Inkjet Bioprinting on Parchment Paper for Hit Identification from Small Molecule Libraries

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Supporting Information

ABSTRACT: In this study, an inkjet bioprinting-based high-throughput screening (HTS) system was designed and applied for the first time to a catecholpyrimidine-based small molecule library to find hit compounds that inhibit c-Jun NH2-terminal kinase1 (JNK1). JNK1 kinase, inactivated MAPKAPK2, and specific fluorescent peptides along with bioink were printed on parchment paper under optimized printing conditions that did not allow rapid evaporation of printed media based on Triton-X and glycerol. Subsequently, different small compounds were printed and tested against JNK1 kinase to evaluate their degree of phosphorylation inhibition. After printing and incubation, fluorescence intensities from the phosphorylated/nonphosphorylated peptide were acquired for the % phosphorylation analysis. The IC50 (inhibitory mol 50) value was determined as 1.55 × 10−15 mol for the hit compound, 22. Thus, this work demonstrated that inkjet bioprinting-based HTS can potentially be adopted for drug discovery process using small molecule libraries, and cost-effective HTS can be expected to be established based on its low nano- to picoliter printing volume.

INTRODUCTION

High-throughput screening (HTS) of small molecule libraries is highly influential in the discovery of hit compounds because it is the starting point in the development of commercial drugs.1 Successful HTS techniques that are routinely used in the discovery process include time-resolved fluorescence resonance energy transfer (FRET),2,3 fluorescence polarization/anisotropy,4,5 alpha Screen,6 scintillation proximity assay,7 chemi-luminescence,8 and cell-free, enzyme-linked immunosorbent assay9 at the molecular and cellular levels. All of these biochemical-based HTS are targeted assays that include reaction components and target molecules that are incubated with synthetic or natural compounds in order to determine the activity of the compound (e.g., activator or inhibitor). Test compounds (small molecules or drugs) usually bind to known molecular targets or unintended off-targets. Such compounds have often led to unexplained reactions, resulting in side effects or drug repositioning opportunities. Drug repositioning is defined as finding new therapeutic activities for already existing drugs including marketed, discontinued, and even synthetic or natural product-based therapeutic agents.10,11 Recently, several studies have identified compounds from a pool of drugs approved for other applications.12,13 This is a profitable approach because the new applications of these compounds build on the available information regarding their pharmacokinetics, safety, and manufacturing.

Recently, we reported the in vitro and in vivo anti-inflammatory ability of 30 unique catechol-functionalized pyrimidine-based small molecules against phosphodiesterase-4B (PDE4B).14,15 Considering the cost-effectiveness of the drug repositioning approach, we intended to discover new potential targets of our synthetic catecholpyrimidine-based small molecule library. In this work, to validate this objective, an inkjet printing-based HTS platform was designed and applied to our 30 synthetic small molecule library. To date, inkjet printing technology has not been applied to screen small molecule libraries in order to find activities with respect to drug targets in the HT manner even though a few molecules have been tested to demonstrate the possibility of screening.16 First, inkjet bioprinting is very attractive for applications for screening of small molecule libraries because its ejection volume reaches the nano-to picoliter range. The use of such an ultrasmall ejection volume is inevitable due to enormous cost reduction expected for the drug discovery process as the number of molecules in the small molecule library increases. In addition, the ejection volume in inkjet bioprinting is highly reproducible, which is quite advantageous for obtaining quantitatively accurate drug efficacy data.

Currently, kinase activity assays depend on the use of expensive reagents and/or instrumentation. On the other hand, inkjet bioprinting can provide a straightforward, low-cost alternative to determine kinase activity through HTS.17,18 Over several decades, inkjet bioprinting technologies have been increasingly used as a tool for the synthesis of small molecules,19 thin film coatings,20 genomics validation,21 and

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NH2-terminal kinase1 (JNK1) and the mitogen-activated protein kinase (MAPK) interact synergistically to inhibit kinase activity. The combination of the bioprinting technique and HTS led to the discovery of new hit compounds that inhibit kinase activity. The optimized composition of bioink is essential for the kinase-to-kinase phosphorylation. The phosphorylation reaction of two different kinase enzymes must be handled extremely carefully for finding a hit compound on the printing substrate. The optimized composition of bioink is essential for the successful HTS. The biomaterials (target enzymes: c-Jun and JNK1) and the enzymes (JNK1 and MAPK) interact synergistically to inhibit JNK1 activity. Taking the HTS approach as a starting point to assess hit kinase inhibitor compounds, 30 small molecules were tested by utilizing the bioprinting-based HTS platform. Our results demonstrate that the hit compound (compound 22) out of 30 small molecules was highly efficient at inhibiting kinase activity in the femtomolar range. Thus, the combination of the bioprinting technique and HTS led to the discovery of catecholpyrimidine small molecules as potential JNK1 inhibitors and represents a new alternative for the screening of synthetic and natural product-based small molecular libraries. These results suggest a bright future for the HTS platform. The bioprinting-based dispersion technique is a promising approach for pharmaceutical applications. The bioprinting-based HTS has several advantages such as low cost due to the nano- to picoliter ejection volume, miniaturized automatic robotic system, and reproducibility and the ease of software-based HT pattern design. Despite these powerful advantages, with the exception of the present work, screening of library compounds for a particular target has not been performed through inkjet bioprinting to date. Figure 1 represents the structure of the pyrimidine-based small molecules. Compounds 22, 26, and 27 were synthesized according to the previously reported procedure. The structure and molecular weight of the compounds were analyzed using NMR and ESI-QTOF mass spectrometry. The detailed synthetic procedures of compounds 22, 26, and 27 are described in the Supporting Information.

Figure 1. Schematic illustration of the JNK1-mediated phosphorylation cascade reaction using the inkjet printing method.

**RESULTS AND DISCUSSION**

**Small Molecules Library for Screening JNK1 Inhibition.** JNK is a serine threonine protein kinase that phosphorylates c-Jun, a component of the transcription factor activator protein-1. JNK activity appears to be critical for both the immune response and for programmed cell death. Hence, therapeutically targeting JNK can provide clinical benefits in disease conditions such as arthritis, inflammatory disease, chronic obstructive pulmonary disease, and myocardial infarction. Recently, we reported on the anti-inflammatory activities of a catechol-functionalized pyrimidine-based small molecule library against PDE4B. Kwak et al. examined the mechanism by which rolufamist (PDE4B inhibitor) inhibits inflammatory mediators such as nuclear factor-κB, p38 mitogen-activated protein kinase, and c-Jun NH2-terminal kinase. Similarly, several other studies have reported the use of PDE4B inhibitors to suppress the activity of JNK/MAPKs. Considering these findings, the catecholpyrimidine-based small molecule library that showed selectivity to PDE4B was tested for multitargeting effect. The bioprinting-based HTS platform was designed and employed to screen the library of novel catecholpyrimidine-based small molecules against kinase.

 Moreover, bioprinting has been used for the direct printing of precise patterns to evaluate the activity of DNA, RNA, protein, enzymes, and cells on various substrates.
Schematic Representation of JNK1-dependent Phosphorylation Reaction. Figure 1 illustrates the scheme for performing the inkjet printing-based JNK1-dependent phosphorylation cascade reaction. To perform the JNK1-dependent phosphorylation reaction, all of the reaction components were sequentially printed on the parchment paper surface and the reaction was carried out. The Ser/Thr 4 peptide was labeled with two fluorophores (donor-coumarin and acceptor-fluorescein) for the FRET pair. This labeled peptide was used to determine the JNK1-dependent phosphorylation cascade reaction on paper. As shown in Figure 1, reaction components, including the synthesized compounds, JNK1 + MAPKAPK2 + Ser/Thr 4 peptide mixture, adenosine triphosphate (ATP), and the development reagent were printed from CMYK cartridges. The reaction occurred on the parchment paper surface. JNK1 activates MAPKAPK2, and in the presence of ATP, the activated MAPKAPK2 transfers a γ-phosphate (γ-PO$_4^{2-}$) group on ATP. γ-PO$_4^{2-}$ is transferred to a single serine or threonine residue in the Ser/Thr peptide substrate. The phosphorylation reaction is inhibited in the presence of the synthesized compound. After the phosphorylation reaction, a development reagent was printed to the reaction spot. The development reagent specifically recognized and cleaved the nonphosphorylated peptide. After cleavage, the fluorescence emission intensities of the phosphorylated and nonphosphorylated peptides were acquired. Based on the fluorescence emission intensity, % phosphorylation was calculated and IM$_{50}$ (inhibitory mole 50) was determined.

Bioink Activity Optimization before the Printing-Based Assay. A FRET peptide (Ser/Thr 4 peptide), tagged with two fluorophores (coumarin and fluorescein), was used to determine the kinase activity on the substrate (parchment paper). The bioink composition based on Triton X-100 and glycerol was optimized for the best printing performance. In particular, the evaporation rate of the printed bioink on the parchment paper is highly important for dealing with consecutive enzymatic reactions such as kinase phosphorylation. The printing condition devoid of evaporation of printed bioink was optimized using glycerol and was utilized to print the assay components. To modify the viscosity, surface tension, and evaporation rate, Triton-X and glycerol were used for the preparation of bioink. Triton-X is used widely as a mild surfactant because it does not affect enzyme activity. Glycerol was used to modify the viscosity and evaporation rate. Nine different concentrations of the 1.33× assay buffer with W/WO surfactant were prepared, and the JNK1 activity was investigated. The control group showed that the phosphorylation efficiency was 36.61%. Bioink solutions of JNK1 (7.5 × 10$^{-7}$ g/mL) + MAPKAPK2 (5.0 × 10$^{-6}$ g/mL) + Ser/Thr 4 peptide (4 μM) mixture, ATP (0.1 mM), and the development reagent in 1.33× assay buffer containing nine concentrations of the surfactant were prepared. Their activities in the prepared 1.33× assay buffer were measured. Figure 2 shows the effect of the surfactant dose on the JNK1 activity. Figure 3 illustrates the % phosphorylation change based on the increased addition (%) of glycerol. For the 0.1% Triton-X dose, there was a negligible decrease in the JNK1 activity. In the presence of the 0.1% Triton-X dose, glycerol was added (1–15%). Because of the viscosity of glycerol, the viscosity of bioink solution was also changed and the glycerol content impacted the JNK1 activity. When 1–4% of glycerol was added to the bioink solution, the JNK1 activity was not altered clearly. However, further addition of glycerol decreased the JNK1 activity, and when more than 5% glycerol was added to the assay buffer, the enzyme activity was decreased vertically as the amount of added glycerol increased.

The printing ability of bioinks was measured prior to the printing-based JNK1 inhibition assay. The printing ability of each bioink composition was detected using an inkjet printer. Table 1 shows the printing ability of the bioinks. The printing was carried out in the presence of 0.1% Triton-X in the bioink. After adding 1–10% of glycerol to the 0.1% Triton-X-containing bioink, printing was performed well. As shown in Table 1, when 15% glycerol was added to the assay buffer, the
bioink was not printed on the parchment paper surface because of its high viscosity. After printing, the spreading patterns of all of the bioink compositions (0−15% glycerol) were observed. At 0% Triton-X of only buffer solution, the printed pattern showed evaporation immediately with small spreading. At 0−10% glycerol with 0.1% Triton-X, there was no spreading or tailing of the printing pattern on the parchment paper. All of the printed patterns were printed clearly and remained without moisture absorption of parchment paper. Furthermore, these phenomena were sustained despite repeated printing on the parchment paper surface. Based on these results, 0.1% Triton-X with 2% glycerol was selected as the bioink media for the inkjet printing-based experiment.

Determination of the JNK1 Inhibition Activity Using Inkjet Printing. The percentage of phosphorylation and inhibitory efficacy of synthesized compounds were acquired through the inkjet bioprinting HTS. As illustrated in Figure 3a, inkjet printing-based JNK1-dependent phosphorylation and JNK1 activity screening was performed. To investigate hit compounds as JNK1 inhibitors, four different synthesized small compounds were used. To check the inhibition activity of small compounds, two groups (control and test) of experiments were performed. To determine the JNK1-dependent phosphorylation efficacy, all of the bioinks were printed on parchment paper. As a control, the printing was carried out with (100% phosphorylation or 0% inhibition condition) and without ATP (0% phosphorylation or 100% inhibition condition) conditions, and phosphorylated-Ser/Thr 4 peptide was printed. The printing pattern was set to be 3 rows by 3 columns for reproducibility, and the pattern size was set by the spot diameter of 1 mm. After printing and incubation, the reaction spot was excited at 400 nm. As shown in Figure 4b, at the 0% inhibition condition, 35.27% of the Ser/Thr 4 peptide was phosphorylated and the emission ratio was 1.84. This result indicates that JNK1 (and MAPKAPK2) is active and can phosphorylate 35.27% of the Ser/Thr 4 peptide on the reaction spot, and 64.73% of the Ser/Thr 4 peptide was cleaved. In the 100% inhibition condition, 0% of the Ser/Thr 4 peptide was phosphorylated and the emission ratio was 3.86. For the phosphorylated-Ser/Thr 4 peptide, 100% of the peptide was phosphorylated and the emission ratio was 0.41%. These observed differences in the % phosphorylation indicate that the phosphorylation cascade reaction was successfully carried out on the parchment paper surface.

As illustrated in Figure 3a, the inkjet printing drug screening assay was performed in order to estimate the JNK1 inhibition ability of 30 small compounds. The compound cartridge (black color cartridge) was changed for the printing of each synthesized compound. After printing a compound, the black cartridge was changed to another black cartridge for the printing of another compound. All black cartridges were cleaned with ethanol before use. The printer head and dispensing part of the black cartridge were cleaned by printing of ethanol in the cartridge. Then, the black cartridge was filled with another synthesized compound along with the identical bioink. The cleaning process of the cartridge was executed automatically using a printer management software. The cleaned cartridge was reused after rapid cleaning. The

| Triton-X | 0% Triton-X | 0.1% Triton-X |
|----------|-------------|---------------|
| glycerol (%) | 0 | 0 | 1 | 2 | 3 | 4 | 5 | 10 | 15 |
| printability | √ | √ | √ | √ | √ | √ | √ | √ | × |

Figure 4. (a) Fluorescence emission of coumarin (445 nm) and fluorescein (525 nm) generated in the reaction spot in the presence of compound 22. (b) Fluorescence emission of coumarin (445 nm) and fluorescein (525 nm) generated in the reaction spot in the presence of compound 26. (c) Fluorescence emission of coumarin (445 nm) and fluorescein (525 nm) generated in the reaction spot in the presence of compound 27.
Table 2. Summary of the Solution Densities of the Bioinks and Their Respective Ejection Volumes/Spot Areas

| density (g/mL) | kinase-peptide mixture | ATP | development solution | compound 22 | compound 26 | compound 27 |
|---------------|------------------------|-----|----------------------|-------------|-------------|-------------|
| CMYK value    | 1.05                   | 1.12| 1.11                 | 1.05        | 1.08        | 1.02        |
| ejected volume (×10−9 L/mm²) | 7.37 ± 0.2 | 6.63 ± 0.17 | 8.15 ± 0.41 | 5.39 ± 0.25 | 5.47 ± 0.48 | 6.1 ± 0.31 |

In summary, a user-friendly bioprinting-based HTS was developed to screen a library of novel catecholpyrimidine-based small molecules, and hit compounds were found to target JNK1. The developed platform showed that a FRET peptide substrate was successfully utilized to determine the phosphorylation percentage, which in turn revealed the inhibitory activity of the synthetic molecules against JNK1. Collectively, the present result demonstrated that the hit compound (compound 22) identified out of 30 small molecules was highly efficient at inhibiting JNK1 activity in the femtomolar range, which was significantly more effective than the reported JNK inhibitors. Herein, bioprinting HTS led to the successful discovery of catecholpyrimidine small molecules as potential JNK1 inhibitors. Thus, the proposed bioprinting-based HTS represents a new option for screening synthetic and natural product-based small molecular libraries.

■ MATERIALS AND METHODS

Chemicals and Materials. All of the assay components including JNK1, inactive MAPKAPK2, ATP (10 mM), Ser/Thr 4 peptide (1 mM), Ser/Thr 4 phosphopeptide (1 mM), phosphorylated-Ser/Thr 4 phosphopeptide (1 mM), and the developing reagent were purchased from Invitrogen (California, USA). Glycerol and Triton-X were purchased from...
Table 3. Number of Printed Moles and Grams of Small Molecules Ejected on Parchment Paper Based on K Value

| K value | Dm0 | ∆m10^−13 | ∆g10^−11 |
|---------|-----|-----------|-----------|
| 5       | 1.55 × 10^−5 | 2.25/10.06 | 0.03/0.44 |
| 10      | 4.50 × 10^−5 | 2.03/9.89 | 0.09/0.44 |
| 15      | 7.50 × 10^−5 | 1.80/8.77 | 0.13/0.44 |
| 20      | 1.09 × 10^−4 | 1.55/7.46 | 0.19/0.44 |
| 25      | 1.33 × 10^−4 | 2.03/12.39 | 0.25/0.44 |
| 30      | 1.55 × 10^−4 | 2.50/17.32 | 0.31/0.44 |
| 40      | 2.00 × 10^−4 | 3.00/22.25 | 0.37/0.44 |

Sigma-Aldrich (MO, USA). All of the chemicals and materials were used without further purification.

**Synthesis of Small Molecules.** The small molecules were synthesized according to a previously reported procedure as explained in detail in the Supporting Information.

**Bioink Preparation.** All of the reagents used in the experiment were freshly prepared prior to the experiment. The S× assay buffer (250 mM of pH 7.5 N-(2-hydroxyethyl)-piperazine-N'-ethanesulfonic acid buffer, 50 mM MgCl2, 5 mM EGTA, 0.05% BRIG-3S) was freshly prepared. JNK1 (7.5 × 10^−7 g/mL), inactive MAPKAPK2 (5.0 × 10^−6 g/mL), ATP (10 mM), Ser/Thr 4 peptide (4 μM), and Ser/Thr 4 phosphopeptidase (4 μM) were prepared using the 1.33× assay buffer. The development solution was prepared using the 1× development buffer. The synthesized small molecule solutions were prepared using a mixture of water and dimethyl sulfoxide (DMSO).

**Bioink Activity Optimization before the Printing-Based Assay.** All reagents used in the experiment were freshly prepared and the amount of DMSO was kept below 2% in order not to affect the activities of the enzymes. All of the solutions were prepared just before the experiment and kept on ice at 0 °C, and the solutions remaining after the experiment were discarded without being reused. The synthesized compounds dissolved in DMSO could be stored at 4 °C for 3 weeks and used for printing. The powders of synthesized compounds were found to be stable up to 2 years at 4 °C. In this work, it was found that at least 1.5 mL solution composed of reagents along with the bioink media was needed in the cartridge for the successful inkjet printing. To modify the viscosity and evaporation velocity, Triton-X and glycerol were used for the bioink preparation. All bioinks were prepared in a 1.33× assay buffer containing 0.1% Triton-X and 1–15% glycerol. Prior to the printing, the phosphorylation activities and printing abilities were measured. JNK1 activity was measured in a 384-well plate. Initially, JNK1 + MAPKAPK2 + Ser/Thr 4 peptide mixtures (5 μL) containing different % of glycerol were transferred to a 384-well plate. ATP (2.5 μL) was added to each well, and the assay plate was placed on a shaker for 30 s to mix the reagents well. The plate was incubated at room temperature (25 °C) for 1.5 h for the cascade phosphorylation. During incubation, the well plate was covered with a transparent sheet to prevent evaporation and contamination of the solution. After incubation, a development solution (5 μL) was added to each well and the assay plate was placed on a shaker for 30 s to mix the reagents. The plate was incubated at room temperature (25 °C) for 1 h for enzymatic cleavage. Then, the well plate was covered with a transparent sheet. Finally, the stop solution (5 μL) was added, and after shaking for 30 s, the fluorescence intensities of coumarin (Ex/Em = 400/445 nm) and fluorescein (Ex/Em = 400/525 nm) were measured using a multilplate reader. Prior to the measurement, the total reaction volume was set to 20 μL after the addition of 1.33× assay buffer (2.5 μL). For the control, with and without ATP conditions experiments were performed, and the emission fluorescence intensity from the phosphorylated-Ser/Thr 4 peptide was acquired. The following two equations were used to calculate the percentage of phosphorylation for the assessment of the inhibition activity of the synthesized compounds.

\[
\text{Emission ratio} = \frac{\text{coumarin emission (445 nm)}}{\text{fluorescein emission (525 nm)}}
\]

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Bioink Printing Ability before the Printing-Based Assay. A set of four cartridges used for the printer (HP Officejet Pro 8100) was modified. The inks present inside the cartridges were removed, and the cartridges were washed 10 times with deionized water and 100% ethanol. To minimize the damage of the cartridge rubber pack during the clearing, the backside of the cartridge was cut and a hole was drilled and the inside was emptied. After the removal of their contents, the four cartridges were dried at room temperature (25°C) overnight. These modified cartridges were used for the experiment.

To determine whether printing is possible based on the composition of all of the bioinks, 1.33× assay buffers containing 0.1% Triton-X and 1–15% of glycerol were prepared. The CMYK (cyan, magenta, yellow, and black) cartridges were filled with each bioink, and bioinks were printed onto the parchment paper surface. The CMYK color values that can verify the ejected volume from the printer head were adjusted using a graphic program (Adobe Photoshop CC), and the printing pattern was set using Microsoft PowerPoint software. The CMYK color codes were converted to RGB color codes, and the color of the printing pattern was set. The pattern was designed using the Microsoft PowerPoint software and each bioink was dispensed onto the printing pattern. All of the printing patterns were designed and were set to 8 rows by 20 columns. The individual printing spots of 8 row by 20 columns correspond to each well in the commercially available well-plate. The dimensions of the row-by-column printing spot can be freely adjusted using the software as required in HTS, for example, 1536 spots and more can be made. All of the color values were set as 100, and the shape of the printing pattern for the reaction was set as a spot with a diameter of 1 mm. All of the bioinks were printed on the each reaction spot, and their printing abilities were identified. The enzymatic stability was found to be stable up to 4 h after the preparation of enzymatic assay solution. Based on this result, the JNK1 inhibition assay was performed.

Determination of JNK1 Inhibition Activity Using Inkjet Printing. To investigate the JNK1-dependent phosphorylation and inhibition activity on parchment paper, all bioinks were printed on parchment paper. First, with and without ATP, 0% inhibition (100% phosphorylation) and 100% inhibition (0% phosphorylation) conditions were measured for the control. The printing patterns were designed as 3 rows by 3 columns for the control assay, 1 mm diameter spot, and 1 mm gap per each spot to spot. To evaluate the inhibitory activity of the synthesized compounds (as JNK1 inhibitors) using inkjet printing, the CMYK color codes settings were fixed at 100, 50, 100, and 100, respectively, in order to print constant amounts of JNK1 + MAPKAPK2 + Ser/Thr 4 phosphopeptide, ATP, phosphorylated-Ser/Thr 4 peptide, development reagent, and 1.33× assay buffer, respectively. The printing patterns were designed as 8 rows by 20 columns with a spot diameter of 1 mm and a gap of 1 mm for each spot. For the synthesized compounds, the color code settings were varied from 5 to 50 in order to vary the amount of the synthesized compounds. Herein, for this experiment, two printing heads and 2 sets of CMYK cartridges were prepared.
For the control assay, the inhibition activity of 100% was evaluated by printing the mixture of JNK1 + MAPKAP2K + Ser/Thr 4 peptide without ATP (Figure 3b—first column), whereas the inhibition activity of 0% was assessed with printing the mixture of JNK1 + MAPKAP2K + Ser/Thr 4 peptide along with ATP (Figure 3b—second column). The phosphorylation of 100% was evaluated by printing the phosphorylated-Ser/Thr 4 peptide (Figure 3b—third column).

After the control assay, inkjet printing-based phosphorylation cascade reactions were performed to determine the JNK1 inhibition activity of the synthesized small compounds. For this assay, the bioink-synthesized compound → JNK1 + MAPKAP2K + Ser/Thr 4 peptide mixture → ATP was sequentially printed on parchment paper at each reaction spot. The assay component-printed papers were incubated for 1 h at room temperature (25 °C). The 1:33x assay buffer was printed once in 20 min to stimulate the phosphorylation cascade reaction on the parchment paper. After incubation, the developing solution was printed on the reaction spot, and the printed papers were incubated for 1 h at room temperature (25 °C). After incubation, fluorescence images [coumarin (Eex/Eem = 400 nm/445 nm) and fluorescein (Eex/Eem = 400 nm/525 nm)] were acquired using a confocal microscope (Leica, TCS SP8). The acquired fluorescence images were analyzed using ImageJ software. The amounts of the assay components printed on each spot were evaluated based on the volume printed per spot and their respective solution densities, as suggested by Song et al.19

■ ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03169.

Synthesis of compounds 27, 28, 29, and 30; operation stability of bioinks; and small molecules and their JNK1 IM30 values (PDF)

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Notes
The authors declare no competing financial interest.

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■ ABBREVIATIONS

HTS, high-throughput screening; JNK1, c-Jun NH2-terminal kinase1; IM30, inhibitory mole 30; PDE4B, phosphodiesterase-4B; CMYK, cyan, magenta, yellow, and black

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