Reprofiling of pyrimidine-based DAPK1/CSF1R dual inhibitors: Identification of 2,5-diamino-4-pyrimidinol derivatives as novel potential anticancer lead compounds

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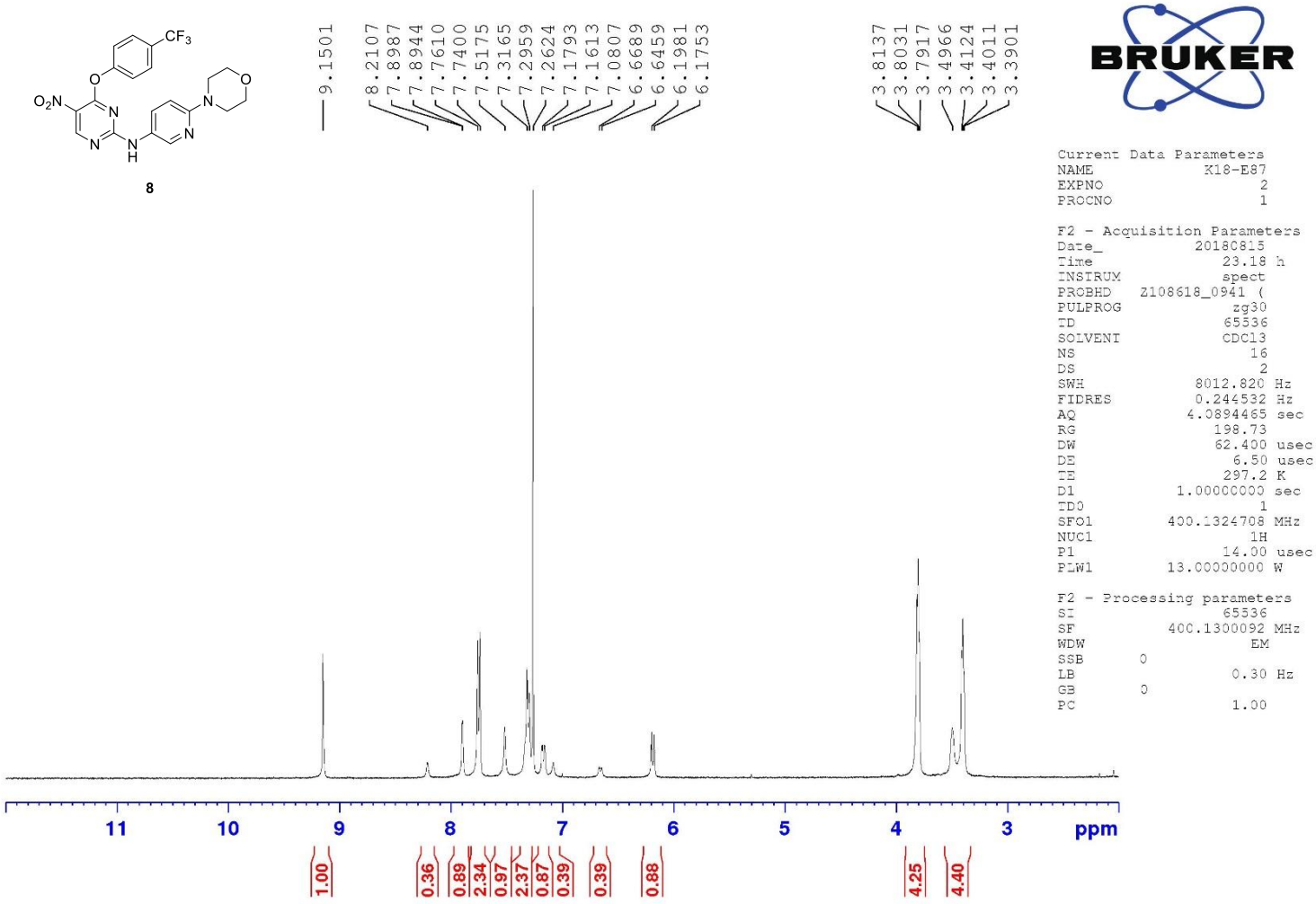
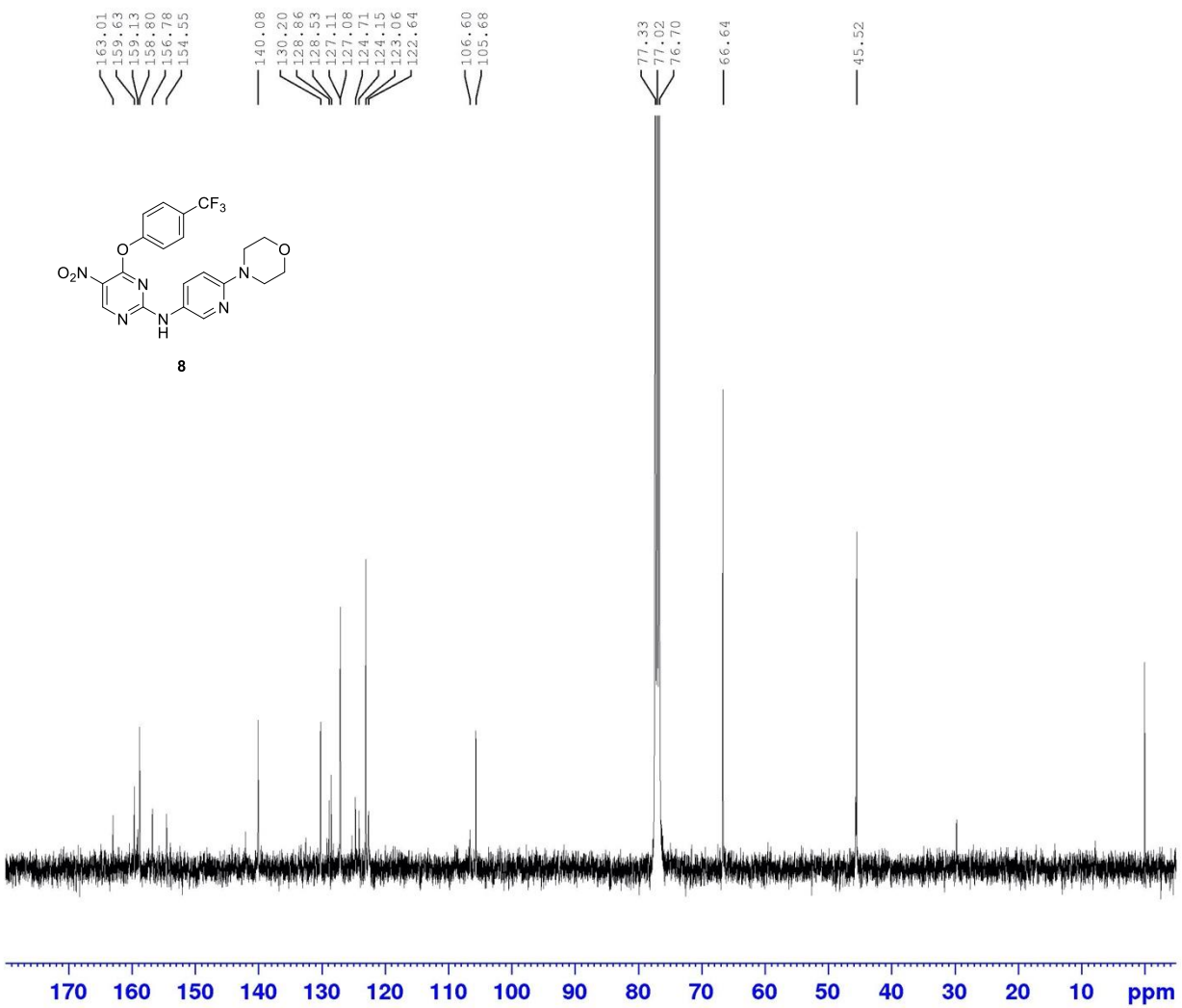
Contents

1. Representative spectral data for the novel compounds.





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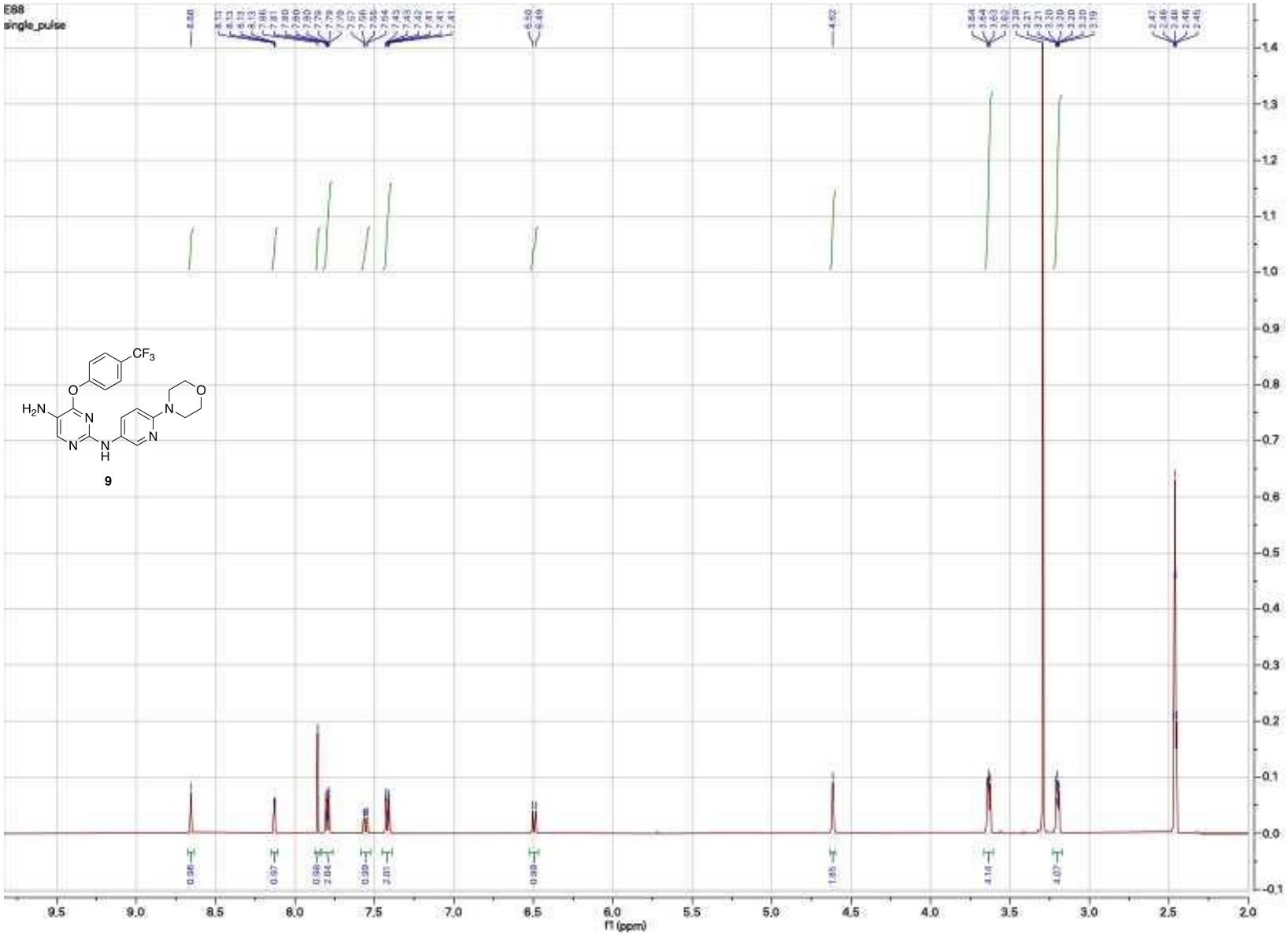
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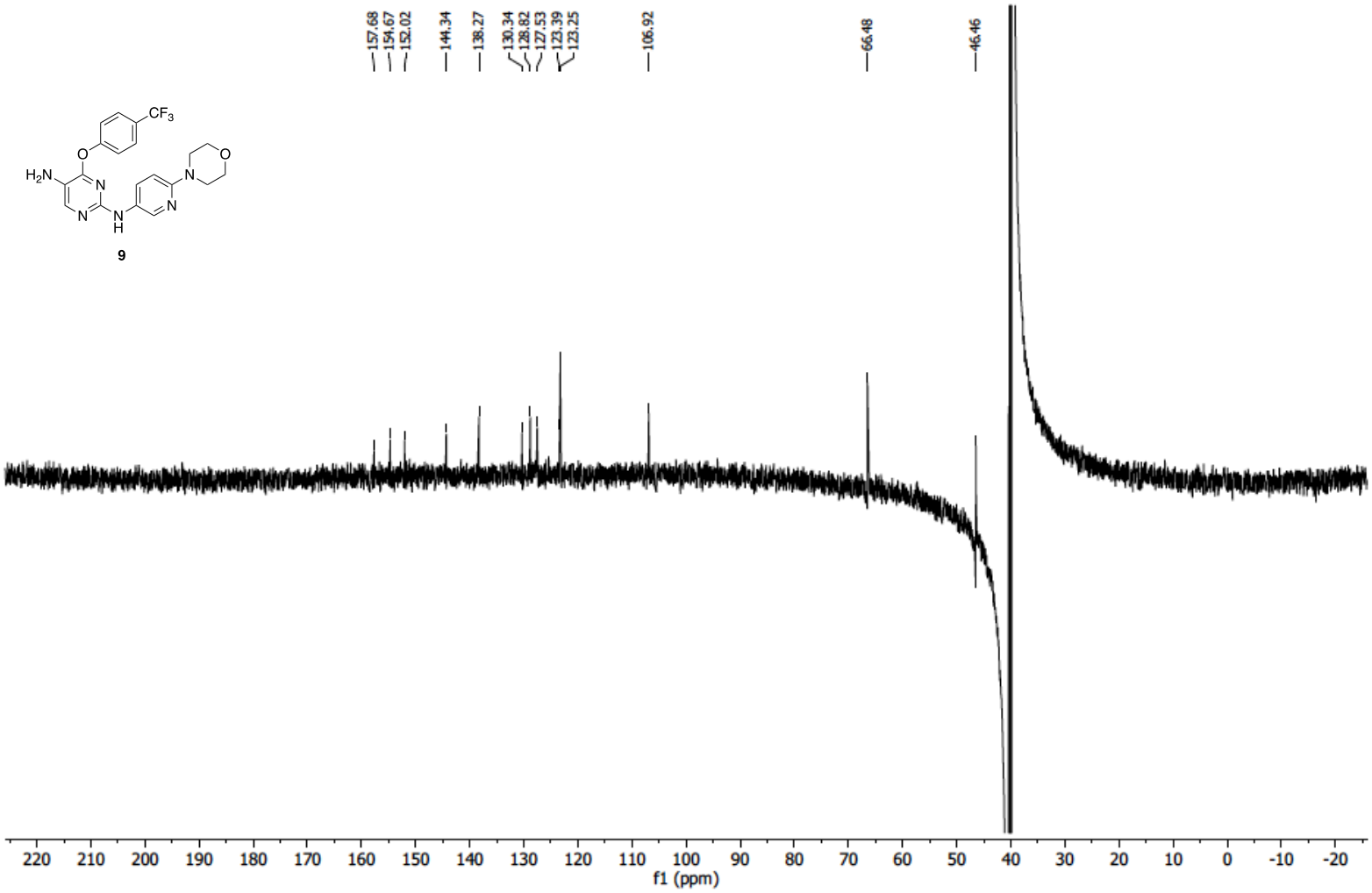
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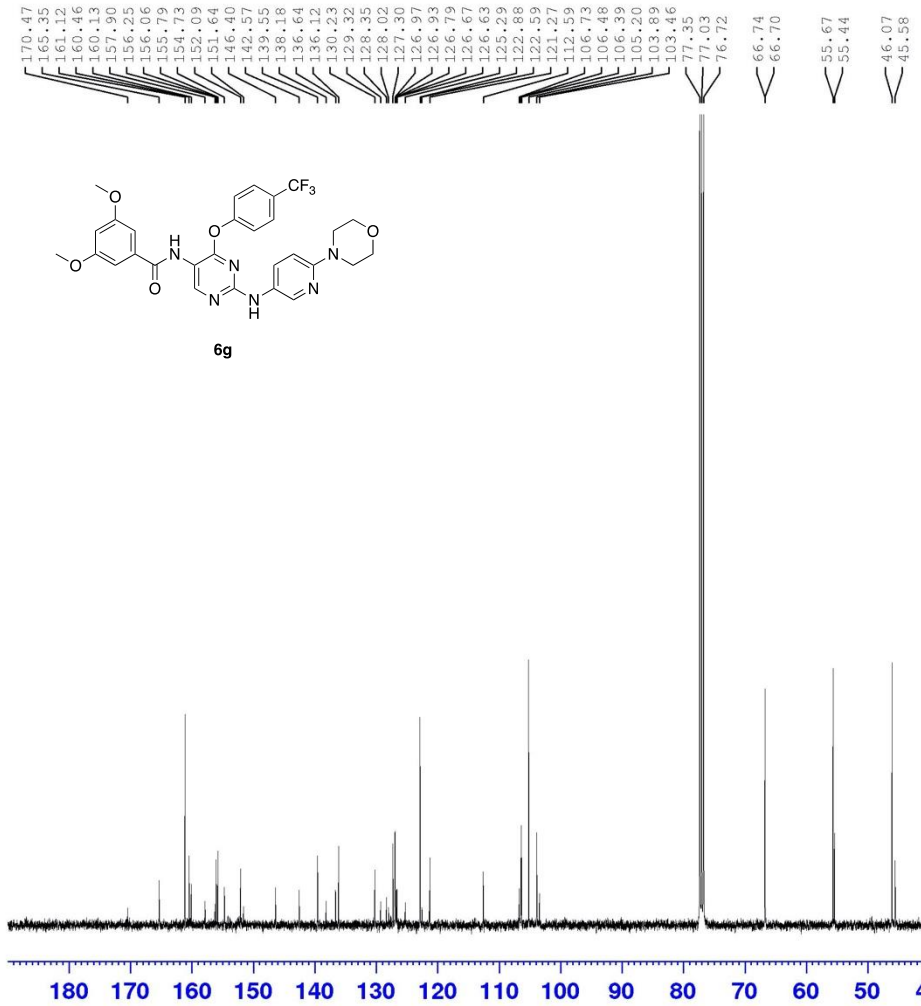

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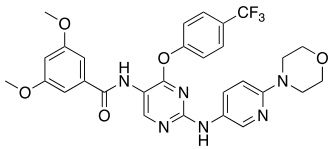

6g

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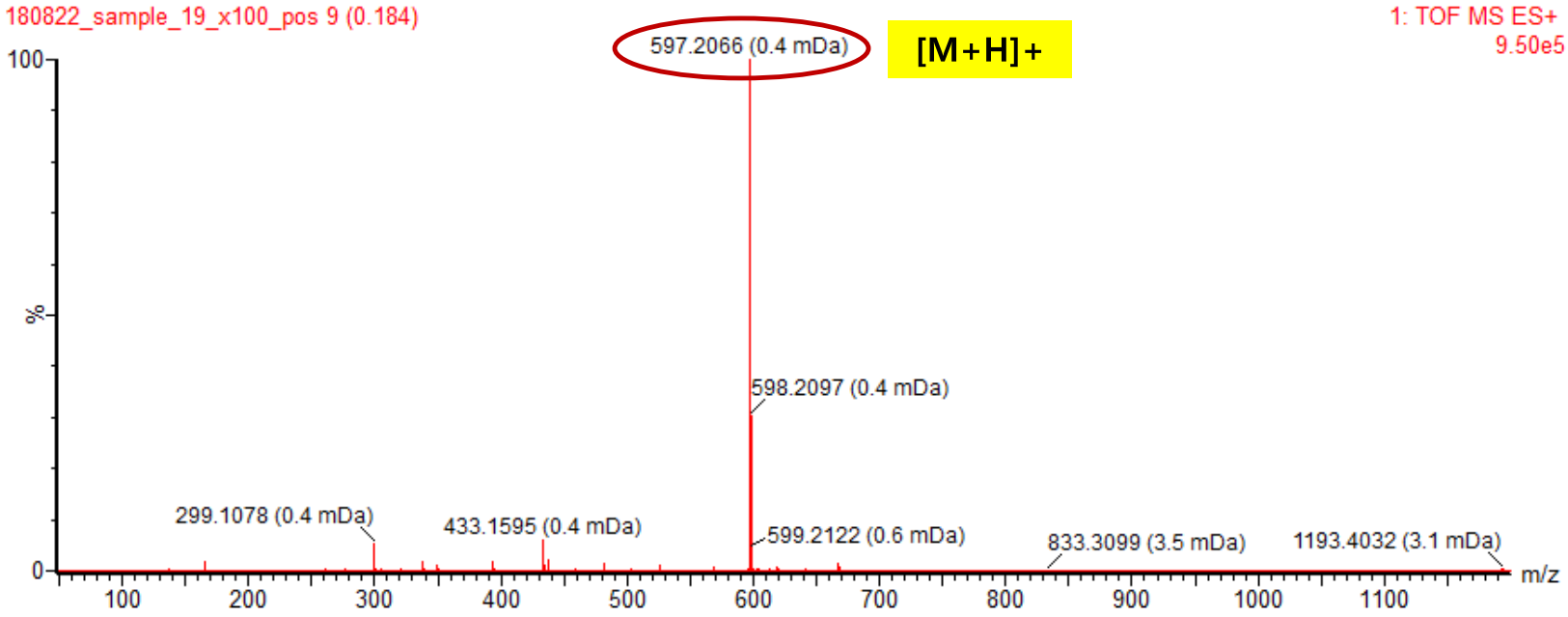

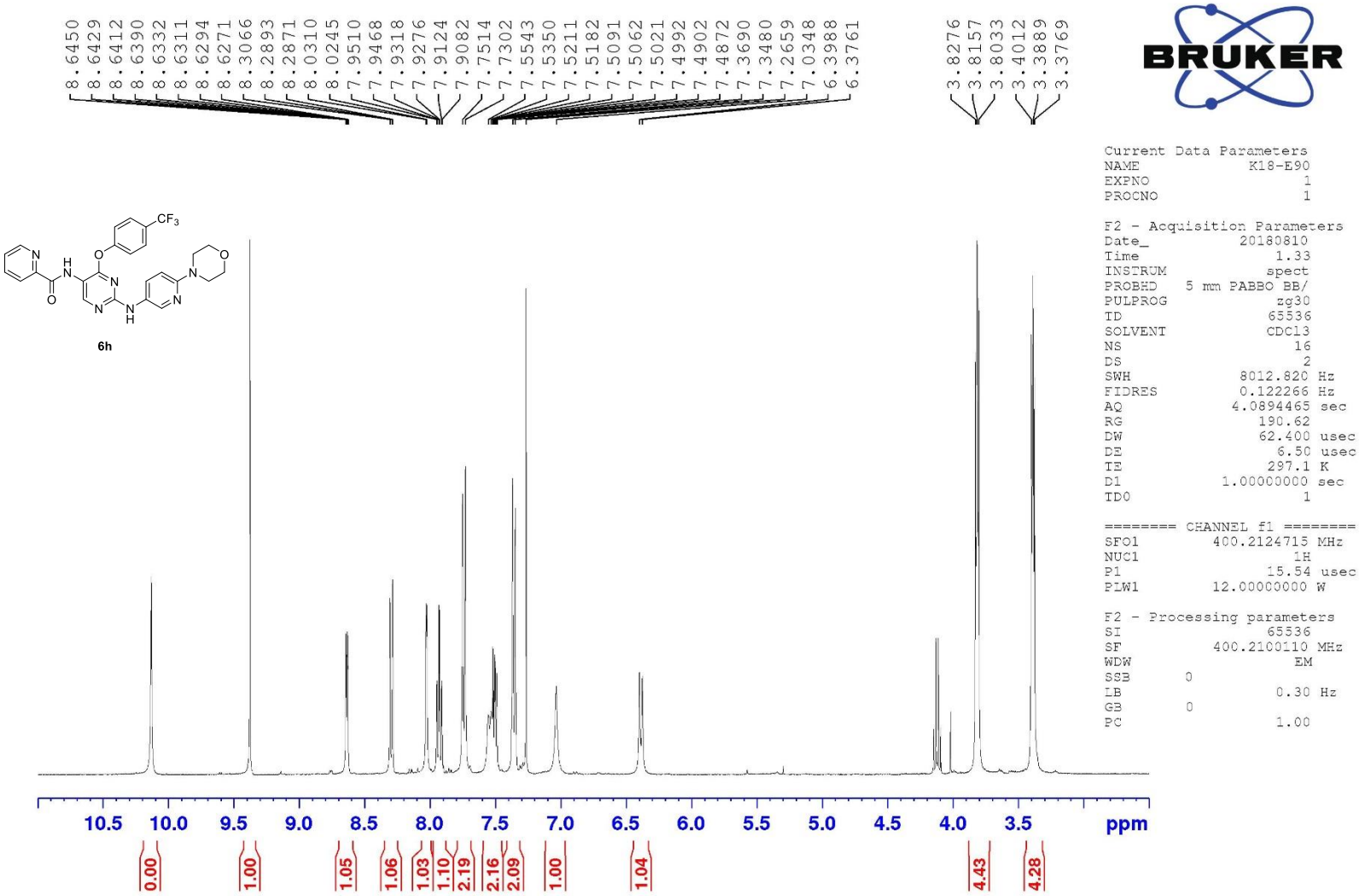
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BRUKER
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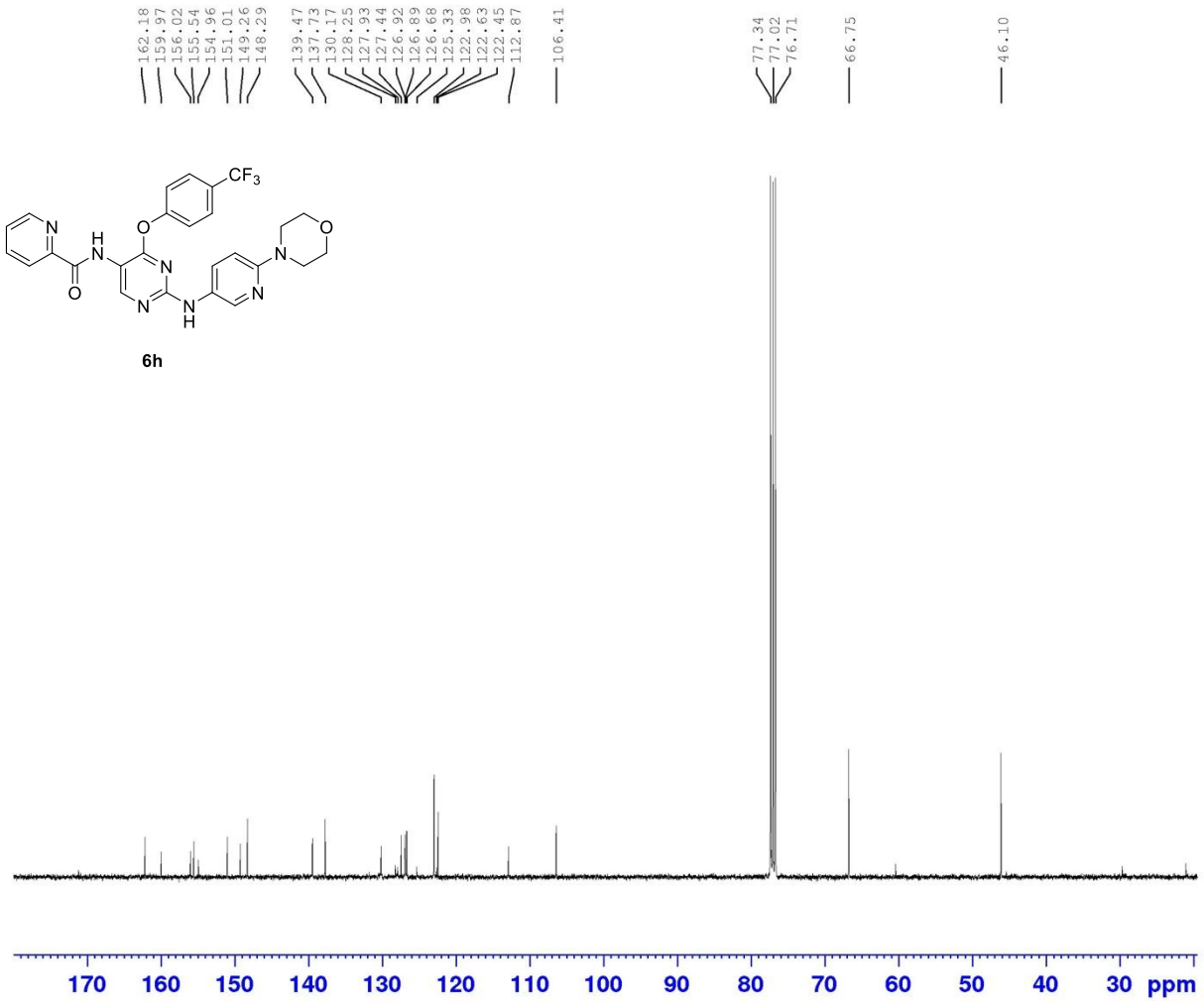

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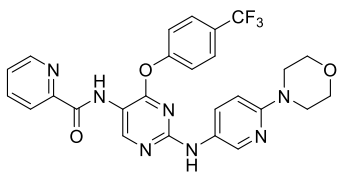
6h



6h

Molecular formula: $C_{26}H_{22}F_3N_7O_3$
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180.0657 (3+)
269.5946 (2+)
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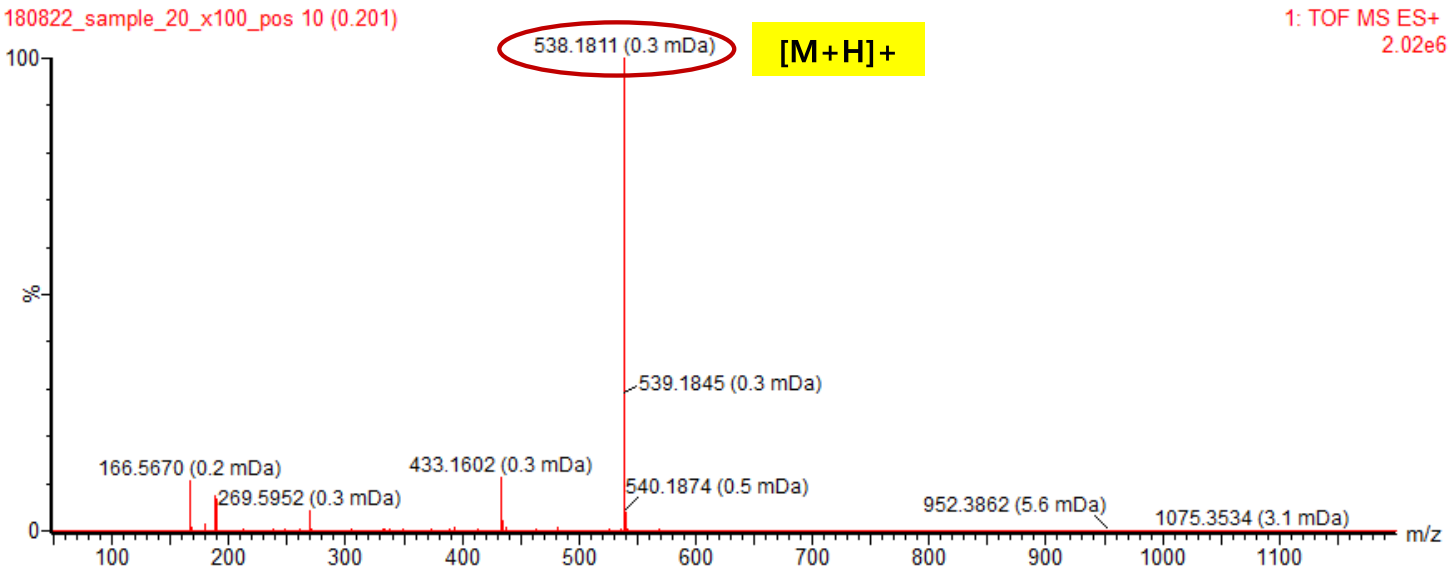


2. Biological experimental data

2.1.In vitro antiproliferative assay using M-NFS-60 cell lines

Materials:

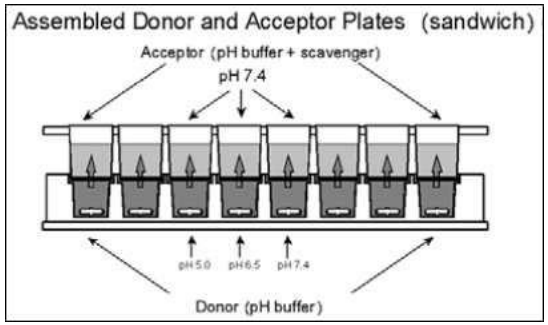
Test compounds and staurosporine were dissolved in DMSO in 10 mM stock. Staurosporine was purchased from Sigma-Aldrich (Saint Louis, MI). Cell Titer-Glo® 2.0 Luminescent cell viability assay reagent was purchased from Promega (Madison, WI). M-NFS-60 cell line was purchased from American Type Culture Collection (Manassas, VA). Cell culture media were as follow: RPMI-1640 + 10% FBS + 20 ng/ml M-CSF. All media are supplemented with 100 µg/mL of penicillin, and 100 µg/mL of streptomycin. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

Procedures:

1. Test compounds and staurosporine were diluted in DMSO solution with 10-dose and 3-fold dilutions in a source plate starting at 10 mM.
2. 25 nL of each test compound or staurosporine was delivered from the source plate to each well of the 384-well cell culture plates by Echo 550.
3. 25 µL of culture media containing 2000 M-NFS-60 cells was added to each of the wells in duplicates of the cell culture plate.
4. The cells were incubated with the compounds at 37°C, 5% CO_2 for 72 hours.

5. 25 µL of Cell Titer Glo 2.0 reagent was added to each well.
6. The contents were mixed on an orbital shaker for 2 min and incubated at room temperature for 15 min to stabilize luminescent signal.
7. Luminescence was recorded by Envision 2104 Multilabel Reader (PerkinElmer, Santa Clara, CA). The number of viable cells in culture was determined based on quantitation of the ATP present in each culture well.
8. The IC_{50} curves were plotted and IC_{50} values were calculated using the GraphPad Prism 4 program based on a sigmoidal dose-response equation.

*2.2.*PAMPA-GIT Assay [1-3]



The effective permeabilities of compound **6e**, and two reference drugs (Verapamil and Ranitidine) were assessed using Double-Sink™ (Pion, GI-PAMPA) via applying a donor 96 well-plate microtitre and an acceptor 96-well filter plate to form a sandwich as illustrated in the figure. Thus, each well is divided into two chambers, separated by a GI-PAMPA membrane. 10 mM DMSO stock

solutions of the tested compounds and reference drugs were prepared and diluted with Prisma buffer solution (pH = 7.4) to provide solutions of 12.5 (**6e**) and 50 μM (reference drugs) concentrations, which were placed in the wells of the donor plate. The acceptor plate containing buffer was placed and allowed to incubate for 4 h at 26 °C. The acceptor plate was removed and the concentration of compounds in the acceptor, the donor, and the reference wells were measured by UV (230–498 nm). The effective permeability (P_e) of each compound was calculated using Pion PAMPA Explorer software (version 3.8) applying calculation basis double sink (acceptor+donor+membrane).The following P_e ranges were established: low penetration of GIT ($P_e < 1.5 \times 10^{-6}$), high penetration of GIT ($P_e > 1.5 \times 10^{-6}$).

References:

[1] M. Kansy, F. Senner, K. Gubernator, Physicochemical High Throughput Screening: Parallel Artificial Membrane Permeation Assay in the Description of Passive Absorption Processes, Journal of Medicinal Chemistry, 41 (1998) 1007-1010.

[2] High-Throughput Measurements of Solubility Profiles, in: Pharmacokinetic Optimization in Drug Research.

[3] A. Avdeef, M. Strafford, E. Block, M.P. Balogh, W. Chambliss, I. Khan, Drug absorption in vitro model: filter-immobilized artificial membranes: 2. Studies of the permeability properties of lactones in Piper methysticum Forst, Eur. J. Pharm. Sci., 14 (2001) 271-280.