Supporting Informaion

Synthesis and Evaluation of *para*-[^{18}F]Fluorocelecoxib for COX-2 Cholangiocarcinoma Imaging

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Experimental

General Information

All reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) Acros (Geal, Belgium), or Tedia (Farfield, OH, USA). Celecoxib was purchased from Matrix Scientific Co. USA. HPLC solvents such as CH_3CH_2OH and CH_3CN were purchased from Avantor Performance Materials. Nylone filters with 0.45 μM pore size were supplied by Waters (Nilford, MA, USA). HPLC solvent was filtrated by using Nylon 66 membranes and nitro-cellulose membrane commercially available from Supelco and Millipole. NMR spectroscopy including ^{1}H-NMR (500 MHz),^{13}C-NMR (125 MHz, DEPT-135) and ^{19}F-NMR (470 or 564 MHz) was performed on a Unity Inova 500 MHz instrument (Varian, USA). Deuterated-solvents employed for NMR spectroscopy including $CDCl_3$ was purchased from Aldrich (St. Louis, MO, USA). Low-Resolution Mass Spectrometry (LRMS) was performed on an ESI-MS spectrometer using a Varian 901-MS Liquid Chromatography Tandem Mass Q-TOF Spectrometer at the Department of Chemistry of National Tsing-Hua University (NTHU) or the Department of Applied Chemistry of National Chio-Tung University (NCTU). High-Resolution Mass Spectrometry (HRMS) was performed using a Varian HPLC (prostar series ESI/APCI) system coupled with a Varian 901-MS (FT-ICR Mass) mass detector and a triple quadrapole instrument.

The gas bottle containing 0.9% F_2/Ne was purchased from SPECTRA GASES, INC. The radiochemistry experiment of [^{18}F]F-**1** and imaging study was performed in Multi Purpose Cyclotron Center at Taipei Veterans General Hospital (TVGH, National PET/Cyclotron Center, NPCC). A mixing gas of [^{18}F]F_2/F_2 was produced on a Scanditronix MC17F PET tracer cyclotron via ^{20}Ne(d,α) nuclear reaction (TVGH, Taiwan). Radiofluorination was carried out in a hot cell under a remote control (Scanditronix Anatech RB III, Uppsala, Sweden). The dilution procedure and concentration was also performed using the same system with the Reacti-vials (14/10, 5.0 mL). The HPLC for purifying radiofluorination product was assembled by Prostar 210 Pump with a 5 mL-loop, semi-preparative column of Develosil ODS-7 5 μm 20×150 mm, Prostar 325

UV–vis detector (λ_{abs} = 260 nm, Varian Corp., Palo Alto, CA, USA) and diode γ-detector (Flow count2000, Bioscan Inc., Washington, USA) in a linear assembly mode. Another HPLC system was used for quality control comprising a NovaPak column (C-18, 4 nm, 3.9×300 mm), Varian Prostar 210 pump, Prostar 325 UV-Vis detector and Bioscan FLOW COUNT gamma detector. Eluents of EtOH/H_2O = 7: 3 and flow rate 0.5 mL/min were used. Packard Cobra II Autogamma counter was used for counting the enzymatic binding assay samples. Another autogamma counter Perkin Elmer, 2470 Automatic Gamma Counter was employed for studies of counting *in-vitro* tracer uptake and competitive inhibition of the racer uptake in COX-2 overexpressed CCA cells. The authentic *ortho*-[^{18}F]F-**1** as a mixture with celecoxib obtained via nonradioactive fluorination with 0.9% F_2/Ne was further purified using HPLC system in Medicinal Chemistry Laboratory at National Tsinghua University. HPLC system comprised of Agilent 1100 series quarternary pump, injection loop (0.5 mL) and UV detector at 260 nm. Binding study employed COX-1 enzyme (5000 units, 200 uL) and COX-2 enzyme (5000 units, 600 uL) purchased from colorimetric COX (ovine) inhibitor screening assay kit (760111, Caymen Chemical). mPGES (500 units, 200 uL) was purchased from Caymen Chemical. Monitoring of the hot-cell synthesis and subsequent manual procedure was performed with a Packard A-100 (BT cell) detector (Capintec Inc., New Jersey, USA). The procedure for separation of the binding mixtures was aided through a blood pressure ball of spirit model P-117 in association with filtration by cartridge set of BAKERBOND Spe Silica Gel (SiOH) disposable Extraction Column.

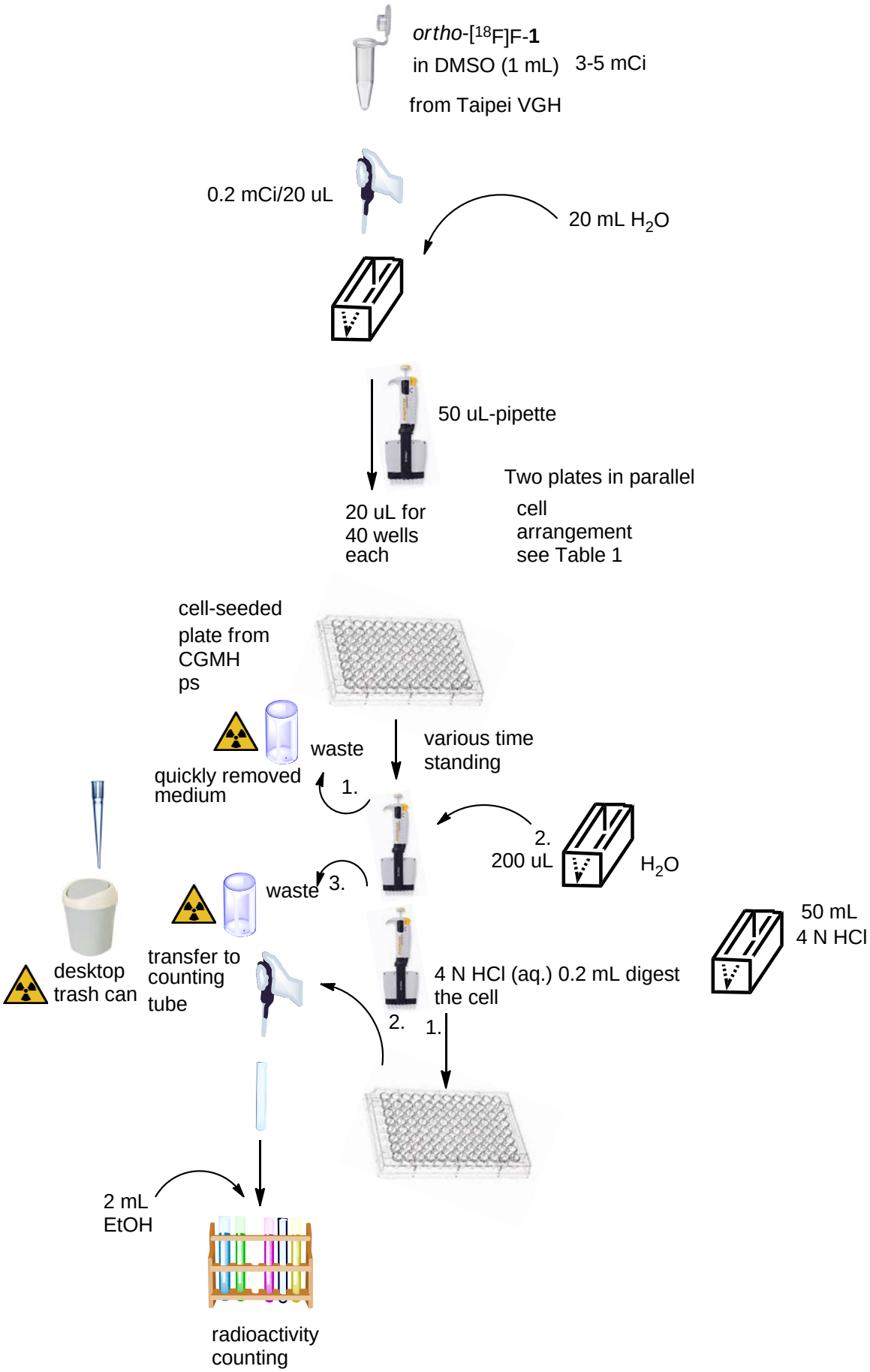


Figure 1. Tracer uptake experimental flowchart

Table 1. Cell-seeding arrangement in tracer accumulation experiment

		COX-2 CCA cell				**CCA cell**				**No Cell**	
		1	2	3	4	5	6	7	8	11	12
2 min	A	F-18	F-18	F-18	F-18	F-18	F-18	F-18	F-18		
10 min	B	F-18	F-18	F-18	F-18	F-18	F-18	F-18	F-18		
30 min	C	F-18	F-18	F-18	F-18	F-18	F-18	F-18	F-18		
1 h	D	F-18	F-18	F-18	F-18	F-18	F-18	F-18	F-18		
2 h	E	F-18	F-18	F-18	F-18	F-18	F-18	F-18	F-18		
No Cell	F										
	G										
	H										

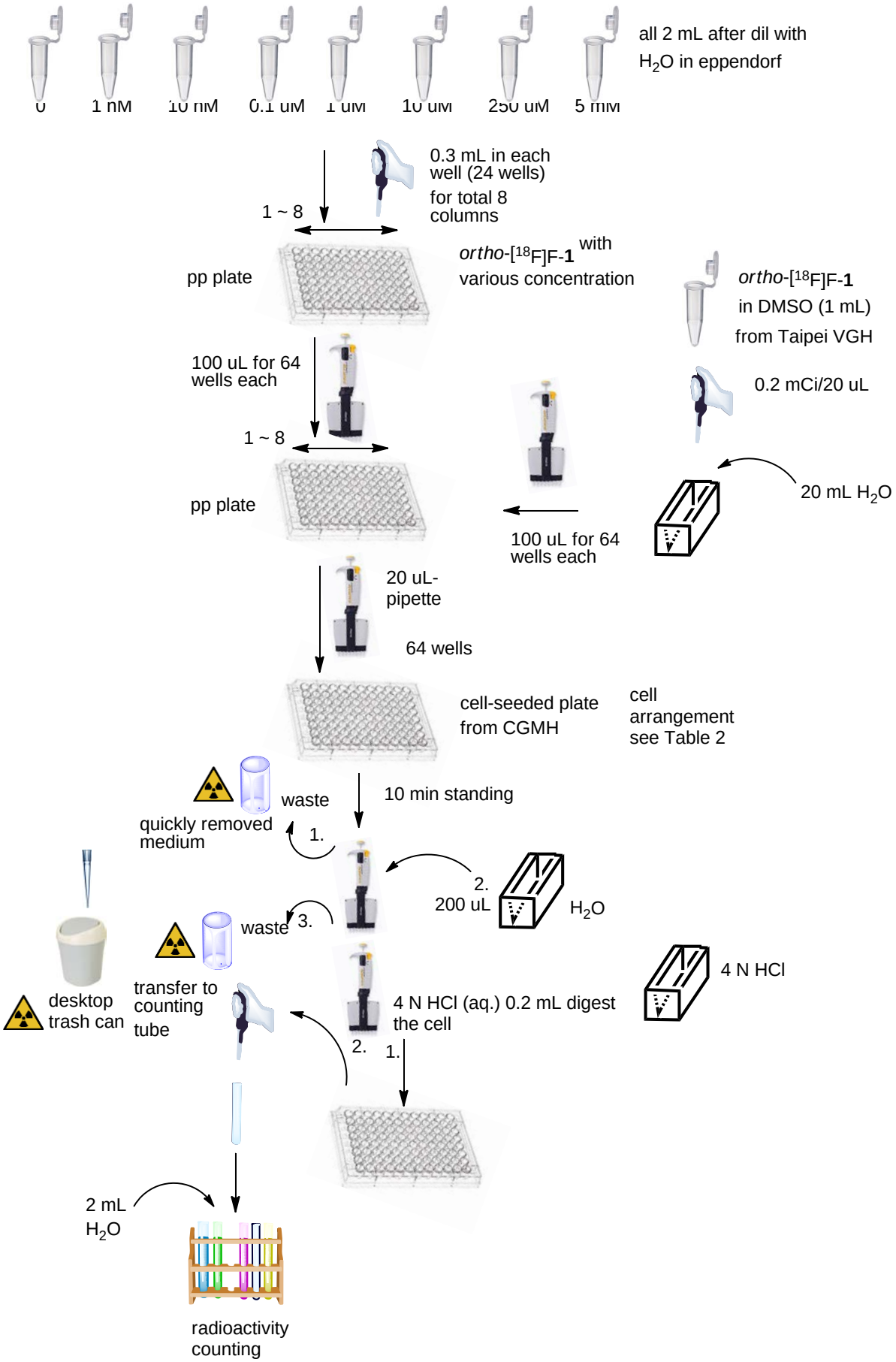
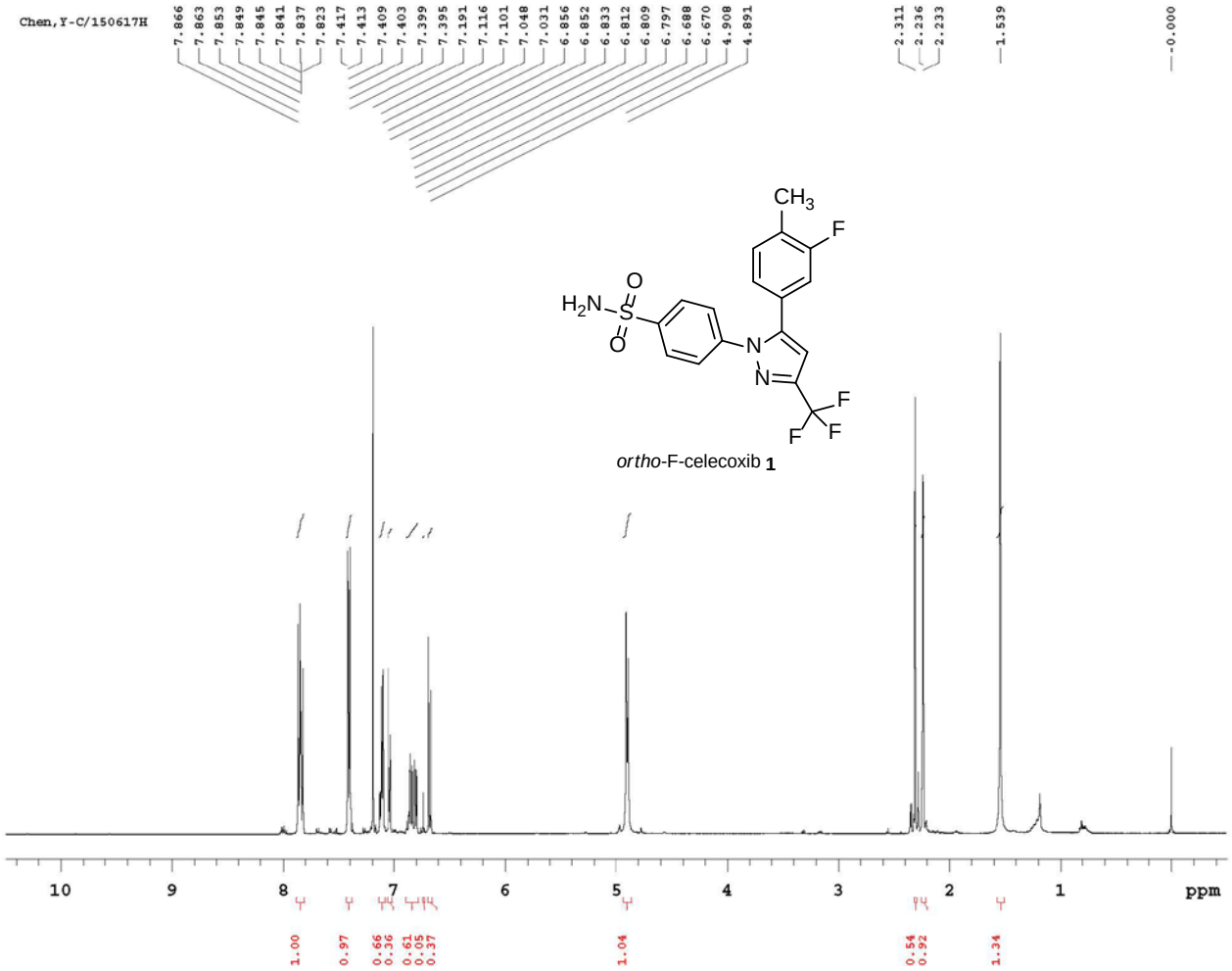


Figure 2. *In vitro* blocking experimental flowchart

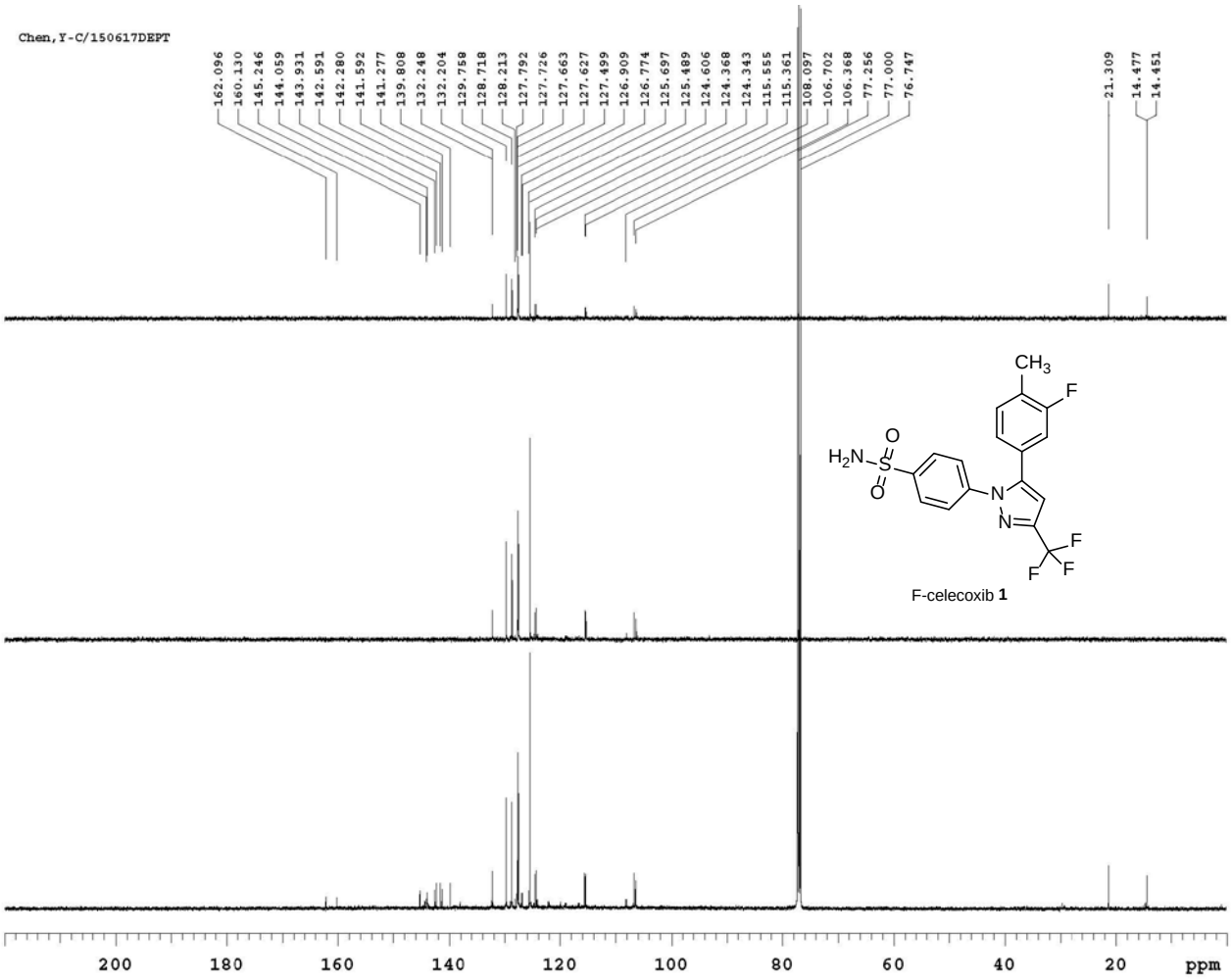
Table 2. Cell-seeding arrangement in blocking experiment (for 10 min) of *ortho*-[^{18}F]F-**1** + celecoxib (0.05 nM ~ 250 μM)

		COX-2 CCA cell				**CCA cell**				**No Cell**			
		1	2	3	4	5	6	7	8	9	10	11	12
0	A												
0.05 nM	B												
0.5 nM	C												
5 nM	D												
50 nM	E												
0.5 uM	F												
12.5 uM	G												
250 uM	H												



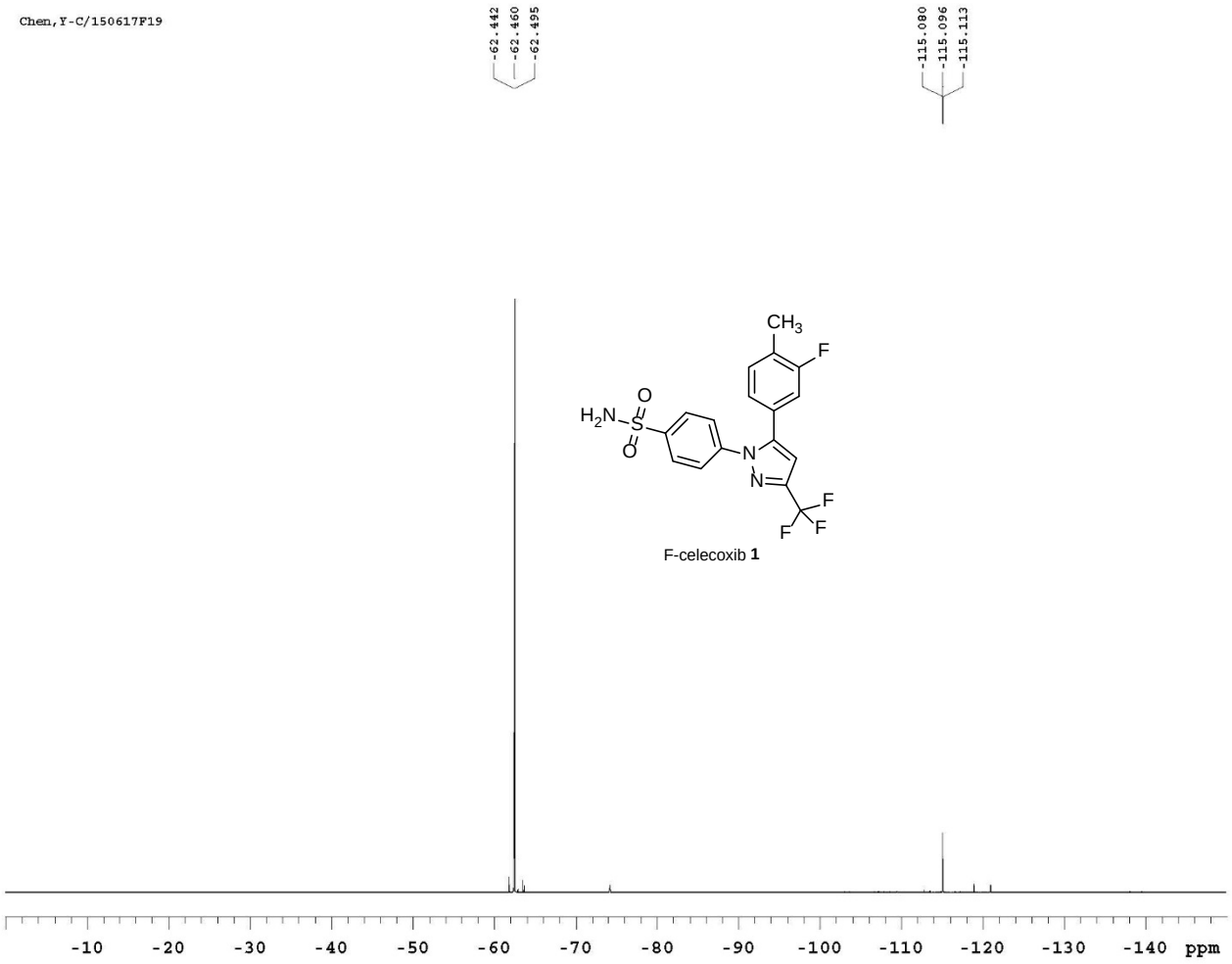
ortho-F-celecoxib **1**

[1]H-NMR of *ortho*-F-celecoxib **1**

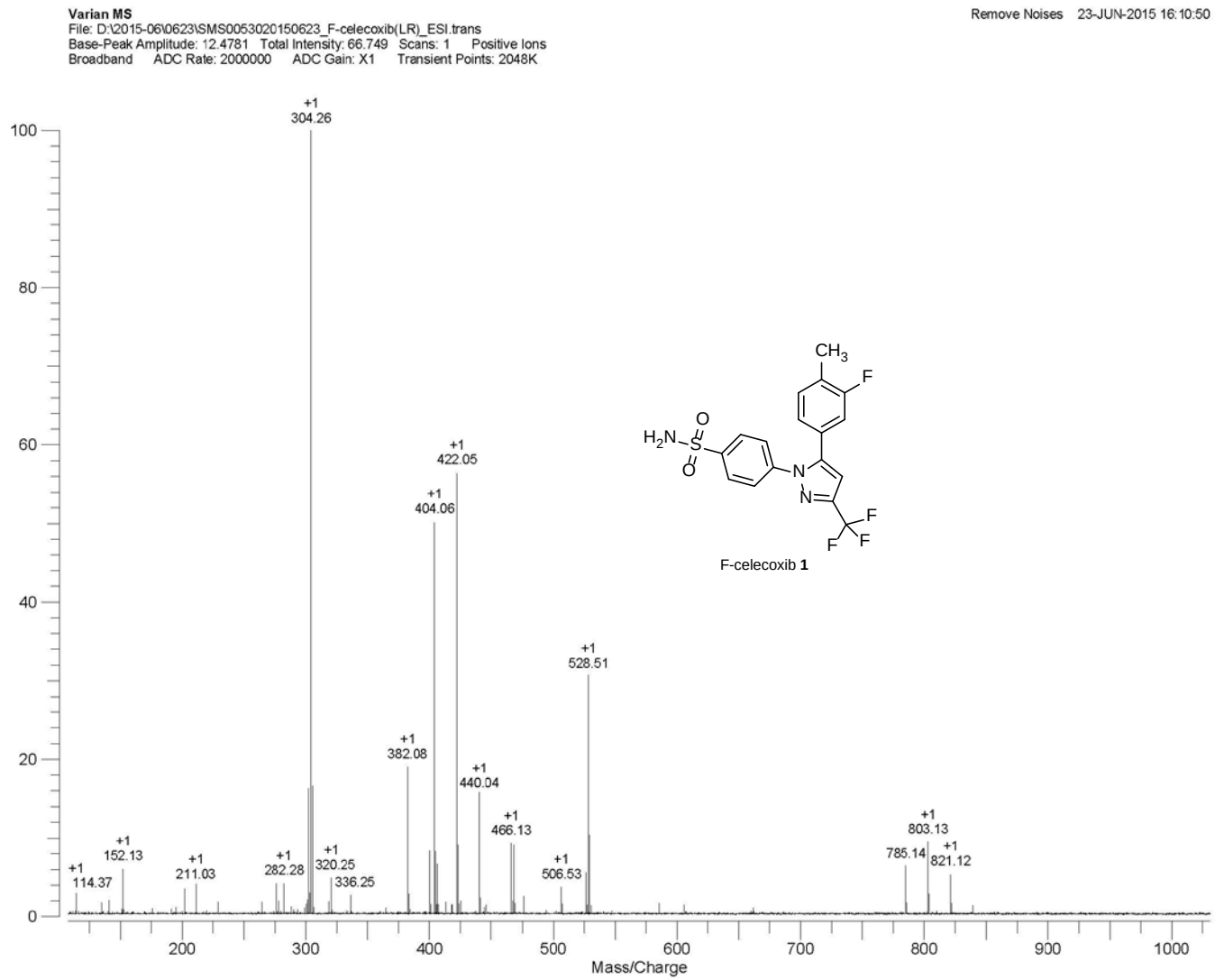


F-celecoxib **1**

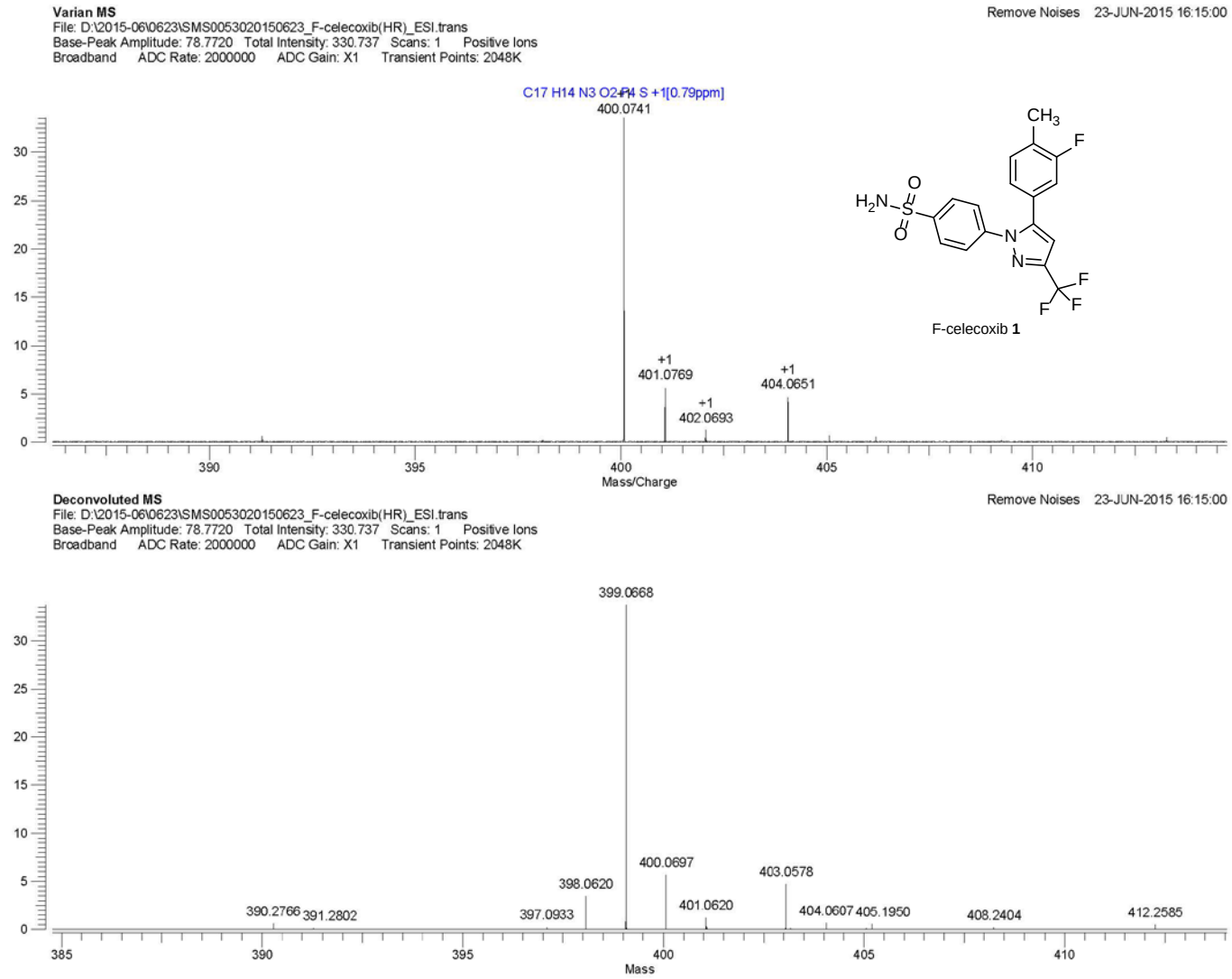
^{13}C-NMR (DEPT-135) of *ortho*-F-celecoxib **1**



^{19}F-NMR of *ortho*-F-celecoxib **1**



LR ESI-MS of *ortho*-F-celecoxib **1**



HR ESI-MS of *ortho*-F-celecoxib **1**