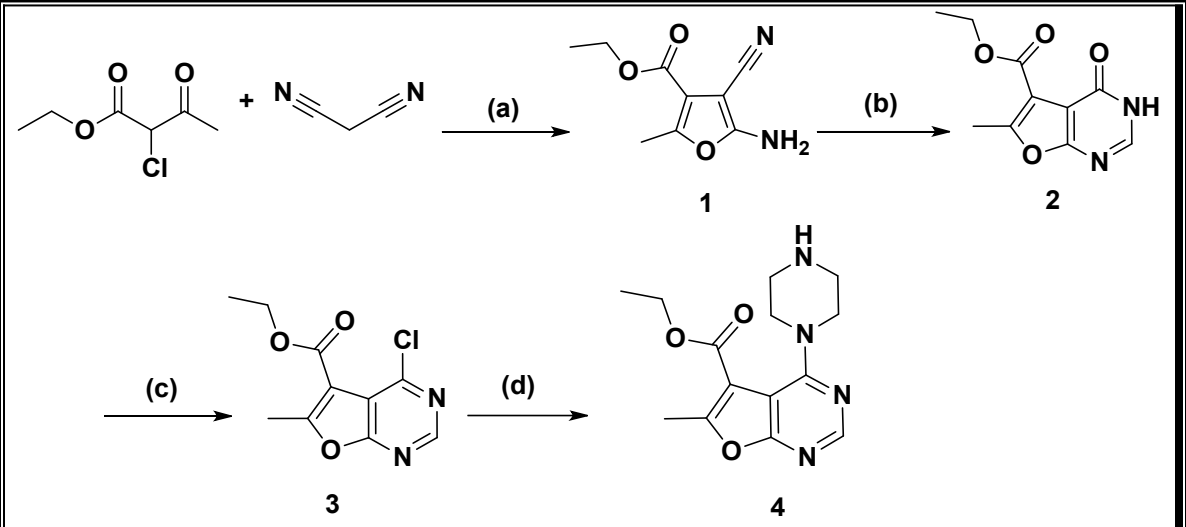
Electronic Supplementary Material (ESI) for RSC Advances.

Designing of new a scaffold for combating pancreatic cancer by targeting PI3K-α

Results and Discussion

1. Chemistry



Scheme 1, Reagents and conditions: (a) NaOEt, rt, 6 hrs, 77%; **(b)** HCOOH, acetic anhydride, reflux, 48 hrs, 80%; **(c)** POCl3, reflux, 3 hrs, 79%; **(d)** anhydrous piperazine, i-PrOH, reflux, 7 hours, 60%.

Table S1: Cell growth percentage of NCI 60 cancer cell lines demonstrated by final compounds *(5a, 5b, 5c, 5d, 7a, 7b, 7c, 7d, 8a, 8b, 8c):*

Cell Growth Percent for the tested compounds

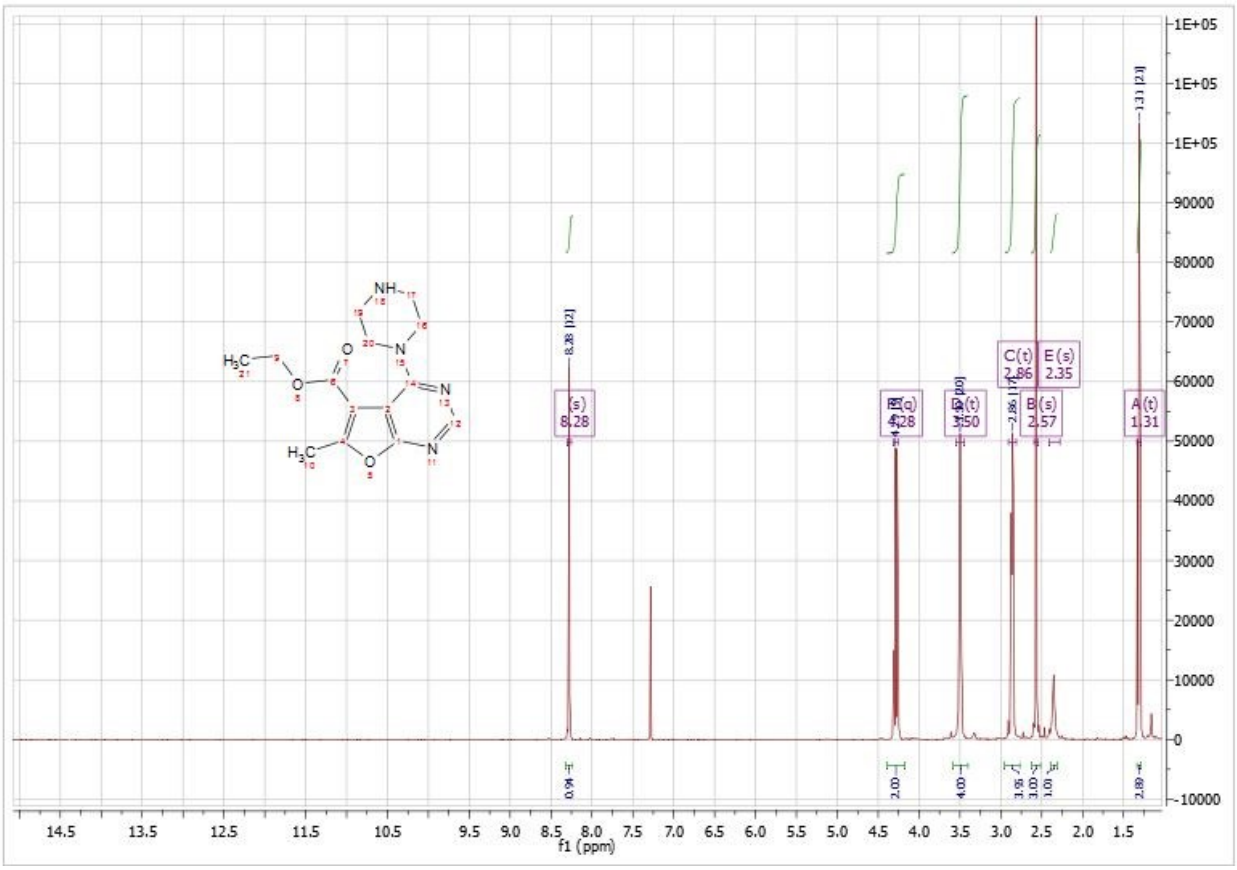
Panel/ Cell line	5a	5b	5c	5d	7a	7b	7c	7d	8a	8b	8c
Leukemia											
CCRF-CEM	98.66	90.19	99.1	91.15	97.28	91.46	76.16	85	84.18	79.76	92.28
HL-60(TB)	101.21	99.29	99.71	100.71	106.85	97.18	73.89	90.33	89.17	93.25	94.67
K-562	103.15	91.1	102.56	91.83	98.24	82.44	53.5	74.41	90.37	92.28	84.95
MOLT-4	100.61	83.93	95.58	91.83	95.75	88.62	59.12	85.2	94.57	92.8	91.3
RPMI-8226	95.92	90.27	98.26	92.31	92.77	81.58	44.88	74.73	80.01	78.82	83.35
SR	100.49	96.76	97.44	87.58	-	84.32	-	72.61	-	88.65	94.51
Non-Small Cell Lung Cancer											
A549/ATCC	107.33	98.05	103.46	102.97	96.36	90.15	85.98	85.27	91.35	96.79	91.99
EKVX	102.6	99.77	101.68	97.08	87.24	88.13	73.99	91.7	81.95	95.48	91.16
HOP-62	92.22	92.89	90.14	98.93	93.77	86.42	90.76	83.9	81.43	83.76	87.21
HOP-92	100.42	99.51	111.95	107.91	81.3	78.91	62.88	88.34	81.81	100.09	83.33
NCI-H226	91.59	87.84	98.16	98.95	88.51	83.28	66.54	79.39	86.7	89.75	87.17
NCI-H23	105.58	94.6	101.56	97.98	92.76	93.93	72.47	94.68	91.6	97.36	90.79
NCI-H322M	100.88	98.41	101.41	97.57	95.01	99.27	88.79	96.43	88.5	94.71	92.28
NCI-H460	108.47	98.28	100.98	101.19	103.62	98.71	91.39	100.64	101.43	82.03	102.79
NCI-H522	91.08	76.24	89.03	82.08	95.27	74.91	77.91	68.68	88.8	81.27	80.9
Colon Cancer											
COLO 205	111.92	107.92	107.04	100.55	102.06	97.99	76.86	99.6	95.38	102.86	104.14
HCC-2998	101.87	116.6	118.74	113.12	106	109.71	104.44	111.37	101.99	112.87	99.26
HCT-116	105.01	94.86	101.24	84.77	98.33	91.58	75.18	83.97	94.6	99.92	97.26
HCT-15	106.41	98.56	100.13	98.32	94.34	95.85	79.8	84.13	83.92	93.14	90.62
HT29	104.56	99.17	107.77	90.86	105.89	95.33	90.19	85.54	101.15	99.74	102.1
KM12	104.5	103.03	98.83	98.98	100.86	95.58	89.93	93.27	96.67	96.07	96.39
SW-620	88.66	84.6	90.2	96.55	100.65	102.46	99.8	92.96	103.57	103.58	90.48
CNS Cancer											
SF-268	95.41	92.9	93.93	94.99	92.59	92.72	90.95	96.74	89.26	95.09	94.12
SF-295	104.86	96.28	103.92	94.31	92.61	88.19	79.94	85.77	80.8	47.36	95.97
SF-539	102.5	100.24	101.24	101.58	96.46	93.45	96.09	84.72	82.38	73.09	91.77
SNB-19	103.39	97.96	105.25	104.36	87.68	97.94	85.41	92.39	84.49	91.4	96.5
SNB-75	84.19	76.38	82.21	86.26	86.53	83.04	93.54	77.56	79.3	49.86	80.48
U251	97.1	100.91	99.21	93.51	97	87.45	89.51	80.18	77.24	71.73	89.66
Melanoma											
LOX IMVI	99.71	94.87	97.11	101.94	96.35	90.54	75.52	88.47	83.52	93.19	90.68
MALME-3M	111.25	112.29	112.22	112.91	104.01	105.23	70.22	105.32	101.72	98.04	99.93

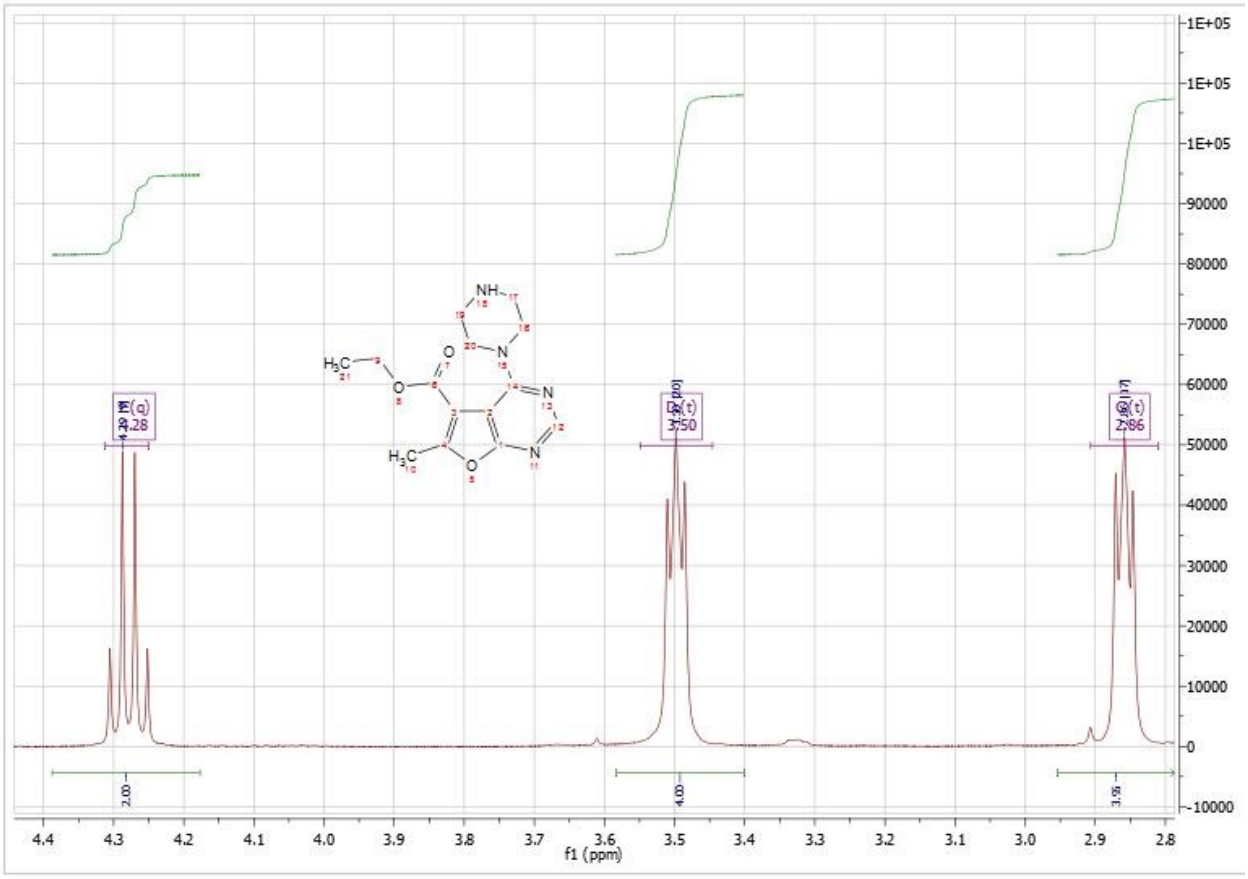
M14 97.97	110.04	105.51	107.11	110.37	95.82	97.93	74.53	100.15	91.23	89.3	104.5
MDA-MB-435	102.76	100.72	101.95	101.7	101.17	103.23	99.77	102.02	101.96	102.46	101.73
SK-MEL-2	105.33	95.62	101.11	96.31	103.87	95.36	93.7	92.31	104.07	96.2	95.27
SK-MEL-28	121.52	116	121.99	116.48	99.07	110.49	85.39	99.89	97.63	106.47	106.74
SK-MEL-5	-	-	-	-	91.56	-	34.78	-	94.03	-	-
UACC-257	104.84	110.29	108.98	107.72	98.02	94.99	78.45	98.59	95.45	106.97	98.03
UACC-62	93.06	87.28	93.13	95.35	85.29	86.84	42.9	80.97	85.59	87.34	87.17
Ovarian Cancer											
IGROV1	97.73	97.7	103.73	96.67	95.73	90.44	73.75	92.09	99.71	106.49	95.39
OVCAR-3	113.17	111.09	112.5	107.25	103.22	100.39	98.43	108.52	95.11	101.97	102.76
OVCAR-4	106.26	78.28	105.47	94.39	96.9	74.98	74.43	84.66	92.55	73.8	85.1
OVCAR-5	101.16	93.76	100.9	95.29	94.04	98.24	96.84	96.59	91.15	93.56	90.01
OVCAR-8	100.8	98.46	102.15	98.7	89.52	87.6	81.46	86.07	93.7	95.47	94.04
NCI/ADR-RES	101.17	92.29	101.99	96.38	95.25	85.71	73.61	88.79	87.23	100.86	90.25
SK-OV-3	106.46	105.69	104.13	102.33	104.46	86.13	85.45	90.25	93.62	85.96	96.95
Renal Cancer											
786-0	100.39	101.46	104.29	103.01	105.67	95.85	99.13	94	98.57	89.16	97.81
A498	-	-	-	-	93.94	-	81.95	-	80.31	-	-
ACHN	99.73	98.74	99.69	99.41	95.65	93.55	85.9	90.78	95.54	101.62	92
CAKI-1	84.66	80.72	84.73	86.34	92.64	82.65	84.27	81.63	93.61	59.52	79.58
RXF 393	113.48	104.87	115.46	115.35	101.45	99.72	92.14	92.74	76.52	90.06	90.76
SN12C	100.43	91.41	97.61	100.53	93.56	94.9	82.67	94.82	92.08	94.88	92.72
TK-10	109.35	105.79	112.08	108.95	106.21	103.35	94.13	105.03	101.16	101.38	100.1
UO-31	77.32	76.97	77.47	73.68	74.37	74.4	48.03	68.36	87.37	94.16	75.12
Prostate Cancer											
PC-3	100.01	88.8	92.64	93.84	89.91	82.23	59.29	84.12	86.19	84.3	91.77
DU-145	110.38	107.11	107.83	110.33	104.91	99.6	87.47	96.32	104.16	105.93	101.43
Breast Cancer											
MCF7	101.78	93.97	103.83	96.93	92.44	92.61	82.97	92.64	93.2	80.44	93.1
MDA-MB-231/ATCC	93.57	90.38	92.94	98.98	95.34	88.61	80.55	84.66	88.01	82.03	89.13
HS 578T	100.66	87.49	91.1	96.99	89.38	81.71	78.76	80.39	62.82	75.19	87.04
BT-549	101.8	103.37	87.37	95.37	96.07	90.82	54.28	96.48	82.74	75.24	92.41
T-47D	95.93	78.39	92.06	90.82	90.54	61.67	47.73	76.24	89.41	85.01	78.67
MDA-MB-468	111.44	90.54	115.41	110.5	84.47	81.86	60.92	91.32	91.47	97.82	85.22
MEAN % Growth inhibition	101.5	95.97	100.86	98.5	95.7	91.07	78.46	89.37	90.41	90.32	92.46

Experimental
Chemistry and analysis

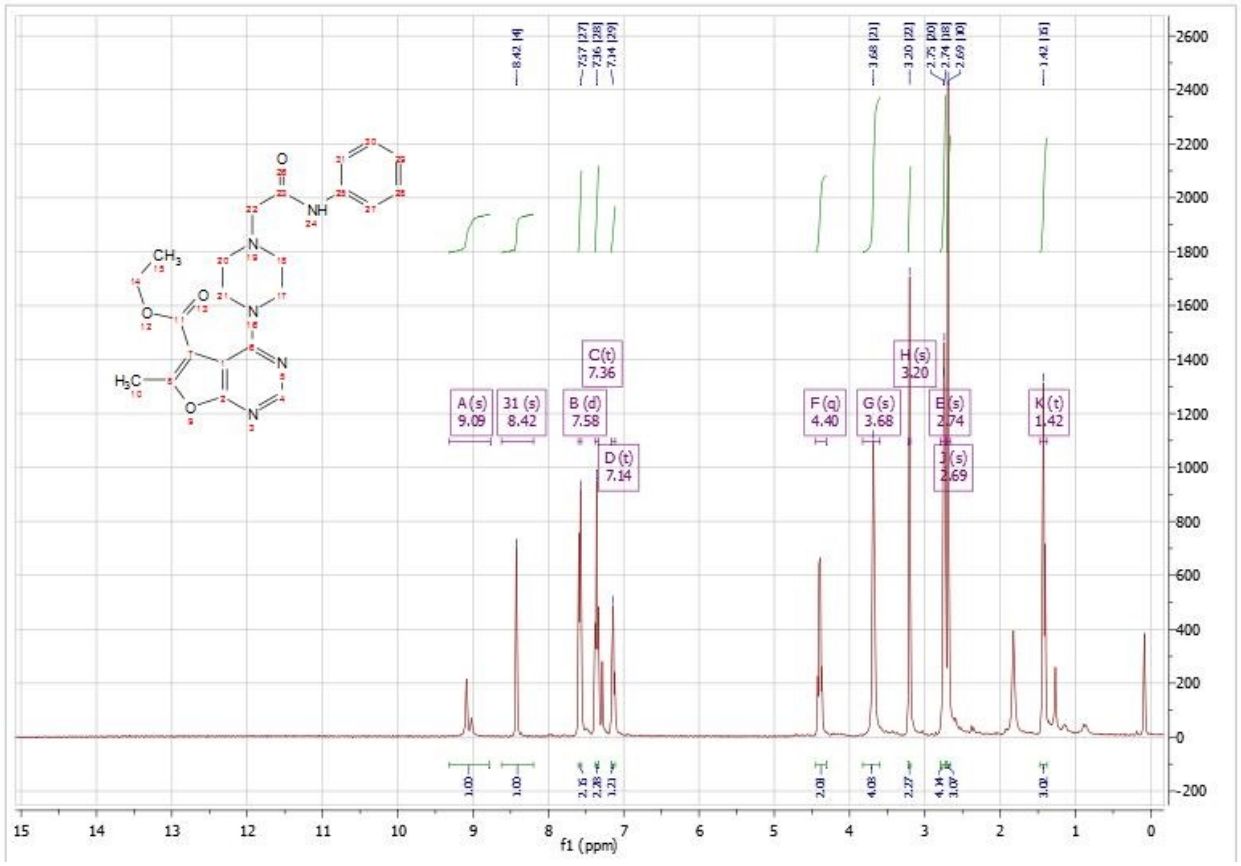
Supplementary Data NMR charts

Ethyl 6-methyl-4-(piperazin-1-yl)furo[2,3-d]pyrimidine-5-carboxylate (4)





Ethyl 6-methyl-4-(4-(2-oxo-2-(phenylamino)ethyl)piperazin-1-yl)furo[2,3-d]pyrimidine-5-carboxylate (5a)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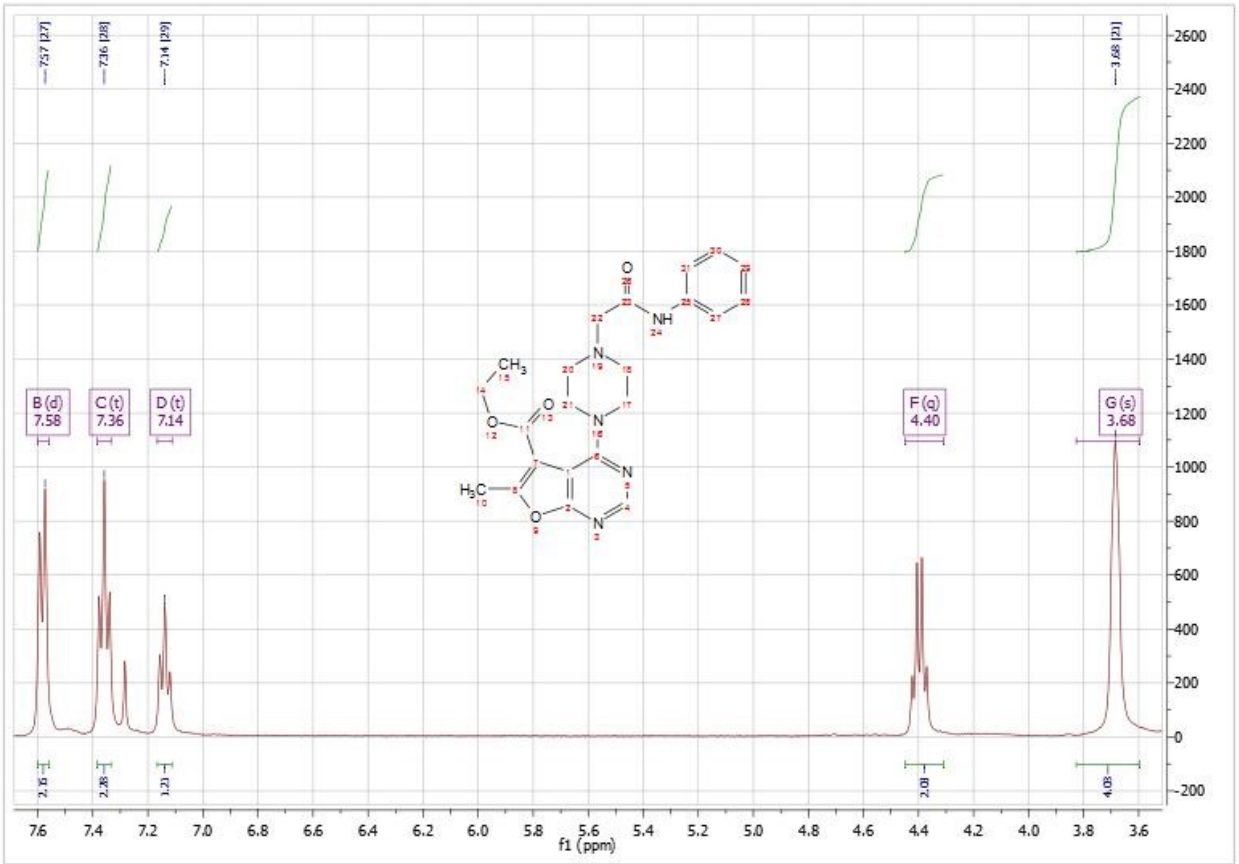
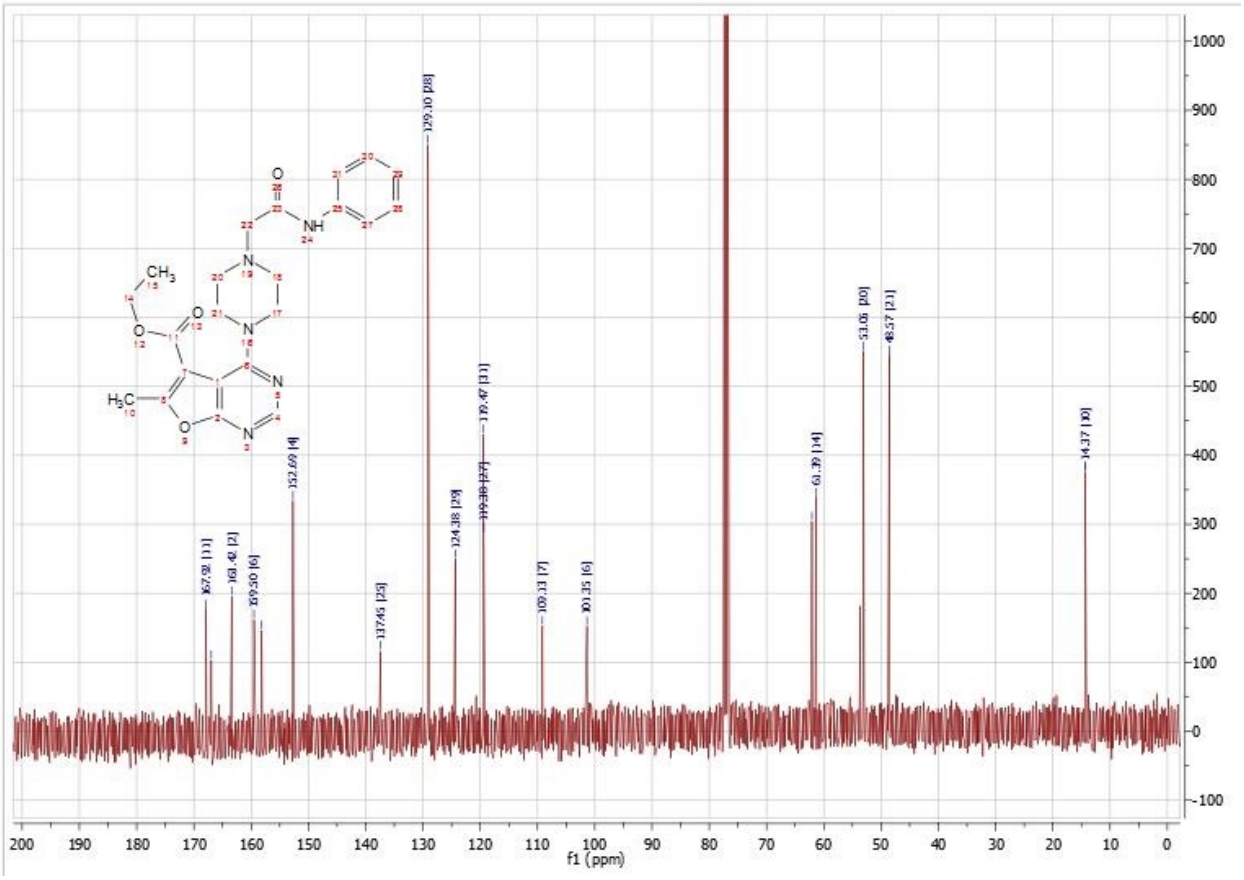
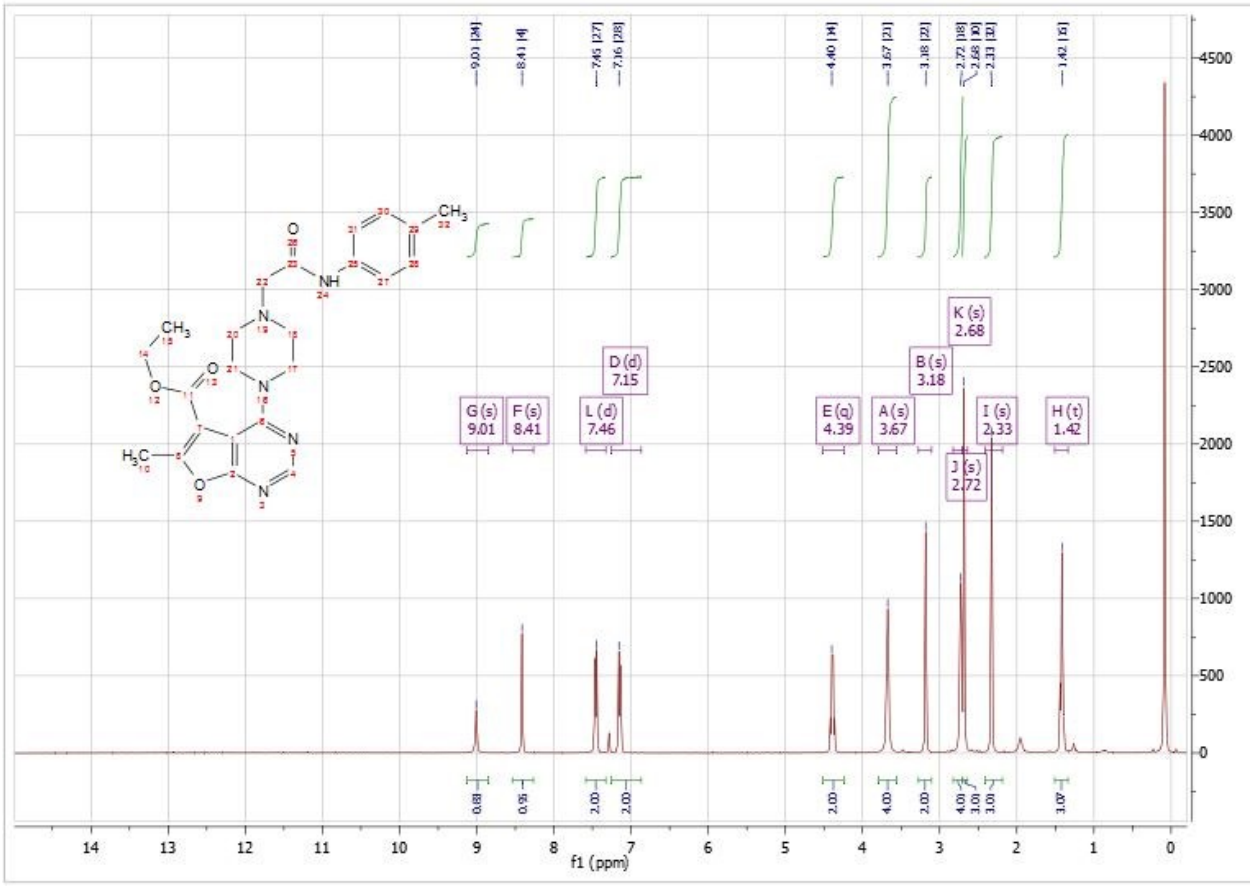
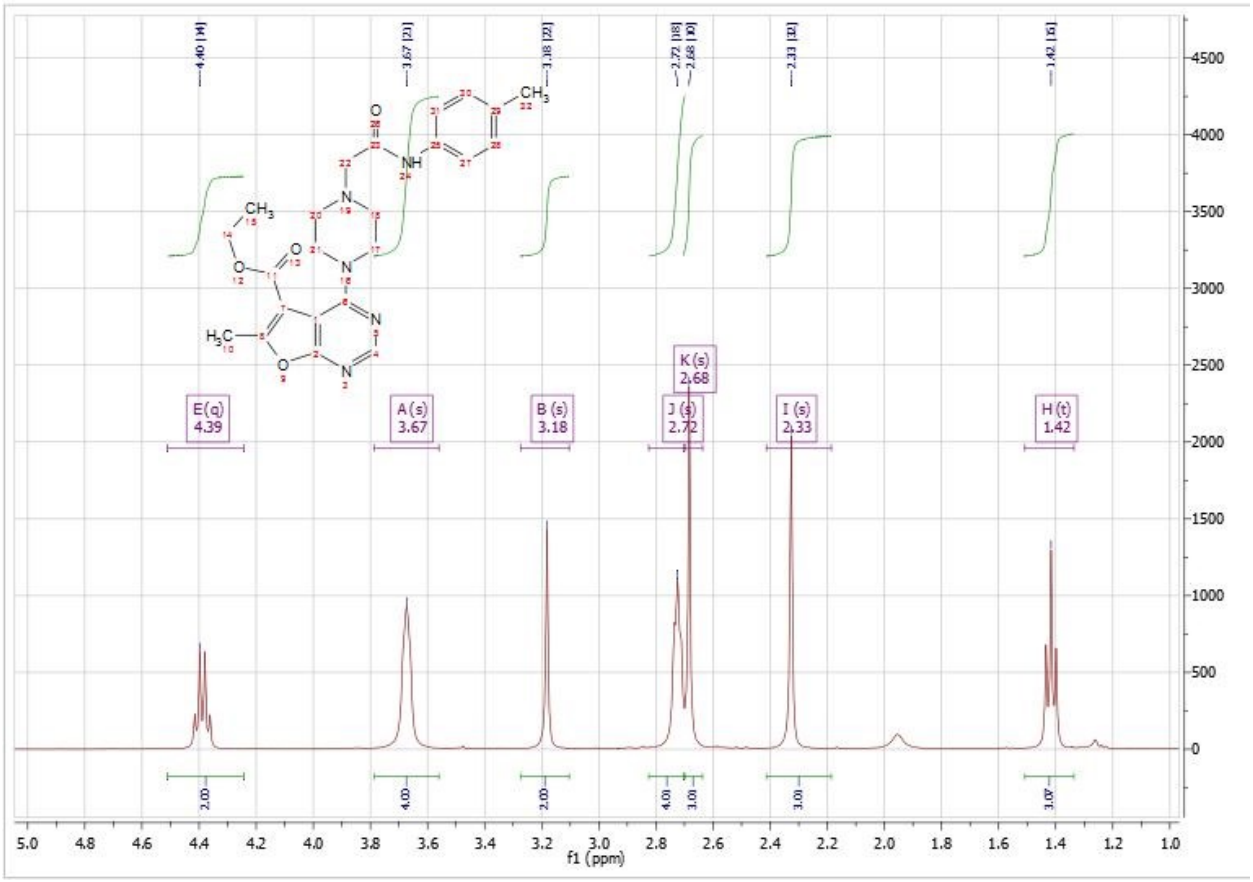
31 (s) 8.42
B (d) 7.58
C (t) 7.36
D (t) 7.14
F (q) 4.40
G (s) 3.68
H (s) 3.20
E (s) 2.74
I (s) 2.69
K (t) 1.42
f1 (ppm)

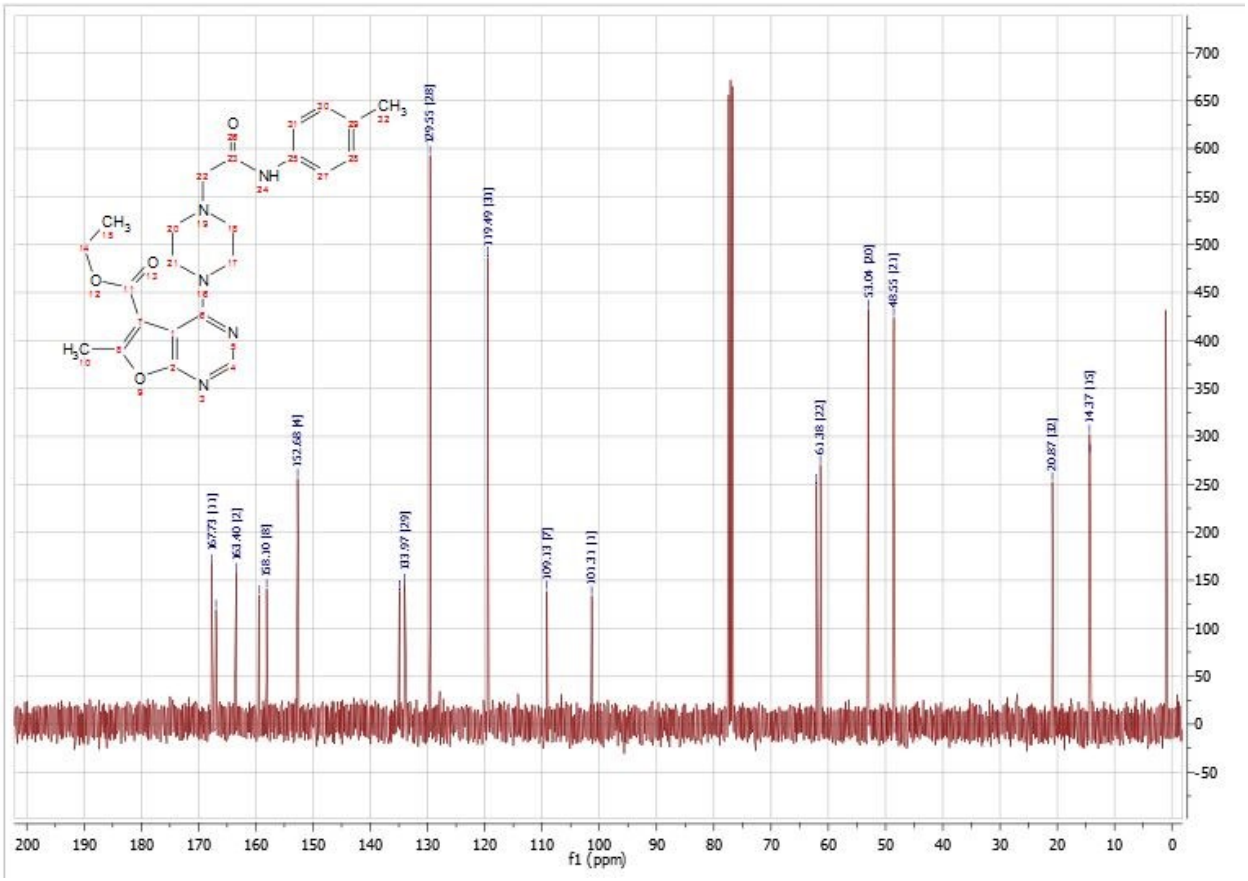

B (d) 7.58
C (t) 7.36
D (t) 7.14
F (q) 4.40
G (s) 3.68
f1 (ppm)



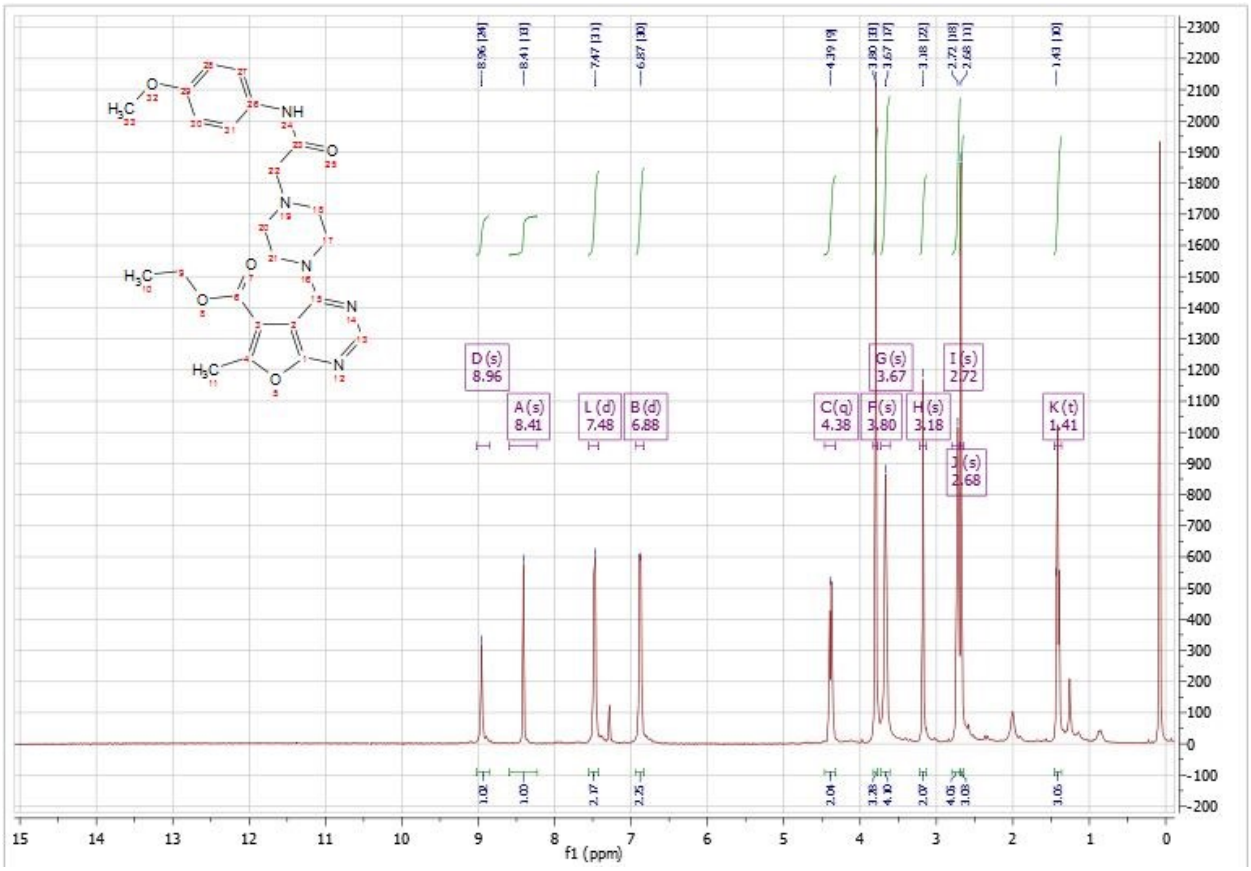
Ethyl 6-methyl-4-(4-(2-oxo-2-(p-tolylamino)ethyl)piperazin-1-yl)furo[2,3-d]pyrimidine-5-carboxylate (5b)

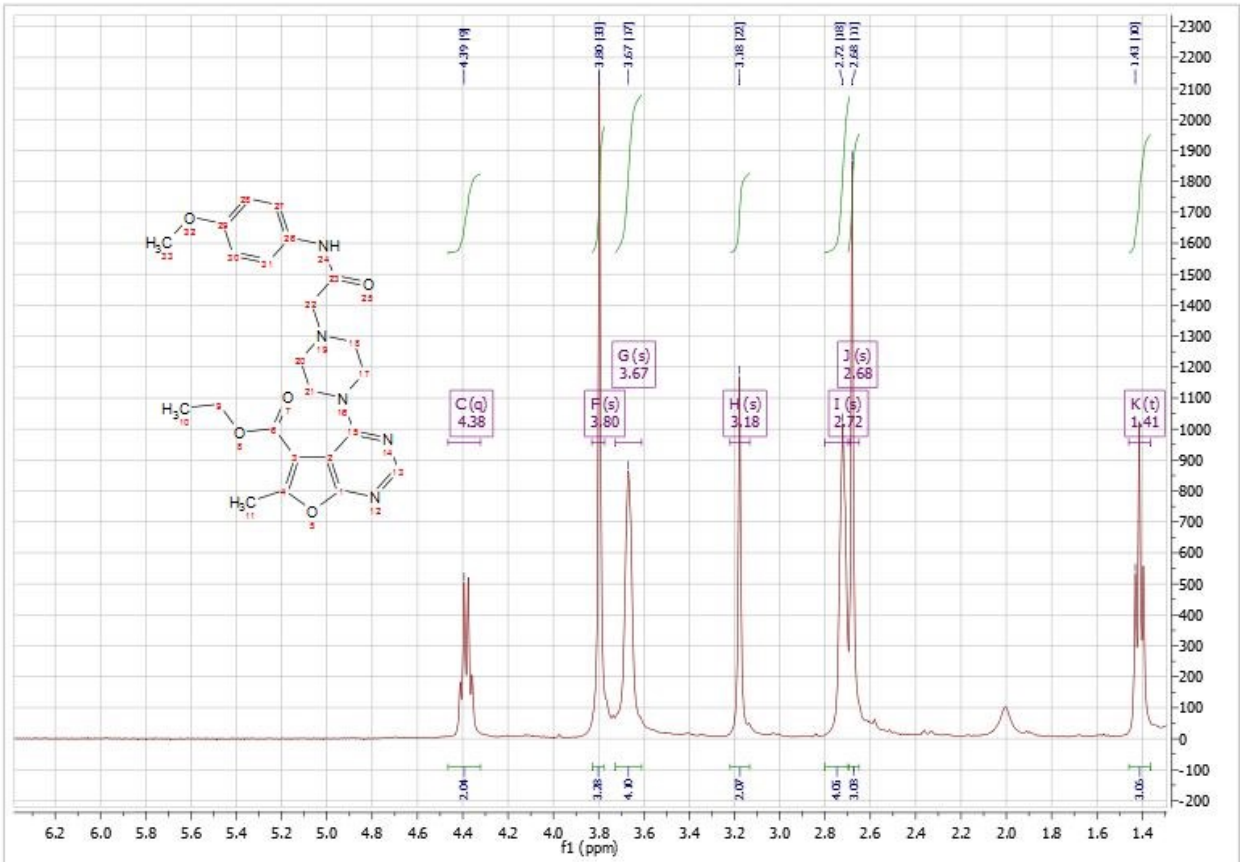

G (s) 9.01
F (s) 8.41
D (d) 7.15
L (d) 7.46
E (q) 4.39
A (s) 3.67
B (s) 3.18
K (s) 2.68
J (s) 2.72
I (s) 2.33
H (t) 1.42
f1 (ppm)

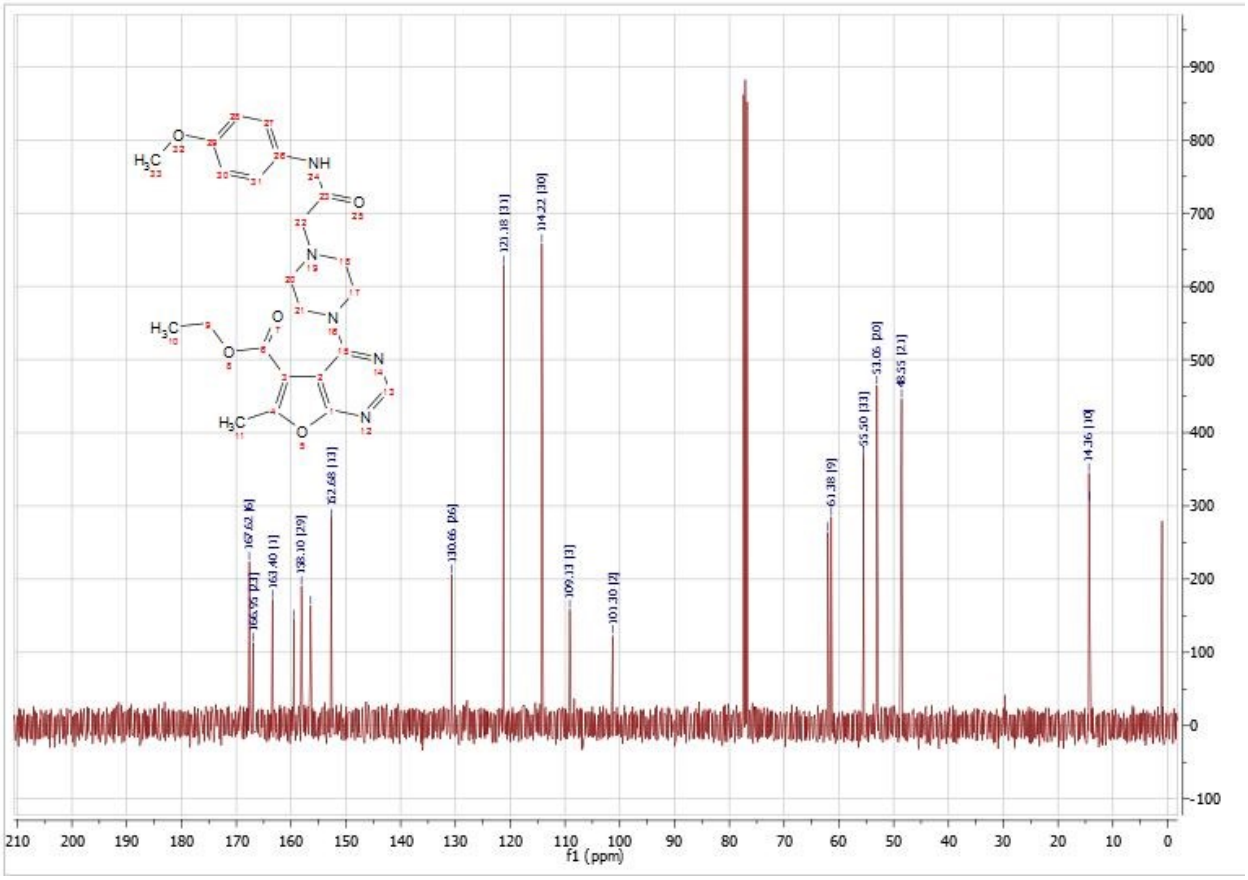

E (q) 4.39
A (s) 3.67
B (s) 3.18
K (s) 2.68
J (s) 2.72
I (s) 2.33
H (t) 1.42
f1 (ppm)



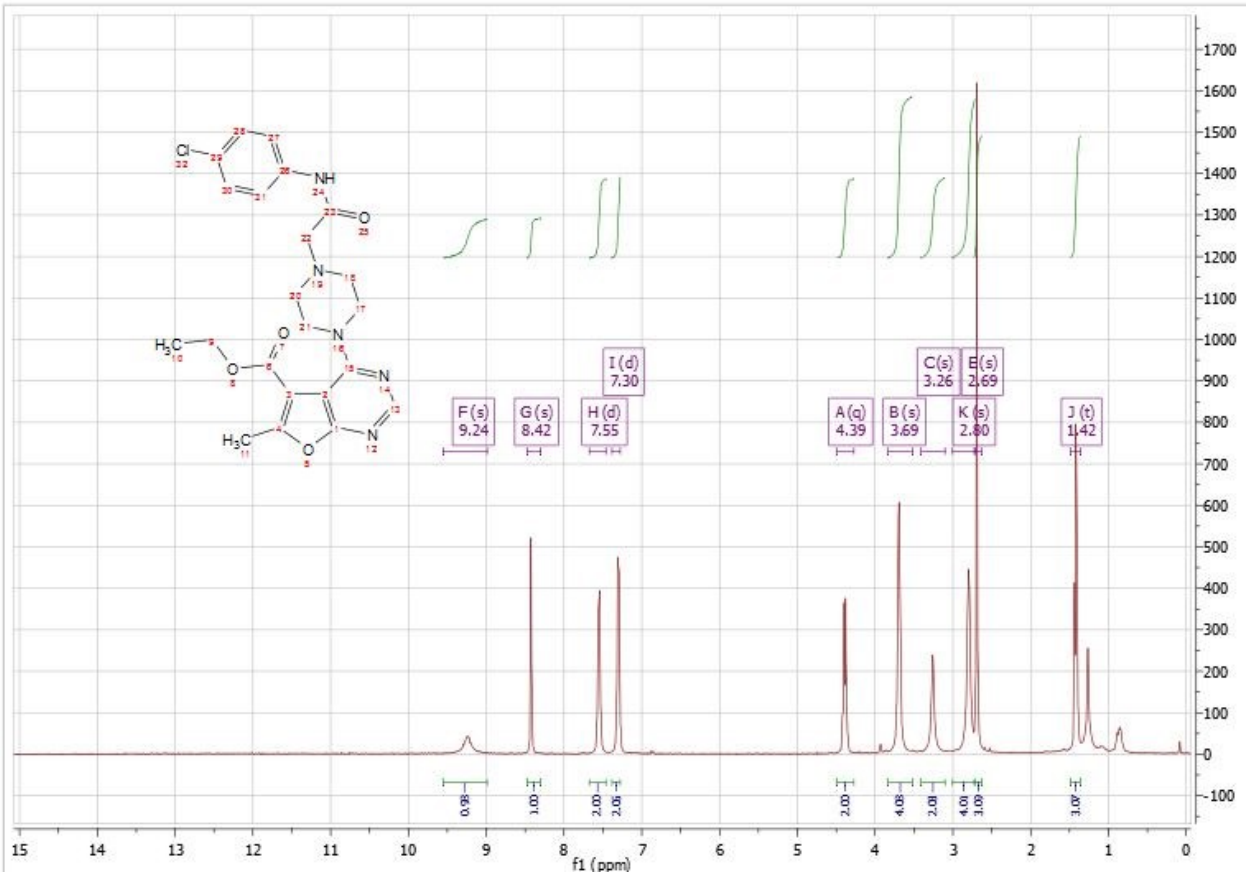
Ethyl 4-(4-(2-((4-methoxyphenyl)amino)-2-oxoethyl)piperazin-1-yl)-6-methylfuro[2,3-d]pyrimidine-5-carboxylate (5c)

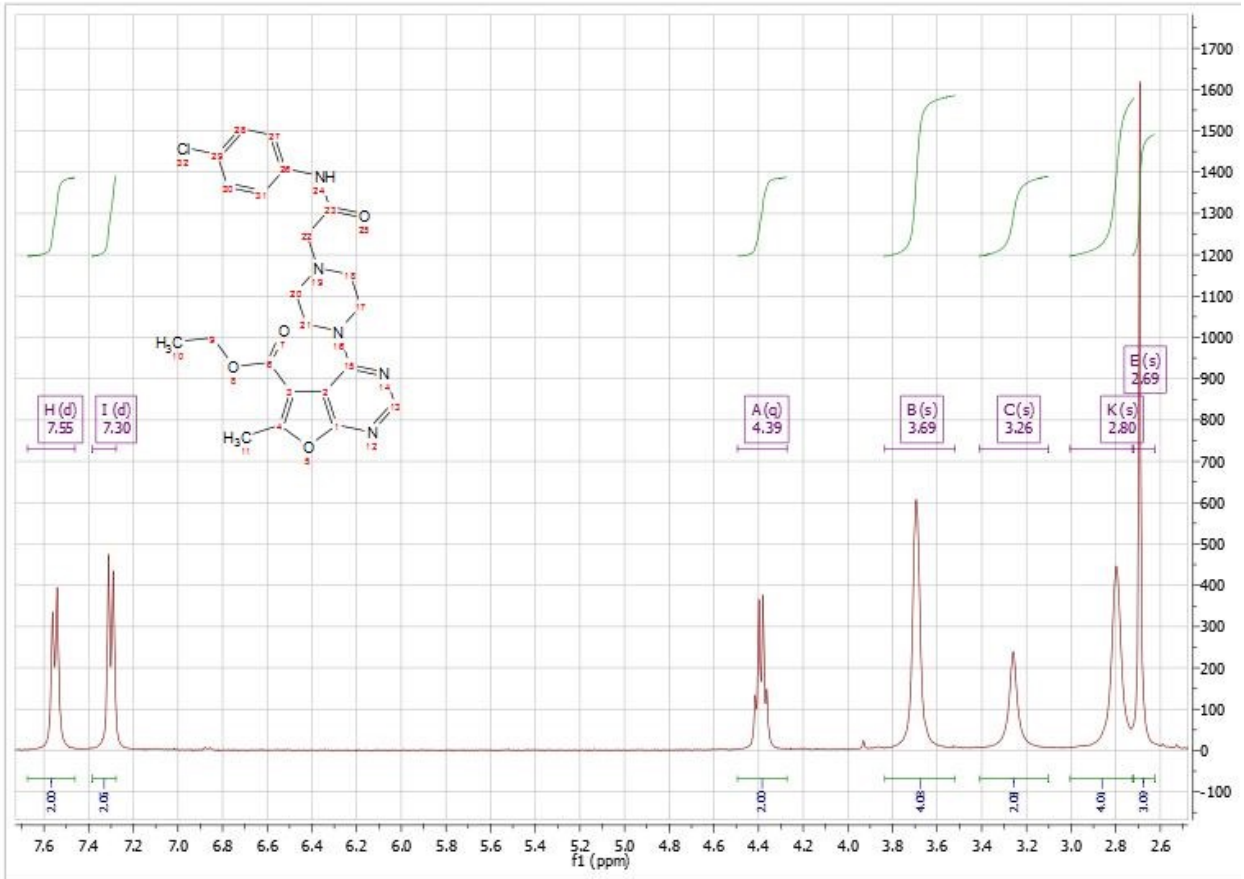

D(s) 8.96
A(s) 8.41
L(d) 7.48
B(d) 6.88
C(q) 4.38
F(s) 3.80
G(s) 3.67
H(s) 3.18
I(s) 2.72
J(s) 2.68
K(t) 1.41
f1 (ppm)

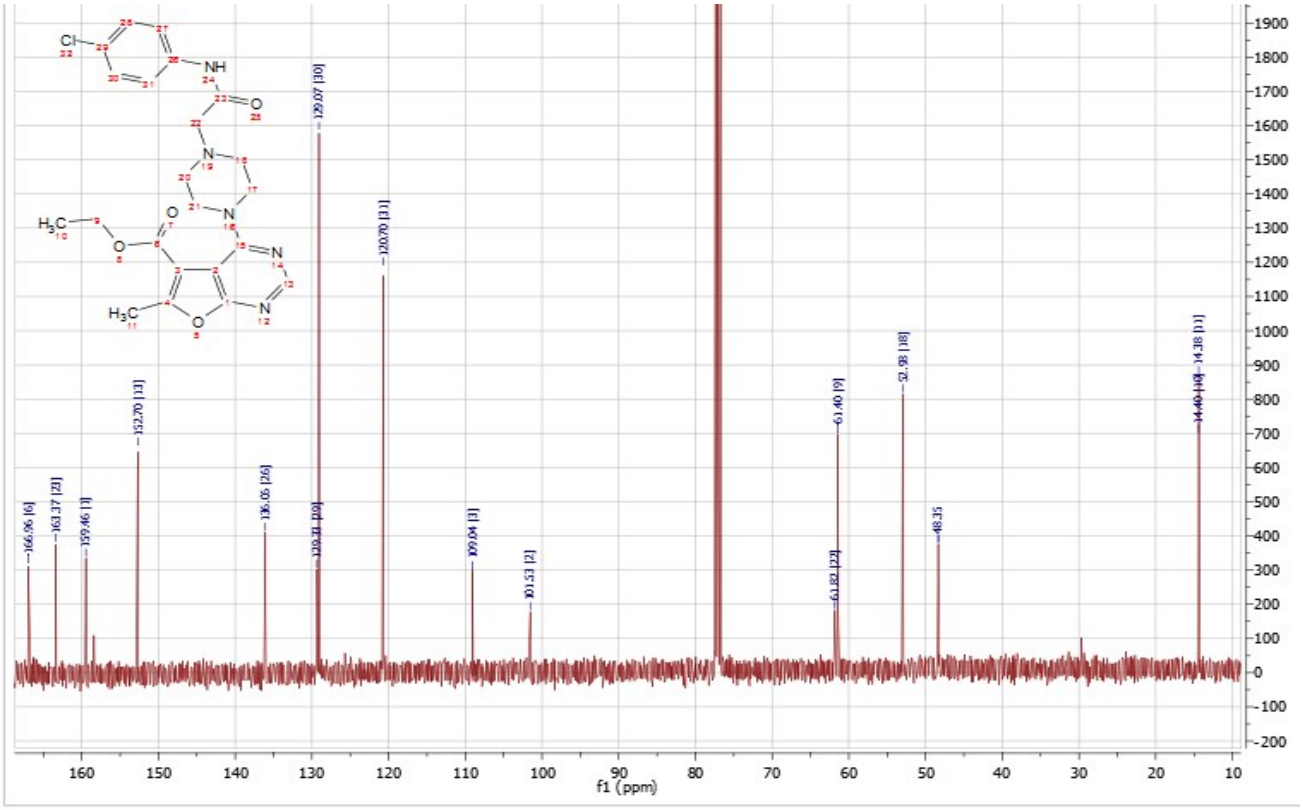

C(q) 4.38
F(s) 3.80
G(s) 3.67
H(s) 3.18
I(s) 2.72
J(s) 2.68
K(t) 1.41
f1 (ppm)



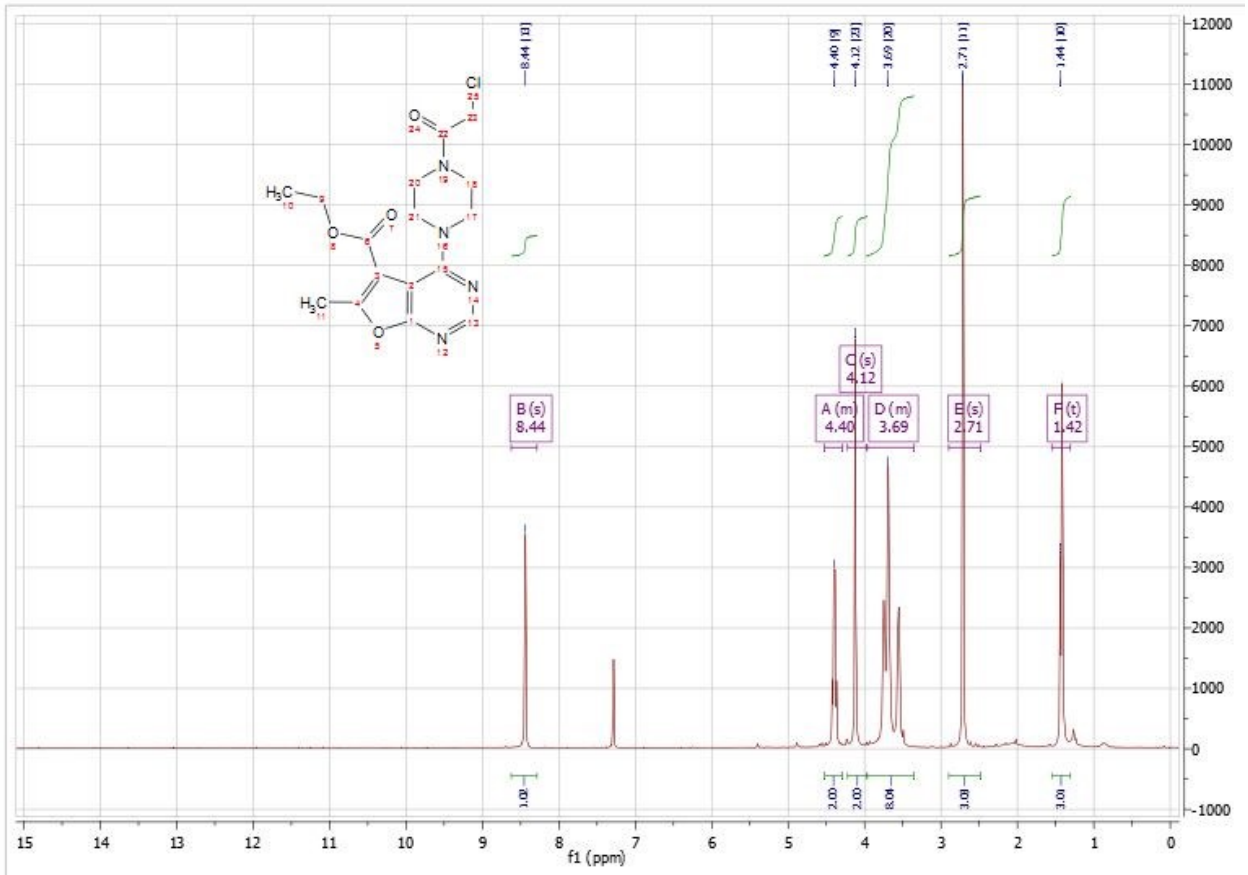
Ethyl 4-(4-(2-((4-chlorophenyl)amino)-2-oxoethyl)piperazin-1-yl)-6-methylfuro[2,3-d]pyrimidine-5-carboxylate (5d)


F(s) 9.24
G(s) 8.42
H(d) 7.55
I(d) 7.30
A(q) 4.39
B(s) 3.69
C(s) 3.26
K(s) 2.80
E(s) 2.69
J(t) 1.42
f1 (ppm)

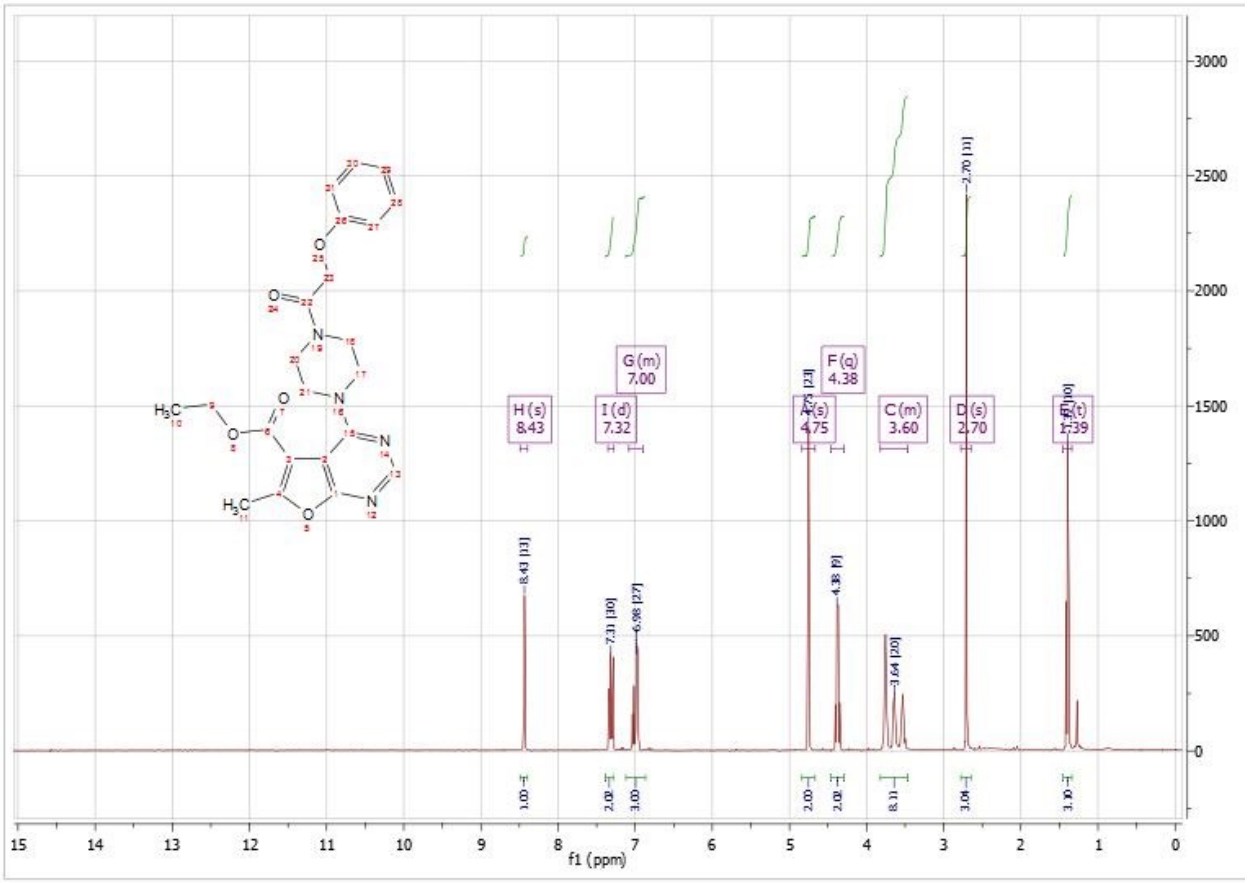

H(d) 7.55
I(d) 7.30
A(q) 4.39
B(s) 3.69
C(s) 3.26
K(s) 2.80
E(s) 2.69
f1 (ppm)

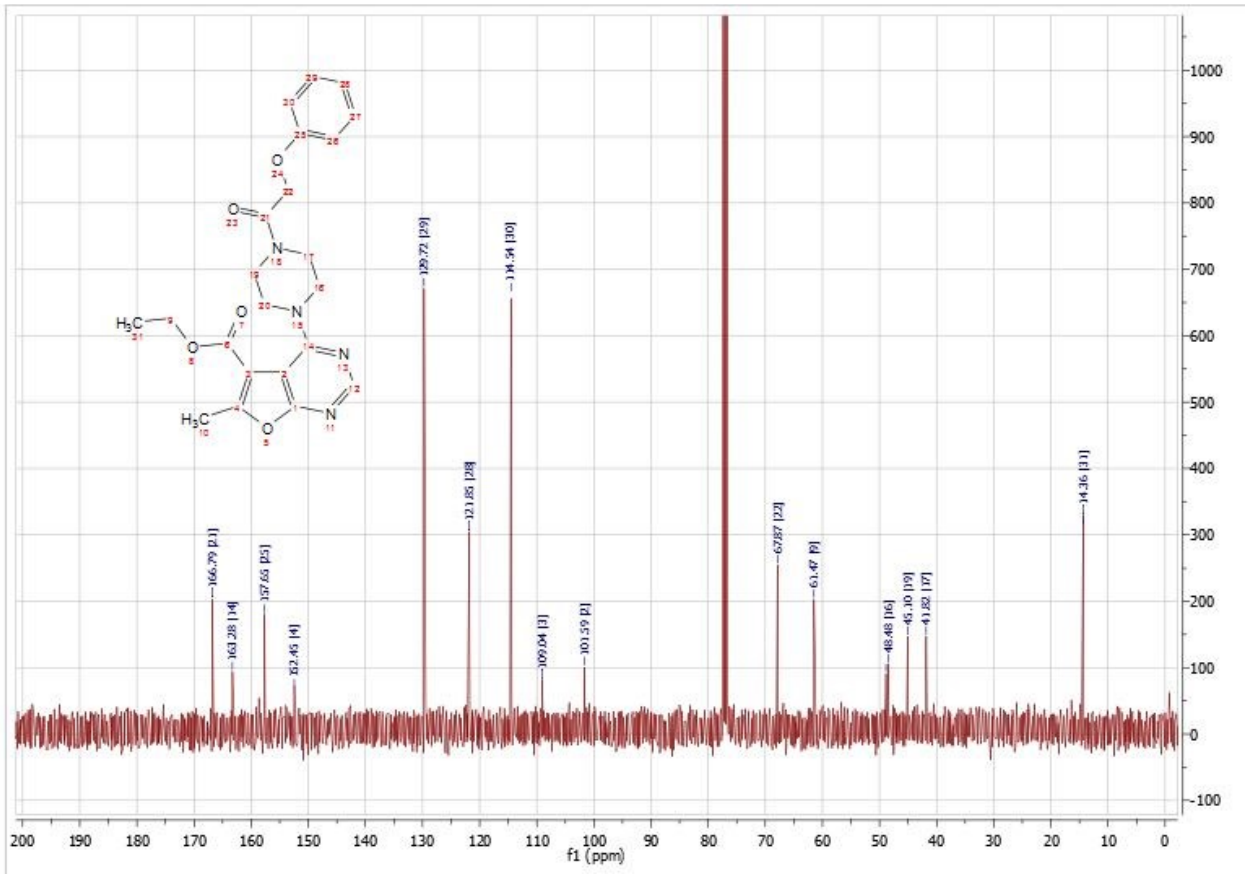


Ethyl 4-(4-(2-chloroacetyl)piperazin-1-yl)-6-methylfuro[2,3-*d*]pyrimidine-5-carboxylate (6)

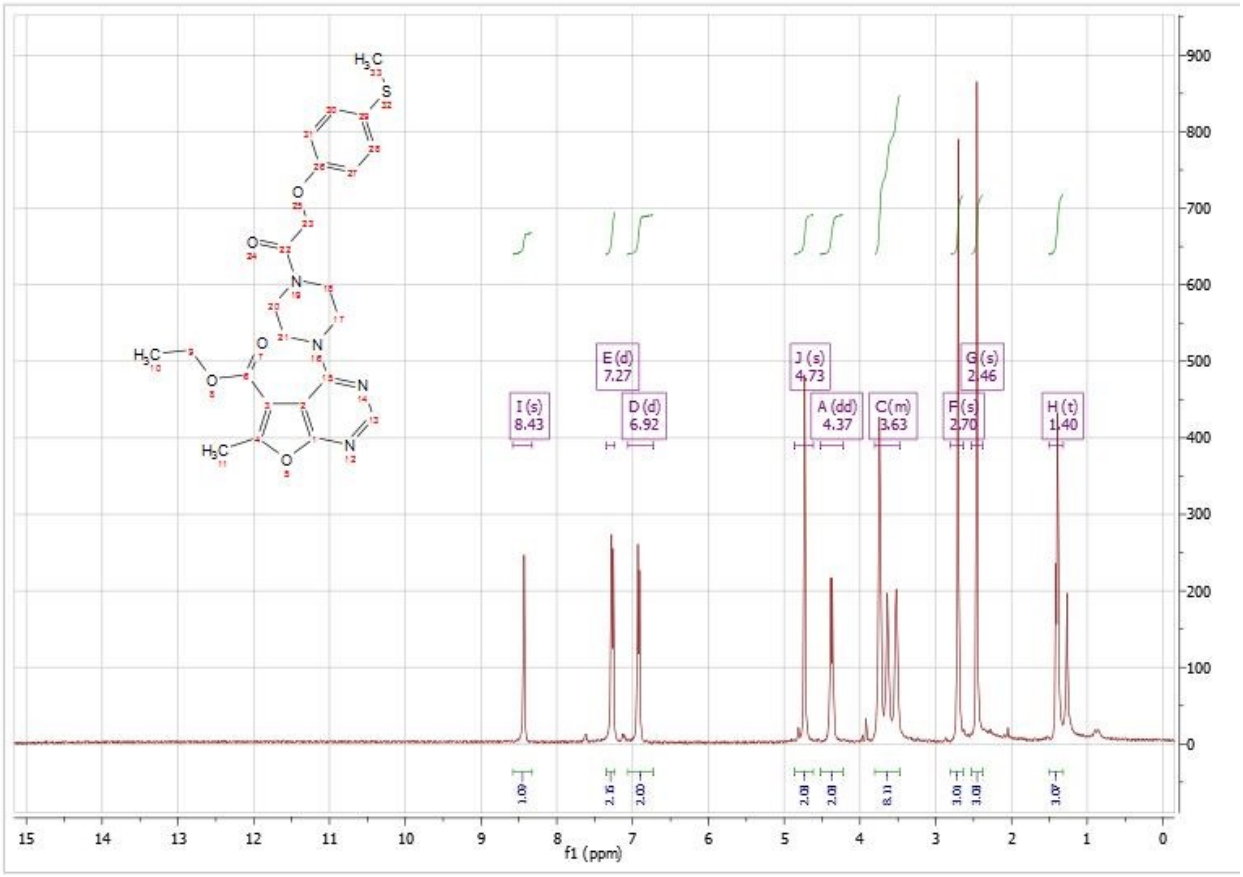


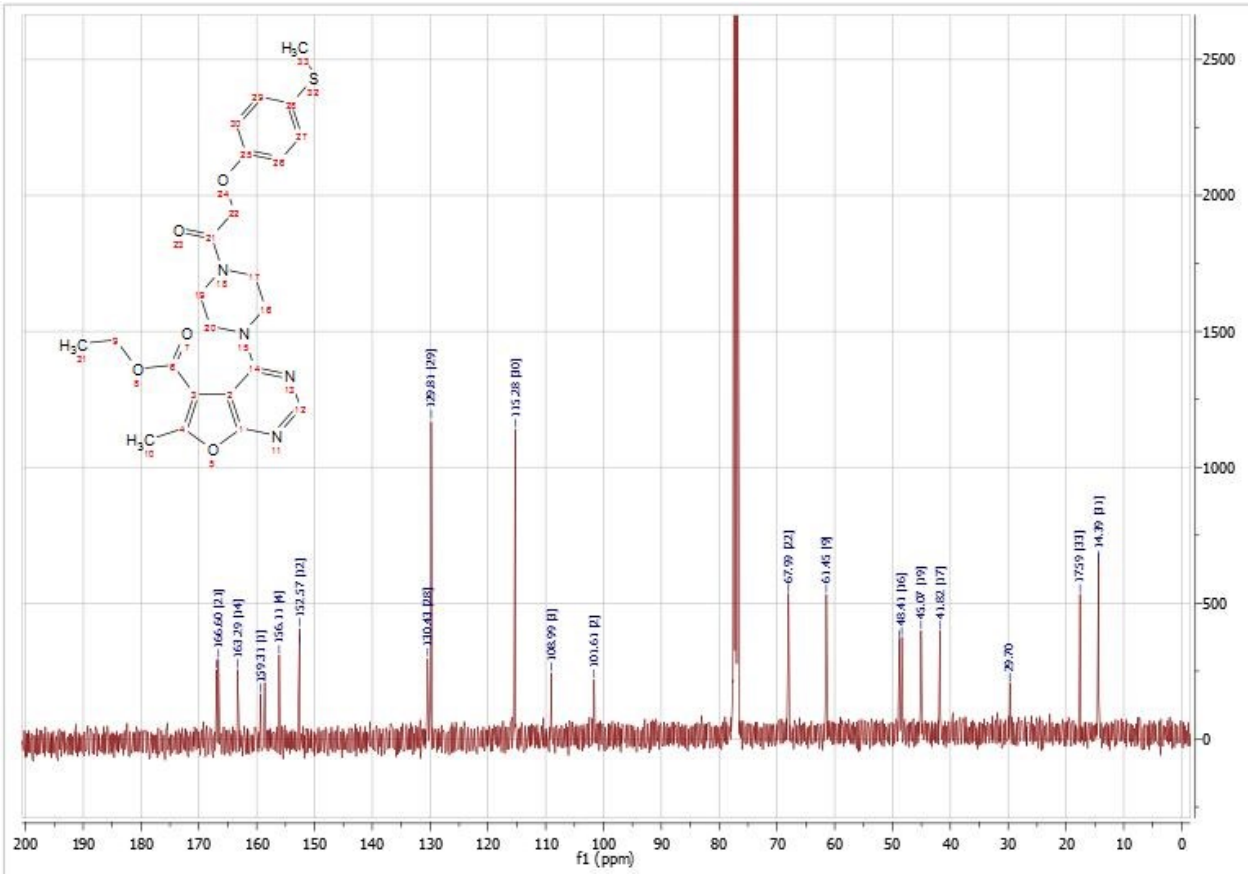
Ethyl 6-methyl-4-(4-(2-phenoxyacetyl)piperazin-1-yl)furo[2,3-d]pyrimidine-5-carboxylate (7a)



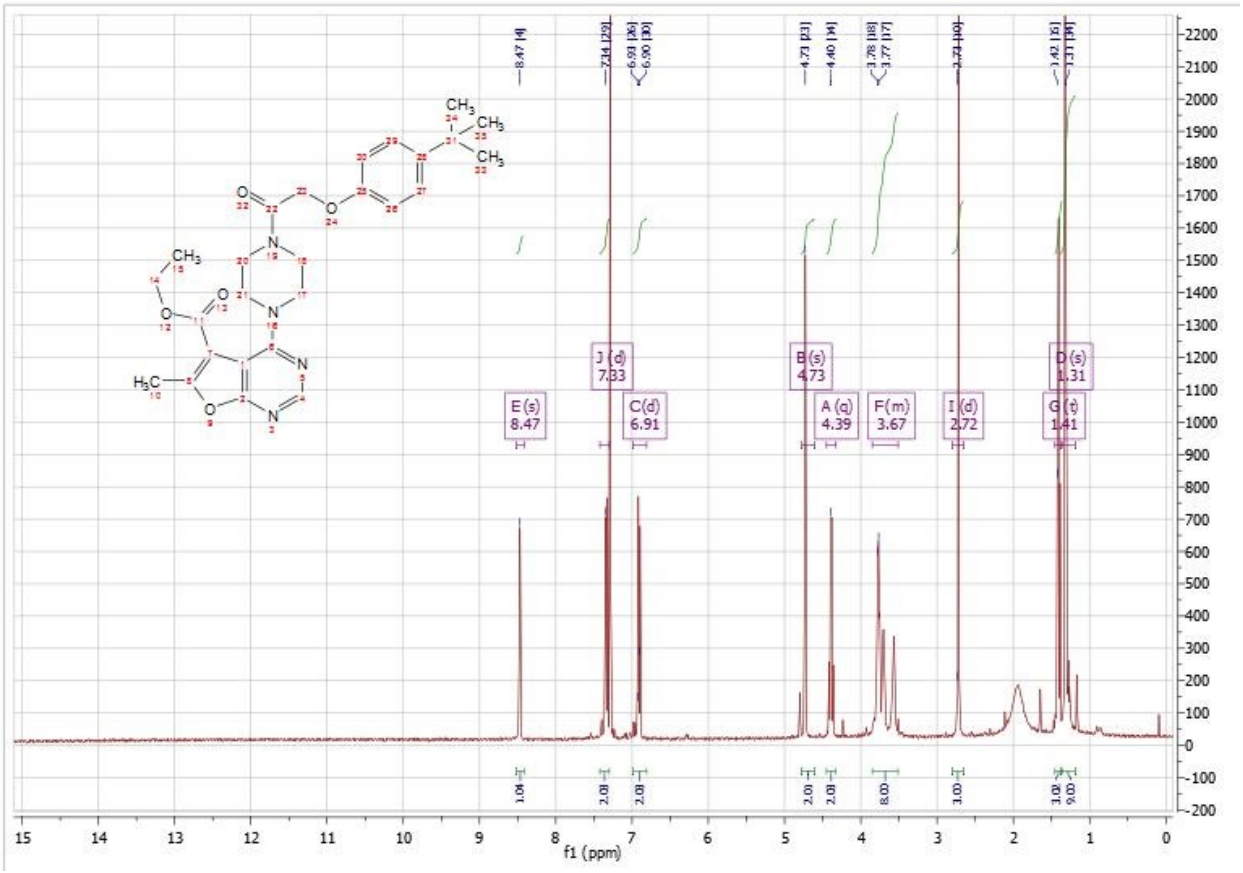


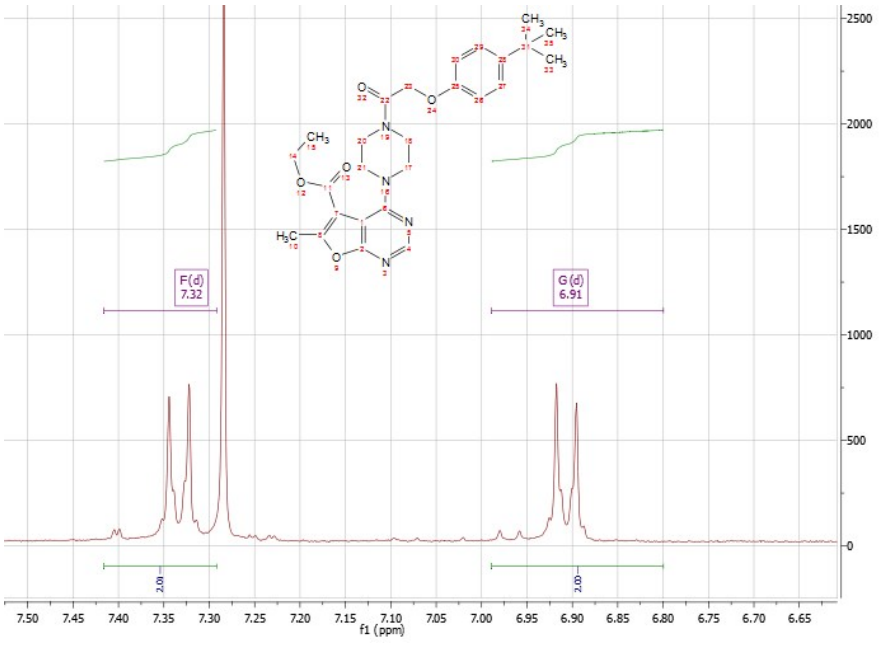
Ethyl 6-methyl-4-(4-(2-(4-(methylthio)phenoxy)acetyl)piperazin-1-yl)furo[2,3-d]pyrimidine-5-carboxylate (7b)

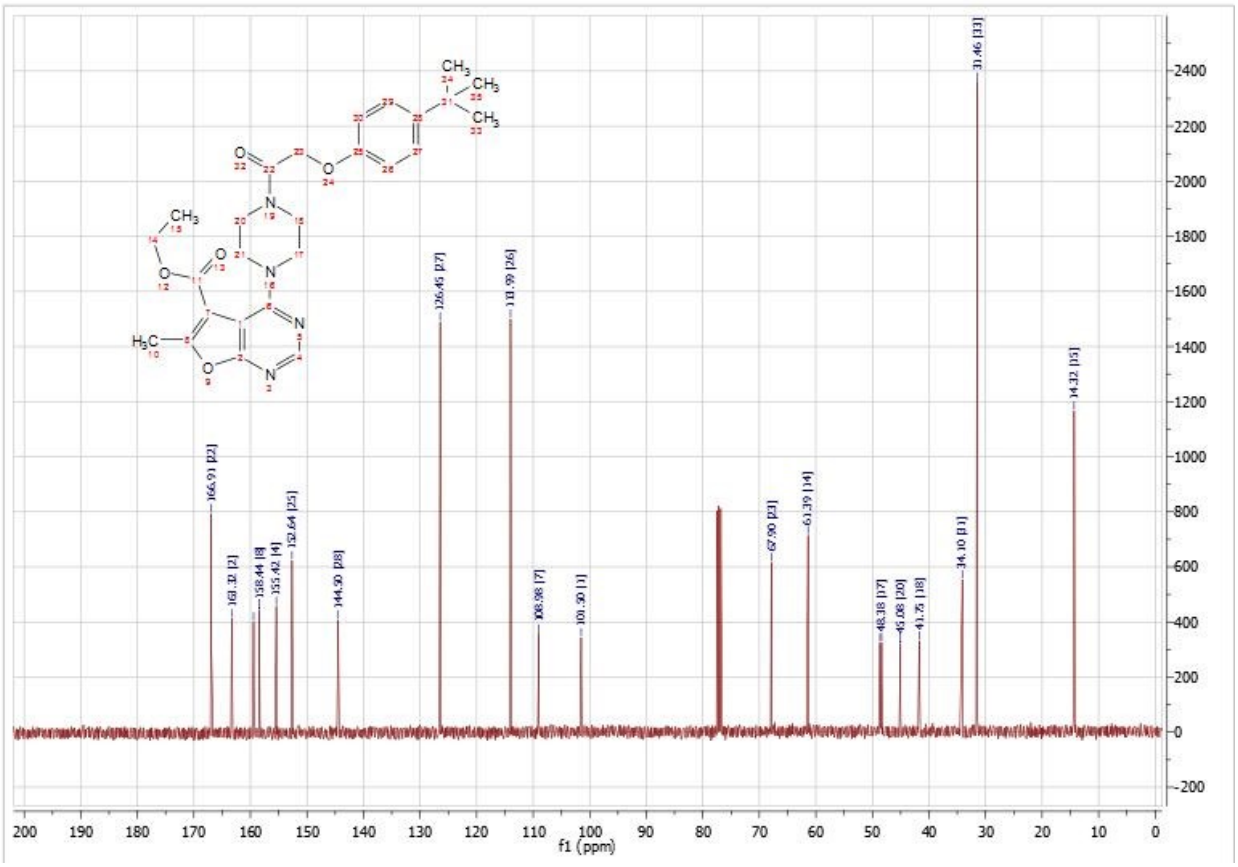

I (s)
8.43
E (d)
7.27
D (d)
6.92
J (s)
4.73
A (dd)
4.37
C (m)
3.63
F (s)
2.70
G (s)
2.46
H (t)
1.40
f1 (ppm)


166.60 [23]
163.29 [14]
159.31 [1]
156.11 [4]
152.57 [12]
130.43 [28]
129.81 [29]
115.28 [10]
108.99 [3]
101.61 [2]
67.99 [22]
61.45 [9]
48.41 [16]
45.07 [19]
41.82 [17]
29.70
17.59 [33]
14.39 [11]
f1 (ppm)

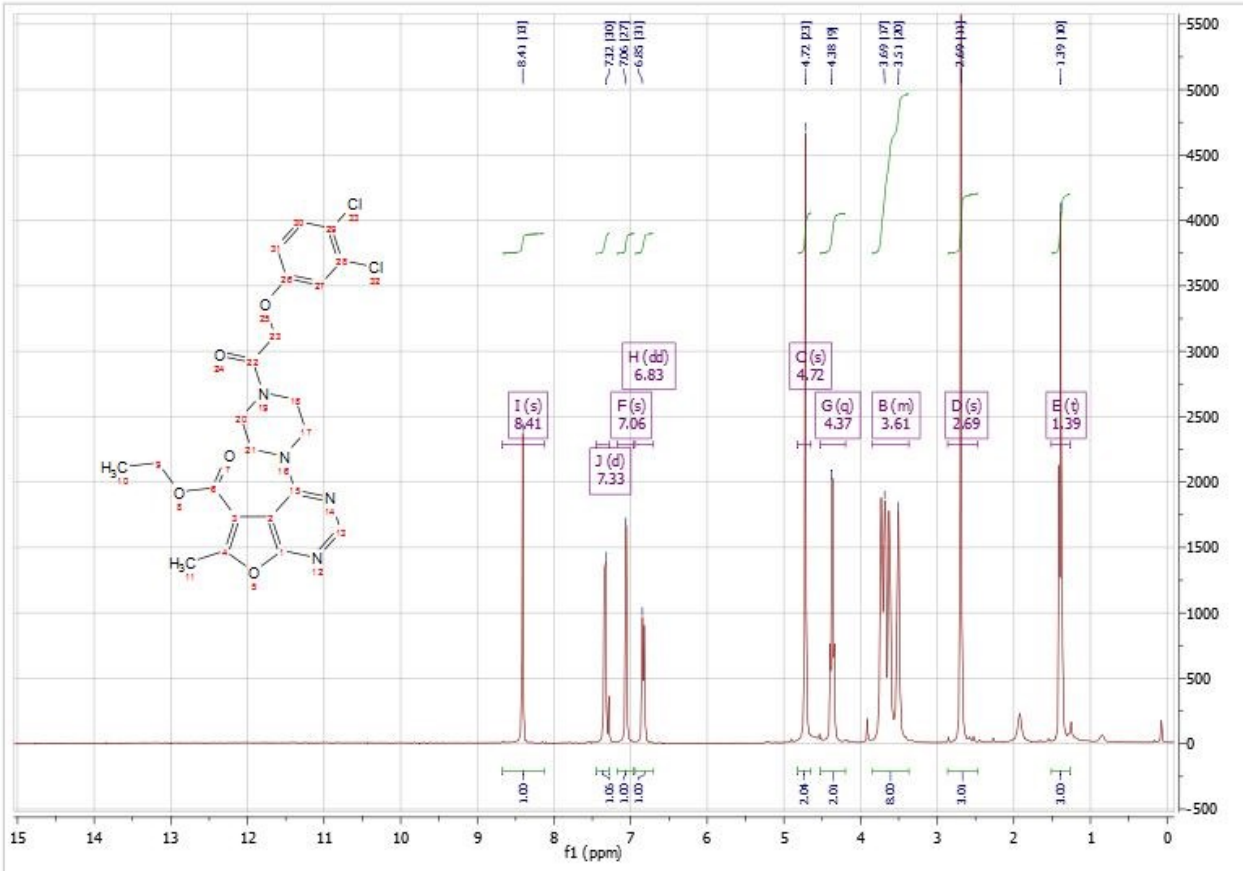
Ethyl 4-(4-(2-(4-(tert-butyl)phenoxy)acetyl)piperazin-1-yl)-6-methylfuro[2,3-d]pyrimidine-5-carboxylate (7c)







Ethyl 4-(4-(2-(3,4-dichlorophenoxy)acetyl)piperazin-1-yl)-6-methylfuro[2,3-d]pyrimidine-5-carboxylate (7d)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H (dd)
6.83
F (s)
7.06
J (d)
7.33
C (s)
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G (q)
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B (m)
3.61
D (s)
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E (t)
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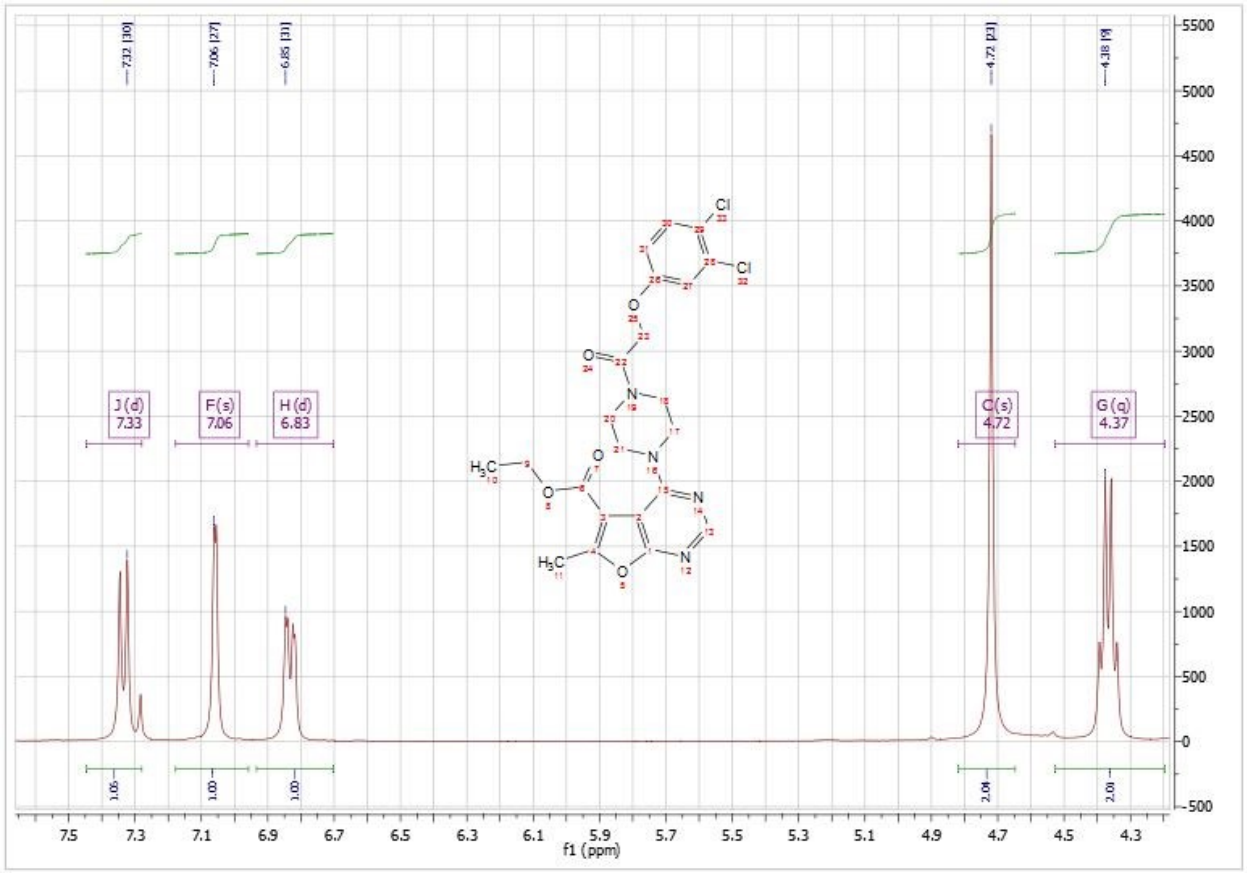
f1 (ppm)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F (s)
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C (s)
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G (q)
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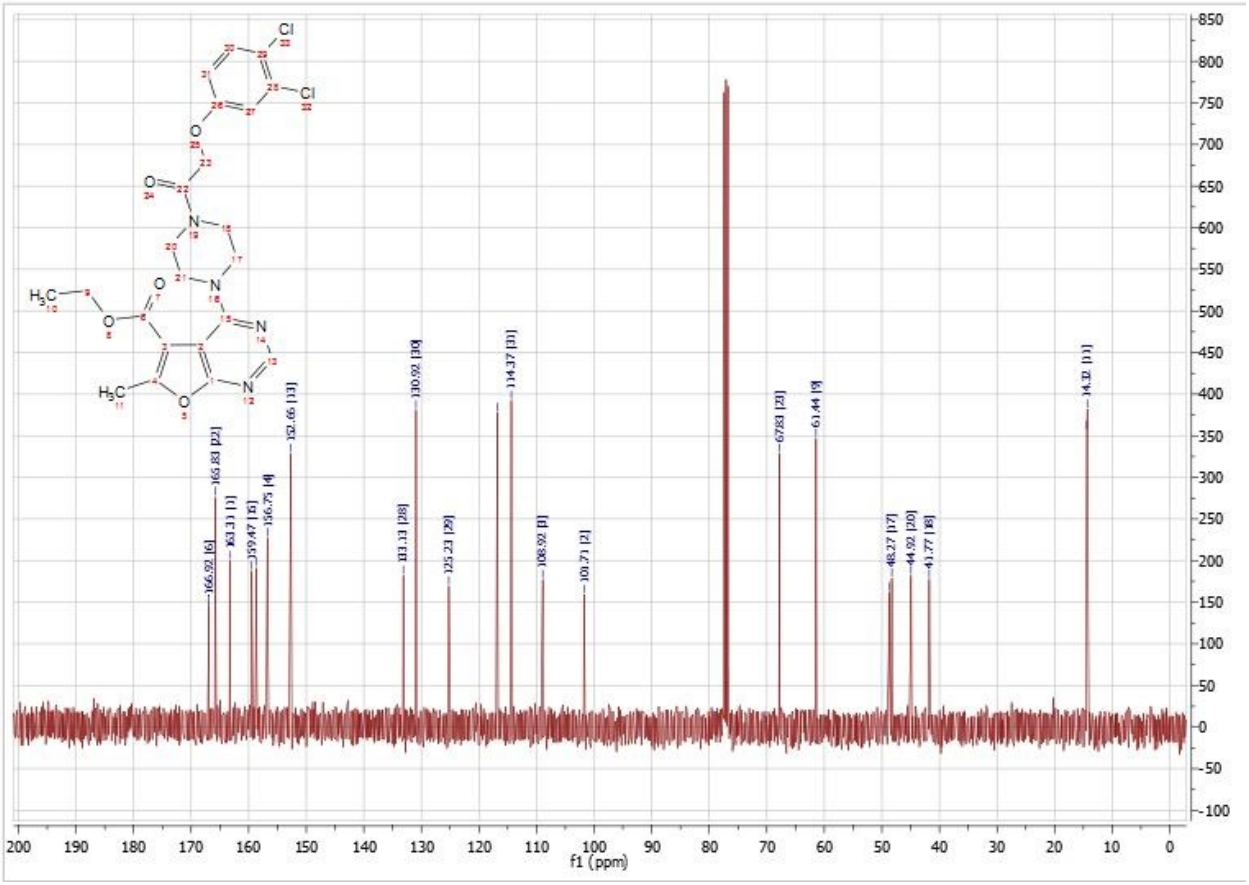
f1 (ppm)



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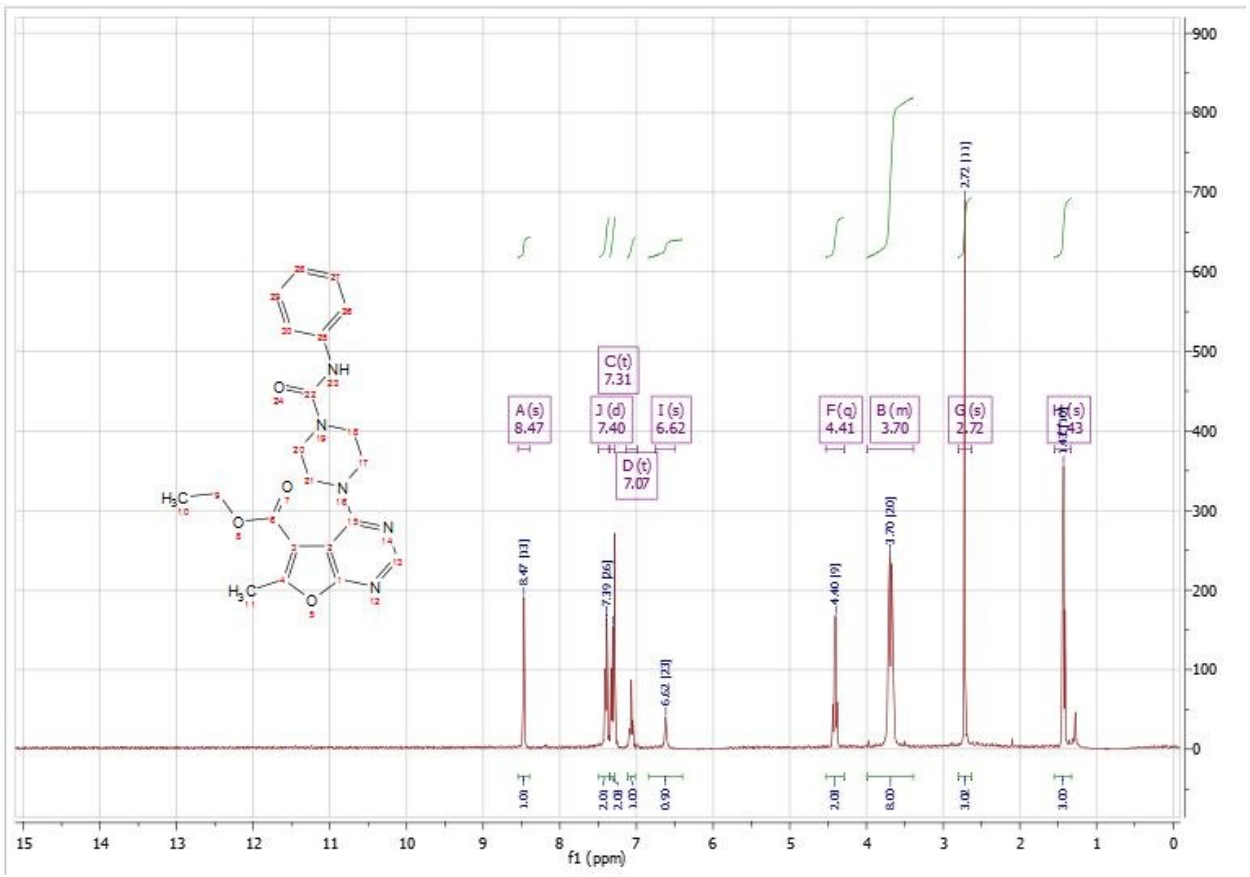
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G(s)
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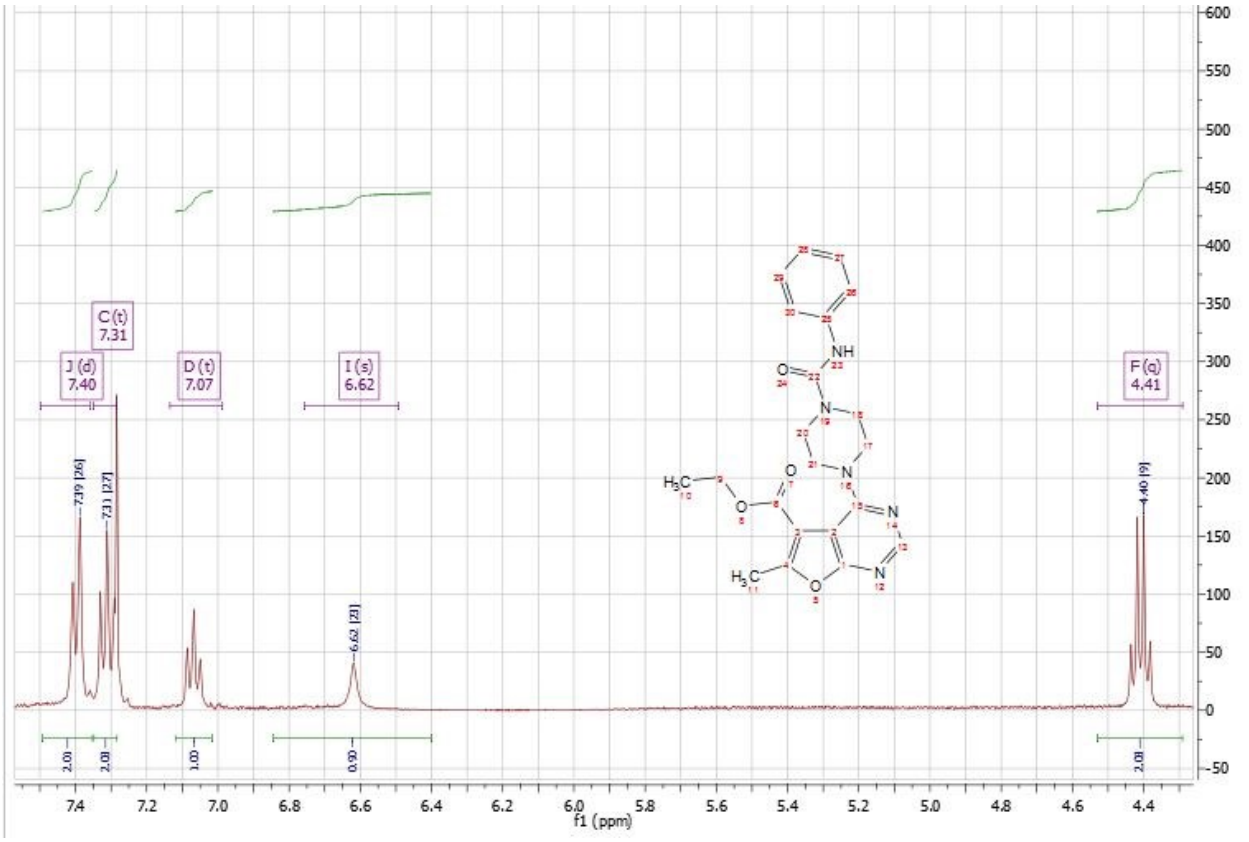
f1 (ppm)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I(s)
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F(q)
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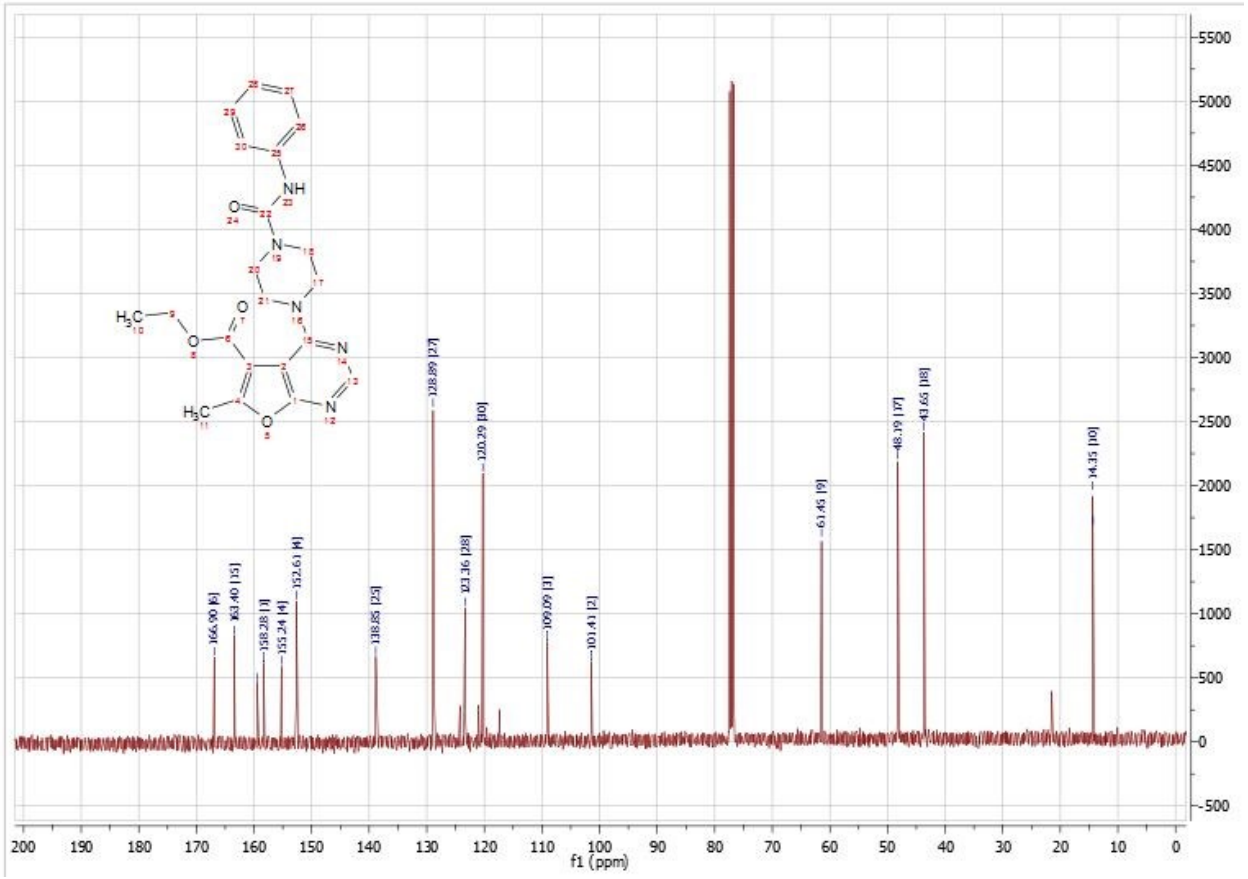
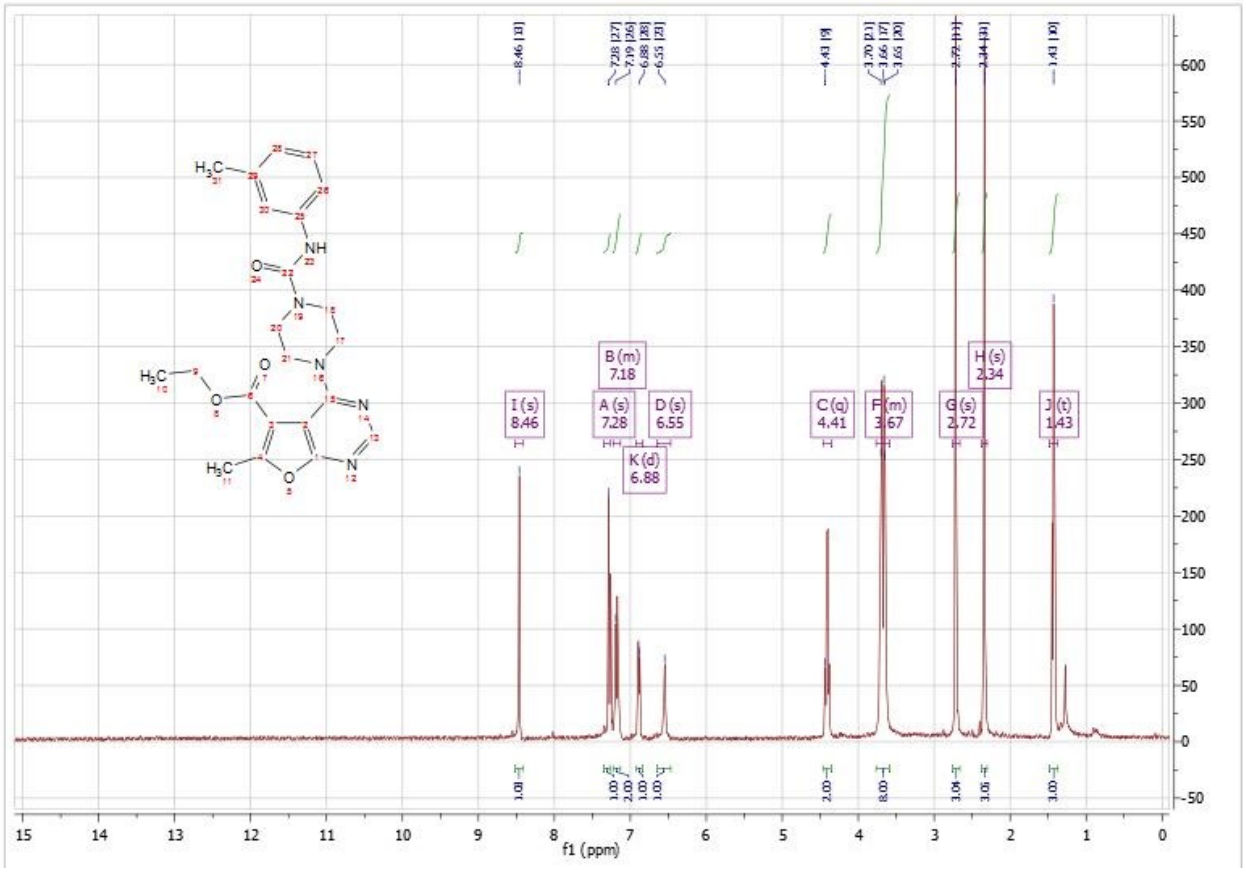
f1 (ppm)



Ethyl 6-methyl-4-(4-(m-tolylcarbamoyl)piperazin-1-yl)furo[2,3-d]pyrimidine-5-carboxylate (8b)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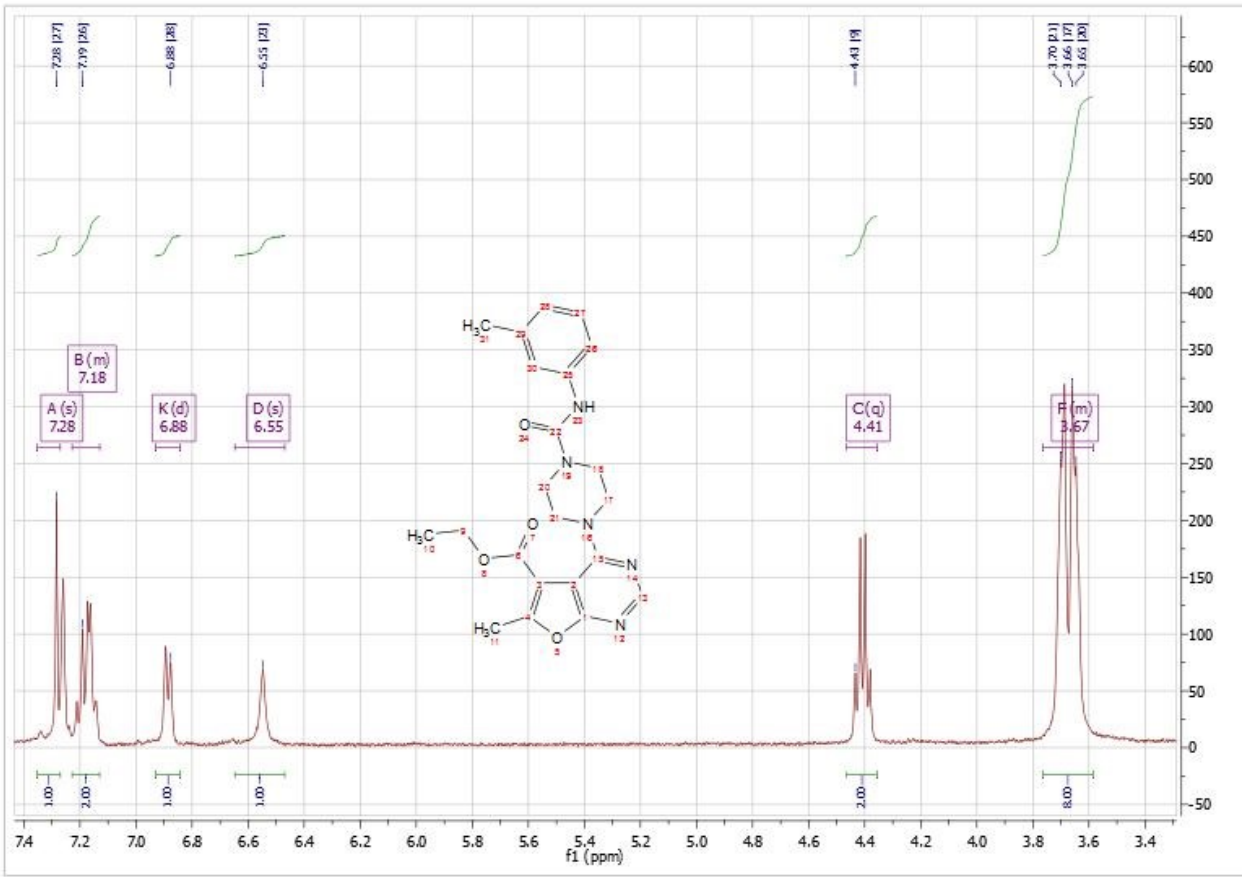
J (t)
1.43
f1 (ppm)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B (m)
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F (m)
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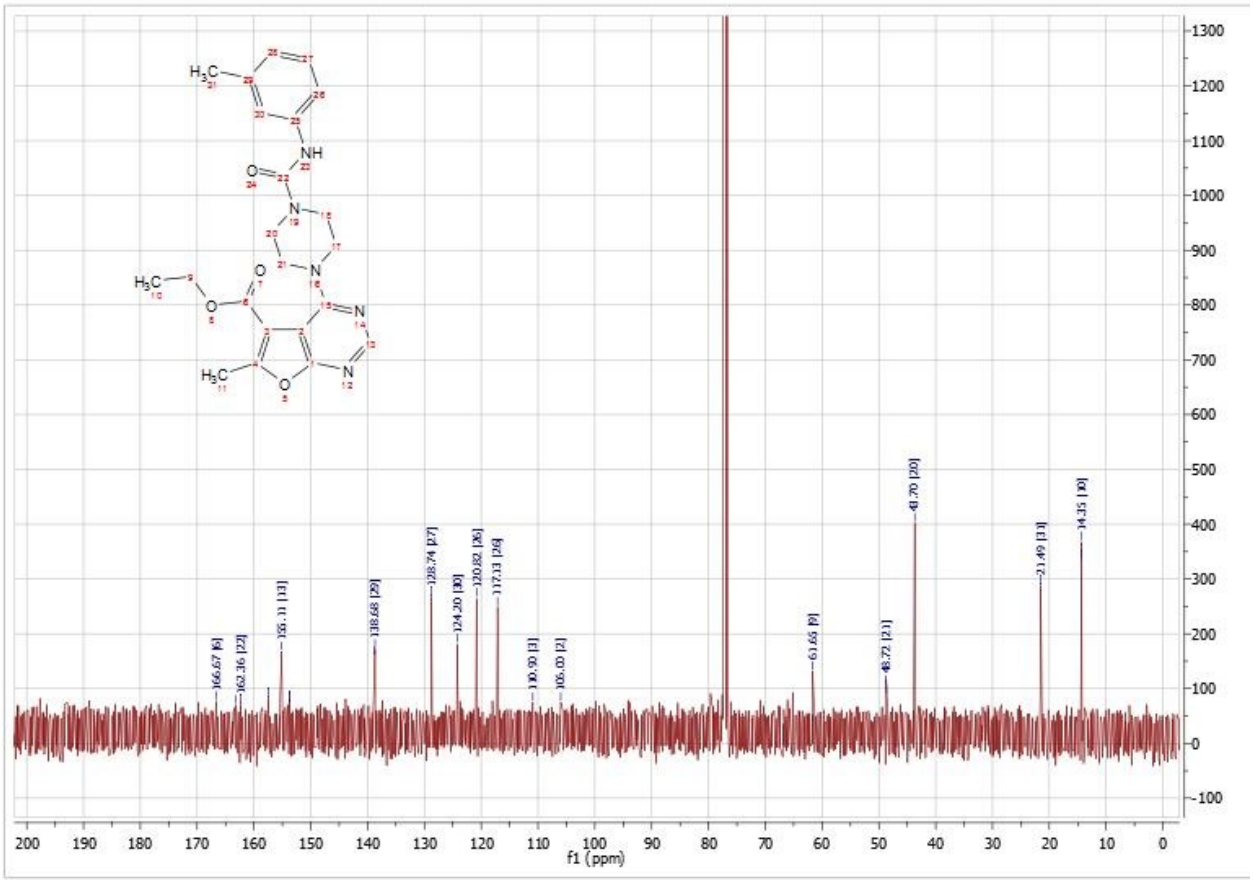
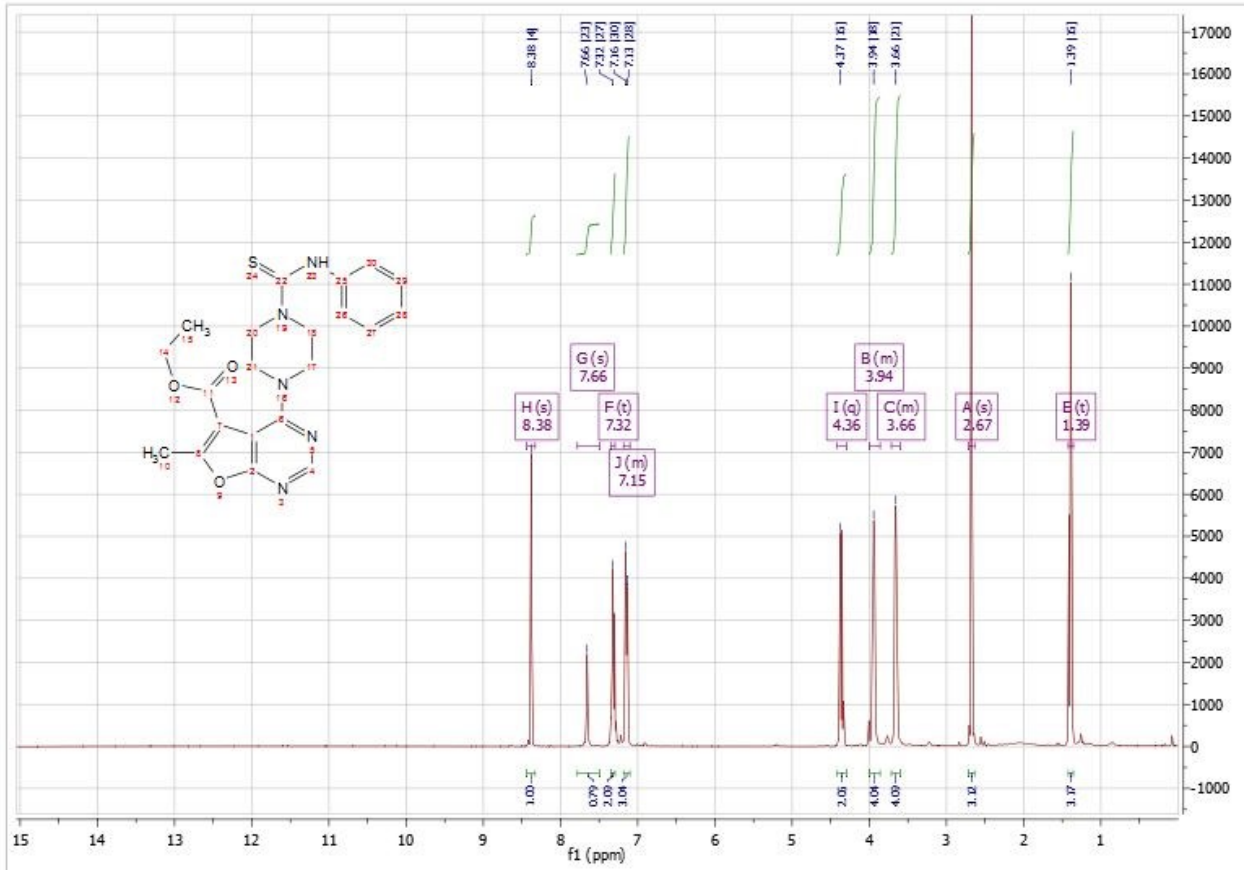
f1 (ppm)



Ethyl 6-methyl-4-(4-(phenylcarbamothioyl)piperazin-1-yl)furo[2,3-d]pyrimidine-5-carboxylate (8c)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G (s)
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J (m)
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I (q)
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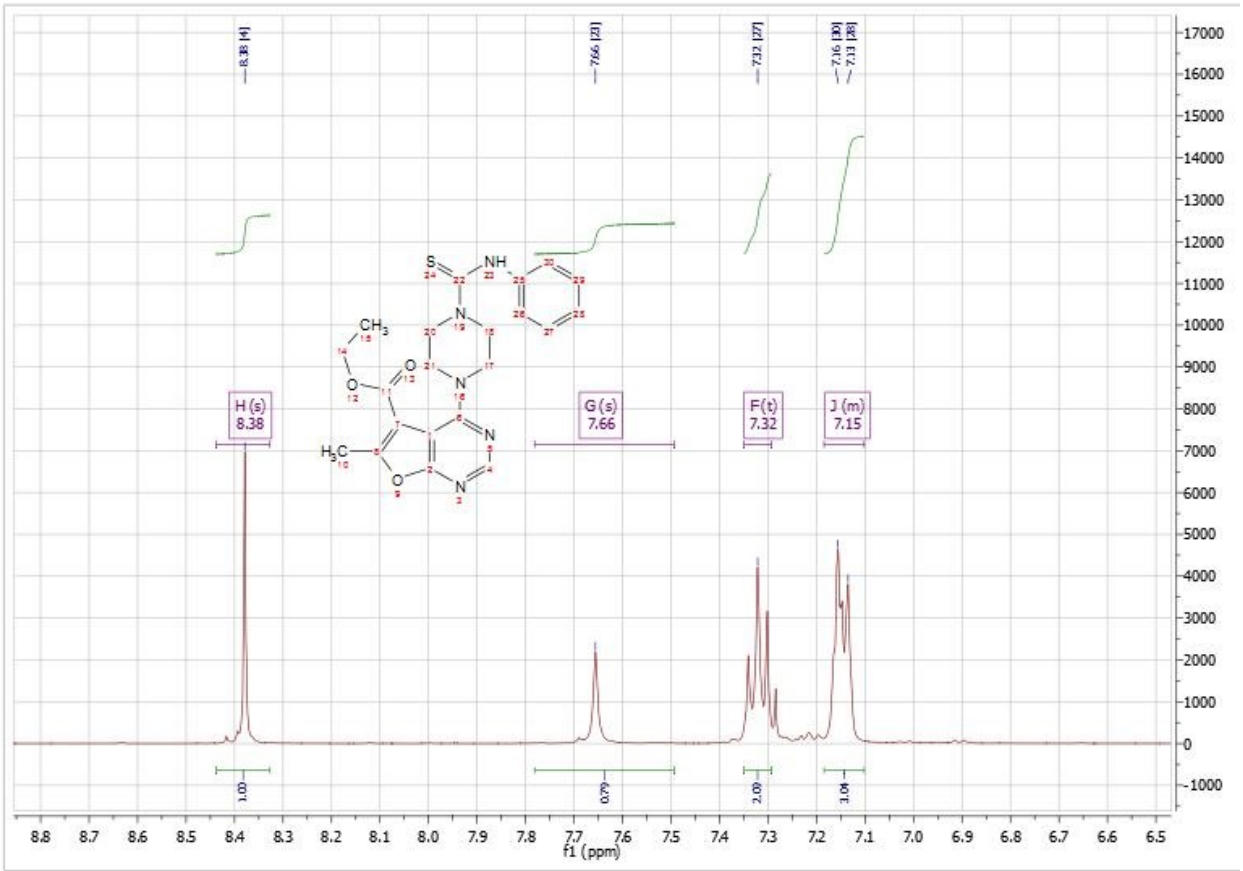
C (m)
3.66
B (m)
3.94
A (s)
2.67
E (t)
1.39
f1 (ppm)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G (s)
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F (t)
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J (m)
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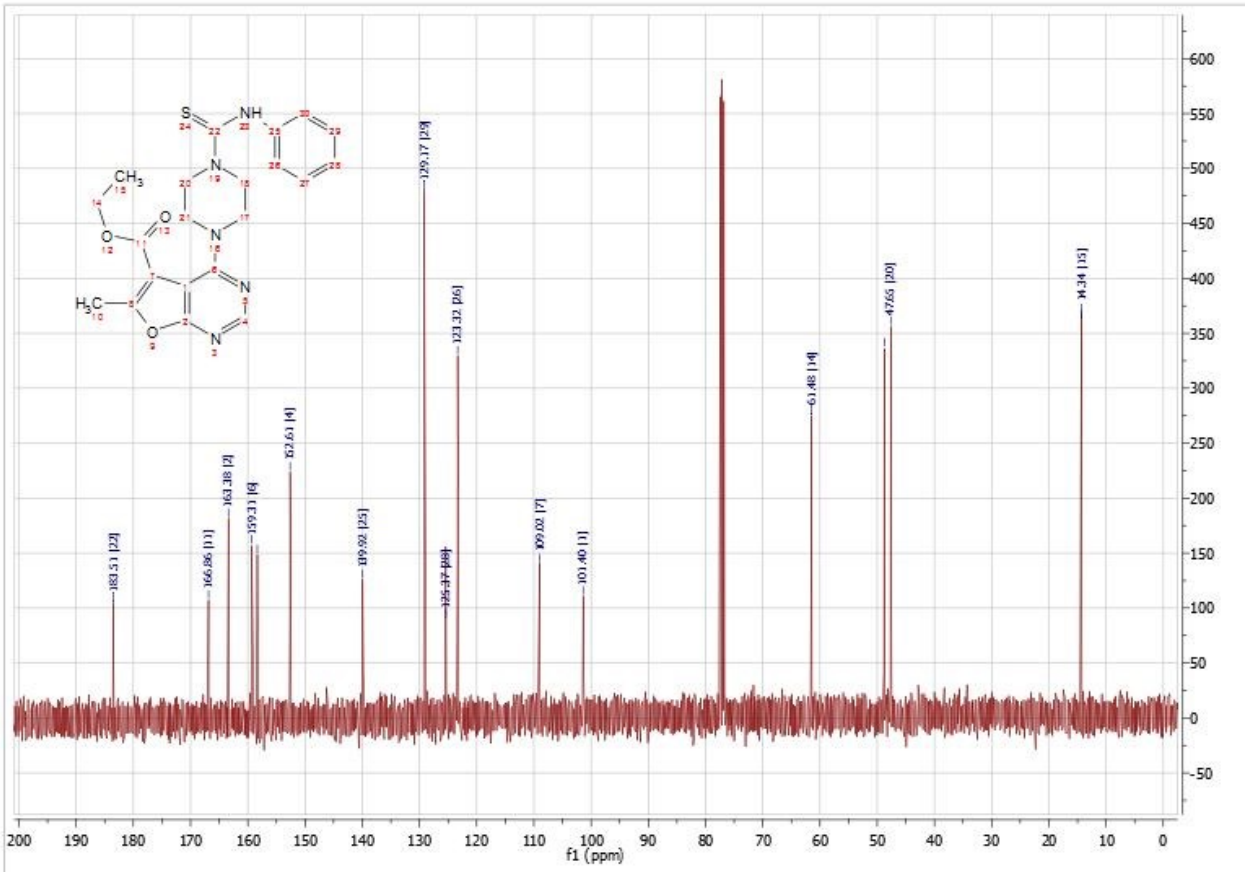
f1 (ppm)



Biological Evaluation assay

In vitro PI3K-α activity

Assay protocol

Assay was performed using the Adapta universal kinase assay, fluorescent based immunoassay detecting ADP using Alexa Fluor™ 647 labeled ADP tracer, ADP formed by the kinase reaction displaces the Alexa Fluor 647 labeled ADP tracer from the antibody, resulting in a decrease in the TR-FRET signal. Determination of the formed ADP is established by calculating the emission ratio by dividing the intensity of the tracer (acceptor) emission by the intensity of the Eu (donor) emission at 615 nm as shown in the equation below.

Test Compounds are screened in 1% DMSO (final) in the well. For 10 point titrations, 3-fold serial dilutions are conducted from the starting concentration of the customer's choosing.

Substrate/Kinase Mixtures; All Substrate/Kinase Mixtures are diluted to a 2X working concentration in the appropriate Kinase Buffer, Lipid substrates are prepared by creating large unilamellar lipid vesicles (LUVs) including a carrier lipid, phosphatidylserine (PS), with a ration "PIP2:PS" of five mole percent L-α-Phosphatidylinositol-4,5-bisphosphate and ninety-five percent phosphatidylserine.

ATP Solution; All ATP Solutions are diluted to a 4X working concentration in water.

ATP Km apparent is previously determined using a radiometric assay

Detection Mix the Detection Mix is prepared in TR-FRET Dilution Buffer, it consists of EDTA (30 mM), Eu-anti-ADP antibody (6 nM) and ADP tracer. It also contains the EC60 concentration of tracer for 5-150 mM ATP.

Assay Protocol;Bar-coded Corning, low volume, white 384-well plate (Corning Cat. #4512)

1. 100 nL – 100X Test Compound in 100% DMSO
2. 2.4 µL – 30 mM HEPES
3. 2.5 µL – 4X ATP Solution
4. 5 µL – 2X Substrate/Kinase Mixture
5. 30-second plate shake
6. 1-minute centrifuge at 1000 x g
7. 60-minute Kinase Reaction incubation at room temperature
8. 5 µL – Detection Mix
9. 30-second plate shake
10. 1-minute centrifuge at 1000 x g
11. 60-minute Detection Mix equilibration at room temperature
12. Read on fluorescence plate reader and analyze the data

PIK3CA/PIK3R1 (p110 alpha/p85 alpha) The 2X PIK3CA/PIK3R1 (p110 alpha/p85 alpha) / PIP2:PS mixture is prepared in 50 mM HEPES pH 7.5, 100 mM NaCl, 0.03% CHAPS, 3 mM MgCl2, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.25 - 2 ng PIK3CA/PIK3R1 (p110 alpha/p85 alpha) and 50 µM PIP2: PS in 32.5 mM HEPES pH 7.5, 50 mM NaCl, 0.015% CHAPS, 1.5 mM MgCl2, 0.5 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of Detection Mix is added.

Data Analysis

ATP/ADP standard curves (SC) are used to calculate the percent ATP conversion of each sample.

Emission Ratio= AF647 Emission (665 nm)/ Europium Emission (615 nm)

$$\% \text{ Conversion} = \left\{ \frac{EC_{50\ SC}}{\left(\frac{Top_{SC} - Bottom_{SC}}{Emission\ Ratio_{Sample} - Bottom_{SC}}\right) - 1\ \hat{}\left(\frac{1}{Hillslope_{SC}}\right)} \right\} * 100$$

$$\% \text{ inhibition} = \left\{ 1 - \frac{\%\ Conversion_{Sample}}{\%\ Conversion_{0\%\ Inhibition\ Ctrl}} \right\} * 100$$

Difference between data Points = $\left|\%\ \text{Inhibition Point 1} - \%\ \text{Inhibition Point 2}\right|$

Test Compound Interference; for each emission wavelength, fluorescence interference is flagged for a compound well that is more than 20% outside the range of the controls.

$$Z' \text{ (using Emission Ratio values)} = 1 - \frac{3 * \text{Stdev}_{0\% \text{ Conv Ctrl}} + 3 * \text{Stdev}_{0\% \text{ Inhibition}}}{|\text{Mean}_{0\% \text{Conv Ctrl}} - \text{Mean}_{0\% \text{ Inhibition}}|}$$

In vitro PANC-1 Anti-proliferative assay

Assay protocol

Paclitaxel a known cancer treatment, is used as control compound. Cells were maintained in RPMI mediasupplemented with 100mg/mL of streptomycin,100units/mLof penicillin and10% of heat –inactivated fetal bovine serum inhumidified, 5% (v/v) CO_2 atmosphere at 37 °C. Cell viability was assessed by SRB assay. Aliquots of 100 µL cell suspension (5x10^3cells) were in 96 well plate sand incubated in complete media for 24h. Cells were treated with another aliquot of 100µL media containing drugs at various concentrations ranging from (0.01, 0.1, 1, 10, 100 µm) . After 72h of drug exposure, cells were fixed by replacing media with 150 µL of 10% TCA and incubated at 4°C for 1h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70µL SRB solution (0.4%w/v) were added and incubated in a dark place at room temperature for 10min. Plates were washed 3 times with 1% acetic acid and allowed to air dry overnight. Then, 150 µL of TRIS (10mM) was added to dissolve protein – bound SRBstain; the absorbance was measured at 540 nm using a BMG LABTECH®-FLUOstar Omega microplate reader (Ortenberg, Germany). [1][2]

In-vitro anti-proliferative activity assay against NCI 60-cell line panel

The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37° C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of

drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37°C, 5 % CO2, 95 % air, and 100 % relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 μl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4 % (w/v) in 1 % acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μl of 80 % TCA (final concentration, 16 % TCA).

Data Analysis

Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as: [(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz [(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

Molecular Modeling study

Molecular modeling was conducted using C-Docker 2.5 software in the interface of Accelry's discovery studio 2.5 (Accelrys Inc., San Diego, CA, USA) at Faculty of pharmacy, Ain Shams university, drug design laboratory.

The X-ray crystal structure of PI3K-α co-crystallized with imidazopyridine- based Compound (I) was obtained from the Protein Data Bank at the Research Collaboration for Structural

Bioinformatics (RCSB) website [www.rcsb.org] (PDB code: 4ZOP) and loaded in Accelry's discovery studio 2.5.

Preparation of the enzyme:

- Open the PDB file of the enzyme in Discovery Studio.
- Delete the unwanted molecules.
- From Tools/ Protein report and utilities/ clean protein. (This step is performed to add hydrogen to the structure of the enzyme and to fix any missing side chains of the amino acids).
- From Tools/ Simulate structure/ Forcefield/ Apply CHARMm forcefield. Due to the addition of hydrogens, clashes occur that should be minimized using a minimization protocol, but before applying this protocol, constraints are applied to the enzyme to keep its 3D structure unchanged.
- From Edit/ Select/ element that doesn't equal hydrogen/ create heavy atoms group.
- From Tools/ Simulate structure/ Create fixed atom constraints on the heavy atoms group.
- From Protocols/ Simulation/ Minimization/ Run.

Identifying the binding pocket:

This step is performed to identify the binding pocket together with the surrounding amino acid residues.

- From Tools/ Define and edit binding site/ Define protein as receptor.
- Select ligand/ Define sphere from selection. The sphere radius is adjusted to ensure that the active site region is covered.

Display lead- protein interactions:

- From Scripts/ Ligand interactions/ show ligand binding site atom. (This step displays the 3D interaction diagram)
- From tools/ Analyze binding site/ Analyze ligand interactions/ Draw ligand interaction diagram/ Draw diagram. (This step displays the 2D interaction diagram).

Docking of the test set: Loading the test-set molecules:

- The test set molecules and the lead compound structures were constructed in ChemDraw, saved as file.mol then loaded in Discovery Studio.

- From Chemistry/ Hydrogens/ add.
- Energy minimization of the test set using Protocol/ General Purpose/ Prepare ligands.
- The ionization pH was adjusted to 7.4, hydrogen atoms were added and no isomers or tautomers were generated from the ligands.

Interactive Docking:

- Once the receptor, ligands and active site have been correctly set up, then the docking procedure can begin.
- From Protocol/ Receptor ligand interaction/ CDOCKER/ Run the protocol.

Validation of the docking process and selection of proper binding pose:

Two validation methods were carried out

a. Comparison of the binding mode of lead compound obtained before docking with the 10 docked poses and selection of the pose with binding pattern similar to the lead before docking.

b. Alignment of the x-ray bioactive conformation of the lead with the best fitted pose of the same compound and calculate the RMSD, from structure/ RMSD.

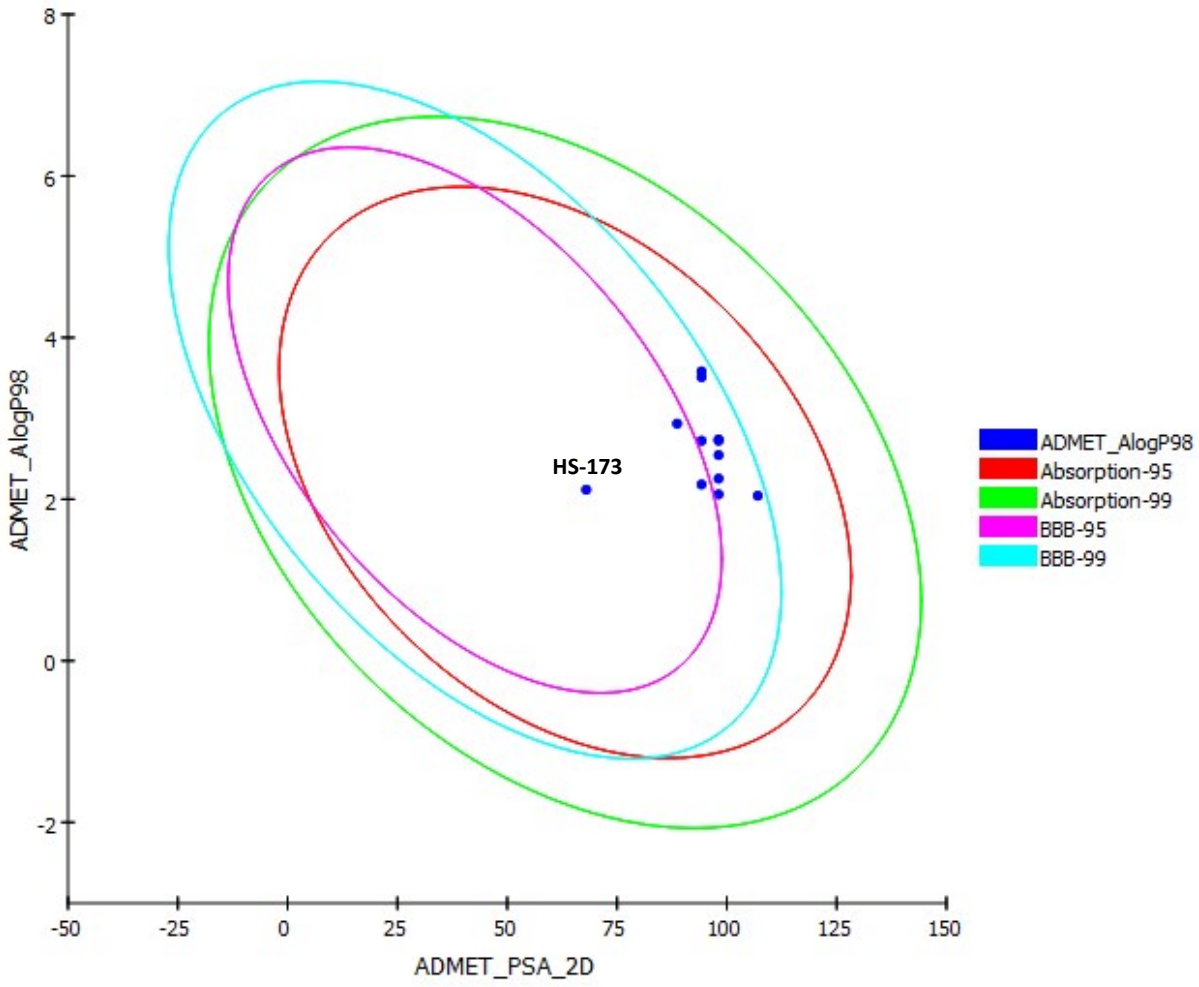
Displaying the docking scores:

- After running the protocol, a report is given about the docked compounds with their docking scores. Ten docking poses were generated for each ligand docked and were thoroughly inspected for getting the best binding mode. The top ranked poses were selected and investigated.
- The docking scores are displayed in energy terms (-CDOCKER Energy). The higher the score (in negative terms), the better the binding affinity.

Displaying the binding pattern of the tested compounds:

- Tools/ Receptor ligand interaction/ Analyze binding site/ Draw ligand interaction diagram.
- For each test compound, the ideal pose in each case is that which have interactions similar to the binding mode of the lead compound.

***In silico* Predictive ADME study for targeted compounds and HS-173**



References:

[1] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 82(13): 1107-1112

[2]Allam RM, Al-Abd AM, Khedr A, Sharaf OA, et al. (2018). Fingolimod interrupts the cross talk between estrogen metabolism and sphingolipid metabolism within prostate cancer cells. Toxicology Letters Apr 11; 291:77-85.