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Development and structure-activity relationship study of SHP2 inhibitor containing 3,4,6-trihydroxy-5-oxo-5H-benzo[7]annulene

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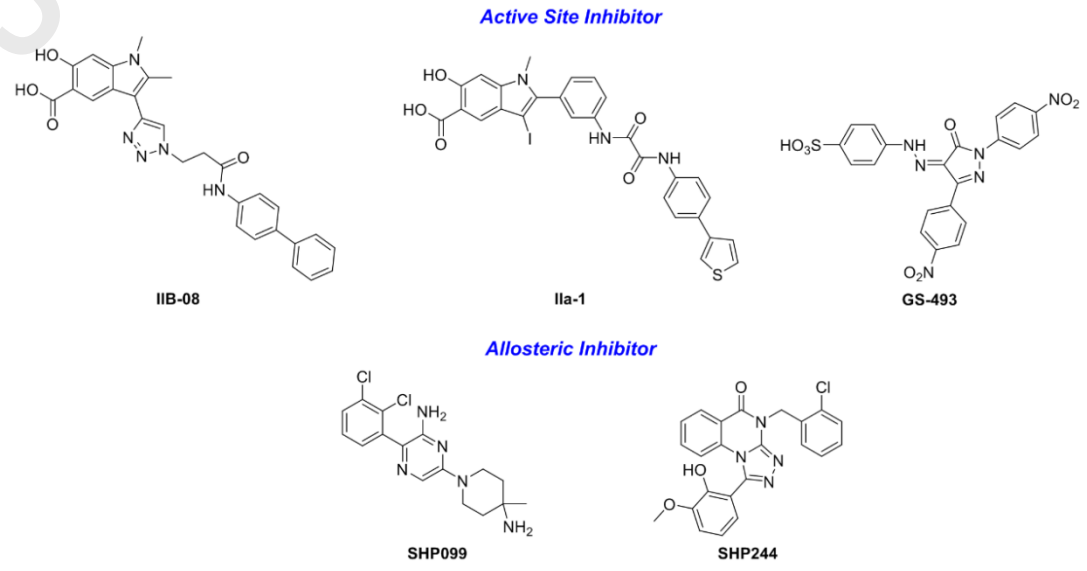
ABSTRACT

SHP2, a non-receptor protein tyrosine phosphatase encoded by PTPN11 gene, plays an important role in the cell growth and proliferation. Activating mutations of SHP2 have been reported as a cause of various human diseases such as solid tumors, leukemia, and Noonan syndrome. The discovery of SHP2 inhibitor can be a potent candidate for the treatment of cancers and SHP2 related human diseases. Several reports on a small molecule targeting SHP2 have published, however, there are limitations on discovery of SHP2 phosphatase inhibitors due to the polar catalytic site environment. Allosteric inhibitor can be an alternative to catalytic site inhibitors. 3,4,6-trihydroxy-5-oxo-5H-benzo[7]annulene **1** was obtained as an initial hit with a 0.097 μM of IC_{50} from high-throughput screening (HTS) study. After the structure-activity relationship (SAR) study, compound **1** still showed the most potent activity against SHP2. Moreover, **1** and **2j** exerted good potency against SHP2 expressing 2D and 3D MDA-MB468.

SHP2 is a PTPN11 gene encoded non-receptor protein tyrosine phosphatase,[1] and regulates cell survival and proliferation through activation of the RAS-ERK.[2,3,4,5,6] It has been suggested that SHP2 interacts with growth factor receptor-bound protein 2 (GRB2) associated binding protein 1 (GAB1), which participates in signal transducer and STAT1 regulation.[7]



SHP tase, epidermal growth factor receptor 2 (HER2) to control tumor development. Activating mutations of SHP2 have been associated in various human diseases, such as several types of leukemia,[8] solid tumors, such as lung, colon, and prostate cancers,[9,10,11] and Noonan syndrome.[12]

Figure 1. Examples of small molecule allosteric- and active-site SHP2 inhibitors

Moreover SHP2 has been reported to bind to immune-inhibitory receptors and participate in the T cell programed cell death/checkpoint pathway (PD-L1/PD-1).[13] SHP2 is known to engage with PD-1, contributing to T cell exhaustion.[14] Anti-PD-1 treatment together with SHP2 deficient T cells shown to be beneficial in treating cancer in vivo.

SHP2 consists of two consecutive phosphotyrosine binding domains (N-SH2 and C-SH2), one catalytic PTP domain, and C-terminal tail. SHP2 stays as an auto-inhibited conformation by intramolecular interactions of N-SH2 domain and PTP domain. However this auto-inhibition is disrupted by binding of bisphosphotyrosyl proteins to SH2 domains, and SHP2 becomes active conformation.[15,16,17]

Several research groups have reported small molecules which are active-site SHP2 inhibitors binding to catalytic PTP domain directly, however, these inhibitors show low potency and poor pharmaceutical properties. Due to the polar nature of the catalytic site of PTPs, the discovery of an active-site targeting small molecule have been challenged.[18,19,20,21] Allosteric inhibition may be a good alternative to overcome the limitations of the PTP catalytic inhibitors. Recently several research groups reported SHP2 allosteric inhibitors,[22,23] SHP099[24,25,26], SHP244,[27] and RMC-4550.[28] These studies show promising development of allosteric PTP inhibitors targeting SHP2 which are potent and orally bioavailable. However, these SHP2 inhibitor candidates show relatively weak anti-cancer potency against cancer cells compare to other potent reagents. This motivates us to search for a more potent SHP2 inhibitor with better anticancer efficacy.

Herein we report the discovery of a SHP2 inhibitor through high-throughput screening and structure-activity relationship study. Moreover we further tested efficacy as a SHP2 inhibitor with selected compounds.

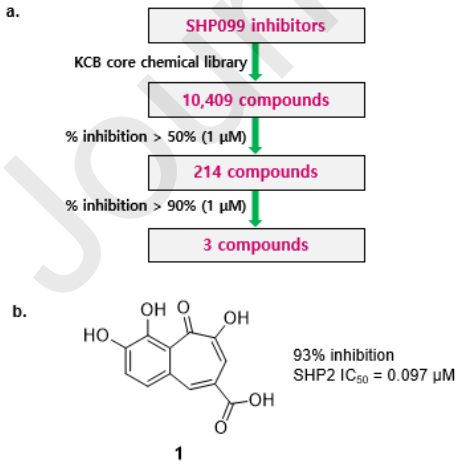
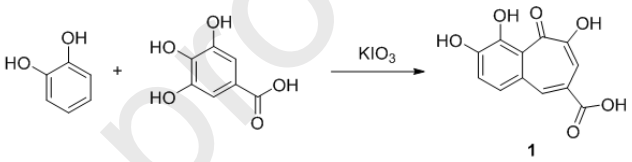


Figure 2. (a) High-throughput screening (HTS) of chemical compounds from Korea Chemical Bank. (b) Structure and biochemical activity of compound **1** from HTS.

High-throughput screening (HTS) was performed to search of a potent SHP2 inhibitor. First we screened 10,409 compounds clinically active compounds and natural compounds. 214 chemicals showed more than 50% inhibition of SHP2 at concentration of 1 μM. After further SHP2 assay screening, three compounds had reliable inhibition activities repeatedly with greater than 90% inhibition at concentration of 1 μM. Among them compound **1** with 3,4,6-trihydroxy-5-oxo-5H-benzo[7]annulene as a core structure showed the most potent SHP2 inhibition with a 0.097 μM of IC_{50} value (Figure 2). The other two compounds are under investigation, so we will not discuss these compounds in this paper including chemical structures.

To confirm the activity, we planned to synthesize **1**. Representative synthetic route is outlined in Scheme 1. Compound **1** is successfully prepared from the corresponding catechol and trihydroxybenzoic acid in presence of KIO_3. The inhibition activity of **1** against SHP2 was tested under the same biochemical assay system, and IC_{50} of 0.097 μM was repeatedly obtained.



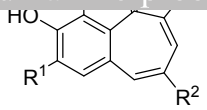
Scheme 1. Synthetic route for the preparation of **1**.

As structure-activity relationship study, we designed and synthesized various 3,4,6-trihydroxy-5-oxo-5H-benzo[7]annulene derivatives. Compounds **2a–2n** with a variety of substituents on rings were prepared through the same or modified synthetic pathway using corresponding starting materials (Scheme 1 and see supporting information) and evaluated SHP2 inhibition (Table 1).

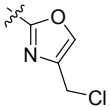
First, carboxylic acid was masked with various alkyl groups, such as a methyl, ethyl or isopropyl, to transfer to esters (**2a-2c,** entries 2-4). Methyl ester **2a** gave 87% inhibition with IC_{50} of 0.161 μM, but other alkyl esters, **2b** and **2c**, did not show good inhibition values. Amide **2d** was also tested, but it only showed 13% inhibition of SHP2. Instead of carbonyl moieties, compounds with a nitrile or an oxazole were also prepared and evaluated, however, they were a lot less potent than **1** (**2e** and **2g**, entries 6 and 8). If carboxylic acid was removed, inhibition of SHP2 was not detected at all (**2f**, entry 7). To explore the scope further, compounds with an additional substituent, such as OMe or F, on a left-side ring were synthesized (entries 9-15). The derivatives with a methoxy group showed almost no inhibition activities against SHP2 (**2h** and **2i**, entries 9 and 10). Compound **2j** which has a fluorine on R^1 position and a carboxylic acid on R^2 positon gave a comparable SHP2 activity, a 90% SHP2 inhibition and 0.277 μM of IC_{50}, to compound **1** (**2j**, entry 11). All the other analogues with a fluorine, however, gave lower inhibition activities (**2k-2n**, entries 12-15).

To examine the role of alcohols as hydrogen bonding interactions, all hydroxyl groups of **1** and **2a** were protected with methyl groups to generate methylated compounds, **3a** and **3b**, respectively (Table 2). Unfortunately SHP2 inhibitions were lost under the same biochemical assay conditions. After the structure-activity relationship study, the parent compound **1** from high-throughput screening was still the best among other small molecules that we prepared. Previously reported

allo ing nitrogen containing heteroaromatic rings with an amine or an alcohol in their structures and these make them possible for hydrogen bonds. Interestingly, compound **1** does not have any functional groups including three hydroxy groups, one ketone, and one carboxylic acid participate in hydrogen bonding interactions.



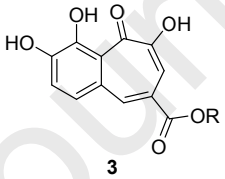
1, 2a-2n

entry	compound	R^1	R^2	Inhibition (%)	SHP2 IC_{50} (μM)	entry	compound	R^1	R^2	Inhibition (%)	SHP2 IC_{50} (μM)
1	**1**	H	CO_2H	93	0.097	9	**2h**	OMe	$CONH_2$	0	ND
2	**2a**	H	CO_2Me	87	0.161	10	**2i**	OMe	CO_2Me	6	8.658
3	**2b**	H	CO_2Et	50	0.985	11	**2j**	F	CO_2H	90	0.277
4	**2c**	H	$CO_2{}^i Pr$	2	4.870	12	**2k**	F	CO_2Me	12	7.552
5	**2d**	H	$CONH_2$	13	4.110	13	**2l**	F	CO_2Et	19	6.544
6	**2e**	H	CN	40	1.230	14	**2m**	F	$CONH_2$	52	0.949
7	**2f**	H	H	0	ND	15	**2n**	F	$CO_2{}^i Pr$	0.47	7.743
8	**2g**	H		29	1.573						

ND = not determined

Table 1. Structure activity relationship study of SHP2 inhibition activities

Three compounds with high potencies against SHP2, **1**, **2a**, and **2j**, were selected for further biological activity study, and a reported allosteric inhibitor, SHP099, was purchased and used as a reference compound for comparisons. MDA-MB-468 viability after treating SHP2 inhibitors were determined (Figure 3a), and IC_{50} of 5.3, 4.6, and 71 μM were obtained, respectively. The data suggest that potency against SHP2 correlates with viability of SHP2 expressed cancer cells. Compounds **1** and **2a** exhibited 5.6 and 4.2 fold lower IC_{50} values compared to SHP099 (29.9 μM of IC_{50}) and these results suggested **1** and **2a** were superior SHP2 expressed cancer cell killing ability. Knockdown of SHP2 with SHP2 siRNA greatly decreases proliferation inhibition of compounds, **1**, **2a**, and **2j**, showing 19.3, 3.0 and 16.3 fold increased in MDA-MB-468 viability in the presence of each compound with 100 μM respectively (Figure 3b, 3c and 3d). This result suggested that **1**, **2a** and **2j** exerted potency against cancer cells through inhibition of SHP2.



3

entry	compound	R	Inhibition (%)	SHP2 IC_{50} (μM)
1	**3a**	H	0	ND
2	**3b**	Me	0	ND

ND = not determined

Table 2. Structure activity relationship study of SHP2 inhibition activities.

To test SHP2 inhibitors on 3D culture, MDA-MB-468 spheroids were fabricated. 20 μM of **1**, **2a**, and **2j,** showed 35.7, 27.5 and 37.2% decrease in 3D MDA-MB-468 viability (Figure 4). IC_{50} values for SHP2 inhibitor SHP099, **1**, **2a** and **2j** were 49.6, 35.8, 62.9 and 39.2 μM respectively. Although exerting decreased anticancer potency (on average 43.7%), compounds **1** and **2j** still showed higher anticancer potency against SHP2 expressing 3D cancer spheroid than SHSP099. Taken together, these data suggest that compounds **1** and **2j** showed stronger potency than known SHP2 inhibitor SHP099 in SHP2 expressing 2D and 3D cancer cells.

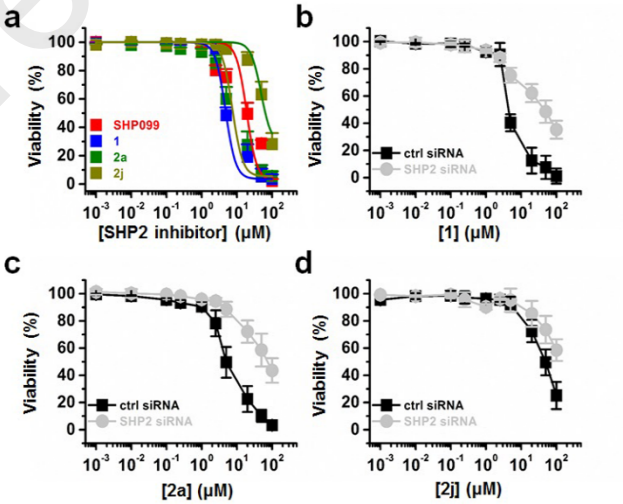
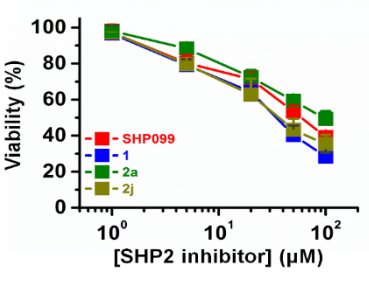


Figure 3. MDA-MB-468 viability (a) in the presence of SHP2 inhibitors (b) with control and SHP2 siRNA with compound **1** (c) compound **2a** (d) compound **2j**.

Figure 4. MDA-MB-468 3D spheroid viability in the presence of SHP2 inhibitors.

We conducted enzyme kinetic study of compound **1** and data is suggesting that compound **1** may noncompetitively inhibit SHP2 (Supplementary Figure 1), therefore the binding mechanism of compound **1** was further studied via molecular



mod
used for docking studies.[29] The crystal structure of SHP2 (PDB code : 5EHR) obtained from the Protein Data Bank was used as the receptor with structural defects fixed by the Protein Preparation Wizard module.

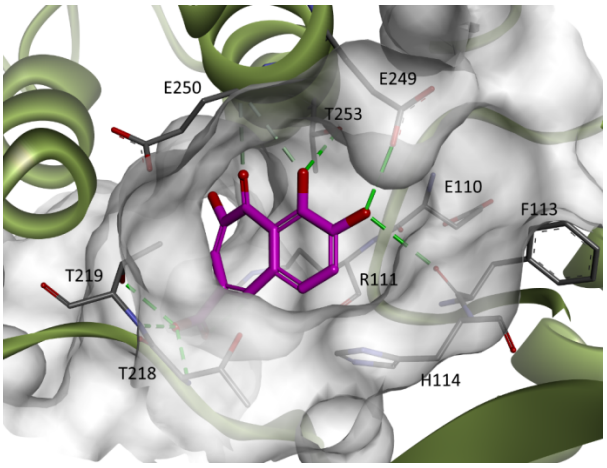


Figure 5. Predicted binding pose of compound **1** (purple sticks) in the allosteric binding site of SHP2. SHP2 is drawn by ribbons along with the interacting residues represented as sticks, and hydrogen-bonding interactions are marked with dashed green lines.

The predicted binding pose of **1** in the allosteric binding site of SHP2 (Figure 5). The activity of **1** was mainly attributed to several strong hydrogen bond interactions and hydrophobic interactions. The carboxylate makes hydrogen bond interactions with Arg111 and nitrogen of Thr218 and Thr219. This hydrogen bond is crucial for the activity, which explains the high activity of **1** and **11**. The hydroxyl groups also make hydrogen bonds with Glu110, Phe113, Glu249 and Thr253. 2-F in compound **11** makes halogen-π interaction with His114. Bigger substituents in **9** and **10** may result in steric collision with His114, resulting in loss of activity.

In conclusion, compound **1** with IC_{50} of 0.097 μM was obtained from high-throughput screening using chemical libraries from Korea Chemical Bank. Efforts by structure-activity relationship to improve SHP2 inhibition activities was not superior to this parent compound. Compound **1** exerted higher potency against SHP2 expressing 2D and 3D MDA-MB468 than SHP099. Further study is underway and will be reported in due course.

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Supplementary Material

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.

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