ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett Letter

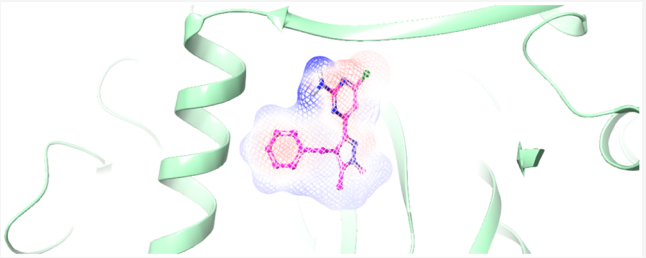
Discovery of TDI-10229: A Potent and Orally Bioavailable Inhibitor of Soluble Adenylyl Cyclase (sAC, ADCY10)

Makoto Fushimi, Hannes Buck, Melanie Balbach, Anna Gorovyy, Jacob Ferreira, Thomas Rossetti, Navpreet Kaur, Lonny R. Levin, Jochen Buck, Jonathan Quast, Joop van den Heuvel, Clemens Steegborn, Efrat Finkin-Groner, Stacia Kargman, Mayako Michino, Michael A. Foley, Michael Miller, Nigel J. Liverton, David J. Huggins,*,‡ and Peter T. Meinke‡

Cite This: *ACS Med. Chem. Lett.* 2021, 12, 1283−1287 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Soluble adenylyl cyclase (sAC) has gained attention as a potential therapeutic target given the role of this enzyme in intracellular signaling. We describe successful efforts to design improved sAC inhibitors amenable for *in vivo* interrogation of sAC inhibition to assess its potential therapeutic applications. This work culminated in the identification of TDI-10229 (**12**), which displays nanomolar inhibition of sAC in both biochemical and cellular assays and exhibits mouse pharmacokinetic properties sufficient to warrant its use as an *in vivo* tool compound.



KEYWORDS: *sAC, Soluble adenylyl cyclase, ADCY10, Inhibitor, Structure-based drug design*

Cyclic AMP (cAMP), a critical second messenger, is involved in a broad range of physiological processes. cAMP is produced from ATP by adenylyl cyclases (ACs) and degraded by catabolizing phosphodiesterases (PDEs). Presently, there are two known distinct classes of adenylyl cyclases in mammals: bicarbonate-regulated soluble adenylyl cyclase (sAC, ADCY10) and G protein-regulated transmembrane adenylyl cyclases (tmACs; ADCY1−9). While PDEs are established drug targets, at this juncture, there exist no therapeutic agents targeting sAC.

In contrast to tmACs, which respond to signals originating in other surrounding cells, sAC functions as an environmental sensor: it is directly regulated by bicarbonate and serves as a physiological $CO_2/HCO_3^-/pH_i$ sensor.[1] sAC is localized in intracellular microdomains and is found distributed through the cytoplasm and in cellular organelles, including inside the nucleus and the mitochondrial matrix.[2] It regulates a diverse array of biological functions, including sperm activation and motility, intraocular pressure, ciliary beat frequency in airways, luminal pH in the epididymis, the mitochondrial electron transport chain, and glucose-stimulated insulin release from β cells of the pancreas.[1,3]

While sAC is ubiquitously expressed, two independently derived sAC knockout (KO) mouse strains show male-specific sterility with only mild other phenotypes (e.g., elevated intraocular pressure).[3−6] Importantly, in humans, loss of sAC function appears similarly benign: two adult males bearing an ADCY10 frameshift variant were sterile but otherwise healthy.[7]

Based on its apparent functions, potent and selective inhibitors of sAC potentially provide a mechanism for therapeutic intervention in multiple disease states, including for hypotony or as an on-demand, nonhormonal contraceptive. While tool compounds, including LRE1 (**1**)[2] and KH7 (**1b**),[8] are known (Figure 1), their properties (weak potencies, low selectivities, poor pharmacokinetic profiles, etc.) render them unsuitable for careful scrutiny of sAC driven pharmacology in cellular or animal models.[9] While additional sAC inhibitors have been described by pharma (primarily via patent disclosures),[10,11] limited data exists regarding their profiles.

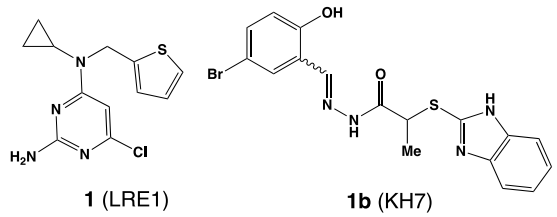


Figure 1. Structures of LRE1 and KH7.

This work describes our efforts to identify compounds, typified by TDI-10229, that combine significantly improved

Received: May 12, 2021
Accepted: July 8, 2021
Published: July 14, 2021

https://doi.org/10.1021/acsmedchemlett.1c00273
ACS Med. Chem. Lett. 2021, 12, 1283−1287

intrinsic potency with the ability to sustain systemic exposure above cell-based IC_{50} values to enable investigations of sAC-mediated biology in preclinical species.

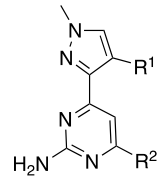
First generation sAC inhibitors, such as KH7, suffered from general cell toxicity and potential non-sAC mediated effects on ATP/cAMP levels that are likely to confound their utility as tool molecules.[2] The more recently discovered LRE1[2] binds in the bicarbonate binding site of sAC, preventing its enzymatic function. LRE1 represented a significant improvement in overall profile relative to KH7 and permitted more extensive interrogation of sAC biology *in vitro*. However, its relatively modest potency in biochemical and cell-based assays suggests that high micromolar levels would be required *in vivo* to elicit sAC-driven pharmacology in translational experiments. LRE1 also showed low oral bioavailability (<5%) in mice, a liability exacerbated by rapid mouse and human microsomal clearance (>768 and 360 μL/(min/mg), respectively), further limiting *in vivo* options.

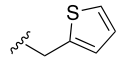
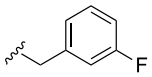
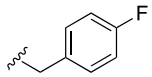
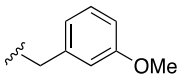
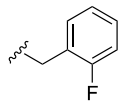
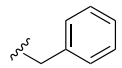
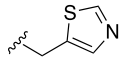
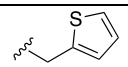
More potent sAC inhibitors with appropriate systemic PK to afford sustained sAC inhibition *in vivo* would represent a significant advance in enabling interrogation of sAC pharmacology *in vivo*. As a low molecular weight (280.8 g/mol) lead, LRE1 represented an auspicious entry point for medicinal chemistry efforts focused on discovering superior sAC inhibitors. Along with improved potency, microsomal stability and pharmacokinetics, we also wished to evolve the LRE1 lead toward more drug-like molecules, lacking the cyclopropylamine and monosubstituted thiophene moieties, which represent potential metabolic liabilities.[12,13]

We leveraged significant structural biology data throughout this project. In the first instance, utilization of free-energy perturbation (FEP) calculations with FEP+[14] based on the X-ray crystal structure of LRE1 bound to sAC[2] led to the design of **2** through scaffold hopping (Table 1). Compound **2** bears a methylpyrazole in lieu of the less desirable cyclopropylamino moiety and yields a 10-fold improvement in potency over LRE1. Medicinal chemistry efforts next focused on modifications around the conserved aminochloropyrimidine headgroup. Unfortunately, this headgroup proved to have extremely narrow SAR, as predicted by FEP+, with very small changes, such as chloro (**6**) to methyl (**10**), leading to a dramatic loss in activity. We then moved on to replacement of the undesirable thiophene ring. It was noted that various aromatic and heteroaromatic moieties were well tolerated on the 4-position methylene of the pyrazole (**3**–**8**), whereas direct attachment of the thiophene ring to the pyrazole (**9**) was not. Of particular note is that substituted phenyl rings, such as the fluorophenyl in compound **6**, yielded similar potency to the thiophene compound **2**.

Addition of a methyl group at the 5-position of the pyrazole was also noted to lead to a small improvement in potency as shown in Table 2 (IC_{50} of 140 nM in **2** vs IC_{50} of 74 nM in **11**). Gratifyingly, the equivalent methyl substitution on the phenyl compound **7** also improved potency and yielded compound **12** with an IC_{50} of 195 nM. Alternative ring systems were also found to retain potency in this context (**13**–**15**), while others abrogated affinity (**16** and **17**). Next, we investigated the effect of substitution at the 5-position of the pyrazole ring (Table 2). Substitution at R^2 on the central pyrazole was found to be sensitive to functionality. For example, the methyl ester substituted analog **18** exhibited higher potency, while other closely related analogs displayed reduced potency (e.g., acid **19** or methyl amide **20**).

Table 1. 4-Substituted Pyrazole Analogs



Cpd	R^1	R^2	Biochemical[a] IC_{50} (nM)
1	LRE1		1500
2		Cl	141
3		Cl	392
4		Cl	342
5		Cl	351
6		Cl	132
7		Cl	989
8		Cl	576
9		Cl	> 1000
10		Me	> 1000

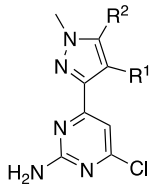
[a]Compounds were tested at minimum in triplicate.

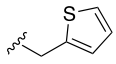
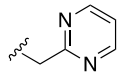
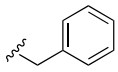
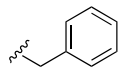
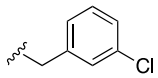
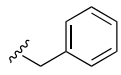
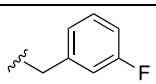
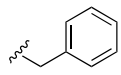
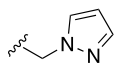
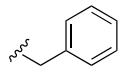
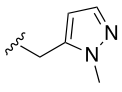
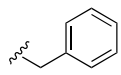
Furthermore, substitution of the R^2 methyl group with hydroxy (**21**) or dimethylamino (**22**) resulted in reduced potency versus methyl (**12**). To analyze the binding details of our improved inhibitors and to aid in further lead optimization, we then obtained crystallographic data with **12** bound to sAC (see Figure 2; Supplementary Table 1; Supplementary Figure 1).

Figure 2 shows the conserved overlap of the two aminochloropyrimidine headgroups. Importantly, the key hydrogen-bonding contacts made by the aminochloropyrimidine headgroup of LRE1 are maintained in **12**. Compared to LRE1, a very subtle rotation of the headgroup of **12** allows it to position its central pyrazole group in the area occupied by LRE1's central amine and additional cyclopropane substituent. The larger pyrazole moiety fills this area more completely and its additional interactions likely contribute to the higher affinities of **2** and **12**. The phenyl group of **12** is also oriented similarly to LRE1's thiophene, albeit with a slightly rotated ring plane. The rotation and larger ring of **12** are accommodated by a minor rearrangement of the interacting Arg176, which previously was observed to adopt multiple different rotamers.[2]

https://doi.org/10.1021/acsmedchemlett.1c00273
ACS Med. Chem. Lett. 2021, 12, 1283−1287

Table 2. 5-Substituted Pyrazole Analogs



Cpd	R^1	R^2	Biochemical[a] IC_{50} (nM)	Cpd	R^1	R^2	Biochemical[a] IC_{50} (nM)
11		Me	74	17		Me	> 10,000 (n=1)
12	 (TDI-10229)	Me	195	18		CO_2Me	28
13		Me	107	19		CO_2H	700 (n = 1)
14		Me	126	20		C(O)NHMe	> 10,000
15		Me	325	21		CH_2OH	1640
16		Me	> 10,000	22		$CH_2N(Me)_2$	> 10,000

[a]Compounds were tested at minimum in triplicate unless otherwise noted.

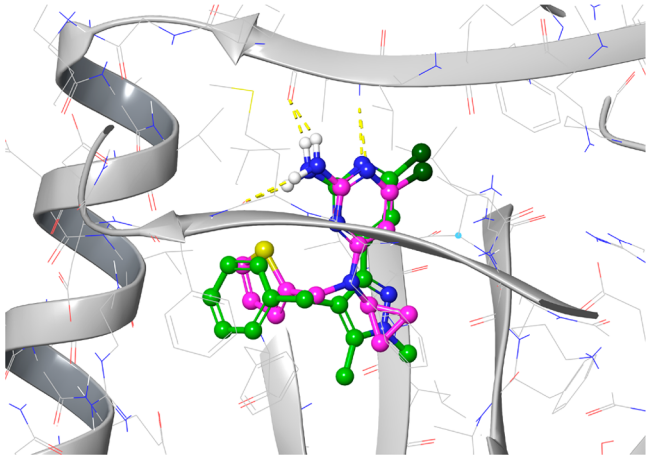


Figure 2. Overlay of crystal structures of sAC complexes with compound **12** and LRE1. The crystal structure of sAC in complex with **12** is displayed as a gray cartoon with gray wires and overlaid with the ligand from the crystal structure of sAC in complex with LRE1. Compound **12** is displayed as atom-colored balls and sticks with green carbons. LRE1 is displayed as atom-colored balls and sticks with magenta carbons.

The protein conformations are otherwise very similar between the two structures.

With sAC inhibitors displaying improved intrinsic potency relative to LRE1 in hand, their activity was evaluated in a cellular context. Given that many compounds exhibited good permeability (parallel artificial membrane permeability assay, PAMPA), it was gratifying to see much improved inhibition of sAC in a cellular assay (Table 3).

Table 3. Cell Activity and Selected Parameters

compd	cell IC_{50}[a] (nM)	PAMPA (nm/s)	HPLC LogD (pH = 7.4)	kinetic solubility (μg/mL)
2	196	258	2.5	6.3
3	225	257	2.8	7.5
11	102	280	2.7	0.38
12	92	274	2.9	1.3

[a]Human 4-4 cell data: compounds were tested at minimum in triplicate.

Of the new analogs generated, TDI-10229 (**12**) was deemed most promising overall in terms of activity coupled with drug-like qualities and was therefore selected for additional characterization. Mouse pharmacokinetic studies of TDI-10229 indicated that this compound, with its 59% bioavailability, is well suited for interrogation of sAC inhibition in an *in vivo* context at reasonable dosages with coverage up to 50-fold over the cell IC_{50} based on free drug concentrations (mouse fu = 0.1, Table 4). Furthermore, TDI-10229 was not cytotoxic at 20 μM (200-fold above its IC_{50} for sAC in cells), it had a low propensity to form reactive intermediates (glutathione trapping study),[16] and it did not show appreciable activity against a panel of 310 kinases and 46 other well-known drug targets

https://doi.org/10.1021/acsmedchemlett.1c00273
ACS Med. Chem. Lett. 2021, 12, 1283−1287

Table 4. Mouse Pharmacokinetic Properties of TDI-10229 (12)

dosage	AUC (μM·h)	C_{max} (μM)	MRT[a] (h)
20 mg/kg (po)	94	15.5	3.95
20 mg/kg (ip)	72	45	2.82

[a]Mean residence time.

(GPCRs, ion channels, and nuclear receptors) [Supporting Information S1]. TDI-10229 also displayed high selectivity for sAC versus the closely related tmAC subtypes.[15]

To summarize, in order to identify an inhibitor suitable for exploration of sAC-driven pharmacology *in vivo*, a set of sAC inhibitors derived from LRE1 were designed and synthesized, efforts facilitated by structural biology input coupled to computational drug design tools. From the novel analogs synthesized, TDI-10229 displayed suitable potency, selectivity, and drug-like properties to facilitate *in vivo* studies of sAC pharmacology in preclinical animal models of disease, including its utility for hypotony or as an on-demand, nonhormonal contraceptive.[3,9,15]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00273.

Synthesis and characterization for compounds **2**–**22**, biology assay methods, ADMET assay methods, mouse pharmacokinetic studies, crystal structure determination, diffraction and refinement statistics, crystal structure of hsAC-cat in complex with TDI-10229 (**12**), and supplementary references (PDF)

Accession Codes

Coordinates and structure factors for the sAC/TDI-10229 crystal structure have been deposited with the worldwide PDB (www.pdb.org) under accession ID 7OVD.

■ AUTHOR INFORMATION

Corresponding Author

David J. Huggins − *Tri-Institutional Therapeutics Discovery Institute, New York, New York 10021, United States; Department of Physiology and Biophysics, Weill Cornell Medicine, New York, New York 10021, United States;* orcid.org/0000-0003-1579-2496; Phone: +1-646-962-6133; Email: dhuggins@tritdi.org; www.tritdi.org

Authors

Makoto Fushimi − *Tri-Institutional Therapeutics Discovery Institute, New York, New York 10021, United States*

Hannes Buck − *Department of Pharmacology, Weill Cornell Medicine, New York, New York 10021, United States*

Melanie Balbach − *Department of Pharmacology, Weill Cornell Medicine, New York, New York 10021, United States*

Anna Gorovyy − *Department of Pharmacology, Weill Cornell Medicine, New York, New York 10021, United States*

Jacob Ferreira − *Department of Pharmacology, Weill Cornell Medicine, New York, New York 10021, United States*

Thomas Rossetti − *Department of Pharmacology, Weill Cornell Medicine, New York, New York 10021, United States*

Navpreet Kaur − *Department of Pharmacology, Weill Cornell Medicine, New York, New York 10021, United States*

Lonny R. Levin − *Department of Pharmacology, Weill Cornell Medicine, New York, New York 10021, United States*

Jochen Buck − *Department of Pharmacology, Weill Cornell Medicine, New York, New York 10021, United States*

Jonathan Quast − *Department of Biochemistry, University of Bayreuth, 95440 Bayreuth, Germany*

Joop van den Heuvel − *Helmholtz-Zentrum für Infektionsforschung, 38124 Braunschweig, Germany*

Clemens Steegborn − *Department of Biochemistry, University of Bayreuth, 95440 Bayreuth, Germany;* orcid.org/0000-0002-0913-1467

Efrat Finkin-Groner − *Tri-Institutional Therapeutics Discovery Institute, New York, New York 10021, United States*

Stacia Kargman − *Tri-Institutional Therapeutics Discovery Institute, New York, New York 10021, United States*

Mayako Michino − *Tri-Institutional Therapeutics Discovery Institute, New York, New York 10021, United States*

Michael A. Foley − *Tri-Institutional Therapeutics Discovery Institute, New York, New York 10021, United States*

Michael Miller − *Tri-Institutional Therapeutics Discovery Institute, New York, New York 10021, United States*

Nigel J. Liverton − *Tri-Institutional Therapeutics Discovery Institute, New York, New York 10021, United States*

Peter T. Meinke − *Tri-Institutional Therapeutics Discovery Institute, New York, New York 10021, United States; Department of Pharmacology, Weill Cornell Medicine, New York, New York 10021, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.1c00273

Author Contributions

‡D.J.H. and P.T.M. contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This work was supported by NIH Grants P50 HD100549 and R01 HD088571 (to J.B. and L.R.L.), DFG grant STE1701/11 (C.S.), F31 AG069501 (T.R.), T32 GM073546-13 (J.F.), and Male Contraceptive Initiative (M.B.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the BESSY beamline staff for excellent technical support and Dr. Tanweer Khan for generating ^{13}C NMR data. The authors gratefully acknowledge the support to the project generously provided by the Tri-Institutional Therapeutics Discovery Institute (TDI), a 501(c)(3) organization. TDI receives financial support from Takeda Pharmaceutical Company, TDI's parent institutes (Memorial Sloan Kettering Cancer Center, The Rockefeller University, and Weill Cornell Medicine) and from a generous contribution from Mr. Lewis Sanders and other philanthropic sources.

■ ABBREVIATIONS

AC, adenylyl cyclase; sAC, soluble adenylyl cyclase; tmAC, transmembrane adenylyl cyclase; po, per os; ip, intraperitoneal

REFERENCES

(1) Rossetti, T.; Jackvony, S.; Buck, J.; Levin, L. R. Bicarbonate, carbon dioxide and pH sensing via mammalian bicarbonate-regulated soluble adenylyl cyclase. *Interface Focus* **2021**, *11*, 20200034.

(2) Ramos-Espiritu, L.; Kleinboelting, S.; Navarrete, F. A.; Alvau, A.; Visconti, P. E.; Valsecchi, F.; Starkov, A.; Manfredi, G.; Buck, H.; Adura, C.; Zippin, J. H.; van den Heuvel, J.; Glickman, J. F.; Steegborn, C.; Levin, L. R.; Buck, J. Discovery of LRE1 as a specific and allosteric inhibitor of soluble adenylyl cyclase. *Nat. Chem. Biol.* **2016**, *12*, 838−844.

(3) Wiggins, S. V.; Steegborn, C.; Levin, L. R.; Buck, J. Pharmacological modulation of the CO2/HCO3-/pH-, calcium-, and ATP-sensing soluble adenylyl cyclase. *Pharmacol. Ther.* **2018**, *190*, 173−186.

(4) Esposito, G.; Jaiswal, B. S.; Xie, F.; Krajnc-Franken, M. A. M.; Robben, T. J. A. A.; Strik, A. M.; Kuil, C.; Philipsen, R. L. A.; van Duin, M.; Conti, M.; Gossen, J. A. Mice deficient for soluble adenylyl cyclase are infertile because of a severe sperm-motility defect. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 2993−2998.

(5) Hess, K. C.; Jones, B. H.; Marquez, B.; Chen, Y.; Ord, T. S.; Kamenetsky, M.; Miyamoto, C.; Zippin, J. H.; Kopf, G. S.; Suarez, S. S.; Levin, L. R.; Williams, C. J.; Buck, J.; Moss, S. B. The ″soluble″ adenylyl cyclase in sperm mediates multiple signaling events required for fertilization. *Dev. Cell* **2005**, *9*, 249−259.

(6) Xie, F.; Garcia, M. A.; Carlson, A. E.; Schuh, S. M.; Babcock, D. F.; Jaiswal, B. S.; Gossen, J. A.; Esposito, G.; van Duin, M.; Conti, M. Soluble adenylyl cyclase (sAC) is indispensable for sperm function and fertilization. *Dev. Biol.* **2006**, *296*, 353−362.

(7) Akbari, A.; Pipitone, G. B.; Anvar, Z.; Jaafarinia, M.; Ferrari, M.; Carrera, P.; Totonchi, M. ADCY10 frameshift variant leading to severe recessive asthenozoospermia and segregating with absorptive hypercalciuria. *Hum. Reprod.* **2019**, *34*, 1155−1164.

(8) Steegborn, C. Structure, mechanism, and regulation of soluble adenylyl cyclases - similarities and differences to transmembrane adenylyl cyclases. *Biochim. Biophys. Acta, Mol. Basis Dis.* **2014**, *1842*, 2535−2547.

(9) Balbach, M.; Fushimi, M.; Huggins, D. J.; Steegborn, C.; Meinke, P. T.; Levin, L. R.; Buck, J. Optimization of lead compounds into on-demand, nonhormonal contraceptives: leveraging a public-private drug discovery institute collaboration. *Biol. Reprod.* **2020**, *103*, 176−182.

(10) Saalau-Bethell, S. M.; Berdini, V.; Cleasby, A.; Congreve, M.; Coyle, J. E.; Lock, V.; Murray, C. W.; O'Brien, M. A.; Rich, S. J.; Sambrook, T.; Vinkovic, M.; Yon, J. R.; Jhoti, H. Crystal structure of human soluble adenylate cyclase reveals a distinct, highly flexible allosteric bicarbonate binding pocket. *ChemMedChem* **2014**, *9*, 823−32.

(11) Buchmann, B.; Harter, M.; Cancho-Grande, Y.; Kosemund, D.; Schirok, H.; Nguyen, D.; Fritsch, M. Azaindoles as Inhibitors of Soluble Adenylate Cyclase. WO 2009/030725 A2, 2009.

(12) Macmillan, D. The use of Derek Nexus to facilitate decision-making in chemical safety assessment. Application of non-animal approaches for decision-making in chemical safety assessment, December 10−11, 2018, NC3Rs, London, UK. https://www.lhasalimited.org/publications/the-use-of-derek-nexus-to-facilitate-decision-making-in-chemical-safety-assessment/5183.

(13) Ellison, C. M.; Madden, J. C.; Judson, P.; Cronin, M. T. D. Using In Silico Tools in a Weight of Evidence Approach to Aid Toxicological Assessment. *Mol. Inf.* **2010**, *29*, 97−110.

(14) Wang, L.; Wu, Y.; Deng, Y.; Kim, B.; Pierce, L.; Krilov, G.; Lupyan, D.; Robinson, S.; Dahlgren, M. K.; Greenwood, J.; Romero, D. L.; Masse, C.; Knight, J. L.; Steinbrecher, T.; Beuming, T.; Damm, W.; Harder, E.; Sherman, W.; Brewer, M.; Wester, R.; Murcko, M.; Frye, L.; Farid, R.; Lin, T.; Mobley, D. L.; Jorgensen, W. L.; Berne, B. J.; Friesner, R. A.; Abel, R. Accurate and Reliable Prediction of Relative Ligand Binding Potency in Prospective Drug Discovery by Way of a Modern Free-Energy Calculation Protocol and Force Field. *J. Am. Chem. Soc.* **2015**, *137*, 2695−2703.

(15) Balbach, M.; Ghanem, L.; Rossetti, T.; Kaur, N.; Ritagliati, C.; Ferreira, J.; Krapf, D.; Molina, L. P.; Santi, C. M.; Hansen, J. N.; Wachten, D.; Fushimi, M.; Meinke, P. T.; Buck, J.; Levin, L. R. Soluble adenylyl cyclase inhibition prevents human sperm functions essential for fertilization. *Mol. Hum. Reprod.* **2021**, submitted for publication. https://www.biorxiv.org/content/10.1101/2021.04.27.441671v1.article-info.

(16) Sameshima, T.; Miyahisa, I.; Yamasaki, S.; Gotou, M.; Kobayashi, T.; Sakamoto, J. High-Throughput Quantitative Intrinsic Thiol Reactivity Evaluation Using a Fluorescence-Based Competitive Endpoint Assay. *SLAS Discov* **2017**, *22*, 1168−1174.

NOTE ADDED AFTER ASAP PUBLICATION

This paper was published ASAP on July 14, 2021, with errors in the Supporting Information. The corrected version was reposted on July 20, 2021.

https://doi.org/10.1021/acsmedchemlett.1c00273
ACS Med. Chem. Lett. 2021, 12, 1283−1287