SUPPORTING INFORMATION

Store-Operated Calcium Entry (SOCE) as a therapeutic target in acute pancreatitis: discovery and development of drug-like SOCE inhibitors

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Chemistry

Synthesis and characterization of azide 73

Reagents and conditions: (a) NaNO$_2$, NaN$_3$, TFA, 0 °C, 2 h, 76%.

4-Azido-3-fluoropyridine, (73). To a solution of 3-fluoropyridin-4-amine 72 (150 mg, 1.33 mmol) in TFA (3.3 mL) NaNO$_2$ (111 mg, 1.61 mmol) was added at 0 °C. After 1 h, NaN$_3$ (1.88 g, 35.2 mmol) was added and the mixture was maintained under vigorous stirring. After 1 h, saturated aqueous NaHCO$_3$ solution was added until pH 8-9 and the aqueous phase was extracted with ethyl acetate (x2). The collected organic layers were dried over sodium sulfate and evaporated to give 4-azido-3-fluoropyridine 73 (139 mg, 1.01 mmol, 76%) as a pale brown oil. $^1$H NMR (300 MHz, CDCl$_3$): δ = 8.40 (d, $J = 3.0$ Hz, 1H), 8.30 (d, $J = 5.2$ Hz, 1H), 7.27 (dd, $J_s = 5.2$ Hz, 3.1 Hz, 1H).

Synthesis and characterization of azide 75

Reagents and conditions: (a) NaN$_3$, DMF, H$_2$O, 80 °C, 16 h, 59%.

Methyl 4-azidopicolinate, (75). Methyl 4-bromopicolinate 74 (300 mg, 1.91 mmol) was solubilized in DMF (2.2 mL) and water (0.11 mL) and NaN$_3$ (149 mg, 2.29 mmol) was added portionwise. The reaction was heated at 80 °C overnight. Then, the mixture was diluted with ethyl acetate and washed with water (x5). The organic phase was dried over sodium sulfate and evaporated to afford compound...
75 as a pale yellow solid (200 mg, 1.13 mmol, 59%). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta = 8.53\) (d, \(J = 5.5\) Hz, 1H), 7.74 (s, 1H), 7.31 (d, \(J = 5.5\) Hz, 1H), 3.89 (s, 3H).

**Synthesis and characterization of alkyne 77**

Reagents and conditions: (a) Ethynyltrimethylsilane, DIPEA, CuI, Pd(PPh\(_3\))\(_2\)Cl\(_2\), DMF, rt, 2 h. (b) TBAF, THF, 0 °C, 30 min, 45%.

**4-Ethynyl-3-fluoropyridine (77).** **Step 1:** 3-Fluoro-4-iodopyridine 76 (118 mg; 0.53 mmol), DMF (1.2 mL), Pd(PPh\(_3\))\(_2\)Cl\(_2\) (35 mg; 0.05 mmol), CuI (9.52 mg; 0.05 mmol), DIPEA (0.36 mL; 2.11 mmol) and ethynyltrimethylsilane (0.22 mL; 1.59 mmol) were added in a Schlenk apparatus under nitrogen atmosphere. After 2 h the mixture was filtered over a pad of celite, the volatile was removed under reduced pressure and the reaction was worked up by dilution with ethyl acetate and washed with water (x1). The organic layer was washed with brine, dried over sodium sulfate and evaporated. The crude product was used in the next step without further purification. **Step 2:** 3-fluoro-4-((trimethylsilyl)ethynyl) pyridine was dissolved in THF (3.3 mL) at 0 °C. After 5 min TBAF (0.62 mL; 0.62 mmol) was added. After 30 min the volatile was removed under reduced pressure and the reaction was worked up by dilution with ethyl acetate and washing with water (x1). The crude material was purified by column chromatography using petroleum ether/ethyl acetate 98:2 and then petroleum ether/ethyl acetate 95:5 as eluents yielding 4-ethynyl-3-fluoropyridine 77 (28.9 mg, 0.23 mmol, 45%) as a brown oil. \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta = 8.50\) (s, 1H), 8.39 (d, \(J = 4.7\) Hz, 1H), 7.38 (d, \(J = 5.5\) Hz, 1H), 3.51 (s, 1H). MS (ESI): \(m/z\): 122 [M + H]\(^+\).
Synthesis and characterization of alkyne 78

![Chemical Structure](image)

Reagents and conditions: (a) Ethynyltrimethylsilane, DIPEA, CuI, Pd(PPh$_3$)$_2$Cl$_2$, toluene dry, 100 °C, 2 h, 99%. (b) CH$_3$COOH, TBAF, THF, 0 °C, 30 min, 93%.

**Methyl 4-ethynlypicolinate, (78). Step 1:** To a solution of methyl 4-bromopicolinate 74 (300 mg, 1.39 mmol) in dry toluene (3 mL), DIPEA (0.48 mL, 2.79 mmol), CuI (24 mg, 0.12 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (29.33 mg, 0.04 mmol) and ethynyltrimethylsilane (0.59 mL, 4.19 mmol) were added in a Schlenk apparatus. The reaction was stirred at reflux for 2 h under nitrogen atmosphere. Then, the mixture was filtered over a pad of celite and rinsed with ethyl acetate. The organic phase was washed with water (x1), dried over sodium sulfate and evaporated. The crude material was purified by column chromatography using petroleum ether/ethyl acetate 8:2 and petroleum ether/ethyl acetate 7:3 as eluents to give methyl 4-((trimethylsilyl)ethynyl)picolinate as a brown oil (322 mg, 1.38 mmol, 99%).

$^1$H NMR (300 MHz; CDCl$_3$): $\delta$ = 8.69 (d, $J = 4.8$ Hz, 1H), 8.14 (s, 1H), 7.54 (d, $J = 4.8$ Hz, 1H), 4.06 (s, 3H), 1.19 (s, 9H).

**Step 2:** methyl 4-((trimethylsilyl)ethynyl)picolinate (173 mg, 0.74 mmol) was dissolved in THF (1.5 mL). The mixture was cooled at 0 °C and CH$_3$COOH (50.9 µL, 0.89 mmol) and TBAF (0.89 mL, 0.89 mmol) were added. The reaction was stirred at 0 °C for 30 min. The volatile was removed under vacuum, ethyl acetate was added and the organic layer was washed with water (x1). After drying over sodium sulfate and evaporation of the solvent, the crude material was purified by column chromatography using petroleum ether/ethyl acetate 6:4 and petroleum ether/ethyl acetate 5:5 as eluents to give compound 78 (111 mg, 0.69 mmol, 93%) as a brown solid. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 8.17 (s, 1 H), 7.96 (d, $J = 6.5$ Hz, 1H), 7.67 (d, $J = 6.5$ Hz, 1H), 7.40 (t, $J = 6.5$ Hz, 1H), 3.92 (s, 3H), 3.12 (s, 1H). MS (ESI): $m/z$: 161 [M + H]$^+$.
NMR Spectra of compounds 31, 34, 35, 36, 38, 40, 50, 56.

31: $^1$H (DMSO-$d_6$), $^{13}$C (DMSO-$d_6$)
34: $^1$H (DMSO-$d_6$), $^{13}$C (DMSO-$d_6$)
35: $^1$H (DMSO-\textit{d}$_6$), $^{13}$C (DMSO-\textit{d}$_6$)
36: $^1$H (DMSO-$d_6$), $^{13}$C (DMSO-$d_6$)
38: $^1$H (DMSO-$d_6$), $^{13}$C (DMSO-$d_6$)
40: $^1$H (DMSO-$d_6$), $^{13}$C (DMSO-$d_6$)
50: $^1\text{H (DMSO-}d_6\text{)}, \quad ^{13}\text{C (DMSO-}d_6\text{)}$
56: $^1$H (DMSO-$d_6$), $^{13}$C (DMSO-$d_6$)
**Figure S1.** Evaluation of the area under the curve (AUC), peak amplitude, and slope of the Ca$^{2+}$-rise in in the absence or presence of the indicated compounds. Graph shows median and IQR of the AUC, peak amplitude and slope of the Ca$^{2+}$-rise. Mann-Whitney U test of compound vs control (\* p<0.0286, ** p<0.0038, *** p<0.0007, **** p<0.0001). Mann-Whitney U test. Mann-Whitney U test of compound vs Synta66 (# = 0.0485).
**In vitro metabolism and pharmacokinetic.**

A Thermo Scientific Q-Exactive Plus system equipped with a Thermo Scientific Vanquish UHPLC system with a binary pump VF-P10, a split sampler VF-A10, and a column compartment VH-C10 were used. Data were acquired and processed using Xcalibur® software.

The operating conditions of the mass spectrometer were as follows: positive mode; sheath gas flow rate, 45 Auxiliary Units (A.U.), auxiliary gas flow rate, 10 A.U.; sweep gas flow rate, 2 A.U.; spray voltage, 3.50 kV; capillary temperature, 300 °C; auxiliary gas heater temperature, 300 °C.

1) **LC-HRMS methods for metabolism studies.**

- Column: *Phenomenex Kinetex C18 100 × 2.1 mm (2.6µm d.p.*) protected with a SecurityGuard® and kept at 40 °C (Torrance, CA, USA).

- Eluent:

  - A: 0.1% formic acid in water.
  - B: methanol.

- Flow rate: 0.250 mL/min.

- Injection volume: 5 µL.

- Gradient program: 0.00 min [B%=20%], 14.00 min [B%=90%], 16.50 min [B%=90%], 17.00 min [B%=20%], 22.00 min [B%=20%].

For metabolic stability assays, samples were acquired in positive full-MS and parallel reaction monitoring (PRM), using the parameters reported in Table S1 and monitoring the ions reported in the inclusion list S2.
Table S1. Parameters used for metabolic stability assays.

| Parameter                  | Value          |
|----------------------------|---------------|
| Microscans                 | 1             |
| Resolution                 | 35.000        |
| AGC target                 | 1×10^6        |
| Maximum IT                 | 120 ms        |
| Number of scan ranges      | 1             |
| Loop count                 | 1             |
| MSX count                  | 1             |
| MSX isocronous ITs         | on            |
| Isolation window           | 1.5 m/z       |
| Fixed first mass           | 180.0 m/z     |
| Collision energy           | CE:37         |
| Mass scan range            | 150-850 m/z   |

Table S2. Ions monitored for metabolic stability assays.

For metabolite characterization of compound 34, samples were analysed in positive full-MS and dd-MS^2 (topN) modes using the parameters reported in Table S3.

Full MS | dd-MS^2 (topN) |
|---------|---------------|
| Microscans | Microscans | 1 |
| Resolution | Resolution | 70.000 | 17.500 |
| AGC target | AGC target | 1×10^6 | 1×10^5 |
| Maximum IT | Maximum IT | 200 ms | 60 ms |
| Number of scan ranges | Loop count | 1 | 4 |
| Scan range | MSX count | 150-850 m/z | 1 |
| TopN | Isolation window | 4.0 m/z |
| Isolation window | Collision energy (CE) | 37 |
| Minimum AGC target | Intensity threshold | 6×10^3 | 1×10^5 |
| Dynamic exclusion | | 3.0 s |

Table S3. Parameters used for metabolite characterization of compound 34.
2) **LC-HRMS methods for pharmacokinetic analysis of compound 34.**

- **Column:** Phenomenex Kinetex C18 150 × 2.1 mm (2.6µm d.p.) protected with a SecurityGuard® and kept at 40 °C (Torrance, CA, USA).

- **Eluent:**
  
  A: 0.1% formic acid in water.
  
  B: acetonitrile.

- **Injection volume:** 5 µL.

- **Analysis** were performed in solvent and flow rate gradient elution (Table S4).

| Time (min) | B%  | Flow [mL/min] |
|------------|-----|---------------|
| 0.00       | 30  | 0.300         |
| 5.00       | 98  | 0.300         |
| 7.00       | 98  | 0.400         |
| 7.50       | 30  | 0.400         |
| 10.00      | 30  | 0.300         |

**Table S4.** Solvent and flow rate gradient elution used for pharmacokinetic analysis of compound 34.

Data were acquired in parallel reaction monitoring (PRM) mode monitoring the ions [M+H]+ 402.14483 (C_{23}H_{19}N_{3}O_{4}) 34 and [M+H]+ 390.12485 (C_{22}H_{16}FN_{3}O_{3}) 40 which was used as internal standard (IS). The operating conditions are reported in Table S5.

| PRM                        |                |
|---------------------------|----------------|
| Microscans                | 1              |
| Resolution                | 35.000         |
| AGC target                | 1×10^5         |
| Maximum IT                | 120 ms         |
| Number of scan ranges     | 1              |
| Loop count                | 1              |
| MSX count                 | 1              |
| MSX isocronous ITs        | on             |
| Isolation window          | 1.5 m/z        |
| Fixed first mass          | 180.0 m/z      |
| Collision energy          | CE:32          |

**Table S5.** Operating conditions used for pharmacokinetic analysis of compound 34.
Purity of lead compounds and thermodynamic aqueous solubility.

A Shimadzu HPLC system (Shimadzu, Kyoto, Japan), consisting of two LC-10AD Vp module pumps, an SLC-10A Vp system controller, an SIL-10AD Vp autosampler, and a DGU-14-A on-line degasser were used for the analysis. The SPD-M10Avp photodiode array detector was used to detect the analytes. LC-Solution 1.24 software was used to process the chromatograms.

- **Column:** Phenomenex Kinetex C18XB, 150 \( \times \) 4.6 mm (5 \( \mu m \) d.p.) protected with a SecurityGuard® (Torrance, CA, USA).

- **Eluent:**
  
  A: 0.2% formic acid in water.
  
  B: 0.2% formic acid in acetonitrile.

- **Flow rate:** 1 mL/min.

- **Injection volume:** 20 \( \mu L \).

- **Wavelength:** 220 and 280 nm

- **Gradient program:** 0.00 min [B%=30%], 10.00 min [B%=90%], 12.50 min [B%=90%], 13.00 min [B%=30%], 18.00 min [B%=30%].
**In vivo PK evaluation of compound 34.**

Table S6. PK parameters of single administration of compound 34 (e.v., 7 mg/kg, n=5)

| Parameter | Unit     | Value   | S.D.   |
|-----------|----------|---------|--------|
| $t_{1/2}$ | h        | 3.21    | 0.57   |
| $T_{\text{max}}$ | h        | 0.20    | 0.18   |
| $C_{\text{max}}$ | μg/L    | 16,834  | 4,829  |
| $V_d$     | L/Kg     | 2.30    | 0.76   |
| $Cl$      | L/h/Kg   | 0.50    | 0.15   |
**Metabolic stability data.**

| 34 | Theoretical [M+H]^+ | Measured [M+H]^+ | Mass shift ppm | RT (min) |
|----|----------------------|-------------------|----------------|----------|
|    | 402.1448             | 402.1444          | 1.08           | 12.917   |

**MS² spectrum**

**Significant fragment ions**

| Mass/Charge (m/z) | Intensity (counts x 10⁶) |
|-------------------|-------------------------|
| P50_001 (F19) #4247 | BS_001 (F19) #4247 |

**M1**

| Theoretical [M+H]^+ | Measured [M+H]^+ | Mass shift ppm | RT (min) |
|----------------------|-------------------|----------------|----------|
| 388.1292             | 388.1287          | 1.24           | 11.971   |

**MS² spectrum**

**Significant fragment ions**

| Mass/Charge (m/z) | Intensity (counts x 10⁶) |
|-------------------|-------------------------|
| P50_002 (F22) #3851 | P50_002 (F22) #3851 |

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R = H or Me
| M2 | Theoretical [M+H]^+ | Measured [M+H]^+ | Mass shift ppm | RT (min) |
|----|---------------------|------------------|----------------|---------|
|    | 404.1241            | 404.1237         | 0.98           | 10.879  |

**MS^3** spectrum

| Significant fragment ions |
|---------------------------|
| ![Fragment ions](image1)  |

| M3 | Theoretical [M+H]^+ | Measured [M+H]^+ | Mass shift ppm | RT (min) |
|----|---------------------|------------------|----------------|---------|
|    | 418.1397            | 418.1390         | 1.79           | 11.815  |

**MS^3** spectrum

| Significant fragment ions |
|---------------------------|
| ![Fragment ions](image2)  |
| G1       | Theoretical [M+H]+ | Measured [M+H]+ | Mass shift ppm | RT (min) |
|----------|--------------------|-----------------|----------------|----------|
| ![Chemical Structure](image) | 578.1769          | 578.1774        | 0.88           | 11.47    |

**MS\textsuperscript{3} spectrum**

**Significant fragment ions**

![MS\textsuperscript{3} Spectrum](image)