# *Supporting Information*

## *Highly potent and selective phosphatidylinositol 4-kinase IIIβ inhibitors as broad-spectrum anti-rhinoviral agents*

Avinash G Vishakantegowda[a,b,1], Dasom Hwang[a,c,1], Prashant Chakrasali[a], Eunhye Jung[a], Joo-Youn Lee[a], Jin Soo Shin[a,*], Young-Sik Jung[a,b,*]

[a] Infectious Diseases Therapeutic Research Center, Korea Research Institute of Chemical Technology, Daejeon 34114, Republic of Korea
[b] Department of Medicinal Chemistry and Pharmacology, University of Science and Technology, Daejeon 34113, Republic of Korea
[c] Laboratory of Veterinary Virology, College of Veterinary Medicine, Chungbuk National University, Cheongju 28644, Republic of Korea

[*] Corresponding authors:
Young-Sik Jung (ysjung@krict.re.kr), Jin Soo Shin (jsshin@krict.re.kr)

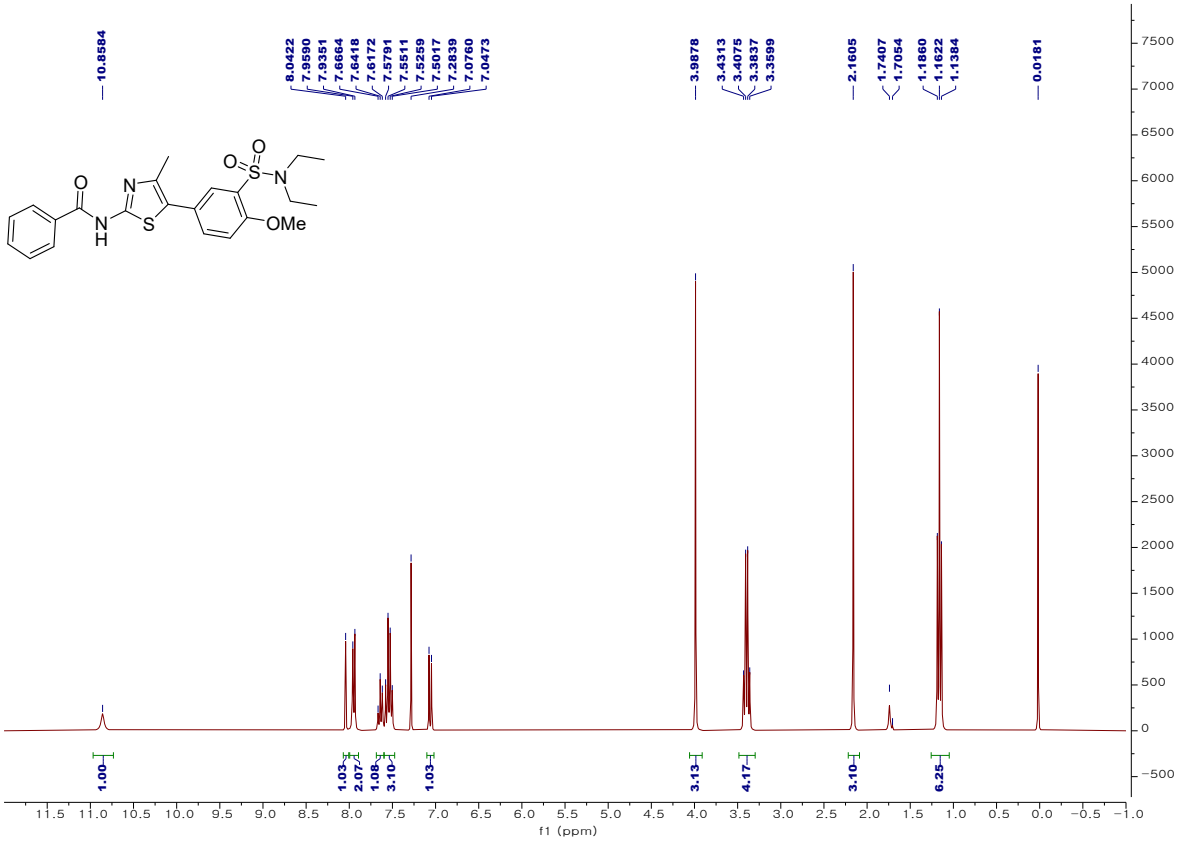
[1] These authors contributed equally to this work.



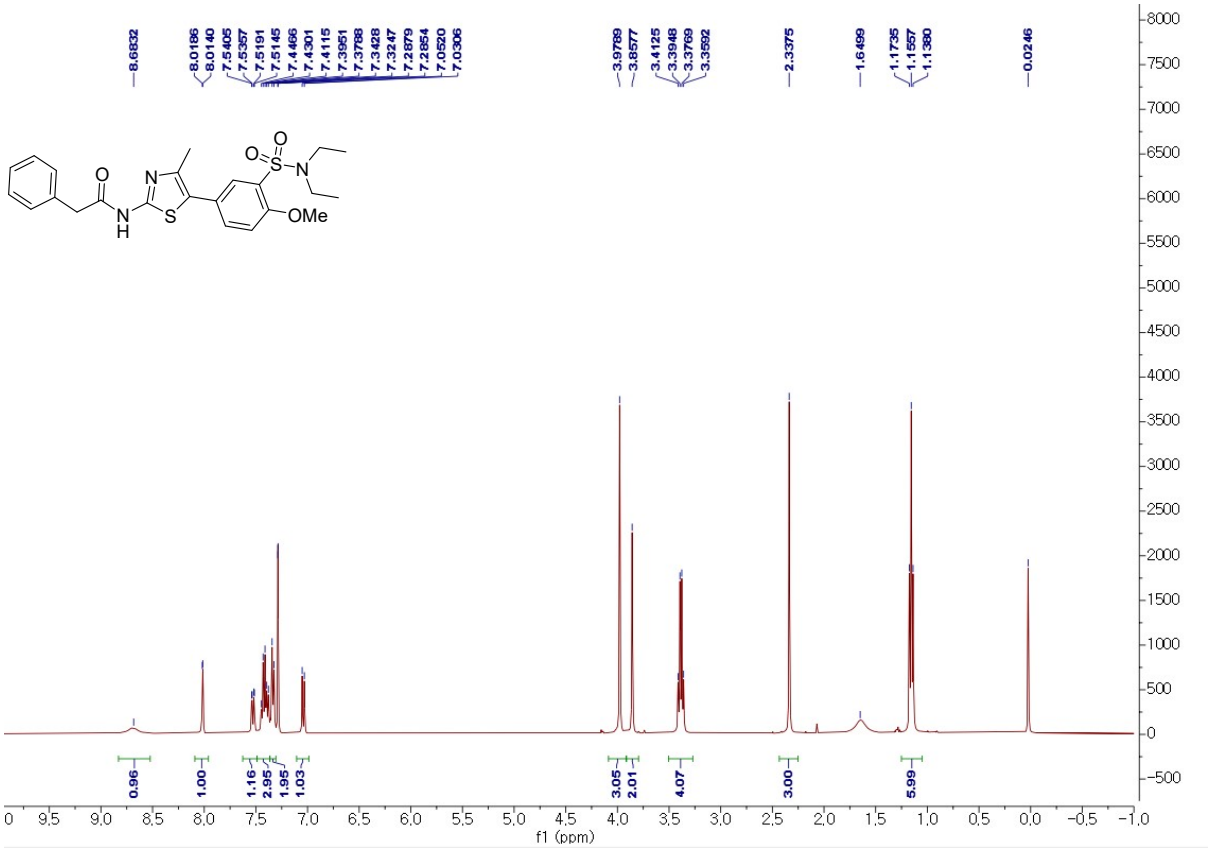
*Abbreviations:* hRVs, human rhinoviruses; PI4KIIIβ, phosphatidylinositol-4-kinase IIIβ; $EC_{50}$, 50% effective concentration; $CC_{50}$, 50% cytotoxic concentration; SI, selectivity index; MOI, multiplicity of infection; SD, standard deviation; DMEM, Dulbecco's modified eagle's medium; $TCID_{50}$, 50% tissue culture infectious dose

# S1. Spectral Copies of $^{1}$H NMR of Compounds

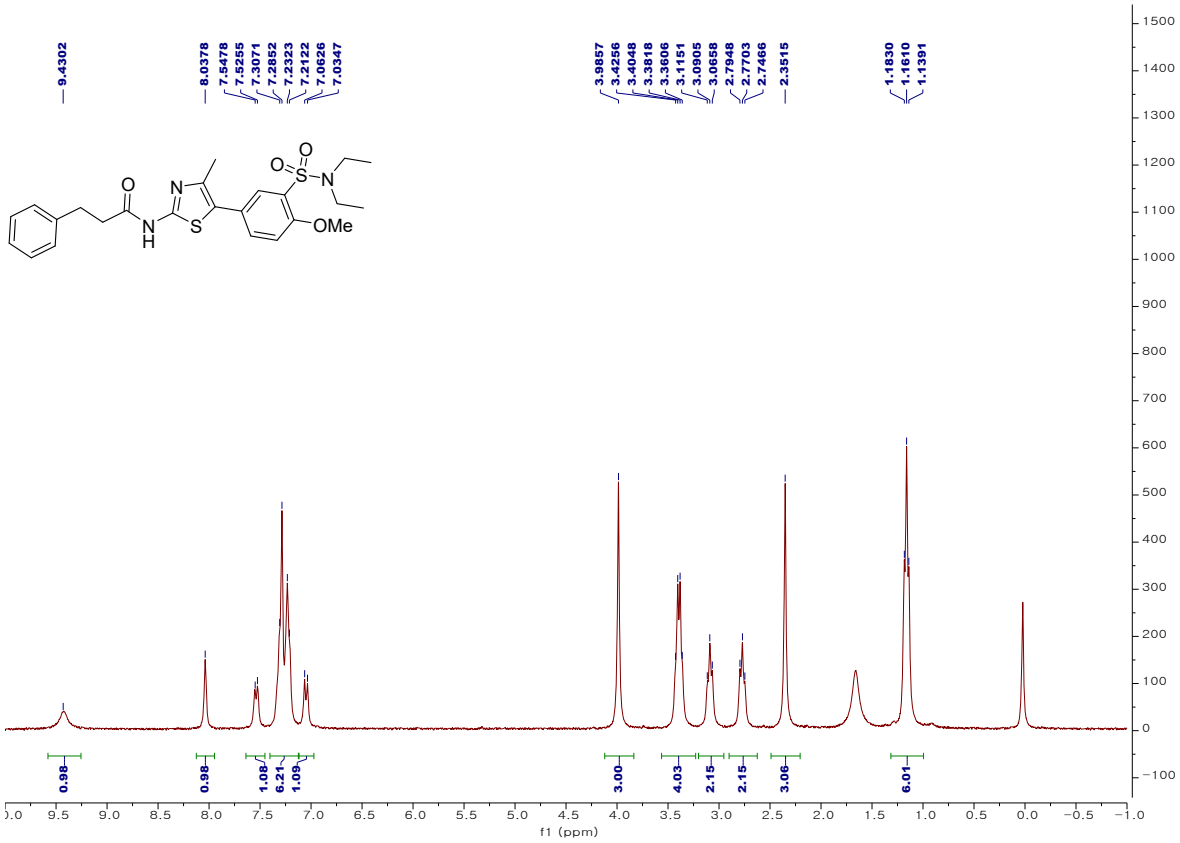
$^{1}$H NMR spectrum of **2** (**KR-27282**)



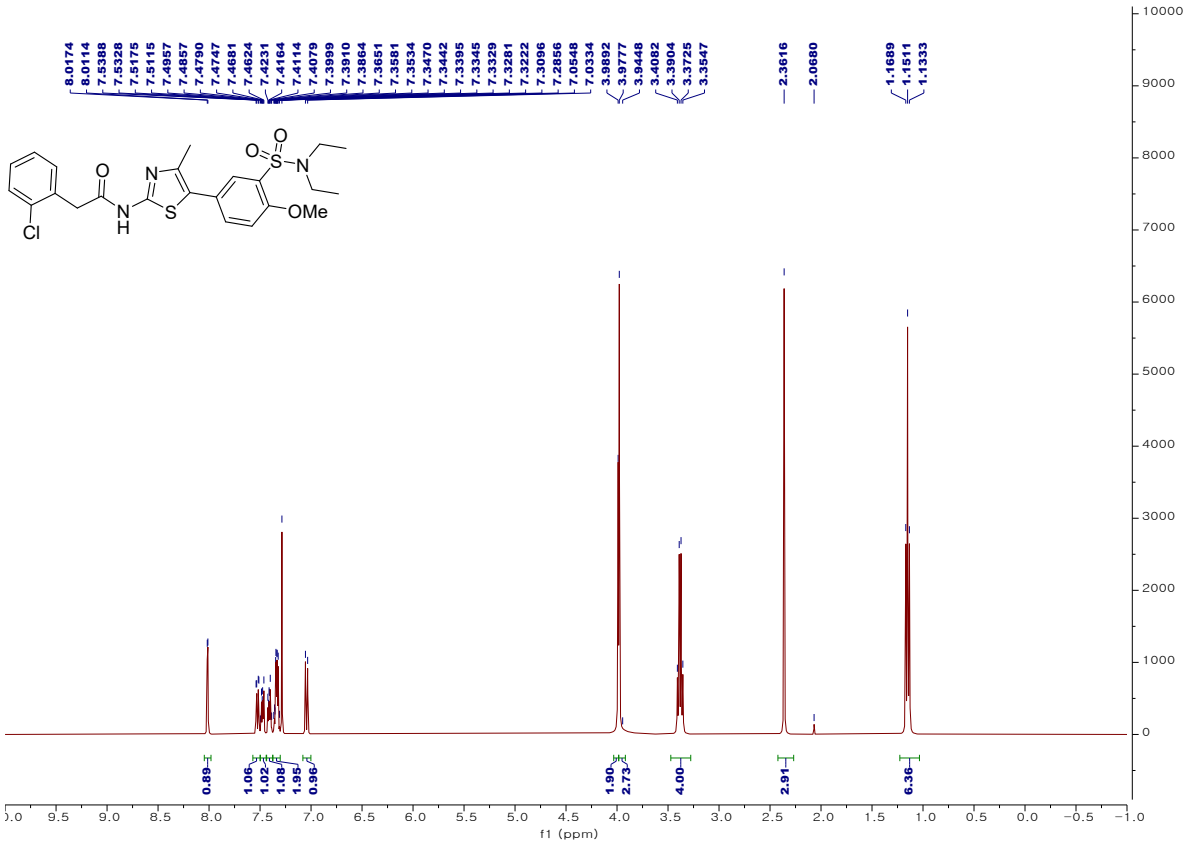
$^{1}$H NMR spectrum of **3** (**KR-27222**)



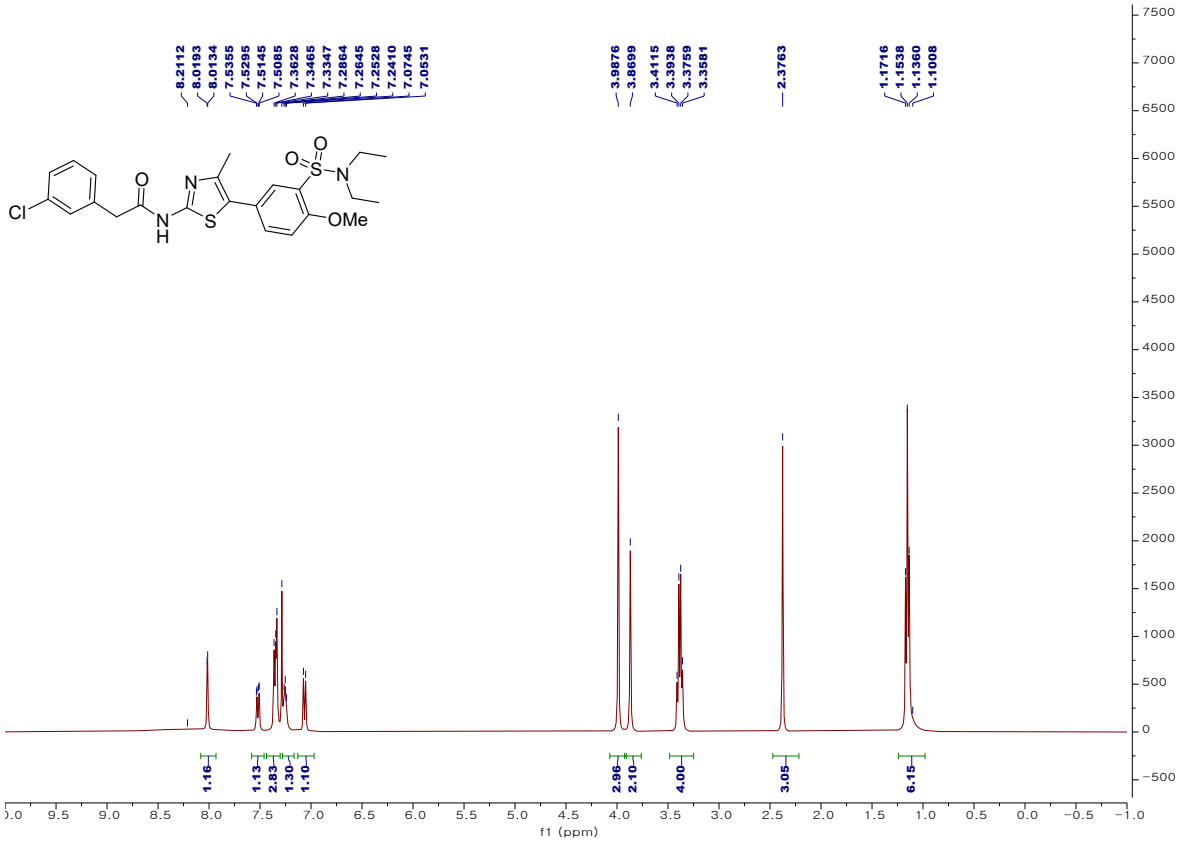
$^{1}$H NMR spectrum of **4** (**KR-27223**)



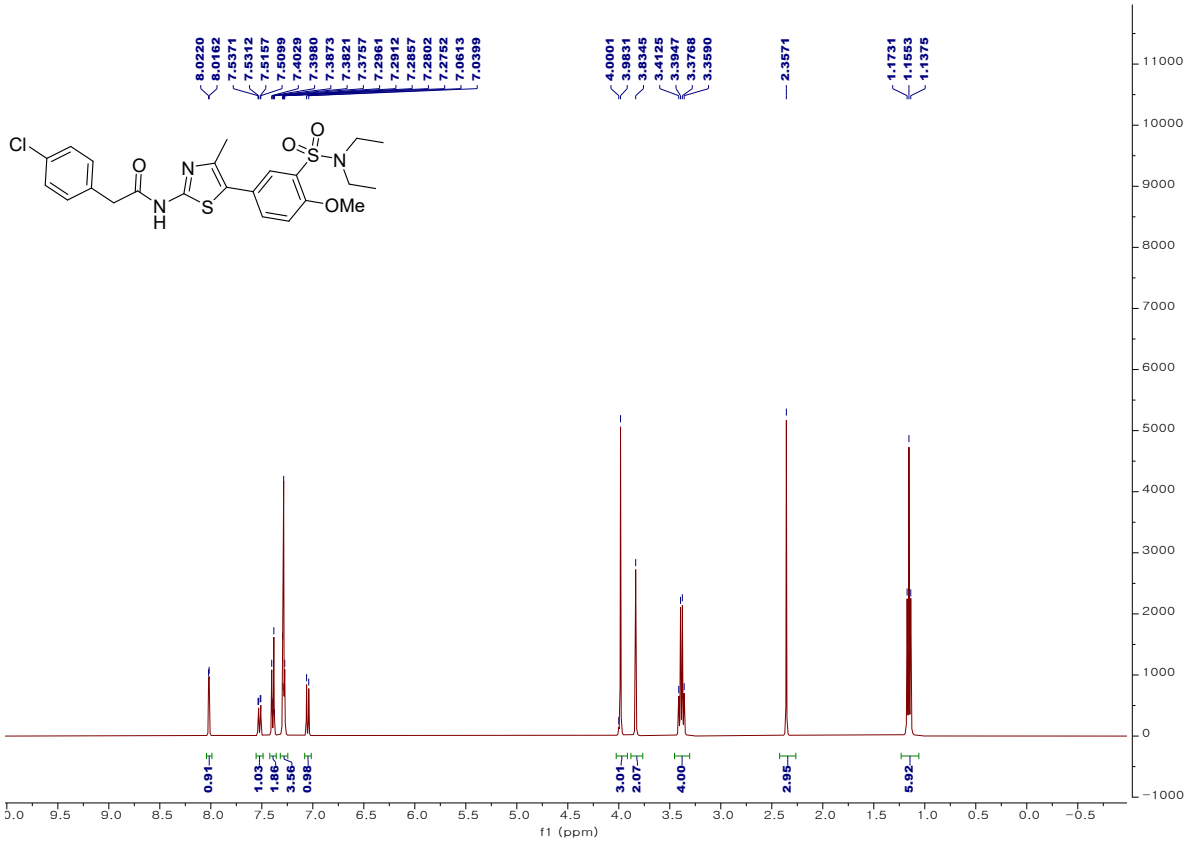
$^{1}$H NMR spectrum of **5a** (**KR-27320**)



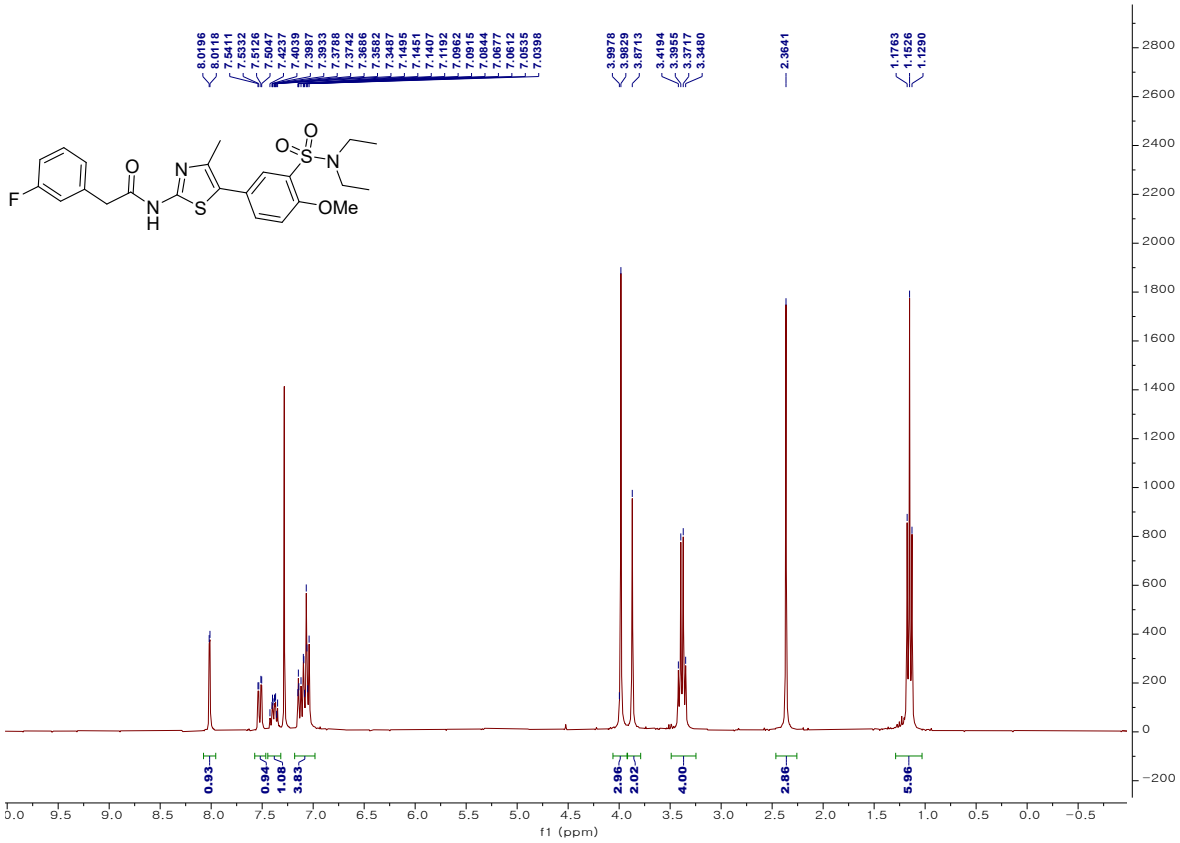
$^{1}$H NMR spectrum of **5b** (**KR-27287**)



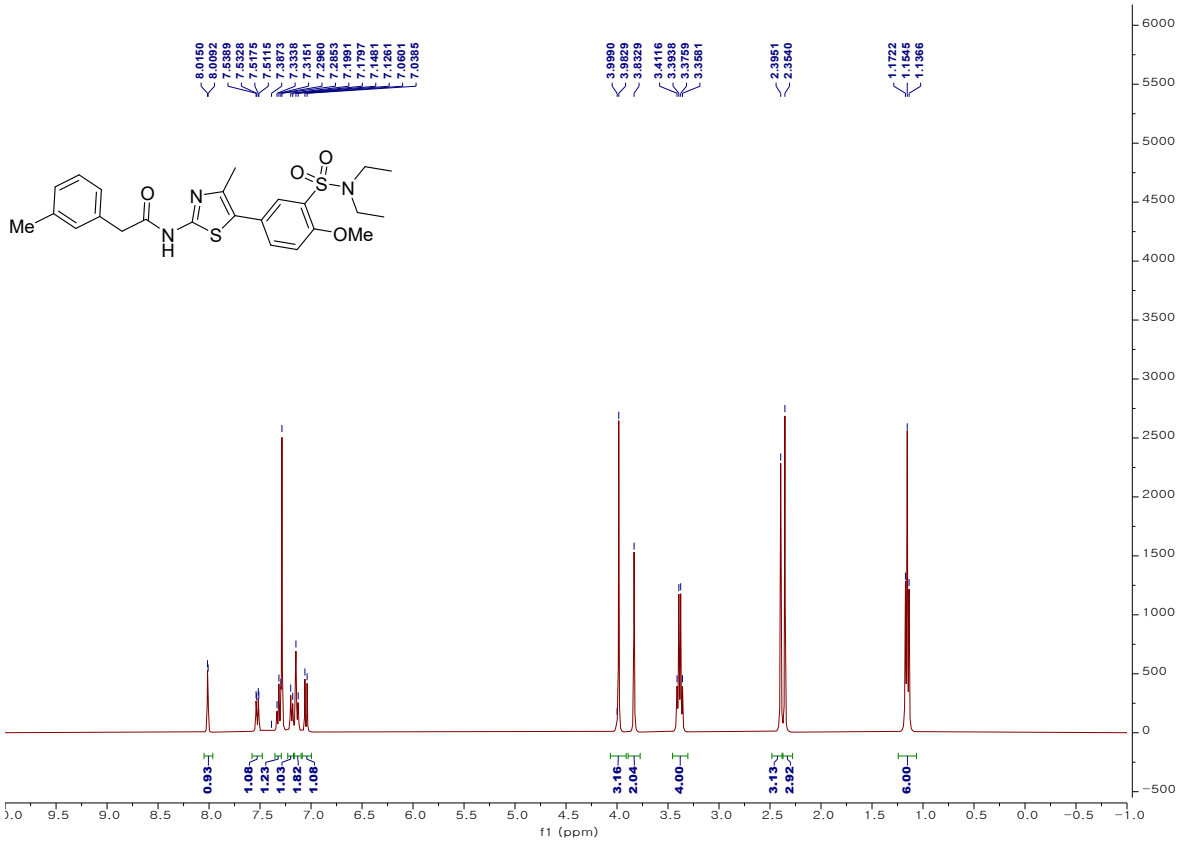
$^{1}$H NMR spectrum of **5c** (**KR-27292**)



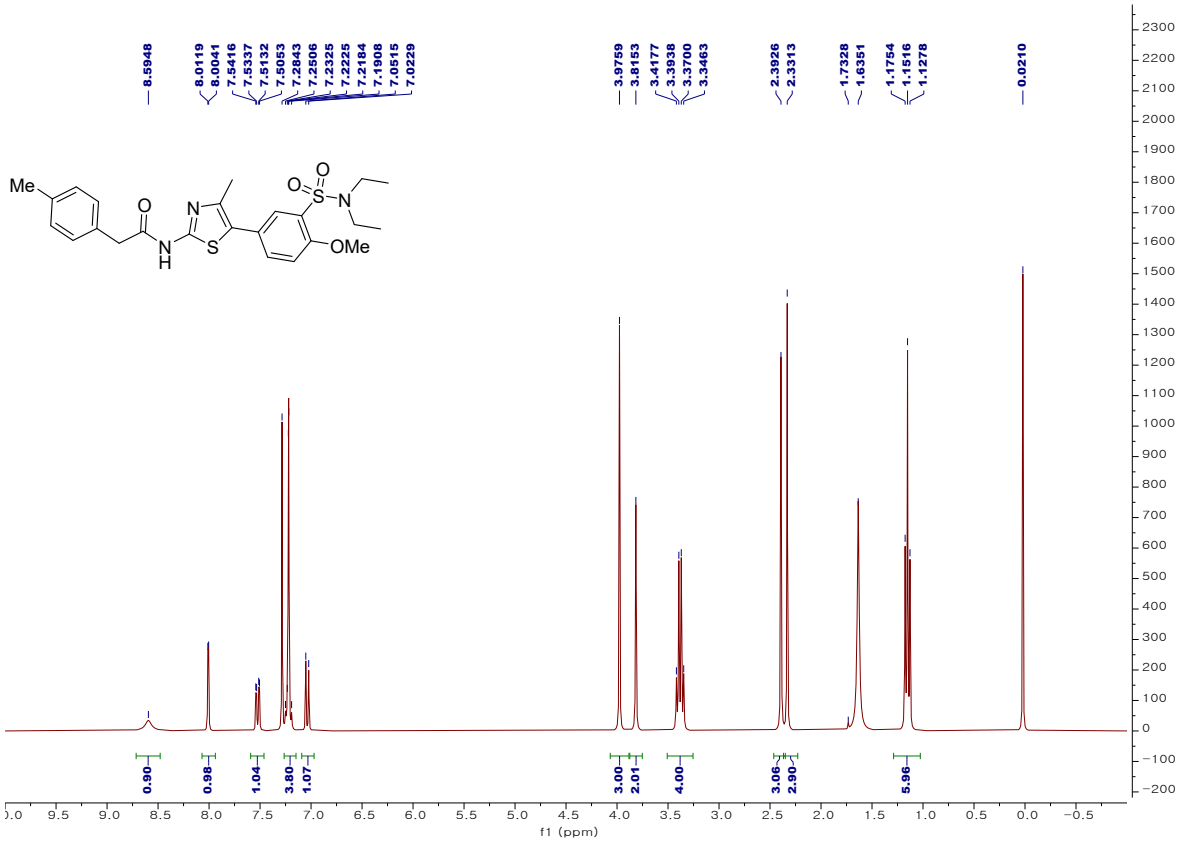
$^{1}$H NMR spectrum of **5d** (**KR-27288**)



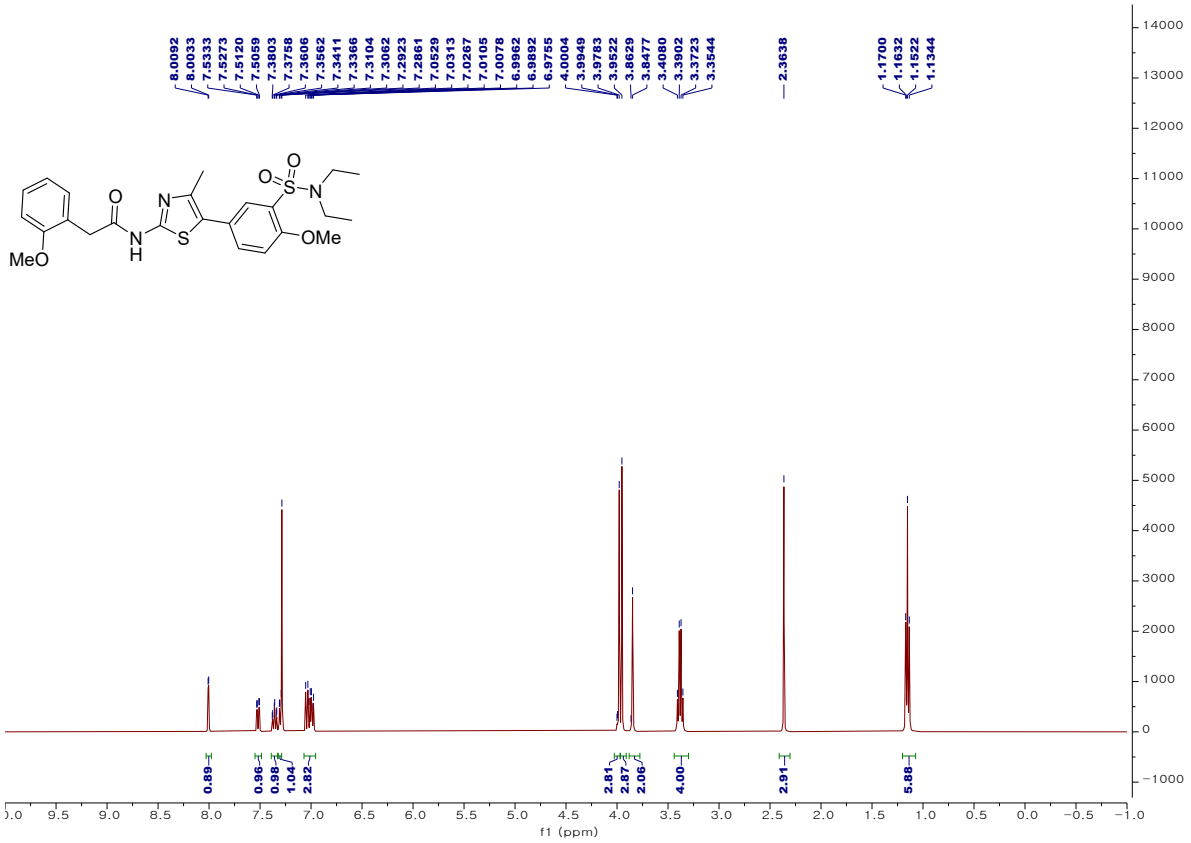
$^{1}$H NMR spectrum of **5e** (**KR-27289**)



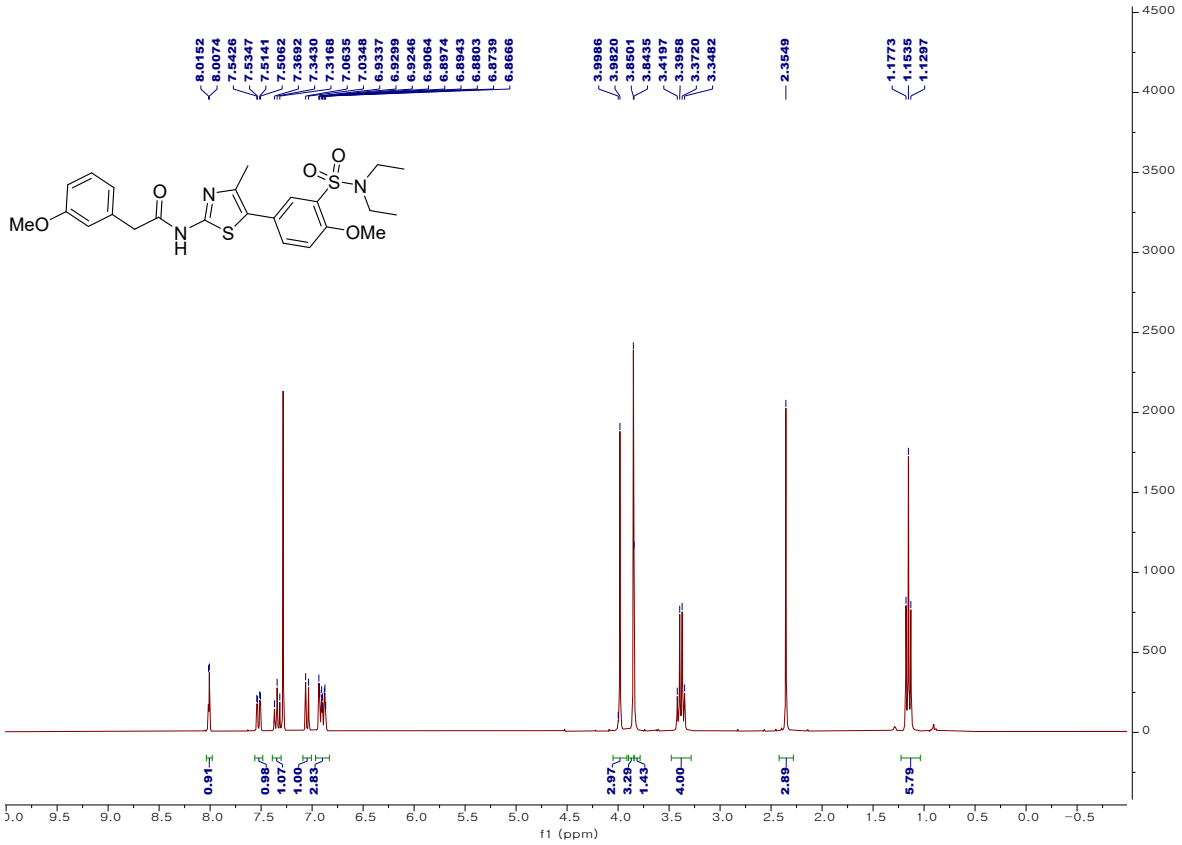
$^{1}$H NMR spectrum of **5f** (**KR-27357**)



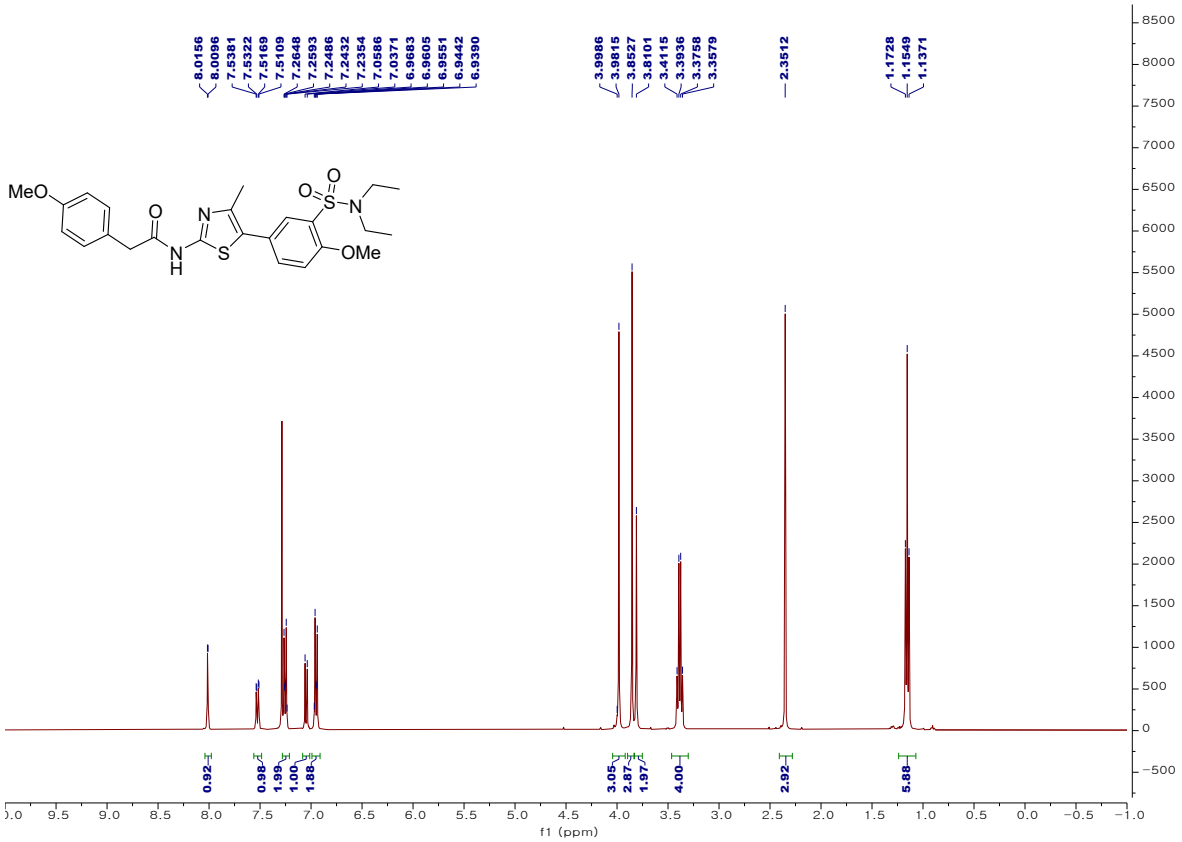
$^{1}$H NMR spectrum of **5g** (**KR-27319**)



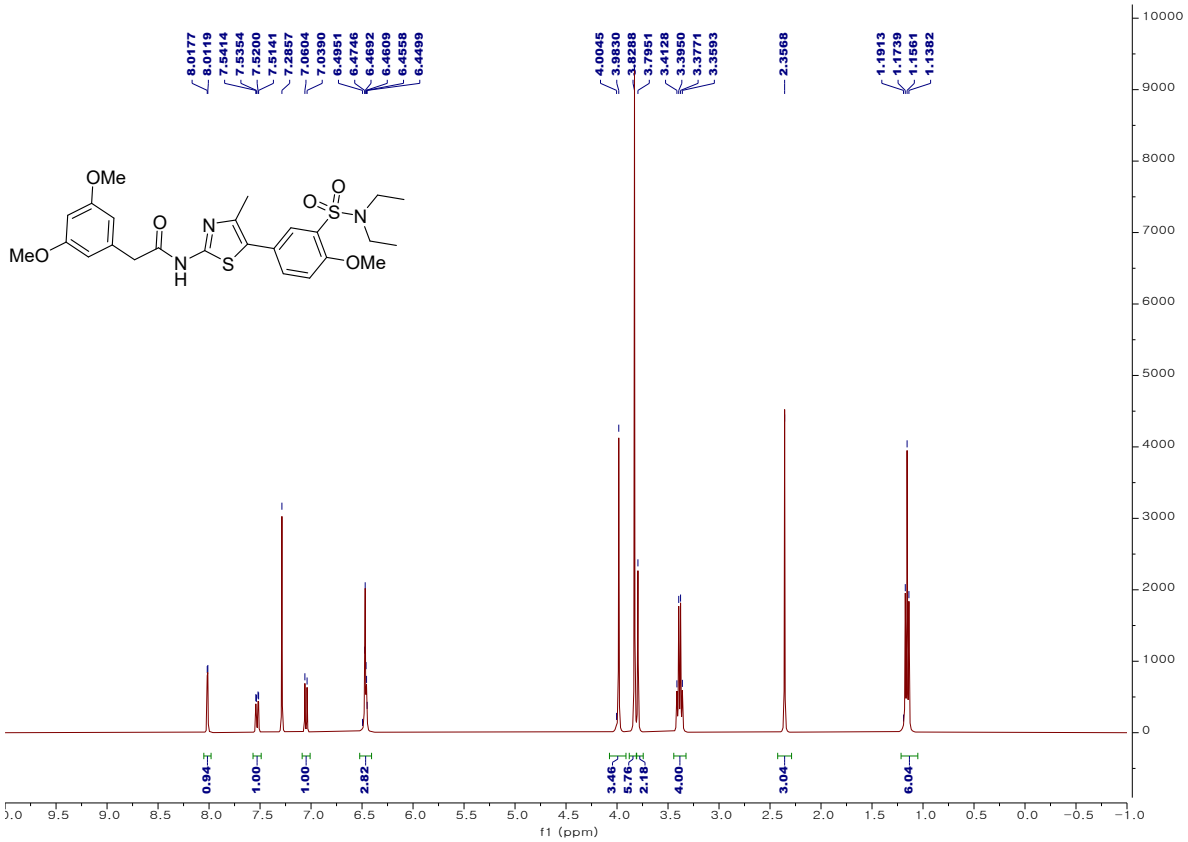
$^{1}$H NMR spectrum of **5h** (**KR-27291**)



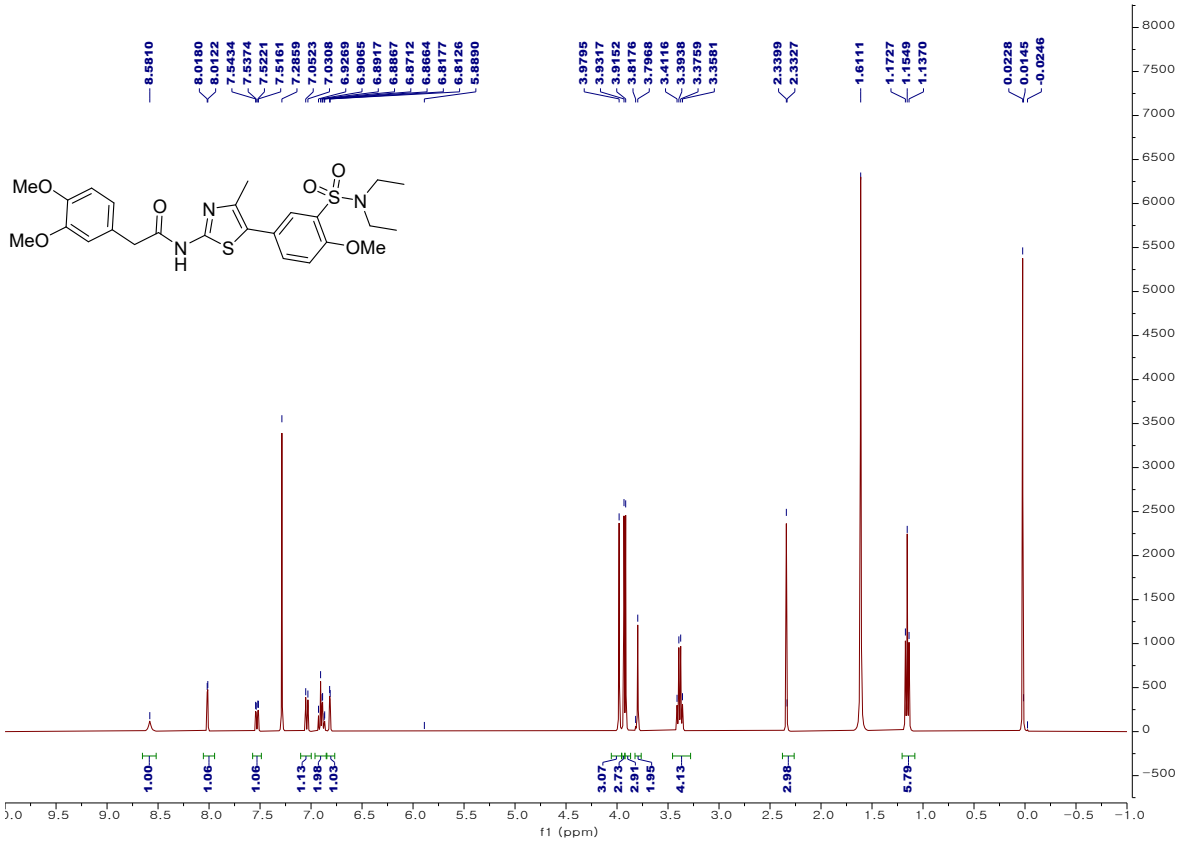
$^{1}$H NMR spectrum of **5i** (**KR-27318**)



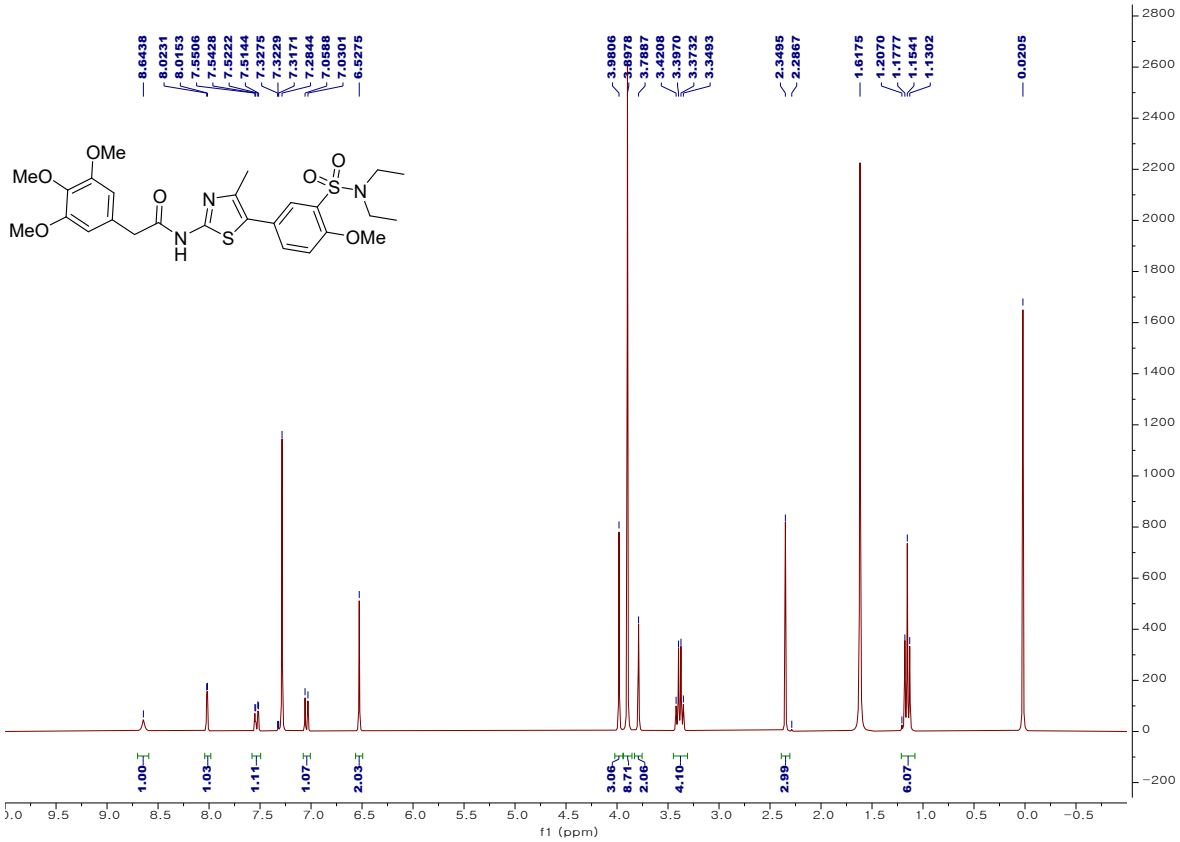
$^{1}$H NMR spectrum of **5j** (**KR-27321**)



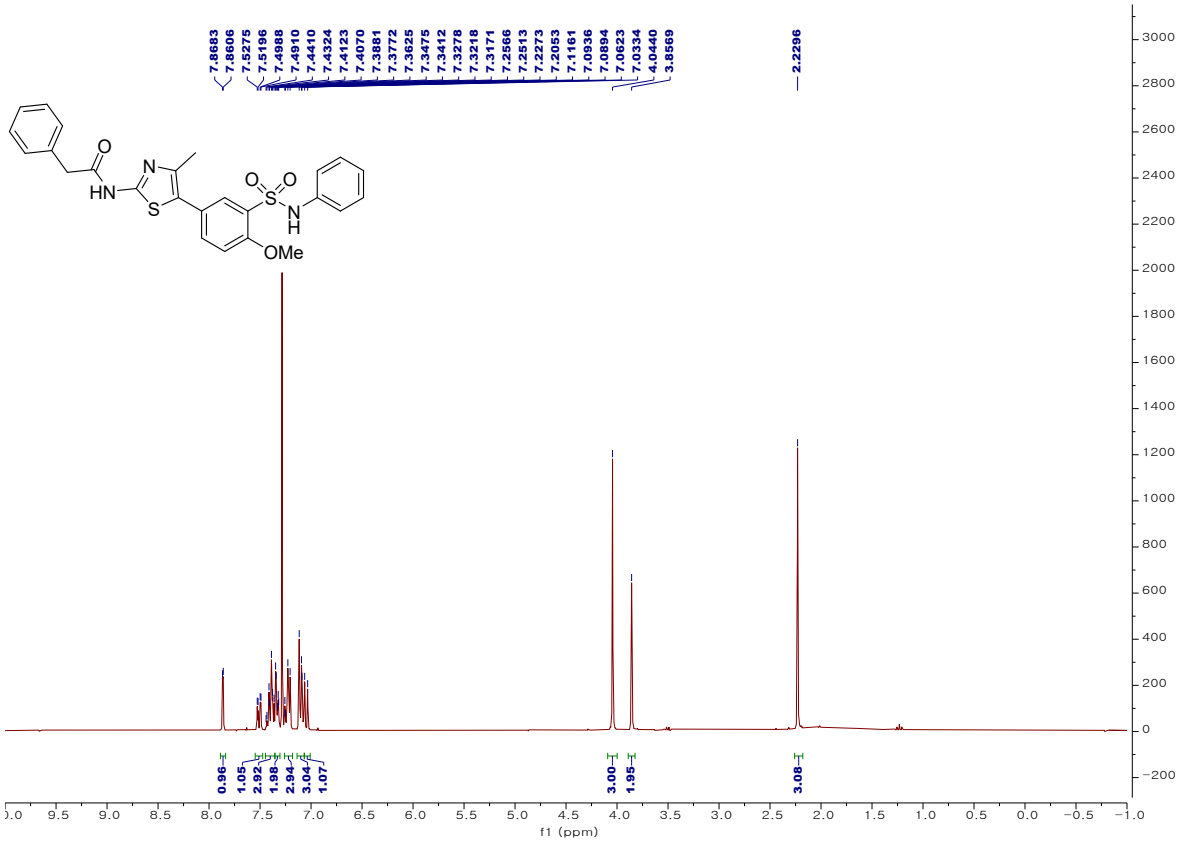
$^{1}$H NMR spectrum of **5k** (**KR-27356**)



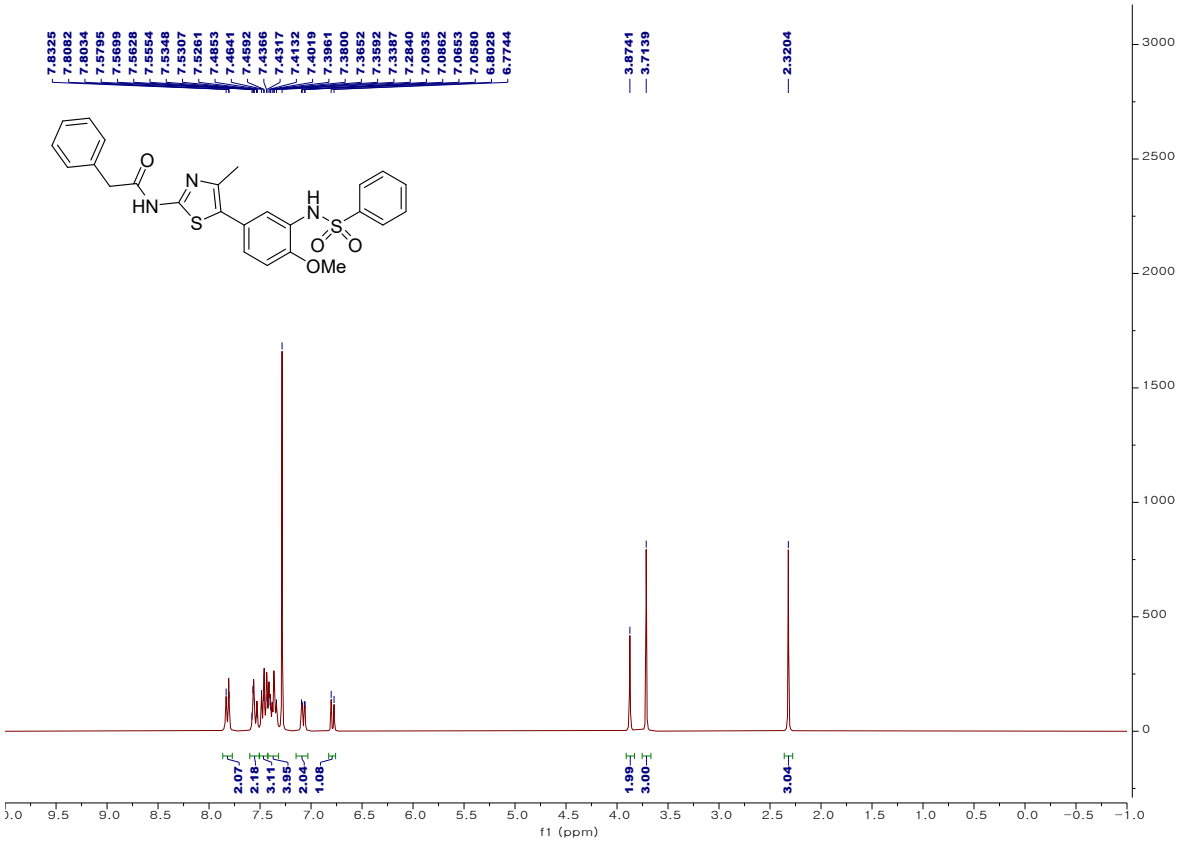
$^{1}$H NMR spectrum of **5l** (**KR-27358**)



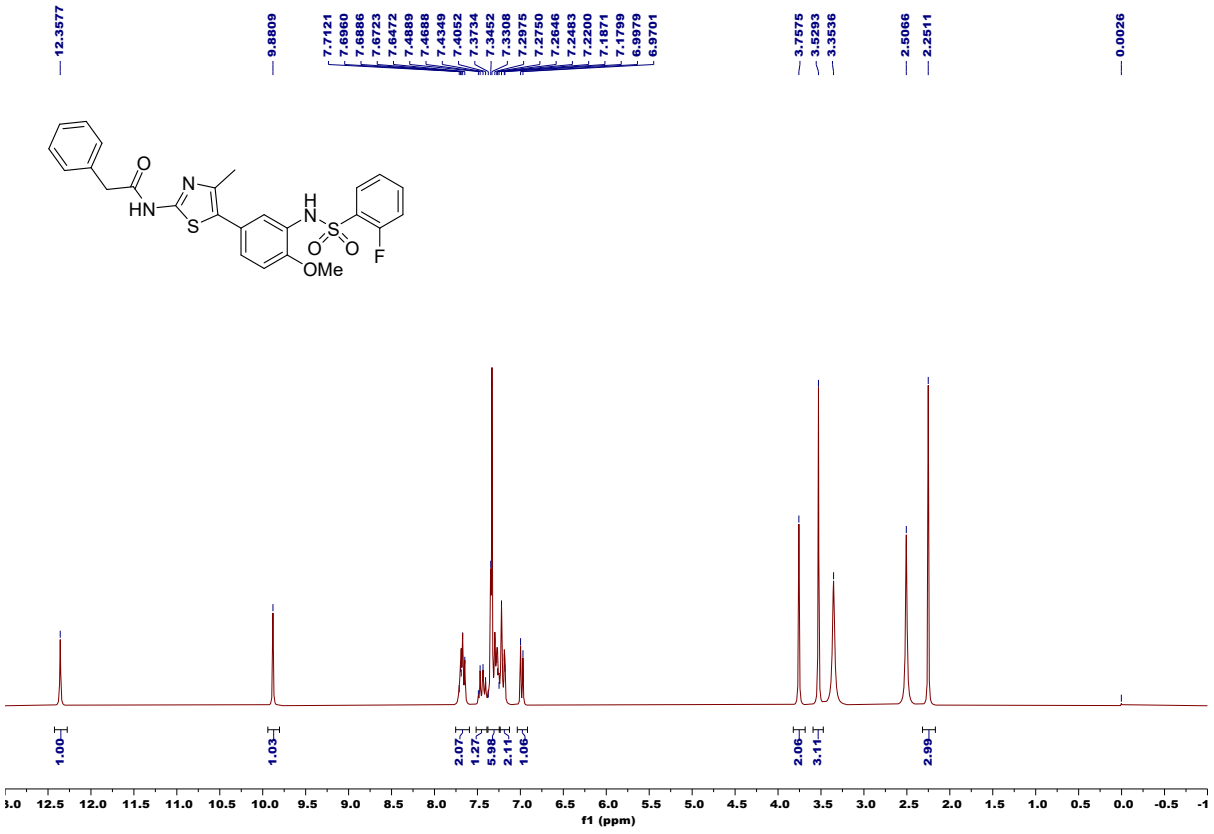
$^{1}$H NMR spectrum of **6** (**KR-27335**)



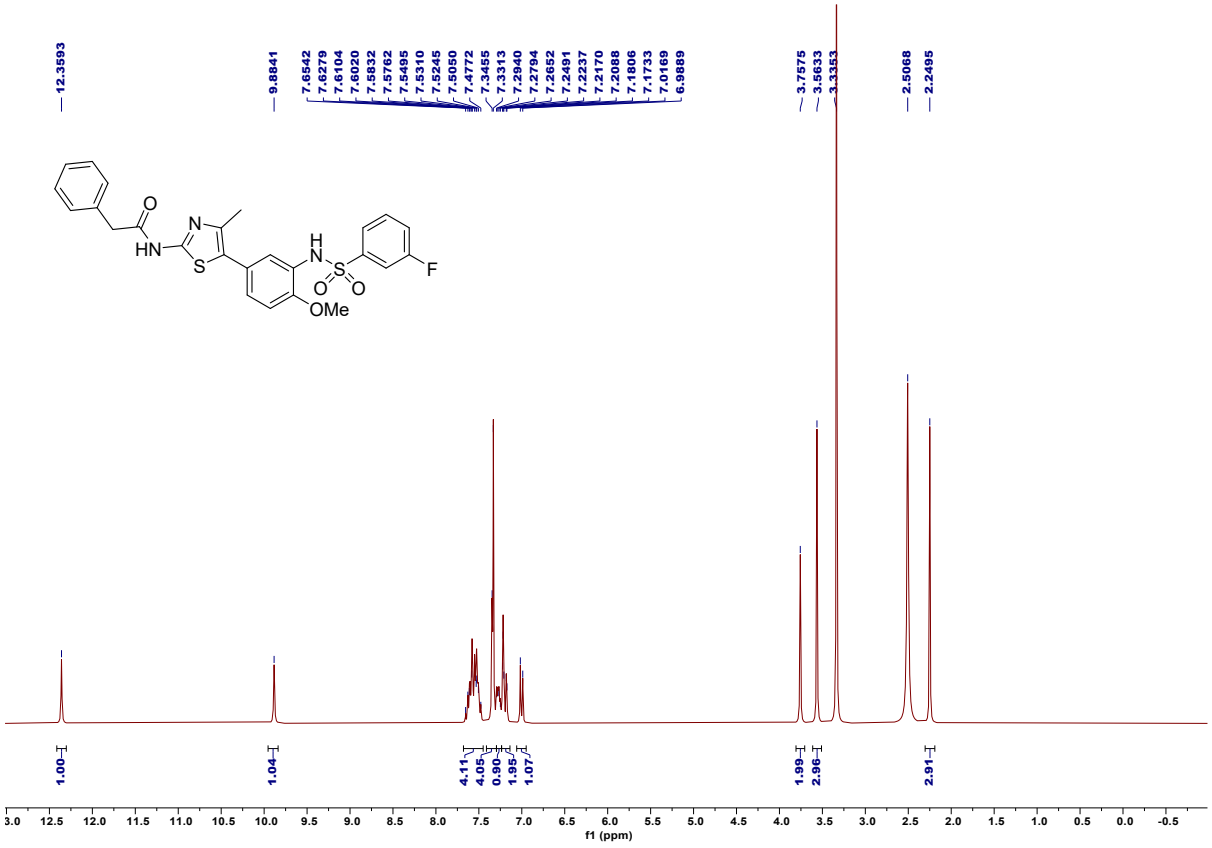
$^{1}$H NMR spectrum of **7a** (**KR-27336**)



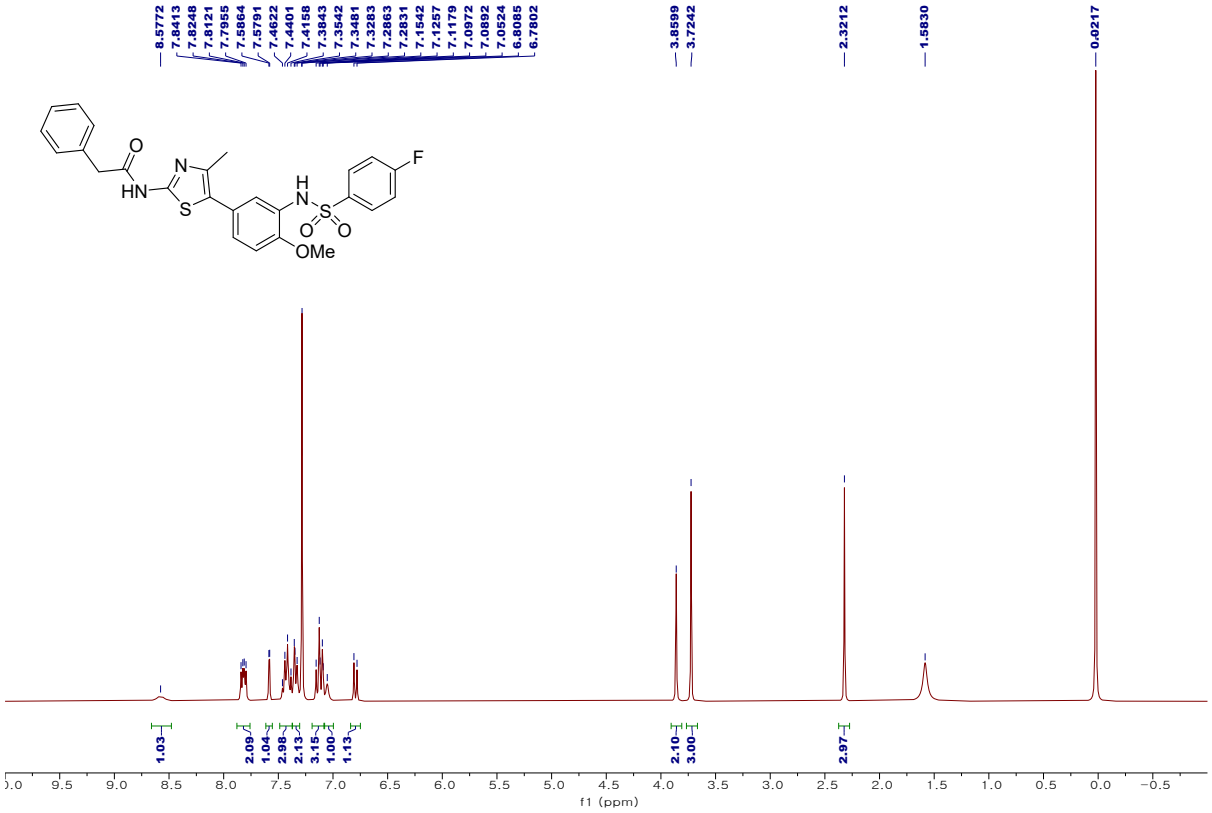
$^{1}$H NMR spectrum of **7b** (**KR-27376**)



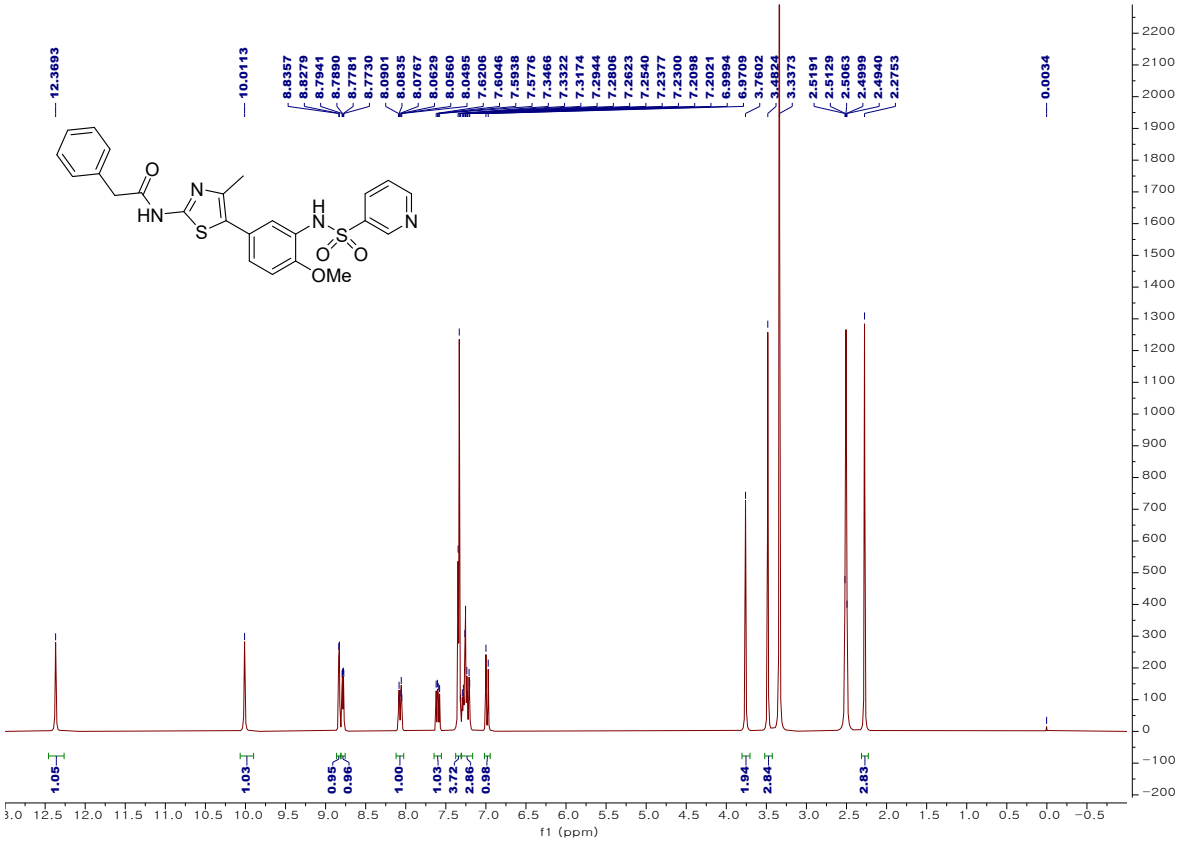
$^{1}$H NMR spectrum of **7c** (**KR-27377**)



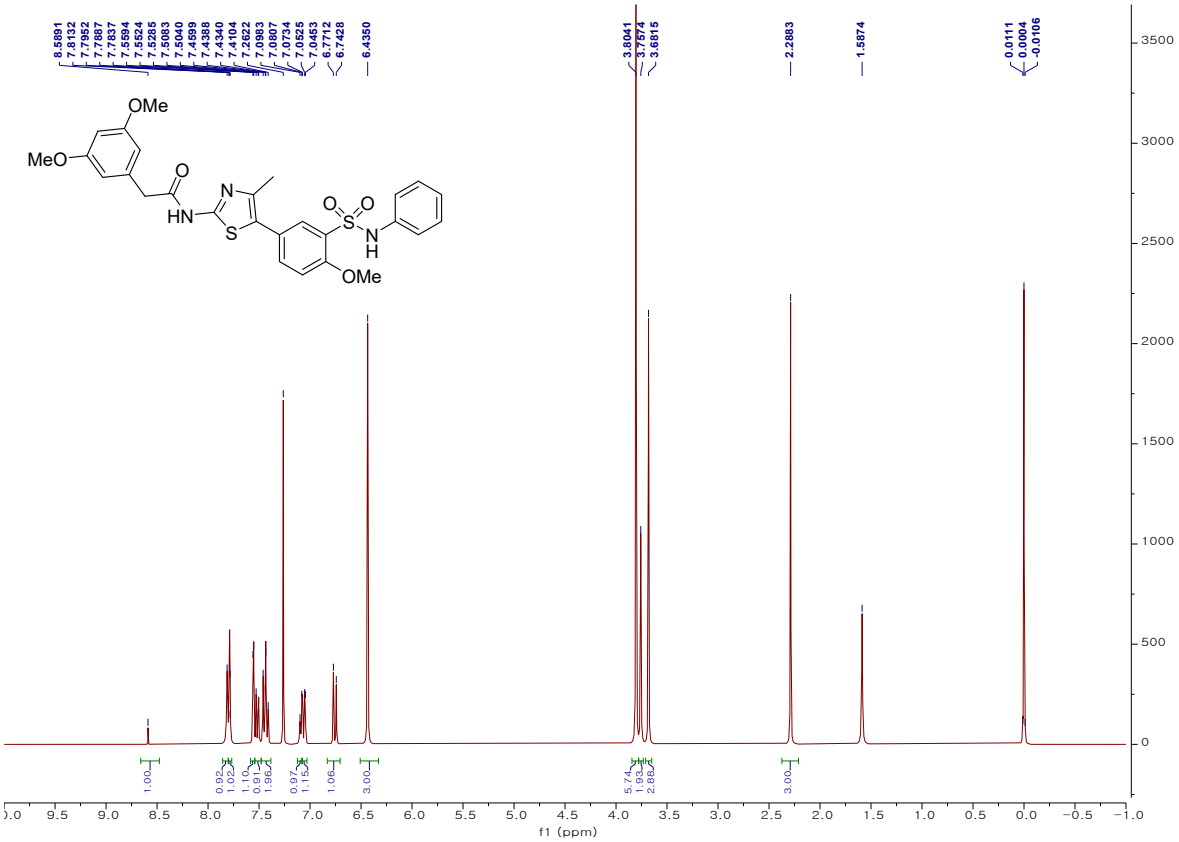
$^{1}$H NMR spectrum of **7d** (**KR-27374**)



$^{1}$H NMR spectrum of **7e** (**KR-27375**)

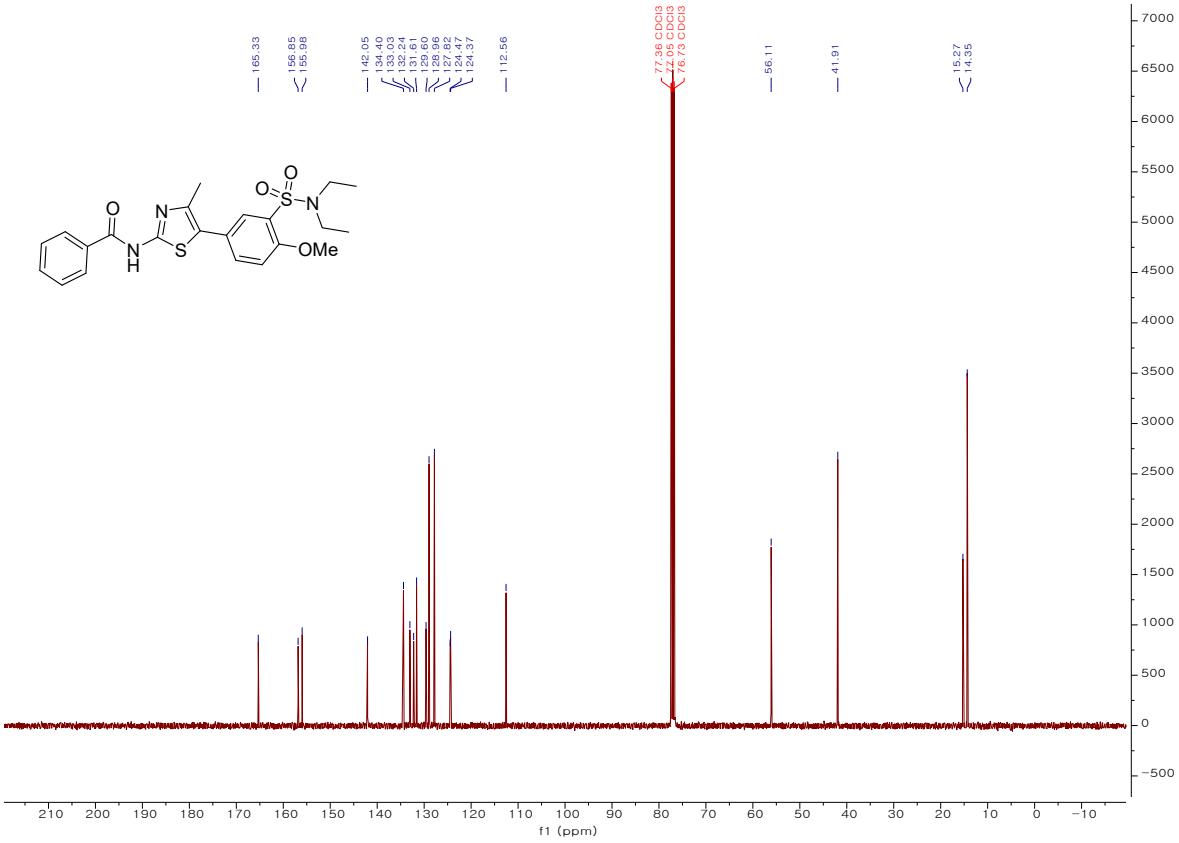


$^{1}$H NMR spectrum of **7f** (**KR-27370**)

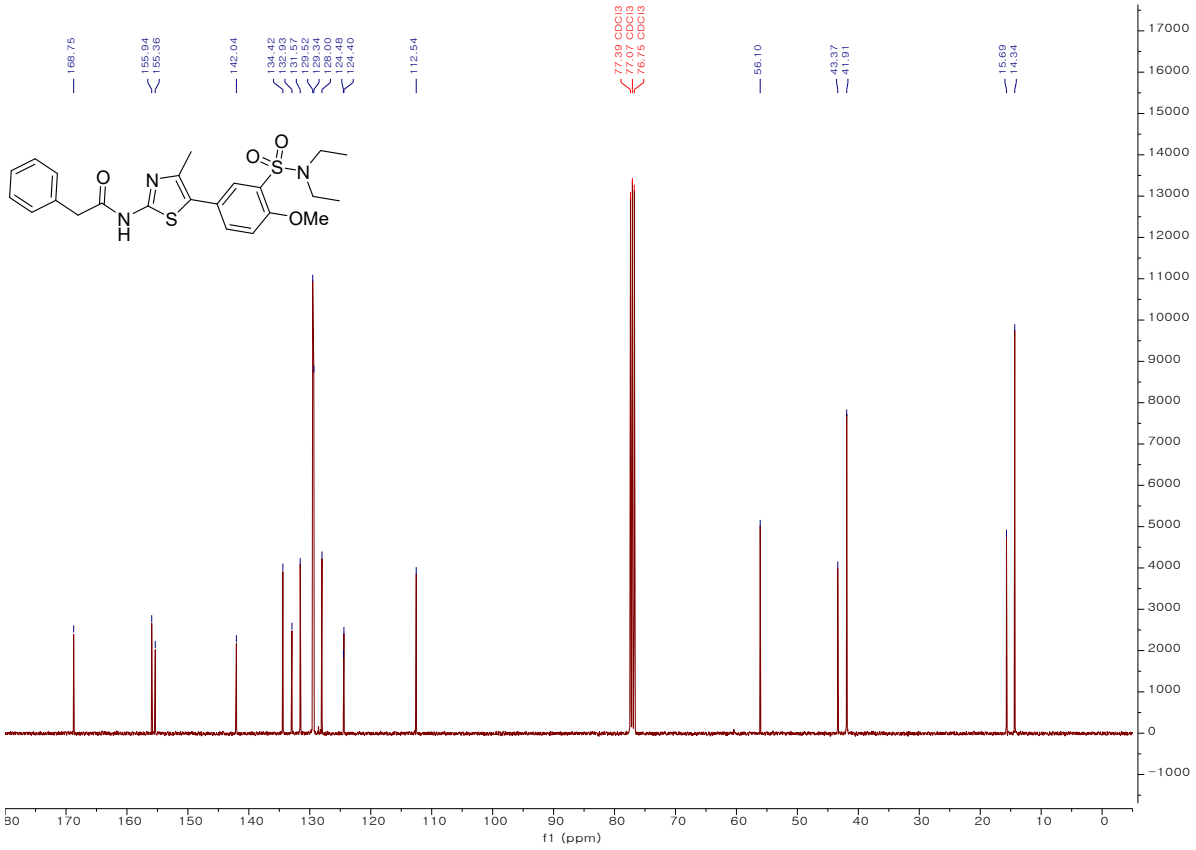


# S2. Spectral Copies of $^{13}$C NMR of Compounds

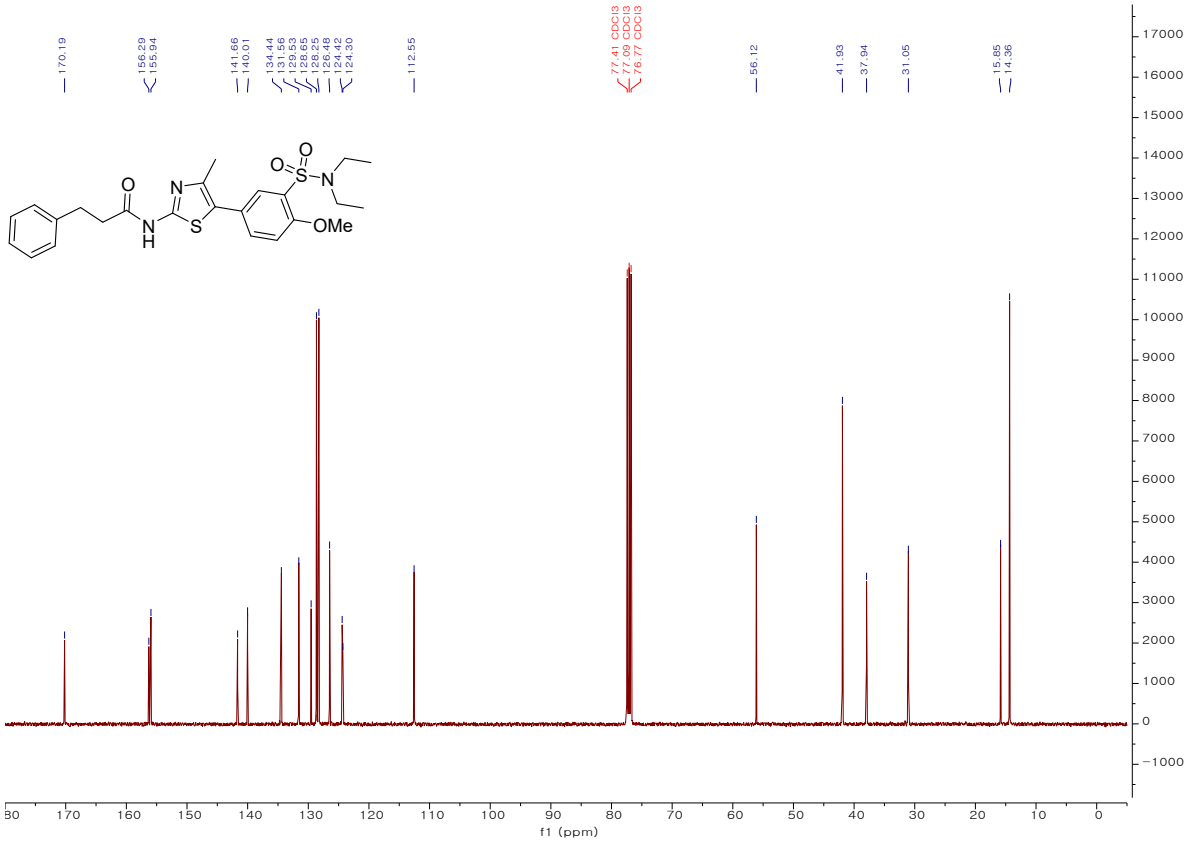
13C NMR spectrum of **2** (**KR-27282**)



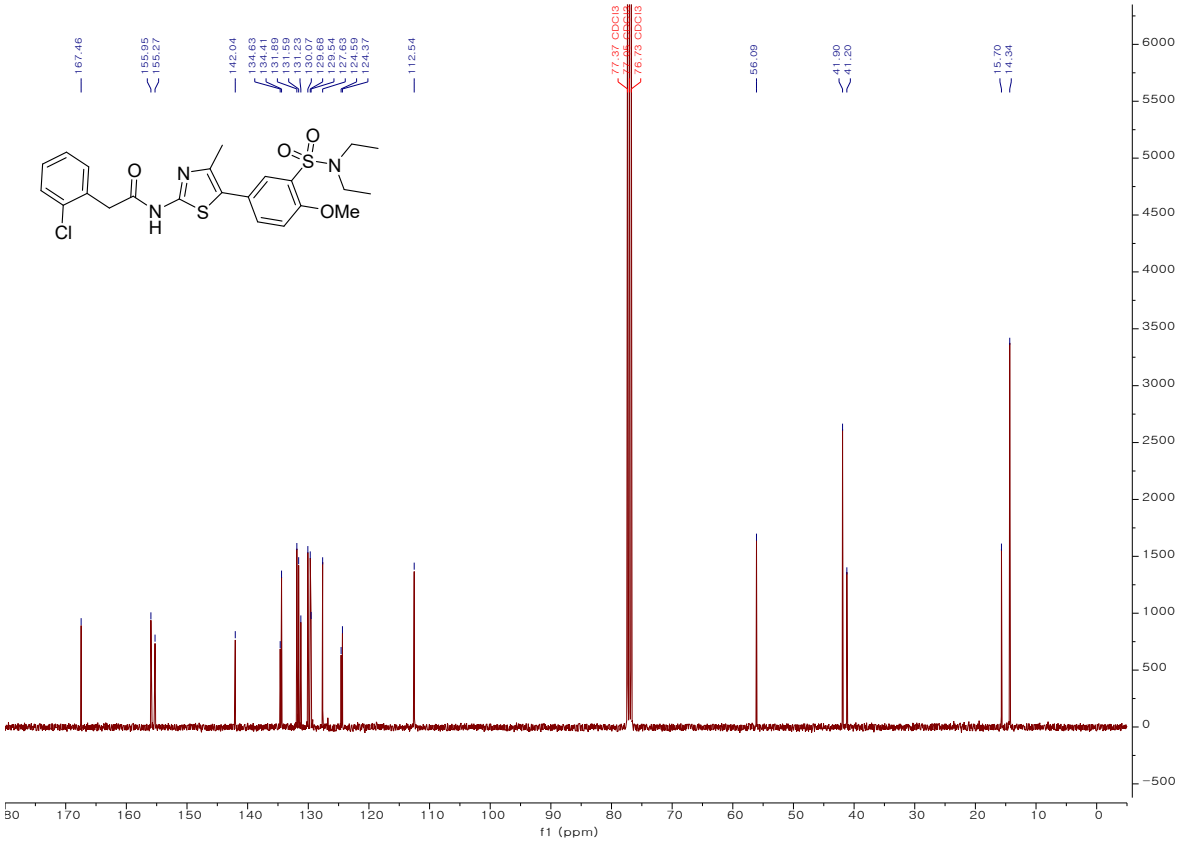
13C NMR spectrum of **3** (**KR-27222**)



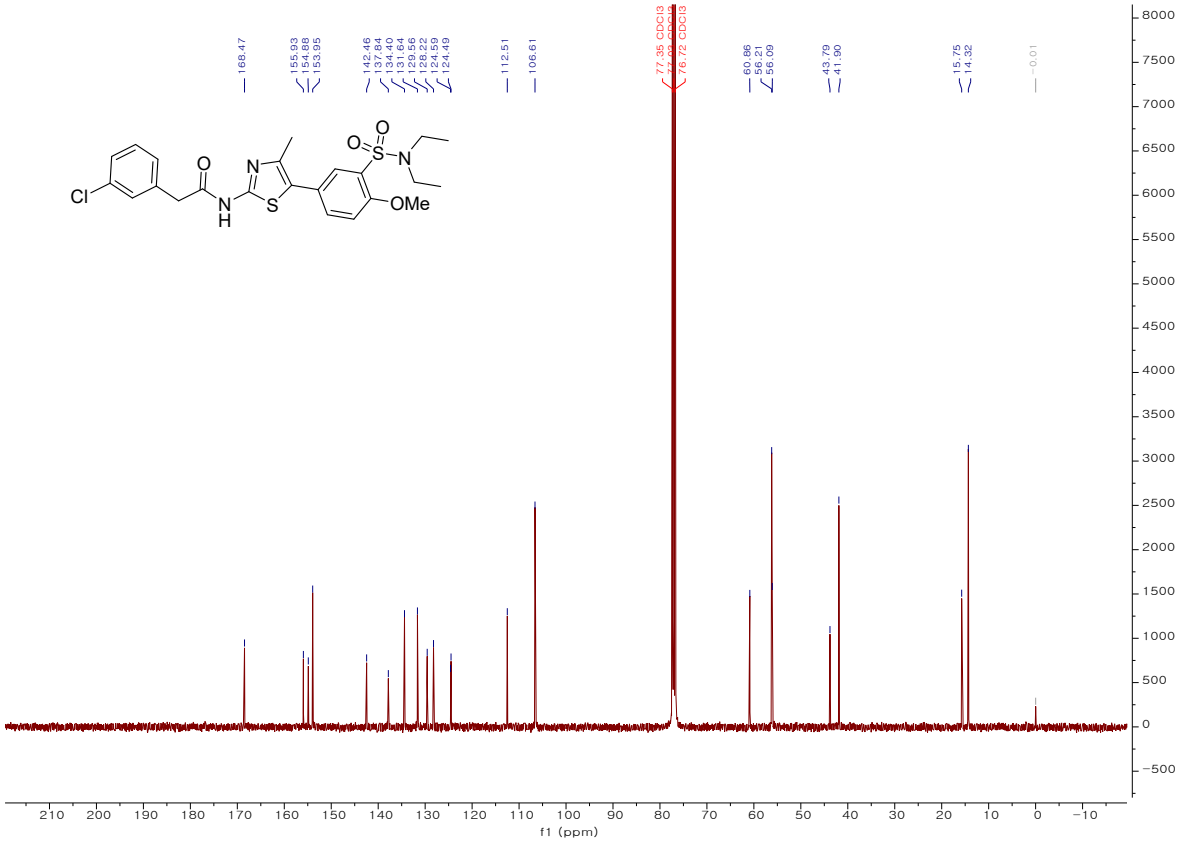
13C NMR spectrum of **4** (**KR-27223**)



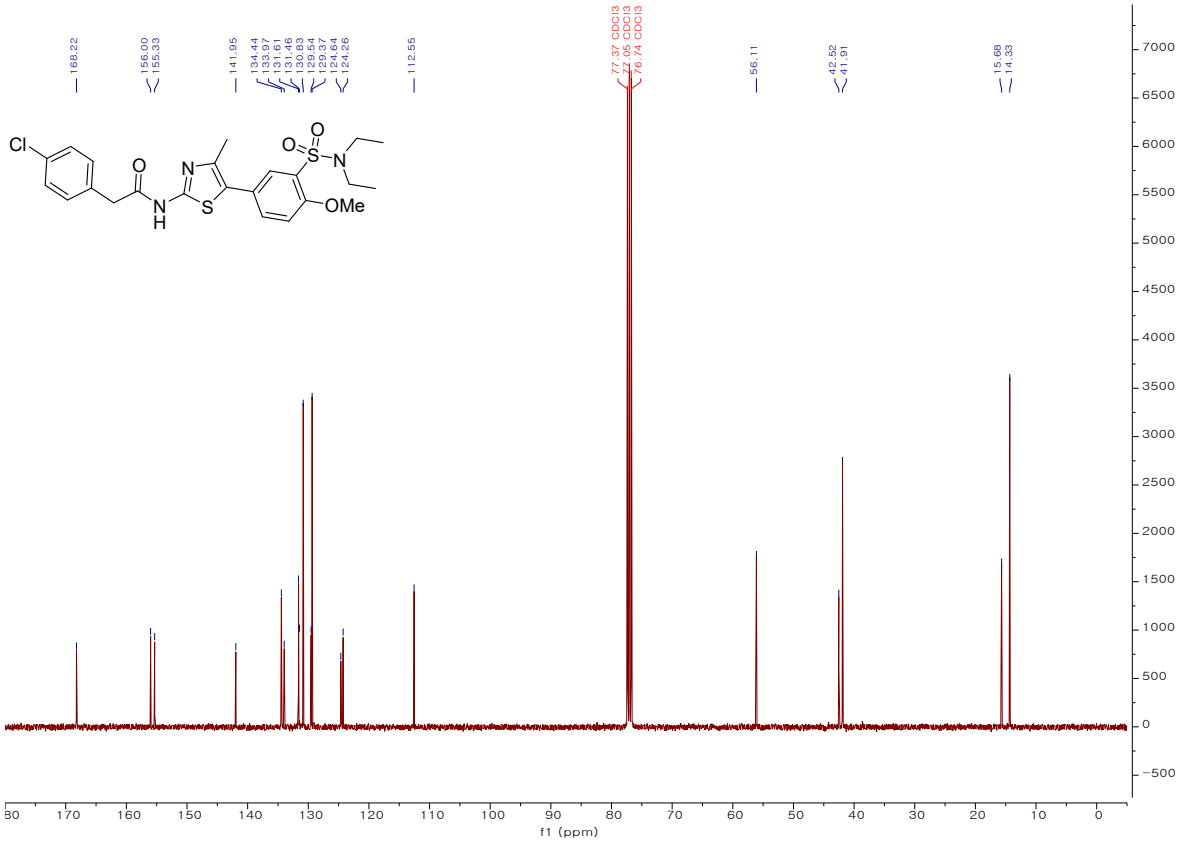
13C NMR spectrum of **5a** (**KR-27320**)



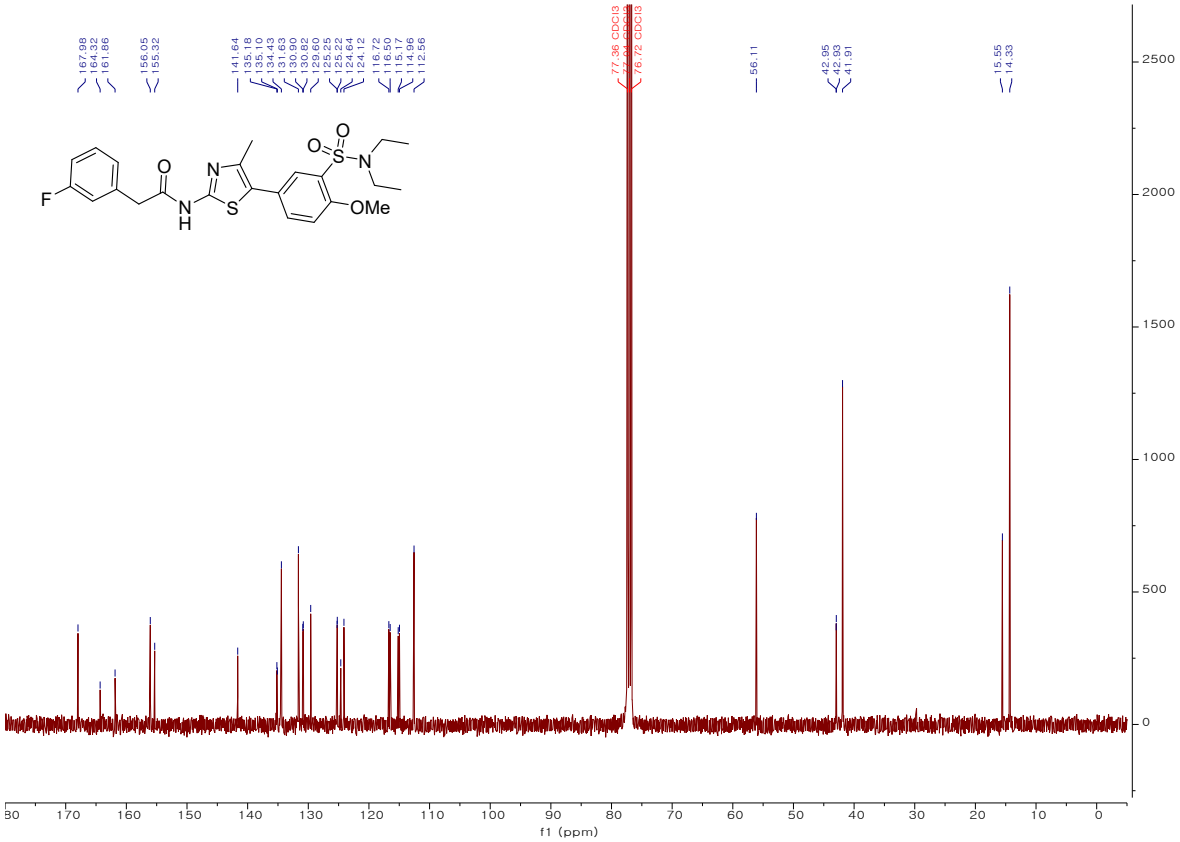
13C NMR spectrum of **5b** (**KR-27287**)



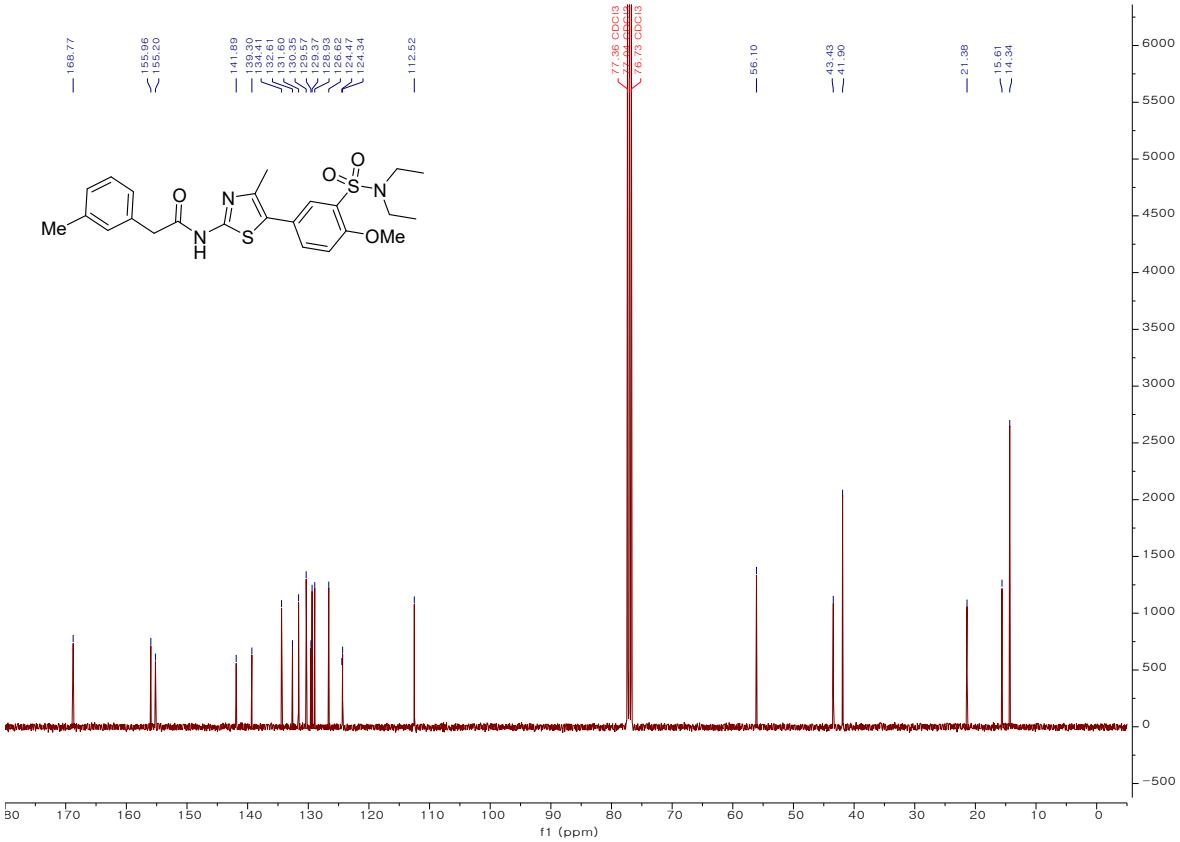
13C NMR spectrum of **5c** (**KR-27292**)



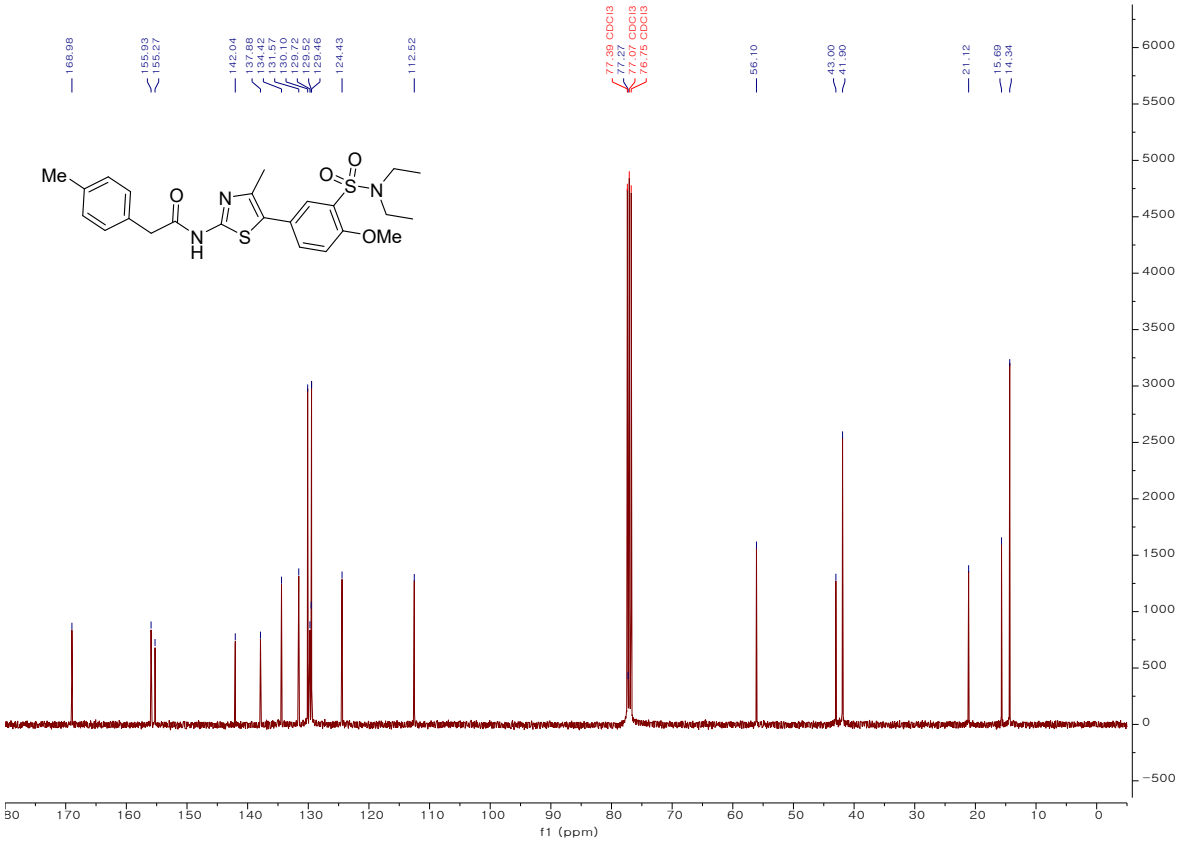
13C NMR spectrum of **5d** (**KR-27288**)



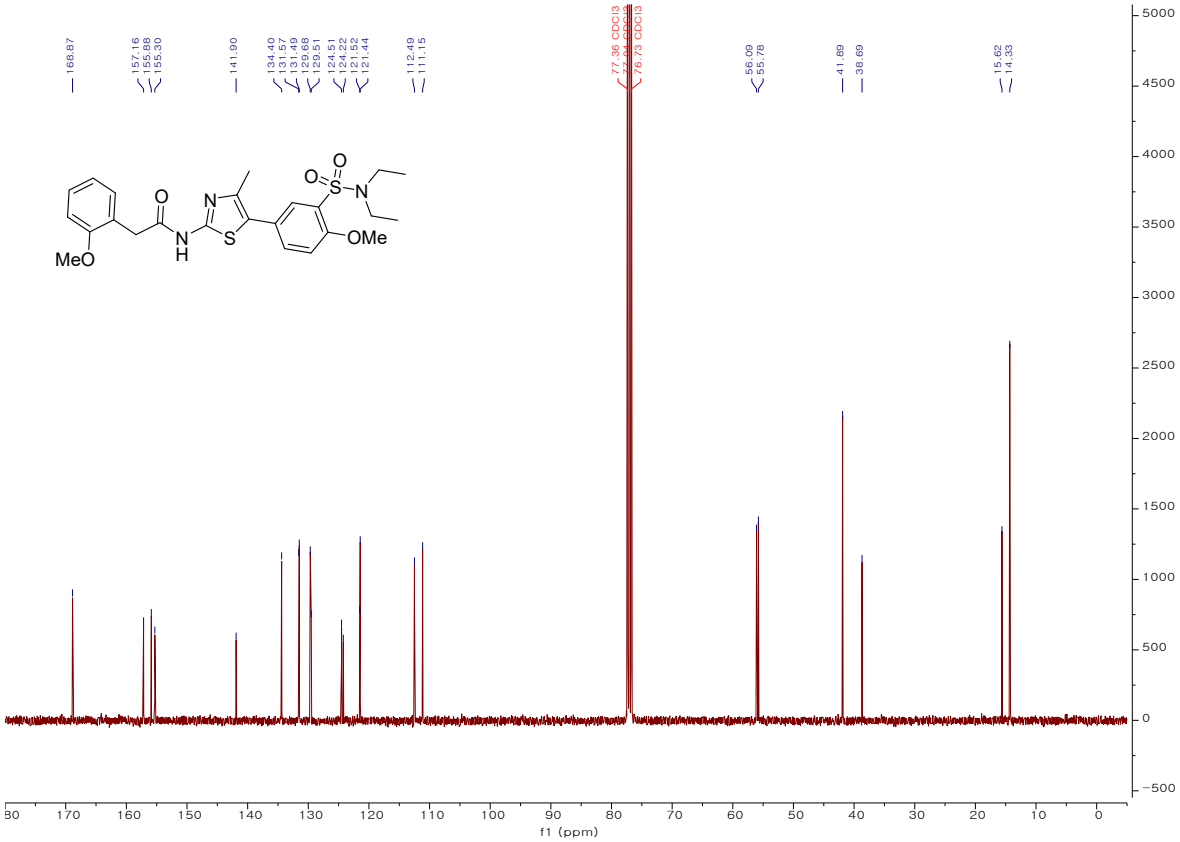
13C NMR spectrum of **5e** (**KR-27289**)



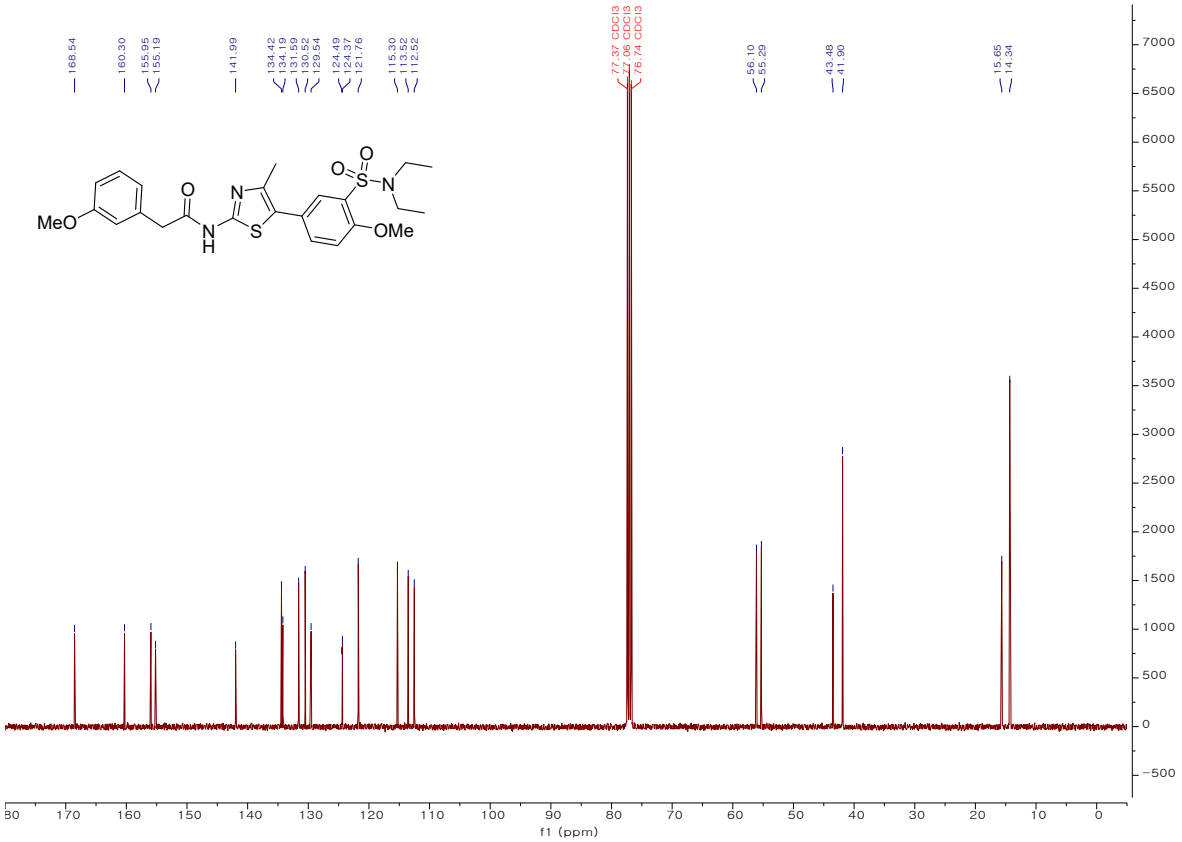
13C NMR spectrum of **5f** (**KR-27357**)



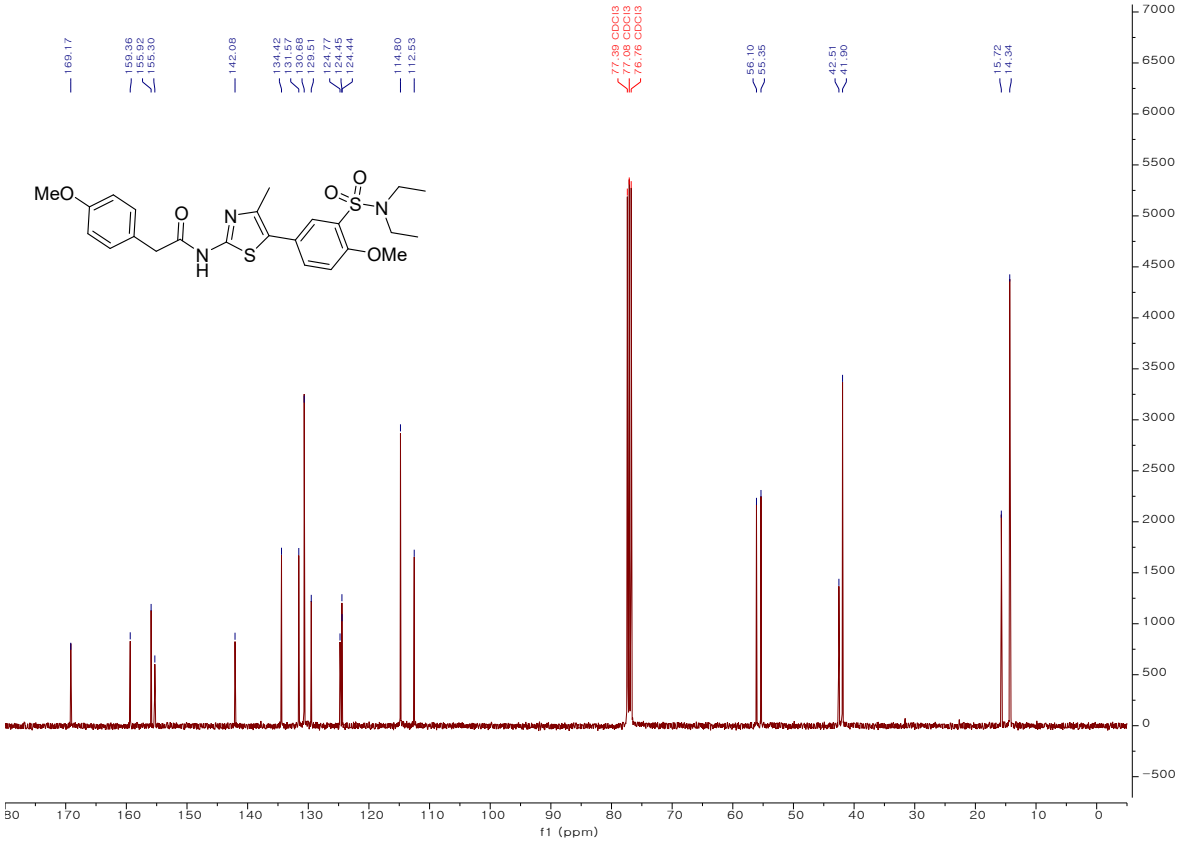
13C NMR spectrum of **5g** (**KR-27319**)



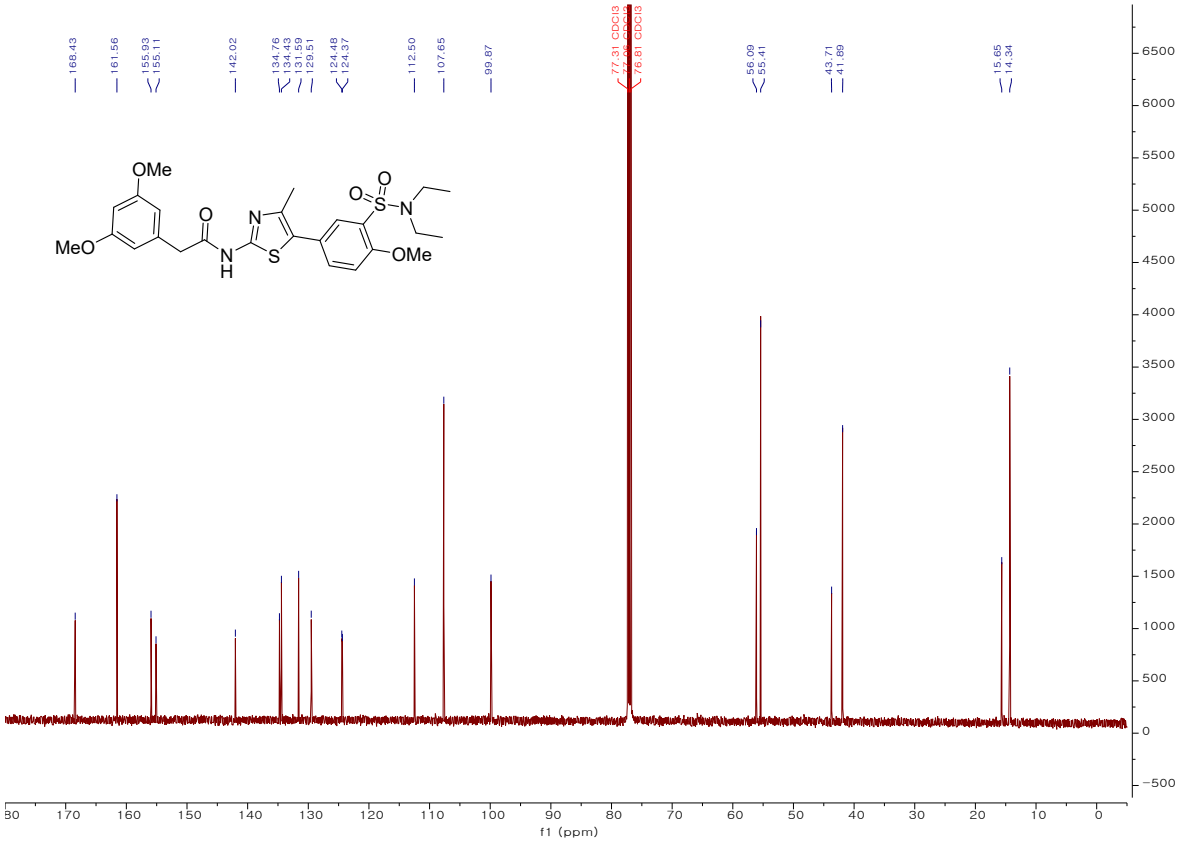
13C NMR spectrum of **5h** (**KR-27291**)



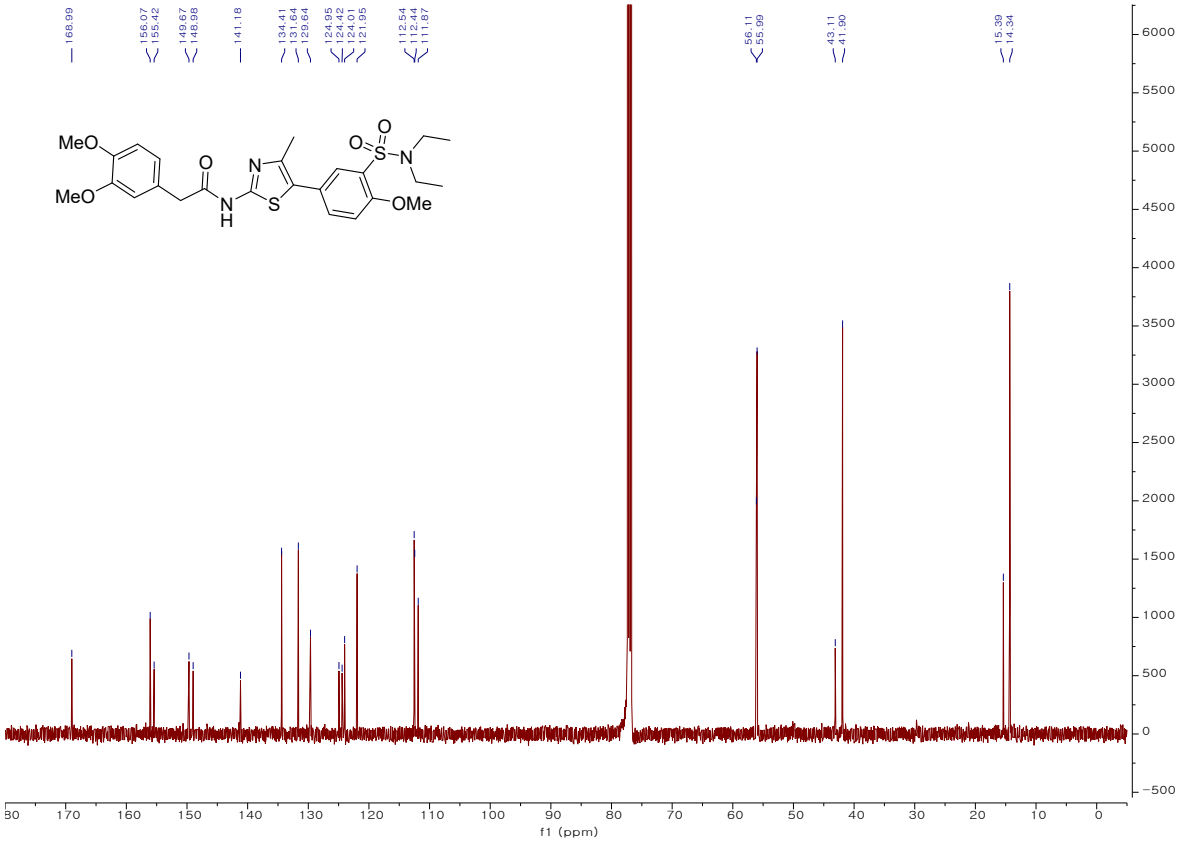
13C NMR spectrum of **5i** (**KR-27318**)



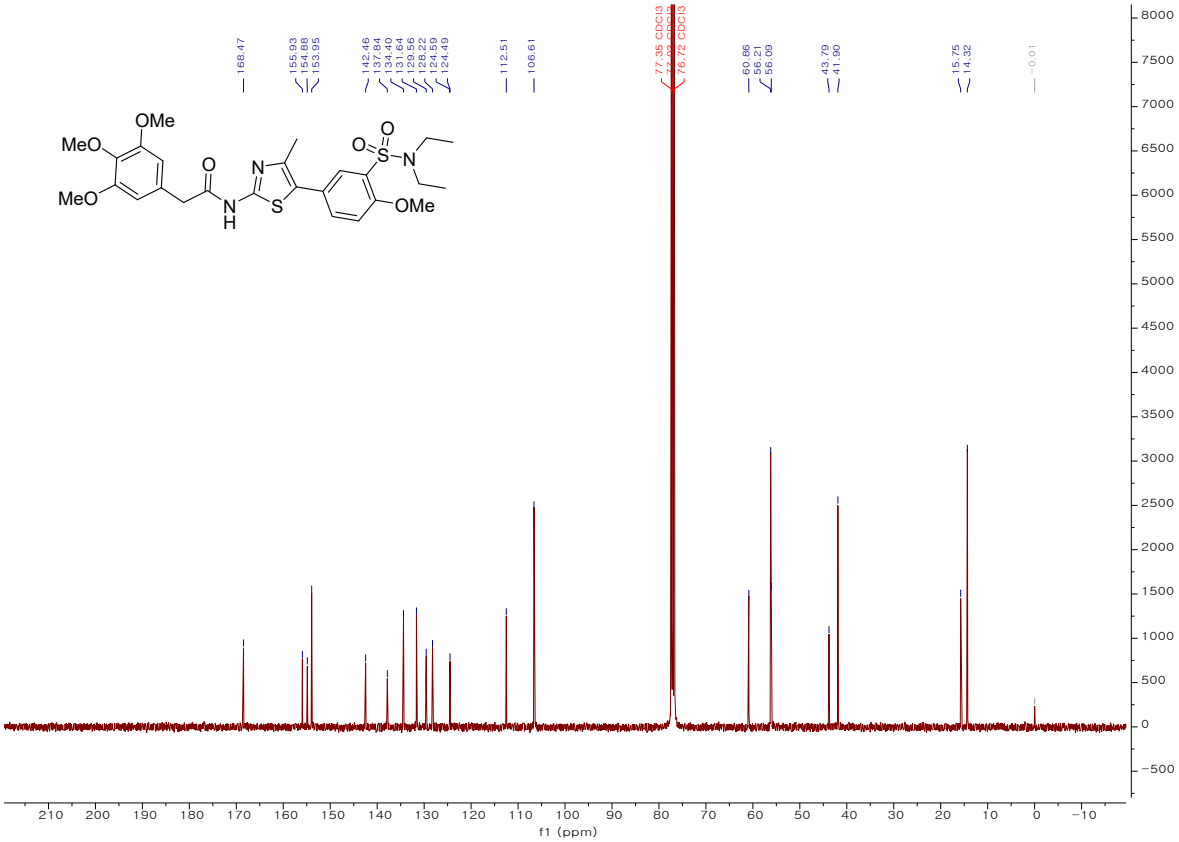
13C NMR spectrum of **5j** (**KR-27321**)



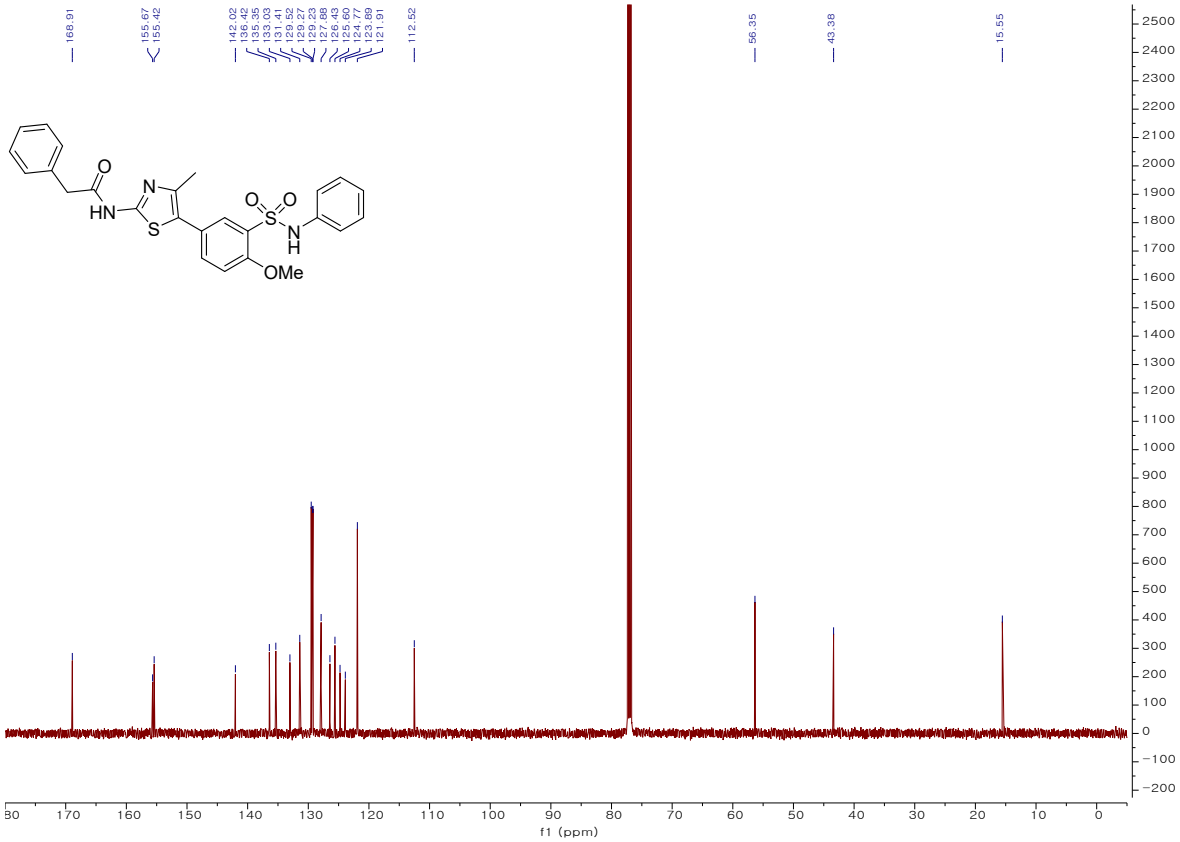
13C NMR spectrum of **5k** (**KR-27356**)



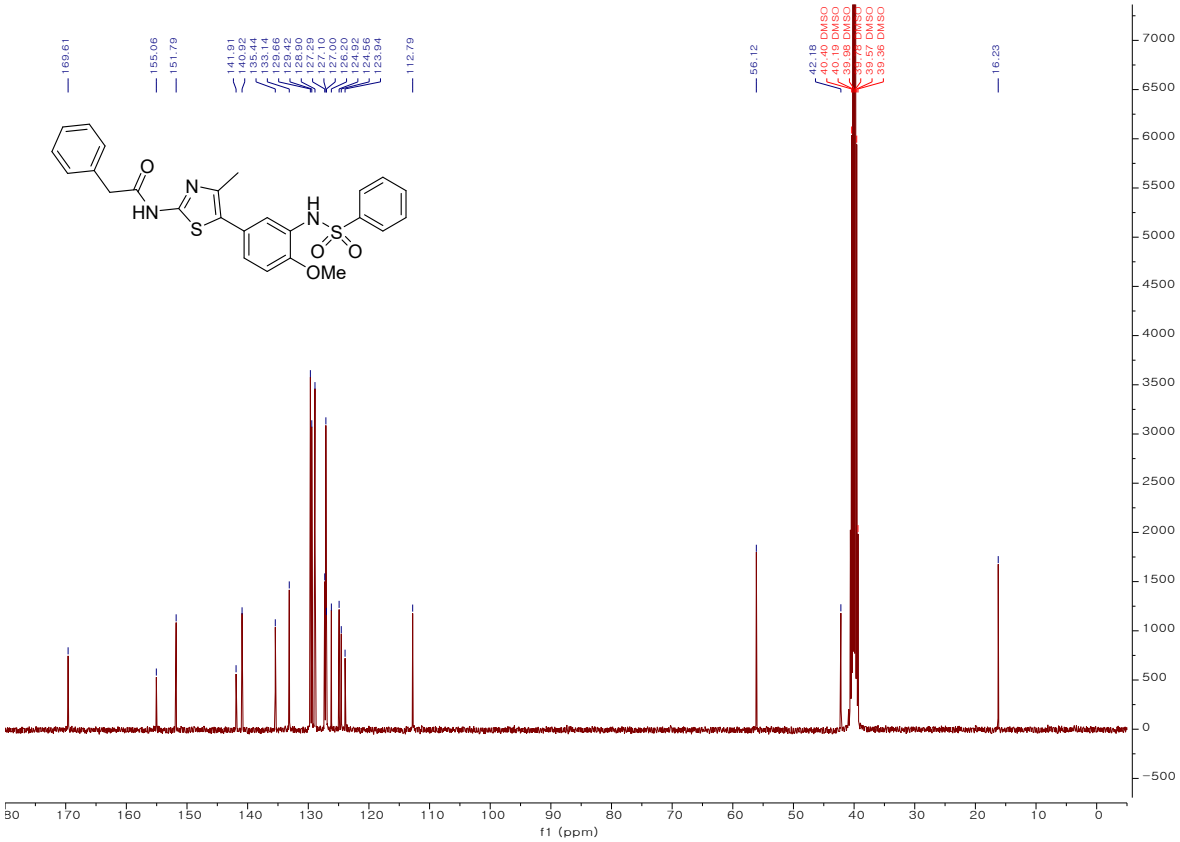
13C NMR spectrum of **5l** (**KR-27358**)



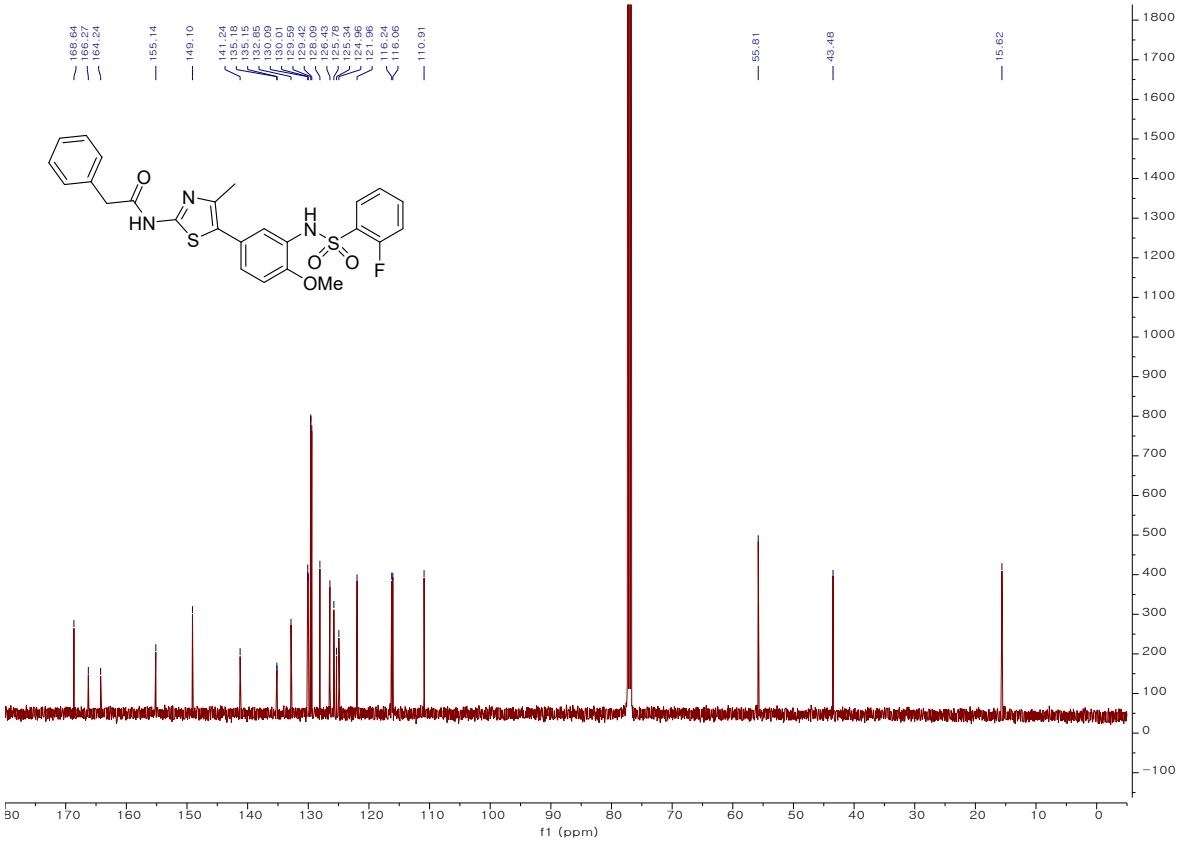
13C NMR spectrum of **6** (**KR-27335**)



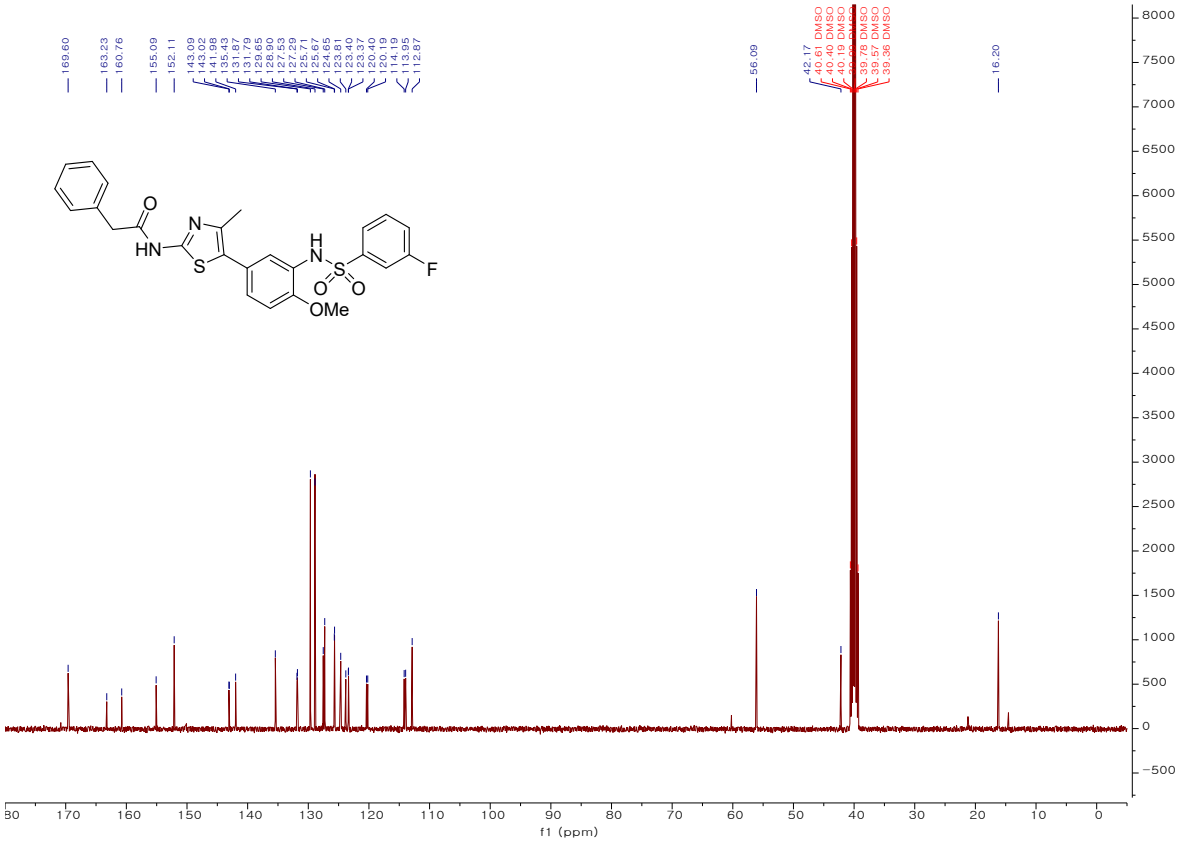
13C NMR spectrum of **7a** (**KR-27336**)



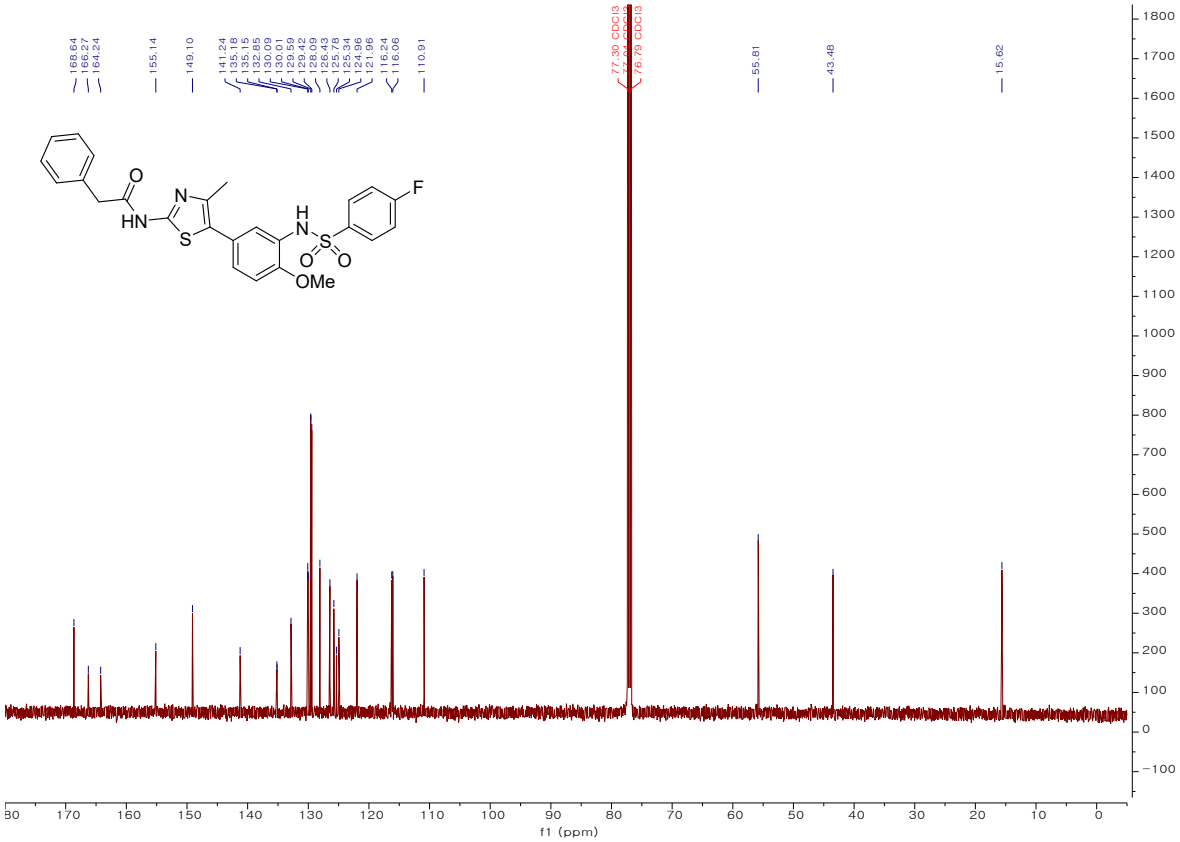
13C NMR spectrum of **7b** (**KR-27376**)



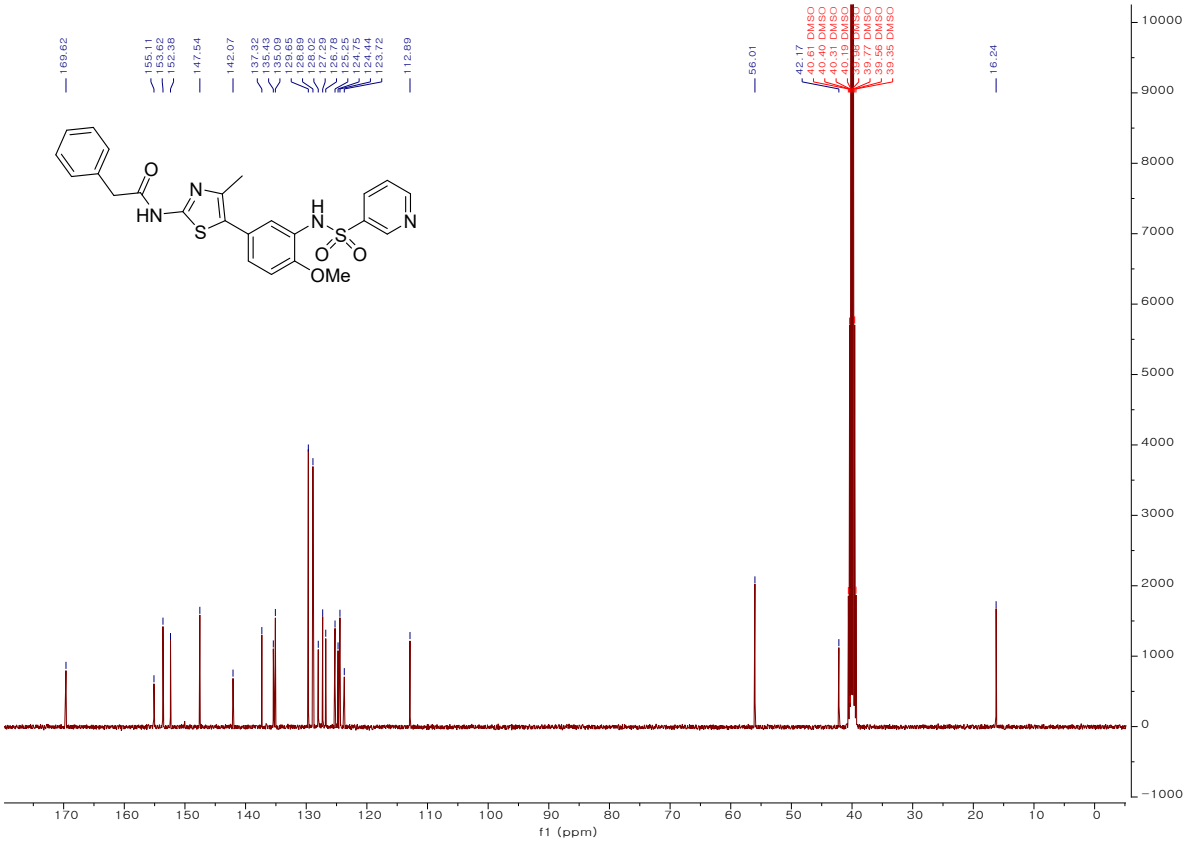
13C NMR spectrum of **7c** (**KR-27377**)



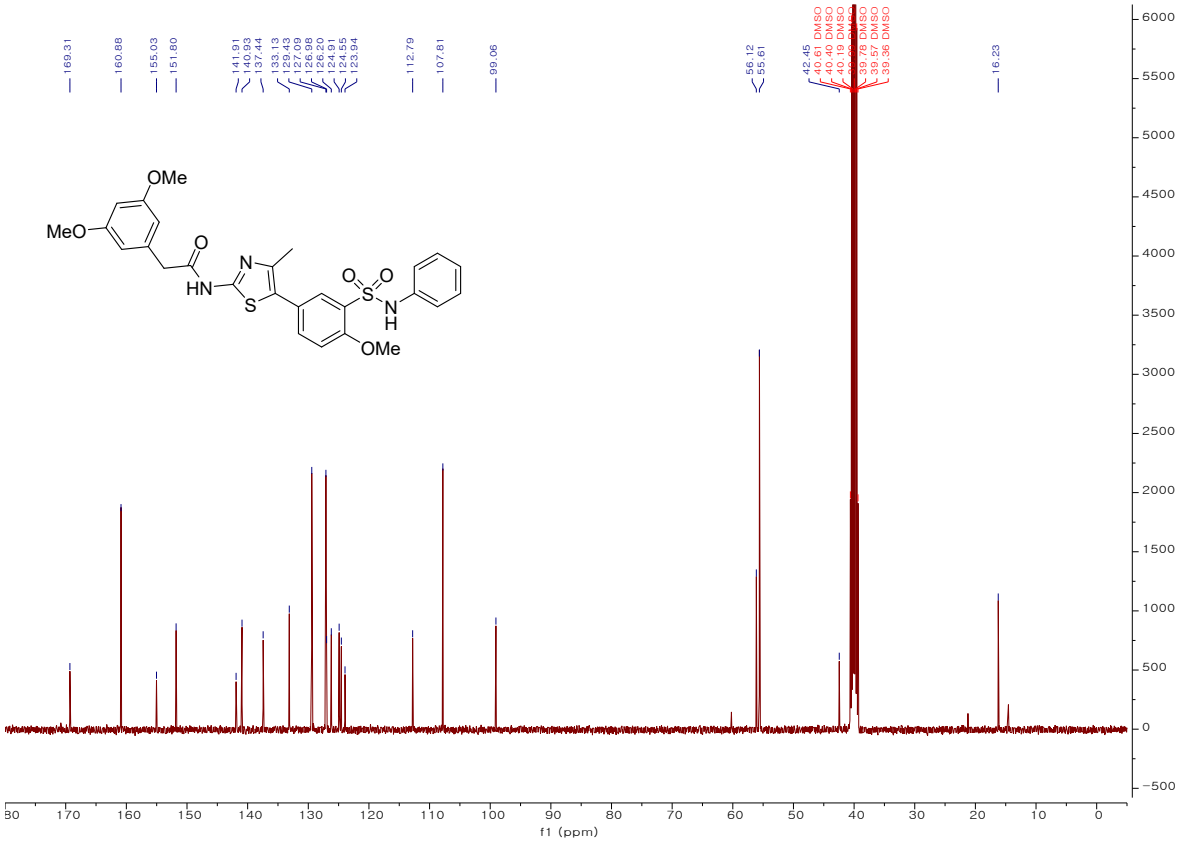
13C NMR spectrum of **7d** (**KR-27374**)



13C NMR spectrum of **7e** (**KR-27375**)

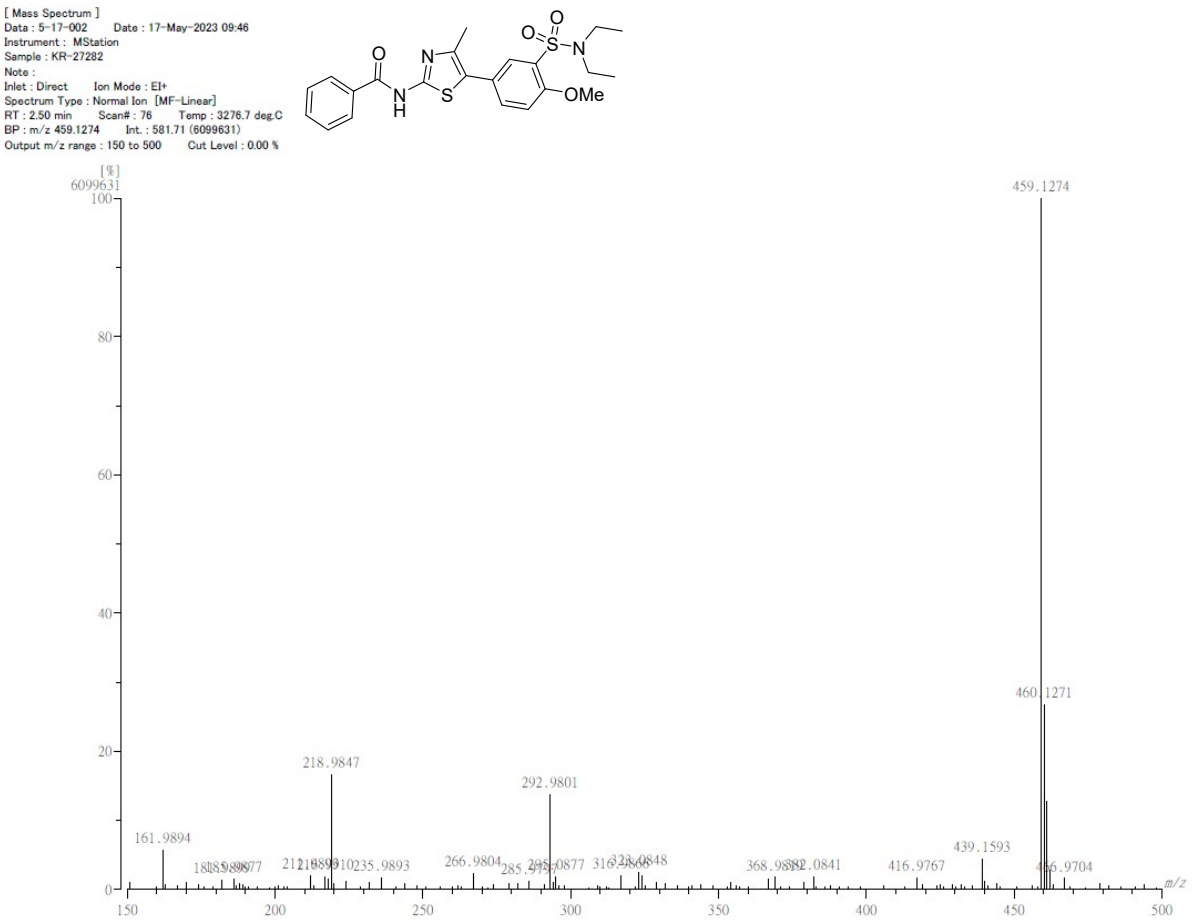


13C NMR spectrum of **7f** (**KR-27370**)

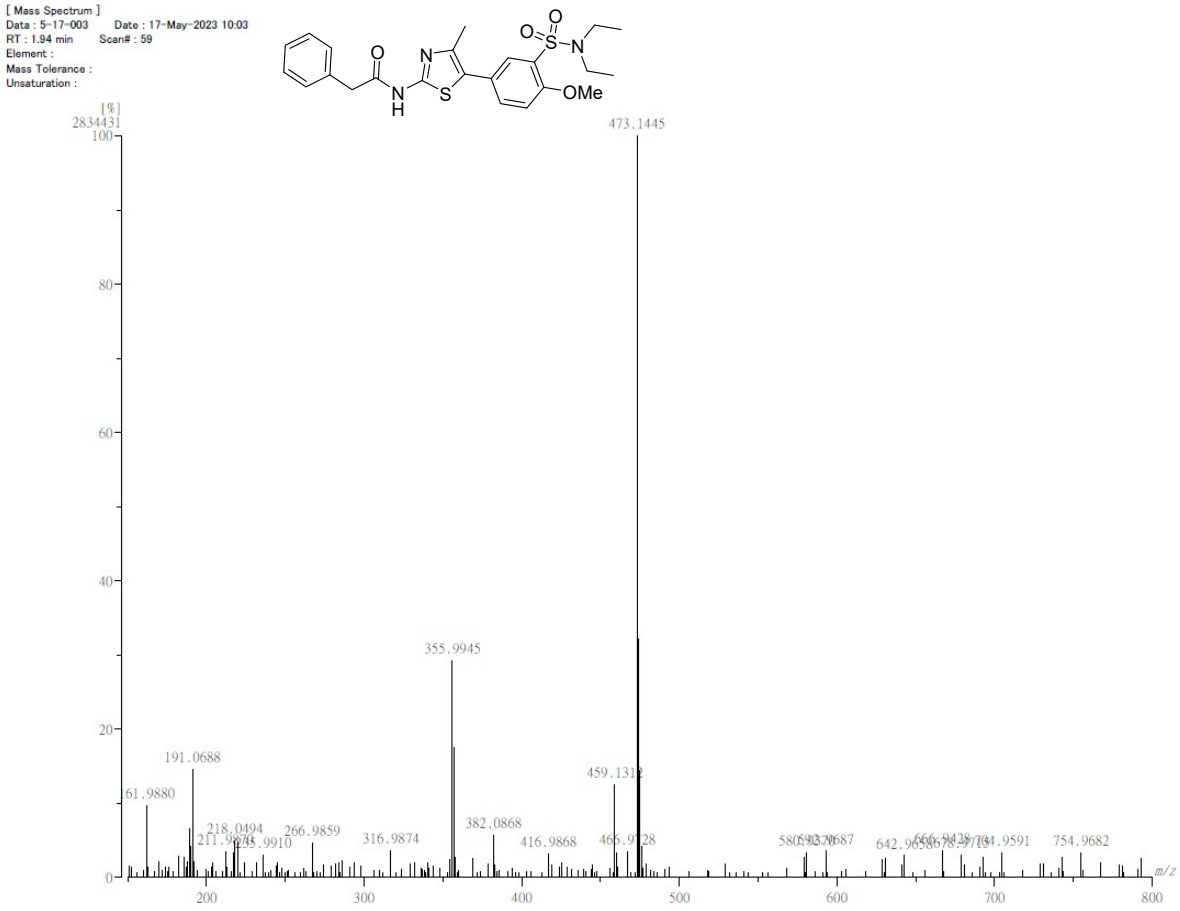


# S3. Spectral Copies of HRMS of Compounds

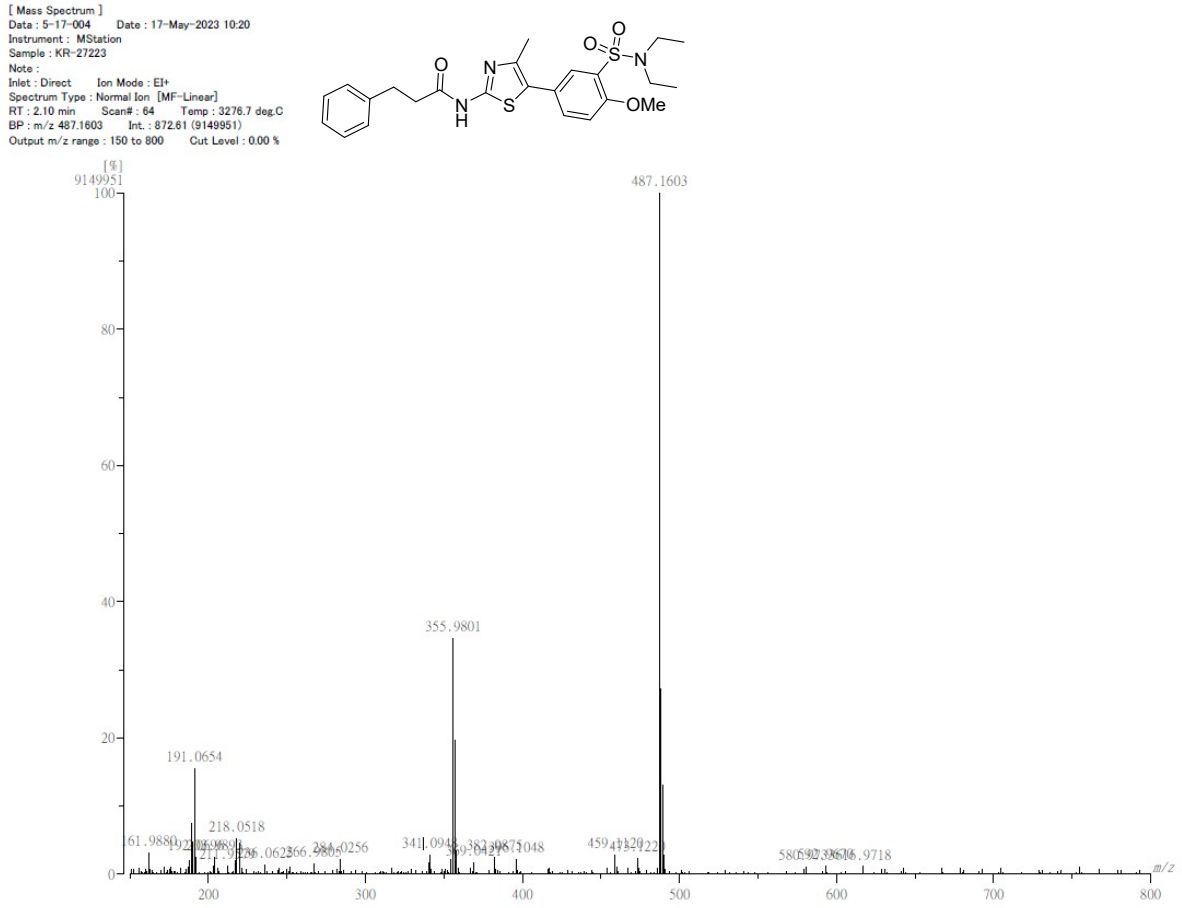
MS spectrum of **2** (**KR-27282**)



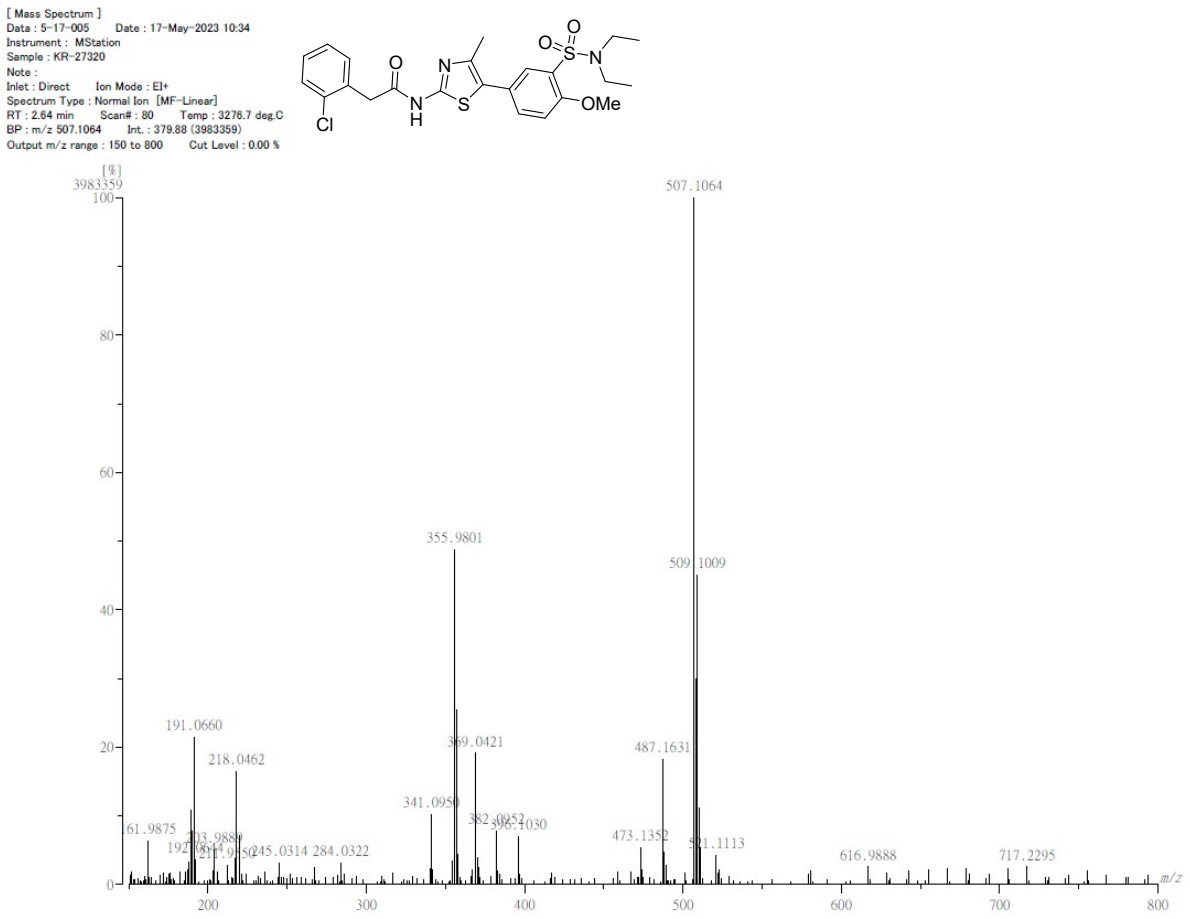
MS spectrum of **3** (**KR-27222**)



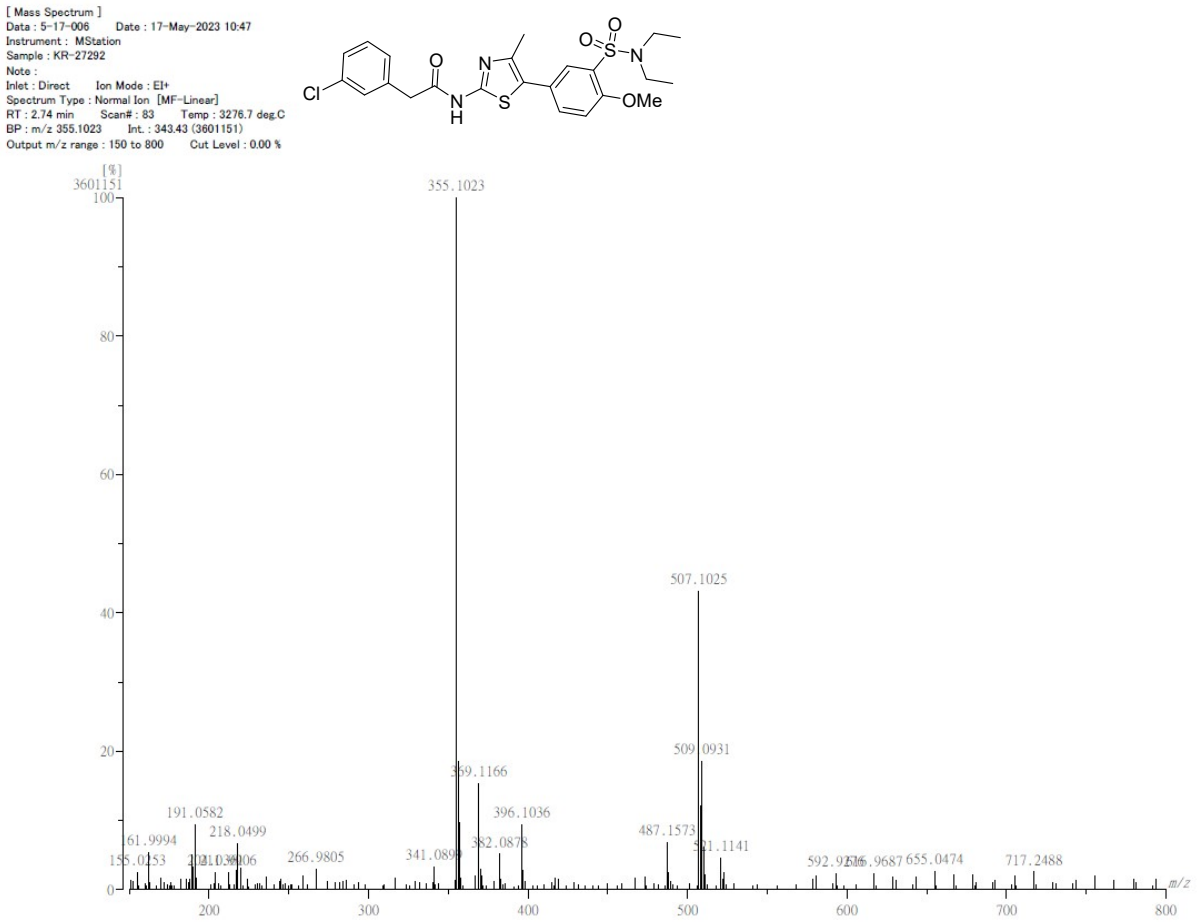
MS spectrum of **4** (**KR-27223**)



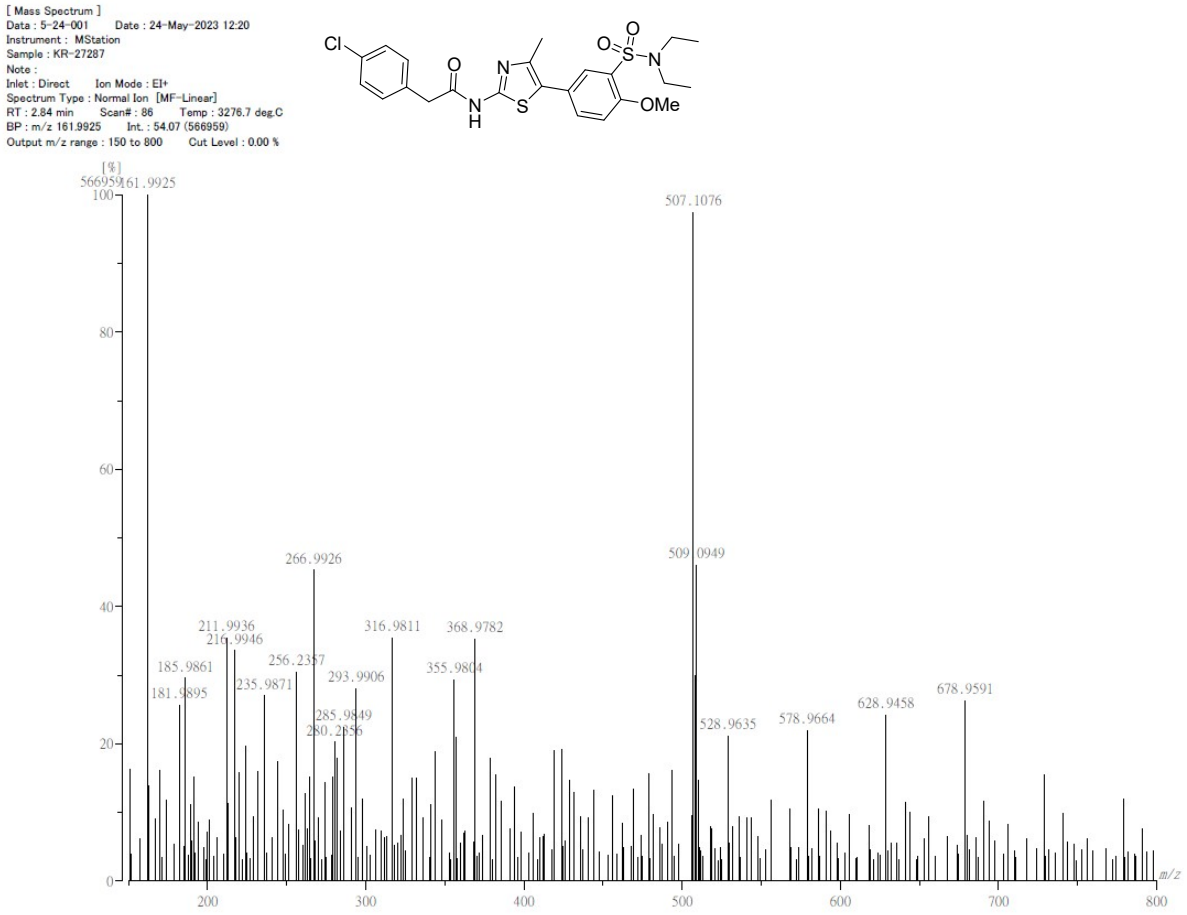
MS spectrum of **5a** (**KR-27320**)



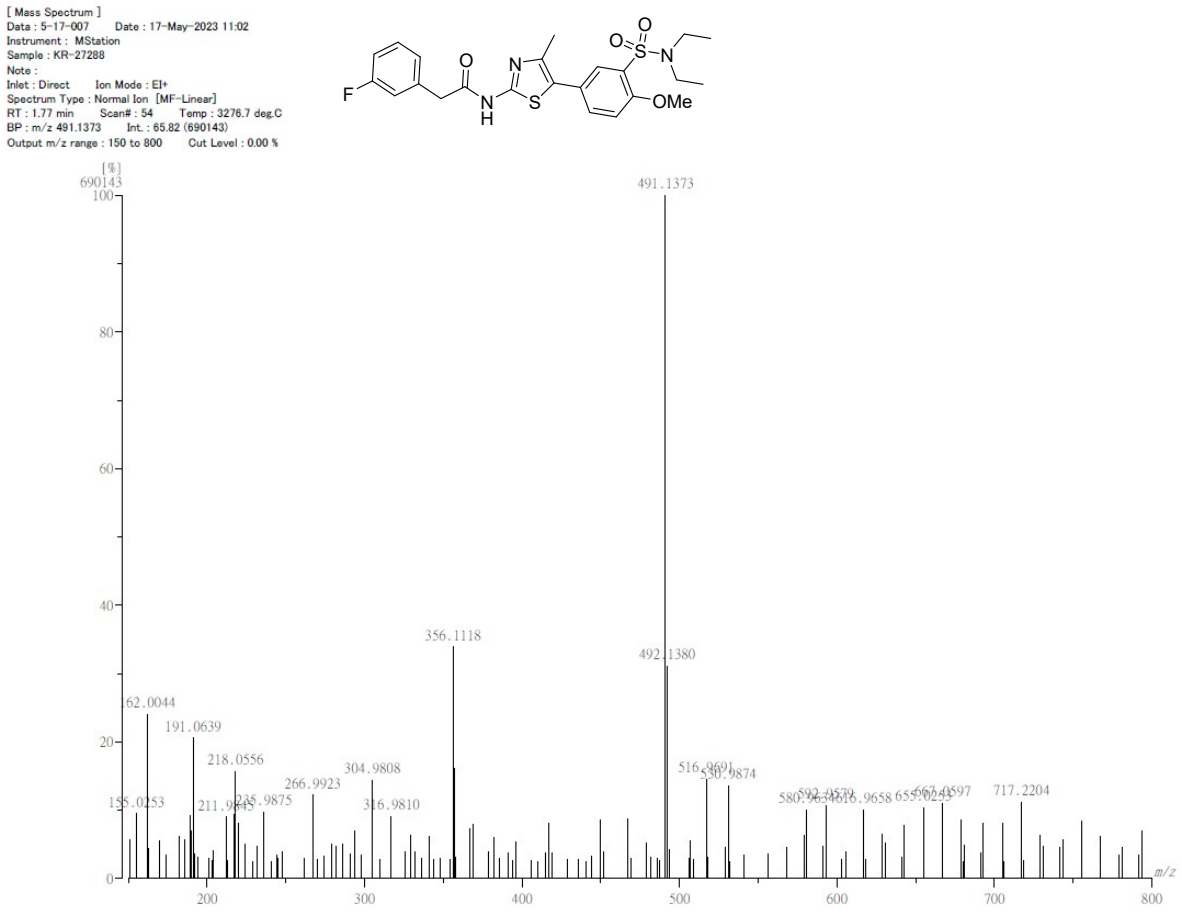
MS spectrum of **5b** (**KR-27287**)



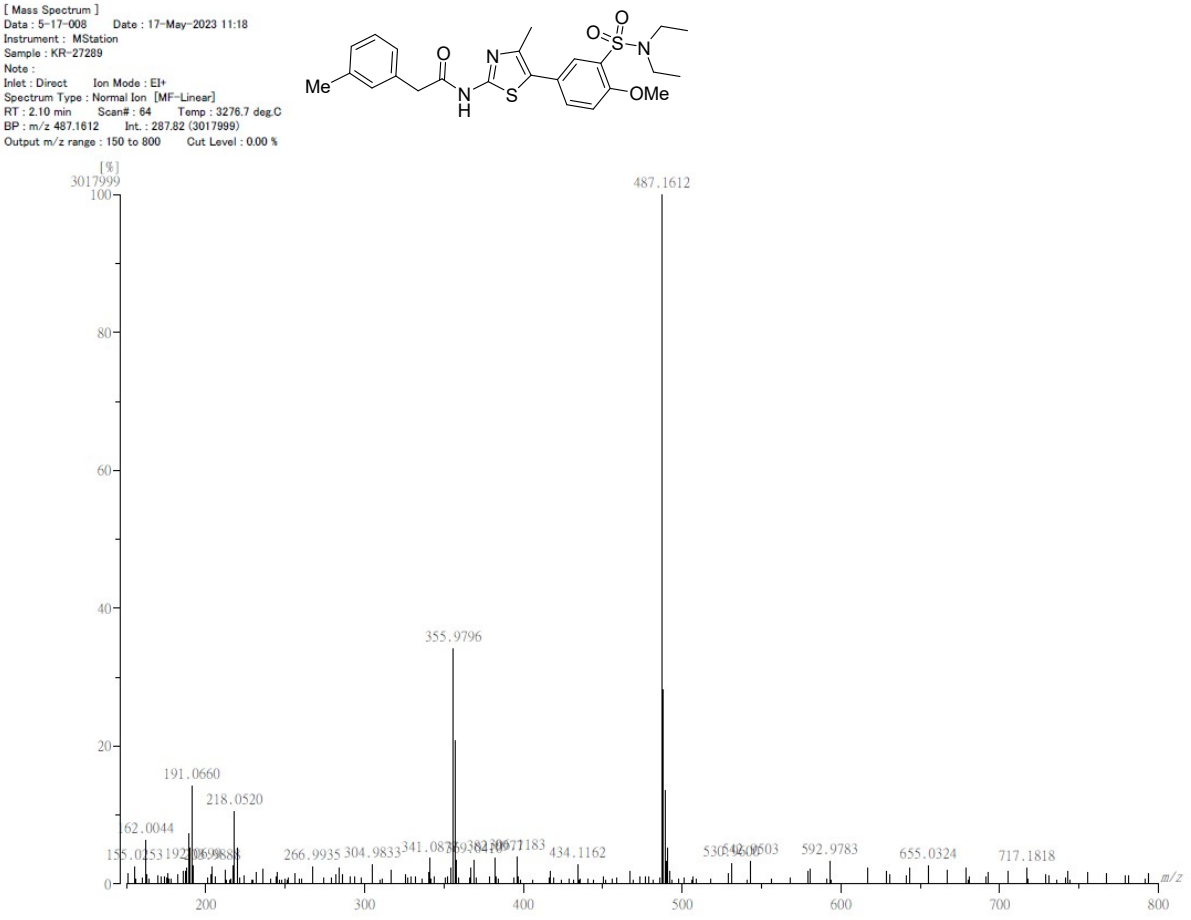
MS spectrum of **5c** (**KR-27292**)



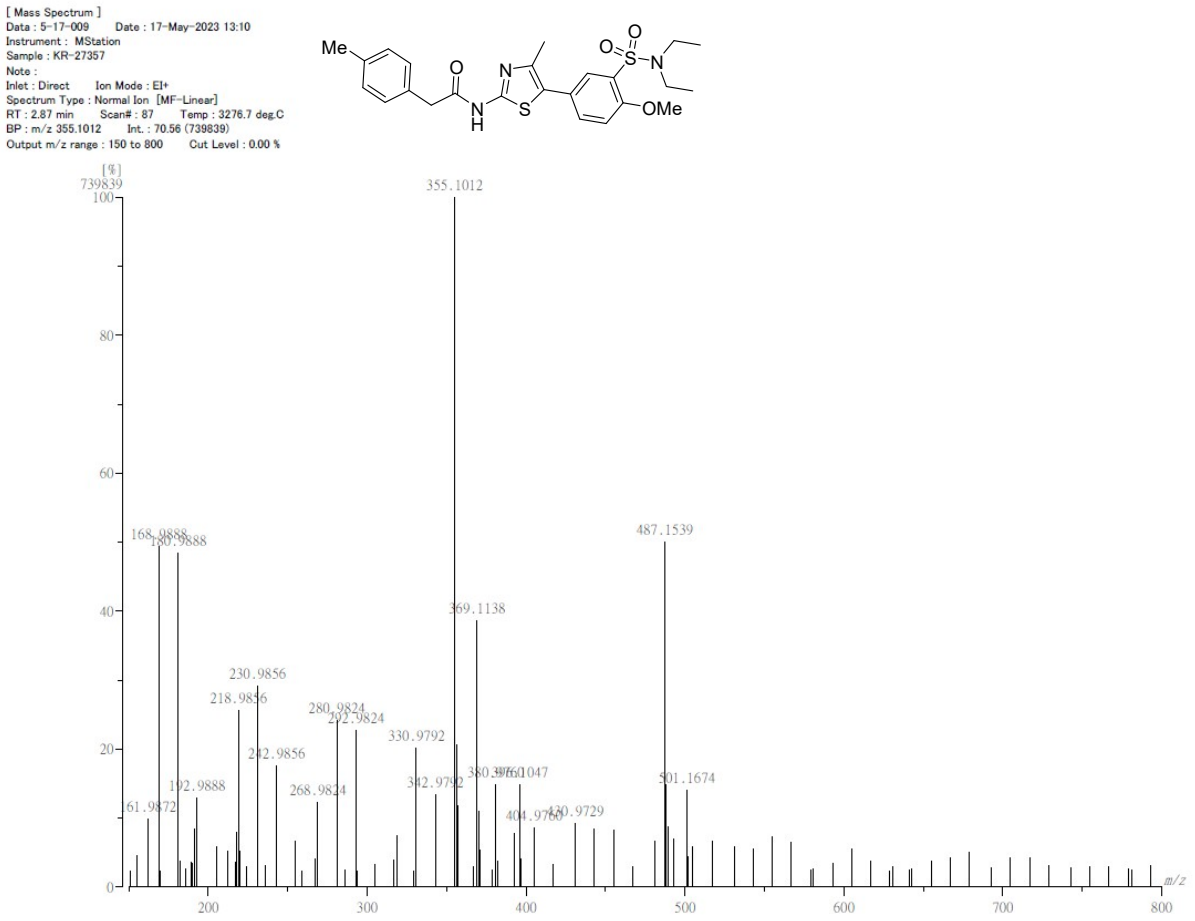
MS spectrum of **5d** (**KR-27288**)



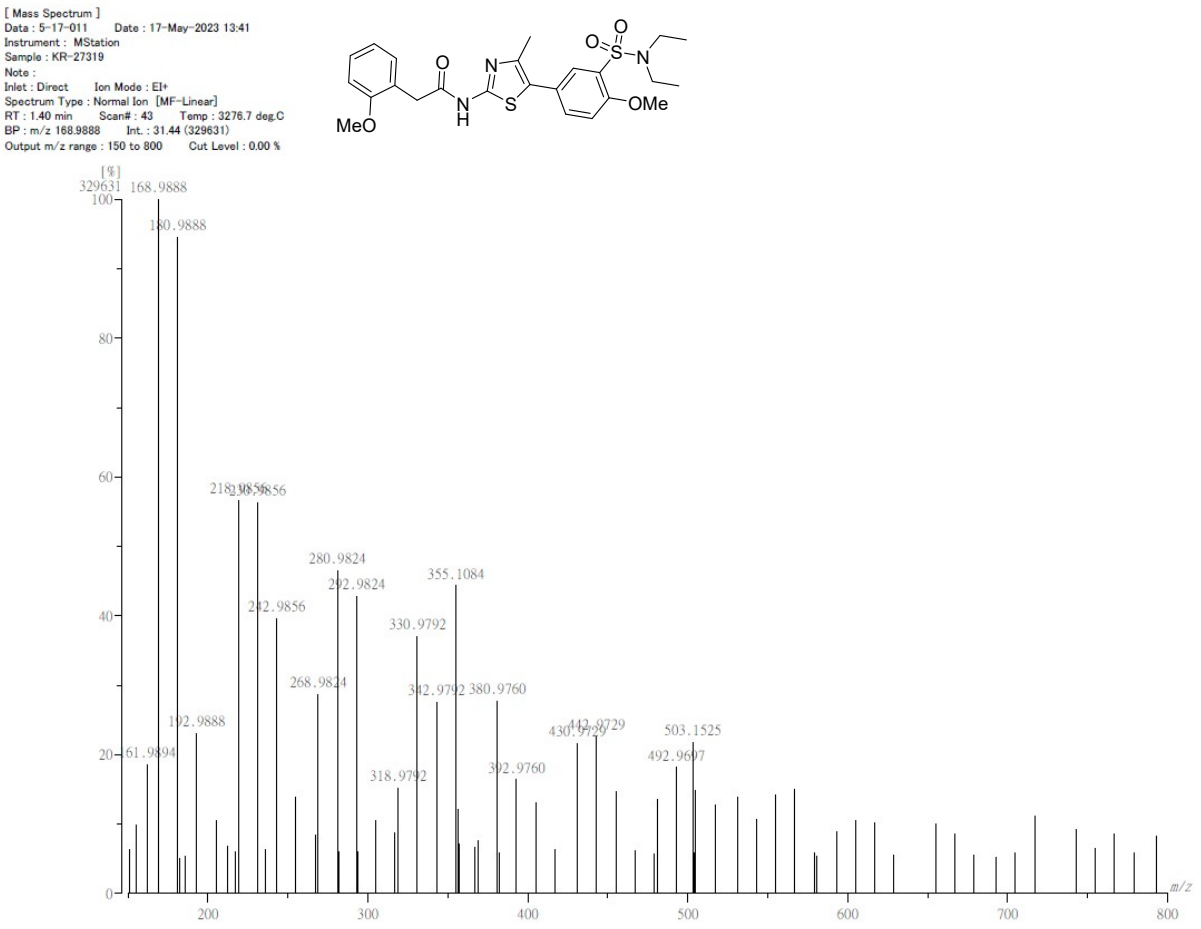
MS spectrum of **5e** (**KR-27289**)



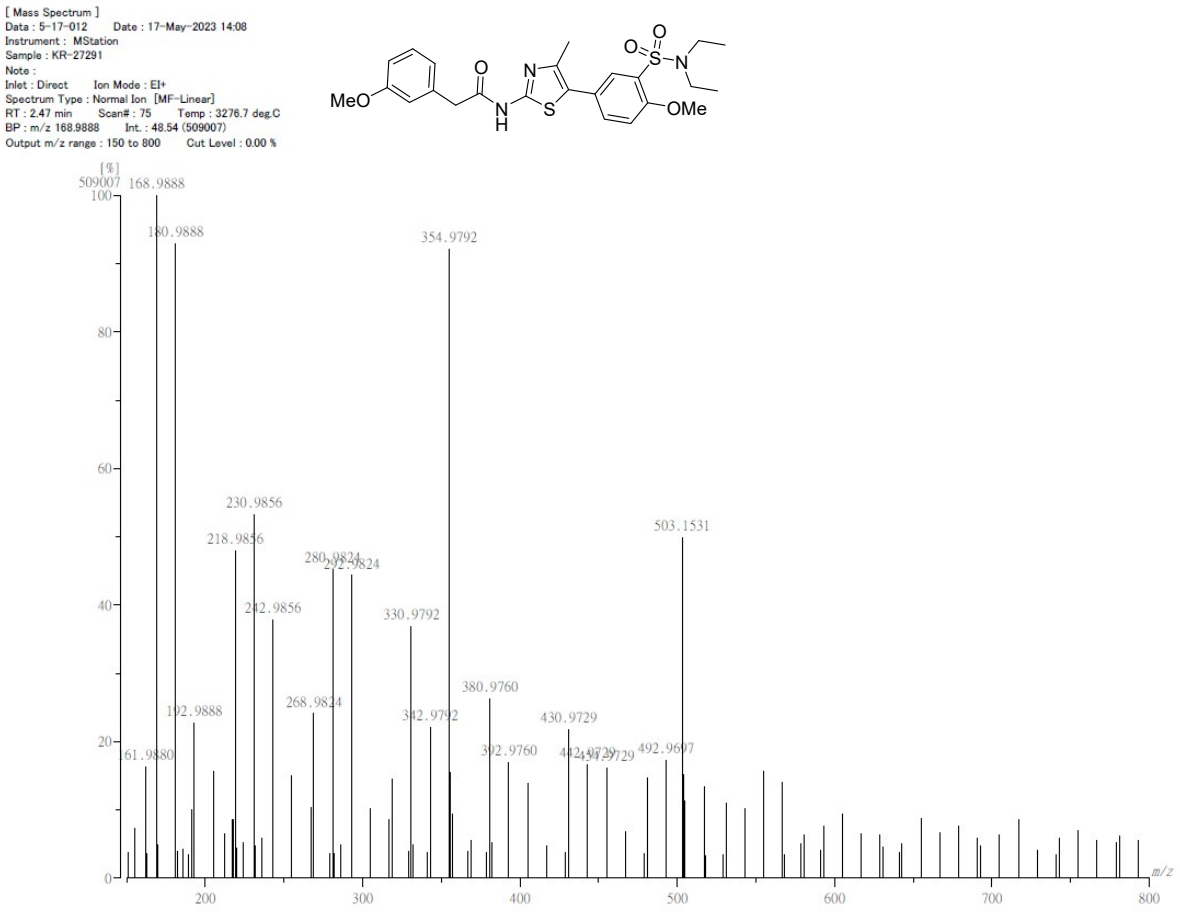
MS spectrum of **5f** (**KR-27357**)



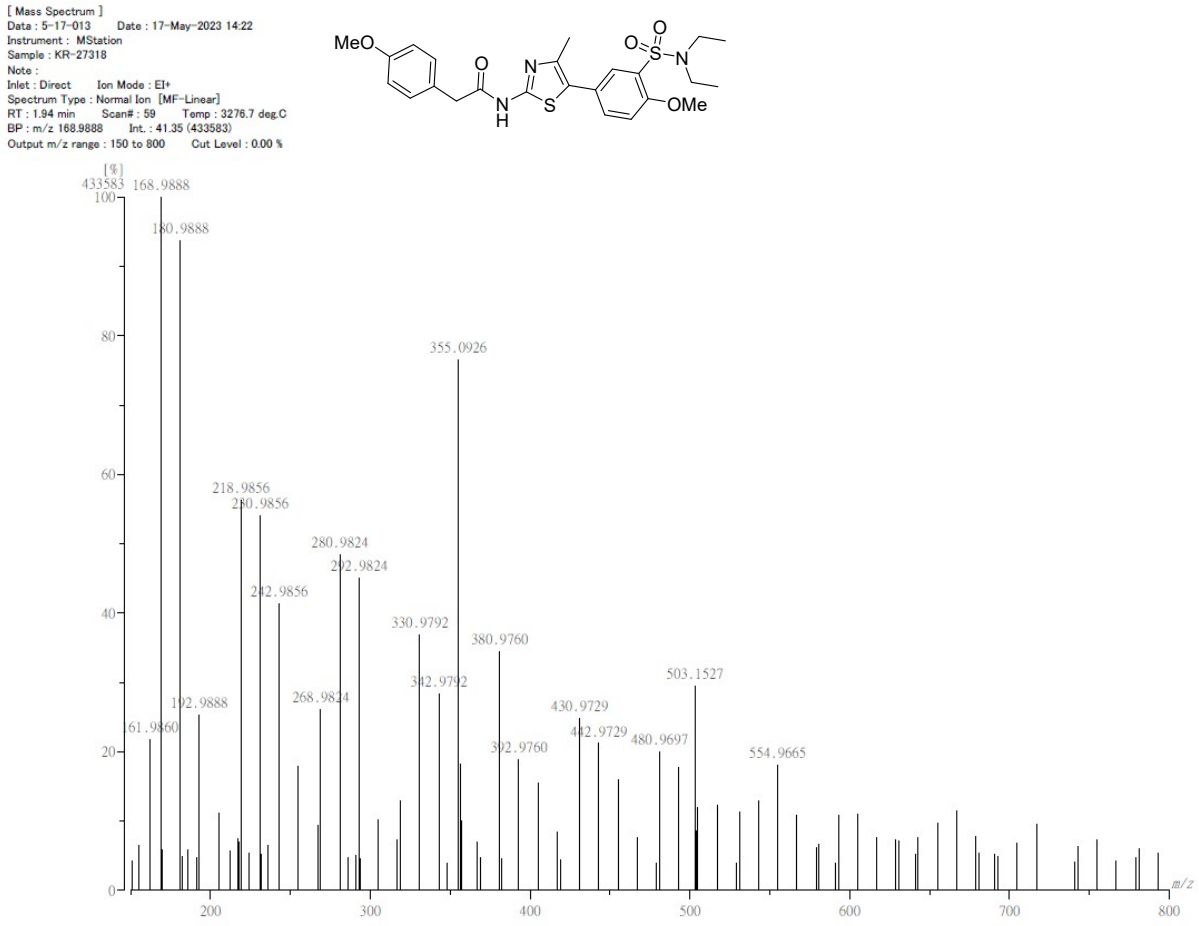
MS spectrum of **5g** (**KR-27319**)



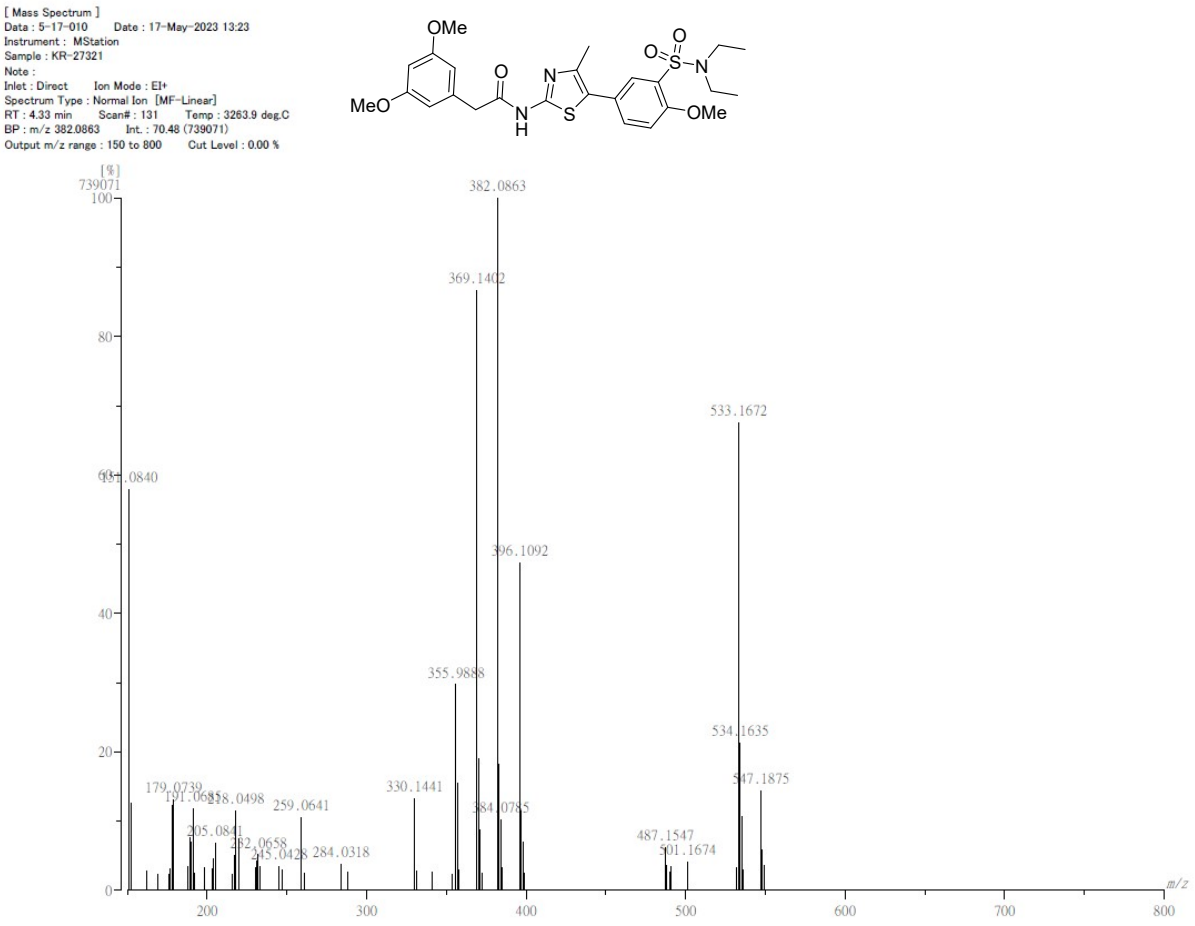
MS spectrum of **5h** (**KR-27291**)



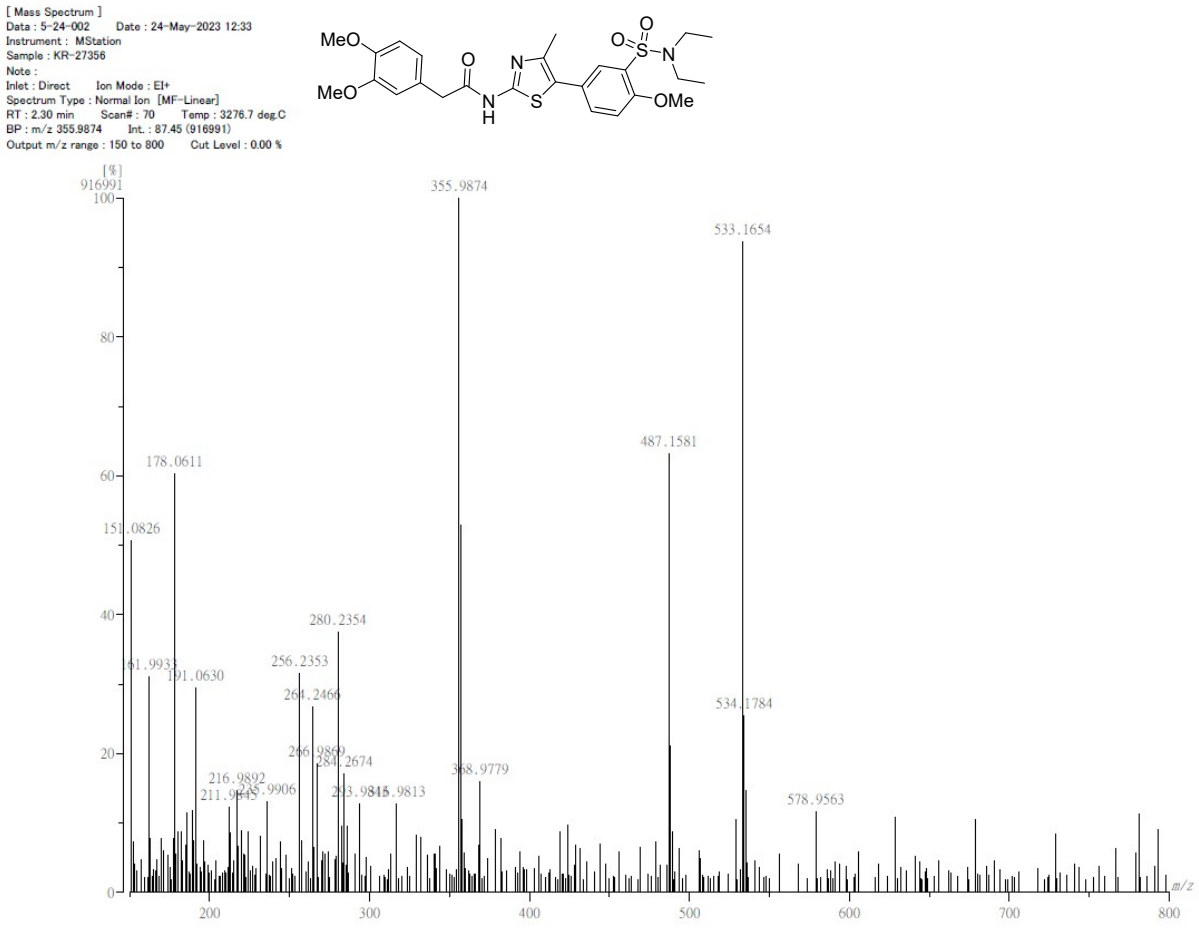
MS spectrum of **5i** (**KR-27318**)



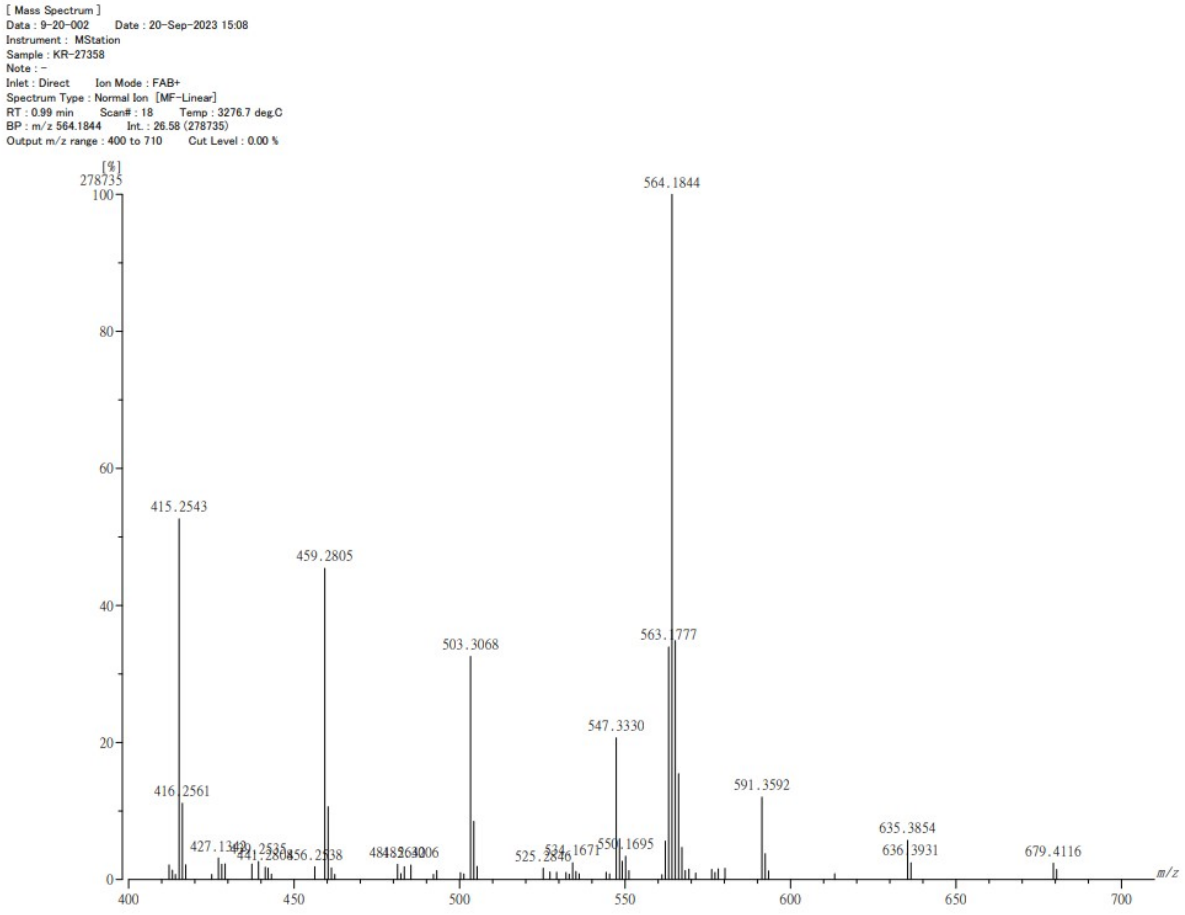
MS spectrum of **5j** (**KR-27321**)



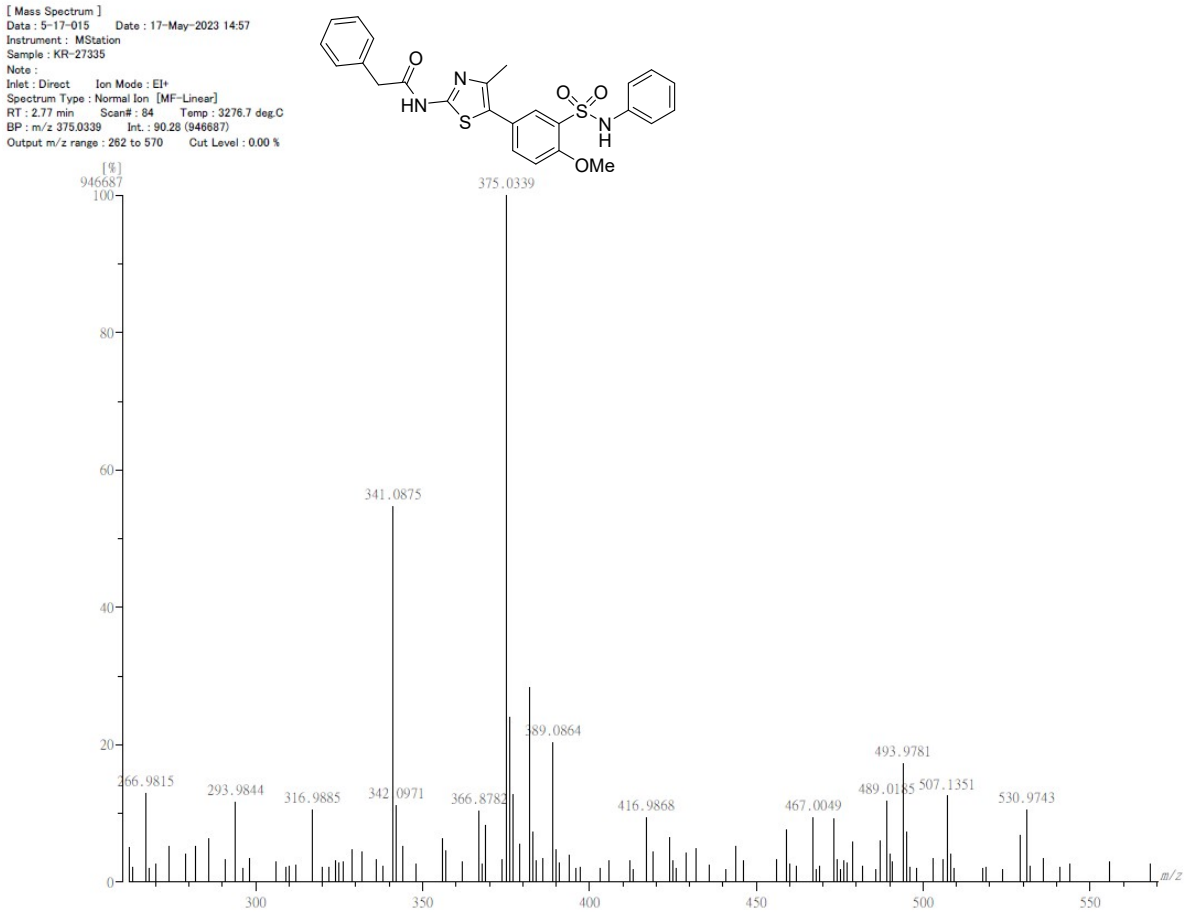
MS spectrum of **5k** (**KR-27356**)



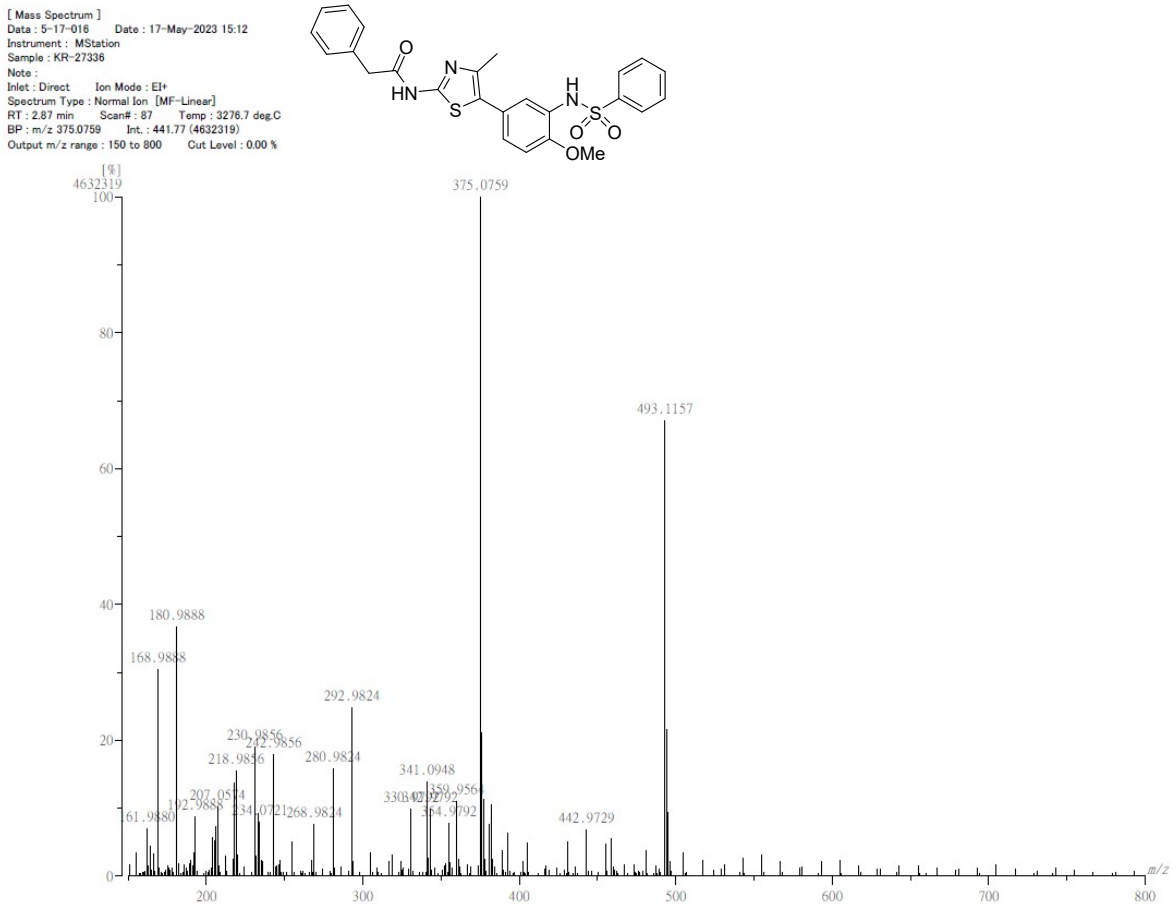
MS spectrum of **5l** (**KR-27358**)



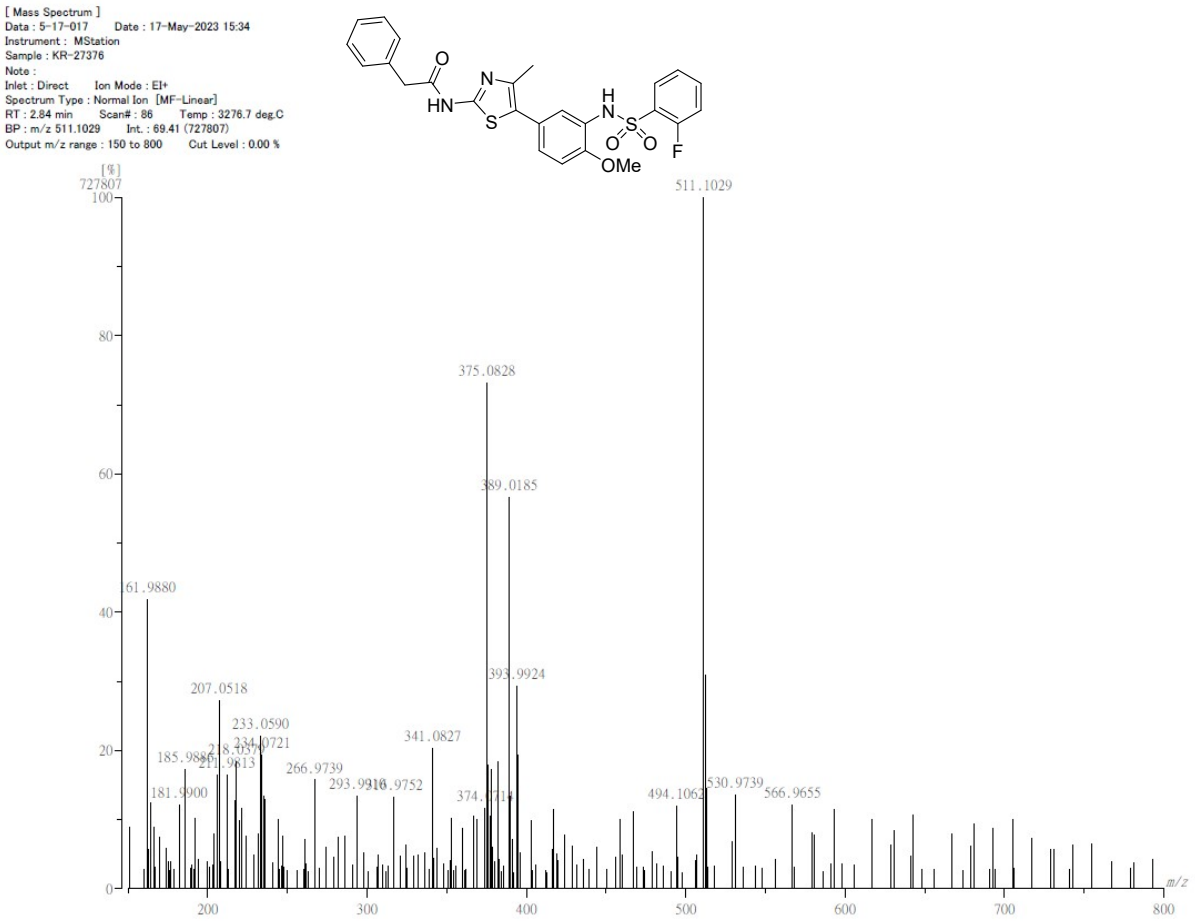
MS spectrum of **6** (**KR-27335**)



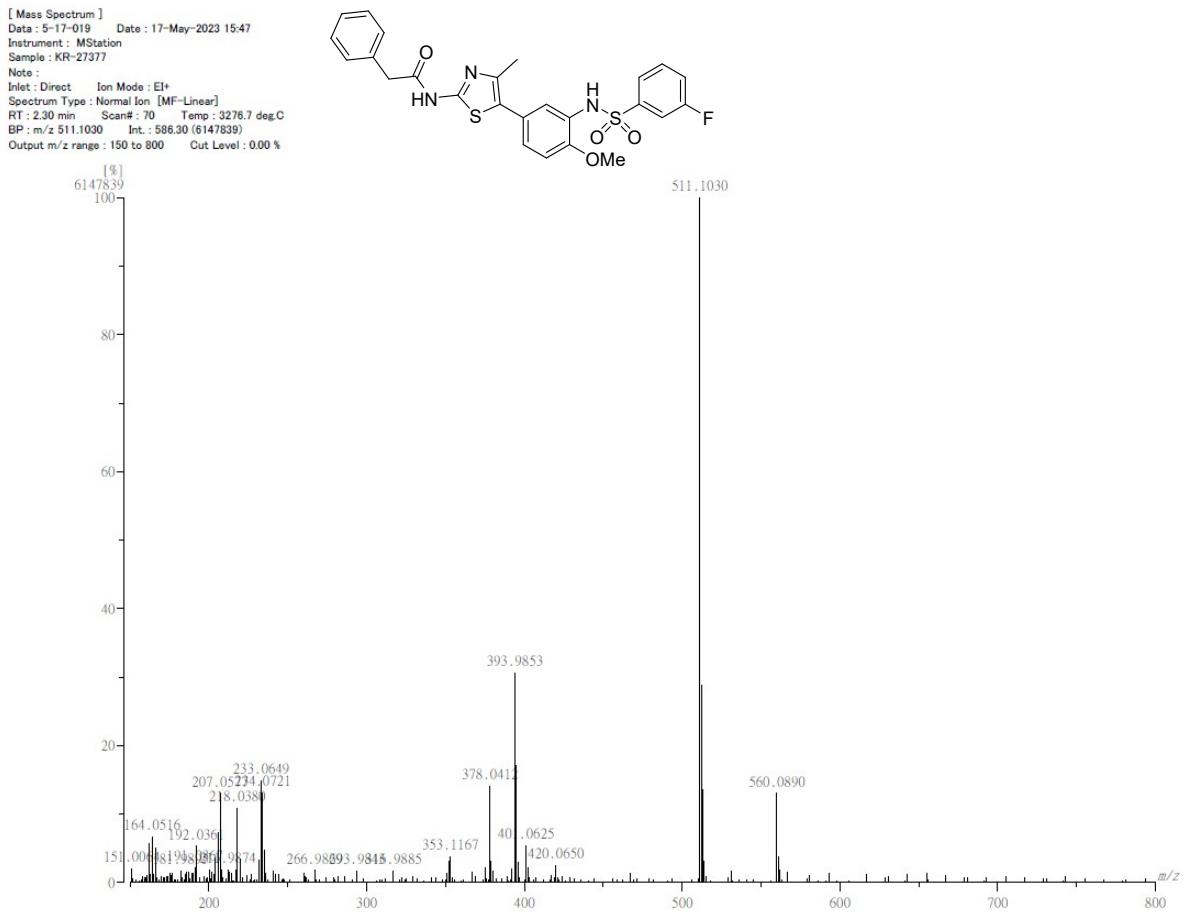
MS spectrum of **7a** (**KR-27336**)



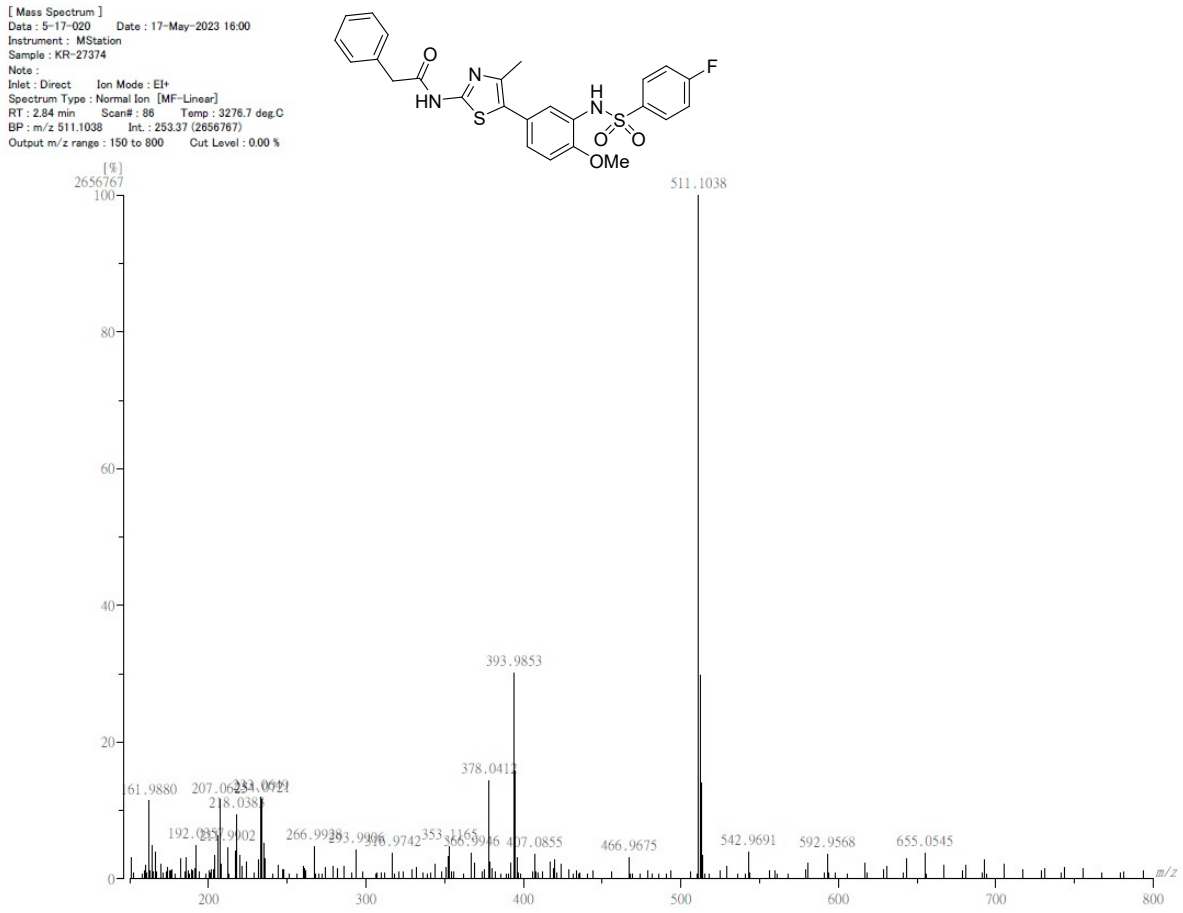
MS spectrum of **7b** (**KR-27376**)



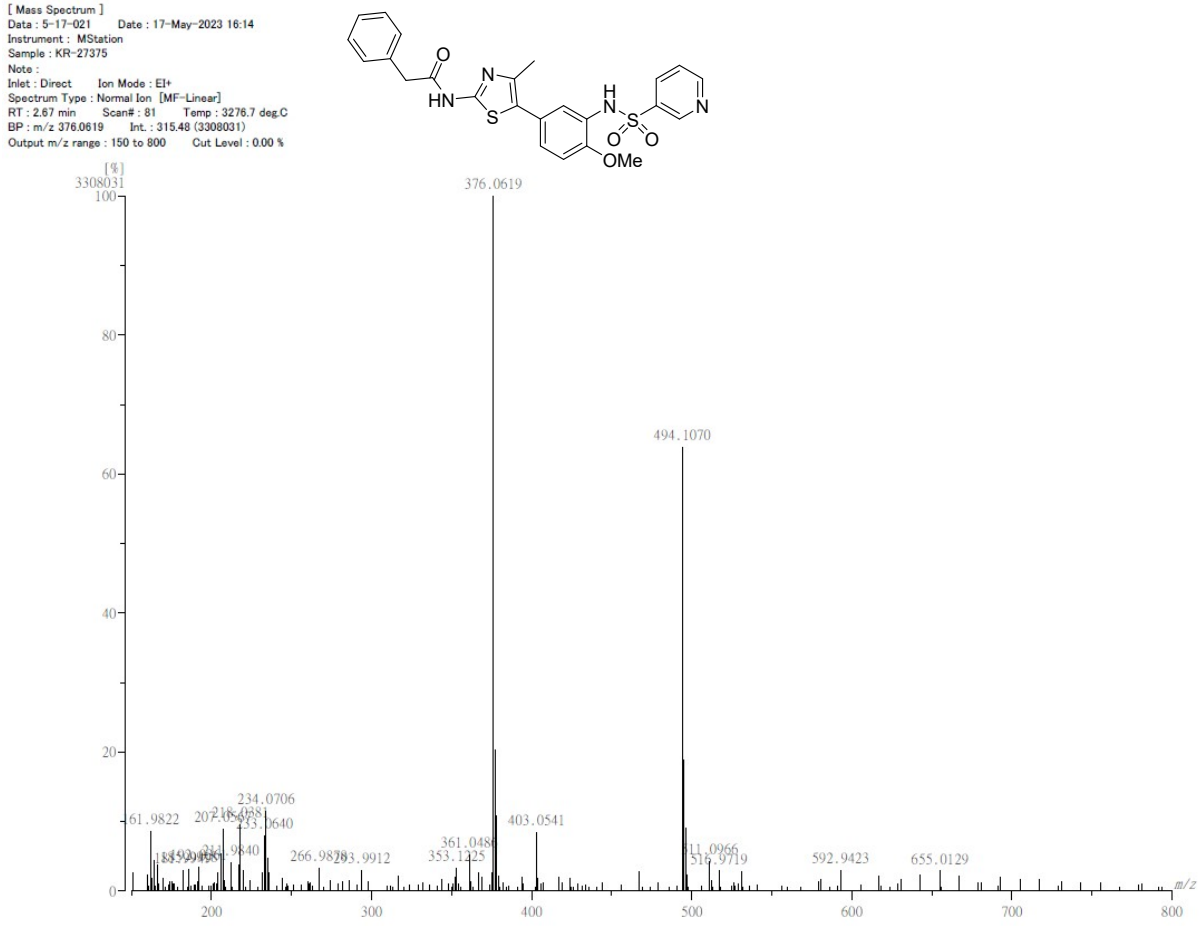
MS spectrum of **7c** (**KR-27377**)



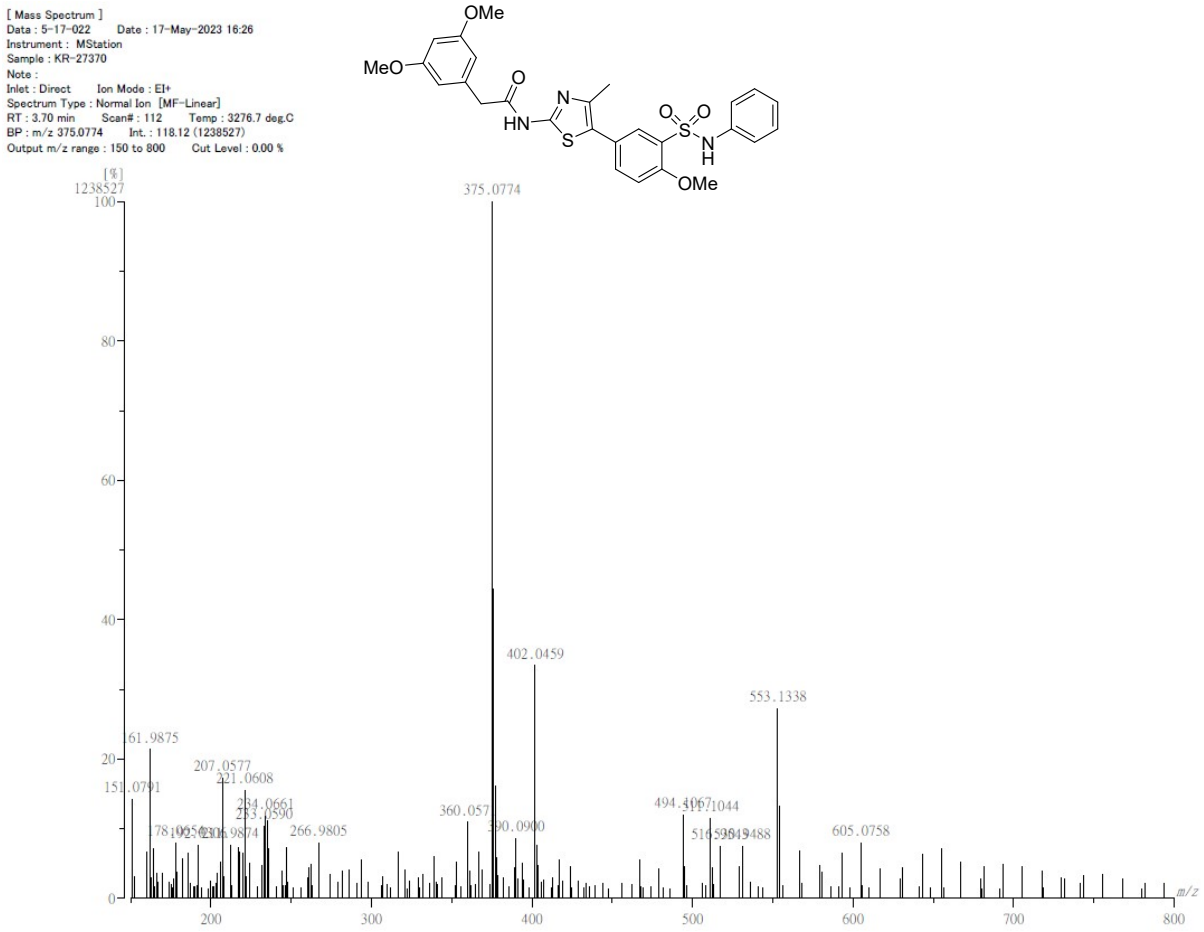
MS spectrum of **7d** (**KR-27374**)



MS spectrum of **7e** (**KR-27375**)



MS spectrum of **7f** (**KR-27370**)



## S4. Antiviral activity of 7f against coronavirus[a]

### Table.

| Virus | Cells | $CC_{50}$ (μM)[b] | $EC_{50}$ (μM)[c] | SI[d] |
|---|---|---|---|---|
| Alpha coronavirus | | | | |
| HCoV-229E | MRC5 | 5.8 | >5.8 | - |
| HCoV-NL63 | LLC-MK2 | >100 | >100 | - |
| FIPv | CRFK | >100 | >100 | - |
| Beta Coronavirus | | | | |
| HCoV-OC43 | MRC5 | 5.8 | >5.8 | - |
| SARS-CoV-2 | Vero | >100 | >100 | - |

[a]All data were obtained from at least two independent experiments, and the mean values ± standard deviations are listed. [b]$CC_{50}$: Cytotoxic concentration (μM) for 50% cell survived, measured by MTT assay. [c]$EC_{50}$: Effective concentration (μM) for 50% inhibition of each virus species, measured by MTT assay. [d]SI: Selectivity index calculated using $CC_{50}/EC_{50}$. [e]Not calculated because the $EC_{50}$ was higher than $CC_{50}$

## S5. Kinase assay

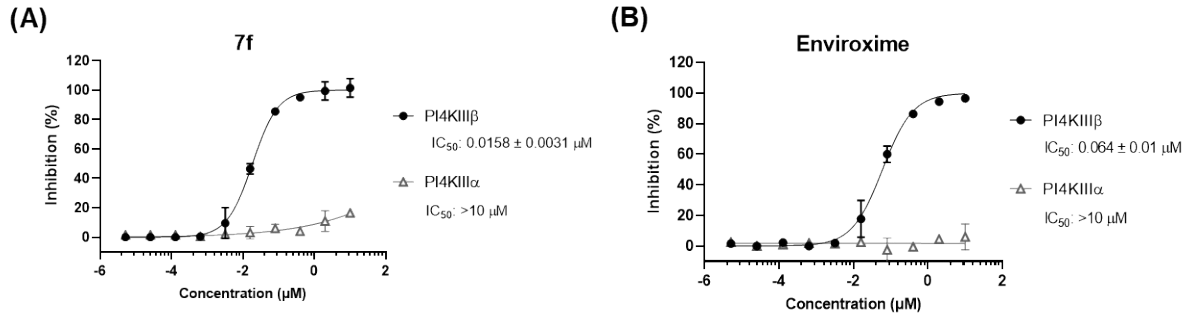
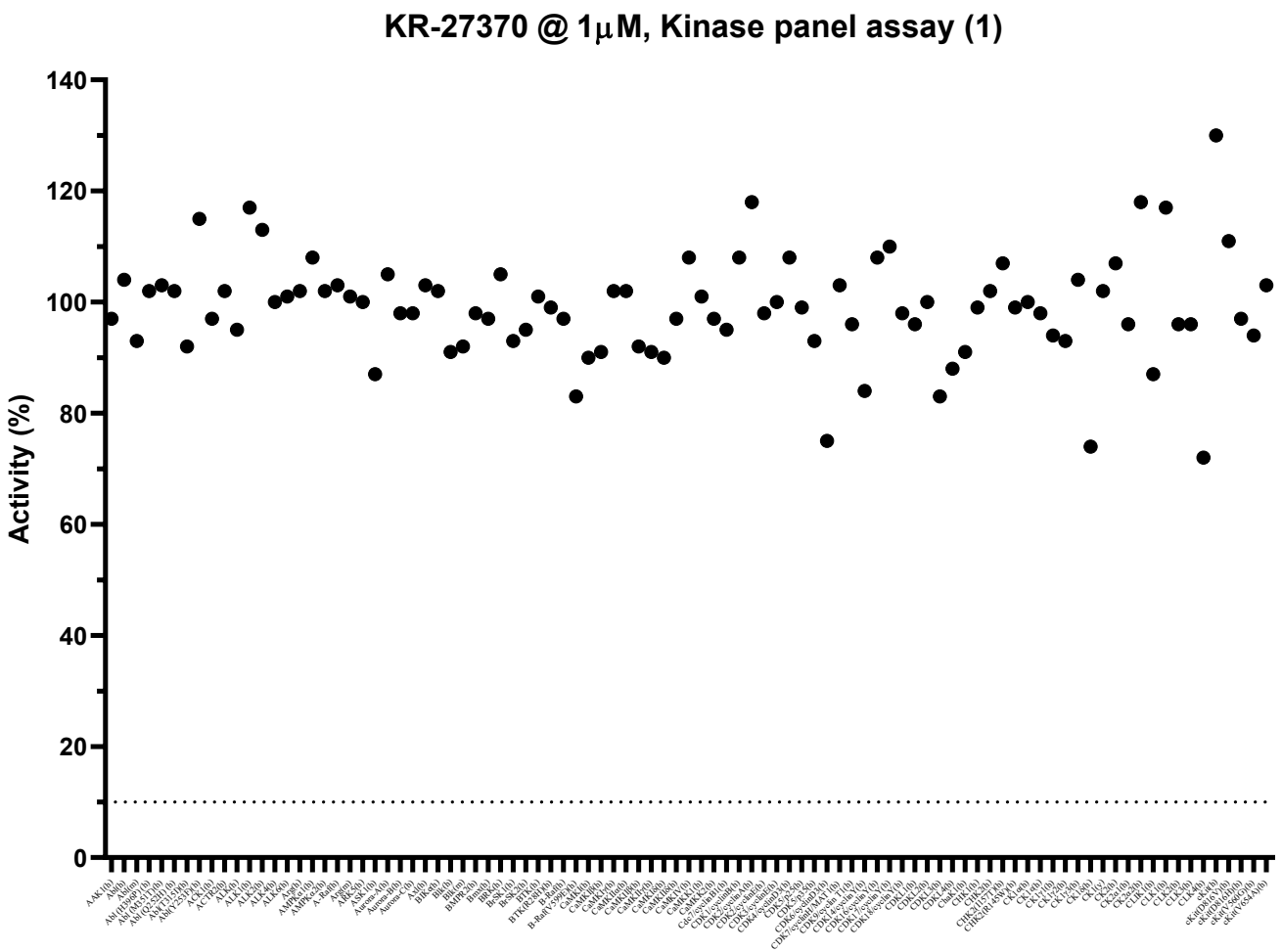
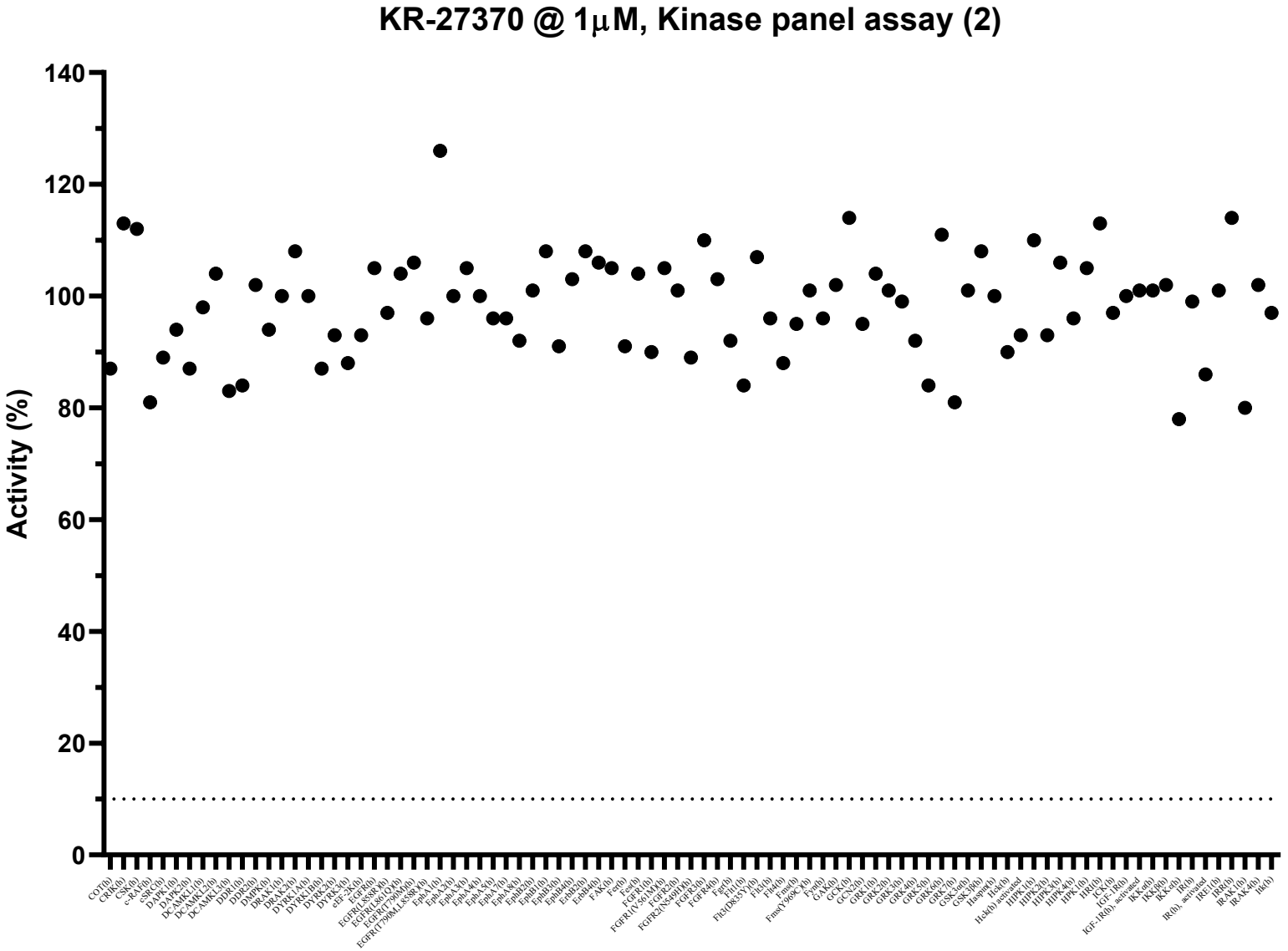


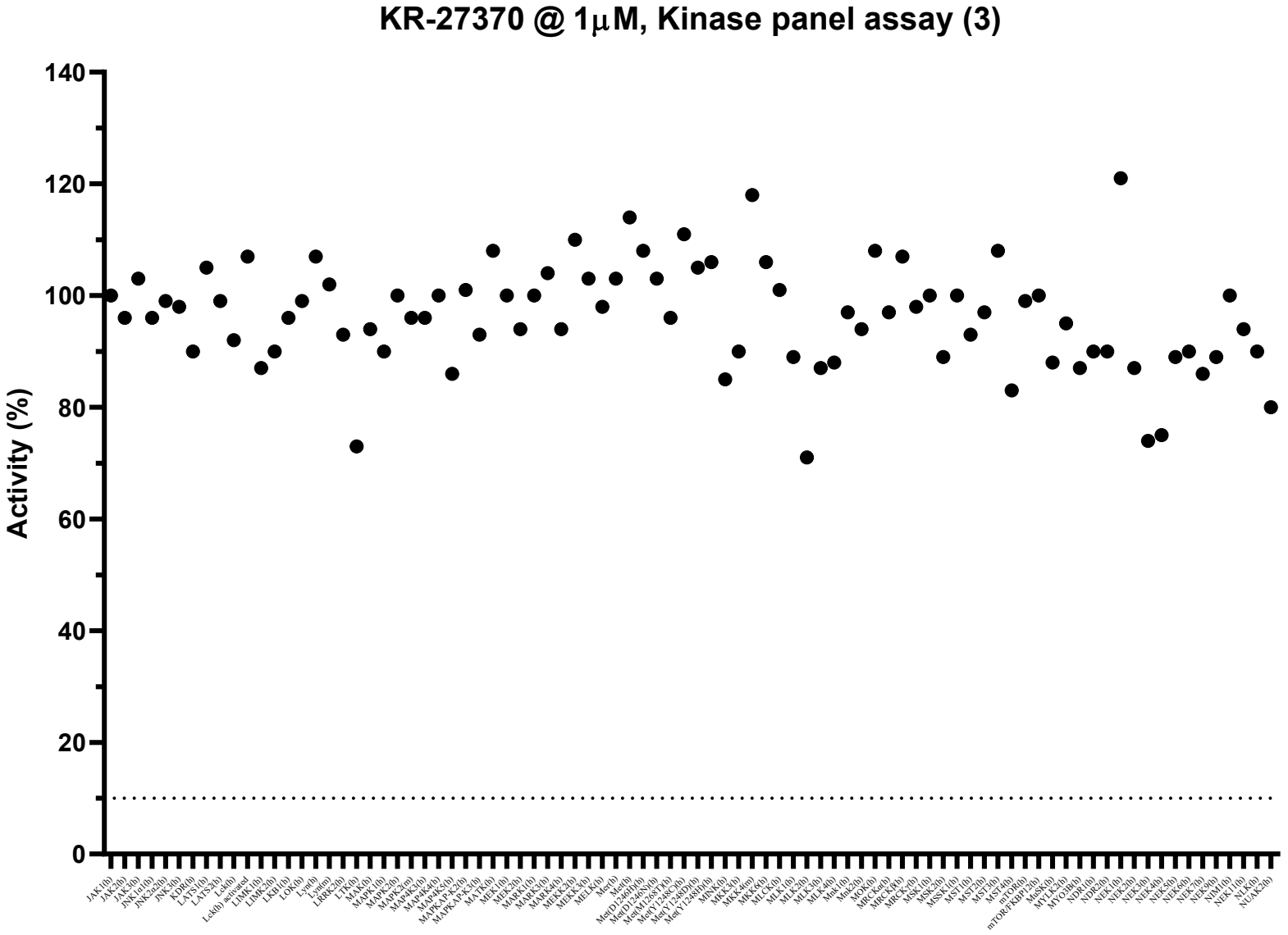

**Figure.** GST-PI4KIII α, β (Invitrogen, Waltham, MA, USA), and PI:PS lipid kinase substrate (Invitrogen) were diluted in Kinase buffer T (Invitrogen). The enzymatic activity of PI4Ks was determined using an ADP-Glo kinase assay kit. Dose-responses of PI4KIIIα (open triangle) and PI4KIIIβ (black circle) in the presence of serially diluted concentrations of **7f** (A) and enviroxime (B). Data represent means (±SD) of at least two dependent experiments performed in duplicate.

## S6. Kinase panel assay

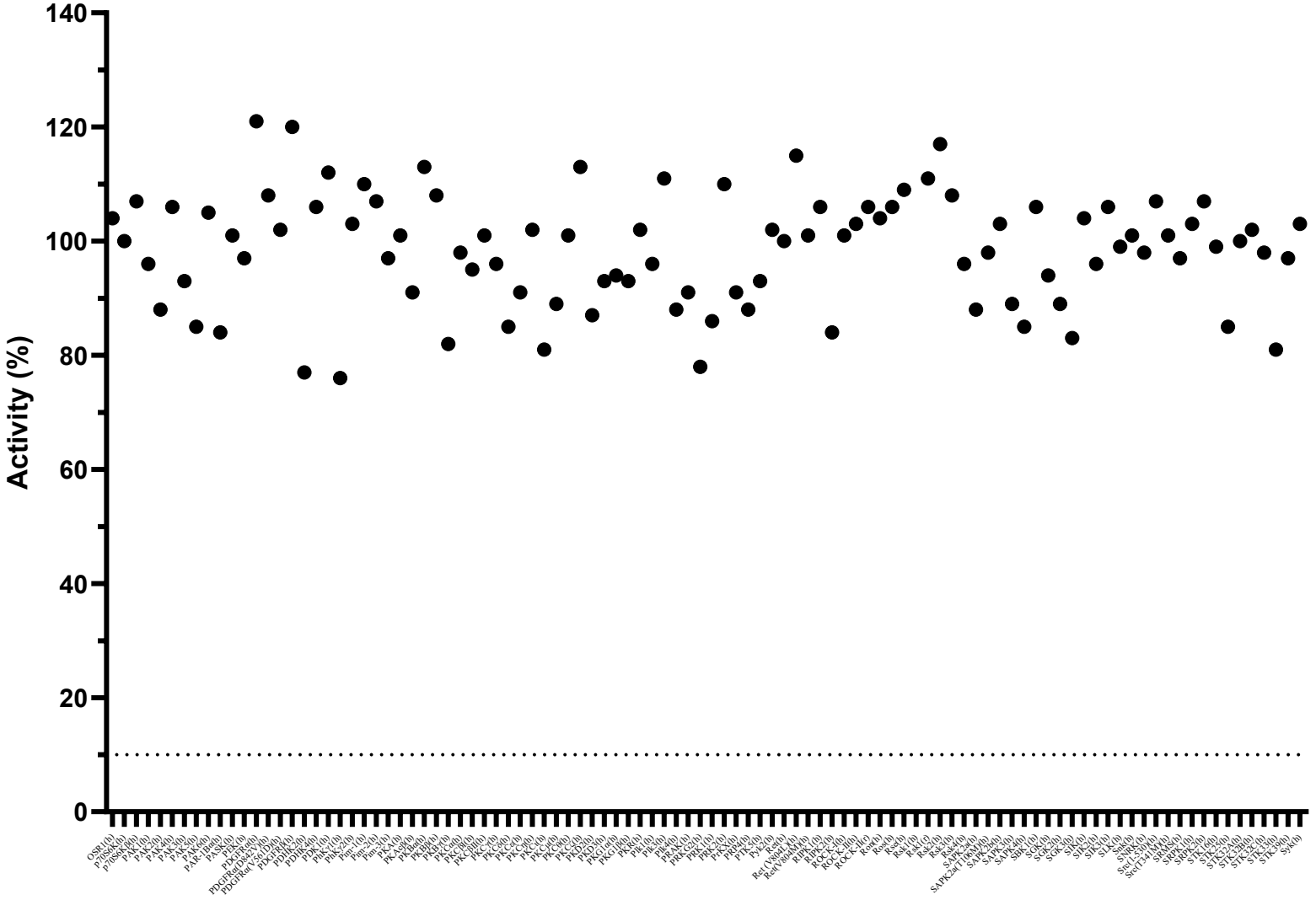
**(A)**

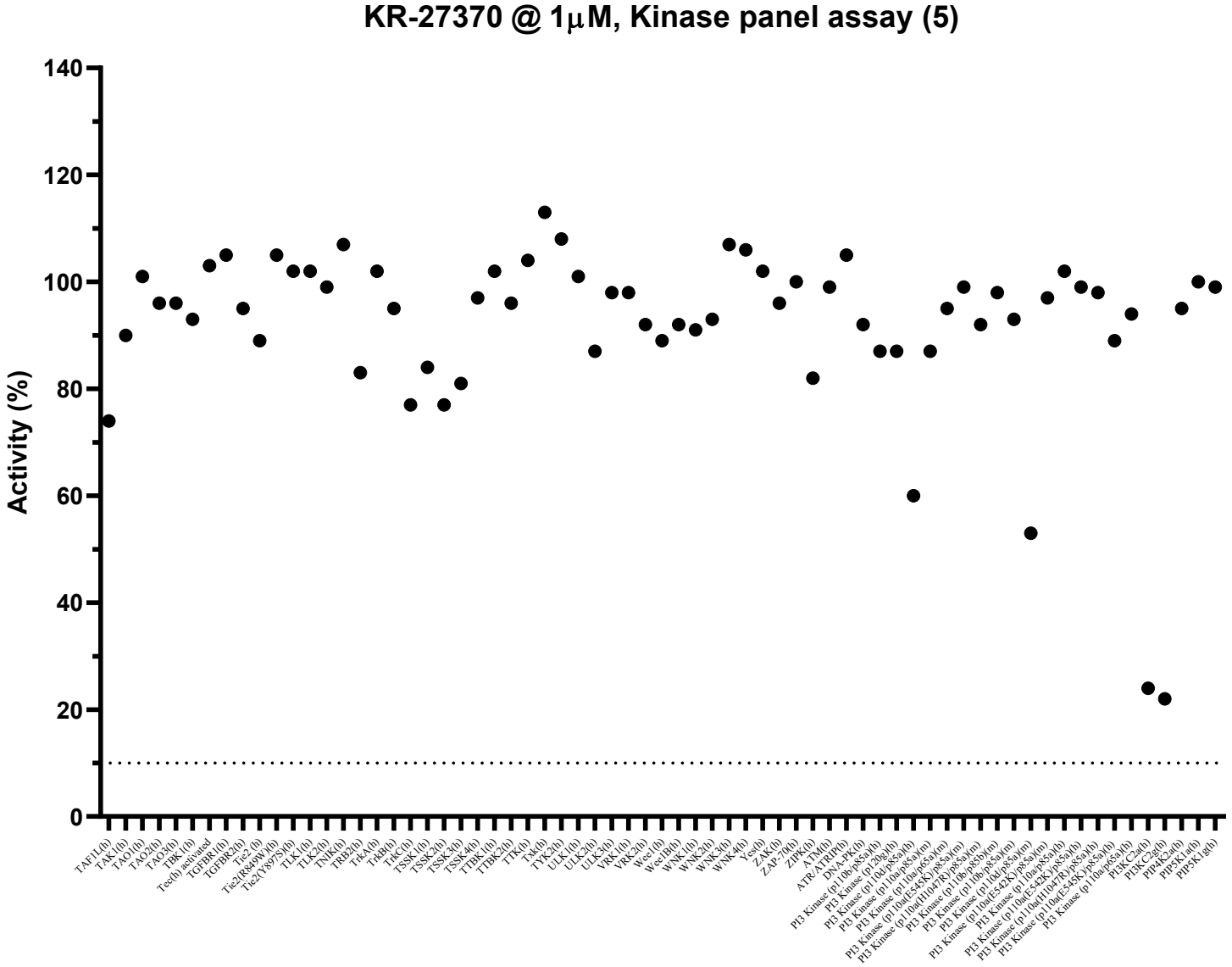



KR-27370 @ 1μM, Kinase panel assay (2)
Activity (%)
140
120
100
80
60
40
20
0
COT(h)
CRK(h)
CSK(h)
c-RAF(h)
SRC(h)
DAPK1(h)
DAPK2(m)
DCAMKL1(h)
DCAMKL2(h)
DCAMKL3(h)
DDR1(h)
DDR2(h)
DMPK(h)
DRAK1(h)
DRAK2(m)
DYRK1A(h)
DYRK1B(h)
DYRK2(h)
DYRK3(h)
eIF-2A(h)
EGFR(E746-A750del)(h)
EGFR(L858R)(h)
EGFR(L861Q)(h)
EGFR(T790M)(h)
EGFR(T790M,L858R)(h)
EphA1(h)
EphA2(h)
EphA3(h)
EphA4(h)
EphA5(h)
EphA7(h)
EphA8(h)
EphB2(h)
EphB1(h)
EphB3(h)
EphB4(h)
ErbB4(h)
FAK(h)
Fer(h)
Fes(h)
rFGFR1(h)
FGFR1(V561M)(h)
FGFR2(h)
FGFR2(N549H)(h)
FGFR3(h)
FGFR4(h)
Fgr(h)
Flt1(h)
Flt3(D835Y)(h)
Flt4(h)
Fms(h)
FmsY969C(h)
Fyn(h)
GAK(h)
GCK(h)
GCK2(h)
GRK1(h)
GRK2(h)
GRK3(h)
GRK4(h)
GRK5(h)
GRK6(h)
GRK7(h)
GSK3a(h)
GSK3b(h)
Haspin(h)
Hck(h)
Hck(h) activated
HIPK1(h)
HIPK2(h)
HIPK3(h)
HIPK4(h)
HIPK1(h)
HIPK(h)
ICK(h)
IGF-1R(h)
IGF-1R(h), activated
IKKa(h)
IKKb(h)
IKKe(h)
IR(h)
IR(h), activated
IRE1(h)
IRR(h)
IRAK1(h)
IRAK4(h)
Ilk(h)


KR-27370 @ 1μM, Kinase panel assay (3)
Activity (%)
140
120
100
80
60
40
20
0
JAK1(h)
JAK2(h)
JAK3(h)
JNK1α1(h)
JNK2α2(h)
JNK3(h)
KDR(h)
LATS1(h)
LATS2(h)
Lck(h)
Lck(h) activated
LIMK1(h)
LIMK2(h)
LKB1(h)
LOK(h)
Lyn(h)
Lyn(m)
LRRK2(h)
LTK(h)
MAK(h)
MAPK1(h)
MAPK2(h)
MAPK3(h)
MAPKAP1(h)
MAPKAP2(h)
MAPKAP3(h)
MAPKAPK2(h)
MAPKAPK3(h)
MARK1(h)
MEK1(h)
MEK2(h)
MEK5(h)
MARK3(h)
MARK4(h)
MEKK2(h)
MEKK3(h)
MELK(h)
Mer(h)
Met(h)
Met(D1246H)(h)
Met(D1246N)(h)
Met(M1268T)(h)
Met(Y1248C)(h)
Met(Y1248D)(h)
Met(Y1248H)(h)
MINK(h)
MKK3(h)
MKK4(m)
MKK6(h)
MLCK(h)
MLK1(h)
MLK2(h)
MLK3(h)
MLK4(h)
Mnk1(h)
Mst1(h)
MOK(h)
MRCKα(h)
MRCKβ(h)
MRCK-γ(h)
MSK1(h)
MSK2(h)
MSSK1(h)
MST1(h)
MST2(h)
MST3(h)
MST4(h)
mTOR(h)
mTOR/FKBP12(h)
MuSK(h)
MYLK2(h)
MYO3B(h)
NDR1(h)
NDR2(h)
NEK1(h)
NEK2(h)
NEK3(h)
NEK4(h)
NEK5(h)
NEK6(h)
NEK7(h)
NEK9(h)
NIM1(h)
NLK(h)
NUAK2(h)

## KR-27370 @ 1μM, Kinase panel assay (4)




KR-27370 @ 1μM, Kinase panel assay (5)
Activity (%)
140
120
100
80
60
40
20
0
TAF1L(h)
TAK1(h)
TAO1(h)
TAO2(h)
TAO3(h)
TBK1(h)
Tec(h) activated
TGFBR1(h)
TGFBR2(h)
Tie2 (h)
Tie2(R849W)(h)
Tie2(Y897S)(h)
TLK1(h)
TLK2(h)
TNIK(h)
TRB2(h)
TrkA(h)
TrkB(h)
TrkC(h)
TSSK1(h)
TSSK2(h)
TSSK3(h)
TSSK4(h)
TTBK1(h)
TTBK2(h)
TTK(h)
Tsk(h)
TYK2(h)
ULK1(h)
ULK2(h)
ULK3(h)
VRK1(h)
VRK2(h)
Wee1(h)
Wee1B(h)
WNK1(h)
WNK2(h)
WNK3(h)
WNK4(h)
Yes(h)
ZAK(h)
ZAP-70(h)
ZIPK(h)
ATM(h)
ATR/ATRIP(h)
DNA-PK(h)
PI3 Kinase (p110b/p85b)(h)
PI3 Kinase (p120g)(h)
PI3 Kinase (p110d/p85b)(h)
PI3 Kinase (p110a/p85a)(m)
PI3 Kinase (p110a[E545K]/p85a)(m)
PI3 Kinase (p110a[H1047R]/p85a)(m)
PI3 Kinase (p110b/p85a)(m)
PI3 Kinase (p110b/p85b)(m)
PI3 Kinase (p110d/p85a)(m)
PI3 Kinase (p110d[E542K]/p85a)(m)
PI3 Kinase (p110a/p85a)(h)
PI3 Kinase (p110a[E542K]/p85a)(h)
PI3 Kinase (p110a[H1047R]/p85a)(h)
PI3 Kinase (p110a/p85b)(h)
PI3 Kinase (p110a[E542K]/p85b)(h)
PI3KC2a(h)
PI3KC2g(h)
PIP4K2a(h)
PIP5K1a(h)
PIP5K1g(h)

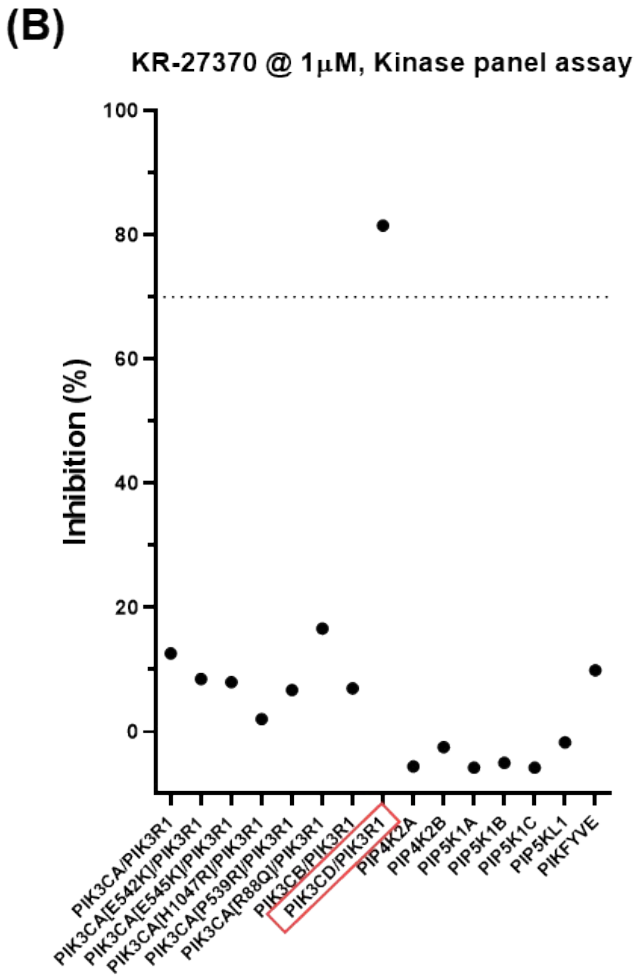



**Figure.** (A) **7f** was tested 435 protein kinases analysis by Eurofins. A Results of 30% or below was established as an inhibitor of the test kinases (PI3KC2α, PI3KC2γ). (B) **7f** was tested 15 protein kinases analysis by Carna biosciences. A Results of 70% or above was established as an inhibitor of the test kinases (PIK3CD/PIK3R1).