Development of a High-Throughput Assay for Inhibitors of the Polo-Box Domain of Polo-Like Kinase 1 Based on Time-Resolved Fluorescence Energy Transfer

Tae Gi Kim, a,b,# Ju Hee Lee, a,b Mi Young Lee, a Ka-Ul Kim, a,c Jeong Hyun Lee, a,c Chi Hoon Park, a,c Byung Ho Lee, a,b and Kwang-Seok Oh,* a,c

a Bio-Organic Science Division, Korea Research Institute of Chemical Technology; 141 Gajeong-ro, Yuseong, Daejeon 34114, Republic of Korea; b Graduate School of New Drug Discovery and Development, Chungnam National University; 99 Daehak-ro, Yuseong, Daejeon 34183, Republic of Korea; and c Department of Medicinal and Pharmaceutical Chemistry, University of Science and Technology; 176 Gajeong-ro, Yuseong, Daejeon 34129, Republic of Korea.

Received April 4, 2017; accepted June 5, 2017

Although enzyme-linked immunosorbent assay (ELISA) technology has been widely accepted for binding assays against the polo-box domain (PBD) of polo-like kinase-1 (Plk1), these assays have a limitation-related heterogeneous procedure, such as multiple incubations and washing steps to apply high-throughput screenings (HTSs). In the present study, a Plk1-PBD binding assay based on time-resolved fluorescence energy transfer (TR-FRET) was developed for HTS of PBD-binding inhibitors. The TR-FRET-based Plk1-PBD binding assay is sensitive and robust and can be miniaturized into the 384-well plate-based format. Compared with the ELISA-based Plk1-PBD binding assay (Z' factor, 0.53; signal-to-background ratio, 4.19), the TR-FRET-based Plk1-PBD binding assay improved the Z' factor (0.72) and signal-to-background ratio (8.16). Using TR-FRET-based Plk1-PBD binding assay, pilot library screening of 1019 natural compounds was conducted and five hit compounds such as haematoxylin, verbascoside, menadione, lithospermic acid and (1,3-dioxolo[4,5-g]isoquinolinium 5,6,7,8-tetrahydro-4-methoxy-6,6-dimethyl-5-[2-oxo-2-(2-pyridinyl)ethyl]-iodide) (DITMD) were identified as Plk1-PBD inhibitor. In a functional assay to validate the hit compounds, five hit compounds exhibited suppression of HeLa cells proliferation. These results suggest that TR-FRET-based Plk1-PBD binding assay can be applied for an efficient and less time-consuming HTS of compound libraries.

Key words polo-like kinase-1; polo-box domain; protein–protein interaction; high-throughput screening

Polo-like kinases (Plks) are well-known conserved subfamily of serine/threonine (Ser/Thr) protein kinases with a role in cell cycle control conserved from yeast to mammals.1,2) To date, five Plk members have been identified. Among them, Plk1 is considered an attractive anti-cancer drug target because its activity can override cellular checkpoints, such as the bipolar spindle formation checkpoint, and induce genetic instability, leading to tumorigenesis in human cells.3,4) Since the overexpression of Plk1 has been documented to be strongly associated with bad prognosis of various cancer types, these kinases are promising targets for anticancer drug development.5,6) Plk1 is characterized by an N-terminal kinase domain and a C-terminal protein–protein interaction (PPI) domain referred to as the polo-box domain (PBD). This PBD is composed of two highly homologous polo boxes (PB1 and PB2) motifs that form a phospho-Ser/Thr-binding module critical for PPIs.7,8) Several potent ATP-competitive Plk1 inhibitors targeting the N-terminal kinase domain have already been reported.9) However, these compounds are not target-specific, because all Plks have structurally similar kinase domains. Thus, discovery of non-ATP-competitive Plk1 inhibitors targeting the C-terminal PBD rather than the kinase domain presents an alternative strategy to inhibit this kinase.10) Although small-molecule drug discovery efforts have mainly focused on enzyme, receptor, and ion-channel targets, increasing effort has focused on the research on PPIs disruptors by applying high-throughput screening (HTS)-compatible protein-binding assays.11,12) Enzyme-linked immunosorbent assay (ELISA) has been consistently used to investigate several PPIs due to its sensitive and reproducibility. Indeed, the ELISA-based Plk1-PBD assay has been widely accepted because this method quantifies Plk1 activity with high sensitivity and specificity. However, this application has significant limitations in HTSs. ELISA involves time-consuming processing such as multiple incubations and washing steps to remove unbound ligands. Given these major inconveniences, a time-resolved fluorescence resonance energy transfer (TR-FRET)-based method has been considered as alternative assay for the HTS of PPI inhibitors.13) Thus, we developed a TR-FRET-based protein binding assay for the HTS of Plk1-PBD binding inhibitors. To our knowledge, the application of a TR-FRET-based Plk1-PBD binding assay has not been previously examined. In present study, we described the development and validation results of a TR-FRET-based Plk1-PBD binding assay and compared it with ELISA-based PBD binding assays. In addition, using established TR-FRET-based Plk1-PBD binding assay, we performed screening approaches in a pilot screen of 1019 natural compounds to identify small molecules that inhibit Plk1-PBD binding to Biotin-DPPLHS-pT-AI (PLHspT).
MATERIALS AND METHODS

Materials Biotinylated PLHST (Biotin-DPPLHSTAI), PLHSpT (Biotin-DPPLHS-pT-AI), and poloboxitide (MAGPMQS-pT-PLNGAKKK) peptides were obtained from Peptron (Daejeon, South Korea). Full-length Human Plk1 expressed as a N-terminal glutathione S-transferase (GST)-fusion protein was obtained from Carna (Kobe, Japan). Streptavidin-XXL665 and anti-GST-cryptate were purchased from Cisbio (Codolet, France). Full-length Human Plk1 fused with enhanced green fluorescent protein (EGFP) was prepared from mitotic HEK293T lysates expressing EGFP-Plk1. Streptavidin-coated 384-well plates, horseradish peroxidase (HRP)-conjugated secondary antibodies, and a 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution were obtained from Thermo Fisher Scientific (Waltham, MA, U.S.A.). To detect bound EGFP-Plk1, an anti-Plk1 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Purpurogallin and poloxin were purchased from TCI America (Portland, OR, U.S.A.) and Sigma-Aldrich (St. Louis, MO, U.S.A.), respectively. All other reagents including MgCl₂, MnCl₂, and dithiothreitol were obtained from Sigma-Aldrich.

TR-FRET-Based Plk1-PBD Binding Assay The binding assay based on TR-FRET to discover inhibitors of Plk1-PBD was performed in Corning 384-well white flat-bottom plates (Corning Life Science, Lowell, MA, U.S.A.). Enzyme, substrate, and compounds were diluted in binding buffer, comprising 50 mM N-(2-hydroxyethyl)piperazine-N′-2-ethanesulfonic acid (HEPES) pH 7.0, 0.02% NaN₃, 0.01% bovine serum albumin (BSA), 0.1 mM Na₂VO₃, 5 mM MgCl₂, and 1 mM dithiothreitol (DTT). GST-fused human Plk1 and biotinylated substrate peptides were mixed at final concentration of 4 ng/well and 0.1 µM, respectively. The total reaction volume was 10 µL, and compounds were preincubated with Plk1 for 10 min before adding the substrate. Binding reactions were conducted for 1 h at 25°C and then 10 µL of detection mixture containing 62.5 nM streptavidin-XXL665 and 2.5 ng/µL of the resulting biotinylated peptide-coated ELISA wells immediately after mixing with the indicated amounts of compounds, and incubated under mild shaking for 1 h at room temperature. Following incubation, ELISA plates were washed 4 times with PBST. To detect bound EGFP-Plk1, plates were incubated for 90 min with 50 µL/well of anti-Plk1 antibody (Santa Cruz Biotechnology) at a concentration of 0.1 µg/mL in blocking buffer and subsequently washed 5 times. Plates were consequently incubated for 1 h with 50 µL/well of HRP-conjugated secondary antibody (Thermo Fisher Scientific) at a 1:2000 dilution in blocking buffer. Plates were washed 5 times with PBST and incubated with 50 µL/well of TMB substrate solution (Thermo Fisher Scientific) for 3 min. Reactions were terminated by the addition of 50 µL/well of 1 N H₂SO₄ stop solution, and optical densities measured at 450 nm using an Envision instrument (PerkinElmer, Inc.). The binding assay was performed in a final volume of 100 µL.

Screening Chemical Library of Natural Compounds The natural products library is a subset of 1019 natural compounds such as terpenes, flavonoids, stilbenes, alkaldoids, lignans and saponins. The natural products library was collected and selected with a natural product-like structure according to molecular diversity criteria, representative of the Korea Chemical Bank (KCB) of the Korea Research Institute of Chemical Technology (KRICT). The natural products library was screened using the TR-FRET-based Plk1-PBD binding assay described above with optimized conditions in 384-well plates in a reaction volume of 20 µL. A final concentration of 10 µM was used for each compound. The reagents including Plk1 enzyme, substrate, streptavidin-XXL665 and anti-GST-cryptate were dispensed by using a Deearc GX8 system (Labcyte Inc., Dublin, Ireland) which enables high-speed low-volume dispensing for HTS. For Z’ factor and signal to background (S/B) ratio determination, negative and positive control were used on each plate with 32 wells in columns 1 and 2, and columns 3 and 4, respectively. Compounds with inhibition higher than 50% in the screen concentration of 10 µM considered hits.

Cell Culture and Viability Assays HeLa cells were cultured at 37°C in Dulbecco’s modified Eagle’s medium (High Glucose) with 2 mM l-glutamine, 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin with 5% CO₂ in a humidified atmosphere. For cell viability assay, HeLa cells were treated with 30 µM test compounds and harvested at 24, 36 and 72 h. Cell viabilities were measured by using a (3-(4,5-dimethylythiazol-2-yl)-2,5-diphenyletrazolium bromide (MTT). Each well of 96-well microplate was added 120 µL serum free media with 200 µg/mL of MTT solution to cell and incubated at 37°C for 2 h. Then the formazan formed was dissolved in dimethyl sulfoxide (DMSO) (200 µL/well). The absorbance was measured using a microplate reader (VICTOR II, PerkinElmer, Inc., Oy, Turku, Finland) at 560 nm. The optical density of formazan formed in control (DMSO) cells was taken as 100% of viability.

Statistical Analysis Statistical data were analyzed by nonlinear curve fitting functions with PRISM version 5.0 (GraphPad Software Inc., San Diego, CA, U.S.A.). Results are expressed as a maximum binding response (Bₘₐₓ) and equilibrium dissociation constant (Kₐ) parameters measured by the one site binding (hyperbola) equation, \( y = B_{max} \times X / (K_a + X) \), and as the IC₅₀ value (concentration causing a half-maximal
inhibition of control activity) determined by non-linear regression analysis of the inhibition–response curves. The Z' factor (representation of the quality of the assay itself) was calculated as $1 - \left( \frac{3 \times SD_{c+} + (3 \times SD_{c-})}{\text{Mean}_{c+} - \text{Mean}_{c-}} \right)$. $SD_{c+}$ and $SD_{c-}$ are the standard deviations of the positive and negative control signals, respectively. $\text{Mean}_{c+}$ and $\text{Mean}_{c-}$ represent the means of the TR-FRET counts of the positive and negative control signals, respectively. The values of cell based assay were expressed as mean$\pm$standard deviation (S.D.) A comparison of results was performed with one-way ANOVA and Dunnett’s multiple comparison tests (Sigma Stat, Jandel Co., San Rafael, CA, U.S.A.). Statistically significances between groups were defined as $p$ values of $<0.05$.

RESULTS

TR-FRET-Based Plk1-PBD Binding Assay

The TR-FRET based Plk1-PBD binding assay was developed using a site-specific biotinylated PLHSpT peptide, a GST-fused Plk1-PBD, streptavidin-XL665, and a europium (Eu) cryptate-labeled anti-GST monoclonal antibody (Fig. 1). The first step in the development of our binding assay was to optimize the concentrations of GST-Plk1 and substrate. With 1 $\mu$M biotinyl-

Fig. 1. TR-FRET-Based Plk1-PBD Binding Assay
Quaternary complexes of PLHSpT, a GST-fused Plk1-PBD, streptavidin-XL665 and Eu cryptate-labeled anti-GST monoclonal antibodies generate proximity for a FRET pair between the donor Eu fluorophore and acceptor fluorophore XL665. When the compound binds to the Plk1-PBD, the Plk1-PBD cannot bind to PLHSpT in a concentration-dependent manner, resulting in a decrease of the TR-FRET signal.

Fig. 2. Dose–Response Curves of Enzyme Titration (A, B) and Substrates Titration (C, D) in TR-FRET Based Plk1-PBD Binding Assay
(A) A fixed concentration of substrates (1 $\mu$M) was incubated with increasing concentration of enzyme preparation (0.2–20ng/well) for 10min. (B) Specific binding was calculated by subtracting nonspecific binding (○) from total binding signals (●). (C) A fixed concentration of GST-Plk1 (4ng/well) was incubated with increasing concentrations of substrate (PLHSpT) preparation (0.01–1$\mu$M) for 60min. (D) Specific binding was calculated by subtracting nonspecific binding (○) from total binding signals (●).
ated PLHSpT, GST-Plk1 titration was performed at various concentrations (from 0.2 to 20 ng/well). The TR-FRET count was correlated with the concentration of GST-Plk1 with a good separation between total and non-specific binding (Fig. 2A) and maximum specific binding was reached in the presence of 20 ng of enzyme (Fig. 2B). Non-specific binding was determined in the presence of 1 µM biotinylated PLHST. Based on the enzyme-dose response curve subtracted with nonspecific binding, GST-Plk1 at 4.4 ± 0.7 ng/well reached half of the maximal response (17.87 ± 0.96 TR-FRET counts). Thus, the TR-FRET based Plk1-PBD binding assay was performed at 4 ng/well of enzyme concentration. In addition, saturation-binding assays were carried out using increasing concentration of substrates to obtain the $K_d$ and $B_{max}$. Figures 2C and D show the dose–response binding curves of PLHSpT to 4 ng/well GST-Plk1. The $K_d$ value of substrates was found to be 0.08 ± 0.01 µM. The maximum binding response under these conditions was 11.85 ± 0.52 TR-FRET counts. Based on these preconditional experiments, the GST-Plk1 and substrate concentrations were established for further screening experiments: 4 ng/well and 0.1 µM, respectively.

**ELISA-Based Plk1-PBD Binding Assay** To define balanced binding conditions, a saturation binding assay was performed with increasing concentrations of GST-Plk1 (from 0.25 to 25 µg/well) for 10 min. Specific binding was calculated by subtracting nonspecific binding (○) from total binding signals (●). A fixed concentration of EGFP-Plk1 (10 µg/well) was incubated with increasing concentrations of substrate (PLHSt or, PLHSpT) preparation (0.001 to 0.1 µM) for 60 min. Non-specific binding was determined in the presence of 0.1 µM biotinylated PLHST. The $K_d$ and $B_{max}$ for EGFP-Plk1 were 12.2 ± 0.74 µg/well and 1.72 ± 0.05, respectively and signal-to-background ratio (S/B) was 4.96. A substrate titration was performed in the presence of various concentrations (from 0.001 to 0.1 µM) of biotinylated PLHSpT peptides incubated with 10 µg/well of EGFP-Plk1, in order to determine the optimal concentration of substrate for the binding assay (Figs. 3C, D). The $K_d$ and $B_{max}$ of the substrates were found to be 0.007 ± 0.002 and 1.09 ± 0.08 µM, respectively. The ELISA-based Plk1-PBD binding assay was performed in the presence of 10 µg/well of EGFP-Plk1 and 0.01 µM substrate.

**Evaluation of Assay Quality and Validity** The quality of our ELISA and TR-FRET-based Plk1-PBD binding assays was evaluated by Z’ factor determination and distribution of S/B data (Fig. 4). The S/B provides an indication of the separation of positive and negative control data that can be used
to estimate the quality of an assay. Binding of the Plk1-PBD showed a better separation of control data in the TR-FRET-based assay compared with that in the ELISA-based assay. The calculated S/B ratios of the ELISA- and TR-FRET-based Plk1-PBD binding assays are 4.19 and 8.16, respectively. The $Z'$ factor analysis is a standard used to appraise the quality of an assay for its possible use as a screening tool entailing a large number of comparisons. The $Z'$ factor for our TR-FRET-based binding assay to Plk1-PBD is 0.72, showing a higher signal stability with a better S/B compared with that for the ELISA-based binding assay ($Z'$ factor: 0.53).

**Evaluation of Known PBD Binding Inhibitors**

To validate our ELISA- and TR-FRET-based Plk1-PBD binding assays with standard compounds, known high-affinity PBD binding inhibitors such as purpurogallin, poloboxtide and poloxin were used to analyze dose-dependent antagonist responses (Fig. 5). Purpurogallin and poloboxtide showed a typical concentration-dependent antagonist response. The IC$_{50}$ values of purpurogallin and poloboxtide in TR-FRET-based Plk1-PBD binding assay were 2.57±0.30 and 6.87±2.65 $\mu$M, respectively, similar to those obtained for the ELISA-based Plk1-PBD binding assay (1.95±0.24 and 5.47±1.19 $\mu$M, respectively). In contrast, poloxin exerted no inhibitory activity in both TR-FRET- and ELISA-based Plk1-PBD binding assays.

**Pilot Library Screening of 1019 Natural Compounds for Inhibition of Plk1-PBD Binding**

Key features of the pilot library screening of 1019 natural compounds were summarized in Table 1. The negative and positive control values were clearly separated with minimum fluctuation (0.86±0.09 and 7.40±0.52, negative and positive control, respectively), resulting S/B ratio of 8.72±0.79. The TR-FRET signal from most compounds followed a normal distribution centered on the negative and positive control (Fig. 6A). $Z'$ factor as an indication of assay robustness in high-throughput format was 0.66±0.05 (Fig. 6B). A total of 39 hit compounds were identified as compounds showing greater than 50% inhibition in the screen concentration of 10 $\mu$M. Among the initial 39 hits, five compounds with greater than 80% inhibition such as haematoxylin, verbascoside, menadione, lisospermic acid and DITMD (1,3-dioxolo[4,5-g]isoquinolinium 5,6,7,8-tetrahydro-4-methoxy-6,6-dimethyl-5-[2-oxo-2-(2-pyridinyl)ethyl]-iodide), were selected for dose-response analysis and showed an IC$_{50}$ ranging from 1.4 to 2.8 $\mu$M (Fig.
Equation: 1

\[ \text{Signal-to-background (S/B) ratio} = \text{Positive control (TR-FRET counts)} / \text{Negative control (TR-FRET counts)} \]

\[ \text{Hit rate (\%)} = \text{Percentage of number of compounds showing greater than 50\% inhibition of the maximum signal} \]

\[ \text{Confirmation rate (\%)} = \text{Percentage of number of cherry-picked hit compounds that were reconfirmed in triplicate experiments using the same cut-off applied in the primary screening} \]

**DISCUSSION**

In the present study, a TR-FRET based Plk1-PBD binding assay was developed to quantify inhibitor potency for the Plk1-PBD/PLHSpT interaction. TR-FRET based assays have been applied to develop assays for various enzyme classes, such as kinases, peptidases, ubiquitin ligases, and deubiquitinases. The TR-FRET technique has greater reliability of time-gate fluorescence measurements with high sensitivity; this greater reliability is based on the log fluorescence emission half-life of lanthanides, characterized by exceptional decay times, allowing efficient discrimination from short-lived background fluorescence from compounds found in biological fluids or from small molecules used in inhibitor screens, its application led to higher SB ratios and excellent robustness. In biological fluids or from small molecules used in inhibitor screens, its application led to higher SB ratios and excellent robustness.

Based on optimized assay conditions, the IC\textsubscript{50} values of purpurogallin and poloboxtide as known Plk1-PBD binding inhibitors were 2.57 and 6.87 \(\mu\text{M}\), respectively, similar to those obtained in the ELISA-based Plk1-PBD binding assay (1.95 and 5.47 \(\mu\text{M}\), respectively). The IC\textsubscript{50} values we obtained for purpurogallin and poloboxtide are in good agreement with previously reported data (2.7\textsuperscript{(15)} and 5.0 \(\mu\text{M}\),\textsuperscript{(23)} respectively).
However, we could not determine the inhibitory activity of poloxin, another known Plk1-PBD binding inhibitor, in both our TR-FRET-based and ELISA-based assays. These results might be due to different binding sites. Liao et al. reported that, unlike purpurogallin and poloboxtide, poloxin may not specifically interact between the Plk1-PBD and a ligand with the p-T78 motif of PBIP1 (PLHSpT).

In this study, two different PPI binding assays (ELISA- and TR-FRET-based) were compared for their performances for quantification of inhibitor potency of the specific interaction between the Plk1 PBD and PLHSpT. The TR-FRET-based Plk1-PBD binding assay showed a significantly greater reliability than the ELISA-based assay, an increased reproducibility, and reduced time needed to perform due to mix-and-read approach. Therefore, the TR-FRET based Plk1-PBD binding assay represents an alternative method for HTS of PBD-binding inhibitors.

Actually, we used this TR-FRET based Plk1-PBD binding assay to analyze the 1019 natural compounds library to identify hit compounds capable of inhibiting the PPI domain of Plk1, polo-box domain. Pilot library screening was performed with a reasonable S/B ratio value (8.72±0.79) and Z’ factors (0.66±0.05) for a HTS. These results demonstrate that our TR-FRET based Plk1-PBD binding assay can achieve the desired sensitivity and reproducibility for a HTS. Among the initial 39 hit compounds, five compounds such as haematoxylin, verbascoside, menadione, lithospermic acid and DITMD, showed greater than 80% inhibition in TR-FRET based Plk1-PBD binding assay.

Fig. 7. Chemical Structures of Hit Compounds as Plk1-PBD Inhibitors

Table 2. Significant Hits from TR-FRET-Based-Plk1-PBD Binding Assay Applied to the Chemical Library of Natural Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
<th>TR-FRET-Based-Plk1-PBD binding assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematoxylin</td>
<td></td>
<td>% inhibition at 10µM</td>
</tr>
<tr>
<td>Verbascoside</td>
<td></td>
<td>91.5</td>
</tr>
<tr>
<td>Menadione</td>
<td></td>
<td>89.2</td>
</tr>
<tr>
<td>DITMD</td>
<td></td>
<td>88.4</td>
</tr>
<tr>
<td>Lithospermic acid</td>
<td>PiK1-PBD binding inhibitor (reference)</td>
<td>82.9</td>
</tr>
</tbody>
</table>

Fig. 8. Suppression of HeLa Cell Proliferation by Hit Compounds

HeLa cells were treated with 30µM five compounds and harvested at indicated time points. Cells treated with DMSO served as the control. Values are mean±S.D. (n=4). *p<0.05, significantly different from the control. a.u.: arbitrary units.
activities such as anti-tumor action in mice,\(^{25}\) promotion of apoptosis in human colorectal cancer,\(^{26}\) induction of tumor tissue necrosis against pancreatic cancer,\(^{27}\) and cytotoxic activity on human breast cancer cells,\(^{28}\) respectively. The pharmacological information of DITMD has not yet been reported. However, its methylenedioxyisoquinolines derivatives were reported to have anti-tumor activity\(^{29}\) and patented as an anti-angiotensin A.\(^{30}\) In Hela cell proliferation assay, reported to have anti-tumor activity and patented as an antioxidant,\(^{31}\) DITMD inhibited cell proliferation more potent than haematoxylin, verbasoside, and lathispermic acid. This different potency could be explained by physico-chemical properties of the compounds such as cell permeability, solubility and stability in media. Plk1 is a critical trigger for M-phase progression and cell proliferation\(^{31,32}\) and Plk1-PBD inhibition alone is sufficient for decreasing proliferation by cell cycle arrest.\(^{33}\) Thus, these results strongly indicate that all five compounds could suppress cell proliferation by inhibition of PPI domain of Plk1, polo-box domain.

In summary, we demonstrated HTS method to screen PLK1-PBD binding inhibitors based on TR-FRET (Time-resolved Fluorescence Resonance Energy Transfer) and identified potent Plk1-PBD inhibitor inducing mitotic arrest in HeLa cells. These TR-FRET-based Plk1-PBD binding assays are considered powerful tools to screen inhibitors of PPI domain of Plk1, polo-box domain, although they have limitations of hot spot analyses for interaction domains and quantitative analyses of bound domains.

Acknowledgments This study was supported by the Bio & Medical Technology Development Program (2014-M3A9A9073788) of the National Research Foundation (NRF). The chemical library used in this study was kindly provided by Korea Chemical Bank (http://www.chembank.org/) of Korea Research Institute of Chemical Technology.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES


