Sperm Toolbox – A selection of small molecules to study human spermatozoa

Franz S. Gruber[a)*], Anthony Richardson [b)*], Zoe C. Johnston [c)*], Rachel Myles [c)], Neil Norcross [b)], David Day [b)], Irene Georgiou[b)], Laura Sesma Sanz [a)], Caroline Wilson [b)], Kevin D. Read [b)], Sarah Martins da Silva [c)] Christopher L. R. Barratt [c)], Ian H. Gilbert [b)], Jason R. Swedlow [a)]

a) Divisions of Computational Biology and Molecular, Cellular and Developmental Biology and National Phenotypic Screening Centre, School of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK

b) Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK

c) Division of Systems Medicine, School of Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, DD19SY, UK

Correspondence:

ORCID
Anthony Richardson: 0009-0005-3690-3409
Rachel Myles: 0009-0000-1667-5879
Irene Georgiou: 0000-0002-6042-9816
Laura Sesma Sanz: 0000-0001-7792-1631
Christopher L.R. Barratt: 0000-0003-0062-9979
Franz S. Gruber: 0000-0003-2008-8460
Zoe C. Johnston: 0000-0003-0904-7166
Caroline Wilson: 0000-0001-9940-0052
Neil R. Norcross: 0000-0001-8050-5217
Sarah Martins de Silva: 0000-0003-2579-4866
Jason R. Swedlow: 0000-0002-2198-1958
Ian H. Gilbert: 0000-0002-5238-1314
Kevin D. Read: 0000-0002-8536-0130

Abstract

Male contraceptive options and infertility treatments are limited. To help overcome these limitations we established a small molecule library as a toolbox for assay development and screening campaigns using human spermatozoa. We have profiled all compounds in our automated high-throughput screening platform and provide the dataset, which allows direct side-by-side comparisons of compound effects and assays using live human spermatozoa.

Main

One of the grand challenges for science and society is the development of novel contraceptives that are safe and deliver control of reproduction to men and women all over the world. In parallel, a deeper understanding of the sperm function and possible interventions for male infertility is required to counter declining birth rates. Recently, the application of advanced technologies has helped to move the human fertility field forward, revealing new potential targets for both contraception and fertility, and new compounds as contraceptive candidates (reviewed by(Anderson and Johnston, 2023; Norcross et al., 2022; Service et al., 2023). A key part of this effort is the development of mechanistic insight and drug candidates that affect the behaviour and properties of human spermatozoa(Balbach et al., 2023; Carlson et al., 2022; Faber et al., 2023; Hau et al., 2022). Towards this end, we have recently developed an automated live human sperm phenotypic, target-agnostic screening platform that can screen 1000s of compounds and identify positive and negative modifiers of sperm function (Gruber et al., 2020; Gruber et al., 2022). These effects can be either positive or negative and therefore starting points for either fertility treatments (including medical assisted reproductive technologies) or contraceptive development. An advantage of using a high-throughput platform is the ability to efficiently compare many compounds under the same assay conditions, within a short amount of time.

To facilitate drug discovery approaches related to contraception or infertility, we assembled a small library containing 84 small molecules, which we have coined the Sperm Toolbox (STB). The idea of the STB is in concordance with similar efforts e.g. the MMV Pandemic Response Box (Samby et al., 2022), the SGC Epigenetic Chemical Probe Collection (Brown and Muller, 2015), or the Drug Repurposing Hub (Corsello et al., 2017) which facilitate deeper understanding of biology or pathology or repurpose approved drugs. We tested the STB compounds in multiple functional sperm assays, for general cytotoxicity and aqueous solubility (Figure 1A). The STB has been assembled to contain previously published compounds and compounds detected in our own screening campaigns (Gruber et al., 2020; Gruber et al., 2022). In addition, we included a few compounds which have been inferred from patent applications or studies of proteins potentially relevant for sperm function (Figure 1B). The focus has been mainly on literature investigating human systems, but compounds tested in animal models, cell-based, or biochemical systems have also been included. The compounds selected in the STB have been described to effect crucial sperm functions e.g. motility, capacitation, acrosome reaction, Ca^{2+} signalling, viability, or are related to other processes (e.g. spermatogenesis, liquefaction, or ejaculation) (Figure 1C). Compound annotations are included in the STB dataset and indicate that Enzymes/Kinases, Receptors, Channels and Transporters are the most represented compound classes. Using information from the drug repurposing hub (Corsello et al., 2017), we generated a protein-protein interaction network of targets related to STB compounds (Figure 1E). This network consists of multiple subclusters related to functions in spermatozoa (Figure 1F) and other biological processes, functions, and pathways (Figure 1G).

78

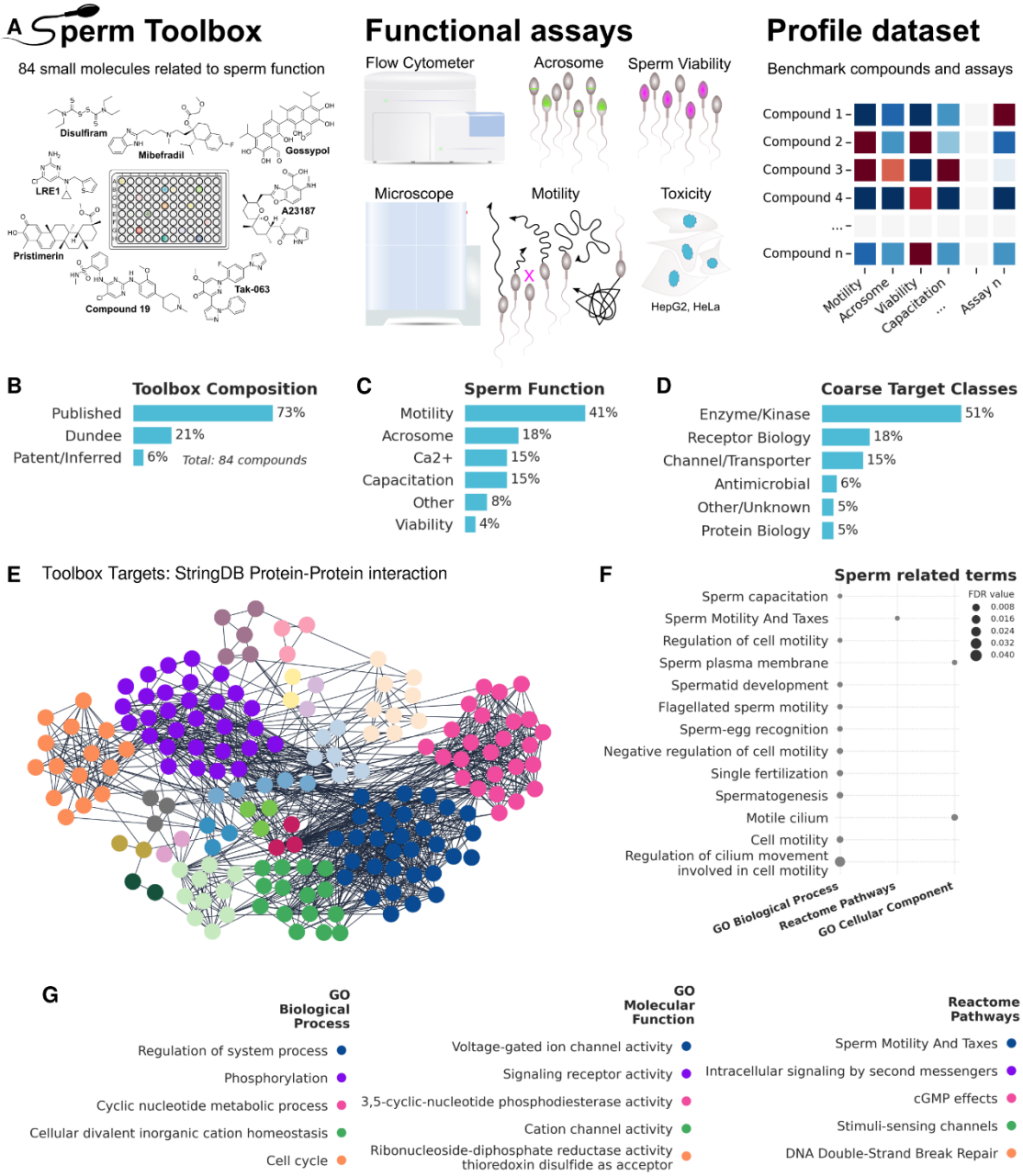


Figure 1

(A) The Sperm Toolbox is a collection of 84 small molecules with published, observed or inferred effect on multiple biological processes in or generation of spermatozoa. Human sperm cells have been tested in various assays, which allows comparison of compounds under the same experimental conditions. (B) Summary of the sperm toolbox compound compositions. (C) Summary of annotated (i.e. published, observed, inferred) sperm function of Toolbox compounds. (D) Coarse classification of Sperm Toolbox target classes. (E) StringDB Protein-Protein interaction networks (Full network, confidence 0.7) of putative targets of Sperm toolbox compounds. Protein targets have been inferred from the repurposing hub. Network has been clustered using MCL clustering in Cyotscape. (F) Sperm related terms (GeneOntology, Reactome) within the network shown in (E). Marker size indicates false discovery rate (FDR). (G) Enriched terms within the biggest subclusters in (E). Redundant terms have been filtered using Cytoscape (cutoff 0.5). Color matches subcluster color in (E)

79

To compare the effects of STB compounds on specific sperm functions, we screened them in multiple phenotypic functional assays. Each sperm function assay has been repeated in multiple donor pools (see Material and Methods), where each pool consists of cells donated from at least 3 different donors to address donor to donor variability. This generates datasets that are more representative of a more general population. Each compound has been tested at two concentrations 10 and 30 µM and normalized to vehicle controls (DMSO). We have modified our assays from the previously published workflow (Gruber et al., 2020) to generate a deeper understanding of compound action (e.g., by increasing incubation time) and to better model *in vivo* conditions (e.g. by introducing conditions which support capacitation, which is a sperm maturation process required for fertilization). Our motility assays, use a high-content microscope to record short timelapse movies and can run in different modes. The first mode, denoted 'motility assay', utilises non-capacitating assay conditions that models the state of spermatozoa after ejaculation. The second mode, denoted 'capacitation assay', utilises capacitating conditions (Extended Figure 1A). Both modes allow sperm motility to be measured. In addition, the capacitation assay also scores hyperactivation (i.e. distinct movement patterns concomitant with capacitation which are crucial for fertilization) and is therefore a marker of capacitation.

The acrosome assays utilize a high-throughput flow cytometer to score for compounds inducing the acrosome reaction, using peanut agglutinin conjugated to a fluorescent dye (labelling the inner acrosomal membrane). As with the motility assays, we have developed two different screening modes, named 'acrosome 1' and 'acrosome 2' (Extended Figure 1A). Both modes are capable of measuring induction of the acrosome reaction. However, in the acrosome 2 assay, a physiological agonist mix of the acrosome reaction is used thus we also also measures inhibition of acrosome reaction (Prajapati et al., 2022).

Additionally, we tested the effect of STB compounds on sperm viability using a complementary live-cell flow-cytometer approach and propidium iodide, which does not permeate intact cell membranes (Extended Figure 1B).

In addition to functional assays using human spermatozoa, we also profiled each STB compound for general cytotoxicity in two cell-lines (HeLa and HepG2). The HeLa-based assay uses the cell painting assay (Bray et al., 2016), while the second assay (HepG2) utilizes a resaruzin based read-out (Baragana et al., 2015). To ensure usability of STB compounds, we also measured aqueous solubility. An overview of additional assays is given in Extended Figure 1C.

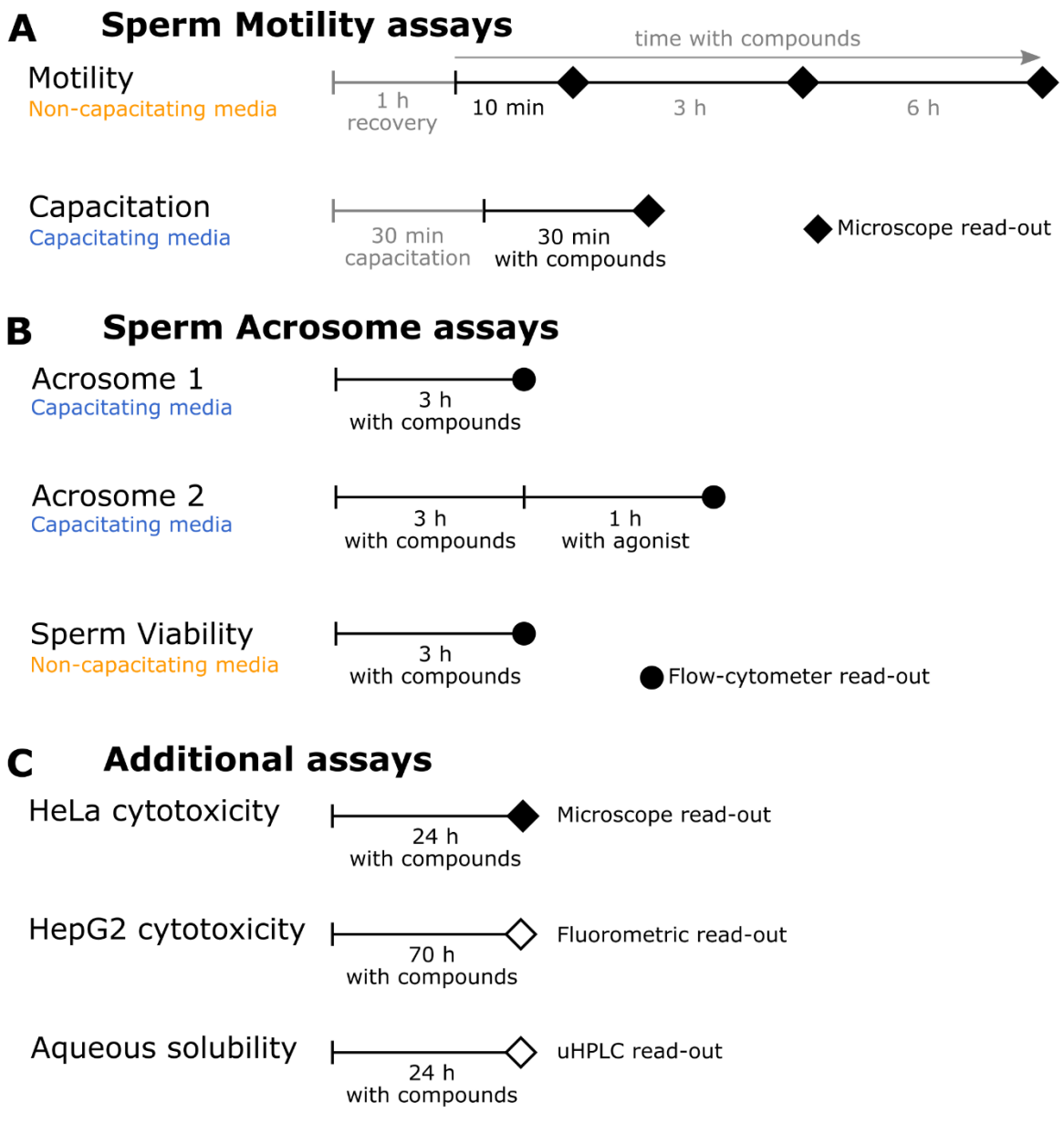
This multi-assay compound profiling dataset has revealed a broader picture of the action of individual compounds, helps find similarities between compounds, and provides an opportunity to compare results from different screening assays. Computational analysis of the profile dataset has the potential to elucidate further sperm biology. Using unsupervised clustering we observed clusters of compounds with a significant reduction of sperm motility and/or increased amounts of acrosome reacted cells (e.g. Alexidine dihydrochloride, Tafenoquin or A23187) (Figure 2A). Some of these compounds also show significant levels of cytotoxicity in HeLa and/or HepG2 cells as well as decreased sperm viability suggesting that their effect on motility and acrosome reaction could be non-specific. However, other clusters show a significantly increased acrosome reaction and hyperactive motility but do not show effects on sperm viability or general cytotoxicity (e.g. LRRK2-IN-1, Clofarabine, Linsitinib, Trequinsin). One example, LRRK2-IN-1, which enhances motility (progressive and hyperactivated), has been described as a selective inhibitor of LRRK2 kinase, which is involved in Parkinson's disease (Deng et al., 2011). This compound shares some structural similarities with two kinase inhibitors in the STB, which decrease sperm motility: Compound 19,

described as a potent Testis-Specific Serine/Threonine Kinase 2 (TSSK2) inhibitor (Hawkinson et al., 2017) and CZC 25146, annotated as a potent LRRK2 inhibitor.

Overall, we observed that a number of compounds show weak or no effects in our assays (Extended figure 2), which stresses an important aspect of the STB: comparing compounds using the same assay conditions. Our screening system is not limited to the buffer system, incubation times or screening concentration used in this study. This flexibility provides an excellent start point to establish control compounds and assay conditions for novel drug discovery campaigns related to contraception and infertility. Our profiled dataset captures well described compounds from the literature and allows direct comparisons to compounds identified in our own screening. We provide the complete dataset as well as web interface for data analysis (https://spermtoolbox.shinyapps.io/spermtoolbox/).

This work represents the first version of the STB. Future revisions could contain newly published compounds modulating functions in spermatozoa e.g., a sAC inhibitor TDI-11861 (Balbach et al., 2023), a CatSper inhibitor RU1968 (Schierling et al., 2023), a SLO3 inhibitor (Lyon et al., 2023) or other contributions from the research community. In addition, the STB could present an important resource for testing and benchmarking newly developed drug screening assay.

In summary the STB is a small library intended to facilitate contraceptive and infertility drug discovery. We have established a unique reference dataset which allows comparing the performance of compounds in multiple assays. This will ultimately give a broader picture of the compounds effect on sperm function.



Extended Figure 1

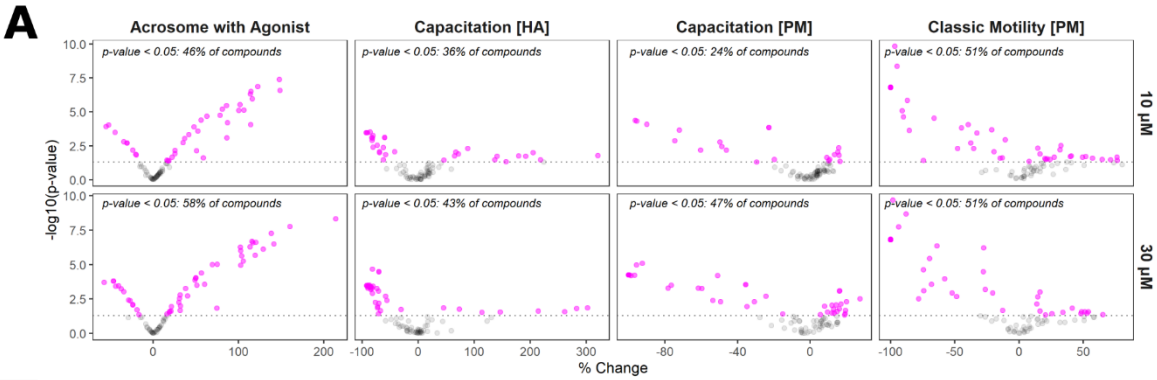
Overview of assays

(A) Diagram showing sperm motility assays run on high-content microscope with compound incubation time/read-out time. Motility assay run under non-capacitating conditions, with a 1 hour recovery phase after preparation of spermatozoa. Capacitation assay run under capacitating conditions. Spermatozoa are capacitated for 30 min prior to compound incubation.

(B) Diagram showing assays run on high-throughput flow cytometer to measure acrosome status and sperm viability. Incubation time with compound, agonist and read-out times are indicated. Acrosome assays run under capaciting conditions. Sperm viability assay run under non-capacitating condition.

(C) Additional assays performed on sperm toolbox compounds.

146



Extended Figure 2

(A) Vulcano plots of Acrosome, Capacitation and Motility assays. Upper panel indicates 10 µM, lower plot panels indicate 30 µM. Incubation times for assays are 3h with compound for Acrosome assay and Classic Motility (PM = progressive motility), and 30 min for the Capacitation assay (HA = hyperactive motility, PM = progressive motility). Dotted line indicates significance levels of 0.05. Any point above dotted line is indicated in magenta.

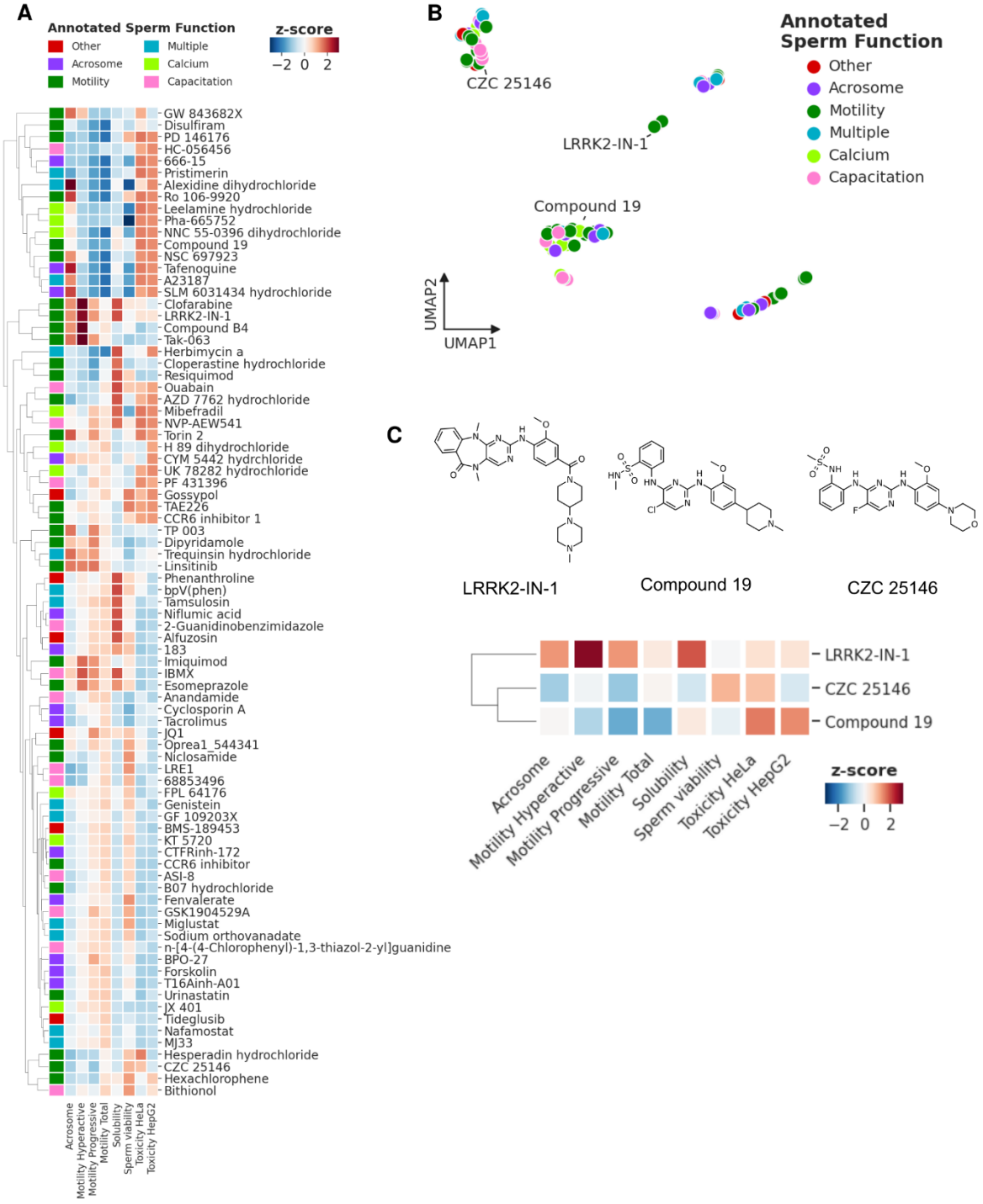


Figure 2

(A) Heatmap showing standardized results (z-score) for every Toolbox compound screened at 30 µM in selected assays: Acrosome 1 (3h with compound then induction with acrosome reaction using an agonist mix), Motility Hyperactive (30 min capacitation then 30 min incubation with compound), Motility Progressive (3h incubation with compounds under non-capacitating conditions), Motility Total (3h incubation with compound under non-capacitating conditions)), Solubility (aqueous solubility of compounds after 24h incubation), Sperm viability (3h with compound then propidium iodide assay), Toxicity HeLa (24h compound incubation then cell painting assay), Toxicity HepG2 (70h incubation

with compound then resaruzin based assay). Heatmap color indicates decrease (blue) or increase (red).

(B) UMAP plot showing groups of compounds with similar assay profile. Color indicates annotated sperm function.

(C) (C) Structures and zoom-in of three examples related to LRRK2 kinase.

Material and Methods

Ethical approval

Written consent was obtained from each donor in accordance with under local ethical approval (University of Dundee, SMED REC 20/45).

Sperm handling

Donated human spermatozoa with normal semen characteristics (concentration, motility; WHO 2010) from healthy volunteers with no known fertility problems were used in this study. Samples were obtained by masturbation after ≥ 48 h of sexual abstinence, liquefied for 30 min at 37 °C, and processed using density gradient centrifugation, to separate cells using 40/80% Percoll (Sigma Aldrich, UK) fractions. Every experiment has been performed on pooled spermatozoa from ≥ 3 donors each.

For experiments in non-capacitating conditions, we used a buffer system slightly modified from (Tardif et al., 2014) using Minimal Essential Medium Eagle (Sigma), supplemented with HEPES (1 M solution, Gibco), sodium lactate (DL-solution, Sigma), sodium pyruvate (100 mM solution, Gibco) and bovine serum albumin (7.5 % solution, Sigma).

For experiments in capacitating conditions, we used a buffer system slightly modified from the HTF from (Prajapati et al., 2022) to a final composition of 97.8 mM NaCl, 4.69 mM KCl, 0.2 mM $MgSO_4$, 0.37 mM KH_2PO_4, 2.04 mM $CaCl_2$, 0.33 mM Na-pyruvate, 12.4 mM Na-lactate, 2.78 mM glucose, 21 mM HEPES, 25 mM $NaHCO_3$ and 3 mg/mL BSA. All buffer components were supplied by Sigma-Aldrich unless otherwise stated and all buffer systems were adjusted to pH 7.4.

Compound handling

Compounds were purchased from vendors indicated in supplementary file x. 10 mM stock solutions in DMSO were generated. Quality control of each compound has been performed (HPLC-MS and NMR).

Compound plates were generated using 10 mM stock solutions of each STB compound in Echo 384-LDV plates (LP-0200). Prior to running experiments, assay ready plates have been generated using Echo acoustic dispensers (550/555). Each compound was screened at 10 and 30 µM final concentration. Plate types used for assays: Motility, HeLa cell painting and Sperm viability (PerkinElmer 384-well PhenoPlate), Acrosome (Greiner 384-PP V-bottom plates).

182

183 **Motility assays**

184 *Classic motility assay:*

185 Cells were rested for 1 h post density gradient centrifugation. 10 µL assay buffer was dispensed into
186 assay ready plates, plates were put onto a shaker instrument for 30 seconds using 1,000 rpm,
187 followed by a short pulse centrifugation. Plates were incubated for 15-30 mins at 37 °C prior to
188 addition of 10 µL of pooled cells (at 1 M/mL concentration). Plates were then incubated in a
189 Yokogawa CV7000 microscope set at 37 °C for 10 min prior to imaging. Imaging a 384-well plate
190 requires ≤ 20 min using the following settings: 2 positions per well, 0.5 sec timelapse movies (3x
191 binning; 11 ms exposure time, 22 ms interval time, 500 intervals). A second and a third timepoint
192 was recorded after 3 h, and 6 h incubation at 37 °C. Data has been processed as described in Gruber
193 et al., 2020.

194

195 *Capacitation assay:*

196 Cells were capacitated for 30 min at 37 °C and 5 % CO2. 10 µL assay buffer was dispensed into assay
197 ready plates, plates were put onto a shaker instrument for 30 seconds using 1,000 rpm, followed by
198 a short pulse centrifugation. Plates were incubated for 15-30 mins at 37 °C and 5% CO2 prior to
199 addition of 10 µL of pooled cells (at 1 M/mL concentration). This was followed by a 20 min
200 compound incubation step at 37 °C and 5 % CO2. Plates were then incubated in our CV7000
201 microscope set at 37 °C and 5% CO2 for 10 min prior to imaging. Imaging a 384-well plate requires ≤
202 20 min using the following settings: 2 positions per well, 0.5 sec timelapse movies (3x binning; 11 ms
203 exposure time, 22 ms interval time, 500 intervals). Data has been processed as described in Gruber
204 et al., 2020.

205

206 **Acrosome assays**

207 In both experimental modes, 20 µL of pooled cells (at 1 M/mL concentration) were capacitated for 3
208 h in presence of compounds at 37 °C and 5% CO2. In mode a, cells were then stained with PNA-
209 Alexa647 (ThermFisher, 1 mg/mL, final dilution 1:1000) and Hoechst dye for 30 min at 37 °C and 5%
210 CO2, followed by 10 min fixation at RT by adding 20 µL of 4% paraformaldehyde (Sigma) in PBS. In
211 mode b, a cocktail of progesterone , prostaglandin A and NH4Cl was added to all compound wells
212 and incubated for an additional 1 h at 37 °C and 5% CO2 to induce acrosome reaction. This was
213 followed by fixation and staining protocol as for mode a. In both modes fixatives and stains were
214 removed and cells were resuspended in PBS, using a 405 platewasher. Plates were then measured
215 on a Novocyte Advanteon high-throughput flow cytometer. Gating of data has been performed using
216 the flow cytometer software to export counts of gated populations.

217

218 **Sperm viability assay**

219 Cells were incubated with compounds for a total of 3h at 37 °C. 20 min prior, propidium iodide was
220 added (1.5 mM solution, Sigma, final dilution 1:2000). Plates were analysed using a Satorius iQue
221 Screener with settings to allow for a sampling time of ≤30 min per 384-well plate. Gating of data has
222 been performed using the flow cytometer software to export counts of gated populations.

223

224 **HeLa cell painting**

225 Cell were incubated with compounds for 24h. Cell painting was performed after Bray et al., 2016
226 with modifications suggested here (Cimini et al., bioRxiv 2022.07.13.499171; doi:
227 https://doi.org/10.1101/2022.07.13.499171):

228 - 10 µL of MitoTracker (Life Technologies, M22426, 1 mM stock in DMSO as recommended by
229 the vendor) is added to 50 µL of media (1:2000 final dilution)
230 - Phalloidin-Alexa594 (Life Technologies, A12381, 66 µM stock in methanol as recommended
231 by the vendor, 1:500 final dilution)
232 - WGA-Alexa594 (Life Technologies, W11262, 5 mg/mL stock, 1:250 final dilution) was used
233 - DAPI (5 mg/mL stock; 1:1000 final dilution) was used to stain DNA
234 - 20 µL of staining solution was used
235 - PBS was used for washing steps

236

237 **HepG2 cytotoxicity assay**

238 HepG2 assay was performed as described previously (Baragana et al., 2015).

239

240 **Aqueous solubility**

241 The aqueous solubility of test compounds was measured using UHPLC, as previously described in
242 (Smith et al., 2022) .

243

244 **Data analysis**

245 Data was normalized to DMSO control wells. Each plate had 16 DMSO wells. A median value of all 16
246 wells was used to calculated percentage change (pct_ch = ((value / DMSO_median) -1) * 100). For
247 sperm viability we calculated fold-change (fc = 1 - (value / DMSO_median)). For each compound we
248 calculated a p-value using a Welch's t-test implemented in scipy (scipy.stats.ttest_ind) comparing a
249 compounds effect to DMSO controls.

250 The following packages have been used for data analysis and visualization:

251 R: Tidyverse, plotly, pHeatmap

252 Python: pandas, numpy, scipy, seaborn, matplotlib, umap

253

254 **Acknowledgements**

255 The authors acknowledge support by the Bill & Melinda Gates Foundation (INV-007117).

256

257 **References**

258 Anderson, D.J., and Johnston, D.S. (2023). A brief history and future prospects of contraception.
259 Science *380*, 154-158.
260 Balbach, M., Rossetti, T., Ferreira, J., Ghanem, L., Ritagliati, C., Myers, R.W., Huggins, D.J., Steegborn,
261 C., Miranda, I.C., Meinke, P.T*., et al.* (2023). On-demand male contraception via acute inhibition of
262 soluble adenylyl cyclase. Nat Commun *14*, 637.
263 Baragana, B., Hallyburton, I., Lee, M.C., Norcross, N.R., Grimaldi, R., Otto, T.D., Proto, W.R.,
264 Blagborough, A.M., Meister, S., Wirjanata, G*., et al.* (2015). A novel multiple-stage antimalarial agent
265 that inhibits protein synthesis. Nature *522*, 315-320.
266 Bray, M.A., Singh, S., Han, H., Davis, C.T., Borgeson, B., Hartland, C., Kost-Alimova, M., Gustafsdottir,
267 S.M., Gibson, C.C., and Carpenter, A.E. (2016). Cell Painting, a high-content image-based assay for
268 morphological profiling using multiplexed fluorescent dyes. Nat Protoc *11*, 1757-1774.
269 Brown, P.J., and Muller, S. (2015). Open access chemical probes for epigenetic targets. Future Med
270 Chem *7*, 1901-1917.
271 Carlson, E.J., Francis, R., Liu, Y., Li, P., Lyon, M., Santi, C.M., Hook, D.J., Hawkinson, J.E., and Georg,
272 G.I. (2022). Discovery and Characterization of Multiple Classes of Human CatSper Blockers.
273 ChemMedChem *17*, e202000499.
274 Corsello, S.M., Bittker, J.A., Liu, Z., Gould, J., McCarren, P., Hirschman, J.E., Johnston, S.E., Vrcic, A.,
275 Wong, B., Khan, M*., et al.* (2017). The Drug Repurposing Hub: a next-generation drug library and
276 information resource. Nat Med *23*, 405-408.
277 Deng, X., Dzamko, N., Prescott, A., Davies, P., Liu, Q., Yang, Q., Lee, J.D., Patricelli, M.P., Nomanbhoy,
278 T.K., Alessi, D.R*., et al.* (2011). Characterization of a selective inhibitor of the Parkinson's disease
279 kinase LRRK2. Nat Chem Biol *7*, 203-205.
280 Faber, E.B., Wang, N., John, K., Sun, L., Wong, H.L., Burban, D., Francis, R., Tian, D., Hong, K.H., Yang,
281 A*., et al.* (2023). Screening through Lead Optimization of High Affinity, Allosteric Cyclin-Dependent
282 Kinase 2 (CDK2) Inhibitors as Male Contraceptives That Reduce Sperm Counts in Mice. J Med Chem
283 *66*, 1928-1940.
284 Gruber, F.S., Johnston, Z.C., Barratt, C.L., and Andrews, P.D. (2020). A phenotypic screening platform
285 utilising human spermatozoa identifies compounds with contraceptive activity. Elife *9*.
286 Gruber, F.S., Johnston, Z.C., Norcross, N.R., Georgiou, I., Wilson, C., Read, K.D., Gilbert, I.H.,
287 Swedlow, J.R., Martins da Silva, S., and Barratt, C.L.R. (2022). Compounds enhancing human sperm
288 motility identified using a high-throughput phenotypic screening platform. Hum Reprod *37*, 466-475.
289 Hau, R.K., Tash, J.S., Georg, G.I., Wright, S.H., and Cherrington, N.J. (2022). Physiological
290 Characterization of the Transporter-Mediated Uptake of the Reversible Male Contraceptive H2-
291 Gamendazole Across the Blood-Testis Barrier. J Pharmacol Exp Ther *382*, 299-312.
292 Hawkinson, J.E., Sinville, R., Mudaliar, D., Shetty, J., Ward, T., Herr, J.C., and Georg, G.I. (2017).
293 Potent Pyrimidine and Pyrrolopyrimidine Inhibitors of Testis-Specific Serine/Threonine Kinase 2
294 (TSSK2). ChemMedChem *12*, 1857-1865.
295 Lyon, M., Li, P., Ferreira, J.J., Lazarenko, R.M., Kharade, S.V., Kramer, M., McClenahan, S.J., Days, E.,
296 Bauer, J.A., Spitznagel, B.D*., et al.* (2023). A selective inhibitor of the sperm-specific potassium
297 channel SLO3 impairs human sperm function. Proc Natl Acad Sci U S A *120*, e2212338120.
298 Norcross, N.R., Georgiou, I., Johnston, Z.C., Gruber, F.S., Swedlow, J.R., Read, K.D., Barratt, C.L., and
299 Gilbert, I.H. (2022). Male contraceptive development: A medicinal chemistry perspective. Eur J Med
300 Chem *243*, 114709.
301 Prajapati, P., Kane, S., McBrinn, R.C., Dean, M.S., Martins da Silva, S.J., and Brown, S.G. (2022).
302 Elevated and Sustained Intracellular Calcium Signalling Is Necessary for Efficacious Induction of the
303 Human Sperm Acrosome Reaction. Int J Mol Sci *23*.
304 Samby, K., Besson, D., Dutta, A., Patra, B., Doy, A., Glossop, P., Mills, J., Whitlock, G., Hooft van
305 Huijsduijnen, R., Monaco, A*., et al.* (2022). The Pandemic Response Box horizontal line Accelerating
306 Drug Discovery Efforts after Disease Outbreaks. ACS Infect Dis *8*, 713-720.

307 Schierling, T., Tosi, B., Eisenhardt, C., Reining, S., Daniliuc, C.G., Brenker, C., Strunker, T., and
308 Wunsch, B. (2023). Synthesis and Functional Characterization of Novel RU1968-Derived CatSper
309 Inhibitors with Reduced Stereochemical Complexity. ACS Pharmacol Transl Sci *6*, 115-127.
310 Service, C.A., Puri, D., Hsieh, T.C., and Patel, D.P. (2023). Emerging concepts in male contraception: a
311 narrative review of novel, hormonal and non-hormonal options. Ther Adv Reprod Health *17*,
312 26334941221138323.
313 Smith, A., Wall, R.J., Patterson, S., Rowan, T., Rico Vidal, E., Stojanovski, L., Huggett, M., Hampton,
314 S.E., Thomas, M.G., Corpas Lopez, V.*, et al.* (2022). Repositioning of a Diaminothiazole Series
315 Confirmed to Target the Cyclin-Dependent Kinase CRK12 for Use in the Treatment of African Animal
316 Trypanosomiasis. J Med Chem *65*, 5606-5624.

317