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Discovery of TRD-93 as a novel DRAK2 inhibitor

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Abstract

Death-associated protein kinase-related apoptosis-inducing protein kinase 2 (DRAK2) has become a promising target for drug development. In search of novel and selective DRAK2 inhibitor motif, in vitro screen kinase assay was established performed using in-house chemical libraries. After through hit triage procedure, N^2-(3,5-dichlorophenyl)-5-fluoro-N^4-methylpyrimidine-2,4-diamine (**1**) was selected as initial hit with structural novelty and drug-likeness. During hit validation, structure–activity relationship of 1 was thoroughly disclosed and **TRD-93** was finally validated as hit for DRAK2 inhibition. **TRD-93** is small (mw = 290) but selectively potent to DRAK2 (IC_{50} = 0.16 μM) over other kinases including DAPK family kinases. Molecular binding model study of **TRD-93** to DRAK2 is also discussed.

KEYWORDS
2-aminophenylpyrimidine, DAPK1, DAPK3, DRAK1, DRAK2, kinase

Death-associated protein kinase-related apoptosis-inducing protein kinase 2 (DRAK2), also known as serine/threonine kinase 17B (STK17B), belongs to the family of death-associated protein kinases (DAPKs) along with DAPK1, DAPK2, DAPK3, and DRAK1.[1] DRAK2 expression is highly enriched in B and T cells for possible immunological responses.[2] DRAK2-deficient knockout mice develop resistance to autoimmune diseases such as experimental autoimmune encephalomyelitis and type I diabetes.[3,4] In addition to this, there is a report that DRAK2 is closely involved in tumorigenesis.[5] Knockdown of *DRAK2* gene resulted in significant tumor growth inhibition in a mouse xenograft animal model. Yang et al.[6] reported that DRAK2 acts as a negative regulator of transforming growth factor-β (TGF-β)-mediated signal transduction pathway: aberrant expression of DRAK2 accelerates the tumorigenesis process by constraining the tumor suppressor activity of TGF-β. Therefore, DRAK2 has become a promising target for drug development. However, only a small number of DRAK2 inhibitors have been reported so far but many of them lack drug-likeness (Figure 1).[7–10]

As an effort to search for novel and selective DRAK2 inhibitor motif, in vitro screen kinase assay was established using the purified recombinant DRAK2 protein and performed using 11 564 compounds from in-house library as depicted in Figure 2. Forty-one compounds were selected as initial hits toward DRAK2. Many of them were classified as *frequent hits* including crizotinib, sunitinib or indirubin scaffolds which are known nonselective kinase inhibitors.[11] One of the singleton hits with structural novelty and drug-likeness was N^2-(3,5-dichlorophenyl)-5-fluoro-N^4-methylpyrimidine-2,4-diamine (**1**) with 0.91 μM inhibitory activity to DRAK2 in biochemical assay. The 2,4-diaminophenylpyrimidine is a well-known kinase inhibitor scaffold having bidentate hinge binding mode.[12] Unlike typical 2,4-diaminophenylpyrimidine, **1** does not have 4-aminophenyl moiety which is known essential for its kinase activities. In particular, **1** is a very small pyrimidine (mw = 287) and has room to derivatize for its potency and properties improvement. Thus, **1** was chosen as an initial hit and hit validation study was executed.

Sangjun Park, Seungmin Kye, and Myoung Eun Jung contributed equally to this study.

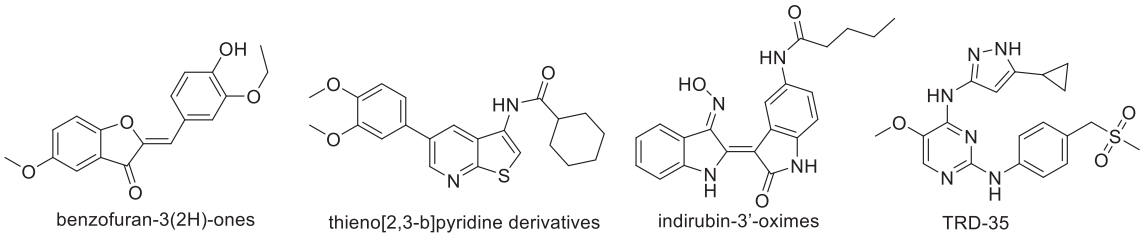
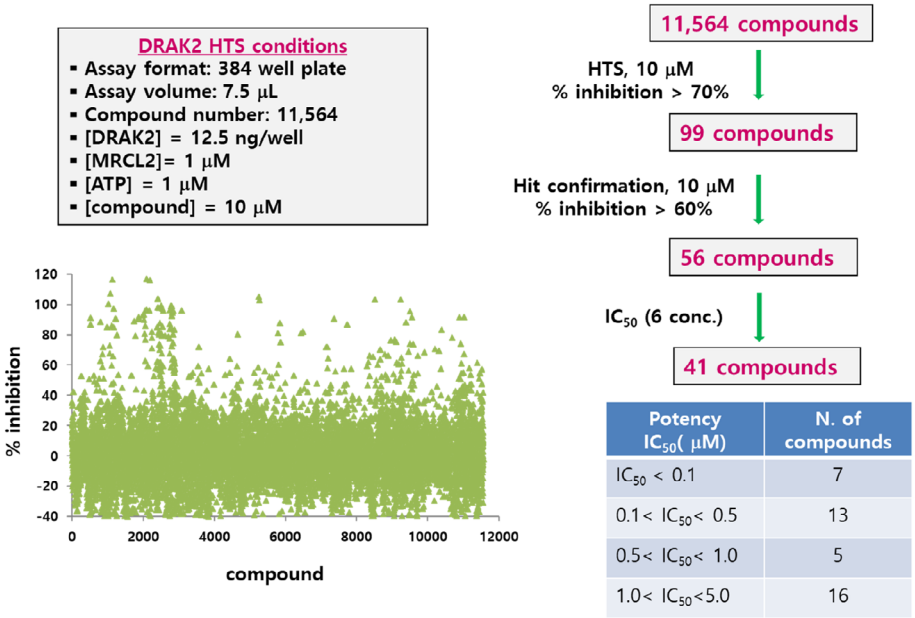
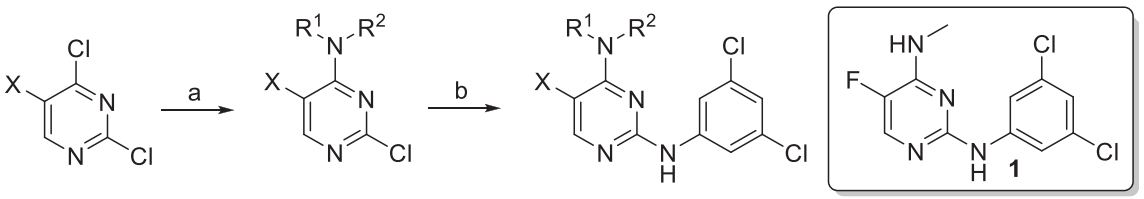


FIGURE 1 Reported DRAK2 inhibitors.



Potency IC_{50}(μM)	N. of compounds
IC_{50} < 0.1	7
0.1< IC_{50}< 0.5	13
0.5< IC_{50}< 1.0	5
1.0< IC_{50}<5.0	16

FIGURE 2 DRAK2 HTS assay protocol and results.

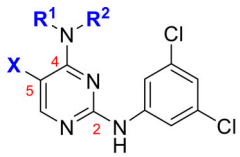


SCHEME 1 Reagents and conditions: (a) R^1R^2NH, isopropanol, 0°C to room temperature, 5 h (40%–80%); (b) 3,5-dichloroaniline, 0.08 N HCl in 2-ethoxyethanol, 100°C, 16 h (33%–73%).

Representative synthetic chemistry is described in Scheme 1 for preparation of **1** and its derivatives. 2,4-Dichloro-5-fluoropyrimidine in isopropanol at 0°C was added methylamine to give 2-chloro-5-fluoro-*N*-methylpyrimidin-4-amine. 2-Chloro-5-fluoro-*N*-methylpyrimidin-4-amine and 3,5-dichloroaniline were coupled in the presence of catalytic amount of hydrogen chloride in 2-ethoxyethanol at 100°C to provide **1** [N^2-(3,5-dichlorophenyl)-5-fluoro-N^4-methylpyrimidine-2,4-diamine] in 56% yield. All the structural analogs were also prepared throughout the same chemistry route without difficulty.

As a part of hit validation processes, structure–activity relationship (SAR) study of **1** was attempted. To survey SAR of **1**, we varied the C5 position while others remain unchanged. As shown in Table 1, rather than **1** which has fluorine at C5, analogs with chlorine (**2**), trifluoromethyl (**3**), or methyl (**4**) were more potent, and derivatives with methoxy (**5**) and hydrogen (**6**) were less potent than **1** in this study. Since chlorine is a preferred substituent at the pyrimidine C5 as kinase inhibitor, we further investigated SAR on C4-amino substituents based on **2**.[12] **TRD-93** (NH_2 on C4) is most potent in this series. Compound **7** bearing an ethylamino at the C4 is less potent and compounds having bigger substituents than ethyl (**8–10**) are all inactive in our assay. It is also noteworthy that dimethylamino analog (**11**) is inactive to DRAK2. Finally, we resurveyed

TABLE 1 DRAK2 biochemical activities of **1** and its analogs.



Entry	X	R^1	R^2	DRAK2 IC_{50} (μM)
1	F	H	Me	0.91
2	Cl	H	Me	0.58
3	CF_3	H	Me	0.22
4	CH_3	H	Me	0.76
5	CH_3O	H	Me	5.3
6	H	H	Me	8
TRD-93	**Cl**	**H**	**H**	**0.16**
7	Cl	H	Et	0.61
8	Cl	H	*i*-Pr	>10
9	Cl	H	*t*-Bu	>10
10	Cl	H	Ph	>10
11	Cl	Me	Me	>10
12	CF_3	H	H	0.12
13	F	H	H	0.87
14	CH_3	H	H	1.6
15	CH_3O	H	H	9.6
16	H	H	H	>10

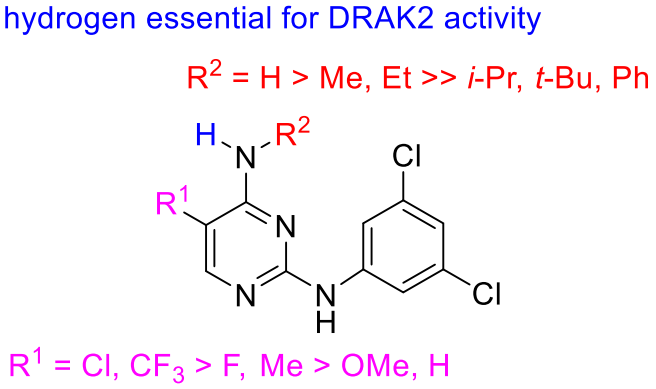


FIGURE 3 Structure–activity relationship summary of **TRD-93** and its analogs to DRAK2.

TABLE 2 Biochemical activities of **TRD-93** to DAPK kinases.

Entry	DRAK2 IC_{50} (μM)	DRAK1 IC_{50} (μM)	DAPK1 IC_{50} (μM)	DAPK3 IC_{50} (μM)
TRD-93	0.16	1.4	>10	>10

the C5 position with free amino at C4 position. In accordance to aforementioned result (**1–6**), trifluoromethyl (**12**) was equipotent to **TRD-93** and fluorine, methyl, methoxy, and hydrogen analogs (**13–16**) were less potent to inactive than **TRD-93** in this series. Throughout this hit validation study, we confirmed **TRD-93** as a validated hit for DRAK2 inhibition and could make below SAR summary described in Figure 3.

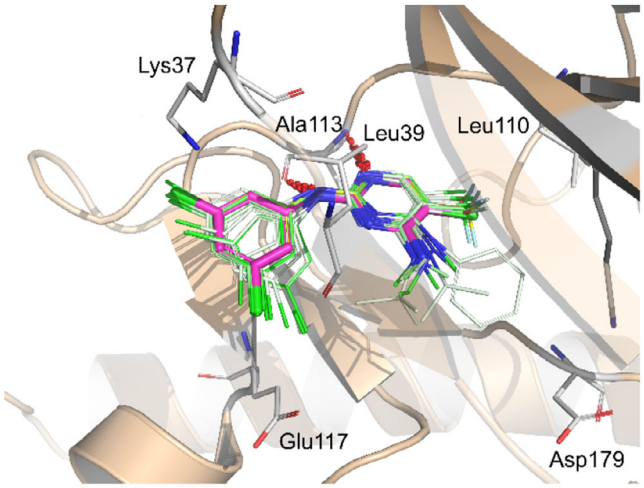


FIGURE 4 Predicted docking pose of ligands in the ligand binding site of DRAK2 (3LM0). Color of Ligand carbon atoms are assigned from green to white with respect to activity respectively. **TRD-93** is depicted in thick magenta sticks.

For selectivity measurement, **TRD-93** is further evaluated over other DAPK family kinases (DRAK1, DAPK1, and DAPK3) as shown in Table 2. Gratifyingly, **TRD-93** retains selectivity over DAPK kinases including DRAK1, DAPK1, and DAPK3 (IC_{50} = 1.4, >10, and >10 μM, respectively).

Kinase profiling assay with **TRD-93** was performed for other kinase selectivity evaluation. **TRD-93** has little to no activities over other kinases profiled including ABL1, EGFR, IGF-1R and INSR at 1 μM kinase profiling assay (97 kinase panel assay done by Eurofins) as summarized in Table S1. Throughout these selectivity studies, **TRD-93** was evaluated as a truly DRAK2 selective inhibitor.

We try to explain DRAK2 mode of inhibition by **TRD-93** using a molecular modeling study. Molecular modeling, including molecular docking, molecular dynamics simulation, and MM-GBSA calculation, was carried out as previously described (figure in Supporting Information Material S1).[13] The crystal structure of DRAK2 (PDB code: 3LM0) was utilized as the receptor structure in this study. The activities of the compounds are mainly ascribed to the strong hydrogen bonds and hydrophobic interactions (Figure 4). The common amino-pyrimidine scaffold makes bidentate hydrogen bonds with main-chain N and O of Ala113 in hinge region. The compounds are also stabilized in the hydrophobic pocket composed of Leu165, Ala60, Tyr112, Leu39, and Val47. Ionic interaction of the dichlorophenyl group with Lys37 and Glu117, and water bridged interactions with Asp179, Glu117 also contribute to the stability of ligand binding. Lack of hydrophobic interaction with gatekeeper Leu110 is detrimental in ligand binding, which is indicated by the loss of experimental activities in ligands with –H (**6**, **16**) and $–OCH_3$ (**5**, **15**) side chains. In addition, unsubstituted

amines in compound **12**, **13**, **14**, **TRD-93** make direct or water-mediated hydrogen bonding with Leu39 contributing to the affinity.

In summary, in search of novel and selective DRAK2 inhibitor motif, in vitro screen kinase assay was established and performed using in-house chemical libraries. After the hit triage procedure, N^2-(3,5-dichlorophenyl)-5-fluoro-N^4-methylpyrimidine-2,4-diamine (**1**) was selected as initial hit with structural novelty and drug-likeness. During hit validation, SAR of **1** was thoroughly disclosed and **TRD-93** was finally validated as a hit for DRAK2 inhibition. **TRD-93** is small (mw = 290) but selectively potent to DRAK2 (IC_{50} = 0.16 μM) over other kinases including DAPK family kinases. Molecular binding model study of **TRD-93** to DRAK2 was discussed, and DAPK kinase family selectivity was tested. With favorable DRAK2 potency and selectivity profile, further lead optimization of **TRD-93** including 2-aminophenyl derivatization is currently underway and will be reported later.

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REFERENCES

[1] H. Sanja, T. Kawai, S. Akira, *J. Biol. Chem.* **1998**, *273*, 29066.
[2] M. A. McGargill, B. G. Wen, C. M. Walsh, S. M. Hendrick, *Immunity* **2004**, *21*, 781.
[3] M. A. McGargill, C. Choy, B. G. Wen, S. M. Hedrick, *J. Immunol.* **2008**, *181*, 7593.
[4] S. J. Ramos, J. B. Hernandez, M. Gatzka, C. M. Walsh, *J. Immunol.* **2008**, *181*, 7606.
[5] D. Kögel, J. H. M. Prehn, K. H. Scheidtmann, *BioEssays* **2001**, *23*, 352.
[6] K. M. Yang, W. Kim, E. Bae, J. Gim, B. M. Weist, Y. Jung, J. S. Hyun, J. B. Hernandez, S. H. Leem, T. Park, J. Jeong, C. M. Walsh, S. J. Kim, *Cell Rep.* **2012**, *2*, 1286.
[7] S. Wang, L. Xu, Y. T. Lu, Y. F. Liu, B. Han, T. Liu, J. Tang, J. Li, J. Wu, J. Y. Li, L. F. Yu, F. Yang, *Eur. J. Med. Chem.* **2017**, *130*, 195.
[8] L. J. Gao, S. Kovackova, M. Sála, A. T. Ramadori, S. De Jonghe, P. Herdewijn, *J. Med. Chem.* **2014**, *57*, 7624.
[9] M. E. Jung, B. J. Byun, H. M. Kim, J. Y. Lee, J. H. Park, N. Lee, Y. H. Son, S. U. Choi, K. M. Yang, S. J. Kim, K. Lee, Y. C. Kim, G. Choi, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2719.
[10] I. Ali, S. Park, M. E. Jung, N. Lee, M. Bibi, C. H. Chae, K.-M. Yang, S.-J. Kim, G. Choi, K. Lee, *Bull. Korean Chem. Soc.* **2020**, *41*, 567.
[11] J. B. Baell, J. W. M. Nissink, *ACS Chem. Biol.* **2018**, *13*, 36.
[12] H. Choe, B. U. Jeon, M. E. Jung, M.-K. Jeon, I. Shin, B. C. Cho, G. Choi, C. H. Chae, K. Lee, *Bull. Korean Chem. Soc.* **2017**, *38*, 1353.
[13] Y. Lee, J. Jang, M. Bibi, K. B. Duggirala, S. H. Ji, J. H. Lee, S. Ahn, J. S. Song, C. H. Chae, S. H. Kim, K. Lee, *Bull. Korean Chem. Soc.* **2021**, *42*, 872.

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