해부 · 생물인류학 제 36 권 제 3 호
Anat Biol Anthropol Vol. 36, No. 3 (2023) pp. 103~110
https://doi.org/10.11637/aba.2023.36.3.103

Check for updates

Original Article

Anti-proliferative Profiling of 6,000 Representative Compounds from the Korean Chemical Bank Diversified Compound Library in De-differentiated Schwann Cells

Mohammad Ashrafuddin Khan[1], Se Yeon Lee[1,2], Youngbuhm Huh[1,2,3], Ja-Eun Kim[2,3,4], Kyo Seon Hwang[2,3,5], Ji Hyun Lee[2,3,5], Hwajin Lee[2,3,6], Yoo Lim Chun[1,7], Na Young Jeong[7], Junyang Jung[1,2,3]

[1]*Department of Anatomy and Neurobiology, College of Medicine, Kyung Hee University*
[2]*Department of Biomedical Science, Graduate School, Kyung Hee University*
[3]*Department of Precision Medicine, Graduate School, Kyung Hee University*
[4]*Department of Pharmacology, College of Medicine, Kyung Hee University*
[5]*Department of Clinical Pharmacology and Therapeutics, College of Medicine, Kyung Hee University*
[6]*Department of Biochemistry and Molecular Biology, College of Medicine, Kyung Hee University*
[7]*Department of Anatomy and Cell Biology, College of Medicine, Dong-A University*

Abstract : Early effective *in vitro* profiling using a vast collection of compounds is crucial to identify hits from large-scale drug screening to find novel therapeutic targets. The usage of molecules with distinct structural properties enhances the possibility of finding fascinating leads and compounds with various biological functions. This study aimed to find effective structures for inhibiting the proliferation of de-differentiated Schwann cells. In this study, we investigated the anti-proliferative effect of 6,000 novel compounds from the Korean Chemical Bank Diversified Compound Library (KCB-DCL) against *in vitro* de-differentiated Schwann cells at a single dose concentration of 30 μM. Their physiochemical properties, as well as hit rates, molecular targets associated with Schwann cell proliferation, such as proto-oncogene tyrosine-protein kinase Src (SRC), and docking of the selected leads, were assessed. We identified 1,420 hits (23.66%) with an impact on cell viability among 6,000 novel diversified compounds. Ten potential leads (**a-j**) were chosen from the hits and subjected to docking analysis. Compound **e** showed the best selectivity toward SRC and had a greater binding affinity (−10.7 kcal/mol) than the well-known SRC inhibitor dasatinib (−7.5 Kcal/mol). These results can provide a foundation for the early stages of drug discovery for the development of novel modulators aimed at SRC receptor proteins in Schwann cells to treat peripheral neurodegenerative diseases.

Keywords : Korean Chemical Bank Diversified Compound Library (KCB-DCL), Anti-proliferative, Schwann cells, Peripheral nerve degeneration, SRC

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning, No. 2021R1A2C1004133 (to N.Y.J.) and No. 2023R1A2C1003763 (to J.J.).
The author(s) agree to abide by the good publication practice guideline for medical journals.
The author(s) declare that there are no conflicts of interest.
Received: June 29, 2022; **Revised:** Agust 28, 2023; **Accepted:** Agust 31, 2023
Co-correspondence to: Na Young Jeong (Department of Anatomy and Cell Biology, College of Medicine, Dong-A University, Seo-gu, Busan 49201, Republic of Korea), Junyang Jung (Department of Anatomy and Neurobiology, College of Medicine, Kyung Hee University, Dongdae-mun-gu, Seoul 02447, Republic of Korea)
E-mail: jnyjjy@dau.ac.kr (N.Y. Jeong), jjung@khu.ac.kr (J. Jung)

ISSN 2671-566X (Online)

INTRODUCTION

The successful screening of very similar chemical compounds is unlikely because molecules featuring a comparable framework will possess comparable physicochemical and biological properties [1]. Thus, a diversified compound library (DCL) was developed. A DCL can be defined as a collection of chemical molecules that can be used for high-throughput screening (HTS) and other drug development processes [2,3]. These compound libraries can be utilized for HTS, high-content screening (HCS), and virtual screening (VS), which are valuable professional tools for drug discovery and groundbreaking indication research [4,5]. Screening vast numbers of compounds covering a larger chemical space evenly (diversity-lead standard) by HTS has been common practice during the early stage of a project over the last decade to identify chemicals with the potential to modulate the target of interest [6,7]. The Korean Chemical Bank (KCB) compound library is a unique library in which the design was driven by protein binding pocket information, scaffold hopping, and novel chemistry. The compounds in this library were selected by a dissimilarity search to provide a wider variety and broader chemical space coverage. All the compounds are drug-like and were screened to remove any inappropriate chemical structures, avoiding false hits. We assessed the anti-proliferative effect of the KCB novel library containing 6,000 compounds against a normal SW10 Schwann cell line to find new lead molecules inhibiting peripheral nerve degeneration (PND), which is regulated by Schwann cell proliferation.

We also looked at the physicochemical properties of the diversified novel compounds in the KCB, such as hit rates and target binding studies. Scientists need to be able to distinguish between promiscuous and harmful drugs when analyzing cell-based and phenotypic assay results. We hope to assist medicinal chemists in selecting the lead study compounds to advance drug development projects by analyzing the activity of compounds in our new library.

MATERIALS AND METHODS

1. Chemicals

All of the novel chemical compounds (6,000 compounds, Table S1) with anti-proliferative activity were obtained from the Korean Chemical Bank, South Korea (https://chembank.org/en/home-4/).

2. Cell culture

The SW10 (CRL-2766) mouse neuronal Schwann cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). SW10 cells were maintained at sub-confluence in Dulbecco Modified Eagle's Medium (DMEM, SH30243.01, Cytiva, Marlborough, MA, USA) supplemented with 1% (v/v) penicillin/streptomycin (30-022-CI, Corning, NY, USA) and 9% (v/v) fetal bovine serum (FBS, SH30919.03, Cytiva, Marlborough, MA, USA) in an incubator at 33°C under a humidified atmosphere of 5% CO_2.

3. Cell viability assay

Cell viability was assessed to determine the anti-proliferative effect of the compounds on SW10 cells by the MTS assay using the Cell Titer 96 AQueous One Solution Cell Proliferation Assay kit (Promega, G3580, Madison, WI, USA), according to the manufacturer's instructions. Briefly, a 100-μL sample of culture medium containing 5×10^3 SW10 cells was distributed in each well of 96-well plates in the presence of the compounds (30 μM) and incubated for 48 h. After incubation, 20 μL of MTS solution reagent was added to each well and reacted for 1 hour. After the reaction, optical density was measured at 492 nm using a microplate spectrophotometer to determine cell viability (51119000, Thermo Fisher Scientific, Waltham, MA, USA). Cell viability was calculated in triplicate for each drug.

4. Molecular docking

We selected ten lead compounds (**a-j**) from the overall hits from the KCB-DCL with high anti-proliferative activity and attempted to assess target binding mechanisms for all of the leads. The AutoDock Vina program [8] was used to simulate docking between a target protein and a ligand. The Open Babel tool [9] was used to convert the docking results data into Protein Data Bank (PDB) format. BIOVIA Discovery Studio Visualizer software [10] was used to visualize the data. 3D structures of the target proteins were obtained from the PDB (http://www.rcsb.org/). Molecular docking between **a-j** and SRC was performed to

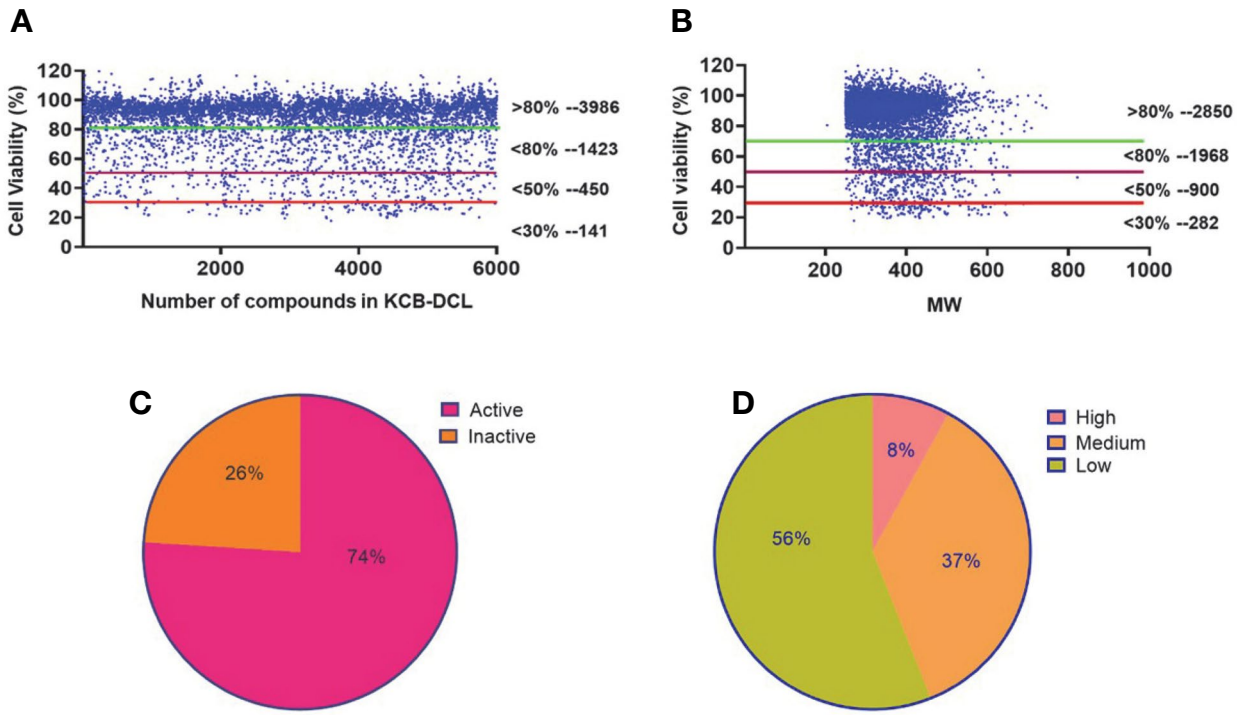

Fig. 1. Drug screening of *in vitro* Schwann cells with KCB-DCL, (A) An illustration of a correlation between the number of compounds in the Korean Chemical Bank Diversified Compound Library (KCB-DCL) and cell viability at various levels of anti-proliferative activity, (B) Molecular weight (MW) vs cell viability, the widely violated parameter in the entire KCB-DCL is MW, (C) A pie chart displaying the percentage of active and inactive compounds, (D) % of compounds in KCB-DCL with high (bright red), medium (pale yellow), and low (green) antiproliferative activity. In X-axis (A, B), ranges of % indicate those of % cell viability of the compounds and the numbers next to the ranges indicate the count number of compounds which are within the range of %.

evaluate the best lead compounds, rule out other options, and evaluate their binding affinity. We employed the Open Babel program to convert 6,000 compounds (ligands) from smile (Table S1) to PDB format after receiving them from the KCB in smile form. The data were then saved in PDBQT format for subsequent docking analysis. The docking scores were compared to those of dasatinib (DAS), a well-known SRC inhibitor, which was used as the positive control.

RESULTS

1. Selection of hits using the MTS assay

Cell viability was assessed by the MTS assay to evaluate the anti-proliferative effects of the compounds selected from the KCB-DCL on SW10 cells. A total of 6,000 compounds were tested at a single concentration of 30 μM, and the percentage (%) of viable cells was estimated as (absorbance of treated cells - absorbance of background) / (absorbance of matched controls - absorbance of background controls) × 100 (%). Treatment of the SW10 cell line with the diverse compounds showed a range of anti-proliferative effects, as shown in Figure 1A, B. We divided 6,000 compounds from three categories of MTS results (high anti-proliferative compounds had maximal responses between 8% and ≤30%, medium anti-proliferative compounds had maximal responses ≤50% and low anti-proliferative compounds had maximal responses of ≤80%, Fig. 1A, B). A total of 1,420 compounds demonstrated activity against SW10 cell lines, corresponding to a consensus hit rate of 26% for the entire KCB-DCL set (Table S1).

The KCB-DCL demonstrated a reasonable rate of anti-proliferative effects (26%), which was consistent with the toxicity of the bulk of the compounds and supported the status and triage of the compounds by counteressays (Fig. 1C). Profiling data can identify hit compounds that are specifically active against SW10 cell lines and chemotype-related activity. Among the identified hits with high

A

Physicochemical properties	% Active compounds
≤ 7HBA	98
≤ 3HBD	96
cLogP ≤ 5	91
MW ≤ 400	86

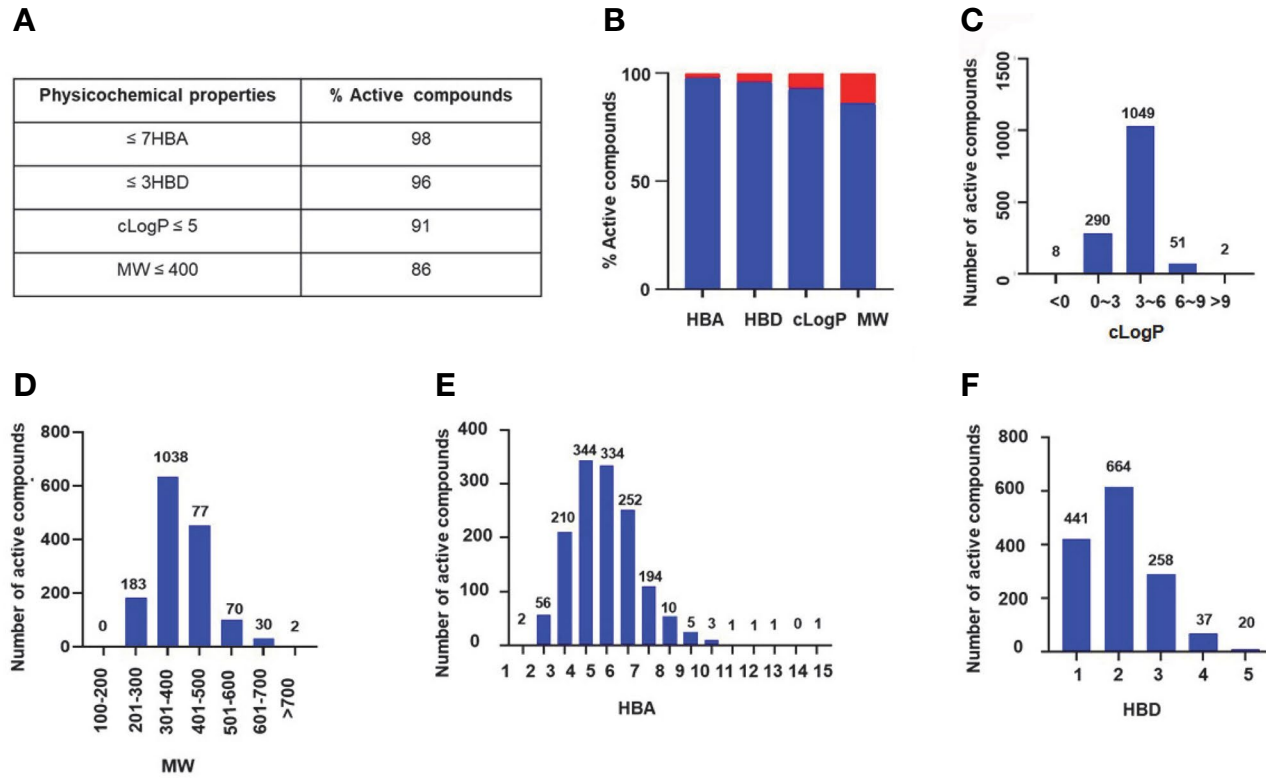

Fig. 2. Graphical analysis of the physiochemical properties of active compounds, (A) Active compounds' (hits) RoCNS% compliance with the Lipinski rule, (B) Frequency of adhere (blue) and violation (red) of the RoCNS parameters by active compounds, (C-F) Graphs showing RoCNS profile of number of active compounds vs physicochemical properties, cLogP (C), molecular weight (MW) (D), hydrogen bond acceptors (HBA) (E), and donors (HBD) (F).

anti-proliferative profiles (≤8%, Fig. 1D), ten diverse compounds with high anti-proliferative values were selected for docking analysis.

2. Physiochemical properties of the active compounds

Simple descriptors like molecular weight (MW), topological polar surface area (TPSA), CLogP, and hydrogen bond donors/acceptors (HBDs/HBAs) are all readily determined in the current framework of the KCB-DCL that was subjected to anti-proliferative evaluation. These parameters are especially important in the context of designing diversified compound libraries. In 1999, Lipinski designed a set of rules that could be used to identify drugs that present good central nervous system (CNS) penetration and gastrointestinal absorption (RoCNS) [11]. Physiochemical properties of synthetic compounds usually represent as MW, TPSA, CLogP, HBDs, and HBAs to elicit a pharmacological or therapeutic effect. To classify some characteristics of the fragments affecting cell de-differentiation, we used the parameters. In this context, we evaluated the applicability of Lipinski's rule for the CNS to our screened KCB-DCL through a percentage analysis of the physicochemical parameters. The results are shown in Figure 2.

About 88% of the KCB-DCL compounds selected for anti-proliferative evaluation complied with the RoCNS criterion, whereas 22% transgressed at least one of the RoCNS-established norms. Figure 2A shows the percentage of active compounds in table form together with each parameter that obeyed RoCNS. Contributions made by the violated parameters included 2% HBAs, 4% HBDs, 9% CLogP, and 14% by MW (Fig. 2B). The highest number (1,049; 74%) of active compounds (hits) (Fig. 2C) with CLogP values were in the range of 3~6, while two compounds had values above 9. Of the active molecules, 1,292 (91%) (Fig. 2A) adhered to the RoCNS criterion for CLogP. This parameter is closely related to bioavailability. With 14% of the active compounds deviating from

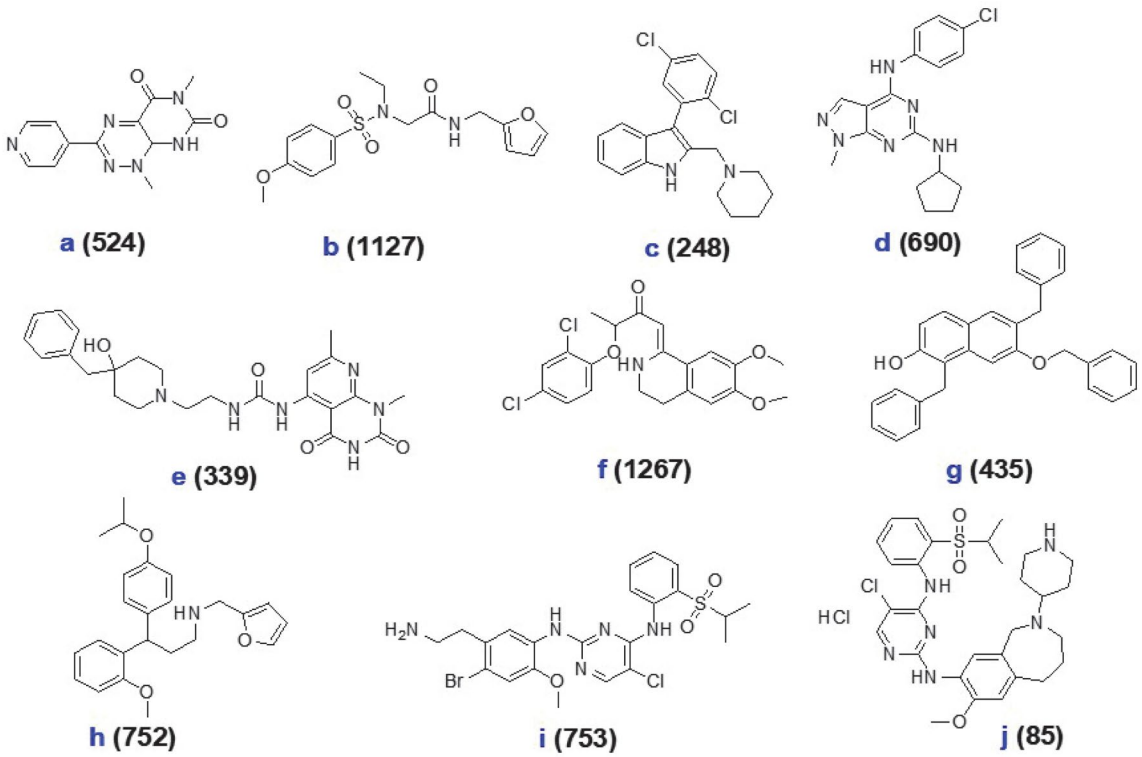

Fig. 3. Top 10 potent compounds structures of the selected leads among the hits **a-j**. The number in the brackets indicates the compound number of KCB-DCL in Table S1. The top ten hits were selected within the tenth from the top of % cell viability among 6,000 compounds.

the RoCNS norm, MW was the most frequently violated criterion. The majority of the active compounds (1,038; 73%) fell within the 300~400 MW range (Fig. 2D). Reduced absorption and lower blood-brain barrier penetration are associated with differences in MW [11]. Suitable ClogP and MW values appear to be the physicochemical characteristics that best indicate the absorption potential of passive processes [12,13]. The majority of the active compounds had HBA sites (98%) as well as HBD sites (96%) (Fig. 2E, F). Ligand binding specificity is significantly affected by these two parameters [14].

3. Active structures for anti-proliferation

As previously addressed, compounds in the KCB-DCL have the best physiochemical properties and a broad range of structural variations. The top ten compounds with potent inhibitory effects on the proliferation of SW10 cells among the hits identified in the KCB-DCL were chosen for *in silico* analysis The key structural characteristics of these selected molecules included the presence of chemical groups like disulfonyl (**b, h, j**), pyrimidine rings (**a, d**), amide urea groups (**b, e**), naphtha rings (**g**), and an indole core with piperazine and dichlorophenyl ring substitutions at the 3 and 4 positions (**c**). Compound (**f**) showed a dimethoxyisoquinoline core with dichlorophenoxy substitution. The majority of the structures were diversified but demonstrated various substituent patterns. The most effective hit compound (**a**, 17.77% cell inhibitory effect) displayed a pyrimidine ring with pyridine substituted at the fifth position. Interestingly, some of the well-known SRC inhibitor drugs have structural similarities [15]. Figure 3 shows the molecular structures of the selected potent lead compounds (**a-j**).

4. Molecular docking for SRC

Non-receptor-type tyrosine kinase SRC is required for an array of signal transduction pathways, including those involved in cell growth, migration, and differentiation. SRC was previously shown to be significantly abundant in peripheral nervous system (PNS) development, falling to lower levels during maturation [15]. The best cell growth inhibition exhibited by the selected lead compounds

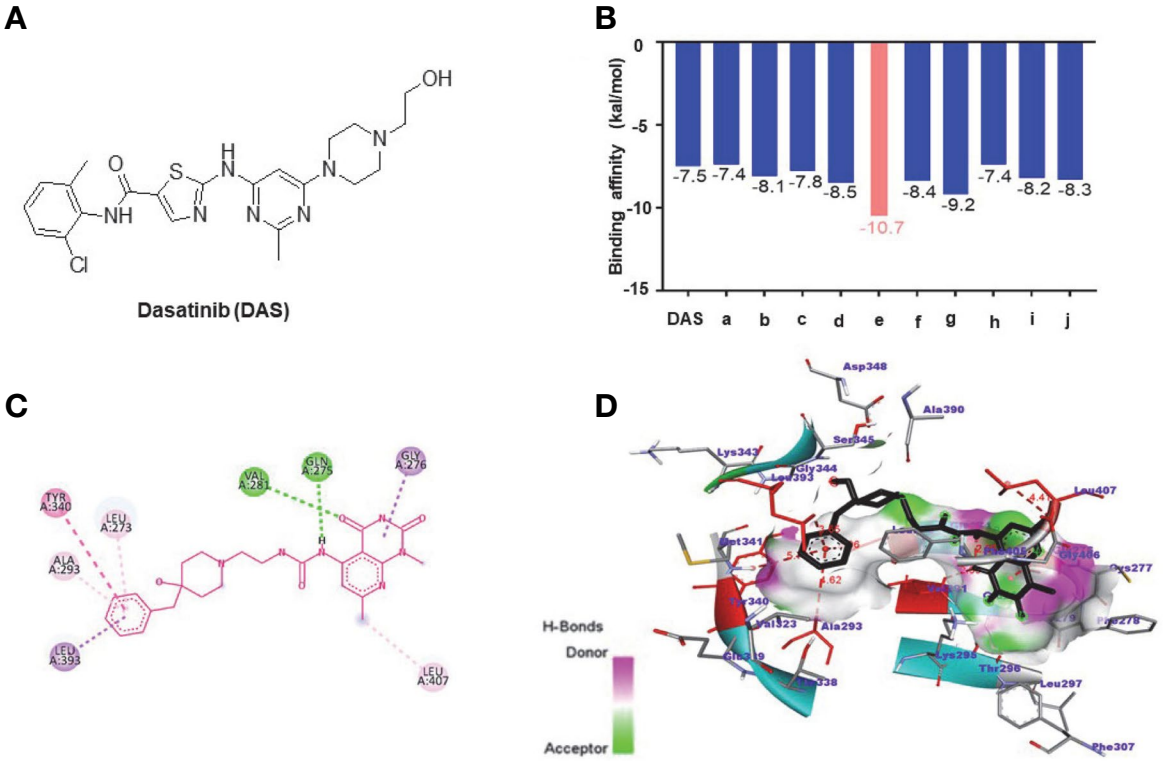

Fig. 4. Docking analysis of the top ten potent compounds that have been chosen, (A) Structure of the Dasatinib (DAS), (B) The binding affinity (kcal/mol) of compounds **a-j** with SRC, (C, D) 2D- and 3D-structure of the most lead compound **e**, (C) and (D), respectively. Conventional hydrogen bond, Val281 and Gln275; π-σ, Leu393 and Gly276; π-π, Tyr340; alkyl, Leu407; π-alkyl, Ala293 and Leu273.

invigorated us to explore the binding mode with SRC (PDB ID: 7WF5) using molecular docking techniques to explain the cause of cell inhibition activity [16]. The selected lead compounds were examined by molecular docking between **a-j** and SRC, considering the role of SRC proteins in cell cycle regulation [17] as a possible therapeutic target in Schwann cells. DAS was used as a positive control drug because this drug has the potential to inhibit the function of these proteins (Fig. 4A) [18,19]. We employed Auto Dock Vina [8] as a docking program. Figure 4B depicts the free binding energy between SRC and the ten selected lead molecules. The greatest affinity for SRC was demonstrated by lead compound **e** (−10.7 kcal/mol), while DAS (−7.5 kcal/mol) exhibited a lower affinity than compound **e**.

SRC proteins were previously reported to be essential for the proliferation, migration, and differentiation of Schwann cells [20]. The cell-inhibitory effect in Schwan cells may be caused by the inhibition of SRC. The docking results of **e** indicated possible π-π, π-σ interactions (Leu393, Tyr340, Gly276) and π-alkyl interactions (π-a) (Ala293 and Leu273) (Fig. 4C, D). Two strong hydrogen bonds with this protein were also observed in this chemical moiety, one with a hydrogen atom of the urea group (O…H-N) (with Gln275) and the other with one of the electronegative oxygen atoms of the dioxopyridol ring (O…HO) (Val281) in the chemical moiety (Fig. 4C, D). These molecular interactions hold compound e in the active socket region of the protein to inhibit further enzymatic action.

DISCUSSION

We were interested in screening the cell-inhibitory action of novel and diverse compounds found in the KCB-DCL because how they influence normal cell lines is unclear. Although this approach is particularly useful for identifying new, unrelated leads that work via comparable pathways, it does not help to elucidate the mechanisms underlying diverse compounds with similar anti-proliferative effects. However, it could assist in finding prom-

ising leads and creating new, better chemical entities. Compared to the exact projection values, the majority of Food and Drug Administration (FDA)-approved CNS drugs deal with randomness [21]. The probability of a compound reaching the site of activity should be higher for those that follow the Lipinski RoCNS and consider its values to be the limits, but does not mean that compounds that violate the rule will not be successful [22]. In view of the stated thought, the majority of compounds in the KCB-DCL adhered to Lipinski rules (88%), with the remainder being dispersed according to the degree of deviation from the value predetermined by the rule (22%).

Our primary objective was to evaluate the effect of 6,000 compounds from the KCB-DCL on cell viability *in vitro* in de-differentiated Schwann cells (SW10 cell line) to identify the hit molecules among the evaluated KCB-DCL and find potent lead molecules among them for novel modulators of PND using target binding affinity, which occurs as a result of irregular Schwan cell proliferation. Docking analysis was applied to the selected hit molecules to choose the best lead molecules that bound most favorably with cell cycle-regulating proteins like SRC in Schwann cells. This research confirmed the significance of the compounds that are active in phenotypic screening, shed light on the potential cell inhibitory activity of novel and diverse libraries, and aids in the early stages of drug discovery for the development of novel SRC protein modulators to treat peripheral neurodegenerative diseases.

PND is essential because it stimulates peripheral nerve regeneration [23]. Schwann cells regulated de-differentiation and proliferation, axon deterioration, demyelination, and macrophage recruitment related to PND [24]. During PND, hyperglycemic conditions in the body induce the malfunction of Schwann cells, and the abnormal Schwann cells lead to irreversible peripheral neurodegenerative diseases such as diabetic peripheral neuropathy. However, there are very few therapies for this medical condition [25]. Thus, our evaluated KCB-DCL represents a group of new, disease-indifferent small compounds, including molecules with drug-like properties, that are intended for the discovery of novel chemical modulators for PND treatment by regulating the proliferation of Schwan cells.

Especially, in drug screening process, cancer cell-line is usually used because of its easy handling and short outcome duration. Because inhibition of cell proliferation by drugs of interest is also easily quantified, in this study, we employed SW10 Schwannoma cells for screening 6,000 representative compounds and obtained several effective hits in short time.

In conclusion, the KCB-DCL was employed for evaluating a variety of novel compounds *in vitro* and *in silico*. Data collected from normal cell lines could be utilized to find novel compounds that are specifically active against proliferating cell lines depending on the target biology. The moderate inhibition of Schwann cell proliferation accompanied by chemotype-related activity and docking studies with target binding analysis were highlighted as the main result of the anti-proliferative profiling of 6,000 KCB-DCL novel compounds. SRC activity inhibition may be the underlying cause of the inhibitory effects on Schwann cells. In comparison to the well-known inhibitor DAS, the potent lead compound **e** demonstrated stronger SRC interaction and higher binding energy release. This anti-proliferative screening approach, which begins with the profiling of physiochemical characteristics and target binding analysis, was demonstrated to be a successful way to identify novel chemical molecules at the initial stages of drug discovery. The findings will open the door to creating new lead chemical modulators in the early stage of drug discovery that may be used to detect the best therapeutic molecules for conditions like peripheral neurodegenerative diseases.

CONFLICTS OF INTEREST

The authors have declared that no competing interest exists.

ACKNOWLEDGMENTS

We thank Won-Joon Eom (Department of Anatomy and Neurobiology, Kyung Hee University, Seoul, Korea) for his valuable discussion.

REFERENCES

1. Johnson MA, Maggiora GM. Concepts and applications of molecular similarity. Wiley. 1990.
2. Huggins DJ, Venkitaraman AR, Spring DR. Rational meth-

ods for the selection of diverse screening compounds. ACS Chem Biol. 2011;6:208-17.
3. Schreiber SL, Kotz JD, Li M, Aubé J, Austin CP, Reed JC, et al. Advancing biological understanding and therapeutics discovery with small-molecule probes. Cell. 2015;161:1252-65.
4. Moustakim M, Felce SL, Zaarour N, Farnie G, McCann FE, Brennan PE. Targetidentification using chemical probes. Meth Enzymol. 2018;610. https://doi.org/10.1016/bs.mie.2018.09.013.
5. Renner S, Popov M, Schuffenhauer A, Roth H-J, Breitenstein W, Marzinzik A, et al. Recent trends and observations in the design of high-quality screening collections. Future Med Chem. 2011;3:751-66.
6. Roy A, McDonald PR, Sittampalam S, Chaguturu R. Open access high throughput drug discovery in the public domain: A mount everest in the making. Curr Pharm Biotechnol. 2010;11:764-78.
7. Nguyen HP, Koutsoukas A, Mohd Fauzi F, Drakakis G, Maciejewski M, Glen RC, et al. Diversity selection of compounds based on 'protein affinity fingerprints' improves sampling of bioactive chemical space. Chem Biol Drug Des. 2013;82:252-66.
8. Trott O, Olson AJ. Autodock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31:455-61.
9. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open babel: An open chemical toolbox. J Cheminform. 2011;3:1-14.
10. Silva NS, Gonçalves LK, Duarte JL, Silva JS, Santos CF, Braga FS, et al. Computational analysis of physicochemical, pharmacokinetic and toxicological properties of deoxyhypusine synthase inhibitors with antimalarial activity. Comput Mol Biosci. 2014;4:47.
11. Lipinski CA, Lombardo F, Dominy BW, Feeney PG. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2012;64:4-17.
12. Wager TT, Hou X, Verhoest PR, Villalobos A. Moving beyond rules: The development of a central nervous system multiparameter optimization (CNS MPO) approach to enable alignment of druglike properties. ACS Chem Neurosci. 2010;1:435-49.
13. Shameer K, Readhead B, Dudley JT, Computational and experimental advances in drug repositioning for accelerated therapeutic stratification. Curr Top Med Chem. 2015;15:5-20.
14. Gribkoff VK, Kaczmarek LK. The need for new approaches in cns drug discovery: Why drugs have failed, and what can be done to improve outcomes. Neuropharmacology. 2017;120:11-9.
15. Creedon H, Brunton VG. Src kinase inhibitors: Promising cancer therapeutics? Crit Rev Oncog. 2012;17. https://doi.org/10.1615/CritRevOncog.v17.i2.20.
16. Meng X-Y, Zhang H-X, Mezei M, Cui M. Molecular docking: A powerful approach for structure-based drug discovery. Curr Comput-Aided Drug Des. 2011;7:146-57.
17. Hossain S, Fragoso G, Mushynski WE, Almazan G. Regulation of peripheral myelination by src-like kinases. Exp Neurol. 2010;226:47-57.
18. Araujo J, Logothetis C. Dasatinib: A potent src inhibitor in clinical development for the treatment of solid tumors. Cancer Treat Rev. 2010;36:492-500.
19. Id Boufker H, Lagneaux L, Najar M, Piccart M, Ghanem G, Body J-J, et al. The src inhibitor dasatinib accelerates the differentiation of human bone marrow-derived mesenchymal stromal cells into osteoblasts. BMC Cancer. 2010;10:1-13.
20. Grove M, Komiyama NH, Nave K-A, Grant SG, Sherman DL, Brophy PG. Fak is required for axonal sorting by Schwann cells. J Cell Biol. 2007;176:277-82.
21. Snell TW, Johnston RK, Srinivasan B, Zhou H, Gao M, Skolnick J. Repurposing FDA-approved drugs for anti-aging therapies. Biogerontology. 2016;17:907-20.
22. Navia MA, Chaturvedi PR, Design principles for orally bioavailable drugs. Drug Discov Today. 1996;1:179-89.
23. Gandhi M, Fargo E, Prasad-Reddy L, Mahoney KM, Isaacs D. Diabetes: How to manage diabetic peripheral neuropathy. Drugs Context. 2022;11. https://doi.org/10.7573/dic.2021-10-2.
24. Grove M, Brophy PJ. Fak is required for schwann cell spreading on immature basal lamina to coordinate the radial sorting of peripheral axons with myelination. J Neurosci. 2014;34:13422-34.
25. Cooke PS, Nanjappa MK, Yang Z, Wang KK. Therapeutic effects of progesterone and its metabolites in traumatic brain injury may involve non-classical signaling mechanisms. Front Neurosci. 2013;7:108.