

## Supplementary Information

### Synthesis, characterization and mechanism of action of anticancer 3-fluoroazetidin-2-ones

Azizah M. Malebari<sup>a,b\*</sup>, Gabriela Duffy Morales<sup>b</sup>, Brendan Twamley<sup>c</sup>, Darren Fayne<sup>d</sup>,  
Mohammed Faraz Khan<sup>d</sup>, Eavan C. McLoughlin<sup>b</sup>, Niamh M. O'Boyle<sup>b</sup>, Daniela M. Zisterer<sup>d</sup>  
and Mary J. Meegan<sup>b\*</sup>

<sup>a</sup> Department of Pharmaceutical Chemistry, College of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia

<sup>b</sup> School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Trinity Biomedical Sciences Institute, 152-160 Pearse Street, Dublin 2, Ireland

<sup>c</sup> School of Chemistry, Trinity College Dublin, Dublin 2, Ireland

<sup>d</sup> School of Biochemistry and Immunology, Trinity College Dublin, Trinity Biomedical Sciences Institute, 152-160 Pearse Street, Dublin 2, Ireland

**Experimental chemistry:** Preparation of [3-(*tert*-Butyldimethylsilyloxy)-4-methoxybenzylidene](3,4,5-trimethoxyphenyl)amine **22**

**Table S1:** Tier-1 Profiling Screen of selected 3-fluoroazetidinones, 3,3-difluoroazetidinones and related compounds

**Table S2:** ADMET and Lipinski Properties for selected 3-fluoroazetidinones, 3,3-difluoroazetidin-2-ones and related compounds

**Table S3:** Growth inhibition of compounds **33**, **37** and **43** in the NCI60 cell line *in vitro* screen (10  $\mu$ M)

**Table S4.** Antiproliferative effects of  $\beta$ -lactam compounds **26**, **32**, **33**, **42** and **43** in MCF-7 human breast cancer cells

**Table S5:** Standard COMPARE Analysis of compound **33** based on one-dose (10  $\mu$ M)

**Table S6:** Standard COMPARE Analysis of compounds **33** based on GI<sub>50</sub> mean graph

**Table S7:** Stability study for compounds **33** and **39**

**Table S8:** Docking Scores for compounds **32**, **33**, **42** and **43**

**Figure S1:** <sup>1</sup>H NMR spectrum of compound **28**

**Figure S2:** <sup>13</sup>C NMR spectrum of compound **28**

**Figure S3:** <sup>1</sup>H NMR spectrum of compound **37**

**Figure S4:** <sup>13</sup>C NMR spectrum of compound **37**

**Figure S5:** <sup>1</sup>H-NMR spectrum of compound **33**

**Figure S6:** <sup>13</sup>C-NMR spectrum of compound **33**

**Figure S7:** <sup>1</sup>H-NMR spectrum of compound **39**

**Figure S8:** <sup>1</sup>H-NMR spectrum of compound **41**

**Figure S9:** <sup>1</sup>H-NMR spectrum of compound **42**

**Figure S10:** <sup>19</sup>F NMR spectrum of compound **23**

**Figure S11:** Cell viability data for 3-fluoro and 3,3-difluoro- $\beta$ -lactam compounds in SW-480 cell line

**Figure S12:** Heat map compound 792959 and macbeccin NSC S330500

**Figure S13:** Protein-ligand interactions for the 3-fluoro and 3,3-difluoro- $\beta$ -lactam compounds **26** and **36**.

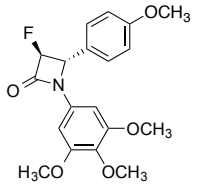
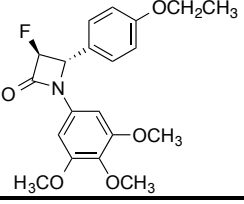
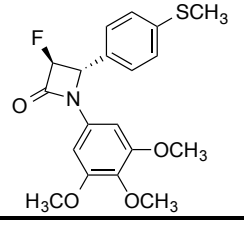
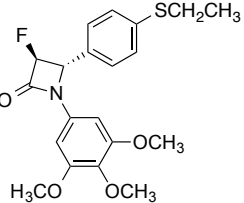
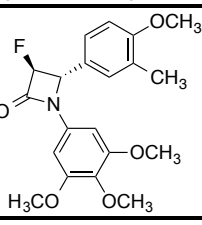
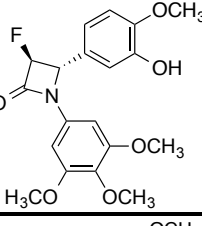
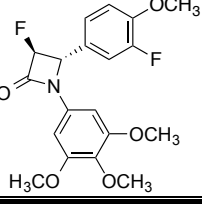
**References**

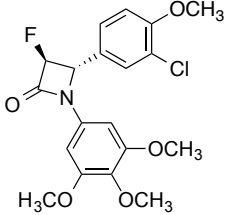
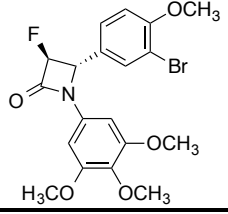
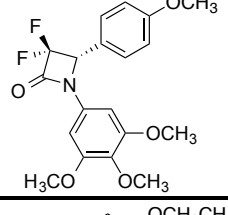
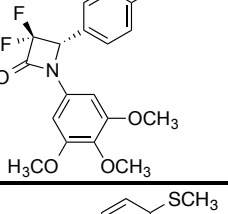
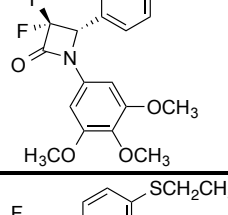
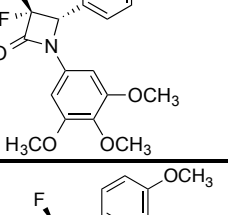
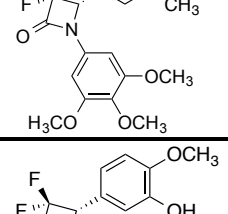
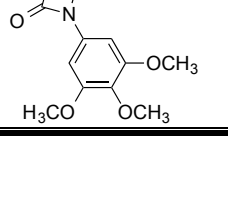
## Experimental chemistry:

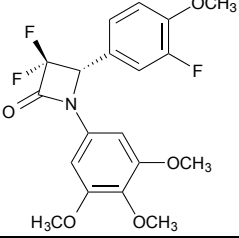
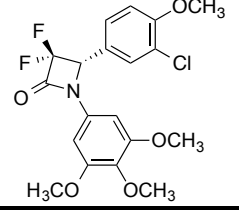
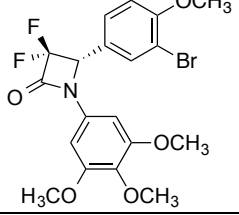
### [3-(*tert*-Butyldimethylsilanyloxy)-4-methoxybenzylidene](3,4,5-trimethoxyphenyl)amine **22**

DBU (8 mmol) was added dropwise to a stirred solution of imine **22** (5 mmol) and *tert*-butyldimethylsilyl chloride (6 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (40 mL) under a nitrogen atmosphere (reaction was continued for 2-4 h until complete as indicated by TLC (eluent, 1:1 hexane/ethyl acetate)). The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and washed with water (100 mL), 0.1 M HCl (50 mL) and saturated aqueous NaHCO<sub>3</sub> (50 mL), before drying over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed *in vacuo* to yield TBDMS-protected imine **22** as amber oil, (yield 52%), purity (HPLC): 100%. Imine **22** was characterised as described previously by us [1], [2]. IR  $\nu_{\max}$  (KBr) cm<sup>-1</sup>: 1618.8, (C=N). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  0.21 (s, 6H, Si(CH<sub>3</sub>)<sub>2</sub>), 1.04 (s, 9H, SiC(CH<sub>3</sub>)<sub>3</sub>), 3.74 (s, 3H, OCH<sub>3</sub>), 3.87 (s, 6H, 2xOCH<sub>3</sub>), 3.93 (s, 3H, OCH<sub>3</sub>), 6.58 (s, 2H, Ar-H), 7.12 (d, 1H, J = 8.2 Hz, Ar-H), 7.48-7.55 (m, 2H, Ar-H), 8.51 (s, 1H, CH=N). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  5.78 Si(CH<sub>3</sub>)<sub>2</sub>, 17.71 SiC(CH<sub>3</sub>)<sub>3</sub>, 24.74 (C(CH<sub>3</sub>)<sub>3</sub>), 55.00 (OCH<sub>3</sub>), 59.25 (OCH<sub>3</sub>), 98.04, 111.22, 121.27, 125.43, 129.50, 135.95, 144.68, 147.73, 153.32 (Ar-C), 158.27 (CH=N). HRMS: Calculated for C<sub>23</sub>H<sub>34</sub>O<sub>5</sub>Si: 432.2216; Found: 432.2213 (M+ H)<sup>+</sup>.

**Table S1: Tier-1 Profiling Screen of selected 3-fluoroazetidin-2-ones, 3,3-difluoroazetidin-2-ones<sup>a</sup>**

| Compound                                                                            | Number | ADMET Solubility <sup>b</sup> | ADMET Solubility Level <sup>c</sup> | ADMET BBB <sup>d</sup> | ADMET BBB Level <sup>e</sup> | ADMET CYP2D6 Prediction <sup>f</sup> | ADMET Hepatotoxic Prediction <sup>g</sup> |
|-------------------------------------------------------------------------------------|--------|-------------------------------|-------------------------------------|------------------------|------------------------------|--------------------------------------|-------------------------------------------|
|    | 26     | -4.1380                       | 2                                   | -0.14200               | 2                            | false                                | true                                      |
|    | 27     | -4.3290                       | 2                                   | -0.034000              | 2                            | false                                | false                                     |
|   | 28     | -4.6260                       | 2                                   | 0.17200                | 1                            | false                                | true                                      |
|  | 29     | -4.8160                       | 2                                   | 0.28000                | 1                            | false                                | true                                      |
|  | 30     | -4.6190                       | 2                                   | 0.0080000              | 1                            | false                                | true                                      |
|  | 32     | -3.8020                       | 3                                   | -0.54600               | 3                            | false                                | true                                      |
|  | 33     | -4.3740                       | 2                                   | -0.079000              | 2                            | false                                | true                                      |

|                                                                                     |           |         |   |           |   |       |       |
|-------------------------------------------------------------------------------------|-----------|---------|---|-----------|---|-------|-------|
|    | <b>34</b> | -4.7750 | 2 | 0.063000  | 1 | false | true  |
|    | <b>35</b> | -4.8480 | 2 | 0.089000  | 1 | false | true  |
|    | <b>36</b> | -4.3990 | 2 | -0.090000 | 2 | false | true  |
|   | <b>37</b> | -4.5850 | 2 | 0.018000  | 1 | false | false |
|  | <b>38</b> | -4.8860 | 2 | 0.22400   | 1 | false | true  |
|  | <b>39</b> | -5.0730 | 2 | 0.33200   | 1 | false | true  |
|  | <b>40</b> | -4.8770 | 2 | 0.060000  | 1 | false | true  |
|  | <b>42</b> | -4.1460 | 2 | -0.49400  | 2 | false | true  |

|                                                                                   |           |         |   |           |   |       |      |
|-----------------------------------------------------------------------------------|-----------|---------|---|-----------|---|-------|------|
|  | <b>43</b> | -4.6320 | 2 | -0.026000 | 2 | false | true |
|  | <b>44</b> | -5.0330 | 2 | 0.11500   | 1 | false | true |
|  | <b>45</b> | -5.1060 | 2 | 0.14100   | 1 | false | true |

<sup>a</sup>Calculated using Pipeline Pilot Professional (v8.5.0.200) BIOVIA, Dassault Systèmes

<sup>b</sup>ADMET Solubility: Log of the water solubility at 25 °C (LogSw)(mol/L)

<sup>c</sup>ADMET Solubility Level: Ranking of the solubility values into the following classes: 0: Extremely Low; 1: Very Low; 2: Low; 3: Good; 4: Optimal; 5: Very Soluble

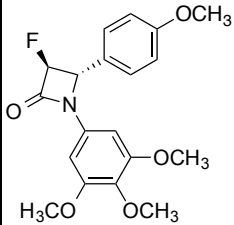
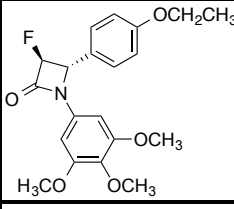
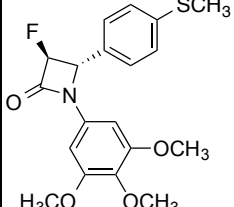
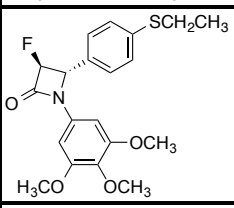
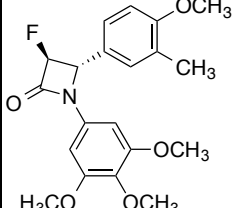
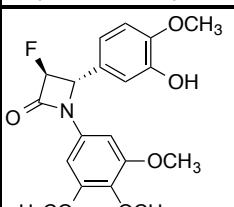
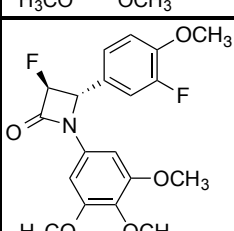
<sup>d</sup>ADMET BBB: Predicts the blood brain barrier penetration of a molecule, defined as the ratio of the concentrations of solute (compound) on the both sides of the membrane after oral administration.

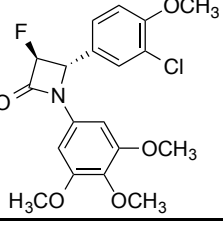
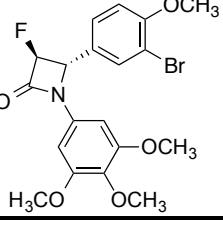
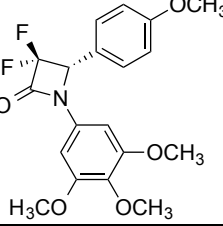
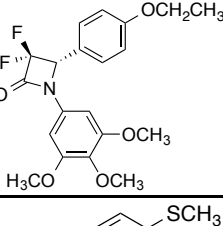
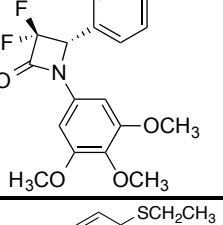
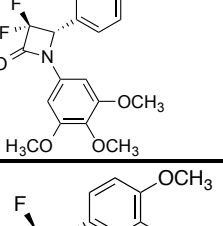
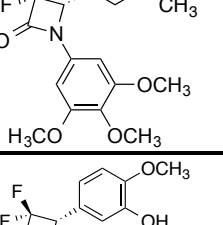
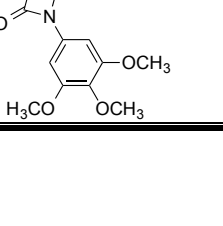
<sup>e</sup>ADMET Blood Brain Barrier Absorption (BBB) Level: Ranking of LogBBB values into one of the following levels: 0: Very High; 1: High; 2: Medium; 3: Low; 4: Undefined (molecule is outside the confidence area of the regression model used to calculate LogBB)

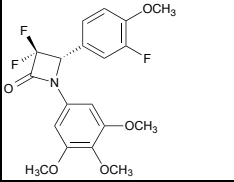
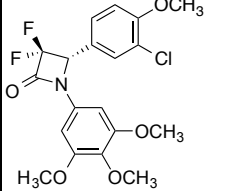
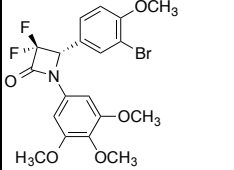
<sup>f</sup>CYP2D6 inhibitor prediction

<sup>g</sup>Human hepatotoxicity prediction

**Table S2: ADMET and Lipinski Properties for Selected 3-fluoroazetidin-2-ones, 3,3-difluoroazetidin-2-ones<sup>a</sup>**

| Compound                                                                            | No. | ADMET Absorption Level <sup>b</sup> | ADMET PPB Prediction <sup>c</sup> | ALogP <sup>d</sup> | MW     | Num HBA | Num HBD | Num Rot Bonds | Molecular Volume | Molecular Polar Surface Area |
|-------------------------------------------------------------------------------------|-----|-------------------------------------|-----------------------------------|--------------------|--------|---------|---------|---------------|------------------|------------------------------|
|    | 26  | 0                                   | true                              | 2.9240             | 361.36 | 5       | 0       | 6             | 127.59           | 57.230                       |
|    | 27  | 0                                   | true                              | 3.2730             | 375.39 | 5       | 0       | 7             | 133.42           | 57.230                       |
|   | 28  | 0                                   | true                              | 3.4830             | 377.43 | 5       | 0       | 6             | 130.68           | 73.300                       |
|  | 29  | 0                                   | true                              | 3.8310             | 391.46 | 5       | 0       | 7             | 136.85           | 73.300                       |
|  | 30  | 0                                   | true                              | 3.4110             | 375.39 | 5       | 0       | 6             | 130.68           | 57.230                       |
|  | 32  | 0                                   | true                              | 2.6820             | 377.36 | 6       | 1       | 6             | 128.96           | 77.460                       |
|  | 33  | 0                                   | true                              | 3.1300             | 379.35 | 5       | 0       | 6             | 128.28           | 57.230                       |

|                                                                                     |           |   |      |        |        |   |   |   |        |        |
|-------------------------------------------------------------------------------------|-----------|---|------|--------|--------|---|---|---|--------|--------|
|    | <b>34</b> | 0 | true | 3.5890 | 395.81 | 5 | 0 | 6 | 130.33 | 57.230 |
|    | <b>35</b> | 0 | true | 3.6730 | 440.26 | 5 | 0 | 6 | 141.65 | 57.230 |
|    | <b>36</b> | 0 | true | 3.0930 | 379.35 | 5 | 0 | 6 | 128.96 | 57.230 |
|   | <b>37</b> | 0 | true | 3.4420 | 393.38 | 5 | 0 | 7 | 134.79 | 57.230 |
|  | <b>38</b> | 0 | true | 3.6520 | 395.42 | 5 | 0 | 6 | 131.71 | 73.300 |
|  | <b>39</b> | 0 | true | 4.00   | 409.45 | 5 | 0 | 7 | 137.88 | 73.300 |
|  | <b>40</b> | 0 | true | 3.5800 | 393.38 | 5 | 0 | 6 | 132.05 | 57.230 |
|  | <b>42</b> | 0 | true | 2.8510 | 395.35 | 6 | 1 | 6 | 130.33 | 77.460 |

|                                                                                   |           |   |      |        |        |   |   |   |        |        |
|-----------------------------------------------------------------------------------|-----------|---|------|--------|--------|---|---|---|--------|--------|
|  | <b>43</b> | 0 | true | 3.2990 | 397.35 | 5 | 0 | 6 | 128.62 | 57.230 |
|  | <b>44</b> | 0 | true | 3.7580 | 413.80 | 5 | 0 | 6 | 131.71 | 57.230 |
|  | <b>45</b> | 0 | true | 3.8420 | 458.25 | 5 | 0 | 6 | 142.34 | 57.230 |

<sup>a</sup>Calculated using Pipeline Pilot Professional (v8.5.0.200) BIOVIA, Dassault Systèmes

<sup>b</sup>ADMET Calculates ADMET Passive Intestinal Absorption properties. Accelrys passive intestinal absorption model. Absorption Level: Ranking of the molecule into one of the following levels: 0: Good; 1: Moderate; 2: Poor; 3: Very Poor

<sup>c</sup>ADMET Plasma Protein Binding (PPB) Prediction: If true, the compound is predicted to be a binder ( $\geq 90\%$ ). Otherwise, it is predicted to be a weak or nonbinder ( $< 90\%$ ).

<sup>d</sup>ChemBioDraw Ultra 13.0.2.3020

**Table S3:** Growth inhibition of compounds **33** (NCI 792959), **37** (NCI 792958) and **43** (NCI 792960) in the NCI60 cell line *in vitro* screen (10  $\mu$ M)<sup>a</sup>

|                                   | Compound <b>33</b> |              | Compound <b>37</b> |              | Compound <b>43</b> |              |
|-----------------------------------|--------------------|--------------|--------------------|--------------|--------------------|--------------|
|                                   | Growth %           | Inhibition % | Growth %           | Inhibition % | Growth %           | Inhibition % |
| <b>Leukaemia</b>                  |                    |              |                    |              |                    |              |
| CCRF-CEM                          | 13.16              | 86.84        | 10.2               | 89.8         | 12.96              | 87.04        |
| HL-60(TB)                         | -14.69             | 114.69       | -15.97             | 115.97       | 0.94               | 99.06        |
| K-562                             | 9.45               | 90.55        | 8.49               | 91.51        | 10.5               | 89.5         |
| <i>MOLT-4</i>                     | 19.7               | 80.3         | 18.14              | 81.86        | 18.67              | 81.33        |
| RPMI-8226                         | 15.32              | 84.68        | 14.64              | 85.36        | 20.78              | 79.22        |
| SR                                | 13.28              | 86.72        | 16.25              | 83.75        | 18.7               | 81.3         |
|                                   |                    |              |                    |              |                    |              |
| <b>Non-Small Cell Lung Cancer</b> |                    |              |                    |              |                    |              |
| A549/A TCC                        | 15.33              | 84.67        | 33.56              | 66.44        | 48.65              | 51.35        |
| EKVX                              | 72.16              | 27.84        | 75.34              | 24.66        | 77.7               | 22.3         |
| HOP-62                            | 31.85              | 68.15        | 40.81              | 59.19        | 38.43              | 61.57        |
| HOP-92                            | 32.28              | 67.72        | 55.62              | 44.38        | 60.84              | 39.16        |
| NCI-H226                          | 58.99              | 41.01        | 71.01              | 28.99        | 75.53              | 24.47        |
| NCI-H23                           | 21.3               | 78.7         | 33.57              | 66.43        | 37.86              | 62.14        |
| NCI-H322M                         | 46.45              | 53.55        | 67.23              | 32.77        | 91.21              | 8.79         |
| NCI-H460                          | 7.06               | 92.94        | 12.73              | 87.27        | 27.15              | 72.85        |
| NCI-H522                          | -8.46              | 108.46       | 19.28              | 80.72        | 17.54              | 82.46        |
|                                   |                    |              |                    |              |                    |              |
| <b>Colon Cancer</b>               |                    |              |                    |              |                    |              |
| COLO 205                          | -68                | 168          | 10.78              | 89.22        | 16.32              | 83.68        |
| HCC-2998                          | 32.93              | 67.07        | 81.66              | 18.34        | 84.2               | 15.8         |
| HCT-116                           | 5.59               | 94.41        | 8.92               | 91.08        | 5.76               | 94.24        |
| HCT-15                            | 13.63              | 86.37        | 22.55              | 77.45        | 19.44              | 80.56        |
| HT29                              | 3.73               | 96.27        | 12.27              | 87.73        | 11.16              | 88.84        |
| KM12                              | 10.73              | 89.27        | 27.97              | 72.03        | 22.9               | 77.1         |
| SW-620                            | 28.73              | 71.27        | 25.47              | 74.53        | 21.22              | 78.78        |
|                                   |                    |              |                    |              |                    |              |
| <b>CNS Cancer</b>                 |                    |              |                    |              |                    |              |
| SF-268                            | 33.13              | 66.87        | 52.38              | 47.62        | 52.94              | 47.06        |
| SF-295                            | 18.61              | 81.39        | 56.32              | 43.68        | 48.29              | 51.71        |
| SF-539                            | 4.17               | 95.83        | 10.75              | 89.25        | 21.65              | 78.35        |
| SNB-19                            | 34.05              | 65.95        | 54.14              | 45.86        | 52.93              | 47.07        |
| SNB-75                            | 50.89              | 49.11        | 55.93              | 44.07        | 64.74              | 35.26        |
| U251                              | 11.86              | 88.14        | 36.19              | 63.81        | 41.61              | 58.39        |

|                        | Compound <b>33</b> |                 | Compound <b>37</b> |                 | Compound <b>43</b> |                 |
|------------------------|--------------------|-----------------|--------------------|-----------------|--------------------|-----------------|
|                        | Growth<br>%        | Inhibition<br>% | Growth<br>%        | Inhibition<br>% | Growth<br>%        | Inhibition<br>% |
| <b>Melanoma</b>        |                    |                 |                    |                 |                    |                 |
| LOX IMVI               | 23.98              | 76.02           | 27.57              | 72.43           | 27.06              | 72.94           |
| MALME-3M               | 74.02              | 25.98           | 63.07              | 36.93           | 64.09              | 35.91           |
| M14                    | 1.91               | 98.09           | 27.45              | 72.55           | 24.24              | 75.76           |
| MDA-MB-435             | 0.35               | 99.65           | 16.83              | 83.17           | 12.92              | 87.08           |
| SK-MEL-2               | 58.83              | 41.17           | 91.78              | 8.22            | 84.61              | 15.39           |
| SK-MEL-28              | 57.74              | 42.26           | 66.8               | 33.2            | 77.22              | 22.78           |
| SK-MEL-5               | -31.46             | 131.46          | 6.76               | 93.24           | 8.08               | 91.92           |
| UACC-257               | 38.74              | 61.26           | 58.69              | 41.31           | 50.01              | 49.99           |
| UACC-62                | 53.45              | 46.55           | 51.61              | 48.39           | 55.46              | 44.54           |
| <b>Ovarian Cancer</b>  |                    |                 |                    |                 |                    |                 |
| IGROV1                 | 31.88              | 68.12           | 37.8               | 62.2            | 44.2               | 55.8            |
| OVCAR-3                | -4.18              | 104.18          | 7.25               | 92.75           | 11.64              | 88.36           |
| OVCAR-4                | 54.92              | 45.08           | 49.59              | 50.41           | 58.34              | 41.66           |
| OVCAR-5                | 43.57              | 56.43           | 66.47              | 33.53           | 78.72              | 21.28           |
| OVCAR-8                | 13.71              | 86.29           | 43.23              | 56.77           | 44.07              | 55.93           |
| NCI/ADR-RES            | 8.4                | 91.6            | 28.94              | 71.06           | 32.94              | 67.06           |
| SK-OV-3                | 33.36              | 66.64           | 71.89              | 28.11           | 75.49              | 24.51           |
| <b>Renal Cancer</b>    |                    |                 |                    |                 |                    |                 |
| 786-0                  | 22.92              | 77.08           | 64.68              | 35.32           | 75.35              | 24.65           |
| A498                   | -5.5               | 105.5           | 92.3               | 7.7             | 97.81              | 2.19            |
| ACHN                   | 32.67              | 67.33           | 55.25              | 44.75           | 60.82              | 39.18           |
| RXF 393                | 28.8               | 71.2            | 54.19              | 45.81           | 49.36              | 50.64           |
| SN12C                  | 33.29              | 66.71           | 50.97              | 49.03           | 54.92              | 45.08           |
| TK-10                  | 64.4               | 35.6            | 103.32             | -3.32           | 105.64             | -5.64           |
| UO-31                  | 43.2               | 56.8            | 51.9               | 48.1            | 53.48              | 46.52           |
| <b>Prostate Cancer</b> |                    |                 |                    |                 |                    |                 |
| PC-3                   | 23.13              | 76.87           | 37.3               | 62.7            | 35.89              | 64.11           |
| DU-145                 | 9.46               | 90.54           | 30.05              | 69.95           | 25.22              | 74.78           |
| <b>Breast Cancer</b>   |                    |                 |                    |                 |                    |                 |
| MCF7                   | 12.83              | 87.17           | 17.19              | 82.81           | 15.06              | 84.94           |
| MDA-MB-231/ATCC        | 19.65              | 80.35           | 46.12              | 53.88           | 54.02              | 45.98           |
| HS 578T                | 25.54              | 74.46           | 37.42              | 62.58           | 21.93              | 78.07           |
| BT-549                 | 15.26              | 84.74           | 44.17              | 55.83           | 34.99              | 65.01           |
| T-47D                  | 74.94              | 25.06           | 70.03              | 29.97           | 65.69              | 34.31           |
| MDA-MB-468             | 23                 | 77              | 34.89              | 65.11           | 44.48              | 55.52           |
|                        |                    |                 |                    |                 |                    |                 |
| Mean                   | 23.66              | 76.34           | 41.05              | 58.95           | 43.57              | 56.43           |

<sup>a</sup>Data obtained from NCI *in vitro* human tumour cell screen 1 dose assay (10 µM). **33** (NCI 792959), **37** (NCI 792958) and **43** (NCI 792960).

[National Cancer Institute Biological Testing Branch; National Cancer Institute; Bethesda, MD; <https://dtp.cancer.gov> (accessed 02/03/2022)].

**Table S4.** Antiproliferative effects of  $\beta$ -lactam compounds **26**, **32**, **33**, **42** and **43** in MCF-7 human breast cancer cells

| Compound                | Antiproliferative activity <sup>a,b</sup> | Log P <sup>c</sup> |
|-------------------------|-------------------------------------------|--------------------|
|                         | MCF-7 Cells                               |                    |
|                         | IC <sub>50</sub> ( $\mu$ M)               |                    |
| <b>26</b>               | 0.312 $\pm$ 0.08                          | 2.92               |
| <b>32</b>               | 0.075 $\pm$ 0.002                         | 2.68               |
| <b>33</b>               | 0.095 $\pm$ 0.004                         | 3.10               |
| <b>42</b>               | 0.321 $\pm$ 0.06                          | 2.85               |
| <b>43</b>               | 1.650 $\pm$ 0.30                          | 3.29               |
| <b>CA-4<sup>b</sup></b> | 0.0035 $\pm$ 0.0002                       | 3.32               |

<sup>a</sup> IC<sub>50</sub> values are half maximal inhibitory concentrations required to inhibit the growth of MCF-7 breast cancer cells. Values represent the mean (SEM (error values 10<sup>-6</sup>) for three experiments performed in triplicate. <sup>b</sup> The IC<sub>50</sub> value obtained for CA-4 in this assay is in agreement with the reported values for CA-4 in MCF-7 and MDA-MB-231 human breast cancer cell lines .[3],[4]. <sup>c</sup>Pipeline Pilot Professional.

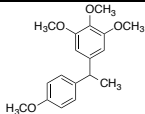
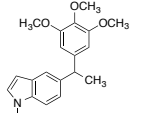
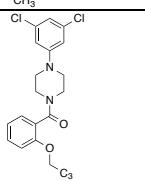
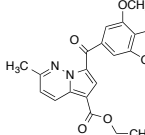
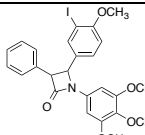
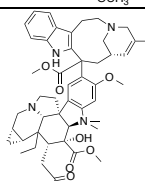
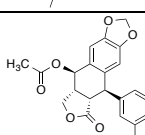
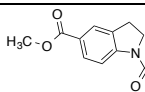
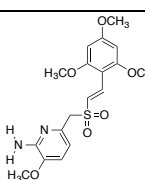
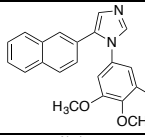
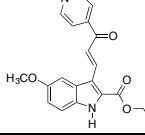
**Table S5:** Standard COMPARE analysis of compound **33** based on one-dose (10  $\mu$ M)<sup>a</sup>

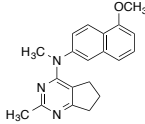
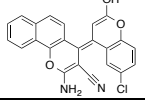
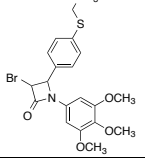
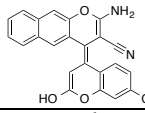
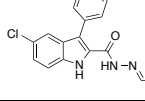
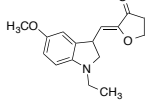
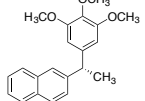
| Rank | Compound            | <i>r</i> |
|------|---------------------|----------|
| 1    | macbecin II         | 0.53     |
| 2    | vincristine sulfate | 0.50     |
| 3    | Floxuridine         | 0.50     |
| 4    | rhizoxin            | 0.49     |
| 5    | tiazofurin          | 0.49     |
| 6    | maytansine          | 0.48     |

<sup>a</sup>The target set was the NCI Standard Database and the target set endpoints were selected to be equal to the seed end points. Standard COMPARE analysis was performed. Correlation values (*r*) are Pearson correlation coefficients. [Reference National Cancer Institute Biological Testing Branch; National Cancer Institute; Bethesda, MD; <https://dtp.cancer.gov> (accessed 02/03/2022)].

The NSC compound reference number 792959 for the seed compound **33** was submitted to the COMPARE website. The reference compounds with most similar GI<sub>50</sub> profile across the NCI60 cell lines, defined as the highest Pearson correlation coefficient (Pearson's *r*). The underlying assumption is that two compounds which are identified as sharing a similar NCI60 profile, they may have a similar mechanism of action.

**Table S6:** Standard COMPARE analysis of compound **33** based on GI<sub>50</sub> mean graph <sup>a</sup>

| Rank | NSC Number | Mechanism of Action                                                                                        | Compound                                                                            | Correlation Coefficient | Reference          |
|------|------------|------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------|--------------------|
| 1    | 791946     | Tubulin polymerization inhibitor                                                                           |    | 0.85                    | [5]                |
| 2    | 791947     | Cytotoxicity, tubulin inhibition                                                                           |    | 0.84                    | [6]                |
| 3    | 794223     | Unknown mechanism of action                                                                                |    | 0.83                    | Data not available |
| 4    | 792769     | Inhibition of colchicine binding site of tubulin.                                                          |    | 0.83                    | [7]                |
| 5    | 792957     | Antiproliferative and antiapoptotic activity, tubulin binding site inhibition                              |   | 0.83                    | [8]                |
| 6    | 791526     | Bisindole alkaloid binds to the microtubule proteins of the mitotic spindle, mitotic arrest and cell death |  | 0.81                    | [9]                |
| 8    | 31708      | Antimitotic action                                                                                         |  | 0.80                    | [10]               |
| 7    | 799732     | Unknown mechanism of action                                                                                | Structure not available                                                             | 0.81                    | Data not available |
| 9    | 705323     | Unknown mechanism of action                                                                                |  | 0.80                    | [11]               |
| 10   | 782882     | Induction of mitotic arrest and apoptosis                                                                  |  | 0.80                    | [12]               |
| 11   | 736992     | Interaction with the colchicine binding site of $\alpha\beta$ -tubulin interface.                          |  | 0.79                    | [13]               |
| 12   | 781551     | Microtubule disruption                                                                                     |  | 0.79                    | [14]               |

|    |        |                                                                           |                                                                                    |      |                    |
|----|--------|---------------------------------------------------------------------------|------------------------------------------------------------------------------------|------|--------------------|
| 13 | 782361 | Microtubule depolymerizing and antitumor activity                         |   | 0.78 | [15]               |
| 14 | 794655 | Unknown mechanism of action                                               |   | 0.78 | Data not available |
| 15 | 792961 | Antiproliferative and antiapoptotic activity                              |   | 0.76 | [16]               |
| 16 | 794658 | Unknown mechanism of action                                               |   | 0.76 | Data not available |
| 17 | 747379 | Caspase activators and apoptosis inducers                                 |   | 0.76 | [17]               |
| 18 | 794639 | Tubulin polymerization inhibition, targets at the colchicine-binding site |   | 0.76 | [18]               |
| 19 | 791945 | Unspecified anticancer activity                                           |  | 0.75 | [19]               |

<sup>a</sup>The target set was the NCI synthetic database and the target set endpoints were selected to be equal to the seed end points. Standard COMPARE analysis was performed. Correlation values (r) are Pearson correlation coefficients.[Reference National Cancer Institute Biological Testing Branch; National Cancer Institute; Bethesda, MD; <https://dtp.cancer.gov> (accessed 02/03/2022)].

**Table S7:** Stability study for compounds **33** and **39**<sup>a</sup>

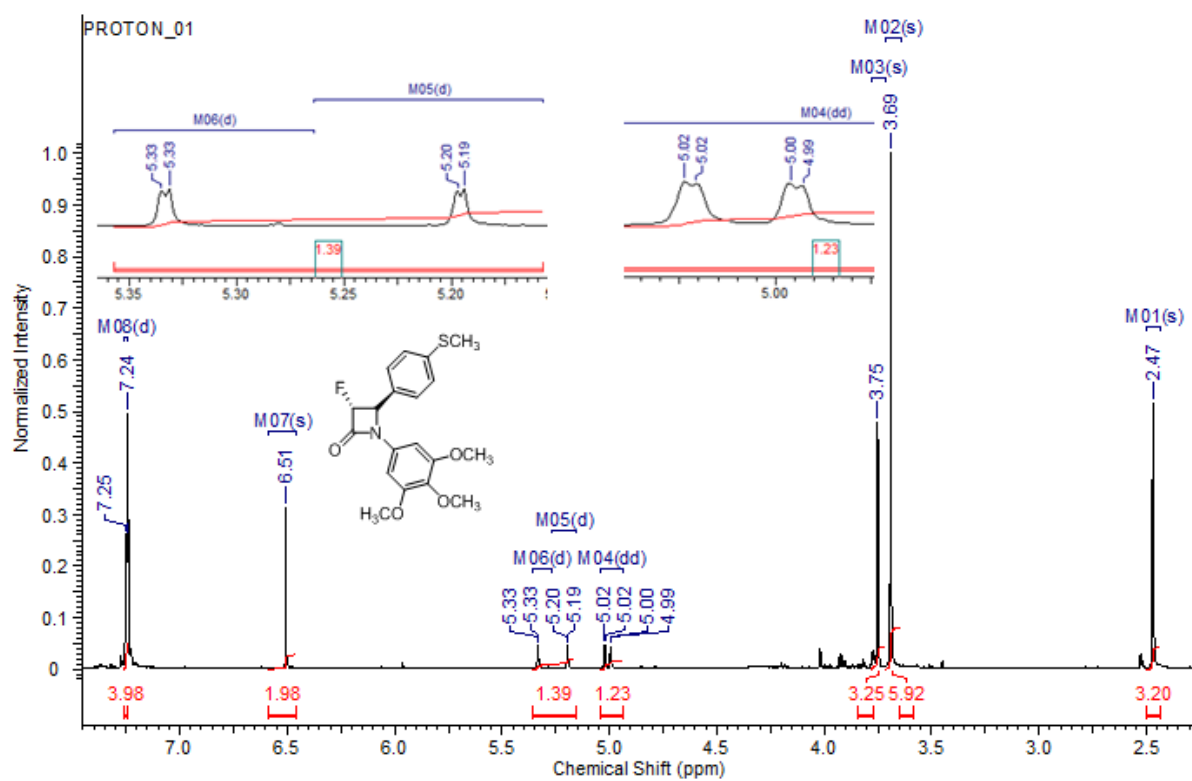
| Conditions                       | Compound <b>33</b><br>(% remaining) | Compound <b>39</b><br>(% remaining) |
|----------------------------------|-------------------------------------|-------------------------------------|
| pH 4                             | 76                                  | 39                                  |
| pH 7.4                           | 87                                  | 50                                  |
| pH 11                            | 26                                  | 19                                  |
| Heat (60°C)                      | 90                                  | 82                                  |
| Light (UV)                       | 88                                  | 61                                  |
| 0.1 M HCl                        | 60                                  | 54                                  |
| 0.1 M NaOH                       | 65                                  | 60                                  |
| 3% H <sub>2</sub> O <sub>2</sub> | 65                                  | 50                                  |

<sup>a</sup> The stability study for compounds **33** and **39** was performed by analytical HPLC, [Symmetry® column (C18, 5 mm, 4.6 x150 mm), Dual Wavelength Absorbance detector (Waters 2487), binary HPLC pump (Waters 1525), and Autosampler (Waters 717 plus)], with mobile phase acetonitrile (70%)/water (30%), flow rate 1 mL/min over 15 min and detection at  $\lambda$  254 nm. Stock solutions of the compounds **33** and **39** (1 mg/mL in mobile phase) were used. Dilutions of 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.0625 mg/mL, 0.03125 mg/mL, 0.015625 and 0.0078 mg/mL were prepared for the calibration curve.

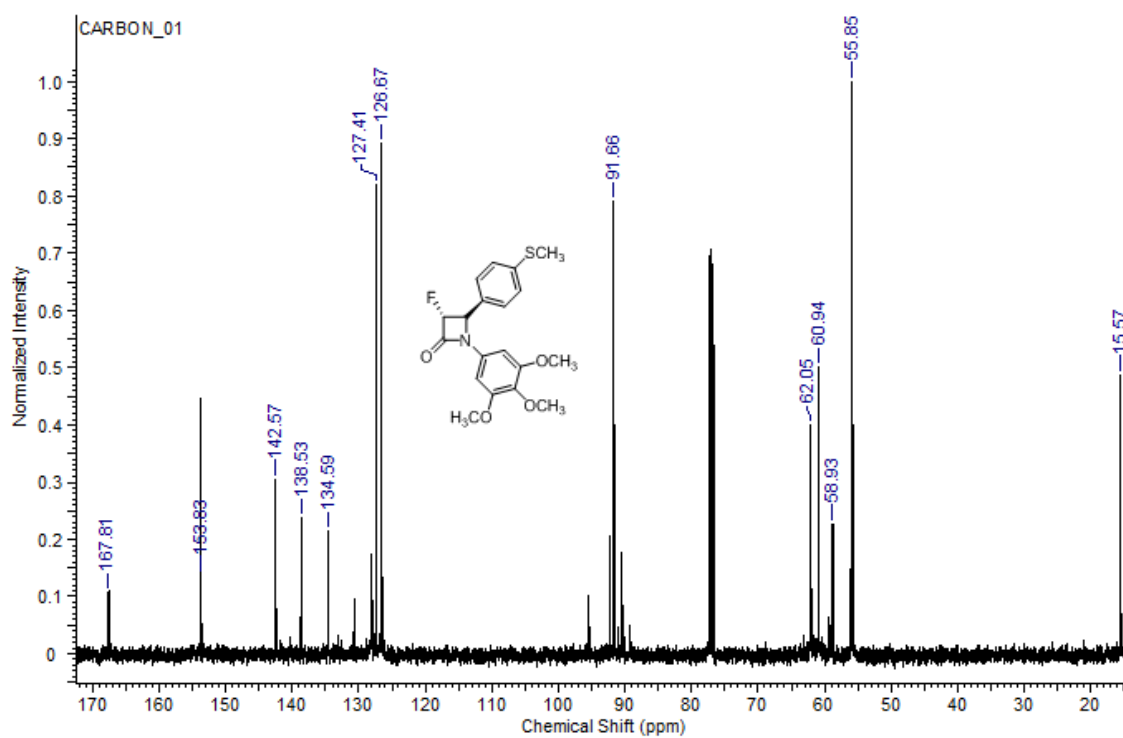
**Table S8:** Docking Scores for compounds **32**, **33**, **42** and **43**

| Compound ID                        | Score <sup>a</sup> |
|------------------------------------|--------------------|
| <b>32</b> (3 <i>S</i> 4 <i>S</i> ) | -9.06              |
| <b>43</b> (4 <i>R</i> )            | -9.04              |
| <b>42</b> (4 <i>S</i> )            | -9.03              |
| <b>33</b> (3 <i>S</i> 4 <i>S</i> ) | -8.90              |
| <b>43</b> (4 <i>S</i> )            | -8.88              |
| <b>32</b> (3 <i>R</i> 4 <i>R</i> ) | -8.47              |
| <b>33</b> (3 <i>R</i> 4 <i>R</i> ) | -8.36              |
| <b>42</b> (4 <i>R</i> )            | -8.22              |

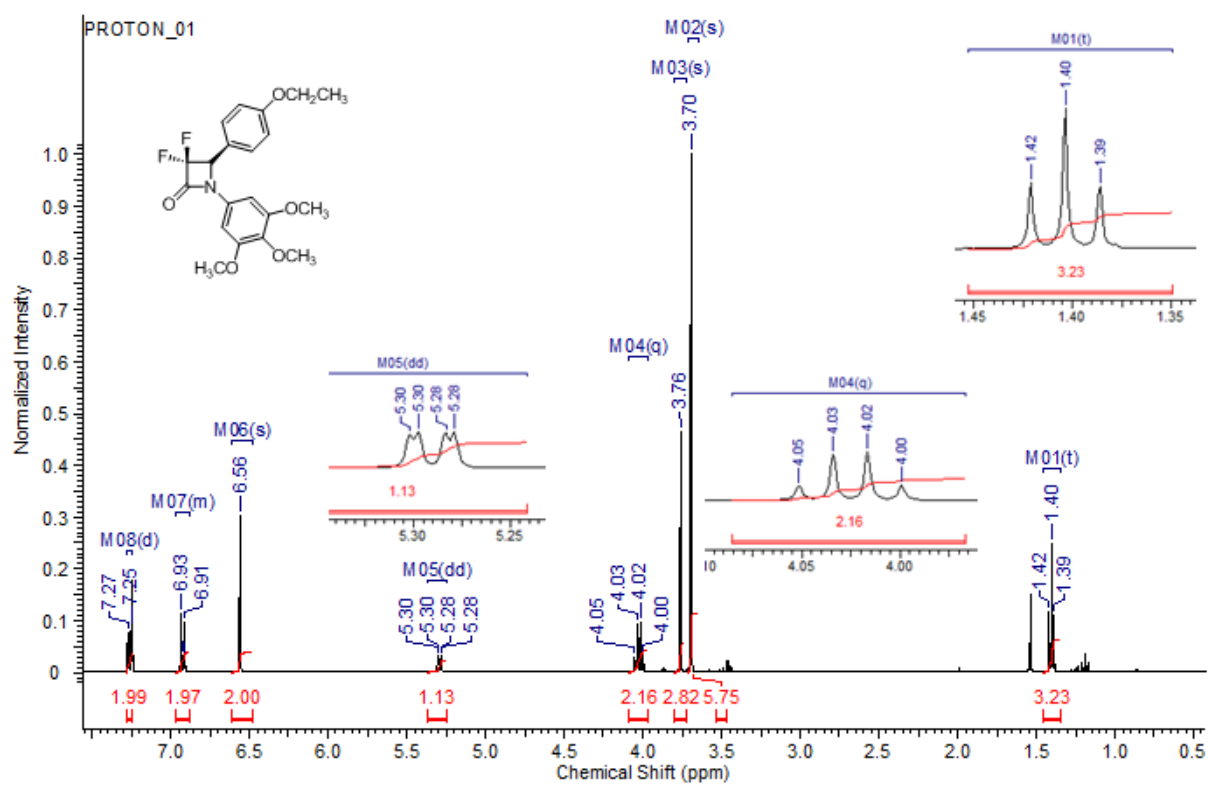
<sup>a</sup>Scores from docking with MOE of the best ranked pose of enantiomers of each compound **32**, **33**, **42** and **43** using the MMFF94x force field



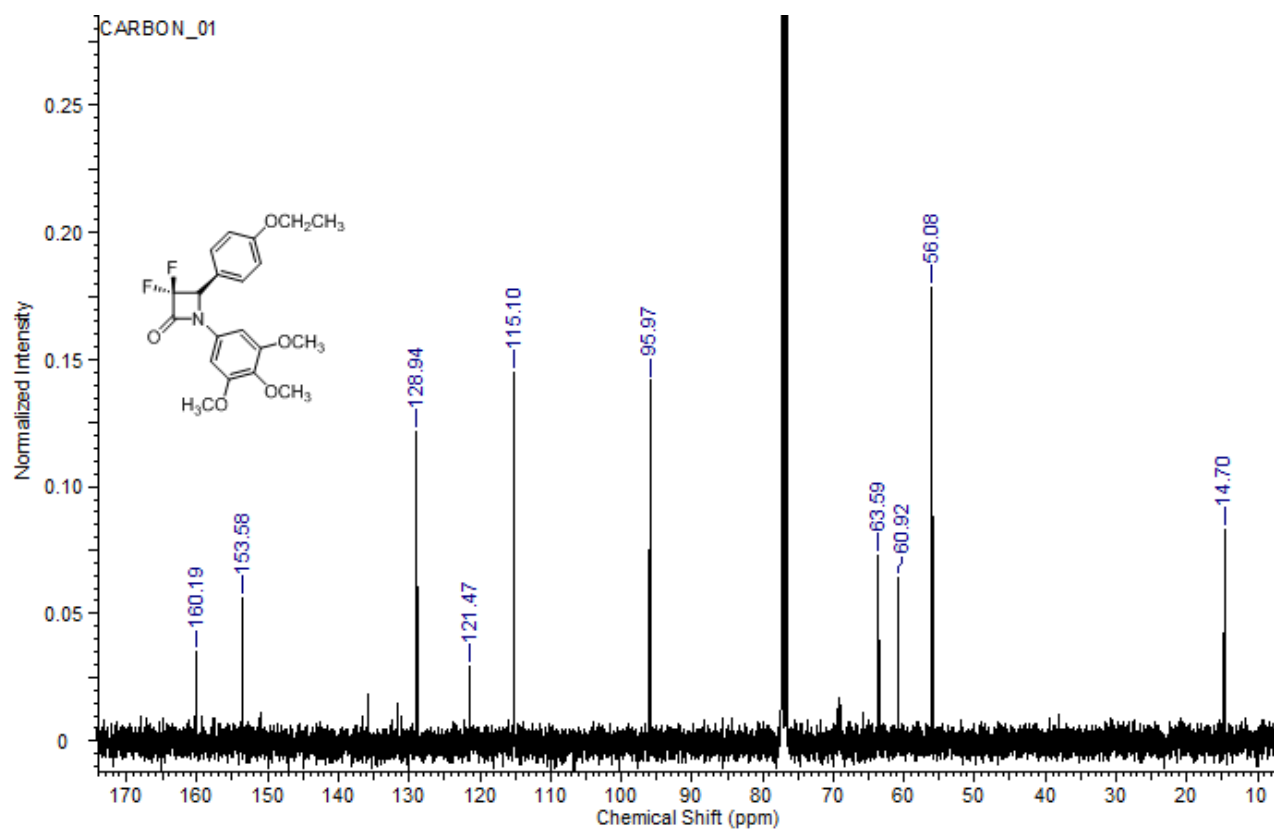
**Figure S1:**  $^1\text{H}$  NMR spectrum of compound **28** (CDCl<sub>3</sub>)



**Figure S2:**  $^{13}\text{C}$  NMR spectrum of compound **28** ( $\text{CDCl}_3$ )



**Figure S3:**  $^1\text{H}$  NMR spectrum of compound **37** ( $\text{CDCl}_3$ )



**Figure S4:**  $^{13}\text{C}$  NMR spectrum of compound **37** ( $\text{CDCl}_3$ )

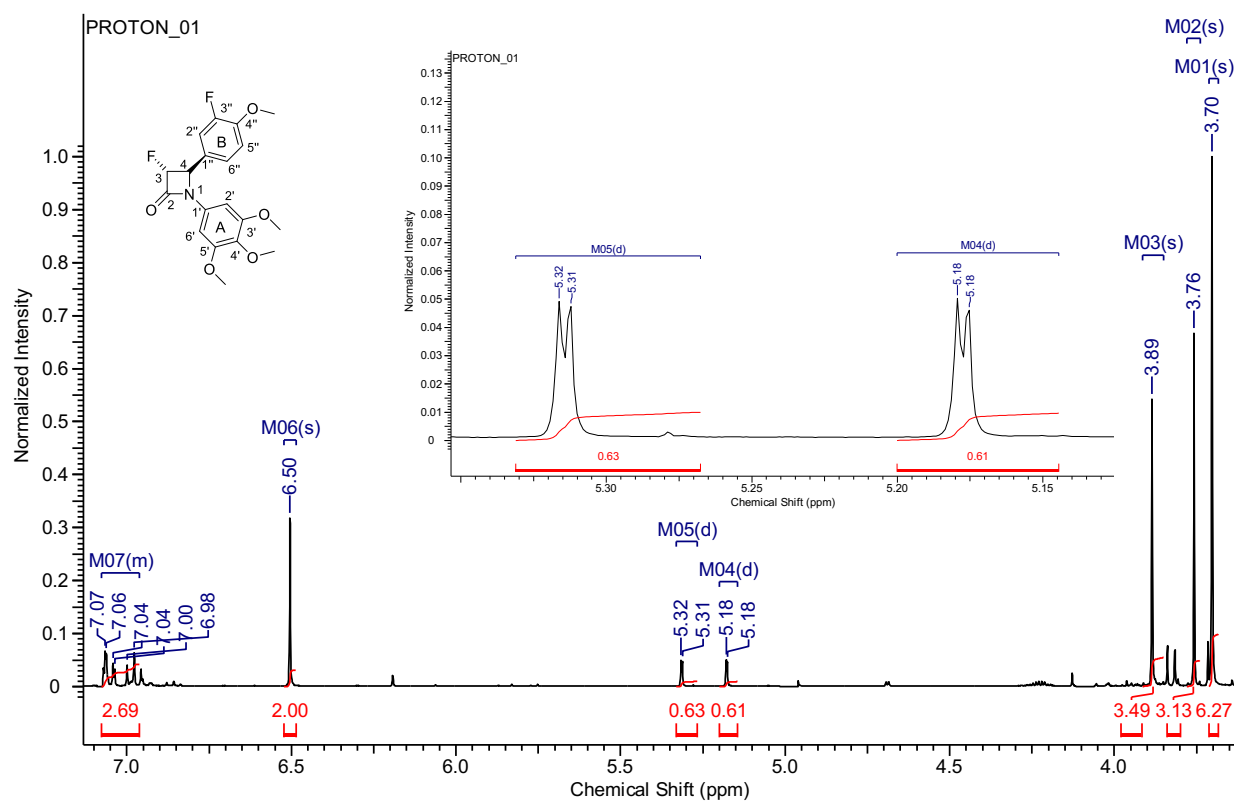
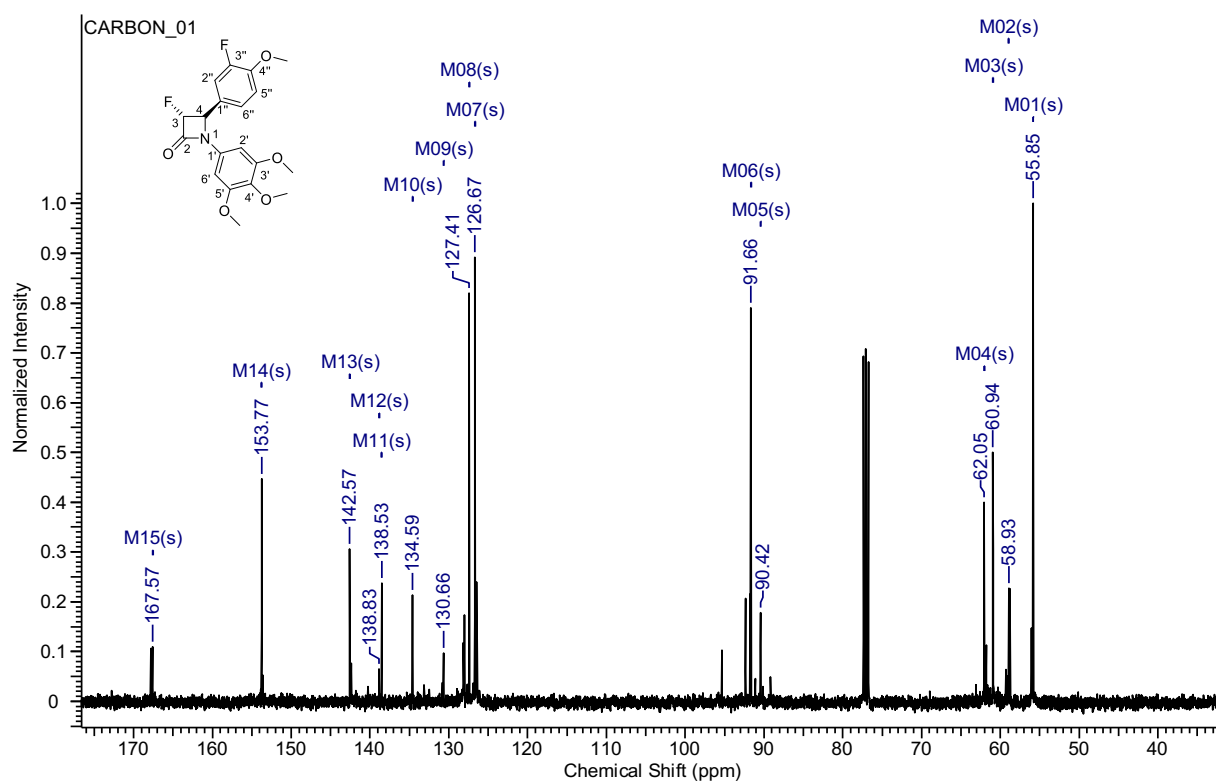
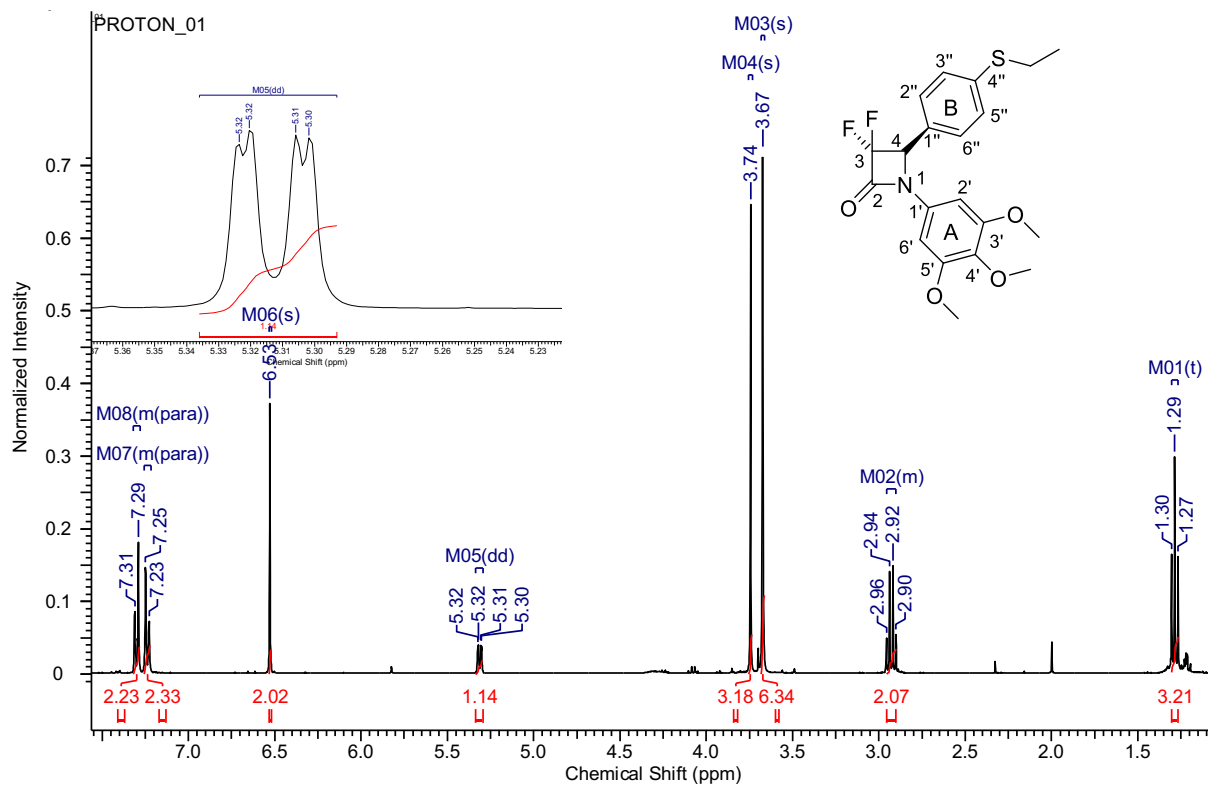


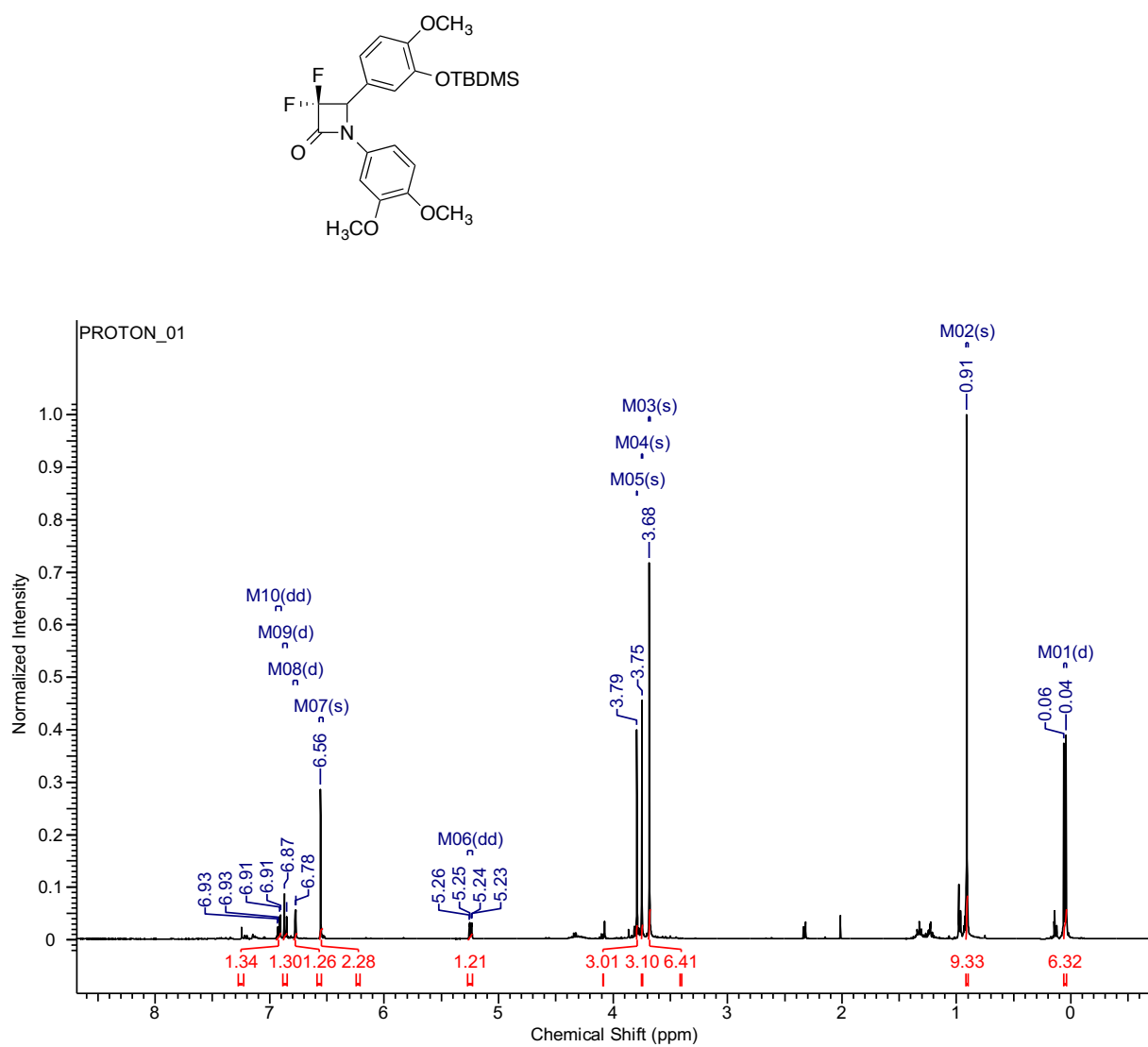
Figure S5: <sup>1</sup>H-NMR spectrum of compound **33** (CDCl<sub>3</sub>)



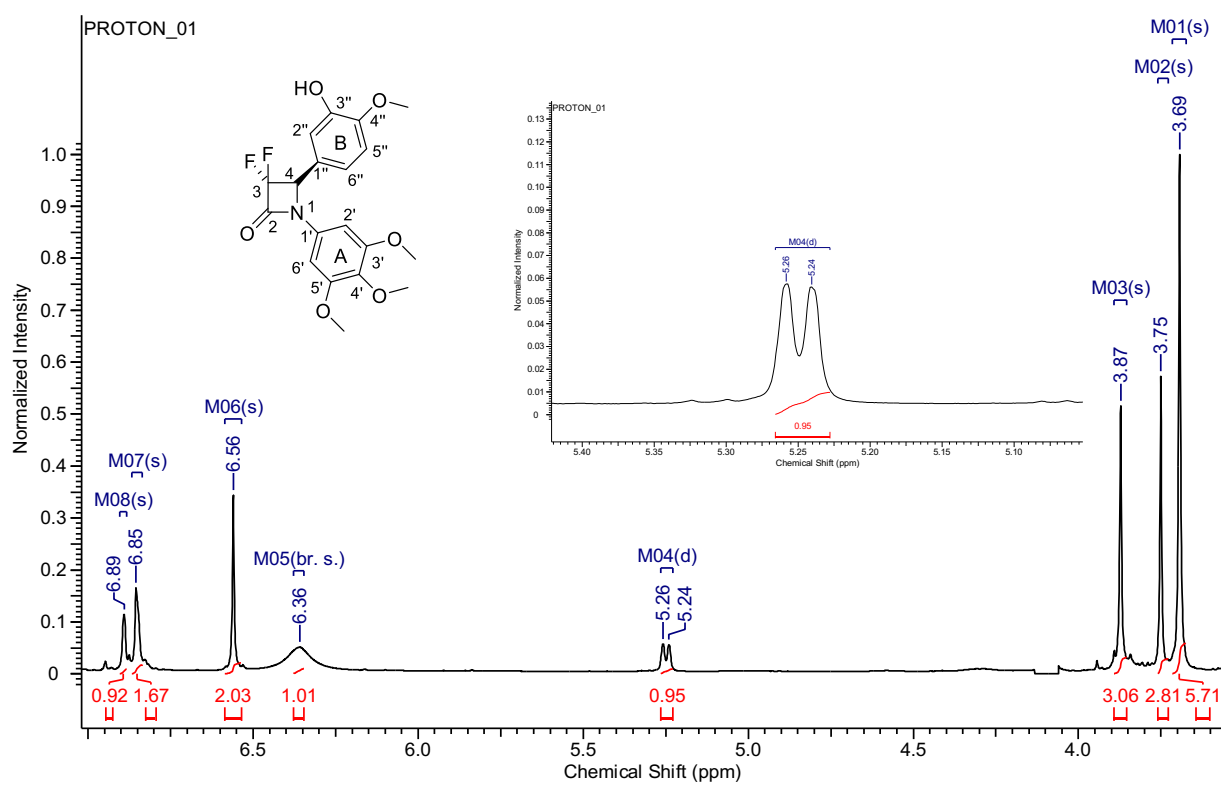
**Figure S6:**  $^{13}\text{C}$ -NMR spectrum of compound **33** ( $\text{CDCl}_3$ )

**Figure S7:**  $^1\text{H}$ -NMR spectrum of compound **39** ( $\text{CDCl}_3$ )

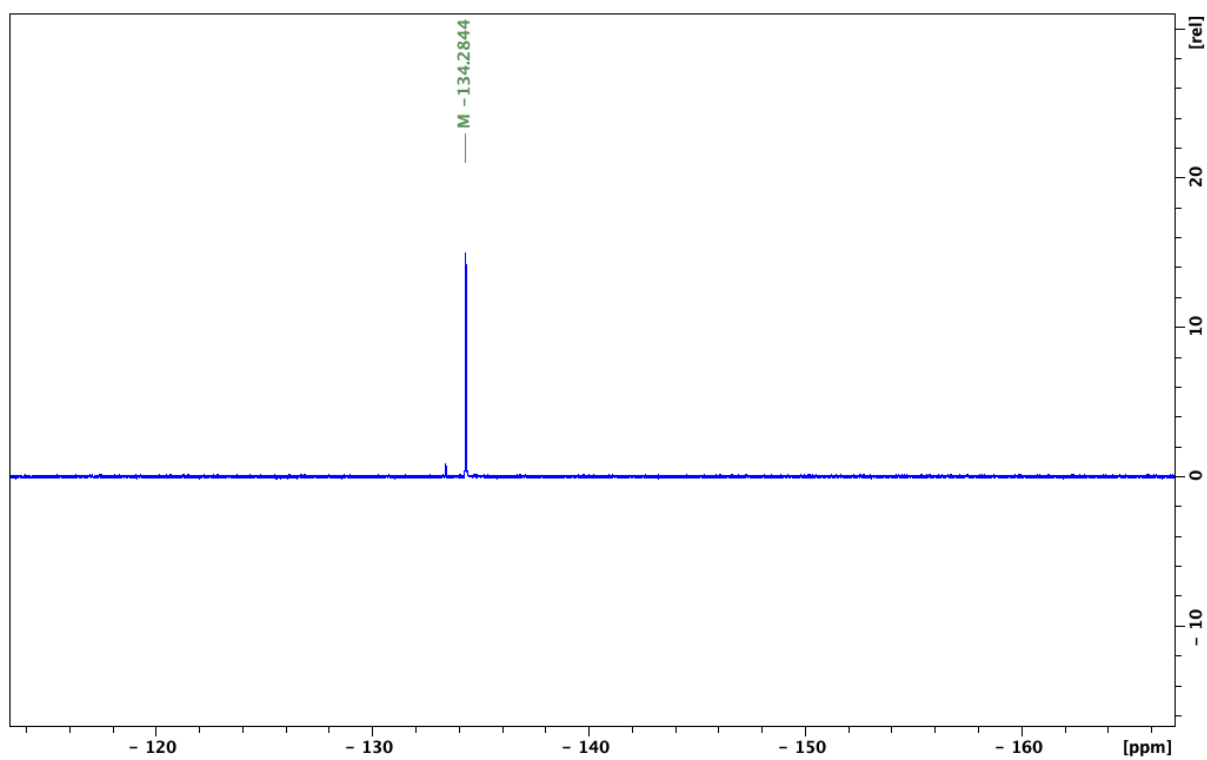




**Figure S8:**  $^1\text{H}$ -NMR spectrum for compound **41** ( $\text{CDCl}_3$ )

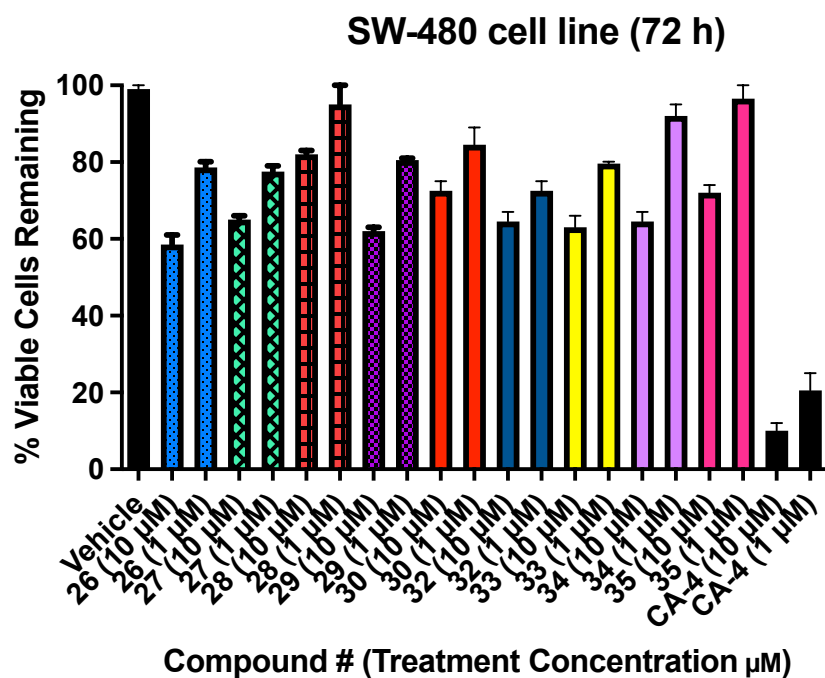


**Figure S9:**  $^1\text{H}$ -NMR spectrum of compound **42** ( $\text{CDCl}_3$ )

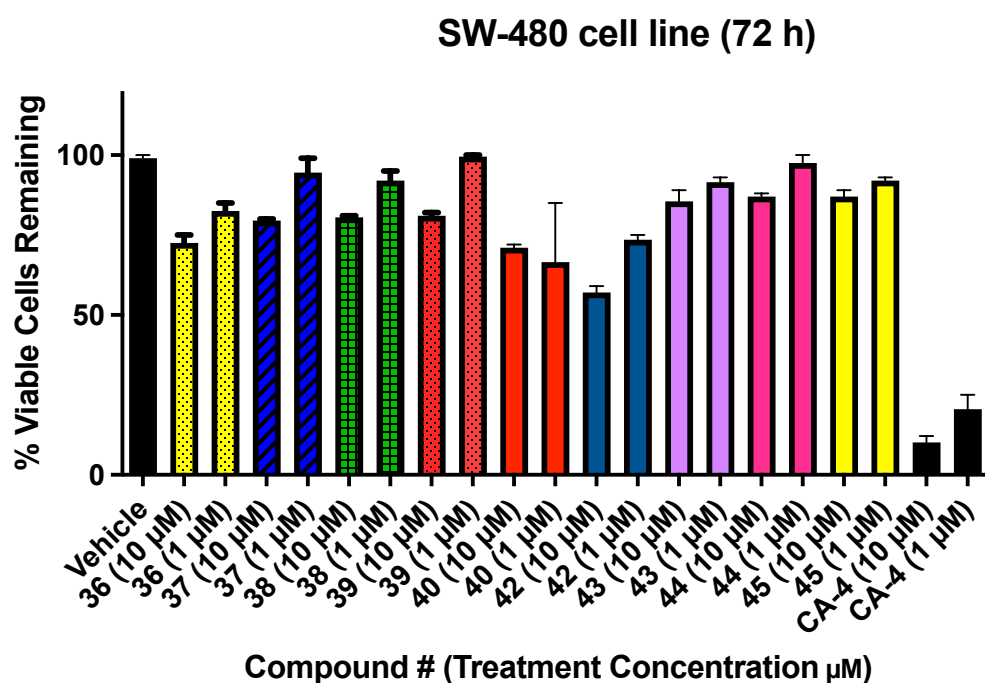


**Figure S10:**  $^{19}\text{F}$  NMR spectrum of compound **23** (376 MHz,  $\text{CDCl}_3$ )

(A)



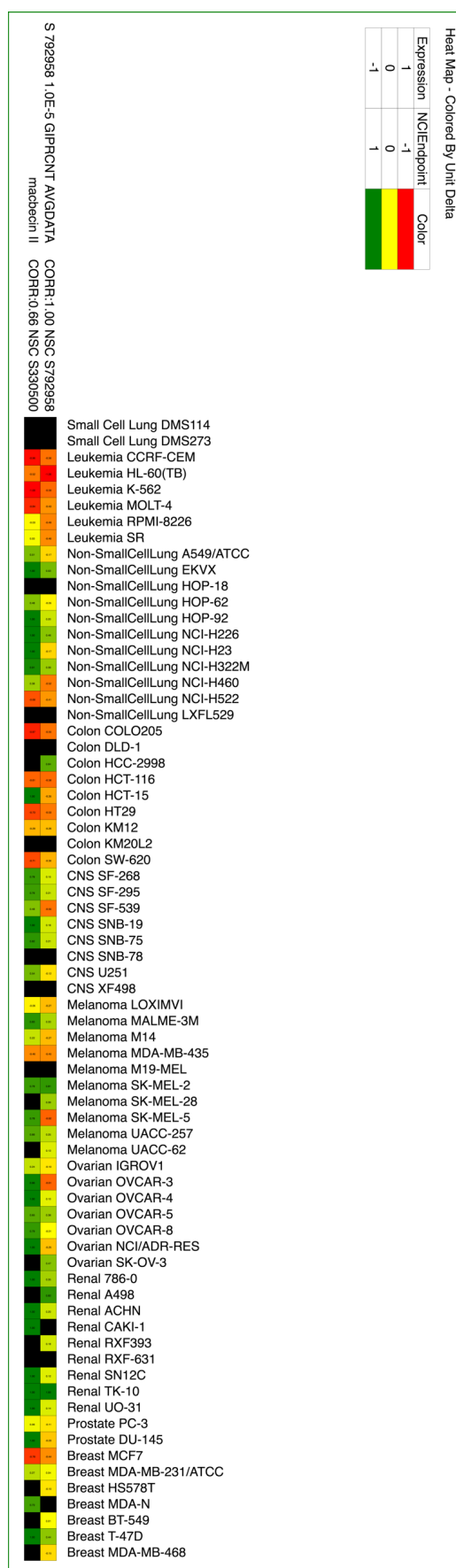
(B)



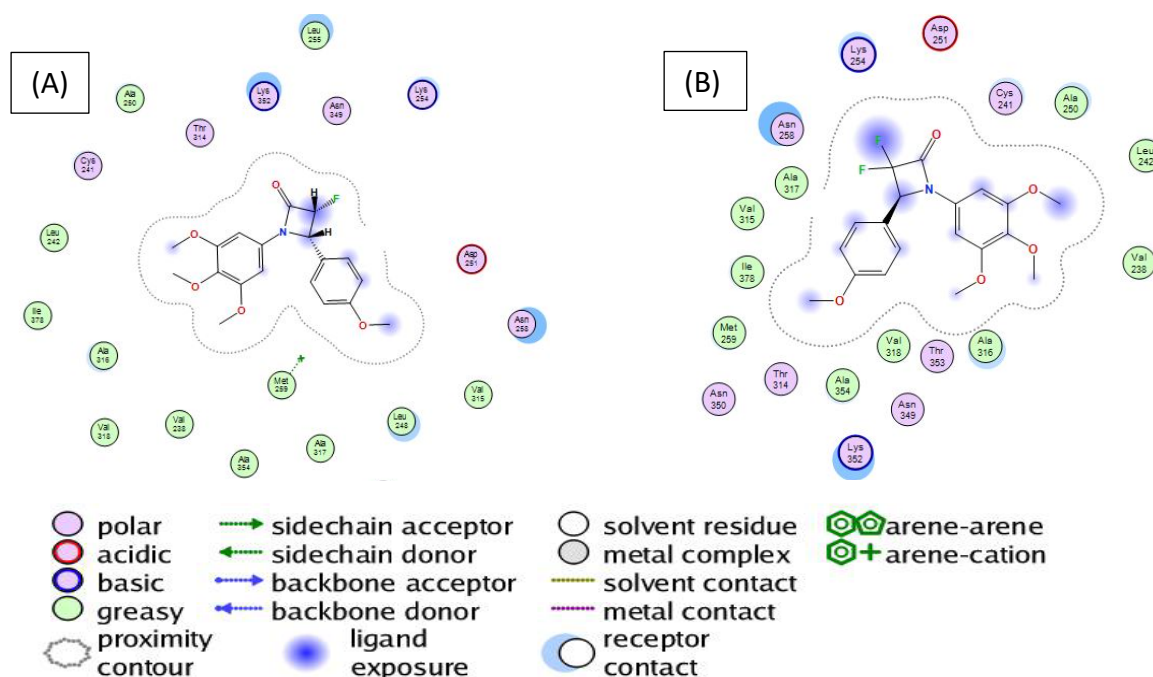
**Figure S11:** Preliminary cell viability data for (A) 3-fluoro  $\beta$ -lactam compounds containing different *para*-substituent ring B 26-29; 3-fluoro  $\beta$ -lactam compounds containing different *meta*-substituent ring B 30, 33-35; (B) 3,3-difluoro  $\beta$ -lactam compounds containing different *para*-substituent ring B 36-39; 3,3-difluoro  $\beta$ -lactam compounds containing different *meta*-substituent

ring B **40, 42-45** in SW-480 cancer cells. The IC<sub>50</sub> value determined for CA-4 in SW480 cells is 0.0063±0.0006 µM.

Cell proliferation of MCF-7 cells was determined with an alamarBlue assay (seeding density  $2.5 \times 10^5$  cells/mL per well for 96-well plates). Compound concentrations of either 10 µM or 1 µM for 72 h were used to treat the cells with control wells containing vehicle ethanol (1% v/v). The mean value for three experiments is shown.



**Figure S12:** Heat map compound 792959 and macbeccin NSC S330500



**Figure S13.** Protein-ligand interactions for the 3-fluoro and 3,3-difluoro- $\beta$ -lactam compounds **26** and **36** with the colchicine-binding site of tubulin.

**(A)** 2D representation of the ligand–protein interactions of compound **26** (red) and Colchicine (blue) in the colchicine binding site interacting with Lys352, Met259, Leu266, Lys254, Asn258 and Leu240 rendered using the LigX module of MOE. **(B)** 2D representation of compound **36** (red) and Colchicine (blue) in the colchicine binding site interacting with Asn258, Lys254, Lys352, Ala316, Cys241 and Ala250 rendered using the LigX module of MOE.

## References

1. O'Boyle, N.M.; Carr, M.; Greene, L.M.; Bergin, O.; Nathwani, S.M.; McCabe, T.; Lloyd, D.G.; Zisterer, D.M.; Meegan, M.J. Synthesis and evaluation of azetidinone analogues of combretastatin a-4 as tubulin targeting agents. *J Med Chem* **2010**, *53*, 8569-8584.
2. Carr, M.; Greene, L.M.; Knox, A.J.; Lloyd, D.G.; Zisterer, D.M.; Meegan, M.J. Lead identification of conformationally restricted beta-lactam type combretastatin analogues: Synthesis, antiproliferative activity and tubulin targeting effects. *Eur J Med Chem* **2010**, *45*, 5752-5766.
3. Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H.M.; Lin, C.M.; Hamel, E. Synthesis and evaluation of analogues of (z)-1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene as potential cytotoxic and antimitotic agents. *J Med Chem* **1992**, *35*, 2293-2306.
4. Messaoudi, S.; Treguier, B.; Hamze, A.; Provot, O.; Peyrat, J.F.; De Losada, J.R.; Liu, J.M.; Bignon, J.; Wdzieczak-Bakala, J.; Thoret, S., *et al.* Isocombretastatins a versus combretastatins a: The forgotten isoca-4 isomer as a highly promising cytotoxic and antitubulin agent. *J Med Chem* **2009**, *52*, 4538-4542.
5. Messaoudi, S.; Hamze, A.; Provot, O.; Treguier, B.; Rodrigo De Losada, J.; Bignon, J.; Liu, J.M.; Wdzieczak-Bakala, J.; Thoret, S.; Dubois, J., *et al.* Discovery of isoerianin analogues as promising anticancer agents. *ChemMedChem* **2011**, *6*, 488-497.
6. Alvarez, R.; Puebla, P.; Diaz, J.F.; Bento, A.C.; Garcia-Navas, R.; de la Iglesia-Vicente, J.; Mollinedo, F.; Andreu, J.M.; Medarde, M.; Pelaez, R. Endowing indole-based tubulin inhibitors with an anchor for derivatization: Highly potent 3-substituted indolephenstatins and indoleisocombretastatins. *J Med Chem* **2013**, *56*, 2813-2827.
7. Popovici, L.; Amarandi, R.M.; Mangalagiu, II; Mangalagiu, V.; Danac, R. Synthesis, molecular modelling and anticancer evaluation of new pyrrolo[1,2-b]pyridazine and pyrrolo[2,1-a]phthalazine derivatives. *J Enzyme Inhib Med Chem* **2019**, *34*, 230-243.
8. Malebari, A.M.; Fayne, D.; Nathwani, S.M.; O'Connell, F.; Noorani, S.; Twamley, B.; O'Boyle, N.M.; O'Sullivan, J.; Zisterer, D.M.; Meegan, M.J. Beta-lactams with antiproliferative and antiapoptotic activity in breast and chemoresistant colon cancer cells. *Eur J Med Chem* **2020**, *189*, 112050.
9. Keglevich, P.H., L.; Dubrovay, Z.; Dékány, M.; Szántay, C.; Kalaus, G.,; Szántay, C. . Bisindole alkaloids condensed with a cyclopropane ring, part 1. 14,15-cyclopropanovinblastine and vincristine. *Heterocycles* **2014** *89*, 653-668.
10. Mojica, M.A.L., A.; Rojas-Sepulveda, A. M.; Marquina, S.; Mendieta-Serrano, M. A.; Salas-Vidal, E.; Villarreal, M. L.; Alvarez, L. . Aryldihydronaphthalene-type lignans from bursera fagaroides var. Fagaroides and their antimitotic mechanism of action *Rsc Adv* **2016**, *6*, 4950-4959.
11. Prakash, O.; Khan, F. Cluster based svr-qsar modelling for hts records: An implementation for anticancer leads against human breast cancer. *Comb Chem High Throughput Screen* **2013**, *16*, 511-521.
12. Lu, T.; Goh, A.W.; Yu, M.; Adams, J.; Lam, F.; Teo, T.; Li, P.; Noll, B.; Zhong, L.; Diab, S., *et al.* Discovery of (e)-3-((styrylsulfonyl)methyl)pyridine and (e)-2-((styrylsulfonyl)methyl)pyridine derivatives as anticancer agents: Synthesis, structure-activity relationships, and biological activities. *J Med Chem* **2014**, *57*, 2275-2291.

13. Bellina, F.; Cauteruccio, S.; Monti, S.; Rossi, R. Novel imidazole-based combretastatin a-4 analogues: Evaluation of their in vitro antitumor activity and molecular modeling study of their binding to the colchicine site of tubulin. *Bioorg Med Chem Lett* **2006**, *16*, 5757-5762.
14. Trabbic, C.J.; Overmeyer, J.H.; Alexander, E.M.; Crissman, E.J.; Kvale, H.M.; Smith, M.A.; Erhardt, P.W.; Maltese, W.A. Synthesis and biological evaluation of indolyl-pyridinyl-propenones having either methuosis or microtubule disruption activity. *J Med Chem* **2015**, *58*, 2489-2512.
15. Xiang, W.; Quadery, T.M.; Hamel, E.; Luckett-Chastain, L.R.; Ihnat, M.A.; Mooberry, S.L.; Gangjee, A. The 3-d conformational shape of n-naphthyl-cyclopenta[d]pyrimidines affects their potency as microtubule targeting agents and their antitumor activity. *Bioorganic & medicinal chemistry* **2021**, *29*, 115887.
16. Malebari, A.M.; Wang, S.; Greene, T.F.; O'Boyle, N.M.; Fayne, D.; Khan, M.F.; Nathwani, S.M.; Twamley, B.; McCabe, T.; Zisterer, D.M., *et al.* Synthesis and antiproliferative evaluation of 3-chloroazetidin-2-ones with antimetabolic activity: Heterocyclic bridged analogues of combretastatin a-4. *Pharmaceuticals (Basel)* **2021**, *14*.
17. Preparation of indole-2-carboxylic acid benzylidenehydrazides and analogs as caspase activators and apoptosis inducers; cai, s.X.; drewe, j.A.; zhang, h.; united state patent us20020091148 (2002-09-12).
18. Xie, Y.; Kril, L.M.; Yu, T.; Zhang, W.; Frasinuk, M.S.; Bondarenko, S.P.; Kondratyuk, K.M.; Hausman, E.; Martin, Z.M.; Wyrebek, P.P., *et al.* Semisynthetic aurones inhibit tubulin polymerization at the colchicine-binding site and repress pc-3 tumor xenografts in nude mice and myc-induced t-all in zebrafish. *Sci Rep* **2019**, *9*, 6439.
19. Taylor, B.L.; Swift, E.C.; Waetzig, J.D.; Jarvo, E.R. Stereospecific nickel-catalyzed cross-coupling reactions of alkyl ethers: Enantioselective synthesis of diarylethanes. *J Am Chem Soc* **2011**, *133*, 389-391.