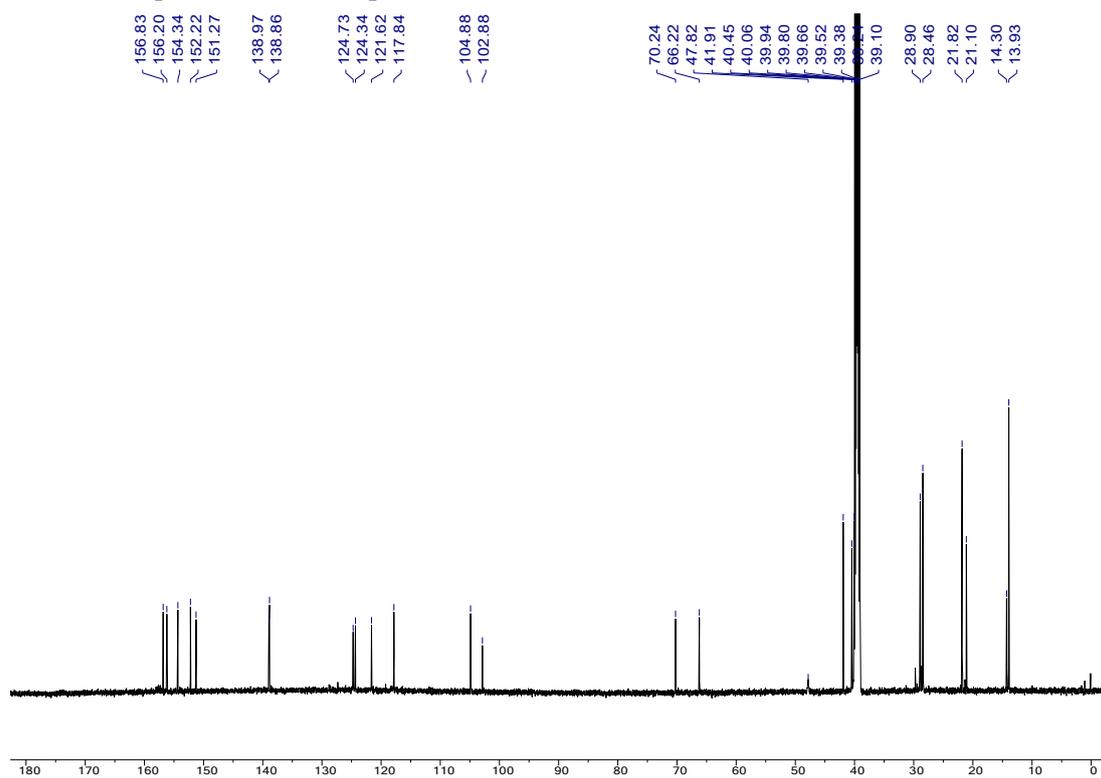
(R)-3-methyl-5-(4-(3-methylmorpholino)-7-(methylsulfonyl)-7H-pyrrolo[2,3-d]pyrimidin-2-yl)phenyl pentylcarbamate (29)



¹H NMR spectrum for compound 29



¹³C NMR spectrum for compound 29



HRMS spectrum for compound 29

A22 #1368 RT: 6.10 AV: 1 NL: 2.64E9
T: FTMS+p ESI Full ms [200.0000-800.0000]

