Discovery and Characterization of Multiple Classes of Human CatSper Blockers

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Figure S2. Representative $I_{\text{CatSper}}$ recordings from healthy human sperm in the absence (red) and presence (purple) of test compound 4a. Baseline signal in high saline (HS) buffer shown in grey. $I_{\text{CatSper}}$ currents recorded in CsDVF elicited by the -80 mV (negative scale) or +80 mV (positive scale) voltage ramp.
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Figure S5. Representative $I_{\text{CatSper}}$ recordings from healthy human sperm in the absence (red) and presence (purple) of test compound 7a. Baseline signal in HS buffer shown in grey. $I_{\text{CatSper}}$ currents recorded in CsDVF elicited by the -80 mV (negative scale) or +80 mV (positive scale) voltage ramp.
Table S1. Inhibition of potassium-activation of CatSper by compounds from each hit series

| ID | Structure | IC<sub>50</sub>, µM | ID | Structure | IC<sub>50</sub>, µM |
|----|-----------|-----------------|----|-----------|-----------------|
| 1a | ![Structure1a](image) | 9.6 | 4b | ![Structure4b](image) | 6.9 |
| 1b | ![Structure1b](image) | 12 | 5a | ![Structure5a](image) | 6.3 |
| 1c | ![Structure1c](image) | 59 | 5d | ![Structure5d](image) | 33 |
| 2a | ![Structure2a](image) | 12 | 5k | ![Structure5k](image) | 18 |
| 2d | ![Structure2d](image) | 12 | 6b | ![Structure6b](image) | 5.1 |
| 3a | ![Structure3a](image) | 7.8 | 7a | ![Structure7a](image) | 11 |
| 4a | ![Structure4a](image) | 9.2 |

CatSper activated by high potassium/high pH buffer. IC<sub>50</sub> values calculated from single experiments.
Table S2. Reduction of $I_{\text{CaSper}}$ current density in human sperm by compounds from series 2 through 7.

| Compound | Current Density (pA/pF) | Inward (–80 mV) | Outward (+80 mV) |
|----------|-------------------------|----------------|-----------------|
| CsDVF    | -20 ± 3                 | 86 ± 12        |                 |
| 2a       | -5.0 ± 0.5              | 23 ± 4         |                 |
| 3a       | -4.0 ± 0.3              | 3.3 ± 2.9      |                 |
| 4a       | -3.4 ± 0.2              | 15 ± 2.4       |                 |
| 5f       | -15 ± 3                 | 35 ± 4         |                 |
| 6b       | -2.5 ± 1.1              | 0.1 ± 1.4      |                 |
| 7a       | -1.2 ± 0.4              | 5.9 ± 1.4      |                 |

CsDVF = Cesium divalent free media. Values are the mean ± SEM of at least 3 independent experiments.
Mass Analysis Report

SAMPLE INFORMATION

Sample Name: 1a
Acq Method Set: Col2_50to500_PosOnly

Acquired: 1/28/2022 8:12:07 PM CST
InjVol: 3.00 uL

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Channel: 214.0nm@10

| RT  | Area  | % Area | Height | Base Peak (m/z) |
|-----|-------|--------|--------|----------------|
| 1   | 2.160 | 116430 | 96.48  | 312.13         |
| 2   | 2.261 | 3612   | 2.99   | 312.11         |
| 3   | 3.452 | 636    | 0.53   | 485.29         |
Sample Name: 2a
Acq Method Set: Col2_50to500_PosOnly
Acquired: 1/28/2022 8:19:07 PM CST
InjVol: 3.00 uL

Channel: 214.0nm@10

| RT  | Area  | % Area | Height | Base Peak (m/z) |
|-----|-------|--------|--------|----------------|
| 1   | 2.152 | 0.37   | 1309   | 279.13         |
| 2   | 2.219 | 98.20  | 288613 | 341.16         |
| 3   | 2.528 | 0.21   | 944    | 179.14         |
| 4   | 2.583 | 0.45   | 1661   | 358.23         |
| 5   | 2.798 | 0.76   | 1555   | 224.09         |
**SAMPLE INFORMATION**

**Sample Name:** 3a  
**Acq Method Set:** Col2_50to500_PosOnly  
**Acquired:** 1/28/2022 10:27:28 PM CST  
**InjVol:** 7.50 uL

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**Graph: Channel: 214.0nm@10**

| RT   | Area  | % Area | Height | Base Peak (m/z) |
|------|-------|--------|--------|-----------------|
| 1    | 2.128 | 0.18   | 1590   | 359.24          |
| 2    | 2.197 | 0.14   | 1234   | 312.17          |
| 3    | 2.268 | 0.01   | 288    | 341.24          |
| 4    | 2.430 | 99.66  | 1098416| 291.23          |
**SAMPLE INFORMATION**

- **Sample Name:** 4a
- **Acq Method Set:** Col2_50to500_PosOnly
- **Acquired:** 1/28/2022 10:34:35 PM CST
- **InjVol:** 7.50 uL

```
| RT    | Area  | % Area | Height | Base Peak (m/z) |
|-------|-------|--------|--------|----------------|
| 1     | 2.962 | 8491   | 1.91   | 221.12         |
| 2     | 3.024 | 432574 | 97.18  | 418.17         |
| 3     | 3.187 | 4053   | 0.91   | 279.14         |
```

Channel: 214.0nm@10
Mass Analysis Report

Sample Information

Sample Name: 5f
Acq Method Set: Col2_50to500_PosOnly
Acquired: 1/28/2022 10:41:39 PM CST
InjVol: 7.50 uL

| RT  | Area   | % Area | Height | Base Peak (m/z) |
|-----|--------|--------|--------|-----------------|
| 1   | 2.267  | 4324   | 0.64   | 365.28          |
| 2   | 2.431  | 674377 | 99.36  | 445.12          |
### SAMPLE INFORMATION

| Sample Name: | 6b |
|-------------|----|
| Acq Method Set: | Col2_50to500_PosOnly |
| Acquired: | 1/28/2022 8:47:06 PM CST |
| InjVol: | 3.00 uL |

![Graph](chart.png)

**Channel:** 214.0nm@10

| RT | Area  | % Area | Height | Base Peak (m/z) |
|----|-------|--------|--------|----------------|
| 1  | 2.212 | 261797 | 91.79  | 123446 368.19  |
| 2  | 2.387 | 12417  | 4.35   | 5882 382.20    |
| 3  | 2.438 | 1349   | 0.47   | 759 368.19     |
| 4  | 2.568 | 2820   | 0.99   | 2823 396.19    |
| 5  | 2.587 | 6844   | 2.40   | 3792 396.21    |
Peak #1

Peak #2

Peak #3

Peak #4

Peak #5
LCMS Analysis Report

Acquired by: System Administrator
Sample Name: YTL-12-6-2-1
Injection Volume: 1
Data File: YTL-12-6-2-1.lcd
Method File: ACN-Water-0.05%TFA-5%B-1.5-3.0MIN(90-900).lcm
Date Acquired: 2022/2/22 17:24:34
Comment: Mobile phaseA:water/0.05%TFA
Mobile phaseB:ACN/0.05%TFA

 Instrument Name: Shimadzu LCMS-2020
<<Pump>>
Mode: Binary gradient
Pump A: LC-20ADXR
Pump B: LC-20ADXR
Total Flow: 1.5000 mL/min
B Conc.: 5.0 %

<<Oven>>
Oven Temperature: 40 C

<<PDA>>
PDA Model: SPD-M20A
Lamp: D2
Start Wavelength: 190 nm
End Wavelength: 400 nm

<<Column>>
Column Name: HALO C18
Length: 30 mm
Internal Diameter: 3.0 mm

<<Interface>>
Interface: ESI
DL Temperature: 250 C
Nebulizing Gas Flow: 1.50 L/min
Heat Block: 250 C
Drying Gas: On

<<MS Parameter>>
Initial Valve Position: -
Start Time: 0.00 min
End Time: 2.85 min
Start m/z: 90.00
End m/z: 900.00
Scan Speed: 3000 u/sec

<<LC Time Program>>
Time   Module   Command   Value
0.01    Pumps    B.Conc    5
2.00    Pumps    B.Conc    100
2.70    Pumps    B.Conc    100
2.75    Pumps    B.Conc    5
3.00    Controller    Stop    -
Retention time: 0.781

Spectrum Mode: Single 0.781(163)  Base Peak: 218.80(2647371)
BG Mode: Averaged 0.431-1.301(93-267) Segment 1 - Event 1
| Parameter          | Value                  |
|--------------------|------------------------|
| ppm                | 0.00 Hz                |
| GB                 | 1024                   |
| LB                 | 0.00 Hz                |
| SSB                | QSINE                  |
| WDW                | 400.130000 MHz         |
| SF                 | 400.1319 MHz           |
| FIQ1               | 400.131885 MHz         |
| PL1W               | 18.64416504 W          |
| PL1                | -3.00 dB               |
| PL1W               | 18.64416504 W          |
| PL1                | -3.00 dB               |
| AQ                 | 0.3686900 sec          |
| RG                 | 203                    |
| DN                 | 180.000 usec           |
| DE                 | 6.50 usec              |
| TE                 | 298.0 K                |
| D0                 | 0.00015976 sec         |
| D1                 | 1.88203502 sec         |
| D8                 | 0.300036000 sec        |
| IN0                | 0.00036000 sec         |
| GB                 | 1024                   |
| LB                 | 0.00 Hz                |
| SSB                | QSINE                  |
| WDW                | 400.130000 MHz         |
| SF                 | 400.1319 MHz           |
| FIQ1               | 400.131885 MHz         |
| PL1W               | 18.64416504 W          |
| PL1                | -3.00 dB               |
| AQ                 | 0.3686900 sec          |
| RG                 | 203                    |
| DN                 | 180.000 usec           |
| DE                 | 6.50 usec              |
| TE                 | 298.0 K                |
| D0                 | 0.00015976 sec         |
| D1                 | 1.88203502 sec         |
| D8                 | 0.300036000 sec        |
| IN0                | 0.00036000 sec         |
| GB                 | 1024                   |
| LB                 | 0.00 Hz                |
| SSB                | QSINE                  |
| WDW                | 400.130000 MHz         |
| SF                 | 400.1319 MHz           |
| FIQ1               | 400.131885 MHz         |
| PL1W               | 18.64416504 W          |
| PL1                | -3.00 dB               |
| AQ                 | 0.3686900 sec          |
| RG                 | 203                    |
| DN                 | 180.000 usec           |
| DE                 | 6.50 usec              |
| TE                 | 298.0 K                |
| D0                 | 0.00015976 sec         |
| D1                 | 1.88203502 sec         |
| D8                 | 0.300036000 sec        |
| IN0                | 0.00036000 sec         |

--- CHANNEL f1 ---

| Parameter | Value       |
|-----------|-------------|
| NUC1      | 1H          |
| P1        | 15.90 usec  |
| PL1       | -3.00 dB    |
| PL1W      | 18.64416504 W |
| SF01      | 400.1318852 MHz |
| ND0       | 1           |
| TD        | 256         |
| SF01      | 400.1319 MHz |
| FIDRES    | 10.856695 Hz |
| SW        | 6.942 ppm   |
| FnMODE    | States−TPPI |
| SI        | 1024        |
| SF        | 400.130000 MHz |
| MDW       | QSINE       |
| SSB       | 2           |
| LB        | 0.00 Hz     |
| GB        | 0           |
| PC        | 1.00        |
| SI        | 1024        |
| MC2       | States−TPPI |
| SF        | 400.130000 MHz |
| MDW       | QSINE       |
| SSB       | 2           |
| LB        | 0.00 Hz     |
| GB        | 0           |
