ACS Medicinal Chemistry Letters

Technology Note

pubs.acs.org/acsmedchemlett

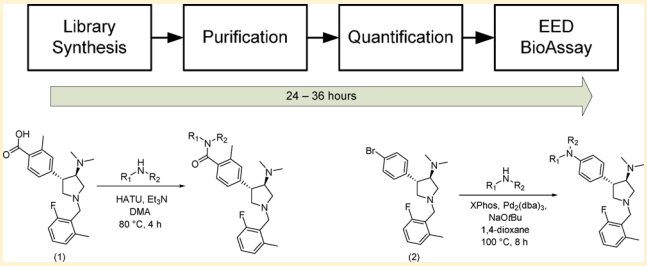
Integrated Platform for Expedited Synthesis−Purification−Testing of Small Molecule Libraries

Aleksandra Baranczak,[†] Noah P. Tu,[†] Jasmina Marjanovic, Philip A. Searle, Anil Vasudevan,* and Stevan W. Djuric

Discovery Chemistry and Technology, AbbVie Inc., 1 North Waukegan Road, North Chicago, Illinois 60064, United States

Supporting Information

ABSTRACT: The productivity of medicinal chemistry programs can be significantly increased through the introduction of automation, leading to shortened discovery cycle times. Herein, we describe a platform that consolidates synthesis, purification, quantitation, dissolution, and testing of small molecule libraries. The system was validated through the synthesis and testing of two libraries of binders of polycomb protein EED, and excellent correlation of obtained data with results generated through conventional approaches was observed. The fully automated and integrated platform enables batch-supported compound synthesis based on a broad array of chemical transformations with testing in a variety of biochemical assay formats. A library turnaround time of between 24 and 36 h was achieved, and notably, each library synthesis produces sufficient amounts of compounds for further evaluation in secondary assays thereby contributing significantly to the shortening of medicinal chemistry discovery cycles.



KEYWORDS: *Automated synthesis, purification, bioassay, automated drug discovery, EED*

Early stage discovery of active small molecules relies on iterative generation of structure−activity relationship (SAR) data. Consolidation of the traditionally lengthy and divided processes of compound synthesis, purification, analysis, and registration, followed by biological testing, has the potential to contribute to accelerated hypothesis validation and discovery of valuable chemical matter.[1] In recent years, several different approaches, predominantly flow technology-based, have been reported, focused on the development of integrated synthesis−purification−bioassay platforms.[2−4] In addition, some of these efforts have incorporated predictive software to design the next round of compounds to be synthesized.[2]

The critical role of automated and integrated systems in providing support to medicinal chemistry programs had been also recognized by our laboratory. We have previously reported an integrated flow synthesis−purification system,[5] an integrated flow synthesis−purification−sample management system,[6] and an integrated microwave synthesis−purification system.[7] Herein, we disclose the development of a fully automated and integrated synthesis−purification−bioassay platform for preparation and evaluation of small molecule libraries. This platform, unlike previously reported systems, enables batch-supported (with integrated microwave) compound synthesis based on a broad array of chemical transformations in conjunction with compound testing in a variety of biochemical assay formats. The platform was validated through the preparation and testing of two libraries of binders of polycomb protein EED.[8−10] The synthesis of these libraries was based on the application of two distinct chemical transformations: acylation and Buchwald coupling. We demonstrated reproducibility and consistency of platform bioassay results with simultaneous significant reduction in time and labor resources as compared with data generated through conventional approaches, i.e., autonomously conducted compound synthesis, purification, and testing.

The integrated platform (Figure 1, Figure S1) comprises a commercially available synthesizer (SWAVE, Chemspeed Technologies) coupled with an in-house developed preparative high-performance liquid chromatography−mass spectrometry (HPLC−MS) system for compound purification, previously described.[7] Addition of fraction analysis by a charged aerosol detector (CAD) for compound quantification, plate evaporator (Ultravap Mistral), liquid handling platform with robotic arm (PerkinElmer JANUS and Twister III), and plate reader (PerkinElmer EnVision) enables biological evaluation of newly synthesized compounds. The complete system is controlled through a combination of AutoSuite software (Chemspeed Technologies), JANUS WinPrep software (PerkinElmer), and an in-house developed application for HPLC−MS−CAD control. Communication between synthesis, purification/analysis, and bioassay platforms is enabled through digital I/O (input−output) lines providing signals for *synthesizer ready*, *HPLC ready*, *HPLC inject start*, and *compound plate available* conditions.

Received: February 9, 2017
Accepted: March 28, 2017
Published: March 28, 2017

DOI: 10.1021/acsmedchemlett.7b00054
ACS Med. Chem. Lett. 2017, 8, 461−465

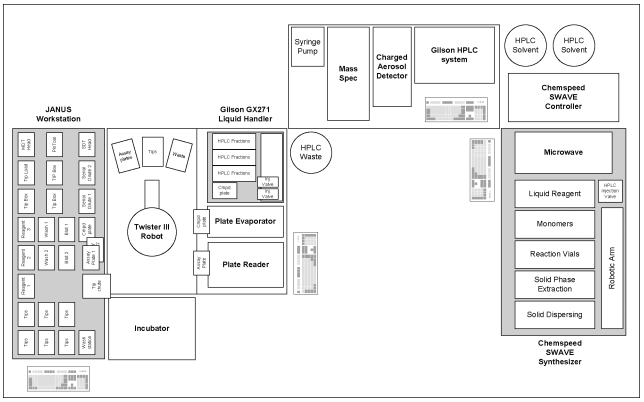


Figure 1. Integrated synthesis−purification−bioassay system (full size version in Supporting Information, Figure S1).

The complete workflow is illustrated in Figure 2. Following MS-triggered preparative HPLC separation of the desired

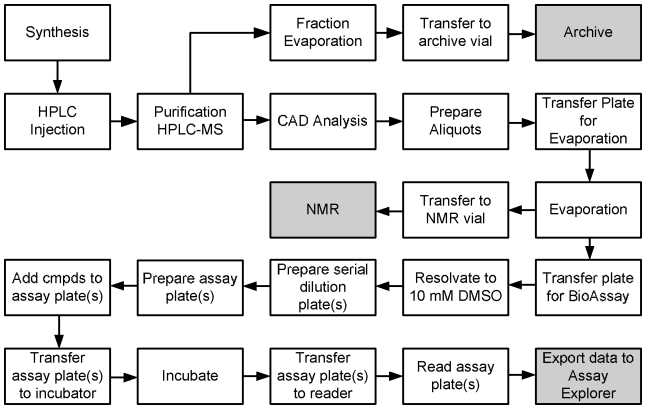


Figure 2. Schematic representation of the workflow for the integrated synthesis−purification−bioassay system.

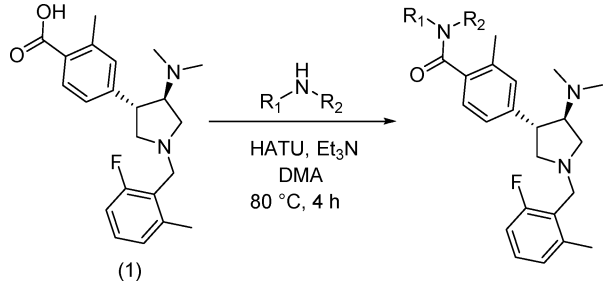
products, a charged aerosol detector (CAD) is utilized to determine the concentration of purified fractions (detailed description of HPLC−MS−CAD integration can be found in the Supporting Information). Upon purification and CAD analysis, two aliquots of a specific amount of each compound are transferred to a 96-well plate. After evaporation of HPLC mobile phase, one aliquot is used for the bioassay, and the second aliquot is used to confirm compound purity by NMR.

A maximum of 48 compounds can thus be synthesized and tested in a single library run; however, the number of tested compounds may be decreased when reference compounds are required to ensure quality control of the bioassay. Reference compounds may be added to a 96-well plate as MeOH solutions to enable accurate transfer and efficient solvent removal (Figure S2A,B). Compound dissolution in DMSO to a specific concentration, followed by serial dilution and bioassay testing are accomplished using the liquid handling platform coupled with plate reader. Activity of synthesized compounds is calculated by curve fitting using Assay Explorer software.

Charged aerosol detection (CAD) has been widely used in the pharmaceutical industry as a "universal" detection technique for the determination of the mass concentration of small molecules.[11,12] The CAD response was calibrated using known concentrations of a control compound (ketoprofen) and validated by comparison with the response from starting core material for each library, compounds **1** and **2** (Figure S3). Although the response for a single compound was very reproducible, variability of the response between different compounds was observed. In the typically observed concentration range for HPLC fractions from library purification (0.5−3.0 mg/mL), the difference in CAD response between compounds was ±40%. The accuracy of the CAD determination for library compounds was also determined by offline quantification of each product fraction using standard gravimetric and NMR spectroscopic analysis, and found to be within ±20% for both libraries. Similar errors have been reported using ELSD for the quantification of compound concentration, without having a significantly adverse effect on the interpretation of final bioassay results.[2−4]

To validate the integration and reliability of the platform, we initiated synthesis and testing of two libraries of EED protein binders. The synthesis of 22-member amide library was based on conditions routinely applied by our laboratory in the synthesis of libraries utilizing acylation reaction and optimized with the use of compound **1** (temperature, duration) (Scheme 1).

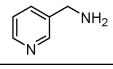
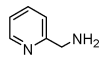
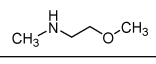
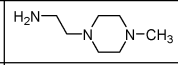
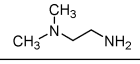
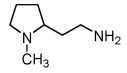
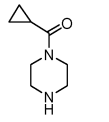
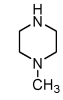
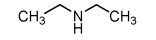
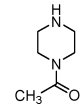
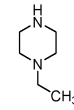
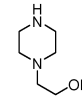
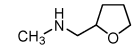
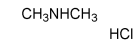
Scheme 1. Synthesis of Amide Library



Amine coupling reagents were dissolved in DMA and placed in 4 mL vials. Solutions of compound **1** in DMA, HATU in DMA, and Et_3N were placed inside the synthesizer. Upon automated addition of all reagents, the reaction solutions were heated at 80 °C for 4 h. Following MS-triggered HPLC separation, the concentration of HPLC fractions of purified compounds was determined by injecting a 50 μL aliquot of the HPLC fraction into the CAD via an analytical HPLC injection valve equipped with a 20 μL sample loop. Based on the CAD determined concentration of pure sample, a calculated volume of the HPLC fraction was dispensed to the 96-well plate containing MeOH solutions of four reference compounds (Figure S2B), giving a known amount of compound for subsequent preparation of 10 mM DMSO stock solution for bioassay. A second aliquot (0.5 mL) of the HPLC fraction was also dispensed in the second half of the 96-well plate for NMR analysis. Upon completion of the entire library synthesis and purification, collected aliquots were dried down using the UltraVap Mistral evaporator with preprogrammed drying protocol (160 min at 60 °C). The plate was then transferred onto the Janus deck by a Twister robotic arm and compounds were dissolved in DMSO to targeted concentration of 10 mM. Next, 90 μL aliquots of each 10 mM DMSO compound stock solution were transferred to a 384-deep well plate and an 11-point, 3-fold dilution was carried out (Figure S2C). Compounds were then subjected to TR-FRET-based binding assay by transferring 50 nL of each compound solution into a 384-well Proxy plate using a Pin Tool (V&P Scientific, Inc.)

DOI: 10.1021/acsmedchemlett.7b00054
ACS Med. Chem. Lett. 2017, 8, 461−465

Table 1. Results of Amide Library Synthesis and Testing

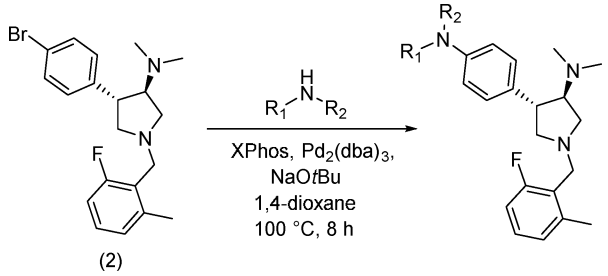
Entry	Monomer	Yield (%)	Integrated IC_{50} [uM]	Offline IC_{50} [uM]
1		71	0.07	0.11
2		8	0.08	0.10
3		34	0.08	0.10
4		29	0.07	0.08
5		37	0.13	0.14
6		6	0.08	0.09
7		57	0.09	0.12
8		51	0.08	0.15
9		34	0.08	0.10
10	 HCl	2	0.34	0.44
11		28	0.14	0.26
12		7	0.31	0.39
13		69	0.26	0.48
14		62	0.10	0.25
15		33	0.18	0.51
16		59	0.29	0.46
17		47	0.27	0.38
18		36	0.11	0.29
19	 HCl	13	0.11	0.18

and adding 10 μL of the assay buffer to each well (DMSO concentration 0.5%). Upon incubation for 1 h at 25 °C, the assay results were detected using the EnVision Plate Reader. To complete characterization of synthesized compounds and confirm their purity, compounds in the second half of the 96-well plate were submitted for NMR analysis. Remaining HPLC fractions were dried-down under vacuum, and the isolated yields were determined offline before sending to a central registration facility for storage.

The synthesis−purification−testing of the 22-member amide library was completed in a total time of 15 h, from initiation of the synthesis to detection of the assay results. Reaction yields varied between 2 and 71% (Table 1). The purity of all compounds was evaluated by NMR, and 3 out of 22 compounds did not meet purity criteria (>90% purity threshold was set), so the results of the bioassay were invalidated for those molecules. A minimum of 0.5 mg of compound (assuming M.W. = 500) is needed to prepare the 10 mM DMSO stock solution for testing. For the 19 compounds found to be pure, sufficient amounts were obtained resulting in an immediate generation of bioassay data through the integrated testing as well as subsequent compound registration for evaluation in secondary assays. The analysis of bioassay results using Assay Explorer showed an excellent agreement between data obtained on the integrated platform and data obtained through autonomous synthesis, purification, and testing of identical library of compounds with the application of identical assay conditions (Table 1, Figure S4). The reliability of the assays was estimated through quantification of minimum significant ratio (acceptable MSR < 3) of four reference compounds tested in parallel.

The synthesis of 33-member aromatic amine library was based on conditions routinely applied by our laboratory in the synthesis of libraries utilizing Buchwald transformation and optimized with the use of compound **2** (temperature, duration) (Scheme 2).

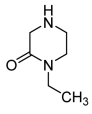
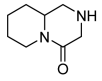
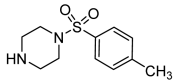
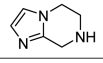
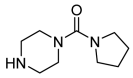
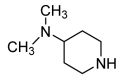
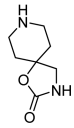
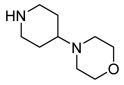
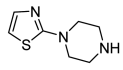
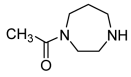
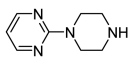
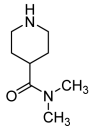
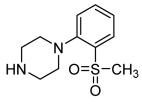
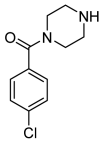
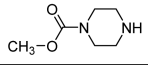
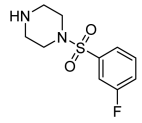
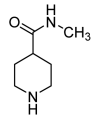
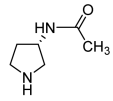
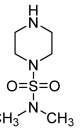
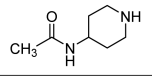
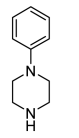
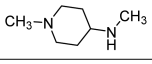
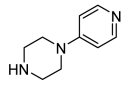
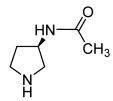
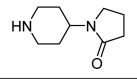
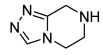
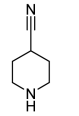
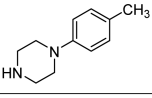
Scheme 2. Synthesis of Aromatic Amine Library



Compound **2** was dissolved in 1,4-dioxane under nitrogen atmosphere and kept in sealed 40 mL vial. Chemspeed synthesizer was filled up with nitrogen gas (20 psi, 10 GPM). The mixture of XPhos, $Pd_2(dba)_3$, and NaO*t*Bu was added via a solid dispensing unit to each of the 4 mL vials containing an amine coupling partner and a stir bar. The vials were sealed and a solution of compound **2** was added. The mixtures were

DOI: 10.1021/acsmedchemlett.7b00054
ACS Med. Chem. Lett. 2017, 8, 461−465

Table 2. Results of Aromatic Amine Library Synthesis and Testing

Entry	Monomer	Yield (%)	Integrated IC_{50} [uM]	Offline IC_{50} [uM]
1		54	0.06	0.12
2		55	0.10	0.17
3		9	0.05	0.08
4		49	0.07	0.10
5		41	0.03	0.04
6		16	0.04	0.10
7		15	0.06	0.14
8		6	0.04	0.06
9		91	0.03	0.04
10		3	0.16	0.27
11		64	0.03	0.07
12		43	0.04	0.07
13		79	0.07	0.19
14		46	0.02	0.04
15		26	0.03	0.04
16		39	0.04	0.05
17		72	0.05	0.11
18		64	0.09	0.24
19		65	0.02	0.06
20		73	0.04	0.16
21		57	0.05	0.09
22		76	0.09	0.35
23		45	0.04	0.14
24		88	0.01	0.01
25		92	0.16	No data
26		60	0.08	0.12
27		68	0.13	0.21
28		82	0.07	0.14
29		70	0.15	0.22

heated at 100 °C for 8 h. Next, the content of each 4 mL vial was transferred to the solid phase extraction (SPE) station and passed through cartridges containing silica gel/diatomaceous earth (1 g, 20:80 w/w). Subsequently, the filtrates were subjected to purification by HPLC. The aforementioned set of steps followed, resulting in generation of bioassay results for this 33-member library.

The synthesis−purification−testing of the aromatic amine library was completed in a total time of 30 h. Reaction yields varied between 3 and 92% (Table 2). The NMR analysis showed that 4 out of 33 compounds did not meet purity criteria, and bioassay results were invalidated for those molecules. For all other remaining compounds, platform bioassay results were found to be in excellent agreement with

DOI: 10.1021/acsmedchemlett.7b00054
ACS Med. Chem. Lett. 2017, 8, 461−465

data obtained through conventional approaches (Table 2, Figure S4)

In summary, we have described a fully automated and integrated platform that consolidates synthesis, purification, quantitation, dissolution, and bioassay testing of small molecule compound libraries. The platform enables batch-supported compound synthesis of up to 48-member libraries and utilizes MS-triggered preparative HPLC with CAD analysis of fractions, for purification/quantitation of compounds. We demonstrate validation of the platform with preparation of two libraries of polycomb protein EED binders. The fully integrated synthesis, purification, and testing of 22-member amide library and 33-member aromatic amine library was achieved in 15 and 30 h, respectively. In comparison, preparation of identical libraries of compounds through conventional approaches, consisting of autonomous synthesis, purification, and testing was conducted in the span of several days reflecting industry standards (an unpublished industry-wide survey of high-throughput chemistry groups revealed an average 7−10 day turnaround for library production and testing).[5] Finally, we observed an excellent correlation of bioassay data between both approaches, and sufficient quantity of each library member was synthesized for purity confirmation by NMR and compound registration for secondary assays contributing to the overall shortening of discovery cycle time.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00054.

Supplemental figures. Full experimental details and characterization of all synthesized compounds. Procedure for *in vitro* characterization of EED binders (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: anil.vasudevan@abbvie.com.

ORCID

Aleksandra Baranczak: 0000-0002-9715-262X

Author Contributions

†These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Ying Wang, Andrew Bogdan, Anurupa Shrestha, Ana Aguirre, and Kathy Sarris for support with library synthesis. Bailin Shaw, Vlasios Manaves, and Michael Karrmann are thanked for critical advice on bioassay implementation. We are grateful to David Blanchard and David Dingle for engineering assistance. All are employees of AbbVie. Yanick Bouchard (PerkinElmer) is thanked for technical support. Brian Warrington (BB Consultants Ltd) is thanked for valuable discussions at the beginning of this project and for providing helpful suggestions throughout. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

■ ABBREVIATIONS

EED, embryonic ectoderm development; SAR, structure−activity relationship; CAD, charged aerosol detector; HPLC, high-performance liquid chromatography; MS, mass spectrometry; SPE, solid phase extraction

■ REFERENCES

(1) Nettekoven, M.; Thomas, A. W. Accelerating drug discovery by integrative implementation of laboratory automation in the work flow. *Curr. Med. Chem.* **2002**, *9*, 2179−2190.

(2) Desai, B.; Dixon, K.; Farrant, E.; Feng, Q.; Gibson, K. R.; van Hoorn, W. P.; Mills, J.; Morgan, T.; Parry, D. M.; Ramjee, M. K.; Selway, C. N.; Tarver, G. J.; Whitlock, G.; Wright, A. G. Rapid discovery of a novel series of Abl kinase inhibitors by application of an integrated microfluidic synthesis and screening platform. *J. Med. Chem.* **2013**, *56*, 3033−3047.

(3) Czechtizky, W.; Dedio, J.; Desai, B.; Dixon, K.; Farrant, E.; Feng, Q.; Morgan, T.; Parry, D. M.; Ramjee, M. K.; Selway, C. N.; Schmidt, T.; Tarver, G. J.; Wright, A. G. Integrated Synthesis and Testing of Substituted Xanthine Based DPP4 Inhibitors: Application to Drug Discovery. *ACS Med. Chem. Lett.* **2013**, *4*, 768−772.

(4) Werner, M.; Kuratli, C.; Martin, R. E.; Hochstrasser, R.; Wechsler, D.; Enderle, T.; Alanine, A. I.; Vogel, H. Seamless integration of dose-response screening and flow chemistry: efficient generation of structure-activity relationship data of beta-secretase (BACE1) inhibitors. *Angew. Chem., Int. Ed.* **2014**, *53*, 1704−1708.

(5) Hochlowski, J. E.; Searle, P. A.; Tu, N. P.; Pan, J. Y.; Spanton, S. G.; Djuric, S. W. An Integrated Synthesis-Purification System to Accelerate the Generation of Compounds in Pharmaceutical Discovery. *J. Flow Chem.* **2011**, *2*, 56−61.

(6) Sutherland, J. D.; Tu, N. P.; Nemcek, T. A.; Searle, P. A.; Hochlowski, J. E.; Djuric, S. W.; Pan, J. Y. An Automated Synthesis−Purification−Sample-Management Platform for the Accelerated Generation of Pharmaceutical Candidates. *J. Lab. Aut.* **2014**, *19*, 176−182.

(7) Tu, N. P.; Sarris, K.; Searle, P. A. An Automated Microwave Assisted Synthesis-Purification System for Rapid Generation of Compound Libraries. *J. Lab. Aut.* **2016**, *21*, 459−469.

(8) Di Croce, L.; Helin, K. Transcriptional regulation by Polycomb group proteins. *Nat. Struct. Mol. Biol.* **2013**, *20*, 1147−1155.

(9) He, Y.; Selvaraju, S.; Curtin, M. L.; Jakob, C. G.; Zhu, H.; Comess, K. M.; Shaw, B.; Galasinski, S.; The, J.; Lima-Fernandes, E.; Szewczyk, M.; Cheng, D.; Klinge, K. L.; Li, H.-Q.; Pliushchev, M.; Algire, M. A.; Maag, D.; Guo, J.; Panchal, S. C.; Sweis, R. F.; Torrent, M.; Bigelow, L. J.; Senisterra, G.; Li, F.; Kennedy, S.; Wu, Q.; Osterling, D. J.; Lindley, D. J.; Gao, W.; Petros, A. M.; Dietrich, J.; Barsyte-Lovejoy, D.; Vedadi, M.; Arrowsmith, C. H.; Chiang, G. G.; Sun, C.; Pappano, W. N.; Buchanan, F. G. The EED protein-protein interaction inhibitor A-395 inactivates the PRC2 complex. *Nat. Chem. Biol.* **2017**, *13*, 389.

(10) Curtin, M. L.; Pliushchev, M. A.; Li, H. Q.; Torrent, M.; Dietrich, J. D.; Jakob, C. G.; Zhu, H.; Zhao, H.; Wang, Y.; Ji, Z.; Clark, R. F.; Sarris, K. A.; Selvaraju, S.; Shaw, B.; Algire, M. A.; He, Y.; Richardson, P. L.; Sweis, R. F.; Sun, C.; Chiang, G. G.; Michaelides, M. R. Amino pyrrolidines as potent and novel protein-protein interaction inhibitors of the PRC2 complex. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 1576.

(11) Almeling, S.; Ilko, D.; Holzgrabe, U. Charged aerosol detection in pharmaceutical analysis. *J. Pharm. Biomed. Anal.* **2012**, *69*, 50−63.

(12) Dixon, R. W.; Peterson, D. S. Development and testing of a detection method for liquid chromatography based on aerosol charging. *Anal. Chem.* **2002**, *74*, 2930−2937.

DOI: 10.1021/acsmedchemlett.7b00054
ACS Med. Chem. Lett. 2017, 8, 461−465